Broad Spectrum Anticancer Activity of Pistagremic Acid

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Abstract

Background: Pistagremic acid; 3-methyl-7-(4,4,10,13,14-pentamethyl-3,2,3,4,5,6,7,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthr-en-17-yl)-oct-3-enoic acid was isolated from the chloroform fraction of Pistacia integerrima. Cytotoxic evaluation against NCI-60 DTP human tumor cell line was performed.

Methods: The anticancer assays for this compound were performed in accordance with the protocol of the Drug Evaluation Branch, of the National Cancer Institute (NCI) Developmental Therapeutic Program (www.dtp.nci.nih.gov) for their anticancer activity in vitro.

Results: It showed broad spectrum antiproliferative activity with an average GI50, and TGI, values 0.103 µM and 0.259 µM, respectively. It also showed significant LC50 value at the average 0.634 µM against all cell lines excluding K-562, RPMI-8226, NCI-H226, and NCI-H460 cell-lines.

Conclusions: Pistagremic acid showed cytotoxicity for all tested cancer cell line, thus it may serve as a potential structure lead for the development of new anticancer drugs.

Keywords: Pistacia integerrima; Pistagremic acid; Antiproliferative agent; Anticancer agent

Introduction

Pistacia integerrima belongs to family Anacardiacea and found in eastern Himalayan range [1]. The leaves, bark and galls of the plant are generally used for the treatment of cough, asthma, fever, vomiting, diarrhea, infection, hyperuricemia, hepatitis and other liver disorders [2-7].

Cancer is one of the notorious and lethal diseases responsible for 7.6 million deaths (approx. 13% of all deaths) with 12.7 million new cases in 2008, that are projected to continue rising, with an estimated 13.1 million deaths with around 22.2 million cases in 2030 [8,9]. Although various types of cancer reported, cancers of the prostate, lung, and liver are the most commonly reported worldwide. There is similar index as the worldwide to developing countries like Pakistan [9].

Researchers have been spending several decades to identify effective agents against cancer which is one of the leading causes of death worldwide. Thus, about 60% of anticancer drugs are structural modification and their analogues from the natural sources such as vinblastine, taxol and camptothecin [10]. However, the management of cancer is still not up to mark and always needs to find out new chemotherapeutic agents. In continuation of our previous investigation on the bioactivities of pistagremic acid (Figure 1) [11], which was evaluated for its cytotoxic effects against NCI 60-cell panel on eight organs (leukemia, non-small cell lung cancer, colon, CNS, melanoma, ovarian, prostate, renal and breast) at National Cancer Institute (NCI) USA, showed significant inhibition of human cancer cell lines.

Materials and Methods

Pistacia integerrima galls were collected from Razagram, Khyber Pakhtunkhwa, Pakistan in February, 2010 and identified as P. integerrima by Prof. Dr. Abdur Rashid, Department of Botany, University of Peshawar. A voucher specimen (RF-895) preserved in the herbarium of the Department of Botany, University of Peshawar, Pakistan.

The plant material was shade-dried at room temperature, grinded into powder and subsequently successively extracted repeatedly (thrice) with methanol (MeOH). The combined methanolic extracts were freed of the solvent under vacuum to obtained thick syrup. Then 400 g of the concentrated methanolic extract was successively partitioned between n-hexane/water, chloroform/water, ethyl acetate/water and butanol/water. The chloroform fraction was concentrated under vacuum pressure and 10 g of it was subjected to silica gel column chromatography, eluting with n-hexane, n-hexane-ethyl acetate (EtOAc) in an increasing order of polarity. As a result, 13 fractions (RF-1 to RF-13) were obtained based on TLC profiles. Fraction RF-4 (90 mg, eluted n-hexane-EtOAc, 82:18) afforded colorless crystals of various sizes which were separated from the solution by decantation. The crystals were re-crystallized with appropriate solvents (n-hexane-acetone, 4:1). The chemical structure of the pure crystalline compound was identified as 2-Methyl-6-(4, 4, 10, 13, 14-pentamethyl-3-xo-2, 3, 4, 5, 6, 7, 10, 11, 12, 13, 14, 15, 16, 17-tetradecahydro-1H-cyclopenta[a] phenanthen-17-yl) hept-2-enoic acid (pistagremic acid) by X-ray crystallography analysis as reported earlier [12].

Anticancer Assay

The anticancer assay against cancer cell lines were conducted at NCI by using Sulphorhodamine B assay [13,14]. These cancer cell lines were grown in RPMI-1640 medium, supplemented with fetal bovine serum (10%) and L-glutamine (2 mM). The culture flasks were kept in CO2.

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Received July 31, 2013; Accepted August 17, 2013; Published August 26, 2013


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(5%) incubator at 37ºC and 100% relative humidity. The adherent cells were obtained using Trypsin–EDTA solution, followed by addition of 10⁶ cells per ml in each well of 96-well plate. The plates were incubated in CO₂ incubator for 24 h prior to addition of tested samples. Aliquots (100 µL) of different dilutions of test extract and compound was added to the appropriate wells and incubated for 48 h. Then cells was fixed in situ by the gentle addition of 50 µL of cold 50% (w/v) trichloroacetic acid (TCA) (final concentration, 10%) and incubated for 30 minutes at 25ºC. The supernatant was discarded, and the plates were washed five times with tap water and air dried overnight. Sulphorhodamine B (SRB) solution (100 µL) at 0.4% (w/v) in 1% acetic acid was added into each well, and plates were incubated for 30 minutes at room temperature. After staining, unbound dye was removed by washing three times with 1% acetic acid and the plates were left to air dry. After 24 hours of drying the bound stain was subsequently solubilized 10 mM trizma base, and the absorbance was recorded in microplate reader at 545 nm by using a microplate ELISA reader (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxicity was recorded as concentration causing 50% growth inhibition.

Growth inhibition of 50% (GI₅₀) was calculated when \[\frac{[T_i - T_z]}{(C - T_z)} \times 100 = 50\]. The compound concentration resulting in total growth inhibition (TGI) was calculated when \(T_i = T_z\). The LC₅₀ indicating a net loss of cells following treatment was calculated when \[\frac{[T_i - T_z]}{T_z} \times 100 = -50\].

Where, \(T_z\) = absorbance measurements at time zero, \(C\) = control growth, and \(T_i\) = test growth in the presence of drug at the five concentration levels.

### Results and Discussion

The cytotoxic activity of pistagremic acid was evaluated using in vitro screening assay against NCI-60 DTP human tumor cell lines (Table 1). The compound was first evaluated at one dose toward 60 cancer lines (concentration 10⁻² mM). The compound showed the potent growth

<table>
<thead>
<tr>
<th>Panel/Cell line</th>
<th>GI₅₀ (µM)</th>
<th>TGI (µM)</th>
<th>LC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leukemia</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CCRF-CEM</td>
<td>0.07</td>
<td>0.25</td>
<td>0.73</td>
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<tr>
<td>HL-60(TB)</td>
<td>0.11</td>
<td>0.27</td>
<td>0.64</td>
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<tr>
<td>K-562</td>
<td>0.09</td>
<td>0.27</td>
<td>-</td>
</tr>
<tr>
<td>MOLT-4</td>
<td>0.13</td>
<td>0.30</td>
<td>0.69</td>
</tr>
<tr>
<td>RPMI-8226</td>
<td>0.07</td>
<td>0.26</td>
<td>-</td>
</tr>
<tr>
<td>SR</td>
<td>0.12</td>
<td>0.30</td>
<td>0.76</td>
</tr>
<tr>
<td>Non-small cell Lung Cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A549/ATCC</td>
<td>0.04</td>
<td>0.18</td>
<td>0.59</td>
</tr>
<tr>
<td>EKVX</td>
<td>0.05</td>
<td>0.18</td>
<td>0.54</td>
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<tr>
<td>HOP-62</td>
<td>0.19</td>
<td>0.34</td>
<td>0.62</td>
</tr>
<tr>
<td>HOP-92</td>
<td>0.06</td>
<td>0.19</td>
<td>0.46</td>
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<tr>
<td>NCI-H226</td>
<td>0.10</td>
<td>0.37</td>
<td>&gt;10</td>
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<tr>
<td>NCI-H23</td>
<td>0.06</td>
<td>0.21</td>
<td>0.58</td>
</tr>
<tr>
<td>NCI-H460</td>
<td>0.07</td>
<td>0.29</td>
<td>&gt;10</td>
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<tr>
<td>NCI-H522</td>
<td>0.07</td>
<td>0.22</td>
<td>0.56</td>
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<tr>
<td>Colon Cancer</td>
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<tr>
<td>COLO 205</td>
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<td>0.32</td>
<td>0.63</td>
</tr>
<tr>
<td>HCC-2998</td>
<td>0.08</td>
<td>0.21</td>
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<tr>
<td>HCT-116</td>
<td>0.08</td>
<td>0.23</td>
<td>0.61</td>
</tr>
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</table>
inhibition against CNS cancer (SF-295) cell-line at GI50 value = 32 nM whereas 193 nM was the highest GI50 value showed against non-small cell lung cancer (HOP-62) cell-lines. Similarly, in terms of lethal concentration 50% (LC50) 45 nM was the lowest value shown against melanoma (UACC-62) cell-lines and over 10000 nM against non-small cell lung cancer (NCI-H226, and NCI-H460) cell-lines. Thus, lung cancer cell-lines showed slight resistance toward this molecule. In dose-dependent response by 10 fold, the typical sigmoidal curve
was observed (Figure 2 and 3) for this compound. Many triterpenes have shown anticancer activity with diverse mechanisms of actions. For example, reactive oxygen species generation, caspase-3 mediated apoptosis [15], inducing oxidative stress and FasL-caspase-3 apoptotic pathway [16]. Triterpenes also have shown to alter the potential across mitochondrial membrane, which may interfere for ATP generation and leading to cell death [17]. A triterpene, maslinic acid found to be activating JNK and p53, leading to activation of caspase-9, which causes mitochondrial degradation and apoptosis [18]. Carboxylic acid present in pistagremic and the core hydrophobic moiety both might have played role to initiation signal and ultimately cell death. Thus, pistagremic acid could showed inhibition against almost all cell-lines and within somewhat narrow range of concentration, and inhibition might be due to same above mentioned mechanism.

Conclusions

In conclusion, we have isolated a broad spectrum anticancer triterpene that exhibited antiproliferative effects at nanomolar range against almost all of the cell lines in the NCI-60 which may serve as a potential lead compound for the development of new anticancer drugs.

Acknowledgments

The authors are grateful to the members of USA National Cancer Institute (NCI), Developmental Therapeutics Program (DTP) for performance of antiproliferative and cytotoxicity evaluations.

References


Figure 2: Dose response curves of pistagremic acid against all cell lines.
Figure 3: Dose response curves of pistagremic acid against the different cell lines of Leukemia, Non-small cell lung cancer, Colon cancer, CNS cancer, Melanoma, Ovarian cancer, Renal cancer, Prostate cancer and Breast cancer.


