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SYNTHESIS, MOLECULAR DOCKING STUDY AND ANTICONVULSANT ACTIVITY OF NOVEL SCHIFF BASES OF 7-AMINO-5-PHENYL-1,3-DIHYDRO-2H-1,4-BENZODIAZEPIN-2-ONE

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ABSTRACT:
The present work describes the synthesis of a novel series of fused heterocyclic azomethine derivatives of 5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one and substituted pyrazole azomethine derivatives of 5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one. The synthesized compounds were evaluated for their anticonvulsant activities in the rat with picrotoxin-induced seizure model and diazepam as reference standard. 7-[(*E*)-(6,8-dimethoxy-3-quinolyl)methyleneamino]-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one(**11d**), 5-phenyl-7-[(*E*)-quinoxalin-2-ylmethyleneamino]-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**11e**), 5-phenyl-7-[(*E*)-[1-phenyl-3-(2-thienyl)pyrazol-4-yl]methyleneamino]-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**11h**) and 7-[(*E*)-[3-(3,5-difluorophenyl)-1-phenyl-pyrazol-4-yl]methyleneamino]-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**11i**) showed 100 % protection at dose 30 mg/kg. Furthermore, rotarod test results demonstrated that none of the screened compounds induced motor deficits in experimental animals. Additionally, the pharmacokinetic, toxicity and physicochemical predictions of the all compounds were calculated *in- silico* by online tools. Outcomes of ADMET studies suggested that the pharmacokinetic parameters of all the synthesized compounds were within the endurable range to become a potential drug candidate as anticonvulsant agents.

KEYWORDS:
Epilepsy, 5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one, Azomethine, Anticonvulsant, ADMET.

INTRODUCTION:

Epilepsy is an ailment of central nervous system in which excessive, hypersynchronous neuronal discharge in the brain results in loss of consciousness and convulsions. Approximately 60 million world populations are suffering from this critical illness, making it one of the most common neurological disorder observed mostly in older people and upper economic class people[i]. A variety of drugs are available for the treatment of epilepsy e.g. Gabapentin,[ii] Pregabalin,[iii] levetiracetam,[iv] Lacosamide[v] and Benzodiazepines.[vi] Treatment of this disease in different age groups with existing medication is challenging, because of poor metabolism and multiple disease problem, including renal failure in the older age group and high rate of metabolism with toxicity in young adults and children.[vii] Therefore, development of novel antiepileptic agents (AEDs) with high efficacy and less toxicity is an utmost importance. Herein, we have considered benzodiazepine (BZD) as active pharmacophore for the further development of novel AEDs due to its broad-spectrum biological activities.[viii] Benzodiazepines (BZDs) augment the outcome of the neurotransmitter gamma-aminobutyric acid (GABA) at the GABA receptor, causing sedative, anticonvulsant and muscle relaxant properties. Some notable example of benzodiazepines derived anticonvulsant agents are Nitrazepam,[ix] Clonazepam, Diazepam,[x] Flunitrazepam, Diclazepam, Phenazepam, Flubromazepam and Lorazepam.[xi] (***Figure 1)***

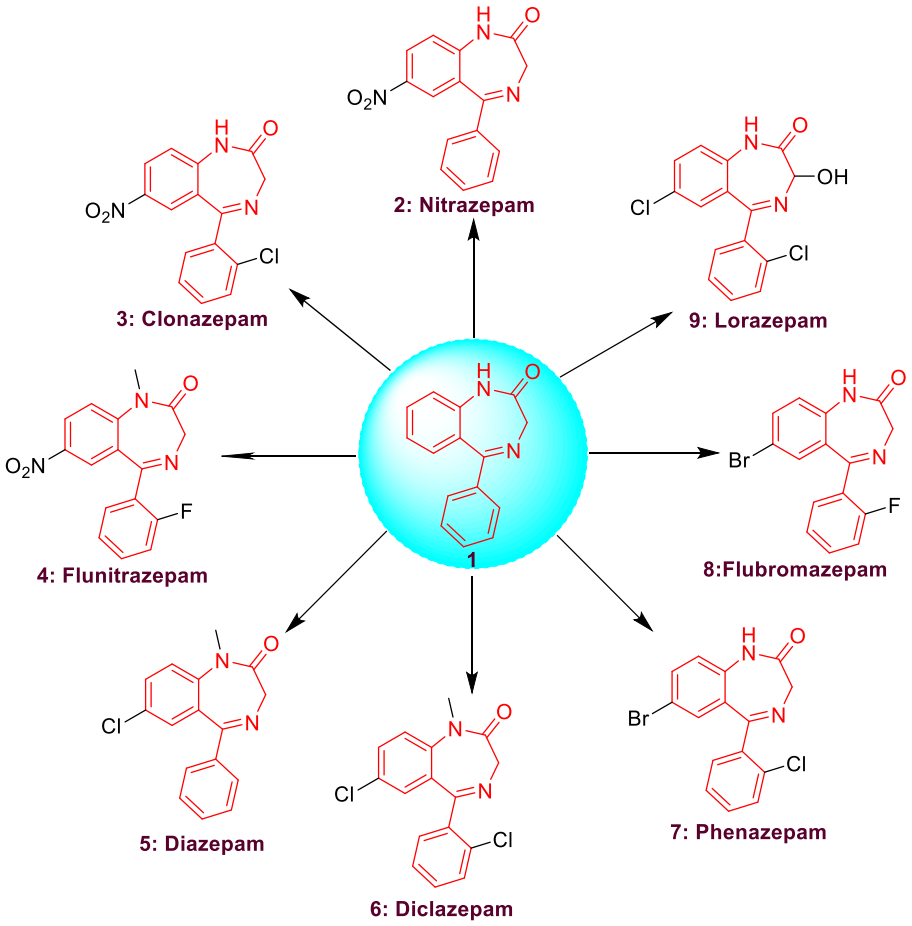


Figure 1 : *The structure of biologically active compounds* ***2*** *–* ***9*** *owing 5-phenyl-1,3-dihydro-2H-1,4-benzodiazepin-2-one skeleton.*

In our ongoing effort to design novel molecules in the development of pharmacotherapies for epilepsy,[xii] we have synthesized a large number azomethine derivative of **10** in which aromatic azomethyl moiety having electron withdrawing or bulky groups at the 7th position showed

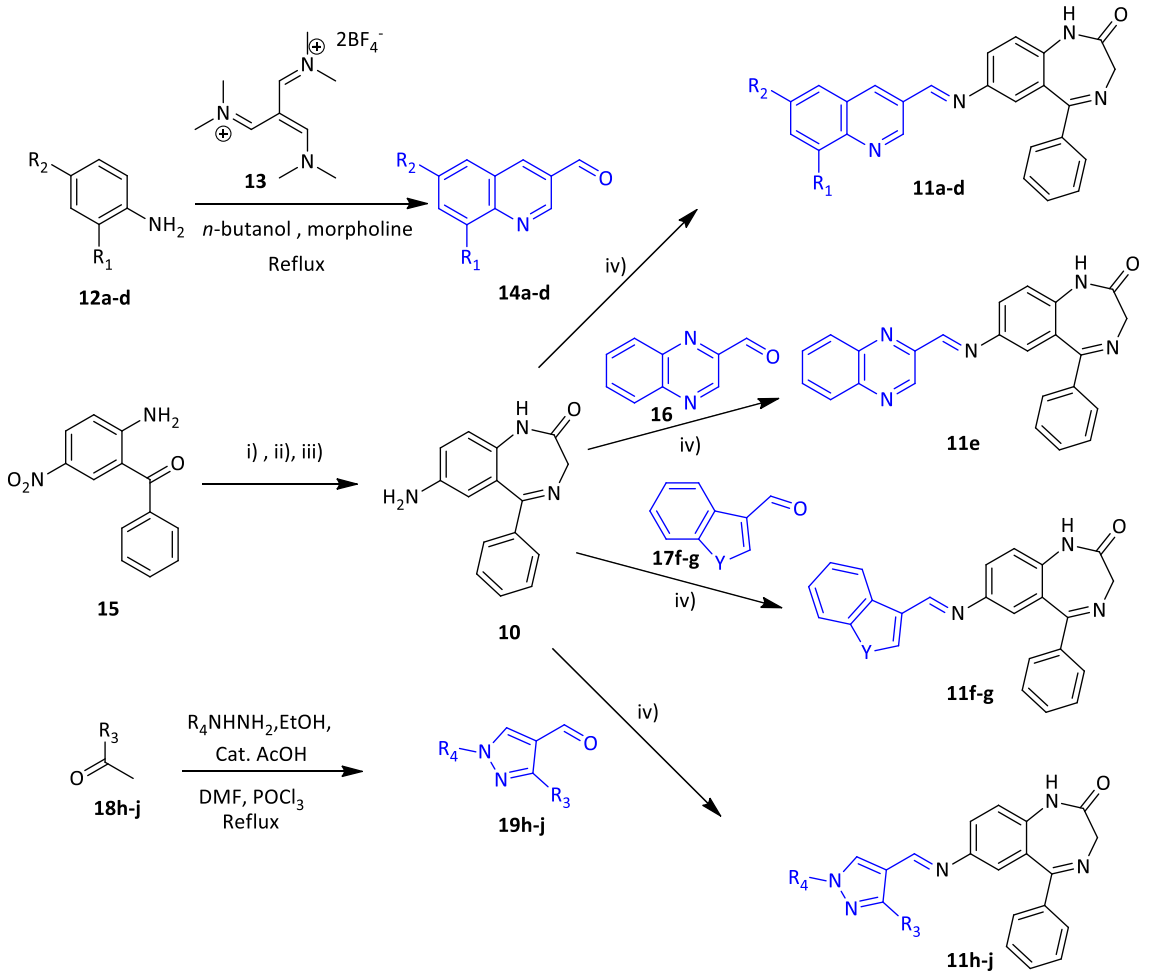
promising activity. In this context and in continuation of our work in medicinal chemistry,[xiii] we have further designed and synthesized a novel series of fused heterocyclic azomethine derivatives of 7-amino-5-phenyl-1,3-dihydro-*2H*-1,4-benzodiazepin-2-one **11a** – **g** and substituted pyrazole azomethine derivatives of 7-amino-5-phenyl-1,3-dihydro-*2H*-1,4-benzodiazepin-2-one **11h** – **j** and screened for their anticonvulsant activities in the rat using picrotoxin-induced seizure model. Furthermore, molecular docking analysis of new synthesized compounds was carried out to examine the binding mode of the interactions. We have also predicted *in-silico* physiochemical and pharmacokinetic properties along with evaluation of *in-vivo* neurotoxicity of synthesized analogues in the rotarod test (NT). Based on the information congregated from pharmacological activities, structure-activity relationship was summarized and potential lead molecules were identified.

RESULTS AND DISCUSSION:

Chemistry

Synthesis of the final targets are shown in ***scheme 1***. The key intermediate 7-amino-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**10**) was synthesized by following the protocol previously reported by us.[xii] Treatment of (2-Amino-5-nitrophenyl) (phenyl)methanone (**15**) with chloroacetyl chloride in toluene afforded *N*-(2-benzoyl-4-nitrophenyl)-2-chloroacetamide, which further reacted with NH_4OAc and hexamethylenetetramine (HMTM) in absolute ethanol to give nitrazepam **2**. Ultrasonication of **2** with $SnCl_2 \cdot 2H_2O$ in ethanol provided 7-amino-5-phenyl-1,3-dihydro-*2H*-1,4-benzodiazepin-2-one (**10**) in good yield.

Synthesis of 3-formyl quinolines **14a** – **d** was achieved by the reaction between "vinamidinium" bis-tetrafluoroborate salt **13** and substituted anilines **12a** – **d** followed by in-situ cyclization and hydrolysis of resulting imino-eneamine salts [xiv].



Scheme 1: *Synthesis route of compounds 11a – j. Reagent and condition: i) Toluene, chloroacetyl chloride, 110 °C, 2 h; ii) NH_4OAc, HMTM, EtOH, 78 °C, 6 h; iii) $SnCl_2.2H_2O$, ultrasonicated, EtOH, 24 °C, 2 h; iv) EtOH, cat. Glacial acetic acid, 78 °C, 1–6 h*

Compounds **19h** – **j** was prepared by reaction of varieties of ketones **18h** – **j** with arylhydrazines in ethanol with catalytic AcOH to give corresponding hydrazones, which then treated with excess Vilsmeier–Haack reagent (DMF-$POCl_3$) to yield pyrazole 4-carbaldehydes **19h** – **j**.[xv]

Synthesis of azomethine derivatives (**11a – j**) were achieved by refluxing 7-amino-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**10**) and appropriate aldehydes in ethanol in the presence of catalytical amount of glacial acetic acid. The spectral methods like ^{1}H-NMR , ^{13}C NMR, IR and high resolution mass spectrometry were used to characterize the structures of the target compounds. The structure of synthesized target compounds is shown in ***figure 2***.

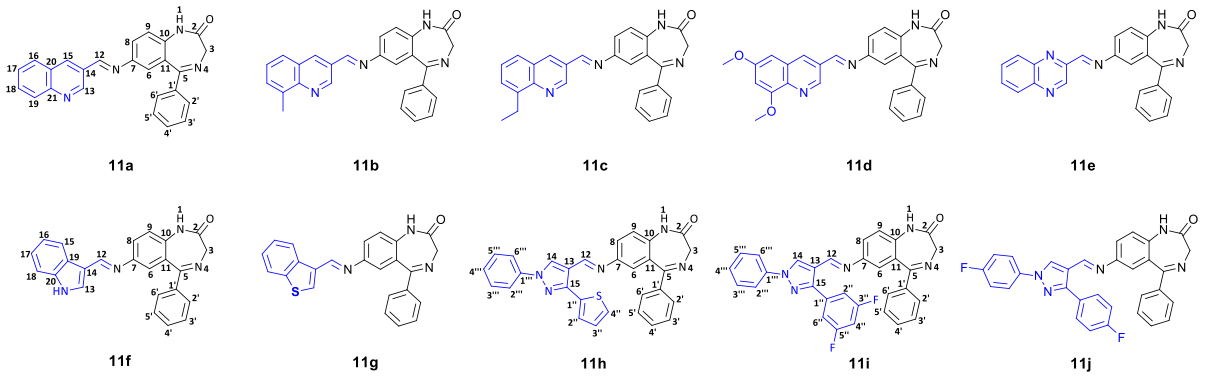


Figure 2: *Synthesized target compounds* ***11a – j****.*

Pharmacology

All the newly synthesized azomethine derivatives were screened in rats and recorded for the duration of convulsion, time of onset of convulsions, convulsion grades and time of onset of tonic-clonic convulsions, where diazepam was used as a reference standard.

Anticonvulsant Activities of Synthesized Compounds (11a – j)

Influence of azomethine derivatives on time of onset of convulsions

In comparison to the convulsive control group, compounds **11a** and **11c** – **i** treated group showed a significant increase ($p<0.001$) in time of onset of convulsions analogous to standard drug diazepam. However, compounds **11b** and **11j** treated groups displayed no change in time of onset of convulsions (***supporting information, figure 1***). In the present study, it was assessed that among all compounds **11d**, **11e**, **11h** and **11i** exhibited a substantial enhancement of time of onset of convulsions as compared to convulsive control group (***Figure 3a***).

Influence of azomethine derivatives on time of onset of tonic clonic convulsions

In comparison to the convulsive control group, compounds **11d** – **i** treated group showed a significant increase ($p<0.001$) in time of onset of tonic-clonic convulsions similar to standard drug diazepam. However, compounds **11a** – **c** and **11j** treated group displayed no change in time of onset of tonic-clonic convulsions (***supporting information, figure 1***). Further, it was observed that derivatives **11d**, **11e**, **11h** and **11i** exhibited a substantial enhancement in the time of onset of tonic-clonic convulsions as compared to the convulsive control group (***Figure 3b***).

Influence of azomethine derivatives on the duration of convulsions

In comparison to the convulsive control group, compounds **11d, 11e, 11h** and **11i** treated group showed a significant decrease ($p<0.01$) in the duration of convulsions similar to standard drug diazepam (***Figure 3c***). However, no change was noticed in the duration of convulsions in other azomethine derivative treated groups (***supporting information, figure 1***).

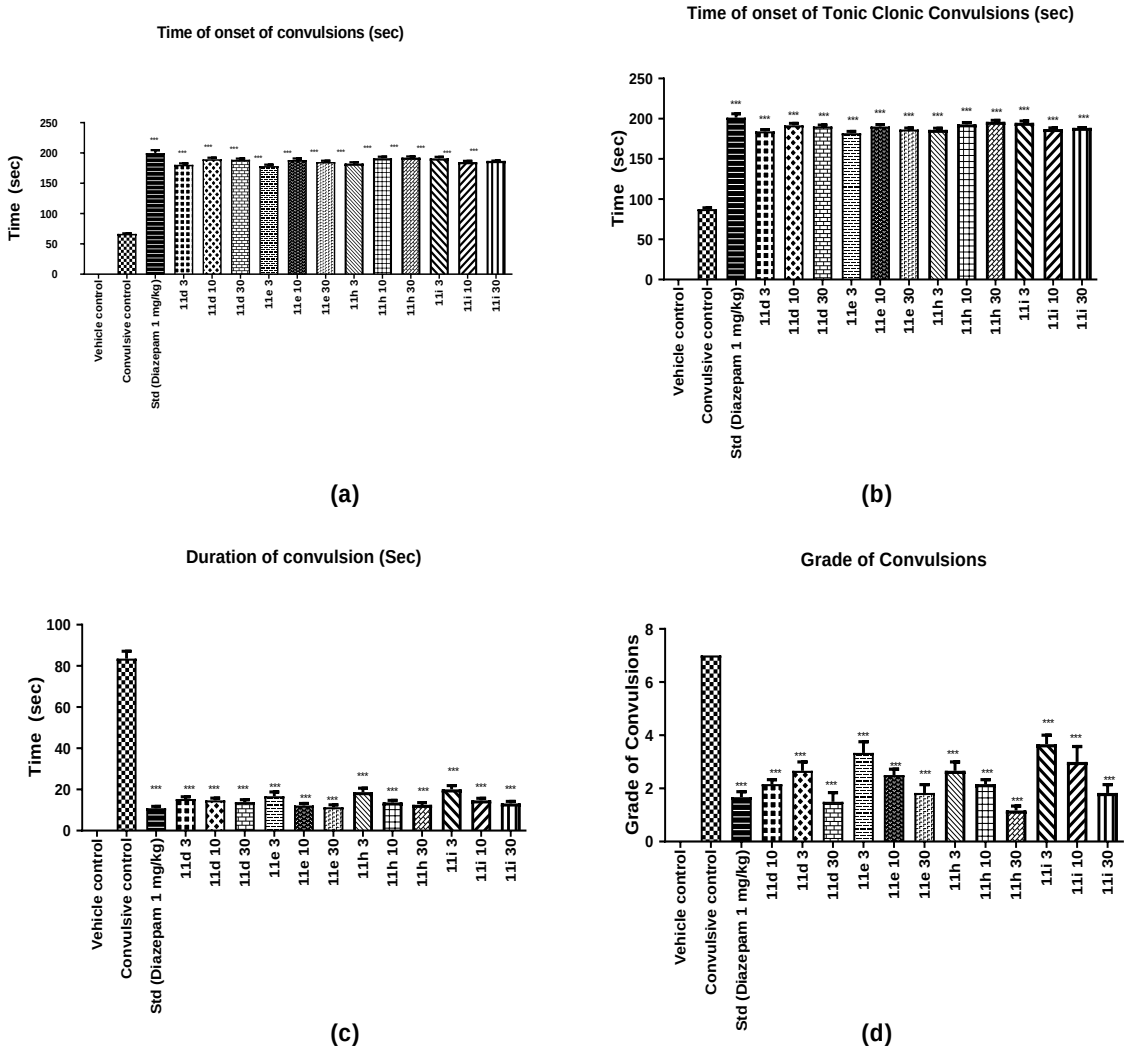


Figure 3: *a) The influence of azomethine derivatives **11d**, **11e**, **11h**, **11i** and standard drug diazepam on the time of onset of convulsions in PTX model in rat. b) The influence of azomethine derivatives **11d**, **11e**, **11h**, **11i** and standard drug diazepam on the time of onset of tonic clonic convulsions in PTX model in rat. c) The influence of azomethine derivatives **11d**, **11e**, **11h**, **11i** and standard drug diazepam on the duration of convulsions in PTX model in rat. d) The influence of azomethine derivatives **11d**, **11e**, **11h**, **11i** and standard drug diazepam on the grade of convulsions in PTX model in rat (Values are represented as mean ±SEM, n = 6, one-way ANOVA followed by Dunnet's t-test, $^{\$\$}p< 0.01$, $^{\$\$\$}p < 0.001$ when compared to vehicle control, *p< 0.05, **p< 0.01, ***p< 0.001 when compared to convulsive control).*

Influence of azomethine derivatives on grade of convulsions

In comparison to the convulsive control group, compounds **11d, 11e, 11h** and **11i** treated group showed a significant decrease (p<0.01) in the grade of convulsions similar to standard drug diazepam (***Figure 3d***). However, other azomethine derivative treated groups have not shown any change in the grade of convulsions (***supporting information, figure 1***). From the whole set of compounds, **11d** and **11h** shows the most potent activity at dose 30 mg/kg as compared to the standard drug diazepam. Current studies, shows the evidence of azomethine derivatives, particularly **11d**, **11e**, **11h** and **11i** are able to ameliorate epileptic seizures induced by

picrotoxin in laboratory animals. The anticonvulsant activity of the novel final compounds is presented in ***Table 1***. Concerning **11a** – **j**, compounds **11c**, **11d**, **11e**, **11h** and **11i** displayed 100 % protection at a dose level of 10 mg/kg for **11c** and 30 mg/kg for rest, compared to diazepam (1 mg/kg).

Table 1*: Anticonvulsant activity of final target compounds in the rat using picrotoxin-induced seizure model and diazepam as reference standard.*

Comp ID/Drug	**Dose (mg/kg)**	**No. of protected animal**	**%Protection**	**ED_{50} (mg/kg) [a]**
Control	0	0	0	0
Diazepam	1	6/6	100	0.14
11a	30	0/6	0	-
	10	0/6	0	
	3	0/6	0	
11b	30	0/6	0	-
	10	0/6	0	
	3	0/6	0	
11c	30	2/6	33.33	-
	10	6/6	100	
	3	2/6	33.33	
11d	30	6/6	100	39.6
	10	4/6	66.66	
	3	4/6	66.66	
11e	30	6/6	100	56.1
	10	5/6	83.33	
	3	4/6	66.66	
11f	30	0/6	0	-
	10	0/6	0	
	3	0/6	0	
11g	30	0/6	0	-
	10	0/6	0	
	3	0/6	0	
11h	30	6/6	100	68.6
	10	4/6	66.66	
	3	4/6	66.66	
11i	30	6/6	100	76.3
	10	3/6	50	
	3	3/6	50	
11j	30	0/6	0	-
	10	0/6	0	
	3	0/6	0	

[a] ED_{50}: Median effective dose

Neurotoxicity screening (NT)

Motor coordination deficits are one of the utmost common adverse effects of antiepileptic drugs (AEDs) and this effect might be a solemn constraint of antiepileptic pharmacotherapy. Here, all final targets at the dose range from 3, 10 and 30 mg/kg those were earlier tested in the picrotoxin-induced seizure model also evaluated for their impact on animals' motor coordination in the rotarod test.[xvi] The outcome gained from the test revealed that none of the evaluated compounds induce motor deficits in experimental animals (***supporting information, figure 2***). Concerning **11a** – **j**, compounds **11c**, **11d**, **11e**, **11h** and **11i**, which displayed 100 % protection, also found 3 to 9 times better at dose 30 mg/kg compared to diazepam (1 mg/kg). The neurotoxicity data of the best compounds of the series and standard drug are presented in ***figure 4***.

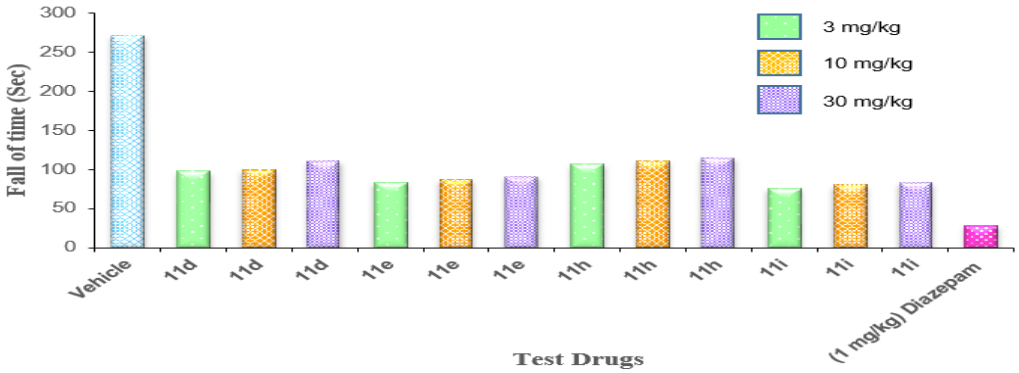


Figure 4: *Comparative locomotor activity (fall of time) of compounds* ***11d***, ***11e***, ***11h*** *and* ***11i*** *with diazepam using rotarod.*

Acute Oral Toxicity Study

The acute toxicity studies of synthesized compounds **11a** – **j** were performed using OECD (Organization of Economic Cooperation and Development) 423 guidelines. Overnight fasted (with free access to water) female Albino Wistar rats of 150 – 180 g were administered stepwise with target compounds **11a** – **j** at dose 5, 50 and 300 mg/kg. The animals were observed continuously for first 4 hours, every half an hour for the next 12 hours and twice in a day for next 14 days. The animals were shown no significant changes in somatomotor activity, mucous membrane, skin, fur, eyes, autonomic, central nervous systems and behavior pattern along with no signs of salivation, tremors, diarrhea, convulsions, lethargy, sleep and coma were noted. There was no mortality was observed.

Quantification studies

The studies in which, the median effective dose (ED_{50}); the dose which brings 50% biological responses in the animals, and median toxic dose (TD_{50}); the dose that gives 50% neurological deficits in the animals were determined. Four active compounds with most promising activity from the set were selected for quantification studies. Diazepam was applied as standard in quantitative assessment. The results of studies in picrotoxin-induced seizure model is presented in ***Table 2***. ED_{50} value of compounds **11d**, **11e** and **11h**; found to be more than diazepam (**11d**, ED_{50} = 39.6 mg/kg, TD_{50} = 330.3 mg/kg; **11e**, ED_{50} = 56.1 mg/kg, TD_{50} = 342.1 mg/kg; **11h**, ED_{50} = 68.6 mg/kg, TD_{50} = 359.4 mg/kg), while their PI values were superior than the used standard diazepam. This indicates the high TD_{50} value of the mentioned compounds. PI value of **11i** indicated that it is less active in comparison to diazepam (ED_{50}=76.3 mg/kg, TD_{50}=351.5 mg/kg, PI=4.61). These values display the good margin of tolerability and safety between doses of anticonvulsants used.

***Table 2**:Quantitative anticonvulsant screening of selected active compounds by picrotoxine-induced seizure model.*

Comp. Id	ED_{50} (mg/kg)[a]	TD_{50} (mg/kg)[b]	PI (TD_{50}/ED_{50})[c]
11d	39.6 (38.9-40.4)	330.3 (329.6-331.1)	8.34
11e	56.1 (54.6-57.6)	342.1 (340.6-343.6)	6.10
11h	68.6 (67.8-69.4)	359.4 (358.6-360.2)	5.24
11i	76.3 (74.9-77.7)	351.5 (350.1-352.9)	4.61
Diazepam[d]	0.14 (0.11-0.17)	0.71 (0.65-0.77)	5.07

[a] ED_{50}: Median effective dose; [b] TD_{50}: Median toxic dose; [c] Protective index (PI): TD_{50}/ED_{50}; [d] ED_{50} data for standard drug diazepam was taken from literature [xvii]; Data in parentheses are the 95 % confidence limits.

***In silico* study**

The physiochemical properties, allied to biological activities are important for designing out the inherent perils of a chemical. The *In-silico* physiochemical properties of the azomethine derivatives **11a** – **j** and reference standard was calculated using molinspiration cheminformatics [xviii] online tool (***Table 3***).

***Table 3:** In-silico physiochemical parameters of azomethine derivatives and the reference anticonvulsant drug Diazepam*

Comp. Id	M.W.[a]	Log P[b]	TPSA[c]	*n*-ROTB[d]	*HBD* [e]	*HBA* [f]	*Lipinski violation*
Rule	< 500	< 5	< 90	< 15	≤ 5	≤ 10	≤ 1
11a	390.14	3.70	66.72	3	1	5	0
11b	404.16	4.10	66.72	3	1	5	0
11c	418.17	4.57	66.72	4	1	5	0
11d	450.16	3.72	85.19	5	1	7	0
11e	391.14	3.75	79.61	3	1	6	0
11f	378.14	3.87	69.62	3	2	5	0
11g	395.10	4.61	53.83	3	1	4	0
11h	487.14	4.82	71.65	5	1	6	0
11i	517.17	5.30	71.65	5	1	6	2
11j	517.17	5.37	71.65	5	1	6	2
5	284.07	2.74	32.67	1	0	3	0

[a] Molecular weight of the compound; [b] Partition coefficient; [c] Topological polar surface area; [d] Number of Rotatable Bonds; [e] Hydrogen bond donor; [f] Hydrogen bond acceptor.

TPSA (topological polar surface area) and LogP a virtuous descriptor characterizing drug absorption, including intestinal absorption, bioavailability and blood-brain barrier penetration. TPSA value >140 $Å^2$ is likely to be deficient at permeating cell membranes and <90 $Å^2$ is necessary for penetration of the blood brain barrier.[xix] TPSA vales for all final target compounds are well within the limit ranging from 54 to 85 $Å^2$.

The other physiochemical properties like molecular weight, number of rotatable bonds, hydrogen bond acceptors and hydrogen bond donors were matched with Lipinski's rule of five,[xx] with exception of compounds **11i** and **11j** which displayed violation of two Lipinski's rules , M.W.: 517.17 and LogP: 5.3. These specification firmly indicate the aptness of the new target compounds as a potential drug like candidates.

In the pharmacokinetics and pharmacodynamics of drugs, solubility of drug plays a crucial role in the drug administration to its absorption and from activity on target part or metabolism excretion from the human body. ADMET prediction of final targets and reference drug were calculated using pkCSM Cambridge online software (***supporting information, Table 1***).[xxi]

Docking Studies

Molecular docking was performed to obtain more insights about interactions between the most active compounds **11d**, **11e**, **11h** and $GABA_A$ receptor active site. A molecular docking study was carried out by Auto Dock 4.2 software, using the implemented empirical free energy function and the Lamarckian Genetic Algorithm (LGA). All the data about binding energies (kcal/mol), number of hydrogen bonds and the number of closest residues around the active site are illustrated (***supporting information Table 2***). A molecular docking score of **11d**, **11e**, **11h** and diazepam were found to be -7.58, -7.42, -8.37 and -6.04 respectively. Notably, the standard drug diazepam interacts via hydrogen bonding with ASN116 amino acid in A chain of $GABA_A$ receptors. Whereas the core ring of diazepam interacts with amino acids, namely ASN88, LEU118 and ARG132 by alkyl interactions. The **11d** shows three hydrogen bonds with TYR160, TYR210 and LYS156 amino acid with $GABA_A$ receptor. The **11e** shows interaction via hydrogen bonding with ASN28 and **11h** shows with PRO97 amino acid. Moreover, the binding pattern and mode of interaction results of compound **11h** at the active site of the $GABA_A$ receptor strongly resemble to the standard drug diazepam. 3D and 2D docking images of **11d**, **11e**, **11h** and diazepam are represented in ***Figure 5***.

Structure activity relationship

Initial structure-activity relationship (SAR) results [xxii,xxiii] revealed that the substitution at the 7th position predominantly modulates the potency of benzodiazepine, whereas any substitutions at positions 6, 8 and 9 may decline the activity. In our previous study, we have shown that the presence of azomethine is also important for activity.[xii] In the present exertion, we synthesized and screened a series of novel azomethine derivatives designed by pharmacophore-based ligand drug design approach **11a** – **j**. Result of *in-vivo* screening of the synthesized compounds found to be encouraging. Pharmacological activity of all the azomethine derivative having fused heterocyclic ring with one hetero atom without any substitution (**11a**, **11f**, **11g**) found to be less active. Compounds having electron donating groups at 8-position of quinoline moiety e.g. 8-ethyl derivative **11c** and 8-methoxy derivative **11d** have shown 100 % protection (***Table-1***) whereas compounds having no substitution at 8 position of quinoline **11a** or compound with poor electron donating group at 8-position **11b** exhibited 0 % protection in the rat using PTX model. On the other hand azomethine derivative having fused heterocyclic ring with two hetero atom without any substitution **11e** have displayed significant anticonvulsant activity. This could be due to the presence of one extra hetero atom, which increases the probability of interaction with the active site. Anticonvulsant activity knowingly altered by electronegativity of substituent's on *N*-pyrazole phenyl ring and substituent's at 3rd position of pyrazole azomethine derivatives. [Potency order: 1-C_6H_5, 3- thienyl (**11h**) > 1-C_6H_5, 3-$C_6H_3F_2$ (**11i**) > 1-C_6H_4F, 3-C_6H_4F (**11j**)]. Analogs **11c**, **11d**, **11e**, **11h** and **11i** were found to be better scaffolds for future modification, which might lead to the potent and safe anticonvulsant agents.

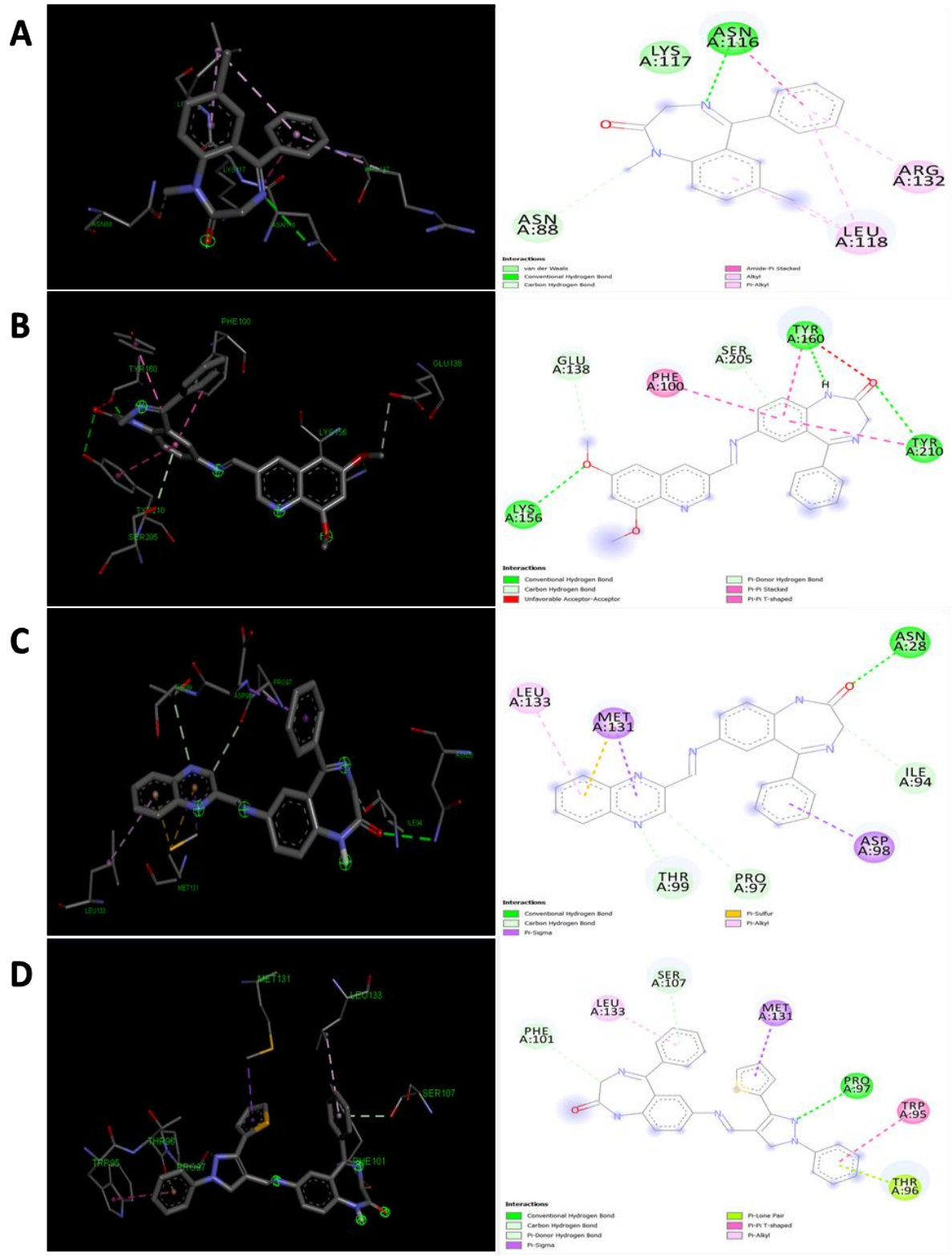


Figure 5: *Molecular docking illustration A) Diazepam, B)* ***11d*** *C)* ***11e****, D)* ***11h***

EXPERIMENTAL SECTION

Chemistry

All the chemical reagent and solvents used for the experiment were commercially purchased and were used without purification. The melting point of all synthesized compounds was measured using SRS-OptiMelt digital melting point apparatus. A Bruker Avance II-400

spectrometer was used to record the 1H and 13C NMR spectra with tetramethylsilane (TMS) as an internal standard. The chemical shifts were given in parts per million (δ) with respective to TMS and coupling constants J is given in Hz. A Shimadzu DRS Prestige 21 was used for to record IR spectra. HRMS analysis performed by Agilent QTOF 6520 mass spectrometer operating at an ionization potential of 70 eV.

Procedure for the synthesis of intermediates (**10**)

Synthesis of the key building block 7-amino-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**10**) was prepared through reported procedure in three steps[xii]

General procedure for the synthesis of Quinoline 3-carbaldehyde (**14a – d**)

Quinoline 3-carbaldehyde (**14a – d**) were prepared through the reported procedure.[xiv]

General procedure for the synthesis of Pyrazole 4-carbaldehyde (**19h** – **j**)

Pyrazole 4-carbaldehyde (**19h – j**) were prepared through the reported procedure.[xv]

General procedure for the synthesis of compounds 11a – j

To a stirred solution of 7-amino-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**10**; 1.0 mmol) in ethanol (7.0 mL) was added aldehydes (1.01 mmol) and a catalytic amount of glacial acetic acid, formed suspension was refluxed for 1 – 6 h, until complete consumption of 7-amino-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one. The reaction mixture was cooled to ambient temperature and the solid collected by filtration under vacuum. The crude solid was thoroughly washed with ethanol (2 × 3 mL), to spare fine powder solid products (**11a – j**) in good yield.

5-Phenyl-7-[(*E*)-3-quinolylmethyleneamino]-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**11a**)

Pale yellow solid; Yield (70 %); m.p: 159 – 161 ^{o}C; IR (KBr) $\tilde{\nu}_{max}$ /cm^{-1}: 2937 – 3196 (C-H), 1680 – 1693 (C=O), 1606 – 1625 (C=N), 1487 (C=C); ^{1}H NMR (DMSO-d$_6$) *δ / ppm*: 4.19 (br. s, 2H, COC**H**$_2$N), 7.20 (d, 1H, *J* = 2.32 Hz, Ar-H), 7.34 (d, 1H, *J* = 8.68 Hz, Ar-H), 7.42 – 7.57 (m, 5H, Ar-H), 7.64 – 7.71 (m, 2H, Ar-H), 7.80 – 7.89 (m, 1H, Ar-H), 8.03 – 8.14 (m, 2H, Ar-H), 8.78 (d, 1H, *J* = 1.40 Hz, Ar-H), 8.85 (s, 1H, -C**H**=N-), 9.39 (d, 1H, *J* = 1.96 Hz, Ar-H), 10.67 (br. s, H–N, D$_2$O exchangeable); ^{13}C NMR (DMSO-d$_6$) *δ / ppm*: 57.1 (C3), 122.1 (C6), 123.2 (C9), 124.7 (C8), 127.0 (C14), 127.1 (C11), 127.4 (C17), 128.3 (C3' and C5'), 128.6 (C20), 128.9 (C16), 129.1 (C19),129.3 (C2' and C6'), 130.3 (C18), 131.1 (C4'), 137.2 (C15), 138.3 (C10), 139.0 (C1'), 145.1 (C7), 148.5 (C21), 149.4 (C13), 159.0 (C12), 169.4 (C5), 170.2 (C2); HRMS (ESI): m/z calcd for $C_{25}H_{19}N_4O^+$: 391.1553 [*M* + H]$^+$, found: 391.1568.

7-[(*E*)-(8-Methyl-3-quinolyl)methyleneamino]-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**11b**)

Pale yellow solid; Yield (70 %); m.p: 247 – 249 ^{o}C; IR (KBr) $\tilde{\nu}_{max}$ /cm^{-1}: 2875 – 3209 (C-H), 1678 (C=O), 1614 (C=N), 1494 (C=C); ^{1}H NMR (CDCl$_3$) *δ / ppm*: 2.84 (s, 3H, CH$_3$), 4.4 (br. s, 2H, COCH$_2$N), 7.22 – 7.26 (m, 2H, Ar-H), 7.39 – 7.54 (m, 5H, Ar-H), 7.59 – 7.67 (m, 3H, Ar-H), 7.75 (d, 1H, *J* = 8.07 Hz, Ar-H), 8.54 (d, 1H, *J* = 1.96 Hz, Ar-H), 8.6 (s, 1H, -CH=N), 8.89 (s, 1H), 9.41 (d, 1H, *J* = 1.83 Hz, Ar-H); ^{13}C NMR (CDCl$_3$) *δ / ppm*: 18.1 (CH3), 56.7 (C3), 122.1 (C6), 123.1 (C9), 125.0 (C8), 126.7 (C17), 127.1 (C16), 127.4 (C14), 128.0 (C11), 128.1 (C20), 128.3 (C3' and C5'), 129.6 (C2' and C6'), 130.4 (C18), 131.4 (C4'), 137.1 (C15), 137.2 (C10), 137.4 (C19), 139.2 (C1'), 146.7 (C7), 148.4 (C21), 148.7 (C13), 158.3 (C12),

170.6 (C5), 171.9 (C2); HRMS (ESI): m/z calcd for $C_{26}H_{19}N_4O^-$: 403.1564 [*M* - H]$^-$, found: 403.1567.

7-[(*E*)-(8-Ethyl-3-quinolyl)methyleneamino]-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**11c**)

Yellow solid; Yield (85 %); m.p. 224 – 226 ºC; IR (KBr) $\tilde{\nu}_{max}$ /cm^{-1}: 2881 – 3197 (C-H), 1678 (C=O), 1612 – 1627 (C=N), 1490 (C=C); ^{1}H NMR (DMSO-d$_6$) *δ / ppm*: 1.29 (t, 3H, *J* = 7.46 Hz, CH_3), 3.23 (q, 2H, -C**H**$_2$-CH_3), 4.19 (br. s, 2H, COC**H**$_2$N), 7.20 (d, 1H, *J* = 2.32 Hz, Ar-H), 7.34 (d, 1H, *J* = 8.68 Hz, Ar-H), 7.42 – 7.61 (m, 6H, Ar-H), 7.65 – 7.71 (m, 2H, Ar-H), 7.92 (d, 1H, *J* = 7.79 Hz, Ar-H), 8.75 (d, 1H, *J* = 2.08 Hz, Ar-H), 8.84 (s, 1H), 9.38 (d, 1H, *J* = 2.08 Hz, Ar-H), 10.66 (br. s, H–N; D_2O exchangeable); ^{13}C NMR (DMSO-d$_6$) *δ / ppm*: 15.2 (CH3), 23.9 (-CH2), 57.0 (C3), 122.1 (C6), 123.1 (C9), 124.6 (C8), 126.9 (C14), 127.0 (C17), 127.1 (C11), 127.2 (C 16), 128.31 (C3' and C5'), 128.33 (C20),129.2 (C2' and C6'), 129.6 (C18), 130.2 (C4'), 137.4 (C15), 138.2 (C10), 138.9 (C19), 142.3 (C1'), 145.2 (C7), 146.8 (C21), 148.4 (C13), 159.0 (C12), 169.3 (C5), 170.2 (C2); HRMS (ESI): m/z calcd for $C_{27}H_{23}N_4O^+$: 419.1866 [*M* + H]$^+$, found: 419.1885.

7-[(*E*)-(6,8-Dimethoxy-3-quinolyl)methyleneamino]-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**11d**)

Yellow solid; Yield (76 %); m.p. 260 – 262 ºC; IR (KBr) $\tilde{\nu}_{max}$ /cm^{-1}: 2881 – 3176 (C-H), 1685 (C=O), 1618 (C=N), 1490 (C=C); ^{1}H NMR (DMSO-d$_6$) *δ / ppm*: 3.93 (s, 3H, OCH_3), 3.99 (s, 3H, -OCH_3), 4.18 (br. s, 2H, COC**H**$_2$N), 6.74 (d, 1H, *J* = 2.20 Hz, Ar-H), 7.05 (d, 1H, *J* = 1.83 Hz, Ar-H), 7.17 (d, 1H, *J* = 2.45 Hz, Ar-H), 7.32 (d, 1H, *J* = 8.68 Hz, Ar-H), 7.42 – 7.56 (m, 5H, Ar-H), 7.64 (dd, 1H, *J* = 8.62, 2.38 Hz, Ar-H), 8.79 (d, 1H, *J* = 1.90 Hz, Ar-H), 8.81 (s, 1H, -C**H**=N-), 9.27 (d, 1H, *J* = 1.96 Hz, Ar-H), 10.63 (br. s, H–N; D_2O exchangeable); ^{13}C NMR (DMSO-d$_6$) *δ / ppm*: 55.7 (OCH_3), 56.1 (OCH_3), 57.0 (C3), 98.9 (C17), 100.0 (C19), 115.0 (C20), 122.0 (C6), 123.0 (C9), 124.6 (C8), 125.9 (C14), 126.9 (C11), 128.3 (C3' and C5'), 129.2 (C2' and C6'), 130.2 (C15), 131.1 (C4'), 138.0 (C10), 138.9 (C1'), 145.3 (C7), 150.2 (C13), 150.9 (C21), 156.2 (C18), 159.0 (C12), 162.5 (C16), 169.3 (C5), 170.2 (C2); HRMS (ESI): m/z calcd for $C_{27}H_{23}N_4O_3^+$: 451.1765 [*M* + H]$^+$, found: 451.1773.

5-Phenyl-7-[(*E*)-quinoxalin-2-ylmethyleneamino]-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**11e**)

Yellow solid; Yield (85 %); m.p. 240 – 242 ºC; IR (KBr) $\tilde{\nu}_{max}$ /cm^{-1}: 2875 – 3203 (C-H), 1681 (C=O), 1610 – 1624 (C=N), 1489 (C=C); ^{1}H NMR (DMSO-d$_6$) *δ / ppm*: 4.20 (br. s, 2H; COC**H**$_2$N), 7.33 (d, 1H, *J* = 2.32 Hz, Ar-H), 7.36 (d, 1H, *J* = 8.80 Hz, Ar-H), 7.42 – 7.58 (m, 5H, Ar-H), 7.82 (dd, 1H, *J* = 8.68, 2.45 Hz, Ar-H), 7.91 – 7.96 (m, 2H, Ar-H), 8.13 – 8.19 (m, 2H, Ar-H), 8.86 (s, 1H, -C**H**=N-), 9.57 (s, 1H, Ar-H), 10.73 (br. s, H–N, D_2O exchangeable); ^{13}C NMR (DMSO-d$_6$) *δ / ppm*: 57.1 (C3), 122.2 (C6), 124.4 (C9), 124.5 (C8), 126.9 (C11), 128.3 (C3' and C5'), 129.0 (C15), 129.2 (C2' and C6'), 129.3 (C18), 130.2 (C16), 130.9 (C17), 131.5 (C4'), 138.9 (C10), 139.2 (C1'), 141.2 (C19), 142.1 (C7), 143.5 (C13), 143.7 (C20), 148.4 (C14), 159.0 (C12), 169.3 (C5), 170.1 (C2); HRMS (ESI): m/z calcd for $C_{24}H_{18}N_5O^+$: 392.1506 [*M* + H]$^+$, found: 392.1519.

7-[(*E*)-1H-Indol-3-ylmethyleneamino]-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**11f**)

Yellow solid; Yield (85 %); m.p. 286 – 288 ºC; IR (KBr) $\tilde{\nu}_{max}$ /cm^{-1}: 2866 – 3197 (C-H), 1685 (C=O), 1618 (C=N), 1490 (C=C); ^{1}H NMR (DMSO-d$_6$) *δ / ppm*: 4.16 (br. s, 2H, COC**H**$_2$N),

7.00 (d, 1H, *J* = 2.26 Hz, Ar-H), 7.12 – 7.29 (m, 3H, Ar-H), 7.41 – 7.57 (m, 7H, Ar-H), 7.96 (d, 1H, *J* = 3.01 Hz, Ar-H), 8.29 (d, 1H, *J* = 7.78 Hz, Ar-H), 8.65 (s, 1H, -C**H**=N-), 10.54 (br. s, H–N-CO, D_2O exchangeable), 11.77 (br. s, H–N, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) *δ / ppm*: 57.0 (C3), 111.9 (C18), 114.8 (C14), 120.9 (C16), 121.7 (C6), 121.9 (C17), 122.0 (C15), 122.8 (C9), 124.5 (C8), 124.6 (C19), 127.0 (C11), 128.2 (C3' and C5'), 129.2 (C2' and C6'), 130.2 (C13), 133.9 (C4'), 136.6 (C10), 137.1 (C20), 139.1 (C1'), 147.4 (C7), 155.8 (C12), 169.5 (C5), 170.2 (C2); HRMS (ESI): m/z calcd for $C_{24}H_{19}N_4O^+$: 379.1553 [*M* + H]$^+$, found: 379.1568.

7-[(*E*)-Benzothiophen-3-ylmethyleneamino]-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**11g**)

Pale yellow solid; Yield (85 %); m.p. 271 – 273 ^{o}C; IR (KBr) $\tilde{\nu}_{max}$ /cm^{-1}: 2883 – 3170 (C-H), 1689 (C=O), 1618 (C=N), 1460 – 1483 (C=C); ^{1}H NMR (DMSO-d_6) *δ / ppm*: 4.18 (br. s, 2H, COC**H**$_2$N), 7.15 (d, 1H, *J* = 2.20 Hz, Ar-H), 7.33 (d, 1H, *J* = 8.68 Hz, Ar-H), 7.41 – 7.53 (m, 5H, Ar-H), 7.55 (d, 2H, *J* = 6.97 Hz, Ar-H), 7.63 (dd, 1H, *J* = 8.62, 2.26 Hz, Ar-H), 8.07 (d, 1H, *J* = 7.21 Hz, Ar-H), 8.47 (s, 1H, -C**H**=N-), 8.82 – 8.89 (m, 2H, Ar-H), 10.62 (br. s, H–N, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) *δ / ppm*: 57.5 (C3), 122.6 (C6), 123.1 (C18), 123.4 (C9), 125.1 (C15), 125.3 (C16), 125.7 (C17), 125.9 (C8), 127.5 (C19), 128.8 (C3' and C5'), 129.7 (C2' and C6'), 130.7 (C13), 133.6 (C14), 136.2 (C11), 138.2 (C10), 138.4 (C4'), 139.5 (C1'), 140.6 (C20), 146.4 (C7), 156.7 (C12), 169.8 (C5), 170.7 (C2); HRMS (ESI): m/z calcd for $C_{24}H_{16}N_3OS^-$: 394.1020 [*M* - H]$^-$, found: 394.1024.

5-Phenyl-7-[(*E*)-[1-phenyl-3-(2-thienyl)pyrazol-4-yl]methyleneamino]-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**11h**)

Pale yellow solid; Yield (85 %); m.p. 241 – 243 ^{o}C; IR (KBr) $\tilde{\nu}_{max}$ /cm^{-1}: 2856 – 3134 (C-H), 1685 (C=O), 1595 – 1618 (C=N), 1496 (C=C); ^{1}H NMR (DMSO-d_6) *δ / ppm*: 4.18 (br. s, 2H, COC**H**$_2$N), 7.08 (d, 1H, *J* = 2.51 Hz, Ar-H), 7.17 (dd, 1H, *J* = 5.02, 3.51 Hz, Ar-H), 7.31 (d, 1H, *J* = 8.78 Hz, Ar-H), 7.36 – 7.60 (m, 9H, Ar-H), 7.65 (dd, 1H, *J* = 5.02, 1.00 Hz, Ar-H), 7.89 (dd, 1H, *J* = 3.76, 1.00 Hz, Ar-H), 7.96 (d, 2H, *J* = 7.83 Hz, Ar-H), 8.66 (s, 1H, Ar-H), 9.13 (s, 1H, -C**H**=N-), 10.59 (br. s, H–N, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) *δ / ppm*: 57.0 (C3), 118.7 (C2''' and 6'''), 119.2 (C13), 122.1 (C6), 122.7 (C9), 124.4 (C8), 126.9 (C11), 127.2 (C2'' and 4'''), 127.9 (C3''), 128.0 (C4''), 128.2 (C3' and C5'), 129.2 (C2' and C6'), 129.6 (C3''' and 5'''), 130.2 (C14), 130.6 (C4'), 133.8 (C15), 137.6 (C10), 138.5 (C1'), 139.0 (C1'''), 146.0 (C1''), 146.6 (C7), 152.6 (C12), 169.4 (C5), 170.1 (C2); HRMS (ESI): m/z calcd for $C_{29}H_{22}N_5OS^+$: 488.1540 [*M* + H]$^+$, found: 488.1545.

7-[(*E*)-[3-(3,5-Difluorophenyl)-1-phenyl-pyrazol-4-yl]methyleneamino]-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**11i**)

White solid; Yield (85 %); m.p. 250 – 252 ^{o}C; IR (KBr) $\tilde{\nu}_{max}$ /cm^{-1}: 3049 – 3196 (C-H), 1689 – 1695 (C=O), 1610 – 1618 (C=N), 1487 – 1500 (C=C), 1126 (C-F); ^{1}H NMR (DMSO-d_6) *δ / ppm*: 4.16 (br. s, 2H, COC**H**$_2$N), 7.05 (d, 1H, *J* = 2.20 Hz, Ar-H), 7.24 – 7.34 (m, 2H, Ar-H), 7.36 – 7.56 (m, 9H, Ar-H), 7.64 (br. d, 2H, *J* = 6.60 Hz, Ar-H), 7.98 (d, 2H, *J* = 7.95 Hz, Ar-H), 8.54 (s, 1H, Ar-H), 9.13 (s, 1H, -C**H**=N-), 10.57 (br. s, H–N, D_2O exchangeable); ^{13}C NMR (DMSO-d_6) *δ / ppm*: 57.0 (C3), 104.5 (t, $^2J_{C,F}$ = 25.68 Hz, C4''), 112.1 (d, $^2J_{C,F}$ = 22.81 Hz, C2''), 112.4 (d, $^2J_{C,F}$ = 22.81 Hz, C6''), 119.5 (C2''' and C6'''), 120.5 (C13), 122.5 (C6), 123.3 (C9), 124.8 (C8), 127.4 (C4'''), 127.9 (C11), 128.7 (C3' and 5'), 129.7 (C2' and C6'), 130.1 (C3''' and C5'''), 130.7 (C14), 131.4 (C4'), 135.8 (t, $^3J_{C,F}$ = 10.32 Hz, C1''), 138.1 (C10), 139.1 (C1'), 139.5 (C1'''), 146.3 (C7), 150.3 (C15), 153.1 (C12), 162.8 (d, $^1J_{C,F}$ =

247.62 Hz, C3''), 162.9 (d, $^{1}J_{C,F}$ = 247.62 Hz, C2''), 169.8 (C5), 170.6 (C2); HRMS (ESI): m/z calcd for $C_{31}H_{22}F_2N_5O^+$: 518.1787 [*M* + H]$^+$, found: 518.1801.

7-[(*E*)-[1,3-Bis(4-fluorophenyl)pyrazol-4-yl]methyleneamino]-5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one (**11j**)

Pale yellow solid; Yield (80 %); m.p. 263 – 265 ^{o}C; IR (KBr) $\tilde{\nu}_{max}$ /cm^{-1}: 2856 – 3132 (C-H), 1685 (C=O), 1608 – 1618 (C=N), 1490 (C=C), 1012 (C-F); ^{1}H NMR (DMSO-d$_6$) *δ / ppm*: 4.16 (br. s, 2H, COC**H**$_2$N), 7.03 (d, 1H; *J* = 2.51 Hz, Ar-H), 7.26 – 7.55 (m, 11H, Ar-H), 7.85 (dd, 2H, *J* = 8.78, 5.52 Hz, Ar-H), 8.04 (dd, 2H, *J* = 9.29, 4.77 Hz, Ar-H), 8.49 (s, 1H, Ar-H), 9.13 (s, 1H, -C**H**=N-), 10.57 (br. s, H–N, D$_2$O exchangeable); ^{13}C NMR (DMSO-d$_6$) *δ / ppm*: 57.5 (C3), 116.0 (d, $^{2}J_{C,F}$ = 22.10 Hz, C3'' and 5''), 116.8 (d, $^{2}J_{C,F}$ = 23.10 Hz, C3''' and 5'''), 120.1 (C13), 121.5 (d, $^{3}J_{C,F}$ = 11.02 Hz, C2''' and 6'''), 122.5 (C6), 123.4 (C9), 124.7 (C8), 127.3 (C11), 128.7 (C3' and 5'), 128.8 (C1''), 129.7 (C2' and C6'), 130.3 (C14), 130.6 (C4'), 131.2 (d, $^{3}J_{C,F}$ = 8.22 Hz; C2'' and 6''), 135.9 (C1'''), 138.0 (C10), 139.5 (C1'), 146.5 (C7), 150.2 (C15), 153.3 (C12), 161.3 (d, $^{1}J_{C,F}$ = 244.40 Hz, C4'''), 162.9 (d, $^{1}J_{C,F}$ = 246.30 Hz, C4''),169.8 (C5), 170.6 (C2); HRMS (ESI): m/z calcd for $C_{31}H_{22}F_2N_5O^+$: 518.1787 [*M* + H]$^+$, found: 518.1795.

Pharmacology

Animals

Adult Albino Wistar rats of either sex weighting 150 – 180 g were obtained from National Institute of Biosciences, Pune and housed for one week at standard laboratory conditions in groups of six animals per cage in the institutional animal house separately. Animals had free access to food and water ad libitum. The Institutional Animal Ethics Committee approved the research protocol and followed the guidelines of Committee for Control and Supervision of Experimentation on Animals (CPCSEA), Government of India on animal experimentation (CPCSEA approval no - IAEC/PCP/PCL03/2020-2021).

Acute oral toxicity study

The acute toxicity studies of synthesized compounds **11a** – **j** were performed using OECD (Organization of Economic Cooperation and Development) 423 guidelines. The acute toxic class method is a step-wise procedure with three animals per step as 5, 50, 300 and 2000 mg/kg body weight. Depending on the mortality or morbidity status of the animals, the next step is decided. In the current experimentation, overnight fasted (with free access to water) female Albino Wistar rats of 150 – 180 g were administered stepwise with 5, 50 and 300 mg/kg. The animals were observed continuously for first 4 hours, every half an hour for the next 12 hours and twice in a day for next 14 days. The animals were observed for changes in somatomotor activity, mucous membrane, skin, fur, eyes, autonomic, central nervous systems and behavior pattern along with no signs of salivation, tremors, diarrhea, convulsions, lethargy, sleep, coma and mortality. The doses 3, 10 and 30 mg/kg were decided for screening of further anti-convulsant activity.

In vivo anticonvulsant activity of target compounds

The animals were screened for the anticonvulsant property in PTX model. This test was used to identify compounds that decrease the seizure threshold. Male albino Wistar rats weighing 150 – 180 g were divided into 13 groups: Group 1 served as normal control and was treated with vehicle 2 % w/v of Tween 80 orally. Group 2 served as convulsive control and received 3.5 mg/kg picrotoxin subcutaneously. Group 3 served as a standard group and was treated with Diazepam 1 mg/kg orally and picrotoxin (3.5 mg/kg subcutaneously). Groups 4 to 13 consisted of 18 animals each and were administered with **11a** – **j** (3, 10 and 30 mg/kg) and picrotoxin

(3.5 mg/kg subcutaneously). The animals were administered orally with respective treatment 24 hours and 1 h before the experimentation. The grade of convulsion, time of onset of tonic clonic convulsions and time of onset of convulsions was documented. 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 evaluated by One-way ANOVA followed by Dunnet's 't' test using GraphPad Prism v. 5 demo. The values are expressed as mean ± SEM, n = 6. $p<0.05$ was considered significant.[xii]

Neurotoxicity screening (NT)

The extent of motor impairment was assessed in rats by the rotarod test. The rats (150 – 180 g) were trained to stay on an accelerating rotarod that rotated at 20 rotations/min and its diameter were 3.2 cm. Only those rats were taken for testing, which could stay on the revolving rod for at least one minute. The test compounds at the doses 3, 10 and 30 mg/kg were administered orally and the time taken to fall off the rotarod was noted. Neurotoxicity was indicated by the inability of the animal to maintain equilibration on a rod for at least one minute. The time spent on the rod is recorded automatically for each animal, the time at which an animal fell off a rod is determined.[xvi]

Quantification studies

For determination of ED_{50} and TD_{50}, groups of animals were orally administered with various doses of the tested compounds until three points were found in the range of 10 – 90 % seizure protection or minimal neurotoxicity. To calculate both ED_{50} and TD_{50} values with 95 % confidence limits, modified Lorke's method was used. The PI's for tested compounds were calculated by following formula: TD_{50}/ED_{50}.[xxiv]

Prediction of *In silico* properties

The physiochemical properties and ADMET (absorption, distribution, metabolism, excretion, and toxicity) prediction plays an important role during the drug discovery, lead identification and optimization. The *In-silico* physiochemical properties of the final target compounds **11a** – **j** and reference standard was calculated using molinspiration chemoinformatics[xviii] online tool, while ADMET properties were predicted using pkCSM Cambridge online software.[xxi]

Docking Studies

Ligand preparation:

The structure of all the ligands was converted into 3D before analysis. The 3D structure later converted from SDF to PDB format using Pymol software. The metals were also removed from the ligand using Pymol software for appropriate docking study. The prepared ligands were saved in PDB format for further docking studies. On the basis of energy minimization the drug binds to effectors/receptors in the most stable form that is the minimum energy form. The active compounds were subjected to conformational analysis and energy minimization using Monte Carlo conformational search. Low energy conformers of all the structures were generated, which was utilized further for analysis.[xxv]

Protein Preparation and Molecular Docking:

The crystal structure of Gamma-aminobutyric acid receptor subunit alpha-1 (PDB ID: 6HUJ) downloaded from PDB database.[xxvi] All the water molecules were removed from the crystal structure of $GABA_A$ receptors. The AutoDock Vina 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 the all compounds **11d**, **11e** and **11h** were executed by AutoDock 4.2 software. A docking grid box was built with 62, 62 and 126 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 analysed using AutoDock 4.2 software as well as in Discovery Studio 4.0.[xxvii–xxix]

CONCLUSIONS

The study reveals the anticonvulsant potential of 5-phenyl-1,3-dihydro-2*H*-1,4-benzodiazepin-2-one azomethine derivatives (**11a** – **j**). The anticonvulsant assessment was performed in rat using PTX model. The results indicated that azomethine derivatives **11c**, **11d**, **11e**, **11h** and **11i** displayed 100 % protection and significant decrease in convulsion grade compared to the control convulsive group. Moreover, there is no hypnotic effect observed at dose 30 mg/kg compared to diazepam (1 mg/kg) by rotarod test. Whereas compounds **11a**, **11b**, **11f**, **11g** and **11j** had shown 100 % mortality of experimental animals in PTX model. Further, *in vivo* acute oral toxicity study and *in silico* ADMET results also supported that the designed azomethine derivatives have a very good pharmacokinetic profile to become a potential drug candidate. Some of the above declared compounds have shown superior protection and may have future commitment. From current study, novel analogs were identified as potential leads. Our upcoming efforts are optimization of the lead molecules to develop potent and safe anticonvulsant agents.

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