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A cinchonidine derivative for photoaffinity labelling of proteins

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SUMMARY: 8-Aminohydrocinchonidine was prepared and coupled with 4-azidosalicylic acid. The resulting amide was labelled with ^{125}I to produce a photoactivatable species for attaching to proteins.

KEYWORDS: 8-Aminohydrocinchonidine, 4-Azidosalicylic acid, Iodine-125, Protein labelling

INTRODUCTION

Photoaffinity labelling is the covalent photoinsertion of a tagged (usually radiolabelled) ligand analogue into a biological receptor. Photoreactive ligands are an important tool in drug discovery and development (1). They can be used to identify drug targets in a complex mixture and to study the affinity and selectivity of the binding of drugs to these targets. They can also be used to generate a molecular map of the ligand-binding site within a particular protein.

4-Azidosalicylic acid (2) is a convenient reagent for derivatizing likely ligands. Upon light activation, reactive nitrenes are generated and these rapidly attach to biomolecules of interest. In addition, the phenolic function allows the efficient introduction of radiolabelled iodine (3). We report here the synthesis of a novel cinchonidine derivative with interesting potential for protein labelling.

RESULTS AND DISCUSSION

The synthetic route is shown in Scheme 1. Hydrocinchonidine 1 [from cinchonidine, as reported for hydroquinine (4)] was nitrated and the 8'-nitro product 2 was reduced with hydrazine and palladium/charcoal. Coupling of the resultant 3 with 4-azidosalicylic acid (ASA) was achieved with 1,1'-carbonyldiimidazole (CDI) as in a related situation (5). However, it transpired that the side-chain hydroxy group coupled preferentially and so it was necessary to use an excess of coupling agents to achieve formation of the ester/amide 4. The product mixture was in fact complex but 4 was isolated by chromatography.

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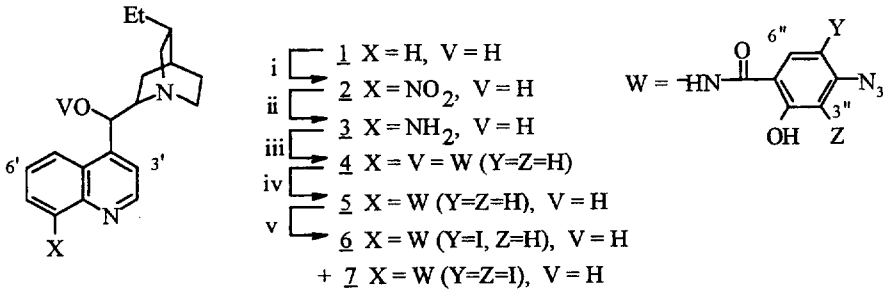

i, fum. HNO_3/c. H_2SO_4/20 °C; ii, N_2H_4/Pd/C/EtOH; iii, ASA/CDI/dioxane; iv, 0.1M $Na_2B_4O_7$/EtOH/H_2O; v, NaI/chloramine T/0.5M K_2HPO_4/DMF

Scheme 1

Intramolecular general base catalysis has been demonstrated in the hydrolysis of salicylate esters at pH 7-10 (6) and so reaction of 4 with aqueous ethanolic sodium borate was used to preferentially remove the ester function; the target amide 5 was separated by chromatography.

A standard sodium iodide/chloramine T iodination procedure was followed (3), and the products were established with unlabelled sodium iodide. Chromatography gave an inseparable mixture of 5"-iodo 6 and 3",5"-diiodo 7 compounds, by electrospray mass spectrometry. The mono compound was the main product, from NMR analysis. Then, iodination with ^{125}I gave a labelled product with the same R_f value.

The radiolabelled species has to date found use in the labelling of the mutidrug resistance protein in cancer research (7), and of binding sites for quinoline antimalarial drugs (8).

EXPERIMENTAL

NMR spectra were recorded in $CDCl_3$, on a Bruker AM-300 spectrometer operating at 300.13 MHz (^1H) and 75.47 MHz (^{13}C). Various standard techniques were used to identify proton-bound carbons in ^{13}C NMR spectra. The electrospray mass spectra were obtained on a VG Bio-Q triple quadrupole mass spectrometer using a water/methanol/acetic acid (50:50:1) mobile phase. Microanalyses were performed at the Campbell Microanalytical Laboratory, University of Otago, New Zealand.

8'-Nitrohydrocinchonidine 2

Compound 1 (1 g) was added portionwise to a mixture of fuming nitric acid (20 mL) and concentrated H_2SO_4 (10 mL), with stirring, at room temperature. The solution was stirred for a further 7 h, then poured onto ice/water (200 mL). This was then neutralized cautiously with 20% NaOH and made alkaline with concentrated NH_4OH. The solid was filtered off, washed with water and allowed to air dry to give 2, suitable for further reaction. A sample was recrystallized from light petroleum (b.p. 90-110 °C), as a white solid, m.p. 152-155 °C [lit. (9) 148 °C].

8'-Aminohydrocinchonidine 3

A mixture of 2 (2 g), 10% Pd/C (0.4 g) and hydrazine hydrate (8 mL) in ethanol (25 mL) was refluxed for 1 h. The catalyst was filtered off, the solvent was removed at reduced pressure, and the residual solid was dissolved in $CHCl_3$ (20 mL) and washed with water (5 mL). The organic layer was dried ($MgSO_4$), and the solvent was removed at reduced pressure, to give the product (1.08 g, 52%), m.p. 161-164 °C [lit.(9) 170 °C]. This was dried at 60 °C/1 mmHg for 16 h prior to being used in further reactions.

A sample was recrystallized from toluene but retained some "toluene of crystallization" (evident in ^1H and ^{13}C NMR spectra), which could not be removed after drying at 60 °C/1 mmHg for 2 days.

Anal. Calcd. for $C_{19}H_{25}N_3O.0.4\ C_7H_8$: C, 75.1; H, 8.2. Found: C, 75.0; H, 8.2%.

N-*[(4-Azido-2-hydroxybenzoyloxy)hydrocinchonidin-8'-yl]-4-azido-2-hydroxybenzamide 4*

A solution of 4-azidosalicylic acid (2) (0.84 g, 5.1 mmol) and 1,1'-carbonyldiimidazole (0.84 g) in anhydrous 1,4-dioxane (15 mL) was heated under reflux for 10 min. and then stirred at room temperature for 1 h. A solution of 3 (0.5 g, 1.61 mmol) in anhydrous 1,4-dioxane (15 mL) was added, the mixture was heated under reflux for 10 min. and then stirred at room temperature overnight. The solvent was removed at reduced pressure and the residue was dissolved in $CHCl_3$ (20 mL), washed successively with 10% $NaHCO_3$ (2 x 20 mL), water (2 x 20 mL), dried ($MgSO_4$) and the solvent was removed at reduced pressure. The residual solid was purified by radial chromatography (silica; $CHCl_3$/MeOH/Et_2NH 19:1:0.1) to give the product (0.51 g, 50%), R_f = 0.74, m.p. 116-120 °C. ^1H NMR δ 0.84, t, J = 7.0 Hz, 3H; 1.2-2.0, m, 8H; 2.25-2.40, m, 1H; 2.59-2.71, m, 1H; 2.95-3.13, m, 2H; 3.4-3.6, m, 1H; 6.58-6.62, m, 4H; 6.71, d, J = 6.7 Hz, 1H; 7.50, d, J = 4.4 Hz, 1H; 7.62, t, 1H; 7.78, d, J = 9.0 Hz, 1H; 7.95, m, 2H; 8.77-8.81, m, 2H; 11.06, s, 1H, NH. ^{13}C NMR δ 12.0, CH_3; 24.2, CH_2; 25.2, CH; 27.7, CH_2; 28.4, CH_2; 37.2, CH; 42.6, CH_2; 58.4, CH_2; 59.7, CH; 75.4, CH; 107.4, CH; 108.2, CH; 108.8, C; 110.2, CH; 110.8, CH; 112.3, C; 116.9, CH; 117.6, CH; 119.2, CH; 125.5, C; 127.8, CH; 127.9, CH; 131.1, CH; 134.3, C; 139.0, C; 145.5, C; 146.0, C; 147.8, CH; 148.0, C; 163.3, C; 167.5, C; 168.5, C. ESMS: *m/z* 634.2 (M+1).

N-*(Hydrocinchonidin-8'-yl)-4-azido-2-hydroxybenzamide 5*

A solution of 4 (0.6 g) in 0.1M sodium borate (24 mL, pH 9.2) and ethanol (60 mL) was heated under reflux for 1.5 h and the solvent was then removed at reduced pressure. The residue was dissolved in water (10 mL) and extracted with $CHCl_3$ (2 x 10 mL). The combined extracts were dried ($MgSO_4$) and the solvent was removed at reduced pressure.The residue was subjected to radial chromatography (silica;

$CHCl_3$/HOAc/MeOH; 9:1:0.3) and the solvent was removed at reduced pressure from the fractions containing compound with $R_f = 0.3$. The residual acetate salt of the product (0.2 g, 44%) was dissolved in $CHCl_3$ and washed successively with 10% $NaHCO_3$ solution (2 x 10 mL), water (10 mL), dried ($MgSO_4$) and the solvent was removed at reduced pressure to give the free base, m.p. softens gradually >150 °C, after recrystallization from light petroleum (b.p. 90-110 °C). ^{1}H NMR (acetate salt—better resolved) δ 0.74, t, J = 7.1 Hz, 3H; 1.10-1.30, m, 3H; 1.60-1.90, m, 2H; 1.95-2.30, m, 4H; 2.08, s, 3H, CH_3CO_2H; 2.65-2.80, m, 1H; 2.95-3.10, m, 1H; 3.20-3.35, m, 1H; 3.35-3.4, m, 1H; 4.38, m, 1H; 6.25, s, 1H, H-9; 6.49, d, J = 1.7 Hz, 1H, H-3"; 6.59, dd, J = 8.4, 1.7 Hz, 1H, H-5"; 7.03, t, 1H, H-6'; 7.41, d, J = 7.9 Hz, 1H, H-5'; 7.63, d, J = 8.4 Hz, 1H, H-6"; 7.72, d, J = 3.4 Hz; 1H, H-3'; 8.39, d, J = 7.4 Hz, 1H, H-7'; 8.69, d, J = 3.4 Hz; 1H, H-2'; 10.74, s, 1H, NH. ^{13}C NMR (free base) δ 11.4, CH_3; 17.7, CH_2; 24.5, CH; 24.9, CH_2; 26.8, CH_2; 35.6, CH; 43.7, CH_2; 56.6, CH_2; 60.5, CH; 66.0, CH; 107.4, CH; 110.2, CH; 111.6, C; 115.9, CH; 116.7, CH; 119.5, CH; 123.9, C; 127.4, CH; 127.5, CH; 133.4, C; 137.7, C; 146.0, C; 147.2, C; 147.8, CH; 163.3, C; 167.4, C. ESMS: *m/z* 473.3 (M + 1).

Anal. (free base). Calcd for $C_{26}H_{28}N_6O_3$: C, 66.1; H, 6.0. Found: C, 66.2; H, 6.3%.

Iodination of 5

These experiments were carried out in reduced light.

(a) NaI (20 mg) was added to a solution of chloramine T (35 mg) in K_2HPO_4 buffer (0.5 M, pH 7.4, 1.5 mL) at room temperature. To this was added compound 5 (30 mg) dissolved in DMF (1.5 mL) and the mixture was stirred for 20 min. Then 10% $NaHSO_3$ solution (1 mL) and water (5 mL) were added and the solid which separated was filtered off. Preparative TLC (silica; $CHCl_3$/HOAc/MeOH; 9:1:0.3) gave a sample of acetate salts, $R_f = 0.43$, primarily the 5"-iodo derivative 6, (from ^{1}H NMR), with a little 3",5"-diiodo compound 7 (from ESMS). The ^{1}H NMR spectrum was similar to that for 5 except the doublet for H-5" was absent and the doublet for H-6" was replaced by a singlet at 8.00 ppm; meta coupling on the H-3" signal was absent. ESMS: *m/z* 599.1 (M+1, mono); 725.0 (M+1, di).

(b) Carrier-free Na^{125}I (10 μL, 1 mCi, 0.5 nmol, Amersham) was added to a solution of chloramine T (10 nmol) in K_2HPO_4 buffer (1M, pH 7.4, 10 μl) at room temperature. To this was added compound 5 (10 nmol) dissolved in DMF (20 μL). After 10 min, 5% $NaHSO_3$ solution (10 μL) was added. TLC as in (a), with autoradiographic visualization, gave an equivalent band. This was extracted with $CHCl_3$/MeOH (4:1). After evaporation of the solvent, the product was dissolved in chloroform, centrifuged to remove any remaining silica, and stored at -20 °C. The estimated overall specific activity was 43 mCi/ μmol.

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