Communications to the Editor

A Potent, Orally Active, Balanced Affinity Angiotensin II AT_1 Antagonist and AT_2 Binding Inhibitor

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The renin angiotensin system (Figure 1) constitutes a proteolytic cascade that results in the formation of the endogenous octapeptide hormone angiotensin II (Ang II).[1] The interaction of Ang II with its receptors leads to vascular smooth muscle contraction, release of noradrenalin, adrenalin, and vasopressin, reduction of renal blood flow and glomerular filtration rate, and increased sodium resorption resulting in a hypertensive response. Ang II may also act as a cellular growth factor and may therefore promote cardiac hypertrophy, arteriosclerosis, and restenosis following vascular injury.[2] Reduction of Ang II levels has been achieved by inhibition of the aspartyl protease renin[3] and the metalloprotease angiotensin converting enzyme (ACE).[4] ACE inhibitors such as captopril and enalapril have become an important therapy for the treatment of essential hypertension. The poor bioavailability of most renin inhibitors has prevented their development, and therefore ACE inhibition has become the method of choice for controlling renin-dependent hypertension. However, ACE is a nonspecific protease that is also responsible for the metabolism of the inflammatory hypotensive hormone bradykinin.[5] The inhibition of ACE results in the potentiation of bradykinin which has been proposed as the cause of the cough and angioneurotic edema that occurs in a small fraction of the population treated with ACE inhibitors.[6]

Antagonists of Ang II constitute a third approach to attenuating the effects of endogenous Ang II without affecting the metabolic processes of ACE. Although a peptidic antagonist saralasin (Sar[1]-Ala[8]-Ang II) has been investigated, it's use beyond a pharmacological tool has been precluded because of poor oral absorption, pharmacokinetics, and partial agonism.[7] The discovery of losartan (1) by Du Pont-Merck[8] and compound 2 (PD 123317) by Parke Davis[9] (Figure 2) led to the characterization of two Ang II receptors: AT_1 (selectively bound and antagonized by losartan) and AT_2 (selectively bound by 2).[10] Other ligands have since been reported that selectively interact with either receptor.[11] The AT_1 and AT_2 receptors are present in varying proportions in many tissues and organs.[11] To date, the majority of the effects of Ang II on the cardiovascular, renal, and central nervous systems are blocked by selective antagonists of the AT_1 receptor.[12] The structure, coupling mechanism, and functional correlates of the AT_2 receptor have not been definitively defined at this time.[12] Antagonism of the AT_1 receptor by losartan has been found to result in as much as a 10-fold increase in circulating Ang II levels through blockade of feedback inhibition of renin release.[13] Although the function of the AT_2 receptor is not known, the opportunity exists for the development of a compound that is an antagonist at both receptors with possible clinical advantages over an AT_1 selective antagonist.[6] Saralasin is an example of a peptidic ligand of both the AT_1 (IC_{50} = 4.8 nM, rat liver) and AT_2 (IC_{50} = 0.9 nM, R3T3 cells) receptors.[14] However, saralasin is a poor tool, due to its poor pharmacological properties and partial agonism. Compounds 3 (BIBS 39) and 4 (BIBS 222) are nonselective ligands of both the AT_1 and AT_2 receptors ($K_i(AT_2)/K_i(AT_1)$ = 17 and 37, respectively; AT_1 K_i = 29 and 20 nM, respectively).[15] However, the difference in binding affinity and the relatively low potency of 3 and 4 suggests that very large doses would be required to ensure that both the

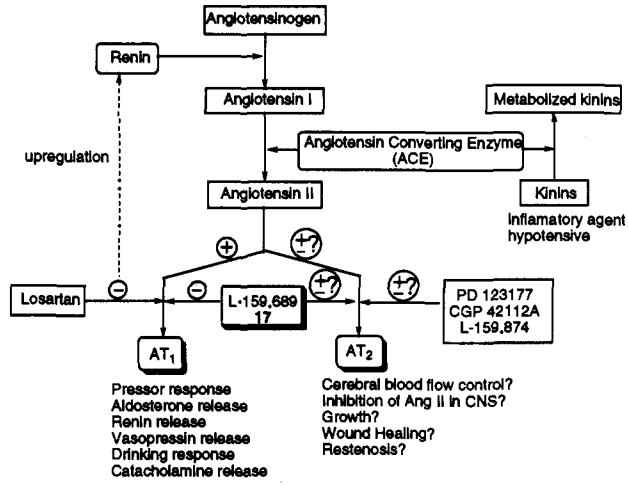

Figure 1. The renin angiotensin system: the receptors and functions. (+) or (−) represents agonism or antagonism, respectively, of the associated receptor.

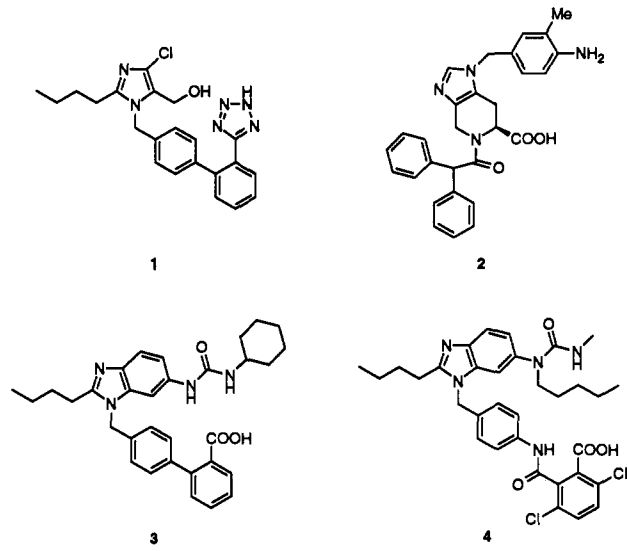

Figure 2. Representative AT_1-selective, AT_2-selective, and nonselective Ang II receptor ligands.

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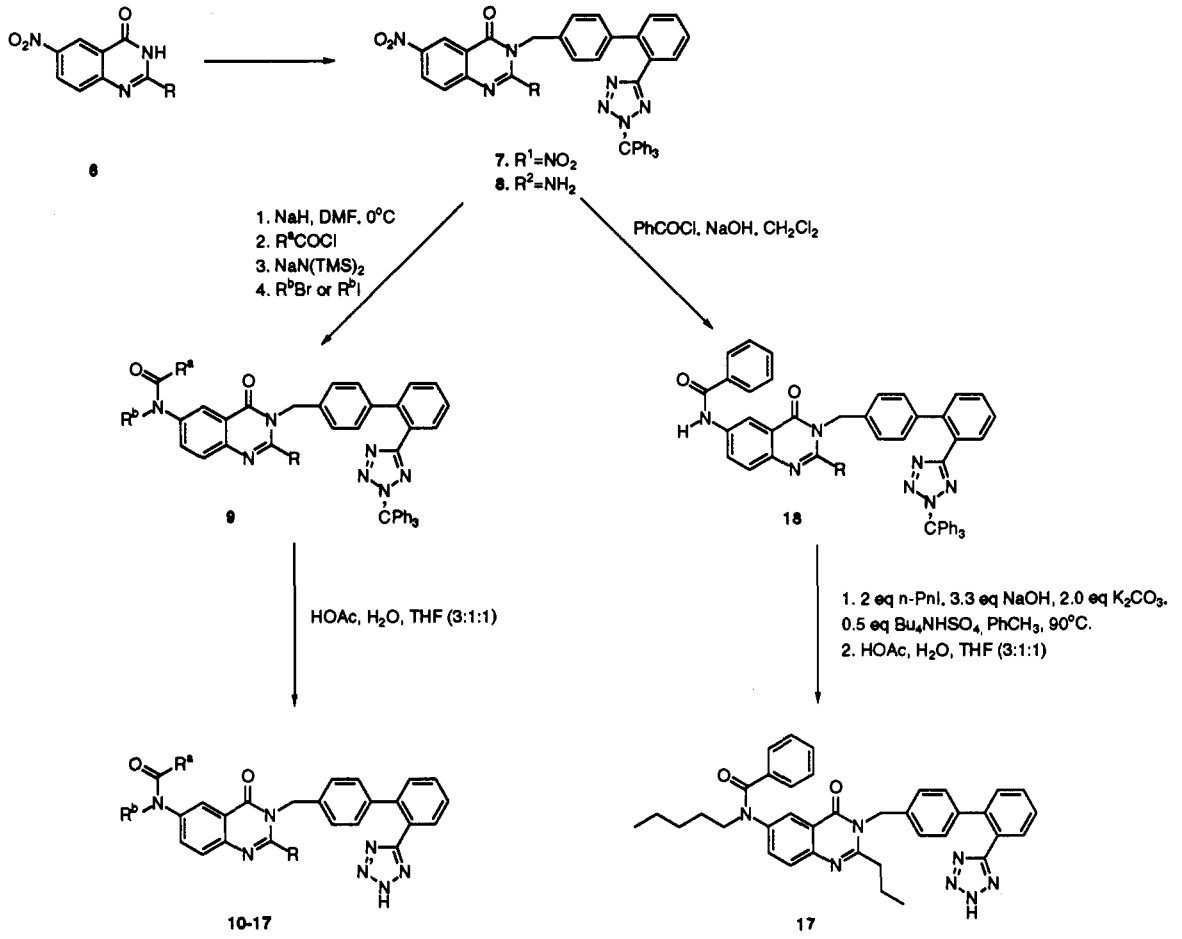
Scheme I. Synthesis of 6-(*N*-Alkyl-*N*-acylamino)quinazolinones



AT_1 and AT_2 receptors are blocked throughout the treatment period.

We previously reported the identification of **5** (L-159,093) (Table II) as a potent, orally-active antagonist of the AT_1 receptor.[16] Although **5** was orally active in rats and rhesus monkeys, compounds with superior bioavailability (e.g., an imidazo[4,5-*b*]pyridine, L-158,809)[17] have been identified in our laboratories. During further studies of the SAR of **5**, we discovered structural modifications that enhanced the binding affinity of the quinazolinone class of antagonists to the AT_2 receptor. We report the identification and initial pharmacological characterization of **17** (L-159,689), a potent antagonist of the AT_1 (IC_{50} = 1.7 nM, rabbit aorta) receptor and binding inhibitor of the AT_2 (IC_{50} = 0.7 nM, rat midbrain) receptor. The high potency, relative binding affinity (IC_{50} AT_2/AT_1 = 0.4), and oral activity of **17** make it a useful pharmacological tool for the study of the functional relevance of the AT_2 receptor.

The synthesis of 6-*N*-alkyl-*N*-acylquinazolinones is illustrated in Scheme I. 2-Alkyl-6-nitroquinazolinone **6** was alkylated under phase-transfer conditions with *N*-(triphenylmethyl)-5-[2-(4′-(bromomethyl)biphenylyl)]-tetrazole to provide the 3-N-alkylated quinazolinone **7** (57% yield). Reduction of **7** by hydrogenation over Raney nickel provided the amine **8**. The *N*-alkyl-*N*-acyl analogues were synthesized in a one-pot reaction: aniline anion formation, acylation with a chloroformate, and re-deprotonation followed by alkylation with an alkyl bromide or iodide to provide the intermediate **9** (in the case of compounds **10** and **11** acylation was followed by deprotection as described below). The tetrazole was deprotected under mild acidic conditions to provide the analogs **10–17** in good overall yield (Table I). Following the identification of **17**, an alternative synthesis was devised. Acylation of **8** under Schotten–Baumann conditions gave **18** in quantitative yield. The most convenient procedure for the N-alkylation of the amide was under phase-transfer conditions to give the precursor to **17** (70% yield). Deprotection provided **17** in 95% yield.

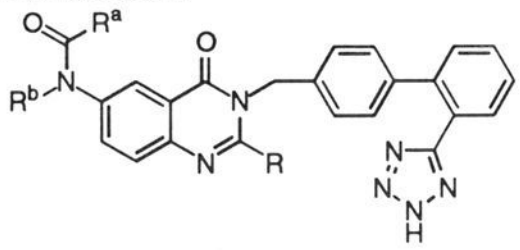
Table I. Structures, Yields, and Characterization of Angiotensin II Antagonists

compd	R	R^a	R^b	analysis[a]	FABMS[b]	% yield[c]
5	Bu	N(Me)-i-Pr	H	C,H,N	551	
10	Bu	O-i-Bu	H	C,H,N	552	46
11	Pr	O-i-Bu	H	C,H,N	538	52
12	Bu	O-i-Bu	Me	C,H,N	566	37
13	Pr	O-i-Bu	Me	C,H,N	556	51
14	Pr	O-i-Bu	Bn	C,H,N	628	57
15	Pr	O-*n*-Pr	Bn	C,H,N	614	59
16	Pr	*n*-Bu	Bn	C,H,N	612	43
17	Pr	Ph	Pn	C,H,N	650	63

[a] All compounds analyzed for C, H, N were within ±0.4% of the calculated formula values. [b] FAB mass spectrum $M^+ + 1$ ion. [c] Overall yield from 6-aminoquinazolinone.

The binding affinity expressed as an IC_{50} for the AT_1 and AT_2 receptors was determined by competitive blockade of the binding of [^{125}I]Sar^{1}Ile^8-Ang II with receptors present in rabbit aorta[18] (AT_1) and rat midbrain[19] (AT_2) tissue, respectively. The most potent 6-substituted quinazolinone previously synthesized and disclosed is **5**, a potent AT_1 selective antagonist as demonstrated by the IC_{50} ratio of AT_2/AT_1 of 45 000. Our approach for improving the oral absorption of this general class of compounds included the N-alkylation of the 6-acylamino

Table II. Binding Affinity to Rabbit Aorta (AT_1) and Rat Midbrain (AT_2) Receptors



compd	R	R^a	R^b	AT_1[a]	AT_2[b]	ratio AT_2/AT_1
5	Bu	N(Me)-i-Pr	H	0.1	4500	45000
10	Bu	O-i-Bu	H	4.0	5350	1337
11	Pr	O-i-Bu	H	3.9	2200	564
12	Bu	O-i-Bu	Me	2.5	2200	880
13	Pr	O-i-Bu	Me	2.1	210	100
14	Pr	O-i-Bu	Bn	4.9	0.6	0.12
15	Pr	O-Pr	Bn	2.5	0.8	0.32
16	Pr	*n*-Bu	Bn	2.4	0.4	0.16
17	Pr	Ph	Pn	1.7	0.7	0.41

[a] IC_{50} (nM) binding [^{125}I]Sar^{1}Ile^8-Ang II to rabbit aorta membranes in absence of BSA. Average of two or more determinations, error ≤ ±2-fold. [b] IC_{50} nM binding [^{125}I]Sar^{1}Ile^8-Ang II to rat midbrain in absence of BSA. Average of two or more determinations, error ≤ ±2-fold.

function, thereby removing a primary amide bond that may have reduced oral absorption, while attempting to maintain solubility by retention of heteroatoms within the 6-substituent. The 2-*n*-butyl carbamate **10** is less AT_1 selective based on the reduction in AT_1 binding affinity. The 2-*n*-propyl carbamate **11** retained AT_1 affinity but gained a factor of 2 in AT_2 affinity. N-Alkylation of **10** and **11** with methyl iodide provided **12** and **13**, and we were surprised to observe a further 10-fold improvement in AT_2 affinity for the 2-propyl analogue **13** whereas the 2-butyl analogue **12** was only 2-fold more potent at AT_2 than the unalkylated **11**. Increasing the size of the *N*-alkyl substituent improved the AT_2 binding affinity without affecting the AT_1 potency. The *N*-benzyl derivative **14** is the optimum *N*-alkyl substituent found in the isobutyl carbamate series ($AT_2/AT_1 = 0.12$). The carbamate itself was modified with little effect on the binding affinity as demonstrated by **15**. The isosteric amide **16** is equipotent with the carbamate **15**. Further optimization of the amide substituent groups led to the moderately AT_2-selective antagonist **17**.

The in vivo activity of analogues of interest was evaluated by assessing the inhibition of the Ang II pressor response to exogenously administered Ang II (0.1 μg/kg iv) in normotensive rats.[20] The carbamate **14** was found to have short duration in this model when administered iv (1.0 mg/kg, 60% peak inhibition of the Ang II pressor response, duration <2 h at over 30% inhibition ($N = 5$)). However, the equipotent amide **16** at 1.0 mg/kg iv elicited 48% peak inhibition of the Ang II pressor response and a duration of action of >6 h ($N = 6$). **17** produced a similar response to that of **16** (70% peak inhibition with duration of >6 h) following dosing at 1.0 mg/kg iv ($N = 7$). **17** was orally absorbed at 3.0 mg/kg, providing 73% inhibition of the Ang II pressor response with a duration of action of >6 h ($N = 5$). These results established that **17** is an orally active angiotensin II antagonist at the AT_1 receptor as determined by the inhibition of the Ang II pressor response in rats.

At this time no biological function can be attributed to the AT_2 receptor, and therefore an assay was developed to detect the presence of AT_1 and AT_2 bioequivalents in

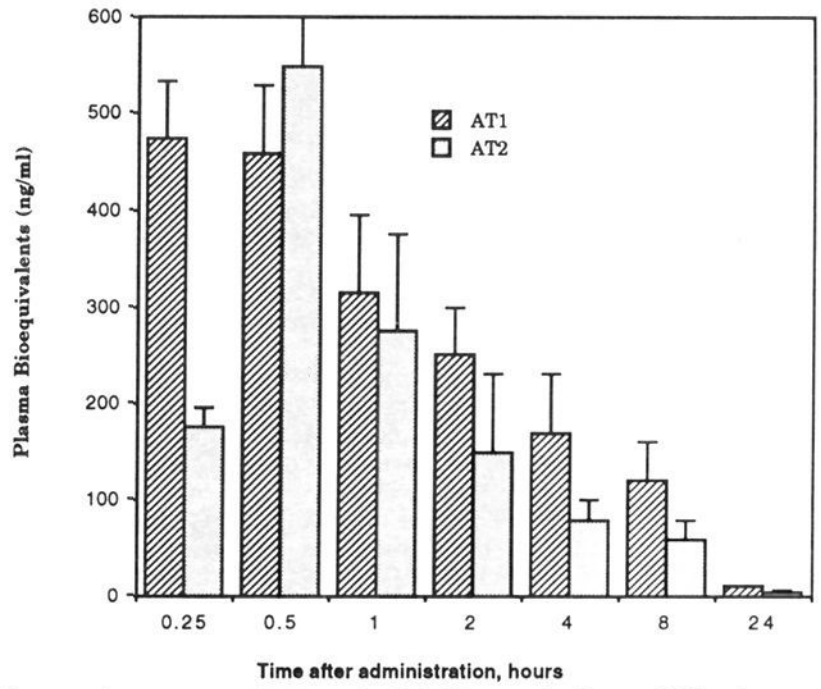


Figure 3. Plasma AT_1 and AT_2 bioequivalents following oral administration of **17** (15.0 mg/kg) to rats.[21] (Apparent concentration of **17** as determined by displacement of [^{125}I]Sar^1-Ile^8-AII from rabbit aorta (AT_1) and rat midbrain (AT_2) receptors.)

plasma by radioligand binding assays at the AT_1 and AT_2 receptors.[21] Following dosing of normotensive rats at 15.0 mg/kg po, plasma samples were drawn and assayed for the concentration of plasma bioequivalents that blocked binding of the nonselective radioligand [^{125}I]Sar^{1}Ile^8-AII from AT_1 and AT_2 receptors. Figure 3 illustrates that the plasma bioequivalents were approximately equal at both the AT_1 and AT_2 receptors throughout the experiment, establishing that balanced AT_1/AT_2 activity was maintained in vivo. The calculated $t_{1/2}$ was 4.2 and 3.4 h at the AT_1 and AT_2 receptors.

Scatchard analysis of [^{125}I]Sar^{1}Ile^8-AII binding with rabbit aorta (AT_1) and bovine cerebellum (AT_2) Ang II receptors in the presence and absence of **17** established that **17** is a competitive and reversible ligand at both receptors.[22] The K_i at the AT_1 and AT_2 receptors is 1.5 and 4.5 nM, respectively.

The ability of Ang II to release aldosterone from the adrenal cortex is an AT_1-mediated response.[23] The dose–response curves for release of aldosterone from rat adrenal cells were determined for Ang II in the presence and absence of 15 nM **17**.[23] The dose–response curve was shifted to the right without a reduction in the maximal response, and no induction of aldosterone release was observed in the absence of Ang II. Estimation of pA_2 values indicated that **17** ($pA_2 = 9.9$) is 100 times more potent than losartan ($pA_2 = 7.9$) in antagonizing aldosterone release in this preparation. **17** is a surmountable antagonist, devoid of agonist properties in Ang II-induced aldosterone release in rat adrenal cells.

Compound **17** is the first example of a potent balanced ligand of both the AT_1 and AT_2 receptors. By in vitro assays we established that **17** is a competitive and reversible ligand at the AT_1 and AT_2 receptors and is an antagonist of AT_1-mediated aldosterone release. By in vivo evaluation we demonstrated that **17** is orally active in rats. The blockade of the AT_1 receptor was determined by antagonism of the Ang II pressor response. The concurrent blockade of the AT_2 and AT_1 receptor is suggested by the maintenance of equivalent concentrations of both AT_2 and AT_1 ligands for greater than 8 h following po administration to rats.

The use of **17** in conjunction with the established AT_1- and AT_2-selective ligands should help to establish the

function of the AT_2 receptor and its place in the renin angiotensin system.

References

(1) Reviewed: Garrison, J. C.; Peach, M. J. Renin and angiotensin. In *Goodman and Gilmans the pharmacological basis of therapeutics*; Gilman, A., Rall, T., Nies, A., et al., Eds.; Pergamon Press: New York, 1990; pp 749–763.

(2) Reviewed: Peach, M. J.; Dostal, D. E. The angiotensin II receptor and the actions of angiotensin II. *J. Cardiovasc. Pharmacol.* **1990**, *16*, Suppl. 4, S25–S30.

(3) Reviewed: Greenlee, W. J. *Renin Inhibitors. Med. Res. Rev.* **1990**, *10*, 173–236.

(4) Reviewed: Wyvratt, M. J.; Patchett, A. A. Recent developments in the design of angiotensin-converting enzyme inhibitors. *Med. Res. Rev.* **1985**, *5*, 483–531.

(5) Erdos, E. G.; Skidgel, R. A. The unusual substrate specificity and the distribution of human angiotensin I converting enzyme. Special lecture. *Hypertension* **1986**, *8*, 40–48.

(6) Levens, N. R.; de Gasparo, M.; Wood, J. M.; Bottari, S. P. Could the pharmacological differences observed between angiotensin II antagonists and inhibitors of angiotensin converting enzyme be clinically beneficial? *Pharmacol. Toxicol.* **1992**, *71*, 241–249.

(7) Moore, A. F.; Fulton, R. W. Angiotensin II antagonists-saralasin. *Drug Dev. Res.* **1984**, *4*, 331–349.

(8) Duncia, J. V.; Chiu, A. T.; Carini, D. J.; Gregory, G. B.; Johnson, A. L.; Price, W. A.; Wells, G. J.; Wong, P. C.; Calabrese, J. C.; Timmermans, P. B. M. W. M. The discovery of potent nonpeptide angiotensin II receptor antagonists: a new class of potent antihypertensives. *J. Med. Chem.* **1990**, *33*, 1312–1329.

(9) Blankley, C. J.; Hodges, J. C.; Klutchko, S. R.; Himmelsbach, R. J.; Chucholowski, A.; Connolly, C. J.; Neergaard, S. J.; Van Nieuwenhze, M. S.; Sebastian, A.; Quinn, J. III; Essenburg, A. D.; Cohen, D. M. Synthesis and structure activity relationships of a novel series of non-peptide angiotensin II receptor binding inhibitors specific for the AT_2 subtype. *J. Med. Chem.* **1991**, *34*, 3248–3260.

(10) Reviewed: Timmermans, P. B. M. W. M.; Chiu, A. T.; Herblin, W. F.; Wong, P. C.; Smith, R. D. Angiotensin II receptor subtypes. *Am. J. Hypertension* **1992**, *5*, 406–410.

(11) AT_1-selective ligands reviewed in the following: (a) Buhlmeyer, P. Angiotensin-II receptor antagonists: patent activity since the discovery of DuP-753. *Curr. Opin. Therapeutic Pat.* **1992**, 1693–1718. (b) Wexler, R. R.; Carini, D. J.; Johnson, A. L.; Wells, G. J.; Chiu, A. T.; Wong, P. C.; Timmermans, P. B. M. W. M. Rationale for the development of Angiotensin II receptor antagonists. *Am. J. Hypertension* **1992**, *5*, 209S–220S. AT_2-selective antagonist CGP42112A: Whitebread, S.; Mele, M.; Kamber, B.; de Gasparo, M. Preliminary biochemical characterization of two angiotensin II receptor subtypes. *Biochem. Biophys. Res. Commun.* **1989**, *163*, 284–291. AT_2 selective piperazine ligands (L-159,874): Wu, M. T.; Ikeler, T. J.; Ashton, W. T.; Chang, R. S. L.; Lotti, V. J.; Greenlee, W. J. Synthesis and structure-activity relationships of a novel series of non-peptide AT_2 selective angiotensin II receptor antagonists; 205th American Chemical Society National Meeting; March 28th–April 2nd, 1993; MEDI No. 100.

(12) Timmermans, P. B. M. W. M.; Benfield, P.; Chiu, A. T.; Herblin, W. F.; Wong, P. C.; Smith, R. D. Angiotensin II receptors and functional correlates. *Am. J. Hypertension* **1992**, *5*, 221S–235S.

(13) Christen, Y.; Waeber, B.; Nussberger, J.; Porchet, M.; Borland, R. M.; Lee, R. J.; Maggon, K.; Shum, L.; Timmermans, P. B. M. W. M.; Brunner, H. R. Oral administration of DuP 753, a specific angiotensin II receptor antagonist, to normal male volunteers: inhibition of pressor response to exogenous angiotensin I and II. *Circulation* **1991**, *83*, 1333–1342. See also: Goldberg, M. R.; Tanaka, W.; Barchowsky, A.; Bradstreet, T. E.; McCrea, J.; Lo, M.-W.; McWilliams, E. J.; Bjornsson, T. D. Effects of Losartan on blood pressure, plasma renin activity, and angiotensin II in volunteers. *Hypertension* **1993**, *21*, 704–713.

(14) Hodges, J. C.; Hamby, J. M.; Blankley, C. J. Angiotensin II receptor binding inhibitors. *Drugs Future* **1992**, *17*, 575–593.

(15) Zhang, J. S.; Entzeroth, M.; Wienen, W. Characterization of BIBS 39 and BIBS 222: two new non peptide angiotensin II receptor antagonists. *Eur. J. Pharmacol.* **1992**, *218*, 35–41.

(16) de Laszlo, S. E.; Allen, E. E.; Quagliato, C. S.; Greenlee, W. J.; Patchett, A. A.; Nachbar, R. B.; Siegl, P. K. S.; Chang, R. S.; Kivlighn, S. D.; Schorn, T. S.; Faust, K. A.; Chen, T. B.; Zingaro, G. J.; Lotti, V. J. Quinazolinones 2. QSAR and in vivo characterization of AT1 selective AII antagonists. Bioorganic and Medicinal *Chem. Lett.* **1993**, *3*, 1299–1304.

(17) Mantlo, N. B.; Chakravarty, P. K.; Ondeyka, D. L.; Siegl, P. K. S.; Chang, R. S.; Lotti, V. J.; Faust, K. A.; Chen, T. B.; Schorn, T. W.; Sweet, C. S.; Emmert, S. E.; Patchett, A. A.; Greenlee, W. J. Potent orally active imidazo[4,5-b]pyridine-based angiotensin II receptor antagonists. *J. Med. Chem.* **1991**, *34*, 2919–2922.

(18) Chang, R. S. L.; Siegl, P. K. S.; Clineshmidt, B. V.; Mantlo, N. B.; Chakravarty, P. K.; Greenlee, W. J.; Patchett, A. A.; Lotti, V. J. In vitro pharmacology of L-158,809, a new highly potent and selective angiotensin II receptor antagonist. *J. Pharmacol. Exp. Ther.* **1992**, *262*, 133–138.

(19) Radioreceptor binding assay utilizing rat midbrain tissues in the absence of BSA and presence of 5.0 mM dithiothreitol to block residual AT_1 receptors as in ref 19.

(20) iv and oral studies as potassium salts (vehicle was saturated $NaHCO_3$ (15%), saline (35%), and distilled water (50%)) as described in the following: Siegl, P. K. S.; Chang, R. S. L.; Mantlo, N. B.; Chakravarty, P. K.; Ondeyka, D. L.; Greenlee, W. J.; Sweet, C. S.; Lotti, V. J. In vivo pharmacology of 17, a new highly potent and selective nonpeptide angiotensin II receptor antagonist. *J. Pharmacol. Exp. Ther.* **1992**, *262*, 139–144.

(21) The femoral vein of male Sprague–Daley rats was cannulated under anesthesia, and the animal was allowed to recover overnight with food and water *ad libitum*. Groups of three rats were used for each time point that plasma levels were to be determined. 17 (15 mg/kg) was dosed orally in 0.5% methylcellulose. At the proper time postdosing the rats were decapitated, and the blood was collected in a 100-mL cup containing 250 μL of 60 mg/mL EDTA. The blood was centrifuged and the plasma collected and frozen. To the plasma samples (0.5 mL) was added 2.5 mL of MeOH, and the resulting samples were mixed and then centrifuged at 3000*g* for 15 min. Aliquots (2.0 mL) were removed and dried with a Speedvac. The dried residues were dissolved in 0.5 mL of DMSO and diluted as required. Aliquots (10 μL) were used for AT_1 and AT_2 [^{125}I]Sar^1-Ile^8-AII binding assays with rabbit aorta[19] and rat midbrain,[20] respectively, and used to quantify the bioequivalents of AT_1- and AT_2-selective binding.

(22) By the method of Scatchard with bovine cerebellum and rabbit aorta tissues in the presence of 2.0 mg/mL BSA. Slightly higher values of K_i over IC_{50} may be due to presence of BSA. K_d values for [^{125}I]Sar^{1}Ile^8-AII were 0.19 and 0.18, respectively.

(23) 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.