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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and biological activities of Schiff bases of gabapentin with different aldehydes and ketones: a structure–activity relationship study

Lingappa Mallesha · Kikkeri N. Mohana · Bantal Veeresh

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Abstract A series of novel gabapentin derivatives **6a–k** and **7a–f** were synthesized, and their biological activities were determined. The chemical structures were confirmed by elemental analyses, UV–visible, FT-IR, and ^{1}H NMR spectral studies. The structure–activity relationships (SAR) for anticonvulsant and antioxidant activities were discussed. Compounds **7a–f** were evaluated for their possible anticonvulsant activity by Maximal Electroshock Seizure (MES) test, and their neurotoxic effects were determined by rotorod test. Majority of the compounds were active in MES tests. Compounds **7b** and **7e** showed good protective effect from seizure when compared to standard drug, phenytoin (100 mg/kg). The same compounds showed no neurotoxicity at the maximum dose administered (100 mg/kg). Most of the novel compounds showed DPPH radical scavenging activity, where compounds **6f**, **6j,** and **7a** were the best radical scavengers (IC_{50} was about 60 μg/ml).

Keywords Gabapentin · Aldehydes · Ketones · Anticonvulsant · Antioxidant

Introduction

Epilepsy is a major neurological disorder affecting a large section of people both male and female throughout the world. Currently available drugs for the treatment of epilepsy are symptomatically effective in only 60–70% of patients. Epilepsy also poses a considerable economic burden on society. The direct costs of epilepsy vary significantly depending on the severity of the disease and the response to treatment. The known potential causes of epilepsy include brain tumors, infections, traumatic head injuries, perinatal insults, developmental malformations, cerebrovascular diseases, febrile seizures, and status epilepticus (Loscher, 2002). Over the years, there has been considerable success in the development of novel antiepileptic drugs (AED) along with new improved formulations. These include older first generation drugs such as carbamazepine, phenobarbitol, and valproic acid and newer second generation drugs such as lamotrigine, vigabatrin, tiagabine, topiramate, gabapentin, and levetiracetam (McCabe, 2000). The selection of an antiepileptic drug for treatment is predicated on its efficacy for the specific type of seizures, tolerability, and safety (Regesta and Tanganelli, 1999; Kwan and Brodie, 2000). Therefore, it is essential to search for newer chemical entities for the treatment of epilepsy.

In recent years, there has been an increased interest in the application of antioxidants to medical treatment as information is constantly gathered linking the development of human diseases to oxidative stress. Free radicals play a role in the pathogenesis of chronic degenerative diseases including cancer, autoimmune, inflammatory, cardiovascular and neurodegenerative diseases, and aging (Cantuti-Castelvetri *et al*., 2000; Surh *et al*., 2001; Vaya and Aviram, 2001; Aruoma, 2003). It is also known that oxidative stress can be induced by a wide range of environmental factors including UV stress, pathogen invasion, herbicide action, and oxygen shortage (Blokhina *et al*., 2003). Owing to these facts, synthetic and natural compounds with potential antioxidant activity are receiving increased attention in biological research, medicine, and pharmacy (Hollman and Katan, 1999).

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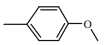
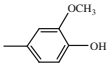
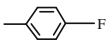
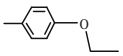
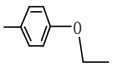
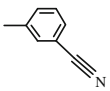
Schiff bases are characterized by the –N=CH– (imine) group, which is important in elucidating the mechanism of transamination and racemisation reactions in biological systems (Lau *et al*., 1999). Due to the great flexibility and diverse structural aspects, a wide range of Schiff bases have been synthesized and their complexation behaviors have been studied (Raman *et al*., 2003). They have been synthesized from a variety of compounds such as amino thiazoles, 2-hydroxy-1-napthalaniline, amino sugars, aromatic aldehydes, ketones, isatin, triazole ring, thiosemicarbazides, amino acids, pyrazolone, etc. (Sridhar and Ramesh, 2002; Piotr and Bogumil, 2002). Antibacterial, antifungal, antitumor, and anticancer activities of some Schiff bases have been reported, and they are active against a wide range of organisms (Sari *et al*., 2003). Some Schiff bases bearing aryl groups or heterocyclic residues possessing excellent biological activities have attracted the attention of many researchers in recent years (Holla *et al*., 2000). The Schiff bases formed from aromatic aldehydes, ketones, and their derivatives are quite stable. Many schiff bases are known to be medicinally important and are used to design medicinal compounds (Capdeville *et al*., 2002).

Gabapentin [1-(aminomethyl) cyclohexaneacetic acid, Neurontin, Gpn] is structurally related to the neurotransmitter gamma-aminobutyric acid (GABA) which has been widely studied for its significant inhibitory action in the central nervous system (Bowery, 1993). Gpn has been advanced for the treatment of neuropathic pain (Rosenberg *et al*., 1997). It is a new generation antiepileptic drug that is used as add-on therapy (Placidi *et al*., 2000) as well as monotherapy (Chadwick *et al*., 1998) in patients with partial seizures (Lima, 2000; Morton and Pellock, 2000). With the introduction of gabapentin and other promising new antiepileptic drugs, safe and effective seizure control may become a reality for an increasing number of adults with epilepsy (Mattson, 1998). In this respect, this article reports the synthesis and biological activities (anticonvulsant activity and antioxidant activity) of a new class of Schiff bases, **6a**–**k** and **7a**–**f**.

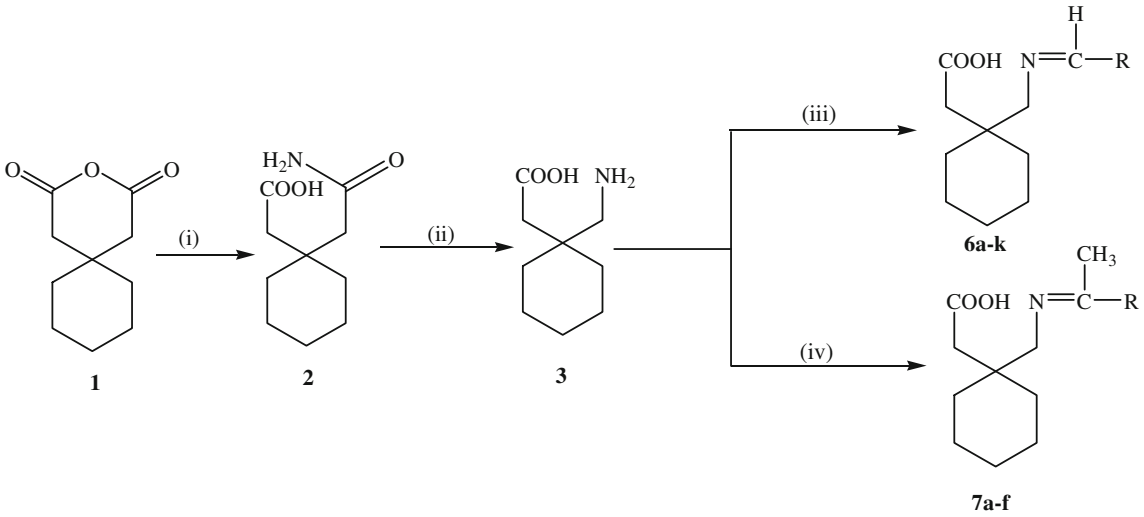
Results and discussion

The reactions of gabapentin with different aldehydes and ketones were carried out in the presence of absolute ethanol. The synthesized compounds were characterized by elemental analyses, UV–visible, FT-IR, and ^{1}H NMR spectral studies. The chemical structures and physical data of all the synthesized compounds are given in Table 1. The elemental analyses data showed good agreement between the experimentally determined values and the theoretically calculated values within ±0.4%. The electronic absorption spectra of the synthesized compounds showed new bands, and the appearance of longer wavelength absorption band in the UV region confirms the formation of compounds. The synthetic route of the compounds is outlined in Scheme 1.

Table 1 Chemical structure, yield, and physical characterization of the synthesized compounds

Compound	R	Yield (%)	m. p. (°C)	λ_{max} (UV–visible, nm)
6a	–H	73	136–139	292
6b	$-CH_3$	78	139–141	285
6c		80	124–126	288
6d		78	118–120	295
6e		76	127–130	340
6f		79	132–135	286
6g		81	135–137	278
6h		84	138–141	287
6i		70	133–135	284
6j		72	135–137	287
6k		85	136–138	289
7a		78	136–138	295
7b		84	137–139	289
7c		80	132–135	281
7d		78	112–114	306
7e		82	113–115	288
7f		76	118–120	286

The absence of NH_2 and C=O absorption bands in the IR spectra confirmed that the synthesized compounds were obtained via condensation. The appearance of a medium to strong absorption band at around 1700 cm^{-1} is due to the stretching vibration of C=N bond formation in the synthesized compounds. The proton spectral data agree with respect to the number of protons and their chemical shifts with the proposed structures. The proton spectral data of the intermediate, gabapentin (**3**) shows resonance at δ 5.52 ppm (s, 2H, NH_2). In all the synthesized compounds, the above resonance disappeared and additional resonances assigned



Scheme 1 Reagents and conditions: (i) NH_3, 5°C, 2 h (ii) NaOBr, NaOH, 55°C, 15 h (iii) different aldehydes, **4a**–**k**, EtOH, 6–8 h (iv) different ketones, **5a**–**f**, EtOH, 6–8 h

to the –CH=N– (δ 6.76–7.14 ppm) and –CH_3–C=N– (δ 2.13–2.15 ppm) were observed, which confirmed the condensation between the amino group and carbonyl group.

Antiepileptic drug research has for several decades focused on identifying new potential drugs based on their anticonvulsant activity against single acute seizures induced by various stimulators, usually in mice and rats. All established antiepileptic drugs have anticonvulsant activity in at least Maximal Electroshock Seizure (MES) model (Loscher and Schmidt, 1994). Thus, this test may in some way distinguish the potential utility of compounds against different seizure types. The anticonvulsant activity of gabapentin has been reported (Fromm, 1994; Gee *et al*., 1996). In this study, the anticonvulsant activity of six newly synthesized gabapentin derivatives, **7a**–**f**, was evaluated by MES model at the dose of 100 mg/kg. The results of MES test are summarized in Table 2. Compounds **7b** and **7e** showed good with anticonvulsant protection of 66.45% and 65.61%. Pyridine group present in **7b** and **7e** appears to be as potent as phenytoin (standard) in the MES tests in rats. Compounds **7a** and **7d** show 12.74% and 24.63% protection, respectively, which are moderate. The relatively lower anticonvulsant potencies of compounds **7c** and **7f** show a protective effect of 6.36% and 0.42%, respectively. Compounds **7a**–**f** were examined for their neurotoxicity using rotorod method (Yogeeswari *et al*., 2005) given in dose 100 mg/kg. Compounds **7b** and **7e** did not exhibit toxicity, whereas compounds **7a**, **7c**, **7d**, and **7f** showed 25% toxicity compared to standard at 2 h of oral administration (Table 3).

The in vitro scavenging assay of DPPH radicals was performed spectrophotometrically (Tapia *et al*., 2004) with ascorbic acid as positive control. Percentages of DPPH radical scavenging activity and IC_{50} values were tabulated

Table 2 Effects of the tested compounds in the maximal electroshock seizure test

Treatment	E/F	% Protection
7a	4.41	12.74[b]
7b	1.58	66.45[a]
7c	4.11	6.36
7d	3.55	24.63[b]
7e	1.62	65.61[a]
7f	4.69	0.42
Standard	1.61	79.29
Control (Vehicle)	4.71	–

Values are expressed as mean ± SE. $n = 6$ animals in each group

E/F = Extension/Flexion [Decrease in ratio of extension phase (in seconds)/flexion phase (in seconds)]

% Protection = (Control-test)/(Control) × 100

[a] $P < 0.01$; [b] $P < 0.05$ when compared to control rats

Table 3 Neurotoxicity screening of the compounds

Compound	Neurotoxicity screen			
	0.5 h	1 h	2 h	4 h
7a	0/4	0/4	1/4	1/4
7b	0/4	0/4	0/4	0/4
7c	0/4	0/4	1/4	1/4
7d	0/4	0/4	0/4	1/4
7e	0/4	0/4	0/4	0/4
7f	0/4	0/4	1/4	1/4
Standard	0/4	1/4	1/4	1/4

The data in the table represent ratio between the numbers of the animals that exhibited neurotoxicity against the number of tested animals

Table 4 DPPH radical scavenging activity of the tested compounds

Compound	Scavenging effect (%)			IC_{50} (μg/ml)
	Concentration of the tested compounds (μg/ml)			
	100	150	200	
6a	30.2	40.6	48.9	–[a]
6b	31.4	41.0	49.5	–[a]
6c	30.1	41.3	49.8	–[a]
6d	49.6	59.8	69.5	94.2
6e	51.0	60.6	71.3	80.5
6f	57.2	64.9	78.2	59.6
6g	47.2	58.0	69.1	104.0
6h	45.1	56.7	66.4	119.4
6i	44.7	53.8	62.8	130.5
6j	55.6	66.4	74.5	60.5
6k	40.0	50.3	59.7	151.2
7a	55.8	66.7	74.6	60.3
7b	31.1	42.5	51.6	194.8
7c	30.6	41.0	49.8	–[a]
7d	32.7	43.8	55.8	175.0
7e	30.8	42.1	51.0	198.2
7f	28.5	37.2	48.4	–[a]
Ascorbic acid	74.1	86.4	99.3	8.6

[a] IC50 values could be determined even at concentration 200 μg/ml

in Table 4. Compounds **6d–k** exhibited highest antioxidant activity with lowest IC_{50} value (<200 μg/ml) when compared with the IC_{50} value of standard (8.6 μg/ml). The percentage scavenging effects of the compound **6f** at 100, 150, 200 μg/ml are 57.2, 64.9, 78.2 and compound **6j** at 100, 150, 200 μg/ml are 55.6, 66.4, 74.5, respectively. The percentage inhibition of the compound **7a** at 100, 150, 200 μg/ml are 55.8, 66.7, 74.6, respectively. Ascorbic acid presented a scavenging effect of 99.3% at the concentration of 200 μg/ml. The moderate inhibition of **7b**, **7d**, and **7e** showed 51.6%, 55.8% and 51.0%, respectively, at 200 μg/ml. Compounds **7c** and **7f** exhibited lower inhibition and did not showed IC_{50} value at 200 μg/ml.

Structure–activity relationships

The initial structure activity relationship can be drawn for the compounds **6a–k** and **7a–f**. It has been reported that, semicarbazones were explored as potent anticonvulsants (Pandeya and Raja, 2002). The basis for the development of semicarbazones has been advocated by citing a binding site hypothesis in which there is (a) a hydrophobic aryl ring, (b) a hydrogen bonding domain, (c) an electron donor acceptor system, and (d) an another hydrophobic aryl ring that are responsible for metabolism.

In our present series of compounds the active compounds **7b** and **7e** possess all the requirements essential for anticonvulsant activity as proposed by Pandeya and Raja. The 2-pyridyl group in **7b** and 3-pyridyl group in **7e** resulted in increased anticonvulsant activity. Both compounds did not exhibit neurotoxicity at highest administered dose. The presence of indole group in **7d** and ethoxybenzene group in **7a** is showed moderate anticonvulsant activity and neurotoxicity. Although benzonitrile group in **7c** and naphthalene group in **7f** were weakly active in the MES test, these compounds contribute to the 25% neurotoxicity at 2 h. From the results it is quite apparent that there are at least four parameters for the activity of anticonvulsant drugs, that is, (i) a lipophilic domain (L, cyclohexane), (ii) the hydrogen donor acceptor site (HBD, carboxylic acid), (iii) an electron donor (D, –C=N–) system, and (iv) a hydrophobic unit (R, substituted aromatic ring). Thus the proposed pharmacophore model for **7b** and **7e** includes all the above factors important for bioactivity (Fig. 1).

The compound **6f** showed higher radical inhibition activity due to the presence of methoxy group (electron donating group) in the aromatic ring (Roopan and Khan, 2009). The compounds **6j** and **7a** bearing an electron donating ethoxy group at para position showed similar antioxidant activity. Electron donating hydroxyl group in **6e** and methoxy group in **6d** showed good antioxidant activity (Jeong *et al.*, 2007). The aromatic ring system with halogens like chlorine or fluorine in **6g**, **6h**, and **6i** was found to be more active than **6b**, **6c**, and **6k**. On the other

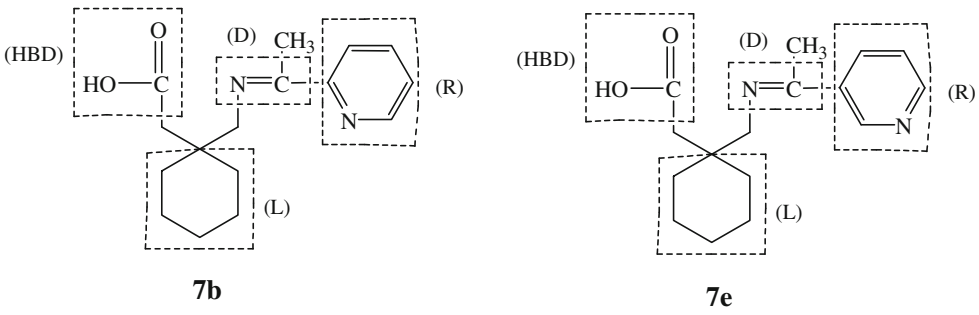


Fig. 1 The pharmacophore model of **7b** and **7e** for anticonvulsant activity

hand, **7a** showed good antioxidant activity due to the presence of electron donating ethoxy group. Pyridyl group in **7b** and **7e** showed similar antioxidant activity. Compounds **7c**, **7d**, and **7f** showed moderate antioxidant activity. The above SAR studies reveal that, the nature of the functional groups is crucial for biological activity.

Conclusion

In conclusion, a series of novel gabapentin derivatives **6a–k** and **7a–f** were synthesized in good yield, characterized by different spectral studies, and their anticonvulsant and antioxidant activities have been evaluated. Compounds **7b** and **7e** showed good anticonvulsant activity with no neurological toxicity. Compounds **6f**, **6j**, and **7a** demonstrated good antioxidant activity. The SAR studies reveal that, both linkage and substituent on phenyl ring are responsible for anticonvulsant and antioxidant activities of these classes of agents. The compounds **7b** and **7e** confirmed the pharmacophore model requirements for activity. On the basis of their activity, these derivatives were identified as viable leads for further studies.

Experimental

Chemistry

All solvents and reagents were purchased from Sigma-Aldrich Chemicals Pvt. Ltd., India**.** Melting points were determined using SELACO-650 hot stage melting point apparatus and were uncorrected. The UV spectra were recorded using Analytik Jena Specord 50 UV–vis spectrophotometer. FT-IR spectra were recorded using a Jasco FTIR-4100 series. ^{1}H NMR spectra were recorded on Shimadzu AMX 400-Bruker, 400 MHz spectrometer using DMSO-d_6 as a solvent, and TMS as internal standard (chemical shift in δ ppm). Elemental (CHNS) analyses were obtained on Vario EL III Elementar.

The target key intermediate, gabapentin (**3**), was synthesized according to the reported procedure (Ashok *et al*., 2008) by the reaction of acid anhydride (**1**) with an aqueous ammonia solution, then Hofmann rearrangement of the obtained monoamide (**2**), followed by acidification and the extraction. The reactions of gabapentin (**3**) with different aldehydes, **4a–k**, and ketones, **5a–f,** were carried out in the presence of ethanol as solvent with a good yield ranging from 70 to 85%. Synthesized molecules **6a–k** and **7a–f** were structurally characterized by ^{1}H NMR, FT-IR, UV–visible, and elemental analyses.

General procedure for the synthesis of gabapentin derivatives **6a–k** and **7a–f**

Equimolar concentrations of different aldehydes/ketones (0.01 mol) and gabapentin (0.01 mol) were stirred for 6–8 h at room temperature in absolute ethanol (25 ml) and then 2–3 drops of concentrated sulfuric acid was added to the mixture. The progress of the reaction was monitored by TLC until the reaction was complete. It was cooled to 0°C, and the precipitate was filtered, washed with diethyl ether, and the residue was recrystallized from ethanol.

2-(1-((Methyleneamino)methyl)cyclohexyl) acetic acid (**6a**)

The general experimental procedure described above afforded **6a**, and the product obtained from gabapentin (**3**) (1.72 g, 0.01 mol) and formaldehyde (**4a**) (0.31 g, 0.01 mol). FT-IR (KBr, cm^{-1}) *v*: 2924 (O–H), 1700 (C=N), 1072 (C–N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.2 (s, br, 1H, OH), 6.76 (s, 2H, CH_2), 2.76 (s, 2H, CH_2), 2.28 (s, 2H, CH_2), 1.38–1.35 (m, 10H, cyclohexyl methylenes group). Anal. calcd. for $C_{10}H_{17}NO_2$ (in %): C, 65.54; H, 9.35; N, 7.64. Found: C, 65.21; H, 9.18; N, 7.38.

2-(1-((Ethylideneamino)methyl)cyclohexyl) acetic acid (**6b**)

The general experimental procedure described above afforded **6b**, and the product obtained from gabapentin (**3**) (1.72 g, 0.01 mol) and acetaldehyde (**4b**) (0.45 g, 0.01 mol). FT-IR (KBr, cm^{-1}) *v*: 2925 (O–H), 1704 (C=N), 1071 (C–N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.3 (s, br, 1H, OH), 7.10 (q, 1H, CH), 2.81 (d, 3H, CH_3), 2.76 (s, 2H, CH_2), 2.28 (s, 2H, CH_2), 1.38–1.34 (m, 10H, cyclohexyl methylenes group). Anal. calcd. for $C_{11}H_{19}NO_2$ (in %): C, 66.97; H, 9.71; N, 7.10. Found: C, 67.11; H, 9.48; N, 7.38.

2-(1-((Benzylideneamino)methyl)cyclohexyl) acetic acid (**6c**)

The general experimental procedure described above afforded **6c**, and the product obtained from gabapentin (**3**) (1.72 g, 0.01 mol) and benzaldehyde (**4c**) (1.10 g, 0.01 mol). FT-IR (KBr, cm^{-1}) *v*: 3024 (C–H), 2924 (O–H), 1702 (C=N), 1461 (C=C), 1068 (C–N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.2 (s, br, 1H, OH), 7.65–7.52 (d, 2H, Ar–H), 7.28–7.21 (m, 3H, Ar–H), 7.13 (s, 1H, CH), 2.75 (s, 2H, CH_2), 2.28 (s, 2H, CH_2), 1.38–1.34 (m, 10H, cyclohexyl methylenes group). Anal. calcd. for $C_{16}H_{21}NO_2$ (in %): C, 74.10; H, 8.16; N, 5.40. Found: C, 73.91; H, 8.28; N, 5.28.

2-(1-((4-Methoxybenzylideneamino)methyl) cyclohexyl)acetic acid (**6d**)

The general experimental procedure described above afforded **6d**, and the product obtained from gabapentin (**3**) (1.72 g, 0.01 mol) and 4-methoxybenzaldehyde (**4d**) (1.23 g, 0.01 mol). FT-IR (KBr, cm^{-1}) *v*: 3024 (C–H), 2925 (O–H), 1702 (C=N), 1461 (C=C), 1070 (C–N). ^1H NMR (DMSO-d_6, 400 MHz) δ: 11.3 (s, br, 1H, OH), 7.53–7.51 (d, 2H, Ar–H), 7.13 (s, 1H, CH), 6.82–6.75 (d, 2H, Ar–H), 3.76 (s, 3H, CH_3), 2.94 (s, 2H, CH_2), 2.29 (s, 2H, CH_2), 1.38–1.36 (m, 10H, cyclohexyl methylenes group). Anal. calcd. for $C_{17}H_{23}NO_3$ (in %): C, 70.56; H, 8.01; N, 4.84. Found: C, 70.71; H, 8.28; N, 4.68.

2-(1-((2-Hydroxy benzylideneamino)methyl) cyclohexyl)acetic acid (**6e**)

The general experimental procedure described above afforded **6e**, and the product obtained from gabapentin (**3**) (1.72 g, 0.01 mol) and 2-hydroxybenzaldehyde (**4e**) (1.23 g, 0.01 mol). FT-IR (KBr, cm^{-1}) *v*: 3054 (C–H), 2924 (O–H), 1704 (C=N), 1461 (C=C), 1073 (C–N). ^1H NMR (DMSO-d_6, 400 MHz) δ: 11.2 (s, br, 1H, OH), 8.50 (s, 1H, OH), 7.41 (d, 1H, Ar–H), 7.28 (t, 1H, Ar–H), 7.13 (s, 1H, CH), 6.88 (t, 1H, Ar–H), 6.75 (d, 1H, Ar–H), 2.96 (s, 2H, CH_2), 2.34 (s, 2H, CH_2), 1.39–1.37 (m, 10H, cyclohexyl methylenes group). Anal. calcd. for $C_{16}H_{21}NO_3$ (in %): C, 69.79; H, 7.69; N, 5.09. Found C-70.01; H-7.48; N, 4.87.

2-(1-((4-Hydroxy-3-methoxybenzylideneamino) methyl)cyclohexyl)acetic acid (**6f**)

The general experimental procedure described above afforded **6f**, and the product obtained from gabapentin (**3**) (1.72 g, 0.01 mol) and 4-hydroxy-3-methoxybenzaldehyde (**4f**) (1.53 g, 0.01 mol). FT-IR (KBr, cm^{-1}) *v*: 3083 (C–H), 2924 (O–H), 1694 (C=N), 1461 (C=C), 1074 (C–N). ^1H NMR (DMSO-d_6) δ: 11.30 (s, br, 1H, OH), 8.43 (s, 1H, OH), 7.13 (d, 1H, Ar–H), 6.97 (s, 1H, CH), 6.65 (s, 1H, Ar–H), 6.23 (d, 1H, Ar–H), 3.18 (s, 3H, CH_3), 2.85 (s, 2H, CH_2), 2.25 (s, 2H, CH_2), 1.42–1.18 (m, 10H, cyclohexyl methylenes group). Anal. calcd. for $C_{17}H_{23}NO_4$ (in %): C, 66.86; H, 7.59; N, 4.59. Found: C, 67.01; H, 7.78; N, 4.87.

2-(1-((2-Chloro-6-fluorobenzylideneamino)methyl) cyclohexyl)acetic acid (**6g**)

The general experimental procedure described above afforded **6g**, and the product obtained from gabapentin (**3**) (1.72 g, 0.01 mol) and 2-chloro-6-fluorobenzaldehyde (**4g**) (1.59 g, 0.01 mol). FT-IR (KBr, cm^{-1}) *v*: 3024 (C–H), 2925 (O–H), 1699 (C=N), 1461 (C=C), 1234 (C–F), 1091 (C–N), 723 (C–Cl). ^1H NMR (DMSO-d_6, 400 MHz) δ: 11.3 (s, br, 1H, OH), 7.21 (t, 1H, Ar–H), 7.13 (s, 1H, CH), 7.11 (d, 1H, Ar–H), 6.87 (d, 1H, Ar–H), 2.76 (s, 2H, CH_2), 2.28 (s, 2H, CH_2), 1.38–1.35 (m, 10H, cyclohexyl methylenes group). Anal. calcd. for $C_{16}H_{19}ClFNO_2$ (in %): C, 61.64; H, 6.14; N, 4.49. Found: C, 61.39; H, 6.19; N, 4.71.

2-(1-((2-Chlorobenzylideneamino)methyl) cyclohexyl)acetic acid (**6h**)

The general experimental procedure described above afforded **6h**, and the product obtained from gabapentin (**3**) (1.72 g, 0.01 mol) and 2-chlorobenzaldehyde (**4h**) (1.41 g, 0.01 mol). FT-IR (KBr, cm^{-1}) *v*: 3060 (C–H), 2924 (O–H), 1699 (C=N), 1462 (C=C), 1092 (C–N), 723 (C–Cl). ^1H NMR (DMSO-d_6, 400 MHz) δ: 11.2 (s, br, 1H, OH), 7.53 (d, 1H, Ar–H), 7.20 (d, 1H, Ar–H), 7.17 (t, 1H, Ar–H), 7.14 (s, 1H, CH), 7.03 (t, 1H, Ar–H), 2.75 (s, 2H, CH_2), 2.28 (s, 2H, CH_2), 1.38–1.34 (m, 10H, cyclohexyl methylenes group). Anal. calcd. for $C_{16}H_{20}ClNO_2$ (in %): C, 65.41; H, 6.86; N, 4.77. Found: C, 65.39; H, 6.59; N, 4.81.

2-(1-((4-Fluorobenzylideneamino)methyl) cyclohexyl)acetic acid (**6i**)

The general experimental procedure described above afforded **6i**, and the product obtained from gabapentin (**3**) (1.72 g, 0.01 mol) (2.78 g, 0.01 mol) and 4-fluorobenzaldehyde (**4i**) (1.25 g, 0.01 mol). FT-IR (KBr, cm^{-1}) *v*: 3060 (C–H), 2925 (O–H), 1699 (C=N), 1462 (C=C), 1234 (C–F), 1077 (C–N). ^1H NMR (DMSO-d_6, 400 MHz) δ: 11.2 (s, br, 1H, OH), 7.65–7.51 (d, 2H, Ar–H), 7.31–7.20 (d, 2H, Ar–H), 7.13 (s, 1H, CH), 2.75 (s, 2H, CH_2), 2.27 (s, 2H, CH_2), 1.38–1.34 (m, 10H, cyclohexyl methylenes group). Anal. calcd. for $C_{16}H_{20}FNO_2$ (in %): C, 69.29; H, 7.27; N, 5.05. Found: C, 69.54; H, 7.39; N, 5.21.

2-(1-((4-Ethoxybenzylideneamino)methyl) cyclohexyl)acetic acid (**6j**)

The general experimental procedure described above afforded **6j**, and the product obtained from gabapentin (**3**) (1.72 g, 0.01 mol) and 4-ethoxybenzaldehyde (**4j**) (1.51 g, 0.01 mol). FT-IR (KBr, cm^{-1}) *v*: 3024 (C–H), 2924 (O–H), 1699 (C=N), 1461 (C=C), 1091 (C–N). ^1H NMR (DMSO-d_6, 400 MHz) δ: 11.1 (s, br, 1H, OH), 7.52–7.41 (d, 2H, Ar–H), 7.13 (s, 1H, CH), 6.71–6.51 (d, 2H, Ar–H), 3.85 (q, 2H, CH_2), 2.75 (s, 2H, CH_2), 2.28 (s, 2H, CH_2), 1.39 (t, 3H, CH_3), 1.38–1.34 (m, 10H, cyclohexyl methylenes group), Anal. calcd. for $C_{18}H_{25}NO_3$ (in %): C, 71.26; H, 8.31; N, 4.62. Found: C, 71.54; H, 8.19; N, 4.41.

2-(1-(((Pyridin-2-yl)methyleneamino)methyl) cyclohexyl)acetic acid (**6k**)

The general experimental procedure described above afforded **6k**, and the product obtained from gabapentin (**3**) (1.72 g, 0.01 mol) and 2-pyridine carboxaldehyde (**4k**) (1.10 g, 0.01 mol). FT-IR (KBr, cm^{-1}) *v*: 3024 (C–H), 2924 (O–H), 1701 (C=N), 1461 (C=C), 1073 (C–N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.3 (s, br, 1H, OH), 8.76 (d, 1H, pyridine-H), 8.11 (d, 1H, pyridine-H), 7.92 (t, 1H, pyridine-H), 7.83 (t, 1H, pyridine-H), 6.97 (s, 1H, CH), 2.76 (s, 2H, CH_2), 2.30 (s, 2H, CH_2), 1.38–1.35 (m, 10H, cyclohexyl methylenes group). Anal. calcd. for $C_{15}H_{20}N_2O_2$ (in %): C, 69.20; H, 7.74; N, 10.76. Found: C, 69.54; H, 7.59; N, 10.41.

2-(1-((1-(4-Ethoxyphenyl)ethylideneamino)methyl) cyclohexyl)acetic acid (**7a**)

The general experimental procedure described above afforded **7a**, and the product obtained from gabapentin (**3**) (1.72 g, 0.01 mol) and 1-(4-ethoxyphenyl)ethanone (**5a**) (1.65 g, 0.01 mol). FT-IR (KBr, cm^{-1}) *v*: 3075 (C–H), 2924 (O–H), 1699 (C=N), 1461 (C=C), 1091 (C–N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.1 (s, br, 1H, OH), 7.57–7.41 (d, 2H, Ar–H), 6.91–6.43 (d, 2H, Ar–H), 3.75 (q, 2H, CH_2), 2.76 (s, 2H, CH_2), 2.29 (s, 2H, CH_2), 2.34 (t, 3H, CH_3), 2.14 (s, 3H, CH_3), 1.37–1.35 (m, 10H, cyclohexyl methylenes group). Anal. calcd. for $C_{19}H_{27}NO_3$ (in %): C, 71.89; H, 8.57; N, 4.41. Found: C, 71.85; H, 8.59; N, 4.21.

2-(1-((1-(Pyridin-2-yl)ethylideneamino)methyl) cyclohexyl)acetic acid (**7b**)

The general experimental procedure described above afforded **7b**, and the product obtained from gabapentin (**3**) (1.72 g, 0.01 mol) and 1-(pyridin-2-yl)ethanone (**5b**) (1.22 g, 0.01 mol). FT-IR (KBr, cm^{-1}) *v*: 3083 (C–H), 2924 (O–H), 1706 (C=N), 1461 (C=C), 1068 (C–N). ^{1}H NMR (DMSO-d_6) δ: 11.31 (s, br, 1H, OH), 8.73 (d, 1H, pyridine-H), 8.01 (d, 1H, pyridine-H), 7.81 (t, 1H, pyridine-H), 7.63 (t, 1H, pyridine-H), 2.78 (s, 2H, CH_2), 2.32 (s, 2H, CH_2), 2.14 (s, 3H, CH_3), 1.38–1.34 (m, 10H, cyclohexyl methylenes group). Anal. calcd. for $C_{16}H_{22}N_2O_2$ (in %): C, 70.04; H, 8.08; N, 10.21. Found: C, 70.21; H, 8.22; N, 10.44.

2-(1-((1,3-Acetylbenzonitrilethylideneamino) methyl)cyclohexyl)acetic acid (**7c**)

The general experimental procedure described above afforded **7c**, and the product obtained from gabapentin (**3**) (1.72 g, 0.01 mol) and 3-acetylbenzonitrile (**5c**) (1.46 g, 0.01 mol). FT-IR (KBr, cm^{-1}) *v*: 3056 (C–H), 2924 (O–H), 2360 (C$\equiv$N), 1699 (C=N), 1461 (C=C), 1078 (C–N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.3 (s, br, 1H, OH), 8.37 (s, 1H, Ar–H), 8.22 (d, 1H, Ar–H), 8.09 (d, 1H, Ar–H), 7.73 (t, 1H, Ar–H), 2.78 (s, 2H, CH_2), 2.32 (s, 2H, CH_2), 2.15 (s, 3H, CH_3), 1.38–1.35 (m, 10H, cyclohexyl methylenes group). Anal. calcd. for $C_{18}H_{22}N_2O_2$ (in %): C, 72.46; H, 7.43; N, 9.39. Found: C, 72.31; H, 7.21; N, 9.58.

2-(1-((1-(1H-indol-3-yl)ethylideneamino)methyl) cyclohexyl)acetic acid (**7d**)

The general experimental procedure described above afforded **7d**, and the product obtained from gabapentin (**3**) (1.72 g, 0.01 mol) and 1-(1H-indol-3-yl)ethanone (**5d**) (1.60 g, 0.01 mol). FT-IR (KBr, cm^{-1}) *v*: 3058 (C–H), 2923 (O–H), 1698 (C=N), 1462 (C=C), 1083 (C–O). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.2 (s, br, 1H, OH), 9.27 (s, 1H, NH), 7.52 (d, 1H, Ar–H), 7.43 (d, 1H, Ar–H), 7.31 (s, 1H, pyrrole-H), 7.13 (t, 1H, Ar–H), 7.10 (t, 1H, Ar–H), 2.75 (s, 2H, CH_2), 2.28 (s, 2H, CH_2), 2.13 (s, 3H, CH_3), 1.36–1.32 (m, 10H, cyclohexyl methylenes group). Anal. calcd. for $C_{19}H_{24}N_2O_2$ (in %): C, 73.05; H, 7.74; N, 8.97. Found: C, 72.81; H, 7.92; N, 9.12.

2-(1-((1-(Pyridin-3-yl)ethylideneamino)methyl) cyclohexyl)acetic acid (**7e**)

The general experimental procedure described above afforded **7e**, and the product obtained from gabapentin (**3**) (1.72 g, 0.01 mol) and 1-(pyridin-3-yl)ethanone (**5e**) (1.22 g, 0.01 mol). FT-IR (KBr, cm^{-1}) *v*: 3012 (C–H), 2924 (O–H), 1698 (C=N), 1461 (C=C), 1088 (C–N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.3 (s, br, 1H, OH), 9.21 (s, 1H, pyridine-H), 8.81 (d, 1H, pyridine-H), 8.37 (d, 1H, pyridine-H), 7.51 (t, 1H, pyridine-H), 2.77 (s, 2H, CH_2), 2.29 (s, 2H, CH_2), 2.14 (s, 3H, CH_3), 1.38–1.36 (m, 10H, cyclohexyl methylenes group). Anal. calcd. for $C_{16}H_{22}N_2O_2$ (in %): C, 70.04; H, 8.08; N, 10.21. Found: C, 70.31; H, 8.21; N, 10.38.

2-(1-((1-(Naphthalen-2-yl)ethylideneamino)methyl) cyclohexyl)acetic acid (**7f**)

The general experimental procedure described above afforded **7f**, and the product obtained from gabapentin (**3**) (1.72 g, 0.01 mol) and 1-(naphthalen-2-yl)ethanone (**5f**) (1.71 g, 0.01 mol). FT-IR (KBr, cm^{-1}) *v*: 3062 (C–H), 2923 (O–H), 1699 (C=N), 1461 (C=C), 1075 (C–N). ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 11.1 (s, br, 1H, OH), 8.50 (s, 1H, Ar–H), 7.41 (d, 1H, Ar–H), 7.31 (d, 1H, Ar–H), 7.20–7.13 (d, 2H, Ar–H), 7.08–6.96 (t, 2H, Ar–H), 2.79 (s, 2H, CH_2), 2.28 (s, 2H, CH_2), 2.14 (s, 3H, CH_3), 1.37–1.35 (m, 10H, cyclohexyl methylenes group). Anal. calcd. for $C_{21}H_{25}NO_2$ (in %): C, 77.98; H, 7.79; N, 4.33. Found: C, 78.24; H, 7.54; N, 4.52.

Biology

Anticonvulsant evaluation

Animals

Male wistar rats procured from National Institute of Nutrition, Hyderabad (190–220 g) were used in this study. The animals were kept in individual cages for 1 week to acclimatize for the laboratory conditions. They were allowed to free access of water and food.

All the experimental procedures were carried out in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. The study was reviewed and approved by the Institutional Animal Ethics Committee, G Pulla Reddy College of Pharmacy, Hyderabad, India.

Maximal electroshock seizure model (MES)

Maximal electroshock seizure model was used in this study to evaluate the anticonvulsant activity of the compounds on male wistar rats. Seizures were induced in rats by delivering electro shock of 150 mA for 0.2 s by means of a convulsiometer through a pair of ear clip electrodes. The test compounds (100 mg/kg) were administered by oral route in the form of solution (The compounds were dissolved in 1% sodium carboxymethyl cellulose), 30 min before the maximal electroshock seizure test. The animals were observed closely for 2 min. The percentage of inhibition of seizure relative to control was recorded and calculated (Vogel and Vogel, 1997). Phenytoin (100 mg/kg) was used as a standard drug.

Neurotoxicity screening

The minimal motor impairment was measured in mice by the rotorod test. The mice were trained to stay on the accelerating rotorod that rotates at 10 revolutions per minute. The rod diameter was 3.2 cm. Trained animals were administered with the test compounds at dose of 100 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the three trails. Phenytoin was used as a standard drug.

Statistical analysis

In this study, data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnet's test to compare the difference between the groups.

Antioxidant activity

The free radical scavenging activity of the synthesized compounds was studied in vitro by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) assay method (Shih and Ke, 2004). Stock solution of the drug was diluted to different concentrations in the range 100–200 μg/ml in methanol. Methanolic solution of the synthesized compounds (2 ml) was added to 0.003% (w/v) methanol solution of DPPH (1 ml). The mixture was shaken vigorously and allowed to stand for 30 min. Absorbance at 517 nm was determined and the percentage of scavenging activity was calculated. Ascorbic acid was used as the standard drug. The inhibition ratio (I%) of the tested compounds was calculated according to the following equation: $I\% = (Ac - As)/Ac \times 100$, where Ac is the absorbance of the control and As is the absorbance of the sample. The concentration of compounds providing 50% scavenging of DPPH (IC_{50}) was calculated from the plot of percentage inhibition against concentration (μg/ml) (Gulcin *et al*., 2004; Elmastas *et al*., 2006). All tests and analyses were done in triplicate and the results were averaged.

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