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THE SAR OF 6-(N-ALKYL-N-ACYL)-2-PROPYL-3-[(2'-TETRAZOL-5-YL)BIPHEN-4-YL)METHYL]-QUINAZOLINONES AS BALANCED AFFINITY ANTAGONISTS OF THE HUMAN AT_1 AND AT_2 RECEPTORS

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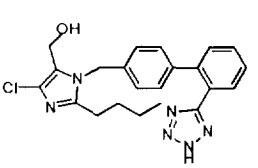
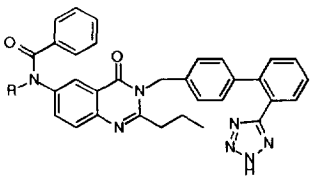
Abstract. Modification of the 6-N-alkyl-N-acyl groups of L-159,689, 6-(N-benzoyl-N-pentyl)-amino-2-propyl-3-[(2'-(tetrazol-5-yl)biphen-4-yl)methyl]quinazolin-4-(3H)one led to the identification of the 6-(N-benzoyl-N-(3-pyridylmethyl)) analog (L-162,537). L-162,537 had improved aqueous solubility and oral bioavailability in the dog. The SAR of this class of AT_1 and AT_2 ligands is discussed.

Introduction

Antagonism of the hypertensive peptide angiotensin II (Ang II)[1] by losartan constitutes a new approach to the treatment of hypertension.[2] Losartan is a selective antagonist of the AT_1 receptor which has been found to be responsible for all the cardiovascular and haemodynamic effects of angiotensin II (Ang II).[3] Ang II also interacts with the AT_2 receptor (or binding protein) for which no functional correlate has been definitively established.[2] Both the rat and human AT_1 and AT_2 receptors have been characterized through the use of selective ligands and have been cloned, sequenced and expressed.[4] Treatment of patients with losartan results in a 2.5 fold increase in the levels of angiotensin II through the blockade of feedback inhibition of the release of renin.[5] The potential exists for increased levels of angiotensin II to interact with unblocked AT_2 receptors. This prompted us to explore the development of Ang II antagonists which block both receptors simultaneously. Previously we described the discovery of the first potent, orally active, balanced affinity antagonist L-159,689 (**1**).[6] Although **1** (Tables 1 and 2) was orally active in rats, the compound was of short duration and was not orally bioavailable in dogs. In the rhesus monkey, long duration of action was elicited i.v. but the Ang II pressor response was not blocked following dosing at 10.0 mg/kg po. The lack of oral bioavailability in species other than the rat precluded consideration of development of **1** as a clinical candidate. We believed that the lack of oral absorption of **1** was due to its high lipophilicity (apparent log P = 5.1) and low aqueous solubility (<1 μg/ml at pH 5.5).



Losartan



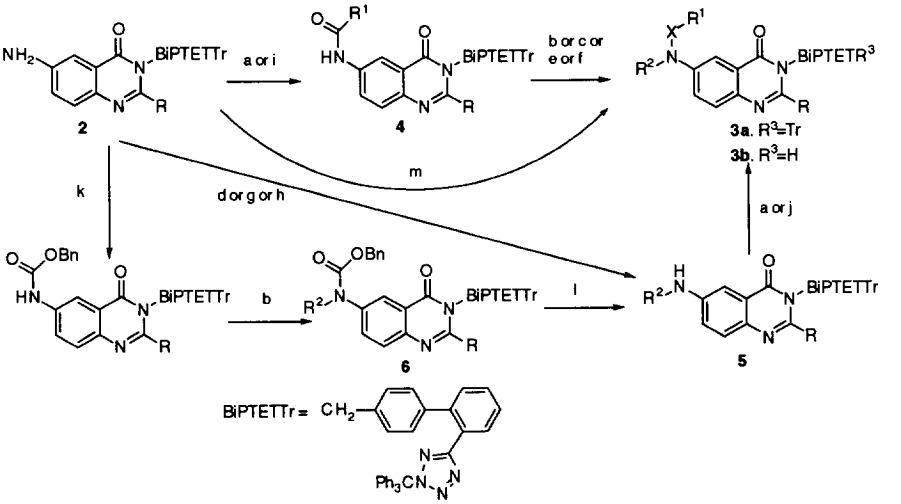
1. R=n-Pn
34. R=CH_2-3-pyr

Introduction of polar functional groups and heterocycles in place of the lipophilic N-n-pentyl and N-benzoyl substituents would lower the log P and increase aqueous solubility thereby possibly improving oral absorption. We wish to describe herein the structure-activity relationships of analogs of (**1**) and the identification of L-162,537 (**34**), an analog with improved pharmacological properties.

Chemistry

The quinazolinone **2** was prepared as described previously.[5] Conversion to the penultimate tetrazole protected intermediates **3a** was accomplished by one of several routes as shown below in Scheme 1. The synthetic approach varied according to the chemical compatibility of the substituents R^1 and R^2, the type and number of analogs required that incorporate a specific substituent and the improvements made in the synthetic methodology during the project. The most direct method (B1) to **3a** was a single reaction vessel conversion of the amine **2** to the intermediate acyl analog **4** which was alkylated *in situ* in the presence of NaH to provide **3a** in 30-70% yield. If several analogs were to be prepared varying only in R^2, the appropriate acylated analog **4** was prepared and purified and then alkylated under a variety of conditions to give **3a** in good yield. Variation of the acyl group while maintaining the same alkyl substituent was accomplished by preparing the secondary amine **5** either directly from **2** or via the carbobenzyloxy intermediate **6**. Acylation of **5** provided **3a**. Deprotection of the trityl protected tetrazole group in **3a** by treatment with acetic acid in water and THF (3:1:1) or HCl (cat)/MeOH gave analogs **3b**, enumerated in Table **1**, following purification by either flash or rotary chromatography.

Scheme 1



Route: B1:m, where X=CO or SO_2; B2: a; b; B3: a, c; B4 a; e; B5: k, b, l, a;i. B6: g, a B7: h, j; B8: d, a; B9: i, f; B10: a, f.
Conditions: a: R^1XCl, Et_3N or iPr_2NEt, DMAP, CH_2Cl_2.;b: $LiNTMS_2$, R^2I or R^2Br or R^2Cl, DMF, 0°C to r.t.; c: Bu_4NHSO_4, toluene, K_2CO_3, R^2I or R^2Br or R^2Cl., 100°C; d: R^2CHO, $PhCH_3$, heat then $NaBH_4$ in dioxane/EtOH. e: NaH, DMF, R^2I or R^2Br or R^2Cl r.t.; f: Bu_4NBr, CH_2Cl_2, 50% NaOH, R^2I or R^2Br or R^2Cl.; g: R^2OTs, K_2CO_3; h: Ph_3Bi, CH_3COOOH, $Cu(OAc)_2$, CH_2Cl_2; i: R^1COOH, EDC, HOBt; j: NaH, amine, R^1COCl; k: a with R^1= OBn, X=CO; l: H_2, 10% Pd/C; m: NaH, amine, R^1COCl, then $LiNTMS_2$, R^2I in DMF.

Results and Discussion

The binding affinity to the rabbit aorta AT_1, rat midbrain AT_2 and rat and human adrenal AT_1 and AT_2 receptors was measured for the antagonists illustrated in Table 1.[7] Single tissue rat and human adrenal assays were only carried out in the case of compounds meeting selective criteria for lipophilicity (log P) and binding affinity.

The influence of the carbonyl group was established through the synthesis of the isosteric, carbonyl transposition analog **7**, the tertiary amine **8** and sulfonamide **9**. The AT_2 binding affinity was improved when the carbonyl group was incorporated within the alkyl substituent instead of the benzyl substituent (**7** vs **1**). The presence of the carbonyl group is essential to good AT_1 affinity as demonstrated by the reduction in AT_1 affinity of **8** and **9** when compared to **1** and **7** respectively. The carbonyl group is also important for binding to the AT_2 receptor as reflected by the poor affinity of amine **8**. The poor affinity for the sulfonamide **9** for the AT_1 receptor indicates that an oxygen atom must not only be pendant to the X group in **3b** but must also be presented correctly within the receptor. This data suggests that the carbonyl oxygen atom of X may be interacting in a hydrogen bond with the AT_1 and AT_2 receptor or the carbonyl group influences the geometry of the pendant groups. Incorporation of a 4-fluoro group into the benzoyl R^1 group of **1** improved AT_2 affinity by seven fold (**10** vs **1**). One approach to reduce the lipophilicity of **1** was to reduce the size of the R^2 alkyl group (**11-12**). One carbon (**11**) could be removed with a concomitant reduction in log P without affecting binding affinity. Further reduction in the R^2 alkyl chain length (**12**) led to a reduction in AT_2 affinity. The thioether **13** lost binding affinity to the AT_1 receptor. Oxidation to the sulfoxide **14** led to a loss in AT_1 and a greater loss in AT_2 affinity which was partially restored on further oxidation to the sulfone **15**. The conformation of the benzamide group was modified by the introduction of a 2-methoxy group in **16** with little effect on binding affinity. An alcohol substituent should reduce lipophilicity and improve solubility, consequently the phenols **18** and **19** were prepared. The precursor of **18**, the O-benzyl ether **17** was surprisingly potent, considering the large size of the group attached to the benzoyl side chain, at both receptors. The phenols **18** and **19** retained affinity to the AT_1 and AT_2 receptor elicited by **1** with a moderate lowering of the log P. Both **18** and **19** were evaluated *in vivo* as the first example of more hydrophilic analogs of **1** (log P **18**=4.7 and **19** = 4.2 vs **1** = 5.1) that met our relative potency criteria.[5] Both analogs had a long duration of action in rats following i.v. administration. Compound **19** was evaluated following p.o. administration and demonstrated negligible oral efficacy. Phosphate prodrugs of alcohols are known to improve water solubility and this approach was applied to the phenol **19** in an attempt to improve oral bioavailability. We were surprised that the binding affinity to both receptors of **20** was largely retained when compared to **19**, although it is possible that phosphatases present in the membrane preparations may have catalyzed the hydrolysis of **20** to **19**. A similar duration of action as in the case of **19** was observed for **20** i.v. A measurable, but significant blockade of the Ang II pressor response was observed following oral administration of **20**, in contrast to **19**, where no blockade was measured, suggesting that the phosphate prodrug was better absorbed.

Heterocyclic replacements of the R^1 phenyl substituent are represented by **21-24**. These compounds retained both AT_1 and AT_2 binding affinity and the log P was reduced in the case of the pyridyl analogs **23** and **24**. Apart from **24**, the i.v. duration of these analogs was short. Replacement of the n-pentyl chain of **1** by more hydrophilic groups was explored with **25-30**. The ester **25** lost some AT_2 affinity but was nearly as potent as **1** at the AT_1 receptor. Hydrolysis of the ester to the acid **26** reduced AT_1 affinity to a small degree (4-fold) but reduced AT_2 affinity by 100-fold over **25**.

Table 1: Structures, binding affinity[a], log P, synthetic method and in vivo properties of analogs of general formula **3b**

#	R^1 X=CO unless stated otherwise	R^2	[b]IC_{50} (nM) AT_1; ratio AT_2/AT_1 rat midbrain/ rabbit aorta		[c]IC_{50} (nM) AT_1; ratio AT_2/AT_1 rat adrenal		[d]IC_{50} (nM) AT_1; ratio AT_2/AT_1 human adrenal		[e]log P	[f]Synth Route	[g]Rat i.v. dur. 1.0 mg/kg	[h]Rat p.o. dur. 1.0 mg/kg
1	Ph	Pn	1.7	0.7	-	-	6.9	1.0	5.1	B1	>3 hr	>3 hr
7	Bu	Bn	2.4	0.2	-	-	5.7	0.1	5.4	B1	>3 hr	>3 hr
8	Ph X=CH_2	Pn	20.0	6.5	-	-	-	-	-	note[i]	-	-
9	Bu X=SO_2	Bn	23.0	0.2	-	-	-	-	-	B1	-	-
10	Ph-4-F	Pn	1.7	0.1	-	-	-	-	5.2	B3	-	-
11	Ph-4-F	Bu	2.3	0.1	-	-	-	-	4.7	B3	-	-
12	Ph-4-F	Pr	1.9	2.0	-	-	-	-	4.3	B3	-	-
13	Ph-4-SMe	Pn	6.4	0.8	-	-	-	-	-	B3	-	-
14	Ph-4-SOMe	Pn	21.0	66.0	-	-	-	-	4.3	ex 13[j]	<3 hr	-
15	Ph-4-SO_2Me	Pn	7.2	6.2	-	-	-	-	4.4	ex 13[k]	-	-
16	Ph-2-OMe	Pn	1.1	1.5	-	-	-	-	5.0	B3	-	-
17	Ph-4-OBn	Pn	8.4	6.7	-	-	-	-	>5.7	B3	-	-
18	Ph-4-OH	Pn	0.9	1.3	0.5	3.1	4.9	5.8	4.7	ex 17[l]	>3 hr	-
19	Ph-4-OH	Bu	1.2	1.3	0.3	17.0	4.0	6.8	4.2	as 18	>3 hr	ND
20	Ph-4-OPO$(ONa)_2$	Bu	2.2	1.9	3.9	5.4	21.4	10.9	2.9	ex 19[m]	>3 hr	<3 hr
21	2-furyl	Pn	3.2	1.7	-	-	-	-	-	B1	<3 hr	-
22	2-thienyl	Pn	2.8	0.2	-	-	-	-	5.1	B3	<3 hr	-
23	3-pyr	Pn	4.5	0.8	-	-	-	-	4.3	B5	<3 hr	-
24	4-pyr	Pn	2.8	1.4	-	-	-	-	4.3	B3	>3 hr	-
25	Ph	-$(CH_2)_3CO_2Et$	2.3	2.3	-	-	-	-	4.1	B3	-	-
26	Ph	-$(CH_2)_3COOH$	9.5	65.3	-	-	-	-	2.3	ex 26[n]	-	-
27	Ph	-$(CH_2)_2O(CH_2)_2OMe$	2.2	6.6	-	-	-	-	3.6	-	-	-
28	Ph	$(CH_2)_2OEt$	1.6	4.9	-	-	-	-	3.9	B10	-	-
29	Ph	-$(CH_2)_4OAc$	1.3	3.7	-	-	-	-	3.3	-	-	-
30	Ph	-$(CH_2)_4OH$	2.6	5.1	-	-	-	-	-	ex 29[n]	-	-
31	Ph	Ph	2.5	7.8	-	-	-	-	-	B7	-	-
32	Ph	-CH_2-Ph	4.6	0.01	0.6	0.1	-	-	4.5	B2	<3 hr	
33	Ph	-CH_2-2-pyr	6.7	0.12	1.3	0.5	5.09	0.2	3.6	B2	-	-
34	Ph	-CH_2-3-pyr	1.7	0.2	0.3	2.1	0.9	1.3	3.4	B2	>3 hr	>3 hr
35	Ph	-CH_2-4-pyr	5.5	0.1	-	-	-	-	3.4	B2	-	-
36	Ph	-CH_2-2-furoyl.	18.5	0.02	-	-	-	-	-	-	-	-
37	Ph	-CH_2-Ph-4-OH	4.9	0.2	0.5	1.5	6.0	0.8	3.6	as 18	-	-
38	Ph	-CH_2-Ph-4-CO_2Me	3.9	0.5	0.8	1.9	9.6	1.7	4.4	B2	<3 hr	-
39	Ph	-CH_2-Ph-4-COOH	6.5	42.6	-	-	-	-	2.4	ex 38[n]	-	-
40	2-pyr	-CH_2-Ph	9.3	0.01	-	-	-	-	3.8	B5	-	-
41	3-pyr	-CH_2-Ph	11.0	0.04	-	-	-	-	3.7	B5	-	-
42	4-pyr	-CH_2-Ph	3.4	0.04	-	-	-	-	3.7	B1	-	-
43	4-Pyr	-CH_2-2-Pyr	9.7	0.9	2.9	1.6	4.4	2.3	2.7	B3	<3 hr	<3 hr
44	2-furyl	-CH_2-2-Pyr	7.5	0.2	2.1	0.3	10.6	0.3	3.2	B2	>3 hr	<3 hr

a. AT_1 and AT_2 potencies are mean values of the results of several independent measurements in fresh preparations of membrane fraction, each run in triplicate. The AT_2/AT_1 ratios were calculated as the ratios of the mean values of the receptor binding affinities. b. Determined in rabbit aorta (AT_1) and rat midbrain tissue (AT_2). c. Determined in rat adrenal tissue: DuP 753 (losartan) and PD 121981 (1 uM) used to block $AT_1$and AT_2 binding respectively. d. Determined in human adrenal tissue as in 'c' in the presence of 2 mg/ml bovine serum albumin. e. Log P estimated by HPLC. f. Synthetic route outlined in Scheme 1. g. Duration of >30% inhibition of the Ang II pressor response where short = < 3 hr, long = > 3 hours following dosing at 1.0 mg/kg i.v. h. As in 'e' following administration at 3.0 mg/kg p.o. i. Compound **2**, $NaCNBH_3$, PhCHO, MeOH then KH, PnBr, DMF. j. HOAC, 1.1 eq. CH_3COOOH. k. excess CH_3COOOH, HOAc. l. **17**, H_2, 10% Pd/C. m. **18** as **3a**, $NaNTMS_2$, $((BnO)_2PO)_2O$ then H_2, 10% Pd/C. n. NaOH, THF, MeOH.

Other analogs incorporating oxygen-containing functions (ethers **27** and **28** and the acetate **29** and the alcohol **30**) were potent AT_1 ligands, but lost a moderate amount of AT_2 affinity when compared to **1**. The increase in the AT_2/AT_1 ratio suggested that these analogs would not have balanced affinity in the rat or human adrenal assays and they were therefore not evaluated further (vide supra). In general, analogs of **1** that incorporate polar atoms or groups had reduced AT_2 affinity in the primary rat midbrain/rabbit aorta assay. Our criterion for balanced affinity in the human adrenal assay was a ratio of less than 1.0 in the primary assay. In order to find analogs with binding affinities that meet this criterion we decided to explore variants of **1** that did not possess the n-pentyl substituent. Replacement of the R^2 pentyl chain by phenyl (**31**) reduced the AT_2 affinity 16 fold. However, incorporation of a benzyl group in place of the pentyl chain (**32**) improved the AT_2 affinity seven-fold over **1**. We believed that this improved AT_2 affinity should enable us to incorporate more polar aromatic and heteroaromatic groups (which tend to reduce AT_2 affinity) while maintaining balanced affinity at the human receptor. To this end, the three isomeric pyridyl methyl analogs **33-35** were prepared. Although somewhat less potent at the AT_1 receptor (excepting **34**), all three analogs maintained AT_2/AT_1 ratios of less than 1.0. The 3-pyridyl isomer, **34** was as potent at the AT_1 receptor as **1** and maintained approximately equal binding affinity to both the AT_1 and AT_2 receptors in all tissues. The in vivo profile of **34** will be discussed below. In contrast to the pyridyl analogs, the furoyl analog **36** was an AT_2 selective ligand. The phenol and ester **37** and **38** had predictable affinities based on our earlier data, and the carboxylic acid **39** lost substantial affinity to the AT_2 receptor, as had been found in the case of the alkyl analog **26**. This result suggests that the presence of an acidic group detracts from AT_2 binding (as in the case of the acid **39**) and thus the pKa of the R^2 group must be lower than that of the phenol **37** to cause reduced AT_2 affinity (in the absence of any conformational or steric effects).

Several analogs incorporating heteroaryl substituents in place of the benzoyl group at R^1 were prepared as illustrated by (**40-42**). A number of analogs were prepared that combined R^1 and R^2 groups that individually reduced log P while retaining binding affinity to both receptors. Of these two examples stand out. The 4-pyridoyl, 2-pyridyl methyl analog **43** was slightly less potent at the AT_1 receptor than **34** and maintained approximately balanced affinity in the rat and human adrenal assays. Compound **43** had the lowest log P (2.7) of all the analogs prepared which maintained good overall binding affinity. Unfortunately, the short i.v. duration of action was reflected in the poor p.o. data. Compound **44**, in which the 4-pyridinoyl R^1 group was replaced by 2-furoyl, was more hydrophobic than **43** and approximately equipotent. The long duration of action of **44** i.v., was not observed following p.o. dosing, probably due to poor absorption.

Table 2 illustrates the improvement in both physical and biological properties that were elicited by the introduction of a 3-pyridyl methyl group (**34**) in place of the N-pentyl group of L-159,689 (**1**). The binding affinity to both receptors was enhanced and the relative affinity was the same (Table 1). The introduction of the protonatable pyridyl methyl group enhanced the water solubility substantially at acidic pH. Solubility in portions of the intestinal tract that are more basic would be maintained through tetrazole salt formation. Ang II pressor response inhibition studies were carried out in the rat and dog. The increased potency of **34** over **1** was reflected in the lower i.v. dose required to inhibit the pressor response for greater than 6 hours. **1** was not orally bioavailable in the dog and was of very short duration i.v. In contrast, in the dog **34** gave reproducible although short duration of action i.v. and was bioavailable following oral administration. **34** was well tolerated in the rhesus monkey. Bioavailability of **34** was determined in the chimp (N=2). Administration of 5.0 mg/kg i.v. was followed by periodic removal of plasma aliquots and determination of plasma bioequivalents of chemical species binding to the AT_1 and AT_2 receptors by radioligand binding assay.[5] The plasma half-life was 14 hours

and 9 hours at the AT_1 and AT_2 receptors respectively. Unfortunately, data from an oral dose of 20 mg/kg indicated that the bioavailability was only approximately 3%.

Table 2

		L-159,689 (1)	L-162,537 (34)
Solubility	pH 1.0	<5.0 μg/ml	150 μg/ml
	pH 5.5	≤1.0 μg/ml	7.0 μg/ml
	pH 8.0	>40 μg/ml	>40 μg/ml
log P		5.1	3.4
Rat	0.3 mg/kg i.v.	-	83% >6 hr
	1.0 mg/kg i.v.	78% >6 hr	-
	3.0 mg/kg p.o.	80% >4 hr	79% >6 hr
Dog	1.0 mg/kg i.v.	73% 0.5 hr	84% 1.5 hr
	3.0 mg/kg p.o.	variable	56% 1.8 hr
Chimp	5.0 mg/kg i.v.	-	$t_{1/2}$ = 14 hr (AT_1); 9 hr (AT_2)
	10 mg/kg p.o.	-	3% bioavailability

Conclusions

The presence of a carbonyl group is preferred at X in the general structure **3b** and may be hydrogen bonding to the receptors or influence the local conformation of pendant groups. Polar functional groups may be incorporated into both the R^1 and R^2 groups of **3b** either individually or in combination to reduce the lipophilicity of the antagonists and modify their physical properties. The AT_2 receptor is more sensitive than the AT_1 receptor to the introduction of polar subsituents. However, acidic groups (pKa<phenol) are detrimental to affinity at both receptors. Lower log P and increased water solubility tended to improve oral bioavailability. L-163,537 (**34**) has improved oral bioavailability over L-159,689 (**1**) in dogs. **34** has a long duration of action i.v. in the chimpanzee but is not orally bioavailable.

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