WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 6.805
Volume 5, Issue 7, 1516-1530. Research Article ISSN 2277– 7105

MOLECULAR MODELLING GUIDED APPROACH FOR *IN SILICO* SCREENING OF SOME HYDRAZONE DERIVATIVES AS GABA-AT INHIBITORS.

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Article Received on
16 May 2016,

Revised on 06 June 2016,
Accepted on 27 June 2016

DOI: 10.20959/wjpr20167-6645

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ABSTRACT

De-novo Hydrazone derivatives were designed through *in silico* studies including Molecular properties prediction, Toxicity risk prediction and by Molecular Docking approaches**.** The hypothetically designed molecules were studied for Lipinski rule of 5 properties. The successful molecules were subjected to toxicity risk prediction by Osiris property calculator. The docking methodology applied in the study was first validated by redocking the Pyridoxal-5-phosphate in active domain of GABA-AT with the co-crystallized one. GABA-AT was explored for the residues imperative for activity by analyzing the binding pattern of vigabatrin and selected compounds of hydrazone derivatives in the active domain. All the selected molecules passed Lipinski rule of five successfully and they were safe. The docking results explored that compound PS12, PS11, PS9 PS6 and PS14 were having significant binding affinity close to vigabatrin, which indicated that these compounds may prove successful anticonvulsant oral candidates.

KEYWORDS: *Hydrazones, GABA-AT, Anticonvulsant, Molecular Modelling, Lipinski's Rule of 5, logP.*

INTRODUCTION

Epilepsy is a chronic non-communicable disorder of the brain that affects people of all ages. Approximately 50 million people worldwide have epilepsy, making it one of the most

common neurological diseases globally.[1] There are approximately 20%–30% of the patients having seizures resistant to the available medical therapies. Despite the major medical need for a new chemotherapeutic agent, drug discovery for anticonvulsants is very challenging.[2] With the help of novel drug discovery methods like computer aided drug design we can design high quality leads which are more likely to succeed in clinical trials.[3]

The search for antiepileptic compounds with a more selective activity and lower toxicity continues to be an area of investigation in medicinal chemistry. A rational drug design process of a new anticonvulsant could be achieved by the identification of new targets through better under-standing of molecular mechanisms of epilepsy. Novel anticonvulsant agents are discovered through conventional screening and/or structure modification rather than a mechanism-driven design.[4]

Gamma amino butyric acid (GABA) has been proposed as a validated target for antiepileptic drugs, because its selective inhibition raises GABA concentration in the brain.10 It is a pyridoxal phosphate (PLP)-dependent homodimeric enzyme, catalyzing reversible transfer of the amino group of GABA to α-ketoglutarate to yield succinic semialdehyde and L-glutamate. c-Amino butyric acid (GABA) is a predominant inhibitory neurotransmitter in mammalian CNS modulating central inhibitory tone via activation of ionotropic $GABA_A$ and $GABA_C$ receptor and G-Protein-coupled $GABA_B$ receptor.[5, 6] c-Amino butyrate aminotransferase (GABA-AT)catalyzes the degradation of GABA to succinic semialdehyde (SSA). Depleted levels of GABA have been shown to cause convulsions.[7] Raising GABA levels in brain have an anticonvulsive effect.[8] GABA-AT is a validated target for antiepileptic drugs because its selective inhibition raises GABA concentration in brain.[9] Numerous strategies exist to elevate GABA levels in the brain. The strategy, which we have taken, involves the inhibition or the inactivation of GABA-AT.[10, 11, 12, and 13] GABA itself is not an effective anticonvulsive agent since it does not cross the blood brain barrier, a protective membrane that prevents xenobiotics from entering the brain.[14] Consequently, a real need exists to develop new anticonvulsant compounds to cover seizures which are so far resistant to presently available drugs. 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 followed by their evaluation by in vitro and in vivo models to discover novel antiepileptic drugs. Several recent successes (pregabalin, brivaracetam) have shown that knowledge of the mechanism of action gives the developer a significant advantage in improving efficacy through increased target potency and selectivity, thereby lowering the potential for dose-related side effects. It is the hope that new generation AEDs with novel mechanisms will increase the likelihood for success in treating a heterogeneous patient population.[15]

GABA–amino transferase (AT) is a homodimer with each subunit containing an active site PLP, covalently bound to LYS329 of chain A via a Schiff base.[16] When the concentration of GABA diminishes below a threshold level in the brain, convulsion results while raising the brain GABA level terminates the seizure. So, due to these features pig GABA-AT [Protein Data Bank code: 1OHV] was taken for interaction studies. The search for antiepileptic compounds with a more selective activity and lower toxicity continues to be an area of investigation in medicinal chemistry. A rational drugdesign process of a new anticonvulsant could be achieved by the identification of new targets through better under- standing of molecular mechanisms of epilepsy. c semialde- hyde (SSA). Depleted levels of GABA have been shown to cause convulsions (Karlsson et al., 1974). Raising GABA levels in brain have an anticonvulsive effect (Krogsgaard– Larsen, 1981). GABA-AT is a validated target for antiep- ileptic drugs because its selective inhibition raises GABA concentration in brain (Storici et al., 1999). Numerous strategies exist to elevate GABA levels in the brain. Thestrategy, which we have taken, involves the inhibition or the inactivation of GABA-AT (Bansal et al., 2010, 2011a, b, c; Nogardy and Weaver, 2005; Silverman and Clift, 2008; Sowaet al., 2005). GABA itself is not an effective anticonvulsive agent since it does not cross the blood brain barrier, a protective membrane that prevents xenobiotics from entering the brain (Silverman et al., 1986). Conse- quently, a real need exists to develop new anticonvulsantcompounds to cover seizures which are so far resistant to presently available drugs. Current marketed antiepileptic drugs consist of a variety of structural classes (lamotrigine, oxcarbazepine, topiramate, gabapentin, and levetiracetam) with different mechanisms of action. These agents typicallyhave non-overlapping efficacy and side effect profiles presenting multiple treatment options for the patient pop- ulation. 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 followed by their evaluation by in vitro and in vivo models to discover novel antiepileptic drugs. Several recent successes (pregabalin, brivaracetam) have shown that knowledge of the mechanism of action gives thedeveloper a significant advantage in improving efficacy through increased target potency and selectivity, thereby lowering the potential for dose-related side effects. It is the hope that new generation AEDs with novel mechanisms will increase the likelihood for success in treating a heterogeneous patient population (Gerlach and Krajewski, 2010.

MATERIAL AND METHODS

Molecular Properties Calculations and Molecular Docking

Molecular properties, mainly hydrophobicity, molecular size, flexibility and the presence of various pharmacophoric features influence the Pharmacokinetic and pharmacodynamics behaviour of molecules in the living organism, including bioavailability. Thus in order to achieve good bioavailable drugs, we have subjected a series of Hydrazone derivatives (PS1-PS14) for the prediction of some basic pharmacokinetic properties under the Lipinski's ''Rule of Five''.

Lipophilicity

All the compounds were subjected to computational study in order to filter the drugs for biological screening. For good membrane permeability logP value should be ≤5.[17] All the title compounds (PS1-PS14) were found to have logP values in the range of 3.39–3.98 except compound PS12 having loP value 5.36.

Absorption, Polar surface area, and ''rule of five" properties

High oral bioavailability is an important factor for the development of bioactive molecules as therapeutic agents. Good intestinal absorption, reduced molecular flexibility (measured by the number of rotatable bonds), low polar surface area or total hydrogen bond count (sum of donors and acceptors), are important predictors of good oral bioavailability.[18] Molecular properties such as membrane permeability and bioavailability is always associated with some basic molecular descriptors such as logP (partition coefficient), molecular weight (MW), or hydrogen bond acceptors and donors counts in a molecule.[19] Lipinski[20] used these molecular properties in formulating his ''Rule of Five". The rule states that most molecules with good membrane permeability have logP ≤ 5, molecular weight ≤500, number of hydrogen bond acceptors ≤10, and number of hydrogen bond donors ≤ 5. This rule is widely used as a filter for drug-like properties. Table 1 contains calculated percentage of absorption

(%ABS), molecular polar surface area (TPSA) and Lipinski parameters of the investigated compounds of the series (PS1-PS14). Magnitude of absorption is expressed by the percentage of absorption. Absorption percent was calculated[21] using the expression: %ABS =109 - 0.345 PSA. Polar surface area (PSA) was determined by the fragment-based method of Ertl and coworkers.[22-23] A poor permeation or absorption is more likely when there are more than 5 H bond donors, 10 H-bond acceptors. Hydrogen-bonding capacity has been also identified as an important parameter for describing drug permeability.[24] The series (PS1-PS14) under investigation had all compounds having hydrogen bond donor and acceptors in considerable range as shown in Table 1.

Number of rotatable bond is important for conformational changes of molecules under study and ultimately for the binding of receptors or channels. It is revealed that for passing oral bioavailability criteria number of rotatable bond should be ≤10. The compounds in this series (PS1-PS14) possess lower range of 'n umber of rotatable bonds' i.e. (3-5) and therefore, exhibit low conformational flexibility.

Molecular polar surface area (TPSA) is a very useful parameter for the prediction of drug transport properties. TPSA is a sum of surfaces of polar atoms (usually oxygen, nitrogen and attached hydrogen) in a molecule. TPSA and volume is inversely proportional to % ABS. All the compounds under study have exhibited good %ABS except D6 having 26% Abs, But all the title compounds (PS1-PS14) followed the Lipinski ''Rule of Five''. The pharmacokinetic parameters were calculated online from Molinspiration Chemoinformatics (http://www.molinspiration.com /cgi-bin/properties) and are given in Table 1.

Osiris Calculations

Structure based drug design is now very routine work as many drug fail to reach clinical phases because of ADME/TOX problem encountered. Therefore prediction of these problems before synthesis is rational approach to minimize cost production of expensive chemicals. The Osiris calculations are tabulated in Table 2. Toxicity risks (mutagenicity, tumorogenicity, irritation, reproduction) and physicochemical properties (cLogP, solubility, drug likeness and drug score) of compounds (PS1-PS14) were calculated by the methodology developed by Osiris.[25] The toxicity risk predictor locates fragments within a molecule, which indicate a potential toxicity risk. Toxicity risk alerts are an indication that the drawn structure may be harmful concerning the risk category specified. The logP value of a compound, which is the logarithm of its partition coefficient between n-octanol and water, is a well-established

measurement of the compound's hydrophilicity. Low hydrophilicities and therefore high logP values may cause poor absorption or permeation. It has been shown that for compounds to have a reasonable probability of good absorption, their logP value must not be greater than 5.0. On this basis, all the compounds PS1-PS10 possessed logP values in the acceptable range.

Aqueous solubility

The aqueous solubility of a compound significantly affects its absorption and distribution characteristics. In general a low solubility goes along with a poor absorption and therefore the general aim is to avoid poorly soluble compounds. Our estimated logS value is a unit stripped logarithm (base 10) of a compound's solubility measured in mol/liter. There are more than 80% of the drugs on the market have a (estimated) logS value greater than -4. In present series the values of logS are around -5. Further, Table-2 shows drug likeness of compounds (PS1-PS14 which is in the acceptable zone to be drug like when compared with standard drug. We have calculated overall drug score (DS) for the compounds PS1-PS14 and compared with that of standard drug Phenytoin. The drug score combines drug likeness, cLogP, logS, molecular weight and toxicity risks in one handy value than may be used to judge the Compound's overall potential to qualify for a drug. This value is calculated by multiplying contributions of the individual properties with the equation (1).

$DS = \prod (1/2+1/2Si) \prod ti$

Where S; $(1/1+e^{ap+b})$

DS is the drug score, Si is the contributions calculated directly from miLogP; logS, molecular weight and drug likeness (pi) via the second equation, which describes a spline curve. Parameters a and b are (1,-5), (1, 5), (0.012, -6) and (1, 0) for cLogP, logS, molecular weight and drug likeness, respectively. The ti is the contributions taken from the four toxicity risk types and the values are 1.0, 0.8 and 0.6 for no risk, medium risk and high risk, respectively. The reported compounds PS1-PS14 showed moderate to good drug score as compared with standard drug used.

Molecular docking studies of the compounds using Pymol/Autodock vina Plugin.

In this step the drug like molecules were subjected to virtual screening for the selected target (GABA) protein to study the drug receptor binding site and their binding mode by analyzing their binding energy using Molecular Modelling Tools and softwares. Modeling studies are required in order to construct molecular models that incorporate all experimental evidences

reported. These models are necessary to obtain a consistent and more precise picture of the biologically active molecules at the atomic level and furthermore, provide new insights that can be used to design novel therapeutic agents. The compounds in the study were subjected to dock in the active domain of GABA protein by using Pymol/Autodockvina Plugin software. Crystal structures of GABA protein in complex with vigabatrin (PDB ID: 1OHV) with resolution 2.4 Å was downloaded from RCSB Protein Data Bank to serve as the docking template.[26] The crystallographic water and ligand molecules were removed from the protein complex. Pymol AutoDockvina plugin developed by Seeliger[27] was used on Linux ubuntu 12.0 installed on Pentium i3workstation. ChemDraw ultra 8.0 software [Chemical Structure Drawing Standard; Cambridge Soft corporation, USA (2003)] was used for construction of compounds which were converted to 3D structures using Chem3D ultra 8.0 software and the constructed 3D structures were energetically minimized by using MOPAC (semiempirical quantum mechanics) with AM1 mozyme geometry, 100 iterations and minimum RMS gradient of 0.10. the structures of the compounds undertaken for the study is shown in Table-3. The redocked pose of the ligand with the co-crystallized structure of the same has been shown in Fig-1.

RESULTS AND DISCUSSIONS

Low hydrophilicities and therefore high logP values may cause poor absorption or permeation. It has been reported by Lipinski, 2001, that for compounds to have a reasonable probability of good absorption, their logP value must not be greater than 5.0. On this basis, most of our compounds possess acceptable logP values and can be considered as oral drugs. All the title compounds (PS1-PS14) were found to have logP values in the range of 3.33 to 3.98 except compound PS2 having logP value 5.36.

According to Veber's rule number of rotatable bonds is important for conformational changes of molecules under study and ultimately for the binding of receptors or channels. It is revealed that for passing oral bioavailability criteria, number of rotatable bond should be <10. The compounds in this series (PS1-PS14) possess lower range of 'number of rotatable bonds' i.e. (2-5) and therefore, exhibit low conformational flexibility. Molecular polar surface area (PSA) is a very useful parameter for the prediction of drug transport properties. PSA is a sum of surfaces of polar atoms (usually oxygen, nitrogen and attached hydrogen) in a molecule. PSA and volume is inversely proportional to %ABS [Remko 2009]. All the title compounds (PS1-PS14) were found to have PSA values in the range of 33.4 to 60.1. PSA and volume is

inversely proportional to % ABS. All the compounds under study have exhibited good % absorption ranging from 88 % to 97 %. All the designation compounds (PS1-PS14) passed the Lipinski ‘‘Rule of Five’’.

Number of hydrogen bond acceptor and donors were calculated for all the molecules in the series. The number of hydrogen bond acceptors and donors were in the range of 3-6 and 0-2 respectively, thus none of the compound violated the Lipinski’s rule in this regard. Further the molecular weights of the compounds taken for the screening were less than 500, thereby all the molecules under consideration have passed Pfizer’s rule of five.

The molecules to be synthesized in future were subjected for prediction of drug score and drug likeness along with the prediction of toxicity risk evaluation. For toxicity risk assessment, Tumorogenicity, Mutagenicity, Irritation and reproductive effect were taken into consideration. Among the series PS1-PS14, All the compounds were safe with respect to Tumorogenecity, Irritation and reproductive effect. But the compounds have shown mild toxicity prediction for mutagenicity, for which the reason can be assigned to the presence of NO_2 group only. Although there are many successful molecules with nitro group such as Nitrazepam, therefore we have planned to go ahead with the present series. Except this all the compounds in the series were safe and do possess acceptable drug likeness and drug score. The compounds PS1-PS14 showed moderate to good drug score as compared with standard drug used. While screening for GABA-AT; docking method was validated by redocking the pyridoxal-5-phosphate (PLP-600) with the GABA-AT protein and the interactions obtained were considered as the standard, to compare with the docking of the other compounds. Redocked structure of PLP in the GABA-AT receptor, as shown in Fig. 1 revealed that the original cocrystallized and docked ligands are overlapping to each other, thereby validating to our docking methodology. The results of docking have been summarized in Table-3. The redocked ligand was found to interact with the Ser-137, Gly-136 and Gln-301 by forming conventional H-bond asshown in Fig3.

When vigabatrin docked with the GABA-AT protein, it had strong binding affinity of -9.5 and formed Hydrogen Bonding with the PLP 600 as shown in fig 4. Interestingly compound 12 has shown binding affinity -8.7, the maximum in the series. Compound PS7 was found to have binding affinity -6.9, the minimum in the series. Thus by and large, it can be concluded that the designed molecules pass the Lipinski’s rule of 5 and veber’s rule. Thereby they are expected to be good oral candidates with low toxicity profile. Further these designed

compounds are also having good affinity with active domain of GABA-AT protein, which forecast the good expected Anticonvulsant activity of these compounds. Finally in large this is worthwhile to go for the wet lab synthesis of these rationally designed compounds.

Table 1: Pharmacokinetic Properties important for good oral bioavailability for the compounds of PS1 series.

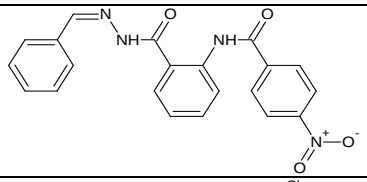
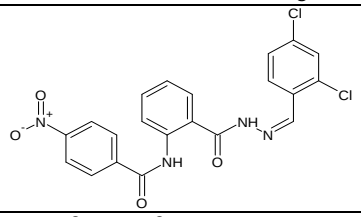
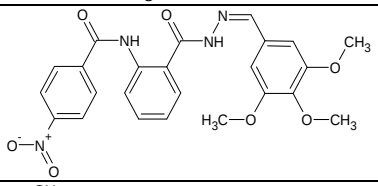
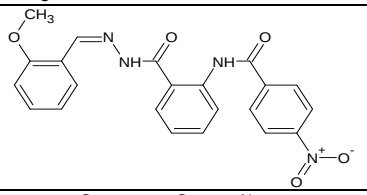
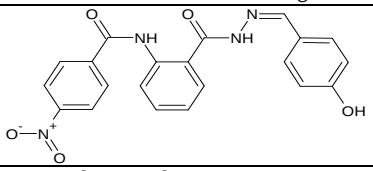
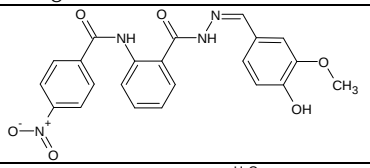
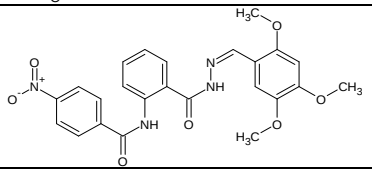
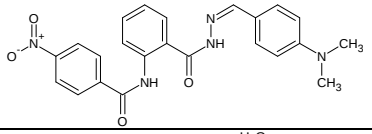
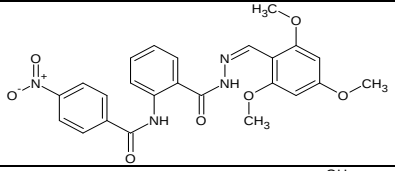
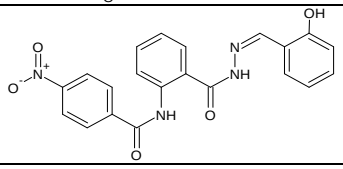
Compds	%ABS	Vol (A3)	TPSA (A2)	NRO TB	HBA	HBD	Log*P*	M W	Lipinski's Violations
Rule	-	-	-	-	<10	<5	≤5	<500	≤1
PS1	97.02	304.64	34.73	3	5	2	3.7	388.12	0
PS2	97.02	336.57	34.73	3	3	0	5.36	456.92	1
PS3	88.65	399.92	58.99	3	6	0	3.48	479.03	0
PS4	94.27	336.24	42.69	4	4	0	3.91	419.01	0
PS5	91.06	315.18	51.99	3	4	1	3.339	405	0
PS6	88.27	348.08	60.1	5	5	1	3.34	435.01	0
PS7	88.63	400.39	59.04	4	6	0	3.87	479.03	0
PS8	96.27	354.19	36.91	4	3	0	3.98	432.04	0
PS9	88.72	399.76	58.79	4	6	0	3.73	479.03	0
PS10	91.07	316.25	51.97	3	4	1	3.59	405	0
PS11	97.02	325.58	34.73	3	3	0	3.94	403.02	0
PS12	94.13	315.18	43.11	2	4	0	3.39	419	0

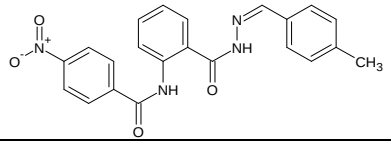
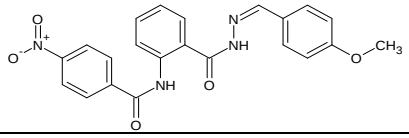
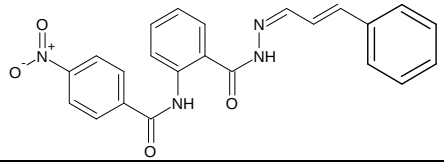
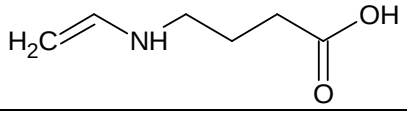
Table 2: Osiris calculation for bioavailability and toxicity prediction of series PS

Compds	BIOAVAILABILITY OF DRUGS				TOXICITY RISK EVALUATION			
	Solubility Score	MW	Drug likeness	Drug	MUT	TUM	IRRIT	RE
PS1	-5.69	388	6.78	0.15				
PS2	-7.17	456	5.78	0.09				
PS3	-5.75	478	3.83	0.14				
PS4	-5.71	418	6.51	0.15				
PS5	-5.4	404	6.62	0.16				
PS6	-5.41	434	6.26	0.16				
PS7	-5.75	478	5.75	0.14				
PS8	-5.73	431	5.26	0.16				
PS9	-5.75	478	6.2	0.14				
PS10	-5.4	404	6.72	0.16				
PS11	-6.04	402	8.21	0.13				
PS12	-5.71	418	6.65	0.15				

Colour of circle indicates level of toxicity; Green: low, Yellow: medium, Red: Highly toxic.

Table3: Docking score of the compounds considered for exhaustive in silico studies.

S.No.	Compd	Structure	Docking Score
1.	PS1		-7.4
2.	PS 2		-7.2
3.	PS 3		-8.4
4.	PS 4		-8.2
5.	PS 5		-8.1
6.	PS 6		-8.5
7.	PS 7		-6.9
8.	PS 8		-8.2
9.	PS 9		-8.6
10.	PS 10		-7.6

11.	PS 11		-8.7
12.	PS 12		-8.9
13	PS14		-8.5
14	Vigabatrin		-9.5

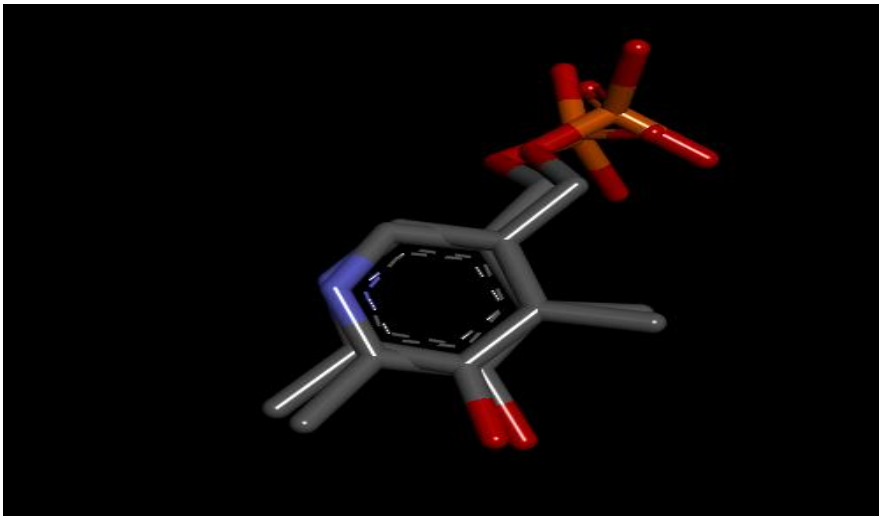


Fig 1: Redocked Structure of PLP.

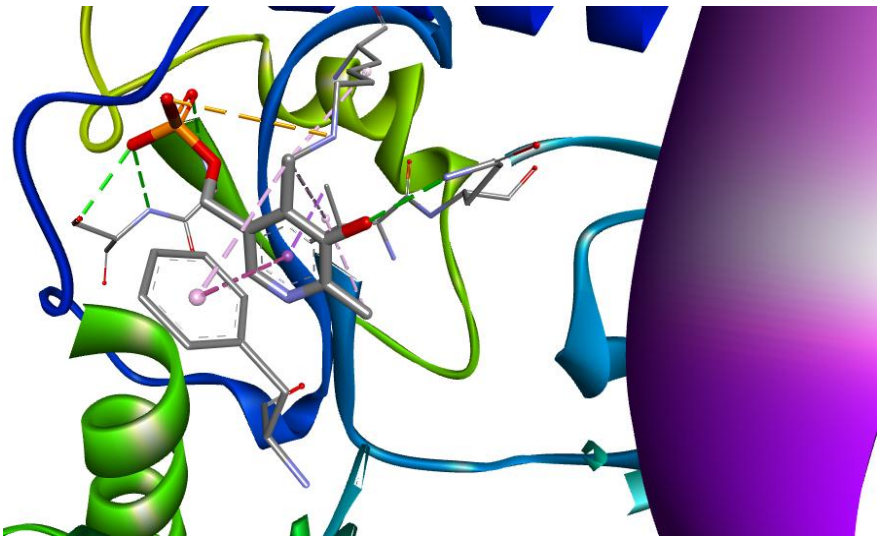


Fig 2: Ligand-receptor Interaction viewed by Discovery studio 2016.

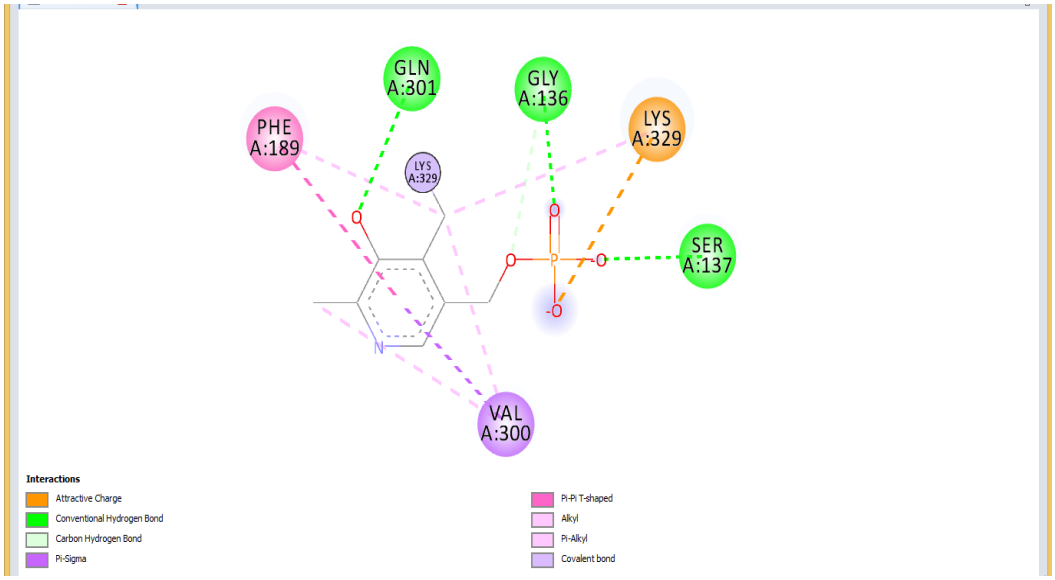


Fig 3: 2D- diagram showing the interaction of PLP with the surrounding residues.

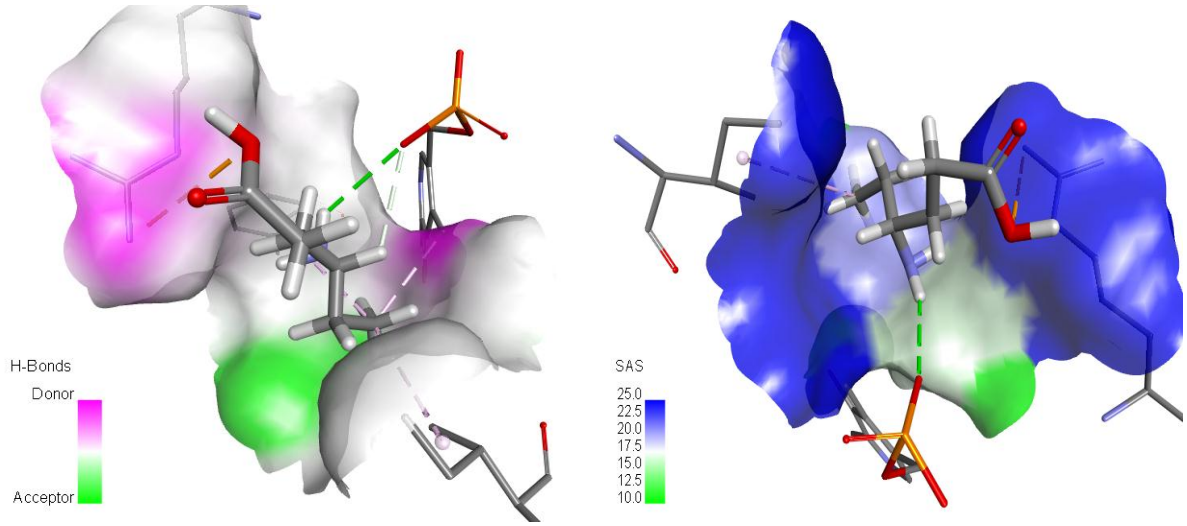


Fig 4: Left figure showing H-bond donor and acceptor surface while right one exhibiting solvent accessible surfaces (SAS).

CONCLUSION

The present work was aimed to design few selected Hydrazone derivatives as tailored GABA-AT inhibitors via Molecular properties prediction and Molecular Modelling studies. Toxicity risk evaluation study was also performed to ensure the safety of the targeted compounds. The compounds designed were first screened for the drug like properties and were filtered on the basis of "Lipinski's rule of 5". They were further subjected to Toxicity risk prediction with the help of Osiris property explorer. The molecular properties were predicted with Molsoft & Molinspiration softwares. Henceforward they were subjected to Molecular docking studies with the help of Autodock/ VinaPymol plugin Software to

understand the binding mode of the rationally designed compounds with the target receptors. All the *denovo* compounds passed the Lipinski rule of 5, with compounds PS2 having one violation only. The toxicity prediction ensured that all the compounds were non-tumorogenic, non-irritating and no effect on reproductive system except having medium risk of mutagenicity in the series which is supposed to be because of the Nitro group present along with the Anil's linkage and can be ignored. Further, docking of the proposed compounds exhibited good binding affinity for compounds PS12, PS11, PS9, PS6 and PS14 which anticipated these compounds to be good oral anticonvulsant compounds. So it is worthwhile to plan the synthesis of the said compounds and the wet lab synthesis is already on the route in our lab.

Conflict of Interest: The authors declare that there is no conflict of interest.

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