Journal of Enzyme Inhibition and Medicinal Chemistry, April 2005; 20(2): 109–113

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Synthesis and antimycobacterial activity of 7-*O*-substituted-4-methyl -2*H*-2-chromenone derivatives *vs Mycobacterium tuberculosis*

SOOK WAH YEE, BINAL SHAH & CLAIRE SIMONS

Medicinal Chemistry Division, Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff CF10 3XF, UK

(Received 3 June 2004; in final form 25 June 2004)

Abstract
A series of 7-*O*-alkoxy-4-methylumbelliferone derivatives were prepared using a convenient one step synthesis. Additionally the bromo- and azido derivatives 7-*O*-(4-bromobutoxy)-, 7-*O*-(6-bromohexyloxy)- and 7-*O*-(6-azidohexyloxy)-4-methylumbelliferone derivatives were prepared. *In vitro* evaluation of antimycobacterial activity determined % inhibition and MIC *vs M. tuberculosis* $H_{37}Rv$ with toxicity (IC_{50}) assessed in VERO cells. The coumarins with longer alkyl chains (nonyl and decyl) showed the optimum inhibitory activity in this series (MIC 3.13 μg/mL) and $IC_{50} > 10$ μg/mL.

Keywords: *7-O*-alkyl-4-methylumbelliferone, antimycobacterial activity, MIC, toxicity (IC_{50})

Introduction

Tuberculosis (TB) is the leading infectious killer of adults and is caused by *Mycobacterium tuberculosis*, an intracellular pathogen that establishes an infection in oxygen-rich alveolar macrophages of the lung [1]. The outer membrane of the mycobacterial cell wall is an important target for antimycobacterium agents, in particular the biosynthesis of cell wall components. The mycobacterial cell wall is very hydrophobic, being composed of long fatty acids (C60-C90), known as mycolic acids, resulting in an efficient barrier to a range of antimycobacterial agents [2]. The mycolic acids are covalently bound to the arabinogalactan and peptidoglycan, which is found between the outer mycolic acid membrane and the lipid bilayer of the plasma membrane. Uptake of any drugs through the outer membrane requires the drugs to be lipophilic in nature although there is evidence of the presence of porin channels in the mycobacterial cell envelope through which both nutrients and drugs could diffuse [3].

Currently TB is treated with agents that target mycolic acid biosynthesis including isoniazid, inhibitors of nucleic acid biosynthesis such as rifampicin which binds and inhibits mycobacterial DNA-dependent RNA polymerase, and the aminoglycoside antibiotic streptomycin which targets protein synthesis [4].

With the increasing incidence of TB [5], in both developing and western countries, and the emergence of multi-drug resistant strains (MDR-TB) [6], the development of new TB therapeutics is very timely. Random screening of compounds from our laboratory identified a lipophilic 7-*O*-alkyl-4-methylumbelliferone as a moderately active antiTB agent. Based on this finding a small series of 7-*O*-alkyl-derivatives were prepared to determine the effect of chain length on inhibitory (MIC) activity.

Materials

All reagents used in the experiments were of general purpose or analytical grade and purchased from Aldrich. Experiments requiring anhydrous conditions were guarded by means of nitrogen balloons. Thin layer chromatography (TLC) was performed using

Correspondence: C. Simons, Medicinal Chemistry Division, Welsh School of Pharmacy, Cardiff University, King Edward VII Avenue, Cardiff CF10 3XF, UK. Tel: 44 02920 876307. Fax: 44 02920 874149. E-mail: simonsc@cardiff.ac.uk

ISSN 1475-6366 print/ISSN 1475-6374 online
DOI: 10.1080/14756360400002015

Merck aluminium backed plates (Kieselgel 60 F_{254}) and visualised by ultra violet (UV) light. Separations by column chromatography were achieved using silica gel (0.0035–0.07 mm, Janssen Chimica, pore diameter ca. 6 nm).

^1H and ^{13}C n.m.r. were recorded on a Brucker Advance DPX300 spectrometer, operating at 300 MHz and 75 MHz respectively, using deuterated solvent ($CDCl_3$). Chemical shifts are given in part per million (ppm) relative to the internal standard tetramethylsilane. Melting points were determined on a Gallenkamp digital melting point apparatus and are uncorrected. Infra red (IR) spectra were determined as a KBr disk using a Perkin Elmer 1600 series FTIR spectrophotometer. Microanalyses were determined by Medac Ltd., Surrey.

Methods

Chemistry

*General method for the preparation of compounds **1**–**8***. To a solution of 4-methylumbelliferone (0.5 g, 2.84 mmol) in anhydrous acetone (25 mL) was added K_2CO_3 (0.78 g, 5.68 mmol). A solution of 1-haloalkane (for compounds **1**–**6**) or dibromoalkane (for compounds **7**–**8**) (11.35 mmol) in anhydrous acetone (15 mL) was then added to the reaction and this mixture was refluxed at 60°C under nitrogen for 10 h. The K_2CO_3 was removed by filtration and the filtrate concentrated under reduced pressure. The resulting residue was diluted with toluene (100 mL) washed with water (75 mL), and the organic layer dried with $MgSO_4$ and concentrated under reduced pressure. Compounds **2**–**6** and **8** were further purified by flash column chromatography.

*7-Butoxy-4-methyl-2H-2-chromenone **1***. Colourless solid (85%), mp 41–43°C. ^1H NMR (d_6-acetone) δ 7.45 (d, J = 8.8 Hz, 1H, H-5), 6.76 (dd, J = 2.4, 8.8 Hz, 1H, H-6), 6.6 (d, J = 2.4 Hz, 1H, H-8), 6.0 (d, J = 1.0 Hz, 1H, H-3), 3.9 (t, J = 6.5 Hz, 2H, CH_2-9), 2.32 (d, J = 1.0 Hz, 3H, CH_3-13), 1.75 (m, 2H, CH_2-10), 1.48 (m, 2 H, CH_2-11), 0.96 (m, 3 H, CH_3-12). ^{13}C NMR δ 163.4 (C=O), 162.0 (C-7), 156.5 (C-8a), 153.8 (C-4), 127.1 (CH-5), 114.4 (C-4a), 113.4 (CH-6), 112.7 (CH-8), 102.1 (CH-3), 69.4 (CH_2-9), 31.0 (CH_2-10), 20.5 (CH_2-11), 19.1 (CH_2-13), 14.7 (CH_3-12). Found: C, 72.27; H, 6.98. $C_{14}H_{16}O_3$ (232.2786) requires: C, 72.39; H, 6.94%.

*7-Hexyloxy-4-methyl-2H-2-chromenone **2***. Purification by flash column chromatography (petroleum ether–ethyl acetate 80:20 v/v) gave the product as an opaque viscous liquid (72%). ^1H NMR δ 7.40 (d, J = 8.8 Hz, 1H, H-5), 6.75 (dd, J = 2.5, 8.8 Hz, 1H, H-6), 6.6 (d, J = 2.5, 1H, H-8), 6.0 (d, J = 1.1 Hz, 1H, H-3), 3.92 (m, 2H, CH_2-9), 2.31 (d, J = 1.1 Hz, 3H, CH_3-15), 1.75 (m, 2H, CH_2-10), 1.40 (m, 2H, CH_2-11), 1.27 (m, 4H, CH_2-12 and CH_2-13), 0.86 (m, 3H, CH_3-14). ^{13}C NMR δ 162.5 (C=O), 161.5 (C-7), 155.5 (C-8a), 153.0 (C-4), 125.9 (CH-5), 113.6 (C-4a), 112.8 (CH-3), 112.0 (CH-6), 101.6 (CH-8), 68.9 (CH_2-9), 31.9 (CH_2-12), 29.3 (CH_2-10), 26.0 (CH_2-11), 23.0 (CH_2-13), 18.9 (CH_3-15), 14.4 (CH_3-14). Found: C, 73.88; H, 7.55. $C_{16}H_{20}O_3$ (260.3322) requires: C, 73.82; H, 7.74%.

*7-Heptyloxy-4-methyl-2H-2-chromenone **3***. Purification by flash column chromatography (petroleum ether–ethyl acetate 75:25 v/v) gave a viscous syrup which crystallised on standing to give a white solid (81%), m.p. 46–48°C. ^1H NMR δ 7.55 (d, J = 8.8 Hz, 1H, H-5), 6.91 (dd, J = 2.5, 8.8 Hz, 1H, H-6), 6.86 (d, J = 2.4 Hz, 1H, H-8), 6.18 (d, J = 1.0 Hz, 1H, H-3), 4.07 (t, J = 6.5 Hz, 2H, CH_2-9), 2.46 (d, J = 1.0 Hz, 3H, CH_3-16), 1.88 (ddd, J = 6.6, 13.5, 14.5 Hz, 2H, CH_2-10), 1.31 (m, 8H, CH_2-11, 12, 13, 14), 0.96 (φt, J = 6.4, 6.9 Hz, 3H, CH_3-15). ^{13}C NMR δ 162.7 (C=O), 161.8 (C-7), 155.7 (C-4), 153.0 (C-8a), 125.9 (CH-5), 113.8 (C-4a), 113.1 (CH-6), 112.2 (CH-8), 101.7 (CH-3), 69.01 (CH_2-9), 32.1 (CH_2-13), 29.5 (CH_2-10), 29.4 (CH_2-12), 26.3 (CH_2-11), 23.0 (CH_2-14), 19.1 (CH_3-16), 14.5 (CH_3-15). Found: C, 74.28; H, 8.06. $C_{17}H_{22}O_3$ (274.3428) requires: C, 74.42; H, 8.08%.

*7-Octyloxy-4-methyl-2H-2-chromenone **4***. Purification by flash column chromatography (petroleum ether–ethyl acetate 1:1 v/v) gave a viscous syrup which crystallised on standing to give a white solid (76%), m.p. 37–40°C. ^1H NMR δ 7.53 (d, J = 8.8 Hz, 1H, H-5), 6.91 (dd, J = 2.5, 8.7 Hz, 1H, H-6), 6.86 (d, J = 2.4 Hz, 1H, H-8), 6.18 (d, J = 1.1 Hz, 1H, H-3), 4.07 (t, J = 13.0 Hz, 2H, CH_2-9), 2.45 (d, J = 1.2 Hz, 2H, CH_3-17), 1.85 (dd, J = 6.8, 14.8 Hz, 2H, CH_2-10), 0.94 (t, J = 6.5 Hz, 3H, CH_3-16). ^{13}C NMR δ 162.7 (C=O), 161.8 (C-7), 155.7 (C-8a), 153.0 (C-4), 125.9 (CH-5), 113.8 (C-4a), 113.1 (CH-3), 112.2 (CH-6), 101.7 (CH-8), 69.1 (CH_2-9), 32.2 (CH_2-10), 29.5 (CH_2-11), 29.6 (CH_2-12), 29.4 (CH_2-13), 26.4 (CH_2-14), 23.1 (CH_2-15), 19.1 (CH_2-16), 14.5 (CH_2-17). Found: C, 75.06; H, 8.39. $C_{18}H_{24}O_3$ (288.3696) requires: C, 74.97; H, 8.39%.

*7-Nonyloxy-4-methyl-2H-2-chromenone **5***. Purification by flash column chromatography (petroleum ether–ethyl acetate 80:20 v/v) gave a viscous syrup which crystallised on standing to give a white solid (62%), m.p. 54–56°C. ^1H NMR δ 7.50 (d, J = 8.8 Hz, 1H, H-5), 6.86 (dd, J = 2.3, 8.8 Hz, 1H, H-6), 6.81 (d, J = 2.2 Hz, 1H, H-8), 6.14 (s, 1H, H-3), 4.02 (t, J = 6.5 Hz, 2H, CH_2-9), 2.41 (d, J = 1.0 Hz, 3H, CH_3-16), 1.83 (ddd, J = 6.6, 13.5, 14.2 Hz, 2H, CH_2-10), 1.45 (m, 2H, CH_2-16), 1.30 (m, 10H, CH_2-11, 12, 13, 14, 15), 0.90 (φt, J = 6.4, 6.9 Hz, 3H, CH_3-17). ^{13}C NMR δ 162.7 (C=O), 161.8 (C-7), 155.7 (C-4), 153.0 (C-8a), 125.9 (CH-5),

113.8 (C-4a), 113.1 (CH-6), 112.2 (CH-3), 101.7 (CH-8), 69.01 (CH_2-9), 32.3, 29.9, 29.8, 29.75, 29.74, 29.4, 26.4 (CH_2-10,11,12,13,14,15), 23.1 (CH_2-16), 19.1 (CH_3-18), 14.6 (CH_3-17). Found: C, 75.32; H, 8.65. $C_{19}H_{26}O_3$ (302.3964) requires: C, 75.46; H, 8.67%.

7-Decyloxy-4-methyl-2H-2-chromenone ***6***. Purification by flash column chromatography (petroleum ether–ethyl acetate 80:20 v/v) gave a viscous syrup which crystallised on standing to give a white solid (64%), m.p. 40–42°C. ^{1}H NMR δ 7.55 (d, $\mathcal{J}$ = 8.8 Hz, 1H, H-5), 6.91 (dd, $\mathcal{J}$ = 2.5, 8.8 Hz, 1H, H-6), 6.86 (d, $\mathcal{J}$ = 2.4 Hz, 1H, H-8), 6.19 (d, $\mathcal{J}$ = 1.1 Hz, 1H, H-3), 4.07 (t, $\mathcal{J}$ = 6.5 Hz, 2H, CH_2-9), 2.46 (d, $\mathcal{J}$ = 1.1 Hz, 3H, CH_3-19), 1.87 (ddd, $\mathcal{J}$ = 6.6, 13.4, 14.6 Hz, 2H, CH_2-10), 1.52 (dd, $\mathcal{J}$ = 6.9, 14.5 Hz, 2H, CH_2-17), 1.36 (m, 12H, CH_2-11, 12, 13, 14, 15, 16), 0.95 (φt, $\mathcal{J}$ = 6.4, 6.9 Hz, 3H, CH_3-18). ^{13}C NMR δ 162.7 (C═O), 161.8 (C-7), 155.7 (C-4), 153.0 (C-8a), 125.9 (CH-5), 113.8 (C-4a), 113.1 (CH-6), 112.2 (CH-3), 101.7 (CH-8), 69.01 (CH_2-9), 32.3, 29.9, 29.8, 29.7, 29.4, 26.4 (CH_2 10, 11, 12, 13, 14, 15, 16), 23.1(CH_2-17), 19.1 (CH_3-19), 14.6 (CH_3-18). Found: C, 75.85; H, 8.94. $C_{20}H_{28}O_3$ (316.4232) requires: C, 75.91; H, 8.92%.

7-(4-Bromobutoxy)-4-methyl-2H-2-chromenone ***7***. Pale yellow solid (83%), mp 39–40°C. ^{1}H NMR δ 7.37 (d, $\mathcal{J}$ = 8.8 Hz, 1H, H-5), 6.70 (dd, $\mathcal{J}$ = 2.5, 8.8 Hz, 1H, H-6), 6.60 (d, $\mathcal{J}$ = 2.5 Hz, 1H, H-8), 5.98 (d, $\mathcal{J}$ = 1.1 Hz, 1H, H-3), 3.90 (t, $\mathcal{J}$ = 6.5 Hz, 2H, CH_2-9), 3.39 (t, $\mathcal{J}$ = 6.6 Hz, 2H, CH_3-12), 2.48 (d, $\mathcal{J}$ = 1.0 Hz, 3H, CH_2-13), 1.91 (m, 4H, CH_2-10, 11). ^{13}C NMR δ 162.3 (C═O), 161.5 (C-7), 155.5 (C-4), 153.1 (C-8a), 126.0 (CH-5), 113.9 (C-4a), 113.1 (CH-6), 112.8 (CH-3), 112.2 (CH-6), 67.9 (CH_2-9), 33.8 (CH_2-12), 33.1 (CH_2-11), 29.7 (CH_2-10), 19.1 (CH_3-13). Found: C, 53.99; H, 4.82. $C_{14}H_{15}BrO_3$ (311.1747) requires: C, 54.04; H, 4.86%.

7-(6-Bromohexyloxy)-4-methyl-2H-2-chromenone ***8***. Purification by flash column chromatography (hexane–ethyl acetate 75:25 v/v) gave a viscous syrup which crystallised on standing to give a white solid (71%), m.p. 58–60°C. ^{1}H NMR δ 7.53 (d, $\mathcal{J}$ = 8.8 Hz, 1H, H-5), 6.91 (dd, $\mathcal{J}$ = 2.5, 8.8 Hz, 1H, H-6), 6.84 (d, $\mathcal{J}$ = 2.4 Hz, 1H, H-8), 6.17 (d, $\mathcal{J}$ = 1.0 Hz, 1H, H-3), 4.06 (t, $\mathcal{J}$ = 6.4 Hz, 2H, CH_2-9), 3.48 (t, $\mathcal{J}$ = 6.7 Hz, 2H, CH_3-14), 2.44 (d, $\mathcal{J}$ = 1.0 Hz, 3H, CH_2-15), 1.92 (m, 4H, CH_2-10, 13), 1.57 (ddd, $\mathcal{J}$ = 3.2, 7.1 Hz, 4H, CH_2-11, 12,). ^{13}C NMR δ 162.5 (C═O), 161.8 (C-7), 155.7 (C-4), 153.0 (C-8a), 125.9 (CH-5), 113.9 (C-4a), 113.1 (CH-6), 112.3 (CH-8), 101.7 (CH-3), 68.7 (CH_2-9), 34.2 (CH_2 13), 32.0 (CH_2 14), 29.2, 28.3, 25.7, (CH_2-10, 11, 12,), 19.1 (CH_3-15). Found: C, 55.29; H, 5.50. $C_{16}H_{19}BrO_3 \cdot 0.4H_2O$ (346.4344) requires: C, 55.47; H, 5.76%.

7-(6-Azidohexyloxy)-4-methyl-2H-2-chromenone ***9***. To a solution of **8** (0.3 g, 0.88 mmol) in anhydrous *N*,*N*-dimethylformamide (10 mL) was added sodium azide (0.115 g, 1.77 mmol) and the resulting orange/red solution heated at 50°C for 2 h. The reaction mixture was poured into water (50 mL) and the resulting yellow solution extracted with dichloromethane (50 mL). The aqueous layer was back extracted with dichloromethane (25 mL) then the organic layers combined, dried with $MgSO_4$ and concentrated under reduced pressure to give the crude product as a yellow/orange syrup. Purification by flash column chromatography (petroleum ether–ethyl acetate 80:20 v/v) gave the product as a viscous yellow syrup, which crystallised on standing to give a cream/yellow solid (0.193 g, 72%), m.p. 36–38° C. IR: 2079.4 cm^{-1} (N_3). ^{1}H NMR δ 7.53 (d, $\mathcal{J}$ = 8.8 Hz, 1H, H-5), 6.89 (dd, $\mathcal{J}$ = 2.5, 8.8 Hz, 1H, H-6), 6.83 (d, $\mathcal{J}$ = 2.45 Hz, 1H, H-8), 6.16 (d, $\mathcal{J}$ = 1.2 Hz, 1H, H-3), 4.06 (t, $\mathcal{J}$ = 6.4 Hz, 2H, CH_2-9), 3.34 (t, $\mathcal{J}$ = 6.8 Hz, 2H, CH_3-10), 2.44 (d, $\mathcal{J}$ = 1.2 Hz, 3H, CH_2-15), 1.88 (dd, $\mathcal{J}$ = 6.5, 8.0 Hz, 2H, CH_2-12), 1.69 (t, $\mathcal{J}$ = 7.0 Hz, 2H, CH_2-11), 1.54 (m, 4H, CH_2-13, 14). ^{13}C NMR δ 162.5 (C═O), 161.8 (C-7), 155.7 (C-4), 153.9 (C-8a), 125.9 (CH-5), 113.9 (C-4a), 113.0 (CH-6), 112.2 (CH-3), 101.7 (CH-8), 68.7 (CH_2-9), 51.8 (CH_2 14), 29.3, 29.2 (CH_2-10 and -13), 26.9, 26.0 (CH_2-11 and -12), 19.1 (CH_3-15). Found: C, 62.80; H, 6.26; N, 13.52. $C_{16}H_{19}N_3O_3 \cdot 0.2H_2O$ (304.9474) requires: C, 63.02; H, 6.41; N, 13.78%.

In Vitro *evaluation of antimycobacterial activity*

All the compounds were tested for antimycobacterial activity at the Tuberculosis Antimicrobial Acquisition & Coordinating facility (TAACF), Birmingham, Alabama, USA.

% Inhibition. Primary screening was conducted at 6.25 μg/mL against *M. tuberculosis* H_{37}Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA) [7]. Compounds exhibiting fluorescence were tested in the BACTEC 460 radiometric system [7].

Minimum inhibitory concentration and cytotoxicity. Compounds demonstrating at least 90% inhibition in the primary screen were re-tested at lower concentrations against *M. tuberculosis* H_{37}Rv to determine the actual minimum inhibitory concentration (MIC) using MABA. The MIC is defined as the lowest concentration effecting a reduction of fluorescence of 90% relative to controls. Compounds displaying MIC ≥ 6.25 μg/mL were

screened by serial dilution to assess cytotoxicity (IC_{50}) in VERO cells starting at 10 × the MIC value. After 72 h exposure, viability was assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 non-radioactive cell proliferation assay [7].

Results and discussions

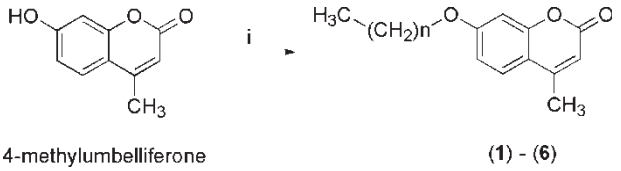
The 7-*O*-alkyl coumarins **1**–**6** were prepared using a one step procedure by reaction of the parent coumarin, 4-methylumbelliferone with the appropriate alkyl halide using potassium carbonate as the base (Scheme 1 and Table I). The reactions proceeded with good yields and the compounds were obtained as low melting point solids (except **2** which was a viscous liquid).

The bromo derivatives **7** and **8** were prepared in a similar manner to coumarins **1**–**6** using an appropriate dibromoalkane, with good yields obtained (Scheme 2). 7-(6-Bromohexyloxy)-4-methyl-2*H*-2-chromenone **8** was subsequently converted to the corresponding 6-azido derivative **9** by reaction with sodium azide in *N*,*N*-dimethylformamide (DMF) (Scheme 2).

In vitro evaluation of antimycobacterial activity, undertaken at the TAACF, determined % inhibition and, where % inhibition was >90%, MIC and toxicity (IC_{50}) data for the novel coumarins (Table II). The parent coumarin 4-methylumbelliferone was devoid of inhibitory activity as were the short chain (**1**, $n = 3$; **2**, $n = 5$) and bromo-substituted (**7**) coumarins.

Coumarin derivatives with a chain length of $n \geq 6$ displayed inhibitory activity, with the longer 7-*O*-alkyl derivatives **5** (nonyl) and **6** (decyl) showing the optimum activity in this series with MIC values of 3.13 μg/mL. The % inhibition data for compound **4** (octyl) appears inconsistent with the trend (increasing activity with increasing chain length) observed and was retested at the TAACF to confirm. Replacement of the terminal chain methyl ($n = 6$) with a bromo or azido moiety resulted in a decrease in MIC.

The compounds were not sufficiently potent to be considered for further evaluation at the TAACF, therefore further studies to determine the mechanism of action have not been performed. Increasing activity with increasing chain length may simply be related to increasing log P (Table III) and therefore an increased ability to penetrate and traverse the outer mycolic acid membrane.



Scheme 1. Reagents and conditions: (i) Alkyl halide (Table I), K_2CO_3, acetone, 60°C, 10 h.

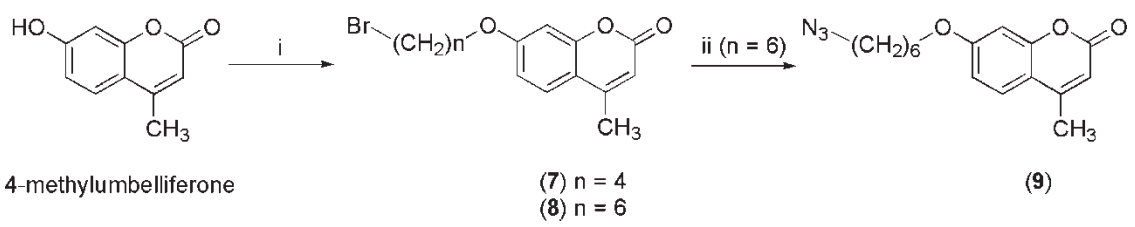
Table I. Yields, Mps and alkyl halide reagents employed for coumarins **1**–**6**.

Compound	Alkyl halide	*n*	Yield (%)	Mp (°C)
1	C_4H_9I	3	85	41–43
2	$C_6H_{13}I$	5	72	–
3	$C_7H_{15}Br$	6	81	46–48
4	$C_8H_{17}I$	7	76	37–40
5	$C_9H_{19}Br$	8	62	54–56
6	$C_{10}H_{21}Br$	9	64	40–42

Table II. *In vitro* evaluation of the antimycobacterial activity of coumarins **1**–**9**.

Compound	% Inhibition[a]	MIC (μg/mL)[b]	IC_{50} (μg/mL)[c]	SI[d]
4-methyl umbelliferone	0	–	–	–
1	0	–	–	–
2	0	–	–	–
3	99	6.25	>10	>1.6
4	82	–	–	–
5	98	3.13	>10	>3.2
6	99	3.13	>10	>3.2
7	0	–	–	–
8	60	>6.25	–	–
9	98	>6.25	–	–
Rifampicin	97–99	0.125	134.98	1080
Isoniazid	nd	0.10	>1000	>10000

[a] % Inhibition determined *vs M. tuberculosis* $H_{37}Rv$ (ATCC 27294).
[b] MIC defined as the lowest concentration effecting a reduction of fluorescence of 90% relative to controls.
[c] Cytotoxicity IC_{50} determined in VERO cells.
[d] Selectivity index defined as IC_{50}/MIC.



Scheme 2. Reagents and conditions: (i) 1,4-dibromobutane ($n = 4$) or 1,6-dibromohexane ($n = 6$), K_2CO_3, acetone, 60°C, 10 h ($n = 4$, 83%; $n = 6$, 71%) (ii) NaN_3, DMF, 50°C, 2 h (72%).

Table III. c Log P data (Crippens fragmentation method[8]) for coumarins **1**–**9**.

Compound	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
c Log P	3.12	3.95	4.37	4.78	5.20	5.62	3.26	4.10	nd

Replacement of the terminal methyl group with a bromo-substituent resulted in a small reduction in the calculated c Log P (determined by Crippens fragmentation [8], CS ChemDraw Ultra 8.0 software), compare coumarin **3** (heptyl, c Log P = 4.37) with the bromo derivative **7** (6-bromohexyl, c Log P = 4.10) (Table III). This suggests that introduction of a polar substituent, Br or N_3, may interfere with uptake or result in interactions with the arabinogalactan or peptidoglycan layer reducing uptake and subsequent inhibitory effect. It is conceivable that these lipophilic coumarin derivatives may disorganise the structure or inhibit the function of the outer membrane, possibly forming pores resulting in a detrimental affect on the integrity of the outer and cytoplasmic membranes with subsequent cell leakage.

Acknowledgements

We thank the Tuberculosis Antimicrobial Acquisition & Coordinating facility (TAACF), Birmingham, Alabama, USA, for antimycobacterial evaluation. One of us (SWY) would like to thank the Nuffield Foundation for an undergraduate research bursary.

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