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Synthesis of *N*-1′, *N*-3′-disubstituted spirohydantoins and their anticonvulsant activities in pilocarpine model of temporal lobe epilepsy

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ABSTRACT

Herein we report the synthesis and anticonvulsant activity of a library of eighteen new compounds that are structural mimics of phenytoin. These class of compounds contain a *N*-1′, *N*-3′-disubstituted spirohydantoin scaffold, where the *N*-1′ and *N*-3′ positions are modified with an alkyl group or aryl group. Of the eighteen compounds synthesized and tested, compound **5c** showed the best anticonvulsant activity. It completely prevented the precursor events of motor seizure in the pilocarpine model of temporal lobe epilepsy. Additionally, ten of the analogs were more effective than phenytoin when compared using the Racine's score in the pilocarpine model. Based on the structure activity relationship (SAR), we concluded that alkyl groups (ethyl, propyl or cyclopropyl) at *N*-3′ position and 4-nitro phenyl group at *N*-1′ position are desirable.

Epilepsy is broadly characterized as a disease that affects the central nervous system (CNS), where episodes of convulsive seizure are typically observed.[1,2] According to the International League Against Epilepsy (ILAE), it is redefined as 'a disease of the brain', in contrast to its previous characterization as a CNS disorder.[3] It is considered the third most common CNS disorder, and according to WHO, approximately 50 million people are currently affected by epilepsy worldwide.[4] Despite the approval of various therapeutic agents for the treatment of epilepsy, current literature reports indicate that there is still a pressing need for more efficacious, and tolerable drug leads.[5] Discovery of diphenylhydantoins (i.e., phenytoin, a sodium ion channel blocker)[6] as antiepileptic agents led to synthesis of several hydantoin analogs that exhibited antiepileptic activity.[7] Subsequently, spirohydantoins and 5,5-(cyclichydrocarbon) hydantoins were also synthesized and shown to exhibit promising anticonvulsant properties[8] (see Fig. 1).

Our research group has a long-standing interest in investigating the spirohydantoin scaffold to generate structural analogs with improved anticonvulsant activity and reduced toxicity.[9–12] From our previous work, we established that modification of *N*-1′ and *N*-3′ positions with various groups enabled us to build a library of compounds, and evaluate the SAR for this particular scaffold.[10] In this manuscript, we are reporting our recent efforts on the synthesis of a new series of *N*-1′,*N*-3′-disubstituted spirohydantoins, and we evaluated their anticonvulsant activities in a pilocarpine model of temporal lobe epilepsy using Racine's score of anticonvulsant activities.[13]

Based on our previous findings, we set out to investigate the effect of various aryl groups on *N*-1′ position, and aliphatic groups on *N*-3′ position on this class of anticonvulsant compounds. A new series of *N*-1′,*N*-3′-disubstituted, 2′H, 3′H, 5′H-spiro[2-benzofuran-1,4′-imidazolidine]-2′, 3, 5′-triones (Table 1) were prepared according to previously established synthetic methods in our lab (Scheme 1).[12] All the final spirohydantoins were made from the corresponding substituted amine and isocyanate.

The synthesis[14–16] began with the preparation of urea derivatives (**3**), and it was subsequently reacted with ninhydrin. The adduct **4** was the advance intermediate and it was exclusively formed as a single regioisomer in 85–90% yield. The regioselectivity was confirmed by ^{1}H NMR spectroscopy. Our group had previously reported the characterization of such adduct using X-ray crystallography and ^{1}H NMR, and confirmed the regioselectivty.[10–12] Nevertheless, we did isolate **4** as a racemic mixture, and was carried forward to the next step. The final transformation involved an oxidative rearrangement of the adduct **4** into the desired spirohydantoin **5**. The rearrangement afforded a single regioisomer of the desired product in 65–75% yield. We proceeded to test the activity of the spirohydantoins as a racemic mixture.

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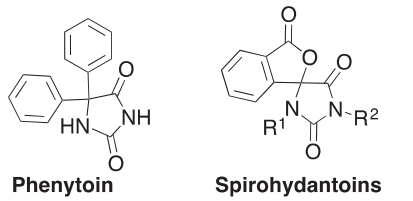


Figure 1. Structures of phenytoin and the spirohydantoins.

Once we had prepared all the derivatives (**5a**–**5s**), we proceeded to evaluate their anticonvulsant activities. They were tested at a dose of 100 mg/kg in a pilocarpine model of temporal lobe epilepsy, a model of status epilepticus.[17–19] Besides synthesized compounds, phenytoin and dimethyl sulfoxide (DMSO) were included as a clinical drug control, and the vehicle control, respectively. In order to compare the activity of newly synthesized compounds to our previously published findings, **5d** was resynthesized and tested in this pilocarpine model. The anticonvulsant activities were evaluated using the average Racine's score[13] at the endpoint of 2 h after injection of pilocarpine. The latency of onset time of motor seizure (Racine's score ⩾ 3), duration of severity (suffering from motor seizure), and survival rates were also determined and recorded.

Ten out of eighteen compounds significantly reduced the average Racine's score from 4.5 to less than 2.0, compared to DMSO (Table 1). Their activities were much better than phenytoin (Racine's score = 3.3). Among them **5b**, **5c**, **5d**, **5g**, **5h**, **5i**, **5m** and **5n** exhibited best activities, with significantly reduced average Racine's score compared to DMSO (Table 1). Their activities were noticeably better than that of phenytoin. To better understand the structure activity relationship of these new compounds, we studied the groups on *N*-1′ and *N*-3′ positions. With an *n*-propyl group in the R^1 position, **5g**, **5h**, and **5i**, where R^2 is substituted with 4-nitrophenyl, 4-chlorophenyl and 2-ethylphenyl, respectively showed improved Racine's score. This comparison indicates that 4-nitrophenyl on R^2 position provides the best activity in the pilocarpine model. In a similar fashion, compounds with cyclopropyl group in the R^1 position, in combination with 4-nitrophenyl (**5m**), 4-chlorophenyl (**5n**), and 2-ethylphenyl (**5o**) were compared. Within this series, the 4-nitrophenyl group provided the best anticonvulsant activity.

In both cases, activities of compounds containing a 4-nitrophenyl group in R^2 position, and having a small hydrophobic group (**5g** and **5m**) are the same in contributing to the lower Racine's score (Racine's score = 0.4) than the compound with phenyl group at R^2 position (**5r**, Racine's score = 3.5). A similar SAR is also confirmed by comparing **5h**, **5n** with **5s**, where 4-chlorophenyl group in R^2 position, in combination with aliphatic groups proved to be more effective.

It is interesting to note that, when a phenyl group is in R^2 position, compounds **5e**, **5k** and **5p** showed potent sedative effect. Rat pups lost righting reflex within 10 min after injection of these compounds, and they did not recover in the following four hours. This is a unique finding from this study, and may provide a new direction for further research in the future.

DMSO and phenytoin had a motor seizure onset time of 6 min and 12 min, respectively, where five of the synthetic spirohydantoins (**5f**, **5g**, **5l**, **5r** and **5s**) considerably delayed the seizure onset time. Interestingly, compound **5r** delayed the onset time from 6 min to 104 min. Moreover, compound **5c** completedly protected the rats from motor seizures.

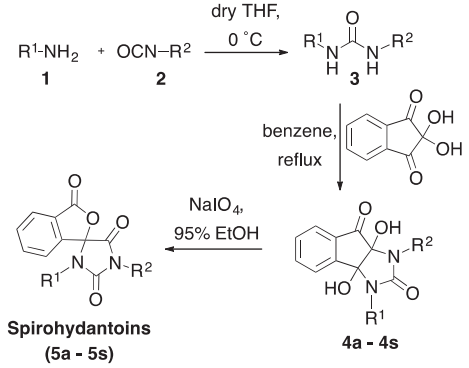
As Pilocarpine induces status epilepticus in our experimental model, we monitor progression of seizure over a 120 min period. After injection of pilocarpine, DMSO group rapidly reached stage 5, the average Racine's score were not decreased in the following two hours which means the rats would suffer from constant seizure. Thus, shortening duration of severity or total prevention of motor seizure is very important. Most tested compounds reduced duration of motor seizures compared to DMSO with 114 min duration. Eight compounds shorten the duration of severity to less than half duration time of severity of phenytoin. For the most promising candidates, we monitored the progression of seizure every 30 min up to 120 min (Table 2). The results indicate that the test animals recovered from seizure within 90–120 min when treated with compounds **5c**, **5d**, **5g** and **5m**. Additionally, compound **5c** was effective in preventing motor seizure induced by pilocarpine, and the rats recovered from the pre-stage of motor seizure within 30 min. As constant seizure occurs in pilocarpine model, the survival rates were also evaluated. Most of the tested compounds increased the survival rate of the rats. They could largely increase survival rate to more than 80% when compared to DMSO (53% survival rate) and phenytoin (67% survival rate).

Table 1
SAR and activity profile of the synthetic spirohydantoins

Compounds	*N*-3′ position (R^1)	*N*-1′-position (R^2)	Racine's score[a,b]
5a	Ethyl	Methyl-4-benzoyl	1.8*
5b	Ethyl	2,6-Diethylphenyl	1.6*
5c	Ethyl	2,4-Dimethoxyphenyl	1.0*
5d	Ethyl	4-Nitrophenyl	1.0**
5e	Propyl	Phenyl	Sedative
5f	Propyl	4-Ethylphenyl	3.5
5g	Propyl	4-Nitrophenyl	0.4***
5h	Propyl	4-Chlorophenyl	0.8**
5i	Propyl	2-Ethylphenyl	0.8**
5j	Propyl	2,6-Diethylphenyl	1.3*
5k	Cyclopropyl	Phenyl	Sedative
5l	Cyclopropyl	4-Ethylphenyl	3.0
5m	Cyclopropyl	4-Nitrophenyl	0.4***
5n	Cyclopropyl	4-Chlorophenyl	1.0***
5o	Cyclopropyl	2-Ethylphenyl	Sedative
5p	Phenyl	Phenyl	Sedative
5q	Phenyl	4-Ethylphenyl	3.0
5r	Phenyl	4-Nitrophenyl	3.5
5s	Phenyl	4-Chlorophenyl	5.0
DMSO			4.5
Phenytoin			3.3

[a] Racine's score: stage 0 —normal nonepileptic activity; stage 1— facial movements, wet dog shakes, and scratching; stage 2— head nodding, tremor; stage 3—forelimb clonus and forelimb extension; stage 4—rearing and salivation; stage 5— falling and status epilepticus. Animals dead from status epilepticus during experiments were recorded as stage 5 in the following observing time points.

[b] One way ANOVA followed by the first post hoc, LSD test was performed and data are expressed, $n = 5$. SEM was not listed. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ are considered to be significantly different from DMSO alone.



Scheme 1. Synthesis of the *N*-1′, *N*-3′-disubstituted spirohydantoins.

Table 2
Progression of status epilepticus induced by pilocarpine following pre-treatment with compounds **5c**, **5d**, **5g** and **5m**

Time (min)	DMSO	Racine's score[a,b]				
		Phenytoin	**5c**	**5d**	**5g**	**5m**
30	4.8	3.0	2.4*	4.4	4.8	4.8
60	4.8	4.5	1.0**	3.2	3.8*	3.8
90	4.5	4.3	1.0**	2.6	1.0**	1.6**
120	4.5	3.3	1.0*	1.0**	0.4***	0.4***

[a] Racine's score: stage 0— normal nonepileptic activity; stage 1— facial movements, wet dog shakes, and scratching; stage 2— head nodding, tremor; stage 3—forelimb clonus and forelimb extension; stage 4—rearing and salivation; stage 5— falling and status epilepticus. Animals dead from status epilepticus during experiments were recorded as stage 5 in the following observing time points.

[b] One way ANOVA followed by the first post hoc, LSD test was performed and data are expressed, $n = 5$. SEM was not listed. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ are considered to be significantly different from the DMSO alone.

In summary, we have successfully synthesized a series of eighteen structural derivatives of *N*-1′, *N*-3′-disubstituted spirohydantoins, which mimic the structure of phenytoin. Based on the SAR, the most active compounds had an alkyl substituent at *N*-3′ position, and a 4-nitrophenyl substituent at *N*-1′ position. In terms of protective effect against pilocarpine-induced seizure, we found that ten compounds (**5a**, **5b**, **5c**, **5d**, **5g**, **5h**, **5i**, **5j**, **5m**, and **5n**) to be more effective than phenytoin. Based on proposed mechanism of action of phenytoin[6], it is likely that the synthetic spirohydantoins function through a similar mode of action. During this study, we were pleased to find that a set of analogs with phenyl group on *N*-1′ position exhibited sedative effects, which may be of interest to researchers in the field who are working on spirohydantoins as agents to target other CNS disorders. Based on these SAR results, we are hopeful that further evaluation of hydrophobic alkyl groups on *N*-3′ position, and electron deficient aryl groups on *N*-1′ position may provide a new direction in improving anticonvulsant activity.

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14. *General procedure for the syntheses of N-1-ethyl, N-3-substituted ureas:* The appropriately substituted isocyanate in 50 ml of dry tetrahydrofuran was added dropwise to the appropriate amine at 0 °C with stirring. The ice-bath was removed after the completion of addition of the isocyanate and the mixture was stirred at room temperature for 30 min. The precipitate formed was isolated via filtration. The product was dried in vacuo and carried forward to the next step without further purification.
15. *General procedure for the synthesis of Indeno [1,2-d] imidazolones (ninhydrin-1,3-disubstituted urea adducts):* Ninhydrin (20–35 mmol) was added to 150 ml of benzene containing equivalent amount of appropriately substituted urea in 500 ml round bottom flask, and heated to reflux for 60 min or more with stirring. The precipitate formed was isolated via filtration. The precipitate was washed with water, and the crude product was dried in vacuo overnight and recrystallized from aqueous methanol (~90%) to a constant melting point. The compounds were isolated in 85–90% yield.
16. *General procedure for the synthesis of N-1′, N-3′-disubstituted spirohydantoins:* sodium periodate, (5–20 mmol) in 50 ml of water was added dropwise to 100 ml of ethanol containing 5–20 mmol of the appropriate urea adduct. The mixture was stirred at room temperature overnight (usually 12–24 h) and the reaction was monitored by TLC. At the end of reaction, inorganic precipitate was removed by filtration. The filtrate was concentrated to one-half of its volume, and the resultant precipitate was collected via filtration and washed with water. The crude product was allowed to dry overnight and recrystallized from ethyl acetate to a constant melting point. The final spirohydantoins were isolated in 65–75% yield. *Spectroscopic data for* ***5c***. MS: (direct insertion) *m*/*z* 382 (M+), 179, 136, 104 (base peak), 76. 1H NMR (400 MHz, DMSO-d_6) δ 8.18–7.68 (m, 4H), 7.42–7.36 (m, 1H), 6.76–6.63 (m, 2H), 3.83 (s, 6H), 3.41–3.36 (m, 1H), 3.15–3.05 (m, 1H), 0.99 (q, $J = 7.2$ Hz, 3H). IR (cm^{-1}): 2940, 2876 (C–H) 1784 (spirolactam carbonyl, C=O), 1731 (ureide, C=O) Anal. Calcd for $C_{22}H_{22}N_2O_4$: C, 69.83; H, 5.86; N, 7.40; Found: C, 69.89; H, 5.90; N, 7.31.
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19. SD Rat pups (14–21 days) received either dimethyl sulfoxide (DMSO) as vehicle control, phenytoin (drug control) or spirohydantoins (test compounds). Phenytoin was the standard drug with dose of 100 mg/kg body weight. Selected compounds were dissolved in DMSO and injections were made ip in a volume not exceeding 1 ml/kg body weight 2 h prior to administration of pilocarpine. Methylscopolamine bromide at a dose of 1 mg/kg ip was injected 30 min prior to administration of pilocarpine. Groups of 5 rats were tested with a 100 mg/kg dose for each compound as a DMSO solution. Control groups received either phenytoin or DMSO. Seizure was scored using Racine's scale as described above.