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Original Research Article

Synthesis and characterization of some new Schiff base derivatives of gabapentin, and assessment of their antibacterial, antioxidant and anticonvulsant activities

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Abstract

Purpose: *To synthesize and characterize some new gabapentin Schiff base derivatives, and to assess their antibacterial, antioxidant and antiepileptic activities.*

Methods: *Four Schiff base derivatives of gabapentin, termed G1, G2, G3 and G4, were synthesized by condensation with benzoin, vanillin, acetophenone, and benzophenone, respectively. Their chemical identities were established by FTIR, ^{1}H NMR and ^{13}C NMR techniques. The new compounds were screened for antibacterial activity using agar well method, antioxidant activity by DPPH assay, and anticonvulsant activity against pentylenetetrazole (PTZ) induced seizures in mice.*

Results: *All the compounds showed antibacterial activity against the test strains to variable degrees, while the parent drug did not exhibit antibacterial activity. The zones of inhibition of compound G2 against Micrococcus luteus (36.2 ± 1.0 mm) and Serratia marcescens (28.2 ± 1.0 mm), and of compound G4 against Stenotrophomonas maltophilia (36.8 ± 1.0 mm) were larger compared to the standard drug, doxycycline, exhibiting zones of inhibition 28.2 ± 1.3, 28.2 ± 0.9 and 20.0 ± 0.9 mm, respectively. In addition, compounds G1 and G2 possessed significantly greater ($p < 0.05$) radical scavenging activity (82.3 ± 1.8 and 92.3 ± 2.2 %, respectively) than the precursor drug, gabapentin (63.2± 2.6 %). The seizure scores for compounds G1 (0.7 ± 0.06) and G2 (0.9 ± 0.07) were comparable ($p > 0.05$) with gabapentin (0.8 ± 0.06), while compounds G3 and G4 were less active ($p < 0.05$) than gabapentin.*

Conclusion: *Compounds G1 and G2 exhibit good antibacterial and antioxidant activities while retaining the anticonvulsant activity of the parent drug, gabapentin, thus making them suitable candidates for further development for the treatment of neurodegenerative pathologies associated with bacterial infections.*

Keywords: *Gabapentin, Antibacterial, Seizures, Antioxidant, Anticonvulsant*

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INTRODUCTION

Schiff bases, named after chemist Hugo Schiff, are formed when any primary amine reacts with a ketone or aldehyde under controlled conditions. A Schiff base is the nitrogen analogue of a ketone or aldehyde in which the carbonyl functional group (C=O) is substituted by an

azomethine (-N=C-) group [1,2]. Schiff base molecules derived from aromatic ketones, aldehydes and their derivatives are quite stable [3].

Schiff bases have been reported to play many important roles in the medicinal field. They have been reported to possess diversified biological activities including anticonvulsant [4], gastro-protective [5], anti-tuberculosis, anticancer, analgesic, anti-inflammatory, antiplasmodial, antibacterial, antifungal and antioxidant [2] activities. The nitrogen atom in azomethine group of Schiff base derivative carries lone pair of electrons in sp^2 hybridized orbital, which can easily interact with several cellular macromolecules by forming hydrogen bond resulting change in cellular functions and possible altered therapeutic activity of the parent molecule [6].

Schiff bases have well-established broad-spectrum antimicrobial and cytotoxic activities [2,7]. Schiff base compounds derived from neuromodulating drugs, such as clobazam [8], lamotrigine [9] and carbamazepine [10] moieties exhibited pronounced antibacterial activities. Gabapentin or 2-(1-(aminomethyl)cyclohexyl) acetic acid, is structurally similar to γ-aminobutyric acid (GABA), and is recommended for treatment of seizures, chronic pain syndromes and diabetic neuropathy [11]. A Schiff base derivative of gabapentin and copper complex have shown good anticancer activity [12].

Some Schiff base derivatives of gabapentin were assessed for their antioxidant and anticonvulsant potential [4]. In recent years, research on the role of reactive oxygen species in the pathogenesis of various human diseases has indicated that antioxidants may be beneficial in the treatment and prevention of several diseases, including neurodegenerative, cardiovascular, autoimmune and infectious diseases. Oxidative stress is an important etiological factor in epileptic seizures and antioxidants may provide neuroprotection to prevent seizures [13]. Antioxidants may also provide protection against pathological consequences of several infectious diseases [14]. Therefore, antioxidant compounds are gaining increased attention in medical research.

The literature survey revealed that apart from the compound G2 [4], synthesis and antioxidant activities of the compounds G1, G3 and G4 have never been reported. In addition, there are presently no studies reporting the antibacterial and anticonvulsant activities of the synthesized compounds. Keeping the above facts in mind and with an interest in developing new lead compounds possessing antibacterial, antioxidant and antiepileptic activities, we synthesized four Schiff's base compounds from gabapentin (G1-G4). The new synthesized compounds were chemically characterized and their antibacterial, antioxidant and anticonvulsant effects were studied using *in vivo* and *in vitro* models.

EXPERIMENTAL

Chemical and reagents

All the chemicals and reagents used in the present study were of analytical grade. Ethanol, vanillin, acetophenone and acetic acid were purchased from Sigma Chemicals USA. 2,2-diphenyl-1-picrylhydrazyl (DPPH), benzophenone, benzoin, ascorbic acid, diazepam and pentylenetetrazole (PTZ) were obtained from Merck. Gabapentin (96%, USP grade) was obtained from Highnoon laboratories Pvt. Ltd Lahore.

Animals

Swiss mice (20 - 25 g; either sex) were used for the evaluation of anticonvulsant activity. The mice were housed in polycarbonate cages at room temperature (23 - 27 °C) under 12 h light-12 h dark cycles at animal house facility of the Department of Pharmacy, the Islamia University Bahawalpur. The guidelines for care and use of laboratory animals issued by National Research Council [15] were complied with and their use was allowed by the institutional ethical committee.

Preparation of Schiff base derivatives

Ethanol solution of gabapentin (1.0 g in 60 mL) was mixed with equimolar quantity of the available aldehyde or ketones in a 250 mL round bottom flask. Glacial acetic acid (2 mL) was used as catalyst. The mixture was refluxed for 4 - 5 h. The product was obtained by pouring the reaction mixture into ice cold water. After filtration it was crystallized from ethanol [16]. The general chemical reaction is described in Figure 1. The four new Schiff base compounds of gabapentin were synthesized by condensation with benzoin, vanillin, acetophenone and benzophenone with respective names of G1, G2, G3 and G4; their formulas as confirmed in the present study are given in Figure 2.

Chemical characterization

Identification of chemical structures and purity of the synthesized compounds was achieved by

already reported methods [12,17]. The solubility of the derived compounds was determined in various solvents, including water, chloroform, DMSO and ethanol by using ultra-sonicator (Model 30H, Elma Schmidbauer GmbH, Germany). Melting points of the derived compounds were determined by adding a small quantity of synthesized compound in a capillary tube and heating the capillary tube in melting point apparatus (Galen Kemp). Infrared spectrum (4000-400 cm^{-1}) of the synthesized compounds was obtained in KBr pellets by means of FTIR spectrophotometer (Bruker Tensor-27 FTIR spectrometer). ^{1}H NMR and ^{13}C NMR spectra of derived compounds were obtained by Bruker 400 spectrophotometer.

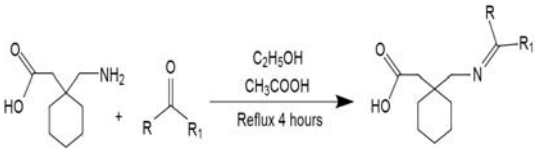

Figure 1: Chemical reaction showing synthesis of gabapentin Schiff base compound

Antimicrobial screening

Antibacterial activity of gabapentin and its derived Schiff base compounds was determined by agar well diffusion method [18] against four strains of Gram positive bacteria (*Bacillus megaterium*, *Bacillus subtilis, Micrococcus luteus* and *Staphylococcus aureus*) and three strains of gram negative bacteria (*Stenotrophomonas maltophilia*, *Serratia marcescens*, and *Escherichia coli*). The Mueller Hinton agar medium in pert plates was sterilized in autoclave and allowed to cool it at about 45°C, 100 µL standard inoculum was aseptically mixed, cooled to 37°C, and 6 mm well were made in the agar plate. The derived compound or the standard drug was dissolved in DMSO to make 0.125, 0.25 and 0.5 mg/mL solution, from which 30 µL of the sample solution was poured in the agar medium wells. The prepared petri dishes were kept at 37°C in incubator for 24 h and zones of inhibition measured in mm. The tests were performed in triplicate. The solvent used did not inhibit bacterial growth (data not shown). Doxycycline was used as a standard drug.

Antioxidant assay

Antioxidant activity of the derivatives was determined by using 2,2-dipheynyl-1-picrylhydrazyl radical (DPPH) assay [19]. The DPPH solution (2.0 ml; 0.2 mg/mL in methanol) and test material solution (1.0 mL; 0.2 mg/mL in methanol) were mixed in a reaction tube and allowed to stand for 30 min at room temperature to complete reaction. When DPPH radical is scavenged, its color is changed from purple to yellow, resulting in a decrease in absorbance at 517 nm. Absorbance of the reaction mixture at 517 nm was taken by using a UV-Visible spectrophotometer. Absorbance of blank sample containing 1.0 ml of methanol and 2.0 mL of DPPH solution was also measured. Inhibition was calculated in terms of scavenging activity (S) as in Eq 1.

$$S\ (\%) = \{(A_b - A_t/A_b)\}100 \ldots\ldots\ldots (1)$$

where A_t and A_b are absorbance of test and blank samples, respectively.

Anticonvulsant activity

Anticonvulsant effects of synthesized compounds were observed *in vivo* by acute intraperitoneal PTZ test. The synthesized compounds were injected into the mice (n = 6) at the doses of 10, 35 and 75 mg/kg, respectively. Thirty min later, mice were injected with 90 mg/kg of PTZ (convulsive dose) and were observed for 60 min for the assessment of PTZ-induced mortality [20].

Once screening in the acute seizure model was done, the next step was the assessment of the effects of the synthesized compounds on PTZ kindling model of epileptogenesis in albino mice [20]. The mice were randomized into four groups (n = 8) and based on results obtained from acute testing, 75 mg/kg doses of the synthesized compounds were selected. All groups except the normal control group received 50 mg/kg sub-convulsive dose of PTZ through intraperitoneal route once on alternate day. The normal control group and the drug control group were given daily dose of 0.25 mL of normal saline and 7.5 mg/kg diazepam, respectively. Similarly, the synthesized compounds were administered once daily to the test groups. The study was carried out for 24 days for the development of epileptogenesis in the PTZ kindling model while a gradual increase and rise in the seizure pattern was observed. After the last treatment dose of PTZ, convulsive score was calculated in each group by using the following criteria: No seizure = 0, initiation of body jerks = 1, manifestation of seizures = 2, generalized seizures with loss of righting reflex = 3, tonic forelimb with loss of righting reflex = 4, and hind limb extension = 5.

Statistical analysis

The results of biological activities are shown as mean or mean ± SEM. Comparison between different groups was made by applying one-way

ANOVA, while p value < 0.05 was taken as level of significance.

RESULTS

Chemical characteristics

The results of chemical characterization of compounds G1, G2, G3 and G4 are given below and their structures are shown in Figure 2.

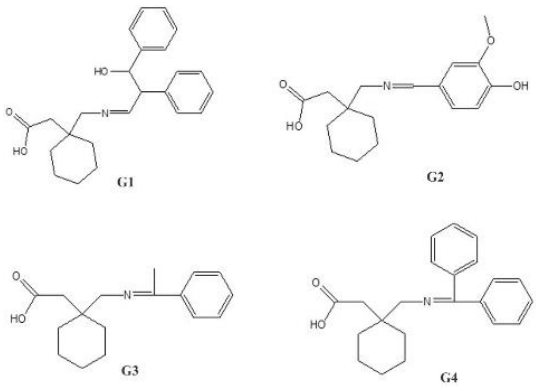

Figure 2: Chemical structures of the synthesized compounds

Spectral characteristics

G1: 2-(1-((3-hydroxy-2,3-diphenylpropylideneamino) methyl) cyclohexyl) acetic acid

Yield 76%; Insoluble in water, but soluble in DMSO, ethanol and chloroform; melting point 172°C-176°C; molecular weight 379.50; molecular formula $C_{24}H_{29}NO_3$; elemental analysis (calculated) C = 75.96, H = 7.70, N = 3.69, O = 12.65. FT-IR v cm^{-1}: 3841 (COOH), 2926 (CH), 1682 (C=O), 1452 (CH=CH), 1657 (C=N), 1253 (C−O) and 3649 (OH).

^{1}H NMR (CDCL3, ppm) δ: 1.38-1.49 m, 1.43-1.46 m, 1.24-1.49 t (−CH_2−), 7.1-7.2 t, 7.0-7.08 t, 7.0-7.19 d, 7.4-7.5 d, 2.0-2.15 s (−CH−), 10.15 s (−OH).

^{13}C NMR (CDCL3, ppm) δ: 22.9 (C-1), 22.9 (C-3/5), 2 26.0 (C-4), 55.8 (C-7), 51.6 (C-8), 162.9 (C-9), 161 (C-10), 36.84 (C-11), 140 (C-12), 123 (C-13/17), 123 (C-14/15), 123 (C-15/16), 77.2 (C-18), 128 (C-19/20), 128 (C-21/22), 128 (C-23).

G2: 2-(1-((4-hydroxy-3-methoxybenzylidene-amino) methyl) cyclohexyl) acetic acid

Yield 75%; Insoluble in water, but soluble in DMSO, ethanol and chloroform; melting point 166°C-169°C; molecular weight 305.37; molecular formula $C_{17}H_{23}NO_4$; elemental analysis (calculated): C = 66.86, H = 7.59, N = 4.59, O = 20.96.

FT-IR v cm^{-1}: 3841 (COOH), 2869 (CH), 1682 (C=O), 1452 (CH=CH), 1682 (C=N), 1253 (C−O), ^{1}H NMR (CDCL3, ppm) δ: 1.40-1.41 m, 1.42-1.43 t, 3.11-3.12 s (−CH_2−), 6.91-7.0 d, 6.8-6.9 s (−CH−), 9.74 s (−OH), 9.74 s (−NH_2), 3.76 s (-CH3-)

^{13}C NMR (CDCL3, ppm) δ: 22.5 (C-1), 36.49 (C-2), 22.5 (C-3), 25.0 (C-4), 36.37(C-6), 55.8 (C-7), 43 (C-8), 177 (C-9), 160 (C-10), 133 (C-11), 114 (C-12), 151 (C-13), 148 (C-14), 117 (C-15), 122 (C-16), 55 (C-17).

G3: 2-(1-((1-phenylethylideneamino) methyl) cyclohexyl) acetic acid

Yield 78%. Insoluble in water, but soluble in DMSO, ethanol and chloroform; melting point 164°C-168°C; molecular weight 273.38, molecular formula $C_{17}H_{23}NO_2$; elemental analysis (calculated): C = 74.69, H = 8.48, N = 5.12, O = 11.70.

FT-IR v cm^{-1}: 3841 (COOH), 2869 (CH), 1682 (C=O), 1452 (CH=CH), 1682 (C=N), 1253 (C−O), ^{1}H NMR (CDCL3, ppm) δ: 1.41-1.42 m, 1.40-1.41 m, 1.2-1.4 t, 2.1-2.5 s, 3.0-3.1 s (−CH_2−), 7.2-7.3 t, 7.5-7.9 d (−CH−), 8.37 s (−OH).

^{13}C NMR (CDCL3, ppm) δ: 22.8 (C-1/3), 36.7 (C-2), 26.0 (C-4), 22.8 (C-5), 36.7 (C-6), 53 (C-7), 43.2 (C-8), 177 (C-9), 178 (C-10), 137 (C-11), 128 (C-12), 126 (C-13), 133 (C-14), 128 (C-15), 128 (C-16), 21 (C-17).

G4: 2-(1-((diphenylmethyleneamino) methyl) cyclohexyl) acetic acid

Yield 73%; Insoluble in water, but soluble in DMSO, ethanol and chloroform; melting point 174°C-176°C; molecular weight 335.50; molecular formula $C_{22}H_{25}NO_2$; elemental analysis (calculated): C = 78.77, H = 7.51, N = 4.18, O = 9.54. FT-IR v cm^{-1}: 3841 (COOH), 2869 (CH), 1682 (C=O), 1452 (CH=CH), 1682 (C=N), 1253 (C−O) ^{1}H NMR (CDCL3, ppm) δ: 1.42-1.44 m, 1.44-1.48 t (−CH_2−), 7.46-7.48 m, 7.24-7.2 t, 7.70.0-7.76 d, 7.6-7.7 d (−CH−), 17.80-7.81s (−OH).

^{13}C NMR (CDCL3, ppm) δ: 22.8 (C-1/5), 36.8 (C-2), 22.8 (C-3), 25.6 (C-4), 36.8 (C-6), 53.6 (C-7), 43.19 (C-8), 178.19 (C-9/10), 137.56 (C-11), 128.28 (C-12/13), 130.4 (C-14), 128.8 (C-15/16), 137.56 (C-17), 128.28 (C-18/19), 132.5 (C-20), 128 (C-21/22).

Antibacterial activity

Antibacterial activity of gabapentin, its Schiff base derivatives and doxycycline were studied using the agar well method at three doses -3.75, 7.5 and 15.0 µg /well and zones of bacterial growth inhibition were measured in mm. The parent drug, gabapentin (G0), gave negative results as it did not inhibit any of the tested organism. Results of antibacterial activity of the tested compounds against gram positive bacterial strains are summarized in Table 1. Effects of the tested compounds against growth of Gram negative bacterial strains are presented in Table 2. All the Schiff base derivatives and the standard drug inhibited bacterial growth to variable extent.

Table 1: Antibacterial activity of gabapentin (G0), its Schiff base derivatives (G1, G2, G3 and G4) and doxycycline against gram positive bacterial strains

Compound	Dose µg/well	Zones of inhibition (mm)			
		Bacillus megaterium	***Bacillus subtilis***	***Micrococcus luteus***	***Staphylococcus aureus***
G0	3.75	-	-	-	-
	7.50	-	-	-	-
	15.0	-	-	-	-
G1	3.75	11.3 ± 0.9	18.2 ± 0.7	14.0 ± 1.2	18.0 ± 1.2
	7.50	14.0 ± 1.2	27.0 ± 0.9	27.0 ± 0.9	27.2 ± 1.3
	15.0	25.0 ± 0.9	33.0 ± 1.2	35.4 ± 1.5	31.8 ± 0.8
G2	3.75	12.3 ± 0.9	20.0 ± 0.3	16.2 ± 1.3	14.0 ± 0.6
	7.50	14.2 ± 0.5	32.2 ± 1.0	29.0 ± 0.9	24.3 ± 1.5
	15.0	27.0 ± 0.9	37.0 ± 0.3	36.2 ± 1.0	34.0 ± 0.3
G3	3.75	12.0 ± 0.6	18.2 ± 0.7	-	12.0 ± 1.2
	7.50	15.2 ± 1.3	20.0 ± 1.2	11.0 ± 1.2	16.2 ± 0.7
	15.0	19.0 ± 0.6	28.2 ± 0.7	20.2 ± 0.7	24.8 ± 0.7
G4	3.75	12.2 ± 0.7	16.0 ± 0.6	15.2 ± 1.3	12.0 ± 0.6
	7.50	18.0 ± 0.9	27.0 ± 0.9	21.0 ± 0.3	20.2 ± 1.3
	15.0	22.2 ± 0.7	33.8 ± 1.0	26.2 ± 0.7	30.0 ± 1.2
Doxycycline	3.75	10.0 ± 0.6	13.0 ± 1.2	12.2 ± 0.7	16.0 ± 0.6
	7.50	15.0 ± 1.2	20.2 ± 0.7	18.2 ± 0.7	25.0 ± 1.2
	15.0	24.8 ± 1.0	32.0 ± 1.2	28.2 ± 1.3	30.0 ± 0.9

Values are mean ± SEM of three observations. (-) indicates no activity, as no inhibition zone noted around the well

Table 2: Antibacterial activity of gabapentin (G0), its Schiff base derivatives (G1, G2, G3 and G4) and doxycycline against gram negative bacterial strains

Compound	Dose µg /well	Zones of inhibition (mm)		
		Stenotrophomonas maltophilia	***Serratia marcescens***	***Escherichia coli***
G0	3.75	-	-	-
	7.50	-	-	-
	15.0	-	-	-
G1	3.75	10.0 ± 1.2	9.0 ± 1.5	19.0 ± 0.6
	7.50	16.2 ± 0.8	17.2 ± 0.7	29.2 ± 0.4
	15.0	25.2 ± 0.5	25.5 ± 1.0	38.2 ± 0.4
G2	3.75	18.0 ± 0.6	10.0 ± 0.6	12.2 ± 0.7
	7.50	21.2 ± 0.7	21.2 ± 0.7	23.2 ± 0.7
	15.0	32.2 ± 1.0	28.2 ± 1.0	29.0 ± 0.9
G3	3.75	18.0 ± 0.6	7.0 ± 0.6	17.2 ± 0.7
	7.50	34.0 ± 0.6	12.0 ± 1.2	24.2 ± 0.4
	15.0	39.7 ± 1.5	21.2 ± 0.7	29.7 ± 1.2
G4	3.75	15.2 ± 1.3	10.0 ± 0.6	11.0 ± 0.6
	7.50	29.0 ± 0.6	18.0 ± 1.2	21.2 ± 0.7
	15.0	36.8 ± 1.0	24.2 ± 0.7	29.8 ± 1.0
Doxycycline	3.75	16.2 ± 0.7	11.2 ± 0.7	15.0 ± 0.6
	7.50	21.2 ± 0.7	15.2 ± 0.7	24.2 ± 0.7
	15.0	28.0 ± 0.9	20.0 ± 0.9	35.2 ± 1.0

Values are mean ± SEM of three observations. (-) indicates no activity, as no inhibition zone noted around the well

Antioxidant assay

The results of DPPH radical scavenging potential of gabapentin and its derivatives are shows Table 3. The compounds G1 and G2 caused greater scavenging, and G3 and G4 caused less scavenging of DPPH free radicals as compared to the parent drug (G0). Ascorbic acid was used as reference standard.

Table 3: The DPPH radical scavenging activity of gabapentin (G0), its derivatives (G1-G4) and ascorbic acid

Compound (200 µg/ml)	DPPH radical scavenging (%)
G0	63.2 ± 2.6
G1	82.3 ± 1.8*
G2	92.3 ± 2.2*
G3	58.4 ± 2.9
G4	32.4 ± 1.6*
Ascorbic acid	96.0 ± 1.2*

Values are mean ± SEM of three observations. *indicates significant (p < 0.05) difference, as compared to G0, as analyzed by one-way ANOVA

Anticonvulsant activity

Administration of PTZ to mice in saline treated control group caused convulsions with 100% morality in mice. All the test compounds showed dose related protection from convulsive deaths in mice, as shown in Table 4.

In 24 days PTZ-induced chemical kindling model, administration of PTZ at 50 mg/kg did not produce seizures on first day of the treatment in either the control or in the test groups. At the end of experiment, average convulsive score for the positive control group was found greater than 4. Other test groups showed protection from PTZ-seizures to various extent as summarized in Table 5 and Figure 3.

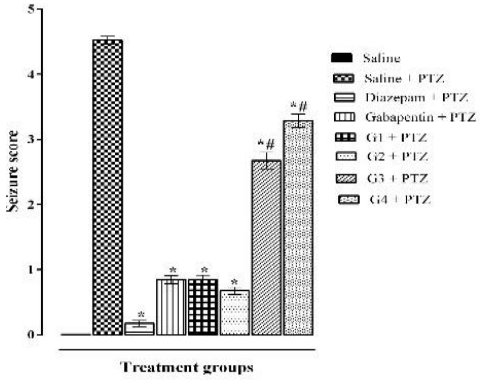

Figure 3: Anticonvulsant activity of different drugs and synthesized compounds in PTZ-evoked chemical kindling model of epileptogenesis in mice. Values are mean ± SEM of eight observations. * indicates significant (p < 0.05) difference from the group received saline + PTZ and # indicates significant difference (p < 0.05) from the group received gabapentin + PTZ, as analyzed by one-way ANOVA

DISCUSSION

The synthesized Schiff bases G1, G2, G3 and G4 were subjected to solubility and melting point determination. The structures of all the compounds were established on the basis of FTIR, ^{1}HNMR and ^{13}CNMR spectral data.

Antibacterial activity of gabapentin, its derived compounds and a standard drug (doxycycline) was studied at three doses by the agar well method, and zones of growth inhibition were measured for the assessment of antibacterial activity of the tested compounds. The parent drug, gabapentin, did not show inhibition of bacterial growth. All the synthesized compounds were found to be active against gram positive and gram-negative bacterial strains.

Table 4: Effects of different compounds upon mortality in mice following PTZ-evoked convulsions

Group/Treatment	Dose	No. of animals with convulsion death	Mortality (%)
Saline	0.25 ml/kg	6/6	100
G1	10 mg/kg	5/6	83
	35 mg/kg	4/6	66
	75 mg/kg	1/6	16
G2	10 mg/kg	5/6	83
	35 mg/kg	4/6	66
	75 mg/kg	1/6	16
G3	10 mg/kg	5/6	83
	35 mg/kg	3/6	50
	75 mg/kg	2/6	33
G4	10 mg/kg	5/6	83
	35 mg/kg	3/6	50
	75 mg/kg	1/6	16

Table 5: Anticonvulsant activity of different drugs and synthesized compounds in PTZ-evoked chemical kindling model of epileptogenesis in mice

Group	Treatment (dose)	Seizure score
Normal control	Normal Saline (0.25 ml/kg)	0
Positive control	Saline (0.25 ml/kg) + PTZ (50 mg/kg)	4.5 ± 0.06
Standard control -1	Diazepam (7.5 mg/kg) + PTZ (50mg/kg)	0.1 ± 0.05*
Standard control -2	Gabapentin (75 mg/kg) + PTZ (50mg/kg)	0.8 ± 0.06*
G1	G1 (75 mg/kg) + PTZ (50 mg/kg)	0.7 ± 0.06*
G2	G2 (75mg/kg) + PTZ (50mg/kg)	0.9 ± 0.07*
G3	G3 (75mg/kg) + PTZ (50mg/kg)	2.7 ± 0.13*#
G4	G4 (7.5mg/kg) + PTZ (50mg/kg)	3.3 ± 0.10*#

Values are mean ± SEM of eight observations. * indicates significant ($p < 0.05$) difference from the positive control group and # indicates significant difference ($p < 0.05$) from the standard control 2 group, as analyzed by one-way ANOVA

Compound G1 showed greater activity against *Micrococcus luteus*, while it was found to possess comparable activity with doxycycline against the *Stenotrophomonas maltophilia, Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus, Serratia marcescens* and *Escherichia coli*.

Compound G2 exhibited greater activity against *Micrococcus luteus* and *Serratia marcescens* when compared to the standard drug. Compound G4 showed larger zones of inhibition against *Stenotrophomonas maltophilia* when compared to the standard drug. Also, compound G3 exhibited larger inhibitory zones against *Stenotrophomonas maltophilia* and *Serratia marcescens* when compared to doxycycline. The mechanism of antibacterial activity of the Schiff base derivatives may involve hydrogen bonding between azomethine group of Schiff bases and various cellular molecules, resulting in an impairment of normal cellular functions [6,7]. Previous studies suggest that Schiff bases have well established biocidal activities [22], so the synthesized Schiff bases may be used for further studies of neurological diseases associated with bacterial infections.

DPPH is a stable radical, which is conveniently used for estimating antioxidant activity of chemicals. Compounds G1 and G2 showed significantly greater DPPH radical scavenging activity (82% and 92%, respectively) compared to gabapentin (63%). The enhanced antioxidant activity of G1 and G2 may be attributed to presence of phenolic hydroxyl and methoxy groups in the molecule [23]. Previous studies suggest that enhanced oxidative stress in brain tissue was involved in the induction of seizures, and PTZ induced seizures are associated with enhanced generation of free radicals in cerebral tissue of animals [24].

In addition, antioxidants are important in attenuating PTZ evoked seizures [25]. Therefore, we selected PTZ induced seizures in mice to study anticonvulsant activity of the synthesized compounds. All the compounds significantly decreased PTZ evoked seizures in mice compared to the saline treated control. Compounds G1 and G2 retained the potent anticonvulsant activity of gabapentin. However, the anticonvulsant activities of G3 and G4 were significantly less compared to gabapentin in the tested model. The proposed pharmacophore model suggests that lipophilic domain, hydrogen donor acceptor site and electron donor system are important for anticonvulsant activity of a molecule [4]. The parent drug and all the synthesized compounds contain these structural elements in their molecules.

However, compounds G3 and G4 have aromatic side chains at azomethine group, which might affect the electron donating activity, resulting decrease in anticonvulsant activity of these compounds as compared to gabapentin. Despite substitution at azomethine group, the compounds G1 and G2 retained anticonvulsant activity of gabapentin, which may be attributed to presence of additional hydroxyl groups, possibly eliminating the free radicals, which are main cause of neurodegenerative pathologies and seizures. Further studies on safety and other models of epilepsy are recommended. The present study may guide toward future development of anti-bacterial and neuromodulating drugs with additional antioxidant activity.

CONCLUSION

The newly synthesized gabapentin derivatives possess appreciable antibacterial and antioxidant activities relative to doxycycline and ascorbic acid, respectively. The anticonvulsant activity of G1 and G2 is comparable to that of the parent drug, gabapentin. Further investigations are recommended to interpret their mechanisms of actions and to demonstrate safety and efficacy in other experimental models.

DECLARATIONS

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. The authors of this paper declare that this work was done by them and all liabilities pertaining to claims relating to the content of this article will be borne by them. MF Saleem, MA Khan and I Ahmad conceptualized the study and contributed in manuscript writing. MF Saleem carried out experimental work, collected data and interpreted the results under the supervision of MA Khan and I Ahmad. N Aslam and U Khurshid contributed in results analysis and manuscript writing. All authors read and approved the manuscript.

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