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QUINAZOLINONES 2: QSAR AND IN VIVO CHARACTERIZATION OF AT1 SELECTIVE AII ANTAGONISTS

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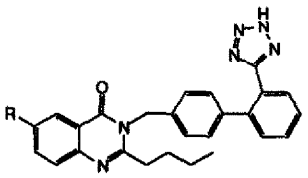
Abstract. The structure activity relationship, linear regression analysis and in vivo evaluation of a series of substituted 2-butyl-3-[(2'-tetrazol-5-yl)biphen-4-yl)methyl]quinazolin-4(1H)-ones as antagonists of the AT_1 receptor for angiotensin II is presented. L-159,093 (2-butyl-6-(N-isopropyl-N-methyl-carbamoyl)amino-3-[(2'-tetrazol-5-yl)biphen-4-yl)methyl]quinazolin-4(1H)-one (IC_{50}=0.1nM rabbit aorta) is shown to be a potent orally active AII antagonist in rats and rhesus.

Introduction

The renin angiotensin system (RAS) is a cascade of proteolytic enzymes (renin and angiotensin converting enzyme (ACE)) that results in the production of the systemic hormone angiotensin II (Ang II). The blockade of the RAS with inhibitors of ACE have demonstrated the effectiveness of the reduction of levels of AII on cardiovascular and kidney haemodynamics, aldosterone production and release, and the reabsorbtion of sodium.[1] Antagonists of Ang II constitute an alternative method of blocking the RAS. Two binding proteins, AT_1 and AT_2 for AII have been characterized by their differential binding to Losartan **1** and PD123177.[2] Further subtypes of these binding proteins may exist.[3] However, all the functional responses to AII described above have been found to be controlled by antagonism of the AT_1 receptor. The previous manuscript outlines the discovery that 6-substituted-2-butyl-3-[(2'-tetrazol-5-yl)biphen-4-yl)methyl] quinazolin-4(1H)-ones **1** are potent ligands of the AT_1 receptor and that the quinazolinone ring constitutes an alternative to the imidazole ring found in Losartan **2**.[4]

We outline below our exploration of the QSAR and and the in vivo pharmacology of 6-substituted quinazolin-4(3H)-ones **3-38** as AT_1 antagonists that resulted in the discovery of L-159,093 (**37**), a highly potent orally active AT_1 (0.1nM rabbit aorta) antagonist.[4]



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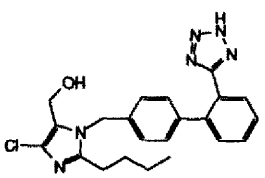

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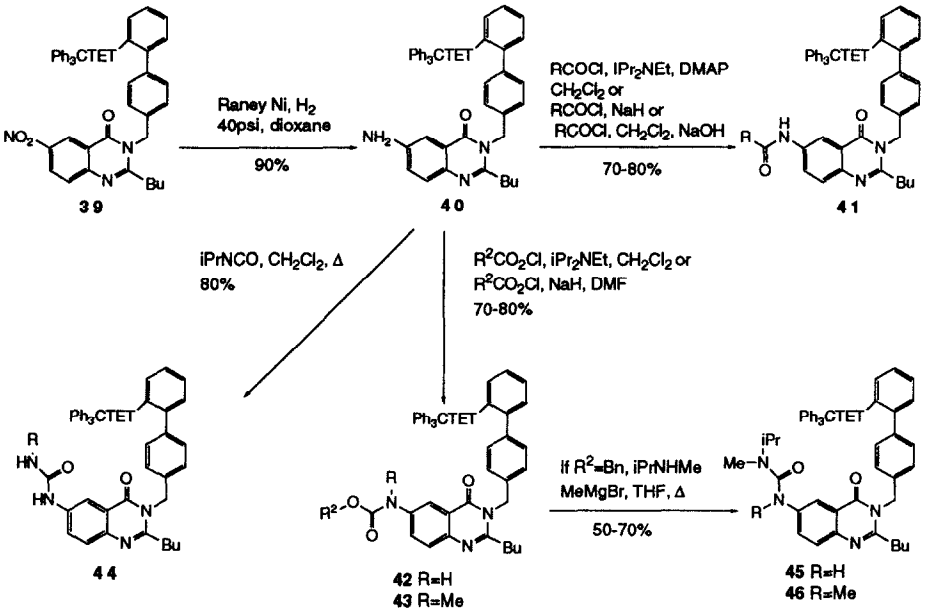
Chemistry

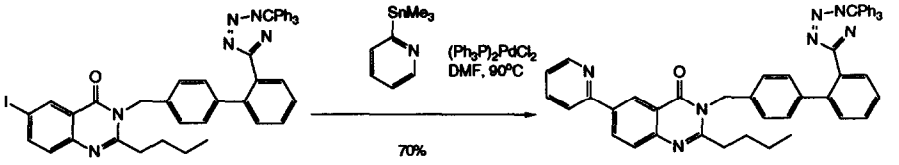
Compounds **3-38** were prepared by one of the three methods oultined in the previous manuscript.[4] The quinazolinones were prepared via acylation of substituted anthranilonitriles followed by basic hydrogen peroxide hydrolysis of the nitrile and cyclization (method A) or via benzoxazone formation from anthranilic acids followed by condensation with ammonium carbonate (method B). Akylation of the quinazolinones with a 2'-protected acid biphenylmethyl bromide gave the precursors to the antagonists. Alternatively, benzoxazone formation was followed by *in situ* condenation with a 2'-protected acid biphenylmethyl amine (method C). The precursors were then modified to give the R group as needed, and deprotected (HCl/MeOH or $HOAc/H_2O/THF$) to give the desired products.

6-amino substituted quinazolinones were prepared as illustrated in Scheme **1**. The nitro quinazolinone **39** (precursor of **12**) was reduced in the presence of Raney nickel at 40 psi hydrogen in dioxane to give the amine **40** (precursor of **25**). Acylation of the amine under standard conditions gave amides **41** (precursor of **30** and **31**). Similarly, acylation with chloroformates gave carbamate **42** (precursor of **32** and **34**). Compound **42** could be converted to N-alkylated carbamates **43** (precursor of **33** and **35**) by means of alkylation of the lithium anion of the carbamate with iodomethane. Urea **44** (the precursor of **36**) was prepared by treatment of **40** with isopropyl isocyanate. N,N-disubstituted ureas as found in **37** and **38** were prepared by addition of the magnesium salt of N-isopropyl-N-methyl amine to the Cbz derivative of **42** to give **45** (precursor of **37**).[5] Alkylation of **45** with iodo methane in the presence of lithium hexamethyldisilazide gave **46** (precursor of **38**).

Scheme 1



The 6-aryl and heteroaryl quinazolinones **23** and **24** were prepared from the trityl protected precursor **47** of **18** via palladium catalyzed coupling with the appropriate stannane as illustrated below for the case of the precursor to **24**.



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The sulfoxide and sulfone **14** and **15** (Table 3) were prepared via hydrogen peroxide oxidation of **13**.

Biology

The in vitro binding affinities of the compounds in Table 1 were determined by their ability to displace the specific binding ligand ^{125}I-Sar^{1}Ile^8-AII from receptors in the rabbit aorta membrane in the presence of 0.2% BSA.[6] It was recognized that the presence of BSA could cause substantial shifts in the IC_{50} binding values.[7] For this reason binding affinity for several antagonists was determined in the absence of BSA. No statistically significant difference was noted between the two assays for this series of antagonists. The errors inherent in the determinations of binding potency are approximately 2-fold.

In vivo activity was evaluated in cannulated normotensive conscious rats by measuring the percent inhibition of the AII pressor response at each dose as a function of time following intravenous or oral administration.[6] The duration of action is expressed as the time for the % inhibition of pressor response to fall below 30% for a single bolus of the drug. The vehicles were mixtures of saturated $NaHCO_3$ (15%), saline (35%), and distilled water 50% for iv and 1N NaOH-0.5% methocel (10:90) for po administration.

Discussion

The previous manuscript had shown that substitution at C-6 of the quinazolinone gave the most potent AT_1 ligands. Tetrazole gave more potent antagonists than did a carboxylic acid by a factor of approximately 30 fold and butyl was found to be the prefered alkyl group at C-2.

Antagonists **3-38** (Table 1) incorporate a set of substituents that span a large range of possible aromatic substituent effects. The alkyl-substitued quinazolinones **9-11** had equal binding affinity for the AT_1 receptor. In general, electron withdrawing groups (**12, 16, 17**) gave reduced binding affinity. However, an exception was found in the case of the methyl sulfone **15** (IC_{50}=2.6nM). Halogen substituents were detrimental to binding affinity (**16-18**). Two quinazolinones which incorporated acidic groups the phenol **19** and triflamide **29** had reduced binding affinities (IC_{50}=27 and 120nM respectively). Since quinazolinone **6** had demonstrated in the 2'-carboxy series that improved binding affinity may be derived from incorporation of an amino substituent, an investigation of 6-amino derivatives was initiated. The 6-amino analog in the tetrazole series **25** (IC_{50}=1.2nM) gave a 5 fold improvement in binding affinity over the unsubstituted analog **8**. Alkyl amines **26-28** gave no further improvement in affinity and amide and carbamate derivatives were also not beneficial (**30-35**). The poor aqueous solubility of the amides and carbamates prompted us to examine urea substituents at C-6. The urea **36** is equipotent with the most potent carbamate **35**. The AT_1 binding affinity was further improved with the the trisubstituted urea **37** (**L-159,093**).

All the antagonists in this study were assayed for AT_2 binding potency and demonstrated affinities of >1000nM.[8]

Table 1: *In Vitro* Binding Data of 1 and Inhibition of AII Pressor Response in Conscious Normotensive Rats

No.	Route	R	IC_{50}, nM.[a]	IC_{50}, nM.[b]	dose mg/kg[c]	iv % inhibition	iv dur. hr	po % inhibition	po dur. hr
3*#	A	H	20	-	-	-	-	-	-
4*#	B	Me	92	-	-	-	-	-	-
5*#	A	NO_2	940	-	-	-	-	-	-
6*#	-	NH_2	33	-	-	-	-	-	-
7*#	-	NHAc	50	-	-	-	-	-	-
8*	A	H	6.0	-	1	40	0.3	NT	NT
					3	70	0.9	54	1.1
					10	98	2.2	NT	NT
9*	B	Me	4.0	1.1	1	28	0	NT	NT
					3	76	2.5	64	4.2
10*	B	iPr	5.0	4.0	1	34	0.3	NT	NT
					3	90	>6	72	>6
11*	C	Et	4.0	-	-	-	-	-	-
12*	A	NO_2	28	-	-	-	-	-	-
13	B	SMe	7.0	-	1	44	0.1	NT	NT
14*	-	SOMe	1.0	0.9	1	89	1.8	NT	NT
15*	-	SO_2Me	3.0	7.6	1	79	0.5	NT	NT
16*	B	F	26	-	-	-	-	-	-
17*	C	Cl	29	-	-	-	-	-	-
18*	A	I	-	12	-	-	-	-	-
19*	B	OH	27	-	-	-	-	-	-
20	-	OCONHiPr	1.0	-	0.3	30	0	NT	NT
21*	B	OMe	5.0	-	1	49	0.2	NT	NT
22	B	OMe(7-OMe)	13		1	30	0.2	NT	NT
23*	-	Ph-4-Cl	-	30	-	-	-	-	-
24*	-	2-Pyr	-	5.0	-	-	-	-	-
25*	-	NH_2	1.3	-	1	93	4.5	32	0.2
26*	-	NHMe	2.0	-	1	94	1.2	NT	NT
27	-	NMe_2	5.0	-	1	55	<0.5	NT	NT
28*	-	NHBn	8.0	1.1	-	-	-	-	-
29	-	$NHSO_2CF_3$	120	-	-	-	-	-	-
30*	-	NHAc	9.0	-	1	97	0.9	NT	NT
31*	-	NHCOBu	2.0	8.9	-	-	-	-	-
32*	-	$NHCO_2iBu$	6.4	1.6	-	-	-	-	-
33*	-	$NMeCO_2iBu$	3.4	1.6	0.3	NT	NT	60	3.5
					1	72	>6	-	-
34*	-	$NHCO_2Bn$	4.3	-	1	84	1	NT	NT
35*	-	$NMeCO_2Bn$	0.83	-	0.3	28	0	NT	NT
36*	-	NHCONHiPr	0.75	0.5	0.3	86	3.5,>6	0	0
37*	-	NHCON(Me)iPr	-	0.1	1	94	>6	84	>5
					0.3	96	4	80	>3.5
					0.1	85	2.5	58	>3
					0.03	15	ND	16	ND
38	-	N(Me)CON(Me)iPr	-	0.58	0.3	70	0.8	NT	NT

#: 2'-carboxy- biphenyl; *: included in linear regression analysis; a: binding affinity rabbit aorta in presence of 0.2% BSA ; b: binding affinity rabbit aorta in absence of 0.2% BSA; c: Inhibition of AII pressor response in normotensive rats. ND=not determined. NT=not tested

QSAR Analysis

To gauge the effects of the various substituents at the 6 position, a quantitative structure activity relationship (QSAR) analysis was made.[9] The compounds included in the study are indicated with a * in Table 1. The choice of compounds was made on the basis of the availability of the required substituent parameters. The average -logIC_{50} (pIC_{50}) was used as the measure of bioactivity; typically, two or more measurements were made for each compound, the largest single-compound sample was four.

Nominal variables were used to account for a possible effect of BSA in the assay medium (*BSA*) and for the acid moiety on the biphenyl (*Acid*); interval variables were used for the R_6-substituent size (V),[10] lipophilicity (π),[11] inductive sigma effect (σ_I),[12] resonance sigma effect (σ_d),[12] and sensitivity of σ_d to electronic demand (σ_e).[12] σ_I values were not available for some of the larger substituents (mostly ureas and carbamates), and therefore we used values for groups with shorter alkyl chains. Multivariate analysis of the datatable showed that there were no correlations among the descriptors.

Quadratic terms for size and lipophilicity were included in the initial regression model which was then refined by a stepwise multiple regression procedure (RS/Explore®).[13] This led to the elimination of *BSA*, V^2, π^2, σ_d, and σ_e.. With the elimination of σ_d and σ_e from the model, an additional 11 observations could be included (substituents for which only σ_I was available) for fitting the reduced model. The final regression equation was:

$$pIC_{50} = 7.63(0.12) + \begin{Bmatrix} -0.782(0.083) & Acid = CO2H \\ +0.782(0.083) & Acid = CN4H \end{Bmatrix} + 0.0113(0.0019)V - 0.406(0.073)\pi - 1.68(0.35)\sigma_I$$

$R^2 = 0.82$, s = 0.36, n = 41, F = 42.0

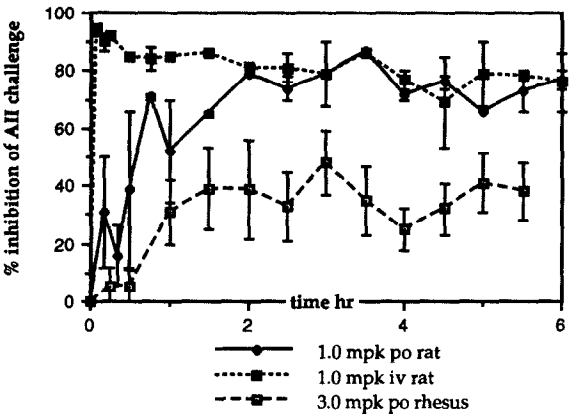
Crossvalidation with 5 groups gave a predictive R^2 of 0.76.

The binding affinity of 6-substituted quinazolinones is, therefore, dependant on the nature of the acidic group attached to the biphenyl and, in general, larger, hyrophilic electron donating substituents provide analogs with enhanced binding affinity. The ureas as a class (**36-38**) and specifically **37** fulfill these properties.

In Vivo Activity

As the SAR was being developed, compounds of interest were evaluated in vivo for inhibition of the AII pressor response in normotensive rats. In general, at a submaximal peak level of pressor inhibition (60-80%) the duration of action (time where % inhibition was maintainted greater that 30%) was short. All compounds that were evaluated attenuated the AII pressor response, suggesting that this class of compounds functionally antagonize the hypertensive response to AII (Table 1). The degree of inhibition and duration of action of the hypertentsive response was not predictable within the series of compounds tested. The 6-isopropyl analog **10** and the 6-methyl analog **9** were both orally active in rats. Antagonist **10** was superior in both % inhibition and duration of action at the same dose although it was marginally less potent *in vitro*.. The shorter duration of **9** may reflect metabolism at the benzylic methyl group. The urea **37** (L-159,093) was 40 fold more potent than **10** and at an equivalent i.v. dose (0.1 mg/kg **37** vs 3 mg/kg **10**) had a shorter duration of action. Both **10** and **37** were evaluated in conscious normotensve rhesus monkeys for inhibition of the AII pressor response at 3 mg/kg po. No response was elicited from **10**, but **37** demonstrated 50% peak inhibition with a duration of action of greater than three hours (Figure 1). The lack of oral activity of **10** at 3 mg/kg in the rhesus monkey may be a reflection of the lower potency of **10**.

Figure 1: Inhibition of Ang II Challenge (0.1ug/kg iv) by **37** in Conscious Normotensive Rats and Rhesus Monkeys



Conclusions

6-substituted-2-butyl-3-[(2'-tetrazol-5-yl)biphen-4-yl)methyl]quinazolin-4(1H)-ones constitute a new class of potent antagonists of angiotensin II at the AT_1 receptor. The in vitro potency may be expressed as an equation wherein the nature of the biaryl acid in combination with large, hydrophilic electron donating substituents gave the most potent compounds. The duration of action of most members of this class of AII antagonists is short in the normotensive rat. However, several members of this series of compounds have good duration of action and are worthy of further evaluation. The most potent compound synthesized within this series, **37** is an orally active antagonist of AII in both normotensive rats and rhesus monkeys.

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9 A full table of biological data and substituent descriptors is available from the authors.

10 Calculated van der Waals' volume ($Å^3$) using our in-house molecular modeling program AMF. The substituent value was determined by calculating the volume of the substituent bound to benzene and then subtracting the calculated volume of benzene (69.981) from it.

11 Calculated log P using version 3.33 of the MedChem software, Pomona College, Claremont, CA. The substituent value was determined by calculating the logP of the substituent bound to benzene and then subtracting the calculated logP of benzene (2.142) from it.

12 The electronic descriptors σ_I, σ_d, and σ_e are applicable for substituents bound to all substrates. See M. Charton, "A General Treatment of Electrical Effects," in *Progress in Physical Organic Chemistry*, volume 16, R.W.Taft, ed.,John Wiley & Sons: New York, 1987.

13 RS/Explore, version 2, BBN Software Products Corp., 10 Fawcett Street, Cambridge, MA 02238.