



Molecular-Docking Study of Pyrimidine analogues against GABA_A Receptor used to design New Antiepileptic agents

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Abstract

Nowadays, a lot of new active substances as antiepileptic agents have been developed. One of the protein targets of antiepileptic is selective gamma-aminobutyric acid (GABA). Selective GABA is the regulator of the central nervous system (CNS) activity. In this research, pyrimidine derivatives were used to design the antiepileptic agent through a selective GABA activation. The potential activity of pyrimidine derivatives could be increased by substitution. The molecular docking of selective GABA activation was required to predict their antiepileptic activity. The molecular docking of pyrimidine derivatives was carried out using AutoDockVina ver.1.1.2. Twenty pyrimidine derivatives were docked into GABA_A with Protein Data Bank (PDB) code 4cof. The interaction was evaluated based on the docking score. Diazepam was used as the reference standard for this research. Twenty pyrimidine derivatives showed the approximate docking score of -6.1 to -8.4 kcal/mol. All twenty pyrimidine derivatives which value that have a greater docking score compared to diazepam used as a standard compound. Derivative P-17 had higher binding energy than other pyrimidine derivatives because it has the smallest docking score. All new pyrimidine derivatives are feasible to synthesize and performed there in vitro evaluation.

Introduction

A convulsion (seizure) is an abnormal event that results from a sudden change in the electrical function of cells in the brain. Epilepsy is a physical condition caused by sudden, brief changes in brain physiology and characterized by the recurrence of seizures. More than 50 million people worldwide have epilepsy, making it one of the most common neurological diseases universally. Approximately 2% of the population suffers from periodic epilepsy, of which around 70% develop it before the age of 18 years. Overall 44% of total cases report before age of 5 years, while 10% of the population experience one seizure in their lifetime.^[1-6] For the treatment of epilepsy, the most commonly preferred antiepileptic drugs help to get relief from the associated symptoms but these drugs could not treat epilepsy completely. Antiepileptic drugs mainly act on ion channels, receptors responsible for opening and closing ion channels like GABA, glutamate, and synthesis and function of neurotransmitters. Therapeutic efficacy of the antiepileptic drug is

overcome by some unwanted side effects such as gastrointestinal disturbance, gingival, drowsiness, ataxia, megaloblastic anemia, hyperplasia, hirsutism etc.[7] In addition, about 30% of patients are refractory to these treatments. In view of the above observations, there is an urgent need to find new anticonvulsant compounds with more selectivity and lower side effect profile. The development of heterocycles as scaffolds, containing a high degree of diversity has become a leading focus in modern drug discovery. Certain possible modifications to the heterocyclic ring by the addition of diverse substituents may lead to new products with better pharmacological profiles. Nitrogen heterocycles are among the most privileged molecular scaffolds of pharmaceuticals, in which pyridine is an important milestone that is present in US Food and Drug Administration (FDA) approved pharmaceuticals. Therefore, it is urgent for researchers to design and discover new drug molecules that possibly offer some of the greatest hopes for success in the present and future era.

Most of the pharmaceutical compounds are based upon heterocycles. An examination of the structures of the highly marketed brand name of the medicines in 2007 discloses that 8 drugs out of the 10 and 71 drugs out of the top 100 drugs belong to the heterocyclic compounds. This is not amazing as heterocycles have dominated medicinal chemistry from the start. Therefore, most of the U.S. Patents through pharmaceutical agencies contain heterocyclic compounds consistent with their importance.[8]

Pyrimidine nucleus exhibited incredible pharmacological properties. Literature indicates that the compounds having pyrimidine nucleus have a broad range of therapeutic activities that include anti-inflammatory, antimicrobial, anticancer, antiviral, anti-HIV, antiprotozoal, antihypertensive, sedative-hypnotics, anticonvulsant and antiallergic. According to Medicinal Chemistry pyrimidine derivatives are very well known for their pharmacological activities. The presence of a pyrimidine nucleus in thymine, cytosine, and uracil, which are important binding blocks of nucleic acids, DNA, and RNA is one important reason for their pharmacological activity. The study indicated that the compounds containing pyrimidine nucleus having a wide range of therapeutic activities, like 5-fluorouracil act as antineoplastic; idoxuridine and trifluridine act as antiviral; zidovudine and stavudine as anti-HIV; trimethoprim, sulfamethazine, and sulphadiazine as antimicrobial; sulphadoxine as antiprotozoal and antimicrobial; minoxidil and prazosin (alpha-adrenergic blocking agent) as antihypertensive; barbiturates e.g. sodium

thiopental as a sedative, hypnotics and anticonvulsant; propylthiouracil as antithyroid; thionylamine as H₁-antihistamine; and toxoflavin and fervennuline as antibiotics.

As a result of the incredible pharmacological activity of pyrimidine derivatives, intensive research has been focused on the anti-inflammatory drug activity of the pyrimidine nucleus. The present work highlights the anticonvulsant activity of pyrimidine and its derivatives.^[9]

Although the exact mechanisms of action of pyrimidine and its derivatives remain unknown, a study in epilepsy indicated that pyrimidine can enhance GABA action. This study's main objective was to examine the pyrimidine derivatives and GABA interaction and identify the importance of GABA activation in epilepsy. Docking analysis was also performed to define the residues involved in pyrimidine binding and down regulatory action on GABA

Material and Methods

Preparation of target protein x-ray structure:

The crystal structure of the human gamma-aminobutyric acid receptor, the GABA (A) R-beta3 homopentamer (PDB ID: 4COF) was selected as the target protein downloaded from <http://www.pdb.org/>.

Design of new Pyrimidin-4(3H)-one derivative:

The role of the new drug development is (i) determining pharmacophore, (ii) manipulating the substituent of pharmacophore (iii) determine the list of new substituents. In this study, Pyrimidin-4(3H)-one is pharmacophore as the anticonvulsant agent. The various substituents selected for designing new compounds consist of -NH₂, -OCH₃, -Cl, -CH₃, -CF₃, -NO₂, -CH(CH₃)₂ etc.

Ligands preparation:

The structures of Pyrimidin-4(3H)-one derivative were drawn by using Chem Draw Ultra 8.0 (Cambridge Soft). The 2D structures of compounds were transformed to the 3D structure using Chem 3D Ultra 8.0. The optimization of molecules and minimization geometry of the ligands was performed using the semi-empirical PM3 method and applying a termination RMS gradient of 0.001 KCal/mol for maximum up to 1000 iterations and saved as PDB format, to be read by the AutoDockvina program.

Molecular Docking Studies

Molecular docking is an attractive scaffold to understand biomolecular drug interactions for the rational drug design and discovery, as well as in the mechanistic study by placing a molecule into the favored binding site of the target-specific region of the target protein mainly in a non-covalent way to form a stable complex of more specificity and potential efficacy. The study of pyrimidine analogs and GABA interaction was evaluated using molecular docking techniques on AutoDockvinaVersion 1.1.2. We used the crystal structure of human GABA_A (code 4COF, <http://www.pdb.org/>) as the target protein. Prior to screening the ligands, the docking protocol was validated by re-docking 4COF ligand into its binding pocket within the GABA_A crystal to obtain the docked pose and rootmean-square distance (RMSD).

Results and Discussion

Virtual screening experiments are the most convenient way to incorporate protein in the docking process by performing docking, using an ensemble of static receptor conformations. Molecular docking is used in modern drug design to help understand the interaction between ligands and receptors. These techniques are supported to the design of novel drug which has specific activity by the mechanism of drug-receptor interaction. Computer-aided drug design (CAAD) helps to identify small molecules by orienting and scoring them in the active binding site of a protein. The docking simulation technique was performed by using AutoDockVina version 1.1.2 with pyrimidine derivatives and they were docked with GABA_A as protein target. This program selected the best docked based on two criteria, such as, ligand binding position and fitness function scores comparison. The parameter to identify the best ligand binding position was the RMSD.

A docking score is a value that reflects the binding energy required to form a bond between the ligand and receptor, which predicts the activity of compounds. It also causes the bond between the ligand and the receptor to be more stable. The binding energy values pyrimidine analogs are shown in Table 1.

Pyrimidin-4(3H)-one derivative:

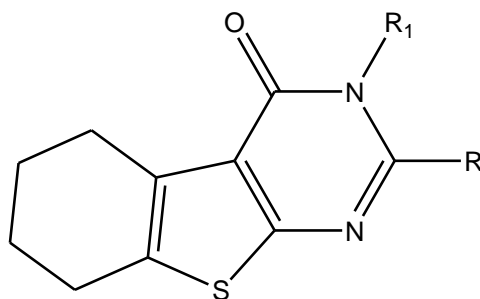
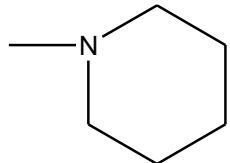
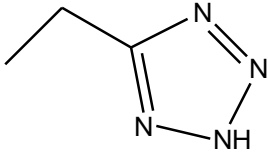
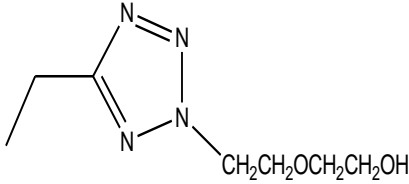
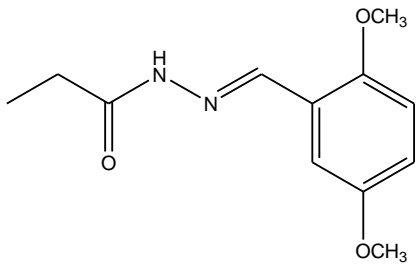
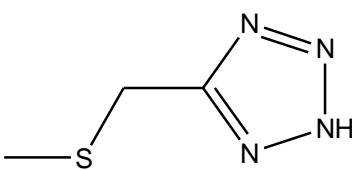
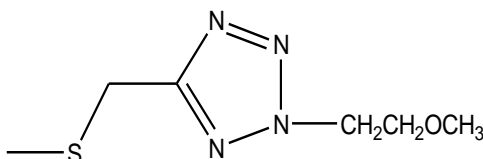
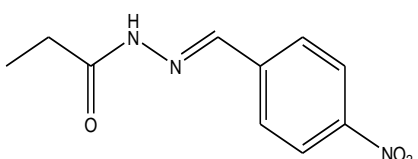
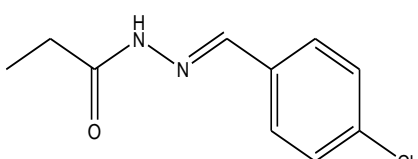
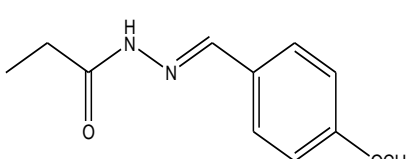
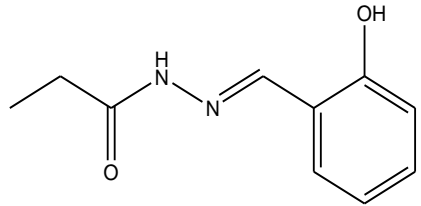
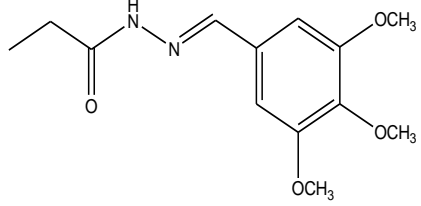
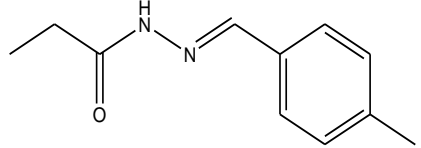
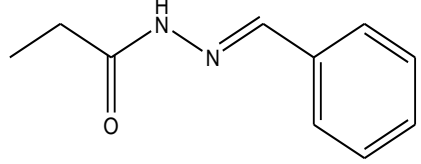
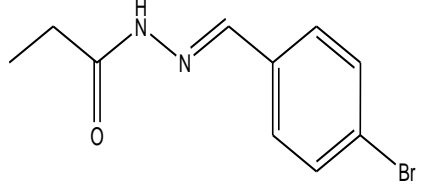


Fig.1: General structure of Pyrimidin-4(3H)-one derivative

Table 1: Docking score of Pyrimidine derivatives with GABA

Ligand	R	R1	Docking Score
P-1	-S=	H	-6.7
P-2	-S-C ₂ H ₅	H	-7.1
P-3		H	-7.8
P-4	-S-C ₂ H ₅	-CH ₂ CN	-7.2
P-5	-S-C ₂ H ₅		-6.8
P-6	-S-C ₂ H ₅		-7.1
P-7	-S-CH ₂ -CN	H	-6.1

P-8	-S-CH ₂ -CN	-CH ₂ COOC ₂ H ₅	-7
P-9	-S-CH ₂ -CN	-CH ₂ CONHNH ₂	-7.8
P-10	-S-CH ₂ -CN		-7.3
P-11		H	-8.1
P-12		H	-7.1
P-13	-S-CH ₂ -CN		-7.8
P-14	-S-CH ₂ -CN		-7.7
P-15	-S-CH ₂ -CN		-8.1

P-16	-S-CH ₂ -CN		-7.1
P-17	-S-CH ₂ -CN		-8.4
P-18	-S-CH ₂ -CN		-8.1
P-19	-S-CH ₂ -CN		-8
P-20	-S-CH ₂ -CN		-8.3
	Std(Diazepam)		-7.5

All pyrimidine derivatives have hydrogen bond interaction with protein residue. One of them which has the lower docking score is compound P-17. This means it has higher binding energy to interact with the target receptor. The interaction of Diazepam in **Figure 2** and compound P-17 with GABA receptor along with hydrogen bonds are shown in **Figure 3**.

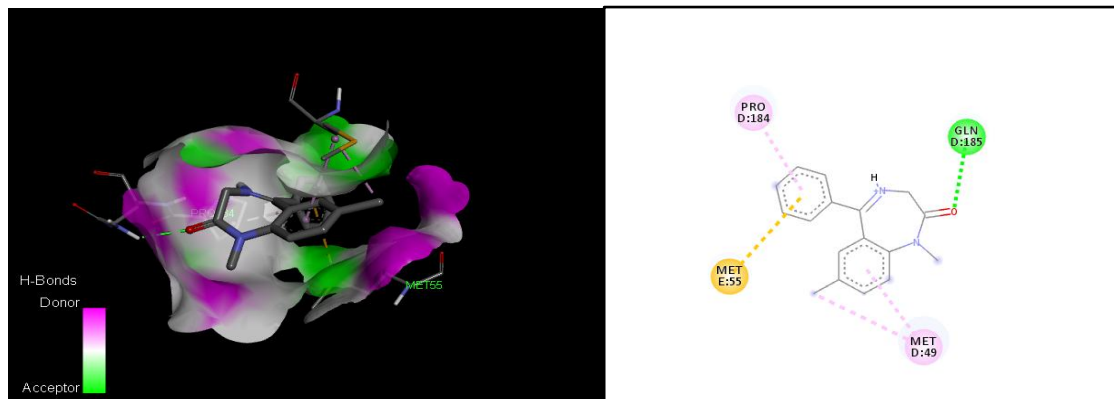


Figure 2: 3D and 2D structure of diazepam interact with GABA

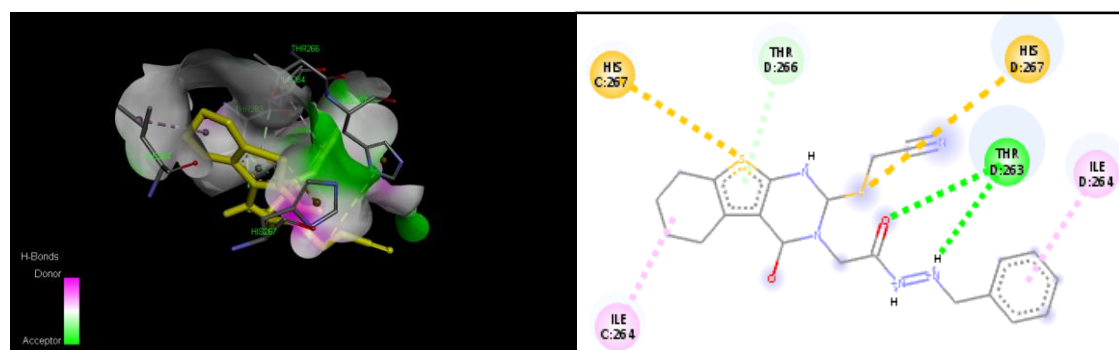


Figure 3: 3D and 2D structure of P17 interact with GABA_A receptor

CONCLUSION:

Twenty molecular structure of Pyrimidin-4(3H)-one derivatives have been docked and score obtained identify the ligands that bind to GABA_A protein structure. The result shows that ten analogues showed a higher docking score than Diazepam used as a standard compound. It means they have higher binding energy interaction with the target receptor. Therefore, these compounds could be considered as potent GABAergic molecules. For further investigation, synthesis and in vitro evaluation is required to get antiepileptic activity.

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