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Radiosynthesis of Antitumor Spliceosome Modulators

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Abstract

A set of novel antitumor agents (the sudemycins) has recently been described that are analogs of the natural product FR901464. We report the radiosynthesis of two of these antitumor drug lead compounds, using a three step procedure: 1) ester hydrolysis, 2) Lindlar's catalyst/tritium gas to give a (*S,Z*)-4-acetoxypent-2-enoic acid derivative, and finally 3) amide bond formation. These labelled analogs are useful in developing a better understanding of the pharmacological properties of this new class of therapeutic lead compounds.

Keywords

sudemycin; alkyne reduction; tritiation; spliceosome; antitumor; Lindlar catalyst

1. Introduction

FR901464 is a natural product isolated from *Pseudomonas sp. No. 2663* and has been shown to inhibit tumour growth in various xenograft models (Nakajima et al., 1996; Nakajima et al., 1996; Nakajima, Sato et al., 1997). FR901464 has been the subject of several successful total synthesis efforts (Thompson et al., 2001; Motoyoshi, et al., 2006; Albert et al., 2007) including the establishment of some structure-activity relationships (SAR) based on the synthesis of new analogs (Thompson et al., 2001; Motoyoshi et al., 2004; Albert et al., 2007; Osman et al., 2011). Most notable among these latter studies was the report of the highly active analogue (meayamycin) by the Koide group (Albert et al., 2007) and their recent very substantial expansion of the SAR work in this area (Osman et al., 2011). Independently from this synthetic work, the Kaida group demonstrated that a methyl ketal derivative of bacterially derived FR901464 (spliceostatin) interacts with the SF3b subunit of the spliceosome, which may in part explain the biological activity of these compounds (Kaida et al., 2007). We have recently reported the synthesis of active synthetic analogs of FR901464 that we call sudemycin E0 and F1 (see Figure 1) (Lagiseti et al., 2008; Lagiseti et al., 2009; Fan et al., 2011). Sudemycin derivatives were designed based on the very similar (or possibly identical) mechanism of action of FR901464 and pladienolide (a structurally unrelated macrolide natural product isolated from *Streptomyces platensis*) (Mizui et al., 2004) that when combined with the known FR901464 SAR data available at the time, allowed us to develop a hypothetical 3D pharmacophore model. Very recently we reported

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that the sudemycins and spliceostatin show dose-dependent and time-dependent alterations in pre-mRNA splicing in certain tumour cell lines *in vitro* and *in vivo* (Fan et al., 2011). The name sudemycin is derived from a combination of the names of the source bacterial genera for these two natural products, *Pseudomonas* and *Streptomyces*.

The sudemycins show significant antitumor activity *in vitro* and *in vivo*, and are currently lead compounds that represent a new class of synthetic antitumor agents that modify pre-mRNA splicing in tumor cells (Lagisetti et al., 2008; Lagisetti et al., 2009; Fan et al., 2011). As part of our preclinical investigation of the cell uptake, protein-binding, *in vivo* absorption, distribution, metabolism, excretion and pharmacokinetic behavior of these drug leads, we desired a radiolabelled version of this class of compounds. It is well known that radiolabelled drug leads offer many advantages in the early drug discovery process (Hesk and McNamara, 2007). We have therefore developed chemistry for the preparation of tritium labelled derivatives of sudemycin, which we describe in this report.

2. Results and Discussion

Our synthesis of tritiated sudemycin F1 (**1a**) and sudemycin E0 (**1b**) starts with the preparation of the (*S,Z*)-4-acetoxypent-2-enoic acid derivatives **2a** and **2b**, via the reaction of alkyne **3a** and **3b** respectively with tritium gas, based on a modification of the literature precedent (Thompson et al., 2001). In order to rehearse the reduction we initially explored the ‘cold’ reduction of acid **3a** and **3b** with deuterium at 1 atm using Lindlar’s catalyst with quinoline in ethanol, as shown in Scheme 1. Following the development of optimized conditions, we found that the resulting *cis* acid **2** coupled with the amine intermediate **4** using HBTU in CH₃CN obtained the desired product **1** in acceptable yield and purity.

This optimized procedure was then applied in the tritiation of **3a** and **3b**, which used a combination of tritium and deuterium (to control the specific activity of the product) in the reduction of acid **3** using a Tritium manifold. All the radiolabelled analogs were purified with HPLC using UV detection and analyzed by FSA-HPLC. The retention time of [³H] sudemycin on the HPLC was identical to the retention time for the deuterated version of this compound from earlier studies, which was confirmed by co-injection of the two compounds having an equal molar concentration and volume (See Figures 2 and 3). The specific activity of the analogs was determined to be ~0.66 Ci/mmol for **1a** and ~0.023 Ci/mmol for **1b**, using scintillation (see Experimental Section).

3. Conclusion

We have successfully developed a practical route to [³H] sudemycin analogs via reduction to form a *cis* radiolabelled pentenoic acid, which is subsequently coupled to the sudemycin intermediate via amide bond formation. These labelled lead compounds will be important tools in the numerous biochemical and pharmacology experiments with this new class of antitumor lead compounds.

4. Experimental

All materials and reagents were used as is unless otherwise indicated. Air- or moisture-sensitive reactions were carried out under a nitrogen atmosphere. Acetonitrile was distilled before use. TLC analysis was performed using glass TLC plates (0.25 mm, 60 F-254 silica gel). Visualization of the developed plates was accomplished by staining with, ceric ammonium molybdate or anisaldehyde followed by heating on a hot plate (120 °C). The deuterated compounds possessed a purity of >90% as determined by ultra performance liquid chromatography on a Waters Acquity UPLC/PDA/ELSD/MS system carried out with a BEH C18 2.1 × 50 mm, 1.7 μm column using gradient elution with solvents A: 10 mM

(NH₄)HCO₃, B: acetonitrile. A PerkinElmer LC was used for the analysis and purification of the radioactive compounds using a Gemini 3.0 × 50 mm, 5μ C18 110A column (analytical) and XBridge Prep C18 10.0 × 100 mm, 5 μm column (semi-preparative). A Beckman Coulter scintillation counter LS6000TA with scintillation fluid, ScintiSafe 30 %, was used to determine the activity of the radioactive compounds. NMR spectra were obtained on Bruker Avance II 400 MHz. The reference values for NMR spectroscopy were d_H 7.26 ppm and d_C 77.0 ppm in CDCl₃ and d_H 2.05 ppm and d_C 206.68 ppm in acetone. The coupling constants deduced in ¹H NMR data cases were obtained by first-order coupling analysis.

Chemistry

(2*s*,5*R*)-2-((2*E*,4*E*)-5-((3*R*,5*S*)-7,7-dimethyl-1,6-dioxaspiro[2.5]octan-5-yl)-3-methylpenta-2,4-dien-1-yl)-1,3-dioxan-5-amine **4**. Prepared according to literature procedure (Lagisetti et al., 2009).

(*S*)-4-((dimethylcarbamoyloxy)pent-2-ynoic acid (**3a**). (*S*)-*tert*-butyl 4-((dimethylcarbamoyloxy)pent-2-ynoate (35 mg, 0.145 mmol) was dissolved in MeOH (2.35 mL) in a 25 mL round bottom flask and cooled in an ice bath. Aqueous LiOH (2.35 mL, 1 N) was added to the reaction which was stirred for 15 min in the ice bath and deemed complete by TLC (20 % EtOAc/hexane). The reaction mixture was concentrated under vacuum at 25 °C. Water (2 mL) was added to the resulting aqueous solution which was washed with EtOAc (4 mL). The aqueous layer was cooled in an ice bath and 1 N HCl was added drop wise to pH 4. The aqueous layer was extracted with EtOAc (3 × 10 mL), dried over Na₂SO₄ and concentrated under vacuum at room temperature. The crude product (19 mg) was purified by flash chromatography, to yield pure product **3a** (2.5 mg) in 9% yield. ¹H NMR (400 MHz, acetone-d₆) δ 5.44 (q, *J* = 6.6 Hz, 1H), 2.92 (s, 6H), 1.49 (d, *J* = 6.7 Hz, 3H). ¹³C NMR (101 MHz, acetone-d₆) δ 159.20, 155.99, 82.17, 80.19, 61.94, 36.61, 36.03, 21.49.

(*S*)-4-acetoxypent-2-ynoic acid **3b**. Prepared according to literature procedure (Thompson et al., 2001).

[²H] (*S,Z*)-4-((dimethylcarbamoyloxy)pent-2-enoic acid (**2a**) [²H]. To a 10 mL round bottom flask containing a stirring suspension of Lindlar's catalyst (1.5 mg, 0.015 mmol) in EtOH (400 μL) at room temperature under nitrogen was added quinoline (1.4 μL, 0.012 mmol). After stirring for 20 min, acid **3a** (21 mg, 0.113 mmol) in EtOH (1.2 mL) was added to the reaction flask then stirred for an additional 20 min before being placed under D₂ at 1 atm. The reaction was stirred overnight and deemed complete by TLC (40 % acetone/hexane). The crude was purified by flash chromatography to yield pure product (6.4 mg) in 30 % yield. ¹H NMR (400 MHz, acetone-d₆) δ 6.12 (q, *J* = 6.5 Hz, 1H), 2.87 (d, *J* = 23.3 Hz, 6H), 1.34 (d, *J* = 6.5 Hz, 3H). ²H NMR (61 MHz, acetone) δ 6.28 (s, 1H), 5.83 (s, 1H). ¹³C NMR (101 MHz, acetone-d₆) δ 167.40, 156.86, 150.40, 120.66, 70.25, 36.87, 36.47, 20.86.

[²H] Sudemycin F1 (**1a**) [²H]. N,N-diisopropylethylamine (DIPEA) (97 μL, 0.557 mmol) was added to a 1 dram vial containing amine **4** (45 mg, 0.139 mmol) dissolved in CH₃CN (500 μL). Crude acid **2a** [²H] (31.2 mg, 0.165 mmol) in CH₃CN (363 μL) was added to the reaction vial. The reaction was cooled in an ice bath and after 5 minutes HBTU (69.7 mg, 0.184 mmol) was added in one portion. The reaction was maintained in the ice bath for 20 min then warmed to room temperature and stirred for 2 hr until deemed complete by TLC (35 % acetone/hexane). EtOAc (2 mL) was added to the reaction mixture which was partitioned with saturated aqueous NaHCO₃ (2 mL). The organic layer was sequentially washed with saturated aqueous NH₄Cl (2 mL), saturated aqueous NaHCO₃ (2 mL), brine (2

mL) then dried over Na_2SO_4 and concentrated under vacuum. The crude was purified by flash chromatography to yield product (20 mg) in 29 % yield. ^1H NMR (400 MHz, CDCl_3) δ 7.94 (d, J = 8.5 Hz, 1H), 6.27 (d, J = 15.8 Hz, 1H), 5.88 (q, J = 6.4 Hz, 1H), 5.59 (dd, J = 15.7, 6.6 Hz, 1H), 5.51 (t, J = 7.3 Hz, 1H), 4.59 (t, J = 5.3 Hz, 1H), 4.50 – 4.41 (m, 1H), 4.06 – 3.87 (m, 5H), 2.90 (s, 6H), 2.57 (s, 2H), 2.53 – 2.47 (m, 2H), 1.99 (d, J = 13.8 Hz, 1H), 1.91 (dd, J = 13.6, 11.7 Hz, 1H), 1.73 (s, 3H), 1.40 (s, 3H), 1.37 (d, J = 6.5 Hz, 3H), 1.28 (s, 3H), 1.23 – 1.18 (m, 1H), 1.15 (dd, J = 13.8, 2.0 Hz, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 165.22, 156.29, 135.68, 135.47, 127.84, 125.88, 102.02, 77.32, 77.20, 77.00, 76.68, 73.02, 70.10, 69.48, 69.29, 55.65, 51.03, 43.77, 42.43, 38.56, 34.17, 31.51, 23.74, 20.40, 12.57. HRMS (ESI) calcd for $\text{C}_{26}\text{H}_{38}\text{D}_2\text{N}_2\text{O}_7$ ($M + \text{Na}$) 517.6098, found 517.2874.

Radiochemistry

$[\text{}^3\text{H}]$ (S,Z)-4-((dimethylcarbamoyl)oxy)pent-2-enoic acid (**2a**). To a 10 mL round bottom flask containing a stirring suspension of Lindlar's catalyst (10.6 mg, 0.100 mmol) in EtOH (2 mL) at room temperature under nitrogen was added quinoline (10.15 μL , 0.086 mmol). After stirring for 20 min, acid **3a** (150 mg, 0.810 mmol) in EtOH (4 mL) was added to the reaction flask and the mixture was stirred for an additional 20 min before being placed under T_2/D_2 on the tritium manifold. With the closed manifold under high vacuum, incorporation of tritium was accomplished by heating the tritium bound uranium source to 350 $^\circ\text{C}$ and allowing the measured manifold pressure to stabilize at 325 mbar. Deuterium was then introduced into the manifold from an alternative source to constitute the make-up mixture, with final pressure reached at 1214 mbar. After subsection of the reaction flask to the T_2/D_2 atmosphere for 18 hrs, a loss of 84 mbar of gas was observed. After purging the reaction to a nitrogen atmosphere, the crude reaction mixture was filtered through celite packed in a filtration tube into a pre-weighed 25 mL round bottom flask. The solvent was removed under vacuum and the crude product (~144 mg) was used in the next step without further purification.

$[\text{}^3\text{H}]$ Sudemycin F1 (**1a**). DIPEA (0.312 mL, 1.793 mmol) was added to a vial containing amine **4** (145 mg, 0.448 mmol) dissolved in CH_3CN (500 μL). The acid **2a** (144 mg, 0.761 mmol) in CH_3CN (363 μL) was added to the reaction vial. The reaction was cooled in an ice bath and after 5 min HBTU (272 mg, 0.717 mmol) was added in one portion. The reaction was stirred in the ice bath for 20 min, warmed to room temperature and stirred for an additional 2 hr before being concentrated *in vacuo* without any additional work-up. The crude material was purified on the semi-prep HPLC to afford 66.3 mg of product **1a** in a total percent yield of 30 %. The total activity was determined from 10 μL of the product stock solution (10 mM in dimethyl sulfoxide). By HPLC and scintillation analysis, the sample has an observed purity of 95 % with a total activity of 85.9 mCi (specific activity of ~0.66 Ci/mmol).

$[\text{}^3\text{H}]$ (S,Z)-4-acetoxypent-2-enoic acid (**2b**). The procedure was the same as reported for **2a** with the exception that the tritium source was heated to 275 $^\circ\text{C}$ to reach a stabilized pressure of 20–22mbar before being brought up to 1008 mbar with deuterium.

$[\text{}^3\text{H}]$ Sudemycin E0 (**1b**). The procedure was the same as reported for **1a**, producing 3.7 mg of final product in 14 % overall yield. The total activity was determined from 10 μL of the product stock solution (10 mM in dimethyl sulfoxide). By HPLC and scintillation analysis, the sample has an observed purity of 95 % with a total activity of 180 μCi (specific activity of ~0.023 Ci/mmol).

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Figure 1.
The structures of FR901464, spliceostatin, meayamycin and sudemycins E0 and F1.

Figure 2.
Analytical HPLC of [^3H] sudemycin F1 (**1a**)

Figure 3.
Analytical HPLC of coinjection of [^3H] sudemycin F1 (**1a**) and [^2H] sudemycin F1 (**1a** [^2H])

a) Tritium/Deuterium, Lindlar's catalyst, quinoline, EtOH; b) **2**, DIPEA, HBTU, CH₃CN

Scheme 1.
Radiosynthesis of sudemycin analogs