Antiinflammatory Activities of 2-Acetyl- and 2-(1-Hydroxyethyl)-4-[3-(dimethylamino)propyl]-3,4-dihydro-3-phenyl-2*H*-1,4-benzothiazine[1]

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The 1,4-benzothiazines represent a series of structurally novel nonsteroidal antiinflammatory agents. Unlike the majority of marketed products, they do not possess any acidic function. The two 1,4-benzothiazines cited in this study demonstrate marked antiinflammatory activity, possess analgesic activity, have low acute oral toxicity in the rat and mouse, and are not ulcerogenic in the fasted rat. These biological activities are of considerable interest; they compare favorably with those of indomethacin, phenylbutazone, and niflumic acid.

Twenty-three compounds of the 1,4-benzothiazine series were synthesized and, based on their activity in the carrageenin-induced edema assay, two of these were selected for further evaluation.[2] The biological activities of these compounds, 2-(1-hydroxyethyl)-4-[3-(dimethylamino)propyl]-3,4-dihydro-3-phenyl-2*H*-1,4-benzothiazine (I) and 2-acetyl-4-[3-(dimethylamino)propyl]-3,4-dihydro-3-phenyl-2*H*-1,4-benzothiazine (II) (Figure 1), were compared with those of indomethacin, phenylbutazone, and niflumic acid (2-[(α,α,α-trifluoro-*m*-tolyl)amino]nicotinic acid).

Experimental Section

The biological activities of the two test compounds and the three standard agents were evaluated in the test procedures described below. Unless indicated otherwise, in each study there were ten animals per experimental group. The ID_{50}, the dose causing a 50% inhibition of reaction, was estimated from dose-response curves.

Carrageenin-Induced Edema. The procedure described by Winter, *et al.*,[3] was used to test agents for antiedema activity. The test compounds were dissolved or suspended in 1% aqueous sodium carboxymethylcellulose (Na CMC) and were administered orally in a volume of 1.0 ml to groups of male Sprague–Dawley rats (seven per group) at doses of 150, 75, and 37.5 mg/kg each. Later (2 hr), the volume of the left hind paw was measured by mercury displacement, and 0.05 ml of a 1% solution of carrageenin in pyrogen-free saline was injected into the plantar surface of the paw. After this injection (3 hr), the volume of the left hind paw was again measured. The ID_{50} was determined for each test compound.

Reversed Passive Arthus Skin Reaction. The reversed passive Arthus reaction was produced according to the procedure described by Goldlust and Schreiber.[4] The intravenous injection of an antigen, bovine serum albumin (BSA), into male albino rabbits was followed immediately by the intradermal injection of rabbit antibody to BSA. Each test compound was administered intradermally as a mixture with the anti-BSA (two sites per rabbit) or intraperitoneally as a saline suspension (two rabbits per ip dose). The volumes of the edematous reactions and the extent of hemorrhage were measured 6 hr after the administration of antigen and antibody. The activities of the compounds were recorded as the per cent inhibition of edema formation, and the effect on development of hemorrhage was observed grossly.

Experimental Allergic Encephalomyelitis. Experimental allergic encephalomyelitis (EAE) was induced in the male Lewis rat by the procedure of Rosenthale and Nagra.[5] An emulsion of guinea pig spinal cord in complete Freund's adjuvant was injected on day 0 by the subplantar route. Rats were treated 12 days by intraperitoneal injection of test compound, starting on day − 1. Control rats (not drug treated) develop fecal impaction, paralysis, and weight loss between days 10 and 14. Animals recover spontaneously between days 18 and 20. The efficacy of each test compound in decreasing the symptoms of EAE was assessed, and the activity was recorded as per cent protection against paralysis.

Adjuvant-Induced Arthritis. Adjuvant arthritis was induced in the male Sprague–Dawley rat by the procedure of Rosenthale and Nagra.[5] On day 0, the volumes of the right and left hind paws (up to and including the lateral malleolus) were measured by mercury displacement; then 0.25 mg of desiccated *Mycobacterium butyricum* (Difco) in 0.05 ml of light mineral oil, U.S.P., was injected into the plantar surface of the left hind paw. The test compounds were dissolved or suspended in 1% aqueous Na CMC and administered by the intraperitoneal route for 20 consecutive days, starting on day 1. Volumes of the right and left hind paws were again measured 21 days later. In addition, the occurrence of lesions and swelling of the forepaws and the presence of nodules on the tail were noted and recorded at that time. The effect of the compound was reported in terms of its per cent inhibition of the locally induced inflammation (injected hind paw) and of the systemically induced inflammation (right hind paw, forepaws, and tail).

Delayed Hypersensitivity Skin Reaction. Male, albino guinea pigs, Hartley strain, were sensitized to *Mycobacterium tuberculosis* by the injection of the organism suspended in Freund's incomplete adjuvant into each hind foot pad and, subcutaneously, into the back of the neck. Three weeks after sensitization, a skin lesion was produced by the intradermal injection of 1.2 μg of pure protein derivative (PPD). Each test compound, suspended in sesame oil, was administered subcutaneously to three–seven guinea pigs per test in five assays at a dose of 50 mg/kg, 30 min before and 5 hr after the injection of the PPD. The standard agents were administered subcutaneously to three guinea pigs per test using the same dosage regimen. The activities of the compounds were recorded as the per cent inhibition of the diameter of the erythematous lesion and of the thickness (cellular induration) of the reaction, measured 24 hr after injection of the antigen.

Synthesis of Hemagglutinin. The effect of the test compounds on the primary immune response was evaluated by the procedure described by Nathan, *et al.*[6] Male Swiss mice were immunized to tanned sheep erythrocytes. Compounds dissolved or suspended in 1% aqueous Na CMC were administered subcutaneously at a dose of 25 mg/kg daily for 4 days starting on the day of immunization. Serum hemagglutinin (antibody) titers were measured 15 days after immunization. The ratio of hemagglutinin titers of the control mice to those of the test mice (C/T) was determined.

Mouse Thymus Cell Culture. Thymus cells of mouse origin incubated in media have been used for testing the activity of compounds as inhibitors of DNA synthesis. The procedure used was adapted from that of Schwartz, *et al.*,[7] which measured the effects of drugs on the synthesis of RNA, DNA, and protein by human peripheral lymphocytes grown in culture. The extent of inhibition of incorporation of thymidine-2-^{14}C (TdR) into an acid-insoluble fraction was measured, and the concentration of compound that caused a 50% inhibition of this incorporation (I_{50}) was calculated.

Erythrocyte-Membrane Stabilization. Fresh canine erythrocytes suspended in 0.15 *M* phosphate buffer, pH 7.4, were lysed by heating at 53° for 20 min. By the procedure described by Brown, *et al.*,[8] the per cent inhibition of heat-induced lysis of canine erythrocytes by the test compound was measured against that of similarly heated control cells not treated with test compounds. The concentration of drug that caused a 50% inhibition of hemolysis (I_{50}) was determined for each compound.

Analgesic Assays. The writhing syndrome[9] and the Randall–Sellito procedure[10] were used to evaluate the analgesic properties of the test compounds.

A. Writhing Test. After the oral administration of the test compound (60 min, suspension in 1% aqueous Na CMC), CF_1 mice were injected intraperitoneally with 0.45% acetic acid, 0.1 ml/10 g of body weight, to elicit the writhing response. The animals were observed

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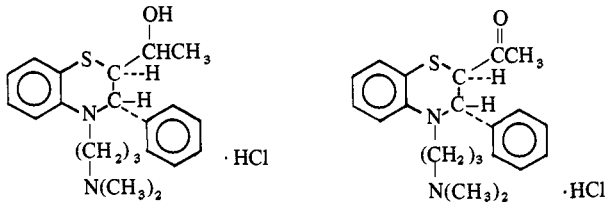

I II

Figure 1. Chemical structure of 2-(1-hydroxyethyl)-4-[3-(dimethylamino)propyl]-3,4-dihydro-3-phenyl-2*H*-1,4-benzothiazine (I) and 2-acetyl-4-[3-(dimethylamino)propyl]-3,4-dihydro-3-phenyl-2*H*-1,4-benzothiazine (II).

for 8 min, and the number of writhing (stretching) responses was determined. The inhibition of the writhing syndrome was expressed as the ID_{50}, the dose of compound that caused a 50% inhibition of response.

B. Randall-Sellito Test. After 0.05 ml of a 0.2% suspension of digitonin (Merck, Darmstadt, Germany) had been injected intramuscularly into the right hind foot of female Sprague–Dawley rats to produce a very marked inflammation, the test compound, suspended or dissolved in water, was administered orally. The pain threshold was measured before and 1 hr after the injection of digitonin, with pain achieved by an increasing compression of the paw. The activity of the compound was expressed as the ID_{50}.

Acute Oral Toxicity in Mice and/or Rats. Aqueous solutions or suspensions of test compounds were given orally as single doses to groups of rats or mice. The oral dose producing 50% lethality (LD_{50}) was determined for each compound.

Ulcerogenic Activity. Male Sprague–Dawley rats were deprived of food pellets, but allowed free access to water containing 5% dextrose, for 48 hr before oral administration (*via* gavage) of the test compound. The compounds were dissolved or suspended in water and were administered orally to six rats each at doses of one-half and two times the oral ID_{50} determined in the carrageenin-induced edema assay. After dosing (6 hr), the animals were sacrificed, and their stomachs were excised and examined grossly for hyperemia, fresh or tarry blood, and erosions (hemorrhagic or nonhemorrhagic) in the rumen and glandular portions. The approximate dose producing gastric erosions in 50% of the rats (the UD_{50}) was estimated graphically.

Results and Discussion

The biological activities of I and II were compared with those of indomethacin, phenylbutazone, and niflumic acid (see Table I). In addition to producing comparable inhibition of carrageenin-induced edema in the rat paw, both compounds inhibited the development of the reversed passive Arthus reaction in the rabbit when administered intraperitoneally. Only I demonstrated activity in this reaction when admixed with the antibody and injected intradermally.

Table I. Comparison of the Biological Activities of 2-(1-Hydroxyethyl)-4-[3-(dimethylamino)propyl]-3,4-dihydro-3-phenyl-2*H*-1,4-benzothiazine (I) and 2-Acetyl-4-[3-(dimethylamino)propyl]-3,4-dihydro-3-phenyl-2*H*-1,4-benzothiazine (II) with Those of Indomethacin, Phenylbutazone, and Niflumic Acid

Test	Dose and route of administration[a]	Measurement	I	II	Indomethacin	Phenylbutazone	Niflumic acid
Carrageenin-induced edema	po	ID_{50}, mg/kg	55	65	7 ± 2.9[b]	102	41 ± 11
Reversed passive Arthus (rabbit)	200 μg id	% inhibition	41 ± 10[c] (36)[d]	12 ± 10 (18)	48 ± 12 (10)	37 ± 11 (4)	52 ± 13 (45)
	25		7	74	50	ND[e]	ND
	50		74	80	ND	ND	ND
	75		55	87	ND	46	54
	100		76	93	ND	ND	ND
	150		ND	ND	ND	30	58
Exptl allergic encephalomyelitis	3	% protection, % survival	ND	ND	44, 100	ND	ND
	15		ND	17, 100	ND	ND	ND
	45		15, 90	69, 80	ND	ND	ND
	60 mg/kg po		ND	ND	ND	ND	26, 100
	90 mg/kg po		ND	ND	ND	ND	100, 10
Adjuvant-induced arthritis	2.5	% inhibition, local/systemic, % survival	ND	ND	71/73, 90	ND	ND
	15		ND	7/40, 60	ND	36/37, 100	39/28, 100
	20		14/21, 100	ND	ND	ND	ND
	30		ND	28/56, 70	ND	53/32, 80	62/41, 100
	40		33/30, 80	ND	ND	ND	ND
	60		34/66, 65	13/61, 40	ND	64/27, 100	69/37, 100
Delayed hypersensitivity skin rxn	2 × 50 mg/kg sc	% inhibition of erythema	11 ± 7[c]	16 ± 7	4	0	11
		% inhibition of induration	34 ± 16	57 ± 16	32	20	20
Hemagglutinin synthesis	25 mg/kg sc	Ratio of control/test	32	64	64	16	8
DNA synthesis		I_{50}, m*M*	0.100	0.025	>0.28	>0.33	0.14
Membrane stabilization		I_{50}, m*M*	0.8	0.6	0.08	0.4	0.04
Analgesic activity							
Writhing	po	ID_{50}, mg/kg	8.4	9.3	0.3	45	44
Randall-Sellito	po	ID_{50}, mg/kg	17	9.5	0.36	11.5	7.5
Acute toxicity							
Rat	po	LD_{50}, mg/kg	800	ND	>12.5 < 15	375	<250
Mouse	po		520	560	ND	ND	ND
Ulcerogenicity, fasted rat	po	UD_{50}, mg/kg	>110	>140	4.6	115	32
Therapeutic index		$\frac{\text{Rat } LD_{50}}{\text{rat } ID_{50} \text{ (CE)}^f}$	14.5	ND	1.8	3.7	<6
Therapeutic index		$\frac{\text{Rat } UD_{50}}{\text{rat } ID_{50} \text{ (CE)}^f}$	>2	>2	≤1	1	1

[a] The compounds were administered ip on a mg/kg basis except where indicated. [b] ID_{50} ± standard deviation where available. [c] Per cent inhibition ± 95% confidence limit where available. [d] Number of rabbits tested. [e] Not determined. [f] ID_{50}, carrageenin-induced edema.

Neither compound nor the standards, administered intradermally or intraperitoneally, had an effect on the development of hemorrhage in the Arthus reaction. Both compounds produced comparable inhibition of local and systemic lesions in adjuvant-induced arthritis in the rat and of paralysis in experimental allergic encephalomylitis. These inhibitions were observed at or near toxic levels, but the toxicity was considered adjuvant-related. Morton and Chatfield[11] had indicated that the adjuvant-induced arthritic rat probably does not detoxify compounds as efficiently as do normal rats because of impaired liver function. When II was tested in normal or adjuvant-treated rats at daily oral doses of 60 mg/kg a marked increase in lethality was observable by the eleventh day in the drug-adjuvant treated group (13/15 deaths), as compared with the drug, nonadjuvant treated group (2/15 deaths). II was more effective than I in decreasing cell induration in the delayed hypersensitivity skin reaction (tuberculin) in the guinea pig, in suppressing hemagglutinin production in the mouse, and in inhibiting TdR uptake by mouse thymus cells in culture. Neither compound was active as a membrane stabilizer. Both compounds demonstrated analgesic activity in the writhing and Randall-Sellito assays, and neither compound produced gastric erosions in the fasted rat. Because of the low acute oral toxicity of I and II in the rat and in the mouse and because they lack ulcerogenic potential, both compounds possess favorable therapeutic indices. These data indicate that both I and II possess antiinflammatory activity of considerable interest; they compare favorably with indomethacin, phenylbutazone, and niflumic acid. Compound I has been selected for further toxicological studies.

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Synthetic Antidiarrheal Agents. 2,2-Diphenyl-4-(4′-aryl-4′-hydroxypiperidino)butyramides

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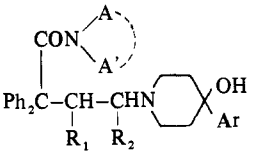
The synthesis of a series of 2,2-diphenyl-4-(4′-aryl-4′-hydroxypiperidino)butyramides and the preliminary evaluation of their antidiarrheal activities are described. Intermediates are (tetrahydro-3,3-diphenyl-2-furylidene)ammonium salts prepared from 4-bromo-2,2-diphenylbutyric acid (**2**). 4-(*p*-Chlorophenyl)-4-hydroxy-*N*,*N*-dimethyl-α,α-diphenyl-1-piperidinebutyramide HCl (**30**, loperamide) and 4-(4-chloro-α,α,α-trifluro-*m*-tolyl)-4-hydroxy-*N*,*N*-dimethyl-α,α-diphenyl-1-piperidinebutyramide HCl (**33**, fluperamide) were approximately two times more potent than diphenoxylate and had a considerably better relative constipating specificity.

As part of a continuing effort to develop novel antidiarrheal agents, a series of 2,2-diphenyl-4-(4′-aryl-4′-hydroxypiperidino)butyramides of formula I were prepared. Diphenoxylate (IIa), a well-known antidiarrheal[1] and potent inhibitor of the peristaltic relfex activity of guinea pig ileum *in vitro*,[2] belongs to a series of 1-(3-cyano-3,3-diphenylpropyl)-4-phenylisonipecotic acid esters. With IIa the aim to synthesize analgesic type compounds devoid of analgesic action, but behaving as highly active inhibitors of gastrointestinal propulsion and defaecation, was achieved. The active metabolite of IIa, difenoxine (IIb),[3] was found to be five times more potent than IIa and to possess a better safety margin.[4-8]

The original objective of this study was to replace the cyano group of II by an amide function, but the approach led invariably to less active or inactive compounds. However, when the carboxyl substituent on the piperidine ring was replaced by a hydroxyl group as well, improvement in activity was found. This modification was surprising, since 4-aryl-4-piperidinols are typical moieties of neuroleptics,



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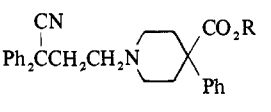

IIa, R = Et
b, R = H

such as haloperidol, trifluperidol, moperone, and clofluperol.[9]

Chemistry. The synthesis of the teritary butyramides I is outlined in Schemes I and II. Ring opening of 2,2-diphenyl-4-hydroxybutyric acid γ-lactone (**1**) with HBr in AcOH afforded 4-bromo-2,2-diphenylbutyric acid (**2**).[10] Subsequent treatment of **2** with $SOCl_2$ and reaction of the intermediate acid chloride with an appropriate secondary amine yielded the corresponding (tetrahydro-3,3-diphenyl-2-furylidene)-ammonium salts III (Table I). Compounds **3** rearranged spontaneously under the reaction conditions. The structure of ammonium salts III was evident from spectral data (Experimental Section) and from their reactivity. Compounds III