Arabian Journal of Chemistry (2016) 9, S342–S347

King Saud University

Arabian Journal of Chemistry

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ORIGINAL ARTICLE

Synthesis, characterization and pharmacological screening of novel benzimidazole derivatives

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Received 8 February 2011; accepted 26 April 2011
Available online 30 April 2011

KEYWORDS

Benzimidazole;
Anticonvulsant activity;
Pharmacological screening

Abstract In present study *o*-phenylenediamine and phenoxyacetic acid were used as starting material through series of steps 2-[2-(phenoxymethyl)-1*H*-benzimidazol-1-yl]acetohydrazide **5** was obtained. Various derivatives of 2-[2-(phenoxymethyl)-1*H*-benzimidazol-1-yl]-N′-[(Z)-phenylmethylidene]acetohydrazide and some compounds containing oxadiazole bearing benzimidazole were synthesized by using various aromatic aldehyde, cyanogens bromide and carbon disulfide/potassium hydroxide. These were elucidated by IR, NMR and elemental analysis and their *in vivo* anticonvulsant screening was performed using MES and scPTZ. Two compounds **7g** and **j** were found to be potent in both the screens and their protective index was found to be better than standard drugs used.

1. Introduction

All the heterocyclic compounds are of great interest in pharmaceutical chemistry. Out of these heterocyclic compounds the benzfused heterocyclic compound, i.e. benzimidazole and its derivatives have wide variety of biological activities like antimicrobial activity (Kumar et al., 2006; Afaf et al., 2000; kazimierczuk et al., 2002; Shetgiri and Kokitkar, 2001; Ansari and Lal, 2009), antiinflammatory-analgesic (Khan and Nandan, 1997; Evans et al., 1996; Taha, 2005), anticancer (Demirayak et al., 2002), CNS depressant (Sharma et al., 1999), androgen receptor antagonist (Raymond et al., 2007), antitubercular (Kaghthara et al., 1999; Foks et al., 2006) and anticonvulsant (Chmirri et al., 1989, 2002; Vostrova et al., 1986; Singh, 1969, 1970). In addition the benzimidazole have played a very important role in the development of theory in heterocyclic chemistry and also extensively in organic synthesis. Benzimidazole nucleus is present in vitamin-B_{12} (Merck index 2001), albendazole, mebendzole and thiabendazole. In this present study some novel derivatives of schiff bases containing benzimidazole nucleus and oxadiazole bearing benzimidazole

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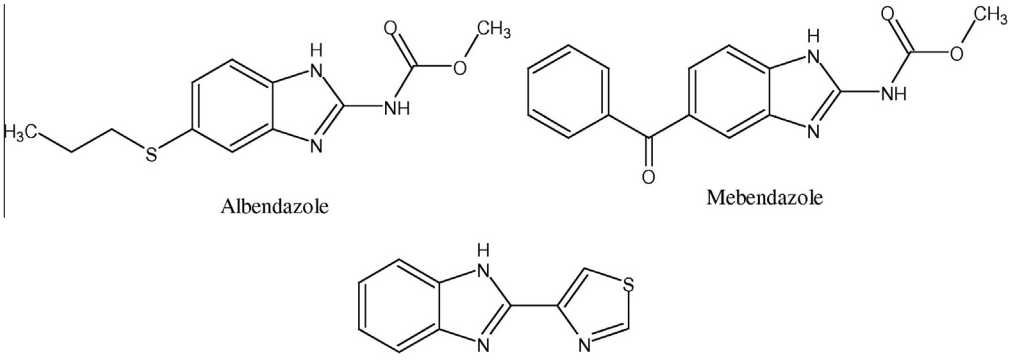
Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

http://dx.doi.org/10.1016/j.arabjc.2011.04.013

derivatives have been synthesized and their antimicrobial and anticonvulsant activity have been established.



2. Experimental

2.1. Instrumentation

The entire chemical reagents which are used in the study were procured locally. The completion of reaction is monitored thin layer chromatography (TLC) using toluene:ethyl acetate:formic acid (5:4:1) and benzene:acetone (9:1) as solvent system. The product is purified by recrystallisation and purity of the compounds was checked by thin layer chromatography (TLC) using silica gel G plates (Merck). The spots were developed in iodine chamber and viewed under UV lamp. Melting points were determined in an open capillary using melting point apparatus and are uncorrected. The proton magnetic resonance (^{1}H NMR) spectra were recorded on a Brucker 300 MHz instrument in DMSO-d_6 using tetramethylsilane as internal standard. The infrared spectra of compounds were recorded in KBr on a Bio Rad FTIR Spectrophotometer.

2.1.1. Procedure for the synthesis of 2-(phenoxymethyl)-1H-benzimidazole **(3)**

A mixture of *o*-phenylenediamine **1** (0.05 mol; 5.40 g) and phenoxyacetic acid **2** (0.05 mol; 7.60 g) in round bottom flask and refluxed in 4 N HCl for 3 h on water bath. After completion of the reaction, the solution was poured onto crushed ice, NaOH solution was added drop wise to neutralize and the resulting solid was filtered, washed with cold water, dried and recrystallized. Yield 80%; m.p. 160–164 °C, IR (KBr) cm^{-1}: 1240 (C—O), 1540 (C═N), 3450 (N—H): ^{1}H NMR (DMSO-d_6) δ ppm: 5.29 (s, 2H, OCH_2), 6.85–7.70 (m, 9H, aromatic), 12.31 (s, 1H, NH). Anal. Cacd for $C_{14}H_{12}N_2O$: C, 74.98; H, 5.39; N, 12.49; O, 7.13. Found: C, 75.0; H, 5.35; N, 12.45; O, 7.10%.

2.1.2. Procedure for the synthesis of ethyl [2-(phenoxymethyl)-1H-benzimidazol-1-yl] acetate **(4)**

To a mixture of 2-(phenoxymethyl)-1*H*-benzimidazole **3** (0.05 mol; 11.2 g) in dry acetone (100 ml), and potassium carbonate (4 g), ethylchloro acetate was added drop wise at room temperature for a period of 20–30 min. The reaction mixture was stirred at room temperature for 10–15 h. The solid thus obtained was filtered off and filtrate was concentrated under reduced pressure. Yield 70%; m.p. 88–92 °C, IR (KBr) cm^{-1}: 1240 (C—O), 1540 (C═N), 1645 (C═O): ^{1}H NMR (DMSO-d_6) δ ppm: 5.29 (s, 2H, OCH_2), 6.70–7.83 (m, 9H, aromatic), 4.17 (m, 2H, CH_2), 1.65 (m, 3H, CH_3). Anal. Cacd for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.85; N, 9.03; O, 15.47. Found: C, 69.65; H, 5.82; N, 9.01; O, 15.42%.

2.1.3. Procedure for the synthesis of 2-[2-(phenoxymethyl)-1H-benzimidazol-1-yl]aceto hydrazide **(5)**

To a mixture of ethyl [2-(phenoxymethyl)-1*H*-benzimidazol-1-yl]acetate **4** (0.01 mol; 3.10 g) and hydrazine hydrate 98% (0.01 mol; 0.49 ml) in alcohol was added and the reaction mixture was refluxed for 4 h. the reaction mixture was cooled and the solid so obtained was filtered, washed with cold water and recrystallized from ethanol. Yield 80%; m.p. 178–180 °C, IR (KBr) cm^{-1}: 1030 (N—N), 1242 (C—O), 1600 (C═N), 1656 (C═O), 3034 (CH—Ar), 3287 (N—H); ^{1}H NMR (DMSO-d_6) δ ppm: 2.52 (s, 1H, NH_2), 4.90 (s, 2H, CH_2), 5.48 (s, 2H, OCH_2), 6.92–7.69 (m, 9H, aroamtic), 9.43 (s, 1H, CONH). Anal. Cacd for $C_{16}H_{16}N_4O_2$: C, 64.85; H, 5.44; N, 18.91; O, 10.80. Found: C, 64.86; H, 5.42; N, 18.89; O, 10.77%.

2.1.3.1. Procedure for the synthesis of 5-{[2-(phenoxymethyl)-1H-benzimidazol-1-yl]methyl}-1,3,4-oxadiazol-2-amine **(6)**.

A methanolic solution of 2-[2-(phenoxymethyl)-1*H*-benzimidazol-1-yl]acetohydrazide **5** (0.0016 mol; 0.4938 g) and cyanogen bromide (0.03 mol; 3.15 g) was refluxed approximately for 5 h. After completion of the reaction, the reaction mixture was cooled at room temperature and poured onto crushed ice. On basification with sodium bicarbonate (5%), a solid mass so separated was washed with water and recrystallized from ethanol. Yield 75%; m.p. 80–84 °C, IR (KBr) cm^{-1}: 1022 (N—N), 1240 (C—O), 1600 (C═N), 3063 (CH—Ar), 3144 (N—H). ^{1}H NMR (DMSO-d_6) δ ppm: 3.63 (s 2H, NH_2), 5.29 (s, 2H, CH_2), 5.39 (s, 2H, OCH_2), 6.94–7.68 (m, 7H, aromatic). Anal. Cacd for $C_{17}H_{15}N_5O_2$: C, 63.54; H, 4.71; N, 21.79; O, 9.96. Found: C, 63.51; H, 4.67; N, 22.79; O, 9.97%.

2.1.4. General procedure for the Synthesis of 2-[2-(phenoxymethyl)-1H-benzimidazol-1-yl]-N'-[substituted phenyl methylidene]acetohydrazide **(7a–j)**

A mixture of 2-[2-(phenoxymethyl)-1*H*-benzimidazol-1-yl]acetohydrazide **(5)** (0.0025 mol; 0.738 g) and benzaldehyde

and its derivative (0.0025 mol) in dimethyl formamide (10 ml) was refluxed for 5 h. After completion of the reaction, the reaction mixture was concentrated, cooled and poured in ice cold water, the precipitate so formed was filtered, dried and recrystallized to give the desired compound.

2.1.4.1. 2-[2-(Phenoxymethyl)-1H-benzimidazol-1-yl]-N'-[(Z)-phenylmethylidene]acetohydrazide ***(7a)***. Yield 70% (Methanol); m.p. 184–188 °C. IR (KBr) cm^{-1}: 1083 (N—N), 1237 (C—O), 1731 (C═N), 3107 (CH—Ar). ^1H NMR (DMSO-d_6) δ ppm: 5.39 (s, 2H, CH_2), 5.61 (s, 2H, OCH_2), 6.91–7.72 (m, 13H, aromatic), 8.21 (s, 1H, CH), 11.79 (s, 1H, CONH). Anal. Cacd $C_{23}H_{20}N_4O_2$: C, 71.86; H, 5.24; N, 14.57; O, 8.32. Found: C, 71.85; H, 5.20; N, 14.55; O, 8.33%.

2.1.4.2. N′-[(Z)-(2-chlorophenyl)methylidene]-2-[2-(phenoxymethyl)-1H-benzimidazol-1-yl]acetohydrazide ***(7b)***. Yield 65% (Methanol); m.p. 174–176 °C. IR (KBr) cm^{-1}: 1081 (N—N), 1242 (C—O), 1731 (C═N), 3115 (CH—Ar), 712 (2-Cl). ^1H NMR (DMSO-d_6) δ ppm: 4.98 (s, 2H, CH_2), 5.31 (s, 2H, OCH_2), 6.83–7.67 (m, 13H, aromatic), 8.01 (s, 1H, CH), 11.09 (s, 1H, CONH). Anal. Cacd for $C_{23}H_{19}ClN_4O_2$: C, 65.95; H, 4.57; N, 13.38; O, 7.64. Found: C, 65.92; H, 4.57; N, 13.28; O, 7.59%.

2.1.4.3. N′-[(Z)-(4-chlorophenyl)methylidene]-2-[2-(phenoxymethyl)-1H-benzimidazol-1-yl]acetohydrazide ***(7c)***. Yield 72% (Methanol); m.p. 220–224 °C. IR (KBr) cm^{-1}: 742 (C—Cl), 1243 (C—O), 1599 (N═C), 1684 (C═O), 3056 (CH—Ar), 3278 (N—H). ^1H NMR (DMSO-d_6) δ ppm: 5.38 (s, 2H, CH_2), 5.61 (s, 2H, OCH_2), 6.91–7.77 (m, 13H, aromatic), 8.02 (s, 1H, CH); 11.84 (s, 1H, CONH). Anal. Cacd for $C_{23}H_{19}ClN_4O_2$: C, 65.95; H, 4.57; N, 13.38; O, 7.64. Found: C, 65.90; H, 4.57; N, 13.31; O, 7.65%.

2.1.4.4. N′-[(Z)-(4-methoxyphenyl)methylidene]-2-[2-(phenoxymethyl)-1H-benzimidazol-1-yl]acetohydrazide ***(7d)***. Yield 70% (Methanol); m.p. 288–192 °C. IR (KBr) cm^{-1}: 1257 (C—O), 1608 (N═C), 1680 (C═O), 3071 (CH—Ar), 3203 (N—H). ^1H NMR (DMSO-d_6) δ ppm: 3.80 (s, 3H, OCH_3), 5.38 (s, 2H, CH_2), 5.59 (s, 2H, OCH_2), 6.89–7.59 (m, 13H, aromatic), 7.98 (s, 1H, CH), 11.66 (s, 1H, CONH). Anal. Cacd for $C_{24}H_{22}N_4O_3$: C 69.55; H, 5.35; N, 13.52; O,11.58. Found: C 69.58; H, 5.33; N, 13.49; O,11.60%.

2.1.4.5. N′-[(Z)-(3-methoxyphenyl)methylidene]-2-[2-(phenoxymethyl)-1H-benzimidazol-1-yl]acetohydrazide ***(7e)***. Yield 63% (Methanol); m.p. 188–190 °C. IR (KBr) cm^{-1}: 1251 (C—O), 1613 (N═C), 1665 (C═O), 3098 (CH—Ar), 3213 (N—H). ^1H NMR (DMSO-d_6) δ ppm: 3.69 (s, 3H, OCH_3), 4.59 (s, 2H, CH_2), 5.23 (s, 2H, OCH_2), 6.71–7.70 (m,13H, aromatic), 8.01 (s, 1H, CH), 9.66 (s, 1H, CONH). Anal. Cacd for $C_{24}H_{22}N_4O_3$: C, 69.55; H, 5.35; N, 13.52; O, 11.58. Found: C, 69.54; H, 5.33; N, 13.49; O, 11.60%.

2.1.4.6. N′-[(Z)-(3,4-dimethoxyphenyl)methylidene]-2-[2-(phenoxymethyl)-1H-benzimida zol-1-yl]acetohydrazide ***(7f)***. Yield 79% (Methanol); m.p. 98–100 °C. IR (KBr) cm^{-1}: 1270 (C—O), 1599 (N═C), 1692 (C═O), 3128 (CH—Ar), 3472 (N—H). ^1H NMR (DMSO-d_6) δ ppm: 3.80 (s, 3H, OCH_3), 5.39 (s, 2H, CH_2), 5.61 (s, 2H, OCH_2), 6.92–7.68 (m 12H, aromatic), 7.95 (s, 1H, CH), 11.69 (s, 1H, CONH). Anal. Cacd for $C_{24}H_{22}N_4O_3$: C, 67.55; H, 5.44; N, 12.60; O, 14.40. Found C, 67.57; H, 5.39; N, 12.65; O, 14.38%.

2.1.4.7. N′-[(Z)-(4-fluorophenyl)methylidene]-2-[2-(phenoxymethyl)-1H-benzimidazol-1-yl]acetohydrazide ***(7g)***. Yield 67% (Methanol); m.p. 156–158 °C. IR (KBr) cm^{-1}: 1078 (N—N), 1225 (C—O), 1746 (C═N), 2982 (CH—Ar), 715 (C—F). ^1H NMR (DMSO-d_6) δ ppm: 5.19 (s, 2H, CH_2), 5.51 (s, 2H, OCH_2), 6.89–7.73 (m, 12H, aromatic), 8.15 (s, 1H, CH), 10.09 (s, 1H, CONH). Anal. Cacd for $C_{23}H_{19}FN_4O_2$: C, 68.65; H, 4.76; F, 4.72; N, 13.92; O, 7.95. Found: C, 68.67; H, 4.69; F, 4.75; N, 13.90; O, 7.96%.

2.1.4.8. N′-[(Z)-(4-aminophenyl)methylidene]-2-[2-(phenoxymethyl)-1H-benzimidazol-1-yl]acetohydrazide ***(7h)***. Yield 65% (Methanol); m.p. 184–186 °C. IR (KBr) cm^{-1}: 1072 (N—N),

Table 1 Anticonvulsant activity and minimal motor impairment data of the compounds **(6, 7a–j, 8).**

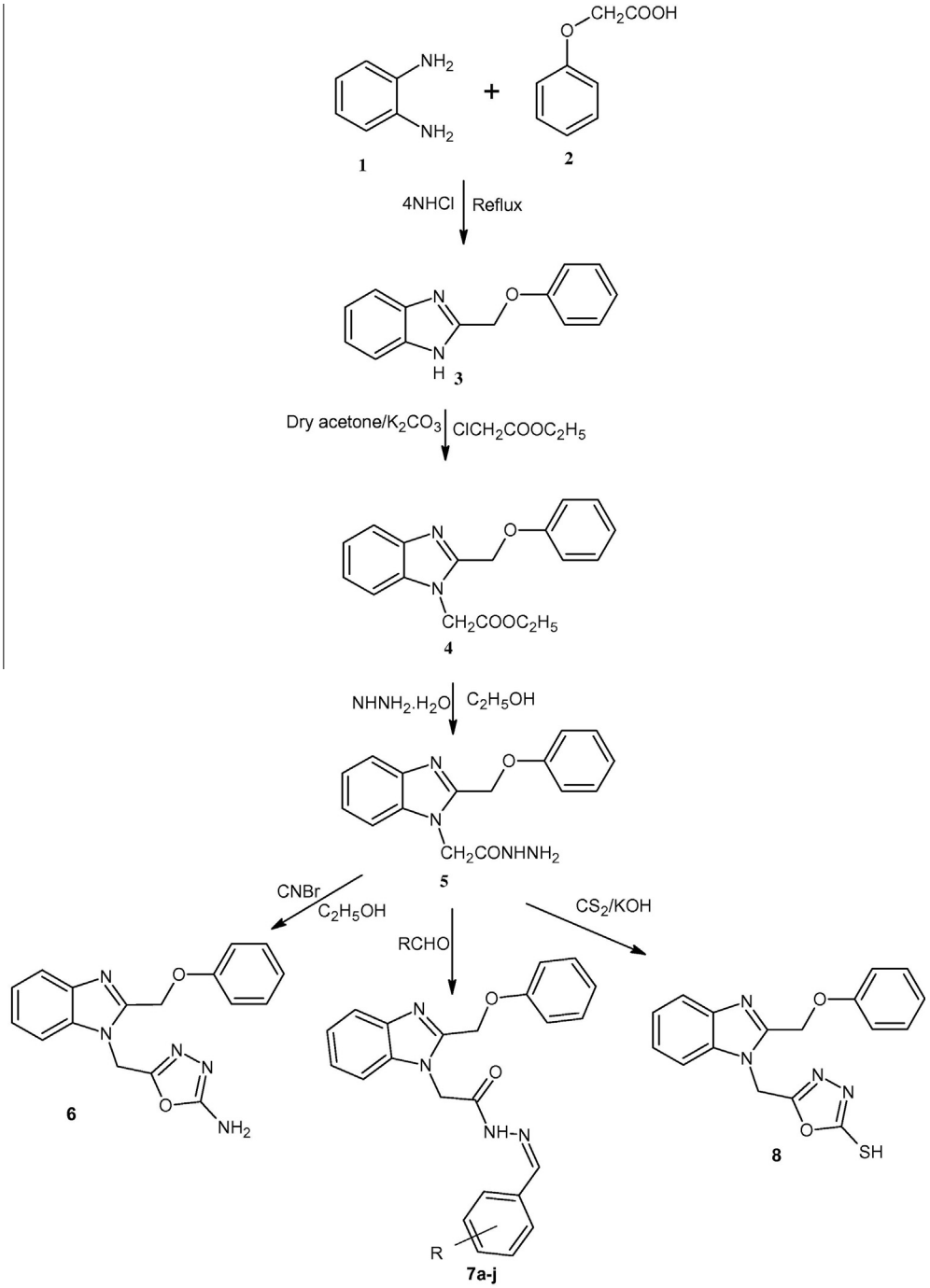
S. No.	Compounds	Intraperitoneal injection in mice[a]					
		MES screen		scPTZ screen		Neurotoxicity screen	
		0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
1	**6**	–	100	–	–	–	100
2	**7a**	300	–	–	100	–	–
3	**7b**	100	300	100	300	300	–
4	**7c**	100	300	–	300	–	300
5	**7d**	–	300	–	300	100	–
6	**7e**	–	300	–	–	–	100
7	**7f**	–	100	–	300	300	–
8	**7g**	30	100	–	100	100	300
9	**7h**	100	300	–	100	–	100
10	**7i**	–	100	–	100	–	300
11	**7j**	30	30	–	100	100	–
12	**8**	100	300	–	100	–	–
13	Phenytoin	30	30	–	–	100	100
14	Ethosuximide	–	–	100	300	–	–

[a] Number of animals used = 6; solvent used – polyethylene glycol; dose – 30, 100, 300 mg/kg i.p. The figures in the table indicate the minimum dose whereby bioactivity was demonstrated in half or more of the mice. The animals were examined at 0.5 and 4 h after injections were administered. Statistical analyses were performed by one way ANOVA techniques followed by Dunnet's test.

1229 (C—O), 1739 (C═N), 2989 (CH—Ar). ^{1}H NMR (DMSO-d_6) δ ppm: 5.17 (s, 2H, CH_2), 5.56 (s, 2H, OCH_2), 6.79–7.77 (m, 12H, aromatic), 8.15 (s, 1H, CH), 10.72 (s, 1H, CONH), 4.01 (s, 2H, aromatic C—NH). Anal. Cacd for $C_{23}H_{21}N_5O_2$: C, 69.16; H, 5.30; N, 17.53; O, 8.01. Found: C, 69.20; H, 5.33; N, 17.54; O, 8.03%.

2.1.4.9. N′-[(Z)-(4,4-dimethylaminophenyl)methylidene]-2-[2-(phenoxymethyl)-1H-benz imidazol-1-yl]acetohydrazide ***(7i)***. Yield 64% (Methanol); m.p. 144–146 °C. IR (KBr) cm^{-1}: 1067 (N—N), 1225 (C—O), 1750 (C═N), 2979 (CH—Ar). ^{1}H NMR (DMSO-d_6) δ ppm: 5.24 (s, 2H, CH_2), 5.62 (s, 2H, OCH_2), 6.59–7.78 (m, 12H, aromatic), 8.12 (s, 1H, CH), 9.02 (s, 1H, CONH), 2.97 (s, 6H, $(NCH_3)_2$). Anal. Cacd for $C_{25}H_{25}N_5O_2$ C, 70.24; H, 5.89; N, 16.38; O, 7.49. Found: C, 70.21; H, 5.90; N, 16.35; O, 7.51%.

2.1.4.10. N′-[(Z)-(4-nitrophenyl)methylidene]-2-[2-(phenoxymethyl)-1H-benzimidazol-1-yl]acetohydrazide ***(7j)***. Yield 69% (Methanol); m.p. 150–152 °C. IR (KBr) cm^{-1}: 1065 (N—N), 1215 (C—O), 1753 (C═N), 2979 (CH—Ar). ^{1}H NMR (DMSO-d_6) δ ppm: 5.20 (s, 2H, CH_2), 5.59 (s, 2H, OCH_2),



R= H, 2-Cl, 4-Cl, 4-OCH_3, 3-OCH_3, 3,4-diOCH_3, 4-F, 4-NH_2, 4,4-dimethylamino, NO_2

Scheme 1

Table 2 Quantitative evaluation of anticonvulsant activity in mice.

Compounds	ED^{a}_{50}		TD^{b}_{50}	PI	
	MES	scPTZ		MES	scPTZ
7g	12.4 (9.0–15.9)	402.6 (277.4–526.3)	502.1 (304.4–694.2)	40.5	1.2
7j	22.1 (13.7–31.0)	432.6 (307.4–556.3)	546.5 (348.8–738.6)	24.7	< 0.2

Number of animals used = 10; solvent used – polyethylene glycol PI protective index = TD_{50}/ED_{50}.
[a] Dose in milligrams per kilogram body weight.
[b] Minimal toxicity which was determined by the rotorod test 30 min after the test drug was administered.

6.62–7.89 (m, 13H, aromatic), 8.09 (s, 1H, CH), 9.04 (s, 1H, CONH). Anal. Cacd for $C_{23}H_{19}N_4No_2O_2$. C, 30.64; H, 2.12; N, 6.21; O, 3.55. Found: C, 30.60; H, 2.10; N, 6.23; O, 3.57%.

2.1.5. Synyhesis of 5-{[2-(phenoxymethyl)-1H-benzimidazol-1-yl]methyl}-1,3,4-oxadiazole-2-thiol ***(8)***

To an ethanolic solution of 2-[2-(phenoxymethyl)-1*H*-benzimidazol-1-yl]aceto hydrazide (0.0025 mol; 0.7388 g) and carbon disulfide (0.005 mol; 0.38 ml), potassium hydroxide solution (30%; 5 ml) was added. The reaction mixture was refluxed on water bath for 4 h. After completion of the reaction, the reaction mixture was cooled at room temperature and poured onto crushed ice, acidified with dilute hydrochloric acid, a solid mass so separated was washed with water and crystallized from methanol.

Yield 64% (Methanol); m.p. 192–194 °C. IR (KBr) cm^{-1}: 1031 (N—N), 1253 (C—O), 1593 (C=N), 3071 (CH—Ar), 2368 (SH). ^{1}H NMR (DMSO-d_6) δ ppm: 3.39 (s 1H, SH), 5.44 (s, 2H, CH_2), 5.81 (s, 2H, OCH_2), 6.94–7.70 (m, 9H, aromatic). Anal. Cacd for $C_{17}H_{14}N_4O_2S$ C, 60.34; H, 4.17; N, 16.56; O, 9.46; S, 9.48. Found: C, 60.33; H, 4.19; N, 16.60; O, 9.44; S, 9.49%.

3. Results and discussion

3.1. Chemistry

2-(Phenoxymethyl)-1*H*-benzimidazole **3** was prepared by refluxing with *o*-phenylenediamine **1** and phenoxyacetic acid **2** in presence of 4NHCl. The compound 3 on reacting with ethylchloroacetate in presence of potassium carbonate in dry acetone gave ethyl [2-(phenoxymethyl)-1*H*-benzimidazol-1-yl]acetate **4** which on treatment with hydrazine hydrate results in 2-[2-(phenoxymethyl)-1*H*-benzimidazol-1-yl]acetohydrazide **5**. The compound **5** gave 5-{[2-(phenoxymethyl)-1*H*-benzimidazol-1-yl]methyl}-1,3,4-oxadiazol-2-amine **6**, 2-[2-(phenoxymethyl)-1*H*-benzimidazol-1-yl]-N′-[substituted phenyl methylidene]acetohydrazide **(7a–j)** and 5-{[2-(phenoxymethyl)-1*H*-benzimidazol-1-yl]methyl}-1,3,4-oxadiazole-2-thiol **8** with cyanogen bromide and carbon disulfide/potassium hydroxide.

3.2. Anticonvulsant activities

The compounds synthesized from 2-[2-(phenoxymethyl)-1*H*-benzimidazol-1-yl]acetohydrazide **5** were screened for their anticonvulsant activity through the MES and PTZ test. The results of the above given test were compared with the standard drugs (phenytoin and ethosuximide) as mentioned in the Table 1. Minimal motor impairment was determined by the rotorod test. The compound **7g** and **j** were found to exhibit most potent anticonvulsant activity. Thus these were further evaluated for their anticonvulsant activity (ED_{50}), Neurotoxicity (TD_{50}) and protective index comparing with their reference standards. The protective index of the compound **7g** and **j** were found to be 40.5 24.7, respectively, which were significantly higher than the standard drugs. The much higher TD_{50} values exhibited by the compound **7g** and **j** indicated that these cause marked lower neurotoxicity than the standard drugs. Structure activity relationship has also been studied, the compound containing *nitro* and *fluoro* group at *para* position was found to be most active in this series. The substitution with electron withdrawing group played a very important role and it follows the order NO_2 < F < Cl. On the other hand the substitution with electron releasing group does not have marked effect on the activity.

4. Conclusion

The present study has identified the anticonvulsant effect of compounds derived from 2-[2-(phenoxymethyl)-1*H*-benzimidazol-1-yl]acetohydrazide. The compound **7g** and **j** has shown potent anticonvulsant activity and lesser neurotoxicity as compared with the standard drugs because NO_2 and F have more electron withdrawing capacity than the other substituents. The protective index of these two compounds was found to be better than standard drugs. Thus the study revealed that these compounds have potential anticonvulsant activity and can be used for further clinical trials (see Scheme 1).

5. Biological methods

5.1. Anticonvulsant screening

5.1.1. Maximal electroshock seizure test (MES test)

The compounds were screened for their anticonvulsant activity by electroshock seizure method (Stables and Kupferberg, 1995). Supramaximal electroshock of current intensity of 50 mA, 60 Hz for 0.2 s duration was applied to mice, with the doses of test compounds 30, 100, 300 mg/kg (Husain et al., 2011). The abolition of the hind limb tonic extensor spasm was recorded as anticonvulsant activity as shown in Table 1.

5.1.2. Pentylenetetrazole-induced seizure test (PTZ test)

The subcutaneous pentylenetetrazole test was performed according to the known protocol (Clark et al., 1984). This method utilizes pentylenetetrazole (75 mg/kg) that produces

seizures in >95% of animals as 0.5% solution subcutaneously in the posterior midline. The animal was observed for 30 min; failure to observe even a threshold seizure (a single episode of clonic spasms of at least 5 s duration) was defined as protection as shown in Table 1. To determine anticonvulsant potency and toxicity, groups of 10 mice were tested with various doses of the drug until at least three points were established between the limits of 90% protection or toxicity and 10% protection or toxicity. The dose of drug required to produce the desired endpoint in 50% of animals (ED_{50}) in each test, the dose eliciting evidence of minimal neurological toxicity in 50% of animals (TD_{50}) are shown in Table 2.

5.1.3. Neurotoxicity screening (NT)

The minimal motor impairment was measured in mice by the rotorod test (Kucukgzuel et al., 2006; Lien et al., 1979). The mice (20–25 g) were trained to stay on an accelerating rotorod that rotates at 10 rotations/min. and its diameter was 3.2 cm. Only those mice were taken for the test which can stay on the revolving rod for at least 1 min. Trained animals were injected i.p. with the test compounds at doses of 300 mg/kg. Neurotoxicity was indicated by the inability of the animal to maintain equilibration on the rod for at least one minute as shown in Table 1.

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