Studies on Nonpeptide Angiotensin II Receptor Antagonists. II.[1] Synthesis and Biological Evaluation of 5*H*-Pyrazolo[1,5-*b*][1,2,4]triazole Derivatives with a C-Linked Oxygen Functional Group at the 6-Position

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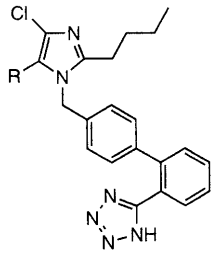
2,7-Diethyl-5*H*-pyrazolo[1,5-*b*][1,2,4]triazole derivatives were synthesized and evaluated for activity as angiotensin II receptor antagonists. Replacement of the C-6 hydrogen with C-linked oxygen functional groups led to derivatives with increased *in vitro* activities. Among these compounds, 2,7-diethyl-5-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]triazole-6-carboxylic acid (2d) showed potent, insurmountable antagonism, but had poor oral potency against angiotensin II-induced pressor response in rats. In order to improve the oral activity, the carboxylic acid function of 2d was converted into a double ester. This modification afforded (±)-1-[(ethoxycarbonyl)oxy]ethyl 2,7-diethyl-5-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]-triazole-6-carboxylate (2f), which was orally active in rats, and produced a dose-dependent decrease in blood pressure when administered orally to conscious furosemide-treated dogs, with *ca.* 3-fold increased potency in comparison with the parent C-6 hydrogen compound.

Key words nonpeptide angiotensin II antagonist; pyrazolo[1,5-*b*][1,2,4]triazole; double ester; antihypertensive

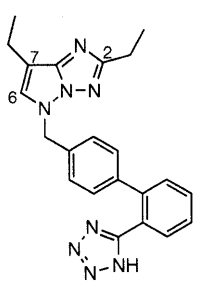
The renin–angiotensin system plays an important role in the regulation of blood pressure through the actions of angiotensin II (AII) and is thus an appropriate target for therapeutic intervention in hypertension.[3] Since the discovery of the first potent and orally active non-peptide AII antagonist, DuP 753 (losartan, COZAAR®),[4] numerous patents and publications on AII antagonists have appeared.[5]

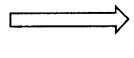
In a previous paper,[1] we reported a series of alkyl-substituted pyrazolo[1,5-*b*][1,2,4]triazole derivatives with AII antagonistic activity. Among these derivatives, 2,7-diethyl-5-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]triazole (**1**) was found to be a highly potent and orally active AII antagonist. From a comparison of the crystal structures of **1** and DuP 753, and the result of a simple conformational analysis of **1**, we postulated that the ethyl groups at the 2- and 7-positions of the pyrazolo[1,5-*b*][1,2,4]triazole ring respectively corresponded to the lipophilic butyl and chloro part of DuP 753. From a structure–activity relationship study of AII antagonists containing the imidazole ring, it appeared that C-linked oxygen functional groups such

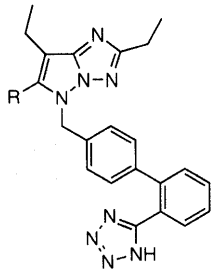


DuP 753 (K salt), R = CH_2OH

EXP3174, R = COOH







R = C-linked oxygen functional groups

1 **2**

Fig. 1

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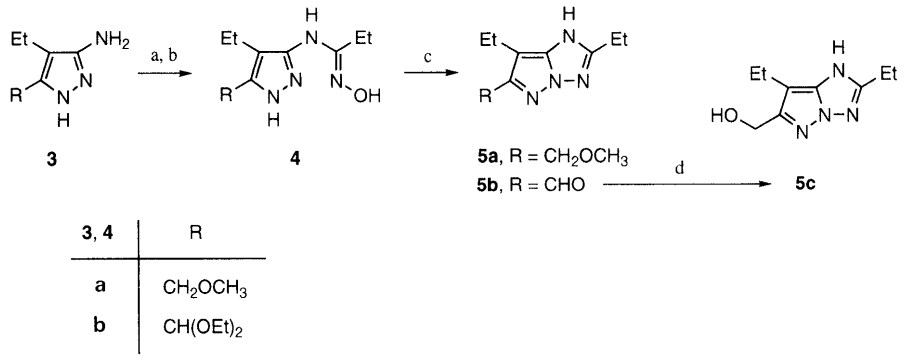
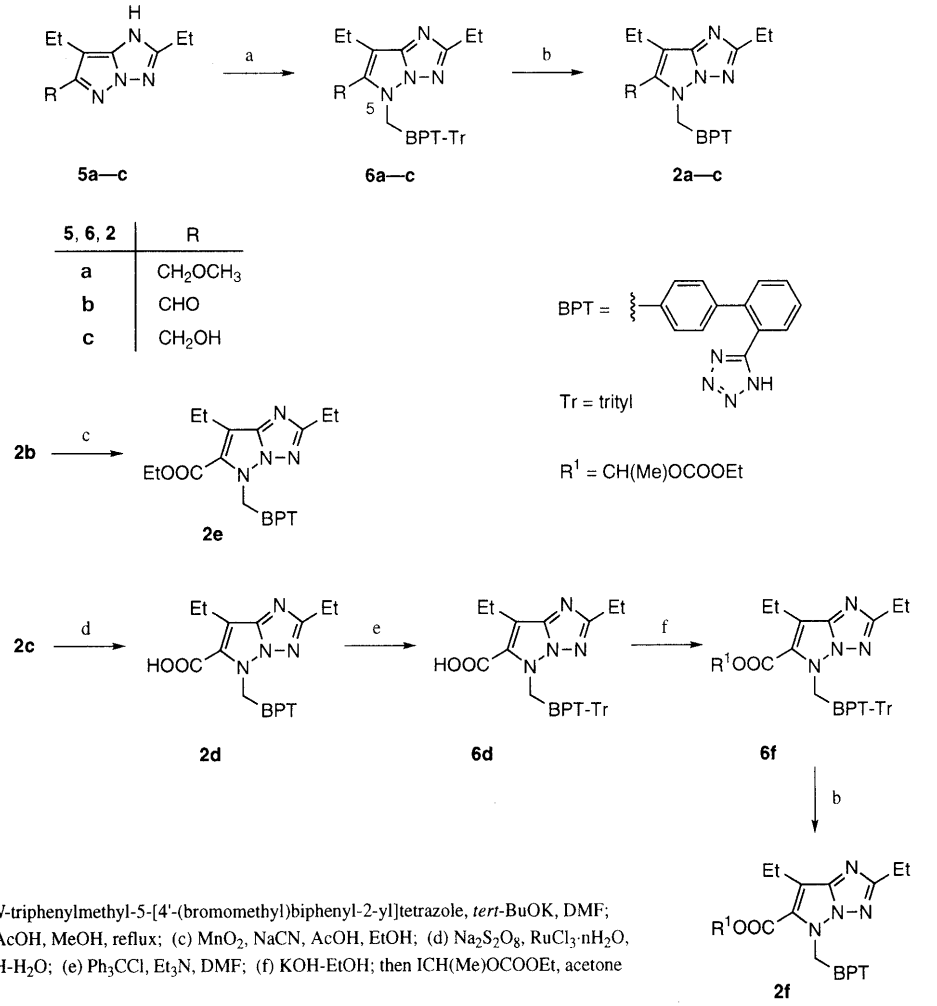


3, 4	R
a	CH_2OCH_3
b	$CH(OEt)_2$

(a) $EtC(OEt)_3$, toluene, reflux; (b) NH_2OH, MeOH; (c) *p*-TsCl, pyridine, *N*,*N*-dimethylacetamide, then pyridine, MeOH, reflux; (d) $NaBH_4$, EtOH

Chart 1



5, 6, 2	R
a	CH_2OCH_3
b	CHO
c	CH_2OH

(a) *N*-triphenylmethyl-5-[4'-(bromomethyl)biphenyl-2-yl]tetrazole, *tert*-BuOK, DMF; (b) AcOH, MeOH, reflux; (c) MnO_2, NaCN, AcOH, EtOH; (d) $Na_2S_2O_8$, $RuCl_3 \cdot nH_2O$, $KOH\text{-}H_2O$; (e) Ph_3CCl, Et_3N, DMF; (f) KOH-EtOH; then ICH(Me)OCOOEt, acetone

Chart 2

as the hydroxymethyl group and the carboxylic acid group at the 5-position of the imidazole ring, which are capable of hydrogen-bonding interaction with the AII receptor, enhance the *in vitro* activity.[4,6] In addition, the major active metabolite of DuP 753, EXP3174, is believed to have a significant role in the antihypertensive effect of DuP 753.[7] These facts prompted us to synthesize and evaluate pyrazolo[1,5-*b*][1,2,4]triazole derivatives (**2**) with a C-linked oxygen functional group at the 6-position.

Chemistry

Starting from the 3-aminopyrazole derivatives (**3**), the construction of the pyrazolo[1,5-*b*][1,2,4]triazole ring was performed according to literature methods[8] (Chart 1). Compound **3** was treated with triethyl orthopropionate, and subsequently reacted with hydroxylamine in methanol to give the *N*-hydroxyamidines (**4**). *O*-Tosylation of the *N*-hydroxyamidine (**4a**) followed by heating in methanol with pyridine afforded the methoxymethyl derivative (**5a**). In a similar manner to that described for **5a**, compound

4b gave the aldehyde (**5b**), with hydrolysis of the acetal moiety. The reduction of **5b** with sodium borohydride in ethanol afforded the hydroxymethyl derivative (**5c**).

Preparation of the target compounds (**2a—f**) was performed as shown in Chart 2. Alkylation reaction of **5a—c** in *N*,*N*-dimethylformamide (DMF) with *N*-triphenylmethyl-5-[4′-(bromomethyl)biphenyl-2-yl]tetrazole[4] using potassium *tert*-butoxide as a base gave the corresponding *N*(5)-isomers (**6a—c**) as the major products. The trityl group of **6a—c** was cleaved by treatment with acetic acid in methanol to yield the final compounds (**2a—c**). The regiochemistry of the *N*(5)-isomers was confirmed by nuclear Overhauser effect (NOE) observations on compounds **2a—c**. The NOE study correlated the benzylic methylene protons to the protons (CH_2OMe, **2a**; CHO, **2b**; CH_2OH, **2c**) at the 6-position of the head.

The aldehyde (**2b**) was treated with manganese dioxide and sodium cyanide in ethanol[9] to give the ethyl ester (**2e**). Oxidation of **2c** with sodium peroxodisulfate in the presence of a catalytic amount of ruthenium chloride in aqueous KOH[10] gave the carboxylic acid (**2d**). Tritylation of **2d** with trityl chloride in DMF in the presence of triethylamine gave the trityltetrazole derivative (**6d**). Alkylation of the potassium salt of **6d** with ethyl 1-iodoethyl carbonate in acetone followed by deprotection of the trityl group provided the double ester (**2f**).

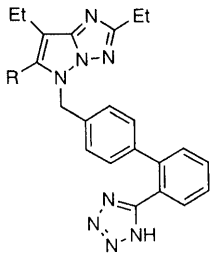
Results and Discussion

The compounds were tested *in vitro* for the ability to inhibit the AII-induced contraction of rabbit thoracic aorta strips. The pharmacological data are summarized in Table 1.

We previously reported that the introduction of an alkyl group at the 6-position of the pyrazolo[1,5-*b*][1,2,4]triazole ring of **1** resulted in reduced activity (**7** *vs.* **1**).[1] In contrast, the methoxymethyl derivative **2a** showed comparable activity to **1**. The aldehyde (**2b**), hydroxymethyl (**2c**), and ethoxycarbonyl (**2e**) compounds showed improved activities relative to their parent compound **1**. These results confirmed that the oxygen function at the 6-position is beneficial to the *in vitro* activity. In the rabbit aorta, the carboxylic acid **2d** caused nonparallel shifts to the right of the concentration–response curve for AII and reduced the maximal response to AII (insurmountable antagonism), as reported with EXP3174.[7a]

An X-ray crystallographic analysis of **2c** was performed (Fig. 2) for comparison of the structure with that of DuP 753. Previously,[1] we showed that the tetrazole rings of the parent **1** and DuP 753 are oriented to opposite sides as a result of the different conformers of each central phenyl ring. In contrast, the conformation revealed in the X-ray crystal structures of **2c** could be superimposed on that of DuP 753 (Fig. 3, Table 2). Of the two ethyl groups of **2c**, the ethyl group at the 2-position, which is more important for the activity of the pyrazolo[1,5-*b*][1,2,4]-triazole derivatives,[1] again mapped to the butyl part of

Table 1. Physical Properties and *in Vitro* AII-Antagonistic Potencies of Compounds **2**



Compd.	R	mp (°C)	Formula[a]	pA_2	pD'_2
DuP 753				8.30	
EXP3174					9.13
1[b]	H			8.74	
7[b]	$CH_2CH_2CH_3$			8.24	
2a	CH_2OCH_3	192—194	$C_{24}N_{26}N_8O$	8.85	
2b	CHO	193—195	$C_{23}H_{22}N_8O \cdot 0.2AcOEt$	9.16	
2c	CH_2OH	179—181	$C_{23}H_{24}N_8O \cdot 0.2H_2O$	9.02	
2d	COOH	Powder	$C_{23}H_{22}N_8O_2 \cdot H_2O$		9.31
2e	$COOCH_2CH_3$	147—149	$C_{25}H_{26}N_8O_2$	9.25	

a) Analytical results were within ±0.4% of the theoretical values. *b*) Ref. 1.

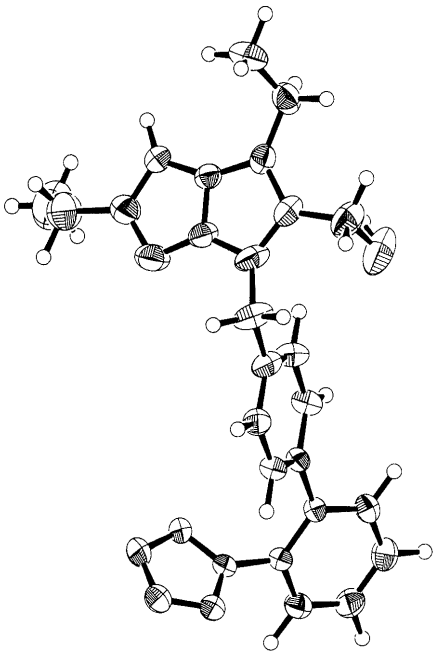


Fig. 2. An ORTEP Drawing of the Molecule of **2c**

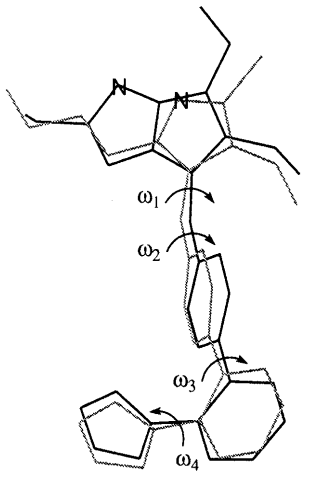


Fig. 3. Superimposition of Crystal Structures of **2c** (Black) and DuP 753 (Gray)

DuP 753.[11] The torsion angles of the bond between the methylene group and the central phenyl ring (*i.e.*, ω_2) are $-167.5°$ for **2c** and 176.7° for DuP 753. The presence of the different conformers of the central phenyl ring in the pyrazolo[1,5-*b*][1,2,4]triazole series (ω_2 was $-112.1°$ for **1**) suggests that the energy cost to rotate the bond between the methylene group and the phenyl ring is low, as we previously calculated for compound **1**.[1] It also appeared that the N-1 atom of the pyrazolo[1,5-*b*][1,2,4]triazole ring of **2c** accepts the acidic proton in the crystal structure. In the imidazole series, the N-3 atom is assumed to be an acceptor during the hydrogen-bonding interaction with the AII receptor.[12] We speculate that the N-1 atom of the pyrazolo[1,5-*b*][1,2,4]triazole ring is able to contribute to a similar interaction.

The compounds were orally evaluated for inhibition of AII-induced pressor response in conscious normotensive rats (Table 3). Administration of **2a** and **2b** caused significant inhibitory activities, but these effects were transient. Compounds **2c** and **2d** had substantially increased *in vitro* activity over **1**, but showed diminished oral potency, probably because of poor bioavailability. The ethyl ester **2e** (30 mg/kg) was found to exert a potent inhibitory activity with a prolonged duration of action compared to **1**. This profile may in part be due to conversion to the parent carboxylic acid **2d** *in vivo* by esterase. In order to improve the oral activity of the acid derivative **2d**, a double ester prodrug **2f** was examined. Such ester residues have been applied to improve the oral absorption of the β-lactam antibiotic, Ampicillin, by masking the highly polar character of its carboxylic acid group.[13] Improvement of the inhibitory activity of **2d** was observed in the double ester **2f** (**2d**, 30 mg/kg *vs.* **2f**, 3 mg/kg).[14] Compound **2f** (30 mg/kg) showed a somewhat slow onset and long-lasting inhibitory activity with a duration of action of >24 h. Its potency was greater than that of DuP 753 and EXP3174.

The oral hypotensive effect of the double ester **2f** was evaluated in furosemide-treated dogs (Fig. 4). Compound **2f** had a dose-dependent hypotensive effect. It showed a *ca.* 3-fold increase in potency compared to **1**, and the maximal decrease in mean blood pressure (MBP) was 37 and 32 mmHg for **2f** (10 mg/kg) and **1** (30 mg/kg), respectively. Compound **2f** showed long-lasting hypotensive activity with a duration of action of >8 h. In this model,

Table 2. Torsion Angles in the Crystal Structures of **2c** and DuP 753

Compd.	ω_1	ω_2	ω_3	ω_4
2c	91.8°	−167.5°	42.0°	61.2°
DuP 753	86.3°	176.7°	40.7°	52.6°

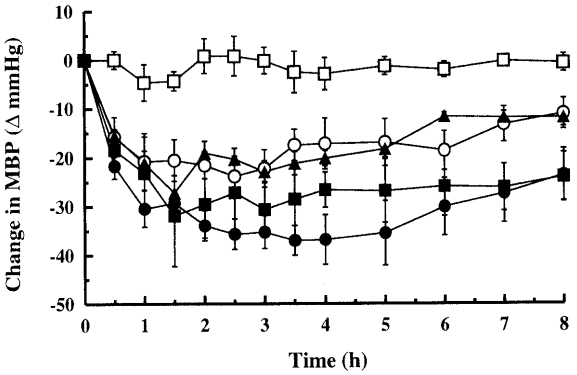
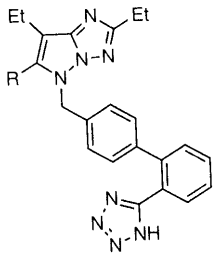


Fig. 4. Oral Hypotensive Activity in Furosemide-Treated Sodium-Depleted Dogs

SEM are indicated by bars. —□— vehicle, $n=4$; —○— **2f**, 3 mg/kg, $n=4$; —●— **2f**, 10 mg/kg, $n=4$; —■— **1**, 30 mg/kg, $n=3$; —▲— DuP 753, 30 mg/kg, $n=4$.

Table 3. *In Vivo* AII-Antagonistic Potencies of Compounds **2**



Compd.	R	Dose (mg/kg *p.o.*)	% inhibition[a]					
			0.5 h	1 h	2 h	4 h	8 h	24 h
DuP 753		30	35	59	66	62	52	18
EXP3174		30	50	43	41	37	27	4
1	H	30	90	90	82	49	19	4
2a	CH_2OCH_3	30	92	85	54	9	N.T.[b]	N.T.
2b	CHO	30	85	67	47	30	24	24
2c	CH_2OH	30	25	23	22	8	N.T.	N.T.
2d	COOH	30	6	12	7	18	34	44
2e	COOEt	10	52	36	29	34	44	54
		30	94	88	77	75	76	71
2f	COOCH(Me)OCOOEt	3	5	19	22	42	42	31
		30	42	63	83	88	91	85

a) Percent inhibition of AII-induced pressor response in conscious normotensive rats after oral administration of test compounds. *b*) Not tested.

2f was *ca.* 10-fold more potent than DuP 753 (**2f**, 3 mg/kg *vs.* DuP 753, 30 mg/kg).

In conclusion, we have developed a novel series of 5*H*-pyrazolo[1,5-*b*][1,2,4]triazole derivatives (**2**) as potent AII antagonists by introducing C-linked oxygen functional groups at the 6-position in the parent compound **1**. Among the compounds in this study, the double ester prodrug **2f** displayed potent and long-lasting AII antagonistic activity when given orally to rats. In a dog model, **2f** showed an increased oral hypotensive effect when compared to DuP 753 and the parent **1**. These results suggest that **2f** may be useful in the therapeutic treatment of AII-related diseases such as hypertension.

Experimental

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL FX-90, a JEOL FX-100, a JNM-LA 300, a JNM-EX 400 or a JNM-GX 500 spectrometer with tetramethylsilane as an internal standard. Mass spectra (MS) were recorded on a Hitachi M-80 (EI) or a JEOL JMS DX-300 (FAB) mass spectrometer. Elemental analysis was performed with a Yanaco MT-5. X-ray diffraction measurements were made with a Rigaku AFC5R diffractometer using Cu $K\alpha$ radiation. Column chromatography was performed on silica gel (Merck Kieselgel 60, 70—230 mesh). Sodium amide (95%) and ruthenium(III) chloride hydrate were purchased from Aldrich Chemical Co.

3-Amino-5-diethoxymethyl-4-ethyl-1*H*-pyrazole (3b) A suspension of sodium amide (95%, 17.2 g, 0.42 mol) in 1 l of liquid ammonia was prepared in a 2 l three-necked round-bottomed flask equipped with a mechanical stirrer with dry ice–methanol cooling. To the stirred suspension, 28.9 g of butyronitrile (0.42 mol) was added over 5 min. After 5 min, ethyl diethoxyacetate (53.3 g, 0.30 mmol) was added over 5 min and stirring was continued for 1 h. Removal of ammonia on a water bath at 40—50 °C under a nitrogen stream afforded the residue, to which were added 150 g of ice–water and 80 ml of diethyl ether. The mixture was acidified with 6 N hydrochloric acid, and the aqueous phase was extracted with 50 ml of diethyl ether. The combined organic extracts were mixed with ethanol (200 ml) and hydrazine hydrate (36.0 ml, 0.74 mol), and diethyl ether was distilled off at atmospheric pressure. The ethanol solution was refluxed overnight, then concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 24 : 1) to give the title compound (52.7 g, 82%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.10 (3H, t, J = 7.5 Hz), 1.21 (6H, t, J = 7.0 Hz), 2.40 (2H, q, J = 7.5 Hz), 3.57 (4H, q, J = 7.0 Hz), 5.35 (3H, br), 5.61 (1H, s). EI-MS m/z: 213 (M^+).

3-Amino-4-ethyl-5-methoxymethyl-1*H*-pyrazole (3a) Compound **3a** was prepared from butyronitrile and ethyl methoxyacetate in a similar manner to that described for compound **3b**.

3a: Yield 57%, oil. ^{1}H-NMR ($CDCl_3$) δ: 1.11 (3H, t, J = 7.6 Hz), 2.36 (2H, q, J = 7.6 Hz), 3.35 (3H, s), 4.41 (2H, s), 5.20—5.90 (3H, m). EI-MS m/z: 155 (M^+).

2,7-Diethyl-1*H*-pyrazolo[1,5-*b*][1,2,4]triazole-6-carboxaldehyde (5b) A solution of compound **3b** (52.6 g, 0.25 mol) and triethyl orthopropionate (49.6 ml, 0.25 mmol) in toluene (500 ml) was refluxed for 4 h. Removal of the solvent under reduced pressure to give a residue, which, without further purification, was treated with a solution of hydroxylamine (0.29 mol) in MeOH (1.5 l). The mixture was stirred for 16 h at room temperature and then concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 19 : 1) to give *N*-hydroxy-*N'*-(5-diethoxymethyl-4-ethyl-1*H*-pyrazol-3-yl)propionamidine (**4b**) (66.6 g, 95%). ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, t, J = 7.6 Hz), 1.12 (3H, t, J = 7.6 Hz), 1.24 (6H, t, J = 7.1 Hz), 2.41—2.48 (4H, m), 3.56—3.61 (4H, m) 5.67 (1H, s). EI-MS m/z: 285 (M^+).

p-Toluenesulfonyl chloride (44.6 g, 0.23 mol) was added to a solution of **4b** (66.5 g, 0.23 mol) and pyridine (37.2 g, 0.47 mol) in *N*,*N*-dimethylacetamide (400 ml) with ice-cooling, and the mixture was stirred for 2 h at room temperature. The reaction mixture was diluted with water (1.2 l) and extracted with $CHCl_3$ (1.2 l). Removal of $CHCl_3$ under reduced pressure afforded the residue, which contained *N*,*N*-dimethylacetamide, and this was refluxed for 2 h in MeOH (1 l) with pyridine (37.2 g, 0.47 mol). The mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 39 : 1) to give a crystalline product, which was washed with a mixture of diisopropyl ether and ethyl acetate (6 : 1) to give 19.5 g (43%) of **5b**, mp 141—142 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.22 (3H, t, J = 7.5 Hz), 1.32 (3H, t, J = 7.5 Hz), 2.82 (4H, q, J = 7.5 Hz), 9.89 (1H, s), 12.86 (1H, br). EI-MS m/z: 192 (M^+). *Anal.* Calcd for $C_9H_{12}N_4O$: C, 56.24; H, 6.29; N, 29.15. Found: C, 55.94; H, 6.29; N, 29.05.

2,7-Diethyl-6-methoxymethyl-1*H*-pyrazolo[1,5-*b*][1,2,4]triazole (5a) Compound **5a** was prepared from **3a** in a similar manner to that described for compound **5b**.

5a: Yield 17%, mp 129—131 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.18 (3H, t, J = 7.6 Hz), 1.28 (3H, t, J = 7.9 Hz), 2.51 (2H, q, J = 7.6 Hz), 2.74 (2H, q, J = 7.9 Hz), 3.21 (3H, s), 4.34 (2H, s), 12.33 (1H, br). EI-MS m/z: 208 (M^+). *Anal.* Calcd for $C_{10}H_{16}N_4O \cdot 0.1H_2O$: C, 57.18; H, 7.77; N, 26.67. Found: C, 57.17; H, 7.69; N, 26.52.

2,7-Diethyl-6-hydroxymethyl-1*H*-pyrazolo[1,5-*b*][1,2,4]triazole (5c) Sodium borohydride (6.25 g, 0.17 mol) was added to a solution of compound **5b** (22.1 g, 0.11 mol) in EtOH (450 ml) with ice-cooling. The mixture was stirred for 1 h at the same temperature, then 1 N hydrochloric acid was added to adjust the pH to 7. The mixture was evaporated *in vacuo*, and the residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 97 : 3—9 : 1) to give a crystalline product, which was washed with diisopropyl ether to give 20.6 g (92%) of **5c**, mp 184—186 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.18 (3H, t, J = 7.6 Hz), 1.27 (3H, t, J = 7.6 Hz), 2.41—2.86 (4H, m), 4.39 (2H, br d), 4.75 (1H, br t), 12.20 (1H, br). EI-MS m/z: 194 (M^+). *Anal.* Calcd for $C_9H_{14}N_4O$: C, 55.65; H, 7.26; N, 28.84. Found: C, 55.49; H, 7.21; N, 28.95.

2,7-Diethyl-6-hydroxymethyl-5-[[2′-[*N*-(triphenylmethyl)tetrazol-5-yl]biphenyl-4-yl]methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]triazole (6c) Compound **5c** (15.1 g, 77.7 mmol) was added to a suspension of potassium *tert*-butoxide (9.72 g, 86.6 mmol) in DMF (500 ml) with ice-cooling. The mixture was stirred for 20 min at the same temperature, then *N*-triphenylmethyl-5-[4′-(bromomethyl)biphenyl-2-yl]tetrazole (53.3 g, 95.6 mmol) was added and the whole was allowed to warm to room temperature. The mixture was stirred overnight, then concentrated under reduced pressure. Water (1.4 l) and AcOEt (1.6 l) were added to the residue and the whole was stirred at room temperature. The crystalline product was collected by filtration and washed successively with AcOEt (150 ml) and water to give the crude product. This was suspended in MeOH (160 ml), and the mixture was heated to reflux, then allowed to cool to room temperature. Filtration afforded 34.6 g (66%) of compound **6c**, mp 194—197 °C (dec.). ^{1}H-NMR ($CDCl_3$) δ: 1.19 (3H, t, J = 7.6 Hz), 1.38 (3H, t, J = 7.6 Hz), 2.37 (2H, q, J = 7.6 Hz), 2.84 (2H, q, J = 7.6 Hz), 4.44 (2H, s), 5.38 (2H, s), 6.91 (6H, d, J = 7.8 Hz), 6.98 (2H, d, J = 8.1 Hz), 7.06 (2H, d, J = 8.1 Hz), 7.23—7.50 (12H, m), 7.90—7.93 (1H, m). FAB-MS m/z: 671 $(M+H)^+$.

2,7-Diethyl-6-methoxymethyl-5-[[2′-[*N*-(triphenylmethyl)tetrazol-5-yl]biphenyl-4-yl]methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]triazole (6a) and 2,7-Diethyl-5-[[2′-[*N*-(triphenylmethyl)tetrazol-5-yl]biphenyl-4-yl]methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]triazole-6-carboxaldehyde (6b) Compounds **6a** and **6b** were prepared from **5a** and **5b**, respectively, in a similar manner to that described for **6c**, except that silica gel column chromatography was used for purification.

6a: Yield 76%, foam. ^{1}H-NMR (DMSO-d_6) δ: 1.19—1.24 (6H, m), 2.56—2.67 (4H, m), 3.21 (3H, s), 4.45 (2H, s), 5.39 (2H, s), 6.85—7.09 (10H, m), 7.30—7.62 (12H, m), 7.78—7.80 (1H, m). FAB-MS m/z: 685 $(M+H)^+$

6b: Yield 54%, mp 144—146 °C (dec.). ^{1}H-NMR ($CDCl_3$) δ: 1.40—1.44 (6H, m), 2.93—3.00 (4H, m), 5.70 (2H, s), 6.90—6.92 (6H, m), 7.04 (2H, d, J = 7.9 Hz), 7.10 (2H, d, J = 7.9 Hz), 7.24—7.48 (12H, m) 7.90—7.92 (1H, m), 9.97 (1H, s). FAB-MS m/z: 669 $(M+H)^+$.

2,7-Diethyl-6-hydroxymethyl-5-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]-methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]triazole (2c) A mixture of **6c** (38.8 g, 57.8 mmol) and acetic acid (74 ml) in MeOH (740 ml) was refluxed for 8 h and then concentrated under reduced pressure. Toluene was added to the residue and subsequently evaporated off. This was repeated 3 times. The resulting residue was crystallized from AcOEt to give **2c** (22.8 g, 92%), mp 179—181 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.21 (3H, t, J = 7.5 Hz), 1.22 (3H, t, J = 7.5 Hz), 2.58 (2H, q, J = 7.5 Hz), 2.63 (2H, q, J = 7.5 Hz), 4.57 (2H, s), 5.46 (2H, s), 7.04 (2H, d, J = 7.9 Hz), 7.16 (2H, d, J = 7.9 Hz), 7.51—7.68 (4H, m). FAB-MS m/z: 429 $(M+H)^+$. *Anal.* Calcd for $C_{23}H_{24}N_8O \cdot 0.2H_2O$: C, 63.93; H, 5.69; N, 25.93. Found: C, 63.80; H, 5.59; N, 25.89.

2,7-Diethyl-6-methoxymethyl-5-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]-methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]triazole (2a) and 2,7-Diethyl-5-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]triazole-6-carboxaldehyde (2b) Compounds **2a** and **2b** were prepared from **6a** and **6b**, respectively, in a similar manner to that described for **2c**.

2a: Yield 56%, mp 192—194 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.22 (6H, t, J=7.6 Hz), 2.60 (2H, q, J=7.6 Hz), 2.65 (2H, q, J=7.6 Hz), 3.24 (3H, s), 4.56 (2H, s), 5.44 (2H, s), 7.05 (2H, d, J=7.9 Hz), 7.14 (2H, d, J=7.9 Hz), 7.51—7.68 (4H, m). FAB-MS m/z: 443 $(M+H)^+$. *Anal.* Calcd for $C_{24}H_{26}N_8O$: C, 65.14; H, 5.92; N, 25.32. Found: C, 65.16; H, 5.97; N, 25.33.

2b: Yield 59%, mp 193—195 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.26 (3H, t, J=7.5 Hz), 1.33 (3H, t, J=7.5 Hz), 2.74 (2H, q, J=7.5 Hz), 2.98 (2H, q, J=7.5 Hz), 5.72 (2H, s), 7.04 (2H, d, J=8.2 Hz), 7.13 (2H, d, J=8.2 Hz), 7.50—7.67 (4H, m), 10.09 (1H, s). FAB-MS m/z: 427 $(M+H)^+$. *Anal.* Calcd for $C_{23}H_{22}N_8O \cdot 0.2AcOEt$: C, 64.37; H, 5.36; N, 25.23. Found: C, 64.54; H, 5.40; N, 25.42.

Ethyl 2,7-Diethyl-5-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]triazole-6-carboxylate (2e) A mixture of **2b** (7.29 g, 17.1 mmol), sodium cyanide (5.12 g, 0.10 mol), manganese dioxide (41.0 g, 0.47 mol), and AcOH (1.5 ml) in EtOH (200 ml) was stirred at room temperature for 18 h. The mixture was diluted with $CHCl_3$ (200 ml) and filtered through Celite. The solvent was removed *in vacuo* and the residue was purified by silica gel column chromatography ($CHCl_3$: EtOH = 23 : 2) to give a foam, to which EtOH (250 ml) and water (200 ml) were added. The mixture was made basic with saturated aqueous $NaHCO_3$ to dissolve the foam, and then concentrated under reduced pressure. The residue was dissolved in water (250 ml) and the mixture was acidified to pH 2 with 1 N hydrochloric acid under ice-cooling. The resulting precipitate was collected by filtration and washed with water to give the title compound (4.04 g, 50%), mp 147—149 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.24—1.31 (9H, m), 2.73 (2H, q, J=7.7 Hz), 2.90 (2H, q, J=7.5 Hz), 4.37 (2H, q, J=7.1 Hz) 5.69 (2H, s), 7.05 (2H, d, J=8.2 Hz), 7.10 (2H, d, J=8.2 Hz), 7.50—7.68 (4H, m). FAB-MS m/z: 471 $(M+H)^+$. *Anal.* Calcd for $C_{25}H_{26}N_8O_2$: C, 63.82; H, 5.57; N, 23.81. Found: C, 63.81; H, 5.66; N, 23.71.

2,7-Diethyl-5-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]triazole-6-carboxylic Acid (2d) Sodium peroxodisulfate (6.46 g, 27.1 mmol) was added to a mixture of **2c** (5.82 g, 13.6 mmol) and $RuCl_3 \cdot nH_2O$ (0.35 g) in 0.67 N aqueous potassium hydroxide solution (180 ml) under ice-cooling. The mixture was stirred overnight at the same temperature. MeOH (2 ml) was added, and stirring was continued at room temperature for 3 h. The mixture was filtered through Celite and the Celite was rinsed with water (50 ml). The filtrate was acidified to pH 2 with 1 N hydrochloric acid under ice-cooling. The resulting precipitate was collected by filtration and washed with water to give the title compound (5.58 g, 93%) as a powder. ^{1}H-NMR (DMSO-d_6) δ: 1.25 (6H, t, J=7.6 Hz), 2.71 (2H, q, J=7.6 Hz), 2.94 (2H, q, J=7.6 Hz), 5.72 (2H, s), 7.04 (2H, d, J=8.3 Hz), 7.09 (2H, d, J=8.3 Hz), 7.50—7.66 (4H, m). FAB-MS m/z: 443 $(M+H)^+$. *Anal.* Calcd for $C_{23}H_{22}N_8O_2 \cdot H_2O$: C, 59.99; H, 5.25; N, 24.33. Found: C, 60.11; H, 4.90; N, 24.52.

2,7-Diethyl-5-[[2′-[*N*-(triphenylmethyl)tetrazol-5-yl]biphenyl-4-yl]-methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]triazole-6-carboxylic Acid (6d) The title compound was prepared from **2d** using a conventional method (trityl chloride/triethylamine/DMF). Yield 83%, mp 173—175 °C (dec.). ^{1}H-NMR (DMSO-d_6) δ: 1.21—1.25 (6H, m), 2.70 (2H, q, J=7.5 Hz), 2.91 (2H, q, J=7.5 Hz), 5.68 (2H, s), 6.85 (6H, d, J=7.3 Hz), 7.01 (2H, d, J=8.1 Hz), 7.04 (2H, d, J=8.1 Hz), 7.30—7.60 (12H, m), 7.79 (1H, d, J=7.3 Hz). FAB-MS m/z: 683 $(M-H)^+$.

(±)-1-[(Ethoxycarbonyl)oxy]ethyl 2,7-Diethyl-5-[[2′-[*N*-(triphenylmethyl)tetrazol-5-yl]biphenyl-4-yl]methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]-triazole-6-carboxylate (6f) A mixture of 1-chloroethyl ethyl carbonate (2.91 g, 19.1 mmol) and sodium iodide (8.60 g, 57.4 mmol) in acetonitrile (65 ml) was stirred for 45 min at 60—70 °C, and then concentrated under reduced pressure. The residue was taken up in diethyl ether, and filtered. The filtrate was evaporated to give crude ethyl 1-iodoethyl carbonate, which was used without further purification. Compound **6d** (7.48 g, 10.9 mmol) was dissolved in 0.5 N ethanolic potassium hydroxide solution (21.8 ml), and the solvent was removed *in vacuo*. A fine suspension of the residue was prepared in acetone (250 ml), and a solution of the crude ethyl 1-iodoethyl carbonate in acetone (30 ml) was added dropwise to it. The mixture was stirred overnight at room temperature, then further ethyl 1-iodoethyl carbonate in an amount equal to that described above was added to the mixture, followed by stirring for 24 h at room temperature. The whole was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (*n*-hexane : AcOEt = 7 : 3) to give **6f** (6.56 g, 75%) as a foam. ^{1}H-NMR ($CDCl_3$) δ: 1.26—1.40 (9H, m), 1.63 (3H, d, J=5.5 Hz), 2.90 (2H, q, J=7.5 Hz), 2.97 (2H, q, J=7.5 Hz), 4.20—4.24 (2H, m), 5.64 (1H, d, J=15.6 Hz), 5.68 (1H, d, J=15.6 Hz), 6.91 (6H, d, J=7.9 Hz), 6.92—7.07 (5H, m), 7.24—7.88 (12H, m), 7.90 (1H, d, J=6.7 Hz). FAB-MS m/z: 801 $(M+H)^+$.

(±)-1-[(Ethoxycarbonyl)oxy]ethyl 2,7-Diethyl-5-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]triazole-6-carboxylate (2f) Compound **2f** was prepared from **6f** in a similar manner to that described for **2c**, except that silica gel column chromatography was used for purification. Yield 92%, mp 80—83 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.09 (3H, t, J=7.6 Hz), 1.24—1.28 (6H, m), 1.63 (3H, d, J=5.5 Hz), 2.64—2.72 (4H, m), 4.14—4.20 (2H, m), 5.72 (1H, d, J=15.8 Hz), 5.77 (1H, d, J=15.8 Hz), 6.90—7.00 (5H, m), 7.40 (1H, d, J=7.9 Hz), 7.50—7.59 (2H, m), 7.89 (1H, d, J=7.9 Hz). FAB-MS m/z: 559 $(M+H)^+$. *Anal.* Calcd for $C_{28}H_{30}N_8O_5 \cdot 0.2H_2O$: C, 59.82; H, 5.45; N, 19.93. Found: C, 59.73; H, 5.40; N, 19.98.

X-Ray Crystallographic Analysis of 2c Suitable crystals ($C_{23}H_{24}N_8O$) for X-ray diffraction studies were formed from EtOH. Crystal data: crystal system, monoclinic; space group, C2/c (#15); lattice parameters, a=23.433(2) Å, b=9.211(2) Å, c=21.146(2) Å, β=108.117(7)°, V=4338(1) Å^3; $D_{calc.}$, 1.312 g/cm^3; Z value, 8; F_{000}, 1808.00; final R value, R=0.066, R_W=0.114.

Antagonism of AII-Contracted Rabbit Aorta Strips The thoracic aorta was isolated from male New Zealand White male rabbits weighing 2.0 to 4.5 kg. The aorta was cleaned of adherent fat and connective tissue, and cut into 3 mm wide and 30 mm long strips. The vascular endothelium was removed by gently rubbing the intimal surface of the vessel. Preparations were mounted in 30 ml organ baths containing Krebs–Henseleit solution (NaCl 118.4, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, $CaCl_2 \cdot 2H_2O$ 2.5, $NaHCO_3$ 25.0, and glucose 11.1 mM) maintained at 37 °C and bubbled with 95% O_2, 5% CO_2. Under a resting tension of 1.5 g, isometric tension changes were recorded on a polygraph (Rikadenki Kogyo, Japan) through a force displacement transducer (Nihon Kohden, Japan). After equilibration for 1 h, a single contractile response curve to the cumulative addition of AII was constructed. The strips were then washed twice and allowed to relax to the baseline tension. Each strip was then incubated for 30 min with several concentrations of test compound and the concentration–response curves for AII were again obtained. The results are expressed as a percentage of the maximal AII response obtained with the first curve, which served as the control. The EC_{50} (the AII concentration that contracted the strip to half the control maximum) for each curve was calculated. Potency data for each compound tested are expressed as the pA_2 (defined as $-\log K_B$, where K_B = (molar concentration of antagonist)/[(EC_{50} with antagonist/EC_{50} without antagonist) − 1]). As EXP3174 and **2d** were found to exert insurmountable AII antagonism, the pD_2' values, *i.e.*, the negative logarithm values of the concentration of the compound which inhibits the maximum response by 50%, were calculated.

Inhibition of Pressor Response to AII in Conscious Normotensive Rats Male Wistar rats aged 16 to 24 weeks were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and the left carotid artery and vein were cannulated with polyethylene tubing for the measurement of blood pressure and the intravenous administration of AII, respectively. The catheter was passed subcutaneously, exteriorized at the neck, and filled with saline containing heparin. The animals were allowed to recover from surgery for at least 3 to 4 d before beginning the experiment. The catheter was connected to a pressure transducer (Nihon Kohden, Japan) and the blood pressure was monitored with a polygraph (Nihon Kohden, Japan). To determine the effect of the compound on the AII-induced pressor response, AII (100 ng/kg) was injected i.v. before the oral administration of the test compound and subsequently at set times. The pressor responses to AII after the administration of the test compound were expressed as percent inhibition of the pressor response before the treatment.

Hypotensive Effect in Furosemide-Treated Sodium-Depleted Dogs Beagle dogs of either sex weighing 8.0 to 13.5 kg were used. The animals were anesthetized with sodium thiopental (30 mg/kg, i.v.). Anesthesia was maintained with 0.5% to 1% halothane in oxygen and room air during surgical operation. Under sterile surgical procedures, the right

femoral artery was exposed. The abdominal aorta was cannulated with polyvinyl tubing *via* the femoral artery. The catheter was passed subcutaneously, exteriorized *via* the neck, and filled with saline containing heparin. The skin incision was closed and the dog was allowed to recover from surgery for at least 3 to 4 d. The dogs received an intramuscular dose and intravenous dose of furosemide (10 mg/kg) at 16 and 2 h before the administration of a test compound, respectively. The animals were deprived of water from 18 h before to 8 h after dosing. The arterial catheter was connected to a pressure transducer (Nihon Kohden, Japan), and MBP was recorded with a polygraph (Nihon Kohden, Japan). MBP were measured before and after oral administration of **2f** (3 and 10 mg/kg), **1** (30 mg/kg) or DuP 753 (30 mg/kg).

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