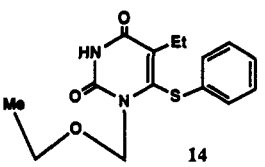
15[29] by the method described. Their activity shown in



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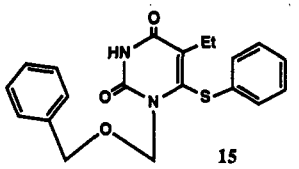

15

Table I, together with that of HEPT, clearly indicates that the initial activity of HEPT has been dramatically improved at this stage. In particular, the EC_{50} value of 15 is comparable to AZT. It should be emphasized that, in terms of CC_{50}, both 5-ethyl analogues are much less cytotoxic than is AZT. When the activity was examined with some AZT-resistant HIV-1 strains, both compounds were equally effective.[30] Their anti-HIV-1 activities were further confirmed by monitoring viral antigen expression in CEM cells as shown in Figure 1.[31] HIV-1 antigen expression was almost completely suppressed at concentrations of 40–800 nM.

It should be mentioned that the analogues synthesized in this study are uniformly inactive against HIV-2,[30] following the original specificity of HEPT. Our recent observation using reverse transcriptase (RT) indicates that 14 and 15 were potent inhibitors of HIV-1 RT, irrespective of the source of enzymes.[30] However, reflecting their lack of activity against HIV-2 in cell cultures, these compounds did not prove inhibitory to HIV-2 RT. These results suggest their mode of action against HIV-1 at RT is clearly distinct from that of AZT. Another point to be emphasized is the effect of the compounds on bone marrow cell proliferation. In our preliminary experiments, AZT suppressed approximately 50% of the colony formation of murine bone marrow progenitor cells at concentration of 1 μM, whereas no such inhibition was observed with 14 and 15 even at 10 μM.

In conclusion, the present study demonstrates the anti-HIV-1 activity originally found in HEPT can be retained, or improved in certain cases, by removing the hydroxyl group. The 5-ethyl-"5'"-deoxy analogues (14 and 15) obtained by further modification at the base moiety are much less toxic than AZT and, in particular, 15 is almost as active as AZT. Considering their effectiveness against AZT-resistant strains of HIV-1 and also their lower toxicity to bone marrow cells, we believe these compounds may constitute highly promising candidates for the chemotherapy of AIDS.

Acknowledgment. Generous financial support (to H. Tanaka) from The Naito Foundation is gratefully acknowledged. This work has also been supported by a British Council Collaborative Research Project (to H. Tanaka and R.T.W.) and in part by the AIDS Basic Research Programme of the European Community and by grants from the Belgian Fonds voor Geneeskundig Wetenschappelijk Onderzoek and the Belgian Geconcerteerde Onderzoeksacties.

Supplementary Material Available: Elemental analyses of compounds 2, 3, 8–12, 14, and 15 (1 page). Ordering information is given on any current masthead page.

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Received January 24, 1991

(29) Physical data for 14 and 15 are as follows. Compound 14: mp 123–125 °C (EtOAc–acetone); MS m/z 306 (M^+); ^1H NMR ($CDCl_3$, 250 MHz) δ 0.99 (t, 3 H, J = 7.4 Hz, 5-CH_2CH_3), 1.10 (t, 3 H, J = 7.0 Hz, OCH_2CH_3), 2.67 (q, 2 H, J = 7.4 Hz, 5-CH_2CH_3), 3.56 (q, 2 H, J = 7.0 Hz, OCH_2CH_3), 5.45 (s, 2 H, OCH_2N), 7.13–7.39 (m, 5 H, SPh), 8.41 (br, 1 H, NH). Compound 15: mp 110–112 °C (EtOAc–hexane); MS m/z 368 (M^+); ^1H NMR ($CDCl_3$, 250 MHz) δ 0.98 (t, 3 H, J = 7.5 Hz, 5-CH_2CH_3), 2.63 (q, 2 H, J = 7.5 Hz, 5-CH_2CH_3), 4.63 (s, 2 H, OCH_2Ph), 5.52 (s, 2 H, OCH_2N), 7.08–7.37 (m, 10 H, 2Ph), 8.25 (br, 1 H, NH).

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(31) The number of HIV-1 antigen-positive cells was measured by indirect immunofluorescence and laser flow cytofluorography with polyclonal antibody used as a probe on day 4 after virus infection, as described previously.[16]

Novel Synthesis and Biochemical Properties of an [^{125}I]-Labeled Photoaffinity Probe for Thromboxane A_2/Prostaglandin H_2 Receptors

Thromboxane A_2 (TXA_2) and prostaglandin H_2 (PGH_2) posses potent proaggregatory, vasoconstrictor, and bronchoconstrictor activities. TXA_2 has been implicated as a pathophysiological mediator in a variety of cardiovascular disorders.[1] As a consequence, there has been considerable interest in the development of TXA_2/PGH_2 receptor antagonists for potential use in some of these pathophysiological disorders. In addition, characterization of TXA_2/PGH_2 receptors at the structural–functional level has begun to be pursued.[2,3] In the past few years, a number of radioligands have been synthesized and used for the characterization of TXA_2/PGH_2 receptors.[4-7] Of the various radioligands, 7-[(2R,2S,3S,5R)-6,6-dimethyl-

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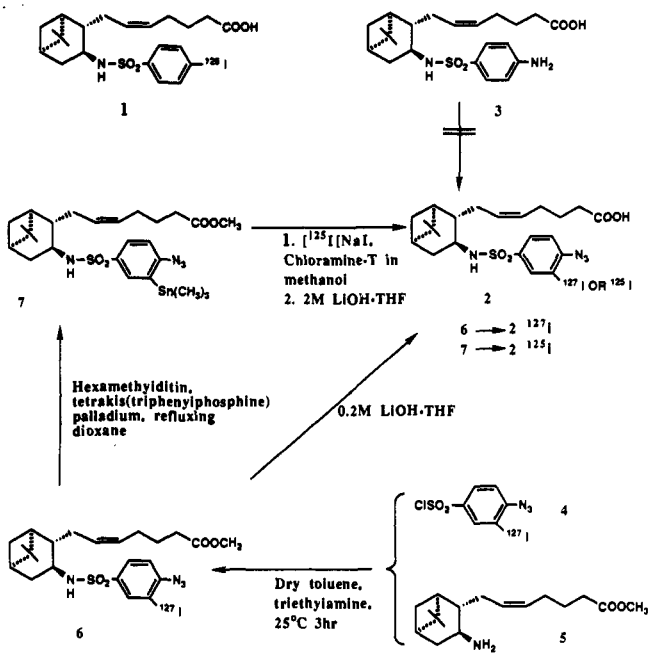
Scheme I



3-[[(4-iodophenyl)sulfonyl]amino]bicyclo[3.1.1]hept-2-yl]-5(*Z*)-heptenoic acid (^{125}I-SAP), **1**, has been shown to bind with high affinity to the TXA_2/PGH_2 receptor in human platelets.[8]

Another technique utilized for the biochemical characterization of receptors is photoaffinity labeling using a radiolabeled photolabile molecule which binds with high affinity to a receptor and can be irreversibly incorporated into the receptor under the influence of ultraviolet light.

Because of the high-affinity binding of **1** to the human platelet receptor and based on previous structure–activity relationships developed for structurally similar norbornyl analogues,[7] we designed as a potential probe the ^{125}I-labeled analogue **2**, 7-[(2*R*,2*S*,3*S*,5*R*)-6,6-dimethyl-3-[[(3-iodo-4-azidophenyl)sulfonyl]amino]bicyclo[3.1.1]hept-2-yl]-5-(*Z*)-heptenoic acid (I-SAP-N_3). Initial attempts to synthesize this analogue via intermediate amine **3** failed. Radioiodination of **3** occurred readily, but conversion to the azide resulted in lactonization of the upper side chain, presumably due to the acid-labile nature of the double bond in these sulfonamide series of compounds. This prompted us to develop an alternative pathway to **2**.

Recent advances in radioiododestannylation suggested that this technique may be applied for the synthesis of **2**.[8,9] The route shown in Scheme I was chosen because the labeling procedure could be performed in the last step under mild nonacidic conditions. To our knowledge the generation of a trialkyl tin moiety in the presence of an aromatic azide has not been attempted. This communication describes the synthesis of a new high-affinity TXA_2/PGH_2 receptor photoaffinity label via trimethyltin derivative **7**.

Synthesis. Commercially available *p*-aminobenzenesulfonic acid was iodinated with ICl[10] and the resulting product purified by preparative HPLC using a 2.54 × 50 cm ODS column with a mobile phase of methanol (80%) and 0.1 M ammonium acetate. The amine was diazotized and converted to the azide by using published procedures.[11] Refluxing the sulfonic acid in sulfonyl chloride gave intermediate **4**.[13] Pinanamine **5**, synthesized by previously published methods,[14] was condensed in anhydrous toluene and triethylamine at room temperature with **4** to yield **6** (80% yield). Hydrolysis in LiOH–THF gave ^{127}I-labeled **2**. Compound **6** also served as the precursor of the ^{125}I-labeled compound via generation of trimethyltin derivative **7** with hexamethylditin and tetrakis(triphenylphosphine)palladium(0) in refluxing dioxane for 3 h. Reaction of this arylstannane derivative with chloramine-T and sodium [^{125}I]iodide in methanol followed by hydrolysis of the ester and HPLC purification yielded [^{125}I]-**2**.[15] The

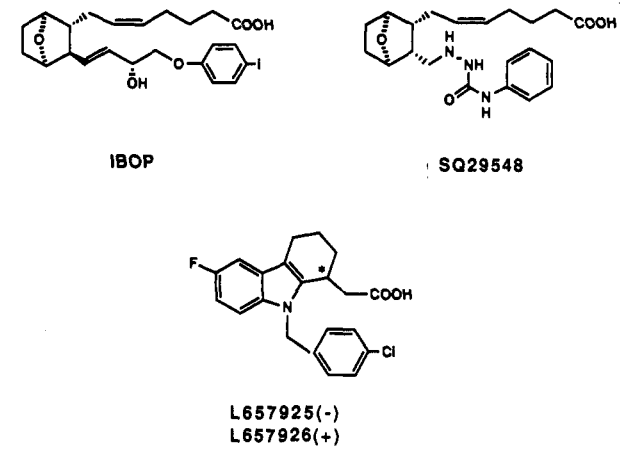


Figure 1. Chemical structures of I-BOP, SQ29548, L657925(-), and its enantiomer, L657926(+).

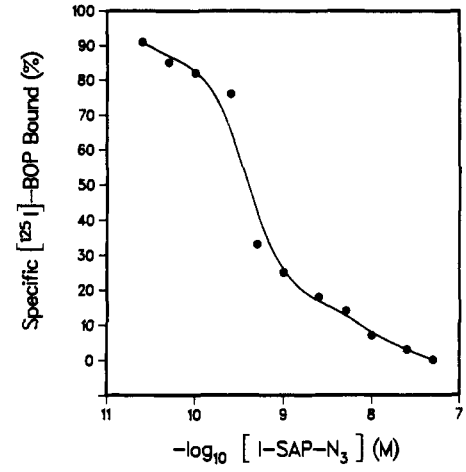


Figure 2. Competition of [^{127}I]-**2** (I-SAP-N_3) for binding sites in human platelet membranes. Platelet membranes (10 μg of protein per tube) were incubated with [^{125}I]IBOP (20 pM), a TXA_2 mimetic, along with varying concentrations of [^{127}I]-**2** in 25 mM MOPS (pH = 6.5) in a final volume of 200 μL. Incubation was for 30 min at 30 °C and stopped by rapid filtration onto GF/C filter paper, which was washed two times with ice-cold assay buffer. Nonspecific binding was defined as the binding remaining in the presence of SQ29548 (10 μM). Data represent the mean of three experiments performed in duplicate.

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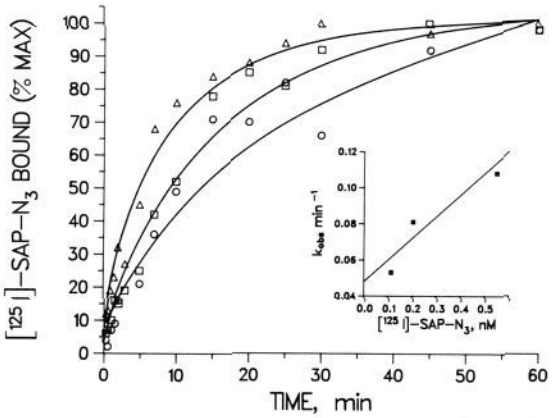

Figure 3. Time course of association of [^{125}I]-**2** (^{125}I-SAP-N_3) to solubilized human platelet membranes. CHAPS-solubilized membranes (20 μg of protein) were incubated with three concentrations of [^{125}I]-**2** for up to 1 h at 30 °C. Nonspecific binding and filtration were carried out as described in the caption for Figure 1, except the GF/C filter paper was prewetted with 0.3% polyethylenimine.[18] The pseudo-first-order rate constants were calculated for each ligand concentration and plotted versus ligand concentration: slope = 0.1125 nM^{-1} min^{-1} = k_1; y intercept = 0.0480 min^{-1} = k_{-1}; K_d = 427 pM = k_{-1}/k_1 (N = 3 for each ligand concentration).

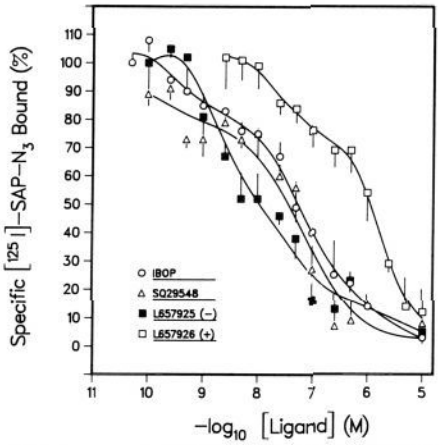

Figure 4. Displacement of [^{125}I]-**2** (^{125}I-SAP-N_3) by compounds which interact at the TXA_2/PGH_2 receptor. Displacement curves were generated with ~100 pM of [^{125}I]-**2** and 20 μg of solubilized platelet membrane protein and various concentrations of inhibitors. The data represent the mean of five separate experiments for each compound. The IC_{50} values were L657925, 11.2 ± 3.2 nM; SQ29548, 35.8 ± 1.7 nM; IBOP, 50.0 ± 6.2 nM; L657926, 1.5 ± 0.1 μM, respectively. Incubation and filtration were carried out as described under in the caption for Figure 2.

product was pure by HPLC and comigrated on TLC plates with authentic unlabeled material; the radiochemical yield based on ^{125}I was 64 ± 3% (N = 4). Importantly, tin derivative **7** is stable and the photoaffinity probe [^{125}I]-**2** can be readily generated from this precursor.

Biochemistry. Competition of [^{127}I]-**2** was carried out with [^{125}I]IBOP (structures of analogues used in this study are shown in Figure 1), a previously described TXA_2/PGH_2 receptor ligand in human platelet membranes and a rapid filtration assay.[5] The K_d value determined from these assays was 382 ± 41 pM (N = 3) (Figure 2). The binding of [^{125}I]-**2** to CHAPS-solubilized platelet membranes was time dependent as is shown in Figure 3. This figure shows the time course of association of [^{125}I]-**2** using three different concentrations of [^{125}I]-**2**. A pseudo-first-order rate constant (k_{obs}) was determined for each concentration of ligand and plotted versus ligand concentration (Figure 3, inset). The slope of this best fit line gave the association rate constant (k_1) and the y intercept provided the dissociation rate constant (k_{-1}). From those values, a K_d of 427 pM was determined ($K_d = k_{-1}/k_1$), which agrees well with the K_d value determined from competition assays.

In order to demonstrate that [^{125}I]-**2** is binding to the TXA_2/PGH_2 receptor, a series of TXA_2 analogues known to interact at the receptor were used to compete with [^{125}I]-**2** binding. These pooled displacement curves are shown in Figure 4. L657925 and L656926 are a pair of enantiomers which have been previously shown to interact at the TXA_2/PGH_2 receptor, with L657925 being about 100 times more potent than L657926.[16] These compounds possessed IC_{50} values of 11.2 nM and 1.5 μM, respectively.

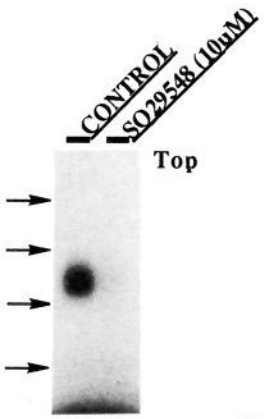

Figure 5. Irreversible incorporation of [^{125}I]-**2** into partially purified TXA_2 receptors. Partially purified receptor was incubated with 1 μCi of [^{125}I]-**2** both in the absence of competing ligand (control lane) and the presence of competing ligand (SQ29548 lane, 10 μM). Following photolysis with a hand-held ultraviolet lamp, the sample was subjected to SDS-PAGE on an 8-16% gradient followed by autoradiography. The arrows indicate the molecular weight marker proteins and are, from the top, 92, 67, 43, and 31 kDa, respectively. The specifically labeled protein band seen in the control lane corresponds to a molecular weight of 50–51 kDa.

IBOP possessed an IC_{50} value of 50 nM while the value of SQ29548 was 35.8 nM.[5,17]

The utility of [^{125}I]-**2** to irreversibly label the TXA_2/PGH_2 receptor is demonstrated in Figure 5. Partially purified receptor from human platelets was incubated with the 1 μCi of [^{125}I]-**2** for 20 min at 30 °C in the absence of a competing ligand (control lane) and the presence of TXA_2 antagonist SQ29548. Following photolysis with ultraviolet light, the sample was subjected to SDS-PAGE followed by autoradiography. A specifically labeled protein band corresponding to 50–51 kDa was observed in the control lane which was abolished in the lane where a competing ligand was present.

(15) **7** (15 nmol) was dissolved in 30 μL of methanol and 1 mCi of sodium [^{125}I]iodide was added, followed by the addition of 5 μL of chloramine-T (5 mg/mL in 200 mM phosphate buffer, pH = 7.5). The reaction proceeded for 4 min at room temperature, and 10 μL of THF and 10 μL of 2 N LiOH were added. After 1 h the mixture was injected into an ODS-3 reverse-phase column utilizing a mobile phase of 80% MeOH and 20% 0.1 M ammonium acetate at a flow rate of 1 mL/min. The product elutes at 6.5–7.0 min under these conditions.

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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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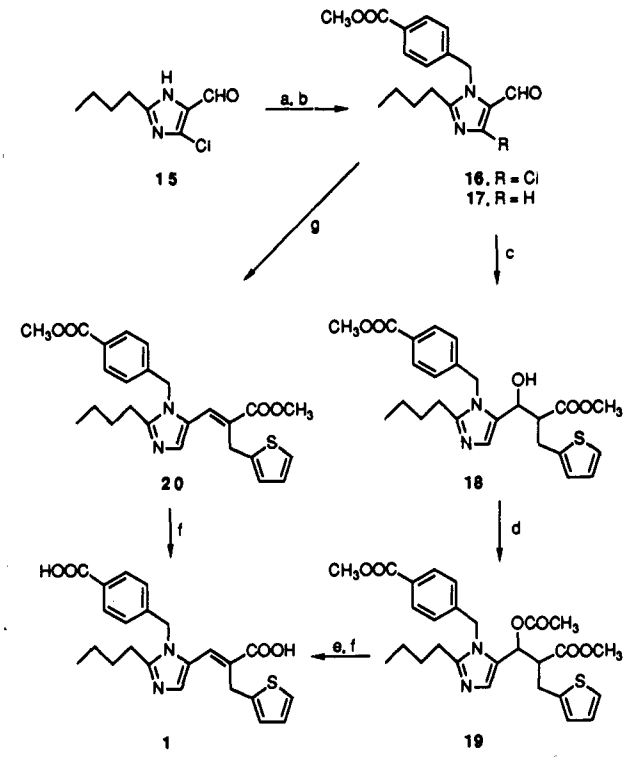
Received December 26, 1990

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

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