ISSN Online: 2349-7777

Virtual Screening of Glycine Analogs: A Structural Insight to NMDA and GABA-AT Receptor

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Received 04 November 2013; accepted in revised form 17 December 2013

Abstract: A series of Glycine analogs (1108 entries) with GABA-AT and NMDA receptor inhibitory activities was subjected to docking methods. Lamarckian Genetic Algorithm was used as search algorithm. The interpretation of docking studies revealed that GABA-AT inhibitory potential of our designed compounds was influenced by number of hydrogen bonding atoms and biphenyl groups in the molecules. Putative interactions between receptor and inhibitors were identified by inspection of docking-predicted poses. This understanding of protein ligand interaction and value of Ki imparts impetus to the rapid development of prospective inhibitors.

Key words: Anticonvulsants, AutoDock, GABA-AT receptor, Glycine, Lamarckian Genetic Algorithm, NMDA receptor.

Introduction

The realization that Glycine receptors are involved in motor reflexes and nociceptive pathways together with the more recent advent of drugs that exhibit some subtype selectivity make the goal of designing selective therapeutic ligands for the glycine receptor that much closer [1]. γ-Aminobutyric acid aminotransferase (GABA-AT), a pyridoxal 5"phosphate-dependent enzyme, is primarily found in the central nervous system and is responsible for the catabolism of the inhibitory neurotransmitter, GABA [2].

Structure based drug design entails knowing at a minimum, the identities of the genes and proteins involved in the disease, the structure of the biological network that they comprise and the features that differentiate the diseased state from healthy state. The past decades have seen an explosion in amount of data required for structure based design of pharmaceutically relevant molecules. This data expansion encompasses both an exponential increase in number of available protein structures and increase in the number of real and hypothetical drug sized molecules available for virtual screening [3].

Computer-Aided Drug Design (CADD) has become an integral part of drug discovery and development efforts in the pharmaceutical and biotechnology industry. QSAR techniques have been used for this purpose for over 50 years. However, since the 1980's, the structure-based design technology has evolved, and today, these techniques are being widely employed and credited for the discovery and design of most of the recent drug products in the market. Due to rapid technological progress in chemistry and bioinformatics, structural biology and computer technology, CADD approaches in molecular docking studies, library design and profiling, high throughput and virtual screening, along with target/structure based *de novo* design, have also become powerful tools and are routinely used in

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the multi-step process of drug discovery. As an emerging technology, CADD accelerates drug development by making use of the accumulated information of existing drugs and biological targets, combined with interdisciplinary information from different fields [4].

We envision the molecular docking platform as being a virtual screening service that would rely on the factual association between drugs and targets [5-10]. AutoDock uses a genetic algorithm to generate the poses of the ligand inside a protein active site. It utilizes the Lamarckian version of GA, where the changes in conformations adopted by molecules after in situ optimization are used as a make up for offspring poses [11]. It applies a Lamarckian model of genetics, in which environment phenotype are reverse transcribed into its genotype and become heritable traits (*sic*). Lamarckian genetic algorithm is the most efficient, reliable, and successful of the three search methods, Monte Carlo simulated annealing, a traditional genetic algorithm, and the Lamarckian genetic algorithm [12].

Glycine is the simplest amino acid and was first identified as a neurotransmitter in 1965. It is found predominantly in the brainstem and spinal cord, but also diffusely throughout the CNS. Like GABA, it is predominantly inhibitory neurotransmitter. Coupled to the *N*-methyl-D-aspartate (NMDA) receptor-channel complex is a strychnine insensitive binding site for glycine. Pharmacological antagonism of glycine binding at this site can produce anticonvulsant activity [13]. GABA-AT is also a validated target for anti-epileptic drugs because its selective inhibition raises GABA concentration in brain [14].

Here we propose a docking based virtual screening strategy for finding effective enzyme inhibitors, keeping glycine pharmacophore.

Experimental

In molecular docking we attempted to predict the structure (or structures) of the intermolecular complex between two molecules i.e, enzyme (protein) and inhibitor (ligand). Docking was performed using Auto Dock Tools® 1.4.6 and MGL Tools® 1.5.4 packages (The Scripps Research Institute, Molecular Graphics Laboratory, 10550 North Torrey Pines Road, CA,92037) running on Red Hat Enterprise Linux 5.0. 3D crystal structures of GABA-AT (Fig 1); PDB code 1OHV[15-17] and NMDA receptor (Fig 2); PDB code 1pb7[18-19] were downloaded from

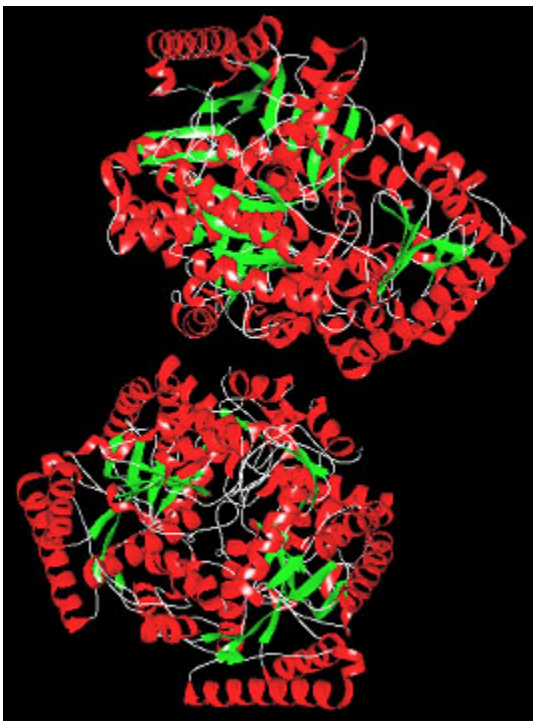

Fig. 1. 3D Crystal structure of GABA-AT

Fig. 2. 3D Crystal structure of NMDA RECEPTOR (1pb7)

Brookhaven protein data bank; PDB[20] (http://www.rcsb.org/pdb) and loaded to python molecular viewer. The non bonded oxygen atoms of water, present in the crystal structure were removed. After assigning the bond orders, missing hydrogen atoms were added, then the partial atomic charges was calculated using Gasteiger-Marsili method [21]. Kollman [22] united atom charges were assigned, non polar hydrogens merged and rotatable bonds were assigned, considering all the amide bonds as non-rotatable. The receptor file was converted to pdbqt format, which is pdb plus 'q' charges and 't' Auto Dock type. (To confirm to the Auto Dock types, polar hydrogens should be present where as non-polar hydrogens and lone pair should be merged, each atom should be assigned Gasteiger partial charges).

Gylcine was selected as template for ligands. Virtual library with more than 1000 entries was designed using Chem Draw Ultra 6.0 [23] (http://www.cambridgesoft.com) and Marvin Sketch 5.0.6.1 [24] (http://www.chemaxon.com) was used for gradient optimization of ligand molecules and conversion in tripos mol2 format. The currently available docking method utilizes the scoring functions in one of the two ways. The first approach uses the full scoring functions to rank a protein ligand conformation. The system is then modified by the search algorithm and the same scoring function is again applied to rank the new structure.

Docking of GABA-AT and NMDA receptor with glycine analogs was carried out using Lamarckian Genetic Algorithm [25], since the other two algorithms (simulated annealing and Genetic algorithm) showed less efficiency utilizes Lamarckian notations. AUTODOCK is a grid based molecular docking method that uses AMBER force field. The receptor is held rigid while the ligand is allowed to flex during the refinement process. A Grid map with 66 × 92 × 72 points, a grid spacing of 0. 6138 A were used. An affinity grid is calculated for each type of the atoms in the substrate, typically carbon, oxygen, nitrogen, hydrogen as well as grid of electrostatic potential using a point charge of +1 as the probe [26-28]. AUTODOCK 4.0 [29-30] uses these interaction maps to generate in ensemble of low energy conformations, thus it uses a Lamarckian Genetic Algorithm as a global optimizer combined with energy minimization as a local search method.

For each new population, a user determined fraction undergoes a local search procedure using random mutation operator where the step size is adjusted to give an appropriate acceptance ratio. The fitness function comprises five terms: a Lennard-Jones potential dispersion/repulsion term, a directional hydrogen bond term, a columbic electrostatic potential, a term proportion to the number of sp^3 bonds in the ligand to represent unfavorable entropy of ligand and a desolvation term.

Results and discussion

The design of new and selective inhibitors of an enzyme is one of the most important applications in contemporary rational drug design. A total of 1108 GLYCINE derivatives were designed retaining the original structure of glycine**.** The result of LGA docking experiments of the Schiffs bases, Acid Hydrazones, Hydrazine Carbonyl analogues of GLYCINE using AutoDock 4.0 and AutoGrid 4.0 are summarized in Table 1.

For each docking experiment, the lowest energy docked conformation was selected from 100 runs. The central processing unit for a single docking experiment took 10-15 minutes, on a 2.19-GHz Intel (R) core2 Duo machine with 2.96GB of RAM and Red Hat Enterprise Linux 5.0 operating system. In order to evaluate accuracy of docking, binding energy and numbers in cluster were used. Ki values (μM) were recorded for lowest energy binding mode. 11 molecules showed better inhibition potential with binding energy greater than -9.00 Kcal/mol. The chemical structures of all the 11 molecules are shown in the figure 3. Modeling and docking analysis revealed the nature of the active site and some key interactions that enabled the binding of glycine analogue to the active site. Among all molecules (1108) screened, the hydrogen bonding of 2-[(E)-[2-methyl-4-phenylphenyl)(2-methyl-5-phenyl-phenyl)methylidene]amino]aceticacid (**S184**), 2-amino-N'-[(1E)-(2-methyl-3phenylphenyl)(2-methyl-4-phenylphenyl) methylidene] aceto-

Table 1. Computationally predicted potencies of all compounds screened

S.No.	Compound code	Binding energy	K_i	Interaction with amino acids	Selectivity observed for
1	S184	-11.02	8.35nM	LYS145, ARG349, MET149, LEU166, PHE161, PHE148, GLU165, PRO178, ARG156, ARG152, TYR180, TRP215	1OHV
2	h183	-10.95	9.39nM	PHE213, TRP215, ARG152, PHE148, TYR180, ASP179, PRO178	1OHV
3	S185	-10.28	28.92nM	ARG349, PHE148, GLY176, PRO178, ARG156, ARG152, MET149, CYS169, PHE161, CYS177	1OHV
4	h184	-10.07	41.60nM	GLU141, ARG349, MET149, PHE148, ASN172, TRP215, PRO178, ARG152, ASP179, TYR180	1OHV
5	138	-9.96	50.40nM	TYR348, ARG349, MET149, PHE148, PRO178, GLY176, PHE161, ARG156, TYR180	1OHV
6	S183	-9.50	108.26nM	ARG349, GLY176, ARG156, ARG152, TYR180, PRO178, GLY176, PHE148	1OHV
7	h13888	-9.42	124.23nM	GLU14, HIS12, VAL19, LEU86, PRO21, GLN61, PRO47, ASN48	1pb7
8	S186	-9.26	161.86nM	ARG349, MET149, PHE148, CYS169, PRO178, ARG156, TYR 180	1OHV
9	184	-9.16	193.50nM	PRO107, PHE111, GLY104, LEU115, LEU355, THR132, LEU120, TYR348, LEU130, PRO347, ILE350, ARG349, PRO344	1OHV
10	h185	-9.14	198.83nM	LYS145, ARG349, GLY195, PHE144, PHE148, PHE213, TRP215, PRO178, TYR180, ASP179	1OHV
11	147	-9.10	214.86nM	GLN61, VAL19, LEU86, ILE11, HIS12, VAL87, PRO47, ASN48, GLY90, HIS57, SER205	1pb7

hydrazide (**h183),** 2-[(E)-[2-methyl-4-phenyl-phenyl)(2-methyl-6-phenylphenyl) methylidene] amino]aceticacid (**S185),** 2-amino-N'-[(1E)-(2-methyl-4phenylphenyl)(2-methylphenyl phenyl) methylidene] aceto hydrazide (**h184)** with ARG152, PHE148, PRO178 appeared to be in

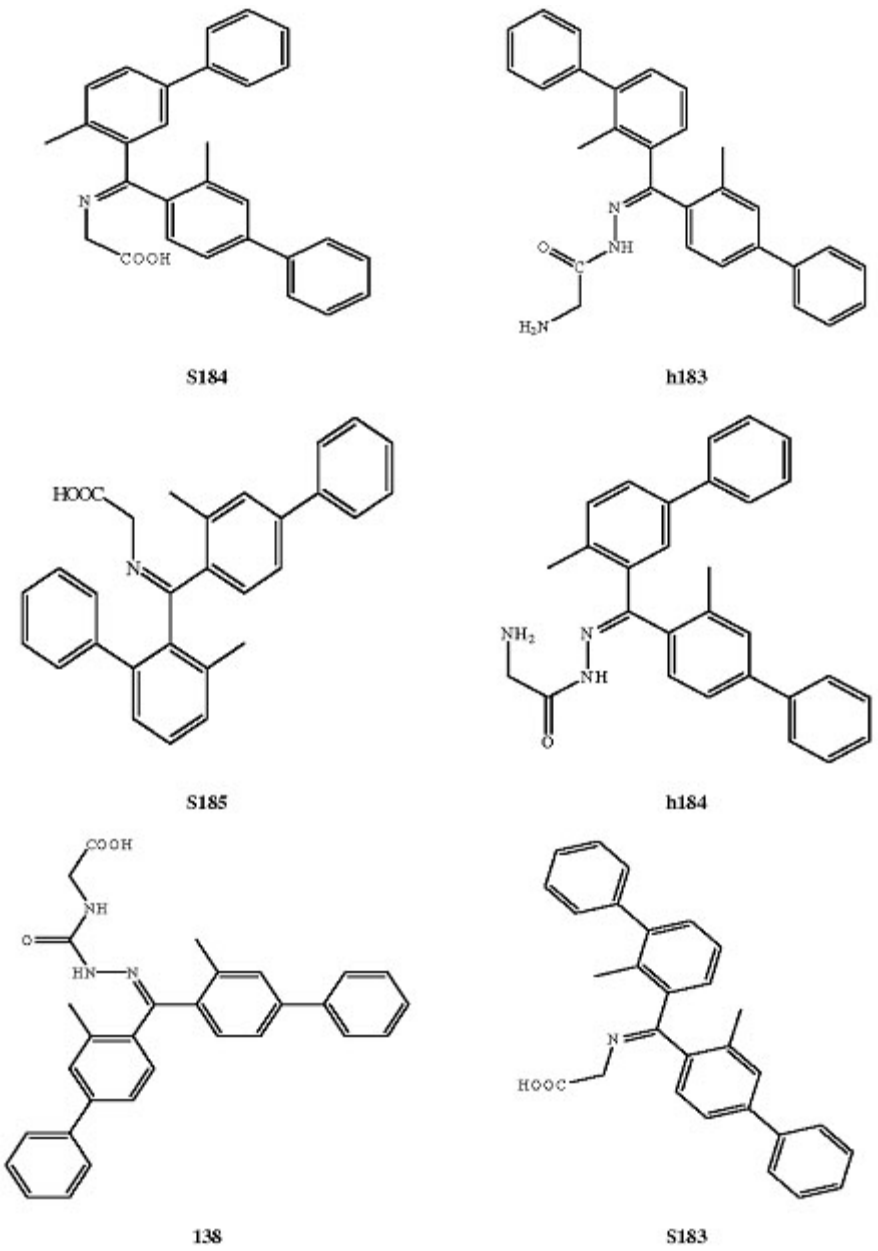

Fig. 3a. Chemical structures of titled compounds

close proximity and explains the high GABA-AT selectivity. Docking poses and binding interactions of **S184** and rest molecules are shown in figure 4.

The compound **S184** showed hydrogen bonding interactions with the residues ARG 349; compound **h183** showed with TRP215; compound **S185** with ARG156 and compound **h184** with ARG349.The results obtained so far showed that 11 molecules among the 1108 possesses better inhibition potential and majority of them were in close proximity ARG152, PHE148, PRO178.

Conclusion

The present study endeavors to peep in to the structural requirements, which is of great pivotal importance for designing new ligands interfering with GABA-AT and showed that new wave of flexible ligand docking program like Auto Dock

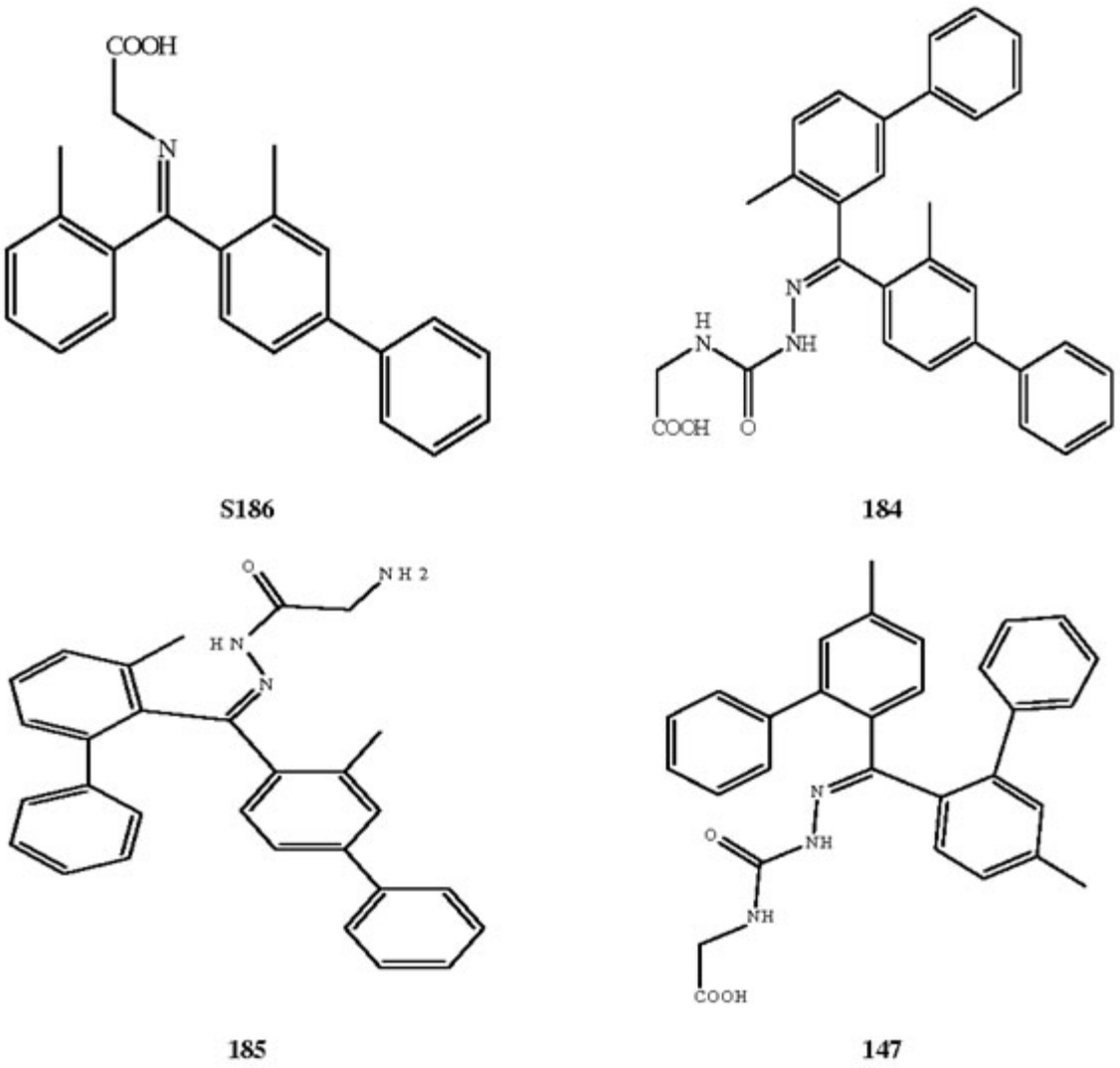

Fig. 3b. Chemical structures of titled compounds

produced unbiased docking of GABA-AT in the enzyme active site. This study contributed molecular insight in to the binding process, There was still significant room for improvement especially for the empirical binding free energy force field and inhibition constant prediction. The presence of aromatic ring was found to play a major role in determining inhibitory activity for GABA-AT. The energy, inhibition constant values and binding interactions revealed from poses provide the clues for the design of new molecules thus gave insight on structural requirement for designing more potent analogues.

These findings would be utilized for synthesizing and evaluating few new novel GABA-AT inhibitors with various substituents at the parent scaffolds.

Acknowledgement(s).

One of the authors Ms. Deepti Pandey likes to thank All India Council for Technical Education, New Delhi for providing fellowship. The authors wish to thank the Chairman, Bharat Institute of Technology, Meerut, Uttar Pradesh, India for providing the necessary facilities for undertaking this research work.

Disclosure

The experimental section described here has been adopted and modified from the work of one of the author (S.K. Bansal *et al*.)

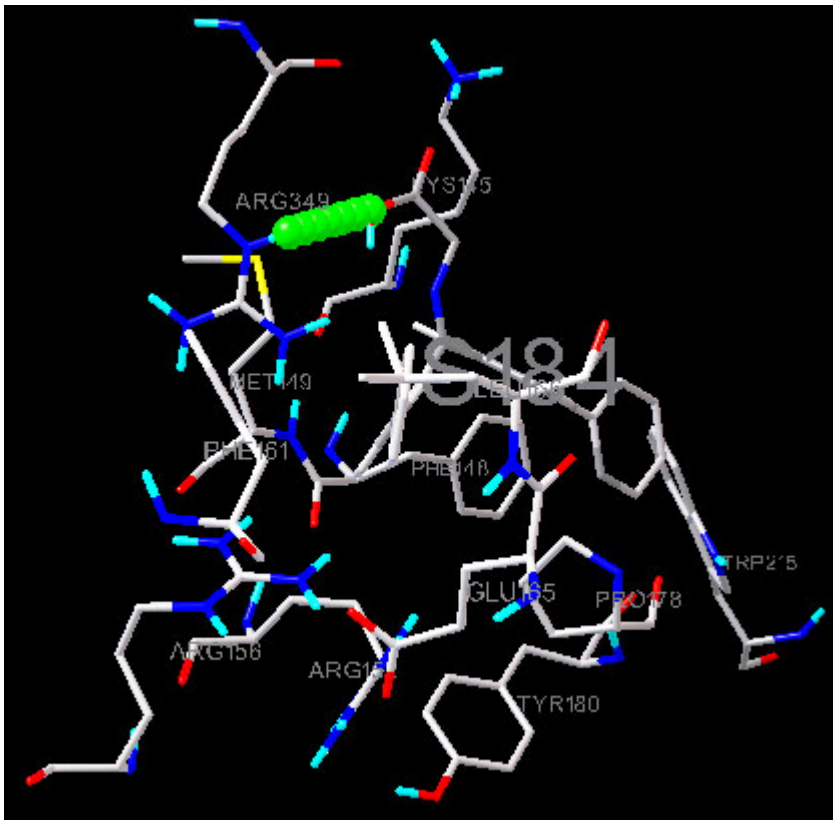

Fig. 4a. Docking Poses and binding interactions of S184

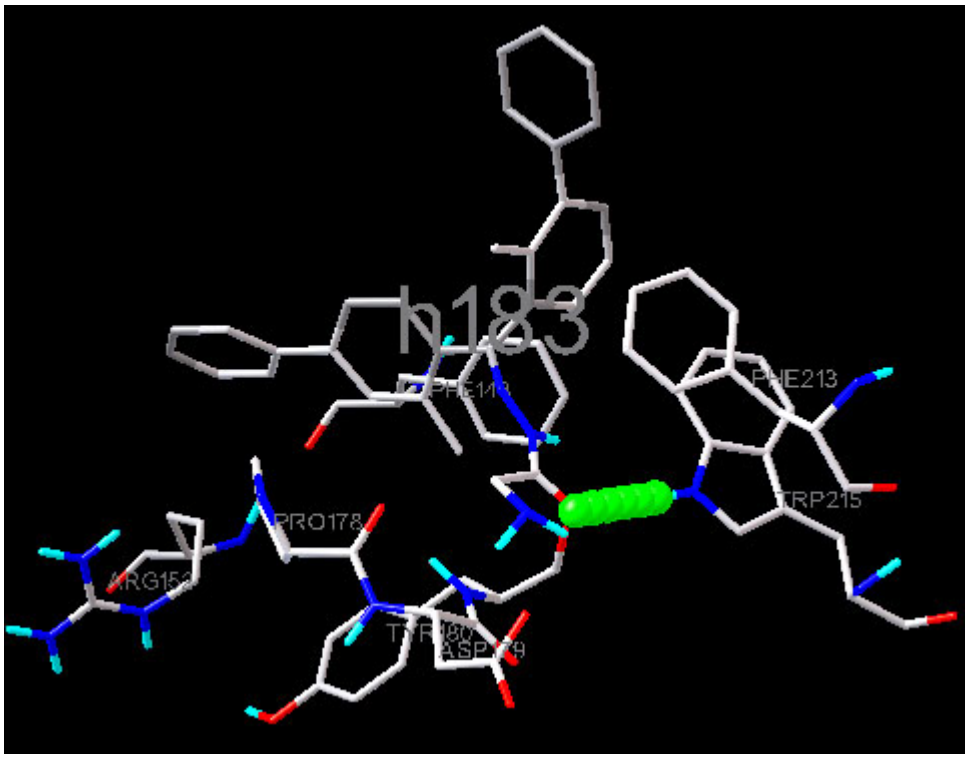

Fig. 4b. Docking Poses and binding interactions of h183

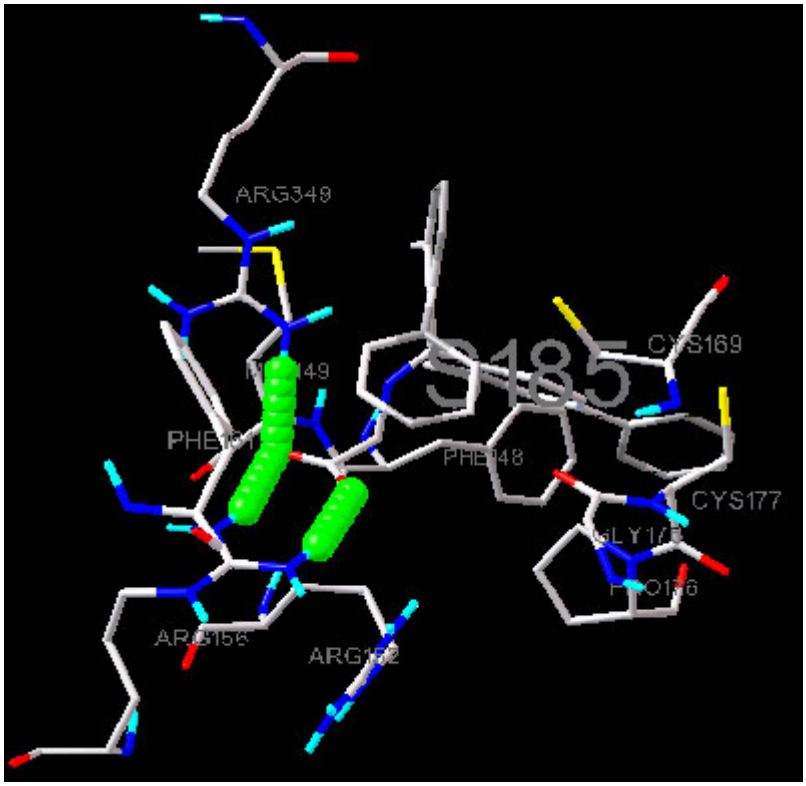

Fig. 4c. Docking Poses and binding interactions of S185

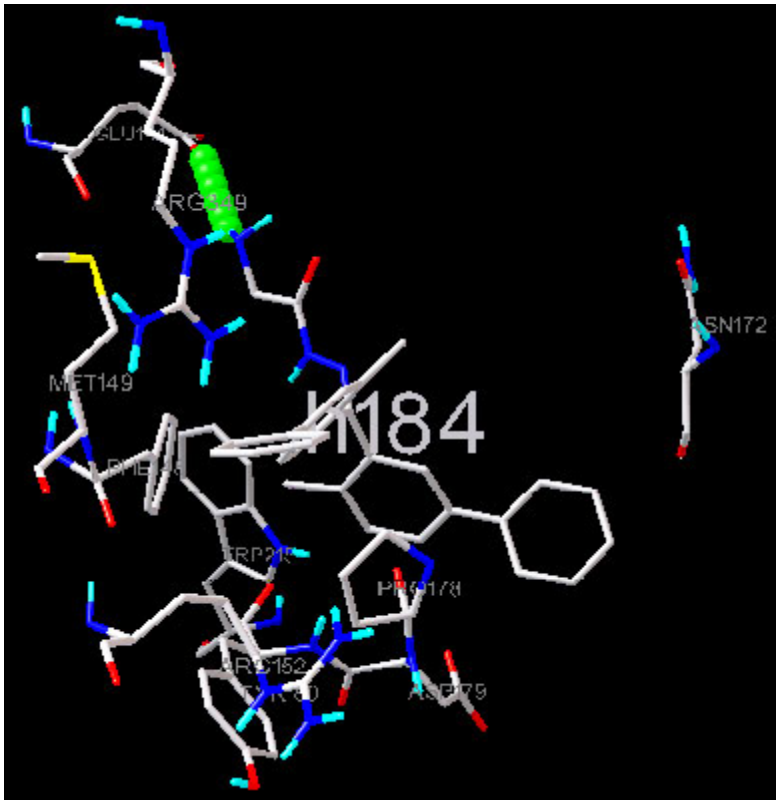

Fig. 4d. Docking Poses and binding interactions of h184

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