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Phenolic derivatives from *Wigandia urens* with weak activity against the chemokine receptor CCR5

Shugeng Cao, Christine Rossant, Siewbee Ng, Antony D. Buss, Mark S. Butler*

MerLion Pharmaceuticals, 59A Science Park Drive, The Fleming, Singapore Science Park, Singapore 118240

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Abstract

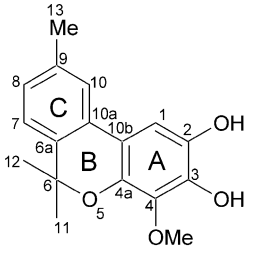
Three compounds, 2,3-dihydroxy-4-methoxy-6,6,9-trimethyl-6H-dibenzo[b,d]pyran (**1**), 8-methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-6-ol (**2**) and 4-methoxy-3-(3-methyl-2-butenyl)-benzoic acid (**3**), have been isolated from *Wigandia urens*. The structures of compounds **1**, **2** and **3** were determined from spectroscopic data and showed activity in a CCR5 assay with IC_{50} values of 33, 46 and 26 μM respectively.

Keywords: *Wigandia urens*; Hydrophyllaceae; Phenolic derivatives; Benzopyran; 2,3-Dihydroxy-4-methoxy-6,6,9-trimethyl-6H-dibenzo[b,d]pyran; 8-Methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-6-ol; 4-Methoxy-3-(3-methyl-2-butenyl)-benzoic acid; CCR5

1. Introduction

Chemokines are small proteins (7–16 kDa) that act through G protein-coupled receptors to regulate a variety of physiological and pathophysiological processes (Kedzierska et al., 2003; Lusso, 2000; Berger et al., 1999). The human immunodeficiency virus Type 1 (HIV-1) uses chemokine receptors (principally CCR5 and CXCR4) as co-receptors with CD4 to gain entry into target cells (Lehner, 2002; Kazmierski et al., 2002). As a consequence, a molecule that binds to the CCR5 receptor could potentially prevent HIV-1 entry into cells, making CCR5 an important target for HIV-1 therapy (De Clerq, 2002; Schwarz et al. 2001).

Wigandia urens (Ruíz & Pav.) HBK (Hydrophyllaceae) is a perennial that grows to a height of 3.0–3.6 m (Gonzalez-Zertuche et al., 2001). A previous study has shown *W. urens* to be a rich source of flavonoids (Wollenweber et al., 1986). An extract derived from the stems of a specimen of *W. urens* collected in Singapore was found to compete effectively with macrophage inflammatory protein (MIP)-1α for binding to human CCR5 using a scintillation proximity assay (SPA). Bioassay guided fractionation using the SPA led to the isolation of two new compounds, **1** and **2**, together with **3**, with IC_{50} values of 33, 46 and 26 μM respectively. Although **3** has been previously synthesised (Folkers and Woodruff, 1963), this is the first report of its isolation as a natural product.



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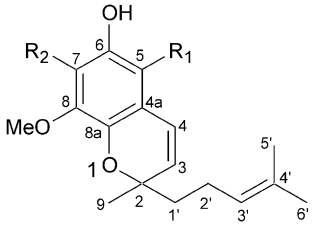
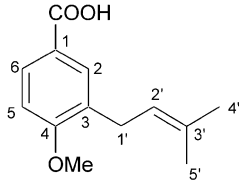


2 $R_1 = R_2 = H$
4 $R_1 = Me$; $R_2 = OMe$



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* Corresponding author. Tel.: +65-6871-9703; fax: +65-6773-7072.
E-mail address: mark@merlionpharma.com (M.S. Butler).

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2. Results and discussion

Compound **1** was obtained as an oil with a molecular formula of $C_{17}H_{18}O_4$ based on (+)-HR ESI mass spectrometry. The ^{1}H NMR spectrum of **1** (Table 1) in DMSO-d_6 showed one aromatic singlet (δ_H 6.91, *s*), three methyl singlets (δ_H 2.31, *s* (3H); 1.52, *s* (6H)), and one methoxy singlet (δ_H 3.73, *s*). In addition, three mutually coupled aromatic protons at δ_H 7.32 (*d*, J = 1.8 Hz), 7.15 (*d*, J = 7.6 Hz) and 7.02 (*dd*, J = 1.8, 7.6 Hz) were assigned to a 1,2,4-trisubstituted aromatic ring system. In the HMBC spectrum of **1** (Fig. 1), H-13 (3H, δ_H 2.31, s) correlated with C-10 (δ_C 121.6), C-9 (δ_C 136.7) and C-8 (δ_C 127.4), while H-8 (δ_H 7.02, *dd*, J = 1.8, 7.6 Hz), H-10 (δ_H 7.32, *d*, J = 1.8 Hz), H_3-11 and H_3-12 (δ_H 1.52, s, 6H) showed HMBC correlations with C-6a (δ_C 135.4). The oxygenated carbon at C-6 (δ_C 76.8) correlated to H-7 (δ_H 7.15, *d*, J = 7.6 Hz) and the *gem*-dimethyl resonances H_3-11 and H_3-12 (δ_H 1.52, *s*), which indicated that C-6 was *para* to the methyl group at C-9. In addition, H-1 (δ_H 6.91, *s*) showed HMBC correlations to not only C-2 (δ_C 138.8), C-10b (δ_C 112.9), C-3 (δ_C 139.6) and C-4a (δ_C 140.4) but also to C-10a (δ_C 128.4), which was correlated with H-7 (δ_H 7.15, *d*, J = 7.6 Hz). This indicated that rings A and C were linked through C-10a and C-10b. The quaternary carbon at C-4 only displayed a single HMBC correlation with the methoxy group (δ_H 3.73, *s*). Selective refocusing of 4-OMe in a 1D NOESY experiment did not show any enhancements, which suggested that the methoxy group was *para* to H-1 (δ_H 6.91, *s*). According to the molecular formula ($C_{17}H_{18}O_4$) of **1**, the substituents at C-2 and C-3 must be hydroxyl groups, and ring B was derived from a C-4aOC-6 linkage. Therefore, the structure of **1**, 2,3-dihydroxy-4-methoxy-6,6,9-trimethyl-6H-dibenzo[b,d]-pyran, was established as shown.

Compound **2**, also identified as a benzopyran, had a pseudomolecular ion $[M-H]^-$ at *m*/z 273.1488 (calc. 273.1491) in a (−)-HR-ESIMS compatible with a molecular formula of $C_{17}H_{22}O_3$. The gross features of its NMR spectra (Table 1) indicated a close structural relationship between **2** and ubichromenol (**4**) (Mukai et al., 1989). The only differences in the ^{1}H NMR spectrum between **2** and **4** were the presence of two *meta*-coupled aromatic protons at δ_H 6.05 (*d*, J = 2.4 Hz, H-5) and 6.27 (*d*, J = 2.4 Hz, H-7) in **2**, instead of a methyl and a methoxy at C-5 and C-7 in **4**. Combined 2D NMR (Fig. 1) experiments defined the structure of **2** as 8-methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-6-ol. The stereochemistry of C-2 in **2** remained undetermined.

Compound **3** ($C_{13}H_{16}O_3$) gave rise to a pseudomolecular ion $[M-H]^-$ at *m*/z 219.1020 (calc. 219.1021). The ^{1}H NMR spectrum ($CDCl_3$) displayed a single methoxy signal at δ_H 3.88, a group of signals for a 1,3,4-trisubstituted aromatic system at δ_H 6.85 (*d*, J = 8.6), 7.83 (*d*, J = 2.2) and 7.93 (*dd*, J = 2.2, 8.6). In addition, the signals at δ_H 5.28 (1H, *m*), 3.30 (2H, d, J = 7.1 Hz), 1.69 (3H, *s*) and 1.73 (3H, *s*) indicated the presence of a 3-methyl-2-butenyl group. A ^{13}C NMR resonance at δ_C 167.5 was indicative of the presence of a carboxyl group. The placement of the carboxyl, methoxy and 3-methyl-2-butenyl groups at C-1, C-4 and C-3 was confirmed by analysis of the HMBC spectrum which showed the following important correlations: 4-OMe/C-4; H-1′/C-4, C-3, C-2, C-2′ and C-3′; H-2 and H-6/1-COOH.

Table 1
NMR spectral data[a] of **1** and **2** in DMSO-d_6

	1		**2**	
Position	^{13}C	^{1}H[b] (mult. J = Hz)	^{13}C	^{1}H[b] (mult. J = Hz)
1	104.0	6.91 (s)		
2	138.8[c]		77.1	
3	139.6[c]		130.8	5.67 (*d*, 9.7)
4	137.7		122.8	6.28 (*d*, 9.7)
4a	140.4[c]		121.6	
5			104.1	6.05 (*d*, 2.4)
6	76.8		150.8	
6a	135.4			
7	123.1	7.15 (*d*, 7.6)	100.9	6.27 (*d*, 2.4)
8	127.4	7.02 (*dd*, 1.8, 7.6)	148.2	
8a			135.0[d]	
9	136.7		25.4	1.26 (3H, s)
10	121.6	7.32 (*d*, 1.8)		
10a	128.4			
10b	112.9			
11	27.0	1.52 (3H, s)		
12	27.0	1.52 (3H, s)		
13	20.8	2.31 (3H, s)		
1′			39.0	1.56 (2H, m)
2′			22.2	1.99 (2H, m)
3′			124.1	5.06 (*dd*, 6.2, 6.7)
4′			130.8	
5′			25.4	1.60 (3H, s)
6′			16.2	1.50 (3H, s)
OMe	60.2	3.73 (3H, s)	54.9	3.67 (3H, s)

[a] Assignments based on COSY, multiplicity-edited HSQC and HMBC experiments.
[b] One proton unless otherwise stated.
[c] Interchangeable.
[d] From HMBC.

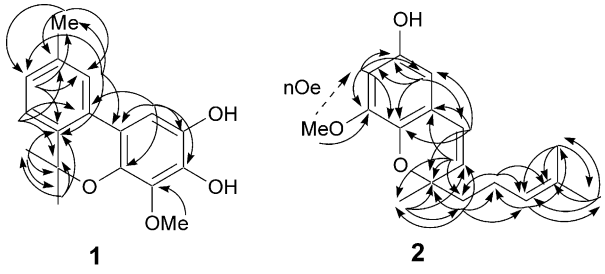

Fig. 1. HMBC correlations of compounds **1** and **2**.

Thus, the structure of **3** was determined as 4-methoxy-3-(3-methyl-2-butenyl)-benzoic acid, a new natural product, which has been previously synthesized (Folkers and Woodruff, 1963).

When tested in the CCR5 assay, compounds **1**, **2** and **3** were active with IC_{50} values of 33, 46 and 26 μM, respectively, which did not warrant further investigation.

3. Experimental

3.1. General procedures

HPLC was performed on Gilson systems complete with Gilson Unipoint v1.90 software, Gilson 170 diode array detector, Gilson 306 pumps and 811c dynamic mixer, Gilson 231 XL sampling injector and Gilson 202 fraction collector. Optical rotations were measured on a Jasco DIP-1000 Digital Polarimeter. UV spectra were measured on an Ultraspec 2000 UV/Visible spectrophotometer, IR spectra were recorded on a Perkin Elmer BioRad FT-IR spectrophotometer. One- and two-dimensional NMR experiments were obtained on a Bruker Avance DRX 500 spectrometer operating at 500 MHz for ^1H and 125 MHz for ^{13}C and spectra referenced to residual protons in the deuterated solvent. High resolution mass spectral data were recorded on a Perspective Biosystems Mariner Biospectrometrey TOF mass spectrometer.

3.2. Plant material

Plant material of *W. urens* (Ruíz & Pav.) HBK (Hydrophyllaceae) was collected in Upper Pierce Reservoir Park of Singapore in September 1994. A voucher specimen of the plant (W30235) has been deposited in the MerLion Pharmaceuticals collection.

3.3. Isolation procedures

Dried stems (50 g) were powdered, extracted with CH_2Cl_2:MeOH (1:1) (500 ml × 2), and the extract concentrated in vacuo. The crude extract (1.0 g) was partitioned using modified Kupchan conditions (Pettit et al., 1985). The localised active CH_2Cl_2 extract (280 mg) was applied to a reverse phase HPLC (12 ml/min; eluted with 50% CH_3CN/H_2O with 0.1% formic acid for 20 min, followed by gradient elution to 100% CH_3CN over 20 min; 150 × 21.2 mm 5 μ HyPURITY Elite C18) to yield 2,3-dihydroxy-4-methoxy-6,6,9-trimethyl-6H-dibenzo[b,d]pyran (**1**, 4 mg, RT 11 min, yield 0.008%), 8-methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-6-ol (**2**, 3 mg, RT 27 min, yield 0.006%) and 4-methoxy-3-(3-methyl-2-butenyl)-benzoic acid (**3**, 5 mg, RT 15 min, yield 0.01%).

3.3.1. 2,3-Dihydroxy-4-methoxy-6,6,9-trimethyl-6H-dibenzo[b,d]pyran (1)

Oil. UV λ_{max} (EtOH) nm (log ϵ): 201 (4.31), 283 (3.69), 327 (3.53); IR (KBr) ν_{max} cm^{-1}: 3459 (br), 2930, 2829, 1460, 1259, 1084; ^1H NMR (500 MHz, DMSO-d_6, Table 1); ^{13}C NMR (125 MHz, DMSO-d_6, Table 1); positive ESIMS m/z: 287 $[M+H]^-$, 255 $[M\text{-}OCH_3]$; negative HRESIMS m/z: 285.1140 (calcd. for $C_{17}H_{17}O_4$, 285.1127, $[M\text{-}H]^-$).

3.3.2. 8-Methoxy-2-methyl-2-(4-methyl-3-pentenyl)-2H-1-benzopyran-6-ol (2)

Oil. $[\alpha]_D^{25}$ −26° (EtOH; *c* 0.026); UV λ_{max} (EtOH) nm (log ϵ): 201 (4.17), 233 (sh) (3.93), 274 (3.63), 335 (3.29); IR (KBr) ν_{max} cm^{-1}: 3435, 2927, 2835, 1593, 1462, 1257; ^1H NMR (500 MHz, DMSO-d_6, Table 1); ^{13}C NMR (125 MHz, DMSO-d_6, Table 1); positive ESIMS m/z: 275 $[M+H]^-$, 219 $[M\text{-}C_4H_7]$; negative HRESIMS m/z: 273.1488 (calcd. for $C_{17}H_{21}O_3$, 273.1491 $[M\text{–}H]^-$).

3.3.3. 4-Methoxy-3-(3-methyl-2-butenyl)-benzoic acid (3)

Oil. UV λ_{max} (EtOH) nm (log ϵ): 211 (3.91), 249 (sh) (3.42), 284 (3.32), 348 (3.14); IR (KBr) ν_{max} cm^{-1}: 3365, 2942, 2835, 1023; ^1H NMR (500 MHz, $CDCl_3$) δ: 1.69 (3H, *s*, H-5′), 1.73 (3H, *s*, H-4′), 3.30 (2H, *d*, $J=7.1$ Hz, H-1′), 3.88 (3H, *s*, OMe), 5.28 (1H, *m*, H-2′), 6.85 (1H, *d*, $J=8.6$ Hz, H-5), 7.83 (1H, *d*, $J=2.2$ Hz, H-2), 7.93 (1H, *dd*, $J=2.2$, 8.6 Hz, H-6); ^1H NMR (500 MHz, DMSO-d_6) δ: 1.62 (3H, *s*, H-5′), 1.67 (3H, *s*, H-4′), 3.22 (2H, *d*, $J=7.1$ Hz, H-1′), 3.81 (3H, *s*, OMe), 5.19 (1H, *m*, H-2′), 7.01 (1H, *d*, $J=8.8$ Hz, H-5), 7.66 (1H, *d*, $J=1.8$ Hz, H-2), 7.77 (1H, *dd*, $J=1.8$, 8.8 Hz, H-6); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 17.1 (q, C-5′), 25.2 (*q*, C-4′), 27.2 (*t*, C-1′), 55.0 (q, OMe), 109.5 (*d*, C-5), 121.0 (*d*, C-2′), 122.4 (*s*, C-1), 128.4 (*d*, C-6), 129.0 (*s*, C-3), 129.5 (*d*, C-2), 132.5 (*s*, C-3′), 161.8 (*s*, C-4), 167.5 (*s*, COOH); positive ESIMS m/z: 221 $[M+H]^-$, 203 [M-OH]; negative HRESIMS m/z: 219.1020 (calcd. for $C_{13}H_{15}O_3$, 219.1021, $[M\text{-}H]^-$).

3.4. Biological assays

CCR5 receptor binding activity was determined in a 96-well SPA format (Cook, 1996) using a $[^{125}I]$-human MIP-1α and membranes prepared from Chinese hamster ovary (CHO) cells overexpressing the human CCR5 receptor. The samples were dissolved in 12.5% aqueous DMSO and incubated with 12 μg of membranes, 0.17 nM $[^{125}I]$-MIP-1α and 0.25 mg Wheat Germ Agglutinin-SPA beads in assay buffer (50 mM Hepes, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1% BSA and a protease inhibitor cocktail) for 5 h at room temperature with shaking. Radioactivity (total binding) was measured after a 2 h bead settling period. Non-specific binding was defined in the presence of 1 μM recombinant human MIP-1α. Human

MIP-1α was also used as a reference compound and had an IC_{50} of 2.7 nM.

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