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THE DESIGN, BINDING AFFINITY PREDICTION AND SYNTHESIS OF MACROCYCLIC ANGIOTENSIN II AT_1 AND AT_2 RECEPTOR ANTAGONISTS.

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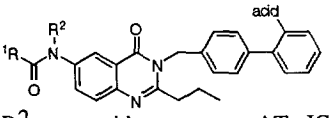
Abstract: Analysis of the SAR of AT_1 selective and AT_1/AT_2 balanced affinity angiotensin II antagonists led to the design of macrocyclic quinazolinone ligands. CoMFA analysis was used to predict the binding affinities of these novel ligands. The synthesis, X-ray crystal structure, binding affinity and the relevance of these studies to the determination of the biologically relevant binding conformation is discussed. Copyright © 1996 Elsevier Science Ltd

Introduction

Many diverse structural classes of analogs of losartan, a potent non-peptidic antagonist of the octapeptide angiotensin II (Ang II), have been identified since its discovery in 1987.[1] Structure-activity relationships have been developed within these classes that characterize the major pharmacophores that contribute to the binding affinity to the AT_1 receptor. Hypotheses have been advanced for the bioactive binding conformation of these ligands based on comparisons with Ang II, X-ray crystal structures of the ligands, calculated low-energy conformations, comparison of low energy conformations of structurally diverse ligands, COMFA studies (vide infra) and the synthesis of conformationally restricted ligands.[2] Three structural classes of ligands for the AT_2 receptor have also been reported, but no hypotheses have been advanced to support a specific binding conformation to the AT_2 receptor.[1] Recently, antagonists with equal affinity to both receptors have been reported.[1]

Table 1



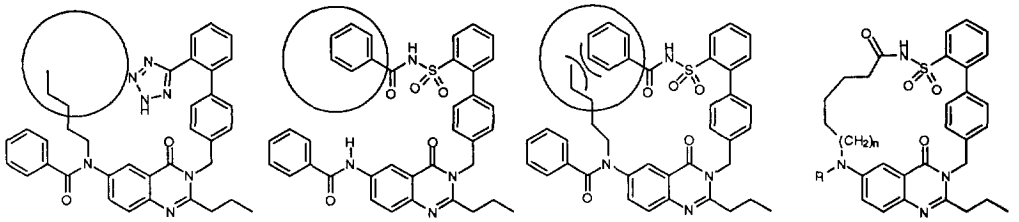
#	R^1	R^2	acid	AT_1 IC_{50}, nM	AT_2 IC_{50}, nM
1	Ph	H	-tetrazole	0.4	3000
2	Ph	n-Pn	-tetrazole	1.5	0.7
3	Ph	n-Pn	-$SO_2NHCOPh$	11.0	350
4	Ph	H	-$SO_2NHCOPh$	1.0	16.0
5	NHiPr	H	-$SO_2NHCOMe$	8.8	630
6	NHiPr	H	-$SO_2NHCOPh$	0.9	18.0

6-amino-2-alkyl-3-[(2'-(tetrazol-5-yl)biphen-4-yl)methyl]-quinazolin-4(3H)-ones can be modified to provide AT_1 selective (**1**)[3] and balanced affinity ligands (**2**).[4] The increase in affinity for the AT_2 receptor of **2** over **1** arises from the presence of two lipophilic 6-amino substituents. Concurrent with these studies, it was found that incorporation of a lipophilic acylsulfonamide in place of the tetrazole group provided enhancement in the AT_2 binding affinity in the imidazopyridine class of Ang II antagonists.[5] Replacement of the tetrazole group in **2** with a benzoyl sulfonamide group in **3** resulted in a 7 and 500-fold loss in AT_1 and AT_2 affinity, respectively. The

affinity to both receptors could be largely restored by removing the 6-N-pentyl substituent in **4**. The pharmacophore responsible for the AT_2 binding affinity of **4** was believed to be the benzoyl sulfonamide group. This hypothesis was supported by the greater reduction in AT_2 binding affinity as compared to AT_1 affinity of the acetylsulfonamide analog **5** as compared to the benzoyl sulfonamide **6**.

We concluded that a pharmacophore (circle) for a lipophillic group exists that contributes to AT_2 affinity in the 2'-tetrazole series (compound **2**) and the 2'-acylsulfonamide series (compound **4**) as shown in Figure 1.[6] The presence of two groups positioned to fill this space in antagonist **3** results in steric conflict, binding conformation disruption and loss in binding affinity at both receptors.

Figure 1.



2 **4** **3** **7**

Since this AT_2 receptor binding site may be occupied by either the acylsulfonamide or a 6-amino group substituent, we believed that a macrocycle **7** containing a lipophilic linking group between the quinazolinone 6-amino group and the acylsulfonamide could be a potent ligand of both the AT_1 and AT_2 receptors.

The ability of compounds **2** and **4** to bind with similar affinity to both receptors suggests that these antagonist may have similar binding interactions with both receptors. However, the conformational flexibility of compounds such as **2** increases the probability that they exist in multiple low-energy conformations. The design of a conformationally restricted macrocyclic analog of **2**, as described above, with affinity for both the AT_1 and AT_2 receptor subtypes would support the hypothesis that a common binding conformation exists at both receptor subtypes.

Molecular Modeling Studies

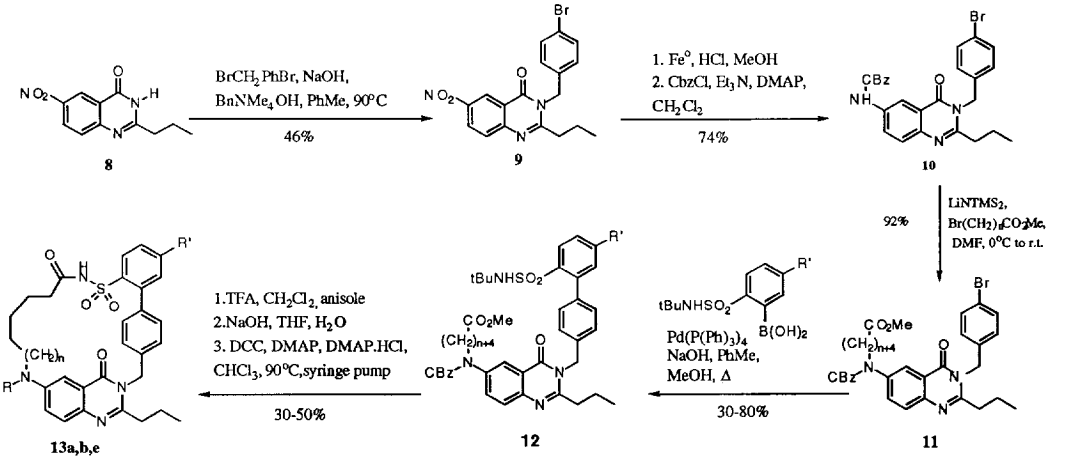
Macrocyclic ligands incorporating an alkyl chain linking group of variable length were evaluated by molecular modeling. Independent CoMFA studies of AT_1 selective and AT_1/AT_2 mixed affinity ligands were used to predict the activity of the macrocyclic quinazolinones at both receptors.[7] For AT_1, the CoMFA superposition was based on a previously derived hypothesis for the receptor bound conformation of Ang II antagonists using the active analog approach.[8] The correlation of this conformational model via CoMFA included 50 diverse quinazolinone and non-quinazolinone antagonists spanning 4 orders of magnitude IC_{50} resulting in a cross validated R^2 of 0.64 (Conventional $R^2 = 0.76$, rms error = 0.66).[9] The binding conformation at the AT_2 receptor used for the CoMFA analysis was derived by positioning the quinazolinone 6-N-alkyl substituent and the 2'-biphenyl acid group in the same region of space as suggested by the SAR studies described above. Analysis over 131 quinazolinones spanning 4 orders of magnitude in IC_{50} AT_2 resulted in a cross validated $R^2 = 0.813$ (conventional $R^2 = 0.944$,

rms error = 0.359).[10] The CoMFA models predicted negligible affects of ring size on AT_1 binding affinity and that a 21 membered ring system would be at least 10 fold less potent than either a 22 or 23 membered ring at the AT_2 receptor (Table 2).

Chemistry and Biology

The preparation of the key intermediate **12** was achieved as shown in Scheme 1. Deprotection of the t-butyl sulphonamide was complicated by associated acid catalyzed deprotection of the Cbz protecting group. The extent of this side reaction was controlled by carefully monitoring of the progression of the reaction. Macrocyclization to **13a, 13b** and **13e** was accomplished under the conditions developed by Keck.[11] Hydrogenation over 10% Pd/C provided the amines **13c** and **13f**. Acylation of **13c** with benzoyl chloride gave **13d**. An X-ray crystal structure of **13e** (from acetonitrile) was obtained to determine the conformation in the solid state (Figure 2).[12]

Scheme 1



The binding affinity to the AT_1 and AT_2 receptors was determined by competitive blockade of ^{125}I-Sar^{1}Ile^8-Ang II binding from rabbit aorta and rat midbrain respectively.[13]

Table 2: Actual and Predicted Binding Affinity to the AT_1 and AT_2 Receptor.

#	n	R	R^1	Actual IC_{50} AT_1 nM	Actual IC_{50} AT_2 nM	Predicted IC_{50} AT_1 nM	Predicted IC_{50} AT_2 nM
13a	1	Cbz	H	23	4000	24	240
13b	3	Cbz	H	1.5	75	9.1	73
13c	3	H	H	0.3	200	9.4	960
13d	3	COPh	H	1.3	49	10	17
13e	3	Cbz	Pr	30	20	-	-
13f	3	H	Pr	3.9	24	-	-

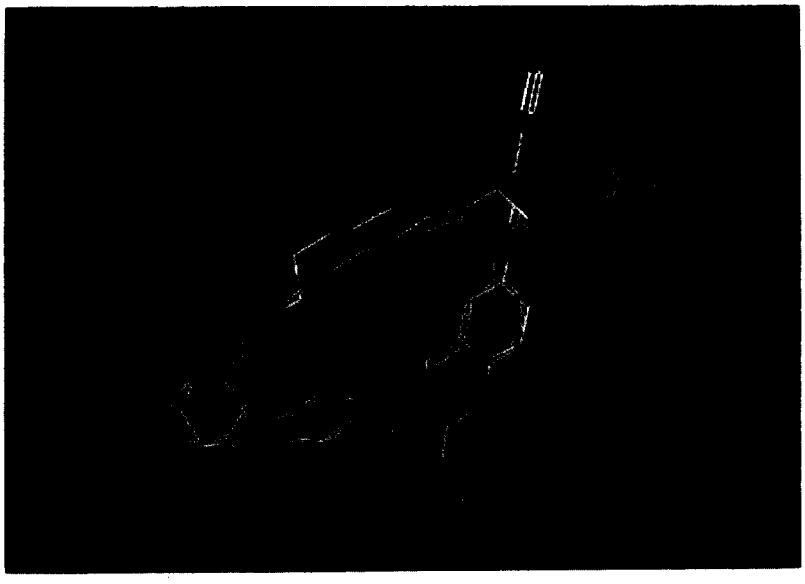
Discussion

Compounds **13a** - **13d** were potent ligands of the AT_1 receptor. The 23 membered ring system in **13b** was more potent than the 21 membered ring **13a**. Removal or alteration of the nitrogen pendant groups in **13c** - **13d** had little effect on AT_1 affinity. The binding affinity to the AT_2 receptor was equally dependent on ring size. Removal of the acyl group from the N-6 position **13c** reduced AT_2 affinity.

We had previously found that 5'-propyl substituents increased AT_2 binding affinity in imidazopyridine antagonists.[14] For this reason analogs **13e** and **13f** were prepared, and were found to be approximately three and ten-fold more potent at the AT_2 receptor and twenty and ten fold less potent at the AT_1 receptor than the 5'-unsubstituted analogs **13b** and **13c**.

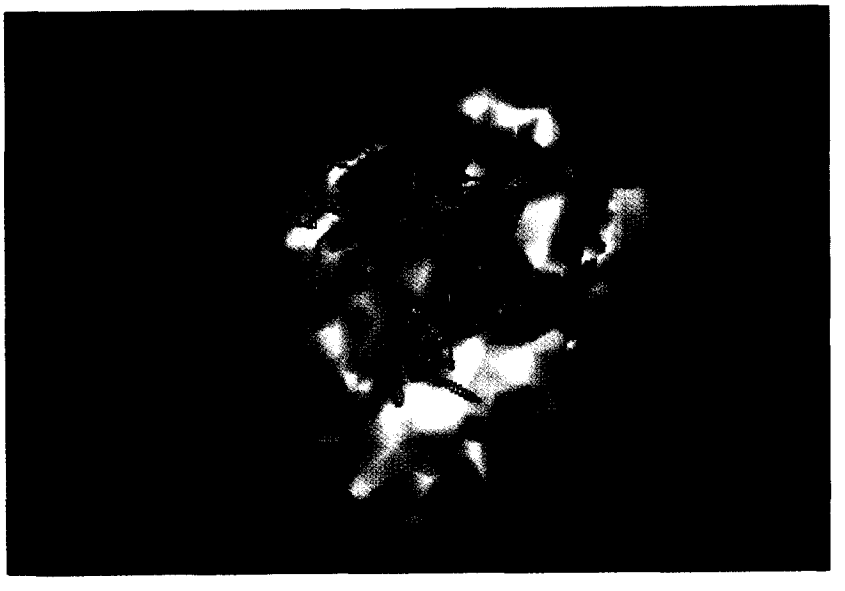
The binding affinity of the synthetic analogs **13a** - **13d** was predicted using the CoMFA models described above. The AT_2 model correctly predicted a decrease in AT_2 affinity for the de-acylated analog **13c** over **13b**. Compound **13d** was more potent at AT_1 and less potent at the AT_2 receptor than had been predicted. While the calculations correctly estimated this *relative* preference, the absolute value of the difference was smaller than observed. Since the CoMFA correlations are sensitive to the conformation of the superposition of the molecules used in their derivation, even minor changes in the actual conformation from that modeled may account for the quantitative errors in the predicted affinities. The X-ray crystal structure of **13e** determined a solid state conformation which compares favorably to the AT_1 and AT_2 models (Figure 2).

Figure 2: Comparison of the modeled geometry of **13b** with the X-ray geometries of **13e**: yellow: conformation from the AT_2 CoMFA study, pink: conformation from the AT_1 CoMFA study, green: X-ray strucutre of **13e**. The rms difference in superposition between the bipheyl quinazolinones as determined crystallographically and as used in the AT1 model (stick) is 0.3Å. For the AT2 model (wire), the rms difference is 0.9 Å.



This qualitative agreement taken with the potency of the macrocycles suggests that the molecular shapes used as the basis for the CoMFA models are relevant to the receptor bound geometry of these ligands at AT_1 and AT_2. The potent binding affinity to both the AT_1 and AT_2 receptors of the conformationally restricted analogs **13b - 13f** suggests that quinazolinones may bind to these receptors in a similar conformation. Figure 3 illustrates the reduction in available conformational space that occurs on introduction of the macrocyclic constraint into the ligand. The activity of these macrocyles provide support for the previously proposed 3D geometry of ligands bound to AT_1. Since the AT_1 COMFA model integrated other classes of known AT_1 ligands, the model may be applicable to other members of the heterocycle-biphenyl acid class of Ang II antagonists as well. Although macrocyclization does not eliminate access to other conformations of low energy, the ability to generate a constrained small molecule which has balanced affinity suggests the possible existence of a common 3D binding geometry of biphenyl ligands to AT_1 *and* AT_2.[15] Conserved regions in the AT_1 and AT_2 receptor sequences may account for such parallelisms. These sequences can also be used to analyze the origins of specific binding to AT_1 and AT_2.

Figure 3: Restriction in accessible space due to macrocyclization. Yellow: surface of the accessible space availailable to the cyclic ligand **13b**. Grey molecules: space occupied by acyclic analog not available to cyclic analog.



Conclusion

Analysis of the SAR of AT_1 selective and balanced affinity AT_1/AT_2 ligands in combination with molecular modeling suggested that macrocyclic ligands might assist in the determination of their bound conformations. The potent binding affinity of macrocycles such as **13e** in combination with its X-ray crystal structure and the CoMFA studies supports the hypothesis for a biologically relevant bound conformation for the

heterocycle-biphenyl class of angiotensin II antagonists. Evidence in support of a related binding conformation at both receptors may suggest that a single binding conformation exists. This would be of great interest in light of the low sequence homology of rat AT_1 and AT_2 receptors.

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12 Compound $C_{43}H_{48}N_4O_6S$, $M_r = 748.949$, triclinic, $P\bar{1}$, $a = 11.8(2)$, $b = 14.37(4)$, $c = 11.96(3)$Å, $\alpha = 97.3(2)$, $\beta = 105.9(4)$, $\gamma = 80.1(5)°$ $V = 1915$ Å^3, $Z = 2$, $D_x = 1.299$ g cm^{-3}, monochromatized radiation λ(Cu K_α) = 1.541838 Å, μ = 1.15 mm^{-1}, $F(000) = 796$, $T = 294$ K. Data were collected on a Rigaku AFC5 diffractometer to a θ limit of 70° which yielded 7441 measured (6974 unique) reflections. There are 1646 unique, observed reflections (with $I \geq 3\sigma(I)$ as the criterion for being observed) out of the total measured. The structure was solved by direct methods (SHELXS-86) (Sheldrick, G.M. Acta Crystallogr., **1990**, A46, 467-473) and refined using full-matrix least-squares on F (SDP-PLUS) (Structure Determination Package Version 3, Enraf-Nonius, Delft, 1985). The final model was refined using 302 parameters and the observed data. The non-hydrogen atoms were refined with a mix of isotropic and anisotropic thermal displacements. The final agreement statistics are: $R = 0.069$, $wR = 0.062$, $S = 1.91$ with $(\Delta/\sigma)_{max} = 0.01$. The least-squares weights were defined using $1/\sigma^2(F)$. The maximum peak height in a final difference Fourier map is 0.28(7) eÅ^{-3} and this peak is without chemical significance. The atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre. The coordinates can be obtained on request from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK.

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