Novel Indolecarboxamidotetrazoles as Potential Antiallergy Agents[1]

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The synthesis and antiallergic potential of a series of novel indolecarboxamidotetrazoles are described. A number of compounds inhibit the release of histamine from anti-IgE-stimulated basophilic leukocytes obtained from allergic donors. Optimal inhibition is exhibited by compounds with 3-alkoxy, 5-methoxy, and 1-phenyl substituents on the indole core structure. Compound **8d** (5-methoxy-3-(1-methylethoxy)-1-phenyl-*N*-1*H*-tetrazol-5-yl-1*H*-indole-2-carboxamide; designated CI-949) is a potent inhibitor of histamine release from human basophils and from guinea pig and human chopped lung.

Histamine, leukotrienes, prostaglandins, and other biologically active mediators, produced and released from mast cells, basophils, and eosinophils, have been implicated in the pathogenesis of asthma and other allergic diseases.[2] The release of these mediators may be initiated by a variety of antigens or other immunologic triggers, which may explain the heterogeneous nature of the allergic patient population. Our lack of understanding of the relative importance of the various cell types and mediators involved has been a roadblock for the successful development of antiasthma drugs. In addition, an incomplete knowledge of the mechanisms of action of currently used drugs for asthma therapy (corticosteroids, disodium cromoglycate (DSCG), and theophylline),[3] as well as the lack of predictive preclinical in vivo models,[4] has hampered new drug development.

DSCG, initially thought to be acting clinically by stabilizing mast cells,[5] has been shown to inhibit the release of histamine only from selected mast cells (rat mast cells and, to a lesser extent, passively sensitized human lung cells).

Mediator release from other types of mast cells or basophils is not inhibited by DSCG.[6] The clinical failures[7] of more potent compounds with activity in the rat passive cutaneous anaphylaxis (PCA) test (a profile similar to that of DSCG) have raised questions about the clinical mechanism of action of DSCG.

We elected to pursue compounds that block the release of multiple mediators from human basophils and mast cells. As a measure of mediator release inhibition, compounds were tested for their ability to block histamine release from antigen-stimulated human basophils. This test model, as developed by Lichtenstein and others,[8,9] has been employed in the evaluation of potential antiallergy drugs of considerable variability in potency and efficacy.[10–14] Inhibition of histamine release from guinea pig and human chopped lung was also evaluated for selected compounds.

The antiallergic activity of a series of furo[3,2-*b*]indoles, including a description of the pharmacology of CI-922 (**1**), has been previously reported.[15–17] Structural modification of **1** led to a series of indolecarboxamidotetrazoles **2**. The synthesis and biological evaluation of these compounds are described in this paper.

Chemistry

The overall synthetic sequence for the preparation of a series of indole-2-carboxamidotetrazoles **8** is shown in Scheme I. (Substituents R_1–R_4 for **3**–**9** are listed in Table IV. All compounds with identical subscripts (a, b, c, etc.) have the same pattern of substituents.)

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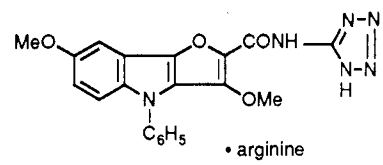
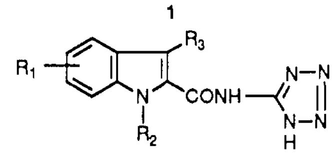


1



2

Alkoxyindole esters **5** were prepared by selective O-alkylation of enolic indole esters **3**. Similar alkylation of N-substituted enolic indole esters **4** provided the related alkoxy esters **6** (methods A and B). Esters **3a**, **3t**, **3x**, **3y**,

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Scheme I

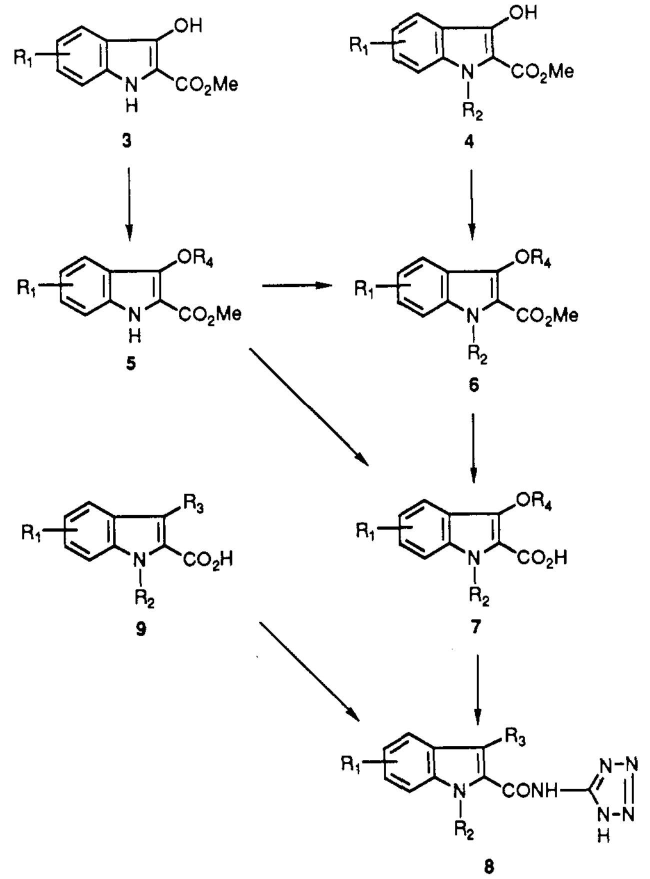
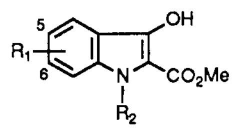


Table I. Intermediate Enolic Indole Esters



no.[a]	mp, °C	cryst solvent	formula	analysis
4n	147–150	$MeOH/H_2O$	$C_{18}H_{17}NO_5$	C, H, N
4u	146–150	Et_2O/hexane	$C_{17}H_{15}NO_4$	C, H, N
4z	170–173	2-methoxyethanol/H_2O	$C_{16}H_{12}ClNO_3$	C, H, N, Cl
4dd	153–155	2-PrOH	$C_{17}H_{15}NO_4$	C, H, N

[a] R_1 and R_2 are as defined in Table IV.

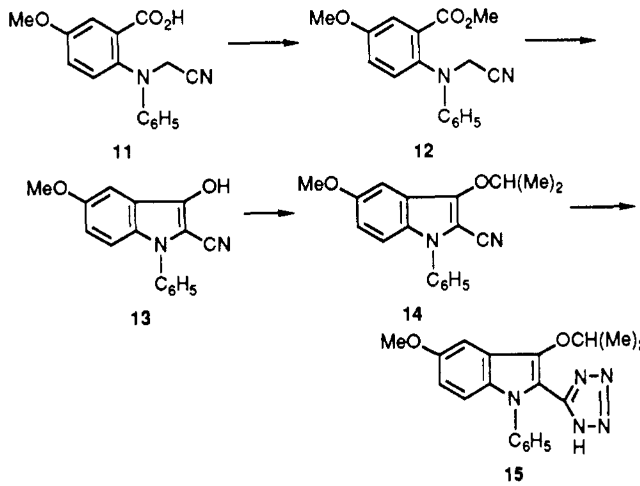
3aa, **4a**, **4q**, **4w**, and **4bb** were prepared as previously described.[18,19] Additional esters (Table I) with a 1-phenyl substituent were prepared by Friedlander's[19,20] procedure from the appropriate 2-(phenylamino)benzoic acid. Reaction of the N-unsubstituted esters **5o** and **5p** with phenylmethyl bromide yielded esters **6r** and **6s**.

Most of the esters **6** were oils or amorphous solids and were utilized crude for conversion to alkoxy acids **7** (Table II) by saponification. Esters **5** were similarly saponified (methods C, D, and E). Alkoxy carboxylic acids **7d**, **7x**, **7y**, and **7aa** and acids **9h**, **9i**, **9j**, and **9m** containing nonalkoxy substituents in the indole 3-position were prepared as previously described.[18,21] In a few cases (**7n**, **7bb**, and **9l**) an analytically pure sample of the intermediate carboxylic acid could not be obtained, and the crude product was converted directly to the (carbonylamino)tetrazole.

(Carbonylamino)tetrazoles **8** were prepared by reaction of 5-aminotetrazole and the coupling reagent 1,1′-carbonylbis(1*H*-imidazole) with carboxylic acids **7** and **9** (methods F and G). Hydroxy (carbonylamino)tetrazoles **8a** and **8v** were obtained by catalytic hydrogenolysis of the corresponding phenylmethoxy compounds **8f** and **8w**. Oxidation of methylthio derivative **8j** provided the methylsulfonyl analogue **8k**.

The preparation of indolyltetrazole **15** is shown in Scheme II. Nitrile ester **12** was obtained by esterification of nitrile carboxylic acid **11**.[19] Cyclization of **12** with base yielded the indole nitrile **13**, and O-alkylation of **13** yielded the alkoxy nitrile **14**. Conversion of **14** to the desired tetrazole **15** was effected with tri-*n*-butylstannyl azide.[22]

Scheme II



Biological Results and Discussion

Indolecarboxamidotetrazoles (Table III) were synthesized with substituent variations on the indole benzene ring (R_1) and at the indole 1- and 3-positions (R_2 and R_3, respectively). Biological activity was initially assessed by the inhibition of histamine release from human leukocytes stimulated by anti-IgE antibody.

Test results for the indolecarboxamidotetrazoles prepared are shown in Table IV. A number of compounds demonstrated potent inhibition of histamine release, and a preliminary structure–activity relationship could be discerned.

When R_1 was 5-methoxy and R_2 was phenyl (the same substituent pattern as that of the indole portion of **1**), an alkoxy substituent in the indole 3-position yielded analogues (**8b**, **8c**, **8d**, and **8f**) with marked inhibitory activity. Replacement of the 3-alkoxy substituent with hydrogen (**8h**), isopropyl (**8i**), hydroxyl (**8a**), 4-nitrophenoxy (**8g**), or methylsulfonyl (**8k**) resulted in a loss of activity. Active compounds (**8j**, **8l**, and **8m**) were also obtained when the indole 3-position oxygen was replaced by sulfur.

When R_1 and R_3 were kept constant as 5-methoxy and 1-methylethoxy, respectively, the desirability of a phenyl substituent on the indole nitrogen (R_2) was evident. The *N*-phenyl analogue (**8d**) showed greater inhibition than the *N*-H (**8p**) or *N*-methyl (**8q**) compounds.

Replacement of the 5-methoxy (R_1) substituent with R_2 held constant as phenyl and R_3 as 1-methylethoxy, in

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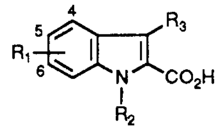
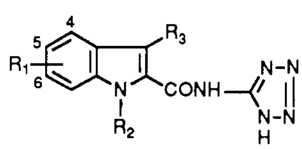
Table II. Intermediate Indolecarboxylic Acids



no.[a]	starting enol ester	alkyl.[b] method	sapon.[c] method	yield,[d] %	mp, °C	cryst solvent	formula	analysis
7b	**4a**	A	D	91	150 dec	acetone/H_2O	$C_{17}H_{15}NO_4$	C, H, N
7c	**4a**	B	D	88	130 dec	MeOH/H_2O	$C_{18}H_{17}NO_4$	C, H, N
7e[e]	**4a**	A	C[f]	36	241–242	*g*	$C_{25}H_{30}NO_4K$	C, H, N[h]
7f	**4a**	A	E	89	123 dec	acetone/H_2O	$C_{23}H_{19}NO_4$	C, H, N
7g	**4a**	*i*	E	30	230 dec	2-methoxyethanol/H_2O	$C_{22}H_{16}N_2O_6$	C, H, N
7o	**3a**	A	E	85	147–150	*g*	$C_{12}H_{13}NO_4$	C, H, N
7p	**3a**	B	E	53	130 dec	EtOH	$C_{13}H_{15}NO_4$	H, N; C[j]
7q	**4q**	B	D	84	110–112	2-methoxyethanol/H_2O	$C_{14}H_{17}NO_4$	C, H, N
7r	**3a**	*i*	E	90	145–147	MeOH/H_2O	$C_{19}H_{19}NO_4$	C, H, N
7s	**3a**	*i*	C	68	128–130	EtOH/hexane	$C_{20}H_{21}NO_4$	C, H, N
7t	**3t**	B	E	66	128–131	*t*-BuOMe/hexane	$C_{19}H_{19}NO_4$	C, H, N
7u	**4u**	B	E	85	113–115	Et_2O/hexane	$C_{19}H_{19}NO_4$	C, H, N
7w	**4w**	B	C	52	132 dec	EtOAc/hexane	$C_{25}H_{23}NO_4$	C, H, N
7z	**4z**	B	D	72	130 dec	EtOAc/hexane	$C_{18}H_{16}ClNO_3$	C, H, N, Cl
7cc	**4bb**	*i*	*i*	89	140–142	EtOAc/hexane	$C_{22}H_{17}NO_3$	C, H, N
7dd	**4dd**	B	E	78	129 dec	Et_2O/hexane	$C_{19}H_{19}NO_4$	C, H, N

[a] R_1, R_2, and R_3 are as defined in Table IV. [b] Examples of general alkylation procedures A and B are given in the Experimental Section. [c] Examples of general saponification procedures C–E are given in the Experimental Section. [d] Yield of unrecrystallized product. [e] Data shown are for the potassium salt of acid **7e**. [f] Heating time was extended to 18 h. [g] The product was not recrystallized. [h] Calculated as the potassium salt hemihydrate. [i] See the Experimental Section for specific procedure. [j] C: calcd, 62.64; found, 63.11.

Table III. Indolecarboxamidotetrazoles



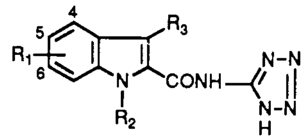
no.[a]	method[b]	yield, %	mp, °C	cryst solvent	formula	analysis
8a	c	52	240 dec	2-methoxyethanol/H_2O	$C_{17}H_{14}N_6O_3$	C, H, N[d]
8b	F	40	235 dec	DMF/H_2O	$C_{18}H_{16}N_6O_3$	C, H, N
8c	G	80	226 dec	2-PrOH/DMF/H_2O	$C_{19}H_{18}N_6O_3$	C, H, N
8d	G	81	227 dec	MeCN/H_2O	$C_{20}H_{20}N_6O_3$	C, H, N
8e	F	45	203–204	EtOAc	$C_{26}H_{32}N_6O_3$	C, H, N
8f	F	54	212 dec	DMF/H_2O	$C_{24}H_{20}N_6O_3$	C, H, N
8g	G	82	235 dec	2-methoxyethanol/H_2O	$C_{23}H_{17}N_7O_5$	C, H, N[e]
8h	G	84	271–274	MeCN/H_2O	$C_{17}H_{14}N_6O_2$	C, H, N
8i	G	78	261–264	MeCN/2-PrOH	$C_{20}H_{20}N_6O_2$	C, H, N
8j	G	63	252–253	MeCN/2-PrOH	$C_{18}H_{16}N_6O_2S$	C, H, N
8k	c	28	263 dec	MeCN/2-PrOH/H_2O	$C_{18}H_{16}N_6O_4S$	C, H, N[d]
8l	G	59	247–250	MeCN/2-PrOH	$C_{20}H_{20}N_6O_2S$	C, H, N, S
8m	G	71	243–245	MeCN/2-PrOH	$C_{23}H_{18}N_6O_2S$	C, H, N, S
8n	G	82	188–190	MeOH/H_2O	$C_{21}H_{22}N_6O_4$	C, H, N
8o	G	68	245 dec	MeCN/DMF/H_2O	$C_{13}H_{14}N_6O_3$	C, H, N
8p	G	68	215 dec	MeCN/H_2O	$C_{14}H_{16}N_6O_3$	C, H, N
8q	F	72	223 dec	2-methoxyethanol/H_2O	$C_{15}H_{18}N_6O_3$	C, H, N
8r	G	92	226 dec	MeCN/H_2O	$C_{20}H_{20}N_6O_3$	C, H, N
8s	G	85	212 dec	*f*	$C_{21}H_{22}N_6O_3$	C, H, N
8t	G	82	254–256	MeCN/2-PrOH	$C_{20}H_{20}N_6O_3$	C, H, N
8u	G	68	205–208	MeCN/2-PrOH	$C_{20}H_{20}N_6O_3$	C, H, N
8v	c	74	243 dec	MeOH/DMF/H_2O	$C_{19}H_{18}N_6O_3$	C, H, N[g]
8w	G	72	205 dec	MeCN/H_2O	$C_{26}H_{24}N_6O_3$	C, H, N
8x	G	91	218 dec	MeCN	$C_{20}H_{20}N_6O_2$	C, H, N
8y	G	92	235 dec	*f*	$C_{19}H_{17}BrN_6O_2$	C, H, N, Br
8z	G	60	228 dec	MeCN/H_2O	$C_{19}H_{17}ClN_6O_2$	C, H, N, Cl
8aa	G	68	240 dec	MeOH/DMF/H_2O	$C_{19}H_{16}Cl_2N_6O_2$	C, H, N, Cl
8bb	G	74	203–205	MeCN/H_2O	$C_{19}H_{18}N_6O_2$	C, H, N
8cc	F	47	208 dec	2-methoxyethanol/H_2O	$C_{23}H_{18}N_6O_2$	C, H, N
8dd	G	60	166 dec	MeCN/2-PrOH	$C_{20}H_{20}N_6O_3$	C, H, N

[a] R_1, R_2, and R_3 are as defined in Table IV. [b] See general methods F and G in the Experimental Section. [c] See the Experimental Section for specific procedure. [d] Calculated as $\cdot H_2O$. [e] N: calcd, 20.80; found, 20.16. [f] The product was not recrystallized. [g] Calculated as $\cdot 0.5H_2O$.

general, resulted in compounds with activity inferior to that of **8d**. The 5-phenylmethoxy (**8w**), 5-chloro (**8z**), and 5,6-dichloro (**8aa**) analogues did retain a reasonable level of activity, while the 6-methoxy (**8u**) showed reduced activity compared to that of **8d**, and the 4-methoxy analogue (**8t**) was inactive.

Replacement of the (carbonylamino)tetrazole function at the indole 2-position with a direct indole–tetrazole bond

Table IV. Inhibition of Histamine Release from Human Basophils by Indolecarboxamidotetrazoles



no.	R_1	R_2	R_3	histamine release: % inhibition[a] 33 μM	10 μM
8a	5-MeO	C_6H_5	OH	In.[b]	
8b	5-MeO	C_6H_5	OMe	74 ± 9 (5)	46 ± 12 (5)
8c	5-MeO	C_6H_5	OEt	73 ± 12 (5)	55 ± 13 (5)
8d	5-MeO	C_6H_6	$OCH(Me)_2$	82 ± 2 (45)	46 ± 3 (43)
8e	5-MeO	C_6H_5	OC_9H_{19}	*c*	
8f	5-MeO	C_6H_5	$OCH_2C_6H_5$	84 (2)	43 (2)
8g	5-MeO	C_6H_5	$O(4\text{-}NO_2C_6H_4)$	In.	
8h	5-MeO	C_6H_5	H	In.	
8i	5-MeO	C_6H_5	$CH(Me)_2$	In.	
8j	5-MeO	C_6H_5	SMe	66 (2)	20 (2)
8k	5-MeO	C_6H_5	SO_2Me	In.	
8l	5-MeO	C_6H_5	$SCH(Me)_2$	50 ± 4 (3)	13 ± 2 (3)
8m	5-MeO	C_6H_5	SC_6H_5	82 (2)	30 (2)
8n	5-MeO	$4\text{-}MeOC_6H_4$	$OCH(Me)_2$	49 (2)	16 (2)
8o	5-MeO	H	OEt	46 (2)	7 (2)
8p	5-MeO	H	$OCH(Me)_2$	62 (2)	17 (2)
8q	5-MeO	Me	$OCH(Me)_2$	45 ± 13 (5)	18 ± 6 (5)
8r	5-MeO	$CH_2C_6H_5$	OEt	55 ± 11 (3)	26 ± 2 (3)
8s	5-MeO	$CH_2C_6H_5$	$OCH(Me)_2$	69 ± 17 (3)	61 ± 28 (3)
8t	4-MeO	C_6H_5	$OCH(Me)_2$	In.	
8u	6-MeO	C_6H_5	$OCH(Me)_2$	50 (2)	24 (2)
8v	5-OH	C_6H_5	$OCH(Me)_2$	In.	
8w	$5\text{-}C_6H_5CH_2O$	C_6H_5	$OCH(Me)_2$	66 (2)	65 (2)
8x	5-Me	C_6H_5	$OCH(Me)_2$	In.	
8y	5-Br	C_6H_5	$OCH(Me)_2$	33 (1)	7 (1)
8z	5-Cl	C_6H_5	$OCH(Me)_2$	72 ± 9 (4)	18 ± 8 (4)
8aa	$5,6\text{-}Cl_2$	C_6H_5	$OCH(Me)_2$	84 (2)	40 (2)
8bb	H	C_6H_5	$OCH(Me)_2$	In.	
8cc	H	C_6H_5	$OCH_2C_6H_5$	In.	
8dd	H	$4\text{-}MeOC_6H_4$	$OCH(Me)_2$	In.	
15				37 (1)	25 (1)

[a] Percent inhibition of basophil histamine release stimulated by anti-IgE. The standard error and (number of experiments) are shown. [b] Inactive (In.) is defined as ≤25% inhibition at a screening concentration of 33 μM. [c] Sample fluorescence interferes with the histamine assay.

(compound **15**) also resulted in reduced activity. In addition, none of the ester or carboxylic acid synthetic intermediates possessed significant inhibitory activity in the basophil test.[23]

Thus, to inhibit histamine release from human basophils, this series of indolecarboxamidotetrazoles must contain an alkylated electron-donating atom in the indole 3-position and be substituted in the 5- or 6-position with an alkoxy or halogen substituent. The 3-alkoxy, 5-methoxy combination appeared to yield the highest potency in blocking histamine release.

Table V is a comparison of **8d**, furo[3,2-*b*]indole **1**, DSCG, and nedocromil, a more potent, second generation version of DSCG.[24] In addition to the basophil assay, these compounds were also evaluated for their ability to inhibit antigen-induced histamine release from guinea pig and human lung mast cells. Compounds **8d** was a potent inhibitor of histamine release in all three models. In contrast, nedocromil is a very weak inhibitor of histamine release from both dispersed lung fragments[25] and basophils. The ability of **8d** to inhibit leukotriene and thromboxane release from mast cells and neutrophils has also been reported recently.[26,27]

Table V. Activity Comparisons in the Basophil and Chopped Lung Models

compound	basophil[a] IC_{50}, μM	guinea pig lung[b] IC_{50}, μM	human lung[a] IC_{50}, μM
8d	15.0	26.7 ± 2.8	16.6 ± 1.8
CI-922	8.5	13.4 ± 1.4	
DSCG	In.[c]	In.[d]	In.[d]
nedocromil	In.[c]	In.[d]	In.[d]

[a] Concentration of drug (μM) inhibiting anti-IgE-stimulated histamine release by 50% of control value. [b] Concentration of drug (μM) inhibiting ovalbumin-stimulated histamine release by 50% of control value. [c] Inactive (In.) is defined as ≤25% inhibition at a screening concentration of 33 μM. [d] Inactive (In.) is defined as ≤25% inhibition at a screening concentration of 75 μM.

Conclusions

A series of novel indolecarboxamidotetrazoles has been prepared. The preliminary antiallergic potential of these compounds was assessed by measuring inhibition of histamine release from human leukocytes stimulated by anti-IgE antibody. A number of compounds showed dose-related activity in this assay. The compounds with the best inhibitory potential contained 3-alkoxy, 5-methoxy, and 1-phenyl substituents on the indole core structure.

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Compound **8d** (now designated CI-949) also inhibited allergic mediator release from guinea pig and human lung. In addition, **8d** is active in several in vivo allergy models,[28] and is currently undergoing clinical trials.

Experimental Section

Melting points were determined on a Mel-Temp or Electrothermal capillary apparatus and are uncorrected. The ^{1}H NMR spectra were determined at 90 MHz on a Varian EM-390, at 100 MHz on an IBM WP100SY, or at 200 MHz on a Varian XL-200 spectrometer with tetramethylsilane as an internal standard. The infrared spectra were recorded as potassium bromide disks on a Digilab FTS-14 or a Nicolet FT-IRMS-1 spectrophotometer. Elemental analyses were provided by the Analytical Chemistry staff of this department. All new compounds yielded spectral data consistent with the proposed structure and microanalyses were within ±0.4% of the theoretical values unless indicated otherwise.

Method A. Methyl 3-Ethoxy-5-methoxy-1*H*-indole-2-carboxylate (5o). A mixture of 11.9 g (0.054 mol) of enol ester **3a**,[18] 8.0 g (0.058 mol) of anhydrous K_2CO_3, and 7.7 mL (9.1 g, 0.059 mol) of Et_2SO_4 in 150 mL of acetone was stirred at reflux for 16 h. The cooled mixture was added to 1.5 kg of ice/H_2O, and the precipitated product was filtered and washed with H_2O. Recrystallization from aqueous MeOH yielded 8.0 g (59%) of analytically pure ester **5o**: mp 118–120 °C; IR (KBr) 3320, 1679, 1483, 1032 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.43 (t, 3 H, CH_2CH_3), 3.87 (s, 3H, OCH_3), 3.95 (s, 3 H, OCH_3), 4.30 (q, 2 H, CH_2CH_3), 6.83–7.33 (m, 3 H, Ar*H*), 8.47 (br s, 1 H, N*H*). Anal. ($C_{13}H_{15}NO_4$) C, H, N.

(Other alkylating agents employed with this procedure were dimethyl sulfate, phenylmethyl bromide, and *n*-nonyl bromide.)

Method B. Methyl 5-Methoxy-3-(1-methylethoxy)-1-phenyl-1*H*-indole-2-carboxylate (6d). A stirred solution of 24.8 g (0.22 mol) of *t*-BuOK in 100 mL of DMSO was cooled in a cold H_2O bath and treated over 30 min with a solution of 44.6 g (0.15 mol) of enol ester **4a**[19] in 100 mL of DMSO. The mixture was stirred for an additional 45 min and then treated in one portion with 25.0 mL (32.8 g, 0.27 mol) of 2-bromopropane. After stirring at room temperature for an additional 48 h, the reaction mixture was added to 2.5 kg of ice/H_2O, acidified with 6.0 N HCl, and extracted with CH_2Cl_2 (4 × 800 mL). The combined organic layers were washed with H_2O (1 × 1.5 L), 5% aqueous $NaHCO_3$ (2 × 1.5 L), and H_2O again. The extracts were dried (Na_2SO_4) and evaporated to leave the product alkoxy ester **6d** as a crude oil containing some residual DMSO. This material was saponified without additional purification.

(This procedure was also employed with diethyl sulfate as the alkylating agent.)

Methyl 1-Phenyl-3-(phenylmethoxy)-1*H*-indole-2-carboxylate (6cc). The N-alkylation procedure described in the preparation of **7r** was also employed for O-alkylation of ester **4bb**[19] with phenylmethyl chloride. From 5.3 g (0.02 mol) of **4bb** there was obtained 3.8 g (54%) of ester **6cc**. A sample recrystallized several times from aqueous MeOH was analytically pure: mp 117–119 °C; IR (KBr) 1725, 1453, 1374, 968 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.67 (s, 3 H, CH_3), 5.27 (s, 2 H, CH_2), 6.90–7.83 (m, 14 H, Ar*H*). Anal. ($C_{23}H_{19}NO_3$) C, H, N.

Method E. 5-Methoxy-1-phenyl-3-(phenylmethoxy)-1*H*-indole-2-carboxylic Acid (7f). A solution of 201 g (0.52 mol) of ester **6f** in 1.0 L of MeOH was treated with a solution of 83 g (1.48 mol) of KOH in H_2O. The mixture was stirred at reflux for 3 h, cooled, and filtered, and the filtrate was added to 7.0 kg of ice/H_2O. Acidification with HOAc precipitated the crude product, which was filtered and washed with water to yield 174 g (89%) of acid **7f**. A sample recrystallized from aqueous acetone was analytically pure: mp 123 °C dec; IR (KBr) 1685, 1493, 1208, 698 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.74 (s, 3 H, CH_3), 5.30 (s, 2 H, CH_2), 6.78–7.57 (m, 13 H, Ar*H*), 8.62 (br s, 1 H, CO_2H). Anal. ($C_{23}H_{19}NO_4$) C, H, N.

5-Methoxy-3-(4-nitrophenoxy)-1-phenyl-1*H*-indole-2-carboxylic Acid (7g). A mixture of 15.0 g (0.050 mol) of enol ester **4a**,[19] 6.96 g (0.050 mol) of anhydrous K_2CO_3, and 7.11 g (0.050 mol) of 1-fluoro-4-nitrobenzene in 90 mL of DMF was stirred and heated on the steam bath for 18 h. The mixture was cooled and added to 750 g of ice/H_2O, and the product was extracted with CH_2Cl_2 (3 × 200 mL). The combined organic layers were washed with H_2O (2 × 300 mL), dried ($MgSO_4$), and evaporated to yield intermediate methyl ester **6g** as an oil containing some residual DMF.

Saponification of the crude intermediate ester **6g** described above by the procedure of method E yielded 6.1 g (30%) of carboxylic acid **7g**. A sample recrystallized from aqueous 2-methoxyethanol was analytically pure: mp 230 °C dec; IR (KBr) 1680, 1490, 1343, 1218 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 3.77 (s, 3 H, CH_3), 6.63–7.67 (m, 10 H, Ar*H*), 8.13–8.35 (m, 2 H, Ar*H*). Anal. ($C_{22}H_{16}N_2O_6$) C, H, N.

3-Ethoxy-5-methoxy-1-(phenylmethyl)-1*H*-indole-2-carboxylic Acid (7r). A suspension of 1.34 g (0.028 mol) of NaH (50% suspension in mineral oil) in 50 mL of DMF was cooled in an ice bath and treated dropwise over 20 min with a solution of 5.9 g (0.024 mol) of ester **5o** in 50 mL of DMF. The ice bath was removed, and the mixture was stirred for 45 min and then treated with 3.4 mL (4.9 g, 0.029 mol) of phenylmethyl bromide. The mixture was stirred for an additional 20 h and then poured into 1.0 kg of ice/H_2O. The solid was filtered and washed with water to yield 6.4 g (79%) of the intermediate methyl ester **6r**, mp 57–59 °C.

Saponification of 5.7 g (0.017 mol) of the crude ester **6r** as described in method E yielded 5.0 g (90%) of carboxylic acid **7r**. A sample recrystallized from aqueous methanol was analytically pure: mp 145–147 °C; IR (KBr) 1674, 1498, 1228, 1037 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.55 (t, 3 H, CH_2CH_3), 3.80 (s, 3 H, OCH_3), 4.53 (q, 2 H, CH_2CH_3), 5.83 (s, 2 H, NCH_2), 6.80–7.41 (m, 8 H, Ar*H*), 10.30 (br s, 1 H, CO_2H). Anal. ($C_{19}H_{19}NO_4$) C, H, N.

5-Methoxy-3-(1-methylethoxy)-1-(phenylmethyl)-1*H*-indole-2-carboxylic Acid (7s). Alkylation of ester **5p**[18] by the procedure described in the preparation of **7r** yielded the intermediate 1-phenylmethyl ester **6s** as an oil. Saponification of **6s** as described in method C and recrystallization of the product from EtOH/hexane gave a 68% overall yield of the analytically pure carboxylic acid **7s**: mp 128–130 °C; IR (KBr) 1673, 1493, 1308, 1228 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.50 (d, 6 H, $CH(CH_3)_2$), 3.87 (s, 3 H, OCH_3), 4.97 (heptet, 1 H, $CH(CH_3)_2$), 5.80 (s, 2 H, CH_2), 6.88–7.47 (m, 8 H, Ar*H*), 10.53 (br s, 1 H, CO_2H). Anal. ($C_{20}H_{21}NO_4$) C, H, N.

Method C. This saponification procedure, in which the K^+ salt of the carboxylic acid product is isolated, has been previously described.[18]

Method D. 5-Chloro-3-(1-methylethoxy)-1-phenyl-1*H*-indole-2-carboxylic Acid (7z). A solution of 14.8 g (0.043 mol) of ester **6z** in 85 mL of MeOH was treated with a solution of 6.4 g (0.11 mol) of KOH in 85 mL of H_2O. The mixture was stirred at reflux for 3 h, cooled, and condensed to one-third of the original volume. The residue was treated with 400 mL of H_2O, and the mixture was extracted with CH_2Cl_2 (3 × 250 mL). The aqueous layer was filtered, cooled in ice, and acidified with 6.0 N HCl. The precipitated product was filtered and washed with water to yield 10.2 g (72%) of crude acid **7z**. A sample recrystallized from EtOAc/hexane was analytically pure: mp 130 °C dec; IR (KBr) 1677, 1497, 1280, 1103 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.48 (d, 6 H, $CH(CH_3)_2$), 4.92 (heptet, 1 H, $CH(CH_3)_2$), 7.00–7.70 (m, 8 H, Ar*H*). Anal. ($C_{18}H_{16}ClNO_3$) C, H, N, Cl.

1-Phenyl-3-(phenylmethoxy)-1*H*-indole-2-carboxylic Acid (7cc). A mixture of 11.9 g (0.033 mol) of ester **6cc** in 200 mL of DMSO was treated with 7.4 g (0.066 mol) of *t*-BuOK. The mixture was stirred and heated at 65 °C for 2 h, cooled, and added to 2.5 kg of ice/H_2O. The solution was filtered, and the filtrate was cooled in ice and acidified with 6.0 N HCl. The precipitated product was filtered and washed with water to yield 9.7 g (89%) of carboxylic acid **7cc**. A sample recrystallized from EtOAc/hexane was analytically pure: mp 140–142 °C; IR (KBr) 1671, 1470, 1276, 1151 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.42 (s, 2 H, CH_2), 6.90–7.84 (m, 14 H, Ar*H*), 8.31 (br s, 1 H, CO_2H). Anal. ($C_{22}H_{17}NO_3$) C, H, N.

3-Hydroxy-5-methoxy-1-phenyl-*N*-1*H*-tetrazol-5-yl-1*H*-indole-2-carboxamide (8a). A solution of 4.4 g (0.010 mol) of (carbonylamino)tetrazole **8f** in 125 mL of THF was subjected to

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catalytic hydrogenation in a Parr apparatus (0.30 g 20% Pd/C catalyst, 25 °C, 52 psi H_2 pressure, 24 h). The mixture was filtered, and the crude product was separated from the spent catalyst by extracting the insoluble material with warm 2-methoxyethanol. The original filtrate and extracts were combined and evaporated. Recrystallization of the residue from aqueous 2-methoxyethanol yielded 1.8 g (52%) of the analytically pure hydroxy (carbonylamino)tetrazole **8a**: mp 240 °C dec; IR (KBr) 1683, 1608, 1497, 1213 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.78 (s, 3 H, CH_3), 6.95–7.48 (m, 8 H, Ar*H*), 10.90 (s, 1 H, O*H* or CON*H*). Anal. ($C_{17}H_{14}N_6O_3{\cdot}H_2O$).

Method F. 5-Methoxy-1-phenyl-3-(phenylmethoxy)-*N*-1*H*-tetrazol-5-yl-1*H*-indole-2-carboxamide (8f). A mixture of 5.2 g (0.014 mol) of acid **7f** and 4.8 g (0.030 mol) of 1,1′-carbonylbis(1*H*-imidazole) in 20 mL of DMF was stirred and heated on the steam bath for 20 min. The mixture was cooled, 1.7 g (0.017 mol) of 5-aminotetrazole monohydrate was added, and heating was continued for an additional 20 min. The cooled reaction mixture was added to 250 g of ice/H_2O and acidified with 4.0 N HCl. The precipitated product was filtered, washed with water, and recrystallized from aqueous DMF to yield 3.3 g (54%) of (carbonylamino)tetrazole **8f**. A sample recrystallized an additional time as above was analytically pure: mp 212 °C dec; IR (KBr) 1669, 1596, 1532, 1211 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.82 (s, 3 H, CH_3), 5.45 (s, 2 H, CH_2), 6.93–7.71 (m, 13 H, Ar*H*), 11.18 (s, 1 H, CON*H*), 15.76 (br s, 1 H, tetrazole N*H*). Anal. ($C_{24}H_{20}N_6O_3$) C, H, N.

5-Methoxy-3-(methylsulfonyl)-1-phenyl-*N*-1*H*-tetrazol-5-yl-1*H*-indole-2-carboxamide (8k). A solution of 1.2 g (0.0032 mol) of (carbonylamino)tetrazole **8j** in 75 mL of H_2O containing 0.27 g (0.0032 mol) of $NaHCO_3$ was treated with a slurry of 2.0 g (0.013 mol) of $KMnO_4$ in 25 mL of acetone. After stirring for 2 h, the mixture was filtered through a bed of Celite filter-aid. The filtrate was cooled in ice and acidified with HOAc to precipitate the sulfone product **8k**, which was filtered and washed with water. Recrystallization from aqueous 2-PrOH/MeCN yielded 0.38 g (28%) of analytically pure sulfone **8k**: mp 263 °C dec; IR (KBr) 1693, 1606, 1408, 1216 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.11 (s, 3 H, SCH_3), 3.73 (s, 3 H, OCH_3), 6.69–7.56 (m, 8 H, Ar*H*), 12.71 (s, 1 H, CON*H*). Anal. ($C_{18}H_{16}N_6O_4S{\cdot}H_2O$) C, H, N.

5-Hydroxy-3-(1-methylethoxy)-1-phenyl-*N*-1*H*-tetrazol-5-yl-1*H*-indole-2-carboxamide (8v). A solution of 3.33 g (0.0071 mol) of (carbonylamino)tetrazole **8w** in 100 mL of THF was subjected to catalytic hydrogenation in a Parr apparatus (0.30 g 20% Pd/C catalyst, 25 °C, 20 psi H_2 pressure, 18 h). After removal of the catalyst by filtration, the filtrate was evaporated. Recrystallization of the residue from aqueous MeOH/DMF yielded 2.0 g (74%) of the analytically pure hydroxy (carbonylamino)tetrazole **8v**: mp 243 °C dec; IR (KBr) 1684, 1603, 1499, 1199 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.43 (d, 6 H, CH(CH_3)$_2$), 4.71 (heptet, 1 H, C*H*(CH$_3$)$_2$), 6.81–7.71 (m, 8 H, Ar*H*), 9.33 (s, 1 H, O*H*), 11.35 (s, 1 H, CON*H*), 16.05 (br s, 1 H, tetrazole N*H*). Anal. ($C_{19}H_{18}N_6O_3{\cdot}0.5H_2O$) C, H, N.

Method G. 5-Chloro-3-(1-methylethoxy)-1-phenyl-*N*-1*H*-tetrazol-5-yl-1*H*-indole-2-carboxamide (8z). A mixture of 8.0 g (0.024 mol) of acid **7z** and 4.5 g (0.028 mol) of 1,1′-carbonylbis(1*H*-imidazole) in 150 mL of MeCN was stirred at reflux for 90 min. The mixture was cooled and treated with 2.5 g (0.029 mol) of anhydrous 5-aminotetrazole followed by 8.2 mL (6.0 g, 0.059 mol) of triethylamine. After heating for an additional 16 h, the mixture was cooled, added to 500 g of ice/H_2O, and acidified with HOAc. The precipitated product was filtered, washed with water, and recrystallized from aqueous MeCN to yield 5.8 g (60%) of the analytically pure (carbonylamino)tetrazole **8z**: mp 228 °C dec; IR (KBr) 1676, 1496, 1280, 1100 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 1.37 (d, 6 H, CH(CH_3)$_2$), 4.74 (heptet, 1 H, C*H*(CH$_3$)$_2$), 7.15–7.56 (m, 7 H, Ar*H*), 7.92 (d, 1 H, #4 Ar*H*), 11.61 (s, 1 H, CON*H*), 16.10 (br s, 1 H, tetrazole N*H*). Anal. ($C_{19}H_{17}ClN_6O_2$) C, H, N, Cl.

Methyl 2-[(Cyanomethyl)phenylamino]-5-methoxybenzoate (12). A mixture of 14.2 g (0.050 mol) of carboxylic acid **11**,[19] 8.0 g (0.058 mol) of anhydrous K_2CO_3, and 6.0 mL (8.0 g, 0.063 mol) of Me_2SO_4 in 400 mL of MeCN was stirred at reflux for 21 h. The cooled mixture was filtered, and the insoluble material was washed with fresh MeCN. The combined filtrates were evaporated, and the residue was recrystallized from aqueous MeOH to yield 13.7 g (92%) of the ester product. A sample recrystallized an additional time as above yielded analytically pure ester **12**: mp 107–109 °C; IR (KBr) 2239 (weak), 1720, 1499, 1211 cm^{-1}; ^1H NMR ($CDCl_3$) δ 3.74 (s, 3 H, CH_3), 3.86 (s, 3 H, CH_3), 4.51 (s, 2 H, CH_2), 6.58–7.44 (m, 8 H, Ar*H*). Anal. ($C_{17}H_{16}N_2O_3$) C, H, N.

3-Hydroxy-5-methoxy-1-phenyl-1*H*-indole-2-carbonitrile (13). A suspension of 8.4 g (0.075 mol) of *t*-BuOK in 200 mL of THF was cooled in ice and treated over 2 h with a solution of 13.7 g (0.046 mol) of ester **12** in 150 mL of THF. The mixture was stirred at room temperature for 48 h and then added to 1.1 kg of ice/H_2O and acidified with HOAc. The precipitated solid was filtered and washed with water. Recrystallization from aqueous MeOH yielded 10.7 g (88%) of the nitrile product. A sample recrystallized an additional time as above yielded analytically pure nitrile **13**: mp 182 °C dec; IR (KBr) 2219, 1599, 1457, 1263 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.77 (s, 3 H, CH_3), 6.97–7.62 (m, 8 H, Ar*H*), 10.86 (s, 1 H, O*H*). Anal. ($C_{16}H_{12}N_2O_2$) C, H, N.

5-Methoxy-3-(1-methylethoxy)-1-phenyl-1*H*-indole-2-carbonitrile (14). Enol nitrile **13** was alkylated with 2-bromopropane by the procedure described in method B. The crude product was purified by flash chromatography (E. Merck 9385 SiO_2; 1:1 CH_2Cl_2 in hexane elution) and then recrystallized from aqueous 2-PrOH. From 6.0 g (0.023 mol) of enol **13** there was obtained 4.8 g (69%) of analytically pure alkoxy nitrile **14**: mp 79–81 °C; IR (KBr) 2203, 1543, 1498, 1262 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.47 (d, 6 H, CH(CH_3)$_2$), 3.86 (s, 3 H, OCH$_3$), 4.90 (heptet, 1 H, C*H*(CH$_3$)$_2$), 6.97–7.58 (m, 8 H, Ar*H*). Anal. ($C_{19}H_{18}N_2O_2$) C, H, N.

5-Methoxy-3-(1-methylethoxy)-1-phenyl-2-(1*H*-tetrazol-5-yl)-1*H*-indole (15). A mixture of 2.97 g (0.0097 mol) of nitrile **14** and 3.6 g (0.011 mol) of tri-*n*-butylstannyl azide[22] in 35 mL of DMF was stirred at reflux for 87 h. The cooled reaction mixture was evaporated, and the residue was dissolved in isopropyl ether. A solution of isopropyl ether saturated with gaseous HCl was added, and the gummy precipitate that formed was removed by extracting with 1.0 N aqueous NaOH (3 × 100 mL). The combined base extracts were washed with CH_2Cl_2 (3 × 150 mL) and then cooled in ice and acidified with HOAc. The precipitated product was removed by extracting with EtOAc (4 × 100 mL). The combined organic layers were back-washed with H_2O (2 × 150 mL), dried (Na_2SO_4), and evaporated to a syrup which crystallized upon standing at room temperature. Recrystallization from hexane/EtOAc yielded 1.3 g (38%) of the analytically pure tetrazole **15**: mp 162–164 °C; IR (KBr) 1606, 1496, 1252, 1066 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.45 (d, 6 H, CH(CH_3)$_2$), 3.87 (s, 3 H, OCH_3), 4.90 (heptet, 1 H, C*H*(CH$_3$)$_2$), 6.92–7.60 (m, 8 H, Ar*H*), 13.17 (br s, 1 H, tetrazole N*H*). Anal. ($C_{19}H_{19}N_5O_2$) C, H, N.

Biological Methods. Human Basophil Test. The basophil procedure has been previously described.[8,14] In brief, whole human blood was obtained from well-characterized allergic donors. After sedimentation of the red cells, the leukocytes were removed, washed, and suspended in buffer solution. Cells were preincubated with the test compound, then challenged with anti-IgE. The released histamine was quantitated by using an automated fluorometric assay. The percent inhibition of histamine release was calculated by comparison of the values from the test drug-treated cells with that of nondrug controls similarly challenged with anti-IgE. As a minimum, each test compound was screened in triplicate at drug concentrations of 33 and 10 μM. An inhibition of $\leq$25% at 33 μM was arbitrarily defined as inactive. Where an IC_{50} value was determined, additional experiments were conducted with threefold concentrations from 1 to 100 μM.

Guinea Pig Chopped Lung Test. Detailed procedures for this test have also been published.[16] Washed aliquots of minced lung from guinea pigs previously sensitized with ovalbumin were incubated (in triplicate) with the test compound or a control buffer solution and then challenged with ovalbumin antigen. Histamine released into the supernatant fluids as well as that remaining in the lung tissue was quantified by an automated fluorometric technique. The effect (percent inhibition) of drug on antigen-induced histamine release was calculated and corrected for spontaneous release from buffer-treated control tissues. Assays were performed at drug concentrations of 0.75, 7.5, and 75 μM in order to calculate IC_{50} values.

Human Chopped Lung Test. Portions of grossly normal-appearing human lung obtained during lobectomy for carcinoma were placed in Tyrode's buffer, dissected free of larger bronchioles and blood vessels, and then chopped with scissors into 25–75-mg fragments. The fragments were washed and then stored overnight in Tyrode's buffer at room temperature. Before use the next day, the tissue was again washed with buffer. Portions of lung tissue (about 400 mg) were placed in each of a series of vials containing buffer at 37 °C. After a 10-min incubation in Tyrode's buffer, test drug or vehicle was added, and 10 min later, the tissue was incubated with anti-IgE in a final dilution of 3:1000. After another 30-min incubation, samples of the supernatant were removed for assay. Histamine was assayed as described above in the guinea pig chopped lung test.

The Role of Position 4 in Angiotensin II Antagonism: A Structure–Activity Study†

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A number of [Sar[1],(pX)Phe[4]]-ANG II and [Sar[1],(pX)Phe[4],Ile[8]]-ANG II analogues were prepared. A good correlation between pX structure in [Sar[1],(pX)Phe[4]]-ANG II and antagonist activity could not be found. However, the data suggest a general trend: Position 4 para substituents that are hydrophilic and capable of donating a hydrogen atom in a hydrogen bond promote agonist activity, while para substituents that are hydrophobic and incapable of donating a hydrogen atom promote antagonist activity. These properties were found to be optimal in the *p*-chloro substituent. The resulting analogue [Sar[1],(pCl)Phe[4]]-ANG II is a potent ANG II antagonist in vivo. The pX substituents that promote antagonist activity in the [Sar[1],(pX)Phe[4]]-ANG II series were unfavorable in [Sar[1],(pX)Phe[4],Ile[8]]-ANG II analogues. ANG II analogues that are antagonists by virtue of an alteration in position 8 require a position 4 agonist side chain. Concurrent modifications of positions 4 and 8 do not give rise to potent antagonists with reduced partial agonist activity.

Among the first reported[1] antagonists of angiotensin II was the analogue [Phe[4],Tyr[8]]-ANG II **3**[2,3] (Table I)[1,3-10] which contained an alteration of the tyrosine in position 4. Other weak antagonists were reported shortly after: [(pF)Phe[4]-ANG II **4**[7] and [Phe[4]]-ANG II **1**[4] (Table I). In that same period antagonists of much greater potency were discovered by alteration of the 8-position: [Ala[8]]-ANG II **9**,[9] [Leu[8]]-ANG II **11**,[11] [Ile[8]]-ANG II **12**,[3] and [Cys[8]]-ANG II **10**.[3]

Substitution of sarcosine for aspartic acid in position 1 was later shown to enhance antagonist action by blocking aminopeptidase action and increasing antagonist affinity[12] as shown in the potent antagonists [Sar[1],Ala[8]]-ANG II **13**[9] and [Sar[1],Ile[8]]-ANG II **14**.[12] Although the development of [Sar[1],X[8]]-ANG II antagonists continued,[13] investigations of [Sar[1],X[4]]-ANG II antagonists were not reported until recently. This may have been due to the lower potency of the [X[4]]-ANG II antagonists compared to the [X[8]]-ANG II antagonists (Table I).

[Sar[1],(SAcm)Phe[4]]-ANG II **5** (Table I) was recently described to be a potent ANG II antagonist by Escher et al.[14] and [Sar[1],(OMe)Tyr[4]]-ANG II **6**, also a potent antagonist, Table I, was recetly reported by Goghari et al.[6] In previous work[5,14] Escher has shown that hydrogen bonding and the inductive effects (electronegativity) of the para substituent are important for optimal agonist activity of position 4 analogues of [Sar[1]]-ANG II. We have been pursuing the structure–antagonist activity relationship of [Sar[1],X[4]]-ANG II antagonists. This paper describes our attempt to optimize the activity of position 4 antagonist analogues.

The partial agonist activity of [Sar[1],Ala[8]]-ANG II **13** and other position 8 modified antagonists has precluded their use as antihypertensive agents[13] in humans. Structural modifications that could reduce such partial agonist activity could improve the therapeutic potential of ANG II antagonists. Since antagonist action may be obtained by modification of either position 4 or position 8, both residues must be responsible for receptor stimulation. Hence, modifications of both positions 4 and 8 in the same peptide may result in the creation of an ANG II antagonist devoid of agonist activity. This possibility was explored in the present study.

† The abbreviations for natural amino acids and nomenclature for peptide structures follow the recommendations for peptide structures of the IUPAC–IUB commission on Biochemical Nomenclature (*J. Biol. Chem.* **1971**, *247*, 977). Abbreviations for nonnative amino acids include Bph = *p*-(dihydroxyboryl)-phenylalanine, (OMe)Tyr = *O*-methyltyrosine, (pF)Phe = *p*-fluorophenylalanine, (SAcm)Phe = *p*-[(acetamidomethyl)thio]-phenylalanine. Other abbreviations in this paper include TEA = triethylamine, TFA = trifluoroacetic acid, DCC = *N,N'*-dicyclohexylcarbodiimide.

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