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BALANCED AT_1 AND AT_2 ANGIOTENSIN II ANTAGONISTS. I. NEW ORALLY ACTIVE 5-CARBOXYL IMIDAZOLYL BIPHENYL SULFONYLUREAS.

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Abstract: A series of substituted imidazolyl biphenyl sulfonylureas have been synthesized. Substitution on the imidazole ring but essentially on the urea side chain significantly increased AT_2 binding with cyclohexylmethyl, cyclopentylmethyl and benzyl as the most effective substituents. Imidazole **13d**, as a representative member of this series, displayed nanomolar binding affinity for both the AT_1 and AT_2 angiotensin II receptor subtypes as well as oral activity.

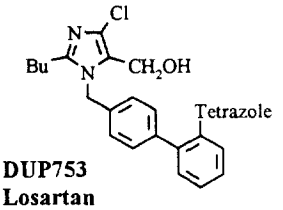
The Renin Angiotensin System (RAS) is known to play an important role in blood pressure regulation and electrolyte homeostasis.[1] Angiotensin II (AII), the biologically active peptide of the RAS, is a potent vasoconstrictor agent and its regulation has been achieved by inhibition of the metalloprotease ACE[2] and of the aspartyl protease renin.[3] More recently, AII receptor antagonists have been investigated as an alternative approach in blocking the hypertensive response to endogenous AII.

Many AII antagonists[4] have been reported since the discovery of Losartan[5] by DuPont. To date, those in development are selective for only one of the two identified AII receptors,[6] the AT_1 subtype, which is responsible for the pressor response and most of the known cardiovascular and renal effects[7] induced by AII. A second AII receptor, the AT_2 subtype, has been identified in various tissues using selective ligands such as PD 123177 [8a] or CGP 42112A.[8b] The physiological action mediated by the AT_2 receptor has not yet been clearly identified even if some groups have shown correlations with renal free water clearance, cerebral blood flow regulation, skin wound healing, collagen synthesis in cardiac fibroblasts and restenosis following vascular injury.[9]

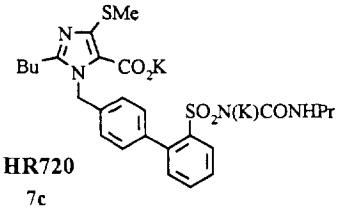
In animal models and in the clinic, blockade of the AT_1 receptor by Losartan led to higher circulating AII levels[10] and the consequences of chronic overstimulation of the AT_2 receptor (positive or negative) are unknown. For this reason, after the discovery of imidazolyl biphenyl sulfonylurea HR 720,[11] our AT_1 selective AII antagonist currently under clinical trials, we focused our attention towards compounds that bind to both AT_1 and AT_2 receptor subtypes.

Nonpeptide AT_1 antagonists with significant affinity for the AT_2 receptor were reported some times ago first by Karl Thomae[12] (BIBS 39: AT_1/AT_2 29/480 nmol) and then by our Hoechst/Roussel group[13] (S 920029). More recently, Merck and DuPont Merck have published [9,14] a series of papers on compounds with high binding affinity for both AT_1 and AT_2 receptor subtypes. Starting from AT_1 selective antagonists in the imidazole,

imidazopyridine and triazolinone series, they showed that considerable enhancement of the AT_2 binding affinity could be achieved by replacement of the tetrazole moiety with a lipophilic acylsulfonamide or sulfonylcarbamate. These publications prompted us to disclose our own results in the imidazolyl biphenyl sulfonylurea series.



DUP753
Losartan

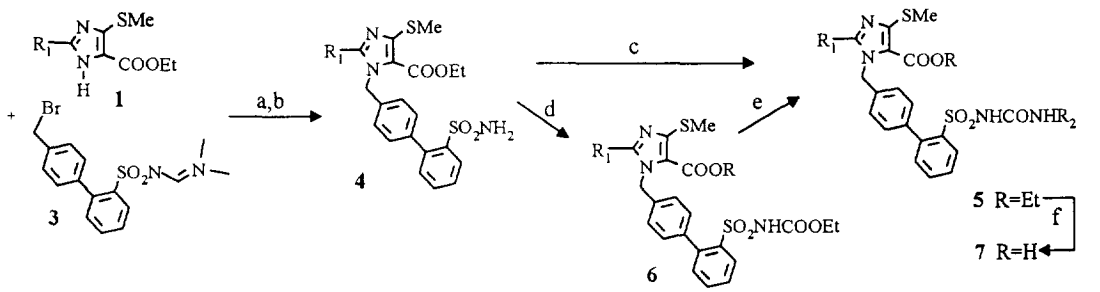


HR720
7c

Chemistry

All compounds prepared for this study were synthesized through a convergent approach by coupling the requisite imidazole[11] **1** with the bromomethyl biphenyl sulfonylamidine[11] **3** as described in scheme I. The major product was the desired N_1 isomer (91 % *vs* 9 % for the N_3 isomer). Sulfonylamidine was cleanly deprotected by refluxing in a 1:1 mixture of concHCl/EtOH and the resulting sulfonamide **4** was converted to sulfonylurea **5** with the appropriate isocyanate in the presence of K_2CO_3. Alternative access to sulfonylureas **5** involved the reaction of ethylsulfonylcarbamate **6** (obtained by reaction of sulfonamide **4** with the ethyl chloroformiate) with the requisite amine in refluxing toluene, without affecting the ethylester group. Sulfonylureas **5** were finally saponified in aqueous ethanolic NaOH solution at room temperature (in order to avoid a possible decarboxylation of the imidazole ring) to give the free acids **7**.

Scheme I

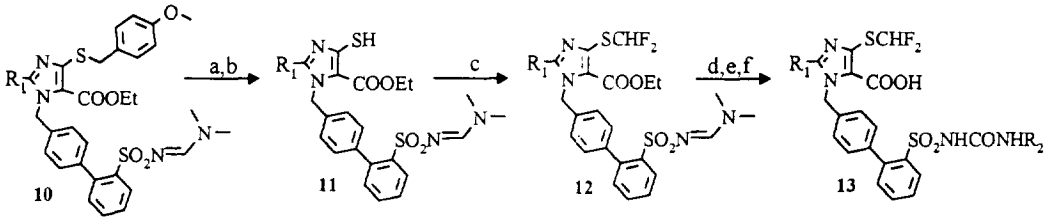


(a) K_2CO_3, DMF, rt; 45h; (b) conc. HCl, EtOH, reflux 2 h; (c) O=C=N-R_2(1.3eq), K_2CO_3(2 eq), acetone, reflux 1h (65-90%); (d) $ClCO_2Et$, K_2CO_3, DME, reflux, 2h; (e) R_2NH_2, toluene, reflux, 15h; (f) 2N NaOH, EtOH, rt, 24h.

The urea moiety of 4-difluoromethylthio imidazoles **13** was analogously prepared from sulfonylamidine **12**. However, the difluoromethylthio group was introduced through a procedure described in scheme II. Treatment of 4-paramethoxybenzylthio imidazole[11]**10** with a 5 to 1 mixture of trifluoroacetic acid/anisole in the presence of mercuric trifluoroacetate led to deprotection of the SH group which was trapped *in situ* as mercury salt. Bubbling

H_2S through an ethyl acetate solution of this salt cleanly generated the thiol **11** which was then treated (after removal of the black precipitate on celite but without any further purification) in DMF with sodium chlorodifluoro acetate in the presence of NaI to afford 4-difluoromethylthio imidazole **12** in 75 % overall yield.

Scheme II



(a) $Hg(CF_3CO_2)_2$, TFA, anisole, 0°C to rt, 20 min; (b) H_2S, EtOAc, rt, 45 min; (c) $ClCF_2CO_2Na$, NaI, DMF, 87°C, 45min; (d) conc. HCl, EtOH, reflux 2 h; ; (e) O=C=N–R_2 (1.3eq), K_2CO_3(2 eq), acetone, reflux 1h; (f) 2N NaOH, EtOH, rt.

Results and discussion.

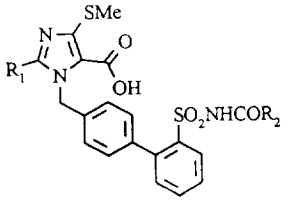
The *in vitro* binding affinities[11] of the compounds described in this paper (Tables I and II) were determined by their ability to displace the specific binding of ^{125}I-AII from rat liver membranes (AT_1 receptors) and rabbit uterus membranes (AT_2 receptors) and are expressed as IC_{50} values. Selected compounds were further evaluated *in vivo* after intravenous and oral administration for their inhibition of the pressor response induced by AII (0.75 μg/kg i.v.) in normotensive pithed rats and are expressed as ID_{50} values[11].

During the course of our search for potent tetrazole isosteres within the imidazole series, we noticed that replacing the tetrazole moiety by the propylsulfonylurea group usually increased the AT_2 affinity by at least two orders of magnitude. Therefore, we decided to investigate the effects on AT_2 affinity of substitution on the urea side chain. Ureas 7 and **13**, close analogs of HR 720, were synthesized. We found that substitution on the urea did not significantly modify the AT_1 binding affinity (most compounds listed in Tables I and II displayed nanomolar or subnanomolar AT_1 affinity) while dramatically improving AT_2 activity.

We first turned our attention to linear alkyl chains on the urea (Table I). The AT_2 binding affinity was enhanced with a longer alkyl chain. The relative order of potency was methyl (**7a**, IC_{50} = 2400 nmol) < ethyl (**7b**, IC_{50} = 1900 nmol) < propyl (**7c**, IC_{50} = 920 nmol) < butyl (**7d**, IC_{50} = 96 nmol). These results are in agreement with those recently published by Merck and DuPont Merck[14c] in their carbamates series where the *n*-butyl group was found to be optimal and where smaller alkyl groups provided less AT_2 activity. However, we observed in our series the highest increase in potency when cyclohexylmethyl and benzyl substituents were used. Thus, N-cyclohexylmethyl (**7e**) and N-benzyl (**7f**) ureas showed comparable binding affinity for the AT_2 receptor (IC_{50} = 17 nmol) with a 6-fold increase when compared to the N-butyl urea (**7d**). As it has already been reported for sulfonylcarbamates and acylsulfonamides, the sulfonylurea side chain appears to occupy a lipophilic

pocket of the AT_2 receptor. Attempts to increase lipophilicity of urea 7 by introduction of a $NHCHPh_2$ group(**7g**) or a NPh_2 group (**7h**) resulted in a 10-fold and 50-fold decrease in potency probably due to steric hindrance and shorter length, respectively. Exchanging the potent benzyl group (**7k**) by the phenyl (**7l**) or para-tolyl groups (**7m**) or replacing the cyclohexylmethyl group (**7j**) with the cyclohexyl group (**7i**) led to a sharp decrease in potency for the AT_2 receptor, illustrating that the methylene spacer is crucial for high binding. Cyclopentylmethyl urea **7p** also displayed high AT_2 affinity similar to that of cyclohexylmethyl urea **7j**.

Table I : SAR of 4-methylthio imidazole



			IC_{50} [a] (nM)		ID_{50} [b] (mg/kg)	
cpds	R1	R2	AT1	AT2	iv	po
7a	Bu	NHMe	1.9	2400	0.27	4.9
7b	Bu	NHEt	0.2	1900	0.17	1.1
7c [c]	Bu	NHPr	0.5	920	0.11	0.7
7d	Bu	NHBu	0.2	97	0.09	0.2
7e	Bu	$NHCH_2c$Hexyl	0.1	17	0.35	NT
7f	Bu	$NHCH_2Ph$	0.1	17	NT	NT
7g	Bu	$NHCHPh_2$	0.4	170	NT	NT
7h	Bu	NPh_2	1.2	850	5.8	NT
7i	Pr	NH*c*Hexyl	0.5	190	NT	NT
7j	Pr	$NHCH_2c$Hexyl	0.1	5.4	0.3	1.5
7k	Pr	$NHCH_2Ph$	0.2	9.5	0.22	1.4
7l	Pr	NHPh	2.3	2200	0.52	NT
7m	Pr	NHPh-4-Me	1.0	367	0.87	NT
7n	Pr	$NHCH_2$-2-Thienyl	0.1	14.3	NT	1.0
7p	Pr	$NHCH_2c$Pentyl	0.1	7.0	NT	1.0
7q	Et	$NHCH_2Ph$	0.1	6.8	1.37	NT
7r	Et	$NHCH_2c$Hex	1.3	1.3	1.84	>10

[a] IC_{50} for inhibition of specific binding of [^{125}I]AII to rat liver (AT1) and rabbit uterus (AT2) membrane preparation (n=2-4).

[b] ED_{50} following intravenous (n=4) or oral (n=18-28) administration to pithed rats for inhibition of pressor response induced by infusion of AII. For details, see ref.11

[c] As its dipotassium salt (HR720)

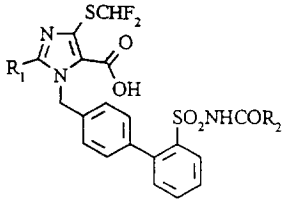
With the two optimized sulfonylurea substitutions in place (cyclohexylmethyl or benzyl urea), our attention was focused on the 2-position of the imidazole ring. 2-ethyl imidazole **7q-r** and 2-propyl imidazole **7j-k** were prepared and compared to 2-butylimidazole **7e-f**. It appeared that introduction of a 2-ethyl or a 2-propyl side chain resulted in a greater AT_2 binding affinity (a 2 to 13-fold increase depending on the urea and 2-substitution with butyl<propyl<ethyl). Thus, 2-ethyl imidazole **7r** showed nanomolar equipotency (1.3 nmol) for both receptor subtypes. However, *in vivo*, after intravenous administration, **7r** (resp **7q**) displayed a 6-fold lower activity when compared to the 2-propyl imidazole **7j** (resp **7k**), indicating that a propyl chain at the 2 position of the imidazole ring seems to be the best compromise for both high AT_1 and AT_2 affinity and *in vivo* efficacy.

In addition, we found that replacement of the methylthio group at the 4-position of the imidazole ring by the difluoromethylthio group resulted in a more than 3-fold improvement in the AT_2 binding affinity [**13a**, **13b** and **13c** (Table II) *vs* **7c**, **7f** and **7e** (Table I), respectively].

Therefore, with the best substitutents in place on the imidazole ring (2-propyl, 4-difluoromethylthio and 5-carboxyl[15]), we decided to further investigate more extensively the substitution on the urea moiety (Table II). We thus prepared the potent cyclohexylmethyl urea **13d** with 1.7 nmol affinity on the AT_2 receptor. Benzyl urea **13e** also exhibited high affinity (5.8 nmol). We then focused on modifications of this benzyl group, knowing that the methylene spacer, as already mentioned, was crucial for AT_2 binding affinity. A longer side chain of two (**13k**) or three (**13l**) methylene units decreased AT_2 binding affinity. Substitution on the phenyl ring retained potency for the p-fluoro substituent but weakened AT_2 binding for the o-chloro (**13g**) and the methylene dioxy (**13i**) substituents. Thiophene (**13m**) and naphthalene (**13j**) were also introduced in place of phenyl (**13e**) but without any improvement on AT_2 binding. All these data confirms that cyclohexyl methyl and benzyl substitution on the urea are optimal for AT_2 potency.

Most of the compounds prepared for this study (Table I and II) were evaluated *in vivo* and, consistent with their high AT_1 binding affinities, they showed potent antihypertensive activity after intravenous and/or oral

Table II : SAR of 4-difluoro methylthio imidazole



			IC_{50} [a] (nM)		ID_{50} [b] (mg/kg)	
cpd	R1	R2	AT1	AT2	iv	po
13a	Bu	Pr	0.5	220	0.09	0.8
13b	Bu	CH_2Ph	0.15	9.0	0.36	2.2
13c	Bu	CH_2*c*Hexyl	0.2	2.5	0.60	1.7
13d	Pr	CH_2*c*Hexyl	0.07	1.7	0.22	1.1
13e	Pr	CH_2Ph	0.2	5.8	0.26	2.0
13f	Pr	CHMePh	0.3	10.6	NT	NT
13g	Pr	CH_2-Ph-2Cl	0.3	42	0.80	NT
13h	Pr	CH_2-Ph-4F	0.1	3.4	0.43	NT
13i	Pr	Piperonyl	0.3	23	0.35	10
13j	Pr	CH_2-2-Naphthyl	0.1	55	NT	NT
13k	Pr	CH_2CH_2Ph	0.1	28	0.64	NT
13l	Pr	$CH_2CH_2CH_2Ph$	0.7	44	0.52	NT
13m	Pr	CH_2-2-Thienyl	0.07	7.8	0.13	3.0
13n	Pr	CH_2CH_2-2-Thienyl	0.03	20	0.33	3.1

[a, b] See Table I for an explanation of tabulated data

administration. Among them, **7j**, **7k**, **7p** and **13d**, which exhibited the highest AT_2 binding affinity within this series (1-10 nmol), displayed comparable ID_{50} after intravenous administrating (0.2-0.3 mg/kg) and proved to be orally active at low doses 1.0-1.5 mg/kg, illustrating that these diacidic antagonists display good apparent bioavailability, similar to HR720.

Conclusion

Beginning with our AT_1 selective antagonist imidazolyl biphenyl sulfonylurea HR 720, we have identified a new series of potent and orally active compounds that bind with high affinity both to the AT_1 and AT_2 receptor subtypes. The substitution of the urea side chain has been found to be crucial for high AT_2 binding while retaining excellent AT_1 affinity with cyclohexylmethyl, cyclopentylmethyl or benzyl as the most effective side chains. The high *in vitro* potency on both receptor subtypes as well as the oral activity of compound **13d** (RU 65868), associated with its structural analogy with the AT_1 selective HR 720, make it useful tool for discovering the advantages of balanced compounds over AT_1 selective antagonists.

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