solution was obtained. It was kept at 25 °C for 18 h, clarified by filtration, and acidified with 0.1 N HCl. The resulting white precipitate was freed from inorganic ions by three cycles of suspension (H_2O, 100 mL)–centrifugation–decantation. The product was dried over P_2O_5 in vacuo at 25 °C: 0.26 g (89%); mp 271 °C dec; NMR, Table I; UV, Table II. Anal. After drying at 150 °C ($C_{22}H_{22}N_4O_3$) C, H, N.

Biological Evaluation

The analogues **1c–g** and also diethyl N^{10}-propargyl-5,8-dideazafolate (**1b**)[8] were tested for their inhibition of purified L1210 thymidylate synthase as previously described.[19] For determination of the inhibition of the growth of L1210 cells in culture the compound was suspended in unsupplemented RPMI 1640 medium that was then adjusted to about pH 9.0 with 1 N NaOH to aid dissolution. After sterile Millipore filtration (0.22 μm) the pH was adjusted back to about 7.5 with sterile 1 N HCl and 0.5 mL of the resulting solution added to 4.5 mL of a suspension of L1210 cells (1 × 10^5 mL^{-1}) and cultured as previously described.[8] The results are expressed in Table III.

Acknowledgment. This work was supported at the Institute of Cancer Research by grants from the Cancer Research Campaign and the Medical Research Council. Dr. G. Hawkes of Queen Mary College and J. Elliot of King's College provided the 400- and 250-MHz NMR spectra under the auspices of the University of London Intercollegiate Research Service. We thank B. Wright for the 90-MHz spectra.

Registry No. **1b**, 76858-74-7; **1c**, 101248-32-2; **1d**, 80014-97-7; **1e**, 101248-33-3; **1f**, 101248-34-4; **1g**, 101248-35-5; **2**, 101248-36-6; **3**, 77766-62-2; **4**, 101248-37-7; **5d**, 27128-94-5; **5g**, 76765-75-8; **6d**, 5854-13-7; **6f**·HCl, 101248-38-8; **6g**, 76765-77-0; **7d**, 80014-88-6; **7f**, 101248-39-9; **7g**, 101248-40-2; **8d**, 80014-78-4; **8f**, 101248-41-3; **8g**, 101248-42-4; **9**, 20637-09-6; **10**, 101248-43-5; **11**, 101248-44-6; HC≡CCH_2Cl, 624-65-7; HC≡CCH_2Br, 106-96-7; diethyl L-aspartate hydrochloride, 16115-68-7; 4-nitrobenzoyl chloride, 122-04-3; ethyl L-alaninate hydrochloride, 1115-59-9; 4-aminohippuric acid, 61-78-9; thymidylate synthase, 9031-61-2.

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Notes

Indolo[2,1-*c*][1,4]benzodiazepines: A New Class of Antiallergic Agents

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A series of 12-(cyclic alkylamino)-6*H*-indolo[2,1-c][1,4]benzodiazepines were synthesized that possess antihistamine and antiserotonin activities as well as ability to inhibit mediator release. Compound **6a**, 12-(4-methyl-1-piperazinyl)-6*H*-indolo[2,1-*c*][1,4]benzodiazepine was a more potent inhibitor of serotonin release than disodium cromoglycate (DSCG) and ketotifen andd approximately equivalent to oxatomide. In the in vivo tests (PCA and ALA), compound **6a** was equivalent or superior to DSCG and oxatomide. These agents have potential for the treatment of a variety of allergic conditions.

The clinical success of disodium cromoglycate (DSCG)[1] in the prophylactic treatment of asthma has stimulated a substantial research effort to discover more potent and orally active inhibitors of mediator release from mast cells and basophils. Consequently, numerous compounds have been described to possess desired preclinical biological properties, and many of these have been proposed to be orally antiallergic agents.[2] The mechanisms of these agents are varied and, in many cases, not totally elucidated. Some have been reported to be pure mediator-release inhibitors (e.g., bufrolin[3]), while others also possess the ability to block receptors for the mediators histamine, serotonin, and/or leukotrienes (e.g., oxatomide,[4] ketotifen,[5] FPL-55712[6]).

Scheme I

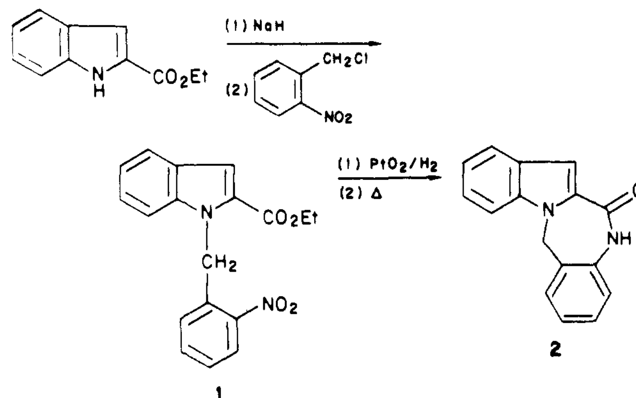

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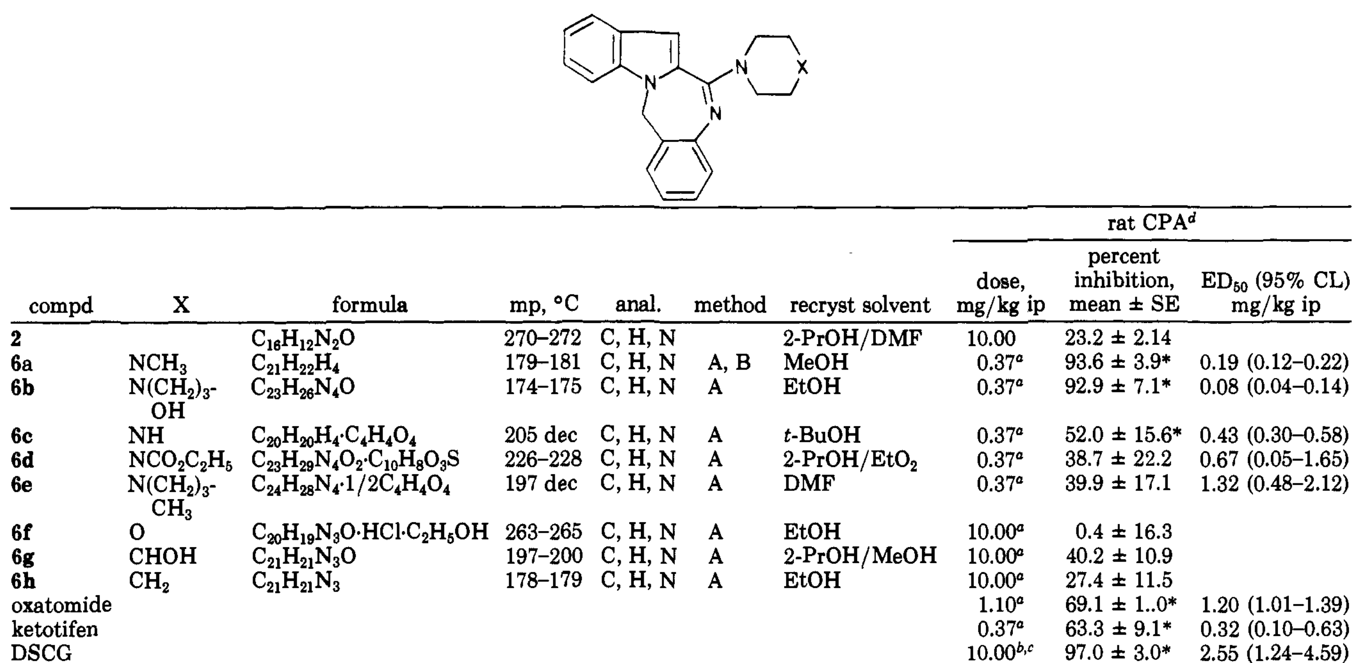
Table I. Inhibition of Rat PCA by 12-(Alkylamino)-6*H*-indolo[2,1-c][1,4]benzodiazepine



compd	X	formula	mp, °C	anal.	method	recryst solvent	rat CPA[d] dose, mg/kg ip	percent inhibition, mean ± SE	ED_{50} (95% CL) mg/kg ip
2		$C_{16}H_{12}N_2O$	270–272	C, H, N		2-PrOH/DMF	10.00	23.2 ± 2.14	
6a	NCH_3	$C_{21}H_{22}H_4$	179–181	C, H, N	A, B	MeOH	0.37[a]	93.6 ± 3.9*	0.19 (0.12–0.22)
6b	$N(CH_2)_3$-OH	$C_{23}H_{26}N_4O$	174–175	C, H, N	A	EtOH	0.37[a]	92.9 ± 7.1*	0.08 (0.04–0.14)
6c	NH	$C_{20}H_{20}H_4 \cdot C_4H_4O_4$	205 dec	C, H, N	A	*t*-BuOH	0.37[a]	52.0 ± 15.6*	0.43 (0.30–0.58)
6d	$NCO_2C_2H_5$	$C_{23}H_{29}N_4O_2 \cdot C_{10}H_8O_3S$	226–228	C, H, N	A	2-PrOH/EtO_2	0.37[a]	38.7 ± 22.2	0.67 (0.05–1.65)
6e	$N(CH_2)_3$-CH_3	$C_{24}H_{28}N_4 \cdot 1/2C_4H_4O_4$	197 dec	C, H, N	A	DMF	0.37[a]	39.9 ± 17.1	1.32 (0.48–2.12)
6f	O	$C_{20}H_{19}N_3O \cdot HCl \cdot C_2H_5OH$	263–265	C, H, N	A	EtOH	10.00[a]	0.4 ± 16.3	
6g	CHOH	$C_{21}H_{21}N_3O$	197–200	C, H, N	A	2-PrOH/MeOH	10.00[a]	40.2 ± 10.9	
6h	CH_2	$C_{21}H_{21}N_3$	178–179	C, H, N	A	EtOH	10.00[a]	27.4 ± 11.5	
oxatomide							1.10[a]	69.1 ± 1..0*	1.20 (1.01–1.39)
ketotifen							0.37[a]	63.3 ± 9.1*	0.32 (0.10–0.63)
DSCG							10.00[b,c]	97.0 ± 3.0*	2.55 (1.24–4.59)

[a] One hour pretreatment. [b] Five minutes pretreatment. [c] iv administration. [d] Asterisk: significantly different from control ($p \leq 0.05$) by Dunnett's test.

In this report, we describe a novel series of agents with an indolo[2,1-*c*][1,4]benzodiazepine nucleus and an amidine functionality, which possess antiallergic properties, including inhibition of mediator release and antagonism of histamine and serotonin. Several of these amidines exhibit potent activity in both the passive cutaneous anaphylaxis (PCA) test in the rat and the active lung anaphylaxis (ALA) test in the guinea pig by both ip and po routes.

Chemistry. The lactam of 6*H*-indolo[2,1-*c*][1,4]-benzodiazepin-12(11*H*)-one (**2**) has not been reported and was prepared by a three-step procedure (Scheme I). Ethyl indole-2-carboxylate was treated with sodium hydride in dimethylformamide, followed by 2-nitrobenzyl chloride. The alkylated nitro product, **1**, was reduced via hydrogenation (PtO_2 or Ra Ni) to obtain an amino ester, which was subsequently cyclized by heating in the presence of 2-hydroxypyridine to lactam **2**.

The 12-(alkylamino)-6*H*-indolo[2,1-*c*][1,4]benzodiazepines (**6**) were prepared from lactam **2** via two methods, as summarized in Scheme II.[7] In method A, lactam **2** was converted by the action of phosphorus pentasulfide to thiolactam **3**. Alkylation of thiolactam **3** with dimethyl sulfate and potassium hydroxide gave methyl thioether **4**, which was reacted with a variety of amines to give amidine products **6**. In method B, reaction of lactam **2** with triethyloxonium tetrafluoroborate[8] gave imino ether **5**, which was reacted with amines in the presence of a catalytic amount of glacial acetic acid to give amidines **6**.

Biological Results and Discussion. The compounds represented in Tables I and II were tested in the rat PCA test. The most potent compounds in the PCA were **6a**–**6c**. As shown in Table II, these compounds, like the reference

Scheme II

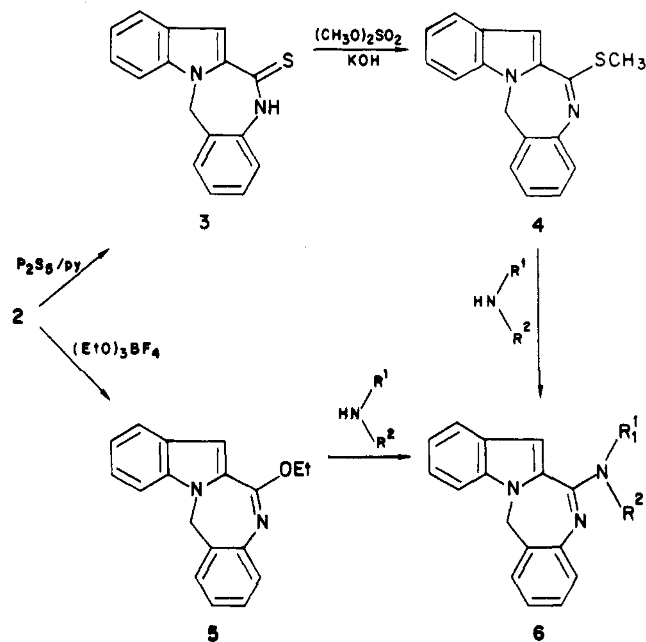


Table II. Effect on PCA, Histamine, and Serotonin

compd	dose, mg/kg ip	percent inhibn, mean ± SE[d] PCA	histamine	serotonin
6a	0.37[a]	93.6 ± 3.9*	58.2 ± 2.9*	50.9 ± 5.6*
	3.3[a]	100.0*	63.3 ± 2.5*	65.9 ± 1.5*
6b	0.12[a]	58.8 ± 25.6	6.8 ± 4.9	24.9 ± 6.0*
	0.37[a]	92.9 ± 7.1*	53.0 ± 9.0*	53.2 ± 4.9*
6c	0.37[a]	52.0 ± 15.6*	14.2 ± 8.3	44.5 ± 7.0*
	3.3[a]	92.7 ± 7.3*	55.7 ± 3.5*	65.8 ± 2.6*
ketotifen	0.37[a]	63.3 ± 9.1*	64.6 ± 5.4*	14.8 ± 9.0
	3.3[a]	92.4 ± 3.2*	62.5 ± 3.8*	35.3 ± 5.1
oxatomide	1.1[a]	69.1 ± 19.0*	38.0 ± 7.2*	35.1 ± 5.2*
	3.3[a]	100.0*	49.3 ± 2.6*	46.5 ± 3.3*
DSCG	1.0[b,c]	37.0 ± 20.1	0.0	11.5 ± 3.5
	10.0[b,c]	97.0 ± 3.0*	8.5 ± 7.4	21.4 ± 5.7

[a] One hour pretreatment. [b] Five minutes pretreatment. [c] iv administration. [d] Asterisk: significantly different from control ($p \leq 0.05$) by Dunnett's test.

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Table III. Active Lung Analphylaxis (ALA) and Serotonin Release Assay

compd	active lung analphylaxis: dose, mg/kg iv	% inhibn, mean ± SE	ED_{50} (95% CL), mg/kg iv	inhibn serotonin rel IC_{50} (95% CL) μM
6a	0.01	41 ± 6	0.01 (0.0009–0.0313)	15.9 (11.6–23.9)
6b	1.0	64 ± 12	0.69 (0.32–1.32)	15.2 (11.5–21.3)
6c	1.0	82 ± 8		7.38 (4.89–14.7)
ketotifen	0.001	57 ± 13	0.002 (0.0001–0.0017)	22.9 (18.4–28.9)
oxatomide	1.0	43 ± 13	1.25 (0.45–7.0)	20.1 (17.4–23.7)
DSCG	100.0	25 ± 19.5		256 (156–530)

Table IV. Inhibition and Duration of Activity after Oral and ip Administration in Guinea Pig ALA

compd	dose, mg/kg	route	percent inhibn time postdosing, h, mean ± SD: 1	7	8	24
6a	1	ip	74 ± 9.4	75 ± 16.2		26 ± 25
ketotifen	1	ip	82 ± 5.4	68 ± 17.6		6.5 ± 39
oxatomide	1	ip	16 ± 32.0	54 ± 31		10.6 ± 62
6a	1	po	51 ± 16.5		87 ± 9	61 ± 20
ketotifen	1	po	68 ± 11.4		73 ± 34	37 ± 17
oxatomide	1	po	-5 ± 19		45 ± 37	25 ± 33

drugs ketotifen and oxatomide, antagonized the effects of histamine and serotonin in rat skin. DSCG, however, inhibited the PCA response but failed to significantly inhibit the response to the biogenic amines.

Since these compounds were demonstrated to attenuate an IgE-mediated cutaneous allergic reaction in the rat, they were further evaluated for antiallergic activity in the guinea pig active lung anaphylaxis (ALA) model. Compounds **6a–6c**, oxatomide, and ketotifen were also active in this model, with ketotifen and **6a** being most potent (Table III). Thus, the activity of these compounds is not a species- or tissue-specific phenomenon.

Since compounds which inhibit degranulation are also active in these in vivo tests, we tested the ability of **6a–6c** to inhibit mediator release. This was accomplished by using the in vivo rat peritoneal cell serotonin release assay. All four compounds were active (Table III). Compounds **6a–6c** were more potent inhibitors of serotonin release than disodium cromoglycate (DSCG) and ketotifen and approximately equivalent to oxatomide. In the in vivo tests (PCA and ALA), compounds **6a–6c** were equivalent or superior to DSCG and oxatomide.

Compound **6a** exhibited both oral and ip activity lasting in excess of 8 h (Table IV).

Conclusions. A novel series of orally active compounds have been described that possess antihistamine and antiserotonin activities as well as the ability to inhibit mediator release. These agents have potential for the treatment of a variety of allergic conditions.

Experimental Section

Melting points were determined on a Thomas Hoover melting point apparatus and are uncorrected. Elemental analyses were done by Atlantic Microanalysis Inc., Atlanta, GA. ^{1}H NMR measurements were obtained on a Varian Associates EM-360, Perkin-Elmer R-32, or JEOL FX60Q spectrometer, and shift values are reported in δ downfield from tetramethylsilane as the internal standard. Infrared spectra were recorded on a Perkin-Elmer 521 spectrophotometer. Mass spectra were recorded on a Finnigan 3300 or Hitachi RMU-6 spectrometer. The procedures reported herein represent general synthetic operations used for preparation of the compounds in Table I.

6*H*-Indolo[2,1-*c*][1,4]benzodiazepin-12(11*H*)-one (2). A mixture of 5.69 g (0.237 mol) of sodium hydride and 200 mL of dry DMF was stirred under an atmosphere of N_2 and cooled with an ice water bath. A solution of 42 g (0.222 mol) of ethyl indole-2-carboxylate in 150 mL of dry *N,N*-dimethylformamide was added with stirring over a period of 1.5 h, and stirring was continued overnight at room temperature. The resulting solution was cooled to -65 °C with a dry ice/acetone bath, and a solution of 50 g (0.029 mol) of 2-nitrobenzyl chloride in 60 mL of dry *N,N*-dimethylformamide was added. After addition, the reaction mixture was allowed to stir at room temperature overnight and then was poured into a mixture of ice and water. A yellow solid was obtained in 64% yield by filtration and recrystallized from ethanol.

The obtained yellow solid (46 g) and 2 g of 10% Pd/C in 450 mL of methanol was hydrogenated under 50 psi of hydrogen pressure at room temperature until the uptake of hydrogen ceased. The catalyst was removed by filtration, and the filtrate was evaporated in vacuo to yield a brown solid that was combined with 300 mL of xylene and 7 g of 2-hydroxypyridine and heated at reflux with a Dean–Stark trap for 48 h. The title compound was obtained by filtration and subsequent recrystallization from 2-propanol/*N,N*-dimethylformamide to give a white solid in 84% yield: mp 270–272 °C; ^{1}H NMR ($CDCl_3/Me_2SO-d_6$) δ 5.29 (s, 2 H), 6.95–7.7 (m, 9 H), 10.25 (br, 1 H); IR (KBr) shows the carbonyl absorption peak at 1665 cm^{-1}. Anal. ($C_{16}H_{12}N_2O$) C, H, N.

6*H*-Indolo[2,1-*c*][1,4]benzodiazepine-12(11*H*)-thione (3). A mixture of 15.84 g (0.064 mol) of 6*H*-indolo[2,1-c][1,4]benzodiazepin-12(11*H*)-one (2), 7.68 g (0.032 mol) of phosphorus pentasulfide, and 150 mL of pyridine was refluxed for 4 h, cooled, and then concentrated on a rotary evaporator to obtain a residue. The residue was treated with 1 M aqueous sodium carbonate to pH 7–7.2. The resulting yellow mixture was stirred for 24 h at room temperature.[9] The title compound, a yellow solid, mp 255–260 °C dec, was obtained by filtration in 65.7% yield: MS, *m/e* 264 (M^+); ^{1}H NMR (Me_2SO-d_6) δ 3.9 (br, 1 H) 5.55 (s, 2 H), 7.1–8.1 (m, 9 H); IR (KBr) carbonyl absorption at 1612 cm^{-1}.

12-(Methylthio)-6*H*-indolo[2,1-*c*][1,4]benzodiazepine (4). To a stirred solution of 1.53 g of 6*H*-indolo[2,1-c][1,4]benzodiazepine-12(11*H*)-thione (3) in 15 mL of dioxane was added simultaneously in four portions during the course of 1 h a solution of 1.93 g of potassium hydroxide in 10 mL of methanol and 2.21 g of dimethyl sulfate in 4 mL of methanol. The mixture was stirred overnight and then concentrated on a rotary evaporator to yield a residue that was dissolved in methylene chloride and then washed with water and saturated sodium chloride solution. Removal of solvent in vacuo gave the title product, a yellow solid, mp 171–173 °C. The product was used without further purification: MS, *m/e* 278 (M^+); IR (KBr) shows a strong absorption peak at 1566 cm^{-1} arising from the C=N group; ^{1}H NMR ($CDCl_3$) δ 2.95 (s, 3 H), 5.2 (s, 2 H), 7.1–7.8 (m, 9 H). Anal. ($C_{17}H_{14}N_2S$), C, H, N, S.

12-(4-Methyl-1-piperazinyl)-6*H*-indolo[2,1-*c*][1,4]benzodiazepine (6a). Method A. A mixture of 4.45 g of 12-(methylthio)-6*H*-indolo[2,1-c][1,4]benzodiazepine (4) and 18 mL of 1-methylpiperazine was heated in a pressure bottle at 230 °C for 12 h. Excess 1-methylpiperazine was removed under reduced pressure. The residue was dissolved in methylene chloride and

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extracted with 5% hydrochloric acid solution. The aqueous solution was neutralized with 1 N sodium hydroxide solution and then extracted with methylene chloride. The extract was dried over sodium sulfate. Removal of solvent gave solid material that was recrystallized from methanol to give the title compound in 50% yield as an off-white solid: mp 179–181 °C; ^{1}H NMR ($CDCl_3$) δ 2.3 (s, 3 H), 2.45–2.55 (t, 4 H), 3.68–3.8 (t, 4 H), 5.02 (s, 2 H), 6.01 (s, 1 H), 6.7–7.6 (m, 8 H); IR ($CHCl_3$) a strong C=N absorption peak at 1582 cm^{-1}; MS, m/e 330 (M^+). Anal. ($C_{21}H_{22}N_4$) C, H, N.

12-Ethoxy-6*H*-indolo[2,1-*c*][1,4]benzodiazepine (5). A solution of 20 g (0.081 mol) of 6*H*-indolo[2,1-c][1,4]benzodiazepin-12(11*H*)-one (3) in 200 mL of methylene chloride was added to a mixture of triethyloxonium tetrafluoroborate in methylene chloride, prepared from 10 mL (0.12 mol) of epichlorohydrin and 20 mL (0.16 mol) of boron trifluoride etherate in 30 mL of anhydrous ether at room temperature. The reaction mixture was stirred at room temperature for 2 days and poured into 400 mL of ice water containing 7.5 g (0.18 mol) of sodium hydroxide. Extraction with chloroform, drying with sodium sulfate, and removal of solvent in vacuo yielded 22.0 g (98% yield) of a tan solid. Recrystallization from ethanol/chloroform yielded 18.6 g of the title compound as a white powder: mp 144 °C; ^{1}H NMR ($CDCl_3$) δ 1.47 (t, 3 H), 4.52 (q, 2 H), 5.07 (s, 2 H), 7.22 (m, 9 H); IR ($CHCl_3$) a C=N absorption at 1641 cm^{-1}. Anal. ($C_{18}H_{16}N_2O$) C, H, N.

12-(4-Methyl-1-piperazinyl)-6*H*-indolo[2,1-*c*][1,4]benzodiazepine (6a). Method B. A mixture of 10.5 g (0.038 mol) of 12-ethoxy-6*H*-indolo[2,1-c][1,4]benzodiazepine (5), 180 g (1.8 mol) of 1-methylpiperazine, and 2.0 mL of glacial acetic acid was heated at reflux temperature for 56 h. Excess amine was removed under vacuum, and the residue was purified by column chromatography (CH_2Cl_2). The obtained solid was recrystallized from EtOH to give an off-white solid (8.5 g, 68% yield) whose IR, NMR, and melting point were identical with that of the product prepared from method A.

Serotonin Release Assay. The procedure employed was as previously described by Chasin et al.[10] (1979). Briefly, rat peritoneal cells were harvested and incubated for 3 h in the presence of [^{3}H]-5-hydroxytryptophan to allow for conversion to [^{3}H]-serotonin. The labeled cells, compound 48/80, and the test compound were incubated together to determine the degree of inhibition of compound 48/80 induced serotonin release.

Passive Cutaneous Anaphylaxis (PCA) in the Rat. In this test, an IgE-mediated wheal was produced in male Sprague–Dawley rats by an intradermal injection of 0.05 mL of high-titer IgE rat antiovalbumin antiserum obtained from rats previously sensitized with ovalbumin.[11] Twenty-four hours postinjection, the rats were challenged intravenously with ovalbumin and Evans Blue dye in saline. Simultaneous intradermal injections of serotonin and histamine were made. Thirty minutes later, the animals were sacrificed and the skin was reflected for analysis of the response. Results are determined by measuring wheal sizes and scoring the color intensities relative to a standard color chart. Percent inhibition was calculated from the following formula: percent inhibition = (1 − mean experimental score/mean control score) × 100. Drugs were administered intraperitoneally 1 h prior to antigen, and the response was evaluated 30 min subsequent to antigen challenge.

Active Lung Anaphylaxis (ALA) in the Guinea Pig. Guinea pigs were sensitized to egg albumin, 1 mg s.c., by *Bordetella pertussis* vaccine (0.5 mL) as an adjuvant.[12] Two weeks after sensitization, the animals were anesthetized with sodium pentobarbital, 65 mg/kg i.p. The trachea, jugular vein, and carotid artery were cannulated for monitoring airway resistance, intravenous administration of test compounds, and measuring mean arterial blood pressure, respectively. The animals were then challenged with egg albumin, 0.5 mg/kg iv, which produced an anaphylactic reaction characterized by severe bronchoconstriction. Compounds were administered either intravenously, orally, or intraperitoneally prior to the antigen challenge, and their effect on bronchoconstriction was evaluated. Compounds were also tested by using the oral route of administration.

Registry No. 1, 99384-70-0; 2, 99384-52-8; 3, 101226-22-6; 4, 101226-23-7; 5, 101226-24-8; 6a, 101226-25-9; 6b, 101226-26-0; 6c, 101226-27-1; 6d, 101226-28-2; 6e, 101226-29-3; 6f, 101226-30-6; 6g, 101226-31-7; 6h, 101226-32-8; 2-nitrobenzyl chloride, 612-23-7; ethyl indole-2-carboxylate, 3770-50-1; 1-methylpiperazine, 109-01-3; 1-(3-hydroxypropyl)piperazine, 5317-32-8; piperazine, 110-85-0; ethyl 1-piperazinecarboxylate, 120-43-4; 1-butylpiperazine, 5610-49-1; morpholine, 110-91-8; 4-hydroxypiperidine, 5382-16-1; piperidine, 110-89-4.

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Structure–Activity Relationships for the Competitive Angiotensin Antagonist [Sarcosine^1,*O*-methyltyrosine^4]angiotensin II (Sarmesin)

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Analogues of the competitive angiotensin antagonist [Sar^1,Tyr(Me)^4]ANG II (sarmesin) in which the sarcosine-1, *O*-methyltyrosine-4, and phenylalanine-8 residues were modified have been synthesized by the solid-phase method. The agonist and antagonist potencies of the 23 peptides synthesized were determined in the rat isolated uterus assay. At position 1, replacement of Sar with Asp, Ala, or Pro gave inactive analogues, and deletion of the N-terminal amino acid produced inactive heptapeptides for all analogues investigated. At position 4, substitution of Tyr with Tyr(Et), D-Tyr, D-Phe, Ile, Thr, or Hyp resulted in inactive analogues, whereas substitution of Phe gave a potent competitive antagonist (pA_2 = 7.9), which retained significant agonist activity (22%). For position 8, [Sar^1,Tyr(Me)^4,Ile^8]ANG II and [Sar^1,Phe^4,Ile^8]ANG II were weaker antagonists (pA_2 = 6.6 and 6.7, respectively) than [Sar^1,Ile^8]ANG II (pA_2 apparent = 8.1) and, moreover, were reversible competitive antagonists. These findings demonstrate that the structural requirements for receptor blockade by sarmesin are remarkably stringent—modifications at positions 1, 4, and 8 markedly reduce the antagonist activity of this peptide.

Antagonists of angiotensin II (ANG II, Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) are traditionally analogues of ANG II with amino acid substitutions at positions 1 and 8,[1] e.g., [Sar^1,Ala^8]ANG II (saralasin), [Sar^1,Ile^8]ANG II. A pre-

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