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Original article

Design, synthesis and anticonvulsant evaluation of novel *N*-(4-substituted phenyl)-2-[4-(substituted) benzylidene]-hydrazinecarbothio amides

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ABSTRACT

Thirty six new *N*-(4-substituted phenyl)-2-[4-(substituted) benzylidene]-hydrazinecarbothioamides were synthesized and evaluated for anticonvulsant activity and neurotoxicity. The anticonvulsant activity was established in three seizure models i.e. MES, scMET and 6 Hz model. The most active compound was 2-[4-(4-chlorophenoxy)benzylidene]-*N*-(4-fluorophenyl)hydrazinecarbothioamide **PC 31** which showed 100% protection at 0.5 h in the 6 Hz test. Compound 2-[4-(4-bromophenoxy) benzylidene]-*N*-(4-bromophenyl) hydrazinecarbothioamide **PC 23** was found to be active in both the MES and 6 Hz test. A computational study was carried out from calculation of a pharmacophore pattern and the 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 the Lamarckian genetic algorithm based on flexible docking studies.

1. Introduction

Epilepsy is one of the most common disorders of the brain, affecting more than 50 million individuals worldwide [1,2]. Epilepsy is a chronic and often progressive disorder characterized by the periodic and unpredictable occurrence of epileptic seizures that are caused by abnormal discharge of cerebral neurons [2]. Epilepsy is not a disease, but a syndrome of different cerebral disorders of the CNS. This syndrome is characterized by paroxysmal, excessive, and hyper synchronous discharges of large numbers of neurons [3]. About 50 million people worldwide have epilepsy with almost 90% of these people being in developing countries [4]. Epilepsy also affects about 4% of individuals over their life time. Despite the development of several new anticonvulsants, over 30% of people with epilepsy do not have seizure control and others do so only at the expense of significant dose related toxicity and peculiar adverse effects that range in harshness from minimal brain impairment and megaloblastic anemia to death from aplastic anemia or hepatic failure [5,6]. These limitations with conventional antiepileptic drugs demand the need for the development of more effective and safer antiepileptic drugs.

Several investigations have recognized aryl thiosemicarbazones as structurally novel class of anticonvulsants [7–10]. In terms of interaction at the binding site, as proposed by Dimmock et al., the pharmacophoric elements were thought to be in the lipophilic aryl ring and hydrogen bonding domain. The attachment of second aryl ring, designated as the distal ring to the proximal aryl ring to increase the van der waal's bonding at the binding site and to increase potency, has also been reported [11,12]. Substitution in the aryl ring by halogens has been found to increase potency in the MES screen [13,14]. To test this hypothesis, the design and synthesis of the titled compounds were carried out by molecular hybridization of the aryl thiosemicarbazide (1) and the 4-(aryloxy)phenyl semicarbazones (2) (Fig. 1). These aryl thiosemicarbazones do not possess the dicarboxamide group as found in conventional antiepileptic drugs e.g. barbiturates, hydantoins, oxazolidiones etc., which may be associated with toxicity and side effects.

The present work reports the synthesis and anticonvulsant activity of *N*-(4-substituted phenyl)-2-[4-(substituted) benzylidene] hydrazinecarbothioamides **PC 1–36**. Their chemical structures were characterized using IR, ^{1}H NMR, MS and elemental

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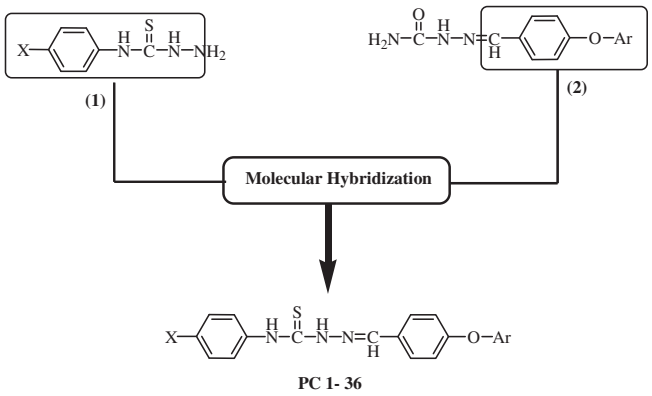


Fig. 1. Concept to design of new *N*-(4-substituted phenyl)-2-[4-(substituted) benzylidene] hydrazinecarbothioamides **PC 1**–**36**.

analysis techniques. All the synthesized titled compounds comprised of the essential pharmacophoric elements (Fig. 2) that are necessary for good anticonvulsant activity as suggested by Unverferth et al. [15]. Their anticonvulsant activity was evaluated by using experimental epilepsy models, i.e., maximal electroshock (MES), subcutaneous metrazole (scMET) and psychomotor seizure (6 Hz) test in mice. The rotorod 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 prediction of pharmacokinetic parameters. In this study, we have used AutoDock 4.0 along with its LGA algorithm for automated flexible ligand docking of compounds with six established epilepsy molecular targets and evaluated docking affinity and count of probable hydrogen bonds.

2. Chemistry

The reaction sequence leading to the formation of the titled compounds, viz. *N*-(4-substituted phenyl)-2-[4-(substituted) benzylidene]-hydrazine carbothioamides **PC 1**–**36** is shown in Scheme 1. The *N*-(4-substituted phenyl) hydrazinecarbothioamides **2a**–**d** were obtained by reacting 4-substituted aniline **1a**–**d** with carbon disulphide in presence of sodium hydroxide and then with hydrazine hydrate. The 4-substituted benzaldehydes **5a**–**i** were prepared by refluxing various substituted phenol **3a**–**i** with 4-fluoro benzaldehyde **4** in *N*,*N*′-DMF in presence of potassium carbonate The *N*-(4-substituted phenyl) hydrazinecarbothioamides **2a**–**d** were refluxed with 4-substituted benzaldehyde **5a**–**i** in the presence of catalytic amount of glacial acetic acid to yield the titled compounds **PC 1**–**36**. Thin layer chromatography (TLC) was run throughout the reactions to optimize the reactions for purity and completion. The physical data for the newly synthesized compounds are presented in Table 1.

The formation of *N*-(4-substituted phenyl) hydrazinecarbothioamides **2a**–**d** from 4-substituted aniline **1a**–**d** was confirmed by its IR and ^{1}H NMR spectral studies. The IR spectra of compound **2b** showed a broad band of $NHNH_2$ group at 3338 cm^{-1} and C=S stretching at 1164 cm^{-1}. Its ^{1}H NMR spectrum showed a singlet

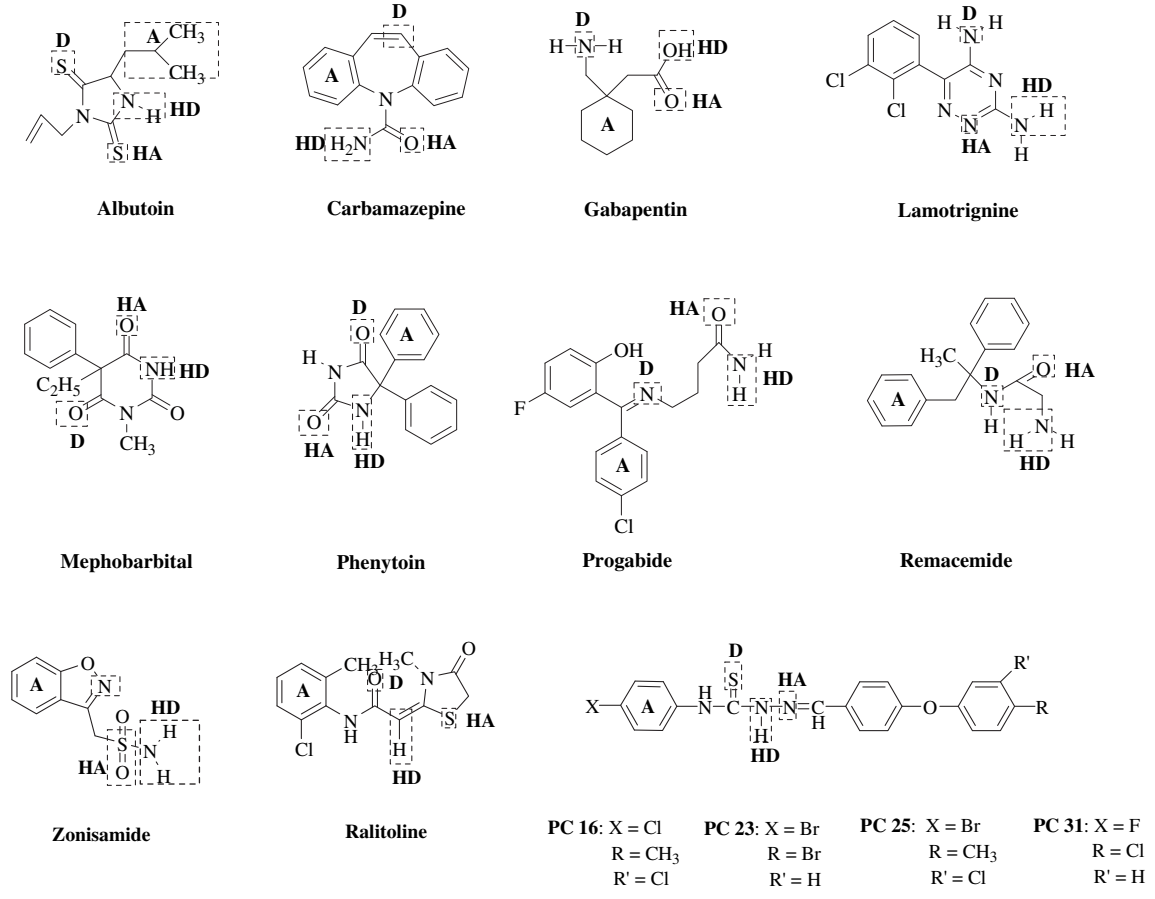
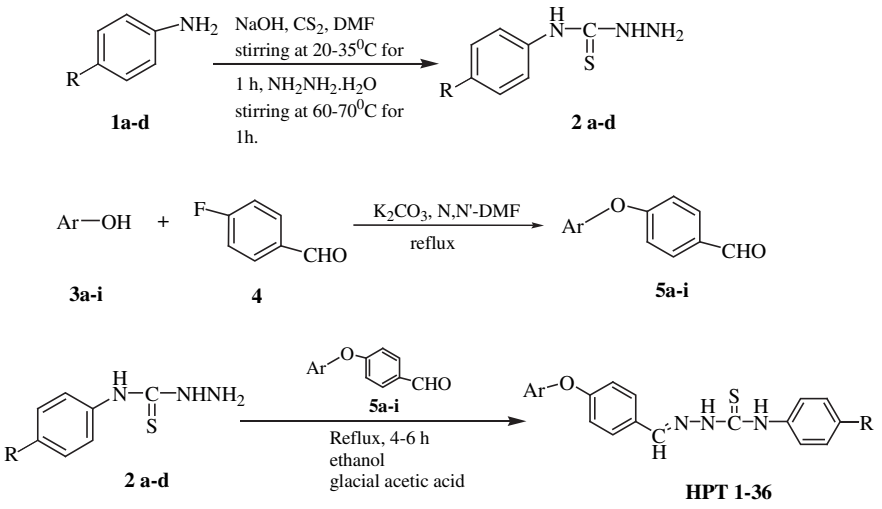
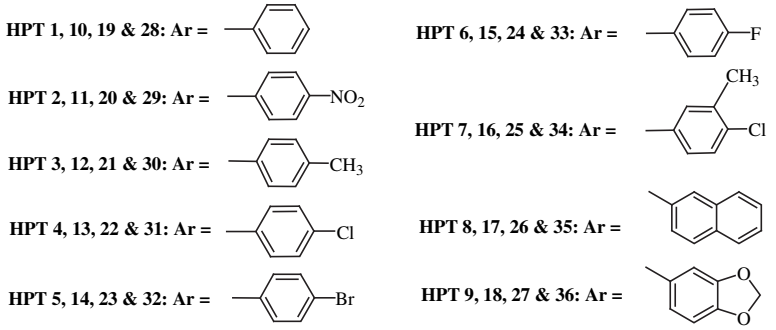


Fig. 2. Pharmacophoric pattern of well-known anticonvulsants.



(**HPT 1-9**, R = H, **HPT 10-18**, R= Cl; **HPT 19-27**, R= Br, **HPT 28-36**, R= F)



Scheme 1. Synthesis of *N*-(4-substituted phenyl)-2-[4-(substituted)benzylidene]- hydrazine carbothioamides **PC 1**–**36**.

of $-NH_2$ protons at δ 4.66 ppm. Four aromatic protons (Ar–H) appeared as a set of signals in the region δ 6.98–7.64 ppm. One proton of NH–N and NH–Ar appeared at δ 9.10 and δ 9.48 ppm respectively were D_2O exchangeable.

The formation of 4-substituted benzaldehydes **5a**–**i** from 4-substituted phenols was confirmed by its IR and ^{1}H NMR spectral studies. The IR spectrum of 4-(4-chloro-phenoxy) benzaldehydes **5d** showed bands at 1662 and 1241 cm^{-1} indicating the presence of C=O *str* and diaryl ether linkage (–O–). The absence of broad band of phenolic –OH at 2900–3000 cm^{-1} confirms the conversion of 4-chloro phenol **3d** to 4-(4-chlorophenoxy) benzaldehydes **5d**. In its ^{1}H NMR spectrum a singlet at δ 9.81 ppm indicating the presence of –CHO group, whereas the absence of a singlet around δ 9.288 ppm for phenolic –OH confirm the conversion of **3d** to **5d**.

The structural assignments to new compounds were based on their elemental analysis and spectral (FT–IR, ^{1}H NMR and mass) data. The IR spectrum of the titled compound **PC 4** showed *N*-H*str* at 3230 and 3117 cm^{-1}, CH = N*str* at 1587 cm^{-1}, diaryl ether linkage (–O–) at 1241 cm^{-1} and C=S*str* at 1165 cm^{-1}. Its ^{1}H NMR spectrum showed a singlet of imine (CH=N) proton that resonated at δ 7.89 ppm. Thirteen aromatic protons (Ar–H) appeared as a set of signals in the region δ 6.97–7.67 ppm. One proton of NH–N and NH–Ar appeared at δ 9.16 and δ 9.85 ppm respectively, were D_2O exchangeable. The mass spectrum showed $M^+ + 1$ peak (m/z) at 382.13 (100.00%) and 384.14 (39.52%) for ^{35}Cl and ^{37}Cl respectively. The presence of CH=N*str* at 1587 cm^{-1} in IR spectrum and a singlet for imine (CH=N) proton at δ 7.89 ppm in ^{1}H NMR spectrum, confirmed the formation of **PC 4**. Further mass spectrum confirmed its purity and molecular weight.

The IR spectrum of the titled compound **PC 13** showed *N*–H$_{str}$ at 3265 and 3142 cm^{-1}, CH=N$_{str}$ at 1589 cm^{-1}, diaryl ether linkage (–O–) at 1243 cm^{-1} and C=S$_{str}$ at 1165 cm^{-1}. Its ^{1}H NMR spectrum showed a singlet of imine (CH=N) proton that resonated at δ 7.83 ppm. Twelve aromatic protons (Ar–H) appeared as a set of signals in the region δ 6.90–7.64 ppm. One proton of NH–N and NH–Ar appeared at δ 9.08 and δ 9.43 ppm respectively were D_2O exchangeable. The mass spectrum showed $M^+ + 1$ peak (m/z) at 416.16 (100.00%), 418.09 (76.62%) and 420.22 (24.30%) for ^{35,35}Cl, ^{35,37}Cl and ^{37,37}Cl respectively. The presence of CH=N$_{str}$ at 1589 cm^{-1} in IR spectrum and a singlet for imine (CH=N) proton at δ 7.83 ppm in ^{1}H NMR spectrum, confirmed the formation of **PC 13**. Further mass spectrum confirmed its purity and molecular weight.

3. Pharmacology

The newly synthesized *N*-(4-substituted phenyl)-2-[4-(substituted)benzylidene] hydrazine carbothioamides **PC 1**–**36** were subjected to anticonvulsant screening according to the anti-convulsant drug development (ADD) program protocol. The profile of anticonvulsant activity was established after i.p. injections into mice and evaluated in the maximal electroshock (MES) and subcutaneous metrazole (scMET) using doses of 30, 100 and 300 mg/kg at two different time intervals. Neurotoxicity was observed by minimal motor impairment which was measured by

Table 1
Physical and elemental analyses data of *N*-(4-substituted phenyl)-2-[4-(substituted)benzylidene]-hydrazinecarbothioamides **PC 1**–**36**.

Compd.	Ar	*R*	Molecular formula (M.W.)	Mp (°C)	Yield (%)	Elemental analysis (%): found (cal.)		
						C	H	N
PC 1	Phenyl	H	$C_{20}H_{17}N_3OS$ (347.43)	146	65	69.11 (69.14)	4.95 (4.93)	12.06 (12.09)
PC 2	4-NO_2 phenyl	H	$C_{20}H_{16}N_4O_3S$ (392.43)	175	71	61.20 (61.21)	4.07 (4.11)	14.22 (14.28)
PC 3	4-CH_3 phenyl	H	$C_{21}H_{19}N_3OS$ (361.46)	202	78	69.75 (69.78)	5.25 (5.30)	11.60 (11.63)
PC 4	4-Cl phenyl	H	$C_{20}H_{16}ClN_3OS$ (381.88)	205	72	62.86 (62.90)	4.21 (4.22)	10.93 (11.00)
PC 5	4-Br phenyl	H	$C_{20}H_{16}BrN_3OS$ (426.33)	212	77	56.30 (56.34)	3.76 (3.78)	9.87 (9.86)
PC 6	4-F phenyl	H	$C_{20}H_{16}FN_3OS$ (365.42)	168	75	65.71 (65.74)	4.39 (4.41)	11.45 (11.50)
PC 7	3-CH_3, 4-Cl phenyl	H	$C_{21}H_{18}ClN_3OS$ (395.91)	222	79	6.67 (63.71)	4.55 (4.58)	10.57 (10.61)
PC 8	Naphthalene-2-yl	H	$C_{24}H_{19}N_3OS$ (397.49)	158	81	72.48 (72.52)	4.80 (4.82)	10.52 (10.57)
PC 9	1,3-benzodioxol-5-yl	H	$C_{21}H_{17}N_3O_3S$ (391.44)	115	71	64.39 (64.43)	4.34 (4.38)	10.68 (10.73)
PC 10	Phenyl	Cl	$C_{20}H_{16}ClN_3OS$ (381.88)	106	62	62.6 (62.90)	4.0 (4.22)	10.9 (11.00)
PC 11	4-NO_2 phenyl	Cl	$C_{20}H_{15}ClN_4O_3$ (426.88)	108	68	56.23 (56.27)	3.3 (3.54)	13.12 (13.12)
PC 12	4–CH_3 phenyl	Cl	$C_{21}H_{18}ClN_3OS$ (395.91)	140	71	63.4 (63.71)	4.3 (4.58)	10.4 (10.61)
PC 13	4-Cl phenyl	Cl	$C_{20}H_{15}Cl_2N_3OS$ (416.32)	150	77	57.67 (57.70)	3.55 (3.63)	10.8 (10.09)
PC 14	4-Br phenyl	Cl	$C_{20}H_{15}BrClN_3OS$ (460.77)	172	74	52.0 (52.13)	3.18 (3.28)	8.99 (9.12)
PC 15	4-F phenyl	Cl	$C_{20}H_{15}ClFN_3OS$ (399.87)	156	72	60.01 (60.07)	3.68 (3.78)	10.49 (10.51)
PC 16	3-CH_3, 4-Cl phenyl	Cl	$C_{21}H_{17}Cl_2N_3OS$ (430.35)	143	76	58.56 (58.61)	3.88 (3.98)	9.68 (9.76)
PC 17	Naphthalene-2-yl	Cl	$C_{24}H_{18}ClN_3OS$ (431.94)	152	78	66.67 (66.74)	4.18 (4.20)	9.66 (9.73)
PC 19	Phenyl	Br	$C_{20}H_{16}BrN_3OS$ (426.33)	143	67	56.2 (56.34)	3.68 (3.78)	9.82 (9.86)
PC 20	4-NO_2 phenyl	Br	$C_{20}H_{15}BrN_4O_3S$ (471.33)	138	71	50.4 (50.97)	3.17 (3.21)	11.81 (11.89)
PC 21	4-CH_3 phenyl	Br	$C_{21}H_{18}BrN_3OS$ (440.36)	170	74	57.23 (57.28)	4.09 (4.12)	9.54 (9.54)
PC 22	4-Cl phenyl	Br	$C_{20}H_{15}BrClN_3OS$ (460.77)	174	72	51.10 (52.13)	3.18 (3.28)	9.10 (9.12)
PC 23	4-Br phenyl	Br	$C_{20}H_{15}Br_2N_3OS$ (505.23)	164	77	47.47 (47.55)	2.86 (2.99)	8.30 (8.32)
PC 24	4- F phenyl	Br	$C_{20}H_{15}BrFN_3OS$ (444.32)	174	70	54.01 (54.06)	3.37 (3.40)	9.43 (9.46)
PC 25	3-CH_3, 4-Cl phenyl	Br	$C_{21}H_{17}BrClN_3OS$ (474.80)	160	72	53.11 (53.12)	3.59 (3.61)	8.84 (8.85)
PC 26	Naphthalene-2-yl	Br	$C_{24}H_{18}BrN_3OS$ (476.39)	158	76	60.48 (60.51)	3.79 (3.81)	8.81 (8.82)
PC 27	1,3-benzodioxol-5-yl	Br	$C_{21}H_{16}BrN_3O_3S$ (470.34)	120	62	53.61 (53.63)	3.41 (3.43)	8.91 (8.93)
PC 28	Phenyl	F	$C_{20}H_{16}FN_3OS$ (365.42)	175	70	65.72 (65.74)	4.40 (4.41)	11.48 (11.50)
PC 29	4-NO_2 phenyl	F	$C_{20}H_{15}FN_4O_3S$ (410.42)	113	74	58.51 (58.53)	3.66 (3.68)	13.63 (13.65)
PC 30	4-CH_3 phenyl	F	$C_{21}H_{18}FN_3OS$ (379.45)	165	71	66.46 (66.47)	4.77 (4.78)	11.05 (11.07)
PC 31	4-Cl phenyl	F	$C_{20}H_{15}ClFN_3OS$ (399.87)	202	70	60.04 (60.07)	3.77 (3.78)	10.50 (10.51)
PC 32	4-Br phenyl	F	$C_{20}H_{15}BrFN_3OS$ (444.32)	162	76	54.05 (54.06)	3.39 (3.40)	9.44 (9.46)
PC 33	4- F phenyl	F	$C_{20}H_{15}F_2N_3OS$ (383.41)	140	69	62.63 (62.65)	3.93 (3.94)	10.94 (10.96)
PC 34	3-CH_3, 4-Cl phenyl	F	$C_{21}H_{17}ClFN_3OS$ (413.90)	196	77	60.92 (60.94)	4.12 (4.14)	10.14 (10.15)
PC 35	Naphthalene-2-yl	F	$C_{24}H_{18}FN_3OS$ (415.48)	192	78	69.37 (69.38)	4.35 (4.37)	10.10 (10.11)
PC 36	1,3-benzodioxol-5-yl	F	$C_{21}H_{16}FN_3O_3S$ (409.43)	115	65	61.58 (61.60)	3.93 (3.94)	10.25 (10.26)

the rotorod test. The results are shown in Table 2. Some compounds were screened in the 6 Hz model to identify their activity at five different time points, ie, 0.25, 0.5, 1.0, 2.0 and 4.0 h after i.p. administration in mice. The results are shown in Table 3. Potential compounds were also subjected to quantification studies in 6 Hz test and the corresponding ED_{50} and TD_{50} reported in Table 4. Some compounds were screened in the acute toxicity test of pilocarpine induced status prevention (PISP) model (Table 5) and *in vitro* hippocampal slice culture neuroprotection assay (NP).

4. Computational study

The pharmacophore pattern studies in which the distance between the various groups postulated as essential for anticonvulsant activity (Fig. 3) 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 (Table 6). A computational study of all 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

Table 2
Anticonvulsant activity (MES and scMET test) and neurotoxicity of *N*-(4-substituted phenyl)-2-[4-(substituted)benzylidene] hydrazinecarbothioamides.

Compound	Intraperitoneal injection in mice[a]						
	MES screen (h)			scMET screen (h)		Neurotoxicity screen (h)	
	0.5	2.0	4.0	0.5	4.0	0.5	4.0
PC 23	–	–	300[a]	–	–	–	–
Phenytoin	30[a]	30[a]	30[a]	–	–	100[a]	100[a]
Sodium valproate	–	–	–	300[a]	–	–	–

[a] Doses of 30, 100 and 300 mg/kg were administered. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The dash (–) indicates absence of activity at maximum dose administered (300 mg/kg).

Table 3
Anticonvulsant activity of some *N*-(4-substituted phenyl)-2-[4-(substituted) benzylidene] hydrazinecarbothioamides by 6 Hz model.

Compound	Dose (mg/kg)	Time (h) to peak effect (*N*/*F*)[a]				
		0.25	0.5	1.0	2.0	4.0
PC 5	100	0/4	1/4	1/4	1/4	0/4
PC 6	100	0/4	0/4	1/4	0/4	0/4
PC 7	100	0/4	1/4	0/4	0/4	0/4
PC 15	100	1/4	1/4	0/4	1/4	1/4
PC 16	100	0/4	1/4	2/4	2/4	0/4
PC 19	100	0/4	1/4	0/4	0/4	0/4
PC 21	100	0/4	1/4	1/4	0/4	0/4
PC 23	100	0/4	3/4	0/4	0/4	0/4
PC 24	100	0/4	1/4	1/4	1/4	0/4
PC 25	100	1/4	3/4	2/4	2/4	0/4
PC 28	100	1/4	1/4	0/4	0/4	0/4
PC 29	100	0/4	2/4	1/4	0/4	0/4
PC 30	100	1/4	2/4	0/4	0/4	0/4
PC 31	100	3/4	4/4	1/4	1/4	1/4
PC 32	100	0/4	1/4	0/4	0/4	0/4
PC 33	100	2/4	1/4	0/4	1/4	0/4
PC 34	100	0/4	1/4	1/4	0/4	0/4
PC 35	100	0/4	0/4	0/4	1/4	1/4

[a] *N*/*F* = number of animals active or toxic over the number tested.

Table 4
Quantification studies of **PC 31**: ED_{50} and TD_{50} values.

Compound	Test	Time (h)	ED_{50} (mg/kg)	95% Confidence interval	Slope	STD Err	PI[a] value
PC 31	6 Hz	0.5	>200	–	–	–	>2.5
	TOX	0.5	>500	–	–	–	

[a] PI value was determined by TD_{50}/ED_{50}.

(Table 7). Calculated miLog *P* for synthesized compounds were then compared with the experimental Log *P* data of these compounds (Table 8). 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. The crystallographic structure of target receptors is given in Fig. 4. Their affinity (Kcal/mol) and count of probable hydrogen bonds were evaluated (Table 9). Docking images of compounds **PC 16**, **PC 23**, **PC 25** and **PC 31** with the target receptors is shown in Fig. 5.

5. Results and discussion

5.1. Anticonvulsant activity and neurotoxicity

Compound **PC 23** was found to be active in the MES test, indicative of the compounds ability to prevent seizure spread. The compound **PC 23** showed 100% protection (1/1, 4.0 h) at a dose of 300 mg/kg. Titled compounds did not exhibit protection in the scMET test, a test used to identify compounds ability to raise seizure threshold. None of the compounds showed neurotoxicity in the highest administered dose (300 mg/kg).

Compounds showed more promising results when tested in the 6 Hz model. Compound **PC 31** was the most active one in this series with protection at two different time points, i.e. 100% (4/4, 0.5 h) and 75% (3/4, 0.25 h) at a dose of 100 mg/kg. Compound **PC 23** showed 75% protection (3/4, 0.5 h) at a dose of 100 mg/kg. Compound **PC 25** showed 75% protection (3/4, 0.5 h) and 50% protection (2/4, 1 h; 2/4, 2.0 h) at a dose of 100 mg/kg. Some compounds showed 50% protection at different time points at a dose of 100 mg/kg viz. **PC 16** (2/4, 1.0 h; 2/4, 2.0 h), **PC 29** (2/4, 0.5 h), **PC 30** (2/4, 0.5 h) and **PC 33** (2/4, 0.25 h). Other compounds showed mild activity in the 6 Hz test. Compound **PC 31** was evaluated in the quantitative 6 Hz test and showed an ED_{50} of >200 mg/kg and TD_{50} > 500 mg/kg at a time of peak effect (TPE) of 0.5 h. The PI value was >2.5.

Compound **PC 25** was subjected to the acute toxicity test of pilocarpine induced status prevention (PISP) model. In the acute toxicity test the compound **PC 25** did not show any toxicity up to 600 mg/kg. Compounds **PC 19** and **PC 31** were evaluated in the primary screen experiment of *in vitro* hippocampal slice culture neuroprotection assay (NP). No significant protection was observed against either KA- or NMDA-induced cytotoxicity.

Table 5
Pilocarpine induced status prevention (PISP) model in acute toxicity test of **PC 25**.

Compound	Test	Dose (mg/kg)	Time (h) (*N*/*F*)[a]				
			0.25	0.5	1.0	2.0	4.0
PC 25	TOX	100	0/2	0/2	0/2	0/2	0/2
	TOX	300	0/2	0/2	0/2	0/2	0/2
	TOX	600	0/2	0/2	0/2	0/2	0/2

[a] *N*/*F* = number of animals toxic over the number tested.

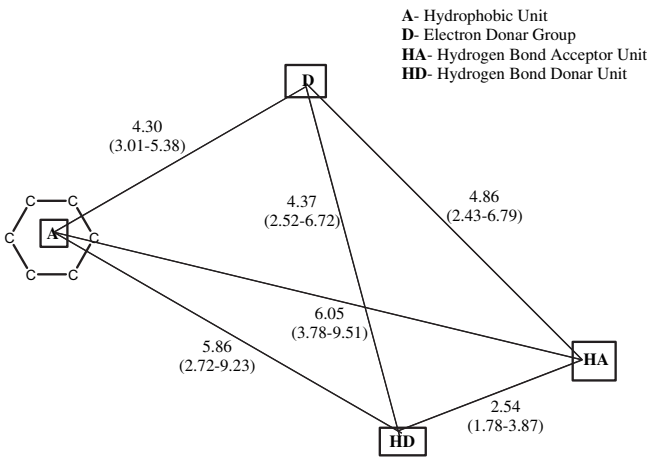


Fig. 3. Four-point 3D pharmacophore model for anticonvulsants derived by using MM3 and CHARMm parametrization (Argus Lab 4.0 and ACD/3D viewer).

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 wellknown and structurally different compounds with anticonvulsant activity- albutoin, carbamazepine, gabapentin, lamotrigine, mephobarbital, phenytoin, progabide, ralitoline, remacemide and zonisamide (Fig. 2) 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 the above mentioned ten well known anticonvulsants and *N*-(4-substituted phenyl)-2-[4-(substituted) benzylidene]-hydrazine carbothioamides using Argus Lab 4.0. Distance between the various structural components essential for activity was determined by the ACD/3D viewer. The crucial structural components that were included in the four-point pharmacophore model 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 (HD). An average distance range for every point was obtained and compared to the *N*-(4-substituted phenyl)-2-[4-(substituted)benzylidene] hydrazine carbothioamides. Now it may be interesting to examine whether the *N*-(4-substituted phenyl)-2-[4-(substituted)benzylidene] hydrazine carbothioamides reflect the conditions of the derived pharmacophore model. Our analysis of the distance relationship showed that *N*-(4-substituted phenyl)-2-[4-(substituted)benzylidene] hydrazine carbothioamides did fulfill the essential demands of the pharmacophore when compared to the average distance requirement.

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 [16]. The percentage of absorption (%ABS) was calculated

Table 6
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
Average 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)
PC 16	6.39	5.07	4.05	1.39	2.61	3.24
PC 23	6.41	5.08	4.02	1.40	2.60	3.26
PC 25	6.40	5.07	4.05	1.41	2.61	3.25
PC 31	6.41	5.08	4.06	1.42	2.62	3.26

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

using TPSA. From all these parameters, it can be observed that all titled compounds exhibited a great %ABS ranging from 77.4 to 93.2%. All compounds of this series except **PC 9** violated Lipinski's parameters. It may be observed from the results that the violation observed in Lipinski's parameters were due to higher Log *P* value than that reported in the rule.

5.2.3. Log P determination

Titled compounds showed dependence of biological activity on lipophillic 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

Table 7
Pharmacokinetic parameters important for good oral bioavailability of compounds *N*-(4-substituted phenyl)-2-[4-(substituted)benzylidene]hydrazine carbothioamide **PC 1–36**.

Compound	% ABS	TPSA (A^2)	n-ROTB	MW	Molecular volume	miLogP	n-OHNH donors	n-OH acceptors	Lipinski's violation
Rule	–	–	–	<500	–	≤5	<5	<10	≤1
PC 1	93.2	45.6	7	347.4	311.7	5.0	2	4	1
PC 2	77.4	91.4	8	392.4	335.1	5.0	2	7	1
PC 3	93.2	45.6	7	361.4	328.3	5.5	2	4	1
PC 4	93.2	45.6	7	381.8	325.3	5.7	2	4	1
PC 5	93.2	45.6	7	426.3	329.6	5.8	2	4	1
PC 6	93.2	45.6	7	365.4	316.7	5.2	2	4	1
PC 7	93.2	45.6	7	395.9	341.8	6.1	2	4	1
PC 8	93.2	45.6	7	397.5	355.7	6.2	2	4	1
PC 9	86.8	64.1	7	391.4	335.7	4.9	2	6	0
PC 10	93.2	45.6	7	381.8	325.3	5.7	2	4	1
PC 11	77.4	91.4	8	426.8	348.6	5.7	2	7	1
PC 12	93.2	45.6	7	395.9	341.8	6.1	2	4	1
PC 13	93.2	45.6	7	416.3	338.8	6.4	2	4	1
PC 14	93.2	45.6	7	460.7	343.1	6.5	2	4	1
PC 15	93.2	45.6	7	399.8	330.2	5.9	2	4	1
PC 16	93.2	45.6	7	430.3	355.4	6.8	2	4	1
PC 17	93.2	45.6	7	431.9	369.3	6.9	2	4	1
PC 18	86.8	64.1	7	425.8	349.2	5.6	2	6	1
PC 19	93.2	45.6	7	426.3	329.6	5.8	2	4	1
PC 20	77.4	91.4	8	471.3	352.9	5.8	2	7	1
PC 21	93.2	45.6	7	440.3	346.2	6.3	2	4	1
PC 22	93.2	45.6	7	460.7	343.1	6.5	2	4	1
PC 23	93.2	45.6	7	505.2	347.5	6.6	2	4	2
PC 24	93.2	45.6	7	444.3	334.5	6.0	2	4	1
PC 25	93.2	45.6	7	474.8	359.7	6.9	2	4	1
PC 26	93.2	45.6	7	476.3	373.6	7.0	2	4	1
PC 27	86.8	64.1	7	470.3	353.5	5.7	2	6	1
PC 28	93.2	45.6	7	365.4	316.7	5.2	2	4	1
PC 29	77.4	91.4	8	410.4	340.0	5.1	2	7	1
PC 30	93.2	45.6	7	379.4	333.2	5.6	2	4	1
PC 31	93.2	45.6	7	399.8	330.2	5.9	2	4	1
PC 32	93.2	45.6	7	444.3	334.5	6.0	2	4	1
PC 33	93.2	45.6	7	383.4	321.6	5.3	2	4	1
PC 34	93.2	45.6	7	413.9	346.8	6.2	2	4	1
PC 35	93.2	45.6	7	415.4	360.6	6.4	2	4	1
PC 36	86.8	64.1	7	409.4	340.6	5.1	2	6	1

%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 8
Log *P* value of *N*-(4-substituted phenyl)-2-[4-(substituted)benzylidene] hydrazinecarbothioamide **PC 1**–**36**.

Compound	Experimental Log *P*	Theoretical miLog *P*
Standard	–	≤5
PC 1	4.9	5.0
PC 2	4.95	5.0
PC 3	5.39	5.5
PC 4	5.52	5.7
PC 5	5.62	5.8
PC 6	5.09	5.2
PC 7	6.03	6.1
PC 8	6.07	6.2
PC 9	4.80	4.9
PC 10	5.64	5.7
PC 11	5.52	5.7
PC 12	5.97	6.1
PC 13	6.20	6.4
PC 14	6.37	6.5
PC 15	5.84	5.9
PC 16	6.59	6.8
PC 17	6.96	6.9
PC 18	5.54	5.6
PC 19	6.38	5.8
PC 20	5.68	5.8
PC 21	6.17	6.3
PC 22	6.24	6.5
PC 23	6.53	6.6
PC 24	5.82	6.0
PC 25	7.89	6.9
PC 26	6.93	7.0
PC 27	5.52	5.7
PC 28	5.09	5.2
PC 29	4.89	5.1
PC 30	5.48	5.6
PC 31	5.78	5.9
PC 32	5.88	6.0
PC 33	5.19	5.3
PC 34	6.07	6.2
PC 35	6.20	6.4
PC 36	4.99	5.1

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. As observed some of the experimental values were in good agreement with the theoretical values. All the titled compounds showed lipophillic character. The Log *P* determined is close to the optimum Log *P* value. Optimum Log *P* value ensures good pharmacokinetic property of drugs. Drugs possessing optimum Log *P* value will be least inhibited in their movement through the aqueous and lipophilic phases of living tissue.

5.2.4. Docking study

In this study, we have used AutoDock 4.0 along with its LGA algorithm for automated flexible ligand docking of compounds **PC 1**–**36** 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 docking affinity (Kcal/mol) and count of probable hydrogen bonds. In this study, compound **PC 16**, **PC 23**, **PC 25** and **PC 31** were selected for docking with six established epilepsy molecular targets. Compound **PC 16** exhibited good binding properties with GABA (A) alpha-1 receptor (affinity value −4.7 kcal/mol and 3 H-bonds), GABA (A) delta receptor (affinity value −6.7 kcal/mol and 2 H-bonds) and glutamate receptor (affinity value −6.0 kcal/mol and 1 H-bonds). Compound **PC 16** showed affinity for Na/H exchanger (affinity value −5.5 kcal/mol), but did not exhibit H-binding properties. Compound **PC 16** did not show affinity and H-bonding with Na channel receptor and T-type calcium channel receptor. Compound **PC 23** exhibited good binding properties with GABA (A) delta receptor (affinity value −6.3 kcal/mol and 2 H-bonds), GABA (A) alpha-1 receptor (affinity value −6.0 kcal/mol and 1 H-bonds), glutamate receptor (affinity value −6.0 kcal/mol and 1 H-bonds) and Na/H exchanger (affinity value −5.4 kcal/mol and 1 H-bonds). Compound **PC 23** did not show affinity and H-bonding with Na channel receptor and T-type calcium channel receptor. Compound **PC 25** exhibited good binding properties with glutamate receptor (affinity

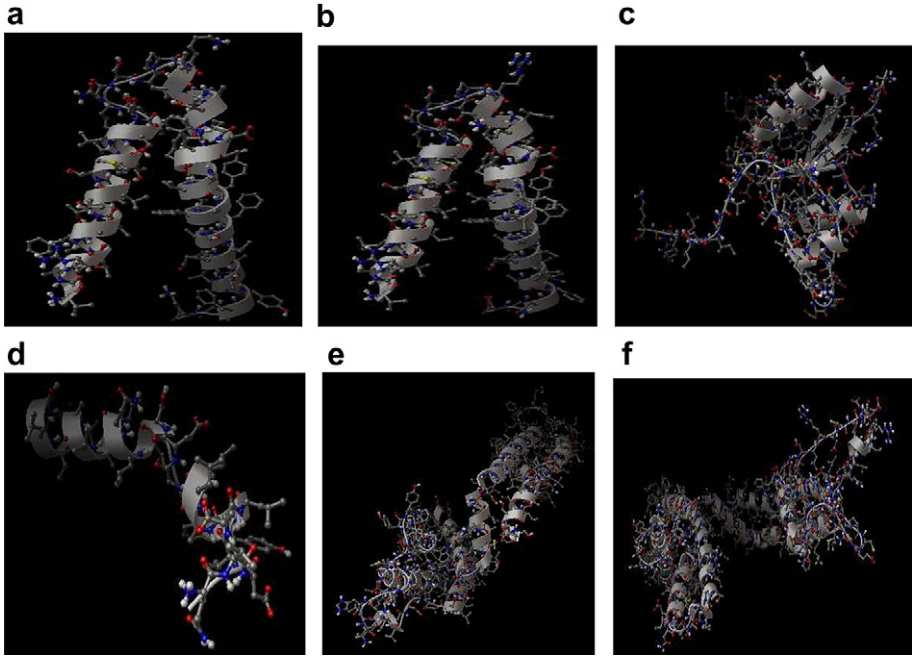


Fig. 4. Crystallographic structure of different receptors used in docking study. (a) GABA (A) alpha-1, (b) GABA(A) delta, (c) glutamate (d) Na/H exchanger (e) Na channel (f) T-type calcium channel receptor.

Table 9
Docking study of compounds **PC 16**, **PC 23**, **PC 25** and **PC 31**.[a]

Ligand	Receptor	Affinity (Kcal/mol)	H-bonds	H-binding ligand			H-binding receptor			
				Element	Atom No.	Type	Residue	Element	Atom No.	Type
PC 16	GABA(A) Alpha-1	−4.7	03	N	11	Donor	Ile 329	O	462	Acceptor
				N	11	Donor	Thr 333	O	501	Both
				N	07	Donor	Thr 333	O	501	Both
	GABA (A) delta	−6.7	02	N	11	Donor	Asp 311	O	317	Acceptor
				N	07	Donor	Asp 311	O	317	Acceptor
	Glutamate	−6.0	01	N	07	Donor	Leu 469	O	250	Acceptor
	Na/H exchanger	−5.5	00	–	–	–	–	–	–	–
	Na channel	0.0	0.0	–	–	–	–	–	–	–
	T-type calcium	0.0	0.0	–	–	–	–	–	–	–
PC 23	GABA(A) alpha-1	−6.0	01	N	07	Donor	Ala 318	O	366	Acceptor
	GABA (A) delta	−6.3	02	N	7	Donor	Asp 311	O	317	Acceptor
				N	7	Donor	Asp 311	O	320	Acceptor
	Glutamate	−6.0	01	N	11	Donor	Leu 455	O	102	Acceptor
	Na/H exchanger	−5.4	01	N	11	Donor	Phe 253	O	100	Acceptor
	Na channel	0.0	0.0	–	–	–	–	–	–	–
	T-type calcium	0.0	0.0	–	–	–	–	–	–	–
PC 25	GABA(A) alpha-1	−5.1	03	N	13	Acceptor	Val 287	N	74	Donor
				N	11	Donor	Thr 283	O	44	Acceptor
				N	07	Donor	Arg 282	O	27	Acceptor
	GABA (A) delta	−6.4	00	–	–	–	–	–	–	–
	Glutamate	−6.9	03	N	07	Donor	Lys 467	O	230	Acceptor
				N	11	Donor	Ser 465	O	213	Acceptor
				N	13	Acceptor	Ser 465	N	209	Donor
	Na/H exchanger	−5.7	01	N	07	Donor	Ala 250	O	76	Acceptor
	Na channel	0.0	0.0	–	–	–	–	–	–	–
	T-type calcium	0.0	0.0	–	–	–	–	–	–	–
PC 31	GABA(A) alpha-1	−6.0	01	N	13	Acceptor	Ser 297	O	168	Both
	GABA (A) delta	−6.2	01	N	11	Donor	Phe 320	O	414	Acceptor
	Glutamate	−6.8	03	N	07	Donor	Lys 467	O	230	Acceptor
				N	11	Donor	Ser 465	O	213	Acceptor
				N	13	Acceptor	Ser 465	N	209	Donor
	Na/H exchanger	−5.5	00	–	–	–	–	–	–	–
	Na channel	0.0	0.0	–	–	–	–	–	–	–
	T-type calcium	0.0	0.0	–	–	–	–	–	–	–

[a] Affinity and count of probable hydrogen bonds were determined by docking studies using Autodock 4.0 software.

value −6.9 kcal/mol and 3 H-bonds), GABA (A) alpha-1 receptor (affinity value −5.1 kcal/mol and 3 H-bonds) and Na/H exchanger (affinity value −5.7 kcal/mol and 1 H-bonds). Compound **PC 25** showed affinity for GABA (A) delta receptor (affinity value −6.4 kcal/mol), but did not exhibit H-binding properties. Compound **PC 25** did not show affinity and H-bonding with Na channel receptor and T-type calcium channel receptor. Compound **PC 31** exhibited good binding properties with glutamate receptor (affinity value −6.8 kcal/mol and 3 H-bonds), GABA (A) delta receptor (affinity value −6.2 kcal/mol and 1 H-bonds) and GABA (A) alpha-1 receptor (affinity value −6.0 kcal/mol and 1 H-bonds). Compound **PC 31** showed affinity for Na/H exchanger (affinity value −5.5 kcal/mol), but did not exhibit H-binding properties. Compound **PC 31** did not show affinity and H-bonding with Na channel receptor and T-type calcium channel receptor.

Docking study results show that the titled compounds exhibited good H-bonding properties with glutamate, GABA (A) alpha, GABA (A) delta and Na/H exchanger, hence emerge as potential anticonvulsants with multiple modes of action.

5.2.5. Structure–Activity relationship

Two-dimensional (2D) modeling and structure activity relationship of potential anticonvulsant agents propose the following essential pharmacophoric features for anticonvulsant activity

- at least one aryl group which acts as a lipophilic domain.
- one or two electron donor atoms.
- and/or an NH group in a special spatial arrangement which acts as a H-bonding domain.
- and a distal hydrophobic center [11].

In the present study various *N*-(4-substituted phenyl)-2-[4-substituted benzylidene] hydrazinecarbothioamides were synthesized in order to investigate the pharmacophoric substituent, responsible for the activity. The 4-substituted phenyl ring acts as a lipophilic domain, C═S group acts as electron donor, NH groups acts as hydrogen bonding domain and the aryl substituents acts as distal hydrophobic center (Fig. 6). Thus it may be stated that compounds **PC 1**–**36** fulfill the essential pharmacophoric requirements for anticonvulsant activity.

In the synthetic design of compounds, the various substituents were placed at positions 7 and 18 of *N*-phenyl-benzylidenehydrazine carbothioamide backbone structure. At position 7, phenyl, 4-nitro phenyl, 4-methyl phenyl, 4-chloro phenyl, 4-bromo phenyl, 4-fluoro phenyl, 4-chloro-3-methyl phenyl, naphthalene-2-yl, 1,3-benzodioxol-5-yl substituents were introduced. While at position 18, the substituent viz. −H, 4-chloro, 4-bromo, 4-fluoro, 4-methyl and 4-methoxy substituents were introduced.

The 4-fluoro substituent at position 18 of *N*-phenyl-benzylidenehydrazine carbothioamide backbone structure yields the most active compounds **PC 28**–**36**. These compounds displayed significant protection with longer duration of action. This observation supports the fact that electron rich substituent at para position of the aryl ring showed increased potency in anticonvulsant screen [11].

The most active compounds of this series are **PC 31**. It has 4-fluoro substituent at position 18 and 4-chloro phenyl substituent at position 7. It was observed that 4-halogenated substituents at position 18 and 4-halogenated phenyl or 4-methyl phenyl substituents at position 7 yield compounds with moderate to significant protection. The order of percentage protection among substituents at position 7 is 4-chloro phenyl $\geq$ 4-methyl phenyl $>$ 4-bromo

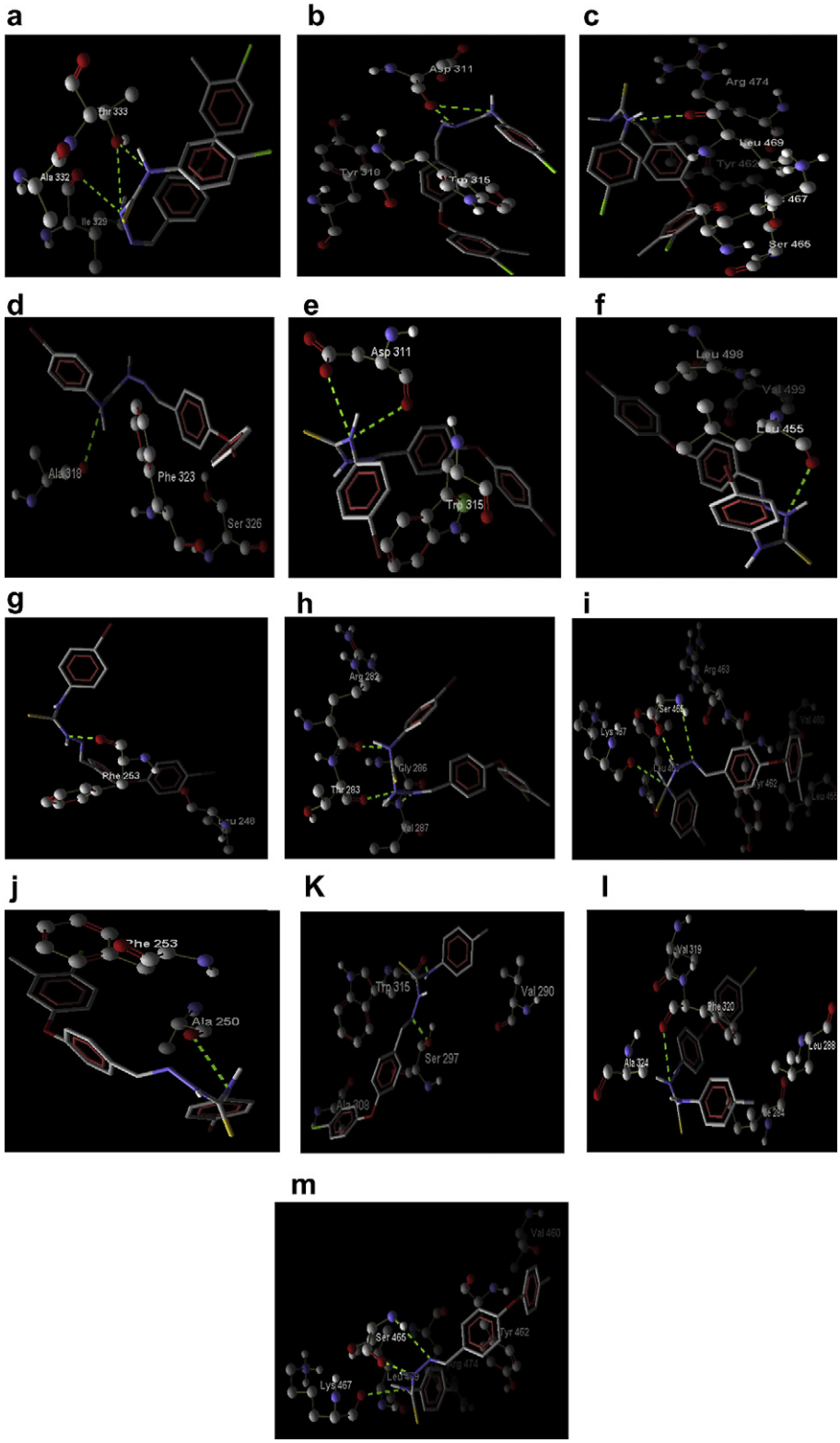


Fig. 5. Docking images of **PC 16**, **PC 23**, **PC 25** and **PC 31**. (a) **PC 16** with GABA(A) alpha-1, (b) **PC 16** with GABA(A) delta, (c) **PC 16** with glutamate receptor (d) **PC 23** with GABA(A) alpha-1 (e) **PC 23** with GABA(A) delta (f) **PC 23** with glutamate receptor, (g) **PC 23** with Na/H exchanger, (h) **PC 25** with GABA(A) alpha-1, (i) **PC 25** with glutamate receptor, (j) **PC 25** with Na/H exchanger, (k) **PC 31** with GABA(A) alpha-1, (l) **PC 31** with GABA(A) delta, (m) **PC 31** with glutamate receptor.

phenyl > 3-methyl-4-chloro phenyl > 4-fluoro phenyl. The 4-H substituent at position 18 and naphthalene-2-yl or 1,3-benzodioxol-5-yl substituent at position 7, yielded inactive compounds.

6. Conclusion

A series of *N*-(4-substituted phenyl)-2-[4-(substituted) benzylidene]-hydrazine carbothioamides were designed,

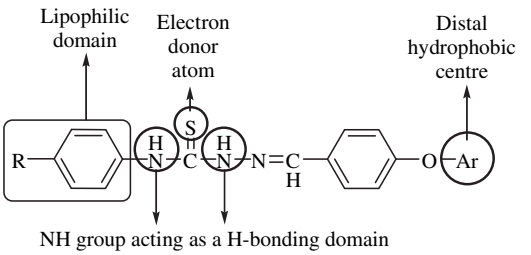


Fig. 6. Essential pharmacophoric requirements of **PC 1**–**36** for anticonvulsant activity.

synthesized, and their anticonvulsant activity and neurotoxicity were evaluated after intraperitoneal administration in three seizure models, which include the MES, scMET and 6 Hz model. A computational study was also carried out, including calculation of pharmacophore pattern, prediction of pharmacokinetic properties and docking studies. The compound **PC 31** displayed significant protection and emerged as a lead in this series. Further, compound **PC 16**, **PC 23** and **PC 25** came out as a potential candidate for further investigation. The results support the fact that substitution in the aryl ring by halogens lead to more active anticonvulsant compounds in comparision to unsubstituted derivatives. Docking study results shows 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 *N*-(4-substituted phenyl)-2-[4-(substituted) benzylidene] hydrazine carbothioamides. However, further studies need to be carried out to ascertain the precise mechanism of action of anticonvulsant activity of these molecules.

7. Experimental protocols

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 F254 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 PerkineElmer 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 (d) and signals were described as singlet (s), doublet (d), triplet (t) and 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 PerkineElmer Model 240C analyzer.

*7.1.1. Synthesis of N-(4-halogenated phenyl) hydrazinecarbothioamides (**2a**–**d**)*

To a solution of 4-halogenated aniline **1a**–**d** (0.01 mol) in DMF (15 ml), sodium hydroxide (0.012 mol) and carbon disulphide (0.01 mol) were added. The mixture was stirred at 20–25 °C for 1 h. To the stirred mixture, hydrazine hydrate (0.03 mol) was added and stirring continued at 60–70 °C for 1 h. On addition of water, a solid separated out which was recrystallized from 90% ethanol. The physical and spectral data of compounds are given below.

*7.1.1.1. N-Phenyl hydrazinecarbothioamides (**2a**).* Yield: 68%; m.p.: 181–183 °C; IR (KBr, cm^{-1}) υ: 3342 ($NHNH_2$), 1163 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 4.68 (s, 2H, NH_2), 6.92–7.66 (a set of signals, 5H, Ar–H), 9.07 (s, 1H, NH–N, D_2O exchangeable), 9.53 (s, 1H, Ar–NH, D_2O exchangeable); MS (*m*/*z*, %): 168.04 (M^+ + 1, 100.00).

*7.1.1.2. N-(4-chloro phenyl) hydrazinecarbothioamide (**2b**).* Yield: 62%; m.p.: 162–164 °C; IR (KBr, cm^{-1}) υ: 3338 ($NHNH_2$), 1164 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 4.66 (s, 2H, NH_2), 6.98–7.64 (a set of signals, 4H, Ar–H), 9.10 (s, 1H, NH–N, D_2O exchangeable), 9.48 (s, 1H, Ar–NH, D_2O exchangeable); MS (*m*/*z*, %): 203.08 (M^+ + 1 for ^{35}Cl, 78.23), 205.02 (M^+ + 1 for ^{37}Cl, 26.52).

*7.1.1.3. N-(4-bromo phenyl) hydrazinecarbothioamides (**2c**).* Yield: 68%; m.p.: 170–172 °C; IR (KBr, cm^{-1}) υ: 3332 ($NHNH_2$), 1167 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 4.62 (s, 2H, NH_2), 6.92–7.66 (a set of signals, 4H, Ar–H), 9.06 (s, 1H, NH–N, D_2O exchangeable), 9.42 (s, 1H, Ar–NH, D_2O exchangeable); MS (*m*/*z*, %): 248.95 (M^+ + 1 for ^{81}Br, 55.23), 246.98 (M^+ + 1 for ^{79}Br, 54.12).

*7.1.1.4. N-(4-fluoro phenyl) hydrazinecarbothioamides (**2d**).* Yield: 73%; m.p.: 181–182 °C; IR (KBr, cm^{-1}) υ: 3336 ($NHNH_2$), 1163 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 4.64 (s, 2H, NH_2), 6.96–7.60 (a set of signals, 4H, Ar–H), 9.03 (s, 1H, NH–N, D_2O exchangeable), 9.47 (s, 1H, Ar–NH, D_2O exchangeable); MS (*m*/*z*, %): 187.03 (M^++ 1, 82.55).

*7.1.2. Synthesis of 4-substituted benzaldehyde (**5a**–**i**)*

A mixture of substituted phenol **3a**–**i** (37.4 mmol), 4-fluoro benzaldehyde 4 (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.

*7.1.2.1. 4-(4-Chloro-phenoxy)-benzaldehyde (**5d**).* Yield: 76%; m.p.: 75 °C; IR (KBr, cm^{-1}) υ: 1662 (C=O), 1241 (–O-); ^{1}H NMR $CDCl_3$, 300 (MHz) δ in ppm: 9.81 (s, 1H, –CHO), 6.22–7.80 (a set of signals, 8H, Ar–H); MS (*m*/*z*, %): 233.03 (M^++1 for ^{35}Cl, 100.00), 235.0 (M^++1 for ^{37}Cl, 34.3).

*7.1.3. Synthesis of N-(4-substituted phenyl)-2-[(4-substituted) benzylidene] hydrazinecarbo- thioamides (**PC 1**–**36**)*

Equimolar quantities (0.01 mol) of 4-substituted benzaldehydes **5 a**–**i** and *N*-(4-substituted phenyl) hydrazinecarbothioamides **2a**–**d** 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. Physical and elemental analysis data of the titled compounds **PC 1**–**36** are depicted in Table 1.

*7.1.3.1. N-(phenyl)-2-(4-phenoxybenzylidene)hydrazinecarbothioamide (**PC 1**).* IR (KBr, cm^{-1}) υ: 3228, 3120 (NH), 1585 (N=CH), 1240 (–O–), 1167 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.88–7.76 (a set of signals, 14 H, Ar–H), 7.85 (s, 1H, CH=N), 9.17 (s, 1H, NH–N, D_2O exchangeable), 9.88 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 348.10 (M^+ + 1, 42.11).

*7.1.3.2. N-(phenyl)-2-[4-(4-nitrophenoxy)benzylidene]hydrazinecarbothioamide (**PC 2**).* IR (KBr, cm^{-1}) υ: 3228, 3116 (NH), 1590 (N=CH), 1520 (N=O), 1243 (–O–), 1166 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.93–7.72 (a set of signals, 13H, Ar–H), 7.87 (s, 1H, CH=N), 9.10 (s, 1H, NH–N, D_2O exchangeable), 9.78 (s, ^{1}H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 393.1 (M^+ + 1, 76.11).

*7.1.3.3. N-(phenyl)-2-[4-(4-methylphenoxy)benzylidene]hydrazinecarbothioamide (**PC 3**).* IR (KBr, cm^{-1}) υ: 3232, 3121 (NH), 1590 (N=

CH), 1247 (–O-), 1168 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 2.36 (s, 3H, CH_3), 6.92–7.63 (a set of signals, 13H, Ar–H), 7.83 (s, 1H, CH=N), 9.18 (s, 1H, NH–N, D_2O exchangeable), 9.88 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 362.15 (M^+ + 1, 100.00).

*7.1.3.4. 2-[4-(4-Chlorophenoxy)benzylidene]-N-(phenyl)hydrazinecarbothioamide (**PC 4**).* IR (KBr, cm^{-1}) υ: 3230, 3117 (NH), 1587 (N=CH), 1241 (–O–), 1165 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.97–7.67 (a set of signals, 13H, Ar–H), 7.89 (s, 1H, CH=N), 9.16 (s, 1H, NH–N, D_2O exchangeable), 9.85 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 382.13 (M^+ + 1 for ^{35}Cl, 100.00), 384.14 (M^+ + 1 for ^{37}Cl, 39.52).

*7.1.3.5. 2-[4-(4-Bromophenoxy)benzylidene]-N-(phenyl)hydrazinecarbothioamide (**PC 5**).* IR (KBr, cm^{-1}) υ: 3230, 3124 (NH), 1593 (N=CH), 1244 (–O–), 1163 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.93–7.69 (a set of signals, 13H, Ar–H), 7.88 (s, 1H, CH=N), 9.12 (s, 1H, NH–N, D_2O exchangeable), 9.77 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 428.08 (M^+ + 1 for ^{81}Br, 100.00), 426.07 (M^+ + 1 for ^{79}Br, 96.60).

*7.1.3.6. N-(phenyl)-2-[4-(4-fluorophenoxy)benzylidene]hydrazinecarbothioamide (**PC 6**).* IR (KBr, cm^{-1}) υ: 3227, 3129 (NH), 1588 (N=CH), 1244 (–O–), 1167 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.92–7.69 (a set of signals, 13H, Ar–H), 7.82 (s, 1H, CH=N), 9.08 (s, 1H, NH–N, D_2O exchangeable), 9.76 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 366.16 (M^+ + 1, 42.89).

*7.1.3.7. 2-[4-(4-chloro-3-methylphenoxy)benzylidene]-N-(phenyl) hydrazinecarbothioamid (**PC 7**).* IR (KBr, cm^{-1}) υ: 3223, 3120 (NH), 1591 (N=CH), 1246 (–O–), 1168 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 2.35 (s, 3H, CH_3), 6.93–7.72 (a set of signals, 12H, Ar–H), 7.81 (s, 1H, CH=N), 9.12 (s, 1H, NH–N, D_2O exchangeable), 9.80 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 396.13 (M^+ + 1 for ^{35}Cl, 100.00), 398.10 (M^+ + 1 for ^{37}Cl, 37.12).

*7.1.3.8. N-(phenyl)-2-[4-(naphthalen-2-yloxy)benzylidene]hydrazinecarbothioamide(**PC 8**).* IR (KBr, cm^{-1}) υ: 3238, 3127 (NH), 1595 (N=CH), 1246 (–O–), 1167 (C=S), 832, 820 (β-naphthyl); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.90–7.88 (a set of signals, 16H, Ar–H and naphthyl–H), 7.84 (s, 1H, CH=N), 9.13 (s, 1H, NH–N, D_2O exchangeable), 10.03 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 398.18 (M+ + 1, 100.00).

*7.1.3.9. 2-[4-(1, 3-benzodioxol-5-yloxy)benzylidene]-N-(phenyl) hydrazinecarbothioamide (**PC 9**).* IR (KBr, cm^{-1}) υ: 3242, 3129 (NH), 1594 (N=CH), 1238 (–O–), 1163 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 5.95 (s, 2H, OCH2), 6.87–7.79 (a set of signals, 12H, Ar–H and benzodioxolyl-H), 7.86 (s, 1H, CH=N), 9.14 (s, 1H, NH–N, D_2O exchangeable), 10.13 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 392.17 (M^+ + 1, 34.14).

*7.1.3.10. N-(4-chlorophenyl)-2-(4-phenoxybenzylidene)hydrazinecarbothioamide (**PC 10**).* IR (KBr, cm^{-1}) υ: 3268, 3148 (NH), 1587 (N=CH), 1241 (–O–), 1167 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.78–7.77 (a set of signals, 13H, Ar–H), 7.80 (s, 1H, CH=N), 9.07 (s, 1H, NH–N, D_2O exchangeable), 9.40 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 382.12 (M^+ + 1 for ^{35}Cl, 100.00), 384.07 (M^+ + 1 for ^{37}Cl, 34.5).

*7.1.3.11. N-(4-chlorophenyl)-2-[4-(4-nitrophenoxy)benzylidene]hydrazinecarbothioamide (**PC 11**).* IR (KBr, cm^{-1}) υ: 3263, 3149 (NH), 1590 (N=CH), 1522 (N=O), 1245 (–O-), 1163 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.91–7.77 (a set of signals, 12H, Ar–H), 7.81 (s, 1H, CH=N), 9.07 (s, 1H, NH–N, D_2O exchangeable), 9.41 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 427.10 (M^+ + 1 for ^{35}Cl, 100.00), 429.05 (M^+ + 1 for ^{37}Cl, 35.16).

*7.1.3.12. N-(4-chlorophenyl)-2-[4-(4-methylphenoxy)benzylidene] hydrazinecarbothioamide (**PC 12**).* IR (KBr, cm^{-1}) υ: 3262, 3153 (NH), 1587 (N=CH), 1247 (–O–), 1168 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 2.35 (s, 3H, CH3), 6.96–7.68 (a set of signals, 12H, Ar–H), 7.79 (s, 1H, CH=N), 9.08 (s, 1H, NH–N, D_2O exchangeable), 9.41 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 396.12 (M^+ + 1 for ^{35}Cl, 100.00), 398.07 (M^+ + 1 for ^{37}Cl, 34.5).

*7.1.3.13. 2-[4-(4-Chlorophenoxy)benzylidene]-N-(4-chlorophenyl) hydrazinecarbothioamide (**PC 13**).* IR (KBr, cm^{-1}) υ: 3265, 3142 (NH), 1589 (N=CH), 1243 (–O–), 1165 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.90–7.64 (a set of signals, 12H, Ar–H), 7.83 (s, 1H, CH=N), 9.08 (s, 1H, NH–N, D_2O exchangeable), 9.43 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 416.16 (M^+ + 1 for ^{35,35}Cl, 100.00), 418.09 (M^+ + 1 for ^{35,37}Cl, 76.62), 420.22 (M^+ + 1 for ^{37,37}Cl, 24.30).

*7.1.3.14. 2-[4-(4-Bromophenoxy)benzylidene]-N-(4-chlorophenyl) hydrazinecarbothioamide (**PC 14**).* IR (KBr, cm^{-1}) υ: 3266, 3147 (NH), 1591 (N=CH), 1244 (–O–), 1163 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.93–7.69 (a set of signals, 12H, Ar–H), 7.80 (s, 1H, CH=N), 9.08 (s, 1H, NH–N, D_2O exchangeable), 9.42 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 461.96 (M^+ + 1 for ^{35,37}Cl and ^{79,81}Br, 100.00), 459.99 (M^+ + 1 for ^{35}Cl and ^{79}Br, 74.6), 463.97 (M^+ + 1 for ^{37}Cl and ^{81}Br, 24.5).

*7.1.3.15. N-(4-chlorophenyl)-2-[4-(4-fluorophenoxy)benzylidene] hydrazinecarbothioamide (**PC 15**).* IR (KBr, cm^{-1}) υ: 3261, 3149 (NH), 1586 (N=CH), 1241 (–O–), 1167 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.92–7.71 (a set of signals, 12H, Ar–H), 7.82 (s, 1H, CH=N), 9.08 (s, 1H, NH–N, D_2O exchangeable), 9.41 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 400.04 (M^+ + 1 for ^{35}Cl, 100.00), 402.06 (M^+ + 1 for ^{37}Cl, 35.8).

*7.1.3.16. 2-[4-(4-Chloro-3-methylphenoxy)benzylidene]-N-(4-chlorophenyl)hydrazinecarbothio amide (**PC 16**).* IR (KBr, cm^{-1}) υ: 3268, 3158 (NH), 1591 (N=CH), 1246 (–O–), 1168 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 2.34 (s, 3H, CH_3), 6.93–7.70 (a set of signals, 11H, Ar–H), 7.81 (s, 1H, CH=N), 9.08 (s, 1H, NH–N, D_2O exchangeable), 9.42 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 430.07 (M^+ + 1 for ^{35,35}Cl, 100.00), 432.06 (M^+ + 1 for ^{35,37}Cl, 66.7), 434.11 (M^+ + 1 for ^{37,37}Cl, 32.4).

*7.1.3.17. N-(4-chlorophenyl)-2-[4-(naphthalen-2-yloxy)benzylidene] hydrazinecarbothioamide (**PC 17**).* IR (KBr, cm^{-1}) υ: 3260, 3143 (NH), 1590 (N=CH), 1246 (–O–), 1164 (C=S), 836, 821 (β-naphthyl); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 7.05–7.88 (a set of signals, 15H, Ar–H and naphthyl–H), 7.92 (s, 1H, CH=N), 9.13 (s, 1H, NH–N, D_2O exchangeable), 10.12 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 432.18 (M^+ + 1 for ^{35}Cl, 100.00), 434.31 (M^+ + 1 for ^{37}Cl, 47.20).

*7.1.3.18. 2-[4-(1,3-benzodioxol-5-yloxy)benzylidene]-N-(4-chlorophenyl)hydrazinecarbothio amide (**PC 18**).* IR (KBr, cm^{-1}) υ: 3258, 3149 (NH), 1597 (N=CH), 1242 (–O–), 1165 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 5.98 (s, 2H, OCH_2), 7.01–7.77 (a set of signals, 11H, Ar–H and benzodioxolyl-H), 7.90 (s, 1H, CH=N), 9.10 (s, 1H, NH–N, D_2O exchangeable), 10.13 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 426.03 (M^+ + 1 for ^{35}Cl, 42.34), 428.06 (M^+ + 1 for ^{37}Cl, 15.27).

*7.1.3.19. N-(4-bromophenyl)-2-(4-phenoxybenzylidene)hydrazinecarbothioamide (**PC 19**).* IR (KBr, cm^{-1}) υ: 3237, 3138 (NH), 1603

(N=CH), 1243 (–O–), 1168 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.76–7.67 (a set of signals, 13H, Ar–H), 7.82 (s, 1H, CH=N), 9.09 (s, 1H, NH–N, D_2O exchangeable), 9.50 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 428.04 ($M^+ + 1$ for ^{81}Br, 100.00), 426.02 ($M^+ + 1$ for ^{79}Br, 97.8).

*7.1.3.20. N-(4-bromophenyl)-2-[4-(4-nitrophenoxy)benzylidene]hydrazinecarbothioamide (**PC 20**).* IR (KBr, cm^{-1}) υ: 3236, 3136 (NH), 1606 (N=CH), 1525 (N=O), 1244 (–O-), 1166 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.73–7.61 (a set of signals, 12H, Ar–H), 7.83 (s, 1H, CH=N), 9.09 (s, 1H, NH–N, D_2O exchangeable), 9.51 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 473.05 ($M^+ + 1$ for ^{81}Br, 100.00), 471.02 ($M^+ + 1$ for ^{79}Br, 98.1).

*7.1.3.21. N-(4-bromophenyl)-2-[4-(4-methylphenoxy)benzylidene] hydrazinecarbothioamide (**PC 21**).* IR (KBr, cm^{-1}) υ: 3257, 3142 (NH), 1586 (N=CH), 1240 (–O–), 1242 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 2.34 (s, 3H, CH_3), 6.83–7.65 (a set of signals, 12H, Ar–H), 7.85 (s, 1H, CH=N), 9.09 (s, 1H, NH–N, D_2O exchangeable), 9.56 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 442.03 ($M^+ + 1$ for ^{81}Br, 100.00), 440.05 ($M^+ + 1$ for ^{79}Br, 98.3).

*7.1.3.22. N-(4-bromophenyl)-2-[4-(4-chlorophenoxy)benzylidene] hydrazinecarbothioamide (**PC 22**).* IR (KBr, cm^{-1}) υ: 3264, 3138 (NH), 1588 (N=CH), 1244 (–O–), 1168 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.86–7.65 (a set of signals, 12H, Ar–H), 7.84 (s, 1H, CH=N), 9.09 (s, 1H, NH–N, D_2O exchangeable), 9.55 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 461.96 ($M^+ + 1$ for ^{35,37}Cl and ^{79,81}Br, 100.00), 459.99 ($M^+ + 1$ for ^{35}Cl and ^{79}Br, 74.6), 463.97 ($M^+ + 1$ for ^{37}Cl and ^{81}Br, 24.5).

*7.1.3.23. 2-[4-(4-bromophenoxy)benzylidene]-N-(4-bromophenyl) hydrazinecarbothioamide (**PC 23**).* IR (KBr, cm^{-1}) υ: 3259, 3145 (NH), 1585 (N=CH), 1241 (–O–), 1163 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.85–7.65 (a set of signals, 12H, Ar–H), 7.85 (s, 1H, CH=N), 9.09 (s, 1H, NH–N, D_2O exchangeable), 9.56 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 505.84 ($M^+ + 1$ for ^{79,81}Br, 61.20), 507.92 ($M^+ + 1$ for ^{81,81}Br, 24.8), 503.86 ($M^+ + 1$ for ^{79,79}Br, 25.7).

*7.1.3.24. N-(4-bromophenyl)-2-[4-(4-fluorophenoxy)benzylidene] hydrazinecarbothioamide (**PC 24**).* IR (KBr, cm^{-1}) υ: 3258, 3142 (NH), 1583 (N=CH), 1240 (–O–), 1168 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.85–7.64 (a set of signals, 12H, Ar–H), 7.84 (s, 1H, CH=N), 9.09 (s, 1H, NH–N, D_2O exchangeable), 9.55 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 446.02 ($M^+ + 1$ for ^{81}Br, 100.00), 444.03 ($M^+ + 1$ for ^{79}Br, 97.8).

*7.1.3.25. N-(4-bromophenyl)-2-[4-(4-chloro-3-methylphenoxy)benzylidene]hydrazinecarbo thioamide (**PC 25**).* IR (KBr, cm^{-1}) υ: 3234, 3135 (NH), 1584 (N=CH), 1241 (–O–), 1165 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 2.36 (s, 3H, CH_3), 6.75–7.64 (a set of signals, 11H, Ar–H), 7.84 (s, 1H, CH=N), 9.09 (s, 1H, NH–N, D_2O exchangeable), 9.51 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 476.04 ($M^+ + 1$ for ^{35,37}Cl and ^{79,81}Br, 100.00), 474.06 ($M^+ + 1$ for ^{35}Cl and ^{79}Br, 74.8), 478.03 ($M^+ + 1$ for ^{37}Cl and ^{81}Br, 31.50).

*7.1.3.26. N-(4-bromophenyl)-2-[4-(naphthalen-2-yloxy)benzylidene] hydrazinecarbothioamide (**PC 26**).* IR (KBr, cm^{-1}) υ: 3265, 3146 (NH), 1589 (N=CH), 1244 (–O–), 1166 (C=S), 836, 821 (β-naphthyl); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.85–7.89 (a set of signals, 15H, Ar–H), 7.84 (s, 1H, CH=N), 9.09 (s, 1H, NH–N, D_2O exchangeable), 9.56 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 478.01 ($M^+ + 1$ for ^{81}Br, 100.00), 476.04 ($M^+ + 1$ for ^{79}Br, 96.50).

*7.1.3.27. 2-[4-(1,3-Benzodioxol-5-yloxy)benzylidene]-N-(4-bromophenyl)hydrazinecarbothio amide (**PC 27**).* IR (KBr, cm^{-1}) υ: 3245, 3133 (NH), 1593 (N=CH), 1240 (–O–), 1168 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 5.96 (s, 2H, OCH_2), 6.88–7.72 (a set of signals, 11H, Ar–H and benzodioxolyl-H), 7.89 (s, 1H, CH=N), 9.13 (s, 1H, NH–N, D_2O exchangeable), 10.17 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 472.02 ($M^+ + 1$ for ^{81}Br, 100.00), 470.05 ($M^+ + 1$ for ^{79}Br, 96.22).

*7.1.3.28. N-(4-fluorophenyl)-2-(4-phenoxybenzylidene)hydrazinecarbothioamide (**PC 28**).* IR (KBr, cm^{-1}) υ: 3138 (NH), 1584 (N=CH), 1240 (–O–), 1165 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.92–7.88 (a set of signals, 13H, Ar–H), 7.82 (s, 1H, CH=N), 9.05 (s, 1H, NH–N, D_2O exchangeable), 9.57 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 366.13 ($M^+ + 1$, 35.87).

*7.1.3.29. N-(4-fluorophenyl)-2-[4-(4-nitrophenoxy)benzylidene] hydrazinecarbothioamide (**PC 29**).* IR (KBr, cm^{-1}) υ: 3147 (NH), 1592 (N=CH), 1527 (N=O), 1241 (–O–), 1166 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.93–7.68 (a set of signals, 12H, Ar–H), 7.85 (s, 1H, CH=N), 9.05 (s, 1H, NH–N, D_2O exchangeable), 9.61 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 411.10 ($M^+ + 1$, 65.78).

*7.1.3.30. N-(4-fluorophenyl)-2-[4-(4-methylphenoxy)benzylidene] hydrazinecarbothioamide (**PC 30**).* IR (KBr, cm^{-1}) υ: 3132 (NH), 1587 (N=CH), 1240 (–O–), 1163 (C=S); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 2.34 (s, 3H, CH_3), 6.92–7.66 (a set of signals, 12H, Ar–H), 7.82 (s, 1H, CH=N), 9.06 (s, 1H, NH–N, D_2O exchangeable), 9.61 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 380.10 ($M^+ + 1$, 100.00).

*7.1.3.31. 2-[4-(4-Chlorophenoxy)benzylidene]-N-(4-fluorophenyl) hydrazinecarbothioamide (**PC 31**).* IR (KBr, cm^{-1}) υ: 3128 (NH), 1586 (N=CH), 1238 (–O–), 1164 (C=S); ^{1}H NMR ($CDCl_3$, 300 Hz) δ in ppm: 6.96–7.65 (a set of signals, 12H, Ar–H), 7.86 (s, 1H, CH=N), 9.04 (s, 1H, NH–N, D_2O exchangeable), 9.61 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 400.12 ($M^+ + 1$ for ^{35}Cl, 100.00), 402.12 ($M^+ + 1$ for ^{37}Cl, 36.80).

*7.1.3.32. 2-[4-(4-Bromophenoxy)benzylidene]-N-(4-fluorophenyl) hydrazinecarbothioamide (**PC 32**).* IR (KBr, cm^{-1}) υ: 3135 (NH), 1589 (N=CH), 1240 (–O–), 1167 (C=S); ^{1}H NMR ($CDCl_3$, 300 Hz) δ in ppm: 6.89–7.68 (a set of signals, 12H, Ar–H), 7.83 (s, 1H, CH=N), 9.07 (s, 1H, NH–N, D_2O exchangeable), 9.56 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 446.02 ($M^+ + 1$ for ^{81}Br, 89.90), 444.03 ($M^+ + 1$ for ^{79}Br, 87.23).

*7.1.3.33. 2-[4-(4-Fluorophenoxy)benzylidene]-N-(4-fluorophenyl) hydrazinecarbothioamide (**PC 33**).* IR (KBr, cm^{-1}) υ: 3135 (NH), 1584 (N=CH), 1239 (–O–), 1165 (C=S); ^{1}H NMR ($CDCl_3$,300 Hz) δ in ppm: 6.90–7.81 (a set of signals, 12H, Ar–H), 7.80 (s, 1H, CH=N), 9.08 (s, 1H, NH–N, D_2O exchangeable), 9.47 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 384.10 ($M^+ + 1$, 36.73).

*7.1.3.34. 2-[4-(4-Chloro-3-methylphenoxy)benzylidene]-N-(4-fluorophenyl)hydrazinecarbothio amide (**PC 34**).* IR (KBr, cm^{-1}) υ: 3135 (NH), 1584 (N=CH), 1241 (–O–), 1163 (C=S); ^{1}H NMR ($CDCl_3$,300 Hz) δ in ppm: 2.35 (s, 3H, CH_3), 6.81–7.62 (a set of signals, 11H, Ar–H), 7.84 (s, 1H, CH=N), 9.07 (s, 1H, NH–N, D_2O exchangeable), 9.54 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 414.06 ($M^+ + 1$ for ^{35}Cl, 100.00), 416.08 ($M^+ + 1$ for ^{37}Cl, 37.80).

*7.1.3.35. N-(4-fluorophenyl)-2-[4-(naphthalen-2-yloxy)benzylidene] hydrazinecarbothioamide (**PC 35**).* IR (KBr, cm^{-1}) υ: 3141 (NH), 1589 (N=CH), 1244 (–O–), 1164 (C=S), 835, 823 (β-naphthyl); ^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm: 6.80–7.86 (a set of signals, 15H, Ar–H

and naphthyl–H), 7.88 (s, 1H, CH=N), 9.11 (s, 1H, NH–N, D_2O exchangeable), 10.10 (s, ^{1}H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 416.13 ($M^+ + 1$, 55.21).

*7.1.3.36. 2-[4-(1,3-Benzodioxol-5-yloxy)benzylidene]-N-(4-fluorophenyl)hydrazinecarbothio amide (**PC 36**).* IR (KBr, cm^{-1}) υ: 3142 (NH), 1595 (N=CH), 1240 (–O–), 1164 (C=S); ^{1}H NMR ($CDCl_3$, 300 Hz) δ in ppm: 5.96 (s, 2H, OCH_2), 6.93–7.67 (a set of signals, 11H, Ar–H and benzodioxolyl–H), 7.88 (s, 1H, CH=N), 9.08 (s, 1H, NH–N, D_2O exchangeable), 10.11 (s, 1H, NH–Ar, D_2O exchangeable); MS (*m*/*z*, %): 410.13 ($M^+ + 1$, 89.70).

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 Institutes 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 mortar pestle to help preparation of suspension. In the preliminary 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 4 h intervals after administration in mice. Selected derivatives were examined for the oral activity in rat MES screen. Further compounds were also tested for their activity in 6 Hz model at five different time points i.e. 0.25, 0.5, 1.0, 2.0 and 4.0 h at a dose of 100 mg/kg administered i.p. Compounds showing significant protection were evaluated for quantification studies in 6 Hz test and ED_{50} reported. Selected derivatives were also evaluated in pilocarpine induced status prevention (PISP) model and *in vitro* hippocampal slice culture neuroprotection assay.

7.2.1. Maximal electroshock (MES) test

For the 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. One compound described in this study was examined for the oral activity in the rat MES screen.

7.2.2. Subcutaneous metrazol seizure threshold (scMET) test

For the 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 convulsions in 97% of animals is injected into a loose fold of skin in the midline of the neck. The animals are placed in 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 rotorod (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. Minimal clonic seizure (6 Hz) test

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. Pilocarpine induced status prevention (PISP) model

Compounds were assessed for pharmacological evaluation of potential activity against nerve agents using the pilocarpine model of epilepsy as an introductory screen. The pilocarpine models are one of the most recognized animal models of status epilepticus (SE).

A. Acute toxicity: To determine acute motor impairment usual starting doses of 100 and 300 mg/kg were administered via the i.p. route to groups of Sprague Dawley rats over several time points. The behavior of the animals was closely observed and recorded over a 4 h period. A minimum number of four (4) rats, two per dose were employed in the acute screen.
B. Status prevention: To determine if the test substance can prevent acute pilocarpine induced status an initial qualitative efficacy screen was performed. Administration of the candidate drug was given to male albino Sprague Dawley rats (150–180 g) via the i.p. route of administration. Then a challenge dose of pilocarpine was administered observing for treatment-effects of the candidate drug. The outcome measures were determined as "protection" or "no protection". The seizure severity was determined using the well established Racine scale.

7.2.6. 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 (PI), a membrane-impermeant compound, is included in all wells of the culture plate. Dying cells have compromised cell membranes, thus PI may diffuse into the cell, intercalate with DNA and fluoresce. Thus, the intensity of the PI 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, zonisamide; a molecular model was suggested on the basis of molecular dynamics distance estimations [17].

7.3.2. Prediction of ADME properties

A computational study of titled compounds was performed for prediction of ADME properties. Polar surface area (TPSA), miLog P, number of rotatable bonds, molecular volume, number of hydrogen donor and acceptor atoms and violations of Lipinski's rule of five [18] were calculated using Molinspiration online property calculation toolkit [19]. Absorption (%ABS) was calculated by: % ABS = 109 − (0.345 × TPSA) [20].

7.3.3. Log P determination

The partition coefficient between octanol and phosphate buffer was determined at room temperature [21]. 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 phase were separated and filtered through membrane filter and drug content in aqueous phase was analysed by UV spectroscopy. Theoretical miLog *P* for synthesized compounds were then compared with the experimental Log *P* data.

7.3.4. Docking study

Compounds **PC 16**, **PC 23**, **PC 25** and **PC 31** 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 among the most important targets in the design and discovery of successful antiepileptic drugs [22]. In the present study, AutoDock 4.0 with its Lamarckian genetic algorithm (LGA) was used for automated flexible ligand docking of **PC 16**, **PC 23**, **PC 25** and **PC 31** with the above mentioned receptors.

The grid maps were calculated using AutoGrid. In all dockings, a grid map with 60 × 60 × 60 points, a grid spacing of 0.375 A ° (roughly a quarter of the length of a carbon–carbon single bond) were used, and the mps were centered on the ligand binding site. For all dockings, 100 independent run with, an initial population of random individuals with a population size of 150 individuals, a maximum number of 2.5×10^6 energy evaluations, maximum number of generations of 27,000, an elitism value of 1 and a number of active torsion of 9 were used. AutoDock 4.0 was used to generate both grid and docking parameter files (i.e., gpf and dpf files) and docking affinity (Kcal/mol) and count of probable H-bonds were determined.

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