Sodium Channel Binding and Anticonvulsant Activities of Hydantoins Containing Conformationally Constrained 5-Phenyl Substituents

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Received August 28, 1989, from the *Department of Chemistry and ‡The Neuropsychiatry Research Program, University of Alabama at Birmingham, Birmingham, AL 35294. Accepted for publication December 4, 1989.

Abstract □ As a preliminary investigation of the importance of the aromatic ring orientation in interactions of 5-phenylhydantoins with the anticonvulsant site on the neuronal voltage-sensitive sodium channel, two isomeric hydantoins containing conformationally constrained phenyl rings and their monocyclic analogues were synthesized. One, a spirohydantoin (**2**) derived from α-tetralone, contains the plane of the phenyl ring in an orientation approximately perpendicular to that for the hydantoin ring. The other, a tricyclic hydantoin (**4**) derived from tetrahydroisoquinoline, contains the plane of the phenyl ring in an orientation roughly coplanar with that for the hydantoin ring. These compounds were evaluated in sodium channel binding and whole animal (mice) anticonvulsant assays. In both assays, **4** was significantly more potent than **2**, suggesting that the anticonvulsant receptor site on the voltage-sensitive sodium channel may require a specific aromatic ring orientation.

The widely used anti-maximal electroshock (anti-MES) anticonvulsant against which new agents continue to be measured is the cyclic imide diphenylhydantoin (DPH, also referred to as phenytoin). Diphenylhydantoin (DPH) and carbamazepine (CAR), a related acyclic urea, have been suggested to cause their anticonvulsant effects by binding at therapeutic concentrations to the neuronal voltage-sensitive sodium channel.[1,2] While other mechanisms of action for DPH have been proposed, binding to sodium channels is voltage and frequency dependent, and neurons that undergo prolonged depolarization or repetitive firing are more completely inhibited, providing an explanation for selective effects on hyperactive versus normal neurons. However, the anticonvulsant site on the voltage-sensitive sodium channel is relatively unstudied, even though it offers significant potential as a target for improved anti-MES anticonvulsants. Compounds with increased selectivity for this site may provide anti-MES anticonvulsants with enhanced potency and fewer side effects. Toward this end, we have been interested in studying the interactions of stereochemically defined compounds with this site.[3,4]

Conformational comparisons of a large number of anti-MES anticonvulsants containing aromatic substituents led to a proposal that the correct orientation of the aromatic rings may be the most important structural feature for activity.[5] In an effort to investigate this possibility for interactions with the sodium channel, we proposed the synthesis and biological evaluations of hydantoins **1–4**. The structures of **1–4** and DPH are illustrated in Figure 1. Compounds 2 and 4 were designed as isomeric tricyclic hydantoins which each constrain the phenyl ring, relative to the hydantoin ring, to different angular relationships. Compounds 1 and 3 were prepared as models of 2 and 4, respectively, which do not use covalent bonding to constrain the phenyl ring orientation.

Using Dreiding models, the range of possible torsional relationships between the phenyl and hydantoin rings of 2 and 4, as shown in Figure 1, was estimated. Using Alchemy

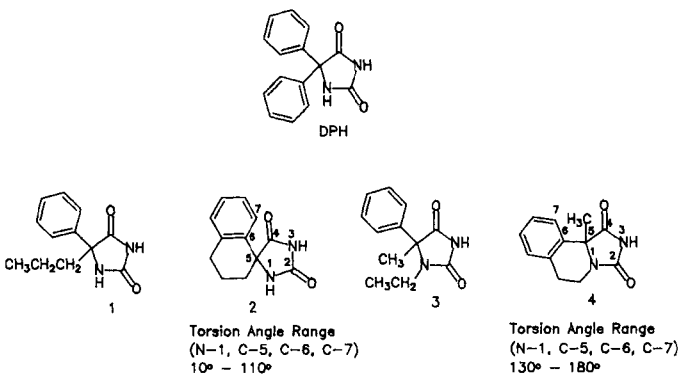

Figure 1—Structures and torsion angle ranges of DPH and the 5-phenylhydantoins synthesized for this study.

2[6] molecular modeling software for the IBM PC, a sampling of low energy conformations within each range was generated, and the relative total energy for each conformation was calculated using the molecular mechanics program of the software; the results are shown in Table I. The stereo view in Figure 2 shows a superimposition of the two lowest energy conformations from this study, **2B** and **4B** (Table I), revealing that upon superimposition of the hydantoin rings, the phenyl rings are held in orientations that are roughly perpendicular to one another.

Here we report that 4 is a more effective binder to the sodium channel than 2, and is also the more effective anti-MES anticonvulsant. This suggests that the sodium channel anticonvulsant site may require a specific aromatic ring orientation.

Table I—Relative Energies and Phenyl Ring Orientations for Selected Low Energy Conformations of Hydantoins 2 and 4.

Hydantoin Conformation[a]	Torsion Angle, ° N-1, C-5, C-6, C-7	Total Potential Energy, kcal/mol[b]
2A	13	6.4
2B	40	3.2
2C	61	3.3
2D	104	6.1
4A	136	5.6
4B	163	5.0
4C	174	5.1

[a] Each conformation is a local energy minimum that resulted from different starting geometries and was generated by the molecular mechanics program in Alchemy 2. [b] Calculated using the molecular mechanics program in Alchemy 2.

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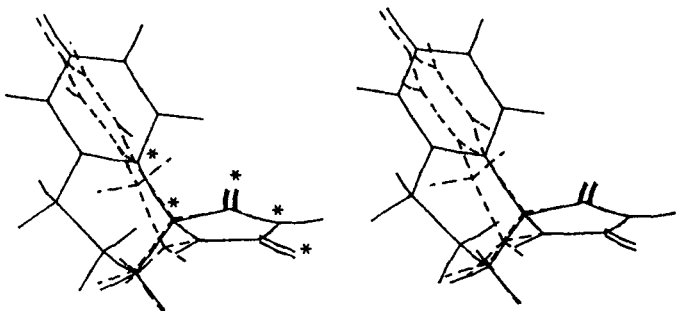


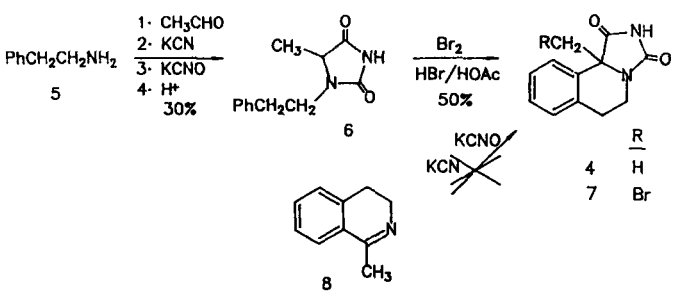
Figure 2—Stereo view (relaxed) for superimposed (starred atoms, RMS difference = 0.047 Å) hydantoins 2B (solid lines) and 4B (broken lines), which illustrates the roughly perpendicular relationship for the orientations of the phenyl rings. (Generated using Alchemy 2, a molecular modeling program for the IBM PC; see ref 6.)

Results and Discussion

Chemistry—Compounds **1** and **2** were synthesized from butyrophenone (97% overall yield) and α-tetralone (91% overall yield), respectively, in the presence of KCN and NH_4CO_3 according to published procedures.[7] Compound **3** was prepared from acetophenone via a modification of a literature method[8] (see *Experimental Section*). Compound **4**, which has not been previously reported, was prepared from phenylethylamine (**5**) according to a literature method[9] for related structures. As shown in Scheme I, the approach to **4** involving imine **8** was unsuccessful, although the cyclization of intermediate hydantoin **6** in the presence of bromine produced **4** in 50% yield. The bromo derivative **7** was the major cyclized product when this latter reaction was carried out under milder conditions. Cyclic imine **8** was also prepared by a published method.[10]

Biology—Hydantoins **1–4**, **6**, and **7** were each evaluated in synaptoneurosomal preparations from rat cerebral cortex as inhibitors of the specific binding of [^{3}H]batrachotoxinin A 20-α-benzoate ([^{3}H]BTX-B) to the voltage-sensitive sodium channel according to our previously reported procedure.[3] The percent inhibition values were initially obtained at a single concentration (either 250 or 500 μM). The IC_{50} values for the more active compounds were then determined from dose–response curves. All experiments were performed in triplicate and included a control which contained 40 μM DPH (the reported[1,2] IC_{50} value).

The above compounds were also evaluated as anticonvulsants in mice (Phases 1 and 2) by the Antiepileptic Drug Development Program of the National Institute of Neurological Disorders and Stroke. Two anticonvulsant assays, a maximal electroshock (MES) test and a subcutaneous metrazol (scMet) test, and a rotorod toxicity assay were employed. Phases 1 and 2 involved ip administration of the compounds as suspensions in 0.5% methylcellulose. Phase 1 was a qualitative assay involving a small number of mice (1–4) at



Scheme I

dose levels of 30, 100, and 300 mg/kg. The more promising compounds from Phase 1 underwent quantification (ED_{50} and TD_{50}) of activities in Phase 2. The details of these procedures have been published.[11,12]

The biological results for **1–4**, synthetic intermediate **6**, and byproduct **7** are given in Table II. As shown, **2** and **7** interacted poorly with the sodium channel, and their activities in the Phase 1 anticonvulsant screen were not sufficient to warrant quantification. Compound **6**, while somewhat more active in the sodium channel assay, was also only marginally active in the qualitative anti-MES screen. These compounds were therefore not further pursued.

In contrast to the constrained phenylhydantoin **2**, which was one of the poorest sodium channel ligands, **4** was one of the most effective binders to the sodium channel (second only to monocyclic model **1**). Compound **4** was also one of the two most potent anti-MES anticonvulsants (along with **1**) in mice (Table II). The ability of the sodium channel assay to predict the relative anti-MES anticonvulsant effects for **2** and **4**, when considered with the observation that the prototypical sodium channel anticonvulsant is the hydantoin DPH, suggests that **4** (and **1**) may cause its anti-MES effects through interaction at this site. While there are other structural differences between **2** and **4**, this study raises the possibility that the sodium channel anticonvulsant binding site may require a specific orientation for the aromatic ring.

Also of interest was the observation that hydantoin **1**, the monocyclic model for spirohydantoin **2**, was more potent than **4** in the sodium channel assay and was equipotent as an anti-MES anticonvulsant (Table II). This is also consistent with a possible conformationally precise aromatic binding site on the sodium channel, since molecular modeling studies (molecular mechanics using Alchemy 2) suggested that the phenyl ring in **1**, due to steric interactions with the bulky propyl substituent, should more highly populate conformations resembling those possible in **4** (and could thus easily assume the conformation required by the receptor). Although **4** does not contain an N-1 hydrogen and is a relatively potent binder to the sodium channel, the greater potency of **1** in the sodium channel assay suggests that the N-1 hydrogen may be involved in interactions with the receptor, possibly via hydrogen bond donation.

The biological results for monocyclic model **3** were more difficult to rationalize, particularly since modeling studies (molecular mechanics using Alchemy 2) did not reveal a clear conformational bias for the phenyl ring. As shown in Table I, **3** was a poor binder to the sodium channel. However, the anti-MES activity for **3**, while less than that of **1** and **4**, was better than anticipated based on the sodium channel binding data.

The study described here is suggestive of sodium channel selectivity, particularly with regard to the correct conformational arrangement of the phenyl ring with respect to the hydantoin ring.

Experimental Section

Compounds **1** and **2** were synthesized as previously reported.[7] Melting points were recorded on an Electrothermal melting point apparatus and are uncorrected. Infrared spectra were obtained on a Beckman Acculab 6 spectrometer, and ^{1}H NMR spectra were recorded on a Varian EM 360 spectrometer at ambient temperature and referenced internally to TMS. The GC/MS studies were performed on a Hewlett Packard 5885 GC/MS. The R_f values were obtained using Kodak Chromagram silica gel F1 sheets (5 × 10 cm, 0.1-mm layer). Elemental analyses were provided by Atlantic Microlab of Atlanta, GA.

[^{3}H]Batrachotoxinin A 20-α-benzoate ([^{3}H]BTX-B), with a specific activity of 50 Ci/mmol, was prepared as described.[13]

1-Ethyl-5-methyl-5-phenylhydantoin (3)—Acetophenone (10.0 g, 0.0833 mol) and cyanotrimethylsilane (8.26 g, 0.0833 mol) in the

Table II—Sodium Channel and Anticonvulsant Assays in Mice

Compound	Na^+ Channel			Anticonvulsant						
				Phase I			Phase II			
	Concentration, μM[a]	Percent Inhibition	IC_{50}, μM	Best Activity, mg/kg			Time, h	MES ED_{50}, mg/kg	scMet ED_{50}, mg/kg	Rotorod TD_{50}, mg/kg
				MES	scMet	Rotorod				
1	250	44	130	30 (1/1)[b]	100 (4/4)	100 (4/4)	0.5	25[c] [22–28]	~85[d]	85 [80–90]
2	500	25	1600	100 (2/3)	300 (4/4)	>300	—	—	—	—
3	500	39	720	100 (3/3)	30 (4/4)	100 (8/8)	0.5	55 [43–66]	24[f] [21–28]	70[f] [54–90]
4	250	51	250	30 (1/1)	100 (4/4)	100 (8/8)	0.5	25 [21–32]	32[c] [16–56]	77[f] [69–90]
6	500	48	—	100 (2/3)	100 (1/1)	300 (4/4)	—	—	—	—
7	500	14	—	100 (2/3)	>300	>300	—	—	—	—
DPH	—	—	40[g]	—	—	—	2.0[h]	10[h] [8–10]	inactive[h]	66[h] [52–72]

[a] Concentration of hydantoin used in assay. [b] Number of animals protected or toxic/number tested. [c] Tested at 1 h. [d] Insufficient compound to complete testing; at 85 mg/kg, 4/8 animals protected. [e] For anticonvulsant data, numbers in brackets refer to 95% confidence intervals. [f] Tested at 0.25 h. [g] References 1 and 2. [h] References 11 and 12.

presence of 10–15 mg of ZnI_2 were stirred under N_2 at room temperature. The exothermic reaction was moderated by cooling in an ice bath in order to maintain the mixture below the boiling point, and stirring was allowed to continue at ambient temperature for a total of 2 h. To the crude trimethylsilylcyanohydrin ether was added Et_2O (100 mL) and 15% HCl (100 mL). This was stirred vigorously at ambient temperature for 2 h. The aqueous layer was removed and washed with ether (2 × 20 mL). This ether layer and the original organic layer were combined and dried (Na_2SO_4). This solution was concentrated on a rotary evaporator to provide the crude cyanohydrin as an oil. This was cooled to 0 °C, anhydrous ethylamine (11.3 g, 0.251 mol) was added, and the mixture was maintained at 0 °C overnight. The cold mixture was slowly added to cold 5% HCl (100 mL), KCNO (7.5 g, 0.092 mol) was added, and the resulting mixture stirred at 0 °C for 1.5 h. This was heated in a boiling water bath; conc. HCL (11 mL) was then added (which produced a white precipitate) and heating was continued for 1 h. The mixture was cooled and filtered, and the collected solid was washed on the filter with water. The solid was dissolved in 5% NaOH (150 mL), the solution was extracted with ether (2 × 20 mL), and the aqueous layer was adjusted to acidic pH with 5% HCl until no additional solid formed. This was filtered to give **3** as a white solid (9.15 g, 50.4%): mp 173–175 °C (ethanol/water; lit.[14] mp 176–177 °C).

10*b*-Methyl-2,5,6,10*b*-tetrahydroimidazo[5,1-*a*]isoquinoline-1,3-dione (4)—To a solution of glacial acetic acid (10 mL), 31% HBr/CH_3CO_2H (4.6 mL), and acetic anhydride (0.5 mL) in a pear-shaped pressure bottle was added hydantoin **6** (10.0 g, 45.9 mmol). To this mixture was added Br_2 (8.0 g, 50 mmol) in a dropwise manner at room temperature over a period of 30 min. The pressure bottle was closed and heated at 60–70 °C for 84 h. The bottle was cooled and opened, and water (200 mL) was added. This mixture was extracted with $CHCl_3$ (3 × 200 mL), the combined organic extracts were dried ($MgSO_4$), and the solution was concentrated on a rotary evaporator to provide **4** (0.82 g, 83%) as a white solid: mp 154–156 °C (benzene); ^1H NMR ($CDCl_3$/DMSO): δ 8.92 (s, 1 H, NH), 7.50–6.82 (m, 4 H, aromatic), 4.62–2.43 (m, 4 H, $ArCH_2CH_2N$), and 1.72 ppm (s, 3 H, CH_3); IR (KBr): 1760 and 1705 (C=O) cm^{-1}; MS (70 ev): *m/e* 216 (M^+).

Anal.—Calc. for $C_{12}H_{12}N_2O_2$: C, 66.65; H, 5.59; N, 12.96. Found: C, 66.52; H, 5.64; N, 12.92.

5-Methyl-1-(2-phenylethyl)hydantoin (6)—To a stirred solution of sodium bisulfite (15 g, 0.15 mol) in water (37 mL) at room temperature was added acetaldehyde (6.7 g, 0.15 mol). This was stirred for 15 min and then warmed to 60 °C for 15 min. After cooling to 35 °C, phenylethylamine (5; 18 g, 0.15 mol) was added and the mixture was maintained at 35 °C for 2 h. A solution of sodium cyanide (7.4 g, 0.15 mol) was slowly added and the mixture was stirred at room temperature for 3 h. Ether (200 mL) was added, the mixture was shaken in a separatory funnel, and the organic layer was removed and concentrated on a rotary evaporator to give a yellow oil. This was cooled in an ice bath and 10% HCl (75 mL) was added over a period of 15 min, maintaining the temperature below 10 °C. To this cold solution was added KCNO (11.8 g, 0.167 mol) in one portion, and stirring was continued at 0 °C for 1.5 h. Concentrated HCl (60 mL) was added, and the cold solution was allowed to stir overnight at room temperature. This was then heated at 90–95 °C for 2.5 h and cooled in an ice bath to provide a white precipitate. This was filtered and washed on the filter with cold water and cold chloroform to yield a white solid (16.4 g, 50%); mp 114–117 °C ($CHCl_3$/hexane); ^1H NMR ($CDCl_3$): δ 8.42 (s, 1 H, NH), 7.18 (s, 5 H, aromatic), 4.10–2.66 (m, 4 H, $PhCH_2CH_2$), 1.82 (m, 1 H, $CHCH_3$), and 1.28 ppm (d, J = 7 Hz, 3 H, CH_3); IR (KBr): 1705 (C=O) cm^{-1}; MS (70 ev): *m/e* 218 (M^+).

Anal.—Calc. for $C_{12}H_{14}N_2O_2$: C, 66.04; H, 6.47; N, 12.84. Found: C, 66.08; H, 6.50; N, 12.80.

10*b*-Bromomethyl-2,5,6,10*b*-tetrahydroimidazo[5,1-*a*]isoquinoline-1,3-dione (7)—A solution of **6** (2.0 g, 9.2 mmol) in glacial acetic acid (15 mL) and 31% HBr/acetic acid (1 mL) containing 2 drops of acetic anhydride was stirred at room temperature in a pear-shaped pressure bottle for 5 h. The mixture was then heated at 55 °C for 24 h and 70 °C for an additional 24 h. The cooled mixture was extracted with $CHCl_3$ (3 × 30 mL), the combined extracts were dried ($MgSO_4$), and the solvent was removed to yield **7** as white crystals (1.5 g, 56%): mp 245–248 °C (benzene); ^1H NMR ($CDCl_3$): δ 11.35 (s, 1 H, NH), 7.85–7.10 (m, 4 H, aromatic), 4.05 (m, 2 H, NCH_2), 3.80 (s, 2 H, CH_2Br), and 3.65–2.50 ppm (m, 2 H, $PhCH_2$); IR (KBr): 1780 and 1705 (C=O) cm^{-1}; MS (70 ev): *m/e* 294 (M^+).

Anal.—Calc. for $C_{12}H_{11}N_2O_2Br$: C, 48.83; H, 3.76; N, 9.49; Br, 27.07. Found: C, 48.70; H, 3.79; N, 9.42; Br, 26.96.

Sodium Channel Binding Assay—We previously described the details of this procedure.[3] Briefly, synaptoneurosomes (which are resealed postsynaptic elements containing attached resealed presynaptic elements, ~980 μg) from rat cerebral cortex were incubated for 40 min at 25 °C with the test compound (250 or 500 μM) in a total volume of 320 μL containing 10 nM [^3H]BTX-B and 50 μg/mL of scorpion venom. Incubations were terminated by dilution with ice cold buffer and filtration through a Whatman GF/C filter paper, and the filters were washed three times with ice cold buffer. Filters were counted in a Beckmann scintillation counter. Specific binding was determined by subtracting the nonspecific binding, which was measured in the presence of 300 μM veratridine, from the total binding of [^3H]BTX-B. All experiments were performed in triplicate and included a control tube containing 40 μM DPH. The IC_{50} values for the more potent compounds were determined from a dose–response curve as previously described.[3]

Anticonvulsant Assays—All whole animal anticonvulsant and neurotoxicity assays were conducted by the Anticonvulsant Drug Development Program of the Epilepsy Branch, National Institute of Neurological Disorders and Stroke. In Phases 1 and 2, compounds were injected intraperitoneally into mice as suspensions in 0.5% methylcellulose. After the time indicated in Table II, the animal was subjected to either a sc Metrazol (sc Met) challenge (85 mg/kg), a maximal electroshock (MES) challenge (produced with 60 cycle AC at 50 mA for 0.2 s via corneal electrodes), or a rotorod toxicity test. Phase 1 was a qualitative assay which utilized small groups of animals (1–4) at dose levels of 30, 100, and 300 mg/kg. Promising compounds from

Phase 1 underwent quantification of activities (ED_{50} and TD_{50}) in Phase 2. The details of these procedures have been published.[11,12]

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Acknowledgments

We thank Annette LaRussa for excellent technical assistance and James Stables of the Antiepileptic Drug Development Program, Epilepsy Branch, National Institute of Neurological Disorders and Stroke, for the whole animal anticonvulsant data. We are also indebted to the National Institutes of Health for generous support of this work (NS23866).