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SUPPLEMENTARY DATA

Supplementary data

Experimental

Materials

Melting points were determined on a Stuart melting point SMP3 capillary apparatus and are uncorrected. ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were obtained on a Bruker Avance 500 instrument. High-resolution mass spectrometry was determined in a Micromass Q-TOF spectrometer.

Compounds **1a-e** and **6a-c** were purchased from Sigma.

The microorganisms *Mycobacterium tuberculosis* $H_{37}Rv$ (ATCC 27294) and *Mycobacterium bovis* BCG Pasteur (ATCC 35734) were used in this work. Murine macrophages RAW264.7 (ATCC TIB71) were employed for assessing the mammalian cytotoxicity of the compounds.

Chemistry

Methyl esterification of coumaric acids. Each coumaric acid **1a-c** (5.00 g, 30.5 mmol) was suspended in 100 mL of methanol, and 5 mL of concentrated sulfuric acid was added. The mixture was refluxed for 20 h. No starting material was detected by TLC and each product was found to be pure by ^{1}H-NMR with no column chromatography required. The yield was greater than 97% for each of the three methyl coumarates. NMR data was analysed and compared with literature.[1,2]

Methyl 4-coumarate (**2a**) mp 119 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.56 (d, *J* = 16.0 Hz, 1H, H-7), 7.34 (d, *J* = 8.5 Hz, 2H, H-2,6), 6.77 (d, *J* = 8.6 Hz, 2H, H-3,5), 6.22 (d, *J* = 16.0 Hz, 1H, H-8), 3.72 (s, 3H, O-Me); ^{13}C-NMR ($CDCl_3$) δ (ppm): 168.8 (C-9), 159.6 (C-4), 145.6 (C-7), 130.2 (C-2,6), 126.1 (C-1), 115.0 (C-3,5), 114.2 (C-8), 51.7 (O-Me).

Methyl 3-coumarate (**2b**) mp 92 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.66 (d, *J* = 16.0 Hz, 1H, H-7), 7.26 (dd, *J* = 9.9, 5.9 Hz, 1H, H-5), 7.09 (d, *J* = 7.7 Hz, 1H, H-6), 7.04 (t, *J* = 2.4 Hz, 1H, H-2), 6.91 (ddd, *J* = 8.1, 2.5, 0.8 Hz, 1H, H-4), 6.42 (d, *J* = 16.0 Hz, 1H, H-8), 3.83 (s, 3H, O-Me); ^{13}C-NMR ($CDCl_3$) δ (ppm): 168.0 (C-9), 156.2 (C-3), 145.1 (C-7), 135.7 (C-5), 130.1 (C-6), 120.7 (C-2), 117.8 (C-4), 117.7 (C-1), 114.6 (C-8), 51.9 (O-Me).

Methyl 2-coumarate (**2c**) mp 136 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.01 (d, *J* = 17.4 Hz, 1H, H-7), 7.74 (dd, *J* = 7.8, 1.5 Hz, 1H, H-6), 7.23 (ddd, *J* = 7.6, 7.8, 1.6 Hz, 1H, H-4), 6.94 (dd, *J* = 7.6, 7.8 Hz, 1H, H-5), 6.83 (d, *J* = 7.9 Hz, 1H, H-3), 6.62 (d, *J* = 16.2 Hz, 1H, H-8), 3.82 (s, 3H, O-Me); ^{13}C-NMR ($CDCl_3$) δ (ppm): 168.4 (C-9), 155.0 (C-2), 140.4 (C-7), 131.4 (C-4), 129.2 (C-6), 121.6 (C-1), 120.9 (C-5), 118.3 (C-8), 116.3 (C-3), 51.8 (O-Me).

***O*-Prenylation and *O*-geranylation of methyl coumarates.** Each methyl ester **2a-c** (1.00 g, 5.61 mmol) was dissolved in 30 mL of acetone and 5 equivalents of potassium carbonate (3.88 g, 28.1 mmol) added to the stirring solution. Three equivalents of dimethylallyl bromide (1.94 mL, 16.8 mmol) (or 3.33 mL, 16.8 mmol of geranyl bromide) was added and the mixture heated at reflux for 20 hours. The solvent was then removed under reduced pressure, and the residue dissolved in 1:1 mixture of dichloromethane/water, the organic layer washed twice with brine, dried over $MgSO_4$, filtered and the solvent evaporated under reduced pressure. The products were purified by column chromatography on silica gel eluting with hexane/ethyl acetate 9:1 mixture. The yields after purification were 91% for *O*-prenyl methyl 4-coumarate, 37% for *O*-prenyl methyl 3-coumarate and 84% for *O*-prenyl methyl 2-coumarate. The yield for *O*-geranyl methyl 4-coumarate was 80%.

***O*-Prenyl methyl 4-coumarate** (**3a**) mp 238 °C (dec); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.66 (d, *J* = 16.0 Hz, 1H, H-7), 7.55 (d, *J* = 8.7 Hz, 2H, H-2,6), 6.95 (d, *J* = 8.7 Hz, 2H, H-3,5), 6.39 (d, *J* = 16.0 Hz, 1H, H-8), 5.47 (t, *J* = 6.6 Hz, 1H, H-2'), 4.58 (d, *J* = 6.5 Hz, 2H, H-1'), 3.79 (s, 3H, O-Me), 1.81 (s, 3H, H-5'), 1.77 (s, 3H, H-4'); ^{13}C-NMR ($CDCl_3$) δ (ppm): 169.7 (C-9), 162.4 (C-4), 146.3 (C-7), 139.1 (C-3'), 131.0 (C-2,6), 128.2 (C-1), 120.8 (C-2'), 116.2 (C-3,5), 115.7 (C-8), 66.0 (C-1'), 52.2 (O-Me), 25.9 (C-5'), 18.3 (C-4').

***O*-Prenyl methyl 3-coumarate** (**3b**)

^{1}H-NMR ($CDCl_3$) δ (ppm): 7.63 (d, *J* = 16.0 Hz, 1H, H-7), 7.25 (dd, *J* = 10.7, 6.1 Hz, 1H, H-5), 7.08 (d, *J* = 7.7 Hz, 1H, H-6), 7.03 (t, *J* = 2.1 Hz, 1H, H-2), 6.92 (dd, *J* = 8.2, 2.1 Hz, 1H, H-4), 6.39 (d, *J* = 16.0 Hz, 1H, H-8), 5.47 (t, *J* = 6.8 Hz, 1H, H-2'), 4.50 (d, *J* = 6.8 Hz, 2H, H-1'), 3.78 (s, 3H, O-Me), 1.78 (s, 3H, H-5'), 1.73 (s, 3H, H-4'); ^{13}C-NMR ($CDCl_3$) δ (ppm): 167.3 (C-9), 159.1 (C-3), 144.8 (C-7), 138.5 (C-3'), 135.6 (C-5), 129.8 (C-6), 120.7 (C-2), 119.3 (C-2'), 117.9 (C-4), 116.8 (C-1), 113.7 (C-8), 64.8 (C-1'), 51.7 (O-Me), 25.8 (C-5'), 18.2 (C-4').

***O*-Prenyl methyl 2-coumarate** (**3c**)

^{1}H-NMR ($CDCl_3$) δ (ppm): 8.02 (d, *J* = 16.2 Hz, 1H, H-7), 7.50 (dd, *J* = 7.7, 1.6 Hz, 1H, H-6), 7.32 (ddd, *J* = 8.4, 7.5, 1.7 Hz, 1H, H-4), 6.94 (ddd, *J* = 7.4, 7.3, 0.9 Hz, 1H, H-5), 6.91 (dd, *J* = 8.4, 0.9 Hz, 1H, H-3), 6.53 (d, *J* = 16.2 Hz, 1H, H-8), 5.50 (t, *J* = 6.6 Hz, 1H, H-2'), 4.59 (t, *J* = 6.6 Hz, 2H, H-1'), 3.79 (s, 3H, O-Me), 1.80 (s, 3H, H-5'), 1.74 (s, 3H, H-4'); ^{13}C-NMR ($CDCl_3$) δ (ppm): 168.0 (C-9), 157.6 (C-2), 140.4 (C-7), 137.8 (C-3'), 131.3 (C-4), 128.8 (C-6), 123.6 (C-1), 120.5 (C-5), 119.5 (C-2'), 118.1 (C-8), 112.4 (C-3), 65.3 (C-1'), 51.5 (O-Me), 25.7 (C-5'), 18.2 (C-4').

***O*-Geranyl methyl 4-coumarate** (**6a**) mp 215 °C (dec); ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.65 (d, *J* = 16.0 Hz, 1H, H-7), 7.46 (d, *J* = 8.7

Hz, 2H, H-2,6), 6.91 (d, *J* = 8.8 Hz, 2H, H-3,5), 6.30 (d, *J* = 16.0 Hz, 1H, H-8), 5.47 (t, *J* = 7.2 Hz, 1H, H-2’), 5.08 (t, *J* = 6.7 Hz, 1H, H-7’), 4.57 (d, *J* = 6.4 Hz, 2H, H-1’), 3.79 (s, 3H, O-Me), 2.15-2.07 (m, 4H, H-5’, H-6’), 1.74 (s, 3H, H-4’), 1.67 (s, 3H, H-10’), 1.60 (s, 3H, H-9’); ^{13}C-NMR ($CDCl_3$) δ (ppm): 167.8 (C-9), 160.7 (C-4), 144.6 (C-7), 141.7 (C-3’), 131.9 (C-8’), 129.7 (C-2,6), 126.9 (C-1), 123.7 (C-7’), 119.0 (C-2’), 115.1 (C-8), 115.0 (C-3,5), 64.5 (C-1’), 51.5 (O-Me), 39.5 (C-5’), 26.2 (C-6’), 25.7 (C-9’), 17.7 (C-10’), 16.7 (C-4’).

Saponification of the methyl ester. Each *O*-prenyl methyl coumarate (250 mg, 1.02 mmol) (250 mg, 0.795 mmol for *O*-geranyl methyl 4-coumarate) was dissolved in 10 mL of a 3:2 mixture of methanol/water and anhydrous LiOH (500 mg, 20.9 mmol) was added and the mixture stirred at room temperature for 14 h. The solvent was then removed under reduced pressure, and the residue was dissolved in water and washed with ethyl acetate. The aqueous layer was collected and acidified with dropwise addition of 5% HCl until no more precipitate was observed to appear. The mixture was extracted with ethyl acetate ensuring that the precipitate dissolved in the organic layer, which was separated and washed with brine. The organic layer was dried with anhydrous magnesium sulfate, filtered and the solvent removed under reduced pressure to yield the coumaric acid. No column chromatography was deemed necessary as judged by TLC and ^{1}H-NMR spectroscopy, except for *O*-prenyl 3-coumaric acid, which was purified on silica gel eluting with an 8:2 mixture of chloroform/methanol. The yields obtained were 96% for *O*-prenyl 4-coumaric acid, 42% for *O*-prenyl 3-coumaric acid and 70% for *O*-prenyl 2-coumaric acid. The yield for *O*-geranyl 4-coumaric acid was 94%.

***O*-Prenyl 4-coumaric acid (4a)** mp 145 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.74 (d, *J* = 16.0 Hz, 1H, H-7), 7.50 (d, *J* = 8.8 Hz, 2H, H-2,6), 6.92 (d, *J* = 8.8 Hz, 2H, H-3,5), 6.31 (d, *J* = 16.0 Hz, 1H, H-8), 5.49 (t, *J* = 6.8 Hz, 1H, H-2’), 4.55 (d, *J* = 6.8 Hz, 2H, H-1’), 1.81 (s, 3H, H-5’), 1.76 (s, 3H, H-4’); ^{13}C-NMR ($CDCl_3$) δ (ppm): 172.6 (C-9), 161.1 (C-4), 146.8 (C-7), 138.8 (C-3’), 130.1 (C-2,6), 126.6 (C-1), 119.1 (C-2’), 115.0 (C-3,5), 114.4 (C-8), 64.9 (C-1’), 25.8 (C-5’), 18.2 (C-4’); HRMS: 233.1187 (experimental M+H), 233.1178 (theoretical M+H).

***O*-Prenyl 3-coumaric acid (4b)** mp 78 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.75 (d, *J* = 16.0 Hz, 1H, H-7), 7.31 (t, *J* = 7.9 Hz, 1H, H-5), 7.14 (d, *J* = 7.7 Hz, 1H, H-6), 7.09 (t, *J* = 1.9 Hz, 1H, H-2), 6.98 (dd, *J* = 8.2, 2.4 Hz, 1H, H-4), 6.43 (d, *J* = 16.0 Hz, 1H, H-8), 5.50 (t, *J* = 6.8 Hz, 1H, H-2’), 4.54 (d, *J* = 6.7 Hz, 2H, H-1’), 1.81 (s, 3H, H-5’), 1.77 (s, 3H, H-4’); ^{13}C-NMR ($CDCl_3$) δ (ppm): 171.7 (C-9), 159.2 (C-3), 147.1 (C-7), 138.7 (C-3’), 135.3 (C-5), 129.9 (C-6), 121.0 (C-2), 119.2 (C-2’), 117.4 (C-4), 117.3 (C-1), 113.8 (C-8), 64.8 (C-1’), 25.8 (C-5’), 18.2 (C-4’); HRMS: 233.1189 (experimental M+H), 233.1178 (theoretical M+H).

***O*-Prenyl 2-coumaric acid (4c)** mp 111 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 8.12 (d, *J* = 16.1 Hz, 1H, H-7), 7.54 (dd, *J* = 7.7, 1.6 Hz, 1H, H-6), 7.35 (ddd, *J* = 8.5, 7.4, 1.7 Hz, 1H, H-4), 6.96 (ddd, *J* = 7.6, 7.5, 0.8 Hz, 1H, H-5), 6.93 (d, *J* = 8.3 Hz, 1H, H-3), 6.55 (d, *J* = 16.1 Hz, 1H, H-8), 5.51 (t, *J* = 6.6 Hz, 1H, H-2’), 4.61 (t, *J* = 6.6 Hz, 2H, H-1’) 1.80 (s, 3H, H-5’), 1.75 (s, 3H, H-4’); ^{13}C-NMR ($CDCl_3$) δ (ppm): 173.0 (C-9), 157.8 (C-2), 142.7 (C-7), 138.0 (C-3’), 131.8 (C-4), 129.2 (C-6), 123.3 (C-1), 120.6 (C-5), 119.4 (C-2’), 117.5 (C-8), 112.5 (C-3), 65.4 (C-1’), 25.8 (C-5’), 18.3 (C-4’); HRMS: 255.1009 (experimental M+Na), 255.0997 (theoretical M+Na).

***O*-Geranyl 4-coumaric acid (6b)** mp 113 °C; ^{1}H-NMR ($CDCl_3$) δ (ppm): 7.75 (d, *J* = 16.0 Hz, 1H, H-7), 7.50 (d, *J* = 8.8 Hz, 2H, H-2,6), 6.92 (d, *J* = 8.8 Hz, 2H, H-3,5), 6.31 (d, *J* = 16.0 Hz, 1H, H-8), 5.48 (t, *J* = 7.2 Hz, 1H, H-2’), 5.09 (t, *J* = 6.8 Hz, 1H, H-7’), 4.58 (d, *J* = 6.5 Hz, 2H, H-1’), 2.15-2.07 (m, 4H, H-5’, H-6’), 1.74 (s, 3H, H-4’), 1.67 (s, 3H, H-10’), 1.60 (s, 3H, H-9’); ^{13}C-NMR ($CDCl_3$) δ (ppm): 172.6 (C-9), 161.1 (C-4), 146.8 (C-7), 141.8 (C-3’), 131.9 (C-8’), 130.0 (C-2,6), 126.6 (C-1), 123.6 (C-7’), 118.9 (C-2’), 115.1 (C-8), 114.4 (C-3,5), 65.0 (C-1’), 39.5 (C-5’), 26.2 (C-6’), 25.7 (C-9’), 17.7 (C-10’), 16.7 (C-4’); HRMS: 301.1804 (experimental M+H) 301.1804 (theoretical M+H).

Antitubercular activity

Minimum inhibitory concentrations (MIC) were determined using the spot culture growth inhibition (SPOTi) assay as described previously.[3,4] Briefly, the compounds were dissolved either in sterile DMSO or water at a concentration of 100 g/L, and dilutions prepared at 80, 60, 40, and 20 g/L. In 24 well plates, 2 µL of the dilutions were dispensed into the wells, and 2 mL of molten Middlebrook 7H10 media supplemented with 0.25% glycerol and 10% OADC (M7H10). For the 96 well plates, the concentrated stock was serially diluted and then 2 µL of the dilutions was dispensed into the wells and 200 µL of M7H10 added. A mid-exponential phase liquid culture of *M. bovis* BCG Pasteur or *M. tuberculosis* $H_{37}Rv$ was diluted and 2 µL (around 500-1000 cells) was dispensed into the middle of each well. The plates were incubated for two weeks at 37 °C in sealed plastic bags and then observed. The MIC was determined as the minimum concentration where no bacterial growth was observed.

RAW264.7 cell toxicity

Cell toxicity was assessed using the mouse macrophage cell line (RAW 264.7) as described previously.[5] Macrophages were grown in 5 mL of RPMI-1640 complete medium (RPMI-1640 medium supplemented with 2 mM L-glutamine and 10% heat-inactivated fetal bovine serum) in 25 cm^2 tissue culture flasks at 37 °C in a humidified, 5% CO_2 incubator. When grown to a confluence of approximately 80%, the cell monolayer was first washed twice with PBS, followed by gentle removal of the supernatant and addition of 5 mL of a cell-lifter mixture (10 mM lidocaine HCl, 10 mM EDTA in PBS). The flask was then incubated at room temperature for 10 min. The collected cells were diluted with an equal volume of complete medium prior to centrifugation at 1000 g for 5 min, and then resuspended in a calculated volume of fresh medium to yield a suspension of 5×10^5 cells/mL. The number of viable cells and the required volume of the medium were determined using a Trypan Blue assay. To perform the cytotoxicity assay, 2 µL of each compound (200 g/L stock solution) was added into 200 µL of RPMI-1640 complete medium in a 96-well cell culture flat-bottom plate. Each

compound was then serially diluted 2-fold in the medium. 100 μL of the prepared cell suspension was subsequently added into each well. The solvent of each compound was used as its control. After 48 h of incubation, the macrophages were washed twice with PBS to remove the compound, and further incubated with fresh medium containing 30 μL of 0.01% resazurin solution at 37 ^{o}C for 24 h. The fluorescence intensity (FI) was measured at λ_{exc}560/λ_{emi}590 nm using a FLUOStar Labtech spectrofluorometer. Finally the half growth inhibitory concentration (GIC_{50}) was determined by considering the control fluorescence as 100% growth.

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