Nonpeptide Angiotensin II Receptor Antagonists. Synthesis and Biological Activity of Benzimidazoles

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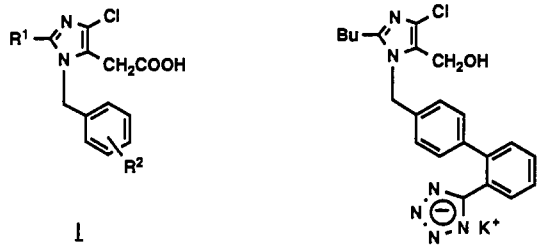
A series of substituted 2-butylbenzimidazoles bearing a biphenylylmethyl moiety at the 1-position was prepared via three synthetic routes and evaluated for angiotensin II (AII) receptor antagonistic activity (in vitro and in vivo). Binding affinity was determined using bovine adrenal cortical membrane. Substitution at the 4-, 5-, or 6-position reduced the affinity relative to that of the unsubstituted compound (**13a**). However, most of the compounds with a substituent at the 7-position showed binding affinity comparable to that of DuP 753 (losartan). In functional studies, a carboxyl group was found to be very important for antagonistic activity against AII. Comparison of 2-butyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-benzimidazole-4-, -5-, -6-, and -7-carboxylic acids (**15a–d**) in an AII-induced rabbit aortic ring contraction assay clearly demonstrated the importance of the substitutional position of the carboxyl group. In an in vivo assay, oral administration of benzimidazole-7-carboxylic acids caused long-lasting inhibition of the AII-induced pressor response in rats. The optimum substituent at the 7-position of the benzimidazole ring was found to be a carboxyl or an ester group. The representative compound, 2-butyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-benzimidazole-7-carboxylic acid (**15d**, CV-11194), inhibited the specific binding of [^{125}I]AII to bovine adrenal cortical membrane with an IC_{50} value of 5.5×10^{-7} M. The AII-induced contraction of rabbit aortic strips was antagonized by CV-11194 (IC_{50} value, 5.5×10^{-11} M), while the compound had no effect on the contraction induced by norepinephrine or KCl. Orally administered CV-11194 at doses of 0.3–10 mg/kg dose-dependently inhibited the AII-induced pressor response in rats and dogs. CV-11194 at 1 mg/kg po reduced blood pressure in spontaneously hypertensive rats (SHR). The three-dimensional molecular structure of CV-11194 was determined by X-ray diffraction.

Introduction

The renin–angiotensin system (RAS) plays an important role in blood pressure regulation and electrolyte homeostasis.[1] Angiotensin II (AII) is the biologically active component of the RAS and is responsible for most of the peripheral effects of this system.

There are two commonly described classes of effective inhibitors of the RAS: renin inhibitors and angiotensin converting enzyme (ACE) inhibitors. In recent years, renin inhibitors with high specificity and affinity for human renin have been reported,[2] but they have yet to be marketed. ACE inhibitors such as captopril, enalapril, and others are very effective for the treatment of most types of hypertension and congestive heart failure.[3] However, their lack of specificity provides a major reason for exploring alternative therapy. Some of the adverse effects of ACE inhibitors such as dry cough and angioedema have been attributed to the multisubstrate action of ACE.[4]

AII receptor antagonists would specifically affect the RAS independently of the source of AII.[5] Saralasin was the first specific peptide antagonist of AII administered to humans, and it was found to reduce blood pressure in hypertensive patients with high renin levels. Unfortunately, long-term antihypertensive treatment was not possible because these peptide antagonists have low oral bioavailability and short duration of action.[6]



I

Ia : R^1=Ph, R^2= 4'-OMe-3'-Me (CV-2973)
Ib : R^1=Bu, R^2=2'-NO_2 (CV-2961)

DuP 753 (losartan)

Figure 1.

Our research efforts during this last decade have focused on finding another way to interfere in the RAS. In 1980, derivatives of benzylimidazole-5-acetic acid (Ia,b) (Figure 1) were found to inhibit both the AII-induced contraction of rabbit aortic strips and the AII-induced pressor response in rats.[7] These compounds were the first nonpeptide AII receptor antagonists, and many researchers soon began studies in an effort to enhance the potency of the prototype. A variety of heterocyclic compounds were synthesized and evaluated as AII receptor antagonists.[8-10] One of the most studied AII antagonists is DuP 753 (losartan) (Figure 1),[8a] which is a chemical modification of the nonpeptide AII receptor antagonist benzylimidazoleacetic acids.

Our strategy of developing more potent AII antagonists than the prototype was to take advantage of the established structure–activity relationships (SAR) of the benzylimidazoleacetic acids and to incorporate a biphenyl group as described by D. J. Carini et al.[8a] We designed imidazole-

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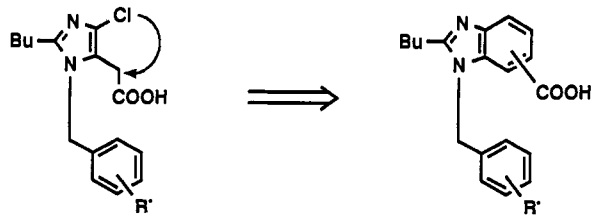


Figure 2. Design of benzimidazolecarboxylic acids.

related heterocyclic compounds with key structural features to exhibit potent AII antagonism: a butyl side chain at the 2-position and a biphenyltetrazole moiety at the 1-position.

A series of azoles such as pyrroles, pyrazoles, and triazoles, not presented here, were prepared and evaluated for AII antagonism. The affinity of these compounds for the AII receptor was comparable to that of DuP 753, but their in vivo AII antagonist activities were less potent. We presumed that this reduction in in vivo activity could be ascribed to the absence of the acetic acid moiety which was found to be essential in the prototype.

We then turned our attention to designing fused heterocycles containing a carboxyl group, particularly benzimidazolecarboxylic acids, by connecting between the methylene group of acetic acid group and the 4-position of imidazole (Figure 2). The present paper describes the synthesis and the SAR of benzimidazoles and emphasizes the significance of a carboxyl group for the potency and oral activity of AII antagonists.[11]

Chemistry

Compounds prepared for this study are shown in Table I, and the synthetic routes are outlined in Schemes I–IV. From among a variety of known synthetic routes for substituted benzimidazoles,[12] we adopted three routes for the synthesis of 2-butyl-1-[[2′-(substituted)biphenyl-4-yl]methyl]benzimidazoles: route i, alkylation of 2-butylbenzimidazoles (**3**); route ii, reductive cyclization of valeroanilides (**12a–h**); and route iii, transformation of the functional groups at the 7-position of benzimidazoles.

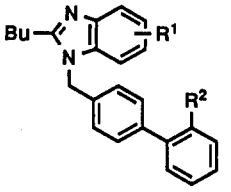
As depicted in Scheme I (route i), alkylation of readily available 2-butylbenzimidazoles (**3**) with 4-(bromomethyl)-biphenyls (**2**)[13] could be carried out in the presence of sodium hydride. These 2-butylbenzimidazoles were prepared via condensation of commercially available 1,2-diaminobenzenes and ethyl valerimidate hydrochloride[14] or reductive cyclization of methyl 3-nitro-2-(*N*-valerylamino)benzoate (**11c**). 2-Butyl-5-methoxybenzimidazole and 2-butyl-5-chlorobenzimidazole led to almost 1:1 mixtures of regioisomers, **5b**, **5c** and **5d**, **5e**, respectively, which were separated by column chromatography. Their structures were assigned based on the fact that in NMR spectra a proton at the 4-position appears at lower field than one at the 7-position due to the anisotropic effect of the C=N double bond in the imidazole moiety.[15] For example, the H-4 proton in **5b** appears at low field (δ 7.29, doublet, J = 2.4 Hz) from H-7 (δ 7.11, doublet, J = 8.8 Hz), and these protons in **5c** appear at δ 7.45 (doublet, J = 8.7 Hz) and δ 7.07 (doublet, J = 2.5 Hz), respectively. However, only one regioisomer, methyl 2-butyl-1-[(2′-cyanobiphenyl-4-yl)methyl]-1*H*-benzimidazole-4-carboxylate (**6a**), was obtained in the case of methyl 2-butylbenzimidazole-4-carboxylate. This selectivity was attributed to steric hindrance caused by the methoxycarbonyl group. Although route i was simple and efficient for analogue synthesis, it sometimes required tedious separation of regioisomers. In addition, 7-substituted benzimidazoles could not be obtained via route i.

Regioselective synthesis was accomplished using route ii in Scheme II. Acylation of aminobenzoates (**7**) with valeryl chloride gave the corresponding (*N*-valerylamino)-benzoates (**8a–f**) in good yield. Treatment of the (*N*-valerylamino)benzoates (**8a–f**) with fuming nitric acid and acetic anhydride afforded regioisomer mixtures in nitrobenzoates, from which the desired nitrobenzoates (**11a–f**) were separated by column chromatography. 6-Methoxy-2-nitroanilide (**11g**) was prepared from 2-amino-3-nitrophenol (**9**) by O-methylation with methyl iodide and potassium carbonate (K_2CO_3) followed by acylation with valeric anhydride in the presence of a catalytic amount of concentrated sulfuric acid (H_2SO_4).[16] The acylation of **10** could not be accomplished with valeryl chloride. The key intermediates, *N*-(4-biphenylylmethyl)-2-nitroanilides (**12a–h**), were obtained by alkylation of **11a–g** with 4-(bromomethyl)biphenyls (**2**) using sodium hydride or K_2CO_3 as a base. Reductive cyclization of **12** was accomplished in good yield with iron powder and concentrated hydrochloric acid in boiling methanol. In this reaction the trityl group of **12e** and **12f** was deprotected to furnish the 2-butyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-benzimidazoles (**14f,g**).

The cyano group of **5a–f**, **6a–e** was converted to a tetrazole group (**13a–f**, **14a–c,e**, **15d**) with NaN_3/NH_4Cl or trimethyltin azide (Me_3SnN_3)[17] (Scheme III). In some cases undesired products were obtained in the reaction with NaN_3/NH_4Cl. For example **6a** gave the methyl ester (**14a**) and the amide (**14h**). In the reaction of 7-carboxylate (**6d**), the ester group was hydrolyzed, and the 1-methyltetrazole derivative (**17**) was formed in 4.7% yield. The structure was confirmed by ^{13}C-NMR and NOE difference spectra. Generally, the reactions proceeded more clearly with Me_3SnN_3 than with NaN_3/NH_4Cl. Alkaline hydrolysis of the benzimidazolecarboxylates (**14a–c,e–g**) afforded the corresponding carboxylic acids (**15a–c,e–g**).

The 2-butylbenzimidazoles possessing a variety of substituents at the 7-position (**14d,i,j**, **18**, **27–33**) were synthesized as shown in Scheme IV (route iii). The carboxylic acid (**15d**) was condensed with alcohol or amine in the presence of sulfuric acid or diethyl phosphorcyanidate (DEPC) to give the corresponding esters (**14d,i,j**) or the amide (**18**). Reduction of the methyl ester (**6b**) to an alcohol (**19**) with sodium borohydride ($NaBH_4$) in MeOH–tetrahydrofuran (THF)[18] followed by chlorination with thionyl chloride ($SOCl_2$) gave a chloride (**20**) which was reacted with nucleophiles (sodium cyanide, sodium methoxide, or dimethylamine) to afford displacement products (**21–23**). The dicyano derivative (**21**) was treated with ethanolic HCl under reflux to give a monoethyl ester (**25**). The cyano group in the biphenyl moiety remained intact under these reaction conditions. The difference in the reactivity of these cyano groups seems to be due to the steric characteristics of the biphenyl group. A 7-methyl derivative (**24**) was obtained by radical reduction of **20** with tributyltin hydride (Bu_3SnH) and benzoyl peroxide (BPO).[19] Demethylation of **5f** with boron tribromide (BBr_3) afforded a 7-hydroxyl derivative (**26**) in 63% yield. These cyano derivatives (**19**, **22–26**) were converted to tetrazoles (**27–32**) as described previously using NaN_3/NH_4Cl or Me_3SnN_3. Alkaline hydrolysis of **31** gave a carboxylic acid (**33**), a one carbon homologue of **15d**.

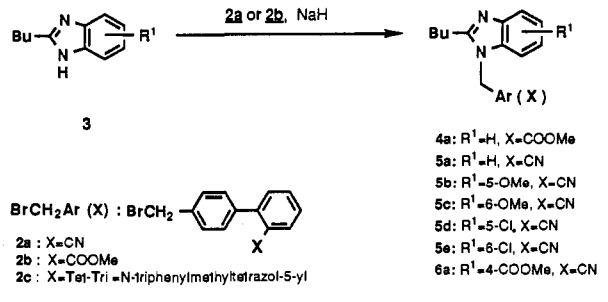
Table I. Inhibitory Effects of AII Receptor Antagonists on Specific Binding of [^{125}I]AII and Pressor Response Induced by AII in Rats



compd	R^1	R^2	in vitro[a] IC_{50}, $\times 10^{-7}$ M	in vivo (po)[b] % inhibn at 3 h/7 h 3 mg/kg	30 mg/kg
13a	H	Tet[c]	9.0	13/19[e]	18/21 (52/17)[d]
13b	5-OMe	Tet	9.1	NT	84/89[f] (60/18)[d]
13c	6-OMe	Tet	11	NT	5/1[f] (77/31)[d]
13d	5-Cl	Tet	15	NT	−8/11 (45/28)[d]
13e	6-Cl	Tet	31	NT	18/21 (23/3)[d]
13f	7-OMe	Tet	28	−6/4	NT
14a	4-CO_2Me	Tet	72	NT	9/11
14b	5-CO_2Me	Tet	7.4	NT	−13/−11[f]
14c	6-CO_2Me	Tet	4.4	NT	0/17
14d	7-CO_2Me	Tet	3.2	78/83	NT
14e	5-Me-7-CO_2Me	Tet	8.7	41/1	NT
14f	5-Cl-7-CO_2Me	Tet	4.4	57/30	NT
14g	6-Me-7-CO_2Et	Tet	9.1	13/15	100/100
14h	4-$CONH_2$	Tet	130	NT	28/15
14i	7-CO_2Et	Tet	14	51/63	NT
14j	7-CO_2Bu	Tet	12	9/−4	78/90
15a	4-CO_2H	Tet	>100	19/19[e]	NT
15b	5-CO_2H	Tet	55	7/−7	NT
15c	6-CO_2H	Tet	90	NT	9/12
15d	7-CO_2H	Tet	5.5	91/85	100/100
15e	5-Me-7-CO_2H	Tet	13	80/63	NT
15f	5-Cl-7-CO_2H	Tet	11	23/5	65/37
15g	6-Me-7-CO_2H	Tet	3.4	40/58	NT
16a	H	CO_2H	11	NT	45/52[f] (20/10)[d]
16b	7-CO_2H	CO_2H	6.6	2/15	46/13
17	7-CO_2H	1-Me-Tet[g]	34	77/75	NT
18	7-CONHi-Pr	Tet	5.4	NT	NT
27	7-CH_2OH	Tet	4.5	16/5	41/22
28	7-CH_2OMe	Tet	6.0	54/63	NT
29	7-CH_2NMe_2	Tet	24	12/10	NT
30	7-Me	Tet	3.3	8/−2	NT
31	7-CH_2CO_2Et	Tet	2.5	24/18	82/55
32	7-OH	Tet	11	2/5	56/32
33	7-CH_2CO_2H	Tet	26	NT	NT
DuP 753			1.5	63/75	NT

[a] Inhibition of specific binding of [^{125}I]AII (0.2 nM) to bovine adrenal cortex. The IC_{50} value is the concentration of compound which inhibits 50% of bound [^{125}I]AII. For details see the Experimental Section. [b] Percent inhibition of the AII (0.1 μg/kg iv)-induced increase in blood pressure in conscious male Sprague–Dawley rats at 3 and 7 h after administration of the test compounds. NT means "not tested". For details see the Experimental Section. [c] Tet: tetrazol-5-yl. [d] Inhibition at 0.5 and 1 h after administration of the test compounds at dose 10 mg/kg iv. [e] Dose 10 mg/kg po. [f] Dose 100 mg/kg po. [g] 1-Me-Tet: 1-methyltetrazol-5-yl.

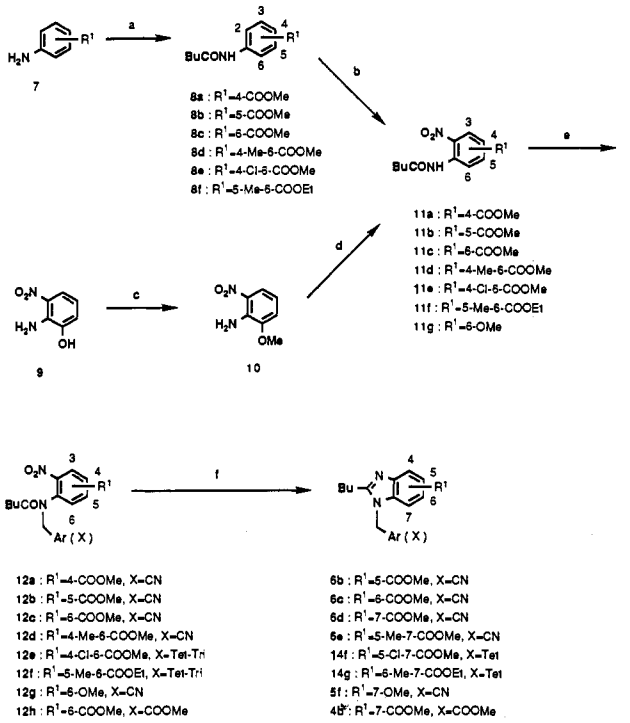
Scheme I (Route i)



Structure–Activity Relationships

Since adrenal cortical tissue has a high density of AII binding sites and has been widely used to study the SAR of various peptide and nonpeptide AII antagonists, bovine adrenal cortical tissue was used to characterize the nonpeptide AII antagonists in this study.[20] Each compound was evaluated for the binding affinity to the AII receptor with respect to the displacement of [^{125}I]AII (0.2 nM) bound to adrenal cortical membranes (Table I). Many compounds were found to have an IC_{50} value (the concentration that displaced 50% of the bound [^{125}I]AII) in the range of 10^{-6}–10^{-7} M. Substitution with a carboxyl (**15d**), methoxycarbonyl (**14d**), ethyl acetate (**31**), methyl (**30**), and hydroxymethyl (**27**) groups at the 7-position increased the affinity relative to unsubstituted **13a**. Substitution at the 4-, 5-, or 6-position (**13b–e**, **14h**) decreased the affinity, except in the case of a methoxycarbonyl group (**14b,c**). The largest difference in receptor binding was observed with the carboxylic acids where the 7-carboxylic acid (**15d**) was more potent than the 4-, 5-, and 6-carboxylic acids (**15a–c**) by 1 order of magnitude. Additional substitution at the 5- or 6-position of the 7-carboxylic acid (**15d**) had no significant effect on binding affinity (**15e–g**). Although the replacement of the tetrazole ring with a carboxyl group afforded compounds with similar affinity (**13a** vs **16a** and **15d** vs **16b**),[21] methylation of the tetrazole ring (**17**) decreased the affinity. We found that appropriate substitution of benzimidazoles results in

Scheme II (Route ii)[a]



[a] (a) BuCOCl, Et_3N; (b) fuming HNO_3, Ac_2O; (c) MeI, K_2CO_3; (d) $(BuCO)_2O$, concentrated H_2SO_4; (e) **2**, K_2CO_3; (f) Fe, concentrated HCl.

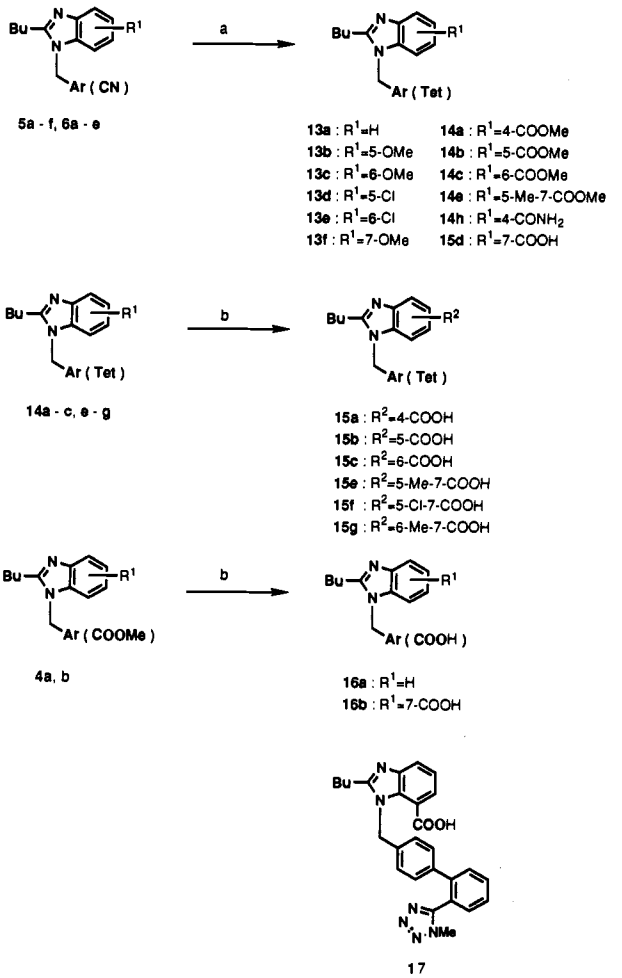
compounds with significantly improved affinity. Some of them have binding affinity comparable to that of DuP 753.

The importance of the substitutional position among the carboxylic acids (**15a–d**) was demonstrated in the case of inhibition of AII-induced contraction in rabbit aortic strips. As shown in Figure 3, the inhibitory effect of 7-carboxylic acid (**15d**) is more potent than those of the other carboxylic acids (**15a–c**) by 2–4 orders of magnitude.

The compounds were also evaluated in vivo for inhibition of the pressor response induced by AII (100 ng/kg iv) in conscious rats, and the data are listed in Table I. When intravenously or orally administered, compounds **13a–e** at 10 mg/kg inhibited pressor response with a very short duration of action, less than 1 h. Enhanced oral activity with a long duration of action was observed with 7-carboxylic acid and its esters (**15d,e,g, 14d–f,i**) at 3 mg/kg po. This is in sharp contrast to the compounds possessing these substituents at the 4-, 5-, or 6-position (**14a–c, 15a–c**) which had little inhibitory effect at 3–100 mg/kg po. Introduction of an additional substituent to the 5- or 6-position of **15d** failed to enhance the antihypertensive potency. Substitution with a hydroxymethyl, methoxymethyl, (ethoxycarbonyl)methyl, or hydroxyl group at the 7-position (**27, 28, 31, 32**) increased the inhibitory activity, but these groups were less effective in vivo than a carboxyl or a ester group. The other substituents examined did not increase the potency. The methyltetrazole derivative (**17**) demonstrated oral activity. Demethylation of **17** in vivo giving **15d** probably occurs rapidly.

These findings indicate that a carboxyl group at the 7-position is essential for good oral activity in the benzimidazole series. The role of this carboxyl group in antihypertensive activity remains unclear. We believe that it causes a secondary conformational change in the AII receptor to strengthen the antagonist–receptor complex

Scheme III[a]



[a] (a) NaN_3, NH_4Cl, DMF or (i) Me_3SnN_3, toluene, (ii) 1 N HCl; (b) 1 N NaOH.

after the initial binding. 2-Butyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-benzimidazole-7-carboxylic acid (**15d**) was selected for further study under the code name of CV-11194.

Biological Activities of CV-11194

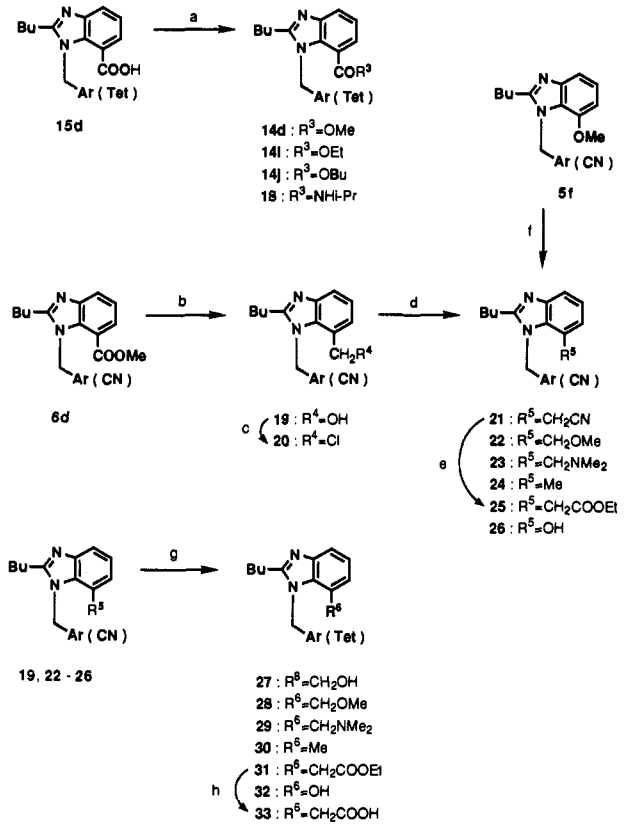
As shown in Figure 4, CV-11194 at concentration from 10^{-9} to 10^{-5} M inhibited the specific binding of [^{125}I]AII to cortical membranes in a concentration related manner. The IC_{50} value was 5.5×10^{-7} M, which is comparable to that of DuP 753.

In isolated rabbit aortic strips, CV-11194 and DuP 753 inhibited AII (10^{-8} M)-induced contraction in a concentration-related manner. The IC_{50} of CV-11194 was 5.5×10^{-11} M and was considerably more potent than that of DuP 753 (Figure 3). CV-11194 had little effect on the contraction induced by norepinephrine (10^{-8} M) and KCl (10^{-8} M).

As shown in Figure 5, oral administration of CV-11194 at doses of 0.3–10 mg/kg produced dose-related inhibition of the pressor response induced by AII (100 ng/kg iv) in conscious normotensive rats. The inhibitory effect of CV-11194 was more potent and longer acting than that of DuP 753.

As shown in Figure 6, CV-11194 at 0.3–10 mg/kg po caused dose-related inhibition of the pressor response to AII (100 ng/kg iv) in dogs. CV-11194 at 10 mg/kg po completely inhibited the pressor response to AII for at

Scheme IV (Route iii)[a]



[a] (a) Alcohol, concentrated H_2SO_4 or DEPC, i-$PrNH_2$, Et_3N; (b) $NaBH_4$, MeOH, THF; (c) $SOCl_2$; (d) nucleophile (NaCN, MeONa, or Me_2NH) or Bu_3SnH, BPO; (e) HCl–EtOH; (f) BBr_3; (g) NaN_3, NH_4Cl, DMF or (i) Me_3SnN_3, toluene, (ii) 1 N HCl; (h) NaOH.

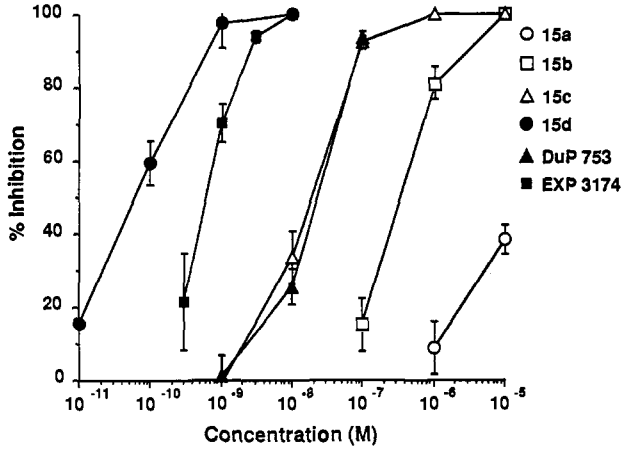


Figure 3. Concentration–inhibition curves of benzimidazole-carboxylic acids, DuP 753, and EXP 3174 on the AII (10 nM)-induced contraction in isolated rabbit aorta.

least 7 h, while the same dose of DuP 753 inhibited the response by only 55% maximally with a short duration of action.

In the spontaneously hypertensive rats (SHR), CV-11194 significantly decreased blood pressure in a dose-dependent manner at 0.3–3 mg/kg po (Figure 7). The antihypertensive effect of CV-11194 lasted for over 7 h. CV-11194 at 0.3 mg/kg po lowered blood pressure as potently as DuP 753 at 3 mg/kg po did.

X-ray Crystallography for CV-11194

X-ray structure analysis of CV-11194 revealed that the molecule is bent almost orthogonally at the methylene

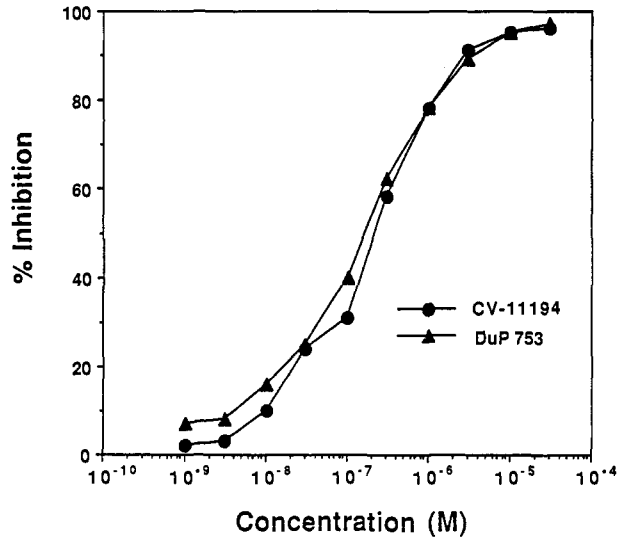


Figure 4. Inhibition of the [^{125}I]AII (0.2 nM) bound to bovine adrenal cortex by CV-11194 (**15d**) and DuP 753.

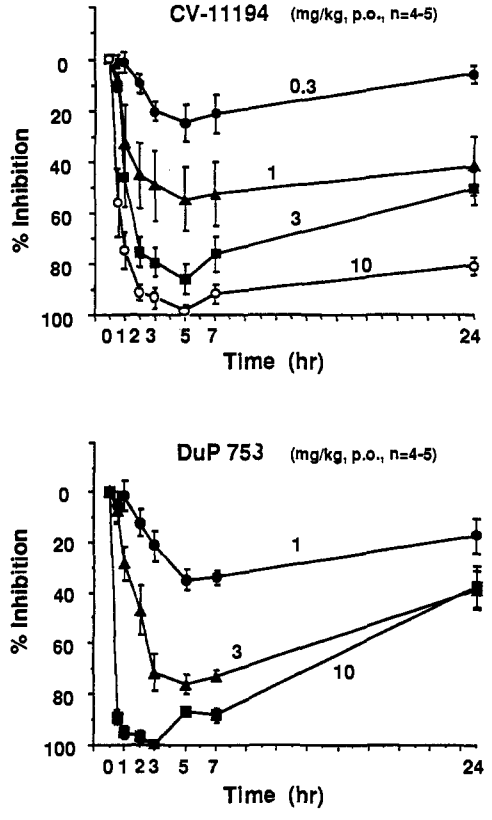


Figure 5. Inhibition of the AII (100 ng/kg iv)-induced pressor response in conscious normotensive rats by CV-11194 (**15d**) and DuP 753.

which connects the biphenyl group and the heterocyclic moiety (Figure 8). The torsion angle C2–N1–C15–C16 is 80.6°, and the angle between the plane of the benzimidazole moiety and the central (1,4-disubstituted) benzene ring is 85.9°. Although the two benzene rings of the unsubstituted biphenyl are coplanar in the crystal state,[22] they are twisted in CV-11194 owing to the presence of a tetrazole ring in the ortho position. The angle of the benzene planes is 48.0°. This geometrical relationship also holds in the case of the tetrazole ring and the terminal benzene ring, since the other benzene is ortho to the tetrazole ring. The angle of the planes is 52.5°. Since the benzene rings are neither coplanar nor perpendicular, there are four possible po-

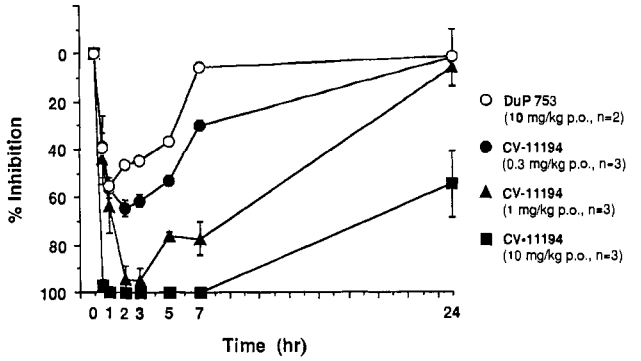


Figure 6. Inhibition of the AII (100 ng/kg iv)-induced pressor response in dogs by CV-11194 (**15d**) (n = 3) and DuP 753 (n = 2).

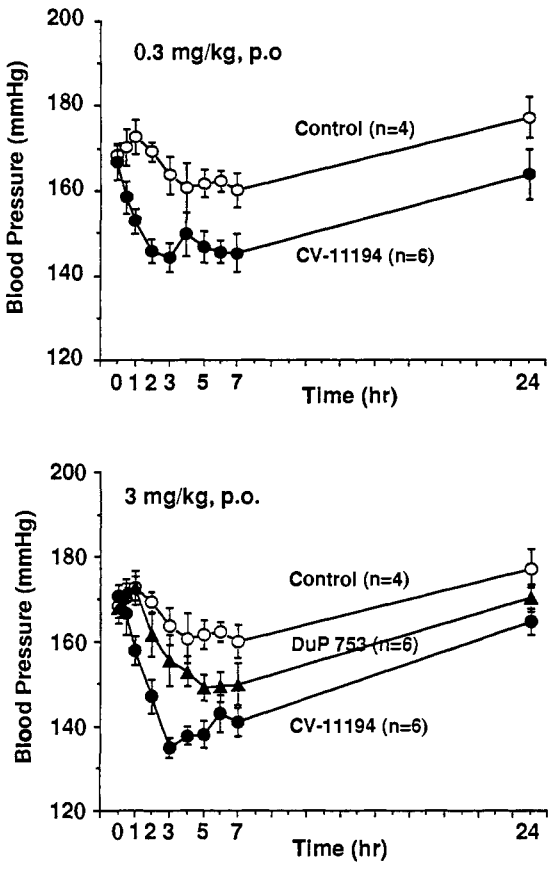


Figure 7. Effects of CV-11194 (**15d**) at 0.3 and 3 mg/kg po and DuP 753 at 3 mg/kg po on mean arterial pressure in conscious SHR.

sitions for the tetrazole ring with respect to the remaining part of the molecule. In fact, these conformations were identified for four other related compounds, not presented here, for which we performed X-ray structure analysis. It seems that there is little energy difference among these conformations, as the intramolecular interaction is weak. It is the intermolecular hydrogen bonding that determines the conformation about the biphenyl linkage in the crystal state. The tetrazole ring donates a hydrogen to a solvent methanol and accepts one from the carboxyl group of another molecule.

Discussion and Conclusion

The binding affinities of the benzimidazoles for the AII receptor showed that adding substituents to the benzene ring failed to cause a drastic change in binding affinity potency, except in the case of substituting at the 4-position (**14a**, **14h**, **15a**) which lowered the potency considerably. This suggests that the presence of a butyl group at the 2-position and a biphenyltetrazole moiety at the 1-position is sufficient for receptor binding and that the benzimidazole moiety, especially around the 5-, 6-, and 7-positions, locates near a large cavity in the receptor where interactions with the receptor are weak. The decreased binding affinity of **14a**, **14h**, and **15a** would be due to increased steric interaction between the substituent at the 4-position and the receptor site.

Also, we have observed that among these benzimidazoles only the compounds having either a carboxyl or an ester group at the 7-position (**14d–g,i,j**, **15d–g**) are potent antagonits in vivo. This propensity was also demonstrated in the AII-induced contraction assay using rabbit aortic strips as well as the AII-induced pressor response assay in rats. Thus, the data indicates that a carboxyl group in addition to the tetrazole ring in the biphenyl part is required, and that the position of the carboxyl group is important for potent functional in vitro and in vivo antagonistic activity. Recently, a major metabolite of DuP 753, EXP 3174,[8a] in rats was identified as the imidazole-5-carboxylic acid analog. This compound is more potent than the parent compound and is likely to contribute to the long duration of the AII inhibitory effect of DuP 753.[8a] Several other diacidic antagonists (SK&F 108566,[8b] DuP 532,[8e] GR 117289,[8f] A-81282,[8g,h] etc.) have also been reported.

In this study little correlation between high binding affinity and functional antagonistic activity (vasocontraction and pressor response) was found. One possible explanation for this discrepancy is the effect of bovine serum albumin (0.25% BSA) used in our binding assay.[23] The binding IC_{50} of **15d** (IC_{50} 5.5 × 10^{-7} M vs 1.0 × 10^{-7} M) differs by a factor of 5.5 in the presence (0.25%) and absence (0.05%) of BSA, but this change is too small to account for its increased functional potency. Another possible explanation is the change in antagonistic pattern. Although the receptor binding affinity of EXP 3174, an insurmountable antagonist, is similar to that of DuP 753, a surmountable antagonist, EXP 3174 exhibits considerably higher antagonistic potency in rabbit aorta.[24] Compound **15d** is also an insurmountable AII antagonist in rabbit aorta.[25]

Our result would indicate that high binding affinity alone is not sufficient to inhibit the functional actions of AII. The importance of a carboxyl group could indicate the presence of a basic site in the receptor to interact with it, but while the interaction contributes greatly to antagonistic potency, it contributes only scarcely to binding affinity. The SAR obtained in this study suggests that the structure of the benzimidazole-7-carboxylic acids can be divided into three parts as shown in Figure 9. The butyl group and the biphenyltetrazole moiety (address domain) will recognize the AII receptor for binding, and the carboxyl group (anchor domain) will act as a functional group to produce potent and long acting antagonism.[26] The benzimidazole ring can be considered an appropriate template to hold the address domain and the anchor domain in the correct arrangement. The fact that the inhibitory potency of CV-11194 was superior to that of EXP 3174 in the vasocontraction assay indicates that a benzimidazole ring is a more preferable template than an imidazole ring to set the pendant carboxyl group in a suitable position.

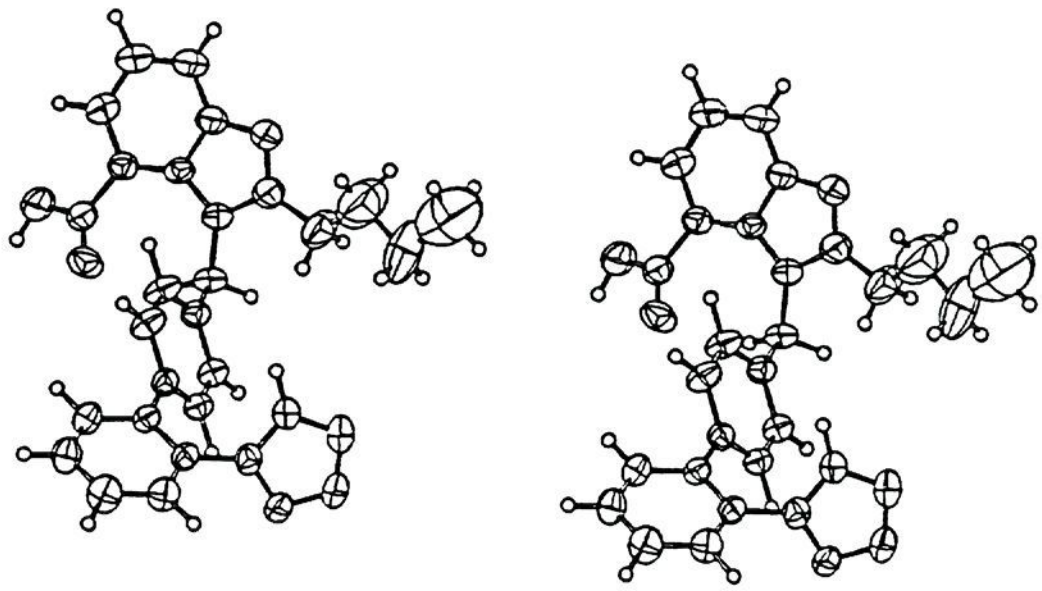


Figure 8. Stereoscopic molecular view of CV-11194 (**15d**). Methanol molecule, solvent of crystallization, was eliminated to simplify the figure.

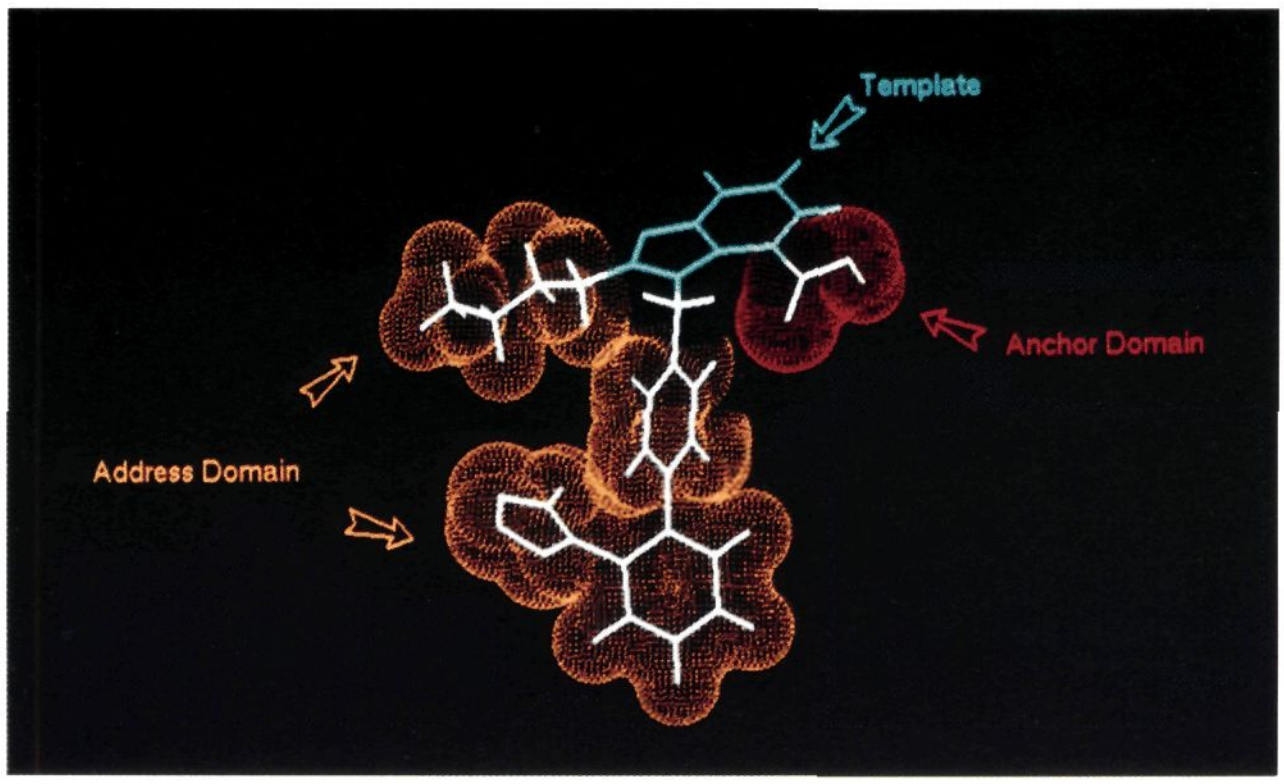


Figure 9. Functional assignment in benzimidazole-7-carboxylic acid AII antagonists. The dot clouds indicate the van der Waals surface of address domain (orange) and anchor domain (red).

The structure of the AII receptor provides some clues to the role of the carboxyl group. AII receptors have been classified as AT_1 or AT_2.[27,28] The AT_1 receptor is a G-protein coupled receptor that mediates biological effects commonly recognized with AII, whereas the function of the AT_2 receptor remains unknown.[29] Several highly similar cDNAs encoding AT_1 receptors have been cloned from bovine adrenal gland,[30] rat vascular smooth muscle,[31] rat kidney,[32] and human liver.[33] Knowledge of the primary amino acid sequence of the AT_1 receptor has permitted structural analysis and definition of seven transmembrane regions. The extracellular loops contain four lysines, two in the first loop and one each in the second and the fourth loop,[33] any of which could be the target for the carboxyl group to induce a secondary conformational change in the receptor to secure the antagonist–receptor complex or to anchor the antagonist.[34] The carboxyl group seems to modify the function of the receptor, although it affects binding affinity only slightly. However, the exact role of the carboxyl group remains unclear, and additional studies are in progress. A more detailed description of the mode of action of this series of antagonists will be described elsewhere.

In conclusion, based on the combination of potent in vitro and in vivo antagonistic activities, CV-11194 was selected for further pharmacological evaluations. CV-11194 is orally active and a more potent and longer acting antagonist than DuP 753. SAR studies revealed that the presence of the carboxyl group at the 7-position is a particularly important structural feature of this compound with regard to its potent functional AII receptor antagonistic activity.

Experimental Section

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on a Hitachi 215 grating infrared spectrophotometer. The proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on either a Varian Gemini-200 (200 MHz) or an EM-390 (90 Mhz) spectrometer. The carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on a JEOL JNM-GX270 (67.8 MHz). Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard, and coupling constants (J) are given in hertz. Column chromatography was performed using silica gel (Wakogel C-300 or Merk Art 9385).

2-Butylbenzimidazole. A mixture of 1,2-phenylenediamine (10.8 g, 100 mmol) and ethyl valerimidate hydrochloride (18.2 g, 110 mmol) in EtOH (100 mL) was stirred for 2 h at room temperature. After evaporation of the solvent in vacuo, the residue was diluted with aqueous $NaHCO_3$. The precipitate was collected by filtration, dried, and recrystallized from EtOAc–

Table II. Physicochemical Data of 2-Butyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]benzimidazoles

compd	synthetic method[a]	yield, %	recryst solvent[b]	mp, °C	formula[c]
13a	C	41	B	235–236 dec	$C_{25}H_{24}N_6$
13b	C	47	B	146–149 dec	$C_{26}H_{26}N_6O_2 \cdot 0.4H_2O$
13c	C	27	B	243–244 dec	$C_{26}H_{26}N_6O_2$
13d	D	38	C	249–250 dec	$C_{25}H_{23}ClN_6 \cdot 0.5H_2O$
13e	D	41	B	216–217	$C_{25}H_{23}ClN_6 \cdot 0.2H_2O$
14a	C	8.8	B	223–224 dec	$C_{27}H_{26}N_6O_2 \cdot 0.3H_2O$
14b	C	34	E	134–136	$C_{27}H_{26}N_7O_2 \cdot 0.5H_2O$
14c	C	41	B	224–225 dec	$C_{27}H_{26}N_6O_2 \cdot 0.2H_2O$
14d	H	56	G	153–155	$C_{27}H_{28}N_6O_2 \cdot 0.4H_2O$
14e	C	12	B	144–145	$C_{28}H_{28}N_6O_2 \cdot 0.1H_2O$
14f	A	74	H	132–133	$C_{27}H_{25}ClN_6O_2$
14g	A	59	J	164–165	$C_{29}H_{30}N_6O_2 \cdot 0.4H_2O$
14h	C	15	C	235–236 dec	$C_{26}H_{25}N_7O \cdot 0.5H_2O$
14i	H	61	E	150–151	$C_{28}H_{28}N_6O_2 \cdot 0.5H_2O$
14j	H	29	H	192–193	$C_{30}H_{32}N_6O_2$
15a	E	78	D	114–118	$C_{26}H_{24}N_6O_2 \cdot 0.2H_2O$
15b	E	27	F	180–182	$C_{26}H_{24}N_6O_2 \cdot 0.6H_2O$
15c	E	58	B	258–259 dec	$C_{26}H_{24}N_6O_2 \cdot 0.5EtOAc$
15d	C	63	B	168–169	$C_{26}H_{24}N_6O_2 \cdot H_2O$
15e	E	71	E	175–178 dec	$C_{27}H_{26}N_6O_2 \cdot 0.5H_2O$
15f	E	72	C	232–234	$C_{26}H_{23}ClN_6O_2 \cdot 0.5H_2O$
15g	E	42	I	298–299	$C_{27}H_{26}N_6O_2 \cdot 0.1H_2O$
16a	E	53	B	230–231	$C_{25}H_{24}N_2O_2$
16b	E	90	C	253–254	$C_{26}H_{24}N_2O_4 \cdot 0.2H_2O$
17	C	4.7	H	133–135	$C_{27}H_{26}N_6O_2$
21	C	56	E	129–130	$C_{29}H_{30}N_6O_2 \cdot 0.4H_2O$
27	C	46	B	152–153	$C_{26}H_{26}N_6O \cdot 0.5EtOAc \cdot 0.1H_2O$
28	D	31	K	175–178	$C_{27}H_{27}N_6ONa \cdot H_2O$
29	D	56	B	178–180 dec	$C_{28}H_{31}N_7 \cdot 2HCl \cdot 0.5EtOAc$
30	C	62	B	222–224	$C_{26}H_{26}N_6 \cdot 0.25EtOAc$
32	D	87	B	189–190	$C_{26}H_{26}N_6O \cdot HCl$
33	E	46	C	170–171	$C_{27}H_{26}N_6O_2$
34	D	46	L	186–188	$C_{25}H_{24}N_6O \cdot 0.5H_2O$

[a] Method A: Fe powder, concentrated HCl. Method C: NaN_3, NH_4Cl, DMF. Method D: (1) Me_3SnN_3, (2) 1 N HCl. Method E: 1 N NaOH. Method F: 1 N HCl. Method H: MeOH, EtOH, or BuOH, concentrated H_2SO_4. [b] A = acetone–MeOH; B = EtOAc–MeOH; C = $CHCl_3$–MeOH; D = acetone–hexane; E = EtOAc; F = MeCN–MeOH; G = EtOH; H = EtOAc–hexane; I = DMF–H_2O; J = EtOAc–diisopropyl ether; K = toluene–EtOAc; L = acetone. [c] All compounds gave satisfactory analyses C, H, N.

hexane to give the title compound (15.9 g, 91.4%) as colorless platelets: mp 153–154 °C (lit. mp 148 °C); ^{1}H NMR ($CDCl_3$) δ 0.89 (3 H, t, *J* = 7), 1.2–1.6 (2 H, m), 1.65–2.00 (2 H, m), 2.91 (2 H, t, *J* = 7), 7.1–7.3 (2 H, m), 7.4–7.6 (2 H, m).

2-Butyl-5-methoxybenzimidazole. The title compound was prepared in 53% yield from 4-methoxy-1,2-phenylenediamine by the procedure described for 2-butylbenzimidazole as pale yellow needles (from diisopropyl ether): mp 95–96 °C; ^{1}H NMR ($CDCl_3$) δ 0.93 (3 H, t, *J* = 7), 1.2–2.0 (4 H, m), 2.89 (2 H, t, *J* = 8), 3.81 (3 H, s), 6.84 (1 H, dd, *J* = 2 and 9), 7.02 (1 H, d, *J* = 2), 7.42 (1 H, d, *J* = 9); IR (KBr) 3200–2300, 1635, 1590, 1490, 1460, 1420, 1260, 1185, 1155, 1025, 840, 830, 790 cm^{-1}. Anal. ($C_{12}H_{16}N_2O$) C, H. N.

2-Butyl-5-chlorobenzimidazole. The title compound was prepared in 78% yield from 4-chloro-1,2-phenylenediamine as colorless needles (from EtOAc–hexane): mp 149–150 °C; ^{1}H NMR ($CDCl_3$) δ 0.90 (3 H, t, *J* = 7), 1.20–1.60 (2 H, m), 1.67–2.00 (2 H, m), 2.92 (2 H, t, *J* = 7.5), 7.17 (1 H, d, *J* = 9), 7.38–7.52 (2 H, m); IR (KBr) 1465, 1445, 1420, 1325, 1290, 1275, 1020, 805, 800 cm^{-1}. Anal. ($C_{11}H_{13}ClN_2$) C, H, N.

Methyl 2-Butylbenzimidazole-4-carboxylate (Method A). Iron powder (1.7 g, 30 mmol) was added portionwise to a mixture of methyl 3-nitro-2-(*N*-valerylamino)benzoate (**11c**) (2.8 g, 10 mmol) and concentrated hydrochloric acid (5.3 mL) in MeOH (35 mL), and the reaction mixture was heated under reflux for 8 h. The insoluble material was filtered off, and the filtrate was concentrated in vacuo. The residue was diluted with water, basified with 6 N NaOH, and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent in vacuo, the residue was purified by flash column chromatography ($CHCl_3$), and the resulting product was recrystallized from diisopropyl ether to give the title compound (1.6 g, 70%) as colorless needles: mp 97–98 °C; ^{1}H NMR ($CDCl_3$) δ 0.93 (3 H, t, *J* = 7.3), 1.37–1.56 (2 H, m), 1.80–1.95 (2 H, m), 2.96 (2 H, t, *J* = 7.7), 4.00 (3 H, s), 7.26 (1 H, t, *J* = 7.7), 7.85 (1 H, dd, *J* = 0.9 and 7.7), 7.91 (1 H, d, *J* = 7.7), 10.13 (1 H, br); IR (KBr) 1715, 1600, 1530, 1435, 1380, 1310, 1270, 1200, 1180, 1145, 1040, 760, 745 cm^{-1}. Anal. ($C_{13}H_{16}N_2O_2$) C, H, N.

Table III. Physicochemical Data of *N*-Phenylvaleramides

compd	yield, %	recryst solvent	mp, °C	formula[a]
8a	58	i-Pr_2O	96–97	$C_{13}H_{17}NO_3$
8b	87	EtOAc–hexane	101–102	$C_{13}H_{17}NO_3$
8c	quant		syrup[b]	
8d	62	hexane	60–61[b]	
8e	99		syrup[b]	
8f	70	hexane	58–59	$C_{15}H_{21}NO_3$

[a] All compounds gave satisfactory analyses C, H, N. [b] The product was used without further purification.

Table IV. Physicochemical Data of *N*-(2-Nitrophenyl)-valeramides

compd	yield, %	recryst solvent	mp, °C	formula[a]
11a	76	EtOAc–hexane	106–107	$C_{13}H_{16}N_2O_5$
11b	36		syrup[b]	
11c	28	i-Pr_2O	61–62	$C_{13}H_{16}N_2O_5$
11d	61	i-Pr_2O	84–85[b]	
11e	60	i-Pr_2O	59–60	$C_{14}H_{18}N_2O_5$
11f	52	EtOAc-hexane	103–104	

[a] All compounds gave satisfactory analyses C, H, N. [b] The product was used without further purification.

Compounds **4b**, **5f**, **6b–e**, **14f**, and **14g** were prepared by essentially the same procedure for the preparation of methyl 2-butylbenzimidazole-4-carboxylate, and their physicochemical data are shown in Tables II and VI.

2-Butyl-1-[(2′-cyanobiphenyl-4-yl)methyl]-1*H*-benzimidazole (5a) (Method B). To a solution of 2-butylbenzimidazole (0.87 g, 5.0 mmol) in DMF (5 mL) was added NaH (60% in oil; 0.24 g, 6.0 mmol) at 0 °C, and the mixture was stirred at the same temperature for 10 min. 4′-(Bromomethyl)-2-cyanobiphenyl (**2a**)

Table V. Physicochemical Data of *N*-[(4-Biphenylyl)methyl]-*N*-(2-nitrophenyl)valeramides

compd	synthetic method[a]	yield, %	recryst solvent	mp, °C	formula[b]
12a	G	quant		syrup[c]	
12b	G	73		syrup[c]	
12c	G	78	EtOAc–hexane	129–130	$C_{28}H_{25}N_3O_5$
12d	G	82	EtOAc–hexane	135–137[c]	
12e	G	32	EtOAc–hexane	141–142	$C_{28}H_{27}N_3O_5$
12f	G	80		syrup[c]	
12g	B	quant		syrup[c]	
12h	B	67		syrup[c]	

[a] Method B: (1) NaH, (2) 3. Method G: 3, K_2CO_3, DMF. [b] All compounds gave satisfactory analyses C, H, N. [c] The product was used without further purification.

(1.4 g, 5.0 mmol) was added to the reaction mixture, and stirring was continued at 0 °C for 1.5 h. The reaction mixture was diluted with water and extracted with EtOAc. The extract was washed with water and dried ($MgSO_4$). After evaporation of the solvent in vacuo, the residue was purified by flash column chromatography (EtOAc–hexane, 1:1) to give **5a** (1.8 g, quant) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.90 (3 H, t, *J* = 7), 1.2–1.6 (2 H, m), 1.65–2.00 (2 H, m), 2.85 (2 H, t, *J* = 7.5), 5.37 (2 H, s), 7.0–7.9 (12 H, m); IR (neat) 2210, 1510, 1475, 1450, 1405, 780, 755, 735 cm^{-1}.

Compounds **4a**, **5b–e**, **6a**, **12g**, and **12h** were prepared by essentially the same procedure for the preparation of **5a**, and their physicochemical data are shown in Tables V and VI.

2-Butyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-benzimidazole-7-carboxylic Acid (15d) and 2-Butyl-1-[[2′-(1-methyltetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-benzimidazole-7-carboxylic Acid (17) (Method C). A mixture of **6d** (3.2 g, 7.6 mmol), NaN_3 (7.4 g, 114 mmol), and NH_4Cl (6.1 g, 114 mmol) in DMF (30 mL) was stirred at 110–120 °C for 3.5 d. The reaction mixture was diluted with water and acidified to pH 3–4 with 1 N HCl. The precipitate was collected by filtration and purified by flash column chromatography ($CHCl_3$–MeOH, 30:1 and then 15:1). The first elute was concentrated in vacuo to give a crystalline product, and the crystals were recrystallized from EtOAc–hexane to give **17** (0.17 g, 4.7%) as colorless needles: mp 133–135 °C; ^{1}H NMR ($CDCl_3$) δ 0.94 (3 H, t, *J* = 7.3), 1.35–1.55 (2 H, m), 1.78–1.93 (2 H, m), 2.96 (2 H, t, *J* = 7.7), 3.15 (3 H, s), 5.82 (2 H, s), 6.81 (2 H, d, *J* = 8.4), 6.97 (2 H, d, *J* = 8.4), 7.25 (1 H, t, *J* = 8.0), 7.48–7.67 (4 H, m), 7.80 (1 H, dd, *J* = 1.0 and 7.6), 7.95 (1 H, dd, *J* = 1.0 and 8.0); ^{13}C NMR (DMSO-d_6) δ 13.55, 21.69, 26.49, 28.71, 33.37, 47.57, 117.82, 120.96, 122.07, 122.55, 124.74, 126.10, 127.87, 128.48, 130.16, 131.12, 131.60, 132.18, 137.30, 137.51, 140.83, 143.41, 154.32, 157.36, 167.50; IR (KBr) 1715, 1520, 1415, 1290, 1260, 1200, 1125, 780, 750 cm^{-1}. The second elute was concentrated in vacuo to give a crystalline product and recrystallization from EtOAc–MeOH gave **15d** (2.3 g, 63%) as colorless prisms: mp 168–169 °C; ^{1}H NMR (DMSO-d_6) δ 0.88 (3 H, t, *J* = 7.2), 1.28–1.46 (2 H, m), 1.65–1.80 (2 H, m), 2.82 (2 H, t, *J* = 7.5), 5.85 (2 H, s), 6.79 (2 H, d, *J* = 8.3), 7.00 (2 H, d, *J* = 8.3), 7.24 (1 H, t, *J* = 7.8), 7.45–7.68 (5 H, m), 7.83 (1 H, dd, *J* = 1.1 and 7.9); IR (KBr) 1720, 1600, 1510, 1455, 1415, 1285, 1255, 1240, 775, 755, 745 cm^{-1}.

Compounds **13a,b,c**, **14a–c,e,h**, **27**, **30**, and **31** were prepared by essentially the same procedure for the preparation of **15d**, and their physicochemical data are shown in Table II.

2-Butyl-5-chloro-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-benzimidazole (13d) (Method D). A mixture of **5d** (0.96 g, 2.4 mmol) and Me_3SnN_3 (1.2 g, 6.0 mmol) in toluene (10 mL) was refluxed for 6 d. The precipitate was collected by filtration and treated with 1 N HCl (3 mL) in EtOH (8 mL) at room temperature for a few minutes. The reaction mixture was diluted with water and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent in vacuo, the residue was recrystallized from $CHCl_3$–MeOH to give **13d** (0.42 g, 38%) as colorless prisms: mp 249–250 °C dec; ^{1}H NMR (DMSO-d_6) δ 0.87 (3 H, t, *J* = 7.4), 1.34 (2 H, m), 1.61–1.76 (2 H, m), 2.81 (2 H, t, *J* = 7.6), 5.50 (2 H, s), 6.99–7.09 (4 H, m), 7.20 (1 H, dd, *J* = 2.0 and 8.6), 7.47–7.70 (7 H, m); IR (KBr) 1500, 1450, 1410, 1000, 785, 760 cm^{-1}.

Compounds **13e**, **28**, **29**, and **34** were prepared by essentially the same procedure for the preparation of **13d** and were shown in Table II.

Sodium 5-[4′-[[2-Butyl-7-(methoxymethyl)benzimidazol-1-yl]methyl]biphenyl-2-yl]tetrazolide (28). 2-Butyl-7-(methoxymethyl)-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-benzimidazole prepared from **22** (0.60 g, 1.5 mmol) and Me_3SnN_3 (1.3 g, 6.0 mmol) by essentially the same procedure for the preparation of **13d** was treated with sodium 2-ethylhexanoate (0.25 g, 1.5 mmol) in EtOAc (30 mL) under reflux. While the mixture was hot, the precipitate formed was collected by filtration and recrystallized from toluene–EtOAc to give the sodium salt (**28**) (0.22 g, 31%) as colorless crystalline powder: mp 189–190 °C; ^{1}H NMR (DMSO-d_6) δ 0.70 (3 H, t, *J* = 7.0), 1.06–1.25 (2 H, m), 1.50–1.65 (2 H, m), 2.49 (2 H, t, *J* = 7.8), 2.86 (3 H, s), 4.21 (2 H, s), 5.27 (2 H, s), 6.41 (2 H, d, *J* = 7.6), 6.73–6.77 (3 H, m), 6.92–7.00 (2 H, m), 7.19–7.30 (2 H, m), 7.37 (1 H, d, *J* = 7.6), 7.62 (1 H, d, *J* = 7.8); IR (KBr) 1510, 1455, 1420, 1405, 1350, 1280, 1080, 740 cm^{-1}.

2-Butyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-benzimidazole-5-carboxylic Acid (15b) (Method E). A mixture of **14b** (0.3 g, 0.60 mmol) and 2 N NaOH (1 mL) in MeOH (5 mL) was heated under reflux for 2 h, and the reaction mixture was concentrated in vacuo. The residue was diluted with water and acidified with 1 N HCl. The precipitate was collected by filtration, dried, and recrystallized from MeCN–MeOH to give **15b** (74 mg, 27%) as a colorless crystalline powder: mp 180–182 °C; ^{1}H NMR (DMSO-d_6) δ 0.88 (3 H, t, *J* = 7.3), 1.28–1.46 (2 H, m), 1.63–1.78 (2 H, m), 2.89 (2 H, t, *J* = 7.7), 5.58 (2 H, s), 7.07 (4 H, s), 7.47–7.70 (5 H, m), 7.86 (1 H, dd, *J* = 1.3 and 8.5), 8.18 (1 H, s); IR (KBr) 1710, 1620, 1450, 1410, 1285, 1210, 775, 755 cm^{-1}.

Compounds **15a,c,e–g**, **16a,b**, and **33** were prepared by essentially the same procedure for the preparation of **15b**, and their physicochemical data are shown in Table II.

Methyl 2-(Valerylamino)benzoate (8c). Valeryl chloride (6.0 g, 50 mmol) was added dropwise to a stirring and ice-cooling mixture of methyl 2-aminobenzoate (7.6 g, 50 mmol) and triethylamine (5.6 g, 55 mmol) in $CHCl_3$ (80 mL). After being stirred at 0 °C for 4 h, the reaction mixture was washed with aqueous $NaHCO_3$ and dried ($MgSO_4$). Evaporation of the solvent in vacuo gave **8c** (12 g, quant) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.95 (3 H, t, *J* = 7), 1.15–1.95 (4 H, m), 2.44 (2 H, t, *J* = 7.5), 3.91 (3 H, s), 7.05 (1 H, t, *J* = 8), 7.53 (1 H, dt, *J* = 2 and 8), 8.02 (1 H, dd, *J* = 2 and 8), 8.74 (1 H, d, *J* = 8); IR (neat) 3320, 1710, 1690, 1600, 1590, 1530, 1445, 1435, 1310, 1295, 1260, 1190, 1175, 1160, 1090, 750, 700 cm^{-1}.

Compounds **8a,b,d–f** were prepared by essentially the same procedure for the preparation of **8c**, and their physicochemical data are shown in Table III.

Methyl 3-Nitro-2-(valerylamino)benzoate (11c). Fuming nitric acid (7.0 mL) was added to ice-cooling acetic anhydride (60 mL), followed by addition of concentrated sulfuric acid (one drop), and **8c** (12 g, 50 mmol) was added to the mixture. After being stirred at room temperature for 4 h, the reaction mixture was diluted with ice–water and extracted with EtOAc. The extract was washed successively with aqueous $NaHCO_3$ and brine and dried ($MgSO_4$). The solvent was evaporated in vacuo, and the residue was purified by flash column chromatography (EtOAc–hexane, 1:8 and then 1:4). The first elute was concentrated in vacuo, and the resulting product was recrystallized from diisopropyl ether to give methyl 5-nitro-2-(valerylamino)benzoate (2.1 g, 15%) as colorless platelets: mp 100–101 °C; ^{1}H NMR ($CDCl_3$) δ 0.97 (3 H, t, *J* = 7.3), 1.35–1.53 (2 H, m), 1.68–1.83 (2 H, m), 4.01 (3 H, s), 8.38 (1 H, dd, *J* = 2.9 and 9.5), 8.94 (1 H, d, *J* = 2.9), 8.98 (1 H, d, *J* = 9.5). The second elute was concentrated in vacuo, and the resulting product was recrystallized from diisopropyl ether to give **11c** (3.9 g, 28%) as colorless needles: mp 61–62 °C; ^{1}H NMR ($CDCl_3$) δ 0.95 (3 H, t, *J* = 7.3), 1.32–1.51 (2 H, m), 1.65–1.80 (2 H, m), 2.46 (2 H, t, *J* = 7.6), 3.97 (3 H, s), 7.30 (1 H, t, *J* = 8.2), 8.10 (1 H, dd, *J* = 1.5 and 8.2), 8.22 (1 H, dd, *J* = 1.5 and 8.2); IR (KBr) 3320, 1730, 1680, 1580, 1535, 1520, 1450, 1365, 1290, 1270, 1210, 1120, 765, 730, 705 cm^{-1}.

Compounds **11a,b,d–f** were prepared by essentially the same procedure for the preparation of **11c**, and their physicochemical data are shown in Table IV.

Table VI. Physicochemical Data of 2-Butyl-1-[(2′-cyanobiphenyl-4-yl)methyl]benzimidazoles

compd	starting materials	synthetic method[a]	yield, %	recryst solvent	mp, °C	formula[b]
4a	**2a, 3**	B	quant		syrup[c]	
4b	**11h**	A	48	i-Pr_2O	78–79	$C_{28}H_{28}N_2O_4$
5a	**2a, 3**	B	quant		syrup[c]	
5b	**2b, 3**	B	44		syrup[c]	
5c	**2b, 3**	B	48		syrup[c]	
5d	**2c, 3**	B	48		syrup[c]	
5e	**2c, 3**	B	35	EtOAc–hexane	124–125	$C_{25}H_{22}ClN_3$
5f	**11g**	A	80	EtOAc–hexane	127–128	$C_{28}H_{25}N_3O$
6a	**2e, 3**	B	82		syrup[c]	
6b	**11a**	A	53		syrup[c]	
6c	**11b**	A	45	EtOAc	166–167	$C_{27}H_{25}N_3O_2$
6d	**11c**	A	90	EtOAc	123–124	$C_{27}H_{25}N_3O_2$
6e	**11e**	A	quant		syrup[c]	

[a] Method A: Fe powder, concentrated HCl. Method B: (1) NaH, (2) **3**. [b] All compounds gave satisfactory analyses C, H, N. [c] The product was used without further purification.

2-Methoxy-6-nitroaniline (10). A mixture of 2-amino-3-nitrophenol (7.7 g, 50 mmol) and K_2CO_3 powder (7.6 g, 55 mmol) in DMF (15 mL) was stirred at room temperature for 30 min, followed by addition of methyl iodide (7.8 g, 55 mmol). The reaction mixture was stirred for a further 5 h, diluted with water, and extracted with EtOAc. The extract was washed with water and dried ($MgSO_4$). After evaporation of the solvent in vacuo, the residue was recrystallized from diisopropyl ether to give **10** (6.9 g, 82%) as dark orange prisms, mp 76–77 °C, which was used for next reaction without further purification: ^{1}H NMR ($CDCl_3$) δ 3.91 (3 H, s), 6.40 (2 H, br), 6.59 (1 H, t, *J* = 7.5), 6.88 (1 H, d, *J* = 7.5), 7.73 (1 H, d, *J* = 7.5).

***N*-(2-Methoxy-6-nitrophenyl)valeramide (11g).** A mixture of **10** (5.9 g, 35 mmol), valeric anhydride (14 g, 77 mmol), and concentrated sulfuric acid (one drop) was stirred at 130–140 °C for 1.5 h. The reaction mixture was diluted with water, bacified with 6 N NaOH, and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent in vacuo, the residue was purified by flash column chromatography ($CHCl_3$), and the product was recrystallized from EtOAc–hexane to give **11g** (3.2 g, 36%) as colorless platelets: mp 113–114 °C; ^{1}H NMR ($CDCl_3$) δ 0.95 (3 H, t, *J* = 7.2), 1.33–1.51 (2 H, m), 1.64–1.79 (2 H, m), 2.42 (2 H, t, *J* = 7.5), 3.94 (3 H, s), 7.14 (1 H, dd, *J* = 1.5 and 8.1), 7.26 (1 H, t, *J* = 8.1), 7.51 (1 H, dd, *J* = 1.5 and 8.1), 7.64 (1 H, br); IR (KBr) 3300, 1670, 1590, 1545, 1520, 1485, 1460, 1430, 1360, 1275, 1055, 800, 735 cm^{-1}. Anal. ($C_{12}H_{16}N_2O_4$) C, H, N.

Methyl 2-[*N*-[(2′-Cyanobiphenyl-4-yl)methyl]valeryl]-amino]-3-nitrobenzoate (12c) (Method G). A mixture of **11c** (3.9 g, 14 mmol), 2-cyano-4′-(bromomethyl)biphenyl (**2a**) (3.8 g, 14 mmol), and K_2CO_3 powder (2.1 g, 15 mmol) in DMF (30 mL) was stirred at room temperature for 15 h. The reaction mixture was diluted with water and extracted with EtOAc. The extract was washed with water and dried ($MgSO_4$). After evaporation of the solvent in vacuo, the residue was purified by flash column chromatography (EtOAc–hexane, 1:3 and then 1:2), and the resulting crystalline product was recrystallized from EtOAc–hexane to give **12c** (5.1 g, 78%) as colorless platelets: mp 129–130 °C; ^{1}H NMR ($CDCl_3$) δ 0.85 (3 H, t, *J* = 7.3), 1.18–1.36 (2 H, m), 1.61–1.72 (2 H, m), 2.08–2.16 (2 H, m), 3.67 (3 H, s), 4.65 (1 H, d, *J* = 14.2), 4.96 (1 H, d, *J* = 14.2), 7.20 (2 H, d, *J* = 8.2), 7.38–7.50 (4 H, m), 7.56–7.68 (2 H, m), 7.75 (1 H, d, *J* = 7.8), 7.98 (1 H, dd, *J* = 1.6 and 8.1), 8.10 (1 H, dd, *J* = 1.6 and 8.1); IR (KBr) 2220, 1735, 1665, 1530, 1430, 1395, 1345, 1290, 1280, 1235, 1200, 1125, 770 cm^{-1}.

Compounds **12a,b,d–f** were prepared by essentially the same procedure for the preparation of **12c**, and their physicochemical data are shown in Table V.

Ethyl 2-Butyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-benzimidazole-7-carboxylate (14i) (Method H). A mixture of **15d** (1.4 g, 3.0 mmol), ethyl orthoformate (4.4 g, 30 mmol), and concentrated sulfuric acid (0.5 mL) in EtOH (40 mL) was heated under reflux for 67 h and then concentrated in vacuo. The residue was diluted with water, adjusted to pH 4 with 1 N NaOH, and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent in vacuo, the residue was purified by flash column chromatography (MeOH–$CHCl_3$, 1:10), and the resulting crystalline product was recrystallized from EtOAc to give **14i** (0.91 g, 61%) as colorless prisms: mp 150–151 °C; ^{1}H NMR (DMSO-d_6) δ 0.82 (3 H, t, *J* = 7.3), 1.17 (3 H, t, *J* = 7.1), 1.21–1.35 (2 H, m), 1.49–1.64 (2 H, m), 2.37 (2 H, t, *J* = 7.7), 4.06 (2 H, q, *J* = 7.1), 5.49 (2 H, s), 6.47 (2 H, d, *J* = 8.2), 6.81 (2 H, d, *J* = 8.2), 6.95–7.10 (2 H, m), 7.28–7.34 (1 H, m), 7.52 (1 H, dd, *J* = 1.5 and 7.1), 7.57–7.67 (2 H, m), 7.97–8.01 (1 H, m); IR (KBr) 1715, 1600, 1520, 1450, 1410, 1370, 1290, 1265, 1220, 1130, 1110, 1040, 755 cm^{-1}.

14d and **14j** were prepared by essentially the same procedure for the preparation of **14i** without orthoester, and their physicochemical data are shown in Table II.

2-Butyl-*N*-isopropyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-benzimidazole-7-carboxamide (18). A mixture of **15d** (0.71 g, 1.5 mmol) and DEPC (90%; 0.82 g, 4.5 mmol) in DMF (6 mL) was stirred at 0 °C for 50 min. Isopropylamine hydrochloride (0.14 g, 1.5 mmol) and triethylamine (0.61 g, 6.0 mmol) were added to the reaction mixture, and stirring was continued at room temperature for a further 2 h. The reaction mixture was diluted with water, adjusted to pH 6 with 1 N HCl, and extracted with EtOAc. The extract was washed with water and dried ($MgSO_4$). After evaporation of the solvent in vacuo, the residue was purified by flash column chromatography (MeOH–$CHCl_3$, 1:10), and the product was recrystallized from MeOH–EtOAc to give **18** (0.31 g, 41%) as colorless prisms: mp 247–249 °C; ^{1}H NMR (DMSO-d_6) δ 0.87 (3 H, t, *J* = 7.2), 0.93 (6 H, d, *J* = 6.6), 1.26–1.44 (2 H, m), 1.62–1.77 (2 H, m), 2.80 (2 H, t, *J* = 7.5), 3.85–3.95 (1 H, m), 5.67 (2 H, s), 6.84 (2 H, d, *J* = 8.1), 6.99 (2 H, d, *J* = 8.1), 7.16–7.24 (2 H, m), 7.44 (1 H, d, *J* = 7.8), 7.51–7.72 (4 H, m), 8.27 (1 H, d, *J* = 7.6); IR (KBr) 1640, 1540, 1510, 1455, 1415, 755, 740 cm^{-1}. Anal. ($C_{29}H_{32}N_7O \cdot 0.2H_2O$) C, H, N.

2-Butyl-1-[(2′-cyanobiphenyl-4-yl)methyl]-7-(hydroxymethyl)-1*H*-benzimidazole (19). MeOH (19 mL) was added dropwise to a mixture of **6d** (10 g, 24 mmol) and $NaBH_4$ (2.2 g, 59 mmol) in THF (100 mL) over 80 min under reflux. The reaction mixture was heated under reflux for further 27 h and concentrated in vacuo. The residue was diluted with water and neutralized with concentrated HCl. The precipitate was collected by filtration and recrystallized from MeOH to give **19** (8.8 g, 93%) as colorless needles: mp 203–204 °C; ^{1}H NMR ($CDCl_3$) δ 0.94 (3 H, t, *J* = 7.3), 1.36–1.55 (2 H, m), 1.79–1.95 (2 H, m), 2.85 (2 H, t, *J* = 7.8), 4.66 (2 H, d, *J* = 4.8), 5.82 (2 H, s), 7.04 (2 H, d, *J* = 8.2), 7.10 (1 H, dd, *J* = 1.4 and 7.4), 7.18–7.26 (1 H, m), 7.40–7.52 (4 H, m), 7.64 (1 H, dt, *J* = 1.6 and 7.7), 7.74–7.82 (2 H, m); IR (KBr) 3200, 2210, 1510, 1480, 1455, 1425, 1410, 1280, 1015, 765, 750 cm^{-1}. Anal. ($C_{26}H_{25}N_3O$) C, H, N.

2-Butyl-7-(chloromethyl)-1-[(2′-cyanobiphenyl-4-yl)methyl]-1*H*-benzimidazole (20). A mixture of **19** (5.4 g, 14 mmol), $SOCl_2$ (8.3 g, 70 mmol), and DMF (one drop) in $CHCl_3$ (80 mL) was heated under reflux for 1 h. The reaction mixture was washed successively with aqueous $NaHCO_3$ and water and dried ($MgSO_4$). After evaporation of the solvent in vacuo, the residue was recrystallized from EtOAc–hexane to give **20** (5.3 g, 92%) as colorless needles: mp 144–145 °C; ^{1}H NMR ($CDCl_3$) δ 0.96 (3

H, t, J = 7.5), 1.38–1.57 (2 H, m), 1.82–1.97 (2 H, m), 2.88 (2 H, t, J = 7.8), 4.60 (2 H, s), 5.78 (2 H, s), 7.07 (2 H, d, J = 8.2), 7.14 (1 H, dd, J = 1.4 and 7.4), 7.21 (1 H, d, J = 7.6), 7.41–7.54 (4 H, m), 7.60–7.69 (1 H, m), 7.75–7.84 (2 H, m); IR (KBr) 2210, 1515, 1480, 1450, 1425, 1400, 1350, 1315, 1280, 760, 745, 690 cm^{-1}. Anal. ($C_{26}H_{24}ClN_3$) C, H, N.

2-Butyl-1-[(2′-cyanobiphenyl-4-yl)methyl]-7-(cyanomethyl)-1*H*-benzimidazole (21). A mixture of **20** (0.83 g, 2.0 mmol) and NaCN (0.12 g, 2.4 mmol) in DMF (10 mL) was stirred at room temperature for 22 h. The reaction mixture was diluted with water and extracted with EtOAc. The extract was washed with water and dried ($MgSO_4$). After evaporation of the solvent in vacuo, the residue was recrystallized from EtOAc to give **21** (0.76 g, 94%) as colorless prisms: mp 180–181 °C; ^{1}H NMR ($CDCl_3$) δ 0.96 (3 H, t, J = 7.3), 1.38–1.57 (2 H, m), 1.81–1.96 (2 H, m), 2.89 (2 H, t, J = 7.7), 3.74 (2 H, s), 5.65 (2 H, s), 7.05 (2 H, d, J = 8.4), 7.14 (1 H, d, J = 7.4), 7.25 (1 H, t, J = 7.7), 7.42–7.56 (4 H, m), 7.65 (1 H, m), 7.74–7.81 (2 H, m); IR (KBr) 2240, 2210, 1590, 1515, 1475, 1455, 1425, 1400, 1340, 1285, 1270, 1195, 880, 830, 780, 760, 750, 740 cm^{-1}. Anal. ($C_{27}H_{24}N_4$) C, H, N.

Ethyl [2-Butyl-1-[(2′-cyanobiphenyl-4-yl)methyl]-1*H*-benzimidazol-7-yl]acetate (25). A solution of **21** (0.76 g, 1.9 mmol) in 3.5 N ethanolic HCl (10 mL) was refluxed for 2.5 h. The reaction mixture was diluted with water, basified with aqueous $NaHCO_3$, and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). After evaporation of the solvent in vacuo, the residue was purified by flash column chromatography (EtOAc–hexane, 1:1) to give **25** (0.9 g, quant) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.94 (3 H, t, J = 7.3), 1.23 (3 H, t, J = 7.2), 1.37–1.55 (2 H, m), 1.80–1.95 (2 H, m), 2.85 (2 H, t, J = 7.8), 3.65 (2 H, s), 4.10 (2 H, q, J = 7.0), 5.73 (2 H, s), 7.01–7.07 (3 H, m), 7.21 (1 H, t, J = 7.7), 7.40–7.68 (5 H, m), 7.71–7.78 (2 H, m); IR (neat) 2210, 1730, 1510, 1475, 1435, 1400, 1365, 1275, 1150, 1040, 760, 735 cm^{-1}.

2-Butyl-1-[(2′-cyanobiphenyl-4-yl)methyl]-7-(methoxymethyl)-1*H*-benzimidazole (22). A solution of **20** (0.83 g, 2.0 mmol) and NaOMe (4.9 M in MeOH; 2 mL) in MeOH (15 mL) was heated under reflux for 6 h. After removal of solvent, the residue was diluted with water and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). The solution was concentrated in vacuo, and the residue was purified by flash column chromatography (EtOAc–hexane, 1:1) to give **22** (0.48 g, 59%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.92 (3 H, t, J = 7), 1.2–2.05 (4 H, m), 2.84 (2 H, t, J = 8), 3.30 (3 H, s), 4.34 (2 H, s), 5.74 (2 H, s), 6.9–7.9 (11 H, m); IR (neat) 2220, 1520, 1480, 1460, 1440, 1425, 1410, 1280, 1190, 760 cm^{-1}.

2-Butyl-1-[(2′-cyanobiphenyl-4-yl)methyl]-7-[(*N*,*N*-dimethylamino)methyl]-1*H*-benzimidazole (23). A solution of **20** (0.65 g, 1.5 mmol) and 50% aqueous dimethylamine (1.5 mL) in EtOH (3 mL) was heated at 80 °C for 4.5 h in sealed tube. The reaction mixture was diluted with water and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). The solution was concentrated in vacuo, and the residue was purified by flash column chromatography (EtOAc–hexane, 1:1 and then 2:1) to give **23** (0.40 g, 63%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.92 (3 H, t, J = 7.3), 1.35–1.53 (2 H, m), 1.81–1.94 (2 H, m), 2.16 (6 H, s), 2.80 (2 H, t, J = 7.9), 3.34 (2 H, s), 6.00 (2 H, s), 6.95–7.01 (3 H, m), 7.16 (1 H, t, J = 7.6), 7.39–7.50 (4 H, m), 7.62 (1 H, m), 7.73–7.77 (2 H, m); IR (neat) 2210, 1515, 1480, 1460, 1440, 1405, 1360, 1330, 1275, 1005, 840, 785, 760 cm^{-1}.

2-Butyl-1-[(2′-cyanobiphenyl-4-yl)methyl]-7-methyl-1*H*-benzimidazole (24). A mixture of **20** (0.62 g, 1.5 mmol), tributyltin hydride (3.0 g, 11 mmol), and catalytic amount of benzoyl peroxide in toluene (20 mL) was heated under reflux for 5.5 h under nitrogen. The reaction mixture was concentrated in vacuo, and the residue was purified by flash column chromatography (EtOAc–hexane, 1:2 and then 1:1). The resulting crystalline product was recrystallized from EtOAc–hexane to give **24** (0.50 g, 88%) as colorless crystals: mp 115–116 °C; ^{1}H NMR ($CDCl_3$) δ 0.93 (3 H, t, J = 7.2), 1.35–1.53 (2 H, m), 1.77–1.92 (2 H, m), 2.51 (3 H, s), 2.82 (2 H, t, J = 7.9), 5.64 (2 H, s), 6.94 (1 H, d, J = 7.4), 7.05 (2 H, d, J = 8.4), 7.15 (1 H, t, J = 7.7), 7.41–7.53 (4 H, m), 7.60–7.68 (2 H, m), 7.76 (1 H, d, J = 8.2); IR (neat) 2210, 1595, 1515, 1480, 1460, 1415, 1400, 1345, 1280, 780, 760, 740 cm^{-1}. Anal. ($C_{29}H_{25}N_3$) C, H, N.

2-Butyl-1-[(2′-cyanobiphenyl-4-yl)methyl]-7-hydroxy-1*H*-benzimidazole (26). Boron tribromide (1.7 g, 6.6 mmol) was added to a solution of **5f** (1.2 g, 3.0 mmol) in CH_2Cl_2 (5 mL) at −72 °C under nitrogen. The reaction mixture was stirred at room temperature for 8 h. Water (5 mL) was added to the reaction mixture, and stirring was continued for a futher 1 h. The reaction mixture was basified with 6 N NaOH and extracted with EtOAc. The extract was washed with brine and dried ($MgSO_4$). The solution was concentrated in vacuo, and the residue was purified by flash column chromatography (EtOAc–hexane, 1:1). The resulting crystalline product was recrystallized from EtOAc–hexane to give **26** (0.69 g, 63%) as colorless prisms: mp 185–186 °C; ^{1}H NMR ($CDCl_3$) δ 0.81 (3 H, t, J = 7.3), 1.22–1.42 (2 H, m), 1.66–1.81 (2 H, m), 2.80 (2 H, t, J = 7.6), 5.81 (2 H, s), 6.71 (1 H, d, J = 7.0), 7.00 (1 H, t, J = 8.0), 7.19–7.26 (3 H, m), 7.38–7.50 (4 H, m), 7.61 (1 H, m), 7.74 (1 H, d, J = 8.4); IR (KBr) 2210, 1650, 1590, 1500, 1475, 1440, 1410, 1365, 1290, 1195, 1160, 1065, 780, 755, 725 cm^{-1}. Anal. ($C_{26}H_{23}N_3O$) C, H, N.

X-ray Structural Analysis of CV-11194 (15d). Crystals of CV-11194 (**15d**) were grown from MeOH. The diffraction experiment was carried out with use of a colorless transparent plate, in a glass capillary, with dimension 0.6 × 0.4 × 0.2 mm. The four-circle diffractometer (Rigaku AFC-5) was used with graphite-monochromated Mo Kα radiation (λ = 0.710 69 Å). The unit cell dimension were determined from angular setting of 25 reflections (2θ values in the range of 32–36°). The crystal data are as follows: $C_{29}H_{24}N_6O_2 \cdot CH_3OH$; MW = 484.56; a = 11.575(3), b = 12.683(4), and c = 9.616(2) Å; α = 105.76(3), β = 98.50(2), and γ = 103.54(2)°; U = 1286.6 (6) Å^3; triclinic; space group P_1; Z = 2; D = 1.251 g/cm^3; μ = 0.91 cm^{-1}. Three-dimensional intensity data were measured by $2\theta - \omega$ scan technique ($2\theta > 30°$). Unique reflections (4539) were measured, of which 3195 with $F_o \geq 3\sigma(F_o)$ were considered as observed. No absorption corrections were applied.

The structure was solved by the direct method using MULTAN program[35] and refined by XTAL system.[36] The most probable phase sets obtained by MULTAN revealed on their E-maps all non-hydrogen atoms except some atoms of the butyl group of CV-11194 (**15d**). The missing atoms were found on the Fourier maps after several cycles of the block-diagonal least-squares refinement using unit weights. Further refinement applying anisotropic thermal parameters to the non-hydrogen atoms produced difference electron density maps showing most of the hydrogen atoms. The hydrogen atom on the tetrazole ring was clearly located on the nitrogen atom next to carbon, which was also confirmed from hydrogen bonding. Thermal vibration of the terminal part of the butyl group was so large that the hydrogen atoms could not be found. In the succeeding refinement using the full-matrix least-squares method all hydrogen atoms were placed at calculated positions of fixed lengths from the bonding atoms and were given an isotropic thermal parameter of B = 20.0 Å. The atomic parameters of hydrogens were not refined in the least-squares calculations, but their position parameters were reset after every cycle. The final R value is 0.100 (R_w = 0.107).

Angiotensin II Receptor Binding Assay. AII receptor binding assay was performed by the modified method of Douglas et al.[37] Briefly, the freshly isolated bovine adrenal cortex was homogenized in 20 times volume of 50 mM Tris-HCl buffer (pH 7.4) containing 5 mM EDTA and 1 mM phenylmethanesulfonyl fluoride. The homogenates were centrifuged at 10000g at 4 °C for 20 min, and the supernatant obtained was centrifuged at 20000g at 4 °C for 30 min. The pellet obtained was used as the source of AII receptors. The pellet was suspended in 50 mM Tris HCl buffer (pH 7.4) containing 5 mM $MgCl_2$ and 0.25% bovine serum albumin. Membrane fraction (20–50 μg of protein), varying concentrations of compound, and [^{125}I]AII (1.85 kBq/50 μL corresponding to 0.2 nM) were incubated with a 100-μL final incubation volume of 50 mM Tris-HCl buffer (pH 7.4) containing 5 mM $MgCl_2$ and 0.25% bovine serum albumin at 25 °C for 60 min. The binding reaction was terminated by addition of 2.5 mL of ice-cold assay buffer. The solution was filtered using a glass filter (Whatman GF/B) to separate the bound and free radioactivity. The radioactivity trapped on the filter was determined with a γ spectrophotometer (Aroka, ARC-600). Nonspecific binding of [^{125}I]AII to the receptor was estimated in the presence of 10 μM unlabeled AII. Assays were performed in duplicate.

Intraassay and interassay IC_{50} values for a given test compound may vary less than 3% and less than 10%, respectively. For **15d** the IC_{50} ($\times 10^{-7}$ M) ± SEM is 5.5 ± 0.88 M (n = 4).

Potency and Selectivity of Angiotensin II Antagonism in Rabbit Aorta. The descending thoracic aorta was isolated from male albino rabbits (2–3 kg) and cut into helical strips 2 mm in width and 20 mm in length. The helical strips were suspended with a load of 2 g in a 20 mL organ bath at 37 °C containing Krebs–Henseleit solution oxygenated continuously with 5% CO_2 in oxygen. The tension induced by addition of agonist was measured isometrically with a force displacement transducer and recorded on a polygraph (Recti-8S, San-ei). After initial resting tension was set, the aortic strips were allowed to equilibrate for 2–3 h. A control contractile response for AII was first determined. The tissue was washed several times until the tension reached base line. After resting for 30–90 min, the tissue was incubated with the compound for 30 min, and then the contractile response for AII (10 nM) was measured. Responses for norepinephrine (10 nM) and KCl (10 nM) were also examined in the presence or absence of the compound to test the specificity. Assays were performed in duplicate.

Effects on Angiotensin-Induced Pressor Response in Conscious Normotensive Rats. On the day before the experiment, male rats (Jcl:Sprague–Dawley, 300–400 g) were anesthetized with sodium pentobarbital (50 mg/kg ip), and the abdominal aorta and vena cava were cannulated with a polyethylene tube (PE-50) via the femoral artery and vein, respectively. The catheters were passed subcutaneously, exteriorized on the neck, and filled with saline-containing heparin. The animals were placed into plastic cages and allowed freedom of movement. The animals were fasted but allowed access freely to drinking water until the experiment. The aortic catheter was connected to a pressure transducer (San-ei 45277, Japan), and blood pressure was monitored on a polygraph (San-ei 7747, Japan). AII (100 ng/kg) was injected into the femoral vein twice during the control period. Inhibitors were then administered orally as a suspension with a small amount of gum arabic. AII challenges were repeated at set times thereafter. The inhibition of the pressor responses to AII was calculated from duplicate experiments. The inhibitory effect (percent inhibition) may vary less than 30%. The data in Figure 5 are also indicative of the variation measured throughout this study for **15d** (n = 4–5) and DuP 753 (n = 4–5).

Effects on Angiotensin-Induced Pressor Response in Conscious Normotensive Dogs. Male beagle dogs, weighing 7.5–11.5 kg, were used. Under sodium pentobarbital (30 mg/kg iv) anesthesia, the dogs were chronically cannulated with a polyethylene tube (PE-100) into the aorta for blood pressure measurement and into the inferior vena cava for injection of drugs. Inhibitors were administered orally (as a capsule). AII (100 ng/kg) was injected iv before and after the inhibitors were administered. The procedures taken thereafter were similar to those taken with rats.

Antihypertensive Effects in Conscious SHR. Male SHR, 19–21 weeks old, were anesthetized with sodium pentobarbital (50 mg/kg ip), and the femoral artery was cannulated with a polyethylene tube (PE-10 fused to PE-50). The catheter was passed subcutaneously to a dorsal site on the neck and was exteriorized. The animals were allowed to recover for 18–24 h individually in plastic cages. The aorta catheter was connected to a pressure transducer (San-ei 45277, Japan), and blood pressure was recorded for 24 h on a polygraph (San-ei 7747, Japan).

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Supplementary Material Available: X-ray data including coordinates, thermal parameters, bond lengths, and bond angles for CV-11194 (**15d**) (7 pages). Ordering information is given on any current masthead page.

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