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SYNTHESIS OF THROMBOXANE RECEPTOR ANTAGONISTS WITH THE POTENTIAL TO RADIOLABEL WITH ^{125}I

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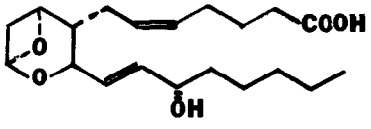
Abstract: The synthesis of several thromboxane receptor antagonists is described along with their ability to inhibit aggregation of human platelets.

Since 1975 when the structure of unstable ($t_{\frac{1}{2}} \simeq 30$ secs at 37°) thromboxane A_2 (TxA_2) 1 was proposed,[1] a variety of stable analogs have been prepared and their pharmacological profiles examined.[2-7] In addition, analogs of the 13-aza series (prostaglandin numbering) have demonstrated antagonistic properties to both platelet aggregation and vascular smooth muscle contraction. For example, ONO-11120 2 is an antagonist of human platelet aggregation and contraction of rat aortic strips when these effects are induced by stable thromboxane agonists.[8]



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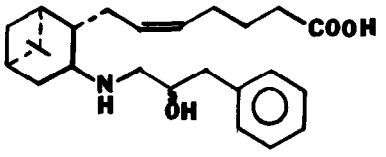

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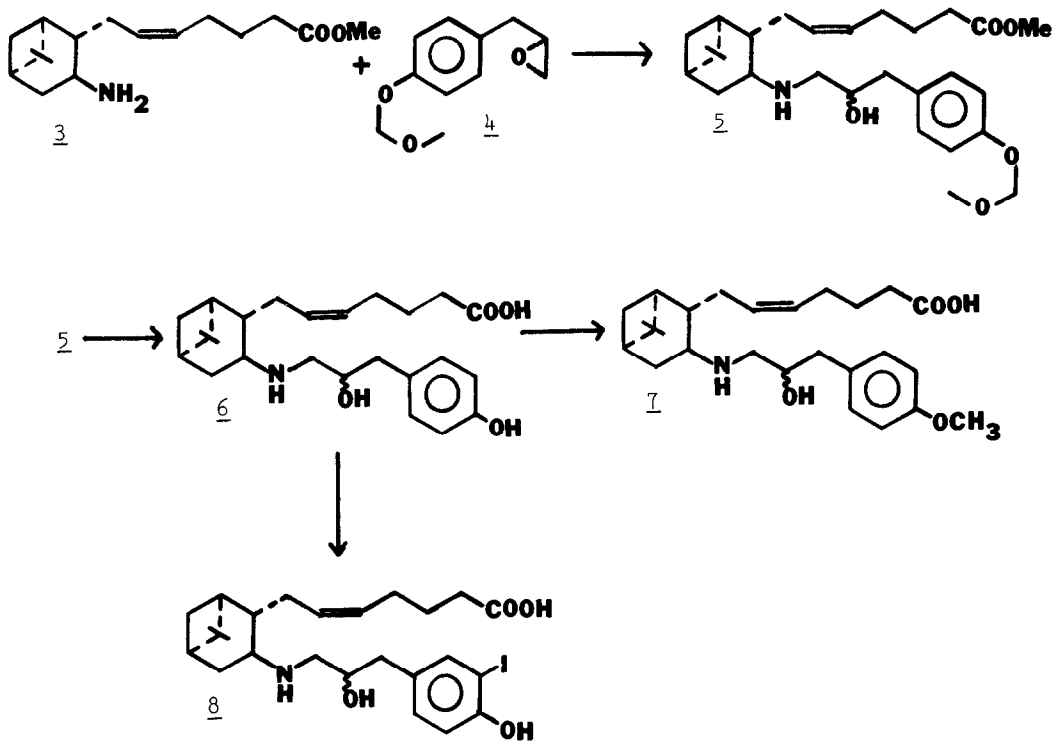
Studies of putative thromboxane receptors, however, have progressed slowly because of a lack of suitable radiolabelled ligands. ^{3}H and ^{125}I are commonly used to radiolabel molecules for binding studies. ^{125}I has the advantage of yielding a much greater specific activity, however, incorporation of the iodine into the molecule may alter the biological activity of the molecule. We have therefore synthesized several analogs of 2 in order

to: (1) further characterize the structure activity relationships of this class of compounds in human platelets and (2) introduce a phenolic group to test the concept that the molecule could be iodinated with either ^{127}I or ^{125}I without loss of activity.

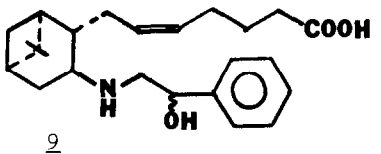
The key intermediate in the synthesis was amine 3 (synthesized by the ONO Pharmaceutical Co., Osaka, Japan). Epoxide 4 was prepared by epoxidation of p-allylphenyl



methoxymethylether with m-chloroperbenzoic acid in CH_2Cl_2 (b.p. 85-92° at 0.5 mmHg; m/z 194 (M^+), 163,151). Para-allylphenyl methoxymethyl ether was prepared according to the method of Kitamura et al.[9] Coupling of amine 3 (0.5 mmole) with 4 (0.55 mmole) in refluxing methanol for 20 hr gave intermediate 5 which was saponified in dilute NaOH followed by gentle warming in dilute HCl to yield pinane derivative 6 (PTA-OH). Purification was achieved on a silica gel column using $CHCl_3$-MeOH as the mobile phase (9:1). The compound 6 (white powder, m.p. 126-128°) was obtained as a C-15 epimeric mixture.

The methyl ester of 6 showed m/z 429 (M^+), 292, 137; fast atom bombardment mass spectrum (FAB-MS) of free acid 6, m/z 416 $(M + H)^+$.

The methylated derivative 7 (PTA-OM) was prepared by treating 6 (10 mg, 25 μmoles) with excess diazomethane in methanol followed by hydrolysis of the ester. Purification by silica gel chromatography ($CHCl_3$:MeOH 9:1) gave a 75% yield of 7 as a glassy solid. The methyl ester of 7 showed m/z 443 (M^+), 292, 151; FAB-MS of free acid 7, m/z 430 $(M+H)^+$. Iodination of 6 (20 mg, 50 μmoles) was carried out by the slow addition of ethanolic I_2 (40 μmoles) to an aqueous ammonia solution of 6. The small quantity of diiodinated material and starting material remaining were separated by chromatography as described for the purification of 6 and 7 to give a 65% yield of 8 (I-PTA-OH) (m.p. 103-105°). The methyl ester of 8 showed m/z 292; FAB-MS of free acid 8, m/z 542 $(M+H)^+$. One other compound in this series was the ω-chain contracted derivative 9 prepared by treating amine 3 with styrene oxide followed by hydrolysis of the ester. A glassy solid (70% yield) was obtained following purification by 9 chromatography. The methyl ester



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of 9 showed m/z 399 (M^+), 292, 107; FAB-MS of free acid 9, m/z 386 $(M+H)^+$.

Inhibition of Human Platelet Aggregation

Compounds 2, 6, 7, 8 and 9 were tested for their ability to antagonize U46619 (1 μM) (a stable thromboxane/endoperoxide mimetic)[10] induced platelet aggregation. I-PTA-OH was equipotent to ONO-11120 whereas PTA-OH and 9 were significantly less potent. PTA-OM was the least potent of the series. These results provide evidence that PTA-OH can be iodinated without loss of biological activity. Therefore, the possibility exists that PTA-OH can be iodinated with ^{125}I and used as a radioactive ligand to characterize

putative thromboxane A_2 receptors. Indeed, we have recently carried out preliminary successful binding studies with ^{125}I-PTA-OH in human platelet membranes (11).

Further biological results together with detailed synthetic work will be reported in full elsewhere.

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