SYNTHESIS OF AN [^{125}I]-AZIDO PHOTOAFFINITY PROBE FOR THE HUMAN PLATELET THROMBOXANE A_2/PROSTAGLANDIN H_2 RECEPTOR

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SUMMARY

The synthesis of [^{125}I]-(1S,2S,3S,5R)-2-(6'-carboxyhex-(2'Z)enyl)-3-(2-hydroxy-3-(4-hydroxy-3-iodophenyl)-1-propylamino)-6,6-dimethylbicyclo[3.1.1]heptane(I-PTA-OH), a high affinity thromboxane A_2/prostaglandin H_2 (TXA_2/PGH_2) receptor antagonist has been previously described. This paper describes the synthesis of an analog of this compound, [^{127}I]- and [^{125}I]-(1S,2S,3S,5R)-2-(6'-carboxyhex-(2'Z)enyl)-3-(2-hydroxy-3-(4-iodo-3-azidophenoxy)-1-propylamino)-6,6-dimethyl-bicyclo[3.1.1]heptane(I-PTA-azido), which has the potential to be used as a photoaffinity probe of the TXA_2/PGH_2 receptor. This compound, which is the highest affinity photoaffinity probe yet described for the human platelet TXA_2/PGH_2 receptor may be a useful tool to characterize this receptor.

Key Words: Thromboxane A_2/prostaglandin H_2 receptors, human platelets, photoaffinity labelling, iodinated azido thromboxane antagonist

INTRODUCTION

Since 1975 when the structure of unstable thromboxane A_2 (TXA_2) 1 was proposed (1), various stable mimetics and antagonists have been prepared and their pharmacological profiles examined (2). These earlier studies have recently culminated in the total synthesis of TXA_2, confirming the originally proposed structure (3). One of the pharmacologic effects of TXA_2, prostaglandin H_2 (PGH_2) and their stable mimetics is platelet aggregation presumably through activation of specific receptors (2). Recently, a specific binding site for the TXA_2/PGH_2 antagonist [^{125}I] PTA-OH (4), 2 has been described in washed human platelets (5) and human platelet membranes (6). Furthermore, this site has been solubilized from human platelet membranes in active form (7) and its hydrodynamic properties determined (8).

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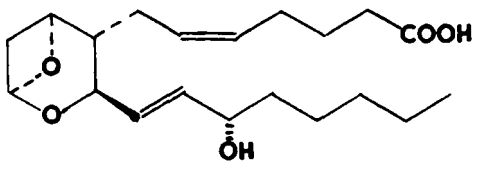

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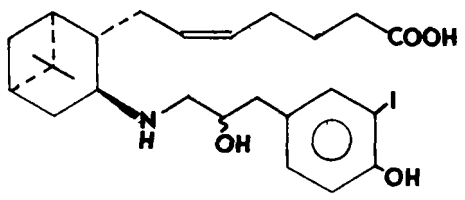

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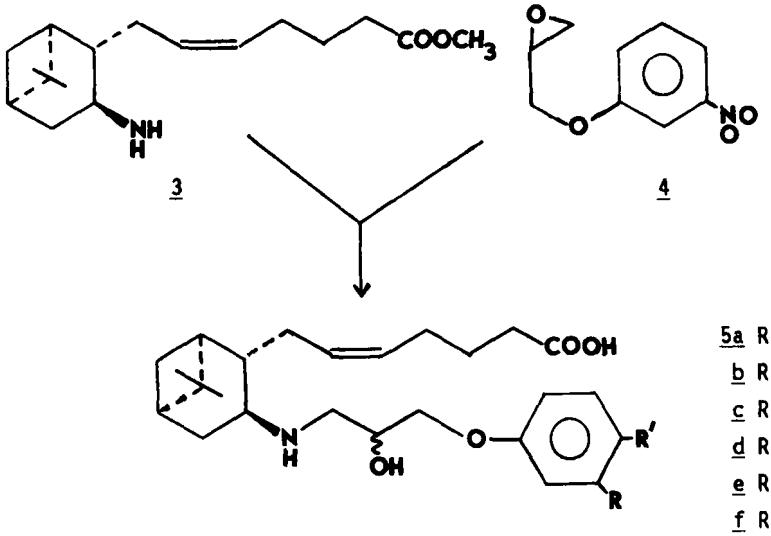
Photoaffinity ligands have been utilized extensively to characterize a variety of receptors. There have been to date only two reports of photoaffinity ligands synthesized for the study of eicosanoid receptors (9,10). In the first report, an aryl diazonium salt was photolyzed in the presence of platelet membranes and irreversable incorporation of the ligand into the TXA_2/PGH_2 receptor was demonstrated. This molecule was not iodinated nor could the affinity for the receptor be demonstrated (9). In the second, an iodinated azido photoaffinity ligand was described but the K_d reported in washed human platelets was 300 nM (10). Previous studies have shown that iodination of a bottom side chain modified prostanoid did not result in loss of biological activity (4,5). Indeed, compound 2 was more potent than its uniodinated precursor (5). Therefore, since ^{125}I possesses a much higher specific activity than tritium, a precursor which could be radio iodinated was preferred over one which could be tritiated. To further the characterization and purification of the TXA_2/PGH_2 receptor we report herein the synthesis of an iodinated azido photoaffinity ligand possessing the highest yet reported affinity for the human platelet TXA_2/PGH_2 receptor.

Figure 1 shows the reaction sequence leading to the precursor amine 5b. Following iodination the amine is converted to an azide. Compound 5c, the amine and 5e, the azide were evaluated for their ability to antagonize U46619 (a stable TXA_2/PGH_2 mimetic) (11) induced platelet aggregation in washed human platelets (5) by constructing dose response curves in the absence and presence of varying concentrations of 5c and 5e. Schild analysis (12) of the data (N=2 for each compound) showed a K_d of 47 and 87 nM for 5c and 10 and 15 nM for 5e. The regression lines of the plots gave a slope of -1.20 and -1.00, respectively and were not significantly different from -1.0, indicating that these compounds are acting in a competitive fashion at the TXA_2/PGH_2 receptor. In radioligand competition studies

using [^{125}I]-PTA-OH, 2, as the tracer, compound 5e possessed a K_d of 64 ± 12 nM (n=3) in washed human platelets. In addition, 5e has been shown to photoincorporate into the human platelet TXA_2/PGH_2 receptor in an irreversible fashion (13).

We have recently synthesized the radioiodinated analog 5f and documented its photoincorporation into the putative TXA_2/PGH_2 receptor. Thus the possibility exists for using this eicosanoid analog as a photoaffinity ligand to further characterize the human platelet TXA_2/PGH_2 receptor. Furthermore, this work demonstrates that the synthesis of other appropriately substituted omega side chain analogs may provide useful tools for the study of other eicosanoid receptors.



5a R = NO_2, R' = H
b R = NH_2, R' = H
c R = NH_2, R' = ^{127}I
d R = NH_2, R' = ^{125}I
e R = N_3, R' = ^{127}I
f R = N_3, R' = ^{125}I

Figure 1

EXPERIMENTAL

Materials

The synthesis of amine 3 (Figure 1) has previously been described (14) and was a gift of ONO Pharmaceutical Company (Osaka, Japan). The structure of all compounds and intermediates was confirmed by ^{1}H NMR spectroscopy (Varian 90 MHz), mass spectroscopy (LKB-9000S) or fast atom bombardment mass spectroscopy (FAB-MS) (Kratos MS-50). The purity was checked by thin layer chromatography on silicagel plates in the following system (v/v): chloroform-methanol (8:2) and by HPLC at 254 nm on a Whatman partisil-5 ODS-3 reverse phase column utilizing 65% methanol-35% 0.1M ammonium acetate as the mobile phase. The final [^{125}I]-derivatives were purified by HPLC using the above described column conditions.

Methods

1-(3-nitrophenoxy) 2,3-epoxypropane 4

Into 100 ml of anhydrous acetone was added 5g (0.036 mole) of 3-nitrophenol (Aldrich), 6.25 g (0.072 mole) of epichlorohydrin and 3g of sodium carbonate. The mixture was allowed to reflux overnight. Water was added (200 ml) to the reaction mixture and the alkaline solution was extracted with chloroform (2 x 100 ml). The combined extracts were dried over $MgSO_4$ and the solvent removed under reduced pressure. The yellow oil was distilled under reduced pressure. The product distilled at 105-108° at 0.5 mm Hg and gave a 52% yield. MS:m/z 195(M^+), 152.

(1S,2S,3S,5R)-2-(6'-carboxyhex-(2'Z)enyl)-3-(2-hydroxy-3-(3-nitrophenoxy)-1-propyl-amino)-6,6-dimethylbicyclo[3.1.1]heptane 5a

Amine 3 (140 mg, 0.5 mmoles) and epoxide 4 (107 mg, 0.55 mmole) were refluxed in anhydrous methanol under an argon atmosphere for 20 hrs. The solvent was removed under reduced pressure and the methyl ester of 5a was hydrolyzed in 5 ml 1:1 tetrahydrofuran: 0.2 N LiOH overnight at room temperature. The solution was neutralized with acetic acid and lyophilized. The solid residue was dissolved in chloroform:methanol (8:2) and applied to a 10 x 2 cm silica gel column. Elution using the same solvent provided a 64% yield of 5a MS: methyl ester, m/z 474(M^+), 322 and 292.

(1S,2S,3S,5R)-2-(6'-carboxyhex-(2'Z)enyl)-3-(2-hydroxy-3-(3-aminophenoxy)-1-propyl-amino)-6,6-dimethylbicyclo[3.1.1]heptane 5b

The nitro compound 5a was reduced to amine 5b using previously described methods (15). 15 mg (32 μmoles) of 5a was dissolved in 1 ml of dilute ammonia and added to a boiling aqueous solution of ferrous sulfate (222 μmoles). Dilute ammonium hydroxide was added over the next five minutes to maintain alkaline conditions. The black precipitate was filtered and the solution was neutralized with acetic acid and lyophilized. The product 5b was purified on a silica gel

column in 44% overall yield from 3. The amine was a glassy solid and consisted of a C-15 epimeric mixture. MS:methyl ester, m/z 444(M^+), 292.

(1S,2S,3S,5R)-2-(6'-carboxyhex-(2'Z)enyl)-3-(2-hydroxy-3-(3-amino-4-iodophenoxy)-1-propylamino)-6,6-dimethylbicyclo[3.1.1]heptane 5c

Amine 5b (10 mg, 23 μmoles) was iodinated in methanol at 0°C by the slow addition of 0.85 equivalents of iodine monochloride dissolved in methanol. Following the addition, the methanol was evaporated and the product was purified on a silica gel column (chloroform:methanol 8:2) to give 85% yield of 5c. This appeared to be the sole product as assessed by HPLC. FAB-MS, m/z 571 $(M+H)^+$.

(1S,2S,3S,5R)-2-(6'-carboxyhex-(2'Z)enyl)-3-(2-hydroxy-3-(3-azido-4-iodophenoxy)-1-propylamino)-6,6-dimethylbicyclo[3.1.1]heptane 5e

The iodinated amine 5c (10 mg, 17 μmoles) was dissolved in 3 N acetic acid and cooled to 0°C. To this solution was added 2 mg (1.5 equivalents) of sodium nitrite and the solution was kept on ice for 10 min. To this was added 3.4 mg (51 μmoles) of sodium azide dissolved in water and the reaction mixture was allowed to stand at room temperature for 30 min. The reaction mixture was lyophilized and purified on a silica gel column (chloroform:methanol, 8:2) to give 75% yield of 5d. FAB-MS:m/z 597 $(M+H)^+$; UV(H_2O): 252, 410; IR(film):2080 cm^{-1}.

RADIOIODINATION

Amine 5b (10 μg) was dissolved in 0.1 M phosphate buffer (20 μl) (pH=7.5) and 2 mCi of Na ^{125}I (carrier free) added. To this solution was added 10 μl (454 ng, 2 equivalents) of a freshly prepared chloramine-T solution (4.5 mg/100 ml). The iodination was allowed to proceed for 2 minutes at room temperature followed by quenching with excess sodium metabisulfite. The reaction mixture was injected onto a Whatman partisil-5 ODS-3 reverse phase column using 65% methanol 35% 0.1 M NH_4 acetate as the mobile phase. The flow rate was 1 ml per min and 1 min fractions were collected. The radiolabel eluted in the fraction at 7 min. The radiolabel

5d migrated with authentic cold standard 5c by thin layer chromatography and HPLC. A yield of 65% with respect to added ^{125}I was obtained.

INTRODUCTION OF AZIDO GROUP

The radioiodinated amine 5d obtained from the HPLC purification was evaporated to dryness under a stream of nitrogen. The residue was taken up in 30 µl of 3 N acetic acid and diazotized at 0°C with 1.5 mole equivalents of sodium nitrite for 5 min. To the diazotized amine was added sodium azide (3 mole equivalents) dissolved in water and the solution was allowed to sit at room temperature for 15 min. This mixture was injected onto the same HPLC column and solvent system described above and 1 min fractions collected. The ^{125}I-PTA-azido, 5f, eluted at 15 min. This product comigrated with cold standard 5e in both thin layer chromatography and HPLC systems. The yield of ^{125}I-PTA-azido from starting ^{125}I-amine was 32%.

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