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Original article

Design & synthesis of *N*′-[substituted] pyridine-4-carbohydrazides as potential anticonvulsant agents

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ABSTRACT

A series of N′-[substituted] pyridine-4-carbohydrazides 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 established after intraperitoneal administration in three seizure models, which include MES, scMET and 6 Hz model. The most active compound of the series was N′-[4-(4-fluorophenoxy)benzylidene]pyridine-4-carbohydrazide PCH 6, which showed a MES ED_{50} value of 128.3 mg/kg and 6 Hz ED_{50} value of 53.3 mg/kg in mice. The median toxic dose (TD_{50}) was 343.6 mg/kg, providing compound PCH 6 with a protection index of 2.67 in the MES test and 6.44 in 6 Hz test. A computational study was also carried out, including calculation of pharmacophore pattern, prediction of pharmacokinetic properties and docking studies.

1. Introduction

Epilepsy is a collective term that includes over 40 different types of human seizure disorders [1]. Approximately 1% of the world population at any one time (≈50 million people world wide) is afflicted with this serious neurological disorder [2]. Although the current drugs provide adequate seizure control in many patients, it is roughly estimated that up to 28–30% of patients are poorly treated with the available antiepileptic drugs (AEDs). Moreover, the current drug therapy is associated with adverse side effects such as drowsiness, ataxia, gastrointestinal disturbances, gingival hyperplasia, hirsutism and megaloblastic anaemia [3,4] and life long medication may be required. These facts necessitate the search for the development of novel anticonvulsant drugs with greater efficacy and fewer side effects.

Hydrazones possessing an azomethine –NHN=CH– proton constitute an important class of compounds for new drug development. In the past decade, hydrazones have been designed as potential anticonvulsants that were structurally dissimilar from very common anticonvulsants containing the dicarboximide function (CONRCO), which may contribute to toxic side effects [5–7]. Isonicotinyl hydrazones were mostly reported as antitubercular [8,9], antidepressant [10] and antimalarial [11]. Iproniazide, a *N*-iso-propyl derivative of isoniazid has mood-elevating effects in patients due to its monoamine oxidase inhibitor activity.

Consistent advances in the design of novel anticonvulsant agents have been obtained through the works of Dimmock and his collegues [12–15], which includes various semicarbazones and hydrazones. Earlier 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 spatial arrangement are to be recommended for anticonvulsant activity [16–18]. Unverferth et al. identified a common pharmacophore model based on some well known voltage-gated sodium channel blockers including phenytoin and lamotrigine [19].

Based on the literature review, we are the first to report the synthesis and anticonvulsant activities of *N*′-[substituted] pyridine-4-carbohydrazides. 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. 1) that are necessary for good anticonvulsant activity as suggested by Unverferth et al. [19]. The essential structural features which could be responsible for an interaction with the active site of voltage-gated sodium channels were a hydrophobic HP unit (R), an electron donor (D) group, and a hydrogen donor/acceptor (HBD) unit [20]. In addition, 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

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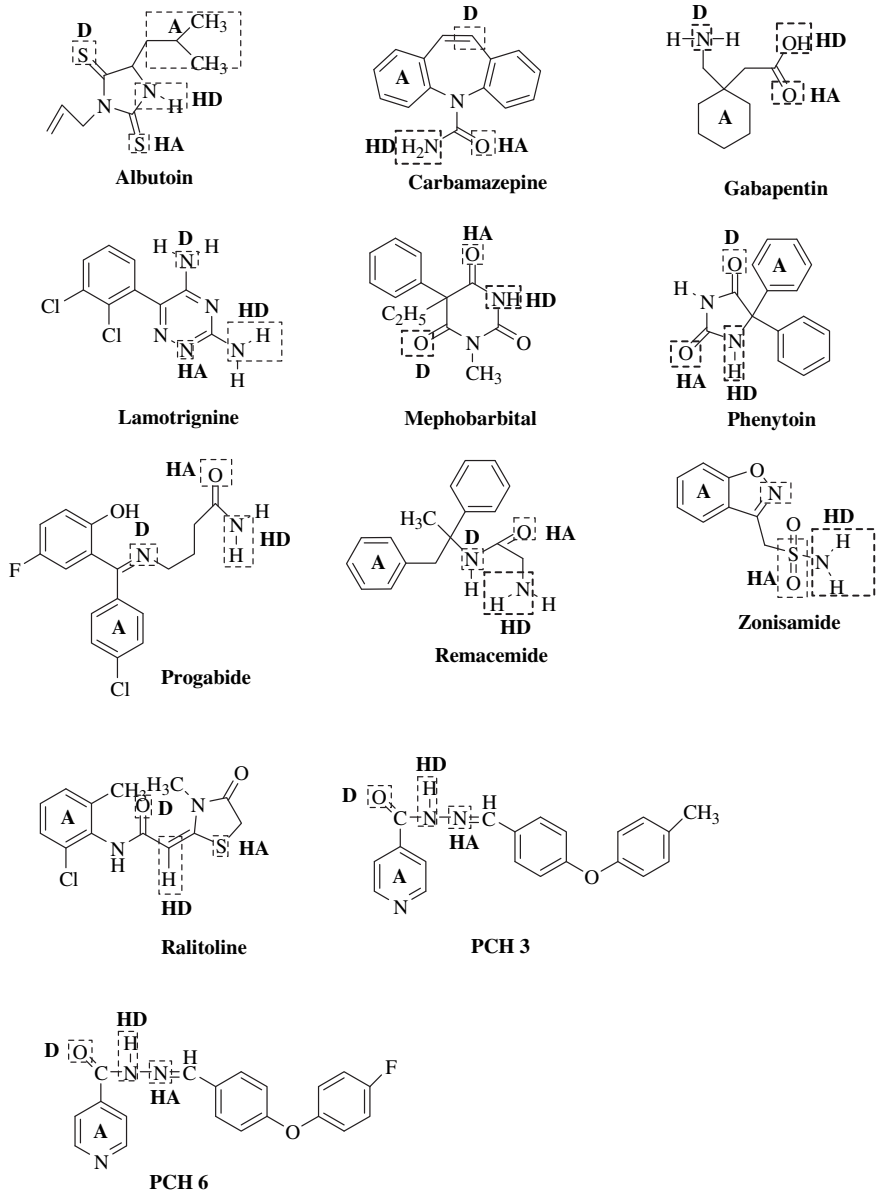


Fig. 1. Pharmacophoric pattern of well-known anticonvulsants.

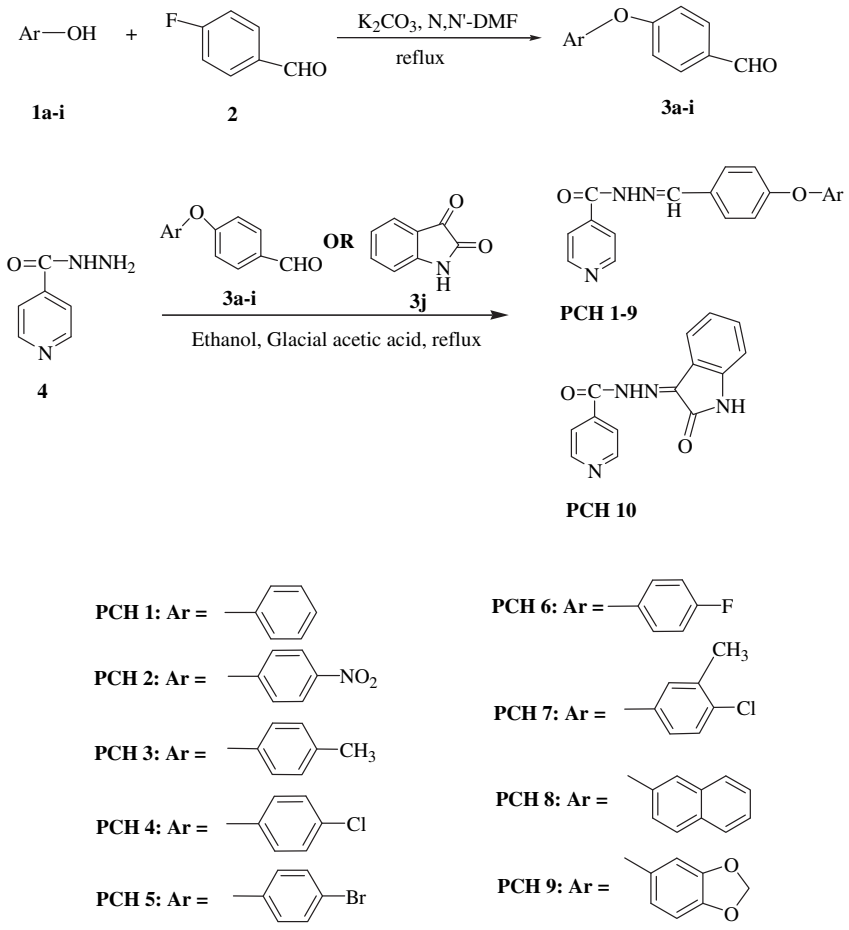
neurotoxicity of the compounds. Computational study was also carried out to highlight the pharmacophore distance mapping, miLog *P* calculation and prediction of pharmacokinetic parameters. In this study, we have used AutoDock 4.0 along with its LGA algorithm for automated flexible ligand docking of 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*′-(substituted) pyridine-4-carbohydrazide **PCH 1**–**10** is shown in Scheme 1. The 4-substituted benzaldehydes **3a**–**i** were prepared by refluxing various substituted phenol **1a**–**i** with 4-fluorobenzaldehyde **2** in *N*,*N*′-DMF in presence of potassium carbonate. The pyridine-4-carbohydrazide **4** was refluxed with various 4-substituted benzaldehyde **3a**–**i** and isatin **3j** in the presence of catalytic amount of glacial acetic acid to yield the titled compounds **PCH 1**–**10**. 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 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 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 *str*, C–H *str* and diaryl ether linkage (–O–). The absence of broad band of phenolic –OH at 2900–3000 cm^{-1} confirm 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 confirm the conversion of **1f** to **3f**.

The IR spectrum of the titled compound **PCH 6** showed C═O *str* at 1651 cm^{-1}, N–H *str* (associated) at 3252 and 3080 cm^{-1}, CH═N *str* at 1605 cm^{-1} and diaryl ether linkage (–O–) at 1255 cm^{-1}. Its ^{1}H NMR spectrum showed a singlet of imine (CH═N) proton at



Scheme 1. Synthesis of *N*′-(substituted) pyridine-4-carbohydrazide **PCH 1**–**10**.

δ 8.359 ppm, two doublet of pyridine protons at δ 8.76 and 6.98 ppm and –NH– proton resonated as a broad singlet at δ 9.86 ppm that was D_2O exchangeable. Aromatic protons appeared as a multiplet in the region δ 7.001–7.885 ppm. The presence of CH═N *str* at 1605 cm^{-1} in IR spectrum and a singlet for imine (CH═N) proton at δ 8.359 ppm confirm the formation of **PCH 6**. Further mass spectrum confirmed their purity and molecular weight.

3. Pharmacology

The newly synthesized *N*′-(substituted) pyridine-4-carbohydrazides **PCH 1**–**10** were subjected to anticonvulsant screening by the anticonvulsant 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 the rotorod test. The results are shown in Table 2. Compound showing significant protection was examined for its oral activity in rat MES screen and result is shown in Table 3. Some compounds were screened in 6 Hz model to identify their 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 4. Potential compounds were also subjected to quantification studies in one or more models i.e. MES, scMET, neurotoxicity and 6 Hz test and the corresponding ED_{50} and TD_{50} reported in Table 5. Some compounds were screened in Pilocarpine Induced Status Prevention (PISP) Model and *In-vitro* Hippocampal Slice Culture Neuroprotection Assay (NP) and results are shown in Tables 6–8.

4. Computational study

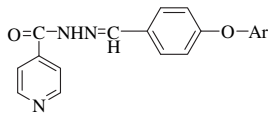
The pharmacophore pattern studies in which distance between the various groups postulated as essential for anticonvulsant activity were done on the 3D optimized structures using ACD/3D viewer version 12.0 and Argus Lab 4.0 Mark A. Thompson Planaria Software LLC. A computational study of all compounds 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* 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 AutoDock 4.0 along with its LGA algorithm for automated flexible ligand docking and affinity (Kcal/mol) and count of probable hydrogen bonds were evaluated.

5. Results and discussion

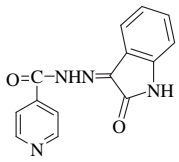
5.1. Anticonvulsant and neurotoxicity evaluation

Some of the synthesized compounds showed protection against MES test, indicative of their ability to inhibit the seizure spread. The

Table 1
Physical data of *N′*-(substituted) pyridine-4-carbohydrazides **PCH 1–10**.



PCH 1-9



PCH 10

Compounds	Ar	Yield (%)	Melting point (°C)	Mol. Formula (Mol. Wt.)	R*f*
PCH 1	Phenyl	76	108	$C_{19}H_{15}N_3O_2$ (317.34)	0.52
PCH 2	4-NO_2-Phenyl	74	224	$C_{19}H_{14}N_4O_4$ (362.34)	0.44
PCH 3	4-CH_3-Phenyl	77	152	$C_{20}H_{17}N_3O_2$ (331.37)	0.54
PCH 4	4-Cl-Phenyl	80	176	$C_{19}H_{14}ClN_3O_2$ (351.79)	0.43
PCH 5	4-Br-Phenyl	79	185	$C_{19}H_{14}BrN_3O_2$ (396.24)	0.41
PCH 6	4- F-Phenyl	82	156	$C_{19}H_{14}FN_3O_2$ (335.33)	0.45
PCH 7	3-Methyl-4-chloro phenyl	76	222	$C_{20}H_{16}ClN_3O_2$ (365.81)	0.48
PCH 8	Naphthalene-2-yl	81	205	$C_{23}H_{17}N_3O_2$ (367.40)	0.40
PCH 9	1,3-benzodioxol-5-yl	74	118	$C_{20}H_{15}N_3O_4$ (361.35)	0.47
PCH 10	–	84	292	$C_{14}H_{10}N_4O_2$ (266.25)	0.50

results are shown in Table 2. Compounds **PCH 3** and **PCH 6** were active in MES test. The compound **PCH 3** showed 100% protection (1/1, 4.0 h) at a dose of 300 mg/kg where as compound **PCH 6** showed 100% protection (1/1, 4.0 h) and 67% protection (2/3, 2.0 h) at a dose of 100 mg/kg. None of the compounds showed protection in scMET test, a test used to identify compounds that elevate seizure threshold. Minor toxicity shown as motor impairment was observed in **PCH 4** (1/2, 4 h) at a dose of 300 mg/kg. None of the other compounds showed neurotoxicity in the highest administered dose (300 mg/kg). Compound **PCH 6** was examined for its oral activity in rat MES screen and showed 25% protection (1/4, 2.0 h) at a dose of 30 mg/kg orally in rats (115–150 g). The results are shown in Table 3.

Compounds showed more promising results when tested in 6 Hz model and the results are shown in Table 4. Compound **PCH 6** was the most active one in this series with protection at three different time points, i.e. 100% (4/4, 0.5 h), 75% (3/4, 0.25 h) and 50% (2/4, 1.0 h) at a dose of 100 mg/kg. Compound **PCH 3** showed 75% protection (3/4, 1.0 h) and 50% protection (2/4, 2.0 h) at a dose of 100 mg/kg. Compound **PCH 10** showed 50% protection (2/4, 0.5 h) at a dose of 100 mg/kg.

Compounds **PCH 3** and **PCH 6** were subjected to quantification studies in MES, scMET, neurotoxicity and 6 Hz test. The results are shown in Table 5. Compound **PCH 6** showed an ED_{50} (95% confidence interval) of 128.3 mg/kg (95–155.9) at a time of peak effect (TPE) of 1h in quantitative MES test. It showed an ED_{50} (95% confidence interval) of >250 mg/kg at a time of peak effect (TPE) of 1 h in quantitative scMET test. It showed a TD_{50} (95% confidence interval) of 343.6 mg/kg (258.6–474.5) at a time of peak effect (TPE) of 8 h in quantitative toxicity test. Compound **PCH 6** showed an ED_{50} (95% confidence interval) of 53.3 mg/kg (28.4–81.4) at a time of peak effect (TPE) of 0.25 h in quantitative 6 Hz evaluation. Compound **PCH 3** was subjected to quantitative 6 Hz evaluation. Compound **PCH 3** showed an ED_{50} of >200 mg/kg at a time of peak effect (TPE) of 1.0 h.

Compounds **PCH 6** and **PCH 10** were subjected to Pilocarpine Induced Status Prevention (PISP) Model. The results are shown in Tables 6 and 7. In acute toxicity test, compound **PCH 10** did not show any toxicity up to 600 mg/kg, but compound **PCH 6** showed toxicity at a dose of 300 mg/kg. Compounds **PCH 6** and **PCH 10** did not exhibit any protection against Pilocarpine Induced Status Prevention model. Compound **PCH 3** was evaluated in the primary screen experiment of *In-vitro* Hippocampal Slice Culture Neuroprotection Assay (NP) and result is shown in Table 8. No significant protection was observed against either KA- or NMDA-induced cytotoxicity.

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

Table 2
Anticonvulsant activity and neurotoxicity of *N′*-(substituted) pyridine-4-carbohydrazides **PCH 1–10**.

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
PCH 1	–	–	–	–	–	–	–
PCH 2	–	–	–	–	–	–	–
PCH 3	–	–	300[b]	–	–	–	–
PCH 4	–	–	–	–	–	–	300[c]
PCH 5	–	–	–	–	–	–	–
PCH 6	–	100[b]	300[b]	–	–	–	–
PCH 7	–	–	–	–	–	–	–
PCH 8	–	–	–	–	–	–	–
PCH 9	–	–	–	–	–	–	–
PCH 10	–	–	–	–	–	–	–
Phenytoin	30	30	30	–	–	100	100
Sodium valproate	–	–	–	300	–	–	–

[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 an absence of activity at maximum dose administered (300 mg/kg).
[b] In the MES screen, compound **PCH 3** showed protection (1/1, 4 h) at a dose of 300 mg/kg. Compound **PCH 6** showed protection (2/3, 2 h) at a dose of 100 mg/kg and protection (1/1, 4 h) at a dose of 300 mg/kg.
[c] Minor toxicity (1/2, 4 h) at a dose of 300 mg/kg was observed with compound **PCH 4**.

Table 3
Evaluation of compound **PCH 6** in the MES test after oral administration (30 mg/kg) to rats.

Compound	Test	Oral administration to rats[a] (h)				
		0.25	0.5	1.0	2.0	4.0
PCH 6[b]	MES	0/4	0/4	0/4	1/4	0/4
	TOX	0/4	0/4	0/4	0/4	0/4

[a] Figure indicate the number of rats out of four which were protected.
[b] In the rat p.o.(test 2), compound **PCH 6** exhibited activity in one animal out of four at 2.0 h.

different compounds with anticonvulsant activity- albutoin, carbamazepine, gabapentin, lamotrigine, mephobarbital, phenytoin, progabide, ralitoline, remacemide and zonisamide (Fig. 1) 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 *N′*-[substituted] pyridine-4-carbohydrazides 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. 2) 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′*-[substituted] pyridine-4-carbohydrazides. Now it may be interesting to examine whether the *N′*-[substituted] pyridine-4-carbohydrazides reflect the conditions of the derived pharmacophore model. Our analyses of the distance relationship showed that *N′*-[substituted] pyridine-4-carbohydrazides did fulfill the essential demands of the pharmacophore when compared to the average distance requirement (Table 9).

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 [21]. The percentage of absorption (%ABS) was calculated using TPSA. From all these parameters, it can be observed that all titled compounds exhibited a great %ABS ranging from 71.3 to 87% (Table 10). None of the 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 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 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 11. As observed some of the experimental values were in good agreement with the theoretical values. All the titled compounds showed lipophillic character.

Table 5
Quantification studies of **PCH 3** and **PCH 6**: ED_{50} and TD_{50} value.

Compound	Test	Time (h)	ED_{50} (mg/kg)	95% Confidence Interval	Slope	STD Err	PI Value[a]
PCH 6	MES	1.0	128.3	95–155.9	6.4	1.7	2.67
	scMET	1.0	>250	–	–	–	–
	TOX	8.0	343.6	258.6–474.5	4.4	1.5	–
	6 Hz	0.25	53.3	28.4–81.4	3	0.8	6.44
PCH 3	6 Hz	1	>200	–	–	–	–

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

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 **PCH 3** and **PCH 6** 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. Compound **PCH 3** have exhibited good binding properties with glutamate receptor (Affinity value −6.1 kcal/mol and 3 H-bonds), GABA (A) delta (Affinity value −6.0 kcal/mol and 2 H-bonds) and GABA (A) alpha-1 receptor (Affinity value −6.3 kcal/mol and 1 H-bonds). The docking images are given in Fig. 3. Compound **PCH 3** does not exhibit binding properties with Na/H exchanger, Na channel receptor and T-type calcium channel receptor. Compound **PCH 6** does not exhibit binding properties with receptors used in the study. The results are shown in Table 12.

6. Conclusion

A series of *N′*-[substituted] pyridine-4-carbohydrazides were designed, 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 **PCH 6** displayed significant protection and emerged as a lead in this series. Further, compound **PCH 3** came out as a potential candidate for further investigation. Furthermore, none of the compounds violated Lipinski's parameters, making them potentially promising agent for epilepsy therapy. Docking study results shows that the

Table 4
Results of anticonvulsant activity of some *N′*-(substituted) pyridine-4-carbohydrazides by 6 Hz model.

Compound	Dose (mg/kg)	Time (h) to peak effect (N/F)*				
		0.25	0.5	1.0	2.0	4.0
PCH 3	100	1/4	1/4	3/4	2/4	0/4
PCH 5	100	0/4	0/4	0/4	1/4	0/4
PCH 6	100	3/4	4/4	2/4	0/4	0/4
PCH 7	100	1/4	0/4	0/4	0/4	0/4
PCH 10	100	1/4	2/4	1/4	0/4	0/4

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

Table 6
Results of Pilocarpine Induced Status Prevention (PISP) Model (Test 71, Acute Toxicity Test) of **PCH 6** and **PCH 10**.

Test	Dose (mg/kg)	Compound	Time (h) (N/F)*				
			0.25	0.5	1.0	2.0	4.0
TOX	100	**PCH 6**	0/2	0/2	0/2	0/2	0/2
		PCH 10	0/2	0/2	0/2	0/2	0/2
TOX	300	**PCH 6**	2/2	2/2	2/2	2/2	1/2
		PCH 10	0/2	0/2	0/2	0/2	0/2
TOX	600	**PCH 10**	0/2	0/2	0/2	0/2	0/2

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

Table 7
Results of Pilocarpine Induced Status Prevention (PISP) Model (Test 71, Response data) of **PCH 6** and **PCH 10**.

Compound	Dose (mg/kg)	Time (h)[a]	N/F	Deaths	Avg.Weight Change (g) ± S.E.M[b]	
					Protected rats	Non- protected rats
PCH 6	200	0.0	0/7	1	–	24.2 ± 1.3
PCH 10	600	0.0	0/8	5	–	20.0 ± 0.0

[a] Post first Stage III seizure.
[b] Weight change 24 h Post first Stage III seizure.

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′*-[substituted] pyridine-4-carbohydrazides. However, further studies need to be carried out to ascertain the precise mechanism of action of anticonvulsant activity of these molecules.

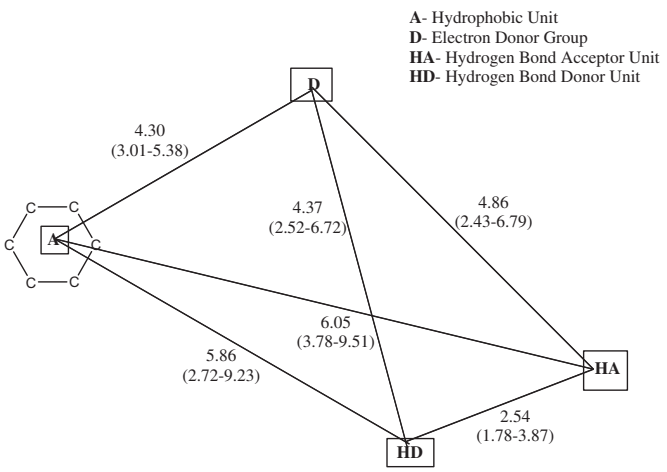


Fig. 2. Four-point 3D pharmacophore model for anticonvulsants derived by using MM3 and CHARMm parametrization (Argus Lab 4.0 and ACD/3D viewer).

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 F_{254} coated aluminium 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 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-fluorobenzaldehyde **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.

7.1.2. Synthesis of N′-(substituted) pyridine-4-carbohydrazide (**PCH 1–10**)

Equimolar quantities (0.01 mol) of 4-substituted benzaldehydes **3a–i**/isatin **3j** and pyridine-4-carbohydrazide **4** 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, elemental analysis and spectral data of the titled compounds **PCH 1–10** are given below.

Table 8
Results of *In-vitro* Hippocampal Slice Culture Neuroprotection Assay (Test 76) of **PCH 3**.

Compound	Excitotoxin	Insult Duration (h)	Primary Screen Results
PCH 3	N-methyl-D-aspartate (NMDA)	4	No neuroprotection observed
	Kainic acid (KA)	4	No neuroprotection observed

*7.1.2.1. N′-(4-phenoxybenzylidene)pyridine-4-carbohydrazide (**PCH 1**).* **IR (KBr, cm^{-1})** *ν*: 3250, 3081 (NH_{str} associated), 1657 ($C{=}O_{str}$), 1597 ($CH{=}N_{str}$), 1240 (–O–). **^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm**: 6.943 and 8.712 (d, 4H, pyridine protons), 7.10–7.87 (m, 9H, Ar-H), 8.353 (s, 1H, CH=N), 10.13 (s, 1H, NH, D_2O exchangeable). **MS (*m/z*, %)**: 318.17 (M^++1, 94.16). **Anal. Calcd**.: C, 71.91; H, 4.76; N, 13.24. Found: C, 71.87; H, 4.73; N, 13.23.

*7.1.2.2. N′-[4-(4-nitrophenoxy)benzylidene]pyridine-4-carbohydrazide (**PCH 2**).* **IR (KBr, cm^{-1})** *ν*: 3256, 3085 (NH_{str} associated), 1657 ($C{=}O_{str}$), 1603 ($CH{=}N_{str}$), 1525 (N=O), 1241 (–O–). **^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm**: 6.943 and 8.721 (d, 4H, pyridine protons), 7.13–7.87 (m, 8H, Ar-H), 8.354 (s, 1H, CH=N), 10.17 (s, 1H, NH, D_2O exchangeable). **MS (*m/z*, %)**: 363.13 (M^++1, 89.17). **Anal. Calcd**: C, 62.98; H, 3.89; N, 15.46. Found: C, 62.92; H, 3.86; N, 15.44.

*7.1.2.3. N′-[4-(4-methylphenoxy)benzylidene]pyridine-4-carbohydrazide (**PCH 3**).* **IR (KBr, cm^{-1})** *ν*: 3253, 3083 (NH_{str} associated), 1655 ($C{=}O_{str}$), 1599 ($CH{=}N_{str}$), 1243 (–O–). **^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm**: 2.348 (s, 3H, CH_3), 6.941 and 8.717 (d, 4H, pyridine protons), 7.11–7.89 (m, 8H, Ar-H), 8.357 (s, 1H, CH=N), 10.20 (s, 1H, NH, D_2O exchangeable). **MS (*m/z*, %)**: 332.26 (M^++1, 100.00). **Anal. Calcd**.: C, 72.49; H, 5.17; N, 12.68. Found: C, 72.47; H, 5.17; N, 12.61.

*7.1.2.4. N′-[4-(4-chlorophenoxy)benzylidene]pyridine-4-carbohydrazide (**PCH 4**).* **IR (KBr, cm^{-1})** *ν*: 3257, 3081 (NH_{str} associated), 1653 ($C{=}O_{str}$), 1601 ($CH{=}N_{str}$), 1241 (–O–). **^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm**: 6.938 and 8.721 (d, 4H, pyridine protons), 7.13–7.91 (m, 8H, Ar-H), 8.352 (s, 1H, CH=N), 9.891 (s, 1H, NH, D_2O exchangeable). **MS (*m/z*, %)**: 352.13 (M^++1 for ^{35}Cl, 100.00), 354.11 (M^++1 for ^{37}Cl, 34.3). **Anal. Calcd**.: C, 64.87; H, 4.01; N, 11.94. Found: C, 64.81; H, 4.03; N, 11.93.

*7.1.2.5. N′-[4-(4-bromophenoxy)benzylidene]pyridine-4-carbohydrazide (**PCH 5**).* **IR (KBr, cm^{-1})** *ν*: 3258, 3086 (NH_{str} associated), 1662 ($C{=}O_{str}$), 1609 ($CH{=}N_{str}$), 1244 (–O–). **^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm**: 6.945 and 8.719 (d, 4H, pyridine protons), 7.13–7.81 (m, 8H, Ar-H), 8.355 (s, 1H, CH=N), 9.921 (s, 1H, NH, D_2O exchangeable). **MS (*m/z*, %)**: 396.12 (M^++1, 100.00), 398.13 (M^++1, 97.3).

Table 9
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)
PCH 3	4.80	4.19	3.99	1.44	2.39	3.77
PCH 6	4.79	4.187	4.00	1.43	2.38	3.77

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

Anal. Calcd.: C, 57.59; H, 3.56; N, 10.60. Found: C, 57.56; H, 3.54; N, 10.57.

*7.1.2.6. N′-[4-(4-fluorophenoxy)benzylidene]pyridine-4-carbohydrazide (**PCH 6**).* **IR (KBr, cm^{-1})** *v*: 3252, 3080 (NH_{str} associated), 1651 ($C{=}O_{str}$), 1605 ($CH{=}N_{str}$), 1255 (-O-). **^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm**: 6.986 and 8.760 (d, 4H, pyridine protons), 7.016–7.885 (m, 8H, Ar-H), 8.359 (s, 1H, CH═N), 9.863 (s, 1H, NH, D_2O exchangeable). **MS (*m/z*, %)**: 336.17 (M^++1, 68.10). **Anal. Calcd**.: C, 68.05; H, 4.21; N, 12.53. Found: C, 68.01; H, 4.20; N, 12.55.

*7.1.2.7. N′-[4-(4-chloro-3-methylphenoxy)benzylidene]pyridine-4-carbohydrazide (**PCH 7**.* **IR (KBr, cm^{-1})** *v*: 3255, 3081 (NH_{str} associated), 1659 ($C{=}O_{str}$), 1607 ($CH{=}N_{str}$), 1237 (−O−). **^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm**: 2.362 (s, 3H, CH_3), 6.938 and 8.711 (d, 4H, pyridine protons), 7.13–7.85 (m, 7H, Ar-H), 8.356 (s, 1H, CH═N), 10.09 (s, 1H, NH, D_2O exchangeable). **MS (*m/z*, %)**: 366.15 (M^++1 for ^{35}Cl, 100.00), 368.11 (M^++1 for ^{37}Cl, 33.7). **Anal. Calcd**.: C, 65.67; H, 4.41; N, 11.49. Found: C, 65.66; H, 4.38; N, 11.46.

*7.1.2.8. N′-[4-(naphthalen-2-yloxy)benzylidene]pyridine-4-carbohydrazide (**PCH 8**).* **IR (KBr, cm^{-1})** *v*: 3247, 3081 (NH_{str} associated), 1661 ($C{=}O_{str}$), 1597 ($CH{=}N_{str}$), 1238 (−O−), 836, 821 (β-naphthyl). **^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm**: 6.939 and 8.707 (d, 4H, pyridine protons), 7.09–7.89 (m, 11H, Ar-H), 8.359 (s, 1H, CH═N), 10.12 (s, 1H, NH, D_2O exchangeable). **MS (*m/z*, %)**: 368.17 (M^++1, 92.38). **Anal. Calcd**.: C, 75.19; H, 4.66; N, 11.44. Found: C, 75.16; H, 4.65; N, 11.41.

*7.1.2.9. N′-{(Z)-[4-(1,3-benzodioxol-5-yloxy)phenyl]methylidene} pyridine-4-carbohydrazide (**PCH 9**).* **IR (KBr, cm^{-1})** *v*: 3246, 3077 (NH_{str} associated), 1654 ($C{=}O_{str}$), 1601 ($CH{=}N_{str}$), 1239 (−O−). **^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm**: 5.989 (s, 2H, CH_2), 6.943 and 8.717 (d, 4H, pyridine protons), 7.14–7.87 (m, 7H, Ar-H), 8.349 (s, 1H, CH═N), 10.02 (s, 1H, NH, D_2O exchangeable). **MS (*m/z*, %)**: 362.15 (M^++1, 89.11). **Anal. Calcd**.: C, 66.48; H, 4.18; N, 11.63. Found: C, 66.41; H, 4.17; N, 11.60.

*7.1.2.10. N′-[(3Z)-2-oxo-1,2-dihydro-3H-indol-3-ylidene]pyridine-4-carbohydrazide (**PCH 10**).* **IR (KBr, cm^{-1})** *v*: 3258, 3080 (NH_{str} associated), 1680 ($C{=}O_{str}$), 1590 ($C{=}N_{str}$). **^{1}H NMR ($CDCl_3$, 300 MHz) δ in ppm**: 6.940 and 8.711 (d, 4H, pyridine protons), 7.19–7.71 (m, 4H, Ar-H), 10.07 (s, 1H, NH, D_2O exchangeable), 12.81 (s, 1H, NH of isatin, D_2O exchangeable). **MS (*m/z*, %)**: 267.13 (M^++1, 95.12). **Anal. Calcd**.: C, 63.15; H, 3.79; N, 21.04. Found: C, 63.11; H, 3.77; N, 21.01.

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 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. 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

Table 10
Pharmacokinetic parameters important for good oral bioavailability of compounds **PCH 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
PCH 1	87.0	63.58	5	331.4	302.896	1	5	0
PCH 2	71.3	109.412	6	376.372	326.23	1	8	0
PCH 3	87.0	63.588	5	345.402	319.457	1	5	0
PCH 4	87.0	63.588	5	365.82	316.432	1	5	0
PCH 5	87.0	63.588	5	410.271	320.782	1	5	0
PCH 6	87.0	63.588	5	349.365	307.828	1	5	0
PCH 7	87.0	63.588	5	379.847	332.993	1	5	0
PCH 8	87.0	63.588	5	381.435	346.888	1	5	0
PCH 9	80.6	82.056	5	375.384	326.826	1	7	0
PCH 10	78.9	87.216	2	266.26	226.486	2	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 11
Log *P* value for compounds **PCH 1-10**.

Compounds	Experimental Log *P*	Theoretical Log *P* (miLog *P*[a])
Rule	–	≤5
PCH 1	3.373	3.477
PCH 2	3.23	3.436
PCH 3	3.652	3.926
PCH 4	3.948	4.155
PCH 5	4.029	4.286
PCH 6	3.496	3.641
PCH 7	4.215	4.532
PCH 8	4.521	4.661
PCH 9	3.197	3.367
PCH 10	1.124	1.045

[a] miLog *P*, logarithm of compound partition coefficient between n-octanol and water calculated as per Molinspiration Online Property Toolkit.

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. 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 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 rat MES screen.

7.2.2. Subcutaneous metrazol seizure threshold (scMET) 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 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 behaviours, 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 was 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

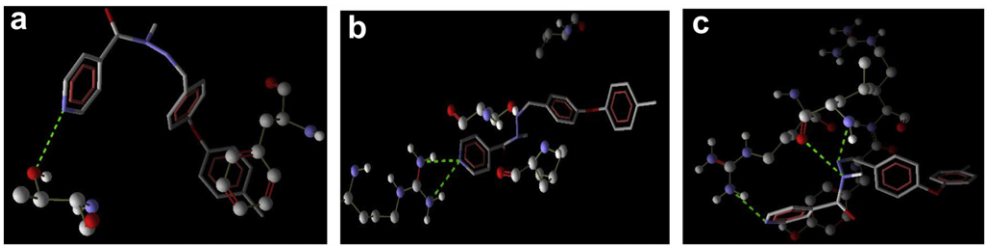


Fig. 3. Docking images (a) **PCH 3** with GABA(A) alpha-1, (b) **PCH 3** with GABA(A) delta, (c) **PCH 3** with Glutamate receptor.

Table 12
Results of docking study of compounds **PCH 3** and **PCH 6**.[a]

Ligand	Receptor	Affinity (Kcal/mol)	H-bonds	H- Binding Ligand			H- Binding Receptor			
				Element	Atom No.	Type	Residue	Element	Atom No.	Type
PCH 3	GABA(A) alpha-1	−6.3	01	N	23	Acceptor	Thr	O	71	Both
	GABA (A) delta	−6.0	02	N	23	Acceptor	Arg	N	165	Donor
				N	23	Acceptor	Arg	N	164	Donor
	Glutamate	−6.1	03	N	23	Acceptor	Arg	N	247	Donor
				N	02	Donor	Leu	O	201	Acceptor
				N	04	Acceptor	Leu	N	198	Donor
	Na/H exchanger	−5.6	NIL	–	–	–	–	–	–	–
	Na channel	−0.0	NIL	–	–	–	–	–	–	–
	T-type calcium	−0.0	NIL	–	–	–	–	–	–	–
PCH 6	GABA(A) alpha-1	−5.8	NIL	–	–	–	–	–	–	–
	GABA (A) delta	−5.4	NIL	–	–	–	–	–	–	–
	Glutamate	−6.2	NIL	–	–	–	–	–	–	–
	Na/H exchanger	−5.5	NIL	–	–	–	–	–	–	–
	Na channel	−0.0	NIL	–	–	–	–	–	–	–
	T-type calcium	−0.0	NIL	–	–	–	–	–	–	–

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

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 [22].

7.3.2. Prediction of ADME properties

A computational study of titled compounds was performed for prediction of ADME properties. Polar surface area (TPSA) [21], miLog *P*, number of rotatable bonds, molecular volume, number of hydrogen donor and acceptor atoms and violations of Lipinski's rule of five [23] were calculated using Molinspiration online property calculation toolkit [24]. Absorption (%ABS) was calculated by: % ABS = 109−(0.345 × TPSA) [25].

7.3.3. Log P determination

The partition coefficient between octanol and phosphate buffer was determined at room temperature [26]. 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 **PCH 3** and **PCH 6** 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 [27]. In present study, AutoDock 4.0 with its Lamarckian genetic algorithm (LGA) was used for automated flexible ligand docking of **PCH 3** and **PCH 6** with above mentioned receptors and docking affinity (Kcal/mol) and count of probable H-bonds were evaluated.

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