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D AND L-N-[(1-BENZYL-1H-IMIDAZOL-5-YL)-ALKYL]-AMINO ACIDS AS ANGIOTENSIN II AT-1 ANTAGONISTS

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Abstract: A series of D and L-N-[(1-benzyl-1H-imidazol-5-yl)-alkyl] - aromatic amino acids (**2** to **9**) and several achiral analogs (**1**,**10**,**11**) were found to be potent AII antagonists (nM range). Among chiral pairs the D isomer had the highest affinity for the binding site. A D-phenylalanine analog, **3**, was the most potent (IC_{50} 3.8 nM) and had activity in vivo similar to SK&F 108566 when given i.v. but was only marginally active when given i.d.

Introduction

Antagonism of the vasoconstricting octapeptide angiotensin II (AII) at the AT-1 receptor as a therapeutic avenue for the treatment of hypertension is now widely recognized.[1] A variety of substituted heterocyclic ring systems have been evaluated for AII antagonist activity.[2] The substituents attached to these rings may vary, but often include an acid sidechain or its metabolic precursor.[3] Molecular modeling studies of the original Takeda compound[4] A, (see scheme for structure) suggested to us that the 5-carboxyl methyl group mimicked the carboxy of the terminal acid of AII and resulted in the discovery of the imidazole-5-acrylic acids as potent, orally active AII antagonists, e.g. SK&F 108566[5] (see scheme for structure). The potent AT-1 binding site affinity of the acrylic acid led us to consider other acid sidechain variants. Modeling suggested that in SK&F 108566 the thienyl carboxylic acid mimicked the Phe^8 of AII, implying that the double bond in this molecule may be an amide or C-N isostere. To avoid amide bond enzymatic hydrolysis, CH_2-N replacements were initially selected as acid sidechain variants. This substitution led to amino acid derivatives and evaluation of the effect of chirality at the AT-1 receptor became possible. While the influence on biological properties of D and L amino acids at position 8 of AII has been investigated by Samanen[7] and others,[8] examples of chiral nonpeptide AII antagonists have been limited.[9] Modeling of the achiral glycine derivative **1** showed that key parts of **1**, e.g. CO_2H, could overlap the corresponding features of SK&F 108566 frozen in the conformation found in the X-ray structure[5] (Figure 1) and provided additional incentive for pursuing this type of compound. Described herein are our early findings of AII antagonist activity encountered with a small series of D and L aromatic amino acids.

Chemistry

Compounds **1** to **9** (Table 1) were prepared in four steps starting from the known[5] imidazole-5-carboxaldehyde **12** (Scheme 1). Thus **12** was converted to the alcohol **13** with $NaBH_4$ and then to the chloride hydrochloride **14** in refluxing $SOCl_2$. Coupling of **14** with the amino acid ester in the presence of Et_3N in methylene chloride gave **15** which was purified by chromatography (silica gel, ethyl acetate/hexane). Hydrolysis with sodium hydroxide in aqueous alcohol followed by treatment with aqueous HCl gave the amino acids **1** to **9**.[8] The achiral N-trisubstituted amino acids, **10** and **11**, were prepared similarly, coupling the known N-(methylaryl)glycine esters **16**[10] and **17**[11] with the chloride **14** to give **18** and **19** followed by aqueous sodium hydroxide hydrolysis.

Biological Results

The in vitro biological data for **1** to **11** and SK&F 108566 were drawn from two separate in vitro assays: a rat mesenteric artery preparation for binding and a rabbit aorta functional assay and are shown in Table 1. Exceptions were **1**,**8** and **9** which were evaluated for inhibition of AII AT-1 binding in LhAT-1D6 cells, the recombinant human AT-1 receptor expressed in a stable cell line[12]. This assay gives similar values to those obtained from rat mesenteric artery [^{125}I] AII binding studies.[13] Compounds **1** to **11** all exhibited significant affinity for the AT-1 receptor ranging in potency from 2.9 nM (**9**) to 673 μM (**6**). Only 5 showed evidence of noncompetitive behavior (aorta). The explanation for this remains unknown but may be a result of conformational changes induced by the methyl group causing 5 to bind at a site and in a manner different from AII. The achiral glycine **1** had modest activity in both the binding (IC_{50} 298 nM) and aorta screens (K_b 335 nM). Among chiral pairs, both enantiomers displayed affinity for the receptor with the D-enantiomer being more potent than the L-enantiomer. α-Methyl substitution (**4** and **5**) resulted in loss of activity compared to H (**2** and **3**). However replacement of phenyl with 3-indole slightly enhanced affinity for binding in the mesentery but not in the aorta functional assay. Unlike the acrylic acid series[5] substitution of phenyl with thienyl (e.g. **3** vs **7**) failed to increase potency. A similar relationship between phenyl and thienyl was seen in the achiral N-trisubstituted compounds **10** and **11** both of which were less potent than their most potent chiral counterparts. Since we postulate that the antagonist amino acid mimics the carboxy terminal phenylalanine of AII, and (D-Phe-8) AII is an AII antagonist while the natural L-Phe-8 is an agonist, it is possible that the imidazole D-amino acid AII antagonists reported in this paper more closely mimic the antagonist conformation of (D-Phe-8) AII than does the L isomer.[14]

Because of its' relative potency, **3** was selected as an initial candidate for further preliminary in vivo evaluation using a previously described model.[15] When given i.v. to conscious normotensive rats, **3** was similar to SK&F 108566 at inhibiting the AII pressor response with similar IC_{50} values (0.08 mg/kg) (Figure 2a). However, upon i.d. administration **3** had only marginal activity at 10 mg/kg and was short-acting compared to SK&F 108566 (Figure 2b). Though speculative, this data suggests **3** may not be well absorbed. A resolution of this observation awaits further investigation.

Scheme 1

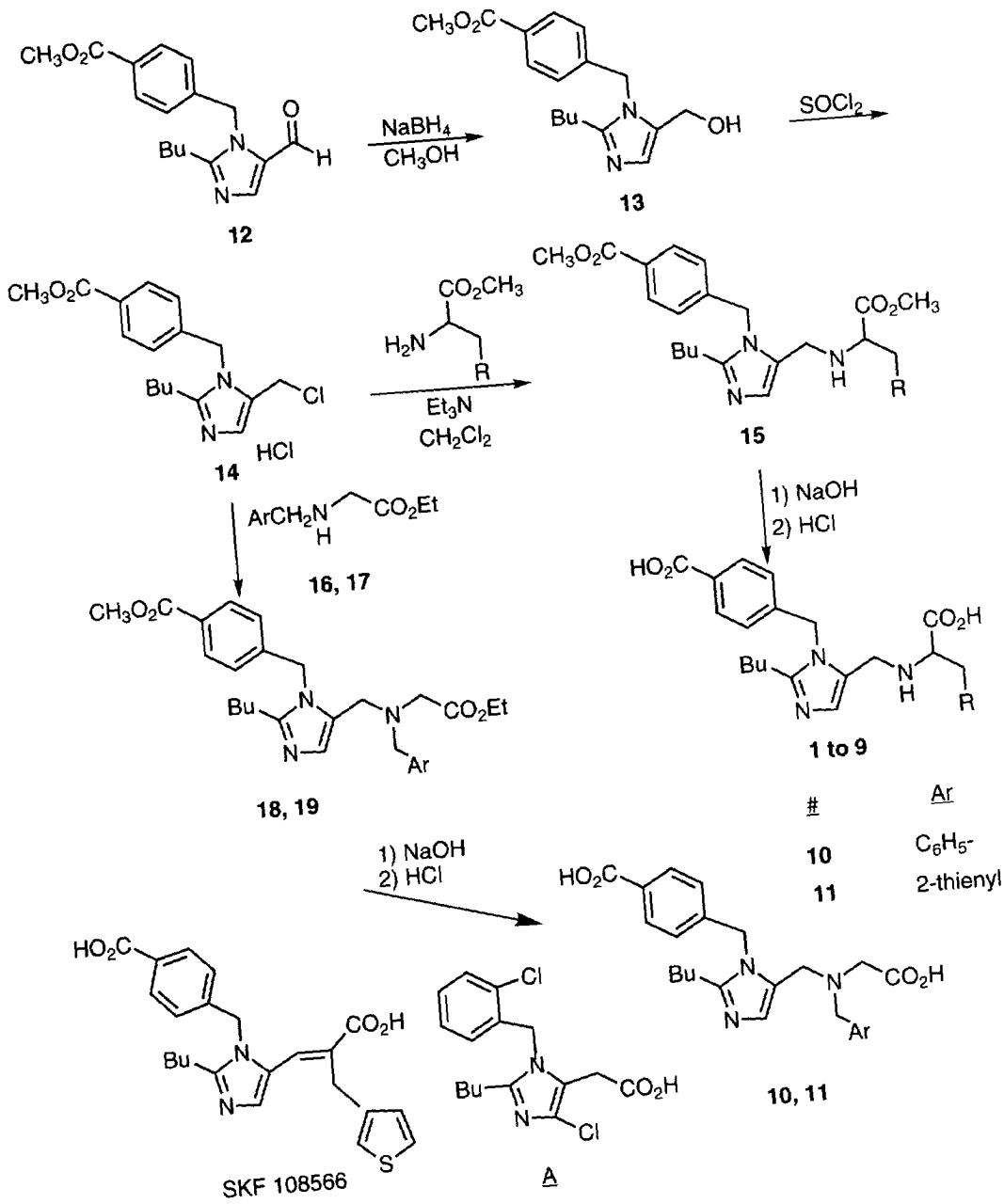
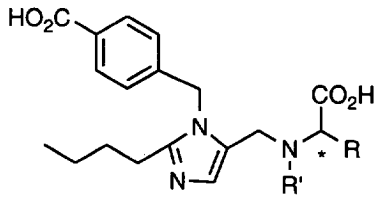

CH3O2C
Bu
N
O
H
12
NaBH4
CH3OH
OH
13
SOCl2
Cl
HCl
14
CO2CH3
H2N
R
Et3N
CH2Cl2
15
CO2CH3
N
H
1) NaOH
2) HCl
HO2C
CO2H
1 to 9
ArCH2N
CO2Et
16, 17
CO2Et
Ar
18, 19
#
Ar
10
C6H5-
11
2-thienyl
1) NaOH
2) HCl
10, 11
SKF 108566
A

Table 1. In Vitro Angiotensin II Antagonist Activity



Compound	R	R'	*	IC_{50}[a] (nM)	K_b[b] (nM)
1	H	H		298[c]	335
2	$C_6H_5CH_2$	H	L	517	125
3	$C_6H_5CH_2$	H	D	3.8	3.6
4	$C_6H_5CHCH_3$	H	L	176	117
5	$C_6H_5CHCH_3$	H	D	28	0.6[d]
6	2-thienyl-CH_2	H	L	673	63
7	2-thienyl-CH_2	H	D	36.7	5.0
8	3-indolyl-CH_2	H	L	48.3[c]	885
9	3-indolyl-CH_2	H	D	2.9[c]	48
10	H	$C_6H_5CH_2$		31	11
11	H	2-thienyl-CH_2		169	2.4
SK&F 108566				1.0	0.21

[a]Inhibition of ^{125}I - AII specific binding to rat mesenteric arteries, n=3-5, as described in ref. 5 except for compounds 1,8 and 9 as noted in footnote c. [b]Inhibition of AII-induced vasconstriction of rabbit aorta, n=3-5 as described in ref. 5 [c]For compounds 1,8 and 9 the data reflect inhibition of AII specific binding to AII AT-1 receptors in the recombinant human AT-1 receptor expressed in LhAT-1D_6 cells, a stable clonal cell line assay as described in ref. 12. The data obtained in this assay are virtually identical to that from the rat mesentery assay in footnote a. [d]Noncompetitive

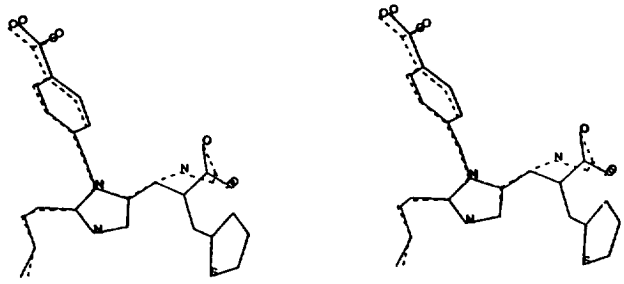


Figure 1. Model of 1(---) overlaid on the crystal structure of SK&F 108566.

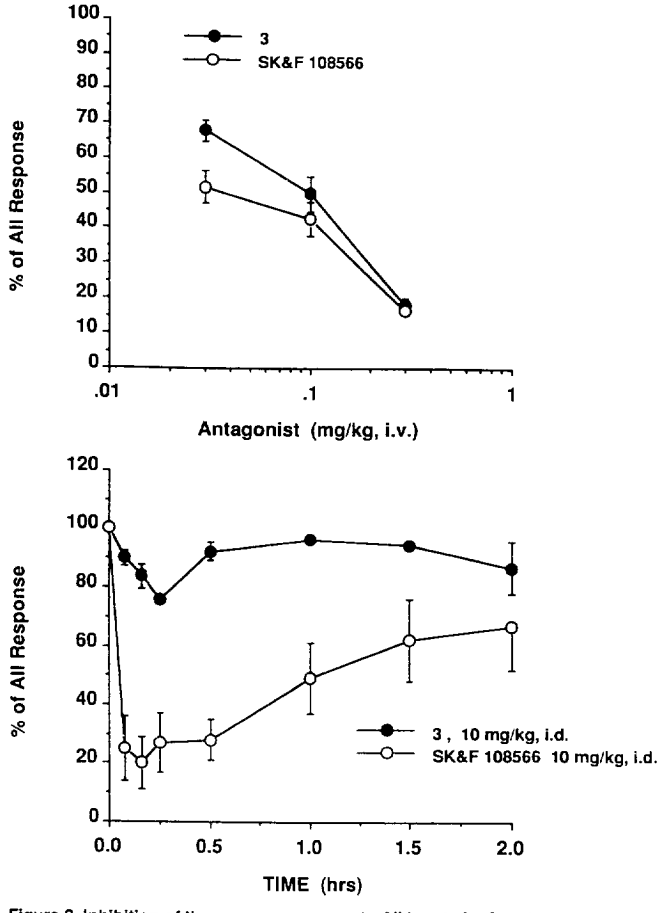


Figure 2. Inhibition of the pressor response to AII in rats by 3 and SK&F 108566 given intravenously (top) or intraduodenally (bottom).

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8. Chiral purity was verified through a combination of ^{1}H NMR chiral shift reagents (esters), chiral HPLC and optical rotation. For example $[\alpha]^{25}$ (1, CH_3OH) for **2** and **3** was +32.5° and -28.9° respectively.

9. However, chiral amino acid amides have been described as AII receptor antagonists including analogs of GR117289, reference 9a and Valsartan, reference 9b. (a) Middlemiss, D.; Watson, S.P.; Ross, B.C.; Dowle, M.D.;Scopes, D.I.C.; Montana, J.G.; Hirst, G.C.; Panchal, T.A.; Paton, J.M.S.; Hubbard, T.; Stuart, G.; Drew, G.M.; Hilditch, A.; Travers, A.; Robertson, M.J.; Hunt, A.A.E.; Palmer, E.; Manchee, G.R. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 2043.
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