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Synthesis and anticonvulsant activity of new 1-[2-oxo-2-(4-phenylpiperazin-1-yl)ethyl]pyrrolidine-2,5-diones

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ABSTRACT

Twenty-two new 1-[2-oxo-2-(4-phenylpiperazin-1-yl)ethyl]pyrrolidine-2,5-diones were synthesized and tested for anticonvulsant activity. Initial anticonvulsant screening was performed using standard maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) screens in mice. Several compounds were tested additionally in the 6-Hz psychomotor seizure model. The neurotoxicity was determined applying the rotarod test. Excluding one compound, all other molecules were found to be effective in at least one seizure model. The most active were 1-(2-oxo-2-{4-[3-(trifluoromethyl) phenyl]piperazin-1-yl}ethyl)pyrrolidine-2,5-dione (**14**), 1-{2-[4-(4-chlorophenyl)piperazin-1-yl]-2-oxo-ethyl}-3-methylpyrrolidine-2,5-dione (**17**), 1-{2-[4-(4-chlorophenyl)piperazin-1-yl]-2-oxoethyl}-3,3-dimethylpyrrolidine-2,5-dione (**23**) and 3,3-dimethyl-1-(2-oxo-2-{4-[3-(trifluoromethyl)phenyl] piperazin-1-yl}ethyl)pyrrolidine-2,5-dione (**26**). These compounds showed high activity in the 6-Hz psychomotor seizure test as well as were active in the maximal electroshock and subcutaneous pentylenetetrazole (**14** and **23**) screens. Initial SAR studies for anticonvulsant activity have been discussed.

Epilepsy is the most prevalent neurological disorder, affecting approximately 50 million people worldwide.[1,2] It is characterized by recurrent seizures of cerebral origin, presenting with episodes of sensory, motor or autonomic phenomenon with or without loss of consciousness. Even though significant advances have been made in epilepsy research, convulsions in 25% of epileptics are inadequately controlled by standard drug therapy.[3,4] In recent times several new drugs, for example, levetiracetam, felbamate, lamotrigine, gabapentin, and topiramate, have been approved to treat epilepsy. Although these drugs have been shown to be effective in epileptic syndromes in a number of patients, their efficacy does not appear to be superior to that of the established antiepileptic drugs. Therefore the ideal antiepileptic should prevent different types of seizures without producing side effects that affect adversely patients' quality of life. Taking into consideration the above continued search for safer, more effective and possibly antiepileptogenic drugs is urgently necessary. The incomplete information on the pathogenesis of human epilepsy and the complex mechanism of action of majority antiepileptic drugs makes it difficult to use rational methodologies of discovery. Conceptually, there are two different methods of obtaining new anticonvulsants namely knowledge-based approaches and screening approaches.[5] Knowledge-based approaches rely on the use of different pharmacophores that were established through the analysis of structural characteristics of clinically effective AEDs as well as other anticonvulsant active compounds. Serendipitous approaches involve a comprehensive screening process that utilizes rodent models. Traditionally, most screening programs employ mice and rats to assess efficacy against either electrically (e.g., maximal electroshock, MES) or chemically (e.g., pentylenetetrazol, bicuculline, or picrotoxin) induced seizures.[6,7] The number of new AEDs currently available, or in development, for the management of epilepsy certainly attests to the success of this approach. However, this method may overlook novel compounds that would be uniquely effective in the therapy-resistant population. One example supporting this hypothesis is provided by levetiracetam, which has demonstrated efficacy in refractory human partial epilepsies. It was found to be inactive against MES and PTZ seizures even at high doses, whereas showed high effectiveness in the 6-Hz psychomotor seizure model of partial epilepsy.[8] Thus it is suggested that the 6-Hz model might be capable for identifying anti-seizure agents with a novel spectrum of activity and unknown mechanism of anticonvulsant action.

It is well documented that one of the important core fragments of anticonvulsants is defined by nitrogen heterocyclic system, usually lactam or imide, with attached phenyl or alkyl groups.[9,10] This common template is found in the structures of first generation anticonvulsants such as ethosuximide or phenytoin, among the newest drugs, for example, levetiracetam or compounds being currently under advanced clinical trials—brivaracetam and seletracetam.

Previous researches from our laboratory have identified pyrrolidine-2,5-diones differently substituted at position-1 and -3 as

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targets for new antiepileptic drugs. Many of them were effective in the maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) screens that are recognized as the 'gold standards' in the early stages of testing.[11–15] The structure–activity relationships studies (SAR) have demonstrated the potent anticonvulsant activity exclusively among the pyrrolidine-2,5-diones containing at the imide nitrogen atom phenylpiperazines with highly electronegative chloro, fluoro or trifluoromethyl substituents. In view of the above results, the present work is an attempt to obtain compounds active not only in the standard MES or *sc*PTZ tests but also in 6-Hz psychomotor seizure model. Therefore a library of twenty-two compounds with the pyrrolidine-2,5-dione system as a core fragment have been synthesized (**9**–**30**). Based on the structures of levetiracetam or brivaracetam, which are model AEDs active in the 6-Hz screen, the respective phenylpiperazines have been introduced as an amide function. Moreover several derivatives with one or two alkyl substituents at position-3 of pyrrolidine-2,5-dione have been obtained. These compounds may be regarded as analogs of brivaracetam with modified propyl group.

The structures of designed compounds along with levetiracetam and brivaracatam are shown in Figure 1.

The intermediates **5**–**8** and final compounds **9**–**30** were synthesized according to Scheme 1. First, the condensation reaction of commercially purchased succinic acid (**1**), 2-methylsuccinic acid (**2**) or 2,2-dimethyl- (**3**) and 2,2-diethyl- (**4**) succinic acids obtained using procedures described elsewhere,[16] with 2-aminoacetic acid yielded in corresponding intermediates **5**–**8**. In the next step **5**–**8** were converted to final compounds (**9**–**30**) in reaction with appropriate secondary amines in the presence of carbonyldiimidazole (CDI). The purities of all molecules were assessed by TLC and gradient HPLC chromatography. The structures were assigned on the basis of ^{1}H NMR, mass spectra and elemental (C, H, N) analysis.

The anticonvulsant activities of **9**–**30** were determined in the maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) tests, as two routine models, after intraperitoneal (ip) injection of the test compounds in mice at doses of 30, 100, and 300 mg/kg.[6] An observation was carried out at two different time intervals—0.5 h and 4 h. The acute neurological toxicity (NT) was determined in the minimal motor impairment rotorod screen. The results along with the data for standard drugs phenytoin and ethosuximide (model substances for MES and *sc*PTZ tests, respectively), are shown in Table 1.

Except of **27** all other compounds showed activity in the MES screen at 100 or 300 mg/kg mainly 0.5 h after administration indicative of their ability to prevent seizure spread.

Eight molecules **10**–**12**, **14**–**16**, **20**, **23**, and **25** were also active at 300 mg/kg in the subcutaneous pentylenetetrazole (*sc*PTZ) screen that enable to identify compounds elevating seizure threshold. In the acute neurotoxicity screen **9**, **13**, **19**, **21**, **22**, **24**, **26**, **27**, **29**, and **30** did not show neurotoxicity at the maximum dose administered (300 mg/kg). The other molecules caused motor impairment at doses of 100 or 300 mg/kg. There was no separation between the anticonvulsant and neurotoxic doses (300 mg/kg) for compounds **16**, **18**, and **28**. The other showed activity in lower doses then neurotoxic properties.

On the basis of mice ip data compounds active at 100 mg/kg in the electrically induced seizures were examined for anticonvulsant activity (MES screen) and neurotoxicity after po. administration in rats at a dose of 30 mg/kg. This screen discloses the time of onset, the approximate time of peak effect (TPE) and the duration of

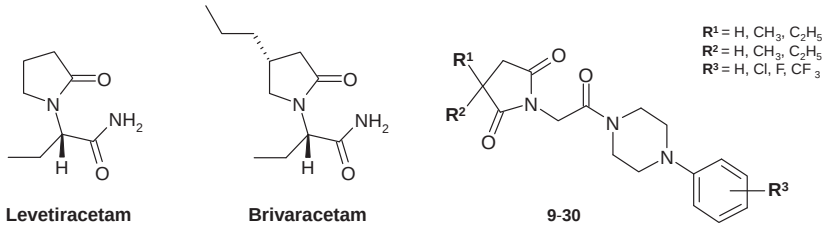
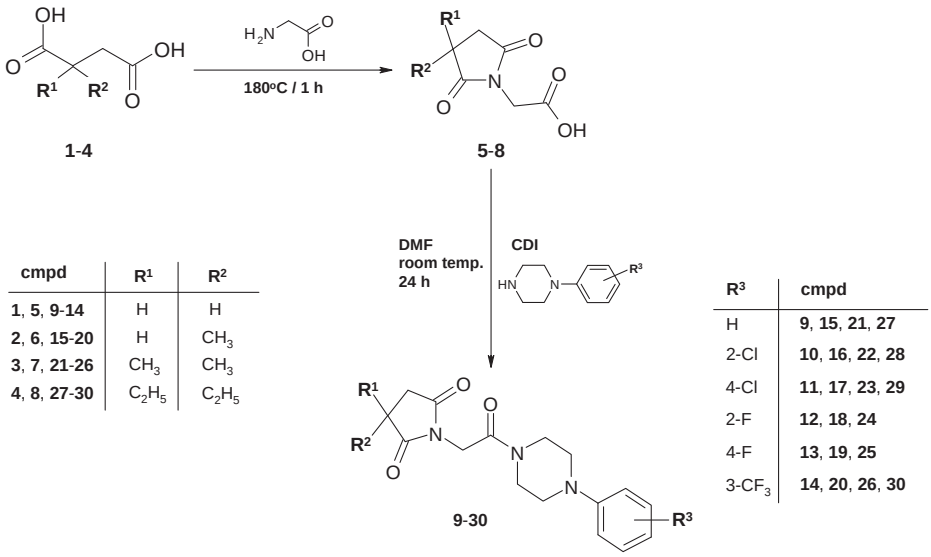


Figure 1. Structures of designed compounds **9**–**30** and model AEDs.



Scheme 1. Synthetic pathways of intermediates **5**–**8** and target compounds **9**–**30**.

Table 1
Anticonvulsant and neurotoxicity screening after ip administration in mice (**9**–**30**)

Compd	R^1	R^2	R^3	Intraperitoneal administration in mice[a]					
				MES[b]		*sc*PTZ[c]		NT[d]	
				0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
9	H	H	H	300	—	—	—	—	—
10	H	H	2-Cl	100	—	300	—	300	—
11	H	H	4-Cl	100	300	300	—	—	300
12	H	H	2-F	300	—	300	—	300	—
13	H	H	4-F	300	300	—	—	—	—
14	H	H	3-CF_3	100	100	—	300	100	300
15	CH_3	H	H	300	—	300	300	300	—
16	CH_3	H	2-Cl	300	—	300	—	300	—
17	CH_3	H	4-Cl	300	100	—	—	—	300
18	CH_3	H	2-F	300	300	—	—	300	—
19	CH_3	H	4-F	300	100	—	—	—	—
20	CH_3	H	3-CF_3	100	100	300	—	300	300
21[g]	CH_3	CH_3	H	300	—	—	—	—	—
22	CH_3	CH_3	2-Cl	300	—	—	—	—	—
23	CH_3	CH_3	4-Cl	100	100	300	—	300	—
24	CH_3	CH_3	2-F	300	—	—	—	—	—
25	CH_3	CH_3	4-F	100	100	300[25]	—	300	—
26	CH_3	CH_3	3-CF_3	100	100	—	—	—	—
27	C_2H_5	C_2H_5	H	—	—	—	—	—	—
28	C_2H_5	C_2H_5	2-Cl	300	—	—	—	300	—
29	C_2H_5	C_2H_5	4-Cl	—	300	—	—	—	—
30	C_2H_5	C_2H_5	3-CF_3	300	300	—	—	—	—
Phenytoin[e]				30	30	—	—	100	100
Ethosuximide[f]				—	—	100	300	—	—

[a] Doses of 30, 100, and 300 mg/kg were administered intraperitoneally. The data indicate the minimum dose effective or neurotoxic in half or more animals tested. The animals were examined at 0.5 and 4.0 h. A dash indicates the absence of anticonvulsant activity and neurotoxicity at the maximum dose administered (300 mg/kg).
[b] Maximal electroshock test.
[c] Subcutaneous pentylenetetrazole test.
[d] Neurotoxicity screening using rotorod test.
[e] Reference drug, data for phenytoin Ref. 17.
[f] Reference drug, data for ethosuximide Ref. 17.
[g] Anti-MES activity at a dose of 100 mg/kg at 0.25 h.

anticonvulsant activity or neurotoxicity. The results are shown in Table 2.

Among these compound **25** was inactive whereas other molecules protected up to 75% (3/4) of animals. The most active were **14** and **17** which showed satisfactory protection from 0.5 h to 4 h after po. administration. These molecules showed comparable activity with phenytoin. The in vivo data in rats confirmed their absorption from gastrointestinal tract and also penetration to central nervous system.

Regardless of rats results the same group of compounds were evaluated for anticonvulsant activity in the 6-Hz psychomotor seizure test in mice after ip administration of dose 100 mg/kg. The 6 Hz stimulation is known as a useful model of therapy-resistant limbic seizures. The results are shown in Table 3.

Among 10 compounds only one derivative—**11** was inactive. Except of less effective compound **10**, the other revealed high activity and protected up to 100% of animals tested in different time intervals. The peaks of 100% (4/4) protection were observed for **14**, **16**, **17**, **20**, **23,** and **25**. Compounds **10**, **19** and **26** protected up to 75% (3/4) of mice.

In summary, library of twenty-two new 1-[2-oxo-2-(4-phenylpiperazin-1-yl)ethyl]pyrrolidine-2,5-diones were synthesized and showed to have appreciable anticonvulsant activity. Except of inactive compound **27** all other molecules were found to be effective in at least one seizure model. The most active were 1-(2-oxo-2-{4-[3-(trifluoromethyl)phenyl]piperazin-1-yl}ethyl)pyrrolidine-2,5-dione (**14**),[19] 1-{2-[4-(4-chlorophenyl)piperazin-1-yl]-2-oxoethyl}-3-methylpyrrolidine-2,5-dione (**17**),[20] 1-{2-[4-(4-chlorophenyl)piperazin-1-yl]-2-oxoethyl}-3,3-dimethylpyrrolidine-2,5-dione (**23**)[21] and 3,3-dimethyl-1-(2-oxo-2-{4-[3-(trifluoromethyl)phenyl]piperazin-1-yl}ethyl)pyrrolidine-2,5-dione (**26**).[22] These compounds showed high activity in the 6-Hz psychomotor seizure test as well as were active in the maximal electroshock and subcutaneous pentylenetetrazole (**14** and **23**) screens. It may suggest their potential effectiveness in different types of epilepsies including therapy-resistant seizures. The SAR studies showed that the most potent are 3-unsubstituted, 3-methyl- and 3,3-dimethyl-pyrrolidine-2,5-diones. The introduction of longer ethyl groups decreases activity. Taking into consideration an amine function the presence of trifluoromethyl group or chloro and fluoro atoms (position-4) at phenylpiperazine moiety are preferential for anticonvulsant properties. It should be noted all of chiral compounds were prepared as racemic mixtures and no attempt has been made to resolve the enantiomers. Due to interesting profile of anticonvulsant activity further investigations

Table 2
Anticonvulsant activity of selected compounds administrated orally in rats (MES screen)

Compd	Oral administration in rats[a]				
	0.25 h	0.5 h	1 h	2 h	4 h
10	0/4	0/4	0/4	0/4	0/4
11	0/4	0/4	0/4	2/4	2/4
14	0/4	2/4	3/4	3/4	3/4
16	3/4	0/4	0/4	0/4	0/4
17	2/4	3/4	3/4	3/4	3/4
19	1/4	2/4	2/4	2/4	1/4
20	0/4	0/4	2/4	2/4	1/4
23	0/4	0/4	0/4	1/4	4/4
25	0/4	0/4	0/4	0/4	0/4
26	1/4	0/4	0/4	0/4	0/4
Phenytoin[b]	1/4	4/4	3/4	3/4	3/4

[a] MES screen, dose of 30 mg/kg was administrated orally. The data indicate: number of rats protected/number of rats tested.
[b] Reference drug, data for phenytoin Ref. 18.

Table 3
Anticonvulsant activity—ip psychomotor seizure test in mice (6-Hz, current 32 mA)

Compd	Intraperitoneal injection into mice[a]				
	0.25 h	0.5 h	1 h	2 h	4 h
10	3/4	2/4	0/4	0/4	0/4
11	0/4	0/4	0/4	0/4	0/4
14	4/4	4/4	3/4	4/4	3/4
16	4/4	2/4	2/4	0/4	0/4
17	3/4	4/4	3/4	1/4	1/4
19	2/4	3/4	3/4	1/4	0/4
20	4/4	4/4	4/4	3/4	0/4
23	4/4	4/4	4/4	1/4	1/4
25	4/4	4/4	3/4	1/4	1/4
26	2/4	3/4	3/4	1/4	2/4

[a] Dose of 100 mg/kg was administrated intraperitoneally. The data indicate: number of mice protected/number of mice tested.

that enable detailed SAR studies in this group derivatives are urgently necessary.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.118.

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19. *Characterization data for* **14**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.82 (4H, s, imide), 3.23 (t, 2H, piperazine, J = 5.13 Hz), 3.31 (t, 2H, piperazine, J = 5.13 Hz), 3.67 (t, 2H, piperazine, J = 5.13 Hz), 3.77 (t, 2H, piperazine, J = 5.13 Hz), 4.36 (2H, s, $-CH_2-$), 7.05–7.16 (3H, m, ArH), 7.38 (1H, t, ArH, J = 7.95 Hz). Anal. calcd for $C_{17}H_{18}F_3N_3O_3$ (369.34): C, 55.28; H, 4.91; N, 11.38. Found: C, 55.30; H, 4.82; N, 11.42. $[M+H]^+$ = 370.15.
20. *Characterization data for* **17**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.40 (3H, t, CH_3, J = 3.59 Hz), 2.43 (1H, d, imide, J = 13.59 Hz), 2.97–3.06 (2H, m, imide), 3.13 (2H, t, piperazine, J = 5.13 Hz), 3.21 (2H, t, piperazine, J = 5.13 Hz), 3.63 (2H, *t*, piperazine, J = 5.0 Hz), 3.75 (2H, t, piperazine, J = 5.13 Hz), 4.33 (2H, s, $-CH_2-$), 6.82–6.87 (2H, m, ArH), 7.21–7.27 (2H, m, ArH). Anal. calcd for $C_{17}H_{20}ClN_3O_3$ (349.81): C, 58.37; H, 5.76; N, 12.01. Found: C, 58.21; H, 5.65; N, 11.94. $[M+H]^+$ = 350.14.
21. *Characterization data for* **23**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.39 (s, 6H, CH_3), 2.65 (s, 2H, imide), 3.14 (t, 2H, piperazine, J = 5.13 Hz), 3.21 (t, 2H, piperazine, J = 5.13 Hz), 3.63 (t, 2H, piperazine, J = 5.13 Hz), 3.74 (t, 2H, piperazine, J = 5.13 Hz), 4.32 (s, 2H, $-CH_2-$), 6.80–6.90 (m, 2H, ArH), 7.18–7.28 (m, 2H, ArH). Anal. calcd for $C_{18}H_{22}ClN_3O_3$ (363.84): C, 59.42; H, 6.09; N, 11.55. Found: C, 59.49; H, 6.15; N, 11.43. $[M+H]^+$ = 364.16.
22. *Characterization data for* **26**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.39 (s, 6H, CH_3), 2.65 (s, 2H, imide), 3.23 (t, 2H, piperazine, J = 5.06 Hz), 3.29 (t, 2H, piperazine, J = 5.06 Hz), 3.66 (t, 2H, piperazine, J = 5.06 Hz), 3.77 (t, 2H, piperazine, J = 5.13 Hz), 4.33 (s, 2H, s, 2H, $-CH_2-$), 7.01–7.17 (m, 3H, ArH), 7.30–7.42 (m, 1H, ArH). Anal. calcd for $C_{19}H_{22}F_3N_3O_3$ (397.39): C, 57.43; H, 5.58; N, 10.57. Found: C, 57.40; H, 5.61; N, 10.60. $[M+H]^+$ = 398.17.