Communications to the Editor

Discovery of a Novel Class of Orally Active, Non-Peptide Angiotensin II Antagonists

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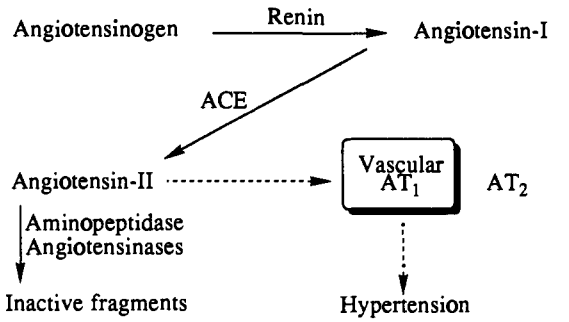
The renin–angiotensin system (RAS) plays a pivotal role in the regulation of blood pressure, volume homeostasis, and salt retention. Angiotensin II, an octapeptide produced by the action of angiotensin converting enzyme (ACE) on angiotensin I, is the most potent pressor agent and the penultimate product of this cascade (Scheme I). In addition to its hypertensive effects, angiotensin II also has mitogenic effects on vascular tissue and stimulates renal tubular sodium reabsorption, implicating it in the etiology of a number of cardiovascular disorders, including chronic renal failure and cardiac hypertrophy.[1] During the early seventies, efforts were mounted to block this pathway at the final step in the cascade via angiotensin II receptor antagonists. This endeavor led to the discovery of a series of peptidic antagonists (e.g. Saralasin) which, when administered iv, produced a short-lived antihypertensive effect in high-renin subjects.[2] The interest in developing these peptide agents as antihypertensives, however, rapidly dwindled due to lack of oral efficacy, short duration of action, and partial agonist activity which caused a transient hypertensive response.

Following a non-peptidic lead by Takeda Pharmaceutical Co. in 1982,[3] Du Pont recently disclosed a novel series of angiotensin II antagonists exemplified by DUP-753.[4] The development of these antagonists demonstrated that intervention of the renin–angiotensin pathway via receptor blockade could lead to a profound antihypertensive effect

Scheme I



with a long duration of action. This antihypertensive activity was demonstrated in both renal artery-ligated rats (RAL) and spontaneously hypertensive rats (SHR). Our interest in the renin–angiotensin system has been long-standing with efforts recently focused on development of a series of novel angiotensin II antagonists for the relevant Type-I receptors (AT_1).[5] In this communication, we present our preliminary SAR work leading to the synthesis of the potent, orally active angiotensin II antagonist, A-81988.

Chemistry. The synthesis of pyrimidine analogs (**10–15**) is described in Schemes II and III. The protected (bromobiphenylyl)tetrazole[6] **1** was reacted with excess propylamine in THF to give **2**. The secondary amine was then coupled to the chloropyrimidine[7] **3** in the presence of triethylamine, and the resulting ester **4** was detritylated with HCl–ethanol followed by hydrolysis with LiOH in refluxing aqueous ethanol to give **14**. Hydrolysis of these esters under the stated condition is sluggish, which may be due to its occlusion by the proximal *N*-alkyl substituent.[8]

For the synthesis of pyrimidine analogs where R^2 = H (**10** and **15**, see Table I), the bromophenyl tetrazole was reacted with excess NaN_3 in DMF followed by reduction of the azide with $LiAlH_4$ in THF to give **5** (Scheme III). The primary amine **5** was then converted to **10** following an analogous method as described in Scheme II for the synthesis of *N*-alkyl analogs (**11–14**).

Unlike chloropyrimidines, chloropyridines were significantly less reactive toward secondary amines (e.g. **2**). As a result, the synthesis was modified to compensate for the

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(1) (a) Peach, M. J. Renin–Angiotensin System: Biochemistry and Mechanism of Action. *Physiol. Rev.* **1977**, *57*, 313–370. (b) Hollenberg, N. K. Pharmacologic Interruption of the Renin–Angiotensin System. *Annu. Rev. Pharmacol. Toxicol.* **1979**, *19*, 559–582. (c) Taubman, M. B.; Berk, B. C.; Izumo, S.; Tsuda, T.; Alexander, R. W.; Nadal-Ginard, B. Angiotensin II Induces *c-fos* mRNA in Aortic Smooth Muscle. *J. Biol. Chem.* **1989**, *264*, 526–530. (d) Schunkert, H.; Ingelfinger, J. R.; Dzau, V. J. Evolving Concepts of the Intrarenal Renin–Angiotensin System in Health and Disease: Contributions of Molecular Biology. *Renal Physiol. Biochem.* **1991**, *14*, 146–154. (e) Dzau, V. J. Significance of the Vascular Renin–Angiotensin Pathway. *Hypertension* **1986**, *8*, 553–557.

(2) (a) Khosla, M. C.; Smeby, R. R.; Bumpus, F. M. Structure Activity Relationship in Angiotensin II Analogs. *Handb. Exp. Pharmacol.* **1974**, *37*, 126–161. (b) Pals, D. T.; Denning, G. S.; Jr.; Keenan, R. E. *Historical* Development of Saralasin. *Kidney Int.* **1979**, *15* (Suppl. 9), S7–S10.

(3) Furakawa, Y.; Kishimoto, S.; Nishikawa, K. U.S. Patent 4,340,598, 1982. Also see U.S. Patent 4,355,040, 1982.

(4) For an extensive review on DUP-753, see: Duncia, J. V.; Carini, D. J.; Chiu, A. T.; Johnson, A. L.; Price, W. A.; Wong, P. C.; Wexler, R. R.; Timmermans, P. B. M. W. M. The Discovery of DUP-753, a Potent, Orally Active Nonpeptide Angiotensin II Receptor Antagonist. *Med. Res. Rev.* **1992**, *12*, 149–191.

(5) (a) Chang, R. S. L.; Lotti, V. J. Two Distinct Angiotensin II Receptor Binding Sites in Rat Adrenal Revealed by New Selective Nonpeptide Ligands. *Mol. Pharmacol.* **1990**, *37*, 347–351. (b) Chiu, A. T.; Herblin, W. F.; McCall, D. E.; Ardecky, R. J.; Carini, D. J.; Duncia, J. V.; Pease, L. J.; Wong, P. C.; Wexler, R. R.; Johnson, A. L.; Timmermans, P. B. M. W. M. Identification of A-II Receptor Subtypes. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 196–203.

(6) For synthesis of this bromide, see: Aldrich, P. E.; Duncia, J. V.; Pierce, M. E. U.S. Patent 4,870,186, 1989.

(7) For the synthesis of chloropyrimidines, see: (a) Yamanaka, H.; Mizugaki, M.; Sagi, M.; Edo, K. Synthesis and Metabolism of 5-Alkylpyrimidine-2-Carboxylic Acids. *Heterocycles* **1979**, *12*, 1323–1326. (b) Peters, E.; Minnemeyer, J.; Spears, A. W.; Tieckelmann, H. The Synthesis of Some 2,4,5-Trisubstituted Pyrimidines. *J. Org. Chem.* **1960**, *25*, 2137–2142. (c) Bredereck, H.; Effenberger, F.; Schweizer, E. H. Neue Synthesen 5-monosubstituierter Pyrimidine. *Chem. Ber.* **1962**, *95*, 803–809.

(8) We have noticed that when R^2 = H (Table I), both pyrimidine and pyridine esters are easier to hydrolyze than when R^2 = alkyl (see Scheme III, 5–14). This led us to believe that R_2 (for both pyrimidine and pyridine analogs) and the carboxy group are close to each other in space. An X-ray analysis of A-81988 (data not shown) clearly lends some support to this hypothesis.

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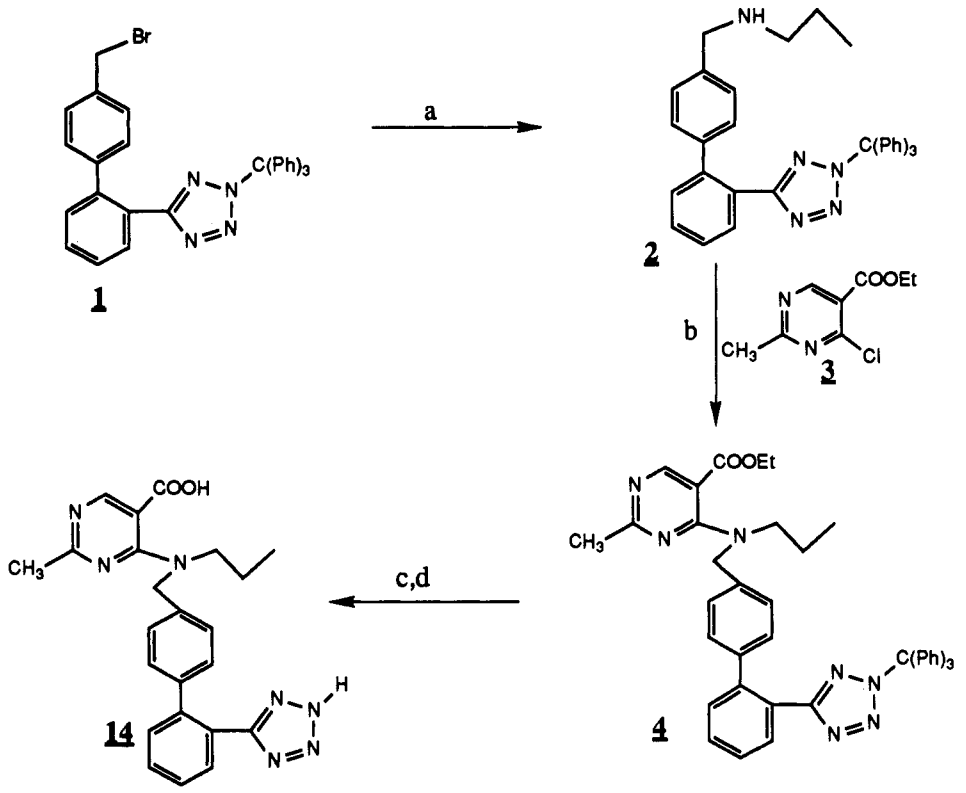
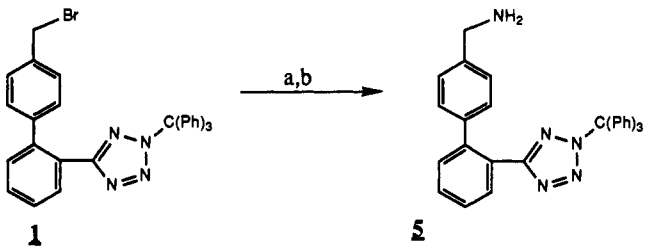
Scheme II [a]



[a] Reagents: (a) 6 equiv of $PrNH_2$ in THF at room temperature for 4 h, 89%; (b) 3, triethylamine (2 equiv) in THF at room temperature, 68%; (c) HCl–EtOH at room temperature for 2 h, 57%; (d) excess LiOH–EtOH–H_2O heated at reflux for 2.5 h, 55%.

Scheme III



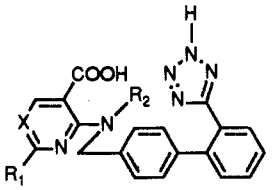
[a] Reagents: (a) 2.5 equiv of NaN_3 in DMF at room temperature for 16 h, 89%; (b) excess $LiAlH_4$ in THF at 0 °C for 30 min gave 5, which was used without purification.

loss of this reactivity. As described in Scheme IV, commercially available chloronicotinic acid[9] was converted to the acid chloride with excess $SOCl_2$ and then treated with ethanol to give the desired ester 7. A high-pressure condensation of the ester with excess propylamine at 100 °C in ethanol gave 8, which was then coupled to the bromide 1 upon formation of the lithium salt with lithium bis(trimethylsilyl)amide. The coupled product 9 was then deprotected to give 18.

Biological Results and Discussion. As shown in Table I, our initial lead structure contained a substituted pyrimidine ring tethered to the biphenyltetrazole moiety (BPT) via an aminomethylene bridge. This exocyclic nitrogen provided an entry into a structurally novel class of AII antagonists (vide DUP-753; Figure 1). Following our preliminary SAR studies, we found that the distance between the heterocycle and the biphenyltetrazole moiety was of paramount importance for the preservation of high

(9) Most of the potent analogs were made from commercially available chloropyridines. For the synthesis of 16, commercially available 2-hydroxy-6-methylpyridine-3-carboxylic acid was first esterified and then treated with PCl_5 to get the corresponding chloropyridine.

Table I



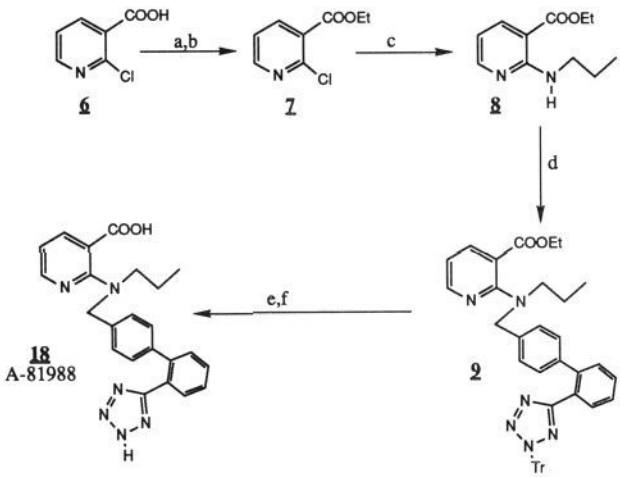
compd	X	R1	R2	pA_2[a]	K_i (nM)[b]
10	N	nBu	H	7.91–7.97 (n = 2)	198 (123–318, n = 2)
11	N	nBu	Me	7.13–7.17 (n = 2)	nd
12	N	Me	nBu	8.81 (n = 1)	15.3 (7.01–33.6, n = 3)
13	N	H	nBu	9.93 (n = 1)	3.13 (1.75–5.56, n = 7)
14	N	Me	nPr	9.60 (n = 1)	2.78 (1.66–4.64, n = 3)
15	N	H	H	5.53–5.69 (n = 2)	nd
16	CH	Me	nBu	8.08–8.22 (n = 2)	122 (n = 1)
17	CH	H	Et	8.70–8.83 (n = 2)	nd
18[c]	CH	H	Pr	10.1–10.7 (n = 16)	0.76 (0.525–1.103, n = 6)
19	CH	H	nBu	9.50 (n = 1)	nd
DUP 753				8.27–8.70 (n = 4)	14.63 (9.53–22.5, n = 8)

[a] AT_1 receptor in isolated rabbit aorta. [b] AT_1 receptor in rat liver; nd (not determined). [c] A-81988. All the biological tests were done with the monopotassium salt of 18. [d] Actual values of two determinations.

potency (data not shown). The presence of a carboxyl group at the 3-position in either ring system was required for high binding affinity (K_i) and in vitro potency (pA_2's). The carboxylic acid modifications to the corresponding esters or amides led to a significant loss of in vitro potency (100–1000-fold).[10]

In the majority of cases, R_1 and R_2 were restricted to linear hydrocarbon chains (see Table I). Initial analogs with n-butyl substitution on the pyrimidine ring (R_1) and with no substitution on the exocyclic nitrogen (R_2 = H) provided us with a first series of moderately potent AII antagonists. However, a significant improvement in potency was realized when the substitution pattern at R_1

Scheme IV



[a] Reagents: (a) $SOCl_2$ neat to acid chloride; (b) EtOH, 95%; (c) $PrNH_2$ (excess) EtOH, 100 °C, 6 h, 85%; (d) 1.2 equiv of $LiN(SiMe_3)_2$ in THF–DMPU at 0 °C and reacted with 1 at room temperature for 1.5 h, 59%; (e) HCOOH in CH_2Cl_2, 86%; (f) NaOH–EtOH, reflux 1.5 h, 90%.

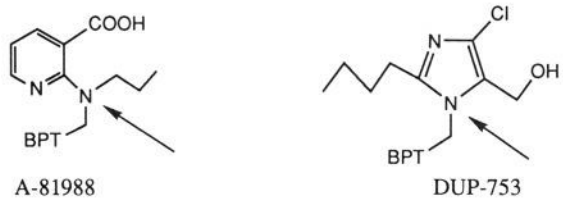


Figure 1.

and R_2 was switched. Further studies established that either an *n*-butyl or an *n*-propyl substitution on the exocyclic nitrogen (R_2) was optimal. On the other hand, no substitution or a methyl group at R_1 was needed for high potency. These modifications led to the discovery of our first highly potent analog **13** with a pA_2 value of 9.93 (isolated rabbit-aorta model).[11] When administered iv at a dose of 0.3–1 mg/kg, the corresponding disodium salt of **13** lowered mean arterial pressure in a dose-dependent manner in the renal artery-ligated (RAL), hypertensive rat.[12,13] However, the oral response of **13** in the RAL rat (1–10 mg/kg, po) was poor both in terms of the antihypertensive effect and also the duration of action. These findings correlated with the compound's (**13**) poor bioavailability (5–8%) in fasted Sprague-Dawley rats.

After screening a number of potent pyrimidine analogs for oral activity without much success, we felt that the problem of poor oral bioavailability might be associated

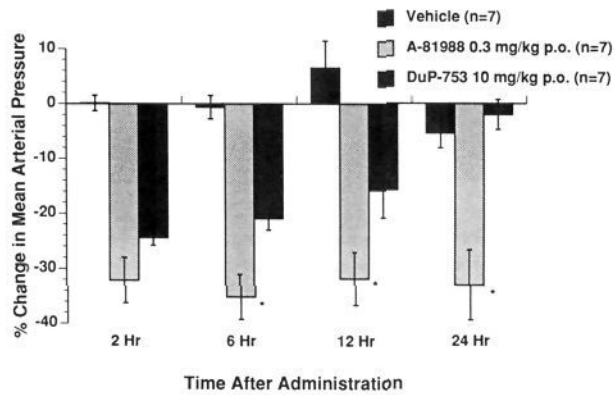


Figure 2. Twenty-four hour oral antihypertensive effects of A-81988 (0.3 mg/kg) and DUP-753 (10 mg/kg) in the conscious renal hypertensive rat. Hypertension in the rat was developed by renal artery ligation as described in refs 12 and 13. Control mean arterial pressure in the rat 6–7 days after renal artery ligation was 165–175 mmHg. Vehicle was a mixture of water and NaCl with a final pH adjusted to 7–7.4. n = number of rats per group. An asterisk indicates different from DUP-753 ($p < 0.05$).

with the presence of a pyrimidine heterocycle. Without introducing major structural changes into the molecule, a series of analogous pyridines was investigated (see **16**–**19** in Table I). Although we observed only a modest gain in in vitro potency with these pyridines, the bioavailability profiles were significantly improved. When administered orally at a dose of 0.3 mg/kg in fasted normotensive Sprague-Dawley (SD) rats, **18** was rapidly absorbed with a bioavailability of 97.2% (±10.4, $n = 8$) and a half-life of 11.4 h.[14]

Compound **18** exhibited high affinity and selectivity for the rat liver AT_1 receptor ($K_i = 0.76$ nM) with virtually no affinity to AT_2 subtype ($K_i > 10\,000$ nM, bovine cerebellum).[15] For the determination of in vitro potency at AT_1 receptor, the isolated rabbit aortic ring preparation was used.[11] Compound **18** shifted the AII curve to the right in a parallel fashion, indicating competitive antagonism with a pA_2 of 10.6 (Schild analysis) and a slope of

(10) Using **12** as a base structure, we synthesized a number of derivatives of the carboxylic acid (esters, amides, and hydroxamic acids). These analogs were found to be weakly active in our in vitro assay ($pA_2 < 7.0$).

(11) For the determination of pA_2 values, we have used the method published by Chiu et al. with the single exception that tissue rings were used instead of strips. (a) Chiu, A. T.; Carini, D. J.; Johnson, A. L.; McCall, D. E.; Price, W. A.; Thoolen, J. M. C.; Wong, P. C.; Taber, R. I.; Timmermans, P. B. M. W. M. Non-peptide Angiotensin-II Receptor Antagonists. II. Pharmacology of S-8308. *Eur. J. Pharmacol.* **1988**, *157*, 13–21. (b) Schild, H. O. pA, A New Scale for the Measurement of Drug Antagonism. *Br. J. Pharmacol.* **1947**, *2*, 189–206.

(12) For iv and po protocols, see refs 12 and 13: Cangiano, J. L.; Rodriquez-Sargent, C.; Martínez-Maldonado, M. Effects of Antihypertensive Treatment on Systolic Blood Pressure and Renin in Experimental Hypertension in Rats. *J. Pharmacol. Exp. Ther.* **1979**, *208*, 310–313.

(13) Wong, P. C.; Price, W. A.; Chiu, A. T.; Duncia, J. V.; Carini, D. J.; Wexler, R. R.; Johnson, A. L.; Timmermans, P. B. M. W. M. Nonpeptide Angiotensin-II Receptor Antagonists. IX. Antihypertensive Activity in Rats in DUP-753, an Orally Active Antihypertensive. *J. Pharmacol. Exp. Ther.* **1990**, *252*, 726–732.

(14) For the determination of bioavailability, the following protocol was used: The pharmacokinetic behavior of A-81988 was evaluated in male Sprague-Dawley derived rats ($n = 4$) following a 1 mg/kg (1 mL/kg solution) bolus iv or po (gavage) dose. Sequential blood samples (10/rat) were obtained from the tail vein of each animal for up to 24 h after dosing. Concentrations of parent drug were determined by reverse-phase HPLC following liquid–liquid extraction (ethyl acetate extraction after acidification of the samples) of the components of interest from the plasma. We found that the bioavailability of A-81988 depended on the oral dose used. As the dose was increased, the bioavailability decreased. Lower dosage (0.3 mg/kg or less, po) led to complete absorption of the drug.

(15) The binding of [^{125}I]saralasin (NEN) to AT_1 receptors in rat liver was performed as described by S. J. Fluharty and L. P. Reagan: Characterization of Binding Sites for the Angiotensin II Antagonist ^{125}I-[Sar^1,Ile^8]-Angiotensin II on Murine Neuroblastoma N1E-115 Cells. *J. Neurochem.* **1989**, *52*, 1393–1400. Rat liver membranes were prepared as described by D. M. Neville, Jr. (Isolation of an Organ Specific Protein Antigen from Cell-surface Membrane of Rat Liver. *Biochim. Biophys. Acta* **1968**, *154*, 540–552) and assayed using 1 μM angiotensin to define nonspecific binding. The binding of [^{125}I]-Tyr^4-angiotensin II (NEN) to AT_2 receptors in bovine cerebellum was performed using a kit (NED-001) obtained from New England Nuclear (NEN) and following a procedure described therein. For both AT_1 and AT_2 receptors, compounds were tested at multiple concentrations as required and analyzed as previously described (Hancock, A. A.; DeLean, A. L.; Lefkowitz, R. J. Quantitative Resolution of beta-adrenergic Receptor Subtypes by Selective Ligand Binding: Application of a Computer Model-fitting Technique. *Mol. Pharmacol.* **1979**, *16*, 1–9. DeLean, A. L.; Ong, H.; Gutkowska, J.; Schiller, P. W.; NcNicoll, N. Evidence for Agonist-induced Interaction of Angiotensin Receptor with a Guanine-nucleotide Binding Protein in Bovine Adrenal Zona Glomerulosa. *Mol. Pharmacol.* **1984**, *26*, 498–508.

0.98. All the analogs listed in Table I exhibited similar competitive antagonism under the same experimental protocol.

As shown in Figure 2, oral administration of 0.3 mg/kg of 18 in the conscious renal hypertensive rat lowered mean arterial blood pressure by 32–35% (control bp 165 ≈ 175 mmHg) for at least 24 h. There was no change in heart rate during the antihypertensive effect of 18.[16] In the same model, a much higher dose of DUP-753 was required (10 mg/kg po) to achieve a modest drop in bp (16–24%; see Figure 2). These data suggest that A-81988 is at least 30 times more potent than DUP-753 and has a much greater duration of action.

(16) Consistent with the finding of antihypertensive effects lasting 24 h, A-81988 (0.3 mg/kg, po) was found to selectively inhibit the vasopressor response to angiotensin II (0.1 μg/kg, iv) by 51–91% for at least 24 h in the conscious normotensive rat. In the same model, we have tested 18 against a host of vasoconstrictors and found that it does not antagonize the vasoconstriction effects induced by norepinephrine, vasopressin, or bradykinin.

In conclusion, we have discovered a novel class of angiotensin II antagonists with high affinity for the type-1 receptor (AT_1). The most potent analog of this series, A-81988, lowers blood pressure in both RAL and SHR rats (SHR data not presented) in a dose-dependent manner with a long duration (>24 h) of action. The overall profile of 18 identifies it as a powerful pharmacological tool for the investigation of a variety of AII mediated pathological conditions.

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Supplementary Material Available: Experimental procedure for the synthesis of all compounds (9 pages). Ordering information is given on any current masthead page.