Studies on Nonpeptide Angiotensin II Receptor Antagonists. I. Synthesis and Biological Evaluation of Pyrazolo[1,5-*b*][1,2,4]triazole Derivatives with Alkyl Substituents

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Alkyl-substituted pyrazolo[1,5-*b*][1,2,4]triazole derivatives were synthesized and evaluated for activity as angiotensin II receptor antagonists. Molecules with the (methylbiphenylyl)tetrazole moiety at N-5 were the preferred compounds. Ethyl substitutions at both C-2 and C-7 resulted in the optimal compound, 2,7-diethyl-5-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]triazole (5n), with a pA_2 value of 8.74 in rabbit aorta. In the *in vivo* tests, 5n inhibited the angiotensin II-induced pressor response in rats after oral administration. This compound also produced a dose-dependent decrease in blood pressure when administered orally to conscious furosemide-treated dogs, having a longer duration of action as compared to DuP 753. These data suggest that 5n may be a useful agent for the treatment of angiotensin II-dependent disease, such as hypertension.

Key words nonpeptide angiotensin II antagonist; antihypertensive; alkyl group; pyrazolo[1,5-*b*][1,2,4]triazole; YM358

The octapeptide angiotensin II (AII) is the primary component of the renin–angiotensin system (RAS) and plays an important role in the regulation of blood pressure, volume homeostasis, and salt retention.[2)] One approach to reduce AII levels is the inhibition of angiotensin converting enzyme (ACE). ACE inhibitors such as captopril and enalapril, which block the conversion of angiotensin I to AII, are widely used in the treatment of hypertension and heart failure.[3)] However, ACE inhibitors also block the metabolism of other peptides such as bradykinin and Substance P, causing undesirable side effects such as dry cough and angioedema.[4)] An alternative and selective method to block RAS is to antagonize the AII receptor directly. Although many peptidic antagonists have been available for a number of years, their clinical use has been limited because of their poor oral activity and partial agonistic activity.[5)] Thus, the discovery by the du Pont group of DuP 753 (losartan, COZAAR®),[6)] the first potent and orally active non-peptide AII antagonist devoid of agonistic activity, was a breakthrough in AII antagonist research.[7)]

On the basis of published work,[6,8)] the features of the structure–activity relationship (SAR) of DuP 753 are as follows. Firstly, the biphenyl acid, especially biphenylyltetrazole (BPT), linked to imidazole by a methylene group is essential for the best binding and oral potency. Secondly, a small alkyl group at the 2-position of the imidazole ring strongly contributes to the binding affinity. It is also reported that the imidazole ring, the chloro group and the hydroxymethyl group in DuP 753 are not essential for binding affinity.[6)] Thus, replacement of the imidazole with other azoles led to 1,3,4-triazole (**1**) and pyrazole (**2**) compounds, albeit with one order of magnitude of loss of binding affinity.[9)] Fusion of a 6-membered ring at C4–C5 bond of the imidazole has produced more potent bicyclic compounds, such as the imidazo[4,5-*b*]pyridine (**3a**, **b**, and L-158 809).[10)] It is interesting that the methyl groups of **3a** and **3b** have a significant effect, producing a 4- to 8-fold increase in binding activity. We considered that these results suggested two key points: 1) The imidazole of DuP 753 can be replaced with a suitably substituted heterocyclic head. 2) At least two lipophilic pockets, which accept the small alkyl groups around the head (*e.g.*, imidazole ring of DuP 753), are present in the AII receptor.

Our effort to find a novel series of orally active nonpeptide AII antagonists was focused on replacing the imidazole moiety of DuP 753 with a bicyclic ring. We designed pyrazolo[1,5-*b*][1,2,4]triazole bearing alkyl groups as the head (Fig. 1). It is considered that pyrazolo[1,5-*b*][1,2,4]triazoles have the structural features of a fused 1,3,4-triazole (**4**) or fused pyrazole (**5**). Furthermore, we assumed that the alkyl groups would be well accepted by the lipophilic pockets in the AII receptor. In this paper, we describe the synthesis and biological properties of this series of compounds, together with a simple conformational analysis of the optimal compound.

Chemistry

The key intermediates (**12**) were prepared by following a literature route[11)] (Chart 1). 3-Amino-1*H*-pyrazole derivatives (**6**) were reacted with the imidate hydrochlorides (**7**) in acetonitrile to give the intermediate amidine hydrochlorides (**8**), which were converted to the *N*-hydroxyamidines (**9**) by treatment with hydroxylamine in methanol (method A). Compounds **6** were also treated with the orthoesters (**10**) in refluxing toluene to give the intermediate imidates (**11**), which were led to **9** in a similar manner to that described for **8** (method B). *O*-Tosylation of the *N*-hydroxyamidines (**9**) followed by heating in methanol with pyridine afforded 1*H*-pyrazolo[1,5-*b*]-[1,2,4]triazole derivatives (**12**).

Preparation of the target compounds (**4**, **5**) was performed as shown in Chart 2 (for convenient discussion of the results, the pyrazolo[1,5-*b*][1,2,4]triazole numbering system is shown). Alkylation of **12** in *N*,*N*-dimethylformamide (DMF) with *N*-triphenylmethyl-5-[4′-(bromomethyl)biphenyl-2-yl]tetrazole[6)] using potassium *tert*-butoxide as a base gave the *N*(1)-isomers (**13**) and

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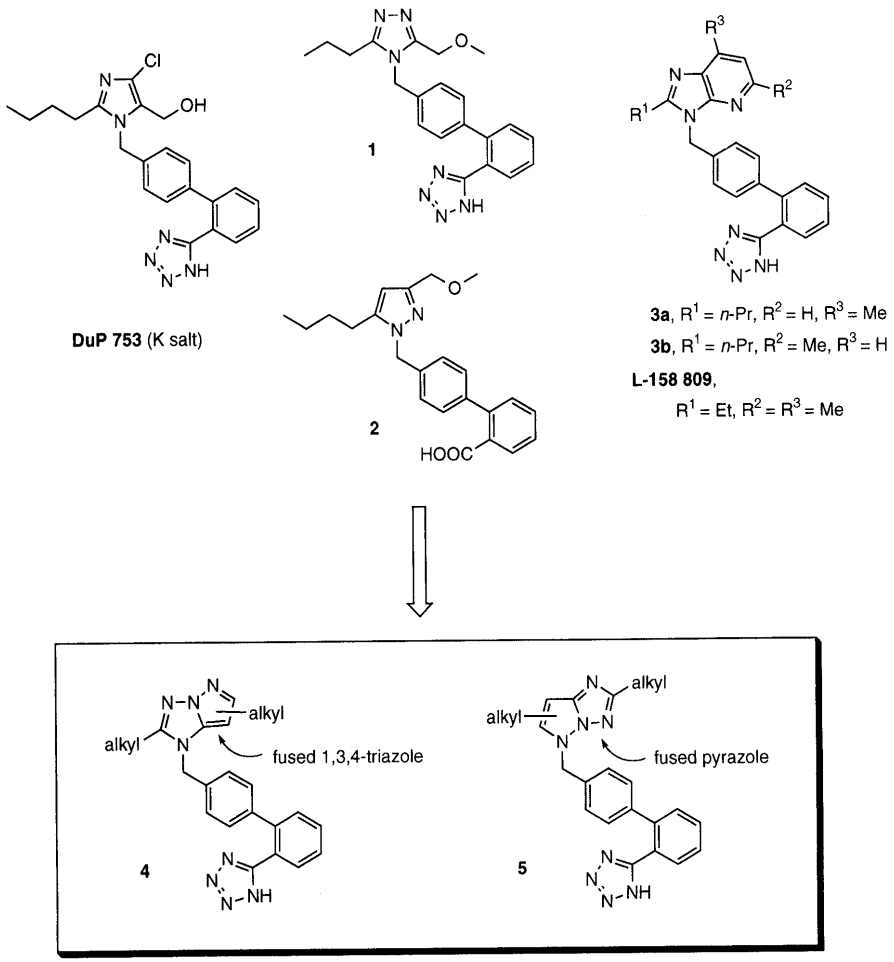
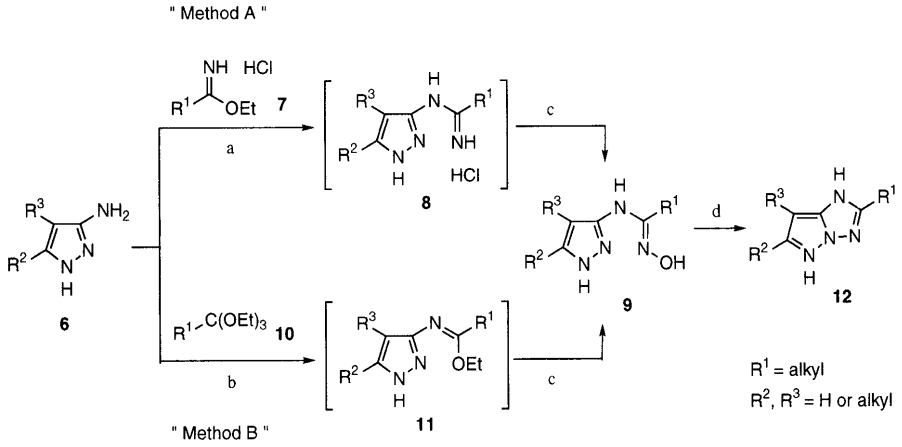


Fig. 1

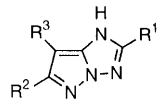


(a) acetonitrile; (b) toluene, reflux; (c) NH_2OH, MeOH; (d) *p*-TsCl, pyridine, *N,N*-dimethylacetamide, then pyridine, MeOH, reflux

Chart 1

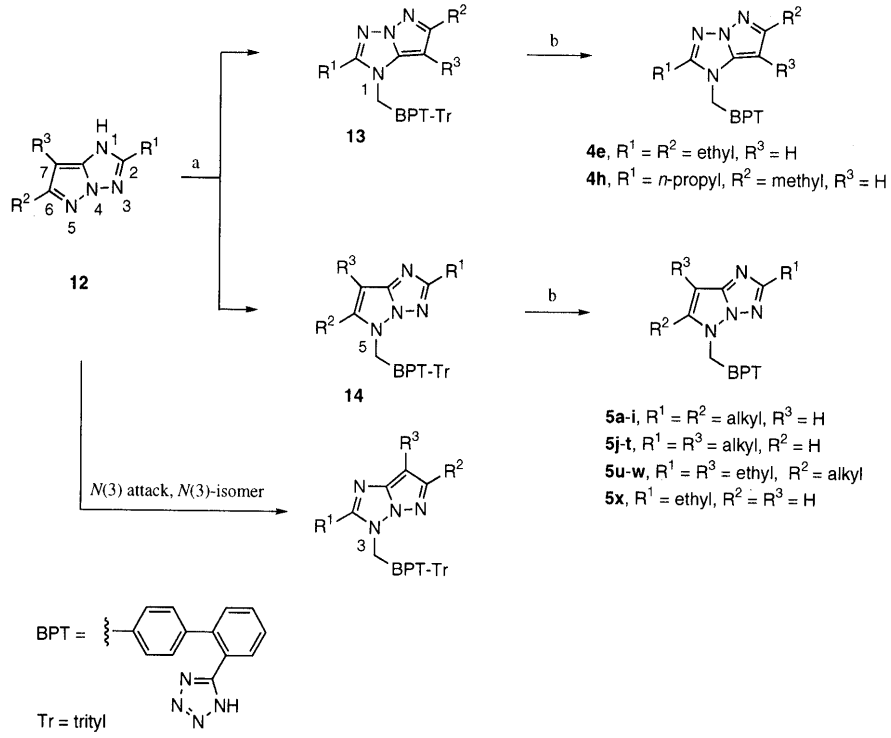
N(5)-isomers (**14**). The isomers could be separated by flash chromatography, and almost invariably the *N*(1)-isomer was the faster-moving spot in silica gel TLC. The ratio of the two isomers was dependent on the nature of the R^3-substituent at the 7-position of the pyrazolo[1,5-*b*][1,2,4]triazole ring. The *N*(1)-isomer (**13**) was obtained in a similar or higher amount compared to the *N*(5)-isomer (**14**), when R^3 is a proton. In contrast, **13** was the minor product when R^3 is an alkyl group, probably because of steric repulsion between the coming alkyl reagent and the R^3-alkyl group. The regiochemistry of the *N*(1)- and *N*(5)-isomers could be confirmed from the ^{1}H-NMR spectra (higher chemical shift of *N*-CH_2 protons for *N*(1)-isomer *vs.* *N*(5)-isomer) and observation of the nuclear Overhauser effect (NOE) in the final compounds (**4**, **5**), which were respectively derived by trityl-deprotection of **13** and **14**. For example, the NOE study for **4h** correlated the *N*(1)-CH_2 protons to H-A and H-B

Table 1. Physical Properties of Compounds **12**



Compd.	R^1	R^2	R^3	Method	Yield (%)[a]	mp (°C)	Formula[b]
12a[c]	Methyl	Methyl	H	A	14	264—266 (dec.)	$C_6H_8N_4$
12b	Methyl	Ethyl	H	A	12	179—181	$C_7H_{10}N_4$
12c	Methyl	*n*-Propyl	H	A	17	196—197	$C_8H_{12}N_4 \cdot 0.1H_2O$
12d	Ethyl	Methyl	H	A	15	206—209	$C_7H_{10}N_4$
12e	Ethyl	Ethyl	H	A	22	164—166	$C_8H_{12}N_4$
12f	Ethyl	*n*-Propyl	H	A	8	161—163	$C_9H_{14}N_4$
12g	Ethyl	*n*-Butyl	H	A	15	144—146	$C_{10}H_{16}N_4$
12h	*n*-Propyl	Methyl	H	A	10	206—207	$C_8H_{12}N_4$
12i	*n*-Propyl	Ethyl	H	A	18	128—130	$C_9H_{14}N_4 \cdot 0.05H_2O$
12j	Methyl	H	Methyl	A	15	258—259 (dec.)	$C_6H_8N_4$
12k	Methyl	H	Ethyl	B	25	202—203	$C_7H_{10}N_4$
12l	Methyl	H	*n*-Propyl	B	16	184—186	$C_8H_{12}N_4$
12m	Ethyl	H	Methyl	A	28	217—218	$C_7H_{10}N_4 \cdot 0.05H_2O$
12n	Ethyl	H	Ethyl	B	45	155—157	$C_8H_{12}N_4 \cdot 0.1H_2O$
12o	Ethyl	H	*n*-Propyl	B	15	153—155	$C_9H_{14}N_4 \cdot 0.05H_2O$
12p	Ethyl	H	*n*-Butyl	B	28	126—128	$C_{10}H_{16}N_4$
12q	*n*-Propyl	H	Methyl	A	18	212—214	$C_8H_{12}N_4$
12r	*n*-Propyl	H	Ethyl	A	19	166—168	$C_9H_{14}N_4$
12s	Ethyl	H	iso-Propyl	B	23	170—173	$C_9H_{14}N_4$
12t	iso-Propyl	H	Ethyl	A	31	191—193	$C_9H_{14}N_4$
12u	Ethyl	Methyl	Ethyl	B	15	171—173	$C_9H_{14}N_4$
12v	Ethyl	Ethyl	Ethyl	A	34	127—129	$C_{10}H_{16}N_4 \cdot 0.1H_2O$
12w	Ethyl	*n*-Propyl	Ethyl	B	12	151—154	$C_{11}H_{18}N_4$
12x	Ethyl	H	H	A	9	146—149	$C_6H_8N_4$

a) Yield calculated from starting 3-amino-1*H*-pyrazoles **6**. *b*) Analytical results were within ±0.4% of the theoretical values. *c*) Ref. 11.



(a) *N*-triphenylmethyl-5-[4′-(bromomethyl)biphenyl-2-yl]tetrazole, *tert*-BuOK, DMF; (b) AcOH, MeOH, reflux

Chart 2

(Fig. 2). On the other hand, NOE was observed between the *N*(5)-CH_2 protons and H-C in **5c**. The NOE study confirmed that *N*(3)-isomers were not formed, though they might theoretically be considered to be formed by the alkylation of 1*H*-pyrazolo[1,5-*b*][1,2,4]triazoles **12**.

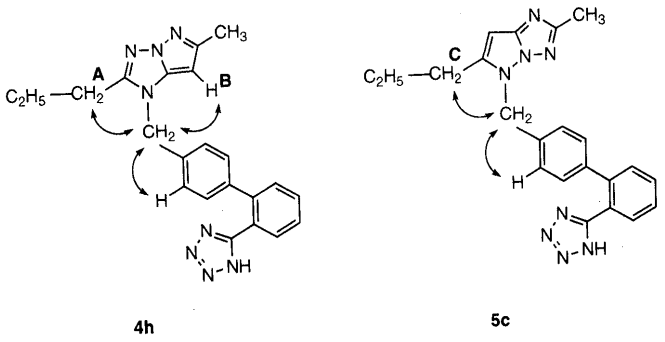


Fig. 2

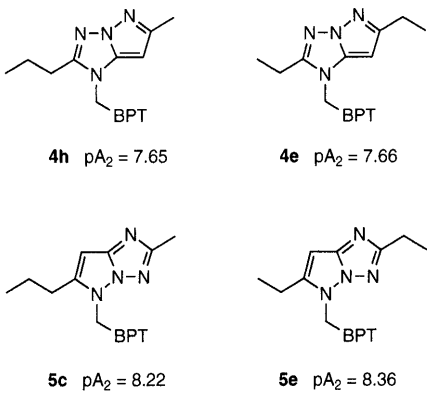
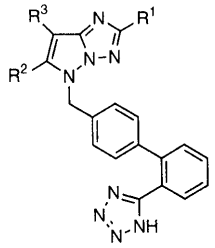


Fig. 3

Table 2. Physical Properties and *in Vitro* AII Antagonistic Potencies of Compounds **5**



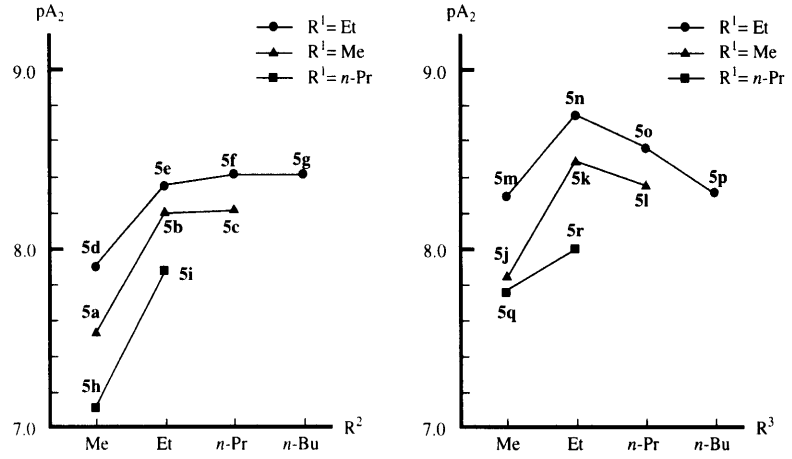
Compd.	R^1	R^2	R^3	Yield (%)[a]	mp (°C)	Formula[b]	pA_2
DuP 753							8.30
5a	Methyl	Methyl	H	25	225—228	$C_{20}H_{18}N_8 \cdot 0.1AcOEt$	7.54
5b	Methyl	Ethyl	H	15	217—219 (dec.)	$C_{21}H_{20}N_8 \cdot 0.45H_2O$	8.21
5c	Methyl	*n*-Propyl	H	12	169—171	$C_{22}H_{22}N_8$	8.22
5d	Ethyl	Methyl	H	25	206—208 (dec.)	$C_{21}H_{20}N_8$	7.91
5e	Ethyl	Ethyl	H	22	180—182	$C_{22}H_{22}N_8 \cdot 0.2H_2O$	8.36
5f	Ethyl	*n*-Propyl	H	19	172—174	$C_{23}H_{24}N_8$	8.42
5g	Ethyl	*n*-Butyl	H	23	183—185	$C_{24}H_{26}N_8$	8.41
5h	*n*-Propyl	Methyl	H	17	175—178	$C_{22}H_{22}N_8 \cdot 0.6H_2O$	7.12
5i	*n*-Propyl	Ethyl	H	25	191—193	$C_{23}H_{24}N_8$	7.88
5j	Methyl	H	Methyl	55	261—265	$C_{20}H_{18}N_8 \cdot 0.5H_2O$	7.84
5k	Methyl	H	Ethyl	36	215—216	$C_{21}H_{20}N_8$	8.50
5l	Methyl	H	*n*-Propyl	22	124—126	$C_{22}H_{22}N_8 \cdot H_2O$[c]	8.36
5m	Ethyl	H	Methyl	55	178—180	$C_{21}H_{20}N_8 \cdot 0.1H_2O$	8.30
5n	Ethyl	H	Ethyl	64	184—186	$C_{22}H_{22}N_8$	8.74
5o	Ethyl	H	*n*-Propyl	57	134—136	$C_{23}H_{24}N_8 \cdot 0.3H_2O$	8.58
5p	Ethyl	H	*n*-Butyl	16	137—139	$C_{24}H_{26}N_8$	8.34
5q	*n*-Propyl	H	Methyl	56	193—196 (dec.)	$C_{22}H_{22}N_8$	7.76
5r	*n*-Propyl	H	Ethyl	59	169—171	$C_{23}H_{24}N_8 \cdot 0.1AcOEt$	8.01
5s	Ethyl	H	iso-Propyl	55	143—145	$C_{23}H_{24}N_8 \cdot 0.2AcOEt$	8.52
5t	iso-Propyl	H	Ethyl	34	110—113	$C_{23}H_{24}N_8 \cdot 0.3H_2O$	8.17
5u	Ethyl	Methyl	Ethyl	64	149—152	$C_{23}H_{24}N_8 \cdot 0.2AcOEt$	8.45
5v	Ethyl	Ethyl	Ethyl	54	207—209 (dec.)	$C_{24}H_{26}N_8$	8.28
5w	Ethyl	*n*-Propyl	Ethyl	48	212—213 (dec.)	$C_{25}H_{28}N_8$	8.24
5x	Ethyl	H	H	18	180—183 (dec.)	$C_{20}H_{18}N_8 \cdot 0.15AcOEt$	7.97

a) Yield calculated from 1*H*-pyrazolo[1,5-*b*][1,2,4]triazoles **12**. *b*) Analytical results were within ±0.4% of the theoretical values unless otherwise noted. *c*) H (Calcd 5.81, Found 5.37).

Results and Discussion

The compounds were tested *in vitro* for the ability to inhibit the AII-induced contraction of rabbit thoracic aorta strips. The pA_2 values were determined in order to compare the relative potencies of the antagonists. For initial evaluation, it was decided to prepare some key compounds (**4h, e, 5c, e**) (Fig. 3). A comparison between *N*(5)-isomers and *N*(1)-isomers, which have the same alkyl groups and BPT group in the same region of space on the head, indicated that the *N*(5)-isomers were rather favorable (pA_2: **5c**, 8.22 *vs.* **4h**, 7.65; **5e**, 8.36 *vs.* **4e**, 7.66), being comparable in potency to DuP 753 (pA_2: DuP 753, 8.30). This preliminary study indicated that imidazole of DuP 753 can be replaced with a suitably alkyl-substituted pyrazolo[1,5-*b*][1,2,4]triazole ring (*e.g.*, *N*(5)-isomers; **5c, e**). Therefore, further work was focused on obtaining the *N*(5)-isomers (**5**).[12]

The *in vitro* pharmacological data for the derivatives are summarized in Table 2. The relationships between the pA_2 values and the alkyl chain length of a series of



(a) 2,6-dialkyl derivatives (**5a-i**) (b) 2,7-dialkyl derivatives (**5j-r**)

Fig. 4. Variation in Potency *in Vitro* with the Alkyl Chain Length on the Pyrazolo[1,5-*b*][1,2,4]triazole Ring

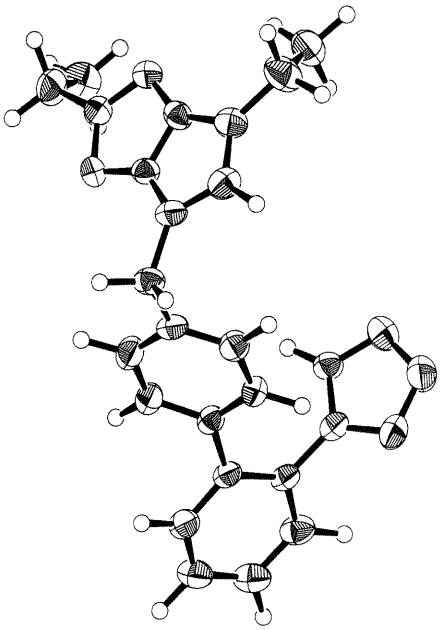


Fig. 5. An ORTEP Drawing of the Molecule of **5n**

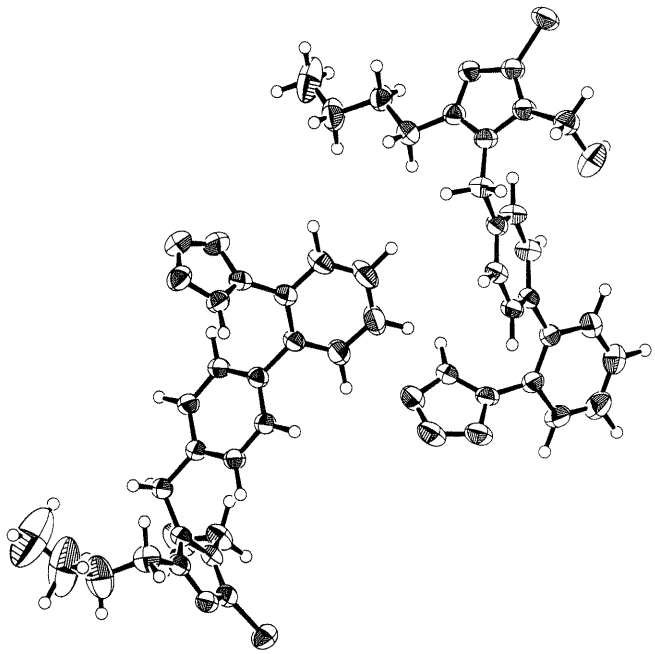


Fig. 6. An ORTEP Drawing of the Molecule of DuP 753

Ethanol solvent of crystallization was eliminated to simplify the figure. Two conformers are present in the crystal lattice; One takes all-*trans* form in the butyl chain (right figure), and another *trans*-gauche form (left figure). Each hydroxy group is placed opposite to the imidazole plane.

2,6-dialkyl derivatives (**5a—i**) and 2,7-dialkyl derivatives (**5j—r**) are shown in Fig. 4. This is helpful to evaluate the most favorable combination of two alkyl groups for the pyrazolo[1,5-*b*][1,2,4]triazole ring.

In the 2,6-dialkyl series (Fig. 4a), the ethyl group appeared to be optimal as the R^1-alkyl group (R^1 = ethyl, **5d—g** compared to R^1 = methyl, **5a—c** or R^1 = *n*-propyl, **5h, i**). The modification of R^2 did not alter the activity, except when R^2 is methyl (**5d**), which seems to be rather unfavorable as compared to the corresponding ethyl, *n*-propyl and *n*-butyl derivatives (**5e—g**). As shown in Fig. 4b, the ethyl group also proved to be optimal as the R^1-alkyl group in the 2,7-dialkyl series (R^1 = ethyl, **5m—p** *vs.* R^1 = methyl, **5j—l** and R^1 = *n*-propyl, **5q, r**), in which the activity varied depending on the length of the R^3-alkyl chain and ethyl gave the maximum pA_2 value of 8.74 (**5n**).

Replacing R^3-ethyl of **5n** with iso-propyl gave **5s** with a small loss of activity. On the other hand, replacing R^1-ethyl of **5n** with iso-propyl led to a compound (**5t**) with *ca.* 4-fold loss of activity, indicating that the lipophilic pocket for the R^1-alkyl group is relatively small. Introduction of R^2-alkyl into **5n** resulted in a decrease of the pA_2 value by 0.29—0.50 as the length of the R^2-alkyl chain was increased (**5u—w**), indicating that steric hindrance between two alkyl groups at the 6- and 7-position is unfavorable. Of note is the finding that removal of the alkyl group from the left part of the head resulted in moderate activity (**5x** *vs. e.g.*, **5e, n**), while in the DuP 753 series the derivative bearing a proton at the 2-position of the imidazole ring is significantly less potent in the binding assay.[8a)]

It was interesting to study the X-ray crystal structure of **5n** in our series (Fig. 5) and to compare it with that of Dup 753 (Fig. 6). Each biphenyl moiety sticks out almost

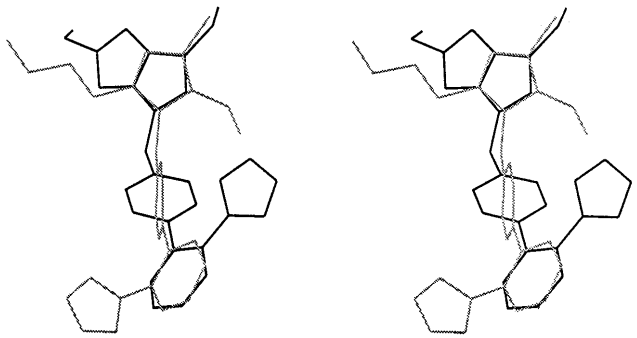
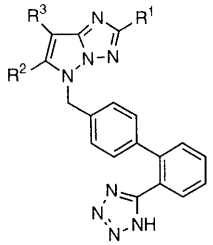


Fig. 7. Superimposition of Crystal Structures of DuP 753 (All-*trans* Form, Gray) and **5n** (Black) (Stereoscopic View)

Table 3. *In Vivo* AII Antagonistic Potencies of Compounds **5**



Compd.	R^1	R^2	R^3	% inhibition[a]			
				1 h	4 h	8 h	12 h
DuP 753				51	88	91	77
5e	Ethyl	Ethyl	H	86	N.T.	8	N.T.
5f	Ethyl	*n*-Propyl	H	76	N.T.	N.T.	N.T.
5g	Ethyl	*n*-Butyl	H	53	N.T.	N.T.	N.T.
5k	Methyl	H	Ethyl	93	81	63	46
5m	Ethyl	H	Methyl	86	53	35	N.T.
5n	Ethyl	H	Ethyl	95	78	72	53
5o	Ethyl	H	*n*-Propyl	96	72	70	21
5p	Ethyl	H	*n*-Butyl	88	22	N.T.	N.T.
5s	Ethyl	H	iso-Propyl	94	84	50	N.T.

a) Percent inhibition of AII-induced pressor response in pithed rats after oral administration of test compounds (30 mg/kg *p.o.*). N.T.: not tested.

orthogonally from the plane of the head. There are two enantiomeric twisted conformations for the biphenyl moiety, and the angles of the benzene planes are −40.1° for **5n**, and 40.7° (all-*trans* form) or 51.4° (*trans*-gauche form) for DuP 753. As shown in Fig. 7, the ethyl group at the 2-position of the pyrazolo[1,5-*b*][1,2,4]triazole ring and the butyl group of the imidazole ring can be placed in the same region of space, while the tetrazole rings orient to opposite sides as a result of the different conformers of the central phenyl ring (lying almost perpendicular). In order to examine available conformers for the central phenyl ring, we have carried out a simple confomational analysis study for **5n** by rotating the bond between the methylene and biphenyl moiety at 3° intervals, using the crystal structure. The calculation was performed using the SYBYL Molecular Modeling Software.[13] Energy calculation for **5n** showed that the bond can rotate 0—360° with an energy cost of less than 2 kcal/mol, easily allowing the compound to adopt a conformation similar to the possible binding conformation of DuP 753. These results and the moderate *in vitro* activity of the mono-ethyl derivative **5x** unexpectedly suggested that the ethyl group at the

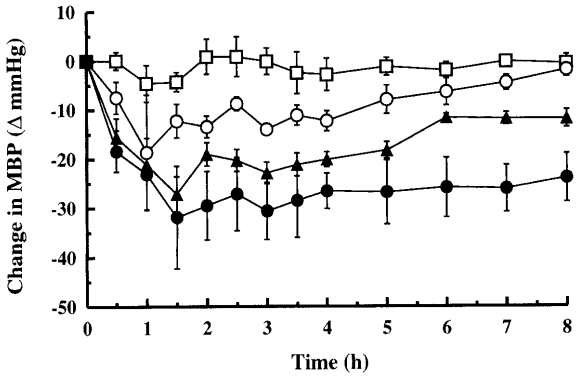


Fig. 8. Oral Hypotensive Activity in Furosemide-Treated Sodium-Depleted Dogs

SEM are indicated for each point. —□— vehicle, $n=4$: —○— **5n**, 10 mg/kg, $n=3$; —●— **5n**, 30 mg/kg, $n=3$; —▲— DuP 753, 30 mg/kg, $n=4$.

2-position of **5n** corresponds to the lipophilic butyl part of DuP753, which is an important structural requirement for AII antagonistic activity. We may also conclude that the ethyl group at the 7-position of **5n**, the chloro group of DuP 753 and the methyl groups of **3a** are accepted by the same lipophilic pocket in the AII receptor.[6]

Some compounds showing high pA_2 value were orally evaluated for inhibition of AII-induced pressor response in pithed rats, and representative results are shown in Table 3. The compounds were given at a dose of 30 mg/kg. Oral potency depends on the alkyl substituents on the pyrazolo[1,5-*b*][1,2,4]triazole ring. 2,7-Dialkyl derivatives proved to be rather more favorable than 2,6-dialkyl derivative (**5k**, **m**—**p**, **s** *vs.* **5e**—**g**) at 1 h after administration of the test compounds. In the 2,7-dialkyl series, the order of activity was ethyl (**5n**) = *n*-propyl (**5o**) > iso-propyl (**5s**) > methyl (**5m**) for R^3-alkyl at 8 h. However, **5o** had a poor potency compared to **5n** at 12 h. In this model, compounds **5** were characterized by the rapid onset of the activity. In contrast, the onset of the activity was slower with DuP 753, presumably due to the delay incurred in the metabolism of DuP 753 to its active component, EXP3174.[14]

Oral hypotensive effects of the optimal compound **5n** was evaluated in furosemide-treated dogs (Fig. 8). Compound **5n** had a dose-dependent hypotensive effect. The values of maximal decrease in mean blood pressure (MBP) at a dose of 30 mg/kg were 32 and 27 mmHg for **5n** and DuP 753, respectively. Compound **5n** showed long-lasting hypotensive activity as compared with DuP 753, with a duration of action of >8 h. In terms of duration of action, DuP 753 appears to be less effective in furosemide-treated dogs than in pithed rats. This species difference of DuP 753 action may involve EXP3174, which is reported to be generated in a lesser amount in dogs than in rats,[15] whereas the influence of metabolism of **5n** in this hypotensive model has not been clarified.

In conclusion, we have identified a novel series of 5*H*-pyrazolo[1,5-*b*][1,2,4]triazole derivatives which are potent and orally active AII antagonists. SAR studies of this series indicate that **5n** is the optimal compound. Based on the results of pharmacokinetic evaluation (data not shown), **5n**-potassium salt (YM358) was selected for further evaluation *in vitro* and *in vivo*. The results will be

reported elsewhere.[16]

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-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%), 3-amino-1*H*-pyrazole and 3-amino-5-methyl-1*H*-pyrazole were purchased from Aldrich Chemical Co. 3-Amino-4-methyl-1*H*-pyrazole was prepared as reported.[17] Other 3-amino-1*H*-pyrazole derivatives **6** were synthesized by the following general method.[18]

General Method for the Synthesis of 3-Amino-1*H*-pyrazole Derivatives (6). 3-Amino-5-ethyl-1*H*-pyrazole[19] A suspension of sodium amide (95%, 39.3 g, 0.96 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. Acetonitrile (39.3 g, 0.96 mol) was added to the stirred suspension over 7 min. After 5 min, methyl propionate (42.0 g, 0.48 mmol) was added over 7 min and stirring was continued for 1 h. Removal of ammonia on a water bath at 40—50 °C under a nitrogen stream afforded a residue, to which was added 300 g of ice–water and 60 ml of diethyl ether. The mixture was acidified with 6 N hydrochloric acid, and the aqueous phase was extracted with 60 ml of diethyl ether. The combined organic extracts were mixed with ethanol (180 ml) and hydrazine hydrate (41.9 g, 0.84 mol), and diethyl ether was distilled off at atmospheric pressure. The resultant ethanol solution was refluxed overnight, then evaporated *in vacuo*. The residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 24 : 1) to give the title compound (27.4 g, 52%) as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, t, J = 7.6 Hz), 2.57 (2H, q, J = 7.6 Hz), 5.36 (3H, br), 5.44 (1H, s). EI-MS m/z: 111 (M^+).

Using this procedure, the following 3-amino-1*H*-pyrazole derivatives were synthesized.

3-Amino-5-*n*-propyl-1*H*-pyrazole[18]: ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, t, J = 7.2 Hz), 1.63 (2H, m), 2.52 (2H, t, J = 7.5 Hz), 5.15 (3H, br), 5.44 (1H, s). EI-MS m/z: 125 (M^+).

3-Amino-5-*n*-butyl-1*H*-pyrazole[20]: ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.80 (7H, m), 2.53 (2H, t, J = 7.4 Hz), 5.43 (1H, s), 5.56 (3H, br). EI-MS m/z: 139 (M^+).

3-Amino-4-ethyl-1*H*-pyrazole[21]: ^{1}H-NMR ($CDCl_3$) δ: 1.16 (3H, t, J = 7.4 Hz), 2.31 (2H, q, J = 7.4 Hz), 5.83 (3H, br), 7.10 (1H, s). EI-MS m/z: 111 (M^+).

3-Amino-4-*n*-propyl-1*H*-pyrazole: ^{1}H-NMR ($CDCl_3$) δ: 0.93 (3H, t, J = 7.2 Hz), 1.37 (2H, m), 2.34 (2H, t, J = 7.5 Hz), 5.93 (3H, br), 7.09 (1H, s). EI-MS m/z: 125 (M^+).

3-Amino-4-*n*-butyl-1*H*-pyrazole: ^{1}H-NMR ($CDCl_3$) δ: 0.80—1.80 (7H, m), 2.30 (2H, t, J = 7.4 Hz), 5.46 (3H, br), 7.10 (1H, s). EI-MS m/z: 139 (M^+).

3-Amino-4-iso-propyl-1*H*-pyrazole: ^{1}H-NMR ($CDCl_3$) δ: 1.18 (6H, d, J = 6.6 Hz), 2.68 (1H, m), 5.80 (3H, br), 7.10 (1H, s). EI-MS m/z: 125 (M^+).

3-Amino-4-ethyl-5-methyl-1*H*-pyrazole: ^{1}H-NMR ($CDCl_3$) δ: 1.08 (3H, t, J = 7.7 Hz), 2.13 (3H, s), 2.30 (2H, q, J = 7.7 Hz), 5.53 (3H, br). EI-MS m/z: 125 (M^+).

3-Amino-4,5-diethyl-1*H*-pyrazole: ^{1}H-NMR ($CDCl_3$) δ: 1.09 (3H, t, J = 7.6 Hz), 1.20 (3H, t, J = 7.6 Hz), 2.32 (2H, q, J = 7.6 Hz), 2.54 (2H, q, J = 7.6 Hz), 5.29 (3H, br). EI-MS m/z: 139 (M^+).

3-Amino-4-ethyl-5-*n*-propyl-1*H*-pyrazole: ^{1}H-NMR ($CDCl_3$) δ: 0.86—1.17 (6H, m), 1.60 (2H, m), 2.32 (2H, q, J = 7.7 Hz), 2.49 (2H, t, J = 7.6 Hz), 5.29 (3H, br). EI-MS m/z: 153 (M^+).

General Method A for the Synthesis of 1*H*-Pyrazolo[1,5-*b*][1,2,4]triazoles (12). 2,6-Diethyl-1*H*-pyrazolo[1,5-*b*][1,2,4]triazole (12e) Ethyl propionimidate hydrochloride (18.9 g, 0.14 mol) was added to a solution of 3-amino-5-ethyl-1*H*-pyrazole (13.5 g, 0.12 mol) in acetonitrile (100 ml) with ice-cooling, and the mixture was stirred overnight at room temperature. The mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 9 : 1—4 : 1) to give 14.3 g (58%) of the crude *N*-(5-ethyl-1*H*-pyrazol-3-yl)propionamidine hydrochloride **8e**. EI-MS m/z: 166 (M^+).

Hydroxylamine hydrochloride (6.75 g, 97.1 mmol) was added to a solution of sodium (2.33 g, 0.10 mol) in MeOH (70 ml) with ice-cooling, and the mixture was stirred for 3 h at room temperature. After removal of the salt by filtration, the filtrate was added to a solution of **8e** (14.2 g, 70.1 mmol) in MeOH (70 ml) in a dropwise manner with ice-cooling. The mixture was stirred overnight at room temperature and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 23 : 2) to give *N*-hydroxy-*N'*-(5-ethyl-1*H*-pyrazol-3-yl)propionamidine **9e** (10.3 g, 81%) as a solid. ^{1}H-NMR (DMSO-d_6) δ: 0.96 (3H, t, J = 7.1 Hz), 1.16 (3H, t, J = 7.6 Hz), 2.31—2.65 (4H, m), 5.73 (1H, s). EI-MS m/z: 182 (M^+).

p-Toluenesulfonyl chloride (10.7 g, 56.1 mmol) was added to a solution of **9e** (10.2 g, 56.0 mmol) and pyridine (4.46 g, 56.4 mmol) in *N*,*N*-dimethylacetamide (50 ml) with ice-cooling, and the mixture was stirred for 1 h at room temperature. The reaction mixture was diluted with water (400 ml) and extracted with $CHCl_3$ (400 ml). Removal of $CHCl_3$ under reduced pressure afforded a residue, which contained *N*,*N*-dimethylacetamide and was refluxed for 2 h in MeOH (250 ml) with pyridine (4.46 g, 56.4 mmol). The mixture was evaporated *in vacuo* and the residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 24 : 1) to give a crystalline product, which was washed with diisopropylether to give 4.38 g (47%) of **12e**. mp 164—166 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.19 (3H, t, J = 7.6 Hz), 1.27 (3H, t, J = 7.6 Hz), 2.48—2.85 (4H, m), 5.55 (1H, s), 12.28 (1H, br). EI-MS m/z: 164 (M^+). *Anal.* Calcd for $C_8H_{12}N_4$: C, 58.52; H, 7.37; N, 34.12. Found: C, 58.42; H, 7.33; N, 34.25.

General Method B for the Synthesis of 1*H*-Pyrazolo[1,5-*b*][1,2,4]triazoles (12). 2,7-Diethyl-1*H*-pyrazolo[1,5-*b*][1,2,4]triazole (12n) A solution of 3-amino-4-ethyl-1*H*-pyrazole (5.00 g, 45.0 mmol) and triethyl orthopropionate (9.05 ml, 45.0 mmol) in toluene (50 ml) was refluxed for 18 h. Removal of the solvent under reduced pressure afforded the crude ethyl *N*-(4-ethyl-1*H*-pyrazol-3-yl)propionimidate (**11n**), which, without further purification, was treated with a solution of hydroxylamine (67.5 mmol) in MeOH (100 ml). The mixture was stirred for 90 min at room temperature and concentrated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 93 : 7) to give *N*-hydroxy-*N'*-(4-ethyl-1*H*-pyrazol-3-yl)propionamidine (**9n**) (7.46 g, 91%). ^{1}H-NMR (DMSO-d_6) δ: 0.86 (3H, t, J = 7.5 Hz), 1.09 (3H, t, J = 7.5 Hz), 2.08—2.43 (4H, m), 7.40 (1H, s). EI-MS m/z: 182 (M^+).

9n (7.41 g, 40.7 mmol) was led to **12n** (3.29 g, 49%) in a similar manner to that described in the preparation of **12e**.

12n: mp 155—157 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.19 (3H, t, J = 7.6 Hz), 1.28 (3H, t, J = 7.6 Hz), 2.50 (2H, q, J = 7.6 Hz), 2.74 (2H, q, J = 7.6 Hz), 7.21 (1H, s), 12.35 (1H br). EI-MS m/z: 164 (M^+). *Anal.* Calcd for $C_8H_{12}N_4 \cdot 0.1H_2O$: C, 57.88; H, 7.41; N, 33.75. Found: C, 57.76; H, 7.32; N, 33.72.

All compounds of formula **12** were prepared from the corresponding 3-aminopyrazole derivatives **6**, in a similar manner to that described for **12e** (method A) or **12n** (method B), and are listed in Tables 1 and 4.

2,6-Diethyl-1-[[2'-[*N*-(triphenylmethyl)tetrazol-5-yl]biphenyl-4-yl]-methyl]-1*H*-pyrazolo[1,5-*b*][1,2,4]triazole (13e) and 2,6-Diethyl-5-[[2'-[*N*-(triphenylmethyl)tetrazol-5-yl]biphenyl-4-yl]methyl]-5*H*-pyrazolo-[1,5-*b*][1,2,4]triazole (14e) **12e** (2.00 g, 12.2 mmol) was added to a suspension of potassium *tert*-butoxide (1.43 g, 12.7 mmol) in DMF (80 ml) with ice-cooling. The mixture was stirred for 15 min at the same temperature, then *N*-triphenylmethyl-5-[4'-(bromomethyl)biphenyl-2-yl]tetrazole (7.47 g, 13.4 mmol) was added and the whole was allowed to warm to room temperature. The mixture was stirred overnight, then concentrated under reduced pressure. Water was added to the residue and the whole was extracted with AcOEt. The organic layer was washed with water, dried over $MgSO_4$ and evaporated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane : AcOEt = 13 : 7—2 : 3) to give 2.60 g (33%) of **13e** and 2.04 g (26%) of **14e**, each as a foam.

13e: ^{1}H-NMR ($CDCl_3$) δ: 1.24 (3H, t, J = 7.6 Hz), 1.29 (3H, t, J = 7.6 Hz), 2.64 (2H, q, J = 7.6 Hz), 2.69 (2H, q, J = 7.6 Hz), 4.92 (2H, s), 5.25 (1H, s), 6.91—6.93 (8H, m), 7.12—7.52 (14H, m), 7.93—7.96 (1H, m). FAB-MS m/z: 641 ($M+H)^+$.

14e: ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, t, J = 7.5 Hz), 1.38 (3H, t, J = 7.5 Hz), 2.53 (2H, q, J = 7.5 Hz), 2.85 (2H, q, J = 7.5 Hz), 5.30 (2H, s), 5.98 (1H, s), 6.87—6.93 (8H, m), 7.09 (2H, d, J = 7.8 Hz), 7.23—7.50 (12H, m), 7.92 (1H, d, J = 7.8 Hz). FAB-MS m/z: 641 ($M+H)^+$.

Table 4. Physical Data for Compounds **12**

Compd.	^{1}H-NMR δ (in DMSO-d_6, J in Hz)	MS m/z (M^+)	Analysis (%) Calcd (Found)		
			C	H	N
12a	2.22 (3H, s), 2.37 (3H, s), 5.51 (1H, s), 12.24 (1H, br)	136	52.93	5.92	41.15
			(52.79	5.92	41.44)
12b	1.19 (3H, t, J=7.6), 2.37 (3H, s), 2.58 (2H, q, J=7.6), 5.53 (1H, s), 12.27 (1H, br)	150	55.98	6.71	37.31
			(55.91	6.74	37.53)
12c	0.91 (3H, t, J=7.3), 1.62 (2H, m), 2.37 (3H, s), 2.53 (2H, t, J=7.3), 5.53 (1H, s), 12.24 (1H, br)	164	57.88	7.41	33.75
			(57.98	7.19	33.86)
12d	1.26 (3H, t, J=7.6), 2.22 (3H, s), 2.73 (2H, q, J=7.6), 5.51 (1H, s), 12.24 (1H, br)	150	55.98	6.71	37.31
			(55.87	6.76	37.57)
12e	1.19 (3H, t, J=7.6), 1.27 (3H, t, J=7.6), 2.48—2.85 (4H, m), 5.55 (1H, s), 12.28 (1H, br)	164	58.52	7.37	34.12
			(58.42	7.33	34.25)
12f	0.91 (3H, t, J=7.3), 1.26 (3H, t, J=7.6), 1.61 (2H, m), 2.53 (2H, t, J=7.3), 2.72 (2H, q, J=7.6), 5.52 (1H, s), 12.26 (1H, br)	178	60.65	7.92	31.43
			(60.49	7.89	31.35)
12g	0.89 (3H, t, J=7.3), 1.27 (3H, t, J=7.6), 1.33 (2H, m), 1.59 (2H, m), 2.56 (2H, t, J=7.6), 2.73 (2H, q, J=7.6), 5.53 (1H, s), 12.29 (1H, br)	192	62.47	8.39	29.14
			(62.35	8.49	29.29)
12h	0.93 (3H, t, J=7.3), 1.71 (2H, m), 2.22 (3H, s), 2.67 (2H, t, J=7.3), 5.51 (1H, s), 12.22 (1H, br)	164	58.52	7.37	34.12
			(58.43	7.35	34.16)
12i	0.94 (3H, t, J=7.3), 1.19 (3H, t, J=7.5), 1.71 (2H, m), 2.59 (2H, q, J=7.5), 2.68 (2H, t, J=7.3), 5.54 (1H, s), 12.28 (1H, br)	178	60.34	7.93	31.28
			(60.16	8.02	31.49)
12j	2.07 (3H, s), 2.38 (3H, s), 7.19 (1H, s), 12.35 (1H, br)	136	52.93	5.92	41.15
			(52.92	6.01	40.99)
12k	1.19 (3H, t, J=7.6), 2.38 (3H, s), 2.49 (2H, q, J=7.6), 7.20 (1H, s), 12.35 (1H, br)	150	55.98	6.71	37.31
			(55.70	6.59	37.22)
12l	0.90 (3H, t, J=7.2), 1.59 (2H, m), 2.32—2.58 (5H, m), 7.20 (1H, s), 12.36 (1H, br)	164	58.52	7.37	34.12
			(58.37	7.39	33.89)
12m	1.27 (3H, t, J=7.6), 2.08 (3H, s), 2.74 (2H, q, J=7.6), 7.19 (1H, s), 12.31 (1H, br)	150	55.65	6.74	37.08
			(55.65	6.75	36.81)
12n	1.19 (3H, t, J=7.6), 1.28 (3H, t, J=7.6), 2.50 (2H, q, J=7.6), 2.74 (2H, q, J=7.6), 7.21 (1H, s), 12.35 (1H, br)	164	57.88	7.41	33.75
			(57.76	7.32	33.72)
12o	0.90 (3H, t, J=7.2), 1.28 (3H, t, J=7.6), 1.37—1.80 (2H, m), 2.46 (2H, t, J=7.4), 2.75 (2H, q, J=7.6), 7.20 (1H, s), 12.32 (1H, br)	178	60.34	7.93	31.28
			(60.35	7.96	30.95)
12p	0.90 (3H, t, J=6.4), 1.19—1.65 (7H, m), 2.47 (2H, t, J=7.3), 2.74 (2H, q, J=7.5), 7.19 (1H, s), 12.30 (1H, br)	192	62.47	8.39	29.14
			(62.21	8.43	29.44)
12q	0.94 (3H, t, J=7.3), 1.72 (2H, m), 2.08 (3H, s), 2.69 (2H, t, J=7.3), 7.18 (1H, s), 12.30 (1H, br)	164	58.52	7.37	34.12
			(58.39	7.34	34.10)
12r	0.95 (3H, t, J=7.3), 1.20 (3H, t, J=7.5), 1.74 (2H, m), 2.50 (2H, q, J=7.5), 2.70 (2H, t, J=7.3), 7.21 (1H, s), 12.33 (1H, br)	178	60.65	7.92	31.43
			(60.49	7.91	31.37)
12s	1.20—1.37 (9H, m), 2.37—3.02 (3H, m), 7.21 (1H, s), 12.31 (1H, br)	178	60.65	7.92	31.43
			(60.64	7.99	31.40)
12t	1.20 (3H, t, J=7.5), 1.32 (6H, d, J=7.0), 2.51 (2H, q, J=7.5), 3.07 (1H, m), 7.22 (1H, s), 12.28 (1H, br)	178	60.65	7.92	31.43
			(60.50	7.69	31.39)
12u	1.16 (3H, t, J=7.6), 1.26 (3H, t, J=7.6), 2.15 (3H, s), 2.44 (2H, q, J=7.6), 2.71 (2H, q, J=7.6), 12.12 (1H, br)	178	60.65	7.92	31.43
			(60.45	7.88	31.72)
12v	1.08—1.36 (9H, m), 2.33—2.85 (6H, m), 12.16 (1H, br)	192	61.89	8.41	28.87
			(61.95	8.50	28.86)
12w	0.90 (3H, t, J=7.3), 1.16 (3H, t, J=7.7), 1.26 (3H, t, J=7.7), 1.57 (2H, m), 2.32—2.84 (4H, m), 12.11 (1H, br)	206	64.05	8.79	27.16
			(63.86	9.03	27.38)
12x	1.28 (3H, t, J=7.6), 2.77 (2H, q, J=7.6), 5.74 (1H, d, J=2.3), 7.40 (1H, d, J=2.3), 12.43 (1H, br)	136	52.93	5.92	41.15
			(53.04	5.93	41.17)

2,6-Diethyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-pyrazolo[1,5-*b*][1,2,4]triazole (4e) A solution of **13e** (1.03 g, 1.61 mmol) and acetic acid (6 ml) in MeOH (54 ml) was refluxed for 3 h and concentrated under reduced pressure. Toluene was added to the residue and evaporated *in vacuo*, and this was repeated 3 times. The resulting residue was crystallized from AcOEt to give **4e** (0.58 g, 91%). mp 198—200 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.16 (3H, t, J=7.6 Hz), 1.24 (3H, t, J=7.4 Hz), 2.57 (2H, q, J=7.6 Hz), 2.83 (2H, q, J=7.4 Hz), 5.21 (2H, s), 5.32 (1H, s), 7.11 (2H, d, J=8.2 Hz), 7.26 (2H, d, J=8.2 Hz), 7.54—7.70 (4H, m). FAB-MS m/z: 399 $(M+H)^+$. *Anal.* Calcd for $C_{22}H_{22}N_8 \cdot 0.1H_2O$: C, 66.02; H, 5.59; N, 27.99. Found: C, 66.18; H, 5.63; N, 27.71.

6-Methyl-2-*n*-propyl-1-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-1*H*-pyrazolo[1,5-*b*][1,2,4]triazole (4h) The preparation of **4h** was carried out from 6-methyl-2-*n*-propyl-1*H*-pyrazolo[1,5-*b*][1,2,4]triazole (**12h**) according to the procedure described for **4e**.

4h: mp 156—157 °C. ^{1}H-NMR (DMSO-d_6) δ: 0.96 (3H, t, J=7.3 Hz), 1.68 (2H, m), 2.20 (3H, s), 2.78 (2H, t, J=7.3 Hz), 5.21 (2H, s), 5.26 (1H, s), 7.11 (2H, d, J=7.9 Hz), 7.24 (2H, d, J=7.9 Hz), 7.54—7.70 (4H, m). FAB-MS m/z: 399 $(M+H)^+$. *Anal.* Calcd for $C_{22}H_{22}N_8$: C, 66.31; H, 5.56; N, 28.12. Found: C, 66.36; H, 5.74; N, 27.74.

2,6-Diethyl-5-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-5*H*-pyrazolo[1,5-*b*][1,2,4]triazole (5e) The preparation of **5e** was carried out from **14e** according to the procedure described for **4e**.

5e: mp 180—182 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.19—1.24 (6H, m), 2.64 (2H, q, J=7.6 Hz), 2.72 (2H, q, J=7.5 Hz), 5.49 (2H, s), 6.06 (1H, s), 7.06 (2H, d, J=9.0 Hz), 7.09 (2H, d, J=9.0 Hz), 7.50—7.68 (4H, m). FAB-MS m/z: 399 $(M+H)^+$. *Anal.* Calcd for $C_{22}H_{22}N_8 \cdot 0.2H_2O$: C, 65.92; H, 5.62; N, 27.87. Found: C, 65.68; H, 5.48; N, 27.80.

All compounds of formula **5** were synthesized from the corresponding **12** according to the procedure described for **5e**, and are listed in Tables 2 and 5.

X-Ray Crystallographic Analysis of 5n Suitable crystals ($C_{22}H_{22}N_8$) for X-ray diffraction studies were formed from MeOH. Crystal data: crystal system, orthorhombic; space group, $P2_12_12_1$(#19); lattice para-

Table 5. Physical Data for Compounds **5**

Compd.	^{1}H-NMR δ (in DMSO-d_6, J in Hz)	MS m/z ($M+H^+$)	Analysis (%) Calcd (Found)		
			C	H	N
5a	2.27 (3H, s), 2.39 (3H, s), 5.47 (2H, s), 6.04 (1H, s), 7.07 (2H, d, J=8.3), 7.10 (2H, d, J=8.3), 7.51—7.68 (4H, m)	371	64.61 (64.39)	5.00 5.02	29.55 29.41)
5b	1.21 (3H, t, J=7.3), 2.28 (3H, s), 2.72 (2H, q, J=7.3), 5.47 (2H, s), 6.05 (1H, s), 7.06 (4H, s), 7.50—7.65 (4H, m)	385	64.25 (64.56)	5.37 5.28	28.55 28.16)
5c	0.93 (3H, t, J=7.3), 1.61 (2H, m), 2.28 (3H, s), 2.69 (2H, t, J=7.7), 5.48 (2H, s), 6.06 (1H, s), 7.06 (4H, s), 7.51—7.68 (4H, m)	399	66.31 (66.08)	5.56 5.53	28.12 27.74)
5d	1.21 (3H, t, J=7.6), 2.38 (3H, s), 2.63 (2H, q, J=7.6), 5.47 (2H, s), 6.03 (1H, s), 7.06 (2H, d, J=7.9), 7.10 (2H, d, J=7.9), 7.51—7.68 (4H, m)	385	65.61 (65.41)	5.24 5.18	29.15 28.85)
5e	1.19—1.24 (6H, m), 2.64 (2H, q, J=7.6), 2.72 (2H, q, J=7.5), 5.49 (2H, s), 6.06 (1H, s), 7.06 (2H, d, J=9.0), 7.09 (2H, d, J=9.0), 7.50—7.68 (4H, m)	399	65.92 (65.68)	5.62 5.48	27.87 27.80)
5f	0.93 (3H, t, J=7.3), 1.21 (3H, t, J=7.6), 1.61 (2H, m), 2.60—2.70 (4H, m), 5.48 (2H, s), 6.06 (1H, s), 7.06 (4H, d, J=1.5), 7.51—7.69 (4H, m)	413	66.97 (66.80)	5.86 5.87	27.16 27.12)
5g	0.88 (3H, t, J=7.3), 1.21 (3H, t, J=7.6), 1.35 (2H, m), 1.57 (2H, m), 2.63 (2H, q, J=7.6), 2.70 (2H, t, J=7.8), 5.49 (2H, s), 6.06 (1H, s), 7.07 (4H, s), 7.50—7.68 (4H, m)	427	67.58 (67.52)	6.14 6.10	26.27 26.15)
5h	0.90 (3H, t, J=7.3), 1.67 (2H, m), 2.38 (3H, s), 2.58 (2H, t, J=7.3), 5.46 (2H, s), 6.02 (1H, s), 7.06 (2H, d, J=7.9), 7.09 (2H, d, J=7.9), 7.48—7.65 (4H, m)	399	64.56 (64.48)	5.71 5.61	27.38 27.38)
5i	0.90 (3H, t, J=7.4), 1.21 (3H, t, J=7.4), 1.67 (2H, m), 2.58 (2H, t, J=7.4), 2.72 (2H, q, J=7.4), 5.48 (2H, s), 6.06 (1H, s), 7.05 (2H, d, J=8.4), 7.08 (2H, d, J=8.4), 7.50—7.68 (4H, m)	413	66.97 (66.97)	5.86 5.92	27.16 26.98)
5j	2.08 (3H, s), 2.28 (3H, s), 5.35 (2H, s), 7.06 (2H, d, J=7.8), 7.17 (2H, d, J=7.8), 7.50—7.66 (5H, m)	371	63.31 (63.67)	5.05 5.13	29.53 29.14)
5k	1.21 (3H, t, J=7.5), 2.28 (3H, s), 2.51 (2H, q, J=7.5), 5.36 (2H, s), 7.06 (2H, d, J=8.3), 7.19 (2H, d, J=8.3), 7.51—7.68 (5H, m)	385	65.61 (65.49)	5.24 5.29	29.15 28.85)
5l	0.89 (3H, t, J=7.3), 1.63 (2H, m), 2.28 (3H, s), 2.46 (2H, t, J=7.6), 5.35 (2H, s), 7.06 (2H, d, J=8.3), 7.17 (2H, d, J=8.3), 7.50—7.68 (5H, m)	399	63.45 (63.28)	5.81 5.37	26.91 26.89)
5m	1.22 (3H, t, J=7.6), 2.09 (3H, s), 2.64 (2H, q, J=7.6), 5.35 (2H, s), 7.06 (2H, d, J=7.8), 7.19 (2H, d, J=7.8), 7.51—7.68 (5H, m)	385	65.55 (65.15)	5.25 5.34	29.01 29.12)
5n	1.21—1.27 (6H, m), 2.52 (2H, q, J=7.5), 2.65 (2H, q, J=7.7), 5.36 (2H, s), 7.07 (2H, d, J=8.6), 7.21 (2H, d, J=8.6), 7.51—7.67 (5H, m)	399	66.31 (66.25)	5.56 5.71	28.12 27.86)
5o	0.90 (3H, t, J=7.3), 1.21 (3H, t, J=7.6), 1.63 (2H, m), 2.47 (2H, t, J=7.3), 2.63 (2H, q, J=7.6), 5.35 (2H, s), 7.06 (2H, d, J=8.1), 7.18 (2H, d, J=8.1), 7.51—7.68 (5H, m)	413	66.10 (66.10)	5.93 5.84	26.81 26.59)
5p	0.89 (3H, t, J=7.3), 1.21 (3H, t, J=7.6), 1.31 (2H, m), 1.60 (2H, m), 2.49 (2H, t, J=7.8), 2.64 (2H, q, J=7.6), 5.35 (2H, s), 7.06 (2H, d, J=8.3), 7.18 (2H, d, J=8.3), 7.51—7.68 (5H, m)	427	67.58 (67.26)	6.14 6.01	26.27 26.15)
5q	0.90 (3H, t, J=7.3), 1.67 (2H, m), 2.09 (3H, s), 2.58 (2H, t, J=7.3), 5.35 (2H, s), 7.05 (2H, d, J=8.2), 7.18 (2H, d, J=8.2), 7.50—7.68 (5H, m)	399	66.31 (66.13)	5.56 5.57	28.12 28.04)
5r	0.90 (3H, t, J=7.6), 1.21 (3H, t, J=7.4), 1.67 (2H, m), 2.51 (2H, q, J=7.4), 2.58 (2H, t, J=7.3), 5.35 (2H, s), 7.05 (2H, d, J=8.3), 7.19 (2H, d, J=8.3), 7.50—7.68 (5H, m)	413	66.71 (66.74)	5.93 5.98	26.60 26.56)
5s	1.20—1.26 (9H, m), 2.64 (2H, q, J=7.6), 2.89 (1H, m), 5.35 (2H, s), 7.07 (2H, d, J=8.1), 7.20 (2H, d, J=8.1), 7.51—7.68 (5H, m)	413	66.46 (66.65)	6.00 5.97	26.05 25.87)
5t	1.21 (3H, t, J=7.6), 1.25 (6H, d, J=6.8), 2.52 (2H, q, J=7.6), 2.96 (1H, m), 5.35 (2H, s), 7.08 (2H, d, J=8.2), 7.21 (2H, d, J=8.2), 7.51—7.68 (5H, m)	413	66.10 (65.93)	5.93 5.77	26.81 26.87)
5u	1.61—1.24 (6H, m), 2.30 (3H, s), 2.49 (2H, q, J=7.5), 2.63 (2H, q, J=7.6), 5.41 (2H, s), 7.05 (2H, d, J=8.6), 7.08 (2H, d, J=8.6), 7.50—7.68 (4H, m)	413	66.46 (66.53)	6.00 5.99	26.05 26.06)
5v	1.05—1.24 (9H, m), 2.51 (2H, q, J=7.5), 2.63 (2H, q, J=7.6), 2.72 (2H, q, J=7.5), 5.43 (2H, s), 7.05 (4H, s), 7.50—7.68 (4H, m)	427	67.58 (67.85)	6.14 6.21	26.27 26.13)
5w	0.89 (3H, t, J=7.3), 1.19—1.23 (6H, m), 1.46 (2H, m), 2.48—2.70 (6H, m), 5.41 (2H, s), 7.04 (4H, t, J=9.0), 7.50—7.68 (4H, m)	441	68.16 (68.20)	6.41 6.50	25.43 25.21)
5x	1.21 (3H, t, J=7.5), 2.64 (2H, q, J=7.5), 5.46 (2H, s), 6.20 (1H, d, J=3.4), 7.07 (2H, d, J=8.3), 7.20 (2H, d, J=8.3), 7.51—7.68 (4H, m), 7.92 (1H, d, J=3.4)	371	64.50 (64.19)	5.04 5.03	29.21 29.33)

meters, a=14.5251(9) Å, b=16.516(2) Å, c=8.2121(9) Å, V=1970.1(3) Å^3; $D_{calc.}$, 1.343 g/cm^3; Z value, 4; F_{000}, 840.00; final R value, R=0.061, R_w=0.096.

X-Ray Crystallographic Analysis of DuP 753 Suitable crystals ($C_{22}H_{23}ClN_6O \cdot 0.5EtOH$) for X-ray diffraction studies were formed from EtOH–AcOEt. Crystal data: crystal system, triclinic; space group, $P\bar{1}$(#2); lattice parameters, a=11.890(2) Å, b=18.118(2) Å, c=11.710(2) Å, α=105.018(9)°, β=91.35(1)°, γ=102.065(9)°, V=2374.8(5) Å^3; $D_{calc.}$, 1.183 g/cm^3; Z value, 4; F_{000}, 888.00; final R value, R=0.084, R_w=0.150.

Molecular Modeling Conformational energy calculations were performed using the SYBYL molecular modeling software,[13] running on an IRIS workstation.

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 gas. 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 washed twice and allowed to relax to the baseline tension. Each strip was then incubated for 30 min with several concentrations of the test compounds 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. EC_{50} (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 $K_B = (\text{molar concentration of antagonist})/([(EC_{50}$ with antagonist$/EC_{50}$ without antagonist$) - 1])$.

Inhibition of Pressor Response to AII in Pithed Rats Male Wistar rats weighing 250 to 400 g (12 to 22 weeks old) were given an oral dose of test compounds (30 mg/kg). At set times after dosing, the rats were anesthetized with ether and pithed by inserting a steel rod through the orbit and foramen magnus down into the spinal canal. Immediately after pithing, the rats were vagotomized bilaterally at the neck and artificially ventilated with room air with a tidal volume of 1 ml/100 g body weight at a rate of 50 breaths/min using a rodent respirator (Shinano, Japan). Blood pressure was measured at the left carotid artery *via* a pressure transducer (Nihon Kohden, Japan) and recorded on a polygraph recorder (Nihon Kohden, Japan). The left femoral vein was cannulated for intravenous administration of AII (1 μg/kg). The pressor response to AII was measured at set times. One animal was used once for each drug. In a separate experiment, the control response to AII was obtained in non-treated animals. Percent inhibition was calculated by the following formula: [(control response − response at set time)/(control response)] × 100.

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 conditions, 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 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 test compounds, 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 was measured before and after oral administration of **5n** (10 mg/kg and 30 mg/kg) or DuP 753 (30 mg/kg).

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References and Notes

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