Synthesis, characterization and evaluation of biological activity of some modified lead molecules

ABSTRACT

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Abstract

The very objective of the present work was to design novel molecules based on reported lead structures for different ailments by docking against important molecular targets using computational methods. The selected molecules were synthesized and characterized using unambiguous protocols with necessary modifications wherever required. These compounds involve mainly polyphenols including curcumin analogues, flavonoids and stereoselective oximes which were subjected to *in vivo* testing. Some of these are being reported as potent anti-bacterials, antifungals, anticancer and anti-Parkinson's disease. This study is likely to be useful in developing these selected molecules as future drugs.

Whole research work is divided in to five chapters. The first chapter starts with the history of drugs describing human struggle to find alternative cures for different diseases since his existence on this planet. The earlier remedies were accidental discoveries. Human experiences transferred through generations have resulted in certain stabilized potent molecules for cure of different diseases. The three phases in Medicinal Chemistry for drug development have been described. The transformation of some lead molecules to effective drugs has been discussed. Lead molecule is a chemical compound that has pharmacological or biological activity likely to be therapeutically useful, but may nevertheless have suboptimal structure that requires modification to fit better to the target; lead drugs offer the prospect of being followed by back-up compounds. Some examples of lead molecules are given in chapter one. We have discussed about the role of polyphenols in human and plants, their origin and biological importance. We have also discussed about curcumin, the yellow pigment of turmeric as a lead molecule used in traditional medicine. Extraction of curcumin from turmeric rhizome, molecular targets of curcumin in human, therapeutic properties of curcumin and limitations of curcumin as a drug have been discussed. A concise account has been given about the tools of bioinformatics used for drug discovery. Computational methods and software's used for drug development are described. Sequential process of *in silico* drug design has been illustrated. A brief account of the work done has been given in chapters two to five.

In chapter two the introduction of Parkinson's disease (PD), its molecular targets including reported literature for its cure have been given. The marketed drugs for the treatment of Parkinson's disease are L-Dopa, Carbidopa and Dopamine. Curcumin is a wonder molecule but has certain limitations to be used as a drug due to its speedy metabolism, biotransformation, quick elimination, inefficient cellular uptake and transport across the blood brain barrier (BBB) resulting in low bioavailability. The literature reports indicate that deficiency of glucose in the brain causes neurogenetic diseases like PD and Alzheimer (AD). Our concept was that glucose moiety covalently bonded with curcumin can serve dual purpose i.e. enhance the uptake of curcumin via BBB barrier and also increase the concentration of glucose in the brain. Therefore, mono and di-glucosides of curcumin were prepared. However the *in silico* study with PD target (α-Synuclein) indicated that curcumin-di-glucoside is more effective as compared to the mono glucoside or curcumin. We synthesized curcumin via aldol condensation of vanillin and acetyl acetone using boric acid, m-xylene and catalytic amount of n-butyl amine. Curcumin was prepared, purified and characterized with ^{1}H NMR, ^{13}C NMR spectrum. The synthesis of mono and di-glucosides of curcumin was carried out by reacting acetobromoglucose, with vanillin followed by nucleophilic substitution reaction with base and phase transfer catalyst, benzyl tri butyl ammonium chloride (BTBAC) in aqueous NaOH and DCM to yield tetra-*O*-acetyl-β-D-glucopyranosyl vanillin. Tetra-*O*-acetyl-β-D-glucopyranosyl vanillin underwent aldol condensation with acetyl acetone in presence of tributyl borate to give B_2O_3 complex .The acetylated mono and di glucosides of curcumin were separated using column chromatography. The deacetylation of the tetra acetates of mono and di glucosides of curcumin was carried out with sodium methoxide in dry methanol at 0 °C, followed by neutralization of the reaction mixture with acidic Dowex resin. Pure mono and di-glucosides were characterized with ^{1}H NMR spectrum. Both glucosides were tested on neuro cell line which indicated that monoglucoside was more effective.

The *in vitro* and *in vivo* bioassay studies of curcumin versus it's mono and di-glucosides against Rotenone (ROT) induced toxicity in N27 cells demonstrated that Curcumin-4-*O*-β-D- monoglucoside has better bioavailability and protective effect than curcumin - diglucoside. Although *in silico* studies indicated that di glucoside has stronger binding with the target but in wet experiment the monoglucoside proved better. The probable reason may be that the free phenolic group of curcumin monoglucoside contributes to its antioxidant activity, the glucoside moiety attached to

the other phenolic helps in enhancing solubility, cellular uptake and bioavailability. According to our knowledge, this is the first report to show that curcumin monoglucoside has a protective effect in ROT-based PD models, suggesting that it could offer better pharmacokinetics and pharmacodynamics compared to curcumin in Parkinsons's disease (PD).

In third chapter, after successful preparation of curcumin as described in previous chapter we synthesized 10 other curcumin derivatives and nano curcumin. Literature reports suggest that active methylene group and the ketone moiety of curcumin are responsible for its initial metabolism. In order to overcome this problem and to improve its pharmacokinetics properties, we studied several synthetic modifications on the carbonyl, active methylene group and side functionalities on aromatic rings. Nitrogen and sulfur containing heterocyclic moieties such as pyrazoles, pyrimidines and thiazoles are well known for their broad-spectrum of pharmacological properties such as antimicrobial, anti-inflammatory, analgesic, enzyme inhibition, antioxidant, and anticancer. These scaffolds have important role in drug designing as important pharmacophores. Therefore towards the synthetic goal, we focused on incorporating heterocyclic moieties i.e. isoxazole, N-substituted pyrazoles, pyrimidine-2(1H)-thione and pyrimidine ring in the molecular scaffold of curcumin. In addition we focused on free phenolic group of curcumin and synthesized sulphate, phosphate and phthalimide glycine and tetra acetyl mono glucoside derivatives. Unambiguous methods were used for synthesis. Based on the *in silico* analysis we executed the chemical synthesis, *in vitro* analysis and validation of the obtained results.

The MIC / IC_{50} values and binding energies of curcumin and its analogues with MipZ and Pyruvate kinase proteins of *Pseudomonas aeruginosa* (Gram negative) and *Staphylococcus aureus* (Gram positive) bacteria respectively have been given in chapter third. We could arrange these compounds in the following order with respect to relative activities towards both Gram positive *(Staphylococcus aureus)* and Gram negative *(Pseudomonas aeruginosa)* bacteria. The IC_{50} of chloramphenicol against both bacteria was considerably less in comparison to the synthesized compounds. From the results obtained it can be concluded that curcumin analogues with the linker between two aromatic rings substituted with nitrogen containing heterocyclic rings e.g. isoxazole, pyrazole, thiazole, thione can prove to be much better drugs for antibacterial activity. Such molecules along with substituents like phosphate or

sulphate substituted at one of the two phenolic hydroxyls of curcumin can prove to be potent anti-inflammatory agents due to their multi-targeted effects. These compounds appear to act by some alternate mechanism too e.g. inhibiting cell division in addition to degradation of bacterial cell wall. These compounds can serve as better antibacterial alternatives, as now a days the microbes become resistant to antibiotics due to their unselective use.

In chapter fourth we have described the design and discovery of new plant based herbal antifungal compounds specifically for inhibiting filamentation in *Candida albicans* and thus attenuate its pathogenicity. These compounds are non-toxic and better solve the problem of drug resistance as well. *Candida albicans* is present in human biome in yeast shape and for most part found in skin and gastrointestinal cavity. It is generally sedate safe in nature, however polymorphic change causes flagellation leading to pathogenicity. The progress of yeast to hyphal shape is called dimorphism. The two structures are critical, however hyphal frame is one more obtrusive for human species.

Our study has revealed four foremost pathways occurring in various cell organelles of *C. albicans*, i.e., glyoxalate pathway, Ras1-pka pathway, Ergosterol pathway and flagging pathways. Further the key components which can possibly influence most extreme pathways have been recorded as Efg1 protein, 2QZX i.e. secreted aspartic proteinase (Sap5), Erg11 and Glyoxalate pathway. We docked *in-silico* all the above mentioned targets with a class of polyphenols such as flavonoids, coumarins, chalcones and curcumin analogs and the best compounds were sorted. The compounds chalcone **(54),** coumarin **(50),** quercetin tetraacetate **(56),** quercetin penta acetate **(55)** and (1E,6E)-1,7-di(1H-indol-3-yl) hepta-1,6-dience-3,5-dione) **(58)** were synthesised using unambiguous methods and further tested *in-vivo* for their sensitivity on the *Candidanian* strain SC5314. We synthesized coumarin **(50)** by Pechmann condensation reaction, chalcone **(54)** via claisen schmidt condensation, quercetin tetra and penta acetates by selective acetylation of quercetin. The synthesis of 1,7-di(1H-indol-3-yl)hepta-1,6-diene-3,5-dione **(58)** was done according to the synthesis of curcumin **(3)**. All these compounds were purified and characterized with ^{1}H NMR, ^{13}C NMR spectra. These were tested for their sensitivity on the *Candidanian* strain SC5314. Quercetin pentacetate **(55),** quercetin tetracetate **(56)** and coumarin **(50)** have been found active in all four targets illustrating their high affinity. The marketed antifungal drugs are mainly azoles e.g Fluconazole has been reported

with many side effects. Including fluconazole most azoles are very toxic for human biome. In such scenario the non-toxic polyphenols seem to show promising results as inhibitors of flagellation, the main cause of pathogenicity in *Candida* species.

In fifth chapter we have prepared stereoselective *Z* isomer of oximes by a novel method. Many methods for synthesis of oximes are reported in literature. Generally a mixture of *E* and *Z* isomers is obtained. Oximes are biologically important. Many oximes are known for their anticancer activity.

We have developed a novel method of synthesis of stereoselective oximes (mainly Z isomer) through direct conversion of substituted aryl/alkyl cyano esters to aryl/alkyl oximes. The alkene-bridged ethyl cyano arylacrylate compounds undergo unexpected C-C bond cleavage with the associated loss of the ethyl cyanoacetate group by Michael addition of hydroxylamine to a benzylidene cyanoacetate followed by a retro Knoevenagel reaction (1, 3 proton shift) without transition-metal catalysis. The significant advantage of the present method is the formation of stereoselective oximes (Mainly *Z* form) since this form is known to be more biologically active. Substituted benzylidene cyano acetates were synthesized via aldol condensation followed by substituted aldehydes with ethyl cyanoacetate using sodium ethoxide as catalyst. Different bases were used as standard, however, sodium ethoxide was found to give maximum yields under optimal conditions. The yields of oximes were in the order heterocyclic>aryl>alkyl. Our new strategy presents one step, time and cost effective cleaner preparation of predominantly *Z* -oximes. These oximes were tested against cancer cell lines MCF-7, A431, A549, PC-3, HepG2, MDAMB-231, L-132, NCIH-520, NCIH-460 .The MTT assay and IC_{50} values indicated that (*Z*)-2,3,4-tri methoxy benzaldehyde oxime **(114)** had maximum antiproliferative activity.

Overall objective of the present work was to develop novel potent molecules from known lead structures by appropriate chemical modification. The molecules were designed by computational methods i.e. docking against selected molecular targets. This was followed by their synthesis using unambiguous methods with necessary modifications wherever required, these compounds were then tested for their activity as compared with standard drugs. We have suggested new future drug like molecules for cure of Parkinson's disease, anti-bacterial, antifungals and anticancer pro-drugs. These molecules can be tested further on animal models for validation. These drugs are likely to prove safe answer to multiple resistance.