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ANTHRAQUINONES: A SCAFFOLD HOPE TO NOVEL γ-AMINO BUTYRIC ACID AMINOTRANSFERASE INHIBITORS

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ABSTRACT

Introduction: γ-amino butyric acid aminotransferase (GABA-AT) is a pyridoxal phosphate (PLP) dependent enzyme that catalyses the degradation of γ-amino butyric acid (GABA). γ-amino butyric acid aminotransferase (GABA-AT) inhibitors are used to treat epilepsy. **Objective:** The aim of this study was to search anthraquinone scaffolds as novel GABA-AT inhibitors using virtual screening based approach. **Materials and Methods:** AutoDock Tools® 1.4.6 and MGL Tools® 1.5.4 software were used to find out binding score, inhibition constant and conformational poses of the ligands inside the active site. AutoDock uses interaction maps to generate ensemble of low energy conformations and AMBER force field to estimate the free energy of binding of a ligand to its target. **Result and Discussion:** Estimated binding energies of top scoring molecules (derivatives of the natural product anthraquinone) were found quite low (e^{-53}M) as compared to that of vigabtrin (-5.5 Kcal/mol). **Conclusion:** These theoretical findings suggesting, the utility of virtual screening as a computational tool as well as significance of anthraquinone scaffolds as potential GABA-AT in-activators.

Keywords: γ-amino butyric acid aminotransferase , anthraquinone, anticonvulsants, virtual screening.

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INTRODUCTION

Epilepsy is a neurological disorder characterized by spontaneous and recurrent seizures with an estimated prevalence of 2-3% in the world population. [1] Although standard therapy permits control of seizure in 80% of these patients, millions have uncontrolled epilepsy. [2] Current marketed antiepileptic drugs consist of a variety of structural classes (lamotrigine, oxcarbazepine, topiramate, gabapentin, and levetiracetam) with different mechanisms of action. These agents typically have non-overlapping efficacy and side-effect profiles presenting multiple treatment options for the patient population. However, approximately 30% of seizure sufferers fail to respond to current therapies. Currently, there is no single drug of choice for treating all types of seizures. One should focus on mechanism-driven discovery of novel compounds. The search for antiepileptic compounds with a more selectivity and lower toxicity continues to be an area of investigation in medicinal chemistry. [3] γ-amino butyric acid aminotransferase (GABA-AT) is a pyridoxal phosphate (PLP) dependent enzyme that catalyses the degradation of γ-amino butyric acid (GABA). The inactivation of GABA-AT has been shown to be an important treatment for epilepsy. [4] The inhibition of GABA-AT has been the target of a great deal of research because of importance of maintaining GABA levels in the prevention of convulsions and for other psychopharmacological effects. Some of the earliest potent inhibitors of GABA-AT to be evaluated were hydrazines and hydroxylamines, which generally had low micromolar or nanomolar inhibition constant.[5] We earlier reported, analogs of GABA [6] [7] and phenyl substituted analogs of β-phenyl ethylidene [8] in search of novel GABA-AT inhibitors. Receptor targeted virtual screening based approach provide few potent molecules. Similarly GABA containing moieties as GABA-AT inhibitors are also reviewed and reported by many research groups. [9] [10] [11] [12] [13] [14] [15] The prototypical example of this class,

vigabatrin (γ-vinyl GABA) shown initial reversible binding to the pyridoxal-5'-phosphate (PLP) cofactor followed by an irreversible step leading to enzyme inactivation. [16] This prompted us to screen anthraquinone-GABA analogs for their GABA-AT inhibitory potential.

MATERIALS AND METHODS

GABA-AT receptor modeling

The receptor model was prepared by using AutoDock 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.

Firstly, the 3D Crystal structure of GABA-AT (Figure 1); PDB code 1OHV, [17] [18] [19] was downloaded from Brookhaven protein data bank (PDB; http://www.rcsb.org/pdb) and loaded to python molecular viewer. The non bonded oxygen atoms of waters, present in the crystal structure were removed. After assigning the bond orders, missing hydrogen atoms were added and the partial atomic charges were calculated using Gasteiger-Marsili method. [20] Kollman [21] 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' AutoDock type. (To confirm to the AutoDock 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).

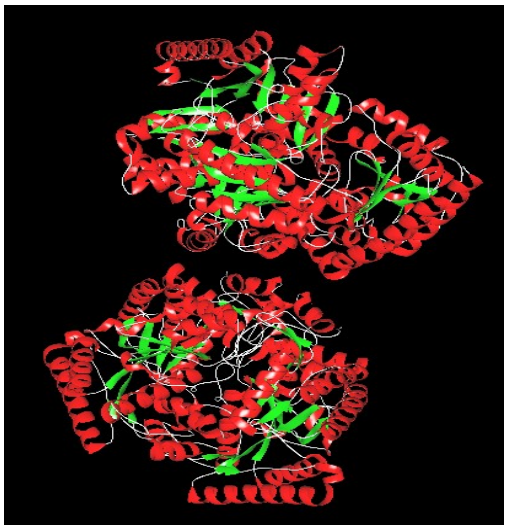


Figure 1: 3D Crystal structure of GABA-AT

Since vigabatrin (Figure 2) form a covalent ternary adduct with the active site LYS 329 of GABA-AT, therefore LYS 329 was included as flexible residue for introducing conformational search of flexible side chain. For the same macromolecule was saved in two files: one containing the formatted, flexible LYS 329 residue and the other all the rest of the residues in the macromolecule.

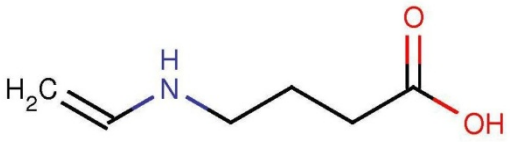


Figure 2: Chemical structure of Vigabatrin

Ligand modeling

ChemDraw Ultra 6.0.1 (Cambridge Soft.Com, 100 Cambridge park drive, Cambridge, MA 02140, USA) was used to draw the 3D structures of different ligand molecules. These were further refined and cleaned in 3D by addition of explicit hydrogens and gradient optimization function of MarvinSketch 5.0.6.1 (Chemaxon Ltd; http://www. Chemaxon.com). All the structures were written in Tripos mol2 file format.

Input molecules files for an AutoDock experiments must confirm to the set of atom types supported by it. AutoDock requires that, ligands give partial atomic charges and AutoDock atom types for each atom; it also requires a description of the rotatable bond in the ligand. AutoDock uses the idea of a tree in which the rigid core of the molecule is a 'root', and the flexible parts are 'branches' that emanate from the root. This set consists of united atom aliphatic carbons, aromatic carbons in cycles, polar hydrogens, hydrogen-bonded nitrogen and directly hydrogen-bonded oxygen among others, each with partial charges. Therefore, pdbqt format was used to write ligands, recognized by AutoDock.

TORSDOF (Torsional Degree of Freedom) is used in calculating the change in the free energy caused by the loss of Torsional degree of freedom upon binding. In the AutoDock 4.0 force field, the TORSDOF value for a ligand is the total number of rotatable bonds in the ligand. This number excludes bonds in rings, bonds to leaf atoms, amide bonds and guanidinium bonds.

Molecular Docking simulations

Prior to actual docking run, AutoGrid 4.0.1, was introduced to pre-calculate grid maps of interaction energies of various atom types. [22] In all dockings, a grid map with 60 × 60 × 60 points, a grid spacing of 0.375 A° (roughly a quarter of the length of a carbon–carbon single bond) were used, and the maps were centered on the ligand binding site. In an AutoGrid procedure, the protein is embedded in a 3D grid and a probe atom is placed at each grid point. The energy of interaction of this single atom with the protein is assigned to the grid point. An affinity grid is calculated for each type of atoms in the substrate, typically carbon, oxygen, nitrogen and hydrogens as well as grid of electrostatic potential using a point charge of +1 as the probe [23] [24]. AutoDock 4.0.1, uses these interaction maps to generate ensemble of low energy conformations [25] [26]. It uses a scoring function based on AMBER force field, and estimates the free energy of binding of a ligand to its target. For each ligand atom types, the interaction energy between the ligand atom and the receptor is calculated for the entire binding site which is discretized through a grid. This has

the advantage that interaction energies do not have to be calculated at each step of the docking process but only looked up in the respective grid maps. Since a grid map represents the interaction energy as a function of the coordinates, their visual inspection may reveal the potential unsaturated hydrogen acceptors or donors or unfavorable overlaps between the ligand and the receptor.

Of the three different search algorithms offered by AutoDock 4.0.1, the Lamarckian Genetic algorithm (LGA) based on the optimization algorithm,[27] was used, since preliminary experiments using other two (Simulated annealing and genetic algorithm) showed that they are less efficient, utilizes (discretized) Lamarckian notation that an adaptations of an individual to its environment can be inherited by its offspring. For all dockings, 100 independent runs with step sizes of 0.2 A^0 for translations and 5 A^0 for orientations and torsions, an initial population of random individuals with a population size of 150 individuals, a maximum number of 2.5×10^6 energy evaluations, maximum number of generations of 27,000, an elitism value of 1, a number of active torsion of 9 were used.

AutoDock Tools® along with AutoDock 4.0.1 and AutoGrid 4.0.1 were used to generate both grid and docking parameter files (i.e. .gpf and .dpf files) respectively.

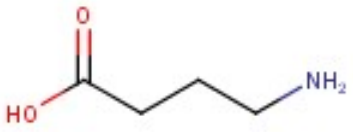


Figure 3: Structure of lead compounds

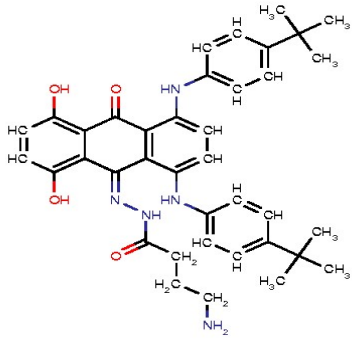


Figure 4a: Structure of SBABA226

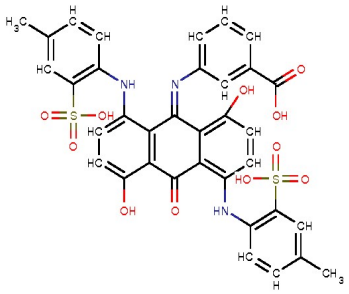


Figure 4b: Structure of AHG225

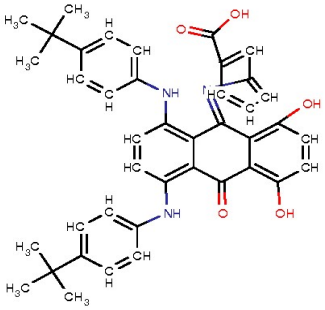


Figure 5a: Structure of SBABA225

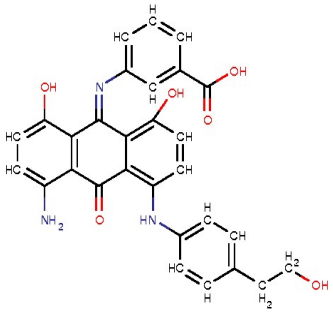
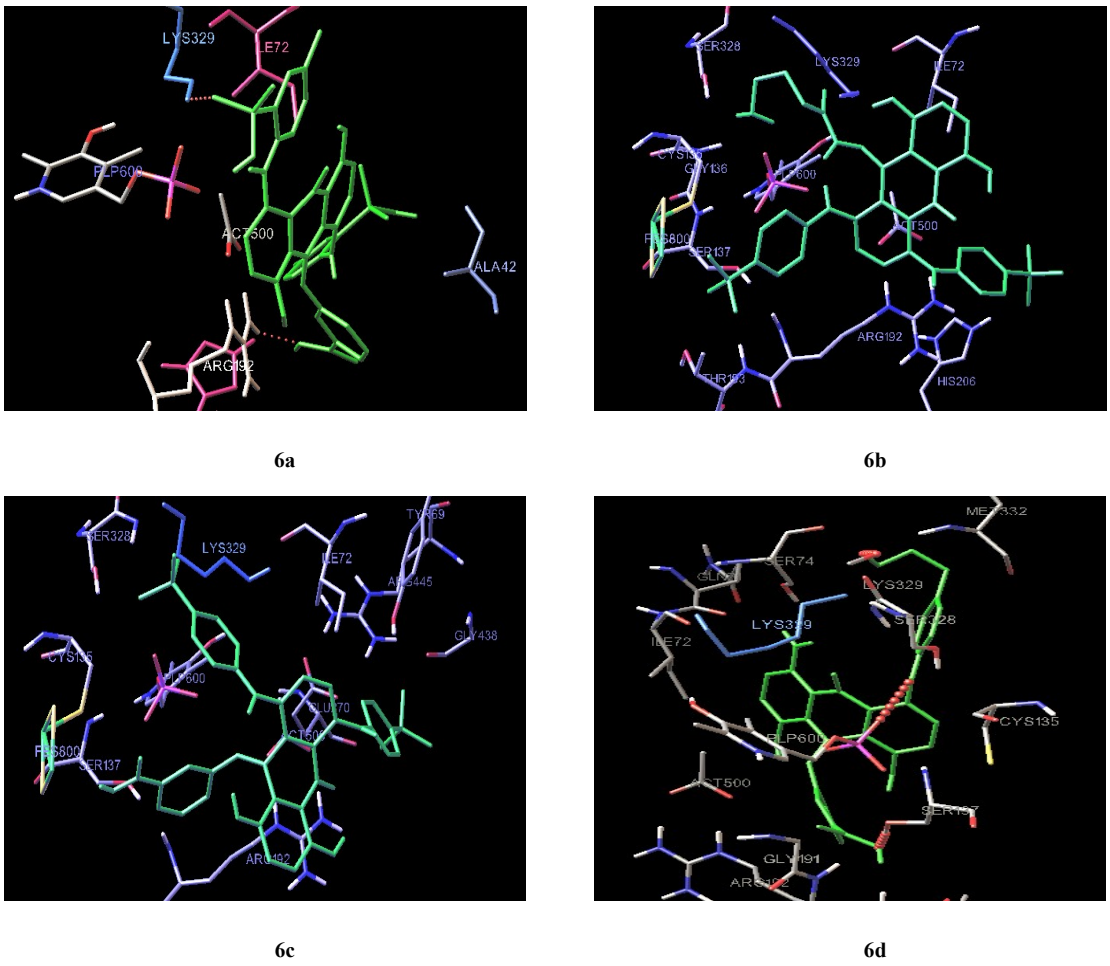


Figure 5b: Structure of SBABA227



6a **6b**

6c **6d**

Figure 6a, 6b, 6c and 6d: Interaction of the titled compounds with GABA-AT. Ligands are shown in green color and hydrogen bond in red dotted lines.

RESULT AND DISCUSSION

As a starting point, we used the lead compound GABA (Figure 3), which was the subject of our earlier work. The present study identified novel compounds that inhibited GABA-AT through structure based in silico screening with docking simulation. In silico screening was performed using AutoGrid 4.1 and AutoDock 4.1 software. 3D structure of protein was downloaded from protein data bank, PDB accession code; IHOV with γ-Ethynyl-GABA (vigabatrin) in the active site pocket and used as a target for the virtual screening. A compound library with 932 entries was tested using slightly modified version of our previously reported method. Interestingly, two compounds, SBABA226 (3-{[(9)-1,5-dihydroxy-4,8-bis[(4-methyl-2-sulfophenyl)amino]-10-oxo-9,10-dihydroanthracen-9-ylidene]amino}benzoic acid) and AHG225 (4-amino-N'-[(9)-1,4-bis[(4-tert-butylphenyl)amino]-5,8-dihydroxy-10-oxo-9,10-dihydroanthracen-9-ylidene] butanehydrazide) shown in Figure 4, have been identified first time to possess extraordinarily low value of inhibition constant (K_i), $4.99e^{-33}$M and 165.94aM (attomolar) respectively. In addition, two more compounds SBABA225 (3-{[(9)-1,4-bis[(4-tert-butylphenyl)amino]-5,8-dihydroxy-10-oxo-9,10-dihydroanthracen-9-ylidene]amino} benzoic acid) and SBABA227 (3-{[(9)-4-amino-1,8-dihydroxy-5-{[4-(2-hydroxyethyl)phenyl]amino}-10-oxo-9,10-dihydroanthracen-9-ylidene]amino}benzoic acid) shown in Figure 5, have exhibited promising value of inhibition constant, 919.09pM (picomolar) and 198.12nM (nanomolar) respectively.

Estimated binding energies of top scoring molecules with GABA-AT were found quite low as compared to that of vigabtrin (-5.5 Kcal/mol). Modeling and docking analysis revealed the nature of the active site and some key interactions that enabled the binding of titled compounds to the active site. Interactions of the titled compounds with GABA-AT are shown in Figure 6. Complex of compound SBABA226 with GABA-AT, hydrogen bonding interaction were found between oxygen of S=O with amino hydrogen of LYS329 (this also allows the positioning of sulfophenyl moiety in near vicinity of ILE72) and oxygen (OH) of

carboxylic group with amino hydrogen of ARG192 (this also allows positioning of amino benzoic acid moiety in the cage formed by ACT, ARG192 and ALA42). Hydroxyl functional group present at 8-position of anthraquinone nucleus favors hydrophobic interactions of its phenyl ring with phenyl ring of HIS206. In compounds AHG225 and SBABA225, no hydrogen bonding was observed. Compound AHG225 was positioned in the cage surrounded by ACT, PLP, ILE72, LYS329, SER328, GLY136, SER137, THR153, ARG192 and HIS206. Anthraquinone nucleus positioned in between amino acids ILE72 and ARG192, preferably favored by hydrophobic interactions. Similarly SBABA225 was covered by the FES, ACT, PLP and amino acids GLU 270, ARG445, ILE72, LYS329, SER328, LYS135, SER137 and ARG192. GLU 270 and LYS329 were in close proximity of the anthraquinone and sulfophenyl moiety moiety respectively. In compound SBABA227, hydrogen bonding interaction were found between hydroxyl group of SER137 with oxygen(OH) of carboxylic group and PLP600 with NH of hydroxyethylphenylamino part of the molecule. Similarly hydrogen bonding interaction was observed in between hydroxyl group of hydroxyethyl part and LYS329. SER329 was intermingled with hydroxyethylphenylamino group. Observations from docking studies explain the high GABA-AT selectivity observed.

CONCLUSION

In summary, we have screened for novel, potent γ-amino butyric acid aminotransferase inhibitors that have anthraqinone structures and obtained the promising results. We, believe that these inhibitors may be proven as magic scaffolds lead compounds. These findings about the epilepsy help the students and researchers to understand aspects of virtual screening to health and disease

Disclosure: The protocol adopted for *insilico* screening of the titled compounds has been adopted and modified from author's previous published work.

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CONFLICT OF INTERESTS

Authors do not have any conflict of interests

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