Synthesis and Structure-Activity Relationships of a Novel Series of Non-Peptide Angiotensin II Receptor Binding Inhibitors Specific for the AT_2 Subtype

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Structure-activity relationships are reported for a novel class of 4,5,6,7-tetrahydro-1*H*-imidazo[4,5-c]pyridine-6-carboxylic acid derivatives that displace ^{125}I-labeled angiotensin II from a specific subset of angiotensin II (Ang II) binding sites in rat adrenal preparations. This binding site is not the Ang II receptor mediating vascular contraction or aldosterone release, but, rather, is one whose function has not yet been fully elucidated. It has been identified in a number of tissues and has a similar affinity for Ang II and its peptide analogues as does the vascular receptor. The non-peptide compounds reported here are uniquely specific in displacing Ang II at this binding site and are inactive in antagonizing Ang II at the vascular receptor or in pharmacological assays measuring vascular effects. PD 123,319 (**79**), one of the most potent compounds, has an IC_{50} of 34 nM. Certain of these compounds may have utility in the definition and study of Ang II receptor subtypes.

Receptors for the peptide hormone angiotensin II (Ang II) mediate a large variety of physiological functions.[1,2] The most prominent of these, and the most studied, is vascular contraction. The central role of Ang II and its receptors in regulating circulatory function in both health and disease[3] is now well-established. This understanding has been advanced considerably with the help of drugs that specifically inhibit the formation of Ang II, such as angiotensin converting enzyme (ACE) inhibitors and renin inhibitors. Drugs of the former class, e.g., captopril and enalapril, have already proved to be very efficacious in treating hypertension and congestive heart failure, and candidate drugs of the latter class have reached clinical evaluation. Such agents, however, are inadequate as tools to study Ang II receptor function directly, especially regarding possible subtypes, since they render Ang II similarly unavailable to all of its receptors.

Ang II receptors have been identified in many tissues other than vascular[4] and have been demonstrated to mediate a variety of additional functions such as hormone release (vasopressin, catecholamines, aldosterone, prostaglandins), fluid transport (kidney, colon), and neurotransmission (brain, sympathetic neurons).[1,2] Furthermore, other studies have led to the identification of Ang II receptors in tissues and organs where their function has not yet been defined (e.g., uterus, ovary, esophagus).[4,5] Finally, the role of Ang II as a growth factor has also been postulated.[6,7]

Progress in the study of such functional diversity has most often come when agonists and antagonists specific for some actions over others have been discovered. The early studies on compounds directly acting via Ang II receptors were productive in defining the structure-activity relationships (SAR) of angiotensin peptide analogues and fragments, and in uncovering specific antagonists based on the peptide structure of Ang II.[2,8] Two things should be appreciated about this early work: first, much of it was developed using assays that measured a functional response, as opposed to a biochemical receptor binding event; and second, the assay responses used to evaluate analogues were primarily reflections of the vascular activity of Ang II (smooth muscle contraction, pressor effects in vivo). The subsequent isolation of receptor preparations was facilitated by these compounds, and the validation of these receptors was based on correlations with the vascular response. As additional peptide analogues became available, they were used to identify and characterize Ang II effects of other kinds, and while the rank ordering of activities of the peptide analogues was frequently similar, some anomalies were noted. One early example was the Ang II response in rat adrenal gland, where medullary receptors mediating catecholamine release showed different relative responses to a series of Ang II analogues than did rabbit aortic strips or adrenal cortical receptors mediating aldosterone release.[9] Another example was the behavior of vascular antagonists such as [Sar^1,Ile^8]Ang II as agonists for some Ang II actions.[10] Several reports exist of different responses to Ang II and Ang III in vascular, adrenal cortical, uterine, and myocardial tissues.[11-14] More recently,

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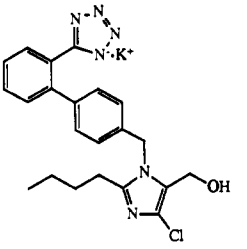
a physiological role has been suggested for Ang II(1-7),[15-17] an Ang II fragment previously thought to be inactive,[1,2] and antagonist peptides based on this fragment have been reported.[18] These and other early observations that pointed to the possible existence of Ang II receptor subtypes could not be probed more deeply due to the lack of appropriate tools, namely, agonists or antagonists of Ang II that were highly selective for these different effects of Ang II. Of the numerous peptide analogues of Ang II prepared over the years, most appear to act nearly equally at both receptor subtypes. Only recently have two peptidic agents with demonstrated subtype selectivity been reported.[19,20] The earliest proposals for designating subtypes were based on the relative potencies of Ang II and Ang III,[21] but these were clouded by differences in methodology and a larger than desirable quantitative variability in results between laboratories in measuring the relative activities of the peptides.

New results in the last few years, however, give hope that Ang II research is poised for a renaissance. Although attempts to isolate and characterize the Ang II receptor by classical methods have proven problematical,[22,23] detailed biochemical work has begun to show firm evidence for different angiotensin receptors.[24] Equally significant have been the reports from Du Pont of a series of specific non-peptide vascular Ang II receptor antagonists, typified by DuP 753.[25-29] Of particular note for the present communication, these compounds have been used to demonstrate the critical nature of receptor assay conditions for Ang II binding experiments, in particular the effect of dithiothreitol (DTT), a frequent additive in such assays.[29] The literature on the use of DTT in binding assays for Ang II has been confusing. Although this disulfide reducing agent has been used in many assay systems to prevent protein degradation, it has clearly been shown to inhibit Ang II binding or abolish Ang II responses in a number of different preparations.[22,23,30,31] In particular, it now seems well-established that DTT at high concentrations alters the structure of the vascular Ang II receptor to prevent agonist binding, whereas there is another class of Ang II binding site that is insensitive to DTT. Further characterization of this latter binding site is in its infancy, and its functional significance, i.e., whether or not it is a true receptor, its distribution, and its relevance to other reports of selective agonists and antagonists, have only begun to be studied. However, it has been shown that standard Ang II agonists and antagonists bind to this second site with affinities similar to those shown for the vascular receptor.[19] DuP 753 binds to this second site with negligible affinity, and thus has served as a defining ligand for the Ang II receptor subtype which mediates most of the known vascular and secretory effects of Ang II. It has



DuP 753

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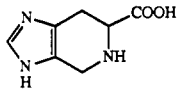
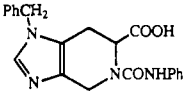
been referred to as the "type 1" or, as more recently proposed, the AT_1 Ang II receptor subtype.[32]

Several years ago, we undertook a project to discover non-peptide Ang II antagonists for possible use as antihypertensive agents. One outcome of this search was the discovery of a series of compounds that specifically compete with Ang II binding at a binding site that is not affected by DTT and which does not mediate any of the usual vascular activities of Ang II. Certain of these compounds have been used to demonstrate the existence of this novel nonvascular Ang II binding site in a variety of tissues, e.g., adrenal[26] (compound 78), uterus[33] (compound 79), and brain[34,35] (compound 36). These compounds have been used as the defining ligands for what has been designated as the "type 2" or AT_2 Ang II binding subtype.[32] The structure–activity relationship (SAR) for Ang II binding displacement and some ancillary biological characterization of these compounds are reported in this paper.

The compounds are derivatives of 4,5,6,7-tetrahydroimidazo[4,5-c]pyridine-6-carboxylic acid (1), the formaldehyde cyclization product of histidine. This material has been isolated as a natural product and given the trivial name spinacine.[36] In particular, 1-substituted-5-acyl analogues were found to have the binding activity of interest. The original inspiration to examine this structural type came from its capability via synthetically accessible modifications to include in a compact form structural elements of the C-terminal tetra- or pentapeptide Y-I-P-H-F sequence of Ang II, which had been shown by early work to be the minimal binding and functional fragment.[2,37] The interest in this structure was further reinforced by a patent report of Ang II antagonist activity in a series of 1-benzylimidazoles.[38] The initial lead, compound 2, had an IC_{50} of 2.1 μM in the rat adrenal binding assay described below.



1 (spinacine)

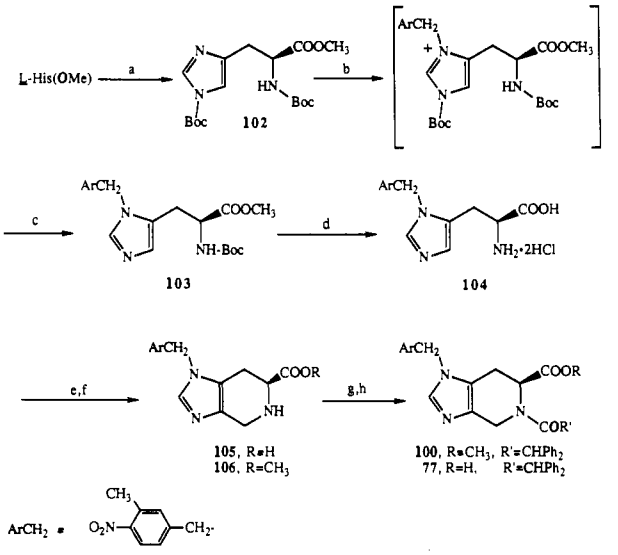


2

Chemistry

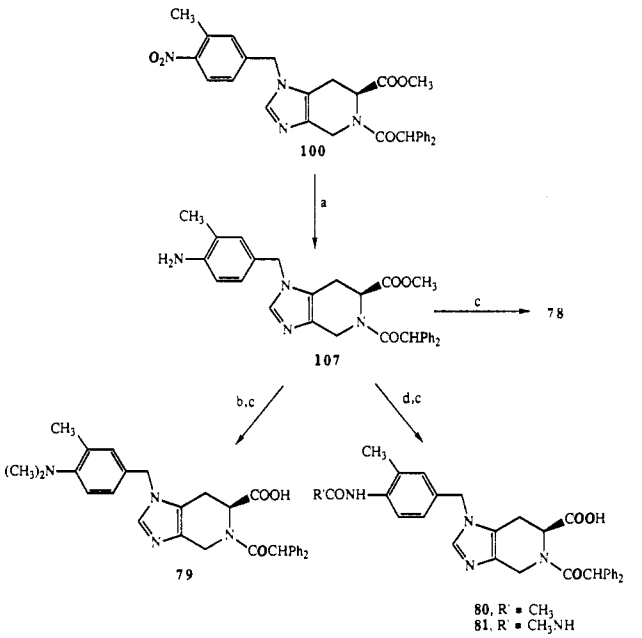
The reaction of histidine with formaldehyde has been known for a long time.[39,40] Under properly controlled acidic conditions, the product of the Pictet–Spengler reaction of histidine (1) can be obtained in excellent yield. We further found that this reaction worked equally well with N^{im}-substituted histidine derivatives.[41] The latter, in turn were available by regiocontrolled synthesis using reported methods or improved procedures which we developed.[41-43] Further description of our work on the

Scheme I.[a] Method A



[a] (a) $(BOC)_2O$; (b) $ArCH_2OTf$, CH_2Cl_2; (c) pH 7, phosphate buffer; (d) 6 N HCl, reflux; (e) CH_2O, H_3O^+; (f) MeOH, H^+; (g) R'COOH, DCC, HOBT; (h) NaOH.

Scheme II.[a] Method B



[a] (a) $SnCl_2$, EtOAc or H_2, RaNi, MeOH, 3 min; (b) CH_2O, $NaBH_4$, HCl, EtOH; (c) NaOH; (d) R'COCl or R'NCO, NEt_3.

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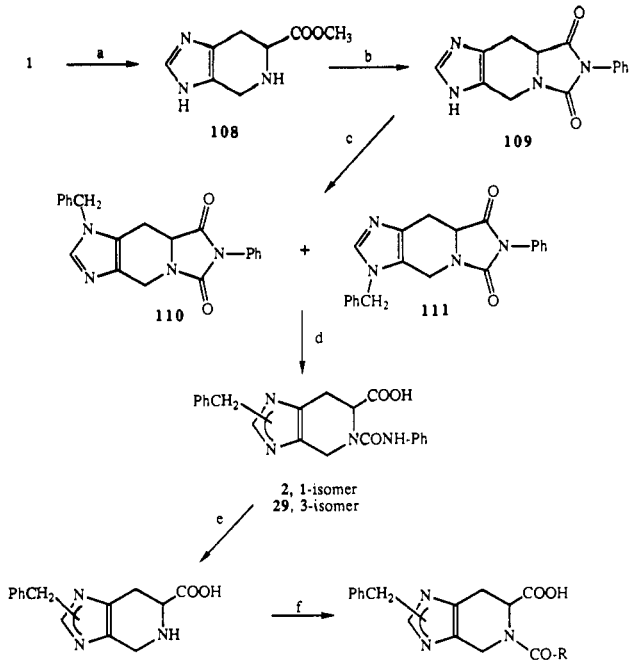
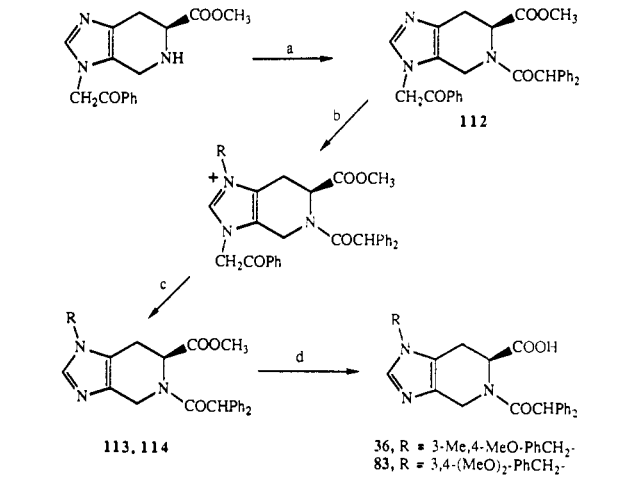
Scheme III.[a] Method C



[a] (a) CH_3OH, HCl; (b) R'NCO, H^+; (c) $PhCH_2Br$, $Me_4N^+OH^-$, DMF, separate isomers; (d) NaOH; (e) KOH, Δ; (f) RCOX, aqueous NaOH.

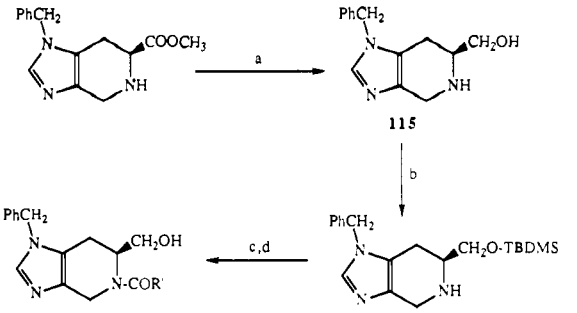
Scheme IV.[a] Method D



[a] (a) $Ph_2CHCOOH$, DCC, HOBT, DMF; (b) RX, CH_3CN; (c) Zn, HOAc; (d) NaOH.

preparation of spinacine and some of its simple derivatives is reported separately.[41] The compounds of interest to the present work were prepared by a variety of routes shown below in Schemes I–V. Scheme I shows a route of great generality for preparing the key intermediate 1-substituted spinacines. Acylation in the final step to provide a wide variety of 5-substituted analogues was carried out by standard peptide-coupling procedures on the methyl esters, followed by mild base hydrolysis to the acids. Further transformations of 4-(nitrobenzyl)-5-acylspinacine methyl esters to give additional analogues not directly accessible are shown in Scheme II. An alternate strategy especially useful when 1-substituted analogues were desired is shown in Scheme IV. Here the 1-alkylation is accomplished at the end of the sequence. An alternative method used early in the program utilized an intermediate phenylhydantoin derivative obtained by reaction of spinacine with phenyl isocyanate (Scheme III). The resulting compound could be alkylated on the imidazole ring under basic conditions

Scheme V.[a] Method E



[a] (a) LAH, THF; (b) TBDMS-Cl, imidazole, THF/DMF; (c) R'COOH, DCC, HOBT, CH_3CN; (d) 2% HF/CH_3CN.

with reactive halides such as benzyl bromide. This method affords a mixture of 1- and 3-substituted analogues. The hydantoins were separated and converted by basic hydrolysis in a stepwise manner, first to a urea analogue, and finally to the substituted spinacine. Acylation was effected in many instances directly on the amino acid by the Schotten–Bauman method.[41] Although the isomer mixture was separable in some cases, hydrolysis was frequently difficult and this approach destroyed optical activity. In addition, angular alkylation in the hydantoin ring occasionally occurred, making product purification difficult. This method was not frequently employed after the regioselective methodologies had been developed. For the latter methods, optical activity was largely preserved in the regioselective pathway, although partial racemization was demonstrated in at least one case. The reduced carboxyl analogues were prepared by the sequence depicted in Scheme V. A procedure analogous to method A was used for the preparation of the 6-descarboxy analogues **86** and **94** with bis-Boc-histamine as starting material instead of bis-Boc-histidine methyl ester.

Biology

A receptor binding assay was developed based on literature recommendations and procedures.[44–46] It is important to note that this assay used whole rat adrenal gland and was run in the presence of 5 mM DTT. This provided a stable and reproducible assay that gave the expected displacement of ^{125}I-Ang II binding for Ang II, Ang III, and the peptide antagonists [Sar^1,Ile^8]Ang II and [Sar^1,Ala^8]-Ang II. IC_{50}'s for displacement were 1.28 ± 0.77, 1.7, 0.31 ± 0.15, and 1.61 ± 0.82 nM, respectively.[37,45] It has been shown by several workers that DTT at these concentrations can abolish the binding of Ang II in some preparations.[22,23,30,31] On the basis of our own work[33] and upon recent receptor characterization studies using these agents and DuP 753,[26,29] it is now clear that the vascular Ang II receptor is not the target of these analogues and that the adrenal binding activity being measured originates from the adrenal medulla, which has been shown to be a rich

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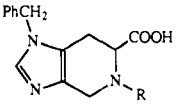
Table I. 1-Benzyl-5-acyl-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acids



no.	R	IC_{50},[a] μM	method[b]	$[\alpha]_D$,[c] deg	mp,[i] °C	formula	anal.[d]
2	CONHPh	2.1	C** [41]	*	270–280	$C_{21}H_{20}N_4O_3 \cdot 0.9H_2O$	C, H, N, H_2O
3	CON(Me)Ph	1.2	C[41]	*	192–195 d	$C_{22}H_{22}N_4O_3 \cdot 0.25H_2O$	C, H, N, H_2O
3a	CON(Me)Ph	1.1	A	nd	173–180	$C_{22}H_{22}N_4O_3 \cdot 0.25H_2O$	C, H, N
4	$CONHCH_2Ph$	11	C	*	182–184	$C_{22}H_{22}N_4O_3$	C, H, N
5	$CON(Me)CH_2Ph$	4.1	C	*	172–174 d	$C_{23}H_{24}N_4O_3$	C, H, N
6	COOPh	4.6	C	*	159–161 d	$C_{21}H_{19}N_3O_4 \cdot 0.12H_2O$	C, H, N, H_2O
7	$COOCH_2Ph$	14.0	C	*	123–127	$C_{22}H_{21}N_3O_4$	H, N; C[e]
7a	$COOCH_2Ph$	4.2	A	16.4, *c* 1.06 DMSO	208–210 d	$C_{22}H_{21}N_3O_4$	C, H, N
8	SO_2CH_2Ph	19	C[41]	*	190–192	$C_{21}H_{21}N_3O_4S$	C, H, N
9	SO_2-4-MeC_6H_4	4.2	C	*	259–261	$C_{21}H_{21}N_3O_4S \cdot 0.25H_2O$	C, H, N; H_2O[f]
10	$CONHCH(CH_3)_2$	21	C	*	170–180 d	$C_{18}H_{22}N_4O_3 \cdot 0.5H_2O$	C, H, N, H_2O
11	$COCH_3$	4% at 10[g]	C[41]	*	225–227	$C_{16}H_{17}N_3O_3$	C, H, N
12	COPh	12% at 10[g]	C[41]	*	236–238	$C_{21}H_{19}N_3O_3$	C, H, N
13	COCOPh	2.4	A	*	244–261	$C_{22}H_{19}N_3O_4$	C, H, N
14	$COCH_2Ph$	1.4	C[41]	*	221–223	$C_{22}H_{21}N_3O_3$	C, H, N
14a	$COCH_2Ph$	0.74	A	17.2 *c* 0.22, MeOH	215–217.5 d	$C_{22}H_{21}N_3O_3$	C, H, N
15	$COCH_2OPh$	4	C	*	205–208	$C_{22}H_{21}N_3O_4$	C, H, N
16	$COCH_2CH_2Ph$	13	C	*	205–207	$C_{23}H_{23}N_3O_3$	C, H, N
17	COCH=CHPh	2.5	C	*	242–244	$C_{23}H_{21}N_3O_3$	C, H, N
18	$COCHPh_2$	1.0	C[41]	*	167–169	$C_{28}H_{25}N_3O_3$	C, H, N
18a	$COCHPh_2$	0.44	A	10.5, *c* 1.1, DMF	220–225 d	$C_{28}H_{25}N_3O_3 \cdot 0.5H_2O$	C, H, N
19	$COCH_2CHPh_2$	1.2	A	−81.4, *c* 1.35, MeOH	239–243	$C_{29}H_{27}N_3O_3 \cdot H_2O$	C, H, N
20	CO(9-fluorenyl)	8.0	C	*	229–231	$C_{28}H_{23}N_3O_3$	C, H, N
21	CO(1-naphthyl)	9.8	A	nd	261–268	$C_{26}H_{23}N_3O_3 \cdot H_2O$	C, H, N
22		0.071	A	18.2, *c* 1.31, MeOH	soft 144–158, 221–225	$C_{27}H_{29}N_3O_3 \cdot 0.5H_2O$	C, H, N
23	$COCH_2(4\text{-}ClC_6H_4)_2$	6.8	A	nd	162–170	$C_{28}H_{23}Cl_2N_3O_3$	C, H, N
24	$COCH_2(4\text{-}CH_3C_6H_4)_2$	1.3	A	7.1, *c* 1.09, MeOH	132–150	$C_{30}H_{29}N_3O_3$	C, H, N
25	$COCH_2(4\text{-}OCH_3C_6H_4)_2$	0.61	A	nd	130–150	$C_{30}H_{29}N_3O_5 \cdot 0.5H_2O$	C, H, N
26	$COCH_2(4\text{-}NO_2C_6H_4)_2$	13	A	1.1, *c* 1.04, MeOH	154–158	$C_{28}H_{23}N_5O_7 \cdot 0.8H_2O$	C, H, N
27	$COCH_2(4\text{-}NH_2C_6H_4)_2$	8.4	A	nd	252–258	$C_{28}H_{27}N_5O_3$	C, H, N
28	$COCH_2(4\text{-}FC_6H_4)_2$	2.1	A	11.3, *c* 0.96, MeOH	152–164	$C_{28}H_{23}F_2N_3O_3 \cdot H_2O$	C, H, N
29	CONHPh, 3-Bn[h]	24% at 10[g]	C	*	soft 152–155, 227–229	$C_{21}H_{20}N_4O_3 \cdot 0.75H_2O$	C, H, N
30	COCH=CHPh, 3-Bn[h]	29	C	*	254–256 d	$C_{23}H_{21}N_3O_3 \cdot HCl$	C, H, N

[a] IC_{50} values determined with four dose levels of each inhibitor in the assay system described in the Experimental Section. [b] Method refers to general route to unacylated spinacine intermediate used; acylation methods are described in ref 41; ** see the Experimental Section for specific details. [c] An asterisk denotes a racemic compound; optically active analogues all have the *S* configuration at position 6; nd = not determined. [d] Analytical results were within ±0.4% of the theoretical values unless otherwise noted. [e] C: calcd, 67.51; found, 67.03. [f] H_2O: calcd, 1.08; found, 1.57. [g] Percent inhibition at a concentration of 10 μM. [h] Imidazole benzyl group at 3-position. [i] A d denotes decomposition.

source of Ang II receptors.[47] Furthermore, it has been shown that DTT selectively deactivates the vascular Ang II receptor, which appears to be the same as the adrenal cortical receptor, and unmasks the nonvascular binding site as a secondary component of Ang II binding. It is this latter component that is inhibited selectively under the conditions of our assay, and the SAR reported here pertains to this activity. The standard rabbit aorta tissue assay[2] and in vivo antihypertensive assays, both in renin-dependent (1K, 2C) Goldblatt hypertensive rats[48] and in spontaneously hypertensive rats (SHR),[49] were used in the initial search for functional activity of the compounds reported here, before their unique properties of the latter were appreciated.

Results and Discussion

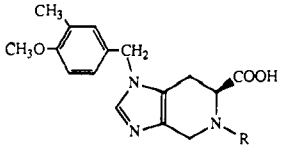
Early analogues of 1-benzylspinacine (Table I) showed that acylation at N^5 with aralkyl acids was needed for activity, and further that there seemed to be a preference for a two-atom spacing between the aryl group and the ring N. These trends can be seen from the compounds listed in Tables I and II (cf. **2** vs **4**, **3** vs **5**, **14** vs **16**, or **36** vs **37**). Most of the compounds in Table I were only obtained in racemic form. The diphenylacetyl analogue **18** stood out as the best substituent of this group from early work (**22** was prepared late in the program). This preference was carried over to the analogues listed in Table II, where the more effective 3-Me,4-OMe-benzyl substituent was employed at N^1. The latter compounds all have the *S* configuration. Purely aliphatic side chains (**34**, **35**) are much less potent than aralkyl analogues of comparable lipophilicity. There is apparently a preference for suitably large groups containing a phenyl moiety and a properly shaped lipophilic moiety. The ability of the aryl group to adopt a favored conformation is evident from the differences in activity observed when it is constrained to adopt

(47) Israel, A.; Masami, N.; Plunkett, L. M.; Saavedra, J. M. High-affinity angiotensin receptors in rat adrenal medulla. *Regul. Pept.* **1985**, *11*, 237–243.

(48) For a description of the method used, see: Blankley, C. J.; Kaltenbronn, J. S.; DeJohn, D. E.; Werner, A.; Bennett, L. R.; Bobowski, G. R.; Krolls, U.; Johnson, D. R.; Pearlman, W. M.; Hoefle, M. L.; Essenburg, A. D.; Cohen, D. M.; Kaplan, H. R. Synthesis and Structure–Activity Relationships of Potent New Angiotensin Converting Enzyme Inhibitors Containing Saturated Bicyclic Amino Acids. *J. Med. Chem.* **1987**, *30*, 992–998.

(49) For a description of the method used, see: Bennett, L. R.; Blankley, C. J.; Fleming, R. W.; Smith, R. D.; Tessman, D. K. Antihypertensive Activity of 6-Arylpyrido[2,3-*d*]pyrimidin-7-amine Derivatives. *J. Med. Chem.* **1981**, *24*, 382–389.

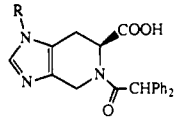
Table II. 1-(3-Methyl-4-methoxybenzyl)-5-acyl-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acids



no.	R	IC_{50},[a] μM	method[b]	$[\alpha]_D$,[c] deg	mp,[h] °C	formula	anal.[d]
31	CON(Me)Ph	0.14	A	nd	132–164 d	$C_{24}H_{26}N_4O_4 \cdot 0.5H_2O \cdot 0.5CH_3CN$	C, H, N
32	$COCH_2Ph$	0.53	A	nd	240–241	$C_{24}H_{25}N_3O_4$	C, H, N
33	COCH(Me)Ph	0.56	A	nd	223 d	$C_{25}H_{27}N_3O_4$	C, H, N
34	$COCH_2$-c-C_6H_{11}	2.0	A	nd	225–236	$C_{24}H_{31}N_3O_4 \cdot CH_3OH$	C, H, N
35	$COCH_2$(1-Ad)	28	A	23.1, *c* 1.09, DMF	210 d	$C_{28}H_{35}N_3O_4$	C, H, N
36	$COCHPh_2$	0.070	A	11.1, *c* 1.16, DMF	220–225 d	$C_{30}H_{29}N_3O_4$	C, H, N
37	$COCH_2CHPh_2$	4.2	A	nd	soft 127, 176–188	$C_{31}H_{31}N_3O_4 \cdot 1.25CH_3OH$	C, H, N
38	COCH=CHPh	2.2	A	−6.44, *c* 1.21, DMF	246–251	$C_{25}H_{25}N_3O_4 \cdot 0.75H_2O$	C, H, N
39	$COCH(Ph)CH_2Ph$	3.5	A	10.1, *c* 1.07, DMF	135–175	$C_{31}H_{31}N_3O_4$	C, H, N
40	CO(9-fluorenyl)	27% at 10[e]	A	−12.8, *c* 1.09, DMF	200–205	$C_{30}H_{27}N_3O_4$	C, H, N
41	$COC(CH_3)Ph_2$	9.5	A	−21.6, *c* 1.06, DMF	210 d	$C_{31}H_{31}N_3O_4$	C, H, N
42		0.085	A	nd	140–149	$C_{30}H_{35}N_3O_4$	C, H, N
43		0.067	A	20.9, *c* 1.16, DMF	190 d	$C_{29}H_{33}N_3O_4$	C, H; N[f]
44		0.11	A	5.6, *c* 1.02, DMF	145 d	$C_{29}H_{33}N_3O_4$	C, H, N
45		0.84	A	21.9, *c* 1.17, MeOH	195 d	$C_{28}H_{31}N_3O_4 \cdot 0.5H_2O$	C, H, N
46	$COCH(4\text{-}FC_6H_4)_2$	2.3	A	13.7, *c* 1.08, MeOH	221–231	$C_{30}H_{27}F_2N_3O_4$	C, H, N, F
47	$COCH(4\text{-}MeC_6H_4)_2$	0.51	A	4.9, *c* 1.09, MeOH	127–149	$C_{32}H_{33}N_3O_4 \cdot H_2O$	C, H, N
48	$COCH(4\text{-}ClC_6H_4)_2$	2.4	A	nd	145–154	$C_{30}H_{27}Cl_2N_3O_4 \cdot 0.5H_2O$	C, H, N, Cl
49	$COCH(4\text{-}OMeC_6H_4)_2$	2.0	A	nd	130–148	$C_{32}H_{33}N_3O_6 \cdot H_2O$	C, H, N
50	$COCH(4\text{-}NO_2C_6H_4)_2$	13	A	nd	152–158	$C_{30}H_{27}N_5O_6 \cdot 0.9H_2O$	C, H; N[g]

[a] IC_{50} values determined with four dose levels of each inhibitor in the assay system described in the Experimental Section. [b] Method refers to general route to unacylated spinacine intermediate used; acylation methods are described in ref 41. [c] All compounds are optically active and have the *S* configuration at position 6; nd = not determined. [d] Analytical results were within ±0.4% of the theoretical values unless otherwise noted. [e] Percent inhibition at a concentration of 10 μM. [f] N: calcd, 8.62; found, 8.15. [g] N: calcd, 11.63; found, 10.21. [h] A d denotes decomposition.

Table III. 1-(Substituted)-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acids



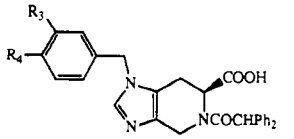
no.	R	IC_{50},[a] μM	method[b]	$[\alpha]_D$,[c] deg	mp,[f] °C	formula	anal.[d]
51	CH_2-c-C_6H_{11}	0.34	A	nd	238–241	$C_{28}H_{31}N_3O_3 \cdot H_2O$	C, H, N
52	CH_2CH_2-c-C_6H_{11}	0.24	A	nd	137–140	$C_{29}H_{33}N_3O_3 \cdot 0.2H_2O$	C, H, N
53	CH_2CH_2(1-Ad)	0.13	A[41]	nd	168–175	$C_{33}H_{37}N_3O_3 \cdot 0.33H_2O$	C, H, N
18a	CH_2Ph	0.44	*e*	*e*	*e*	*e*	*e*
54	CH_2CH_2Ph	0.24	D	1.1, *c* 1.1	238–240 d	$C_{29}H_{27}N_3O_3 \cdot 0.2H_2O$	C, H, N
55	$CH_2CH_2CH_2Ph$	0.7	D	2.4, *c* 1.1	125–140	$C_{30}H_{29}N_3O_3 \cdot 0.33H_2O$	C, H, N
56	$CH(CH_3)Ph$	1.1	D	4.8, *c* 2	239–245 d	$C_{29}H_{27}N_3O_3$	C, H, N
57	$CHPh_2$	0.11	D	2.0, *c* 1	220–240 d	$C_{34}H_{29}N_3O_3 \cdot 0.33H_2O$	C, H, N
58	CH_2-(2-naphthyl)	0.27	D	3.5, *c* 1	180 d	$C_{32}H_{27}N_3O_3$	C, H, N
59	CH_2-(3-pyridyl)	0.12	D	3.3, *c* 1.2	250 d	$C_{27}H_{24}N_4O_3$	C, H, N

[a] IC_{50} values determined with four dose levels of each inhibitor in the assay system described in the Experimental Section. [b] Method refers to general route to general route described in the text and the Experimental Section. [c] All compounds are optically active (determined in DMF) and have the *S* configuration at position 6; nd = not determined. [d] Analytical results were within ±0.4% of the theoretical values unless otherwise noted. [e] See Table I. [f] A d denotes decomposition.

specific orientations (cf. **36** vs **40–45**). Substituents on the diphenylacetyl moiety are uniformly detrimental to potency (**23–28** and **46–50**). Finally, there was a strong indication that 1-substitution on the imidazole ring was preferred to 3-substitution (**2** and **17** vs **29** and **30**).

Tables III and IV show the effect of varying the 1-substituent in the 5-diphenylacetyl series. Again, bulky, lipophilic groups give good activity with no apparent preference in this region for an aromatic ring with specific spacing relative to the spinacine ring (Table III). Examination of aryl substitution in the 1-benzyl series, however, revealed that striking improvements in potency could be obtained with variations in both the nature and pattern of substitution. In particular, occupation of both the 3- and 4-positions gave highly potent analogues, and a clear preference for strongly electron donating groups, especially in the 4-position, was seen. Thus, the most potent analogues had a 3-Me or -Et group combined with a 4-amino,

Table IV. 1-(Substituted-benzyl)-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acids



no.	R_3	R_4	IC_{50},[a] μM	method[b]	$[\alpha]_D$,[c] deg	mp,[h] °C	formula	anal.[d]
18a	H	H	0.44	*e*	*e*	*e*	*e*	*e*
60	H	OCH_3	0.29	A	nd	150–160 d	$C_{29}H_{27}N_3O_4$	C, H, N
61	H	F	0.45	A	nd	158–162	$C_{28}H_{24}FN_3O_3 \cdot 0.7H_2O$	C, H, N, F
62	H	NO_2	0.61	A	nd	210–215 d	$C_{28}H_{24}N_4O_5$	C, H, N
63	H	NH_2	0.15	A	nd	214–216 d	$C_{28}H_{26}N_4O_3$	C, H, N
64	H	$NHCOCH_3$	0.35	B	nd	254–256	$C_{30}H_{28}N_4O_4 \cdot H_2O$	C, H, N
65	H	NMe_2	0.085	A	nd	166–170	$C_{30}H_{30}N_4O_3$	C, H, N
66	H	NEt_2	0.29	A	nd	177–205	$C_{32}H_{34}N_4O_3$	C, H; N[f]
67	H	CF_3	0.19	A	nd	225–227 d	$C_{29}H_{24}F_3N_3O_3 \cdot 0.5H_2O$	C, H, N
68	CH_3	H	0.15	D	nd	134–145	$C_{29}H_{27}N_3O_3 \cdot 0.33H_2O$	C, H, N
69	CF_3	H	0.46	D	nd	215–225	$C_{29}H_{24}F_3N_3O_3$	C, H, N
70	Cl	H	1.2	D	7.9, *c* 0.93	137–152	$C_{28}H_{24}ClN_3O_3 \cdot 0.1H_2O$	C, H, N, Cl
71	Br	H	0.65	D	8.5, *c* 1.1	195–215 d	$C_{28}H_{24}BrN_3O_3 \cdot 0.33H_2O$	C, H, N, Br
72	OCH_3	H	0.12	D	9.9, *c* 1.1	213–216 d	$C_{29}H_{27}N_3O_4 \cdot 0.33H_2O$	C, H, N
73	CH_3	CH_3	0.30	D	9.2, *c* 2.1	238–240	$C_{30}H_{29}N_3O_3 \cdot 0.33H_2O$	C, H, N
74	CH_3	H(5-CH_3)	0.14	D	3.9, *c* 1.0	140–158	$C_{30}H_{29}N_3O_3 \cdot 0.5H_2O$	C, H, N
75	CH_3	OH	0.056	A	12.4, *c* 1.08	>200 d	$C_{29}H_{27}N_3O_4 \cdot 0.5H_2O$	C, H, N
36	CH_3	OCH_3	0.070	*g*	*g*	*g*	*g*	*g*
76	CH_3	H(6-OCH_3)	0.72	D**	5.09, *c* 1.06	135–154	$C_{30}H_{29}N_3O_4$	C, H, N
77	CH_3	NO_2	0.29	A**	2.3, *c* 1.12	190–192 d	$C_{29}H_{26}N_4O_5$	C, H, N
78	CH_3	NH_2	0.066	A**	15.9, *c* 1.03	200–205 d	$C_{29}H_{28}N_4O_3 \cdot 0.25H_2O$	C, H, N
79	CH_3	NMe_2	0.034	A**	19.0, *c* 0.97	>200 d	$C_{31}H_{32}N_4O_3$	C, H, N
80	CH_3	$NHCOCH_3$	0.089	B	7.9, *c* 2.0	175–180	$C_{31}H_{30}N_4O_4 \cdot 0.33H_2O$	C, H, N
81	CH_3	$NHCONHCH_3$	0.044	B	8.5, *c* 2.1	198–201 d	$C_{31}H_{31}N_5O_4 \cdot 0.33H_2O$	C, H, N
82	CH_2CH_3	OCH_3	0.050	D	8.8, *c* 1.01	137–146	$C_{31}H_{31}N_3O_4$	C, H, N
83	OCH_3	OCH_3	0.098	D**	6.0, *c* 1.0	140–150	$C_{30}H_{29}N_3O_5 \cdot 0.33H_2O$	C, H, N
84	F	OCH_3	0.26	D	11.03, *c* 1.07	170–195	$C_{29}H_{26}FN_3O_4$	C, H, N, F
85	NO_2	OCH_3	0.051	A	nd	183–217	$C_{29}H_{26}N_4O_6 \cdot 1.5H_2O$	C, H, N

[a] IC_{50} values determined with four dose levels of each inhibitor in the assay system described in the Experimental Section. [b] ** See the Experimental Section for specific details. [c] All compounds are optically active (determined in DMF) and have the *S* configuration at position 6; nd = not determined. [d] Analytical results were within ±0.4% of the theoretical values unless otherwise noted. [e] See Table I. [f] N; calcd, 10.60; found, 10.11. [g] See Table II. [h] A d denoted decomposition.

-amide, or -oxy function (**75**,**78**–**82**). A QSAR analysis for these substituted-benzyl analogues (Table VII) confirms these observations by showing highly significant correlations with a combination of total electron donating character (*R*) and an indicator variable for the presence of a 3-alkyl substituent (IND_3A) (eqs 1–4). The correlation

	n	R^2	*s*	*F*	
$\log(1/IC_{50}) = 6.56 - 0.49R$	30	0.21	0.37	7.3	(1)
$\log(1/IC_{50}) = 6.46 - 0.41R + 0.31IND\_3A$	30	0.34	0.35	7.1	(2)
$\log(1/IC_{50}) = 7.00 - 0.40R + 0.32IND\_3A - 0.16\pi$	30	0.43	0.33	6.6	(3)
$\log(1/IC_{50}) = 6.46 - 0.44R + 0.40IND\_3A - 0.14\pi$	29	0.56	0.28	10.6	(4)

matrix of Table VIII shows the independence of considered and included parameters. Liphophilicity, as measured by π,[50] is barely significant in eqs 3 and 4 and carries a negative sign. This may suggest that either a size or lipophilicity optimum has been surpassed by the larger analogues in the set. Substituent molar refractivity (MR) shows no significant correlation with activity. The one analogue, **76**, with a substituent adjacent to the benzylic methylene bridge to the imidazole ring, was an outlier in this analysis, suggesting a negative influence for substitution in this region. Equation 4, lacking this compound, was the best obtainable with this set of data.

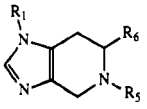
Variations in the group at C^6 were examined briefly (Table V). Carboxylic acid isomers with *S* configuration at this position were found to be more active than their racemic counterparts. In the cases where both enantiomers were prepared, the (*R*)-carboxylic acid analogues of **18a**, **87**, **79**, and **97** were seen to be less active than the corresponding *S* isomers. (The measured value for **97** is inflated due to the presence of up to 18% of the more active *S* isomer **79**). Methyl esters were routinely about 10-fold less active than the corresponding acids. The reduced alcohol analogues were more variable in their potency relative to the corresponding acids, but again were usually substantially less active than the acids. Two examples entirely lacking a 6-substituent (**86**, **94**) showed weak activity. One amide example, **89**, was inactive.

In general, the effect of substituents at various positions was only partly additive, and many cases were observed where the activity of a particular combination was no better than the worst of the groups combined. This seemed especially true of the bis(4-substituted)diphenylacetyl compounds, which did not show the activity enhancement provided by 1-(substituted-benzyl) groups in other subseries. Rather precise requirements for optimal binding exist for the whole molecule, and the sensitivity to changes in one region of the molecule increases as another portion is optimized.

In view of the original rationale for examining this series, it is of interest to note that several of the SAR trends noted here show rough parallels to reported SAR for Ang II peptides. The behavior of the 6-position COOH function relative to esters and alcohols is quite similar to that of the required C-terminal COOH of Ang II in agonists.[51] The proximity of a required aromatic group of proper

(50) Pomona College MedChem software, v 3.54., Daylight Systems, Inc.

Table V. Additional Analogues with Position 6 Variations



no.	R_1	R_5	R_6	IC_{50},[a] μM	method[b]	$[\alpha]_D$,[c] deg	mp, °C	formula	anal.[d]
86	CH_2Ph	$COCHPh_2$	H	6.1	**	*	134–136	$C_{27}H_{25}N_3O$	C, H, N
87	CH_2Ph	$COCHPh_2$	COOH (*R*)	1.2	A	−11.0, *c* 1.06, DMF	149–154	$C_{28}H_{25}N_3O_3 \cdot 0.5H_2O$	C, H, N
88	CH_2Ph	$COCH_2Ph$	COOMe	8.8	C[40]	*	127–129	$C_{29}H_{27}N_3O_3$	C, H, N
89	CH_2Ph	CONHPh	$CONH_2$	14% at 10[e]	C[40]	*	273–277	$C_{21}H_{21}N_5O_2 \cdot 0.13CHCl_3$	C, H, N, Cl
90	CH_2Ph	$COCH_2Ph$	CH_2OH	5.2	E	nd	71–81	$C_{22}H_{23}N_3O_2 \cdot 0.25H_2O$	C, H, N
91	CH_2Ph	CON(Me)Ph	CH_2OH	1.6	E	70.5, *c* 1.04, MeOH	65–75	$C_{22}H_{24}N_4O_2 \cdot 0.3CHCl_3$	C, H, N
92	CH_2Ph		CH_2OH	6.7	E	7.60, *c* 1.04, DMF	105–110	$C_{31}H_{31}N_3O_4$	C, H, N
93	CH_2Ph	$COCHPh_2$	CH_2OH	2.1	E	49.3, *c* 1.03, DMF	223–230	$C_{28}H_{27}N_3O_2 \cdot 0.25H_2O$	C, H, N
94	3-Me,4-$OMeC_6H_3CH_2$	$COCHPh_2$	H	4.4	***	*	101–114	$C_{30}H_{29}N_3O_3 \cdot H_2O$	C, H, N
95	3-Me,4-$OMeC_6H_3CH_2$	CONMePh	CH_2OH	0.52	E	68.0, *c* 1.01, DMF	137–140	$C_{24}H_{28}N_4O_3$	C, H, N
96	3-Me,4-$OMeC_6H_3CH_2$	$COCHPh_2$	CH_2OH	0.37	E	16.3, *c* 1.16, DMF	164–170	$C_{30}H_{31}N_3O_3 \cdot 0.5H_2O$	C, H, N
97	3-Me,4-$NMe_2C_6H_3CH_2$	$COCHPh_2$	COOH (*R*)[f]	0.097	A	−14.4 *c* 1.05, DMF	255–256 d	$C_{31}H_{32}N_4O_3 \cdot 0.4H_2O$	C, H, N
98	3-Me,4-$NMe_2C_6H_3CH_2$	$COCHPh_2$	COOMe	0.35	A	3.1, *c* 2, DMF	165 d	$C_{32}H_{34}N_4O_3 \cdot 2HCl \cdot 0.33H_2O$	C, H, N
99	3-Me,4-$NMe_2C_6H_3CH_2$	$COCHPh_2$	CH_2OH	0.20	E	12.0, *c* 1.08, DMF	178–185	$C_{31}H_{34}N_4O_2$	C, H, N
100	3-Me,4-$NO_2C_6H_3CH_2$	$COCHPh_2$	COOMe	5.1	A	14.2, *c* 2, $CHCl_3$	168–171	$C_{30}H_{28}N_4O_5$	C, H, N
101	3-Me,4-$NO_2C_6H_3CH_2$	$COCHPh_2$	CH_2OH	2.0	E	nd	187–195	$C_{29}H_{28}N_4O_4$	C, H; N[g]

[a] IC_{50} values determined with four dose levels of each inhibitor in the assay system described in the Experimental Section. [b] **See the Experimental Section for specific details; ***same method as for 86. [c] An asterisk denotes racemic compound; all others have the *S* configuration at position 6, unless noted; nd = not determined. [d] Analytical results were within ±0.4% of the theoretical values unless otherwise noted. [e] Percent inhibition at a concentration of 10 μM. [f] Chiral HPLC analysis indicated this to be a 18:82 mixture of *S*:*R* enantiomers. [g] N: calcd, 11.88; found, 11.17. [h] A d denotes decomposition.

Table VI. Brain vs Adrenal Binding

	IC_{50}, μM			IC_{50}, μM	
no.	adrenal	brain	no.	adrenal	brain
79	0.034	0.21	**68**	0.15	1.6
18a	0.44	3.8	**96**	0.37	0.13
78	0.066	0.58	**55**	0.70	0.40
20	8.0	19.0	Ang II	0.0013	0.004
23	6.8	8.2	[Sar[1],Ala[8]]Ang II	0.0016	0.0051
67	0.19	0.8	[Sar[1],Ile[6]]Ang II	0.00035	0.0001
53	0.13	0.17			

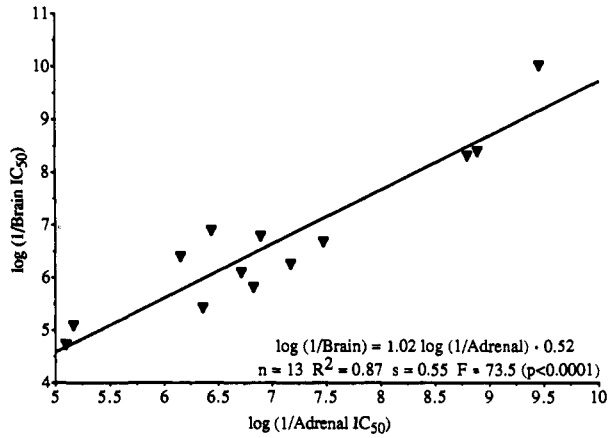


Figure 1. Brain vs adrenal gland binding (data of Table VI plotted).

spacing and shape to this COOH further strengthens the view that the 5,6-region of the spinacines may be recognized by the region of the receptor interacting with the C-terminal residue of Ang II, especially agonists. Further analogy comes with a recent report that [Sar[1],Diphenylalanine[8]]AngII is an even more potent agonist than Ang II itself.[52] The spinacine ring system would then have the spacer function of His[6]-Pro[7] in Ang II, possibly picking up a H-bonding interaction of His[6] as well. The spinacine 1-position could then be viewed as interacting with receptor binding regions recognizing either or both of Tyr[4]-Ile[5]. The bulk-tolerant nature of the Ile[5] site[53,54] could accommodate either aromatic or aliphatic residues at N^1. The substituted aromatic groups might, alternatively, access the Tyr[4] site. Especially striking is the evident preference for electron-donating groups in the benzyl ring of the spinacine analogues. The QSAR analysis closely parallels observations made by Guillemette et al.[55] for [X[4]]Ang II agonists. Interestingly, a similar correlation does not seem to hold for [X[4]]Ang II antagonists.[56]

An early indication of the unique character of these compounds came when several of the most potent of them were examined for their ability to inhibit angiotensin binding in other tissues. In most cases, negative results were obtained. For example, PD 123319 (**79**) showed no

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Table VII. Compounds and Parameters Used in QSAR Analysis

									eq 4	
no.	IC_{50}	log $(1/IC_{50})$	π^a	MR/10[a]	σ^b	F^b	R^b	IND_3A[c]	predicted	residual
18a	0.44	6.357	0	0	0	0	0	0	6.46	−0.11
36	0.07	7.155	0.42	1.081	−0.34	0.22	−0.64	1	7.08	0.07
58	0.27	6.569	1.17	1.688	0.04	0.03	0.01	0	6.29	0.27
59	0.12	6.921	−1.48	−0.211	0.73	0.65	0.15	0	6.61	0.31
60	0.29	6.538	−0.08	0.617	−0.27	0.26	−0.51	0	6.70	−0.16
61	0.45	6.347	0.14	0.016	0.06	0.43	−0.34	0	6.59	−0.25
62	0.61	6.215	−0.26	0.726	0.78	0.67	0.16	0	6.43	−0.22
63	0.15	6.824	−1.23	0.369	−0.66	0.02	−0.68	0	6.93	−0.11
64	0.35	6.456	−0.98	1.332	0	0.28	−0.26	0	6.72	−0.26
65	0.085	7.071	0.20	1.296	−0.83	0.10	−0.92	0	6.84	0.23
66	0.29	6.538	1.09	2.224	−0.90	0.01	−0.91	0	6.71	−0.17
67	0.19	6.721	0.88	0.510	0.54	0.38	0.19	0	6.26	0.46
68	0.15	6.824	0.50	0.464	−0.07	−0.04	−0.13	1	6.85	−0.02
69	0.46	6.337	0.88	0.510	0.43	0.38	0.19	0	6.26	0.08
70	1.2	5.921	0.71	0.491	0.37	0.41	−0.15	0	6.43	−0.51
71	0.65	6.187	0.86	0.777	0.39	0.39	−0.17	0	6.42	−0.23
72	0.12	6.921	−0.08	0.617	0.12	0.26	−0.51	0	6.70	0.22
73	0.3	6.523	1.00	0.928	−0.24	−0.08	−0.26	1	6.83	−0.31
74	0.14	6.854	1.00	0.928	−0.14	−0.08	−0.26	1	6.83	0.02
75	0.056	7.252	−0.17	0.617	−0.44	0.25	−0.77	1	7.22	0.03
76	0.72	6.143	0.42	1.081	−0.34	0.22	−0.64	1	*7.08*[d]	
77	0.29	6.538	−0.04	1.189	0.71	0.63	0.03	1	6.85	−0.32
78	0.066	7.180	−0.73	0.833	−0.73	−0.02	−0.81	1	7.32	−0.14
79	0.034	7.469	0.69	1.760	−0.90	0.06	−1.05	1	7.22	0.25
80	0.089	7.051	−1.29	1.796	−0.07	0.24	−0.39	1	7.21	−0.16
81	0.044	7.357	−1.10	2.165	−0.31	−0.32	−0.16	1	7.09	0.27
82	0.05	7.301	0.95	1.545	−0.34	0.19	−0.61	1	6.99	0.31
83	0.098	7.009	−0.55	1.234	−0.15	0.52	−1.02	0	6.99	0.02
84	0.26	6.585	0.20	0.632	0.07	0.69	−0.85	0	6.81	−0.22
85	0.051	7.292	−0.32	1.342	0.44	0.93	−0.35	0	6.66	0.63

[a] Calculated by Pomona MedChem CLOGP program, v. 3.54; π = CLOGP(X) − CLOGP(18a); MR = CMR(X) − CMR(18a); CLOGP(18a) = 3.295, CMR(18a) = 13.103. [b] Taken from ref 59; sum for all substituents. [c] Presence of methyl or ethyl group at meta position to ring attachment. [d] Compound not used in derivation of eq 4; predicted value.

Table VIII. Correlation Matrix (r) of Parameters and Activity (n = 30)

	π	MR/10	σ	F	R	IND_3A
π	1					
MR/10	0.10	1				
σ	−0.05	−0.43	1			
F	−0.16	−0.30	0.68	1		
R	0.04	−0.36	0.80	0.18	1	
IND_3A	0.05	0.34	−0.35	−0.44	−0.19	1
log $(1/IC_{50})$	−0.03	0.39	−0.45	−0.21	−0.45	0.45

activity in rat adrenal cortical preparations in the absence of DTT, rat aorta, rat mesentery, rat liver, or rat pituitary Ang II receptor preparations. We have reported that binding is observed in rat uterus,[33] and others have observed binding in rat ovaries.[57] Preliminary studies in our hands in rat brain under conditions reported by Snyder et al.[45] showed that although the brain preparation was slightly less sensitive than adrenal to the spinacines, Ang II binding inhibition activity was observed for analogues in the series possessing several structure variations with potencies which paralleled those found in the adrenal (Table VI, Figure 1). Selectivity of one of the compounds reported in this paper (**36**) for brain vs adrenal has also been reported by others.[34,35]

Selected compounds of this series were examined for their ability to inhibit binding in other receptor assays. No significant inhibition was found in dopamine (haloperidol), adenosine A1 (cyclopentyladenosine) or A2 (*N*-ethyladenosinecarboxamide), α_1-adrenergic (WB-4101), α_2-adrenergic (clonidine), calcium (nitrendipine), benzodiazepine (flunitrazepam), 5-HT_2 (ketanserin), or muscarinic (quinuclidine benzilate) receptor binding assays below 10 μM. Additionally, no inhibition was observed of cyclooxygenase, angiotensin converting enzyme, human renin, or phosphodiesterase 1. Therefore the binding inhibition activity observed is quite specific for a unique Ang II binding subtype. The question of whether these compounds are agonists or antagonists must await the demonstration of a functional correlate of binding at the AT_2 subtype so that their ability to block or mimic an Ang II action at this site can be assessed.

In spite of the potent Ang II binding inhibition of some of these compounds, it was further found that none of them antagonized Ang II-induced contractions in rabbit aortae at concentrations commensurate with their binding potency. In vivo, attempts to find blood pressure lowering effects were generally not promising, although some of the compounds did cause very modest (10–20%) reductions of blood pressure in renal hypertensive rats, following 2–3 days dosing at 30–100 mg/kg. This behavior is in marked contrast to the Du Pont series of selective vascular antagonists such as DuP 753 that show the expected antagonist activity in both of these functional assays.[25,28] Although work is currently in progress to find a functional consequence of the binding inhibition of these compounds, this has so far not met with success. At present, these compounds can best be utilized as tools to dissect some of the aspects of Ang II actions. The discovery of this novel Ang II binding subtype also suggests that the existing peptide receptor binding SAR for angiotensin agonists and antagonists needs to be reexamined with respect to subtype selectivity, especially regarding compounds with "novel" or unusual properties or SAR. On the other hand, some analogues previously judged to be inactive in vascular

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assays may yet show some affinity for this new Ang II binding site. In particular, binding assays employing rat uterus or brain appear to have a substantial proportion of Ang II binding attributable to the nonvascular binding subtype.[19,25,33-35]

In conclusion, we report here a novel type of non-peptide Ang II receptor binding inhibitor that appears to interact with a newly discovered Ang II binding site, which has been referred to as the "type 2" or AT_2 angiotensin receptor.[32] This site has been demonstrated to exist in several tissues and to show a similar affinity for Ang II and its peptide analogues as the more well-known vascular receptor. The function of this binding site has yet to be determined. The compounds described here are very specific discriminators for this receptor subtype and should prove to be of use in further studies of Ang II receptor pharmacology and, perhaps also, physiology. Compounds **36** (PD121,981), **78** (PD123,177), and **79** (PD123,319) are all well-suited for this purpose.

Experimental Section

General. All reagents were of commercial quality from freshly opened containers. Reagent-quality solvents were used without further purification. Analytical TLC plates and silica gel (230–400 mesh) were purchased from EM Reagents. Melting points were taken using a MelTemp apparatus and are uncorrected. All temperatures are reported in degrees Celsius and pressures are reported in mmHg. Room temperature is 25 ± 3 °C. Elemental analyses were obtained by using a Perkin-Elmer 240 elemental analyzer and observed rotations at the Na D line were obtained at 25 °C by using a Perkin-Elmer 240 polarimeter. Proton NMR (^{1}H NMR) spectra were obtained using either a Varian XL 200-MHz spectrometer or a Varian EM-390 90-MHz spectrophotometer and are reported in ppm downfield from Me_4Si. IR spectra were obtained with a Nicolet IR80 spectrophotometer and are reported in inverse centimeters. Mass spectra were obtained by using a VG Model 7070E/HF spectrometer with either DEI or FAB ionization and are reported in atomic mass units.

Method A. a. *N*-[(1,1-Dimethylethoxy)carbonyl]-3-[(3-methyl-4-nitrophenyl)methyl]-L-histidine, Methyl Ester (103). A solution of triflic anhydride (5.55 mL, 33 mmol) in CH_2Cl_2 (50 mL) was chilled to −75 °C under N_2 and treated dropwise over 10 min with a solution composed of 3-methyl-4-nitrobenzyl alcohol (5.52 g, 33 mmol), $(iPr)_2NEt$ (5.75 mL, 33 mmol), and CH_2Cl_2 (50 mL). The resulting mixture was stirred 30 min at −75 °C, and then it was treated with a solution of **102**[42] (11.07 g, 30 mmol) in CH_2Cl_2 (50 mL). The reaction was allowed to warm slowly to room temperature for over 8 h and then was poured into 0.25 M potassium phosphate buffer (pH 7, 300 mL). The resulting mixture was stirred vigorously for 1.5 h. CH_2Cl_2 (250 mL) was added and the organic layer was separated, washed with more of the same buffer, and dried over $MgSO_4$. Evaporation gave a gum that was purified by flash chromatography on silica gel, eluting with $CHCl_3$ then $CHCl_3$–MeOH (99:1) to give **103** (7.8 g, 62%) as a foam upon evaporation: ^{1}H NMR ($CDCl_3$) δ 7.97 (d, 1 H, 5-Ar), 7.53 (s, 1 H, H-2), 7.02 (m, 2 H, 2- + 6-Ar), 6.89 (s, 1 H, H-5), 5.14 (m, 3 H, $ArCH_2$ + NH), 4.40 (q, 1 H, CH), 2.93 (d, 2 H, CH_2), 2.56 (s, 3 H, $ArCH_3$), 1.41 (s, 9 H, tBu). Two batches of this material were combined and used without further characterization below.

b. 3-[(3-Methyl-4-nitrophenyl)methyl]-L-histidine, Dihydrochloride (104). A solution of **103** (13.7 g, 32.8 mmol) in MeOH (20 mL) was treated with 6 N HCl (300 mL) and heated at reflux for 90 min. The resulting solution was allowed to cool to room temperature and evaporated. The residue was dissolved in H_2O (100 mL) and the solution was treated with activated charcoal, filtered through Celite, and evaporated to afford **104** (13.5 g, quantitative) as a wet gum: ^{1}H NMR (D_2O) δ 8.97 (s, 1 H, H-2), 8.10 (d, 1 H, 5-Ar), 7.65 (s, 1 H, H-5), 7.40 (s, 1 H, 2-Ar), 7.37 (d, 1 H, 6-Ar), 5.62 (s, 2 H, $ArCH_2$), 4.04, (t, 2 H, CH), 3.35 (m, 2 H, CH_2). This material was used without further characterization below.

c. (*S*)-4,5,6,7-Tetrahydro-1-[(3-methyl-4-nitrophenyl)methyl]-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acid, Dihydrochloride (105). A solution of **104** (from above, 13.5 g, ca. 32.8 mmol) was treated with 36% aqueous CH_2O (8 mL, 96 mmol) and stirred first at room temperature for 30 min and then at reflux for 90 min. The resulting solution was cooled on an ice bath and evaporated to a gum. The gum was treated with absolute EtOH (50 mL) and warmed on a steam bath until crystallization began. The resulting slurry was further diluted with iPrOH (50 mL) and chilled on an ice bath. After several hours, **105** was collected by filtration and dried at ca. 25 mmHg, 70 °C overnight to afford a pale yellow solid (10.5 g, 83%): mp 260–263 °C dec with gas evol; ^{1}H NMR (D_2O) δ 9.02 (s, 1 H, H-2), 8.05 (d, 1 H, 5-Ar), 7.36 (s, 1 H, 2-Ar), 7.33 (d, 1 H, 6-Ar), 5.58 (AB, 2 H, $ArCH_2$), 4.59 (q, 2 H, H-4), 4.42 (t, 1 H, H-6), 3.37 (dd, 1 H, H-7), 2.99 (dd, 1 H, H-7′); IR (KBr) ν 1706 (COOH); MS (FAB thioglycerol) *m/e* 317 (M^+ + 1); $[\alpha]^{23}_D$ −50.6° (*c* 2.13, H_2O). Anal. ($C_{15}H_{16}N_4O_4 \cdot 2HCl$) C, H, N.

d. (*S*)-4,5,6,7-Tetrahydro-1-[(3-methyl-4-nitrophenyl)methyl]-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acid, Methyl Ester (106). A suspension of **105** (9.25 g, 24.0 mmol) in MeOH (400 mL) was treated with $(MeO)_3CH$ (50 mL) and saturated with anhydrous HCl. The resulting suspension was heated at reflux for 24 h, during which time complete dissolution occurred. Evaporation of the solution gave a foam that was treated with cold 10% Na_2CO_3 (100 mL) and extracted into CH_2Cl_2 (4 × 100 mL). The combined organic fractions were dried over $MgSO_4$ and evaporated to a viscous syrup which slowly crystallized on standing. The resulting crystalline mass was triturated with ether and collected by filtration to give **106** (6.60 g, 83.3%) as a pale yellow solid: mp 100–104 °C; ^{1}H NMR ($CDCl_3$) δ 8.95 (d, 1 H, 5-Ar), 7.48 (s, 1 H, H-2), 7.00 (m, 2 H, 2- + 6-Ar), 5.06 (s, 2 H, $ArCH_2$), 3.98 (m, 2 H, H-4), 3.74 (m + s, 4 H, H-6 + CO_2Me), 2.69 (m, 2 H, H-7), 2.57 (s, 3 H, $ArCH_3$), 2.21 (br, 1.5 H, NH^+, H_2O); IR (KBr) ν 3315 (NH), 1733 (CO_2Me); MS (DEI) *m/e* 330 (M^+), 271 (base peak, $M^+ - CO_2Me$); $[\alpha]^{23}_D$ −26.0° (*c* 1.02, DMF). Anal. ($C_{16}H_{18}N_4O_4 \cdot 0.25H_2O$) C, H, N.

e. (*S*)-5-(Diphenylacetyl)-4,5,6,7-tetrahydro-1-[(3-methyl-4-nitrophenyl)methyl]-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acid, Methyl Ester (100). A mixture of DCC (16.7 g, 81.0 mmol), HOBT·H_2O (10.9 g, 81.0 mmol), and diphenylacetic acid (17.2 g, 81.0 mmol), was dissolved in CH_3CN (150 mL) and stirred for 15 min at room temperature. The resulting suspension was treated with **106** (25.4 g, 77.0 mmol), diluted with CH_3CN (125 mL), and stirred for 48 h at room temperature. Solid was removed by filtration and the filtrate was evaporated. The residue was partitioned between 10% Na_2CO_3 (300 mL) and CH_2Cl_2 (500 mL). Tbe organic layer was dried over $MgSO_4$ and evaporated. The major product was isolated by flash chromatography on silica gel, eluting with $CHCl_3$ to $CHCl_3$–MeOH (99:1) to afford a gum upon evaporation of the solvents. The gum was dissolved in MeOH (100 mL) and the resulting solution was concentrated to a slurry on a rotary evaporator. The slurry was cooled to complete crystallization, diluted with ether and filtered to give **100** (31.9 g, 79%): mp 168–171 °C; ^{1}H NMR ($CDCl_3$) δ 7.97 (m, 2 H, 5-Ar), 7.47 and 7.45 (s, 1 H, H-2), 7.26 (m, 10 H, PhH), 7.02 (m, 2 H, 2- + 6-Ar), 6.03 and 5.35 (d, 1 H, H-6), 5.38 and 5.02 (s, 1 H, $COCHPh_2$), 5.08 (s, 2 H, $ArCH_2$), 5.21 and 4.78 (d, 1 H, H-4), 5.00 and 4.27 (d, 1 H, H-4′), 3.60 and 3.57 (s, 3 H, CO_2Me), 3.13 and 2.89 (d, 1 H, H-7), 2.70 and 2.18 (dd, 1 H, H-7′), 2.58 (s, 3 H, $ArCH_3$) (Doubling of resonances is due to *E*- and *Z*-amide conformers. Conformers are present at ca. 7:1 ratio in $CDCl_3$.); IR (KBr) ν 1749 (CO_2Me), 1650 (CON); MS (FAB, thioglycerol) *m/e* 525 (M^+ + 1); $[\alpha]^{23}_D$ +14.2° (*c* 2.04, $CHCl_3$). Anal. ($C_{30}H_{28}N_4O_5$) C, H, N.

f. (*S*)-5-(Diphenylacetyl)-4,5,6,7-tetrahydro-1-[(3-methyl-4-nitrophenyl)methyl]-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acid (77). A solution of **100** (1.50 g, 2.85 mmol) in a mixture of THF (9 mL) and MeOH (4 mL) was treated at room temperature with 1 N NaOH (3.1 mL, 3.1 mmol). After 2 h, 1 N HCl (3.1 mL, 3.1 mmol) was added and the reaction mixture was diluted with MeOH (25 mL) and H_2O (5 mL). The resulting solution was concentrated at reduced pressure until a thick slurry existed. The slurry was diluted with H_2O and the precipitate was collected by filtration. The resulting colorless solid was dried at ca. 25 mmHg, 70 °C, overnight to give **77** (1.3 g, 89%): mp 158–165 °C (glass), >190 °C dec with gas evol; ^{1}H NMR (DMSO-d_6) δ 12.95 (br, 1 H, COOH), 7.97 (d, 1 H, 5-Ar), 7.75 and 7.73 (s, 1 H, H-2),

7.25 (m, 12 H, 2- + 6-Ar + PhH), 5.71 and 5.25 (complex, 4 H, $COCHPh_2$ + H-6 + $ArCH_2$), 4.94 and 4.78 (d, 1 H, H-4), 4.22 and 3.98 (d, 1 H, H-4'), 3.08 and 2.99 (d, 1 H, H-7), 3.69 and 3.07 (dd, 1 H, H-7'), 2.49 (s, 3 H, $ArCH_3$) (Doubling of resonances is due to *Z*- and *E*-amide conformers. Conformers are present at ca. 2:1 ratio in DMSO-d_6.); IR (KBr) ν 1730 (COOH), 1654 (CON); MS (FAB, thioglycerol) *m/e* 511 (M^+ + 1), 1021 (2 M^+ + 1); $[\alpha]^{23}_D$ +2.3° (*c* 1.12, DMF). Anal. ($C_{29}H_{26}N_4O_5$) C, H, N.

Method B. a. (*S*)-1-[(4-Amino-3-methylphenyl)methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acid, Methyl Ester (107). Procedure 1. A solution of **100** (5.95 g, 11.3 mmol) in EtOAc (150 mL) was treated with $SnCl_2 \cdot 2H_2O$ powder (15.4 g, 68.2 mmol) and heated at reflux for 2 h. The reaction mixture was cooled to room temperature and treated with saturated aqueous $NaHCO_3$ (500 mL), with vigorous stirring to break up lumps formed. The organic layer was separated from the aqueous layer, and solids which were washed with EtOAc (4 × 350 mL). The combined organic layers were dried over $MgSO_4$ and evaporated. The residue was purified by flash chromatography on silica gel, eluting with $CHCl_3$–MeOH (99:1) to give **107** as a colorless foam (5.0 g, 89.6%) after evaporation of solvents and drying at 0.5 mmHg, at room temperature, overnight: ^{1}H NMR ($CDCl_3$) δ 7.35 (m, 11 H, H-2 + PhH), 6.82 (s, 1 H, 2-Ar), 6.80 (d, 1 H, 6-Ar), 6.61 (d, 1 H, 5-Ar), 5.99 and 4.99 (d, 1 H, H-6), 5.34 and 4.97 (s, 1 H, $COCHPh_2$), 4.87 and 4.82 (d, 2 H, $ArCH_2$), 5.22 and 4.77 (d, 1 H, H4), 4.29 and 4.19 (d, 1 H, H-4'), 3.66 (br, 2 H, NH_2), 3.57 and 3.52 (s, 3 H, CO_2Me), 3.20 and 2.99 (d, 1 H, H-7), 2.70 and 2.27 (dd, 1 H, H-7'), 2.13 (s, 3 H, $ArCH_3$) (Doubling of resonances is due to *Z*- and *E*-amide conformers. Conformers are present at ca. 7:1 ratio in $CDCl_3$.).

Procedure 2. A solution of **100** (25.6 g, 51.8 mmol), in a mixture of THF (100 mL) and MeOH (500 mL) was treated with Raney nickel (3.0 g), and hydrogenated at 50 psi for 6 h at room temperature. The catalyst was removed by filtration and the filtrate was evaporated to a slightly colored gum. Flash chromatography as above gave a quantitative yield of **107**, which was identical by NMR to that produced by procedure 1. The **107** produced by both methods was used without further characterization below.

b. (*S*)-1-[(4-Amino-3-methylphenyl)methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acid (78). A solution of **107** (2.0 g, 4.05 mmol) in a mixture of THF (15 mL) and MeOH (5 mL) was treated at room temperature with 1 N NaOH (4.5 mL, 4.5 mmol). The reaction was stirred for 2 h and then 1 N HCl (4.5 mL, 4.5 mmol) was added. The reaction mixture was evaporated and the residue was purified by preparative reversed-phase chromatography (Waters Prep 500, using one C18 PrepPak), eluting with CH_3CN–H_2O (30:70). Fractions containing pure **78** were evaporated to give a colorless solid (1.4 g, 72%): mp 157–160 °C (glass), >200 °C dec with gas evol; ^{1}H NMR (DMSO-d_6) δ 12.95 (br, 1 H, COOH), 7.58 (s, 1 H, H-2), 7.33 (m, 10 H, PhH), 6.78 (m, 2 H, 2- + 6-Ar), 6.55 (d, 1 H, 5-Ar), 5.73 and 5.29 (d, 1 H, H-6), 5.70 and 4.91 (s, 1 H, $COCHPh_2$), 5.82 (complex, 3 H, $ArCH_2$ + H-4), 4.19 and 3.98 (d, 1 H, H-4'), 3.30 (br , 2.5 H, NH_2 + H_2O), 3.18 and 3.02 (d, 1 H, H-7), 2.70 and 2.19 (dd, 1 H, H-7'), 1.99 (s, 3 H, $ArCH_3$) (Doubling of resonances is due to *Z*- and *E*-amide conformers. Conformers are present at ca. 2:1 ratio in DMSO-d_6.); IR (KBr) ν 3450 and 3370 (NH_3^+), 1638 (br, COO, CON); MS (FAB, thioglycerol) *m/e* 481 (M^+ + 1). $[\alpha]^{23}_D$ +15.9° (*c* 1.30, DMF). Anal. ($C_{29}H_{28}N_4O_3 \cdot 0.25H_2O$) C, H, N.

c. (*S*)-1-[[4-(Dimethylamino)-3-methylphenyl]methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acid (79). A solution of **107** (4.50 g, 9.37 mmol) in absolute EtOH (180 mL) was treated sequentially with 37% aqueous H_2CO (3.87 mL, 46.4 mmol), 1 N HCl (9.37 mL, 9.37 mmol), and $NaCNBH_3$ (1.44 g, 22.9 mmol). The resulting solution was stirred for 15 min and then a mixture of EtOH (18 mL) and 1 N HCl (18.7 mL, 18.7 mmol) was added dropwise over 1.5 h. The reaction mixture was stirred at room temperature overnight and then TFA (2.2 mL) was added. Evaporation gave an off-white solid was purified by reversed-phase chromatography (Waters Prep 500, using two C18 PrepPaks), eluting with CH_3CN–0.1% TFA (aqueous) (25:75). Pure product fractions were concentrated at reduced pressure to remove CH_3CN, and the resulting aqueous solution was frozen and lyophilized to give the ditrifluoroacetate salt of **79** as a fluffy white solid (6.0 g, 83%): ^{1}H NMR (D_2O) δ 8.82 and 8.68 (s, 1 H, H-2), 7.75 and 7.69 (d, 1 H, 5-Ar), 7.25 (complex, 12 H, 2- + 6-Ar + PhH), 5.7–4.7 (complex, 13.5 H, H-6, H-4, $COCHPh_2$, $ArCH_2$, and HDO), 4.51 and 4.39 (d, 1 H, H4'), 3.31 (s, 6 H, NMe_2), 3.16 and 2.98 (d, 1 H, H-7), 2.87 and 2.24 (dd, 1H, H-7'), 2.52 and 2.46 (s, 3 H, $ArCH_3$) (Doubling of resonances is due to *Z*- and *E*-amide conformers. Conformers are present at ca. 3:2 ratio in D_2O.); IR (KBr) ν 3440 (OH), 1676 (br, CO); MS (FAB, thioglycerol) *m/e* 1017 (2 M^+ + 1), 509 (M^+ + 1), 509 (M^+ + 1); $[\alpha]^{23}_D$ +6.0° (*c* 1.03, MeOH). Anal. ($C_{31}H_{32}N_4O_3 \cdot 2CF_3CO2H \cdot 2H_2O$) C, H, N.

The free base could be isolated by neutralizing an aqueous solution of this salt (1.9 g) with 1 N NaOH to pH 6.0. The supernatant was decanted from a gummy solid. The latter was dissolved in 25 mL of MeOH and treated with 10 mL H_2O, and the solution was concentrated at reduced pressure until turbidity was observed. Filtration removed a small amount of gray impurity. Further concentration and dilution with water gave a precipitate which adhered to the wall of the flask. The supernatant was again decanted and the residue was recrystallized from CH_3CN to give 0.7 g of pure **79**: mp >200 °C dec with gas evol; ^{1}H NMR (DMSO-d_6) δ 7.62 (s, 1 H, H-2), 7.25 (complex, 10 H, PhH), 5.71 and 5.27 (d, 1 H, H-6), 5.69 and 4.91 (s, 1 H, $COCHPh_2$), 4.98 (m, 2 H, $ArCH_2$), 4.88 and 4.73 (d, 1 H, H4), 4.18 and 3.97 (d, 1 H, H4'), 3.17 and 3.01 (d, 1 H, H-7), 2.68 and 2.18 (m, 1 H, H-7'), 2.60 and 2.58 (2 s, 6 H, NMe_2), 2.20 (s, 3 H, $ArCH_3$) (Doubling of resonances is due to *Z*- and *E*-amide conformers. Conformers are present at ca. 2:1 ratio in DMSO-d_6.); IR (KBr) ν 1656 (br, COO and CON); MS (FAB, thioglycerol) *m/e* 532 (M^+ + Na), 509 (base peak, M^+ + 1); $[\alpha]^{23}_D$ +19.0° (*c* 0.97, DMF). Anal. ($C_{31}H_{32}N_4O_3$) C, H, N.

d. 5-(Diphenylacetyl)-4,5,6,7-tetrahydro-1-[[3-methyl-4-[[(methylamino)carbonyl]amino]phenyl]methyl]-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acid (81). A solution of **107** (3.0 g, 6.0 mmol) in CH_2Cl_2 (25 mL) was treated with methyl isocyanate (0.40 mL, 6.8 mmol) and stirred under nitrogen for 30 h at 23 °C. The resulting solution was evaporated and the residue was purified by flash chromatography on silica gel, eluting with $CHCl_3$–MeOH (97:3) to afford the methyl ester of **81** (3.3 g, 99.4%) as a foam upon evaporation of solvents: ^{1}H NMR ($CDCl_3$) δ 7.47 (d, 1 H, 5-Ar), 7.42 (s, 1 H, H-2), 7.25 (m, 10 H, PhH), 6.88 (m, 2 H, 2- + 6-Ar), 6.35 (s, 1 H, ArNH), 5.95 (d, 1 H, H-6), 5.37 and 4.90 (s, 1 H, $COCHPh_2$), 5.23 and 4.77 (d, 1 H, H-4), 4.97 (m, 1 H, MeNHCO), 4.95 (s, 2 H, $ArCH_2$), 4.25 and 4.07 (d, 1 H, H-4'), 3.55 and 3.48 (s, 3 H, CO_2CH_3), 3.10 and 2.90 (d, 1 H, H-7), 2.73 (d, 3 H, CH_3N), 2.67 and 2.30 (dd, 1 H, H-7'), 2.15 and 2.12 (s, 3 H, $ArCH_3$) (Doubling of resonances is due to *Z*- and *E*-amide conformers. Conformers are present at ca. 7:1 ratio in $CDCl_3$.). This material was used without further purification in the next step.

The conversion of the methyl ester obtained above to **81** (78% yield) was accomplished using a procedure analogous to that described above for the saponification of **107** to **78**: mp 198–201 °C dec with gas evol; ^{1}H NMR ($CDCl_3$) δ 7.78 (d, 1 H, 5-Ar), 6.76 (m, 2 H, H-2 + CONHAr), 7.23 (m, 10 H, PhH), 6.93 (m, 2 H, 2- + 6-Ar), 6.43 (m, 1 H, NH), 5.73 and 5.30 (d, 1 H, H-6), 5.70 and 4.93 (s, 1 H, $COCHPh_2$), 4.99 (m, 2 H, CH_2Ar), 4.90 and 4.75 (d, 1 H, H-4), 4.20 and 3.99 (d, 1 H, H-4'), 3.40 (br, H_2O), 3.20 and 3.03 (d, 1 H, H-7), 2.67 (m, 3.7 H, CH_3N + H-7' (major conformer)), 2.15 (m + s, 3.3 H, $ArCH_3$ + H-7' (minor conformer)) (Doubling of resonances is due to *Z*- and *E*-amide conformers. Conformers are present at ca. 2:1 ratio in DMSO-d_6.); IR (KBr) ν 1648 (br, CO); MS (FAB, thioglycerol) *m/e* 1613 (3 M^+ + 1), 1075 (2 M^+ + 1), 538 (base peak, M^+ + 1); $[\alpha]^{23}_D$ +8.5° (*c* 2.08, DMF). Anal. ($C_{31}H_{31}N_5O_4 \cdot 0.33H_2O$) C, H, N.

Method C. a. (*S*)-8a,9-Dihydro-7-phenyl-3*H*-diimidazo[1,5-*a*:4',5'-*d*]pyridine-6,8(4*H*,7*H*)-dione (109). A mixture of **108**[41] (12.7 g, 50 mmol), Et_3N (15.19 g, 50 mmol), and DMF (150 mL) was treated with phenyl isocyanate (5.95 g, 50 mmol) and stirred at room temperature for 2 h. The $Et_3N \cdot HCl$ was removed by filtration and the filtrate was concentrated at reduced pressure. Water (200 mL) was added to the resulting residue to give a solid that was recrystallized from MeOH–H_2O to afford **109** (9.2 g, 69%): mp 133–136 °C; ^{1}H NMR ($CDCl_3$) δ 7.66 (s, 1 H, H-2), 7.55–7.35 (m, 5 H, PhH), 4.76 (d, 1 H, H4), 4.59 (m, 1 H, H-8a),

4.25 (d, 1 H, H-4'), 3.15–2.89 (complex, 2 H, H-9); IR (KBr) ν 1720, 1775 (CO); MS (DEI) m/e 268 (M^+); $[\alpha]^{23}_D$ −201.3° (c 1.05, MeOH). Anal. ($C_{14}H_{12}N_4O_2 \cdot 0.75H_2O$), C, H, N, H_2O.

b. 8a,9-Dihydro-7-phenyl-1-(phenylmethyl)-1*H*-diimidazo[1,5-*a*:4',5'-*d*]pyridine-6,8(4*H*,7*H*)-dione (110) and 8a,9-Dihydro-7-phenyl-3-(phenylmethyl)-3*H*-diimidazo[1,5-*a*:4',5'-*d*]pyridine-6,8(4*H*,7*H*)-dione (111). A solution of **109** (27.3 g, 100 mmol) in DMF (60 mL) was treated with a solution of tetramethylammonium hydroxide in MeOH (20 wt %, 46.5 g, 102 mmol) and stirred a few minutes before benzyl bromide (17.4 g, 100 mmol) was added dropwise. After 30 min the separated solids were filtered and washed with DMF and H_2O to give 13.7 g of crude **110** which was further purified by recrystallization from DMF to yield 9.2 g (25.1%) as a colorless solid: mp 277–279 °C; ^{1}H NMR (TFA-*d*) δ 7.68 (s, 1 H, H-2), 7.52 (m, 6 H, PhH), 7.32 (m, 4 H, PhH), 5.47 (AB, 2 H, NCH_2Ar), 5.35 (d, 1 H, H-4), 4.82 (q, 1 H, H-8a), 4.61 (d, 1 H, H-4'), 3.55 (d, 1 H, H-9), 3.05 (dd, 1 H, H-9'); IR (KBr) ν 1720, 1775 (CO); MS (DEI) m/e 358 (M^+). Anal. ($C_{21}H_{18}N_4O_2$) C, H, N.

Water (200 mL) was added to the DMF filtrate above to precipitate a crude solid. The dried solid was triturated with acetone (200 mL), and solids were removed by filtration. The filtrate was concentrated and the residue was recrystallized from DMF to give pure **111**: mp 227–229 °C; ^{1}H NMR (TFA-*d*) δ 8.56 (s, 1 H, H-2), 7.42 (m, 6 H, PhH), 7.25 (m, 4 H, PhH), 5.35 (AB, 2 H, NCH_2Ph), 5.05 (d, 1 H, H-4), 4.70 (m, 1 H, H-8a), 4.20 (d, 1 H, H-4'), 3.55 (dd, 1 H, H-9), 3.15 (dd, 1 H, H-9'); IR (KBr) ν 1716, 1775 (CO). MS (DEI) m/e 358 (M^+). Anal. ($C_{21}H_{18}N_4O_2$) C, H, N.

c. 4,5,6,7-Tetrahydro-5-[(phenylamino)carbonyl]-1-(phenylmethyl)-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acid (2). A suspension of **110** (0.90 g, 2.5 mmol), 1 N NaOH (10 mL, 10 mmol), and MeOH (30 mL) was heated for 30 min at reflux. The resulting solution was concentrated at reduced pressure and the residual syrup was treated with glacial acetic acid (0.60 g, 10 mmol). The resulting precipitate was collected by filtration, washed with H_2O, and recrystallized from warm MeOH (ca. 40 °C, 10 mL) to afford **2** (0.55 g, 59%): mp 100° (softens), 270–280 °C; ^{1}H NMR (D_2O + NaOD) δ 7.58 (s, 1 H, H-2), 7.08–7.34 (m, 10 H, PhH), 4.88–5.08 (m, 3 H, NCH_2Ph, H-6), 4.40 (AB, 1 H, H-4), 3.46 (d, 1 H, H-7), 3.14 (d, 1 H, H-7'); IR (KBr) ν 1655, 1740 (CO); MS (FAB) 377 ($M^+ + 1$). Anal. ($C_{21}H_{20}N_4O_3 \cdot 0.9H_2O$) C, H, N, H_2O.

d. 4,5,6,7-Tetrahydro-5-[(phenylamino)carbonyl]-3-(phenylmethyl)-3*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acid (29). Hydrolysis of **111** to **29** was achieved using an identical procedure for the conversion of **110** to **2**: mp softens 152–155 °C, 227–229 °C; ^{1}H NMR (D_2O + NaOD) δ 7.61 (s, 1 H, H-2), 7.08–7.34 (m, 10 H, PhH), 4.7–5.0 (m, 3 H, NCH_2Ph, H-6), 4.31 (AB, 1 H, H-4), 3.19 (d, 1 H, H-7), 2.86 (d, 1 H, H-7'); IR (KBr) ν 1745 (w), 1665, 1630 (CO); MS (FAB) m/e 377 ($M^+ + 1$). Anal. ($C_{21}H_{20}N_4O_3 \cdot 0.75H_2O$) C, H, N.

Method D. a. (*S*)-5-(Diphenylacetyl)-4,5,6,7-tetrahydro-1-[(4-methoxy-3-methylphenyl)methyl]-1*H*-imidazo[4,5-*c*]-pyridine-6-carboxylic Acid, Methyl Ester (113). A solution of **112**[41] (4.0 g, 8.1 mmol) in CH_3CN (80 mL) was treated with 4-methoxy-3-methylbenzyl chloride (1.72 g, 10.1 mmol) and heated at reflux for 2 days. The resulting solution was evaporated to a gum which was dissolved in CH_2Cl_2 (10 mL) and added dropwise to vigorously stirred Et_2O (300 mL). The precipitate was collected by filtration and dried overnight at ca. 25 mmHg, 30 °C, to give a crude imidazolium salt (4.76 g) which was dissolved in glacial acetic acid (50 mL), diluted with MeOH (50 mL), treated with Zn dust (10 g), and sonicated for 5 h. A stream of N_2 was bubbled through the reaction mixture to provide additional agitation. The resulting mixture was filtered to remove excess Zn which was rinsed with CH_2Cl_2. The filtrate was further diluted with CH_2Cl_2 (300 mL) and neutralized by dropwise addition of 10% aqueous Na_2CO_3 (300 mL) with vigorous stirring. The organic layer was separated, dried over $MgSO_4$, and evaporated. Flash chromatography on silica gel, eluting with $CHCl_3$ to $CHCl_3$–MeOH (98:2), gave **113** (2.1 g, 50%) as a foam upon evaporation of the solvents: ^{1}H NMR ($CDCl_3$) δ 7.44 (s, 1 H, H-2), 7.30 (m, 10 H, PhH), 6.94 (m, 2 H, 2- + 6-Ar), 6.79 (d, 1 H, 5-Ar, 6.02 (d, 0.86 H, H-6 (major conformer)), 5.40 and 5.32 (s, 1 H, $COCHPh_2$), 5.2–4.7 (complex, 3.14 H, $ArCH_2$ + H-4 + H-6 (minor conformer)), 4.29 and 4.21 (d, 1 H, H-4'), 3.85 (s, 3 H, $ArOCH_3$), 3.57 and 3.52 (s, 3 H, CO_2CH_3), 3.20 and 2.99 (d, 1 H, H-7), 3.70 and 3.23 (dd, 1 H, H-7'), 3.21 (s, 3 H, $ArCH_3$) (Doubling of resonances is due to *E*- and *Z*-amide conformers. Conformers are present at ca. 7:1 ratio in $CDCl_3$.). This material was used without further characterization below.

b. (*S*)-5-(Diphenylacetyl)-4,5,6,7-tetrahydro-1-[(4-methoxy-3-methylphenyl)methyl]-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acid (36). A solution of **113** (1.20 g, 2.35 mmol) in THF (8 mL) and MeOH (4 mL) was treated with 1 N NaOH (2.7 mL, 2.7 mmol) and stirred at room temperature for 4 h. The organic solvents were evaporated at reduced pressure, and the residue was redissolved in H_2O (8 mL) and treated with 1 N HCl (2.7 mL, 2.7 mmol). The resulting precipitate was collected by filtration and dried at ca. 25 mmHg, 75 °C, for 72 h to give **36** (0.8 g, 68.8%): mp 135–140 °C (glass), 220–225 °C dec; ^{1}H NMR (DMSO-d_6) δ 13.0 (br, 1 H, COOH), 7.61 (s, 1 H, H-2), 7.27 (m, 10 H, PhH), 7.0–6.9 (m, 3 H, 2- + 5- + 6-Ar), 5.68 (t, 1.7 H, $COCHPh_2$ + H-6 (major conformer)), 5.26 (d, 0.3 H, H-6 (minor conformer)), 5.03 and 4.97 (d, 2 H, CH_2Ar), 4.87 and 4.71 (d, 1 H, H-4), 4.19 and 3.95 (d, 1 H, H-4'), 3.77 and 3.75 (s, 3 H, $ArOCH_3$), 3.16 and 2.99 (d, 1 H, H-7), 2.63 (dd, 0.7 H, H-7' (major conformer)), 2.10 (m, 3.3 H, $ArCH_3$ + H-7 (minor conformer)) (Doubling of resonances is due to *Z*- and *E*-amide conformers. Conformers are present at ca. 2:1 ratio in DMSO-d_6.); IR (KBr) ν 1626 (COOH), 1654 (CON), MS (FAB, thioglycerol) m/e 496 ($M^+ + 1$); $[\alpha]^{23}_D$ +11.1° (c 1.16, DMF). Anal. ($C_{30}H_{29}N_3O_4$) C, H, N.

(*S*)-1-[(3,4-Dimethoxyphenyl)methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acid (83). a. (*S*)-1-[(3,4-Dimethoxyphenyl)methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acid, Methyl Ester (114). A solution of 3,4-dimethoxybenzyl chloride (1.5 g, 8.0 mmol), **112** (2.5 g, 5.1 mmol), and CH_3CN (25 mL) was heated at reflux overnight. After cooling to room temperature, the resulting solution was added dropwise to vigorously stirred ether (400 mL). Precipitate was collected by filtration and dissolved in a mixture of MeOH (40 mL) and glacial acetic acid (40 mL). Zn dust (8 g) was added and the mixture was sonicated for 2 h while bubbling a stream of N_2 through the solution for additional agitation. Excess Zn was removed by filtration and the filtrate was diluted with CH_2Cl_2 (400 mL) and neutralized by dropwise addition of 10% aqueous Na_2CO_3. The organic layer was separated, dried over $MgSO_4$, and evaporated. Flash chromatography on silica gel, eluting with $CHCl_3$ to $CHCl_3$–MeOH (99:1) gave **114** (1.6 g, 60%) as a foam upon evaporation of solvents: ^{1}H NMR ($CDCl_3$) δ 7.5–7.1 (m, 11 H, H-2 + PhH), 6.83 (d, 1 H, 6-Ar), 6.61 (m, 2 H, 2- + 5-Ar), 5.99 (d, 0.86 H, H-6 (major conformer)), 5.38 (s, 0.86 H, $COCHPh_2$ (major conformer)), 5.35–4.85 (complex, 2.42 H, CH_2Ar + (H-6, $COCHPh_2$, H-4) (minor conformer)), 4.78 (d, 0.86 H, H-4 (major conformer)), 4.29 and 4.20 (d, 1 H, H-4'), 3.89 and 3.83 (2 s, 6 H, $ArOCH_3$), 3.58 and 3.52 (s, 3 H, CO_2Me), 3.17 and 2.94 (d, 1 H, H-7), 2.70 and 2.22 (dd, 1 H, H-7') (Doubling of resonances is due to *Z*- and *E*-amide conformers. Conformers are present at ca. 7:1 ratio in $CDCl_3$.). This material was used without further characterization below.

b. (*S*)-1-[(3,4-Dimethoxyphenyl)methyl]-5-(diphenylacetyl)-4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic Acid (83). A solution of **114** (1.6 g, 3.0 mmol) in a mixture of THF (10 mL) and MeOH (3 mL) was treated with 1 N NaOH (3.3 mL, 3.3 mmol) and stirred at room temperature for 90 min. Organic solvents were evaporated at reduced pressure, and the residue was dissolved in H_2O (40 mL) and treated dropwise with 1 N HCl (3.3 mL, 3.3 mmol). The aqueous layer was decanted from a gummy precipitate, and then the precipitate was dissolved in MeOH and evaporated to a glass. This glass was suspended in water and sonicated to give **83** (1.3 g, 84%) as a white solid after drying at ca. 25 mmHg, 50 °C, overnight: mp 140–150 °C (glass); ^{1}H NMR (DMSO-d_6) δ 13.0 (br, 1 H, COOH), 7.70 and 7.67 (s, 1 H, H-2), 7.25 (m, 10 H, PhH), 6.88 (m, 2 H, 2- + 6-Ar), 6.65 (m, 1 H, 5-Ar), 5.72 and 5.30 (d, 1 H, H-6), 5.70 (s, 0.7 H, $COCHPh_2$ (major conformer)), 5.10–4.85 (complex, 2.6 H, CH_2Ar + $COCHPh_2$, H-4 (minor conformer)), 4.72 (d, 0.7 H, H-4 (major conformer)), 4.19 and 3.98 (d, 1 H, H-4'), 3.75 and 3.70 (2 s, 6 H, $ArOCH_3$), 3.5–3.1 (br, H_2O), 3.19 and 3.03 (d, 1 H, H-7), 2.67 and

2.10 (dd, 1 H, H-7'); IR (KBr) ν 1727 (COOH), 1654 (CON); MS (FAB, thioglycerol) m/e 512 ($M^+ + 1$); $[\alpha]^{23}_D$ +6.0° (c 1.04, DMF). Anal. ($C_{30}H_{29}N_3O_5 \cdot 0.33H_2O$) C, H, N.

Method E. a. (*S*)-4,5,6,7-Tetrahydro-1-(phenylmethyl)-1*H*-imidazo[4,5-*c*]pyridine-6-methanol, Hydrochloride (115). To a solution of (*S*)-4,5,6,7-tetrahydro-1-(phenylmethyl)-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic acid, methyl ester[41] (2.7 g, 0.011 mol), in 50 mL of dry THF was added 0.38 g (0.11 mol) of LAH. The resulting mixture was stirred overnight at room temperature under nitrogen and then quenched by successive additions of 0.4 mL of H_2O, 0.4 mL of 15% NaOH solution, and 1.1 mL of H_2O. Filtration and concentration gave a pale yellow solid. This was converted to the hydrochloride salt for characterization by dissolving in methanolic HCl and precipitating with ether to afford 1.4 g (49%) of solid product. An analytical sample of **115** was obtained upon recrystallization from CH_3CN: mp 190–192 °C; ^{1}H NMR (DMSO-d_6) δ 9.6 (br s, 2 H, NH_2^+), 7.85 (s, 1 H, H-2), 7.1–7.5 (m, 5 H, Ph), 5.6 (br s, 1 H, OH), 5.15 (AB, 2 H, $ArCH_2$), 4.0 (q, 2 H, CH_2O), 3.2–3.8 (m, 3 H, H-4, H-6), 2.75 (ABC, 2 H, H-7); $[\alpha]^{23}_D$ −2.1° (c 1.04, MeOH). Anal. ($C_{14}H_{17}N_3O \cdot HCl$) C, H, N.

b. (*S*)-5-(Diphenylacetyl)-4,5,6,7-tetrahydro-1-(phenylmethyl)-1*H*-imidazo[4,5-*c*]pyridine-6-methanol (93). To a solution of **115** (free base, 25.4 g, 0.11 mol) in 50 mL of dry THF was added 17.9 g (0.26 mol) of imidazole and 34.7 g (0.23 mol) of *tert*-butyl dimethylsilyl chloride. Then 100 mL of DMF was added to achieve solution and this was stirred at 40 °C for 5 h. The resulting mixture was concentrated at reduced pressure and the residue was extracted with a 20% CH_2Cl_2–EtOAc mixture. The organic layer was washed successively with water and dilute $NaHCO_3$ solution and then dried and concentrated to give 30.3 g of oily *O*-monosilyl ether. This was observed to give a single spot by TLC (10% MeOH/$CHCl_3$) and was used in the next step without further purification.

To a solution of 2.4 g (12 mmol) of DCC in 50 mL of CH_3CN was added 2.48 g (12 mmol) of diphenylacetic acid followed by 1.6 g (12 mmol) of HOBT·H_2O. After this was stirred for 15 min at room temperature, 3.2 g (9 mmol) of the silated amine prepared above was added and stirring was continued overnight at room temperature. The solids were filtered, and the solution was concentrated at reduced pressure. The residue was taken up in EtOAc and washed twice with saturated $NaHCO_3$ solution and dried over $MgSO_4$. Concentration gave an oil which was dissolved in 100 mL of 2% HF in CH_3CN. After 8 h of stirring at room temperature, the mixture was concentrated, the residue taken up in EtOAc, and the solution washed with saturated $NaHCO_3$, dried, and concentrated. Product **93** crystallized and was filtered: mp 223–230 °C; IR (KBr) ν 1647 (CON); MS (DEI) m/e 437 (M^+), 406 ($M^+ - 31$), 318, 270, 242, 212, 185, 167; $[\alpha]^{23}_D$ +49.3° (c 1.03, DMF). Anal. ($C_{28}H_{27}N_3O_2 \cdot 0.25H_2O$) C, H, N.

5-(Diphenylacetyl)-4,5,6,7-tetrahydro-1-(phenylmethyl)-1*H*-imidazo[4,5-*c*]pyridine (86). A solution of 9.8 g (35.7 mmol) of N^{im}-benzylhistamine[58] in 400 mL of 1 N HCl was treated with 8.9 mL (107 mmol) of 36% aqueous formaldehyde. The mixture was stirred at room temperature for 30 min, then at reflux for 4.5 h. Concentration of the cooled mixture to dryness gave the crude 1-(phenylmethyl)-1*H*-imidazo[4,5-c]pyridine dihydrochloride as a gum. The product was purified by dissolving in EtOH (twice) and evaporating to dryness. A hygroscopic white solid was obtained after trituration with cold EtOH: ^{1}H NMR (D_2O) δ 8.7 (s, 1 H, H-2), 7.1–7.35 (m, 5 H, Ph), 5.25 (s, 2 H, $PhCH_2$), 4.3 (AB, 2 H, H-4), 3.4 (t, 2 H, H-6), 2.75 (br t, 2 H, H-7).

A suspension of 4.0 g (19.3 mmol) of DCC, 2.6 g (19.3 mmol) of 1-HOBT·H_2O, and 40.8 g, (19.3 mmol) of diphenylacetic acid in 55 mL of CH_3CN was stirred at room temperature for 20 min and then added to a solution of 5.2 g (18.2 mmol) of the imidazopyridine dihydrochloride obtained above and prestirred for 30 min in 55 mL of CH_3CN containing 6.7 mL (38.5 mmol) of diisopropylamine. An additional 60 mL of CH_3CN was used to complete the transfer, and stirring was continued for 48 h at room temperature. The reaction mixture was filtered and the filtrate was concentrated to dryness. The residue was partitioned between $CHCl_3$ and 0.25 M phosphate buffer solution (pH 7). The organic layer was washed with 10% Na_2CO_3 and phosphate buffer and then dried and concentrated at reduced pressure to give a yellow gum. This was purified by column chromatography over silica gel, eluting the desired product with a 98:2 $CHCl_3$–MeOH mixture. Dissolution of the gum in CH_2Cl_2 and evaporation gave 5.3 g (71%) of **86** as an off-white solid: mp 134–136 °C; ^{1}H NMR (DMSO-d_6) δ 7.0–7.8 (m, 16 H, 3 Ph + H-2), 5.3 and 5.2 (2 s, 1 H, $COCHPh_2$), 4.9–5.15 (m, 2 H, CH_2Ar), 4.6 and 4.85 (2 br s, 2 H, H-4), 4.0 and 3.75 (2 m, 2 H, H-6), 2.55 and 2.05 (2 m, 2 H, H-7) (Doubling of resonances is due to *Z*- and *E*-amide conformers. Conformers are present at ca. 1:1 ratio in DMSO-d_6.); IR (KBr) ν 1639 (CON); MS (DEI) m/e 407 (M^+), 316, 288, 240. Anal. ($C_{27}H_{25}N_3O$) C, H, N.

Receptor Binding Assay. Following ref 44, rat adrenal glands were collected in ice-cold Hepes buffer, pH 7.4, containing dextrose and serum albumin. After trimming and mincing, the tissue was dispersed in 20 mM $NaHCO_3$. The filtered homogenate was centrifuged, and the 10000–100000*g* fraction was used for studies of Ang II binding. The assay medium was prepared by mixing 40 μL of extract (containing 20–50 μg of membrane protein) with 40 μL of a buffer (50 mM in Tris-HCl, 120 mM NaCl, 5 mM dithiothreitol, and 0.8% bovine serum albumin) with 20–40 pg (17–34 fM) of ^{125}I-Ang II. (New England Nuclear) dissolved in 20 μL of H_2O for a total volume of 100 μL. Varying concentrations of reference agents or test compounds were added to the incubation medium to generate binding inhibition curves. Ang II binding was corrected for nonspecific binding in the presence of 1.0 μM unlabeled Ang II. Binding studies were performed in duplicate at 22–25 °C for 30 min. Separation of bound and free labeled angiotensin was achieved by Millipore filtration using HAWP nitrocellulose filters (0.45 μM), and filters were counted using a γ-spectrophotometer (Packard). Receptor binding is expressed as a ratio of specific counts bound to the protein in the presence of test compounds or of various inhibitor concentrations to specific counts bound in their absence. The IC_{50} value was determined for the purposes of this study, typically from four to five points spanning the concentration inhibiting binding by 50%. Individual points were determined with an average error of ±5% and the replication error for IC_{50} determination between experiments was within 50%. For studies of brain binding, rat brain tissue was prepared following the procedure of ref 45, and the assay was run in a manner identical to that described above.

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