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Synthesis and Evaluation of 3-[(2,4-Dioxo-1,3,8-triazaspiro[4.6]undec-3-yl)methyl]benzonitrile Derivatives as Potential Anticonvulsants

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New 3-[(2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl)methyl]benzonitrile derivatives **8**–**37** were synthesized and their pharmacological activities were 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) and pentylenetetrazole (PTZ) test. Compounds **11**, **18**, **31**, and **32** showed significant and protective effect on seizure, when compared with the standard drug valproate. The same compounds were found to exhibit advanced anticonvulsant activity as well as lower neurotoxicity than the reference drug. From this study, it is quite apparent that there are at least three parameters for the activity of anticonvulsant drugs, that is, a lipophilic domain, a hydrophobic center, and a two-electron donor.

Keywords: Anticonvulsant / Maximal electroshock seizure / Neurotoxicity / Triazaspirobenzonitriles

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Introduction

Epilepsy, with an incidence of 0.5–1% of the population, is second only to stroke as the most common derangement of the central nervous system [1]. 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 [2, 3]. Over the years, there has been considerable success in the development of novel antiepileptic drugs (AED) along with new improved formulations. These include older ''first generation'' drugs such as carbamazepine, phenobarbitol, valproic acid, and newer, ''second generation'' drugs such as lamotrigine, vigabatrin, tiagabine, topiramate, gabapentin, and levetiracetam [4, 5]. The selection of an antiepileptic drug for the treatment is predicated on its efficacy for the specific type of seizures, tolerability, and safety [6, 7]. Therefore, it is essential to search for newer chemical entities for the treatment of epilepsy. Quite recently, spirohydantoin analogs have become an emerging new class of potent anticonvulsants [8].

Hydantoins, a class of cyclic imides, have been demonstrated to possess good anticonvulsant property [9, 10]. Depending on the nature of substitution on the hydantoin ring, a wide range of other pharmacological properties, e.g., antihypertensive [11], herbicidal [12], antitumor [13], anti-HIV [14], antibacterial [15] and antiviral [16] activities, have also been identified.

In this study, the authors have reported the synthesis of a new class of spirohydantoin analogs, such as 1,3,8-triazaspirohydantoins as anticonvulsant agents.

Results and discussion

Chemistry

The synthetic pathway being utilized in the preparation of 3-[(2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl)methyl]benzonitrile derivatives is outlined as shown in Scheme 1. The synthesis begins by protection [17] of piperidin-4-one hydro-

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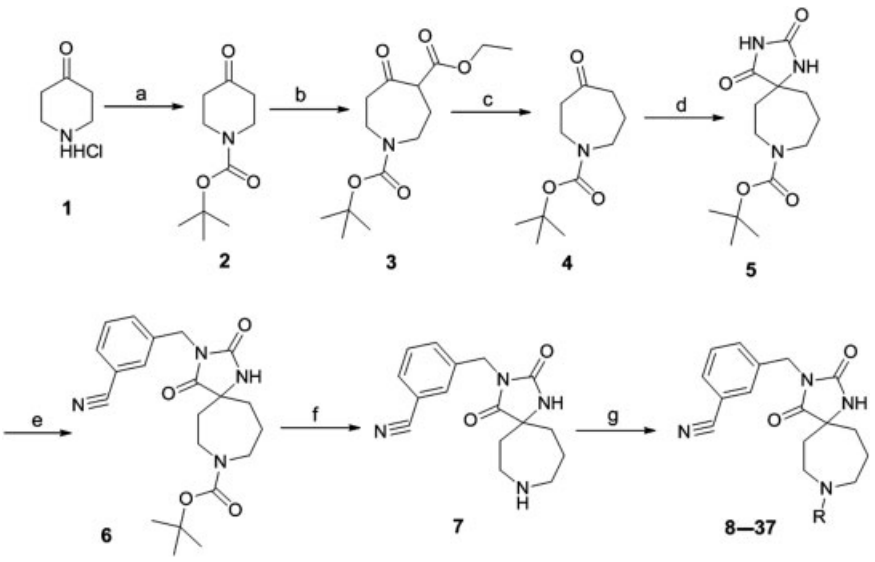

Scheme 1. Synthetic pathway of targeted compounds **8**–**37**. Reagents and conditions: (a) Boc anhydride, triethylamine, dichloromethane, rt, 16 h; (b) BF3 etherate, ethyl diazoacetate, ether, −20°C, 1 h; (c) lithium hydroxide, tetrahydrofuran, water, 80°C, 18 h; (d) ammonium carbonate, sodium cyanide, ethanol/water, 20°C, 24 h, 50°C, 24 h; (e) anhydrous potassium carbonate, 3-(bromomethyl)-benzonitrile, acetonitrile, reflux, 6 h; (f) dioxane in HCl, 20°C, 4 h, sodium carbonate solution, 20°C,1 h; (g) R = sulfonyl chloride/acid chloride, triethylamine, dichloromethane, 20°C, 16 h.

chloride **1** with di-*tert*-butyl dicarbonate (Boc anhydride) using dry dichloromethane as a solvent. The increase of the carbon chain length [18] was carried out by BF_3 etherate, ethyl diazoacetate, and ether as solvent at −20°C to form seven membered ring **3**. The hydrolysis and decarboxylation [19] were carried out using lithium hydroxide, tetrahydrofuran and water as the solvents to get compound **4**. Under Bucherer-Bergs condition [20, 21], construction of azaspiro bicyclic hydantoin **5** was made. The reaction was carried out in aqueous ethanolic media using sodium cyanide and ammonium carbonate at heating temperature. The introduction of the substituent aryl groups at *N*-3 position of hydantoin ring was achieved *via* selective *N*-alkylation reaction by reacting aryl halide in presence of potassium carbonate and acetonitrile solvent [22, 23]. Target key intermediate **7** was accomplished by deprotection of Boc group from compound **6** with dioxane in HCl and followed by basification with sodium carbonate solution [24]. The aim of the **7** was introduced to respective sulfonyl chlorides/acid chlorides at the *N*-position of triazaspiro bicyclic moiety to lead to the desired compounds **8**–**37** for structure–activity relationship (SAR) study [25, 26]. This was furnished by normal condensation reaction with good yield. The formation of the hydantoin ring was confirmed by ^{1}H NMR and mass spectra. The *N*-substitution of substituted 3-[(2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl)methyl]benzonitrile with different acid chlorides and sulfonyl chlorides was confirmed by the disappearance of the NH group in ^{1}H NMR, mass spectra, and IR data. The products obtained were purified by column chromatography using dichloromethane/methanol as an eluent. The chemical structures, physical data, and yield of all the synthesized compounds are given in Table 1.

Pharmacology

In past years, the discovery and development of anticonvulsant drugs has been the noticeable research field. The search for new compounds combining strong anticonvulsant activity is in progress. Many amide and sulfonamide derivatives were prepared as potent anticonvulsant drugs and the SAR studies have been reported [27]. In the present study, the anticonvulsant activities of 30 newly synthesized triazaspirohydantoin derivatives such as amides and sulfonamides were determined using chemically induced PTZ and electrically induced MES model of seizures.

The screening was performed at 1.0 mmol/kg of all synthesized compounds. The results are presented in Table 2. The preliminary anticonvulsant screening showed that compounds **22**, **23**, **24**, **29**, **34**, and **36** were inactive; however, many of these compounds were active against PTZ at 1.0 mmol/kg, among which compounds **11**, **18**, **31**, and **32** presented 100% protection. Compounds **16**, **19**, and **33** offered 66% protection. Compounds **8**, **9**, **10**, **12**, **13**, **15**, **16**, **17**, **19**, **20**, **25**, **26**, **27**, **28**, **30**, **32**, **33**, and **35** showed moderate protective effect in the MES and compounds **11**, **18**, and **31** were found to be 100% protective. Majority of the compounds, except **22**, **23**, **24**, **29**, **34**, and **36**, were active in

Table 1. Chemical structure, yield and melting point of the compounds **8**–**37**.

Compound	R	Yield (%)	m.p. (°C)
8	3,4-$(H_3C)_2C_6H_3SO_2$	67	182
9	2-$H_3CC_6H_4SO_2$	67	180
10	3-$BrC_6H_4SO_2$	65	194
11	2-$FC_6H_4SO_2$	69	188
12	2,5-$(H_3C)_2C_6H_3SO_2$	68	180
13	4-$H_3CC_6H_4SO_2$	69	182
14	3-$ClC_6H_4SO_2$	68	198
15	4-F,2-$H_3CC_6H_3SO_2$	70	192
16	4-$H_3COC_6H_4SO_2$	68	184
17	4-$H_3CC_6H_4$ CH_2SO_2	63	182
18	3-$FC_6H_4SO_2$	67	189
19	3,5-$(H_3C)_2C_6H_3SO_2$	67	180
20	4-$H_5C_2C_6H_4SO_2$	63	184
21	3-$H_3CC_6H_4SO_2$	68	181
22	2-$NCC_6H_4SO_2$	65	224
23	2-$ClC_6H_4CH_2ONHCO$	68	202
24	4-ClC_6H_4ONHCO	65	204
25	4-$CH_3C_6H_4NHCO$	72	186
26	$C_6H_5CH_2NHCO$	67	189
27	2-FC_6H_4NHCO	71	195
28	3-$CH_3C_6H_4NHCO$	70	187
29	3-ClC_6H_4NHCO	70	198
30	C_6H_5NHCO	72	181
31	3-FC_6H_4NHCO	71	196
32	4-FC_6H_4NHCO	68	195
33	C_6H_5ONHCO	73	187
34	4-ClC_6H_4NHCO	68	199
35	2-$CF_3C_6H_4NHCO$	68	214
36	2-ClC_6H_4NHCO	67	199
37	4-$(CH_3)_3CHC_6H_4NHCO$	68	192

Table 2. Anticonvulsant activity of the new synthesized compounds (1.0 mmol/kg) and valproate (300 mg/kg).

Compounds	MES[a] (% protection)	PTZ[b] (% protection)
Valproate	100	100
8	60	47
9	50	33
10	50	30
11	100	100
12	66	39
13	55	31
14	33	0.0
15	65	44
16	67	66
17	56	41
18	100	100
19	62	66
20	59	31
21	43	28
22	0.0	0.0
23	0.0	0.0
24	0.0	0.0
25	56	39
26	47	33
27	66	33
28	57	31
29	0.0	0.0
30	66	45
31	100	100
32	66	100
33	50	66
34	0.0	0.0
35	51	27
36	0.0	0.0
37	45	25

[a] Maximal electroshock seizure test.
[b] Pentylenetetrazol test.

MES tests making them useful for a broad spectrum of seizure types. The bioactivity in the MES test, exhibited by sulfonamide and amide derivatives such as **8**–**37**, when the hydrogen group at the *N*-position was replaced by a phenyl ring, substitution of a small lipophilic group like fluoro group to the phenyl ring increases the anticonvulsant activity of **11**, **18**, **31**, and **32**.

The compounds that exhibited significant activity were subsequently subjected to a quantitative determination of the median effective dose (ED_{50}) and toxic dose (TD_{50}) in mice. Results of the quantitative test for selected compounds, along with the data on the current antiepileptic drugs, are shown in Table 3. Interestingly, the ED_{50} values of the selected compounds were found to be smaller compared to the reference anticonvulsant drug at molar doses. Among the tested compounds, 3-[(2,4-dioxo-8-(3-fluorophenyloyl)-1,3,8-triazaspiro[4.6]-undec-3-yl)methyl]benzonitrile (**31)** was the most active and promising compound in this work. It possessed strong ED_{50} of 0.22 mmol/kg and TD_{50} of 0.70 mmol/kg in the PTZ-induced seizure. This compound **31** was found to exhibit better activity than that of valproate, which is a commercially available drug. It showed a protective index (PI) of 3.07, which was twofold higher compared to valproate having the PI value 1.54. Table 3 reveals an ED_{50} of 0.33 mmol/kg and a TD_{50} of 0.58 mmol/kg for **18**, as compared with 0.47 and 0.68 mmol/kg, respectively, for **32**. Thus, the PI of **18** is slightly greater than the PI of **32**. Compound **32**, with an ED_{50} value of 0.47 mmol/kg, was a little weaker than valproate, and it possessed lower neurotoxicity with PI value of 1.44.

MES and PTZ model of seizures were most widely used in the search for new AEDs, suggesting that it really possesses a good anticonvulsant profile. PTZ is a most frequently used substance as well as an acute experimental model in the preliminary screening to test potential anticonvulsant drugs. The mechanism by which PTZ is believed to exert its action is by acting as an antagonist at the GABA receptor complex. Several biochemical hypotheses have been advanced involving the inhibition of the GABAergic system and the system of

Table 3. Comparison of the anticonvulsant activity (ED_{50}), acute neurotoxic effects (TD_{50}), median lethal dose (LD_{50}), therapeutic and protective indexes of the most promising anticonvulsant new synthesized compounds and standards in mice.

Compound	ED_{50} (mmol/kg)	TD_{50} (mmol/kg)	LD_{50} (mmol/kg)	Therapeutic index	Protective index
Valproate	1.65	2.56	3.00	1.81	1.54
11	0.24	0.56	1.10	4.42	2.26
18	0.33	0.58	0.91	2.73	1.75
31	0.22	0.70	1.17	5.16	3.07
32	0.47	0.68	1.04	2.19	1.44

ED_{50} = median effective dose providing anticonvulsant protection in 50% of mice against pentylenetetrazole (PTZ) induced seizures.
TD_{50} = median toxic dose producing minimal neurological toxicity in 50% of mice subjected to the Chimney test.
LD_{50} = median lethal dose that causes 50% mortality in mice.
Therapeutic index = LD_{50}/ED_{50}.
Protective index = TD_{50}/ED_{50}.

the excitatory amino acid glutamate and aspartate [28]. It was reported that the MES test was correlated to the generalized tonic–clonic seizures and to a certain extent, partial convulsions in humans and, the PTZ test to the generailized absence seizure [29]. So, these two kinds of seizure tests are very meaningful for clinical prediction of the anticonvulsant drug candidates.

For several decades, antiepileptic drugs research has focused on identifying new potential drugs based on their anticonvulsant activity against single acute seizures induced by various stimulators, usually in mice. All established antiepileptic drugs have anticonvulsant activity in at least the MES model [30]. Thus, this test may, in some way distinguish the potential utility of compounds against different seizure types. Hence, there is an increase in anticonvulsant activity by our molecular modifications in the sulfonamide and amide series anticonvulsants. Dimmock *et al.* [31, 32] has proposed a binding site hypothesis for these compounds eliciting anticonvulsant activity.

All the synthesized title compounds were comprised of the essential pharmacophoric elements (Fig. 1) that are necessary for good anticonvulsant activity as suggested by Dimmock and Baker.

A scrutiny for certain selected structures for active anticonvulsants has been shown to possess a hydrophobic unit (R), an electron donor group (D), and a hydrogen donor–acceptor unit (HBD). In the present study, a series of the active compounds possess all these requirements, which are essential for anticonvulsant activity as proposed by Dimmock and others. Thus, our new proposal for a pharmacophore model includes not only three factors but also an additional hydrophobic binding site R shown in Fig. 1 for bioactivity. From the results of this study, the following structure–activity relationships could be derived. On the one hand, the substitution pattern at different positions of the sulfonamide and amides was compared with fluoro (**11**, **18**, **27**, **31**, and **32**) *versus* chloro (**14**, **23**, **24**, **29**, **34**, and **36**). This emphasises that the lipophilic fluorine on the phenyl ring plays a key role in the anticonvulsant activity and chloro decreases the activity. On the other hand, the cyano (**22**) electron withdrawing moiety is practically inactive in both the MES and PTZ tests.

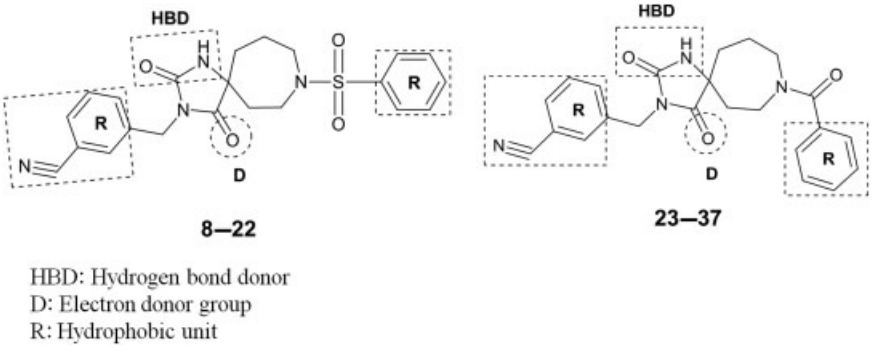

Figure 1. The essential structure elements for the pharmacophore of Dimmock and Baker are indicated by rectangles.

Conclusion

In conclusion, anticonvulsants have greatly improved the lives of people with epilepsy. Approximately 70% of patients can achieve complete freedom from seizures with appropriate treatment [33]. From this study, it is quite apparent that there are at least four parameters for the activity of anticonvulsant drugs, which are, (i) a lipophilic domain, (ii) a distal hydrophobic center, (iii) a two-electron donor system, and (iv) a hydrogen donor–acceptor unit. Lipophilicity appears to govern the MES and PTZ activity. If there is a lipophilic moiety, then MES activity is favored. The synthesized compounds confirmed all the four pharmacophore model requirements for their activity. Compounds **11**, **18**, **31**, and **32** with fluorine group showed anticonvulsant activity in MES and PTZ tests as compared with compounds without fluorine substituents. These four compounds also showed optimum lipophilicity and lesser neurotoxicity than some standard drugs. They may act as lead molecules for future investigations.

Experimental

Chemistry

Melting points were determined in one end open capillary tubes on a Buchi 530 melting point apparatus and are uncorrected. The ^{1}H NMR spectra were recorded on a Bruker Avance −400 MHz NMR instrument using TMS as an internal standard and DMSO-d_6 as a solvent. Chemical shifts are given in parts per million (δ-scale) and coupling constants are given in Hertz. Mass spectra were recorded on a Perkin–Elmer LC–MS PE Sciex API/65 spectrophotometer. IR spectra were recorded using KBr on a 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 pre-coated TLC plates were used. Elemental analysis (C, H, and N) was undertaken with a Perkin–Elmer model 240C analyzer.

*Synthesis of tert-butyl 4-oxopiperidine-1-carboxylate (**2**)*

A mixture of piperidin-4-one hydrochloride **1** (4 g, 29.49 mmol), triethylamine (5.96 g, 58.99 mmol), and Boc anhydride (7.08 g, 32.44 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), dried over Na_2SO_4, and concentrated *in vacuo* to give the crude product which was recrystallized from hexane to get the pure product **2**. White color solid: (5.20 g, 88%); m.p.: 75°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 3.58–3.59 (m, 4H), 2.63–2.64 (m, 4H), 1.44 (s, 9H); MS: *m/z* 199.2 (M^+), 200.2 (M+1); Anal. calcd. for $C_{10}H_{17}NO_3$: C, 60.28; H, 8.60; N, 7.03%. Found: C, 60.20; H, 8.56; N, 7.07%.

*Synthesis of 1-tert-butyl 4-ethyl 5-oxoazepane-1,4-dicarboxylate (**3**)*

A mixture of *tert*-butyl 4-oxopiperidine-1-carboxylate **2** (5.2 g, 26.10 mmol) and dry diethylether (52 mL) was stirred at −20°C. Borontrifluoroetherate (4.81 g, 33.93 mmol) and ethyl diazoacetate (4.50 g, 39.15 mmol) were added dropwise simultaneously, stirred for 1 h. After completion of the reaction (TLC), it was brought to 0°C and 30% potassium carbonate solution was added till it was basic. The reaction mixture was extracted with ethylacetate (3 × 50 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the crude product which was purified by column chromatography over silica gel (60–120 mesh) using hexane/ethylacetate (7:3) as an eluent to get pure product **3**. Colorless liquid: (4.80 g, 64%); ^{1}H NMR: 400 MHz, DMSO-d_6: δ 4.24 (q, J = 9.40 Hz, 2H), 3.73 (t, J = 8.67 Hz, 1H), 3.54–3.56 (m, 4H), 2.56–2.62 (m, 4H), 1.44 (s, 9H), 1.30 (t, J = 9.23 Hz, 3H); MS: *m/z* 270.3 (M^+), 271.3 (M+1); Anal. calcd. for $C_{13}H_{20}NO_5$: C, 61.95; C, 57.76; H, 7.46; N, 5.18%. Found: C, 57.71; H, 7.40; N, 5.12%.

*Synthesis of tert-butyl 4-oxoazepane-1-carboxylate (**4**)*

Reactants of 1-*tert*-butyl 4-ethyl 5-oxoazepane-1,4-dicarboxylate **3** (4.8 g, 16.82 mmol) in tetrahydrofuran (20 mL) and lithium hydroxide (2.41 g, 100.94 mmol) in water (7 mL) were mixed and the reaction mixture was heated to 80°C for 18 h and cooled to room temperature. After completion of the reaction (TLC), the reaction mixture was extracted with ethylacetate (3 × 50 mL), dried over anhydrous Na_2SO_4 and concentrated *in vacuo* to give the crude product which was purified by column chromatography over silica gel (60–120 mesh) using hexane/ethylacetate (6:4) as an eluent to get pure product **4**. White color solid: (3.3 g, 92%); m.p.: 145°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 3.57–3.59 (m, 4H), 2.64–2.66 (m, 4H), 1.66–1.68 (m, 2H), 1.45 (s, 9H); MS: *m/z* 213.3 (M^+), 214.3 (M+1); Anal. calcd. for $C_{11}H_{19}NO_3$: C, 61.95; H, 8.98; N, 6.57%. Found: C, 61.95; H, 8.98; N, 6.57%.

*Synthesis of tert-butyl 2,4-dioxo-1,3,8-triazaspiro[4.6]undecane-8-carboxylate (**5**)*

Accurately weighed amount of *tert*-butyl 4-oxoazepane-1-carboxylate **4** (3.3 g, 15.47 mmol) in ethanol (25 mL) and ammonium carbonate (2.52 g, 34.03 mmol) were taken in water (25 mL). A solution of sodium cyanide (1.6 g, 32.79 mmol) in water (10 mL) was added dropwise and the reaction mixture was stirred at room temperature for 24 h at 50°C and cooled to room temperature. After completion of the reaction (TLC), solid was filtered, washed with water, and dried *in vacuo* to get hydantoin **5**. White color solid: (3.8 g, 86%); m.p.: 219°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 10.63 (s, 1H), 8.41 (s, 1H), 3.45–3.69 (m, 2H), 3.06–3.18 (m, 2H), 1.79–1.82 (m, 3H), 1.67–1.67 (m, 3H), 1.40 (s, 9H); MS: *m/z* 283.3 (M^+), 284.3 (M+1); Anal. calcd. for $C_{13}H_{21}N_3O_4$: C, 55.11; H, 7.47; N, 14.83%. Found: C, 55.07; H, 7.42; N, 14.85%.

*Synthesis of tert-butyl 3-(3-cyanobenzyl)-2,4-dioxo-1,3,8-triazaspiro[4.6]undecane-8-carboxylate (**6**)*

tert-Butyl 2,4-dioxo-1,3,8-triazaspiro[4.6]undecane-8-carboxylate **5** (3.8 g, 13.41 mmol), anhydrous potassium carbonate (2.77 g, 20.11 mmol) and 3-(bromomethyl)benzonitrile (2.89 g, 14.75 mmol) in acetonitrile (40 mL) were refluxed for 6 h and, after completion of the reaction (TLC), cooled to room temperature and filtered. Filtrate was concentrated under vacuum to give the crude product and it was purified by column chromatography over silica gel (60–120 mesh) using chloroform/methanol (9:1) as an eluent to get pure product **6**. White color solid: (4.5 g, 84%); m.p.: 212°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.90 (s, 1H),

7.77 (q, J = 1.20 Hz, 1H), 7.76 (d, J = 1.68 Hz, 2H), 7.55–7.55 (m, 2H), 4.56 (s, 2H), 3.28 (t, J = 6.76 Hz, 2H), 3.14 (q, J = 3.80 Hz, 2H), 2.20 (q, J = 10.12 Hz, 2H), 2.07 (q, J = 6.28 Hz, 2H), 1.85 (t, J = 4.72 Hz, 2H), 1.39 (s, 9H); MS: m/z 398.5 (M^+), 399.5 (M+1); Anal. calcd. for $C_{21}H_{26}N_4O_4$: C, 63.30; H, 6.58; N, 14.06%. Found: C, 63.25; H, 6.51; N, 14.02%.

*Synthesis of 3-[(2,4-dioxo-1,3,8-triazaspiro[4.6]-undec-3-yl)methyl]benzonitrile (**7**)*

Dioxane in HCl (50 mL) was mixed with *tert*-butyl 3-(3-cyanobenzyl)-2,4-dioxo-1,3,8-triazaspiro[4.6]undecane-8-carboxylate **6** (4.5 g, 11.29 mmol) and cooled to 0°C. Then, the mixture was allowed to 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, extracted with dichloromethane (3 × 50 mL). Dried over Na_2SO_4 and concentrated *in vacuo* to get pure product **7**. White color solid: (2.8 g, 83%); m.p.: 198°C; ^{1}H NMR: 400 MHz, DMSO-d_6: 400 MHz, DMSO-d_6: δ 8.90 (s, 1H), 8.72 (s, 1H), 7.77 (q, J = 1.20 Hz, 1H), 7.76 (d, J = 1.68 Hz, 2H), 7.55–7.55 (m, 2H), 4.56 (s, 2H), 3.28 (t, J = 6.76 Hz, 1H), 3.14 (q, J = 3.80 Hz, 3H), 2.20 (q, J = 10.12 Hz, 1H), 2.07 (q, J = 6.28 Hz, 2H), 1.85 (t, J = 4.72 Hz, 3H); MS: m/z 298.3 (M^+), 299.3 (M+1); Anal. calcd. for $C_{16}H_{18}N_4O_2$: C, 64.41; H, 6.08; N, 18.78%. Found: C, 64.37; H, 6.01; N, 18.72%.

*General procedure for the synthesis of triazaspiro sulfonamides (**8**–**22**)*

The reactants such as 3-[(2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl)methyl]benzonitrile **7** (100 mg 0.33 mmol), triethylamine (50.88 mg, 0.50 mmol), and sulfonyl chloride (0.37 mmol) in dichloromethane (4 mL) were mixed and stirred at room temperature for 16 h. After completion of the reaction (TLC), it was quenched with saturated sodium bicarbonate solution, extracted with dichloromethane (3 × 4 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the crude product which was purified by column chromatography on silica employing dichloromethane/methanol (9:1) as an eluent to obtain pure white solid (**8**–**22**).

*3-{[8-(3,4-Dimethylphenyl)sulfonyl-2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl]methyl}benzonitrile (**8**)*

White color solid: (104 mg, 67%); mp = 182°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 7.76 (s, 1H), 7.75 (s, 1H), 7.67 (s, 1H), 7.48–7.57 (m, 4H), 7.37 (d, J = 8.08 Hz, 1H), 4.56 (s, 2H), 3.32–3.54 (m, 1H), 3.20 (s, 1H), 3.07 (q, J = 9.48 Hz, 2H), 2.30 (d, J = 2.32 Hz, 6H), 1.70–2.05 (m, 6H); MS: m/z 466.6 (M^+), 467.6 (M+1); IR (KBr) 1344 (S=O), 1281 (S=O) cm^{-1}; Anal. calcd. for $C_{24}H_{26}N_4O_4S$: C, 61.78; H, 5.62; N, 12.01%. Found: C, 61.78; H, 5.62; N, 12.01%.

*3-{[8-(2-Methylphenyl)sulfonyl-2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl]methyl}benzonitrile (**9**)*

White color solid: (101 mg, 67%); mp = 180°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.86 (s, 1H), 7.77 (s, 3H), 7.53–7.58 (m, 3H), 7.38–7.45 (m, 2H), 4.58 (s, 2H), 3.23–3.58 (m, 4H), 2.53 (s, 3H), 1.79–1.99 (m, 6H); MS: m/z 452.5 (M^+), 453.5 (M+1); IR (KBr) 1345 (S=O), 1284 (S=O) cm^{-1}; Anal. calcd. for $C_{23}H_{24}N_4O_4S$: C, 61.05; H, 5.35; N, 12.38%. Found: C, 61.01; H, 5.33; N, 12.32%.

*3-{[8-(3-Bromophenyl)sulfonyl-2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl]methyl}benzonitrile (**10**)*

White color solid: (112 mg, 65%); mp = 194°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.82 (s, 1H), 7.92 (d, J = 7.44 Hz, 2H), 7.81 (t, J = 6.64 Hz, 1H), 7.76 (d, J = 6.92 Hz, 1H), 7.68 (s, 1H), 7.52–7.61 (m, 3H), 4.57 (s, 2H), 3.60 (q, J = 4.12 Hz, 1H), 3.34 (q, J = 0.72 Hz, 1H), 3.16 (q, J = 5.88 Hz, 2H), 1.74–1.98 (m, 6H); MS: m/z 517.4 (M^+), 518.4 (M+1); IR (KBr) 1342 (S=O), 1280 (S=O) cm^{-1}; Anal. calcd. for $C_{22}H_{21}BrN_4O_4S$: C, 51.07; H, 4.09; N, 10.83%. Found: C, 51.01; H, 4.04; N, 10.80%.

*3-{[8-(2-Fluorophenyl)sulfonyl-2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl]methyl}benzonitrile (**11**)*

White color solid: (106 mg, 69%); mp = 188°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.85 (s, 1H), 7.83 (d, J = 7.28 Hz, 1H), 7.68–7.80 (m, 3H), 7.47–7.58 (m, 3H), 7.41 (t, J = 7.68 Hz, 1H), 4.57 (s, 2H), 3.62 (q, J = 4.00 Hz, 1H), 3.45 (q, J = 0.72 Hz, 1H), 3.24 (q, J = 7.24 Hz, 2H), 1.74–1.99 (m, 6H); MS: m/z 456.5 (M^+), 457.5 (M+1); IR (KBr) 1347 (S=O), 1283 (S=O) cm^{-1}; Anal. calcd. for $C_{22}H_{21}FN_4O_4S$: C, 57.88; H, 4.64; N, 12.27%. Found: C, 57.81; H, 4.58; N, 12.20%.

*3-{[8-(2,5-Dimethylphenyl)sulfonyl-2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl]methyl}benzonitrile (**12**)*

White color solid: (107 mg, 68%); mp = 180°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.84 (s, 1H), 7.76 (d, J = 6.76 Hz, 1H), 7.68 (s, 1H), 7.56 (t, J = 9.76 Hz, 3H), 7.34 (q, J = 7.76 Hz, 2H), 4.58 (s, 2H), 3.59 (q, J = 4.76 Hz, 1H), 3.29–3.38 (m, 1H), 3.23 (q, J = 8.24 Hz, 2H), 2.47 (s, 3H), 2.34 (s, 3H), 1.78–1.98 (m, 6H); MS: m/z 466.6 (M^+), 467.6 (M+1); IR (KBr) 1336 (S=O), 1278 (S=O) cm^{-1}; Anal. calcd. for $C_{24}H_{26}N_4O_4S$: C, 61.78; H, 5.62; N, 12.01%. Found: C, 61.70; H, 5.56; N, 12.04%.

*3-{[8-(2-Methylphenyl)sulfonyl-2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl]methyl}benzonitrile (**13**)*

White color solid: (105 mg, 69%); mp = 182°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.87 (s, 1H), 7.77 (s, 3H), 7.53–7.58 (m, 3H), 7.38–7.45 (m, 2H), 4.58 (s, 2H), 3.60 (q, J = 4.12 Hz, 1H), 3.34 (q, J = 0.72 Hz, 1H), 3.16 (q, J = 5.88 Hz, 2H), 2.34 (s, 3H), 1.74–1.98 (m, 6H); MS: m/z 452.5 (M^+), 453.5 (M+1); IR (KBr) 1345 (S=O), 1284 (S=O) cm^{-1}; Anal. calcd. for $C_{23}H_{24}N_4O_4S$: C, 61.05; H, 5.35; N, 12.38%. Found: C, 61.01; H, 5.30; N, 12.31%.

*3-{[8-(3-Chlorophenyl)sulfonyl-2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl]methyl}benzonitrile (**14**)*

White color solid: (108 mg, 68%); mp = 198°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.82 (s, 1H), 7.81 (d, J = 1.64 Hz, 1H), 7.75–7.80 (m, 3H), 7.66 (q, J = 7.88 Hz, 2H), 7.55 (d, J = 7.04 Hz, 2H), 4.57 (s, 2H), 3.60 (q, J = 3.80 Hz, 1H), 3.36 (q, J = 9.20 Hz, 1H), 3.16 (q, J = 10.40 Hz, 2H), 1.74–1.98 (m, 6H); MS: m/z 472.9 (M^+), 474 (M+1); IR (KBr) 1341 (S=O), 1282 (S=O) cm^{-1}; Anal. calcd. for $C_{22}H_{21}ClN_4O_4S$: C, 55.87; H, 4.48; N, 11.85%. Found: C, 55.87; H, 4.48; N, 11.85%.

*3-{[8-(4-Fluoro-2-methylphenyl)sulfonyl-2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl]methyl}benzonitrile (**15**)*

White color solid: (111 mg, 70%); mp = 192°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.86 (s, 1H), 7.81–7.85 (m, 1H), 7.74–7.77

(m, 2H), 7.68 (q, J = 6.52 Hz, 2H), 7.56 (t, J = 7.00 Hz, 2H), 4.58 (s, 2H), 3.57 (d, J = 3.76 Hz, 1H), 3.32 (q, J = 4.80 Hz, 1H), 3.25 (q, J = 8.00 Hz, 2H), 2.53 (s, 3H), 1.78–1.99 (m, 6H); MS: *m/z* 470.5 (M^+), 471.5 (M+1); IR (KBr) 1346 (S=O), 1287 (S=O) cm^{-1}; Anal. calcd. for $C_{23}H_{23}FN_4O_4S$: C, 58.71; H, 4.93; N, 11.91%. Found: C, 58.65; H, 4.90; N, 11.84%.

*3-{[8-(4-Methoxyphenyl)sulfonyl-2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl]methyl}benzonitrile (**16**)*

White color solid: (107 mg, 68%); mp = 184°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.80 (s, 1H), 7.72 (q, J = 13.88 Hz, 3H), 7.67 (s, 1H), 7.54 (q, J = 9.08 Hz, 2H), 7.13 (d, J = 8.68 Hz, 2H), 4.56 (s, 2H), 3.84 (s, 3H), 3.52 (q, J = 5.32 Hz, 1H), 3.29 (q, J = 8.48 Hz, 1H), 3.14 (q, J = 9.40 Hz, 2H), 1.71–1.96 (m, 6H); MS: *m/z* 468.5 (M^+), 469.5 (M+1); IR (KBr) 1340 (S=O), 1281 (S=O) cm^{-1}; Anal. calcd. for $C_{23}H_{24}N_4O_5S$: C, 58.96; H, 5.16; N, 11.96%. Found: C, 58.91; H, 5.10; N, 11.93%.

*3-{[8-(4-Methylbenzyl)sulfonyl-2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl]methyl}benzonitrile (**17**)*

White color solid: (98 mg, 63%); mp = 182°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.83 (s, 1H), 7.92 (d, J = 7.44 Hz, 2H), 7.81 (t, J = 6.64 Hz, 1H), 7.76 (d, J = 6.92 Hz, 1H), 7.67 (s, 1H), 7.52–7.61 (m, 3H), 4.57 (s, 2H), 4.22 (s, 2H), 3.60 (q, J = 4.12 Hz, 1H), 3.34 (q, J = 0.72 Hz, 1H), 3.16 (q, J = 5.88 Hz, 2H), 2.34 (s, 3H), 1.73–1.97 (m, 6H); MS: *m/z* 466.6 (M^+), 467.6 (M+1); IR (KBr) 1337 (S=O), 1277 (S=O) cm^{-1}; Anal. calcd. for $C_{24}H_{26}N_4O_4S$: C, 61.78; H, 5.62; N, 12.01%. Found: C, 61.71; H, 5.67; N, 12.04%.

*3-{[8-(3-Fluorophenyl)sulfonyl-2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl]methyl}benzonitrile (**18**)*

White color solid: (102 mg, 67%); mp = 189°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.82 (s, 1H), 7.75 (d, J = 6.84 Hz, 1H), 7.68–7.73 (m, 2H), 7.62–7.65 (m, 2H), 7.52–7.60 (m, 3H), 4.57 (s, 2H), 3.59 (q, J = 3.84 Hz, 1H), 3.35 (q, J = 8.92 Hz, 1H), 3.16 (t, J = 13.28 Hz, 2H), 1.73–1.98 (m, 6H);); MS: *m/z* 456.5 (M^+), 457.5 (M+1); IR (KBr) 1347 (S=O), 1283 (S=O) cm^{-1}; Anal. calcd. for $C_{22}H_{21}FN_4O_4S$: C, 57.88; H, 4.64; N, 12.27%. Found: C, 57.82; H, 4.57; N, 12.22%.

*3-{[8-(3,5-Dimethylphenyl)sulfonyl-2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl]methyl}benzonitrile (**19**)*

White color solid: (105 mg, 67%); mp = 180°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.86 (s, 1H), 7.76 (d, J = 6.76 Hz, 1H), 7.67 (s, 1H), 7.56 (t, J = 9.76 Hz, 3H), 7.34 (q, J = 7.76 Hz, 2H), 4.56 (s, 2H), 3.59 (q, J = 4.76 Hz, 1H), 3.29–3.38 (m, 1H), 3.23 (q, J = 8.24 Hz, 2H), 2.46 (s, 3H), 2.33 (s, 3H), 1.78–1.98 (m, 6H); MS: *m/z* 466.6 (M^+), 467.6 (M+1); IR (KBr) 1336 (S=O), 1278 (S=O) cm^{-1}; Anal. calcd. for $C_{24}H_{26}N_4O_4S$: C, 61.78; H, 5.62; N, 12.01%. Found: C, 61.71; H, 5.57; N, 12.04%.

*3-{[8-(4-Ethylphenyl)sulfonyl-2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl]methyl}benzonitrile (**20**)*

White color solid: (99 mg, 63%); mp = 184°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.81 (s, 1H), 7.77 (d, J = 7.00 Hz, 1H), 7.70 (t, J = 3.76 Hz, 3H), 7.52–7.58 (m, 2H), 00007.46 (d, J = 8.20 Hz, 2H), 4.57 (s, 2H), 3.54 (q, J = 3.80 Hz, 1H), 3.33 (t, J = 12.84 Hz, 1H), 3.11 (q, J = 8.52 Hz, 2H), 2.55 (q, J = 1.68 Hz, 2H), 1.73–1.97 (m, 6H), 1.21 (t, J = 7.60 Hz, 3H); MS: *m/z* 466.6 (M^+), 467.6 (M+1); IR (KBr) 1337 (S=O), 1277 (S=O) cm^{-1}; Anal. calcd. for $C_{24}H_{26}N_4O_4S$: C, 61.78; H, 5.62; N, 12.01%. Found: C, 61.72; H, 5.67; N, 12.06%.

*3-{[8-(3-Methylphenyl)sulfonyl-2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl]methyl}benzonitrile (**21**)*

White color solid: (103 mg, 68%); mp = 181°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.82 (s, 1H), 7.78 (t, J = 1.40 Hz, 1H), 7.74 (q, J = 8.32 Hz, 1H), 7.50–7.61 (m, 6H), 4.58 (s, 2H), 3.56 (q, J = 3.84 Hz, 1H), 3.33 (d, J = 6.64 Hz, 1H), 3.12 (q, J = 9.12 Hz, 2H), 2.39 (s, 3H), 1.73–1.98 (m, 6H); MS: *m/z* 452.5 (M^+), 453.5 (M+1); IR (KBr) 1345 (S=O), 1284 (S=O) cm^{-1}; Anal. calcd. for $C_{23}H_{24}N_4O_4S$: C, 61.05; H, 5.35; N, 12.38%. Found: C, 60.92; H, 5.30; N, 12.32%.

*3-{[8-(2-Cyanophenyl)sulfonyl-2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl]methyl}benzonitrile (**22**)*

White color solid: (101mg, 65%); mp = 224°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.84 (s, 1H), 7.81 (d, J = 1.64 Hz, 1H), 7.75–7.80 (m, 3H), 7.66 (q, J = 7.88 Hz, 2H), 7.55 (d, J = 7.04 Hz, 2H), 4.58 (s, 2H), 3.60 (q, J = 3.80 Hz, 1H), 3.36 (q, J = 9.20 Hz, 1H), 3.16 (q, J = 10.40 Hz, 2H), 1.74–1.99 (m, 6H); MS: *m/z* 463.5 (M^+), 464.5 (M+1); IR (KBr) 1341 (S=O), 1282 (S=O) cm^{-1} ; Anal. calcd. for $C_{23}H_{21}N_5O_4S$: C, 59.60; H, 4.57; N, 15.11%. Found: C, 59.56; H, 4.51; N, 15.09%.

*General procedure for the synthesis of triazaspiro carboxylate (**23–37**)*

A mixture of 3-[(2,4-dioxo-1,3,8-triazaspiro[4.6]undec-3-yl)-methyl]benzonitrile **7** (100 mg 0.33 mmol), triethylamine (50.88 mg, 0.50 mmol) and acid chloride (0.37 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, extracted with dichloromethane (3 × 4 mL), dried over Na_2SO_4 and concentrated *in vacuo* to give the crude product which was purified by column chromatography on silica employing dichloromethane/methanol (9:1) as an eluent to obtain pure white solid (**23–37**).

*(2-Chlorobenzyl)-3-(3-cyanobenzyl)-2,4-dioxo-1,3,8-triazaspiro[4.6]undecane-8-carboxylate (**23**)*

White color solid: (106 mg, 68%); mp = 102°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.94 (s, 1H), 7.77 (d, J = 1.48 Hz, 1H), 7.74 (t, J = 23.40 Hz, 1H), 7.48–7.57 (m, 4H), 7.38 (q, J = 2.28 Hz, 2H), 5.17 (s, 2H), 4.59 (s, 2H), 3.60 (q, J = 4.12 Hz, 1H), 3.34 (q, J = 0.72 Hz, 1H), 3.16 (q, J = 5.88 Hz, 2H), 1.74–1.98 (m, 6H); MS: *m/z* 432.5 (M^+), 433.5 (M+1); IR (KBr) 1670 (C=O) cm^{-1}; Anal. calcd. for $C_{24}H_{24}N_4O_4$: C, 66.65; H, 5.59; N, 12.96%. Found: C, 66.60; H, 5.54; N, 12.91%.

*(4-Chlorophenyl)-3-(3-cyanobenzyl)-2,4-dioxo-1,3,8-triazaspiro[4.6]undecane-8-carboxylate (**24**)*

White color solid: (99 mg, 65%); mp = 204°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.96 (s, 1H), 7.78 (d, J = 1.60 Hz, 1H), 7.75 (t, J = 11.40 Hz, 2H), 7.49–7.58 (m, 3H), 7.38 (q, J = 2.28 Hz, 2H), 4.58 (s, 2H), 3.60 (q, J = 4.12 Hz, 1H), 3.34 (q, J = 0.72 Hz, 1H), 3.16 (q, J = 5.88 Hz, 2H), 1.74–1.98 (m, 6H); MS: *m/z* 452.9 (M^+), 454 (M+1); IR (KBr) 1675 (C=O) cm^{-1}; Anal. calcd. for $C_{23}H_{21}ClN_4O_4$: C, 61.00; H, 4.67; N, 12.37%. Found: C, 61.09; H, 4.61; N, 12.33%.

*3-[(2,4-Dioxo-8-(4-methylphenyloyl)-1,3,8-triazaspiro[4.6]undec-3-yl)methyl]benzonitrile (**25**)*

White color solid: (101 mg, 72%); mp = 186°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.96 (s, 1H), 7.76 (d, J = 6.52 Hz, 1H), 7.69 (d, J = 6.96 Hz, 1H), 7.55 (t, J = 9.76 Hz, 2H), 7.30 (d, J = 7.84 Hz, 2H), 7.23 (d, J = 7.84 Hz, 2H), 4.59 (s, 2H), 3.58–3.68 (m, 1H), 3.39–3.42 (m, 1H), 3.32–3.38 (m, 2H), 2.33 (s, 3H), 1.71–1.86 (m, 6H); MS: m/z 416.5 (M^+), 417.5 (M+1); IR (KBr) 1677 (C=O) cm^{-1}; Anal. calcd. for $C_{24}H_{24}N_4O_3$: C, 69.21; H, 5.81; N, 13.45%. Found: C, 69.17; H, 5.74; N, 13.40%.

*3-[(2,4-Dioxo-8-(benzylmethyloyl)-1,3,8-triazaspiro[4.6]undec-3-yl)methyl]benzonitrile (**26**)*

White color solid: (94 mg, 67%); mp = 189°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.98 (s, 1H), 7.77 (d, J = 8.00 Hz, 1H), 7.69 (d, J = 4.00 Hz, 1H), 7.57 (q, J = 4.00 Hz, 2H), 7.39–7.45 (m, 5H), 4.60 (s, 2H), 3.70 (s, 2H), 3.59–3.69 (m, 1H), 3.39–3.42 (m, 1H), 3.32–3.41 (m, 2H), 1.79–1.89 (m, 6H); MS: m/z 416.5 (M^+), 417.5 (M+1); IR (KBr) 1658 (C=O) cm^{-1}; Anal. calcd. for $C_{24}H_{24}N_4O_3$: C, 69.21; H, 5.81; N, 13.45%. Found: C, 69.14; H, 5.70; N, 13.41%.

*3-[(2,4-Dioxo-8-(2-fluorophenyloyl)-1,3,8-triazaspiro[4.6]undec-3-yl)methyl]benzonitrile (**27**)*

White color solid: (100 mg, 71%); mp = 195°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.98 (s, 1H), 7.67–7.78 (m, 2H), 7.46–7.57 (m, 3H), 7.38–7.42 (m, 1H), 7.27–7.32 (m, 2H), 4.60 (s, 2H), 3.57–3.67 (m, 1H), 3.34–3.40 (m, 1H), 3.31–3.32 (m, 2H), 1.72–1.89 (m, 6H); MS: m/z 420.4 (M^+), 421.4 (M+1); IR (KBr) 1671 (C=O) cm^{-1}; Anal. calcd. for $C_{23}H_{21}FN_4O_3$: C, 65.70; H, 5.03; N, 13.33%. Found: C, 65.67; H, 5.06; N, 13.26%.

*3-[(2,4-Dioxo-8-(3-methylphenyloyl)-1,3,8-triazaspiro[4.6]undec-3-yl)methyl]benzonitrile (**28**)*

White color solid: (98 mg, 70%); mp = 187°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.99 (s, 1H), 7.76 (d, J = 6.52 Hz, 1H), 7.69 (d, J = 6.96 Hz, 1H), 7.55 (t, J = 9.76 Hz, 2H), 7.30 (d, J = 7.84 Hz, 2H), 7.23 (d, J = 7.84 Hz, 2H), 4.60 (s, 2H), 3.58–3.69 (m, 1H), 3.39–3.43 (m, 1H), 3.33–3.37 (m, 2H), 2.41 (s, 3H), 1.70–1.89 (m, 6H); MS: m/z 416.5 (M^+), 417.5 (M+1); IR (KBr) 1677 (C=O) cm^{-1}; Anal. calcd. for $C_{24}H_{24}N_4O_3$: C, 69.21; H, 5.81; N, 13.45%. Found: C, 69.18; H, 5.77; N, 13.40%.

*3-[(2,4-Dioxo-8-(3-chlorophenyloyl)-1,3,8-triazaspiro[4.6]undec-3-yl)methyl]benzonitrile (**29**)*

White color solid: (103 mg, 70%); mp = 198°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.95 (s, 1H), 7.76 (d, J = 6.64 Hz, 1H), 7.73 (d, J = 22.72 Hz, 1H), 7.54 (q, J = 6.84 Hz, 4H), 7.48 (d, J = 10.40 Hz, 1H), 7.37 (d, J = 7.36 Hz, 1H), 4.60 (s, 2H), 3.58–3.68 (m, 1H), 3.41–3.44 (m, 1H), 3.32–3.33 (m, 2H), 1.78–1.89 (m, 6H); MS: m/z 436.9 (M^+), 438 (M+1); IR (KBr) 1679 (C=O) cm^{-1}; Anal. calcd. for $C_{23}H_{21}ClN_4O_3$: C, 63.23; H, 4.84; N, 12.82%. Found: C, 63.18; H, 4.79; N, 12.76%.

*3-[(2,4-Dioxo-8-(phenyloyl)-1,3,8-triazaspiro[4.6]undec-3-yl)methyl]benzonitrile (**30**)*

White color solid: (97 mg, 72%); mp = 181°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.97 (s, 1H), 7.76 (d, J = 6.44 Hz, 1H), 7.69 (d, J = 9.12 Hz, 1H), 7.54 (q, J = 6.96 Hz, 2H), 7.38–7.44 (m, 5H), 4.59 (s, 2H), 3.59–3.68 (m, 1H), 3.39–3.42 (m, 1H), 3.32–3.35 (m, 2H), 1.73–1.89 (m, 6H); MS: m/z 402.4 (M^+), 403.4 (M+1); IR (KBr) 1667 (C=O) cm^{-1}; Anal. calcd. for $C_{23}H_{22}N_4O_3$: C, 68.64; H, 5.51; N, 13.92%. Found: C, 68.57; H, 5.45; N, 13.90%.

*3-[(2,4-Dioxo-8-(3-fluorophenyloyl)-1,3,8-triazaspiro[4.6]undec-3-yl)methyl]benzonitrile (**31**)*

White color solid: (100 mg, 71%); mp = 196°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.88 (s, 1H), 7.76 (t, J = 6.48 Hz, 1H), 7.68 (d, J = 9.20 Hz, 1H), 7.46–7.56 (m, 3H), 7.25 (q, J = 5.52 Hz, 3H), 4.59 (s, 2H), 3.89 (d, J = 11.84 Hz, 1H), 3.57 (q, J = 4.00 Hz, 1H), 3.36 (q, J = 5.52 Hz, 2H), 1.71–1.92 (m, 6H); MS: m/z 420.5 (M^+), 421.5 (M+1); IR (KBr) 1672 (C=O) cm^{-1}; Anal. calcd. for $C_{23}H_{21}FN_4O_3$: C, 65.70; H, 5.03; N, 13.33%. Found: C, 65.67; H, 5.07; N, 13.27%.

*3-[(2,4-Dioxo-8-(4-fluorophenyloyl)-1,3,8-triazaspiro[4.6]undec-3-yl)methyl]benzonitrile (**32**)*

White color solid: (96 mg, 68%); mp = 195°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.87 (s, 1H), 7.76 (d, J = 5.20 Hz, 2H), 7.68 (d, J = 7.16 Hz, 2H), 7.47–7.56 (m, 2H), 7.26 (t, J = 8.84 Hz, 2H), 4.59 (s, 2H), 3.90 (d, J = 18.04 Hz, 1H), 3.44 (d, J = 13.64 Hz, 1H), 3.28–3.41 (m, 2H), 1.72–1.87 (m, 6H); MS: m/z 420.5 (M^+), 421.5 (M+1); IR (KBr) 1675 (C=O) cm^{-1}; Anal. calcd. for $C_{23}H_{21}FN_4O_3$: C, 65.70; H, 5.03; N, 13.33%. Found: C, 65.69; H, 5.01; N, 13.29%.

*(Phenyl)-3-(3-cyanobenzyl)-2,4-dioxo-1,3,8-triazaspiro[4.6]undecane-8-carboxylate (**33**)*

White color solid: (102 mg, 73%); mp = 187°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.96 (s, 1H), 7.76 (d, J = 6.44 Hz, 1H), 7.69 (d, J = 9.12 Hz, 1H), 7.54 (q, J = 6.96 Hz, 2H), 7.39–7.45 (m, 5H), 4.58 (s, 2H), 3.59–3.69 (m, 1H), 3.39–3.41 (m, 1H), 3.32–3.36 (m, 2H), 1.74–1.89 (m, 6H); MS: m/z 418.4 (M^+), 419.4 (M+1); IR (KBr) 1667 (C=O) cm^{-1}; Anal. calcd. for $C_{23}H_{22}N_4O_4$: C, 66.02; H, 5.30; N, 13.39%. Found: C, 66.06; H, 5.25; N, 13.31%.

*3-[(2,4-Dioxo-8-(4-chlorophenyloyl)-1,3,8-triazaspiro[4.6]undec-3-yl)methyl]benzonitrile (**34**)*

White color solid: (99 mg, 68%); mp = 199°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.96 (s, 1H), 7.76 (d, J = 5.60 Hz, 1H), 7.68 (d, J = 7.88 Hz, 1H), 7.55 (d, J = 6.80 Hz, 1H), 7.50 (d, J = 8.40 Hz, 3H), 7.43 (q, J = 8.60 Hz, 2H), 4.59 (s, 2H), 3.89 (d, J = 14.44 Hz, 1H), 3.40 (d, J = 10.00 Hz, 1H), 3.30 (d, J = 19.68 Hz, 2H), 1.71–1.87 (m, 6H); MS: m/z 436.9 (M^+), 438 (M+1); IR (KBr) 1679 (C=O) cm^{-1}; Anal. calcd. for $C_{23}H_{21}ClN_4O_3$: C, 63.23; H, 4.84; N, 12.82%. Found: C, 63.16; H, 4.77; N, 12.79%.

*3-[(2,4-Dioxo-8-(2-trifluoromethylphenyloyl)-1,3,8-triazaspiro[4.6]undec-3-yl)methyl]benzonitrile (**35**)*

White color solid: (108 mg, 68%); mp = 214°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.96 (s, 1H), 7.84 (d, J = 56.72 Hz, 1H), 7.68 (d, J = 8.32 Hz, 1H), 7.55 (d, J = 6.80 Hz, 2H), 7.50 (d, J = 8.40 Hz, 2H), 7.43 (q, J = 8.60 Hz, 2H), 4.59 (s, 2H), 3.95 (d, J = 13.96 Hz, 1H), 3.40 (d, J = 13.20 Hz, 1H), 3.31 (d, J = 11.76 Hz, 2H), 1.69–1.87 (m, 6H); MS: m/z 470.4 (M^+), 471.4 (M+1); IR (KBr) 1680 (C=O) cm^{-1}; Anal. calcd. for $C_{24}H_{21}F_3N_4O_3$: C, 61.27; H, 4.50; N, 11.91%. Found: C, 61.21; H, 4.47; N, 11.86%.

*3-[(2,4-Dioxo-8-(2-chlorophenyloyl)-1,3,8-triazaspiro[4.6]undec-3-yl)methyl]benzonitrile (**36**)*

White color solid: (98 mg, 67%); mp = 199°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.95 (s, 1H), 7.77 (d, J = 1.28 Hz, 1H), 7.68 (d,

J = 12.76 Hz, 2H), 7.56 (d, J = 6.80 Hz, 2H), 7.42–7.53 (m, 3H), 4.59 (s, 2H), 4.00 (d, J = 6.84 Hz, 1H), 3.35 (d, J = 16.40 Hz, 1H), 3.22–3.26 (m, 2H), 1.69–1.89 (m, 6H); MS: m/z 436.9 (M^+), 438 (M+1); IR (KBr) 1679 (C=O) cm^{-1}; Anal. calcd. for $C_{23}H_{21}ClN_4O_3$: C, 63.23; H, 4.84; N, 12.82%. Found: C, 63.16; H, 4.77; N, 12.79%.

*3-[(2,4-Dioxo-8-(4-tert-butylphenyloyl)-1,3,8-triazaspiro[4.6]undec-3-yl)methyl]benzonitrile (**37**)*

White color solid: (104 mg, 68%); mp = 192°C; ^{1}H NMR: 400 MHz, DMSO-d_6: δ 8.97 (s, 1H), 7.86 (d, J = 8.48 Hz, 1H), 7.76 (d, J = 6.44 Hz, 1H), 7.69 (s, 1H), 7.56 (t, J = 6.68 Hz, 1H), 7.51 (d, J = 8.52 Hz, 1H), 7.48 (d, J = 36.96 Hz, 1H), 7.32 (t, J = 12.40 Hz, 2H), 4.59 (s, 2H), 3.91 (d, J = 17.00 Hz, 1H), 3.41 (d, J = 19.28 Hz, 1H), 3.33–3.35 (m, 2H), 1.73–1.86 (m, 6H), 1.28 (s, 9H); MS: m/z 470.4 (M^+), 471.4 (M+1); IR (KBr) 1669 (C=O) cm^{-1}; Anal. calcd. for $C_{24}H_{21}F_3N_4O_3$: C, 61.27; H, 4.50; N, 11.91%. Found: C, 61.24; H, 4.47; N, 11.88%.

Pharmacology

The anticonvulsant activity was evaluated by maximal electroshock seizure (MES) and pentylenetetrazole (PTZ) tests. Mice (18–20 g) were procured from the 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 to 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.

Anticonvulsant activity

Maximal electroshock seizure model was used in the present study to evaluate the anticonvulsant activity of the drugs on mice. Seizures were induced in mice by delivering electroshock of 150 mA for 0.2 s by means of a convulsiometer through a pair of ear clip electrodes. The test compounds were administered intraperitoneally (i.p.) at dose of 1.0 mmol/kg 30 min before the seizure induction. The animals were observed closely for 2 min. The percentage of inhibition of seizure relative to control was recorded and calculated [34]. The PTZ test was carried out by the i.p. injection of a convulsant dose of PTZ (100 mg/kg). Seizures and tonic–clonic convulsions, hypnosis, and death were recorded. Sodium valproate (200 mg/kg) was used as reference drug.

The neurological toxicity

The acute neurotoxicity of the selected compounds was assessed according to a method described by Boissier *et al.* [35]. In this test, the animals had to climb backwards up the tube. Motor impairment was indicated by the inability of the animals to climb backward up the transparent tube within 30 s. The neurotoxic effects of the tested compounds were expressed as their median toxic doses (TD_{50} values), representing the doses at which the investigated compounds impaired motor coordination in 50% of the animals.

Quantification studies

Anticonvulsant activity was expressed in terms of the median effective dose (ED_{50}), that is, the dose of drug required to produce the biological responses in 50% of animals; neurotoxicity was expressed as the median toxic dose (TD_{50}). Groups of six mice each were given a range of i.p. doses of the selected drug until at least four points were established in the range of 10–90% seizure protection or minimal observed neurotoxicity [36]. From the plot of these data, the respective ED_{50} and TD_{50} values, slope of the regression line, and standard error of the slope were calculated by means of a computer program based on the method described by Finney [37].

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