Synthesis and Skeletal Muscle Relaxant Activity of 3-(Aminoacyl)-1-[[[5-(substituted phenyl)-2-furanyl]methylene]amino]-2,4-imidazolidinediones

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Abstract □ A series of 3-(aminoacyl)-1-[[[5-(substituted phenyl)-2-furanyl]methylene]amino]-2,4-imidazolidinediones was synthesized and evaluated for skeletal muscle relaxant activity. All compounds were active by the intravenous route, and nine of 11 were active orally. One compound was very active when evaluated by the mouse Straub tail and rotarod tests; its efficacy index (ED_{50} rotarod/ED_{50} Straub tail) was 2.0, while its therapeutic index (LD_{50}/ED_{50} Straub tail) was >225.

Keyphrases □ 3-Aminoacyl-substituted 2,4-imidazolidinediones—synthesis and evaluation for skeletal muscle relaxant activity, mice □ Dantrolene sodium—related compounds synthesized and evaluated for skeletal muscle relaxant activity, mice □ Skeletal muscle relaxant activity—dantrolene sodium-related compounds synthesized and evaluated for activity, mice □ Muscle, skeletal—relaxant properties evaluated in dantrolene-related compounds, mice

Several 2,4-imidazolidinediones have skeletal muscle relaxant activity (1). One member of this series, dantrolene[1], caused skeletal muscle relaxation by a unique mechanism, acting directly on the skeletal muscle (2, 3). It has been hypothesized that dantrolene sodium induces muscle relaxation by inhibiting the release of Ca^{2+} from the sarcoplasmic reticulum (4, 5).

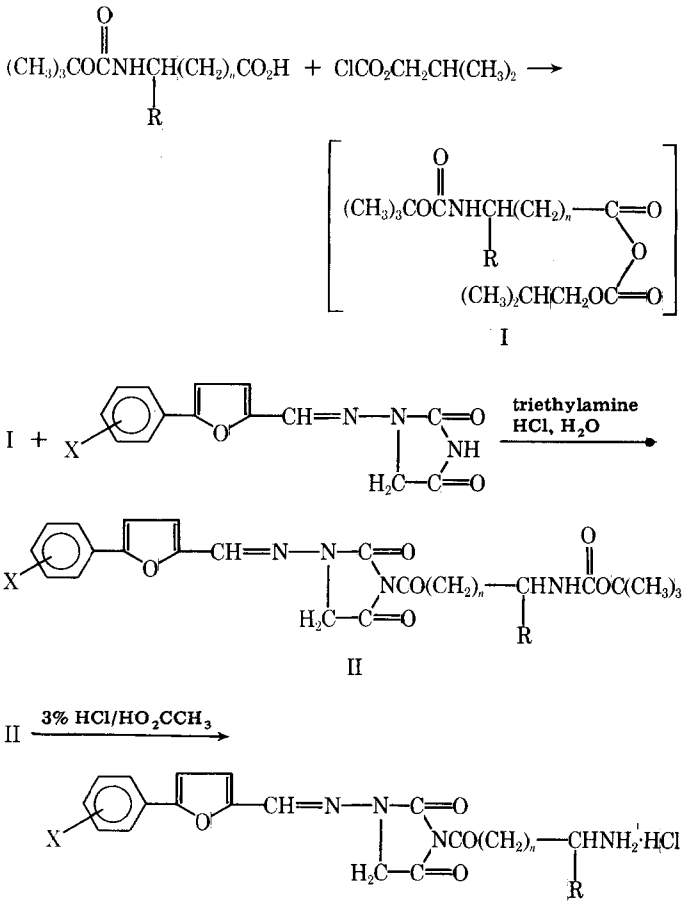
This paper presents the syntheses (6) and skeletal muscle relaxant activity of a series of 3-aminoacyl compounds related to dantrolene.

EXPERIMENTAL[2]

The imidazolidinediones selected as starting materials were prepared by a literature method (1). The title compounds (Table I) were prepared by the general procedure shown in Scheme I and as exemplified by the following typical reaction.

3 -(3-Aminopropionyl)-1-[[[5-(4-nitrophenyl)-2-furanyl]methylene]amino]-2,4-imidazolidinedione Hydrochloride (IIIa)—*N-tert*-Butoxycarbonyl-β-alanine (14 g, 0.075 mole) in dimethylformamide (350 ml) was cooled to 0°, and triethylamine (21 ml, 0.15 mole) was added rapidly. The solution was maintained at from −5 to −10° while isobutyl chlorocarbonate (10.5 ml, 0.075 mole) was added. The stirred solution was kept at −5° for 10 min, and then solid 1-[[[5-(4-nitrophenyl)-2-furanyl]methylene]amino]-2,4-imidazolidinedione (21 g, 0.068 mole) was added over 5 min. The temperature was maintained at from −5 to 0° for 30 min, and then the solution was stirred for 1 hr without cooling. The dimethylformamide solution was poured into a stirred solution of ice water (3.0 liters) containing concentrated hydrochloric acid (50 ml). The resulting yellow solid was collected and air dried. Recrystallization from acetonitrile (2.5 liters) yielded 17 g (51%) of intermediate product, mp 213–214°.

The 3-[3-(*N-tert*-butoxycarbonylamino)-1-oxopropyl-1-[[[5-(4-nitrophenyl)-2-furanyl]methylene]amino]-2,4-imidazolidinedione (17 g, 0.035 mole) was added to 3% hydrochloric acid–acetic acid (400 ml) at 15°, and the mixture was stirred for 5 hr. The mixture was filtered, and the collected solid was washed with nitromethane (200 ml) and ether (100 ml). The solid was dried in a 60° oven overnight, yielding 14 g of IIIa (94%, 48% overall), mp 211–212°; IR: 2.8–4.0 (broad N–H), 5.6, 5.7, 5.8, 6.25, 6.6, and 7.5 μ.



IIIa–IIIk

Scheme I—See Table I for specific values for X, R, and n.

Pharmacology—*Gross Observation*—Groups of three unfasted male mice[3], 20–27 g, were used. The drugs were administered perorally at doses of 50–800 mg/kg as a 2% concentration suspended in 0.5% methylcellulose 4000 cps[4]. Gross observation for pharmacological effects were made for 2 hr, utilizing a rating of drug effect signs similar to that described by Irwin (7). Muscle relaxation was rated on a scale from 0 to 4: 0, no effect; 1, slight; 2, moderate; 3, marked; and 4, extreme.

Pithed Rat Gastrocnemius Muscle Preparation—The method used for direct stimulation of the gastrocnemius muscle in rats[5] (n = 4) was the same as that described previously (8). The test drugs were administered intravenously at logarithmically spaced doses in tetrahydrofurfuryl alcohol[6] or dimethyl sulfoxide[7]. Solvent effects accounted for a <20% decrease in the twitch response at the highest volume tested.

Rotarod Testing in Mice—A method similar to that described by

[1] Morton-Norwich Products.

[2] Melting points were taken in a Mel-Temp apparatus in open capillary tubes and are uncorrected. IR spectra were determined as Nujol mulls on a Perkin-Elmer 137B spectrophotometer.

[3] Taconic Farms, TAC:(SW)fBr.

[4] Methocel, Dow Chemical Co.

[5] Taconic Farms, TAC:SD/NfBr.

[6] Eastman Kodak Co.

[7] McKesson and Robbins.

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Table I—Physical Properties of IIIa–IIIk

Compound	X	n	R	Yield, %	Melting Point	Formula	Analysis, %	Calc.	Found	IR of Carbonyls, μ
IIIa	4-NO_2	1	H	48	211–212°	$C_{17}H_{15}N_5O_6$·HCl	C	48.41	48.44	5.6, 5.7, 5.8
							H	3.82	3.95	
							N	16.61	16.92	
IIIb	3-Cl, 4-Cl	1	H	45	199–202°	$C_{17}H_{14}Cl_2N_4O_4$·HCl	C	45.81	45.81	5.55, 5.7, 5.8
							H	3.39	3.33	
							N	12.57	12.45	
IIIc	4-F	1	H	28	195–197°	$C_{17}H_{14}FN_4O_4$·HCl	C	51.72	51.96	5.6, 5.7, 5.8
							H	4.08	4.18	
							N	14.19	13.94	
IIId	4-Cl	1	H	82	242–245°	$C_{17}H_{15}ClN_4O_4$·HCl	C	49.77	49.90	5.55, 5.7, 5.8
							H	3.69	3.87	
							N	13.66	13.48	
IIIe	3-Cl, 4-Cl	0	CH_3	25	192–196°	$C_{17}H_{14}Cl_2N_4O_4$·HCl	C	45.81	45.98	5.55, 5.7, 5.8
							H	3.39	3.41	
							N	12.57	12.65	
IIIf	4-CN	1	H	62	193–194°	$C_{18}H_{15}N_5O_4$·HCl	C	53.08	53.55	5.55, 5.65, 5.8
							H	4.01	4.05	
							N	17.43	17.30	
IIIg	3-Cl, 4-Cl	4	H	28	205–207°	$C_{20}H_{20}Cl_2N_4O_4$·HCl	C	49.24	49.24	5.5, 5.65, 5.8
							H	4.34	4.30	
							N	11.49	11.51	
IIIh	3-Cl, 4-Cl	0	$C_6H_5CH_2$	52	152–160°	$C_{23}H_{18}Cl_2N_4O_4$·HCl	C	52.94	52.66	5.6, 5.7, 5.8
							H	3.67	3.85	
							N	10.74	10.67	
IIIi	4-OCH_3	1	H	40	190° dec.	$C_{18}H_{18}N_4O_5$·HCl	C	53.14	53.41	5.6, 5.7, 5.85
							H	4.71	4.93	
							N	13.77	13.77	
IIIj	3-Cl, 4-F	1	H	28	191–193°	$C_{17}H_{14}ClFN_4O_4$·HCl	C	47.57	47.50	5.55, 5.7, 5.8
							H	3.52	3.88	
							N	13.05	13.00	
IIIk	3-CF_3, 4-Cl	1	H	49	198–199°	$C_{18}H_{14}ClF_3N_4O_4$·HCl	C	45.11	45.26	5.5, 5.7, 5.8
							H	3.15	3.43	
							N	11.69	11.45	

[a] Compounds IIIa–IIIk showed the higher energy absorptions of the ring C=O at 5.55–5.6 μ upon acylation and loss of tautomerism; in contrast, the starting material for IIIa, for example, exhibited C=O values at 5.68 and 5.85 μ(1).

Table II—Skeletal Muscle Relaxant Activity of a Series of 3-(Aminoacyl)-1-[[[5-(substituted phenyl)-2-furanyl]methylene]amino]-2,4-imidazolidinediones

Compound	Gastrocnemius Twitch Tension, % inhibition[a]	Maximum Muscle Relaxation Score[b] (at mg/kg po)	Straub Tail ED_{50}[c,i]	Rotarod ED_{50}[d,i]	Muscle Relaxant Efficacy Index[e]	LD_{50}[f,i]	Therapeutic Index[g]
IIIa	−87 (1.5)	4 (400)	18 (33–37)	33.6 (21–49)	2.0	>4000	>225
IIIb	−70 (4.5)	3 (200)	38 (22–64)	19.7 (14–28)	0.5	460 (449–471)	11
IIIc	−45 (1.7)	2 (800)	90 (58–138)	18.3 (2–66)	0.2	226 (221–244)	3
IIId	−81 (2.2)	2 (800)	201 (104–362)	17.1 (12–24)	0.1	646 (525–763)	3
IIIe	−69 (0.4)	4 (400)	70 (34–159)	64.2 (34–99)	0.9	1055 (785–1391)	15
IIIf	−86 (1.6)	2 (800)	367 (206–491)	27.5 (18–42)	0.4	155 (115–196)	0.4
IIIg	−74 (2.6)	3 (400)	48 (32–72)	<5.0	<0.1	—	—
IIIh	−65 (1.8)	0 (200)	—	—	—	—	—
IIIi	−50 (3.3)	0 (800)	—	—	—	—	—
IIIj	−74 (2.6)	3 (800)	291 (116–76972)	33.0 (29–38)	0.1	314 (290–329)	1.1
IIIk	−74 (1.3)	3 (800)	85 (55–128)	19.0 (13–29)	0.2	589 (<560–>682) (indeterminate)	6.9
Dantrolene sodium	−80 (1.0)	4 (400)	37 (20–66)	62.0 (27–262)	1.7	1370 (888–1853)[h]	37
Clodanolene sodium	−78 (3.7)	4 (400)	22 (7–60)	22.0 (17–37)	1.7	742 (299–1204)[h]	38

[a] Percent inhibition of electrically induced contractions of the rat gastrocnemius muscle at a cumulative dose of 36.6 mg/kg iv. The figure in parentheses is the standard error of the mean. [b] Where 1 = slight, 2 = moderate, 3= marked, and 4= severe drug effect as measured by gross observation in mice and the dose (in parentheses) at which this occurred. [c] Intraperitoneal dose of drug causing inhibition of morphine-induced Straub tail in 50% of the mice. [d] Intraperitoneal dose of drug causing loss of motor coordination (rotarod) in 50% of the mice. [e] ED_{50} rotarod/ED_{50} Straub tail. [f] Intraperitoneal dose of drug causing death in 50% of the mice within 72 hr. [g] LD_{50}/ED_{50} Straub tail. [h] See Ref. 8. [i] The figures in parentheses are the 95% confidence limits. Muscle relaxant testing was not completed on compounds which were not effective orally (IIIh, IIIi) or in which the Rotarod ED_{50} was below the Straub Tail ED_{50} (IIIg).

Dunham and Miya (9) was used to test the effect of drugs on motor coordination. Male mice[3] (n = 10/group) were trained to walk the revolving rod (20 rpm) for over 1 min. The test drugs were administered intraperitoneally in 0.5% methylcellulose at logarithmically spaced doses to the previously trained mice, and performance trials were conducted 30 min following drug administration. The inability of an animal to stay on the rotarod for more than 30 sec was considered a positive drug effect. The ED_{50} values (rotarod) (the drug dose that caused 50% of the animals to fall off the rotarod within 30 sec) were calculated using the method of Litchfield and Wilcoxon (10).

Straub Tail in Mice—The method used in these experiments was similar to that reported by Ellis and Carpenter (11). Male mice[3] (n = 10/group) were used for Straub tail testing. The drugs were administered intraperitoneally in 0.5% methylcellulose 20 min after morphine[8] (15 mg/kg sc) injection. The drugs were judged effective if they caused the elevated Straub tail to lay flat on the table. The compounds were administered in logarithmically spaced doses, and ED_{50} values (dose of the

[8] Mallinckrodt.

drug causing the elevated tail to lay flat in 50% of the animals) were obtained by the method of Litchfield and Wilcoxon (10).

LD_{50} in Mice—The test compounds were administered perorally in 0.5% methylcellulose to groups of unfasted male mice[3] (n = 10/group). The animals were held in cages for 72 hr, and the deaths were recorded. The LD_{50} values (drug dose causing death in 50% of the animals) were calculated using the method of Litchfield and Wilcoxon (10).

RESULTS AND DISCUSSION

The synthesized compounds (6) exhibited a better absorption and solubility profile than the parent compound, dantrolene. Eleven compounds were synthesized and evaluated for skeletal muscle relaxant activity (Table II). In the pithed rat gastrocnemius muscle preparation, all compounds were effective intravenous skeletal muscle relaxants (>50% inhibition of the electrically induced twitch contraction) (Table II).

Compounds III*a* and III*f* were also potent inhibitors of the gastrocnemius twitch tension. These compounds were the most effective in that the maximal inhibition of the twitch response was 86–87% whereas dantrolene sodium and clodanolene sodium only yielded a maximal inhibition of 80%.

Gross observation in mice identified nine of the 11 compounds as orally effective muscle relaxants. Among these nine (muscle relaxant score of >1 at doses up to 800 mg/kg), III*a* and III*e* were the most effective, producing muscle relaxant scores of 4. Compounds III*b*, III*g*, III*j*, and III*k* were slightly less effective, producing muscle relaxant scores of 3; III*b* produced a 3 level of muscle relaxation at 200 mg/kg.

Lowering of the Straub tail previously was shown (11) to result from relaxation of the sacrococcygeal muscles at the base of the tail. Several different drugs can produce this muscle relaxation (11). However, the muscle relaxant properties of a drug are useful only when they can be separated in a dose-dependent manner from its neurotoxic properties (motor incoordination). Motor incoordination is an unacceptable consequence of skeletal muscle relaxant therapy and, therefore, is a limiting factor in determining muscle relaxant efficacy. In the Straub tail model, the muscle relaxant dose is identified as St ED_{50}; the motor incoordination dose is identified with the rotarod test (Rr ED_{50}). Comparing these two doses as a ratio, ED_{50} rotarod:ED_{50} Straub tail, gives an indication of the separation between the two dose ranges (*i.e.*, the greater the ratio, the more separation between the two doses).

Compound III*a* yielded a muscle relaxant efficacy index of 2.0, and all other compounds produced muscle relaxant efficacy indexes of <1.0. Additionally, when III*a* was evaluated for acute toxicity, no deaths were seen up to 4000 mg/kg, and the resultant therapeutic index was >225.

The difference in muscle relaxant efficacy indexes between III*a* and other muscle relaxants of this type (dantrolene sodium and clodanolene sodium), including the compounds in Table II, is small but consistent. Compound III*a* is a potent, direct-acting skeletal muscle contraction antagonist (inhibition of the directly induced gastrocnemius twitch tension). The muscle relaxant efficacy index and acute therapeutic index values reflect a high degree of efficacy and safety.

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Formation of Methyl Ester of Salicyluric Acid during Quantitation of Salicyluric Acid in Urine by High-Pressure Liquid Chromatography

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Abstract □ The formation of the methyl ester of salicyluric acid was observed during the quantitation of salicyluric acid and other salicylate metabolites in urine by high-pressure liquid chromatography. This methyl ester formation caused artificially low values for salicyluric acid and high values for salicylic acid.

Keyphrases □ Salicyluric acid—methylation during high-pressure liquid chromatography □ Methylation—salicyluric acid in urine, high-pressure liquid chromatography □ High-pressure liquid chromatography—salicyluric acid in urine, methylation

Several high-pressure liquid chromatographic (HPLC) assays for salicylic acid and its metabolites, salicyluric acid and gentisic acid, in serum and urine were reported recently (1–8). Review of these methods indicates that certain precautions must be taken to prevent sublimation losses of salicylic acid during evaporation (6) and decom-

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