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A POTENT LONG-ACTING IMIDAZOLE-5-ACRYLIC ACID ANGIOTENSIN II AT-1 RECEPTOR ANTAGONIST

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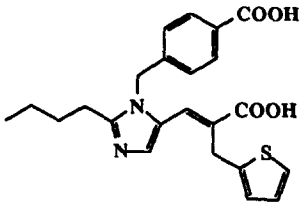
Abstract: SB 203220 (**9**), the naphthyl analog of SK&F 108566 (**1**), is a potent long-acting AT- 1 antagonist. In comparison to **1**, which is a competitive antagonist, **9** is a partially insurmountable antagonist with a slower receptor off-rate, a greater resistance to tissue washout, and is more highly protein bound.

In previous work[1,2,3,4,5] we have described the design, synthesis, and angiotensin II antagonist activity of **1**, (E)-α-[[2-butyl-1-[(4-carboxyphenyl)methyl]-1H-imidazol-5-yl]methylene]-2-thiophene-propanoic acid (SK&F 108566). This compound was designed starting from **2** (CV-2947)[6] using



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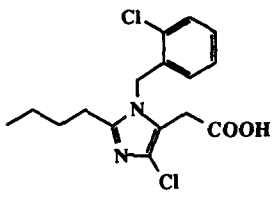

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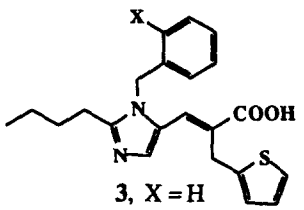


3, X = H

4, X = Cl

an overlay hypothesis with a postulated bioactive conformation of angiotensin II to suggest design strategy. A striking feature of **2** is the orthogonal relationship of the phenyl and imidazole rings enhanced by the 2-chloro substituent of the phenyl. In the imidazole-5-(2-thienylmethyl) acrylic acid series, the 2-chloro analog **4** has 10-fold greater receptor affinity than the unsubstituted phenyl analog **3** (440 nM versus 4530 nM). Here also the 2-chloro substituent causes the phenyl and imidazole rings to be orthogonal as evidenced by NMR data: for **3**, all the phenyl hydrogen peaks are at δ 7.29-7.38 while the 6-hydrogen of the phenyl of **4** gives rise to a doublet at δ 6.38, and thus is clearly shielded by the imidazole pi electron cloud. In the case of **1**, the 2- and 6-hydrogens of the phenyl give rise to an NMR peak which is a doublet at δ 7.07 coupled to the 3,5-hydrogen signal which is a doublet at δ 7.98. This suggests that for **1** and **3** in solution the phenyl and imidazole ring conformation could be less constrained in an orthogonal conformation than in **4**, and that compounds with a more constrained orthogonal conformation might have enhanced receptor affinity.

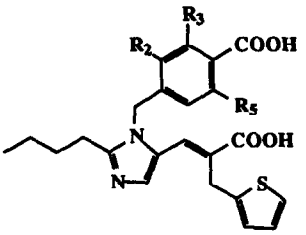
Compound **5** (Table 1) was prepared in order to obtain an analog of **1** in which the phenyl and imidazole rings were conformationally constrained in an orthogonal manner similar to that of **2** and **4**. While this 2-chloro-4-carboxy analog had receptor affinity similar to that of **1**, in the functional assay it was 10-fold more potent in inhibiting the AII induced vasoconstriction of rabbit aorta. However, the 3-chloro analog **6** had receptor affinity and activity in the functional assay similar to that of **1**. The 6-hydrogen of the phenyl of **5** gives rise in the NMR to a doublet at δ 6.45 while the signal for the corresponding hydrogen of **6** occurs at δ 6.9 suggesting that in **5** the phenyl and imidazole rings are more nearly orthogonal than in **6**. In previous work[2] we had found that the 2,3-dichloro analog of **4** had about an order of magnitude greater receptor affinity than **4**. This and the activities seen for **5** and **6** suggested the synthesis of **7**, the 2,3-dichloro analog of **1**. This compound showed about 6 times improved receptor affinity over **1** although it had similar functional activity. Interestingly, the 2,5-dichloro analog **8** had 220 times less receptor affinity than **7**, suggesting a unique property for the adjacent chlorine atoms.

The 2,3-dichlorophenyl arrangement in **7** is reminiscent of dichloroisoproterenol which was a predecessor of propranolol in which a naphthalene was substituted for the 2,3-dichlorobenzene. This suggested that the 2,3-dichlorobenzene and the naphthalene are bioisosteres. A similar replacement starting with **7** gave **9 (SB 203220)** which is a particularly interesting compound. It was prepared as shown in Scheme 1 by methods similar to those previously reported for **1** and **5** - **8**. Methyl 4-bromomethylnapthalene-1-carboxylate **11** was prepared from 1-bromo-4-methylnapthalene **10** by standard methods. Reaction of this with 2-butyl-4-iodoimidazole-5-carboxaldehyde[7] **12** in the presence of K_2CO_3 in dimethylformamide gave the desired regio-isomer **13** in high yield. Hydrogenolytic de-iodination gave the aldehyde **14** which was reacted with the half-acid half-ester **15** in a Knoevenagel condensation to give the acrylic acid ester **16.** Alkaline hydrolysis of this gave the desired dicarboxylic acid **9** whose structure was confirmed by x-ray crystallography. As shown in Figure 1, in the crystal the imidazole and naphthalene rings are orthogonal. This relationship of the two rings is also seen in solution by NMR. Thus the 2-hydrogen of the naphthyl ring of **9** gives rise to a doublet at δ 6.33 which is coupled to a doublet at δ 7.99 arising from the adjacent 3-hydrogen. The signal from the 2-and 6-hydrogens of **1** is substantially downfield suggesting that the naphthyl ring of **9** is more restrained in an orthogonal conformation than the phenyl ring of **1**.

Compound **9** under the usual assay conditions showed almost 12 times less receptor affinity than **1**, but was 4 times more potent in the functional rat aorta assay. It had similar potency to **1** in inhibiting the pressor response to exogenous angiotensin II in the conscious rat on both i.v. and i.d. dosing with a somewhat longer duration of action on i.d. dosing than **1**. Compound **9** was highly selective towards AII in that it had no effect on the pressor response to vasopressin or norepinephrine. In renin-dependent hypertensive rats, **9** dosed at 10 mg/kg i.d. lowered mean arterial blood pressure from 150 to 112 mm Hg with a duration of action of 14 hours. In this protocol **1** had similar potency, but the duration of action

was only 1.5 hr. In the angiotensin I infused dog, **9** given at 10 mg/kg p.o. inhibited the pressor effect for up to 20 hours, in comparison to the 14 hour duration of action for **1**. However the maximum inhibition of the pressor effect of angiotensin I was similar for both compounds at doses of 3 and 10 mg/kg p.o. Both **1** and **9** dosed at 10 mg/kg p.o. lowered mean blood pressure about 35 mm Hg for more than 12 hours in the 2-kidney, 1-clip Goldblatt renin dependent hypertensive dog.

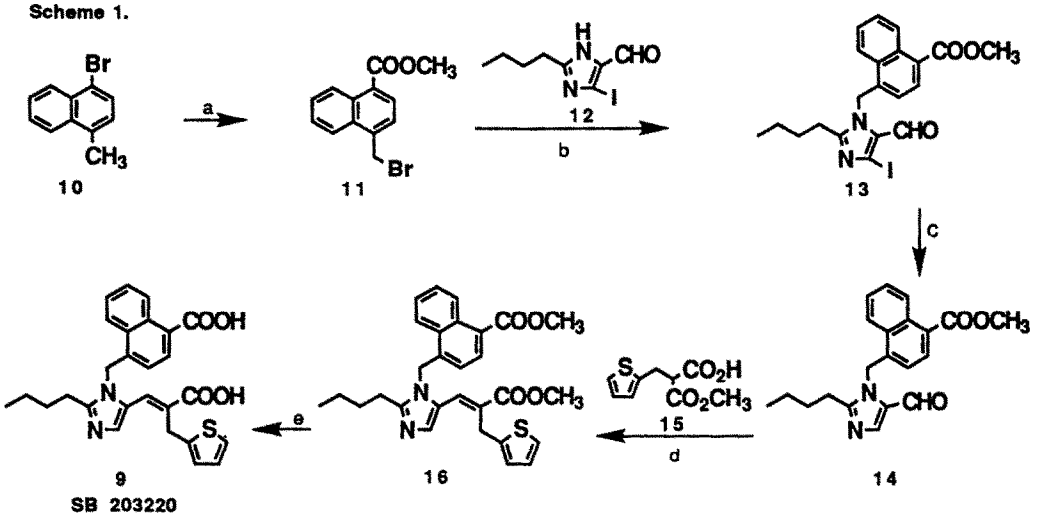
Table 1. In Vitro and In Vivo Angiotensin II Antagonist Activity



Compound	R_2	R_3	R_5	IC_{50}[a] (nM)	K_b[b] (nM)	ID_{50}[c](mg/kg) i.v.	i.d.	$t_{1/2}$[d](dose)
1	H	H	H	1.0	0.21	0.08	5.5	85(10)
5	Cl	H	H	1.45	0.02	0.06	2.4	150(10)
6	H	Cl	H	1.04	0.13	0.12	16	108(30)
7	Cl	Cl	H	0.16	0.18	0.04	5.4	96(10)
8	Cl	H	Cl	35.4	1.58	0.68	-	-
9			H	11.8	0.059[e]	0.05	3.8	145(10)

[a]Inhibition of [^{125}I] AII specific binding to rat mesenteric arteries n = 3 - 5, as described in ref. 4. [b]Inhibition of AII-induced vasconstriction of rabbit aorta, n = 3 - 5, as described in ref. 4. [c]Dose that produced a 50% inhibition of the pressor response to AII in conscious normotensive rats, n = 3 - 5, as described in ref. 4. [d]Time in minutes after intraduodenal dosing for the pressor response of AII to recover 50% of its initial effect. [e]Measured at 30% inhibition rather than the 50% used for the other compounds.

Scheme 1.



a, (1) n-BuLi, CO_2, (2) CH_3OH, HCl, (3) NBS, $(C_6H_5COO)_2$; b, K_2CO_3, DMF; c, Pd/C, H_2, KOAc; d, piperidine; e, KOH, EtOH - H_2O.

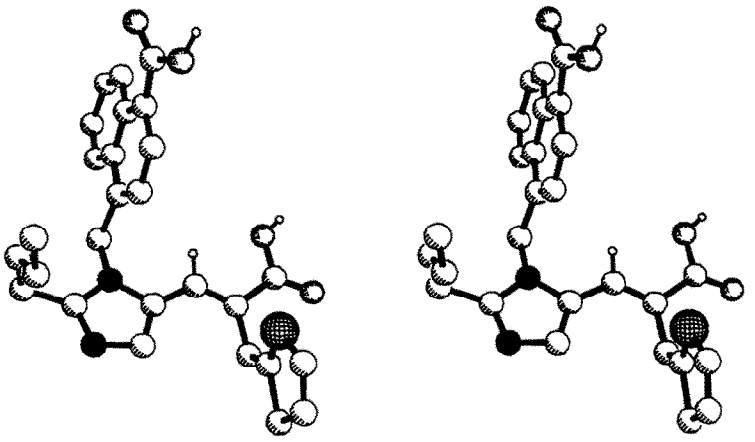
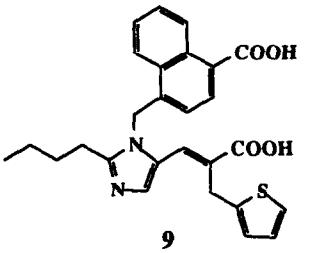


Figure I. Stereodrawing of the x-ray structure of **9**. Oxygens are dotted, nitrogens are solid, and the sulfur is cross-hatched.



9

A rationale for the longer duration of action of **9** in comparison to that of **1** may be found in more detailed *in vitro* studies. The binding studies were repeated in the presence of 0.25% bovine serum albumin (BSA) and in the absence of BSA. The receptor affinity for **9** decreased from 1.2 nM in the absence of BSA to 31 nM in the presence of BSA, while the corresponding values for **1** were 2 nM and 7.3 nM. The relative potencies in the absence of BSA correlated better with the rabbit aorta data and also suggested that the longer duration of action of **9** may be due to high binding to serum albumin and slow release. In addition, rat adrenal cortical membranes pretreated with **1** and then extensively washed bound the same quantity of AII as similarly washed control membranes. However, pretreatment with **9** decreased by 71% the amount of AII bound by similarly washed membranes suggesting that **9** had a slower dissociation rate from the receptor. This was confirmed in experiments in which the dissociation of labeled drug from receptor was measured[8,9]. Thus **1** was completely dissociated from the receptor in 15 minutes at 25° while even after 2 hours at 30° about 35% of **9** was still bound to the receptor.

Also, in the rabbit aorta functional assay **9** exhibited partially insurmountable antagonism. Thus **9** at 1-1000 nM produced parallel rightward shifts of the AII concentration response curve while reducing the maximal contractile response 35% at all drug concentrations. This is unlike classical non-competitive receptor antagonists which decrease the maximal response with increasing dose. The K_b (apparent) value shown in Table 1 was calculated at the EC_{30} response, and in view of the abnormal dose-concentration curves is not a classical K_b. The reversibility of **1**- and **9**- mediated antagonism of the contractile response to AII was investigated by replacing the bath media of the drug treated aortic rings every 5-10 minutes. Although the effect of both compounds was reversed, the washout of **1** was complete in 30 minutes while with **9** some drug effect was still apparent at 180 minutes. The above data suggests that although the longer duration of action of **9** in comparison to **1** may be ascribed in part to sequestering of drug in plasma protein followed by slow release, another important factor may be slow dissociation from the receptor.

The marked *in vitro* differences between **1** and **9** suggests that they might be binding to the receptor in a different manner. We have suggested[3] that **1** binds to the AT-1 receptor in a manner so that the 4-carboxybenzyl mimics the Tyr-4 of AII while the acrylic acid carboxyl and thienylmethyl mimic the Phe-8 of AII. In contrast, we have postulated that in the biphenyltetrazole class of imidazole angiotensin II antagonists the tetrazole and the phenyl ring to which it is attached may mimic the Phe-8 carboxyl and

phenyl ring of AII, while the imidazole-5-hydroxymethyl or carboxyl aligns with the Tyr-4 phenol. Similarly, in **9** the 4-carboxyl of the naphthalene and the benzo ring of the naphthalene may mimic the Phe-8 suggesting that the 2'-biphenyltetrazole and the 4-carboxynaphthalene may function as bioisosteres.

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