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A NEW CLASS OF BALANCED AT_1/AT_2 ANGIOTENSIN II ANTAGONISTS: QUINAZOLINONE AII ANTAGONISTS WITH ACYLSULFONAMIDE AND SULFONYLCARBAMATE ACIDIC FUNCTIONALITIES

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Abstract. The structure activity relationships of a series of 2-alkyl-6-(acylamino)-3-[((2'-acylaminosulfonyl)biphenyl-4-yl)methyl]quinazolin-4-(3H)-ones were studied in order to identify balanced angiotensin II antagonists capable of potent binding to both AT_1 and AT_2 angiotensin receptor subtypes. The optimization of the substitution pattern led to the discovery of a potent, balanced quinazolinone antagonist **L-162,393**, which displayed long lasting blockade of angiotensin pressor response in rats, dogs and rhesus monkeys.

Introduction

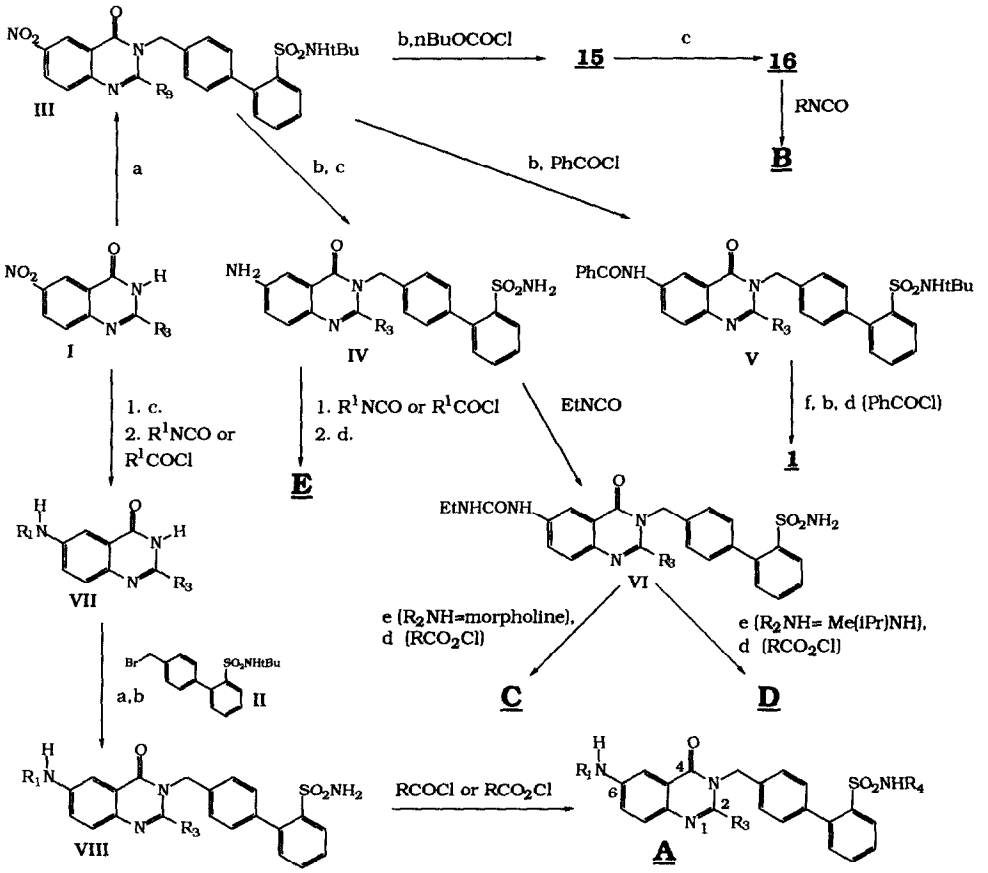
The use of angiotensin II (AII) antagonists has recently become an alternative approach to renin or ACE inhibitors in blocking the hypertensive response to endogenous AII [1]. DuP 753 (losartan) and other AII antagonists have been widely tested in various models of animal hypertension and have been shown to block AII induced blood pressure increases in different species [2]. These antagonistshave been demonstrated to be selective antagonists of one of the AII receptors, a G-protein coupled receptor now designated AT_1. The biphasic binding of some AT_1 selective antagonists in some tissues led to the discovery of a second AII receptor subtype now known as the AT_2 receptor. The identification and studies of the AT_1 and A T_2 subtypes were possible due to the development of selective AT_1 antagonists and AT_2 selective antagonists such as CGP42112A (peptidic) and PD123177 (nonpeptidic) [3]. Many functional responses have been identified for the AT_1 receptor (vasopressor response , aortic smooth muscle contraction, salt and water excretion from kidney, stimulation of adrenal aldosterone secretion), which is the predominant AII receptor in vascular tissue and liver, and is also widespread in other tissues where it is coexpressed with the AT_2 subtype. The role of the AT_2 receptor, which has been found to predominate in tissues such as rat midbrain [4], human uterus [5] and, more recently, in canine pancreas [6], is much less clear. Recently, however, AT_2 receptor-mediated effects of AII have been proposed in postangioplasty restenosis in rats [7], skin wound healing in rats [8] and collagen synthesis in human cardiac fibroblasts [9]. Due to increased plasma renin activity and AII levels resulting from administration of an AT_1 selective antagonist such as losartan [10], yet-unidentified *in vivo* responses mediated through the unblocked AT_2 receptor are possible. Thus, simultaneous blockade of both receptors might prove advantageous in the treatment of hypertension. We have investigated the design of a "balanced" AII antagonist capable of equipotently blocking AII binding to both receptors. Two benzimidazole-based AII antagonists BIBS-39 and BIBS-222 have been described by Karl Thomae [11], which showed K_iAT_2/K_iAT_1 ratios of 17 and 37 respectively. However the AT_2 potencies of these antagonists (K_i~0.5 μM) were modest. A recent publication from our group [12] revealed a more potent series of 2-propyl-6-(N-alkyl-N-acylamino)-3-[((2'-tetrazol-5-yl)biphen-4-yl)methyl-quinazolin-4(3H)-ones derived

balanced AII antagonists with AT_2/AT_1 IC_{50} ratios close to or below unity and IC_{50} values at the nanomolar level. Although antagonists in this series showed effective blockade of AII pressor response in rats, their evaluation in rhesus monkeys failed to demonstrate oral activity. In the present publication, the development of a related series of balanced quinazolinone AII antagonists with acylsulfonamide and sulfonylcarbamate acidic functionalities in place of the tetrazole are described which have improved *in vivo* profiles (both sulfonamide [13a] and sulfonylcarbamate [13b] acidic functionalities have previously shown favorable properties in other series of AII antagonists).

Chemistry

The synthetic strategies leading to the quinazolinone AII antagonists are schematically outlined in Scheme 1 along with the specific synthetic routes (**A-E**) employed for particular compounds **1-27** shown in Table 1.

Scheme 1.



a. K_2CO_3. II. DMF; b. TFA/CH_2Cl_2;c. H_2, 10% Pd/C; d.RCOCl or RCO_2Cl or RNCO or RCOimidazole as appropriate; e. R_2NH, heat; f. NaH, DMF, benzyl bromide

The 6-nitroquinazolinone **I** [14] was alkylated with the bromomethyl biphenyl derivative **II** [13a] in DMF in the presence of powdered potassium carbonate (the O-alkylated byproduct was not separated at this stage from the major 3-N alkylation product **III**, but was later decomposed in the trifluoroacetic acid deprotection step into easily separable products).

The intermediate **III** was used in several different sequences of transformations, which differ essentially only in the order of the transformations performed, leading to the same general type of final AII antagonist structure (routes **B-E**). Some difficulties were encountered in the synthesis of N',N'-dialkyl quinazolinone ureas such as **8** or **22**. Since the 6-amino function of quinazolinone **IV** was found to be quite unreactive towards carbamoyl chlorides, an alternative method was developed for the synthesis of these analogs. The N'-ethylurea **VI** was reacted at elevated temperature with a large excess of i-Pr(Me)NH (with pyridine as cosolvent) or with neat morpholine to give the corresponding transaminated urea intermediates, which were then transformed into the final AII antagonists (routes **C** and **D**). Alternatively, the 6-position of **I** was first elaborated into urea or amide functionality, and the resulting intermediate **VII** was alkylated with the bromomethyl derivative **II** yielding (after TFA deprotection) the sulfonamide **VIII**. Still another sequence of steps was employed for the synthesis of **1** which is the only example with tertiary amide functionality at the 6-position of the quinazolinone. The sulfonamide-protecting t-butyl group was retained in intermediate **V** to minimize the unwanted sulfonamide alkylation during the benzamide alkylation step. The benzylation of **V** furnished the desired benzylation product, albeit in low yield, without any need for additional protecting group manipulation.

Discussion

We have previously developed a series of quinazolinone biphenyltetrazole analogs as balanced AII antagonists [12]. Due to their short duration of action after i.v. and oral administration to dogs and lack of oral activity in monkeys, we turned our attention towards quinazolinones with sulfonamide based acidic functionality. At the outset of the study, we employed the 6-(N-acyl-N-alkylamino) substituent, which had been found to be crucial for high AT_2 activity in the quinazolinone biphenyltetrazole series. Although the binding potency of antagonist **1** was nearly balanced (IC_{50} AT_2/AT_1=2.2), its binding affinities were found to be ~ 2 orders of magnitude lower than those of similar tetrazole derivatives. After several unsuccessful attempts to increase the potencies of such 6-(N-acyl-N-alkylamino) substituted quinazolinones, a breakthrough came when the secondary amide **2** was found to have improved AT_1 potency without suffering a large increase in IC_{50} AT_2/AT_1 ratio. Unfortunately, the potassium salt of **2** had the disadvantage of very low solubility. The diacetyl analog **11** although more soluble (as its potassium salt) than **2**, showed ~ 20 fold lower binding to both receptors. Among acylsulfonamides tested the best IC_{50} AT_2/AT_1 ratio was achieved with the 3-Me-2-furoyl acyl group **7** which had been previously used in triazolinone AII antagonists [15].

We found that an acidic sulfonylcarbamate of the appropriate sidechain length confers greater AT_2 activity than does a benzoyl sulfonamide (cf. **3** *vs.* **5**; **2** *vs.* **26**). The optimal carbamate sidechain length was found to be C_4, and extending it further did not improve AT_2 binding (cf. **4** *vs.* **5**), and was even found to be deleterious in some cases (cf. **22** *vs.* **24**). The carbamate sidechain appears to occupy a lipophilic pocket of the AT_2 receptor since heteroatoms are not well tolerated (cf. **6** *vs.* **5**). Branching of the end of the sidechain resulted in a small additional increase in AT_2 binding (cf. **8** *vs.* **10**; **14** *vs.* **21**) and resulted in an AII antagonist **10** with the lowest IC_{50} AT_2/AT_1 ratio in the series.

With the optimized sulfonylcarbamate functionality in place, attention was focused on the substituents on the quinazolinone ring. The presence of amide type functionality at the 6-position was found to be necessary for high AT_2

affinity (cf. **15** and **16** *vs.* **17**) , but not for AT_1 binding. Comparison of **18** and **25** illustrates that both amides and isosteric ureas have equivalent affinity to each receptor.

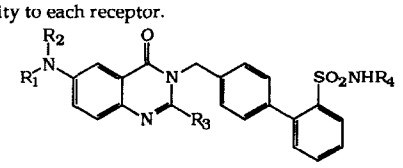


Table 1. *In Vitro* Activity Data of Quinazolinone Angiotensin II Antagonists

Method		R_3	R_2	R_1	R_4	IC_{50}^a (AT_1) nM	IC_{50}^b (AT_2) nM	AT_2/AT_1 ratio
1	E	Et	CH_2Ph	COPh	COPh	180	400	2.2
2	A	Pr	H	COPh	COPh	0.99	16	16
3	E	Bu	H	CONHiPr	COPh	0.15	12	80
4	E	Bu	H	CONHiPr	CO_2n-Pn	0.17	2.9	17
5	E	Bu	H	CONHiPr	CO_2n-Bu	0.15	3.3	22
6	E	Bu	H	CONHiPr	CO_2-(2-OMe-Et)	0.32	13	41
7	E	Bu	H	CONHEt	CO-2-(3-Me-Furan-2-yl)	1 1	8.9	8.1
8	D	Bu	H	CON(Me)iPr	CO_2n-Bu	0.24	2.4	10
9	E	Bu	H	CONHEt	CO_2n-Bu	0.24	7.4	31
10	D	Bu	H	CON(Me)iPr	CO_2-(3-Me-Bu)	0.33	1.6	4.9
11	E	Pr	H	COMe	COMe	16	290	18
12	E	Pr	H	CONHiPr	CO_2n-Bu	0.12	3.6	30
13	E	Pr	H	CONHiPr	CO_2n-Pr	0.25	20	80
14	E	Pr	H	CONHEt	CO_2n-Bu	0.18	6.3	35
15	B	Pr	O	O	CO_2n-Bu	15	580	39
16	B	Pr	H	H	CO_2n-Bu	0.86	170	200
17	E	Pr	H	CONHMe	CO_2n-Bu	0.26	10	38
18	B	Pr	H	CONHn-Pr	CO_2n-Bu	0.18	3.6	20
19	B	Pr	H	CONHn-Bu	CO_2n-Bu	0.25	4.6	18
20	A	Pr	H	CO(2-Furan-yl)	CO_2-(3-Me-Bu)	0.36	5.0	14
21	E	Pr	H	CONHEt	CO_2-(3,3-di-Me-Bu)	0.43	4.4	10
22	C	Pr	H	$CON(CH_2CH_2)_2O$	CO_2-(3-Me-Bu)	0.25	2.8	11
23	D	Pr	H	CON(Me)iPr	CO_2n-Bu	0.21	3.2	15
24	C	Pr	H	$CON(CH_2CH_2)_2O$	CO_2-(4-Me-Pn)	0.39	8	20
25	E	Pr	H	COn-Bu	CO_2n-Bu	0.19	5.3	28
26	A	Pr	H	COPh	CO_2-(3-Me-Bu)	0.35	2.1	6.1
27	B	Pr	H	CONHPh	CO_2-(3-Me-Bu)	0.55	15	28

a. Binding affinities determined in rabbit aortic membrane preparation as described previously [16]; b. Binding affinities determined in rat midbrain membrane preparation [17] with addition of DTT to abolish AT_1 receptor binding .

Longer aliphatic sidechains at the N' nitrogen atom of the 6-position urea had only small positive effect on AT_2 binding (cf. **17** vs. **14**, **18** and **19**). The N'-(Me)iPr urea substituent, employed previously in the AT_1 selective tetrazole quinazolinone L-159,093 [18], was superior to other substituents in providing both potency and balanced activity (cf. **5** and **9** with **8**). The N'-(Me)iPr urea functionality also proved favorable for *in vivo* activity as exemplified by **8** (**L-162,393**, *vide infra*).

In Vivo Activity

Several balanced quinazolinone antagonists (as their potassium salts) were found to inhibit pressor response to AII in conscious, normotensive rats after intravenous or oral administration (see ref. [15] for *in vivo* testing methodology). The quinazolinone **8** (**L-162,393**) distinguished itself with an outstanding *in vivo* activity profile, blocking AII pressor response in rats (1 mg/kg i.v. or p.o.) with a duration of action exceeding 6 hours (Fig.1).

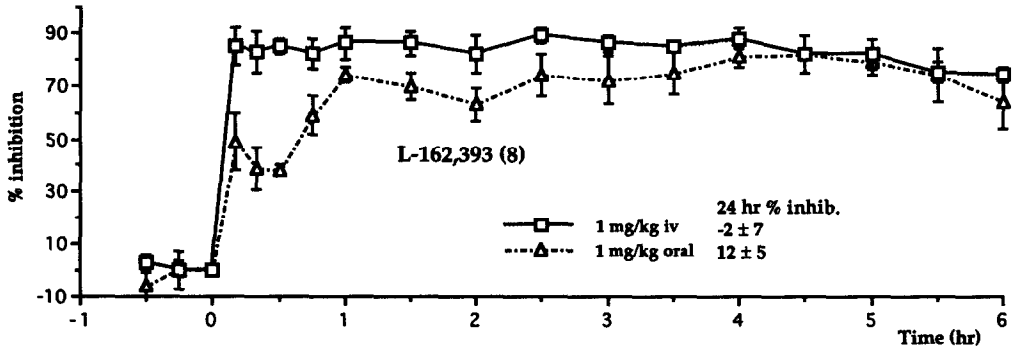


Fig. 1. Inhibition of AII induced response (mean arterial blood pressure increase) after i.v. and oral administration of L-162,393 (8) to normotensive rats vs time.

This antagonist was superior to other quinazolinones when administered orally to dogs, and also showed oral activity in rhesus monkeys (Table 2).

Table 2

In vivo AII pressor response blocking activity of **L-162,393** (potassium salt) in conscious rats, dogs and rhesus monkeys.

Rat		**% max. inhibition**	**duration**	**n**
1.0 mg/kg	i.v.	97 ± 3	>6 hr	(4)
0.1 mg/kg	i.v.	64 ± 5	>4 hr	(6)
i.v. ED_{50}=0.067 mg/kg				
1.0 mg/kg	p.o.	90 ± 5	>5.5 hr	(4)
0.3 mg/kg	p.o.	72 ± 4	>3 hr	(4)
p.o. ED_{50}=0.22 mg/kg				
Dog				
0.3 mg/kg	i.v.	99 ± 1	>6 hr	(4)
3.0 mg/kg	p.o.	96 ± 4	>24 hr	(2)
Monkey				
0.3 mg/kg	i.v.	86 ± 6	>5 hr	(4)
3.0 mg/kg	p.o.	71 ± 6	>6 hr	(4)

Conclusions

The quinazolinone AII antagonists described in the present publication represent a new class of potent angiotensin II binding inhibitors capable of blocking both AT_1 and AT_2 receptors. Due to this particular characteristic, such antagonists may prove to have an advantage over AT_1 selective antagonists in the treatment of hypertension. Some representatives of this series have outstanding oral activity in various species. Of these, L-162,393 shows a nearly balanced binding profile (IC_{50} AT_2/AT_1 = 10) and is orally active in three species (rats, dogs and monkeys). Efforts to develop more potent, fully balanced AII antagonists are underway.

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