Bioorganic & Medicinal Chemistry Letters 27 (2017) 2579–2582

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel bioactive molecules from *Lentzea violacea* strain AS 08 using one strain-many compounds (OSMAC) approach

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ARTICLE INFO

Article history:
Received 16 January 2017
Revised 1 March 2017
Accepted 24 March 2017
Available online 25 March 2017

Keywords:
Actinomycetes
Lentzea violacea
Bioactive compounds
Antitumor and antimicrobial activity

ABSTRACT

A new eudesmane sesquiterpenoid (**1**), and a new homologue of virginiae butanolide E (**2**) along with butyl isobutyl phthalate (**3**) were isolated from, actinomycete-*Lentzea violacea* strain AS08 isolated from north western Himalayas by stressing on modified one strain-many compounds (OSMAC) method. The structures of the new compounds were elucidated by extensive spectroscopic analyses including 1D, 2D NMR along with HR-ESI-MS and FT-IR data. Herein, a distinctive method was added for inspecting secretory profile of the strain by quantification of extract value of cell free supernatant in different types of culture media fallowed by HPLC profiling of respective extracts, which revealed a highly altered metabolic profile of the strain and formed the base for the selection of media. The compounds **1** and **2** showed moderate activity against Gram negative (MIC ~32–64 μg ml^{-1}) in comparison to Gram positive bacterial pathogens. Compound **1** exhibited significant activity in human cancerous cell lines (IC_{50} ~19.2 μM).

Actinomycete genera represent the microbial factories for production of wide range of metabolites with extensive biological activities.[1] Around the globe a small portion of the existing ecosystems have been systematically searched for isolation of actinomycetes for discovery of novel chemical scaffolds.[2,3] During isolation of actinomycetes from soils of untapped Himalayan ecosystems for their chemical diversity and screening in drug discovery paradigms, as part of our programme, we encountered a rare actinomycete strain, characterised as *Lentzea violacea* strain AS08**.** Preliminarily activity of crude extract and no report on molecules from this strain became inspiration for further investigation. The discouragements owing to re-isolation of previously isolated molecules it became imperative to adopt new dereplication methods for production of novel bioactive secondary metabolites.[4,5] So for dereplication, we modified one strain-many compounds **(**OSMAC) method for estimation of secondary metabolites, wherein mass of organic extracts obtained with respect to different growth media was quantified and correlated with their HPLC chromatograms for the chemical diversity. The selective fermentation conditions were set for large scale culture to gain mass of extract for isolation of molecules. The study describes the isolation, structural elucidation, anti-bacterial and anti-tumor activities of compounds **1**–**3** (Fig. 1). The bioactive actinomycete strain, AS08, was isolated and selected during screening of soil samples from high altitude, cold regions of Thajiwas glacier located in north western Himalayas. The strain displayed whitish vegetative and yellowish aerial mycelial colours and also produced green soluble pigment on incubation after 15 days in CYPS media. 16S rRNA gene sequence (1419 bp) revealed that the strain belongs to genus *Lentzea* and species *violacea* as evident from the high similarity to 16S rRNA gene sequences from *Lentzea violacea* (Accession No. FJ486311.1) and *Lentzea violacea* strain F173540 (Accession No. EU593726.1).

Taking together the physical characteristics and the nucleotide sequence of the corresponding 16S rRNA, the actinomycete was identified as *Lentzea violacea* strain AS08. The nucleotide sequence of rRNA gene sequences of the isolate-*Lentzea violacea* was submitted to NCBI under gene bank accession number KX444628.1, and phylogenetic analysis was carried out (Supplementary Fig. S16).

As the strain was isolated from cold climate areas, duplicate cultures of strain were grown in CYPS broth at different

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http://dx.doi.org/10.1016/j.bmcl.2017.03.075
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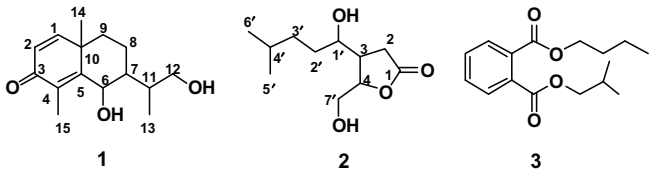

Fig. 1. Structures of isolated compounds: –an eudesmane sesqui terpenoid, **1**; a new homologue of virginiae butanolide E, **2** and butyl isobutyl phthalate, **3**.

temperatures to observe effect of temperature on its growth rate. Maximum growth was observed at 28°C and the strain exhibited poor growth at 37 °C and 4 °C (Fig. 2a) by determining the dry weight at interval of every third day. Thus, strain appeared to be psychrotolerant mesophile with respect to its growth at optimum temperature.

For dereplication studies, so as to observe the chemical changes of the strain by alteration of the culture media,[6] the ethyl acetate extracts were acquired by growing strain on three distinct media types: CYPS, SCP-1 and SC (For compositions see supplementary information). Here in our attempt, we determined the amount of ethyl acetate extracts of cell free supernatant in different culture media and then followed the HPLC analysis of respective extracts**.** Thus, the extract value signifying actual assessable load of the secondary metabolites was taken along with HPLC profiles for deeper insights about chemical diversity. This was added as adjustment to method as described by Tormo et al.[7] i.e., extract with large no of peaks and least overlapping HPLC fingerprint was selected. Using this method highest quantity of extract was observed in SC medium (Fig. 2b).

The analysis of HPLC results illustrated that maximum increase in number of peaks was also observed in SC medium in comparison to other media types (supplementary Figs. S13–S15). So, SC medium was selected for large scale culture for the isolation of bioactive metabolites. Further, the TLC profile of SC media extract indicated large number of minor compounds, which could not be isolated in amounts required for structural elucidation. The scaled-up fermented broth of the strain cultured on SC medium, yielded crude extract (2.5 g) which was subjected to fractionation and isolation of compounds by silica gel column chromatography (Supplementary experimental section).

Compound **1** was obtained as white crystalline solid with m.p; 158–160 °C. It exhibited a molecular ion peak at 251.1633 $[M+H]^+$ in HRESIMS, consistent with molecular formula of $C_{16}H_{12}O_6$. IR spectrum exhibited strong bands at 3380 and 1658 cm^{-1} indicating the presence of hydroxyl and conjugated carbonyl group.

The ^{1}H NMR spectrum of the compound **1** showed signals at δ_H 6.82 (d, J = 8.4 Hz, 1H), 6.12 (d, J = 8.4 Hz, 1H) assignable to α, β unsaturated double bond conjugated with carbonyl, 4.65 (d, J = 9.4 Hz, 1H); 3.48 (d, J = 7.4 Hz, 2H) are assignable to —*C*HOH and —CH_2OH respectively. The spectra showed the signals for three methyl groups, two as singlets δ_H 2.23 (s, 3H) assignable to CH_3C═C—, 1.28 to (s, 3H) CH_3C, and one methyl doublet δ_H 0.94 (d, J = 5.6 Hz, 3H) assignable to CH_3CH—. HSQC/^{13}C NMR-DEPT spectrum exhibited the presence of fifteen carbon signal including three methyls, three methylene, five methine and four quaternary carbons. δ_C 188.55 represents carbonyl, 158.32 and 124.42 are assignable to two sp^2 methine carbons, 163.15 and 128.47 represent two sp^2 quaternary carbons, 71.96 and 65.54 represent oxygenated methine and methylene carbons respectively (Table 1; Supplementary Figs. S1–S4). The structure was further confirmed by extensive 2D NMR analysis and the key correlations observed in COSY and HMBC are shown in Fig. 3. Key COSY correlations were observed between H-1 (δ_H 6.82) and H-2 (δ_H 6.12), between H-6 (δ_H 4.65) H-7 (δ_H 1.86), H-11 (δ_H 2.40) and between H-12 (δ_H 3.48). Some key HMBC correlations were observed between H-1 (δ_H 6.82) and C-3 (δ_C 188.5), C-10 (δ_C 42.2), C-14 (δ_C 10.2); between H-2 (δ_H 6.12) and C-3 (δ_C 188.5), C-4 (δ_C 128.6), C-5 (δ_C 163.1); between H-6 (δ_H 71.56) and C-5 (δ_C 163.1), C-7 (δ_C 33.7), between H-9 (δ_H 1.61) and C-10 (δ_C 42.2): between H-12 (δ_H 3.48) and C-7 (δ_C 33.7), C-11 (δ_C 46.6), C-13 (δ_C 9.4). Based on the complete spectroscopic description including ^{1}H NMR, HSQC, COSY, HMBC, HRESIMS and UV, the compound **1** was confirmed to be an eudesmane type sesquiterpenoid named as 8-hydroxy-7-(1-hydroxypropan-2-yl)-1,4a-dimethyl-5,6,7,8-tetrahydronaphthalen-2(4aH)-one. The Compound is newly isolated to best of our knowledge**.** The novel sesquiterpene derivatives[8–10] with different biological activities have been reported from actinomycetes.

Compound **2** was obtained as colourless sticky oil, soluble in chloroform, methanol and ether. It exhibited a molecular ion peak at 217.1432 $[M+H]^+$ in HRESIMS, consistent with molecular formula of $C_{11}H_{20}O_4$. IR spectrum exhibited strong bands at 3386 and 1652 cm^{-1} indicating the presence of hydroxyl and carbonyl group. The ^{1}H NMR spectrum of the compound **2** showed signals at δ 2.18 (m, 2H), 2.79 (ddd, J = 2.4, 2.3, 2.8 Hz), 4.54 (m, 1H); 4.15 (m, 1H); 3.89 (dd, 1H, J = 2.4 Hz each) and 3.68 (dd, 1H, J = 2.4 Hz each), 1.37 (m, 1H) 1.34 (m, 2H), 1.15 (m, 2H), 0.86 (s, 3H), 0.88 (s, 3H),while ^{13}C/HSQC spectra indicated exhibited 11 signals at δ 178.01, 79.37, 69.75, 64.42, 46.71, 34.96, 32.66, 28.13, 22.56, 22.52, 22.50 (Table 1; Supplementary Figs. S5–S8). The structure was strongly supported by COSY spectra (Fig. 4).

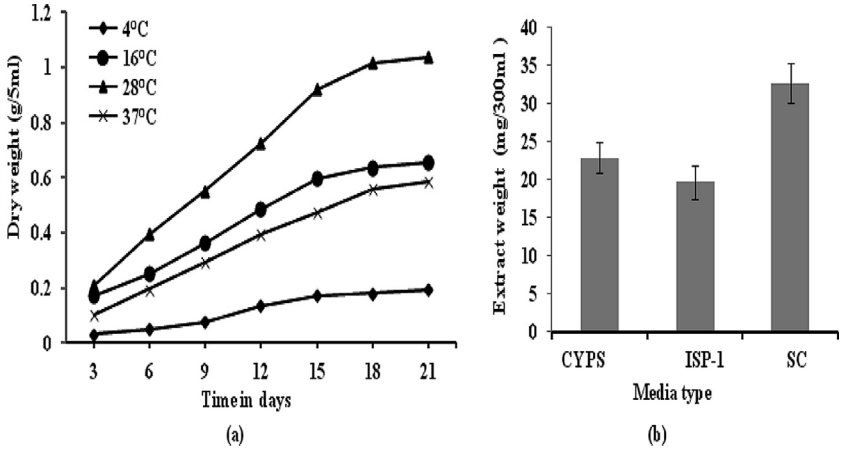

Fig. 2. Temperature profiling and media optimization. (a) Growth observed by dry weight in mg/5 ml of *Lentzea violacea* AS08 cultured at the temperatures 4 °C, 16 °C, 18 °C and 37 °C (b) ethyl extract-value of *Lentzea violacea* AS08 in mg/300 ml in different media (CYPS, ISP-1 and SC).