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Design, synthesis, and anticonvulsant activity of *N*-phenylamino derivatives of 3,3-dialkyl-pyrrolidine-2,5-diones and hexahydro-isoindole-1,3-diones

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Abstract—In the present study on the development of new anticonvulsants, the library of differently substituted *N*-phenylamino pyrrolidine-2,5-dione and hexahydro-isoindole-1,3-dione derivatives was synthesized. The anticonvulsant activity of all the compounds was evaluated using the maximal electroshock (MES) and pentylenetetrazole (*sc*PTZ) screens, which are the most widely employed seizure models for early identification of candidate anticonvulsants. Their neurotoxicity was determined applying the rotorod test. The pharmacological results revealed that the majority of compounds were effective in electrical (MES) and/or pentylenetetrazole induced seizure (*sc*PTZ) models. The quantitative in vivo anticonvulsant evaluation of *N*-phenylamino-3,3-dimethyl-pyrrolidine-2,5-dione (**15**), conducted at the time of peak pharmacodynamic activity (TPE), showed the MES ED_{50} value of 69.89 mg/kg in rats. The median toxic dose (TD_{50}) was 500 mg/kg, providing compound **15** with a protective index (TD_{50}/ED_{50}) of 7.15 in the MES test.

1. Introduction

Epilepsy, a common neurological disorder characterized by recurrent spontaneous seizures arising from excessive electrical activity in some portion of the brain, is a major, worldwide, public health problem, which affects approximately 1% of the population.[1] Despite the increasing understanding of the pathogenesis of seizures up to 30% of epilepsies are poorly treated with the available antiepileptic drugs (AEDs). Moreover, a large number of new AEDs marked during recent years did not change the proportion of patients responding to the treatment and many of those medications cause serious side effects, which include ataxia, nausea, mental dulling, and hepatotoxicity.[2,3] Therefore, with all of the above findings in mind the continued search for safer and more effective AEDs is urgently necessary.

One of the important core fragments of anticonvulsants is defined by a nitrogen heteroatomic system, usually a cyclic imide, at least of one carbonyl group and phenyl or alkyl substituents attached to the heterocyclic system.[4–6] This common template is present in the structures of older generation of AEDs such as phenobarbital (**1**), primidone (**2**) or phenytoin (**3**) (Fig. 1), which are known to be active against maximal electroshock seizure test (MES), the experimental animal model for generalized tonic–clonic epilepsy. The extensive SAR studies of AEDs revealed, however, that the phenyl substituents attached at the heteroatomic imide or cyclic amide ring are not necessary to retain the anticonvulsant activity. An example of such molecules is ethosuximide (**4**), recently marked AED–levetiracetam (**5**) or compounds being currently under the clinical trials, namely brivaracetam (ucb 34714) (**6**) and seletracetam (ucb 44212) (**7**)[7–10] (Fig. 1). In contrast to the drugs described above these molecules were inactive in the MES screen. They revealed protection in the subcutaneous pentylenetetrazole seizure test (*sc*PTZ)-**4**, the animal model of absence epilepsy, or in secondarily generalized motor seizures screen in corneally kindled mice, **5**–**7**, which is claimed to detect compounds that can act to prevent the development of seizures. Such substances could be termed 'antiepileptogenetic'.[11]

Previous studies from our laboratory have demonstrated the potent anticonvulsant activity among the *N*-phenylamino-azaspiranes, from which

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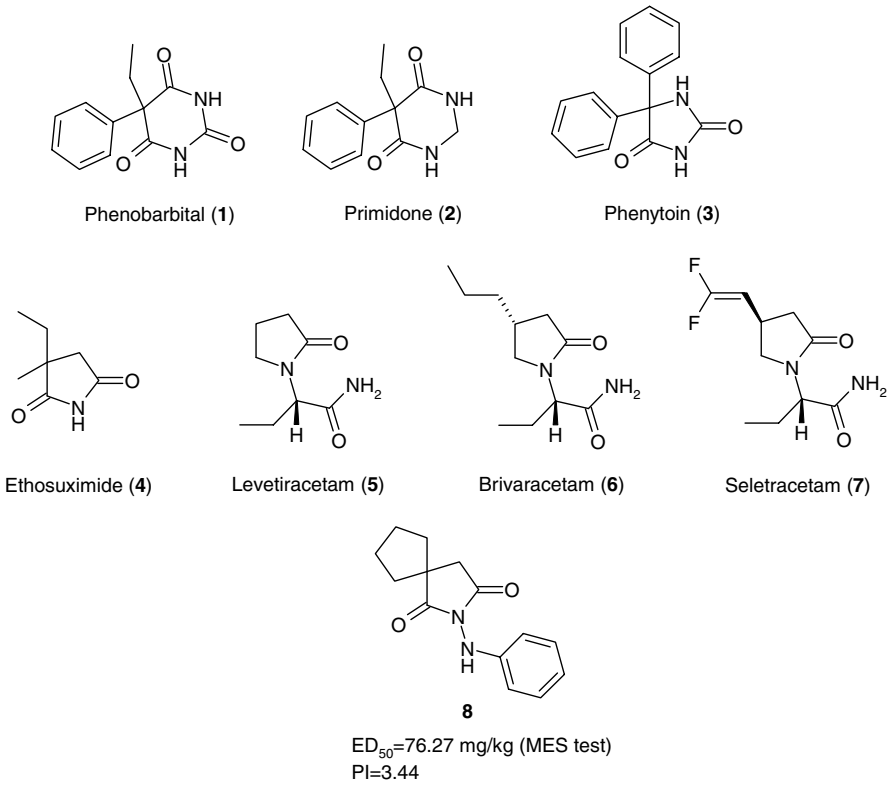


Figure 1. Structures of known anticonvulsants and model compound **8**.

the *N*-phenylamino-2-azaspiro[4.4]nonane-1,3-dione (**8**) showed ED_{50} = 76.27 mg/kg and a protective index PI (TD_{50}/ED_{50}) of 3.44 in the MES test in rats.[12,13] Following these results, as part of our efforts to design new anticonvulsant agents which would be active both in MES and in *sc*PTZ tests and according to the foregoing effective in different types of seizures, in the present study we have synthesized a library of compounds with *N*-phenylamino pyrrolidine-2,5-dione system as a core fragment. At the position-3 of the succinimide ring we have introduced different alkyl substituents, which approximate the designed molecules to the structure of ethosuximide, one of the not numerous drugs effective in absence epilepsy.[14] On the other hand, we have changed additionally the manner of connection between the cyclohexane ring and the imide system from the described spiro carbon atom for two common carbon atoms observed for bicyclic hexahydro-isoindole-1,3-diones.[12]

2. Results and discussion

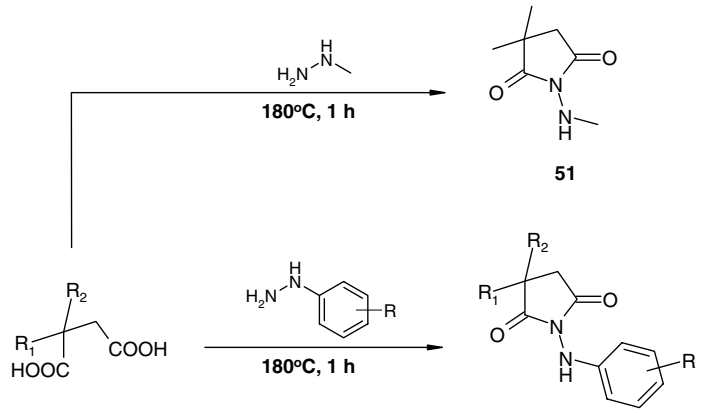
2.1. Chemistry

The synthesis of compounds **15**–**51** was accomplished as presented in Scheme 1. The starting materials for **15**–**44**, 2,2-dimethyl- (**9**), 2-ethyl-2-methyl- (**10**), 2-methyl-2-propyl- (**11**), 2-butyl-2-methyl- (**12**) or 2,2-diethyl- (**13**) succinic acid derivatives were prepared by the using methods reported elsewhere.[15,16] Compounds **45**–**50** were synthesized by use of commercially available 1,2-cyclohexanedicarboxylic anhydride (Merck, Darmstadt, Germany) (**14**), as reaction substrate. Titled imides **15**–**51** were obtained in a simple one-pot cyclization reaction of the prepared 2,2-dialkyl-succinic acids (**9**–**13**) or 1,2-cyclohexanedicarboxylic anhydride (**14**) with appropriately substituted phenyl-hydrazines (**15**–**50**) or methyl-hydrazine (**51**) by heating them at ca. 180 °C for 1 h. The crude products were crystallized from isopropanol and purified by column chromatography, giving the final compounds in yields ranging from 61% to 81%. Their purity was assessed by TLC and gradient HPLC chromatography. The structures of compound synthesized were confirmed by both spectral and elemental analysis. The detailed physical and analytical data are listed in Section 4.

2.2. Anticonvulsant activity

The preclinical discovery and development of new chemical agents for the treatment of epilepsy are based mainly on the use of predictable animal models. At the present time there are three in vivo screens used routinely that include the maximal electroshock seizure (MES), the subcutaneous pentylenetetrazole (*sc*PTZ) and the kindling model. From these tests the MES and *sc*PTZ screens are recognized as the 'gold standards' in the early stages of testing.[17,18]

The profile of anticonvulsant activity of all the compounds was established in the maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) tests, after intraperitoneal injection into mice at doses of 30, 100, and 300 mg/kg. An observation was carried out at two different time intervals, namely 0.5 and 4 h.



9: R_1=R_2=CH_3, cmpd **15-21**, **51**

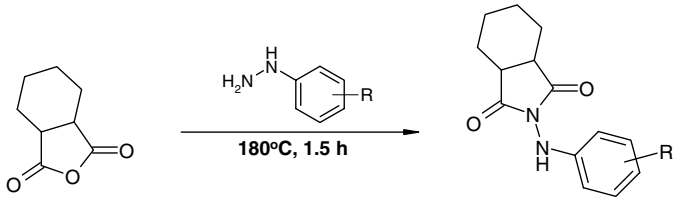
10: R_1=CH_3, R_2=C_2H_5, cmpd **22-29**

11: R_1=CH_3, R_2=C_3H_7, cmpd **30-34**

12: R_1=CH_3, R_2=C_4H_9, cmpd **35-38**

13: R_1=C_2H_5, R_2=C_2H_5, cmpd **39-44**

R	cmpd
H	**15**, **22**, **39**
2-CH_3	**23**
4-CH_3	**16**, **24**, **40**
2-Cl	**17**, **25**, **30**, **35**, **41**
3-Cl	**18**, **26**, **31**
4-Cl	**19**, **27**, **32**, **36**, **42**
2,4-Cl	**20**, **28**, **33**, **37**, **43**
4-Br	**21**, **29**, **34**, **38**, **44**



R	cmpd
H	**45**
4-CH_3	**46**
2-Cl	**47**
3-Cl	**48**
4-Cl	**49**
2,4-Cl	**50**

Scheme 1. General method for the synthesis of succinimides **15**–**51**.

Several molecules were also screened at 1 h (**21**, **28**, and **43**) and 2 h (**42**). The acute neurological toxicity (NT) was determined in the minimal motor impairment-rotorod screen. The results are shown in Table 1.

The initial anticonvulsant evaluation showed that excluding molecules **36**, **39**, **44**, **46**, **47**, and **51**, devoid of anticonvulsant activity, all other derivatives were effective in ip MES and/or *sc*PTZ screens.

Compounds that revealed protection in the MES test, indicative of the ability of substance to prevent seizure spread, at a dose of 100 mg/kg include **17**–**21**, **27**, **28**, **32**, **34**, **37**, **42**, **43**, and **50**, whereas molecules **15**, **16**, **22**–**26**, **29**–**31**, **35**, **40**, and **45** provided anti-MES protection at a dose of 300 mg/kg. The majority of these compounds showed activity only at 0.5 h, indicating that they have rapid onset and short duration of anticonvulsant action. From these series **19**, **21**, **28**, **32**, **34**, and **43** were active in the MES model both at 0.5 and at 4 h, whereas **20** and **50** showed activity only at 4 h. Derivatives **21**, **28**, **42**, and **43** revealed the anti-MES activity at a dose of 100 mg/kg in different time periods, namely 1 h (**21**, **28**, and **43**) and 2 h (**42**), additionally.

Compounds **15**–**17**, **19**, **20**, **22**, **24**, **27**–**35**, **37**, **38**, **41**–**43**, **45**, **48**, and **49** were found to be active in the *sc*PTZ test, which identify substances elevating seizure threshold.

Table 1. The results after intraperitoneal administration to mice

Compound	Intraperitoneal injection in mice[a]						$\log k_w$
	MES[b]		*sc*PTZ[c]		NT[d]		
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	
15	300	—	300	—	100	—	1.471
16	300	—	300	—	300	—	1.642
17	100	—	300	—	30	—	1.876
18	100	—	—	—	300	—	2.071
19	100	300	100	300	300	300[14]	2.080
20	—	100	100	—	100	—	2.179
21[f]	300	300	—	—	300[14]	—	1.864
22	300	—	300	—	100	—	1.695
23	300	—	—	—	100	100	1.864
24	300	—	300	—	300	—	1.928
25	300	—	—	—	300	—	2.029
26	300	—	—	—	300	—	2.183
27	100	—	300	—	100	300	2.201
28[f]	300	300	100	300	30	300[14]	2.334
29	300	—	300	100	300[14]	—	2.024
30	300	—	300	—	300	—	2.364
31	300	—	300	—	100	—	2.401
32	100	300	300	—	100	300[14]	2.445
33	—	—	100	—	—	—	2.647
34	100	300	300	—	300	300	2.403
35	300	—	300	—	300	—	2.566
36	—	—	—	—	300	—	2.638
37	100	—	100	—	100[14]	—	2.896
38	—	—	300	—	300	—	2.539
39	—	—	—	—	300	—	1.826
40	300	—	—	—	100[14]	—	1.999
41	—	—	300	—	100	—	2.244
42[f]	—	300	100	300	300	300	2.314
43[f]	300	300	100	—	100	—	2.552
44	—	—	—	—	—	—	2.221
45	300	—	300	—	—	—	1.937
46	—	—	—	—	300	—	2.028
47	—	—	—	—	—	—	2.162
48	—	—	300	—	300	—	2.173
49	—	—	100	—	—	—	2.224
50		100	—	—	300	300	2.425
51	—	—	—	—	—	—	ND
Phenytoin[e]	30	30	—	—	100	100	
Ethosuximide[e]	—	—	100	300	—	—	

ND, not determined.

Response comments:[14] unable to grasp rotarod.

[a] Doses of 30, 100, and 300 mg/kg were administered. The data indicate the minimum dose whereby bioactivity was demonstrated. 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 (rotorod test).

[e] Reference drugs, data for phenytoin and ethosuximide Ref. 19.

[f] Molecules **21**, **28**, **43** showed anti-MES activity at a dose of 100 mg/kg at 1 h, **42** at 2 h.

Among these molecules, **19**, **28**, and **42** showed anti-*sc*PTZ activity at the dose of 100 and 300 mg/kg at time periods 0.5 and 4 h that was equivalent to ethosuximide used as reference anticonvulsant drug. Comparable results were obtained for **20**, **33**, **37**, **43**, and **49** providing protection at a dose of 100 mg/kg at 0.5 h as well as **29** that was active at doses of 100 mg/kg (4 h) and 300 mg/kg at 0.5 h. The other derivatives **15**–**17**, **22**, **24**, **27**, **30**–**32**, **34**, **35**, **38**, **41**, **45**, and **48** showed activity against *sc*PTZ screen at a dose of 300 mg/kg at 0.5 h.

As shown in Table 1, the majority of compounds were effective both in MES and in *sc*PTZ tests; therefore these molecules may be useful in treating not only generalized tonic–clonic epilepsy but also absence seizures. It is also noteworthy that in each series of derivatives the most lipophilic compounds were the most potent and possessed the longest duration of anticonvulsant activity. The lipophilic properties for all the compounds were measured experimentally by the use of the RP-HPLC method and expressed as log k_w parameters (Table 1).

In the neurotoxicity screen (NT), **33**, **44**, **45**, **47**, **49**, and **51** did not show neurotoxicity in the maximum dose administered (300 mg/kg). Among these molecules **44**, **47**, and **51** were inactive additionally. Compounds **16**, **18**, **19**, **21**, **24**–**26**, **29**, **30**, **34**–**36**, **38**, **39**, **42**, **46**, **48**, and **50** were less neurotoxic than phenytoin and exhibited motor impairment at the dose of 300 mg/kg at 0.5 h and also 4 h (**19**, **26**, **32**, **34**, **42**, and **50**). The other derivatives revealed neurotoxicity at a dose of 100 mg/kg (**15**, **20**, **22**, **23**, **27**, **31**, **32**, **37**, **40**, **41**, and **43**) or 30 mg/kg, namely **17** and **28**. Compounds **33**, **45**, and **49** emerged as anticonvulsants without neurotoxic properties.

On the basis of the data obtained in ip screen in mice and according to Anticonvulsant Screening Project (ASP) disposition, six compounds **15**, **21**, **27**, **28**, **29**, and **45** were selected and examined for their anticonvulsant activity in the MES screen as well as neurotoxicity after po administration into rats at a dose of 30 mg/kg. The results obtained are presented in Table 2.

As can be seen from these data, compounds **27**, **28**, and **45** were moderately effective in rat MES oral screen and protected only 25% of tested animals at the time of 0.5 h (**28** and **45**), 1 h (**27**), 2 h (**28**) or 4 h (**27**). Compound **15** provided 25% at 0.25 h and one peak of 50% protection at time point of 1 h. The other derivatives were more effective and showed the total duration of anticonvulsant activity within 0.5–4 h (**21**), whereas **29** was active in all time periods. Compound **21** protected 50% of rats at the time of 2 and 4 h as well as 25% of animals tested

Table 2. Anticonvulsant activity (MES test) of selected compounds administrated orally to rats

Compound	Oral administration to rats[a]				
	0.25 h	0.5 h	1 h	2 h	4 h
15	1	0	2	0	0
21	0	1	1	2	2
27	0	0	1	0	1
28	0	1	0	1	0
29	3	2	1	1	1
45	0	1	0	0	0
Phenytoin[b]	1	4	3	3	3

[a] The data in the oral MES screen indicate the number of rats of four that were protected at a dose of 30 mg/kg.

[b] Reference drug, data from Ref. 32.

at 0.5 and 1 h. Derivative **29** showed the peak of 75% protection at 0.25 h and was also effective in 50% and 25% of animals at 0.5 or 1 h, 2 and 4 h, respectively. These compounds were less active and showed shorter duration of satisfactory action than phenytoin. They were non-toxic when given orally.

Three molecules **33**, **38**, and **45**, which were active only in the ip *sc*PTZ (**33** and **38**) or in both MES and *sc*PTZ screens (**45**) in mice, were evaluated in the subcutaneous pentylenetetrazole test after oral administration into rats. Initially a dose of 50 mg/kg was employed and the anticonvulsant activity was assessed in several time points (Table 3). The results obtained were compared with standard drug ethosuximide, which is the model substance effective in this screen. As can be seen, **33** protected 50% of tested animals at 0.5 h and 25% at 0.25 and 4 h, whereas **38** and **45** showed 25% protection at time periods 0.5 and 0.25 h, respectively. These molecules were less active than ethosuximide and did not exhibit also neurotoxicity at the tested dose of 50 mg/kg.

Selected compounds **35** and **38** were chosen for the evaluation of anticonvulsant activity in the 6-Hz test. The selection was made randomly as a part of the search of molecules providing anti 6-Hz protection among chemically diversified compounds pursued in the NIH/NINDS. The 6-Hz screen has been validated as a model of therapy-resistant epilepsy, recently. It was not used widely because of its lack of clinical validity since the hydantoins such as phenytoin failed to show protective activity. Nevertheless, the clinically effective antiepileptic drug levetiracetam, which is not active in the conventional MES and *sc*PTZ tests, does exhibit protective activity in the 6-Hz model. This suggested that the 6-Hz model might be capable for identifying antiseizure agents with a novel spectrum of activity and unknown mechanism of anticonvulsant action.[20] The results obtained for compounds **35** and **38** are shown in Table 4.

As can be seen from the above data, **35** and **38** administrated intraperitoneally at a dose of 100 mg/kg into mice protected 50% (**35**) or 25% (**38**) of animals tested at 0.25 h.

Compound **15** was chosen for quantification of the pharmacological parameters (ED_{50} and TD_{50}). The quantitative evaluation of the MES median effective dose (ED_{50}) and toxic dose (TD_{50}) was performed at previously estimated time of peak effect (TPE) after oral administration into rats. Results of the quantitative tests for compound **15**, along with the data for the standard drug-phenytoin, are shown in Table 5.

In the rat oral MES screen compound **15** revealed an ED_{50} value of 69.89 mg/kg and a TD_{50} of 500 mg/kg, resulting in protection index (TD_{50}/ED_{50}) of 7.15. The time to peak effect for this molecule was 0.5 h. Compound **15** was less active than reference drug.

According to the ASP dispositions compounds **18** and **46** were assessed for potential activity against nerve agents using the Pilocarpine Induced Status Prevention (PISP) screen, which is one of the animal models of status epilepticus (SE). The outcome measures are determination of 'protection' or 'no-protection'. The results are shown in Table 6.

In the Pilocarpine Induced Status Prevention (PISP) screen compound **18** was found to be protective in 7/8 animals at time-zero using the dose of 65 mg/kg, whereas **46** protected 6/7 rats at a dose of 450 mg/kg. It is noteworthy that molecule **46** was inactive in ip. MES and *sc*PTZ in mice but displayed the anticonvulsant activity in the PISP model. To determine the acute motor impairment doses of 30, 100, and 300 mg/kg were administrated via the ip. route. Two rats per each dose were employed. The animals were observed in the following time periods: 0.25, 0.5, 1, 2, and 4 h. The result of acute toxicity screens revealed that both compounds **18** and **46** were negligibly neurotoxic and caused the motor impairment at a dose of 300 mg/kg within the time range 0.25–2 h (**18**) or 0.25–1.0 h (**46**).

2.3. Structure–activity relationships

The results of the preliminary anticonvulsant screening revealed that the most active were compounds with

Table 3. Anticonvulsant activity (*sc*PTZ test) of selected compounds administrated orally to rats

Compound	Oral administration to rats[a]				
	0.25 h	0.5 h	1 h	2 h	4 h
33	1	2[25]	0	0	1
38	0	1	0	0	0
45	1	0	0	0	0
Ethosuximide[b]	0	2	1	1	0

Response comments:[25] Myoclonic jerks.
[a] Dose of 50 mg/kg was administrated. The data in the oral *sc*PTZ screen indicate the number of rats of four that were protected.
[b] Reference drug, data from Ref. 19.

Table 4. Anticonvulsant evaluation, psychomotor seizure test (6 Hz) after intraperitoneal injection into mice

Compound	Intraperitoneal injection into mice[a]				
	0.25 h	0.5 h	1 h	2 h	4 h
35	0	2	0	0	0
38	0	1	0	0	0

[a] Dose of 100 mg/kg was administrated. The data indicate the number of mice of four that were protected.

Table 5. Quantification studies of compound **15** in the MES and neurotoxicity test (NT) in rats after oral administration

Compound	Route	TPE[a] (h)	MES ED_{50}[b] (mg/kg)	NT TD_{50}[b] (mg/kg)	PI[c]
15	Oral	0.5	69.89 ± 1.64	500	7.15
Phenytoin[d]	Oral	2.0	23.2 ± 4.28	>500	>21.6

[a] Time to peak effect.
[b] Compound **15** was examined at 0.5 h after administration and results are represented as means ± SEM at 95% confidence limit (MES, maximal electroshock test; NT, neurotoxicity).
[c] Protection index (TD_{50}/ED_{50}).
[d] Reference drug, data from Ref. 32.

Table 6. Pilocarpine Induced Status Prevention (PISP) model

Compound	Dose (mg/kg)	Time (h)[a]	Protected rats	Non-protected rats	Average weight change ± SEM (g)[b]	
					Protected rats	Non-protected rats
18	65	0	7	8	15 ± 0.0	12.9 ± 2.2
46	450	0	6	7	10.2 ± 1.2	12.0 ± 0.0

The results for compounds **18** and **46** after ip administration (Test 71).
[a] Post-first stage III seizure.
[b] Weight change 24 h post-first stage III seizure.

two methyl; ethyl-methyl or methyl-propyl substituents at the position-3 of pyrrolidine-2,5-dione ring. The replacement of the methyl group into the ethyl substituent among the *N*-phenylamino derivatives of 3,3-diethyl-pyrrolidine-2,5-diones (**39**–**44**) or the introduction of the longer butyl chain into position-3 of succinimide moiety (**35**–**38**) decreased the activity. The similar effect was observed in the case of removal of one 3-alkyl group, namely for *N*-phenylamino-3-methylpyrrolidine-2,5-dione, that may be recognized as close analogue of the most active compound **15**.[21] The anticonvulsant properties depended also on the kind and location of the substituents at the phenyl ring. In general, the most effective were molecules with highly electronegative chloro atoms at position-2,4 (**20**, **28**, **33**, **37**, **43**, and **50**) or -4 (**19**, **27**, **32**, and **42**). The replacement of the chloro substituents into isosteric bromo atoms caused similar pharmacological properties. The respective *ortho*- and *meta*-chloro derivatives as well as compounds with methyl substituents and unsubstituted molecules were less active. Furthermore, comparison of results obtained previously for the *N*-phenylamino-2-azaspiro[4.4]nonane- and [4.5]decane-1,3-diones[12] and compounds described herein, which may be recognized as analogues or respective spirosuccinimides with degraded cycloalkyl ring showed that such modification substantially increased the anticonvulsant activity. This structural feature seems to be especially important for anti-*sc*PTZ protection. The change of connection of cyclohexane ring with pyrrolidine-2,5-dione moiety from spiro carbon atom to two common carbon atoms among the hexahydro-isoindole-1,3-diones (**45**–**50**) did not influence the anticonvulsant activity. The lack of anticonvulsant activity for molecule **51**, which may be recognized as an analogue of active compound **15**, proved that the presence of the aromatic moiety at the imide nitrogen is crucial for the anticonvulsant properties of such type of compounds.

3. Conclusion

The results obtained revealed that number of *N*-phenylamino-3,3-dialkyl-pyrrolidine-2,5-diones and hexahydro-isoindole-1,3-diones were effective in the MES and he *sc*PTZ screens. The most active was *N*-phenylamino-3,3-dimethyl-pyrrolidine-2,5-dione that showed the ED_{50} value of 69.89 mg/kg and a protective index (TD_{50}/ED_{50}) of 7.15 in the MES test in rats. The anticonvulsant activity depended on the kind and position of substituents at the aryl moiety as well as the length of alkyl substituents at the position-3 of succinimide moiety. Several molecules were effective in the 6-Hz model, a model of therapy-resistant epilepsy as well as the Pilocarpine Induced Status Prevention (PISP) screen that is recognized as the animal model of status epilepticus.

4. Experimental

4.1. Chemistry

All the chemicals and solvents were purchased from Merck (Darmstadt, Germany) and were used without further purification. Melting points (mp) were determined in open capillaries on a Büchi 353 melting point apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. The purity and homogeneity of the compounds were assessed by TLC and gradient HPLC chromatography. The thin-layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} aluminum sheets (Merck, Darmstadt, Germany), using developing system consisting of chloroform/acetone (9:1, v/v). Spots were detected by their absorption under UV light ($\lambda = 254$ nm) and by visualization with 0.05 mol I_2 in 10% HCl. Analytical HPLC were run on a Waters Alliance HPLC instrument, equipped with a Chromolith SpeedROD column (4.6 × 50 mm). Standard conditions were eluent system A (water), system B (acetonitrile). A flow rate of 5 ml/min and a gradient of (0–100%) B over 5 min were used, detection 214 nm. Retention times (t_r) are given in minutes. Elemental analysis for C, H, and N were carried out by a micromethod using the elemental Vario EI III Elemental analyser (Hanau, Germany). The results of elemental analyses were within ±0.4% of the theoretical values. ^{1}H NMR spectra were obtained in a Varian Mercury spectrometer (Varian Inc., Palo Alto, CA, USA), in $CDCl_3$, operating at 300 MHz. Chemical shifts are reported in δ values (ppm) relative to TMS $\delta = 0$ (^{1}H), as internal standard. The *J* values are expressed in Hertz (Hz). Signal multiplicities are represented by the following abbreviations: s (singlet), br s (broad singlet), d (doublet), t (triplet), dd (double doublet), td (triple doublet), ddd (double double doublet), and m (multiplet). For chosen compounds **15**, **17**, **20**, **21**, **23**, **25**, **28**, **36**, **42**, **43**, and **49** the mass spectra (MS) were recorded on AMD—604 Mass Spectrometer operating at 70 eV.

4.1.1. General procedure for the synthesis of compounds 15–51. To a suspension of 0.01 mol of 2,2-dimethyl-succinic acid (**9**), 2-ethyl-2-methyl-succinic acid (**10**), 2-methyl-2-propyl-succinic acid (**11**), 2-butyl-2-methyl-

succinic acid (**12**), 2,2-diethyl-succinic acid (**13**) or 1,2-cyclohexanedicarboxylic anhydride (Merck, Darmstadt, Germany) (**14**) in 10 ml of water, the appropriately substituted phenyl-hydrazines (compounds **15**–**50**) or methyl-hydrazine (compound **51**) was gradually added. The mixture was heated in an oil bath with simultaneous distillation of water. After complete removal of the water the temperature of the reaction mixture rose up to 180 °C and was maintained for 1 h. The crude products were crystallized from isopropanol. The obtained solid residues were purified by column chromatography on Silica gel 60 (Merck, Darmstadt, Germany) using chloroform/acetone mixture (9:1, v/v), as a solvent. After evaporation of the solvents the oil products were recrystallized from isopropanol.

4.1.1.1. *N*-Phenylamino-3,3-dimethylpyrrolidine-2,5-dione (15).[†] White powdery crystals. Yield: 76%; HPLC (t_R 1.99 min); mp 127–129 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (s, 6H, $-CH_3$, $-CH_3$), 2.68 (s, 2H, imide), 6.11 (br s, 1H, NH), 6.75–6.78 (m, 2H, $C_{2,6}$-ArH), 6.96–7.01 (m, 1H, C_4-ArH), 7.22–7.27 (m, 2H, $C_{3,5}$-ArH); MS *m*/*z* 219 $[M+1]^+$, 218 $[M]^+$; $C_{12}H_{14}N_2O_2$ (218.26).

4.1.1.2. *N*-(4-Methylphenyl)-amino-3,3-dimethylpyrrolidine-2,5-dione (16). White powdery crystals. Yield: 70%; HPLC (t_R 2.27 min); mp 116–118 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (s, 6H, $-CH_3$, $-CH_3$), 2.26 (s, 3H, Ar-4CH_3), 2.65 (s, 2H, imide), 6.05 (br s, 1H, NH), 6.69–6.72 (m, 2H, $C_{3,5}$-ArH), 7.03–7.06 (m, 2H, $C_{2,6}$-ArH); $C_{13}H_{16}N_2O_2$ (232.28).

4.1.1.3. *N*-(2-Chlorophenyl)-amino-3,3-dimethylpyrrolidine-2,5-dione (17). White powdery crystals. Yield: 79%; HPLC (t_R 2.36 min); mp 138–140 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.43 (s, 6H, $-CH_3$, $-CH_3$), 2.70 (s, 2H, imide), 6.53 (dd, 1H, C_6-ArH, J = 1.54 Hz, J = 8.21 Hz), 6.58 (br s, 1H, NH), 6.91 (td, 1H, C_4-ArH, J = 1.45 Hz, J = 8.08 Hz), 7.11–7.17 (m, 1H, C_5-ArH), 7.33 (dd, 1H, C_3-ArH, J = 1.38 Hz, J = 7.97 Hz); MS *m*/*z* 254 $[M+2]^+$, 252 $[M]^+$; $C_{12}H_{13}ClN_2O_2$ (252.70).

4.1.1.4. *N*-(3-Chlorophenyl)-amino-3,3-dimethylpyrrolidine-2,5-dione (18). White powdery crystals. Yield: 70%; HPLC (t_R 2.47 min); mp 70–72 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (s, 6H, $-CH_3$, $-CH_3$), 2.69 (s, 2H, imide), 6.13 (br s, 1H, NH), 6.64 (ddd, 1H, C_6-ArH, J = 0.72 Hz, J = 2.26 Hz, J = 8.13 Hz), 6.72 (t, 1H, C_2-ArH, J = 2.08 Hz), 6.95 (ddd, 1H, C_4-ArH, J = 0.78 Hz, J = 1.82 Hz, J = 7.97 Hz), 7.16 (t, 1H, C_5-ArH, J = 8.05 Hz); $C_{12}H_{13}ClN_2O_2$ (252.70).

4.1.1.5. *N*-(4-Chlorophenyl)-amino-3,3-dimethylpyrrolidine-2,5-dione (19). White powdery crystals. Yield: 81%; HPLC (t_R 2.41 min); mp 148–150 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.41 (s, 6H, $-CH_3$, $-CH_3$), 2.68 (s, 2H, imide), 6.10 (br s, 1H, NH), 6.68–6.73 (m, 2H, $C_{2,6}$-ArH), 7.18–7.22 (m, 2H, $C_{3,5}$-ArH); $C_{12}H_{13}ClN_2O_2$ (252.70).

4.1.1.6. *N*-(2,4-Dichlorophenyl)-amino-3,3-dimethylpyrrolidine-2,5-dione (20). White powdery crystals. Yield: 76%; HPLC (t_R 2.77 min); mp 158–160 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.42 (s, 6H, $-CH_3$, $-CH_3$), 2.70 (s, 2H, imide), 6.46 (d, 1H, C_6-ArH, J = 8.72 Hz), 6.52 (br s, 1H, NH), 7.12 (dd, 1H, C_5-ArH, J = 2.28 Hz, J = 8.70 Hz), 7.34 (d, 1H, C_3-ArH, J = 2.28 Hz); MS *m*/*z* 290 $[M+4]^+$, 288 $[M+2]^+$, 286 $[M]^+$; $C_{12}H_{12}Cl_2N_2O_2$ (287.15).

4.1.1.7. *N*-(4-Bromophenyl)-amino-3,3-dimethylpyrrolidine-2,5-dione (21). White powdery crystals. Yield: 78%; HPLC (t_R 2.52 min); mp 157–159 °C; δ 1.41 (s, 6H, $-CH_3$, $-CH_3$), 2.68 (s, 2H, imide), 6.10 (br s, 1H, NH), 6.63–6.68 (m, 2H, $C_{2,6}$-ArH), 7.32–7.36 (m, 2H, $C_{3,5}$-ArH); MS *m*/*z* 299 $[M+2]^+$, 297 $[M]^+$; $C_{12}H_{13}BrN_2O_2$ (297.15).

4.1.1.8. *N*-Phenylamino-3-ethyl-3-methyl-pyrrolidine-2,5-dione (22). White powdery crystals. Yield: 73%; HPLC (t_R 2.24 min); mp 68–70 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H, $-CH_3$, J = 7.46 Hz), 1.38 (s, 3H, $-CH_3$), 1.62–1.88 (m, 2H, $-CH_2-$), 2.53 (d, 1H, imide, J = 18.37 Hz), 2.75 (d, 1H, imide, J = 18.38 Hz), 6.17 (br s, 1H, NH), 6.75–6.79 (m, 2H, $C_{2,6}$-ArH), 6.95–7.01 (m, 1H, C_4-ArH), 7.21–7.27 (m, 2H, $C_{3,5}$-ArH); $C_{13}H_{16}N_2O_2$ (232.28).

4.1.1.9. *N*-(2-Methylphenyl)-amino-3-ethyl-3-methylpyrrolidine-2,5-dione (23). White powdery crystals. Yield: 67%; HPLC (t_R 2.49 min); mp 96–98 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (t, 3H, $-CH_3$, J = 7.46 Hz), 1.39 (s, 3H, $-CH_3$), 1.66–1.89 (m, 2H, $-CH_2-$), 2.35 (s, 3H, Ar-2CH_3), 2.55 (d, 1H, imide, J = 18.36 Hz), 2.77 (d, 1H, imide, J = 18.37 Hz), 6.03 (br s, 1H, NH), 6.50 (d, 1H, C_6-ArH, J = 8.21 Hz), 6.89 (td, 1H, C_4-ArH, J = 1.11 Hz, J = 7.44 Hz), 7.06–7.13 (m, 2H, $C_{3,5}$-ArH); MS *m*/z 247 $[M+1]^+$, 246 $[M]^+$; $C_{14}H_{18}N_2O_2$ (246.31).

4.1.1.10. *N*-(4-Methylphenyl)-amino-3-ethyl-3-methylpyrrolidine-2,5-dione (24). White powdery crystals. Yield: 69%; HPLC (t_R 2.51 min); mp 99–101 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.93 (t, 3H, $-CH_3$, J = 7.46 Hz), 1.37 (s, 3H, $-CH_3$), 1.63–1.87 (m, 2H, $-CH_2-$), 2.26 (s, 3H, Ar-4CH_3), 2.51 (d, 1H, imide, J = 18.35 Hz), 2.73 (d, 1H, imide, J = 18.36 Hz), 6.06 (br s, 1H, NH), 6.70–6.73 (m, 2H, $C_{2,6}$-ArH), 7.03–7.06 (m, 2H, $C_{3,5}$-ArH); $C_{14}H_{18}N_2O_2$ (246.31).

4.1.1.11. *N*-(2-Chlorophenyl)-amino-3-ethyl-3-methylpyrrolidine-2,5-dione (25). White powdery crystals. Yield: 71%; HPLC (t_R 2.62 min); mp 116–118 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97 (t, 3H, $-CH_3$, J = 7.46 Hz), 1.40 (s, 3H, $-CH_3$), 1.64–1.90 (m, 2H, $-CH_2-$), 2.56 (d, 1H, imide, J = 18.43 Hz), 2.78 (d, 1H, imide, J = 18.42 Hz), 6.54 (dd, 1H, C_6-ArH, J = 1.37 Hz, J = 8.11 Hz), 6.59 (br s, 1H, NH), 6.91 (td, 1H, C_4-ArH, J = 1.43 Hz, J = 7.77 Hz), 7.11–7.16 (m, 1H, C_5-ArH), 7.33 (dd, 1H, C_3-ArH, J = 1.35 Hz,

[†] Compound **15** was described by Levy,[22] mp 131–132 °C, no spectral data available.

J = 7.96 Hz); MS m/z 268 $[M+2]^+$, 266 $[M]^+$; $C_{13}H_{15}ClN_2O_2$ (266.73).

4.1.1.12. *N*-(3-Chlorophenyl)-amino-3-ethyl-3-methyl-pyrrolidine-2,5-dione (26). White powdery crystals. Yield: 71%; HPLC (t_R 2.65 min); mp 103–105 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (t, 3H, –CH_3, J = 7.46 Hz), 1.39 (s, 3H, –CH_3), 1.63–1.89 (m, 2H, –CH_2–), 2.55 (d, 1H, imide, J = 18.43 Hz), 2.77 (d, 1H, imide, J = 18.43 Hz), 6.12 (br s, 1H, NH), 6.65 (ddd, 1H, C_6-ArH, J = 0.89 Hz, J = 2.30 Hz, J = 8.13 Hz), 6.73 (t, 1H, C_2-ArH, J = 2.08 Hz), 6.95 (ddd, 1H, C_4-ArH, J = 0.88 Hz, J = 1.90 Hz, J = 7.98 Hz), 7.16 (t, 1H, C_5-ArH, J = 8.05 Hz); $C_{13}H_{15}ClN_2O_2$ (266.73).

4.1.1.13. *N*-(4-Chlorophenyl)-amino-3-ethyl-3-methyl-pyrrolidine-2,5-dione (27). White powdery crystals. Yield: 66%; HPLC (t_R 2.65 min); mp 80–82 °C; ^{1}H NMR (300 MHz, $CDCl_3$): ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.94 (t, 3H, –CH_3, J = 7.45 Hz), 1.38 (s, 3H, –CH_3), 1.67–1.88 (m, 2H, –CH_2–), 2.53 (d, 1H, imide, J = 18.43 Hz), 2.75 (d, 1H, imide, J = 18.42 Hz), 6.11 (br s, 1H, NH), 6.70–6.74 (m, 2H, $C_{2,6}$-ArH), 7.19–7.21 (m, 2H, $C_{3,5}$-ArH); $C_{13}H_{15}ClN_2O_2$ (266.73).

4.1.1.14. *N*-(2,4-Dichlorophenyl)-amino-3-ethyl-3-methyl-pyrrolidine-2,5-dione (28). White powdery crystals. Yield: 77%; HPLC (t_R 2.99 min); mp 138–140 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (t, 3H, –CH_3, J = 7.45 Hz), 1.39 (s, 3H, –CH_3), 1.63–1.89 (m, 2H, –CH_2–), 2.56 (d, 1H, imide, J = 18.46 Hz), 2.78 (d, 1H, imide, J = 18.47 Hz), 6.48 (d, 1H, C_6-ArH, J = 8.70 Hz), 6.53 (br s, 1H, NH), 7.11 (dd, 1H, C_5-ArH, J = 2.28 Hz, J = 8.69 Hz), 7.35 (d, 1H, C_3-ArH, J = 2.29 Hz); MS m/z 304 $[M+4]^+$, 302 $[M+2]^+$, 300 $[M]^+$; $C_{13}H_{14}Cl_2N_2O_2$ (301.17).

4.1.1.15. *N*-(4-Bromophenyl)-amino-3-ethyl-3-methyl-pyrrolidine-2,5-dione (29). White powdery crystals. Yield: 66%; HPLC (t_R 2.71 min); mp 122–124 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H, –CH_3, J = 7.44 Hz), 1.38 (s, 3H, –CH_3), 1.64–1.88 (m, 2H, –CH_2–), 2.54 (d, 1H, imide, J = 18.46 Hz), 2.75 (d, 1H, imide, J = 18.46 Hz), 6.07 (br s, 1H, NH), 6.65–6.68 (m, 2H, $C_{2,6}$-ArH), 7.34–7.38 (m, 2H, $C_{3,5}$-ArH); $C_{13}H_{15}BrN_2O_2$ (311.18).

4.1.1.16. *N*-(2-Chlorophenyl)-amino-3-methyl-3-propyl-pyrrolidine-2,5-dione (30). White powdery crystals. Yield: 67%; HPLC (t_R 2.90 min); mp 96–98 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (t, 3H, –CH_3, J = 7.21 Hz), 1.19–1.80 (m, 7H, –C_2H_4–, –CH_3), 2.57 (d, 1H, imide, J = 18.42 Hz), 2.80 (d, 1H, imide, J = 18.42 Hz), 6.53 (dd, 1H, C_6-ArH, J = 1.42 Hz, J = 8.11 Hz), 6.58 (br s, 1H, NH), 6.91 (td, 1H, C_4-ArH, J = 1.42 Hz, J = 7.76 Hz), 7.11–7.17 (m, 1H, C_5-ArH), 7.32 (dd, 1H, C_3-ArH, J = 1.40 Hz, J = 7.97 Hz). $C_{14}H_{17}ClN_2O_2$ (280.76).

4.1.1.17. *N*-(3-Chlorophenyl)-amino-3-methyl-3-propyl-pyrrolidine-2,5-dione (31). White powdery crystals. Yield: 67%; HPLC (t_R 2.95 min); mp 58–60 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (t, 3H, –CH_3, J = 7.21 Hz), 1.20–1.80 (m, 7H, –C_2H_4–, –CH_3), 2.56 (d, 1H, imide, J = 18.42 Hz), 2.78 (d, 1H, imide, J = 18.41 Hz), 6.10 (br s, 1H, NH), 6.65 (ddd, 1H, C_6-ArH, J = 0.70 Hz, J = 2.26 Hz, J = 8.15 Hz), 6.73 (t, 1H, C_2-ArH, J = 2.09 Hz), 6.95 (ddd, 1H, C_4-ArH, J = 0.74 Hz, J = 1.80 Hz, J = 7.96 Hz), 7.16 (t, 1H, C_5-ArH, J = 8.05 Hz); $C_{14}H_{17}ClN_2O_2$ (280.76).

4.1.1.18. *N*-(4-Chlorophenyl)-amino-3-methyl-3-propyl-pyrrolidine-2,5-dione (32). White powdery crystals. Yield: 66%; HPLC (t_R 2.91 min); mp 90–92 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H, –CH_3, J = 7.20 Hz), 1.18–1.78 (m, 7H, –C_2H_4–, –CH_3), 2.54 (d, 1H, imide, J = 18.43 Hz), 2.77 (d, 1H, imide, J = 18.42 Hz), 6.10 (br s, 1H, NH), 6.70–6.73 (m, 2H, $C_{2,6}$-ArH), 7.19–7.22 (m, 2H, $C_{3,5}$-ArH); $C_{14}H_{17}ClN_2O_2$ (280.76).

4.1.1.19. *N*-(2,4-Dichlorophenyl)-amino-3-methyl-3-propyl-pyrrolidine-2,5-dione (33). White powdery crystals. Yield: 73%; HPLC (t_R 2.91 min); mp 108–110 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (t, 3H, –CH_3, J = 7.20 Hz), 1.20–1.79 (m, 7H, –C_2H_4–, –CH_3), 2.57 (d, 1H, imide, J = 18.46 Hz), 2.79 (d, 1H, imide, J = 18.46 Hz), 6.47 (d, 1H, C_6-ArH, J = 8.70 Hz), 6.52 (br s, 1H, NH), 7.12 (dd, 1H, C_5-ArH, J = 2.09 Hz, J = 2.49 Hz), 7.34 (d, 1H, C_3-ArH, J = 2.28 Hz); $C_{14}H_{16}Cl_2N_2O_2$ (315.2).

4.1.1.20. *N*-(4-Bromophenyl)-amino-3-methyl-3-propyl-pyrrolidine-2,5-dione (34). White powdery crystals. Yield: 66%; HPLC (t_R 2.98 min); mp 115–117 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 3H, –CH_3, J = 7.20 Hz), 1.18–1.78 (m, 7H, –C_2H_4–, –CH_3), 2.55 (d, 1H, imide, J = 18.43 Hz), 2.77 (d, 1H, imide, J = 18.42 Hz), 6.07 (br s, 1H, NH), 6.65–6.67 (m, 2H, $C_{2,6}$-ArH), 7.34–7.37 (m, 2H, $C_{3,5}$-ArH); $C_{14}H_{17}BrN_2O_2$ (325.21).

4.1.1.21. *N*-(2-Chlorophenyl)-amino-3-butyl-3-methyl-pyrrolidine-2,5-dione (35). White powdery crystals. Yield: 64%; HPLC (t_R 3.17 min); mp 78–80 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.91 (t, 3H, –CH_3, J = 7.05 Hz), 1.17–1.82 (m, 9H, –C_3H_6–, –CH_3), 2.56 (d, 1H, imide, J = 18.42 Hz), 2.78 (d, 1H, imide, J = 18.42 Hz), 6.55 (dd, 1H, C_6-ArH, J = 1.41 Hz, J = 8.12 Hz), 6.59 (br s, 1H, NH), 6.91 (td, 1H, C_4-ArH, J = 1.42 Hz, J = 7.76 Hz), 7.11–7.17 (m, 1H, C_5-ArH), 7.33 (dd, 1H, C_3-ArH, J = 1.38 Hz, J = 7.96 Hz); $C_{15}H_{19}ClN_2O_2$ (294.78).

4.1.1.22. *N*-(4-Chlorophenyl)-amino-3-butyl-3-methyl-pyrrolidine-2,5-dione (36). White powdery crystals. Yield: 66%; HPLC (t_R 3.15 min); mp 94–96 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, 3H, –CH_3, J = 7.06 Hz), 1.14–1.80 (m, 9H, –C_3H_6–, –CH_3), 2.55 (d, 1H, imide, J = 18.43 Hz), 2.76 (d, 1H, imide, J = 18.43 Hz), 6.08 (br s, 1H, NH), 6.71–6.74 (m, 2H, $C_{2,6}$-ArH), 7.19–7.22 (m, 2H, $C_{3,5}$-ArH); MS m/z 297 $[M+2]^+$, 295 $[M]^+$; $C_{15}H_{19}ClN_2O_2$ (294.78).

4.1.1.23. *N*-(2,4-Dichlorophenyl)-amino-3-butyl-3-methyl-pyrrolidine-2,5-dione (37). White powdery crystals. Yield: 69%; HPLC (t_R 3.45 min); mp 68–70 °C;

^{1}H NMR (300 MHz, $CDCl_3$): δ 0.91 (t, 3H, –CH_3, J = 7.01 Hz), 1.15–1.81 (m, 9H, –C_3H_6–, –CH_3), 2.56 (d, 1H, imide, J = 18.46 Hz), 2.78 (d, 1H, imide, J = 18.47 Hz), 6.48 (d, 1H, C_6-ArH, J = 8.70 Hz), 6.52 (br s, 1H, NH), 7.11 (dd, 1H, C_5-ArH, J = 2.26 Hz, J = 8.69 Hz), 7.34 (d, 1H, C_3-ArH, J = 2.28 Hz); $C_{15}H_{18}Cl_2N_2O_2$ (329.23).

4.1.1.24. *N*-(4-Bromophenyl)-amino-3-butyl-3-methyl-pyrrolidine-2,5-dione (38). White powdery crystals. Yield: 67%; HPLC (t_R 3.22 min); mp 104–106 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.90 (t, 3H, –CH_3, J = 7.05 Hz), 1.14–1.79 (m, 9H, –C_3H_6–, –CH_3), 2.54 (d, 1H, imide, J = 18.43 Hz), 2.76 (d, 1H, imide, J = 18.43 Hz), 6.09 (br s, 1H, NH), 6.65–6.68 (m, 2H, $C_{2,6}$-ArH), 7.33–7.37 (m, 2H, $C_{3,5}$-ArH); $C_{15}H_{19}BrN_2O_2$ (339.23).

4.1.1.25. *N*-Phenylamino-3,3-diethyl-pyrrolidine-2,5-dione (39). White powdery crystals. Yield: 73%; HPLC (t_R 2.49 min); mp 68–70 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.93 (t, 6H, –CH_3, –CH_3, J = 7.46 Hz), 1.61–1.88 (m, 4H, –CH_2–, –CH_2–), 2.63 (s, 2H, imide), 6.19 (br s, 1H, NH), 6.76–6.79 (m, 2H, $C_{2,6}$-ArH), 6.94–7.00 (m, 1H, C_4-ArH), 7.21–7.25 (m, 2H, $C_{3,5}$-ArH); $C_{14}H_{18}N_2O_2$ (246.31).

4.1.1.26. *N*-(4-Methylphenyl)-amino-3,3-diethyl-pyrrolidine-2,5-dione (40). White powdery crystals. Yield: 75%; HPLC (t_R 2.71 min); mp 76–78 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.92 (t, 6H, –CH_3, –CH_3, J = 7.45 Hz), 1.63–1.85 (m, 4H, –CH_2–, –CH_2–), 2.26 (s, 3H, Ar-4CH_3), 2.61 (s, 2H, imide), 6.06 (br s, 1H, NH), 6.70–6.74 (m, 2H, $C_{2,6}$-ArH), 7.03–7.05 (m, 2H, $C_{3,5}$-ArH); $C_{15}H_{20}N_2O_2$ (260.34).

4.1.1.27. *N*-(2-Chlorophenyl)-amino-3,3-diethyl-pyrrolidine-2,5-dione (41). White powdery crystals. Yield: 67%; HPLC (t_R 2.82 min); mp 106–108 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.96 (t, 6H, –CH_3, –CH_3, J = 7.47 Hz), 1.64–1.90 (m, 4H, –CH_2–, –CH_2–), 2.66 (s, 2H, imide), 6.56 (dd, 1H, C_6-ArH, J = 1.45 Hz, J = 8.12 Hz), 6.60 (br s, 1H, NH), 6.90 (td, 1H, C_4-ArH, J = 1.46 Hz, J = 7.69 Hz), 7.10–7.15 (m, 1H, C_5-ArH), 7.32 (dd, 1H, C_3-ArH, J = 1.38 Hz, J = 7.96 Hz); $C_{14}H_{17}ClN_2O_2$ (280.76).

4.1.1.28. *N*-(4-Chlorophenyl)-amino-3,3-diethyl-pyrrolidine-2,5-dione (42). White powdery crystals. Yield: 72%; HPLC (t_R 2.85 min); mp 113–115 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.92 (t, 6H, –CH_3, –CH_3, J = 7.46 Hz), 1.64–1.88 (m, 4H, –CH_2–, –CH_2–), 2.63 (s, 2H, imide), 6.12 (br s, 1H, NH), 6.70–6.75 (m, 2H, $C_{2,6}$-ArH), 7.19–7.22 (m, 2H, $C_{3,5}$-ArH); MS *m*/*z* 283 $[M+2]^+$; 281 $[M]^+$; $C_{14}H_{17}ClN_2O_2$ (280.76).

4.1.1.29. *N*-(2,4-Dichlorophenyl)-amino-3,3-diethyl-pyrrolidine-2,5-dione (43). White powdery crystals. Yield: 71%; HPLC (t_R 3.18 min); mp 98–100 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.95 (t, 6H, –CH_3, –CH_3, J = 7.46 Hz), 1.66–1.88 (m, 4H, –CH_2–, –CH_2–), 2.65 (s, 2H, imide), 6.49 (d, 1H, C_6-ArH, J = 8.71 Hz), 6.53 (br s, 1H, NH), 7.11 (dd, 1H, C_5-ArH, J = 2.27 Hz, J = 8.71 Hz), 7.34 (d, 1H, C_3-ArH, J = 2.28 Hz); MS *m*/*z* 318 $[M+4]^+$, 316 $[M+2]^+$, 314 $[M]^+$; $C_{14}H_{16}Cl_2N_2O_2$ (315.20).

4.1.1.30. *N*-(4-Bromophenyl)-amino-3,3-diethyl-pyrrolidine-2,5-dione (44). White powdery crystals. Yield: 72%; HPLC (t_R 2.92 min); mp 136–138 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.92 (t, 6H, –CH_3, –CH_3, J = 7.46 Hz), 1.64–1.88 (m, 4H, –CH_2–, –CH_2–), 2.63 (s, 2H, imide), 6.12 (br s, 1H, NH), 6.65–6.69 (m, 2H, $C_{2,6}$-ArH), 7.33–7.36 (m, 2H, $C_{3,5}$-ArH); $C_{14}H_{17}BrN_2O_2$ (325.21).

4.1.1.31. *N*-Phenylamino-hexahydro-isoindole-1,3-dione (45).[‡] White powdery crystals. Yield: 74%; HPLC (t_R 2.32 min); mp 156–158 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.47–1.53 (m, 4H, cyclohexane), 1.81–1.97 (m, 4H, cyclohexane), 2.95–3.03 (m, 2H, imide), 6.09 (br s, 1H, NH), 6.76–6.80 (m, 2H, $C_{2,6}$-ArH), 6.94–7.00 (m, 1H, C_4-ArH), 7.21–7.27 (m, 2H, $C_{3,5}$-ArH); $C_{14}H_{16}N_2O_2$ (244.30).

4.1.1.32. *N*-(4-Methylphenyl)-amino-hexahydro-isoindole-1,3-dione (46). White powdery crystals. Yield: 70%; HPLC (t_R 2.55 min); mp 136–138 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.48–1.51 (m, 4H, cyclohexane), 1.84–1.91 (m, 4H, cyclohexane), 2.26 (s, 3H, –CH_3), 2.96–2.99 (m, 2H, imide), 6.00 (br s, 1H, NH); 6.70–6.74 (m, 2H, $C_{2,6}$-ArH), 7.03–7.06 (m, 2H, $C_{3,5}$-ArH); $C_{15}H_{18}N_2O_2$ (258.32).

4.1.1.33. *N*-(2-Chlorophenyl)-amino-hexahydro-isoindole-1,3-dione (47). White powdery crystals. Yield: 74%; HPLC (t_R 2.64 min); mp 156–158 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.49–1.55 (m, 4H, cyclohexane), 1.82–1.97 (m, 4H, cyclohexane), 2.98–3.06 (m, 2H, imide), 6.55–6.58 (m, 2H, 1H, NH, 1H, C_6-ArH), 6.90 (td, 1H, C_4-ArH, J = 1.42 Hz, J = 7.72 Hz), 7.10–7.16 (m, 1H, C_5-ArH), 7.32 (dd, 1H, C_3-ArH, J = 1.37 Hz, J = 7.96 Hz); $C_{14}H_{15}ClN_2O_2$ (278.74).

4.1.1.34. *N*-(3-Chlorophenyl)-amino-hexahydro-isoindole-1,3-dione (48). White powdery crystals. Yield: 70%; HPLC (t_R 2.69 min); mp 168–170 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.50–1.57 (m, 4H, cyclohexane), 1.84–1.96 (m, 4H, cyclohexane), 3.00–3.04 (m, 2H, imide), 6.07 (br s, 1H, NH), 6.65 (ddd, 1H, C_6-ArH, J = 0.88 Hz, J = 2.29 Hz, J = 8.12 Hz), 6.73 (t, 1H, C_2-ArH, J = 2.07 Hz), 6.95 (ddd, 1H, C_4-ArH, J = 0.88 Hz, J = 1.90 Hz, J = 7.98 Hz), 7.17 (t, 1H, C_5-ArH, J = 8.04 Hz); $C_{14}H_{15}ClN_2O_2$ (278.74).

4.1.1.35. *N*-(4-Chlorophenyl)-amino-hexahydro-isoindole-1,3-dione (49). White powdery crystals. Yield: 73%; HPLC (t_R 2.63 min); mp 188–190 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.48–1.53 (m, 4H, cyclohexane), 1.85–1.93 (m, 4H, cyclohexane), 2.98–3.01 (m, 2H, imide), 6.04 (br s, 1H, NH), 6.72–6.75 (m, 2H, $C_{2,6}$-

[‡] Compound **45** was described by Baddar et al.,[23] mp 173–174 °C, no ^{1}HNMR data available.

ArH), 7.19–7.22 (m, 2H, $C_{3,5}$-ArH); MS *m/z* 281 $[M+2]^+$, 279 $[M]^+$; $C_{14}H_{15}ClN_2O_2$ (278.74).

4.1.1.36. *N*-(2,4-Dichlorophenyl)-amino-hexahydro-isoindole-1,3-dione (50). White powdery crystals. Yield: 69%; HPLC (t_R 2.99 min); mp 165–167 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.51–1.58 (m, 4H, cyclohexane), 1.81–1.98 (m, 4H, cyclohexane), 3.00–3.04 (m, 2H, imide), 6.49–6.52 (m, 2H, 1H, NH, 1H, C_6-ArH), 7.11 (dd, 1H, C_5-ArH, J = 2.25 Hz, J = 8.90 Hz), 7.34 (d, 1H, C_3-ArH, J = 2.29 Hz); $C_{14}H_{14}Cl_2N_2O_2$ (313.19).

4.1.1.37. *N*-Methylamino-3,3-dimethyl-pyrrolidine-2,5-dione (51). White powdery crystals. Yield: 61%; mp 61–63 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.34 (s, 6H, $-CH_3$, $-CH_3$), 2.54 (s, 2H, imide), 2.72 (s, 3H, $-CH_3$), 3.02 (br s, 1H, NH); $C_7H_{12}N_2O_2$ (156.19).

4.2. Lipophilicity determination

The analytical system consisted of a Waters (Milford, USA) instrument, equipped with 515 HPLC pumps, a 600S flow controller, 486 Tunable Absorbance Detector and injector: Rheodyne 7161 valve (Cotati, CA, USA). The measurements were performed on a LiChrospher C_{18} end-capped column, 250 × 4.6 mm (Merck, Darmstadt, Germany). Acetonitrile for measurements was of gradient grade and was purchased from Merck (Darmstadt, Germany). The water was filtrated under vacuum a 0.45 μm HA Millipore filter (Millipore, Milford, MA, USA). For chromatographic analysis stock solutions of 1.0 mg/ml of the samples in acetonitrile/water (1:1, v/v) were prepared and filtrated through 0.2 μm Millipore filter (Millipore, Milford, MA, USA). These solutions were kept in Eppendorf tubes at room temperature. Isocratic runs were carried out with mixtures containing increasing acetonitrile volume fractions from 40% to 95% v/v in 5% increment. Flow rate 1 ml/min. The injection volume was 5μl in all cases. Uracil (Merck, Darmstadt, Germany) was used as the unretained compound to determine the dead volume at the different acetonitrile concentrations in the mobile phase. The retention times were measured at room temperature by the UV detector at the λ_{max} of the analytes (214 nm). Three sets of measurements were conducted for each compound and the mean value was used for further calculations. The capacity factors log k were calculated by us of equation: $k = (t_r - t_0)/t_0$, where t_r and t_0 are the retention times for solute and unretained compound (uracil), respectively. The log k values were extrapolated to 100% water concentration that enabled the estimation of relative retention parameters log k_w. This procedure utilized an equation: $\log k = \log k_w - S\varphi$, where S is the slope, φ is the volume fraction of the organic modifier. The log k_w parameters characterize the partition of the compound between a non-polar hydrocarbon stationary phase and pure water.[24,25]

4.3. Pharmacology

The initial anticonvulsant evaluation was performed within the Antiepileptic Drug Development (ADD) Program in Epilepsy Branch, National Institutes of Health, National Institute of Neurological Disorders and Stroke (NIH/NINDS), Bethesda, MD, USA, by using procedures described elsewhere.[26,27]

Male albino mice (CF-1 strain) and male albino rats (Sprague–Dawley) were used as experimental animals. The animals were housed in metabolic cages and allowed free access to food and water. The compounds were suspended in 0.5% methylcellulose/water mixture.

Phase I studies in mice involved two convulsant tests: maximal electroshock seizure test (MES), subcutaneous pentylenetetrazole seizure test (*sc*PTZ) and rotorod test for neurological toxicity (NT).

4.3.1. The maximal electroshock test (MES). In the MES screen, an electrical stimulus of 0.2 s in duration (50 mA in mice and 150 mA in rats at 60 Hz) is delivered via corneal electrodes primed with an electrolyte solution containing an anesthetic agent.

4.3.2. The subcutaneous pentylenetetrazole seizure test (*sc*PTZ). This screen utilizes a dose of pentylenetetrazole (85 mg/kg in mice and 70 mg/kg in rats) that produces clonic seizures lasting for a period of at least five seconds in 97% (CD_{97}) of animals tested. At the anticipated time of testing the convulsant is administered subcutaneously.

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. The results are presented in Table 1.

Selected derivatives were administrated orally into rats using four animals at a fixed dose of 30 mg/kg (MES test) and 50 mg/kg (*sc*PTZ test) (Phase VIa). This screen discloses the time of onset, the approximate time of peak effect (TPE) and the duration of anticonvulsant activity. For both doses the motor impairment was studied in parallel. Rats were tested at five time periods ranging from one quarter to 4 h post-substance administration. The results are shown in Tables 2 and 3.

4.3.3. The neurological toxicity (NT). induced by compound was detected in mice or rats using standardized rotorod test.[28] Untreated control mice or rats, when placed on the rod, can maintain their equilibrium for a prolonged time period. The acute motor impairment can be demonstrated by the inability of the animal to maintain equilibrium for a given time.

4.3.4. Quantification studies. The quantitative determination of ED_{50} and TD_{50} values for **15** was performed at previously estimated time of peak effect after oral administration into rats. Groups of eight rats received various doses of the compound until at least three points were established in the range of 10–90% seizure protection or minimal neurotoxicity. From the plot of the data obtained, the respective ED_{50} and TD_{50} values, 95% confidence intervals, slope of the regression line and standard error of the slope were calculated by means of a computer program written at NINDS/NIH. The results are shown in Table 4.

4.3.5. The 6-Hz model. This screen was carried out according to the protocol originally described by Brown et al.[29] and more recently by Barton et al.[20] and Kaminski et al.[30] It is an alternative electroshock paradigm that uses low-frequency (6 Hz), long-duration (3 s) electrical stimulation. Corneal stimulation (0.2 ms-duration monopolar rectangular pulses at 6-Hz for 3 s) was delivered by a constant-current device. During the stimulation, mice were manually restrained and released into the observation cage immediately after the current application. The seizures manifest in 'stunned' posture associated with rearing, forelimb, automatic movements and clonus, twitching of the vibrissae and Straub-tail. The duration of the seizure activity ranges from 60 to 120 s in untreated animals. At the end of the seizure, animals resume their normal exploratory behavior. The experimental end point is protection against the seizure. The animal is considered to be protected if it resumes its normal exploratory behavior within 10 s from the stimulation.[30]

4.3.6. The Pilocarpine Induced Status Prevention (PISP) model. The male albino rats (Sprague–Dawley, 150–180 g) were used as experimental animals. The compounds were administrated via the ip route of administration. Then a challenge dose of pilocarpine is given observing for treatment-effects of the substance tested. The seizure severity is determined using the well-known Racine scale,[31] as follows: (I) immobility, eye closure, twitching of vibrissae, sniffing, and facial clonus; (II) head nodding associated with more severe facial clonus; (III) clonus of one of the fore limbs; (IV) rearing often accomplished by bilateral forelimb clonus and (V) all of the above plus loss of balance and falling, accomplished by generalized clonic seizures. The anticonvulsant activity of compounds **18** and **46** was assessed at time zero, namely the time from the first stage III seizures (Test 71). The outcome measures are determination of 'protection' or 'no protection'.

4.4. Statistical analysis

Statistical analyses were performed using the computer program Statistica version 5, 1997 Edition, Copyright© Statsoft, Inc. 1984–1997.

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