Conclusion

In this communication we have described an efficient synthesis of an ^{125}-labeled photoaffinity probe of high affinity for human platelet TXA_2/PGH_2 receptors. The high affinity of this probe and its irreversible incorporation into the TXA_2 receptor should make it a useful tool for the study of this receptor. This synthesis was accomplished via an exchange of the ^{127}I isotope with a trimethyltin group in the presence of an azide group followed by an electrophilic destannylation using [^{125}I]ICl generated with chloramine-T. This novel approach should prove to be generally applicable to the synthesis of other photoaffinity probes where generation of an azide under acid conditions is not possible due to the presence of other acid-labile groups in the molecule. In addition, the exchange can be performed as a final step and in good yields, eliminating the need to generate an azide in the final step, which is commonly done for ^{125}I-labeled azides.

Acknowledgment. We thank Ms. Charlene Bourke-Walters for the preparation of this manuscript. This work was supported in part by NIH HL36838 and HL07260.

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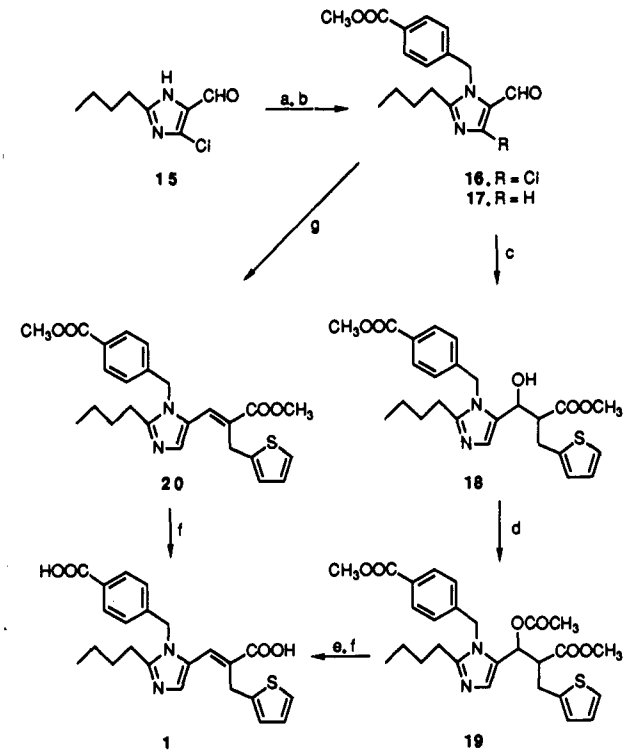
Received December 26, 1990

1-(Carboxybenzyl)imidazole-5-acrylic Acids: Potent and Selective Angiotensin II Receptor Antagonists

The renin–angiotensin system (RAS) plays an important role in the regulation of blood pressure and fluid and electrolyte balance. Blockade of the renin–angiotensin system by inhibiting the biosynthesis of the effector hormone, angiotensin II (AII), with angiotensin converting enzyme (ACE) inhibitors has been shown to be clinically effective in the treatment of hypertension, congestive heart failure, and, potentially, chronic renal failure.[1] However, ACE inhibitors, in addition to their effects on the RAS, inhibit bradykinin metabolism, and as a result may produce cough and angioedema. An alternative, and perhaps more selective approach to interfering with the RAS, is to inhibit the binding of AII to its receptor. Such an antagonist would be expected to exhibit similar therapeutic effects as the ACE inhibitors, but may lack the undesirable side effects related to bradykinin potentiation.[2] Although a number of peptide analogues of AII have been reported to have AII receptor antagonist properties, all have retained partial agonist properties and have lacked oral bioavailability.[1] More recently, several groups have described nonpeptide AII receptor antagonists that show promise as inhibitors of the RAS.[3,4] In this communication, we describe the design, synthesis, and pharmacological characterization of the (4-carboxybenzyl)imidazole-5-acrylic acid 1 (SK&F 108566), a highly potent, selective non-peptide AII receptor antagonist which was designed to mimic the C-terminal region of AII.

Scheme I[a]



[a] (a) Methyl 4-(bromomethyl)benzoate, K_2CO_3, DMF (89%); (b) H_2, 5% Pd–C, KOAc, MeOH (97%); (c) methyl 3-(2-thienyl)-propionate, LDA, THF (98%); (d) Ac_2O, DMAP, CH_2Cl_2 (94%); (e) DBU, toluene (94%); (f) KOH, EtOH, H_2O (83%); (g) monomethyl (2-thenyl)malonate, piperidine, pyridine, toluene (40%).

The benzylimidazole 2 (Table I) was reported by the Takeda group[4] to be an AII antagonist. A detailed evaluation of this compound indicated that it was a specific, albeit weak, competitive antagonist of AII, which not only inhibited the pressor effect of AII in normotensive rats but in addition lowered blood pressure in renin-dependent hypertensive rats. In developing a strategy for enhancing affinity in this class of AII antagonists, we postulated that

(1) Corvol, P. New Therapeutic Prospects of Renin-Angiotensin System Inhibition. *Clin. Exp. Hypertens.-Theory Practice* **1989**, *A11* (Suppl. 2), 463–470.
(2) Chin, H. L.; Buchan, D. A. Severe Angioedema after Long-Term Use of an Angiotensin-Converting Enzyme Inhibitor. *Ann. Intern. Med.* **1990**, *112*, 312–313.
(3) (a) Dunica, J. V.; Chiu, A. T.; Carini, D. J.; Gregory, G. B.; Johnson, A. L.; Price, W. A.; Wells, G. J.; Wong, P. C.; Calabrese, J. C.; Timmermans, P. B. M. W. M. The Discovery of Potent Nonpeptide Angiotensin II Receptor Antagonists: A New Class of Potent Antihypertensives. *J. Med. Chem.* **1990**, *33*, 1312–1329. (b) Wong, P. C.; Chiu, A. T.; Price, W. A.; Thoolen, M. J. M. C.; Carini, D. J.; Johnson, A. L.; Taber, R. I.; Timmermans, P. B. M. W. M. Nonpeptide Angiotensin II Receptor Antagonists. I. Pharmacological Characterization of 2-*n*-Butyl-4-chloro-1-(2-chlorobenzyl)imidazole-5-acetic acid, sodium salt (S-8307). *J. Pharmacol. Exp. Ther.* **1989**, *247*, 1–7. (c) Wong, P. C.; Price, W. A.; Chiu, A. T.; Carini, D. J.; Dunica, J. V.; Johnson, A. L.; Wexler, R. R.; Timmermans, P. B. M. W. M. Nonpeptide Angiotensin II Receptor Antagonists: Studies with EXP9270 and DuP 753. *Hypertension* **1990**, *15*, 823–834. (d) Wong, P. C.; Price, W. A.; Chiu, A. T.; Wong, N. Y.; Dunica, J. V.; Carini, D. J.; Johnson, A. L.; Timmermans, P. B. M. W. M. EXP-6803, A Nonpeptide Angiotensin II Receptor Antagonist. *Cardiovasc. Drug Rev.* **1989**, *7*, 285. (e) Chiu, A. T.; Dunica, J. V.; McCall, D. E.; Wong, P. C.; Price, W. A.; Thoolen, M. J. M. C.; Carini, D. J.; Johnson, A. L.; Timmermans, P. B. M. W. M. Nonpeptide Angiotensin II Receptor Antagonists. III. Structure–Function Studies. *J. Pharmacol. Exp. Ther.* **1989**, *250*, 867–874.
(4) Furukawa, Y.; Kishimoto, S.; Nishikawa, K. Hypotensive Imidazole-5-acetic Acid Derivatives. U.S. Patent 4 355 040, 1982.

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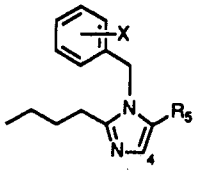
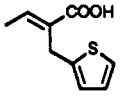
Table I. Angiotensin II Antagonist Activity of Imidazole-5-acrylic Acids



no.	structure X	structure R_5	mp, °C	in vitro IC_{50},[a] nM	in vitro K_B,[b] nM	in vivo:[c] iv ID_{50}, mg/kg
2	2-Cl (imidazole-4-Cl)		167–169.5	43000	2700	30 ± 3.5
3	2-Cl		158–161	12000	1900	22.5
4	2-Cl		178–179	8900	810	15 ± 0.72
5	2-Cl		176–177.5	2600	64	14 ± 3.3
6	2-Cl		176–177[e]	440	51	3.6 ± 0.42
7	2-OH	*d*	181–183	90	34	
8	4-OH-3-CH_3	*d*	150–152	21	33	
9	2-NO_2	*d*	205–206	31	57	2.6 ± 0.38
10	4-NO_2	*d*	198–200	620	50	
11	2-COOH	*d*	209–210	6000	2250	
1	4-COOH (SK&F 108566)	*d*	260–261	1.0	0.21	0.08 ± 0.005
12	2-Cl-4-COOH	*d*	230–231[f]	1.45	0.02	0.06 ± 0.005
13	[Sar^1]AII			1.3	*g*	
14	[Sar^1,Thi^8]AII			0.11	2.5	

[a] Inhibition of [^{125}I]AII specific binding to rat mesenteric membranes, n = 3–5. See footnote 10 for experimental details. [b] Inhibition of AII-induced vasoconstriction of rabbit aorta; n = 3–5. See footnote 11 for experimental details. Compounds 1, 2, and 6 were tested for and exhibited competive inhibition and had no effect on KCl-, norepinephrine-, or endothelin-induced vasoconstriction. [c] Dose that produced 50% inhibition of the pressor response to AII in conscious normotensive rates. See footnote 12 for experimental details. [d] Same R_5 substituent as above. [e] Hemihydrate. [f] Hydrate. Loses water at 140–143° C. [g] Agonist.

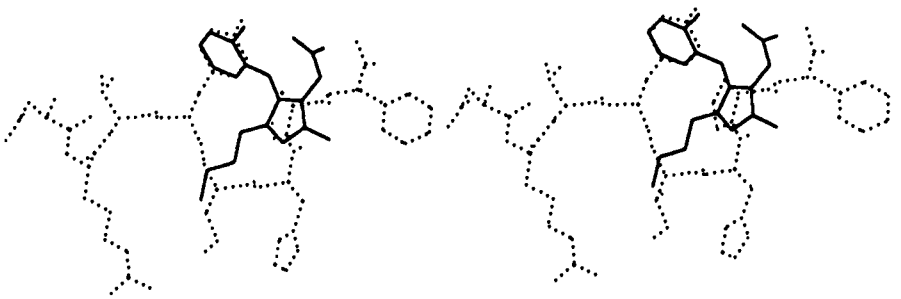
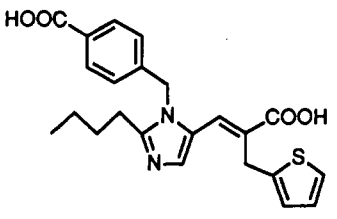


Figure 1. Stereoplot of an overlay of an energy minimized conformation of 2 (solid) on a postulated bioactive conformation of AII (dotted).



1

H - Asp - Arg - Val - Tyr - Ile - His - Pro - Phe - OH

Angiotensin II

2 was mimicking critical elements of AII. NMR and molecular modeling studies[5] indicated that the 2-chloro substituent on the phenyl ring of 2 directs the imidazole and phenyl rings into an orthogonal relationship. In the Fermandjian model[6] for a bioactive conformation of AII, the tyrosine side chain similarly projects from the peptide backbone in an orthogonal manner, suggesting an overlay in which the benzyl of 2 mimics the tyrosine side chain. An alternative model to relate 2 to the Fermandjian conformation would have the *N*-benzyl side chain and carboxyl of 2 mimicking the carboxy terminal Phe^8 of AII. However, compounds prepared based on these overlays showed no significant improvement in activity over 2. These disappointing results led us to consider whether the *N*-benzyl and carboxyl groups of 2 could correspond to the Tyr^4 aromatic side chain and Phe^8 carboxyl group, respectively, of AII. Although such an overlay would be inconsistent with the Fermandjian model, it is possible to place AII in a conformation, consistent with peptide SAR[6] and acceptable ϕ/ψ angles, which would place these two residues in relatively close proximity. An overlay of 2 with AII in such a conformation is shown in Figure 1.

Consideration of this model, albeit hypothetical, suggested a number of structural modifications of 2 which

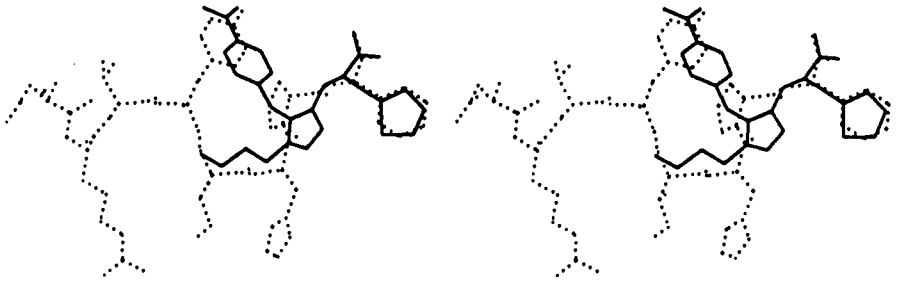


Figure 2. Stereoplot of an overlay of 1 (solid) on a postulated bioactive conformation of AII (dotted).

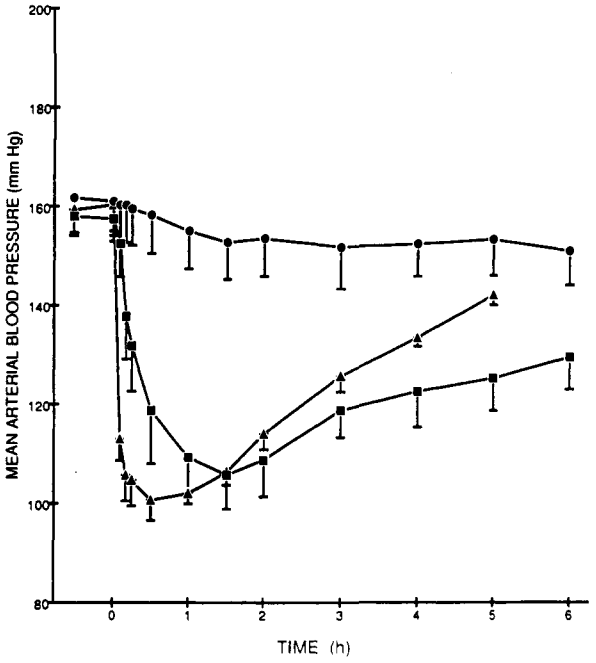


Figure 3. Effect of 3 mg/kg iv, $n = 3$ (triangles) and 10 mg/kg po, $n = 4$ (squares) administration of 1 on blood pressure in the AI-infused dog. Conscious normotensive dogs that were trained to stand quietly in a sling were infused intravenously over the course of the experiment with 100 ng/kg min of AI to elevate mean blood pressure from about 100 mmHg to 160 mmHg. Vehicle treatment, $n = 4$ (circles), results in a slight downward trend over time.

might enhance affinity. In order to extend the acid side chain to approximate better the Tyr[4]-Phe[8] separation, but limit conformational freedom, the trans 5-acrylic acid **4** was prepared and found to be more potent than **2** (Table I). Addition of an α-benzyl group to the acrylic acid side chain (**5**) to mimic better the Phe[8] side chain of AII resulted in a further increase in affinity. The thienyl analogue **6**, in addition to having 5-fold more binding affinity than the corresponding benzyl analogue **5**, exhibited enhanced oral activity as an antagonist of AII-induced hypertension in the rat. This increase in affinity of **6** over **5** is consistent with the 12-fold better binding affinity subsequently observed for [Sar[1],Thi[8]]AII (**14**) versus [Sar[1]]AII (**13**).

The effect of changing the functionality on the *N*-benzyl ring was explored (Table I). A hydroxy group, especially in the 4-position (**8**), and a nitro group in the 2-position (**9**) gave compounds with substantially improved binding affinity. However, the 4-carboxy analogue **1** as well as the 2-chloro-4-carboxy compound **12** exhibited significantly increased activity.[7] This dramatic potency of **1** and **12** would suggest that the aryl carboxyl group is picking up a new binding site in the receptor, and perhaps is mimicking the Tyr[4] phenol. In such a model[8] (Figure 2), the acrylic acid carboxyl and thienyl groups then align with the corresponding elements of Phe[8] and the 2-butyl lies in the hydrophobic region near Ile[5]. Finally, the N–C–N imidazole region and the acrylic acid double bond may mimic peptide amide bonds.

The synthesis of **1** is shown in Scheme I. Alkylation of 4-chloro-5-formyl-2-butylimidazole[4] **15** with *p*-carbomethoxybenzyl bromide gave the required 1-benzyl 5-aldehyde **16** with high regioselectivity. The use of the chloroimidazole in this reaction is essential to achieve high regioselectivity; the deschloro analogue of **15** affords a 1:1 mixture of isomeric *N*-benzyimidazoles. Reaction of **17** with the lithium enolate of methyl 3-(2-thienyl)propionate gave **18** as a mixture of diastereomers, each of which afforded the same trans olefin **20** on acetylation followed by base-catalyzed elimination. Alternatively, **17** could be converted to the diester **20** in a single step by Knoevenagel reaction with the half-ester of methyl (2-thienyl)methylmalonate. The regio- and stereochemistry of **1** was established by NOE and X-ray crystallography. The other imidazoles in Table I were prepared by similar procedures.

Compound **1** exhibited competitive inhibition of [^{125}I]AII binding to rat mesenteric artery membranes (IC_{50} = 1.0 nM), and competitive inhibition of AII-induced vasoconstriction of rabbit aorta (K_B = 0.21 nM). It is highly selective for the AII-1 receptor and shows little affinity (IC_{50} > 1000 nM) for the AII-2 receptor[9] present in human uterus, bovine brain or ovary, or rat brain. The high potency of **1** is also observed in vivo. In the normotensive rat, **1** exhibited potent inhibition of the pressor response

(5) Energy minimization calculations were carried out with use of MAXIMIN in Sybyl Version 3.4 (Tripos, Inc.).

(6) Smeby, R. R.; Fermandjian, S., Conformation of Angiotensin II. *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins, 5*; Weinstein, B., Ed.; Marcel Dekker, Inc.: New York, 1978; p 117–162. Peach, M. J. Structural features of angiotensin II which are important for biologic activity. *Kidney Int.* **1979**, *15*, S-3. Samanen, J.; Narindray, D.; Adams, W.; Cash, T.; Yellin, T.; Regoli, D. Effects of D-Amino Acid Substitution on Antagonist Activities of Angiotensin II Analogs. *J. Med. Chem.* **1988**, *31*, 510–516. Samanen, J.; Hempel, J. C.; Narindray, D., Regoli, D. A position seven analog of angiotensin II with potent antagonist activity. *Peptides Chemistry and Biology, Proc. 10th Amer. Peptide Symp.*; Marshall, G. R. Ed.; Escom Science Publishers: Leiden, 1988; pp 137–139. Samanen, J.; Cash, T.; Narindray, D.; Brandeis, E.; Yellin, T.; Regoli, D. The Role of Position 4 in Angiotensin II Antagonism: A Structure–Activity Study. *J. Med. Chem.* **1989**, *32*, 1366–1370.

(7) The NMR of **12** as well as that of the other members of the 2-chlorobenzyl series suggested that the imidazole and benzyl rings are orthogonal. This implies that the bioactive form of **1** may also have orthogonal benzyl and imidazole rings.

(8) See ref 3 for a comprehensive bibliography pertaining to other proposed AII models.

to AII (ID_{50} = 0.08 mg/kg iv). In dogs, infusion of AI at 100 ng/kg min results in a significant elevation of blood pressure (Figure 3). Compound 1 at 3 mg/kg iv or 10 mg/kg po lowered blood pressure substantially. Most significantly, at 10 mg/kg po, this reduction persisted for at least 6 h. Agonist activity was not observed for 1 either in vitro or in vivo.

Compound 1 is a novel, highly potent, orally active AII-1 selective competitive antagonist lacking agonist activity. On the basis of this profile, it has been selected as a potential candidate for clinical investigation for the treatment of hypertension, renal failure, and congestive heart failure.

(9) Chiu, A. T.; Herblin, W. F.; McCall, D. E.; Ardecky, R. J.; Carini, D. J.; Duncia, J. V.; Pease, L. J.; Wong, P. C.; Wexler, R. R.; Johnson, A. L.; Timmermans, P. B. M. W. M. Identification of Angiotensin II Receptor Subtypes. *Biochem. Biophys. Res. Commun.* **1989**, *165*, 196–203.

(10) The radioligand binding assay was a modification of a method previously described (Gunther, S.; Gimbrone, M. A.; Alexander, R. W. Identification and Characterization of the High Affinity Vascular Angiotensin II Receptor in Rat Mesenteric Artery. *Circ. Res.* **1980**, *47*, 278–286). A particulate fraction from rat mesenteric arteries was incubated in Tris buffer with 80 pM of [^{125}I]angiotensin II with or without angiotensin II antagonists for 1 h at 25 °C. The incubation was terminated by rapid filtration and receptor-bound [^{125}I]angiotensin II trapped on the filter was quantitated with a gamma counter. The potency of angiotensin II antagonists was expressed as the IC_{50}, which is the concentration of antagonist to displace 50% of the total specifically bound angiotensin II.

(11) The ability of the compounds to antagonize angiotensin II induced vasoconstriction was examined in the rabbit aorta. Ring segments were cut from the rabbit thoracic aorta and suspended in organ baths containing physiological salt solution. The ring segments were mounted over metal supports and attached to force displacement transducers which were connected to a recorder. Cumulative concentration response curves to angiotensin II were performed in the absence of antagonist or following a 30-min incubation with antagonist. Antagonist dissociation constants (K_B) were calculated by the dose ratio method using the mean effective concentrations.

(12) Rats were prepared with indwelling femoral arterial and venous catheters. (Gellai, M.; Valtin, H. Chronic vascular constrictions and measurements of renal function in conscious rats. *Kidney Int.* **1979**, *15*, 419–426). Two to three days following surgery the rats were placed in a restrainer, and blood pressure was continuously monitored from the arterial catheter with a pressure transducer and recorded on a polygraph. The change in mean arterial pressure in response to intravenous injections of 250 ng/kg angiotensin II was compared at various time points prior to and following the administration of the compounds intravenously at doses of 3–300 mg/kg. The dose of compound needed to produce 50% inhibition of the control pressor response to angiotensin II (IC_{50}) was used to estimate the potency of the compounds.

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Received September 28, 1990

Additions and Corrections

1990, Volume 33

Anette M. Johansson,* Karin Fredriksson, Uli Hacksell, Cor J. Grol, Kjell Svensson, Arvid Carlsson, and Staffan Sundell: Synthesis and Pharmacology of the Enantiomers of *cis*-7-Hydroxy-3-methyl-2-(dipropylamino)tetralin.

Page 2926. The general structure in Table I should be changed to