Communications to the Editor

A Potent, Orally Active, Balanced Affinity Angiotensin II AT₁ Antagonist and AT₂ 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 renin³ 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 (Sar¹-Ala⁸-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-Merck⁸ and compound 2 (PD 123317) by Parke Davis⁹ (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 AT₁



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.

receptor.¹² The structure, coupling mechanism, and functional correlates of the AT₂ receptor have not been definitively defined at this time.¹² Antagonism of the AT₁ 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 AT₂ 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 AT₁ selective antagonist.⁶ Saralasin is an example of a peptidic ligand of both the AT_1 (IC₅₀) = 4.8 nM, rat liver) and AT_2 (IC₅₀ = 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 AT₁ and AT₂ 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

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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 AT₁ 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 (IC₅₀ = 1.7 nM, rabbit aorta) receptor and binding inhibitor of the AT_2 (IC₅₀ = 0.7 nM, rat midbrain) receptor. The high potency, relative binding affinity (IC₅₀ $AT_2/AT_1 = 0.4$), 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

 Table I. Structures, Yields, and Characterization of Angiotensin

 II Antagonists

compd	R	Rª	R⁵	analysis ^a	FABMS ^b	% yield•
5	Bu	N(Me)-i-Pr	Н	C,H,N	551	
10	Bu	O-i-Bu	н	C,H,N	552	46
11	Pr	O-i-Bu	н	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	\mathbf{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 $\pm 0.4\%$ of the calculated formula values. ^b FAB mass spectrum M⁺ + 1 ion. ^c Overall yield from 6-aminoquinazolinone.

under mild acidic conditions to provide the analogs 10-17in 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.

The binding affinity expressed as an IC₅₀ for the AT₁ and AT₂ receptors was determined by competitive blockade of the binding of [¹²⁵I]Sar¹Ile⁸-Ang II with receptors present in rabbit aorta¹⁸ (AT₁) and rat midbrain¹⁹ (AT₂) tissue, respectively. The most potent 6-substituted quinazolinone previously synthesized and disclosed is 5, a potent AT₁ selective antagonist as demonstrated by the IC₅₀ ratio of AT₂/AT₁ of 45 000. Our approach for improving the oral absorption of this general class of compounds included the N-alkylation of the 6-acylamino 15

16

17

Pr

Pr

Pr

O-Pr

n-Bu

Ph

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



 a IC₅₀ (nM) binding [¹²⁵I]Sar¹Ile⁸-Ang II to rabbit aorta membranes in absence of BSA. Average of two or more determinations, error $\leq \pm 2$ -fold. b IC₅₀ nM binding [¹²⁵I]Sar¹Ile⁸-Ang II to rat midbrain in absence of BSA. Average of two or more determinations, error $\leq \pm 2$ -fold.

Bn

Bn

Pn

2.5

2.4

1.7

0.8

0.4

0.7

0.32

0.16

0.41

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 AT₂ 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 AT₂ affinity for the 2-propyl analogue 13 whereas the 2-butyl analogue 12 was only 2-fold more potent at AT₂ than the unalkylated 11. Increasing the size of the N-alkyl substituent improved the AT₂ 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 AT₂-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 μ g/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 AT₁ 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 [¹²⁵I]Sar¹-Ile⁸-AII from rabbit aorta (AT₁) and rat midbrain (AT₂) receptors.)

plasma by radioligand binding assays at the AT₁ and AT₂ 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 [¹²⁵I]Sar¹Ile⁸-AII from AT₁ and AT₂ receptors. Figure 3 illustrates that the plasma bioequivalents were approximately equal at both the AT₁ and AT₂ receptors throughout the experiment, establishing that balanced AT₁/AT₂ activity was maintained in vivo. The calculated $t_{1/2}$ was 4.2 and 3.4 h at the AT₁ and AT₂ receptors.

Scatchard analysis of $[^{125}I]$ Sar¹Ile⁸-AII binding with rabbit aorta (AT₁) and bovine cerebellum (AT₂) 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 AT₁ and AT₂ receptors is 1.5 and 4.5 nM, respectively.

The ability of Ang II to release aldosterone from the adrenal cortex is an AT₁-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.

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- (19) Radioreceptor binding assay utilizing rat midbrain tissues in the absence of BSA and presence of 5.0 mM dithiothreitol to block residual AT₁ receptors as in ref 19.
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- (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 $250 \,\mu$ L 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 3000g 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 μ L) were used for AT₁ and AT₂ [¹²⁵I]Sar¹-Ile8-AII binding assays with rabbit aorta¹⁹ and rat midbrain.²⁰ respectively, and used to quantify the bioequivalents of AT1- and AT₂-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 IC₅₀ may be due to presence of BSA. K_d values for [125I]Sar1Ile8-AII were 0.19 and 0.18, respectively.
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