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Original article

Design, synthesis and potential 6 Hz psychomotor seizure test activity of some novel 2-(substituted)-3-{[substituted]amino}quinazolin-4(3*H*)-one

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ABSTRACT

Thirty new 2-(substituted)-3-{[substituted]amino}quinazolin-4(3*H*)-one were designed and synthesized keeping in view the structural requirement of pharmacophore and evaluated for anticonvulsant activity and neurotoxicity. The anticonvulsant activity of the titled compounds was assessed using the 6 Hz psychomotor seizure test. The most active compound of the series was 3-({(*E*)-[3-(4-chloro-3-methylphenoxy)phenyl]methylidene}amino)-2-phenylquinazolin-4(3*H*)-one **PhQZ 7**, which showed 100% protection (4/4, 0.5 h) and 75% protection (3/4, 0.25 h) at a dose of 100 mg/kg in mice. A computational study was carried out for calculation of pharmacophore pattern and prediction of pharmacokinetic properties. Titled compounds have also exhibited good binding properties with epilepsy molecular targets such as glutamate, GABA (A) delta and GABA (A) alpha-1 receptors, in Lamarckian genetic algorithm based flexible docking studies.

1. Introduction

Quinazolin-4(3*H*)-one **1** and their derivatives constitute an important class of heterocyclic compounds and are shown to have potent CNS activities such as anticonvulsant [1–5] and CNS depressant [6,7]. A literature survey revealed that the presence of a substituted aromatic ring at position 3 and a methyl group at position 2 on quinazolin-4(3*H*)-one nucleus is a necessary requirement for CNS depression and anticonvulsant activities. Modification of the methyl group by some other chemical moiety yielded structural analogs with potent CNS activity. Methaqualone **2** (2-methyl-3-o-tolyl-4(3*H*)-quinazolinone) is a well known sedative-hypnotic containing quinazolin-4(3*H*)-one nucleus. In fact, these evidences suggest that the quinazolin-4(3*H*)-one nucleus possess a pharmacophoric character for CNS activity. In addition, the 4-(aryloxy) phenyl semicarbazones **3** (Fig. 1) were reported as potential anticonvulsant [8]. The design and synthesis of the titled compounds were carried out with two objectives: the first was the molecular hybridization of Methaqualone, having Quinazolin-4(3*H*)-one nucleus and 4-(aryloxy) phenyl semicarbazones (Fig. 2). The second objective was the replacement of methyl group at position 2 of synthesized compounds by phenyl and n-propyl group and to report their effect on anticonvulsant activity. The second objective was based upon the hypothesis that methyl group at the second position of quinazolin-4(3*H*)-one is not always necessary for the CNS activity and other groups when placed at this position can also lead to potent CNS active agents [9,10]. The 2-substituted quinazolin-4(3*H*)-one was also coupled with isatin, a well known anticonvulsant.

Based on the literature review, we are the first to report the synthesis and anticonvulsant activities of 2-(substituted)-3-{[substituted]amino}quinazolin-4(3*H*)-one. Their chemical structures were characterized using IR, ^{1}H NMR, MS and elemental analysis techniques. All the synthesized titled compounds comprised of the essential pharmacophoric elements (Fig. 3) that are necessary for good anticonvulsant activity as suggested by Unverferth et al. [11]. In addition, their anticonvulsant activity was evaluated by using 6 Hz psychomotor seizure test in mice. The rotarod assay was performed in mice to evaluate the neurotoxicity of the compounds. Computational study was also carried out to highlight the pharmacophore distance mapping, log P calculation and pharmacokinetic parameters. In this study, we have used Autodoc 4.0 along with its LGA algorithm for automated flexible ligand docking of compounds with six established epilepsy molecular targets and evaluated their affinity and hydrogen bonding.

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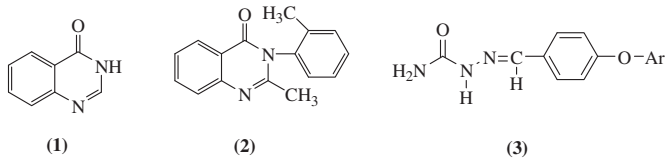


Fig. 1. Chemical structure of quinazolin-4(3*H*)-one (1), Methaqualone (2) and 4-(aryloxy)phenyl semicarbazone **(3)**.

2. Chemistry

The reaction sequence leading to the synthesis of titled compounds, viz. 2-(substituted)-3-{[substituted]amino}quinazolin-4(3*H*)-one (**PhQZ 1–10**, **MtQZ 1–10** and **PrQZ 1–10**) is shown in Scheme 1. The 4-substituted benzaldehydes **3a–i** were prepared by refluxing various substituted phenol **1a–i** with 4-fluoro benzaldehyde **2** in *N,N*-DMF in presence of potassium carbonate. The 2-phenyl-4*H*-3,1-benzoxazin-4-one **5** was obtained by reacting anthranilic acid **4** with benzoyl chloride in pyridine. The 2-methyl-4*H*-3,1-benzoxazin-4-one **6** was obtained by refluxing anthranilic acid **4** in acetic anhydride. Anthranilic acid **4** was refluxed in butyric anhydride to obtain *N*-propyl anthranilic acid **7**, which was further refluxed with acetic anhydride to obtained 2-propyl-4*H*-3,1-benzoxazin-4-one **8**. The 2-substituted-4*H*-3,1-benzoxazin-4-ones (**5**, **6**, **8**) were converted to their corresponding amines i.e., 3-amino-2-(substituted)-quinazolin-4(3*H*)-one (**9**, **10**, **11**) by reacting with hydrazine hydrate. The 3-amino-2-(substituted)-quinazolin-4(3*H*)-ones (**9, 10, 11**) were condensed with various 4-substituted benzaldehyde **3a–i**/isatin **3j** to yield the titled compounds **PhQZ 1–10**, **MtQZ 1–10** and **PrQZ 1–10**. Thin layer chromatography (TLC) was run throughout the reactions to optimize the reactions for purity and completion. The physical and elemental analyses data of the titled compounds are summarized in Table 1.

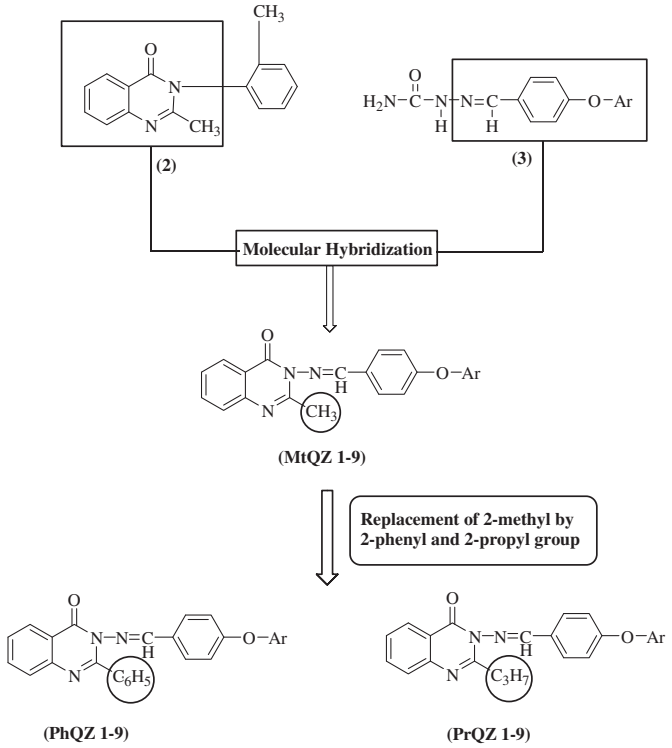


Fig. 2. Rational concept to new 2-(substituted)-3-{[substituted]amino}quinazolin-4(3*H*)-ones **PhQZ 1–10**, **MtQZ 1–10** and **PrQZ 1–10**.

The structural assignments to new compounds were based on their elemental analysis and spectral (FT-IR, ^{1}H NMR and mass) data. The formation of 4-substituted benzaldehydes **3a-i** from 4-substituted phenols **1a-i** was confirmed by its IR and ^{1}H NMR spectral studies. The IR spectrum of 4-(4-fluorophenoxy) benzaldehydes **3f** showed bands at 1665, 3035 and 1240 cm^{-1} indicating the presence of C=O stretching, C–H stretching and diaryl ether linkage (–O–). The absence of broad band of phenolic –OH at 2900–3000 cm^{-1} confirms the conversion of 4-fluoro phenol **1f** to **3f**. In its ^{1}H NMR spectrum a singlet at δ 9.87 ppm indicating the presence of –CHO group, where as absence of a singlet around δ 9.288 ppm for phenolic –OH confirms the conversion of **1f** to **3f**.

The IR spectrum of the titled compound **PhQZ 7** over the 3067 cm^{-1} showed multiple weak absorption peak corresponding to Qu-H and Ar–H stretching vibration. The strong absorption at 1685 cm^{-1} is due to the C=O stretching vibration and the moderate intensity absorption at 1596 cm^{-1} corresponds to a CH=N stretching vibration. The 1447–1526 cm^{-1} absorptions are due to the skeleton vibration of the aryl and heterocyclic rings. The strong absorption at 1240 cm^{-1} corresponds to diaryl ether linkage (O). The absorption peaks at 755 and 699 cm^{-1} arise due to the phenyl substitution at 2-position in the quinazolinone. Its ^{1}H NMR spectrum showed a singlet at δ 8.45 ppm due to the proton attached to the imine carbon. A set of signals appear between δ 6.82 and 8.39 ppm corresponds to aromatic and quinazolinone protons. The methyl protons appear at δ 2.36 ppm. The presence of CH=N stretching vibration at 1596 cm^{-1} in IR spectrum and a singlet for proton attached to the imine carbon at δ 8.45 ppm confirm the formation of **PhQZ 7**. Further mass spectrum confirmed their purity and molecular weight.

3. Pharmacology

The newly synthesized 2-(substituted)-3-{[substituted]amino} quinazolin-4(3*H*)-one derivatives (**PhQZ 1–10**, **MtQZ 1–10**, **PrQZ 1–10**) were subjected to anticonvulsant screening according to anticonvulsant drug development (ADD) program protocol. The profile of anticonvulsant activity of six titled compounds was established after i.p. injections into mice and compounds were evaluated in the maximal electroshock (MES) and subcutaneous metrazole (scMET) model using doses of 30, 100 and 300 mg/kg at two different time intervals. The twenty four titled compounds were screened in 6 Hz psychomotor seizure test to identify their anticonvulsant activity at five different time points, i.e., 0.25 h, 0.5 h, 1.0 h, 2.0 h and 4.0 h after i.p. administration in mice. The results are shown in Table 2. Compound showing potential activity was also subjected to quantification studies in 6 Hz test and neurotoxicity. The corresponding an ED_{50} and a TD_{50} were reported in Table 3. The most active compound was screened in *in-vitro* hippocampal slice culture neuroprotection assay (NP) and results are shown in Table 4. Neurotoxicity was observed by minimal motor impairment which was measured by the rotarod test.

4. Computational study

The pharmacophore pattern studies in which distance between the various groups postulated as essential for anticonvulsant activity were done on the 3D optimized structures using ACD/3D viewer version 12.0 and Argus Lab 4.0 Mark A. Thompson Planaria Software LLC. A computational study of all titled compounds was performed for prediction of ADME properties such as absorption (% ABS), polar surface area (TPSA), milog P, number of rotatable bonds, and violations of Lipinski's rule of five by using molinspiration online property calculation toolkit. Calculated milog P for synthesized compounds were then compared with the experimental log P

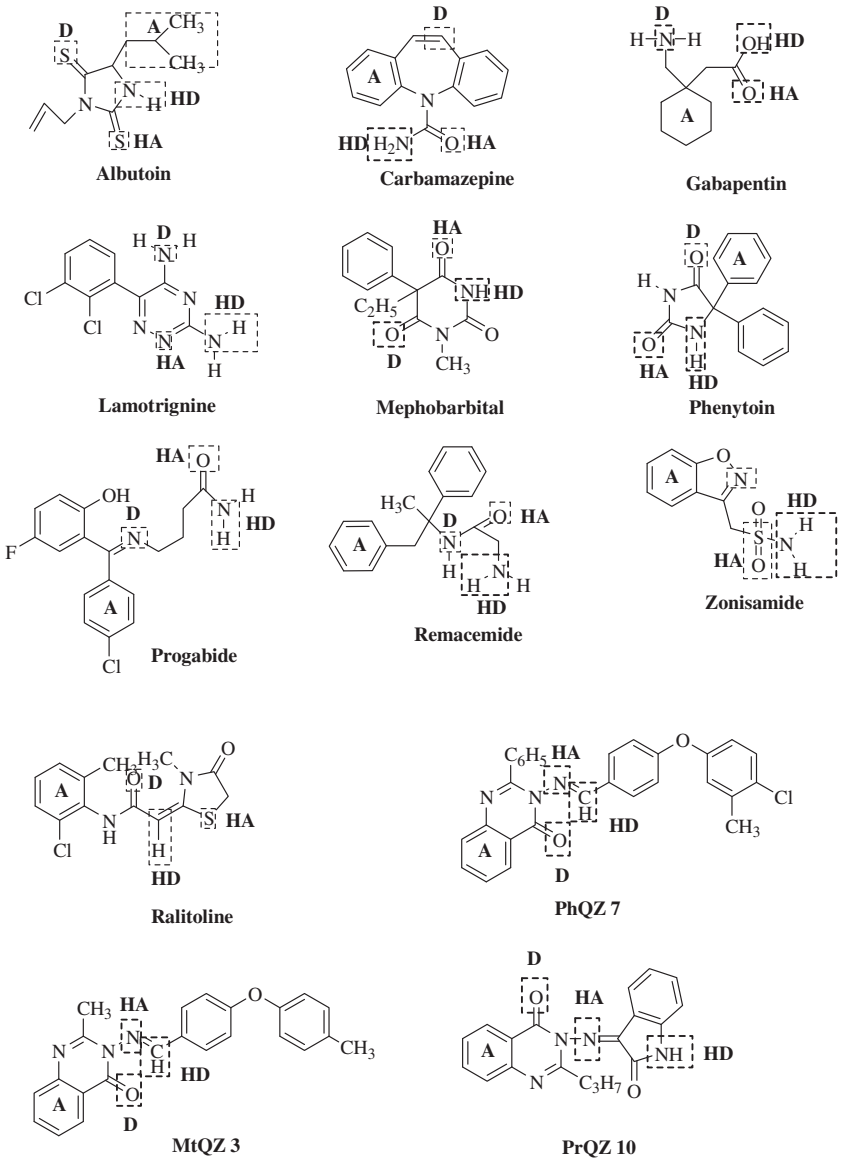


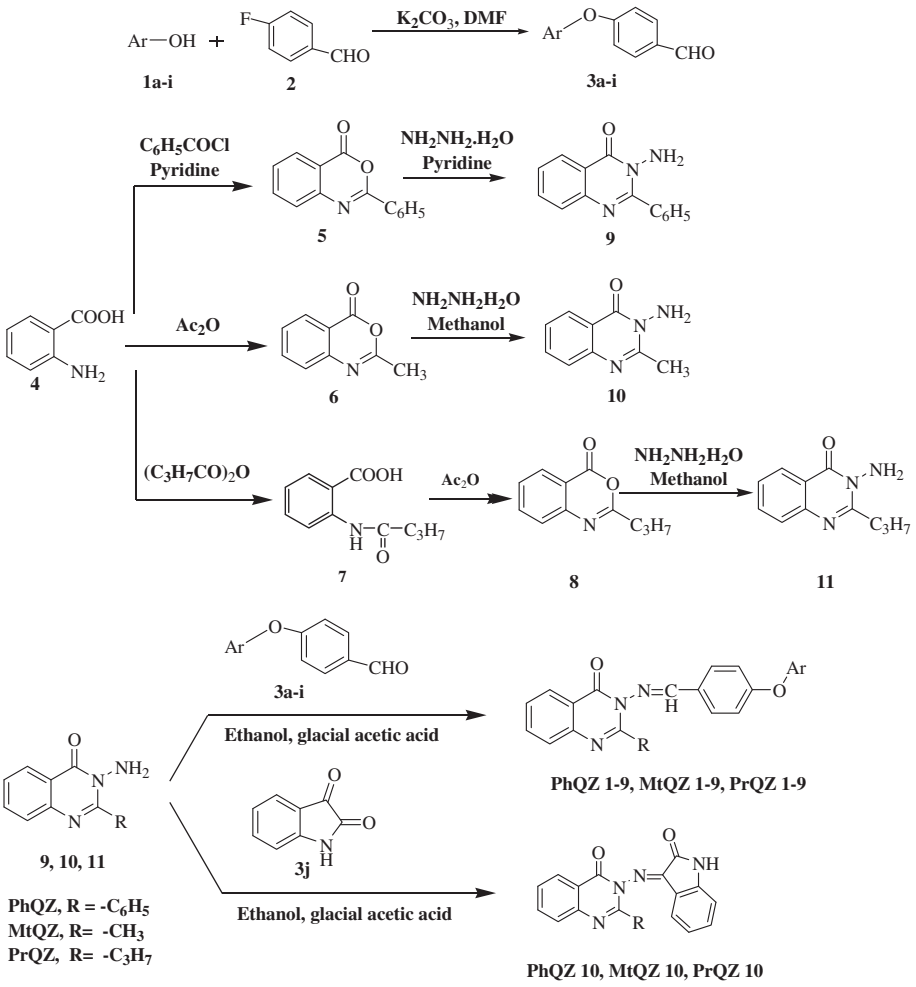
Fig. 3. Pharmacophoric pattern of well-known anticonvulsants and 2-(substituted)-3-{[substituted]amino}quinazolin-4(3*H*)-one **PhQZ 7**, **MtQZ 3** and **PrQZ 10**.

data of these compounds. Docking study of titled compounds was performed with six established epilepsy molecular targets namely GABA (A) alpha-1 receptor, GABA (A) delta receptor, glutamate receptor, Na/H exchanger, Na channel receptor, T-type calcium channel receptor by using Autodoc 4.0 along with its LGA algorithm for automated flexible ligand docking and affinity (kcal/mol) and hydrogen bonding were evaluated.

5. Result and discussion

5.1. Anticonvulsant and neurotoxicity evaluation

The six newly synthesized 2-(substituted)-3-{[substituted] amino}quinazolin-4(3*H*)-one derivative (**PhQZ 1–4, PhQZ 8** and **10**) were screened in the maximal electroshock (MES), subcutaneous metrazole (scMET) model to identify their anticonvulsant activity. None of them showed activity in MES and scMET screen. The rest twenty six titled compounds were screened in 6 Hz psychomotor seizure test and the results were found promising. 6 Hz psychomotor seizure or minimal clonic seizure test was used to identify the anticonvulsant activity of compounds at five different time points, i.e., 0.25 h, 0.5 h, 1.0 h, 2.0 h and 4.0 h after i.p. administration in mice at a dose of 100 mg/kg. The 6 Hz psychomotor seizure test is used to assess a compound's efficacy against electrically induced seizures but uses a lower frequency (6 Hz) and longer duration of stimulation (3 s). It is used to identify clinically useful AEDs that are ineffective in the standard MES and scMET tests but still have anticonvulsant activities *in-vivo*. Neurotoxicity was observed by minimal motor impairment which was measured by the rotarod (neurotoxicity) test. The results of screening at five different points are summarized in Table 2. As observed from the results of various tested 2-(substituted)-3-{[substituted]amino} quinazolin-4(3*H*)-one derivatives **PhQZ 7**, **MtQZ 3** and **PrQZ 10** showed good activity with significant protection. Compound **PhQZ 7** showed 100% protection (4/4, 0.5 h), 75% protection (3/4, 0.25 h) and 25% protection (1/4, 1.0 and 2.0 h) at a dose of 100 mg/kg. Compound **PhQZ 6** showed 50% protection (2/4, 0.25 and 0.5 h) and 25% protection (1/4, 1.0 h) at a dose of 100 mg/kg. Compound **MtQZ**



Scheme 1. Synthesis of 2-(substituted)-3-{[substituted]amino}quinazolin-4(3*H*)-one (**PhQZ 1**–**10**, **MtQZ 1**–**10** and **PrQZ 1**–**10**).

3 showed 75% protection (3/4, 0.5 h), 50% protection (2/4, 0.25 and 1.0 h) and 25% protection (1/4, 2.0 h) at a dose of 100 mg/kg. Compound **MtQZ 7** showed 50% protection (2/4, 0.25 and 0.5 h) and 25% protection (1/4, 1.0 h) at a dose of 100 mg/kg. Compounds **MtQZ 5** and **8** showed 50% protection (2/4, 0.5 h) at a dose of 100 mg/kg. Compound **MtQZ 6** showed 50% protection (2/4, 1.0 h) at a dose of 100 mg/kg. Compound **PrQZ 10** showed 75% protection (3/4, 0.25 h), 50% protection (2/4, 0.5 and 1.0 h) and 25% protection (1/4, 2.0 h) at a dose of 100 mg/kg. Compound **PrQZ 5** showed 50% protection (2/4, 0.25 and 1.0 h) and 25% protection (1/4, 0.5 h) at a dose of 100 mg/kg. Compound **PrQZ 8** showed 50% protection (2/4, 0.25 and 0.5 h) and 25% protection (1/4, 1.0 h) at a dose of 100 mg/kg. Compounds **PrQZ 3, 4** and **7** showed 50% protection (2/4, 0.5 h), (2/4, 0.25 h) and (2/4, 0.25 h) respectively at a dose of 100 mg/kg. Other compounds showed mild to moderate activity at single or two or more time points. None of the compounds showed neurotoxicity in the highest administered dose.

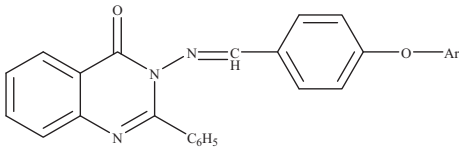
Compound **PhQZ 7** was most active compound of the series and subjected to quantitative 6 Hz evaluation and biological response evaluated at 50, 100, 200 and 350 mg/kg. It showed an ED_{50} and a TD_{50} of >350 mg/kg at a time of peak effect (TPE) of 0.5 h in quantitative 6 Hz and toxicity test (Table 3). Compound **PhQZ 7** was evaluated in the primary screen experiment of *in-vitro* hippocampal slice culture neuroprotection assay (NP) but no significant protection was observed against either KA or NMDA induced cytotoxicity (Table 4).

5.2. Computational study

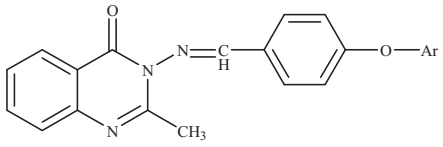
5.2.1. Distance mapping

The present work involves the correlation of the structural requirement of well known and structurally different anticonvulsant compounds with the titled compounds. The two-dimensional (2D) modeling on anticonvulsants has identified that at least one aryl unit, one or two electron donor atoms, and/or an NH group in a special spatial arrangement is recommended for anticonvulsant activity. In the present study, the 10 well known and structurally different compounds with anticonvulsant activity-albutoin, carbamazepine, gabapentin, lamotrigine, mephobarbital, phenytoin, progabide, ralitoline, remacemide and zonisamide (Fig. 3) with different mechanisms of action, were selected so as to propose a generalized pharmacophore model. The pharmacophore group's distance estimation was done by molecular mechanics calculation with the force fields based on both CHARMm force fields and MM3 parametrization. In the present work, energy minimization was performed on above mentioned ten well known anticonvulsants and 2-(substituted)-3-{[substituted]amino}quinazolin-4(3*H*)-one derivatives using Argus Lab 4.0. Distance between the various structural components essential for activity was determined by ACD/3D viewer. The crucial structural components that were included in the four-point pharmacophore model (Fig. 4) were the aryl ring center or the lipophilic group (A), an electron donor atom (D), a hydrogen bond acceptor (HA), and a hydrogen bond donor

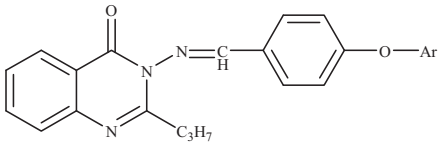
Table 1
Physical and elemental analyses data of (i) 2-phenyl-3-{[substituted]amino}quinazolin-4(3*H*)-ones **PhQZ 1-9,** (ii) 2-methyl-3-{[substituted]amino}quinazolin-4(3*H*)-ones **MtQZ 1-9,** (iii) 2-propyl-3-{[substituted]amino}quinazolin-4(3*H*)-ones **PrQZ 1-9**, and (iv) 3-[(2-oxo-1,2-dihydro-3H-indol-3-ylidene)amino]-2-(substituted)quinazolin-4(3*H*)-one **PhQZ 10, MtQZ 10, PrQZ 10**.



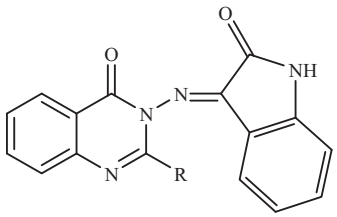
PhQZ 1-9



MtQZ 1-9



PrQZ 1-9



PhQZ 10, MtQZ 10, PrQZ 10

Compd.	Ar	R	Molecular Formula (M. W.)	Mp (°C)	Yield[a] (%)	Elemental analysis (%): Found (cal.)		
						C	H	N
PhQZ 1	Phenyl	-	$C_{27}H_{19}N_3O_2$ (417.46)	120-122	70	77.63 (77.68)	4.54 (4.59)	10.06 (10.07)
PhQZ 2	4-NO_2 phenyl	-	$C_{27}H_{18}N_4O_4$ (462.46)	203-204	68	70.07 (70.12)	3.90 (3.92)	12.08 (12.12)
PhQZ 3	4-CH_3 phenyl	-	$C_{28}H_{21}N_3O_2$ (431.49)	149-151	74	77.91 (77.94)	4.87 (4.91)	9.71 (9.74)
PhQZ 4	4-Cl phenyl	-	$C_{27}H_{18}ClN_3O_2$ (451.90)	228-230	64	71.72 (71.76)	4.02 (4.01)	9.24 (9.30)
PhQZ 5	4-Br phenyl	-	$C_{27}H_{18}BrN_3O_2$ (496.35)	110-112	68	65.30 (65.33)	3.65 (3.66)	8.41 (8.47)
PhQZ 6	4- F phenyl	-	$C_{27}H_{18}FN_3O_2$ (435.45)	182-184	72	74.48 (74.47)	4.13 (4.17)	9.61 (9.65)
PhQZ 7	3-CH_3, 4-Cl phenyl	-	$C_{28}H_{20}ClN_3O_2$ (465.93)	183-185	70	72.17 (72.18)	4.29 (4.33)	9.01 (9.02)
PhQZ 8	Naphthalene-2-yl	-	$C_{31}H_{21}N_3O_2$ (467.52)	207-208	73	79.60 (79.64)	4.54 (4.53)	8.93 (8.99)
PhQZ 9	1,3-benzodioxol-5-yl	-	$C_{28}H_{19}N_3O_4$ (461.47)	101-102	61	72.83 (72.88)	4.11 (4.15)	9.10 (9.11)
MtQZ 1	Phenyl	-	$C_{22}H_{17}N_3O_2$ (355.39)	118-120	68	74.31 (74.35)	4.80 (4.82)	11.83 (11.82)
MtQZ 2	4-NO_2 phenyl	-	$C_{22}H_{16}N_4O_4$ (400.39)	175-176	71	65.95 (66.00)	4.01 (4.03)	13.97 (13.99)
MtQZ 3	4-CH_3 phenyl	-	$C_{23}H_{19}N_3O_2$ (369.42)	196-198	72	74.72 (74.78)	5.14 (5.18)	11.35 (11.37)
MtQZ 4	4-Cl phenyl	-	$C_{22}H_{16}ClN_3O_2$ (389.83)	178-180	58	67.74 (67.78)	4.11 (4.14)	10.76 (10.78)
MtQZ 5	4-Br phenyl	-	$C_{22}H_{16}BrN_3O_2$ (434.29)	177-178	61	60.82 (60.84)	3.68 (3.71)	9.69 (9.68)
MtQZ 6	4- F phenyl	-	$C_{22}H_{16}FN_3O_2$ (373.38)	196-197	66	70.75 (70.77)	4.30 (4.32)	11.24 (11.25)
MtQZ 7	3-CH_3, 4-Cl phenyl	-	$C_{23}H_{18}ClN_3O_2$ (403.86)	131-133	68	68.37 (68.40)	4.46 (4.49)	10.39 (10.40)
MtQZ 8	Naphthalene-2-yl	-	$C_{25}H_{18}N_3O_2$ (392.43)	165-167	72	76.51 (76.52)	4.65 (4.62)	10.67 (10.71)
MtQZ 9	1,3-benzodioxol-5-yl	-	$C_{23}H_{17}N_3O_4$ (399.40)	106-107	55	69.16 (69.17)	4.26 (4.29)	10.50(10.52)
PrQZ 1	Phenyl	-	$C_{24}H_{21}N_3O_2$ (383.44)	80-82	67	75.17 (75.18)	5.51 (5.52)	10.97 (10.96)
PrQZ 2	4-NO_2 phenyl	-	$C_{24}H_{20}N_4O_4$ (428.44)	137-138	65	67.25 (67.28)	4.69 (4.71)	13.04 (13.08)
PrQZ 3	4-CH_3 phenyl	-	$C_{25}H_{23}N_3O_2$ (397.47)	118-119	62	75.51 (75.54)	5.80 (5.83)	10.55 (10.57)
PrQZ 4	4-Cl phenyl	-	$C_{24}H_{20}ClN_3O_2$ (417.89)	103-105	56	68.93 (68.98)	4.80 (4.82)	10.07 (10.06)
PrQZ 5	4-Br phenyl	-	$C_{24}H_{20}BrN_3O_2$ (462.34)	102-103	59	62.31 (62.35)	4.37 (4.36)	9.07 (9.09)
PrQZ 6	4- F phenyl	-	$C_{24}H_{20}FN_3O_2$ (401.43)	78-80	62	71.80 (71.81)	5.01 (5.02)	10.44 (10.47)
PrQZ 7	3-CH_3, 4-Cl phenyl	-	$C_{25}H_{22}ClN_3O_2$ (431.91)	86-88	58	69.50 (69.52)	5.13 (5.13)	9.70 (9.73)
PrQZ 8	Naphthalene-2-yl	-	$C_{28}H_{23}N_3O_2$ (433.50)	101-102	68	77.55 (77.58)	5.31 (5.35)	9.68 (9.69)
PrQZ 9	1,3-benzodioxol-5-yl	-	$C_{25}H_{21}N_3O_4$ (427.45)	92-94	54	70.24 (70.25)	4.91 (4.95)	9.84 (9.83)
PhQZ 10	-	C_6H_5	$C_{22}H_{14}N_4O_2$ (366.37)	234-236	77	72.09 (72.12)	3.81 (3.85)	15.28 (15.29)
MtQZ 10	-	CH_3	$C_{17}H_{12}N_4O_2$ (304.30)	116-118	79	67.07 (67.10)	3.94 (3.97)	18.38 (18.41)
PrQZ 10	-	n-C_3H_7	$C_{19}H_{16}N_4O_2$ (332.36)	173-174	75	68.62 (68.66)	4.83 (4.85)	16.83 (16.86)

[a] Solvent used for recrystallization – ethanol : water (9:1).

(HD). An average distance range for every point was obtained and compared to the 2-(substituted)-3-{[substituted]amino}quinazolin-4(3*H*)-one derivatives. Now it may be interesting to examine whether the 2-(substituted)-3-{[substituted]amino}quinazolin-4(3*H*)-one derivatives reflect the conditions of the derived pharmacophore model. Our analyses of the distance relationship showed that 2-(substituted)-3-{[substituted]amino}quinazolin-4(3*H*)-one derivatives did fulfill the essential demands of the pharmacophore when compared to the average distance requirement (Table 5).

5.2.2. Prediction of ADME properties

A computational study for prediction of ADME properties of titled compounds was performed. Topological polar surface area (TPSA), i.e., surface belonging to polar atoms, is a descriptor that was shown to correlate well with passive molecular transport through membranes and, therefore, allows prediction of transport properties of drugs in the intestines and blood–brain barrier crossing [12]. The percentage of absorption (%ABS) was calculated using TPSA. From all these parameters, it can be observed that all

Table 2
6 Hz anticonvulsant activity and neurotoxicity of 2-(substituted)-3-{[substituted] amino}quinazolin-4(3*H*)-one **PhQZ 1–10, MtQZ 1–10, PrQZ 1–10**.

Compound	Time (h) to peak effect (N/F)[a]					Toxicity[a]
	0.25	0.5	1.0	2.0	4.0	
PhQZ 6	2/4	2/4	1/4	0/4	0/4	0/4
PhQZ 7	3/4	4/4	1/4	1/4	0/4	0/4
PhQZ 9	1/4	0/4	0/4	0/4	0/4	0/4
MtQZ 1	0/4	1/4	0/4	0/4	0/4	0/4
MtQZ 2	1/4	1/4	1/4	1/4	0/4	0/4
MtQZ 3	2/4	3/4	2/4	1/4	0/4	0/4
MtQZ 5	0/4	2/4	1/4	1/4	0/4	0/4
MtQZ 6	0/4	1/4	2/4	1/4	0/4	0/4
MtQZ 7	2/4	2/4	1/4	0/4	0/4	0/4
MtQZ 8	0/4	2/4	0/4	0/4	0/4	0/4
MtQZ 9	0/4	1/4	0/4	0/4	0/4	0/4
MtQZ 10	0/4	1/4	0/4	0/4	0/4	0/4
PrQZ 1	0/4	1/4	1/4	0/4	0/4	0/4
PrQZ 2	1/4	0/4	1/4	0/4	0/4	0/4
PrQZ 3	0/4	2/4	0/4	0/4	0/4	0/4
PrQZ 4	2/4	0/4	0/4	0/4	0/4	0/4
PrQZ 5	2/4	1/4	2/4	0/4	0/4	0/4
PrQZ 6	1/4	0/4	0/4	0/4	0/4	0/4
PrQZ 7	2/4	1/4	1/4	1/4	0/4	0/4
PrQZ 8	2/4	2/4	1/4	0/4	0/4	0/4
PrQZ 10	3/4	2/4	2/4	1/4	0/4	0/4

[a] N/F = number of animals active or toxic over the number tested at a dose of 100 mg/kg.

titled compounds exhibited a great %ABS ranging from 73.7 to 89.5% (Table 6). Furthermore, compounds **PhQZ 1–9; MtQZ 4, 5** and **8** and **PrQZ 1–9** violated only one Lipinski's parameter. None of the other compounds violated Lipinski's parameters, making them potentially promising agents for epilepsy therapy.

5.2.3. log P determination

Titled compounds showed dependence of biological activity on lipophilic character in a congeneric series. In particular, for drugs acting on central nervous system to be potent, they have to cross blood–brain barrier (BBB), thus potency has been correlated with optimum lipophilicity (log P) near 2. In this study, we attempted to correlate the anticonvulsant activity of congeners with their calculated log *P* value. The experimental log *P* values were determined using the octanol–phosphate buffer method. The data is presented in Table 7. As observed some of the experimental values were in good agreement with the theoretical values. All the titled compounds showed lipophilic character.

5.2.4. Docking studies

In this study, we have used Autodoc 4.0 along with its LGA algorithm for automated flexible ligand docking of compounds **PhQZ 7**, **MtQZ 3** and **PrQZ 10** with six established epilepsy molecular targets namely GABA (A) alpha-1 receptor, GABA (A) delta receptor, glutamate receptor, Na/H exchanger, Na channel receptor, T-type calcium channel receptor and evaluated their affinity (kcal/mol) and hydrogen bonding. **PhQZ 7** has exhibited good binding properties with glutamate receptor (Affinity value −6.9 kcal/mol and 2H-bonds), GABA (A) alpha-1 receptor (Affinity value −6.8 kcal/mol and 1H-bond) and GABA (A) delta receptor (Affinity value −7.1 kcal/mol and 1H-bond). It also showed affinity with Na/H exchanger (Affinity value −5.5 kcal/mol and 0H-bond). Compound **MtQZ 3** has exhibited good binding properties with glutamate receptor (Affinity value −7.8 kcal/mol and 4H-bonds) and GABA (A) alpha-1 receptor (Affinity value −7.2 kcal/mol and 1H-bond). It also showed affinity with GABA (A) delta receptor (Affinity value −7.2 kcal/mol and 0H-bond) and Na/H exchanger (Affinity value −6.3 kcal/mol and 0H-bond). Compound **PrQZ 10** has exhibited good binding properties with glutamate receptor (Affinity value −6.9 kcal/mol and 2H-bond). It also showed affinity with GABA (A) alpha-1 receptor (Affinity value −5.9 kcal/mol and 0H-bond), GABA (A) delta receptor (Affinity value −5.8 kcal/mol and 0H-bond), and Na/H exchanger (Affinity value −4.9 kcal/mol and 0H-bond). Compounds **PhQZ 7**, **MtQZ 3** and **PrQZ 10** did not show affinity and binding with Na channel and T-type calcium channel receptor. The docking study results of **PhQZ 7**, **MtQZ 3** and **PrQZ 10** with these six receptors are shown in Table 8. The docking images are shown in Fig. 5. Docking study results show that the titled compounds exhibited good affinity and binding properties with glutamate, GABA (A) alpha-1 and GABA (A) delta receptors. Affinity was also observed with Na/H exchanger without H-bond.

Table 3
Quantitative 6 Hz evaluation: ED_{50} value and biological response data.

Compd.	Test	Time (h)	ED_{50}(mg/kg)	ED_{50} biological response			
				Dose (mg/kg)			
				50	100	200	350
PhQZ 7	6 Hz	0.5	>350	1/8	3/8	1/8	2/8
	TOX	0.5	>350	–	–	–	0/8

Table 4
Results of *in-vitro* hippocampal slice culture neuroprotection assay (Test 76).

Compound	Excitotoxin	Insult duration (h)	Primary screen results
PhQZ 7	N-methyl-D-aspartate (NMDA)	4	No neuroprotection observed
	Kainic acid (KA)	4	No neuroprotection observed

5.3. Structure–activity relationship (SAR)

In the present study, various 2-(substituted)-3-{[substituted] amino}quinazolin-4(3*H*)-ones were synthesized by using different substituent, in order to investigate the pharmacophoric substituent, responsible for better activity. In the synthetic design, the various substituents were introduced at positions 6 and 20 of quinazolin-4(3*H*)-one backbone structure. At position 6, we introduced methyl, phenyl and n-propyl group, chosen on the basis of literature and hypothesis as discussed in Introduction section. At

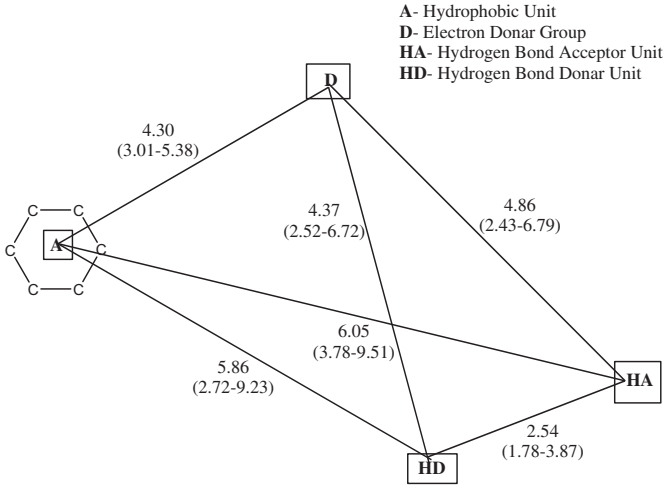


Fig. 4. Four-point 3D pharmacophore model for anticonvulsants derived by using MM3 and CHARMm parametrization (Argus Lab 4.0 and ACD/3D viewer).

Table 5
Distance range between the essential structural elements A, D and HA–HD.[a]

Compounds	A–HA	A–HD	A–D	HA–HD	HD–D	HA–D
Albutoin	5.37	2.72	4.51	2.72	4.03	5.40
Carbamazepine	4.28	4.28	4.25	2.33	5.75	5.67
Gabapentin	4.26	4.93	3.83	2.23	3.57	4.50
Lamotrigine	5.30	7.42	4.54	2.42	4.94	4.25
Mephobarbital	3.78	5.50	4.81	2.34	4.63	5.23
Phenytoin	6.20	4.01	4.35	2.63	3.88	5.17
Progabide	9.51	9.23	3.79	2.41	6.72	6.79
Ralitoline	8.30	5.55	4.56	2.75	2.52	4.85
Remacemide	7.51	8.75	5.38	3.87	3.96	2.43
Zonisamide	6.02	6.22	3.01	1.78	3.71	4.31
Av distance	6.05	5.86	4.30	2.54	4.37	4.86
(**range**)	(3.78–9.51)	(2.72–9.23)	(3.01–5.38)	(1.78–3.87)	(2.52–6.72)	(2.43–6.79)
PhQZ 7	5.26	6.06	3.81	1.37	4.28	2.93
MtQZ 3	5.28	6.13	3.96	1.37	4.26	2.90
PrQZ 10	5.27	7.90	3.93	3.57	5.71	2.90

[a] Distances calculated for 3D optimized structures using MM3 and CHARMm parameterization (Argus Lab 4.0 and ACD/3D viewer).

position 20, the substituent viz. phenyl, 4-nitro phenyl, 4-methyl phenyl, 4-chloro phenyl, 4-bromo phenyl, 4-flouro phenyl, 4-chloro-3-methyl phenyl, naphthalene-2-yl, 1,3-benzodioxol-5-yl were introduced. Keeping in view, the pharmacophoric character of isatin for anticonvulsant activity, the isatinyl moiety was also attached to position N-11. These substituents were chosen based on the literature review and computational study.

The most active compound **PhQZ 7** has phenyl at position 6 and 4-chloro-3-methyl phenyl at position 20. It has been observed that at position 6, the presence of phenyl was more significant than methyl and n-propyl, and yields more active compounds. This observation support the hypothesis that methyl group at second position of quinazolin-4(3*H*)-one is not always necessary for the CNS activity and other groups when placed at this position can also lead to potent CNS active agents. In addition, at position 20, the presence of halogenated phenyl was more significant than phenyl, non-halogenated phenyl, naphthalene-2-yl, 1,3-benzodioxol-5-yl substituents. This observation clearly indicates the effect of electronic factors on activity. The 4-chloro-3-methyl phenyl substituent is most active one and also increases the duration of action. This increase in duration of action might be because of blockade of metabolism at position 25 by methyl group. The presence of isatinyl at position N-11 and n-propyl at position 6, also yields the active compound **PrQZ 10** with good duration of action. The presence of naphthalene-2-yl and 1,3-benzodioxol-5-yl substituent at position 20 does not yield any active compound.

Table 6
Pharmacokinetic parameters important for good oral bioavailability of compounds **PhQZ 1–10**, **MtQZ 1–10**, **PrQZ 1–10**.[a]

Compound	%ABS	TPSA (A^2)	n-ROTB	MW	MV	n-OHNH donors	n-ON acceptors	Lipinski's violations
Rule	–	–	–	<500	–	<5	<10	≤1
PhQZ 1	89.5	56.49	5	417.47	374.7	0	5	1
PhQZ 2	73.7	102.3	6	462.47	398.04	0	8	1
PhQZ 3	89.5	56.50	5	431.50	391.26	0	5	1
PhQZ 4	89.5	56.50	5	451.91	388.24	0	5	1
PhQZ 5	89.5	56.50	5	496.37	392.59	0	5	1
PhQZ 6	89.5	56.50	5	435.46	379.63	0	5	1
PhQZ 7	89.5	56.50	5	465.94	404.80	0	5	1
PhQZ 8	89.5	56.50	5	467.53	418.70	0	5	1
PhQZ 9	83.1	74.97	5	461.48	398.63	0	7	1
PhQZ 10	81.4	80.12	2	366.38	314.85	1	6	0
MtQZ 1	89.5	56.50	4	355.39	319.85	0	5	0
MtQZ 2	73.7	102.3	5	400.39	343.19	0	8	0
MtQZ 3	89.5	56.50	4	369.42	336.41	0	5	0
MtQZ 4	89.5	56.50	4	389.84	333.39	0	5	1
MtQZ 5	89.5	56.50	4	434.29	337.74	0	5	1
MtQZ 6	89.5	56.50	4	373.39	324.78	0	5	0
MtQZ 7	89.5	56.50	4	405.88	356.16	0	5	0
MtQZ 8	89.5	56.50	4	405.46	363.85	0	5	1
MtQZ 9	83.1	74.96	4	399.41	343.78	0	7	0
MtQZ 10	81.4	80.12	1	304.31	260.00	1	6	0
PrQZ 1	89.5	56.49	6	383.45	353.46	0	5	1
PrQZ 2	73.7	102.3	7	428.45	376.79	0	8	1
PrQZ 3	89.5	56.50	6	397.48	370.02	0	5	1
PrQZ 4	89.5	56.50	6	417.90	366.99	0	5	1
PrQZ 5	89.5	56.50	6	462.35	371.34	0	5	1
PrQZ 6	89.5	56.50	6	401.44	358.39	0	5	1
PrQZ 7	89.5	56.50	6	431.92	383.55	0	5	1
PrQZ 8	89.5	56.50	6	433.51	397.45	0	5	1
PrQZ 9	83.1	74.96	6	427.46	377.39	0	7	1
PrQZ 10	81.4	80.12	3	332.36	293.61	1	6	0

[a] %ABS, percentage of absorption; TPSA, topological polar surface area; n-ROTB, number of rotatable bonds; MW, molecular weight; MV, molecular volume; n-OHNH, number of hydrogen bond donors; n-ON, number of hydrogen bond acceptors.

Table 7
log *P* value for titled compounds **PhQZ 1–10, MtQZ 1–10, PrQZ 1–10**.

Compounds	Experimental log *P*	Theoretical log *P* (milog *P*[a])
Rule	–	≤5
PhQZ 1	6.134	6.357
PhQZ 2	6.123	6.316
PhQZ 3	6.291	6.805
PhQZ 4	6.381	7.035
PhQZ 5	6.394	7.166
PhQZ 6	6.212	6.521
PhQZ 7	6.517	7.411
PhQZ 8	6.526	7.54
PhQZ 9	6.012	6.247
PhQZ 10	4.032	4.371
MtQZ 1	4.012	4.329
MtQZ 2	4.013	4.288
MtQZ 3	4.321	4.777
MtQZ 4	4.598	5.007
MtQZ 5	4.643	5.138
MtQZ 6	4.212	4.493
MtQZ 7	4.412	4.812
MtQZ 8	5.221	5.512
MtQZ 9	4.011	4.219
MtQZ 10	2.232	2.343
PrQZ 1	5.202	5.404
PrQZ 2	5.178	5.363
PrQZ 3	5.451	5.853
PrQZ 4	5.782	6.082
PrQZ 5	5.878	6.213
PrQZ 6	5.221	5.568
PrQZ 7	6.019	6.459
PrQZ 8	6.123	6.587
PrQZ 9	4.823	5.294
PrQZ 10	3.234	3.418

[a] milog *P*, logarithm of compound partition coefficient between n-octanol and water calculated as per molinspiration online property toolkit.

6. Conclusion

A series of 2-(substituted)-3-{[substituted]amino}quinazolin-4(3*H*)-one derivatives were designed, synthesized, and their anticonvulsant activity was evaluated. The titled compounds displayed the significant 6 Hz psychomotor seizure test activity. The neurotoxicity evaluation was done by rotarod test. A computational study was also carried out, including calculation of pharmacophore pattern, prediction of pharmacokinetic properties and docking studies. The compound **PhQZ 7** displayed 100% protection and emerged as a lead in this series. Further, compounds **MtQZ 3** and **PrQZ 10** came out as a potential candidate for further investigation. Compounds **PhQZ 6**, **MtQZ 5–8**, **PrQZ 3–5 and PrQZ 7** and **8** exhibited moderate protection. None of the compound showed neurotoxicity in rotarod test. Furthermore, **PhQZ 10**; **MtQZ 1–3**, **6**, **7**, **9**, **10**; and **PrQZ 10** also did not show Lipinski's violation, making them potentially promising agent for epilepsy therapy. Docking study results show that the compounds exhibited good binding properties with glutamate, GABA (A) delta and GABA (A) alpha-1 receptor. The docking study data strongly support the assumption that these receptors may be involved in observed anticonvulsant activity of 2-(substituted)-3-{[substituted]amino}quinazolin-4(3*H*)-one derivatives. However, further studies need to be carried out to ascertain the precise mechanism of anticonvulsant activity of these molecules.

7. Experimental protocol

7.1. Chemistry

All the chemicals and solvents, purchased from Merck (India), Spectrochem (India), Himedia (India) and S. d. Fine were used without further purification. The progress of reaction was monitored by thin layer chromatography, performed on a silica gel 60 F_{254} coated aluminum sheet. The melting points were determined by using Thomas–Hoover melting point apparatus and are uncorrected. The FT-IR spectra were recorded on Perkin–Elmer Spectrum BX-II Spectrophotometer. The ^{1}H NMR spectra were recorded on Bruker 300 MHz High Resolution NMR spectrometer using TMS as an internal standard. Chemical shifts were reported in ppm (δ) and signals were described as singlet (s), doublet (d), triplet (t) and

Table 8
Docking results of compounds **PhQZ 7, MtQZ 3** and **PrQZ 10**.[a]

Ligand	Receptor	Affinity (kcal/mol)	H-bonds	H-binding ligand			H-binding receptor			
				Element	Atom No.	Type	Residue	Element	Atom No.	Type
PhQZ 7	GABA (A) alpha-1	−6.8	01	N	19	Donor	Phe323	O	407	Acceptor
	GABA (A) delta	−7.1	01	N	19	Donor	Lue325	O	458	Acceptor
	Glutamate	−6.9	02	O	08	Acceptor	Try 456	O	146	Both
				N	16	Acceptor	Ser 554	N	1067	Donor
	Na/H exchanger	−5.5	00	–	–	–	–	–	–	–
	Na channel	−0.0	00	–	–	–	–	–	–	–
	T-type calcium	−0.0	00	–	–	–	–	–	–	–
MtQZ 3	GABA (A) alpha-1	−7.2	01	O	00	Acceptor	Lys339	N	567	Donor
	GABA (A) delta	−7.2	00	–	–	–	–	–	–	–
	Glutamate	−7.8	04	N	08	Donor	Pro531	O	846	Acceptor
				N	08	Donor	Thr533	O	865	Both
				O	00	Acceptor	Try459	O	146	Both
				N	11	Donor	Try459	O	146	Both
	Na/H exchanger	−6.3	00	–	–	–	–	–	–	–
	Na channel	−0.0	00	–	–	–	–	–	–	–
	T-type calcium	−0.0	00	–	–	–	–	–	–	–
PrQZ 10	GABA (A) alpha-1	−5.9	00	–	–	–	–	–	–	–
	GABA (A) delta	−5.8	00	–	–	–	–	–	–	–
	Glutamate	−6.9	02	O	25	Acceptor	Try459	O	146	Both
				N	16	Acceptor	Thr533	O	865	Both
	Na/H exchanger	−4.9	00	–	–	–	–	–	–	–
	Na channel	−0.0	00	–	–	–	–	–	–	–
	T-type calcium	−0.0	00	–	–	–	–	–	–	–

[a] Affinity and H-bonds calculations were determined by docking studies using Autodoc 4.0 software.

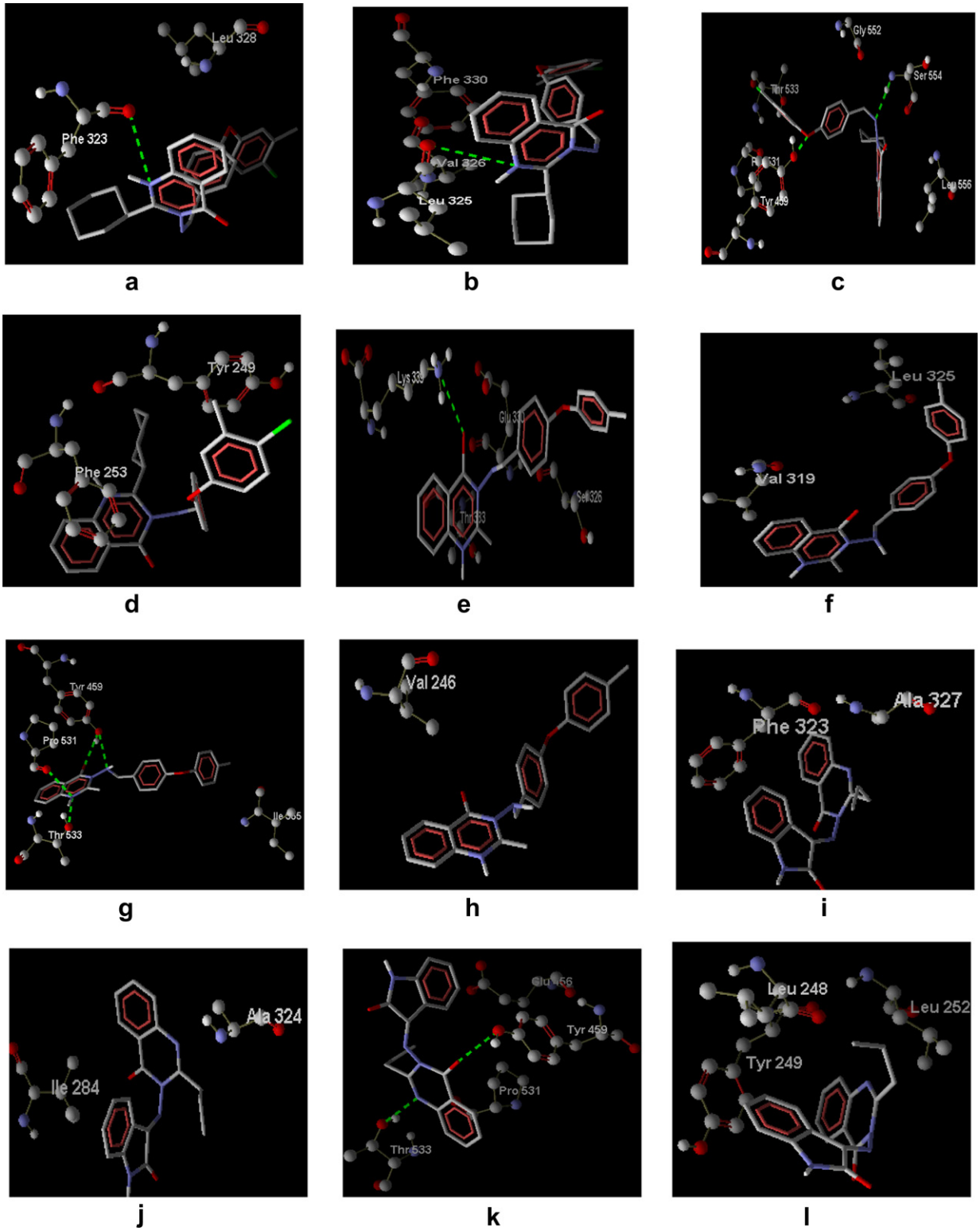


Fig. 5. Docking Images. (a) **PhQZ 7** with GABA (A) alpha-1 receptor, (b) **PhQZ 7** with GABA (A) delta receptor, (c) **PhQZ 7** with glutamate receptor, (d) **PhQZ 7** with Na/H exchanger; (e) **MtQZ 3** with GABA (A) alpha-1 receptor, (f) **MtQZ 3** with GABA (A) delta receptor, (g) **MtQZ 3** with glutamate receptor, (h) **MtQZ 3** with Na/H exchanger; (i) **PrQZ 10** with GABA (A) alpha-1 receptor, (j) **PrQZ 10** with GABA (A) delta receptor, (k) **PrQZ 10** with glutamate receptor, (l) **PrQZ 10** with Na/H exchanger.

multiplet (m). All exchangeable protons were confirmed by addition of D_2O. The mass spectra were recorded on a Waters Micromass ZQ 2000 mass spectrometer. Elemental analysis (C, H, N) was undertaken with Perkin–Elmer Model 240C analyzer.

*7.1.1. Synthesis of 4-substituted benzaldehyde (**3a–i**)*

A mixture of substituted phenol **1a–i** (37.4 mmol), 4-fluoro benzaldehyde **2** (37.4 mmol) and potassium carbonate (38.8 mmol) in *N,N*-dimethylformamide (30 ml) was refluxed for 16–18 h under nitrogen. After cooling, the product was extracted from the reaction mixture and purified by chromatography.

4-(4-Fluoro-phenoxy)-benzaldehyde (**3f**): Yield: 70%; m.p.: 41 °C; IR (KBr, cm^{-1}) υ: 1665 (C=O), 1240 (O); ^1H NMR ($CDCl_3$, 300 MHz) δ: 9.87 (s, 1H, CHO), 6.83–7.87 (a set of signals, 8H, Ar-H); MS (*m*/*z*, %): 216.17 ($M^+ + 1$, 67.2)

*7.1.2. Synthesis of 2-phenyl-4H-3,1-benzoxazin-4-one (**5**)*

To a stirred solution of anthranilic acid **4** (0.05 mol) in pyridine (50 mL), benzoyl chloride (0.05 mol) was added drop wise, maintaining the temperature near 0–6 °C for 1 h. The reaction mixture was stirred for another 3 h at room temperature until a solid product was separated. The reaction mixture was neutralized with saturated sodium bicarbonate solution and the pale yellow solid which separated was filtered, washed with water and recrystallised from ethanol. Yield: 86%; mp. 112–114 °C; IR (KBr, cm^{-1}) υ: 3033 (Qu-H, Ar-H), 1680 (C=O), 1602 (C=N), 1178 (C–O); ^1H NMR ($CDCl_3$, 300 MHz) δ: 6.88–8.18 (m, 9H, Qu-H and Ar-H), MS (*m*/*z*, %): 224.06 ($M^+ + 1$, 54.27).

*7.1.3. Synthesis of 2-methyl-4H-3,1-benzoxazin-4-one (**6**)*

A mixture of anthranilic acid **4** (0.01 mol) and acetic anhydride (0.1 mol) was refluxed on gentle flame for 2 h. The excess acetic anhydride was distilled off under reduced pressure and the residue was dissolved in petroleum ether and kept aside for 1 h, to yield **6**. Yield: 78%; mp: 180–182 °C; IR (KBr, cm^{-1}) υ: 1665 (C=O), 1605 (C=N), 1169 (C–O); ^1H NMR ($CDCl_3$, 300 MHz) δ: 6.91–8.09 (m, 4H, Qu-H), 2.39 (s, 3H, CH_3); MS (*m*/*z*, %): 162.11 ($M^+ + 1$, 34.12).

*7.1.4. Synthesis of 2-propyl-4H-3,1-benzoxazin-4-one (**8**)*

Anthranilic acid **4** (0.1 mol) was refluxed with butyric anhydride for 3 h. Excess of anhydride was distilled off by vacuum distillation to obtain the precipitate of *N*-propyl anthranilic acid **7**, which was further refluxed with acetic anhydride for 2 h to yield **8**. Yield: 72%; mp: 58–60 °C; IR (KBr, cm^{-1}) υ: 1732 (C=O), 1608 (C=N), 1160 (C–O); ^1H NMR ($CDCl_3$, 300 MHz) δ: 6.84–8.28 (m, 4H, Qu-H), 2.71 (t, 2H, CH_2Qu), 1.83 (sextet, 2H, CH_2Me), 1.03 (t, 3H, CH_3); MS (*m*/*z*, %): 190.05 ($M^+ + 1$).

*7.1.5. Synthesis of 3-amino-2-phenylquinazolin-4(3H)-one (**9**)*

To a stirred solution of **5** (0.05 mol) in pyridine (20 mL), 80% hydrazine hydrate (0.15 mol) was added. The reaction mixture was stirred and refluxed for 30 min. After cooling, the crude product was obtained by filtration and recrystallized from 80% ethanol to afford **9** as a white solid. yield: 86%; m.p. 178–180 °C; IR (KBr, cm^{-1}) υ: 3423 (NH_2), 3033 (C–H, Ar-H), 1687 (C=O), 1591 (C=N); ^1H NMR ($CDCl_3$, 300 MHz) δ: 6.81–8.20 (m, 9H, Qu-H and Ar-H), 4.67 (s, 2H, Qu-NH_2); MS (*m*/*z*, %): 238.12 ($M^+ + 1$, 38.16).

*7.1.6. Synthesis of 3-amino-2-(methyl/propyl) quinazolin-4(3H)-one (**10**, **11**)*

A mixture of 2-methyl/propyl-4*H*-3,1-benzoxazin-4-one (**6**/**8**) (0.01 mol) and 80% hydrazine hydrate (0.03 mol) in methanol was refluxed for 2–3 h and cooled. The separated solid was recrystallized from ethanol.

3-Amino-2-methyl-quinazolin-4(3H)-one (**10**) Yield: 82%; mp: 139–141 °C; IR (KBr, cm^{-1}) υ: 3310-3262 (NH_2), 1678 (C=O), 1617 (C=N), 1595 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) δ: 6.71–8.18 (m, 4H, Qu-H), 4.63 (s, 2H, NH_2), 2.38 (s, 3H, CH_3); MS (*m*/*z*, %): 176.08 ($M^+ + 1$, 38.13).

3-Amino-2-propyl-quinazolin-4(3H)-one (**11**) Yield: 77%; mp: 84–86 °C; IR (KBr, cm^{-1}) υ: 3304–3260 (NH_2), 1672 (C=O), 1617 (C=N), 1602 (C=C); ^1H NMR ($CDCl_3$, 300 MHz) δ: 6.78–8.27 (m, 4H, Qu-H), 4.68 (s, 2H, NH_2), 2.73 (t, 2H, CH_2Qu), 1.82 (sextet, 2H, CH_2Me), 1.07 (t, 3H, CH_3); MS (*m*/*z*, %): 204.08 ($M^+ + 1$, 43.18).

*7.1.7. Synthesis of 2-(substituted)-3-{[substituted]amino} quinazolin-4(3H)-one (**PhQZ 1–PhQZ** , **MtQZ 1–10**, **PrQZ 1–10**)*

Equimolar quantities (0.01 mol) of 4-substituted benzaldehydes **3a–i**/isatin **3j** and 3-amino-2-(substituted)-quinazolin-4(3*H*)-one (**9, 10, 11**) were dissolved in warm ethanol containing 0.5 ml of glacial acetic acid. The reaction mixture was refluxed for 4–6 h and set aside. The resultant solid was washed with ethanol and recrystallized from 90% ethanol. The physical data and elemental analysis data of the titled compounds **PhQZ 1–10, MtQZ 1–10, PrQZ 1–10** are presented in Table 1. The spectral data of titled compounds **PhQZ 1–10, MtQZ 1–10, PrQZ 1–10** are given below.

*7.1.7.1. 2-Phenyl-3-{[(E)-(3-phenoxyphenyl)methylidene]amino}quinazolin-4(3H)-one (**PhQZ 1**).* IR (KBr, cm^{-1}) υ: 3046 (Qu-H, Ar-H), 1687 (C=O), 1599 (N=CH), 1446–1524 (C=C, benzene and Qu-ring), 1243 (O), 754, 696 (mono substituted benzene); ^1H NMR ($CDCl_3$, 300 MHz) δ: 8.43 (s, 1H, CH=N), 6.83–8.17 (a set of signals, 18H, Qu-H and Ar-H); MS (*m*/*z*, %): 418.16 ($M^+ + 1$, 57.31).

*7.1.7.2. 3-({(E)-[3-(4-Nitrophenoxy)phenyl]methylidene}amino)-2-phenylquinazolin-4(3H)-one (**PhQZ 2**).* IR (KBr, cm^{-1}) υ: 3058 (Qu-H, Ar-H), 1682 (C=O), 1594 (N=CH), 1525 (N=O), 1449–1528 (C=C, benzene and Qu-ring), 1247 (O), 759, 696 (mono substituted benzene); ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.47 (s, 1H, CH=N), 6.78–8.11 (a set of signals, 17H, Qu-H and Ar-H); MS (*m*/*z*, %): 463.16 ($M^+ + 1$, 37.11).

*7.1.7.3. 3-({(E)-[3-(4-Methylphenoxy)phenyl]methylidene}amino)-2-phenylquinazolin-4(3H)-one (**PhQZ 3**).* IR (KBr, cm^{-1}) υ: 3030 (Qu-H, Ar-H), 1662 (C=O), 1599 (N=CH), 1450–1540 (C=C, benzene and Qu-ring), 1243 (O), 751, 702 (mono substituted benzene); ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.31 (s, 1H, CH=N), 6.92–8.04 (a set of signals, 17H, Qu-H and Ar-H), 2.35 (s, 3H, CH_3); MS (*m*/*z*, %): 432.12 ($M^+ + 1$, 21.22).

*7.1.7.4. 3-({(E)-[3-(4-Chlorophenoxy)phenyl]methylidene}amino)-2-phenylquinazolin-4(3H)-one (**PhQZ 4**).* IR (KBr, cm^{-1}) υ: 3041 (Qu-H, Ar-H), 1690 (C=O), 1596 (N=CH), 1449–1528 (C=C, benzene and Qu-ring), 1246 (O), 756, 697 (mono substituted benzene); ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.44 (s, 1H, CH=N), 6.82–8.18 (a set of signals, 17H, Qu-H and Ar-H); MS (*m*/*z*, %): 452.14 ($M^+ + 1$ for ^{35}Cl, 61.20), 454.10 ($M^+ + 1$ for ^{37}Cl, 19.3).

*7.1.7.5. 3-({(E)-[3-(4-Bromophenoxy)phenyl]methylidene}amino)-2-phenylquinazolin-4(3H)-one (**PhQZ 5**).* IR (KBr, cm^{-1}) υ: 3039 (Qu-H, Ar-H), 1681 (C=O), 1594 (N=CH), 1451–1528 (C=C, benzene and Qu-ring), 1246 (O), 757, 693 (mono substituted benzene); ^1H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.49 (s, 1H, CH=N), 6.82–8.27 (a set of signals, 17H, Qu-H and Ar-H); MS (*m*/*z*, %): 498.08 ($M^+ + 1$ for ^{81}Br, 43.12), 496.03 ($M^+ + 1$ for ^{79}Br, 40.43).

*7.1.7.6. 3-({(E)-[3-(4-Fluorophenoxy)phenyl]methylidene}amino)-2-phenylquinazolin-4(3H)-one (**PhQZ 6**).* IR (KBr, cm^{-1}) υ: 3058 (Qu-H, Ar-H), 1686 (C=O), 1597 (N=CH), 1449–1528 (C=C, benzene

and Qu-ring), 1241 (O), 759, 696 (mono substituted benzene); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.44 (s, 1H, CH=N), 6.82–8.19 (a set of signals, 17H, Qu-H and Ar-H); MS (*m*/*z*, %): 436.15 ($M^+ + 1$, 61.23).

*7.1.7.7. 3-({(E)-[3-(4-Chloro-3-methylphenoxy)phenyl]methylidene} amino)-2-phenylquinazolin-4(3H)-one (**PhQZ 7**).* IR (KBr, cm^{-1}) υ: 3067 (Qu-H, Ar-H), 1685 (C=O), 1596 (N=CH), 1447–1526 (C=C, benzene and Qu-ring), 1240 (O), 755, 699 (mono substituted benzene); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.45 (s, 1H, CH=N), 6.82–8.39 (a set of signals, 16H, Qu-H and Ar-H), 2.36 (s, 3H, CH_3); MS (*m*/*z*, %): 466.11 ($M^+ + 1$ for ^{35}Cl, 67.70), 468.08 ($M^+ + 1$ for ^{37}Cl, 21.56).

*7.1.7.8. 3-({(E)-[3-(Naphthalen-2-yloxy)phenyl]methylidene} amino)-2-phenylquinazolin-4(3H)-one (**PhQZ 8**).* IR (KBr, cm^{-1}) υ: 3061 (Qu-H, Ar-H), 1682 (C=O), 1591 (N=CH), 1440–1519 (C=C, benzene and Qu-ring), 1244 (O), 836, 821 (β-naphthyl), 754, 696 (mono substituted benzene); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.43 (s, 1H, CH=N), 6.83–8.31 (a set of signals, 20H, Qu-H, naphthyl-H and Ar-H); MS (*m*/*z*, %): 468.16 ($M^+ + 1$, 32.89).

*7.1.7.9. 3-({(E)-[3-(1,3-Benzodioxol-5-yloxy)phenyl]methylidene} amino)-2-phenylquinazolin-4(3H)-one (**PhQZ 9**).* IR (KBr, cm^{-1}) υ: 3049 (Qu-H, Ar-H), 1685 (C=O), 1597 (N=CH), 1449–1521 (C=C, benzene and Qu-ring), 1247 (O), 755, 699 (mono substituted benzene); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.41 (s, 1H, CH=N), 6.71–8.27 (a set of signals, 16H, Qu-H, sesamol-H and Ar-H), 5.98 (s, 2H, CH_2); MS (*m*/*z*, %): 462.16 ($M^+ + 1$, 23.10).

*7.1.7.10. 3-[(2-Oxo-1,2-dihydro-3H-indol-3-ylidene)amino]-2-phenylquinazolin-4(3H)-one (**PhQZ10**).* IR (KBr, cm^{-1}) υ: 3261 (NH), 3068 (Qu-H, Ar-H), 1687 (C=O), 1589 (N=C), 1446–1528 (C=C, benzene and Qu-ring), 757, 698 (mono substituted benzene); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 12.81 (s, 1H, NH of Isatin, D_2O exchangeable), 6.92–8.19 (a set of signals, 13H, Qu-H, Isatin-H and Ar-H); MS (*m*/*z*, %): 367.12 ($M^+ + 1$, 64.65).

*7.1.7.11. 2-Methyl-3-{[(E)-(3-phenoxyphenyl)methylidene]amino} quinazolin-4(3H)-one (**MtQZ 1**).* IR (KBr, cm^{-1}) υ: 3049 (Qu-H, Ar-H), 1667 (C=O), 1594 (N=CH), 1467–1558 (C=C, benzene and Qu-ring), 1240 (O), 754, 696 (mono substituted benzene); ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.93 (s, 1H, CH=N), 6.93–8.28 (a set of signals, 13H, Qu-H and Ar-H), 2.63 (s, 3H, Qu-CH_3); MS (*m*/*z*, %): 356.09 ($M^+ + 1$, 47.11).

*7.1.7.12. 2-Methyl-3-({(E)-[3-(4-nitrophenoxy)phenyl]methylidene} amino)quinazolin-4(3H)-one (**MtQZ 2**).* IR (KBr, cm^{-1}) υ: 3058 (Qu-H, Ar-H), 1672 (C=O), 1594 (N=CH), 1521 (N=O), 1466–1561 (C=C, benzene and Qu-ring), 1244 (O); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.95 (s, 1H, CH=N), 6.88–8.25 (a set of signals, 12H, Qu-H and Ar-H), 2.62 (s, 3H, Qu-CH_3); MS (*m*/*z*, %): 401.11 ($M^+ + 1$, 57.04).

*7.1.7.13. 2-Methyl-3-({(E)-[3-(4-methylphenoxy)phenyl]methylidene}amino)quinazolin-4(3H)-one (**MtQZ 3**).* IR (KBr, cm^{-1}) υ: 3067 (Qu-H, Ar-H), 1670 (C=O), 1591 (N=CH), 1471–1566 (C=C, benzene and Qu-ring), 1243 (O); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.91 (s, 1H, CH=N), 6.97–8.30 (a set of signals, 12H, Qu-H and Ar-H), 2.64 (s, 3H, Qu-CH_3), 2.37 (s, 3H, Ar-CH_3); MS (*m*/*z*, %): 370.10 ($M^+ + 1$, 33.20).

*7.1.7.14. 3-({(E)-[3-(4-Chlorophenoxy)phenyl]methylidene}amino)-2-methylquinazolin-4(3H)-one (**MtQZ 4**).* IR (KBr, cm^{-1}) υ: 3061 (Qu-H, Ar-H), 1673 (C=O), 1596 (N=CH), 1469–1568 (C=C, benzene and Qu-ring), 1241 (O); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.94 (s, 1H, CH=N), 6.92–8.28 (a set of signals, 12H, Qu-H and Ar-H), 2.63 (s, 3H, Qu-CH_3); MS (*m*/*z*, %): 390.11 ($M^+ + 1$ for ^{35}Cl, 66.22), 392.07 ($M^+ + 1$ for ^{37}Cl, 21.8).

*7.1.7.15. 3-({(E)-[3-(4-Bromophenoxy)phenyl]methylidene}amino)-2-methylquinazolin-4(3H)-one (**MtQZ 5**).* IR (KBr, cm^{-1}) υ: 3049 (Qu-H, Ar-H), 1676 (C=O), 1594 (N=CH), 1465–1562 (C=C, benzene and Qu-ring), 1245 (O); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.90 (s, 1H, CH=N), 6.87–8.27 (a set of signals, 12H, Qu-H and Ar-H), 2.64 (s, 3H, Qu-CH_3); MS (*m*/*z*, %): 436.02 ($M^+ + 1$ for ^{81}Br, 32.07), 434.05 ($M^+ + 1$ for ^{79}Br, 32.14).

*7.1.7.16. 3-({(E)-[3-(4-Fluorophenoxy)phenyl]methylidene}amino)-2-methylquinazolin-4(3H)-one (**MtQZ 6**).* IR (KBr, cm^{-1}) υ: 3055 (Qu-H, Ar-H), 1671 (C=O), 1597 (N=CH), 1462–1568 (C=C, benzene and Qu-ring), 1240 (O); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.94 (s, 1H, CH=N), 6.84–8.29 (a set of signals, 12H, Qu-H and Ar-H), 2.67 (s, 3H, Qu-CH_3); MS (*m*/*z*, %): 374.08 ($M^+ + 1$, 41.12).

*7.1.7.17. 3-({(E)-[3-(4-Chloro-3-methylphenoxy)phenyl]methylidene} amino)-2-methylquinazolin-4(3H)-one (**MtQZ 7**).* IR (KBr, cm^{-1}) υ: 3064 (Qu-H, Ar-H), 1675 (C=O), 1593 (N=CH), 1467–1568 (C=C, benzene and Qu-ring), 1243 (O); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.92 (s, 1H, CH=N), 6.82–8.31 (a set of signals, 11H, Qu-H and Ar-H), 2.65 (s, 3H, Qu-CH_3), 2.37 (s, 3H, CH_3); MS (*m*/*z*, %): 404.09 ($M^+ + 1$ for ^{35}Cl, 47.67), 406.10 ($M^+ + 1$ for ^{37}Cl, 15.7).

*7.1.7.18. 2-Methyl-3-({(E)-[3-(naphthalen-2-yloxy)phenyl]methylidene}amino)quinazolin-4(3H)-one (**MtQZ 8**).* IR (KBr, cm^{-1}) υ: 3061 (Qu-H, Ar-H), 1672 (C=O), 1591 (N=CH), 1469–1570 (C=C, benzene and Qu-ring), 1244 (O), 834, 823 (β-naphthyl); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.95 (s, 1H, CH=N), 6.83–8.34 (a set of signals, 15H, Qu-H, naphthyl-H and Ar-H), 2.67 (s, 3H, Qu-CH_3); MS (*m*/*z*, %): 393.11 ($M^+ + 1$, 41.22).

*7.1.7.19. 3-({(E)-[3-(1,3-Benzodioxol-5-yloxy)phenyl]methylidene} amino)-2-methylquinazolin-4(3H)-one (**MtQZ 9**).* IR (KBr, cm^{-1}) υ: 3057 (Qu-H, Ar-H), 1671 (C=O), 1597 (N=CH), 1471–1568 (C=C, benzene and Qu-ring), 1243 (O); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.91 (s, 1H, CH=N), 6.79–8.21 (a set of signals, 11H, Qu-H, sesamol-H and Ar-H), 5.93 (s, 2H, CH_2), 2.65 (s, 3H, Qu-CH_3); MS (*m*/*z*, %): 400.13 ($M^+ + 1$, 33.21).

*7.1.7.20. 2-Methyl-3-[(2-oxo-1,2-dihydro-3H-indol-3-ylidene) amino]quinazolin-4(3H)-one (**MtQZ 10**).* IR (KBr, cm^{-1}) υ: 3263 (NH), 3066 (Qu-H, Isatin-H), 1684 (C=O), 1587 (N=C), 1465–1568 (C=C, benzene and Qu-ring); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 12.83 (s, 1H, NH of isatin, D_2O exchangeable), 6.95–8.22 (a set of signals, 8H, Qu-H and isatin-H), 2.63 (s, 3H, Qu-CH_3); MS (*m*/*z*, %): 305.06 ($M^+ + 1$, 61.40).

*7.1.7.21. 3-{[(E)-(3-Phenoxyphenyl)methylidene]amino}-2-propylquinazolin-4(3H)-one (**PrQZ 1**).* IR (KBr, cm^{-1}) υ: 3049 (Qu-H, Ar-H), 1685 (C=O), 1602 (N=CH), 1447–1521 (C=C, benzene and Qu-ring), 1241 (O), 751, 697 (mono substituted benzene); ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 8.41 (s, 1H, CH=N), 6.80–8.27 (a set of signals, 13H, Qu-H and Ar-H), 2.46 (t, 2H, CH_2Qu), 1.80 (sextet, 2H, CH_2Me), 1.01 (t, 3H, CH_3); MS (*m*/*z*, %): 384.10 ($M^+ + 1$, 27.13).

*7.1.7.22. 3-({(E)-[3-(4-Nitrophenoxy)phenyl]methylidene}amino)-2-propylquinazolin-4(3H)-one (**PrQZ 2**).* IR (KBr, cm^{-1}) υ: 3062 (Qu-H, Ar-H), 1677 (C=O), 1604 (N=CH), 1521 (N=O), 1451–1525 (C=C, benzene and Qu-ring), 1244 (O); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.43 (s, 1H, CH=N), 6.73–8.23 (a set of signals, 12H, Qu-H and Ar-H), 2.48 (t, 2H, CH_2Qu), 1.85 (sextet, 2H, CH_2Me), 1.00 (t, 3H, CH_3); MS (*m*/*z*, %): 429.08 ($M^+ + 1$, 34.10).

*7.1.7.23. 3-{(E)-[3-(4-Methylphenoxy)phenyl]methylidene}amino-2-propylquinazolin-4(3H)-one (**PrQZ 3**).* IR (KBr, cm^{-1}) υ: 3042 (Qu-H, Ar-H), 1668 (C=O), 1607 (N=CH), 1445–1542 (C=C, benzene and Qu-ring), 1241 (O); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.35 (s, 1H, CH=N), 6.81–8.24 (a set of signals, 12H, Qu-H and Ar-H), 2.47 (t, 2H, CH_2Qu), 2.37 (s, 3H, Ar-CH_3), 1.86 (sextet, 2H, CH_2Me), 1.06 (t, 3H, CH_2CH_3); MS (*m*/*z*, %): 398.11 ($M^+ + 1$, 38.81).

*7.1.7.24. 3-({(E)-[3-(4-Chlorophenoxy)phenyl]methylidene}amino)-2-propylquinazolin-4(3H)-one (**PrQZ 4**).* IR (KBr, cm^{-1}) υ: 3061 (Qu-H, Ar-H), 1683 (C=O), 1608 (N=CH), 1444–1527 (C=C, benzene and Qu-ring), 1242 (O); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.47 (s, 1H, CH=N), 6.87–8.28 (a set of signals, 12H, Qu-H and Ar-H), 2.48 (t, 2H, CH_2Qu), 1.87 (sextet, 2H, CH_2Me), 1.05 (t, 3H, CH_3); MS (*m*/*z*, %): 418.14 ($M^+ + 1$ for ^{35}Cl, 43.12), 420.08 ($M^+ + 1$ for ^{37}Cl, 14.02).

*7.1.7.25. 3-({(E)-[3-(4-Bromophenoxy)phenyl]methylidene}amino)-2-propylquinazolin-4(3H)-one (**PrQZ 5**).* IR (KBr, cm^{-1}) υ: 3049 (Qu-H, Ar-H), 1680 (C=O), 1599 (N=CH), 1450–1526 (C=C, benzene and Qu-ring), 1240 (O); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.47 (s, 1H, CH=N), 6.78–8.26 (a set of signals, 12H, Qu-H and Ar-H), 2.47 (t, 2H, CH_2Qu), 1.81 (sextet, 2H, CH_2Me), 1.01 (t, 3H, CH_3); MS (*m*/*z*, %): 462.05 ($M^+ + 1$ for ^{79}Br, 28.31), 464.07 ($M^+ + 1$ for ^{81}Br, 27.11).

*7.1.7.26. 3-({(E)-[3-(4-Fluorophenoxy)phenyl]methylidene}amino)-2-propylquinazolin-4(3H)-one (**PrQZ 6**).* IR (KBr, cm^{-1}) υ: 3052 (Qu-H, Ar-H), 1684 (C=O), 1596 (N=CH), 1446–1530 (C=C, benzene and Qu-ring), 1242 (O); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.43 (s, 1H, CH=N), 6.80–8.25 (a set of signals, 12H, Qu-H and Ar-H), 2.47 (t, 2H, CH_2Qu), 1.82 (sextet, 2H, CH_2Me), 1.00 (t, 3H, CH_3); MS (*m*/*z*, %): 402.11 ($M^+ + 1$, 34.55).

*7.1.7.27. 3-({(E)-[3-(4-Chloro-3-methylphenoxy)phenyl]methylidene}amino)-2-propylquinazolin-4(3H)-one (**PrQZ 7**).* IR (KBr, cm^{-1}) υ: 3062 (Qu-H, Ar-H), 1678 (C=O), 1603 (N=CH), 1444–1526 (C=C, benzene and Qu-ring), 1244 (O); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.42 (s, 1H, CH=N), 6.80–8.31 (a set of signals, 11H, Qu-H and Ar-H), 2.44 (t, 2H, CH_2Qu), 2.38 (s, 3H, Ar-CH_3), 1.82 (sextet, 2H, CH_2Me), 1.06 (t, 3H, CH_2CH_3); MS (*m*/*z*, %): 432.11 ($M^+ + 1$ for ^{35}Cl, 47.17), 434.10 ($M^+ + 1$ for ^{37}Cl, 15.25).

*7.1.7.28. 3-({(E)-[3-(Naphthalen-2-yloxy)phenyl]methylidene}amino)-2-propylquinazolin-4(3H)-one (**PrQZ 8**).* IR (KBr, cm^{-1}) υ: 3063 (Qu-H, Ar-H), 1676 (C=O), 1595 (N=CH), 1442–1517 (C=C, benzene and Qu-ring), 1241 (O), 835, 823 (β-naphthyl); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.48 (s, 1H, CH=N), 6.81–8.26 (a set of signals, 15H, Qu-H, naphthyl-H and Ar-H), 2.44 (t, 2H, CH_2Qu), 1.81 (sextet, 2H, CH_2Me), 1.05 (t, 3H, CH_3); MS (*m*/*z*, %): 434.16 ($M^+ + 1$, 22.48).

*7.1.7.29. 3-({(E)-[3-(1,3-Benzodioxol-5-yloxy)phenyl]methylidene}amino)-2-propylquinazolin-4(3H)-one (**PrQZ 9**).* IR (KBr, cm^{-1}) υ: 3057 (Qu-H, Ar-H), 1681 (C=O), 1603 (N=CH), 1455–1520 (C=C, benzene and Qu-ring), 1244 (O); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 8.42 (s, 1H, CH=N), 6.78–8.26 (a set of signals, 11H, Qu-H, sesamol-H and Ar-H), 5.96 (s, 2H, CH_2), 2.46 (t, 2H, CH_2Qu), 1.84 (sextet, 2H, CH_2Me), 1.06 (t, 3H, CH_3); MS (*m*/*z*, %): 428.07 ($M^+ + 1$, 34.23).

*7.1.7.30. 3-[(2-Oxo-1,2-dihydro-3H-indol-3-ylidene)amino]-2-propylquinazolin-4(3H)-one (**PrQZ 10**).* IR (KBr, cm^{-1}) υ: 3212 (NH), 3062 (Qu-H, Isatin-H), 1673 (C=O), 1611 (N=C), 1466–1529 (C=C, benzene and Qu-ring); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 10.87 (s, 1H, NH of Isatin, D_2O exchangeable), 6.77–8.24 (a set of signals, 8H, Qu-H and Isatin-H), 2.42 (t, 2H, CH_2Qu), 1.82 (sextet, 2H, CH_2Me), 1.03 (t, 3H, CH_3); MS (*m*/*z*, %): 333.10 ($M^+ + 1$, 21.14).

7.2. Pharmacology

The evaluation of anticonvulsant activity and neurotoxicity was carried out by the Epilepsy Branch, National Institute of Neurological Disorder and Stroke, National Institute of Health, Bethesda, USA following the reported procedures.

Male albino mice (CF-1 strain, 18–25 g) and male albino rats (Sprague–Dawley, 100–150 g) were used as experimental animals. The synthesized derivatives were suspended in 0.5% methyl cellulose and the test compound is usually manipulated with a motar pestle to help preparation of suspension. The titled compounds (**PhQZ 1–4, PhQZ 8** and **10**) were subjected to anticonvulsant screening by MES and scMET model. The titled compounds (**PhQZ 5–7** and **9**, **MtQZ 1–10** and **PrQZ 1–10**) were subjected to anticonvulsant screening by 6 Hz psychomotor seizure test. In the anticonvulsant screening by MES and scMET tests, each compound was administered as an i.p. injection at three dose levels (30, 100 and 300 mg/kg) and anticonvulsant and neurotoxic effects were assessed at 30 min and 4h intervals after administration. Similarly in 6 Hz psychomotor seizure test screening was performed at five different time points i.e., 0.25 h, 0.5 h, 1.0 h, 2.0 h and 4.0 h at a dose of 100 mg/kg administered i.p. Neurotoxicity was observed by minimal motor impairment which was measured by the rotarod (neurotoxicity) test. Compounds showing significant protection were evaluated for quantification studies in 6 Hz test and ED_{50} and protection index (PI) value reported. Selected derivatives were also evaluated in *in-vitro* hippocampal slice culture neuroprotection assay.

7.2.1. Maximal electroshock (MES) test

For MES test, 60 Hz of alternating current (50 mA in mice, 150 mA in rats) is delivered for 0.2 s by corneal electrodes which have been primed with an electrolyte solution containing anesthetic agent (0.5% tetracaine HCl). An animal is considered protected from MES-induced seizures upon abolition of hind limb tonic extensor component of the seizure.

7.2.2. Subcutaneous metrazol (scMET) seizure threshold test

For scMET test animals are pretreated with various doses of the test compound. At a previously determined TPE of the test compound the dose of metrazol which will induce convulsion in 97% of animals is injected into a loose fold of skin in the midline of the neck. The animals are placed in an isolation cage to minimize stress and observed for the next 30 min to see the absence of seizure. An episode of clonic spasms, approximately 3–5 s of the fore and/or hind limbs, jaws or vibrissae was taken as the end point. Animals which do not meet this criterion were considered protected.

7.2.3. Neurotoxicity-minimal motor impairment (MMI)

Minimal motor impairment was measured by the rotarod (neurotoxicity) test. When a mouse is placed on a rod that rotates at a speed of 6 rpm, the animal can maintain its equilibrium for a long period of time. The compound was considered toxic if the treated animal falls off this rotating rod 3 times during 1 min period.

7.2.4. 6 Hz psychomotor seizure test

6 Hz psychomotor seizure test or minimal clonic seizure (6 Hz) test was used to assess compound's efficacy against electrically induced seizures but used a lower frequency (6 Hz) and longer duration of stimulation (3 s). Test compounds were pre-administered to mice via i.p. injection. At varying times, individual mice (four mice per time point) were challenged with sufficient current delivered through corneal electrodes to elicit a psychomotor seizure in 97% animals (32 mA for 3 s). The untreated mice would

display seizure characterized by a minimal clonic phase followed by stereotyped, automatistic behaviors, described originally as being similar to the aura of human patients with partial seizure. Animals not displaying this behavior are considered to be protected. Most potent derivatives were tested quantitatively in the 6 Hz study and ED_{50} reported.

7.2.5. In-vitro hippocampal slice culture neuroprotection assay (NP): primary screen experiment

The "Primary Screen Experiment" is a qualitative assessment of the ability of a compound to prevent excitotoxic cell death. Organotypic hippocampal slice cultures are treated with N-methyl-D-aspartate (NMDA) or kainic acid (KA) to induce neuronal cell death. Propidium iodide, a membrane-impermeant compound, is included in all wells of the culture plate. Dying cells have compromised cell membranes, thus propidium iodide may diffuse into the cell, intercalate with DNA and fluoresce. Thus, the intensity of the propidium iodide fluorescence is proportional to the amount of cell death in the individual slices. Hippocampal slice cultures are treated with the excitotoxin alone, or where indicated above, with the excitotoxin and either one or two investigational compounds at the concentrations indicated. If neuroprotection occurs as a consequence of the added compound, slice cultures will have a visibly reduced fluorescent intensity when compared to the slice cultures that have been treated with the excitotoxin alone.

7.3. Computational study

7.3.1. Distance mapping

The pharmacophore pattern studies in which distance between the various groups postulated as essential for anticonvulsant activity were done on the 3D optimized structures using ACD/3D viewer version 12.01 and Argus Lab 4.0 Mark A. Thompson Planaria Software LLC. In conformational analysis of the ten clinically effective, well known and structurally different anticonvulsant drugs such as albutoin, carbamazepine, gabapentin, lamotrigine, mephobarbital, phenytoin, progabide, ralitoline, remacemide, and zonisamide; a molecular model was suggested on the basis of molecular dynamics distance estimations [13].

7.3.2. Calculation of physicochemical parameters

A computational study of titled compounds was performed for prediction of ADME properties. Polar surface area (TPSA) [12], milog P, number of rotatable bonds, molecular volume, number of hydrogen donor and acceptor atoms and violations of Lipinski's rule of five [14] were calculated using Molinspiration online property calculation toolkit [15]. Absorption (%ABS) was calculated by: $\%ABS = 109\text{-}(0.345 \times TPSA)$ [16].

7.3.3. log P determination

The partition coefficient between octanol and phosphate buffer was determined at room temperature [17]. 10 mL of octanol and 10 mL phosphate buffer were taken in a glass stoppered graduated tube and 5 mg of accurately weighed compound was added. The mixture was then shaken with the help of mechanical shaker for 24 h at room temperature and then transferred to a separating funnel and allowed to dynamic equilibrate for 6 h. The aqueous and octanol phases were separated and filtered through membrane filter and drug content in aqueous phase was analyzed by UV spectroscopy. Theoretical milog P for synthesized compounds was then compared with the experimental log P data.

7.3.4. Docking study

Compounds **PhQZ 7**, **MtQZ 3** and **PrQZ 10** were selected as ligands for docking studies with six established epilepsy receptors namely GABA(A) alpha-1, GABA(A) delta, glutamate, Na/H exchanger, Na channel and T-type calcium channel receptor. These receptors are the most important molecular targets in the design and discovery of successful antiepileptic drugs [18]. In the present study, AutoDock 4.0 with its Lamarckian genetic algorithm (LGA) was used for automated flexible ligand docking of **PhQZ 7**, **MtQZ 3** and **PrQZ 10** with above mentioned receptors and affinity (kcal/mol) and H-bond properties were evaluated.

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