Anticonvulsant Properties of Selected Pyrrolo[2,3-*d*]pyrimidine-2,4-diones and Intermediates

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Abstract □ A series of pyrrolo[2,3-*d*]pyrimidine-2,4-diones and intermediates was tested for anticonvulsant activity in mice. Eleven of the 15 compounds possessed anticonvulsant activity against pentylenetetrazol-induced convulsions. One compound afforded more anticonvulsant protection against pentylenetetrazol than did trimethadione (67 and 50%, respectively). A suspension of this compound was found to be as effective as a solution in producing anticonvulsant activity. The results suggest that the nitrile analogs were more potent compared to the carbamyl analog due to higher lipid solubility.

Keyphrases □ Pyrrolo[2,3-*d*]pyrimidine-2,4-diones and intermediates—anticonvulsant activity, structure–activity relationships □ Anticonvulsant activity—pyrrolo[2,3-*d*]pyrimidine-2,4-diones and intermediates, structure–activity relationships □ Structure–activity relationships—pyrrolo[2,3-*d*]pyrimidine-2,4-diones and intermediates, anticonvulsant activity

Many clinically useful anticonvulsants such as the hydantoins (1), succinimides (2), oxazolidinediones (3), and barbiturates (4) incorporate a cyclic imide structure. A series of substituted pyrrolopyrimidinediones synthesized in this laboratory (5) also contain a cyclic imide structure and, therefore, were postulated to possess possible anticonvulsant activity. Preliminary information from this laboratory confirmed the hypothesis and demonstrated that several of the pyrrolopyrimidinediones possessed anticonvulsant activity against pentylenetetrazol-induced seizures in mice.

In addition, some clinically useful anticonvulsants such as the propanediol carbamates (6) and acetylureas (7) contain a linear carbamate or urea function. Since intermediates in the synthesis of pyrrolopyrimidinediones contained carbamate functions, these compounds also were predicted to possess anticonvulsant activity.

Johnson *et al.* (8) reported the synthesis of a series of 2-aminopyrrole analogs of lidocaine that possessed cardiovascular depressant properties devoid of central nervous system (CNS) motor stimulation (9,10). Since one compound appeared to possess CNS depressant properties, it also was predicted to possess anticonvulsant activity.

The purpose of this study was to examine the anticonvulsant activity and structure–activity relationship for selected pyrrolo[2,3-*d*]pyrimidine-2,4-diones and related intermediates.

EXPERIMENTAL[1]

The synthesis of the compounds (I*b*, Ic, IIc–II*i*, III*a*, and III*b*) (Table I) used in this study was reported previously (5). The intermediates (I*a*, II*a*, and II*b*) were synthesized by the following procedure.

Chemistry—*2-(2-Chlorocarbethoxyamido)-3-cyano-4-methyl-5-benzylpyrrole* (*Ia*)—A solution of 2-amino-3-cyano-4-methyl-5-benzylpyrrole (16.5 g, 0.078 mole) (11) in acetone (100 ml) and pyridine (6.8 g, 0.086 mole) was stirred in an ice bath with the dropwise addition of 2-chloroethyl chloroformate (12.3 g, 0.086 mole). After the addition was complete, the bath was removed, and the solution was stirred at room temperature for 30 min. The solution was diluted with ice water (400 ml), and the precipitate was collected and air dried. The crude carbamate was recrystallized from 300 ml of methanol–water (2:1) to yield beige crystals (22.2 g, 89.5%). Recrystallization from methanol yielded beige crystals that were homogeneous on TLC with ethyl acetate (R_f 0.55), mp 110–111°; IR (KBr): 3560, 3300, 2200, 1715, 1630, 1240, and 1020 cm^{-1}.

Anal.—Calc. for $C_{16}H_{16}ClN_3O_2$: C, 60.47; H, 5.08; Cl, 11.16; N, 13.22. Found: C, 60.58; H, 5.13; Cl, 11.05; N, 13.25.

2-(2-Chlorocarbethoxyamido) -3- carbamyl-4-methyl-5-benzylpyrrole (*IIa*)—The carbamate (I*a*) (20.0 g, 0.063 mole) was added to 85% phosphoric acid (300 ml) that was preheated to 110°. The resulting solution was stirred for 7 min at 110–130° and then was poured over ice (1000 g). Potassium hydroxide (100 g) dissolved in water was added, and the precipitate was collected, washed with water, and air dried. The crude product (14.0 g, 66.3% yield) was recrystallized from 95% ethanol (500 ml), followed by recrystallization from methanol to yield pale-pink crystals that were homogeneous on TLC with ethyl acetate (R_f 0.45), mp 172–173°; IR (KBr): 3500, 3420, 3340, 3300, 1715, 1640, 1600, 1580, 1560, 1390, and 1210 cm^{-1}.

Anal.—Calc. for $C_{16}H_{18}ClN_3O_3$: C, 57.23; H, 5.40; Cl, 10.56; N, 12.51. Found: C, 57.30; H, 5.44; Cl, 10.55; N, 12.51.

2-(2,2,2-Trichlorocarbethoxyamido) -3- carbamyl-4,5-dimethylpyrrole (*IIb*)—A solution of 2-(2,2,2-trichlorocarbethoxyamido)-3-cyano-4,5-dimethylpyrrole (23.4 g, 0.0753 mole) in concentrated sulfuric acid (50 ml) was stirred at 100° for 6 min and then was poured slowly into 800 g of ice water (1:1). The precipitate was collected, washed with water, and air dried. The product was recrystallized from methanol (800 ml) to yield pale-pink crystals (18.0 g, 72.7% yield) that were homogeneous on TLC with ethyl acetate (R_f 0.60), mp > 300°; IR (KBr): 3525, 3360, 3280, 1730, 1650, 1590, 1570, 1560, 1400, 1200, 810, 750, and 720 cm^{-1}.

Anal.—Calc. for $C_{10}H_{12}Cl_3N_3O_3$: C, 36.55; H, 3.68; N, 12.79. Found: C, 36.46; H, 3.72; N, 12.85.

Pharmacology—*Animal Preparation*—Male HA/ICR mice[2], 15–30 g, were used. Compounds tested for anticonvulsant activity were prepared as 3% suspensions containing polysorbate 80[3] (0.5%) and acacia (0.5%) in water and were injected intraperitoneally. Solutions for intravenous administration (6%) were prepared by dissolving the compound in 30% propylene glycol and adjusting the pH to 8.0 with sodium hydroxide. Intravenous injections were administered *via* the tail vein using a 27-gauge needle and a mouse holder.

Maximal Electroshock Seizure Test—Mice were administered 50 mamp of alternating current[4] (60 Hz) for 200 msec through corneal electrodes (12). Several drops of 0.9% NaCl were instilled in each eye prior to application of the electrodes. Abolition of the tonic hindlimb extensor component of the seizure was defined as anticonvulsant activity.

Phenytoin[5] was used as the reference anticonvulsant for maximal electroshock. Three-hour pretreatment with phenytoin (10 mg/kg sc) produced 100% protection against maximal electroshock seizures in mice (12). The procedure was modified by using a 45-min pretreatment with a dose of 30 mg/kg ip (suspension) to produce 100% protection.

Pentylenetetrazol Seizure Test—Pentylenetetrazol[6] was administered subcutaneously at a dose of 85 mg/kg (ED_{97}) to elicit a clonic seizure lasting at least 5 sec (12). Trimethadione[7] was used as the reference

[1] IR spectra were determined on a Beckman AccuLab 4 spectrophotometer using the potassium bromide technique. Melting points were obtained using a Thomas-Hoover capillary apparatus and are uncorrected. TLC was performed using Eastman chromatogram sheets, type 6060 (silica gel), and the sheets were developed in an iodine chamber. Carbon, hydrogen, chlorine, and nitrogen values were obtained from Atlantic Microlab, Atlanta, Ga.

[2] Sprague–Dawley, Madison, Wis.
[3] Tween 80, ICI Americas, Wilmington, Del.
[4] Wahlquist electroshock apparatus, Provo, Utah.
[5] Dilantin, Parke-Davis, Detroit, Mich.
[6] Metrazol, Sigma Chemical Co., St. Louis, Mo.
[7] Tridione, Abbott Laboratories, North Chicago, Ill.

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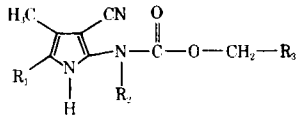
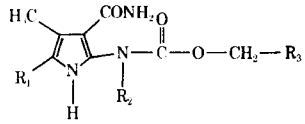
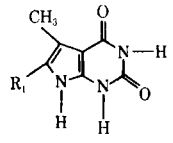
Table I—Anticonvulsant Activity and Rotorod Toxicity of Selected Pyrrolo[2,3-*d*]pyrimidine-2,4-diones and Intermediates [a]

Compound	R_1	R_2	R_3	Protection against Pentylenetetrazol-Induced Convulsions[b], %	Rotorod Toxicity[c], %
					
I*a*	$CH_2C_6H_5$	H	CH_2Cl	33	0
I*b*	C_2H_5	H	CH_3	67	83
I*c*	$CH_2C_6H_5$	H	CH_3	17	0
					
II*a*	$CH_2C_6H_5$	H	CH_2Cl	17	0
II*b*	CH_3	H	CCl_3	0	0
II*c*	CH_3	H	CH_3	17	0
II*d*	CH_3	CH_3	CH_3	0	0
II*e*	C_2H_5	H	CH_3	33	33
II*f*	iso-C_4H_9	H	CH_3	17	0
II*g*	C_6H_5	H	CH_3	0	0
II*h*	$CH_2C_6H_4$-*p*-OH	H	CH_3	17	0
II*i*	$CH_2C_6H_5$	H	CH_3	0	0
					
III*a*	C_2H_5			17	0
III*b*	$CH_2C_6H_5$			17	0
IV[d]				17	0
Control[e]				0	0
Trimethadione (400 mg/kg)				50	0
Phenytoin (30 mg/kg)				—	0

[a] Male mice were injected with 30 mg/kg ip (suspension) of test compound and evaluated for maximal electroshock protection. All compounds were injected 45 min before administration of 50 mamp of alternating current for 200 msec. None of the compounds exhibited any protection against maximal electroshock-induced convulsions. Phenytoin, 30 mg/kg ip, provided 100% protection (n = 6). [b] Male mice were injected with 400 mg/kg ip of test compounds (suspension) 45 min before 85 mg of pentylenetetrazol/kg sc. Percent protection equals the percentage of animals protected against seizures induced by 85 mg of pentylenetetrazol/kg (ED_{97}) (n = 6). [c] Previously trained male mice received 400 mg/kg ip of test compounds (suspension) 45 min before placement on the rotorod for 90 sec. Rotorod toxicity is defined as the percentage of animals that did not remain on the rod for at least 90 sec (n = 6). [d] 2-Diethylaminoacetamido-3-carbamyl-4-methyl-5-benzylpyrrole hydrochloride (8, 9). [e] Control mice received 3.84 ml of suspension vehicle/kg (0.5% polysorbate 80 and 0.5% acacia in water).

Table II—Effect of Injection Route and Solubilization of I*b* on Anticonvulsant Activity

Convulsive Method[a]	Dose of I*b* and Route	Preparation[b]	Protection[c], %	Rotorod Toxicity[d], %
Electroshock	Control[e], ip	Solution	0	—
	30 mg/kg ip or iv	Solution	0	0
	30 mg/kg ip	Suspension	0	0
Pentylenetetrazol	Control, ip	Solution	0	—
	50 mg/kg ip or iv	Solution	0	0
	400 mg/kg ip	Solution	83	83
	400 mg/kg ip	Suspension	67	83

[a] Male mice were injected with 30 mg of I*b*/kg ip (suspension) and evaluated for maximal electroshock protection. Compound I*b* was injected 45 min before administration of 50 mamp of alternating current for 200 msec. Male mice were injected with I*b* 45 min before 85 mg of pentylenetetrazol/kg sc (ED_{97}) (n = 6). [b] Solution of I*b* was prepared in 30% propylene glycol. Suspension of I*b* was prepared as described in *Experimental* (n = 6). [c] Percent protection equals the percentage of animals protected against seizures induced by 85 mg of pentylenetetrazol/kg (ED_{99}) or electroshock (n = 6). [d] Rotorod toxicity is defined as the percentage of animals that did not remain on the rod for at least 90 sec (n = 6). [e] Control = 3.84 ml/kg of 30% propylene glycol (1.2 g/kg of propylene glycol).

pentylenetetrazol antagonist. At a pretreatment time of 45 min, 400 mg/kg of trimethadione suspension given intraperitoneally protected 50% of the animals against pentylenetetrazol-induced convulsion.

Test compounds were administered intravenously or intraperitoneally at a dose of 50 or 400 mg/kg 45 min before injection of pentylenetetrazol. Anticonvulsant activity was recorded if clonic spasms were not observed within 30 min after the pentylenetetrazol administration. The data were reported as the percentage of animals protected against seizures induced by 85 mg of pentylenetetrazol/kg given subcutaneously.

Rotorod Test—Previously trained mice were placed on a 2.54-cm diameter rubber-coated plastic rod (rotorod) rotating at 6 rpm before and 45 min after administration of the test compound. Rotorod toxicity was defined as the percentage of animals that did not remain on the rod for at least 90 sec.

RESULTS AND DISCUSSION

A test compound dose of 400 mg/kg was chosen for comparison with the anticonvulsant ED_{50} dose (400 mg/kg) of trimethadione (Table I). Suspensions of the test compounds were administered intraperitoneally due to solubility problems. At a dose of 400 mg/kg ip, 11 of the 15 test compounds protected against pentylenetetrazol-induced seizures (Table I). Compound I*b* was more active than trimethadione (67 *versus* 50% protection, respectively). Compounds II*e* and I*a* were slightly less active than trimethadione (33 *versus* 50% protection, respectively). Compounds I*c*, II*a*, II*c*, II*f*, II*h*, III*a*, III*b*, and IV exhibited slight anticonvulsant activity (17%).

Phenytoin at a dose of 30 mg/kg ip afforded 100% protection against maximal electroshock seizures. Since the test compounds were insoluble,

suspensions of the compounds including phenytoin were prepared. None of the test compounds exhibited any protection against maximal electroshock seizures at 40 mg/kg ip (suspension, footnote *a*, Table I). Compounds II*e* and I*b* produced definite signs of neurological toxicity (33 and 83%, respectively) as measured on the rotorod at 400 mg/kg (Table I). No CNS toxicity was observed with any other test compound.

The anticonvulsant evaluation indicated that compounds possessing a nitrile group at the 3-position (I*a*–I*c*) were more potent compared to compounds possessing a carbamyl group at that position (II*a*–II*i*). Introduction of the more polar carbamyl group (13) in II*a*–II*i* resulted in decreased lipid solubility compared to I*a*–I*c*, which contain the nitrile group. Decreased lipid solubility probably retards the passage of these compounds into the CNS.

The effect of the administration route and solubilization method on the most potent compound (I*b*) was examined further (Table II). Suspensions of I*b* were administered intraperitoneally due to solubility limitations. Suspensions then were compared to the anticonvulsant effects produced by propylene glycol solutions of I*b* when it was administered intraperitoneally or intravenously.

In the electroshock studies, 30 mg of I*b*/kg was chosen since phenytoin produced 100% protection against maximal electroshock seizures when it was administered as an intraperitoneal suspension. Variation in neither the injection route (intraperitoneal *versus* intravenous) nor the means of solubilization (suspension *versus* solution) was effective in producing anticonvulsant activity.

Compound I*b*, 75 mg/kg, was lethal following intravenous administration. Gross observation of the animals following this dose indicated that death was probably due to cardiovascular toxicity. Therefore, 50 mg/kg iv was selected as the maximum allowable dose for comparative purposes. Unfortunately, at 50 mg of I*b*/kg, variation in the administration route (intravenous *versus* intraperitoneal) was ineffective in producing protection against pentylenetetrazol-induced seizures.

Since 400 mg of I*b*/kg ip (suspension) afforded 67% protection in the pentylenetetrazol seizure test, 400 mg/kg ip of I*b* then was administered as a solution in propylene glycol for comparison (Table II). The completely dissolved solution of I*b* appeared to be slightly more effective than the intraperitoneally injected suspension of I*b* (83 *versus* 67% protection, respectively). However, the extent of rotorod toxicity appeared equivalent (83%). Therefore, complete solubilization of I*b* was concluded to be unnecessary for anticonvulsant activity.

Drugs that are useful in petit mal seizures are effective in elevating the threshold of electroshock- and drug-induced convulsions (14). Drugs used for grand mal epilepsy do not significantly affect the threshold of electrically induced seizures. None of the test compounds exhibited anticonvulsant activity against maximal electroshock but did block pentylenetetrazol-induced seizures. The active pyrrolopyrimidinediones and intermediates probably exert their activity through an elevation of the convulsive threshold, similar to trimethadione (14).

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High-Pressure Liquid Chromatographic Analysis of Pramoxine Hydrochloride in High Lipoid Aerosol Foam Dosage Form

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Abstract □ A rapid and quantitative method for the determination of pramoxine hydrochloride by high-pressure liquid chromatography is presented. The drug is extracted as the salt from a preparation with a high lipoid composition by partitioning it to the aqueous phase of an ether–methanol–water–acetic acid system. The extract is chromatographed on an octadecylsilane bonded packing with a methanol–water–acetic acid–methanesulfonic acid mobile phase. The time required for each separation is ~6 min. Analytical recoveries of 100.4 ± 1.5% were obtained.

Keyphrases □ Pramoxine hydrochloride—analysis, high-pressure liquid chromatography, high lipoid aerosol foam dosage form □ High-pressure liquid chromatography—analysis, pramoxine hydrochloride, high lipoid aerosol foam dosage form □ Anesthetics, topical—pramoxine hydrochloride, high-pressure liquid chromatographic analysis, high lipoid aerosol foam dosage form

Pramoxine hydrochloride, a widely used topical anesthetic, can present analytical difficulties due to its surfactant behavior. Its hydrophilic and lipophilic properties result in substantial matrix effects from common pharmaceutical excipients, particularly when the drug is incorporated into a high lipoid content base.

The conventional analytical method described in NF XIV (1) is based on nonspecific, nonaqueous titrimetry and spectrophotometric determinations. TLC[1] was used for qualitative analysis. Mario and Meehan (2) used the drug as an internal standard for a GLC assay of cough–cold preparations. Analysis of the high lipoid composition by GLC in this laboratory resulted in lengthy sample preparation and 30-min separations.

This study was undertaken to develop a rapid and reliable method for the determination of pramoxine hydrochloride in high lipoid preparations. The method was re-

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