SYNTHESIS AND EVALUATION OF SEROTONIN 5-HT LIGANDS

Abstract of Thesis

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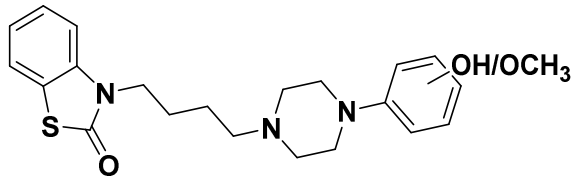
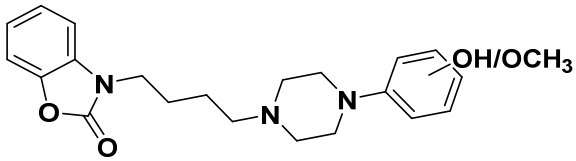
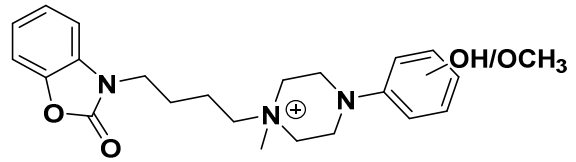
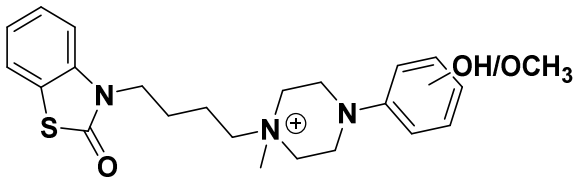
Abstract

The thesis entitled **"SYNTHESIS AND EVALUATION OF SEROTONIN 5-HT LIGANDS"** consists of five chapters. The main objective of the thesis is to synthesize the ligands active for 5-HT_7 serotonin sub-receptors. The proposed ligands contained three different moieties: the benzoxazolone / benzothiazolone moiety, the 4-carbon alkyl chain spacer, and the arylpiperazinyl moiety. All three moieties were conjugated together in such a way that the terminal positions were occupied by benzoxazolone / benzothiazolone and arylpiperazinyl moieties, as shown in Table 1.

Table 1: Ligands for 5-HT_7/5-HT_{1A} receptors

Series I		**1** ‘*o*’-OH, **4** ‘*o*’-OCH_3 **2** ‘*m*’-OH, **5** ‘*m*’-OCH_3 **3** ‘*p*’-OH, **6** ‘*p*’-OCH_3
Series II		**7** ‘*o*’-OH, **10** ‘*o*’-OCH_3 **8** ‘*m*’-OH, **11** ‘*m*’-OCH_3 **9** ‘*p*’-OH, **12** ‘*p*’-OCH_3
Series III		**13** ‘*o*’-OH, **16** ‘*o*’-OCH_3 **14** ‘*m*’-OH, **17** ‘*m*’-OCH_3 **15** ‘*p*’-OH, **18** ‘*p*’-OCH_3
Series IV		**19** ‘*o*’-OH, **22** ‘*o*’-OCH_3 **20** ‘*m*’-OH, **23** ‘*m*’-OCH_3 **21** ‘*p*’-OH, **24** ‘*p*’-OCH_3

Total 24 compounds of four series as shown in table 1 were synthesized and characterized by ^{1}H-NMR (Nuclear Magnetic Resonance), ^{13}C-NMR, and mass spectrometry. All these compounds showed binding affinity in the range of < 20nm.

Prior to the clinical application of the synthesized ligands, it is necessary to understand their potential as a drug molecule, which was evaluated by their pharmacokinetic analysis. In pharmacokinetic study the major aspect is ADME (absorption, distribution, metabolism and elimination) and the protein which plays a major role in this process is serum albumin. Serum albumin interacts with a variety of

ligands, which includes hormones, drugs, fatty acids, and other compounds, influencing their pharmacokinetic behavior. The major source of drug transportation is its binding efficacy to protein present in plasma, the binding of drug to protein directly affects its pharmacokinetic behavior. BSA (bovine serum albumin) with two tryptophan residues and 76 percent sequence similarity with HSA (human serum albumin) was considered as a standard protein. The binding aspects of synthesized ligands with serum protein were explored using spectroscopic and *cheminformatic* methodologies.

UV-Vis and fluorescence spectroscopic studies were performed to determine the binding constant between the BSA and drug molecule which also tells about the possible interaction mechanism. The correlation between the energetics of interaction and computational studies were performed to know about the driving forces responsible for the process of interaction. During interaction process, the changes in 2D structure (α-helix and β-sheet) of serum protein were identified by CD spectroscopy. The effect of synthesized derivatives on esterase-like property of BSA for drug hydrolysis, which is an important aspect of metabolic activity, was performed by esterase-like assay. The outline of the thesis is shown in Fig.1.

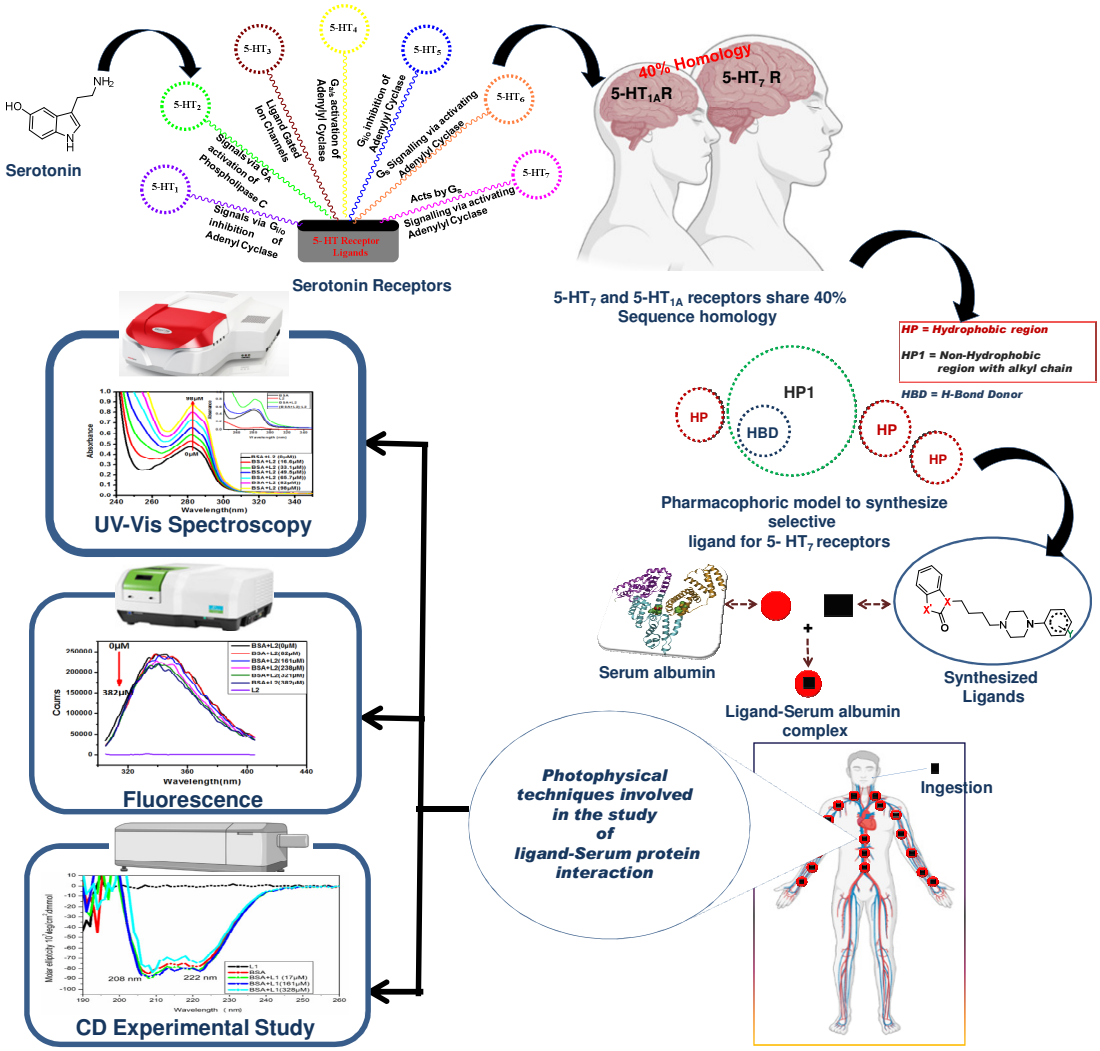


Fig. 1: Outline of synthetic and photophysical approach

Chapter 1: This chapter comprised of overview of 5-HT receptor subtypes in nervous system. Among the seven serotonin receptor subtypes, we have mainly focused on the study of 5-HT_{1A}/ 5-HT_7 receptor ligands from various chemical classes. 5-HT_{1A} and 5-HT_7 are the first and last members of the serotonin receptor family. These receptors are mainly responsible for modulating the pathophysiological and neuronal functions of the body and are distributed in brain areas such as hypothalamus, amygdala, pre-frontal cortex, and hippocampus etc. 5-HT_{1A}/ 5-HT_7 receptors share nearly 40% homology with each other, hence it is difficult to synthesize the selective ligands for either of these receptors. To overcome this limitation and to synthesize the selective ligands for 5-HT_7 receptors, the Lopez-Rodriguez *et al.* in 2003, Vermeulen *et al.* in

2004, Kolaczkowski *et al.* in 2006, and Kim *et al.* in 2014 developed several pharmacophore models as shown in Fig. 2.

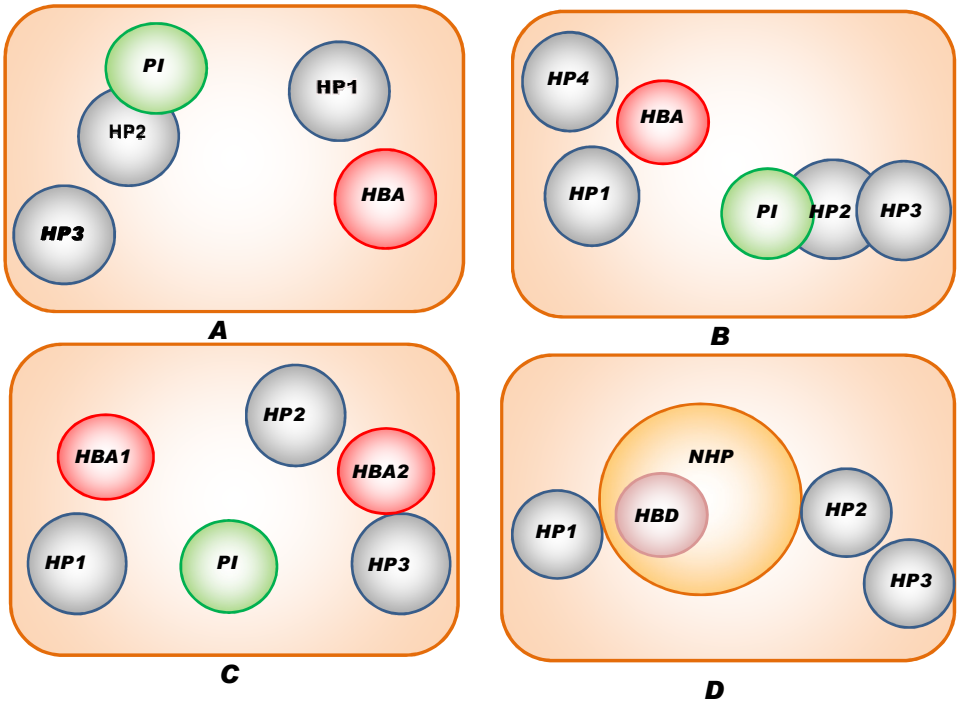


Fig.2: Pharmacophore models proposed by A)- Lopez-Rodriguez *et al.* in 2003 B)- Vermeulen *et al.* in 2004 C)- Kolaczkowski *et al.* in 2006 D)- Kim *et al.* in 2014

Where, HP denotes the hydrophobic regions, PI represents a positive charge, HBA represents a hydrogen bond acceptor, HBD denotes the H-bond donor, and NHP denotes the non-hydrophobic regions.

The structure of BSA was described in the second section of this chapter (Fig.3). It consists of 583 amino acids in total, which are divided into three subdomains of similar origin: domains I, II, and III, which have 1-183, 184-376, and 377-583 amino acid residues, respectively. BSA also has three hydrophobic ligand binding sites in subdomains IIA (198-582 residues), IIIA (307-582 residues), and IB (115-184 residues). The synthesized ligands bind efficiently at sites IIA and IIIA, but there is no binding interaction observed at site IB. The BSA is a major plasma protein and also acts as a source of transportation of drugs, so the binding efficacy of drugs with BSA is directly related with the pharmacokinetics of the drugs.

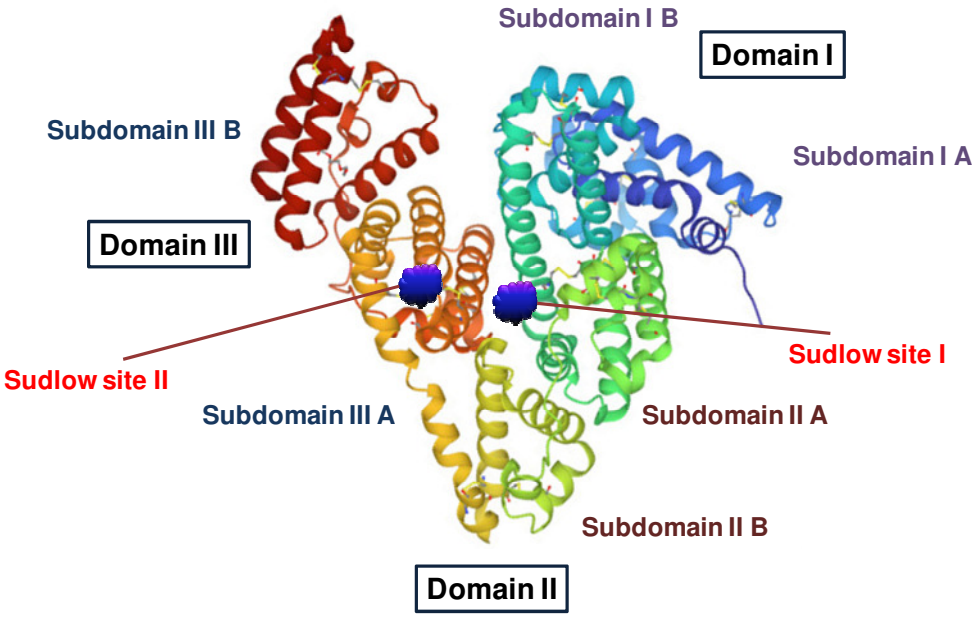


Fig. 3: Structure of BSA

Chapter 2: This chapter is about the synthesis of the designed compounds based on benzothiazolone/ benzoxazolone and arypiperazinyl nucleus as shown in Fig. 4. These compounds were categorized into four different series as shown in table 1. Series III and IV were proposed to give these ligands more hydrophilicity, allowing them to cross the blood-brain barrier and target serotonin receptors in the CNS. The synthesized ligands were further characterized by ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry.

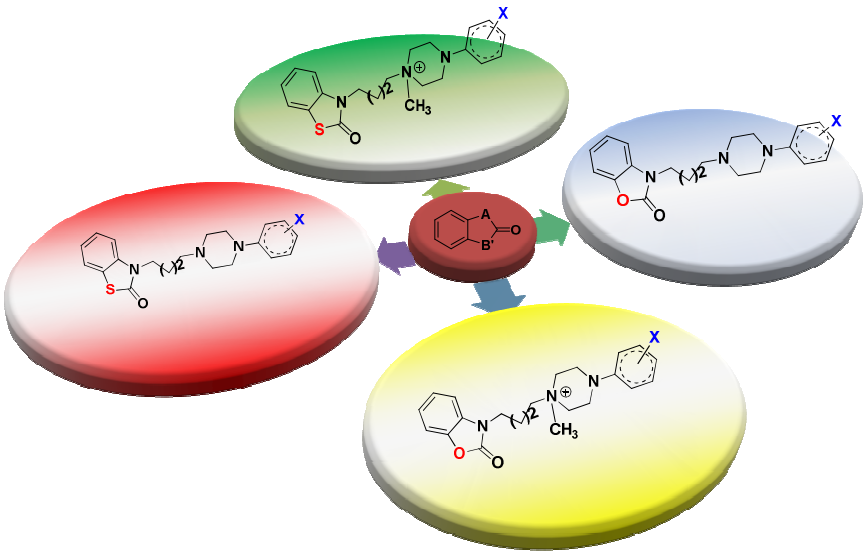


Fig. 4: Representative figure of synthesized ligands (Series I-IV)

Chapter 3: This chapter involves the study of binding interaction mechanism between the compounds of series I & II with BSA. This chapter is divided into two parts; Chapter 3A and Chapter 3B. The overview of the study is being shown by Fig. 5

Chapter 3A: This chapter includes the photophysical and *cheminformatic* study of compounds of series I (arylpiperazinyl-butyl benzothiazolone derivatives). The binding constant between the BSA and these derivatives was found to be the moderate, indicating reversible nature of binding. The binding stoichiometry (n) varied between 0.6-1.6 which demonstrates the occurrence of negative as well as positive cooperativity in the interaction mechanism. The sign of ΔG explained the spontaneity of the process. The *cheminformatic* study revealed the predominance of H-bonding and hydrophobic interactions in the binding interaction mechanism.

Chapter 3B: The drug-likeliness behavior of compounds from series II (arylpiperazinyl-butyl) benzoxazolone was investigated in this chapter via chemico-biological interactions to determine whether these derivatives will go far enough at the active site. The hill equation was used to calculate the binding constant between this class of derivatives and BSA. It was observed that these compounds have a higher affinity than compounds from series I for BSA, implying that the compounds from series I will be more readily available in free form. These outcomes were consistent with our previous research that benzothiazolone derivatives have a higher binding affinity (K_i) than benzoxazolones, indicating that compounds from both series are active at serotonin receptors and have a moderate range of binding with BSA.

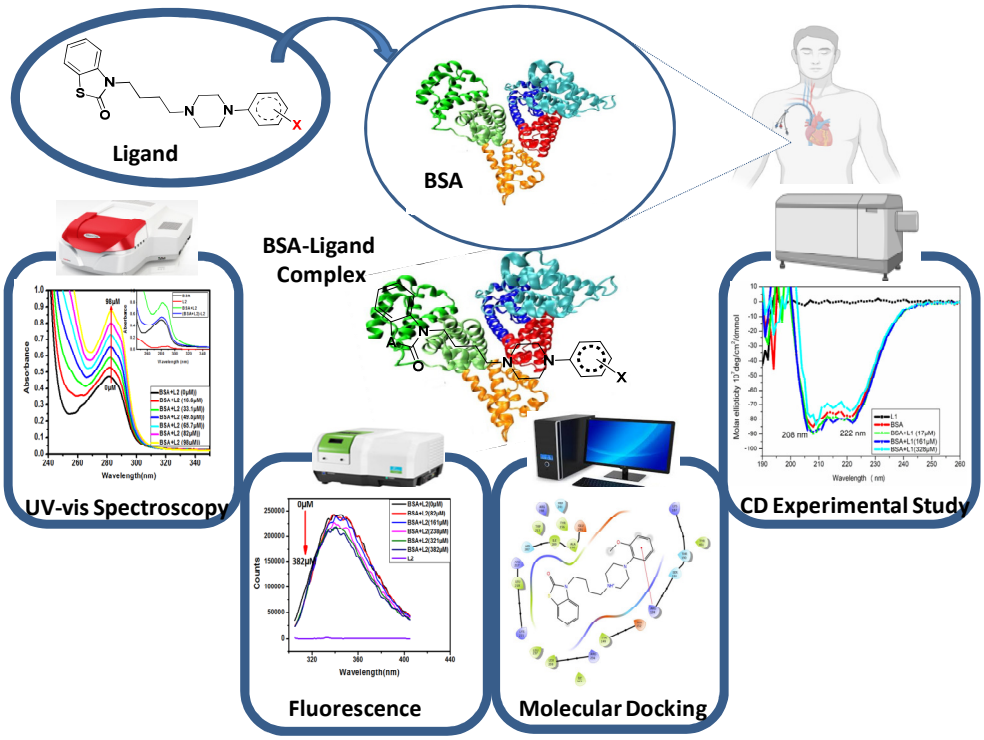


Fig. 5: Overview of the processes involved in binding interaction of Series I & II.

Chapter 4: This chapter explored the pharmacokinetics and pharmacodynamics of compounds of series III & IV via interaction with transport protein BSA as shown in Fig. 6. It consists of two different parts; Chapter 4A and Chapter 4B.

Chapter 4A: This chapter explores the pharmacokinetics of compounds of series III (butyl-benzoxazolone substituted piperazinium derivatives) which were synthesized by methylating the *N*-atom of piperazine moiety. The pharmacokinetics of these compounds was determined with the help of multi spectroscopic techniques and molecular docking. These compounds showed a mild interaction with BSA. Thermodynamic and molecular docking studies suggested the involvement of non-covalent forces (Van der Waals and H-bonding) in the transport mechanism.

Chapter 4B: This chapter focused on the interaction mechanism for transport of series IV (butyl-benzothiazolone substituted piperazinium derivatives) compounds on binding with BSA. The mechanism of binding interaction was enthlapically driven, and only mild interaction existed between BSA and these compounds. Among all the four series of compounds these compounds showed the least affinity with BSA and maximum binding affinity was shown by the compounds of series II.

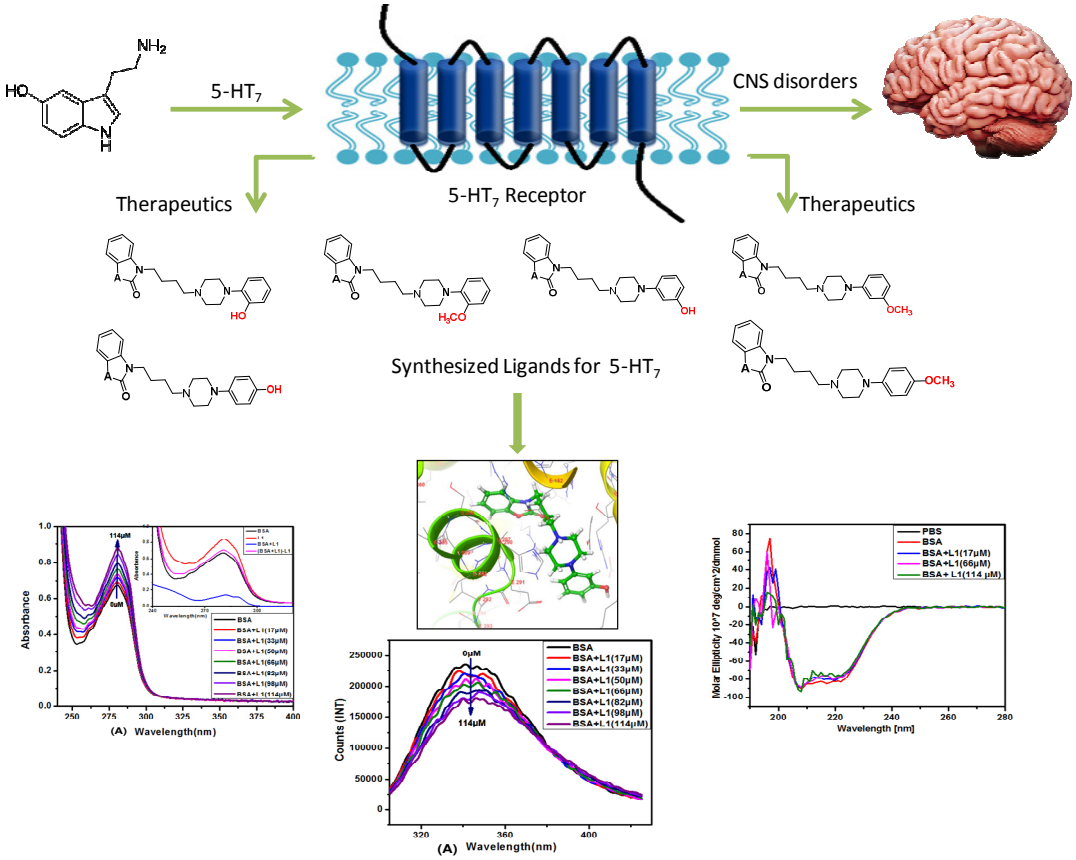


Fig. 6: Chemico-biological interactions for compounds of series III & IV

Chapter 5: This chapter explored the effect of all the synthesized derivatives on the esterase-like property of BSA, with *p*-NPA (*p*-nitrophenyl acetate) as a substrate. Here, the varying concentration of the substrate (50μM, 75 μM and 100 μM) has been used, and the concentration of the synthesized ligands was varied from lower to higher range (17μM-114μM) with fixed BSA (15 μM) concentration as shown in Fig. 7. During these studies compounds 1, 2, 3, 6, 9, 12, 14, and 15 showed enhanced esterase-like activity of BSA on all concentrations, though the extent of enhancement varied. Similarly, compounds 8, 13, 19, 20, 21, 22, and 24 decreased the catalytic activity on all concentration, but at a different extent. Some of the compounds such as 7, 10, 11, 16, 17, 18, 22, and 23 altered their behavior either as inhibitor to activator or vice versa with concentration variation.

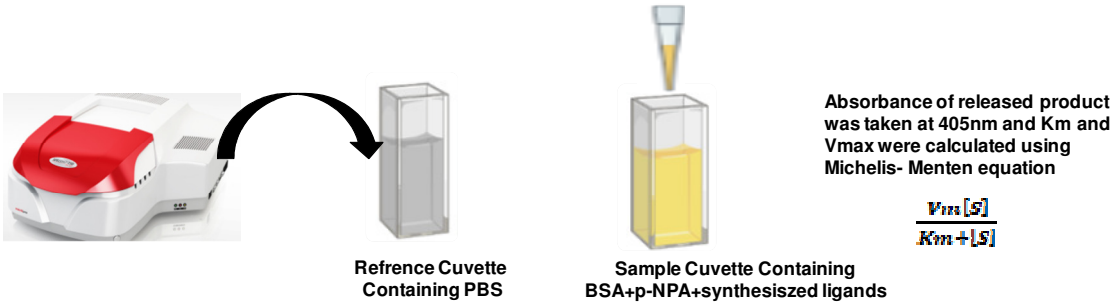


Fig. 7: Methodology involved in esterase-like assay of BSA

Conclusion

A total of 24 compounds were synthesized on the basis of previously reported pharmacophore specific for 5-HT_7 receptors. These compounds were categorized into four different series on the basis of their skeleton. To explore the pharmacokinetics and therapeutic window of any newly designed compounds, its binding study with serum protein is an important aspect. For our study we have used BSA as a reference serum protein. The binding of drugs with serum protein must be reversible in nature, so it also decides the dosage of drugs. The binding interaction mechanism of synthesized compounds with BSA was carried out with the help of different spectroscopic techniques (UV-Vis, fluorescence, and circular dichroism) and molecular docking. Finally, the biological aspect of the synthesized derivatives was explored with the help of *p*-NPA esterase assay to estimate hydrolyzing capability under *in-vivo* conditions.

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