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DEVELOPMENT OF ANGIOTENSIN II ANTAGONISTS WITH EQUIPOTENT AFFINITY FOR HUMAN AT_1 AND AT_2 RECEPTOR SUBTYPES.

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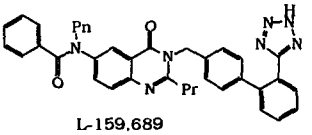
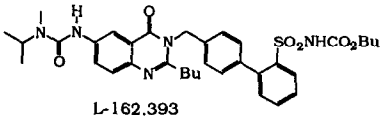
Abstract. The quinazolinone sulfonylcarbamate L-163,579 (9) is a potent, balanced antagonist of the binding of angiotensin II (Ang II) to human AT_1 and AT_2 receptors. This antagonist produces a long-lasting blockade of Ang II-induced pressor response in both rats and dogs after oral administration.

Introduction

The continuing high level of interest in angiotensin II (Ang II) antagonists is due to their therapeutic potential as alternatives to the well established antihypertensive ACE inhibitors.[1] Several different classes of potent Ang II antagonists have shown efficacy in animal models of hypertension and some are currently undergoing clinical trials.[2] To date, Ang II antagonists in development are selective for the AT_1 receptor which has been determined to be responsible for all the known physiological effects of Ang II.[3] The AT_2 receptor, of unknown physiological function, present in many tissues and selectively expressed at defined stages of development, has remained unblocked.[3] Clinical trials of losartan (an AT_1 selective Ang II antagonist) have shown that the plasma concentration of Ang II is increased ten fold due to the blocking of feedback inhibition release of renin.[4] The potential for increased concentrations of Ang II in the presence of receptor subtypes of unknown clinical significance to lead to possible side effects has prompted the development of antagonists that bind to both the AT_1 and AT_2 receptors.



L-159,689
IC_{50}:
AT_1= 1.5 nM (rabbit aorta)
AT_2= 0.7 nM (rat midbrain)
AT_1= 7.0 nM (human adrenal)
AT_2= 7.0 nM (human adrenal)

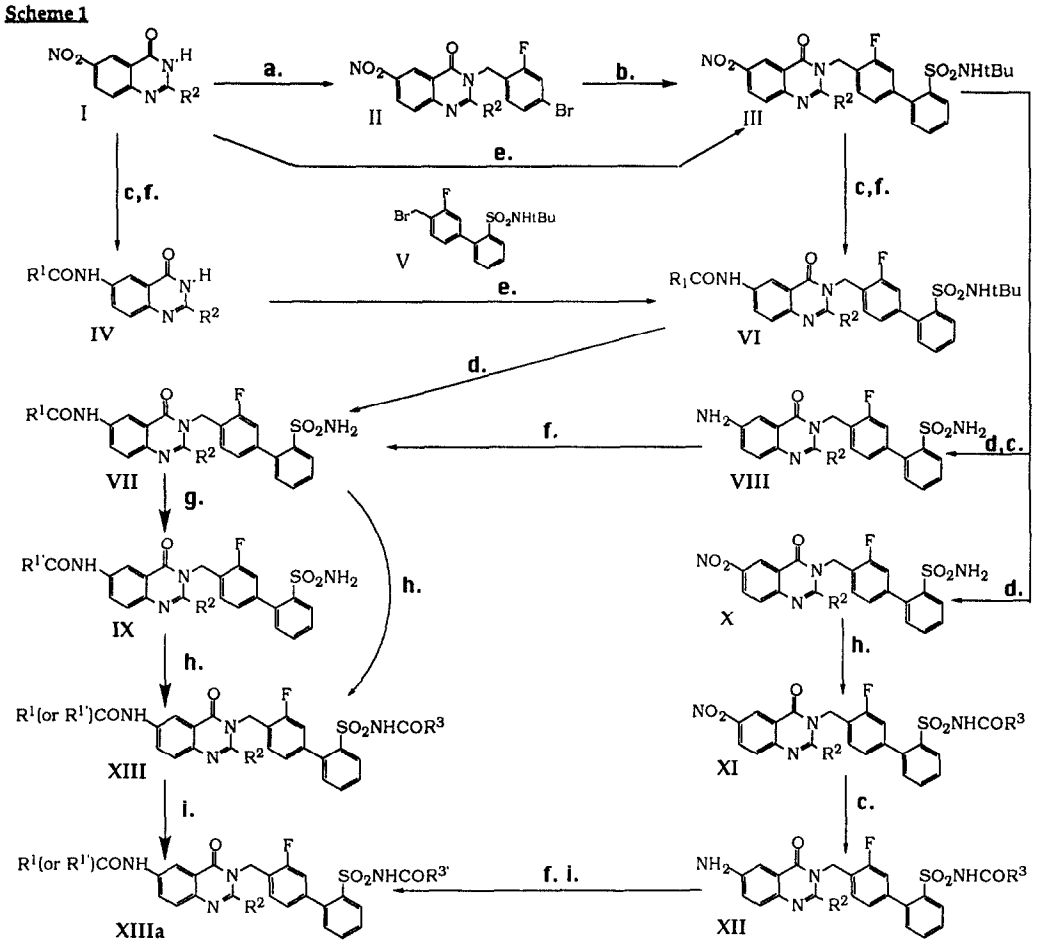


L-162,393
IC_{50}
AT_1= 0.24 nM (rabbit aorta
AT_2= 2.4 nM (rat midbrain)
AT_1= 4.0 nM (human adrenal)
AT_2= 60.0 nM (human adrenal)

6-Amino-2-alkyl-3-[(2'-(tetrazol-5-yl)biphen-4-yl)methyl]-quinazolinones can be modified to selectively antagonize the AT_1 receptor[5] and to antagonize the AT_1 receptor and bind with equal affinity to the AT_2 receptor as shown above for L-159,689.[6] Furthermore, this structural class may be modified to provide AT_2 selective binding inhibitors.[7] The AT_2 binding affinity of compounds such as L-159,689 is largely derived from the presence of two lipophilic substituents attached to the 6-amino group, and the presence of a linear or non-linear three carbon alkyl group at C2. We have shown previously that incorporation of an acyl aminosulfonyl or carbamoylaminosulfonyl group in place of the 2'-tetrazole substituent and in the absence of a disubstituted 6-amino group provided compounds with AT_2/AT_1 IC_{50} ratios of approximately 10, as illustrated by L-162,393.[8] The binding affinity ratios of angiotensin antagonists were also assessed in rat and human adrenal tissue.[9] The

binding affinity ratios in these tissues were generally increased although the extent of the increase depended on the structural class. In the case of the acylaminosulfonamide **2** the ratio of the AT_2/AT_1 IC_{50} increased from 10 to 15. Since no in vivo functional response assay existed to determine the extent of AT_2 receptor blockade, our goal for the AT_2/AT_1 IC_{50} ratio was less than or equal to 1.0 in human tissue. From our collaboration with the the DuPont Merck group we had learnt that a 2-fluoro-2'-acylaminosulfonyl-biphenyl group provided enhancement of AT_2 binding affinity over the 2-unsubstituted 2'-acylaminosulfonyl-biphenyl group.[10] We describe herein the incorporporation of the 2-fluoro-2'-acylaminosulfonyl-biphenyl group into the quinazolinone class of Ang II antagonists and the identification of L-163,579 (**9**) as an antagonist of the AT_1 receptor that binds with equal affinity to the AT_2 receptor.

Scheme 1



Synthetic routes: A: a,b,c,d,f,g,h.; B: c,f,e,d,g,h.; C: c,f,e,d,h.; D: c,f,e,d,g,h.;E. c,f,e,d,g,h,ı., F: e,d,c,f,h.; G : e,d,h,c,f.
a. 4-bromo-2-fluorobenzylbromide/K_2CO_3/DMF ; b. 2-(SO_2NHt-Bu)-PhB$(OH)_2$/$(Ph_3P)_4Pd$/toluene/ethanol/aq. NaOH; c H_2 10% Pd/C ; d. TFA/anisole ; e. 4-bromomethyl-3-fluoro-2'-(t-butylaminosulfonyl)-biphenyl (V)/K_2CO_3/DMF ; f . R^1NCO or R^1COCl or $COCl_2$ followed by i-Pr(Me)NH , g. heating in morpholine or ıPr(Me)NH / pyridine, h R^3COCl/DMAP/pyridine ; ı. heating in $R^{3'}OH$ (neat)

Chemistry

The synthetic methodology applied to the fluorobiphenyl quinazolinone antagonists was analogous to that described previously.[8] The synthetic routes A- G leading from the corresponding 2-alkyl-6-nitroquinazolinones **II** to the Ang II antagonists of general structure **XIII** or **XIIIa** are outlined in Scheme 1. Suzuki coupling of **II** with 4-bromo-2-fluorobenzylbromide provided intermediate **III** (R^3=Bu) which was then converted via Route A into several antagonists.[11] However, the variable yields of **III** prompted the development of alternative routes leading from 2-alkyl-6-nitroquinazolinones **I** to biphenyl intermediates **III** or **VI** which did not suffer from the apparent incompatibility of the nitro group with Suzuki coupling conditions. The biphenyl alkylating reagent **V** was obtained by coupling 4-bromo-2-fluorotoluene to 2-(SO_2NHt-Bu)-phenylboronic acid followed by NBS bromination. Alkylation of nitroquinazolinones **I**, or the more advanced intermediate **IV** (Scheme 1 step e.) furnished the respective N-alkylated quinazolinones **III** and **VI** in high yields. In both cases the N-alkylation product was accompanied by the O-alkylation by-product, which was removed by acidolysis during the deprotection of the t-butylsulfonamide with trifluoracetic acid (Scheme 1 step d.). The high yielding, one pot synthesis of 6-iPr(Me)NCONH-quinazolinone ureas by sequential treatment of 6-aminoquinazolinone **VIII** with phosgene and isopropylmethylamine was a considerable improvement over the previously reported method described for L-161,393 (Scheme 1 step f.).[8a] Other ureas were obtained either by direct condensation with the corresponding isocyanates (Scheme 1 step f.) or by transamination at elevated temperatures (Scheme 1 step g.). The sulfonyl carbamate functionality of **XIII** was found to be considerably more reactive towards such nucleophilic displacement and therefore the alcoholysis of **XIII** leading to the transalkoxylated sulfonylcarbamate **XIIIa** was conveniently used in some cases (Scheme 1 step i.).

Discussion

Antagonists were evaluated for binding affinity to AT_1 and AT_2 receptors in a variety of species and tissues as illustrated in Table 1. The AT_2 potency enhancing effect of the fluorine atom at the 2-position of the biphenyl was found to be effective in the quinazolinone series.[10] The rat midbrain/rabbit aorta IC_{50} AT_2/AT_1 ratios of **1**, **3** and **4** (Table 1) were found to be consistently 3-4 fold lower than the desfluoro analogs reported previously (modeling and NMR studies provided some insight into the nature of this fluorine effect and will be the subject of a separate publication).[12] Unfortunately, the human adrenal ratio IC_{50} AT_2/AT_1 = 8.7 of the fluorinated analog of L-161,393 (Table 1, entry **1**), although improved, was still unsatisfactory (desired value of IC_{50} AT_2/AT_1 = 1 *vide supra*). In general, antagonists with a 2-butyl or 2-propyl substituents (**1-6**), although highly potent towards both receptors, displayed IC_{50} AT_2/AT_1 ratios greater than 1.0. Only the combination of a fluorine atom substitution and shorter C-2 sidechain (**7-21**) provided a variety of balanced affinity Ang II antagonists. This observation is illustrated by comparing the C-2 homologs **2** and **9**, **4** and **16**, **5** and **14**, **3** and **10**, **3** and **20**. Within the 2-ethyl series with the same R^3 group but differing R^1 substituents (**7**, **8**, **9**) the binding affinities were very similar, although small differences in relative affinities to the receptor subtypes gave rise to changes in IC_{50} AT_2/AT_1 ratios. Within a series of 2-ethyl analogs with the same R^1 but different R^3 groups (**9-13**) similar binding affinities to the receptors are found except for the isobutyloxymethylene analog **11**, the affinity of **11** for the AT_1 receptor being substantially less than that found for the other members of the series. The morpholine urea present in **16** and **17** and the pyridyl amide in **18** illustrate the considerable variation of R^1 that may be introduced without compromising affinity. Introduction of a 4-ethyl group into analogs bearing the 2'-N-benzoylaminosulfonyl acidic function provided antagonists with improved binding affinity (cf **13**, **14** and **15**).[13] Among R^3 substituents, the isobutoxyacetyl was found to confer more AT_2 potency relative to AT_1 than any other type of sidechain. The use of a isobutoxyacetyl R^3 group provided the most balanced antagonist **6** in the 2-propyl series and the most AT_2 selective antagonist in the 2-ethyl series **11**. It is worth noting that while 2-butyl and 2-propyl substituted antagonists show higher

AT_2/AT_1 ratios in human adrenal than in rat adrenal, the situation in the 2-ethyl and 2-methyl substituted series is reversed. The sensitivity of relative receptor binding affinity may reflect the known differences in receptor primary structure.[14] The analog L-163,579 (**9**) was selected for in-depth evaluation due to its balanced potency in the human adrenal, relatively high *in vitro* activity and promising pharmacological properties in preliminary *in vivo* testing. IC_{50} values in other human tissue receptors were also determined for **9** and the affinities were found to be balanced to both receptors from kidney (IC_{50} AT_1=1.8nM, AT_2=1.7nM) and aorta (IC_{50} AT_1=4.1nM, AT_2=5.1nM) tissue.

In vivo Activity

Preliminary *in vivo* evaluation of some of the fully balanced antagonists shown in Table 1 allowed us to select **9** as the most interesting representative of the series. It has shown, in its potassium salt form, high i.v. activity in the inhibition of the Ang II induced pressor response in both rats (1.0 mg/kg iv. max. inhib. 91 ± 3% , 6hr. inhib. 62 ± 7%) and in dogs (1.0 mg/kg iv: max. inhi. 89 ± 2% , 6hr inhib. 48±1%).[15]

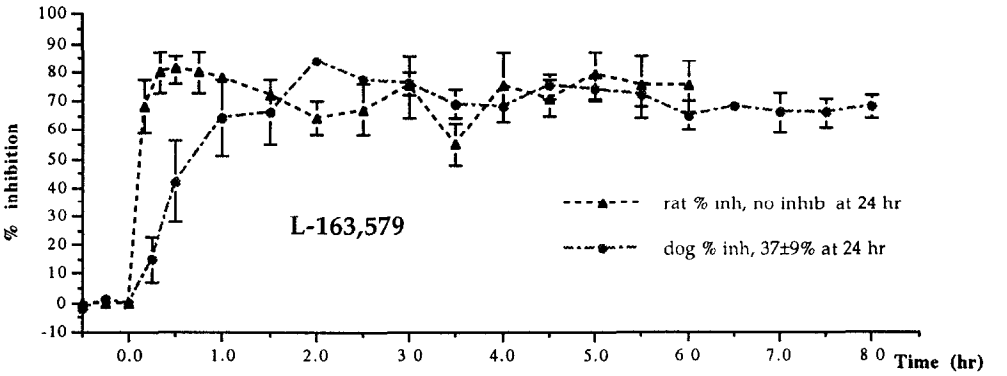


Fig.1 Inhibition of AII challenge by 3 mg/kg dose of potassium salt of L-163,579 in concious normotensive rats (n=2) and dogs (n=2) administered orally in 0.5 % methocel solution.

The ED_{50} values determined in rats were 0.29 and 0.76 mg/kg following i v. and po administration respectively. In both rats and dogs L-163,579 displayed potent, long lasting Ang II pressor response inhibitory activity when dosed orally at 3mg/kg as shown in Figure 1. At the same dose, 8 hr oral bioavailability was found to be 23% in rats and 17% in dogs (AUC, concentration measurement by AT_1 radioligand binding assay). In the absence of a functional response for blockade of the AT_2 receptor L-163,579 bioequivalents were determined by $AT_1$and AT_2 receptor binding assay of plasma samples withdrawn periodically following po and iv dosing in rats and dogs.[16] The study confirmed that in vivo AT_1 and AT_2 binding affinity was maintained throughout the experiment.

Conclusions

Structural modification of L-162,393, a prototypical AT_1/AT_2 balanced affinity quinazolinone Ang II antagonist which incorporates sulfonylcarbamate acidic functionality has led to considerable improvement in attaining balanced affinity towards the human Ang II receptor subtypes. Incorporation of a fluorine atom in the biphenyl moiety and truncation of the 2-position alkyl substituent resulted in a number of balanced Ang II antagonists with AT_2/AT_1 IC_{50} ratios =1 or less.

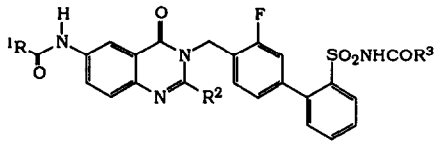


Table 1. *In vivo* activities (IC_{50}) of balanced quinazolinone antagonists in rabbit, rat and human tissue Ang II receptors.[a]

	R^2	synth route	R^1	R^3	Rabbit AT_1[b] nm	AT_2/AT_1	Rat AT_2[c] nm	Rat adrenal AT_1 nm	AT_2/AT_1	AT_2 nm	Human adrenal[d] AT_1 nm	AT_2/AT_1	AT_2 nm
1	Bu	A	iPr(Me)N-	n-butoxy-	0.19	*2.7*	0.44	0.25	*3.2*	0.77	0.89	*8.7*	7.7
2	Bu	A	iPr(Me)N-	2-cyclopropylethoxy-	0.13	*2.5*	0.29	0.23	*1.5*	0.36	1.2	*7.6*	7.8
3	Bu	A	iPr(Me)N-	3-Me-butoxy-	0.23	*1.3*	0.21	0.14	*1.5*	0.22	1.9	*4.4*	8.0
4	Pr	B	$O(CH_2CH_2)_2N$-	3-Me-butoxy-	0.24	*2.3*	0.48	0.13	*4.3*	0.50	0.71	*4.7*	3.3
5	Pr	C	iPrNH-	2-F-4-Et-Ph-	0.20	*1.4*	0.28	0.11	*2.3*	0.21	0.84	*5.3*	4.4
6	Pr	C	iPrNH-	i-butyl-O-CH_2-	0.52	*1.7*	1.0	0.17	*2.1*	0.38	1.5	*2.3*	3.5
7	Et	C	EtNH-	2-cyclopropylethoxy-	0.73	*0.87*	0.60	0.42	*2.3*	0.88	12	*0.77*	9.8
8	Et	C	iPrNH-	2-cyclopropylethoxy-	0.92	*0.61*	0.56	0.64	*0.92*	0.80	7.2	*0.90*	6.5
9	Et	F	iPr(Me)N-	2-cyclopropylethoxy-	0.57	*1.2*	0.39	0.50	*1.83*	0.77	8.1	*0.87*	6.2
10	Et	F	iPr(Me)N-	3-Me-butoxy-	0.62	*0.33*	0.20	0.30	*1.1*	0.30	10	*0.90*	9.2
11	Et	F	iPr(Me)N-	i-butyl-O-CH_2-	2.2	*0.10*	0.21	2.0	*0.30*	0.61	65	*0.18*	11
12	Et	C	iPr(Me)N-	$Me_2C{=}CHCH_2O$-	0.77	*0.97*	0.75	0.39	*2.7*	1.04	14.7	*0.62*	9.3
13	Et	C	iPr(Me)N-	4-Et-Ph-	0.54	*1.6*	0.70	0.38	*2.5*	0.93	5.8	*1.6*	8.9
14	Et	C	iPrNH-	2-F-4-Et-Ph-	0.19	*1.3*	0.25	0.14	*2.5*	0.34	1.6	*2.0*	3.1
15	Et	C	EtNH-	2-F-Ph-	4.0	*0.46*	1.6	3.9	*1.4*	4.5	59	*1.1*	65
16	Et	B	$O(CH_2CH_2)_2N$-	3-Me-butoxy-	2.0	*0.16*	0.31	0.66	*1.5*	0.95	18	*0.58*	10
17	Et	E	$O(CH_2CH_2)_2N$-	n-butoxy-	3.8	*0.38*	1.3	1.3	*2.2*	2.5	33	*1.0*	32
18	Et	G	2-pyridyl-	3-Me-butoxy-	2.3	*0.79*	1.8	1.3	*2.5*	3.8	85	*0.47*	37
19	Me	D	iPrNH	3-Me-butoxy-	1.2	*0.59*	0.70	0.5	*1.2*	0.63	15	*0.93*	11
20	Me	C	iPr(Me)N-	3-Me-butoxy-	2.3	*0.45*	1.05	0.58	*1.24*	0.72	26.5	*0.47*	12.5
21	Me	D	$(Me)_2N$-	2-cyclopropylethoxy-	2.3	*3.3*	6.6	2.3	*1.2*	2.4	12	*0.96*	12

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 mean values of ratios obtained when side by side determinations of AT_1 and AT_2 were run each time. b. Determined in rabbit aorta membrane preparation.[17] c. Determined in rat midbrain membrane preparation.[18] d. Determined in human adrenal membrane preparation stabilized with 2 mg/ml of bovine serum albumin (BSA). For AT_1 and AT_2 potency determination DuP 753 and PD 121981 (1 μm) were used to eradicate competing binding to $AT_1$and AT_2 respectively. Higher IC_{50} values obtained in the human adrenal receptors were due to nonspecific binding of antagonists to BSA. Several antagonists were tested in the absence of BSA using cloned AT_1 human receptors expressed in CHO cells, and had IC_{50} values 30-60 fold lower.

Relatively small structural modifications gave rise to changes in relative receptor binding affinities. L-163,579, a fully balanced Ang II antagonist, was shown to be a potent, orally active inhibitor of Ang II-induced pressor response in conscious normotensive rats and dogs. L-163,579 maintained balanced binding affinity following iv and po administration in rats and dogs.

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