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Research Article

Synthesis of PNU-159548 labelled with ^{14}C and ^{2}H

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Summary

The cytotoxic antitumor compound PNU-159548 (**1**) has been labelled with ^{14}C and ^{2}H. A three-step sequence starting from [14-^{14}C]idarubicin (**2a**) led to radiochemically pure (>98%) [14-^{14}C]PNU-159548 with a specific activity of 1.13 GBq/mmol. The synthesis of [$^{2}H_4$]PNU-159548 was carried out in a similar manner starting from [1,1,2,2-$^{2}H_4$]2-bromoethanol (**3b**). Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: PNU-159548; anthracyclines; alkycyclines; deuterium; carbon-14; antitumor agent

Introduction

PNU-159548 (4-demethoxy-3′-deamino-3′-aziridinyl-4′-methylsulphonyl-daunorubicin **(1)**) belongs to the alkycycline class, a novel family of cytotoxic antitumor agents obtained from anthracyclines. By comparison with idarubicin (4-demethoxydaunorubicin hydrochloride (**2**)), a well-known anthracycline extensively used in a number of polychemotherapy regimens,[1–3] PNU-159548 (**1**) has two structural modifications in the sugar moiety: an aziridyl ring in the C-3′ position and a methylsulphonyl group at the C-4′ position. These differences play

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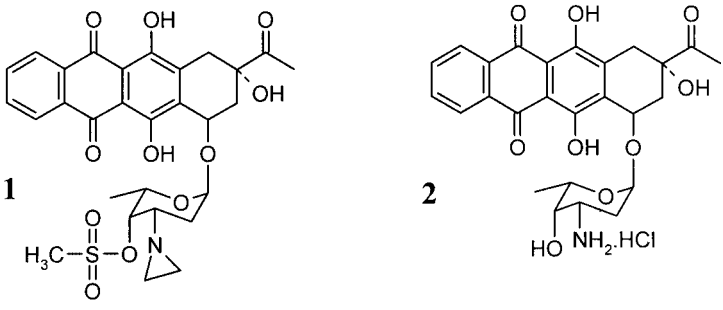
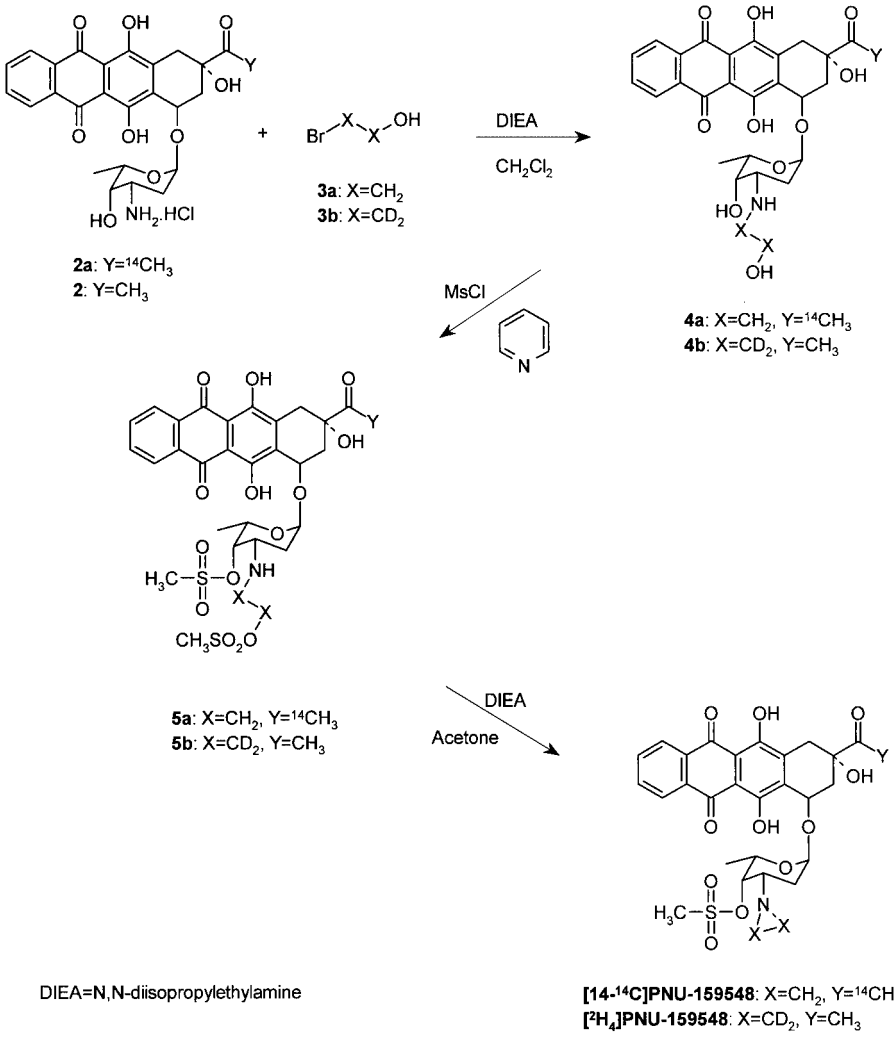


Scheme 1.

an important part in the mechanism of action and have implications for the pharmacology of the compound.[4,5] The preparation of a specifically labelled ^{14}C form of PNU-159548 was required for *in vivo* and *in vitro* metabolism studies. Previous studies carried out with [14-^{14}C]anthracyclines indicated that the C-14 position of the anthracyclines is suitable for the ^{14}C introduction.[6–9] This information, as well as the availability in our laboratories of a suitable amount of the radiolabelled precursor [14-^{14}C]idarubicin (**2a**), prompted us to label PNU-159548 in the same position. Moreover, a similar procedure was applied also to prepare the stable isotope labelled version which was required in order to develop an LC-MS assay. In this paper the syntheses of both the ^{14}C and ^{2}H labelled forms of the title compound are reported.

Discussion and results

The synthetic route used to introduce ^{14}C at the C-14 position and ^{2}H in the aziridyl ring of the title compound is outlined in the Scheme. The reaction of the ^{14}C-labelled idarubicin **2a** with a 30 molar excess of 2-bromoethanol (**3a**) in methylene chloride in the presence of *N*,*N*-diisopropylethylamine (DIEA) gave the intermediate **4a**. The corresponding dimethylsulphonyl derivative **5a** was then obtained by reacting **4a** with methanesulphonyl chloride (MsCl) in anhydrous pyridine. The aziridinyl ring closure was achieved by stirring **5a** in acetone in the presence of DIEA affording the crude [14-^{14}C]PNU-159548. After purification by flash-chromatography, [14-^{14}C]PNU-159548 was obtained, $>98\%$ radiochemically pure with a specific activity of 1.13 GBq/mmol. The overall radiochemical yield was 27% from **2a**. The preparation of ^{2}H-labelled PNU-159548 was performed in a similar manner starting from [1,1,2,2-$^{2}H_4$]2-bromoethanol (**3b**) and



Scheme 2.

idarubicin (**2**). [$^{2}H_4$]PNU-159548 was obtained in 98% chemically pure form and an isotopic enrichment of 98%.

Experimental

General methods

4-Demethoxy-[14-^{14}C]daunorubicin hydrochloride **2a** was supplied by the Metabolism & Pharmacokinetics Group, Tsukuba Research

Laboratories, Pharmacia & Upjohn, Japan. [1,1,2,2-$^{2}H_4$]2-Bromoethanol (**3b**) was purchased from C/D/N Isotopes. All solvents and reagents were of analytical grade and were used without purification unless otherwise indicated. Radioactivity measurements were performed on a Tri-Carb 2100 TR liquid scintillation analyser (Packard) using Rialuma (Lumac System) as liquid scintillation cocktail. TLC and radio-TLC measurements were made using a Packard Bioscan System 200 imaging scanner and silica gel Merck F254 plates (20 × 5 cm, 0.25 mm thick) in $CHCl_3$:MeOH:H_2O 150:42:6 (v/v) (system A) and CH_2Cl_2:MeOH:AcOH:H_2O 30:4:1:0.5 (v/v) (system B). Chemical purities were determined by HPLC performed at 25°C using a series-200 pump (Perkin-Elmer) equipped with a LC-295 UV/VIS detector (Perkin-Elmer) and PE-Nelson Turbochrom 4.0 software. Radiochemical purities were determined using an A-515TR radio-HPLC analyser (Packard) equipped with a 0.5 ml homogeneous cell (liquid scintillation cocktail: Ultima Flo-M (Packard); ratio to HPLC effluent: 3/1), under the following conditions: Inertsil ODS-3 column (250 × 4.6 mm I.D.; particle size 5 μm, supplied by Alltech) eluting with CH_3CN:MeOH 4:3 (A) and 2.78 g/l KH_2PO_4 at pH 6.4 with TEA (B) mixtures (isocratic at 60% A for 20 min, linear gradient from 60% A to 85% A over 20 min, isocratic at 85% A for 15 min, linear gradient from 85% A to 60% A over 7 min); flow rate 1 ml/min; wavelength 254 nm. The purification of the final compound was performed by flash chromatography on a silica gel column according to the following procedure: the column was packed with toluene, washed with methylene chloride (about 1 column volume), loaded with the crude PNU-159548, dissolved in the minimum amount of methylene chloride, then eluted with toluene:acetone 8:1 (v/v).

*4-Demethoxy-3′-N-(2-hydroxyethyl)-[14-^{14}C]daunorubicin (**4a**)*

The compound **2a** (180.56 MBq, 0.1598 mmol) was dissolved in anhydrous methylene chloride (3 ml), then 2-bromoethanol (**3a**) (340 μl, 4.794 mmol) and DIEA (83.5 μl, 0.4794 mmol) were added. The reaction mixture was stirred at 36°C for 48 h. At the end of the reaction (determined by radio-HPLC and by radio-TLC, system A), the solution was evaporated to dryness giving a red oil which was then dissolved in methylene chloride (10 ml). The obtained solution was extracted with water (2 × 5 ml) then the red aqueous phase was adjusted to pH 7.4 with 5% $NaHCO_3$ solution. After extraction with methylene

chloride until the solution became colourless (3×5 ml), the organic phases were collected, dried over anhydrous sodium sulphate and evaporated to dryness. The residue was dissolved in methylene chloride (2 ml) and the solution was added to a mixture of ethyl acetate: acetone 1:1 (v/v) (10 ml). A red flaky precipitate was obtained which was filtered through a D4 sintered-glass filter. The red filtrate was added to *n*-hexane (10 ml) yielding an additional amount of precipitate that was filtered and added to the first precipitate. The solid was dissolved in methylene chloride:methanol 4:1 (v/v) (10 ml) and after solvent evaporation to dryness compound **4a** was obtained (158.73 MBq, 0.1406 mmol, 72.7% radiochemically pure by radio-HPLC, 63% radiochemically pure by radio-TLC, system A). This compound was used without further purification in the next step. The radiochemical yield was 88% from **2a**.

*4-Demethoxy-3′-N-(2-methanesulphonylethyl)-4′-O-methanesulphonyl-[14-^{14}C]daunorubicin (**5a**)*

To a cooled (-10°C) solution of **4a** (158.73 MBq, 0.1406 mmol) in dry pyridine (1.6 ml, 20 mmol) methanesulphonyl chloride (43.6 µl, 0.5625 mmol) was added with stirring under nitrogen and the reaction mixture kept at $+5$° for 2.5 h. At the end of the reaction (determined by radio-HPLC and radio-TLC, system B), the cold bath was removed, methylene chloride (10 ml) and water (2×5 ml) were added and the reaction mixture stirred for 5 min. The organic phase was then washed with an acidic aqueous solution (pH $= 5$ with 0.1 M sulphuric acid; 2×5 ml) in order to reduce the pyridine content and then washed with water (2×5 ml). After drying over anhydrous sodium sulphate and solvent evaporation to dryness, intermediate **5a** was recovered and immediately used in the next step.

4-Demethoxy-3′-deamino-aziridinyl-4′-O-methanesulphonyl-[14-^{14}C]daunorubicin ([14-^{14}C]PNU-159548)

To a solution of **5a** (158.73 MBq, 0.1406 mmol) in acetone (40 ml), DIEA (56.4 µl, 0.3234 mmol) was added. The reaction mixture was stirred at 36°C for 48 h. At the end of the reaction (determined by radio-HPLC and radio-TLC, system B) the solution was evaporated to dryness, the residue dissolved in methylene chloride (2 ml) and added to *n*-hexane (10 ml). The obtained red flaky precipitate was filtered through a D4 sintered-glass filter, washed with *n*-hexane and dissolved in methylene

chloride. After solvent evaporation to dryness, the crude final compound was purified by flash-chromatography (see General methods). [14-^{14}C]PNU-159548 (48.99 MBq) was obtained in 98% radiochemically pure form (by radio-HPLC, Rt = 38.4 min), with a specific activity of 1.13 GBq/mmol. The overall radiochemical yield was 27% from **2a**.

*4-Demethoxy-3′-N-(2-[$^{2}H_4$]hydroxyethyl)daunorubicin (**4b**)*

[1,1,2,2-$^{2}H_4$]2-bromoethanol **(3b)** (0.83 ml, 11.25 mmol) and DIEA (196.4 µl, 1.125 mmol) were added in turn to a solution of **2** (200 mg, 0.375 mmol) in dry methylene chloride (7.6 ml). The reaction mixture was stirred at 36°C for 48 h. At the end of the reaction (determined by HPLC and TLC, system A), the solution was evaporated to dryness giving a red oil which was then dissolved in methylene chloride (20 ml). The solution was extracted with water (2 × 5 ml) and the red aqueous phase was adjusted to pH 7.4 with a 5% aqueous $NaHCO_3$ solution. After extraction with methylene chloride until the solution was colourless (4 × 10 ml), the organic phases were collected, dried over anhydrous sodium sulphate and evaporated to dryness. The residue was dissolved in methylene chloride (2 ml) and the solution was added dropwise to a mixture of ethyl acetate:acetone 1:1 (v/v) (10 ml). A red flaky precipitate was obtained which was filtered through a D4 sintered-glass filter. The red filtrate was added to *n*-hexane (15 ml) yielding an additional amount of precipitate that was filtered and added to the first precipitate. The solid was then dissolved in methylene chloride:methanol 4:1 (v/v) (10 ml) and, after solvent evaporation to dryness compound **4b** (196 mg, 0.359 mmol, 89.7% chemically pure by HPLC) was obtained and used without further purification in the next step. The chemical yield was 96% from **2**.

*4-Demethoxy-3′-N-(2-methanesulphonyl[$^{2}H_4$]ethyl)-4′-O-methanesulphonyl Daunorubicin (**5b**)*

Methanesulphonyl chloride (111.2 µl, 1.437 mmol) was added to a cooled (−10°C) solution of **4b** (196 mg, 0.359 mmol) in dry pyridine (4 ml) with stirring under nitrogen. The reaction mixture was kept at +5° for 2.5 h. At the end of the reaction (determined by HPLC and TLC, system B) the cold bath was removed, methylene chloride (15 ml) and water (2 × 6 ml) were added and the reaction mixture stirred for 5 min. The organic phase was washed with an acidic aqueous solution

(pH = 5 with 0.1 M sulphuric acid; 3 × 6 ml) in order to reduce the pyridine content. The organic layer was washed with water (2 × 6 ml), dried over anhydrous sodium sulphate and evaporated to dryness giving intermediate **5b** which was immediately used in the next step.

4-Demethoxy-3′-deamino-[$^2H_4$]aziridinyl-4′-O-methanesulphonyl-daunorubicin ([$^2H_4$]PNU-159548)

To a solution of **5b** (252 mg, 0.359 mmol) in acetone (90 ml), DIEA (144 µl, 0.826 mmol) was added. The reaction mixture was stirred at 36°C for 48 h. At the end of the reaction (determined by HPLC and TLC, system B) the solution was evaporated to dryness, the residue dissolved in methylene chloride (3 ml) and added dropwise to *n*-hexane (10 ml). The red flaky precipitate was filtered through a D4 sintered-glass filter, washed with *n*-hexane and dissolved in methylene chloride (10 ml). The organic solution was evaporated to dryness affording the crude final compound (144 mg). After purification by flash chromatography (see General methods), [$^2H_4$]PNU-159548 (about 80 mg) was obtained, > 98% chemically pure (by HPLC, Rt = 38.4 min). The overall yield from **2** was approximately 43%. MS (ESI–MS): *m/z* 628 (4, $[MNa]^+$); *m/z* 606 (100, $[MH]^+$); *m/z* 333 (4, $[M\text{-}C_9^2H_4H_{14}O_6]^+$); *m/z* 291 (11, $[M\text{-}C_{11}^2H_4H_{17}O_7]^+$. NMR (^{1}H NMR; $CDCl_3$; 500 MHz): 1.36 δ (d, J = 6.4 Hz, 6′CH_3); 1.50 δ (m, 3′ H); 1.81 δ (dd, J = 4.7 and 13.6 Hz, 2′ Heq); 2.10 δ (m, 2′ Hax); 2.11 δ (dd, J = 4.0 and 14.9 Hz, 8 Hax); 2.31 δ (d, J = 14.9, 8 Heq); 2.39 δ (s, $COCH_3$); 3.01 δ (d, J = 19 Hz, 10 Hax); 3.21 δ (s, CH_3SO_2); 3.23 δ (d, J = 19.0, 10 Heq); 4.09 δ (q, J = 6.4 Hz, 5′H); 4.45 δ (s, 9 OH); 4.75 δ (s, 4′ H); 5.28 δ (dd, J = 2.2 and 4.0 Hz, 7 H); 5.53 δ (d, J = 3.6 Hz, 1′ H); 7.85 δ (m, 2 H + 3 H); 8.37 δ (m, 1 H + 4 H); 13.35 δ (s, 11 OH); 13.60 δ (s, 6 OH).

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