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Saccharonol B, a new cytotoxic methylated isocoumarin from *Saccharomonospora azurea* ☆

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ABSTRACT

From an antimicrobial gram-positive actinomycete strain of *Saccharomonospora azurea* (MTCC11714) isolated from high altitude soil of Kargil (J&K, India), a new isocoumarin saccharonol B (**2**) along with two known compounds viz. saccharonol A (**1**) and piericidin A_3 (**3**) was isolated and characterized. The structure of the new compound was established based on extensive 2D-NMR data. Saccharonol B (**2**) exhibited mild antimicrobial activity against a standard panel of microorganisms *Staphylococcus aureus* ATCC 29213, *Candida albicans* ATCC 90028, and *Aspergillus fumigatus* MTCC 1811 with MIC values in the range of 128–248 μg/mL. Saccharonol B (**2**) and piericidin A_3 (**3**) showed selective cytotoxic activity against human pancreatic carcinoma cell line (MIAPaCa-2) with IC_{50} values of 9 and 8 μM, respectively. Mechanistic studies indicated that saccharonol B (**2**) arrests S-phase of the cell cycle and causes dose-dependent loss of mitochondrial potential in MIAPaCa-2 cells.

Microorganisms isolated from soil samples have emerged as an important source of novel chemical scaffolds and biologically important secondary metabolites.[1] A new strain of *Saccharomonospora azurea* (MTCC11714) (Synonym: *Saccharomonospora caesia*) a gram-positive, soil-dwelling filamentous bacterium[2] belonging to the family *Pseudonocardiaceae*[3] was isolated from high altitude soil samples of Kargil (India). Previous chemical investigations[4] on the genus *Saccharomonospora* have led to the isolation and identification of antibiotic metabolites viz. antibiotic AB 65[4a] and sakyomicin E.[4b] Antibiotic AB 65 is reported to be active against gram-positive bacteria, mycobacteria, fungi, and yeasts whereas sakyomicin E is known to possess anticancer activity. *S. azurea* is also used for the commercial production of antibiotics primycins.[5] The chemical structures of primycins and sakyomicin E are shown in Figure 1.

Isocoumarin is an important class of natural products widely occurring in plant kingdom and is known to exhibit a wide range of biological activities[6] including antimicrobial[6f] and anti-cancer[6g,7] properties. This class of natural products has a rare occurrence among microorganisms, and very few isocoumarins are reported so far. Herein, we report isolation of two isocoumarins saccharonols A and B (**1** and **2**) and pyridyl compound piericidin A_3 (**3**) from an actinomycete strain of *Saccharomonospora azurea* (MTCC11714) isolated from the high altitude soil of Kargil. Saccharonol B (**2**) and piericidin A_3 (**3**) showed significant cytotoxicity in human pancreatic carcinoma cell line (MIAPaCa-2). The structures of compounds **1** and **2** were established by detailed spectroscopic analysis, including extensive examination of 2D-NMR (^1H–^1H COSY, HMQC, and HMBC) data whereas compound **3** was characterized by comparing the data with the literature values.[8] All three compounds were screened for antimicrobial (against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Candida albicans* ATCC 90028, and *Aspergillus fumigatus* MTCC 1811) and cytotoxic (in prostate, pancreatic, and leukemia cell lines) activities. The mechanistic evaluation of the new compound **2** was also

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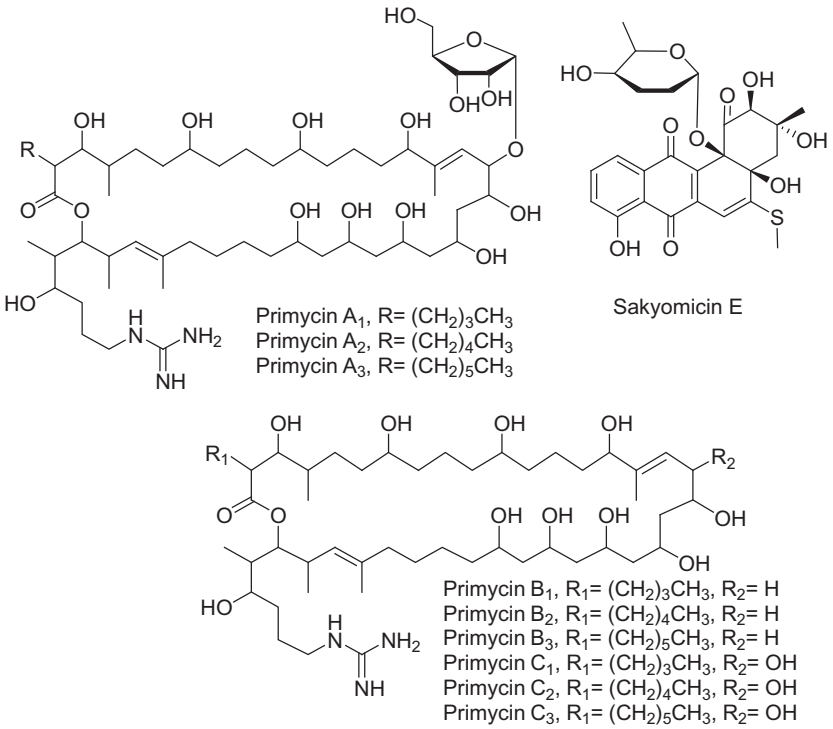

Figure 1. Chemical structures of known secondary metabolites of genus *Saccharomonospora.*

carried out to investigate the possible mechanism of action of its cytotoxicity.

During our investigations, a large number of actinomycetes (>100) were isolated from the soil collected from the high altitude region of Kargil. All isolated actinomycetes were screened for antimicrobial activity against a panel of microbes such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis*, and *Candida albicans* by agar diffusion method. From the antimicrobial screening, an actinomycete IIIMA-1414 showed a promising zone of inhibition for *E. coli* (14 mm), *S. aureus* (18 mm), *M. luteus* (14 mm), *B. subtilis* (20 mm), and *C. albicans* (24 mm) and therefore this strain was selected for the isolation of secondary metabolites. The soil isolate of actinomycete IIIMA-1414 strain is gram-positive, spore-forming filamentous bacterium and identified as *Saccharomonospora azurea* by 16s rDNA methodology. During identification, the ~1.4 kb rDNA fragment was amplified using high–fidelity PCR polymerase. The PCR product was sequenced bi-directional using the forward, reverse, and internal primers. The microbe was found most similar to *Saccharomonospora azurea.*[9] This microbial strain IIIMA-1414 has been deposited in a repository of Microbial type Culture Collections & Gene bank (MTCC) at IMTECH Chandigarh, India and assigned the accession no. MTCC11714. The rDNA 1376 bp sequences were deposited at NCIB; gb-admin@ncbi.nlm.nih.gov (GeneBank accession no. KC855265).

Saccharomonospora azurea (MTCC11714) was grown in 7 L fermenter using CYPS (casein starch medium without agar) keeping the agitation 300 rpm, temperature 28 °C and air 1 vvm for 120 h. After termination of fermenter, methanol (450 mL) was added to the fermentation broth (4.5 L) and filtered. The filtrate was then extracted following the NCI protocol and passed through Dianion HP-20 resin. Sephadex LH-20 size-exclusion column chromatography of MeOH extract using 80% MeOH/acetone as a mobile phase led to the isolation of two isocoumarins, saccharonols A (**1**) and B (**2**) and one pyridyl compound piericidin A_3 (**3**).[10] The occurrence of isocoumarins and piericidin class of compounds together in microbial sources was reported earlier (from *Streptomyces* sp.).[11]

Saccharonol A (**1**) was isolated as a yellow solid with a molecular formula of $C_{10}H_9O_4$ established from a high-resolution ESIMS measurement of the $[M+H]^+$ peak at *m/z* 193.0495. The ^1H and ^{13}C NMR values (Tables 1 and 2) were indicative of a isocoumarin structure. The ^1H NMR spectrum in DMSO-d_6 showed the presence of highly deshielded deuterium exchangeable signal at δ 11.00 ppm for one proton, indicating the presence of H-bonded –*OH* group. Two doublets at δ 6.34 and 6.31 ppm with coupling constants of 2.02 and 2.15 Hz, indicated the presence of two aromatic protons located meta to each other. Two singlets at δ 6.48 and

Table 1
^1H NMR data of saccharonols A and B (**1** and **2**) and piericidin A_3 (**3**)[a]

Position	Saccharonol A (**1**)	Saccharonol B (**2**)	Piericidin A_3 (**3**)
	^1H (*J*, Hz)		
1			3.38 d (8.0)
2			5.42–5.35 m
4	6.48 s	6.16 s	2.80 d (8.0)
5	6.34 d (2.02)	6.40 s	5.64–5.58 m
6			6.12 d (12.0)
7	6.31 d (2.15)		
8			5.22 d (8.0)
9			2.36–2.33 m
10			3.66 d (8.0)
12			5.42–5.35 m
13			2.36–2.33 m
14			0.87 d (8.0)
15			0.87 d (8.0)
3-CH_3	2.21 s	2.25 s	1.80 s
7-CH_3			1.64 s
9-CH_3			0.81 s
11-CH_3			1.75 s
8-OH	11.00 s	11.32 s	
6-OCH_3		4.03 s	
2′-CH_3			2.09 s
4′-OCH_3			3.85 s
5′-OCH_3			3.95 s

[a] Chemical shift values in ppm.