ISOLATION, CHEMICAL MODIFICATION AND CYTOTOXIC EVALUATION OF ATRANORIN, THE MAJOR METABOLITE OF THE FOLIOSE LICHEN *PARMOTREMA MELANOTHRIX*

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The foliose lichen, *Parmotrema melanothrix* has been chemically screened for the first time and isolated the depside atranorin (**1**) in significant quantity (2 %) along with methyl 2,4-dihydroxy-3,6-dimethylbenzoate (**2**) and methyl 3-formyl-2,4-dihydroxy-6-methylbenzoate (**3**). In view of its abundance, interesting structural features and significant biological profile, atranorin was subjected to chemical modification and synthesised five diverse analogues in very good yields (70~93%). The synthesised analogues along with the three isolated compounds were evaluated for their cytotoxic potential against a panel of six human cancer cell lines using MTT assay. Among the tested compounds, **1a** showed enhanced activity than the parent compound (**1**) against almost all the tested cell lines. Significantly, **1a** showed highest activity (IC_{50} = 15.19 μM) against prostate cancer cell line (DU145). The results indicate that complete protection of the phenolic hydroxyls in atranorin as acetates enhances the cytotoxicity, especially against DU 145.

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Introduction

Lichens are highly productive natural sources with a conglomerate of two organisms such as fungi and algae or cyanobacteria.[1] They can survive in a wide range of habitats and harsh environmental conditions such as low temperatures, prolonged darkness, drought and continuous light. These extreme conditions encourage the lichens to produce biologically active secondary metabolites such as depsides, depsidones, xanthones, dibenzofurans and chromones.[2] Most of these secondary metabolites are unique to lichens and are produced biosynthetically via the polyketide pathway.[3]

Lichen substances exhibit a wide range of biological activities such as antimicrobial, anti-inflammatory, HIV-1 integrase inhibitory, antioxidant, antipyretic and antiproliferative or cytotoxic.[4,5] The genus *Parmotrema*, belongs to the Parmeliaceae family, is the largest one in the lichen kingdom. The lichens of this family are characterized by the development of a foliose growth.[6]

The major secondary metabolites of *Parmotrema* genus are depsides, which possess two or more aromatic rings joined through ester linkages. Interestingly these metabolites are reported with several biological activities such as antimicrobial, antiviral, antiprotozoal, insecticidal, antitermite, cytotoxic, antioxidant, wound healing, antiherbivore, analgesic and anti-inflammatory.[7-9] Among the various *Parmotrema* lichens, *P. melanothrix* is unexplored both chemically and biologically. In a preliminary study different polar extracts *P. melanothrix* were tested and reported to exhibit antioxidant and antibacterial activity against *S. viridians*, *S. aureus* and Acinetobacter.[10] Virtually, no systematic chemical examination has been done on this species except the preliminary phytochemical screening of Spielmann and Marcelli who identified atranorin and protopraesorediosic acid by performing some spot tests.[11] As, there are no systematic chemical and biological studies were carried so far on *P.melanothrix*, we have now taken up its detailed chemical screening and chemical modification of atranorin, its major metabolite to identify potent cytotoxic leads.

Experimental

General experimental procedure

Silica gel 60 F254 TLC glass plates (Merck) were used to monitor purity of the isolated compounds and all the reactions. Column chromatography was carried out by using Acme grade silica gel (60-120 mesh). Melting points were determined on Buchi melting point apparatus and are uncorrected. IR spectra were recorded on Nicollet 740 FTIR spectrophotometer using KBr pellets. ^{1}H and ^{13}C NMR spectra were recorded on Brucker 300 MHz or Varian 500 MHz in $CDCl_3$ with TMS as internal standard. Chemical shifts were expressed as Hertz (Hz). HRMS spectra were recorded on Agilent-ESI QTOF or JEOL mass spectrometers.

Lichen material

The lichen, *Parmotrema melanothrix* (250 g) was collected from rocks (saxicolous) in Tirumala Hills (13.67820N, 79.35220E), Tirupathi, Andhra Pradesh, India at an altitude of 976 meters (3,202 ft). The collected lichen material was identified according to their morphological characteristics.[12]

Extraction and isolation

The lichen material was washed under tap water to remove any foreign matter. It was shade dried and powdered in a pulveriser. The powdered lichen (250 g) was extracted with acetone (1 L) at room temperature overnight. Concentration of acetone solubles under vacuum to one quarter volume and keept at room temperature overnight followed by filtration yielded a brown coloured solid (5.5 g, 2.2 %) and an extract (5 g, 2 %). The brown colour solid on recrystallisation using hexane-ethanol solvent system, yielded pure compound **1** (5 g, 2 %). Repetitive Si gel column chromatographic separation of the acetone extract (5 g) afforded compounds **2** (1.0 g, 0.4 %) and **3** (0.5 g, 0.2 %).

3-Hydroxy-4-(methoxycarbonyl)-2,5-dimethylphenyl 3-formyl-2,4-dihydroxy-6-methylbenzoate (atranorin, 1)

Yield 2 %, m.p. 192-196 ^{0}C. IR (KBr): 3450, 1770, 1730, 1650, 1580,1260, 1145 cm$^{-1.1}$H NMR (300 MHz, $CDCl_3$) δ = 2.09 (s, 3H), 2.54 (3H, S), 2.69 (s, 3H), 3.98 (s, 3H), 6.40(1H,S) 6.51(s, 1H), 10.36 (1H,S),11.96 (1H,S), 12.51 (s, 1H), 12.56 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 9.60, 24.24, 25.80, 52.56, 103.06, 108.78, 110.49, 113.07, 116.241, 117.01, 140.09, 152.21, 152.65, 163.09, 167.708, 169.30, 169.90, 172.41, 194.04. HRMS (ESI) *m/z* calcd. for $C_{19}H_{17}O_8$ $[M-H]^+$ 373.0923, found 373.0917.

Methyl 2,4-dihydroxy-3,6-dimethylbenzoate (2)

Yield 0.4 %, m.p 143-145 ^{0}C. IR (KBr): 3404, 3082, 2943, 1627, 1500, 1446, 1424, 1368 cm^{-1}. ^{1}H NMR (300 MHz, CDCl3) δ = 2.10 (s, 3H), 2.45 (s, 3H), 3.92 (s, 3H), 6.20 (s, 1H), 12.04 (s, 1H); ^{13}C NMR (125 MHz, CDCl3) δ = 7.37, 23.79, 51.53, 104.88, 108.29, 110.28, 139.85, 157.83, 162.82, 172.38. HRMS (ESI) *m/z* calcd. for $C_{10}H_{13}O_4$ $[M+H]^+$ 197.0814, found 197.0810.

Methyl 3-formyl-2,4-dihydroxy-6-methylbenzoate (3)

Yield 0.2 %, m.p 145-149 ^{0}C, ^{1}H NMR (300 MHz, $CDCl_3$) δ = 2.53 (s, 3H), 3.96 (s, 3H), 6.29 (s, 3H), 10.34 (s, 1H), 12.40 (s, 1H), 12.87 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 24.93, 52.03, 103.5, 108.1, 111.8, 152.0, 166.3, 168.0, 171.7, 193.6; HRMS (ESI): *m/z* calcd. for $C_8H_7O_3$ $[M-H]^+$ 151.0395, found 151.0394.

Preparation of 4-((3-acetoxy-4-(methoxycarbonyl)-2,5 dimethylphenoxy)carbonyl)-2-(diacetoxymethyl)-5-methyl-1,3-phenylene diacetate (1a).

A mixture of compound **1** (0.05 g, 0.13 mmol), acetic anhydride (5 mL) and pyridine (catalytic amount) was stirred at room temperature for 12 h. The reaction mixture was poured into 20 mL of ice water. The brown colored solid was collected by filtration and washed with ice water. The reaction mixture was diluted with water and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulphate, concentrated under reduced pressure and chromatographed over silica gel column to afford compound **1a** as brown colored crystals (0.075 g, 93 %), m.p. 98-102 ^{0}C. IR (KBr): 2951 (C-H), 1776 ($OCOCH_3$), 1728 ($OCOCH_3$) cm$^{-1.}$ ^{1}H NMR (500 MHz, $CDCl_3$) δ = 2.01 (s, 3H, C8^1-CH_3), 2.06 (s, 3H, C9^1-CH_3), 2.30 (s, 3H, C9-CH_3), 2.301 (s, 3H, C4-$COCH_3$), 2.39 (s, 3H, C2-$COCH_3$), 2.57 (s, 3H, C3^1-$COCH_3$), 3.89 (s, 3H, C7^1-OCH_3), 6.94 (s, 3H, C5-H), 7.01(s, 1H, C6^1-H), 7.97 (s, 1H, HCOO); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 10.01 (C3^1), 20.05 (C4-$COCH_3$), 20.37 (C2-$COCH_3$), 20.46 (C3^1-$COCH_3$), 20.67 (C8-$COCH_3$), 20.80 (C8-$COCH_3$), 21.80 (C8-$COCH_3$), 21.31 (C9^1-$COCH_3$), 52.17 (C7^1-OCH_3), 83.45 (C8), 119.61 (C4^1), 121.36 (C2^1), 123.71 (C6^1), 124.28 (C3), 124.48 (C5), 136.24 (C5^1), 141.88 (C6), 148.26 (C3^1) 148.9 (C2), 150.23 (C4), 150.99 (C1^1), 162.81(C8-C=O), 166.42 (C4-C=O), 168.27 (C2-C=O), 168.32 (C3^1-C=O), 168.66 (C7), 168.93 (C7^1). HRMS (ESI): *m/z* calcd. for $C_{29}H_{30}NaO_{14}$ $[M+Na]^+$, 625.1528, found 625.1549.

Preparation of 3-methoxy-4-(methoxycarbonyl)-2,5-dimethylphenyl 3-formyl-2, 4-dimethoxy-6-methylbenzoate (1b)

To atranorin **1** (0.1 g , 0.26 mmol) in dry acetone (5 mL), was added methyl iodide (0.049 ml, 0.80 mmol) and K_2CO_3 (0.1 g, 0.80 mmol) and stirred the reaction mixture for 12 h. In the process of purification, acetone was evaporated under vacuum conditions and the reaction mixture was diluted with water and extracted with ethyl acetate (3x10 mL). The combined organic layer was dried over anhydrous sodium sulphate followed by concentration under reduced pressure and chromatography over silica gel column afforded compound **1b** as colourless crystals (0.08g, 72 %), m.p. 109-112 ^{0}C. IR (KBr): 3421 (C-H), 2940 (C-H), 2864 (C-H), 1740 (C=O) cm$^{-1.}$ ^{1}H NMR, (300 MHz, $CDCl_3$) δ = 2.1(s, 3H, C8^1- CH_3), 2.3 (s, 3H, C9-CH_3), 2.5 (s, 3H, C9^1-CH_3), 3.8 (s, 3H, C4-OCH_3), 3.9 (s, 3H, C2-OCH_3), 3.9 (s, 3H, , C5^1-OCH_3), 6.6 (s, 1H, C5-H), 6.8 (s, 1H, C6^1-H), 10.4 (s, 1H, C8-CHO); ^{13}C NMR, (125 MHz, $CDCl_3$) δ = 9.58 (C8^1) , 19.13 (C9^1), 20.91 (C9), 52.20 (C7^1-OCH_3), 56.26 (C4-OCH_3), 61.51 (C2-OCH_3), 64.51 (C3^1-OCH3), 109.14 (C3), 116.35 (C1,), 119.10 (C5), 121.36 (C4^1), 122.12 (C6^1), 126.62 (C2^1), 134.62 (C5^1), 145.20 (C6), 150.61(C1^1), 156.70 (C3^1), 160.50 (C2), 163.31 (C4), 165.12 (C7), 168.24 (C7^1), 187.9 (C8). HRMS (ESI): *m/z* calcd. for $C_{22}H_{25}O_8$ $[M+H]^+$, 417.1544, found 417.1562.

Preparation of 3-methoxy-4-(methoxycarbonyl)-2,5-dimethylphenyl 3-(hydroxymethyl)-2,4-dimethoxy-6-methylbenzoate (1c)

Compound **1b** (0.05 g, 0.12 mmol) was reduced to its corresponding alcohol by using sodium borohydride (0.004 g, 0.12 mmol) in methanol at 0 ^{0}C, the reaction mixture was stirred for 1h. Methanol was removed under reduced pressure after the reaction is over. The reaction mixture was diluted with water and extracted with ethyl acetate (3x10 mL). The organic layer was dried over anhydrous sodium sulphate, concentrated under reduced pressure and chromatographed over silica gel column to afford compound **1c** as colorless crystals (0.042 g, 83 %), m.p: 104-107 ^{0}C. IR (KBr): 3422 (O-H), 2923 (C-H) cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 2.19 (s, 3H, C8^1-CH_3), 2.30 (s, 3H, C9-CH_3), 2.49 (s, 3H, C9^1-CH_3), 3.81 (s, 3H, C4-OCH_3), 3.91 (s, 3H, C2-OCH_3), 3.93 (s, 3H, C7^1- OCH_3), 3.94 (s, 3H, C3^1-OCH_3), 4.75 (s, 2H, C8- CH_2), 6.60 (s, 1H, C5-H), 6.81 (s,

3H, C6^{1}-H); ^{13}C NMR (125 MHz, $CDCl_3$) δ = 9.34 (C8^{1}), 18.92 (C9^{1}), 20.16 (C9), 54.88 (C7^{1}-OCH_3), 55.58 (C4-OCH_3), 61.74 (C2-OCH_3), 63.94 (C3^{1}-OCH_3), 108.33 (C5), 118.97 (C6^{1}), 120.03 (C4^{1}), 121.89 (C2^{1}), 126.22 (C1), 134.31 (C6), 138.18 (C5^{1}), 150.55 (C1^{1}), 156.45 (C3^{1}), 157.14 (C4), 159.85 (C2), 165.66 (C7), 168.08 (C7^{1}); HRMS (ESI): *m/z* calcd. for C22H26NaO8 $[M+Na]^+$, 441.152, found 441.1554.

Preparation of 3-(allyloxy)-4-(methoxycarbonyl)-2,5-dimeth-ylphenyl 2,4-bis(allyloxy)-3-formyl-6-methylbenzoate (1d)

To atranorin **1** (0.05 g, 0.13 mmol) in analytical grade acetone solvent (5 mL), was added K_2CO_3 (0.05 g, 0.40 mmol) and allyl bromide (0.03 ml, 0.40 mmol) and stirred the reaction mixture at room temperature for 12h. After completion of the reaction as indicated by TLC, the reaction mixture was extracted with ethyl acetate (3x10 mL) and the combined organic layer was washed with water. The resultant organic layer was dried over anhydrous sodium sulphate. The dried organic layer was concentrated under reduced vapor pressure and chromatographed over silica gel column to afford compound **1d** as colorless crystals (0.055 g, 83 %), m.p. 82-86 ^{0}C; IR (KBr): 2923 (C-H), .2853 (C-H), 1646 (C=C), 1597 (C=C) cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 2.16 (s, 3H, , C8^{1}), 2.29 (s, 3H, S,), 2.50 (s, 3H, C9^{1}), 3.90 (s, 3H, C7^{1}-OCH_3), 4.42(dt, 2H, J= 5.49, 1.39, C4-OCH_2), 4.59 (dt, 2H, J= 5.95, 1.2, C2-OCH_2), 4.69 (dt, 2H, J= 5.03,1.5, C3^{1}-OCH_2), 5.24-5.28 (m, 2H, C4-allyl protons), 5.35-5.41 (m, 3H, C2- allyl protons), 5.47-5.52 (m, 1H, C4-allyl proton), 6.10-6.17 (m, 3H, C2- allyl protons); ^{13}C NMR, (125 MHz, $CDCl_3$) δ = 10.3 (C8^{1}), 19.5 (C9^{1}), 21.1 (C9), 70.0 (C4-O-C), 75.6 (C2-O-C), 78.3 (C3^{1}-O-C), 110.5 (C4^{1}), 117.7 (C2^{1}), 118.7 (C4-allyl carbon), 119.0 (C2- allyl carbon), 119.6 (C3^{1}- allyl carbon), 132.0 (C4- allyl carbon), 133.3 (C2- allyl carbon), 133.6 (C3^{1}- allyl carbon), 134.8 (C5^{1}), 145.0 (C6), 150.8 (C1^{1}), 155.8 (C2), 159.1 (C4), 162.5 (C3^{1}), 165.5 (C7), 168.5 (C7^{1}), 188.2 (C8). HRMS (ESI): *m/z* calcd. for C28H30O8Na $[M+Na]^+$ 517.1838, found 517.1830.

Preparation of 3-hydroxy-4-(methoxycarbonyl)-2,5-dimethyl-phenyl 2,4-dihydroxy-6-methyl-3-((2-phenylhydrazono)methyl) benzoate (1e)

Phenyl hydrazine (0.014 g, 0.013 mmol) and sodium acetate (0.013 g, 0.16 mmol) were added slowly to atranorin (0.05g, 0.13mmol) in DCM (5 mL). The reaction mixture was diluted with water and extracted with dichloromethane (3x10 mL). The Organic layer was dried over anhydrous sodium sulphate and evaporated under reduced pressure. The resultant residue was purified over silica gel column to afford compound **1e** as colorless crystals (0.051 g, 82 %), m.p. 170-172 ^{0}C. IR (KBr): 3419 (C=N), 3338 (C-N), 2922

sodium sulphate and evaporated under reduced pressure. The resultant residue was purified over silica gel column to afford compound **1e** as colorless crystals (0.051 g, 82 %), m.p. 170-172 ^{0}C. IR (KBr): 3419 (C=N), 3338 (C-N), 2922

(C-H), 2854 (C-H), 1654 (C=C, aromatic), 1565 (C=C, aromatic), 1491 (C=C, aromatic) cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$) δ = 2.10 (s, 3H, C2^{1}) , 2.54 (s,3H, C6-CH_3), 2.66 (s, 3H, C5^{1}-CH_3), 6.47 (s, 1H, C5-H), 6.52 (s, 1H, C6^{1}-H), 6.91-6.96 (m, 3H, aromatic), 7.29-7.32 (m, 2H, aromatic), 8.37 (s, 1H, C8-H); ^{13}C NMR, (125 MHz, $CDCl_3$) δ = 9.34 (C8^{1}), 24.0 (C9^{1}), 102.78 (C1), 105.34 (C2^{1}), 110.03 (C3), 112.53 (C5), 112.74 (NH-Ar), 116.21 (C6^{1}), 116.88 (C4^{1}), 120.84 (NH-Ar), 129.52 (NH-Ar), 136.84 (NH-Ar), 139.70 (NH-Ar), 143.38 (NH-Ar), 144.31 (C8), 152.32 (C1^{1}),162.82(C2), 163.69 (C4), 170.16 (C7), 172.22 (C7^{1}); HRMS (ESI): *m/z* calcd. for $C_{25}H_{25}N_2O_7$ $[M+H]^+$ 465.1662, found 465.1653.

Cytotoxicity Assay

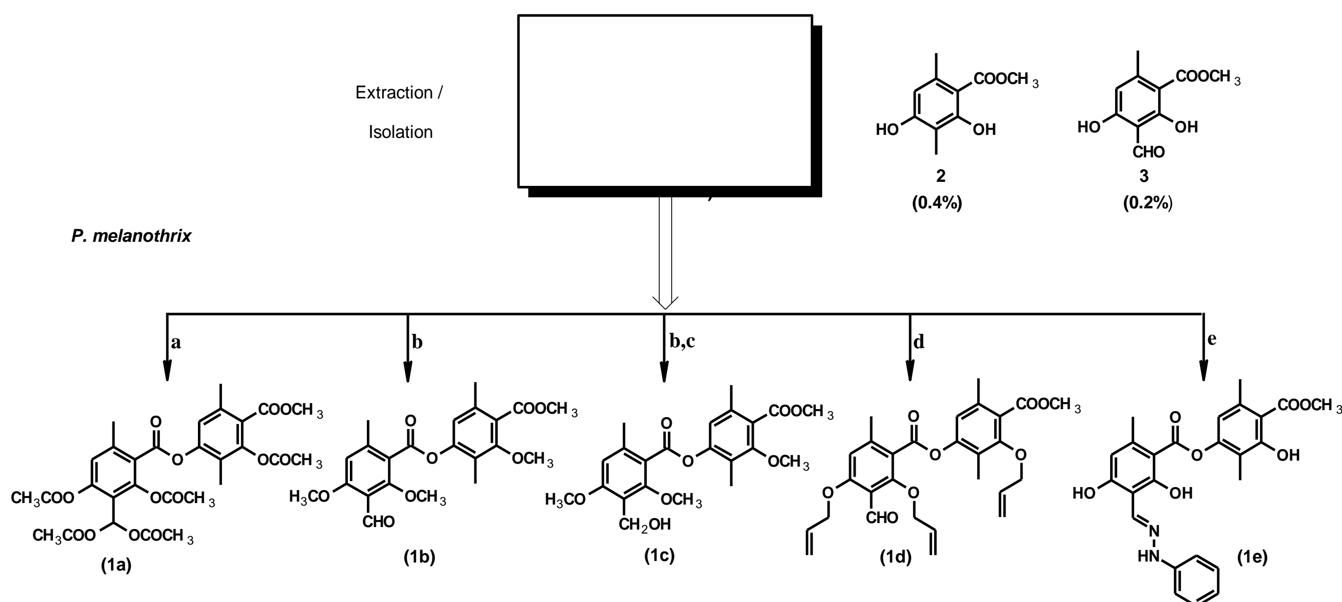
The above isolated and synthesized compounds were screened for anticancer activity against five different cell lines including A549 (Lung Cancer), DU145 (Prostate Cancer), MCF-7(Breast Cancer), SiHa (Cervical Cancer), U87MG (Glioblastoma) cell lines. The cells were plated at a density of 5 × 10^{3} cells per well in a 96 well plate supplemented with 10 % FBS and after 24 h of incubation at 37 ºC and 5 % CO_2, they were treated with respective concentration of the compounds dissolved in the culture media with vehicle controls and known standards for 48 h.

Cell viability was determined by adding 100 µL of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide, MTT reagent (0.5 mg mL^{-1}) dissolved in serum free media added to each well and incubated for 4 h. Then the media was aspirated and the formazan crystals were dissolved in 200 µL of DMSO and absorbance was taken at 570 nm in multimode plate reader (BioTek Instruments, Synergy 4, and Winooski, VT)). The percent cell inhibition in treated cells was calculated by normalizing the cells with 0 % inhibition with control group. Then the compounds which exhibited the percentage inhibition of more than 50 % at a concentration of 150 µM in the initial screening were further screened for dose response curve with series of 7 concentrations starting from initial 150 µM. MTT was performed as described previously and IC_{50} values were determined from DRC plot by linear regression method. Graph was plot between different concentration and percentage Inhibition. All the values were expressed as Mean ± SEM in three different experiments in which each treatment was performed in triplicates.

Results and discussion

Chemistry

P. melanothrix was collected from the lower temperate region of Tirumala Hills (13.65000 N 79.420 E) located in Andhra Pradesh, India. This region is having more saxicolous kind of *P. melanothrix*. Before carrying out extraction, it was washed with fresh water to remove foreign matter. The lichen material was shade dried, powdered in a pulveriser and extracted with acetone at room temperature overnight. The extract was concentrated to one quarter volume and left overnight at room temperature. Filtration of the extract yielded a brown coloured solid (2.2 %) and filtrate (2 %). The solid on recrystallisation from hexane-ethanol solvent system, yielded a single and pure compound **1** (Scheme 1). The residue obtained on evaporation of the filtrate, was subjected to silica gel flash column chromatography with n-hexane and ethyl acetate solvents as eluents to yield two pure compounds **2** and **3** (Scheme 1).



Scheme 1. Isolation of natural products from *P. melanothrix* and synthesized atranorin derivatives.

Table 1. Biological profile of atranorin derivatives over cancer cells.

S.No.	IC50 (µM)					
	A549	**DU145**	**HT-29**	**MCF-7**	**SiHa**	**U87MG**
1	96.70±0.92	52.03±5.72	45.16±4.66	71.9±4.66	86.41±3.48	>100
1a	29.25±4.40	15.19±2.90	19.16±0.72	30.35±1.54	22.07±2.55	16.96±1.83
1b	99.91±6.00	26.34±2.21	24.74±3.73	50.79±2.40	54.49±3.05	>100
1c	>100	42.7±2.42	63.04±5.97	67.09±2.06	>100	>100
1d	74.66±6.25	22.12±2.37	48.3±6.99	96.91±5.15	>100	>100
1e	58.96±1.70	27.52±5.36	>100	55.93±5.05	>100	>100
2	87.28±7.34	35.93±3.15	>100	84.53±4.91	83.93±6.18	80.44±6.88
3	>100	>100	>100	>100	>100	>100
Docetaxel	0.04	0.64	0.019	0.034	0.247	0.106

The structures of the isolated compounds were elucidated by their spectral data (^{1}H & ^{13}C NMR, IR and HRMS). Compound **1** was obtained as colourless crystals and its molecular formula was deduced as $C_{19}H_{18}O_8$ from its HRMS, which showed the molecular ion peak at m/z 373.0917 [M-H]$^+$.

The IR spectrum of the compound showed the absorption bands at 3450 and 1730 cm^{-1} corresponding to hydroxyl and ester functionalities respectively. ^{1}H and ^{13}C NMR spectra of the compound revealed its identity as atranorin. Its structure was further confirmed by comparing its physical and spectral data with reported values.[13] Atranorin was earlier isolated from few lichen species belonging to *Cladina*, *Evernia* and *Pseudoevernia* genera.[14-16] However, its present isolation assumes significance as it is now isolated in high yield (2 %). Compound **2** was obtained as colourless crystals (0.4 %). Its molecular formula was deduced as $C_{10}H_{12}O_4$ by ESI-HRMS, which showed the protonated molecular ion at m/z 197.0810 [M+H]$^+$. Its IR spectrum showed the absorption bands at 3404 and 1627 cm^{-1} corresponding to hydroxyl and ester functionalities respectively. Its ^{1}H and ^{13}C NMR spectra identifies its structure as methyl 2, 4-dihydroxy-3, 6-dimethylbenzoate. Its identity was further confirmed by comparing its spectral data with the reported values.[17] Compound **3** was obtained as colourless crystals (0.2 %) and its molecular formula was deduced as $C_{10}H_{10}O_5$ from ESI-HRMS, which showed the molecular ion peak at *m/z* 211.0608 [M+H]$^+$. Its IR spectrum showed absorption bands for hydroxyl (3404 cm^{-1}) and aldehydic carbonyl (1644 cm^{-1}) functionalities. Its ^{1}H and ^{13}C NMR spectra reveals its identity as methyl 3-formyl-2,4-dihydroxy-6-methylbenzoate.[18] Among the isolated metabolites, atranorin (**1**) was obtained in significant quantity (2%). It is to mention here that atranorin was reported to exhibit potent biological activities such as anticancer,[19] anti-inflammatory (Inhibition of LTB_4 biosynthesis).[20] Further atranorin was reported to be non toxic to normal cells and has an added advantage as a promising scaffold for making diverse analogues to identify potent bio-active lead molecules. Hence, compound **1** was fine tuned by chemical modification of its active functional groups (2 phenolic hydroxyls and 1 aldehydic group) and synthesised five diverse analogues as shown in scheme 1 (**1a**-**1e**). The structures of the synthesised analogues were confirmed by their spectral data (^{1}H and ^{13}C NMR, IR, HRMS). Treatment of **1** with acetic anhydride in presence of pyridine afforded compound **1a** in 93% yield. Its structure was confirmed by spectral data. The IR spectrum of compound **1a** showed sharp peaks at 1776 cm^{-1} and 1728

cm^{-1} corresponding to the carbonyl group in acetoxyl and ester functionalities. The ^{1}H NMR displayed peaks between δ 2.01 ~ 2.39, corresponding to the methyl protons of acetyl group. The ^{13}C NMR spectrum of compound **1a** showed peaks between δ 20.05-20.80 and δ 162.81- 168.66 corresponding to the methyl and carbonyl carbons of acetoxyl groups. The HRMS spectrum of compound **1a** showed the corresponding sodiated $[M+Na]^+$ molecular ion at *m/z* 625.1549 confirming its structure. Compound **1** when treated with alkyl halides (methyl iodide or allyl bromide) in presence of K_2CO_3 in acetone yielded the corresponding tri alkyl ethers (**1b** and **1d**) in good yield (72 & 83 %). The unsaturated ether (**1d**) showed the characteristic peaks in IR spectrum at 1597, 1458 and 1407 cm^{-1} confirming the presence of olefin groups. Its ^{1}H NMR spectrum showed the characteristic peaks between δ = 4.42-5.52 and δ = 6.10-6.17 corresponding to the methylene olefinic protons. The ^{13}C NMR spectrum while confirming these observations exhibited the carbon signal between δ 119.0-133.6. The HRMS spectrum of the compound confirmed its structure by exhibiting the sodiated molecular ion at *m/z* 517.1830 [M+Na]+ corresponding to $C_{28}H_{30}O_8Na$. Compound **1c** was obtained by the treatment of **1b** with $NaBH_4$ in presence of methanol in 83 % of yield. The IR spectrum of compound **1c** showed the characteristic absorption band at 3422 cm^{-1} corresponding hydroxyl group. The ^{1}H and ^{13}C NMR spectra of compound **1c** confirmed the absence of aldehydic group and presence of a hydroxy methylene group in the compound. The HRMS spectra of compound **1c** further confirmed its structure by exhibiting the sodiated molecular ion $[M+Na]^+$ at *m/z* 441.1554. The analogue **1e** was obtained, when compound **1** was treated with phenyl hydrazine in dichloromethane and sodium acetate to yield the product **1e** in 82 % yield. The structure of compound **1e** was identified by its spectral data, which exhibited absorption band at 1645 cm^{-1} corresponding to the imine functionality in IR spectrum and showed diagnostic signals at δ 8.37 and between δ = 7.29-7.32 in ^{1}H NMR spectrum corresponding to imine and aryl ring protons respectively. This further supported by the ^{13}C NMR spectrum, which showed characteristic signals δ = 143.38 corresponding to the imine carbons. The HRMS of compound **1e** showed $[M+H]^+$ molecular ion peak at *m/z* 465.1653 confirming its structure.

Cytotoxicity

The three isolated compounds (**1-3**) and five synthesized analogues **1a-1e** (Scheme-1), were screened for their cytotoxic potential against five human cancer cell lines such as A549 (lung cancer), DU145 (prostate cancer), MCF-7 (breast cancer), SiHa (cervical cancer), U87MG (glioblastoma) using docetaxel by employing MTT assay.[20] Analysis of data, presented in Table 1, revealed that the synthesised compounds showed enhanced activities than parent compound **1**. The cytotoxicity found to be increased when the three phenolic hydroxyls and aldehyde functionality were acylated as acetates (**1a**) against DU145 (IC_{50} = 15.19±2.90 μM), U87MG (IC_{50} = 16.96±1.83μM), HT-29 (IC_{50} = 19.16±0.72μM), SiHa (IC_{50} = 22.07±2.55 μM), A549 (IC_{50} = 29.25±4.40 μM) and MCF-7 (IC_{50} = 30.35±1.54 μM). When the methyl groups were introduced in compound **1** in the form of ethers (1b), cytotoxicity found enhanced against HT-29 (IC_{50} = 24.74±3.73μM) and DU145 ((IC_{50} = 26.34±2.21μM) cell lines. Interestingly, compound (**1d**) with allyl ether groups showed enhanced cytotoxic activity against the prostate cancer cell line DU145 ((IC_{50} = 27.52±5.36μM). Similarly, the phenyl hydrazone (**1e**) showed improved cytotoxicity against prostate cancer cell line DU145 ((IC_{50} = 22.12±2.37μM) than the parent compound **1**.

Conclusion

In conclusion, chemical screening of *P. melanothrix* resulted in the isolation three orcinol based metabolites (**1-3**) with the depside, atranorin (2 %) as the major metabolite. In view of its abundance and interesting skeletal features, compound (**1**) was subjected to chemical modification and synthesised five diverse analogues (**1a-1e**). Among the synthesised compounds, **1a** showed enhanced cytotoxicity against almost all the cancer cell lines screened with highest activity against the prostate cancer cell line with an IC_{50} of 15.19μM.

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