Antimycobacterial 1,4-napthoquinone natural products from *Moneses uniflora*

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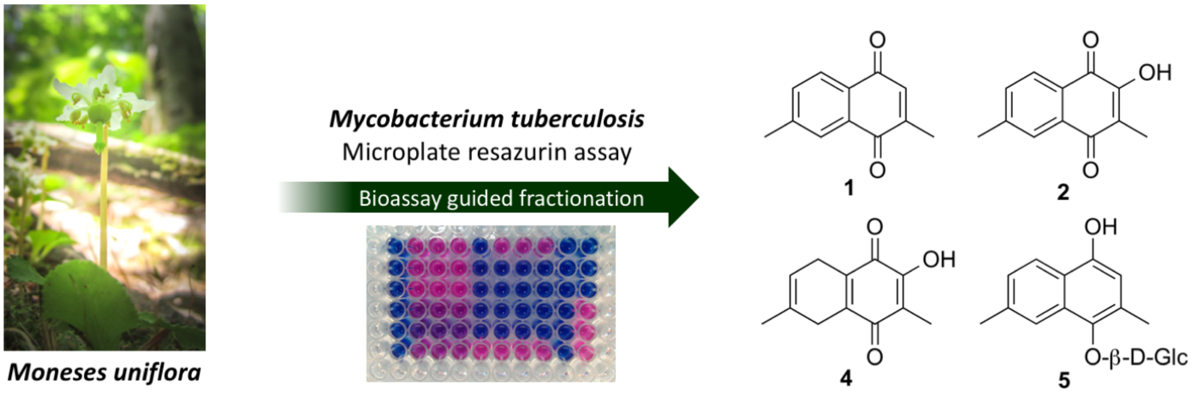
Graphic Abstract



Abstract

A new 1,4-naphthoquinone derivative, 5,8-dihydro-3-hydroxychimaphilin (**4**) and five known compounds (**1**, **2** and **5**–**7**) were isolated from an extract of the Canadian medicinal plant *Moneses uniflora* that significantly inhibited the growth of *Mycobacterium tuberculosis* H37Ra. The structure of **4** was established through analysis of NMR and MS data and the absolute configuration of the glycone of **5** was determined by chemical transformation and comparison with standards prepared from D- and L-glucose. All compounds isolated were screened against *Mycobacterium tuberculosis* (H37Ra) and the mammalian HEK 293 cell line and, with the exception of compounds **5** and **7**, exhibited marked selectivity in their bioactivity: Compound **1** exhibited potent antimycobacterial activity (IC_{50} of 5.4 μM) and moderate cytotoxicity (IC_{50} of 30 μM); compounds **2**, **4** and **6** showed moderate antimycobacterial activity (IC_{50} values from 28 – 47 μM) without affecting the viability of mammalian cells; compound **5** displayed moderate activity in both assays (IC_{50} values of 44 and 55 μM respectively); and compound **7** was not active in either assay. These data suggest that the *Moneses* napthaquinone derivatives elicit biological responses in mycobacterial and mammalian cells through disparate modes of action that warrant further investigation.

Keywords

Antimycobacterial activity; Medicinal plant; *Moneses uniflora*; *Mycobacterium tuberculosis*; Naphthoquinone; Naphthohydroquinone glycoside

1. Introduction

Moneses uniflora (L.) Gray (Ericaceae), commonly known as the one-flowered wintergreen, single delight or wood nymph, is a small (3 – 10 cm high) perennial herb with a circumboreal distribution across the northern hemisphere (Freeman, 2009). It is common in cool, moist coniferous woods across Canada (Freeman, 2009; Hinds, 2000) and has been used by the First Nations peoples for a variety of medicinal purposes that include the treatment of tuberculosis (MacKinnon et al., 2009) and symptoms associated with the disease (Moerman, 1998, 2009). Indeed, Towers and co-workers reported that an extract of *M. uniflora* collected from the Haida Gwaii archipelago off the north coast of British Columbia, Canada, showed antimycobacterial activity when tested at high titre in a disk diffusion screening assay against *Mycobacterium tuberculosis* and *Mycobacterium avium* (20 mg per disk; McCutcheon et al. 1997); although the extract and three antibiotic naphthoquinone constituents, chimaphilin, 3-hydroxychimaphilin and 8-chlorochimaphilin (**1** – **3**), did not display activity against the same *Mycobacterium* spp. when tested at lower amounts (100 µg per disk; Saxena et al. 1996). In our hands, however, a methanolic extract of *M. uniflora* collected on the east coast of Canada in New Brunswick showed significant activity against *M. tuberculosis* H37Ra in a microplate-based screening assay (mean growth inhibition ± SD = 99.6 ± 0.8 % at 100 µgmL^{-1}; O'Neill et al. 2014) and prompted us to further investigate this plant. Bioassay guided fractionation of the extract led to the isolation of a new 1,4-naphthoquinone derivative, 5,8-dihydro-3-hydroxychimaphilin (**4**), three known 1,4-naphthoquinone derivatives (**1**, **2** and **5**) and two coumarins (**6** and **7**).

2. Results and Discussion

Compound **4** was isolated as orange needles that gave a sodiated molecular (M + Na^+) ion at *m/z* 227.0679, (calcd for $C_{12}H_{12}O_3Na^+$: 227.0684) in the positive ion HRESIMS, consistent with a molecular formula of $C_{12}H_{12}O_3$ and implying seven degrees of unsaturation. The ^{13}C NMR spectrum showed 12 resonances (Table 1) that were indicative of a dimethylated naphthoquinone scaffold (Lee et al., 2001; Saxena et al., 1996). The presence of two methylenes [δ 24.6 (C-5, t) and 3.01 (H_2-5, m) and 29.2 (C-8, t) and 2.99 (H_2-8, m) respectively] and a phenol [δ 150.9 (C-3, s) and 6.91 (3-OH, bs)] suggested that **4** was a hydroxylated analogue of 5,8-dihydrochimaphilin (**8**; Lee et al. 2001). HMBC correlations observed for 3-OH (to C-2, C-3 and C-4), 2-Me (to C-1, C-2 and C-3), H_2-5 (to C-4a, C-6, C-7, and C-8a) and H_2-8 (to C-4a, C-5, C-6, C-7, and C-8a) together with the H_2-5–H-6–7-Me–H_2-8 spin system revealed by the COSY spectrum allowed **4** to be identified as 5,8-dihydro-3-hydroxychimaphilin.

Compound **5** was isolated as an optically active ($[\alpha]^{25}_{D}$= –9.2) amorphous solid that was determined to have the molecular formula $C_{18}H_{22}O_7$ from HRESIMS data (M+Na^+ ion at *m/z* 373.1258; calcd for $C_{18}H_{22}O_7Na^+$: 373.1263). The structure and absolute stereochemistry of **5** was established to be 4-hydroxy-2,7-dimethylnaphthylene-1-*O*- β-D-glucopyranoside with the planar structure being revealed through analysis of 1D and 2D NMR data (Table 2) and the configuration of the glucose moiety assigned by comparison of the specific rotation of the α-1-methoxy-2,3,4,6-*O*-acetylglucopyranoside obtained from methanolysis and acetylation of the natural product ($[\alpha]^{25}_{D}$= +68) with α-1-methoxy-2,3,4,6-*O*-acetyl derivatives prepared from D- and L-glucose ($[\alpha]^{25}_{D}$= +184 and $[\alpha]^{25}_{D}$= –175 respectively). Although **5** was first reported as a natural product of *Chimaphila umbellata* in the patent literature in 2002 (Hwang, 2002), details of its isolation did not appear until 2015 and, whilst it was assumed to be the D-glucoside, both the NMR data and the configuration of the glycone remained to be assigned (Shin et al., 2015). Compounds **1**, **2**, **6** and **7** were identified as chimaphilin (Saxena et al., 1996), 3-hydroxychimaphilin (Saxena et al., 1996), isofraxetin (Artem'eva et al., 1973a; Artem'eva et al., 1973b; Zhou et al., 2017), and umbelliferone (Gottlieb et al., 1979; Timonen et al., 2011; Zolek et al., 2003) by HRMS and comparison of their NMR data with literature values.

The antimycobacterial activity and cytotoxicity of the compounds isolated from *M. uniflora* was evaluated *in vitro* against *M. tuberculosis* (H37Ra) and immortalized human embryonic kidney (HEK 293) cells (Table 3). With the exception of umbelliferone, all of the compounds inhibited the growth of *M. tuberculosis*; 3-hydroxychimaphilin (**2**), 5,8-dihydro-3-hydroxychimaphilin (**4**), 4-hydroxy-2,7-dimethylnaphthylene-1-*O*-β-D-glucopyranoside (**5**) and isofraxetin (**6**) were moderately antimycobacterial whilst chimaphilin (**1**) exhibited makedly increased activity (*M. tuberculosis*: MIC 20 µM, IC_{50} 5.4 µM) comparable to that reported for the most active antimycobacterial naphthoquinone natural products (Salomon and Schmidt, 2012).

1,4-Naphthoquinones are well known to manifest widespread cytototoxic effects through the generation of reactive oxygen species, intracellular redox cycling and alkylation of glutathione as well as nucleophilic sites in nucleic acids and proteins (Klotz et al., 2014; Kumagai et al., 2012), and these largely unspecific mechanisms have been used to rationalize antimycobacterial activity of quinones and napthoquinones in the past (Tran et al., 2004). However, if the antimycobacterial activity of the *Moneses* napthoquinones was affected by the action of one or more of these general mechanisms, we would expect them to display similar profiles of bioactivity in both of our assays rather than the marked differences that we observed. Whilst all of the napthaquinones exhibited significant antimycobacterial activity, only the naphthoquinones lacking a hydroxyl substituent at C-3 (**1** and **5**) displayed any appreciable level of cytotoxicity when evaluated against the HEK 293 cell line. This structure activity relationship is in accordance with previous findings (D'Arcy et al., 1987; Inbaraj and Chignell, 2004; Klaus et al., 2010; Klotz et al., 2014), and, given that both **1** and **5** are C-2 methyl substituted, it would follow that the mechanism of cytotoxicity exhibited in the HEK 293 cells is predominantly redox-based rather than a consequence of Michael-type alkylation of nucleophilic cellular targets (Inbaraj and Chignell, 2004; Kumagai et al., 2012). The marked difference observed in our bioassay data therefore suggests that the antimycobacterial activity of the napthoquinones isolated from *M. uniflora* is being elicited through a more specific mechanism of action such as DNA gyrase inhibition (Karkare et al., 2013) that may warrant further in-depth study.

3. Experimental

3.1 General Experimental Procedures

All solvents for extraction and fractionation were purchased from Fisher Scientific (Ottawa, ON, Canada). NMR solvents were purchased from Sigma-Aldrich (Oakville, ON, Canada). Flash chromatography was performed using a Biotage Flash + chromatography system on KP-Sil 25+S silica cartridges (40−63 µm, 60 Å) and size exclusion chromatography was performed with Sephdex LH-20 (25-100 µm). Semi-preparative normal phase HPLC was performed on a Waters 510 pump, a Waters R401 refractive index detector and a Phenomenex Luna silica column (250 × 10 mm, 10u, 100Å) at a flow rate of 4.0 mL/min. Optical rotations were determined on a Rudolph autopol III polarimeter at 589 nm using a 5 cm sample cell. IR spectra were recorded on a Perkin Elmer Spectrum Two FT-IR spectrometer as thin films on a sodium chloride disk. NMR spectra were recorded on an Agilent 400-MR DD2 instrument in $CDCl_3$ or CD_3OD and were calibrated to residual protonated solvent resonances (δ_H 7.260 and 3.310; δ_C 77.160 and 49.000, respectively). HRMS was recorded on a Thermo LTQ Exactive instrument with an ESI source. Antimycobacterial susceptibility tests were performed using modified Middlebrook 7H9 broth base (BBL™ MGIT™, Becton Dickinson, Mississauga, Ontario) in non-tissue culture treated, low-binding, black 96-well microtitre plates sealed with polyester films (50 μm). Fluorometric readings (in relative fluorescence units, RFU) were recorded using a Molecular Devices Gemini EM dual-scanning microplate spectrofluorometer with a 530 nm excitation filter and a 590 nm emission filter operating in top-scan mode.

3.2 Plant Material

Whole plants of *M. uniflora* were collected by hand from Saint Léonard, New Brunswick, Canada (47°16.024' N, 67°43.874' W) in October, 2013. The whole plants were washed with water to remove debris, freeze dried and stored at –20℃. Species identification was confirmed by Dr. Stephen Clayden (New Brunswick Museum Herbarium; voucher number NBM VP-37097).

3.3 Extraction and isolation

The freeze dried *M. uniflora* (16 g) was ground to a powder and exhaustively extracted in MeOH for 8 hours using a Soxhlet apparatus to obtain a crude extract (5.5 g) that exhibited significant antimycobacterial activity and was subjected to bioassay-guided fractionation. A portion of the crude extract (4.4 g) was fractionated using a modified Kupchan solvent-solvent partition to give five fractions (Li et al., 2012). The antimycobacterial hexane, CH_2Cl_2 and EtOAc fractions were further separated to obtain pure compounds. The hexane fraction (511 mg) was subjected to silica flash column chromatography eluting from 100% hexanes to 100% EtOAc with 10% increment resulting eight fractions (H_1- H_8). A portion of fraction H_3 (46 of 93 mg) was subjected to normal phase HPLC (9:1 hexane/EtOAc) to obtain **1** (23 mg) and **2** (6 mg). The CH_2Cl_2 fraction (654 mg) was subjected to Sephadex LH-20 (1:1 CH_2Cl_2/MeOH) to give ten fractions (D_1 – D_{10}). A portion of fraction D_7 (47 of 72 mg) was subjected to normal phase HPLC (17:3 hexane/EtOAc) to obtain **4** (12 mg). A portion of fraction D_8 (30 of 85 mg) was subjected to normal phase HPLC (33: 17 hexane/EtOAc) to give **6** (5 mg) and **7** (9 mg). A portion of the EtOAc fraction (176 of 712 mg) was subjected to Sephadex LH-20 (1:1 CH_2Cl_2/MeOH) resulting ten fractions (E_1 – E_{10}). Fraction E_4 (20 mg) was subjected to reverse phase HPLC (17:33 MeOH/H_2O) to obtain **5** (11 mg).

*3.3.1 Chimaphilin (**1**).* Yellow needles; IR (thin film) ν_{max} 2956, 2924, 1667, 1599, 1298 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, *J* = 7.9 Hz, 1H, H-5), 7.87 (dq, 1.8, 0.6 Hz, 1H, H-8), 7.50 (ddq, *J* = 7.9, 1.8, 0.7 Hz, 1H, H-6), 6.79 (q, *J* = 1.5 Hz, 1H, H-3), 2.48 (bs, 3H, 7-Me), 2.17 (d, *J* = 1.5 Hz, 3H, 2-Me), consistent with literature values (Kosuge et al., 1985; Saxena et al., 1995; Saxena et al., 1996); ^{13}C NMR (100 MHz, $CDCl_3$) δ 186.0 (s, C-1), 185.1 (s, C-4), 148.0 (s, C-2), 144.8 (s,C-7), 135.8 (d, C-3), 134.5 (d, C-6), 132.2 (s, C-8a), 130.2 (s, C-4a), 127.0 (d, C-5) , 126.4 (d, C-8), 22.0 (q, 7-Me), 16.6 (q, 2-Me), data were consistent with literature values (Kagawa et al., 1992; Kosuge et al., 1985; Saxena et al., 1995; Saxena et al., 1996); ESIHRMS *m/z* 187.0755 ($M + H^+$), calcd for ($C_{12}H_{11}O_2 + H^+$), 187.0759.

3.3.2 3-Hydroxychimaphilin (2). Yellow needles; IR (thin film) ν_{max} 3364, 2919, 2850, 1698, 1646 cm^{-1}; ^{1}H NMR (CDCl$_3$) δ 7.94 (d, *J* = 7.8 Hz, 1H, H-5), 7.90 (dq, 1.7, 0.6 Hz, 1H, H-8), 7.45 ((ddq, *J* = 7.8, 1.7, 0.7 Hz, 1H, H-6), 7.35 (bs, 1H, 3-OH), 2.48 (bs, 3H, 7-Me), 2.08 (s, 3H, 2-Me), consistent with literature values (Saxena et al., 1995; Saxena et al., 1996);^{13}C NMR (100 MHz, CDCl$_3$) δ 185.4 (s, C-1), 181.0 (s, C-4) 153.3 (s, C-3), 146.4 (s, C-7), 133.6 (d, C-6), 133.0 (s, C-8a), 127.4 (d, C-8), 127.2 (s, C-4a), 126.5 (d, C-5), 120.2 (s, C-2), 22.2 (q, 7-Me) 8.8 (q, 2-Me), consistent with literature values (Saxena et al., 1995; Saxena et al., 1996); ESIHRMS *m/z* 203.0704 (M + H$^+$), calcd for ($C_{12}H_{11}O_3$ + H$^+$), 203.0708.

3.3.3 5,8-Dihydro-3-hydroxychimaphilin (4). Orange needles; IR (thin film) ν_{max} 3392, 2898, 1652, 1383, 1355 cm^{-1}; ^{1}H and ^{13}C NMR see table 1; ESIHRMS *m/z* 227.0679 (M + Na$^+$), calcd for ($C_{12}H_{12}O_3$ + Na$^+$), 227.0684.

*3.3.4 4-Hydroxy-2,7-dimethylnaphthylene-1-O-β-D-glucopyranoside (**5**).* Purple amorphous solid; $[\alpha]^{25}_{D}$= –9.2 (c 0.8, MeOH); IR (thin film) ν_{max} 3353, 2923, 1660, 1294, 1075 cm^{-1}; ^{1}H and ^{13}C NMR see table 2; ESIHRMS *m/z* 373.1258 (M + Na$^+$), calcd for ($C_{18}H_{22}O_7$ + Na$^+$), 373.1263.

*3.3.5 Isofraxetin (**6**).* White needles; IR (thin film) ν_{max} 3345, 2927, 1692, 1582, 1121 cm^{-1}; ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.61 (d, *J* = 9.5, 1H, H-4), 6.53 (s, 1H, H-8), 6.28 (d, *J* = 9.5, 1H, H-3) 3.94 (s, 3H, 7-OMe), consistent with literature values (Artem'eva et al., 1973a; Artem'eva et al., 1973b); ^{1}H NMR (400 MHz, CD$_3$OD) δ 7.83 (d, *J* = 9.4, 1H, H-4), 6.71 (s, 1H, H-8), 6.21 (d, *J* = 9.4, 1H, H-3) 3.89 (s, 3H, 7-OMe), consistent with literature values (Zhou et al., 2017);

^{13}C NMR (100 MHz, CD_3OD, assignments marked with asterisks may be interchanged) δ 163.7 (s, C-2), 147.1 (s, C-7), 146.7 (d, C-4), 140.7 (s, C-8a*), 140.6 (s, C-5*), 134.1 (s, C-6), 112.7 (d, C-3), 112.2 (s, C-4a), 101.1 (d, C-8), 56.8 (q, 7-OMe); ESIHRMS *m/z* 209.0445 (M + H^+), calcd for ($C_{10}H_9O_5$ + H^+), 209.0450.

3.3.6 Umbelliferone (7). Yellow needles; IR (thin film) ν_{max} 3171, 1687, 1610, 1131 cm^{-1};^{1}H NMR (400 MHz, CD_3OD) δ 7.84 (bd, *J* = 9.5 Hz, 1H, H-4), 7.44 (d, *J* = 8.5 Hz, 1H, H-5), 6.79 (dd, *J* = 8.5, 2.3 Hz, 1H, H-6), 6.70 (bd, *J* = 2.3 Hz, 1H, H-8), 6.18 (d, *J* = 9.5 Hz, 1H, H-3), consistent with literature values (Timonen et al., 2011); ^{13}C NMR (100 MHz, CD_3OD) δ 163.7 (s, C-2), 163.2, (s, C-7) 157.2 (s, C-8a), 146.0 (d, C-4), 130.7 (d, C-5), 114.5 (d, C-6), 113.1 (s, C-4a), 112.3 (d, C-3), 103.4 (d, C-8), consistent with literature values (Gottlieb et al., 1979; Timonen et al., 2011; Zolek et al., 2003); ESIHRMS *m/z* 163.0390 (M + H^+), calcd for ($C_9H_7O_3$ + H^+), 163.0395.

*3.4 Determination of the absolute stereochemistry of **5***

Compound **5** (7 mg) was dissolved in 10 mL of anhydrous MeOH, acetyl chloride (0.2 mL) was added and the reaction mixture was stirred under reflux for two hours. The reaction mixture was concentrated *in vacuo* and the residue partitioned between water and EtOAc. The aqueous fraction was lyophilized to give the methyl glucoside (4 mg) that was stirred overnight with acetic anhydride (3 mL) and DMAP (1 mg) in anhydrous pyridine (9 mL) at room temperature. EtOAc (30 mL) was added to the reaction mixture before it was washed with 1M HCl (3 × 40 mL), saturated sodium bicarbonate solution (3 × 40 mL) and distilled H_2O (3 × 40 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give an 8:3 mixture (by ^{1}H NMR) of the peracetylated α-and β-methylglucosides [anomeric proton resonances at δ 4.95 (d, 3.7 Hz) and 4.43 (d, 7.9 Hz) respectively]. Normal phase HPLC (7:3 hexane/EtOAc) of the anomers gave α-1-methoxy-2,3,4,6-*O*-acetylglucose (4 mg) and β-1-methoxy-2,3,4,6-*O*-acetylglucose (1 mg) that afforded ^{1}H NMR data identical to literature values (Grayson et al., 2005; van Well et al., 2005). Commercial D-glucose and L-glucose (20 mg each) were treated in the same manner to give α-D-1-methoxy-2,3,4,6-*O*-acetylglucose, β-D-1-methoxy-2,3,4,6-*O*-acetylglucose, α-L-1-methoxy-2,3,4,6-*O*-acetylglucose and β-L-1-methoxy-2,3,4,6-*O*-acetylglucose. The specific rotation obtained for the α-1-methoxy-2,3,4,6-*O*-acetylglucose obtained from **5** {$[\alpha]^{25}_{D}$= +68 (*c* 0.37, $CHCl_3$)} was compared to those obtained for the peracetylated α-methylglucosides prepared from D- and L-glucose {$[\alpha]^{25}_{D}$= +184 (*c* 0.95, $CHCl_3$) and $[\alpha]^{25}_{D}$= –175 (*c* 1.00, $CHCl_3$) respectively} and confirmed the D-configuration of the glucose moiety in **5**.

3.4.1 α -1-Methoxy-2,3,4,6-*O*-acetylglucose. ^{1}H NMR ($CDCl_3$, assignments marked with asterisks or daggers may be interchanged): δ 5.48 (dd, *J* = 10.2, 9.5 Hz, 1H, H-3*), 5.07 (dd, *J* = 10.2, 9.5 Hz, 1H, H-4*), 4.95 (d, *J* = 3.7 Hz, 1H, H-1), 4.90 (dd, *J* = 10.2, 3.7 Hz, 1H, H-2), 4.26 (dd, *J* = 12.3, 4.7 Hz, 1H, H-6a), 4.11 (dd, 1H, *J* = 12.3, 2.2 Hz, 1H, H-6b), 3.99 (ddd, *J* = 10.2, 4.7, 2.2 Hz, 1H, H-5), 3.41 (s, 3H, 1-OMe), 2.10 (s, 3H, 3-OAc†), 2.08 (s, 3H, 4-OAc†), 2.03 (s, 3H, 2-OAc†), 2.01 (s, 3H, 6-OAc†).

3.4.2 β -1-Methoxy-2,3,4,6-*O*-acetylglucose. ^{1}H NMR ($CDCl_3$, assignments marked with asterisks may be interchanged): δ 5.21 (t, *J* = 9.5 Hz, 1H, H-3), 5.10 (dd, *J* = 10.0, 9.5 Hz, 1H, H-4), 4.99 (dd, *J* = 9.5, 7.9 Hz, 1H, H-2), 4.43 (d, *J* = 7.9 Hz, 1H, H-1), 4.28 (dd, *J* = 11.9, 4.6 Hz, 1H, H-6a), 4.15 (dd, 1H, *J* = 11.9, 2.5 Hz, 1H, H-6b), 3.70 (ddd, *J* = 10.0, 4.6, 2.5 Hz, 1H, H-5), 3.51 (s, 3H, 1-OMe), 2.09 (s, 3H, 3-OAc*), 2.05 (s, 3H, 4-OAc*), 2.03 (s, 3H, 2-OAc*), 2.01 (s, 3H, 6-OAc*).

3.5 Biological Assays

Antimycobacterial activity against *M. tuberculosis* H37Ra (ATCC 25177) was evaluated using the microplate resazurin assay, as previously described (O'Neill et al., 2014). Cytotoxicity was evaluated against HEK 293 cells (ATCC CRL-1573) using the CellTiter-Blue cell viability assay, as previously described (Carpenter et al., 2012). All assays were run in triplicate. The MIC of a compound was considered to be the lowest concentration at which it inhibited mycobacterial growth by more than a mean value of 90% (Collins and Franzblau, 1997). Absolute median inhibitory concentrations (IC_{50}s) were estimated by four-parameter logistic (4PL) regression (Sebaugh, 2011) using GraphPad Prism (version 7.0c).

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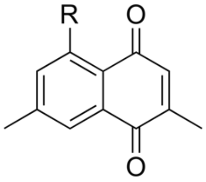
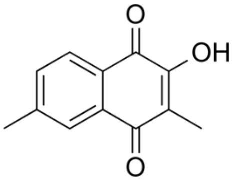


1: R = H
3: R = Cl



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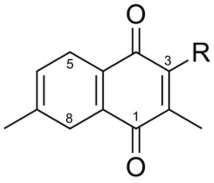
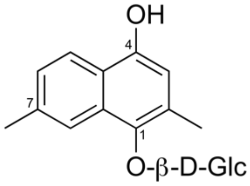
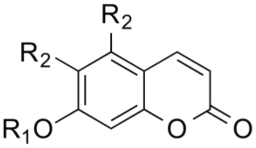


4: R = OH
8: R = H



5



6: R_1 = CH_3; R_2 = OH
7: R_1= R_2 = H

Table 1. NMR Spectroscopic data of 5,8-dihydro-3-hydroxychimaphilin (4)[a]

Position	δ_C, mult.	δ_H (int., mult, *J* in Hz)	HMBC	COSY
1	187.6, s			
2	116.9, s			
3	150.9, s			
4	183.0, s			
5	24.6, t	3.01 (1H, m) 3.06 (1H, m)	4a, 6, 7, 8a	6
6	116.6, d	5.48 (1H, m)		5, 8, 7-Me
7	130.5, s			
8	29.2, t	2.99 (1H, m) 3.04 (1H, m)	4a, 5[b], 6, 7, 8a	6, 7-Me
8a	141.9, s			
4a	135.1, s			
2-Me	8.2, q	1.94 (3H, s)	1, 2, 3	
7-Me	23.1, q	1.77 (3H, m)	6, 7, 8	6, 8
3-OH		6.91 (1H, s)	1[b], 2, 3, 4	

[a] Recorded in $CDCl_3$ at 400 MHz for ^1H and 100 MHz for ^{13}C.

[b] Weak correlation

Table 2. NMR spectroscopic data of 4-Hydroxy-2,7-dimethylnaphthylene-1-*O*-β-D-glucopyranoside (5)[a]

Position	δ_C, mult.	δ_H (int., mult, *J* in Hz)	HMBC	COSY
1	143.5, s			
2	129.5, s			
3	110.7, d	6.55 (1H, s)	1, 4, 8a, 2-Me	2-Me
4	151.0, s			
5	123.2, d	7.98 (1H, d, 8.5)	4, 4a, 7	6
6	126.9, d	7.18 (1H, dd, 8.5, 1.6)	5, 8, 8a, 7-Me	5, 8, 7-Me
7	136.7, s			
8	122.4, d	8.20 (1H, bs)	1, 6, 7-Me, 8a	6, 7-Me
8a	124.1, s			
4a	130.5, s			
1'	106.4 d	4.74 (1H, d, 7.8)	1, 3'	2'
2'	75.9, d	3.64 (1H, m)	1', 3'	1', 3', 5'
3'	78.1, d	3.46 (1H, m)	2', 4'	2'
4'	71.8, d	3.43 (1H, m)	3', 4'	5'
5'	77.8, d	3.09 (1H, ddd, 9.3, 5.2, 2.5)		4', 6'
6'	62.9, t	3.65 (1H, d, 11.7, 5.2) 3.75 (1H, dd, 11.7, 2.5)	4', 5'	5'
2-Me	17.8, q	2.44 (3H, s)	1, 2, 3	
7-Me	22.0, q	2.48 (3H, bs)	6, 7, 8	6, 8

[a] Recorded in CD_3OD at 400 MHz for ^1H and 100 MHz for ^{13}C.

Table 3. Biological activities (MIC and IC$_{50}$ values in µM [µg/mL]) of 1, 2 and 4 – 7

Compounds	*Mycobacterium tuberculosis* H37Ra		HEK 293 cells
	MIC	IC$_{50}$ (95% CI)[a]	IC$_{50}$ (95% CI)
1	20	5.44 (5.08 – 5.800)	30.2 (26.1 – 35.0)
	[3.125]	[1.01 (0.95 – 1.080]	[5.63 (4.85 – 6.52)]
2	125	47.3 (42.1 – 53.0)	>100
	[25]	[9.56 (8.52 – 10.7)]	[>50]
4	125	28.2 (27.1 – 29.3)	>100
	[25]	[5.75 (5.53 – 5.98)]	[>50]
5	300	43.5 (40.4 – 46.8)	54.7 (41.2 – 71.58)
	[100]	[15.2 (14.2 – 16.4)]	[19.1 (14.4 – 25.1)]
6	250	32.8 (29.6 – 36.3)	>100
	[50]	[6.83 (6.16 – 7.56)]	[>50]
7	>1000	>100	>100
	[>200]	[>50]	[>50]
Rif[b]	6.25×10^{-2}	5.24×10^{-3} (4.74×10^{-3}– 5.80×10^{-3})	ND[c]
	[5.00×10^{-2}]	[4.31×10^{-3} (3.90×10^{-3}– 4.77×10^{-3})]	
DPT[b]	ND	ND	38.4 (21.2 – 69.9)
			[15.3 (8.45 – 27.6)]

[a] CI = confidence interval.

[b] Positive control: Rif = rifampin; DPT = deoxypodophyllotoxin.

[c] ND = Not determined.

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