genated Krebs-Henseleit medium containing 0.5% urethane at room temperature.

The vertebrae were dissected away from the cord and the dura removed. The dorsal and ventral roots were separated by hemisection of the spinal ganglia. The cord was hemisected sagittally, and each half was placed on a small square of disposable nappy-linear material mounted on a wire mesh grid and transferred to the superfusion block. The cord was superfused (1 mL/min) with medium, and DC and evoked potential differences between the cut end of a ventral root (usually L4 or L5) and the body of the cord were recorded. The system was earthed via a third electrode. Bipolar stainless steel hook electrodes were used to stimulate the corresponding dorsal root to evoke ventral root potentials (VRP), which were displayed after amplification, on an oscilloscope (Gould 4100) and pen recorder (Kipp and Zonen BD9). Supramaximal square wave stimuli were used at a rate of 1 pulse/60 s.

Agonists were applied to the cord in superfusion fluid in 2-mL doses, and antagonists were superfused continuously in the medium for at least 15 min before further testing of agonists. The responses to the agonists were observed as changes in the DC potential between the ventral root and the body of the cord.

Guinea Pig Ileum Assay. Guinea pigs (Dunkin-Hartley, 300–350 g) of both sexes were killed by stunning and exsanguination. The distal ileum was dissected out and suspended in a 5-mL organ bath containing Krebs solution at 37 °C and gassed with 95% oxygen–5% carbon dioxide. Agonist dose-response curves were constructed with use of a 3-min cycle allowing 1-min agonist contact time, doses being washed from the bath following application. To assess antagonist activity, agonist dose-responses curves were constructed in the presence of antagonist (1–10 μM, 15-min contact time) and the contractile responses were recorded with an isotonic tranducer connected to a Kipp and Zonen BD9 chart recorder.

Studies of Substance P Analogues on Rabbit Blood Pressure Responses. Rabbits of either sex (1.5–3.0 kg) were anesthetized with halothane. The trachea was cannulated, and cannulae were also inserted into a carotid artery and femoral vein for blood pressure recording and drug injections, respectively. Dose-response relations were constructed for iv bolus doses of substance P and substance P(6–11)-hexapeptide and also acetylcholine chloride (ACh). The hypotensive responses were more markedly apparent as reductions of diastolic blood pressure than of systolic blood pressure. Suitable agonist doses were selected for examination of the effects of the antagonist peptides, the latter being administered iv as slow infusion, over 20-min periods. The percentage changes in the maximal hypotensive responses to the agonists following antagonist doses are shown in Table IV.

Registry No. 1, 101809-76-1; 2, 101809-77-2; 3, 101809-78-3; 4, 101809-79-4; 5, 101809-80-7; 6, 101914-71-0; 7, 101809-81-8; 8, 101834-52-0; 9, 101914-72-1; 10, 101809-82-9; 11, 101809-83-0; 12, 101809-84-1; 13, 101809-85-2; 14, 101809-86-3; 15, 101809-87-4; 16, 101809-88-5; 17, 101809-89-6; 18, 101809-90-9; 19, 101809-91-0; 20, 101809-92-1; 21, 101809-93-2; 22, 101809-94-3; 23, 101809-95-4; 24, 101809-96-5; 25, 101809-97-6; 26, 101809-98-7; 27, 101809-99-8; 28, 101810-00-8; 29, 101810-01-9; 30, 101810-02-0; 31, 101810-03-1; 32, 101810-04-2; 33, 101914-73-2; 34, 76260-78-1; 35, 101810-05-3; 36, 101810-06-4; 37, 101810-07-5; 38, 101810-08-6; 39, 101810-09-7; 40, 101810-10-0; 41, 101810-11-1; 42, 101810-12-2; 43, 101810-13-3; 44, 80434-86-2; SP, 33507-63-0; SP(6–11)-hexapeptide, 51165-07-2; carbachol, 51-83-2; L-glutamic acid, 56-86-0.

Antagonists of Substance P. Further Modifications of Substance P Antagonists Obtained by Replacing either Positions 7, 9 or 7, 8 and 11 of SP with D-Amino Acid Residues

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Antagonists of SP and the C-terminal (6–11)-hexapeptide have been obtained by multiple D-amino acid substitutions in various positions of SP and by protecting the N^{α}-Arg^1 and N^{ϵ}Lys^3 amino groups with benzyloxycarbonyl groups. On the guinea pig ileum a number of these antagonized both SP and the hexapeptide. Except [N^{α}-Z-Arg^1,D-Pro^2,N^{ϵ}-Z-Lys^3,Asn^5,Arg^6,D-Phe^7,D-Trp^9]-SP-OMe (**4**) and the corresponding amide **7**, which were more potent antagonists of SP than the hexapeptide, all the others, e.g., [N^{α}-Z-Arg^1,D-Pro^{2,4},N^{ϵ}-Z-Lys^3,D-Phe^{7,8},Sar^9,D-Met^{11}]-SP-OMe (**9**), [N^{α}-Z-Arg^1,D-Pro^{2,4},N^{ϵ}-Z-Lys^3,D-Phe^{7,8},Sar^9,MeLeu^{10},D-Met^{11}]-SP-OMe (**11**), were more potent antagonists of the hexapeptide. On the rat spinal cord preparation, most of the antagonists were only active against the hexapeptide. A few antagonized SP, but these also reduced carbachol or both carbachol and glutamate responses. Two of the antagonists, [D-Pro^2,Asn^5,Lys^6,D-Phe^7,D-Trp^9]-SP-OMe (**2**) and [Boc-D-Pro^4,D-Phe^{7,8},Sar^9,D-Met^{11}]-SP(4–11)-OMe (**10**), were inactive on the ileum but still antagonized the hexapeptide on the spinal cord. The smallest peptides to antagonize SP and the hexapeptide were two heptapeptides, **6** and **21**, [Z-Asn^5,Arg^6,D-Phe^{7,8},Gly$^9\psi$(CH$_2$S)D-Leu^{10},D-Met^{11}]-SP(5–11)-OMe (**21**) being more potent than **6**. None of the antagonists showed significant analgesic activity without side effects. Some of the antagonists were shown to release histamine from isolated rat peritoneal cells.

Since the discovery of substance P (SP),[1,2] a number of physiological and pharmacological effects have been ascribed to this peptide and these have recently been reviewed.[3-5] Specific antagonists of SP are therefore needed to investigate the pharmacology of SP and also to define the role of various receptors that have recently been proposed.[6,7]

A number of SP antagonists have recently been reported.[8-11] These have been obtained by substituting

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D-amino acid residues either in the entire undecapeptide sequence or in the C-terminal octapeptide sequence of SP.

In an earlier publication we described the synthesis and biological activities of a number of SP analogues.[11] By carrying out at least two D-amino acid substitutions in the SP molecule, two types of Substance P antagonists were obtained. One of these types, e.g., [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8},D-Met^{11}]-SP-OMe and [D-Trp^{7,8,9}]-SP-OMe, antagonized SP and SP(6–11)-hexapeptide on the guinea pig ileum but only the hexapeptide on the rat spinal cord preparation. The other type of antagonists, e.g., [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Phe^{7,8},D-Met^{11}]-SP-OMe and [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Pro^{9,10}]-SP-OMe, had no effect against SP or the hexapeptide on the guinea pig ileum but were potent antagonists of the hexapeptide on the rat spinal cord preparation. Further modifications of [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Phe^{7,8},D-Met^{11}]-SP-OMe and [D-Trp^{7,8,9}]-SP-OMe reported earlier[11] and [D-Pro^2,D-Phe^7,D-Trp^9]-SP (an antagonist reported by Folkers et al.)[12] have now been attempted. The main aim of the work was to improve the potency of the antagonists and also to change the specificity in a way so that SP may be antagonized on the rat spinal cord preparation.

The two glutamine residues in positions 5 and 6 of [D-Pro^2,D-Phe^7,D-Trp^9]-SP were replaced by Asn^5,Lys^6 or Asn^5,Arg^6 residues. The Asn^5,Lys^6 and Asn^5,Arg^6 residues are present in positions 5 and 6 of physalaemin and phyllomedusin, respectively. In D-Phe^{7,8},D-Met^{11} series of compounds, positions 9 and/or 10 were substituted with Sar^9 or Sar^9 and MeLeu^{10}, or Gly$^9\psi$(CH$_2$S)Leu^{10}, or Gly$^9\psi$(CH$_2$S)D-Leu^{10} residues. D-Pro residues in positions 2 and/or 4 with Asn^5,Arg^6 residues in positions 5 and 6 were also incorporated in some of the antagonists. In D-Trp^{7,8,9} series of analogues Leu^{10} and Met^{11} residues were replaced with D-Leu^{10} and D-Met^{11} residues. Since in the earlier series of antagonists the analogues with a C-terminal methyl ester were better antagonists than the corresponding amides,[11] most of the analogues have only been prepared as methyl esters.

Synthesis

All of the analogues listed in Table I were prepared by the solid-phase method[13] with a Beckmann Model 990 automated peptide synthesizer. The protected peptides were assembled on a hydroxymethyl ester resin support by using the strategy described earlier.[11] The protected peptides were then cleaved from the resin by transesterification to give the methyl esters, which were rigorously purified by counter-current distribution or silica gel column chromatography or by gel filtration on LH-20 in dimethylformamide. The bis(benzyloxycarbonyl) groups were cleaved by trifluoroacetic acid–thioanisole treatment,[14] and the deprotected peptides were further purified by chromatography on CM-52 or Bio-Gel P-4. All of the peptides were homogeneous on TLC in several solvent systems and gave correct amino acid analysis after acid or base hydrolysis.

The Glyψ(CH$_2$S)Leu and Glyψ(CH$_2$S)D-Leu residues were prepared by modifying the procedures reported earlier.[15,16] These earlier methods for the synthesis of H-Glyψ(CH$_2$S)Leu-OH (both isomers) involved the use of a large excess (10 equiv) of aqueous sodium bicarbonate solution as the solvent in the condensation of 2-aminoethanethiol hydrochloride and (*R*)- or (*S*)-2-bromo-4-methylpentanoic acid. Thus in order to prepare these pseudodipeptides on a reasonable scale, large volumes of aqueous bicarbonate are required (typically a 25-mmol reaction requires ca. 500 mL of 0.5 M aqueous bicarbonate). Reducing the amount of aqueous bicarbonate from 10 to 3 equiv allowed for preparations on the 300-mmol scale with 1800 mL of 0.5 M sodium bicarbonate solution (method A). The overall yield, melting point, and optical rotations were in agreement between the two reaction conditions. The workup procedure included a crude six or eight separating funnel CCD with butan-1-ol–water. In this way the unreacted excess cysteamine and the bulk of the inorganic salts were removed thus making the ion-exchange chromatography step more manageable. The pseudodipeptides could also be prepared with use of aqueous DMF as solvent and triethylamine (3 equiv) as base (method B). The product from this reaction precipitated out and was washed and recrystallized to acceptable purity, thus eliminating the CCD and ion-exchange procedures.

Biological Activity

The analogues listed in Table I were tested for agonist and antagonist activity on the guinea pig ileum, and the rat spinal cord preparation and the results are summarized in Table II.

The antagonist activity on the ileum was tested against SP, SP(6–11)-hexapeptide, and acetylcholine. The results in Table II are expressed as dose ratios that were calculated from the ratio of the EC_{50} (effective concentration producing 50% of the maximum response) values of the agonist in the presence of the antagonist to the EC_{50} value of the agonist alone. At least two ileum preparations were used for each compound and dose ratios of less than two were considered insignificant. The analogues did not show any agonist activity up to the dose tested and were also inactive as antagonists of acetylcholine.

The antagonist activity of the compounds was also tested against SP, SP(6–11)-hexapeptide, glutamate, and carbachol on a neonatal rat hemisected spinal cord preparation.[17] The above agonists caused depolarizations of the ventral roots, which were measured as amplitude of response and area of response. The effects of the antagonists were expressed as percentage change in response amplitude and area, but since both of these changes were similar, only results for amplitude changes are given in Table II. At least two spinal cord preparations were used for each compound, and less than 10% change in response amplitude was not considered significant.

A few antagonists were tested for their ability to release histamine from rat peritoneal mast cells by using a

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Table I. Physical and Chemical Characteristics of Substance P Analogues

compound	purification[a]	TLC[b] R_f(A)	R_f(B)	R_f(C)	R_f(K)	amino acid analysis[c] (16-h acid digest) Arg	Pro	Lys	Glu	Phe	Leu	Met	Trp	Asp	Glyψ-(CH_2S)-Leu
[N^{α}-Z-Arg^1,D-Pro^2,N^{ϵ}-Z-Lys^{3,6},Asn^5,D-Phe^7,D-Trp^9]-SP-OMe (1)	CCD (A, B) (K 14 in B)	0.62	0.68	0.20	0.64	0.96	2.0	2.04		2.06	1.02	0.95	0.96	1.01	
[D-Pro^2,Asn^5,Lys^6,D-Phe^7,D-Trp^9]-SP-OMe (2)	CM-52 (0.01–0.1 M AcOH)	0.14	0.30	0.0	0.0	0.97	2.01	2.08		1.95	1.0	0.95	0.97	1.04	
[N^{α}-Boc-Arg^1,D-Pro^2,N^{ϵ}-Z-Lys^3,Asn^5, Arg^6,D-Phe^7,D-Trp^9]-SP-OMe (3)	CCD (A, B) (K 2.6 in B)	0.67	0.77	0.15	0.57	2.03	2.03	1.05		2.02	1.03	0.96	0.95	1.02	
[N^{α}-Z-Arg^1,D-Pro^2,N^{ϵ}-Z-Lys^3,Asn^5,Arg^6, D-Phe^7,D-Trp^9]-SP-OMe (4)	CCD (B) (K 1.16)	0.31	0.63	0.0	0.59	2.08	1.94	1.01		1.95	1.04	0.97	0.95	0.96	
[D-Pro^2,Asn^5,Arg^6,D-Phe^7,D-Trp^9]-SP-OMe (5)	Bio-Gel P-4 (0.001 M HCl)	0.14	0.37	0.0	0.08	1.97	2.0	1.02		1.96	1.04	0.98	0.96	0.97	
[Boc-Asn^5,Arg^6, D-Phe^7,D-Trp^9]-SP(5–11)-OMe (6)	silica column chromatography[d]	0.73	0.77	0.33	0.69	1.02				1.96	1.02	0.94	0.98	0.98	
[N^{α}-Z-Arg^1-D-Pro^2,N^{ϵ}-Z-Lys^3,Asn^5,Arg^6,D-Phe^7,D-Trp^9]-SP (7)	CCD (B) (K 0.68)	0.32	0.65	0.0	0.66	2.02	2.05	1.03		2.0	1.0	0.96	0.96	1.03	
[D-Pro^2,Asn^5,Arg^6,D-Phe^7,D-Trp^9]-SP (8)	CM-52 (0.1–2 M AcOH)	0.06	0.36	0.0	0.0	1.97	2.0	0.98		1.98	0.98	1.02	0.98	1.04	
[N^{α}-Z-Arg^1,D-Pro^{2,4},N^{ϵ}-Z-Lys^3,D-Phe^{7,8},Sar^9,D-Met^{11}]-SP-OMe (9)	silica column chromatography[e]	0.45	0.59	0.23	0.83	0.98	1.97	0.96	1.96	2.0	1.01	0.99			
[Boc-D-Pro^4,D-Phe^{7,8},Sar^9,D-Met^{11}]-SP-(4–11)-OMe (10)	silica column chromatography[f]	0.69	0.73	0.55	0.96		0.98		g	1.96	1.04	0.97			
[N^{α}-Z-Arg^1,D-Pro^{2,4},N^{ϵ}-Z-Lys^3,D-Phe^{7,8}, Sar^9,MeLeu^{10},D-Met^{11}]-SP-OMe (11)	CCD (C) (K 3.0)	0.46	0.67	0.20	0.82	0.96	2.02	1.01	2.04	1.95		0.95			
[D-Pro^{2,4},D-Phe^{7,8},Sar^9,MeLeu^{10},D-Met^{11}]-SP-OMe (12)	CM-52 (0.1–2 M AcOH)	0.16	0.37	0.09	0.0	0.96	2.03	0.97	2.04	2.02		0.96			
[Boc-D-Pro^4,D-Phe^{7,8},Sar^9,MeLeu^{10},D-Met^{11}]-SP-(4–11)-OMe (13)	silica column chromatography[f]	0.66	0.72	0.49	0.96		1.07		g	2.0		0.99			
[N^{α}-Z-Arg^1,D-Pro^{2,4},N^{ϵ}-Z-Lys^3,D-Phe^{7,8}, Gly$^9\psi$(CH_2S)Leu^{10},D-Met^{11}]-S-P-OMe (14)	CCD (D)	0.43	0.64	0.23	0.73	0.97	2.02	0.96	2.06	2.01		0.96			0.98
[D-Pro^{2,4},D-Phe^{7,8},Gly$^9\psi$(CH_2S)Leu^{10},D-Met^{11}]-S-P-OMe (15)	CM-52 (0.1–2 M AcOH)	0.2	0.39	0.0		0.97	2.02	0.98	1.97	2.08		0.97			0.93
[Boc-D-Pro^4,D-Phe^{7,8},Gly$^9\psi$(CH_2S)Leu^{10},D-Met^{11}-SP(4–11)-OMe (16)	CCD (E)	0.86	0.76	0.60	0.96		1.01		2.08	1.97		0.97			0.96
[N^{α}-Z-Arg^1,D-Pro^{2,4},N^{ϵ}-Z-Lys^3,D-Phe^{7,8},Gly$^9\psi$-(CH_2S)D-Leu^{10},D-Met^{11}]-SP-OMe (17)	CCD (D) (K 1.82)	0.56	0.67	0.29	0.85	0.98	2.01	1.02	2.0	1.98		0.97			0.97
[D-Pro^{2,4},D-Phe^{7,8},Gly$^9\psi$(CH_2S)D-Leu^{10},D-Met^{11}]-SP-OMe (18)	CM-52 (0.1–2 M AcOH)	0.20	0.39	0.0		0.98	2.02	0.98	1.96	2.02		0.98			0.96
[N^{α}-Z-Arg^1,D-Pro^2,N^{ϵ}-Z-Lys^3,Asn^5,Arg^6,D-Phe^{7,8},Gly$^9\psi$(CH_2S)D-Leu^{10},D-Met^{11}]-SP-OMe (19)	CCD (F) (K 2.83)	0.34	0.62	0.0	0.57	1.96	2.02	0.97		1.96		0.97		1.02	1.04
[Boc-D-Pro^4,D-Phe^{7,8},Gly$^9\psi$(CH_2S)D-Leu^{10},D-Met^{11}]-SP(4–11)-OMe (20)	CCD (G)	0.86	0.76	0.60	0.96		1.01		2.03	1.98		0.98			1.02
[Z-Asn^5,Arg^6,D-Phe^{7,8},Gly$^9\psi$(CH_2S)D-Leu^{10},D-Met^{11}]-SP(5–11)-OMe (21)	LH-20, DMF	0.51	0.62	0.76	0.55	0.97				1.95		1.03		1.03	1.01
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8},D-Leu^{10},D-Met^{11}]-SP-OMe (22)	CCD (H) (K 0.6)	0.57	0.63	0.50	0.64	0.95	2.1	0.98	2.1		0.96	0.97	1.94		
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8,9},D-Leu^{10},D-Met^{11}]-SP-OMe (23)	CCD (G) (K 0.3)	0.56	0.62	0.50	0.65	0.99	2.02	1.03	1.98		0.98	0.95	2.89		
[N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8,9},D-Leu^{10},D-Met^{11}]-SP (24)	CCD (G) (K 0.18)	0.60	0.65	0.52	0.70	0.98	2.0	1.03	1.97		1.0	0.97	2.9		

[a] Details of the counter-current systems (CCD) are described in the Experimental Section. Partition coefficient values (K) were obtained from the actual counter-current runs. [b] See Experimental Section for details of the solvent systems. [c] Trp figures were obtained from base hydrolysis. [d] Chloroform–methanol–water–acetic acid (55:40:10:1) was used for elution. [e] Methanol (5, 10%) in chloroform and chloroform–methanol–water–acetic acid (55:40:1:1) were used as eluting solvents. [f] Methanol (2, 5, 10%) in chloroform were used as eluting solvents. [g] Sarcosine and glutamic acid peaks overlapped.

Table II. Biological Activities of Substance P Analogues

	guinea pig ileum			rat spinal cord				
		antagonist activity (dose ratio)			antagonist activity (% change in amplitude response)			
compd	concn, μM	SP	SP(6–11)	concn, μM	SP	SP(6–11)	Glut	Carb
1	1	5.0	9.3	21	+17	−44	−15	
	4.5	11.4	10.8	43	0	−54		
2	5	<2	<2	10	+9	−54	+8	
	10	<2	<2					
3	1	3.7	3.1	20	+17	−85	−9	
	5	2.8	5.1					
4	5	14.0	3.2	5	−29	−73	−3	−52
	10	31.1	11.1	10	+3	−75	−1	−51
				50	−37	−85	+20	
5	5	2.2	2.2					
	10	6.0	4.0					
6	1	<2	4.4	10	+15	−56	−3	−26
	5	2.1	16.0	50	−17	−79	+11	−40
7	5	6.5	12.7	5	0	−7		
	10	66.2	46.1	50	−39	−100	−11	−30
8	5	<2	<2					
	10	<2	<2					
9	5	11.2	24.0	5	0	−70	−15	−12
	10	25.3	67.4	25	−21	−100	−24	−39
				50	−20	−100	−36	
10	5	<2	<2	25	+7	−29		
	10	<2	<2	50	−12	−57	−4	
11	5	7.7	17.5	0.5	+12	−28	+6	−20
	10	6.8	51.3	1.0	+5	−57	0	−24
				5.0	0	−71	0	−30
				10	+16	−92	−35	−58
12	5	<2	<2					
	10	2.2	2.5					
13	5	<2	<2					
	10	<2	<2					
14	5	4.5	21.6	5	−29	−71	+5	−19
	10	15.2	49.3	50	−67	−84	−5	−50
15	5	2.7	2.5					
	10	3.6	3.4					
16	5	<2	<2	5	+42	−7		
	10	<2	<2	50	−62	−80	−50	
17	0.5	2.4	4.2	0.5	+7	−13	−7	−20
	1.0	3.4	20.1	1.0	+2	−48	−12	−33
	5.0	38.0	41.0	5.0	−2	−72	−23	−46
	10.0	52.0	80.0	10.0	+10	−83	−31	−48
				50.0	−23		−25	−50
18	5	<2	3.1					
	10	<2	19.0					
19	5	18.5	54.5					
	10	20.0	68.0					
20	5	<2	3.6	5	+46	−28		
	10	2.3	15.3	50	−54	−74	−43	
21	5	5.8	69.0					
	10	18.2	225					
22	5	15.0	34.0					
	10	17.2	37.0					
23	5	6.2	7.2					
	10	9.8	10.7					
24	5	3.9	5.9					
	10	6.9	6.9					
25	10	10.5	90	10	+19	−33	−3	−6
				25	+18	−77	−13	
				50	+8.3	−75	+11	−24

fluorimetric assay[18] and also for the analgesic activity in the mouse acetylcholine abdominal constriction test.[19,20]

Results and Discussion

(a) In Vitro Studies. The [D-Pro^2,D-Phe^7,D-Trp^9] series of compounds with further amino acid substitutions in positions 5 and 6 (1–8) are listed first in Table II followed by the [D-Phe^{7,8},D-Met^{11}] series of compounds (**9–21**). Compounds with D-Trp residues in position 7, 8 (**22**) or 7, 8, and 9 (**23, 24**) are listed at the end. The biological activities of these analogues were compared with [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Trp^{7,8},D-Met^{11}]-SP-OMe (**25**) reported earlier.[11]

In the first series of compounds (1–8) the N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3 analogues (**1, 4, 7**) antagonized SP and the hexapeptide on the guinea pig ileum. The corresponding analogues without the benzyloxycarbonyl groups (**2, 5, 8**) were either inactive or marginally active at 10 μM. In this aspect the above analogues were similar to **25** and other

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[D-Trp^{7,8},D-Met^{11}] series of compounds reported earlier.[11] The analogues containing Asn^5,Arg^6, [N^α-Z-Arg^1,D-Pro^2,N^ϵ-Z-Lys^3,Asn^5,Arg^6,D-Phe^7,D-Trp^9]-SP-OMe (**4**) and N^α-Z-Arg^1,D-Pro^2,N^ϵ-Z-Lys^3,Asn^5,Arg^6,D-Phe^7,D-Trp^9]-SP (**7**), were considerably more potent than the corresponding Asn^5,Lys^6 analogue (**1**). Compounds **4** and **7** were somewhat more potent against SP than the hexapeptide on the ileum, and in this respect both of these analogues were different from **25**, which was much more potent against the hexapeptide. On the rat spinal cord preparation, compounds **1**, **4**, and **7** like compound **25** did antagonize SP(6–11)-hexapeptide. The responses to glutamate were not affected. Unlike [N^α-Z-Arg^1,N^ϵ-Z-Lys^3,D-Trp^{7,8},D-Met^{11}]-SP-OMe, compounds **4** and **7** also antagonized SP (~40% reduction in response amplitude at 50 μM), but at the same concentration both these compounds also affected carbachol to a similar extent (30–50% reduction in response amplitude).

The C-terminal heptapeptide analogue [Boc-Asn^5,Arg^6,D-Phe^7,D-Trp^9]-SP(5–11)-OMe (**6**) antagonized primarily the hexapeptide on the ileum and the spinal cord.

In the D-Phe^{7,8},D-Met^{11} series of compounds (**9–21**), again the bis(benzyloxycarbonyl) compounds [N^α-Z-Arg^1,D-Pro^{2,4},N^ϵ-Z-Lys^3,D-Phe^{7,8},Sar^9,MeLeu^{10},D-Met^{11}]-SP-OMe (**11**), [N^α-Z-Arg^1,D-Pro^{2,4},N^ϵ-Z-Lys^3,D-Phe^{7,8},Gly$^9\psi$(CH$_2$S)-Leu^{10},D-Met^{11}]-SP-OMe (**14**), and [N^α-Z-Arg^1,D-Pro^{2,4},N^ϵ-Z-Lys^3,D-Phe^{7,8},Gly$^9\psi$(CH$_2$S)D-Leu^{10},D-Met^{11}]-SP-OMe (**17**) were much more potent than the corresponding compounds without the benzyloxycarbonyl groups (**12**, **15**, and **18**). Compounds **11**, **14**, and **17** were potent antagonists of SP and the hexapeptide on the ileum whereas compound **12** was inactive at 10 μM, **15** was marginally active, and **18** was only a moderate antagonist of the hexapeptide. As antagonists of SP and the hexapeptide compounds, **11**, **14**, and **17** were similar to **25** except that **17** was much more potent against SP than compound **25** (dose ratio 52 and 10.5, respectively, at 10 μM). On the rat spinal cord preparation the three analogues (**11**, **17**, and **25**) were without any significant effect against SP and were antagonists of the hexapeptide. Only compounds **11** and **17** were more potent (92% and 83% reduction in response amplitude, respectively, at 10 μM) than **25** (33% reduction in response amplitude at 10 μM and 75% at 50 μM), but **25** had no significant effect against glutamate and carbachol whereas **11** and **17** affected glutamate and carbachol at 10 μM. Up to 5 μM, **11** did not affect glutamate. [N^α-Z-Arg^1,D-Pro^{2,4},N^ϵ-Z-Lys^3,D-Phe^{7,8},Gly$^9\psi$(CH$_2$S)-Leu^{10},D-Met^{11}]-SP-OMe (**14**) behaved somewhat differently from compounds **11**, **17**, and **25**. It antagonized both SP and the hexapeptide on the spinal cord preparation (29% and 67% reduction in SP response amplitude at 5 and 50 μM, respectively) without affecting glutamate, but responses to carbachol were depressed (19% and 50% at 5 and 50 μM, respectively).

The C-terminal (4–11)-octapeptide fragments, [Boc-D-Pro^4,D-Phe^{7,8},Sar^9,D-Met^{11}]-SP(4–11)-OMe (**10**) and [Boc-D-Pro^4,D-Phe^{7,8},Sar^9,MeLeu^{10},D-Met^{11}]-SP(4–11)-OMe (**13**) were inactive on guinea pig ileum but [Boc-D-Pro^4,D-Phe^{7,8},Gly$^9\psi$(CH$_2$S)D-Leu^{10},D-Met^{11}]-SP(4–11)-OMe (**20**) showed some antagonist activity against the hexapeptide. The C-terminal heptapeptide derivative [Z-Asn^5,Arg^6,D-Phe^{7,8},Gly$^9\psi$(CH$_2$S)D-Leu^{10},D-Met^{11}]-SP(5–11)-OMe (**21**) was considerably more potent (dose ratio 18.2 and 225 against SP and the hexapeptide, respectively, at 10 μM). The heptapeptides **6** and **21** are the smallest peptide antagonists obtained from this series of compounds, **21** being much more potent than **6**.

The D-Trp-containing antagonists, [N^α-Z-Arg^1,N^ϵ-Z-Lys^3,D-Trp^{7,8},D-Leu^{10},D-Met^{11}]-SP-OMe (**22**), [N^α-Z-Arg^1,N^ϵ-Z-Lys^3,D-Trp^{7,8,9},D-Leu^{10},D-Met^{11}]-SP-OMe (**23**), and N^α-Z-Arg^1,N^ϵ-Z-Lys^3,D-Trp^{7,8,9},D-Leu^{10},D-Met^{11}]-SP (**24**), were only tested on the guinea pig ileum. Compound **22** was moderately active (dose ratio 17.2 and 37.0 against SP and the hexapeptide, respectively), but **23** and **24** were much less potent.

When the antagonist activity of [N^α-Z-Arg^1,N^ϵ-Z-Lys^3,D-Trp^{7,8},D-Met^{11}]-SP-OMe (**25**) on the guinea pig ileum (dose ratio 10.5 against SP and 90 against the hexapeptide) is compared with the other antagonists reported here, the following points emerge.

(a) The majority of the above antagonists have a profile similar to compound **25** in that the analogues are more potent against the hexapeptide. A number of such antagonists, e.g., [N^α-Z-Arg^1,D-Pro^{2,4},N^ϵ-Z-Lys^3,D-Phe^{7,8},Sar^9,D-Met^{11}]-SP-OMe (**9**), [N^α-Z-Arg^1,D-Pro^{2,4},N^ϵ-Z-Lys^3,D-Phe^{7,8},Gly$^9\psi$(CH$_2$S)D-Leu^{10},D-Met^{11}]-SP-OMe (**17**), are almost equipotent to **25** as antagonists of the hexapeptide. The other antagonists of this type, e.g., N^α-Z-Arg^1,D-Pro^{2,4},N^ϵ-Z-Lys^3,D-Phe^{7,8},Sar^9,MeLeu^{10},D-Met^{11}]-SP-OMe (**11**), [N^α-Z-Arg^1,D-Pro^{2,4},N^ϵ-Z-Lys^3,D-Phe^{7,8},Gly$^9\psi$(CH$_2$S)Leu^{10},D-Met^{11}]-SP-OMe (**14**), and [N^α-Z-Arg^1,N^ϵ-Z-Lys^3,D-Trp^{7,8},D-Leu^{10},D-Met^{11}]-SP-OMe (**22**), were somewhat less potent. A comparison of compounds **9** with **11** and **17** with **14** suggests that replacement of the Leu^{10} residue with MeLeu and Gly$^9\psi$(CH$_2$S)D-Leu^{10} residue with Gly$^9\psi$(CH$_2$S)Leu^{10} residues results in reduced potency.

(b) Two of the antagonists, [N^α-Z-Arg^1,D-Pro^2,N^ϵ-Z-Lys^3,Asn^5,Arg^6,D-Phe^7,D-Trp^9]-SP-OMe (**4**) and [N^α-Z-Arg^1,D-Pro^2,N^ϵ-Z-Lys^3,Asn^5,Arg^6,D-Phe^7,D-Trp^9]-SP (**7**), have a profile different from **25**. Compounds **4** and **7** were less potent antagonists (dose ratio 11.1 and 46.1, respectively, at 10 μM) than compound **25** (dose ratio 90 at 10 μM) against the hexapeptide, but both of these analogues were much more potent antagonists of SP (dose ratio 31.1 and 66.2 at 10 μM) compared to **25** (dose ratio 10.5 at 10 μM). This change in profile that appears to be due to the Asn^5,Arg^6 substitutions can also be seen in another weak antagonist, [D-Pro^2,Asn^5,Arg^6,D-Phe^7,D-Trp^9]-SP-OMe (**5**), but not in [N^α-Boc-Arg^1,D-Pro^2,N^ϵ-Z-Lys^3,Asn^5,Arg^6,D-Phe^7,D-Trp^9]-SP-OMe (**3**).

A comparison of the antagonist profile of **25** with other antagonists on the rat spinal cord preparation also shows some interesting differences.

(a) Some of the antagonists, e.g., [N^α-Z-Arg^1,D-Pro^2,N^ϵ-Z-Lys^{3,6},Asn^5,D-Phe^7,D-Trp^9]-SP-OMe (**1**), [N^α-Boc-Arg^1,D-Pro^2,N^ϵ-Z-Lys^3,Asn^5,Arg^6,D-Phe^7,D-Trp^9]-SP-OMe (**3**), [N^α-Z-Arg^1,D-Pro^{2,4},N^ϵ-Z-Lys^3,D-Phe^{7,8},Sar^9,MeLeu^{10},D-Met^{11}]-SP-OMe (**11**), have a biological profile similar to that of **25**, i.e., they all antagonize SP and the hexapeptide on the guinea pig ileum and only the hexapeptide on the rat spinal cord. Responses to SP on the spinal cord were potentiated.

(b) Unlike **25**, [D-Pro^2,Asn^5,Lys^6,D-Phe^7,D-Trp^9]-SP-OMe (**2**) and [Boc-D-Pro^4,D-Phe^{7,8},Sar^9,D-Met^{11}]-SP(4–11)-OMe (**10**) were inactive on the guinea pig ileum, but both of these antagonized the hexapeptide on the spinal cord. In this respect, **2** and **10** were similar to [N^α-Z-Arg^1,N^ϵ-Z-Lys^3,D-Pro^{9,10}]-SP-OMe and [N^α-Z-Arg^1,N^ϵ-Z-Lys^3,D-Phe^{7,8},D-Met^{11}]-SP-OMe reported earlier[11] but less potent. Compound **2** showed 54% reduction in response amplitude at 10 μM and compound **10** showed 57% reduction in response amplitude at 50 μM, and in comparison [N^α-Z-Arg^1,N^ϵ-Z-Lys^3,D-Pro^{9,10}]-SP-OMe gave 67% reduction in response amplitude at 5 μM and completely abolished the

Table III. Histamine-Releasing Activity of the SP Antagonists

compd	histamine release: IC_{50}, M	compd	histamine release: IC_{50}, M
1	$>5 \times 10^{-4}$	11	1.2×10^{-5}
4	6×10^{-7}	14	3.3×10^{-5}
6	5×10^{-4}	17	2.5×10^{-5}
7	5×10^{-7}	[D-Pro^2,D-Trp^{7,9}]-SP	4.0×10^{-6}
9	5.5×10^{-5}		

hexapeptide responses at 50 μM.

(c) A number of antagonists, e.g., [N^{α}-Z-Arg^1,D-Pro^2,N^{ϵ}-Z-Lys^3,Asn^5,Arg^6,D-Phe^7,D-Trp^9]-SP-OMe (**4**), [N^{α}-Z-Arg^1,D-Pro^2,N^{ϵ}-Z-Lys^3,Asn^5,Arg^6,D-Phe^7,D-Trp^9]-SP (**7**), [N^{α}-Z-Arg^1,D-Pro^{2,4},N^{ϵ}-Z-Lys^3,D-Phe^{7,8},Sar^9,D-Met^{11}]-SP-OMe (**9**), [N^{α}-Z-Arg^1,D-Pro^{2,4},N^{ϵ}-Z-Lys^3,D-Phe^{7,8},Gly$^9\psi$(CH$_2$S)Leu^{10},D-Met^{11}]-SP-OMe (**14**), [Boc-D-Pro^4,D-Phe^{7,8},Gly$^9\psi$(CH$_2$S)Leu^{10},D-Met^{11}]-SP(4-11)-OMe (**16**), and [Boc-D-Pro^4,D-Phe^{7,8},Gly$^9\psi$(CH$_2$S)D-Leu^{10},D-Met^{11}]-SP(4–11)-OMe (**20**), like compound **25** antagonized SP and the hexapeptide on the ileum, but unlike **25** all these compounds also antagonized SP on the spinal cord. Compounds **4**, **7**, and **14** antagonized SP and the hexapeptide without any significant effect on glutamate, but responses to carbachol were depressed. Analogues **9**, **16**, and **20** also depressed glutamate response.

(d) Compounds **4** and **7**, which were more potent as antagonists of SP than the hexapeptide on the guinea pig ileum, were more potent against the hexapeptide on the spinal cord. Both **4** and **7** antagonized SP on the spinal cord without affecting glutamate responses although carbachol responses were depressed. None of the analogues mentioned above antagonized SP on the spinal cord without affecting either carbachol or both carbachol and glutamate.

Histamine Release. SP and its shorter fragments have been shown to be involved in histamine release,[21,22] and recently one of the SP antagonists, [D-Pro^4,D-Trp^{7,9,10}]-SP(4-11)-octapeptide, has also been shown to release histamine above 10 μM concentration.[23] This histamine-releasing property of the SP antagonists may be undesirable if such compounds were to be used as drugs. We have, therefore, studied the effects of some of the antagonists on histamine release from isolated rat peritoneal mast cells using the procedure of Fretwell et al.[18] A range of antagonist concentrations were used to produce dose–response curves. EC_{50} values were calculated from these, and the results are summarized in Table III. All of the undecapeptide analogues in which the amino groups were protected with benzyloxycarbonyl groups and only the guanidino function of Arg^1 was free (**1**, **9**, **11**, **14**, and **17**) were only weakly active ($IC_{50} > 5 \times 10^{-4}$ to 1.2×10^{-5}). The heptapeptide analogue (**6**) with a free guanidino function was also weakly active, but two other antagonists, **4** and **7**, which had two unprotected guanidino groups, were much more potent ($IC_{50} \sim 5 \times 10^{-7}$). [D-Pro^2,D-Trp^{7,9}]-SP, which had the α-amino and the guanidino groups of Arg^1 unprotected, was also a potent releaser of histamine.

Analgesic Activity. A number of antagonists reported here were tested for analgesic activity in the acetylcholine-induced abdominal constriction test. The compounds were dissolved in cremophor/dimethyl sulfoxide, diluted with 0.9% NaCl, and administered intravenously to groups of 10 mice at a dose of 5–20 mg/kg. Four minutes later rotorod coordination was tested for 1 min, followed immediately by an injection of acetylcholine (2.5 mg/kg ip). No convincing analgesia was observed. Although inhibition of writhing (50–100%) was observed with several of the compounds, behavioral depression or loss of coordination was always associated with it. Paralysis of the hind limbs was also seen in a number of animals. No correlation between side effects and histamine release was observed.

Conclusions

The three antagonists of SP and the hexapeptide, [D-Pro^2,D-Phe^7,D-Trp^9]-SP-OMe, [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Phe^{7,8},D-Met^{11}]-SP-OMe, and [D-Trp^{7,8,9}]-SP-OMe, can be modified by various modifications in a number of other positions with retention of antagonist activity. The potency and selectivity in a number of these antagonists were significantly altered by benzyloxycarbonyl groups in positions 1 and 3 and Asn^5-Arg^6, Sar,^{9} Sar^9-MeLeu^{10}, Gly$^9\psi$(CH$_2$S)Leu^{10}, and Gly$^9\psi$(CH$_2$S)D-Leu^{10} replacements.

The benzyloxycarbonyl groups on N$^{\alpha}$-amino group of Arg^1 and N^{ϵ}-amino group of Lys^3 appear to be very important for the antagonist potency in the D-Phe^7,D-Trp^9 and D-Phe^{7,8}D-Met^{11} series of compounds. All of the analogues with benzyloxycarbonyl groups were potent antagonists of SP and the hexapeptide, and in comparison the compounds without these groups were either inactive or only marginally active. Compound **3** with only one benzyloxycarbonyl group on the N^{ϵ}-amino group of Lys^3 and a *tert*-butyloxycarbonyl group on the N^{α}-amino group of Arg^1 was much less active than the corresponding bis-(benzyloxycarbonyl) compound **4**.

The replacement of the two glutamine residues in positions 5 and 6 by Asn^5-Arg^6 residues along with the benzyloxycarbonyl groups in positions 1 and 3 not only increased the potency of these analogues but also changed the antagonist profile. Two such analogues, **4** and **7**, were more potent against SP than the hexapeptide on the ileum. In heptapeptide analogues, **6** and **21**, this change in profile was not seen; both of these were much more potent against the hexapeptide. Compounds **4** and **7** also showed antagonist activity against SP on the spinal cord. It would, therefore, appear that other substituents in positions 1–4 were also very important along with positions 5 and 6 for the increased potency against SP in undecapeptide analogues.

In the D-Phe^{7,8},D-Met^{11} series of compounds, positions 9 and 10 appear to be very important for potency and selectivity along with positions 7 and 8. [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Phe^{7,8},D-Met^{11}]-SP-OMe was earlier shown to be inactive on the guinea pig ileum against SP and the hexapeptide but was a potent antagonist of the hexapeptide (78% reduction in response amplitude at 33.5 μM) on the spinal cord. The corresponding D-Trp^{7,8} analogue (**25**) was active on the ileum and the spinal cord. When the Gly^9 residue alone or Gly^9-Leu^{10} residues together are replaced with Sar^9 (**9**), Sar^9-MeLeu^{10} (**11**), Gly$^9\psi$(CH$_2$S)Leu^{10} (**14**), or Gly$^9\psi$(CH$_2$S)D-Leu^{10} (**17**), all of the analogues, unlike [N^{α}-Z-Arg^1,N^{ϵ}-Z-Lys^3,D-Phe^{7,8},D-Met^{11}]-SP-OMe, had antagonist activity against SP and the hexapeptide on the ileum. Although Sar^9 (**9**) and Sar^9-MeLeu^{10} (**11**) analogues showed very little effect against SP on the spinal cord, compound **14** antagonized SP as well. The effects seen in position 9 and 10 modified analogues may also be partly due to D-proline substitution in positions 2 and 4 since compounds **9**, **11**, **14**, and **17** all have D-Pro^{2,4} residues. For strict comparison [N^{α}-Z-Arg^1,D-Pro^{2,4},N^{ϵ}-Z-Lys^3,D-Phe^{7,8},D-Met^{11}]-SP-OMe would have to be made and tested.

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The effect of changing Leu[10] residue on antagonist activity can also be seen in D-Trp[7,8,9] series of compounds. [N^{α}-Z-Arg[1],N^{ϵ}-Z-Lys[3],D-Trp[7,8,9]]-SP-OMe and the corresponding amide were inactive on the ileum, but the D-Leu[10] analogues (**23**, **24**) show antagonist activity against SP and the hexapeptide on the ileum.

From the above results, substitutions in a number of positions of the antagonists appear to be making a substantial contribution to the potency and selectivity of these antagonists, but since the results have been derived from three series of compounds, it is not possible to generalize these findings at present.

Although no convincing analgesic activity was seen in any of the analogues, it may be due to the side effects associated with these compounds. It is possible that the side effects are a combination of histamine release and the effects of these antagonists against glutamate- and carbachol-sensitive systems. Until more potent and selective antagonists of SP are available, it would be difficult to assess the role of SP in pain or other physiological systems.

Experimental Section

Symbols and abbreviations used follow the IUPAC–IUB recommendations.[24] As eight compounds reported in this paper have a backbone-CONH-replaced by -CH_2S-, attention is drawn to the current nomenclature for this change involving the use of the prefix ψ (for pseudo). Thus Glyψ(CH_2S)Leu indicates that in the dipeptide the normal -CONH- amide bond has been replaced by -CH_2S-, generating a pseudodipeptide. Other abbreviations used and data on TLC systems are as in the previous paper.[11] Systems used for CCD purifications referred to in Table I are as follows: A, methanol–acetic acid–water–ethyl acetate (2.5:1:5:8, v/v); B, butan-1-ol–acetic acid–water–ethyl acetate–cyclohexane (3:1:5:3:3, v/v); C, propan-2-ol–acetic acid–water–ethyl acetate (2.5:1:6:6, v/v); D, methanol–acetic acid–water–chloroform–carbon tetrachloride (8:1:2:7:6, v/v); E, methanol–water–chloroform–carbon tetrachloride (3:1:3:1, v/v); F, methanol–acetic acid–water–chloroform–carbon tetrachloride (5:1:4:8:2, v/v); G, methanol–water–chloroform–carbon tetrachloride (8:2:5:5, v/v); H, ethanol–acetic acid–water–ethyl acetate–petrol ether (60–80 °C) (2.5:1:5:8:2, v/v); J, methanol–water–ethyl acetate–cyclohexane (3:5:8:1, v/v).

Peptide Synthesis. All analogues were prepared on the hydroxymethyl Merrifield resin (1% cross-linked) by the previously described solid-phase protocol with a Beckman 990 automated peptide synthesizer.[11] Following the format of the previous paper, synthetic details are given for the assembly of a protected peptide resin, for the transesterification reaction, and for the removal of the two Z groups. Also included are the synthetic details relating to the two pseudodipeptides and their Boc derivatives used in this study.

Preparation of Z-Arg-D-Pro-Lys(Z)-D-Pro-Gln-Gln-D-Phe-D-Phe-Glyψ(CH_2S)D-Leu-D-Met-resin. Boc-D-Met-Resin (0.28 mmol of Met/g of resin, 2.5 g, 0.7 mmol) was placed in the Teflon reaction vessel of the synthesizer. Deblocking, neutralization, and coupling reactions were carried out following the previously described protocol.[11] The N^{ϵ}-amino group of lysine was protected by the Z group, and arginine was introduced as Z-Arg(HCl)-OH. The fully extended peptide resin was washed with propan-2-ol and methanol and dried in a vacuum oven at 40 °C (3.4 g).

Preparation of Z-Arg-D-Pro-Lys(Z)-D-Pro-Gln-Gln-D-Phe-D-Phe-Glyψ(CH_2S)D-Leu-D-Met-OMe (17). The protected peptide resin obtained above (3.4 g) was suspended in freshly distilled DMF (50 mL)–MeOH (50 mL). Triethylamine (10 mL) was added, and the suspension was gently stirred at room temperature overnight. The insoluble resin was removed by filtration and washed with warm (50 °C) DMF (2 × 25 mL) and MeOH (1 × 30 mL). The combined filtrate and washings were evaporated, and the crude compound was purified by CCD with use of system D (723 mg, 63%).

Preparation of H-Arg-D-Pro-Lys-D-Pro-Gln-Gln-D-Phe-D-Phe-Glyψ(CH_2S)D-Leu-D-Met-OMe (18). The di-Z peptide ester (**17**) from above (478 mg, 0.29 mmol) was deprotected by a TFA (10 mL)–thioanisole (4 mL)[14] treatment as described previously.[11] The crude product was converted to acetate salt by passing through a column of Bio-Rad AG1-X2 resin (acetate form, 50 mL) and then purified by chromatography on Whatman CM-52 resin with a gradient of 2 M AcOH (400 mL) vs. 0.1 M AcOH (400 mL) (359 mg, 90%).

Preparation of the Pseudodipeptides. Method A. H-Glyψ(CH_2S)D-Leu-OH. (*S*)-(−)-2-bromo-4-methylpentanoic acid [58.5 g, 300 mmol; $[\alpha]^{22}_D$ −38.9° (*c* 2, MeOH)] was dissolved in 0.5 M sodium bicarbonate (1800 mL, 900 mmol), which had been nitrogen purged for 30 min. Still under nitrogen purge, 2-aminoethanethiol hydrochloride (102.2 g, 900 mmol) was added and the reaction solution stirred for 1 h and then stoppered and left for 2 days. The solution was acidified with 6 N HCl and then concentrated to about 750 mL. Butan-1-ol (750 mL) was added, and after partitioning, the aqueous phase was transferred in a counter-current fashion through eight separating funnels, each containing butan-1-ol (500 mL) saturated with water. The organic phases were washed eight times with water (200 mL) saturated with butan-1-ol. In this CCD system the inorganic salts and excess unreacted 2-aminoethanethiol were retained in the aqueous phases while the product partitioned into the organic phases with a slight tail-back into the aqueous phases. The combined organic phases were evaporated to an oil, which was dissolved in water (750 mL) and neutralized by the addition of 2 N NaOH before it was passed down a column of Bio-Rad AG1-X2 resin (hydroxide form; 360 mL) in two batches. The column was washed with water and the product was eluted with a gradient of 10% AcOH (1 L) vs. water (1 L). The products from both columns were combined and recrystallized from aqueous ethanol: mp 207–211 °C; $[\alpha]^{23}_D$ + 22.62° (*c* 1.5, H_2O); R_f(A) 0.45, R_f(B) 0.5, R_f(K) 0.44 (25.8 g, 45%) [lit.[15] mp 205–210 °C; $[\alpha]^{22}_D$ +24.1° (*c* 2, H_2O)]. Anal. ($C_8H_{17}NO_2S$) C, H, N, S.

Method B. H-Glyψ(CH_2S)Leu-OH. 2-Aminoethanethiol hydrochloride (43.69 g, 385 mmol) in water (50 mL) was purged with nitrogen for 30 min and then added to an ice-cold solution of (*R*)-(+)-2 bromo-4-methylpentanoic acid [25 g, 128 mmol; $[\alpha]^{22}_D$ +39.6° (*c* 1, MeOH)] in DMF (200 mL) also under nitrogen purge. Triethylamine (53.9 mL, 385 mmol) in DMF (40 mL) was added to the chilled reaction solution, and stirring under nitrogen was continued for 30 min. Solid started to separate, which after about 1.5 h became a solid mass. This solid was collected by filtration (slow) and washed with cold ethanol and then with ether. The product was recrystallized from methanol–propan-2-ol: mp 207–209 °C; $[\alpha]^{22}_D$ −23.9° (*c* 1, H_2O), R_f(A) 0.46, R_f(B) 0.5, R_f(K) 0.44 (10.6 g, 43%) [lit.[15] mp 205–210 °C; $[\alpha]^{22}_D$ −23.2° (*c* 2, H_2O)]. Anal. ($C_8H_{17}NO_2S$) C, H, N, S.

Preparation of Boc-Glyψ(CH_2S)Leu-OH. Di-*tert*-butyl dicarbonate (Fluka AG; 5.0 g, 22.7 mmol) in 5% aqueous *tert*-butyl alcohol (5 mL) was added dropwise to a stirred solution of H-Glyψ(CH_2S)Leu-OH (3.41 g, 17.8 mmol) in 2 N NaOH (8.9 mL, 17.8 mmol) and *tert*-butyl alcohol (25 mL) at 50 °C. The reaction was stirred at 50 °C for a further 2 h and then allowed to stand overnight at room temperature. The Boc derivative was then isolated as a dicyclohexylamine salt by the procedure commonly used for the synthesis of such compounds (8.0 g, 95%), mp 145–147 °C. Anal. ($C_{25}H_{48}N_2O_4S$) C, H, N, S.

Preparation of Boc-Glyψ(CH_2S)D-Leu-OH. This was prepared exactly as the L isomer above and likewise converted to the dicyclohexylamine salt, mp 145–147 °C (18.3 g, 90%). Anal. ($C_{25}H_{48}N_2O_4S$) C, H, N, S.

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Registry No. **1**, 101835-61-4; **2**, 101835-62-5; **3**, 101835-63-6; **4**, 101835-64-7; **5**, 101835-65-8; **6**, 101835-66-9; **7**, 101835-67-0; **8**, 101835-68-1; **9**, 101835-69-2; **10**, 101835-70-5; **11**, 101835-71-6; **12**, 101858-57-5; **13**, 101835-72-7; **14**, 101835-73-8; **15**, 101835-74-9; **16**, 101835-75-0; **17**, 101915-11-1; **18**, 101915-12-2; **19**, 101835-76-1;

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20, 101915-13-3; **21**, 101835-77-2; **22**, 101835-78-3; **23**, 101835-79-4; **24**, 101835-80-7; **25**, 33507-63-0; [D-Pro^2, D-Trp^{7,9}]-SP, 80434-86-2; SP(6–11)-hexapeptide, 51165-07-2; (S)-(−)-*i*-PrCH$_2$CH(Br)CO$_2$H, 28659-87-2; (*R*)-(+)-*i*-PrCH$_2$CH(Br)CO$_2$H, 429890-28-3; H-Glyψ(CH$_2$S)-D-Leu-OH, 66386-09-2; H-Glyψ(CH$_2$S)Leu-OH, 61844-81-3; Boc-Glyψ(CH$_2$S)Leu-OH, 101835-81-8; Boc-Glyψ(CH$_2$S)-D-Leu-OH, 101835-83-0; carbachol, 51-83-2; L-glutamate, 56-86-0; histamine, 51-45-6; 2-aminoethanethiol, 60-23-1.

Synthesis of 2-(4-Substituted-1-piperazinyl)benzimidazoles as H_1-Antihistaminic Agents

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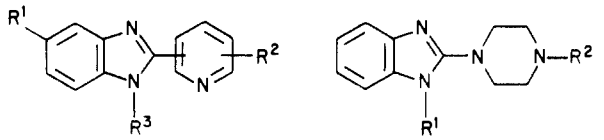
A series of 2-(4-substituted-1-(homo)piperazinyl)benzimidazoles was prepared and tested for H_1-antihistaminic activity in vitro and in vivo. Most of the compounds showed antihistaminic activity and some of the 1-[2-(substituted-oxy)ethyl] derivatives exhibited potent activity. In a structure–activity comparison it was found that the oxygen atom in the 2-(substituted-oxy)ethyl group at the 1-position of the benzimidazole nucleus played an important role for potent antihistaminic activity, especially in vivo. One of the most potent compounds, 1-(2-ethoxyethyl)-2-(4-methyl-1-homopiperazinyl)benzimidazole (**69**), was 39 times more potent than chlorpheniramine maleate in H_1-antihistaminic activity in vivo and was selected for clinical evaluation. The structure of compound **69** is of interest because it provides only a single aromatic unit linked through a chain to a basic nitrogen, while most H_1-antihistaminic agents have structures that comprise a double-aromatic unit linked through a chain to a basic tertiary amino group.

In the previous paper[1] from our laboratory, we reported the synthesis and biological evaluation of 2-(substituted-pyridinyl)benzimidazoles (**1**) as a novel type of nonacidic antiinflammatory agent. In continuing our study we found a patent[2] concerning the synthesis of 2-(4-substituted-1-piperazinyl)benzimidazole derivatives (**2**), some of which possessed antiinflammatory and analgesic activity.



1 **2**

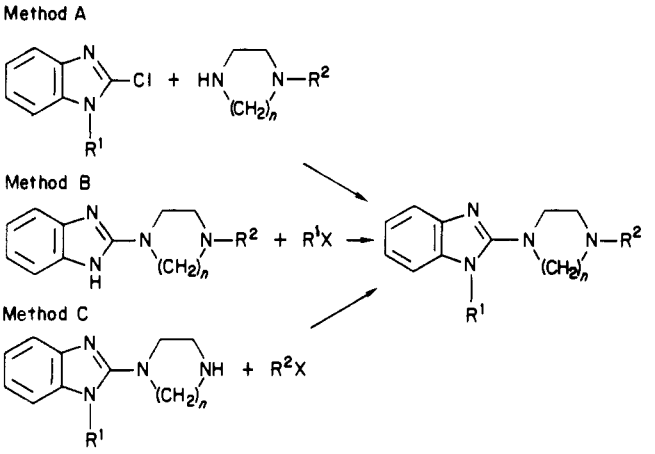
The similarity of structures and pharmacological activities between compounds **1** and **2** urged us to evaluate pharmacological profiles of compounds **2** and compare them with ours. To our surprise, results of our pharmacological screening tests revealed that some of the compounds possessed potent H_1-antihistaminic activity in addition to antiinflammatory activity.

We were interested in the combination of the structure and H_1-antihistaminic activity of compounds **2** and planned to synthesize the derivatives in order to investigate a new type of H_1-antihistaminic agent; the structure of compounds **2** was appreciably different from that of typical H_1-antihistaminic agents, and only a few derivatives were mentioned in the patent.

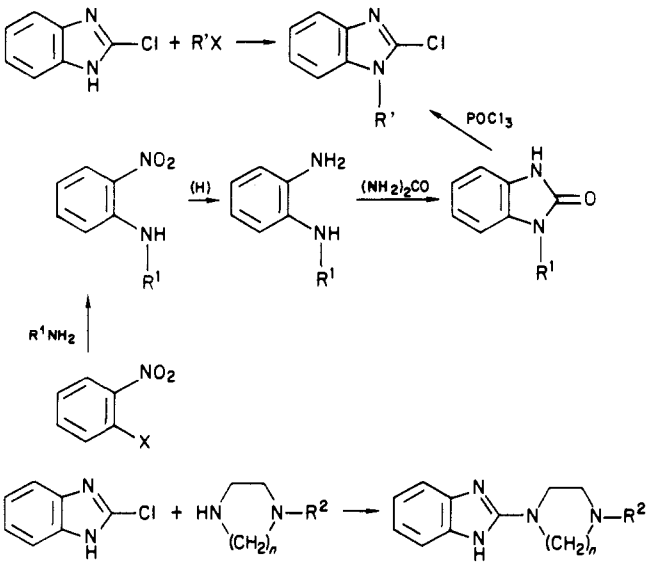
In this paper we report the synthesis of 2-(4-substituted-1-(homo)piperazinyl)benzimidazoles and the results of their pharmacological screening tests for H_1-antihistaminic activity in guinea pigs. Structure–activity relationships are also discussed.

Chemistry. Test compounds (Table II) were for the most part prepared by methods A, B, or C, as shown in Scheme I. In method A, 1-substituted-2-chlorobenzimidazoles were treated with appropriate (homo)piperazine derivatives to give the desired compounds. In method B,

Scheme I



Scheme II



2-(4-substituted-1-(homo)piperazinyl)benzimidazoles were alkylated at the 1-position of the benzimidazole nucleus to afford the desired compounds. And in method C, the desired compounds were prepared by alkylation at the

(1) Tsukamoto, G.; Yoshino, K.; Kohno, T.; Ohtaka, H; Kagaya, H.; Ito, K. *J. Med. Chem.* **1980**, *23*, 734.
(2) Kodama, T.; Takai, A.; Nakabayashi, M.; Watanabe, I.; Sadaki, H.; Kodama, T.; Abe, N.; Kurokawa, A. (Toyama Chemical Co.) Japan Kokai Patent 126 682, 1975; *Chem. Abstr.* **1976**, *84*, 44060h.

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