Cytotoxicity of 6-acetonyldihydrochelerythrin, arnottianamide and 6-(2-hydroxypropyl)-dihydrochelerythrine towards human cancer cell lines

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Received 27 November 2012; accepted (revised) 18 February 2014

The alkaloids, 6-acetonyldihydrochelerythrin \textbf{1} and arnottianamide \textbf{2} have been isolated for the first time from conical prickles on the stem bark of \textit{Zanthoxylum rhetsa} (Roxb.) DC. These compounds have been investigated for their cytotoxicity against seven cancer cell lines of various origins. The compound \textbf{1} is proved to be more potent as an anticancer agent and is very specific to the colon cancer cell line, SW-480 and cervical cancer cell line, HeLa. Compound \textbf{2} is more active against breast cancer cell lines. The change in anticancer activity of \textbf{1} after conversion to 6-(2-hydroxypropyl)-dihydrochelerythrine \textbf{3} has also been evaluated and a decrease in activity is observed.

**Keywords:** 6-Acetonyldihydrochelerythrin, arnottianamide, 6-(2-hydroxypropyl)-dihydro chelerythrin, \textit{Zanthoxylum rhetsa}, anticancer

\textit{Zanthoxylum} is a common component of rain forest vegetation belonging to the \textit{Rutaceae} family. Traditional healers have used different species of \textit{Zanthoxylum} for the treatment of tooth ache, urinary and venereal diseases, rheumatism and lumbago. The plants belonging to this genus have been subjected to various previous phytochemical investigations and the metabolites isolated from them include alkaloids, aliphatic and aromatic amines, amides, lignans, coumarins, sterols, carbohydrate residues, etc. Some of these metabolites are reported to be cytotoxic, molluscicidal, anticonvulsant, antisickling, anesthetic, antibacterial, antihypertensive and anti inflammatory\textsuperscript{1}.

\textit{Zanthoxylum rhetsa}, one among the genera \textit{Zanthoxylum}, is a deciduous evergreen tree with pale corky bark, covered with conical prickles on stems and branches, usually found in the evergreen forests and other parts of peninsular India and in different regions of Southern Asia\textsuperscript{2}. The fruits and stem bark of this plant are used traditionally as a stimulant, astringent, stomachic and digestive and prescribed for urinary infection, dyspepsia, heart troubles, tooth ache, asthma and bronchitis\textsuperscript{3}. The seed oil is effective in cholera and is useful as an antiseptic, disinfectant and anti-inflammatory agent\textsuperscript{4}. Compounds isolated from \textit{Z. rhetsa} include dihydroavicine, rhetsinine\textsuperscript{5}, N-methylflindersine, zanthobungeanine, dictamine, ruteacarpine, \textgreek{f}-agarine, skimmianine, evodiamine, canthin-6-one\textsuperscript{6}, rhetsine, rhetine, chelerythrine\textsuperscript{6} and hydroxyevodiamine\textsuperscript{7} from bark, arborine and dictamine from fruits\textsuperscript{8} and ruteacarpine from seeds\textsuperscript{9}. The two alkaloids, \textit{viz}, 6-acetonyldihydrochelerythrin
1 and arnottianamide 2 were isolated from this plant for the first time and were investigated for their cytotoxicity towards cancer cells. In order to compare the anticancer activity due to functional group transformation, 6-(2-hydroxypropyl)-dihydrochelerythrine 3 was synthesized from 1.

Results and Discussion

The compound 1 was obtained as a white crystalline solid giving blue fluorescence in UV at 366 nm, during elution with 20% ethyl acetate. It gave a positive test with Dragendorff’s reagent and 2,4-dinitro-phenylhydrazine and a yellow spot on spraying the plate with 20% aqueous sulphuric acid and heating to 110°C. This result suggested the compound to be an alkaloid with a carbonyl group. The melting point of the compound is 188°C and the optical rotation $[\alpha]_D ^{19} = -132^\circ$ (c 0.1g, CHCl₃)

The molecular mass of the compound, from the positive HREIMS is 405.14936, indicating a molecular formula of C₂₁H₂₃NO₃ for which the calculated molecular mass is 405.15761. This molecular formula corresponds to 6-acetonyldihydrochelerythrin earlier isolated from different plants. All the spectra of this compound were identical to that reported for 6-acetonyldihydrochelerythrin. This conclusion is supported by an intense peak at m/z 381.12088 corresponding to the molecular formula C₁₃H₁₀NO₆ for which the calculated molecular mass is 381.12123. The odd mass and the reaction with Dragendorff’s reagent indicate the compound to be an alkaloid.

The ¹H NMR spectrum displayed, in the aromatic region, two singlets at δ values 7.08 and 7.19 corresponding to H-1 and H-4. There are also two pairs of ortho-coupling doublets at δ 7.72 and 7.31 corresponding to H-5 and H-6 and δ 6.54 and 6.80 corresponding to H-11 and H-12 respectively in this region. The spectrum showed two methoxyl group protons at δ 3.90 and 3.92 and exhibited a singlet integrating as two protons at δ 6.08 typical of a methylenedioxy group signal. Moreover, the ¹H NMR spectrum also showed the three proton singlet at δ 3.01 and one proton singlet at δ 8.16 which can be attributed to N-methyl formamide of which the corresponding carbons appeared at δ 33.2 and 164.5 on ¹³C NMR spectrum. The rest of the signals in ¹³C NMR spectrum (125 MHz, DMSO-$d_6$) for the compound are at δ 55.9 (OCH₃), 61.3 (OCH₃), 99.3 (CH), 101.5 (CH₂), 104.1 (CH), 104.3 (CH), 118.6 (C), 125.0 (CH), 127.4(CH), 127.5 (CH), 128.8 (C), 131.3 (C), 133.4 (C), 135.6 (C), 135.8(C), 146.7 (C), 148.1(C), 149.3 (C) and 152.0 (C). These evidences combined with the HMBC, HMQC and DEPT spectra of the compound and comparison of the data from the earlier report proves the compound to be arnottianamide. This is the first report of this compound from Z. rhetsa.

The compound 3 was obtained by reduction of the carbonyl group of 1 using sodium borohydride (Scheme I). The reduction of 1 with NaBH₄ was performed affording the racemic alcohol 3 as a white solid. This hitherto unknown compound melted at 210°C and furnished a molecular ion peak at m/z 407.04189 corresponding to the molecular formula.
Table 1 — Comparison of IC50 values of the compounds 1, 2 and 3

<table>
<thead>
<tr>
<th>Cell line</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa</td>
<td>52.07</td>
<td>75.25</td>
<td>62.94</td>
</tr>
<tr>
<td>SK-BR-3</td>
<td>297.61</td>
<td>68.49</td>
<td>166.66</td>
</tr>
<tr>
<td>Hep G2</td>
<td>161.29</td>
<td>200.00</td>
<td>511.24</td>
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<tr>
<td>HCT 116</td>
<td>107.38</td>
<td>200.00</td>
<td>122.60</td>
</tr>
<tr>
<td>SW480</td>
<td>29.74</td>
<td>175.00</td>
<td>133.97</td>
</tr>
<tr>
<td>A375</td>
<td>500.00</td>
<td>75.00</td>
<td>874.12</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>71.00</td>
<td>61.00</td>
<td>82.00</td>
</tr>
</tbody>
</table>

C23H28NO5 (calculated 407.17326). 1H NMR (500 MHz, CDCl3) was very similar to 1 and the δ value 4.02-4.10 (1H, m,-C(OH)H-) confirms the reduction.

On analysis of the anticancer activity of the compounds, it was found that among the three compounds, 1 was the most potent. (Table I). It is very interesting to note that this compound is very specific to the colon cancer cell line, SW-480 and the cervical cancer cell line, HeLa while it is ineffective towards cancer cells of other tissue origin. Among the seven cancer cell lines studied, the cervical cancer cell line HeLa was sensitive to all the compounds (Table I) and showed moderate IC50 values in response to all the three compounds (Table I). The Hepato carcinoma cell line, HEPG2 was the most resistant (Table I) among the cell lines studied and none of these drugs could induce cytotoxicity in this liver cancer cell line (IC50 value range, 156.25 µM – 625 µM). Among the colon cancer cell lines, HCT 116 was resistant to all the compounds studied, whilst 1 induced cytotoxicity in SW480 (IC50-29.74µM). The reason for the differential action of these compounds in cancer cells of same tissue origin has to be studied further. Similarly, among the breast cancer cell lines, SKBR3 was moderately sensitive only to 2 (IC50-68.49µM) while MDAMB 231, another breast carcinoma cell line was moderately sensitive to all the compounds studied (Table I) out of which compound 2 (IC50-61µM) produced maximum cytotoxicity.

Similarly, 2 caused moderate cytotoxicity in the melanoma and cervical cancer cell lines (IC50-75µM). It is also noteworthy that the melanoma cell line is highly resistant to the compounds 1 and 3 (IC50-500µM and 874.12 µM respectively). Further studies are required to find out the mechanism behind the variation in their cytotoxicity towards cancer cells of various tissue origins.

Experimental Section

Melting points were measured on a Büchi SMP-20 melting point apparatus using one-side open capillary tubes and are uncorrected. NMR spectra were recorded on a Bruker ARX 500 instrument at RT. Mass spectra were recorded on FINNEGAN MAT 8200 and FISON MD 800 instruments.

Plant material

The plant material was collected from the botanical garden, University of Calicut. It was authenticated by Dr. A. K. Pradeep, Department of Botany, University of Calicut. A voucher specimen of the plant material has been deposited in the specially maintained herbarium in the Department of Chemistry, University of Calicut.

Extraction and isolation

The prickles on the stem bark (5.0 kg) was coarsely powdered and successively extracted with petroleum ether and acetone (60-80°C, 3 × 7 L) respectively. The combined extracts in each solvent system was filtered and concentrated to 200 mL. The concentrated acetone extract, after removal of the solvent, yielded a dark green residue (98 g) which was then fractionated by column chromatography over silica gel using mobile phase gradients of ethyl acetate in petroleum ether (from 0% up to 100% ethyl acetate).

6-Acetonyldihydrochelerythrin, 1. White crystalline solid with blue flourescence in UV light, m.p. 188°C. 1H NMR (500 MHz, CDCl3): δ 2.06 (3H,
The cervical cancer cell line HeLa, hepatocarcinoma cell line, Hep G2, the breast cancer cell lines SK-BR-3 and MDA-MB-231, colon cancer cell lines, HCT 116 and SW480 and the melanoma cell line, A375 were used for the antitumor activity. All these cell lines were obtained from NCCS, Pune and were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, along with 100 units/mL penicillin, 50 µg/mL streptomycin and 1 µg/mL of amphotericin-B and were maintained at 37°C in a humidified atmosphere of 5% CO2 and 95% air.

For the cytotoxicity experiments, cells (3×10^4 /well) were seeded in 0.2 mL of the medium (DMEM with 10% FBS) in 96-well plates. After overnight incubation, various concentrations of the compounds (10-100 µM) were added to the cells and after 72 hr, the percentage of viable cells in the wells was determined by MTT assay. 16

Briefly, 72 hr after the drug treatment, the drug containing media was removed and fresh media containing 25 µL of MTT solution (5 mg/mL in PBS) and 75 µL of complete medium were added to wells and incubated for 2 hr. At the end of incubation, MTT lysis buffer (20% sodium dodecyl sulphate in 50% dimethyl formamide) was added to the wells (100 µL /well) and incubated for another 1 hr at 37°C. At the end of incubation, the optical densities at 570 nm of treated samples/ A and 570 of control samples were measured using a plate reader (Bio-Rad). The relative cell viability in percentage was calculated as the percentage of treated samples/ control samples × 100.

Acknowledgement
One of the authors (PMS) is thankful to UGC, New Delhi for financial assistance under SAP.

References
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