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Anticonvulsant activity of reaction products of 5,5-diphenylhydantoin with substituted methylene bromides

Some derivatives of 5,5-diphenylhydantoin (phenytoin) were synthesized by the alkylation of phenytoin with substituted methylene bromides. The hydantoins were evaluated for possible anticonvulsant activity in the maximal electroshock (MES)- and subcutaneous pentylenetetrazole (ScMet)-induced seizures and for neurotoxicity in the rotorod test in mice and rats.

Key Words: Anticonvulsants; Hydantoins; Methylene bromides

Received: July 4, 2001 [FP608]

Introduction

Epilepsy is a symptom of excessive temporary neuronal discharge, characterized by discrete recurrent episodes, in which there is a disturbance of movement, sensation, behavior, perception, and/or consciousness. It has been estimated that 0.5 to 1% of the population is affected by some form of epilepsy [1,2].

Anticonvulsant drugs display their activity by different mechanisms. The drugs can influence the ion transport across cell membranes, or inhibitory or excitatory neurotransmitter systems [3].

Although many treatments for epilepsy are available, more efforts should be devoted to novel approaches. The quest[4] to discover more efficacious new anticonvulsant drugs with fewer adverse effects and the potential to provide the patient with a better quality of life prompted the preparation and biological evaluation of the derivatives of hydantoins. The compounds obtained were evaluated for anticonvulsant activity in the maximal electroshock (MES)- and subcutaneous pentylenetetrazole (ScMet)-induced seizures and for neurotoxicity in the rotorod test in mice and rats.

Table 1. Physical data.

Cmpd	Reaction time	Crystallizing solvent	Melting point
3a	4 h	Ethanol	137–138 °C (Lit[6]:137–138°C)
3b	36 h	Hexane–Ethyl acetate	194–195 °C
3c	30 h	Hexane–Ethyl acetate	180–181 °C
4	5 h	Hexane–Ethyl acetate	237–239 °C

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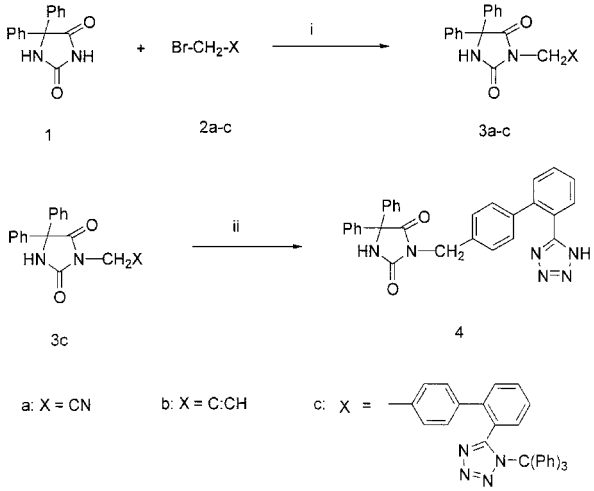

Scheme. i = K_2CO_3, DMF, 25 °C; or KOH, EtOH, reflux; ii = MeOH, reflux, 5 h.

Chemistry

The hydantoins were synthesized as outlined in the scheme below. Alkylation of N-3 of the hydantoin was accomplished with the appropriate methylene bromide. In the synthesis of 5,5-diphenyl-3-(acetonitrilyl)-hydantoin **3b**, the yield was higher when alkylation was executed with potassium hydroxide. The removal of the trimethyl moiety from **3c** to give **4** was accomplished in refluxing methanol. This is in agreement with a recent study on the de-protection or removal of the triphenylmethyl moiety[5]. The assignment of all structures was established on the basis of IR, NMR, mass spectrometry, and microanalysis.

Pharmacology

The four compounds **(3a–c, 4)** were screened for anticonvulsant activity according to standard procedures, which involves the maximal electroshock seizure test (MES test)

0365-6233/01/1111/0366 $17.50 +.50/0

Table 2. Anticonvulsant activity in the MES test and the ScMet test and toxicity in the rotorod test of hydantoins following intraperitoneal administration to mice. The numbers are expressed as animals protected/animals tested.

Cmpd	Dose	MES		ScMet		Toxicity		Class [a]
	[mg/kg]	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h	
3a	30	0/1	0/1	0/1	0/1	0/4	0/2	1
	100	0/3	3/3	0/1	0/1*	0/8	0/4	
	300	0/1	1/1	0/1	0/1*	0/4	2/2	
3b	3	–	0/4	–	–	–	0/4	1
	10	–	0/4	–	–	–	0/4	
	30	0/1	1/1	0/1	0/1	0/4	0/2	
	100	1/3	3/3	0/1	0/1	0/8	2/4	
	300	1/1	1/1	0/1	0/1	2/4	1/2	
3c	30	0/1	0/1	0/1	0/1*	0/4	0/2	3
	100	0/3	0/3	0/1*	0/1*	0/8	0/4	
	300	0/1	1/1	0/1*	0/1	0/4	0/2	
4	30	0/1	0/1	0/1	1/5*	0/4	0/2	1
	100	0/3	0/3	0/1	1/5*	0/8	0/4	
	300	0/1	0/1	0/1	0/1	0/4	0/2	

*Death following clonic seizures. [a] Classification according to reference [7]. Class 1, active at 100 mg/kg; Class 2, active at 300 mg/kg; Class 3, inactive.

Table 3. Quantitative anticonvulsant (TD_{50}, ED_{50} MES, and PI) activity of **3b** and standard anticonvulsants after intraperitoneal administration to mice. The values are expressed in mg/kg. 95% confidence intervals are listed in brackets, while the time of test is listed in square brackets.

Cmpd	TD_{50} (rotorod)	ED_{50} (MES)	PI (TD_{50}/ED_{50})
3b	82.4 (68.8–98.6) [2h]	48.8 (34.6–62.7) [2h]	1.7
Phenytoin [a]	42.8 (36.4–47.5) [2h]	6.48 (5.65–7.24) [2h]	6.6
Valporate [a]	483 (412–571) [1/4h]	287 (237–359) [1/4h]	1.7

[a] Data from references [7,9].

Table 4. Anticonvulsant activity of **3a** and **3b**. Test results in rats (30 mg/kg p.o.).

Cmpd	Test	Time (h)				
		0.25	0.50	1.0	2.0	4.0
3a	MES	0/4	0/4	0/4	0/4	4/4
	TOX	0/4	1/4	0/4	0/4	0/4
3b	MES	2/4	0/4	1/4	2/4	2/4
	TOX	0/4	0/4	0/4	0/4	0/4

and the seizure threshold test with subcutaneous pentylenetetrazole (scMet test). The acute neurological toxicity was determined in the rotorod test. The results of the screening in mice are summarized in Table 2. Only compounds **3a** and **3b** displayed an effect in the maximal electroshock seizure test at a dose of 100 mg/kg with **3b** also exhibiting an effect at a lower dose of 30 mg/kg. Fifty percent of the numbers of mice used were neurotoxic at a minimum dose of 100 mg/kg. Death following clonic seizures was recorded in some cases with the subcutaneous pentylenetetrazole (scMet test). The anticonvulsant activity recorded for compound **4** in the scMet test with corresponding absence of acute neurotoxicity was low.

Additional evaluation of **3a** and **3b** in rats at a dose of 30 mg/kg (p.o.) at MES over a period of 4 h revealed the time for peak activity. Both compounds were significantly not neurotoxic in the rotorod test. The time for peak activity for **3a** was 4 h while **3b** displayed more or less the same level of activity throughout the period. No anticonvulsant activity was however observed for **3b** after 50 min.

However, in comparison to some known antiepileptic drugs, the protective index (PI) in the MES test for **3b** and valproate is the same. The median toxic dose (TD_{50}) for **3b** as well as effective dose (ED_{50}) were correspondingly high compared to phenytoin. The median toxic dose (TD_{50}) of **3b** was 2-fold of phenytoin while the median effective dose was 7.5-fold less active than phenytoin. The modification of phenytoin by alkylation with subtituted methylene bromides therefore did not appreciably enhance the activity of phenytoin.

Acknowledgments

The anticonvulsant and toxicity testing by Dr. J. P. Stables at the NINDS Epilepsy Branch, National Institutes of Health, Rockville, MD, USA is gratefully acknowledged.

Experimental

Chemistry

Melting points were determined with a Kofler hot-stage microscope and were reported uncorrected. The reactions and purity of the products were monitored by TLC. Nuclear Magnetic Resonance spectra were recorded on a Varian Gemini 200 (TMS); infra-red spectra were measured on a Perkin-Elmer type 457 and the mass spectra were determined using a Varian MAT 44S, EI: 70 eV. Propargyl bromide and bromoacetonitrile were obtained from Aldrich while *N*-(triphenyl)-5-[4-(bromomethyl)-biphenyl-2-yl]-tetrazole was synthesized according to a known procedure[8].

Alkylation of 5,5-diphenyl-3-prop-2-ynylhydantoin

Method I: A mixture of substituted methylene bromide (0.02 mol), 5,5-diphenyl-3-prop-2-ynylhydantoin (0.02 mol), and potassium hydroxide (0.02 mol) was refluxed in 30 ml ethanol. Standard work-up procedures was carried out and the compound crystallized from appropriate solvent.

Method II: Substituted methylene bromide (3.97 mmol) was added to a stirred suspension of 5,5-diphenyl-3-prop-2-ynylhydantoin (3.97 mmol) and potassium carbonate (3.97 mmol) in 20 ml *N*,*N*-dimethylformamide at room temperature. Standard work-up procedures were executed and the compound crystallized from appropriate solvent.

*5,5-Diphenyl-3-prop-2-ynylhydantoin **3a***

Method I: Propargyl bromide was added to 5,5-diphenyl-3-prop-2-ynylhydantoin and gave after work-up fine needles of 5,5-diphenyl-3-prop-2-ynylhydantoin **3a** (5.1 g, 76%).

*5,5-Diphenyl-3-(acetonitrilyl)-hydantoin **3b***

Method II: 0.92 g (80%) ; IR (KBr): ν (cm^{-1}) = 3390 (NH), 1775, 1730 (C=O), 1600; ^{1}H NMR (Acetone d_6): δ = 4.72 (s, 2H, CH_2), 7.48 (brs, 10H, Ar-H), 8.89 (brs, 1H, N-H). MS: *m/z* = 292 [M^++ 1] (12), 291 [M^+] (60), 262 (69), 235 (7), 208 (20), 180 (100), 165 (26), 152 (6), 104 (21), 77 (21), 51 (9); $C_{17}H_{13}N_3O_2$ (291.31). Calc. C 70.09 H 4.50 N 14.43; found C 70.20 H 4.54 N 14.30.

*5,5-Diphenyl-3-{-(-[2′-(N-triphenylmethyl)-tetrazol-5-yl]-biphenyl-4-yl)-methyl}-hydantoin **3c***

Method II: 2.10 g (72%) IR (KBr): ν (cm^{-1}) = 3200 (NH), 3090, 1760, 1730 (C=O) 1600. ^{1}H NMR ($CDCl_3$): δ = 4.58 (s, 2H, CH_2), 6.87–6.89 (d, *J* = 8.1Hz, 6H, Ar-H), 7.00–7.10 (d, *J* = 8.2Hz, 2H, Ar-H), 7.07–7.09 (d, *J* = 8.2Hz, 2H, Ar-H), 7.19–7.32 (m, 23H, Ar-H), 7.41–7.45 (m, 2H, Ar-H), 7.89–7.91 (d, *J* = 7.8Hz, 1H, Ar-H). MS: *m/z* [FAB] = 728 [M^+] ; $C_{48}H_{36}N_6O_2$ (728.86). Calc. C 79.10 H 4.98 N 11.53; found C 79.04 H 5.10 N 11.40.

*5,5-Diphenyl-3-[-(2′-tetrazol-5-yl)-biphenyl-4-yl]-methyl-hydantoin **4***

5,5-Diphenyl-3-{-(-[2′-(*N*-triphenylmethyl)-tetrazol-5-yl]-biphenyl-4-yl)-methyl}-hydantoin **3c** (1.0g, 1.37 mmol) was refluxed in 25 ml methanol for 5h. A column chromatography of the crude product (dichloromethane/ethyl acetate 2:1) gave 5,5-diphenyl-3-[-(2′-tetrazol-5-yl)-biphenyl-4-yl]-methyl-hydantoin **4** as needles. 0.47g (72%), IR (KBr): ν (cm^{-1}) = 3380 (NH), 3090, 1780, 1720 (C=O) 1600, 1500, 1400, 960, 760, 700; ^{1}H NMR (DMSO-d_6): δ = 4.60 (s, 2H, CH_2), 7.0 (d, *J* = 8.2Hz, 2H, Ar-H), 7.10 (d, *J* = 8.1Hz, 2H, Ar-H), 7.34–7.38 (m, 10H, Ar-H), 7.57 (t, *J* = 7.8Hz, 2H, Ar-H), 7.67 (d, *J* = 7.8Hz, 2H, Ar-H), 9.80 (s, 1H, NH); MS: *m/z* = 486 [M^+](2), 485[M^+–1](3), 458(3), 443(2), 251(22), 233(18), 208(33), 178(100), 104(51), 77(48), 51(9). $C_{29}H_{22}N_6O_2$ (486.54). Calc. C 71.59 H 4.56 N 17.27; found C 71.50 H 4.44 N 17.14.

Pharmacology

Anticonvulsant testing was provided by the Antiepileptic Drug Development Program, Epilepsy Branch, Division of Convulsive, Developmental and Neuromuscular Disorders, National Institutes of Health, according to standard procedures [6] and included the MES test and the seizure scMet test. In the MES test, an electrical stimulus of 50 mA was delivered for 0.2 sec via corneal electrodes to mal CF1 mice at 30 minutes and 4 h after the administration of the compounds. Blockade of the tonic extension of the hind limbs was considered protection against seizures. For the scMet test a convulsant dose of 85 mg/kg of pentylenetetrazole dissolved in saline was injected in a loose fold of skin on the back of the neck and the animals were isolated and observed for 30 minutes. Absence of clonic spasms for at least 5 sec indicated the elevation of the pentylenetetrazole-induced seizure threshold. The acute neurological toxicity was determined in the rotorod test where the animal was placed on a rod rotating at 6 rpm. Neurological deficiency was indicated by inability to maintain equilibrium for 1 min in each of 3 trials. For all these evaluations the compounds were dissolved or suspended in 0.5 % aqueous methyl cellulose.

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