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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and potential anticonvulsant activity of new 5,5-cyclopropanespirohydantoin derivatives

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Abstract In this study, sixteen new 5,5-cyclopropanespirohydantoin derivatives were synthesized and tested for anticonvulsant activity using maximal electroshock (MES), subcutaneous pentylenetetrazole screens. Their neurotoxicity was determined by the rotarod test. Two compounds **7f** and **7n** showed promising anticonvulsant activities in both models employed for anticonvulsant evaluation. The most active compound **7f** showed the MES-induced seizures with ED_{50} value of 8.5 mg/kg and TD_{50} value of 381.7 mg/kg after intraperitoneally injection to mice, which provided compound **7f** with a protective index (TD_{50}/ED_{50}) of 44.9 in the MES test.

Keywords 5,5-Cyclopropanespirohydantoin · MES · *sc*PTZ · Neurotoxicity

Introduction

Epilepsy is a group of long-term neurological chronic disorders characterized by the onset of spontaneous convulsant and nonconvulsant seizures that result from neuronal hyperexcitability and hypersynchronous neuronal firing (Fisher *et al.*, 2005). Over 50 million people worldwide are affected by epilepsy, and many of patients suffer from epilepsy over their lifetime (Brown and Holmes, 2001; Yogeeswari *et al.*, 2007). Despite the development of several new antiepileptic drugs (AEDs), the treatment of epilepsy remains still inadequate and the patients suffer from many side effects such as headache, ataxia, hepatotoxicity, gingival hyperplasia and nausea (Perucca *et al.*, 2007; Smith *et al.*, 2007; Kwan and sander, 2004; James and Lesley, 2005; Zaccara *et al.*, 2007; Emilio, 1999).

The commonly used classic AEDs showed that most of these compounds included three essential fragments for anticonvulsant activity, namely an amide function, an electron-donating group and an aryl group (Bojic *et al.*, 1996; Bialer, 2006) (Fig. 1). Using the above data, our laboratory has focused on 5,5-cyclopropanespirohydantoin as a new anticonvulsant pharmacophore, and the previous work exhibited some of these compounds including 5,5-cyclopropanespirohydantoin fragment showed more potent anticonvulsant activity than standard drug phenytoin (He *et al.*, 2010a, b, 2012a, b, c) (Fig. 2).

Above these facts and in continuation of our research program on design and synthesis of new antiepileptic agents, the new 5,5-cyclopropanespirohydantoin derivatives have been obtained in order to search for safer and more effective AEDs (Fig. 3). The obtained results showed that the most potent compounds were **7f** and **7n**, which were chosen for further pharmacological screening (ED_{50} and TD_{50}).

Results and discussion

The synthetic strategy for target compounds (**7a–p**) was executed as outlined in Scheme 1. Compound **1** was obtained by reacting isobutyraldehyde with diethyl

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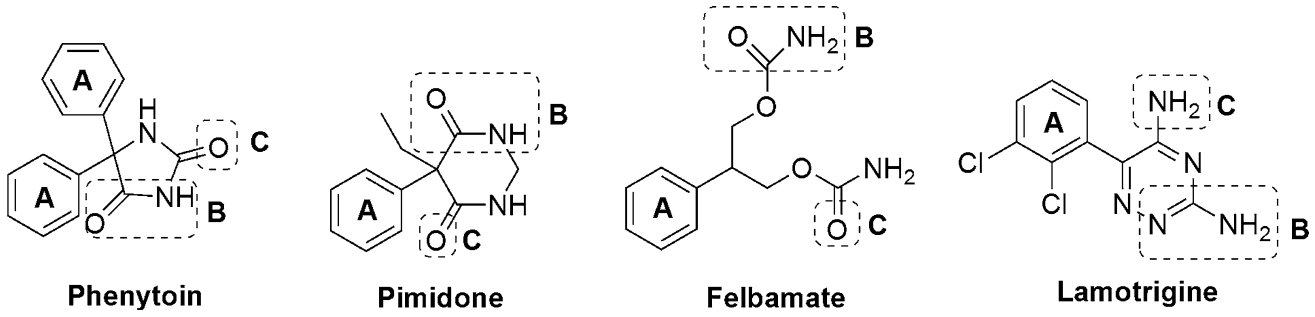


Fig. 1 Structure of anticonvulsant drugs with vital structural fragments. *A* aryl group, *B* amide function, *C* electron-donating group

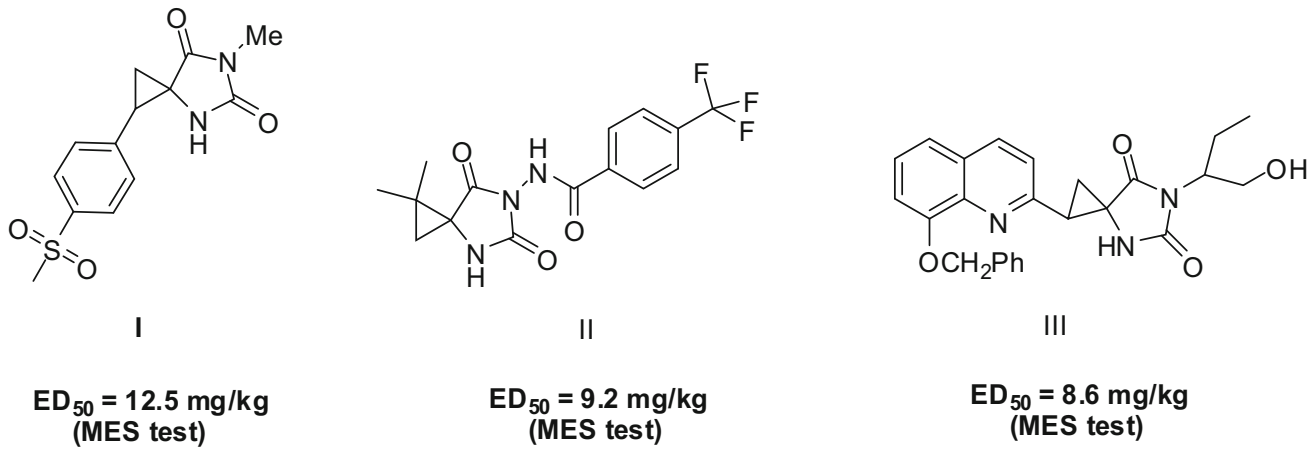
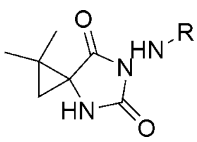


Fig. 2 Structure of compounds **I**, **II** and **III**

Fig. 3 Structure of title compounds

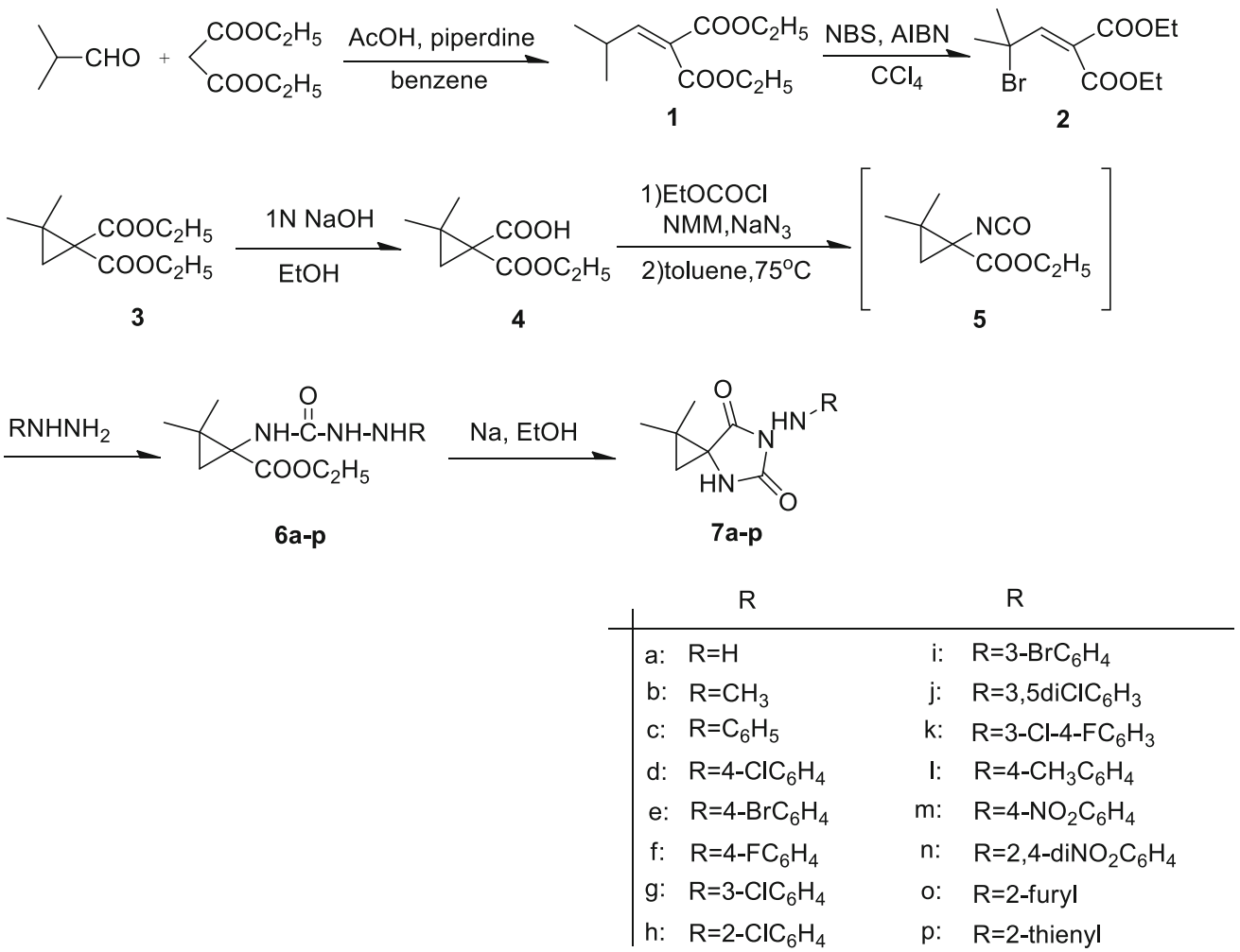


malonate in the presence of acetic acid and piperidine. Then, bromination of compound **1** with azobisisobutyronitrile (AIBN) and N-bromosuccinimide (NBS) in CCl_4 afforded the desired bromo compound **2**. Diethyl 2,2-dimethylcyclopropane-1,1-dicarboxylate **3** was synthesized by a Michael initiated ring closure (MIRC) reaction according to the previous report (VERHÉ *et al.*, 1978). Then, monoester **4** was synthesized after monosaponification in a 1-N NaOH/ethanol (1.1 equiv) solution at room temperature for 12 h (He *et al.*, 2012a, b, c). This was then converted to corresponding acyl azide by using ethyl chloroformate in the presence of *N*-methyl morpholine (NMM) followed by reaction with sodium azide in a one-pot synthesis. The isocyanate **5** was successfully generated by a Curtius reaction in situ on heating the acyl azide in toluene solution at 75 °C. Isocyanate **5** was allowed to react directly with various hydrazines without isolation. The desired compounds **6a**–**p** were readily obtained. Finally, those compounds **6a**–**p** cyclized on treatment with Na (1 equiv) in EtOH and provided N-3-amino-substituted spirohydantoins **7a**–**p** with cyclopropane ring in good yields (yield ≥80 %). The compounds were checked by TLC, ^{1}H NMR, ^{13}C NMR and MS.

All the experimental protocols were carried out following the standard procedures proposed by the NIH anticonvulsant drug development (ADD) program, via the anticonvulsant screening project (ASP). The initial evaluation (phase I) included the maximal electroshock seizure (MES), the subcutaneous pentylenetetrazole (*sc*PTZ) and neurotoxicity.

The compounds **7a**–**p** were administrated intraperitoneally (ip) into the mice using dose of 30, 100 and 300 mg/kg, and the observations were taken at two different time intervals (0.5 and 4.0 h). Neurotoxicity was measured by the rotarod test. The calculated logP (Clogp) values were calculated using the software in ACD Labs 8.0 version. The results are shown in Table 1.

The initial anticonvulsant evaluation indicated that all the compounds were effective in ip MES and/or *sc*PTZ screens. In the MES test, all of the compounds showed protection in half or more of the tested mice after 0.5 h except **7a**, **7b**, **7o** and **7p**, indicative of their ability to prevent seizure spread.



Scheme 1 Steps for the synthesis of compounds **7a–p**

Compounds that were active at 100 mg/kg after 0.5 h in MES test included **7c**, **7d**, **7e** and **7k** indicative of their good ability to protect from seizure spread at a higher dose. Among these compounds, **7e** and **7k** were also active at the same dose after 4.0 h. This showed that these compounds have quick onset and long duration of action at relatively higher dose. From these series, **7f** and **7n** showed anti-MES activity at the dose of 30 mg/kg at time periods 0.5 h, and the most active compound **7n** was active in the MES test at both 0.5 and 4.0 h in the same dose that was equivalent to phenytoin used as reference anticonvulsant drug.

The *sc*PTZ screen showed that compounds **7c**, **7e**, **7f**, **7g**, **7h**, **7k**, **7m** and **7n** were found to be active after 0.5 h and/or 4.0 h, and the other derivatives devoid of anticonvulsant activity. Compounds **7f** and **7n** were active after 4.0 h at the dose of 300 mg/kg, and the other compounds showed no activity.

In the neurotoxicity screen, compounds **7b, 7d**, **7e**, **7f**, **7j**, **7m** and **7n** did not show any neurotoxicity in the maximum dose administered (300 mg/kg). Compounds **7p** revealed neurotoxicity at a dose of 100 mg/kg. The majority of these compounds exhibited less neurotoxic than phenytoin.

Compounds **7f** and **7n** were selected for the quantification of the pharmacological parameters (ED_{50} and TD_{50}). Results of the quantitative test for these compounds, along with the data on the standard drugs (phenytoin, carbamazepine, phenobarbital and valproate), are reported in Table 2. In the mice MES screen, the tested compounds showed a higher protective index (PI) than all the standard drugs. In the mice ip *sc*PTZ screen, compound **7f** gave an ED_{50} of 61.8 mg/kg and a TD_{50} of 381.7 mg/kg, resulting in a high protection index (PI), that is, TD_{50}/ED_{50}, of 5.8 when compared to phenobarbital and valproate.

Table 1 Anticonvulsant activity and neurotoxicity of compounds **7a–n** administered intraperitoneally to mice

Compounds	Intraperitoneal injection in mice[a]						ClogP[b]
	MES[c]		*sc*PTZ[d]		Neurotoxicity[e]		
	0.5 h	4.0 h	0.5 h	4.0 h	0.5 h	4.0 h	
7a	–	300	–	–	300	–	−0.55 ± 0.67
7b	–	300	–	–	–	–	−0.22 ± 0.67
7c	100	–	–	300	–	300	1.53 ± 0.67
7d	100	–	–	–	–	–	2.52 ± 0.68
7e	100	300	100	–	–	–	2.70 ± 0.70
7f	30	100	100	300	–	–	1.98 ± 0.70
7g	300	300	–	300	–	300	2.56 ± 0.68
7h	300	–	300	–	–	300	2.04 ± 0.68
7i	300	–	–	–	–	300	2.74 ± 0.70
7j	300	–	–	–	–	–	3.61 ± 0.68
7k	100	300	–	300	–	–	3.12 ± 0.72
7l	300	–	–	–	–	300	1.99 ± 0.67
7m	300	300	300	–	–	–	1.99 ± 0.68
7n	30	30	300	300	–	–	2.18 ± 0.69
7o	–	–	–	–	300	300	1.37 ± 0.69
7p	–	–	–	–	300	300	1.88 ± 0.69
Phenytoin[f]	30	30	–	–	100	100	2.52 ± 0.38
Ethosuximide[g]	–	–	100	300	–	–	0.38 ± 0.46

[a] 30, 100 and 300 mg/kg of doses were administered ip. The figures in the table indicate the minimal dose whereby bioactivity was demonstrated in half or more of the mice. The animals were examined at 0.5 and 4.0 h after injection was administered. A dash indicates an absence of activity at maximum dose administered (300 mg/kg)

[b] ClogP was calculated using software ACD Labs 8.0 version

[c] Maximal electroshock test

[d] Subcutaneous pentylenetetrazole test

[e] Neurotoxicity screening (rotarod test)

[f] Data from Ref. (Dimmock *et al.*, 1995)

[g] Data from Ref. (Rajak *et al.*, 2009)

Conclusion

In summary, the present studies revealed that numbers of 3-amino-substituted 5,5-cyclopropanespirohydantoin derivatives were effective in the MES and/or scPTZ screens. The most active was 6-(4-fluorophenylamino)-1,1-dimethyl-4,6-diazaspiro[2.4]heptane-5,7-dione (7f) which showed ED50 value of 8.5 mg/kg and a protective index (TD50/ED50) of 44.9 in the MES test in mice. This compound showed greater ED50 and lower TD50 to standard drugs.

Experimental

Chemistry

All chemicals and solvents were purchased from commercial sources. Solvents and reagents were dried and purified according to the literature methods. Melting points were uncorrected and measured on an XT-4 apparatus. ^{1}H-NMR and ^{13}C-NMR spectra were obtained on a Bruker AV400 apparatus in DMSO-d_6 and $CDCl_3$ with TMS as internal standard. The mass spectra (MS) were recorded on AMD-604 mass spectrometer operating at 70 eV.

*Synthesis of 1-(ethoxycarbonyl)-2,2-dimethylcyclopropanecarboxylic acid (**4**)*

Compound **4** was synthesized as described previously in our laboratory (He *et al.*, 2010a, b).

To a solution of compound **3** (10 mmol) in anhydrous ethanol (50 mL) was added 1 N sodium hydroxide (25 mL, 1.1 equiv, 25 mmol), and the resulting mixture was stirred at room temperature for 12 h. EtOH was removed under reduced pressure, water was added to the residue, and the mixture was acidified by means of a saturated $KHSO_4$ and extracted with ethyl acetate (3 × 30 mL). The combined

Table 2 Phase II quantitative anticonvulsant evaluation in mice (test drug administered ip)

Compounds	ED_{50}^{a}		TD_{50}^{b}	PI[c]	
	MES	*sc*PTZ		MES	*sc*PTZ
7f	8.5 (5.8–14.1)[d]	61.8 (41.8–96.2)	381.7 (369.3–416.2)	44.9	5.8
7n	14.3 (10.1–11.9)	126.1 (101.2–153.2)	441.5 (401.2–474.5)	30.8	3.5
Phenytoin[e]	9.5 (8.1–10.4)	>300	65.5 (52.5–72.9)	6.9	<0.22
Carbamazepine[e]	8.8 (5.5–14.1)	>100	71.6 (45.9–135)	8.1	<0.22
Phenobarbital[e]	21.8 (21.8–25.5)	13.2 (5.8–15.9)	69 (62.8–72.9)	3.2	5.2
Valproate[e]	272 (247–338)	149 (123–177)	426 (369–450)	1.6	2.9

Number of animals used: 10; solvent used: polyethylene glycol (0.1 mL, ip)

[a] Dose in milligrams per kilogram body mass

[b] Minimal toxicity which was determined by rotarod test 30 min after the test drug was administered

[c] Protection index (TD_{50}/ED_{50})

[d] Data in parentheses are the 95 % confidence limits

[e] Data from references (Ucar *et al.*, 1998)

extracts were dried over Na_2SO_4 and evaporated to give products **4** without further purification.

*General method for the synthesis of compounds **6a–p***

Compounds **6a–p** were synthesized as described previously in our laboratory (He *et al.*, 2012a, b, c).

Compound **4** (10 mmol) was dissolved in dry THF (30 mL) and cooled to -15 °C. After the addition of $ClCOOC_2H_5$ (11 mmol) and N-methylmorpholine (NMM) (12 mmol), the mixture was stirred for 20 min. A solution of NaN_3 (25 mmol) in H_2O was added and stirred for 1 h at −10 °C. The solution was then diluted with H_2O and extracted with EtOAc. The organic layers were washed with brine, dried over Na_2SO_4 and concentrated under reduced pressure to give crude acyl azide. This crude acyl azide could be further purified by a flash column chromatography (PE-EtOAc, 4:1, $R_f = 0.6$). Purified acyl azide was dissolved in toluene (30 mL), and the resulting solution was heated to 75 °C under stirring. After gas evolution had stopped, toluene was removed under reduced pressure to afford isocyanate **5** as clear oil. This isocyanate **5** was directly used in the next step without further purification. Hydrazine (10 mmol) was added to a stirred suspension of isocyanate **5** in dry THF (40 mL) at room temperature for 1 h. The solvent was removed under reduced pressure to give products **6** and were recrystallized as white solid.

*Ethyl 1-(hydrazinecarboxamido)-2,2-dimethylcyclopropanecarboxylate (**6a**)* Yield: 83 %. White solid. Mp: 125–127 °C. IR (KBr, cm^{-1}): 3376 (N–H), 1712 (C=O), 1664 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 0.80 (d, 1H, $J = 4.92$ Hz, Cpr-CH), 1.14(s, 6H, $2CH_3$), 1.16(t, 3H, $J = 7.04$ Hz, CH_3), 1.51 (d, 1H, $J = 4.92$ Hz, Cpr-CH), 4.06 (q, 2H, $J = 7.04$ Hz, CH_2), 4.96–5.23(m, 2H, NH_2), 7.18(br, 1H, NH), 8.31(brs, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100 Hz): δ 13.1, 18.3, 19.8, 28.4, 30.4, 43.5, 62.3, 163.0, 172.1. ESI-MS: 216.2 ($[M + H]^+$).

*Ethyl 2,2-dimethyl-1-(2-methylhydrazinecarboxamido) cyclopropanecarboxylate (**6b**)* Yield: 85 %. White solid. Mp: 130–131 °C. IR (KBr, cm^{-1}): 3352 (N–H), 1729 (C=O), 1672 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.79 (d, 1H, $J = 4.88$ Hz, Cpr-CH), 1.13 (s, 6H, $2CH_3$), 1.18 (t, 3H, $J = 7.04$ Hz, CH_3), 1.50 (d, 1H, $J = 4.88$ Hz, Cpr-CH), 2.38 (s, 3H, NCH_3), 4.05 (q, 2H, $J = 7.04$ Hz, CH_2), 7.14 (brs, 1H, NH), 8.04 (brs, 1H, NH), 8.89 (brs, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100 Hz): δ 13.2, 18.4, 19.8, 27.9, 31.2, 43.2, 46.3, 61.1, 162.8, 171.4. ESI-MS: 230.2 ($[M + H]^+$).

*Ethyl 2,2-dimethyl-1-(2-phenylhydrazinecarboxamido)cyclopropanecarboxylate (**6c**)* Yield: 85 %. White solid. Mp: 101–102 °C. IR (KBr, cm^{-1}): 3359 (N–H), 1703 (C=O), 1668 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.79 (d, 1H, $J = 4.80$ Hz, Cpr-CH), 1.14 (s, 6H, $2CH_3$), 1.18 (t, 3H, $J = 7.04$ Hz, CH_3), 1.52 (d, 1H, $J = 4.80$ Hz, Cpr-H), 4.05 (q, 2H, $J = 7.04$ Hz, CH_2), 7.22 (brs, 1H, NH), 7.44–7.54 (m, 2H, $C_{2,6}$-ArH), 7.56–7.58 (m, 1H, C_4-ArH), 7.89–8.00 (m, 2H, $C_{3,5}$-ArH), 8.86 (brs, 1H, NH), 9.89 (brs, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100 Hz): δ 13.2, 18.4, 19.8, 27.9, 31.2, 43.2, 61.1, 126.4, 128.4, 129.3, 132.1, 162.8, 171.4. ESI-MS: 292.2 ($[M + H]^+$).

*Ethyl 1-(2-(4-chlorophenyl)hydrazinecarboxamido)-2,2-dimethylcyclopropanecarboxylate (**6d**)* Yield: 88 %. White solid. Mp: 87–89 °C. IR (KBr, cm^{-1}): 3346 (N–H), 1692 (C=O), 1652 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.79 (d, 1H, $J = 4.12$ Hz, Cpr-CH), 1.14 (s, 6H, $2CH_3$), 1.19 (t, 3H, $J = 7.12$ Hz, CH_3), 1.54 (d, 1H, $J = 4.12$ Hz,

Cpr-H), 4.04 (q, 2H, $J = 7.12$ Hz, CH_2), 7.13 (brs, 1H, NH), 7.55 (d, 2H, $J = 8.48$ Hz, $C_{3,5}$-ArH), 7.87 (d, 2H, $J = 8.48$ Hz, $C_{2,6}$-ArH), 8.45 (brs, 1H, NH), 9.56 (brs, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100 Hz): δ 13.2, 18.4, 19.8, 27.9, 31.2, 43.2, 61.1, 126.4, 128.4, 129.3, 132.1, 162.8, 171.4. ESI-MS: 325.2 ($[M + H]^+$), 327.2($[M + 2H]^{2+}$).

Ethyl 1-(2-(4-bromophenyl)hydrazinecarboxamido)-2,2-dimethylcyclopropanecarboxylate ***(6e)*** Yield: 85 %. White solid. Mp: 92–93 °C. IR (KBr, cm^{-1}): 3359 (N–H), 1703 (C=O), 1668 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.78 (d, 1H, $J = 4.42$ Hz, Cpr-CH), 1.12 (s, 6H, $2CH_3$), 1.18 (t, 3H, $J = 7.14$ Hz, CH_3), 1.52 (d, 1H, $J = 4.42$ Hz, Cpr-H), 4.06 (q, 2H, $J = 7.16$ Hz, CH_2), 7.22 (brs, 1H, NH), 7.65 (d, 2H, $J = 8.44$ Hz, $C_{3,5}$-ArH), 7.85 (d, 2H, $J = 8.44$ Hz, $C_{2,6}$-ArH), 8.45 (brs, 1H, NH), 9.88 (brs, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100 Hz): δ 13.4, 18.1, 20.2, 28.1, 33.2, 41.4, 64.2, 122.7, 129.4, 131.2, 134.7, 163.3, 172.2. ESI-MS: 370.1 ($[M + H]^+$), 372.1($[M + 2H]^{2+}$).

Ethyl 1-(2-(4-fluorophenyl)hydrazinecarboxamido)-2,2-dimethylcyclopropanecarboxylate ***(6f)*** Yield: 80 %. White solid. Mp: 132–133 °C. IR (KBr, cm^{-1}): 3377 (N–H), 1743 (C=O), 1672 (C=O).^{1}H-NMR ($CDCl_3$, 400 MHz): 0.78 (d, 3H, $J = 4.86$ Hz, Cpr-CH), 1.12 (s, 6H, $2CH_3$), 1.19 (t, 3H, $J = 7.04$ Hz, CH_3), 1.51 (d, 1H, $J = 4.86$ Hz, Cpr-CH), 4.06 (q, 2H, $J = 7.04$ Hz, CH_2), 7.04 (brs, 1H, NH), 7.27–7.33 (m, 2H, $C_{3,5}$-ArH), 7.92–7.96 (m, 2H, $C_{2,6}$-ArH), 8.88 (brs, 1H, NH), 9.56 (brs, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100 Hz): δ 13.1, 18.4, 19.7, 28.3, 33.6, 42.1, 63.7, 116.2, 128.0, 157.3, 161.6, 163.7, 171.8. ESI-MS: 310.2 ($[M + H]^+$).

Ethyl 1-(2-(3-chlorophenyl)hydrazinecarboxamido)-2,2-dimethylcyclopropanecarboxylate ***(6g)*** Yield: 80 %. White solid. Mp: 95–96 °C. IR (KBr, cm^{-1}): 3376 (N–H), 1721 (C=O), 1668 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.78 (d, 1H, $J = 4.88$ Hz, Cpr-CH), 1.12 (s, 6H, $2CH_3$), 1.18 (t, 3H, $J = 7.08$ Hz, CH_3), 1.46 (d, 1H, $J = 4.88$ Hz, Cpr-CH), 4.08 (q, 2H, $J = 7.08$ Hz, CH_2), 7.10–7.14(m, 4H, $C_{2,4,5,6}$-ArH), 7.25 (brs, 1H, NH), 8.40 (brs, 1H, NH), 9.25 (brs, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100 Hz): δ 13.2, 18.6, 20.2, 28.9, 34.2, 42.5, 64.9, 119.6, 122.1, 128.5, 131.1, 135.7, 150.3, 164.1, 172.2. ESI-MS: 325.2 ($[M + H]^+$), 327.2$[M + 2H]^{2+}$.

Ethyl 1-(2-(2-chlorobenzoyl)hydrazinecarboxamido)-2,2-dimethyl cyclopropanecarboxylate ***(6h)*** Yield: 82 %. White solid. Mp: 101–102 °C. IR (KBr, cm^{-1}): 3374 (N–H), 1712 (C=O), 1677 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.80 (d, 1H, $J = 4.92$ Hz, Cpr-CH), 1.15 (s, 6H, $2CH_3$), 1.17 (t, 3H, $J = 7.04$ Hz, CH_3), 1.52 (d, 1H, $J = 4.92$ Hz, Cpr-CH), 4.06 (q, 2H, $J = 7.04$ Hz), 6.99 (brs, 1H, NH), 7.41–7.52 (m, 4H, ArH), 8.56 (brs, 1H, NH), 9.27 (brs, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100 Hz): δ 12.6, 17.9, 20.0, 28.4, 33.9, 41.9, 65.2, 123.1, 126.8, 128.5, 129.0, 131.4, 146.1, 163.9, 171.8. ESI-MS: 325.2 ($[M + H]^+$).

Ethyl 1-(2-(3-bromophenyl)hydrazinecarboxamido)-2,2-dimethyl cyclopropanecarboxylate ***(6i)*** Yield: 80 %. White solid. Mp: 87–88 °C. IR (KBr, cm^{-1}): 3371 (N–H), 1708 (C=O), 1669 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.77 (d, 1H, $J = 4.88$ Hz, Cpr-CH), 1.13 (s, 6H, $2CH_3$), 1.18 (t, 3H, $J = 7.14$ Hz, CH_3), 1.49 (d, 1H, $J = 4.88$ Hz, Cpr-CH), 4.06 (q, 2H, $J = 7.14$ Hz, CH_2), 7.18 (brs, 1H, NH), 7.25–7.30(m, 4H, $C_{2,4,5,6}$-ArH), 8.15 (brs, 1H, NH), 9.35 (brs, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100 Hz): δ 12.6, 17.9, 20.0, 28.4, 33.9, 41.9, 65.2, 123.1, 126.8, 128.5, 129.0, 131.4, 146.1, 163.9, 171.8. ESI-MS: 370.1 ($[M + H]^+$), 372.1 ($[M + 2H]^{2+}$).

Ethyl 1-(2-(3,5-dichlorophenyl)hydrazinecarboxamido)-2,2-dimethyl cyclopropanecarboxylate ***(6j)*** Yield: 88 %. White solid. Mp: 104–106 °C. IR (KBr, cm^{-1}): 3356 (N–H), 1712 (C=O), 1678 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.77 (d, 1H, $J = 4.74$ Hz, Cpr-CH), 1.14 (s, 6H, $2CH_3$), 1.20 (t, 3H, $J = 7.22$ Hz, CH_3), 1.50 (d, 1H, $J = 4.74$ Hz, Cpr-CH), 4.09 (q, 2H, $J = 7.22$ Hz, CH_2), 6.85 (brs, 1H, NH), 7.92–7.18 (m, 3H), 8.58 (brs, 1H, NH), 9.35 (brs, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100 Hz): δ 12.9, 18.2, 20.3, 27.9, 34.2, 42.5, 66.7, 117.14, 122.82, 135.13, 140.72, 164.2, 171.4. ESI-MS: 361.2 ($[M + H]^+$), 363.2 ($[M + 2H]^{2+}$).

Ethyl 1-(2-(3-chloro-4-fluorophenyl)hydrazinecarboxamido)-2,2-dimethyl cyclopropanecarboxylate ***(6k)*** Yield: 83 %. White solid. Mp: 129–130 °C. IR (KBr, cm^{-1}): 3376 (N–H), 1745 (C=O), 1690 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.78 (d, 1H, $J = 4.88$ Hz, Cpr-CH), 1.12 (s, 6H, $2CH_3$), 1.18 (t, 3H, $J = 7.18$ Hz, CH_3), 1.51 (d, 1H, $J = 4.88$ Hz, Cpr-CH), 4.09 (q, 2H, $J = 7.18$ Hz), 6.56–6.72 (m, 3H, $C_{2,5,6}$-ArH), 7.35 (brs, 1H, NH), 8.29 (brs, 1H, NH), 9.47 (brs, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100 Hz): δ 13.3, 18.8, 20.5, 28.3, 34.8, 41.9, 66.9, 123.1, 126.8, 128.5, 129.0, 131.4, 146.1, 163.5, 172.1. ESI-MS: 344.1 ($[M + H]^+$). 346.1 ($[M + 2H]^{2+}$).

Ethyl 2,2-dimethyl-1-(2-p-tolylhydrazinecarboxamido) cyclopropanecarboxylate ***(6l)*** Yield: 88 %. White solid. Mp: 122–123 °C. IR (KBr, cm^{-1}): 3374 (N–H), 1743 (C=O), 1688 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.80 (d, 1H, $J = 4.88$ Hz, Cpr-CH), 1.15 (s, 6H, $2CH_3$), 1.21 (t, 3H, $J = 7.14$ Hz, CH_3), 1.52 (d, 1H, $J = 4.88$ Hz, Cpr-CH), 2.89(s, 3H, Ar-CH_3), 4.08 (q, 2H, $J = 7.14$ Hz, CH_2), 7.41 (brs, 1H, NH), 7.29 (d, 2H, $J = 8.00$ Hz, $C_{3,5}$-ArH), 7.82 (d, 2H, $J = 8.00$ Hz, $C_{2,6}$-ArH), 8.47 (brs, 1H, NH), 9.72 (brs, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100 Hz): δ 14.5, 20.1.8, 21.5, 22.8, 29.1, 35.9, 42.5, 67.4, 120.8, 130.0, 133.5, 136.1, 156.9, 172.4. ESI-MS: 306.2 ($[M + H]^+$).

*Ethyl 2,2-dimethyl-1-(2-(4-nitrophenyl)hydrazinecarboxamido) cyclopropanecarboxylate (**6m**)* Yield: 80 %. Yellow solid. Mp: 138–139 °C. IR (KBr, cm^{-1}): 3368 (N–H), 1721 (C=O), 1682 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.86 (d, 1H, $J = 4.88$ Hz, Cpr-CH), 1.16 (s, 6H, $2CH_3$), 1.22 (t, 3H, $J = 7.20$ Hz, CH_3), 1.53 (d, 1H, $J = 4.88$ Hz, Cpr-CH), 4.12 (q, 2H, $J = 7.20$ Hz, CH_2), 7.27 (brs, 1H, NH), 7.87 (d, 2H, $J = 8.28$ Hz, $C_{3,5}$-ArH), 8.07 (d, 2H, $J = 8.28$ Hz, $C_{2,6}$-ArH), 8.54 (brs, 1H, NH), 9.43 (brs, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100 Hz): δ 14.9, 20.2, 22.7, 27.4, 29.1, 44.5, 61.4, 117.6, 125.8, 141.3, 147.4, 155.6, 171.2. ESI-MS: 337.2 ($[M + H]^+$).

*Ethyl 1-(2-(2,4-dinitrophenyl)hydrazinecarboxamido)-2,2-dimethylcyclopropanecarboxylate (**6n**)* Yield: 75 %. Yellow solid. Mp: 157–158 °C. IR (KBr, cm^{-1}): 3388 (N–H), 1766 (C=O), 1682 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.80 (d, 1H, $J = 4.92$ Hz, Cpr-CH), 1.16 (s, 6H, $2CH_3$), 1.22 (t, 3H, $J = 7.14$ Hz, CH_3), 1.54 (d, 1H, $J = 4.92$ Hz, Cpr-CH), 4.09 (q, 2H, $J = 7.14$ Hz, CH_2), 7.28 (dd, 1H, $J_1 = 8.84$ Hz, $J_2 = 2.52$ Hz), 7.33 (brs, 1H, NH), 7.46 (d, 1H, $J = 2.52$ Hz), 7.58 (d, 1H, $J = 8.84$ Hz), 8.15 (brs, 1H, NH), 9.36 (brs, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100 Hz): δ 14.2, 21.4, 22.9, 28.3, 30.1, 42.1, 63.2, 118.3, 125.2, 129.3, 131.5, 133.6, 149.9, 157.6, 172.1. ESI-MS: 382.1 ([M + H] +).

*Ethyl 1-(2-(2-furan-2-yl)hydrazinecarboxamido)-2,2-dimethylcyclopropanecarboxylate (**6o**)* Yield: 70 %. White solid. Mp: 92–93 °C. IR (KBr, cm^{-1}): 3287 (N–H), 1736 (C=O), 1698 (C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.78 (d, 1H, $J = 4.26$ Hz, Cpr-CH), 1.17 (s, 6H, $2CH_3$), 1.22 (t, 3H, $J = 7.20$ Hz, CH_3), 1.59 (d, 1H, $J = 4.26$ Hz, Cpr-CH), 4.13 (q, 2H, $J = 7.20$ Hz, CH_2), 7.16–7.24 (m, 3H, ArH), 7.72 (brs, 1H, NH), 8.56 (brs, 1H, NH), 9.67 (brs, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100 Hz): δ 14.1, 20.7, 22.6, 29.1, 31.5, 44.5, 65.7, 111.4, 143.5, 149.7, 149.9, 155.3, 170.9. ESI-MS: 282.3 ([M + H] +).

*Ethyl 1-(2-(2,-thiophen-2-yl)hydrazinecarboxamido)-2,2-dimethylcyclopropanecarboxylate (**6p**)* Yield: 75 %. White solid. Mp: 104–105 °C. IR (KBr, cm^{-1}): 3372 (N–H), 1788(C=O), 1690(C=O). ^{1}H-NMR ($CDCl_3$, 400 MHz): 0.81 (d, 1H, $J = 4.76$ Hz, Cpr-CH), 1.14 (s, 6H, $2CH_3$), 1.25 (t, 3H, $J = 7.24$ Hz, CH_3), 1.54 (d, 1H, $J = 4.76$ Hz, Cpr-CH), 4.06 (q, 2H, $J = 7.24$ Hz, CH_2), 7.28–7.45 (m, 3H, ArH), 7.65 (brs, 1H, NH), 8.85 (brs, 1H, NH), 9.76 (brs, 1H, NH). ^{13}C-NMR ($CDCl_3$, 100 Hz): δ 14.4, 21.2, 22.3, 28.3, 30.9, 42.7, 66.3, 109.2, 139.5, 147.2, 148.1, 154.7, 169.5. ESI-MS: 298.4 ([M + H] +).

*General method for the synthesis of compounds 7**a**–**p***

Compounds **7a**–**p** were synthesized as described previously in our laboratory (He *et al.*, 2012a, b, c)

*6-Amino-1,1-dimethyl-4,6-diazaspiro[2.4]heptane-5,7-dione (**7a**)* Yield: 83 %. White solid. Mp: 112–113 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.19 (d, 1H, $J = 4.22$ Hz, Cpr-H), 1.25 (s, 3H, CH_3), 1.29 (s, 3H, CH_3), 1.44 (d, 1H, $J = 4.22$ Hz, Cpr-H), 4.33 (br, 2H, NH_2), 7.75 (br, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 Hz): δ 18.8, 22.3, 26.1, 26.4, 49.8, 157.7, 175.5. ESI-MS: 169.3 ($[M + H]^+$).

*1,1-Dimethyl-6-(methylamino)-4,6-diazaspiro[2.4]heptane-5,7-dione (**7b**)* Yield: 85 %. White solid. Mp: 133–134 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.17 (d, 1H, $J = 4.08$ Hz, Cpr-H), 1.23 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.44 (d, 1H, $J = 4.08$ Hz, Cpr-H), 3.01(s, 3H, NCH_3), 4.21 (br, 1H, NH), 7.88 (br, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 Hz): δ 18.4, 22.8, 26.2, 26.4, 48.5, 49.3, 155.6, 176.1. ESI-MS: 184.2 ($[M + H]^+$).

*1,1-Dimethyl-6-(phenylamino)-4,6-diazaspiro[2.4]heptane-5,7-dione (**7c**)* Yield: 88 %. White solid. Mp: 115–116 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.18(d, 1H, $J = 3.44$ Hz, Cpr-CH), 1.25(s, 3H, CH_3), 1.28(s, 3H, CH_3), 1.42(d, 1H, $J = 3.44$ Hz, Cpr-CH), 7.28–7.35(m, 2H, $C_{2,6}$-ArH), 7.45–7.48(m, 1H, C_4-ArH), 7.55(brs, 1H, NH), 7.79–7.83(m, 2H, $C_{3,5}$-ArH), 9.88(brs, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 Hz): δ 18.5, 22.6, 27.4, 28.2, 48.5, 126.4, 128.3, 129.5, 132.5, 160.6, 172.1. ESI-MS: 246.3 ($[M + H]^+$).

*6-(4-Chlorophenylamino)-1,1-dimethyl-4,6-diazaspiro[2.4]heptane-5,7-dione (**7d**)* Yield: 80 %. White solid. Mp: 152–153 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.18(d, 1H, $J = 4.92$ Hz, Cpr-CH), 1.27(s, 3H, CH_3), 1.30(s, 3H, CH_3), 1.33(d, 1H, $J = 4.92$ Hz, Cpr-CH), 7.62(d, 2H, $J = 8.54$ Hz, $C_{3,5}$-ArH), 7.66(brs, 1H, NH), 7.91(d, 2H, J = 8.52 Hz, $C_{2,6}$ = ArH), 10.08(brs, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 Hz): δ 18.2, 22.9, 26.9, 28.1, 49.3, 124.2, 130.3, 131.2, 152.7, 161.3, 171.5. ESI-MS: 280.7 ($[M + H]^+$). 282.1 ($[M + 2H]^{2+}$).

*6-(4-Bromophenylamino)-1,1-dimethyl-4,6-diazaspiro[2.4]heptane-5,7-dione (**7e**)* Yield: 80 %. White solid. Mp: 158–159 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.08(d, 1H, $J = 4.96$ Hz, Cpr-CH), 1.17(s, 3H, CH_3), 1.26(s, 3H, CH_3), 1.38(d, 1H, $J = 4.96$ Hz, Cpr-CH), 7.65(d, 2H, $J = 8.52$ Hz, $C_{3,5}$-ArH), 7.73 (brs, 1H, NH), 7.88 (d, 2H, $J = 8.52$ Hz, $C_{2,6}$-ArH), 10.12 (brs, 1H, NH). ^{13}C-NMR

(DMSO-d_6, 100 Hz): δ 18.8, 22.4, 27.4, 28.6, 49.6, 121.3, 132.1, 132.5, 154.9, 162.6, 173.1. ESI-MS: 325.2 ($[M + H]^+$), 327.2 ($[M + 2H]^{2+}$).

*6-(4-Fluorophenylamino)-1,1-dimethyl-4,6-diazaspiro[2.4]heptane-5,7-dione (**7f**)* Yield: 85 %. White solid. Mp: 162–163 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.16 (d, 1H, $J = 5.58$ Hz, Cpr-CH), 1.23(s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.36 (d, 1H, $J = 4.92$ Hz, Cpr-CH), 7.44–7.58 (m, 4H, ArH), 8.80 (brs, 1H, NH), 10.94 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 Hz): δ 18.5, 22.6, 27.8, 28.2, 48.5, 116.3, 128.1, 128.3, 157.2, 161.3, 172.4. ESI-MS: 264.3 ($[M + H]^+$).

*6-(3-Chlorophenylamino)-1,1-dimethyl-4,6-diazaspiro[2.4]heptane-5,7-dione (**7g**)* Yield: 80 %. White solid. Mp: 142–143 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.12 (d, 1H, $J = 4.46$ Hz, Cpr-CH), 1.16 (s, 3H, CH_3), 1.19 (s, 3H, CH_3), 1.39 (d, 1H, $J = 4.46$ Hz, Cpr-CH), 7.41–7.47 (m, 2H, ArH), 7.66–7.71(m, 2H, ArH), 8.88 (brs, 1H, NH), 10.42 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 Hz): δ 18.7, 22.2, 27.1, 28.4, 49.5, 121.0, 122.8, 125.9, 131.3, 135.4, 155.0, 164.4, 172.3. ESI-MS: 280.7 ($[M + H]^+$), 282.7 ($[M + 2H]^{2+}$).

*6-(2-Chlorophenylamino)-1,1-dimethyl-4,6-diazaspiro[2.4]heptane-5,7-dione (**7h**)* Yield: 83 %. White solid. Mp: 154–155 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.16 (d, 1H, $J = 5.52$ Hz, Cpr-CH), 1.24(s, 3H, CH_3), 1.31(s, 3H, CH_3), 1.37 (d, 1H, $J = 5.52$ Hz, Cpr-CH), 7.39–7.56 (m, 4H, ArH), 8.84 (brs, 1H, NH), 11.15 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 Hz): δ 18.4, 22.6, 27.1, 28.3, 49.6, 124.0, 126.8, 127.8, 128.7, 131.3, 150.1, 164.4, 171.0. ESI-MS: 280.7 ($[M + H]^+$).

*6-(3-Bromophenylamino)-1,1-dimethyl-4,6-diazaspiro[2.4]heptane-5,7-dione (**7i**)* Yield: 82 %. White solid. Mp: 109–110 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.13(d, 1H, $J = 4.46$ Hz, Cpr-CH), 1.22 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.34 (d, 1H, $J = 4.46$ Hz, Cpr-CH), 7.41–7.46(m, 2H, ArH), 7.99–8.12(m, 2H, ArH), 8.45 (brs, 1H, NH), 11.72 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 Hz): δ 18.8, 22.3, 26.7, 28.1, 48.8, 121.3, 122.4, 125.6, 132.1, 135.2, 157.1, 166.2, 171.4. ESI-MS: 325.2 ($[M + H]^+$), 327.2 ($[M + 2H]^{2+}$).

*6-(3,5-Dichlorophenylamino)-1,1-dimethyl-4,6-diazaspiro[2.4]heptane-5,7-dione (**7j**)* Yield: 85 %. White solid. Mp: 129–130 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.13 (d, 1H, $J = 5.22$ Hz, Cpr-CH), 1.16 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.38 (d, 1H, $J = 5.22$ Hz, Cpr-CH), 7.54 (s, 2H, $C_{2,6}$-ArH), 7.66 (s, 1H, C_4-ArH), 8.68 (brs, 1H, NH), 11.22 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 Hz): δ 23.7, 27.0, 32.1, 32.8, 51.3, 130.7, 132.5, 139.1, 140.1, 159.9, 177.1. ESI-MS: 315.1 ($[M + H]^+$), 317.1 ($[M + 2H]^{2+}$).

*6-(3-Chloro-4-fluorophenylamino)-1,1-dimethyl-4,6-diazaspiro[2.4]heptane-5,7-dione (**7k**)* Yield: 80 %. White solid. Mp: 136–137 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.09 (d, 1H, $J = 4.78$ Hz, Cpr-CH), 1.14 (s, 3H, CH_3), 1.27 (s, 3H, CH_3), 1.38 (d, 1H, $J = 4.78$ Hz, Cpr-H), 7.66 (s, 1H, C_2-ArH), 8.18 (m, 2H, $C_{4,5}$-ArH), 8.35 (brs, 1H, NH), 10.67 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 Hz): δ 21.4, 23.3, 27.1, 28.5, 51.2, 118.2, 125.4, 129.1, 131.8, 133.8, 150.2, 163.2, 172.0. ESI-MS: 319.1 ($[M + H]^+$), 321.1 ($[M + 2H]^{2+}$).

*1,1-Dimethyl-6-(p-tolylamino)-4,6-diazaspiro[2.4]heptane-5,7-dione (**7l**)* Yield: 90 %. White solid. Mp: 103–104 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.16 (d, 1H, $J = 4.00$ Hz, Cpr-CH), 1.24 (s, 3H, CH_3), 1.30 (s, 3H, CH_3), 1.37 (d, 1H, $J = 4.00$ Hz, Cpr-CH), 2.41(s, 3H, Ar-CH_3), 7.32 (d, 2H, $J = 8.80$ Hz, $C_{3,5}$-ArH), 7.91 (d, 2H, $J = 8.80$ Hz, $C_{2,6}$-ArH), 8.77 (brs, 1H, NH), 10.88 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 Hz): δ 18.5, 22.6, 28.1, 48.5, 55.7, 114.7, 124.6, 127.9, 157.6, 159.4, 172.7. ESI-MS: 260.3 ($[M + H]^+$).

*1,1-Dimethyl-6-(4-nitrophenylamino)-4,6-diazaspiro[2.4]heptane-5,7-dione (**7m**)* Yield: 80 %. Yellow solid. Mp: 128–129 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.16 (d, 1H, $J = 4.12$ Hz, Cpr-CH), 1.25 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.37 (d, 1H, $J = 4.12$ Hz, Cpr-CH), 7.69 (d, 2H, $J = 8.88$ Hz, $C_{3,5}$-ArH), 8.02 (d, 2H, $J = 8.88$ Hz, $C_{2,6}$-ArH), 8.90 (brs, 1H, NH), 10.88 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 Hz): δ 19.0, 22.2, 27.4, 27.7, 48.4, 124.7, 127.4, 139.0, 145.3, 155.1, 172.3. ESI-MS: 291.2 ($[M + H]^+$).

*6-(2,4-Dinitrophenylamino)-1,1-dimethyl-4,6-diazaspiro[2.4]heptane-5,7-dione (**7n**)* Yield: 80 %. Yellow solid. Mp: 144–156 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.13 (d, 1H, $J = 4.12$ Hz, Cpr-CH), 1.22 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.33 (d, 1H, $J = 4.12$ Hz, Cpr-CH), 7.46 (s, 1H, C_3-ArH), 7.91–8.02 (m, 2H, $C_{5,6}$-ArH), 8.53 (brs, 1H, NH), 11.82 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 Hz): δ 19.1, 23.1, 27.6, 27.9, 48.3, 123.9, 125.5, 126.7, 138.8, 142.2, 144.9, 156.3, 171.6. ESI-MS: 335.1 ($[M + H]^+$).

*6-(Furan-2-ylamino)-1,1-dimethyl-4,6-diazaspiro[2.4]heptane-5,7-dione (**7o**)* Yield: 82 %. White solid. Mp: 102–103 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.12 (d, 1H, $J = 4.52$ Hz, Cpr-CH), 1.22 (s, 3H, CH_3), 1.28 (s, 3H, CH_3), 1.34 (d, 1H, $J = 4.52$ Hz, Cpr-CH), 6.68–6.69 (m, 1H, ArH), 7.86–7.87 (m, 1H, ArH), 7.95–8.03 (m, 1H, ArH), 8.89 (brs, 1H, NH), 10.84 (brs, 1H, NH). ^{13}C-NMR

(DMSO-d_6, 100 Hz): δ 19.1, 20.3, 27.9, 28.5, 48.4, 108.6, 111.1, 142.2, 148.9, 163.7, 169.7. ESI-MS: 236.2 ($[M + H]^+$).

*1,1-Dimethyl-6-(thiophen-2-ylamino)-4,6-diazaspiro[2.4]heptane-5,7-dione (**7p**)* Yield: 82 %. White solid. Mp: 109–110 °C. ^{1}H-NMR (DMSO-d_6, 400 MHz): 1.15 (d, 1H, $J = 4.32$ Hz, Cpr-CH), 1.26 (s, 3H, CH_3), 1.32 (s, 3H, CH_3), 1.36 (d, 1H, $J = 4.32$ Hz, Cpr-CH), 7.12–7.14 (m, 1H, ArH), 7.89–7.91 (m, 1H, ArH), 8.01–8.03 (m, 1H, ArH), 8.33 (brs, 1H, NH), 11.54 (brs, 1H, NH). ^{13}C-NMR (DMSO-d_6, 100 Hz): δ 18.9, 22.4, 27.1, 29.6, 49.0, 107.3, 109.2, 137.4, 147.6, 165.4, 170.7. ESI-MS: 252.3 ($[M + H]^+$).

Pharmacology

Anticonvulsant tests were established by the Antiepileptic Drug Development Program, Epilepsy Branch, National Institute of Neurological and Communicative Disorders and Stroke, National Institute of Health in Bethesda, USA (Swinyard *et al.*, 1952). Male Kunming mice (20 ± 2.0 g) were used as experimental animals. All the animals were purchased from Wuhan University Laboratory Animal Center. The animals were approved by the Animal Care Committee of Wuhan University. The tested compounds were injected intraperitoneally to mice as a suspension in 0.5 % methylcellulose at the dose of 30, 100 and 300 mg/kg to one to four mice. The anticonvulsant activity of the tested compounds was detected by both electrical and chemoconvulsant seizure tests. Phenytoin and ethosuximide were chosen as the standard drugs for the comparison. The neurological toxicity was determined in the rotarod test (Krall *et al.*, 1978).

MES—maximal electroshock seizure pattern test

For the MES test according to the method of Swinyard (Swinyard, 1969). In the MES screen, an electrical stimulus of 0.25 s in duration (50 mA in mice) is delivered via corneal electrodes primed with an electrolyte solution containing an anesthetic agent.

PTZ-induced seizure test

This screen utilizes a dose of pentylenetetrazole that produces clonic seizures lasting for a period of at least 5 s in 97 % (CD97) of animals tested (Vamecq *et al.*, 1998), and pentylenetetrazole was dissolved in sufficient 0.9 % saline to allow subcutaneous injections to mice. All the compounds were injected intraperitoneally into mice at the dose levels of 30, 100 and 300 mg/kg with anticonvulsant activity and neurotoxicity assessment at 0.5 and 4 h after administration.

Neurotoxicity screening

Minimal motor impairment was measured in mice by using standardized rotarod test (Dunham *et al.*, 1957). The mouse was placed on a 1 in. diameter knurled plastic rod rotating at 6 rpm. The acute motor impairment can be demonstrated by the inability of the animal to maintain equilibrium on a rod for at least 1 min.

Quantification studies

All quantitative in vivo anticonvulsant and neurotoxicity studies were conducted in terms of the median effective dose (ED_{50}) and the median toxic dose (TD_{50}). Groups of at least eight mice were given with various doses of the candidate drug until at least two points were established between the limits of 100 % protection or minimal toxicity and 0 % protection or minimal toxicity (White *et al.*, 2002). The dose of drug required to produce the desired end point in 50 % of animals in each test, the 95 % confidence interval, the slope of the regression line and standard error of the slope was then calculated by a computer program based on the method described by Finney (Finney, 1971).

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References

Bialer M (2006) New antiepileptic drugs that are second generation to existing antiepileptic drugs. Expert Opin Investig Drugs 15:637–647

Bojic U, Elmazar MMA, Hauck RS, Nau H (1996) Further branching of valproate related carboxylic acids reduces the teratogenic activity, but not the anticonvulsant effect. Chem Res Toxicol 9:866–870

Brown TR, Holmes GI (2001) Primary care: epilepsy. N Engl J Med 344:1145–1151

Dimmock JR, Pandey SN, Quail JW, Pugazhenthi U, Allen TM, Kao GY, Balzarini J, DeClercq E (1995) Evaluation of the semicarbazones, thiosemicarbazones and bis-carbohydrazones of some aryl alicyclic ketones for anticonvulsant and other biological properties. Eur J Med Chem 30:303–314

Dunham NW, Miya TA, Edwards LD (1957) The pharmacological activity of a series of basic esters of mono- and dialkylmalonic acids. J Am Pharm Assoc 46:64–66

Emilio P (1999) The clinical pharmacokinetics of the new antiepileptic drugs. Epilepsia 40(Suppl. 9):7–13

Finney DJ (1971) Statistical logic in the monitoring of reactions to therapeutic drugs. Methods Inf Med 10:237–245

Fisher RS, Emde Boas W, Blume W, Elger C, Genton P, Lee P, Engel J Jr (2005) Epileptic seizures and epilepsy: definitions proposed by the International League against epilepsy (ILAE) and the International Bureau for epilepsy (IBE). Epilepsia 46:470–472

He X, Qiu G, Yang J, Xiao Y, Wu Z, Qiu G, Hu X (2010a) Synthesis and anticonvulsant activity of new 6-methyl-1-substituted-4,6-diazaspiro[2.4]heptane-5,7-diones. Eur J Med Chem 45:3818–3830

He X, Zhong M, Zhang T, Wu W, Wu Z, Yang J, Xiao Y, Pan Y, Qiu G, Hu X (2010b) Synthesis and anticonvulsant activity of N-3-arylamide substituted 5,5-cyclopropanespirohydantion derivatives. Eur J Med Chem 45:5870–5877

He X, Zhong M, Zhang T, Yang J, Wu Z, Xiao Y, Guo H, Qiu G, Hu X (2012a) Synthesis and anticonvulsant activity of 1-(8-(benzyloxy)quinolin-2-yl)-6-substituted-4,6-diazaspiro[2,4]heptane-5,7-diones. Eur J Med Chem 48:338–346

He X, Zhong M, Zhang T, Wu W, Wu Z, Xiao Y, Hu X (2012b) Synthesis and anticonvulsant activity of ethyl 1-(2-arylhydrazinecarboxamido)-2,2-dimethylcyclopropanecarboxylate derivatives. Eur J Med Chem 54:542–548

He X, Zhong M, Yang J, Wu Z, Xiao Y, Guo H, Hu X (2012c) Synthesis and anticonvulsant activity of 1-(2-(8-(benzyloxy)quinolin-2-yl)-1-butyrycyclopropyl)3-substituted urea derivatives. Chem Biol Drug Des 79:771–779

James ES, Lesley J (2005) Zonisamide. CNS Drugs 19:347–367

Krall RL, Penry JK, White BG, Kupferberg HJ, Swinyard EA (1978) Antiepileptic drug development: II. Anticonvulsant drug screening. Epilepsia 19:409–428

Kwan P, Sander JW (2004) The natural history of epilepsy: an epidemiological view. J Neurol Neurosurg Psychiatry 75:1376–1381

Perucca E, French J, Bialer M (2007) Development of new antiepileptic drugs: challenges, incentives, and recent advances. Lancet Neurol 6:793–804

Rajak H, Deshmukh R, Aggarwal N, Kashaw S, Kharya MD, Mishra P (2009) Synthesis of novel 2,5-disubstituted 1,3,4-thiadiazoles for their potential anticonvulsant activity: pharmacophoric model studies. Arch Pharm 342:453–461

Smith M, Wilcox KS, White HS (2007) Discovery of antiepileptic drugs. Neurotherapeutics 4:12–17

Swinyard EA (1969) Laboratory evaluation of antiepileptic drugs. Epilepsia 10:107–119

Swinyard EA, Brown WC, Goodman LS (1952) Comparative assays of antiepileptic drugs in mice and rats. J Pharmacol Exp Ther 106:319–330

Ucar H, Van derpoorten K, Cacciaguerra S, Spampinato S, Stables JP, Depovere P, Isa M, Masereel B, Delarge J, Poupaert JH (1998) Synthesis and anticonvulsant activity of 2 (3H)-benzoxazolone and 2 (3H)-benzothiazolone derivatives. J Med Chem 41:1138–1145

Vamecq J, Lambert D, Poupaert JH, Masereel B, Stables JP (1998) Anticonvulsant activity and interactions with neuronal voltage-dependent sodium channel of analogs of ameltolide. J Med Chem 41:3307–3313

Verhé R, De Kimpe N, De Buyck L, Courtheyn D, Schamp N (1978) Preparation of 2,2-dialkylcyclopropanes geminally substituted with electron-withdrawing groups. Synthesis 1978:530–532

White HS, Woodhead JH, Wilcox KS, Stables JP, Kupferberg HJ, Wolf HH, Levy RH, Mattson RH, Meldrum BS, Perucca E (2002) Antiepileptic drugs. Lippincott Williams & Wilkins Publishers, New York, pp 36–48

Yogeeswari P, Ragavendran JV, Sriram D (2007) Neuropathic pain: strategies in drug discovery and treatment. Expert Opion Drug Discover 2:169–184

Zaccara G, Franciotta D, Perucca E (2007) Idiosyncratic adverse reactions to antiepileptic drugs. Epilepsia 48:1223–1244