New Cytotoxic Alkylated Anthraquinone Analogues from a Soil Actinomycete *Streptomyces* sp. WS-13394

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Four new alkylated anthraquinone analogues (1–4) were isolated from a soil actinomycete *Streptomyces* sp. WS-13394. The structures of compounds 1–4 were elucidated to be 1,4,6-trihydroxy-8-alkylated-anthraquinones by means of spectroscopic methods, including UV, one dimensional (1D), 2D-NMR and MS spectrometry. All compounds showed activities against BGC-823 and MCF-7 with IC₅₀ from 0.99 to 3.54 µg/mL, while 2 exhibited cytotoxicity against HepG2, A875, BGC-823 and MCF-7 with IC₅₀ 2.29, 4.90, 0.99, and 1.66 µg/mL, respectively.

Key words: alkylated anthraquinone; *Streptomyces* sp. WS-13394; actinomycete; cytotoxic

Anthraquinones, having a wide distribution in nature, were well-known for their antimicrobial, antitumor, anti-inflammatory and antiviral activities.¹ As reported in the literature, some of the alkylated 9,10-anthraquinone showed significant activity of antitumor, such as R1128 B, isolated from the cultured broth of *Streptomyces* sp. No. 1128, showed significant activity of antitumor, such as R1128 B, isolated from the cultured broth of *Streptomyces* sp. No. 1128, showed significant activity of antitumor, such as R1128 B, isolated from the cultured broth of *Streptomyces* sp. No. 1128, showed significant activity of antitumor.

Results and Discussion

Compound 1, obtained as a red pigment, had a molecular formula of C₁₇H₁₄O₅, inferred by its high resolution-electrospray ionization-mass spectra (HR-ESI-MS) (m/z 321.0737, [M+Na]⁺), requiring eleven degrees of unsaturation. The UV spectrum of 1 exhibited absorption bands at 466, 277, and 225 nm, highly suggesting an anthraquinone chromophore.⁶ The ¹H-NMR spectrum of 1 (Table 1) showed two ortho-coupling aromatic protons at δ₂ 7.24 (1H, d, J=9.3 Hz, H-2) and 7.20 (1H, d, J=9.3 Hz, H-3), and two meta-coupling ones at δ₂ 7.57 (1H, d, J=2.5 Hz, H-5) and 6.98 (1H, d, J=2.5 Hz, H-7). In addition, a propyl moiety contributed by two methylene signals at δ₂ 1.35 (2H, t, J=7.7 Hz, H-1) and 1.67 (2H, m, H-2'), and a methyl signal at δ₂ 1.01 (3H, t, J=7.3 Hz, H-3') were also observed in the ¹H-NMR spectrum. In the ¹³C-NMR spectrum of 1 (Table 2), 14 sp² carbon signals, including four quaternary sp² carbon signals in the low-field region at δ₂ 152.0, 158.1, 158.2 and 164.2, and two carbonyl carbon signals at δ₂ 188.2 and 189.2, suggesting the presence of a tetra-substituted anthraquinone core. As required by the molecular formula, three hydroxyl groups and a propyl group account for the substituents on the anthraquinone ring. The substituents and their location on the anthraquinone ring were further established by analysis of the correlation spectroscopy (COSY) and heteronuclear multiple bond correlation (HMBC) spectra of 1 (Fig. 2). COSY correlations between three aliphatic protons (H-1'/H-2' and H-2'/H-3'), combined with HMBC correlations from H-1' to C-2' (25.1), C-3' (14.7), C-8 (152.0) and C-8a (123.9), and H-2' to C-1' (39.3), C-3' and C-8 established the location of a propyl group at C-8, which in turn indicated the hydroxy groups were located at C-1, C-4 and C-6, respectively. This deduction was corroborated by the HMBCs of H-2 and H-3 to C-1 (158.2).

Fig. 1. Structures of Compounds 1–4

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and C-4 (158.2), H-5 and H-7 to C-6 (164.2), and COSY correlations between H-2 and H-3. As a result, 1 was established to be 1,4,6-trihydroxy-8-propyl-9,10-anthraquinone.

Compound 2, isolated as red powder, had a molecular formula of C_{18}H_{16}O_{5}, as established by HR-ESI-MS (m/z 335.0894, [M+Na]^+), one more methylene (CH$_2$) than 1. The $^1$H- and $^{13}$C-NMR data were almost identical with those of 1 (Tables 1, 2), except for the resonances for the alkyl substituent at C-8. NMR data (Tables 1, 2) of three methylenes [δ$_H$ 3.15 (2H, t, $J=7.7$), 3.08 (2H, t, $J=7.6$), 3.05 (2H, m), 3.12 (2H, t, $J=7.7$)] and one methyl [δ$_H$ 1.01 (3H, t, $J=7.3$), 1.02 (3H, d, $J=6.7$)] showed the existence of a butyl group. HMBC correlations (Fig. 2) from H-1 to C-8 (152.2) and C-8a (123.9) indicated that the butyl moiety was located at C-8. Therefore, the structure of 2 was established as 1,4,6-trihydroxy-8-propyl-9,10-anthraquinone.

Compounds 3 and 4, both obtained as red powder, gave the same [M+Na]$^+$ ion at m/z 349.1051 in HR-ESI-MS spectrum,
which was consistent with the molecular formula of C_{19}H_{18}O_{5}. Comparison of NMR spectra (Tables 1, 2) data of compounds 3 and 4 with those of 1 and 2 indicated differences in the alkyl substituent at C-8. Five aliphatic carbon including two methylenes and one methyl, and COSY correlations (Fig. 2) (H-1/uni2032H-2, H-2/uni2032H-3', H-3'/uni2032H-4' and H-3'/uni2032H-5'), suggested the existence of an isopentyl group in compound 3. In contrast, resonances attributed to aliphatic carbons of four methylenes and one methyl, and COSY correlations (Fig. 2) of H-1'/uni2032H-2'/H-3'/uni2032H-4'/H-5' showed the presence of a penty1 moiety in compound 4. The full structures of 3 and 4 were further confirmed by heteronuclear single quantum coherence (HSQC) and HMBC experiments. Finally, structures of 3 and 4 were formulated as 1,4,6-trihydroxy-8-isopentyl-9,10-anthaquinone and 1,4,6-trihydroxy-8-pentyl-9,10-anthaquinone respectively.

Compounds 1–4 were examined for their cytotoxic activities against four human tumor cells lines. All compounds showed activities against BGC-823 and MCF-7 with IC_{50} from 0.99 to 3.54 µg/mL, while 2 exhibited cytotoxicity against HepG2, A875, BGC-823 and MCF-7 with IC_{50} 2.29, 4.90, 0.99, and 1.66 µg/mL respectively (Table 3).

**Experimental**

**General Experimental Procedures** NMR spectra, including HSQC, HMBC and COSY, were recorded on a Bruker AVANCE-500 instrument with tetramethylsilane (TMS) as an internal standard (Bruker BioSpin group, German). ESI-MS and HR-ESI-MS data were obtained on a Waters LC-MS (Waters Corporation, U.S.A.) and Thermo Q-T of Micromass (Thermo Electron Corporation, U.S.A.) spectrometers, respectively. Preparative HPLC was carried on a Waters 2767 Auto-purification System (Waters Corporation, U.S.A.) coupled with a diode array detector (DAD) detector, using Sunfire Prep C_{18} OBD (5 µm, 19×250 mm/10×250 mm, Ireland) column.

**Microorganism** The soil actinomycete WS-13394 was isolated from a soil sample collected from Shimen County, Hunan Province, China, in January 2007. Analysis of 16S ribosomal RNA (rRNA) sequence of WS-13394 revealed 99% identity to Streptomyces sp. VTU E-062996. A voucher strain was preserved at Hubei Biopesticide Engineering Research Center, Hubei Academy of Agricultural Sciences, in Wuhan, China.

**Cultivation and Extraction** Seed fermentations of strain WS-13394 were carried out in ISP-2 medium (glucose 4.0 g/L, malt extract 10.0 g/L, yeast extract 4.0 g/L, agar 20.0 g/L, ad-
References