Synthesis and Evaluation of Novel Pyrazolo[1,5-*a*]pyrimidine Derivatives as Nonpeptide Angiotensin II Receptor Antagonists

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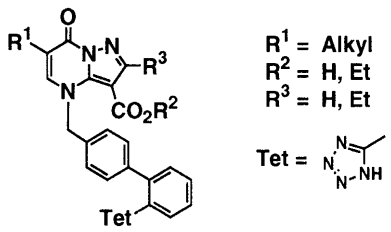
A novel series of 6-alkyl-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carboxylic acid derivatives was prepared as angiotensin II (AII) receptor antagonists. When evaluated in an *in vitro* binding assay using COS cells transfected with a cDNA encoding a human AT_1 angiotensin II receptor, the compounds in this series showed K_i values in the range of 0.4—4.0 nM. In anesthetized spontaneously hypertensive rats (SHRs), administration of the 6-propyl derivative 4d (1 mg/kg, i.v.) reduced the mean blood pressure (MBP) by a maximum of more than 30 mmHg from the normal value.

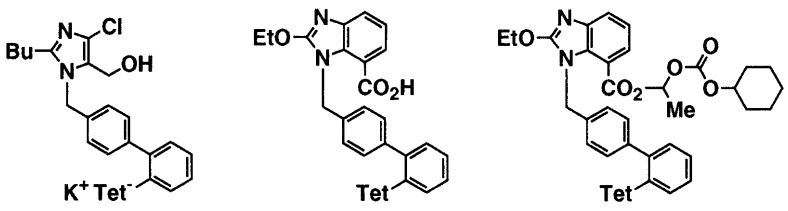
Key words pyrazolo[1,5-*a*]pyrimidine; angiotensin II; receptor antagonist; antihypertensive agent; synthesis

Angiotensin converting enzyme (ACE) inhibitors are widely used for the clinical treatment of hypertension and cardiac failure. However, ACE inhibitors also block the metabolism of bradykinin, causing side effects such as dry coughing. Angiotensin II (AII) receptor antagonists (AII antagonists) are therefore of interest as a potential new class of drugs that would interact with the renin–angiotensin system, since AII antagonists blocking the final action site in the system are expected not to have the undesirable side effects.[1] The search for nonpeptidic AII antagonists was started from a weakly active compound patented by Takeda in 1982.[2] Since DuPont reported the first orally active AII antagonist, DuP 753 (losartan), various nonpeptidic antagonists have been described.[3] Recently, Takeda has disclosed a more potent compound, CV-11974, and its prodrug TCV-116.[4] Structure–activity relationship investigations of CV-11974 showed that a carboxyl group at the 7-position of benzimidazole scarcely contributed to the binding affinity, but played an important role in the antagonistic activity.[4] These results suggest that an ionic interaction of the carboxyl group with a supposed cationic site of the AII receptor would act as a secondary factor controlling potent and long-lasting antagonistic activity, rather than being a crucial factor for the strong receptor binding. We have searched for another heterocyclic system which might be able to place a carboxyl group appropriately at the cationic site. In this study, a new series of 3-carboxypyrazolo[1,5-*a*]-pyrimidine derivatives were designed and synthesized, to see whether the carboxyl group on the heterocycle has a similar effect.

Chemistry

Target compounds **4a**—**g** were synthesized as shown in Chart 1. We chose various aliphatic esters as starting materials for the synthesis of the pyrazolo[1,5-*a*]pyrimidine rings. Initially, these esters were formylated with ethyl formate using two equivalents of potassium *tert*-butoxide as a base. Without purification, the formyl intermediates were reacted with ethyl 3-amino-4-pyrazolecarboxylate[5] to afford the corresponding ethyl 6-alkyl-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carboxylates **1a**—**d**, **f**, **g**. The 2-ethyl derivative **1e** was also prepared using ethyl 3-amino-5-ethyl-4-pyrazolecarboxylate. Their





DuP 753 (losartan) CV-11974 TCV-116

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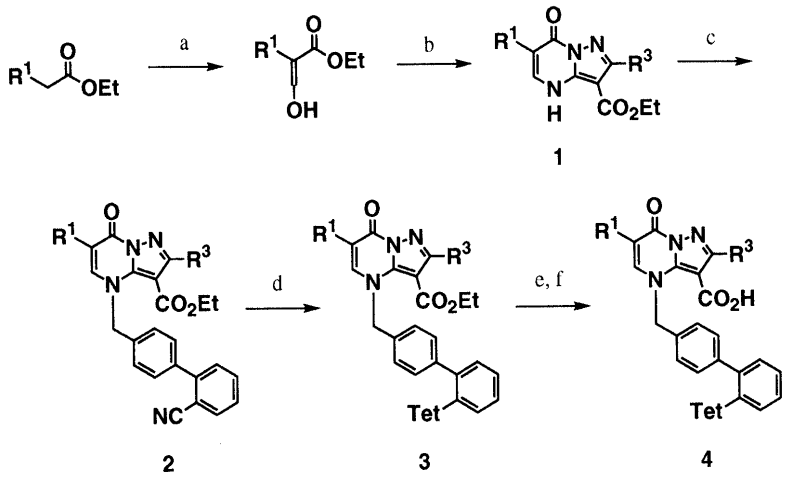
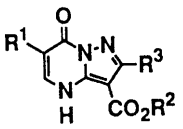


reagents: (a) HCO_2Et / *tert*-BuOK / THF; (b) HOAc / 3-amino-4-carbethoxypyrazole derivative / EtOH; (c) 4'-(bromomethyl)biphenyl-2-carbonitrile / NaH / DMF; (d) Me_3SnN_3 / xylene; (e) 4 N aq. NaOH / EtOH; (f) 1 N aq. HCl

Chart 1

Table 1. Physicochemical Data for the Pyrazolo[1,5-*a*]pyrimidines **1**



Compd.	R^1	R^2	R^3	Yield (%)[a]	mp (°C)	Formula[b]
1a	*n*-Butyl	Et	H	76	293 (dec.)	$C_{13}H_{17}N_3O_3$
1b	Ethyl	Et	H	66	>300	$C_{11}H_{13}N_3O_3$
1c	Ethoxy	Et	H	18	295 (dec.)	$C_{11}H_{13}N_3O_4$
1d	*n*-Propyl	Et	H	46	>300	$C_{12}H_{15}N_3O_3$
1e	*n*-Propyl	Et	Et	75	209—210	$C_{14}H_{19}N_3O_3$
1f	*n*-Pentyl	Et	H	53	282 (dec.)	$C_{14}H_{19}N_3O_3$
1g	Cyclopropyl	Et	H	48	>300	$C_{12}H_{13}N_3O_3$

a) See the experimental section for representative procedures. *b*) Analytical results were within ±0.3% of the theoretical values.

physicochemical data are shown in Table 1. Deprotonation of **1** with sodium hydride in *N*,*N*-dimethylformamide (DMF) followed by addition of 4'-(bromomethyl)biphenyl-2-carbonitrile gave **2**. The regiochemistry of **2** was finally determined by the X-ray crystallographic analysis of ethyl 6-propyl-7-oxo-4-[[2'-(1-trityl-1*H*-tetrazol-5-yl)-biphenyl-4-yl]methyl]-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-4-carboxylate, which was prepared from **3d** with trityl chloride. The N^4 alkylated products **2** were heated with two equivalents of trimethyltin azide, then hydrolyzed with concentrated aqueous HCl to afford the tetrazoles **3**. Finally, alkaline hydrolysis of **3** gave the carboxylic acids **4**.

Biological Results and Discussion

Compounds **3a—d**, **f**, **g** and **4a—g** were evaluated as AII antagonists by testing the potency to displace [^{125}I]-AII binding to COS cells transfected with a cDNA encoding a human AT_1 angiotensin II receptor. The K_i values in this assay are listed in Table 2.

Some of the 3-ethoxycarbonyl derivatives **3** showed relatively high activity, although ionic interaction with the AII receptor could not be expected for these compounds. On the other hand, the hydrolysis of the ester group, affording the 3-carboxyl derivatives **4**, led to over 10-fold increase in the binding affinity. Therefore, it was thought that the binding affinity of the compounds is greatly enhanced by the ionic interaction of the carboxyl group with the receptor.

Comparison of the alkyl chain at the 6-position suggested that small substituents were disadvantageous to the receptor binding for both the 3-ethoxycarbonyl derivatives **3** and the 3-carboxyl derivatives **4**. The 6-ethoxy derivative **3c** showed weak affinity in comparison with the 6-propyl derivative **3d**, which had a substituent of a similar size, suggesting that an ethoxy substituent at the 6-position reduced the binding activity. On the contrary, no significant difference was observed between **4c** and **4d**, that is, the binding activity was not influenced by substitution with the ethoxy group. Introduction of an ethyl group at the 2-position (**4e**) slightly reduced the binding activity.

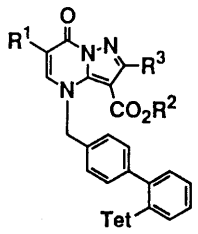
The 3-carboxyl derivatives **4a—f** were also evaluated using rat liver membrane to show K_i values in the range of 1.7—3.4 nM. The influence of the variation of the alkyl substituent at the 6-position was negligible, with the exception of the cyclopropyl derivative **4g**.

All compounds **4a—g** were evaluated for intravenous antihypertensive activity[6] in anesthetized spontaneously hypertensive rats (SHRs) and for oral antihypertensive activity in conscious SHRs.

At a dosage of 1.0 mg/kg i.v., the most potent compound **4d** induced a 35 mmHg decrease in the mean blood pressure (MBP), and **4b—g** also reduced MBP in the range of 20—30 mmHg from the normal value at the maximal point. However, the 2-butyl derivative **4a** was much less active in spite of its high binding activity, and administration of 10 mg/kg was required for a 20 mmHg decrease in MBP.

In oral administration of the compounds in this series

Table 2. Physicochemical Data and *in Vitro* AII Antagonist Potencies of the Pyrazolo[1,5-*a*]pyrimidine Derivatives



Compound	R^1	R^2	R^3	mp (°C)	Formula[a]	Receptor assay, K_i (nM)[b]	
						Human	Rat
3a	*n*-Butyl	Et	H	223—225	$C_{27}H_{27}N_7O_3$	8.5	
3b	Ethyl	Et	H	205—207	$C_{25}H_{23}N_7O_3$	150.0	
3c	Ethoxy	Et	H	238—240	$C_{25}H_{23}N_7O_4$	20.0	
3d	*n*-Propyl	Et	H	245—246	$C_{26}H_{25}N_7O_3$	6.2	
3e[c]	*n*-Propyl	Et	Et	Oil		N.T.	
3f	*n*-Pentyl	Et	H	214—215	$C_{28}H_{29}N_7O_3$	5.9	
3g[d]	Cyclopropyl	Et	H	Powder	$C_{26}H_{23}N_7O_3$	56.0	
4a	*n*-Butyl	H	H	252—254	$C_{25}H_{23}N_7O_3$	0.85	2.5
4b	Ethyl	H	H	188—190	$C_{23}H_{19}N_7O_3 \cdot C_2H_5OH$	4.0	2.4
4c	Ethoxy	H	H	227—229	$C_{23}H_{19}N_7O_4 \cdot 0.1H_2O$	1.2	1.7
4d	*n*-Propyl	H	H	253—255	$C_{24}H_{21}N_7O_3 \cdot 0.2H_2O$	0.68	2.5
4e	*n*-Propyl	H	Et	239—241	$C_{26}H_{25}N_7O_3 \cdot 0.2H_2O$	1.1	2.5
4f	*n*-Pentyl	H	H	240—242	$C_{26}H_{25}N_7O_3$	0.36	3.4
4g	Cyclopropyl	H	H	183—185	$C_{24}H_{19}N_7O_3 \cdot 0.2H_2O$	2.4	10.0
CV-11974						0.3	1.5

a) Analytical results were within ±0.3% of the theoretical values. *b*) K_i values represent an average of two or more determinations from separate assays. *c*) N.T.: not tested. *d*) Determined by HR-SIMS.

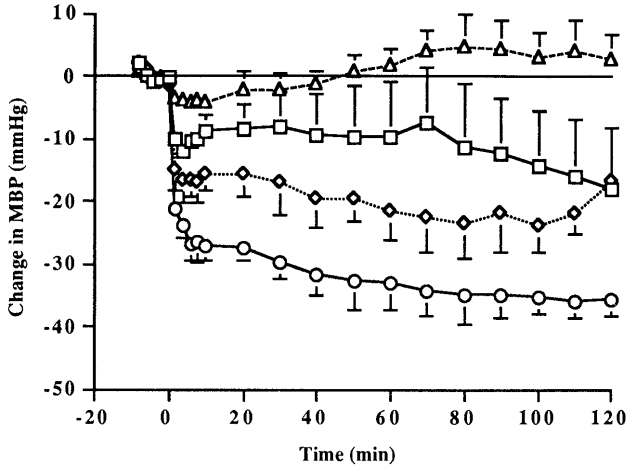


Fig. 1. Intravenous Antihypertensive Activity in Anesthetized SHR of **4d** ($n=3$)

Values represent the means±S.E.M. ---△---, saline; —□—, 0.03 mg/kg; ···◇···, 0.1 mg/kg; —○—, 1.0 mg/kg.

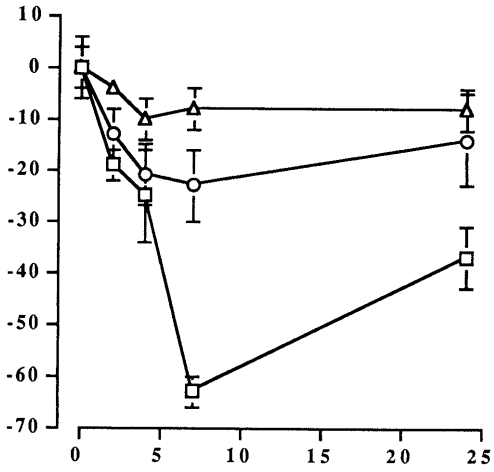


Fig. 2. Oral Antihypertensive Activity in Conscious SHR of **4d** (10 mg/kg, $n=3$) and TCV-116 (0.1 mg/kg, $n=3$)

Values represent the means±S.E.M. —△—, saline; —○—, **4d**; —□—, TCV-116.

(**4a**—**g**), however, no marked decrease in blood pressure was observed. Even with the most potent derivative **4d**, 10 mg/kg was required to cause a 20 mmHg decrease of MBP (Fig. 2). The ester derivatives **3a**—**d**, which were expected to show higher oral bioavailability than **4a**—**d**, also showed weak activity.

In conclusion, a new series of 3-carboxypyrazolo[1,5-*a*]-pyrimidine derivatives has been synthesized, through a short and straightforward route compared with that used for CV-11974, which required multiple steps because of the difficulty in direct *N*-alkylation of the 3-carboxybenzimidazole at the desired position. This series of compounds showed high-affinity AII receptor binding. Furthermore, a significant difference of binding affinity was observed between the 3-ethoxycarbonyl derivatives **3** and the 3-carboxyl derivatives **4**, suggesting that the ionic interaction of the carboxyl group with AII receptor is important for strong binding affinity of this heterocycle, in contrast with the case of the 7-carboxybenzimidazole derivatives. In the *in vivo* assay, however, the compounds did not show strong or long-lasting antihypertensive activity comparable to that of TCV-116.

Experimental

General Procedures Melting points were determined on a Yanagimoto hot plate micro melting point apparatus without correction. Mass spectra were recorded on a Hitachi M-60 instrument. ^{1}H-NMR spectra were recorded on a Varian VXR-200 spectrometer in $CDCl_3$ unless otherwise noted. Chemical shifts are reported as δ values with respect to tetramethylsilane (TMS) as an internal standard. Signal multiplicities are represented by s (singlet), d (doublet), t (triplet), q (quartet), br (broad), and m (multiplet). Abbreviations are as follows: Tet, tetrazole. Column chromatography was done on Kieselgel 60 (E. Merck, 230—400 mesh). Organic extracts were dried over $MgSO_4$.

Ethyl 6-Butyl-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carboxylate (1a) A solution of ethyl hexanoate (1.44 g, 10.0 mmol) and ethyl formate (1.60 g, 21.6 mmol) in tetrahydrofuran (THF) (5 ml) was added dropwise to a solution of potassium *tert*-butoxide (2.50 g, 22.3 mmol) in THF (20 ml) at room temperature under nitrogen. The mixture was stirred for 3 h at room temperature. After removal of the solvent, the residue was partitioned between ether (20 ml) and ice water (20 ml). The aqueous layer was made acidic with concentrated aqueous HCl, and extracted with ether (20 ml × 2). The organic layer was dried and then concentrated *in vacuo* to give 1.19 g of oily product. This was taken up in EtOH (5 ml), then ethyl 3-amino-4-pyrazolecarboxylate (700 mg, 4.51 mmol) and AcOH (1 ml) were added, and the mixture was refluxed for 12 h. It was allowed to cool to room temperature, and the resulting precipitate was collected by filtration and washed with EtOAc to afford 900 mg (76%) of **1a** as white crystals, mp 293 °C (dec.) (EtOH). ^{1}H-NMR ($CDCl_3$–CD_3OD) δ: 0.95 (3H, t, J=7.0 Hz), 1.39 (3H, t, J=7.0 Hz), 1.32—1.65 (4H, m), 2.55 (2H, t, J=7.0 Hz), 4.36 (2H, q, J=7.0 Hz), 7.53 (1H, s), 8.18 (1H, s). *Anal.* Calcd for $C_{13}H_{17}N_3O_3$: C, 59.30; H, 6.51; N, 15.96. Found: C, 59.40; H, 6.53; N, 16.01.

Ethyl 6-Alkyl-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carboxylate (1b—g) The preparation of **1b—g** was carried out according to the procedure described for **1a**.

Ethyl 6-Butyl-4-[(2′-cyanobiphenyl-4-yl)methyl]-7-oxo-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carboxylate (2a) A suspension of **1a** (520 mg, 1.97 mmol) in DMF (20 ml) was heated at 60 °C for 15 min under nitrogen, and allowed to cool to room temperature. NaH (90 mg, 2.25 mmol; 60% dispersion in oil) and 4′-(bromomethyl)biphenyl-2-carbonitrile (700 mg, 2.57 mmol) were successively added, and the resulting mixture was stirred for 4 h at room temperature. It was then poured into ice water and extracted with EtOAc (20 ml × 3). The organic extract was washed with H_2O and brine, and dried. The crude product was purified by column chromatography on silica gel with *n*-hexane–EtOAc (v/v, 1/1) to give 660 mg (73.5%) of **2a** as white crystals, mp 135—136 °C (EtOAc–*n*-hexane). ^{1}H-NMR ($CDCl_3$) δ: 0.92 (3H, t, J=7.4 Hz), 1.35 (3H, t, J=7.2 Hz), 1.24—1.68 (4H, m), 4.29 (2H, q, J=7.2 Hz), 6.02 (2H, s), 7.24—7.80 (9H, m), 8.32 (1H, s). *Anal.* Calcd for $C_{27}H_{26}N_4O_3$: C, 71.35; H, 5.57; N, 12.33. Found: C, 71.44; H, 5.85; N, 12.01.

Ethyl 6-Butyl-7-oxo-4-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-4,7-dihydropyrazolo[1,5*a*]pyrimidine-3-carboxylate (3a) A suspension of **2a** (560 mg, 1.23 mmol) and trimethyltin azide (510 mg, 2.48 mmol) in xylene (10 ml) was heated at 110 °C for 24 h. After cooling, the resulting precipitate was collected by filtration, washed several times with xylene,

Table 3. Physicochemical Data for **1b—g**

Compd.	Analysis (%): Calcd (Found)			^{1}H-NMR ($CDCl_3$–CD_3OD)
	C	H	N	
1b	56.16	5.57	17.86	1.25 (3H, t, J=7.6 Hz), 1.40 (3H, t, J=7.0 Hz), 2.60 (2H, q, J=7.6 Hz), 4.37 (2H, q, J=7.0 Hz),
	(56.16	5.60	17.81)	7.57 (1H, s), 8.12 (1H, s)
1c	52.59	5.22	16.72	1.30 (3H, t, J=6.8 Hz), 1.31 (3H, t, J=7.0 Hz), 4.05 (2H, q, J=6.8 Hz), 4.30 (2H, q, J=7.0 Hz),
	(52.44	5.31	16.72)	7.65 (1H, s), 8.18 (1H, s)
1d	57.82	6.07	16.86	0.98 (3H, t, J=7.6 Hz), 1.40 (3H, t, J=7.0 Hz), 1.58—1.76 (2H, m), 2.54 (2H, t, J=7.0 Hz), 4.37
	(57.52	6.12	16.84)	(2H, q, J=7.0 Hz), 7.56 (1H, s), 8.18 (1H, s)
1e	60.63	6.91	15.15	0.97 (3H, t, J=7.0 Hz), 1.33 (3H, t, J=7.6 Hz), 1.40 (3H, t, J=7.2 Hz), 1.52—1.75 (2H, m), 2.54
	(60.71	6.71	15.23)	(2H, t, J=8.0 Hz), 2.99 (2H, t, J=7.6 Hz), 4.34 (2H, q, J=7.2 Hz), 7.40 (1H, d, J=5.6 Hz), 9.65 (1H, d, J=5.6 Hz)
1f	60.63	6.91	15.15	0.90 (3H, t, J=6.8 Hz), 1.40 (3H, t, J=7.0 Hz), 1.20—1.45 (4H, m), 1.50—1.75 (2H, m), 2.56
	(60.59	6.88	15.21)	(2H, t, J=7.0 Hz), 4.37 (2H, q, J=7.0 Hz), 7.55 (1H, s), 8.18 (1H, s)
1g	58.29	5.30	16.99	0.65 (2H, m), 1.03 (2H, m), 1.45 (3H, t, J=7.2 Hz), 1.92 (1H, m), 4.44 (2H, q, J=7.2 Hz), 7.65
	(58.41	5.33	17.13)	(1H, s), 8.48 (1H, s)

Table 4. Physicochemical Data for **3b—g**

Compd.	Analysis (%): Calcd (Found)			^{1}H-NMR ($CDCl_3$)
	C	H	N	
3b	63.96	4.94	20.88	1.18 (3H, t, J=7.4 Hz), 1.35 (3H, t, J=7.0 Hz), 2.57 (2H, q, J=7.4 Hz), 4.28 (2H, q, J=7.0 Hz),
	(63.93	5.14	20.63)	5.91 (2H, s), 7.05 and 7.15 (2H × 2, ABq, J=8.4 Hz), 7.42—7.62 (4H, m), 7.73 (1H, d, J=7.0 Hz), 8.27 (1H, s)
3c	61.84	4.78	20.20	1.36 (3H, t, J=7.0 Hz), 1.41 (3H, t, J=7.0 Hz), 4.05 (2H, q, J=7.0 Hz), 4.29 (2H, q, J=7.0 Hz),
	(61.59	4.90	20.20)	5.93 (2H, s), 7.13 (4H, s), 7.36—7.78 (5H, m), 8.33 (1H, s)
3d	64.58	5.21	20.28	0.95 (3H, t, J=7.3 Hz), 1.36 (3H, t, J=7.2 Hz), 1.53—1.73 (2H, m), 2.52 (2H, t, J=7.6 Hz), 4.28
	(64.48	5.27	20.23)	(2H, q, J=7.2 Hz), 5.91 (2H, s), 7.07—7.17 (4H, m), 7.32—7.61 (4H, m), 7.32—7.61 (4H, m), 7.75—7.79 (1H, m), 8.30 (1H, s)
3e				0.90 (3H, t, J=7.2 Hz), 1.18 (3H, t, J=7.4 Hz), 1.30 (3H, t, J=7.2 Hz), 1.40—1.65 (2H, m), 2.44 (2H, t, J=7.6 Hz), 2.87 (2H, t, J=7.4 Hz), 4.24 (2H, q, J=7.2 Hz), 5.66 (2H, s), 6.98 and 7.10 (2H × 2, ABq, J=8.4 Hz), 7.32 (1H, s), 7.34—7.60 (3H, m), 7.91—7.94 (1H, m)
3f	65.74	5.71	19.17	0.86 (3H, t, J=6.6 Hz), 1.20—1.39 (7H, m), 1.42—1.64 (2H, m), 2.52 (2H, t, J=7.8 Hz), 4.27
	(65.82	5.83	19.07)	(2H, q, J=7.1 Hz), 5.90 (2H, s), 7.08 and 7.15 (2H × 2, ABq, J=8.6 Hz), 7.32 (1H, s), 7.39—7.60 (3H, m), 7.88 (1H, dd, J=7.2, 1.6 Hz), 8.24 (1H, s)
3g[a]	$[M+H]^+$ 482.1940 (482.1940)			0.59 (2H, m), 0.88 (2H, m), 1.32 (3H, t, J=7.0 Hz), 1.86 (1H, m), 4.24 (2H, q, J=7.0 Hz), 5.89 (2H, s), 7.00 and 7.08 (2H × 2, ABq, J=8.4 Hz), 7.37—7.57 (4H, m), 7.89 (1H, dd, J=7.4, 1.6 Hz), 8.14 (1H, s)

a) Determined by HR-SIMS: Calcd (Found).

Table 5. Physicochemical Data for **4b—g**

Compd.	Analysis (%): Calcd (Found)			^{1}H-NMR (DMSO-d_6)
	C	H	N	
4b	61.59	5.17	20.11	1.06 (3H, t, J=7.0 Hz), 1.14 (3H, t, J=7.4 Hz), 2.47 (2H, q, J=7.4 Hz), 3.44 (2H, q, J=7.0 Hz),
	(61.65	5.35	19.99)	6.00 (2H, s), 7.05 and 7.15 (2H×2, ABq, J=8.6 Hz), 7.27—7.75 (4H, m), 8.03 (1H, s), 8.24 (1H, s)
4c	59.92	4.24	21.27	1.30 (3H, t, J=7.0 Hz), 3.99 (2H, q, J=7.0 Hz), 6.00 (2H, s), 7.05 and 7.15 (2H×2, ABq,
	(59.80	4.31	21.32)	J=8.4 Hz), 7.35—7.70 (4H, m), 8.05 (1H, s), 8.25 (1H, s)
4d	62.79	4.70	21.36	0.89 (3H, t, J=7.4 Hz), 1.49—1.61 (2H, m), 2.42 (2H, t, J=7.5 Hz), 6.00 (2H, s), 7.06 and 7.13
	(62.86	4.82	21.40)	(2H×2, ABq, J=8.6 Hz), 7.40—7.70 (4H, m), 8.04 (1H, s), 8.25 (1H, s)
4e	64.11	5.26	20.13	0.89 (3H, t, J=7.2 Hz), 1.19 (3H, t, J=7.4 Hz), 1.42—1.62 (2H, m), 2.41 (2H, t, J=7.8 Hz), 2.84
	(64.11	5.43	19.93)	(2H, q, J=7.4 Hz), 5.81 (2H, s), 7.06 (4H, s), 7.49—7.67 (4H, m), 8.03 (1H, s)
4f	64.58	5.21	20.28	0.85 (3H, t, J=6.6 Hz), 1.20—1.40 (4H, m), 1.40—1.65 (2H, m), 2.44 (2H, t, J=7.6 Hz), 6.00
	(64.32	5.36	20.37)	(2H, s), 7.05 and 7.13 (2H×2, ABq, J=8.2 Hz), 7.40—7.70 (4H, m), 8.05 (1H, s), 8.24 (1H, s)
4g	62.33	4.36	21.20	0.61 (2H, m), 0.94 (2H, m), 1.90 (1H, m), 5.90 (2H, s), 7.04 and 7.11 (2H×2, ABq, J=8.4 Hz),
	(62.27	4.59	21.02)	7.25—7.57 (4H, m), 7.77 (1H, dd, J=7.4, 1.6 Hz), 8.30 (1H, s)

and then suspended in EtOH (10 ml). A 1 N aqueous HCl solution (4 ml) was added, and the reaction mixture was stirred for 1 h at room temperature. After removal of the solvent, the residue was partitioned between CH_2Cl_2 and ice water. The aqueous layer was extracted with CH_2Cl_2 (10 ml×2). The combined organic layer was dried and concentrated *in vacuo*. The residue was purified by chromatography on silica gel. Elution with $CHCl_3$–MeOH (v/v, 20/3) afforded 420 mg (68.5%) of **3a** as white crystals, mp 223—225 °C (EtOH–EtOAc). ^{1}H-NMR ($CDCl_3$) δ: 0.88 (3H, t, J=7.4 Hz), 1.32 (3H, t, J=7.0 Hz), 1.20—1.62 (4H, m), 2.49 (2H, t, J=7.8 Hz), 4.25 (2H, q, J=7.4 Hz), 5.90 (2H, s), 7.00 and 7.05 (2H×2, ABq, J=9.0 Hz), 7.33—7.58 (4H, m), 7.87—7.92 (1H, m), 8.17 (1H, s). *Anal.* Calcd for $C_{27}H_{27}N_7O_3$: C, 65.18; H, 5.47; N, 19.71. Found: C, 65.17; H, 5.55; N, 19.60.

Ethyl 6-Alkyl-7-oxo-4-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carboxylate (3b—g) The preparation of **3b—g** was carried out according to the procedure described for **3a**.

6-Butyl-7-oxo-4-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carboxylic Acid (4a) A suspension of **3a** (370 mg, 0.74 mmol) in EtOH (10 ml) was treated with 4 N aqueous NaOH (2.5 ml), and the mixture was refluxed for 1 h. After removal of the solvent, the residue was dissolved in H_2O. The aqueous solution was washed with EtOAc and made acidic with 1 N aqueous HCl (12 ml). The resulting precipitate was collected by filtration, washed with H_2O, and dried under reduced pressure at 70 °C to give 290 mg (83%) of **4a** as white crystals, mp 252—254 °C (MeOH). ^{1}H-NMR (DMSO-d_6) δ: 0.89 (3H, t, J=7.0 Hz), 1.20—1.62 (4H, m), 2.44 (2H, t, J=7.4 Hz), 5.99 (2H, s), 7.06 and 7.12 (2H×2, ABq, J=8.6 Hz), 7.50—7.68 (4H, m), 8.05 (1H, s), 8.24 (1H, s). *Anal.* Calcd for $C_{25}H_{23}N_7O_3$: C, 63.96; H, 4.94; N, 20.88. Found: C, 63.75; H, 5.14; N, 20.76.

6-Alkyl-7-oxo-4-[[2′-(1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-4,7-dihydropyrazolo[1,5-*a*]pyrimidine-3-carboxylic Acid (4b—g) The preparation of **4b—g** was carried out according to the procedure described for **4a**.

X-Ray Crystallographic Analysis of Ethyl 6-Propyl-7-oxo-4-[[2′-(1-trityl-1*H*-tetrazol-5-yl)biphenyl-4-yl]methyl]-4,7-dihydropyrazolo[1,5-*a*]-pyrimidine-4-carboxylate The title compound was prepared from **3d** using a conventional method (trityl chloride/triethylamine/THF), mp 166—167 °C (EtOAc). ^{1}H-NMR ($CHCl_3$) δ: 0.89 (3H, t, J=7.3 Hz), 1.32 (3H, t, J=7.6 Hz), 1.50—1.65 (2H, m), 2.39 (2H, t, J=7.6 Hz), 4.25 (2H, q, J=7.0 Hz), 5.84 (2H, s), 6.85—7.00 (8H, m), 7.10—7.20 (3H, m), 7.20—7.40 (11H, m), 7.45—7.50 (2H, m), 7.90—8.00 (1H, m), 8.05 (1H, s). *Anal.* Calcd for $C_{45}H_{39}N_7O_3$: C, 74.76; H, 5.42; N, 13.51. Found: C, 74.51; H, 5.53; N, 13.25. Crystal data: triclinic; space group $P\bar{1}$, a=10.660(1) Å, b=20.422(1) Å, c=9.056(1) Å, α=93.25(1)°, β=108.74(1)°, γ=89.97(1)°, V=1863.8(4) Å^3, Z=2, D_x=1.29 g/cm^3, μ(Cu-K_α)=6.28 cm^{-1}. A crystal with dimensions of 0.3×0.1×0.3 mm was mounted to a Rigaku AFC-5R diffractometer with graphite-monochromated Cu-K_α radiation. The data were measured using the ω–2θ scan technique to a maximum 2θ value of 140.2°. Of the 7265 reflections which were collected, 6783 were unique. The data were corrected for Lorentz and polarization effects. The structure was solved by direct methods. Non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement was based on 4935 observed reflections [$I>3.0\sigma(I)$] and 496 variable parameters and converged (largest parameter was 0.08 times its esd) with unweighted and weighted agreement factors of R=0.063 and R_w=0.054. The maximum and minimum peaks on the final difference Fourier map corresponded to 0.33 and −0.33 e Å^{-3}, respectively. All calculations were performed using the teXsan crystallographic software package of Molecular Structure Corporation (1992).

AII Receptor Binding Assay Using COS Cells A cDNA encoding human AT_1 AII receptor, donated by Dr. Inagami (Vanderbilt Univ., U.S.A.), was inserted into the mammalian expression vector pcDNAI (Invitrogen). COS-7 cells were plated in 175-cm^2 flasks and grew to 80% confluency after 3 d. The cells were then transfected with 40 μg of DNA in 150 μl of lipofectin reagent (Gibco). Two or three days after transfection, the binding assay was done as described previously.[7] In brief, cell suspensions (1.2×10^6 cell/ml), dispersed with 0.025% trypsin/1 mM EDTA, were inclubated at 25 °C for 60 min in 0.2 ml of Hepes (20 ml): buffered Hanks' solution containing 1 mg/ml phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, 10 μg/ml pepstatin A, 250 μg/ml bacitracin, 10 μg/ml soybean trypsin inhibitor and 0.1 mM amastatin with 0.1 nM [^{125}I]AII (81.4 TBq/mmol, New England Nuclear) in the absence or presence of non-radioactive compounds. Each binding reaction was terminated by addition of 2.5 ml of ice-cold 50 mM Tris–HCl (pH 7.4), followed by rapid filtration through a GF/C glass fiber filter under reduced pressure. The filters were then quickly washed 4 more times with 2.5 ml of the Tris buffer, and the radioactivity retained on the filter was counted. Nonspecific binding, determined in the presence of 10^{-6} M non-radiolabeled AII, was 5—10% of the total binding. K_i values were calculated from the equation $K_i=IC_{50}/(1+[L]/K_d)$, where IC_{50}=the concentration causing 50% inhibition of specific [^{125}I]AII binding, [L]=[^{125}I]AII concentration, and K_d=the dissociation constant for [^{125}I]AII (0.46 nM).

AII Receptor Binding Assay Using Rat Liver Membranes Preparation of rat liver membranes was performed according to the method of Chiu *et al.*[8] The incubation mixture (0.1 ml) contained 50 mM Tris–HCl (pH 7.4), 0.1 mM amastatin, 1 mM α-phenylmethanesulfonyl fluoride (PMSF), and membranes (0.03—0.05 mg of protein) with 0.2—0.3 nM [^{125}I]AII in the absence or presence of non-radioactive compounds. The equilibrium binding studies were carreid out according to the procedure using COS cells.

Evaluation of Antihypertensive Activity in Anesthetized SHRs Male SHR (280—350 g) were anesthetized with pentobarbital sodium (60 mg/kg i.p.). Arterial and venous catheters were surgically implanted. Briefly, a polyethylene catheter was placed in the femoral artery to record the arterial pressure of the anesthetized animals. Another polyethylene catheter was inserted into the femoral vein to administer the vehicle (saline, polyethylene glycol or 1% gum arabic) or test compound. The rats received 0.4 mg/kg/min i.v. pentobarbital sodium infused continuously over the experimental period to maintain anesthesia. The arterial catheter was connected to a pressure transducer coupled with a polygraph to monitor the arterial pressure. After a 1-h stabilization period, the rats were given the vehicle or test compound intravenously and their blood pressure was monitored for 1 h.

Evaluation of Antihypertensive Activity in Conscious SHRs Male SHR

(280—350 g) were anesthetized with pentobarbital sodium (60 mg/kg i.p.). An arterial catheter was surgically implanted. Briefly, a polyethylene catheter was placed in the femoral artery; this catheter was used to record the arteirla pressure of the conscious, freely moving animals. The catheter was tunneled subcutaneously to the back of the head. After the rats had completely recovered from anesthesia (at least 2.5 h after surgery), the arterial catheter was connected to a pressure transducer-coupled polygraph to monitor the arterial pressure. After a 1-h stabilization period, rats were given the vehicle (saline, polyethylene glycol or 1% gum arabic) or test compound by gavage and the blood pressure was monitored for 4 h.

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