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L-161,638: A POTENT AT_2 SELECTIVE QUINAZOLINONE ANGIOTENSIN II BINDING INHIBITOR

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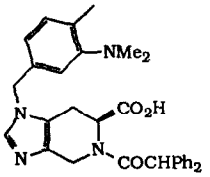
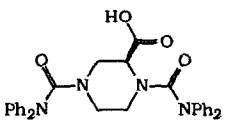
Abstract. The relative AT_1 and AT_2 binding affinities of 6-amino-2-alkyl-3-[((2'-tetrazol-5-yl)biphenyl-4-yl)methyl]quinazolin-4-(3H)-ones can be manipulated by changing the substitution at positions C-2 and N-6 of the quinazolinone nucleus. L-161,638 was identified as a potent orally bioavailable and AT_2 selective angiotensin II binding inhibitor.

Introduction

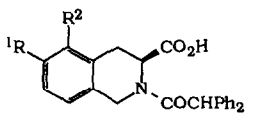
The renin-angiotensin system has attracted considerable interest due to its importance as a regulator of blood pressure. The interaction of angiotensin II (Ang II) with the AT_1 subtype of Ang II transmembrane receptors is known to trigger intracellular events responsible for the elevation of blood pressure.[1] Since the discovery of losartan,[2] the first potent nonpeptide AT_1 antagonist capable of blocking this interaction, many other potent nonpeptide antagonists of the AT_1 receptor have been discovered.[3] The biphasic binding of AT_1 receptor antagonists in some tissues lead to the identification of a second Ang II receptor subtype known as the AT_2 receptor.[4] Studies of the distribution of the AT_2 receptor and its physiological role have been possible due to the sensitivity of the AT_1 receptor to reducing agents such as dithiothreitol and to the availability of AT_2 selective binding inhibitors such as CGP42112A (peptidic)[5] and PD123319 (nonpeptidic).[6] More recently L-159,686,[7] PD 126055 [8] and an analog from DuPont Merck have achieved good AT_2 affinity.[9] Although the importance of AT_2 receptor mediated effects are not yet clear, the continued interest in this aspect of Ang II physiology is represented by recent publications on AT_2 receptor cloning and expression,[10] and possible functional correlates.[11]



PD 123319
AT_2 IC_{50}=6.9 nM
AT_1/AT_2=>10^5



L-159,686
AT_2 IC_{50}=1.5 nM
AT_1/AT_2=>10^5



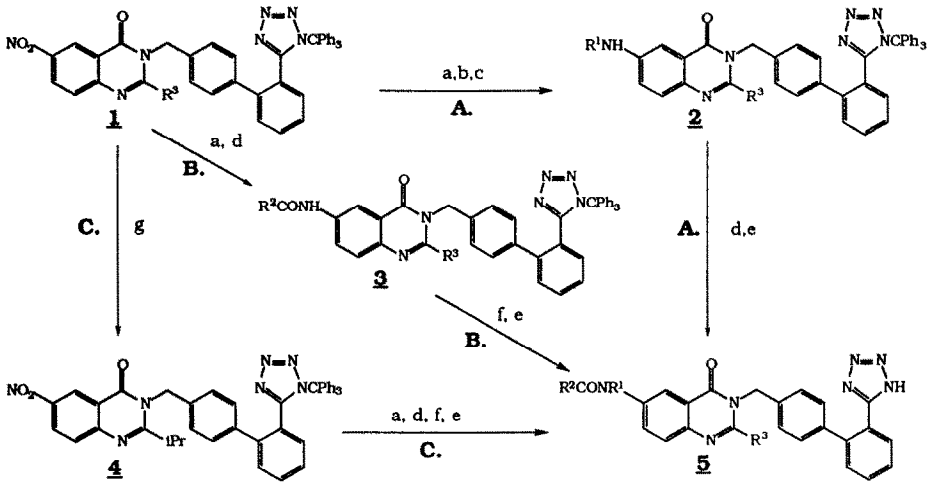
PD 126055
R^1=OMe R^2=OBn
AT_2 IC_{50}=0.58nM
AT_1/AT_2=>10^5

Du Pont
R^1= Ph R^2=H
AT_2 IC_{50}=30.0 nM
AT_1/AT_2=>10^5

The selective blockade of AT_1 receptors results in the blockade of inhibitory feedback receptors controlling renin release leading to an increase in Ang II levels.[12] The possibility that unblocked AT_2 receptors could lead to unwaranted side-effects has prompted the investigation of balanced affinity blockers of both receptors. We have recently reported the identification of selective antagonists of the AT_1 receptor [13] and also of antagonists with equal affinity for both AT_1 and AT_2 receptors[14] the most potent of which are illustrated by **1** and **2** (Table 1). The SAR derived from these studies suggested that AT_2-selective binding inhibitors could be derived by further modifications of this system. We describe herein the identication of L-161,638 (**13**), the most potent AT_2 receptor ligand discovered to date.

Chemistry

Scheme 1



a. Raney Ni, H_2; b. R^1CHO; c. $NaBH_4$; d. R^2COCl; e. 3:1:1 AcOH:THF:H_2O; f. NaH, R^1Br, DMF; g. **1** (R^3= Et) , MeI, 50% NaOH, CH_2Cl_2, Bu_4NBr.

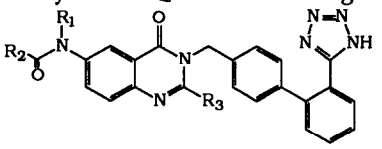
The synthetic strategy applied to the quinazolinone Ang II antagonists was analogous to that described previously for other series of AT_1 selective and balanced quinazolinone AII antagonists.[13,14] The 6-nitroquinazolinone intermediates **1** were converted into the final antagonists of the general structure **5** by several alternative routes as shown in Scheme 1. Route A consisted of reduction of the 6-nitro group in the presence of Raney Ni catalyst (use of Pd on carbon results in partial deprotection of the tetrazole nitrogen), followed by reductive alkylation to provide intermediate **2**. Acylation of the 6-amino group and acetic acid deprotection of the tetrazole produced the antagonists **5**. In route B, the 6-amino group was first acylated and the resulting amides **3** were alkylated and deprotected. Route C was applied only to the synthesis of the 2-

isopropyl quinazolinone by alkylation of the 2-position α–carbon of **1** (R_3=Et) to furnish the intermediate **4** which, in a sequence of steps identical to route B, was converted into the corresponding antagonist **5**.

Biology

The in vitro binding affinities of the compounds in Table 1 were determined by their ability to block the binding of the AT_1 and AT_2 ligand ^{125}I-Sar^{1}Ile^8-AII to receptors in rabbit aorta membrane (AT_1)[15] and rat midbrain (AT_2)[16] (the latter in the presence of dithiothreitol to prevent any residual AT_1 binding). Because of the errors inherent in the determinations of binding potencies, affinities which differ by 2-fold are not considered significant. In vivo studies were carried out in conscious normotentsive rats by iv and po administration at 5 mg/kg. Since no functional response can yet be measured for blockade of the AT_2 receptor, plasma samples were withdrawn periodically and the concentration of species binding to the AT_2 receptor was established by the AT_2 radioligand binding study described above. [17]

Table 1. *In Vitro* Activity Data of Quinazolinone Angiotensin II Antagonists



	R_1	R_2	R_3	AT_1[a] IC_{50}(nm)	AT_2[b] IC_{50}(nm)	selectivity (AT_1/AT_2 ratio)	synth. route[c]
1. (L-159,093)	H	i-Pr(Me)N	Bu	0.1	4500	0.00002	-
2. (L-159,689)	Pn	Ph	Pr	1.7	0.70	2.4	-
3.	Bn	i-BuO	Bu	5.1	33.00	0.2	-
4.	Bn	i-BuO	Pr	4.9	0.60	8.2	-
5.	Bn	i-BuO	Et	160.0	3.30	48.5	-
6.	$(CH_3)_2CCHCH_2$	Ph	Et	79.0	1.00	79.0	B
7.	Bn	Ph	Et	72.0	0.22	327.3	A
8.	Bn	4-pyr	Et	170.0	1.00	170.0	A
9.	2-Cl-Bn	Ph	Et	410.0	0.46	891.3	B
10.	Bn	Ph	Me	590.0	1.40	421.4	B
11.	2-Cl-Bn	Ph	Me	820.0	0.74	1108.1	B
12.	Bn	Ph	i-Pr	1200.0	0.35	3428.6	C
13.(**L-161,638**)	Bn	2-thienyl	Et	200.0	0.06	3333.3	B
14.	Bn	2-thienyl	iPr	42.0	0.24	175.0	B

a. Binding affinities determined in rabbit aortic membrane preparation as described previously; b. Binding affinities determined in rat midbrain membrane preparation with addition of DTT to abolish AT_1 receptor binding; c. For unspecified synthetic route see ref. 13 and 14.

Discussion

Our previous studies had established that the relative AT_1 and AT_2 binding affinities could be manipulated by changing the substitution at position C-2 and substituents on the 6-amino group on the quinazolinone nucleus. Investigation of balanced affinity ligands indicated that the AT_2 binding affinity could be enhanced by the incorporation of two lipophilic groups into a 6-amido substituent.[8] Comparision of the AT_2 selectivities of quinazolinones differing in the lengths of the aliphatic sidechains at the 2-position (cf **3**, **4** and **5**) indicated that alkyl groups shorter than n-propyl reduced AT_1 affinity while AT_2 affinity was maintained (for the case of **4** and **5**).[18] In this study directed toward an AT_2 selective antagonist, we found that 6-N-benzyl-N-benzoyl-amino rather than 6-N-alkyl-N-benzoyl-amino substitution enhanced AT_2 binding affinity. Among the benzyl group substitutions tested, the 2-chloro substituent was found to be consistent in increasing AT_2 selectivity (cf. **7** with **9** and **10** with **11**), providing 1000 fold selectivity for AT_2 affinity (**9** and **11**). Use of a isopropyl substituent at the 2 position resulted in still further selectivity increases (**12**). Heterocyclic replacements for the benzoyl group were examined, and a significant increase in AT_2 affinity was provided by the 2-thienyl amide L-161,638 (**13**). The 2-isopropyl analog **14** did not show improved selectivity, due to a considerably lower potency towards the AT_2 receptor.

Table 2. Oral bioavailabilities and $T_{1/2}$ of some quinazolinone AT_2 selective antagonists

	R_1	R_2	R_3	Oral bioavailability (%)	$T_{1/2}$	selectivity (AT_1/AT_2 ratio)	log P
6	$(CH_3)_2CCHCH_2$	Ph	Et	23	3 hr	79	4.2
8	Bn	4-pyr	Et	12	4 hr	170	3 4
10	Bn	Ph	Me	86	4 hr	420	3 5
12	Bn	Ph	i-Pr	20	2 hr	3400	4.5
13	Bn(L-161,638)	2-thienyl	Et	53	3 hr	3300	4.1

Although no functional assay is available to study the in vivo response to the blockade of AT_2 receptors, the oral bioavailability (over 8 hr) and duration (expressed as T 1/2 values) in rats was determined for some of the most potent and selective compounds in order to assess their potential as pharmacological tools in future investigations (Table 2). Although the compounds tested had similar plasma half-life, variation was observed in their oral bioavailability values. With the exception of the isonicotinoyl amide **8**, an inverse correlation was observed between oral bioavailability and log P value. The low bioavailability of **8** may be due to its zwitterionic character at neutral pH. The high potency and selectivity of **13**, in combination with its excellent oral bioavailability in rats, led to its selection for further studies focused on the function of the AT_2 receptor.

Conclusions

Appropriate substitution of 2-alkyl-6-amino-3-[(2'-tetrazol-5-yl)biphen-4-yl)methyl)]quinazo--lin-4(3H)-ones allows the relative binding affinity to the AT_1 and AT_2 receptors to be adjusted to provide selective ligands for either receptor or ligands that bind with equal affinity to both receptors. Potent AT_2 selective ligands are formed when the heterocycle is substituted at C-2 with one or two carbon alkyl groups, or branched alkyl substituents and when the N-6 group is a N-benzoyl-N-benzyl group or equivalent pharmacophore. Several members of this class are shown to be orally bioavailable and have good duration of action. Of these, compound **13** (L-161,638) was chosen for further study on account of its high AT_2 binding affinity and favorable pharmacological properties.

References and Notes

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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**, 749-763.
2. 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. J. Med. Chem. **1990**, *33*, 1312-13292.
3. Timmermans, P. B. M. W. M.; Wong, P. C.; Chiu, A. T.; Herblin, W. F.; Benfield, P.; Carini, D. J.; Lee, R. J.; Wexler, R. R.; Saye, J. M.; Smith, R. D. Pharmacological Reviews **1993**, *45*, 205-251.
4. Dudley, D. T; Panek, R. L.; Major, T. C; Lu, G. H.; Bruns, R. F.; Klinkefus, B. A.; Hodges, J. C.; Weishaar, R. E. Mol. Pharmacol., **1990**, *38*, 370-377.
5. Whitebread, S.; Mele, M.; Kamber, R.; de Gaspzro, M. Biochem. Biophys. Res. Comm. **1989**, *163*, 284-291.
6. 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. ; Quin III, J. ; Essenberg, A. D.; Cohen, D. M. J. Med. Chem. **1991**, *34*, 3248-3260.
7. Wu, M. T.; Ikeler, T. J.; Ashton, W. T.; Chang, R. S. L.; Lotti, V. J.; Greenlee, W. J. Biorg. Med. Chem. Lett. **1993**, *3*, 2023-2028.
8. Klutchko, S. Hamby, J. M.; Hodges, J. C. Bioorg. Med. Chem. Lett. **1993**, *4*, 57-62.
9. VanAtten, M. K.; Ensinger, C. L.; Chiu, A. T.; McCall, D. E.; Nguyen, T. T.; Wexler, R. R.; Timmermans, P. B. M. W. M. J. Med. Chem. **1993**, *36*, 3985-3992.
10. Mukoyama, M; Nakajima, M.; Horiuchi, M; Sasamura, H; Pratt, R. E.; Dzau, V. J. J. Biol. Chem. **1993**, *268*, 24539-24542 and Kambayashi, Y; Bardhan, S.; Takahashi, K; Tsuzuki, S.; Inui, H.; Hamakubo, T.; Inagami, T. J. Biol. Chem. **1993**, *268*, 24543-24546.
11. Timmermans, P. B. M. W. M.; Benfield, P.; Chiu, A. T.; Herblin, W. F.; Wong, P. C.; Smith, R. D. Am J. Hypertension **1992**, *5*, 221S-235S.

12. 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. Circulation, **1991**, *83*, 1333-1342.
13. 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. Bioorg. Med. Chem. Lett. **1993**, 3, 1299-1304.
14. de Laszlo, S. E.; Quagliato, C. S; Greenlee, W. J.; Patchett, A. A.; Chang, R. S. L; Lotti, V. J.; Chen, T-B; Scheck, S. A.; Faust, K. A.; Kivlighn, S. S.; Schorn, T. S.; Zingaro, G. J.; Siegl, P. K. S. J. Med. Chem. **1993**, *36*, 3207-3210
15. Chang, R. S. L.; Siegl, P. K. S.; Clineshmidt, B. V.; Mantlo, N. B.; Chakravarty, P. S.; Greenlee, W. J.; Patchett, A. A.; Lotti, V. J. J. Pharmacol. Exp. Ther. **1992**, *262*, 133-138.
16. Radioreceptor assay utilzing rat midbrain tissues in the absence of BSA and presence of 5.0 mM dithiothreitol to block residual AT_1 receptors as in reference 15.
17. A complete description of the in vivo protocol may be found in reference 14.
18. We had observed before that AT_2 binding affinity was reduced when C-2 was larger than n-propyl as discussed in reference 14.

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