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Synthesis and Evaluation of Anticonvulsant Activity of Some Schiff Base of 1,4-Benzodiazepine Amine

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Abstract: A variety of 1,4-benzodiazepine azomethines (3a-n) and 1,4-benzodiazepine benzamide (4) were prepared, characterized and evaluated for the anticonvulsant activity in the rat using picrotoxin-induced seizure model. The prepared 1,4-benzodiazepine azomethine derivative emerged potentially anticonvulsant molecular scaffolds exemplified by compounds, 7-(4-nitrophenyl)methyleneamino-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (3c), 7-[4-(dimethylamino)phenyl]methyleneamino-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (3e), 7-[(4-bromo-2,6-difluoro-phenyl)methyleneamino]-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (3l) and 7-[3-(4-fluorophenyl)-1-phenyl-pyrazol-4-yl]methyleneamino-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (3n). All these four compounds have shown substantial decrease in the wet dog shake numbers and grade of convulsions with respect to the standard drug diazepam. Most active compound, 7-[4-(dimethylamino)phenyl]methyleneamino-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (3e) exhibits 74 % protection against convulsion which was higher than standard drug diazepam. Furthermore, to identify the binding mode of the interaction amongst the target analogs and binding site of the benzodiazepine receptor, molecular docking study and molecular dynamic simulation were carried out. Additionally, *in silico* pharmacokinetic and toxicity predictions of target compounds were carried out using AdmetSAR tool. Results of ADMET studies suggest that the pharmacokinetic parameters of all the target compounds were within the acceptable range to become a potential drug candidate as antiepileptic agents.

Keywords: Epilepsy; 1,4-benzodiazepines; Azomethine; Anticonvulsant; Molecular docking.

Introduction

Epilepsy is a disorder of central nervous system in which excessive and rapid neuronal discharge in the brain results in loss of consciousness and convulsions. Approximately 60 million world populations are suffering from this critical ailment, making it one of the most common neurological diseases.[1] There are many antiepileptic drugs (AEDs) available for the cure epilepsy. Depending upon the mechanism of the actions, currently available marketed AEDs classified into three major categories, which includes inhibition of sodium channel function (e.g. phenytoin, carbamazepine),[2] inhibition of calcium channel function (e.g. ethosuximide)[3] and enhancement of GABA action (e.g. benzodiazepines, barbiturates).[4, 5] Other mechanism includes inhibition of glutamate release, the block of glutamate receptor and the combination of the above actions, often coupled with an additional mechanism (e.g. valproic acid, gabapentin).[6] Despite, almost 30 % patient's compliance is not observed to currently available antiepileptic treatment, while in some critical cases multidrug therapy is required. This leads to significant side effects, like toxicity of neurons, depression, central nervous system (CNS) related disorders, cosmetic (gingival hyperplasia) as well as severe hepatotoxicity and megaloblastic anaemia.[7, 8] Therefore, there is an important necessity to develop novel antiepileptic agent with a high degree of selectivity and specificity.

In recent years, the discovery of the novel antiepileptic agents primarily focused on the reduction of seizures in symptomatic fashion. Literature survey suggests that pharmacophore-based ligand drug design is an excellent approach for the development of novel antiepileptic agents.[9] It relies on the knowledge of old and new anticonvulsant active drugs that interact to the biological target of interest. The main aim of this approach is the structural modification of known AEDs, with the acuity of designing molecules with a more efficacious and comparatively less or no critical side effect compare to available AEDs.[10] Here, we have selected benzodiazepine as active pharmacophore (**Figure 1**) for the development of novel AEDs. Benzodiazepines are one of the privileged class of nitrogen containing heteroaromatic compound in the area of pharmaceuticals and mainly acting on the central nervous

system. Among the benzodiazepines, 1,4-benzodiazepine derivatives are the most abundant and have a well time-honoured position in antiepileptic therapy. [11, 12] In addition to their established anticonvulsant activity, [13] 1,4-benzodiazepine also demonstrates many other important biological activities such as, anti-anxiety, [14] hypnotic, [15] NNRTI of HIV-1 reverse transcriptase, [16–18] anti-arrhythmic agents, [19, 20] antiproliferative, [21] anti-platelet anti-ulcer, [22] vasopressin antagonist activity [23] as well as anti-inflammatory agents. [24] The early structure-activity relationship (SAR) study [25, 26] indicated that the potency of benzodiazepine is mainly determined by substitution at 7th position, whereas any substitutions at positions 6, 8 and 9 may decrease the activity. SAR study also promotes the presence of carbonyl function at 2nd position, phenyl ring at 5th position, the presence of double bond between 4th and 5th position in diazepine ring **B** and no alkyl substitution or a less bulky alkyl group at N-1, as shown in **Figure 1**. On the other hand, compounds containing azomethine group in the structure are known as Schiff bases represents a vital class of medicinally important compounds. [27–29] Thus, a strong hypothesis was proposed that combination of 1,4-benzodiazepine as pharmacophore along with azomethine at the C7-position would also display potent anticonvulsant effects.

Thus, we have decided to react 7-amino nitrazepam (**2**) with substituted aryl aldehydes **8a-n** to form a library of Schiff bases (**Figure 2**) and evaluate their anticonvulsant activity. Though some Schiff bases of 7-amino nitrazepam (**2**) known in the literature, [30] those were never evaluated for anticonvulsant activity.

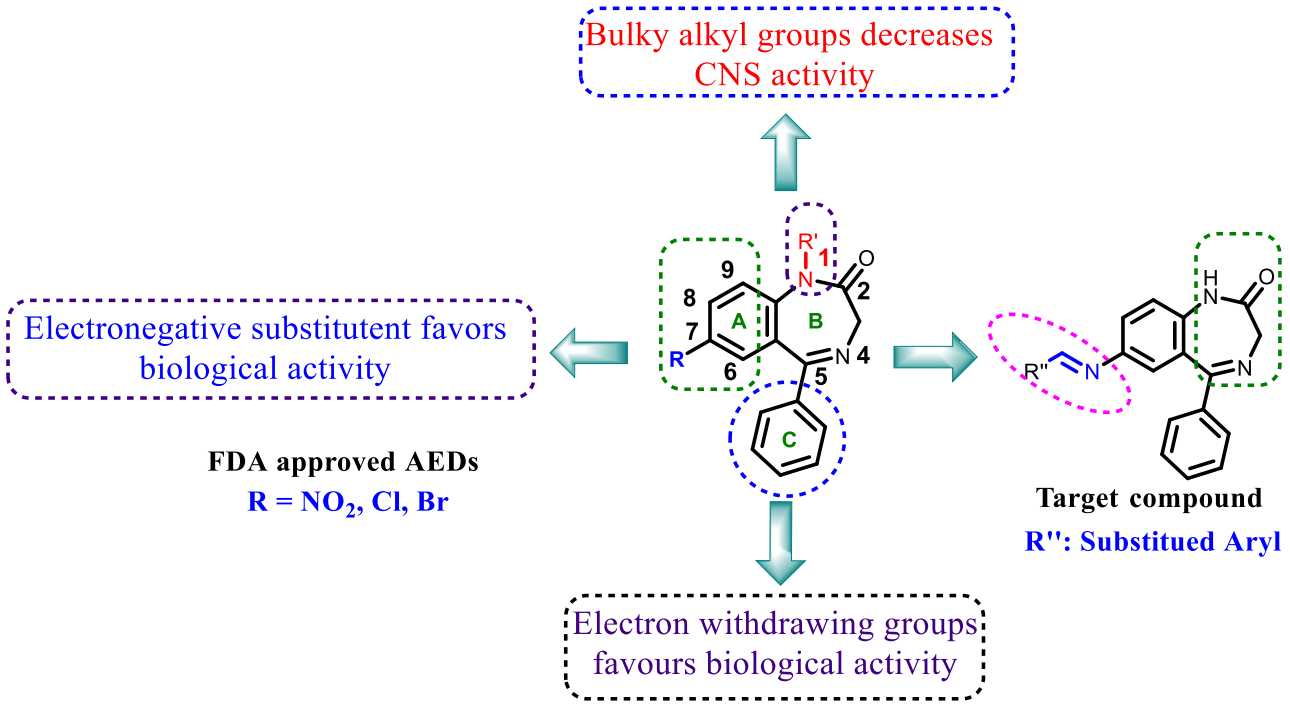


Figure 1 Molecular hybridization of 1,4-benzodiazepine and azomethine having drug potency.

In this research work, we have reported rational design, synthesis and anticonvulsant activity of some 1,4-benzodiazepine azomethine analogue **3a-n** and a benzamide derivative of 1,4-benzodiazepine **4**. Further, molecular docking of the synthesized compounds was carried out on the $GABA_A$ receptor to identify the binding mode of the interactions. Moreover, *in-silico* ADMET and the molecular property predictions of all the derivatives were reported. Based on the information gathered from pharmacological activity and molecular docking, SAR was summarized and potential lead molecules were identified.

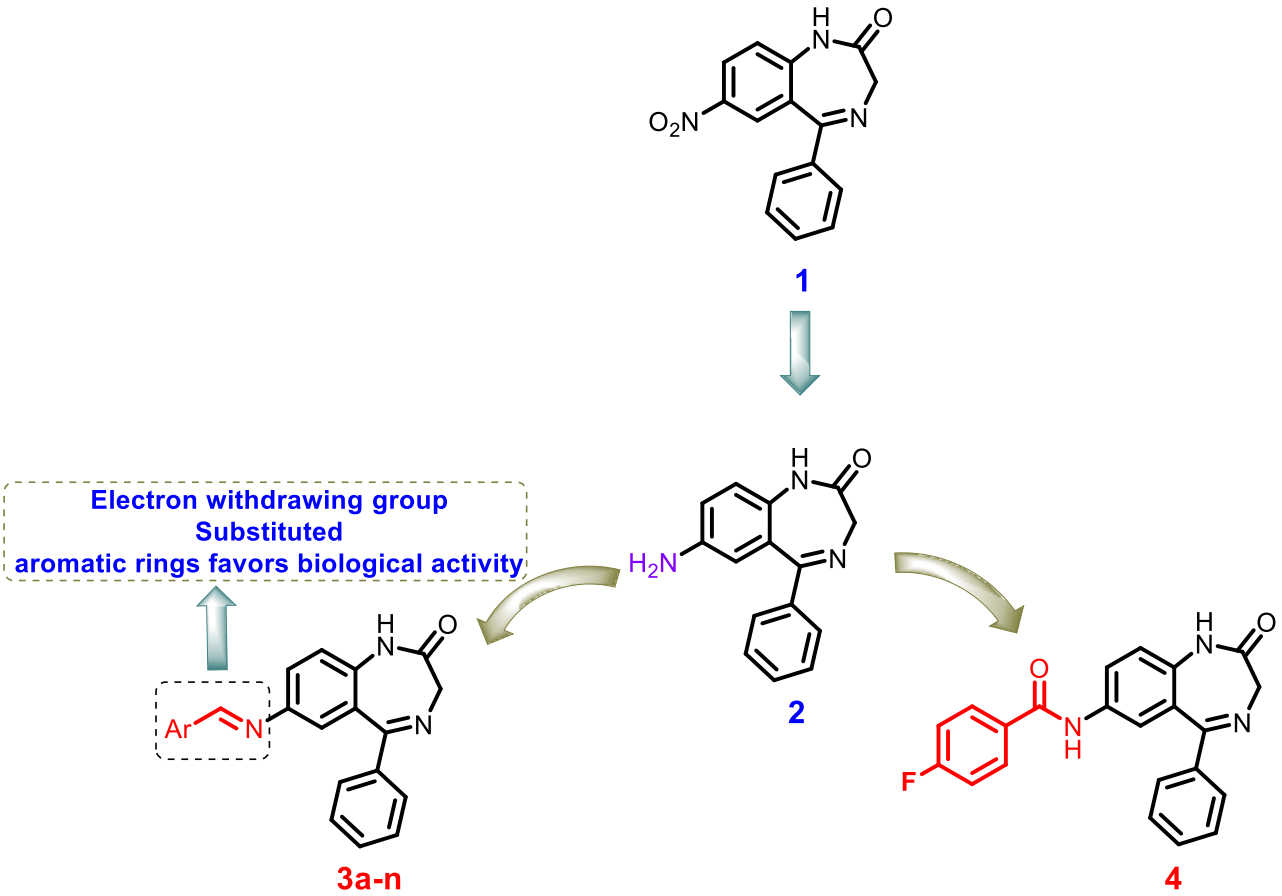
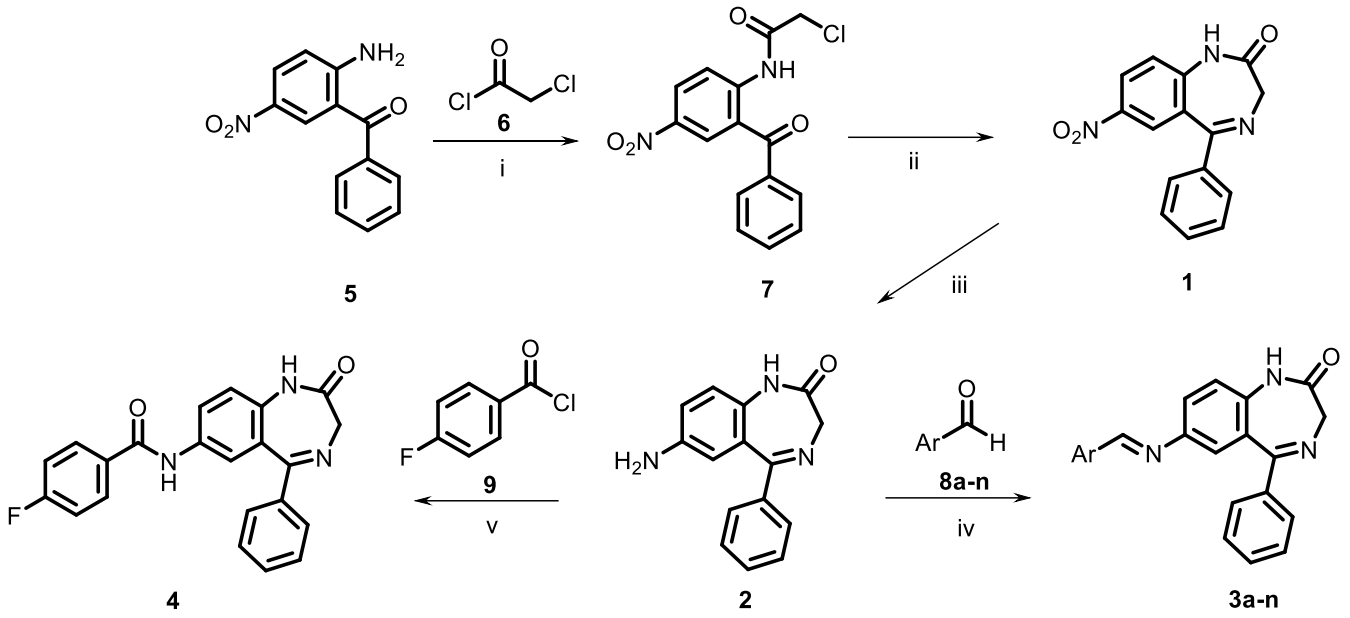


Figure 2 Design strategies for the synthesis of azomethine derivative of 1,4-benzodiazepine as anticonvulsant agents.

Results and Discussion

Chemistry

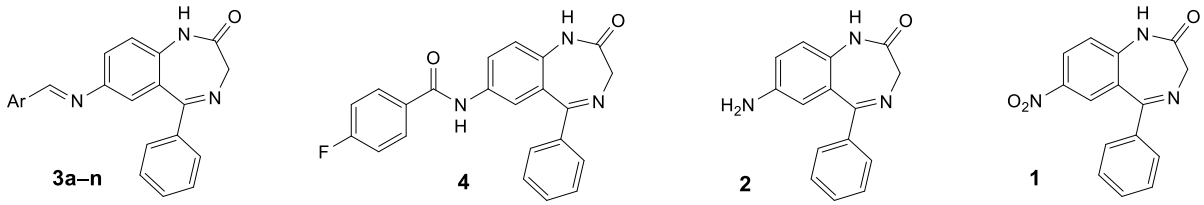
Target compounds **3a-n** and **4** were synthesized according to **scheme 1**. Synthesis of the key building block 7-amino nitrazepam (**2**) was achieved in three steps starting from commercial 2-amino-5-nitro-benzophenone (**5**). Acylation of 2-amino-5-nitro-benzophenone (**5**) with chloroacetyl chloride **6** in toluene at 110 °C afforded 2-chloroacetamido-5-nitrobenzophenone (**7**). [31] Amide **7** in the presence of hexamethylenetetramine (HMTM) and NH_4OAc in absolute ethanol at 78 °C gave nitrazepam **1**. [32] Reduction of nitrazepam **1** with $SnCl_2.H_2O$ in EtOH ensued in the formation of 7-amino nitrazepam (**2**). [33] Azomethine derivative of 1,4-benzodiazepine **3a-n** were synthesized by the reaction of 7-amino nitrazepam (**2**) and appropriate aldehydes **8a-n** in ethanol at 78 °C with catalytic amount of glacial acetic acid. Amide **4** was obtained by the coupling of 7-amino nitrazepam (**2**) and 4-fluoro benzoyl chloride (**9**) in the presence of triethyl amine in CH_2Cl_2. The structures of the target compounds were characterized by spectral methods. The physicochemical data of synthesized analogues are depicted in **Table 1.**



8a, **3a**; Ar = C_6H_5, **8b**, **3b**; Ar =4-FC_6H_4, **8c**, **3c**; Ar = 4-$NO_2C_6H_4$, **8d**, **3d**; Ar = 4-$CH_3CO_2C_6H_4$
8e, **3e**; Ar = 4-$N(CH_3)_2C_6H_4$, **8f**, **3f**; Ar = 2-F,3-BrC_6H_3, **8g**, **3g**; Ar = 2-F,4-BrC_6H_3
8h, **3h**; Ar = 2-Cl,4-BrC_6H_3, **8i**, **3i**; Ar = 2-F,4-$CF_3C_6H_3$, **8j**, **3j**; Ar = 3-NO_2,4-FC_6H_3
8k, **3k**; Ar = 2,4,6-FC_6H_2, **8l**, **3l**; Ar = 2,6-F,4-BrC_6H_2, **8m**, **3m**; Ar = 1-naphthyl,
8n, **3n**; Ar = 3-(4-FC_6H_4)-1-C_6H_5-1H-pyrazole

Scheme 1 *Reagents and conditions*: (i) Toluene, 110 °C, 2 h, 98 %; (ii) HMTM, NH_4OAc, EtOH, 78 °C, 6 h, 71 %; (iii) $SnCl_2.2H_2O$, EtOH, ultrasonicated, 24 °C, 2 h, 90 %; (iv) EtOH, cat. Glacial CH_3COOH, 78 °C, 1-6 h; (v) Et_3N, CH_2Cl_2, 24 °C, 2 h, 65 %.

Table 1 Physicochemical data to assess Lipinski's rule of five and BBB permeability of the synthesized compounds.



Compound	M.W.[a]	Log *P*[b]	*BBB Permiability*[c]	*HBD*[d]	*HBA*[e]
3a	339.14	4.05	0.9931	1	3
3b	357.13	4.21	0.9952	1	4
3c	384.12	2.93	0.9982	1	4
3d	397.14	3.87	0.9580	1	5
3e	382.18	4.33	0.9641	1	4
3f	435.04	5.04	0.9914	1	4
3g	435.04	5.04	0.9914	1	4
3h	451.01	5.44	0.9903	1	3
3i	425.12	5.13	0.9911	1	7

3j	402.11	3.06	0.9750	1	5
3k	393.11	4.52	0.9926	1	6
3l	453.03	5.19	0.9914	1	5
3m	389.15	5.05	0.9931	1	3
3n	499.18	6.31	0.9945	1	6
4	373.12	3.15	0.9920	2	4
2	251.10	1.38	0.9844	2	3
1	281.08	1.56	0.9802	3	6

[a] Molecular weight of the compound, [b] partition coefficient, [c] Blood brain barrier permeability, [d] Hydrogen bond donor, [e] Hydrogen bond acceptor

Pharmacology

All the synthesized 1,4-bezodiazepine (BZD) derivatives were screened for the anticonvulsant property in PTZ induced seizure model in rats. This test was used to identify compounds that decrease the seizure threshold. Here, time of onset of convulsions, time of onset of tonic clonic convulsions and convulsion grades were recorded in rat for all the target compounds.

Anticonvulsant activities of synthesized compounds (**3a-n, 4 and 2**)

Effect of BZD derivatives in Time of onset of Convulsions

There was a significant increase ($p<0.001$) in time of onset of convulsions of compound **3b** to **3n** and **4** treated groups similar to standard drug diazepam as compared to the convulsive control group. However, there was no change in the onset of convulsions in **3a** and **2** treated groups. In the current study (**Figure 3(a)**), it was detected that compounds **3c**, **3e**, **3l** and **3n** display a significant enhancement of time of onset of convulsions as compared to

convulsive control.

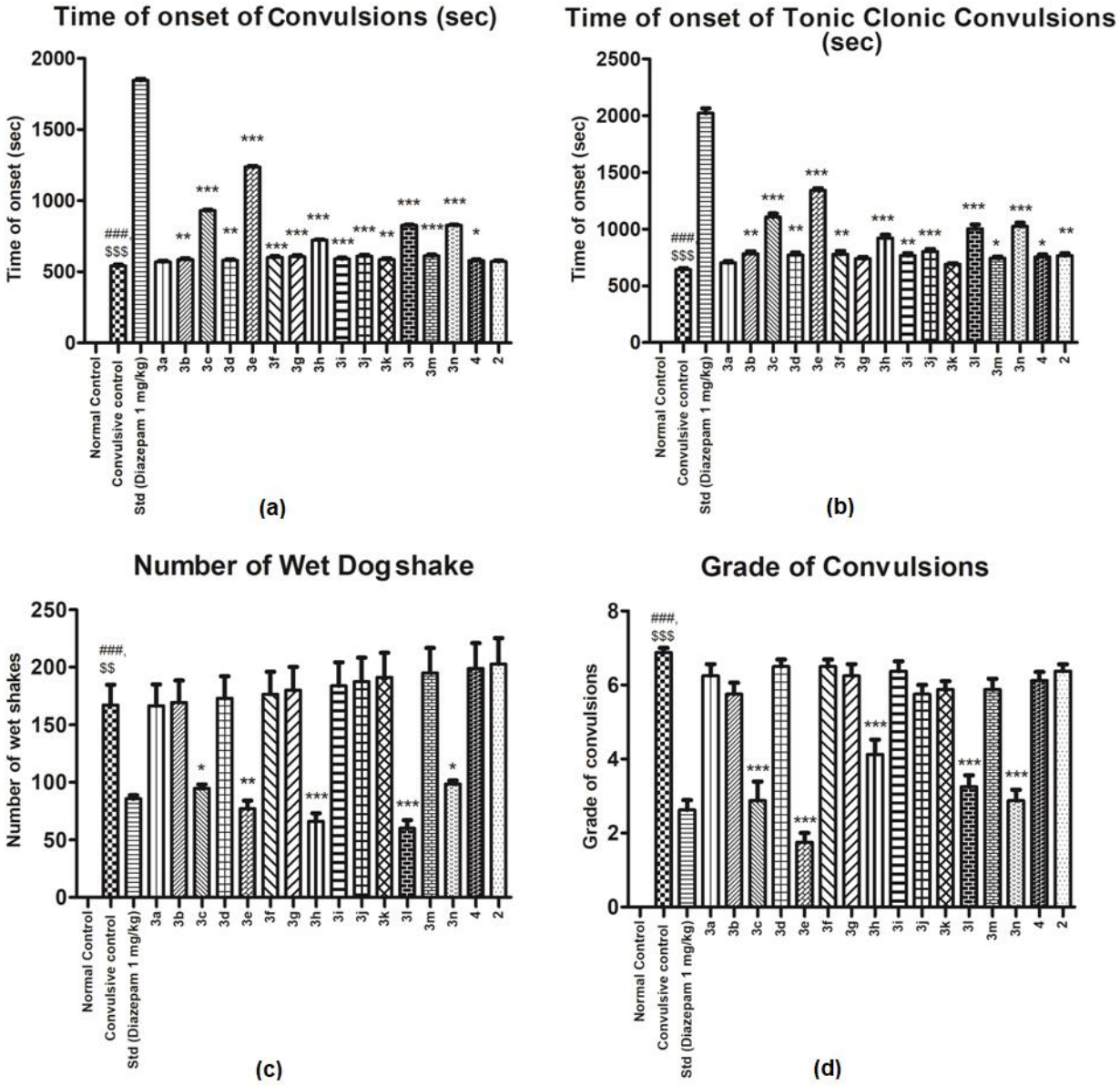


Figure 3 a) Effect of compounds **3a**-**3n, 4, 2** and standard drug diazepam on time of onset of convulsions in PTZ induced seizure model in rat. **b**) Effect of compounds **3a**-**3n, 4, 2** and standard drug diazepam on time of onset of tonic clonic convulsions in PTZ induced seizure model in rat. **c**) Effect of compounds **3a**-**3n, 4, 2** and standard drug diazepam on number of wet dog shake in PTZ induced seizure model in rat. **d**) Effect of compounds **3a**-**3n, 4, 2** and standard drug diazepam on grade of convulsions in PTZ induced seizure model in rat. (Values are represented as mean ± SEM, n = 8, One-way ANOVA followed by Dunnet's 't' test, $$p<0.01, $$$p<0.001 when compared to Normal control, *p<0.05, **p<0.01, ***p<0.001 when compared to Convulsive control).

Effect of BZD derivatives in Time of onset of Tonic-Clonic convulsions

There was a significant increase (p<0.001) in time of onset of tonic-clonic convulsions in the compounds **3a** to **3n, 4** and **2** treated group similar to standard drug diazepam as compared to the, convulsive control group. Further, (**Figure 3 (b)**), it was observed that derivatives **3c, 3e, 3l, 3n** show a significant increase in the time of onset of tonic-clonic convulsions as compared to the convulsive control group.

Effect of BZD derivatives in wet dog shake

There was a significant decrease (p< 0.01) in wet dog shake numbers in the compounds **3c, 3e, 3h, 3l,** and **3n** treated groups similar to standard drug diazepam treated group as compared to the convulsive control group. However, no change was noticed in the wet dog shakes in other BZD derivative treated groups (**Figure 3 (c)**). Further, it was noted that there are compounds **3e, 3h** and **3l** shown superior activity as compared to standard drug diazepam.

Effect of BZD derivatives in Grade of Convulsions

There was a significant decrease (p<0.01) in the grade of convulsions in the compound **3c, 3e, 3l** and **3n** treated group similar to standard drug diazepam treated the group as compared to the convulsive control group. However, there was no change in the grade of convulsions in other BZD derivative treated groups (**Figure 3(d)**). From the entire set of compounds, **3e** shows the most potent activity as compared to standard drug diazepam. In the current

research work, it is evident that BZD derivatives specifically **3c, 3e, 3l** and **3n** were able to ameliorate the epileptic seizures induced by PTZ in laboratory animals.

Here, in PTZ-induced acute clonic convulsion model, most active compound **3c, 3e, 3l** and **3n** display maximum protection against convulsion in the range of 52 % to 74 %. (**Figure 4**) While, Diazepam show 61.90 % protection against convulsion. The results of anti-convulsion activity strongly revealed compound **3e** show better activity compare to diazepam with 74.40 % protection against convulsion.

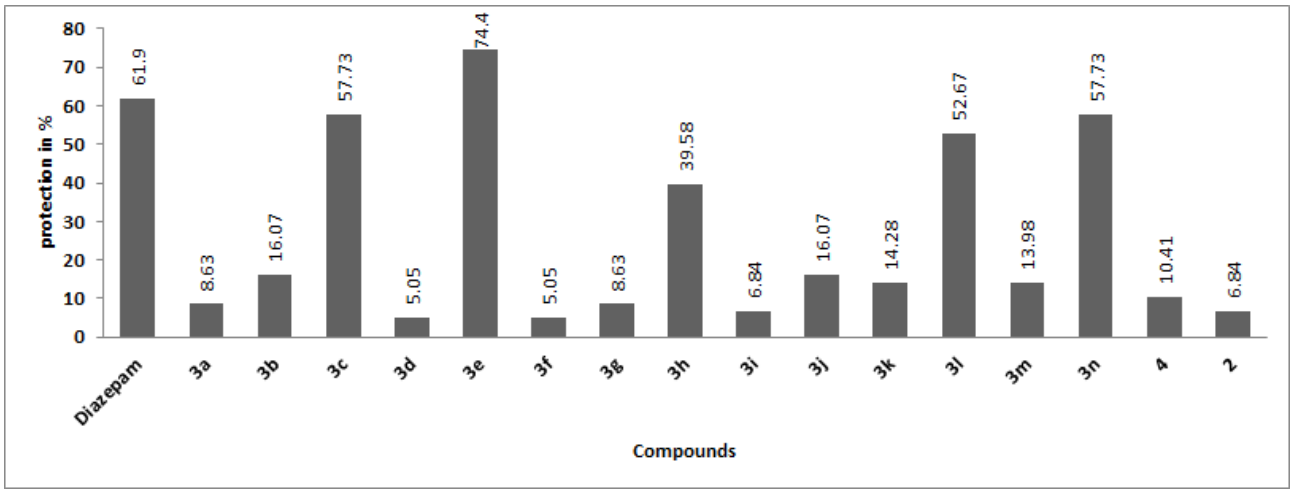


Figure 4 Anticonvulsant activity of the tested compounds using PTZ model.

Acute oral toxicity study

Notably, no significant change in respiratory, reflexes, ocular signs, muscle tone, skin, cardiovascular and gastrointestinal parameters was observed in **3a-3n, 4, 2** treated groups with a comparison to a normal control group of animals administered with the vehicle. However, there was a considerable reduction in motor activity in **3a-3n, 4, 2** treated groups when compared to a normal control group of animals administered with the vehicle. The animals treated with compounds **3c, 3e, 3h, 3l** and **3n** at the doses of 50 mg/kg body weight has been found to have lost the righting reflex which indicated that treatment with **3c, 3e, 3h, 3l** and **3n** at 50 mg/kg caused a hypnotic effect in the rat.

In Silico molecular docking study

Molecular docking study was accomplished for the identification of the binding mode of interaction and to confirm whether the anticonvulsant activity of compounds is mediated via the BZD receptor. Using the gradient optimization algorithm and an empirical scoring function the molecular docking of most active analogue **3e** and standard drug Diazepam in the active site of $GABA_A$ receptor were carried out by AutoDockVina tool. Molecular docking score of diazepam and most active compound **3e** were found to be -8.904 and -8.265 respectively. Notably, a standard drug diazepam interacts via hydrogen bonding with Ser204 amino acid in A chain of $GABA_A$ receptor. While, core ring of diazepam interacts with amino acids namely Thr206, Ala79, Leu140, Tyr159 and Thr142 by hydrophobic interactions. In **Figure 5**, the most important residues in the binding mode of diazepam are Thr206, Tyr 209, His101, Tyr159, and Phe77 are presented. Further, π-π interactions were also observed with Tyr159 and Tyr209 amino acids respectively. Most active compound **3e** was witnessed to bind at the active site of the $GABA_A$ receptor with similar binding mode as observed in the diazepam. In compound **3e**, hydrogen bonding interaction was noticed with His101 While, hydrophobic interaction was detected with amino acids namely Leu, Ser, Asp, Thr, Val, Tyr. Further, π- π interactions were noted with Phe77, Tyr209 and Tyr159 amino acids as similar to that of standard drug diazepam. Moreover, the results of the interaction of compound **3e** at the active site of the $GABA_A$ receptor strongly revealed that it's binding pattern and mode of interaction resembles the standard drug diazepam. 2D interaction of diazepam and compound **3e** is represented in **Figure 5** respectively.

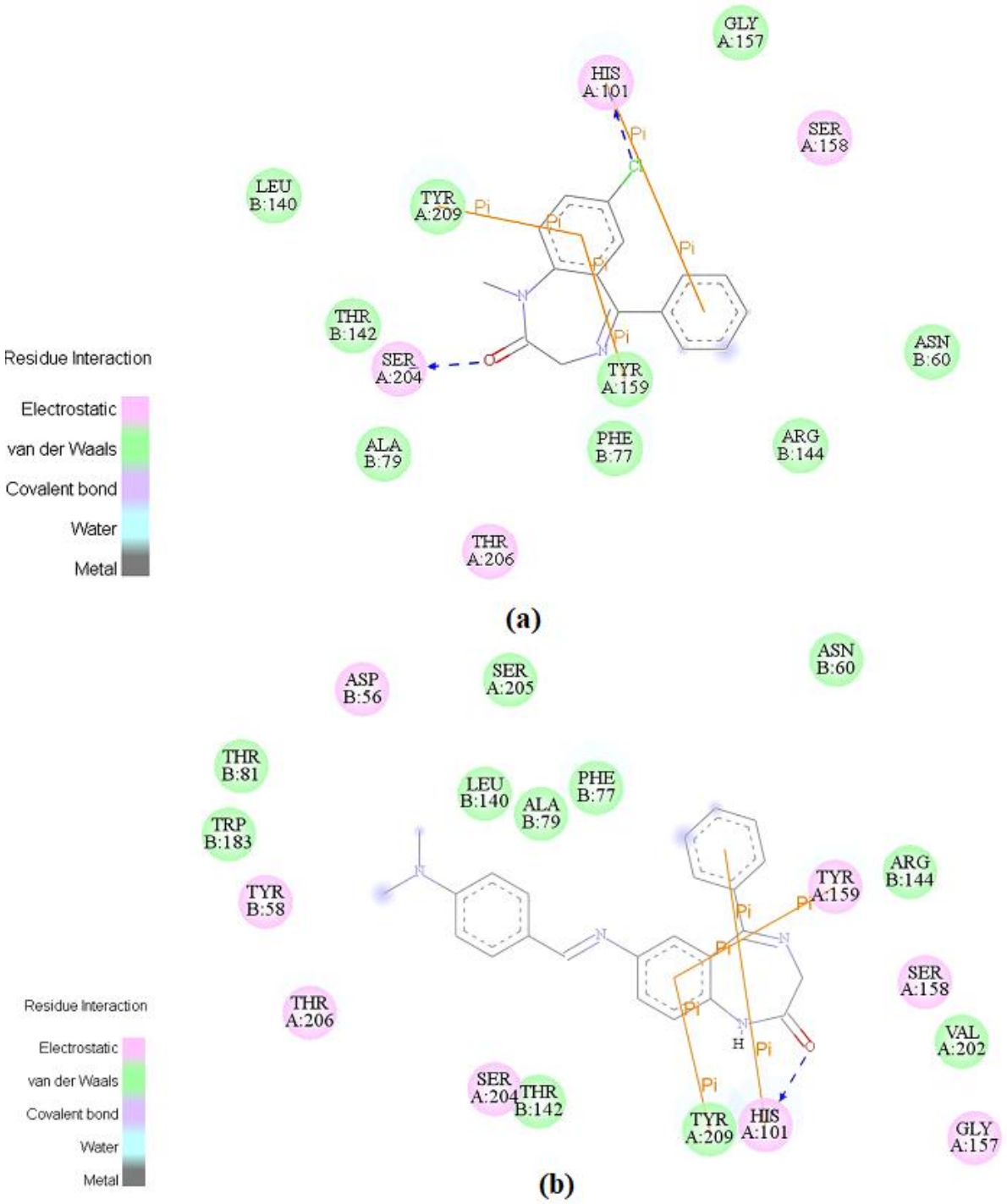


Figure 5 a) 2D interaction of diazepam with active site of $GABA_A$ receptor. **b)** 2D interaction of most active compound **3e** with active site of $GABA_A$ receptor.

Molecular dynamics simulations

Molecular dynamics study was carried out to check the stability of the most active compound **3e** and diazepam on $GABA_A$ receptor. For the analysis of trajectories of molecular dynamics simulation RMSD and radius of gyration calculated and explored. RMSD value of compound **3e** and diazepam are depicted in **Figure 6**. The RMSD trajectories clearly demonstrated that complex with compound **3e** and diazepam were equilibrated in the range of RMSD value 0.25 to 0.5 nm. The results of RMSD value indicate that ligand was not fluctuating after binding to the active site of receptor. Further, binding pattern of most active compound **3e** and diazepam was almost similar. Further, the rigidity of protein-ligand complexes can be explained using the radius of gyration (Rg) parameter obtained from MD simulation trajectories. The Rg values were recorded from the trajectories and plotted against time and depicted in **Figure 7**. Rg trajectories in **Figure 7** suggest that all system was little bit fluctuating during the simulation process in the case of compound **3e** as well as diazepam.

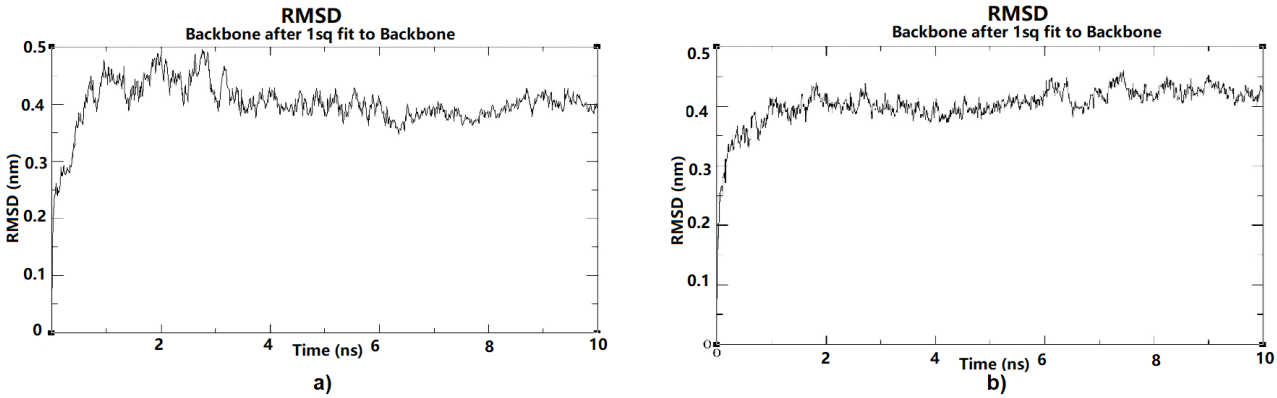


Figure 6 a) RMSD vs. time of protein-ligand complexes for compound **3e b**) RMSD vs. time of protein-ligand complexes for diazepam

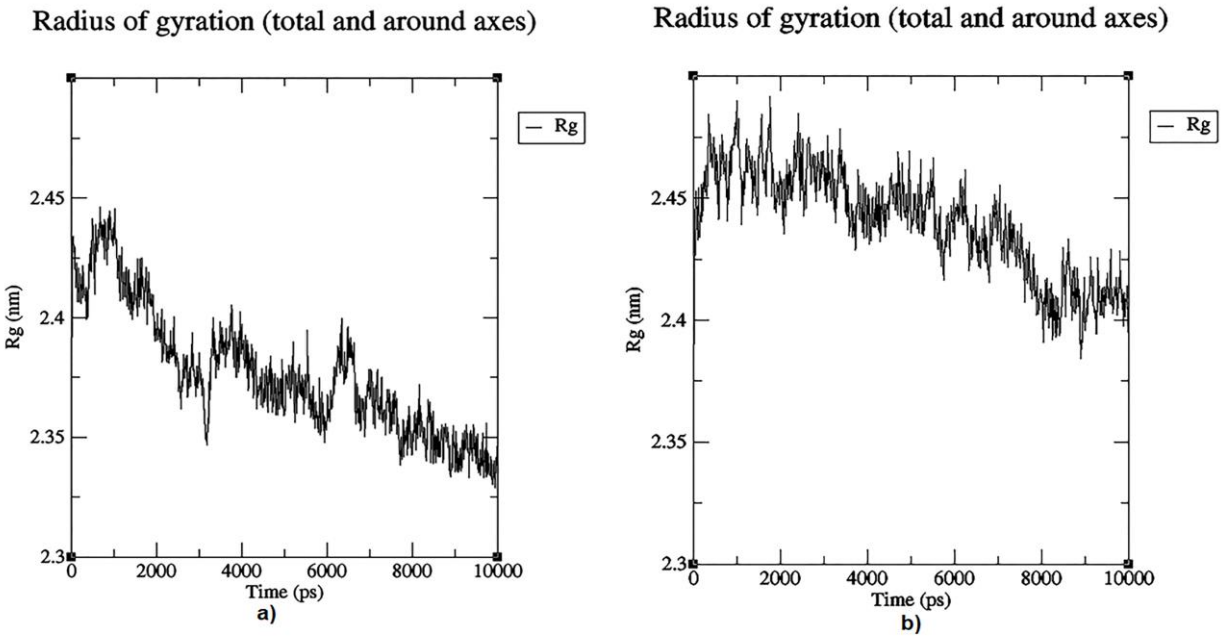


Figure 7 a) Radius of gyration vs. time of protein-ligand complexes for compound **3e b**) Radius of gyration vs. time of protein-ligand complexes for diazepam.

ADMET properties prediction of the target analogues

Drug solubility is one of the important parameter which plays an essential role in the pharmacokinetics and pharmacodynamics of the drug. It plays a role from the administration of drug, absorption and also during the activity on targeted part or metabolism and excretion from the human body. ADMET prediction of target analogs was accomplished by the use of online software AdmetSAR. Different permeability parameters such as Blood Brain Barrier (BBB) penetration, Caco-2 cell permeability, Human Intestinal Absorption (HIA), renal organic cation transport and AMES toxicity test were predicted. P-glycoprotein is one of the membrane efflux transporters having an important role in the determining the absorption, distribution, metabolism, excretion, and toxicological behaviours of some drugs and molecules in development and also plays a major role in the multidrug resistance (MDR) phenomenon. P-gp substrates recognition at the early stages of the drug discovery process is very important. Cytochrome enzymes (CYPs) are involved in the metabolism and biosynthesis of endogenous molecules. So, the study of metabolism-related parameter provides hints to overcome any sign of toxicity. Results suggest that all the synthesized analogs were able to absorb from human intestine and there were no signs of toxicity or mutagenicity in the compounds. Results of ADMET study is depicted in **Table 2**. (Supporting data)

SAR summarization based on molecular docking and pharmacological study

The biological study delivered preliminary insight regarding SAR. Results of pharmacological activity suggested that the presence of azomethine functionality with suitably substituted aromatic ring plays a significant role in the biological activity. All the synthesized compounds contain same 1,4-benzodiazepine amine moiety which, did not show anticonvulsant activity. Whereas, 1,4-benzodiazepine amine attached to aromatic moiety having electron withdrawing or bulky groups at 7th position via azomethine demonstrated significant anticonvulsant activity. In the compounds **3c, 3e, 3l** and **3n** where electron withdrawing groups such as Br or NO_2 and bulky group like dimethyl amine present at the *para*- position of phenyl ring or substituted

pyrazole lead to a substantial decrease in the grade of convulsion (**Figure 8**). In case of unsubstituted phenyl ring or *ortho*- and *meta*- position substituted aromatic ring present in the compounds **3a, 3f, 3g, 3i, 3k** revealed moderate to poor biological activity.

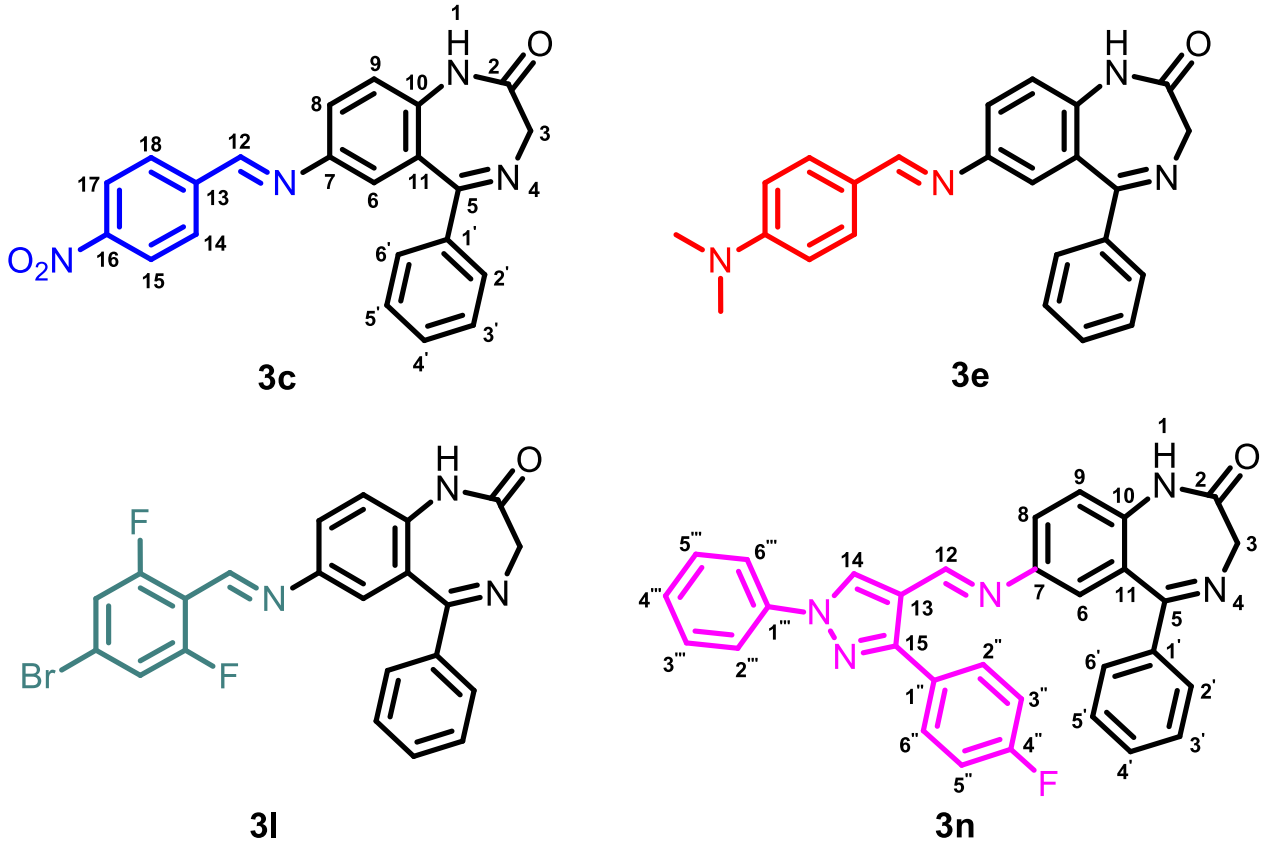


Figure 8 Structures of most active compounds.

Conclusions

The primary aim of the present research work was to develop novel 1,4-benzodiazepine derivatives as anticonvulsant agents. The preliminary anticonvulsant evaluation was performed in rats using classical Picrotoxin induced epileptic model. From the entire dataset, compounds **3c, 3e, 3l** and **3n** displayed significantly better anticonvulsant activity in comparison to standard drug diazepam. Further, there was also a substantial decrease in grade of convulsions in **3c, 3e, 3l** and **3n** treated groups when compared to the convulsive control group. Additionally, molecular docking study was undertaken to retrieve information regarding the mode of interaction at the active site. Further, *in silico* ADMET results and *in- vivo* acute oral toxicity study also indicated that the designed azomethine derivatives have very good pharmacokinetic profile to become a potential drug candidate. Based on these results, we have identified novel analogs as potential leads. Our future endeavor is the optimization of the lead molecules to find potent and safe anticonvulsant agents.

Experimental Section

Chemistry

All the solvents and reagents were purchased commercially and were used without further purification. SRS-OptiMelt digital melting point apparatus was employed to determine the melting point of all synthesized compounds and were uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance II-400 spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift values are given in ppm (δ) relative to TMS and coupling constants *J* are given in Hz. Agilent QTOF 6520 mass spectrometer operating at an ionization potential of 70 eV were used to perform the HRMS analysis. IR spectra were recorded on Shimadzu DRS Prestige 21. Partition coefficient (log P) values were calculated using the CS ChemDraw Ultra version 12.01, by CambridgeSoft.Com.

Procedure for the synthesis of intermediates (7, 1 and 2)

Synthesis of 2-chloroacetamido-5-nitrobenzophenone (7)

To a stirred solution of 2-amino-5-nitro-benzophenone (**5**) (2.42 g, 10 mmol) in toluene (24 mL) under nitrogen, was added chloroacetyl chloride **6** (10 mmol), heated to reflux and allowed to stir at the same temperature for 2 h. The reaction mixture was then cooled to room temperature and

concentrated in vacuum. The crude was purified by crystallization from ethanol (24 mL) at room temperature to give 2-chloroacetamido-5-nitrobenzophenone (**7**) as white crystalline solid (3.11 g), Yield: 98 %, M.p. 175–176 °C.

Synthesis of 7-nitro-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (1)

To a stirred solution of 2-chloroacetamido-5-nitrobenzophenone (**7**) (3.5 g, 11 mmol) in ethanol (50 mL) were added hexamethylenetetramine (HMTM; 3.4 g, 24 mmol) and ammonium acetate (1.86 g, 24 mmol). The reaction mixture was then heated to reflux and permitted to stir at the same temperature for 6 h. The volatiles were evaporated, water (35 mL) was added and the resulting suspension was stirred at 60 °C for 30 min. The suspension was cooled to 15 °C, filtered and dried under vacuum. To the crude product was added toluene (10 mL) and heated at 70 °C for 30 min. The obtained suspension was cooled to 10 °C, filtered, and the solid was washed with cold toluene (2× 3 mL) to afford 7-nitro-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (**1**) as yellow solid (2.2 g), Yield: 71 %, M.p. 224–225 °C.

Synthesis of 7-amino-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (2)

A mixture of 7-nitro-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (**1**) (2.81 g, 10 mmol) and tin chloride dihydrate (10.4 g, 55 mmol) in ethanol (30 mL) was ultrasonicated at room temperature for 2 h. The reaction mixture was diluted with water (120 mL), made alkaline with aqueous ammonia and extracted with dichloromethane (3 × 100 mL). The combined organic layer was dried over sodium sulphate and concentrated under reduced pressure to give 7-amino-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (**2**) as a yellow solid (2.2 g).

7-amino-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (2)

Yellow solid, Yield: 90 %. M.p. 244 – 245 °C. IR (KBr, cm^{-1}): 3329 – 3337 (N-H), 2849 – 3204 (C-H), 1676 (C=O), 1570 – 1603 (C=N), 1500 (C=C), 1340 (C-N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.05 (*br. s*, $H_2C(3)$); 5.18 (*br. s*, H_2N, D_2O exchangeable); 6.40 (*d*, *J* = 2.20 Hz, H–C(6)); 6.78 (*dd*, *J* = 8.56, 2.32 Hz, H–C(8)); 6.93 (*d*, *J* = 8.56 Hz, H–C(9)); 7.38 – 7.57 (*m*, H–C(2', 3', 4', 5' and 6')); 10.05 (*br. s*, H–N, D_2O exchangeable). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 57.0 (C(3)); 113.5 (C(6)); 118.1 (C(8)); 122.1 (C(9)); 127.3 (C(11)); 128.1 (C(3' and 5')); 129.2 (C(10)); 129.2 (C(2' and 6')); 129.9 (C(4')); 139.5 (C(1')); 144.0 (C(7)); 169.7 (C(5)); 170.0 (C(2)). HR-MS: 252.1138 ([*M* + H]$^+$, $C_{15}H_{14}N_3O^+$; calc. 252.1131).

General procedure for the synthesis of compounds (3a–n)

A mixture of the appropriate aromatic aldehydes (**8a–n**) (1.01 mmol) and 7-amino-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (**2**) (1.0 mmol) in ethanol (7.0 mL) and few drops of glacial acetic acid was refluxed for 2-4 h, until 7-amino-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one is fully consumed. The reaction mixture was cooled to room temperature and the precipitates formed were collected by suction filtration. The crude product was washed thoroughly with ethanol (2 × 3 mL), to afford fine powder solid products (**3a–n**) in good yield.

7-(benzylideneamino)-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (3a)

Off-white solid, Yield: 80 %. M.p. 123 – 124 °C. IR (KBr, cm^{-1}): 2831 – 3061 (C-H), 1662 – 1680 (C=O), 1568 – 1628 (C=N), 1489 (C=C). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.16 (*br. s*, $H_2C(3)$); 7.08 (*d*, *J* = 2.01 Hz, H–C(6)); 7.31 (*d*, *J* = 8.78 Hz, H–C(9)); 7.40 – 7.59 (*m*, H–C(2', 3', 4', 5', 6', 8, 15, 16 and 17)); 7.87 (*d*, *J* = 6.56 Hz, H–C(14 and 18)); 8.59 (*s*, H–C(12)); 10.61 (*br. s*, H–N, D_2O exchangeable). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 57.1 (C(3)); 122.0 (C(9)); 122.9 (C(6)); 124.5 (C(8)); 126.9 (C(11)); 128.3 (C(15 and 17)); 128.7 (C(3' and 5')); 128.8 (C(2' and 6')); 129.2 (C(14 and 18)); 130.2 (C(16)); 131.6 (C(4')); 135.8 (C(10)); 137.9 (C(13)); 139.0 (C(1')); 145.5 (C(7)); 161.1 (C(12)); 169.4 (C(5)); 170.2 (C(2)). HR-MS: 340.1458 ([*M* + H]$^+$, $C_{22}H_{18}N_3O^+$; calc. 340.1444).

7-(4-fluorophenyl)methyleneamino-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (3b)

Yellow solid, Yield: 85 %. M.p. 221 – 222 °C. IR (KBr, cm^{-1}): 1682 (C=O), 1587 – 1626 (C=N), 1487 (C=C), 1014 (C-F). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.17 (*br. s*, $H_2C(3)$); 7.08 (*d*, *J* = 2.20 Hz, H–C(6)); 7.26 – 7.58 (*m*, H–C(2', 3', 4', 5', 6', 8, 9, 15 and 17)); 7.93 (*dd*, *J* = 8.56, 5.84 Hz, H–C(14 and 18)); 8.59 (*s*, H–C(12)); 10.61 (*br. s*, H–N, D_2O exchangeable). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 57.0 (C(3)); 115.7 (C(15)); 116.0 (C(17)); 122.0 (C(9)); 122.8 (C(6)); 124.6 (C(8)); 126.9 (C(11)); 128.3 (C(3' and 5')); 129.2 (C(2' and 6')); 130.2 (C(4')); 130.9 (C(14)); 131.0 (C(18)); 132.5 (C(13)); 137.9 (C(10)); 138.9 (C(1')); 145.4 (C(7)); 159.7 (C(12)); 164.0 (*d*, ^1J(C,F) = 247.84 Hz, C(16)); 169.3 (C(5)); 170.2 (C(2)). HR-MS: 358.1365 ([*M* + H]$^+$, $C_{22}H_{17}FN_3O^+$; calc. 358.1350).

7-(4-nitrophenyl)methyleneamino-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (3c)

Yellow solid, Yield: 85 %. M.p. 281 – 282 °C. IR (KBr, cm^{-1}): 2955 – 3186 (C-H), 1670 (C=O), 1599 – 1628 (C=N), 1489 – 1495 (C=C), 1342 and 1520 (NO_2). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.17 (*br. s*, $H_2C(3)$); 7.19 (*d*, *J* = 2.08 Hz, H–C(6)); 7.33 (*d*, *J* = 8.68 Hz, H–C(9)); 7.40 – 7.55 (*m*, H–C(2', 3', 4', 5' and 6')); 7.65 (*dd*, *J* = 8.56, 2.20 Hz, H–C(8)); 8.12 (*d*, *J* = 8.68 Hz, H–C(14 and 18)); 8.31 (*d*, *J* = 8.68 Hz, H–C(15 and 17)); 8.78 (*s*, H–C(12)); 10.66 (*br. s*, H–N, D_2O exchangeable). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 57.1 (C(3)); 122.1 (C(9)); 123.5 (C(6)); 123.9 (C(15 and 17)); 124.7 (C(8)); 126.9 (C(11)); 128.3 (C(3' and 5')); 129.2 (C(2' and 6')); 129.6 (C(14 and 18)); 130.3 (C(4')); 138.6 (C(10)); 138.9 (C(1')); 141.3 (C(13)); 144.6 (C(7)); 148.8 (C(16)); 159.1 (C(12)); 169.3 (C(5)); 170.2 (C(2)). HR-MS: 385.1306 ([*M* + H]$^+$, $C_{22}H_{17}N_4O_3^+$; calc. 385.1295).

Methyl- 4-(2-oxo-5-phenyl-1,3-dihydro-1,4-benzodiazepin-7-yl)iminomethylbenzoate (3d)

Yellow solid, Yield: 76 %. M.p. 209 – 210 °C. IR (KBr, cm^{-1}): 1724 (ester C=O), 1676 (amide C=O), 1605 – 1618 (C=N), 1491 (C=C), 1109 and 1275 (C-O). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.87 (*s*, OCH_3); 4.17 (*br. s*, $H_2C(3)$); 7.15 (*br. s*, H–C(6)); 7.31 (*d*, *J* = 8.56 Hz, H–C(9)); 7.39 – 7.56 (*m*, H–C(2', 3', 4', 5' and 6')); 7.62 (*d*, *J* = 7.96 Hz, H–C(8)); 7.95 – 8.15 (*m*, H–C(14, 15, 17 and 18)); 8.70 (*s*, H–C(12)); 10.63 (*br. s*, H–N, D_2O exchangeable). ^{13}C NMR (100 MHz, DMSO-d_6)

δ: 52.3 (OCH_3); 57.1 (C(3)); 122.1 (C(9)); 123.3 (C(6)); 124.6 (C(8)); 127.0 (C(11)); 128.3 (C(3' and 5')); 128.8 (C(2' and 6')); 129.3 (C(14 and 18)); 129.5 (C(15 and 17)); 130.3 (C(4')); 131.7 (C(16)); 138.3 (C(10)); 138.9 (C(1')); 139.8 (C(13)); 144.0 (C(7)); 160.0 (C(12)); 165.8 (C=O, ester); 169.3 (C(5)); 170.2 (C(2)). HR-MS: 398.1512 ([*M* + H]$^+$, $C_{24}H_{20}N_3O_3^+$; calc. 398.1499).

7-[4-(dimethylamino)phenyl]methyleneamino-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (3e)

Yellow solid, Yield: 70 %. M.p. 248 – 249 °C. IR (KBr, cm^{-1}): 1686 (amide C=O), 1585 – 1610 (C=N), 1481 (C=C), 1362 (C-N, aromatic). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.98 (*s*, $N(CH_3)_2$); 4.15 (*br. s*, $H_2C(3)$); 6.73 (*d*, *J* = 8.80 Hz, H–C(15 and 17)); 6.97 (*d*, *J* = 2.20 Hz, H–C(6)); 7.26 (*d*, *J* = 8.68 Hz, H–C(9)); 7.40 – 7.56 (*m*, H–C(2', 3', 4', 5', 6' and 8)); 7.67 (*d*, *J* = 8.80 Hz, H–C(14 and 18)); 8.36 (*s*, H–C(12)); 10.53 (*br. s*, H–N, D_2O exchangeable). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 39.6 ($N(CH_3)_2$); 57.0 (C(3)); 111.3 (C(15 and 17)); 121.9 (C(9)); 122.4 (C(6)); 123.5 (C(13)); 124.4 (C(8)); 126.9 (C(11)); 128.2 (C(3' and 5')); 129.2 (C(2' and 6')); 130.1 (C(4')); 130.3 (C(14 and 18)); 136.9 (C(10)); 139.0 (C(1')); 146.5 (C(7)); 152.4 (C(16)); 160.2 (C(12)); 169.4 (C(5)); 170.1 (C(2)). HR-MS: 383.1878 ([*M* + H]$^+$, $C_{24}H_{23}N_4O^+$; calc. 383.1866).

7-[(3-bromo-2-fluoro-phenyl)methyleneamino]-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (3f)

Yellow solid, Yield: 72 %. M.p. 222 – 223 °C. IR (KBr, cm^{-1}): 1684 (C=O), 1610 (C=N), 1491 (C=C), 1016 (C-F). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.31 (*br. s*, $H_2C(3)$); 6.98 – 7.06 (*m*, H–C(17)); 7.10 (*d*, *J* = 1.24 Hz, H–C(6)); 7.14 – 7.27 (*m*, H–C(8)); 7.30 – 7.42 (*m*, H–C(3', 4', 5' and 9)); 7.46 – 7.61 (*m*, H–C(2', 6' and 16)); 7.98 (*t*, *J* = 6.72 Hz, H–C(18)); 8.58 (*s*, H–C(12)); 9.57 (*br. s*, H–N, D_2O exchangeable). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 56.7 (C(3)); 109.6 (C(15)); 122.2 (C(9)); 123.5 (C(17)); 124.7 (C(6)); 124.9 (C(13)); 125.4 (C(8)); 126.9 (C(4')); 128.0 (C(11)); 128.3 (C(3' and 5')); 129.7 (C(2' and 6')); 130.4 (C(18)); 136.4 (C(16)); 137.4 (C(10)); 139.1 (C(1')); 146.6 (C(7)); 153.2 (C(12)); 159.0 (*d*, ^1J(C,F) = 252.22 Hz, C(14)); 170.7 (C(5)); 172.0 (C(2)). HR-MS: 436.0457 ([*M* + H]$^+$, $C_{22}H_{16}BrFN_3O^+$; calc. 436.0455).

7-[(4-bromo-2-fluoro-phenyl)methyleneamino]-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (3g)

Off-white solid, Yield: 70 %. M.p. 221 – 222 °C. IR (KBr, cm^{-1}): 2843 – 3178 (C-H), 1678 (C=O), 1599 – 1608 (C=N), 1492 (C=C), 1012 (C-F). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.16 (*br. s*, $H_2C(3)$); 7.14 (*d*, *J* = 2.32 Hz, H–C(6)); 7.30 (*d*, *J* = 8.68 Hz, H–C(9)); 7.41 – 7.54 (*m*, H–C(2′, 3', 4', 5', 6′ and 17)); 7.62 (*dd*, *J* = 8.62, 2.38 Hz, H–C(8)); 7.71 (*dd*, *J* = 10.21, 1.65 Hz, H–C(15)); 7.97 (*t*, *J* = 8.13 Hz, H–C(18)); 8.68 (*s*, H–C(12)); 10.63 (*br. s*, H–N, D_2O exchangeable). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 57.0 (C(3)); 119.5 (C(15)); 122.1 (C(9)); 122.7 (C(13)); 123.5 (C(6)); 124.3 (C(8)); 125.5 (C(16)); 126.9 (C(11)); 128.2 (C(17)); 128.2 (C(3' and 5')); 129.2 (C(4')); 129.2 (C(2' and 6')); 130.2 (C(18)); 138.4 (C(10)); 138.9 (C(1')); 145.0 (C(7)); 152.8 (C(12)); 161.6 (*d*, ^1J(C,F) = 255.86 Hz, C(14)); 169.3 (C(5)); 170.2 (C(2)). HR-MS: 436.0456 ([*M* + H]$^+$, $C_{22}H_{16}BrFN_3O^+$; calc. 436.0455).

7-[(4-bromo-2-chloro-phenyl)methyleneamino]-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (3h)

Yellow solid, Yield: 75 %. M.p. 219 – 220 °C. IR (KBr, cm^{-1}): 2885 – 3180 (C-H), 1697 (C=O), 1606 – 1620 (C=N), 1487 (C=C), 694 (C-Cl), 586 (C-Br). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.17 (*br. s*, $H_2C(3)$); 7.11 (*d*, *J* = 2.08 Hz, H–C(6)); 7.31 (*d*, *J* = 8.56 Hz, H–C(9)); 7.39 – 7.54 (*m*, H–C(2′, 3', 4', 5' and 6′)); 7.56 – 7.67 (*m*, H–C(8 and 15)); 7.84 (*d*, *J* = 1.59 Hz, H–C(17)); 8.00 (*d*, *J* = 8.56 Hz, H–C(14)); 8.75 (*s*, H–C(12)); 10.64 (*br. s*, H–N, D_2O exchangeable). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 57.1 (C(3)); 122.1 (C(9)); 123.6 (C(6)); 124.3 (C(8)); 125.4 (C(11)); 126.9 (C(16)); 128.2 (C(3' and 5')); 129.2 (C(2' and 6')); 129.7 (C(4')); 130.2 (C(15)); 130.8 (C(14)); 131.8 (C(10)); 132.2 (C(17)); 135.8 (C(13)); 138.5 (C(1')); 138.9 (C(18)); 144.9 (C(7)); 155.7 (C(12)); 169.2 (C(5)); 170.2 (C(2)). HR-MS: 452.0155 ([*M* + H]$^+$, $C_{22}H_{16}BrClN_3O^+$; calc. 452.0160).

7-[2-fluoro-4-(trifluoromethyl)phenyl]methyleneamino-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (3i)

Pale yellow solid, Yield: 77 %. M.p. 228 – 229 °C. IR (KBr, cm^{-1}): 2895 – 3196 (C-H), 1680 (C=O), 1612 – 1620 (C=N), 1492 (C=C), 1132 (C-F). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.17 (*br. s*, $H_2C(3)$); 7.18 (*d*, *J* = 1.36 Hz, H–C(6)); 7.32 (*d*, *J* = 8.56 Hz, H–C(9)); 7.39 – 7.55 (*m*, H–C(2′, 3', 4', 5' and 6′)); 7.64 – 7.68 (*m*, H–C(8 and 15)); 7.81 (*d*, *J* = 10.40 Hz, H–C(17)); 8.23 (*t*, *J* = 7.52 Hz, H–C(18)); 8.78 (*s*, H–C(12)); 10.66 (*br. s*, H–N, D_2O exchangeable). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 57.0 (C(3)); 113.9 (C(15)); 121.5 (C(17)); 121.7 (CF_3); 122.1 (C(9)); 123.8 (C(6)); 124.5 (C(8)); 126.9 (C(11)); 127.0 (C(13)); 128.3 (C(3' and 5')); 129.1 (C(18)); 129.2 (C(2' and 6')); 130.2 (C(4')); 132.8 (C(16)); 138.7 (C(10)); 138.9 (C(1')); 144.7 (C(7)); 152.6 (C(12)); 161.5 (*d*, ^1J(C,F) = 253.67 Hz, C(14)); 169.3 (C(5)); 170.2 (C(2)). HR-MS: 426.1239 ([*M* + H]$^+$, $C_{23}H_{16}F_4N_3O^+$; calc. 426.1224).

7-[(4-fluoro-3-nitro-phenyl)methyleneamino]-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (3j)

Pale yellow solid, Yield: 80 %. M.p. 258 – 259 °C. IR (KBr, cm^{-1}): 3063 – 3288 (C-H), 1691 (C=O), 1612 (C=N), 1487 (C=C), 1342 and 1531 (NO_2), 1012 (C-F). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.18 (*br. s*, $H_2C(3)$); 7.17 (*d*, *J* = 2.32 Hz, H–C(6)); 7.33 (*d*, *J* = 8.68 Hz, H–C(9)); 7.40 – 7.55 (*m*, H–C(2′, 3', 4', 5' and 6′)); 7.62 (*dd*, *J* = 8.68, 2.32 Hz, H–C(8)); 7.71 (*dd*, *J* = 11.13, 8.80 Hz, H–C(17)); 8.29 (*ddd*, *J* = 8.65, 4.31, 2.08 Hz, H–C(18)); 8.61 (*dd*, *J* = 7.46, 1.96 Hz, H–C(14)); 8.73 (*s*, H–C(12)); 10.65 (*br. s*, H–N, D_2O exchangeable). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 57.0 (C(3)); 119.2 (C(17)); 122.1 (C(9)); 123.1 (C(6)); 124.8 (C(8)); 126.0 (C(14)); 126.9 (C(11)); 128.3 (C(3' and 5')); 129.2 (C(2' and 6')); 130.3 (C(4')); 133.00 (C(13)); 135.6 (C(18)); 137.2 (C(15)); 138.4 (C(10)); 138.9 (C(1')); 144.5 (C(7)); 156.0 (*d*, ^1J(C,F) = 265.34 Hz, C(16)); 158.0 (C(12)); 169.3 (C(5)); 170.2 (C(2)). HR-MS: 403.1216 ([*M* + H]$^+$, $C_{22}H_{16}FN_4O_3^+$; calc. 403.1201).

5-phenyl-7-[(2,4,6-trifluorophenyl)methyleneamino]-1,3-dihydro-1,4-benzodiazepin-2-one (3k)

Pale yellow solid, Yield: 75 %. M.p. 241 – 242 °C. IR (KBr, cm^{-1}): 3034 – 3196 (C-H), 1687 (C=O), 1587 – 1620 (C=N), 1483 (C=C), 1043 (C-F). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.16 (*br. s*, $H_2C(3)$); 7.06 (*d*, *J* = 2.32 Hz, H–C(6)); 7.26 – 7.57 (*m*, H–C(2′, 3', 4', 5', 6′, 8, 9, 15 and 17)); 8.53 (*s*, H–C(12)); 10.62 (*br. s*, H–N,

D_2O exchangeable). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 57.0 (C(3)); 101.6 (*t*,^2J(C,F) = 26.78 Hz, C(15 and 17)); 110.4 (*t*,^2J(C,F) = 12.84 Hz, C(13)); 122.1 (C(9)); 122.8 (C(8)); 124.3 (C(6)); 126.9 (C(11)); 128.2 (C(3' and 5')); 129.2 (C(2' and 6')); 130.2 (C(4')); 138.3 (C(10)); 139.0 (C(1')); 145.7 (C(7)); 150.9 (C(12)); 161.8 (*dd*, ^1J(C,F) = 256.59 Hz, ^3J(C,F) = 15.67 Hz, C(14 and 18)); 163.4 (*dt*, ^1J(C,F) = 250.76 Hz, ^3J(C,F) = 16.77 Hz, C(16)); 169.3 (C(5)); 170.2 (C(2)). HR-MS: 394.1176 ([*M* + H]$^+$, $C_{22}H_{15}F_3N_3O^+$; calc. 394.1162).

7-[(4-bromo-2,6-difluoro-phenyl)methyleneamino]-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (3l)

Pale yellow solid, Yield: 70 %. M.p. 228 – 229 °C. IR (KBr, cm^{-1}): 2970 – 3049 (C-H), 1680 (C=O), 1612 (C=N), 1494 (C=C), 1045 (C-F). ^1H NMR (400 MHz, DMSO-d_6) δ: 4.17 (*br. s*, $H_2C(3)$); 7.08 (*d*, *J* = 2.20 Hz, H–C(6)); 7.31 (*d*, *J* = 8.56 Hz, H–C(9)); 7.40 – 7.62 (*m*, H–C(2′, 3', 4', 5', 6′, 8, 15 and 17)); 8.54 (*s*, H–C(12)); 10.63 (*br. s*, H–N, D_2O exchangeable). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 57.0 (C(3)); 112.7 (C(13)); 116.2 (C(15)); 116.4 (C(17)); 122.1 (C(9)); 122.9 (C(8)); 124.3 (C(6)); 124.7 (C(16)); 126.9 (C(11)); 128.2 (C(3' and 5')); 129.2 (C(2' and 6')); 130.2 (C(4')); 138.5 (C(10)); 138.9 (C(1')); 145.6 (C(7)); 151.0 (C(12)); 161.0 (*d*, ^1J(C,F) = 266.79 Hz, C(14 and 18)); 169.3 (C(5)); 170.2 (C(2)). HR-MS: 454.0358 ([*M* + H]$^+$, $C_{22}H_{15}BrF_2N_3O^+$; calc. 454.0361).

7-[1-naphthylmethyleneamino]-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (3m)

Yellow solid, Yield: 72 %. M.p. 251 – 252 °C. IR (KBr, cm^{-1}): 2947 – 3053 (C-H), 1689 (C=O), 1614 (C=N), 1489 (C=C). ^1H NMR (400 MHz, DMSO-d_6) δ: 4.19 (*br. s*, $H_2C(3)$); 7.20 (*d*, *J* = 1.84 Hz, H–C(6)); 7.35 (*d*, *J* = 8.68 Hz, H–C(9)); 7.40 – 7.73 (*m*, H–C(2′, 3', 4', 5', 6′, 2'', 3'', 8 and 15)); 8.01 (*d*, *J* = 7.95 Hz, H–C(1'')); 8.09 (*d*, *J* = 8.07 Hz, H–C(4'')); 8.13 (*d*, *J* = 7.21 Hz, H–C(16)); 9.15 (*d*, *J* = 8.32 Hz, H–C(14)); 9.20 (*s*, H–C(12)); 10.63 (*br. s*, H–N, D_2O exchangeable). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 57.1 (C(3)); 122.0 (C(4")); 123.1 (C(9)); 124.4 (C(3")); 124.6 (C(6)); 125.4 (C(8)); 126.3 (C(15)); 127.0 (C(11)); 127.5 (C(1")); 128.3 (C(3' and 5')); 128.7 (C(2")); 129.3 (C(2' and 6')); 130.2 (C(14)); 130.4 (C(4')); 130.7 (C(18)); 130.8 (C(17)); 132.1 (C(16)); 133.4 (C(10)); 137.9 (C(13)); 139.0 (C(1')); 146.0 (C(7)); 160.9 (C(12)); 169.4 (C(5)); 170.2 (C(2)). HR-MS: 390.1612 ([*M* + H]$^+$, $C_{26}H_{20}N_3O^+$; calc. 390.1601).

7-[3-(4-fluorophenyl)-1-phenyl-pyrazol-4-yl]methyleneamino-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (3n)

Pale yellow solid, Yield: 88 %. M.p. 267 – 268 °C. IR (KBr, cm^{-1}): 1685 (C=O), 1593 – 1622 (C=N), 1496 (C=C), 1012 (C-F). ^1H NMR (400 MHz, DMSO-d_6) δ: 4.16 (*br. s*, $H_2C(3)$); 7.03 (*d*, *J* = 2.08 Hz, H–C(6)); 7.26 – 7.54 (*m*, H–C(2′, 3', 4', 5', 6′, 2'', 6'', 3''', 4''', 5''', 8 and 9)); 7.84 (*dd*, *J* = 8.44, 5.62 Hz, H–C(3'' and 5'')); 7.98 (*d*, *J* = 7.95 Hz, H–C(2''' and 6''')); 8.48 (*s*, H–C(14)); 9.11 (*s*, H–C(12)); 10.58 (*br. s*, H–N, D_2O exchangeable). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 57.1 (C(3)); 115.4 (C(3")); 115.6 (C(5")); 118.8 (C(2"' and 6"')); 119.6 (C(13)); 122.0 (C(9)); 122.9 (C(6)); 124.2 (C(8)); 126.9 (C(11)); 127.2 (C(4"')); 128.2 (C(3' and 5')); 128.4 (C(1")); 129.2 (C(2' and 6')); 129.6 (C(3"' and 5"')); 129.7 (C(14)); 130.1 (C(4')); 130.7 (C(2")); 130.8 (C(6")); 137.5 (C(10)); 138.8 (C(1')); 139.0 (C(1"')); 146.0 (C(7)); 151.6 (C(15)); 152.8 (C(12)); 162.4 (*d*, ^1J(C,F) = 244.20 Hz, C(4")); 169.4 (C(5)); 170.1 (C(2)). HR-MS: 500.1894 ([*M* + H]$^+$, $C_{31}H_{23}FN_5O^+$; calc. 500.1881).

Synthesis of 4-fluoro-N-(2-oxo-5-phenyl-1,3-dihydro-1,4-benzodiazepin-7-yl)benzamide (4)

To a stirred solution of 7-amino-5-phenyl-1,3-dihydro-1,4-benzodiazepin-2-one (**2**) (1.2 mmol) and triethyl amine (2.4 mmol) in dichloromethane (3.0 mL) under nitrogen 4-fluorobenzoyl chloride (**9**) (1.32 mmol) was added at 24 °C and stirred for 2 h. The reaction mixture was quenched with water and the precipitate formed was collected by filtration. The crude solid was washed thoroughly with dichloromethane (2 × 2 mL) and water (2 × 2 mL) and dried to obtain 4-fluoro-N-(2-oxo-5-phenyl-1,3-dihydro-1,4-benzodiazepin-7-yl)benzamide (**4**) as off-white solid.

4-fluoro-N-(2-oxo-5-phenyl-1,3-dihydro-1,4-benzodiazepin-7-yl)benzamide (4)

Off-white solid, Yield: 65 %. M.p. 285 – 286 °C. IR (KBr, cm^1): 2839 – 3063 (C-H), 1683 and 1647 (C=O), 1608 (C=N), 1500 (C=C), 1012 – 1028 (C-F). ^1H NMR (400 MHz, DMSO-d_6) δ: 4.14 (*br. s*, $H_2C(3)$); 7.24 (*d*, *J* = 9.03 Hz, H–C(9)); 7.33 (*t*, *J* = 8.22 Hz, H–C(15 and 19)); 7.42 – 7.56 (*m*, H–C(2′, 3', 4', 5' and 6′)); 7.71 (*d*, *J* = 2.26 Hz, H–C(6)); 7.95 – 8.06 (*m*, H–C(8, 16 and 18)); 10.35 (*br. s*, H–N(12), D_2O exchangeable); 10.49 (*br. s*, H–N(1), D_2O exchangeable); ^{13}C NMR (100 MHz, DMSO-d_6) δ: 57.0 (C(3)); 115.2 (C(16)); 115.4 (C(18)); 121.3 (C(9)); 121.4 (C(6)); 123.7 (C(8)); 126.4 (C(11)); 128.3 (C(3' and 5')); 129.3 (C(2' and 6')); 130.2 (C(15)); 130.3 (C(19)); 130.4 (C(4')); 130.9 (C(14)); 133.9 (C(10)); 135.5 (C(1')); 139.0 (C(7)); 164.1 (*d*, ^1J(C,F) = 247.90 Hz, C(17)); 164.4 (C(13)); 169.3 (C(5)); 170.2 (C(2)). HR-MS: 374.1307 ([*M* + H]$^+$, $C_{22}H_{17}FN_3O_2^+$; calc. 374.1299).

PHARMACOLOGY

In vivo anticonvulsant activity of target compounds

Adult male Wistar rats (180-200g) were purchased from National Institute of Biosciences, Pune and housed for one week at the institute animal house separately in groups of six animals per cage at standard laboratory conditions. Animals had free access to food and water ad libitum. The research protocol was approved by the Institutional Animal Ethics Committee and performed in accordance with the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India on animal experimentation (CPCSEA approval no - CPCSEA/PCP/PCL03/2018). The rats were divided into 19 groups of 8 animals each. Group 1 served as normal control and was treated with vehicle 2 % w/v of Tween 80 orally. Group 2 served as Picrotoxin convulsive control and was treated with vehicle 2 % w/v of Tween 80 and Picrotoxin was administered at 3 mg/kg i.p. Group 3 served as a standards group and received Diazepam 1 mg/kg orally. Group 4 to 19 served as **3a-3n**, **4** and **2** (2.5 mg/kg). The animals were administered orally with respective treatment 24 hours before the experimentation and at 1 hour before the experimentation. After which, the Picrotoxin was injected at a dose of 3 mg/kg, i.p. to all the animals except normal control group. The time of onset of convulsions, their severity was

scored; a number of wet dog shakes and time of appearance of tonic response as well as the death of animals, if any were noted. The following were the guidelines used to grade the severity of convulsions: 0- No convulsive behavior, 1-head or body twitching, 2- clonic forelimb convulsion, 3 –rearing, 4- falling back, 5- when 3 or 4 last for more than 5 min, 6- tonus, 7 –convulsion for more than 10 min. The data were analyzed by One-way ANOVA followed by Dunnet's 't' test using GraphPad Prism v. 5 demos. The values are expressed as mean ± SEM, n = 8. $p<0.05$ was considered significant. [34–36]

Acute oral toxicity study

Adult female Wistar rats (180-200g) were used to carry out an acute toxicity study. As per OECD (Organization for economic co-operation and development) guidelines 423, acute oral toxicity study was carried out for compounds **3a** to **3n**, **4** and **2**. The animals were alienated into 17 groups of 3 animals each as vehicle control and compounds **3a-3n**, **4**, **2** groups. Overnight fasted healthy female Wistar Albino rats (n = 3) were administered orally compound **3a-3n**, **4**, **2** (in 2 % w/v Tween 80) at a dose of 5 mg/kg body weight. After dosing, the animals were observed individually at least once during the first 30 min, and thereafter periodically during the first 24 h, with special attention given during the first 4 h, and subsequently daily thereafter, for a total of 14 days. Here, different signs of toxicity such as changes in skin and fur, eyes, mucous membrane, respiratory, CNS activity, and the behavior pattern were observed. Main Attention was focused to observe tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. As per OECD guidelines, acute toxicity test was repeated with doses of 50 mg/kg body weight.

In silico molecular docking

Ligand Preparation

The structures of all the derivatives were converted into 3D before analysis. Merck Molecular Force Field (MMFF) with distance-dependent dielectric function and energy gradient of 0.001 kcal/mol with iteration limit to 10,000 was utilized to perform energy optimization of all the molecules. [37] On the basis of energy minimization is that the drug binds to effectors/receptors in the most stable form, i.e., the minimum energy form. The optimized compounds were subjected to conformational analysis and energy minimization using Monte Carlo conformational search. Low energy conformers of all the structures were generated and which was utilized further for analysis. The conformational analysis includes moving the atoms of a molecule in such a way that the total energy of the system is reduced based on empirical representation of the interaction energy of the atoms of a molecule. MMFF method was used to minimize each atom in the designed ligands. [38]

Protein preparation and molecular docking

Ernst *et al*. have reported the homology model of the diazepam-bound $GABA_A$ receptor which was retrieved from the supplementary material of their published paper. [39] All the water molecules were removed from the crystal structure of $GABA_A$ receptor. AutoDockVina tool was used for the protein synthesis and Grid generation. Polar hydrogens were added into the structure and Gasteiger charges were computed and applied accordingly. Missing residues in the proteins were also added at the time of preparation. Molecular docking study of Diazepam and most active compound **3e** were executed with AutoDockVina tool. A docking grid box was built with 40, 40 and 40 points in x = 43.640, y = 43.866 and z = 9.3290 directions. Using the gradient optimization algorithm and an empirical scoring function the molecular docking was conducted to generate the best binding affinity or fitness of protein-ligand binding poses between compounds as $GABA_A$ receptor. The best binding conformations of ligands were selected and analyzed using AutoDockVina Tool as well as in Discovery Studio 4.0.

Molecular dynamics simulation

Molecular dynamics simulation was carried out to understand the dynamic behavior of protein-ligand complex. Here, best docked complexes of compound **3e** and diazepam with $GABA_A$ receptor were used for MD simulation study using Gromacs 5.1.1. [40, 41] 7.0 pH was used to fix the protonation states to their normal states. The CHARMM27 all atoms force field was used for the simulation. To setup the system protein was surrounded by a cubic water box of SPC_3 water molecules and it was extended 10 Å from the protein. The topology of the small molecules was generated using the SwissParam. As a part of system setup, the periodic boundary condition (PBC) was applied in all around the molecule. Suitable numbers of Na+ and Cl- charges were used to neutralize the system. Each system was minimized using the steepest descent algorithm for 10,000 steps. For each docked complex 1 ns of time span MD simulation was performed at constant pressure (1 atm) and temperature (300 K). At each 1 ps the iterations were documented to analysis of MD simulations. The root mean-square deviation (RMSD) and radius of gyration were used to analyze the performance of each system. [42]

Prediction of ADMET properties for the designed derivatives

In the process of drug discovery, during the lead identification and optimization ADMET (absorption, distribution, metabolism, excretion, and toxicity) prediction plays an important role. ADMET properties were predicted using the online tool AdmetSAR. [43] It is free software available online which was accessed on 10th September 2018. It provides all the data related to ADMET properties.

Supplementary Material

Supporting information for this article given separately.

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Author Contribution Statement

PN performed all experiments, purified all compounds, analyzed the data and summarized the results. AS and KD tested all compounds for their anticonvulsant activity. TA helped in the compiling the data of the manuscript. SG, MG and SNS conceived and designed this research and wrote the manuscript. All authors have contributed to the final version and approved the final manuscript.

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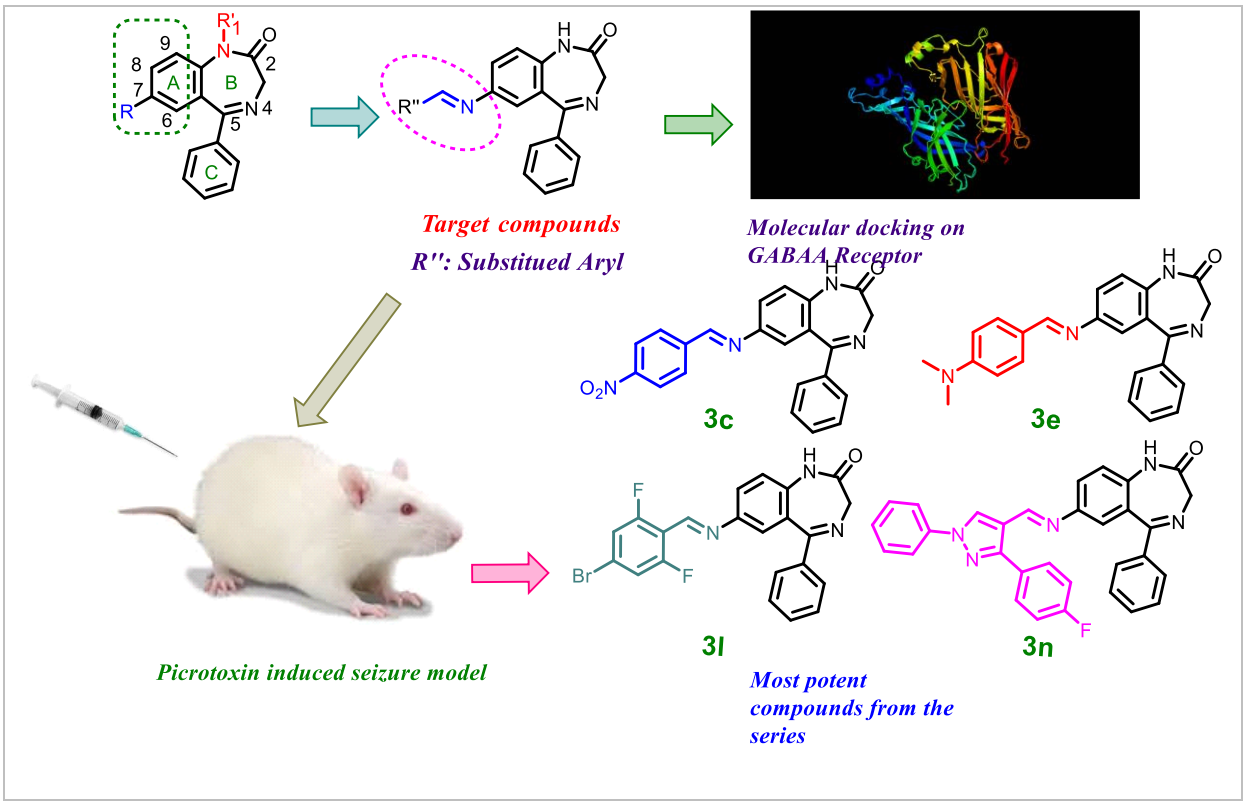

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