6,7-*O*,*O*-Demethylene-6,7-*O*,*O*-dimethyl-4β-(4″-fluoroanilino)-4-desoxypodophyllotoxin (27): 178 mg; mp 240–243 °C, crystals from ethanol–acetone; $[\alpha]^{25}_D$ −95° (*c* = 0.30, acetone); IR (KBr) 3360, 2940, 1770, 1600, 1570, 1510, and 1460 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.97 (t, 2 H, H-3″,5″), 6.75 (s, 1 H, H-5), 6.55 (s, 1 H, H-8), 6.51 (t, 2 H, H-2″,6″), 6.33 (s, 2 H, H-2′,6′), 4.66 (m, 2 H, H-1,4), 4.40 (t, 1 H, H-11), 4.01 (t, 1 H, H-11), 3.86 (s, 3 H, OCH_3-6), 3.83 (s, 3 H, OCH_3-4′), 3.82 (s, 3 H, OCH_3-7), 3.75 (s, 6 H, OCH_3-3′,5′), 3.20 (dd, *J* = 13.7, 5.0 Hz, 1 H, H-2), and 3.02 (m, 1 H, H-3). Anal. ($C_{29}H_{30}FNO_7 \cdot {}^1/_2H_2O$) C, H, N.

6,7-*O*,*O*-Demethylene-6,7-*O*,*O*-dimethyl-4′-*O*-demethyl-4β-(4″-fluoroanilino)-4-desoxypodophyllotoxin (31): 45 mg; mp 221–224 °C; $[\alpha]^{25}_D$ −93° (*c* = 0.25, acetone); IR (KBr) 3380, 2940, 1760, 1600, 1510, and 1460 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.96 (t, 2 H, H-3″,5″), 6.74 (s, 1 H, H-5), 6.55 (s, 1 H, H-8), 6.50 (t, 2 H, H-2″,6″), 6.34 (s, 2 H, H-2′,6′), 5.43 (s, 1 H, OH-4′), 4.65 (m, 2 H, H-1,4), 4.39 (t, 1 H, H-11), 4.00 (t, 1 H, H-11), 3.86 (s, 3 H, OCH_3-6), 3.82 (s, 3 H, OCH_3-7), 3.75 (s, 6 H, OCH_3-3′,5′), 3.18 (dd, *J* = 14.7, 4.9 Hz, 1 H, H-2), and 3.01 (m, 1 H, H-3). Anal. ($C_{28}H_{28}FNO_7 \cdot {}^1/_4H_2O$) C, H, N.

Biological Assay. Assays for the inhibition of human DNA topoisomerase II and the cellular protein-linked DNA breaks as well as the cytotoxicity in KB cells were carried out according to the procedures described previously.[24]

Acknowledgment. The authors thank Mike Fisher of the Cancer Research Center, UNC-Chapel Hill for KB cell culture assay. This work was supported by grants from the American Cancer Society CH-370 (K. H. L.) and the National Cancer Institute CA 44358 (Y. C. C.).

Registry No. **2**, 518-28-5; **3**, 1174-97-6; **4**, 138456-90-3; **5**, 138355-75-6; **6**, 138355-76-7; **7**, 6559-91-7; **8**, 138456-91-4; **9**, 1178-09-2; **10**, 138456-92-5; **11**, 138355-77-8; **12**, 138355-78-9; **13**, 138355-79-0; **14**, 138355-80-3; **15**, 138355-81-4; **16**, 138355-82-5; **17**, 138355-83-6; **18**, 138355-84-7; **19**, 138355-85-8; **20**, 138355-86-9; **21**, 138355-87-0; **22**, 138355-88-1; **23**, 138355-89-2; **24**, 138355-90-5; **25**, 138355-91-6; **26**, 138355-92-7; **27**, 138355-93-8; **28**, 138355-94-9; **29**, 138355-95-0; **30**, 138355-96-1; **31**, 138355-97-2; **32**, 127882-73-9; **33**, 127882-59-1; **34**, 127882-56-8; **35**, 125830-36-6; NH_2Ph, 62-53-3; *p*-$NH_2C_6H_4NO_2$, 100-01-6; *p*-$NH_2C_6H_4CO_2C_2H_5$, 94-09-7; *p*-$NH_2C_6H_4CN$, 873-74-5; *p*-$NH_2C_6H_4F$, 371-40-4; *m*-$NH_2C_6H_4OH$, 591-27-5; DNA topoisomerase II, 80449-01-0.

(24) Lee, K. H.; Imakura, Y.; Haruna, M.; Beers, S. A.; Thurston, L. S.; Dai, H. J.; Chen, C. H.; Liu, S. Y.; Cheng, Y. C. Antitumor Agents. 107. New Cytotoxic 4-Alkylamino Analogues of 4′-Demethylepipodophyllotoxin As Inhibitors of Human DNA Topoisomerase II. *J. Nat. Prod.* **1989**, *52* (3), 606–613.

New Nonpeptide Angiotensin II Receptor Antagonists. 1. Synthesis, Biological Properties, and Structure–Activity Relationships of 2-Alkyl Benzimidazole Derivatives

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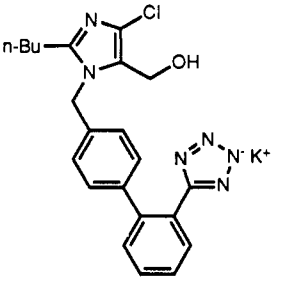
On the basis of an extension of the literature lead 1, a series of benzimidazoles have been synthesized and shown to be angiotensin II (AII) receptor antagonists. The structure–activity relationships of these new antagonists have been explored and the key binding interactions defined. Molecular mechanics calculations were carried out on analogues of imidazole AII antagonists and conformationally restricted analogues were synthesized. The benzimidazole antagonists displaced AII in binding studies in vitro with IC_{50} values in the range 10^{-5}–10^{-7} M and antagonized the hypertensive effects of AII in vivo (rats) following intravenous administration with ED_{50} values in the range of 5–20 mg/kg.

Angiotensin II (AII), a powerful endogenous vasconstrictor produced by the renin–angiotensin system (RAS), is a major regulator of blood pressure in mammals.[1] Blockade of the RAS, through inhibition of angiotensin converting enzyme (ACE), has provided an effective means of lowering blood pressure in the majority of hypertensive patients.[2] However, ACE not only cleaves angiotensin I to produce AII, but also hydrolyzes a variety of other biologically significant peptides.[3] Thus, alternative and potentially more selective approaches to blockade of the RAS have been sought, such as inhibitors of the more specific enzyme renin.[4] Another obvious target has been receptor antagonists of AII itself. Until recently all potent AII receptor antagonists reported have been peptide analogues of AII[5,6] and have suffered from the problems normally associated with peptides such as poor oral absorption, short plasma half lives and rapid clearance.[7] In addition many exhibit partial agonism.[7] Therefore the recent discovery by the Du Pont group of a series of imidazole derivatives which are potent, nonpeptide AII antagonists has provided an important advance in the area.[8]

The most studied compound of this new class of AII antagonists, DuP 753, is currently undergoing clinical eval-



DUP 753

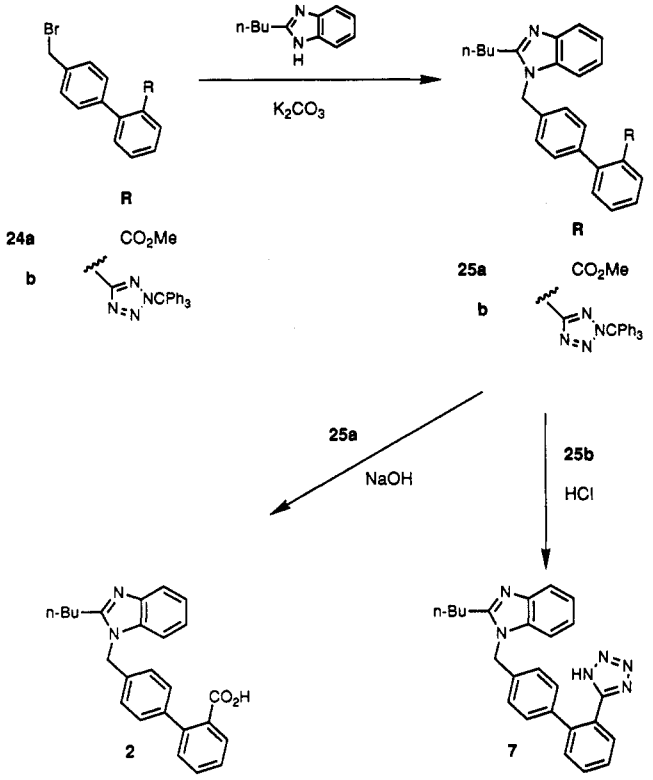
† ICI Pharmaceuticals Group.

(1) (a) Valloton M. B. The Renin-Angiotensin System. *Trends Pharmacol. Sci.* **1987**, *8*, 69–74. (b) Ferrario, C. M. The Renin–Angiotensin System. Importance in Physiology and Pathology. *J. Cardiovasc. Pharmacol.* **1990**, *15* (suppl. 3), 51–55.

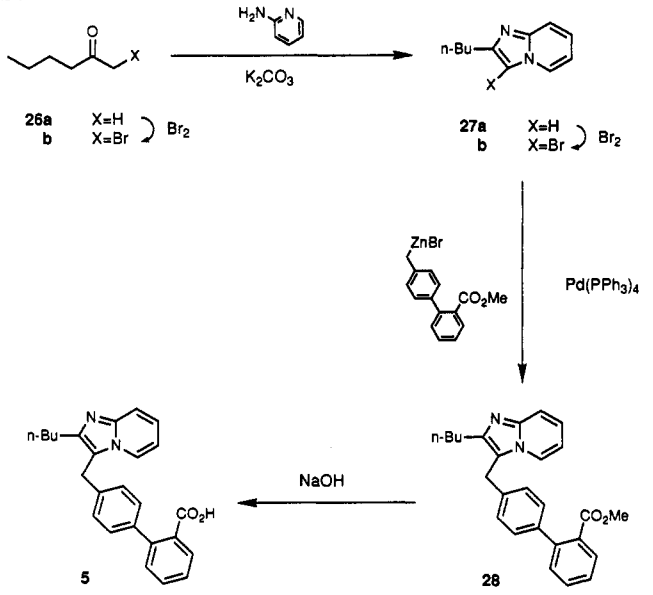
(2) For a review, see: Wyratt, M. J.; Patchett, A. A. Recent Developments in the Design of Angiotensin-Converting Enzyme Inhibitors. *Med. Res. Rev.* **1985**, *5*, 483–531.

0022-2623/92/1835-0877$03.00/0

Scheme I

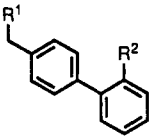


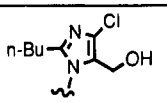
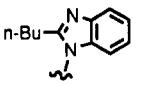
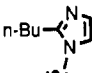
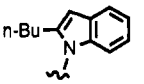
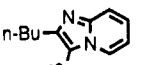
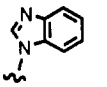
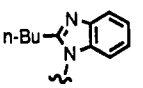
Scheme II



uation for the treatment of hypertension.[9] We report here on our early studies in which we sought to develop an understanding of the structure activity relationships of these new imidazole AII antagonists. This work also led

Table I. Characterization and AII Receptor Binding of 1–6



compd	R^1	R^2	mp, °C	formula[a]	IC_{50}, μM
1		CO_2H	166–167	$C_{22}H_{23}ClN_2O_3 \cdot H_2O$	0.48
2		CO_2H	225–227	$C_{25}H_{24}N_2O_2 \cdot 0.25H_2O$	2.3
3		CO_2H	193–194	$C_{21}H_{22}N_2O_2 \cdot 0.25H_2O$	9.4
4		CO_2H	109–111	$C_{26}H_{25}NO_2 \cdot 0.25H_2O$	51
5		CO_2H	182–185	$C_{25}H_{24}N_2O_2 \cdot 0.5H_2O$	7.4
6		CO_2H	233–234	$C_{21}H_{16}N_2O_2$	140
7		tetrazol-5-yl	230–232	$C_{25}H_{24}N_6$	0.096

[a] All compounds exhibited NMR and mass spectra consistent with structure and gave satisfactory analyses C, H, N (±0.4%).

(3) (a) Erdos, E. G.; Skidgel, R. A. The Unusual Substrate Specificity and the Distribution in Human Angiotensin I Converting Enzyme. *Hypertension.* **1986**, *8* (Suppl. I), I-34–I-37. (b) Palenker, J.; Lentzen, H.; Brandt, U. Enkephalin Degradation by Enkephalinergic Neuroblastoma Cells. Involvement of Angiotensin-Converting-Enzyme. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1984**, *325*, 214–217. (c) Skidgel, R. A.; Engelbrecht, S.; Johnson, A. R.; Erdos, E. G. Hydrolysis of Substance P and Neurotensin by Converting Enzyme an Neutral Endopeptidase. *Peptides* **1984**, *5*, 769–776. (d) Yokosawa, H.; Ugura, Y.; Ishii, S. J. Purification and Inhibition by Neuropeptides of Angiotensin-Converting Enzyme from Rat Brain. *Neurochemistry* **1983**, *41*, 403–410.

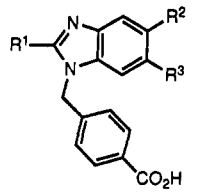
(4) Greenlee, W. J. Renin Inhibitors. *Med. Res. Rev.* **1990**, *10*, 173–236.

(5) For key examples of peptide AII antagonists see: (a) Pals, D. T.; Masucci, F. D.; Sipos, F.; Denning, G. S., Jr. A Specific Competitive Antagonist of the Vascular Action of Angiotensin II. *Circ. Res.* **1971**, *29*, 664–672. (b) Scanlon, M. N.; Matsoukas, J. M.; Franklin, K. J.; Moore, G. J. A New Approach to Angiotensin Antagonists: Methylation of the Tyrosine Hydroxyl in Angiotensin II. *Life Sci.* **1984**, *34*, 317–321. (c) Bovy, P. R.; Trapani, A. J.; McMahon, E. G.; Palomo, M. A Carboxy-Terminus Truncated Analogue of Angiotensin II, [Sar[1]] angiotensin II-(1-7)-Amide Provides an Entry to a New Class of Angiotensin II Antagonists. *J. Med. Chem.* **1989**, *32*, 520–522.

(6) For the first report of weak nonpeptide AII antagonists and their subsequent pharmacological characterization see: (a) Furukawa, Y.; Kishimato, S.; Nishikawa, K. Hypotensive Imidazole-5-acetic Acid Derivatives. U. S. Patent 4 355 040, 1982. (b) Wong, P. C.; Chiu, A. T.; Price, W. A.; Thoolen, M. J. M. C.; Carini, D. A.; Johnson, A. L.; Taber, R. I.; Timmermans, P. B. M. W. M. Nonpeptide Angiotensin II Receptor Antagonists. 1. Pharmacological Characterization of 2-*n*-Butyl-4-chloro-1-(2-chlorobenzyl)imidazole-5-acetic acid, sodium salt (S-8307). *J. Pharmacol. Exp. Ther.* **1988**, *247*, 1–7. (c) Chiu, A. T.; Carini, D. J.; Johnson, A. L.; McCall, D. E.; Price, W. A.; Thoolen, M. J. M. C.; Wong, P. C.; Taber, R. I.; Timmermans, B. M. W. M. Non-peptide Angiotensin II Receptor Antagonists. II. Pharmacology of S-8308. *Eur. J. Pharmacol.* **1988**, *157*, 13–21.

(7) (a) Streten, D. H. P.; Anderson, G. H., Jr. In *Handbook of Hypertension, Vol 5: Clinical Pharmacology of Antihypertensive Drugs*; Doyle, A. E., Ed.; Elsevier, 1984; pp 246–271. (b) Bumpus, F. M.; Khosla, M. C. In *Hypertension: Physiopathology and Treatment*; Genest, J., Koiw, E., Kuckel, O., Eds.; McGraw-Hill: New York, 1977, pp 183–201.

Table II. Effect of Substituents in the Benzimidazole



compd	R^1	R^2	R^3	mp, °C	formula[a]	IC_{50}, μM
8	*n*-Bu	H	H	229–231	$C_{19}H_{20}N_2O_2 \cdot 0.25H_2O$	1.8
9	*n*-Pr	H	H	208–210	$C_{18}H_{18}N_2O_2 \cdot 0.25H_2O$	11
10	*n*-Hex	H	H	191–193	$C_{21}H_{24}N_2O_2$	0.64
11	*n*-Bu	F	H	202–204	$C_{19}H_{19}FN_2O_2 \cdot 0.25H_2O$	4.9
12	*n*-Bu	H	F	202–204	$C_{19}H_{19}FN_2O_2$	7.1
13	*n*-Bu	Cl	Cl	244–246	$C_{19}H_{18}Cl_2N_2O_2$	1.6

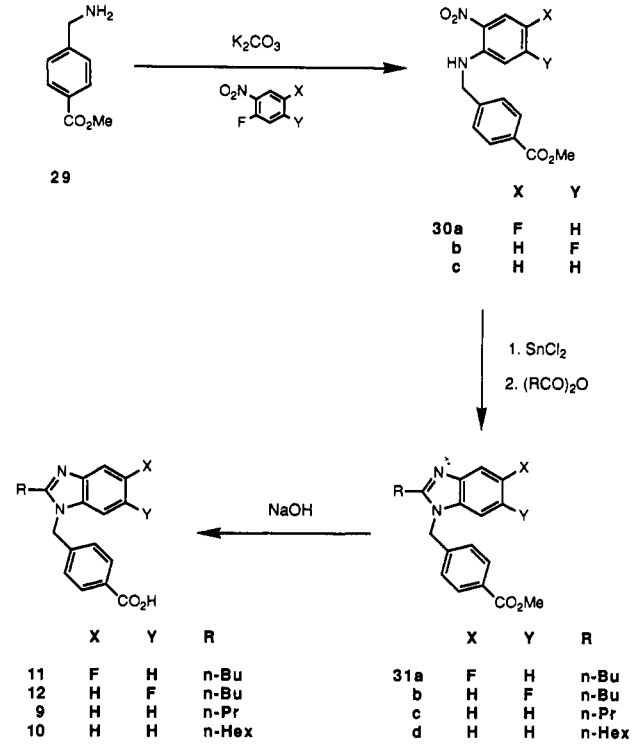
[a] All compounds exhibited NMR and mass spectra consistent with structure and gave satisfactory analyses C, H, N (±0.4%).

to the discovery of new benzimidazole AII antagonists, and we also report the synthesis, biological evaluation, and structure–activity relationships of these compounds.

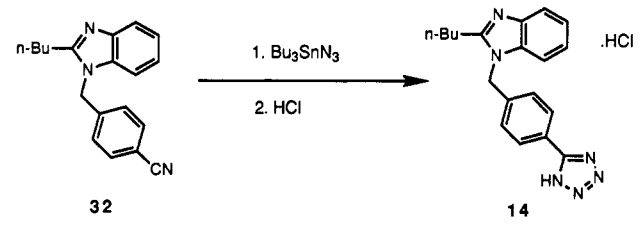
Chemistry

The compounds in Table I were synthesized as shown in Schemes I and II. The (bromomethyl)biphenyl ester **24a**[8a] was a starting material for all the biphenylcarboxylic acids **2–6** which, except for **5**, were prepared by the two-step procedure illustrated for acid **2** (Scheme I). The heterocycles were prepared by literature procedures[10] and were alkylated under standard conditions using potassium carbonate as base. Alkaline hydrolysis of the resulting

Scheme III



Scheme IV



methyl esters gave the final products **2–4** and **6**. Tetrazole **7** was prepared by alkylation of the trityl-protected biphenyl tetrazole **24b**[11] under the standard conditions followed by acid-promoted detritylation to give **7** (Scheme I). The imidazopyridine **5** was prepared as shown in Scheme II. Hexan-2-one **26a** was selectively brominated[12] to yield after fractional distillation the primary bromide **26b**. Condensation of **26b** with 2-aminopyridine gave the imidazopyridine **27a**. Bromination of **27a** provided bromide **27b** which served as the electrophilic partner for a palladium-catalyzed coupling reaction[13] with the (biphenylmethyl)zinc reagent prepared by the reaction of bromo ester **24a** with activated zinc powder. Alkaline hydrolysis of the resulting ester **28** gave **5**.

The compounds **8** and **13** (Table II) and **15–21** (Table III) were prepared in an analogous manner to acid **2** (Scheme I) by alkylation of the benzimidazole with the

(8) (a) Carini, D. J.; Duncia, J. J. V. Angiotensin II Receptor Blocking Imidazoles. European Patent Application 253 310, 1988. (b) Duncia, J. V.; Chui, A. T.; Carini, D. J.; Gregory, G. B.; Johnson, A. L.; Price, W. A.; Wells, G. J.; Wong, P. C.; Calabrese, J. C.; Timmermans, P. B. M. W. M. The Discovery of Potent Nonpeptide Angiotensin II Receptor Antagonists: A New Class of Potent Antihypertensives. *J. Med. Chem.* **1990**, *33*, 1312–1329. (c) Carini, D. J.; Duncia, J. V.; Johnson, A. L.; Chui, A. T.; Price, W. A.; Wong, P. C.; Timmermans, P. B. M. W. Nonpeptide Angiotensin II Receptor Antagonists: *N*-[(Benzyloxy)benzyl]imidazoles and Related Compounds as Potent Antihypertensives. *J. Med. Chem.* **1990**, *33*, 1330–1336. (d) Wong, P. C.; Price, W. A., Jr.; Chui, A. T.; Carini, D. J.; Duncia, J. V.; Johnson, A. L.; Wexler, R. R.; Timmermans, P. B. M. W. M. Nonpeptide Angiotensin II Receptor Antagonists. Studies with EXP9270 and DuP 753. *Hyptertension* **1990**, *15*, 823–834. (e) Carini, D. J.; Duncia, J. V.; Aldrich, P. E.; Chui, A. T.; Johnson, A. L.; Pierce, M. E.; Price, W. A.; Santella, J. B., III; Wells, G. J.; Wexler, R. R.; Wong, P. C.; Yoo, S-E; Timmermans, P. B. M. W. M. Nonpeptide Angiotensin II Receptor Antagonists: The Discovery of a series of *N*-(Biphenylmethyl)imidazoles as Potent, Orally Active Antihypertensives. *J. Med. Chem.* **1991**, *34*, 2525–2547.

(9) (a) Chui, A. T.; McCall, D. E.; Price, W. A.; Wong, P. C.; Carini, D. J.; Duncia, J. V.; Johnson, A. L.; Wexler, R. R.; Yoo, S. E.; Timmermans, P. B. M. W. M. Nonpeptide Angiotensin II Receptor Antagonists VII. Cellular and Biochemical Pharmacology of DuP 753. *J. Pharmacol. Exp. Ther.* **1990**, *252*, 711–718. (b) Wong, P. C.; Price, W. A.; Chui, A. T.; Duncia, J. V.; Carini, D. J.; Wexler, R. R.; Johnson, A. L.; Timmermans, P. B. M. W. M. Nonpeptide Angiotensin II Receptor Antagonists VIII. Characterization of Functional Antagonism displayed by DuP 753, an Orally Active Antihypertensive Agent. *J. Pharmacol. Exp. Ther.* **1990**, *252*, 719–725.

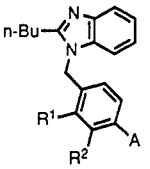
(10) (a) Phillips, M. A. The Formation 2-Substituted Benzimidazoles. *J. Chem. Soc.* **1928**, 2393–2399. (b) Curtis, N. J.; Brown, R. S. An Easily Introduced and Removed Protecting Group for Imidazole Nitrogen: A Convenient route to 2-Substituted Imidazoles. *J. Org. Chem.* **1980**, *45*, 4038–4040. (c) Sakamoto, T.; Kondo, Y.; Yamanaka, H. *Heterocycles* **1986**, *24*, 31–32.

(11) Aldrich, P. E.; Pierce, M. E.; Duncia, J. J. V. Tetrazole Intermediates to Antihypertensive compounds. European Patent Application 291 969, 1988.

(12) Gaudry, M.; Marquet, A. Enolisation des Cetones Dissymetriques III. Acces Facile aux Bromomethylcetones par Bromation en Presence de Methanol. *Tetrahedron* **1970**, *26*, 5611–5615. (See also: *Org. Synth.* **55**, 24–27.)

(13) Negishi, E.; King, A. O.; Okukado, N. Selective Carbon-Carbon Bond Formation via Transition Metal Catalysis 3. A Highly Selective Synthesis of Unsymmetrical Biaryls and Diarylmethanes by Nickel- or Palladium-Catalysed Reaction of Aryl and Benzyl Zinc Derivatives with Aryl Halides. *J. Org. Chem.* **1977**, *42*, 1821–1823.

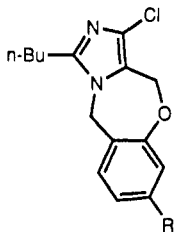
Table III. Effect of Substituents in the Tolyl Ring



compd	R^1	R^2	A	mp, °C	formula[a]	IC_{50}, μM
8	H	H	CO_2H	229–231	$C_{19}H_{20}N_2O_2 \cdot 0.25H_2O$	1.8
14	H	H	tetrazol-5-yl	186–187	$C_{19}H_{20}N_6 \cdot HCl$	5.6
15	F	H	CO_2H	224–226	$C_{19}H_{19}FN_2O_2$	0.86
16	Br	H	CO_2H	242–244	$C_{19}H_{19}BrN_2O_2$	3.7
17	OCH_3	H	CO_2H	198–200	$C_{20}H_{22}N_2O_3$	18
18	NO_2	H	CO_2H	225–228	$C_{19}H_{19}N_3O_4 \cdot 0.25H_2O$	1.8
19	H	CH_3	CO_2H	191–192	$C_{20}H_{22}N_2O_2 \cdot 0.25H_2O$	5.4
20	H	Br	CO_2H	207–208	$C_{19}H_{19}BrN_2O_2$	3.9
21	H	OCH_3	CO_2H	186–188	$C_{20}H_{22}N_2O_3$	12

[a] All compounds exhibited NMR and mass spectra consistent with structure and gave satisfactory analyses C, H, N (±0.4%).

Table IV. Characterization and AII Binding Affinity of Benzoxazepines **22** and **23**



compd	R	mp, °C	formula[a]	IC_{50}, μM
22	2-$PhCO_2H$	227–228	$C_{22}H_{21}ClN_2O_3$	8.5
23	CO_2H	182–184	$C_{16}H_{17}ClN_2O_3 \cdot 0.25H_2O$	15

[a] All compounds exhibited NMR and mass spectra consistent with structure and gave satisfactory analyses C, H, N (±0.4%).

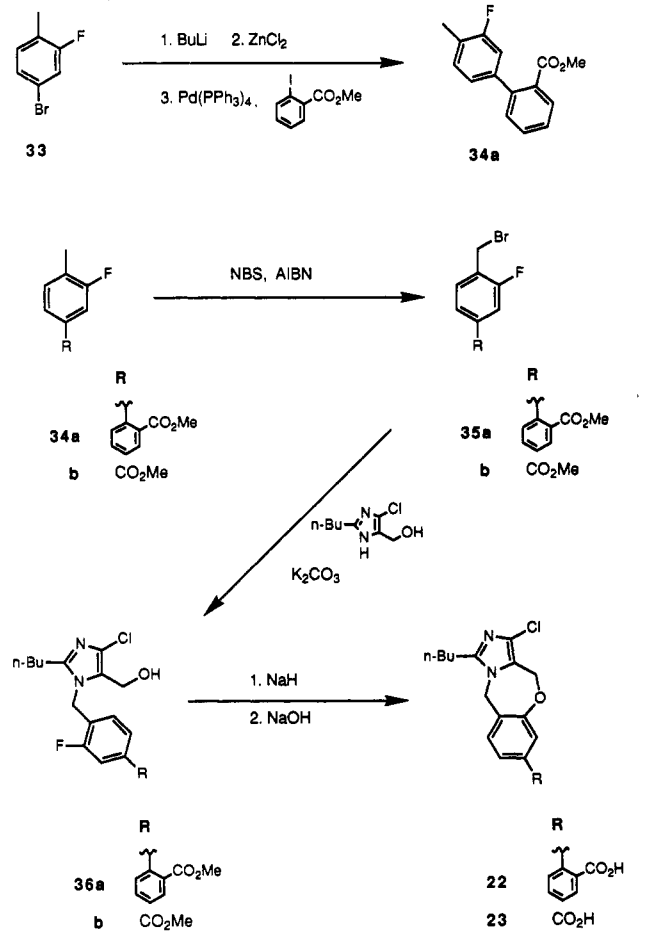
appropriate methyl 4-(bromomethyl)benzoate ester[14] followed by basic ester hydrolysis.

The variously substituted benzimidazoles **9–11** were obtained by the route shown in Scheme III. The aminomethyl ester **29** was treated with the appropriate 2-fluoronitrobenzene derivatives in the presence of potassium carbonate to yield the nitroanilines **30a–c**. Reduction of the nitro group with tin(II) chloride[15] followed by reaction with the appropriate alkanoic anhydride in the presence of *p*-toluenesulfonic acid afforded the benzimidazole esters **31a–d** which were hydrolyzed as before to give the carboxylic acids **9–11**.

The tetrazole **14** was prepared from the nitrile **32** which was obtained by an analogous method to benzimidazole **25a** starting from 4-(bromomethyl)benzonitrile. Reaction of **32** with tributyltin azide in refluxing toluene followed by treatment with hydrochloric acid yielded the benzimidazole **14** (Scheme IV).

The benzoxazepine **22** (Table IV) was prepared from bromofluorotoluene **33** via several steps (Scheme V). Lithiation of **33** followed by transmetalation with zinc chloride and palladium-catalyzed reaction[13] with methyl 2-iodobenzoate provided the fluorobiphenyl ester **34a**. Radical bromination of the benzylic methyl group of **34a**,

Scheme V



under standard conditions, led to bromomethyl ester **35a**. Alkylation of 2-butyl-4-chloro-5-(hydroxymethyl)imidazole[6a] with **35a** leads to both possible imidazole regioisomers. The major product **36a** was easily separated from the more polar minor isomer by flash chromatography. Treatment of **36a** with sodium hydride in refluxing xylene resulted in cyclization with displacement of fluoride ion. Subsequent addition of aqueous alkali led to ester hydrolysis and the production of the acid **22**. The cyclization and hydrolysis reactions could not be performed in the reverse order; the acid resulting from the hydrolysis of ester **36a** did not cyclize under similar or more forcing conditions. This observation leads us to suggest that the key cyclization in the preparation of **22** proceeds by an

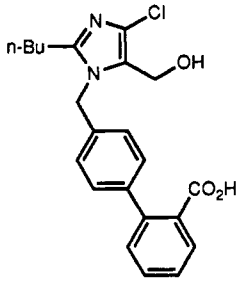
(14) The syntheses of the benzylic bromides utilized in this paper are described: Roberts, D. A.; Russell, S. T. Heterocyclic Compounds. European Patent Application 0 399 732, 1990.

(15) Bellamy, F. D.; Ou, K. Selective Reduction of Aromatic Nitro Compounds with Stannous Chloride in Non-Acidic and Non-Aqueous Medium. *Tetrahedron Lett.* **1984**, *25*, 839–842.

intramolecular S_N2Ar mechanism, rather than via a benzyne-type intermediate. The same reactivity was shown in the route to benzoic acid **23** which was prepared in an analogous manner to **22** from the fluoro ester **34b** (Scheme V).

Discussion

At the start of our studies, the imidazole **1**[16] was prepared as a representative example of the recently reported nonpeptide AII antagonists.[8] Our first aim was to define the key structural elements of the imidazole **1** which were critical to its binding to the AII receptor, and then to gain some understanding of the role of these structural features in their interaction with the AII receptor. We first turned our attention to the contribution of the heterocyclic portion to the binding affinity of **1**. In an attempt to simplify the heterocycle, particularly from a synthetic viewpoint, we prepared the benzimidazole **2** and we were pleased to find that the AII antagonist properties of **1** (guinea pig adrenal binding IC_{50} 0.48×10^{-6} M, rabbit aorta pA_2 7.1)[17] were largely maintained in **2** (IC_{50} 2.3×10^{-6} M, pA_2 7.2). From



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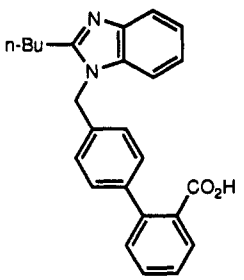


2

this result we concluded that the chlorine and hydroxymethyl groups were not critical to binding, and that the bicyclic heterocycle could be accommodated by the AII receptor without significant loss of affinity. A series of heterocyclic variants based on the benzimidazole **2** were then prepared to explore the importance to binding affinity of the various features of the heterocyclic portion of **2** (Table I). First, the roles of the hydrophobic, hydrocarbon portions of **2** were investigated by preparation of compounds **3** and **6**. Removal of the benzenoid fragment of **2**, to give the imidazole **3**, led to only a small drop in affinity, while the contribution of the 2-butyl group was shown to be much more significant as demonstrated by the very weak affinity of the unsubstituted benzimidazole **6**. The roles of the nitrogen atoms of the benzimidazole group were explored in compounds **4** and **5**. The basic N-3 nitrogen was shown to play an important role in binding by the 25-fold drop in the binding affinity of indole **4** compared to benzimidazole **2**, while the moderate fall in binding affinity shown by the imidazopyridine **5** indicated a less critical contribution of the N-1 nitrogen of **2**.

Turning attention to the N-1 substituent of **2**, replacement of the carboxyl group (of **2**) with a tetrazole (compound **7**) led to a 20-fold improvement in affinity mirroring similar changes in the original imidazole series.[8d] Replacements for the biphenyl acid portion of **2**, which impart similar binding, were sought. The critical contribution of the carboxyl group to the binding affinity of imidazole AII antagonists such as **1** has been demonstrated[8b] and we confirmed that a suitably placed acidic group, ionized at physiological pH, is important for good binding. In our search for an alternative to the biphenyl group, we prepared a variety of compounds with different aromatic spacer groups between carboxylic acid and heterocycle (data not shown). Only the 4-toluic acid derivative **8** retained activity comparable to the biphenyl acid **2** (Table II). Molecular modeling indicated that the biphenyl and toluic acids place the acid group in the same region of space with minimal change in conformation around the linking methylene groups. We concluded, therefore, that in the biphenyl and toluic acid series the ionized acid group binds to the same positively charged site on the receptor.

The effect of varying and introducing new substituents in toluic acid **8** was explored (Tables II and III). A variety of branched and heteroatom-substituted analogues of the 2-butyl group in **8** were prepared (data not shown) but all possessed much lower levels of activity. Only the 2-*n*-alkyl derivatves retained significant binding activity, with the 2-*n*-hexylbenzimidazole **10** showing a slight increase in affinity. Similar structure–activity relationships were found around the 2-substituent in both biphenyl and toluic acid benzimidazole series and similarly stringent requirements at this position were reported for the initial imidazole antagonists.[8b] Therefore, we concluded that in all these series the heterocycles are binding to the receptor in a similar manner in which the 2-alkyl groups of the heterocycle bind to a constrained, hydrophobic pocket of the receptor.

The effect of lowering the basicity of the benzimidazole group to a value closer to that of the 4-chloro-5-(hydroxymethyl)imidazole group of the lead compound **1** was investigated. Introduction of halogen substituents in the 5- and 6-positions of the benzimidazoles **8** significantly reduced the pK_a of the benzimidazoles **11–13**.[18] However, the binding affinity of compounds **11–13** was little altered (Table II). For all the antagonists prepared, the basic nitrogen of the heterocyclic group is substantially unprotonated at physiological pH and it would appear therefore that the role of this nitrogen on binding to the receptor is that of a hydrogen-bond acceptor.

In an attempt to optimize the binding affinity of toluic acid antangonist **8**, the effect of introducing substituents into the benzene ring bearing the carboxylic acid of **8** was also investigated (Table III, **15–21**). In general, the binding shown was similar to that of the parent acid **8**, although introduction of electron-donating substituents (**17**, **19**, and **21**) led to reduced affinity while a 2-fluoro substituent (**15**) produced a small improvement in affinity. Replacing the carboxyl group of **8** with a tetrazole did not lead to the improvement in activity seen in the biphenyl series (Table I compounds **2** and **7**); the tolyl tetrazole **14** displayed binding affinity slightly inferior to the toluic acid **8** (Table III).

Finally, the conformational preference about the methylene group linking the heterocycle and phenylene ring was

(16) Imidazole **1** was given the number EXP7711 by the Du Pont group. For pharmacological characterization of EXP7711 see: (a) Timmermanns, P. B. M. W. M.; Carini, D. J.; Chui, A. T.; Duncia, J. V.; Price, W. A.; Wells, G. J.; Wong, P. C.; Wexler, R. R.; Johnson, A. L. Nonpeptide Angiotensin II Receptor Antagonists: A Novel Class of Antihypertensive Agents. *Blood Vessels* **1990**, *27*, 295–300. (b) Koeple, J. P.; Bovy, P. R.; McMahon, E. G.; Olins, G. M.; Reitz, D. B.; Salles, K. S.; Schuh, J. R.; Trapani, A. J.; Blaine, E. H. Central and Peripheral Actions of a Nonpeptide Angiotensin II Receptor Antagonist. *Hypertension* **1990**, *15*, 841–847.

(17) See experimental section for procedures. Binding experiments in whole guinea pig adrenals indicated the presence of only angiotensin II receptors of subtype 1.[22] No type 2 receptors were detected.

(18) The measured macroscopic basic pK_a for compound **1** was 3.66 and for compound **8** was 5.87. Calculated values for compounds **11–13** were as follows: **11**, 5.35; **12**, 5.35; **13**, 4.50.

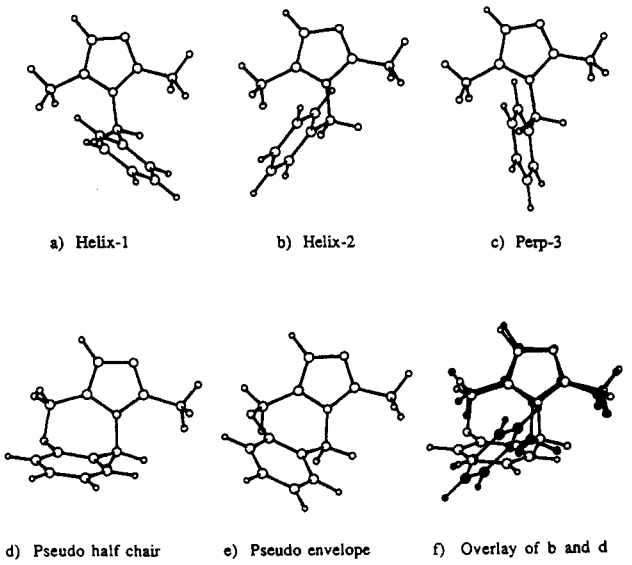


Figure 1.

considered. As described later, molecular modeling studies were carried out to gain some indication of the conformational preferences about this methylene group in the imidazole and benzimidazole AII antagonists. Both heterocycles gave similar results leading to a model of the preferred conformations. On the basis of this model, conformational constrained heterocycles were sought that could explore experimentally the possible binding conformation of these new AII antagonists. Benzoxazepines **22** and **23** were chosen as modeling indicated that the rigid tricycle of these compounds might lock the conformation around the linking methylene group to mimic a speculated receptor binding conformation. Both showed moderate binding affinity but at a lower level to that of **1** and **2**. The moderate activity shown by **22** could indicate that a conformation accessed by its tricyclic ring system is reasonably close to but not coincident with the binding conformation adopted by **1** when it interacts with the AII receptor. Alternatively, it is possible that the locked conformation of **22** is close to optimal but that the additional ring is not readily accommodated by the AII receptor.

Molecular Modeling Studies

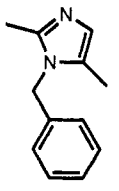
The energetically favorable conformations of imidazole **1** were explored using molecular mechanics calculations to probe the conformational preferences around the methylene linking the heterocyclic and biphenyl portions of the molecule. As a model for the phenyl–imidazole linkage of **1**, the simplified imidazole **37** was considered. The conformational analysis of **37** is analogous to that of diphenylmethane.[19] Molecular mechanics were performed using an in-house program AESOP.[20] The calculations on **37** suggested two helical conformations of nearly equal energy as minima (Figure 1, parts a and b). One of these, Helix-1, was found to be the conformation observed for **1** in the crystal by X-ray diffraction.[21] A third conformation with imidazole and phenyl rings perpendicular, Perp-3 (Figure 1c), was found to be low-energy transition state (0.5 kcal/mol above Helix-1 and -2) between the two helical conformations. The alternative transition state, the Gable conformation (not shown) was found to be higher in energy at 1.5 kcal/mol above either helix. Thus molecular mechanics predicts two low-energy conformations of **37**, Helix-1 and Helix-2. Analogous results were obtained from modeling 1-benzyl-2-methylbenzimidazole.

Conformationally restricted analogues of **37** with geometries similar to Helix-1 or Helix-2 were sought with the aim of designing more potent AII antagonists by eliminating the loss of rotational entropy that antagonists such as **2** make on binding to the AII receptor. Thus, molecular mechanics calculations were performed on **38**, a cyclized analogue of **37**. Conformational analysis of **38** indicated that two minima were present (AESOP). The pseudo-half-chair (Figure 1d) was found to be the lowest in energy with the pseudoenvelope (Figure 1e) 1.4 kcal/mol higher in energy. The preferred pseudo-half-chair conformation was overlayed with both Helix-1 and Helix-2 of **37**. It was found that good overlap was obtained with Helix-2, but not with Helix-1. In the former overlay (Figure 1f), the atoms of the imidazole rings overlay closely, while the torsions around the linking methylenes and the directional orientation of the phenyl rings are similarly close. However, the phenyl rings do not overlay in a coplanar manner but lie at 50° to each other.

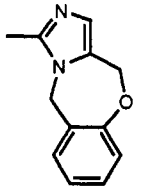
Table V. ED_{50} Values[a] (Iv Administration in AII Infused Rats)

compd	in vitro IC_{50}, μM	ED_{50}[a] ± SEM, mg/Kg
2	2.3	13.8 ± 2.0[b]
7	0.096	5.2 ± 0.9[c]
8	1.8	12.6 ± 2.7[c]
10	0.64	>20[c]
11	4.9	9.1 ± 5.8[d]
16	3.7	11.1 ± 5.6[c]
17	18	16.1 ± 4.7[c]
20	3.9	5.0 ± 2.6[d]

[a] Dose resulting in 50% inhibition of AII pressor response. [b] n = 7. [c] n = 4. [d] n = 3.



37



38

(19) Barnes, J. C.; Paton, J. D.; Damewood, J. R., Jr.; Mislow, K. Crystal and Molecular Structure of Diphenylmethane. *J. Org. Chem.* **1981**, *46*, 4975–4979 and references therein.

(20) Molecular mechanics calculations were performed using AESOP-2.3.[23] Molecular models were constructed graphically using an in-house program ENIGMA.

(21) Crystal structure of **1** determined by Dr. M. McPartlin, unpublished.

(22) (a) Chui, A. T.; Herblin, W. F.; McCall, D. E.; Ardecky, R. J.; Carini, D. J.; Duncia, J. V.; Pease, L. J.; Wong, P. C.; Wexler, R. R.; Johnson, A. L.; Timmermans, P. B. M. W. M. Identification of Angiotensin II Receptor Subtypes. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 196–203. (b) Chan, R. S. L.; Lotti, V. J. Two Distinct Angiotensin II Receptor Binding Sites in Rat Adrenal Revealed by New Selective Nonpeptide Ligands. *Mol. Pharmacol.* **1990**, *37*, 347–351. (c) Whitebread, S.; Mele, M.; Kamber, B.; de Gasparo, M. Preliminary Biochemical Characterization of Two Angiotensin II Receptor Subtypes. *Biochem. Biophys. Res. Commun.* **1989**, *163*, 284–291.

(23) AESOP is an in-house molecular mechanics program, ICI Americas, Wilmington, DE 19897, derived in part from BIGSTRN-3 (QCPE 514): Nachbar, R.; Mislow, K. *QCPE Bulletin* **1986**, *6*, 96. AESOP employs the MM2 force field parameters: see, Allinger, N. L. *QCPE Bulletin* **1980**, *12*, 395.

In conclusion, molecular modeling predicts that analogues of 1 cyclized as in 38 would possess a low-energy conformation similar to Helix-2, one of the two predicted low-energy conformations of 1.

In Vivo Evaluation

Selected compounds were tested for AII antagonist activity in vivo in an AII infused, normotensive rat model. In this test, the antagonists were administered intravenously and potencies for inhibiting the increase in blood pressure induced by AII infusion were determined and expressed as ED_{50} values (Table V).

The compounds tested showed substantial antagonism of the hypertensive effects of AII in the dose range of 5–20 mg/kg. In general, the in vivo results (Table V) broadly reflect the relative potencies determined by the in vitro binding assay. However, two exceptions are evident. The lower activity of the *n*-hexylbenzimidazole **10** is probably a reflection of an unfavorable increase in lipophilicity relative to the *n*-butyl analogue 8 possibly leading to higher protein binding and greater partitioning into unfavorable compartments. While the origin of the greater than expected activity of bromo-substituted toluic acid **20** is less obvious.

When these compounds were administered orally in an AII infused, normotensive rat model at a dose of 50 mg/kg only marginal effects on blood pressure were seen.

Summary

This paper describes new nonpeptide antagonists of the vasoconstrictor AII. On the basis of the literature lead **1**, the benzimidazole AII antagonist **2** was synthesized. An investigation to determine the key structural features of **2** responsible for its AII receptor binding was undertaken and structure–activity relationships were developed. It was found that a *p*-toluic acid could replace the biphenyl acid of **2**, and a series of toluic acid derivatives were prepared.

These studies enabled the development of a structure–activity relationship which defined those elements of **2** and its analogues which have a critical role in binding. These features were (a) an *n*-alkyl subtituent, 3–6 carbons in length at the 2-position of the benzimidazole; (b) a basic nitrogen probably acting as a hydrogen-bond acceptor at the 3-position of the benzimidazole; and (c) a suitably positioned acidic group, ionized at physiological pH. Benzimidazoles possessing all these features demonstrated good AII antagonist properties, with IC_{50} values for inhibition of AII receptor binding in the range of 10^{-5}–10^{-7} M, and ED_{50} values for inhibiting AII induced rises in blood pressure in the range of 5–20 mg/kg following intravenous administration in the rat. However, the oral activity of compounds in this series was poor, only marginal effects being seen at a dose of 50 mg/kg.

Experimental Section

Melting points were determined in open glass capillary tubes with a Buchi apparatus and are uncorrected. ^1H NMR spectra were recorded on Bruker WM200 or WM250 instruments and are reported as δ values (parts per million) relative to tetramethylsilane as internal standard. Chemical ionization mass spectra (CIMS) were recorded on a VG 12-12 quadrupole or a VG 70-250 SE spectrometer. Fast-atom bombardment mass spectra (FABMS) were determined on a VG ZAB 2-SE spectrometer.

Tetrahydrofuran (THF) and xylenes were dried by distillation from calcium hydride. *N,N*-Dimethylformamide (DMF) was dried over 4-Å molecular sieves. All evaporations were carried out at below 50 °C by using a Buchi rotary evaporator. Flash chromatography was performed on silica (Merck Kieselgel: Art. 9385).

Methyl 4′-[(2-Butyl-1*H*-benzimidazol-1-yl)methyl]biphenyl-2-carboxylate (25a). A mixture of 2-butylbenzimidazole (2.6 g, 14.9 mmol), methyl 4-(bromomethyl)biphenyl-2-carboxylate[8a] (**24a**) (5.0 g, 16.4 mmol), and potassium carbonate (1.55 g, 11.2 mmol) in DMF (40 mL) was heated at 100 °C for 3 h. CH_2Cl_2 (200 mL) was added, and insoluble material was removed by filtration. The filtrate was concentrated, and the residue was purified by flash chromatography eluting with hexane/ethyl acetate/triethylamine (60:40:1 v/v) to give **25a** (2.7 g, 45%) as a clear oil: ^1H NMR ($CDCl_3$) 0.95 (t, 3 H), 1.45 (sextet, 2 H), 1.85 (quintet, 2 H), 2.89 (t, 2 H), 3.62 (s, 3 H), 5.39 (s, 2 H), 7.08 (d, 2 H), 7.15–7.6 (complex m, 8 H), 7.75–7.85 (m, 2 H).

4′-[(2-Butyl-1*H*-benzimidazol-1-yl)methyl]biphenyl-2-carboxylic Acid (2). Aqueous sodium hydroxide solution (1 M, 6.5 mL) was added to a solution of the ester (**25a**) (2.0 g, 5.0 mmol) in ethanol (20 mL). The solution was heated under reflux for 3 h, and then volatile material was removed by evaporation. The residue was dissolved in water (20 mL) and the solution acidified to pH 4 with 20% aqueous citric acid. The precipitated solid was collected and recrystallized from aqueous methanol to give **2** (0.83 g, 43%) as a white solid: mp 225–227 °C; ^1H NMR (DMSO-d_6) 0.88 (t, 3 H), 1.38 (sextet, 2 H), 1.73 (quintet, 2 H), 2.85 (t, 2 H), 5.51 (s, 2 H), 7.0–7.75 (complex m, 12 H), 12.6 (br s, 1 H); CIMS *m/e* 385 $(M + H)^+$. Anal. ($C_{25}H_{25}N_2 \cdot 0.25H_2O$) C, H, N.

2-Butyl-1-[(2′-1*H*-tetrazol-5-ylbiphenyl-4-yl)methyl]benzimidazole (7). 2-Butyl-1-[[2′-[2-(triphenylmethyl)-2*H*-tetrazol-5-yl]biphenyl-4-yl]methyl]benzimidazole (**25b**) (400 mg) was dissolved in an 8 M solution of hydrogen chloride in dioxane (5 mL), and water (0.5 mL) was added. The solution was allowed to stand for 1.5 h and then volatile material was removed by evaporation. The residue was partitioned between saturated aqueous $NaHCO_3$ (10 mL) and ether (10 mL). The aqueous phase was separated, washed with ether (3 × 10 mL), and acidified to pH 4 and 20% aqueous citric acid. The precipitated solid was collected and recrystallized from EtOAc to give **7** (94 mg, 37%) as a white solid: mp 230–232 °C; ^1H NMR (DMSO-d_6) 0.87 (t, 3 H), 1.36 (sextet, 2 H), 1.70 (quintet, 2 H), 2.81 (t, 2 H), 5.47 (s, 2 H), 7.04 (s, 4 H), 7.15 (m, 2 H), 7.4–7.7 (complex m, 6 H); FABMS *m/e* 407 $(M - H)^-$, 379. Anal. ($C_{25}H_{24}N_6$) C, H, N.

1-Bromohexan-2-one (26b). Bromine (32 g) was added dropwise to a stirred solution of hexan-2-one (**26a**, 20 g) in methanol (120 mL) at −10 °C. The mixture was then stirred at 0 °C for 45 min followed by 45 min at 10 °C. Water (60 mL) and then concentrated sulfuric acid (100 mL) were added (care exotherm), and the mixture stirred overnight at room temperature. Water (180 mL) was added and the mixture was extracted with ether (4 × 100 mL). The combined extracts were washed with aqueous $NaHCO_3$ (50 mL) and water (2 × 50 mL). The extracts were dried ($CaCl_2$), the solvent was evaporated, and the residue was distilled under reduced pressure to give **26b** (18.2 g, 51%): bp 73–78 °C (18 mgHg); ^1H NMR ($CDCl_3$) 0.93 (t, 3 H), 1.35 (sextet, 2 H), 1.60 (quintet, 2 H), 2.66 (t, 2 H), 3.88 (s, 2 H).

2-Butylimidazo[1,2-*a*]pyridine (27a). The bromo ketone (**26b**) (5.70 g), 2-aminopyridine (2.30 g), and potassium carbonate (2.53 g) were added to ethanol (80 mL) and the mixture refluxed for 3 h. The mixture was cooled and filtered, and the solvent was removed by evaporation. The residue was purified by flash chromatography eluting with hexane/ethyl acetate/triethylamine (60:30:1 v/v) to give **27a** (2.14 g, 50%) as an oil: ^1H NMR ($CDCl_3$), 0.97 (t, 3 H), 1.44 (sextet, 2 H), 1.77 (quintet, 2 H), 2.79 (t, 2 H), 6.72 (t, 1 H), 7.11 (dd, 1 H), 7.33 (s, 1 H), 7.53 (d, 1 H), 8.04 (d, 1 H).

3-Bromo-2-butylimidazo[1,2-*a*]pyridine (27b). A solution of bromine (1.76 g) in water (40 mL) was added dropwise to a stirred solution of imidazopyridine (**27a**) in ethanol (25 mL) at room temperature. The mixture was stirred at room temperature for 4 h. The ethanol was removed by evaporation and the aqueous residue made basic with $NaHCO_3$ and extracted with CH_2Cl_2 (3 × 30 mL). The extracts were dried ($MgSO_4$), the solvent was evaporated, and the residue was purified by flash chromatography eluting with hexane/ethyl acetate/triethylamine (70:30:1 v/v) to give **27b** (1.85 g, 73%) as an oil: ^1H NMR ($CDCl_3$) 0.96 (t, 3 H), 1.43 (sextet, 2 H), 1.78 (quintet, 2 H), 2.79 (t, 2 H), 6.88 (td, 1 H), 7.19 (ddd, 1 H), 7.54 (dd, 1 H), 8.05 (dd, 1 H).

Methyl 4′-[(2-Butylimidazo[1,2-*a*]pyridin-3-yl)methyl]biphenyl-2-carboxylate (28). Activated zinc dust (327 mg) was added to a stirred solution of methyl 4′-(bromomethyl)biphenyl-2-carboxylate[8a] (**24a**) (610 mg) in THF (2.5 mL) under argon at room temperature. The mixture was stirred at room

temperature for 3 h, at which time this showed no (bromomethyl)biphenyl starting material remained. The bromoimidazopyridine (**27b**) (253 mg) in dry THF (1.5 mL) was then added followed by a solution of tris(dibenzylideneacetone)dipalladium(0) (23 mg) and tri-*o*-tolylphosphine (45 mg) in THF (1 mL). The reaction mixture was stirred at room temperature for 1 h, then heated at reflux for 2 h, and finally stirred for 16 h at room temperature. Water (5 mL) and ether (15 mL) were added, and the organic phase was separated and then dried ($MgSO_4$). The solvent was removed by evaporation and the residue purified by flash chromatography eluting with hexane/ethyl acetate/triethylamine (60:40:1 v/v) to give **28** (146 mg, 37%) as an oil: ^1H NMR ($CDCl_3$) 0.95 (t, 3 H), 1.45 (sextet, 2 H), 1.82 (quintet, 2 H), 2.85 (t, 2 H), 3.61 (s, 3 H), 4.31 (s, 2 H), 6.70 (td, 1 H), 7.05–7.9 (complex m, 11 H).

4′-[(2-Butylimidazo[1,2-*a*]pyridin-3-yl)methyl]biphenyl-2-carboxylic Acid (5). The title compound was prepared from **28** by the same procedure described for the preparation of **2**. The product was recrystallized from aqueous methanol to give **5** (55 mg, 39%) as a white solid: mp 182–185 °C; ^1H NMR (DMSO-d_6) 0.90 (t, 3 H), 1.36 (sextet, 2 H), 1.69 (quintet, 2 H), 2.76 (t, 2 H), 4.35 (s, 2 H), 6.81 (td, 1 H), 7.1–7.6 (complex m, 9 H), 7.68 (dd, 1 H), 8.12 (d, 1 H); CIMS *m/e* 385 $(M + H)^+$. Anal. ($C_{25}H_{24}N_2O_2 \cdot 0.5H_2O$) C, H, N.

Methyl 4-[[(4-Fluoro-2-nitrophenyl)amino]methyl]benzoate (30a). A mixture of methyl 4-(aminomethyl)benzoate (**29**) (0.50 g, 3.0 mmol), 2,5-difluoronitrobenzene (0.40 g, 2.5 mmol) and potassium carbonate (0.35 g, 2.5 mmol) in DMF (5 mL) was heated at 100 °C for 5 h. The mixture was cooled, CH_2Cl_2 (20 mL) was added, and insoluble material was removed by filtration. The solvent was evaporated and the residue recrystallized from MeOH/EtOAc to give **30a** (0.54 g, 70%) as orange needles: mp 144–146 °C; ^1H NMR ($CDCl_3$) 3.92 (s, 3 H), 4.61 (d, 2 H), 6.70 (dd, 1 H), 7.17 (ddd, 1 H), 7.62 (d, 2 H), 7.93 (dd, 1 H), 8.04 (d, 2 H), 8.36 (br s, 1 H). Anal. ($C_{15}H_{13}FN_2O_4$) C, H, N.

Methyl 4-[(2-Butyl-5-fluoro-1*H*-benzimidazol-1-yl)methyl]benzoate (31a). A mixture of powdered tin(II) chloride dihydrate (1.0 g, 4.4 mmol) and the nitro compound (**30a**) (266 mg, 0.88 mmol) in methanol (7 mL) was heated under reflux for 10 h. The solvent was evaporated, and EtOAc (20 mL) and 1 M NaOH solution (15 mL) were added to the residue. The mixture was filtered through Celite, and the aqueous layer was separated and extracted with EtOAc (2 × 20 mL). The combined extracts were washed with brine (20 mL), dried ($MgSO_4$), and evaporated. The crude diamine residue was then dissolved in toluene (7 mL) and valeric anhydride (0.2 mL, 1.0 mmol), and valeric acid (0.1 mL, 0.9 mmol) and *p*-toluenesulfonic acid monohydrate (190 mg, 1.0 mmol) were added. The mixture was heated under reflux for 10 h. EtOAc (20 mL) was added and the solution washed with $NaHCO_3$ solution. The organic phase was dried ($MgSO_4$) and evaporated. The residue was purified by flash chromatography eluting with hexane/ethyl acetate/triethylamine (60:40:1) to give **33a** (142 mg, 48%) as an oil: ^1H NMR ($CDCl_3$) 0.91 (t, 3 H), 1.41 (sextet, 2 H), 1.81 (quintet, 2 H), 2.81 (t, 2 H), 3.90 (s, 3 H), 5.37 (s, 2 H), 6.85–7.05 (complex m, 2 H), 7.08 (d, 2 H), 7.44 (dd, 1 H), 7.98 (d, 2 H).

4-[(2-Butyl-5-fluoro-1*H*-benzimidazol-1-yl)methyl]benzoic Acid (11). The title compound was prepared from **31a** by the same procedure described for the preparation of **2**. The product was recrystallized from aqueous methanol to give **11** (90 mg, 66%) as a white solid: mp 202–204 °C; ^1H NMR (DMSO-d_6), 0.85 (t, 3 H), 1.34 (sextet, 2 H), 1.68 (quintet, 2 H), 2.80 (t, 2 H), 5.57 (s, 2 H), 7.00 (td, 1 H), 7.16 (d, 2 H), 7.3–7.5 (m, 2 H), 7.88 (d, 2 H), 12.9 (br s, 1 H); FABMS *m/e* 325 $(M - H)^-$, 191. Anal. ($C_{19}H_{19}FN_2O_2 \cdot 0.25H_2O$) C, H, N.

2-Butyl-1-[(4′-1*H*-tetrazol-5-ylphenyl)methyl]benzimidazole Hydrochloride (14). 2-Butyl-1-[(4-cyanophenyl)methyl]benzimidazole (**32**) (0.72 g) was added to a stirred solution of tributyltin azide (2.02 g) in toluene (30 mL), and the mixture was then refluxed for 48 h. The solvent was removed by evaporation, and the residue was purified by flash chromatography eluting with CH_2Cl_2/MeOH (9:1 v/v). The product free base was dissolved in ethanol and precipitated by addition of ethereal hydrogen chloride and then recrystallized (ethanol/ether) to give **14** (0.60 g, 65%) as white solid: mp 186–187 °C; ^1H NMR (DMSO-d_6) 0.93 (t, 3 H), 1.43 (sextet, 2 H), 1.82 (quintet, 2 H), 3.29 (t, 2 H), 5.92 (s, 2 H), 7.5–7.7 (complex m, 4 H), 7.8–7.95 (complex m, 2 H), 8.16 (d, 2 H); FABMS *m/e* 331 $(M - H)^-$, 173. Anal. ($C_{19}H_{20}N_6 \cdot HCl$) C, H, N.

Methyl 3′-Fluoro-4′-methylbiphenyl-2-carboxylate (34a). A 1.6 M solution of butyllithium in hexane (15.7 mL) was added slowly to a solution of 4-bromo-2-fluorotoluene (**33**) (4.725 g) in THF (25 mL) at −78 °C under argon. The mixture was stirred for 0.5 h then a 1.0 M solution of zinc chloride in ether (25 mL) was added and the mixture stirred a further 10 min at −78 °C. Tetrakis(triphenylphosphine)palladium(0) (290 mg) and methyl 2-iodo benzoate (5.9 g) were added sequentially. The mixture was then allowed to warm to room temperature and then heated under reflux for 18 h. The reaction was cooled, and 0.1 M hydrochloric acid (30 mL) and ether (30 mL) were added. The aqueous layer was separated and extracted with ether (30 mL). The combined extracts were dried ($MgSO_4$) and the solvent evaporated. The residue was purified by flash chromatography eluting with hexane/ethyl acetate (15:1 v/v) to give **34a** (4.24 g, 77%) as a clear oil: ^1H NMR ($CDCl_3$) 2.31 (d, 3 H), 3.68 (s, 3 H), 6.95 (s, 1 H), 6.99 (d, 1 H), 7.18 (t, 1 H), 7.34 (d, 1 H), 7.40 (t, 1 H), 7.52 (t, 1 H), 7.81 (d, 1 H).

Methyl 4′-(Bromomethyl)-3′-fluorobiphenyl-2-carboxylate (35a). A mixture of the biphenyl ester (**34a**) (4.1 g, 16.8 mmol), *N*-bromosuccinimide (3.14 g, 17.6 mmol), and AIBN (100 mg) in CCl_4 (40 mL) was heated at reflux for 2 h under argon. The mixture was cooled and insoluble material removed by filtration. The filtrate was evaporated and the residue purified by flash chromatography eluting with hexane/ethyl acetate (15:1 v/v) to give **35a** (3.55 g, 65%) as an oil: ^1H NMR ($CDCl_3$) 3.70 (s, 3 H), 4.47 (s, 2 H), 7.1–7.5 (m, 7 H).

Methyl 3′-Fluoro-4′-[[2-butyl-4-chloro-5-(hydroxymethyl)-1*H*-imidazol-1-yl]methyl]biphenyl-2-carboxylate (36a). The title compound was prepared by the method described for ester (**25a**) starting with 2-butyl-4-chloro-5-(hydroxymethyl)imidazole[6a] (1.20 g, 6.4 mmol) and the bromomethyl ester (**36a**) (1.90 g, 5.9 mmol). The crude product was purified by flash chromatography eluting with hexane/ethyl acetate (3:2 v/v) to give **36a** (770 mg, 30%) as a clear oil: ^1H NMR ($CDCl_3$), 0.89 (t, 3 H), 1.36 (sextet, 2 H), 1.68 (quintet, 2 H), 2.60 (t, 2 H), 3.67 (s, 3 H), 4.57 (s, 2 H), 5.29 (s, 2 H), 6.74 (t, 1 H), 7.04 (m, 2 H), 7.30 (dd, 1 H), 7.44 (td, 1 H), 7.54 (td, 1 H), 7.87 (dd, 1 H); CIMS *m/e* 431 $(M + H)^+$.

2-(3-Butyl-1-chloro-5*H*,11*H*-imidazo[5,1-*c*][1,4]benzoxazepin-8-yl)benzoic Acid (22). A mixture of the ester **36a** (770 mg, 1.79 mmol) and sodium hydride (80 mg of a 60% dispersion in oil, 2.0 mmol) in xylenes (12 mL) was heated at reflux for 20 h. The reaction was then allowed to cool and 1.7 M NaOH solution (1.5 mL) and EtOH (5 mL) were added, and the mixture was heated at 100 °C for a further 3 h. Volatile material was removed by evaporation and the residue partioned between ether (10 mL) and water (10 mL). The aqueous layer was separated and acidified to pH 3–4 with 20% citric acid solution. The precipitated solid was recrystallized from MeOH/acetone/water to give **22** (529 mg, 75%) as an off-white solid: mp 227–228 °C; ^1H NMR (DMSO-d_6) 0.89 (t, 3 H), 1.33 (sextet, 2 H), 1.57 (quintet, 2 H), 2.74 (t, 2 H), 5.28 (s, 2 H), 5.45 (s, 2 H), 6.77 (d, 1 H), 6.88 (dd, 1 H), 7.3–7.6 (complex m, 4 H), 7.69 (dd, 1 H), 12.7 (br s, 1 H); CIMS *m/e* 397 $(M + H)^+$. Anal. ($C_{22}H_{21}ClN_2O_3$) C, H, N.

Angiotensin Receptor Binding Methodology. The approach used was a conventional ligand binding assay based on the interaction of monoiodinated angiotensin II with a washed membrane fraction prepared from guinea pig adrenal glands.

Fresh guinea pig adrenal glands were trimmed free from fat and were rinsed thoroughly in ice-cold physiological saline. All subsequent procedures were at 3–4 °C unless otherwise stated. Pooled tissue was minced with scissors and then homogenized in 20 volumes of 20 mM *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid/sodium hydroxide (HEPES/NaOH) buffer, pH 7.1, using a Polytron PT-10 homogenizer to obtain a fine suspension. The homogenate was centrifuged at 1000*g* for 10 min and the supernatant further centrifuged at 63000*g* for 10 min. The membrane pellet was washed three times by resuspension in the above buffer and centrifugation at 63000*g* and was finally resuspended in 20 volumes of 20 mM HEPES/NaOH buffer, pH 7.1, containing 10 mM magnesium chloride and 100 mM sodium chloride. Aliquots of the membrane suspension were stored frozen

in liquid nitrogen until required.

Test compounds were dissolved and diluted as necessary in dimethyl sulfoxide (DMSO). The drug solutions were then diluted in assay buffer (20 mM HEPES/NaOH, pH 7.1, containing 10 mM magnesium chloride, 100 mM sodium chloride and 0.2% (w/v) bovine serum albumin) such that the DMSO concentration was 3% (v/v). A 50 μL aliquot of each diluted drug solution was incubated for 90 min at room temperature (nominally 20 °C) with 50 μL of monoiodinated angiotensin II [(3-[^{125}I]iodotyrosyl)-angiotensin II, 74 TBq/mmol, (Amersham International plc)] and 50 μL of membrane suspension containing approximately 50 μg membrane protein. Following incubation, receptor-bound radioactivity was separated from free by rapid filtration over glass-fiber mats (Skatron Ltd, Newmarket, UK). Receptor-bound radioactivity trapped on the filter mats was quantified using a gamma counter (Pharmacia/Wallac 1277 Gammamaster).

Specific angiotensin binding was defined as the difference between total binding and the nonspecific binding which was determined in the presence of excess (0.3 μM) nonradioactive angiotensin II. Specific angiotensin binding in the presence of drug was compared to the control specific binding in the presence of vehicle. Results were calculated as percentage displacement relative to the control. IC_{50} values (concentration for 50% displacement of the specifically bound monoiodinated angiotensin II) were determined from the relationship between percentage inhibition and drug concentration. Test concentrations were chosen to bracket the expected IC_{50} and to cover at least 4 orders of magnitude.

Under the assay conditions used, the total binding and nonspecific binding were typically in the range 0.48–0.85 fmol and 0.02–0.04 fmol per 150 μL, respectively. The concentration of monoiodinated angiotensin used in the assay was 0.1 nM and the IC_{50} for its displacement by authentic angiotensin II was 0.77 ● 0.46 nM (mean ± standard deviation, n = 400). The presence of 1% DMSO in the incubation mixture had no significant effect on angiotensin binding to the adrenal receptor.

pA_2 Determination in the in Vitro Rabbit Aorta. New Zealand White rabbits (2–4 kg) were deeply anaesthetized with sodium pentobarbitone, the descending thoratic aorta was removed, cut into helical strips and mounted in 20-mL organ baths containing Krebs bicarbonate solution at 37 °C and bubbled with 5% CO_2. Resting tension was set at 1.0 g and the aortic strips were allowed to equilibrate for 120 min, after which a cumulative dose–response curve to angiotensin II was constructed. Following a 60-min washout period, an angiotensin II antagonist was added to the bath and 60 min later, a second angiotensin II dose–response curve was constructed. The pA_2 values of antagonists were determined according to the method of Schild.

Intravenous ED_{50} Determination in the Rat. Male Alderley Park Wistar rats (200–250 g) were prepared with indwelling carotid artery and jugular vein catheters under Saffan (alphaxolone/alphadolone) anaesthesia. The following day, arterial blood pressure was measured while angiotensin II was infused intravenously at a dose of 1 μg/kg^{-1} min^{-1}, which increased mean aterial pressure by approximately 50 mmHg. During the angiotensin II infusion, incremental doses of an angiotensin II antagonist were administered intravenously and the ED_{50} of the antagonist determined.

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5-(Aminomethyl)-3-aryldihydrofuran-2(3*H*)-ones, a New Class of Monoamine Oxidase-B Inactivators

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Both *cis*- and *trans*-5-(aminomethyl)-3-aryldihydrofuran-2(3*H*)-one hydrochloride salts (**9** and **10**) were synthesized efficiently in a 5-step sequence from arylacetic acids. Both compounds were found to be irreversible inactivators of monoamine oxidase B. These compounds constitute the first members of a new class of monoamine oxidase inactivators.

5-(Aminomethyl)-3-aryl-2-oxazolidinones (**1**) were reported to be selective inactivators of monoamine oxidase B (EC 1.4.3.4)[1] and therefore to have the potential to be useful adjuncts to L-dopa treatment for Parkinson's disease.[2] Recently, we investigated the mechanism of inactivation of monoamine oxidase by **1**[3] and concluded that inactivation arises from attachment of the enzyme to the α-position of the aminomethyl side chain (**2**, X = amino acid residue). The stability of this seemingly unstable adduct **2** was proposed to be the result of the electron-

Table I. Kinetic Constants for the Inactivation of Monoamine Oxidase-B by 5-(Aminomethyl)-3-aryldihydrofuran-2(3*H*)-ones

compd	k_{inact}, min^{-1}	K_I, mM	k_{inact}/K_I min^{-1} mM^{-1}
9a	2.57×10^{-3}	4.2	6.1×10^{-4}
10a	2.14×10^{-3}	5.1	4.2×10^{-4}
9b	4.44×10^{-3}	6.67	6.7×10^{-4}
10b	23×10^{-3}	18.2	12.6×10^{-4}
1 (R = Me, R′ = H)[11]	0.8×10^{-3}	12.5	0.71×10^{-4}

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