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Molecular Docking Studies of Dihydropyridazin-3(2*H*)-one Derivatives as Anticonvulsant Agents

Sushil Prasad, Sukhbir Lal Khokra and Manish Devgun✉

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ABSTRACT

Molecular docking is the identification of ligand's correct binding geometry *i.e.* pose in the binding site and estimation of its binding affinity for rational design of drug molecule. The current study endeavored the high throughput *in silico* screening of 56 derivatives of dihydropyridazin-3(2*H*)-one docked with human cytosolic branched chain amino transferase using PyRx-virtual screening tool. Out of 56 compounds, almost all the test compounds showed very good binding affinity score. Gabapentin was used as standard drug which shows binding affinity of -6.2. On the basis of H-bond interactions, compounds **3**, **9**, **11**, **25**, **26**, **31**, **34**, **39**, **47**, **48**, **51**, **54**, **56** were found to be potent outcome for anticonvulsant activity. Compounds **11**, **25**, **39**, **56** showed excellent H-bond interactions with protein active site, Among which compound **11** showed the outstanding interactions with acceptable bond length 2.34, 2.57, 2.62, 3.03 Å.

KEYWORDS

Molecular docking, Dihydropyridazin-3(2*H*)-one, Amino transferase, PyRx-virtual, Gabapentin.

INTRODUCTION

Arrival of new medication into a market will take an average of 10-15 years and about US $2 billion. Traditional approaches of drug discovery relied on chemical entities, which were obtained from natural products and the whole process was time consuming and perhaps less economical [1]. These drawbacks allows the shift of traditional approach to combinatorial and *in silico* approach, which supported the assistance in structural information [2]. These new advancements play essential role in reducing expense and the hour of early drug discovery [3]. Drug design typically require the new model of small ligands that are similar in shape and charge to the active site of bio-molecular target in which it binds. Drug design is an innovative method for discovering potential medicines dependent on the biological target or another ligand molecule. Modern drug research includes the detection of screening impacts, medicinal chemistry and enhancement of these impacts through growing sensitivity, selectivity (to decrease the risk for side effects), potency and oral bioavailability. When a compound satisfies all upper criteria, product discovery process should continue to clinical trials. Computer assisted drug design (CADD) has drawn various synthetic organic chemists to its focus. Computer

Author affiliations:

Institute of Pharmaceutical Sciences, Kurukshetra University, Kurukshetra-136119, India

✉To whom correspondence to be addressed:

E-mail: manishdevgun@gmail.com

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assisted drug development techniques have greatly improved the drug discovery process and also reduced the cost involved in the entire process. Tools including combinatorial library development, virtual high performance screening, quantitative structure activity and structure property relationship, molecular docking, ADME/tox analysis and comparative modeling, *etc.* have developed a fundamental part of modern drug discovery process.

Nowadays, QSAR research is accompanied by drug design and growth, optimizing pharmacokinetic and pharmacodynamics properties. Structure and ligand based drug design are important methodology of virtual screening in computer aided drug design. Structure based methodology relies on information of 3D-structure of protein obtained *via* NMR, X-ray crystallography and any other technique while ligand based methodology is based on pharmacophore modelling and quantitative structural activity relationship (QSAR) [4]. The molecular docking technique determines binding interaction between protein molecule and optimum conformation of ligand, so that overall energy of system get minimized and stable complex is formed [5]. Ideally, computational docking gives prediction about binding affinity and interaction of ligand with protein's active site before the compound is synthesized. Hence, reduce the cost of money and material for the synthesis.

Molecular docking is an interesting framework to comprehend biomolecular interaction for rational design of drug molecule. The docking investigation is done by putting a ligand into the favoured restricting site of the target protein, predominantly in a non-covalent manner to form a steady and stable complex of adequate potency and greater specificity. There are two significant objectives of molecular docking. The first is to accurately recognize the optimum conformational mode of a given ligand in the dynamic or restricting pocket of protein. The second is to effectively rank a group of ligands in agreement to their decided binding affinities. Molecular docking requires broad testing of ligand conformations for a ligand in the binding pocket of a protein and in this way creates enormous number of potential modes that situates a ligand inside the dynamic site of enzyme. A decent positioning algorithm samples all the possible binding modes while the scoring framework positions every one of the arrangements and recognizes the most probable restricting or binding mode of the ligand.

Types of drug design

Structure based drug designing: It depends on the understanding of 3D structure of protein acquired through NMR, X-ray crystallography and any other technique. If the target protein is not at reach, homology modelling on that protein is created on the basis of experimentally related protein in that series. This also encouraged the rapid development in structure based drug design [4].

Two methods-first includes "finding" ligand for a given protein also called as database searching. The primary benefit of this technique is that it saves manual efforts to get novel leads. Second is "building" ligand also known as receptor based drug designing. The main advantage of this method includes ligands which are not present in any of the database must be proposed.

Ligand-based drug designing: It depends on the information of another ligands that binds to the same pocket of interest. These molecules gives idea about a pharmacophore requirement which explains the minimum structure qualification that would be possessed by a ligand molecule for binding to the biological target. Additionally, QSAR in which correlation between experimentally observed characteristics and calculated properties must be obtained. The QSAR assumes an indispensable part in the prediction of activity of new analogues [6].

Categories of molecular docking

Lock and key docking: The enzyme particularity towards its substrate depends on the two segments that are substrate and enzyme. Substrate fit into enzyme entirely like a key in a lock. This lock and key relationship briefly conceptualized the embodiment of enzyme-substrate association where the enzyme depicts the lock and the substrate portrays the key. In such frameworks, it is a necessity that the key(substrate) fit appropriately into the active site of the lock (enzyme) for useful productivity to happen. Keys (substrates) that are excessively little, excessively enormous or with mistakenly situated scores and notches, won't find a way into the lock. Both ligand and receptor are assumed rigid and are closely bound. This model also explains 3d complementarity between receptor and ligand [7].

Induced fit docking: Here receptor site is assumed to be adjustable in nature. The ligand binds flexibly at the active site of receptor with maximum binding affinity, implementing the concept of complementarity between ligand and protein molecule [8].

Ensemble docking: Based on complexity and flexibility of conformational state of proteins. Many protein structures utilized as associate degree for docking with ligand [9,10]. Basically, the nerve cells (neurons) of brain speak with each other by electrical signals transmission from one cell to another. These signals demonstrates various activities like seeing, feeling, listening, muscle movement, reasoning and so forth. A seizure happens when the firing of neurotransmitter is abnormalized either in a detached space of the mind or all through the mind. Epilepsy is a neurological paroxysmal cerebral dysarrythmia portrayed by intermittent unconstrained seizures, either in cerebral side of the brain (general seizures) or limited to some parts of the cerebral hemisphere(incomplete or partial seizures) [11,12]. In the event, if entire cerebrum of brain is included, the electrical imbalance is called generalized seizure. This sort of seizure also used to be known as a grand mal seizure. The most conspicuous indication of a grand mal seizure is the transient muscles rigidity (tonic phase) which ultimately turns into clonic phase followed by depression of CNS and prolonged sleep.

If entire cerebrum is not involved, the electric imbalance generated is called absence seizure and indication s are jerking movement of head, arm and blinking movement of eyelids followed by complete loss of consciousness. Currently epilepsy management is still a major problem due to uncontrolled seizures in some types of epilepsy. A long time treatment is required even throughout a lifetime. As per recent studies of World Health Organization (WHO), approximately 2.4 million yearly cases are epilepsy infected in around the world [13] and about 70

million people worldwide are epilepsy suffering till now, among which 90% of people are residing in developing countries [14,15]. At present, 40% of patients have a genetic background that contributes to different epilepsy. Epilepsy cause considerable effect on health, social wellness and quality of life as compared to healthy individuals [16].

Grandmal (generalized tonic-clonic) and petit mal (absence seizure) are the most common types of epilepsy

Grandmal epilepsy: A grandmal seizure normally starts from right or left side of the hemisphere, which spread and advance into a two-sided tonic-clonic seizure. It is a major form of epilepsy usually begins in childhood or during puberty which lasts upto 1-2 min and illustrated as tonic clonic activity by complete loss of cognizance. It starts with transient muscles rigidity (tonic phase) which ultimately turns into clonic phase followed by depression of CNS function and prolonged sleep [17]. Grandmal seizures are further categorized into motor and non-motor seizures. The International League Against Epilepsy (ILAE) sorted grandmal seizures under seizures belonging to generalized in onset [18].

Petitmal epilepsy: A petitmal seizure makes you gaze into space and jerking movement of head, arm are observed followed by complete loss of consciousness. It can likewise be called absence seizure. Petitmal seizures are further categorized into typical and atypical seizures. This type of seizure can happen frequently and even occur many times per day. It is generally occured in kids and normally don't cause any long term issues. These kinds of seizures may be set off by a spell of hyperventilation.

The field of antiepileptic drug development has gotten very unique in the recent years. It imparts many promising research studies and there is a proceeding with interest for new antiepileptic agents as it has not been feasible to control each sort of seizure with presently accessible antiepileptic drugs. Numerous patients that accomplish seizures control with drugs, experience the ill effects of medicine instigated neurotoxicity with number of side effects which includes sleepiness, gastric disturbance, cerebellar ataxia, vitamin B_{12} deficiency, *etc.* [19]. A number of researches explored the presence of aryl group with one or more electron donating groups or presence of imino group in six membered ring is vital for anticovulsant activity. Pyridazinone are group of heterocyclic organic compound with six membered ring structure that show broad pharmacological activity such as anticonvulsant, anti-inflammatory, antimalarial, antifungal, anticonvulsant, antibacterial *etc.* [19-24] and also have wide range of applications in many prescription drugs as main building blocks. The medicinal value of pyridazinones has guided to create specific derivatives; the substitution may be organized in pharmacophoric pattern for showing better pharmacological activity.

This results in synthesis of novel pyridazinones derivatives and evaluation of their anticonvulsant activity. The greater part of medication used today as anticonvulsants contain ureide moiety like barbiturates, benzodiazepines, oxazolidinediones, succinimides and so on [25-28]. The pyridazinone derivatives show wide range of biological activities and may act as biological active pharmacophore in medicinal chemistry [29]. This moiety may help in finding new medications of likely restorative therapeutic value. To find significant anticonvulsant activity, molecular docking can be performed which predicts binding affinity scores of different ligands interacting with protein and their interactions at protein active site. The basic structure of 4,5-dihydropyridazin-3(2*H*)-one is shown below in Fig. 1. In this article, we have hypothetically designed different 4,5-dihydropyridazin-3(2*H*)-one derivatives and contrasted their anticonvulsant specificity with gabapentin, taken as standard [30].

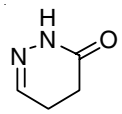


Fig. 1. Basic structure of 4,5-dihydropyridazin-3(2*H*)-one

Target for anticonvulsant drugs

In silico docking investigation of 56 compounds gave us an idea regarding novels responsible for anticonvulsant activity. The results obtained demonstrated that all examined ligands have comparable orientation and position inside putative binding site of human cytosolic branched chain amino transferase protein (PDB ID-2A1H), which serves as passage channel for substrate to active site. There is strong connection between affinity of ligand towards protein and binding free energy which can contribute in understanding and interpreting the activity of ligand by various possible mechanisms.

EXPERIMENTAL

Finding of optimum binding mode of ligand to receptor site is the main objective of docking. Docking studies has been performed with a group of theoretic pyridazinone derivatives using PyRx-virtual screening tool on protein with PDB ID-2A1H. The protein structure has been imported from https://www.rcsb.org/structure/2A1H. The structure of various 4,5-dihydropyridazin-3(2*H*)-ones analogues are shown in Table-1.

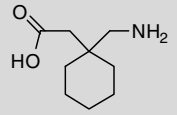
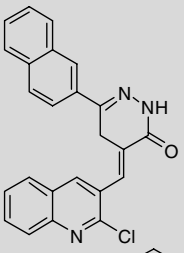
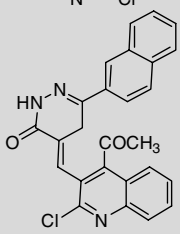
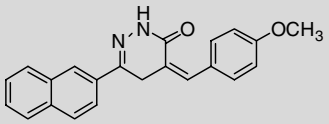
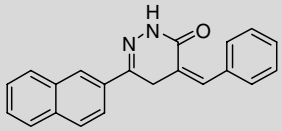
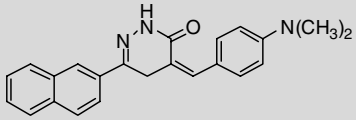
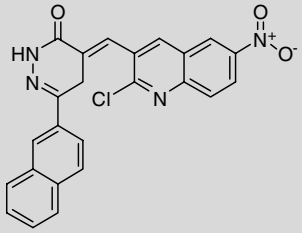
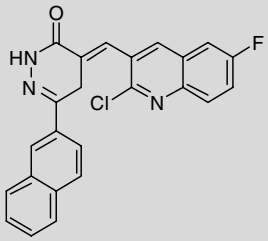
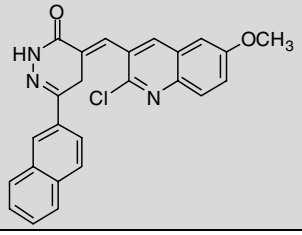
Steps involved:

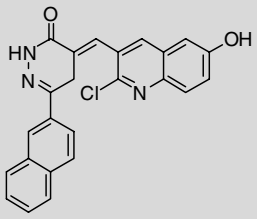
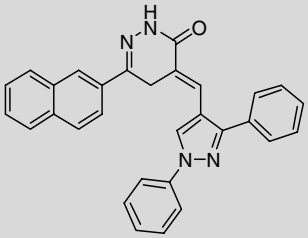
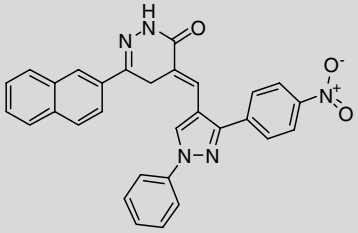
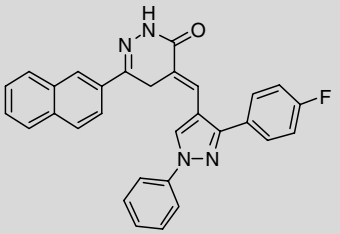
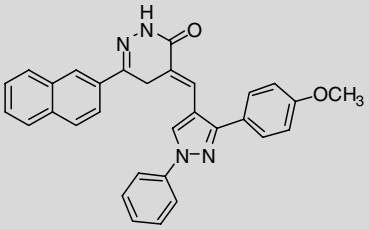
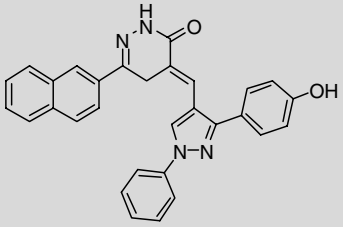
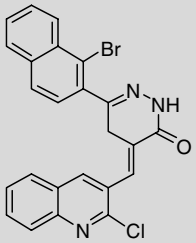
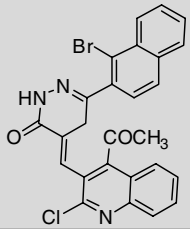
- Importing a protein file and protein preparation
- Preparation of ligands using MarvinSketch 5.11.0
- Detecting cavities of protein molecules
- Execution of docking through Vina Wizard
- Determination of various poses of ligand on protein molecule
- Determination of binding affinity scores
- Determination of protein residue responsible for binding and hydrogen bond interaction

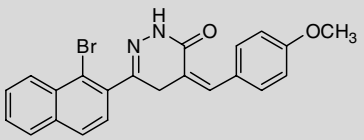
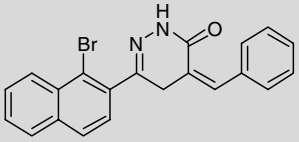
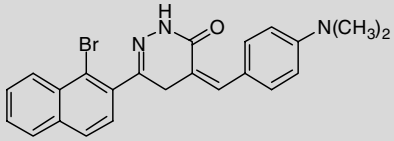
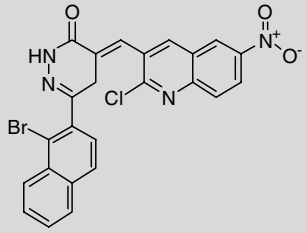
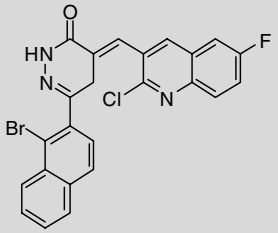
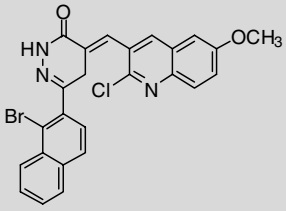
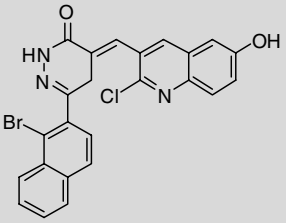
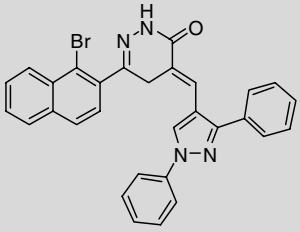
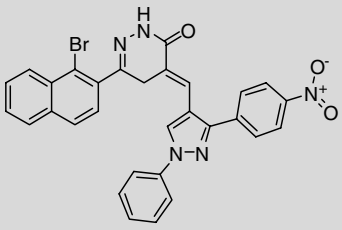
Compound selection: Based on literature data, we chose 56 hypothetical compounds and docking was performed using protein (PDB ID-2A1H) for antiepileptic activity using PyRx-virtual screening tool. The selection of these 56 compounds is based on broad literature study of compounds containing pyridazinone nucleus and on the basis of this literature study, the best outcomes for binding to the protein active site were accounted and hypothesized. The structure of 56 hypothetical compounds are mentioned in Table-1.

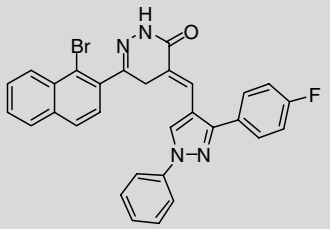
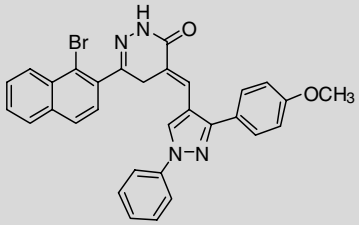
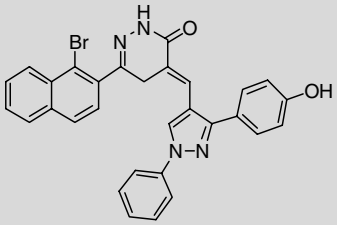
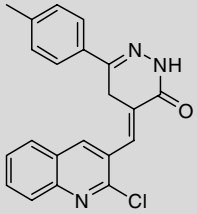
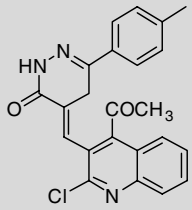
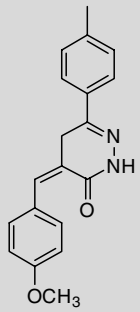
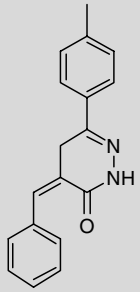
Preparation of ligand: Preparation of ligand molecules were done by Chem Draw Professionals 15.0 and Marvin Sketch. The molecules were converted into 2D and then 3D using build

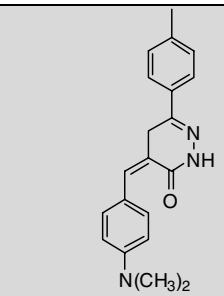
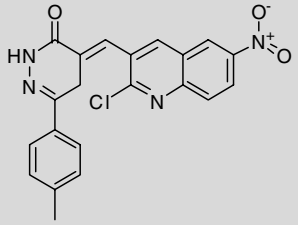
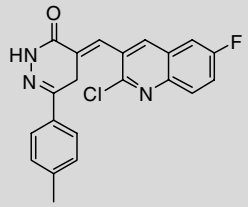
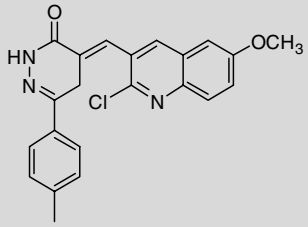
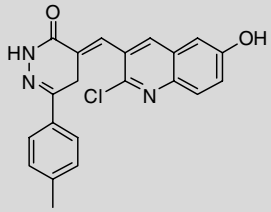
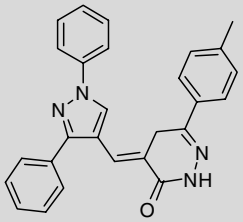
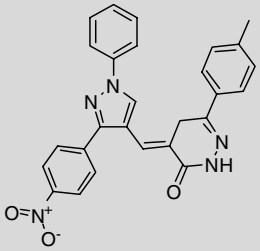
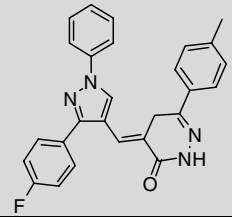
TABLE-1
LIGAND-RECEPTOR INTERACTION DATA OF DIHYDROPYRIDAZIN-3(2*H*)-ONE
ON PDB ID: 2A1H USING PyRx-VIRTUAL SCREENING TOOL

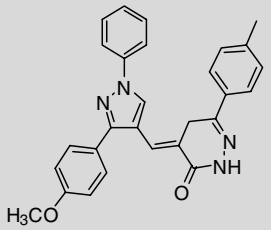
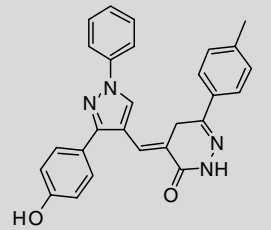
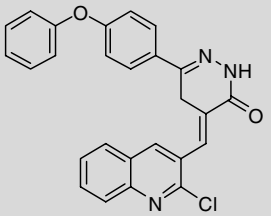
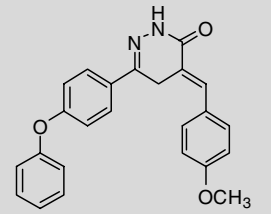
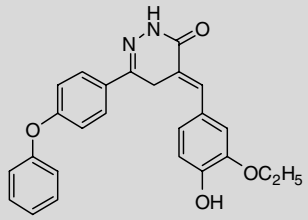
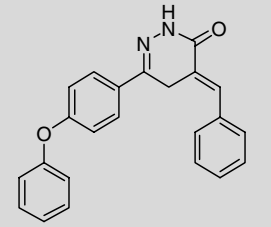
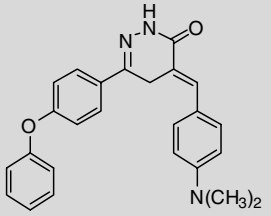
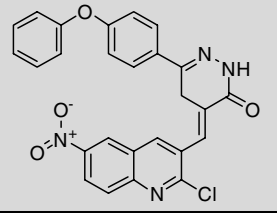
Compound	Structure	Interaction of amino acid having shortest bond length (*indicates H-bond interacting amino acid)	Number of hydrogen bond interactions	Binding affinity score
Gabapentin		Thr240A(2.60)* Plp400A(3.57)	1	-6.2
1		Tyr173B(4.31) Tyr70A(5.21) Val155A(2.36)* Ala314B(3.69) Phe75B(4.86)	1	-10.4
2		Val155A(2.37)* Ala314B(3.24) Phe75B(4.87) Tyr70A(5.25) Tyr173B(4.29)	1	-11.1
3		Thr240A(2.31)* Thr210A(2.84)* Val155B(4.62) Ala314A(4.94) Tyr173A(4.71) Phe30A(4.47)	2	-9.9
4		Thr240A()*	1	-9.6
5		Phe30A(4.47) Ala314A(4.75) Leu28A(3.48)*	1	-9.6
6		Val170A(5.38) Ala314A(3.96) Lys202A(3.24) Thr313A(3.47) Val155B(3.01)* Phe30A(4.75) Tyr173A(4.79)	1	-11.1
7		Phe30B(4.66) Tyr173B(4.61) Tyr141B(2.21)* Ala314B(3.81) Plp400B(4.31)	1	-10.2
8		Ala314B(3.45) Val29B(4.68) Plp400B(5.29)	0	-11.1

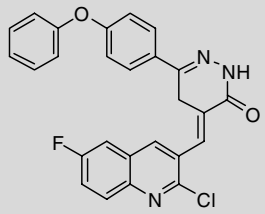
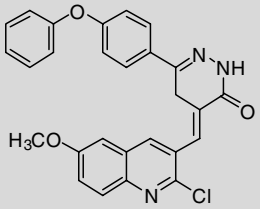
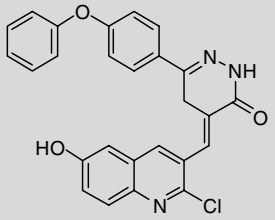
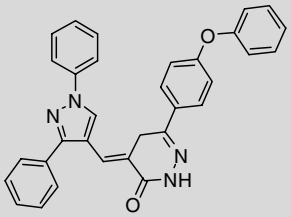
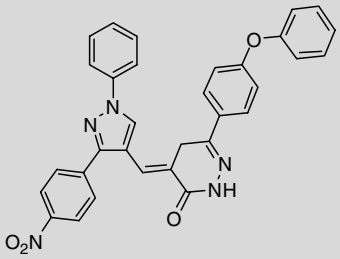
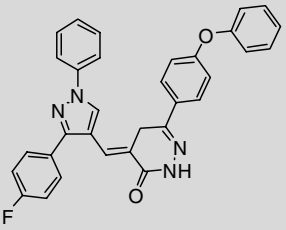
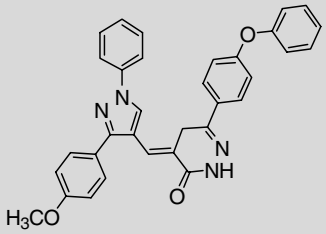
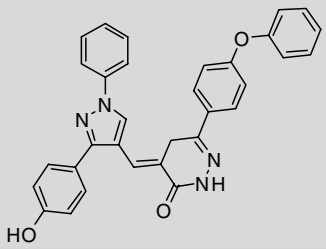
9		Tyr141B(1.85)* Val155A(2.83)* Phe30B(4.54) Tyr173B(4.62) Ala314B(3.20) Plp400B(4.08)	2	-10.8
10		Tyr173A(2.18)* Ala314A(3.67) Phe30A(5.00)	1	-10.1
11		Tyr70B(2.34)* Arg143A(2.57)* Tyr141A(2.62)* Thr240A(3.03)* Phe30A(2.79) Ala314A(4.61)	4	-10.5
12		Thr210B(3.09) Thr240B(2.41)* Val155A(4.75) Tyr173B(4.40) Phe30B(5.16) Ala314B(4.75)	1	-10.3
13		Ala314A(4.34) Phe30A(5.06) Arg143A(4.34) Val155B(5.04)	0	-10.7
14		Phe30A(5.01) Ala314A(3.66)* Tyr173A(2.18)	1	-10.4
15		Ala314A(3.07) Phe30A(2.97) Val155B(5.37)	0	-10.2
16		Gln224B(3.22)* Val155A(4.45) Phe30B(5.06) Plp400B(4.70) Ala314B(4.11) Tyr173B(4.77)	1	-10.3

17		Val29A(3.94) Ala314A(4.49)	0	-8.6
18		Phe30A(3.30) Tyr173A(4.98) Ala314A(5.48) Arg143A(4.61)	0	-9.2
19		Val29A(5.02) Ala314A(4.66) Phe30A(4.72) Thr240A(3.39)	0	-9.2
20		Ala314A(4.60) Val155B(5.33) Lys79A(1.99)* Phe30A(4.90) Ala172A(5.03) Val170A(5.14) Val29A(4.60)	1	-10.5
21		Gly171A(3.07) Val155B(5.32) Ala314A(3.89) Val29A(4.77) Tyr173A(5.28) Phe30A(5.26) Val170A(5.05)	0	-10.1
22		Ala135B(4.37) Leu28B(5.20) Pro175B(4.43)	0	-9.3
23		Val155B(5.31) Tyr173A(4.93) Gly171A(3.06)* Val170A(4.94) Ala172A(4.93) Ala314A(4.82) Val29A(4.53)	1	-10
24		Ala314A(4.10)* Val155B(2.68) Phe30A(4.89) Val29A(4.59)	1	-9.9
25		Thr240A(2.70)* Ala314A(4.83) Arg143A(2.64)* Lys202A(3.23) Plp400(2.70)* Phe30A(2.80) Tyr173A(4.85) Leu218A(3.80)	3	-10.4

26		Tyr173B(4.07)* Gln214B(2.41) Val155A(4.89) Thr210B(3.17) Thr240B(2.05)* Ala314B(4.78) Phe30B(5.20)	2	-10.9
27		Phe30A(2.66) Ala314A(4.54)	0	-10.1
28		Leu218A(3.78) Thr240A(2.45)* Ala314A(4.78) Phe30A(2.68)	1	-10.6
29		Val155A(4.66) Leu153A(3.21) Plp400B(4.28) Ala314B(3.17) Phe30B(4.59) Tyr173B(4.59)	0	-9.0
30		Leu28B(2.38)* Val29B(4.55) Ala135B(3.58) Ala172B(2.70) Pro175B(5.27) Val170B(5.42) Tyr173B(2.43)	1	-9.0
31		Tyr141B(5.36) Plp400B(3.72) Ala314B(4.16) Thr240B(2.53)* Gln224B(2.50)* Val155A(3.5)	2	-8.5
32		Tyr173A(5.08) Phe30A(3.84) Arg143A(4.49)	0	-8.6

33		Val155B(5.21) Tyr70B(5.03) Ala314A(4.78) Phe30A(4.26) Leu28A(2.42)*	1	-8.3
34		Arg143A(3.02)* Ala314A(3.54) Val155B(2.30)* Val225A(4.49) Trp227A(4.88) Lys202A(3.20) Phe30A(5.16)	2	-10.5
35		Arg143A(4.70) Ala314A(3.36) Phe30A(3.39) Gly154B(3.71) Lys202A(3.57) Plp400A(2.58)* Val155B(4.91) Val225A(4.40) Trp227A(4.65)	1	-10.3
36		Thr240A(2.30)* Met241A(3.53) Ala314A(3.90) Leu153B(3.33) Tyr173B(5.29)	1	-10
37		Val155B(2.19)* Ala314A(3.19) Phe30A(4.94) Tyr173A(4.87)	1	-9.2
38		Val155B(5.10) Val225A(4.52) Trp227A(4.56) Arg143A(4.20) Ala314A(5.25) Phe30A(4.57)	0	-10.5
39		Arg143A(2.56)** Tyr141A(2.84)* Ala314A(4.76) Tyr173A(5.07) Phe30A(2.90) Val29A(4.75) Val211A(4.43) Val155B(4.25)	3	-10.1
40		Val155B(5.13) Val225A(4.45) Trp227A(4.56) Arg143A(4.21) Phe30A(4.54) Ala314A(5.11)	0	-10.6

41		Val155B(5.14) Phe75A(4.84) Ala314A(4.73)	0	-10.4
42		Val155B(5.10) Val225A(4.44) Trp227A(4.55) Arg143A(4.37) Ala314A(5.05) Phe30A(4.46)	0	-10.3
43		Tyr173A(1.91)* Ala314A(4.88)	1	-9.7
44		Phe30B(4.25) Tyr173B(4.70) Ala314B(3.87) Plp400B(4.83)* Thr313B(2.31)	1	-9.1
45		Gly77(3.70) Phe30(4.25) Tyr173(4.70) Plp400(2.46)*	1	-10
46		Tyr70B(5.79) Phe75A(5.28) Ala314A(4.88) Phe30A(4.05) Val170A(4.97)	0	-9.7
47		Ala314(5.47) Gly171(3.71) Leu28(3.60) Thr240(2.55)* Val155(2.85)*	2	-9.1
48		Lys79A(2.08)* Phe30A(5.08) Val170A(4.98) Ala172A(4.85) Leu28A(2.35)* Val29A(3.79) Ala314A(3.26)	2	-10.3

49		Tyr70B(5.85) Arg143A(4.23) Phe75A(5.36) Ala314A(4.93) Tyr173A(4.04)*	1	-10.2
50		Tyr173A(4.59) Gly171A(2.05)* Ala314A(3.57) Val155B(5.45)	1	-9.8
51		Pro175B(5.25) Phe30B(4.49) Tyr173B(4.77) Ala314B(3.20) Plp400B(4.14) Tyr141B(1.94)* Val155A(2.33)*	2	-9.9
52		Phe30B(3.68) Tyr173B(4.36) Thr240B(2.67)* Plp400B(4.14) Ala314B(3.22)	1	-10.4
53		Ala314A(4.01) Phe30A(4.91) Val170A(5.19) Val29A(3.68) Val155B(2.46)*	1	-10.5
54		Gly171A(2.62)* Val170A(3.69) Tyr173A(2.33)* Phe30A(4.97) Ala314A(4.55)	2	-10.4
55		Gln224A(3.39)* Ala314A(4.82) Tyr173A(3.78) Phe30A(5.02) Val170A(4.83)	1	-10.7
56		Gly171A(2.47)* Tyr173A(2.27)* Thr240A(2.57)* Ala314A(4.62)	3	-10.5

and optimize method. The obtained structure will be saved in PDB format. This step involved preparation of ligand molecules and were assigned bond, bond order, hybridization charges, free hydrogens and flexible torsions. The generated 3D structure was imported in PyRx-virtual screening tool for docking.

Set of hypothetical compounds: The docking study was performed with a set of hypothetical 4,5-dihydropyridazin-3(2*H*)-one derivatives. The structure of all 56 compounds with binding affinity scores are illustrated in Table-1.

Protein preparation: The protein used for docking was imported from https://www.rcsb.org/structure/2A1H and protein preparation was done by using discovery studio 2021 client software. All water molecules, co-crystallized ligand and other chemical compound were removed from main protein structure. Different interactions are considered to calculate the binding affinity score between receptor and ligands.

Docking: Traditional approaches were extremely high-ticket, more time-consumption and fewer economical to discover a modern clinical drug [5]. To overcome disadvantage of ancient strategies, simpler and rational strategies are introduced that deem virtual screening, supported the provision of structure details. Virtual screening approaches are also known as a structure and ligand based product design technique. The structural approach to drug, explains molecular linking up while ligand based approaches discuss interaction between quantitative structure response (QSAR) and pharmacophore modeling. Docking method describes the connection between the material and target molecule.

Here all the designed ligands and reference drugs were assigned within Vina Wizard and then Autodock wizard and necessary bond, bond order, hybridization, polar charges, *etc*. were allotted using corresponding softwares. Docking of ligand was performed by generating a number of conformation of ligand within the active site and score of different conformation within active site should be note down on the basis of various interactions between ligand and receptor.

Binding affinity is generally influenced by non-covalent intermolecular interactions such as vander Waal interactions, electrostatic interactions, hydrophobic interactions and hydrogen bonding between two molecules. Likewise, binding affinity between a ligand and receptor's active site might also be influenced by the presence of different other molecules.

RESULTS AND DISCUSSION

In silico docking investigation of 56 derivatives of 4,5-dihydropyridazin-3(2*H*)-one gave us an idea regarding novels responsible for antiepileptic activity. The output of molecular docking is related with protein-ligand interaction and the determination of optimum conformation of that ligand in binding pocket. The outcome of protein ligand interaction along with standard drug have been summed up in tabular form as binding affinity score, number of H-bondings and amino acid interactions. The negative binding energies of ligand indicates stable binding interaction between receptor and ligands. More negative is the binding free energy score, more favourable is the pose for binding to protein active site. There is strong connection between affinity of ligand towards protein and binding free energy, which can contribute in understanding and interpreting the activity of ligand by various possible mechanisms.

Binding free energy calculations: PyRx-Virtual screening tool software, which was used to calculate relative binding energies of all docked protein-ligand complexes with default parameters. Binding free energy is calculated by using the following equation:

$$\Delta G_{bind} = G_{complex} - (G_{protein} + G_{ligand})$$

where ΔG_{bind} is the binding free energy, $G_{complex}$ is free energy of complex, $G_{protein}$ and G_{ligand} is free energy of the target protein and ligand, respectively.

The current study includes docking of 56 compounds using PDB(2A1H) with gabapentin as standard drug. All the test compounds showed better binding affinity score as compare to standard drug (Fig. 2). Most of test compounds showed excellent number of hydrogen bonding interactions as compare to gaba-pentin On the basis of H-bond interactions of these 56 comp-ounds, compounds **3**, **9**, **11**, **25**, **26**, **31**, **34**, **39**, **47**,

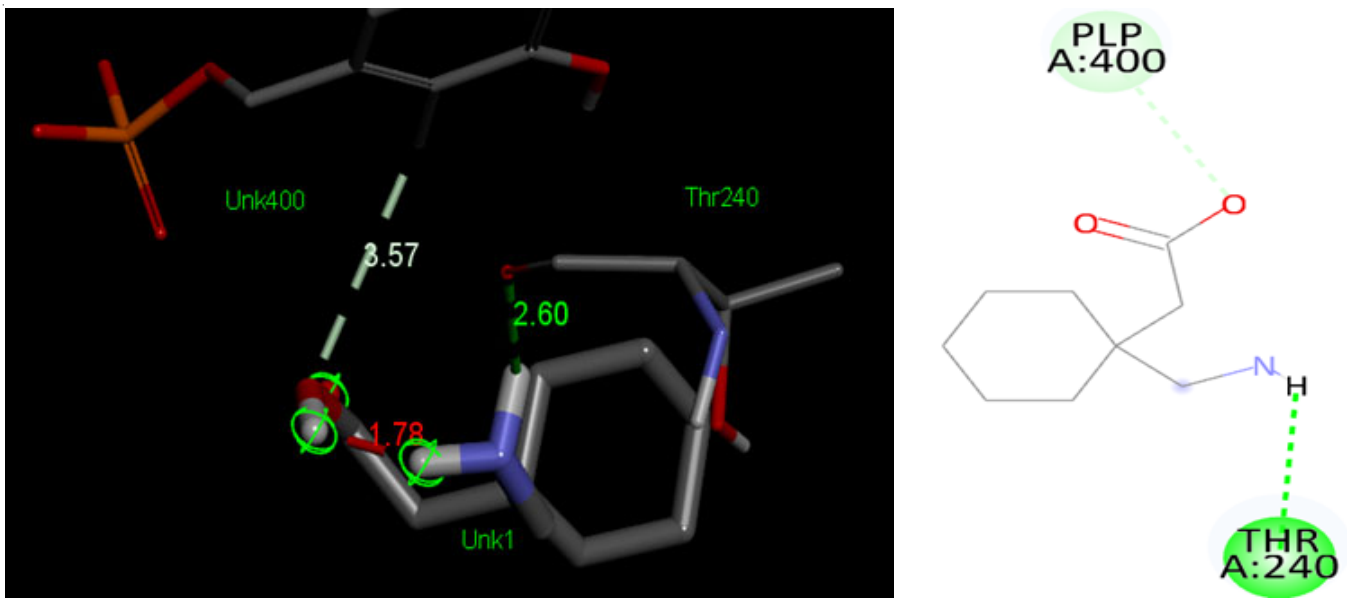


Fig. 2. Interaction of gabapentin (standard) with amino acids Thr240A(2.60)*; Plp400A(3.57)

48, **51**, **54**, **56** were found to be potent outcome for anti-convulsant activity. Compounds **11**, **25**, **39**, **56** showed excellent H-bond interactions with protein active site (Figs. 3-6). Among which compound **11** showed the outstanding interactions with acceptable bond length 2.34; 2.57; 2.62; 3.03 Å. The molecular docking results with binding free energy calculations are depicted in Table-1.

Conclusion

The results obtained demonstrated that all examined ligands have comparable orientation and position inside

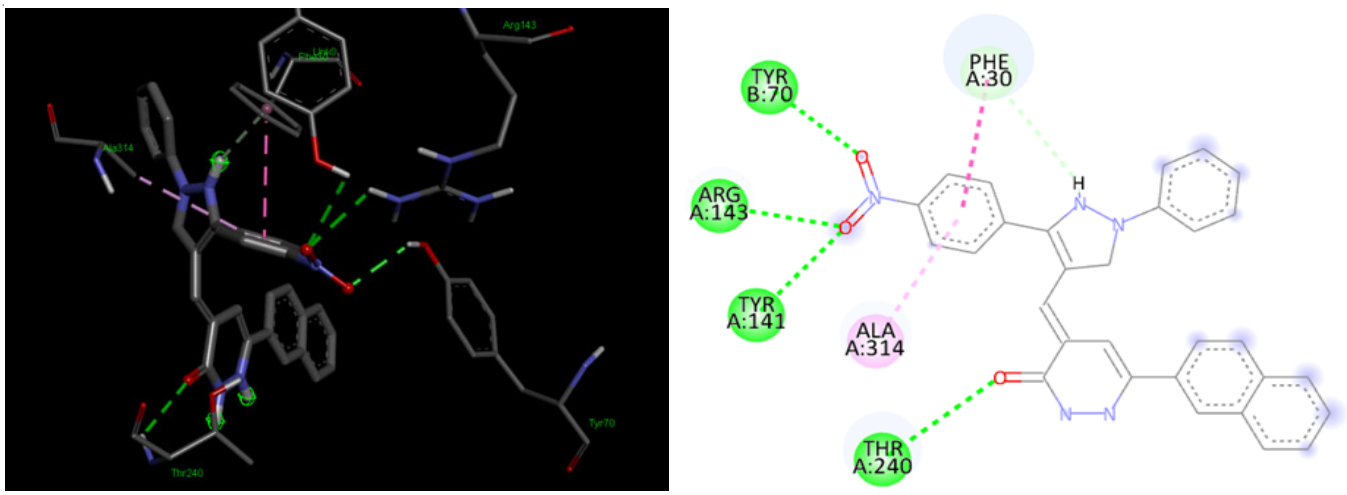


Fig. 3. Interaction of compound **11** with amino acids Tyr70B(2.34)*; Arg143A(2.57)*; Tyr141A(2.62)*; Thr240A(3.03)*; Phe30A(2.79); Ala314A(4.61)

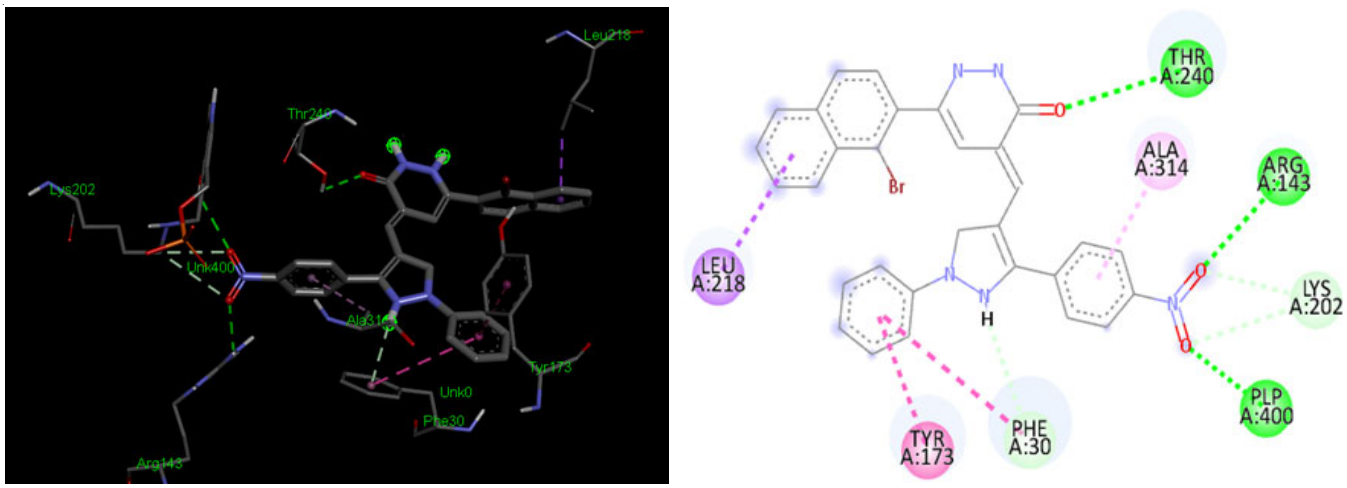


Fig. 4. Interaction of compound **25** with amino acids Thr240A(2.70)*; Ala314A(4.83); Arg143A(2.64)*; Lys202A(3.23); Plp400(2.70)*; Phe30A(2.80); Tyr173A(4.85); Leu218A(3.80)

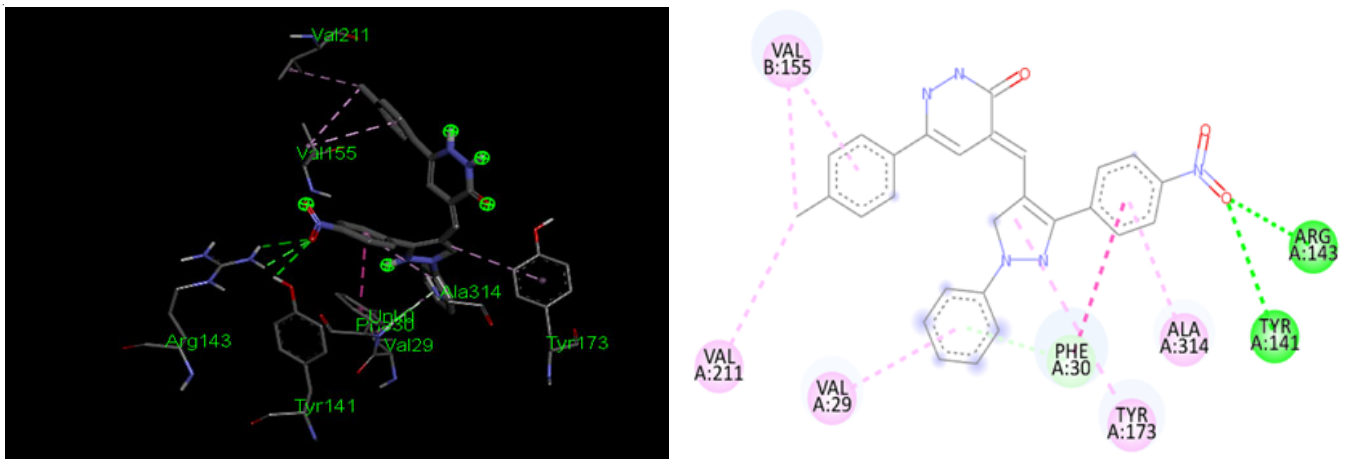


Fig. 5. Interaction of compound **39** with amino acids Arg143A(2.56)**; Tyr141A(2.84)*; Ala314A(4.76); Tyr173A(5.07); Phe30A(2.90); Val29A(4.75); Val211A(4.43); Val155B(4.25)

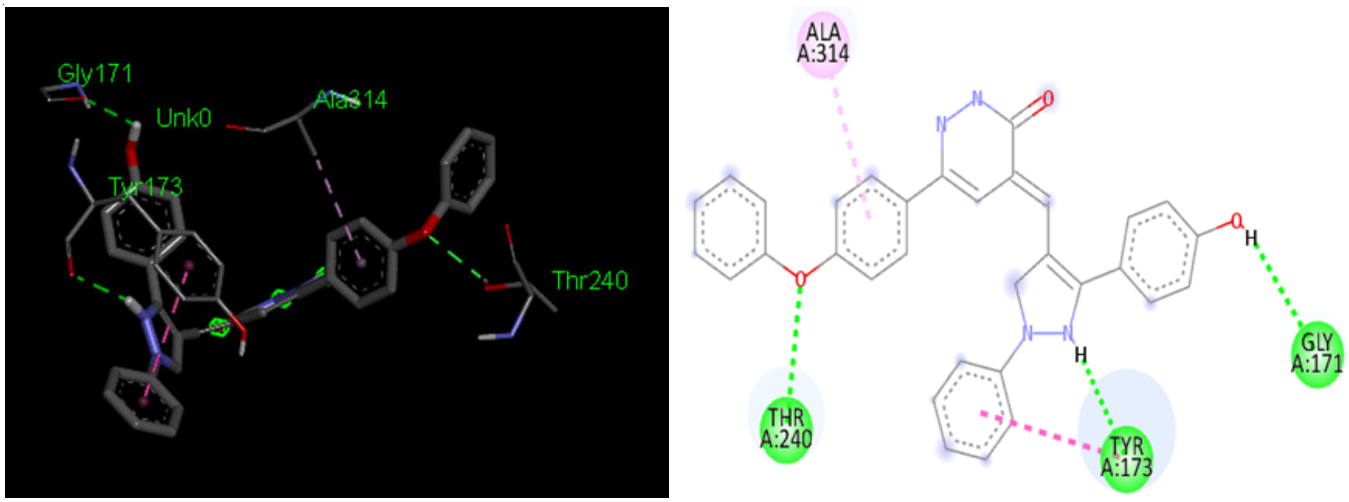


Fig. 6. Interaction of compound **56** with amino acids Gly171A(2.47)*; Tyr173A(2.27)*; Thr240A(2.57)*; Ala314A(4.62)

putative binding site of human cytosolic branched chain amino transferase protein (PDB ID-2A1H) which serves as passage channel for substrate to active site. There is strong connection between affinity of ligand towards protein and binding free energy which can contribute in understanding and interpreting the activity of ligand by various possible mechanisms. Also, the geometry of ligand-receptor complex assumes fundamental part in forming drug activity. This *in silico* study also provides great idea to the researchers looking for novel acting agents for this CNS disorder. It may be well suggested that 2A1H protein might be tighly engaged with pyridazinone analogues bearing benzylidene substituents and aromatic ring accordingly and based on data, the ultimate compounds are need to be synthesized for further exploration.

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