Indian J Med Res 137, February 2013, pp 391-393

Correspondence

Evaluation of bioactive compounds produced by *Nocardia levis* MK-VL_113 & *Streptomyces tendae* TK-VL_333 for cytotoxic activity

Sir,

Natural products are the logical starting point for discovering new drugs as evidenced by the discovery of penicillin[1]. More than 60 per cent of approved antitumour drugs are derived from natural compounds[2,3]. Drugs with anticancer properties including anthracycline, bleomycin, actinomycin and mitomycin have been isolated from actinobacteria[4,5]. We have earlier reported isolation and characterization of bioactive metabolites for the first time from *Nocardia levis* MK-VL_113 and *Streptomyces tendae* TK-VL_333[6-10]. In this study these bioactive metabolites were tested for cytotoxicity which is the pre-requisite assay for testing anticancer activity.

For the extraction and purification of bioactive metabolites produced by *N. levis* and *S. tendae*, the pure cultures of the strains grown in seed medium (Yeast extract-malt extract-dextrose broth) were transferred individually to optimized fermentation media (Sucrose-tryptone broth for *N. levis*[6] and galactose-tyrosine broth for *S. tendae*[7]) under aseptic conditions. The fermentation process was turned off after 96 h of incubation and the culture broths were collected, extracted with ethyl acetate and evaporated to vacuum. The crude residues thus obtained were subjected to purification by using chromatographic techniques (silica gel column, thin layer and semi-preparative high performance liquid chromatography) and the structure of the pure bioactive compounds was elucidated and confirmed on the basis of Fourier transform infrared (FTIR), Electron impact mass (EIMS)/Electron spray ionization mass (ESIMS) spectrometry and nuclear magnetic resonance (^{1}H NMR and ^{13}C NMR) spectroscopy[8-10].

Screening of secondary metabolites obtained from *N. levis* led to the identification of bioactive compounds namely 1-phenylbut-3-ene-2-ol, bis-(2-ethylhexyl) phthalate, bis-(5-ethylheptyl) phthalate and a partially purified fraction containing phenylethyl alcohol, dibutyl phthalate and 1,2-benzenedicarboxylic acid, 3-nitro[8,9]. Five bioactive compounds namely 1*H*-indole-3-carboxylic acid, 2,3-dihydroxy-5-(hydroxymethyl) benzaldehyde, 4-(4-hydroxyphenoxy) butan-2-one, acetic acid-2-hydroxy-6-(3-oxo-butyl)-phenyl ester and 8-methyl decanoic acid were purified from the culture broth of *S. tendae*[10]. No information is available on the production of these bioactive metabolites by *Streptomyces*. Hence, these bioactive metabolites isolated from *N. levis* MK-VL_113 and *S. tendae* TK-VL_333 were tested for cytotoxic activity using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5,-diphenyltetrazolium bromide) assay[11].

Cell lines *viz.*, U-937 (Human leukemic monocyte lymphoma cell line) and HL-60 (Human promyelocytic leukemia cell line) procured from National Centre for Cell Science, Pune, India were cultured on RPMI-1640 (Hi-media®, Mumbai) medium supplemented with 10 per cent (v/v) foetal bovine serum, 1 mM $NaHCO_3$, 2 mM L-glutamine and penicillin-streptomycin in a humidified atmosphere (95%) with 5 per cent CO_2 at 37°C. Cells of U-937 and HL-60 (2 x 10^4 cells per well) were seeded in each well of 96-well microtiter plate containing 0.1 ml of medium. After overnight incubation, the cells were treated with different test concentrations of bioactive compounds (0-200 µg/ml) of the strains at identical conditions with three replicates of each concentration. After 24 h of incubation, the cell viability was assessed by adding 10 µl of MTT (5 mg/ml) per well and the plates were incubated in a CO_2 incubator at 37°C for 4 h. The formazan crystals formed in the cells were dissolved with 100 µl of 0.1 per cent DMSO and the rate of colour development was measured at 570 nm in a spectrophotometer (Spectra MAX Plus, Molecular

Table. Cytotoxic activity of the bioactive compounds produced by *N. levis* MK-VL_113 and *S. tendae* TK-VL_333

Bioactive compound	Cytotoxic activity (IC_{50} µg/ml)	
	Cell lines	
	U-937	HL-60
NL_1	99.14±0.54	111.15±0.82
NL_2	86.21±0.92	119.89±0.61
NL_3	101.5±1.23	132.7±0.75
NL_4	135.5±0.81	162.5±0.36
T_1	76.5±0.43	92.5±0.9
T_2	125.8±0.65	156.02±1.13
T_3	152.9±0.94	190.71±0.55
T_4	119.5±0.6	132.78±1.4
T_5	91.18±0.45	95.09±0.95
Etoposide (positive control)	10.56±0.7	1.077±0.06

Values are the means ± SD of three replicates
NL_1, 1-phenylbut-3-ene-2-ol; NL_2, bis-(2-ethylhexyl) phthalate; NL_3, bis-(5-ethylheptyl) phthalate; NL_4, partially purified fraction; T_1, 1*H*-indole-3-carboxylic acid; T_2, 2,3-dihydroxy-5-(hydroxymethyl) benzaldehyde; T_3, 4-(4-hydroxyphenoxy) butan-2-one; T_4, acetic acid-2-hydroxy-6-(3-oxo-butyl)-phenyl ester; T_5, 8-methyl decanoic acid

Devices, supported by SOFTmax PRO-3.0, USA). The inhibition of cell viability (IC_{50}) was determined with reference to that of etoposide (standard).

Bioactive compounds produced by *N. levis* and *S. tendae* showed cytotoxic activity against U-937 and HL-60 cell lines. Among these, NL_1 and NL_2 from *N. levis* and T_1 and T_5 from *S. tendae* exhibited high cytotoxicity when compared to the others (Table). However, their activity was less when compared to etoposide (positive control). Actinobacteria particularly *Streptomyces* spp. are known to produce a number of potent cytotoxic compounds including anthracyclines, FCE 21424, FCE 24366 and FCE 24367[12], saptomycins[13], cremeomycin[14], clecarmycins[15], actinomycins G_2-G_6[16], moromycins A and B, saquayamycin B and fridamycin D[17] while brasiliquinones A, B and C[18], nocardiones A and B[19] and chemomicin A[20] were recorded from *Nocardia* spp. Keeping the potentiality of the actinobacterial metabolites as anticancer agents, the bioactive metabolites of *N. levis* and *S. tendae* showing moderate cytotoxic activity against the cell lines, U-937 and HL-60 may be used as model systems for the preparation of anticancer drugs by testing further anticancer assays.

Acknowledgment

The first author (AK) thanks Indian Council of Medical Research (ICMR), New Delhi, for providing financial assistance in the form of senior research fellowship.

Kavitha Alapati & Vijayalakshmi Muvva*
Department of Botany & Microbiology
Acharya Nagarjuna University
Guntur 522 510, India
**For correspondence*:
profmvijayalakshmi@rediffmail.com

References

1. Wedge DE, Camper ND. Connections between agrochemicals and pharmaceuticals. In: Cutler SJ, Cutler HG, editors. *Biologically active natural products: pharmaceuticals*. Washington, DC: CRC Press; 1999. p. 1-15.
2. Cragg G, Newman D, Snader K. Natural products in drug discovery and development. *J Nat Prod* 1997; *60* : 52-60.
3. Newman DJ, Cragg GM, Snader KM. Natural products as sources of new drugs over the period 1981-2002. *J Nat Prod* 2003; *66* : 1022-37.
4. Rocha AB, Lopes RM, Schwartsmann G. Natural products in anticancer therapy. *Curr Opin Pharmacol* 2001; *11* : 364-9.
5. Newman DJ, Cragg GM. Advanced preclinical and clinical trials of natural products and related compounds from marine sources. *Curr Med Chem* 2004; *11* : 1689-709.
6. Kavitha A, Vijayalakshmi M. Cultural parameters affecting the production of bioactive metabolites by *Nocardia levis* MK-VL_113. *J Appl Sci Res* 2009; *5* : 2138-47.
7. Kavitha A, Vijayalakshmi M. Influence of cultural conditions on the production of bioactive metabolites by *Streptomyces tendae* TK-VL_333. *Res J Biotechnol* 2009; *4* : 56-64.
8. Kavitha A, Prabhakar P, Vijayalakshmi M, Venkateswarlu Y. Production of bioactive metabolites by *Nocardia levis* MK-VL_113. *Lett Appl Microbiol* 2009; *49* : 484-90.
9. Kavitha A, Prabhakar P, Narasimhulu M, Vijayalakshmi M, Venkateswarlu Y, Venkateswara Rao K, *et al*. Isolation, characterization and biological evaluation of bioactive metabolites from *Nocardia levis* MK-VL_113. *Microbiol Res* 2010; *165* : 199-210.
10. Kavitha A, Prabhakar P, Vijayalakshmi M, Venkateswarlu Y. Purification and biological evaluation of the metabolites produced by *Streptomyces tendae* TK-VL_333. *Res Microbiol* 2010; *161* : 335-45.
11. Mosmann T. Rapid calorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *J Immunol Methods* 1983; *65* : 55-63.

12. Cassinelli G, Arlandini E, Ballabio M, Bordoni T, Geroni C, Giuliani F, *et al*. New biosynthetic anthracyclines related to barminomycins incorporating barbiturates in their moiety. *J Antibiot* 1990; *43* : 19-28.

13. Abe N, Nakakita Y, Nakamura T, Enoki N, Uchida H, Munekata M. New antitumor antibiotics, saptomycins. I. Taxonomy of the producing organism, fermentation, HPLC analysis and biological activities. *J Antibiot* 1993; *46* : 1530-5.

14. McGuire JN, Wilson SR, Rinehart KL. Cremeomycin, a novel cytotoxic antibiotic from *Streptomyces cremeus*. Structure elucidation and biological activity. *J Antibiot* 1995; *48* : 516-9.

15. Fujii N, Katsuyama T, Kobayashi E, Hara M, Nakano H. Clecarmycins, new antitumor antibiotics produced by *Streptomyces*: Fermentation, isolation and biological properties. *J Antibiot* 1995; *48* : 768-72.

16. Bitzer J, Gesheva V, Zeeck A. Actinomycins with altered threonine units in the beta-peptidolactone. *J Nat Prod* 2006; *69* : 1153-7.

17. Abdel-Fattah MS, Kharel MK, Hitron JA, Baig I, Rohr J. Moromycins A and B: Isolation and structure elucidation of *C*-glycosylangucycline type antibiotics from *Streptomyces* sp. KYOO2. *J Nat Prod* 2008; *71* : 1569-73.

18. Nemoto A, Tanaka Y, Karasaki Y, Komaki H, Yazawa K, Mikami Y. Brasiliquinones A, B and C, new benz[a] anthraquinone antibiotics from *Nocardia brasiliensis*. I. Producing strain, isolation and biological activities of the antibiotics. *J Antibiot* 1997; *50* : 18-21.

19. Otani T, Sugimoto Y, Aoyagi Y, Igarashi Y, Furumai T, Saito N, *et al*. New Cdc25B tyrosine phosphate inhibitors, nocardiones A and B, produced by *Nocardia* sp. TP-A0248: Taxonomy, fermentation, isolation, structural elucidation and biological properties. *J Antibiot* 2000; *53* : 337-44.

20. Sun CH, Wang Y, Wang Z, Zhou JQ, Jin WZ, You HG, *et al*. Chemomicin A: A new angucyclinone antibiotic produced by *Nocardia mediterranei* subsp. *kanglensis* 1747-64. *J Antibiot* 2007; *60* : 211-5.