Design, Synthesis and Evaluation of Novel Agents to Treat Alzheimer's Disease

THESIS

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by

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1. Introduction

Alzheimer's disease (AD) is a remarkably, and to date inexplicably, most common neurodegenerative disorder. It usually affects the population of 65 years and older. The population suffering from AD is projected to expand with increase in life expectancy. As per National Institute on Aging, USA, 5.3 million Americans have been reported to suffer from AD in 2015 with 5.1 million being from the age group of 65 and older. It bears an annual cost of approximately $226 billion– a cost that does not address the impact of the disease on families, individuals, and society. Currently, treatment of Alzheimer's disease includes acetylcholinesterase (AChE) inhibitors for mild to moderate cases, and NMDA (*N*-methyl-D-aspatarte)-receptor antagonists for the treatment of moderate to severe Alzheimer dementia. The number of drugs available for the AD treatment are few despite the large population suffering from AD. Till date only four drugs (tacrine, donepezil, rivastigmine and galantamine) have been approved for AD treatment by US Food and Drug Administration (FDA). These drugs give symptomatic relief and do not stop disease progression as these act on downstream events of disease process. There is therefore an unmet need to design drugs that directly act on the enzymes involved in disease etiology.

Amyloid plaques and neurofibrillary tangles (NFTs) are considered to be the main pathologies for AD. These are further characterized by neuroinflammation and neuronal dysfunction ultimately leading to neuronal death. These pathologies are described through amyloid cascade hypothesis, according to which, Aβ [a fragment of the amyloid precursor protein (APP)] plays the key role. APP is a large transmembrane protein whose sequential cleavage by secretases (α, β, and γ) generates Aβ-42. Major portion of APP is cleaved by α-secretases in non-amyloidogenic pathway while minor portion of APP is cleaved by β-secretases resulting in the production of soluble APPβ and a C-99 long C-terminal fragment. This fragment is further cleaved by γ-secretase within the transmembrane domain to generate Aβ-42. As compared to the other Aβ isoforms (Aβ-39 and Aβ-40), Aβ-42 is more hydrophobic and fibrillogenic, which renders it the ability to aggregate to oligomers, accumulate in the brain and initiate a cascade of events that lead to neuronal dysfunctioning, neurodegeneration and neuronal fatality. BACE-1 inhibition can lead to reduction of Aβ-42 production. BACE-1 knockout mice were not seen to manifest Aβ production and also did not show any negative health effects. Furthermore, the crystal structure of BACE-1 co-crystallized with different ligands, reveals the basic requirements and the key features needed for enzyme inhibition. It proposes the necessity of a group that acts as H-bond

donor, so that it can form H-bonding interactions with catalytic Asp228 and Asp32. Further, hydrophobic pockets (S1, S2´ and S3) are key regions for β-secretase inhibition. S1 hydrophobic cleft is formed by the side chains of Tyr71, Phe108, Trp115, Ile118, and Leu30. Like S1, S3 is also a largely hydrophobic pocket formed by side chains of Trp115 and Ile110, as well as main chains of Gln12, Gly11, Gly230, Thr231, Thr232 and Ser35 while S2´ comprises of Ile126, Trp76, Val69, Arg128, Tyr198 amino acid residues. Altogether, the evidences gathered from tissue culture and animal studies and knowledge of active site interactions make BACE-1 the most important target.

In the last decade several drug discovery strategies have been exploited in the search for BACE-1 inhibitors as potential anti-AD drug candidates. The inhibitors with therapeutic potential would require, besides good potency and pharmacokinetic properties, low molecular weight (<500 daltons) and high lipophilicity in order to penetrate the blood-brain barrier. Research aimed at the discovery of BACE-1 inhibitors has been strengthened by the large amount of available information, particularly, on the proteasic domain which is structurally well-defined, opening new opportunities for rational drug design. More than 550 BACE-I patent citations can be found on Scifinder for last 5.5 years which is indicative of the efforts that have been put till date to overcome the challenges in the design of BACE-I inhibitors. Substrate-based methods have been used as the starting point for developing BACE-1 inhibitors. Many substrate-based peptidomimetic inhibitors were also developed by big pharmaceutical companies and academic research groups. Unfortunately, despite their nanomolar affinity *in vitro*, these peptidomimetic BACE-1 inhibitors did not present a valuable pharmacokinetic profile (i.e., large size, poor brain permeability, short half-life *in-vivo*, and low oral availability) making them unsuitable drug candidates. However, most of non-peptidic BACE-1 inhibitors were developed using fragment-based lead generation method. Many big pharmaceutical companies employed High Throughput Screening (HTS) to identify hit compounds from different chemical libraries. In contrast to HTS, which uses libraries of relatively high molecular weight compounds, the fragment-based drug discovery (FBDD) approach takes advantage of libraries comprising more diverse and smaller-sized compounds (fragments) to identify hits that can be efficiently developed into potent leads with drug-like properties. After the hit identification, chemical modifications result in lead compounds and further these leads are optimized into suitable drug candidates. While substrate-based BACE-1 inhibitors usually show high potency and selectivity, their poor oral availability and permeability across the blood-brain barrier frequently makes them unsuitable

drug candidates. By contrast, the HTS method has the advantage of generating hits with high diversity, smaller size, and more drug-like properties (i.e., oral availability and brain penetration). However, the hit rate of HTS tends to be extremely low and the hits generally have lower potency and selectivity than substrate-based inhibitors.

2. Aims and objectives

The present thesis work was aimed to design, synthesize and evaluate BACE-1 inhibitors to treat Alzheimer's disease. It was also decided to generate structurally diverse library of compounds to obtain leads of different structural scaffolds. Previously, many groups have worked on focused libraries around the lead compounds and have not succeeded due to several reasons that include structural complexity, poor pharmacokinetics and poor brain-barrier penetration. For these reasons, small molecular inhibitors (having low molecular weight) were the focus of this work. Further, advances in computational techniques have allowed the researchers to speculate on probable success in their efforts. The availability of several PDB crystal structures also gave enough idea about binding interactions in the BACE-1 active site. Therefore, the present work adopted *in silico* guided approach to design the small molecular, structurally diverse libraries.

To achieve the aim, following steps were envisaged:

Step 1: To design *in silico* database of small molecular, structurally diverse compounds using structure-based design approach and to predict their binding in the BACE-1 enzyme active site.

Step 2: To synthesize *in silico* designed compounds showing significant binding affinity by different synthetic approaches followed by purification and characterization.

Step 3: To screen synthesized compounds for their *in vitro* BACE-1 inhibitory potential.

Step 4: To analyze the results and derive structure activity relationship of these compounds.

3. Design of BACE-1 inhibitors

Since the aim was to look at small molecules, prototypes from literature having moderate BACE-1 inhibition were chosen. Following the reductionist approach, initially, a structure similar to prototype was docked and the poses were analyzed. Further, a library of compounds having electron donating and electron withdrawing substitutents on the selected structure was designed. Using the docking results of first library, design of further libraries to overcome the problems was envisioned. Docking was performed using Molegro Virtual Docker (MVD) and Glide (Schrodinger). The docking process using 2OHP was validated using extracted ligand (6IP-

389) and further validated using external data set of 20 molecules reported as BACE-1 inhibitors by different research groups. Further, in order to envisage oral bioavailability, *in silico* determination of drug likeliness (OSIRIS property explorer) and few molecular descriptors (JChem for Excel) was performed. BBB permeability was predicted using online BBB permeation prediction software available at http://www.cbligand.org/BBB/. The designed compounds were synthesized and synthesized compounds were tested *in vitro* for their ability to inhibit BACE-1. Further, *in vitro* - *in silico* correlation was drawn.

3.1. Design of Acridin-9-yl hydrazide derivatives as BACE-1 inhibitors

BACE-1 belongs to the family of aspartyl proteases. For designing BACE-1 inhibitors, it was decided to start with known aspartyl protease inhibitors. This strategy has been used for various other targets and there has been reasonable success. Further, it was expected to show activity by similar interactions in the active site. Hydrazinyl acridine derivatives have been reported to inhibit two aspartyl proteases Plasmepsin-II and Cathepsin-D, *in silico* as well as *in vitro*. The hydrazinyl acridine derivatives were reported to occupy S1 and S3 subsites of plasmepsin-II and cathepsin-D. In plasmepsin-II, only one of the aspartate was hydrogen bonded with –NH- of the ligand while in cathepsin-II none could be bonded. The activity of acridine derivatives on BACE-1 has not been reported. Taking this into account, design of acridine derivatives as BACE-1 inhibitors was attempted.

Almost all the designed analogues showed that the enzyme-inhibitor complex was primarily stabilized by hydrogen bonds between the hydrazide part of the inhibitor and Asp32. The acridinyl moiety showed π-π stacking with Tyr71, occupied the S2´ region while the phenyl ring was buried in S1 cavity. It showed significant interactions and accommodation of substrate binding clefts.

3.2 Design of *N*-Phenyl-2-[(phenylsulfonyl)amino]acetamide derivatives as BACE-1 inhibitors

From the docking simulation study of acridin-9-yl-hydrazide derivatives, it was observed that, although these compounds were able to occupy S1 and S2' active site, S3 cavity was not fully occupied. Moreover, these could interact with only one of the aspartate instead of aspartate dyad. These results were concurrent to those observed for Plasmepsin-II and Cathepsin-D. Therefore, it was decided to design compounds which could show interactions with aspartate dyad as well as extend in S3 cavity. As acridine derivatives were also predicted to have

mutagenicity, modifications in acridines were not considered. Instead, literature reports for compound showing these properties were searched.

Various reports have indicated that sulfonamide derivatives have good CNS penetration. Gerritz et al have reported acyl guanidine derivatives as BACE-1 inhibitors. Replacement of acyl guanidine portion in reported compounds with sulfonyl-amino-acetamide motif was done to check the effect on BACE-1 inhibition. Substituted sulfonamide derivatives possessing two aromatic moieties separated by a sulfonyl-amino-acetamide linker were designed. Designed compounds displayed key interactions with aspartate dyad and also occupied the S1 region well. Further, these derivatives showed few interactions with S3 region amino acids like Thr231 and Thr232.

3.3 Design of Substituted pyrimidine derivatives as BACE-1 inhibitors

From the second series, it was deduced that due to the flexibility of 5-atom chain linker separating two phenyl rings, *N*-Phenyl-2-[(phenylsulfonyl)amino]acetamides formed 'U' shape, flipping the orientation towards S2´ cavity than S3. The aim was therefore to occupy the S3 cavity along with S1 cavity retaining the desired interactions. This could be achieved by reducing linker size and inducing rigidity in the linker separating aromatic rings on both sides. Gianpaolo et al have reported substituted 2-aminoimidazole derivatives substituted with N-aromatic and C4 aromatic groups as BACE-1 inhibitors. In view of this, substituted pyrimidine derivatives were considered as BACE-1 inhibitors because, the 2-substitution was expected to interact with aspartic dyad and aromatic rings at 4th and 6th position would occupy targeted S1 and S3 pockets. The pyrimidine ring would provide 3-atom rigid linker to prevent flipping of the phenyl rings and orient the substitution at 2nd position in the centre of catalytic aspartate dyad. While 2-amino pyrimidines with various substituents were first designed, it was also thought to replace the 2-amino group of pyrimidine with -hydroxyl and -thiol groups. Since –amino group can be bioisosterically replaced with –hydroxyl and –thiol, it was expected that these will retain or enhance the desired interactions. For most of the compounds, following interactions were seen: (i) the guanidinium functionality interacted with catalytic aspartates *i.e.* Asp32 and Asp228; (ii) ring B formed hydrophobic interactions with Phe108, Leu30 and Ile110 in S1 cavity; (iii) ring B also formed π-π stacking with Trp115 in S1 cavity; (iv) ring A established hydrophobic interactions with Thr231 and Gly230 in S3 cavity; (v) ring A also showed π-π stacking with Tyr71.

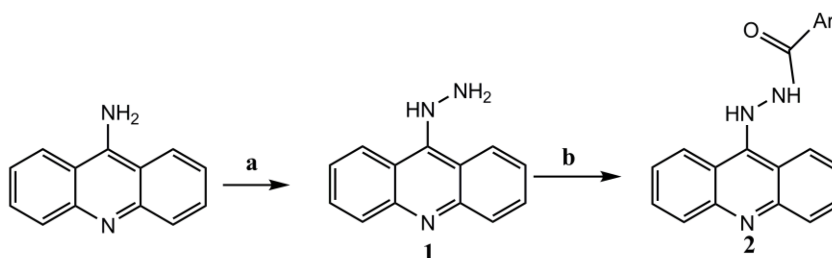
3.4 Design of Substituted allylidene hydrazinecarboximidamide derivatives as BACE-1 inhibitors

In (sulfonylamino)acetamide series, the linker for separating two aromatic rings was 5 atom long and it was noticed that ring A occupies S2' cavity and ring B is oriented towards S1 cavity. Thus, it did not occupy S3 cavity as due to flexibility, it formed 'U' shape in active site. In substituted pyrimidine series, having guanidinyl moiety present in pyrimidine ring to impart rigidity, it was observed that 5 atom linker is not essential and the substitution at 2 position is essential for binding with aspartate dyad. Therefore, another series of allylidene hydrazinecarboximidamide derivatives was envisaged, which had aminoguanidine substitution on a short 3 atom linker to bind with aspartate dyad and the two aromatic groups on either side of linker to bind S1 and S3 cavity. Thus, it had limited flexibility as compared to [sulfonyl)amino]acetamide series and had guanidinyl moiety of substituted pyrimidine series separated from linker by an amino group. Exploring these structures with different substituents on ring A and ring B revealed the occupance of substrate binding cavities and interactions with active site amino acids. a library of 31 allylidene hydrazinecarboximidamide derivatives was designed. All the compounds displayed higher docking score revealing strong hydrogen bonding interactions with catalytic aspartate dyad and the aromatic rings properly accommodated in S1 and S3 substrate binding clefts.

4. Synthesis

4.1 Synthesis of Acridin-9-yl hydrazide derivatives

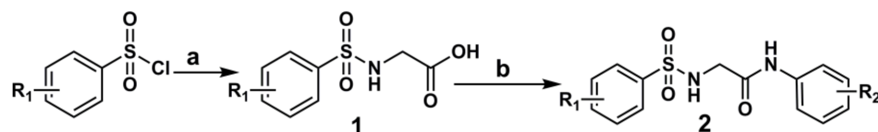
A dataset of 12 acridin-9-yl hydrazide compounds was designed. To synthesize, following scheme was followed:



Scheme 1: Reagents and conditions: (a) $NaNO_2$, HCl, -5 to 0^0C , 1h; $SnCl_2$, HCl (b) HOBt, EDC-HCl, THF, TEA, substituted carboxylic acids, 0^0C , 12 h.

4.2 Synthesis of *N*-Phenyl-2-[(phenylsulfonyl)amino]acetamide derivatives

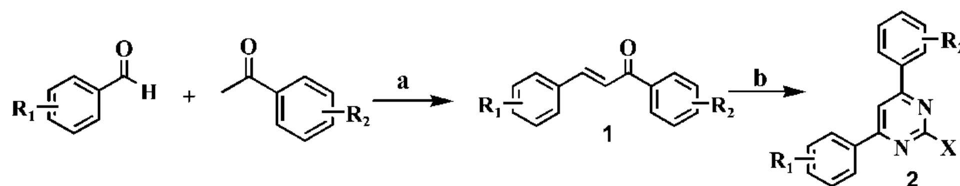
To synthesize *N*-Phenyl-2-[(phenylsulfonyl)amino]acetamide derivaties, following scheme was used. Total of 33 compounds were synthesized.



Scheme 2: Reagents and conditions: (a) $NaHCO_3$, glycine, 80^0C, 2-3 h (b) HOBt, EDC-HCl, DCM, TEA, substituted aniline, 0^0C, 12 h.

4.3 Synthesis of substituted pyrimidine derivatives

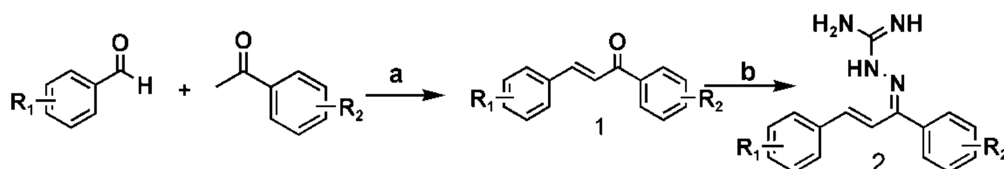
Based upon the computational studies, the substituted pyrimidine derivatives were synthesized as given in the Scheme 3.



Scheme 3: Reagents and conditions: (a) NaOH, EtOH, rt, 4-6 h (b) NaOH, urea/thiourea/guanidine-HCl, EtOH, 70^0C, 2-8 h.

4.4 Synthesis of allylidene hydrazinecarboximidamide derivatives

Allylidene hydrazinecarboximidamide derivatives were prepared using scheme 4 given below. Scheme 3 and scheme 4, both involve chalcone preparation in first step.



Scheme 4: Reagents and conditions: (a) NaOH, EtOH, rt, 4-6 h (b) Aminoguanidine-HCl, Conc. HCl, THF, Reflux 20-24 h.

5. *In vitro* screening of BACE-1 Inhibitors and *in vitro- in silico* correlation

For studying the BACE-1 inhibitory potency of all the synthesized compounds, *in vitro* enzyme inhibition assay was done. The concentration used was 10µM as the compounds used for designing library had been reported to have maximum inhibition at around 10µM concentration. From amongst designed compounds, those showing maximum inhibitory potential were also subjected to IC_{50} value determination. This section also describes the *in silico* - *in vitro* correlation.

5.1 Acridin-9-yl hydrazide derivatives

As indicated by docking scores as well as in vitro BACE-1 inhibition, it was observed that electron donating substitution on benzoyl moiety is preferred over electron withdrawing substituents.

5.2 *N*-Phenyl-2-[(phenylsulfonyl)amino]acetamide derivatives

Overall, it was observed that substituents on ring A matter more than substituent on ring B. Further, it can be said that bulkier the ring substituent, better is the activity. Both the substituents complement each other and adjusting the bulkiness would improve BACE-1 inhibition. In this series, compound 2.17 showed highest % inhibition and hence was studied for IC_{50} determination. It showed IC_{50} of 7.90µM, indicating that it has comparable potency to the prototype compound reported by Gerritz *et al*. Advantage of this compound is that it is much smaller to reported prototype.

5.3 Substituted pyrimidine derivatives

Overall, it was observed that replacing phenyl with heterocyclic ring as ring A, in general, weakens the activity. Taking into account the inhibition profile and interaction patterns of all these compounds, it can be proposed that 2-amino pyrimidines having substitution of electron withdrawing (such as –nitro) or moderately activating groups (such as benzyloxy) at *meta* position on ring A with electron withdrawing substituents on ring B at *ortho and para* position are beneficial for BACE-1 inhibitory activity. Excellent interaction pattern observed for compound 2.13G was concomitant with BACE-1 inhibition where it displayed maximum inhibition of 73.91% at 10µM concentration. Therefore, it was tested further and was found to have IC_{50} of 6.92µM.

Structure activity relationship study suggests that our hypothesis of moderately rigid structure with functionalities to bind with catalytic aspartates and aromatic residues to occupy active site regions was correct.

5.4 Allylidene hydrazinecarboximidamide derivatives

For this series, the docking scores as well as % inhibition values were higher. This can be attributed to favourable interactions with the catalytic aspartates and cavity occupance. In the perspective of SAR studies, it can be suggested that moderately flexible 3 atom linker with substituted aromatic residues on both side and hydrogen bond donor to bind aspartate dyad may results in active compounds. It is beneficial to incorporate electron poor groups on para position of ring B which renders capability to form strong π-π stacking with Tyr71. Inclusion of functional groups on ring A interacting with S3 region amino acids (Thr231, Thr232, Gly230,

Gly11) are key contributors to increased potency of the ligand. Compound C23A and C24A had highest inhibition of 61.18% and 78.23% respectively, which is concurrent with docking interactions. Compound C24A was studied further and was found to have IC_{50} of 6.42µM, which confirms our hypothesis that for BACE-1 inhibition, 3 atom linker with substituted aromatic residues on either side and hydrogen bond donor to bind aspartate dyad is crucial.

6. Summary and conclusion

To conclude, the specific contribution of the present work can be given as:

1. It complements the understanding that structure-based design followed by computer aided drug discovery is helpful technique in achieving higher hit rates.
2. The study helps in identifying the potential binding modes for the design of BACE-1 inhibitors. It was observed that moderately rigid structure with groups for interacting with aspartate dyad, occupying S1 as well as S3 cavity is essential.
3. The most active compound, C24A displayed all the desired interactions and hence shows IC_{50} value of 6.42µM.
4. The study can be a basis for indicating that small, low molecular weight ligands as compared to those reported in the literature can also inhibit the BACE-1 enzyme. These compounds may also penetrate the blood brain barrier as indicated by *in silico* prediction. It can be proposed that many compounds reported in this thesis will have increased brain permeability due to low logP values, low molecular weight and low polar surface area.
5. Compounds C24A represents a suitable starting point for extensive modifications for hit-to-lead conversion and eventually lead generation to effectively inhibit BACE-1 enzyme.