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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and structure–activity relationship studies on novel 8-amino-3-[2-(4-fluorophenoxy)ethyl]-1,3-diazaspiro[4.5]decane-2,4-dione derivatives as anticonvulsant agents

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Abstract A series of novel 8-amino-3-[2-(4-fluorophenoxy)ethyl]-1,3-diazaspiro[4.5]decane-2,4-dione derivatives **7–36** was synthesized and their pharmacological activity was determined with the objective to better understand their structure–activity relationship (SAR) for anticonvulsant activity. All the compounds 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 **24**, **27**, and **34** showed a significant and protective effect on seizure, when compared with standard drug phenytoin. The compounds having amide bond showed moderate protective effect on MES induced seizures compared to sulfonamide.

Keywords Spirohydantoins · Acid chloride · Sulfonyl chloride · Anticonvulsant · Epilepsy

Introduction

Epilepsy is a common neurological affliction characterized by excessive temporary neuronal discharge that affects about 1 % of the world's population (Pessah *et al.,* 2009). It is affecting a large section of people both male and female across the world. Every year approximately 250,000 new cases are added to this figure. Epilepsy also poses a considerable economic burden on the society. The direct costs of epilepsy vary significantly depending on the severity of the disease and the response to the treatment. The known potential causes of epilepsy include brain tumors, infections, traumatic head injuries, perinatal insults, developmental malformations, cerebrovascular diseases, febrile seizures, and status epileptics (Loscher, 2002). Many patients have seizures that are resistant to the available medical therapies. Although 70–80 % of epileptics are currently controlled by a variety of drugs, seizure protection is often accompanied by numerous side effects including drowsiness, ataxia, gastrointestinal disturbances, gingival hyperplasia, hirsutism, and megaloblastic anemia (Davies-Jones, 1988; Bialer *et al.,* 2004).

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, phenobarbital, valproic acid, and newer, "second generation" drugs such as lamotrigine, vigabatrin, tiagabine, topiramate, gabapentin, and levetiracetam (Patel *et al.,* 2006; Mc Cabe, 2000). The selection of an antiepileptic drug for the treatment is predicated on its efficacy for the specific type of seizures, tolerability, and safety (Hwang and Kim, 2008; Kwan and Brodie, 2000). Therefore, it is essential to search for newer chemical entities for the treatment of epilepsy. A review on the new structural entities having anticonvulsant activity has recently appeared (Cosford *et al.,* 1998). Quite recently, spirohydantoin analogs have become an emerging new class of potent anticonvulsants (Zha *et al.,* 2004).

Hydantoins, a class of cyclic imides, have been demonstrated to possess good anticonvulsant property (Merritt and Putnam, 1938; Hassell *et al.,* 1980). Depending on the nature

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of substitution on the hydantoin ring, a wide range of other pharmacological properties, e.g., antihypertensive (Edmunds *et al.,* 1995), herbicidal (Hanessian *et al.,* 1995), anti-HIV (Comber *et al.,* 1992), antibacterial (Oh *et al.,* 1995), and antiviral (Kim *et al.,* 2001) activities, have also been identified. This prompted the authors to prepare a new class of spirohydantoin analogs, such as 1,3-diazaspirohydantoins and study their anticonvulsant activity through maximal electroshock seizure (MES) model, and their neurotoxic effects were determined by rotorod test.

Experimental section

General

The starting materials and reagents were obtained from commercial suppliers and were used without further purification. Melting points were determined in one end open capillary tubes on a Buchi 530 melting point apparatus and are uncorrected. The ^{1}H and ^{13}C NMR spectra were recorded on Bruker Advance 400 MHz NMR instrument using TMS as internal standard and DMSO-d_6/$CDCl_3$ as solvent. Chemical shift are given in parts per million (δ-scale) and coupling constant are given in Hertz. Mass spectra were recorded on Perkin–Elmer LC–MS PE Sciex API/65 Spectrophotometer. IR spectra were recorded using KBr on 8400S Shimadzu Fourier Transform Spectrophotometer (ν_{max} in cm^{-1}). Silica gel column chromatography was performed using Merck 7734 silica gel (60–120 mesh) and Merck made TLC plate. Elemental analysis (C, H, and N) was undertaken with Perkin-Elmer model 240C analyzer and the data showed good agreement between the experimentally determined values and the theoretically calculated values within ±0.4 %.

Synthesis

*Synthesis of tert-butyl (4-hydroxycyclohexyl) carbamate (**2**)*

A mixture of 4-aminocyclohexanol hydrochloride (4 g, 26.47 mmol), triethylamine (5.35 g, 52.95 mmol), and Boc anhydride (5.77 g, 26.47 mmol) in dry dichloromethane (40 mL) was stirred at room temperature for 16 h. After completion of the reaction (TLC), the reaction mixture was poured into water and extracted with dichloromethane (3 × 40 mL), the product was dried over anhydrous Na_2SO_4, and concentrated in vacuo to give the crude product which was recrystallized from hexane to get the pure product. White color solid: (5.10 g, 90 %); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.38 (s, 1H), 3.54 (m, 1H), 3.17 (m, 1H), 2.10 (d, J = Hz, 1H), 1.77–1.78 (m, 2H), 1.62–1.63 (m, 4H), 1.53–1.56 (m, 2H), 1.43 (s, 9H). MS: *m/z* 215.3 (M^+), 216.3 (M + 1).

*Synthesis of tert-butyl (4-oxocyclohexyl)carbamate (**3**)*

A mixture of dimethyl sulfoxide (5.54 g, 71.04 mmol) and dichloromethane (50 mL) was stirred at −78 °C. Oxalyl chloride (4.51 g, 35.53 mmol) was added dropwise, stirred for 1 h, *tert*-butyl (4-hydroxycyclohexyl)carbamate (5.1 g, 23.68 mmol) in dichloromethane (25 mL) was added at −78 °C, and allowed to be stirred for 2 h. After completion of the reaction (TLC), it was quenched with triethylamine (11.98 g, 118.44 mmol). The reaction mixture was poured into water, extracted with dichloromethane (3 × 40 mL), the product was dried over Na_2SO_4, and concentrated in vacuo to get the crude product which was purified by column chromatography over silica gel (60–120 mesh) using hexane/ethyl acetate (6:4) as eluent to get the pure product. *Colorless liquid*: (4.30 g, 85 %); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.40 (s, 1H), 3.90 (m, 1H), 1.77–1.78 (m, 2H), 1.62–1.63 (m, 4H), 1.53–1.54 (m, 2H), 1.42 (s, 9H); MS: *m/z* 213.3 (M^+), 214.3 (M + 1).

*Synthesis of tert-butyl (2,4-dioxo-1,3-diazaspiro[4.5] dec-8-yl)carbamate (**4**)*

A mixture of *tert*-butyl (4-oxocyclohexyl)carbamate (4.3 g, 20.16 mmol) and ammonium carbonate (4.26 g, 44.35 mmol) was taken in ethanol (25 mL) and water (25 mL). A solution of sodium cyanide (2.07 g, 42.34 mmol) in water (10 mL) was added dropwise and the reaction mixture was stirred at room temperature for 24 h and heated to 50 °C for 24 h and cooled to room temperature. After completion of the reaction (TLC), the solid was filtered, washed with water (100 mL), and dried in vacuo to get the hydantoin. White color solid: (4.5 g, 79 %); ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.61 (s, 1H), 8.48 (s, 1H), 6.72 (s, 1H), 3.17 (s, 1H), 1.77–1.78 (m, 2H), 1.62–1.63 (m, 4H), 1.53–1.56 (m, 2H), 1.43 (s, 9H); MS: *m/z* 283.3 (M^+), 284.3 (M + 1).

*Synthesis of tert-butyl {3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5]dec-8-yl}carbamate (**5**)*

A mixture of *tert*-butyl (2,4-dioxo-1,3-diazaspiro[4.5]dec-8-yl)carbamate (4.5 g, 15.88 mmol), anhydrous potassium carbonate (3.07 g, 22.23 mmol), and 1-(2-bromoethoxy)-4-fluorobenzene (4.17 g, 19.05 mmol) in acetonitrile (50 mL) was refluxed for 6 h. After completion of the reaction (TLC), it was cooled to room temperature and filtered. Filtrate was concentrated under vacuo to give the crude product which was then purified by column chromatography over silica gel (60–120 mesh) using chloroform/methanol (9:1) as eluent to get the pure product. White color solid: (5.4 g, 81 %); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.93 (s, 1H), 7.24–7.25 (m, 2H), 7.14–7.14 (m, 2H), 6.76 (d, J = 5.48 Hz, 1H), 4.51 (s, 2H), 3.21 (s, 1H), 1.80–1.80 (m, 2H), 1.68–1.69 (m, 2H),

1.54–1.57 (m, 2H), 1.43–1.45 (m, 2H), 1.39 (s, 9H); MS: *m/z* 391.4 (M^+), 392.4 (M + 1).

*Synthesis of 8-amino-3-[2-(4-fluorophenoxy)ethyl]-1,3-diazaspiro[4.5]decane-2,4-dione (**6**)*

A reactant *tert*-butyl{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5]dec-8-yl}carbamate (5.4 g, 12.87 mmol) was taken in dioxane (30 mL), cooled to 0 °C, and dioxane in HCl (50 mL) was added and stirred at room temperature for 4 h. After completion of the reaction (TLC), dioxane was removed under vacuum and the reaction mixture was neutralized with sodium carbonate solution, the product was extracted with dichloromethane (3 × 50 mL) and dried over Na_2SO_4, and finally concentrated in vacuo to get the pure product. White color solid: (3.4 g, 82 %); ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.84 (s, 1H), 7.23–7.24 (m, 2H), 7.13–7.13 (m, 2H), 6.02 (m, 2H), 4.50 (s, 2H), 2.60–0.00 (m, 1H), 2.19 (d, J = 8.00 Hz, 2H), 1.66–1.69 (m, 4H), 1.50–1.53 (m, 2H), 1.25–1.29 (m, 2H); MS: *m/z* 291.3 (M^+), 292.3 (M + 1).

*General procedure for the synthesis of **7–36***

A mixture of 8-amino-3-[2-(4-fluorophenoxy)ethyl]-1,3-diazaspiro[4.5]decane-2,4-dione (100 mg 0.31 mmol), triethylamine (0.46 mmol), and sulfonyl chlorides/acid chlorides (0.34 mmol) in dichloromethane (4 mL) was stirred at room temperature for 16 h. After completion of the reaction (TLC), it was quenched with saturated sodium bicarbonate solution; the product was extracted with dichloromethane (3 × 4 mL), dried over Na_2SO_4, and concentrated in vacuo to get the crude product which was next purified by column chromatography on silica employing dichloromethane/methanol (9:1) as elutant to obtain the pure product.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5]dec-8-yl}-3,4-dimethyl benzenesulfonamide (**7**)*

White color solid: (102 mg, 67 %); mp = 180 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.87 (s, 1H), 7.63 (s, 1H), 7.61 (s, 1H), 7.50–7.52 (m, 1H), 7.30 (d, J = 7.96 Hz, 1H), 7.07–7.08 (m, 2H), 6.85–6.86 (m, 2H), 4.07 (t, J = 5.76 Hz, 2H), 3.67 (t, J = 5.76 Hz, 2H), 3.33 (s, 1H), 2.26 (d, J = 10.72 Hz, 6H), 1.28–1.59 (m, 8H); MS: *m/z* 489.6 (M^+), 490.6 (M + 1); IR (KBr) 3,361 (N–H), 1,344 (S=O), 1,281 (S=O)cm^{-1}; Calcd for $C_{24}H_{28}FN_3O_5S$: C 58.88, H 5.76, N 8.58 %; Found: C 58.81, H 5.70; N, 8.52 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5]dec-8-yl}-2-methylbenzene sulfonamide (**8**)*

White color solid: (105 mg, 71 %); mp = 178 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.85 (s, 1H), 7.86 (s, 1H), 7.84 (s, 1H), 7.45–7.45 (m, 1H), 7.33–7.36 (m, 2H), 7.08–7.11 (m, 2H), 6.86–6.87 (m, 2H), 4.07 (t, J = 5.16 Hz, 2H), 3.67 (t, J = 6.00 Hz, 2H), 3.37 (s, 1H), 2.56 (s, 3H), 1.30–1.63 (m, 8H); MS: *m/z* 475.5 (M^+), 476.5 (M + 1); IR (KBr) 3,362 (N–H), 1,345 (S=O), 1,284 (S=O)cm^{-1}; Calcd for $C_{23}H_{26}FN_3O_5S$: C 58.09, H 5.51, N 8.84 %; Found: C 58.01, H 5.47, N, 8.80 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5]dec-8-yl}-2-bromobenzene sulfonamide (**9**)*

White color solid: (114 mg, 68 %); mp = 204 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.89 (s, 1H), 7.96 (d, J = 8.88 Hz, 2H), 7.80 (d, J = 7.84 Hz, 2H), 7.54 (t, J = 7.88 Hz, 1H), 7.10 (q, J = 2.20 Hz, 2H), 6.87 (q, J = 4.40 Hz, 2H), 4.08 (t, J = 5.72 Hz, 2H), 3.68 (t, J = 5.76 Hz, 2H), 3.39 (s, 1H), 1.32–1.61 (m, 8H); MS: *m/z* 540.4 (M^+), 541.4 (M + 1); IR (KBr) 3,357 (N–H), 1,342 (S=O), 1,280 (S=O)cm^{-1}; Calcd for $C_{22}H_{23}BrFN_3O_5S$: C 48.90, H 4.29, N 7.78 %; Found: C 48.85, H 4.24, N 7.71 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5]dec-8-yl}-2-fluobenzene sulfonamide (**10**)*

White color solid: (104 mg, 70 %); mp = 175 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.88 (s, 1H), 8.10 (d, J = 8.28 Hz, 1H), 7.80 (d, J = 1.56 Hz, 1H), 7.71 (t, J = 1.00 Hz, 1H), 7.35–7.35 (m, 2H), 7.08–7.10 (m, 2H), 6.86–6.86 (m, 2H), 4.08 (t, J = 5.72 Hz, 2H), 3.67 (t, J = 5.84 Hz, 2H), 3.34 (s, 1H), 1.45–1.63 (m, 8H); MS: *m/z* 479.5 (M^+), 480.5 (M + 1); IR (KBr) 3,368 (N–H), 1,347 (S=O), 1,283 (S=O) cm^{-1}; Calcd for $C_{22}H_{23}F_2N_3O_5S$: C 55.11, H 4.83, N 8.76 %; Found: C 55.08, H 4.81, N 8.72 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5]dec-8-yl}-2,5-dimethyl benzenesulfonamide (**11**)*

White color solid: (107 mg, 70 %); mp = 180 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.84 (s, 1H), 7.67–7.69 (m, 2H), 7.18–7.20 (m, 1H), 7.09–7.10 (m, 1H), 6.99–6.99 (m, 2H), 6.86–6.89 (m, 2H), 4.06 (t, J = 5.76 Hz, 2H), 3.67 (t, J = 5.76 Hz, 2H), 3.33 (s, 1H), 2.45 (s, 3H), 2.25 (s, 3H), 1.51–1.59 (m, 2H), 1.46 (t, J = 4.32 Hz, 2H), 1.33 (d, J = 9.06 Hz, 2H), 1.18 (t, J = 7.22 Hz, 2H); MS: *m/z* 489.6 (M^+), 490.6 (M + 1); IR (KBr) 3,355 (N–H), 1,336 (S=O), 1,278 (S=O) cm^{-1}; Calcd for $C_{24}H_{28}FN_3O_5S$: C 58.88, H 5.76, N 8.58 %; Found: C 58.81, H 5.70, N 8.51 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5]dec-8-yl}-4-methylbenzene sulfonamide (**12**)*

White color solid: (102 mg, 69 %); mp = 176 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.83 (s, 1H), 7.32–7.33 (m, 4H),

7.22 (d, $J = 7.88$ Hz, 1H), 7.08–7.08 (m, 2H), 6.89–6.93 (m, 2H), 4.12 (t, $J = 5.72$ Hz, 2H), 3.72 (t, $J = 5.76$ Hz, 2H), 3.34 (s, 1H), 2.45 (s, 3H), 1.18–1.90 (m, 8H); MS: *m/z* 475.5 (M^+), 476.5 (M + 1); IR (KBr) 3,362 (N–H), 1,342 (S=O), 1,281 (S=O) cm^{-1}; Calcd for $C_{23}H_{26}FN_3O_5S$: C 58.09, H 5.51, N 8.84 %; Found: C 58.02, H 5.48, N 8.82 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5] dec-8-yl}-3-chlorobenzene sulfonamide (**13**)*

White color solid: (108 mg, 70 %); mp = 190 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.88 (s, 1H), 7.96 (s, 1H), 7.81–7.82 (m, 1H), 7.75–7.78 (m, 1H), 7.66–7.69 (m, 1H), 7.61 (t, $J = 7.92$ Hz, 1H), 7.08–7.12 (m, 2H), 6.85–6.89 (m, 2H), 4.08 (t, $J = 5.68$ Hz, 2H), 3.68 (t, $J = 5.76$ Hz, 2H), 3.33 (s, 1H), 1.32–1.58 (m, 8H); MS: *m/z* 496 (M^+), 497 (M + 1); IR (KBr) 3,354 (N–H), 1,341 (S=O), 1,282 (S=O) cm^{-1}; Calcd for $C_{22}H_{23}ClFN_3O_5S$: C 53.28, H 4.67, N 8.47 %; Found: C 53.21, H 4.60, N 8.42 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5] dec-8-yl}-4-fluoro-2-methyl benzenesulfonamide (**14**)*

White color solid: (114 mg, 74 %); mp = 186 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.85 (s, 1H), 7.84–7.92 (m, 2H), 7.24 (t, $J = 9.00$ Hz, 2H), 7.10 (t, $J = 8.72$ Hz, 2H), 6.86–6.89 (m, 2H), 4.07 (t, $J = 5.72$ Hz, 2H), 3.67 (t, $J = 5.72$ Hz, 2H), 3.34 (s, 1H), 2.56 (s, 3H), 1.59 (q, $J = 10.56$ Hz, 3H), 1.42 (d, $J = 11.04$ Hz, 3H), 1.32 (d, $J = 8.88$ Hz, 1H), 1.18 (d, $J = 11.12$ Hz, 1H); MS: *m/z* 493.5 (M^+), 494.5 (M + 1); IR (KBr) 3,364 (N–H), 1,346 (S=O), 1,287 (S=O) cm^{-1}; Calcd for $C_{23}H_{25}F_2N_3O_5S$: C 55.97, H 5.11, N 8.51 %; Found: C 55.97, H 5.11, N 8.51 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5] dec-8-yl}-4-methoxybenzene sulfonamide (**15**)*

White color solid: (103 mg, 67 %); mp = 181 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.87 (s, 1H), 7.19–7.74 (m, 2H), 7.61 (d, $J = 7.88$ Hz, 1H), 7.06–7.12 (m, 4H), 6.84–6.89 (m, 2H), 4.07 (t, $J = 5.84$ Hz, 2H), 3.75 (s, 3H), 3.67 (t, $J = 5.80$ Hz, 2H), 3.34 (s, 1H), 1.16–1.57 (m, 8H); MS: *m/z* 491.5 (M^+), 492.5 (M + 1); IR (KBr) 3,359 (N–H), 1,340 (S=O), 1,281 (S=O) cm^{-1}; Calcd for $C_{23}H_{26}FN_3O_6S$: C 56.20, H 5.33, N 8.55 %; Found: C 56.36, H 5.30, N 8.51 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5] dec-8-yl}-4-methylbenzyl sulfonamide (**16**)*

White color solid: (112 mg, 74 %); mp = 167 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.83 (s, 1H), 7.23–7.23 (m, 2H), 7.10–7.17 (m, 5H), 6.89–6.93 (m, 2H), 4.21 (d, $J = 1.84$ Hz, 2H), 4.12 (t, $J = 5.64$ Hz, 2H), 3.72 (t, $J = 5.68$ Hz, 2H), 3.43 (s, 1H), 2.28 (s, 3H), 1.65 (d, $J = 11.84$ Hz, 1H), 1.49–1.59 (m, 6H), 1.37 (d, $J = 11.00$ Hz, 1H); MS: *m/z* 489.6 (M^+), 490.6 (M + 1); IR (KBr) 3,352 (N–H), 1,337 (S=O), 1,277 (S=O) cm^{-1}; Calcd for $C_{24}H_{28}FN_3O_5S$: C 58.88, H 5.76, N 8.58 %; Found: C 58.81, H 5.72, N 8.50 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5] dec-8-yl}-3-fluobenzene sulfonamide (**17**)*

White color solid: (105 mg, 70 %); mp = 175 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.87 (s, 1H), 7.93–7.95 (m, 1H), 7.58–7.65 (m, 3H), 7.48 (t, $J = 1.52$ Hz, 1H), 7.08–7.12 (m, 2H), 6.85–6.88 (m, 2H), 5.08 (t, $J = 5.72$ Hz, 2H), 3.68 (t, $J = 11.40$ Hz, 2H), 3.38 (s, 1H), 1.57–1.60 (m, 8H); MS: *m/z* 479.5 (M^+), 480.5 (M + 1); IR (KBr) 3,362 (N–H), 1,346 (S=O), 1,287 (S=O) cm^{-1}; Calcd for $C_{22}H_{23}F_2N_3O_5S$: C 55.11, H 4.83, N 8.76 %; Found: C 55.09, H 4.81, N 8.73 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5] dec-8-yl}-3,5-dimethylbenzene sulfonamide (**18**)*

White color solid: (108 mg, 71 %); mp = 182 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.88 (s, 1H), 7.40–0.00 (m, 3H), 7.07–7.14 (m, 2H), 6.84–6.91 (m, 4H), 4.10 (t, $J = 5.68$ Hz, 2H), 3.70 (t, $J = 5.84$ Hz, 2H), 3.37 (s, 1H), 2.29 (s, 6H), 1.41–1.52 (m, 8H); MS: *m/z* 489.6 (M^+), 490.6 (M + 1); IR (KBr) 3,364 (N–H), 1,347 (S=O), 1,280 (S=O) cm^{-1}; Calcd for $C_{24}H_{28}FN_3O_5S$: C 58.88, H 5.76, N 8.58 %; Found: C 58.82, H 5.71, N 8.52 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5] dec-8-yl}-4-ethylbenzene sulfonamide (**19**)*

White color solid: (113 mg, 74 %); mp = 169 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.86 (s, 1H), 7.70–7.72 (m, 3H), 7.69–7.70 (m, 2H), 7.08–7.12 (m, 2H), 6.85–6.89 (m, 2H), 4.07 (t, $J = 5.76$ Hz, 2H), 3.67 (t, $J = 5.84$ Hz, 2H), 3.34 (s, 1H), 2.65 (q, $J = 7.68$ Hz, 2H), 1.41–1.59 (m, 8H), 1.19 (t, $J = 7.20$ Hz, 3H); MS: *m/z* 489.6 (M^+), 490.6 (M + 1); IR (KBr) 3,360 (N–H), 1,341 (S=O), 1,283 (S=O) cm^{-1}; Calcd for $C_{24}H_{28}FN_3O_5S$: C 58.88, H 5.76, N 8.58 %; Found: C 58.84, H 5.72, N 8.51 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5] dec-8-yl}-3-methylbenzene-sulfonamide (**20**)*

White color solid: (107 mg, 72 %); mp = 179 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.87 (s, 1H), 7.58–7.71 (m, 2H), 7.35–7.44 (m, 3H), 7.08–7.13 (m, 2H), 6.85–6.88 (m, 2H), 4.07 (t, $J = 11.40$ Hz, 2H), 3.67 (t, $J = 5.76$ Hz, 2H), 3.34 (s, 1H), 2.30 (s, 3H), 1.51–1.58 (m, 2H), 1.46 (t, $J = 4.36$ Hz, 2H), 1.33 (d, $J = 9.04$ Hz, 2H), 1.18 (t,

$J = 7.28$ Hz, 2H); MS: *m/z* 475.5 (M^+), 476.5 (M + 1); IR (KBr) 3,358 (N–H), 1,339 (S=O), 1,278 (S=O) cm^{-1}; Calcd for $C_{23}H_{26}FN_3O_5S$: C 58.09, H 5.51, N 8.84 %; Found: C 58.02, H 5.47, N 8.80 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5]dec-8-yl}-2-cyanobenzene sulfonamide (**21**)*

White color solid: (105 mg, 69 %); mp = 221 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.86 (s, 1H), 8.34 (d, $J = 8.00$ Hz, 1H), 8.04–8.09 (m, 2H), 7.90 (t, $J = 7.80$ Hz, 1H), 7.81 (t, $J = 7.64$ Hz, 1H), 7.10 (t, $J = 8.80$ Hz, 2H), 6.86–6.89 (m, 2H), 4.32 (t, $J = 7.68$ Hz, 2H), 3.68 (t, $J = 5.68$ Hz, 2H), 3.39 (s, 1H), 1.67 (t, $J = 12.75$ Hz, 2H), 1.43–1.52 (m, 2H), 1.34 (d, $J = 9.28$ Hz, 2H), 1.26 (d, $J = 1.24$ Hz, 2H); MS: *m/z* 486.5 (M^+), 487.5 (M + 1); IR (KBr) 3,369 (N–H), 1,368 (S=O), 1,287 (S=O) cm^{-1}; Calcd for $C_{23}H_{23}FN_4O_5S$: C 56.78, H 4.77, N 11.52 %; Found: C 56.73, H 4.71, N 11.48 %.

*2-Chlorobenzyl{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5]dec-8-yl}carbamate (**22**)*

White color solid: (108 mg, 71 %); mp = 191 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.00 (s, 1H), 8.35 (d, $J = 8.04$ Hz, 1H), 7.84 (q, $J = 8.60$ Hz, 2H), 7.53 (q, $J = 11.04$ Hz, 2H), 7.05–7.09 (m, 2H), 6.87–6.90 (m, 2H), 4.80 (s, 2H), 4.09–4.14 (m, 2H), 3.69 (t, $J = -8.32$ Hz, 2H), 1.35–1.86 (m, 8H); MS: *m/z* 489.9 (M^+), 491 (M + 1); IR (KBr) 3,368 (N–H), 1,670 (C=O) cm^{-1}; Calcd for $C_{24}H_{25}ClFN_3O_5$: C 58.84, H 5.14, N 8.58 %; Found: C 58.78, H 5.10, N 8.51 %.

*4-Chlorophenyl{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5]dec-8-yl}carbamate (**23**)*

White color solid: (112 mg, 76 %); mp = 198 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.01 (s, 1H), 8.35 (d, $J = 8.04$ Hz, 1H), 7.84 (q, $J = 8.60$ Hz, 2H), 7.53 (q, $J = 11.04$ Hz, 2H), 7.05–7.08 (m, 2H), 6.86–6.90 (m, 2H), 4.09–4.14 (m, 3H), 3.69 (t, $J = 8.32$ Hz, 2H), 1.35–1.86 (m, 8H); MS: *m/z* 475.9 (M^+), 477 (M + 1); IR (KBr) 3,372 (N–H), 1,675 (C=O) cm^{-1}; Calcd for $C_{23}H_{23}ClFN_3O_5$: C 58.05, H 4.87, N 8.83 %; Found: C 58.01, H 4.84, N 8.80 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5]dec-8-yl}-4-methylbenzamide (**24**)*

White color solid: (101 mg, 74 %); mp = 213 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.00 (s, 1H), 8.18 (d, $J = 8.12$ Hz, 1H), 7.72 (d, $J = 8.16$ Hz, 2H), 7.24 (d, $J = 8.04$ Hz, 2H), 7.05–7.09 (m, 2H), 6.87–6.91 (m, 2H), 4.15 (s, 1H), 4.11 (t, $J = 5.76$ Hz, 2H), 3.71 (t, $J = 5.84$ Hz, 2H), 2.34 (s, 3H), 1.13–1.85 (m, 8H); MS: *m/z* 439.5 (M^+), 440.5 (M + 1); IR (KBr) 3,378 (N–H), 1,677 (C=O) cm^{-1}; Calcd for $C_{24}H_{26}FN_3O_4$: C 65.59, H 5.96, N 9.56 %; Found: C 65.54, H 5.91, N 9.50 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5]dec-8-yl}-2-phenylacetamide (**25**)*

White color solid: (103 mg, 75 %); mp = 200 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.00 (s, 1H), 8.51 (d, $J = 276.68$ Hz, 1H), 7.44–7.46 (m, 5H), 7.07 (t, $J = 8.76$ Hz, 2H), 6.87–6.90 (m, 2H), 4.14 (s, 1H), 4.11 (t, $J = 5.72$ Hz, 2H), 3.71 (t, $J = 5.80$ Hz, 2H), 3.40 (s, 2H), 1.65–1.75 (m, 8H); MS: *m/z* 439.5 (M^+), 440.5 (M + 1); IR (KBr) 3,364 (N–H), 1,658 (C=O) cm^{-1}; Calcd for $C_{24}H_{26}FN_3O_4$: C 65.59, H 5.96, N 9.56 %; Found: C 65.54, H 5.90, N 9.51 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5]dec-8-yl}-2-fluorobenzamide (**26**)*

White color solid: (100 mg, 72 %); mp = 202 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.99 (s, 1H), 8.26 (d, $J = 8.04$ Hz, 1H), 7.49–7.54 (m, 2H), 7.24–7.28 (m, 2H), 7.06–7.10 (m, 2H), 6.88–6.91 (m, 2H), 4.10–4.13 (m, 3H), 3.71 (t, $J = 5.84$ Hz, 2H), 1.41–1.70 (m, 8H); MS: *m/z* 443.4 (M^+), 444.4 (M + 1); IR (KBr) 3,369 (N–H), 1,671 (C=O) cm^{-1}; Calcd for $C_{23}H_{23}F_2N_3O_4$: C 62.30, H 5.23, N 9.48 %; Found: C 62.27, H 5.20, N 9.42 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5]dec-8-yl}-3-methylbenzamide (**27**)*

White color solid: (99 mg, 72 %); mp = 213 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.01 (s, 1H), 8.23 (d, $J = 8.16$ Hz, 1H), 7.61 (q, $J = 4.00$ Hz, 2H), 7.33 (d, $J = 4.96$ Hz, 2H), 7.08 (t, $J = 8.68$ Hz, 2H), 6.88–6.92 (m, 2H), 4.13 (q, $J = 5.68$ Hz, 3H), 3.72 (t, $J = 5.84$ Hz, 2H), 2.35 (s, 3H), 1.43–1.86 (m, 8H); MS: *m/z* 439.5 (M^+), 440.5 (M + 1); IR (KBr) 3,373 (N–H), 1,670 (C=O) cm^{-1}; Calcd for $C_{24}H_{26}FN_3O4$: C 65.59, H 5.96, N 9.56 %; Found: C 65.51, H 5.89, N 9.51 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5]dec-8-yl}-3-chlorobenzamide (**28**)*

White color solid: (104 mg, 73 %); mp = 231 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.01 (s, 1H), 8.41 (d, $J = 7.96$ Hz, 1H), 7.87 (s, 1H), 7.79 (d, $J = 7.76$ Hz, 1H), 7.60 (t, $J = 0.92$ Hz, 1H), 7.50 (t, $J = 7.88$ Hz, 1H), 7.08 (t, $J = 8.64$ Hz, 2H), 6.88–6.91 (m, 2H), 4.12 (q, $J = 1.00$ Hz, 3H), 3.72 (t, $J = 5.76$ Hz, 2H), 1.33–1.87 (m, 8H); MS: *m/z* 459.9 (M^+), 461 (M + 1); IR (KBr) 3,376 (N–H), 1,679 (C=O) cm^{-1}; Calcd for $C_{23}H_{23}ClFN_3O_4$: C 60.07, H 5.04, N 9.14 %; Found: C 60.01, H 5.02, N 9.10 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5] dec-8-yl}benzamide (**29**)*

White color solid: (100 mg, 76 %); mp = 189 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.02 (s, 1H), 8.28 (d, J = 8.04 Hz, 1H), 7.82 (d, J = 7.48 Hz, 2H), 7.51 (d, J = 7.20 Hz, 1H), 7.45 (t, J = 10.88 Hz, 2H), 7.08 (t, J = 8.76 Hz, 2H), 6.90 (q, J = 4.32 Hz, 2H), 4.10–4.17 (m, 3H), 3.72 (t, J = 5.76 Hz, 2H), 1.34–1.87 (m, 8H); MS: *m/z* 425.5 (M^+), 426.5 (M + 1); IR (KBr) 3,365 (N–H), 1,667 (C=O) cm^{-1}; Calcd for $C_{23}H_{24}FN_3O_4$: C 64.93, H 5.69, N 9.88 %; Found: C 64.89, H 5.61, N 9.84 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5] dec-8-yl}-3-fluorobenzamide (**30**)*

White color solid: (106 mg, 77 %); mp = 202 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.98 (s, 1H), 8.26 (d, J = 8.04 Hz, 1H), 7.49–7.54 (m, 2H), 7.24–7.27 (m, 2H), 7.06–7.11 (m, 2H), 6.89–6.91 (m, 2H), 4.11–4.13 (m, 3H), 3.71 (t, J = 5.84 Hz, 2H), 1.40–1.71 (m, 8H); MS: *m/z* 443.4 (M^+), 444.4 (M + 1); IR (KBr) 3,370 (N–H), 1,672 (C=O) cm^{-1}; Calcd for $C_{23}H_{23}F_2N_3O_4$: C 62.30, H 5.23, N 9.48 %; Found: C 62.28, H 5.21, N 9.43 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5] dec-8-yl}-4-fluorobenzamide (**31**)*

White color solid: (102 mg, 74 %); mp = 202 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.00 (s, 1H), 8.29 (d, J = 8.08 Hz, 1H), 7.87–7.90 (m, 2H), 7.26–7.30 (m, 2H), 7.05–7.09 (m, 2H), 6.87–6.90 (m, 2H), 4.12 (q, J = 5.72 Hz, 3H), 3.71 (t, J = 5.84 Hz, 2H), 1.35–1.86 (m, 8H); MS: *m/z* 443.4 (M^+), 444.4 (M + 1); IR (KBr) 3,371 (N–H), 1,674 (C=O) cm^{-1}; Calcd for $C_{23}H_{23}F_2N_3O_4$: C 62.30, H 5.23, N 9.48 %; Found: C 62.27, H 5.20, N 9.41 %.

*Phenyl{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro [4.5]dec-8-yl}carbamate (**32**)*

White color solid: (107 mg, 78 %); mp = 188 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.02 (s, 1H), 8.35 (d, J = 8.04 Hz, 1H), 7.84 (q, J = 8.60 Hz, 2H), 7.53 (q, J = 11.04 Hz, 2H), 7.04–7.08 (m, 3H), 6.86–6.90 (m, 2H), 4.09–4.14 (m, 3H), 3.69 (t, J = 8.32 Hz, 2H), 1.35–1.86 (m, 8H); MS: *m/z* 441.5 (M^+), 442.5 (M + 1); IR (KBr) 3,366 (N–H), 1,669 (C=O) cm^{-1}; Calcd for $C_{23}H_{24}FN_3O_5$: C 62.58, H 5.48, N 9.52 %; Found: C 62.50, H 5.42, N 9.48 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5] dec-8-yl}-4-chlorobenzamide (**33**)*

White color solid: (108 mg, 75 %); mp = 231 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.00 (s, 1H), 8.35 (d, J = 8.04 Hz, 1H), 7.84 (q, J = 1.80 Hz, 2H), 6.93 (q, J = 1.84 Hz, 2H), 7.05–7.09 (m, 2H), 6.87–6.90 (m, 2H), 4.12 (q, J = 5.72 Hz, 3H), 3.71 (t, J = 5.80 Hz, 2H), 1.35–1.86 (m, 8H); MS: *m/z* 459.9 (M^+), 461 (M + 1); IR (KBr) 3,376 (N–H), 1,677 (C=O) cm^{-1}; Calcd for $C_{23}H_{23}ClFN_3O_4$: C 60.07, H 5.04, N 9.14 %; Found: C 60.04, H 5.01, N 9.10 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5] dec-8-yl}-2-(trifluoromethyl) benzamide (**34**)*

White color solid: (112 mg, 73 %); mp = 217 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.99 (s, 1H), 8.43 (d, J = 8.08 Hz, 1H), 7.63–7.77 (m, 3H), 7.47 (d, J = 7.44 Hz, 1H), 7.08 (d, J = 2.40 Hz, 2H), 6.88–6.91 (m, 2H), 4.11 (q, J = 5.12 Hz, 3H), 3.72 (t, J = 5.84 Hz, 2H), 1.44–1.84 (m, 8H); MS: *m/z* 493.5 (M^+), 494.5 (M + 1); IR (KBr) 3,378 (N–H), 1,680 (C=O) cm^{-1}; Calcd for $C_{24}H_{23}F_4N_3O_4$: C 58.42, H 4.70, N 8.52 %; Found: C 58.38, H 4.67, N 8.49 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5] dec-8-yl}-2-chlorobenzamide (**35**)*

White color solid: (106 mg, 74 %); mp = 231 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.00 (s, 1H), 8.38 (d, J = 8.12 Hz, 1H), 7.37–7.49 (m, 4H), 7.08 (q, J = 2.61 Hz, 2H), 6.88–6.91 (m, 2H), 4.11 (q, J = 6.48 Hz, 3H), 3.71 (t, J = 5.88 Hz, 2H), 1.41–1.85 (m, 8H); MS: *m/z* 459.9 (M^+), 461 (M + 1); IR (KBr) 3,372 (N–H), 1,676 (C=O) cm^{-1}; Calcd for $C_{23}H_{23}ClFN_3O_4$: C 60.07, H 5.04, N 9.14 %; Found: C 60.01, H 4.97, N 9.10 %.

*N-{3-[2-(4-fluorophenoxy)ethyl]-2,4-dioxo-1,3-diazaspiro[4.5] dec-8-yl}-4-tert-butylbenzamide (**36**)*

White color solid: (110 mg, 73 %); mp = 249 °C; ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.00 (s, 1H), 8.18 (d, J = 8.16 Hz, 1H), 7.74 (d, J = 8.44 Hz, 2H), 7.45 (d, J = 8.48 Hz, 2H), 7.07 (t, J = 8.76 Hz, 2H), 6.87–6.90 (m, 2H), 4.09–4.12 (m, 3H), 3.71 (t, J = 5.80 Hz, 2H), 1.65–1.75 (m, 8H), 1.28 (s, 9H); MS: *m/z* 481.6 (M^+), 482.6 (M + 1); IR (KBr) 3,366 (N–H), 1,669 (C=O) cm^{-1}; Calcd for $C_{27}H_{32}FN_3O_4$: C 67.34, H 6.70, N 8.73 %; Found: C 67.28, H 6.67, N 8.70 %.

Pharmacology

The anticonvulsant activity was evaluated by MES test and neurotoxicity screening. Albino mice (18–20 g) were procured from National Institute of Nutrition, Hyderabad. The animals were kept in individual cages for 1 week to

acclimatize for laboratory conditions. They were allowed 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.

MES model

Maximal electroshock seizure (MES) model was used in the present study to evaluate the anticonvulsant activity of the drugs on mice. Seizures were induced in mice 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 30 min before the MES 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, p.o) 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 min. The rod diameter was 3.2 cm. Trained animals were given an intraperitoneally (ip) injection of the test compounds at dose of 100 mg/kg. Neurotoxicity was indicated by the inability of animal to maintain equilibrium on the rod for at least 1 min in each of the three trails.

Statistical analysis

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

Results

Pharmacological activity

Anticonvulsant assays were performed for all the synthesized compounds **7–36.** The compounds were injected intraperitoneally into mice and evaluated in the maximal electroshock (MES) and neurotoxicity screens, using dose of 100 mg/kg and the observations were made at four different time intervals (0.5, 1, 2, and 4 h). The results are presented in Tables 1 and 2.

The initial anticonvulsant evaluation showed that all the compounds, except **12** and **19** exhibited significant and protective effects on seizure when compared to vehicle treated mice. Compounds **24**, **27**, and **34** were able to protect seizure effect significantly higher than others and this effect was similar when compared to standard drug treated mice. Compounds **7, 11, 18**, **22**, **23**, **25**, **26**, **28**, **29**, **30**, **31**, **32**, **33**, **35**, and **36** showed moderate protective effect on seizure. Compounds **8**, **9**, **10**, **13**, **14**, **15**, **16**, **17**, **20**, and **21** showed lower protective effect on seizure. Majority of the compounds, except **7**, **12**, and **19** were active in MES tests making them useful for broad spectrum of seizure types.

The bioactivity in MES test was exhibited by sulfonamide derivatives **7–21**, when the hydrogen atom at the position-21 was replaced by a phenyl ring; substitution with small lipophilic group like fluoro, chloro at the ortho, meta, and para position of the phenyl ring was found to decrease the anticonvulsant activity; and strongly electron-withdrawing group like cyano at the 2-position of the phenyl ring caused a decrease in activity. However, substitution with two methyl groups at the phenyl ring of **7, 11,** and **18** resulted in increased activity. Similarly, in amide derivatives such as **22–36**, when the hydrogen atom at the 21-position was replaced by a phenyl ring the activity increased but substitution with small electron-withdrawing group on the phenyl ring resulted in moderate anticonvulsant activity. Compounds **24, 27,** and **34** exhibited activity at 100 mg/kg in MES test. Evidently, this distal hydrophobic center alters the bioavailability of the compounds.

In the neurotoxic experiments, the compounds were administrated by oral route at 100 mg/kg in mice. The tested compounds did not showed neurotoxicity at 0.5 and 1 h. The potent compounds **24, 27,** and **34** did not show neurotoxicity. Compounds **26, 30,** and **31** showed 50 % toxicity after 4 h of oral administration.

Discussion

Chemical synthesis

The synthetic pathway utilized in the preparation of 8-amino-3-[2-(4-fluorophenoxy)ethyl]-1,3-diazaspiro[4.5]decane-2,4-dione derivatives is outlined in Scheme 1. A variety of combinatorial approaches have been described by which pharmacophoric groups were attached to such relativity rigid scaffold. The synthesis begins with the protection of 4-aminocyclohexanol hydrochloride **1** with Boc anhydride using dry dichloromethane as a solvent (Wenya *et al.,* 2010). The oxidation reaction was carried by Swern oxidation (Ting *et al.,* 2005) using dichloromethane, dimethylsulfoxide, oxalyl chloride, and triethylamine as the reagents at −78 °C, to form ketone **3**. Under Bucherer–Bergs condition, construction of azaspiro bicyclic hydantoin **4** was made (Brown *et al.,* 1997; Scott *et al.,* 1997).

Table 1 Results and effects of the compounds **7–36** in the maximal electroshock seizure

Intraperitoneal injection in mice[a]		
Treatment	E/F[b]	% Protection
Control (vehicle)	7.69	–
7	7.61	42.61
8	6.69	13.0
9	7.12	7.41
10	7.31	4.94
11	4.31	43.95
12	7.54	1.14
13	7.34	4.55
14	7.44	3.25
15	6.98	9.23
16	6.64	12.6
17	7.19	6.5
18	4.24	43.12
19	7.58	1.23
20	7.34	4.55
21	7.28	4.51
22	3.02	48.82
23	3.13	59.29
24	1.57	79.58[c]
25	3.19	47.21
26	1.72	48.12
27	1.51	78.62[c]
28	3.76	58.61
29	3.11	55.78
30	1.69	58.02
31	1.58	57.94
32	2.98	56.89
33	3.59	49.83
34	1.59	79.32[c]
35	3.89	49.41
36	3.12	48.15

Values are expressed as mean ± SE, $n = 6$ animals (mice) in each group

[a] 100 mg/kg of dose were administered ip

[b] E/F = extension/flexion [decrease in ratio of extension phase (in seconds)/flexion phase (in seconds)]

[c] $p < 0.05$ when compared to standard drug treated mice

Table 2 Neurotoxicity screening of synthesized compounds **7–36** in the rotorod test

Compound	Neurotoxicity screen	
	2 h	4 h
Standard	0/4	0/4
7	0/4	0/4
8	0/4	0/4
9	0/4	0/4
10	0/4	0/4
11	0/4	0/4
12	0/4	0/4
13	1/4	1/4
14	0/4	0/4
15	0/4	0/4
16	1/4	1/4
17	0/4	0/4
18	0/4	0/4
19	0/4	0/4
20	0/4	0/4
21	0/4	0/4
22	0/4	0/4
23	0/4	0/4
24	0/4	0/4
25	0/4	0/4
26	2/4	2/4
27	0/4	0/4
28	1/4	1/4
29	0/4	0/4
30	2/4	2/4
31	2/4	2/4
32	0/4	0/4
33	1/4	1/4
34	0/4	0/4
35	0/4	0/4
36	0/4	0/4

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

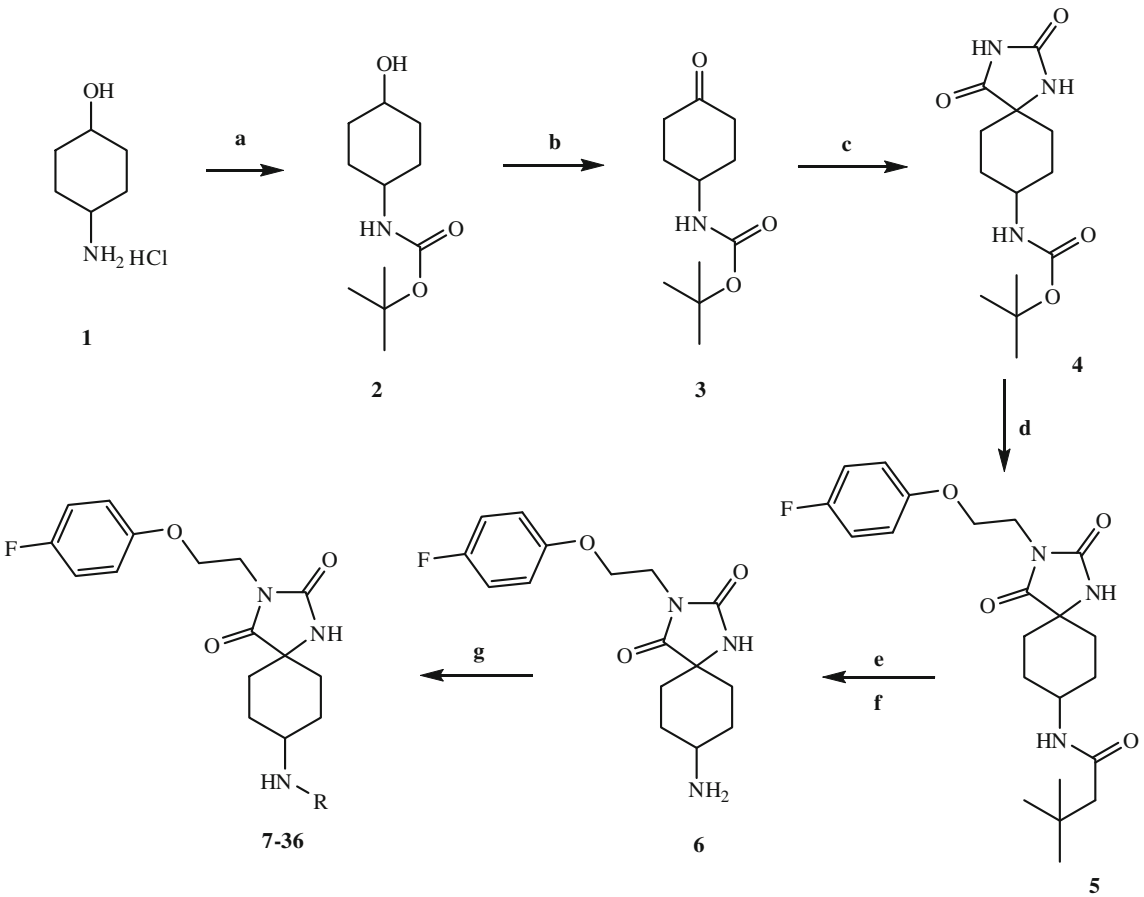
The reaction was carried out in aqueous ethanolic medium using sodium cyanide and ammonium carbonate at elevated temperature. The introduction of the substituent aryl groups at *N*-3 position of hydantoin ring was achieved via selective *N*-alkylation reaction by reacting with aryl halide in the presence of potassium carbonate and acetonitrile solvent (Zha *et al.,* 2004; Carmen *et al.,* 1984). Target key intermediate **6** was accomplished by deprotection of Boc group from compound **5** with dioxane in HCl, followed by basification with sodium carbonate solution (Anna and Andrzej, 2003). The aim of the step 6 was to introduce respective sulfonyl chloride/acid chloride at the *N*-position of azaspiro bicyclic moiety to lead the desired compounds **7–36** for structure–activity relationship (SAR) study (Susumu *et al.,* 1990; Guillaume *et al.,* 2010). This was furnished by normal nucleophilic substitution reactions with good yield. The formation of the hydantoin ring was confirmed by ^{1}H NMR and Mass spectral studies.

The *N*-substitution of substituted 8-amino-3-[2-(4-fluorophenoxy)ethyl]-1,3-diazaspiro [4.5]decane-2,4-dione

with different acid chlorides/sulfonyl chlorides was confirmed by the disappearance of NH_2 group in ^1H NMR and IR spectra. The products obtained were purified by column chromatography using hexane and ethyl acetate (8:2) as an eluents. The absence of NH_2 absorption bands in the IR spectra confirmed that the synthesized compounds were obtained via condensation. The appearance of strong absorption band at around 3,350 cm^{-1} is due to the stretching vibration of N–H band in the synthesized compounds. The proton NMR spectral data agreed with respect to the number of protons and their chemical shifts with the proposed structures. The proton spectral data of the intermediate, hydantoin **6** showed resonance at δ 6.02 ppm (m, 2H, NH_2). In all the synthesized compounds, the above resonance disappeared and additional resonances assigned to the –NH–S=O (d 7.96–7.94 ppm) and –NH–C=O (d 8.42–8.40 ppm) were observed, which confirmed the condensation between the amino group and carbonyl group. The chemical structures, physical data, and yield of all the synthesized compounds are given in Table 3.

Pharmacological activity

In past years the discovery and development of anticonvulsant drugs have been the noticeable research fields. The search for newer compounds combining strong anticonvulsant activity is in progress. Many amide and sulfonamide derivatives have been discovered as potent anticonvulsant drugs and the SAR studies have been reported (Siddiqui *et al.,* 2007). In the present study, the anticonvulsant activity of 30 newly synthesized 8-amino-3-[2-(4-fluorophenoxy)ethyl]-1,3-diazaspiro[4.5]decane-2, 4-dione derivatives of amides and sulfonamides in MES models of seizures in mice was investigated. For several decades, antiepileptic drug research has 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 AED have anticonvulsant activity in at least MES model (Loscher and Schmidt, 1994). Thus, this test may in some way distinguish the potential utility of compounds against



Scheme 1 Synthesis of 8-amino-3-[2-(4-fluorophenoxy)ethyl]-1, 3-diazaspiro[4.5]decane-2,4-dione (**7–36**). Reagent and condition (*a*) Boc anhydride, triethylamine, dichloromethane, rt, 16 h; (*b*) oxalyl chloride, triethylamine, dichloromethane/dimethylsulfoxide, −78 °C, 4 h; (*c*) ammonium carbonate, sodium cyanide, ethanol/water, rt, 24 h, 50 °C, 24 h; (*d*) anhydrous potassium carbonate, 1-(bromomethyl)-2-(difluoromethoxy)benzene, acetonitrile, reflux, 6 h; (*e*) dioxane in HCl, rt 4 h; (*f*) sodium carbonate solution, rt, 1 h; (*g*) R = sulfonyl chloride/acid chloride, triethylamine, dichloromethane, rt, 16 h

Table 3 Chemical structure, yield, and melting point of the compounds **7–36**

Compound	R	Yield (%)	m.p. (°C)
7	3,4-$(H_3C)_2C_6H_3SO_2$	67	180
8	2-$H_3CC_6H_4SO_2$	71	178
9	3- $BrC_6H_4SO_2$	68	204
10	2- $FC_6H_4SO_2$	70	175
11	2,5-$(H_3C)_2C_6H_3SO_2$	70	180
12	4-$H_3CC_6H_4SO_2$	69	176
13	3- $ClC_6H_4SO_2$	70	190
14	4-F,2-$H_3CC_6H_3SO_2$	74	186
15	4-$H_3COC_6H_4SO_2$	67	181
16	4-$H_3CC_6H_4$ CH_2SO_2	74	167
17	3-$FC_6H_4SO_2$	70	175
18	3,5-$(H_3C)_2C_6H_3SO_2$	71	182
19	4-$H_5C_2C_6H_4SO_2$	74	169
20	3-$H_3CC_6H_4SO_2$	72	179
21	2-$NCC_6H_4SO_2$	69	221
22	2-$ClC_6H_4CH_2ONHCO$	71	191
23	4-ClC_6H_4ONHCO	76	198
24	4-$CH_3C_6H_4NHCO$	74	213
25	$C_6H_5CH_2NHCO$	75	200
26	2-FC_6H_4NHCO	72	202
27	3-$CH_3C_6H_4NHCO$	72	213
28	3-ClC_6H_4NHCO	73	231
29	C_6H_5NHCO	76	189
30	3-FC_6H_4NHCO	77	202
31	4-FC_6H_4NHCO	74	202
32	C_6H_5ONHCO	78	188
33	4-ClC_6H_4NHCO	75	231
34	2-$CF_3C_6H_4NHCO$	73	217
35	2-ClC_6H_4NHCO	74	231
36	4-$(CH_3)_3CHC_6H_4NHCO$	73	249

different seizure types. In the present series of compounds, many of them showed good anticonvulsant activity at 100 mg/kg compared to phenytoin. So, there is an increase in anticonvulsant activity by molecular modifications described in the present work. Regarding the sulfonamide and amide series, Dimmock and others (Dimmock and Baker, 1994; Dimmock *et al.,* 2000) have proposed a binding site hypothesis for these compounds eliciting anticonvulsant activity.

A scrutiny for certain selected structures for active anticonvulsants has been shown to possess a hydrophobic unit (R), an electron donor group (D), and hydrogen bond domain unit (HBD). In the present series of compounds, the active compounds possess all the requirements essential for anticonvulsant activity as proposed by Dimmock and others. Thus, our new proposal for a pharmacophore model includes factors which are responsible for bioactivity is shown in Fig. 1. From the results of this study, the following SARs could be derived. On the one/other hand, the substitution pattern at different position of the sulfonamides was compared: dimethyl (**7**, **11**, and **18**) versus fluoro (**10** and **17**) versus chloro (**13**) versus methyl (**8, 12**, and **20**). This emphasizes that the hydrophobic and lipophilic domains in the molecule are responsible for the potent anticonvulsant activity. In addition, comparing the effect of electron donating (**7, 8, 11, 12, 15, 18**, and **19**) and electron-withdrawing (**13** and **21**) groups on the substituted benzene sulfonamides, those with electron donating substitutions showed potent anticonvulsant activity. On the other hand, the presence of methyl group at positions 2 and 5 on the phenyl moiety increased the activity.

The substitution pattern at different positions of the amides was compared: hydrogen (**29**) versus methyl (**24** and **27**) versus fluoro (**26, 30,** and **31**) versus chloro (**28** and **33**). This emphasizes that the hydrophobic and lipophilic

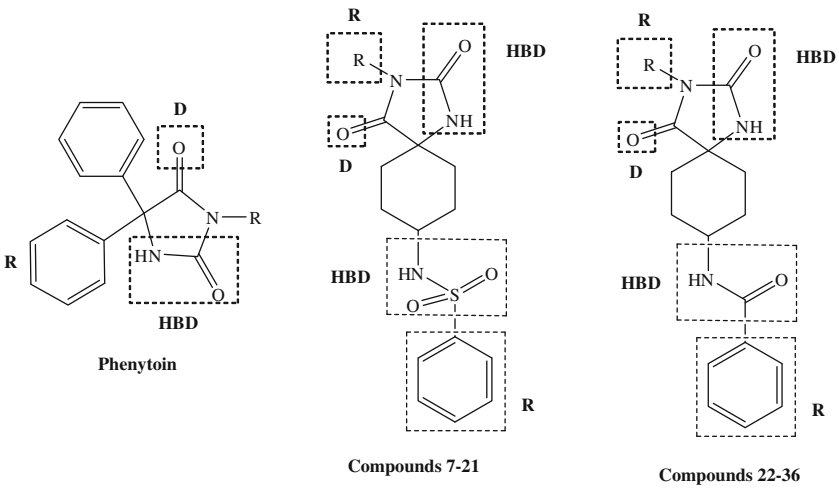


Fig. 1 Anticonvulsant agent showing essential pharmacophoric elements present in their structure, *HBD* hydrogen bond domain; D = electron donor; R = hydrophobic unit

domains in the molecule are responsible for the potent anticonvulsant activity. In addition, comparing the effect of electron donating (**24, 27,** and **36**) and electron-withdrawing (**28** and **33**) groups on substituted amides, those with electron donating substitutions showed potent anticonvulsant activity. On the other hand, the presence of methyl at positions 3 and 4 on the phenyl moiety increased the activity.

The compounds **7–36** practically did not reduce the number of tonic seizures in the MES test. Fluorine and chlorine compounds behaved differently in electrically and chemically induced seizure models, showing no effect in the MES test.

Conclusion

Anticonvulsants have greatly improved the lives of people with epilepsy. Approximately 70 % of the patients can achieve complete freedom from seizures with appropriate treatment (Scheuer and Pedley, 1997). In this study, all the compounds having amide bond showed better anticonvulsant activity compared to sulfonamide. Methyl substituted amides **24** and **23** and dimethyl substituted sulfonamides **7, 11**, and **18** showed significant protective effects on MES induced seizures. Similarly, it is quite apparent that there are at least three parameters for the activity of anticonvulsant drugs, that is, (i) a lipophilic domain, (ii) a distal hydrophobic center, and (iii) an *electron-withdrawing* center (–CN=). Hydrophobic size appears to govern the MES activity. The presence of methyl group showed improved MES activity. Compounds **24, 27,** and **34** without a fluoro or chloro group showed anticonvulsant activity in MES test as compared with compounds **12, 13,** and **17** with fluoro or chloro substituent. The synthesized compounds confirmed the pharmacophore model requirements for the activity such as (A) hydrophobic domain; (B) a lipophilic domain; (C) electron *withdrawing* moiety; and (D) electro donor acceptor in all the synthesized compounds.

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