Synthesis and characterization of some biologically active nitrogen containing compounds: Development of new chemotherapeutic agents

THESIS

SUBMITTED TO

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A CENTRAL UNIVERSITY)
LUCKNOW

BABASAHEB
BHIMRAO
AMBEDKAR
UNIVERSITY

LUCKNOW

प्रज्ञा शील करुणा

ESTABLISHED 1996

FOR THE DEGREE OF

Doctor of Philosophy

IN

APPLIED CHEMISTRY

Submitted by

Akhilesh Kumar Shukla

(M.Sc. Chemistry, CSIR-NET)
(Enrolment No- 101/15)

Under the supervision of

Dr. Jyoti Pandey

Assistant Professor

DEPARTMENT OF APPLIED CHEMISTRY
SCHOOL FOR PHYSICAL SCIENCES
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
(A Central University, NAAC Accredited 'A' Grade)
VIDYA VIHAR, RAEBARELI ROAD, LUCKNOW-226 025

2020

Dedicated to

My Parents

Whose inimitable love and affection stands before me as a constant source of energy & inspiration in every walk of my life.....

DECLARATION

I, hereby declare that the research work embodied in this thesis entitled "**Synthesis and characterization of some biologically active nitrogen containing compounds: Development of new chemotherapeutic agents**" is original research work done by me under the supervision of **Dr. Jyoti Pandey, Assistant Professor, Department of Applied Chemistry, School for Physical Sciences, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, U.P., India**. It has not been submitted in part or full for any other diploma or degree in any other University. In this Thesis, matter written, data presented are original and if any plagiarism, is the sole responsibility of the student Mr. Akhilesh Kumar Shukla. If any allegations/queries/questions arise regarding the thesis, I will be solely responsible and answerable.

I also declare that the thesis is essentially free from all kinds of plagiarism.

Akhilesh Shukla

Akhilesh Kumar Shukla
Research Scholar
Department of Applied Chemistry
BBAU Lucknow

CERTIFICATE

This is to certify that the thesis entitled "**Synthesis and characterization of some biologically active nitrogen containing compounds: Development of new chemotherapeutic agents**" submitted by **Mr. Akhilesh Kumar Shukla,** M.Sc. (Chemistry) is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

The Thesis submitted to Babasaheb Bhimrao Ambedkar University satisfies all the requirements as stipulated in the *Doctor of Philosophy (Ph.D.) regulations - 1999 as amended in ~~2008/2010~~/2013/~~2016/2019~~* and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the University.

Date: 28/01/XX

Place: Lucknow

28/01/XX

Supervisor

Head of the Department

28/01/2020

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW
प्रज्ञा शील करुणा
ESTABLISHED 1996

बाबासाहेब भीमराव अम्बेडकर विश्वविद्यालय

(केन्द्रीय विश्वविद्यालय)

विद्या विहार, रायबरेली रोड, लखनऊ–226025

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

(A Central University)

Vidya Vihar, Raebareli Road, Lucknow-226025

Letter No.- 149 **/COE/BBAU/2017** **Dated:** 28/07/2017

Ph.D. Course Work Certificate

This is to certify that **Akhilesh Kumar Shukla**, Enrollment No. 101/15 Ph.D. Research Scholar, Department of Applied Chemistry of the University has successfully completed his Ph.D. Course work in the examination held during December, 2015.

(A.K. Maurya)
Deputy Registrar (Exam.)

ACKNOWLEDGEMENTS

Milestones of life are achieved, not only by individual efforts but with blessing, guidance and help of parents, teachers, friends, elders, and our mighty Lord. Many people have contributed directly and indirectly to my education and research. Here I will express my gratitude for all of them.

First, I want to thank especially my parents, for allowing me the possibility of a great education. My parent's dedication and encouragement are invaluable and without their support, I would not be writing this note.

I would like to express my immense gratitude to my supervisor ***Dr. Jyoti Pandey*** *who encouraged and supported my efforts, knowledgeableguidance during my doctoral research venture. Her scholarly suggestions, immense interest, constant help and affectionate behavior have been a beacon of light for me. Her suggestions will remain with me as an infinite source of scientific learning throughout my life. Her observations and comments helped me to establish the overall direction of the research and to move forward. I am indebted to her more than she knows.*

I wish to convey my profound thank to ***Prof. Sanjay Singh****, Vice-chancellor BabasahebBhimraoAmbedkar University for providing the necessary facilities during the course of the work and giving me the opportunity to work in the University. I would also like to thank* ***Prof. Kaman Singh*** Head*, School for Physical & Decision Sciences, Babasaheb Bhimrao Ambedkar Universityfor providing the necessary facilities and suggestions during the entire period of my work.*

I am deeply indebted to ***Dr. Rama P. Tripathi,*** *Dean & Course Coordinator (Medicinal Chemistry), NIPER-Raebareli and former Chief Scientist & Prof. AcSIR CSIR-CDRI, Lucknow for their crucial contribution and ever-ready help, which made them a backbone of this research and so to this thesis.*

*I owe my sincere thanks to**Prof. Gajanan Pandey,Dr. Anjani Tiwari**, Dr. Shailesh Kumar, Dr. Preeti Gupta, and Dr. Jawahar Lal Jat,DAC Division,BBAU for their crucial contribution and ever-ready help during my research period.*

*I heartily acknowledge my CSIR-CDRI senior colleagues, friends and juniorsDr. A. Ajay, Dr.N. Anand, Dr. Munna. P. Gupta, Dr. Rahul Sharma, Dr.KapilUpadhyaya, Dr. G. Ramakrishna, Dr.Ravi K. Thakur, Dr.Kartikey Singh, Dr. Udai K. Patel and Navendu Pathak, for their support and constructive research environment. I am also indebted to **Smt. PremaTripathi** for her constant support, motivation, valuable suggestions and providing me family like atmosphere.*

*It is an honor for me to thank all my teachers since my childhood days to M.Sc. who had taught me to come this far and each one had a contribution in shaping my persona and intellect over a period of my lifetime. I am especially thankful to my teachers especially, **Prof. V.K. Panday**, Prof. A. K. S. Chauhan, Prof. R. N. Pathak, Prof. R. M. Naik, Prof. Naveen Khare, Prof. Anil Mishra, Prof. V. K. Sharma, Prof. Padam Kant,Dr. A.D.Mishra, Dr. N.P.Tripathi, Dr. A.K.Singh and Dr. Abhinav Kumar sir for their motivation and suggestions.*

*Friendship is a god given virtue and a valuable asset. I pay my heartiest thanks to my dear friends specially **Dr.Vishwa Deepak Tripathi, Dr. Lalit P.Gupta, Dr. Puneet Gupta, Dr.Avinash Tiwari, Dr.Anand Pandey, Vikram Mishra,Dr.Nisha Yadav,Dr.Manish Yadav, Dr. Pankaj Nag, Dr. Shivam Maurya,Dr. Tripurari pujan, Kamal Verma, Saurabh babu,Dr. Aniruddh P. Chaudhary,Dr. Atul Upadhyay, Ranjan Singh,Prashant Mishra, Santosh Mishra** without whom it was near impossible to fulfill my objective.*

*I also would like to thank my senior **Dr.Manoj K.Shrivash, Dr. Deepak Kumar, Dr. Abhishek Verma** and juniors Baldev, Preeti Kanchan, Huda Khanam in BBAU for providing ever possible help.*

*The driving forces for accomplishment of my endeavor are my beloved parents **Shri Jata Shanker Shukla** & **Smt Kanti Shukla**, who extended every care, moral support and affection not only in this period but during my entire span of life. Once again i pay my sincere obeisance to my cherished parents without whom it was near impossible to fulfill my objective.*

*I am particularly grateful to my wife, **Smt. Vijaylakshmi**, for the encouragement and support that she provided during the research and so to this thesis.I will remain indebted to my loving all brother & sisterMadhulika,Anuradha, Renu, Acharya Avinash,Achyut,Ashutosh, Savita, Sarita, Adarsh, Arvind, Anshima,Saurabh, Anshuman, Aviralfortheir care, affection, encouragement and support.I am thankful to my sweet little son "**Ramendra**" and daughter "**Artika**" for their beautiful smile, who always prompts me to achieve the goal. Many more have helped me in this endeavor and I would like to thank them all with an excuse of not able to accommodate their names.*

Last but not the least, financial assistance in terms of research fellowship by UGC, New Delhi is gratefully acknowledged.

*I am thankful to the **Babasaheb Bhimrao Ambedkar University, Lucknow** for allowing me to submit this work in the form of a thesis. I owe my most sincere gratitude to the Almighty for giving me such a great combination of family, friends and teachers.*

Finally, I would like to thank everybody who was important to the successful realization of thesis, as well as expressing my apology that I could not mention personally one by one.

Thanks to all

(Akhilesh Kumar Shukla)

PREFACE

The discovery of novel and efficacious human therapeutics is one of humanity's most crucial responsibilities. In an ideal world, no education would be absolute without some exposure to the ways in which new medicines are discovered and developed.

Medicinal chemistry is a multidisciplinary science together with synthetic organic chemistry, natural products chemistry, enzymology, chemical biology and computational method. It is concerned with the design and synthesis of new chemical entities in order to produce new medicines for the prevention and treatment of human diseases. It also includes the study of available drugs, their biological properties and their structure activity relationship study.

One of the widely accepted approaches in drug design is the synthesis of library of compounds based on the existing knowledge of biological targets or synthesis of analogues of existing drugs or lead molecules. Human genome sequence has enabled the understanding of the genetic and molecular bases of diseases and the identification of new molecular targets, followed by their validations for drug development. The drug is most commonly an organic small molecule which activates or inhibits the function of biomolecules such as protein which in terms results in a therapeutic benefit to the patient.

The most abundant source of organic materials has revolutionized the drug discovery process from heterocylces along with carbohydrates also. A great deal of drug molecules has been developed and tremendous works are in progress from heterocycles. The developments of heterocycle hybrids as information carrier during various biological processes such as trafficking of different biomolecules, modulation of protein function, energy storage, intercellular adhesion, signal transduction, malignant transformation, viral and bacterial cell surface recognition, as well as involvement in selective binding and several other molecular recognition phenomenon make them superb in

pharmaceutical, medicinal and biological sciences.Drug discovery is the multidisciplinary and multistep laboratories processes to tackle one of the biggest fundamental issues in health sciences are the design and creation of smarter, safer and better drugs against different diseases such as malaria, tuberculosis, cancer, diabetes,HIV, etc. is fundamental goal of medicinal chemists.

The work embodied in this thesis has been carried out in the Department of Chemistry, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow, Uttar Pradesh226025, India during the period 2015 to 2019. The thesis is divided into four chapters.

Chapter 1: Illustrates an overview on '*Hydroxamate analogues: Recent progress in the synthesis of compound & their therapeutic importance'*. This review describes in brief the synthesis and other properties of currently available hydroxamates based drug analogues developed in the past 50 years as anticancer/antitumor drugs.

Chapter 2: Describes the*'Synthesis of N-hydroxycinnamide derivatives and their bioevaluation'*byutilizing the Horner-Wadsworth-Emmons (HWE) olefination of different aromatic aldehydes with triethylphosphonoacetate (TEPA) in the presence of LiOH in THF at ambient temperature andthe acrylate derivatives with hydroxylamine hydrochloride in the presence of KOH in methanol at 0-5 °C results in the formation of respective N-hydroxyacrylamide in moderate to excellent yields.The synthesized hydroxamate analogues were evaluated for their anti-cancer activity in both MCF-7 (ER+ve) and MDA-MB-231 (ER-ve) cells and HEK-293 cells using MTT assay.

Chapter3: Describes the '*Synthesis of novel purine nucleoside analogues and their biological evaluation*'with a view todeveloped a new series of glycoconjugates comprised of three core structural units: (i) a sugar moiety, (ii) 1, 2, 3-triazole moiety and (iii) 2-amino-6-chloropurine unit. The sugar provides a common drug template while the 2-amino-6-chloropurine unit acts as an important pharmacophore for antifungal activity and the 1,2,3-triazole linker is

a bio-isostere of the amide bond serves as a biocompatible, non-labile covalent spacer between the sugar and 2-amino-6-chloropurine unit them as antifungals. These compounds were screened for their *in-vitro* anti-fungal activity against *Aspergillus niger*and *Aspergillusterreus.*

Chapter4: Describes the '*Synthesis and bioevaluation of novel glycosyl 1, 2, 3-1H-triazolyl methyl benzamide derivative*'involving CuAAc of the N-propargylated 2-amino-N-(prop-2-yn-1-yl)benzamide/2-hydoxy-N-(prop-2-yn-1-yl) benzamide and 2-(4-methylphenylsulfonamido)-N-(prop-2-yn-1-yl) benzamide derivatives with different sugar azides in the ambient condition in excellent yields. The synthesised glycohybrids were screened for their anti-diabetic activity.

AKHILESH KUMAR SHUKLA

LIST OF ABBREVIATIONS

Ac	:	Acetyl
AcOH	:	Acetic acid
AcOBu	:	n-Butyl acetate
AcCl	:	Acetyl chloride
Ac_2O	:	Acetic anhydride
ALL	:	Acute lymphoblastic leukemia
Aq	:	Aqueous
bs	:	Broad singlet (in NMR)
$CuSO_4$	:	Copper sulphate
CH_3CN	:	Acetonitrile
cm^{-1}	:	1/centimeter
COSY	:	Correlation spectroscopy
$CDCl_3$	:	Deuterated chloroform
DNA	:	Deoxyribonucleic acid
DMF	:	*N*,*N*-Dimethylformamide
DCE	:	1,2-Dichloroethane
DMSO	:	Dimethylsulfoxide
DCM	:	Dichloromethane
DBU	:	1,8-Diazabicyclo [5.4.0] undec-7-ene
DHFR	:	Dihydrofolatereductase
DMSO-d6	:	Deuterateddimethlysulphoxide
DMAP	:	4-(Dimethylamino)pyridine
DIBAL	:	Diisobutylaluminium hydride
dd	:	Doublet of doublet (NMR)
EtOAc	:	Ethyl acetate
EtOH	:	Ethanol
Et_3N	:	Triethyl amine
Et_2O	:	Diethyl ether
ESIMS	:	Electro Spray IonizationMass Spectrometry
equiv.	:	Equivalent
FDA	:	Food and drug administration
g	:	Gram
hCNT	:	HumanConcentrativeNucleosideTransporter
hENT	:	Human Equilibrative Nucleoside Transporter

HPLC	:	High-performance liquid chromatography
HIV	:	Human immunodeficiency virus
Hz	:	Hertz
HMDS	:	Hexamethyldisilazane
IR	:	Infra-red
IC_{50}	:	Inhibitory concentration 50%
J	:	Coupling constant (in NMR)
K_2CO_3	:	Potassium carbonate
m	:	Multiplet (in NMR)
MDS	:	Myelodysplastic syndrome
mL	:	Millilitre (s)
mmol	:	millimole (s)
m.p.	:	Melting point
m/z	:	Mass to charge ratio
MeOH	:	Methyl alcohol
mg	:	Milligram
MsCl	:	Methanesulfonyl chloride
MeCN	:	Acetonitrile
NMR	:	Nuclear magnetic resonance
NaOMe	:	Sodium methoxide
NaOEt	:	Sodium ethoxide
NaOH	:	Sodium hydroxide
NaN_3	:	Sodium azide
Na_2SO_4	:	Sodium sulphate
PNP	:	Purine nucleosidephosphorylase
ppm	:	Parts per million
$POCl_3$	:	Phosphorus oxy chloride
PhSH	:	Thiophenol
PTSA	:	Para toluene sulfonic acid
P_2O_5	:	Phosphorus pentaoxide
Pf	:	Plasmodium falciparum
q	:	Quartet
Q-TOF	:	Quadrupole time of flight
Rf	:	Retention Factor
RT/rt	:	Room temperature
RNA	:	Ribonucleic acid

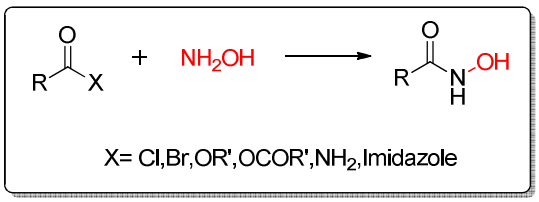
s	:	Singlet (in NMR)
THF	:	Tetrahydrofuran
TLC	:	Thin Layer Chromatography
t-BuOH	:	Tertiary butanol
TMS	:	Tetra methyl silane
t	:	Triplet
Ts	:	*p*-Toluenesulphonyl
TsCl	:	*p*-Toluenesulphonyl Chloride
µM	:	micromolar(s)
µ	:	Micro
WHO	:	World health organization
Zn	:	Zinc
BuLi	:	Butyl lithium
CuI	:	Copper iodide

Chapter-1

Hydroxamate analogues: Recent progress in the synthesis of compound and their therapeutic importance

1.1.INTRODUCTION

Hydroxamic acids or hydroxamate form a class of compounds which display interesting chemical and biological properties, containing the functional group–CONHOH[1-3]. They are the amide derivatives, where the hydrogen atom of NH_2 group has been replaced by an OH group. Hydroxamates form a class of compounds which display interesting chemical and biological properties. Among the several possible synthetic methods for the preparation of hydroxamic acids, two approaches, which have been used in most of the cases, are (i) reaction of acyl halides with hydroxylamine and (ii) reactions of acids or esters with hydroxylamine (**Scheme 1**).



Scheme .1 General synthesis of hydroxamates

Both synthetic pathways correspond to acyl substitution where the nucleophile is the hydroxylamine as free base and the leaving group can be either halides X– or the R′O– depending upon the starting compound. These are the most used and reliable methods which are currently applied for the preparation of known as well as new hydroxamic acids. Hydroxamates mainly exist in two tautomer forms: keto-form and oxime-form, as shown in **Figure 1.**

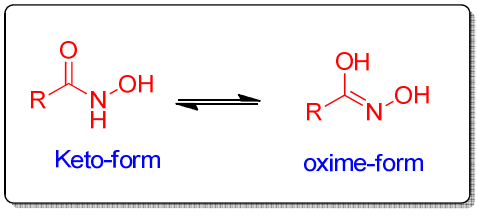
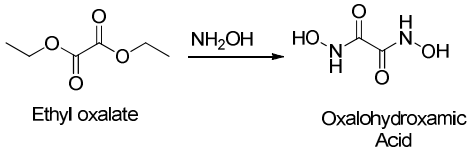


Figure.1. Tautomerism in hydroxamates

Their carbonyl and*N*-hydroxy groups are capable of forming a complex with different metals in a bidentate fashion, making them excellent ligands for a number of metals of biological significance including zinc, iron and nickel. Among these metals, zinc is one of the most frequently occurring metals in metalloenzymes (>300 enzymes) particular in zinc-dependent endopeptidases [4, 5], matrix metalloproteases (MMPs) [6, 7], and histone deacetylases (HDACs) [8-10], which play substantial roles in cancer therapy in previous decades.

1.2 . History

The chemistry of hydroxamic acids or hydroxamates initiated in 1869 when H. Lossenisolatedoxalohydroxamic acid from the reaction between ethyl oxalate and hydroxylamine (**Scheme 2.**)[11]. Although these compounds have been known for over 149 years, their synthesis continues to occupy plenty of pages of scientific journals dealing with new synthetic approaches and possible applications in organic chemistry as well as medicinally bioactive molecules. A number of review articles dealing with the chemistry of hydroxamic acids [12-14]. One of the earliest is by Lossen, which summarizes the status of nearly 25 years of research done by Lossen's group. The work published in Chemical Reviews in 1943 represents the first complete review dealing with the nomenclature, synthesis, and chemistry of hydroxamic acid. Thirty years later, an updated chapter by Sandler and Karo was published in organic functional group preparation, a book edited by Academic Press in 1972 [15].



Scheme 2. Synthesis of oxalohydroxamic acid

1.3.Current status

After decades of very successful drug discovery and development, drug discovery has highlighted the diverse biological and pharmacological properties of key pharmacophore. Pharmaceutical industry scaled-down hydroxamates research in the late 1990s in favour of automated high throughput screening (HTS) of compound libraries. Compound libraries assembled with the aid of combinatorial chemistry were thought to produce more hits than 'old fashioned' hydroxamates. Despite this decline in the use of hydroxamates in drug discovery, newly marketed drugs derived from hydroxamates hold about the same share as before. The numerous and successful discoveries of compounds in the early times of modern drug discovery were quite exclusively based on the traditional use of the hydroxylamine hydrochloride. In accordance with their nature, hydroxamates derive molecules is an upsurge in the demand for drug discovery and development.In view of their remarkable significance, tremendous efforts have been devoted to access a large variety of molecules through significant and convenient strategies. Next, we discuss the recent development in drug discovery has emphasized the diverse biological and pharmacological properties as an anticancer [16, 17], antimalarial [18-20], cardiovascular [21, 22], anti-HIV [23-25], anti-inflammatory/antiallergic agents [26, 27], anti-bacterial activities(**Figure 2**)[28-30].

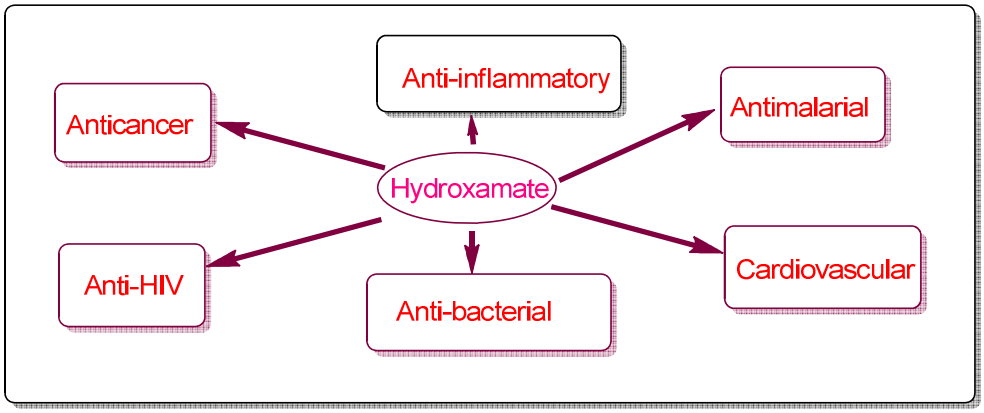


Figure 2. Various medicinal property associated with hydroxamate moiety**.**

1.4. Hydroxamates: Classification

Hydroxamatecan be categorized into two categories, i.e. simple or complex, depending upon the chemical structure and ease of digestion of hydroxamate-

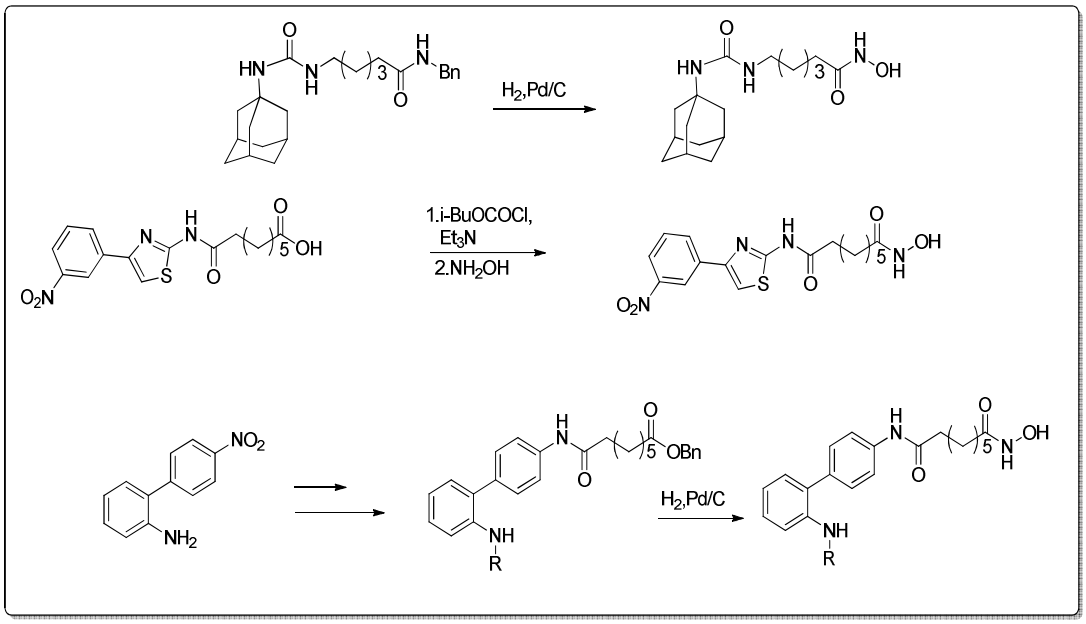
1.4.1. Hydroxamic acid having aliphatic chain spacers

Histone deacetylase inhibitors having aliphatic linker can be further separated into dissimilar subclasses on the basis of branching and saturation of alkyl chains.

1.4.2. Hydroxamic acid having a straight-chain aliphatic spacer

Generally all SAHA style hydroxamic acid require common structural characteristics: zinc-binding group (ZBG) in the catalytic pocket, opposite capping group, and straight-chain alkyl, vinyl, or aryl linker involving the two [31]. These functional groups cooperate with three relatively preserved regions of the catalytic pocket of HDACs [32]. The linker in the hydroxamic acid and amide must be at least five carbon atoms in length, by lengths of five and six being optimal. Introducing an alkyl spacer length of 2, 3, or 4 condensed both enzyme inhibitory and anti-proliferative potency. This is due to the transformed orientation of terminal aryl group of the inhibitors, which leads to a significant reduction in the biological activity [33-35].

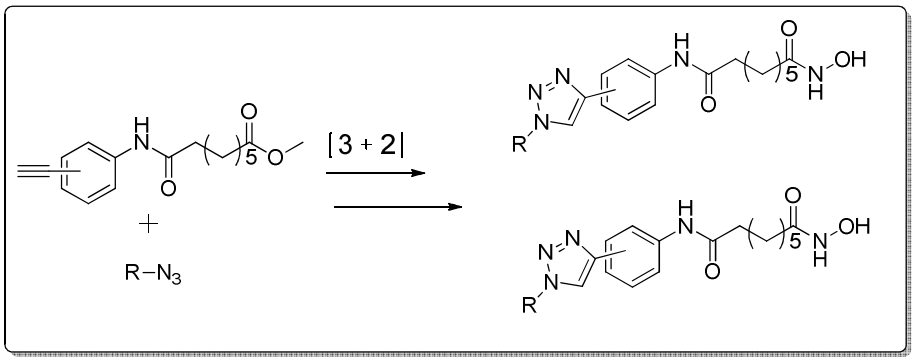
Kozikowski*et al.* also measured the nature of the ZBG present in the HDACi to achieve specific therapeutic endpoints [36]. They associated the ability of HDACi containing either a hydroxamate or a mercaptoacetamide as the zinc-binding group, and revealed that some of the mercaptoacetamide based HDACi are fully protective, however, the hydroxamates, indicated toxicity at higher concentrations. The consequences of these findings were reliable with the possibility that the mercaptoacetamide-based HDACi interact with an unlike subset of the HDAC isozymes or otherwise, interact selectively with only the cytoplasmic HDACs that are decisive for defence from oxidative stress (**Scheme 3**)[37, 38].



Scheme 3. Synthesis of HDAC inhibitors containing hydroxamate or mercaptoacetamide group.

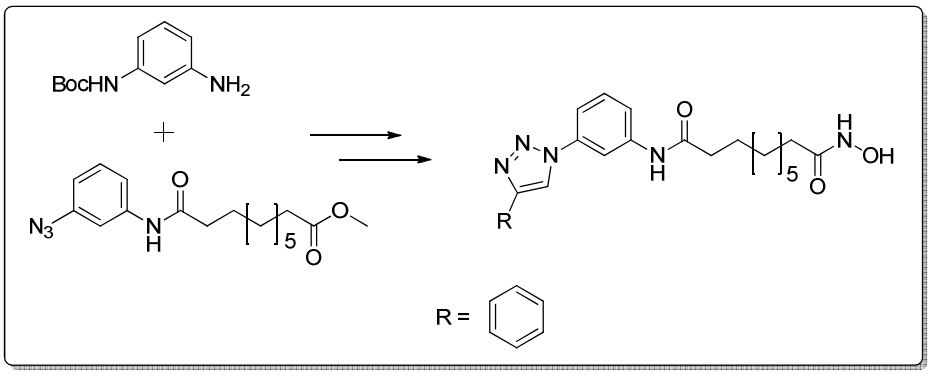
Chen *et al.* reported that the cap region of a set of triazolylphenyl-based HDACi and displayed that the nature of substitution on the phenyl ring played important role in their selectivity for HDAC1 versus HDAC6 (**Scheme 4**). The triazolyl ring combined with the phenyl ring system showed the inhibition of

HDAC6(IC_{50} 1.9 nM), this compound embodied a valuable research tool and a candidate for further chemical modifications [39].



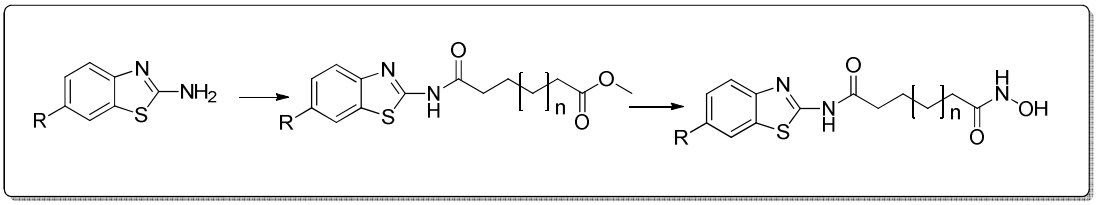
Scheme 4. Synthesis of triazolylphenylbasedHDACi.

He *et al.* distinctly reported 1-aryl-1H-[1, 2, 3] triazolylphenyl based analogues and tested for their anti-proliferative activities and HDAC isoform selectivity against pancreatic cell lines (**Scheme 5**). It was found to reenergize the expression of CDK inhibitor proteins and to conquer pancreatic cancer cell growth *in vivo*. One of the compounds was found to be a very effective inhibitor of cancer cell growth *in vitro* with the lowest IC_{50} value of 20 nM against MiaPaca-2 cell. The replacement of phenyl ring of SAHA with heterocyclic nucleus like benzothiazole formed active analogues of SAHA [40].



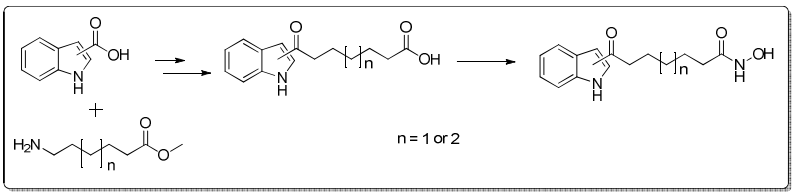
Scheme 5. Synthesis of triazolyl-phenyl basedHDACi.

Oanh*et al.* observed that numerous compounds with six carbon-bridge linking benzothiazole moiety and hydroxamic functional groups exhibited good inhibitory activity against HDAC3 and HDAC4 at as low as 1 mg/ml and showed potent cytotoxicity against five cancer cell lines (SW620, MCF-7, PC3, AsPC-1 and NCI-H460) with average IC_{50} values of 0.81 mg/ml, almost equipotent to SAHA (**Scheme 6**)[41, 42].



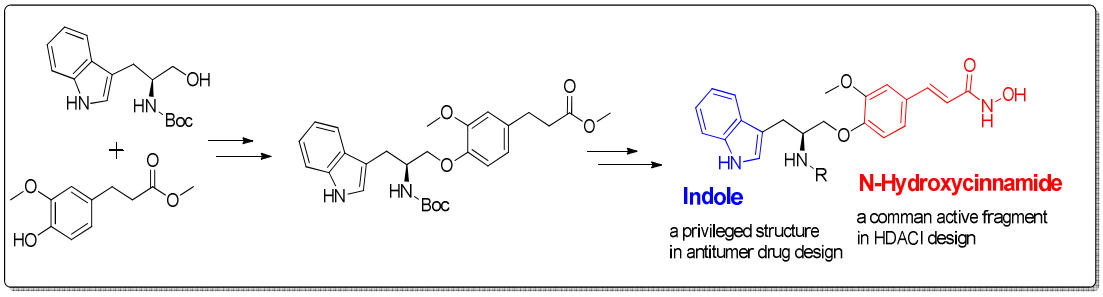
Scheme 6. Synthesis of six carbon-bridge linkedbenzothiazole based HDACi.

An indole amide moiety at the terminus with a sequence of hydroxamic acid-based HDACi synthesized by Dai *et al.* Introduction of a methyl group on the indole nitrogen caused a 3-fold decline in activity (**Scheme 7**). Compounds with a 2-indole amide moiety were initiate as the most active inhibitors between the different regioisomer. This tendency was also seen in the 3-indolyl analogues. However, the activity fading was reversed when a benzyl group was introduced to the nitrogen atom. Introduction of substituents on the indole ring further improved the potency and produced a series of very effective inhibitors with noteworthy anticancer activity. Removal of amide linking unit from indole ring also produced active compounds [43].



Scheme 7. Synthesis of indole amide moiety based HDACi.

Gianniniet *al.* planned and established the bis-(indolyl) methane moiety that can be synthesized a sequence of hydroxamic acids (**Scheme 8**). This bis-(indolyl) methane moiety can be used as a valid surface recognition cap in the design of HDACi. SAR analysis has resolved the relevance of the bis-(indolyl) methane moiety is more potent than the mono-indolyl analogue (3-indolyl derivative is less potent than bis-(indolyl) analogue) [44].



Scheme 8. Synthesis of bis-(indolyl) methane-based HDACi.

Pandey J. *et al* synthesized *N*-Hydroxycinnamamide derivatives and evaluate them for their anticancer activity against human triple-negative breast cancer cell line MDA-MB-231, MCF-7 and Non-malignant origin cell line, HEK-293 (human embryonic kidney) **Figure 3.**[45]. Several compounds exhibit potential anticancer activity, targeting EGFR. The interaction and docking image of compound 1, 2 and 3 with EGFR target protein is shown below:

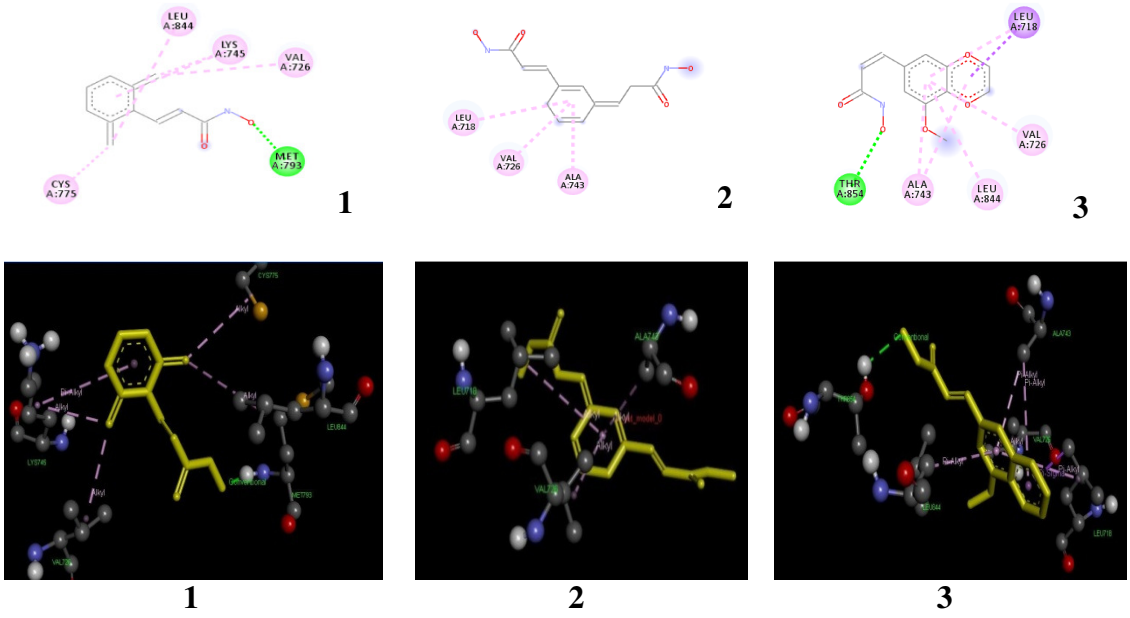
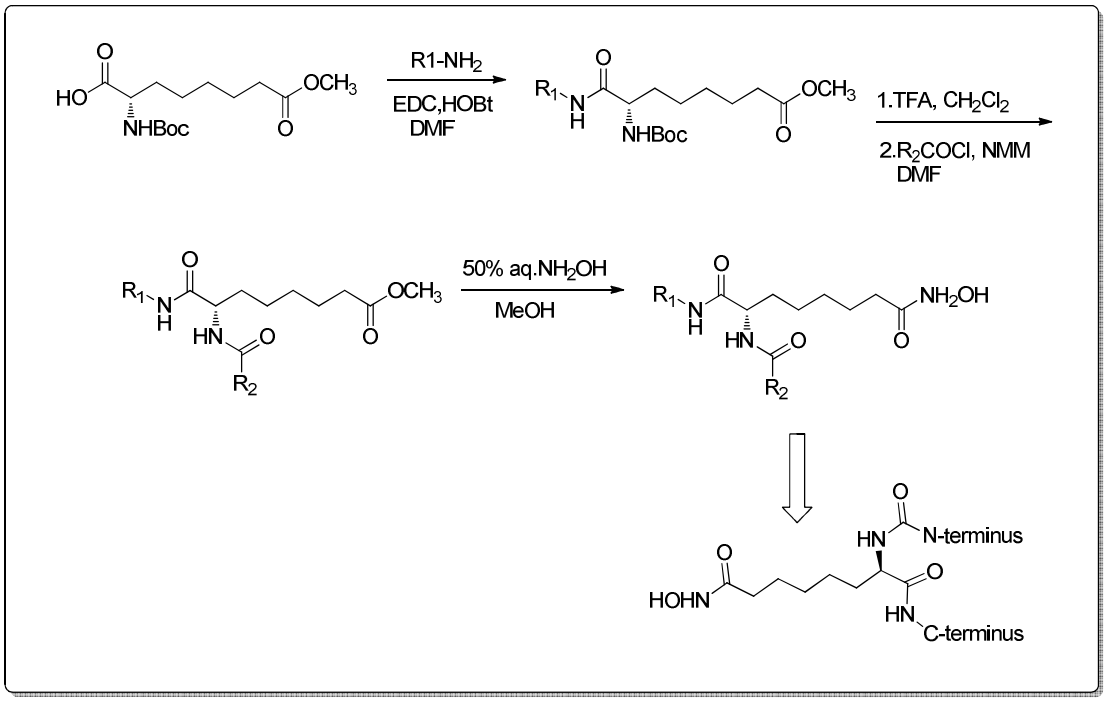


Figure 3. Interaction and docking image of compound 1, 2and 3 with EGFR target protein

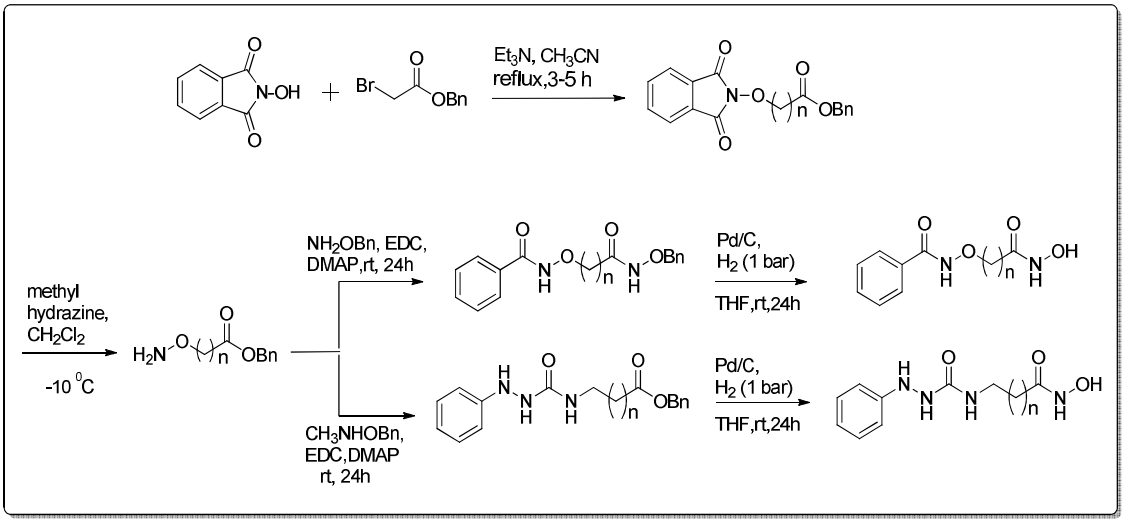
An effective novel class of HDACi of amino suberoyl hydroxamic acids (ASHAs) were explained by Belvedere *et al.*They fused aminoquinolide and anilide cap group-containing compounds. These compounds showed cell proliferation and HDAC inhibitory events at nanomolar concentrations and have been shown to inhibit tumour growth in both colon and breast xenograft models. A common trend in HDAC inhibitory strength of 6-aminoquinolide > 8- aminoquinolide>anilide was observed (**Scheme 9**)[46].



Scheme 9. Synthesis of HDACi bearing fused aminoquinolide and anilidecap group.

In order to understand the connecting units of dissimilar effect, Wang *et al.* observed novel sulfonylurea and acyl urea linking unit attached with alkyl hydroxamates (**Scheme 10**)[47]. The acyl urea moiety has extra hydrogen bond acceptors and donors as compared to amide or urea; therefore the orientation of the linker and cap group may change, thus influencing strength as well as isoform-selectivity. *N*-alkylated acyl urea and urea linked straight-chain hydroxamates offered much reduced HDAC potency due to opposed interactions at the binding pocket rim region. Bromine, naphthalene, phenyl and chloro aniline substituted cap groups showed 10–20-fold enhanced HDAC1 activity as compared to SAHA. Structure-activity relationship (SAR) was recognized for the length of linear chain linker and substitutions on the benzolylurea group. They also showed that the bulkier linker can directly interact with the hydrophobic pocket of the HDAC enzyme. In general, these compounds showed good stability (t1/2 >30 min) in the human liver microsomal

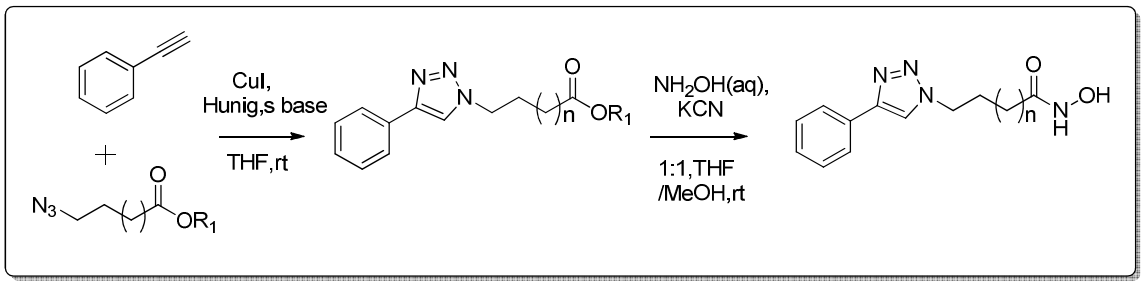
assay. To see the outcome of oxygen as connecting unit, a series of aryloxyalkanoic acid hydroxyamides was synthesized for their HDAC inhibitory activity. Scientists merged oxygen atom between aromatic cap group and alkyl spacer. Some compounds were found to be more effective *in vitro* than trichostatin A (IC_{50} = 3 nM). The strengths of aryloxyalkanoic acid hydroxyamides showed that neither an amide group (as present in trichostatin A and SAHA) nor a rigid (alkylene) chain is vital for low nanomolar HDAC enzyme inhibition. Marek *et al.* establisheda novel alkoxyamide connecting unit linker with potent hydroxamate-based HDAC inhibitor[48]. They confirmed that the insertion of alkoxyamide connecting unit linker region donates to the selectivity. This may be due to a charge-assisted hydrogen bond between the alkoxyamide nitrogen and the carboxylate group/sulphur atom of surrounding amino acid or additional polarization of the N-H bond due to the presence of the N-alkoxy moiety [49].



Scheme 10. Synthesis of HDACi having sulfonylurea and acyl urea linking unit

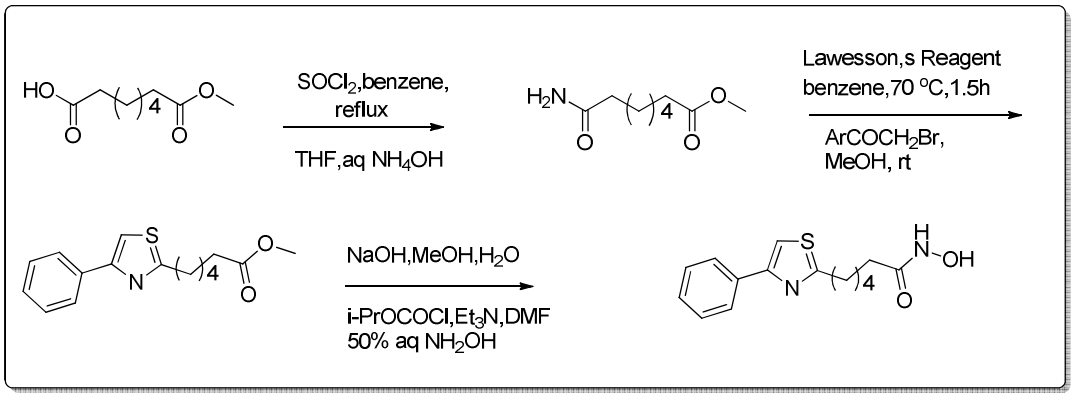
Chen *et al.* combined a 1, 2, 3-triazole ring as a surface recognition cap group-linking moiety in SAHA-like HDACi. SAR study directed that triazole-linked

hydroxamates exhibited a cap group dependent inclination for either five- or six-methylene spacer groups (**Scheme 11)**. Using ‘‘click’’ chemistry (Huisgen cyclo-addition reaction), several triazole-linked SAHA-like hydroxamates were synthesized. Docking study on histone deacetylase-like protein (HDLP) with reverence to SAHA into HDLP presented that these compounds have likings for two dissimilar binding pockets at the protein surface. They confirmed that the triazole ring defined attached to the phenyl cap group was more potent than the triazole ring and is divided from the cap group by a methylene group. This result specifies that the triazole ring is definitely an active participant in the interface of this class of compound with the HDAC active site [50, 51].



Scheme 11. Synthesis of HDACi having triazole ring as a surface recognition cap.

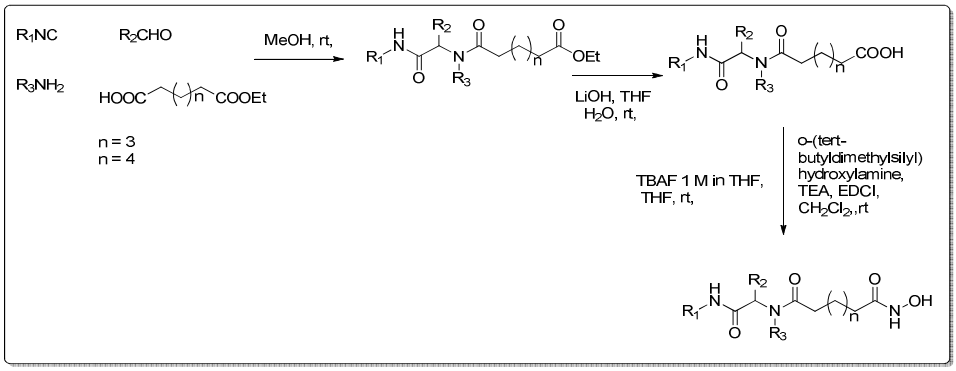
In advance studies on HDACi, Dai *et al.* fused a sequence of structurally different and effective HDACi, in which a five-membered heteroaromatic assembly assigns the part to the hydrophobic group (**Scheme 12)**. These compounds are considerably more effective than SAHA in the HDAC enzymatic assay and presented inspiring anticancer activity beside the growth of human HT-1080 fibrosarcoma and human MDA-435 breast carcinoma cell lines [52].



Scheme 12. Synthesis of HDACi having a five-membered heteroaromatic ring.

Bigioni*et al.* established a series of novel compounds that contain amide linking unit in the ring system and alkyl linker was likely attached to the nitrogen of amide. They designed and synthesized a chain of 5, 11- dihydrodibenzo [b, e] azepine-6-ones alkylated on the amide nitrogen with an alkyl chain bearing hydroxamic acids moiety at the end.They discovered that the biphenyl methyl fortunate fragment recognition style as a new series of HDACi[53]. The endocyclic ketone was accepted, but was not valuable to the binding affinity; meanwhile, it caused a three-fold drop in the IC_{50}. The effect on cell activity was more affected probably due to reduced permeability.

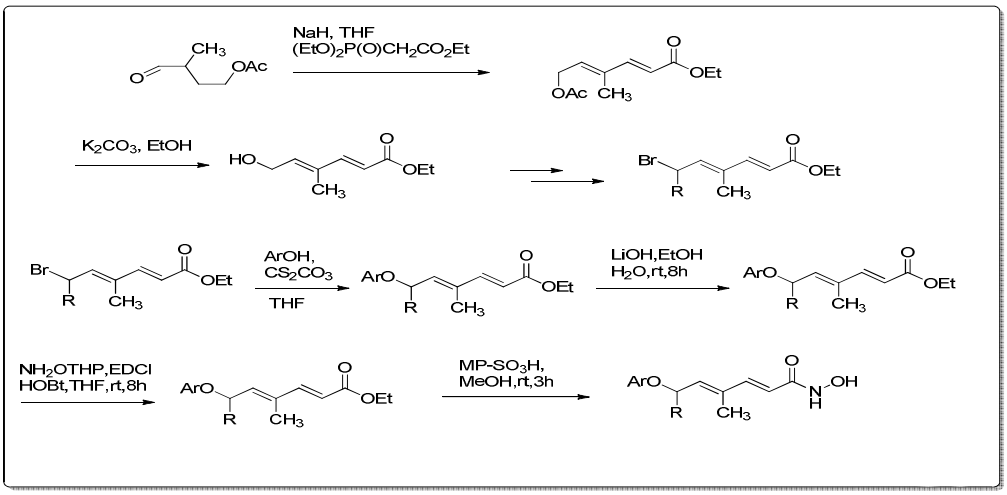
Zinc-chelating moiety was synthesized by Grolla*et al.* by using Ugi-reaction.The main reaction in the synthesis of the anticipated HDACi with Ugi-reaction transforms to the α-aminoacylamides exhibiting as ester function. One of the compounds that show improved inhibitory strengths compared to SAHA, representing the hindered lipophilic deposit grafted on the peptide framework of the α-aminoacylamides that can be favourable in the contact with the enzyme. Most of the compounds having the hydroxamate moiety displayed cytotoxic activity similar to that of SAHA (**Scheme 13)**. [54].



Scheme 13. Synthesis of HDACi via ugi reaction.

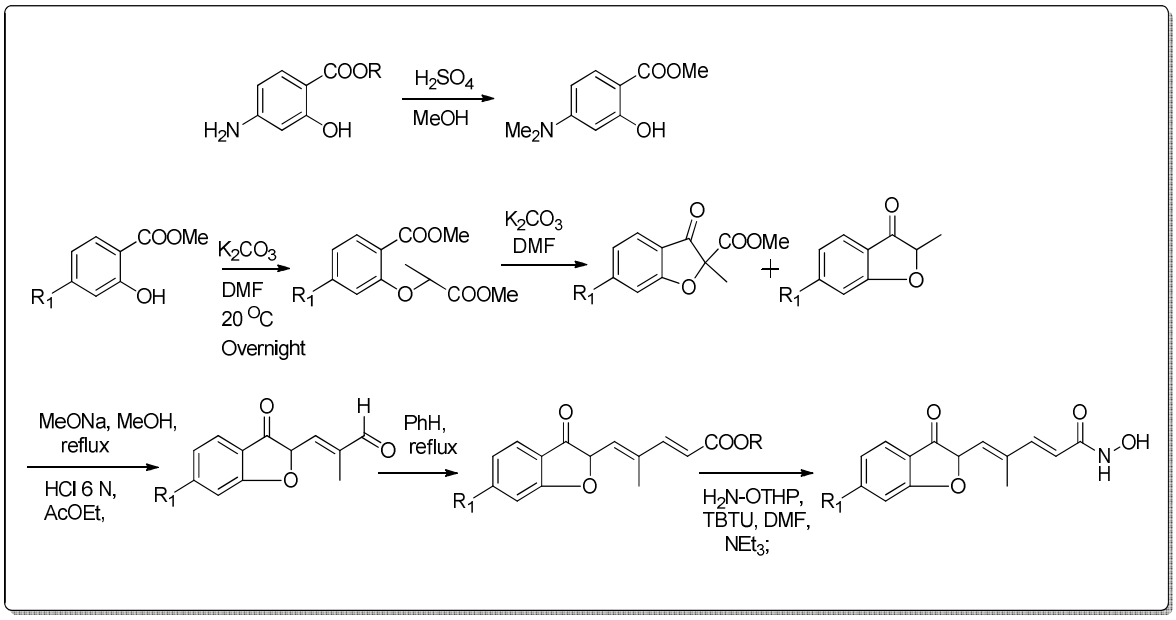
1.4.3 Hydroxamate having branched-chain aliphatic spacer

In order to enhance the potency of larger HDAC inhibitors based on TSA or SAHA Pabba*et al.* synthesized series of TSA analogues having either an aryl ether or sulphone functionality as linking unit with the overlaying group (**Scheme 14**), a branched-chain diene linker and carboxylic or hydroxamic acid terminal group accomplished of binding to the zinc residue confined within the active site of HDAC enzyme [55]. They find out the manifold increase in HDAC inhibitory action with the emergency of large arylsulphone group with the resultant aryl ether.



Scheme 14. Synthesis of TSA analogues having either aryl ether or sulphone functionality as linking unit.

To overview the (R)-methyl assembly and of the diene function, which are found in TSA, into the amide bond similarities, does not result in an improved HDAC inhibitory strength. Charrier *et al.* described the synthesis of new benzofuranonesand projected them against NCI-H661 non-small cell lung cancer cells. They established that the benzofuranones cap section does not have the desired alignment typical for hydrophobic interactions (**Scheme 15)**[56].



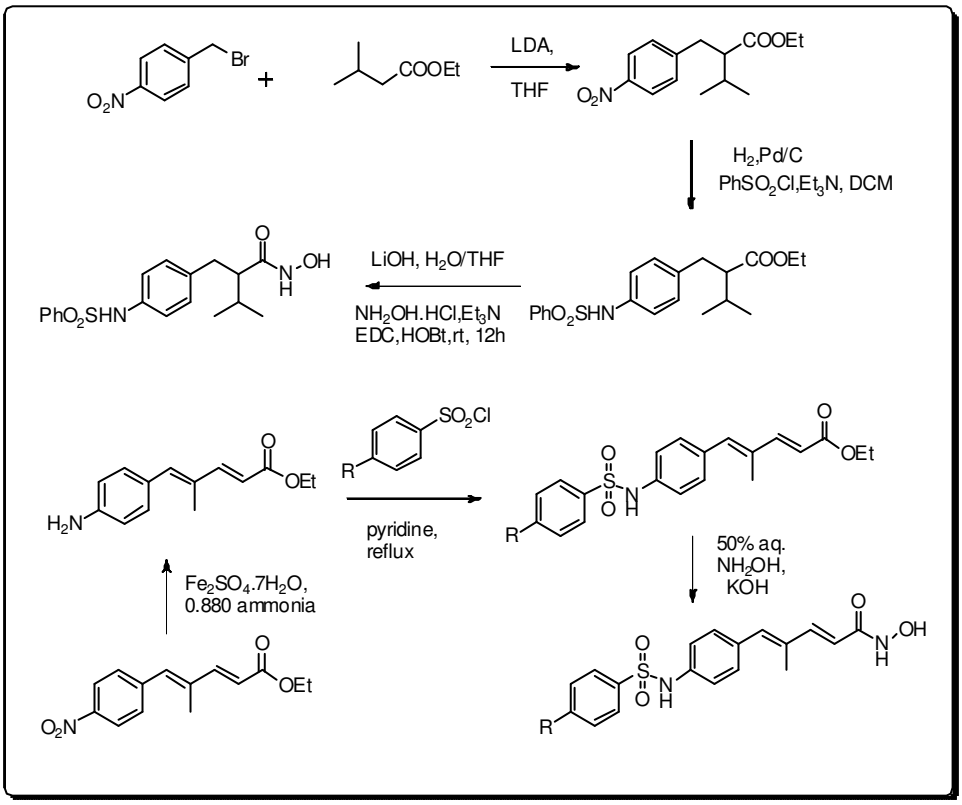
Scheme 15. Synthesis of benzofuranones based HDACi.

1.5. Hydroxamic acid-based HDACi having cyclic spacers

1.5.1. HDACi having phenyl ring cyclic spacers

An innovative chain of HDACi having sulphonamide group as an assembly unit and aromatic spacer displayed auspicious activity in HDAC inhibitory assay and antiproliferative activity. These complexes characterized by a cinnamic spacer plugged with a substituted phenyl group. Most compounds displayed an antiproliferative activity similar to that of SAHA. At equitoxic concentrations, the

established compounds were more effective than SAHA in tempting apoptotic cell death. The 4-phenyl-cinnamic acid framework was found necessary for good cytotoxic activity on dissimilar cell lines. Modification of the position of the side chain, of the proximal ring with a cyclohexyl, led to a considerable decrease in the activity. Further optimization of this arrangement by substitution of the terminal aromatic ring produced HDACi with good *in vitro* and *in vivo* activities. *N*-methylation of sulphonamide reduced the activity. For HDAC inhibitory activity, the best chain length between the aryl and the hydroxamate was established to be two carbons. These studies established that steric bulk is not acceptable near the zinc-binding site. It was detected that double bond similarities possess more than a 10-fold boosted potency related with the triple bond equivalent oxamflatin. The effectiveness of arylpenta-2, 4-dienoic acid hydroxyamides suggest that neither the keto group nor the complete seven-carbon chain in trichostatin A is dynamic for effective enzyme inhibition. Assimilation of a 4-methyl group in unsaturated chain existing between ZBG and phenyl linker improved *in vitro* potency over two orders of magnitude; a significant observation that it has similar potency with trichostatin A. Replacement on *p*-position of cap assembly with chloro group equivalent exhibited a greater strength for HDAC inhibition and MTT assay than the 3,4-dichloro and 2,4-dichloro products. A more electron-withdrawing group existing in *p*-position appears to be harmful for potency (**Scheme 16**).



Scheme 16. Synthesis ofhydroxamic acid-based HDACi having cyclic spacers.

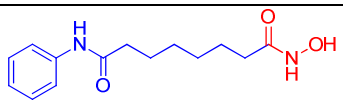
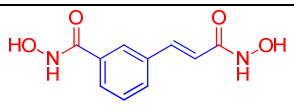
1.6. Application of hydroxamates:

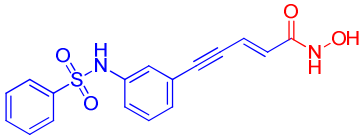
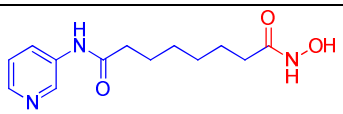
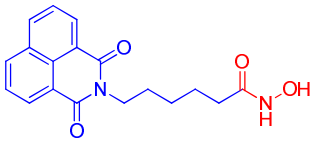
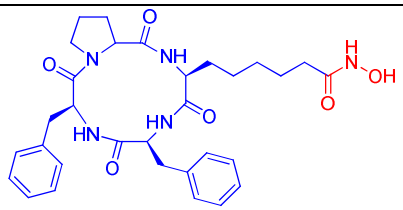
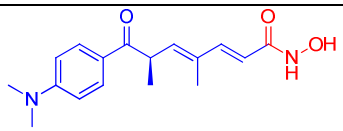
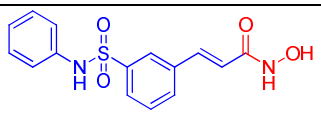
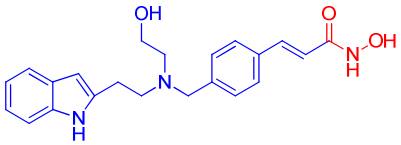
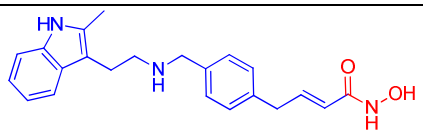
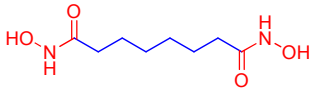
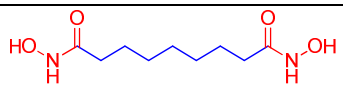
1.6.1. Hydroxamates as histone deacetylase inhibitor (HDACi)

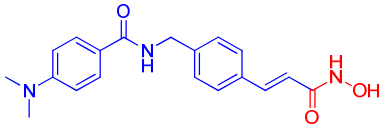
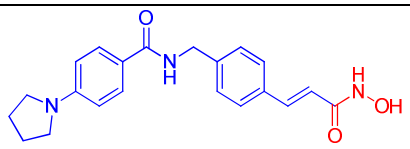
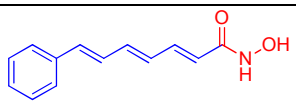
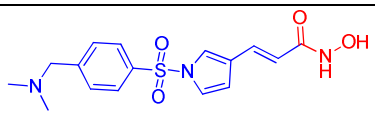
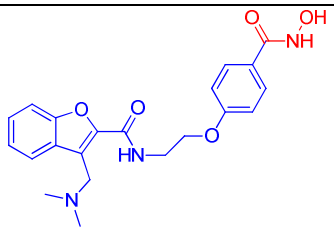
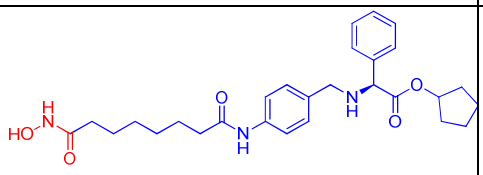
Hydroxamic acidsor hydroxamatesare the utmost extensively discovered class of HDACi. Essential features of hydroxamic acid-based molecules are the polar hydroxamic group, a four–six-carbon hydrophobic methylene spacer (CU, polar connection unit), a second polar site, and a terminal hydrophobic group. Contemporary in most HDACi, the CU can network with amino acids in the channel, and a four or six-carbon component hydrophobic spacer (linker), permitting the zinc-binding group to effect and compound with the zinc ion hindering the enzyme [57]. HDAC inhibitors can encourage cell cycle arrest, apoptosis and cellular differentiation, as well as hinder angiogenesis.Suberoylanilidehydroxamic acid

(SAHA, Vorinostat), the sample of this kind of HDACi, efficiently inhibits HDAC1, 2, 3, 4, 6, 7, and 9, and shows lower potency contrary to HDAC8 [58-60]. At the molecular level, the antiproliferative properties of SAHA include the accumulation of acetylated histones, succeeding in transcriptional activation and reduction of cyclin B1, c-myc, and cyclin D1 levels (independent of the active β-catenin pathway), foremost to induction of apoptosis, G_2/M cell cycle arrest, and cell differentiation. Trichostatin A was the core natural product revealed to display HDACi activity. TSA hinders HDAC1, 2, and 3, and HDAC4, 6, 7, and 9 HDACs at single-digit nanomolar level, being less effective against HDAC8. Data from a wide range of studies submit that TSA causes apoptosis in tumourcells; however, in normal cells, it mainly arrests cell cycle progression. Further SAHA and TSA, supplementary hydroxamate-based complexes have been designed, such as belinostat (PXD-101), panobinostat (LBH-589), and dacinostat (LAQ824) [61].

Table 1. Histone deacetylase inhibitors

Name	**Structure**	Activity*			
		HDAC	**Cell culture**	**Animal tumour Models**	**Clinical**
Suberoylanilide hydroxamic acid (SAHA)		Y	Y	Y	Y
m-Carboxy cinnamic acid bishydroxamic acid (CBHA)		Y	Y	Y	-

Oxamflatin		Y	Y	Y	-
Pyroxamide		Y	Y	Y	Y
Scriptaid		Y	Y	-	-
TPX-HA analogue (CHAP)		Y	Y	Y	-
Trichostatin A (TSA)		Y	Y	Y	-
Belinostat (PXD-101)		Y	Y	Y	Y
Dacinostat (LAQ-824)		Y	Y	Y	-
Panobinostat (LBH-589)		Y	Y	Y	Y
Subericbis-hydroximic acid (SBHA)		Y	Y	-	-
Azelaicbis-hydroximic		Y	Y	Y	-

acid (ABHA)					
SK-7041		Y	Y	-	-
SK-7068		Y	Y	-	-
CG1521		Y	Y	-	-
Resminostat (4SC-201)		Y	Y	Y	Y
Abexinostat (PCI-24781)		Y	Y	Y	-
Tefinostat (CHR-2845)		Y	Y	Y	Y

*'Y' designates that the complex has been revealed to have activity in hindering partially purified histone deacetylase (HDAC), development of altered cells in culture and *in vivo* tumour progress in animal studies.

1.6.2 Hydroxamic acid derivatives as tyrosinase inhibitor and antioxidant

Hydroxycinnamic acids such as p-coumaric acid (4-hydroxy-cinnamic acid), caffeic acid (3, 4- dihydroxy-cinnamic acid), sinapic acid (3, 5-dimethoxy-4-hydroxy-cinnamic acid) andferulic acid (3-methoxy-4-hydroxy-cinnamic acid) are well-known antioxidants, which are distributed in many plants and found in fruits, tea, coffee, and

wine. They exhibit strong antioxidant activity because they can have resonance stabilized phenoxy radicals after the hydrogen radical is abstracted due to the phenolic nucleus and extended side chain conjugation. In particular, CA, *p*-CoA and dihydrocaffeic acid (DHCA) possessing phenol or catechol moiety have drawn attention due to their multiple biological and pharmaceutical activities [62-64].

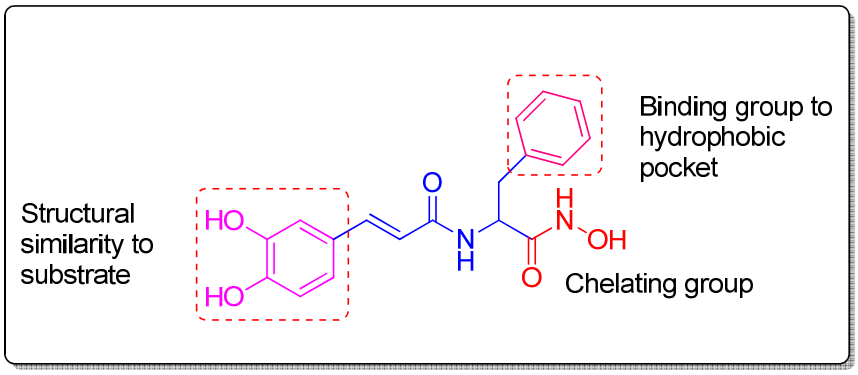


Figure 4. CA-Phe-NHOH

1.6.3 Hydroxamates as an MMP-inhibitor

Matrix metalloproteinase (MMPs) are concerned with different biological processes. They are accountable for tissue remodel and degradation of the extracellular matrix proteins; as well as gelatin, collagens, elastins, proteoglycans and matrix glycoproteins [7, 65]. MMPs are well studied to be the precursor of the metastatic process, which signal gravity in cancer evolution. MMP inhibitors are interesting molecules because they are able to limit this step of invasiveness and regulate cancer cells malignancy. Such a mode of action is exhibited by Ilomastat, batimastat, cipemastat, periostat and marimastat are all hydroxamate compounds used in the treatment of cardiovascular diseases. More precisely, MMPs exert their activity by cleaving amide bonds of peptides due to the presence of water, itself linked to the zinc (II) ion of the active site [7]. Hydroxamic acid derivatives can chelate the zinc ion

replacing the water and leading to enzyme inactivation, which explains the antitumor effects of these molecules.

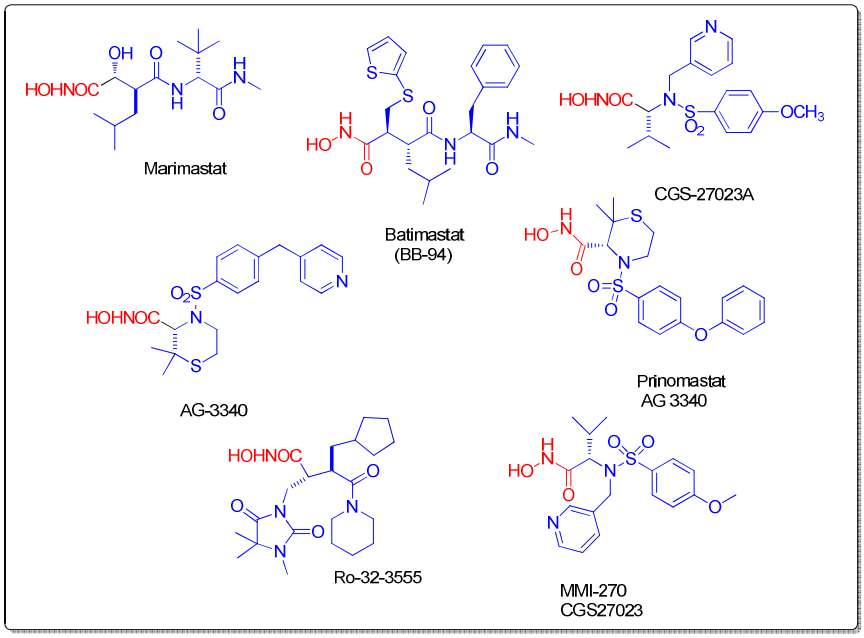


Figure5: Several MMP inhibitors are in the clinical trial

1.6.4 Hydroxamate based anti-parasitic agents

Parasites classified as macroparasites (can be seen through naked eyes) such as helminths and microparasites, which are smaller such as virus, bacteria and protozoa. Parasites feed on the host for their nutrition requirement and lead to several fatal diseases like malaria, filarial,leishmaniasis, trichomoniasisand so on. Anti-parasitic agents are a class of medication used for the treatment of disease caused by parasites. These are known to target parasites responsible for the disease either inhibiting their growth or by destroying them [20, 66].

1.7. Parasitic diseases of humans

There are a number of parasitic pathogens that cause disease in humans, and for many diseases severity is linked with the immune status of the host. For example, infection

with *Plasmodiumfalciparum*, the parasite that causes most malaria-related deaths, may be asymptomatic or cause only mild symptoms in adults living and in malaria-endemic regions that have acquired immunity to this pathogen. By contrast, children under the age of 5 in malaria-endemic regions are at most risk of severe malarial disease and death, primarily because of their undeveloped immune systems. Women undergoing their first pregnancy are more susceptible to a severe form of malaria than their non-pregnant counterparts or women from the same regions have had multiple pregnancies and immune-compromised people such as those with HIV/AIDS. Likewise, infection with *Toxoplasmagondii*, the causative agent of toxoplasmosis, is usually self-limiting but can pose a more serious threat or be fatal to the foetus of women who first contract the disease while pregnant. Toxoplasma parasites can also cause serious opportunistic infections in immune-compromised people those with AIDS, or in people undergoing chemotherapy or organ transplants. Relationships between immune status and human diseases caused by helminths, such as schistosomiasis, are also complex and may be negatively associated with HIV infection[66, 67].

In addition to the influence of pre-existing immunity or immune status, interactions between parasites and their hosts are complex, confounded by many factors, including parasite virulence, age and nutritional status, and co-infection. These factors often combine in resource-poor settings and pose significant challenges in the fight against major human parasitic pathogens. Other challenges facing researchers engaged in anti-parasitic drug discovery include difficulty dissecting the molecular mechanisms underlying parasite resistance and identification and validation of new drug targets. Drug discovery is often hindered by the complex lifecycles of human-infecting

parasites (more than one host, distinct morphological forms, and asexual and sexual replication), the genetic divergence of parasites, and the inability to genetically manipulate and biochemically characterize many species. However, in the past decade, sequencing and annotation of the complete genomes of many of the major parasites that infect humans have begun to improve our understanding of the biology of these parasites.

1.7.1 Functional role of HDACs in major human parasites

HDACs have been identified in all the major human parasitic pathogens. This section will focus on key functional findings available for characterized or partially characterized HDACs. Plasmodium HDACs only one of the three identified class-I/II HDAC homologues have been investigated in detail for *Plasmodium falciparum*. In silico homology modelling of *Pf*-HDAC1 predicts a high level of conservation of the active-site tunnel, but differences at the entrance to the active-site tunnel compared with human HDACs[68]. These differences may explain the better in vitro growth inhibition activity of some HDAC inhibitors for *P. falciparum* compared with mammalian cells. The functional role(s) of *Pf*-HDAC1 in *P. falciparum* has not yet been directly demonstrated; however, as discussed below, the consequence of HDAC inhibitor treatment of *P. falciparum* parasites is beginning to be elucidated and it is likely PfHDAC1 is involved in the post-translational modification of histones and therefore control of gene expression. Whereas little is known about the other class-I/II *P. falciparum* HDACs, the two class-III homologues have been better studied. *Pf*-Sir2A, which has both histone deacetylase and ADP-ribosyltransferase activity, and *Pf*-Sir2B are classified as type-III and type-IV sirtuins, respectively. *Pf*-Sir2A has a role in maintaining *P. falciparum* telomere length, heterochromatin establishment in

sub-telomeric regions, and the regulation of a subset of *P. falciparum* virulence genes involved in antigenic variation and cytoadhesion/pathogenesis. *Pf*-Sir2B also silences a subset of these *P. falciparum* genes, however, a set controlled by a different type of promoter than those regulated by *Pf*-Sir2A. While neither *Pf*-Sir2 is essential for *P. falciparum* growth. Their role in regulating virulence gene expression makes them potential targets for anti-disease therapies, for example by interfering with infected erythrocyte cytoadhesion to host cell receptors that mediate severe forms of the diseases and/or by blocking malaria parasite immune evasion [69].

1.7.2Antimalarial agents

Malaria is a mosquito-borne parasitic disease caused by parasitic protozoa (a group of single-celled microorganisms) belonging to genus *Plasmodium*. Malaria transmitted through the biting of infected female *Anopheles* mosquito through their saliva. A number of antimalarial drugs are available that can help to prevent or interrupt malaria like chloroquine and its derivatives pyrimethamine, sulphonamideetc.[70]

Siderophores, a Greek word meaning “iron carrier” are strong iron-chelating compounds secreted by microorganisms such as fungi, bacteria and grasses, some of them contain hydroxamates. As it is evident that malaria parasite entails Fe^{3+} during the erythrocytic phase of its life cycle, hydroxamates can be effectively used to stop the growth of malarial parasites [71].

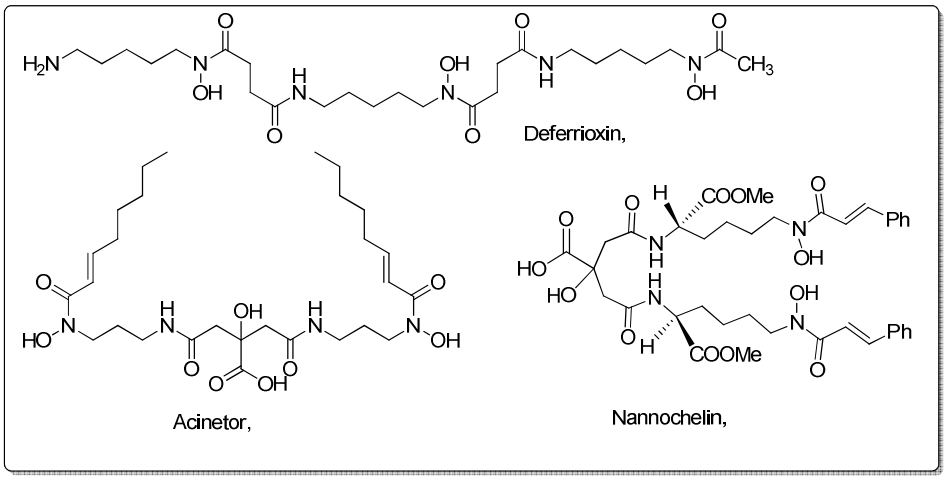


Figure 6. Structures hydroxamates siderophores

Based on the above observation, Tripathi group has synthesized a series of glycosylhydroxamates, among them two compounds, displayed good antimalarial activity. It was also observed that amine substituent is pivotal for the antimalarial activity and compounds with furanose moiety are more active than compounds with galactopyranosyl ring derivative[72].

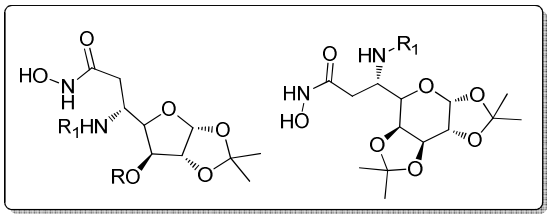


Figure 7. Some representative carbohydrate-based antimalarial agents.

Parasitic diseases cause significant global morbidity and mortality, particularly in underdeveloped regions of the world. There is no licensed vaccine available for any human parasitic disease and drug resistance is compromising the efficacy of many available anti-parasitic drugs. This is driving drug discovery research on new agents with novel modes of action. Histone deacetylase (HDAC) inhibitors are being

investigated as drugs for a range of diseases, including cancers and infectious diseases such as HIV/AIDS, and several parasitic diseases. In this review, we want to focus on the current state of knowledge of HDAC inhibitors targeted to the major human parasitic diseases malaria,toxoplasmosis, leishmaniasis schistosomiasis and trypanosomiasis. Insights are provided into the unique challenges that will need to be considered if HDAC inhibitors are to be progressed towards clinical development as potential new anti-parasitic drugs.

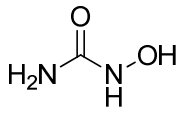
1.7.3. Anti-HCV agent

HCV is a major cause of chronic hepatitis, which affects three to four million people worldwide annually. Chronic hepatitis C is associated with a high risk for development of liver cirrhosis and hepatocellular carcinoma. However, this treatment promotes a sustained viral response in only about 50% of the patients. Therefore, new efficient antiviral therapies are in great demand. HCV is a positive-strand RNA virus whose genome consists of 9600 base pairs encoding 3 structural and 7 non-structural proteins. Two key viral enzymes *NS3 protease* and *NS5B polymerase* remain the most popular targets for the design of new anti-HCV drugs; however, rapid resistance development to the agents targeting these enzymes justifies the search and characterization of new proteins involved in the viral life cycle, that could be affected by small molecules[73, 74].

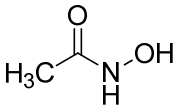
1.7.4. As an Anti-HIV agent

Efforts to find effective anti-HIV-1 chemotherapy have been focused on the development of chemicals that inhibit viral proteins, which are essential for HIV replication. The most important limitation of this therapeutic approach is the rapid generation of mutated quasi-species of HIV-1 resistant to those inhibitors. It has been

suggested that a combination of antiviral chemotherapy with some inhibitors of cellular proteins may greatly improve anti-HIV-I treatment. Among these proteins, the cellular enzyme ribonucleoside diphosphate reductase may be an important target, since this enzyme can be inhibited by compounds of the hydroxamate family such as hydroxyurea (HU). HU is a free radical quencher that inhibits the cellular enzyme ribonucleoside diphosphate reductase and in so doing, reduces the levels of a deoxyribonucleotide. Hydroxyurea has been used over the last 30 years for the treatment of human diseases such as chronic myelogenalleukemia, myeloproliferative syndromes and more recently sickle cell anaemia. Moreover, HU inhibits HIV-1 DNA synthesis in activated peripheral blood lymphocytes by decreasing the amount of intracellular deoxynucleotides. Combination of hydroxyureawith the nucleoside analogue generated a synergistic inhibitory effect without increasing toxicity[75, 76].



1



2

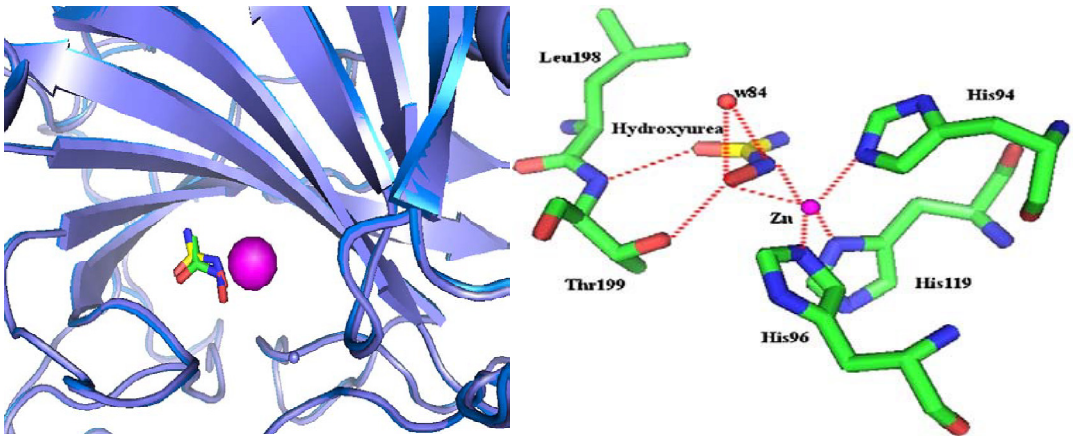


Figure8:(a) *N*-Hydroxyurea interactions when bound to the active site of hCA II.

(b) Superposition of the hCA II–$H_2NCONHOH$ **1** (in yellow) and hCA II–$CH_3CONHOH$ **2**(in green) adducts.

1.7.5 Antibacterial agents

In recent years, there has been a growing interest in developing bacterial peptide deformylase (PDF) inhibitors as novel antibiotics. PDF is an iron-containing metalloenzyme, which catalyses the removal of the *N*-formyl group from the terminal methionine residue of nascent proteins.PDF is essential for both Gram-positive and Gram-negative bacteria since deformylation is a necessary step to complete protein biosynthesis and maturation. Due to its wide distribution in bacteria and its absence in mammalian cells, PDF represents an attractive target for the discovery of broad-spectrum antibacterial drugs[77].Many PDF inhibitors have been reported in recent years.Most of the compounds with sufficient potency and antibacterial activity share a common structure[78]. Two structural features are constantly recurrent and seem to account for most of the binding energy; a metal chelating group X, most commonly a hydroxamate or *N* –formylhydroxylamine, and a *N*-alkyl (usually *N*-butyl) residue at P10 mimicking the methionine side chain and fitting into the deep S10 hydrophobic pocket in the PDF active site. Examples are the naturally occurring antibiotic actinonin (1) and the reverse hydroxamate from British Biotech BB-83698 (2),currently in phase I clinical trials[79]. However, since most of the known inhibitors still have significant peptide characteristics, there are some concerns about their selectivity and in vivo metabolic stability. Here we wish to report the synthesis and preliminary in vitro evaluation of a new series of non-peptidic PDF inhibitors having an isoxazole-3-hydroxamic acid as central core (3). The binding mode of this

class of inhibitorsis investigated by comparison with the crystal structure of the actinonin–PDF complex[80, 81]

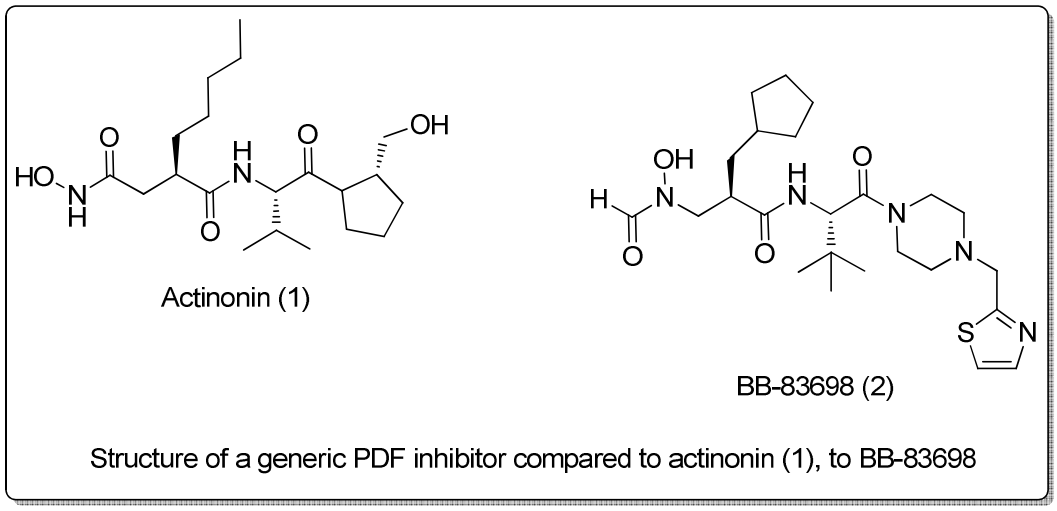


Figure 9. Structure of a generic PDF inhibitor compared to actinonin (1), to BB-83698

1.7.6. As botulinum neurotoxin protease inhibitors

Botulinum neurotoxins are one of the most toxic proteins currently known. Based on a recently identified potent lead structure, 2,4-dichlorocinnamic acid hydroxamatethe typical BoNT poisoning by accidental food consumption is rare in modern society, serious threats have emerged with the possibility of its use as a biological weapon[82, 83].

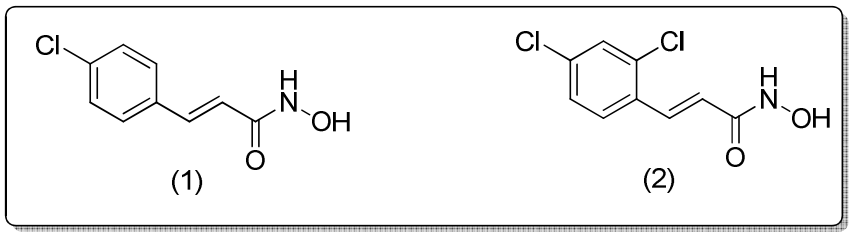


Figure 10. Structures of potent BoNT/A LC inhibitors, 2-chlorocinnamic acid hydroxamate (1) and 2,4-dichlorocinnamic acid hydroxamate (2).

1.7.7. As potential anti-leukemic agents

Synthesis of novel 1,4-benzodiazepine ring characterized by histone deacetylase inhibitors (HDACi) used as the cap, joined through an amide function or a triple bond as connection units, to a linear alkyl chain bearing the hydroxamate function as Zn^{2+}-chelating group. Biological tests performed in human acute promyelocyticleukaemia NB_4 cells showed that new hybrids can induce histone H_3/H_4 acetylation, growth arrest, and also apoptosis. Notably, chiral compounds exhibit stereo selective activity[84].

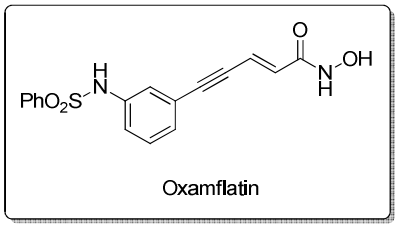
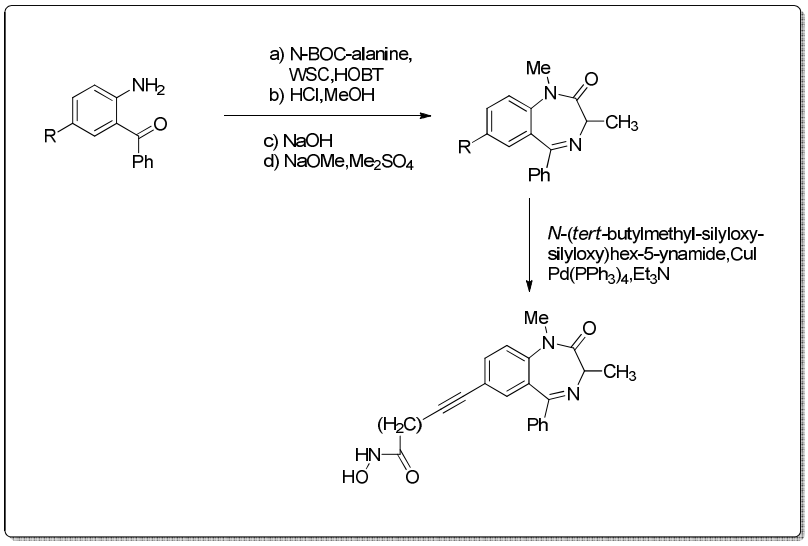


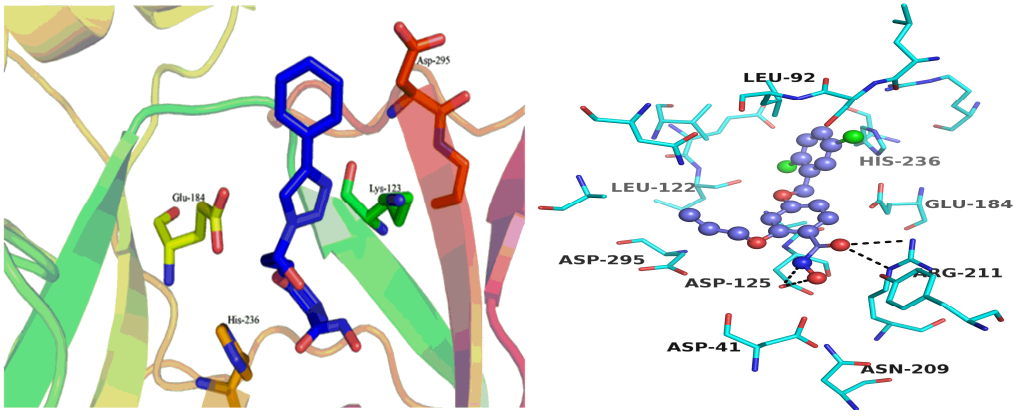
Figure 11. Structure of Oxamflatin



Scheme 17. Synthesis 1,4-benzodiazepine ring containing HDACi.

1.7.8. As potential LigA inhibitors

Tuberculosis (TB), one of the major public health threat and is an important infectious disease, causing high morbidity and mortality worldwide. The hydroxamates were detected as potential LigA inhibitors after an *in silico* screening experimentation by an effective fragment database and AUTODOCK 3.05. Furthermore, Hydroxamic acid-derived compound with NAD^+ binding site of *Mycobacterium tuberculosis* DNA LigA (MtuLigA) were found to be promisingly active [85].



(a) **(b)**

Figure.12. NAD^+ binding site of *Mycobacterium tuberculosis* DNA LigA (MtuLigA)

1.8. Hydroxamates nucleus based drugs available in the market

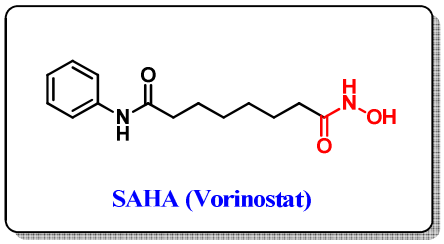
Here we briefly describe the selected examples of successfully marketed drugs that are derivatives of hydroxamate compounds, or synthetic molecules for which the lead was a natural product. The focus here is on some more recent drugs that had a major impact on human lives, while the more historical and well-known examples are as follows:

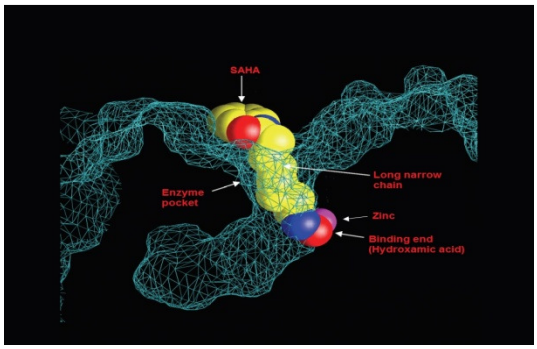
1.8.1. Vorinostat (SAHA, trade name, Zolinza) FDA approved in 2006

Vorinostat also known as Suberoylanilidehydroxamic acid,abbreviated as SAHA (**Figure 13**)[86-88], the hydroxamic acid-based drug for the treatment of cutaneous manifestation of T-cell lymphoma[89, 90].Lymphoma is a cancer of the lymphocytes (white blood cells) that comprise the lymphatic system. It has mainly two forms, namely Hodgkin lymphoma and non-Hodgkin lymphoma[91, 92], structurally belonging to the class of hydroxamic acid-containing hybrid polar molecules that suppress the proliferation of cancer cells *in-vitro* and reduce the growth of experimental tumours*in-vivo*[93-95]. There are on-going clinical trials of SAHA, and for haematological malignancies, vorinostat can be given orally with a maximum tolerated dose of 400 mg once daily or 200 mg twice daily, but the doselevel can be increased up to 600 mg in solid tumours[96]. SAHA, trichostatin, and butyrate are inhibitors of nuclear histone deacetylases (HDAC)[93, 97, 98]. Inhibitors of HDAC increase the expression of a variety of genes, accounting for their antitumor effects. For example, SAHA increases the expression of genes driving cell cycle, tumour suppression, differentiation, and apoptosis. SAHA also binds to S3 protein in the cytosol, a component of the ribosome. In addition to suppressing tumour growth, inhibitors of HDAC may affect other intracellular regulatory pathways. Trichostatin for example, increases viral expression, including that of HIV-1.Vorinostat, a histone deacetylase inhibitor, is currently marketed for the treatment of cutaneous T cell lymphoma (CTCL), a type of skin cancer [99]. It is used for treating patients having a tumour characterized by proliferation of neoplastic cells. Vorinostat, a second-generation polar-planar compound, binds to the catalytic domain of the histone deacetylases (HDACs)[100]. Actually, histone deacetylases (HDACs) are a

group of enzymes that remove acetyl groups from histones and regulate expressions of tumour suppressor genes. This allows the hydroxamic moiety to chelate Zn^{+} ion located in the catalytic pockets of HDAC, thereby inhibiting deacetylation and leading to an accumulation of both hyperacetylated histones and transcription factors. Hyper acetylation of histone proteins results in the up-regulation of the cyclin-dependent kinase p21, followed by G1 arrest. Hyperacetylation of non-histone proteins such as tumour suppressor p53, alpha-tubulin, and heat-shock protein 90 produces additional anti-proliferative effects. This agent also induces apoptosis and sensitizes tumour cells to cell death processes. Vorinostat crosses the blood-brain barrier.

Vorinostat is a broad inhibitor of HDAC activity and inhibits class I and class II HDAC enzymes [101]. Based on crystallographic studies, it has been seen that vorinostat binds to the zinc atom of the catalytic site of the HDAC enzyme with the phenyl ring of vorinostat projecting out of the catalytic domain onto the surface of the HDAC enzyme[102]. On binding to the HDAC enzyme, there is the accumulation of acetylated proteins including histones, which in turn manifests in multiple cellular effects [103-106].Vorinostat has too been concerned having an importance on other types of cancers, such as advanced solid tumours, brain metastasis, melanoma, lung cancer, refractory colorectal, pancreatic, and multiple myeloma. In the dealings of its target, Vorinostat inhibits Class I, II and IV HDAC proteins, however not the NAD+-dependent Class III HDAC[107-109].





(a) **(b)**

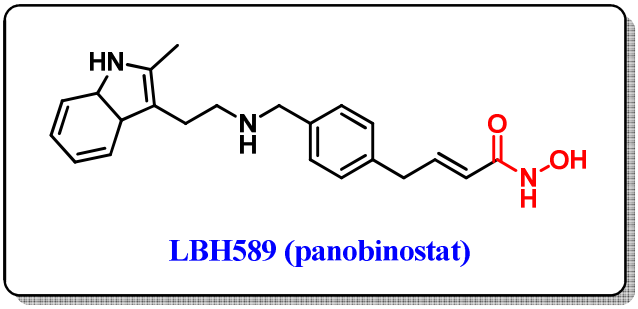
Figure 13.(a) Structure of SAHA (vorinostat) (b) Structural representation of SAHA (vorinostat) bound to an HDAC-like protein

1.8.2. Panobinostat (LBH-589, trade name, Farydak)FDA approved in February 2015

Panobinostat (LBH-589) is an experimental drug developed by Novartis for the treatment of various cancers[110-112]. It acts as a non-selective histone deacetylase inhibitor. It is being tested against Hodgkin's Lymphoma, cutaneous T cell (CTCL) [113, 114] and other types of malignant disease in phase III clinical trials, against myelodysplastic syndromes, breast cancer and prostate cancer in phase II trials and against chronic myelomonocyticleukaemia in phase I trial.

The reported mean terminal half-life of panobinostat is approximately 16 hours. A subset of patients with CTCL that were part of a larger trial of patients (n = 32) with various solid tumours or CTCL, treated with panobinostat, showed a response in 8 of 10 patients (two complete responses, four partial responses, and two disease stabilizations. No objective responses were observed in patients with malignancies other than CTCL (as part of the larger trial with 32 patients), although five patients with other tumours did achieve disease stabilization [115]. Most patients

with CTCL were treated with 20mg of panobinostat on Monday, Wednesday and Friday (one patient was treated with 30 mg). The responses included one disease stabilization, four partial responses, and two complete responses. Both complete responses occurred after cessation of panobinostat administration [92]. The expression of 23 specific genes was altered in all patients tested (n = 6) after treatment with panobinostat. Panobinostat administration was associated with cardiac toxicities, mainly electrocardiogram changes in a study of patients with haematological malignancies in which 13 of 15 patients had AML. Extensive cardiac conduction evaluation is being undertaken in many studies without a clear association of cardiac toxicity to date. Other adverse effects associated with panobinostat treatment included fatigue, hypokalemia, anorexia, thrombocytopenia, nausea, vomiting, and fatigue. Panobinostat is under further clinical investigation for patients with haematological malignancies as a single agent or in combination with a variety of treatments. Current combinational therapies include idarubicin, bortezomib, and melphalan[116-118].

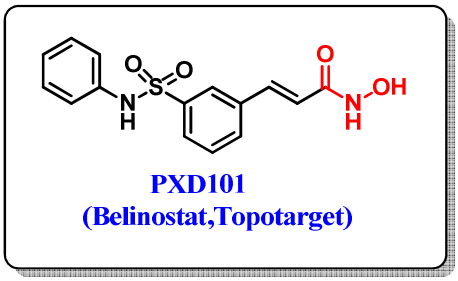


LBH589 (panobinostat)

1.8.3.Belinostat (PXD-101,trade name Beleodaq) FDA approved in July 2014

Belinostat has a sulphonamide-hydroxamate structure, acts by inhibiting potential of hydroxamic acid of class I and II histone deacetylase (HDAC) enzymes, which adjust acetylation intensities of histone and non-histone proteins [119]. Acetylated histones and other proteins are accumulated by Belinostat by producing

cell cycle arrest or apoptosis of various transformed cells *in-vitro* [120]. HDACs can control the appearance of tumour suppressor genes and actions of transcriptional factors occupied in cancer instigation and progression through adjustment of DNA or the structural constituents of chromatin. SoHDACIs are recognized to provoke cell death in malignant cells in the course of multiple mechanisms, counting up-regulation of death receptors and initiation of cell cycle arrest.*In vitro*, belinostat caused the accumulation of acetylated histones and other proteins increased the expression of tumour-suppressor genes. It ultimately induces cell cycle arrest, inhibition of angiogenesis and/or apoptosis of some transformed cells. This agent may sensitize drug-resistant tumour cells to other antineoplastic agents, possibly through a mechanism involving the down-regulation of thymidylate synthase [121-124].

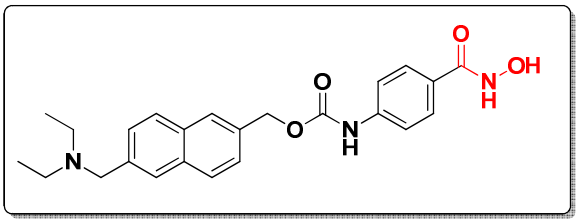


PXD101
(Belinostat,Topotarget)

1.8.4.Givinostat(ITF2357)

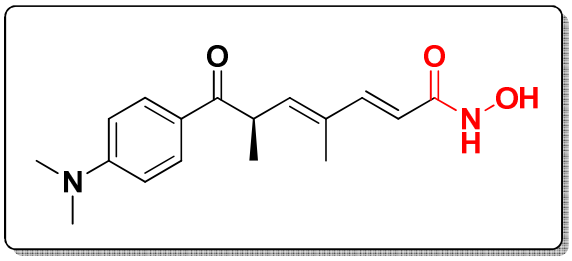
Givinostator **gavinostat (ITF2357)**is hydroxamic acid-containing HDAC inhibitor which revealed potential clinical benefits in patients with anti-inflammatory, antiangiogenic, chronic lymphocytic leukaemia, Hodgkin's lymphoma and anti-neoplastic activities. A phase II study was conducted to evaluate in several clinical studies and the safety and efficacy of givinostat in patients including studies for the treatment of $JAK2^{V617F}$ positive myeloproliferative neoplasm's (MPN) diseases, a type of blood cancer, periodic fever syndrome, systemic-onset juvenile idiopathic arthritis and for the treatment of polycythaemia vera[8, 125, 126].In 2013, this

designation was assigned by the FDA for the treatment of Duchene's muscular dystrophy and for the treatment of Becker's muscular dystrophy [127].



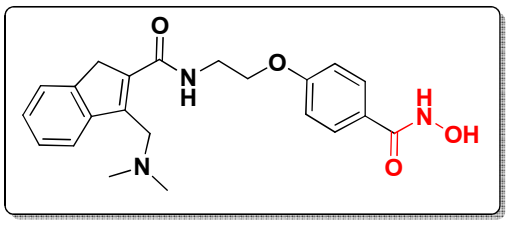
1.8.5. Trichostatin A

Trichostatin A (TSA) is also one of the first natural hydroxamic acid or hydroxamate compounds that serve as an antifungal antibiotic isolated from the actinomycete*Streptomyces hygroscopicus*that was found to inhibit HDACs (i.e.sirtuins) [128, 129]. TSA is a member of a larger class of histone deacetylase inhibitors(HDIs or HDACIs) that have a broad spectrum of epigenetic activities [130]. It also inhibits the eukaryotic cell cycle during the beginning of the growth stage. It can be used to alter gene expression by interfering with the removal of acetyl groups from histones (histone deacetylases, HDAC) and therefore altering the ability of DNA transcription factors to access the DNA molecules inside chromatin. Other mechanisms may include the activity of HDIs to induce cell differentiation, thus acting to "mature" some of the differentiated cells found in tumours. HDIs have multiple effects on non-histone effect or molecules, so the anti-cancer mechanisms are truly not understood at this time [131-134]. TSA was initially proposed to be a pan-cellular HDAC inhibitor with movement against HDACs 1-7 and 9 in nanomolar concentrations, and HADC 8 in micro molar concentrations. In spite of the pronounced activity of TSA, it was excluded as a clinical drug due to its many side effects such as apoptosis, non-transformed cells and increased DNA damage [135].



1.8.6.Abexinostat

Abexinostat formerly known as PCI-24781 is a novel second generation phenyl hydroxamic acid based drug that showed broad spectrum anticancer activities in preclinical studies and is in Phase II clinical trials for β-cell lymphoma [136]. Pre-clinical study suggests the potential for treatment of different types of cancer as well. Orally bio-available HDAC inhibitor that has previously been shown to have activity*in vitro* and *in vivo* against a broad array of cancers, including hematopoietic malignancies, bone and soft-tissue sarcomas [137]. Western blotting analysis showed the cleavage of caspase-3 and PARP, indicating apoptosis as a primary mechanism of action. Further studies with xenograft mouse models indicated increased survival among animals treated with a combination of abexinostat and bortezomib. Additionally, it acts as a potent radio sensitizing agent and is synergistic with cytotoxic chemotherapy, such as doxorubicin in preclinical models [138-141].



1.9. Conclusion

Hydroxamate is an unambiguous and essential scaffold that involved in a variety of fundamental biological processes and pathological situations. Diversity with respect to structure, stereochemistry and functional groups along with better ADMET properties create the ideal platform for their use in drug discovery and development.This review describes in brief the synthesis and evaluation of hydroxamatesanalogues developed in the past years with multiple therapeutic properties such as anti-cancer, anti-malarial, cardiovascular, anti-HIV, anti-inflammatory and anti-bacterial activities. With the advancement of research and technology, isolation and analysis in order to comprehend the chemistry of diverse and complex hydroxamates are creating opportunities for revolutionary augmentation in understanding at the molecular level. The global importance of hydroxamates analogues has stemmed both from the development of novel compounds with broad applications and mechanisms of action, enabling pharmacological intervention to potentiate for further progress in this plausible biologically desirable pharmaceutical agents.

1.10. REFERENCES:

[1] Miller MJ. Syntheses and therapeutic potential of hydroxamic acid based siderophores and analogs. Chemical Reviews. 1989;89:1563-79.

[2] Hider RC, Kong X. Chemistry and biology of siderophores. Natural product reports. 2010;27:637-57.

[3] Muri EM, Nieto MJ, Sindelar RD, Williamson JS. Hydroxamic acids as pharmacological agents. Current medicinal chemistry. 2002;9:1631-53.

[4] Jacobsen JA, Jourden JLM, Miller MT, Cohen SM. To bind zinc or not to bind zinc: an examination of innovative approaches to improved metalloproteinase inhibition. Biochimica et Biophysica Acta (BBA)-Molecular Cell Research. 2010;1803:72-94.

[5] Jacobsen FE, Lewis JA, Cohen SM. A new role for old ligands: discerning chelators for zinc metalloproteinases. Journal of the American Chemical Society. 2006;128:3156-7.

[6] Tu G, Xu W, Huang H, Li S. Progress in the development of matrix metalloproteinase inhibitors. Current medicinal chemistry. 2008;15:1388-95.

[7] Verma RP, Hansch C. Matrix metalloproteinases (MMPs): chemical–biological functions and (Q) SARs. Bioorganic & medicinal chemistry. 2007;15:2223-68.

[8] Bantscheff M, Hopf C, Savitski MM, Dittmann A, Grandi P, Michon A-M. Chemoproteomics profiling of HDAC inhibitors reveals selective targeting of HDAC complexes. Nature biotechnology. 2011;29:255.

[9] Marks PA, Miller T, Richon VM. Histone deacetylases. Current opinion in pharmacology. 2003;3:344-51.

[10] Kozikowski AP, Chen Y, Gaysin A, Chen B, D'Annibale MA, Suto CM. Functional differences in epigenetic modulators superiority of mercaptoacetamide-based histone deacetylase inhibitors relative to hydroxamates in cortical neuron neuroprotection studies. Journal of medicinal chemistry. 2007;50:3054-61.

[11] Chauhan S. The Phthalhydroxamate Ligand and its Organotellurium (IV) Chloride Complexes: Preparation and Spectral Studies.

[12] Kurzak B, Kozłowski H, Farkas E. Hydroxamic and aminohydroxamic acids and their complexes with metal ions. Coordination Chemistry Reviews. 1992;114:169-200.

[13] Mizukami S, Nagata K. Metal complexes of hydroxamic acid analogs. Coordination Chemistry Reviews. 1968;3:267-78.

[14] Kovacic P, Edwards CL. Hydroxamic acids (therapeutics and mechanism): chemistry, acyl nitroso, nitroxyl, reactive oxygen species, and cell signaling. Journal of Receptors and Signal Transduction. 2011;31:10-9.

[15] Graham N. Polymer synthesis: Vol 1: Stanley R. Sandler and Wolf Karo Academic Press, New York, 1974, 572 pp.£ 18.95. Elsevier; 1975.

[16] Kelly WK, Marks PA. Drug insight: histone deacetylase inhibitors-development of the new targeted anticancer agent suberoylanilide hydroxamic acid. Nature Reviews Clinical Oncology. 2005;2:150.

[17] Butler LM, Agus DB, Scher HI, Higgins B, Rose A, Cordon-Cardo C. Suberoylanilide hydroxamic acid, an inhibitor of histone deacetylase, suppresses the growth of prostate cancer cells in vitro and in vivo. Cancer research. 2000;60:5165-70.

[18] Holland KP, Elford HL, Bracchi V, Annis CG, Schuster SM, Chakrabarti D. Antimalarial activities of polyhydroxyphenyl and hydroxamic acid derivatives. Antimicrobial agents and chemotherapy. 1998;42:2456-8.

[19] Hynes JB. Hydroxylamine derivatives as potential antimalarial agents. 1. Hydroxamic acids. Journal of medicinal chemistry. 1970;13:1235-7.

[20] Giannini G, Battistuzzi G, Vignola D. Hydroxamic acid based histone deacetylase inhibitors with confirmed activity against the malaria parasite. Bioorganic & medicinal chemistry letters. 2015;25:459-61.

[21] Zhao L, Chen C-N, Hajji N, Oliver E, Cotroneo E, Wharton J. Histone deacetylation inhibition in pulmonary hypertension: therapeutic potential of valproic acid (VPA) and suberoylanilide hydroxamic acid (SAHA). Circulation. 2012:Circulationaha. 112.103176.

[22] Colussi C, Berni R, Rosati J, Straino S, Vitale S, Spallotta F. The histone deacetylase inhibitor suberoylanilide hydroxamic acid reduces cardiac arrhythmias in dystrophic mice. Cardiovascular research. 2010;87:73-82.

[23] Archin NM, Espeseth A, Parker D, Cheema M, Hazuda D, Margolis DM. Expression of latent HIV induced by the potent HDAC inhibitor suberoylanilide hydroxamic acid. AIDS research and human retroviruses. 2009;25:207-12.

[24] Plewe MB, Butler SL, R. Dress K, Hu Q, Johnson TW, Kuehler JE. Azaindole hydroxamic acids are potent HIV-1 integrase inhibitors. Journal of medicinal chemistry. 2009;52:7211-9.

[25] Tanis SP, Plewe MB, Johnson TW, Butler SL, Dalvie D, DeLisle D. Azaindole N-methyl hydroxamic acids as HIV-1 integrase inhibitors-II. The impact of physicochemical properties on ADME and PK. Bioorganic & medicinal chemistry letters. 2010;20:7429-34.

[26] Musser JH, Kubrak DM. Quinolinyl benzene hydroxamic acids as anti-inflammatory/antiallergic agents. Google Patents; 1988.

[27] Tanaka K, Matsuo K, Nakanishi A, Hatano T, Izeki H, Ishida Y. Syntheses and anti-inflammatory and analgesic activities of hydroxamic acids and acid hydrazides. Chemical and pharmaceutical bulletin. 1983;31:2810-9.

[28] Güneş H, Coşar G. Synthesis of some hydroxamic acid derivatives of benzimidazole and their antibacterial and antifungal activities. Arzneimittel-Forschung. 1992;42:1045-8.

[29] Cvetni Z. Antimicrobial activity of N-phthaloylamino acid hydroxamates. Acta Pharm. 2005;55:387-99.

[30] Pathak A, Blair VL, Ferrero RL, Mehring M, Andrews PC. Bismuth (III) benzohydroxamates: powerful anti-bacterial activity against Helicobacter pylori and hydrolysis to a unique Bi 34 oxido-cluster [Bi 34 O 22 (BHA) 22 (H-BHA) 14 (DMSO) 6]. Chemical Communications. 2014;50:15232-4.

[31] Giannini G, Cabri W, Fattorusso C, Rodriquez M. Histone deacetylase inhibitors in the treatment of cancer: overview and perspectives. Future medicinal chemistry. 2012;4:1439-60.

[32] Zhang L, Lei J, Shan Y, Yang H, Song M, Ma Y. Recent progress in the development of histone deacetylase inhibitors as anti-cancer agents. Mini reviews in medicinal chemistry. 2013;13:1999-2013.

[33] Miller TA, Witter DJ, Belvedere S. Histone deacetylase inhibitors. Journal of medicinal chemistry. 2003;46:5097-116.

[34] Schäfer S, Saunders L, Eliseeva E, Velena A, Jung M, Schwienhorst A. Phenylalanine-containing hydroxamic acids as selective inhibitors of class IIb histone deacetylases (HDACs). Bioorganic & medicinal chemistry. 2008;16:2011-33.

[35] Oger F, Lecorgne A, Sala E, Nardese V, Demay F, Chevance S. Biological and biophysical properties of the histone deacetylase inhibitor suberoylanilide hydroxamic acid are affected by the presence of short alkyl groups on the phenyl ring. Journal of medicinal chemistry. 2010;53:1937-50.

[36] Dow GS, Chen Y, Andrews KT, Caridha D, Gerena L, Gettayacamin M. Antimalarial activity of phenylthiazolyl-bearing hydroxamate-based histone deacetylase inhibitors. Antimicrobial agents and chemotherapy. 2008;52:3467-77.

[37] Butler KV, Kalin J, Brochier C, Vistoli G, Langley B, Kozikowski AP. Rational design and simple chemistry yield a superior, neuroprotective HDAC6 inhibitor, tubastatin A. Journal of the American Chemical Society. 2010;132:10842-6.

[38] Segretti MC, Vallerini GP, Brochier C, Langley B, Wang L, Hancock WW. Thiol-based potent and selective HDAC6 inhibitors promote tubulin acetylation and T-regulatory cell suppressive function. ACS medicinal chemistry letters. 2015;6:1156-61.

[39] Li Y, Luo X, Guo Q, Nie Y, Wang T, Zhang C. Discovery of N 1-(4-((7-Cyclopentyl-6-(dimethylcarbamoyl)-7 H-pyrrolo [2, 3-d] pyrimidin-2-yl) amino) phenyl)-N 8-hydroxyoctanediamide as a Novel Inhibitor Targeting Cyclin-dependent Kinase 4/9 (CDK4/9) and Histone Deacetlyase1 (HDAC1) against Malignant Cancer. Journal of medicinal chemistry. 2018;61:3166-92.

[40] He R, Chen Y, Chen Y, Ougolkov AV, Zhang J-S, Savoy DN. Synthesis and biological evaluation of triazol-4-ylphenyl-bearing histone deacetylase inhibitors as anticancer agents. Journal of medicinal chemistry. 2010;53:1347-56.

[41] Huong TTL, Huong PT, Thao TP, Dung PTP, Oanh DTK, Huong NTM. Exploration of some indole-based hydroxamic acids as histone deacetylase inhibitors and antitumor agents. Chemical Papers. 2017;71:1759-69.

[42] Ali R, Siddiqui N. Biological aspects of emerging benzothiazoles: a short review. Journal of Chemistry. 2013;2013.

[43] Dai Y, Guo Y, Guo J, Pease LJ, Li J, Marcotte PA. Indole amide hydroxamic acids as potent inhibitors of histone deacetylases. Bioorganic & medicinal chemistry letters. 2003;13:1897-901.

[44] Giannini G, Marzi M, Di Marzo M, Battistuzzi G, Pezzi R, Brunetti T. Exploring bis-(indolyl) methane moiety as an alternative and innovative CAP group in the design of histone deacetylase (HDAC) inhibitors. Bioorganic & medicinal chemistry letters. 2009;19:2840-3.

[45] Shukla AK, Shrivash MK, Tripathi VD, Konwar R, Pandey J. Identification of N-Hydroxycinnamamide analogues and their bio-evaluation against breast cancer cell lines. Biomedicine & Pharmacotherapy. 2018;107:475-83.

[46] Belvedere S, Witter DJ, Yan J, Secrist JP, Richon V, Miller TA. Aminosuberoyl hydroxamic acids (ASHAs): a potent new class of HDAC inhibitors. Bioorganic & medicinal chemistry letters. 2007;17:3969-71.

[47] Wang H, Lim Z-Y, Zhou Y, Ng M, Lu T, Lee K. Acylurea connected straight chain hydroxamates as novel histone deacetylase inhibitors: Synthesis, SAR, and in vivo antitumor activity. Bioorganic & medicinal chemistry letters. 2010;20:3314-21.

[48] Marek L, Hamacher A, Hansen FK, Kuna K, Gohlke H, Kassack MU. Histone deacetylase (HDAC) inhibitors with a novel connecting unit linker region reveal a

selectivity profile for HDAC4 and HDAC5 with improved activity against chemoresistant cancer cells. Journal of medicinal chemistry. 2013;56:427-36.

[49] Stenzel K, Hamacher A, Hansen FK, Gertzen CG, Senger J, Marquardt V. Alkoxyurea-based histone deacetylase inhibitors increase cisplatin potency in chemoresistant cancer cell lines. Journal of medicinal chemistry. 2017;60:5334-48.

[50] Chen PC, Patil V, Guerrant W, Green P, Oyelere AK. Synthesis and structure–activity relationship of histone deacetylase (HDAC) inhibitors with triazole-linked cap group. Bioorganic & medicinal chemistry. 2008;16:4839-53.

[51] C Mwakwari S, Patil V, Guerrant W, K Oyelere A. Macrocyclic histone deacetylase inhibitors. Current topics in medicinal chemistry. 2010;10:1423-40.

[52] Dai Y, Guo Y, Curtin ML, Li J, Pease LJ, Guo J. A novel series of histone deacetylase inhibitors incorporating hetero aromatic ring systems as connection units. Bioorganic & medicinal chemistry letters. 2003;13:3817-20.

[53] Bigioni M, Ettorre A, Felicetti P, Mauro S, Rossi C, Maggi CA. Set-up of a new series of HDAC inhibitors: the 5, 11-dihydrodibenzo [b, e] azepin-6-ones as privileged structures. Bioorganic & medicinal chemistry letters. 2012;22:5360-2.

[54] Grolla AA, Podesta V, Chini MG, Di Micco S, Vallario A, Genazzani AA. Synthesis, biological evaluation, and molecular docking of Ugi products containing a zinc-chelating moiety as novel inhibitors of histone deacetylases. Journal of medicinal chemistry. 2009;52:2776-85.

[55] Pabba C, Gregg BT, Kitchen DB, Chen ZJ, Judkins A. Design and synthesis of aryl ether and sulfone hydroxamic acids as potent histone deacetylase (HDAC) inhibitors. Bioorganic & medicinal chemistry letters. 2011;21:324-8.

[56] Charrier C, Clarhaut J, Gesson J-P, Estiu G, Wiest O, Roche J. Synthesis and modeling of new benzofuranone histone deacetylase inhibitors that stimulate tumor suppressor gene expression. Journal of medicinal chemistry. 2009;52:3112-5.

[57] Vanhaecke T, Papeleu P, Elaut G, Rogiers V. Trichostatin A-like hydroxamate histone deacetylase inhibitors as therapeutic agents: toxicological point of view. Current medicinal chemistry. 2004;11:1629-43.

[58] Venugopal B, Evans T. Developing histone deacetylase inhibitors as anti-cancer therapeutics. Current medicinal chemistry. 2011;18:1658-71.

[59] Gryder BE, Sodji QH, Oyelere AK. Targeted cancer therapy: giving histone deacetylase inhibitors all they need to succeed. Future medicinal chemistry. 2012;4:505-24.

[60] Librizzi M, Caradonna F, Cruciata I, Debski J, Sansook S, Dadlez M. Molecular Signatures Associated with Treatment of Triple-Negative MDA-MB231 Breast Cancer Cells with Histone Deacetylase Inhibitors JAHA and SAHA. Chemical research in toxicology. 2017;30:2187-96.

[61] Chhabra S. Novel proteasome inhibitors and histone deacetylase inhibitors: progress in myeloma therapeutics. Pharmaceuticals. 2017;10:40.

[62] Kwak S-Y, Yang J-K, Choi H-R, Park K-C, Kim Y-B, Lee Y-S. Synthesis and dual biological effects of hydroxycinnamoyl phenylalanyl/prolyl hydroxamic acid derivatives as tyrosinase inhibitor and antioxidant. Bioorganic & medicinal chemistry letters. 2013;23:1136-42.

[63] Lin Y-S, Chen S-H, Huang W-J, Chen C-H, Chien M-Y, Lin S-Y. Effects of nicotinic acid derivatives on tyrosinase inhibitory and antioxidant activities. Food chemistry. 2012;132:2074-80.

[64] Alam N, Yoon KN, Lee KR, Shin PG, Cheong JC, Yoo YB. Antioxidant activities and tyrosinase inhibitory effects of different extracts from Pleurotus ostreatus fruiting bodies. Mycobiology. 2010;38:295-301.

[65] Jani M, Tordai H, Trexler M, Bányai L, Patthy L. Hydroxamate-based peptide inhibitors of matrix metalloprotease 2. Biochimie. 2005;87:385-92.

[66] Andrews KT, Haque A, Jones MK. HDAC inhibitors in parasitic diseases. Immunology and cell biology. 2012;90:66-77.

[67] Mathew RT. The M1AAP and M17LAP aminopeptidases of Plasmodium falciparum malaria: McGill University Montreal, Quebec; 2014.

[68] T Andrews K, N Tran T, P Fairlie D. Towards histone deacetylase inhibitors as new antimalarial drugs. Current pharmaceutical design. 2012;18:3467-79.

[69] Engel JA, Jones AJ, Avery VM, Sumanadasa SD, Ng SS, Fairlie DP. Profiling the anti-protozoal activity of anti-cancer HDAC inhibitors against Plasmodium and Trypanosoma parasites. International Journal for Parasitology: Drugs and Drug Resistance. 2015;5:117-26.

[70] Trenholme K, Marek L, Duffy S, Pradel G, Fisher G, Hansen FK. Lysine acetylation in sexual stage malaria parasites is a target for antimalarial small molecules. Antimicrobial agents and chemotherapy. 2014:AAC. 02721-13.

[71] Elizabeth C, Abate CM. Cultural Factors Affecting Heavy Metals Removal by Actinobacteria. Actinobacteria: Application in Bioremediation and Production of Industrial Enzymes. 2013:26.

[72] K Tiwari V, C Mishra R, Sharma A, P Tripathi R. Carbohydrate based potential chemotherapeutic agents: recent developments and their scope in future drug discovery. Mini reviews in medicinal chemistry. 2012;12:1497-519.

[73] Kozlov MV, Kleymenova AA, Romanova LI, Konduktorov KA, Kamarova KA, Smirnova OA. Pyridine hydroxamic acids are specific anti-HCV agents affecting HDAC6. Bioorganic & medicinal chemistry letters. 2015;25:2382-5.

[74] Kozlov MV, Kleymenova AA, Romanova LI, Konduktorov KA, Smirnova OA, Prasolov VS. Benzohydroxamic acids as potent and selective anti-HCV agents. Bioorganic & medicinal chemistry letters. 2013;23:5936-40.

[75] Torres G. Hydroxyurea, a potential new anti-HIV agent. GMHC treatment issues: the Gay Men's Health Crisis newsletter of experimental AIDS therapies. 1995;9:7.

[76] Mayhew CN, Phillips JD, Greenberg RN, Birch NJ, Elford HL, Gallicchio VS. In Vivo and In Vitro Comparison of the Short□Term Hematopoietic Toxicity Between Hydroxyurea and Trimidox or Didox, Novel Ribonucleotide Reductase Inhibitors with Potential Anti-HIV-1 Activity. Stem Cells. 1999;17:345-56.

[77] Hackbarth CJ, Chen DZ, Lewis JG, Clark K, Mangold JB, Cramer JA. N-alkyl urea hydroxamic acids as a new class of peptide deformylase inhibitors with antibacterial activity. Antimicrobial agents and chemotherapy. 2002;46:2752-64.

[78] Chen D, Hackbarth C, Ni Z, Wu C, Wang W, Jain R. Peptide deformylase inhibitors as antibacterial agents: identification of VRC3375, a proline-3-alkylsuccinyl hydroxamate derivative, by using an integrated combinatorial and medicinal chemistry approach. Antimicrobial agents and chemotherapy. 2004;48:250-61.

[79] Jain R, Sundram A, Lopez S, Neckermann G, Wu C, Hackbarth C. α-Substituted hydroxamic acids as novel bacterial deformylase inhibitor-based antibacterial agents. Bioorganic & medicinal chemistry letters. 2003;13:4223-8.

[80] Jain R, Chen D, White R, Patel D, Yuan Z. Bacterial peptide deformylase inhibitors: a new class of antibacterial agents. Current medicinal chemistry. 2005;12:1607-21.

[81] Cali P, Naerum L, Mukhija S, Hjelmencrantz A. Isoxazole-3-hydroxamic acid derivatives as peptide deformylase inhibitors and potential antibacterial agents. Bioorganic & medicinal chemistry letters. 2004;14:5997-6000.

[82] Čapková K, Yoneda Y, Dickerson TJ, Janda KD. Synthesis and structure–activity relationships of second-generation hydroxamate botulinum neurotoxin a protease inhibitors. Bioorganic & medicinal chemistry letters. 2007;17:6463-6.

[83] Šilhár P, Silvaggi NR, Pellett S, Čapková K, Johnson EA, Allen KN. Evaluation of adamantane hydroxamates as botulinum neurotoxin inhibitors: synthesis, crystallography, modeling, kinetic and cellular based studies. Bioorganic & medicinal chemistry. 2013;21:1344-8.

[84] Salmi-Smail C, Fabre A, Dequiedt F, Restouin A, Castellano R, Garbit S. Modified cap group suberoylanilide hydroxamic acid histone deacetylase inhibitor derivatives reveal improved selective antileukemic activity. Journal of medicinal chemistry. 2010;53:3038-47.

[85] Kukshal V, Mishra M, Ajay A, Khanam T, Sharma R, Dube D. Synthesis and bioevaluation of aryl hydroxamates distinguishing between NAD+ and ATP-dependent DNA ligases. MedChemComm. 2012;3:453-61.

[86] Gronkiewicz K. Therapeutic potential of the anti-fibrotic drug Suberanilohydroxamic acid (SAHA) in canine corneal fibrosis: University of Missouri--Columbia; 2016.

[87] Butler MS. Natural products to drugs: natural product-derived compounds in clinical trials. Natural product reports. 2008;25:475-516.

[88] Ganai SA. Strategy for enhancing the therapeutic efficacy of histone deacetylase inhibitor dacinostat: the novel paradigm to tackle monotonous cancer chemoresistance. Archives of pharmacal research. 2015:1-11.

[89] Shabason JE, Camphausen K. HDAC inhibitors in cancer care. Oncology. 2010;24:180.

[90] GroZiNGer C. sL sCHreiBer. 2002. Deacetylase enzymes: biological functions and the use of small-molecule inhibitors. Chem Biol.9:3-16.

[91] Rizvi MA, Evens AM, Tallman MS, Nelson BP, Rosen ST. T-cell non-Hodgkin lymphoma. Blood. 2006;107:1255-64.

[92] Ellis L, Pan Y, Smyth GK, George DJ, McCormack C, Williams-Truax R. Histone deacetylase inhibitor panobinostat induces clinical responses with associated alterations in gene expression profiles in cutaneous T-cell lymphoma. Clinical Cancer Research. 2008;14:4500-10.

[93] Leoni F, Zaliani A, Bertolini G, Porro G, Pagani P, Pozzi P. The antitumor histone deacetylase inhibitor suberoylanilide hydroxamic acid exhibits antiinflammatory properties via suppression of cytokines. Proceedings of the National Academy of Sciences. 2002;99:2995-3000.

[94] Mei S, Ho AD, Mahlknecht U. Role of histone deacetylase inhibitors in the treatment of cancer. International journal of oncology. 2004;25:1509-19.

[95] Marchion D, Münster P. Development of histone deacetylase inhibitors for cancer treatment. Expert review of anticancer therapy. 2007;7:583-98.

[96] Varasi M, Thaler F, Abate A, Bigogno C, Boggio R, Carenzi G. Discovery, synthesis, and pharmacological evaluation of spiropiperidine hydroxamic acid based derivatives as structurally novel histone deacetylase (HDAC) inhibitors. Journal of medicinal chemistry. 2011;54:3051-64.

[97] Vigushin DM, Coombes RC. Histone deacetylase inhibitors in cancer treatment. Anti-cancer drugs. 2002;13:1-13.

[98] Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone deacetylase inhibitors. Nature reviews Drug discovery. 2006;5:769.

[99] Falkenberg KJ, Johnstone RW. Histone deacetylases and their inhibitors in cancer, neurological diseases and immune disorders. Nature reviews Drug discovery. 2014;13:673.

[100] Ganai SA. Histone deacetylase inhibitor pracinostat in doublet therapy: a unique strategy to improve therapeutic efficacy and to tackle herculean cancer chemoresistance. Pharmaceutical biology. 2016;54:1926-35.

[101] Richon V. Cancer biology: mechanism of antitumour action of vorinostat (suberoylanilide hydroxamic acid), a novel histone deacetylase inhibitor. British journal of cancer. 2006;95:S2.

[102] Wagner FF, Weïwer M, Lewis MC, Holson EB. Small molecule inhibitors of zinc-dependent histone deacetylases. Neurotherapeutics. 2013;10:589-604.

[103] Wang C, Henkes LM, Doughty LB, He M, Wang D, Meyer-Almes F-J. Thailandepsins: bacterial products with potent histone deacetylase inhibitory activities and broad-spectrum antiproliferative activities. Journal of natural products. 2011;74:2031-8.

[104] Andrianov V, Gailite V, Lola D, Loza E, Semenikhina V, Kalvinsh I. Novel amide derivatives as inhibitors of histone deacetylase: design, synthesis and SAR. European journal of medicinal chemistry. 2009;44:1067-85.

[105] Gryder BE, Akbashev MJ, Rood MK, Raftery ED, Meyers WM, Dillard P. Selectively targeting prostate cancer with antiandrogen equipped histone deacetylase inhibitors. ACS chemical biology. 2013;8:2550-60.

[106] Chen L, Petrelli R, Gao G, Wilson DJ, McLean GT, Jayaram HN. Dual inhibitors of inosine monophosphate dehydrogenase and histone deacetylase based on a cinnamic hydroxamic acid core structure. Bioorganic & medicinal chemistry. 2010;18:5950-64.

[107] Marks P. Discovery and development of SAHA as an anticancer agent. Oncogene. 2007;26:1351.

[108] Lane AA, Chabner BA. Histone deacetylase inhibitors in cancer therapy. Journal of clinical oncology. 2009;27:5459-68.

[109] Xu W, Parmigiani R, Marks P. Histone deacetylase inhibitors: molecular mechanisms of action. Oncogene. 2007;26:5541.

[110] Strickler JH, Starodub AN, Jia J, Meadows KL, Nixon AB, Dellinger A. Phase I study of bevacizumab, everolimus, and panobinostat (LBH-589) in advanced solid tumors. Cancer chemotherapy and pharmacology. 2012;70:251-8.

[111] Omene C, Kalac M, Wu J, Marchi E, Frenkel K, O'Connor OA. Propolis and its active component, caffeic acid phenethyl ester (CAPE), modulate breast cancer therapeutic targets via an epigenetically mediated mechanism of action. Journal of cancer science & therapy. 2013;5:334.

[112] Anne M, Sammartino D, Barginear MF, Budman D. Profile of panobinostat and its potential for treatment in solid tumors: an update. OncoTargets and therapy. 2013;6:1613.

[113] Wilcox RA. Cutaneous T□cell lymphoma: 2014 Update on diagnosis, risk estratification, and management. American journal of hematology. 2014;89:837-51.

[114] Ahmad Ganai S. Panobinostat: The small molecule metalloenzyme inhibitor with marvelous anticancer activity. Current topics in medicinal chemistry. 2016;16:427-34.

[115] Atadja P. Development of the pan-DAC inhibitor panobinostat (LBH589): successes and challenges. Cancer letters. 2009;280:233-41.

[116] Stimson L, La Thangue NB. Biomarkers for predicting clinical responses to HDAC inhibitors. Cancer letters. 2009;280:177-83.

[117] Shao W, Growney JD, Feng Y, O'connor G, Pu M, Zhu W, et al. Activity of deacetylase inhibitor panobinostat (LBH589) in cutaneous T-cell lymphoma models: Defining molecular mechanisms of resistance. International journal of cancer. 2010;127:2199-208.

[118] Li X, Zhang J, Xie Y, Jiang Y, Yingjie Z, Xu W. Progress of HDAC inhibitor panobinostat in the treatment of cancer. Current drug targets. 2014;15:622-34.

[119] Poole RM. Belinostat: first global approval. Drugs. 2014;74:1543-54.

[120] Finn PW, Loza E, Carstensen E. The Discovery and Development of Belinostat. Successful Drug Discovery. 2016:31-57.

[121] Markozashvili D, Ribrag V, Vassetzky YS. Histone deacetylase inhibitors and epigenetic regulation in lymphoid malignancies. Investigational new drugs. 2015;33:1280-91.

[122] Ganguly BB. Small-molecule Inhibitors of Epigenetic Mutations as Compelling Drugtargets for Myelodysplastic Syndromes. Current cancer drug targets. 2017;17:586-602.

[123] Rodrigues DA, Ferreira-Silva GA, Ferreira AC, Fernandes RA, Kwee JK, Sant'Anna CM. Design, synthesis, and pharmacological evaluation of novel *N*-acylhydrazone derivatives as potent histone deacetylase 6/8 dual inhibitors. Journal of medicinal chemistry. 2016;59:655-70.

[124] Hamm CA, Costa FF. Epigenomes as therapeutic targets. Pharmacology & therapeutics. 2015;151:72-86.

[125] Rambaldi A, Dellacasa CM, Finazzi G, Carobbio A, Ferrari ML, Guglielmelli P. A pilot study of the HistoneDeacetylase inhibitor Givinostat in patients with JAK2V617F positive chronic myeloproliferative neoplasms. British journal of haematology. 2010;150:446-55.

[126] Joosten LA, Leoni F, Meghji S, Mascagni P. Inhibition of HDAC activity by ITF2357 ameliorates joint inflammation and prevents cartilage and bone destruction in experimental arthritis. Molecular medicine. 2011;17:391.

[127] Consalvi S, Mozzetta C, Bettica P, Germani M, Fiorentini F, Del Bene F. Preclinical studies in the mdx mouse model of duchenne muscular dystrophy with the histone deacetylase inhibitor givinostat. Molecular medicine. 2013;19:79.

[128] Sekhavat A, Sun J-M, Davie JR. Competitive inhibition of histone deacetylase activity by trichostatin A and butyrate. Biochemistry and Cell biology. 2007;85:751-8.

[129] Adcock I. HDAC inhibitors as anti□inflammatory agents. British journal of pharmacology. 2007;150:829-31.

[130] Yoshida M, Horinouchi S, Beppu T. Trichostatin A and trapoxin: novel chemical probes for the role of histone acetylation in chromatin structure and function. Bioessays. 1995;17:423-30.

[131] Yi J, Luo J. SIRT1 and p53, effect on cancer, senescence and beyond. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics. 2010;1804:1684-9.

[132] Huang X-B, Li J, Zheng L, Zuo G-H, Han K-Q, Li H-Y. Bioinformatics analysis reveals potential candidate drugs for HCC. Pathology & Oncology Research. 2013;19:251-8.

[133] Baradari V, Huether A, Höpfner M, Schuppan D, Scherübl H. Antiproliferative and proapoptotic effects of histone deacetylase inhibitors on gastrointestinal neuroendocrine tumor cells. Endocrine-related cancer. 2006;13:1237-50.

[134] Florea A-M, Büsselberg D. Breast cancer and possible mechanisms of therapy resistance. Journal of Local and Global Health Science. 2013:2.

[135] Rodríguez-Paredes M, Esteller M. Cancer epigenetics reaches mainstream oncology. Nature medicine. 2011;17:330.

[136] Sholler GS, Currier EA, Dutta A, Slavik MA, Illenye SA, Mendonca MCF. PCI-24781 (abexinostat), a novel histone deacetylase inhibitor, induces reactive oxygen species-dependent apoptosis and is synergistic with bortezomib in neuroblastoma. Journal of cancer therapeutics & research. 2013;2:21.

[137] Evens AM, Balasubramanian S, Vose JM, Harb W, Gordon LI, Langdon R. A phase I/II multicenter, open-label study of the oral histone deacetylase inhibitor abexinostat in relapsed/refractory lymphoma. Clinical Cancer Research. 2015:clincanres. 0624.2015.

[138] Raha P, Thomas S, Thurn KT, Park J, Munster PN. Combined histone deacetylase inhibition and tamoxifen induces apoptosis in tamoxifen-resistant breast cancer models, by reversing Bcl-2 overexpression. Breast Cancer Research. 2015;17:26.

[139] Vaiserman AM, Pasyukova EG. Life extension in Drosophila by histone deacetylase inhibitors. Life Extension: Springer; 2015. p. 245-64.

[140] Ling Y, Wang X, Wang C, Xu C, Zhang W, Zhang Y. Hybrids from Farnesylthiosalicylic Acid and Hydroxamic Acid as Dual RasRelated Signaling and Histone Deacetylase (HDAC) Inhibitors: Design, Synthesis and Biological Evaluation. ChemMedChem. 2015;10:971-6.

[141] Pham-The H, Le-Thi-Thu H. Integrating structure and ligand-based approaches for modelling the histone deacetylase inhibition activity of hydroxamic acid derivatives. Asian J Pharm Clin Res. 2018;11:198-206.

Chapter-2

Synthesis of N-hydroxycinnamide derivatives and their bio-evaluation

2.1. INTRODUCTION

Cancer is one of the mostsevere public health issue around the globe according to the World Health Organization (WHO)[1]. Among various type of cancers breast cancer is one of the major causes of cancer death among women worldwide. Due to its complex cancer biology, it is necessary to use multiple therapeutic modalities. So far, the conventional treatments for breast cancer are surgical intervention, hormonal therapy, radiotherapy and chemotherapy. It is merely responsible for 20-25% of all cancer cases and 15-18% of cancer deaths among women [2]. Although, the emergence of drugs such as Tamoxifen and Toremifene makes chemotherapy a viable choice for breast cancer patients. The development of drug resistance and severe side effects are unresolved problems in clinical oncology [3]. Therefore, the search for novel anti-cancer compounds with improved features is needed. In recent oncology research, different breast cancer cell lines have been applied by investigators for drug discovery purposes and among these cells estrogen non-dependant MDA-MB-231 is one of the most extensively used model [4].Hydroxamic acids or hydroxamates are carboxylic acid or aldehyde analogues where –COOH group or –CHO group has been replaced by CONHOH or –CONH-R[5, 6].Hydroxamic acids are well knowneffective molecules in the field of cancer chemotherapy and as a mutagenic agent. Several hydroxamates based drugsare functioning very good in clinicsfor cancer chemotherapy such asSAHA[7], PXD-101(Belinostat&Topotarget) [8] and LBH-589(Panobinostat)[9] which are approved by the U.S. Food and Drug Administration (FDA) in October 2006, July 2014 and February 2015, respectively(**Figure 1**).

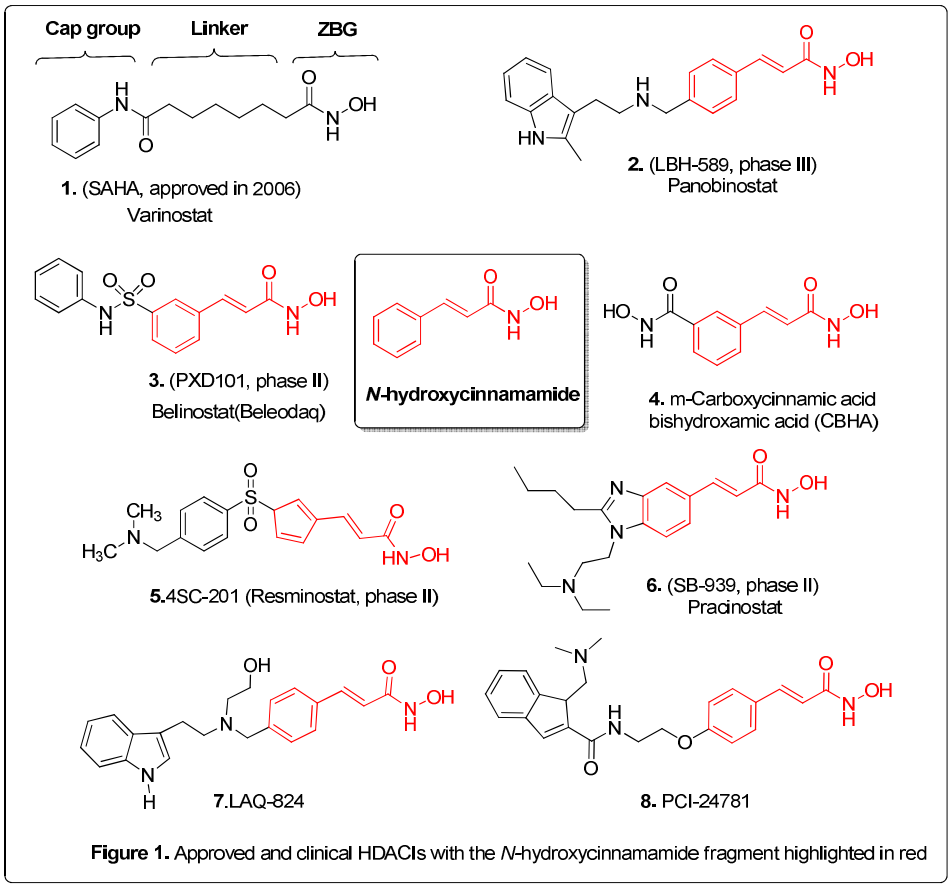


Figure 1. Approved and clinical HDACIs with the *N*-hydroxycinnamamide fragment highlighted in red

There are some other hydroxamate based molecules are in clinical trials such as SB-939 (phase II)[10] and 4SC-201 (Rasminostat, phase II)[11],m-carboxycinnamic acid bishydroximic acid (CBHA) [12].Among various derivatives of hydroxamic acid, SAHA (Suberoyl Anilide Hydroxamic Acid) is considered as a potent anticancer agent. These molecules posses very good chelating ability.This chelating property make them very favourable for enzyme inhibition and therefore,hydroxamates are of great chemotherapeutic importance. Due to these special properties hydroxamates are very interesting group for scientists from all over the world. Many research groups have synthesized different hydroxamic acid moieties aswell known inhibitors of matrix metalloproteinases (MMPs)[13], peptidyldeformylases[14],adenylyl cyclases (ACs)[15],inosine monophosphate dehydrogenase (IMPDH)[16], histone deacetylase (HDAC)[17], carbonic anhydrases [18],tumor necrosis factor converting enzyme

(TACE, ADAM17)[19]and TNF-α–converting enzyme[20].One research group reported anti-leukemic activity[21] in hydroxamic acids.Aza indole hydroxamic acid derivatives are known to possess potent anti-HIV activities[22], while sulfonamido hydroxamates are good anti-osteoarthritis agents[23].In addition to these interesting properties this moiety is also present in many growth factors[24], food additives[25], anti-biotics[26], anti-tumors[27],anti-fungals[28], cell division factors[29] and enzyme inhibitors[30]. They also haveshown inhibition against melanogenesis[31].As many enzymes are inhibited by hydroxamates, several physiological processes are affected by this versatile class[32]. Some molecules with hydroxamic acid functionality have also been reported as NO donors[33] and the acetylated hydroxamates derivatives can act as effective aspirin analogues by prostaglandin H_2synthase inhibition[34, 35].

There are several hydroxamic acid derivatives that are either in preclinical or clinical studies against MMP inhibitors and some of them are listed below (**Figure 2**).

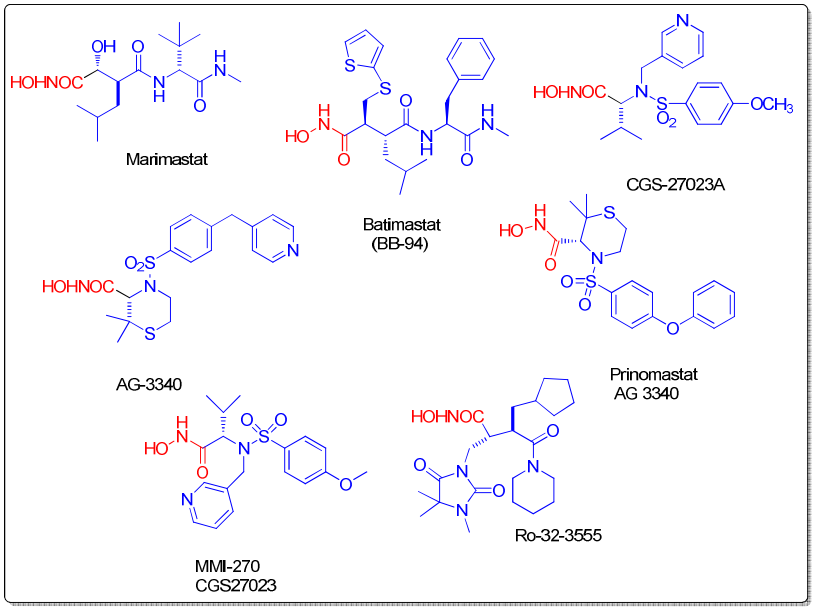


Figure 2. MMP inhibitors in clinical trial

Peptidyl deformylase is a metalloenzymes containing hydroxamate moiety has recently been recognized to utilize Fe^{++} as the catalytic metal for *N*-formyl hydrolysis during peptide biosynthesis. The Fe^{3+} is co-ordinated to two histidine residues, one cysteine residueand a molecule of water, which can donate a proton to Glu133 (**Figure 3**).

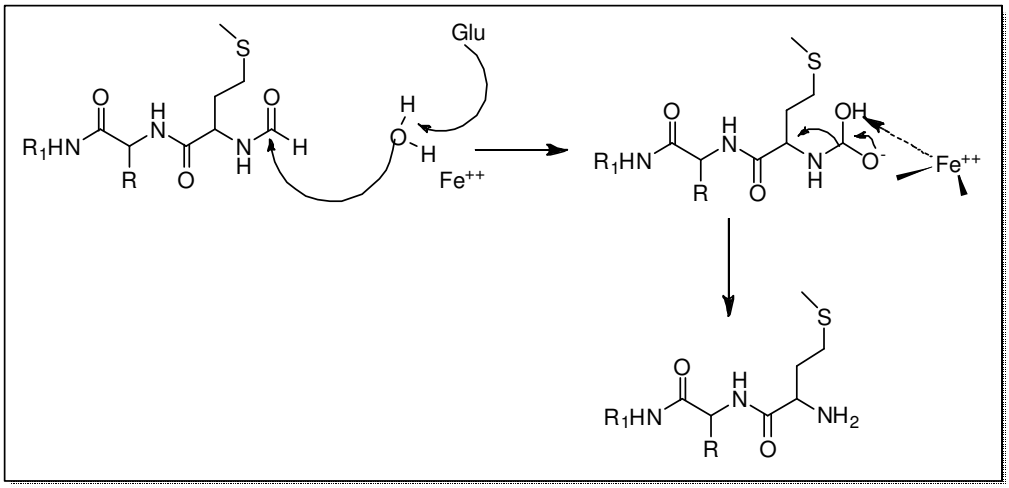


Figure 3.

As shown in **Figure 3,** peptidyl deformylase is responsible for the co-translational removal of the *N*-formyl methionine in the protein biosynthesis during the chain elongation of peptides[36]. This enzyme has been identified as a target to develop new drugs. Hydroxamates are known to inhibit peptidyl deformylase where hydroxamate attached to the molecule can interact with the metal ion forming a transition state structure, which corresponds to formyl methionyl peptide bound to a ferrous ion at the site of the deformylase enzyme[37].

Hydroxamic acids are also known as mainsynthons in the resinproduction[38].Hydroxamic acids are natural, low molecular weight, highly specific Fe-chelating siderophores (**Figure 4**)[39]. Triazolyl hydroxamates have been

reported to be active against pancreatic cancer cell line and inhibit the growth of *Plasmodium falciparum*[40].

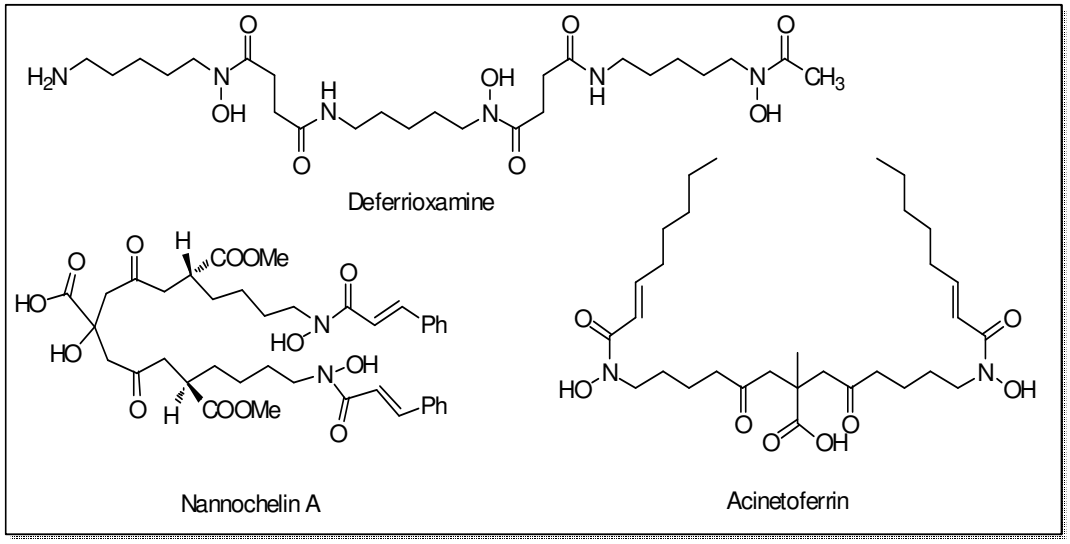


Figure 4. Hydroxamate based natural siderophores

Mammalian serine racemase (SR), an enzyme responsible for the biosynthesis of the neurotransmitter D-serine, activates the *N*-methyl-D-aspartate (NMDA) receptors in the CNS. The above enzyme is inhibited by hydroxamic acids[41].They can also be used as bioisosteres of carboxylic acid, being weak acids[42].Hydroxamates are known inhibitors of the enzyme Ribonucleotide reductase (RNR) of *Plasmodium* which catalyzes the reduction of ribonucleotides to deoxyribonucleotides, the first and rate-limiting step for *de novo* synthesis of deoxyribonucleoside-triphosphates and hence RNA has been proved as safe target [43].The hydroxamates have also shown very good anti- trypanosomal activity by inhibition of the α-glycerophosphate oxidase system, cysteine proteases, 6-phosphogluconate dehydrogenase enzyme[44-46].

In recent years, hydroxamic acid derivatives have attracted increasing attention for their potentialtowards various etiological factors associated with

cancer[47]. Our main objective to evaluate this single functional moiety which not only fit in the receptor but also create a diversified activity towards various diseases.They have also been used as acyl cation equivalents for the preparation of carbonyl compounds and *N*-methoxyamides as precursors of *N*-methoxy-*N*-acylnitrenium ions in electrophilic aromatic substitutions and as precursor for *β*-lactams synthesis[48]. Different *N*-benzoxy amides as precursor of hydroxamic acids, are known for different biological activities including anti-inflammatory[49],anti-asthmatic[30], anti-metastatic[50], anti-biotic[51],psychotropic[52], insecticidal[53], acaricidal[54]and nematocidal activity[55].

2.2.Earlier methods of synthesis

Hydroxamic acids or hydroxamates have been prepared earlier by both conventional solution phase synthesis &solid phase synthesis. Recently microwave assisted synthesis is also reported. Primarily they have been prepared by reaction involving amines or carboxylic acids or their derivatives few of the methods are briefly described below.

2.2.1.Solution phase synthesis

2.2.1.1. By reaction of Oximes:

Earlier hydroxamates synthesis was reported by reductive acylation of oximes. These oximes on reaction with triethylsilyl hydride and acid chloride results *N*-acyl hydroxamate in good yields (**Figure 5**)[56].

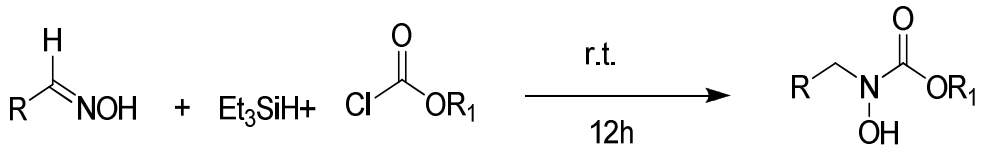


Figure 5

2.2.1.2. By ring opening of dicyanooxiranes:

α-Halo hydroxamic acid derivative were synthesized in good yields by reaction of dicyano oxirane with hydroxyl amines in presence of lithium bromide (**Figure 6**)[57].

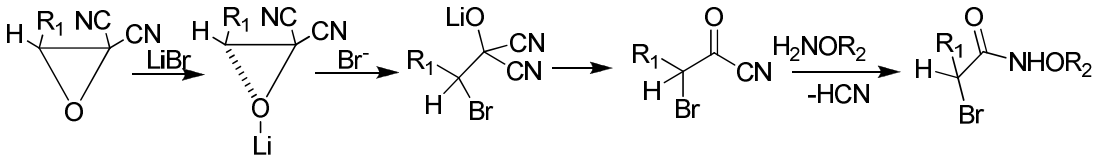


Figure 6

2.2.1.3. By reaction of amines with benzoyl peroxide

Perkins, et al. reported synthesis of *N*-Alkylated hydroxamic acid [58]in good yields by reaction of different amines followed by subsequent reaction with benzoyl peroxide, acetyl chloride and ammonia (**Figure 7**).

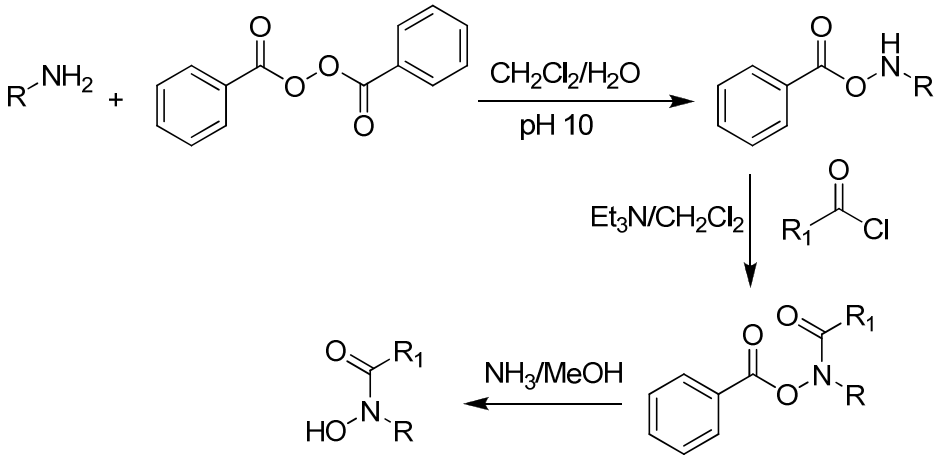


Figure 7

2.2.1.4. By diamine:

The reaction of 1,3-propanediamine and di-*tert*-butyl dicarbonate afforded 3-(*tert*-butoxycarbonylamino) propylamine which was dissolved in a biphasic carbonate buffer (pH 10.5). The latter gave desired benzoyloxy amine. Further acylation with

trans-2-octenoyl chloride resulted in *N*-(3-(*tert*-butoxycarbonylamino)propyl)-*N*-(benzoyloxy)-2(*E*)-octenamide and latter on further deprotection gave the respective hydroxamic acid in good yield (**Figure 8**)[59].

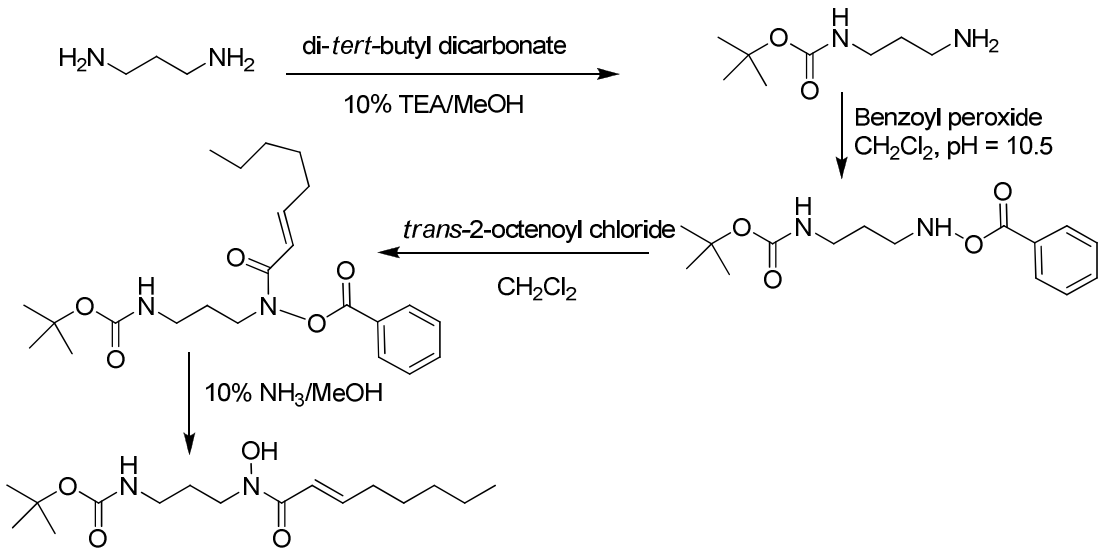


Figure 8

2.2.1.5. By *O*-benzylhydroxylamine hydrochlorides:

Reaction of *O*-benzylhydroxylamine hydrochlorides with 2-acylpyridazin-3(2*H*)-ones in the presence of triethyl amine or amberlite IRA-67 in acetonitrile is reported to give respective *O*-alkyl hydroxamides in good to excellent yields (**Figure 9**)[60, 61].

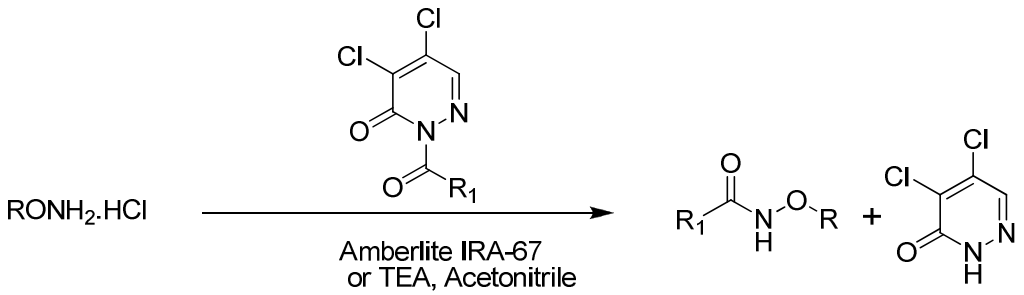


Figure 9

2.2.1.6. By ethyl malonyl chloride:

Slow addition of ethyl malonyl chloride to a mixture of *O*-protected *N*-methyl hydroxyl amine in presence of Et_3N (1 equiv) afforded *O*-silylated hydroxamic acid in good yield (**Figure 10**)[62].

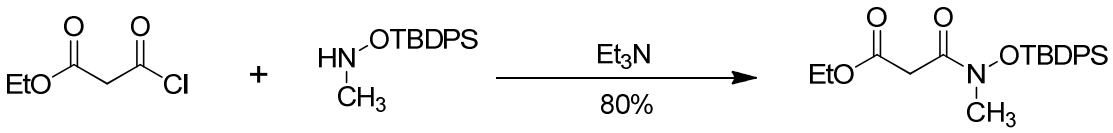


Figure 10

2.2.1.7. By MgO mediated synthesis from acid chlorides:

Hydroxylamine hydrochloride in methanol/water was treated with MgO followed by addition of a solution of Fmoc-amino acid chloride in THF and MgO, to get the respective amino acid derived hydroxamic acid in good yields (**Figure 11**)[63].

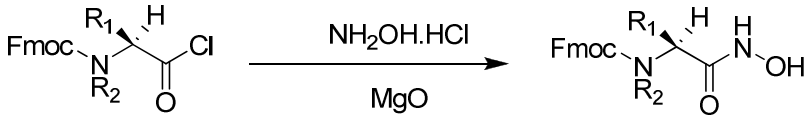


Figure 11

2.2.2. From Carboxylic acids and their derivatives

2.2.2.1.By α, β unsaturated acids:

Reactions of α, β unsaturated carboxylic acids with trityloxyamine in the presence of1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt)gave an intermediate (**A**). The latter on deprotection on treatment with trifluoroacetic acid (TFA)/triethyl silane (Et_3SiH) in

dichloromethane(CH_2Cl_2)gave the respective hydroxamate in good yields. (**Figure 12**)[57].

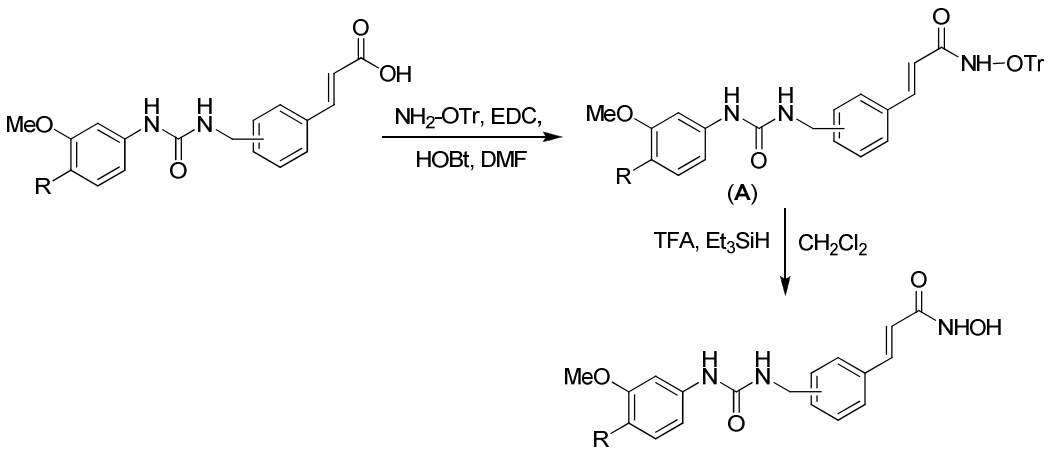


Figure 12

2.2.2.2. By pyridyl carboxylate:

Reaction of pyridylcarboxylate with sequential reaction of LiOH, oxalyl chloride, and hydroxyl amine gave the respective hydroxamates in good yields. The latar is an important matrix metalloproteinases inhibitor (**Figure 13**)[64, 65].

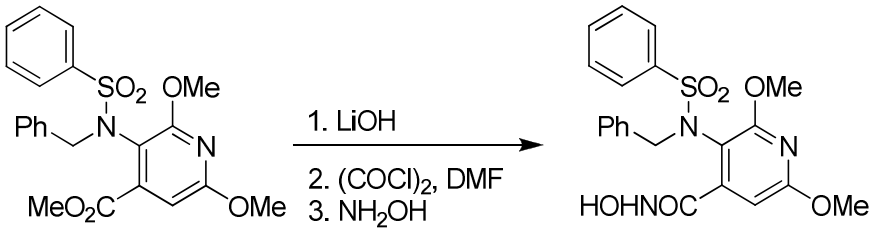


Figure 13

2.2.2.3. By ademantyl carboxylic acid

Admantane based hydroxamates were prepared by Chang, et.al[66]. The method involves conversion of ademantyl carboxylic acid to admantylcarbaxamide by reaction with 4-amino acid methyl benzoate followed by treatment with sodium

hydroxide to give the respective acid. The latter on reaction with ethylchloroformate followed by treatment with hydroxyl amine gave corresponding hydroxamate (**Figure 14**).

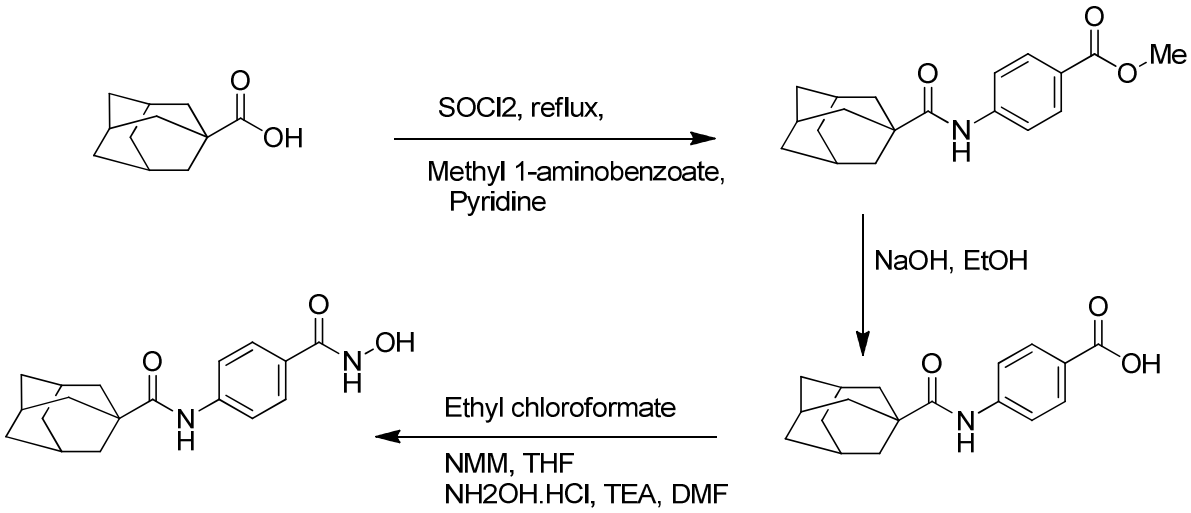


Figure 14

Similarly 5-arylisoxazole-3-carboxylic acid methyl esters were converted to the respective hydroxamic acids in good yields by heating with hydroxylamine hydrochloride in the presence of KOH. The compounds were identified as selective Histone Deacetylase Inhibitors (HDACIs) (**Figure 15**)[67].

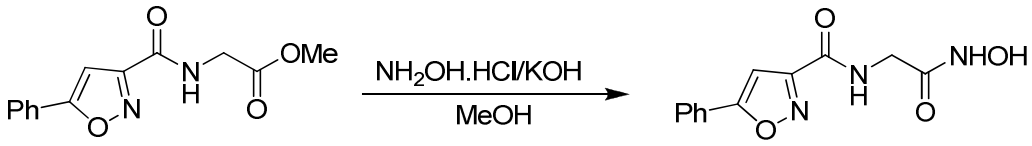


Figure 15

2.2.2.4.By using PyBrop and hydroxyl amine:

Carboxylic acids were activated with PyBrop (bromo-tris-pyrrolidinophosphonium-hexa-fluorophosphate), followed by addition of *N*-methyl

hydroxyl amine in dichloromethane to get the hydroxamic acid in good yield (**Figure 16**)[32].

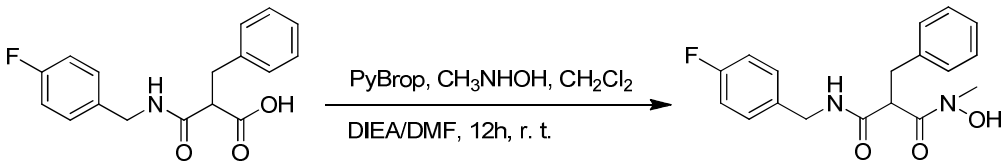


Figure 16

2.2.2.5. By using HOBt and EDC as a coupling reagent:

Hydroxamic acids were synthesized in good yields from carboxylic acids by using EDC/HOBt as coupling reagent, followed by attack of *O*-benzyl hydroxyl amine in THF at 0 ºC (**Figure 17**)[68].

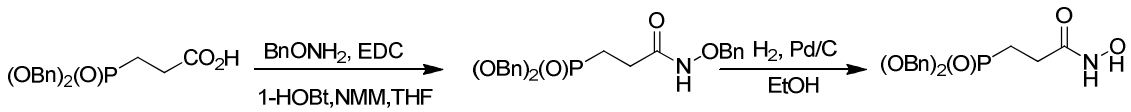


Figure 17

2.2.2.6. By coupling of carboxylic acids with hydroxylamines

A one pot procedure for the synthesis of hydroxamates from carboxylic acid has been developed recently.

2.2.2.7. By using TCT (2, 4, 6-Trichlorotriazine) as coupling reagent:

It involves reaction of carboxylic acid with cyanuric chloride in the presence of *N*-Methylmorpholine (NMM), Dimethyl aminopyridine (DMAP) as catalyst followed by treatment with $NH_2OH.HCl$ to give the respective hydroxamates (**Figure 18**)[69].

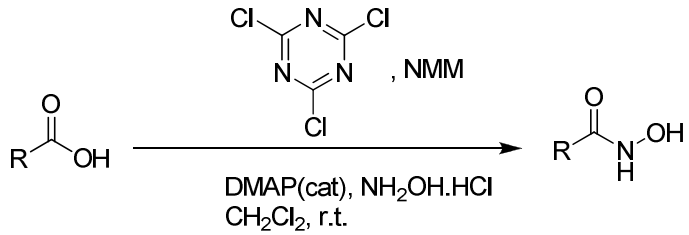


Figure 18

2.2.2.8. By coupling of acid with hydroxylamine using phosphoric acid diethyl ester 2-phenylbenzimidazol-1-yl ester:

Reaction of carboxylic acid with *O*-alkyl hydroxylamine hydrochloride in the presence of phosphoric acid diethyl ester 2-phenylbenzimidazol-1-yl ester in DMF provides *O*-alkyl hydroxamic acids in excellent yield (**Figure 19**)[70].

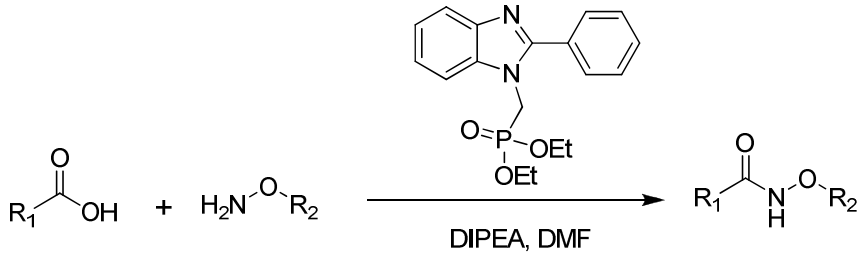


Figure 19

2.2.2.9. By multicomponent reaction:

The multicomponent reaction of *O*-Benzyl hydroxylamine, isovaleraldehyde, acetic acid, and cyclohexyl isocyanide in the presence of $ZnCl_2.Et_2O$ yielded hydroxamic acid in good yield (**Figure 20**)[62].

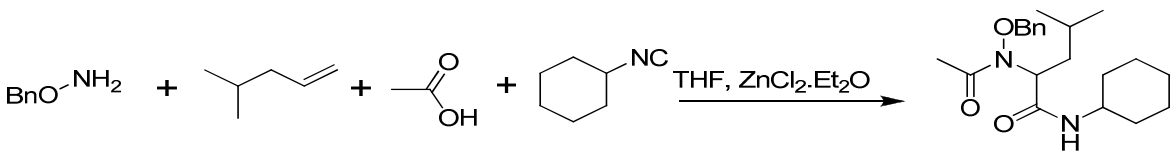


Figure 20

2.2.2.10.By using mixed phosphoric anhydrides (PPAA):

Hydroxamic acids could be achieved in moderate to good yield by reaction of carboxalic acid with hydroxyl amine hydrochloride in the presence of mixed phosphoric anhydride (PPAA) and triethylamine in acetonitrile (**Figure 21**)[71].

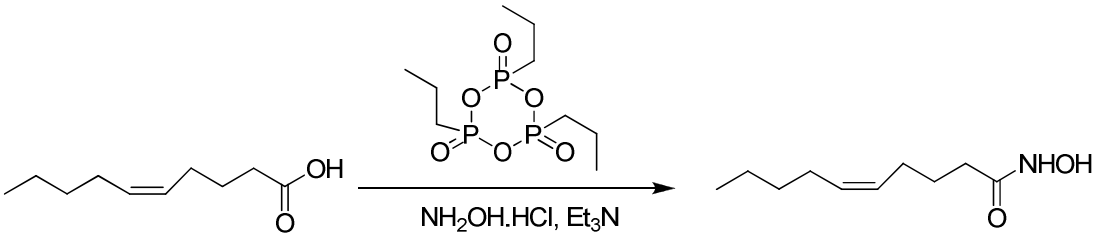


Figure 21

2.2.2.11. By amino acids:

Protected amino acids on reaction with hydroxyl amine in general resulted in hydroxamate. Supuranet. al.,[59, 72] have shown that α-amino acid on Boc protection followed by reaction with NH_2OH in (1,3-diisopropylcarbodiimide) DICand subsequent deprotection of Boc group resulted in desired hydroxamates, which is a potent carbonic anhydrase inhibitor (**Figure 22**).

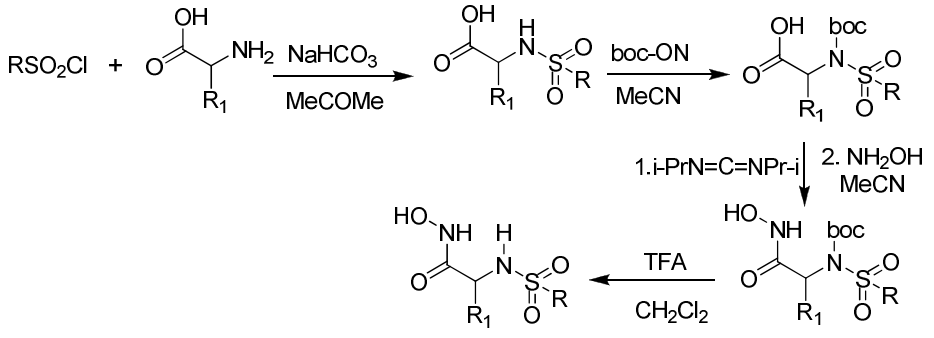


Figure 22

O-Acylation of *tertiary* butyl hydroxyl amine with *N*-acetyl glycine or alanine using EDC, yielded the respective hydroxyl amine esters. Treatment of

hydroxylamine ester withFmoc-amino acid chlorides gave protected hydroxamic acids in good yields (**Figure 23**)[61, 73].

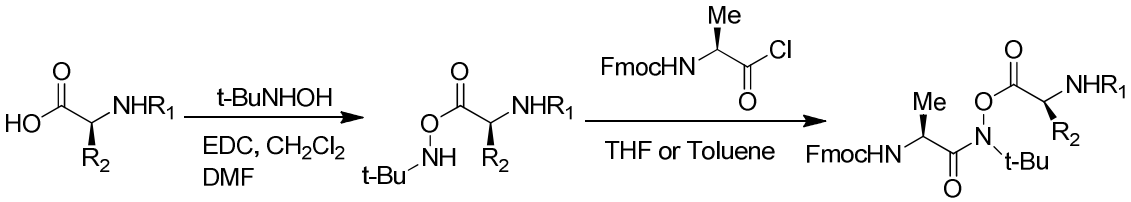


Figure 23

2.2.2.12.By using dimethyl ester:

N-Alkyl hydroxyl amide methyl esters quarate were produced in good yield by reaction of dimethyl ester with 1.1 equivalent of free base and *N*-alkyl hydroxyl amines in MeOH at ambient temperature (**Figure 24**)[74].

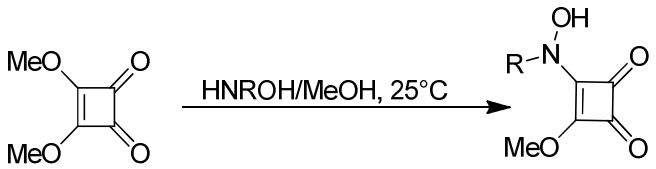


Figure 24

2.2.3. From aldehydes

2.2.3.1. By the amidation of aldehydes with nitroso compounds:

Direct amidation of aldehydes was carried by *N*-heterocyclic carbene (NHC) catalysed reaction of aldehyde with nitroso compound in the presence of DBU as base to give the respective hydroxamic acids in good yields (**Figure 25**) [75].

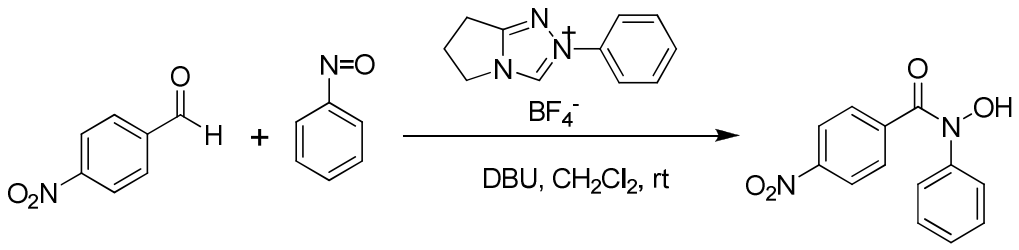


Figure 25

2.2.3.2. ByAngeli Rimini's reaction:

In this method solid supported *N*-hydroxy benzene sulfonamide was dipped in THF followed by treatment with sodium methoxide in methanol and subsequent reaction with different aldehydes to give the respective hydroxamic acid in good yields (**Figure 26**) [76].

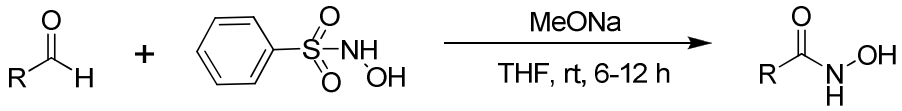


Figure 26

2.2.4. From ketones

2.2.4.1. By cyclic ketones:

Treatment of cyclobutanone or cyclopentanone with *N*-hydroxy benzenesulfonamide under basic conditions yields the ring-expanded cyclic hydroxamic acid (**Figure 27**) [77].

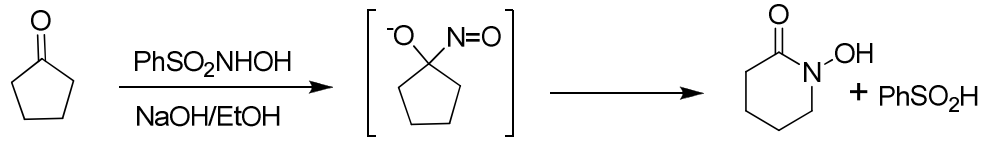


Figure 27

2.2.4.2. By *β*-carbonyl compounds:

The synthesis of hyroxamic acid derivatives was achieved in good yield by heating β-carbonyl compounds with hydroxyl amine (**Figure 28**) [78].

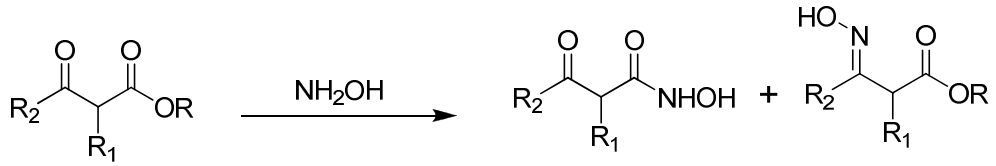


Figure 28

2.2.4.3. By Lewis acid catalysed reaction:

Stereo controlled synthesis of hydroxamic acid was achieved in good yield by ring opening of 3-aza-2-oxabicyclo [2.2.1]hept-5-ene systems followed by attack of nucleophiles. Stereo selection occurs during attack of nucleophile depending upon the size of nucleophile [79]. Attack of methanol provides *anti*-1,4-hydroxamic acid product **I**, in addition to minor amounts of the *syn*-1,4- (**II**) and *anti*-1,2-products (**III**). By changing the solvent from methanol to 2-propanol, the preference for *anti*-1,4-product (**I**) formation decreased. However with increasing nucleophile size, a greater amount of *syn*-1,4-product (**II**) was formed (**Figure 29**).

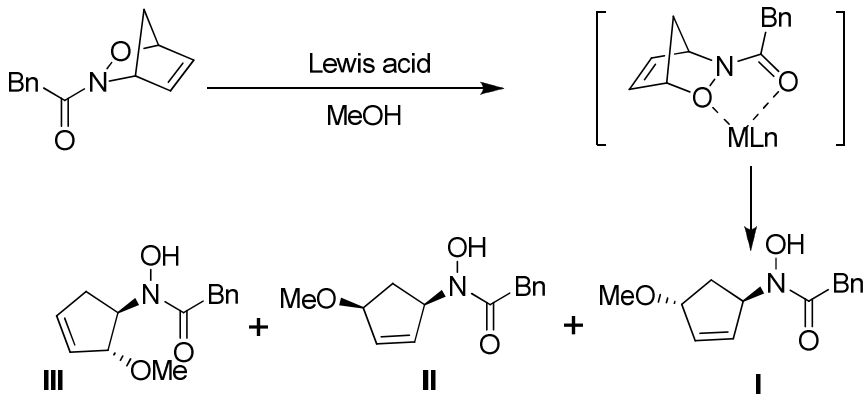


Figure 29

2.2.5. By solid phase approach

Several hydroxamates have been prepared by using solid phase support and few of them are described below.

2.2.5.1. By HMBA-AM resin as solid support:

Hydroxymethyl benzamide (HMBA-AM) resin bound ester, loaded with small quantity of potassium cyanide, was converted to the respective hydroxamic acids by reaction of hydroxyl amine in THF/MeOH solution in good yields (**Figure 30**)[80].

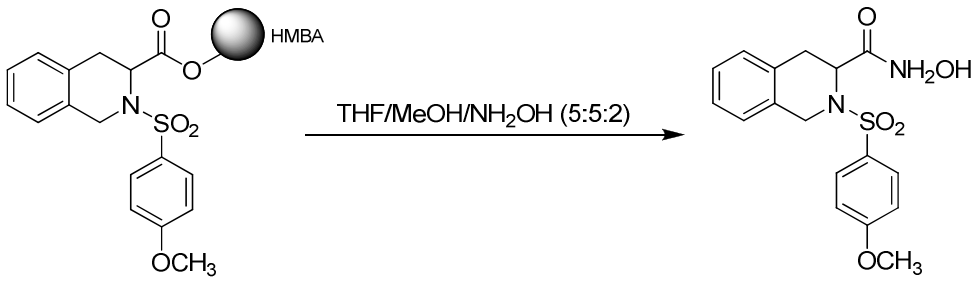


Figure 30

2.2.5.2. By wang resin as solid support:

Acylation of *N*-methyl, *O*-polymer linked hydroxyl amine, by *in situ*-prepared symmetrical anhydrides of *N*-Fmoc protected amino acids, followed by removal of the Fmoc group, yielded polymer-supported hydroxamates which was further modified chemically in the side chain. The liberated amino group was reacted with sulfonyl chlorides to provide resin-bound sulfonamides. A final Mitsunobu reaction or electrophilic substitution on *N*-alkylated sulfonamide gave the polymer-supported compounds which on further deprotection converted respective hydroxamic acids in good yields (**Figure 31**)[81]**.**

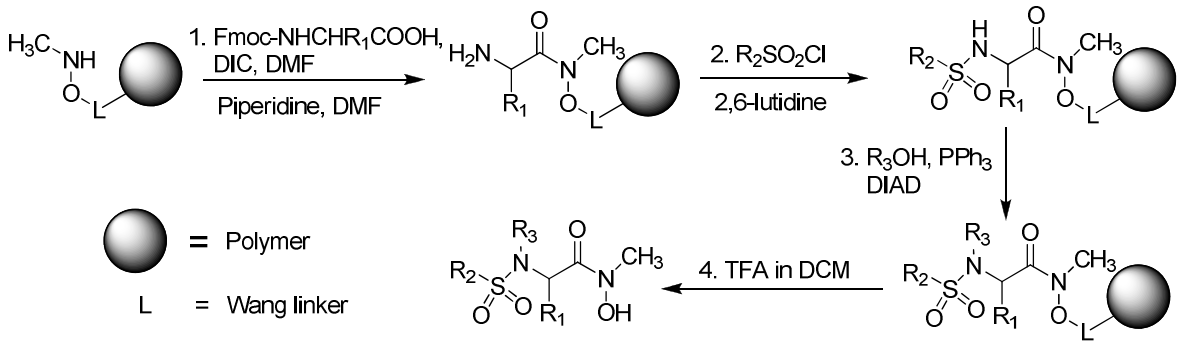


Figure 31

2.2.5.3. By ROMPGEL supported synthesis:

ROMPGEL are *N*-hydroxy succinimide containing (ROM-polymers) polymers have excellent quality and loading capacity and have been successfully used for the synthesis of hydroxamic acid, carbamates and amides. The method involves reaction of hydroxyl amine hydrochloride with triethyl amine in presence of ROMPGEL to get hydroxamic acid in quantitative yield (**Figure 32**)[82].

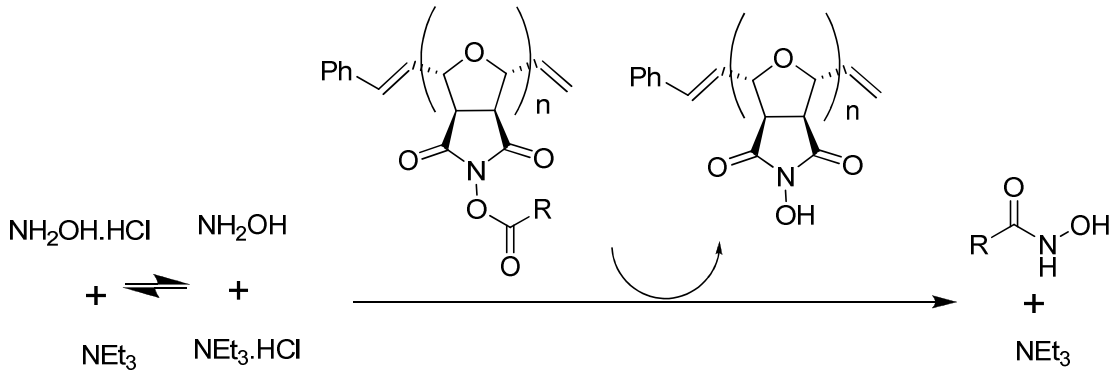


Figure 32

2.2.5.4. By *N*-Linked hydroxyl amine resin supported synthesis:

N-linked hydroxylamine resin derived from Merrifield resin was coupled with carboxylic acids under standard carbodiimide coupling condition, followed by acidolytic cleavage, to give the desired hydroxamic acids in good yield (**Figure 33**)[83].

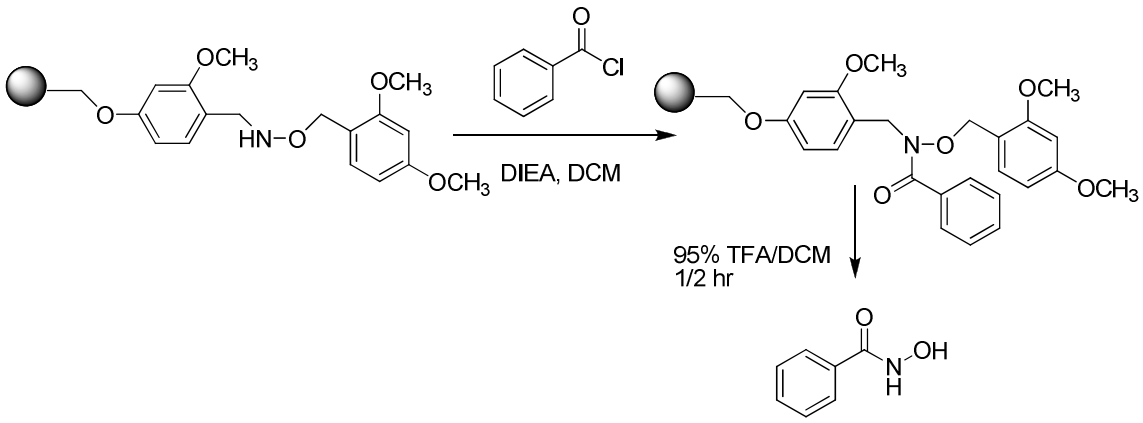


Figure 33

2.2.5.5. By hydroxythiophenol resin supported synthesis:

Several hydroxamates were prepared in good yields by reacting hydroxyl thiophenol resin bound carboxylic acids with aquous hydroxylamine solution in dichloromethane at room temperature for 17-24 h (**Figure 34**)[84].

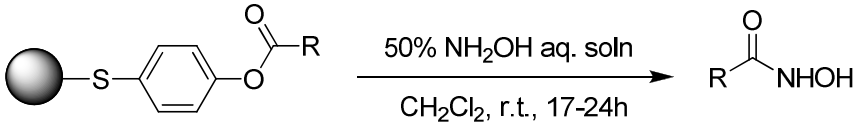


Figure 34

2.2.6. Microwave assisted synthesis:

2.2.6.1. Synthesis ofthiacalix[4]arene hydroxamic acid

Initially,*p-tert*-butyl phenol, was transformed to *p-tert*-Butylthiacalix[4]arene using elemental sulfur S_8 and NaOH in tetra ethylene glycol dimethyl ether (TEGDME)[85]. *p-tert*-Butylthiacalix[4]arene,on treatment with Phenol/$AlCl_3$ in toluene at 80 ºC gave an intermediate (**A**), which on nitration with KNO_3/$AlCl_3$ in the presence of TEGDME followed by di-t-butylation gave a trinitroderivative[86], which on partial reduction under microwave led to the formation of respective hydroxamic acid in good yields (**Figure 35**)[87].

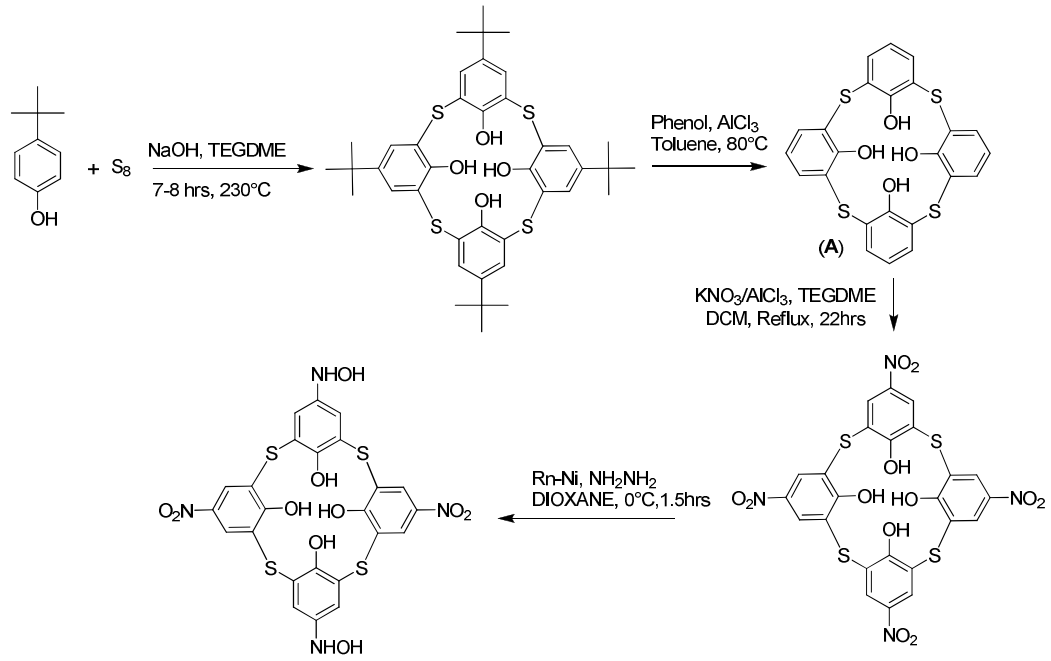


Figure 35

2.2.6.2.MW assisted Weinrebamidation of lactones

One pot Baeyer–Villiger oxidation of cycloheptanone and Weinrebamidation of lactones, with trimethylaluminum solution (Me_3Al) and benzylhydroxylamine hydrochloride ($BnONH_2{\cdot}HCl$) as nucleophile at 0^o C for 2h into the corresponding hydroxamate derivative and further elaboration of terminal –OH group into aminohydroxamate derivative(**Figure 36**) [88].

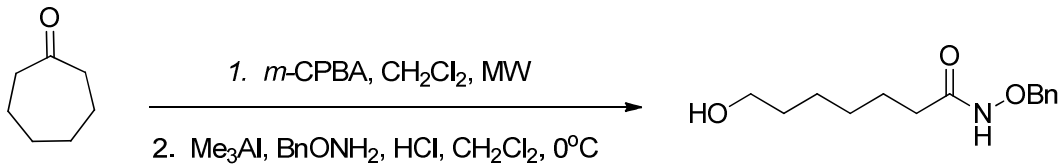


Figure 36

2.2.7. From naturally occurring compounds

2.2.7.1 Frommannitol:

Stereochemically pure acyclic *O*-benzyl hydroxamates (**XIII**) were prepared starting from D-mannitol. Adopting a selective protection strategy followed by

tosylation gave an intermediate (**II**). The latter on monomesylation gave monomesylate**III,** which was treated with sodium azide in HMPA to afford the azide derivative **IV,** which in turn was oxidized with NMO in the presence of catalytic amounts of TPAP followed by deprotection to give a ketotriol**V** [89]. Selective protection of the primary hydroxy group with TBS followed by reduction of the azide group and subsequent intramolecular reductive amination with 5% rhodium on alumina under an atmospheric pressure (15 psi) gave an intermediate pyrrolidine derivative**VI**. Cbz protection of NH group followed by benzylation led to the formation of benzyl ether. The TBS group on the primary hydroxyl group was then removed by TBAF to give alcohol **VII**in three steps. Subsequent oxidation of alcohol followed by reaction of *O*-benzylhydroxyl amine gave *O*-benzyl hydroxamic acid in good yield (**Figure 37**)[90].

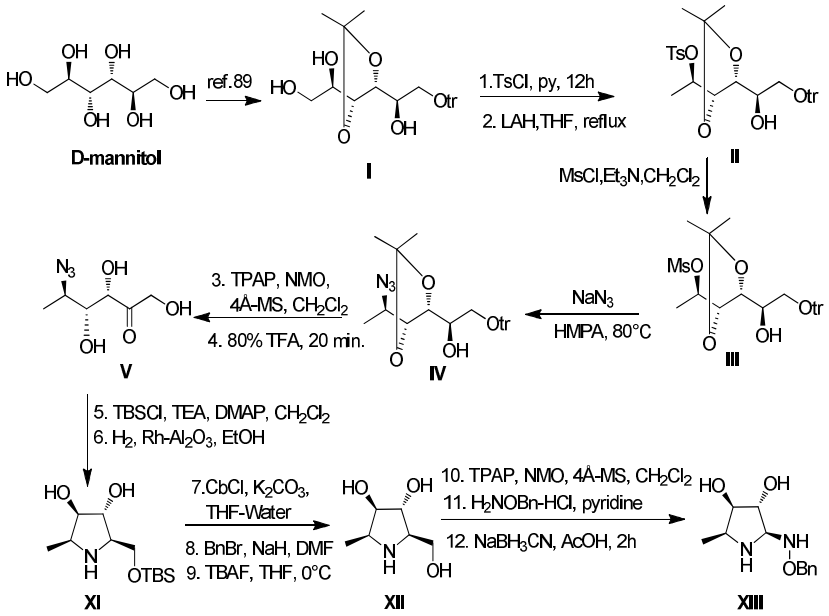


Figure 37

2.2.7.2.From (-)-dimethyl 2,3-*O*-isopropylidene-D-tartrate:

(-)-Dimethyl 2,3-*O*-isopropylidene-D-tartrate on selective reduction with sodium borohydride in pyridine gave a monoester (**A**). The latter on phosphorylation

with dibenzylphosphoroiodidate (DBPI) gave benzylphosphate monoester, which on treatment with hydroxylamine, in presence of sodium methoxide/methanol gave the hydroxamic acid derivative (**B**). The protecting group was cleaved by reduction with H_2/Pd-C in methanol to give the desired hydroxamates in stereochemically pure form (**Figure 38**)[78].

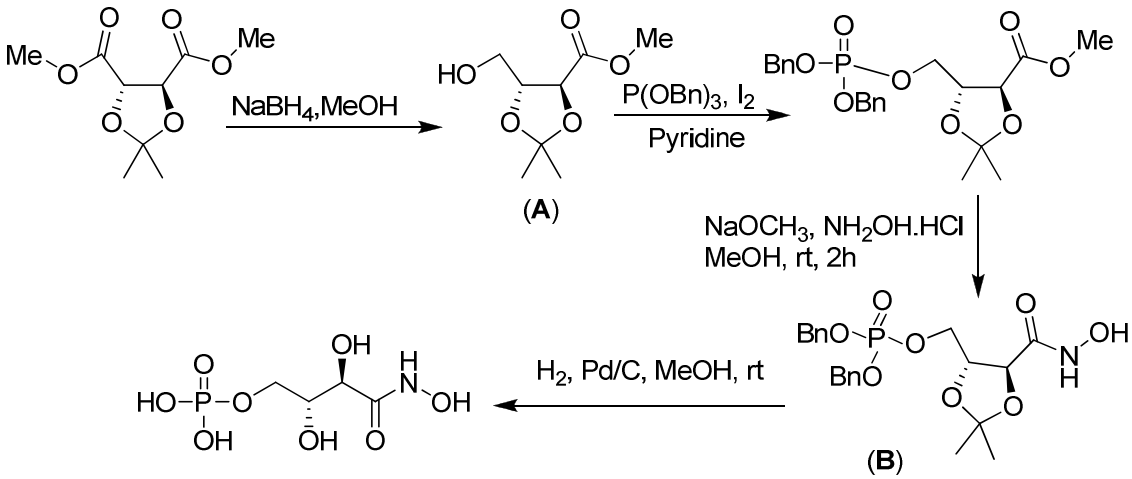


Figure 38

2.2.8. Some other important method for the synthesis of hydroxamates:

Riva etal., [91] have developed the synthesis of the suberoylalanide hydroxamic acid (SAHA), a clinical candidate in good yield**.** The method involves reaction of suberoyl chloride with a mixture of aniline and sodium carbonate in THF/H_2O. The contents were simultaneously pumped out at room temperature into a reactor to get methyl suberanilate. A mixture of crude methyl suberanilate and aqueous hydroxyl amine in methanol was again reacted with a stream of 2 equiv of sodium methoxide in MeOH at 90 °C, pumped for 50 min in to the reactor, to avoid unnecessary column chromatography purification. A silica-supported quaternary amine (ISOLUTE PE-AX) was added in this technique to filter out all the impurities (**Figure 39**).

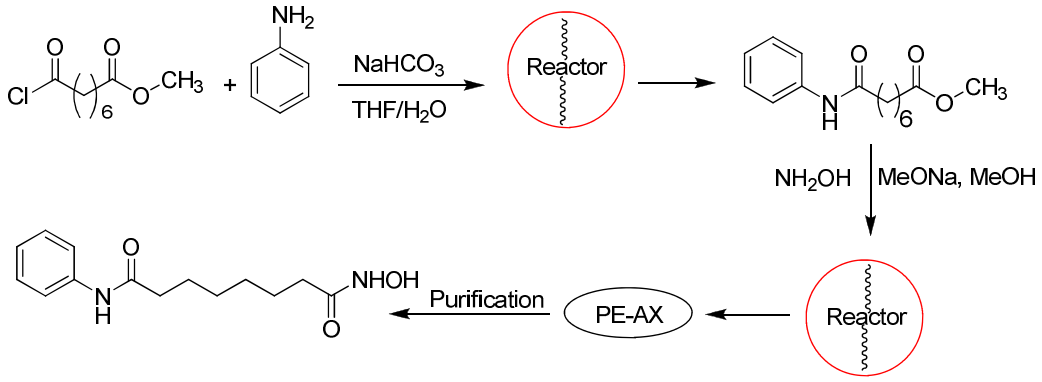


Figure 39

2.2.9. Frommethyl phenyl sulfone:

Treatment of methyl phenyl sulfone (1.0 equiv) with (LiHMDS) Lithium hexamethyldisilane (2.0 equiv) in THF at -78 °C followed by addition of *N*-benzyloxycarbamate(1.0 equiv) afforded the corresponding protected hydroxamic acid in good yields (**Figure 40**)[92].

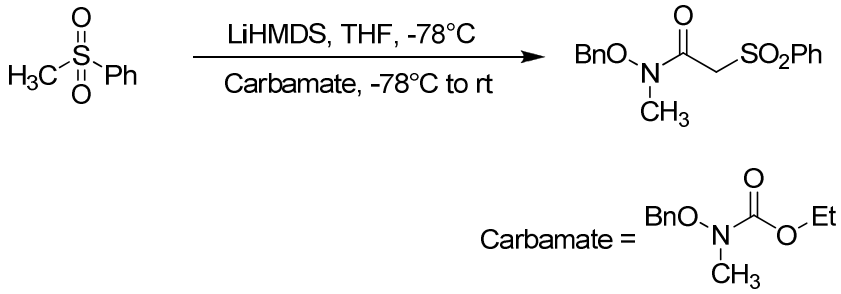


Figure 40

2.2.10. From*N*-Acyloxazolidinones:

Treatment of the achiral oxazolidinones with a variety of hydroxylamines in the presence of catalytic amount of $Sm(OTf)_3$ in THF provides corresponding hydroxamic acidin chemoselectively manner (no racemisation reported) in moderate to good yields depending on the amines (**Figure 41**)[93].

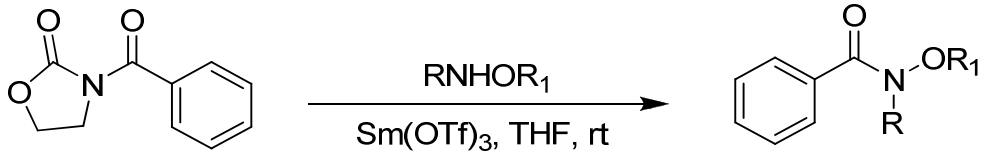


Figure 41

2.3.Basis of present work:

As described above,hydroxamic acids or hydroxamates unit is important for eliciting antiproliferative activity. However, many of these compounds are associated with drawbacks such as high toxicity, poor solubility and high cost of synthesis and drug resistance.They have also been used as acyl cation equivalents for the preparation of carbonyl compounds and *N*-methoxyamides as precursors of *N*-methoxy-*N*-acylnitrenium ions in electrophilic aromatic substitutions and as precursor for β-lactams synthesis [94, 95].

It is evident from literature survey that hydroxamic acid derivatives have attracted scientists for their potential as highly efficacious in combating various etiological factors associated with cancer [96].Therefore, there is an urgent need of molecule which can circumvent these problems and display potent anti-tumor activity.Keeping all these facts in mind we have synthesised a series of *N*-hydroxycinnamamidederivative may play a major rolein the synthesis of drug like molecules as they are structurally and stereochemically diverse molecules and due to their involvement in various biological process they are suitable for the generation of chemical libraries for drug discovery and development.

2.4. Present work:

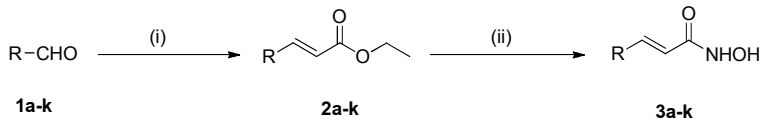
Present work demonstrates synthesis of hydroxamic acids by aldehyde using HWE reaction andsynthesis of 2-*O*-alkyl benzhydroxamic acids and finally their biological evaluation against cancer cell lines.

2.5. Synthesis of hydroxamic acid derivatives:

Compounds synthesised in this section are described below:

(*E*)-3-(4-chlorophenyl)-*N*-hydroxyacrylamide (**3a**), (*E*)-3-(2, 6-dichlorophenyl)-*N*-hydroxyacrylamide (**3b**), (*E*)-3-(pyridin-4-yl)-*N*-hydroxyacrylamide (**3c**) (*E*)-3-(4-(1*H*-imidazol-1-yl)phenyl)-*N*-hydroxyacrylamide(**3d**),(2*E*,2*E'*)-3,3'-(1,3-phenylene)bis*N*-hydroxy acrylamide (**3e**),(2*E*,2*E'*)-3,3'-(1,4-phenylene)bis*N*-hydroxy acrylamide (**3f**),(*E*)-3-(4-(benzyloxyphenyl)-*N*-hydroxyacrylamide(**3g**),(*E*)-3-(3,4,5-trimethoxyphenyl)-*N*-hydroxy-acrylamide (**3h**), (*E*)-3-(4-(2-(hydroxyamino)-2-oxoethoxyphenyl-*N*-hydroxy acrylamide(**3i**), (*E*)-3-(1-methyl-1*H*-indol-3-yl)-*N*-hydroxy acrylamide (**3j**), (*E*)-3-(furan-2-yl)-*N*-hydroxyacrylamide (**3k**),2-(allyloxy)-4-(benzyloxy)-*N*-hydroxybenzamide (**4e**).

The starting acrylate derivatives (**2a-k**) were prepared by the Horner-Wadsworth-Emmons (HWE) olefination of different aromatic aldehydes (**1a-k**) with triethylphosphonoacetate in the presence of LiOH in THF at ambient temperature[97]. The reaction of acrylate derivatives **(2a-k)** with hydroxylamine hydrochloride in the presence of KOH in methanol at 0-5 °C results in the formation of respective *N*-hydroxyacrylamide (**3a-k**) in moderate to excellent yields (**Scheme 1**).



1a-k **2a-k** **3a-k**

1-3a, R = 4-chlorophenyl
1-3b, R = 2,6-dichlorophenyl
1-3c, R = pyridin-4-yl
1-3d, R = 4-(1*H*-imidazol-1-yl)phenyl
1-3e, R = isophthalaldehyde
1-3f, R = terephthalaldehyde
1-3g, R= 4-hydroxybenzaldehyde
1-3h, R = 3,4,5-trimethoxybenzaldehyde
1i-3i, R = 4-hydroxybenzaldehyde
1-3j, R = 1*H*-indole-3-carbaldehyde
1-3k, R = furan-2-carbaldehyde

Reagents and conditions (i) LiOH (1.1eq), TEPA (1.1eq), THF, RT
(ii) $NH_2OH.HCl$ (5 eq), KOH (10 eq), CH3OH, 0°C- RT.

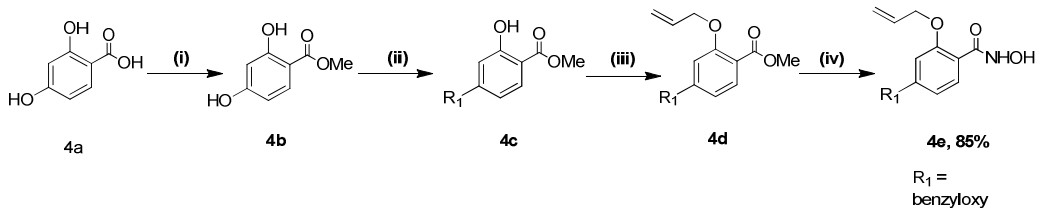
Scheme 1: **Synthesis of *N*-Hydroxycinnamamide derivatives**

Structure of of the above *N*-Hydroxycinnamamide (**3a)** was established on the basis of spectroscopic data. IR spectrum of the compound exhibited a absorption band at 3466 cm^{-1} and 1677 cm^{-1} for OH and carbonyl group of hydroxamic acid. ESIMS of the compound showed a peak at m/z 198.03 $[M+H]^+$ corresponding to molecular formula of the compound. In ^1H NMR spectrum both the methyls of methoxy group were observed as broad singlet at δ 3.43. Aromatic protons were appeared as doublet, singlet and doublet at δ 7.19, 7.04 and at 6.46 respectively. In ^{13}C NMR spectrum C=O of amide linkage was observed at δ 167.8, while quaternary aromatic carbons was appeared at 152.6 and 148.2. Aromatic carbons were appeared at the range of 110.4–123. Methyl carbons of the two methoxy groups were observed at δ 55.8 and 55.7 respectively.

Similarly reaction of methyl esters of the carboxylic acids (**2a-k**)[98-103]on reaction with hydroxyl amine hydrochloride in presence of KOH led to the formation of respective N-hydroxy benzamides **3a-k** in good yields. Structures of all the compounds were established on the basis of spectroscopic data. IR spectrum of the compounds exhibited absorption bands in the range of 3300-3450 cm^{-1} and 1660-1680 cm^{-1} for OH and carbonyl group of hydroxamic acid functionalities. ESIMS of the compounds showed $[M+H]^+$ peaks corresponding to their molecular formulae. In ^1H NMR spectra of the compounds, aromatic protons appeared as doublet, singlet and multiplets in the range of δ 8.00-6.40. In ^{13}C NMR spectrum, C signals of C=O of amide linkage were observed in the range of δ 165-172.8, while the aromatic carbons were visible in the range of 110.4–135.5.

2.6. Synthesis of 2-*O*-alkyl benzhydroxamic acids:2-(allyloxy)-4-(benzyloxy)-*N*-hydroxybenzamide(4e)

The compounds methyl 2,4-dihydroxybenzoic acid (**4a**)was esterified with methanol in presence of 20 mol% of concentrated sulphuric acid to give methyl benzoate(**4b**)which onchemoselectivebenzylation with benzyl bromidein acetone in the presence of anhydrous K_2CO_3 and catalytic amount of tetrabutylammonium bromide (TBAB)gave methyl 4-(benzyloxy)-2-hydroxybenzoate (**4c**) in 90% yield. The latter (**4c**) on allylation with allyl bromide in refluxing THF in the presence of anhydrous K_2CO_3 and a catalytic amount of tetrabutylammonium bromide (TBAB) resulted in methyl 2-(allyloxy)-4-(benzyloxy)benzoate (**4d**) [104].Finally, the methyl benzoate derivative (**4d**) on reaction with hydroxylamine hydrochloride in the presence of solid KOH in methanol at 0-5 °C led to the formation ofdesired 2-(allyloxy)-4-(benzyloxy)-*N*-hydroxybenzamide(**4e**) in 85% yield (**Scheme 2**).



Reagents and conditions (i) MeOH, 20% H_2SO_4, reflux (ii) Benzyloxy bromide, K_2CO_3, Acetone, RT, (iii) allyl bromide, K_2CO_3, TBAB, THF, reflux (iv) $NH_2OH.HCl$, KOH, MeOH, 0-30 °C.

Scheme 2: **Synthesis of *O*-alkyl benzamide derivative**

The structure of compound **4b** was established on the basis of spectroscopic data and it was identical in all respects to the compounds reported earlier [105]. ESIMS showed a peak at m/z 298.1 $[M+H]^+$corresponding to its molecular formula $C_{18}H_{18}O_4$. The IR absorptions at ν_{max}= 1717 cm^{-1}indicated the presence of the COOMe group. The ^1H NMR spectrum of compound **4d** showed the methylene protons of OCH_2 as a singlet at δ 5.13 and benzylic protons of-OCH_2 as a doublet at δ 4.67-4.66 while three

methyl protons of -OCH_3 were observed as singlet in at δ 3.81. The aromatic protons were visible as double doublet at δ 7.15 with coupling constant J = 7.7 Hz, and as multiplets at the range of δ 6.54-6.26 and δ 6.15-6.02. In the ^{13}C NMR spectrum of compound **4d**, methylene (OCH_2) carbon was observed at δ 72.1 while -CH_3 carbons of -OCH_3 were visible at δ 52.3 and aromatic carbons were observed at their usual chemical shifts.

The structure of compound **4e** was established on the basis of spectroscopic data. ESIMS spectrum showed a peak at m/z 300.1 $[M+H]^+$corresponding to its molecular formula $C_{17}H_{18}NO_4$. In IR, absorptions band of OH and NH, appears at ν_{max}= 3448cm^{-1} and 3021cm^{-1} respectively. ^1H NMR spectrum of compound **4e** was similar to the compound **4d** with absence of signal for methoxy group protons and appearance of -NH protons at δ 9.08. In the ^{13}C NMR spectrum of compound **4e**, carbon of amide linkage was observed at δ 166.6, while other carbons were visible at their usual chemical shifts. The details are given in experimental section.

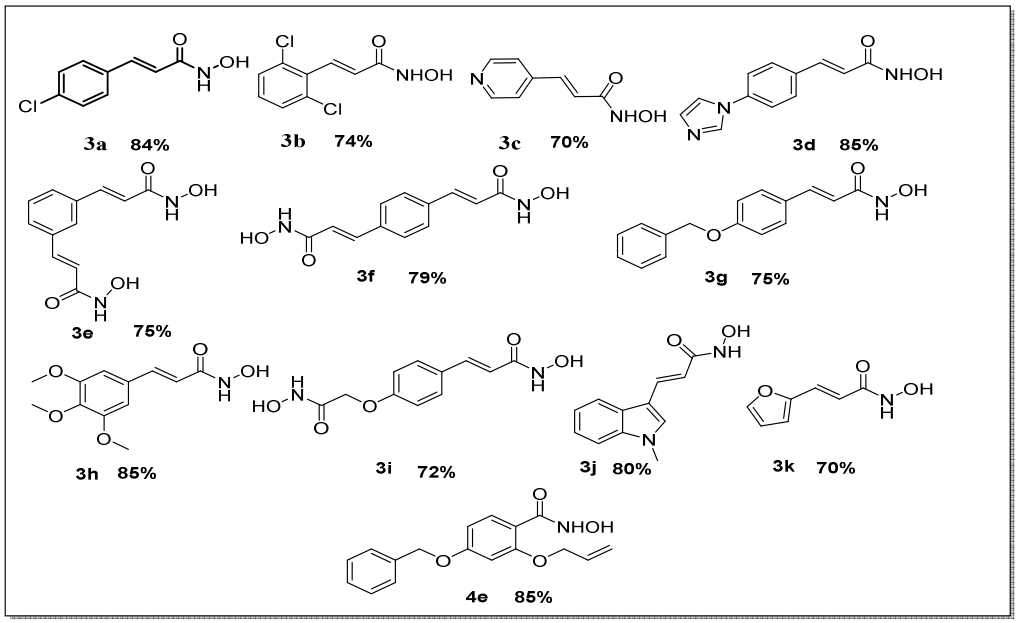


Figure 42. Library of synthesized hydroxamates

2.7. Biological activity

All the synthesized compounds were screened for anti-cancer activity in both MCF-7 (ER+ve) and MDA-MB-231 (ER-ve) cells and HEK-293 cells using MTT assay and results are shown in table 1.

Table 1. Anti-proliferative effect of synthesized compounds with cell line MCF-7, MDA-MB-231 and HEK-293.

Code	**MCF-7** IC_{50} μM	**MDA-MB-231** IC_{50} μM	**HEK-293** IC_{50} μM
3a	>50	>50	>50
3b	30±2.26	13±2.62	>50
3c	>50	45±1.43	>50
3d	>50	39±3.45	>50
3e	>50	42±2.37	>50
3f	>50	41±3.12	>50
3g	34.3±1.54	35±3.45	>50
3h	31±3.24	5±2.16	>50
3i	>50	41±2.54	>50
3j	>50	43±3.43	>50
3k	42±2.32	>50	>50
4e	45±3.34	37±3.12	>50
Tamoxifen	10±1.32	15±2.04	50±2.18

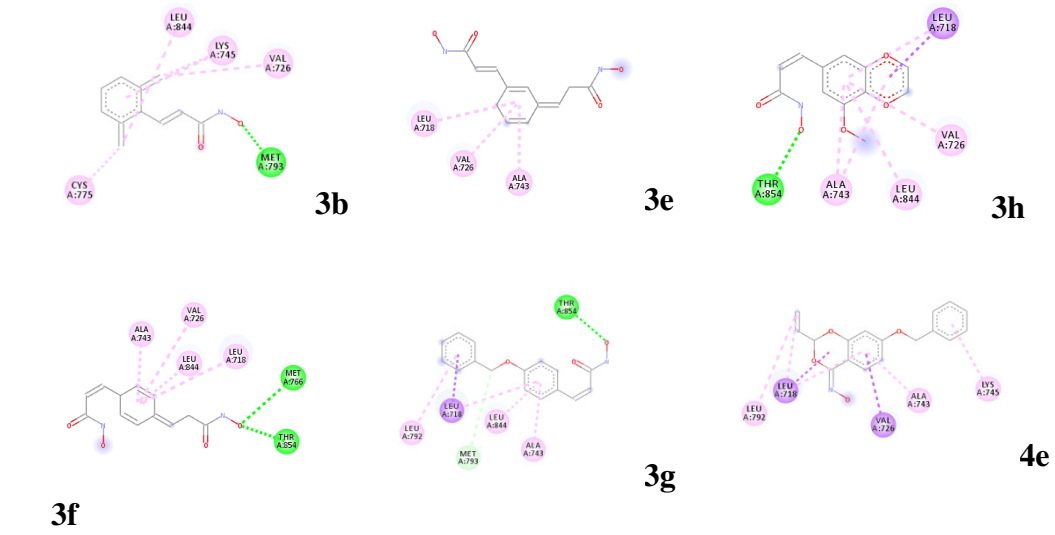
2.8. Docking study on EGFR protein target of breast cancer

2.8.1. In silico molecular docking analysis

We have analyzed the synthesized compounds through docking study using Autodock 4.2 to exploit the possible interactions between ligands and EGFR kinase. The analysis predicted that compound **3b** interacted amino acids (Leu $_{844}$, Lys_{745}, Val_{726}, Met_{793}, and Cys_{775}) in EGFR protein which could be the potential target site for binding of **3b** by formation of hydrogen bond with chloro and hydroxamic acid groups of **3b**. The **3e** interacted with amino acids (Leu_{718}, Val_{726} and Ala_{743}). **3f** interacted with amino acids (Ala $_{743}$, Val $_{726}$, Leu $_{844}$, Met_{766} and Thr_{854}). **3g** interacted with amino acids (Leu_{844}, Leu_{792}, Leu $_{718}$, Ala_{743}, Thr_{854} and Met_{793}). **3h** interacted with amino acids (Thr_{854}, Ala_{743}, Leu_{718}, Val_{726} and Leu_{844}). and compound **4e**interacted with amino acids (Leu_{792}, Leu_{718}, Val_{726}, Ala_{743} and Lys_{745})(**Figure43**) in EGFR protein.

Table 2: Analysis of the interaction between breast cancer target and synthesized molecules .

Ligands	Docking energies (Kcal/mol)	Ligands	Docking energies (Kcal/mol)
3a	-6.0	3g	-7.4
3b	-6.1	3h	-6.7
3c	-5.5	3i	-6.9
3d	-6.7	3j	-6.9
3e	-7.0	3k	-5.3
3f	-7.1	7	-7.7



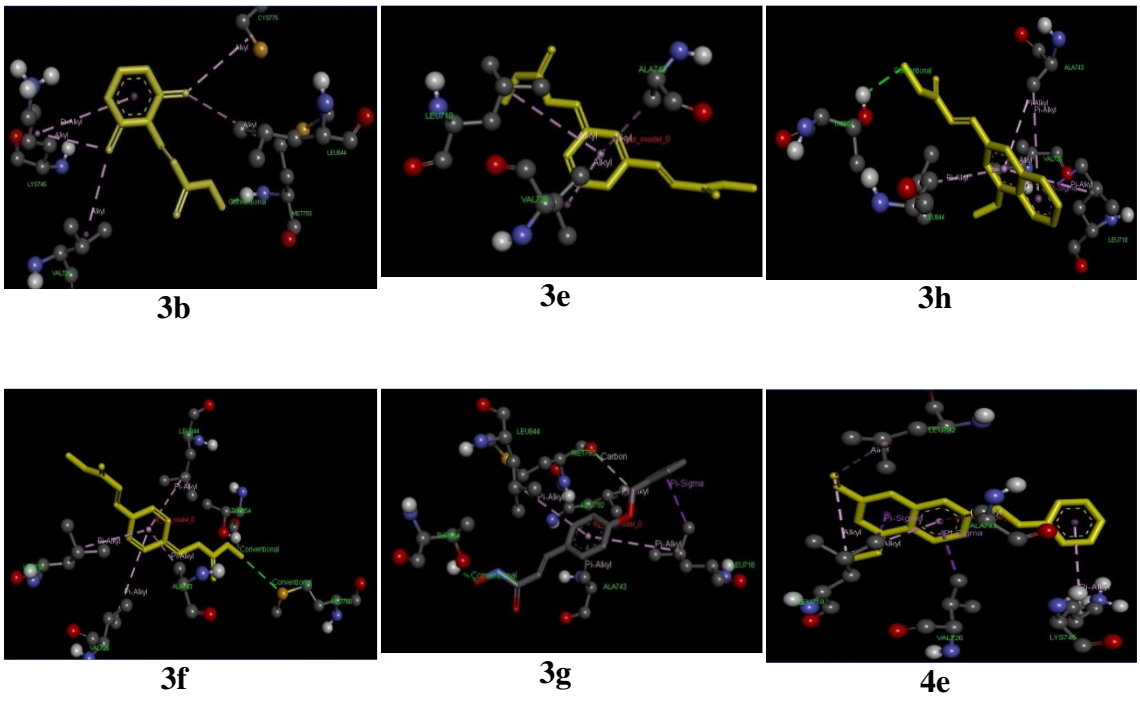


Figure 43. Interaction with EGFR target protein,2D &3D. Structure of docking image of compounds**3b**, **3e**, **3f**, **3g**, **3h** and **4e**.

2.9. Results and discussions:

Among all the synthesized compounds, **3b** and **3h** shows potent anticancer activity against breast cancer cell line. **3b** and **3h** shows potent anticancer activity against MCF-7 and MDA-MB-231 but its activity is more pronounced in aggressive breast cancer model (MDA-MB-231 cells) with IC_{50} 13µM and 5µM respectively. Out of the series compound 3h was the most active and potent analogue with lowest IC_{50} against MDA-MB-231 cells. Interestingly, compound 3hshows no cytotoxicity against HEK-293 cells suggesting that compound 3h was probably the safest compound of this series with specific activity against cancer cells.

Compound **3h** *arrest progression of cells at G0/G1 check point*

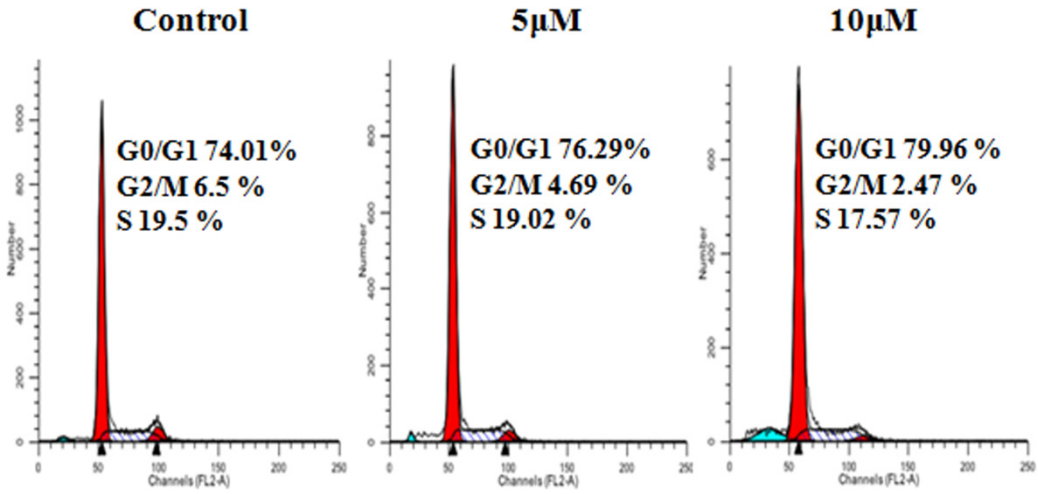


Figure 44.Effect of compound **3h** on cell cycle progression in MDA-MB-231 cells. Cells were treated with compound **3h** for 24h washed with PBS, stained with PI and analyzed by flow cytometry. Quantification of flow cytometry data has been shown as percent of total cells.

Compound **3h** dose dependently inhibited progression of cell cycle at G0/G1 check point (**Figure 44**). Furthermore, dose dependent increase of sub-diploid

populations were also increased which indicate possible DNA fragmentation and apoptosis of MDA-MB-231 cells due to compound **3h**treatment.

Compound **3h** *induces DNA damage and apoptosis in MDA-MB-231 cells*

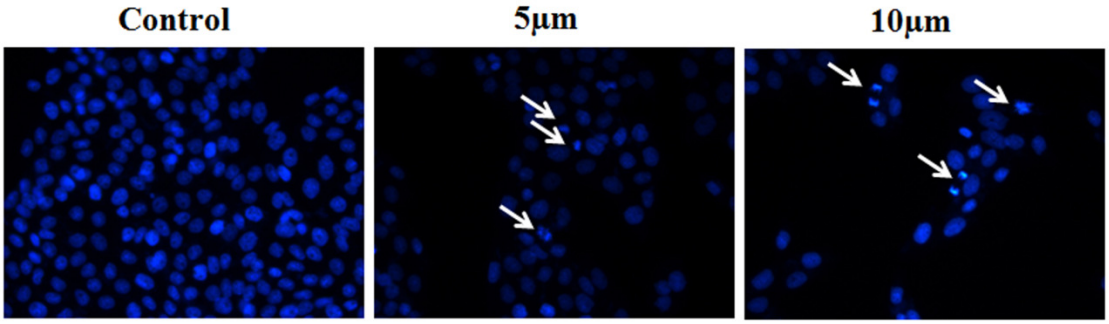


Figure 45.Effect of compound **3h** on nuclear morphology in MDA-MB-231 cells. Cells were treated with compound **3h** for 24h washed with PBS, fixed with paraformaldehyde, stained with Hoechst-33358 and analyzed by fluorescent microscopy.

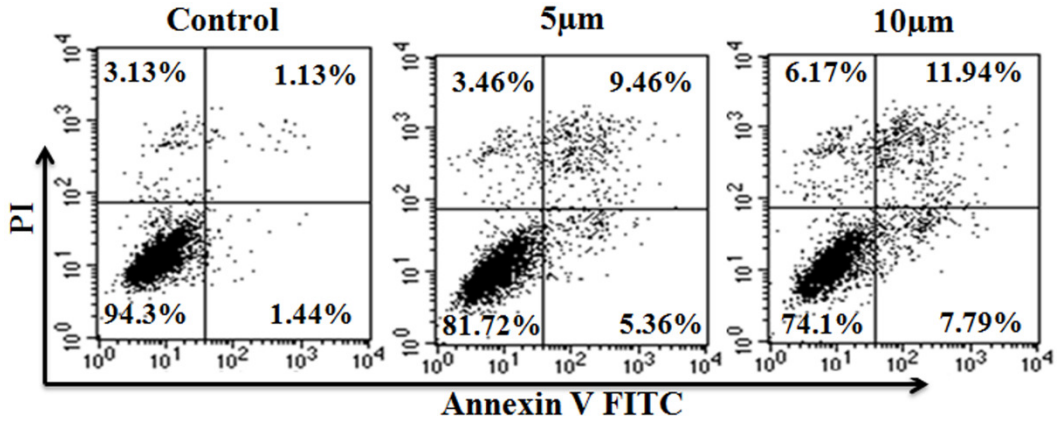


Figure 46. Effect of compound **3h**on apoptosis in MDA-MB-231 cells. Cells were treated with compound **3h**for 24h washed with PBS, stained with AnnexinV-FITC/PI and analyzed by flow cytometry. Quantification of flow cytometry data has been shown as percent of total cells.

If any compound treatment causes DNA damage and fragmentation, altered nuclear morphology of MDA-MB-231 cells can be monitored with DNA binding fluorescent Hoechst stain under florescence microscopy. In this case, compound **3h**induced dose-dependent DNA fragmentations in treated group as compared to non-treated vehicle control clearly indicating cells were undergoing apoptosis due to compound exposure (**Figure 45**). This data was further confirmed by flow cytometry using AnnexinV-FITC/PI dual staining technique. Results showed that compound **3h**induces apoptosis dose dependently in MDA-MB-231 cells as compared to non-treated vehicle control cells (**Figure 46**).

Compound **3h** *induce MMP loss in MDA-MB-231 cells*

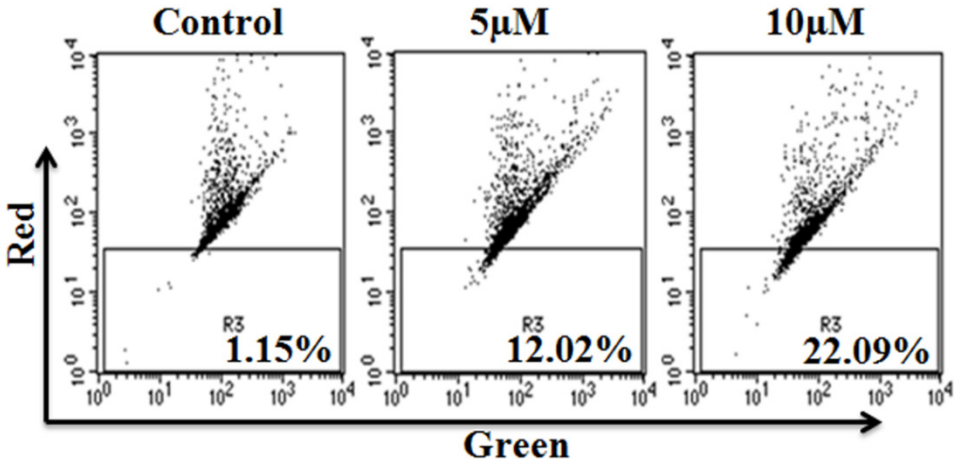


Figure 47.Effect of compound **3h** on MMP in MDA-MB-231 cells. Cells were treated with compound **3h** for 24h, washed with PBS, stained with JC-1 and analyzed by flow cytometry. Quantification of flow cytometry data has been shown as percent of total cells.

Further, effect of compound **3h** on mitochondria as alterations in MMP was carried out using membrane potential sensitive dye JC-1. Any increase in intensity of green fluorescence from red fluorescence of JC-1dye stained cells indicates mitochondrial depolarization. Flow cytometric data indicate increase of green

fluorescence in MDA-MB-231 cells with treatment of increasing concentration of compound **3h** (**Figure 47**). This clearly indicates significant loss of MMP in MDA-MB-231 cells due to compound **3h** treatment.

Compound **3h** *induce ROS generation in MDA-MB-231 cells*

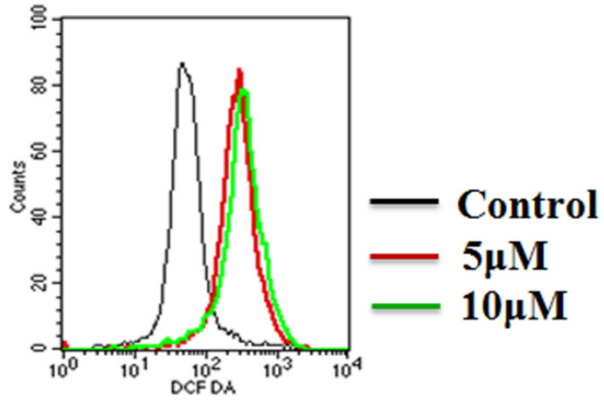


Figure 48. Effect of compound **3h** on ROS generation in MDA-MB-231 cells. Cells were treated with compound **3h** for 24h washed with PBS, stained with DCFH-DA and analyzed by flow cytometry.

Reactive oxygen species (ROS) is a well-known mediator of apoptosis and majority of chemotherapeutics detrimental to cancer cells due to their ROS inducing capacity [106, 107].As compound **3h** induces apoptosis, it is possible that it might induce ROS in MDA-MB-231 cells. Therefore, effect of compound **3h** on ROS generation in MDA-MB-231 cells was evaluated by flow cytometry using DCFH-DA dye. Flow cytometric data revealed that treatment of compound **3h** significantly induced ROS generation compared to non-treated vehicle control MDA-MB-231 cells (**Figure 48**).

2.10.Experimental section

All chemicals, reagents and solvents were purchased from SigmaAldrich and Merck. Thin layer chromatography was performed usingsilica gel 60 F254 plates with

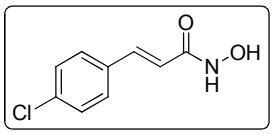
detecting agent iodine vapours, Merck silica gel (60–120 mesh) was used for column chromatography. IRspectra were recorded as thin films or in chloroform solution with aPerkin–Elmer Spectrum RX-1 (4000–450 cm^{-1}) spectrophotometer. ^{1}Hand ^{13}C NMR spectra were recorded on a Brucker DRX-400 and 101MHz in DMSO-d_6. Chemical shift values are reported in ppm relative to TMS as internal reference, unless otherwise stated; s (singlet), d(doublet), t (triplet), m(multiplet); J in hertz. FAB mass spectra wereperformed using a mass spectrometer Jeol SX-102 and ESI mass spectrawith Quattro II (Micromass). Melting points were obtained manually bycapillary methods and are uncorrected. Elemental analysis were performedon a Perkin–Elmer 2400 II elemental analyzer. The organicextracts were dried over anhydrous Na_2SO_4 and evaporation of thesolvent was carried out on a rotary evaporator under reduced pressure.

2.10.1.General synthesis of hydroxamic acid

To the stirring a mixture of desired acrylate (1 mmol) in methanol added with hydroxylamine hydrochloride (5 mmol) and KOH(10 mmol) at 0-5 °C, it was stirred at room temperature until consumption of the starting material (according to TLC). After the completion of reaction, the solvent was evaporated under reduced pressure to give a crude mass, which was purified by column (SiO_2, 60-120 mesh) using a gradient of EtOAc /hexane as eluent to give the pure compounds **3a-k**, **4e** in excellent yields.

2.10.2. (*E*)-3-(4-chlorophenyl)-*N*-hydroxyacrylamide (3a)

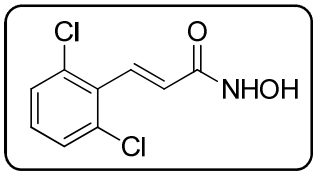
It was obtained by the reaction of ethyl-3-(4-chlorophenyl)acrylate **2a**(0.5 g, 2.37 mmol), hydroxyl amine hydrochloride (0.824 g, 11.8 mmol) and KOH(1.32 g, 23.7 mmol) in methanol at 0-5 ºCto room temperature to gave the corresponding acrylamide **3a** in 84% yield (0.85 g) as a white



solid, mp 140-142 ºC; IR(ν_{max},cm^{-1}): 3416, 3039, 1650, 1090, 754.^{1}H NMR (400 MHz, DMSO-d_6); δ_H 9.57-9.31 (1H, brs, NH) 7.60-7.58 (2H, d, *J* = 8.3 Hz, Ar-H), 7.46-7.44 (2H, d, *J* = 8.3 Hz, Ar-H), 7.41 (1H, brs, CH), 6.49-6.45 (1H, m), 2.08 (1H, s, OH).^{13}C NMR (100 MHz, DMSO-d_6); δ_C 162.9(CO), 137.5(Ar-C), 134.3(Ar-C), 134.1(Ar-C), 129.6(Ar-C), 129.4(Ar-C), 120.2.HRMS: Calcd. Accurate mass for ($C_9H_9ClNO_2$), 198.0316. Found.198.0313 $[M+H]^+$

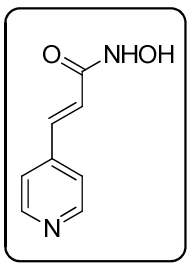
2.10.3. (*E*)-3-(2, 6-dichlorophenyl)-*N*-hydroxyacrylamide(3b)

It was obtained by the reaction of ethyl-3-(2,6-dichlorophenyl) acrylate **2b** (0.5 g, 2.03 mmol), hydroxyl amine hydrochloride (0.708 g, 10.15 mmol) and KOH(1.14 g, 20.3 mmol) in methanol at 0-5 °C to room temperature to gave the corresponding acrylamide **3b** in 74% yield (0.85 g) as a white solid, mp 142-144 ºC; IR (ν_{max}, cm^{-1}); 3684, 3411, 1602, 1523, 757.^{1}H NMR (400 MHz, DMSO-d_6); δ_{H10}.31-10.05 (1H, brs, NH), 7.54 (2H, m, Ar-H), 7.49-7.45 (1H, d, *J* = 16.0 Hz), 7.39-7.35 (1H, t, *J* = 7.8 Hz) 6.55-6.51 (1H, d, *J* = 15.9 Hz). ^{13}C NMR (100 MHz, DMSO-d_6); δ_C 162.0(CO), 134.1(Ar-C), 132.4(Ar-C), 132.0(Ar-C), 130.8(Ar-C), 129.5(Ar-C), 128.1. HRMS:Calcd. Accurate mass for ($C_9H_8Cl_2NO_2$); 231.9927. Found.231.9940 $[M+H]^+$



2.10.4. (*E*)-3-(pyridin-4-yl)-*N*-hydroxyacrylamide (3c)

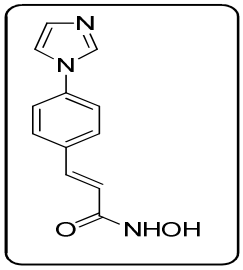
It was obtained by the reaction of ethyl-3-(pyridin-4-yl)acrylate **2c** (0.5 g, 2.82 mmol), hydroxyl amine hydrochloride (0.980 g, 14.1mmol) and KOH(1.58 g, 28.2 mmol) in methanol at 0-5 °C to room temperature to gave the corresponding acrylamide **3c** in 70% yield (0.77 g) as a yellow solid, mp 156-158 ºC; IR (ν_{max}, cm^{-1}); 3435, 2855, 1611, 1512, 1400, 756, 669.^{1}H NMR (400 MHz, DMSO-d_6); δ_H 10.97 (1H, brs, NH), 8.60-8.65 (2H, d, *J* = 5.9 Hz, Ar-H), 7.53-7.51 (2H, d, *J* = 6.0 Hz, Ar-H), 7.44-7.40 (1H, d,



J = 15.8 Hz), 6.70-6.67 (1H, d, *J* = 15.8 Hz).^{13}C NMR (100 MHz, DMSO-d_6); δ_C 161.7(CO), 150.2(Ar-C), 141.9(Ar-C), 135.8(Ar-C), 123.6(Ar-C), 121.5. HRMS:Calcd. Accurate mass for ($C_8H_9N_2O_2$), 165.0659. Found.166.0672 $[M+H]^+$

2.10.5. (*E*)-3-(4-(1*H*-imidazol-1-yl) phenyl)-*N*-hydroxyacrylamide(3d)

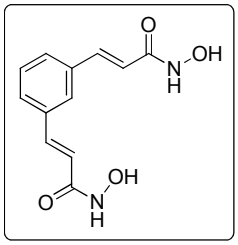
It was obtained by the reaction of (*E*)-ethyl 3-(4-(1*H*-imidazol-1-yl)phenyl)acrylate **2d** (1.0 g, 4.12 mmol), hydroxyl amine hydrochloride (1.434 g, 20.63 mmol)



KOH(2.31 g, 40.27 mmol) in methanol at 0-5 °C to room temperature to gave the corresponding acrylamide **3d** in 85% yield (0.85 g) as a light brown solid, mp 158-160 ºC; IR (ν_{max}, cm^{-1}): 3435, 2855, 1611, 1512, 1400, 756, 669.^{1}H NMR (400 MHz, DMSO-d_6); δ_H 10.21-9.64(1H, brs, NH), 8.31 (1H, m, Ar-H), 7.78 (1H, m, Ar-H),7.70 (4H, m, Ar-H) 7.50-7.46 (1H, d, *J* = 15.9 Hz, CH), 7.12 (1H, s, Ar-H), 6.53-6.49 (1H, d, *J* = 16.0, CH).^{13}C NMR (100 MHz, DMSO-d_6); δ_C 163.0(CO), 137.7(Ar-C), 137.4(Ar-C), 135.9(Ar-C), 133.8(Ar-C), 130.5(Ar-C), 129.4(Ar-C), 120.8, 120.0, 118.2. HRMS:Calcd. Accurate mass for ($C_{12}H_{12}N_3O_2$), 230.0924. Found: 230.0924 $[M+H]^+$

2.10.6. (2*E*, 2*E'*)-3,3'-(1,3-phenylene)-bis-*N*-hydroxyacrylamide(3e)

It was obtained by the reaction of diethyl-1,3-(3-phenyl)acrylate**2e** (1.0 g, 3.55 mmol), hydroxyl amine hydrochloride (1.20 g, 18.75 mmol) and KOH(1.92 g, 3.45

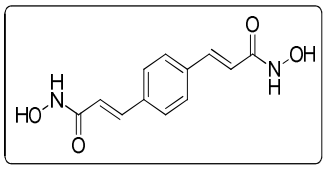


mmol) in methanol at 0-5 °C to room temperature to gave the corresponding acrylamide **3e** in 75% yield (0.75 g) as a brown solid, mp 123-125 ºC; IR (ν_{max}, cm^{-1}): 3415, 2865, 1655, 1570, 1295, 858, 755, 659.^{1}H NMR (400 MHz, DMSO-d_6) δ_H10.04 (2H, brs, NH), 7.84 (1H, s,Ar-H), 7.57-7.55 (2H, d, *J* = 7.4 Hz, Ar-H), 7.48-7.44 (2H, d, J = 16.0 Hz, CH), 7.42 (1H, s,Ar-H), 6.58-6.54 (2H, d, J = 15.76 Hz, CH).^{13}C NMR

(100 MHz, DMSO-d_6) δ 163.0 (CO), 138.0(Ar-C), 135.9(Ar-C), 128.5(Ar-C), 127.3(Ar-C), 120.4(Ar-C), 39.2.HRMS: Accurate mass for ($C_{12}H_{13}N_2O_4$), 249.0870. Found.249.0872 $[M+H]^+$

2.10.7. (2*E*,2*E'*)-3,3'-(1,4-phenylene)-bis-*N*-hydroxyacrylamide(3f)

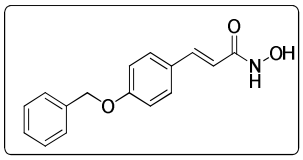
It was obtained by the reaction of diethyl-1,4-(3-phenyl)acrylate**2f** (1.0 g, 3.52 mmol), hydroxyl amine hydrochloride (1.16 g, 17.65 mmol) and KOH(1.87 g, 3.55 mmol) in



methanol at 0-5 °C to room temperature to gave the corresponding acrylamide **3f** in 79% yield (0.79 g) as a brown solid, mp 124-126 ºC;IR (ν_{max}, cm^{-1}); 3515, 2955, 1661, 1580, 1305, 765, 679.^{1}H NMR (400 MHz, DMSO-d_6); δ_H9.92 (2H, brs, NH), 7.66-7.54 (4H, m,Ar-H), 7.43-7.41 (2H, d, *J* = 10.2 Hz, CH), 6.55-6.52 (2H, d, *J* = 11.0 Hz, CH).^{13}C NMR (100 MHz, DMSO-d_6); δ_C 163.0 (CO), 138.1(Ar-C), 138.0(Ar-C), 136.2(Ar-C), 134.3(Ar-C), 131.6(Ar-C), 129.5(Ar-C), 128.5 (Ar-C), 127.1, 120.2, 39.8. HRMS:Calcd. Accurate mass for ($C_{12}H_{13}N_2O_4$), 249.0870. Found.249.0867 $[M+H]^+$.

2.10.8. (*E*)-3-(4-(benzyloxyphenyl)-*N*-hydroxyacrylamide (3g)

It was obtained by the reaction of (*E*)-ethyl 3-(4-(benzyloxyphenyl)acrylate **2g** (1.0 g, 3.35 mmol), hydroxyl amine hydrochloride (1.16 g, 16.75 mmol) and KOH(1.87 g,

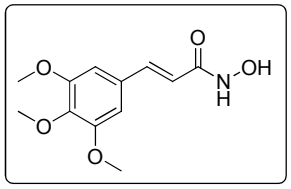


3.35 mmol) in methanol at 0-5 °C to room temperature to gave the corresponding acrylamide **3g** in 75% yield (0.75 g) as a brown solid, mp 123-125 ºC;IR (ν_{max}, cm^{-1}): 3409, 3104, 1669, 1537, 1491, 1232, 747, 641.^{1}H NMR (400 MHz, DMSO-d_6); δ_H 10.55 (1H, brs, NH),7.52-7.50 (2H, d, J = 8.1 Hz, Ar-H), 7.46-7.38 (5H, m, Ar-H) 7.35-7.33 (2H, d, *J* = 8.0 Hz, Ar-H), 6.41-6.37 (1H, d, J = 16.56 Hz, CH), 6.35-6.32 (1H, d, J = 15.68 Hz, CH), 5.14 (2H, s, OCH_2).^{13}C NMR (100 MHz, DMSO-d_6); δ_C 159.8 (CO),

143.9(Ar-C), 138.4(Ar-C), 137.2(Ar-C), 128.9(Ar-C), 128.3(Ar-C), 128.0(Ar-C), 117,1(Ar-C), 115.6(Ar-C), 69.7. HRMS: Calcd. Accurate mass for ($C_{16}H_{16}NO_3$), 270.1125. Found.270.1116$[M+H]^+$.

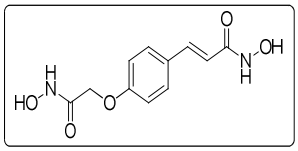
2.10.9. (*E*)-3-(3,4,5-trimethoxyphenyl)-*N*-hydroxy acrylamide (3h)

It was obtained by the reaction of (*E*)-ethyl 3-(3,4,5-trimethoxyphenyl)acrylate **2h** (1.0 g, 3.35 mmol), hydroxyl amine hydrochloride (1.16 g, 16.75 mmol) and KOH(1.87 g, 3.35 mmol) in methanol at 0-5 °C to room temperature to gave the corresponding acrylamide **3h** in 85% yield (0.85 g) as a brown solid, mp 120-122 ºC, IR(ν_{max},cm^{-1}): 3392, 2919, 1673, 1507, 1290, 703. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.57 (1H, brs, NH) 7.42-7.39 (2H, d, *J* = 9.56 Hz, Ar-H), 6.99-6.96 (1H, d, J = 15.72 Hz, CH), 6.45-6.41 (1H, d, J = 15.88 Hz, CH), 3.81 (s, 6H), 3.68 (s, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ 153.5 (CO), 139.1(Ar-C), 138.9(Ar-C), 130.9(Ar-C), 118.8(Ar-C), 105.4, 60.5, 56.3.HRMS. Calcd. Accurate mass for ($C_{12}H_{16}NO_5$), 254.1023. Found: 254.1032 $[M+H]^+$.



2.10.10.(*E*)-3-(4-(2-(hydroxyamino)-2-oxoethoxy)phenyl-*N*-hydroxy acrylamide (3i)

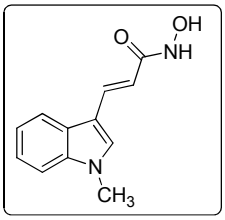
It was obtained by the reaction of (*E*)-ethyl 3-(4-(2-ethoxy-2-oxoethoxy) phenyl acrylate **2i** (1.0 g, 3.35 mmol), hydroxyl amine hydrochloride (1.16 g, 16.75 mmol) and KOH(1.87 g, 3.35 mmol) in methanol at 0-5 °C to room temperature to gave the corresponding acrylamide **3i** in 72% yield (0.72 g) as a brown solid, mp 122-124 ºC; IR(ν_{max},cm^{-1}): 3402, 3019, 1680, 1290, 767, 658.^{1}H NMR (400 MHz, DMSO-d_6); δ_H 10.71 (1H, brs, NH), 9.06 (1H, brs, NH) 7.52-7.50 (2H, d, *J* = 6.64 Hz, Ar-H), 7.43-7.39 (1H, d, J = 15.72 Hz, CH), 6.91 (2H, m, Ar-H), 6.36-6.32 (1H, d, J = 1 Hz, CH), 4.50 (2H,



s,OCH_2). ^{13}C NMR (100 MHz, DMSO-d_6); δ_C164.5 (CO), 163.5 (CO), 159.2 (CO), 138.3(Ar-C), 129.4(Ar-C), 128.5(Ar-C), 117.3(Ar-C), 115.5(Ar-C), 66.2, 40.5, 40.3, 40.1, 39.9, 39.7, 39.5, 39.3.HRMS: Calcd. Accurate mass for ($C_{13}H_{15}N_2O_5$), 279.0975. Found.279.0972 $[M+H]^+$

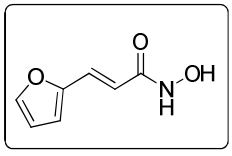
2.10. 11.(*E*)-3-(1-methyl-1*H*-indol-3-yl)-*N*-hydroxy acrylamide (3j)

It was obtained by the reaction of (*E*)-ethyl 3-(1-methyl-1*H*-indol-3-yl)acrylate **2j** (1.0 g, 3.35 mmol), hydroxyl amine hydrochloride (1.16 g, 16.75 mmol) and KOH(1.87 g, 3.35 mmol) in methanol at 0-5 °C to room temperature to gave the corresponding acrylamide **3j** in 80% yield (0.80 g) as a brown solid, mp 125-127 ºC; IR (ν_{max}, cm^{-1}): 3449, 3129, 2830, 1664, 1507, 1215.^1H NMR (400 MHz, DMSO-d_6) δ 11.90 (s, 1H, NH), 7.53-7.51 (2H, d, *J* = 8.12 Hz, Ar-H), 7.30-7.26 (1H, d, J = 16.2 Hz, CH), 7.23 (1H, s, Ar-H), 7.21-7.19 (2H, d, J = 7.92 Hz, Ar-H), 6.32-6.28 (1H, d, J = 15.92 Hz, CH), 3.82 (3H, s, CH_3).^{13}C NMR (100 MHz, DMSO-d_6) δ 169.0 (CO), 138.3(Ar-C), 138.2(Ar-C), 135.2(Ar-C), 125.9(Ar-C), 122.9(Ar-C), 121.5(Ar-C), 120.3, 112.6, 111.1, 40.6, 33.3.HRMS: Calcd. Accurate mass for ($C_{12}H_{13}N_2O_2$), 217.0972.Found.217.0978 $[M+H]^+$



2.10.12.(*E*)-3-(furan-2-yl)-*N*-hydroxyacrylamide (3k)

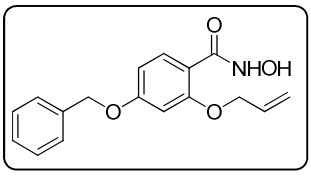
It was obtained by the reaction of (E)-ethyl 3-(furan-2-yl)acrylate **2k** (0.5 g, 2.82 mmol), hydroxyl amine hydrochloride (0.980 g, 14.1mmol) and KOH(1.58 g, 28.2 mmol) in methanol at 0-5 °C to room temperature to gave the corresponding acrylamide **3k** in 70% yield (0.77 g) as a yellow solid, mp 156-158 ºC, IR(ν_{max},cm^{-1}): 3412, 3129, 1683, 1510, 652. ^1H NMR (400 MHz, DMSO-d_6) δ 9.86 (1H, brs, NH), 7.75 (s, 1H), 7.28-7.25 (1H, d, J = 15.6 Hz, CH), 6.75 (s, 1H), 6.56 (s, 1H),6.28-6.24 (1H, d, J = 15.6 Hz,



CH). ^{13}C NMR (100 MHz, DMSO-d_6) δ 163.0 (CO), 151.3(Ar-C), 145.1(Ar-C), 126.1(Ar-C), 116.8(Ar-C), 114.1, 112.8. HRMS. Calcd. Accurate mass for ($C_7H_8NO_3$), 154.0499. Found: 154.0489 $[M+H]^+$.

2.10.13. 2-(allyloxy)-4-(benzyloxy)-*N*-hydroxybenzamide (4e)

It was obtained by the reaction of methyl-2-allyloxy-4-benzyloxybenzoate **4d** (1.0 g, 3.35 mmol), hydroxyl amine hydrochloride (1.16 g, 16.75 mmol) and KOH(1.87 g, 3.35 mmol) in methanol at 0-5 °C to room temperature to gave the corresponding acrylamide **4e** in 85% yield (0.85 g) as a brown solid, mp 124-126 ºC; IR (ν_{max}, cm^{-1}): 3399, 3019, 1641, 1607, 1491, 1421, 1215, 1182, 757, 669.^{1}H NMR (400 MHz, DMSO-d_6); δ_H 9.08 (1H, brs, NH), 7.60-7.57 (1H, m, Ar-H) 7.45-7.43 (2H, d, *J* = 7.1 Hz, Ar-H), 7.41-7.37 (2H, t, *J* = 7.0 Hz, Ar-H), 7.35-7.31 (1H, m, Ar-H), 6.68-6.67 (2H, m, Ar-H), 6.08-5.99 (1H, m, C*H*=CH_2), 5.41-5.36 (1H, dd, J_1= 17.2 Hz, J_2= 1.1 Hz, C*H*=CH_2), 5.28-5.25 (1H, d, *J* = 10.5 Hz, C*H*=CH_2), 5.13 (2H, s, OCH_2),4.67-4.66 (2H, d, *J* = 4.9 Hz, OCH_2). ^{13}C NMR (100 MHz, DMSO-d_6); δ_C 163.6 (CO), 161.7 (CO), 157.3 (CO), 137.0(Ar-C), 133.6(Ar-C), 128.9 (Ar-C), 128.4(Ar-C), 128.2 (Ar-C), 118.4, 115.3, 106.9, 100.9, 69.9, 69.3.HRMS. Calcd. Accurate mass for ($C_{17}H_{18}NO_4$), 300.1230. Found: 300.1221 $[M+H]^+$.



2.11. Biological evaluation

2.11.1.*Cell culture:*

Breast Cancer cell lines, MDA-MB-231 and MCF-7 were originally obtained from American type of cell culture collection (ATCC), USA and stock was maintained in laboratory. Non-malignant origin cell line, HEK-293 (human embryonic kidney) was obtained from institutional cell repository of animal tissue culture facility. Cells were cultured in CO_2 incubator at 37°C with 5% CO_2 and 95%

humidity in RPMI-1640 growth medium supplemented with 10% FBS and 1% of antibiotic and antimycotic solution.

2.11.2.*Cell Viability Assay:*

MTT (3-(4,5-dimethylthiazol-2yl)-2,5-diphenyl tetrazolium bromide) assay was performed to determine cell viability upon compound exposure as per method described earlier. In brief, cells @ $1x10^4$/well were seeded in 96 well microculture plates in 100µl RPMI-1640 medium and incubated for 24 hours at 37°C in a CO_2 incubator. Compounds were diluted to required concentration in phenol red free RPMI-1640 medium supplemented with 2% FBS. After 24 hours of incubation, cells were treated with desired concentration of compounds and respective vehicle alone control for 24 hours. At the end of incubation, 10µL (5 mg/mL) of MTT (Sigma, USA) was added and incubated for another 3h. Finally, supernatant was carefully discarded and 200µl of DMSO was added to dissolve formazan crystals under gentle shaking in plate shaker (Biosan, USA) and absorbance at 570nm wavelength was recorded in a microplate reader (Microquant, BioTek).

2.11.3.*Cell Cycle Analysis:*

Effect of **compound 3h** of cell cycle phase distribution was measured with flow cytometry technique using propidium iodide (PI) staining method. For this, $1x10^6$ MDA-MB-231 cells were seeded in six well plates and allowed to grow for 24h. Next day, cells were treated with **compound 3h** for another 24h. At the end of that incubation peroid, cells were harvested, washed with PBS and fixed with 70% chilled ethanol. Fixed cells were washed with PBS and stained with PI (30 µg/mL) in PBS containing 10 µg/mL RNase A for 30 min at room temperature in dark condition. DNA content of the cells was measured using a FACS Calibur flow cytometer using

FACScan (Becton Dickinson) and percentages of cells in each phases of cell cycle were calculated.

2.11.4. ***Hoechst staining:***

To determine effect of **compound 3h** on nuclear morphology, fluorescent microscopy was carried out using Hoechst staining. $1x10^6$ MDA-MB-231 cells were seeded in 6-well culture plates for 24h. After another 24h of treatment with **compound 3h**, cells were washed with PBS, fixed with 4% paraformaldehyde for 15 minutes and subsequently cells were permeabilized with 3% paraformaldehyde containing 0.1% tritonX-100 for 10 minutes. Finally, cells were stained with 1µg/ml of Hoechst-33358 (Sigma, USA) for 30 minutes at room temperature, washed with PBS and photographed using fluorescent microscopy.

2.11.5. ***Apoptosis Assay:***

Effect of **compound 3h** on cell death was carried out using flow cytometry based Annexin V-FITC/PI apoptosis kit (Sigma, USA). $1x10^6$ MDA-MB-231 cells were seeded in 6-well culture plates for 24h, followed by treatment with **compound 3h** for another 24h. After 24h of treatment, cells were harvested, washed with PBS and stained with Annexin V-FITC/PI kit for 10 minutes. Finally, samples were analyzed by flow cytometry (FACScan, Becton Dickinson).

2.11.6.*Mitochondrial Membrane Potential Analysis:*

To analyze effect of **compound 3h** on mitochondrial membrane potential, $1x10^6$ MDA-MB-231 cells were seeded in 6-well culture plates and grown for 24h. Grown cell monolayers were treated with **compound 3h** for another 24h. At the end of treatment, cells were harvested, washed with PBS and incubated with 5µg of JC-1 dye for 30 minutes at room temperature, followed by washing and re-suspension in

300µl PBS. Finally, JC-1 stained MDA-MB-231 cells were analyzed by flow cytometry using FACScan (Becton Dickinson).

2.11.7.*Reactive Oxygen Species (ROS) Analysis:*

ROS was measured in terms of reactive oxygen-induced fluorescence of DCF-DA, basically a non-fluorescent cell staining dye. For this, $1x10^6$ MDA-MB-231 cells were seeded in 6-well plates for 24h, followed by treatment with **compound 3h** for 24h. At the end of incubation, cells were harvested, washed with PBS and then fixed with chilled absolute methanol. Cells were stained with 10µg/mL DCFH-DA and incubated at room temperature in dark condition for 30 min. After incubation, cells were centrifuged and re-suspended in 300µl PBS and analyzed by flow cytometry (FACScan, Becton Dickinson, USA).

2.12. Conclusion:

The present study investigates the anticancer effects of*N*-Hydroxycinnamamide derivatives in human breast cancer cells. Molecular docking results showed that **3b, 3e, 3f, 3g, 3h** and **7**could act on the activesites of pro-apoptotic proteins EGFR to induce apoptotic death of cancer cells. Out of 12 compounds screened**compound3b** and **compound 3h** exhibits the most potent activity against aggressive breast cancer cells (MDA-MB-231) with IC_{50}13µM and 5µM respectively. **Compound 3h** inhibits cell cycle progression by accumulating cells at G0/G1 checkpoints. Moreover, **compound 3h** promotes DNA fragmentation and induction of apoptosis. Furthermore, loss of mitochondrial membrane potential induced by **compound 3h**. One of the major mechanisms of action of **compound 3h** for its anti-cancer activity is probably the induction of ROS in cancer cells thereby inducing apoptotic cell deaths in cancer cells.

2.13.REFERENCES:

[1] Mai A, Rotili D, Massa S, Brosch G, Simonetti G, Passariello C. Discovery of uracil-based histone deacetylase inhibitors able to reduce acquired antifungal resistance and trailing growth in Candida albicans. Bioorganic & medicinal chemistry letters. 2007;17:1221-5.

[2] Samuni Y, Flores-Santana W, Krishna MC, Mitchell JB, Wink DA. The inhibitors of histone deacetylase suberoylanilide hydroxamate and trichostatin A release nitric oxide upon oxidation. Free Radical Biology and Medicine. 2009;47:419-23.

[3] Park M, Rizzo CJ. Stereocontrolled de Novo Synthesis of β-2 '-Deoxyribonucleosides. The Journal of organic chemistry. 1996;61:6092-3.

[4] Farràs J, del Mar Lleó M, Vilarrasa J, Castillón S, Matheu M, Solans X. New bicyclic nucleosides related to 6-azaisocytidine. Tetrahedron letters. 1996;37:901-4.

[5] Whittaker M, Floyd CD, Brown P, Gearing AJ. Design and therapeutic application of matrix metalloproteinase inhibitors. Chemical Reviews. 1999;99:2735-76.

[6] Caldarelli M, Habermann J, Ley SV. Synthesis of an array of potential matrix metalloproteinase inhibitors using a sequence of polymer-supported reagents. Bioorganic & medicinal chemistry letters. 1999;9:2049-52.

[7] Kumar B, Yadav A, Lang JC, Teknos TN, Kumar P. Suberoylanilide hydroxamic acid (SAHA) reverses chemoresistance in head and neck cancer cells by targeting cancer stem cells via the downregulation of nanog. Genes & cancer. 2015;6:169.

[8] Foss F, Advani R, Duvic M, Hymes KB, Intragumtornchai T, Lekhakula A. A Phase II trial of Belinostat (PXD101) in patients with relapsed or refractory peripheral or cutaneous T-cell lymphoma. British journal of haematology. 2015;168:811-9.

[9] Atadja P. Development of the pan-DAC inhibitor panobinostat (LBH589): successes and challenges. Cancer letters. 2009;280:233-41.

[10] Chu Q-C, Nielsen T, Alcindor T, Gupta A, Endo M, Goytain A. A phase II study of SB939, a novel pan-histone deacetylase inhibitor, in patients with translocation-associated recurrent/metastatic sarcomas—NCIC-CTG IND 200. Annals of Oncology. 2015;26:973-81.

[11] Khan O, La Thangue NB. HDAC inhibitors in cancer biology: emerging mechanisms and clinical applications. Immunology & Cell Biology. 2012;90:85-94.

[12] Song Y, Hai T, Wang Y, Guo R, Li W, Wang L. Epigenetic reprogramming, gene expression and in vitro development of porcine SCNT embryos are significantly improved by a histone deacetylase inhibitor—m-carboxycinnamic acid bishydroxamide (CBHA). Protein & cell. 2014;5:382-93.

[13] Gowravaram MR, Johnson JS, Delecki D, Cook ER, Tomczuk BE, Ghose AK. Inhibition of matrix metalloproteinases by hydroxamates containing heteroatom-based modifications of the P1'group. Journal of medicinal chemistry. 1995;38:2570-81.

[14] Thorarensen A, Douglas Jr MR, Rohrer DC, Vosters AF, Yem AW, Marshall VD. Identification of novel potent hydroxamic acid inhibitors of peptidyl deformylase and the importance of the hydroxamic acid functionality on inhibition. Bioorganic & medicinal chemistry letters. 2001;11:1355-8.

[15] Pierre S, Eschenhagen T, Geisslinger G, Scholich K. Capturing adenylyl cyclases as potential drug targets. Nature reviews Drug discovery. 2009;8:321.

[16] Shu Q, Nair V. Inosine monophosphate dehydrogenase (IMPDH) as a target in drug discovery. Medicinal research reviews. 2008;28:219-32.

[17] Glaser KB, Staver MJ, Waring JF, Stender J, Ulrich RG, Davidsen SK. Gene expression profiling of multiple histone deacetylase (HDAC) inhibitors: defining a common gene set produced by HDAC inhibition in T24 and MDA carcinoma cell lines. Molecular cancer therapeutics. 2003;2:151-63.

[18] Supuran CT. Carbonic anhydrases: catalytic and inhibition mechanisms, distribution and physiological roles. Carbonic anhydrase: CRC press; 2004. p. 13-36.

[19] Tsakadze NL, Sithu SD, Sen U, English WR, Murphy G, D'Souza SE. Tumor necrosis factor-α-converting enzyme (TACE/ADAM-17) mediates the ectodomain cleavage of intercellular adhesion molecule-1 (ICAM-1). Journal of Biological Chemistry. 2006;281:3157-64.

[20] Horiuchi K, Kimura T, Miyamoto T, Takaishi H, Okada Y, Toyama Y. Cutting edge: TNF-α-converting enzyme (TACE/ADAM17) inactivation in mouse myeloid cells prevents lethality from endotoxin shock. The Journal of Immunology. 2007;179:2686-9.

[21] Konopleva M, Watt J, Contractor R, Tsao T, Harris D, Estrov Z. Mechanisms of antileukemic activity of the novel Bcl-2 homology domain-3 mimetic GX15-070 (obatoclax). Cancer research. 2008;68:3413-20.

[22] Plewe MB, Butler SL, R. Dress K, Hu Q, Johnson TW, Kuehler JE. Azaindole hydroxamic acids are potent HIV-1 integrase inhibitors. Journal of medicinal chemistry. 2009;52:7211-9.

[23] Xie XW, Wan RZ, Liu ZP. Recent Research Advances in Selective Matrix Metalloproteinase-13 Inhibitors as Anti-Osteoarthritis Agents. ChemMedChem. 2017;12:1157-68.

[24] Hain T. Neuro-ophthalmology and neuro-otology. Psychiatry. 2001;71:8-12.

[25] Leoni F, Zaliani A, Bertolini G, Porro G, Pagani P, Pozzi P. The antitumor histone deacetylase inhibitor suberoylanilide hydroxamic acid exhibits antiinflammatory properties via suppression of cytokines. Proceedings of the National Academy of Sciences. 2002;99:2995-3000.

[26] Penke B, Toth G, Varadi G. Analogue and Conformational Studies on Peptides, Hormones and Other Biologically Active Peptides. AMINO ACIDS PEPTIDES AND PROTEINS. 2003;34:55-148.

[27] Komatsu Y, Tomizaki K-y, Tsukamoto M, Kato T, Nishino N, Sato S. Cyclic hydroxamic-acid-containing peptide 31, a potent synthetic histone deacetylase inhibitor with antitumor activity. Cancer research. 2001;61:4459-66.

[28] Jackman JE, Fierke CA, Tumey LN, Pirrung M, Uchiyama T, Tahir SH. Antibacterial agents that target lipid a biosynthesis in gram-negative bacteria inhibition of diverse UDP-3-O-(R-3-hydroxymyristoyl)-N-acetylglucosamine deacetylases by substrate analogs containing zinc binding motifs. Journal of Biological Chemistry. 2000;275:11002-9.

[29] Onishi HR, Pelak BA, Gerckens LS, Silver LL, Kahan FM, Chen M-H. Antibacterial agents that inhibit lipid A biosynthesis. Science. 1996;274:980-2.

[30] Reddy AS, Kumar MS, Reddy GR. A convenient method for the preparation of hydroxamic acids. Tetrahedron Letters. 2000;41:6285-8.

[31] Park Y-J, Kim T-J, Cho Y-H, Jung Y-J, Im H-J, Song K-S. EPR investigation on a quantitative analysis of Eu (II) and Eu (III) in LiCl/KCl eutectic molten salt. Bulletin of the Korean Chemical Society. 2008;29:127-9.

[32] Scozzafava A, Supuran CT. Carbonic anhydrase and matrix metalloproteinase inhibitors: sulfonylated amino acid hydroxamates with MMP inhibitory properties act as efficient inhibitors of CA isozymes I, II, and IV, and N-hydroxysulfonamides inhibit both these zinc enzymes. Journal of medicinal chemistry. 2000;43:3677-87.

[33] Marmion CJ, Murphy T, Docherty JR, Nolan KB. Hydroxamic acids are nitric oxide donors. Facile formation of ruthenium (II)-nitrosyls and NO-mediated

activation of guanylate cyclase by hydroxamic acids. Chemical Communications. 2000:1153-4.

[34] Loll P, Sharkey C, O'Connor S, Dooley C, O'Brien E, Devocelle M. O-acetylsalicylhydroxamic acid, a novel acetylating inhibitor of prostaglandin H2 synthase: structural and functional characterization of enzyme-inhibitor interactions. Molecular pharmacology. 2001;60:1407-13.

[35] Dooley C, Devocelle M, McLoughlin B, Nolan K, Fitzgerald D, Sharkey C. A novel family of hydroxamate-based acylating inhibitors of cyclooxygenase. Molecular pharmacology. 2003;63:450-5.

[36] Meinnel T, Mechulam Y, Blanquet S. Methionine as translation start signal: a review of the enzymes of the pathway in Escherichia coli. Biochimie. 1993;75:1061-75.

[37] Tripathi R, Mishra R, Dwivedi N, Tewari N, Verma S. Current status of malaria control. Current medicinal chemistry. 2005;12:2643-59.

[38] Fournand D, Vaysse L, Dubreucq E, Arnaud A, Galzy P. Monohydroxamic acid biosynthesis. Journal of Molecular Catalysis B: Enzymatic. 1998;5:207-11.

[39] Wang QX, King J, Phanstiel IV O. An Improved Synthesis of O-Benzoyl Protected Hydroxamates. The Journal of Organic Chemistry. 1997;62:8104-8.

[40] Chen Y, Lopez-Sanchez M, Savoy DN, Billadeau DD, Dow GS, Kozikowski AP. A series of potent and selective, triazolylphenyl-based histone deacetylases inhibitors with activity against pancreatic cancer cells and Plasmodium falciparum. Journal of medicinal chemistry. 2008;51:3437-48.

[41] Hoffman HE, Jirásková J, Cígler P, S□anda M, Schraml J, Konvalinka J. Hydroxamic acids as a novel family of serine racemase inhibitors: mechanistic

analysis reveals different modes of interaction with the pyridoxal-5′-phosphate cofactor. Journal of medicinal chemistry. 2009;52:6032-41.

[42] Marmion CJ, Griffith D, Nolan KB. Hydroxamic Acids– An Intriguing Family of Enzyme Inhibitors and Biomedical Ligands. European Journal of Inorganic Chemistry. 2004;2004:3003-16.

[43] Holland KP, Elford HL, Bracchi V, Annis CG, Schuster SM, Chakrabarti D. Antimalarial activities of polyhydroxyphenyl and hydroxamic acid derivatives. Antimicrobial agents and chemotherapy. 1998;42:2456-8.

[44] Clarkson Jr AB, Grady RW, Grossman SA, McCallum RJ, Brohn FH. Trypanosoma brucei brucei: a systematic screening for alternatives to the salicylhydroxamic acid-glycerol combination. Molecular and biochemical parasitology. 1981;3:271-91.

[45] Gupta D. Irreversible peptidomimetic inhibitors of plasmepsin-II as potential antimalarial agents: Wayne State University; 2007.

[46] Dardonville C, Rinaldi E, Barrett MP, Brun R, Gilbert IH, Hanau S. Selective inhibition of Trypanosoma brucei 6-phosphogluconate dehydrogenase by high-energy intermediate and transition-state analogues. Journal of medicinal chemistry. 2004;47:3427-37.

[47] Pal D, Saha S. Hydroxamic acid–A novel molecule for anticancer therapy. Journal of advanced pharmaceutical technology & research. 2012;3:92.

[48] Bailén MA, Chinchilla R, Dodsworth DJ, Nájera C. Direct synthesis of hydroxamates from carboxylic acids using 2-mercaptopyridone-1-oxide-based thiouronium salts. Tetrahedron Letters. 2001;42:5013-6.

[49] Casini A, Scozzafava A, Supuran CT. Sulfonamide derivatives with protease inhibitory action as anticancer, anti-inflammatory and antiviral agents. Expert Opinion on Therapeutic Patents. 2002;12:1307-27.

[50] Griffith D, Cecco S, Zangrando E, Bergamo A, Sava G, Marmion CJ. Ruthenium (III) dimethyl sulfoxide pyridinehydroxamic acid complexes as potential antimetastatic agents: synthesis, characterisation and in vitro pharmacological evaluation. JBIC Journal of Biological Inorganic Chemistry. 2008;13:511-20.

[51] Inouye Y, Okada H, Nakamura S. Hydroxamic Acid Antimycotic Antibiotics: A New Group of Antibiotics. Annals of the New York Academy of Sciences. 1988;544:180-2.

[52] Chowdhury N, Dasgupta S, Singh NP. Photoinduced DNA cleavage by anthracene based hydroxamic acids. Bioorganic & medicinal chemistry letters. 2012;22:4668-71.

[53] Bravo HR, Copaja SV, Argandoña VH. Chemical basis for the antifeedant activity of natural hydroxamic acids and related compounds. Journal of agricultural and food chemistry. 2004;52:2598-601.

[54] King WF. Fungicidal tin salts of thienyl and furyl hydroxamic acids, compositions, and method of use therefor. Google Patents; 1986.

[55] Zasada I, Meyer S, Halbrendt J, Rice C. Activity of hydroxamic acids from Secale cereale against the plant-parasitic nematodes Meloidogyne incognita and Xiphinema americanum. Phytopathology. 2005;95:1116-21.

[56] Wu P-L, Sun C-J. A simple method for the preparation of N-substituted hydroxamic acids by reductive acylation of oximes. Tetrahedron letters. 1991;32:4137-8.

[57] Boukhris Sd, Souizi A. Simple syntheses of hydroxamic acids and their conversion into α-hydroxy and α-amino acids. Tetrahedron Letters. 2000;41:2559-62.

[58] Grierson L, Perkins MJ. Synthesis of novel N-(primary) alkylhydroxamic acids. Tetrahedron letters. 1993;34:7463-4.

[59] Wang QX, Phanstiel O. Total synthesis of acinetoferrin. The Journal of Organic Chemistry. 1998;63:1491-5.

[60] Ali B, Zukerman-Schpector J, Ferreira FP, Shamim A, Pimenta DC, Stefani HA. Lewis-acid catalyzed N-acyliminium ion cyclodimerization: synthesis of symmetrical 1, 4-dioxanes. Tetrahedron Letters. 2015;56:1153-8.

[61] Kim JJ, Park YD, Kim HK, Cho SD, Kim JK, Lee SG. Convenient Preparation of O-Alkylhydroxamic Acids Using 2-Acylpyridazin-3 (2 H)-ones. Synthetic communications. 2005;35:731-8.

[62] Hoffman RV, Madan S. Synthesis and reactions of 2-substituted ethyl N-alkylmalonylhydroxamic acids. The Journal of organic chemistry. 2003;68:4876-85.

[63] Ech-Chahad A, Minassi A, Berton L, Appendino G. An expeditious hydroxyamidation of carboxylic acids. Tetrahedron letters. 2005;46:5113-5.

[64] Monbaliu J-CM, Masschelein KG, Stevens CV. Electron-deficient 1-and 2-azabuta-1, 3-dienes: a comprehensive survey of their synthesis and reactivity. Chemical Society Reviews. 2011;40:4708-39.

[65] Suzuki T, Nagano Y, Kouketsu A, Matsuura A, Maruyama S, Kurotaki M. Novel inhibitors of human histone deacetylases: design, synthesis, enzyme inhibition, and cancer cell growth inhibition of SAHA-based non-hydroxamates. Journal of medicinal chemistry. 2005;48:1019-32.

[66] Gopalan B, Ponpandian T, Kachhadia V, Bharathimohan K, Vignesh R, Sivasudar V. Discovery of adamantane based highly potent HDAC inhibitors. Bioorganic & medicinal chemistry letters. 2013;23:2532-7.

[67] Supuran CT, Casini A, Scozzafava A. Protease inhibitors of the sulfonamide type: anticancer, antiinflammatory, and antiviral agents. Medicinal research reviews. 2003;23:535-58.

[68] Gediya LK, Chopra P, Purushottamachar P, Maheshwari N, Njar VC. A new simple and high-yield synthesis of suberoylanilide hydroxamic acid and its inhibitory effect alone or in combination with retinoids on proliferation of human prostate cancer cells. Journal of medicinal chemistry. 2005;48:5047-51.

[69] Flipo M, Charton J, Hocine A, Dassonneville S, Deprez B, Deprez-Poulain R. Hydroxamates: relationships between structure and plasma stability. Journal of medicinal chemistry. 2009;52:6790-802.

[70] Lim NC, Morton MD, Jenkins HA, Brückner C. Squaric acid N-hydroxylamides: synthesis, structure, and properties of vinylogous hydroxamic acid analogues. The Journal of Organic Chemistry. 2003;68:9233-41.

[71] Zinglé C, Kuntz L, Tritsch D, Grosdemange-Billiard C, Rohmer M. Isoprenoid biosynthesis via the methylerythritol phosphate pathway: structural variations around phosphonate anchor and spacer of fosmidomycin, a potent inhibitor of deoxyxylulose phosphate reductoisomerase. The Journal of Organic Chemistry. 2010;75:3203-7.

[72] Mastrolorenzo A, Scozzafava A, Supuran CT. 4-Toluenesulfonylureido derivatives of amines, amino acids and dipeptides: a novel class of potential antitumor agents. European journal of pharmaceutical sciences. 2000;11:325-32.

[73] Leggio A, Belsito E, De Luca G, Di Gioia M, Leotta V, Romio E. One-pot synthesis of amides from carboxylic acids activated using thionyl chloride. RSC Advances. 2016;6:34468-75.

[74] Braslau R, Axon JR, Lee B. Synthesis of N-Hydroxy Peptides: Chemical Ligation of O-Acyl Hydroxamic Acids. Organic letters. 2000;2:1399-401.

[75] Wong FT, Patra PK, Seayad J, Zhang Y, Ying JY. N-heterocyclic carbene (NHC)-catalyzed direct amidation of aldehydes with nitroso compounds. Organic letters. 2008;10:2333-6.

[76] Porcheddu A, Giacomelli G. Angeli– Rimini's Reaction on Solid Support: A New Approach to Hydroxamic Acids. The Journal of organic chemistry. 2006;71:7057-9.

[77] Banerjee R, King SB. Synthesis of Cyclic Hydroxamic Acids through– NOH Insertion of Ketones. Organic letters. 2009;11:4580-3.

[78] Katritzky AR, Barczynski P, Ostercamp D, Yousaf TI. Mechanisms of heterocyclic ring formations. 4. A carbon-13 NMR study of the reaction of. beta.-keto esters with hydroxylamine. The Journal of Organic Chemistry. 1986;51:4037-42.

[79] Surman MD, Miller MJ. Regio-and Stereochemically Controlled Formation of Hydroxamic Acid Containing a nti-or s yn-1, 4-Cycloalkenols from Acylnitroso-Derived Diels– Alder Adducts. The Journal of organic chemistry. 2001;66:2466-9.

[80] Ho CY, Strobel E, Ralbovsky J, Galemmo RA. Improved solution-and solid-phase preparation of hydroxamic acids from esters. The Journal of organic chemistry. 2005;70:4873-5.

[81] Stanger KJ, Krchňák V. Polymer-supported N-derivatized, O-linked hydroxylamine for concurrent solid-phase synthesis of diverse N-alkyl and N– H hydroxamates. Journal of combinatorial chemistry. 2006;8:435-9.

[82] Barrett AG, Cramp SM, Roberts RS, Zecri FJ. A ROMPGEL-supported N-hydroxysuccinimide: A host of acylations with minimal purification. Organic letters. 2000;2:261-4.

[83] Yin Z, Low K, Lye P. N-Linked Hydroxylamine Resin: Solid-Phase Synthesis of Hydroxamic Acids. Synthetic communications. 2005;35:2945-50.

[84] Choi J, Park JG, Pang Y-P. Convenient synthesis of a library of discrete hydroxamic acids using the hydroxythiophenol (Marshall) resin. Tetrahedron letters. 2008;49:1103-6.

[85] Kumagai H, Hasegawa M, Miyanari S, Sugawa Y, Sato Y, Hori T. Facile synthesis of p-tert-butylthiacalix [4] arene by the reaction of p-tert-butylphenol with elemental sulfur in the presence of a base. Tetrahedron letters. 1997;38:3971-2.

[86] Desroches C, Parola S, Vocanson F, Perrin M, Lamartine R, Létoffé J-M. Nitration of thiacalix [4] arene using nitrosium nitrate complexes: synthesis and characterization of tetranitro-, tetraamino-, and tetra (4-pyridylimino) tetrahydroxythiacalix [4] arene. New Journal of Chemistry. 2002;26:651-5.

[87] Agrawal Y, Pancholi J. Synthesis of thia-calix [4] arene hydroxamic acids by microwave irradiation. Synthetic Communications®. 2008;38:2446-58.

[88] Randino R, Cini E, D'Ursi AM, Novellino E, Rodriquez M. Facile Baeyer–Villiger oxidation of cyclic ketones: conventional versus microwave-assisted approach. Tetrahedron letters. 2015;56:5723-6.

[89] Xia J, Hui Y-Z. The stereospecific synthesis of mixed-acid phospholipids with polyunsaturated fatty acid from D-mannitol. Tetrahedron: Asymmetry. 1997;8:451-8.

[90] Takayanagi M, Flessner T, Wong C-H. A strategy for the solution-phase parallel synthesis of N-(pyrrolidinylmethyl) hydroxamic acids. The Journal of organic chemistry. 2000;65:3811-5.

[91] Riva E, Gagliardi S, Mazzoni C, Passarella D, Rencurosi A, Vigo D. Efficient continuous flow synthesis of hydroxamic acids and suberoylanilide hydroxamic acid preparation. The Journal of Organic Chemistry. 2009;74:3540-3.

[92] Liu Y, Jacobs HK, Gopalan AS. Reactions of N-Benzyloxycarbamate Derivatives with Stabilized Carbon Nucleophiles: A New Synthetic Approach to Polyhydroxamic Acids and Other Hydroxamate-Containing Mixed Ligand Systems. The Journal of organic chemistry. 2009;74:782-8.

[93] Giacomelli G, Porcheddu A, Salaris M. Simple one-flask method for the preparation of hydroxamic acids. Organic letters. 2003;5:2715-7.

[94] Franklin T, Snow G. Antifungal, antiprotozoal and antiviral agents. Biochemistry of antimicrobial action: Springer; 1989. p. 137-60.

[95] Schaeffer HJ, Bhargava PS. Enzyme inhibitors. V. The syntheses of 6-substituted-(9-hydroxyalkyl) purines and their evaluation as inhibitors of adenosine deaminase. Biochemistry. 1965;4:71-6.

[96] De Clercq E, Holý A. Acyclic nucleoside phosphonates: a key class of antiviral drugs. Nature reviews Drug discovery. 2005;4:928.

[97] Ramakrishna KKG, Gunjan S, Shukla AK, Pasam VR, Balaramnavar VM, Sharma A. Identification of novel phenyl butenonyl C-glycosides with ureidyl and sulfonamidyl moieties as antimalarial agents. ACS Medicinal Chemistry Letters. 2014;5:878-83.

[98] Lauterbur PC. Some applications of C13 nuclear magnetic resonance spectra to organic chemistry. Annals of the New York Academy of Sciences. 1958;70:841-57.

[99] Sieiro C, Sanchez-Palacios A, Calle P, De La Vega J. ESR study and ab initio calculations of some methylbenzoates derivatives. Journal of Molecular Structure. 1986;142:427-30.

[100] Li J-T, Li H-Y, Li H-Z. An efficient synthesis of aryloxyacetic acid and arylthioacetic acid esters. Journal of Chemical Research. 2004;2004:416-7.

[101] Banerjee A, Sengupta S, Adak MM, Banerjee GC. Use of phosphorus pentoxide: Esterification of organic acids. The Journal of Organic Chemistry. 1983;48:3106-8.

[102] Vieira TO, Green MJ, Alper H. Highly regioselective anti-markovnikov palladium-borate-catalyzed methoxycarbonylation reactions: unprecedented results for aryl olefins. Organic letters. 2006;8:6143-5.

[103] Ram RN, Charles I. Selective esterification of aliphatic nonconjugated carboxylic acids in the presence of aromatic or conjugated carboxylic acids catalysed by NiCl2. 6H2O. Tetrahedron. 1997;53:7335-40.

[104] Kukshal V, Mishra M, Ajay A, Khanam T, Sharma R, Dube D. Synthesis and bioevaluation of aryl hydroxamates distinguishing between NAD+ and ATP-dependent DNA ligases. MedChemComm. 2012;3:453-61.

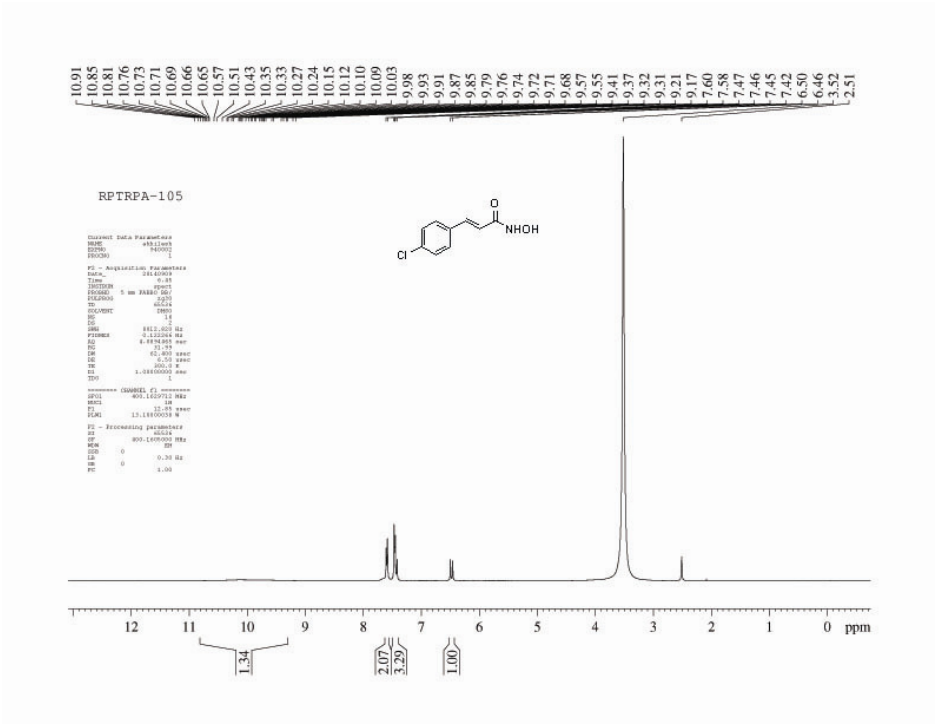
[105] Katritzky AR, Singh SK, Cai C, Bobrov S. Direct synthesis of esters and amides from unprotected hydroxyaromatic and-aliphatic carboxylic acids. The Journal of organic chemistry. 2006;71:3364-74.

[106] Le GT, Abbenante G, Becker B, Grathwohl M, Halliday J, Tometzki G. Molecular diversity through sugar scaffolds. Drug discovery today. 2003;8:701-9.

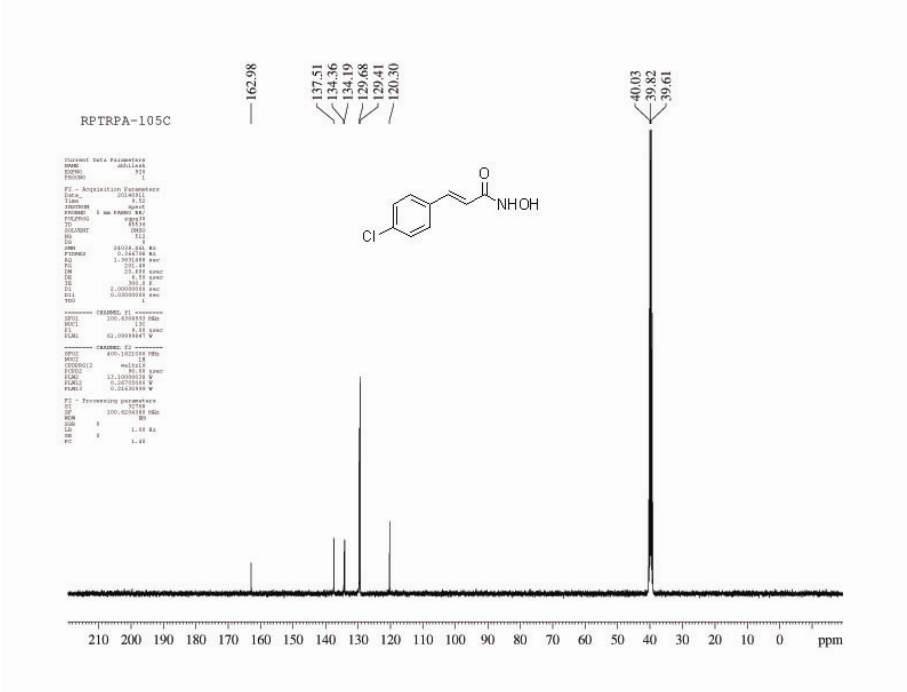
[107] Tietze LF, Bell HP, Chandrasekhar S. Natural product hybrids as new leads for drug discovery. Angewandte Chemie International Edition. 2003;42:3996-4028.

2.13. Copies of ^{1}H NMR and ^{13}C NMR Spectra of Selected Compounds:

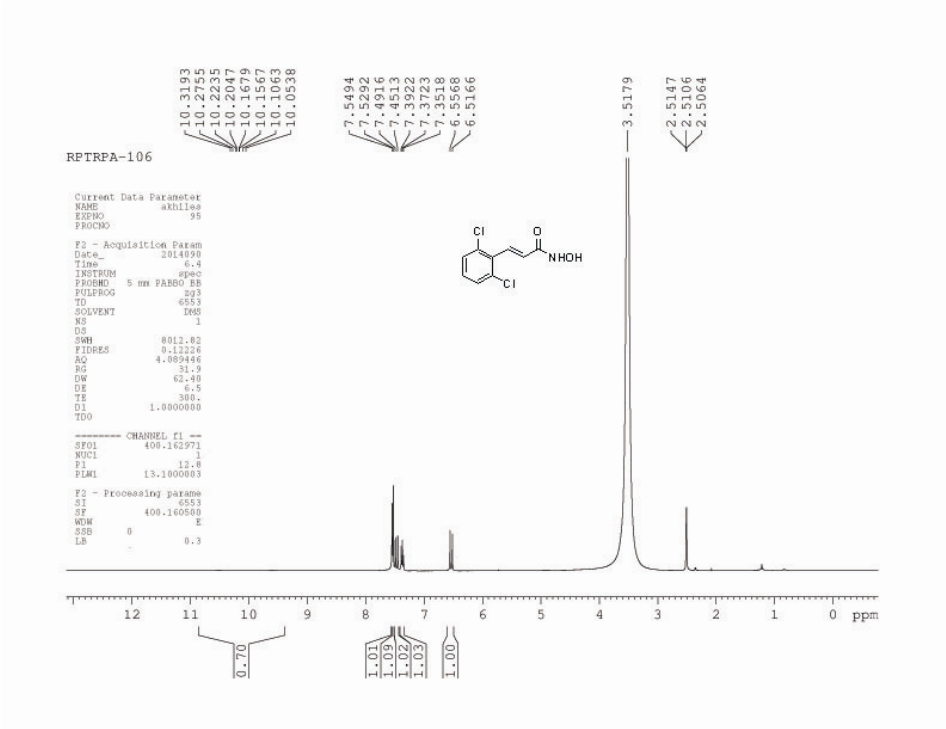
^{1}H NMR spectra of compound **3a**



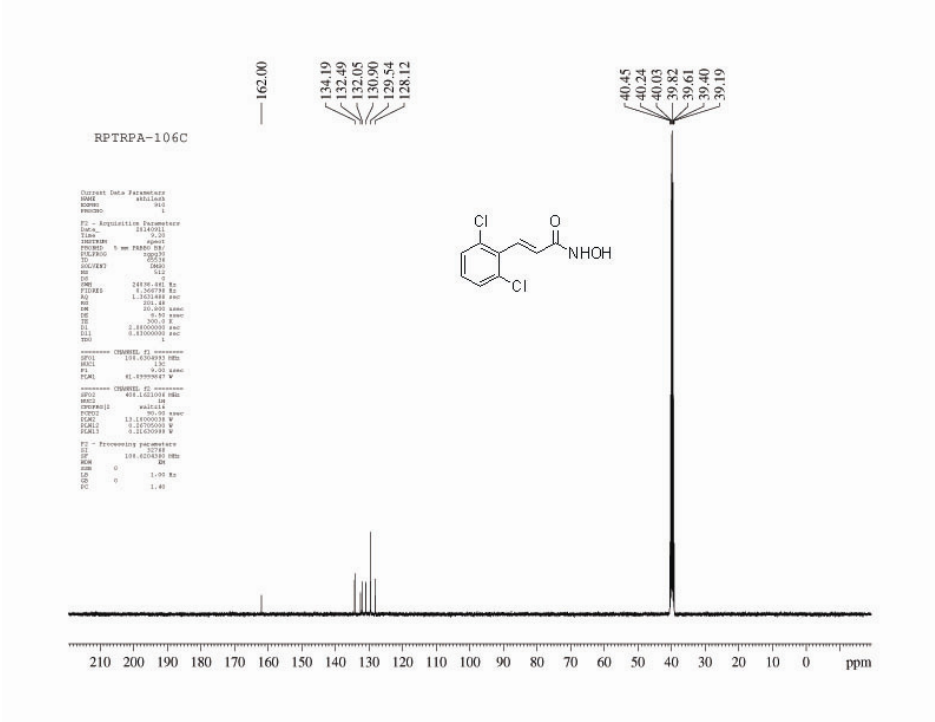
^{13}C NMR of compound **3a**



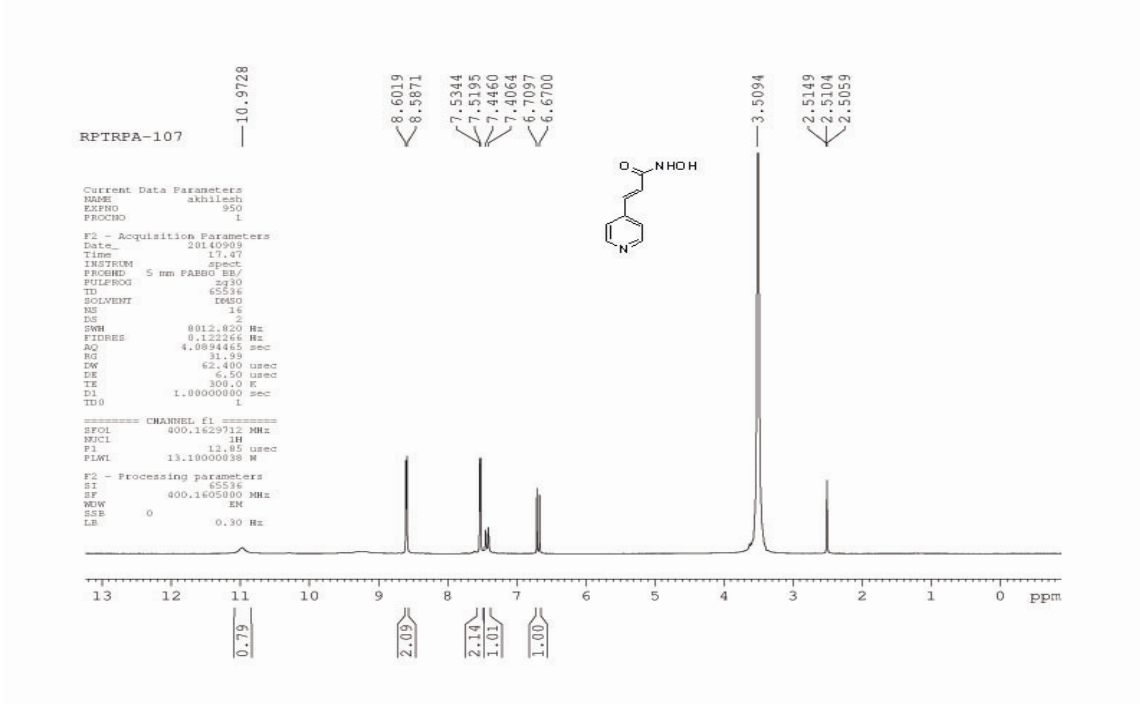
^{1}H NMR spectra of compound **3b**



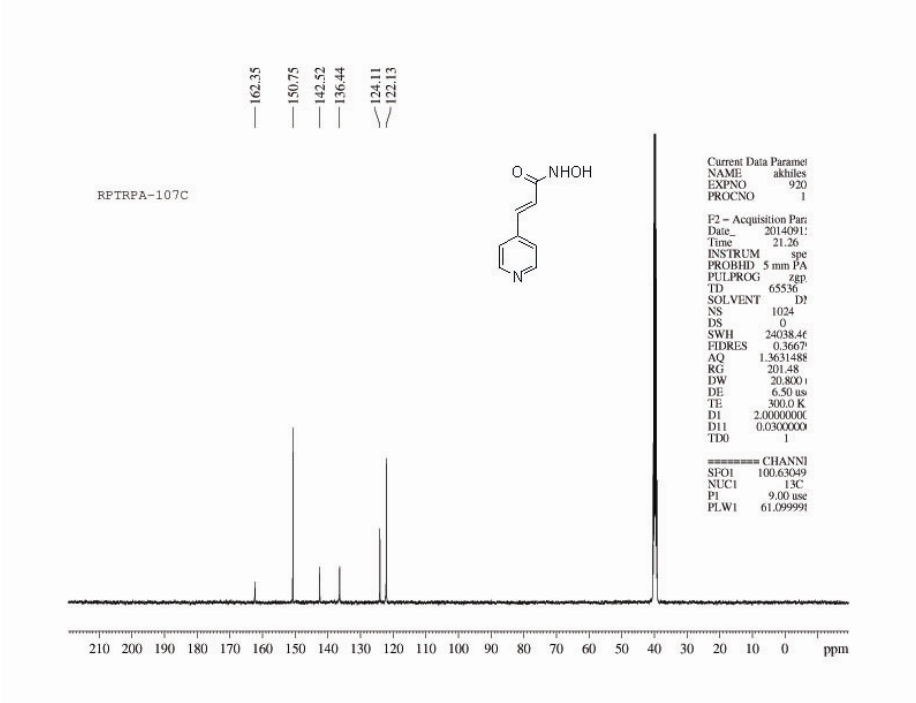
^{13}C NMR of compound **3b**



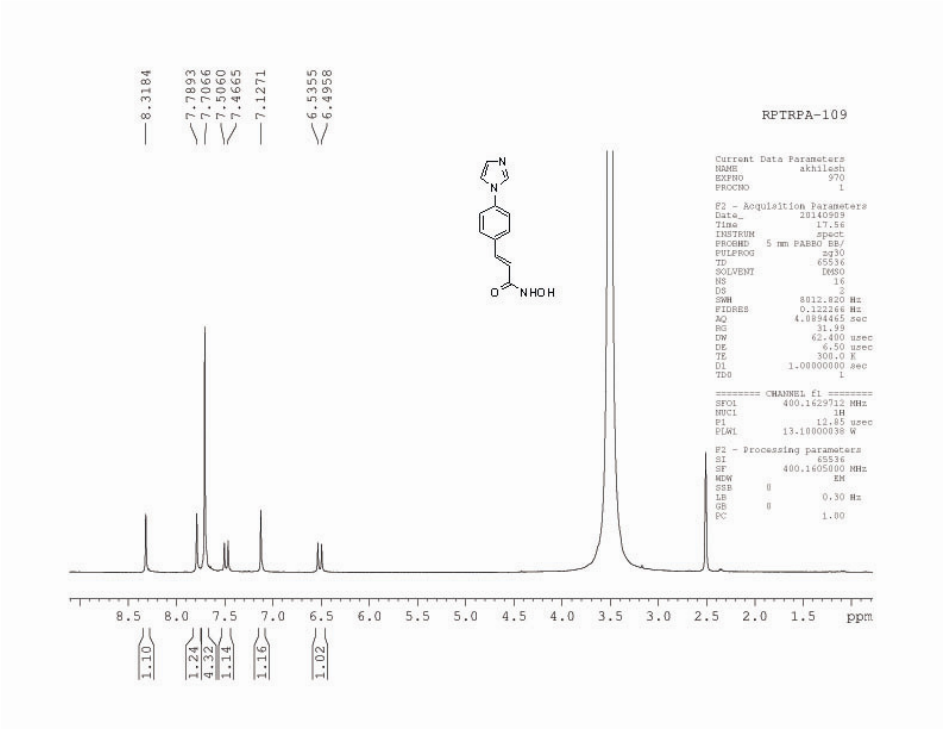
^{1}H NMR spectra of compound **3c**



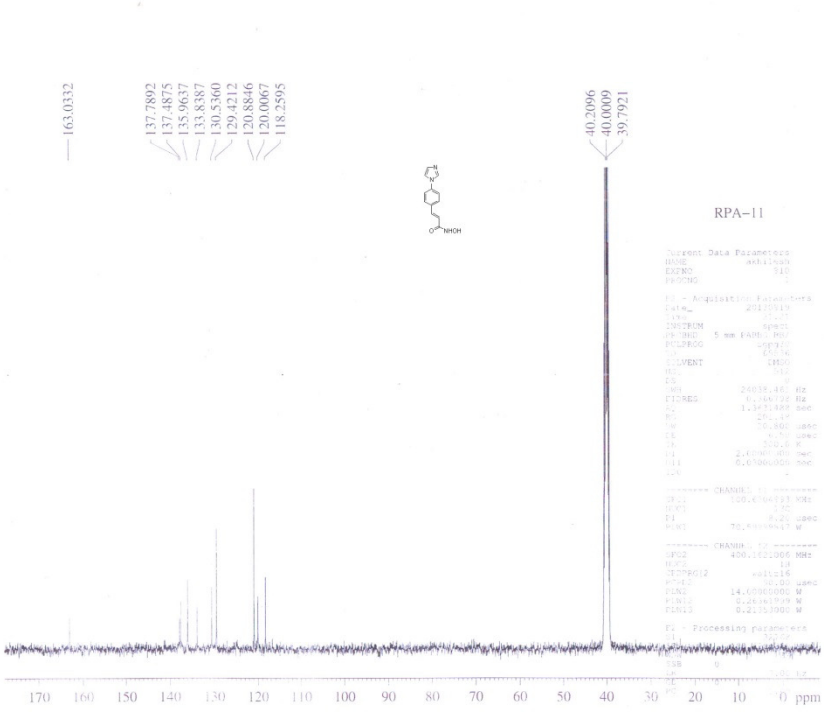
^{13}C NMR of compound **3c**



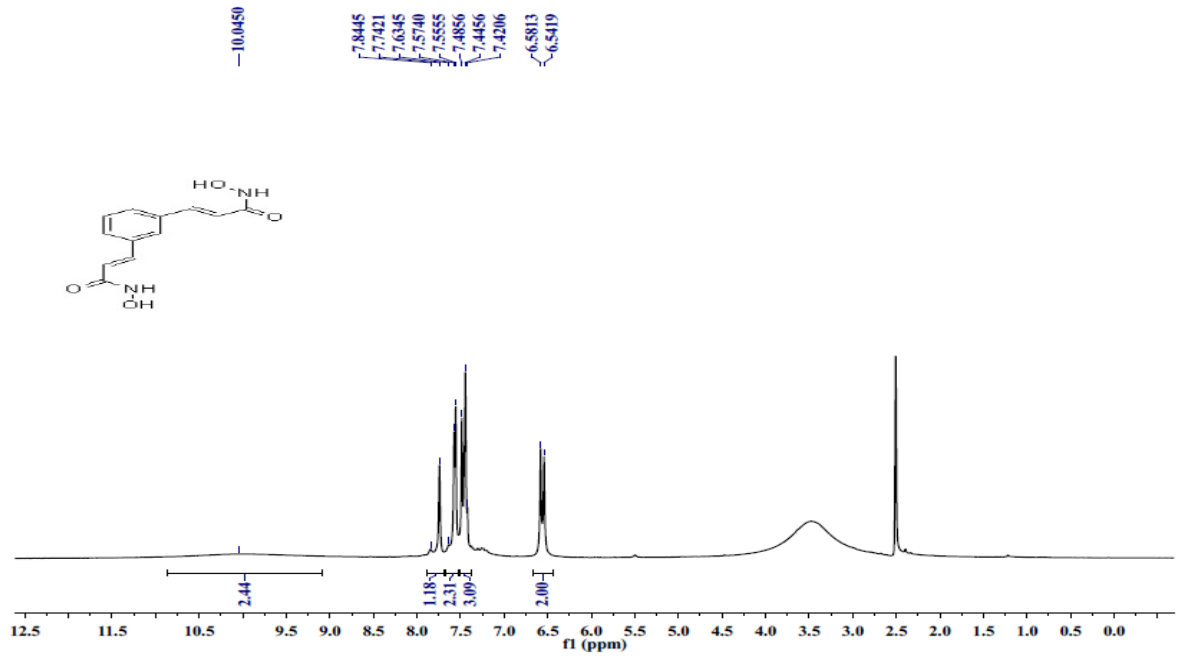
^{1}H NMR spectra of compound **3d**



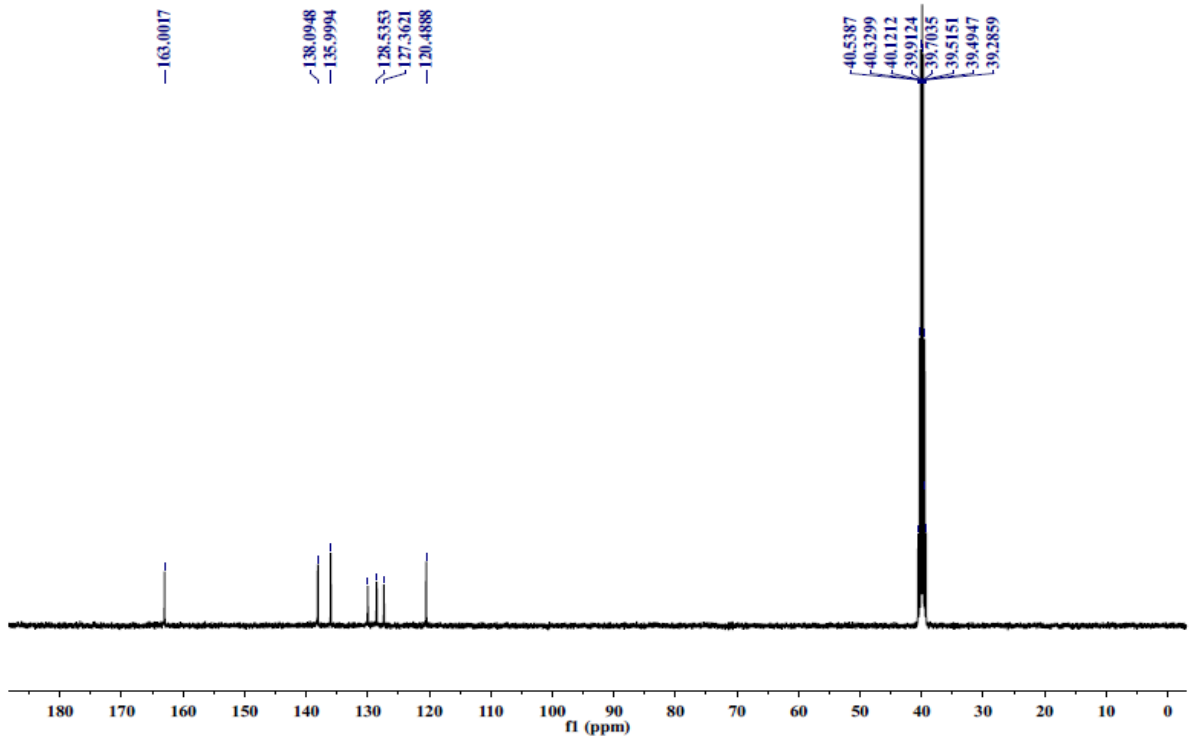
^{13}C NMR of compound **3d**



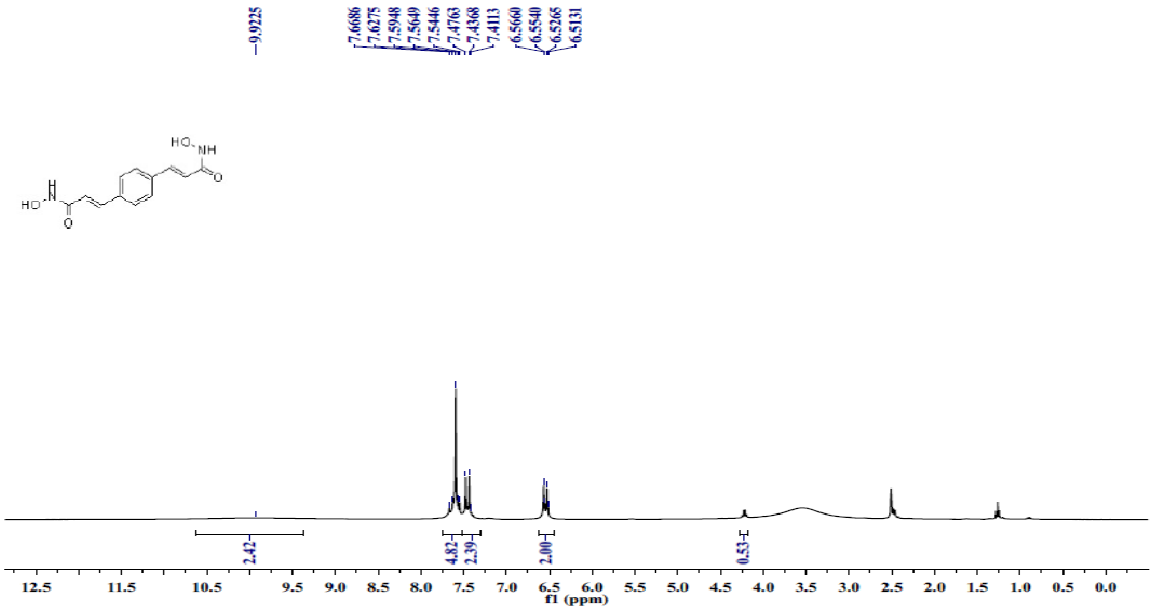
^{1}H NMR spectra of compound(**3e**)



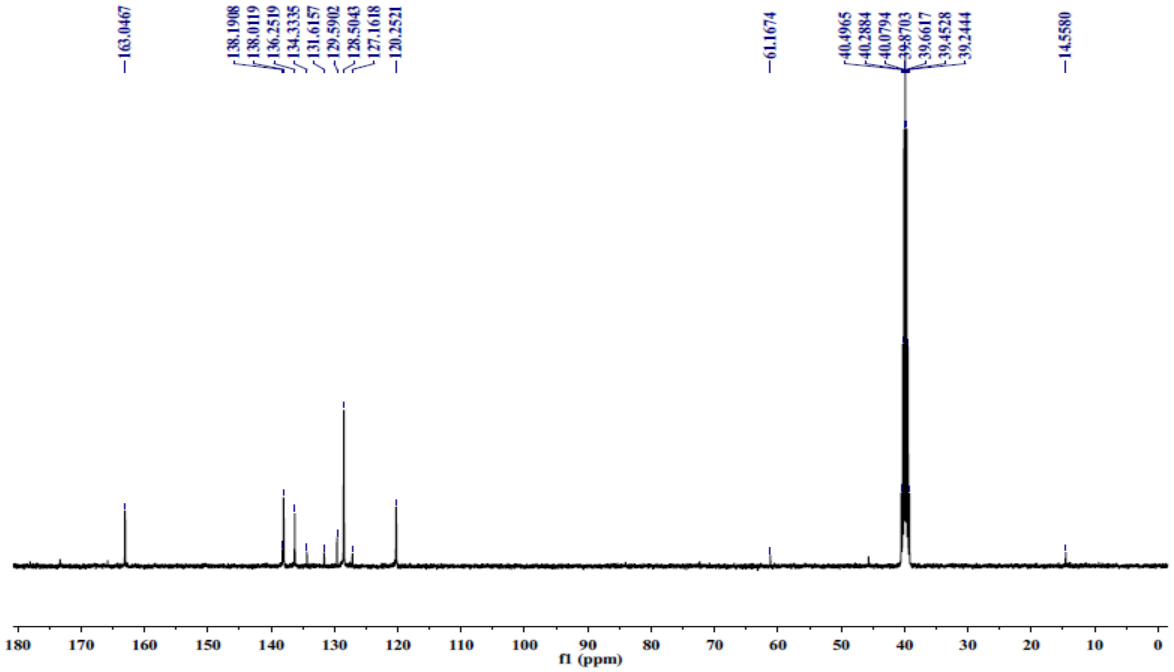
^{13}CNMR spectra of compound(**3e**)



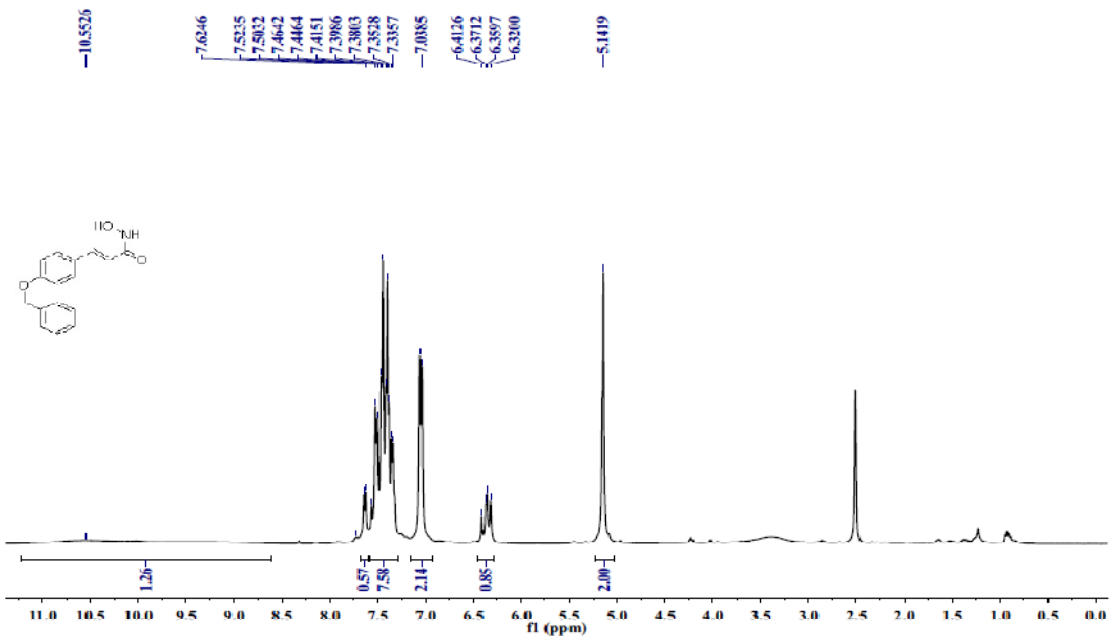
^{1}H NMR spectra of compound(**3f**)



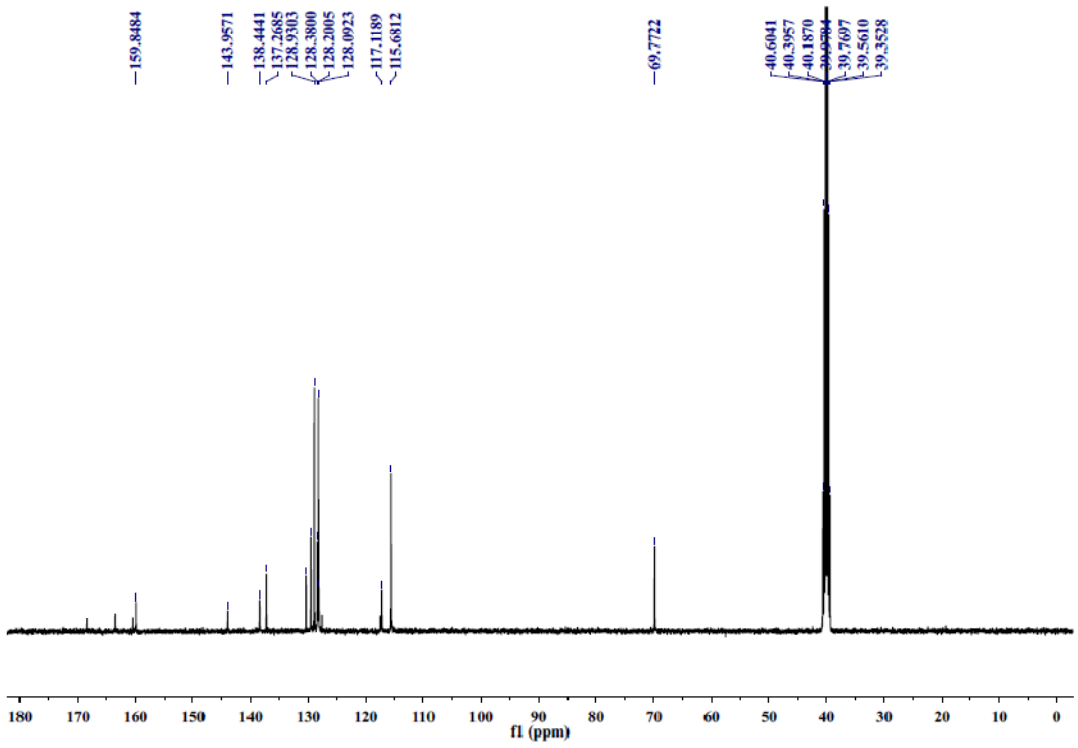
^{13}C NMR spectra of compound(**3f**)



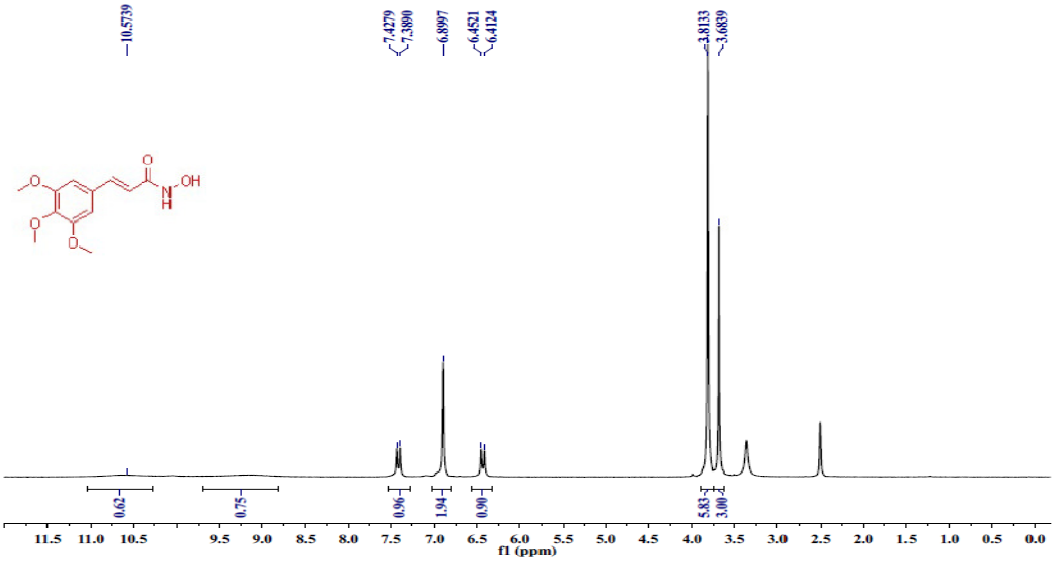
^{1}H NMR spectra of compound(**3g**)



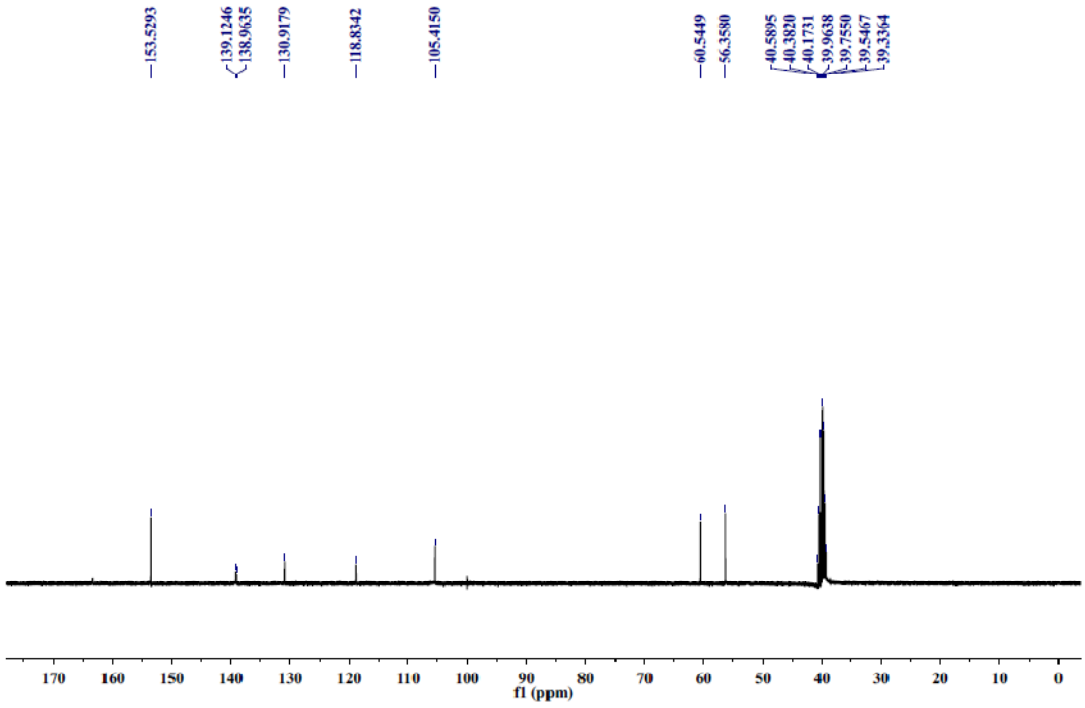
^{13}CNMR spectra of compound(**3g**)



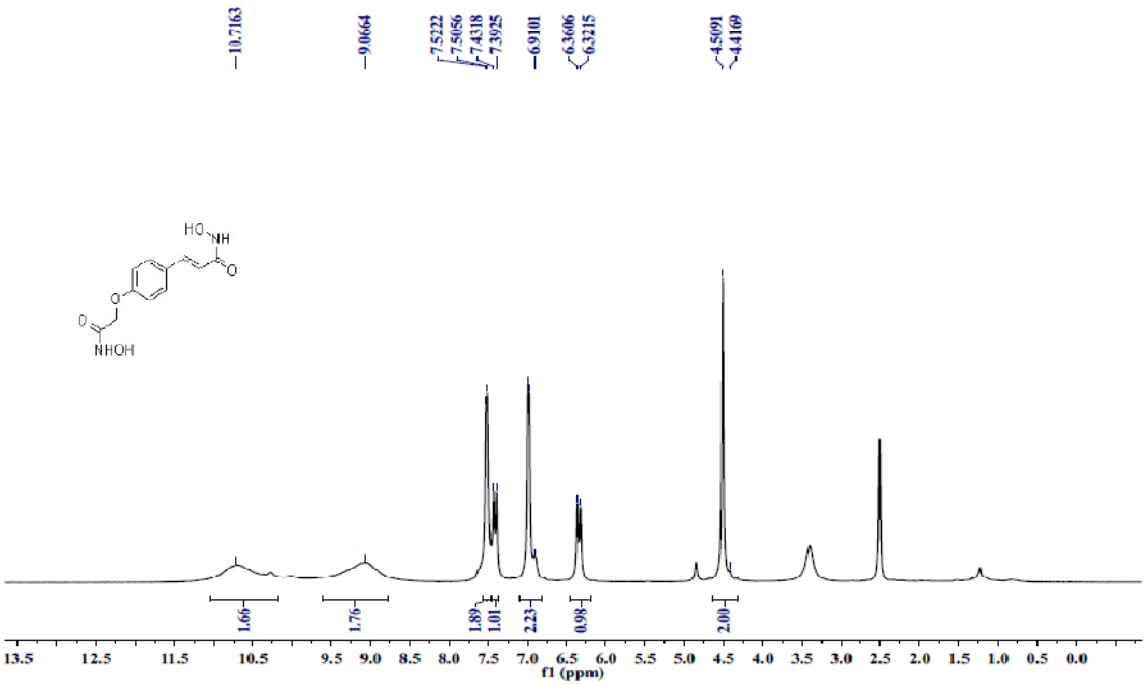
^{1}HNMR spectra of compound(**3h**)



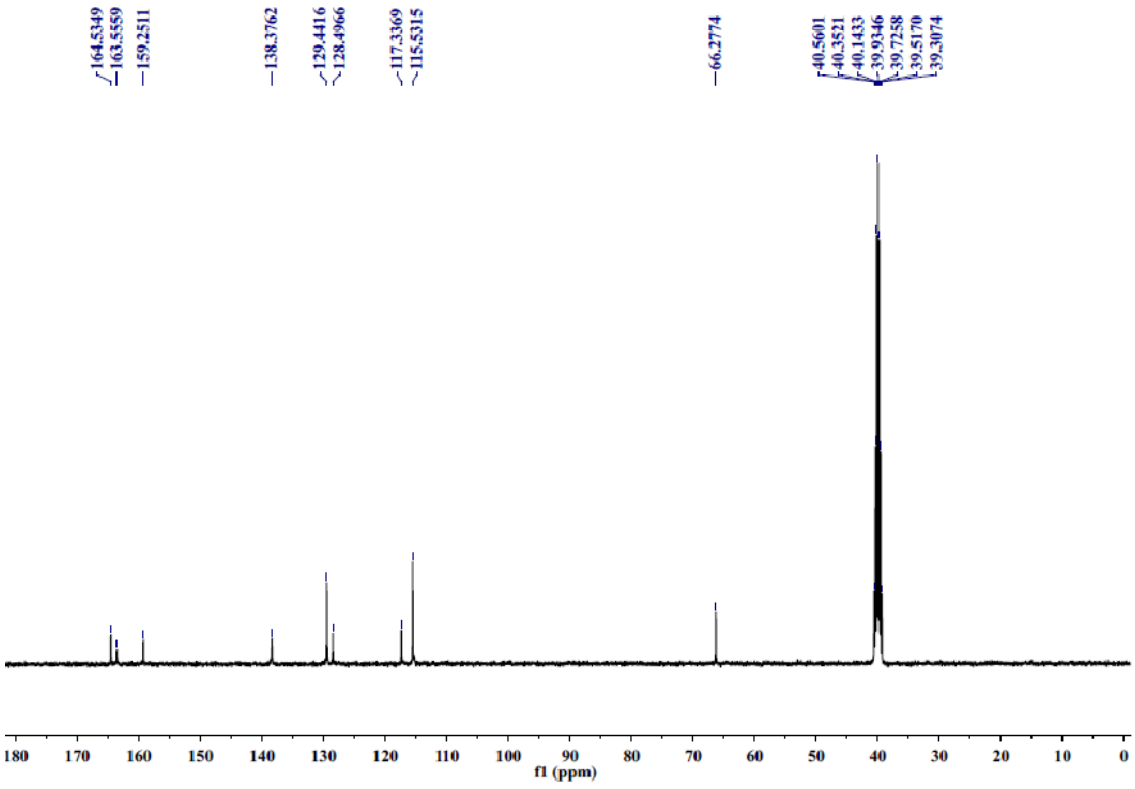
^{13}C NMR spectra of compound(**3h**)



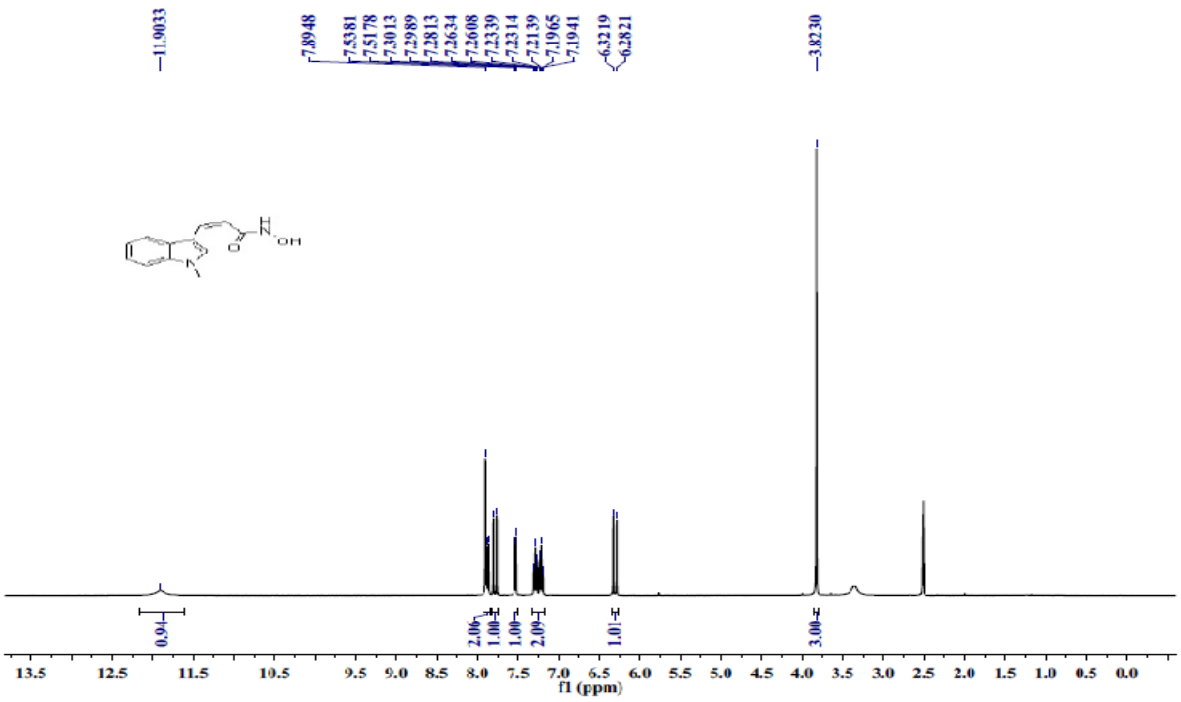
^{1}H NMR spectra of compound(**3i**)



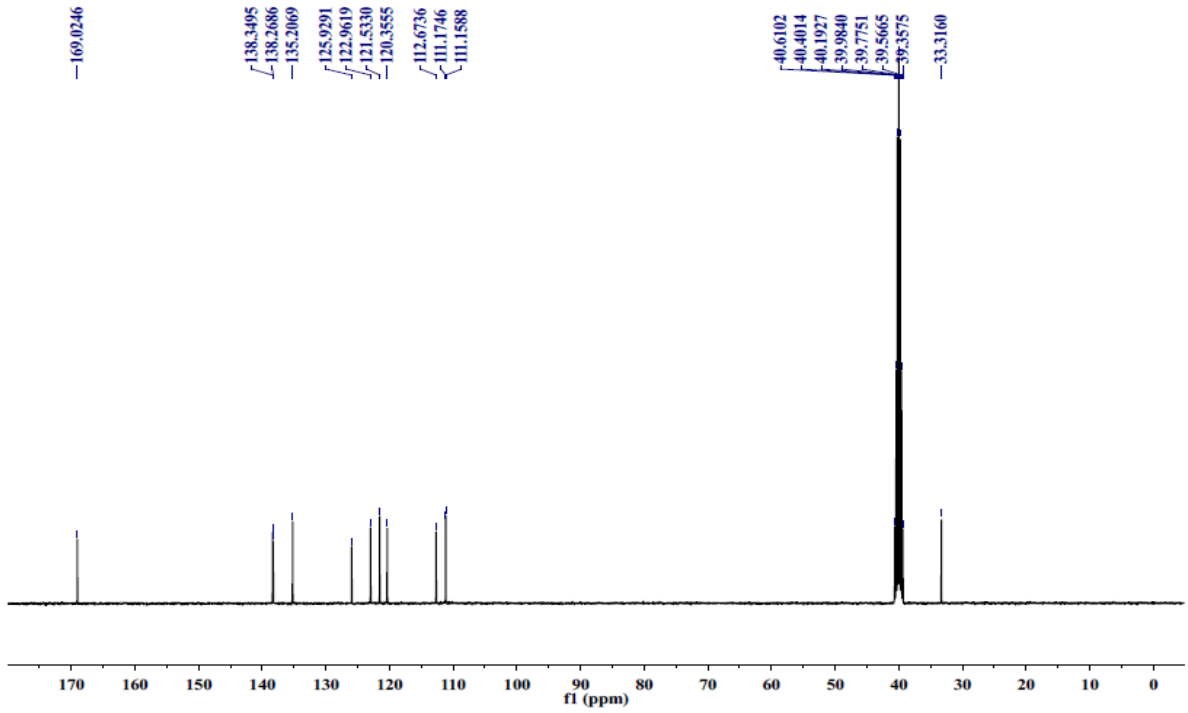
^{13}CNMR spectra of compound(**3i**)



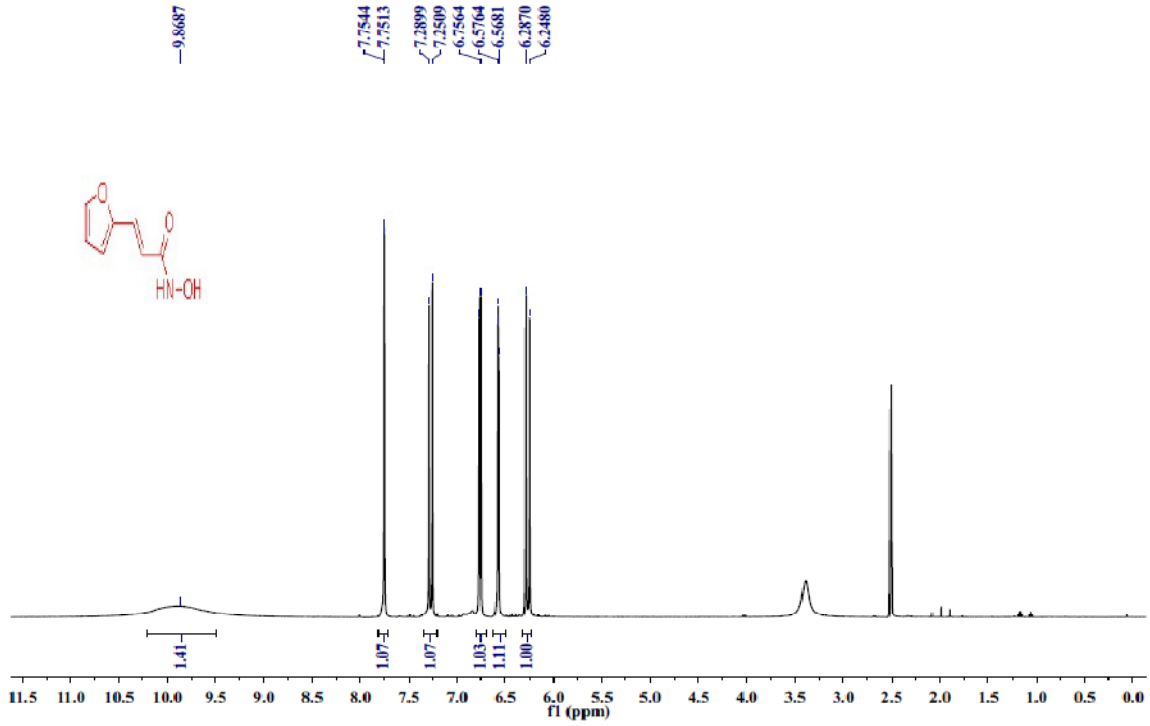
^{1}H NMR spectra of compound(**3j**)



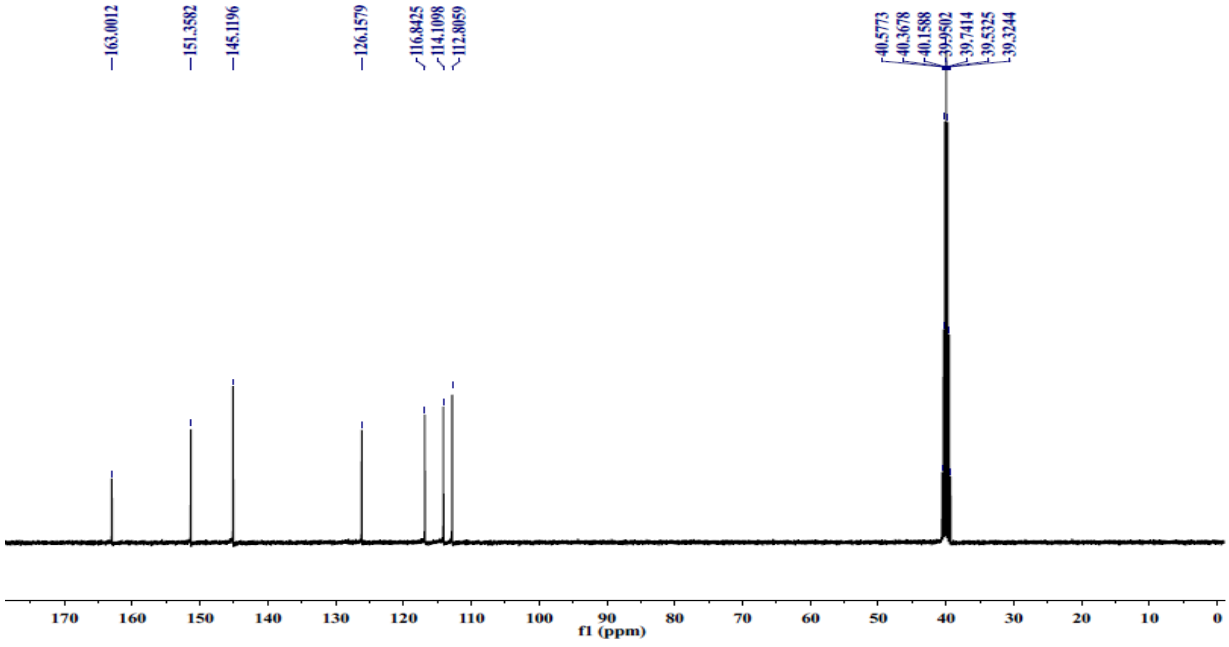
^{13}C NMR spectra of compound(**3j**)



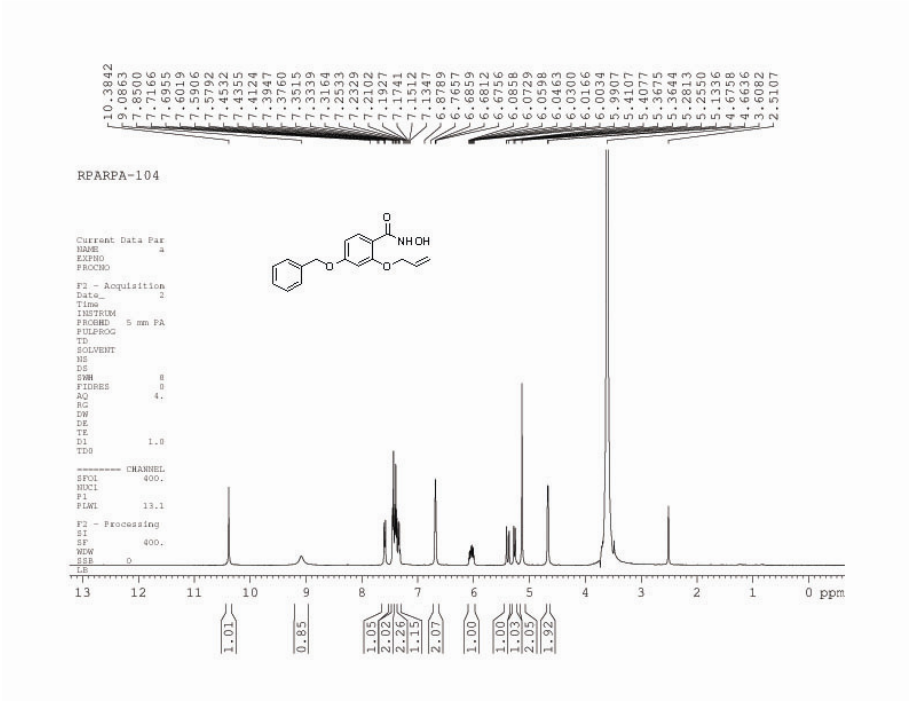
^{1}H NMR spectra of compound(**3k**)



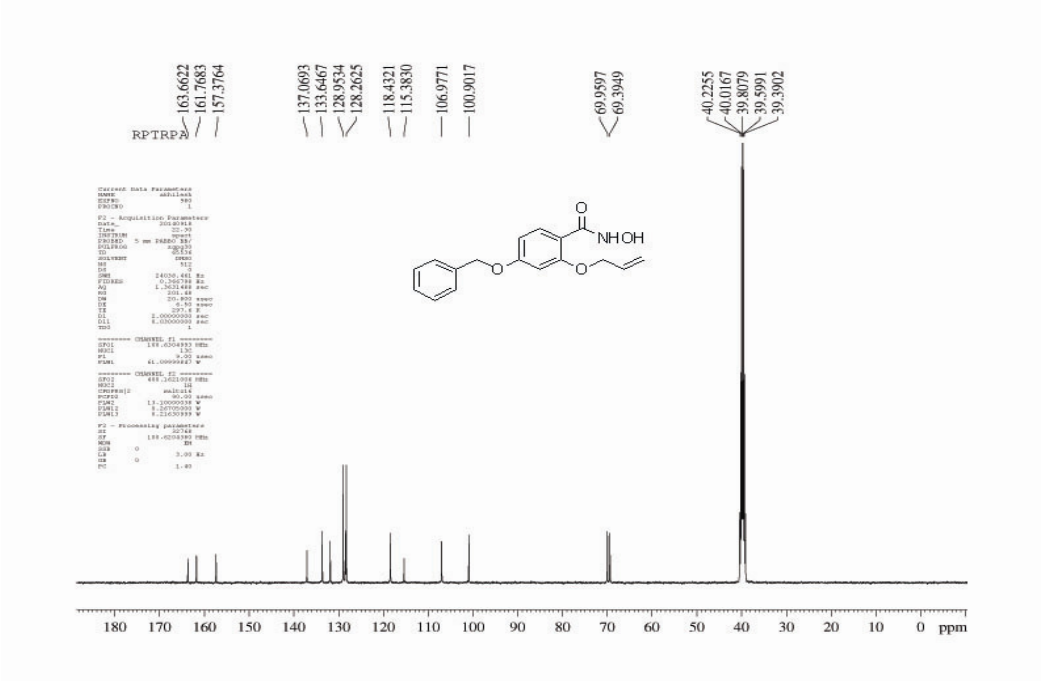
^{13}C NMR spectra of compound(**3k**)



^{1}H NMR spectra of compound (**4e)**



^{13}C NMR spectra of compound **(4e)**



Chapter-3

Synthesis of novel purine nucleoside analogues and their biological evaluation

3.1 INTRODUCTION

Nucleosides are considered as fundamental building blocks of nucleic acids and endowed with a broad spectrum of biological activity[1, 2].These analogues are synthetically modified compounds which are being synthesized to mimic the natural nucleosides and help in exploiting cellular metabolism and inhibiting cell division and viral replication when incorporated into various cellular processes like DNA and RNA synthesis, cell signalling, enzyme regulation and metabolic process[3].In addition to this, these analogues can interact with the essential enzymes and inhibit their action as human and viral polymerases, kinases, ribonucleotide reductase, DNA methyltransferases and purine, pyrimidine nucleoside phosphorylase. Sugar moieties covalently linked with some other biomolecules like proteins, peptides, lipids etc. are of considerable interest due to their involvement in complex biological processes and highly selective molecular recognition [4].They are very crucial in cellular recognition events, including signal transduction, cell adhesion and inflammation, immune response, tumour metastasis and viral & bacterial infections. Consequently, multivalent glycohybrids attached with heterocyclic pharmacophore are of great importance in medicinal chemistry and drug discovery [5]. Thus Sugar moieties attached with heterocyclic framework opens new doors for the facile and successful construction of a wide range of bioactive molecules [6] (**Figure 1**).

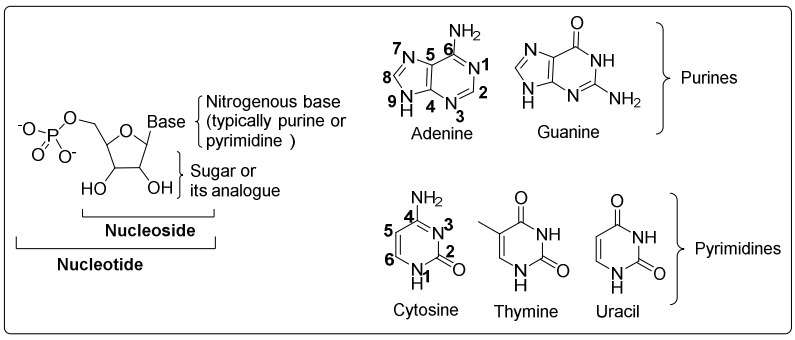


Figure 1. Structural representation of naturally occurring nucleosides and nucleotides.

Nucleosides, especially the purine adenosine, have important biological roles, and purines have been a fruitful source of inspiration for medicinal chemists for many years [7, 8]. As a result, a broad variety of bioactive purine derivatives has been designed, which has led to the launch of several powerful drugs with diverse applications [9, 10]. In that respect, major advances have been made concerning the treatment of viral infections by purine derivatives, as exemplified by the well-known drugs acyclovir(anti-herpes) [11, 12] and abacavir (anti-HIV) [13], tubercidin (anti-parasitic) [14], cladribine (anti-cancer) [15], synguanol (antiviral) [16], oxytocin-A (antibiotic) [17] and MDL73811 (anti-malarial) [18]. (**Figure 2**) Besides their versatile role purine-based heterocycles also possess significant therapeutic potentials serving as inhibitors of Hsp90 [19, 20], Src kinase [21] etc.

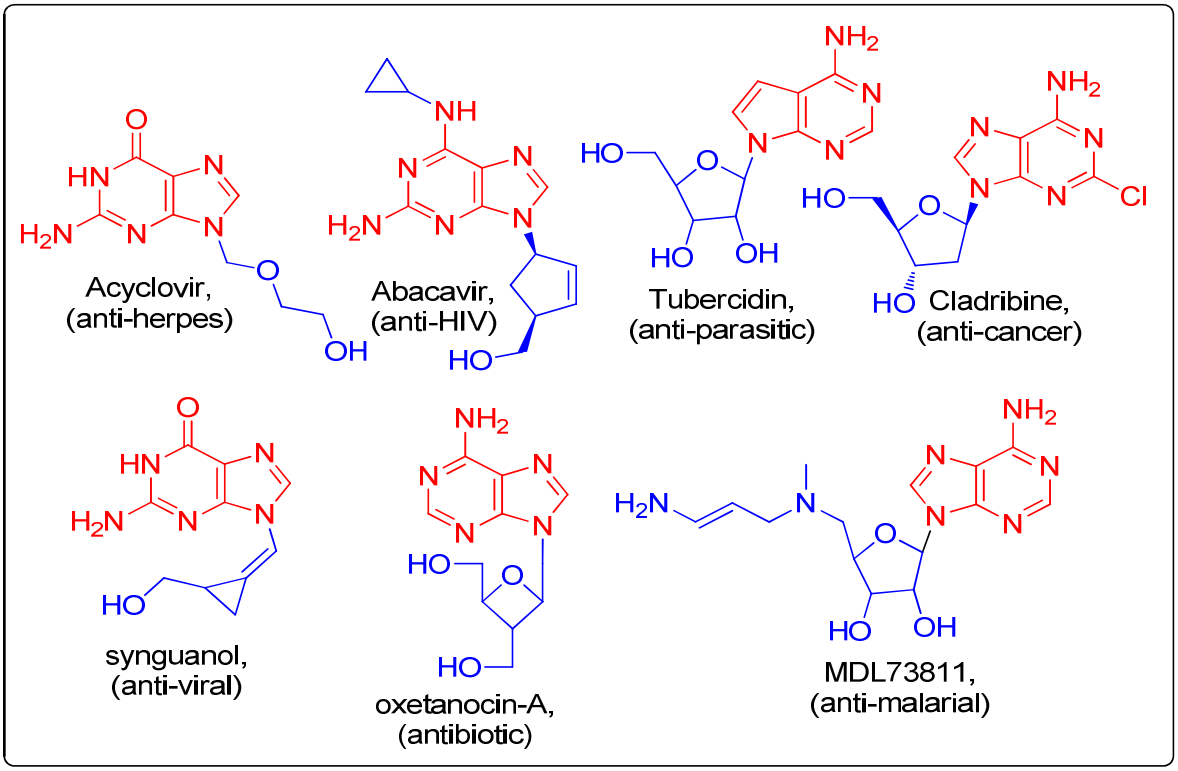


Figure 2: Some of the purine based well-known drugs.

Carbohydrates play a perfect tool in this context. They present a rigid core with a number of functional (hydroxyl or amino) groups in defined spatial orientations and they provide a series of scaffolds in which all possible isomers either occurs naturally or are available by the inversion of individual positions. Moreover, employment of sugar moieties in drug development is not limited to a passive role as a scaffold supporting pharmacophoric groups, but they can also actively influence pharmacology and pharmacokinetic properties. In fact, sugars appended to natural pharmaceutically important products are known to influence drug solubility, target recognition, toxicity, and mechanism of action. They often dictate their biological activity and, thus, the development of rapid methods to diversify natural product sugar ligands is anticipated to have a great effect on the search for new therapeutics. Inspired by these very special properties associated with sugars various 1, 2, 3-triazole-linked nucleoside, nucleotide, and oligonucleotide mimics that are of exciting biological values have been successfully synthesized in high yields and fully characterized with various spectroscopic and spectrometric techniques.

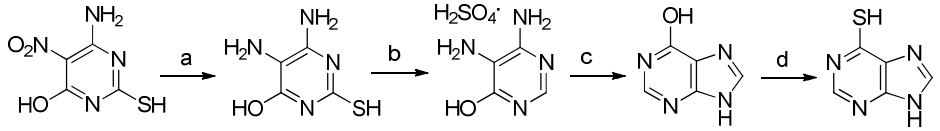
3.2.Earlier methods ofsynthesis

Purine nucleosides have been prepared earlier by various chemists. Some of the methods are briefly described below:

3.2.1. Synthesis of 6-Mercaptopurine

Earlier before 1953 when 6-Mercaptopurine was approved by the FDA, Elion's group found that 6-Mercaptopurine (**1**)was showing potent anticancer activities against different varieties of rodent tumours. It was approved for the treatment of pediatric acute lymphocytic leukaemia (ALL). It was synthesized by Elion and Hitchings in 1952, starting from 4-amino-6-hydroxy-5-nitrosopyrimidine-2-thiol, which was reduced, dethiolated and cyclized to give hypoxanthine. The hypoxanthine thus

obtained was treated with P_2S_5 to give the desired 6-Mercaptopurine (**1**) (**Figure 3**) [22].

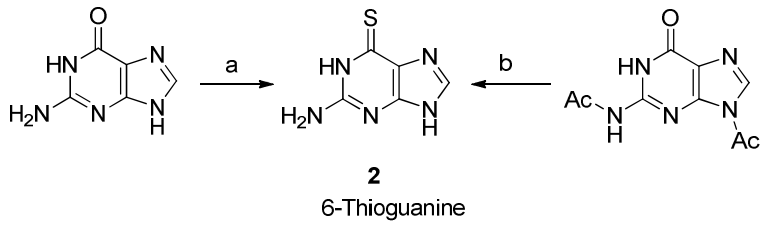


Reagents and conditions: (a) $Na_2S_2O_4$, H_2O, 60-70 °C, 80-85%; (b) Raney Ni, Na_2CO_3, H2O, reflux, 2 h then H_2SO_4, 88%; (c) HCO_2H, reflux, 2 h then aq NaOH, AcOH, 93%; (d) P_2S_5, tetralin, 190-200 °C, 12 h, 40%

Figure 3

3.2.2. Synthesis of 6-Thioguanine

6-Thioguanine (**2**) was approved by FDA in 1966 and mainly used to treat acute myelogenous leukaemia. It is a type of cancer in which abnormal white blood cells of myeloid lineage is involved which divides rapidly and interferes with normal white blood cell production. Elion and Hitchings havesynthesized 6-Thioguanine (**2**) in the year 1954[23] by converting Guanine to 6-Thioguanine **2** by treatment with P_2S_5 in refluxing pyridine. 6-Thioguanine **2** can also be synthesized by sulfuration of the diacylated guanine with P_2S_5, followed by deprotection(**Figure 4**)[24].



Reagents and conditions: (a) P_2S_5, Pyridine, reflux, 2.5 h, 32%; (b) P_2S_5, pyridine hydrochloride, pyridine, 110 °C, 4 h, then HCl, H_2O, pH-4, recrystallization, 75%.

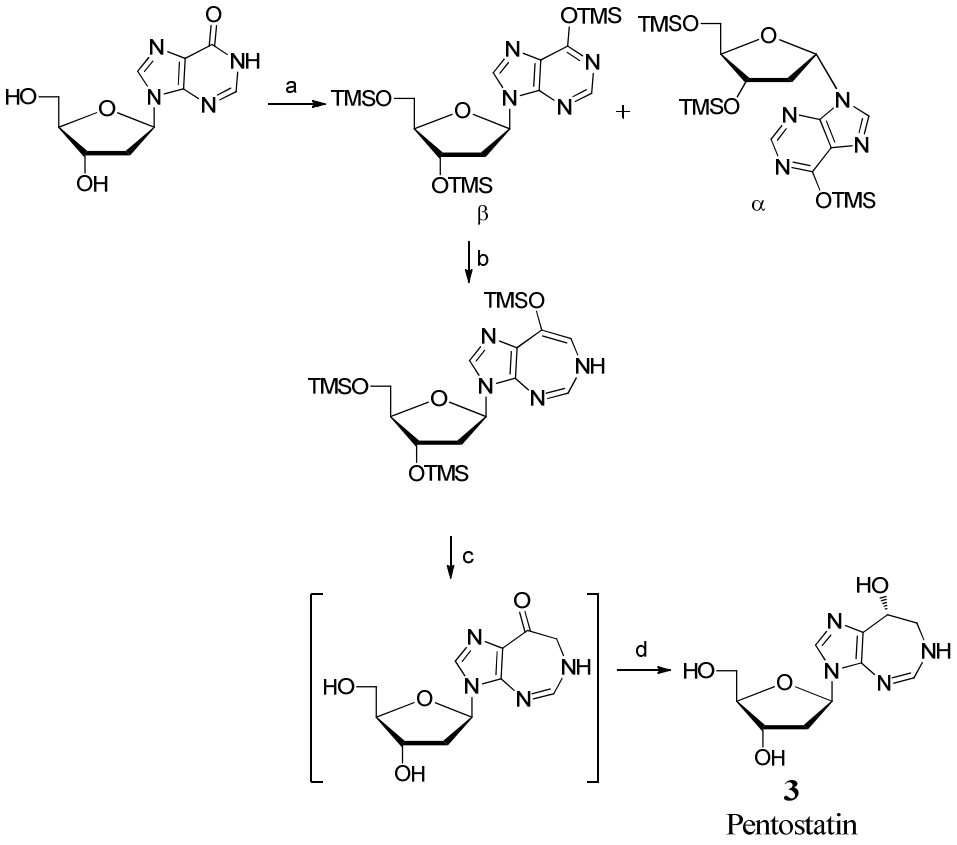
Figure 4

3.2.3. Synthesis of Pentostatin

Pentostatin (**3**) is an antibiotic and was isolated from a culture broth of Streptomyces antibiotics. It is also an inhibitor of mammalian adenosine deaminase[25, 26].It is the

only anticancer nucleoside analogueapproved by the FDA in 1991, which is active without metabolism. It also targets the enzyme cellular methyltransferases, interfering with the ability of the cell to methylate both DNA and mRNA[27, 28].Pentostatin (**3**) is basically used in the treatment of hairy cell leukaemia and is efficacious against lymphoid malignancies having high adenosine deaminase activities[29, 30].

Phiasivongsa and Redkar have synthesized Pentostatin (**3**) by performing a ring expansion of protected 2′-deoxyinosine using diazomethane, followed by deprotection and reduction giving a diastereomeric mixture of Pentostatin (**3**)(**Figure 5**) [31].



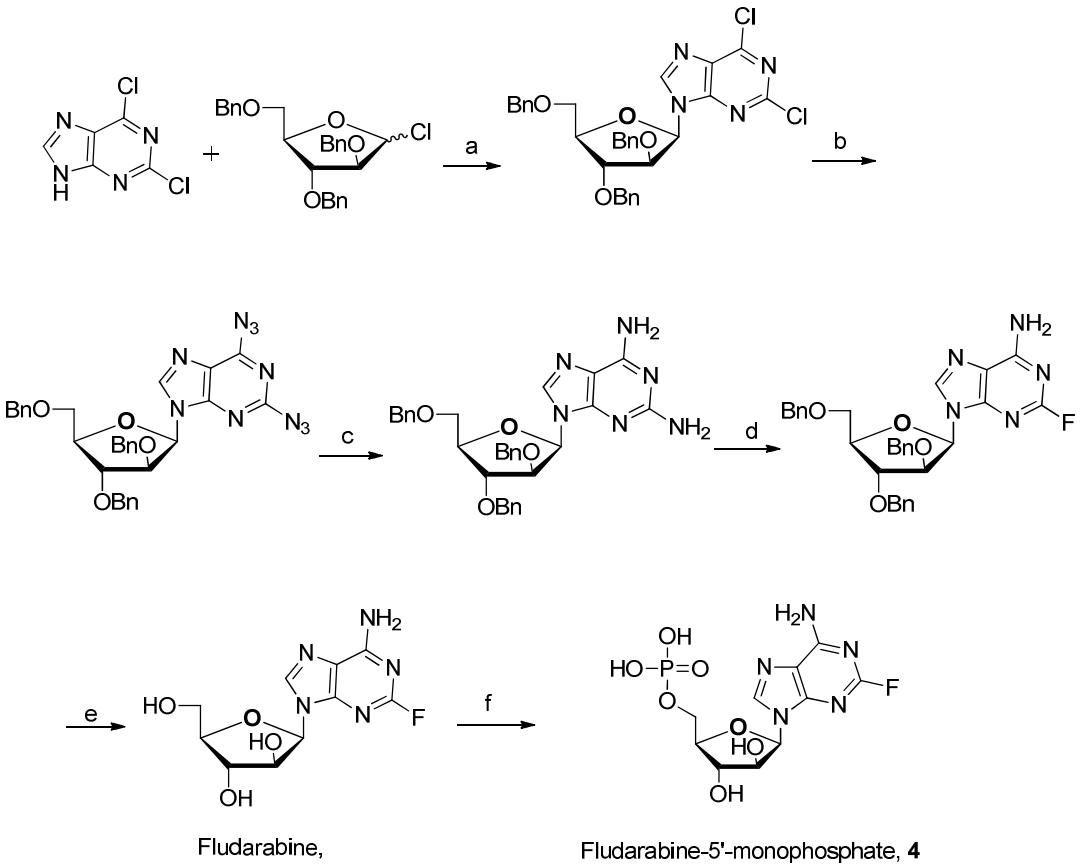
Reagents and conditions: (a) $(Me_3Si)_2NC(O)CF_3$, pyr, MeCN, 12 h; (b) $BF_3{\cdot}Et_2O$, DCM, 0 °C, 30 min; (c) TBAF, THF, 0 °C, 2 h; (d) $NaBH_4$, MeOH, H_2O, rt, 1 h.

Figure 5

3.2.4. Synthesis of Fludarabine and Fludarabine-5′-monophosphate

Fludarabine-5′-monophosphate (**4**) is used as a drug for the treatment of chronic lymphoid leukaemia and hairy cell leukaemia [32].The drug was approved by the FDA in 1991 after being subsequently explored in clinical trials.

Montgomery and Hewson have reported the discovery synthesis of Fludarabine in 1991(**Figure 6**)[33]. The key substrate chlorosugar was prepared from D-ribose by treatment of methanolic sulfuric acid to form methyl-β-D-ribofuranoside, followed by 3′,5′-*O*-benzylation and subsequent hydrolysis, *p*-nitrobenzylation, and chlorination of the anomeric position.The required β-anomer was obtained by glycosylation of 2,6-dichloropurine and chlorosugar. Subsequent treatment of with sodium azide followed by catalytic reduction gave the intermediate diaminopurine. Balz-Schiemann reaction was carried out to introduce the fluorine atom at N^2 of purine ring followed by debenzylation resulting in the formation of fludarabine. Fludarabine-5′-monophosphate (**4**) was finally prepared by treatment of fludarabine with phosphoryl chloride and triethyl phosphate [34].



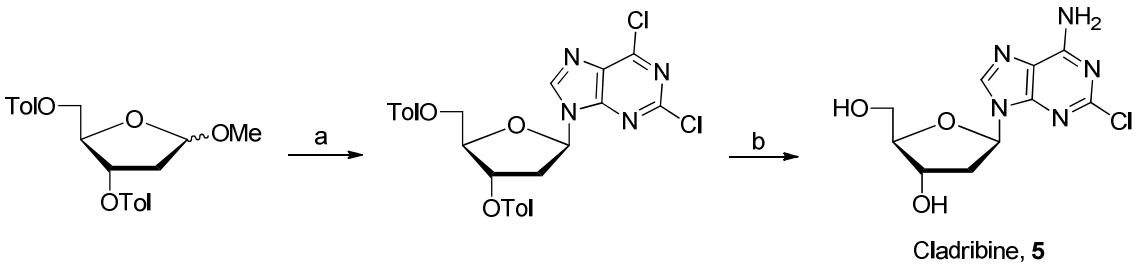
Reagents and conditions: (a) $Hg(CN)_2$, $CaSO_4$, $MeNO_2$, reflux, 3 h, 11%; (b) NaN_3, EtOH, H_2O, 1 h, 98%; (c) Pd/C, H_2, EtOH, rt, 6 h, 75%; (d) HBF_4, $NaNO_2$, $CHCl_3$, -10 °C, 40 min, 36%; (e) Na, NH_3, 34%; (f) $POCl_3$, $PO(OEt)_3$, 0 °C, 3.5 h, 96%.

Figure 6

3.2.5. Synthesis of Cladribine

Deficiency of inherited adenosine deaminase has been reported to cause lymphopenia, an immunodeficiency in human beings[35]. Further, it has been clarified by Carson *et al.* that a deoxyadenosine analogue inhibiting adenosine deaminase activity could be effective in the treatment of lymphocytic malignancies[36].Later in collaboration with John Montgomery, Carson designed 2-chloro-2′-deoxyadenosine (cladribine, **5**), which exhibited important cytotoxicity in malignant T lymphocytes[36]. Cladribine (**5**) is transported inside the cells by both hCNT and hENT. It was approved by the FDA in 1992 for the treatment of hairy cell leukaemia [37].

A simple non-stereoselective fusion glycosylation synthesis of cladribine (**5**) was reported by Christensen *et al*[38]in 1972 from diprotected sugar. The sugar derivative was prepared by treatment of 2′-deoxyribose in methanol with a catalytic amount of acid followed by *p*-toluoyl protection. The *β*-anomer of the glycosylated productthus obtained was treated with methanolic ammonia, leading to the formation of Cladribine (**5**) (**Figure 7**).



Reagents and conditions: (a) 2, 6-dichloropurine, melt, then dichloroaceticacid, and fusion under vacuum; (b) NH_3/NaOMe.

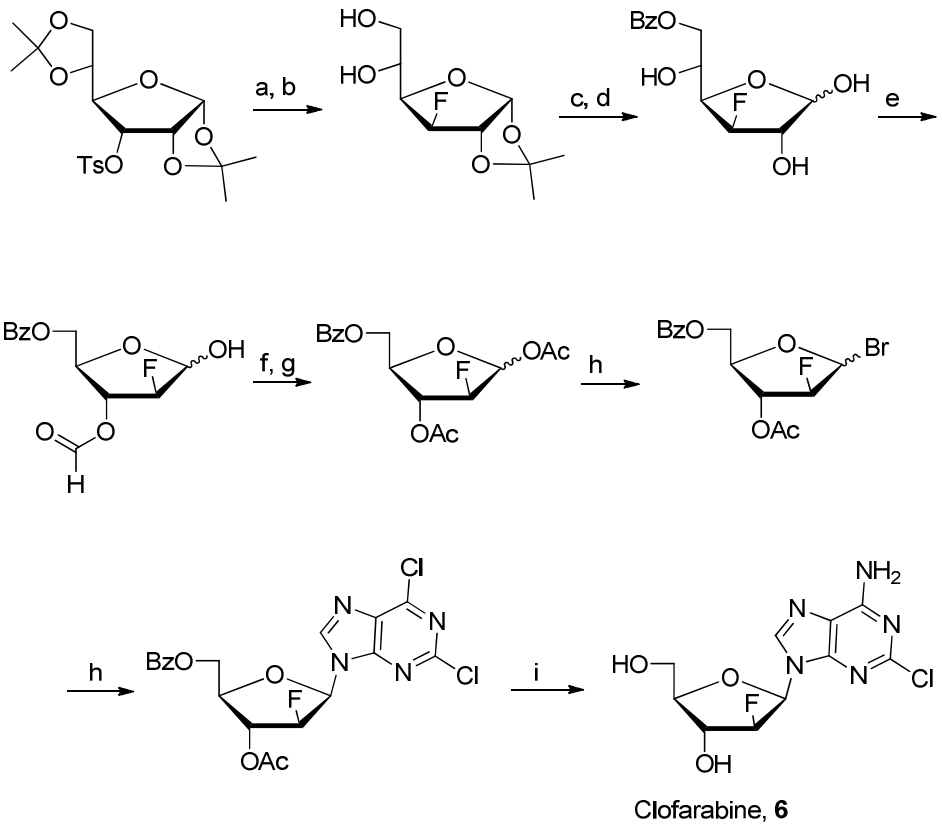
Figure 7

3.2.6. Synthesis of Clofarabine

Clofarabine (**6**) is a purine derived second-generation nucleoside analogue, which was synthesized to overcome the limitations of fludarabine-5′-monophosphate (**5**). The drug was approved by FDA in 2004 in order to treat the refractory pediatric acute lymphoblastic leukaemia [39, 40]. It has been shown to have longer intracellular retention time with good activity.

The synthesis began in the early 1990s when Montgomery *et al.*have prepared protected sugar intermediateto synthesize clofarabine (**6**) (**Figure 8**)[41, 42].The diprotected tosylate was synthesized by isopropylidene protection and tosylation of *α*-D-glucofuranose, which was stereoselectively fluorinated followed by selective deprotection, giving the fluorinated sugar. Then benzoylation of the 5′-OH was done

followed by deprotection to give the lactol. Further, oxidative cleavage and subsequent rearrangement affordedwhich was then converted to bromosugar. Glycosylation of 2,6-dichloropurine with was done and nucleoside derivative was separated by chromatography. Treatment with ethanolic ammonia led to form impure clofarabine (**6**), which when treated with LiOH in MeCN/H_2O afforded pure clofarabine (**6**).



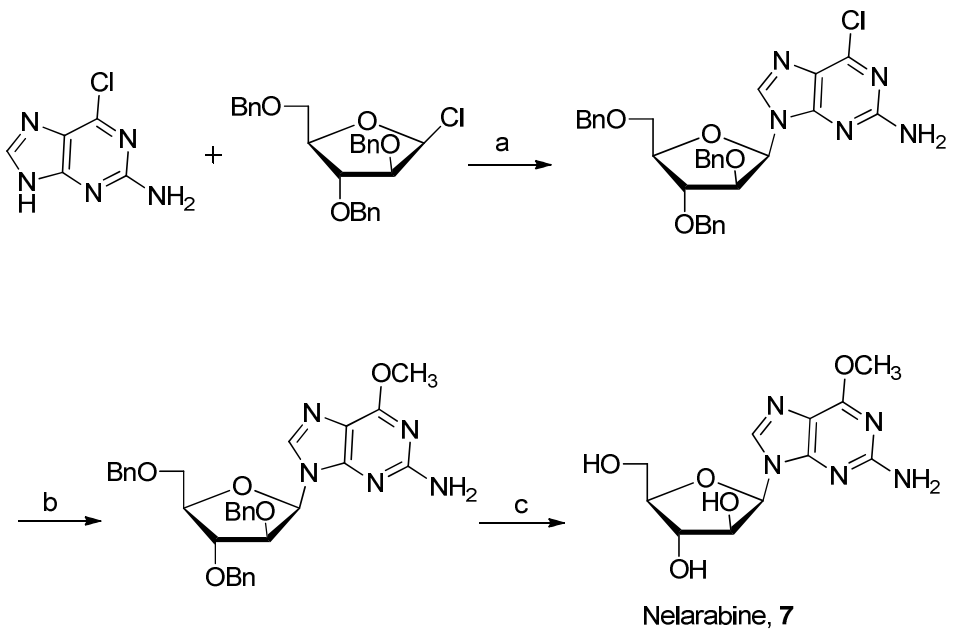
Reagents and conditions: (a) KF, acetamide, 210 °C, 62%; (b) MeOH-0.7% H_2SO_4 (1:1 v/v); (c) BzCl in DCM, pyr, -15 °C, 80%; (d) Amberlite IR-120 (H+), H_2O/dioxane, 80 °C, 78%; (e) KIO_4, H_2O; (f) NaOMe, MeOH; (g) Ac_2O, pyr, 80%; (h) HBr/HOAc, DCM; (i) 2,6-dichloropurine, DCE, 100 °C, 32% then NH_3/EtOH, steel bomb, 3 days, solvent switch to MeCN/H_2O, LiOH, 42%.

Figure 8

3.2.7. Synthesis of Nelarabine

Nelarabine (**7**)is a prodrug of 9-*β*-D-arabinofuranosyl guanine (araG) and has been recentlyapprovedby the FDA in 2005, for the treatment of relapse T cell acute lymphocytic leukaemia and lymphoblastic lymphoma[43].

Zong *et al.*[44] havesynthesized nelarabine (**7**)starting from the glycosylation of 2-amino-6-chloropurine with 1-chloro-2,3,5 tri-*O*-benzyl-arabinofuranoseform glycosylated product. Nucleophilic displacement of 6-chloro with methoxy group was done to afford the benzylated intermediate followed by debenzylation by catalytic transfer hydrogenation with the *in situ* hydrogen source coming from ammonium formate, gave the desired nelarabine (**7**)(**Figure 9**).



Reagents and conditions: (a) NaH, MeCN; (b) MeONa, MeOH; (c) Pd/C, $HCOONH_4$, MeOH, reflux, 90 min, 91%.

Figure 9

3.3. Basis of Work

As described above, nucleoside analogues having modificationin base and sugar units are significantly important due to their chemotherapeutic potential and diagnostic

applications in the living system[45]. Purine nucleoside analogues have been used clinically for almost five decades [3, 46, 47]. Purine nucleosides and their analogues have been mainly scrutinized due to their potential activity as cytotoxic [39], immunostimulator [48], antiviral [49], antifungal and antibacterial agents [50], enzyme inhibitors [51]. Further, the purine nucleus is found in a wide variety of biologically active molecules including nucleotides, enzyme cofactors, intracellular second messengers, and pharmaceutical agents [52-54]. The 2-amino-6-chloro-9*H*-purine scaffold belongs to an important pharmacophoric template that can be further modified to yield highly potent and lead compound.

Methods for preparing analogues of these important molecules generally rely on substitution of the intact purine ring system. However, modification of the core is not a straightforward process, here we have designed and synthesized novel 2-amino-6-chloropurine based nucleoside analogues selectively functionalized at C-2 amino group [55-57]. We have utilized click chemistry for the generation of triazole linker in order to link purine and sugar moiety. Due to the possibility of various diversity points in sugar structures, we were able to synthesize a library of purine based nucleosides having varying sugar moieties.

In this context, carbohydrates play a major role due to their involvement in various biological processes. They are suitable for the generation of chemical libraries for drug discovery and development [58-60], several carbohydrates based molecules were designed and developed as antifungal therapeutics. Carbohydrates are associated with the stigma of weak bonding and its inability to cross the cell membrane and therefore have been excluded in drug discovery in native form for a long time [61]. To improve the bioavailability, the prodrug strategy is used to mask the polar hydroxyl groups by hydrophobic acyl groups [3, 62, 63]. After reaching in blood, these esters

are hydrolyzed by enzymes into its native form [64].Over the last decade, there has been a great interest in the synthesis of bioconjugates using1,2,3-triazole as a linker. 1, 2, 3-triazole a bioisostere of amide bond is an important pharmacophore and such compounds are useful in material sciences and chemical biology.

Keeping all these facts in mind we embarked upon a new series of glycoconjugates comprised of 3 core structural units: (i) a sugar moiety, (ii) 1, 2, 3-triazole moiety and (iii) 2-amino-6-chloropurine unit. The sugar provides a common drug template while the 2-amino-6-chloropurineunit acts as an important pharmacophore for inhibition and the 1,2,3-triazole linker is a bioisostere of the amide bond serves as a biocompatible, nonlabile covalent spacer between the sugar and 2-amino-6-chloropurineunit (**Figure 10**).

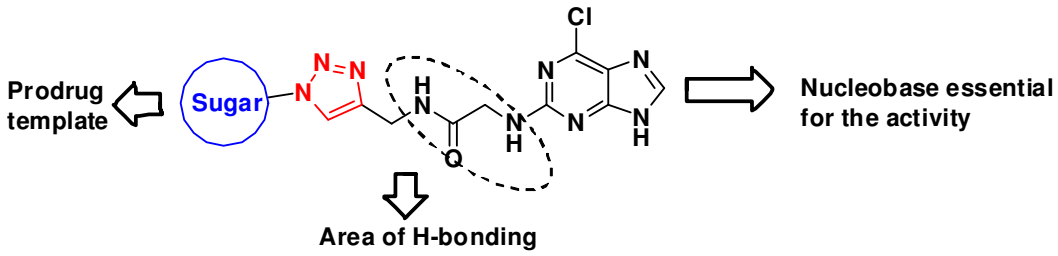


Figure 10. Design of 2-amino-6-chloropurinebased glycoconjugates

3.4 Present work:

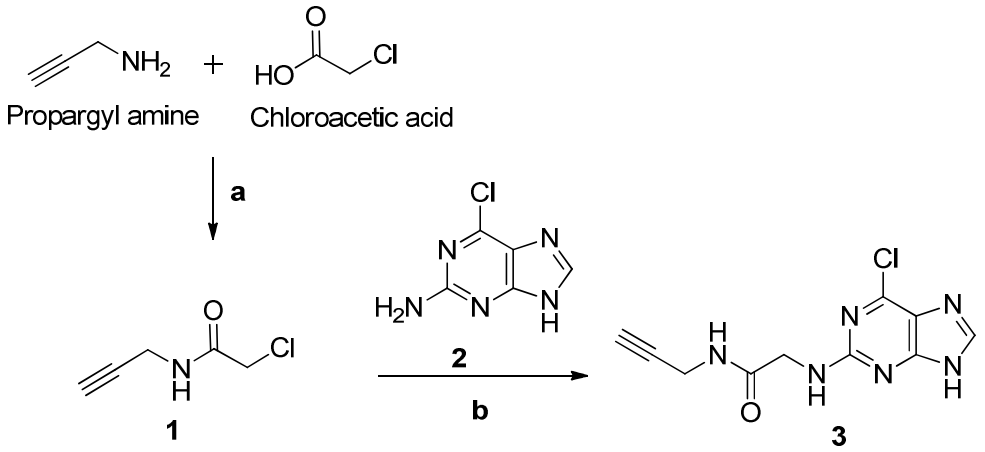
The present work describes the synthesis of novel purine nucleoside analogues having bases 2-amino-6-chloropurineand triazole with different sugar as well as aryl azides resulting triazole by Cu (I)-catalysed azide-alkyne 1, 3-dipolar cycloaddition reaction. The library of synthesized nucleoside analogues were evaluated for antifungalactivity. The newly synthesized compounds are listed below:

2-[(6-chloro-9*H*-purin-2-yl) amino]-*N*-(prop-2-yn-1-yl) acetamide(**3**), 2-[{6-chloro-9*H*-purin-2-yl} amino] -*N*-[{1´-(1´´deoxy-2´´, 3´´, 4´´, 6´´- tetra-*O*-acetyl-β-D-glucopyranos-1´´yl)-1´*H*-1´,2´,3´- triazol-4´-yl}methyl]- acetamide(**5a**),2-[{6-chloro-9*H*-purin-2-yl} amino] -*N*-[{1´-(1´´deoxy-2´´, 3´´, 4´´, 6´´- tetra-*O*-acetyl-β-D-galactopyranos-1´´yl)-1´*H*-1´,2´,3´- triazol-4´-yl}methyl]- acetamide(**5b**), 2-[{6-chloro-9*H*-purin-2-yl} amino] -*N*-[{1´-(1´´deoxy-2´´, 3´´, 4´´, 6´´- tetra-*O*-acetyl-β-D-mannopyranos-1´´yl)-1´*H*-1´,2´,3´- triazol-4´-yl}methyl]- acetamide (**5c**), 2-[{6-chloro-9*H*-purin-2-yl} amino] -*N*-[{1´-(1´´deoxy-2´´, 3´´, 4´´, 6´´- tetra-*O*-acetyl-β-D-ribopyranos-1´´yl)-1´*H*-1´,2´,3´- triazol-4´-yl}methyl]- acetamide (**5d**), 2-[{6-chloro-9*H*-purin-2-yl} amino] -*N*-[{1´-(1´´deoxy-2´´, 3´´, 4´´, 6´´- tetra-*O*-acetyl-β-D-cellobios-1´´yl)-1´*H*-1´,2´,3´- triazol-4´-yl}methyl]- acetamide (**5e**), 2-[{6-chloro-9*H*-purin-2-yl} amino] -*N*-[{1´-(6´´deoxy-1´´, 2´´, 3´´, 4´´- di-*O*-isopropylidene-α-D-galactopyranos-6´´-yl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]acetamide (**5f**), 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-[{1´-(5´´deoxy-1´´,2´´-*O*-isopropylidene-α-D-xylofuranos-5´´-yl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]- acetamide (**5g**), 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-[{1´-(β-D-glucopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]- acetamide (**6a**), 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-[{1´-(β-D-galactopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]- acetamide (**6b**), 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-[{1´-(β-D-mannopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl] acetamide (**6c**),2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-[{1´-(β-D-ribopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]- acetamide (**6d**), 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-[{1´-(β-D-cellobiopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]- acetamide (**6e**), 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (**8a**),2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-((1-(2-bromobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (**8b**), 2-[{6-chloro-9*H*-

purin-2-yl} amino]-*N*-((1-(4-bromobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (**8c**), 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-((1-(4-fluorobenzyl)-1*H*-1, 2, 3-triazol-4-yl) methyl) acetamide (**8d**), 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-((1-(4-(trifluoromethyl) benzyl)-1*H*-1, 2, 3-triazol-4-yl) methyl) acetamide (**8e**), 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-((1-(4-nitrobenzyl)-1*H*-1, 2, 3-triazol-4-yl) methyl) acetamide (**8f**),2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-((1-(2-phenoxyethyl)-1*H*-1, 2, 3-triazol-4-yl)methyl)acetamide (**8g**)

3.5. Synthesis of purine derived nucleoside analogues

Among the various methodologies reported for synthesis of purine nucleoside analogs, the Cu (I)-catalyzed Huisgen alkyne-azide cycloaddition (CuAAC, also known as 'click reaction')[65] has apparently been one of the most frequently used reactions. The synthesis of triazoles using 'click chemistry' has contributed to a renaissance in the chemistry of azides as building blocks toward higher complexity glycoconjugates [66]. We started our synthetic journey with an objective of synthesis of purine based triazole containing nucleoside analogues, and then corresponding aryl triazole analogues. Primarily, the intermediate 2-chloro-N-propynyl-acetamide was synthesized by coupling of chloroacetic acid and propargyl amine by reported HOBt amide-coupling protocol. The second step in the synthetic strategy is the synthesis of purine derived nucleoside analogues employs *N*-alkylation of2-amino-6-chloropurine **2** at the amino group with the 2-chloro-*N*-propynyl-acetamide **1** by heating these two reactants at 80 °C using strong base sodium hydride in DMF. (**Scheme1**) Thus, we end up with the intermediate purine derived alkyne **3** in good yield. The synthesized alkyne analogue was further precisely characterized by spectroscopic (^{1}H, ^{13}C NMR) data.



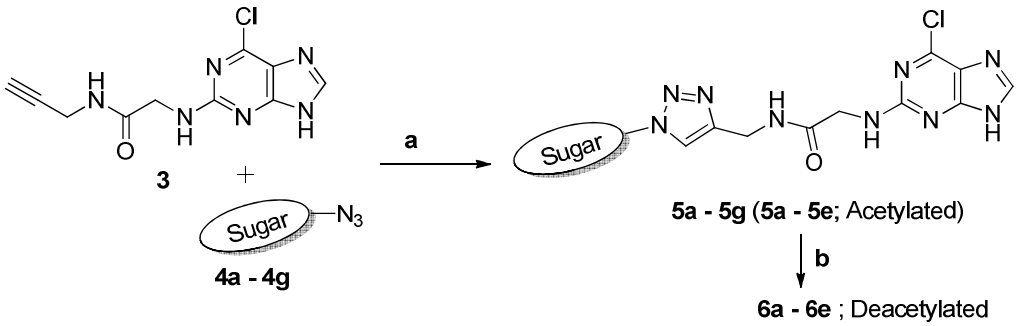
Reagent and condition: a) HOBT, DIPC, dichloromethane, 0°C to rt, 12 h, 75-86% b) 2-amino-6-chloropurine, NaH, DMF, rt (2h) to 80 °C (8h), 90-95%.

Scheme 1. Synthesis of purine derived alkyne 2-((6-chloro-9*H*-purin-2-yl) amino)-*N*-(prop-2-yn-1-yl) acetamide

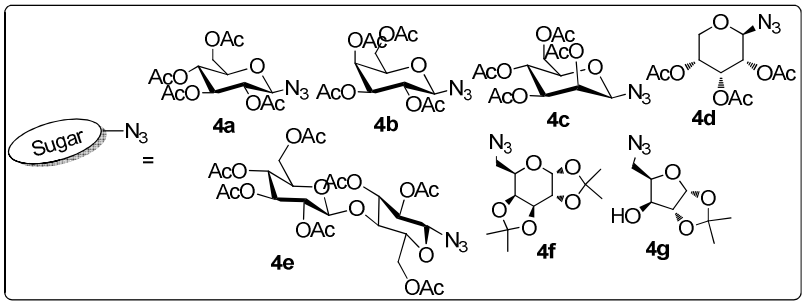
The structure of the product **3**was established by their spectroscopic data. ESIMS of the compound displays *m/z* = 264 as $[M+H]^+$ peak corresponding to its molecular formulae $C_{10}H_9ClN_6O$. In IR spectrum, characteristic absorption peaks observed at 3372 cm^{-1} for -NH, 1670 cm^{-1} for carbonyl (-NHC=O). In the ^1H NMR spectrum, the two exchangeable NH_2protons were observed at δ 5.43 (bs, 2H, -NH_2) and the amide -NH proton was visible at δ_H 8.73-8.71 (m, 1H, N-9), while the alkynyl proton was visible at δ 3.16 besides other usual protons at their usual chemical shift. In ^{13}C NMR spectrum, the peaks at δ 166.5 accounted the amide group carbon (-NH-CO-) along with other usual signals. The reaction of 2-amino-6-chloropurine **2** at the amino group with the 2-chloro-*N*-propynyl-acetamide (**1**)the above reaction conditions led to the formation of 2-((6-chloro-9*H*-purin-2-yl) amino)-*N*-(prop-2-yn-1-yl) acetamide (**3**) in good yield (**Scheme 2**).

The sugar azides **4a-4g** were prepared and characterized following earlier reported methods[4, 5, 67, 68]. Thus, having purine derived alkyne **3** and sugar azides in hand,

the well known 'Click' reaction was performed in solvent system *t*-BuOH/H_2O (1:1) taking equimolar quantities of the reactants, $CuSO_4.5H_2O$ (10 mol%) and sodium ascorbate (20 mol%) at ambient temperature (Scheme 2). The reactions were monitored (TLC) up to the completion of the reaction. Thus various 1, 2, 3-triazole-linked purine nucleoside analogues have been successfully synthesized in high yields. The respective products **5a**-**5e**were isolated and characterized based on their ^1H, ^{13}C and Mass spectral data.These compoundswere isolated to Zemplen deacetylation with NaOMe/MeOH at room temperature which led to the formation of the deacetylated purine nucleoside analogues **6a-6e**, respectively in good yields. (**Scheme 2**)

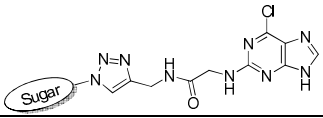
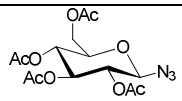
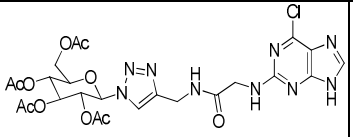
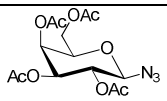
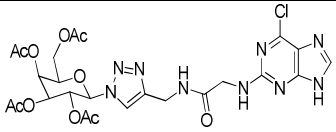
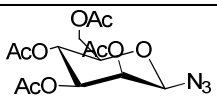
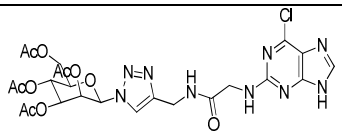
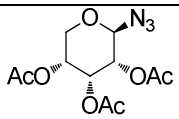
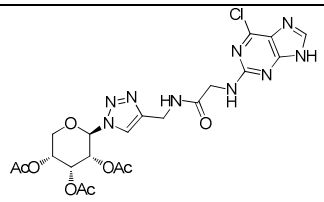
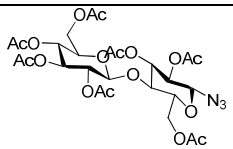
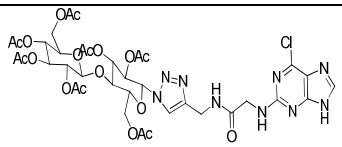
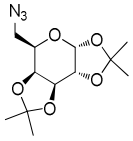
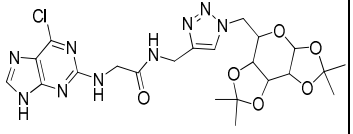
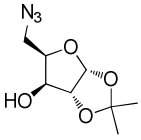
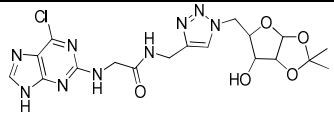


Reagent and condition: a) CuSO4.5H2O (10 mol %), sodium ascorbate (20 mol %), t-BuOH: H2O (1:1 v/v), rt, 4 h, 65-75 % b) NaOMe, MeOH, rt, 0.5 h, 80-85%.



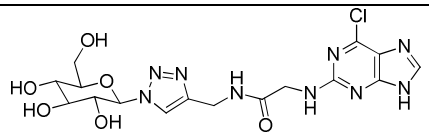
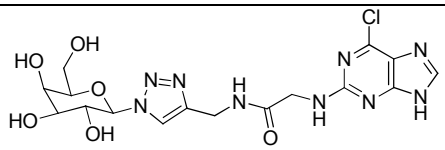
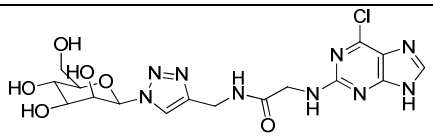
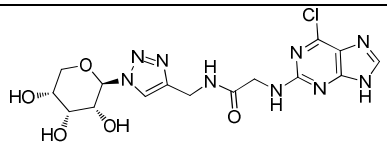
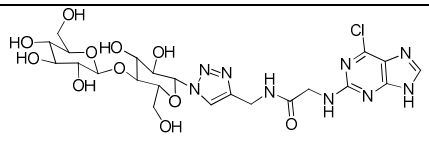
Scheme 2: Synthesis of purine derived nucleoside analogues.

Table 1: Preparation of nucleoside analogues (**5a-g**) by CuAAC reactions of N^1-purine alkyne (**3**) with azido sugars (**I**-**VII**)

Entry	N^1- Purine alkyne	Sugar Azides	Products 	Yield (%)
1.	**3**	 **I**	 **5a**	84
2.	**3**	 **II**	 **5b**	86
3.	**3**	 **III**	 **5c**	75
4.	**3**	 **IV**	 **5d**	75
5.	**3**	 **V**	 **5e**	81
6.	**3**	 **VI**	 **5f**	85
7.	**3**	 **VII**	 **5g**	88

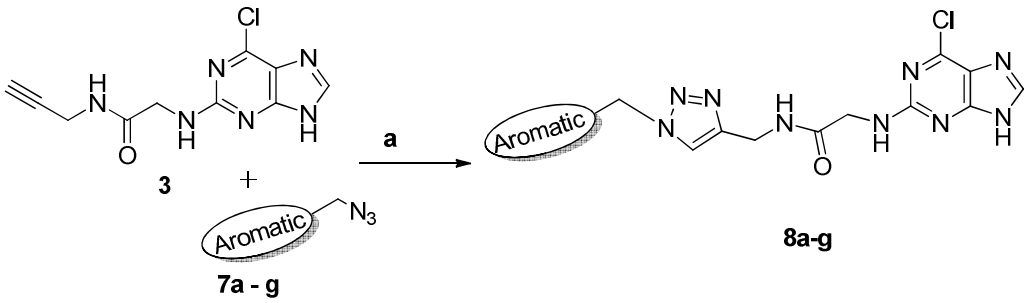
Further, the acetylated nucleoside analogues **5a**-**e** were subjected to Zemplen deacetylation with NaOMe/MeOH at room temperature which led to the formation of the deacetylated purine nucleoside analogues **6a-e**, respectively in good yields. (**Scheme 1**, Table 2).

Table 2: Synthesized deacetylated compounds **6a-e**fromacetylated products**5a-e**

Entry	Acetylated substrates	Deacetylated Products	Yield (%)
1.	**5a**	 **6a**	81
2.	**5b**	 **6b**	84
3.	**5c**	 **6c**	90
4.	**5d**	 **6d**	83
5.	**5e**	 **6e**	77

As described in **Scheme 2**, similarly the aromatic azides **7a**-**g** was prepared according to the literature and the respective azide was subjected to CuAAC 1, 3-dipolar cycloaddition with the alkyne **3** which led to the formation of final products **8a-g** in good yields (**Scheme3**). To the stirring solution of synthesized purine alkyne **3**(1 mmol) and the corresponding benzyl azide **7a-g** (1 mmol) in *t*-butanol+water (1:1, 20 mL), was added $CuSO_4.5H_2O$ (10 mol %) and Na-ascorbate (20 mol %) and stirred

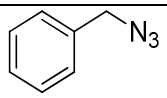
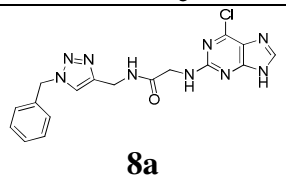
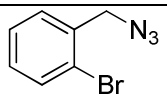
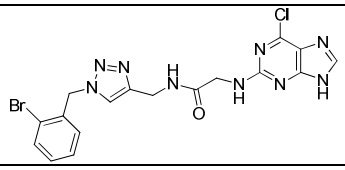
the reaction mixture at room temperature till the reaction completes (usually 3-4 h). After completion of the reaction (on TLC), it was extracted with ethyl acetate and washed with water and brine solution. The organic layer was dried (anhd. Na_2SO_4) and evaporated under reduced pressure to give a crude mass. The latter was purified by silica gel (60-120 mesh) column chromatography using chloroform: methanol (9:1) as eluent to give the solid white compound and characterized with the help of ^1H, ^{13}C and Mass spectral data.

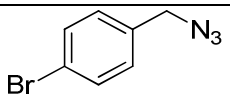
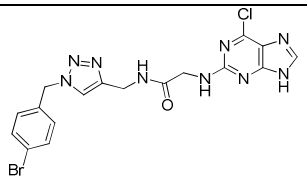
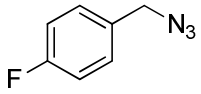
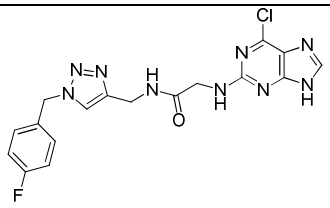
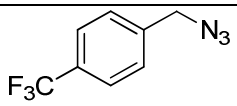
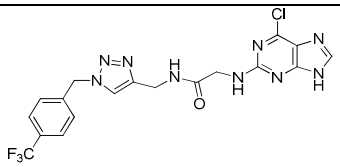
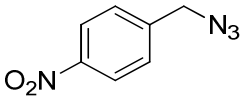
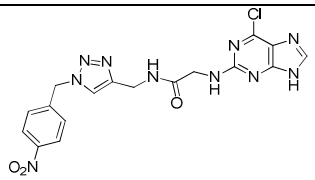
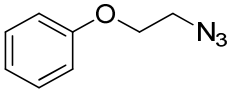
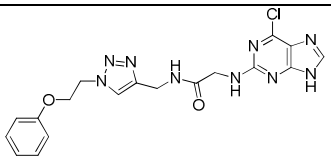


Reagent and Conditions: a) $CuSO_4.5H_2O$ (10 mol %), sodium ascorbate (20 mol %), t-BuOH: H_2O (1:1 v/v), RT, 4 h, 73-85%

Scheme 3: Synthesis of *N*-9 unprotected 6-chloro-8-arylpurines 2-((6-chloro-9*H*-purin-2-yl) amino)-*N*-(prop-2-yn-1-yl) acetamide

Table 3: Preparation of nucleoside analogues (**8a-g**) by CuAAC reactions of N^1-purine alkyne (**3**) with aromatic azide (**7a-g**)

Entry	N^1- Purine alkyne	Aromatic Azides	Products	Yield (%)
1.	**3**	 **7a**	 **8a**	83
2.	**3**	 **7b**		73

			8b	
3.	**3**	 **7c**	 **8c**	77
4.	**3**	 **7d**	 **8d**	74
5.	**3**	 **7e**	 **8e**	80
6.	**3**	 **7f**	 **8f**	71
7.	**3**	 **7g**	 **8g**	75

3.6. Biological study

All the synthesized nucleosides were evaluated for their antifungal activity (MIC_{50})against***Aspergillus*** **sp.**

Compound	**MIC_{50}(µg/L)**	
	Aspergillusniger	***Aspergillusstirus***
Control	**-**	**-**
6b	0.021	0.0424
6e	0.212	0.318
8e	0.318	0.212
8f	0.0424	0.106

Compound	**Laccase activity**	
	***Aspergillus* niger**	***Aspergillusstirus***
Control	9.71±0.46	10.75±0.66
6b	22.08±1.38	16.72±0.69
6e	10.37±0.85	8.76±.64
8e	14.09±1.11	12.22±.35
8f	11.93±1.01	15.30±.69

Compound	**MnP activity**	
	***Aspergillus* niger**	***Aspergillusstirus***
Control	61.53±3.28	61.16±.91
6b	79.82±12.92	79.15±1.57
6e	83.16±2.25	79.91±1.80
8e	67.11±1.32	72.29±1.37
8f	62.85±0.79	66.50±1.24

Compound	**Lignin peroxidase activity**	
	***Aspergillus* niger**	***Aspergillusstirus***
Control	9.59±0.55	9.46±1.20
6b	20.30±1.33	17.31±1.31
6e	18.04±0.45	16.01±0.33
8e	7.96±0.37	7.43±1.07
8f	11.98±0.73	14.26±0.62

3.6.1. Organism and culture condition

Fungi *Aspergillus niger* and *Aspergillus stirus* were procured from the Department of Environmental Science, Babasaheb Bhimrao Ambedkar University, Lucknow. The fungal strains were cultured in Erlenmeyer flasks (200 ml) containing potato dextrose broth and the culture was maintained at 30±2 ^{0}C in the laboratory. Pure fungal inoculum (ml) was inoculated in 200ml Erlenmeyer flasks containing 25 ml potato dextrose broth (PDB). The broth was prepared by dissolving 24 gm PDB in one litre distilled water followed by autoclave to ensure the complete eradication of microbes. The inoculated cultures were kept for growth and acclimatization for 72h under laboratory condition. After 72 h, 1-2 ml inoculants applied in freshly prepared PDB (25 ml). After 5 days of the treatment, cultures were centrifuged (3000 rpm for 10 min) and analyze for the enzymatic activity (Laccase, Lignin peroxidase and Mn peroxidase).

3.6.2. Results

3.6.2.1. Purine nucleoside derivatives and antifungal effect

The antifungal activity in term of minimum inhibitory concentration (MIC_{50}) in fungi Aspergillus sp.treated with different nucleoside derivates compound viz., **6b, 8f,**

8eand **6e**were exhibited a different degree of inhibition (50% reduction in growth) at different concentration. The nucleoside derivative **6b** exhibited the highest inhibition of fungal growth at the concentration of 0.021 µg/L showing its potent antifungal activity with *Aspergillus niger*. However, in the case of *A. stirus,* it was 0.042. Compounds **8e** in *Aspergillus niger* and **6e** in *A. stirus*expressed least inhibition of fungal growth at the concentration of 0.318 µg/L in both the fungus as compared to other compounds. Overall results showed that nucleoside derivative **6b** and **8f** showed comparatively high antifungal activity in comparison to other compounds.

3.6.2.2. Nucleoside derivatives and enzyme activity

The extracellular enzymes vi,z Laccase, MnP and LiP activity in fungi*A. niger* and *A stirus* were assayed. Maximum laccase activity was observed with compound **6b** in both fungi as compared to their respective control which was increased by 2.27 fold and 55% respectively. However, minimum laccase activity observed with **6e** in both the fungi. The fungal activity with nucleoside derivative **8f** and **8e** in *A. niger* was 11.93±1.01 U/ml and 14.09±1.11U/ml, respectively, while with *A. stirus*, it was 15.30±.69 and 12.22±.35 U/ml respectively.

The activity of enzyme manganese peroxidase was found to be least influenced in fungus *A. niger* treated with **8f**, however, maximum activity was observed in fungus treated with **6e** as compared to control. The fungal MnP activity against other nucleoside derivatives viz., **6b** and **8e** were in the order of 79.82±12.92 and 67.11±1.32, respectively with their control. In the case of fungus *A. stirus*, maximum activity was observed with **6e** (30.65%) followed by **6b** (29.41%), **8e** (18.19%) and **8f** (8.73%) in comparison to control.

In the case of enzyme lignin peroxidase, maximum activity was observed in both the fungi *A. niger* and *A. stirus* treated with nucleoside derivative **6b** in

comparison to control. The activity of fungi*A. niger* treated with other derivatives was in order of **6e** (18.04±0.45U/ml), **8f** (11.98±0.73U/ml) and **8e** (7.96±0.37U/ml) and with *A. stirus,* it was **6e** (16.01±0.33U/ml), **8f** (14.26±0.62U/ml) and **8e** (7.43±1.07 U/ml) in comparison to control.

3.6.2.3. Discussion

Infections caused by fungi have gained much attention in recent decades due to its huge impact on the emerging population of animal and wildlife[69, 70]. *Aspergillus* fungi and its pathogenicity are the most common known diseases in the world. The drug available for the treatment of *Aspergillus* laden disease falls into 4 categories pyrimidine, polyene echinocandin and azole drugs. Off these, azoles are the first preference drugs in the management and prophylaxis of aspergillosis[71]. In agriculture and biomedical science control of fungal invasion and their pathogenicity represents a serious confront towards to the increasing number of immune-compromised patients and antifungal resistant strains.

Antifungal activity in the present study against nucleoside derivative compounds demonstrates the degree of inhibition of growth by supplementation of the compound. MIC_{50} (50% inhibition in growth of the fungi) at the low dose of a compound represents the high degree of susceptibility and most effective towards mycotoxicity with lower survival rate and can be used as an antifungal agent. The nucleoside derivative **6b** showed maximum toxicity as the lower dose which may be ascribed to degradation of membrane lipid and protein, disruption of the cell wall and cell lysis [72].Similar results of antifungal activity against the compound of nucleoside derivatives were also represented by various authors [73-75].

Laccase, MnP and LiP are ligninolytic and extracellular oxidoreductase enzymes and have the ability to oxidizes a wide range of aromatic and non-aromatic

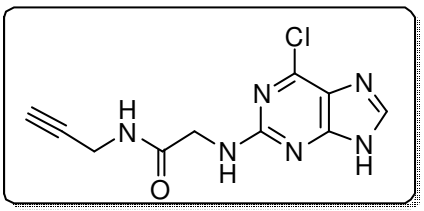
compounds through a chain reaction of radical oxidation [76]. Increase in extracellular enzyme activity (laccase, MnP and LiP) in fungi towards nucleoside compounds explains the oxidizing capacity of fungi to degrade the compound which results into decrease in the growth of fungus leading to the death of the fungi [77]. Degradation of compounds and subsequent production of intermediate and other compound causes cellular toxicity and death of the microorganisms [78, 79] reported that MnP covert the Mn^{2+} to very reactive Mn^{3+}, which in turn oxidizes a broad range of phenolic as well as non-phenolic substrates[80]. However, reduced activity of extracellular enzymes signifies that the substrate binds to fungi and adsorb on the cellular biomass with diminished antifungal activity.

Laccases are first ligninolytic enzymes secreted by the fungus to the surrounding environment and oxidize low molecular-weight organic compounds and capacity to produce phenoxy radicals as the mediators[81].Nucleoside compounds induced enhanced LiP and MnP activities in fungi *A. niger* and *A.stirus* in present study reveals the degradation of lignin present in the cell wall of fungi which leads to death of the fungi [82].

3.7. Experimental Section

3.7.1. General procedure for the preparation of 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-(prop-2-yn-1-yl) acetamide (3)

The solution containing 2-amino-6- chloro-1*H*-purine (1 equiv.) and NaH (1.5 equiv.) in DMF (30 mL) was stirred at room temperature for 2 hours under moisture-free conditions. 2-Chloro-N-propynyl-acetamide (1 equiv.) was then added and the reaction mixture was stirred at 80 °C for 10-12 hours. After the



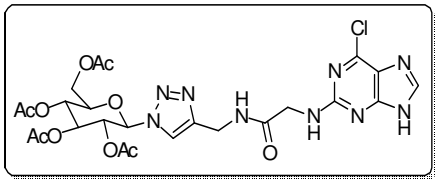
reaction was complete (on TLC), reaction mixture was evaporated and washed with ethyl acetate and water. The organic layer was separated and evaporated to dryness and the crude obtained was purified by column chromatography ($CHCl_3$-MeOH = 9:1) to give pure compound **3**as white solid. Yield 57%, 0.969 g, mp 109-110 $^{\circ}$C; R_f 0.6 (8:2, $CHCl_3$-MeOH); (c 0.1, MeOH); IR (ν_{max}, cm^{-1}): 3393, 2145, 1670, 1528, 1352, 1090, 733, 650.^{1}H NMR (400 MHz, DMSO-d_6):δ_H 8.73-8.71 (m, 1H, N-9),8.06 (s, 1H, C-8 H),6.91 (s, 2H),4.78 (s, 2H),3.92-3.90 (m, 2H),3.18-3.17 (t, J = 2.61 Hz, 1H),^{13}C NMR (100 MHz , DMSO-d_6):δ_C 166.5 (CO), 160.3 (Ar-C), 154.9 (Ar-C), 149.7 (Ar-C), 144.5 (Ar-C), 123.5 (Ar-C), 81.1, 73.9 (-OCH_2), 45.3, 28.6; HRMS: $[M+H]^+$.Calcd. Accurate mass for ($C_{10}H_9ClN_6O$): 347.0903. Found 347.0911

3.7.2. General procedure for the synthesis of nucleoside analogues 5a-5g:

The synthesized purine alkyne **3** (1 mmol) and the respective glycosyl azide (1 mmol) were taken in a 50 ml round bottomed flask containing tert-Butanol and water (1:1 ratio, 20 ml) and set to stir at room temperature. To the stirring reaction mixture was added sodium ascorbate (20 mol %) and $CuSO_4.5H_2O$ (10 mol %) one by one. The reaction mixture was stirred for 3-4 h till the completion of the reaction (TLC). The reaction mixture was then extracted with EtOAc and water. The organic layer was dried (anhd. Na_2SO_4) and evaporated under reduced pressure to give a crude mass. The latter was purified by silica gel (60-120 mesh) column chromatography using chloroform: methanol (9:1) as eluent to give the solid white compound titled below.

3.7.3.2-[{6-chloro-9*H*-purin-2-yl} amino] -*N*-[{1´-(1´´deoxy-2´´, 3´´, 4´´, 6´´- tetra-*O*-acetyl-β-D-glucopyranos-1´´yl)-1´H-1´,2´,3´- triazol-4´-yl}methyl]- acetamide (5a)

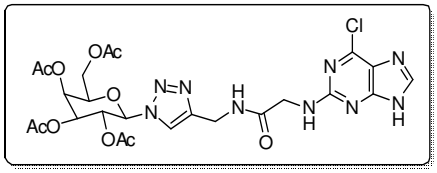
It was obtained by the reaction of alkyne **3** (0.5 g, 2.70 mmol) and glycosyl azide **I**1 g, 2.70 mmol) according to the procedure **3.7.2** as a white solid, yield 84%, 0.61 g,



mp 121-122°C;R_f 0.5 (9:1, Chloroform:methanol);IR (KBr, cm^{-1}) 3026, 1745, 1685, 1544, 740, 670.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.83-8.80 (m, 1H), 8.26 (s, 1H), 8.06 (s, 1H), 6.92 (s, 2H), 6.35 (d, J = 9.21 Hz, 1H), 5.68-5.64 (t, J = 9.35 Hz, 1H), 5.57-5.52 (t, J = 9.35 Hz, 1H), 5.20-5.16 (t, J = 9.76 Hz, 1H), 4.79 (s, 2H), 4.36-4.35 (m, 3H), 4.17-4.13 (m, 1H), 4.09-4.06 (m, 1H), 2.04-1.98 (m, 9H), 1.79 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 170.5, 170.0, 169.8, 168.9 (4 × -$COCH_3$), 166.6, 160.3 (-CO), 154.9 (Ar-C), 149.7 (Ar-C), 145.3 (Ar-C), 144.5 (Ar-C), 123.5 (Ar-C), 122.7 (Ar-C), 84.2 (C-1´´), 73.8 (C-5´´), 72.7 (C-3´´), 70.5 (C-4´´), 67.9 (C-2´´), 62.2 (C-6´´), 45.4 (-CH_2), 20.9, 20.8, 20.7, 20.4 (4 × -$OCOCH_3$); HRMS: $[M+H]^+$.Calcd. Accurate mass for ($C_{24}H_{28}ClN_9O_{10}$): 638.1648. Found 638.1674

3.7.4. 2-[{6-chloro-9*H*-purin-2-yl} amino] -*N*-[{1´-(1´´deoxy-2´´, 3´´, 4´´, 6´´- tetra-*O*-acetyl-β-D-galactopyranos-1´´yl)-1´H-1´,2´,3´- triazol-4´-yl}methyl]- acetamide (5b)

It was obtained by the reaction of alkyne **3** (0.5 g, 2.70 mmol), and glycosyl azide **II** (1 g, 2.70 mmol) according to the procedure **3.7.2**as a white solid, yield 86%, 0.62 g,

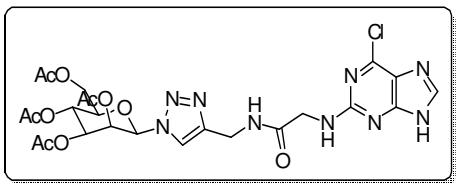


mp 122-123 °C;R_f 0.5 (9:1, Chloroform:methanol);IR (KBr, cm^{-1}) 3150, 1752, 1690, 1560, 756.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.81-8.79 (m, 1H), 8.17 (s, 1H), 8.06 (s, 1H), 6.91 (s, 2H), 6.26 (d, J = 9.35 Hz, 1H), 5.64-5.59 (t, J = 9.35 Hz, 1H), 5.47-5.43 (m, 2H), 4.80 (s, 2H), 4.59-4.56 (t, J = 5.69 Hz, 1H), 4.37 (d, J = 5.39 Hz, 2H), 4.16 (dd, J_1 = 5.10 Hz, J_2 = 11.69

Hz, 1H), 4.04 (dd, J_1 = 4.35 Hz, J_2 = 11.69 Hz, 1H), 2.19 (s, 3H), 2.00 (s, 3H), 1.95 (s, 3H), 1.82 (s, 3H), ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 170.4, 170.3, 169.9, 169.0 (4 × -$COCH_3$), 166.6, 160.3 (-CO), 154.9 (Ar-C), 149.7 (Ar-C), 145.2 (Ar-C), 144.5 (Ar-C), 123.5 (Ar-C), 122.9 (Ar-C), 84.6 (C-1´´), 73.4 (C-5´´), 70.9 (C-3´´), 68.1 (C-4´´), 67.8 (C-2´´), 62.0 (C-6´´), 45.4 (-CH_2), 20.9, 20.8, 20.7, 20.4 (4 × -$OCOCH_3$); HRMS: $[M+H]^+$ Calcd. Accurate mass for ($C_{24}H_{28}ClN_9O_{10}$): 638.1648. Found 638.1696.

3.7.5.2-[{6-chloro-9*H*-purin-2-yl} amino] -*N*-[{1´-(1´´deoxy-2´´, 3´´, 4´´, 6´´- tetra-*O*-acetyl-β-D-mannopyranos-1´´yl)-1´H-1´,2´,3´- triazol-4´-yl}methyl]- acetamide (5c)

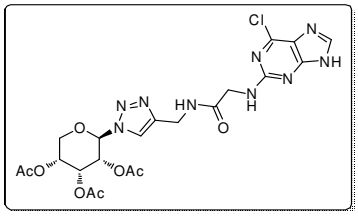
It was obtained by the reaction of alkyne **3**(0.5 g, 2.70 mmol)and glycosyl azide **III**(0.77 g, 2.70mmol) according to the procedure **3.7.2** as a white solid, yield 75%,



0.24 g, mp120-122°C;R_f 0.5 (9:1, Chloroform:methanol);IR (KBr, cm^{-1}) 3156, 3026, 1755, 1670, 1580, 745, 650.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.85-8.82 (m, 1H), 8.16 (s, 1H), 8.06 (s, 1H), 6.92 (s, 2H), 6.43 (s, 1H), 5.83 (dd, J_1 = 3.80 Hz, J_2 = 9.43 Hz, 1H), 5.71 (m, 1H), 5.28-5.23 (t, J = 9.43 Hz,1H), 4.80 (s, 2H), 4.40-4.35 (m, 2H), 4.25 (dd, J_1 = 5.05 Hz, J_2 = 12.63 Hz, 1H), 4.02 (dd, J_1 = 2.16 Hz, J_2 = 12.51 Hz, 1H), 3.89-3.86 (m, 1H), 2.16 (s, 3H), 2.05 (s, 3H), 1.99 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 170.5, 170.4, 169.9, 169.8 (4 × -$COCH_3$), 166.7, 160.2 (-CO), 154.8 (Ar-C), 149.7 (Ar-C), 145.3 (Ar-C), 144.5 (Ar-C), 124.6 (Ar-C), 123.5 (Ar-C), 83.1 (C-1´´), 71.8 (C-5´´), 68.8 (C-3´´), 68.1 (C-4´´), 65.7 (C-2´´), 61.9 (C-6´´), 45.4 (-CH_2), 20.98, 20.9, 20.88, 20.8 (4 × -$OCOCH_3$); HRMS: $[M+H]^+$ Calcd. Accurate mass for ($C_{24}H_{28}ClN_9O_{10}$): 638.1648. Found 638.1466.

3.7.6. 2-[{6-chloro-9*H*-purin-2-yl} amino] -*N*-[{1´-(1´´deoxy-2´´, 3´´, 4´´, 6´´- tetra-*O*-acetyl-β-D-ribopyranos-1´´yl)-1´H-1´,2´,3´- triazol-4´-yl}methyl]- acetamide (5d)

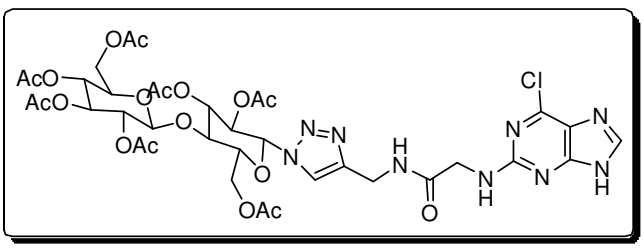
It was obtained by the reaction of alkyne **3** (0.5 g, 2.70 mmol) and glycosyl azide **IV** (0.58 g, 2.70 mmol) according to the procedure **3.7.2** as a white solid, yield 75%, 0.24



g, mp 119-121 °C;R_f 0.5 (9:1, Chloroform:methanol);IR (KBr, cm^{-1}) 3140, 1739, 1644, 1565, 1120, 760, 630.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.83-8.81 (m, 1H), 8.22 (s, 1H), 8.06 (s, 1H), 6.93 (s, 2H), 6.14 (d, J = 9.11 Hz, 1H), 5.72 (s, 1H), 5.67 (dd, J_1 = 2.78 Hz, J_2 = 9.06 Hz, 1H), 5.21-5.17 (m, 1H), 4.79 (s, 2H), 4.36-4.35 (m, 2H), 4.04-3.94 (m, 2H), 2.19 (s, 3H), 2.01 (s, 3H), 1.82 (s, 3H), . ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 170.3, 169.7, 169.1 (3 × -$COCH_3$), 166.6, 160.3 (-CO), 154.9 (Ar-C), 149.7 (Ar-C), 145.3 (Ar-C), 144.5 (Ar-C), 123.5 (Ar-C), 82.7 (C-1´´), 71.5 (C-5´´), 68.3 (C-3´´), 67.5 (C-4´´), 63.2 (C-2´´),45.4 (-CH_2), 20.9,20.7, 20.5 (3 × -$OCOCH_3$); HRMS: $[M+H]^+$ Calcd. Accurate mass for ($C_{21}H_{24}$ ClN_9O_8): 566.1436. Found 566.1478.

3.7.7. 2-[{6-chloro-9*H*-purin-2-yl} amino] -*N*-[{1´-(1´´deoxy-2´´, 3´´, 4´´, 6´´- tetra-*O*-acetyl-β-D-cellobios-1´´yl)-1´H-1´,2´,3´- triazol-4´-yl}methyl]- acetamide (5e)

It was obtained by the reaction of alkyne **3** (0.5 g, 2.70 mmol) and glycosyl azide

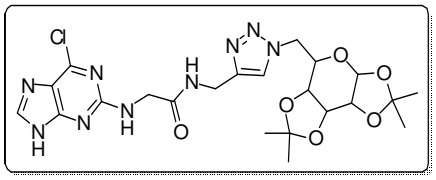


V(0.69 g, 2.70 mmol)according to the procedure **3.7.2** as a white solid, yield 81%, 0.24 g, mp 121-122 °C; R_f 0.5 (9:1, Chloroform:methanol);IR (KBr, cm^{-1}) 3103, 2993, 1742, 1684, 1575, 1020, 739.^{1}H

NMR (400 MHz, DMSO-d_6): δ_H 8.81-8.79 (m, 1H), 8.15 (s, 1H), 8.05 (s, 1H), 6.92 (s, 2H), 6.25 (d, J = 9.16 Hz, 1H), 5.55-5.51 (t, J = 9.32 Hz,1H), 5.45-5.41 (t, J = 9.44 Hz,1H), 5.32-5.27 (t, J = 9.44 Hz,1H), 4.94-4.89 (m, 2H), 4.78 (s, 2H), 4.70-4.66 (m, 1H), 4.41-4.33 (m, 3H), 4.28-4.20 (m, 2H), 4.08-3.95 (m, 4H), 2.06 (s, 3H), 2.03 (s, 3H), 1.99-1.98 (m, 9H), 1.93 (s, 3H), 1.78 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 170.7, 170.5, 170.1, 169.9, 169.6, 169.5, 169.0 (7 × -$COCH_3$) , 166.6, 160.3 (-CO), 154.8 (Ar-C), 149.7 (Ar-C), 145.2 (Ar-C), 144.5 (Ar-C), 123.5 (Ar-C), 122.7 (Ar-C), 100.1 (Ar-C), 84.1 , 79.6 (C-1´´), 76.4 , 74.9 (C-5´´), 72.6 71.6, (C-3´´), 70.9 70.6 (C-4´´) , 68.2, 62.7 (C-2´´), 62.0, (C-6´´), 45.4 (-CH_2), 34.7, 21.1, 20.9, 20.8, 20.7, 20.6, 20.4 (7 × -$OCOCH_3$); HRMS: $[M+H]^+$ Calcd. Accurate mass for ($C_{36}H_{44}$ ClN_9O_{18}): 926.2493. Found 926.2499.

3.7.8. 2-[{6-chloro-9*H*-purin-2-yl} amino] -*N*-[{1´-(6´´deoxy-1´´, 2´´, 3´´, 4´´- di-*O*-isopropylidene-α-D-galactopyranos-6´´-yl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]acetamide (5f)

It was obtained by the reaction of alkyne **3** (0.5 g, 2.70 mmol) and glycosyl azide **VI** (0.63 g, 2.70 mmol) according to the procedure **3.7.2**. as a white solid, yield 85%,

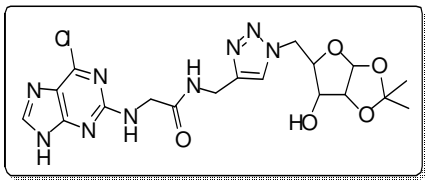


0.24 g, mp 121-122 °C; R_f 0.5 (9:1, Chloroform:methanol);IR (KBr, cm^{-1}) 3065, 1682, 1534, 1038, 758, 623.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.81-8.78 (m, 1H), 8.05 (1H, s, H-5´), 7.92 (s, 1H), 6.91 (s, 2H), 5.41 (1H, d, J = 4.86 Hz, H-6), 4.78 (s, 2H), 4.68 (dd, J_1 = 2.34 Hz, J_2 = 7.86 Hz, 1H), 4.58 (dd, J_1 = 2.97 Hz, J_2 = 14.14 Hz, 1H), 4.41-4.36 (m, 2H), 4.34-4.31 (m, 3H), 4.22-4.20 (m, 1H), 1.42 (3H, s, CH_3), 1.33-1.32 (6H, s, CH_3), 1.23 (3H, s, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 166.5 (-CO), 160.2 (Ar-C), 154.8 (Ar-C), 149.6 (Ar-C),

144.5 (Ar-C), 144.3 (Ar-C), 124.0 (Ar-C), 123.5 (Ar-C), 109.2 (C-1´´), 108.5 (C-4´´), 95.9 (C-3´´), 71.0 (C-2´´), 70.6 (C-5´´), 70.1, 67.2 (C-6´´), 50.5, 45.4, 34.8, 26.4, 26.1, 25.3, 24.7; HRMS: $[M+H]^+$ Calcd. Accurate mass for ($C_{22}H_{28}$ ClN_9O_6): 550.1851. Found 550.1892.

3.7.9. 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-[{1´-(5´´deoxy-1´´,2´´-*O*-isopropylidene-α-D-xylofuranos-5´´-yl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]-acetamide (5g)

It was obtained by the reaction of alkyne **3** (0.5 g, 2.70 mmol) and glycosyl azide **VII** (0.63 g, 2.70 mmol) according to the procedure **3.7.2** as a white solid, yield 88%, 0.32 g, mp 121-122 °C; R_f 0.5 (9:1, Chloroform:methanol);IR (KBr, cm^{-1}) 3026, 1752, 1644, 730.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.79-8.77 (1H, brs, NH), 8.06 (1H, s, H-5´), 7.97 (s, 1H) , 6.91 (s, 2H), 5.88 (d, J = 3.53 Hz, 1H H-6), 5.64 (d, J = 4.77 Hz, 1H H-5), 4.79 (2H, m, H-1´´, H-3´´), 4.62 (dd, J_1 = 3.52 Hz, J_2 = 13.98 Hz, 1H), 4.49-4.44 (2H, m, H-6´),, 4.39-4.34 (3H, m, H-4´´, H-5´´), 4.08 (1H, m, OH), 1.34 (3H, s, CH_3), 1.23 (3H, s, CH_3); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 166.5 (-CO), 160.2 (Ar-C), 154.8 (Ar-C), 149.6 (Ar-C),, 144.5 (Ar-C), 124.1, 123.5 (Ar-C), 111.2 (Ar-C), 104.9 (Ar-C), 85.4 (C-1´´), 79.6 (C-5´´), 74.1 (C-3´´), 49.4 (C-4´´), 45.4 (C-2´´), 34.8, 27.1, 26.4; HRMS: $[M+H]^+$ Calcd. Accurate mass for ($C_{18}H_{22}$ ClN_9O_5): 480.1432. Found 480.1444.



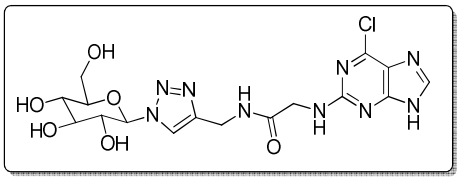
3.7.10. General procedure for the synthesis of deacetylated nucleoside analogues 6a-6e:

The respective acetylated nucleoside analogue (**5a**-**5e**) was mixed with NaOMe (a pinch of sodium metal in methanol) in methanol and the stirring continued till the

reaction was completed (30 min, TLC). After completion the reaction mixture was neutralized by Amberlite IR-120-H^+ ion-exchange resin, followed by filtration (the resin is washed several times with methanol) and evaporation up to dryness, afforded the titled compound.

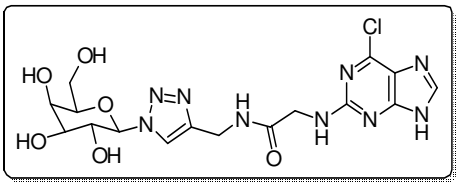
3.7.11. 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-[{1´-(β-D-glucopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]- acetamide (6a)

It was obtained by treating the acetylated compound **5a** (0.2 g, 0.31 mmol) with NaOMe in methanol, to give the fully deacetylated compound **6a** (0.12 g, 81%)according to the procedure **3.7.10** as a white solid, mp 121-123 °C; R_f 0.5 (5:5, Hexane-EtOAc); IR (ν_{max}, cm^{-1}):3368, 3058, 1690, 1611 1522, 1226, 694.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.83-8.80 (m, 1H), 8.13-8.09 (d, *J* = 14.98 Hz, 2H), 5.53-5.51 (d, *J* = 9.31 Hz, 1H), 4.80 (s, 2H), 4.37-4.36 (m, 2H), 3.78-3.68 (m, 6H), 3.45-3.37 (m, 4H), 3.25-3.21 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 166.6 (-CO), 160.2 (Ar-C), 155.0 (Ar-C), 149.7 (Ar-C), 144.6 (Ar-C), 123.6 (Ar-C), 122.7 (Ar-C), 87.8 (C-1´´), 80.4 (C-5´´), 77.5 (C-3´´), 72.5 (C-4´´), 70.0 (C-2´´), 61.2 (C-6´´), 45.4 (-CH_2), 34.7; HRMS: $[M+H]^+$ Calcd. Accurate mass for ($C_{16}H_{20}ClN_9O_6$): 470.1225. Found 470.1234.



3.7.12. 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-[{1´-(β-D-galactopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]- acetamide (6b)

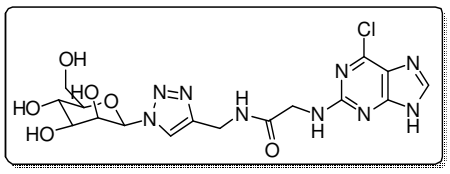
It was obtained by treating the acetylated compound **5b** (0.2 g, 0.27 mmol) with NaOMe in methanol, to give the fully deacetylated compound **6b** (0.13 g, 84%) according to the procedure **3.7.10** as a white solid, mp 121-122°C; R_f 0.5 (5:5, Hexane-EtOAc); IR (ν_{max}, cm^{-1}):3420, 1695 (C=O),



1640, 1524, 1026, 762, 664.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.83-8.80 (m, 1H), 8.09-8.08 (m, 2H), 5.49 (d, J = 9.28 Hz, 1H), 4.81 (s, 2H), 4.38-4.36 (m, 2H), 4.04-3.99 (m, 5H), 3.77-3.69 (m, 3H), 3.56-3.45 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 166.5 (-CO), 160.2 (Ar-C), 154.9 (Ar-C), 149.7 (Ar-C), 144.7 (Ar-C), 123.5 (Ar-C), 122.3 (Ar-C), 88.5 (C-1´´), 78.8 (C-5´´), 74.2 (C-3´´), 69.8 (C-4´´), 68.8 (C-2´´), 60.8 (C-6´´), 45.4 (-CH_2), 34.8; HRMS: $[M+H]^+$, Calcd. Accurate mass for ($C_{16}H_{20}ClN_9O_6$): 470.1225. Found 470.1297.

3.7.13. 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-[{1´-(β-D-mannopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl] acetamide (6c)

It was obtained by treating the acetylated compound **5c** (0.2 g, 0.29 mmol) with NaOMe in methanol, to give the fully deacetylated compound **6c** (0.115 g, 90%)

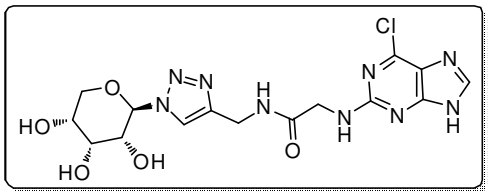


according to the procedure **3.7.10** as a white solid, mp 123-124°C; R_f 0.5 (5:5, Hexane-EtOAc); IR (ν_{max}, cm^{-1}):3380, 2995, 1689, 1585 1105, 794, 625.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.83-8.81 (m, 1H), 8.09-8.08 (m, 2H), 5.90 (d, J = 4.23 Hz, 1H), 4.80 (s, 2H), 4.38-4.37 (m, 4H), 4.01 (s, 2H), 3.87-3.85 (m, 3H), 3.64-3.54 (m, 4H), 3.39-3.35 (m, 1H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 166.5 (-CO), 160.2 (Ar-C), 154.8 (Ar-C), 149.6 (Ar-C), 144.6 (Ar-C), 144.5 (Ar-C), 122.5 (Ar-C), 123.3 (Ar-C), 86.0 (C-1´´), 78.8 (C-5´´), 71.6 (C-3´´), 68.6 (C-4´´), 68.1 (C-2´´), 61.1 (C-6´´), 45.4 (-CH_2), 34.7; HRMS: $[M+H]^+$, Calcd. Accurate mass for ($C_{16}H_{20}ClN_9O_6$): 470.1225. Found 470.1231.

3.7.14. 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-[{1´-(β-D-ribopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]- acetamide (6d)

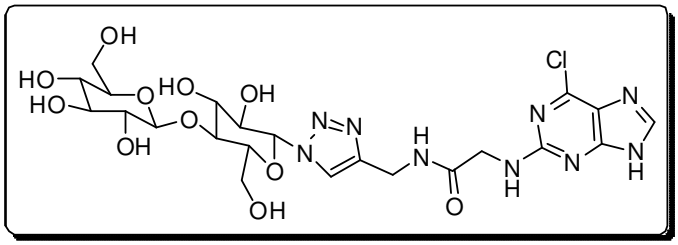
It was obtained by treating the acetylated compound **5d** (0.2 g, 0.3 mmol) with NaOMe in methanol, to give the fully deacetylated compound **6d** (0.125 g, 83%)

according to the procedure **3.7.10** as a white solid, mp 122-123 °C; R_f 0.5 (5:5, Hexane-EtOAc); IR (ν_{max}, cm^{-1}):3405, 3060, 1678, 1590, 1125, 764, 640.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.84-8.81 (t, J = 5.34 Hz,1H), 8.10 (d, J = 5.11 Hz, 2H), 5.63 (d, J = 9.17 Hz, 1H), 4.79 (s, 2H), 4.36-4.35 (d, J = 5.34 Hz, 2H), 4.02-3.97 (m, 4H), 3.70-3.65 (m, 4H), 3.61-3.58 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 166.5 (-CO), 160.3 (Ar-C), 154.8 (Ar-C), 149.6 (Ar-C), 144.5 (Ar-C), 123.4 (Ar-C), 122.7 (Ar-C), 85.3 (C-1´´), 71.5 (C-5´´), 69.6 (C-3´´), 66.9 (C-4´´), 65.6 (C-2´´), 45.4 (-CH_2), 34.7; HRMS: $[M+H]^+$, Calcd. Accurate mass for ($C_{15}H_{18}ClN_9O_5$): 440.1119. Found 440.1148.



3.7.15. 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-[{1´-(β-D-cellobiopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]- acetamide (6e)

It was obtained by treating the acetylated compound **5e** (0.2 g, 0.3 mmol) with NaOMe in methanol, to give the fully deacetylated compound **6e** (0.116 g, 77%) according to the procedure **3.7.10** as a white solid, mp 119-120°C; R_f 0.5 (5:5, Hexane-EtOAc); IR (ν_{max}, cm^{-1}):3353, 3043, 1696, 1597, 1059, 776, 681.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.86-8.83 (m, 1H), 8.13-8.10 (m, 2H), 5.63 (d, J = 9.37 Hz, 1H), 4.80 (s, 2H), 4.37-4.32 (m, 9H), 3.86-3.82 (t, J = 9.06 Hz, 2H), 3.78-3.72 (m, 3H), 3.65-3.55 (m, 4H), 3.52-3.39 (m, 2H), 3.26-3.17 (m, 2H), 3.10-3.01 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C166.5 (C=O), 160.3 (Ar-C), 154.8 (Ar-C), 149.6 (Ar-C), 144.6 (Ar-C), 144.5 (Ar-C), 123.4 (Ar-C), 122.6 (Ar-C), 103.6 (Ar-C), 87.4, 80.1 (C-1´´), 78.2, 77.3 (C-5´´), 76.9, 75.7



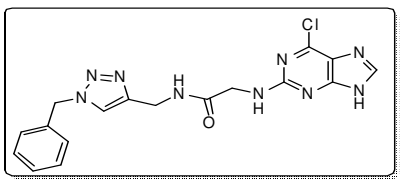
(C-3´´), 73.8, 72.2 (C-4´´), 70.5, 61.5 (C-2´´), 60.4 (C-6´´), 45.4 (-CH_2), 34.7; HRMS: $[M+H]^+$, Calcd. Accurate mass for ($C_{22}H_{30}ClN_9O_{11}$): 632.1753. Found 632.1788.

3.7.16. General procedure for the synthesis of different substituted benzyl-triazolyl-purinyl aminoacetamide 8a-8g:

To the mixture of Purine alkyne **3** (1 mmol) and the corresponding benzyl azide **7a-g** (1 mmol) in *t*-butanol+water (1:1, 20 mL), $CuSO_4.5H_2O$ (10 mol %) and Na-ascorbate (20 mol %)was added followed by stirring at room temperature up to completion of reaction (usually 3-4 h). After completion (monitored by TLC), the reaction mixture was extracted with ethyl acetate and washed with water and then brine solution. The organic layer was dried (anhd. Na_2SO_4) and evaporated under reduced pressure yielding oily mass. The latter was purified by silica gel (60-120 mesh) column chromatography using chloroform: methanol (9:1) as elutant to give the solid white compound titled below.

3.7.17. 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-((1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (8a)

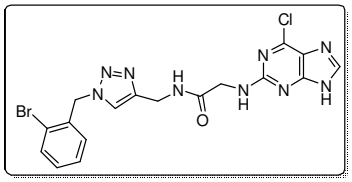
It was obtained by the reaction of alkyne **3** (0.2 g, 0.35 mmol) and benzyl azide **7a** (0.035 ml, 0.35 mmol) according to the procedure **3.7.15** as a white solid, yield 83%,



0.25 g, mp 120-122 °C; R_f 0.5 (9:1, Chloroform:methanol);IR (KBr, cm^{-1}) 3123, 2920, 1682, 1564, 760.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.73 (S, 1H), 8.04 (s, 1H), 7.98 (s, 1H), 7.38 (m, 5H), 6.91 (s, 2H), 5.57 (s, 2H), 4.76 (s, 2H), 4.33 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 166.5, 160.2, 154.8, 149.7, 144.9, 144.5, 136.5, 129.2 (2C), 128.6, 128.4 (2C), 123.6, 123.5, 53.2, 45.4, 34.8; HRMS: $[M+H]^+$, Calcd. Accurate mass for ($C_{17}H_{16}ClN_9O$): 398.1166. Found 398.1194.

3.7.18. 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-((1-(2-bromobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (8b)

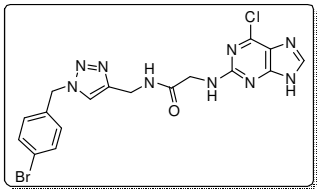
It was obtained by the reaction of alkyne **3** (0.2 g, 0.35 mmol) and benzyl azide **7b** (0.08 g, 0.35 mmol) according to the procedure **3.7.15** as a white solid, yield73%,



0.33 g, mp 121-122 °C; R_f 0.5 (9:1, Chloroform:methanol);IR (KBr, cm^{-1}) 3076, 1687, 1534, 830, 754.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.78-8.75 (m, 1H), 8.05 (s, 1H), 7.98 (s, 1H), 7.71 (dd, J_1 = 1.11 Hz, J_2 = 7.91 Hz, 1H), 7.43-7.39 (m, 1H), 7.34-7.30 (m, 1H), 7.18 (dd, J_1 = 1.62 Hz, J_2 = 7.62 Hz, 1H), 6.92 (s, 2H), 5.67 (s, 2H), 4.78 (s, 2H), 4.36-4.35 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 166.5, 160.2, 154.8, 149.7, 144.8, 144.5, 135.4, 133.3, 130.9, 130.8, 128.7, 124.0, 123.5, 123.3, 53.3, 45.4, 34.8; HRMS: $[M+H]^+$, Calcd. Accurate mass for ($C_{17}H_{15}BrClN_9O$): 476.0271. Found 476.0298.

3.7.19. 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-((1-(4-bromobenzyl)-1*H*-1,2,3-triazol-4-yl) methyl) acetamide (8c)

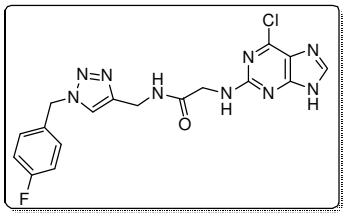
It was obtained by the reaction of alkyne **3** (0.2 g, 0.35 mmol) and benzyl azide **7c** (0.063 g, 0.35 mmol) according to the procedure **3.7.15** as a white solid, yield77%,



0.28 g, mp 122-123 °C; R_f 0.5 (9:1, Chloroform:methanol);IR (KBr, cm^{-1}) 3103, 2953, 1686, 1574, 802, 755.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.74 (S, 1H), 8.05 (s, 1H), 7.99 (s, 1H), 7.58 (d, J = 7.96 Hz, 2H), 7.28 (d, J= 7.96 Hz, 2H), 6.92 (s, 2H), 5.56 (s, 2H), 4.77 (s, 2H), 4.33 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 166.5, 160.2, 155.0, 149.7, 145.0, 144.6, 135.9, 132.1, (2C), 130.7 (2C), 123.6, 121.9, 52.4, 45.4, 34.8; HRMS: $[M+H]^+$, Calcd. Accurate mass for ($C_{17}H_{15}BrClN_9O$): 476.0271. Found 476.0343.

3.7.20. 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-((1-(4-fluorobenzyl)-1*H*-1,2,3-triazol-4-yl) methyl) acetamide (8d)

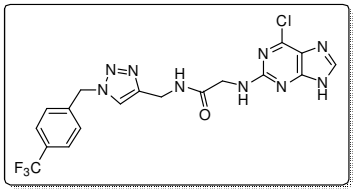
It was obtained by the reaction of alkyne **3** (0.2 g, 0.35 mmol) and benzyl azide **7d** (0.06 g, 0.35 mmol) according to the procedure **3.7.15** as a white solid, yield74%,



0.23 g, mp 118-119°C; R_f 0.5 (9:1, Chloroform:methanol);IR (KBr, cm^{-1}) 3108, 2904, 1692, 1554, 820, 739.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.75-8.73 (m, 1H), 8.06 (s, 1H), 7.99 (s, 1H), 7.41-7.37 (m, 2H), 7.23-7.19 (m, 2H), 6.93 (s, 2H), 5.57 (s, 2H), 4.77 (s, 2H), 4.34-4.32 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 166.6, 163.5, 161.1, 160.2, 149.8, 145.1, 132.6, 132.5, 130.8 (2C), 130.7, 123.5, 116.1, 115.9, 52.5, 45.4, 34.8; HRMS: $[M+H]^+$, Calcd. Accurate mass for ($C_{17}H_{15}ClFN_9O$): 416.1072. Found 416.1092.

3.7.21. 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-((1-(4-(trifluoromethyl) benzyl)-1*H*-1,2,3-triazol-4-yl) methyl) acetamide (8e)

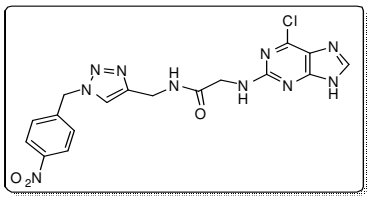
It was obtained by the reaction of alkyne **3** (0.2 g, 0.35 mmol) and benzyl azide **7e** (0.058 g, 0.35 mmol) according to the procedure **3.7.15** as a white solid, yield 80%,



.28 g, mp 120-121 °C; R_f 0.5 (9:1, Chloroform:methanol);IR (KBr, cm^{-1}) 3126, 2965, 1678, 1534, 810, 743.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.75-8.73 (m, 1H), 8.05-8.03 (m, 2H), 7.46 (d, J = 8.48 Hz, 2H), 7.39 (d, J = 8.35 Hz, 2H), 6.92 (s, 2H), 5.63 (s, 2H), 4.77 (s, 2H), 4.34 (d, J= 5.31 Hz, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 166.6, 160.2, 154.8, 149.7, 148.5, 145.0, 144.5, 135.9, 130.5 (3C), 123.7, 123.5, 121.8 (2C), 52.3, 45.4, 34.8; HRMS: $[M+H]^+$, Calcd. Accurate mass for ($C_{18}H_{15}ClF_3N_9O$): 466.1040. Found 466.1086.

3.7.22. 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-((1-(4-nitrobenzyl)-1*H*-1,2,3-triazol-4-yl) methyl) acetamide (8f)

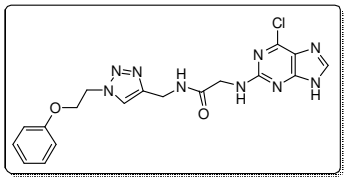
It was obtained by the reaction of alkyne **3** (0.2 g, 0.35 mmol) and benzyl azide **7f** (0.064 g, 0.35 mmol) according to the procedure **3.7.15** as a white solid, yield71%,



0.235 g, mp 119-120 °C; R_f 0.5 (9:1, Chloroform:methanol);IR (KBr, cm^{-1}) 3159, 2985, 1691, 1572, 1324, 750, 657.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.78-8.75 (m, 1H), 8.25 (d, J = 8.44 Hz, 2H), 8.07-8.05 (m, 2H), 7.54 (d, J = 8.44 Hz, 2H), 6.92 (s, 2H), 5.77 (s, 2H), 4.78 (s, 2H), 4.36-4.35 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 166.6, 160.2, 154.8, 149.7, 147.7, 145.2, 144.5, 143.9, 129.5 (2C), 124.3 (2C), 124.0, 123.5, 52.3, 45.5, 34.8; HRMS: $[M+H]^+$, Calcd. Accurate mass for ($C_{17}H_{15}ClN_{10}O_3$): 443.1017. Found 443.1044.

3.7.23. 2-[{6-chloro-9*H*-purin-2-yl} amino]-*N*-((1-(2-phenoxyethyl)-1*H*-1,2,3-triazol-4-yl) methyl) acetamide (8g)

It was obtained by the reaction of alkyne **3** (0.2 g, 0.35 mmol) and benzyl azide **7g**



(0.058 g, 0.35 mmol) according to the procedure **3.7.15** as a white solid, yield 75%, 0.24 g, mp 122-123°C; R_f 0.5 (9:1, Chloroform:methanol);IR (KBr, cm^{-1}) 3145, 2948, 1699, 1602, 1270, 768, 674.^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.77 (S, 1H), 8.09 (s, 1H), 8.03 (s, 1H), 7.29-7.26 (t, J = 7.74 Hz, 2H), 6.96-6.90 (m, 5H), 4.78 (s, 2H), 4.76-4.73 (t, J = 4.81 Hz, 2H), 4.38-4.34 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 166.5, 160.3, 158.2, 155.1, 149.8, 144.8, 130.0 (3C), 124.0, 121.5, 115.0 (3C), 66.5, 49.5, 45.5, 34.8; HRMS: $[M+H]^+$, Calcd. Accurate mass for ($C_{18}H_{18}ClN_9O_2$): 428.1272. Found 428.1298.

3.8. Biological evaluation:

3.8.1. Materials and Methods:

All the experiments were performed in the laboratory of DES, BBAU,Lucknow. After 72h of growth, stock solution of different salt viz.**6b**, **6e**, **8e** and **8f**were prepared (17 mg/ml). The stock solution of the each salts were prepared in dimethyl sulphoxide solvent. 1µL, 2 µL, 5 µL, 10 µL, 15µL and 20 µL of salts (stock solution)of different concentration were applied separately in each flask containing 25 ml nutrient broth to analyze the antifungal and enzyme activity of the compounds. A control devoid of salts was served as control.

3.8.2. Antifungal activity

The growth of microorganism were measured by measuring optical density (OD) after 24 h, 48 h and 72 h of the growthwith the help of spectrophotometer of wavelength of 600 nm. Antifungal activity in the form of percentage inhibition (Inhibitory concentration; IC_{50}) was analyzed on the basis of % decrease in density of treated fungal culture as compared to control.

3.8.3. Preparation of crude enzymes extract:

The crude extract to access the activities of different cellular enzymes were prepared by homogenizing the fungal biomass in sodium potassium tartrate buffer (pH-5.0). After homogenization, samples were centrifuged at 10000 rpm for 10 min in control laboratory conditions. The supernatant obtained were used for analysis of enzyme activity.

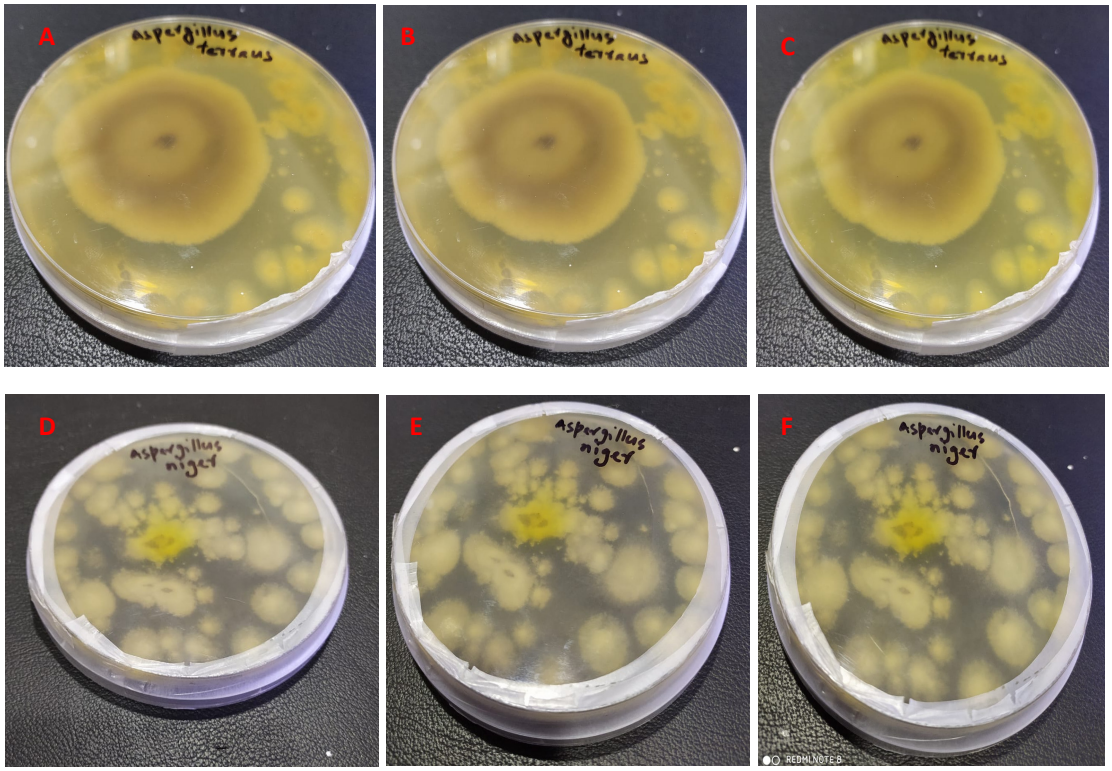


Figure 11. Culture plate of fungi *A. niger* **(A-C)** and *A. terreus* **(D-F)**

3.8.4. Laccase activity

Laccase activity was assayed by adding 1 ml of 1 mM of guaiacol prepared in o.5 mM of sodium phosphate buffer (pH 6.0) to 1 ml of the crude enzyme [83]. Mix the sample and incubate at 30 ℃ for 10 min. The absorption was recorded at the wavelength of 495nm.

3.8.5. MnP activity

MnP (MnP; EC 1.11.1.13) activity in fungus *Aspergillus niger* and *Aspergillus terreus*were analyzed by following the protocol of Arora and Gill 2005[84]300 µl crude enzyme was mixed with a reaction mixture containing 0.1 ml of 1mM $MnSO_4$, 0.1 ml of 1mM H_2O_2 and 0.25 ml of 1mM guaiacol. After the mixing, incubate for the 5 minutes in room temperature. The MnP activity was assayed by reading the absorbance at 465 nm using a spectrophotometer.

3.8.6. LiP activity

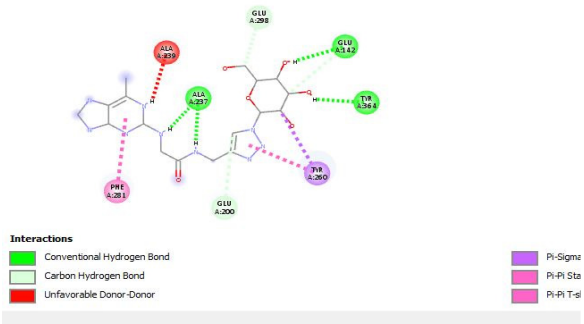
LiP (EC 1.11.1.14) activity in fungus *Aspergillus niger* and *Aspergillus terreus* were analyzed by following the methods ofMagalhães, 1996, Bholay, 2012 [85, 86]. 100µl of the crude enzyme was mixed with a reaction mixture containing 1.5 ml of 0.1mM sodium tartrate buffer (pH 2.5), 0.5 ml of 2mM veratryl alcohol and 0.2 ml of 0.4 mm H_2O_2. The mixture was allowed to stand at room temperature for the 10 minutes and the absorption was recorded at 310 nm.

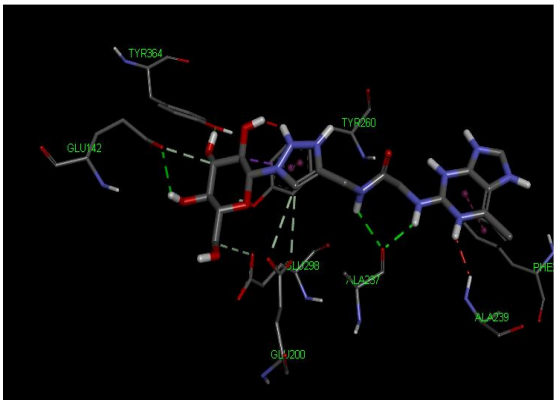
3.9. Molecular docking study

Active sites on the *β*-galactosidase(PDB Id: 5ifp) is determined using online tool CASTp3.0. Active site A: 200 as proton donor and site A: 298 as nucleophile respectively. Docking energy calculated with Autodock 4.2.6, best-fit docked model with lowest minimum binding energy of (ΔG) has been chosen to reveal the molecular interaction between our design compounds and *β*-galactosidase. Molecular docking study have found that compound **6b, 8f, 8e** and **6e** binding energy is calculated -9.4, -9.4, -9.1 and –9.3 kcal/mol, whereas, Interaction between active site of β-galactosidase with designed molecule, **6b** was interacted (ALA) 339, (GLU) 298, (GLU) 142, (TYR) 364, (TYR) 260, (GLU) 200, (PHE) 281, (ALA) 297; **8f** was interacted (GLU) 142, (TYR) 260, (PHE) 281, (PRO) 261, (Pro) 278, (SER) 238, (GLU) 200, (GLU) 298; **8e** was (GLU) 275, (PRO) 278, (ASP) 276, (GLU) 200, (GLU) 298, (TYR) 260, (GLU) 142, (TYR) 806,(SER) 238,(PHE) 281and **6e** (ALA) 259, (HIS) 257, (ASP) 258, (SER) 238, (GLU) 200, (GLU) 298, (GLU) 142, (TYR) 260, (GLY) 275, (ASN) 280. (Figure) The other surrounding amino acids depicted hydrophobic, Pi-Pi, Pi-Sigma interaction with **6b,8f, 8e** and **6e**. Therefore, *in silico* molecular docking results are promised with the results obtained *in vitro* study.

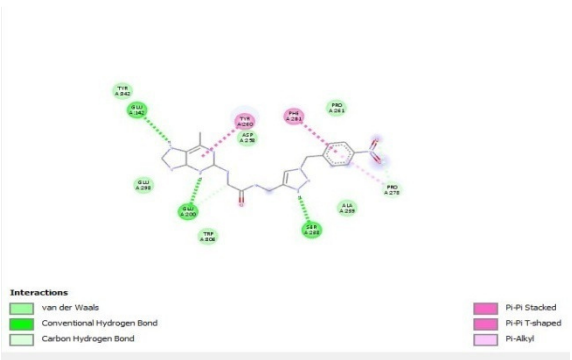
Table 4: Analysis of the interaction between β-galactosidase and synthesized molecules

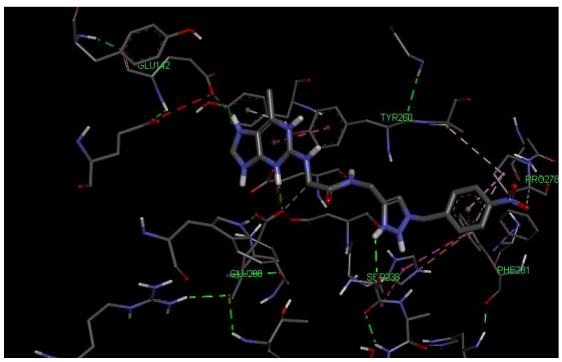
Ligands	Docking energies (Kcal/mol)	Ligands	Docking energies (Kcal/mol)
5a	-7.4	**6d**	-7.7
5b	-7.6	**6e**	-9.4
5c	-7.1	**8a**	-7.9
5d	-7.5	**8b**	-8
5e	-6.8	**8c**	-8.8
5g	-7.0	**8d**	-7.
6a	-8.9	**8e**	-8.9
6b	-9.4	**8f**	-9.3
6c		**8f**	



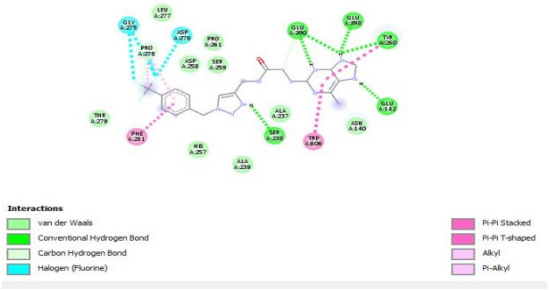


6b **6b**

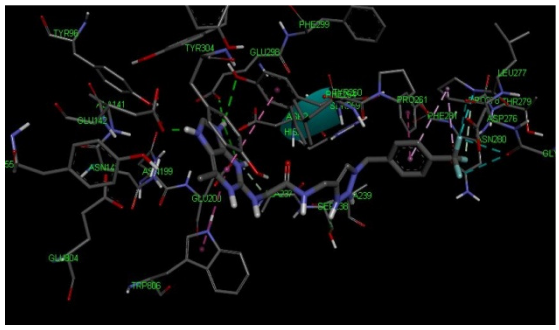




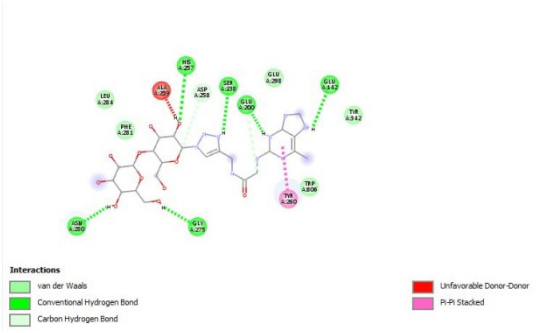
8f **8f**



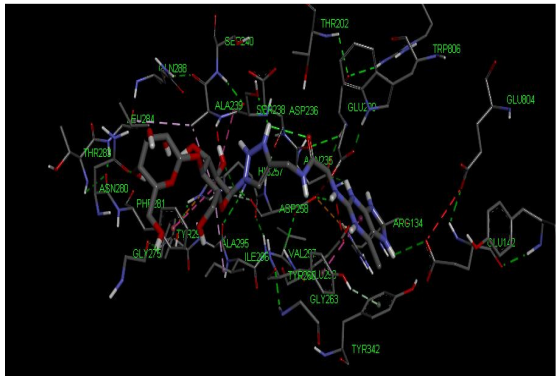
8e



8e



6e



6e

Figure 12: 3D and 2D diagram of compound **6b, 8f, 8e**and **6e** interaction between β-galactosidase.

3.10. Conclusion

In present study, we have demonstrated the synthesis of asmall library based on2-amino-6-chloropurine based glycoconjugates which was further screened for the antifungal activity against *Aspergillusniger*.Thus our finding in present work discloses the hidden antifungal property of purine based glycoconjugates and also gives an insight in the mechanism of action. This work may become an important lead in future antifungal drug discover project.

3.11. REFERENCES:

[1] Baker D, Chu C. Nucleosides and nucleotides as antitumor and antiviral agents: Springer; 2013.

[2] Townsend LB. Chemistry of nucleosides and nucleotides: Springer Science & Business Media; 2013.

[3] Jordheim LP, Durantel D, Zoulim F, Dumontet C. Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. Nature reviews Drug discovery. 2013;12:447.

[4] Anand N, Jaiswal N, Pandey SK, Srivastava A, Tripathi RP. Application of click chemistry towards an efficient synthesis of 1, 2, 3-1H-triazolyl glycohybrids as enzyme inhibitors. Carbohydrate research. 2011;346:16-25.

[5] Singh BK, Yadav AK, Kumar B, Gaikwad A, Sinha SK, Chaturvedi V. Preparation and reactions of sugar azides with alkynes: synthesis of sugar triazoles as antitubercular agents. Carbohydrate research. 2008;343:1153-62.

[6] Tiwari VK, Mishra BB, Mishra KB, Mishra N, Singh AS, Chen X. Cu-catalyzed click reaction in carbohydrate chemistry. Chemical reviews. 2016;116:3086-240.

[7] Rideout JL. Nucleosides, nucleotides and their biological applications: Academic Press; 2012.

[8] Newman DJ. Natural products as leads to potential drugs: an old process or the new hope for drug discovery? Journal of medicinal chemistry. 2008;51:2589-99.

[9] Wu F, Li P, Hu D, Song B. Design, synthesis, and antiviral activity of novel purine derivatives containing 1, 4-pentadien-3-one moiety. Research on Chemical Intermediates. 2016;42:7153-68.

[10] Celik GD, Disli A, Oner Y, Acik L. Synthesis of some novel amino and thiotetrazole purine derivatives and investigation of their antimicrobial activity and DNA interactions. Medicinal Chemistry Research. 2013;22:1470-9.

[11] Hostetler KY. Nucleoside analogues. Google Patents; 1996.

[12] Mitra AK. Acyclovir-peptide analogs. Google Patents; 2010.

[13] Sriram D, Yogeeswari P, Myneedu NS, Saraswat V. Abacavir prodrugs: microwave-assisted synthesis and their evaluation of anti-HIV activities. Bioorganic & medicinal chemistry letters. 2006;16:2127-9.

[14] Mooberry SL, Stratman K, Moore RE. Tubercidin stabilizes microtubules against vinblastine-induced depolymerization, a taxol-like effect. Cancer letters. 1995;96:261-6.

[15] Robak T, Korycka A, Robak E. Older and new formulations of cladribine. Pharmacology and clinical efficacy in hematological malignancies. Recent patents on anti-cancer drug discovery. 2006;1:23-38.

[16] Wu Z, Zhou S, Zemlicka J. Stereoselective Approach to the Z-Isomers of Methylenecyclopropane Analogues of Nucleosides: A New Synthesis of Antiviral Synguanol. Nucleosides, Nucleotides and Nucleic Acids. 2009;28:165-74.

[17] Kinight C. Oxytocin: An alternative to antibiotic for treating mastitis, Hannuch Research Insitute. Year book. 1999.

[18] Too K, Brown DM, Bongard E, Yardley V, Vivas L, Loakes D. Anti-malarial activity of N6-modified purine analogues. Bioorganic & medicinal chemistry. 2007;15:5551-62.

[19] Taldone T, Chiosis G. Purine-scaffold Hsp90 inhibitors. Current topics in medicinal chemistry. 2009;9:1436-46.

[20] Kim S-H, Bajji A, Tangallapally R, Markovitz B, Trovato R, Shenderovich M. Discovery of (2 S)-1-[4-(2-{6-amino-8-[(6-bromo-1, 3-benzodioxol-5-yl) sulfanyl]-9 H-purin-9-yl} ethyl) piperidin-1-yl]-2-hydroxypropan-1-one (MPC-3100), a purine-based Hsp90 inhibitor. Journal of medicinal chemistry. 2012;55:7480-501.

[21] Metcalf I, van Schravendijk M, Dalgarno DC, Sawyer TK. Targeting protein kinases for bone disease: discovery and development of Src inhibitors. Current pharmaceutical design. 2002;8:2049-75.

[22] Elion GB, Burgi E, Hitchings GH. Studies on condensed pyrimidine systems. IX. The synthesis of some 6-substituted purines. Journal of the American Chemical Society. 1952;74:411-4.

[23] Elion GB, Hitchings GH. The synthesis of 6-thioguanine. Journal of the American Chemical Society. 1955;77:1676.

[24] Teo S. Eastern Westerns: Film and Genre Outside and Inside Hollywood: Routledge; 2017.

[25] Woo PW, Dion HW, Lange SM, Dahl LF, Durham LJ. A novel adenosine and ara-a deaminase inhibitor,(R)-3-(2-deoxy-β-D-erythro-pentofuranosyl)-3, 6, 7, 8-tetrahydroimidazo [4, 5-d][1, 3] diazepin-8-ol. Journal of Heterocyclic Chemistry. 1974;11:641-3.

[26] Shih-Fong C, Stoeckler JD, Parks Jr RE. Transport of deoxycoformycin in human erythrocytes: Measurement by adenosine deaminase titration and radioisotope assays. Biochemical pharmacology. 1984;33:4069-79.

[27] Smaaland R, Schanche J-S, Kvinnsland S, HØstmark J, Ueland PM. Methylthioadenosine phosphorylase in human breast cancer. Breast cancer research and treatment. 1987;9:53-9.

[28] Hermes M, Osswald H, Kloor D. Role of S-adenosylhomocysteine hydrolase in adenosine-induced apoptosis in HepG2 cells. Experimental cell research. 2007;313:264-83.

[29] Grever MR, Doan CA, Kraut EH. Pentostatin in the treatment of hairy-cell leukemia. Best Practice & Research Clinical Haematology. 2003;16:91-9.

[30] Kurzrock R. Therapy of T cell lymphomas with pentostatin. Annals of the New York Academy of Sciences. 2001;941:200-5.

[31] Phiasivongsa P, Redkar S. Synthesis and manufacture of pentostatin and its precursors, analogs and derivatives. Google Patents; 2005.

[32] Gerrie AS, Zypchen LN, Connors JM. Fludarabine and rituximab for relapsed or refractory hairy cell leukemia. Blood. 2012;119:1988-91.

[33] Montgomery J, Hewson K. Nucleosides of 2-fluoroadenine. Journal of medicinal chemistry. 1969;12:498-504.

[34] Montgomery JA, Shortnacy AT. Prodrug derivatives of 9β-D-arabinofuranosyl-2-fluoroadenine. Google Patents; 1982.

[35] Grunebaum E, Cohen A, Roifman CM. Recent advances in understanding and managing adenosine deaminase and purine nucleoside phosphorylase deficiencies. Current opinion in allergy and clinical immunology. 2013;13:630-8.

[36] Carson DA, Wasson DB, Kaye J, Ullman B, Martin DW, Robins RK. Deoxycytidine kinase-mediated toxicity of deoxyadenosine analogs toward malignant human lymphoblasts in vitro and toward murine L1210 leukemia in vivo. Proceedings of the National Academy of Sciences. 1980;77:6865-9.

[37] Parker WB, Shaddix SC, Chang C-H, White EL, Rose LM, Brockman RW. Effects of 2-chloro-9-(2-deoxy-2-fluoro-β-D-arabinofuranosyl) adenine on K562

cellular metabolism and the inhibition of human ribonucleotide reductase and DNA polymerases by its 5′-triphosphate. Cancer research. 1991;51:2386-94.

[38] Christensen LF, Broom AD, Robins MJ, Bloch A. Synthesis and biological activity of selected 2, 6-disubstituted (2-deoxy-. alpha.-and-. beta.-D-erythro-pentofuranosyl) purines. Journal of medicinal chemistry. 1972;15:735-9.

[39] Robak P, Robak T. Older and new purine nucleoside analogs for patients with acute leukemias. Cancer treatment reviews. 2013;39:851-61.

[40] Locke F, Agarwal R, Kunnavakkam R, Van Besien K, Larson R, Odenike O. A novel clofarabine bridge strategy facilitates allogeneic transplantation in patients with relapsed/refractory leukemia and high-risk myelodysplastic syndromes. Bone marrow transplantation. 2013;48:1437.

[41] Montgomery JA, Shortnacy-Fowler AT, Clayton SD, Riordan JM, Secrist III JA. Synthesis and biological activity of 2'-fluoro-2-halo derivatives of 9-. beta.-D-arabinofuranosyladenine. Journal of medicinal chemistry. 1992;35:397-401.

[42] Montgomery JA, Shortnacy AT, Carson DA, Secrist III JA. 9-(2-Deoxy-2-fluoro-. beta.-D-arabinofuranosyl) guanine: a metabolically stable cytotoxic analogue of 2'-deoxyguanosine. Journal of medicinal chemistry. 1986;29:2389-92.

[43] Cohen MH, Johnson JR, Massie T, Sridhara R, McGuinn WD, Abraham S. Approval summary: nelarabine for the treatment of T-cell lymphoblastic leukemia/lymphoma. Clinical Cancer Research. 2006;12:5329-35.

[44] Shelton J, Lu X, Hollenbaugh JA, Cho JH, Amblard F, Schinazi RF. Metabolism, biochemical actions, and chemical synthesis of anticancer nucleosides, nucleotides, and base analogs. Chemical reviews. 2016;116:14379-455.

[45] Thalassitis A, Hadjipavlou-Litina DJ, Litinas KE, Miltiadou P. Synthesis of modified homo-N-nucleosides from the reactions of mesityl nitrile oxide with 9-

allylpurines and their influence on lipid peroxidation and thrombin inhibition. Bioorganic & medicinal chemistry letters. 2009;19:6433-6.

[46] Galmarini CM, Mackey JR, Dumontet C. Nucleoside analogues and nucleobases in cancer treatment. The lancet oncology. 2002;3:415-24.

[47] Bonate PL, Arthaud L, Cantrell Jr WR, Stephenson K, Secrist III JA, Weitman S. Discovery and development of clofarabine: a nucleoside analogue for treating cancer. Nature reviews Drug discovery. 2006;5:855.

[48] Zacharie B, Gagnon L, Attardo G, Connolly TP, St-Denis Y, Penney CL. Synthesis and activity of 6-substituted purine linker amino acid immunostimulants. Journal of medicinal chemistry. 1997;40:2883-94.

[49] De Clercq E, Holý A. Acyclic nucleoside phosphonates: a key class of antiviral drugs. Nature reviews Drug discovery. 2005;4:928.

[50] Franklin T, Snow G. Antifungal, antiprotozoal and antiviral agents. Biochemistry of antimicrobial action: Springer; 1989. p. 137-60.

[51] Schaeffer HJ, Bhargava PS. Enzyme inhibitors. V. The syntheses of 6-substituted-(9-hydroxyalkyl) purines and their evaluation as inhibitors of adenosine deaminase. Biochemistry. 1965;4:71-6.

[52] Hatse S, De Clercq E, Balzarini J. Role of antimetabolites of purine and pyrimidine nucleotide metabolism in tumor cell differentiation. Biochemical pharmacology. 1999;58:539-55.

[53] Santiago FS, Khachigian LM. Nucleic acid based strategies as potential therapeutic tools: mechanistic considerations and implications to restenosis. Journal of molecular medicine. 2001;79:695-706.

[54] Sridhar R, Hanson-Painton O, Cooper DR. Protein kinases as therapeutic targets. Pharmaceutical research. 2000;17:1345-53.

[55] Toyota A, Katagiri N, Kaneko C. Synthesis of Nucleosides and Related Compounds. Part 32. The Alkylation of 2-Amino-6-chloropurine with Alcohols by Mitsunobu Reaction for a Synthesis of Carbocyclic Guanosine Analogs. ChemInform. 1993;24:no-no.

[56] Vince R, Hua M. Synthesis and anti-HIV activity of carbocyclic 2', 3'-didehydro-2', 3'-dideoxy 2, 6-disubstituted purine nucleosides. Journal of medicinal chemistry. 1990;33:17-21.

[57] BALSIGER RW, MONTGOMERY JA. Synthesis of Potential Anticancer Agents. XXV. Preparation of 6-Alkoxy-2-aminopurines2. The Journal of organic chemistry. 1960;25:1573-5.

[58] Gordon EM, Barrett RW, Dower WJ, Fodor SP, Gallop MA. Applications of combinatorial technologies to drug discovery. 2. Combinatorial organic synthesis, library screening strategies, and future directions. Journal of medicinal chemistry. 1994;37:1385-401.

[59] Le GT, Abbenante G, Becker B, Grathwohl M, Halliday J, Tometzki G. Molecular diversity through sugar scaffolds. Drug discovery today. 2003;8:701-9.

[60] Tietze LF, Bell HP, Chandrasekhar S. Natural product hybrids as new leads for drug discovery. Angewandte Chemie International Edition. 2003;42:3996-4028.

[61] Moreno-Mendieta S, Guillen D, Hernandez-Pando R, Sanchez S, Rodriguez-Sanoja R. Potential of glucans as vaccine adjuvants: A review of the α-glucans case. Carbohydrate polymers. 2017;165:103-14.

[62] Jana S, Mandlekar S, Marathe P. Prodrug design to improve pharmacokinetic and drug delivery properties: challenges to the discovery scientists. Current medicinal chemistry. 2010;17:3874-908.

[63] Peterson LW, McKenna CE. Prodrug approaches to improving the oral absorption of antiviral nucleotide analogues. Expert opinion on drug delivery. 2009;6:405-20.

[64] Pendri A, Conover CD, Greenwald RB. Antitumor activity of paclitaxel-2′-glycinate conjugated to poly (ethylene glycol): a water-soluble prodrug. Anti-cancer drug design. 1998;13:387-95.

[65] Jahnz-Wechmann Z, Framski G, Januszczyk P, Boryski J. Bioactive fused heterocycles: Nucleoside analogs with an additional ring. European journal of medicinal chemistry. 2015;97:388-96.

[66] VANDAMME E. Microbial synthesis of D-ribose: metabolic deregulation and fermentation process. Advances in applied microbiology. 1997;44:167.

[67] Singh N, Pandey S, Tripathi RP. Regioselective [3+ 2] cycloaddition of chalcones with a sugar azide: easy access to 1-(5-deoxy-D-xylofuranos-5-yl)-4, 5-disubstituted-1H-1, 2, 3-triazoles. Carbohydrate research. 2010;345:1641-8.

[68] Thakur RK, Mishra A, Ramakrishna K, Mahar R, Shukla SK, Srivastava A. Synthesis of novel pyrimidine nucleoside analogues owning multiple bases/sugars and their glycosidase inhibitory activity. Tetrahedron. 2014;70:8462-73.

[69] Fisher MC, Henk DA, Briggs CJ, Brownstein JS, Madoff LC, McCraw SL. Emerging fungal threats to animal, plant and ecosystem health. Nature. 2012;484:186.

[70] Meyer V, Andersen MR, Brakhage AA, Braus GH, Caddick MX, Cairns TC. Current challenges of research on filamentous fungi in relation to human welfare and a sustainable bio-economy: a white paper. Fungal biology and biotechnology. 2016;3:6.

[71] Hagiwara D, Watanabe A, Kamei K, Goldman GH. Epidemiological and genomic landscape of azole resistance mechanisms in Aspergillus fungi. Frontiers in microbiology. 2016;7:1382.

[72] Deane EE, Whipps JM, Lynch JM, Peberdy JF. The purification and characterization of a Trichoderma harzianum exochitinase. Biochimica et Biophysica Acta (BBA)-Protein Structure and Molecular Enzymology. 1998;1383:101-10.

[73] Chowdhury S, Bhuiyan M, Ozeki Y, Kawsar SMA. Simple and rapid synthesis of some nucleoside derivatives: structural and spectral characterization. Current Chemistry Letters. 2016;5:83-92.

[74] Thomson J, Lamont I. Nucleoside analogues as antibacterial agents. Frontiers in microbiology. 2019;10:952.

[75] Doncel GF, Parang K, Agarwal HK. Substituted nucleoside derivatives with antiviral and antimicrobial properties. Google Patents; 2016.

[76] Songulashvili G, Flahaut S, Demarez M, Tricot C, Bauvois C, Debaste F. High yield production in seven days of Coriolopsis gallica 1184 laccase at 50 L scale; enzyme purification and molecular characterization. Fungal biology. 2016;120:481-8.

[77] Kiiskinen L-L, Kruus K, Bailey M, Ylösmäki E, Siika-aho M, Saloheimo M. Expression of Melanocarpus albomyces laccase in Trichoderma reesei and characterization of the purified enzyme. Microbiology. 2004;150:3065-74.

[78] Hofrichter M. lignin conversion by manganese peroxidase (MnP). Enzyme and Microbial technology. 2002;30:454-66.

[79] Kapich AN, Steffen KT, Hofrichter M, Hatakka A. Involvement of lipid peroxidation in the degradation of a non-phenolic lignin model compound by manganese peroxidase of the litter-decomposing fungus Stropharia coronilla. Biochemical and biophysical research communications. 2005;330:371-7.

[80] Miyata N, Iwahori K, Fujita M. Manganese-independent and-dependent decolorization of melanoidin by extracellular hydrogen peroxide and peroxidases from Coriolus hirsutus pellets. Journal of fermentation and bioengineering. 1998;85:550-3.

[81] Maciel MJM, Ribeiro HCT. Industrial and biotechnological applications of ligninolytic enzymes of the basidiomycota: A review. Electronic Journal of Biotechnology. 2010;13:14-5.

[82] Dashtban M, Schraft H, Syed TA, Qin W. Fungal biodegradation and enzymatic modification of lignin. International journal of biochemistry and molecular biology. 2010;1:36.

[83] Weenink XO, Punt PJ, Van Den Hondel CA, Ram AF. A new method for screening and isolation of hypersecretion mutants in Aspergillus niger. Applied microbiology and biotechnology. 2006;69:711-7.

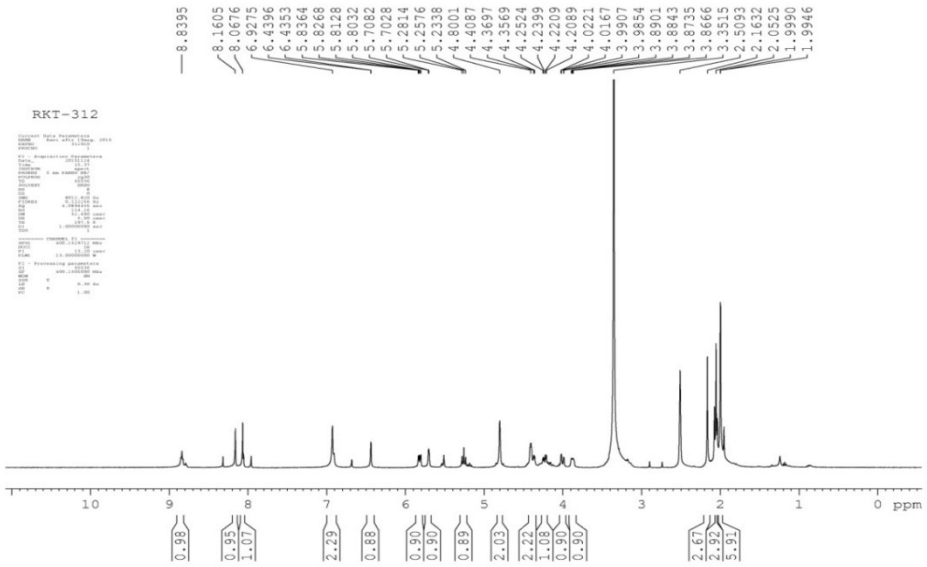
[84] Arora DS, Gill PK. Production of ligninolytic enzymes by Phlebia floridensis. World Journal of Microbiology and Biotechnology. 2005;21:1021-8.

[85] Magalhães DB, de Carvalho MEA, Bon E, Neto JSA, Kling SH. Colorimetric assay for lignin peroxidase activity determination using methylene blue as substrate. Biotechnology techniques. 1996;10:273-6.

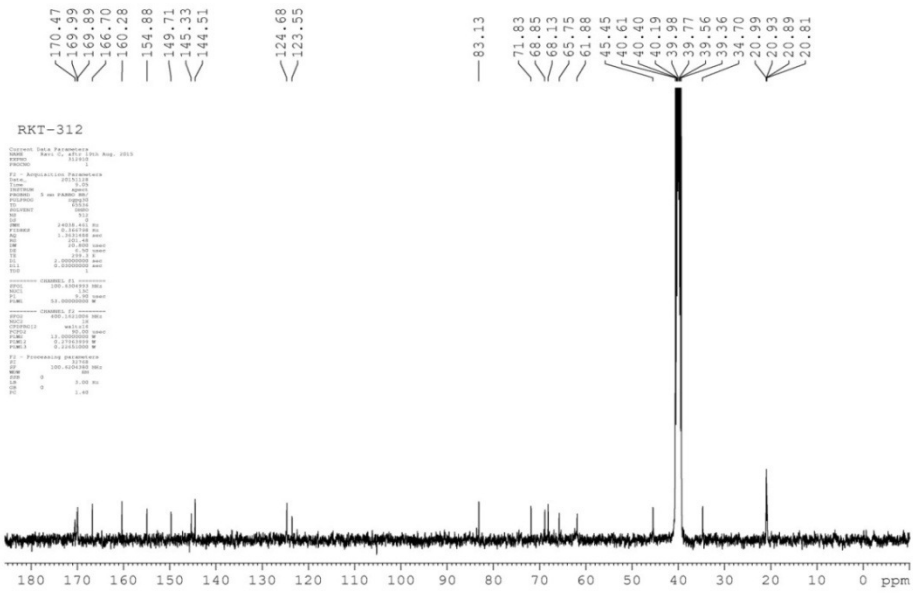
[86] Bholay A, Borkhataria BV, Jadhav PU, Palekar KS, Dhalkari MV, Nalawade P. Bacterial Lignin Peroxidase: A Tool for Biobleaching and Biodegradation of Industrial Effluents. Universal Journal of Environmental Research & Technology. 2012;2.

^{1}H and ^{13}C data of few selected purine based nucleoside scaffolds derivatives

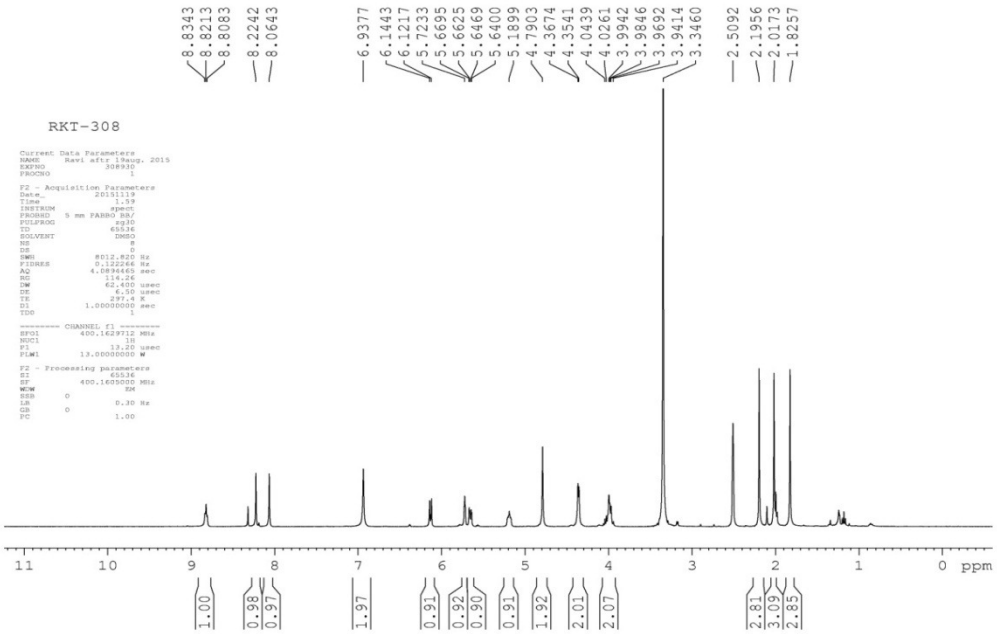
^{1}H of 2-[{6-chloro-9*H*-purin-2-yl}amino] -*N*-[{1´-(1´´deoxy-2´´, 3´´, 4´´, 6´´- tetra-*O*-acetyl-β-D-mannopyranos-1´´yl)-1´H-1´,2´,3´- triazol-4´-yl}methyl]- acetamide (5c)



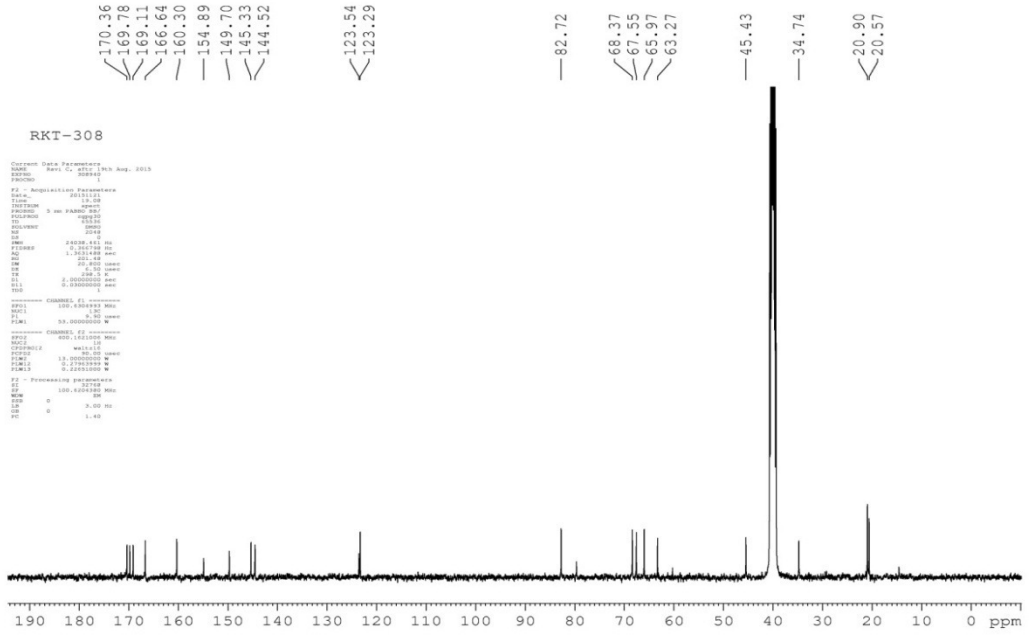
^{13}C of 2-[{6-chloro-9*H*-purin-2-yl}amino] -*N*-[{1´-(1´´deoxy-2´´, 3´´, 4´´, 6´´- tetra-*O*-acetyl-β-D-mannopyranos-1´´yl)-1´H-1´,2´,3´- triazol-4´-yl}methyl]- acetamide (5c)



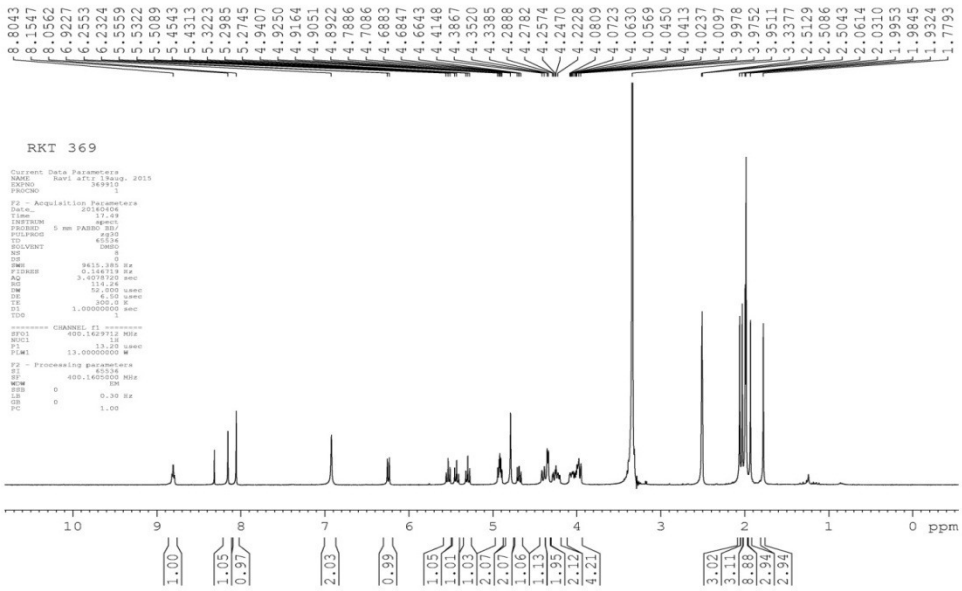
^{1}H of 2-[{6-chloro-9*H*-purin-2-yl}amino] -*N*-[{1´-(1´´deoxy-2´´, 3´´, 4´´, 6´´- tetra-*O*-acetyl-β-D-ribopyranos-1´´yl)-1´H-1´,2´,3´- triazol-4´-yl}methyl]- acetamide (5d)



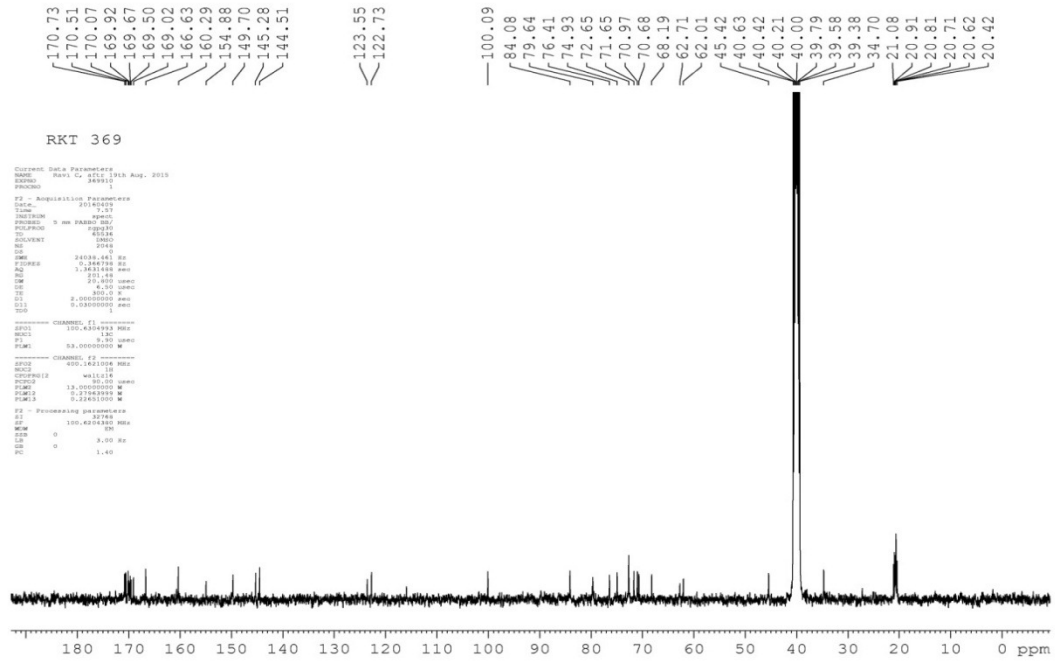
^{13}C of 2-[{6-chloro-9*H*-purin-2-yl}amino] -*N*-[{1´-(1´´deoxy-2´´, 3´´, 4´´, 6´´- tetra-*O*-acetyl-β-D-ribopyranos-1´´yl)-1´H-1´,2´,3´- triazol-4´-yl}methyl]- acetamide (5d)



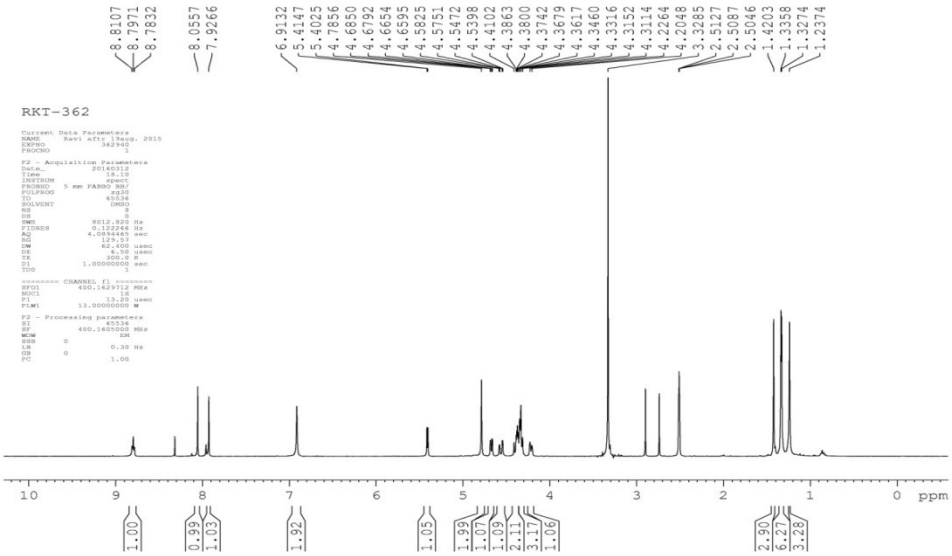
^{1}H of 2-[{6-chloro-9*H*-purin-2-yl}amino] -*N*-[{1´-(1´´deoxy-2´´, 3´´, 4´´, 6´´- tetra-*O*-acetyl-β-D-cellobios-1´´yl)-1´H-1´,2´,3´- triazol-4´-yl}methyl]- acetamide (5e)



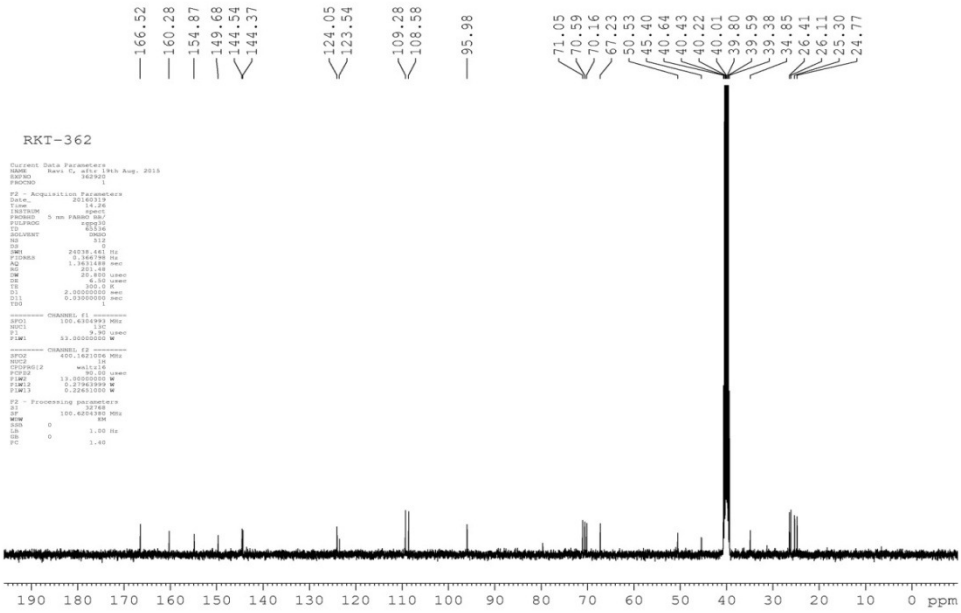
^{13}C of 2-[{6-chloro-9*H*-purin-2-yl}amino] -*N*-[{1´-(1´´deoxy-2´´, 3´´, 4´´, 6´´- tetra-*O*-acetyl-β-D-cellobios-1´´yl)-1´H-1´,2´,3´- triazol-4´-yl}methyl]- acetamide (5e)



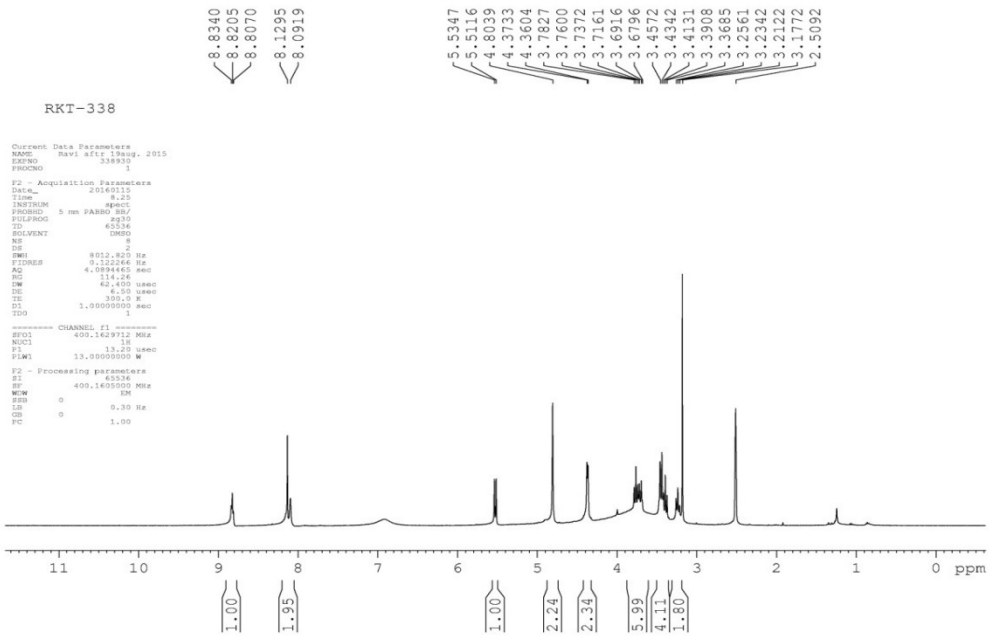
^{1}H of 2-[{6-chloro-9*H*-purin-2-yl}amino] -*N*-[{1´-(6´´deoxy-1´´, 2´´, 3´´, 4´´- di-*O*-isopropylidene-α-D-galactopyranos-6´´-yl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]acetamide (5f)



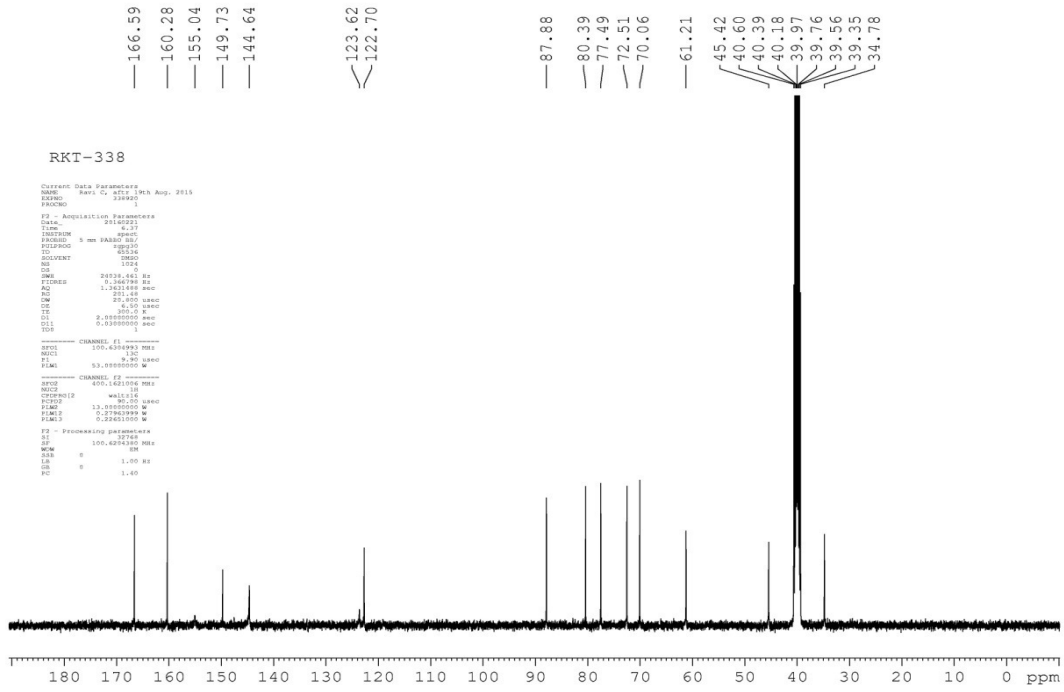
^{13}C of 2-[{6-chloro-9*H*-purin-2-yl}amino] -*N*-[{1´-(6´´deoxy-1´´, 2´´, 3´´, 4´´- di-*O*-isopropylidene-α-D-galactopyranos-6´´-yl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]acetamide (5f)



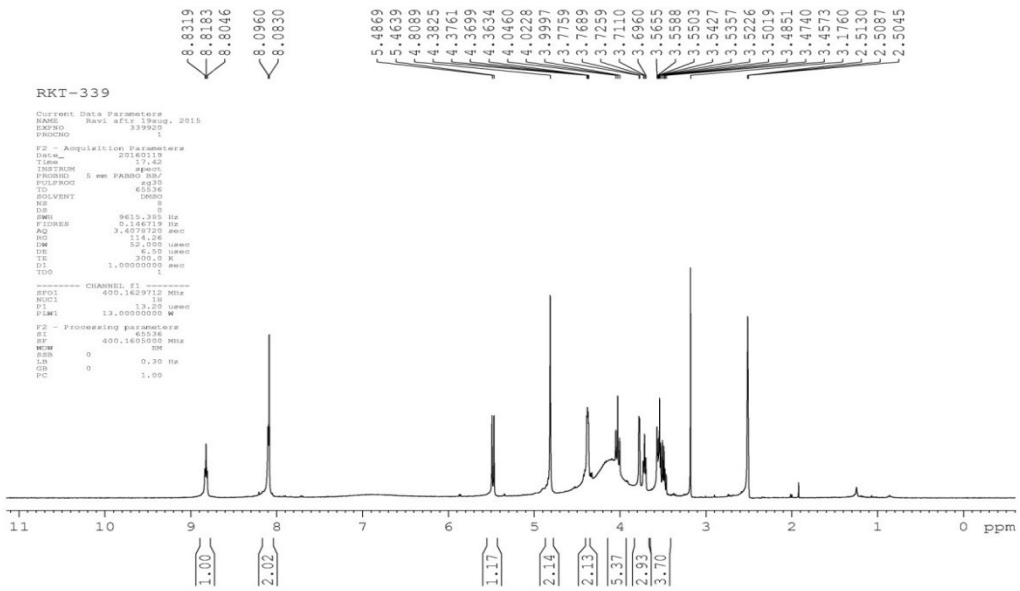
^{1}H of 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-[{1´-(β-D-glucopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]- acetamide (6a)



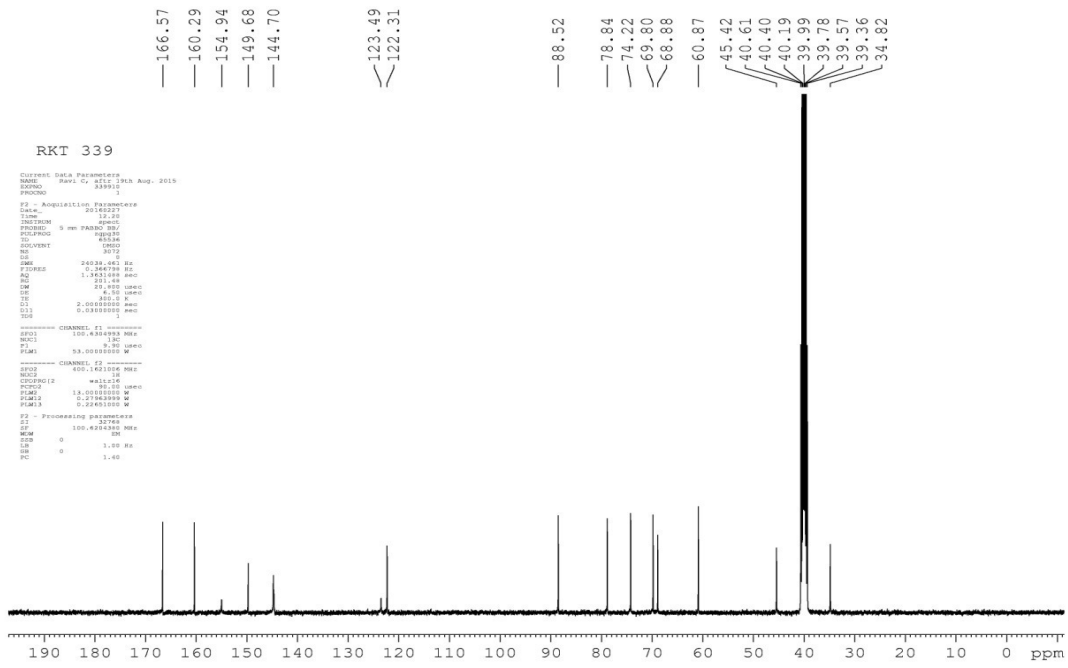
^{13}C of 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-[{1´-(β-D-glucopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]- acetamide (6a)



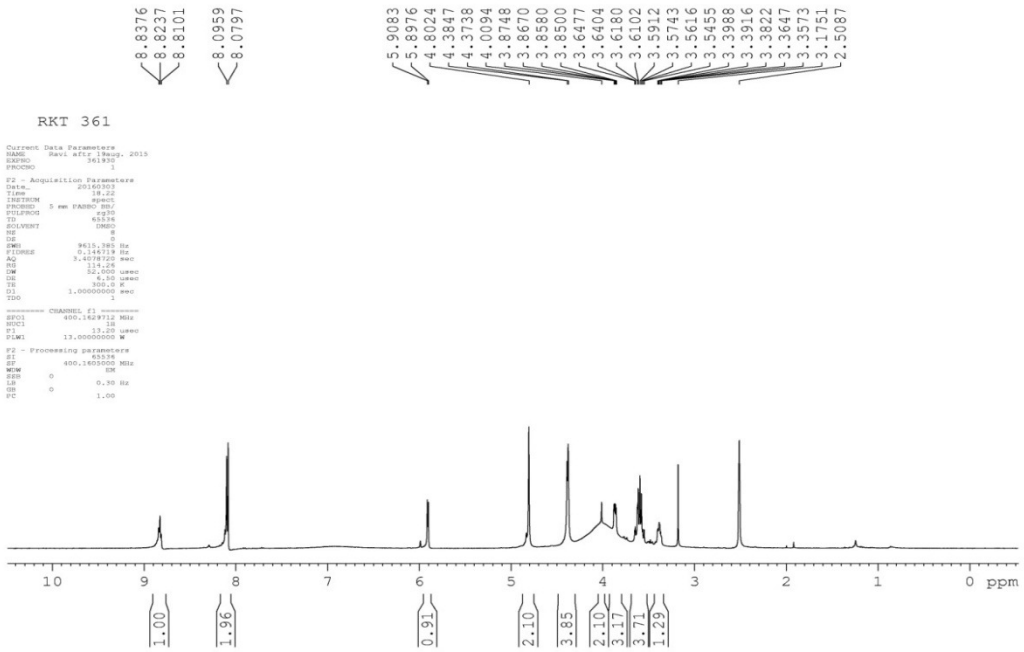
^{1}H of 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-[{1´-(β-D-galactopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]- acetamide (6b)



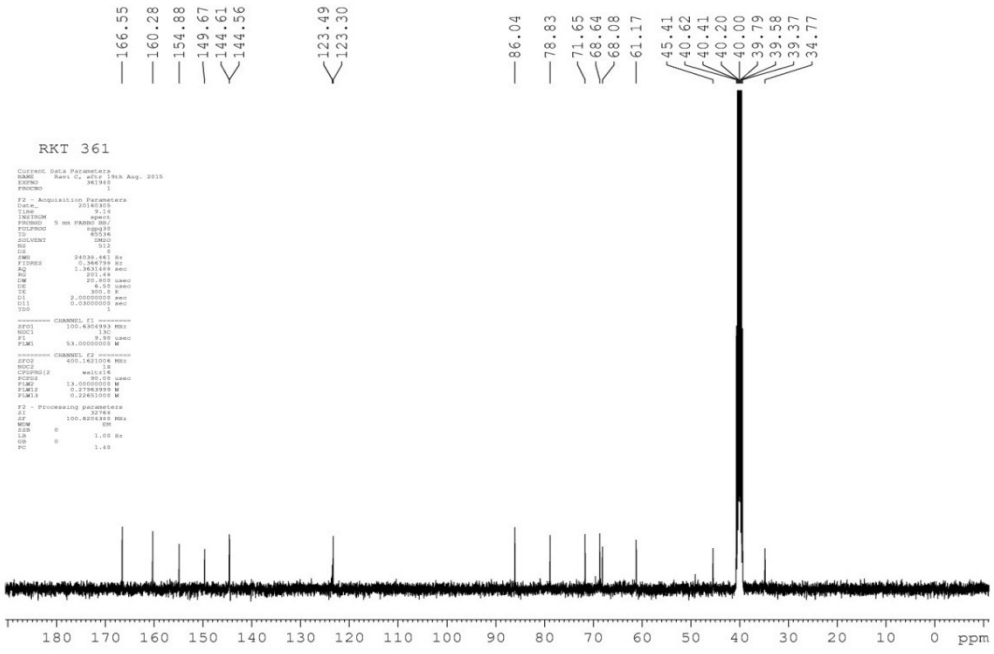
^{13}C of 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-[{1´-(β-D-galactopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]-acetamide(6b)



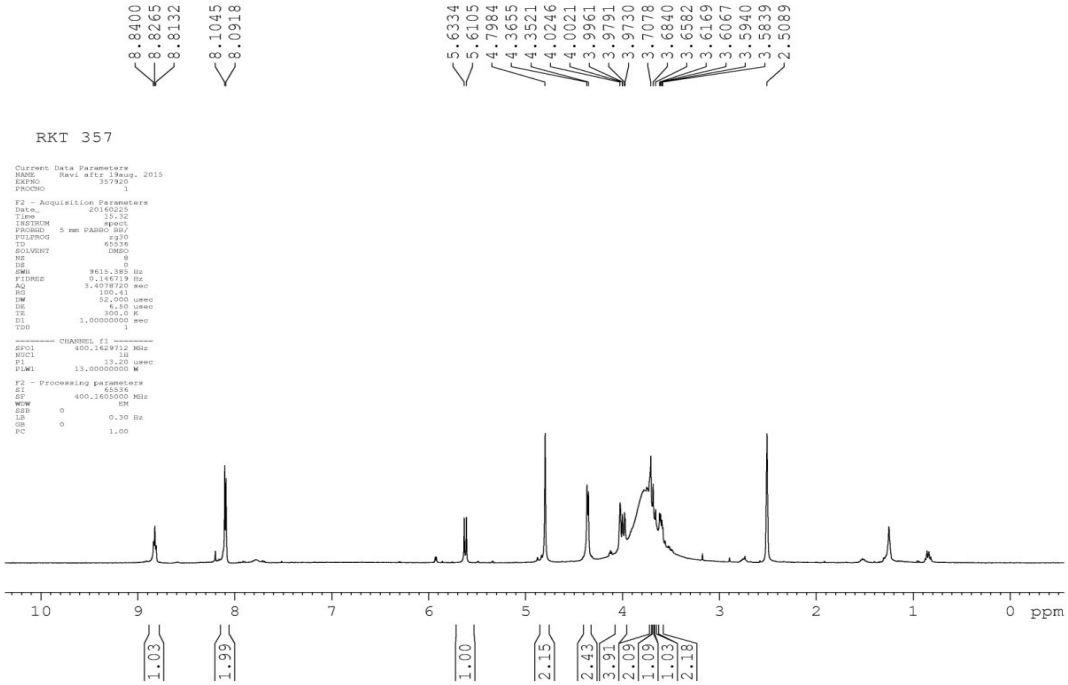
^{1}H of 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-[{1´-(β-D-mannopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl] acetamide (6c)



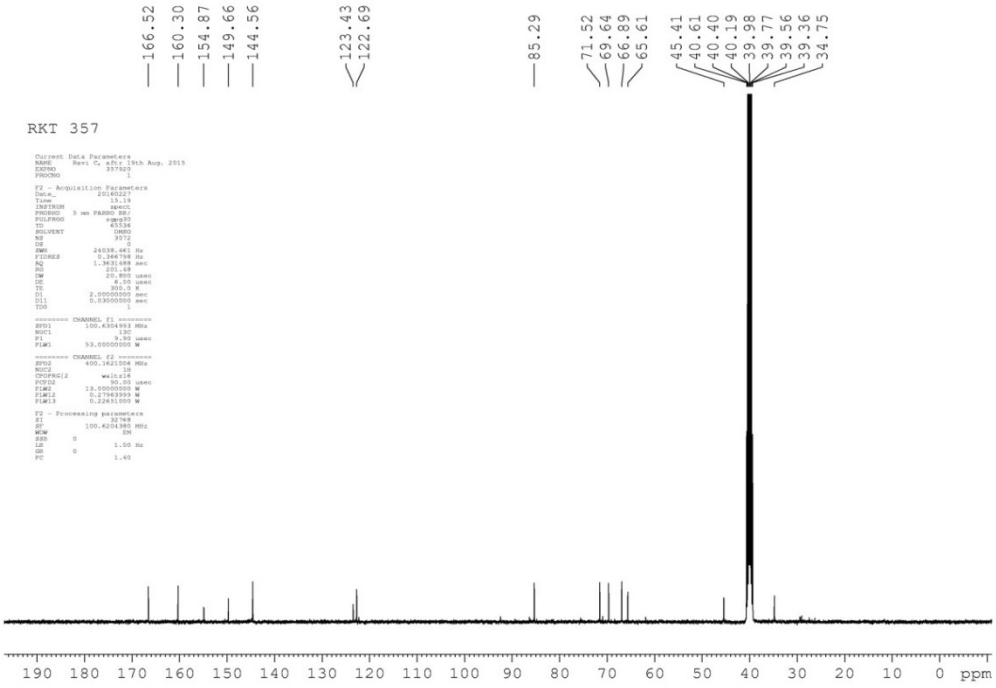
^{13}C of 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-[{1´-(β-D-mannopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl] acetamide (6c)



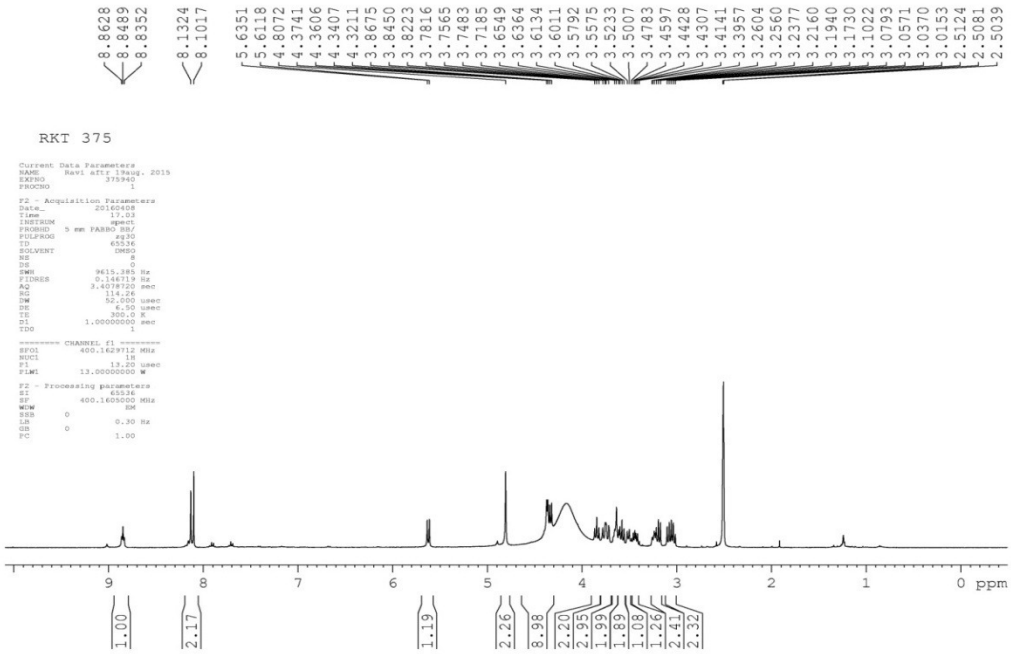
^{1}H of 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-[{1´-(β-D-ribopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]- acetamide (6d)



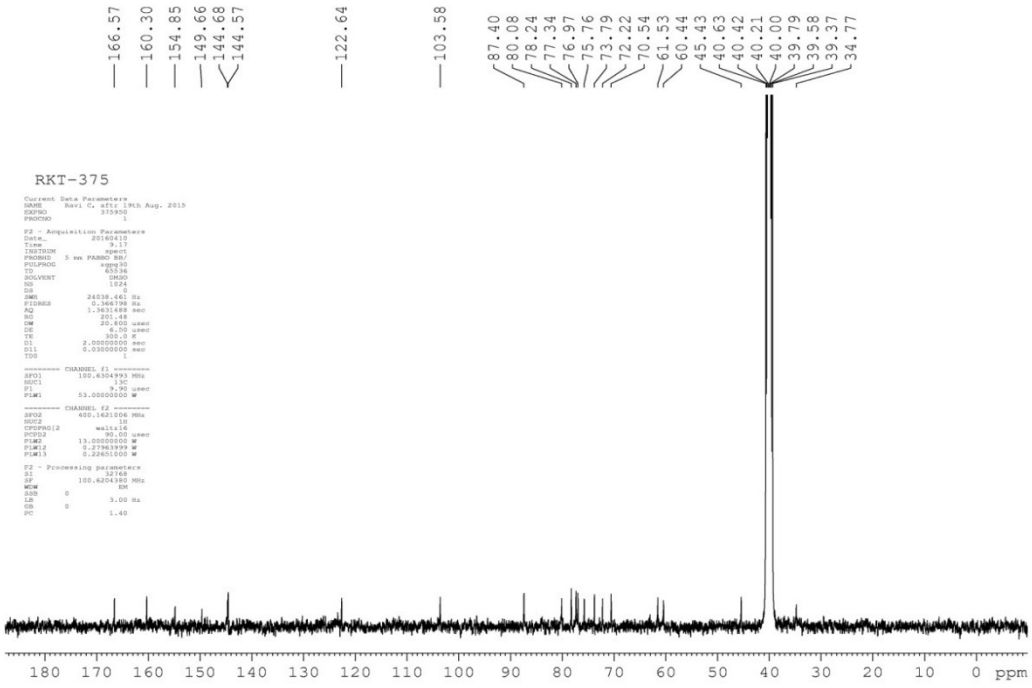
^{13}C of 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-[{1´-(β-D-ribopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]-acetamide(6d)



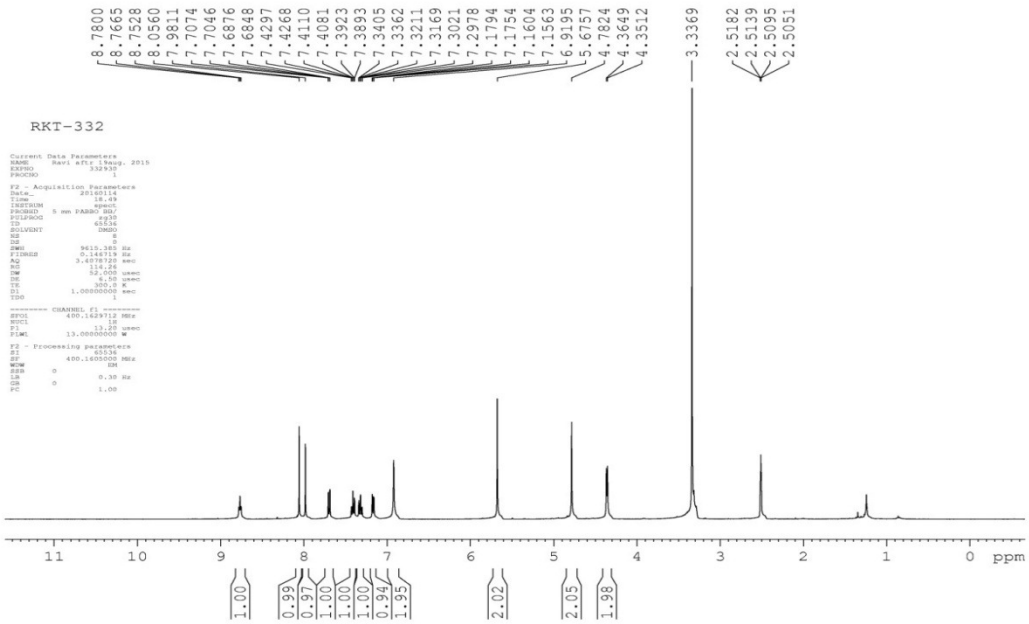
^{1}H of 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-[{1´-(β-D-cellobiopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]- acetamide (6e)



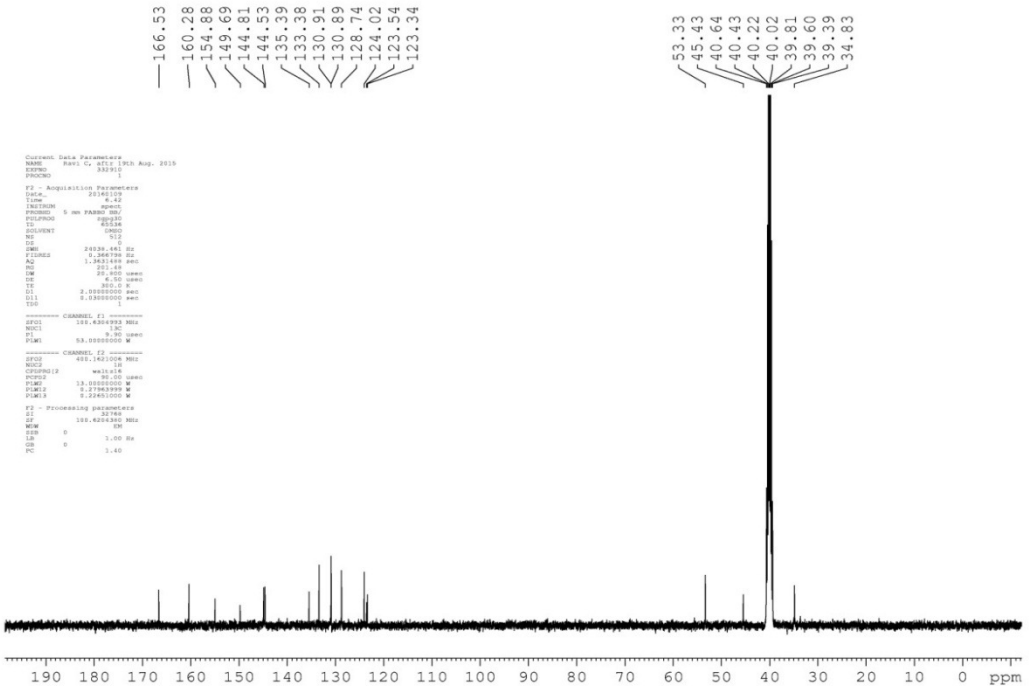
^{13}C of 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-[{1´-(β-D-cellobiopyranosyl)-1´H-1´,2´,3´-triazol-4´-yl}methyl]- acetamide (6e)



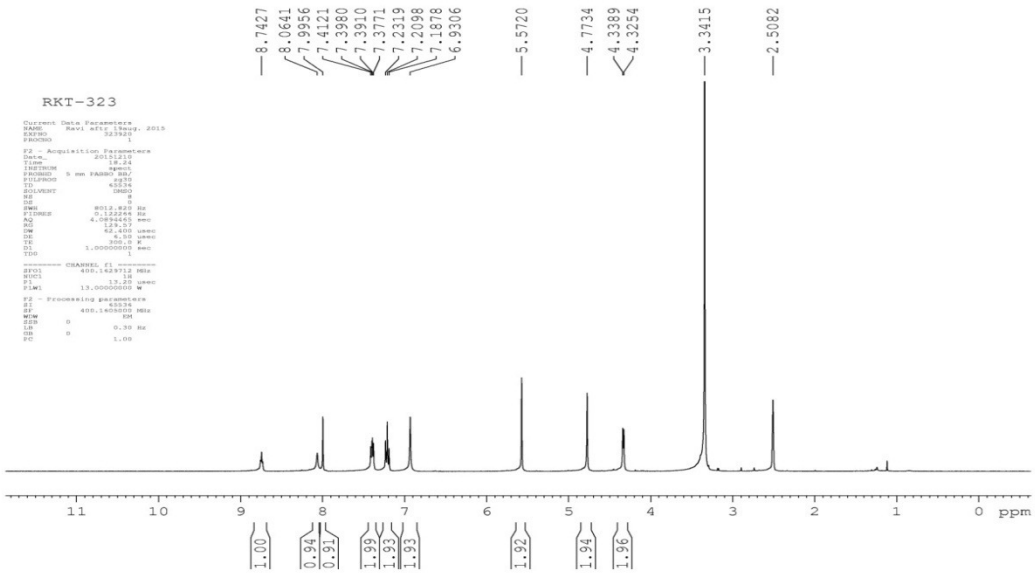
^{1}H of 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-((1-(2-bromobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide(8b)



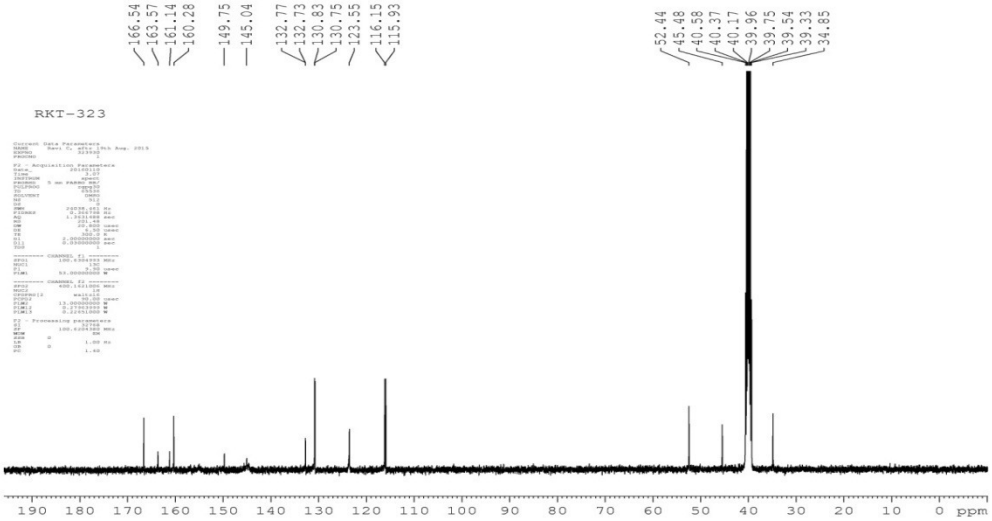
^{13}C of 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-((1-(2-bromobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide(8b)



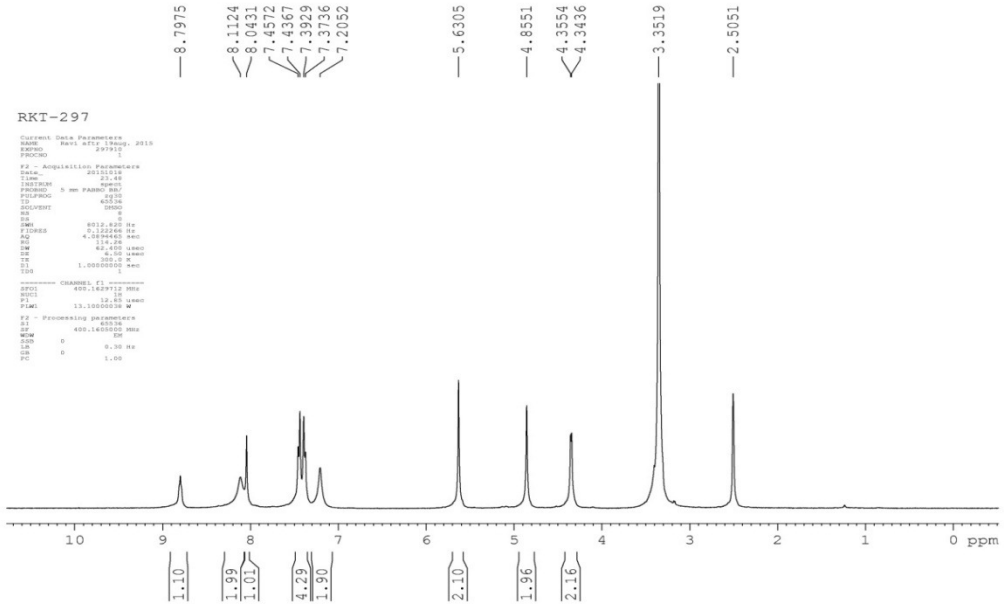
^{1}H of 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-((1-(4-fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (8d)



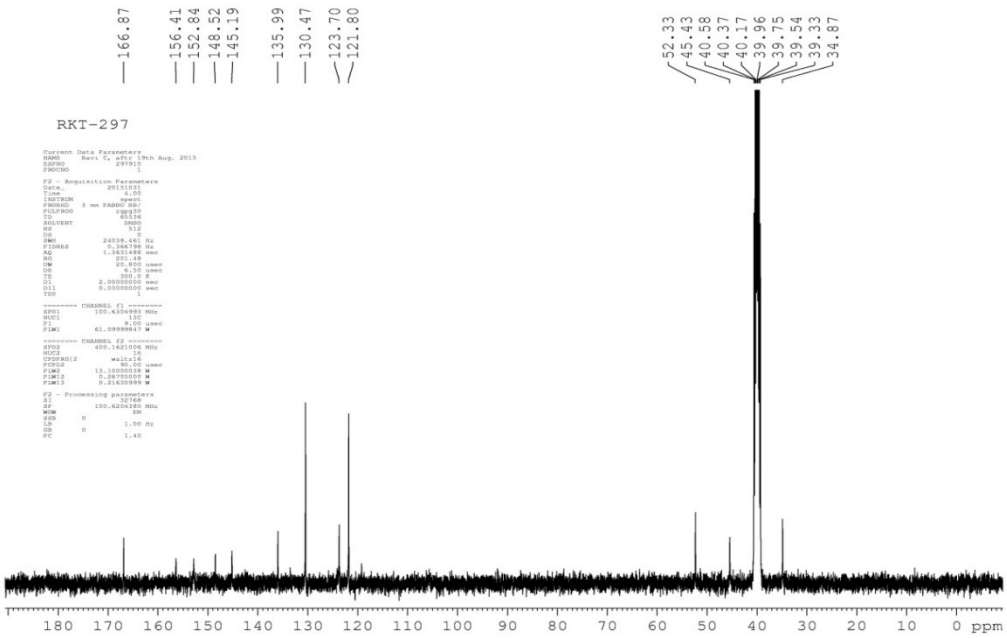
^{13}C of 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-((1-(4-fluorobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (8d)



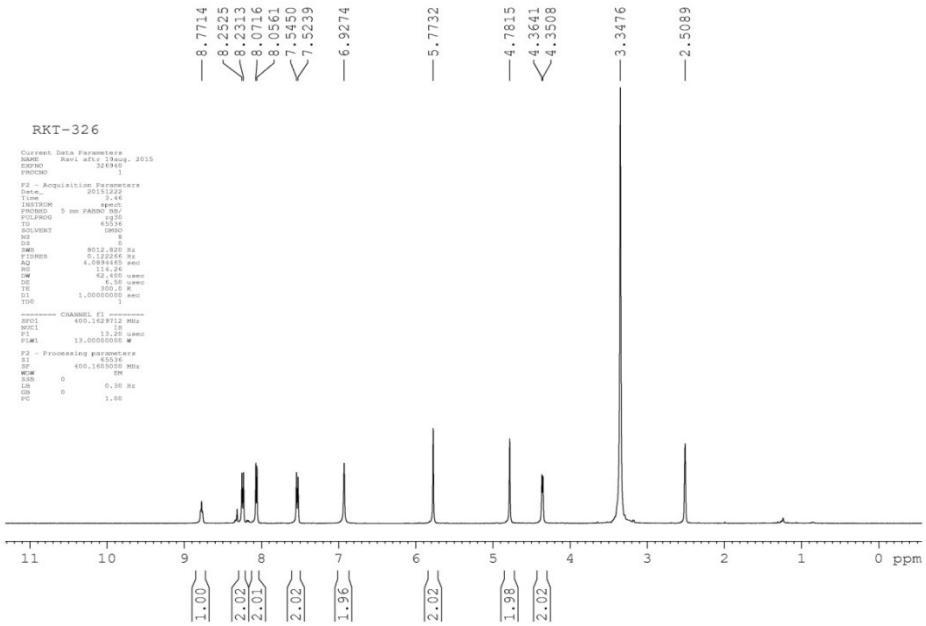
^{1}H of 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-((1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (8e)



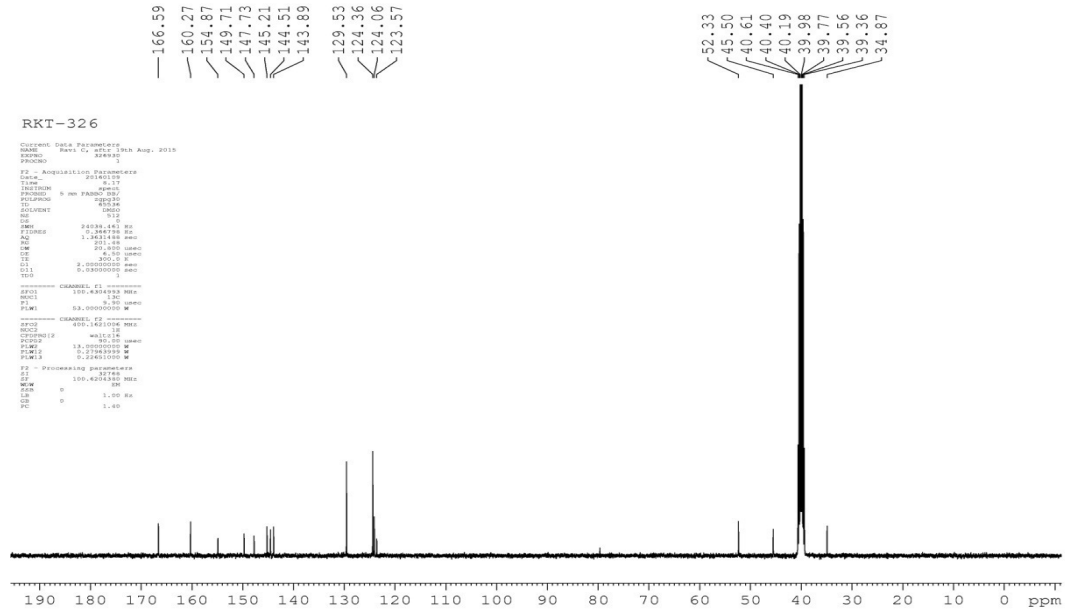
^{13}C of 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-((1-(4-(trifluoromethyl)benzyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (8e)



^{1}H of 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-((1-(4-nitrobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (8f)



^{13}C of 2-[{6-chloro-9*H*-purin-2-yl}amino]-*N*-((1-(4-nitrobenzyl)-1*H*-1,2,3-triazol-4-yl)methyl)acetamide (8f)



Chapter-4

Synthesis and bioevaluation of novel glycosyl 1,2,3-1H-triazolyl methyl benzamide derivative

4.1. Introduction

Diabetes mellitus (DM) is a chronic disease of global commons, which tends to have problems with adherence[1, 2]. Adherence with medication, diet, and exercise, and blood glucose self–monitoring is quite challenging[3].Due to consumption of carbohydrate-enriched diet, metabolic and heterogeneous disorder causing high blood-glucose level, leading to hyperglycaemia [4]. As per the Global report on Diabetes (2018) by World Health Organization, diabetes was the seventh major cause of death in 2016 [5, 6].

Carbohydrates form the largest group of naturally occurring compounds in nature and also crucial due to their pivotal role in medicinal chemistry and drug discovery [7-9]. They are also known for their essential function in development, recognition, growth, function and survival of living cells and organisms. Many drug molecules including several antibiotics and few anti-diabetic medicines contain terminal sugar moieties which are necessary for their biological action [10].Sugar molecules are benefitted with immense structural diversity which makes it appropriate for library generation of small heterocycles in search of lead molecules[11, 12]. They can introduce multiple functionalities in restricted steps. According to estimation there are more than 194 million people are suffering from diabetes globally that will increase to 333 million by 2025 [5].α-glucosidaseis a membrane-bound enzyme that catalyzes the hydrolysis of the glycosidic bond at the non-reducing terminal of the sugar, resulting in the release of free glucose into the digestive tract. They are responsible for glucose absorption and increase the glucose concentration in blood. Inhibition of α-glucosidase could diminish the rate of carbohydrates assimilation and concealment of postprandial hyperglycaemia [13]. Along these, α-glucosidase has been regarded as significant objective for the discovery and development of novel

anti-diabetic drugs. As of now, there are three A-glucosidase inhibitors like acarbose, voglibose, and miglitol, which have been utilized in clinical treatment of type-2 diabetes mellitus[14, 15].

Benzamide moiety is very significant class of nitrogen heterocycles and considered as privileged structures in drug discovery owing to their important roles as key building blocks in the synthesis of many drugs[16]. This heterocyclic nucleus is linked with diverse range of pharmacological activities such as anti-hypertensive[17], anti-bacterial[18], anti-inflammatory[19], anti-cancer[20], analgesic[21],anti-histamine[22], CNS stimulant[23] and anti-diabetic activities[24].Hence, we tried to couple the benzamide nucleus with terminal sugar moieties by applying a suitable and biologically significant linker. 1,2,3-Triazoles are also an essential scaffold in drug discovery and may an important role in development of several molecules which exhibiting important biological activities such as anti-fungal[25],anti-tubercular[26, 27] anti-cancer[28], anti-HIV[29, 30] anti-bacterial[25] anti-viral[31]anti-Alzheimer[32]anti-mycobacterial[33]and glycosidase inhibitors[34-36].

4.2 Carbohydrate as potential glycosidase inhibitors

Glycosidases are widespread enzymes involved in the biosynthesis of oligosaccharides at cell surfaces (Golgi apparatus and endoplasmic reticulum) by selectively cleaving the glycosidic bond in glycoproteins and play a vital role in the digestion of dietary carbohydrates. Glycosidases and glycotransferases constitute around 1-3% of the genome of most organisms [37]. Since these enzymes control the hydrolysis of carbohydrates, which is biologically widespread process, therefore glycosidase inhibitors have many potential applications as therapeutic agents. Inhibition of these enzymes can alter cell-cell or cell-virus recognition processes.

Iminosugars have emerged as a versatile tool for the development of therapeutic agents [38]. Development of iminosugars has gained momentum after their discovery as glycosidase inhibitors. The easy access of these iminosugars has led to the evolution of new anti-diabetics, anti-HIV, antivirals and also novel therapeutics for the treatment of genetic disorders. L-enantiomers are less explored as compared to the D-iminosugars. Finding of D'Alonzo et al. demonstrated the role of L-iminosugars in the inhibition mechanism of specific enzymes and enzyme-inhibitor interactions and has garnered attention of medicinal chemists [39].

4.2.1 Glycosidase inhibitors: Antidiabetic effects

Hydrolytic cleavage of glycosidic bonds during digestion is an important feature of glycosidases to controls sugar level in blood. It is very fruitful for the treatment of type 2 insulin independent diabetes mellitus (NIDDM). For example, Acarbose (**1**), an anti-diabetic drug referred as pseudotetra-saccharide derived from the culture broth of *Actinoplanes* strain SE_{50}, used to treat type-2 diabetes mellitus and, in some countries, prediabetes. Acarbose (**1**) reversibly binds at the α-glycosidse enzymes in the brush border of intestinal mucosa and pancreatic α-amylase. Inhibition of these enzymes prevents the hydrolysis of oligosaccharides which in turn reduces the level of blood glucose. Miglitol (**2**), an oral anti-diabetic drug acts by preventing the α-glucosidase enzymes to hydrolyze carbohydrate into simpler forms which can be absorbed by the body. Therefore, it lowers the post-prandial hyperglycaemia. Emiglitata (**3**), derivative with the ethyl benzoate moiety makes it more lipophilic than miglitol but inhibitory profile is not much convincing. Voglibose (**4**) is α-glucosidse inhibitor used for the treatment of diabetes mellitus by lowering the post-prandial blood glucose levels. Thus, α-glucosidase inhibitors exerts their anti-diabetic effects by inhibiting the pancreatic α-amylase which are responsible for the hydrolysis

of complex starch into oligosaccharides in the small intestine and also by inhibiting the membrane bound α-glucosidase, which hydrolyse the oligosaccharides into simple sugars (glucose and other monosaccharides) and hence lower blood glucose level. Because of their specific mechanism of action as mentioned above, they are associated with several side effects such as abdominal distension, stomach pain, flatulence, diarrhoea etc. as undigested carbohydrates reach colon and got digested by intestinal bacteria leading to these side effects.

Miglustat (*N*-butyl-deoxynojirimycin, **5**) is a first iminosugar drug extracted from plant and microorganisms and approved by FDA in Europe in 2002 [40] and the United States in 2003 [41] for treatment of type-1 Gaucher disease marketed under the trade name Zavesca. It was developed by Oxford GlycoSciences and marketed by Actelion (**Figure.1**).

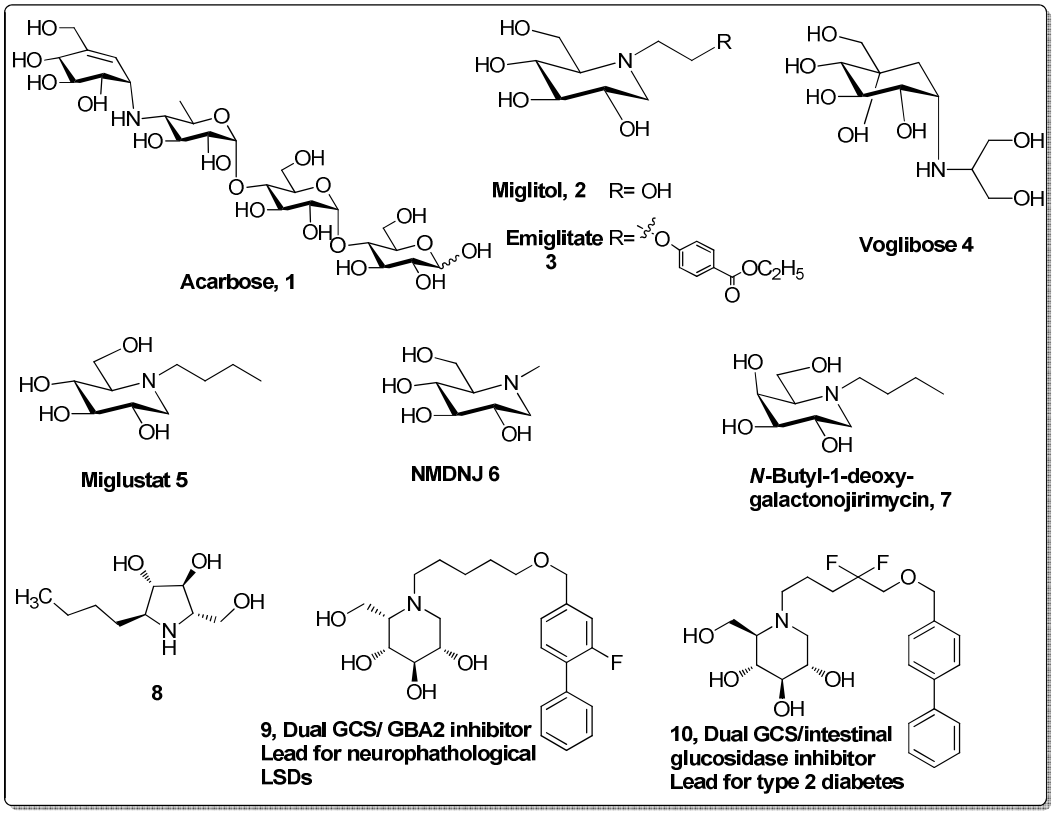


Figure 1. Structures of some glycosidase inhibitors with anti-diabetic effect

Miglustat (**5**) is recommended for symptomatic patients with mild to moderate type gaucher disease where enzyme replacement therapy is unsuitable. It is approved for the treatment of progressive neurological complications in people with Niemann-Pick disease, type C (NPC) in Japan, Canada and Europe. Other carbohydrate analogues including*N*-methyl-1-deoxy-nojirimycin (**6**) and *N*-butyl-1-deoxy-galactonojirimycin (**7**) are also used for the treatment of gaucher disease and fibery disease. Kato et al. described a potent class of pyrrolidine based carbohydrate analogue, i.e. α-1-*C*-butyl-1,4-dideoxy-1,4-imino-L-arabinitol (α-1-*C*-butyl-LAB, **8**) as α-glucosidase inhibitors[42]. It has promising glucose lowering activity for treating postprandial hyperglycaemia. It selectively inhibits intestinal α-glucosidase enzyme and lowers down some of the undesired side effects associated with other anti-diabetic compounds [43-48].Recently Amar et al. described the *N*-alkylated deoxinojirimycin derivatives as dual glycosylceramide synthase/ neutral glycosyl ceramidase inhibitors. Biphenyl substituted L-iodo-configured deoxinojirimycin derivative (**9**) is known as selective for glucosylceramidase and the non-lysosomal glucosylceramidase, and considered as lead for the treatment of neuropathological lysosomal storage disorders, whereas their D-gluco counterpart (**10**) is also a potent inhibitor of intestinal glycosidases and regarded as a prime candidate for type-2 diabetes therapeutics [49].

4.2.2. Mechanism of action of α-glucosidase inhibitors

Diabetes is a group of metabolic diseases characterized by high concentration of glucose in blood. A glycosidic bond is a covalent chemical bond that connects two simple sugars by means of an oxygen atom. Many polysaccharides are formed by monosaccharides joined byα or β-glycosidic bonds. During the digestion process these bonds are hydrolyzed by α-glucosidase liberating the carbohydrate subunits as nutrients.

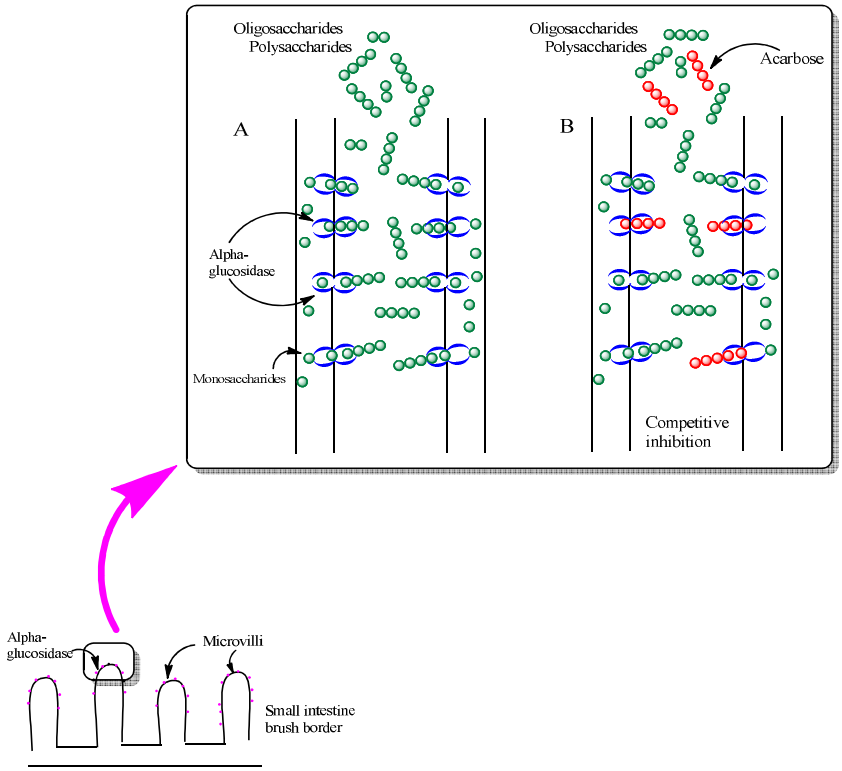


Figure 2. Mechanism of action of α-glucosidase inhibitors.

The mechanism of action is based on the competitive inhibition of enzymes required for the digestion of carbohydrates (**Figure 2**). By competitive and reversible inhibition of intestinal α-glycosidases, acarbose slows down carbohydrate digestion and prolongs the overall carbohydrate digestion time, thus, reducing the rate of glucose absorption leading to the suppression of post-prandial hyperglycaemia [50, 51].

4.3. Earlier methods of synthesis of triazole

Sugar triazoles have earlier been prepared by the reaction of propargyl derivatives and azides under 1,3-dipolar cycloaddition reactions or click conditions. Few of the important methods are discussed methods below:

4.3.1. Synthesis of 1-glycosyl-4-phenyl triazoles

Rossi et al.[52]have reported the synthesis of 1-glycosyl-4-phenyltriazoles attached to the anomeric carbon of glycosyl unit. The glycohybrid with galactose unit showed a moderate inhibition of *Escherichia coli* β-galactosidase (ECG), while the glycohybrid with glucose unit showed better inhibitionof bovine liver galactosidase (BLG). These glycohybrids were synthesized by copper(I) catalyzed [3+2] cycloaddition reaction of the appropriate acetyl-protected β-azidoglycosides with phenylacetylene. The acetylated analogue when subjected to Zemplen deprotection involving afforded the required triazolyl glycohybrids as single regioisomers (**Figure 3**).

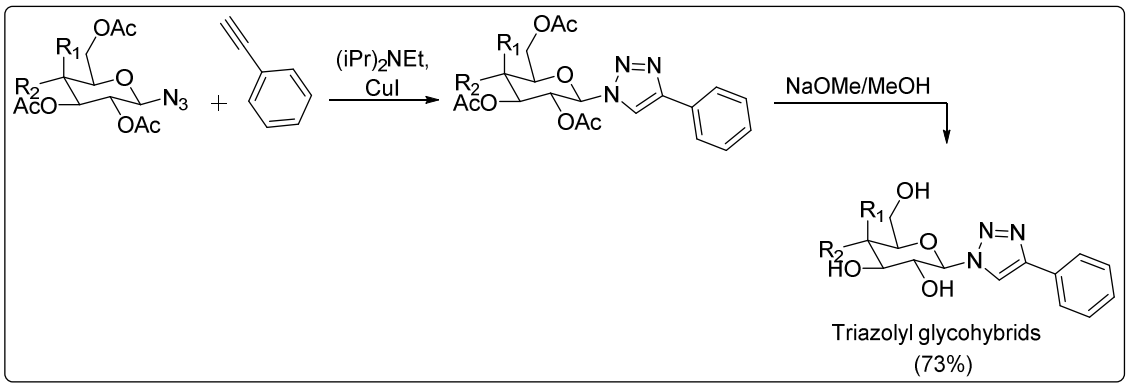


Figure 3

4.3.2. Synthesis of septanosyl 1,2,3-triazoles

Castro et al.[53] have synthesized a family of septanosyl1,2,3-triazoles from a carbohydratebased oxepine in four steps. Epoxidation of the starting oxepine was done bydimethyldioxirane (DMDO) followed by trapping of the intermediate 1,2-anhydroseptanose with sodium azide, to give the β-substituted glycosyl azide. The obtained septanosylazide was then reacted with different alkynes under thermal Huisgen dipolar cycloaddition or copper(I) catalyzed reaction. Hydrogenolysis of the obtained benzyl protected analogues resulted in the formation of required substituted

septanosyl-triazoles(**Figure 4**). The compounds were evaluated for various glycosidases, but no inhibition was observed up to a concentration of 1mM. The lack of activity in septanosyl-triazoles prevented authors a substantive analysis on the importance of seven-membered ring for glycosidase inhibitors.

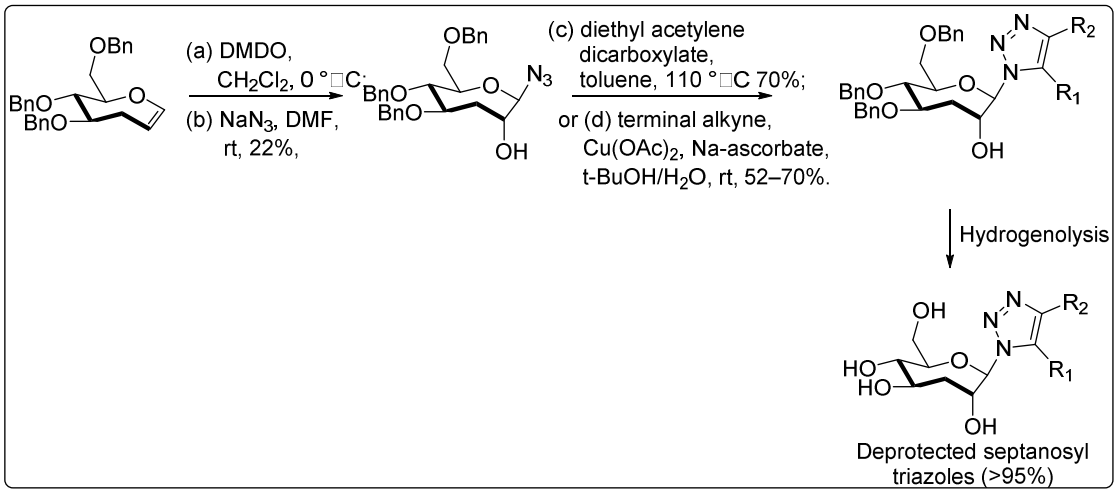


Figure 4

4.3.3.Synthesis of novel 1,2,3-1*H*-triazolyl glycohybrids

Tripathi et al.[54, 55] have reported the synthesis of coumarin based triazolyl glycohybrids using CuAAC reaction of 4-*O*-propargylcoumarin with glycosyl azides in fair to good yields (**Figure 5**).The propargyloxy coumarin was prepared by reaction of 4-hydroxy coumarin with propargyl bromide, in presence of K_2CO_3 and TBAB as catalyst in acetone at room temperature for 4-5h. The synthesizd compounds were evaluated for *α-glucosidase* enzyme inhibition and found to show good inhibitory activity at 100 μM.

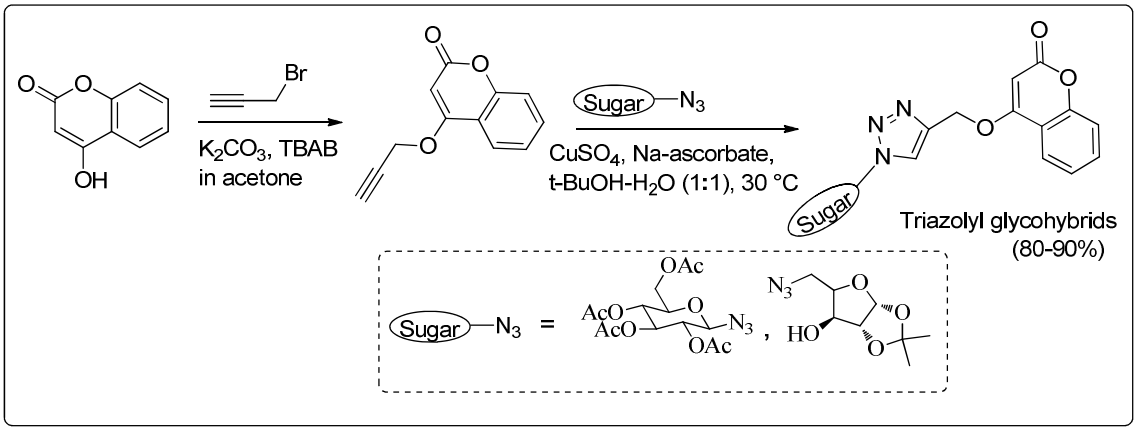


Figure 5

4.3.4. Synthesis of triazole-fused iminosugars

Heightman et al.[56] have reported the synthesis of triazole fused iminosugars. The ring opened alkyne isomers of sugar were accomplished in three steps involving the oxidation of tri-*O*-benzyl-L-xylofuranoseto the aldonolactone followed by addition of (trimethylsilyl)ethynyl-lithium and subsequent reduction of the resulting hemiacetals with sodium borohydride. The ring opened alkyne isomers were regioselectivelybenzylated followed by tosylation and further treated the resulting tosylates with sodium azide in DMSO at 110 ºC; to give the benzyl protected triazolo iminosugar *via* intramolecular cycloaddition of the intermediate azidoalkynes. Debenzylation of the resulting compounds gave the desired compounds(**Figure 6**). These compounds were evaluated for glycosidase inhibitory effect, and the compound with *gluco* configuration showed the best activity with an IC_{50} value of 2 mM for the *β*-glucosidase.

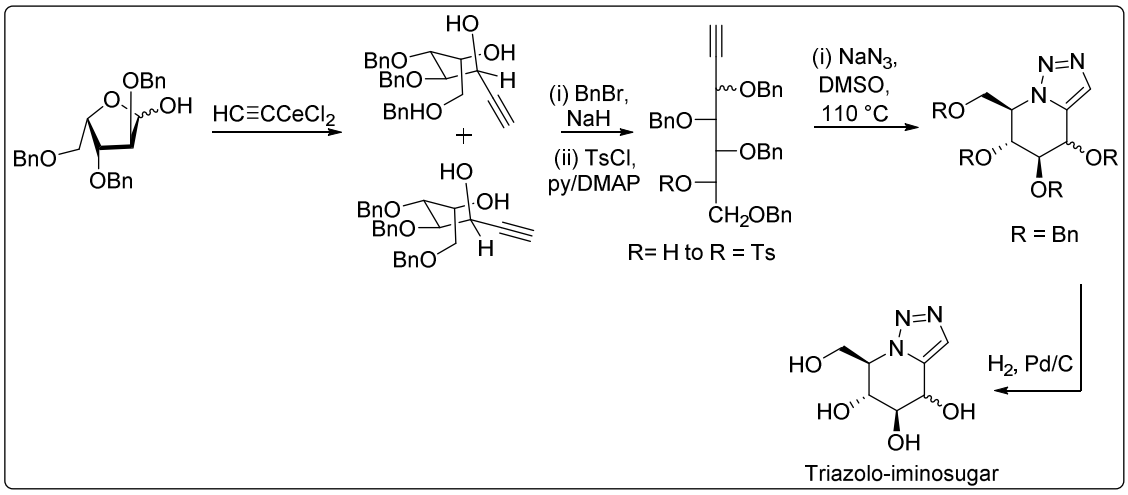


Figure 6

4.3.5. Synthesis of oxazolidinones containing glycosyltriazoles under sonication

Zhang et al.[57] has reported by an efficient, practical and convenient synthesis of 1,2,3-triazole-fused oxazolidinone-carbohydrate conjugates via sonication as shown in (**Figure 7**).

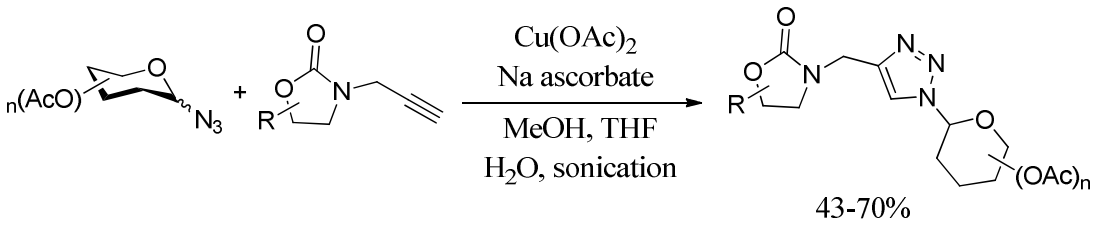


Figure 7

4.3.6. One-pot synthesis of *O*-glycosyltriazolesfrom D-glucal.

Saeeng et al.[58] has reported an efficient and convenient method for the synthesis of 2,3-unsaturated-glycosyl triazoles via tandem *O*-glycosylation using iodine promoter and a mild copper-catalyzed click reaction as shown in (**Figure8**).

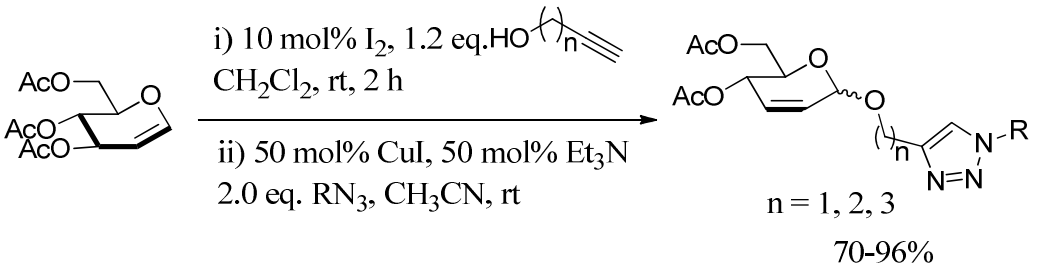


Figure 8

4.3.7. Synthesis of furano-triazolesby copper (I)-catalyzed 1,3-dipolar cycloaddition of azido alkynes.

Chandrasekhar et al.[59] has reported the construction of furano-triazole macrocyclic compounds from furanose sugar substrates via click conditions as shown in (**Figure9**).

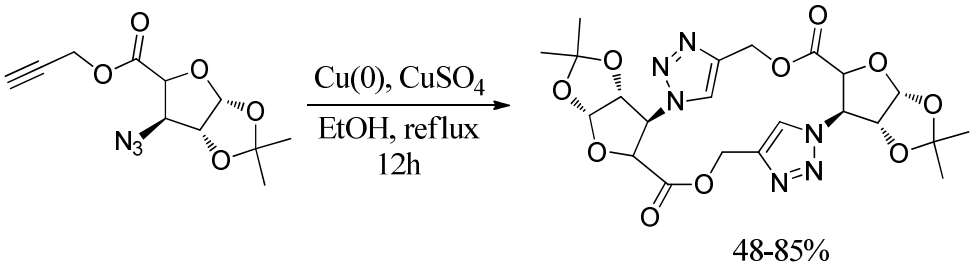


Figure 9

4.3.8. Synthesis of furan-appended glycosyltriazoles

Mukherjee et al.[60]has reported a novel $Cu(OTf)_2$/Cu powder-mediated one-pot reaction for synthesizing furan-based glycosyl triazoles from the D-glucal as shown in (**Figure 10**).This method was very useful in the construction of other highly substituted heterocyclic natural product skeletons.

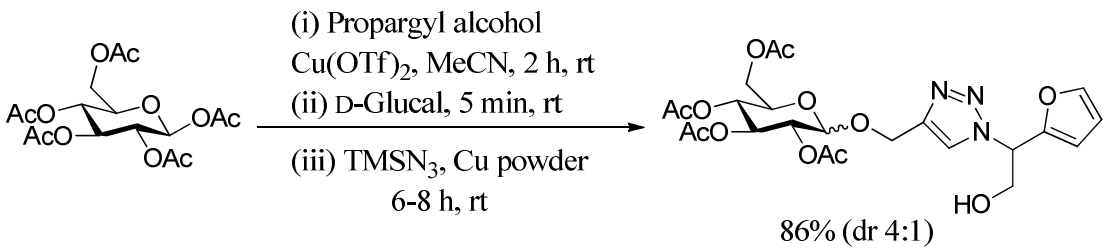


Figure 10

4.3.9. One-pot synthesis of triazole-linked glycoconjugates

Wang et al.[61] has developed a one-pot glycosylation method for the synthesis of 1,2,3-triazole bridged glycoconjugates from readily available unprotected saccharides or saccharide acetates via Cu(I) catalyzed Huisgen 1,3-dipolar cycloaddition reaction as shown in (**Figure 11**). This one-pot process proceeded well in the synthesis of polyvalent glycomolecules such as dendrimers.

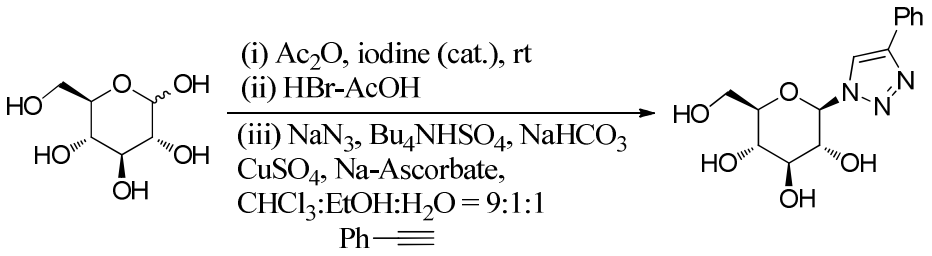


Figure 11

4.3.10. Synthesis of a triazole-linked *C*-disaccharides through the copper(I)-catalyzedazide-alkyne coupling in ionic liquids.

Marra et al.[62]hasreported by the 1,4-disubstituted triazole-linked *C*-disaccharides through the copper(I)-catalyzedazide-alkyne click reaction in ionic liquids (ILs) as shown in (**Figure 12**).

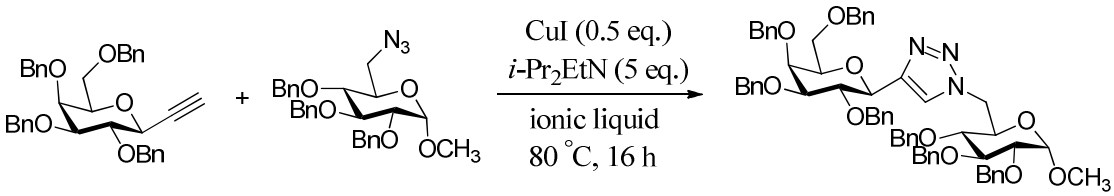


Figure 12

4.3.11. Synthesis of *C*-glycosyl-bis-1,2,3-triazole derivatives from D-glucal

Shamim et al.[63] has developed an efficient and microwave-assisted synthesis of *C*-glycosyl-bis-1,2,3-triazoles from a 3,4,6-tri-*O*-acetyl-D-glucal.These bis-triazole derived glycosides were very attractive for biological evaluation and as synthetic intermediates (**Figure 13**).

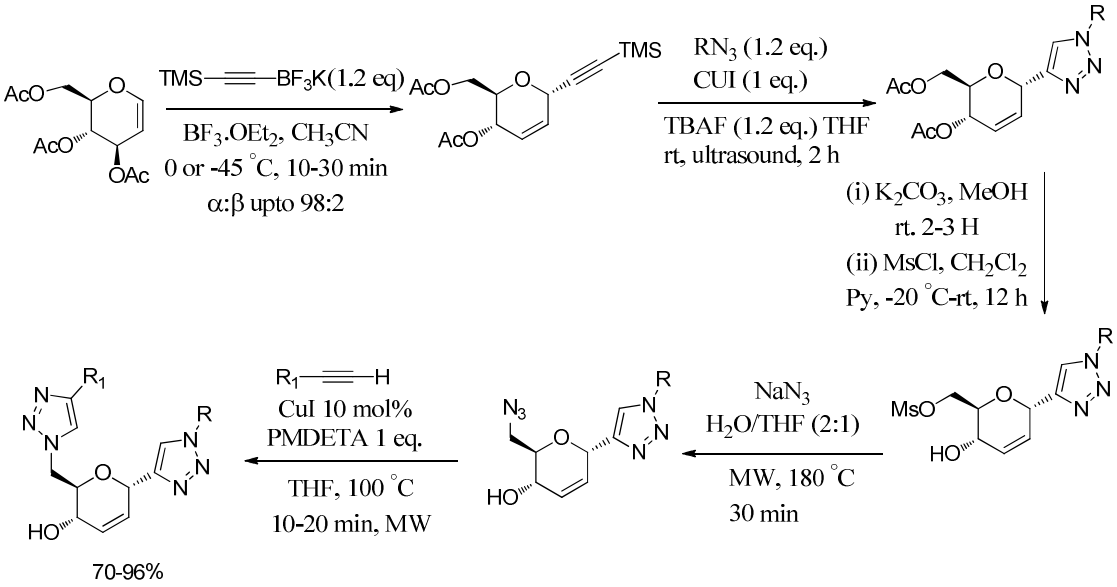


Figure 13

4.3.12. Synthesis of 1,2,3-triazole-fused tetracyclic compounds

Hotha et al [64] has reported the convenient synthesis of 1,2,3-triazole-fused tetracyclic compounds through intramolecular 1,3-dipolar cycloaddition of carbohydrate-derived azido-alkynes (**Figure 14**).

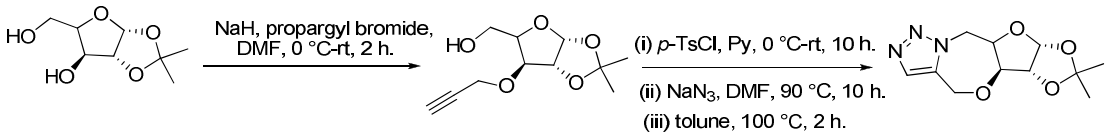


Figure 14

4.3.13. Synthesis of *C*-glycosyltriazoles via 1,3-dipolar cycloaddition reaction

Dondoni et al.[65] has developed the new class of unnatural *C*-glycosyl amino acids featuring a triazole moiety between the sugar and amino acid entities through the 1,3-dipolar cycloaddition reaction (**Figure 15**).

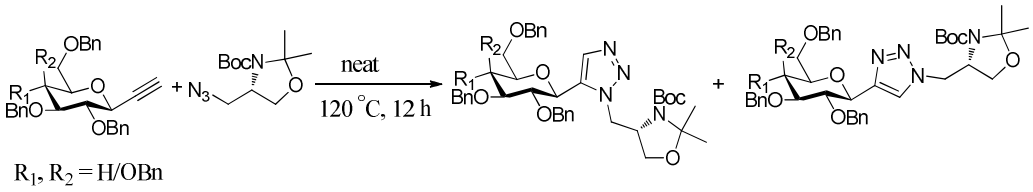


Figure 15

4.3.14. An efficient synthesis of glycosyltriazoles with two or more than two sugar units

Tripathi et al.[54] has developed the novel glycohybrid triazoles using CuAAC reaction of alkynyl glycosides with sugar azides in good yields. A few of the glycohybrids showed promising inhibitory activities against α-glucosidase, glycogen phosphorylase and glucose-6-phosphatase enzymes (**Figure 16**).

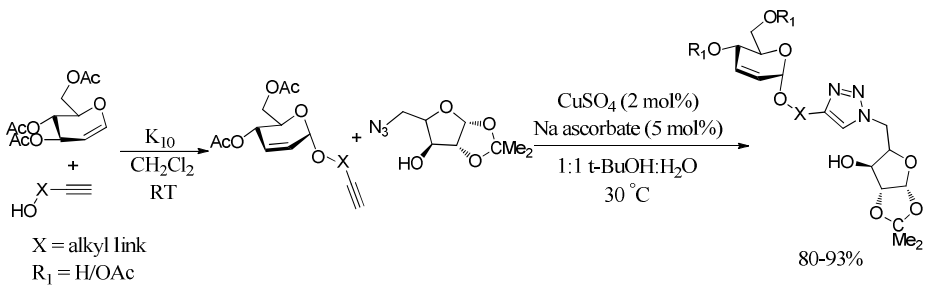


Figure 16

4.3.15. Synthesis of monomeric triazolophanes through the Cu(I)-catalyzed cycloaddition

Bhattacharya et al.[66] has developed a strained monomeric 12 to 17 membered triazolophanes through the Cu(I)-catalyzed intramolecular cycloaddition of an azide and alkyne incorporating an aromatics, furanoses and peptidic moieties (**Figure 17**).

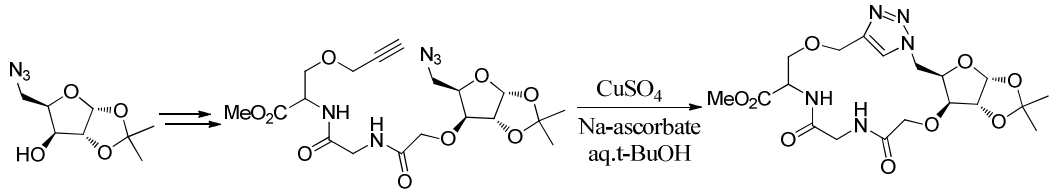


Figure 17

4.3.16. Synthesis of glycosyltriazole benzene sulphonamide

Wilkinson et al.[67] has reported a series of glycosyltriazole benzene sulphonamides from the different sugar azides and 4-ethynyl benzene sulphonamide via the Cu(I)-catalyzed 1,3-dipolar cycloaddition reaction (**Figure 18**).Many of these glycosyltriazoles exhibited potent carbonic anhydrases (CA) inhibitory activity.

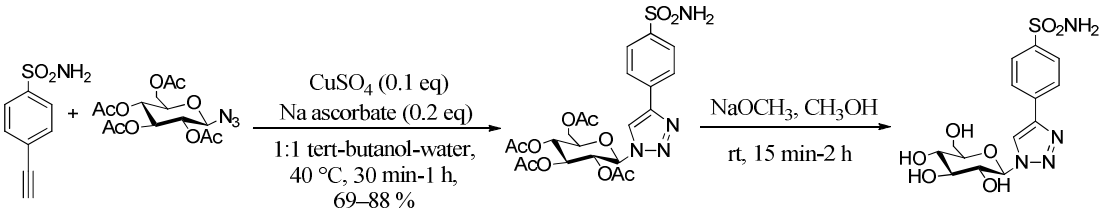


Figure 18

4.4. Copper(I) catalyzed azide-alkyne cycloaddition (CuAAC)

The well-known method to access 1,4-disubstituted 1,2,3-triazoles is the copper-catalyzedazide-alkyne cycloaddition (CuAAC) [68-70], which was first introduced by Meldal[68] and Sharpless [71]. In this reaction, an azide is basically reacted with an

alkyne in a [3+2] Cycloaddition manner using a Cu (I) as catalyst. The azide-alkyne cycloaddition reaction was introduced by Huisgen[72], in which the substrates were heated to give a mixture of both 1,4-disubstituted and 1,5-disubstituted 1,2,3-triazoles. This result led to the contradiction of some of the requirements of "click reaction". In the year 2002, Sharpless and Meldal independently observed the formation of the 1,4-regioisomer when Cu (I) catalyst was used in the azide-alkyne cycloaddition reaction [68, 71]. The Cu (I) catalytic system helps in accelerating the reaction allowing it to proceed at room temperature. The Cu (I) catalyst can be easily generated *in situ* by combining copper (II) sulphate together with sodium ascorbate. Sodium ascorbate acts as a reducing agent, which converts *in situ* copper (II) into copper (I). The regioselective formation of only 1,5-disubstituted 1,2,3-triazole has been also reported by Jia et al.by using Ru (II) catalyst in the cycloaddition (**Figure 19**) [73].

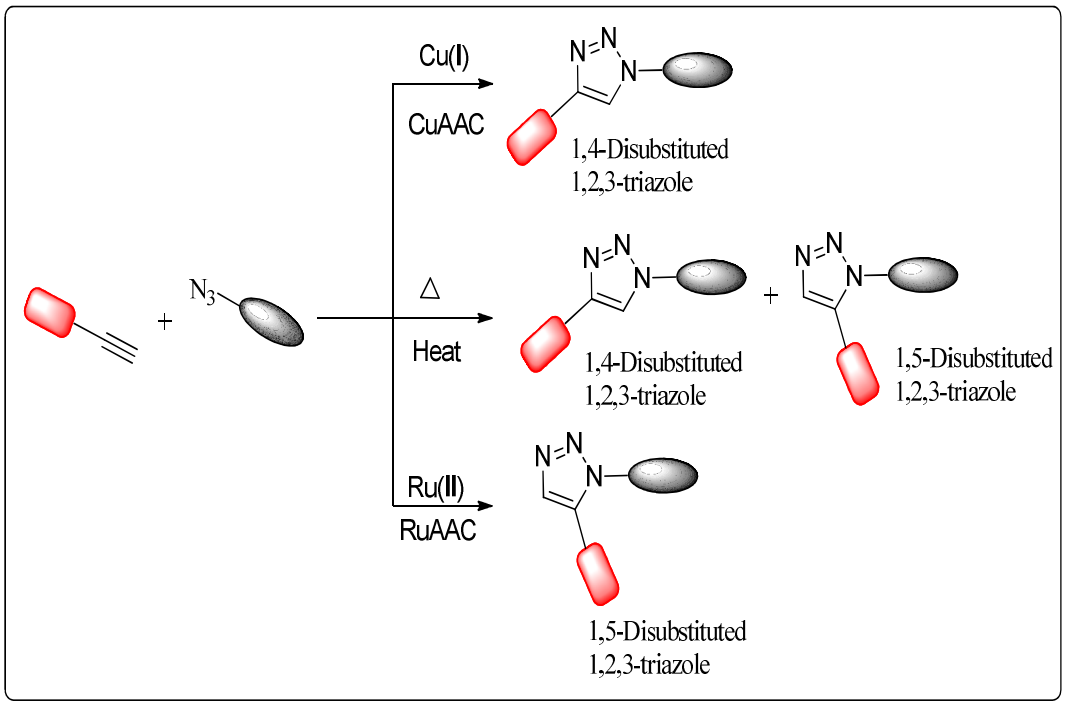


Figure 19: 1,3-Dipolar cycloaddition of azide andalkyne.

4.5. Basis of present work

It is the proven fact in literature that the presence of sugar functionality in drug like molecules alters the bioavailability and efficacy of molecules drastically. Hence, medicinal chemists have tremendous burden of developing new molecules with anti-diabetic potential. We hypothesize to couple terminal sugar moiety with aglycon pharmacophore in order to get better pharmacological property. Keeping the above facts on priority, we were prompted to synthesize a novel series of glycohybrids triazoles, consisting of benzamides, triazole and sugars and their inhibition potential was studied against α-glucosidase enzyme in a quest for new anti-diabetic agents (**Figure 20**).

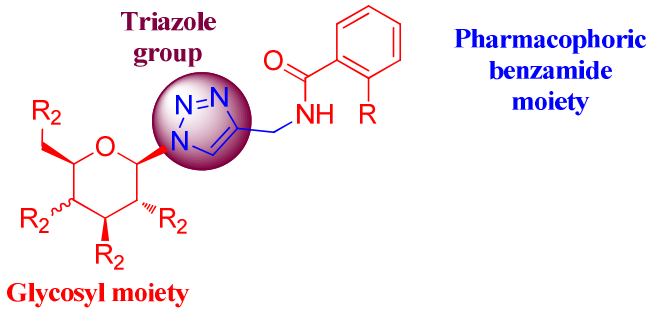


Figure 20. Designing structure for novel glycosyl-triazole benzamides

Compounds having triazolyl unit (1,2,3- and 1,2,4-triazole) possesses important biological activities such as antibacterial, antifungal, anti-tubercular, anti-HIV and anticancer[77]. Several 1,2,3-triazoles have been reported in the literature as potent α-*glucosidase* inhibitors[78, 79].The 1,2,3-triazole unit is the surrogate of peptide bond and imparts advantage to bioactive molecules in terms of stability and biological activity[80] (**Figure 21**).

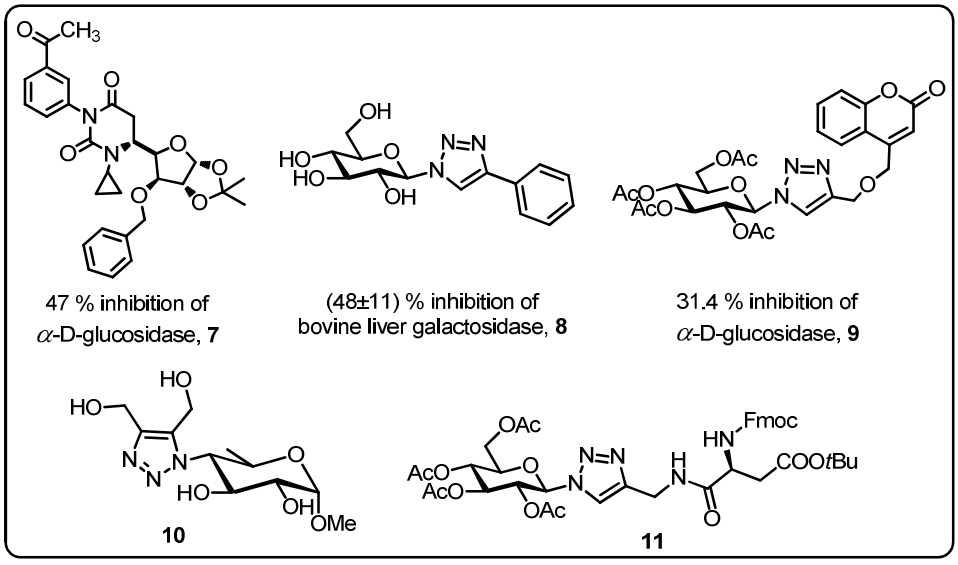


Figure 21: Some of the triazolyl sugar hybrids with significant *α-glucosidase* inhibition.

4.6. Present Work:

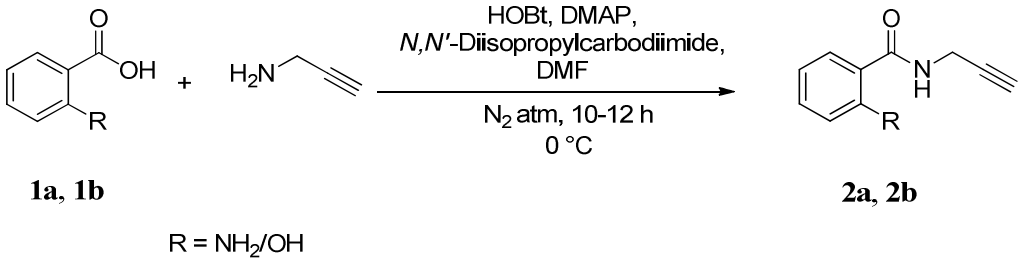
The present study describes the synthesis of novel triazolyl based glycosyl methyl benzamide derivative with different sugar azides in the ambient condition in excellent yields. All the synthesized compounds evaluated for theα-*glucosidase* inhibitory activity. The compounds synthesized in this section are listed below:

2-amino-*N*-(prop-2-yn-1-yl) benzamide **(2a)**,2-hydroxy-*N*-(prop-2-yn-1-yl) benzamide **(2b)**,2-(4-methylphenylsulfonamido)-*N*-(prop-2-yn-1-yl) benzamide **(2c)**,2-amino-*N*-[(1-(1′-deoxy-2′, 3′, 4′, 6′-tetra-*O*-acetyl-*β*-D-glucopyranos-1′-yl)]-1*H*-1, 2, 3-triazol-4-yl) methyl-benzamide **(3a)**,2-amino-*N*-[(1-(1′-deoxy-2′, 3′, 4′, 6′-tetra-*O*-acetyl-*β*-D-galactopyranose-1′-yl)]-1*H*-1, 2, 3-triazol-4-yl) methyl)-benzamide **(3b)**,2-hydroxy-*N*-[(1-(1′-deoxy-2′, 3′, 4′, 6′-tetra-*O*-acetyl-*β*-D-glucopyranos-1′-yl)]-*1H*-1,2,3-triazol-4′-yl)methyl-benzamide **(3c)**,2-hydroxy-*N*-[(1-(1′-deoxy-2′,3′,4′,6′-tetra-*O*-acetyl-*β*-D-galactopyranose-1′-yl)]-*1H*-1, 2, 3-triazol-4-yl)methyl-benzamide**(3d)**,4-methyl-*N*-[(1-(1′-deoxy-*β*-D-glucopyranos-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzenesulphonamide(**3e**),4-methyl-*N*-[(1-(1′-deoxy-*β*-D-

galactopyranose-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzenesulphonamide **(3f)**,2-amino-*N*-[(1-(1′-deoxy-*β*-D-glucopyranos-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzamide **(4a)**,2-amino-*N*-[(1-(1′-deoxy-*β*-D-galactopyranose-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzamide**(4b)**,2-hydroxy-*N*-[(1-(1′- deoxy-*β*-D-glucopyranos-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzamide**(4c)**,2-hydroxy-*N*-[(1-(1′-deoxy-*β*-D-galactopyranose-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzamide **(4d)**,4-methyl-*N*-[(1-(1′-deoxy-*β*-D-glucopyranos-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzenesulphonamide **(4e)**,4-methyl-*N*-[(1-(1′-deoxy-*β*-D-galactopyranose-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzenesulphonamide **(4f)**.

4.7. Synthesis of 2-amino-benzamide

2-Amino-*N*-propargyl benzamides (**2a** and **2b**) were prepared from commercially available anthranilic acid (**1a**) and 2-hydroxy-benzoic acid (**1b**) following earlier reported protocols[81] as shown in **Scheme 1**. The structures were established on the basis of their spectroscopic data[82]. The flask was degassed and then filled with N_2 (balloon), and HOBt (1mmol), 4- dimethylaminopyridine (DMAP, 1 mmol) were added to the reaction mixture. Then *N*,*N*'-Diisopropylcarbodiimide (DIPC, 1 mmol) was added to the reaction mixture in dropwise manner. After 10 mins, propargyl amine (1.1 mmol) was added and the reaction was stirred overnight at ambient temperature to get the desired 2-amino-*N*-(prop-2-yn-1-yl) benzamide (**2a**) &2-hydoxy-*N*-(prop-2-yn-1-yl) benzamide (**2b**) in good yield respectively (**Scheme 1**).

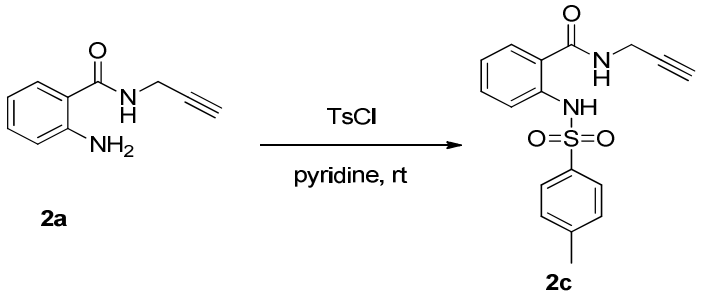


Scheme 1. Synthesis of 2-amino-*N*-propargyl benzamide derivatives.

The structure of the product **2a** was established by their spectroscopic data. ESIMS of the compound displays *m/z* = 175 as $[M+H]^+$ peak corresponding to its molecular formulae $C_{10}H_{11}N_2O$. In IR spectrum, characteristic absorption peaks observed at 3372 cm^{-1} for amine, 3019 cm^{-1} for -NH, 1648 cm^{-1} for carbonyl (-NHC=O). In the ^1H NMR spectrum, the two exchangeable NH_2 protons were observed at δ 5.43 (bs, 2H, $-NH_2$) and the amide -NH proton was visible at δ 6.25 (bs, 1H, -NH) while the alkynyl proton was visible at δ 2.16 besides other usual protons at their usual chemical shift. In ^{13}C NMR spectrum, the peaks at δ 168.8 accounted the amide group carbon (-NH-CO-) along with other usual signals. Similarly, the reaction of 2-hydroxy-benzoic acid (**1b**) with propargyl amine the above reaction conditions led to the formation of 2-hydroxy-*N*-(prop-2-yn-1-yl) benzamide (**2b**) in 88% yield (**Scheme 1**).

4.7.3.Synthesis of 2-(4-methylphenylsulfonamido)-*N*-(prop-2-yn-1-yl) benzamide (2c)

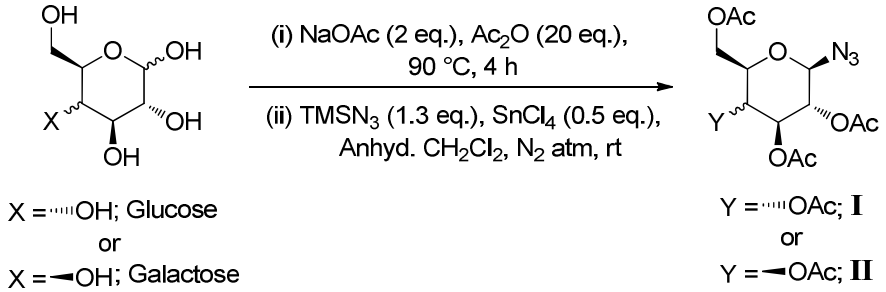
2-(4-methylphenylsulfonamido)-*N*-(prop-2-yn-1-yl) benzamide (**2c**) were prepared by following earlier reported protocol[83] as shown in **Scheme 2**. To a solution of the 2-amino-*N*-(prop-2-yn-1-yl) benzamide (**2a**, 1.0 equiv) in pyridine (1 M) at room temperature, *p*-toluenesulfonyl chloride (1.05 equiv) was added. The reaction mixture was allowed to stir at room temperature overnight. The reaction mixture was quenched with water, and the aqueous layer was extracted with dichloromethane. The combined organic extracts were washed with aqueous copper sulphate. The organic layer was then dried over magnesium sulphate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography to afford the desired 2-(4-methylphenylsulfonamido)-*N*-(prop-2-yn-1-yl) benzamide (**2c**) in 79% yield (**Scheme 2**).



Scheme 2. Synthesis of 2-(4-methylphenylsulfonamido)-N-(prop-2-yn-1-yl) benzamide derivative.

4.7.4. Synthesis of glycosyl azide and triazolylbenzamide

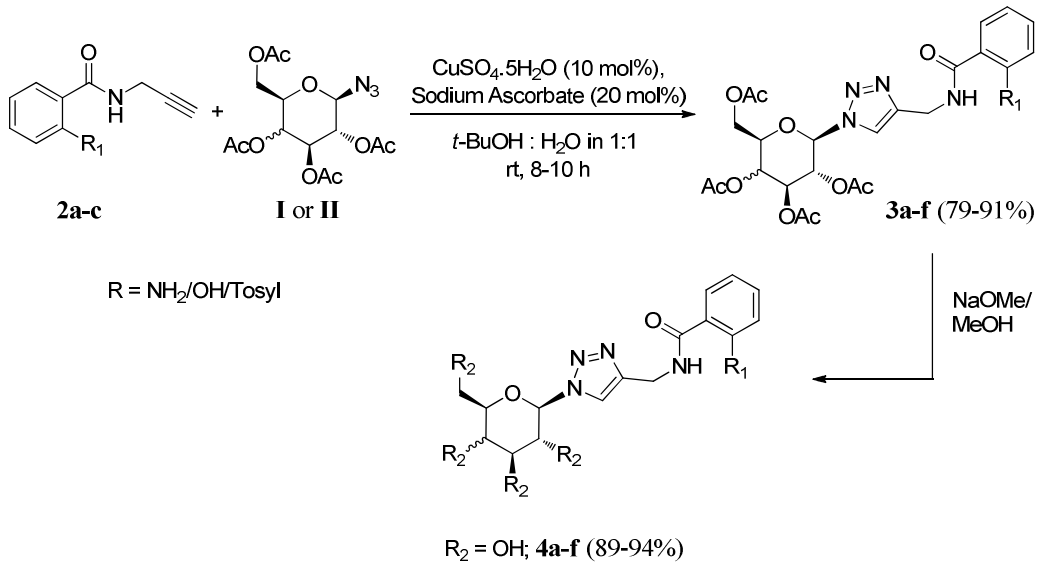
The glycosylazides (**I** and **II**) were prepared from commercially available glucose and galactose following the methods already reported[84] in the literature as shown in **Scheme 3**. The structures were established on the basis of their spectroscopic data. These were identical in all respects to those reported earlier[85, 86].



Scheme 3. Synthesis of the glycosylazides

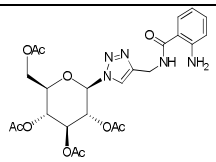
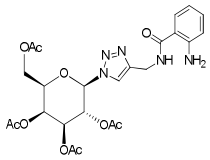
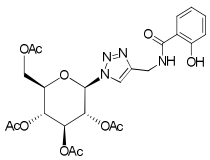
The strategy for the synthesis of glycosyltriazolylbenzamide is depicted in **Scheme 4**. Having the 2-phenyl-3-propargyl-benzamide (**2c**) and glycosylazides (**I** and **II**) in our hand the CuAAC reactions were performed in *t*-BuOH/H_2O (1:1) using equimolar quantities of the reagents, $CuSO_4.5H_2O$ (10 mol%) and sodium ascorbate (20 mol%) at ambient temperature to afford epimeric mixtures of peracetyl glycosyl triazolylbenzamide (**3a-f**) in good yields (**Scheme 4**, **Table 1**). Propargylbenzamides

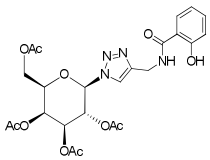
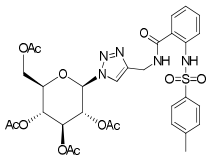
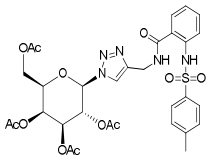
and glycosylazidesselectively gave only one regioisomer, 1,4-disubstituted triazole via 1,3-dipolar cycloaddition reaction.



Scheme 4. Synthesis of glycosyl benzamide

Table 1. Peracetylatedglycosyltriazolyl2-amino-benzamide (**4a-f**) from glycosyl azides and 2-amino-benzamide.

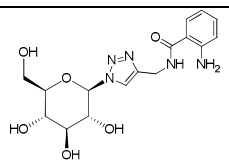
Entry	2-amino-benzamide	Glycosyl Azide	Peracetylatedglycosylbenzamide	Yield (%)[a]
1	**2a**	**I**	 **3a**	80
2	**2a**	**II**	 **3b**	78
				

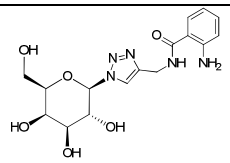
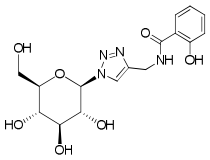
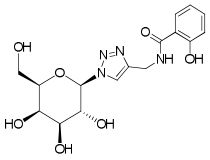
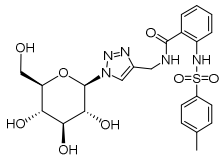
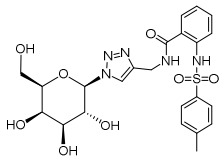
3	**2b**	**I**	**3c**	77
4	**2b**	**II**	 **3d**	75
5	**2c**	**I**	 **3e**	73
6	**2c**	**II**	 **3f**	71

[a] *Isolated yield as pure product*

The Zemplen deacetylation of the above peracetylatedglycosyltriazolyl2-amino-benzamide (**3a-f**) with NaOMe/MeOH at room temperature led to the formation of the deacetylatedglycosyltriazolyl2-amino-benzamide (**4a-f**) respectively in good yields (**Scheme 4**,**Table 2**).

Table 2: Synthesized deacetylated compounds **4a-f**from peracetylatedglycosyltriazolyl2-amino benzamide (**3a-f)**

Entry	**Peracetylated substrates**	**Deacetylated products**	**Yield (%)[a]**
1	**3a**	 **4a**	94

2	**3b**	 **4b**	92
3	**3c**	 **4c**	93
4	**3d**	 **4d**	91
5	**3e**	 **4e**	92
6	**3f**	 **4f**	90

[a] *Isolated yield as pure product*

4.8.Experimental section

Commercially available reagent grade synthetic compounds were utilized as got. Each and every reaction were monitored by TLC on E. Merck Kieselgel 60 F254, with detection by UV light, spraying 20% alkaline $KMnO_4$ solution as well as spraying with 4% ethanolic H_2SO_4. Column chromatography was performed on Silica Gel (60–120 mesh, E. Merck). IR spectra were recorded as thin films or in KBr solution with a Perkin–Elmer Spectrum RX-1 (4000–450 cm^{-1}) spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on Bruker DRX 400 MHz, and 100 MHz

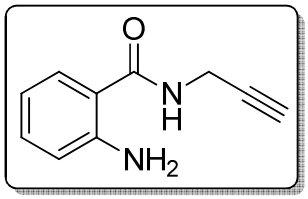
instruments, respectively, in $CDCl_3$ and DMSO-d_6. The chemical shift values are reported in ppm with respect to TMS (tetramethylsilane) as the internal reference, unless otherwise generally expressed; s (singlet), d (doublet), t (triplet), dd (double doublet), m (multiplet); J in Hertz. ESI mass spectra were performed utilizing a Quattro II (Micromass) instrument. Optical rotations were estimated in a 1.0-dm tube with a Rudolf Autopol III and Horiba polarimeters in $CHCl_3$ and MeOH.

4.8.1. General procedure for the compounds 2a-b

Anthranilic acid/2-hydroxy-benzoic acid (**1a, 1b**, 1 mmol) was added to a 100 mL round bottom flask in dry *N*,*N*-dimethylformamide (20 mL) equipped with ice bath. The flask was degassed, then filled with N_2 (balloon), and HOBt (1mmol), 4-dimethyl amino pyridine (DMAP, 1 mmol) added to the reaction mixture. Then *N*,*N'*-Diisopropylcarbodiimide(DIPC, 1 mmol) was added to the solution in drop wise. After 10 mins, propargyl amine (1.1 mmol) was added to the reaction mixture and stirred overnight at ambient temperature. The crude mixture was taken up in water (50 mL) and extracted with ethyl acetate (3 × 40 mL). The combined organic extracts were washed with brine (50 mL), dried over sodium sulphate, filtered, concentrated, and purified by column chromatography (1:1 EtOAc:Hexanes) to afford desired compounds **2a-b**.

4.8.2. 2-amino-*N*-(prop-2-yn-1-yl) benzamide (2a)

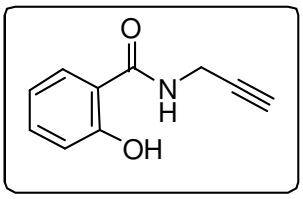
It was obtained by the reaction of anthranilic acid **1a** (5.0 g, 36.49 mmol) with HOBt (4.92 g, 36.49 mmol), DMAP (4.45 g, 36.49 mmol), DIPC (5.67 mL, 36.49 mmol) and propargyl amine (2.56 mL, 40.14 mmol) in dry DMF (20 mL) in 89% yield (5.65 g) as a white solid; mp 87 ºC; IR (ν_{max}, cm^{-1}): 3372, 3019,1648, 1586, 1403, 1215 and 757.^{1}H NMR (400 MHz, $CDCl_3$+CCl_4): δ 7.24 (m, 1H, Ar-H), 7.12 (m, 1H, Ar-H),



6.57 (m, 2H, Ar-H), 6.25 (bs, 1H, -NH), 5.43 (bs, 2H, $-NH_2$), 4.10 (m, 2H, $-CH_2$), 2.16 (m, 1H, -CH). ^{13}C NMR (100 MHz, $CDCl_3$+CCl_4): δ 168.8 (C=O), 148.9 (Ar-C), 132.6 (Ar-C), 127.2 (Ar-C), 117.3 (Ar-C), 116.5 (Ar-C), 114.9 (Ar-C), 79.7, 71.7 (-CH), 29.4 ($-CH_2$). M.F: $C_{10}H_{11}N_2O$ $[M+H]^+$; ESIMS: m/z 175.

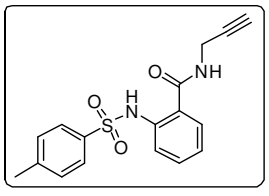
4.8.3. 2-hydroxy-*N*-(prop-2-yn-1-yl) benzamide (2b)

It was obtained by the reaction of salicylic acid **1b** (5.0 g, 34.70 mmol) with HOBt (4.88 g, 34.70 mmol), DMAP (4.41 g, 34.70 mmol), DIPC (5.32 mL, 34.70 mmol) and propargyl amine (2.46 mL, 34.12 mmol) in dry DMF (20 mL) in 88% yield (5.42 g) as a white solid; mp 86-88 ºC; IR (ν_{max}, cm^{-1}): 3372, 3019, 1648, 1586, 1403, 1215 and 757.^1H NMR (400 MHz, $CDCl_3$+CCl_4): δ 7.24 (m, 1H, Ar-H), 7.12 (m, 1H, Ar-H), 6.57 (m, 2H, Ar-H), 6.25 (bs, 1H, -NH), 5.43 (bs, 2H, $-NH_2$), 4.10 (m, 2H, $-CH_2$), 2.16 (m, 1H, -CH). ^{13}C NMR (100 MHz, $CDCl_3$+CCl_4): δ 168.8 (C=O), 148.9 (Ar-C), 132.6 (Ar-C), 127.2 (Ar-C), 117.3 (Ar-C), 116.5 (Ar-C), 114.9 (Ar-C), 79.7, 71.7 (-CH), 29.4 ($-CH_2$). M.F: $C_{10}H_9NO_2$ $[M+H]^+$; ESIMS: m/z 175.



4.8.4. 2-(4-methylphenylsulfonamido)-*N*-(prop-2-yn-1-yl) benzamide (2c)

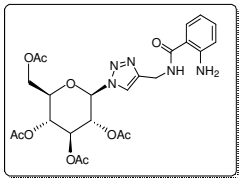
It was obtained by the reaction the 2-amino-*N*-(prop-2-yn-1-yl) benzamide (**2a**, 2.0 g, 13.28 mmol) in pyridine (5ml) at room temperature with *p*-toluenesulfonyl chloride (5.05 ml) wad added. The reaction mixture was allowed to stir at room temperature overnight and extracted with DCM to give the crude product, which was purified by column chromatography (20% EtOAc/Hexane) to give the title compound (**2c**) in79% yield (g) as a white solid; mp 87 ºC; IR (ν_{max}, cm^{-1}): 3376, 3028, 1644, 1307, 1215, 1173, 907 and 669.^1H NMR (400 MHz, $CDCl_3$+CCl_4): δ7.76 (bs, 1H, -NH), 7.54 (m, 2H, Ar-H), 7.32 (m, 2H, Ar-H), 7.12 (m, 2H, Ar-H), 6.94(m, 2H, Ar-H), 4.10 (m, 2H, -



CH_2), 2.36 (m, 3H, -CH_3), 2.16 (m, 1H, -CH). ^{13}C NMR (100 MHz, $CDCl_3$+CCl_4): δ 169.3 (C=O), 149.7 (Ar-C), 132.8 (Ar-C), 127.2 (2C, Ar-C), 118.3 (2C, Ar-C), 117.5 (Ar-C), 115.9 (Ar-C), 79.7, 71.7 (-CH), 29.3 (-CH_2), 21.6 (-CH_3);M.F: $C_{17}H_{16}N_2O_3S$ $[M+H]^+$; ESIMS: m/z 329.09.

4.8.5. 2-amino-*N*-[(1-(1′-deoxy-2′, 3′, 4′, 6′-tetra-*O*-acetyl-*β*-D-glucopyranos-1′-yl)]-1*H*-1, 2, 3-triazol-4-yl) methyl-benzamide (3a)

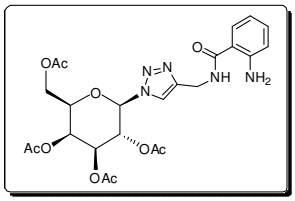
It was obtained by the reaction of **2a** (0.50 g, 1.89 mmol), azido sugar **I** (0.72 g, 1.89 mmol), $CuSO_4.5H_2O$ (0.042 g, 0.16 mmol) and sodium ascorbate (0.071 g, 0.35 mmol) in 1:1 tert-Butanol-water (40 mL) in 80% yield (1.03 g) as a white solid; mp 106-108 ºC; IR (ν_{max}, cm^{-1}): 3290, 2349, 1607, 1523, 1245, 1217, 1182, 758 and 669.^{1}H NMR (400 MHz, $CDCl_3$+CCl_4): δ 7.83 (s, 1H, triazolyl-H), 7.35 (m, 1H, Ar-H), 7.26 (m, 1H, Ar-H), 6.81 (bs, 1H, -NH), 6.66 (m, 2H, Ar-H), 5.82 (d, *J* = 9.2 Hz, 1H, H-1´), 5.52-5.47 (m, 2H, H-2´, H-3´), 5.25 (m, 1H, H-4´), 4.74 (m, 2H, -CH_2), 4.24-4.09 (m, 2H, H-5´, H-6′), 2.22 (s, 3H, -$OCOCH_3$), 2.03 (s, 3H, -$OCOCH_3$), 2.00 (s, 3H, -$OCOCH_3$), 1.87 (s, 3H, -$OCOCH_3$).^{13}C NMR (100 MHz, DMSO-d_6): δ 170.4 (C=O), 170.3 (C=O), 169.9 (C=O), 168.9 (C=O), 150.2 (C=O), 146.3(Ar-C), 132.3 (Ar-C), 128.6 (Ar-C), 122.7 (Ar-C), 116.8 (Ar-C), 115.0 (Ar-C), 114.6, 84.6, 73.4, 70.9 (-CH), 68.0, 67.8, 62.0, 34.8, 20.9(-CH_2), 20.8(-CH_3), 20.7(-CH_3), 20.4(-CH_3). HRMS: Calcd. Accurate mass for ($C_{24}H_{29}N_5O_{10}$): 548.1914. Found 548.1956 $[M+H]^+$.



4.8.6. 2-amino-*N*-[(1-(1′-deoxy-2′, 3′, 4′, 6′-tetra-*O*-acetyl-*β*-D-galactopyranose-1′-yl)]-1*H*-1, 2, 3-triazol-4-yl) methyl)-benzamide (3b)

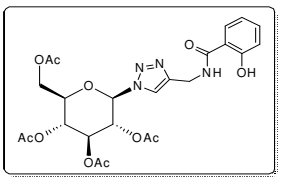
It was obtained by the reaction of **2a** (0.50 g, 1.89 mmol), azido sugar **II** (0.72 g, 1.89 mmol), $CuSO_4.5H_2O$ (0.042 g, 0.16 mmol) and sodium ascorbate (0.071 g, 0.35 mmol) in 1:1 tert-butanol-water (40 mL) in 78% yield (1.08 g) as a yellow solid; mp

118-120 ºC; IR (ν_{max}, cm^{-1}): 3292, 2365, 1610, 1535, 1230, 1216, 1184, 771 and 669.^{1}H NMR (400 MHz, $CDCl_3$+CCl_4): δ 7.88 (s, 1H, triazolyl-H), 7.37 (m, 1H, Ar-H), 7.20 (m, 1H, Ar-H), 6.91 (bs, 1H, -NH), 6.66 (m, 2H, Ar-H), 5.82 (d, *J* = 9.2 Hz, 1H, H-1´), 5.52-5.47 (m, 2H, H-2´, H-3´), 5.25 (m, 1H, H-4´), 4.74 (m, 2H, -CH_2), 4.24-4.09 (m,2H, H-5´, H-6′), 2.22 (s, 3H, -$OCOCH_3$), 2.03 (s, 3H, -$OCOCH_3$), 2.00 (s, 3H, -$OCOCH_3$), 1.87 (s, 3H, -$OCOCH_3$).^{13}C NMR (100 MHz, DMSO-d_6): δ 170.4 (C=O), 170.3 (C=O), 169.9 (C=O), 168.9 (C=O), 150.2 (C=O), 146.3 (Ar-C), 132.3 (Ar-C), 128.6(Ar-C), 122.7 (Ar-C), 116.8 (Ar-C), 115.0 (Ar-C), 114.6 (Ar-C), 84.6, 73.4, 70.9 (-CH), 68.0, 67.8, 62.0, 34.8(-CH_2), 20.9(-CH_3), 20.8(-CH_3), 20.7(-CH_3), 20.4(-CH_3). HRMS: Calcd. Accurate mass for ($C_{24}H_{29}N_5O_{10}$): 548.1914. Found 548.1952 $[M+H]^+$.



4.8.7. 2-hydroxy-*N*-[(1-(1′-deoxy-2′, 3′, 4′, 6′-tetra-*O*-acetyl-*β*-D-glucopyranos-1′-yl)]-*1H*-1,2,3-triazol-4′-yl) methyl-benzamide (3c)

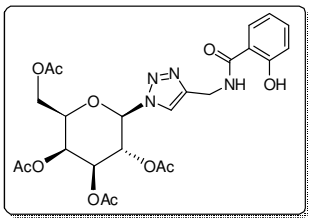
It was obtained by the reaction of **2b** (0.53 g, 1.85 mmol), azido sugar **I** (0.73 g, 1.89 mmol), $CuSO_4.5H_2O$ (0.042 g, 0.16 mmol) and sodium ascorbate (0.071 g, 0.35 mmol) in 1:1 tert-Butanol-water (40 mL) in 77% yield (1.06 g) as a white solid; mp 111-113 ºC; IR (ν_{max}, cm^{-1}): 3449, 2395, 1620, 1534, 1230, 1218, 1185 and 754.^{1}H NMR (400 MHz, DMSO-d_6): δ 12.40 (m, 1H, -NH), 9.22 (s, 1H, triazolyl-H), 7.37 (m, 1H, Ar-H), 7.20 (m, 1H, Ar-H), 6.66 (m, 2H, Ar-H),6.20 (m, 1H, -OH), 5.82 (d, *J* = 9.2 Hz, 1H, H-1´), 5.52-5.47 (m, 2H, H-2´, H-3´), 5.25 (m, 1H, H-4´), 4.74 (m, 2H, -CH_2), 4.24-4.09 (m, 2H, H-5´, H-6′), 2.22 (s, 3H, -$OCOCH_3$), 2.03 (s, 3H, -$OCOCH_3$), 2.00 (s, 3H, -$OCOCH_3$), 1.87 (s, 3H, -$OCOCH_3$).^{13}C NMR (100 MHz, DMSO-d_6): δ 170.0, 169.5, 169.2, 168.4(4 × -$COCH_3$), 160.8(-CO), 145.4(Ar-C), 133.8(Ar-C), 128.0(Ar-C), 122.4(Ar-C), 118.6(Ar-C), 117.6(Ar-C), 115.2(Ar-C), 96.0(C-1′), 84.4(C-5′),



79.0(C-3′), 78.6(C-4′), 73.9(C-2′), 72.8(C-6′), 70.5, 67.9, 61.9, 40.3(-CH_2), 39.8, 34.8,20.8, 20.6, 20.5, 20.2(4 × -$OCOCH_3$); HRMS: Calcd. Accurate mass for ($C_{24}H_{28}N_4O_{11}$): 549.1755. Found 549.1792 $[M+H]^+$.

4.8.8. 2-hydroxy-*N*-[(1-(1′-deoxy-2′,3′,4′,6′-tetra-*O*-acetyl-*β*-D-galactopyranose-1′-yl)]-*1H*-1, 2, 3-triazol-4-yl)methyl-benzamide (3d)

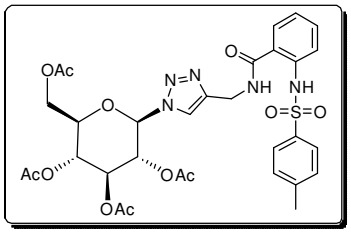
It was obtained by the reaction of **2b** (0.53 g, 1.85 mmol), azido sugar **II** (0.73 g, 1.89 mmol), $CuSO_4.5H_2O$ (0.042 g, 0.16 mmol) and sodium ascorbate (0.071 g, 0.35 mmol) in 1:1 tert-Butanol-water (40 mL) in 75% yield (1.07 g) as a yellow solid; mp 120-122 ºC; IR (ν_{max}, cm^{-1}): 3434, 2381, 1625, 1537, 1226, 1217, 1184, 753 and 659.^{1}H NMR (400 MHz, $CDCl_3$): δ 12.18(m, 1H, -NH), 7.90 (s, 1H, triazolyl-H), 7.53 (m, 1H, Ar-H), 7.47 (m, 1H, Ar-H), 7.37 (m, 2H, Ar-H),6.82 (m, 1H, -OH), 5.83(d, *J* = 9.2 Hz, 1H, H-1´), 5.52-5.47 (m, 2H, H-2´, H-3´), 5.26 (m, 1H, H-4´), 4.78 (m, 2H, -CH_2), 4.24-4.09 (m, 2H, H-5´, H-6′), 2.22 (s, 3H, -$OCOCH_3$), 2.03 (s, 3H, -$OCOCH_3$), 2.00 (s, 3H, -$OCOCH_3$), 1.87 (s, 3H, -$OCOCH_3$).^{13}C NMR (100 MHz, $CDCl_3$): δ 169.9, 169.7, 168.4, 168.7(4 × -$COCH_3$), 161.7(-CO), 144.8(Ar-C), 134.1(Ar-C), 125.8(Ar-C), 121.3(Ar-C), 118.5(Ar-C), 114.0(Ar-C), 86.3(C-1′), 77.2(C-5′), 76.9(C-3′), 74.0(C-4′), 70.6(C-2′), 68.0(C-6′), 34.6(-CH_2), 20.5, 20.5, 20.4, 20.1(4 × -$OCOCH_3$); HRMS: Calcd. Accurate mass for ($C_{24}H_{28}N_4O_{11}$): 549.1755 Found 549.1796 $[M+H]^+$.



4.8.9. 4-methyl-*N*-[(1-(1′-deoxy-*β*-D-glucopyranos-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzene sulphonamide (3e)

It was obtained by the reaction of **2c** (0.1 g, 0.66 mmol), azido sugar **I** (0.25 g, 0.66 mmol), $CuSO_4.5H_2O$ (0.02 g, 0.06 mmol) and sodium ascorbate (0.03 g, 0.13 mmol)

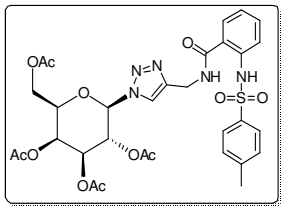
in 1:1 tert-butanol-water (20 ml) in 73% yield (0.32 g) as a white solid; m.p. 86-88ºC;IR (ν_{max}, cm^{-1}): 3245, 2371, 1658, 1510, 1309, 1227, 1169 and 768.^{1}H NMR



(400 MHz, DMSO-d_6): δ 10.74 (s, 1H, -NH), 7.91 (s, 1H, triazolyl-H), 7.61 (m, 3H, Ar-H), 7.40 (m, 2H, Ar-H), 7.26 (m, 2H, Ar-H), 7.11 (m, 2H, Ar-H), 6.98 (m, 2H, Ar-H), 5.88 (d, *J* = 9.1 Hz, 1H, H-1´),5.48-5.42 (m, 2H, H-2´, H-3´), 5.26 (m, 1H, H-4´), 4.60 (m, 2H, -CH_2),4.29-4.12 (m, 2H, H-5´, H-6′),2.32 (s, 3H, -$OCOCH_3$), 2.06(s, 3H, -$OCOCH_3$), 2.03(s, 3H, -$OCOCH_3$), 1.86(s, 3H, -$OCOCH_3$).^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2(-CO), 169.7(-CO), 169.0(-CO), 168.6(-CO), 168.3(-CO), 143.3, 139.0 (Ar-C), 136.6 (Ar-C), 132.6 (Ar-C), 129.4 (Ar-C), 127.2, 127.0 (Ar-C), 123.3 (Ar-C), 121.0, 120.9 (Ar-C), 96.1, 85.8, 77.2, 76.9, 76.6, 75.2, 72.6, 70.4, 67.6, 61.3, 35.1, 21.4, 20.5, 20.4, 20.1. HRMS: Calcd. Accurate mass for ($C_{31}H_{35}N_5O_{12}S$): 702.2003. Found 702.2042 $[M+H]^+$.

4.8.10.4-methyl-*N*-[(1-(1′-deoxy-*β*-D-galactopyranose-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzene sulphonamide (3f)

It was obtained by the reaction of **2c** (0.1 g, 0.66 mmol), azido sugar **II** (0.25 g, 0.66 mmol), $CuSO_4.5H_2O$ (0.02 g, 0.06 mmol) and sodium ascorbate (0.03 g, 0.13 mmol)

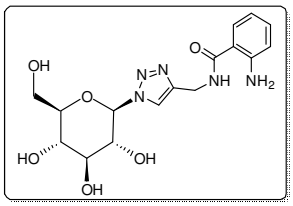


in 1:1 tert-butanol-water (20 ml) in 71% yield (0.30 g) as a white solid; m.p. 86-88ºC;IR (ν_{max}, cm^{-1}): 3242, 2374, 1661, 1512, 1307, 1228, 1163 and 768.^{1}H NMR (400 MHz, DMSO-d_6): δ 10.74 (s, 1H, -NH), 7.94 (s, 1H, triazolyl-H), 7.64 (m, 3H, Ar-H), 7.43 (m, 2H, Ar-H), 7.26 (m, 2H, Ar-H), 7.13 (m, 2H, Ar-H), 6.99 (m, 2H, Ar-H), 5.85 (d, *J* = 9.1 Hz, 1H, H-1´),5.57-5.52 (m, 2H, H-2´, H-3´), 5.26 (m, 1H, H-4´), 4.63 (m, 2H, -CH_2), 4.25-4.19 (m, 2H, H-5´, H-6′), 2.32 (s, 3H, -$OCOCH_3$), 2.23 (s, 3H, -$OCOCH_3$), 2.01 (s, 3H, -$OCOCH_3$), 1.88 (s, 3H, -$OCOCH_3$).^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0,

169.8, 169.5, 168.8(4 × -$COCH_3$), 168.3 (-CO), 144.4 (Ar-C), 143.3 (Ar-C), 139.0 (Ar-C), 136.7 (Ar-C), 132.6 (Ar-C), 129.4 (Ar-C), 127.3 (Ar-C), 127.2 (Ar-C), 127.0 (Ar-C), 123.3, 121.4 (Ar-C), 121.0, 121.0 (Ar-C), 96.1, 86.3, 77.2, 76.9, 76.6, 74.1, 70.7, 68.0, 66.7, 61.0, 42.1, 35.1, 23.5, 21.4, 20.1, 20.5, 20.4, 20.2. HRMS: Calcd. Accurate mass for ($C_{31}H_{35}N_5O_{12}S$): 702.2003. Found 702.2045 $[M+H]^+$.

4.8.11. 2-amino-*N*-[(1-(1′-deoxy-*β*-D-glucopyranos-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzamide (4a)

It was obtained by treating the acetylated compound **3a** (0.40 g, 0.63 mmol) with NaOMe in methanol, in 94% yield (0.27 g) as a white solid p 83-85 ºC;$[\alpha]_D^{25}$ 0.4 (c

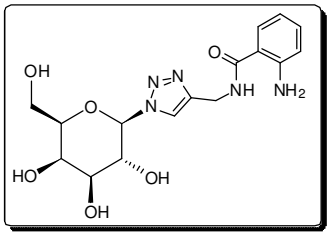


0.1, CH_3OH); IR (ν_{max}, cm^{-1}): 3411, 3019, 1638, 1215 and 758.^{1}H NMR (400 MHz, DMSO-d_6): δ 8.74 (m, 1H, -NH), 8.08 (s, 1H, triazolyl-H), 7.53 (d, J = 6.9 Hz, 1H, Ar-H), 7.16 (m, 1H, Ar-H,), 6.70 (d, J = 8.0 Hz, 1H, Ar-H), 6.53 (t, *J* = 7.7 Hz, 1H, Ar-H), 6.41 (bs, 2H, -NH_2), 5.51 (d, J = 9.3 Hz, 1H, H-1´), 5.38 (m, 1H, -OH), 5.26 (m, 1H, -OH), 5.17 (m, 1H, -OH),4.68 (m, 1H, -OH), 4.48 (m, 2H, -CH_2), 3.78-3.59 (m, 4H, H-2′, H-3′, H-4′, H-5′), 3.25 (m, 2H, H-6′).^{13}C NMR (100 MHz, DMSO-d_6): δ 169.3 (C=O), 150.1 (Ar-C), 145.8 (Ar-C), 132.3 (Ar-C), 128.6 (Ar-C), 122.4(Ar-C), 116.8 (Ar-C), 115.1 (Ar-C), 114.5 (Ar-C), 87.8 (C-1´), 80.3 (C-5´), 77.4 (C-3´), 72.4 (C-4´), 69.9 (C-2´), 61.1 (C-6´), 34.8 (-CH_2). HRMS: Calcd. Accurate mass for ($C_{16}H_{21}N_5O_6$): 380.1492. Found 380.1531 $[M+H]^+$.

4.8.12.2-amino-*N*-[(1-(1′-deoxy-*β*-D-galactopyranose-1′-yl)]-*1H*-1,2,3-triazol-4-yl) methyl-benzamide (4b)

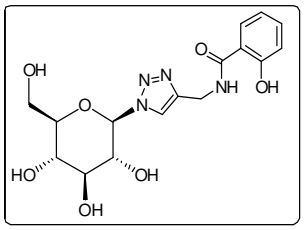
It was obtained by treating the acetylated compound **3b** (0.40 g, 0.63 mmol) with NaOMe in methanol, in 92% yield (0.26 g) as a white solid; mp64-66 ºC;$[\alpha]_D^{25}$ 1.3 (c 0.1, CH_3OH); IR (ν_{max}, cm^{-1}): 3400, 3019, 1644, 1215 and 769.^{1}H NMR (400 MHz,

DMSO-d_6): δ 8.77 (m, 1H, -NH), 8.04 (s, 1H, triazolyl-H), 7.53 (m, 1H, Ar-H), 7.16 (m, 1H, Ar-H), 6.70 (m, 1H, Ar-H), 6.53 (m, 1H, Ar-H), 5.47 (d, *J* = 9.1 Hz, 1H, H-1´), 4.48 (m, 2H, -CH_2), 4.02 (m, 2H, H-5′, -OH), 3.75 (m, 3H, 3 × -OH), 3.70 (m, 4H, H-2′, H-3′, H-4´, H-6´), 3.17 (bs, 2H, -NH_2).^{13}C NMR (100 MHz, DMSO-d_6): δ 169.3 (C=O), 150.1 (Ar-C), 145.9 (Ar-C), 132.3, 128.6 (Ar-C), 122.0, 116.9 (Ar-C), 115.1 (Ar-C), 114.5 (Ar-C), 88.4 (C-1´), 78.8 (C-5´), 74.1 (C-3´), 69.8 (C-4´), 68.9 (C-2´), 60.8 (C-6′), 34.9.HRMS: Calcd. Accurate mass for ($C_{16}H_{21}N_5O_6$): 380.1492. Found 380.1528 $[M+H]^+$.



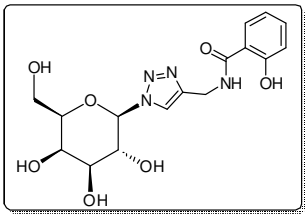
4.8.13.2-hydroxy-*N*-[(1-(1′-deoxy-*β*-D-glucopyranos-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzamide (4c)

It was obtained by treating the acetylated compound **3c** (0.40 g, 0.60 mmol) with NaOMe in methanol in 93% yield (0.27 g) as a white solid; mp 84-86 ºC;$[\alpha]_D^{25}$ – 2.4 (c 0.1, CH_3OH); IR (ν_{max}, cm^{-1}): 3670, 3401, 3018, 1644, 1215 and 757.^{1}H NMR (400 MHz, DMSO-d_6): δ 8.74 (m, 1H, -NH), 7.94 (s, 1H, triazolyl-H), 7.53 (m, 1H, Ar-H), 7.15 (m, 1H, Ar-H), 6.72 (m,1H, Ar-H), 6.52 (m, 1H, Ar-H), 6.43 (bs, 1H, -OH), 5.88 (d, J = 9.3 Hz, 1H, H-1´), 5.38 (m, 1H, -OH), 5.26 (m, 1H, -OH), 5.17 (m, 1H, -OH),4.68 (m, 1H, -OH), 4.48 (m, 2H, -CH_2), 3.78-3.59 (m, 3H, H-2′, H-3′, H-4′), 3.25 (m, 2H, H-5′, H-6′)^{13}C NMR (100 MHz, DMSO-d_6): δ 169.2(C=O), 150.1(Ar-C), 145.8(Ar-C), 132.2(Ar-C), 128.6(Ar-C), 123.8(Ar-C), 116.8(Ar-C), 114.7(Ar-C), 111.2, 104.9, 85.4, 79.6, 74.0, 49.2, 34.9(-CH_2), 27.0, 26.HRMS: Calcd. Accurate mass for ($C_{18}H_{23}N_5O_5$): 390.1699. Found 390.1735 $[M+H]^+$.



4.8.14.2-hydroxy-*N*-[(1-(1′-deoxy-*β*-D-galactopyranose-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzamide (4d)

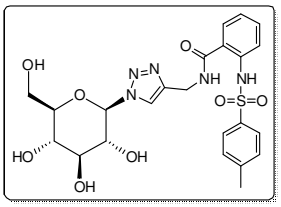
It was obtained by treating the acetylated compound **3d** (0.40 g, 0.58 mmol) with NaOMe in methanol in 91% yield (0.26 g) as a yellow solid; mp 73-75 ºC;$[\alpha]_D^{25}$ – 1.6



(c 0.1, CH_3OH); IR (ν_{max}, cm^{-1}): 3408, 3019, 1753, 1644, 1215 and 769.^{1}H NMR (400 MHz, DMSO-d_6): δ 12.43 (s, 1H, -NH), 9.35 (s, 1H, triazolyl-H), 8.13 (s, 1H, Ar-H), 7.90 (m, 1H, Ar-H), 7.43 (m, 1H, Ar-H), 7.39 (m, 1H, Ar-H), 6.92 (d, *J* = 9.1 Hz, 1H, H-1´), 5.48 (m, 2H, -CH_2), 5.21 (m, 1H, -OH), 4.59 (m, 2H, H-5′, -OH), 4.09 (m, 3H, 3 × -OH), 3.75-3.34 (m, 5H, H-2′, H-3′, H-4´, H-6´), ^{13}C NMR (100 MHz, DMSO-d_6): δ 169.2(-CO), 160.3(Ar-C), 144.9(Ar-C), 134.2(Ar-C), 128.4(Ar-C), 122.2(Ar-C), 119.1(Ar-C), 117.8(Ar-C), 115.7(Ar-C), 88.5(C-1′), 78.8(C-5′), 74.1(C-3′), 69.7(C-4′), 68.9(C-2′), 60.8(C-6′), 40.6(-CH_2). HRMS: Calcd. Accurate mass for ($C_{16}H_{20}N_4O_7$): 381.1332. Found 381.1372 $[M+H]^+$.

4.8.15. 4-methyl-*N*-[(1-(1′-deoxy-*β*-D-glucopyranos-1′-yl)]-*1H*-1,2,3-triazol-4-yl) methyl-benzenesulphonamide (4e)

It was obtained by treating the acetylated compound **3e** (0.40 g, 0.59 mmol) with NaOMe in methanol in 92% yield (0.26 g) as a white solid; mp 88-90 ºC;$[\alpha]_D^{25}$ – 1.2

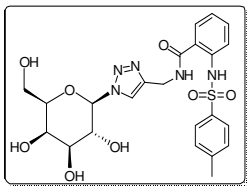


(c 0.1, CH_3OH); IR (ν_{max}, cm^{-1}): 3408, 3019, 1753, 1644, 1215 and 769.^{1}H NMR (400 MHz, DMSO-d_6): δ 11.56 (s, 1H, -NH), 9.33 (s, 1H, triazolyl-H), 8.17 (s, 1H, Ar-H), 7.74 (m, 1H, Ar-H), 7.63 (m, 2H, Ar-H), 7.46 (m, 2H, Ar-H), 7.30 (m, 2H, Ar-H),7.11 (d, *J* = 9.1 Hz, 1H, H-1´), 5.55 (m, 2H, -CH_2), 5.52 (m, 1H, -OH), 4.50 (m, 2H, H-5′, -OH), 3.80 (m, 3H, 3 × -OH), 3.76-3.67 (m, 4H, H-2′, H-3′, H-4´, H-6´),2.31 (s, 3H, -CH_3).^{13}C NMR (100 MHz, DMSO) δ 168.5 (C=O), 144.6 (Ar-C), 144.3 (Ar-C), 138.8(Ar-C), 136.0 (Ar-C), 133.1 (Ar-C), 130.3 (Ar-C), 128.9,

127.2 (Ar-C), 123.8, 122.7 (Ar-C), 119.9 (Ar-C), 87.8, 80.3, 77.4, 72.4, 70.0, 40.4, 40.2, 40.0, 39.8, 39.8, 39.5, 39.3, 39.1, 35.1, 21.4. HRMS: Calcd. Accurate mass for ($C_{23}H_{27}N_5O_8S$): 534.1580. Found 534.1621 $[M+H]^+$.

4.8.16.4-methyl-*N*-[(1-(1′-deoxy-*β*-D-galactopyranose-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzenesulphonamide (4f).

It was obtained by treating the acetylated compound **3f** (0.40 g, 0.59 mmol) with NaOMe in methanol in 90% yield (0.25 g) as a white solid; mp 83-85 ºC;$[\alpha]_D^{25}$ – 5.4



(c 0.1, CH_3OH); IR (ν_{max}, cm^{-1}): 3408, 3019, 1753, 1644, 1215 and 769.^{1}H NMR (400 MHz, DMSO-d_6): δ 11.60 (s, 1H, -NH), 9.34 (s, 1H, triazolyl-H), 8.15 (s, 1H, Ar-H), 7.75 (m, 1H, Ar-H), 7.63 (m, 2H, Ar-H), 7.51 (m, 2H, Ar-H), 7.31 (m, 2H, Ar-H), 7.13 (d, *J* = 9.1 Hz, 1H, H-1´), 5.51 (m, 2H, -CH_2), 4.51 (m, 1H, -OH), 4.08 (m, 2H, H-5′, -OH), 3.78(m, 3H, 3 × -OH), 3.57-3.45 (m, 4H, H-2′, H-3′, H-4´, H-6´),2.31 (s, 3H, -CH_3).^{13}C NMR (100 MHz, DMSO) δ 168.5 (C=O), 144.6 (Ar-C), 144.3(Ar-C), 138.9 (Ar-C), 136.1 (Ar-C), 133.1 (Ar-C), 130.3 (Ar-C), 128.9 (Ar-C), 127.2 (Ar-C), 123.8 (Ar-C), 122.3 (Ar-C), 120.6 (Ar-C), 120.0 (Ar-C), 88.5, 78.8, 74.2, 69.8, 68.8, 60.8, 49.0, 40.5, 40.3, 40.1, 39.9, 39.7, 39.5, 39.3, 35.2, 23.7, 21.4. HRMS: Calcd. Accurate mass for ($C_{23}H_{27}N_5O_8S$): 534.1580. Found 534.1618 $[M+H]^+$.

4.9. Biological evaluation

Screening of all the synthesized glycosyl 1,2,3-1H-triazolyl methyl benzamide derivatives were screened for the *α-glucosidase* enzyme inhibition. The compounds dissolved in DMSO were evaluated for their inhibitory potential against the *α-glucosidase* enzyme at 10 μM. Determination of percentage of inhibition was done with respect to control. The results of *α-glucosidase* enzyme inhibitory activity of all the compounds have been shown in **Table 3** and evidently, most of the triazolo-methyl benzamide derivative with deacetylated sugar unit displayed better *α-*

*glucosidase*enzyme inhibition than that of analogues with acetylated sugar unit. Among all the compounds screened, 6 compounds (**4a, 4b, 4c, 4d, 4e** and **4f)** showed promising α-glucosidase enzyme inhibitory activities (**Table 3**). The deacetylated glycosyl methyl benzamide derivative**4f** showed most promising activity with 42.9% inhibition against *α-glucosidase* enzyme while the acetylated glycosyl methyl benzamide derivative**3d** also exhibited good inhibition (26.9%) of enzyme. The deacetylated compounds **4d** and **4c** proved to be potent *α-glucosidase* enzyme inhibitors with 40.6% and 39.7% inhibition, respectively. Other two deacetylated analogues **4b** and **4a** showed 36.9% and 35.7% moderate inhibition of the enzyme, respectively. Compounds **4e** showed enzyme inhibitory activity with 24.6% inhibition, respectively.

Table 3:*α-glucosidase*inhibitory activity (10 μM) of the synthesized benzamide analogues

S.No.	**Code**	% *α-glucosidase* inhibition
1.	**3a**	5.18
2.	**3b**	6.21
3.	**3c**	22.7
4.	**3d**	26.9
5.	**3e**	15.7
6.	**3f**	17.3
7.	**4a**	**35.7**
8.	**4b**	**36.9**
9.	**4c**	**39.7**
10.	**4d**	**40.6**
11.	**4e**	**24.6**
12.	**4f**	**42.9**
13.	**Acarbose**	**53.4**

4.9.1. Materials & Methods:

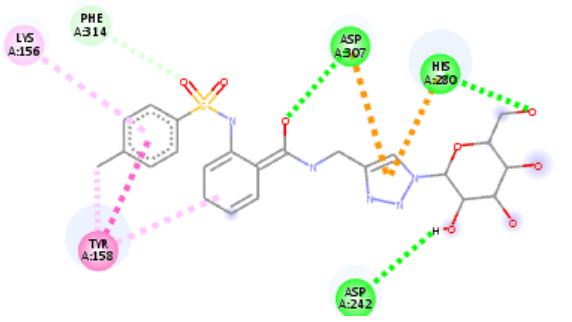
α-glucosidase inhibitory activity of test compounds was measured following the modified method as described by Pistia- Brueggeman and Hollingsworth (2001)[87].The desired test compound in 100 μl of 10% DMSO was pre-incubated with 500 μl of purified *α-glucosidase* in phosphate buffer (50 mM, pH 6.8) for 10 min at 37 ºC in a shaking water bath before the addition of 200 μl of glutathione (1.0 mg/ml and 200 μl of p-nitro-phenylglucoside and incubation at 37 ºC for a further period of 20 min. The enzyme reaction was stopped by addition of 500 μl of sodium bicarbonate solution (100 mM) and the color of p-nitro-phenol formed was measured at 405 nm in a double beam spectrophotometer (Thermo). The % inhibition on *α-glucosidase* was calculated as below:

% inhibition = ΔOD test sample/ ΔODcontrol x 100 – 100

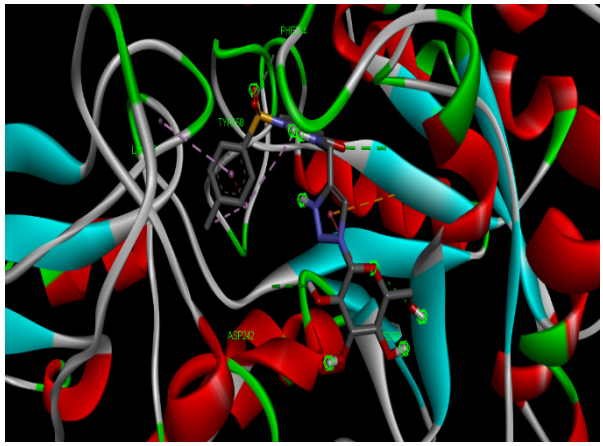
4.10. Molecular docking study

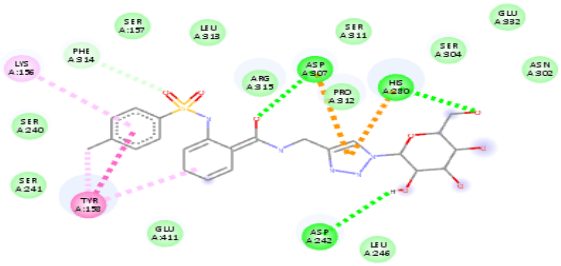
Active sites on the *α-glucosidase*(PDB Id: 3A4A) is determined using online tool CASTp3.0. Aspartic acid (Asp) 215 and glutamate (Glu) 277 are located in active site of *α-glucosidase* which are characterized as nucleophilic and proton donor respectively. Active site of the enzyme identified as mutagenic site in some amino acids which are present at located of (Asp) 215, (VAL) 216, (Gly) 217 and (Ser) 218.Docking energy calculated with Autodock 4.2.6, best-fit docked model with lowest minimum binding energy of (ΔG) has been chosen to reveal the molecular interaction between acarbose and *α* glucosidase. As well as our designed molecule study and we have found that compound **4c, 4d** and **4f**binding energy is calculated-9.4, -9.5 and -8.4 kcal/mol, whereas, acarbose interacted at the active site of *α-glucosidase* with ΔG of -8.7 kcal/mol. Interaction between active site of *α-glucosidase* enzyme with designed molecule, **4c** was interacted (LYS) 156, (PHE) 314, (TYR) 158, (ASP) 357, (PRO) 312, (HIS) 280, and (ASP) 242, **4d**was interacted

(LYS) 156, (ARG) 315, (PHE) 314, (HIS) 280, (ASP) 307 and (TRY) 158, **4f** was interacted (SER) 311, (PRO) 312, (GLN) 279, (GLU) 277, (TYR) 72, (ARG) 442, (ASP) 352 and (TYR) 158.(**Figure 2**) The other surrounding amino acids depicted hydrophobic and Pi-Pi interaction with **4c**,**4d** and **4f** compound. Therefore, *in silico* molecular docking results of **4c**, **4d**and **4f**arepromised with the results obtained in enzyme kinetic study *in vitro*.

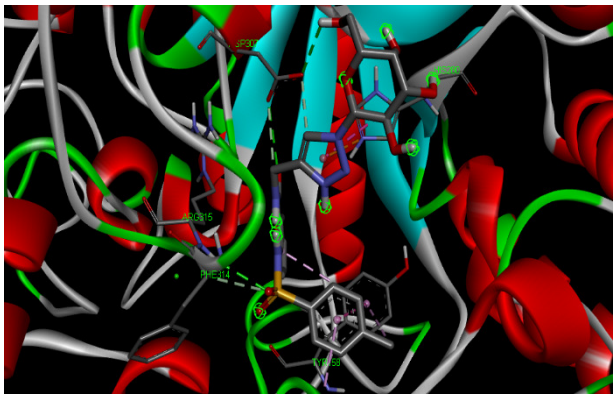


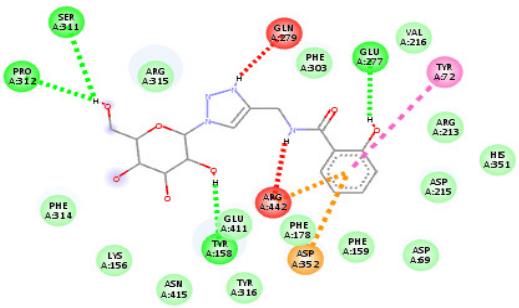
4c





4d





4f

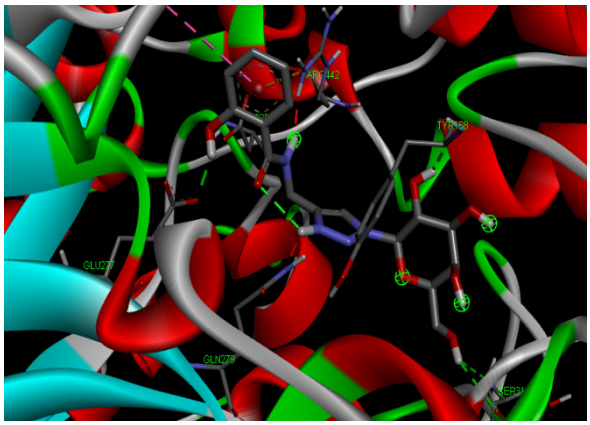


Figure 2. 2D & 3D Representation of the interactions between *α-glucosidase* and glycosyl 1,2,3-1H-triazolyl methyl benzamide (**4c**, **4d and 4f**)

4.11. Conclusion

In conclusion, we have synthesized novel glycosyl methyl benzamide analogues with 1,4-regioselectivity employing the well-known CuAAC reaction of the propylated benzamides with different sugar azides in ambient condition in very good yields. These compounds were evaluated for α-glucosidase enzyme inhibitory activity and three compounds **4f**, **4d** and**4c** exhibited 42.9%, 40.6%, and 39.7%inhibition, respectively as compared to standard drug acarbose having 53.4 % inhibition of the enzyme. Thus, these glycosyl methyl benzamide analogues hold potential to be developed as antidiabetic agents.

4.12. REFERENCES:

[1] Grant RW, Devita NG, Singer DE, Meigs JB. Polypharmacy and medication adherence in patients with type-2 diabetes. Diabetes care. 2003;26:1408-12.

[2] Albuquerque C, Correia C, Ferreira M. Adherence to the therapeutic regime in person with type-2 diabetes. Procedia-Social and Behavioral Sciences. 2015;171:350-8.

[3] Bonger Z, Shiferaw S, Tariku EZ. Adherence to diabetic self-care practices and its associated factors among patients with type-2 diabetes in Addis Ababa, Ethiopia. Patient preference and adherence. 2018;12:963.

[4] Meena SN, Naik MM, Ghadi SC, Tilve SG. α-Glucosidase inhibition activity and in silico study of 2-(benzo [d][1, 3] dioxol-5-yl)-4H-chromen-4-one, a synthetic derivative of flavone. Bioorganic & medicinal chemistry. 2018.

[5] Cho N, Shaw J, Karuranga S, Huang Y, da Rocha Fernandes J, Ohlrogge A. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes research and clinical practice. 2018;138:271-81.

[6] Zimmet P, Alberti KG, Magliano DJ, Bennett PH. Diabetes mellitus statistics on prevalence and mortality: facts and fallacies. Nature Reviews Endocrinology. 2016;12:616.

[7] Kren V, Martínková L. Glycosides in medicine:"The role of glycosidic residue in biological activity". Current medicinal chemistry. 2001;8:1303-28.

[8] Bertozzi CR, Kiessling LL. Chemical glycobiology. Science. 2001;291:2357-64.

[9] Hein CD, Liu X-M, Wang D. Click chemistry, a powerful tool for pharmaceutical sciences. Pharmaceutical research. 2008;25:2216-30.

[10] K Tiwari V, C Mishra R, Sharma A, P Tripathi R. Carbohydrate based potential chemotherapeutic agents: recent developments and their scope in future drug discovery. Mini reviews in medicinal chemistry. 2012;12:1497-519.

[11] Tiwari VK, Mishra BB, Mishra KB, Mishra N, Singh AS, Chen X. Cu-catalyzed click reaction in carbohydrate chemistry. Chemical reviews. 2016;116:3086-240.

[12] Horne G, Wilson FX, Tinsley J, Williams DH, Storer R. Iminosugars past, present and future: medicines for tomorrow. Drug discovery today. 2011;16:107-18.

[13] Hara T, Nakamura J, Koh N, Sakakibara F, Takeuchi N, Hotta N. An importance of carbohydrate ingestion for the expression of the effect of α-glucosidase inhibitor in NIDDM. Diabetes Care. 1996;19:642-7.

[14] Toeller M. α-Glucosidase inhibitors in diabetes: efficacy in NIDDM subjects. European Journal of Clinical Investigation. 1994;24:31-5.

[15] Martin AE, Montgomery PA. Acarbose: an α-glucosidase inhibitor. American Journal of Health-System Pharmacy. 1996;53:2277-90.

[16] Amini-Rentsch L, Vanoli E, Richard-Bildstein S, Marti R, Vilé G. A Novel and Efficient Continuous-Flow Route To Prepare Trifluoromethylated N-Fused

Heterocycles for Drug Discovery and Pharmaceutical Manufacturing. Industrial & Engineering Chemistry Research. 2019;58:10164-71.

[17] Jiang L, Wang M-Y, Wan F-X, Qu Z-Q. Synthesis and biological activity of tri-substituted 1, 2, 4-triazoles bearing benzimidazole moiety. Phosphorus, Sulfur, and Silicon and the Related Elements. 2015;190:1599-605.

[18] Ertan T, Yildiz I, Ozkan S, Temiz-Arpaci O, Kaynak F, Yalcin I. Synthesis and biological evaluation of new N-(2-hydroxy-4 (or 5)-nitro/aminophenyl) benzamides and phenylacetamides as antimicrobial agents. Bioorganic & medicinal chemistry. 2007;15:2032-44.

[19] Pau A, Boatto G, Palomba M, Asproni B, Cerri R, Palagiano F. Synthesis of N-[4-(alkyl) cyclohexyl]-substituted benzamides with anti-inflammatory and analgesic activities. Il Farmaco. 1999;54:524-32.

[20] Tandon R, Singh I, Luxami V, Tandon N, Paul K. Recent Advances and Developments of in vitro Evaluation of Heterocyclic Moieties on Cancer Cell Lines. The Chemical Record. 2019;19:362-93.

[21] Amin K, Kamel M, Anwar M, Khedr M, Syam Y. Synthesis, biological evaluation and molecular docking of novel series of spiro [(2H, 3H) quinazoline-2, 1′-cyclohexan]-4 (1H)-one derivatives as anti-inflammatory and analgesic agents. European Journal of Medicinal Chemistry. 2010;45:2117-31.

[22] Cale Jr AD, Gero TW, Walker KR, Lo YS, Welstead Jr WJ, Jaques LW. Benzo-and pyrido-1, 4-oxazepin-5-ones and-thiones: Synthesis and structure-activity relationships of a new series of H1-antihistamines. Journal of medicinal chemistry. 1989;32:2178-99.

[23] Irwin I, Palme M, Becker C, Druzgala P. Stereoisomeric compounds and methods for the treatment of gastrointestinal and central nervous system disorders. Google Patents; 2012.

[24] Charaya N, Pandita D, Grewal AS, Lather V. Design, synthesis and biological evaluation of novel thiazol-2-yl benzamide derivatives as glucokinase activators. Computational biology and chemistry. 2018;73:221-9.

[25] Wang X-L, Wan K, Zhou C-H. Synthesis of novel sulfanilamide-derived 1, 2, 3-triazoles and their evaluation for antibacterial and antifungal activities. European journal of medicinal chemistry. 2010;45:4631-9.

[26] Singh A, Biot C, Viljoen A, Dupont C, Kremer L, Kumar K. 1H-1, 2, 3-triazole-tethered uracil-ferrocene and uracil-ferrocenylchalcone conjugates: Synthesis and antitubercular evaluation. Chemical biology & drug design. 2017;89:856-61.

[27] Zhang S, Xu Z, Gao C, Ren Q-C, Chang L, Lv Z-S. Triazole derivatives and their anti-tubercular activity. European journal of medicinal chemistry. 2017;138:501-13.

[28] Ma N, Wang Y, Zhao B-X, Ye W-C, Jiang S. The application of click chemistry in the synthesis of agents with anticancer activity. Drug design, development and therapy. 2015;9:1585.

[29] Mohammed I, Kummetha IR, Singh G, Sharova N, Lichinchi G, Dang J. 1, 2, 3-triazoles as amide bioisosteres: discovery of a new class of potent HIV-1 Vif antagonists. Journal of medicinal chemistry. 2016;59:7677-82.

[30] Whiting M, Muldoon J, Lin YC, Silverman SM, Lindstrom W, Olson AJ. Inhibitors of HIV-1 protease by using in situ click chemistry. Angewandte Chemie International Edition. 2006;45:1435-9.

[31] da Silva FdC, do Carmo Cardoso MF, Ferreira PG, Ferreira VF. Biological Properties of 1H-1, 2, 3-and 2H-1, 2, 3-Triazoles. Chemistry of 1, 2, 3-triazoles: Springer; 2014. p. 117-65.

[32] Lewis WG, Green LG, Grynszpan F, Radić Z, Carlier PR, Taylor P. Click chemistry in situ: acetylcholinesterase as a reaction vessel for the selective assembly of a femtomolar inhibitor from an array of building blocks. Angewandte Chemie International Edition. 2002;41:1053-7.

[33] Emmadi NR, Bingi C, Kotapalli SS, Ummanni R, Nanubolu JB, Atmakur K. Synthesis and evaluation of novel fluorinated pyrazolo-1, 2, 3-triazole hybrids as antimycobacterial agents. Bioorganic & medicinal chemistry letters. 2015;25:2918-22.

[34] Périon R, Ferrières V, Garcia-Moreno MI, Mellet CO, Duval R, Fernández JMG. 1, 2, 3-Triazoles and related glycoconjugates as new glycosidase inhibitors. Tetrahedron. 2005;61:9118-28.

[35] Slámová K, Marhol P, Bezouška K, Lindkvist L, Hansen SG, Křen V. Synthesis and biological activity of glycosyl-1H-1, 2, 3-triazoles. Bioorganic & medicinal chemistry letters. 2010;20:4263-5.

[36] Wang G, Peng Z, Wang J, Li J, Li X. Synthesis and biological evaluation of novel 2, 4, 5-triarylimidazole–1, 2, 3-triazole derivatives via click chemistry as α-glucosidase inhibitors. Bioorganic & medicinal chemistry letters. 2016;26:5719-23.

[37] Asano N. Sugar-mimicking glycosidase inhibitors: bioactivity and application. Cellular and molecular life sciences. 2009;66:1479-92.

[38] Davies GJ, Gloster TM, Henrissat B. Recent structural insights into the expanding world of carbohydrate-active enzymes. Current opinion in structural biology. 2005;15:637-45.

[39] D'Alonzo D. Guaragna A. Palumbo G. Curr Med Chem. 2009;16:473.

[40] Lyseng-Williamson KA. Miglustat: a review of its use in Niemann-Pick disease type C. Drugs. 2014;74:61-74.

[41] Pastores GM, Barnett NL, Kolodny EH. An open-label, noncomparative study of miglustat in type I Gaucher disease: efficacy and tolerability over 24 months of treatment. Clinical therapeutics. 2005;27:1215-27.

[42] Natori Y, Sakuma T, Watanabe H, Wakamatsu H, Kato A, Adachi I. Catalytic asymmetric synthesis of stereoisomers of 1-Cn-butyl-LABs for the SAR study of α-glucosidase inhibition. Tetrahedron. 2019;75:2866-76.

[43] Asano N. Glycosidase inhibitors: update and perspectives on practical use. Glycobiology. 2003;13:93R-104R.

[44] Truscheit E, Frommer W, Junge B, Müller L, Schmidt DD, Wingender W. Chemistry and biochemistry of microbial α-glucosidase inhibitors. Angewandte Chemie International Edition in English. 1981;20:744-61.

[45] Neufeld EF. Lysosomal storage diseases. Annual review of biochemistry. 1991;60:257-80.

[46] Tifft CJ, Proia RL. Stemming the tide: glycosphingolipid synthesis inhibitors as therapy for storage diseases. Glycobiology. 2000;10:1249-58.

[47] Platt FM, Neises GR, Dwek RA, Butters TD. N-butyldeoxynojirimycin is a novel inhibitor of glycolipid biosynthesis. Journal of Biological Chemistry. 1994;269:8362-5.

[48] Danzin C, EHRHARD A, Ducep J. Deoxynojirimycin derivatives as slow-binding and tight-binding inhibitors of sucrase and isomaltase. Abstracts of papers of the american chemical society: amer chemical soc 1155 16th st, nw, washington, DC 20036; 1989. p. 21-CARB.

[49] Ghisaidoobe AT, van den Berg RJ, Butt SS, Strijland A, Donker-Koopman WE, Scheij S. Identification and development of biphenyl substituted iminosugars as improved dual glucosylceramide synthase/neutral glucosylceramidase inhibitors. Journal of medicinal chemistry. 2014;57:9096-104.

[50] Bischoff H. The mechanism of α-glucosidase inhibition in the management of diabetes. Clinical and investigative medicine Medecine clinique et experimentale. 1995;18:303-11.

[51] Tundis R, Loizzo M, Menichini F. Natural products as α-amylase and α-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update. Mini reviews in medicinal chemistry. 2010;10:315-31.

[52] Rossi LL, Basu A. Glycosidase inhibition by 1-glycosyl-4-phenyl triazoles. Bioorganic & medicinal chemistry letters. 2005;15:3596-9.

[53] Castro S, Cherney EC, Snyder NL, Peczuh MW. Synthesis of substituted septanosyl-1, 2, 3-triazoles. Carbohydrate research. 2007;342:1366-72.

[54] Anand N, Jaiswal N, Pandey SK, Srivastava A, Tripathi RP. Application of click chemistry towards an efficient synthesis of 1, 2, 3-1H-triazolyl glycohybrids as enzyme inhibitors. Carbohydrate research. 2011;346:16-25.

[55] Singh N, Pandey S, Tripathi RP. Regioselective [3+ 2] cycloaddition of chalcones with a sugar azide: easy access to 1-(5-deoxy-D-xylofuranos-5-yl)-4, 5-disubstituted-1H-1, 2, 3-triazoles. Carbohydrate research. 2010;345:1641-8.

[56] Heightman TD, Locatelli M, Vasella A. Synthesis of Fused Triazoles as Probes for the Active Site of Retaining β-Glycosidases: From Which Direction Is the Glycoside Protonated? Helvetica chimica acta. 1996;79:2190-200.

[57] Zhang J, Chen H-N, Chiang F-I, Takemoto JY, Bensaci M, Chang C-WT. Sonication-Assisted Library Synthesis of Oxazolidinone– Carbohydrate Conjugates. Journal of combinatorial chemistry. 2007;9:17-9.

[58] Dubey N, Sharma P, Kumar A. Clay-supported Cu (II) catalyst: An efficient, heterogeneous, and recyclable catalyst for synthesis of 1, 4-disubstituted 1, 2, 3-triazoles from alloxan-derived terminal alkyne and substituted azides using click chemistry. Synthetic Communications. 2015;45:2608-26.

[59] Chandrasekhar S, Rao CL, Nagesh C, Reddy CR, Sridhar B. Inter and intramolecular copper (I)-catalyzed 1, 3-dipolar cycloaddition of azido-alkynes: synthesis of furanotriazole macrocycles. Tetrahedron letters. 2007;48:5869-72.

[60] Yousuf SK, Taneja SC, Mukherjee D. Multicomponent cascade transformation of d-glucal to furan-appended triazole glycoconjugates. The Journal of organic chemistry. 2010;75:3097-100.

[61] Chittaboina S, Xie F, Wang Q. One-pot synthesis of triazole-linked glycoconjugates. Tetrahedron letters. 2005;46:2331-6.

[62] Marra A, Vecchi A, Chiappe C, Melai B, Dondoni A. Validation of the copper (I)-catalyzed azide– alkyne coupling in ionic liquids. Synthesis of a triazole-linked C-disaccharide as a case study. The Journal of organic chemistry. 2008;73:2458-61.

[63] Shamim A, Souza FB, Trossini GH, Gatti FM, Stefani HA. Synthesis of C-glycosyl-bis-1, 2, 3-triazole derivatives from 3, 4, 6-tri-acetyl-D-glucal. Molecular diversity. 2015;19:423-34.

[64] Hotha S, Anegundi RI, Natu AA. Expedient synthesis of 1, 2, 3-triazole-fused tetracyclic compounds by intramolecular Huisgen ('click') reactions on carbohydrate-derived azido-alkynes. Tetrahedron letters. 2005;46:4585-8.

[65] Dondoni A, Giovannini PP, Massi A. Assembling heterocycle-tethered C-glycosyl and α-amino acid residues via 1, 3-dipolar cycloaddition reactions. Organic letters. 2004;6:2929-32.

[66] Ray A, Manoj K, Bhadbhade MM, Mukhopadhyay R, Bhattacharjya A. Cu (I)-catalyzed cycloaddition of constrained azido-alkynes: Access to 12-to 17-membered monomeric triazolophanes incorporating furanoside rings. Tetrahedron letters. 2006;47:2775-8.

[67] Wilkinson BL, Innocenti A, Vullo D, Supuran CT, Poulsen S-A. Inhibition of carbonic anhydrases with glycosyltriazole benzene sulfonamides. Journal of medicinal chemistry. 2008;51:1945-53.

[68] Meldal M, Tornøe CW. Cu-catalyzed azide− alkyne cycloaddition. Chemical reviews. 2008;108:2952-3015.

[69] Gramlich PM, Wirges CT, Manetto A, Carell T. Postsynthetic DNA Modification through the Copper-Catalyzed Azide–Alkyne Cycloaddition Reaction. Angewandte Chemie International Edition. 2008;47:8350-8.

[70] Hein JE, Fokin VV. Copper-catalyzed azide–alkyne cycloaddition (CuAAC) and beyond: new reactivity of copper (I) acetylides. Chemical Society Reviews. 2010;39:1302-15.

[71] Rostovtsev VV, Green LG, Fokin VV, Sharpless KB. A stepwise huisgen cycloaddition process: copper (I)-catalyzed regioselective "ligation" of azides and terminal alkynes. Angewandte Chemie International Edition. 2002;41:2596-9.

[72] Huisgen R. Kinetics and mechanism of 1, 3-dipolar cycloadditions. Angewandte Chemie International Edition in English. 1963;2:633-45.

[73] Zhang L, Chen X, Xue P, Sun HH, Williams ID, Sharpless KB. Ruthenium-catalyzed cycloaddition of alkynes and organic azides. Journal of the American Chemical Society. 2005;127:15998-9.

[74] Bock VD, Hiemstra H, Van Maarseveen JH. CuI-catalyzed alkyne–azide "click" cycloadditions from a mechanistic and synthetic perspective. European Journal of Organic Chemistry. 2006;2006:51-68.

[75] Liang L, Astruc D. The copper (I)-catalyzed alkyne-azide cycloaddition (CuAAC)"click" reaction and its applications. An overview. Coordination Chemistry Reviews. 2011;255:2933-45.

[76] Singh MS, Chowdhury S, Koley S. Advances of azide-alkyne cycloaddition-click chemistry over the recent decade. Tetrahedron. 2016;72:5257-83.

[77] Agalave SG, Maujan SR, Pore VS. Click chemistry: 1, 2, 3-triazoles as pharmacophores. Chemistry–An Asian Journal. 2011;6:2696-718.

[78] da Rocha DR, Santos WC, Lima ES, Ferreira VF. Synthesis of 1, 2, 3-triazole glycoconjugates as inhibitors of α-glucosidases. Carbohydrate research. 2012;350:14-9.

[79] Ferreira SB, Sodero AC, Cardoso MF, Lima ES, Kaiser CR, Silva Jr FP. Synthesis, biological activity, and molecular modeling studies of 1 h-1, 2, 3-triazole derivatives of carbohydrates as α-glucosidases inhibitors. Journal of medicinal chemistry. 2010;53:2364-75.

[80] Kolb HC, Sharpless KB. The growing impact of click chemistry on drug discovery. Drug discovery today. 2003;8:1128-37.

[81] Sharma A, Sharma S, Tripathi RP, Ampapathi RS. Robust turn structures in α-β cyclic tetrapeptides induced and controlled by carbo-β3 amino acid. The Journal of organic chemistry. 2012;77:2001-7.

[82] Twin H, Batey RA. Intramolecular hetero Diels– Alder (Povarov) approach to the synthesis of the alkaloids luotonin a and camptothecin. Organic letters. 2004;6:4913-6.

[83] Braun M-G, Katcher MH, Doyle AG. Carbofluorination via a palladium-catalyzed cascade reaction. Chemical Science. 2013;4:1216-20.

[84] Goebel M, Nothofer H-G, Ugi I. A facile synthesis of per-*O*-alkylated glycono-δ-lactones from per-*O*-alkylated glycopyranosides and a novel ring contraction for pyranoses. Tetrahedron. 1997;53:3123-34.

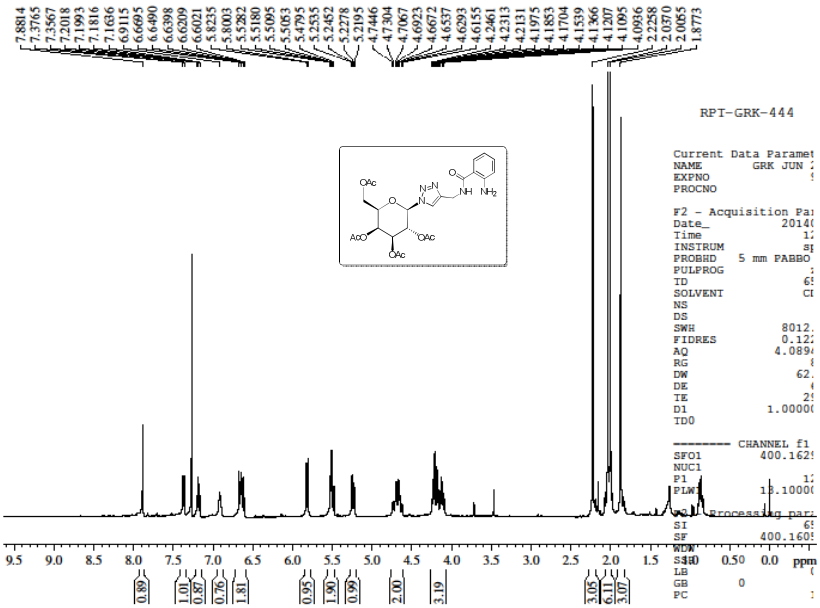
[85] Masuda M, Shimizu T. Synthesis of Novel α, ω-Type 1 Glucosamide and Galactosamide Bolaamphiphiles. Journal of carbohydrate chemistry. 1998;17:405-16.

[86] Thakur RK, Mishra A, Ramakrishna K, Mahar R, Shukla SK, Srivastava A. Synthesis of novel pyrimidine nucleoside analogues owning multiple bases/sugars and their glycosidase inhibitory activity. Tetrahedron. 2014;70:8462-73.

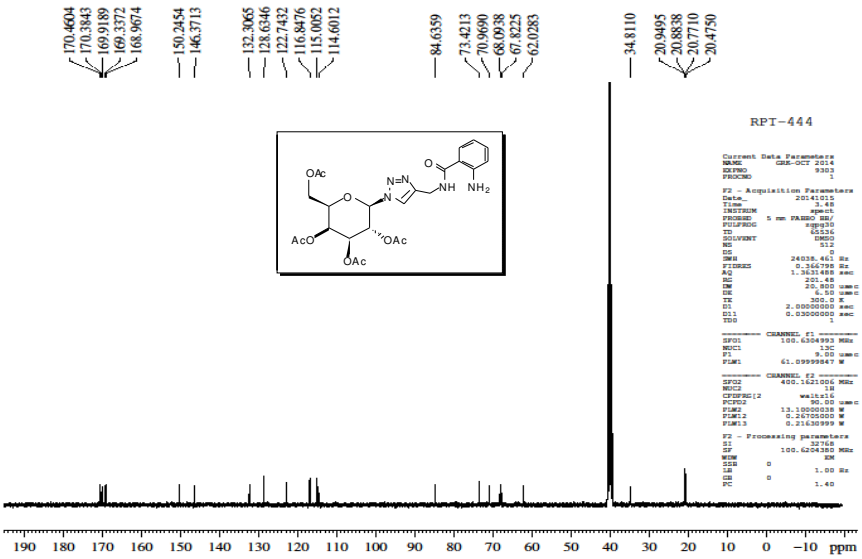
[87] Pistia-Brueggeman G, Hollingsworth RI. A preparation and screening strategy for glycosidase inhibitors. Tetrahedron. 2001;57:8773-8.

4.13. Scanned copy of ^{1}H NMR and ^{13}C NMR Spectra of Selected Compounds glycosyl-1,2,3-1*H*-triazolyl methyl benzamide derivative:

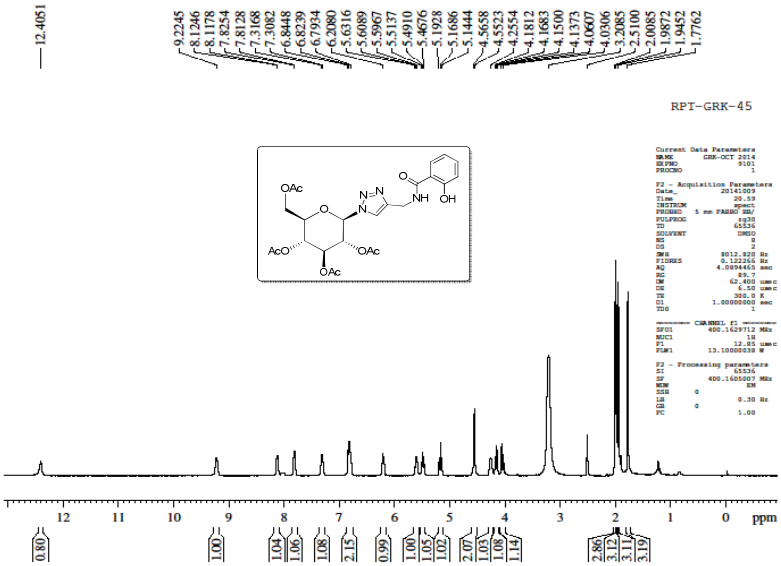
^{1}HNMR spectra of2-amino-*N*-[(1-(1′-deoxy-2′, 3′, 4′, 6′-tetra-*O*-acetyl-*β*-D-galactopyranose-1′-yl)]-1*H*-1, 2, 3-triazol-4-yl) methyl)-benzamide **(3b)**



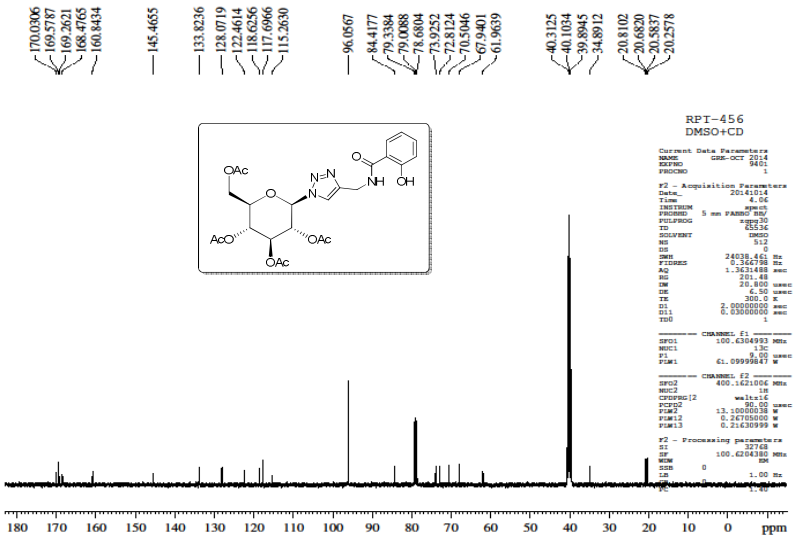
^{13}C NMR spectraof 2-amino-*N*-[(1-(1′-deoxy-2′, 3′, 4′, 6′-tetra-*O*-acetyl-*β*-D-galactopyranose-1′-yl)]-1*H*-1, 2, 3-triazol-4-yl) methyl)-benzamide **(3b)**



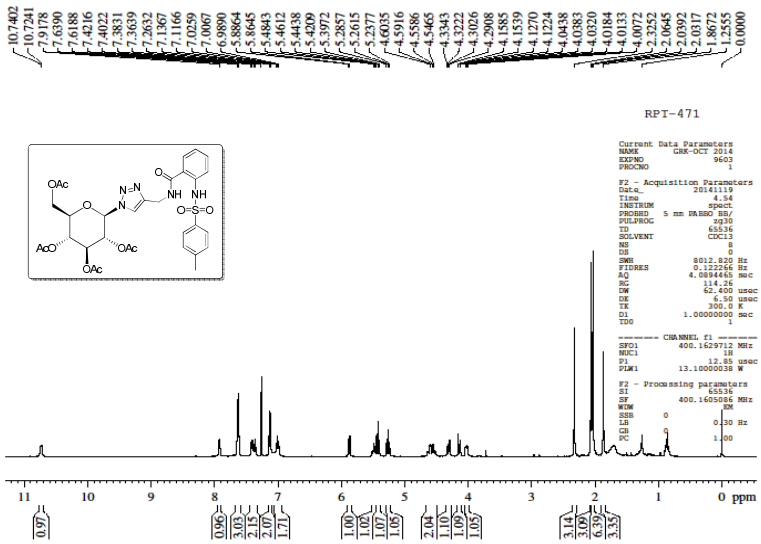
^{1}H NMR spectra of2-hydroxy-*N*-[(1-(1′-deoxy-2′, 3′, 4′, 6′-tetra-*O*-acetyl-*β*-D-glucopyranos-1′-yl)]-*1H*-1,2,3-triazol-4′-yl)methyl-benzamide (3c)



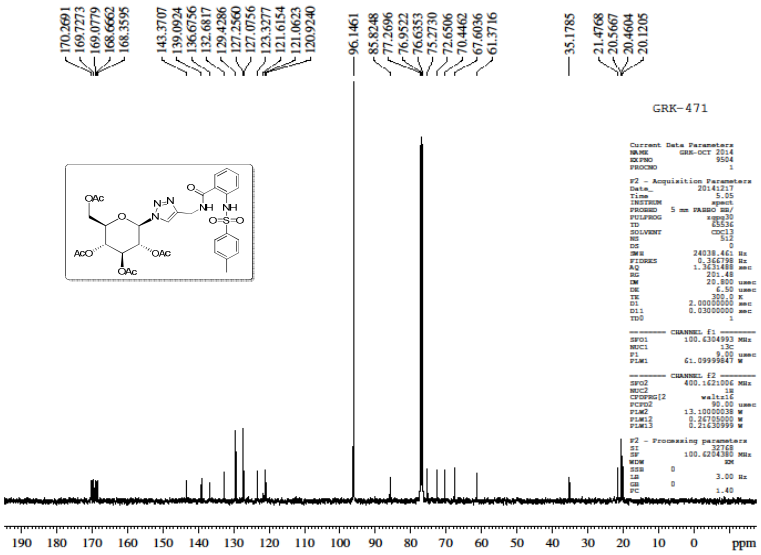
^{13}C of 2-hydroxy-*N*-[(1-(1′-deoxy-2′, 3′, 4′, 6′-tetra-*O*-acetyl-*β*-D-glucopyranos-1′-yl)]-*1H*-1,2,3-triazol-4′-yl)methyl-benzamide (3c)



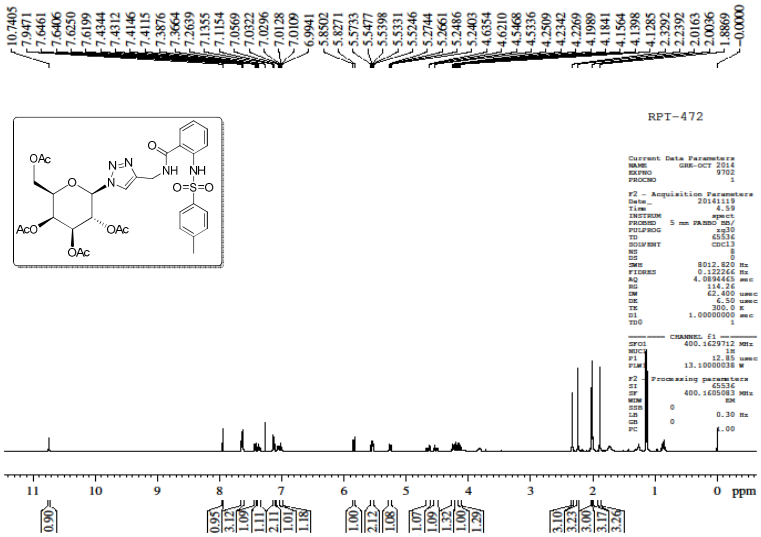
^{1}H NMR spectra of 4-methyl-*N*-[(1-(1′-deoxy-*β*-D-glucopyranos-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzenesulphonamide (3e)



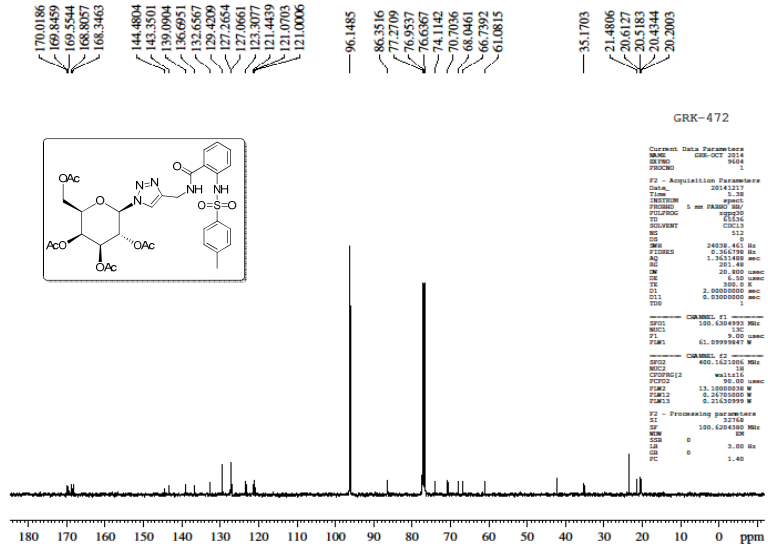
^{13}C NMR spectra of 4-methyl-*N*-[(1-(1′-deoxy-*β*-D-glucopyranos-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzenesulphonamide **(3e)**



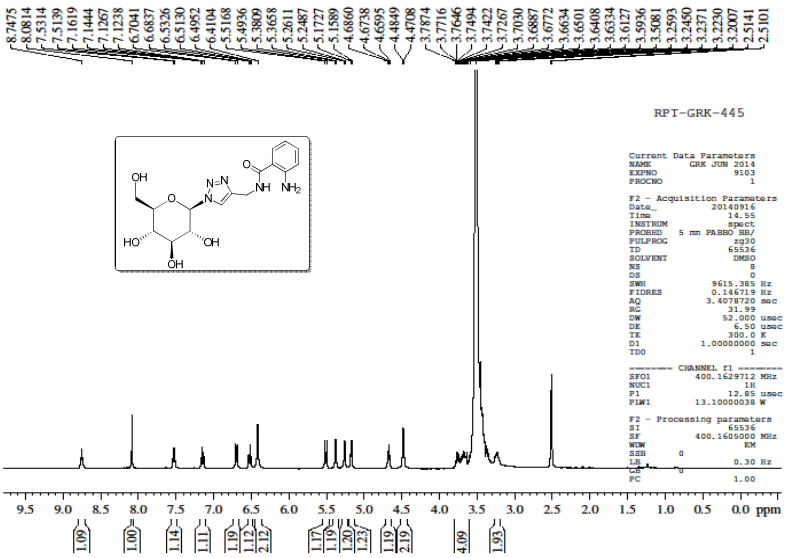
^{1}H NMR spectra of 4-methyl-*N*-[(1-(1′-deoxy-*β*-D-galactopyranose-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzenesulphonamide (3f)



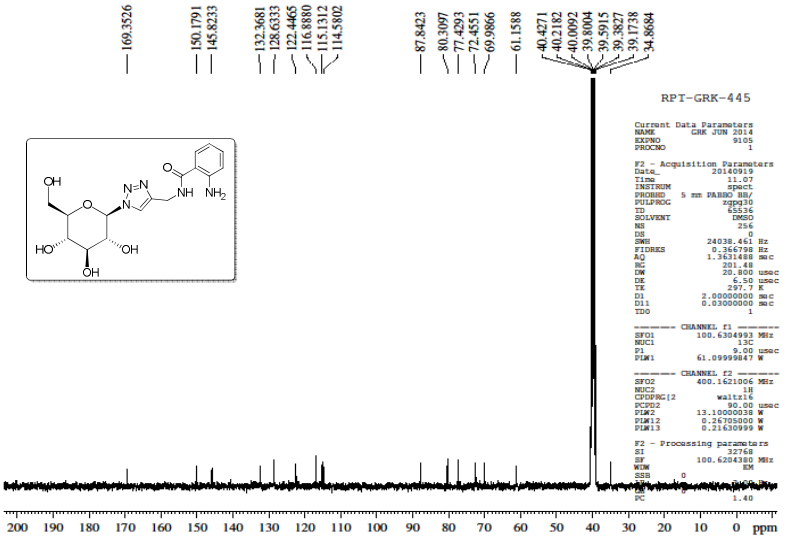
^{13}C NMR spectra of of 4-methyl-*N*-[(1-(1′-deoxy-*β*-D-galactopyranose-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzenesulphonamide**(3f)**



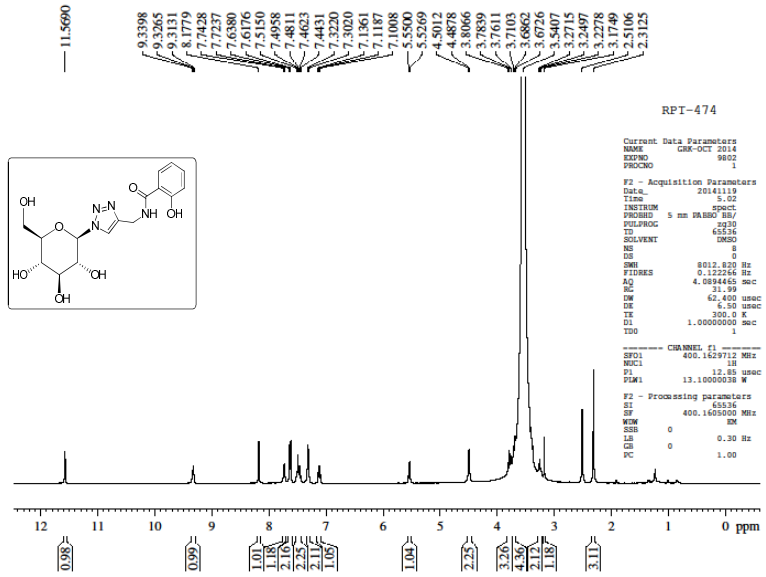
^{1}H NMR spectra of of 2-amino-*N*-[(1-(1′-deoxy-*β*-D-glucopyranos-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzamide (4a)



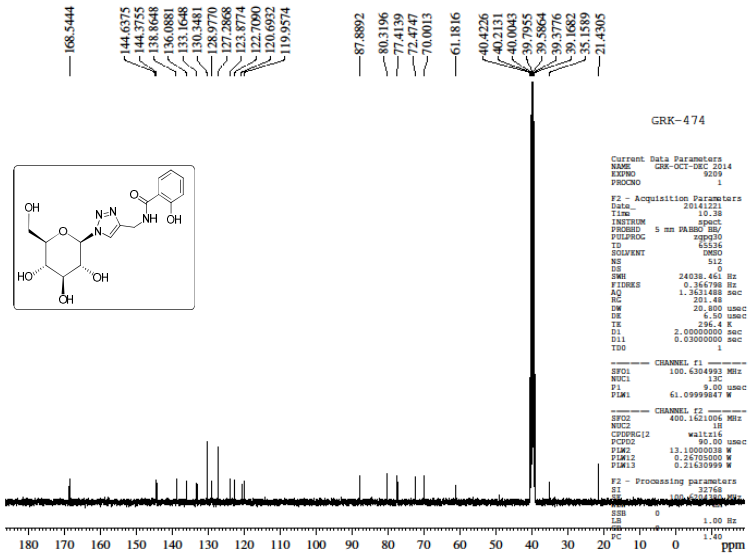
^{13}C NMR spectra of of 2-amino-*N*-[(1-(1′-deoxy-*β*-D-glucopyranos-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzamide **(4a)**



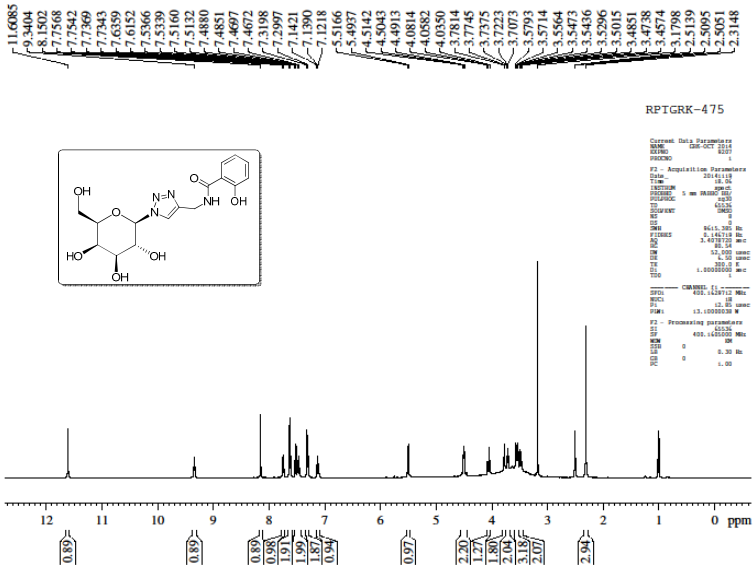
^{1}H NMR spectra of of 4-methyl-*N*-[(1-(1′-deoxy-*β*-D-glucopyranos-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzenesulphonamide (4c)



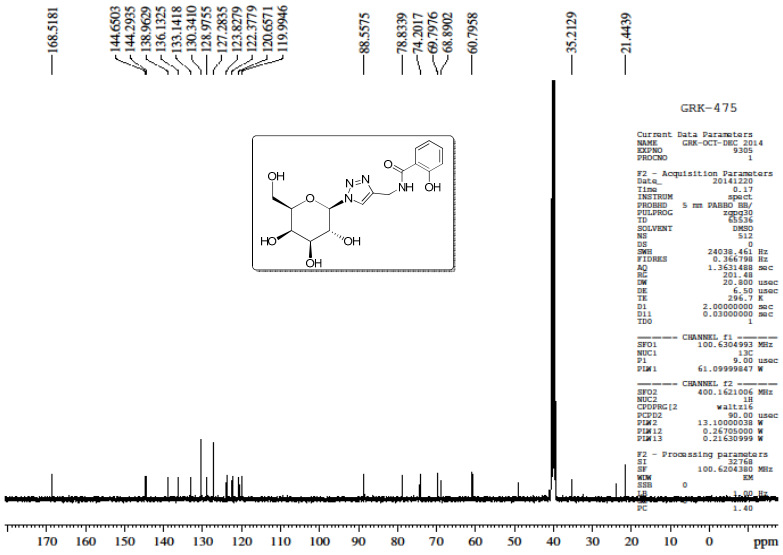
^{13}C NMR spectra of 4-methyl-*N*-[(1-(1′-deoxy-*β*-D-glucopyranos-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzenesulphonamide **(4c)**



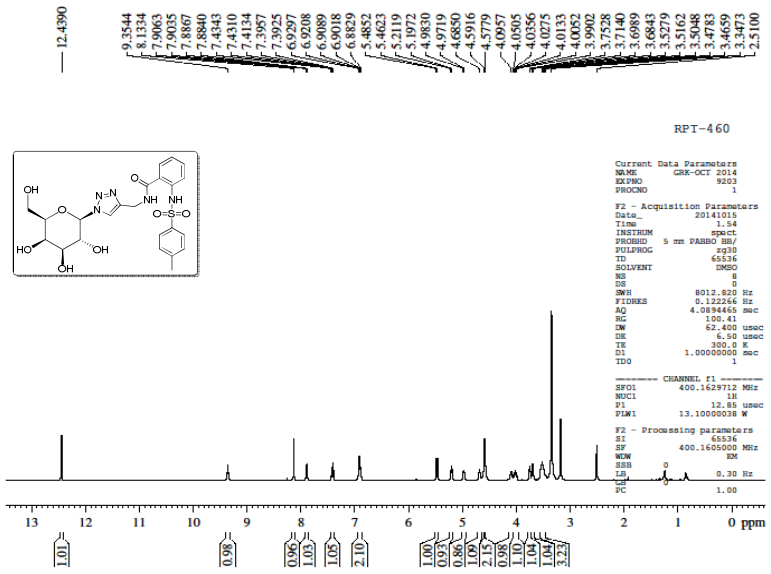
^{1}H NMR spectra of4-methyl-*N*-[(1-(1′-deoxy-*β*-D-galactopyranose-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzenesulphonamide (4d)



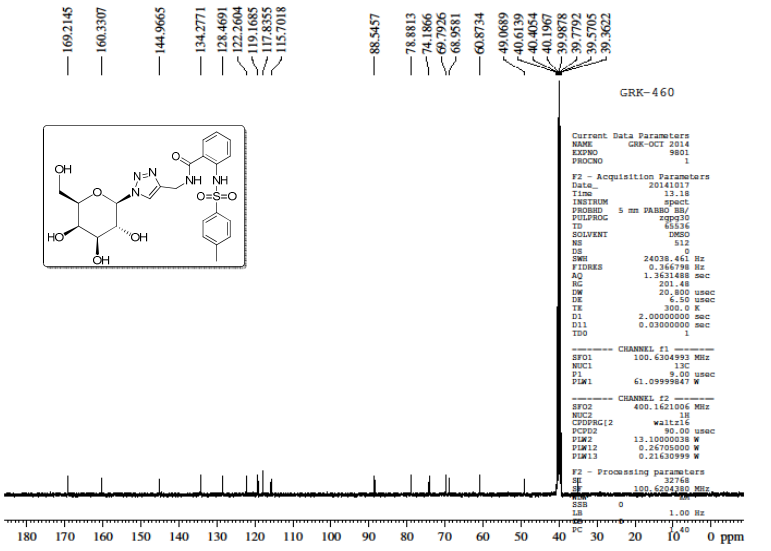
^{13}C NMR spectra of 4-methyl-*N*-[(1-(1′-deoxy-*β*-D-galactopyranose-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzenesulphonamide**(4d)**



^{1}H NMR spectra of 2-hydroxy-*N*-[(1-(1′-deoxy-*β*-D-galactopyranose-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzamide (4f)



^{13}C NMR spectra of 2-hydroxy-*N*-[(1-(1′-deoxy-*β*-D-galactopyranose-1′-yl)]-*1H*-1,2,3-triazol-4-yl)methyl-benzamide **(4f)**



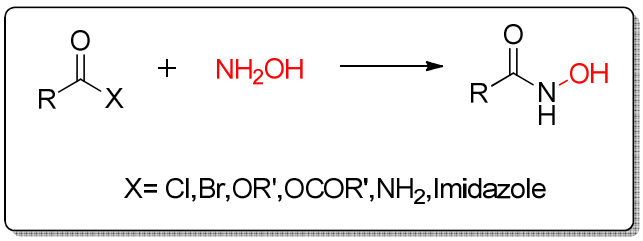
Summary

Synthesis and characterization of some biologically active nitrogen containing compounds: Development of new chemotherapeutic agents

The work of the present thesis has been divided into following **four chapters**:

Chapter-1: *Hydroxamate analogues: Recent progress in the synthesis of compound & their therapeutic importance.*

Hydroxamic acids or hydroxamate form a class of compounds which display interesting chemical and biological properties, containing the functional group –CONHOH. They are the amide derivatives, where the hydrogen atom of NH group has been replaced by an OH group. Hydroxamates form a class of compounds which display interesting chemical and biological properties. Among the several possible synthetic methods for the preparation of hydroxamic acids, two approaches, which have been used in most of the cases, are (i) reaction of acyl halides with hydroxylamine and (ii) reactions of acids or esters with hydroxylamine (**Scheme 1**).



Scheme .1 General synthesis of hydroxamates

Both synthetic pathways correspond to acyl substitution where the nucleophile is the hydroxylamine as free base and the leaving group can be either halides X– or the R′O– depending upon the starting compound. These are the most used and reliable methods which are currently applied for the preparation of known as well as new

hydroxamic acids. They mainly have two tautomers: keto-form and oxime-form, as shown in **Figure 1.**

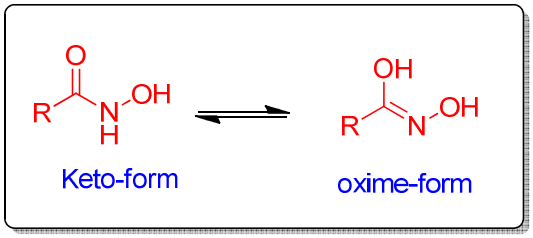


Figure.1 Tautomerism in hydroxamates

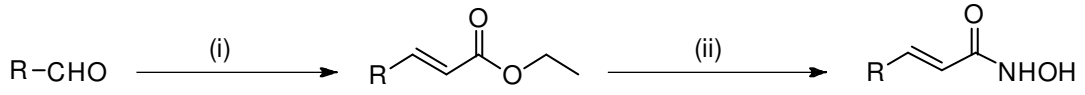
Their carbonyl and *N*-hydroxy groups are capable of forming complex metals in a bidentate fashion, making them excellent ligands for a number of metals of biological significance including zinc, iron, and nickel. Among these metals, zinc is one of the most frequently occurring metals in metalloenzymes (>300 enzymes) particular in zinc-dependent endo-peptidases matrix metalloproteases (MMPs) and histone deacetylases (HDACs) which play substantial roles for cancer therapy in previous decades.

Chapter-2: *Synthesis of N-hydroxycinnamide derivatives and their bio-evaluation*

Cancer is one of the most severe public health issues around the globe according to the World Health Organization (WHO). Among various type of cancers breast cancer is also one of the major causes of cancer death among women worldwide. Due to its complex cancer biology, it is necessary to use multiple therapeutic modalities. So far, the conventional treatments for breast cancer are surgical intervention, hormonal therapy, radiotherapy and chemotherapy. It is merely responsible for 20-25% of all cancer cases and 15-18% of cancer deaths among women.

Hydroxamic acids or hydroxamates are carboxylic acid or aldehyde analogues where –COOH group or –CHO group has been replaced by CONHOH or –CONH-R. Hydroxamic acids are well known effective molecules in the field of cancer chemotherapy and as a mutagenic agent. Several hydroxamates based drugs are functioning very good in clinics for cancer chemotherapy such as SAHA, PXD-101 (Belinostat,Topotarget) and LBH-589 (Panobinostat), which are approved by the U.S. Food and Drug Administration (FDA) in October 2006, July 2014 and February 2015, respectively.

The starting acrylate derivatives (**2a-k**) were prepared by the Horner-Wadsworth-Emmons (HWE) olefination of different aromatic aldehydes (**1a-k**) with triethylphosphonoacetate in the presence of LiOH in THF at ambient temperature. The reaction of acrylate derivatives **(2a-k)** with hydroxylamine hydrochloride in the presence of KOH in methanol at 0-5 °C results in the formation of respective *N*-hydroxy acrylamide (**3a-k**) in moderate to excellent yields (**Scheme 1**).



1a-k **2a-k** **3a-k**

1a, **2a**, **3a, 84 %**; R = 4-chlorophenyl
1b, 2b, 3b, 74 %; R = 2,6-dichlorophenyl
1c, **2c**, **3c, 70 %**; R = pyridin-4-yl
1d, **2d, 3d, 85 %**; R = 4-(1*H*-imidazol-1-yl)phenyl
1e, 2e, 3e, 75 %; R = isophthalaldehyde
1f, 2f, 3f, 79 %; R = terephthalaldehyde
1g, 2g, 3g, 75 %; R= 4-hydroxybenzaldehyde
1h, 2h, 3h, 85 %; R = 3,4,5-trimethoxybenzaldehyde
1i, 2i, 3i, 72 %; R = 4-hydroxybenzaldehyde
1j, 2j, 3j, 80 %; R = 1*H*-indole-3-carbaldehyde
1k, 2k, 3k, 85 %; R = furan-2-carbaldehyde

Scheme 1: **Synthesis of *N*-Hydroxycinnamamide derivatives,**

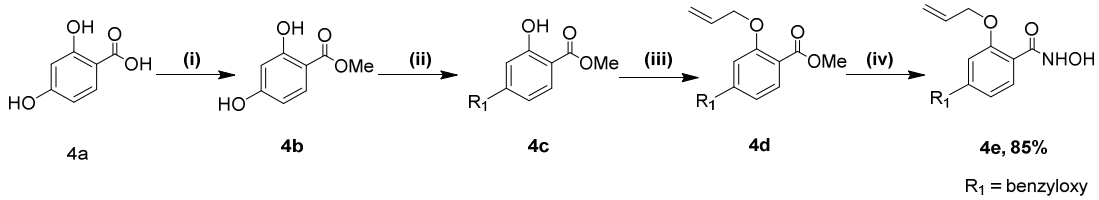
Reagents and conditions (i) LiOH (1.1eq), TEPA (1.1eq), THF, RT (ii) $NH_2OH.HCl$ (5 eq), KOH (10 eq), CH_3OH, 0°C- RT.

Synthesis of 2-*O*-alkyl benzhydroxamic acids:

Following 2-*O*-alkyl benzhydroxamic acids were synthesized in this section:

2-(allyloxy)-4-(benzyloxy)-*N*-hydroxy benzamide (4e),

The compounds methyl 2, 4-dihydroxybenzoate (**4b**) on chemoselective benzylation with benzyl bromide in acetone in the presence of anhydrous K_2CO_3 and catalytic amount of tetra-butyl ammonium bromide **(TBAB)** gave methyl 4-(benzyloxy)-2-hydroxybenzoate (**4c**) in 90% yield. The latter (**4c**) on allylation with allyl bromide in refluxing THF in the presence of anhydrous K_2CO_3 and a catalytic amount of tetra-butyl ammonium bromide (TBAB) resulted in methyl 2-(allyloxy)-4-(benzyloxy) benzoate (**4d**). Finally, the methyl benzoate derivative (**4d**) on reaction with hydroxylamine hydrochloride in the presence of solid KOH in methanol at 0-5 °C led to the formation of desired 2-(allyloxy)-4-(benzyloxy)-*N*-hydroxy benzamide(**4e**) in 85% yield (**Scheme 2**).



Scheme 2: **Synthesis of *O*-alkyl benzamide derivative**

Reagents and conditions (i) MeOH, 20% H_2SO_4, reflux (ii) Benzyloxy bromide, K_2CO_3, Acetone, RT, (iii) allyl bromide, K_2CO_3, TBAB, THF, reflux (iv) $NH_2OH.HCl$, KOH, MeOH, 0-30 °C.

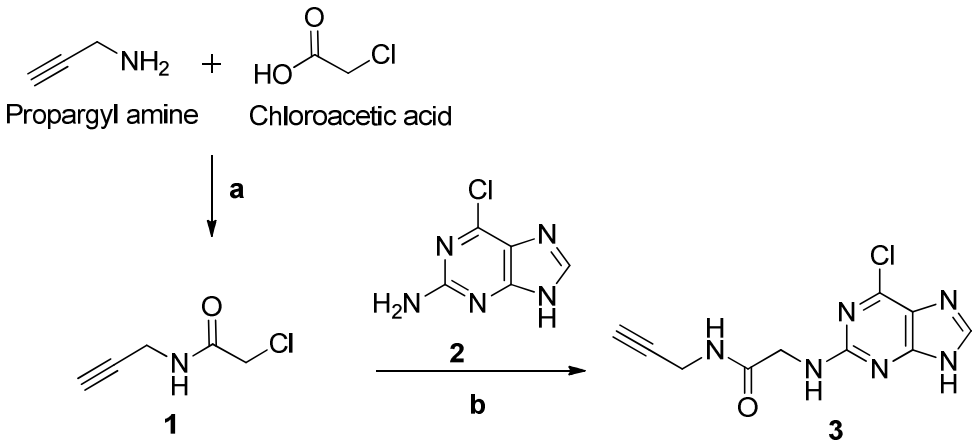
Chapter-3: Synthesis of novel purine nucleoside analogues and their biological evaluation

Nucleosides are considered as fundamental building blocks of nucleic acids and endowed with a broad spectrum of biological activity. These analogues are

synthetically modified compounds which are being synthesized to mimic the natural nucleosides and help in exploiting cellular metabolism and inhibiting cell division and viral replication when incorporated into various cellular processes like DNA and RNA synthesis, cell signaling, enzyme regulation and metabolic process. In addition to this, these analogues can interact with the essential enzymes and inhibit their action as human and viral polymerases, kinases, ribonucleotide reductase, and purine, pyrimidine nucleoside phosphorylase, DNA methyl transferases. Sugar moieties covalently linked with some other biomolecules as proteins, peptides, lipids etc. are of considerable interest due their involvement in complex biological processes and highly selective molecular recognition. They are very crucial in cellular recognition events, including signal transduction, cell adhesion and inflammation, immune response, tumour metastasis and viral & bacterial infections. Consequently multivalent glycohybrids attached with heterocyclic pharmacophore are great importance in medicinal chemistry and drug discovery. Thus Sugar moieties attached with heterocyclic framework opens new doors for the facile and successful construction of wide range of bioactive molecules.

Among the various methodologies reported for synthesis of purine nucleoside analogs, the Cu (I)-catalyzed Huisgen alkyne-azide cycloaddition (CuAAC, also known as 'click reaction') has apparently been one of the most frequently used reactions. The synthesis of triazoles using 'click chemistry' has contributed to a renaissance in the chemistry of azides as building blocks toward higher complexity glycoconjugates. We started our synthetic journey with an objective of synthesis of purine based triazole containing nucleoside analogues, and then corresponding aryl triazole analogues. Primarily, the intermediate 2-chloro-N-propynyl-acetamide was synthesized by coupling of Chloroacetic acid and propargyl amine by reported HOBt

amide-coupling protocol. The second step in the synthetic strategy is the synthesis of purine derived nucleoside analogues employs *N*-alkylation of 2-amino-6-chloropurine **2** at the amino group with the 2-chloro-*N*-propynyl-acetamide **1** by heating these two reactants at 80 °C using strong base sodium hydride in DMF. (**Scheme 1**) Thus, we end up with the intermediate purine derived alkyne **3** in good yield. The synthesized alkyne analogue was further precisely characterized by spectroscopic (^{1}H, ^{13}C NMR) data.

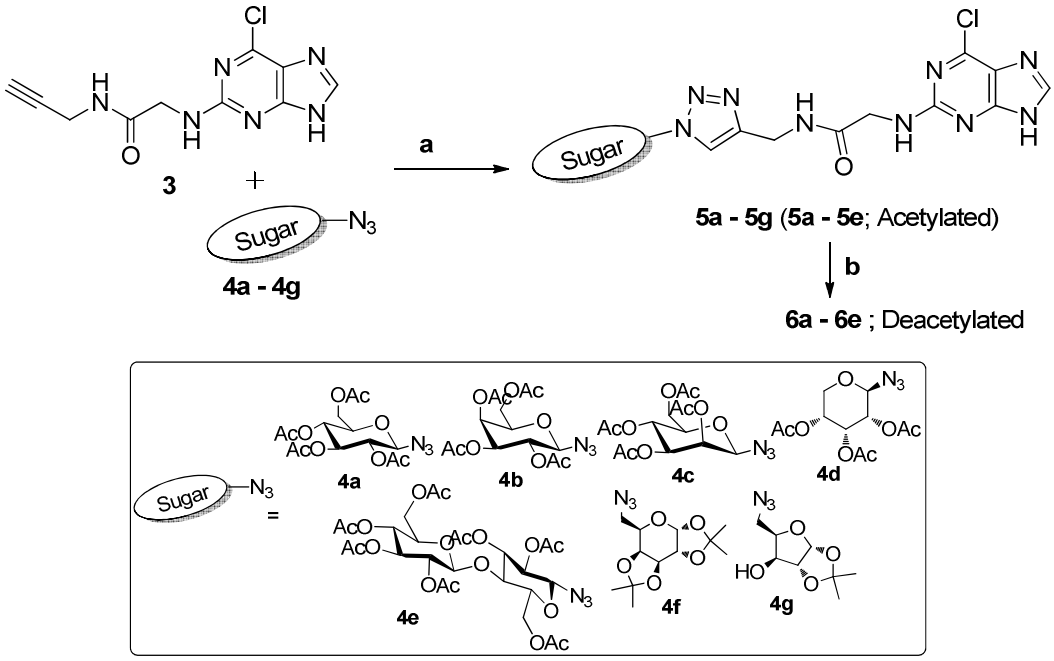


Scheme 1: Synthesis of purine derived alkyne 2-((6-chloro-9*H*-purin-2-yl) amino)-*N*-(prop-2-yn-1-yl) acetamide **a**) HOBT, DIPC, dichloromethane, 0°C to rt, 12 h, 75-86% **b**) 2-amino-6-chloropurine, NaH, DMF, rt (2h) to 80 °C (8h), 90-95%.

Synthesis of purine derived nucleoside analogues

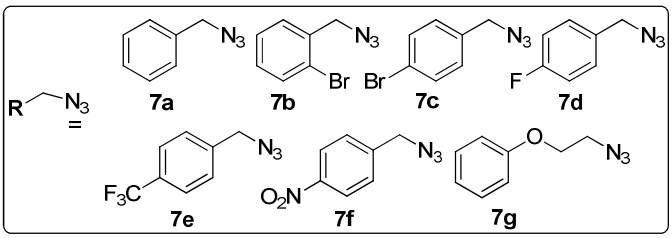
The sugar azides **4a-4g** were prepared and characterized following earlier reported methods. Thus, having purine derived alkyne **3** and sugar azides in hand, the well known 'Click' reaction was performed in solvent system *t*-BuOH/H_2O (1:1) taking equimolar quantities of the reactants, $CuSO_4.5H_2O$ (10 mol%) and sodium ascorbate (20 mol%) at ambient temperature (**Scheme 2**). The reactions were monitored (TLC) up to completion of reaction. Thus various 1, 2, 3-triazole-linked purine nucleoside analogues have been successfully synthesized in high yields. The respective products **5** were isolated and characterized based on their ^{1}H, ^{13}C and Mass spectral data.

Further, the acetylated nucleoside analogues **5a**-**5e** were subjected to Zemplen deacetylation with NaOMe/MeOH at room temperature which led to the formation of the deacetylated purine nucleoside analogues **6a-6e**, respectively in good yields.



Scheme 2: Synthesis of purine derived nucleoside analogues. **a**) $CuSO_4.5H_2O$ (10 mol %), sodium ascorbate (20 mol %), *t*-BuOH**:** H_2O (1:1 v/v), rt, 4 h, 65-75 % **b**) NaOMe, MeOH, rt, 0.5 h, 80-85%.

As described in Scheme 1, similarly the aromatic azides **7a**-**7g** was prepared according to the reported procedure. The respective azide was subjected to CuAAC 1, 3-dipoar cycloaddition with the alkyne **3**. (**Scheme 3**) As described in Scheme 2, Click reaction conditions were maintained which led to the formation of final products **8a-8g** in good yields. The products were isolated and characterized with the help of ^{1}H, ^{13}C and Mass spectral data.



Scheme 3: Synthesis of *N*-9 unprotected 6-chloro-8-arylpurines 2-((6-chloro-9*H*-purin-2-yl) amino)-*N*-(prop-2-yn-1-yl) acetamide. **a**) $CuSO_4.5H_2O$ (10 mol %), sodium ascorbate (20 mol %), *t*-BuOH**:** H_2O (1:1 v/v), RT, 4 h, 75-86%.

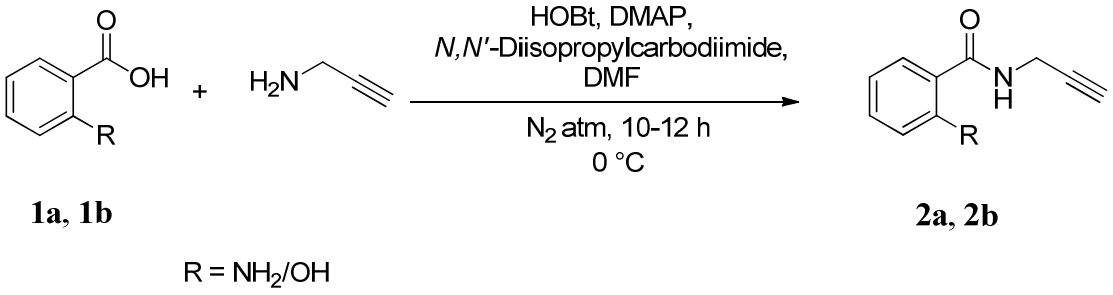
Chapter-4: Synthesis and bioevaluation of novel glycosyl 1, 2, 3-1H-triazolyl methyl benzamide derivatives

Diabetes mellitus (DM) is a chronic disease of global commons, which tends to have problems with adherence. Adherence with medication, diet, and exercise, and blood glucose self–monitoring is quite challenging. Due to consumption of carbohydrate-enriched diet, metabolic and heterogeneous disorder causing high blood-glucose level, leading to hyperglycemia. As per the Global Report on Diabetes (2018) by World Health Organization, diabetes was the seventh major cause of death in 2016. Carbohydrates form the largest group of naturally occurring compounds in nature and also crucial due to their pivotal role in medicinal chemistry and drug discovery. They are also known for their essential function in development, recognition, growth, function and survival of living cells and organisms. Many drug molecules including several antibiotics and few anti-diabetic medicines contain terminal sugar moieties which are necessary for their biological action.

Benzamide moiety is very significant class of nitrogen heterocycles are considered as privileged structures in drug discovery owing to their important roles as key building blocks in the synthesis of a many drugs. This heterocyclic nucleus is linked with diverse range of pharmacological activities such as antihypertensive,

antibacterial, anti-inflammatory, anticancer, analgesic, antihistamine, CNS stimulant and antidiabetic activities. Hence we tried to couple the benzamide nucleus with terminal sugar moieties by applying a suitable and biologically significant linker. 1,2,3-Triazoles are also an essential scaffold in drug discovery and development as several molecules with this moiety exhibited important biological activities such as antifungal, antitubercular, anticancer, anti-HIV, antibacterial, antiviral, anti-Alzheimer, anti-mycobacterial and glycosidase inhibitors.

2-Amino-*N*-propargyl benzamides (**2a** and **2b**) were prepared from commercially available anthranilic acid (**1a**) and 2-hydroxy-benzoic acid (**1b**) following earlier reported protocols as shown in **Scheme 1**. The structures were established on the basis of their spectroscopic data. The flask was degassed and then filled with N_2 (balloon), and HOBt (1mmol), 4- dimethylaminopyridine (DMAP, 1 mmol) were added to the reaction mixture. Then *N*, *N*'-Diisopropylcarbodiimide (DIPC, 1 mmol) was added to the reaction mixture in drop wise manner. After 10 mins, propargyl amine (1.1 mmol) was added and the reaction was stirred overnight at ambient temperature to get the desired 2-amino-*N*-(prop-2-yn-1-yl) benzamide (**2a**) 2-hydoxy-*N*-(prop-2-yn-1-yl) benzamide (**2b**) in good yield (**Scheme 1**).



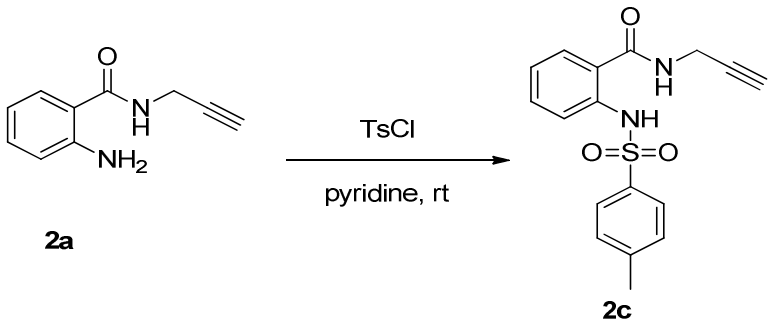
Scheme 1. Synthesis of 2-amino-*N*-propargyl benzamide derivatives.

The structure of the product **2a** was established by their spectroscopic data. ESIMS of the compound displays *m/z* = 175 as $[M+H]^+$ peak corresponding to its molecular

formulae $C_{10}H_{11}N_2O$. In IR spectrum, characteristic absorption peaks observed at 3372 cm^{-1} for amine, 3019 cm^{-1} for -NH, 1648 cm^{-1} for carbonyl (-NHC=O). In the ^1H NMR spectrum, the two exchangeable NH_2 protons were observed at δ 5.43 (bs, 2H, -NH_2) and the amide -NH proton was visible at δ 6.25 (bs, 1H, -NH) while the alkynyl proton was visible at δ 2.16 besides other usual protons at their usual chemical shift. In ^{13}C NMR spectrum, the peaks at δ 168.8 accounted the amide group carbon (-NH-CO-) along with other usual signals. Similarly, the reaction of 2-hydroxy-benzoic acid (**1b**) with propargyl amine the above reaction conditions led to the formation of 2-hydroxy-*N*-(prop-2-yn-1-yl) benzamide (**2b**) in good yield (**Scheme 1**).

Synthesis of 2-(4-methylphenylsulfonamido)-*N*-(prop-2-yn-1-yl) benzamide (2c)

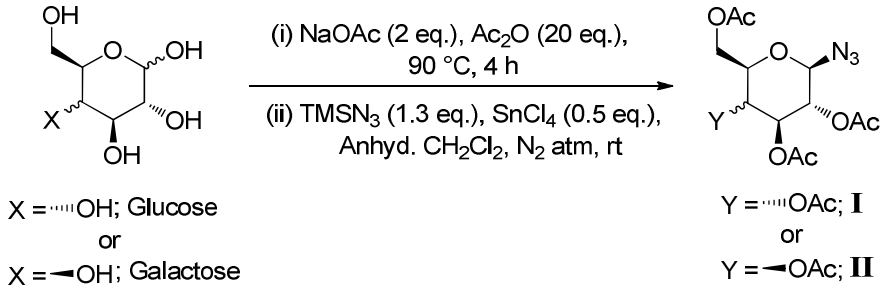
2-(4-methylphenylsulfonamido)-*N*-(prop-2-yn-1-yl) benzamide (**2c**) were prepared by following earlier reported protocols as shown in (**Scheme 2)**. To a solution of the 2-amino-*N*-(prop-2-yn-1-yl) benzamide (**2a**, 1.0 equiv) in pyridine (1 M) at room temperature, *p*-toluenesulfonyl chloride (1.05 equiv) was added. The reaction mixture was allowed to stir at room temperature overnight. The reaction mixture was quenched with water, and the aqueous layer was extracted with dichloromethane. The combined organic extracts were washed with aqueous copper sulphate. The organic layer was then dried over magnesium sulphate, filtered, and concentrated under reduced pressure. The residue was purified by column chromatography to afford the desired 2-(4-methylphenylsulfonamido)-*N*-(prop-2-yn-1-yl) benzamide (**2c**) in 79% yield (**Scheme 2**).



Scheme2. Synthesis of 2-(4-methylphenylsulfonamido)-*N*-(prop-2-yn-1-yl) benzamide derivative.

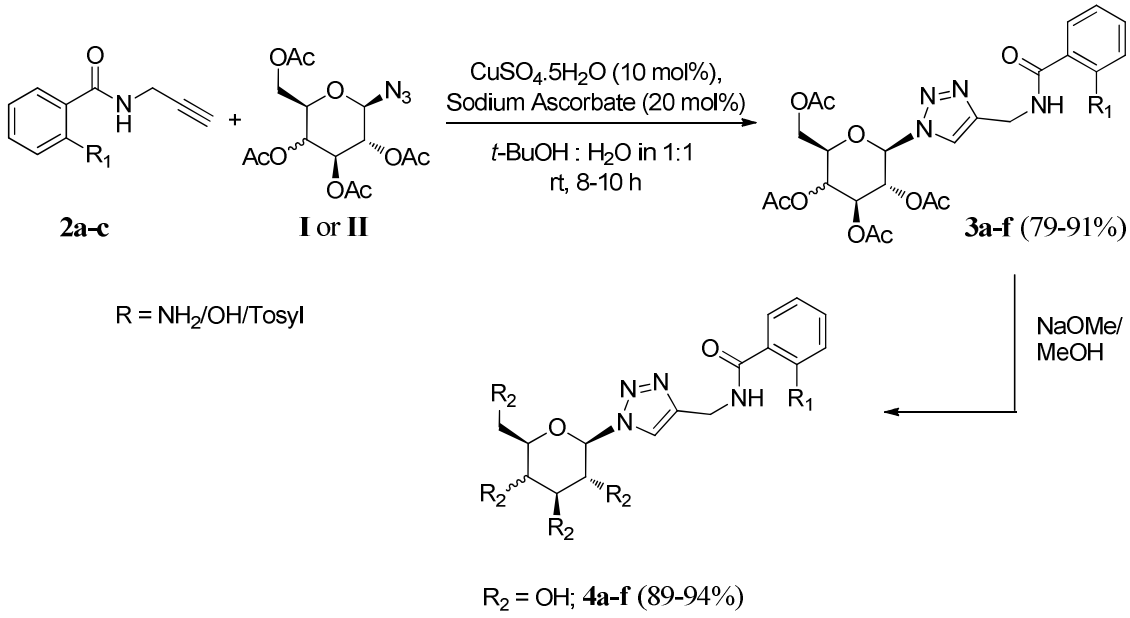
Synthesis of glycosyl triazolyl benzamide

The glycosyl azides (**I** and **II**) were prepared from commercially available glucose and galactose following the methods already reported in the literature as shown in (**Scheme 3**). The structures were established on the basis of their spectroscopic data. These were identical in all respects to those reported earlier.



Scheme 3. Synthesis of the glycosyl azides

The strategy for the synthesis of glycosyl triazolyl benzamide is depicted in **Scheme 4**. Having the 2-phenyl-3-propargyl-benzamide (**2-c**) and glycosyl azides (**I** and **II**) in our hand the CuAAC reactions were performed in *t*-BuOH/H_2O (1:1) using equimolar quantities of the reagents, $CuSO_4.5H_2O$ (10 mol%) and sodium ascorbate (20 mol%) at ambient temperature to afford epimeric mixtures of peracetyl glycosyl-triazolyl benzamide (**3a-f**) in good yields (**Scheme 4**). Propargyl benzamides and glycosyl azides selectively gave only one regioisomer, 1, 4-disubstituted triazole via 1, 3-dipolar cycloaddition reaction.



Scheme 4. Synthesis of glycosyl benzamide

In conclusion, we have synthesized novel glycosyl methyl benzamide analogues with 1, 4-regioselectivity employing the well-known CuAAC reaction of the propylated benzamides with different sugar azides in ambient condition in very good yields. These compounds were evaluated for α-glucosidase enzyme inhibitory activity and three compounds **4f**, **4d** and **4c** exhibited 42.9%, 40.6%, and 39.7% inhibition, respectively as compared to standard drug acarbose having 53.4 % inhibition of the enzyme. Thus, these glycosyl methyl benzamide analogues hold potential to be developed as antidiabetic agents.

Publications & Conferences

List of Research Publications

1. Identification of N-Hydroxycinnamamide analogues and their bio-evaluation against breast cancer cell lines. **Akhilesh Kumar Shukla,** Hamidullah, MK Shrivash, VD Tripathi, R Konwar, J Pandey. ***Biomedicine & Pharmacotherapy*** **(2018)**, 107, 475-483. **ISSN**-07533322
2. Study of developments of biologically active Quinazolinones derivatives: A review. Aniruddh Prasad Chaudhary, **Akhilesh Kumar Shukla**, Jyoti Pandey, Padam Kant ***Chemistry & Biology Interface***, **(2018)**, 8, 2, 62-83. **ISSN-** 22494820
3. Synthesis, characterization and antimicrobial evaluation of N-(4-oxo-2phenyl/thiophenyl quinazoline-3(4*H*)-yl)-1H-indole-2 or 3-carboxamide derivatives. Aniruddh Prasad Chaudhary, **Akhilesh Kumar Shukla**, Padam Kant ***Chemistry & Biology Interface***, **(2018)**, 8, 6, 359-372. **ISSN-** 22494820
4. Design and Synthesis of Novel Heterocyclic Curcumin Analogues as Anticancer Agents and Filarial Topoisomerase II Inhibitors. V.D.Tripathi and **Akhilesh Kumar Shukla.** ***Asian Journal of Organic & Medicinal Chemistry*** **(2018)**, 4, 3,149-153. **ISSN**-24568937
5. Regioselective Three Component Domino Synthesis of Polyhydrospiro [indoline-3, 3'- pyrrolizine]-2-one *via* [3+2] Cycloaddition Reaction. V.D.Tripathi and **Akhilesh Kumar Shukla** and H.S.Mohammed. ***Asian Journal of Organic Chemistry*** **(2019),** 31, 3, 613-616. **ISSN**-09707077
6. Identification of Novel Phenyl Butenonyl *C*-Glycosides with Ureidyl and Sulfonamidyl Moieties as Antimalarial Agents, K. K. G. Ramakrishna, S. Gunjan, **Akhilesh Kumar Shukla**, V. R. Pasam, V. M. Balaramnavar, A. Sharma, S. Jaiswal, J. Lal, R. Tripathi, Anubhooti, R. Ramachandran and Rama Pati Tripathi ***ACS Medicinal Chemistry Letters.*** **(2014)**, *5*, 878-883. **ISSN**-19485875
7. Development of novel glycosyl-1, 2, 3-1*H*-triazolyl methyl benzamide derivatives as inhibitor of α-glucosidase: Synthesis, characterization and *In-silico* study. **Akhilesh Kumar Shukla**, KKG Ramakrishna, MK Shrivash and Jyoti Pandey (Communicated).

8. Design, synthesis and *In-silico* evaluation of novel 1*H*-1, 2, 3-triazolyl-methyl-acetamide-D-glucose conjugates as antifungal agents. **Akhilesh Kumar Shukla**, Ravi. K.Thakur, MK Shrivash and Jyoti Pandey (Communicated).

Book Chapter:

1. Carbohydrates based chemotherapeutics: A frontier in drug discovery and development. S. Mishra, K. Upadhyaya, K. B. Mishra, **Akhilesh Kumar Shukla**, Rama P. Tripathi, and Vinod K. Tiwari. *SNPC **Elsevier book chapter*** **Book: SNPC49C-9780444636010,** Chapter-10, **(2016)**, Page 308-355**, Studies in Natural Products Chemistry, Vol. 49.http://dx.doi.org/10.1016/B978-0-444-63601-0.00010-7.**
2. Hydroxamates based chemotherapeutics: Frontier in drug discovery and development, Jyoti Pandey and **Akhilesh Kumar Shukla** (Under revision).

Presentations in Scientific Conferences:

1. Participation and Poster Presentation in **International Symposium** on "Emerging Frontiers in Carbohydrate Chemistry and Glycobiology" CARBO-XXXIV on **5th-7th December-2019** at Department of Chemistry, University of Lucknow,Lucknow Organised by Association of Carbohydrate Chemists and Technologists, India [**ACCTI**] and NIPER-Raebareli.
2. Participation and Oral Presentation in **Global Conference** On The Control Of Green House Gases At The Source By Physical And Chemical Technology on **22nd-24th**, **April-2019** at Department of Chemistry, School of Physical & Decision Sciences (SPDS) at Babasaheb Bhimrao Ambedkar University (A Central University), Vidya Vihar, Raebareli Road Lucknow-226025, UP, India.
3. Participation and Poster Presentation in **International Symposium** on "CHEMICAL SCIENCES: NATIONAL AND GLOBAL POSPECTIVE" on **29th-31st October-2018** at Lucknow Christian Degree College, Golaganj, Lucknow-226018.

4. Participation and Poster Presentation in **International Symposium** on "International Conference on Emerging Trends in Chemical Sciences (**ICETCS)** on **24th-25th February-2018 at** Deen Dayal Upadhyaya Gorakhpur University,Gorakhpur-273009.
5. Participation and Poster Presentation in **International Symposium** on "International Conference on Updates in Cancer Prevention and Research" [**ICUCPR-2017**] & Satellite Conference on Translational Pharmaceutical Research: Trends and Implication on **14th-16th& 20th February-2017** at Babasaheb Bhimrao Ambedkar University (A Central University), Vidya Vihar, Raebareli Road Lucknow-226025, UP, India.
6. Participation and Poster Presentation in National Seminar on "Role of analytical techniques in advanced scientific research" (**ATASR-2016**) **19-20 March, 2016** at National P.G.College, Lucknow, UP, India.
7. Participation and Poster Presentation in 6th **International Symposium** on "Current Trends in Drug Discovery & Research **25th-28th February 2016** at CSIR-CDRI, Lucknow, UP, India.
8. Participation and Poster Presentation in 3rd Lucknow Science Congress and **National Conference** on "Science for Society: An Interdisciplinary Approach" **31st Oct-2nd November-2015** at Babasaheb Bhimrao Ambedkar University (A Central University), Vidya Vihar, Raebareli Road Lucknow-226025, UP, India.
9. Participation and Poster Presentation in **National Conference** on Innovative Methods in Chemistry Education [IMCE-2015] & National Convention of Chemistry Teachers [**NCCT-2015**], **8th-10th October 2015** at Babasaheb Bhimrao Ambedkar University, Lucknow, UP, India.
10. Participation and Poster Presentation in 21st ISCB **International Conference** (ISCBC-2015), **25th-28th February-2015** at CSIR-Central Drug Research Institute, Lucknow, UP, India.
11. Participation and Poster Presentation in **International Conference** on The Ramanbhai Foundation 7th International Symposium on Current Trends in Pharmaceutical Sciences: "Advances in New Drug Discovery and Development" **2nd-4th February-2015** held at YMCA International Centre, S. G. Highway, Ahmedabad and Gujarat, India.

12. Participation and Poster Presentation in **International Conference** on challenges in chemistry and biology of Carbohydrates CARBO-XXVIII ,**20th-22th, Janaury-2014,** Dehra Dun, India Organised by Association of Carbohydrate Chemists and Technologists [**ACCTI**], India.
13. Participation and Poster Presentation in 5th**International symposium** on Drug Development for Orphan/Neglected Diseases (**CTDDR-2013**), **26th-28th February-2013**, at CSIR-Central Drug Research Institute, Lucknow, UP, India.
14. Participation and Poster Presentation in **International Conference** on Chemistry and Materials Prospects and Perspectives (**ICCMPP-2012**), **14th-16th December-2012**, at Babasaheb Bhimrao Ambedkar University (A Central University), Vidya Vihar, Raebareli Road Lucknow-226025, UP, India.
15. Participation and Poster Presentation in **International Conference**& Humboldt Kolleg on Recent aspects of Organic/Organometallic Compounds and their Usefulness in Materials and Industries on **03rd-06th January-2012** Organised by Chemistry Department, University of Lucknow, Lucknow, UP, India.

Biomedicine & Pharmacotherapy 107 (2018) 475–483

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

journal homepage: www.elsevier.com/locate/biopha

Identification of *N*-Hydroxycinnamamide analogues and their bio-evaluation against breast cancer cell lines

Akhilesh Kumar Shukla[a], Hamidullah[b,c,d], Manoj Kumar Shrivash[a], Vishwa Deepak Tripathi[e], Rituraj Konwar[c,d], Jyoti Pandey[a,*]

[a] Department of Chemistry, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow 226025, UP, India
[b] Department of Dermatology, University of Wisconsin Madison, WI 53706, USA
[c] Endocrinology Division, CSIR-Central Drug Research Institute (CDRI), Sector 10, Jankipuram Extension, Sitapur Road, Lucknow 226031, India
[d] Academy of Scientific and Innovative Research (AcSIR), CSIR-Central Drug Research Institute Campus, Lucknow 226031, India
[e] Department of Chemistry, Maharani Kalyani College Laheriasarai, Lalit Narayan Mithila University, Darbhanga 846001, Bihar, India

ARTICLE INFO

Keywords:
Breast cancer
MDA-MB-231
MCF-7
N-Hydroxycinnamamide
HEK-293

ABSTRACT

The present study demonstrates the identification of *N*-hydroxycinnamamide derivatives and their anticancer potential against human triple-negative breast cancer cell line MDA-MB-231, MCF-7 and non-malignant origin cell line, HEK-293 (human embryonic kidney). MTT assay was studied with HEK-293 cell line. Anticancer potential of the *N*-hydroxycinnamamide derivatives were compared with marked drug Tamoxifen through in vitro study. The compound numbers **3b** and **3h** exhibit most potent activity against antagonistic breast cancer cells (MDA-MB-231) with IC_{50} **13μM** and **5μM** respectively. **Compound 3h** promotes DNA fragmentation and induction of apoptosis. Furthermore, loss of mitochondrial membrane potential induced by **compound 3h**. The major mechanism of **compound 3h** for anti-breast cancer activity was probably initiation of reactive oxygen species (ROS) in cancer cells thereby persuading apoptotic cell deaths in cancer cells.

1. Introduction

Cancer is one of the most severe public health issue around the globe according to the World Health Organization (WHO) [1]. Among various type of cancers breast cancer is also one of the major causes of cancer death among women worldwide. Due to its complex cancer biology, it is necessary to use multiple therapeutic modalities. So far, the conventional treatments for breast cancer are surgical intervention, hormonal therapy, radiotherapy and chemotherapy. It is merely responsible for 20–25% of all cancer cases and 15–18% of cancer deaths among women [2]. Although the emergence of drugs such as Tamoxifen and Toremifene makes chemotherapy a viable choice for breast cancer patients, the development of drug resistance and severe side effects are unresolved problems in clinical oncology [3]. Therefore, the search for novel anti-cancer compounds with improved features is needed. In recent oncology research, different breast cancer cell lines have been applied by investigators for drug discovery purposes and among these cells estrogen non-dependant MDA-MB-231 is one of the most extensively used model [4].

Hydroxamic acids or hydroxamates are carboxylic acids or aldehyde analogues where −COOH group or −CHO group has been replaced by −CONHOH or −CONHR [5]. Hydroxamic acids are well known as efficacious molecules in the field of cancer chemotherapy and as a mutagenic agent. Several hydroxamates based drugs are functioning very good in clinics for cancer chemotherapy such as SAHA [6,7], PXD-101 (Belinostat,Topotarget) [8] and LBH-589 (panobinostat) [9], which are approved by the U.S. Food and Drug Administration (FDA) in October 2006, July 2014 and February 2015, respectively (Fig. 1). There are some other hydroxamate based molecules are in clinical trials, such as m-carboxycinnamic acid bishydroximic acid (CBHA) [10], SB-939 (phase II) [11] and 4SC-201 (Resminostat, phase II) [12].

Among various derivatives of hydroxamic acid, SAHA (Suberoylanilide Hydroxamic Acid) is considered as a potent anticancer agent [13]. These molecules possess very good chelating ability [14]. This chelating property makes them very favourable for enzyme inhibition and therefore hydroxamates possess a special place in cancer drug discovery research. Due to these special properties hydroxamates are very interesting group for scientists from all over the world. Several research groups have synthesized different hydroxamic acid moieties with well-known inhibitors of matrix metalloproteinases (MMPs) [15], peptidyldeformylases [16], adenylyl cyclases (ACs) [17], inosine monophosphate dehydrogenase (IMPDH), histone deacetylase (HDAC)

* Corresponding author.
E-mail address: drjyotibbau@gmail.com (J. Pandey).

https://doi.org/10.1016/j.biopha.2018.08.015
Received 11 June 2018; Received in revised form 24 July 2018; Accepted 6 August 2018

AJOMC-P149

ARTICLE

www.asianpubs.org

Design and Synthesis of Novel Heterocyclic Curcumin Analogues as Anticancer Agents and Filarial Topoisomerase II Inhibitors

Vishwa Deepak Tripathi[1,✉] and Akhilesh Kumar Shukla[2]

Asian Journal of Organic & Medicinal Chemistry

Volume: 3 Year: 2018
Issue: 4 Month: October–December
pp:
DOI: https://doi.org/10.14233/ajomc.2018.AJOMC-P149

Received:
Accepted:
Published:

ABSTRACT

A series of substituted curcumin analogues have been designed and synthesized *via* condensation reaction of benzaldehydes and dehydroacetic acid. Synthesized molecules were further evaluated for their inhibitory activity against various cancer cell lines. Most of the synthesized compounds were significantly inhibited the growth of these cell lines. Ten most active compounds in the series were further screened to check their inhibitory effect against filarial topoisomerase II enzyme. All the compounds screened against topoisomerase II exhibited excellent inhibition upto percentage inhibition more than 95 %. Further, the structure-activity relationships of the evaluated compounds reveals that among the synthesized compounds, nitro substituted chalcones **5** and **8** were the most active compounds having IC_{50} value of 5.49 and 4.46 µM against A549 (*lung carcinoma*) cell lines, respectively and significantly inhibited *S. cervi* Topoisomerase II activity upto more than 95 %.

KEYWORDS

Curcumin, Anticancer, Topoisomerases II, Chalcones.

Please provide all the spectral data and other physico-analytical data of compounds from 4-22

INTRODUCTION

In spite of the recent progress and development cancer chemotherapy, there is still need for new compounds of therapeutic interest to bring this disease under control. A large number of modern drugs have been developed from natural sources, especially from plants [1]. Natural product derive or inspired molecules forms a large group of compounds with anticancer activity. A variety of naturally occuring compounds such as curcumin, paclitaxol, vinblastin, combretastatin A-4, desmosdumotin C and colchicine are well known anticancer agents.

Among them curcumin is the compound possessing a large number of biological activities and is most abundant in nature. Curcumin, is the yellow pigment extracted from the rhizoma of *Curcuma longa*, is the pharmacologically active substance of turmeric. By tradition, turmeric has been used for many ailments, particularly as an anti-inflammatory agent, and curcumin has been identified as the active principle of turmeric [2]. Curcumin is non-toxic and has a variety of positive pharmacological effects as anti-inflammatory, anti-oxidative and antiseptic properties have been reported and displayed good pharma-

Author affiliations:

[1]Department of Chemistry, Lalit Narayan Mithila University, M.K. College, Darbhanga-846003, India
Department of Applied Chemistry, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow-226025, India

✉To whom correspondence to be addressed:

Fax:
Tel:
E-mail: vishwadeepak66@gmail.com

Available online at: http://ajomc.asianpubs.org

Asian Journal of Chemistry; Vol. 31, No. 3 (2019), 613-616

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2019.21737

Regioselective Three Component Domino Synthesis of Polyhydrospiro[indoline-3,3'-pyrrolizine]-2-one *via* [3+2] Cycloaddition Reaction

VISHWA DEEPAK TRIPATHI[1,*], AKHILESH KUMAR SHUKLA[2] and HASAN SHAMRAN MOHAMMED[3]

[1]Department of Chemistry, M.K. College (Lalit Narayan Mithila University), Darbhanga-846003, India
[2]Department of Applied Chemistry, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow-226025, India
[3]Department of Chemistry, Science College, Al-Qadisiyah University, Al-Qadisiyah, Iraq

*Corresponding author: E-mail: vishwadeepak66@gmail.com

Received: 3 October 2018; *Accepted*: 12 November 2018; *Published online*: 31 January 2019; AJC-19255

In present work, we have reported the synthesis and characterisation of novel hexahydrospiro[indoline-3,3′-pyrrolizine]-2-one derivatives in good to excellent yields *via* [3+2] cycloaddtion reaction in regioselective manner. These compounds were synthesized *via* multicomponent reaction of substituted 3-cinnamoyl-4-hydroxy-6-methyl-2*H*-pyran-2-one, isatin, L-proline at room temperature. All the synthesized hexahydrospiro molecules were characterized by ^{1}H and ^{13}C NMR, IR spectra, mass spectra and elemental analysis. Regioselectivity in synthesized molecules were also explained on the basis of secondary orbital interactions. A simple and facile methodology is developed which has great importance in synthetic chemistry.

Keywords: Spiropyrrolidine, Dehydroacetic acid, Isatin, Multicomponent reaction, Cycloaddition.

INTRODUCTION

In the past several years, significant advances have been achieved on the development of new synthetic methods to access spiroindole derivatives. Spiro compounds with attached indole moiety having all-carbon quaternary stereogenic center has attracted synthetic organic chemists due to its fascinating structure and biological importance [1-3]. These polyhydro heterocyclic compounds are important targets among synthetic as well as medicinal chemist due to its wide range of pharmacological properties. Spiroindoles and their derivatives have facile structural similarity the core unit of many naturally occurring molecules that possess significant biological activities, which include spasmolitic, diuretic, anticoagulant, anticancer, and antianaphylactic activities [4].

Therefore, a number of protocols have been developed to synthesize these structural frameworks *via* cycloaddition reaction [5]. The [3+2] cycloadditions have special place in synthetic chemistry as well for theoretical chemist because it constitutes one of the most fundamental reactions for regioselective construction of 5-membered heterocyclic compounds [6-8]. The reaction of azomethine ylides with various dipolarophils forms highly substituted pyrrolidines derivatives [9,10]. Enhancing the efficiency and manoeuvrability of reaction is a challenge in organic synthesis. For a molecule with fascinating structure and excellent pharmacological properties always promotes synthetic chemists to develop easy and ecofriendly synthesis [11]. Spirocyclic systems containing one carbon atom common to two rings are structurally interesting and asymmetric characteristic of the molecule due to the chiral spiro carbon which is one of the important criteria of the biological activities [12,13]. The presence of sterically constrained spiro structure in various natural products also adds to the interest in the investigations of spiro compounds [14]. The spirooxindole ring system forms the core structure of many pharmacological agents and alkaloids [15]. For example, spirotryprostatin (Fig. 1), a natural product isolated from the fermentation of *Aspergillus funigatus*, has been identified as a novel inhibitor of microtubule assembly [16]. Natural product isopteropodine [17] (Fig. 1) has been shown to modulate the function of muscarinic and serotonin receptors. It has been observed that incorporation of more than one bioactive heterocyclic moiety into a single framework may result into the production of novel heterocycles with enhanced bioactivity [18-20]. Spirooxindoles have been reported to behave as aldose reductase,

Chemistry & Biology Interface, 2018, 8, 6, 359-372

RESEARCH PAPER ISSN: 2249 –4820

CHEMISTRY & BIOLOGY INTERFACE

An official Journal of ISCB, Journal homepage; www.cbijournal.com

Synthesis, characterization and antimicrobial evaluation of N-(4-oxo-2phenyl/ thiophenylquinazolin-3(*4H*)-yl)-1H-indole-2 or 3-carboxamide derivatives

Aniruddh Prasad Chaudhary[a], Akhilesh Kumar Shukla[b] and Padam Kant[a]*

[a]*Department of Chemistry University of Lucknow, Lucknow 226007*
[b]*Department of Chemistry, Babasaheb Bhimrao Ambedkar University (A Central University), Lucknow-226025,UP, India.*
E-mail: kant_padam@lkouniv.ac.in, anirudhraj0002@gmail.com
Received 4 July 2018, Accepted; 28 December 2018

Abstract: A series of novel N-(4-oxo-2phenyl/thiophenylquinazolin-3(*4H*)-yl)-1H-indole-2 or 3-carboxamide derivatives were prepared and screened for their antimicrobial activities against different strains of bacteria and fungi. The most potent antibacterial and antifungal activity was exhibited by compound **5d & 5h** with halogen substituted ring in the respective series compared to the compounds bearing other electron donating or withdrawing groups. The synthesized compounds were characterized with the help of spectroscopic techniques including IR, ^{1}H-NMR, ^{13}C-NMR and Mass spectra.

Keywords: Quinazolinone, carboxamide derivatives, antibacterial and antifungal activity

Introduction:

Infectious diseases caused by microorganisms and their emergence to resistance pose a serious threat to present and future populations. [1-5] Thus there is an urgent need for new antimicrobial agents. Quinazolinone derivatives, one of the most active classes of compounds, possess a wide spectrum of biological activities [6-10] asantitumor [11-15], anti-inflammatory, [16-17] anticonvulsant, [18] antioxidants, [19] analgesic [20-22] and antimicrobial [23-30].

On the other hand, the indole framework represents a "privileged" structural motif commonly found in pharmaceutical drugs and natural products.[31]It is an important bicyclic heterocyclic nucleus and it is formed by the fusion of pyrrol and benzene ring at α, β position through sharing of one double bond [32-33].Several substituted indole are reported in literature which showssignificant antibacterial, analgesic, anti-inflammatory, antipyretic,antitumor, anti-hypertensive, anti-depressant, antagonist, anti-emetic and antiviral

ACS Medicinal Chemistry Letters

Letter

pubs.acs.org/acsmedchemlett

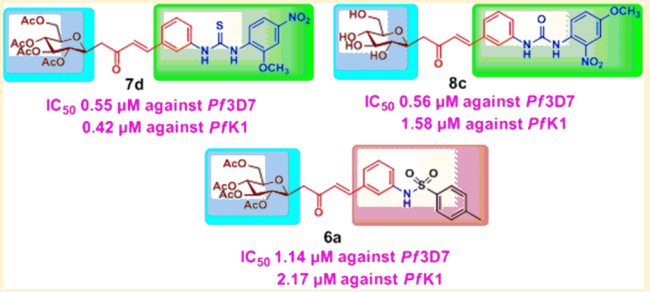
Identification of Novel Phenyl Butenonyl *C*-Glycosides with Ureidyl and Sulfonamidyl Moieties as Antimalarial Agents

K. Kumar G. Ramakrishna,[‡] Sarika Gunjan,[§] Akhilesh Kumar Shukla,[‡] Venkata Reddy Pasam,[‡] Vishal M. Balaramnavar,[‡] Abhisheak Sharma,[∥] Swati Jaiswal,[∥] Jawahar Lal,[∥,†] Renu Tripathi,*[,§,†] Anubhooti,[#] Ravishankar Ramachandran,[†,#] and Rama Pati Tripathi*[,‡,†]

[†]Academy of Innovative Science and Research, [‡]Medicinal and Process Chemistry Division, [§]Parasitology Division, [∥]Pharmacokinetics & Metabolism Division, and [#]Molecular and Structural Biology Division, CSIR-Central Drug Research Institute (CSIR-CDRI), Sector 10, Jankipuram Extension, Sitapur Road, Lucknow 226031, India

Supporting Information

ABSTRACT: A new series of *C*-linked phenyl butenonyl glycosides bearing ureidyl(thioureidyl) and sulfonamidyl moieties in the phenyl rings were designed, synthesized, and evaluated for their *in vitro* antimalarial activities against *Plasmodium falciparum* 3D7 (CQ sensitive) and K1 (CQ resistant) strains. Among all the compounds screened the *C*-linked phenyl butenonyl glycosides bearing sulfonamidyl moiety (**5a**) and ureidyl moiety in the phenyl ring (**7d** and **8c**) showed promising antimalarial activities against both 3D7 and K1 strains with IC_{50} values in micromolar range and low cytotoxicity offering new HITS for further exploration.



KEYWORDS: *Antimalarial agent, phenyl sulfonamides, diarylureides, Plasmodium falciparum*

Malaria, the most severe parasitic disease, infects more than 500 million people and continues to kill around one million children each year.[1] Most of the malarial infections and deaths are due to *Plasmodium falciparum* and *Plasmodium vivax* species. Although an arsenal of very effective antimalarial drugs have been used to control this disease, the culprit *P. falciparum* has developed resistance to nearly all available antimalarial drugs.[2] Artemisinin, the last line of defense against multidrug resistant malaria parasites in some parts of the world became resistance in present circumstance.[3,4] The recent emergence of resistance necessitates the search for new antimalarial drugs, which overcome the resistance and act through novel mechanisms.

The dihydropteroate synthase (DHPS), hemoglobin degradation enzymes, and shikimate pathway enzymes have been identified for the novel potential targets for new antimalarial drugs in the past decade.[5] Very recently a class of hemoglobin degradation enzymes, plasmepsins, has been discovered as a validated drug target and diphenyl ureas are known to inhibit this enzyme and display antimalarial activity.[6] Several other urea derivatives exhibit potent antimalarial activity.[7−10] *Plasmodium falciparum* hexose transporter (PfHT) plays a very important role in malaria parasites as a critical enzyme for glucose uptake and the survival of the parasite.[11] Simple 3-O-alkyl/alkenyl glucosides were shown to inhibit the PfHT and good antimalarial activity. Indeed, glucose is an essential energy substrate in many parasites, and they can undergo a metabolic shift *in vivo*, switching from predominately glycolytic metabolism to metabolism of alternative carbon sources through induction of gene sets combined with function of mitochondria and apicoplast.[12] Glucose delivery, however, is crucial for parasite survival and may also be critical for metabolic diversion of this key substrate from host tissues and thereby aggravating the disease processes.[13] Diphenyl propenones (chalcones), however, also exhibit antimalrial activity,[7,14−22] and malaria trophozoite cysteine protease has been proposed as possible target for this class of compound.[14,18] Phenyl urenyl chalcones also exhibit antimalrail activity via multiple mechanisms.[7]

Inspired by the above facts we thought to design and synthesize compounds based on sugars having *C*-linked phenyl propenone moiety and diphenyl urea units together to get hitherto unreported antimalarial agents (Figure 1). In order to further analyze the feature requirement of these molecules in 3D space, we analyzed the common features through HipHop algorithm.[23] The HipHop algorithm finds the common feature pharmacophore model among the set of the highly active ligands and thus referred as qualitative model without the use of the activity data representing the 3D arrangement of the essential features important for the specific activity. HypoGen on the converse deals with the development of quantitative pharmacophore model and requires biological activities with at least 3−4 orders of difference; therefore, in this work, HipHop

Received: March 27, 2014
Accepted: June 2, 2014
Published: June 2, 2014

INTERNATIONAL CARBOHYDRATE CONFERENCE (CARBO-XXXIV)

Centenary Year Celebrations

"Emerging Frontiers in Carbohydrate Chemistry and Glycobiology"

December 5-7, 2019

Certificate

This is to certify that

AKHILESH KUMAR SHUKLA
BBAU, LUCKNOW, INDIA

PARTICIPATED AND PRESENTED POSTER

in the International Carbohydrate Conference (CARBO-XXXIV) held at Department of Chemistry, University of Lucknow, Lucknow, India.

Dr. Desh Deepak	**Prof. Ashok Prasad**	**Prof. Naveen Khare**
Convener, CARBO-XXXIV	Secretary, ACCTI	President, ACCTI & Organising Secretary, CARBO-XXXIV

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

This event has been proposed on the occasion of the
WORLD EARTH DAY-22 April 2k19 By
Department of Chemistry, School of Physical & Decision Sciences (SPDS)
Babasaheb Bhimrao Ambedkar University (A Central University)
Vidya Vihar, Raebareli Road Lucknow - 226025, India
In Association With The Asian Association of Sugarcane Technologists' (AASCT)

GCGHGSPCT-2k19
GLOBAL CONFERENCE ON THE CONTROL OF GREENHOUSE GASES AT THE SOURCE BY PHYSICAL AND CHEMICAL TECHNOLOGY

Global Conference On The Control Of Green House Gases At The Source By Physical And Chemical Technology

22 - 24, April - 2019

Certificate of Participation

This is to certify that Prof./ Dr./ ~~Mr.~~/ Ms Akhilesh Kumar Shukla

from Babasaheb Bhimrao Ambedkar University (A Central University) Lucknow, India

has participated and contributed in the conference as

Chairman of Session/ Plenary Speaker/ Distinguished Speaker/ Oral/ Poster Presentor/Volunteer

A K Tiwari

(Dr. Anjani K. Tiwari)
Organizing Secretary

Kaman

(Prof. Kaman Singh)
Chairman/Convener

DEEN DAYAL UPADHYAYA GORAKHPUR UNIVERSITY, GORAKHPUR

International Conference
on
Emerging Trends in Chemical Sciences
(ICETCS)

Certificate

This is to certify that Prof./Dr./Mr./Ms. Akhilesh Kumar Shukla *of*

BBAU, Lucknow *has*

participated and presented a paper (oral/poster) in the Conference held on February 24-25, 2018.

He/She was awarded Nil *prize in poster presentation.*

S. K. Sengupta
Convenor

O. P. Pandey
Head & Organizing Secretary

Organised by DEPARTMENT OF CHEMISTRY, D.D.U. Gorakhpur University, Gorakhpur-273 009

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW
प्रज्ञा शील करुणा
ESTABLISHED 1996

International Conference on Updates in Cancer Prevention and Research (ICUCPR-2017)

&

Satellite Conference on Translational Pharmaceutical Research: Trends and Implications

14th – 16th & 20th February 2017

Certificate

This is to certify that Prof./Dr./Mr./Ms. Akhilesh K Shukla

has chaired the session/presented an invited lecture/oral presentation/poster presentation/participated entitled "Synthesis & biological anticancer agents"

in ICUCPR & IPCBBAU-2017 at Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh.

Anand Prakash
Convener

R.C. Sobti
Vice Chancellor & Patron

6th International Symposium
on
"Current Trends in Drug Discovery & Research"

25th - 28th February 2016

CTDDR-2016

Certificate

This is to certify that Dr./Mr./Ms. Akhilesh Kumar Shukla

has participated/ presented a paper (oral/poster) in the

CTDDR-2016 symposium held at CSIR-CDRI, Lucknow

(Arun Kumar Sinha)
Organizing Secretary

(Madhu Dikshit)
Chairperson

CSIR - Central Drug Research Institute, Lucknow
(Council of Scientific & Industrial Research)
Web: cdriindia.org

NATIONAL SEMINAR

On

"ROLE OF ANALYTICAL TECHNIQUES IN ADVANCED SCIENTIFIC RESEARCH"

(ATASR-2016)

March 19-20, 2016

CERTIFICATE

This is to certify that Prof/ Dr/ Mr/ Ms .....AKHILESH KUMAR SHUKLA..... affiliated with .....Baba Bhimrao Ambedkar University..... actively participated in the scientific deliberations and also delivered ~~invited lecture~~ / participated or presented ~~paper~~ / poster entitled .....“Hydroximic acid – A novel molecule for anticancer therapy.”.....

during the National Seminar on **“Role of Analytical Techniques in Advanced Scientific Research” (ATASR-2016) organized by Department of Chemistry, National Post Graduate College (An Autonomous College of Lucknow University, NAAC 'A' Grade, CPE),** from March 19-20, 2016.

Prof. S. P. Singh
Patron & Principal

Dr. Vikas Singh
Convener

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
LUCKNOW
प्रज्ञा शील करुणा
ESTABLISHED 1996

3rd Lucknow Science Congress and National Conference

on

"Science For Society : An Interdisciplinary Approach"

31st October - 2nd November 2015

Certificate

This is to certify that Prof./Dr./Mr./Ms./ Akhilesh Kumar Shukla has chaired the session/ participated/volunteered/presented Model/Poster/Invited Lecture/Research paper entitled Pyrrolidines Importance in Medicinal Chemistry in the 3rd Lucknow Science Congress & National Conference on "Science for Society : An Interdisciplinary Approach", organized by Babasaheb Bhimrao Ambedkar University from 31st October to 2nd November, 2015.

R. C. Sobti
Vice Chancellor & Patron

Kamal Jaiswal
Convenor

URKUND

Urkund Analysis Result

Analysed Document: Thesis 24Jan2020.pdf (D62916197)
Submitted: 1/24/2020 8:02:00 AM
Submitted By: gbl.bbau@gmail.com
Significance: 1 %

Sources included in the report:

https://www.deepdyve.com/lp/royal-society-of-chemistry/synthesis-and-bioevaluation-of-aryl-hydroxamates-distinguishing-ALWk8D4c7I
https://www.researchgate.net/publication/328499055_Design_synthesis_and_in_vitro_biological_evaluation_of_novel_benzimidazole_tethered_allylidenehydrazinylmethylthiazole_derivatives_as_potent_inhibitors_of_Mycobacterium_tuberculosis
66ca84ad-8555-4999-b171-dc350748fcf9
de8af415-1673-4633-80d6-87ad98a167e4
https://www.researchgate.net/publication/237056832_The_design_and_synthesis_of_a_new_class_of_RTKHDAC_dual-targeted_inhibitors
https://link.springer.com/article/10.1007/s41061-019-0243-6

Instances where selected sources appear:

17