

# Reversal of multidrug resistance and antitumor promoting activity of 3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyirin isolated from *Pistacia integerrima*

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**Abstract:** The bioactive triterpenoid 3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyirin (**1**) has been isolated from multiple plant sources. In this study, chloroform fraction of *Pistacia integerrima* extract was processed for the isolation of the compound. The compound identity was confirmed by advanced spectroscopy technique. X-ray crystallography was applied for molecular structure confirmation. In addition, compound **1** was screened for its activity on reversal of MDR (multidrug resistance) mediated by P-gp (P-glycoprotein). This was accomplished by using rhodamine123 exclusion on multidrug-resistant human ABCB1 gene transfected mouse T-lymphoma cell line. Outcomes revealed that MDR reversing effect was comparable to verapamil as positive control *in vitro*. Treatment of TPA-induced tumor promotion with 3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyirin led to reduction in the applied anti-tumor promotion experiment. The chemo-preventive effect of 3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyirin was comparable to curcumin as positive control based on the reduction of immediate early tumor antigen expression. Molecular docking by applying Autodock Vina 1 and i-GEMDOCK v 2.1 tools indicated that compound **1** gives good docking results, as determined by their fitness score and specificity. Moreover, results showed that compound **1** isolated from *Pistacia integerrima* precisely attached to a region where co-crystallized ligand for receptor previously existed. Our findings may explain the use of *Pistacia integerrima* plant extracts as an anticancer agent in folk medicine.

## Introduction

The most important difficulty in chemotherapy and in the cure of cancer are the resistance pattern of cancer cells known as multidrug resistance (MDR) (Szabó and Molnar, 2016).

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The resistance of Cancer cells to anticancer agents is developed through several mechanisms. One of such mechanisms is the overexpression of the ATP-binding cassette (ABC) transporters. These transporters are actually largest family of proteins that are bound to the membrane and bind with ATP (Leonard *et al.*, 2003; Gottesman and Ambudkar, 2001). In energy dependent manner, ABC efflux transporters extrude amphipathic compounds against the concentration gradient. A number of ATP-binding cassette transporters play a physiological role, i.e., protection of liver, kidney and brain (Gottesman and Ambudkar, 2001; Sarkadi *et al.*, 2006; Szakács *et al.*, 2006).

P-glycoprotein is the first known drug efflux encoded with the gene ABCB1. This protein consists of 1,280 amino acids and two transmembrane domains (Szakács *et al.*, 2006). Additionally, in several human cancers the overexpression of this protein occurs and can eject a wide-ranging of drugs, i.e., antibiotics, anticancer, antidepressants, and others (Caraci *et al.*, 2011). So, it decreases drug accumulation in multidrug-resistant cells. Some drugs have been proposed to suppress the activity of ABCB1, e.g., tamoxifen, dexniguldipine, valsopodar and tariquidar (Lopez and Martinez-Luis, 2014; Germann *et al.*, 1993). In carcinogenesis studies, assessment of EBV-EA inhibition is employed as preliminary screening model for in-vitro antitumor enhancing potential of different chemopreventive agents. EBV is a herpes family virus capable of several cancers (such as gastric cancer, nasopharyngeal carcinoma, Hodgkin's lymphoma, among others). Presence of antibody to the EA of EBV indicates that EBV is active, replicating and capable of cancerous transformation. So, any inhibitor of the EA is considered to have anticancer effect (Kapadia *et al.*, 2000).

MRP1 (multidrug-resistant protein-1) was initially described in doxorubicin-resistant lung cancer cells. Without expression of ABCB1, it displays a multi-drug resistant phenotype (Cole *et al.*, 1992). MRP1 shows overexpression in intestines, blood-brain barrier, & oral mucosa (He *et al.*, 2011); however, among the organ in lung its expressed higher and this may have a protective role in air contamination and toxin enter through inhalation (Sakamoto *et al.*, 2013). The physiological substrates of multidrug-resistant protein-1 are reported to be glutathione conjugates leukotriene C<sub>4</sub>, bile acid, and folic acid. It showed resistance against, methotrexate, vincristine, and etoposide doxorubicin (Cole and Deeley, 2006). Approximately 31.6% of lung tumors have been identified to have MRP1 expression and showed weak response to cisplatin therapy with paclitaxel, gemcitabine and vinorelbine (Li *et al.*, 2009).

Furthermore, Doyle *et al.*, (1998) first cloned the BCRP (Breast Cancer Resistance Protein) through MCF-7 (drug-resistant breast cancer cell line). Breast cancer resistance protein with a size of 72 kDa is a half transporter member of the ATP-binding cassette transporter subfamily G (ABCG2). Owing to its presence in tissues including liver, placenta, ovary, colon, small intestine, prostate gland, and brain, the expression of BCRP overlaps largely with ABCB1 (Doyle *et al.*, 1998). Similarly, it was noted that overexpression is often linked with the resistance of large

number of anticancer compound, i.e., mitoxantrone, antifolates, flavopiridol camptothecins and anthracyclines (Assaraf, 2006; Bihorel *et al.*, 2007; Robey *et al.*, 2007). A number of publications have dealt with the large occurrences of drug efflux mechanism in cancer region. Number of literature described significant relation overexpression of MRP1 or ABCB1 and improper treatment response in leukemia & solid tumors (Larkin *et al.*, 2004; Damiani *et al.*, 2006; Brinkhuis *et al.*, 2002), whereas others literature have also cited a prognostic relationship for BCRP overexpressions (Nampoothiri *et al.*, 2008).

*Pistacia integerrima* is a well-known member of family Anacardiaceae family, which is commonly known as Kakar sigghi in Eastern Himalayan (Ismail *et al.*, 2011), at an altitude of 2.4 to 3.6 km. This is a medium sized deciduous plant with folkloric health modulating importance for a number of ailments. Previous studies have indicated that this plant has anti-inflammatory, expectorant, blood purifier, gastroprotective, anti-asthmatic, antidiarrheal properties (Uddin *et al.*, 2011; Ahmad *et al.*, 2010). Particularly, the gall of *P. integerrima* tree has been traditionally used to cure diarrhea, asthma, psoriasis, fever, liver disorders, and oxidative stress, etc. (Uddin *et al.*, 2012a; Uddin *et al.*, 2012b; Ullah *et al.*, 2014). The barks of *P. integerrima* are also used in the folkloric system for the treatment of cough, asthma, fever, diarrhea, snake bite as well as jaundice (Rahman *et al.*, 2011). Amyrin type of terpenes has been reported for significant anticancer and cytotoxic activity (Wen *et al.*, 2018; Mishra *et al.*, 2016). 3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyrin is a bioactive amyrin type of triterpenoids which has documented for significant  $\beta$ -secretase,  $\alpha$ -glucosidases activity (Bawazeer *et al.*, 2020a). 3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyrin has also documented for anti-inflammatory, muscle relaxation, gastrointestinal, and antipyretic potential (Rauf *et al.*, 2016a; Bawazeer *et al.*, 2020a). In continuation of our phytochemical and pharmacological investigations of *P. integerrima* extracts, we have isolated the triterpenoid 3-oxo-6  $\beta$ -hydroxy-  $\beta$ -amyrin (1), also  $\beta$ -sitosterol and stigmasterol from the chloroform fraction of the plant extract. This compound has been identified as 3-oxo-6 $\beta$ -hydroxy-  $\beta$ -amyrin based on 2-D NMR and single X-ray crystallography techniques. Accordingly, the current finding was performed to discover the reversal ability of the isolated compound for multidrug-resistance in mouse lymphoma cells.

## Materials and Methods

### Chemicals and equipment

All the chemicals as well reagents used in this screening test were commercially grade. Melting points were calculated through Bicoth (Bibby Scientific limited, UK) melting point apparatus. We recorded FT-IR spectra as KBr disks, from 400 to 4000 cm<sup>-1</sup>, on Nicolet 380 FT-IR spectrophotometer (Thermo Scientific, UK), and UV-Vis spectra, 200 to 700 nm, were acquired with the aid of a Hitachi-U-3200 (Japan) instrument with chloroform solutions (1-cm cell). <sup>1</sup>H-NMR (500 MHz), <sup>13</sup>C-NMR (125 MHz), HMBC (500 MHz), and HSQC (600 MHz) spectra were acquired with a NMR spectrometer (AVANCEIII AV600) equipped with a

Cryoprobe in solvent (CDCl<sub>3</sub>) and employed with TMS (internal standard). Chemical shifts were reported as ppm (parts per million) and expressed as  $\delta$  units, while the values for J (coupling constant) were expressed in Hz (Hertz). In order to obtain EI-mass spectral data mass spectrometer (JMS-HX-110; JEOL) was employed; EI source 70 eV. Column chromatography was performed with the help of Merck silica gel-60 having dimensions as 0.063 to 0.200 mm, whereas aluminum TLC-plates in which silica gel was pre-coated with F254 (fluorescence indicator) was supplied by Fluka.

#### Plant collection

The bark of *Pistacia integerrima* was procured from village Razagram, KPK, Pakistan (Feb, 2010). Identification and authentication were done by a botanist, Prof. Dr. Abdur Rashid, University of Peshawar (UOP), Pakistan. The voucher specimen (Bot. 20037(PUP)) was submitted to herbarium at the Department of Botany, UOP, Pakistan.

#### Preparation of extract

After collection, the *P. integerrima* bark was dried under shade at 25°C followed by grinding to form uniform powdery sample. This powder was then subjected to methanol for extraction purposes (Bawazeer, 2020b). The extraction was repeated three times. Solvents were evaporated from the methanolic extracts, under reduced pressure provided a syrupy liquid (400 g). The syrup liquid substance was sequentially fractionated between butanol-water, chloroform-water, n-hexane-water, and ethyl acetate-water. Afterwards, chloroform (CHCl<sub>3</sub>) fraction was concentrated by rotary evaporation using anhydrous Na<sub>2</sub>SO<sub>4</sub> and resultant residue obtained weighed 98.6 g. A small quantity (10 gram) of CHCl<sub>3</sub> extract was processed through column chromatography (silica gel based) and was initially eluted using n-hexane followed by elution with mixture of n-hexane and ethyl acetate solvent system in an increasing polarity manner. Ten fractions (F-1 to F-10) of the eluted liquid were collected. On TLC profiling, the fraction F-3 (60 mg; eluted with n-hexane-ethyl acetate, 82:18, v/v) resulted colorless crystals (compound 1). Later by decantation process, these crystals were separated from the solution. Finally, compound 1 was identified and characterized as 3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyrin by different spectroscopic techniques such as <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, IR, and mass spectral data, the data was compared to literature and found identical (Wang *et al.*, 2005).

#### In-vitro EVA-EA (Epstein-Barr-Virus early antigen) activation induction assay

Compound 1, 3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyrin was evaluated *in vitro* against EBV-EA, activation assay. Concisely, Raji cells (lymphoblastoid cells) derived from Burkitt's lymphoma carried by EBV genome. These cells were cultured using RPMI-1640 medium provided 10% fetal bovine serum (FBS). Using an indirect immunofluorescence technique, smears from cell suspension were made (Kapadia *et al.*, 2000; Wang *et al.*, 2006). EBV-EA activation was less than 0.1% in experimented subline of Raji cells. For 48 hours, cells were incubated in medium (1 mL) containing butanoic

acid (4 mM), TPA (12-O-tetradecanoylphorbol-13-acetate) [32 pM = 20 ng in 2  $\mu$ L dimethyl sulfoxide (DMSO)] and test compounds (various amount) dissolved in 2  $\mu$ L of DMSO. By indirect immunofluorescence assay the EBV-EA-inducing cells were stained. A minimum of 500 cells were calculated for each assay, and number of positive (stained cells) were noted. EBV-EA induction (average) of experimented biomolecule (compound-1) was determined in ratio relative to the control experiment (100%), processed with butanoic acid (4 mM) and 12-O-tetradecanoylphorbol-13-acetate (32 pM). Around 35% was calculated the EBV-EA induction. By using trypan blue staining method, viability of treated Raji cells was noted where viability of cells for TPA-positive control was >80%. So, just those compounds showing induction <80% (% of control) of the EBV-active cells (having cell viability >60%) were capable of inhibiting the activation due to promoter substances. Curcumin was used as a standard compound (Wang *et al.*, 2006; Zhang *et al.*, 2013).

#### MDR reversal assay

The following procedure was applied on mouse lymphoma cells for determination of reversal MDR of compound 1. Medium (McCoy's 5A) consists of 10% inactivated (by heat) horse serum provided with antibiotics and L-glutamine was employed to grow L5178 and L5178Y MDR cell lines. As mentioned earlier by Rauf *et al.* (2015b), the L5178 mouse T-cell lymphoma parent cells were stably infected with pHa MDR1/A retrovirus. The L5178Y (MDR1-expressing cell line) was selected by culturing the transfected cells with colchicine containing medium. Then, Cells were distributed using aliquots (0.5 mL) into Eppendorf tubes after being adjusted to a density of  $2 \times 10^6$  cells per mL and suspended in the McCoy's 5A medium (serum-free). Final concentrations (4  $\mu$ g/ml) of test compounds and positive control (verapamil) were used. Verapamil was used as a positive control as it is a chemosensitizer & Ca<sup>+2</sup> channel blocker that inhibits multidrug resistance gene product and therefore reduces MDR1-mediated drug resistance. At room temperature, incubation of samples were carried out for 10 minutes prior to use. Then, added 10  $\mu$ L of rhodamine-123, (5.20  $\mu$ M) in samples to employed as an indicator. Cells were further incubated for 20 minutes and washed (two-times), and re-suspended in Phosphate buffered solution (0.5 mL) for examination. With the aid of a flow cytometer i.e., Partec CyFlow (Germany) we measured the fluorescence of cell population, using DMSO as the solvent as well as control. Using this technique, percent FI (Fluorescence Intensity) was measured for treated parental and MDR-cell lines in comparison to untreated cells. On the basis of FI values, fluorescence activity ratio (FAR) was calculated using the following equations (Rauf *et al.*, 2016b).

$$FAR = \frac{MDR_{treated} / MDR_{control}}{parental_{treated} / parental_{control}}$$

#### Computational studies

Protein data bank (PDB) was used for the retrieval purposes of crystalline structure of mice P-gp (coded as: 4Q9L)

(Sussman *et al.*, 1998). The 3-D structure of P-gp was subjected to an energy minimization process with the aid of a program known as Swiss-PDB viewer (version 4.1.0) (Guex and Peitsch, 1997). For docking studies, Avogadro's software & Chem sketch was used for the preparation of ligands structures (Hanwell *et al.*, 2012). Similarly, i-GEMDOCK (version 2.1) & Autodock Vina were used for docking purposes (Trott and Olson, 2010; Hsu *et al.*, 2011). For optimization of docking method co-crystallized inhibitor of P-glycoprotein was used (Shityakov and Förster, 2014). Two software namely LIGPLOT plus (version 1.4.5) and discovery studio visualizer was used subsequently for docking analysis (Rauf *et al.*, 2015b; Azam *et al.*, 2013).

## Results

### Characterization of compound 1

Compound 1, was purified as white crystals. Spectral data employed that the chemical structure of 1 (Fig. 1) has been identified as 3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyrin. For confirmation, single X-ray crystallography was carried out, which confirmed the structure (Fig. 2).

### Effect in EVA-EA (Epstein-Barr-Virus early antigen) activation induction assay

Tab. 1 presents the results of the EVB-EA activation induction assay. Our findings reveal that compound 1 caused significant antitumor promotion activity at various test concentrations with an IC<sub>50</sub> value of 458  $\mu$ g/mL. However, the standard

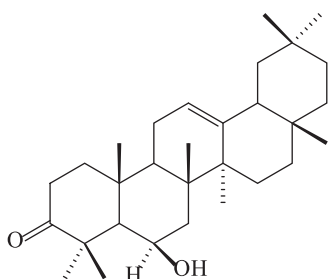


FIGURE 1. The structure of compound 1 (3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyrin).

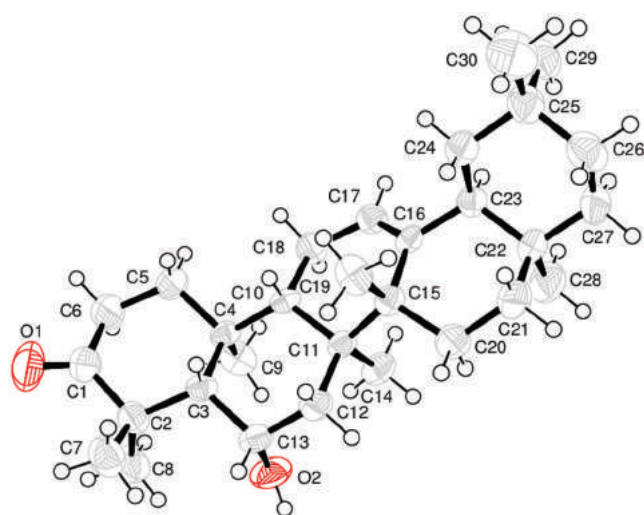


FIGURE 2. X-ray crystallographic image of compound (1).

compound, curcumin was more potent with IC<sub>50</sub> of 340  $\mu$ g/mL. Viability rate of the Raji cells shown by tested compound at a concentration of 1000 (mol ratio/32 pmol TPA) was 60%. Additionally, its effect on MDR mouse lymphoma cell line was also evaluated, results of which are displayed in Tab. 3. These results show that compound 1 exhibits a remarkable effect on MDR-mouse lymphoma cells. Therefore, compound 1 may have a possible chemopreventive effect.

### Reversal of MDR in mouse lymphoma cells effects

3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyrin (1) was also assessed for its properties on the reversion of multi-drug resistance mediated by P-gp using rhodamine-123 exclusion study on MDR human ABCB1 gene-transfected mouse T-lymphoma cell line. *In vitro* results revealed that MDR reversing effect was comparable to verapamil as positive control. The short time experiment exhibited that isolated compound 1 was an active MDR-modulator. Verapamil-a chemosensitizer & Ca<sup>2+</sup> channel blocker-acted as a positive control in current experimentation (Tab. 2).

### Petra/Osiris/Molinspiration (POM) Analyses of compound 1

Presented in Figs. 3 and 4 are the results pertaining to molecular properties predicted for compound 1 such as TPSA, GPCR (G-protein-coupled receptors), ligand and ICM (a protein modeling and design method). Results reveal that compound 1 has limited violation (NV (Number of Violations) = 1) of five rules of Lipinski. In conclusion, compound 1 has potential bioactivity as Nuclear Receptor Ligand and Enzyme Inhibitor (NRL = 0.68 and EI = 0.63 respectively). The bioactivity scores of compound 1 were found to be in accordance with the standard scores of the standard drugs (Tab. 3).

### Docking analysis

Our results confirm that free energies of the studied compound are a bit higher than those of Rhodamine123 (Tab. 3). This implies that certain structural features of 3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyrin may be the reason to its inhibitory properties on P-glycoprotein from mice. Moreover the predicated docked orientation of compound; 3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyrin shown by sticks red color, the co-crystallized ligand of the receptor is shown by the cyan color while the standard compound Rhodamine 123 is shown by green color in the binding site of P-gp (Fig. 4). Furthermore, as depicted in Fig. 5, docking interactions of 3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyrin indicate hydrophobic interactions (total: 08) and no hydrogen bonding. The hydrophobic bondings reported come from residues Met68, Leu64, Phe332, Ile336, Gln343, Met945, Tyr949, and Met982. These hydrophobic contacts of 3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyrin are responsible for its binding capacity to the P-gp receptor.

## Discussions

In-silico screening has been proven to be a vital tool for discovering new inhibitors against receptors. Purposely, we conducted docking studies to recognize the inhibiting



TABLE 1

Percent Inhibitory Effects on the induction of Epstein-Barr Virus Early Antigen (EBV-EA) by standard compound (curcumin) and isolated compound 1 (3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyrin)

Samples	Concentration (mol ratio/TPA) % to control (% viability)				
	1000	500	100	10	IC50
<b>Compound 1</b>	12.4 $\pm$ 0.4(60)	47.3 $\pm$ 1.5	75.1 $\pm$ 2.2	100 $\pm$ 0.6	458
Curcumin	0 $\pm$ 0.1(60)	22.8 $\pm$ 0.2	81 $\pm$ 0.6	100 $\pm$ 0.6	340

TPA (32 pmol) = 100%. Negative cont. 0%. Values represent the relative percentage to the positive control, with TPA (32 pmol, 20 ng) representing 100% induction at four different concentrations in terms of molar ratio/32 pmol TPA. Data are expressed as mean  $\pm$  S.D (n = 3). IC50 values represent the mole ratio of compounds, relative to TPA, required to inhibit 50% of the positive control activated with 32 pmol TPA. Values in parentheses are percentage viability of Raji cells. (In all other experiments, viability was >80%.)

TABLE 2

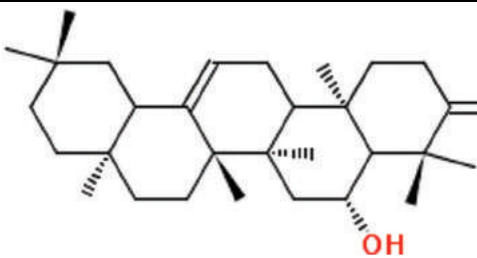
Effect of 3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyrin on reversal of multidrug resistance in MDR mouse lymphoma cells

Sample	(final concentration) $\mu$ g/mL	FSC*	SSC*	Mean	FAR*	Peak Ch*
PAR* MEAN	–	2224	643	68.15	–	68.5
MDR MEAN	–	2326	914	<b>1.64</b>	–	1.54
Verapamil	10	2329	711	21.9	<b>13.35</b>	27.4
3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyrin	4	2282	769	205	<b>125</b>	237
DMSO	0.2%	2247	759	1.02	<b>0.62</b>	0.931

\*PAR = Parental cell line; FSC = Forward scatter analysis; SSC = Side scatter analysis; FAR = Fluorescence ratio for treated/untreated samples; Peak ch = Fluorescence peak channel.

TABLE 3

Molinspiration calculations of Bioactivity Scores (BS) of compound 1. <sup>[a]</sup> Compound 1 has potential bioactivity as Nuclear receptor ligand and enzyme inhibitor (NRL = 0.68 and 0.63 respectively)

Molecular properties calculation		Optimized structure	Bioactivity scores calculation	
TPSA	37		GPCR ligand	0.18
MW	440.71		Ion channel modulator	-0.09
nON	2		Kinase inhibitor	-0.51
nOHNH	1		Nuclear receptor	<b>0.68</b> <sup>[a]</sup>
nviolations	1		Protease inhibitor	0.07
Volume	463		Enzyme inhibitor	<b>0.63</b> <sup>[a]</sup>

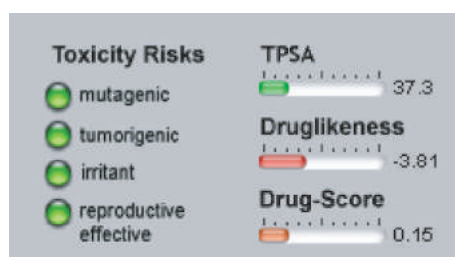
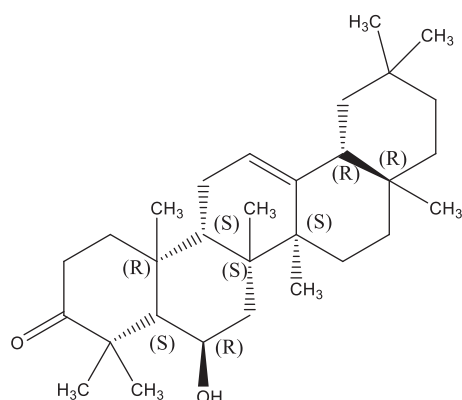
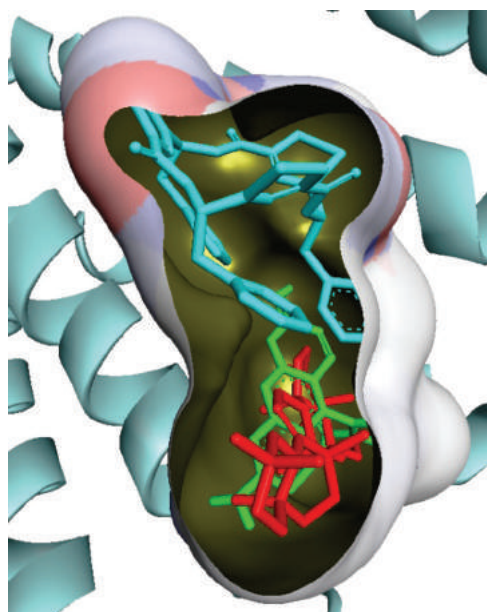


FIGURE 3. Osiris calculations of drug likeness of compound 1. Toxicity Risks ( ■: not toxic, ■: slightly toxic, ■: highly toxic). Molecular Weight (M.W. < 500 g/mole) is in perfect accordance with Lipinski 5 rules but cLogP > 5.



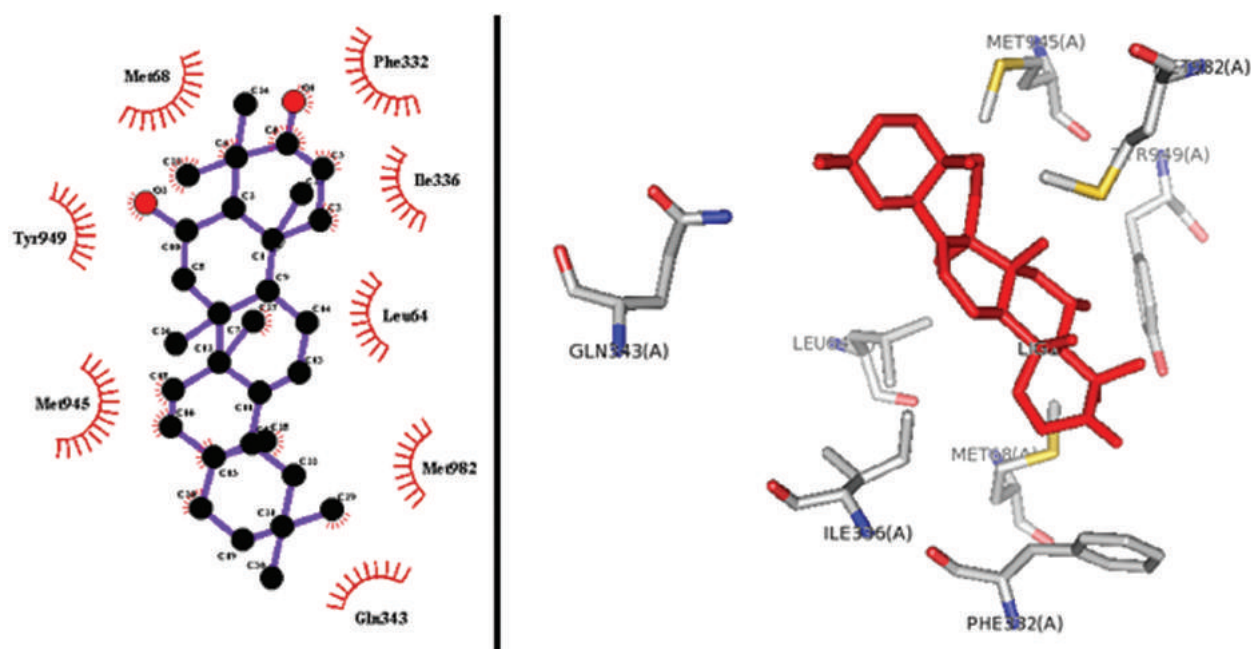
**FIGURE 4.** Predicted docked orientation of compound 3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyrin shown by sticks red color, the co-crystallized ligand of the receptor is shown by the cyan color while the standard compound Rhodamine 123 is shown by green color in the binding site of P-gp.

potential of 3-oxo-6 $\beta$ -hydroxy-  $\beta$ -amyrin with the P-gp. Results from docking analysis revealed that 3-oxo-6 $\beta$ -hydroxy-  $\beta$ -amyrin gives good docking results on both docking software. As displayed in Figs. 4 and 5, the results showed that compound 1 isolated from *Pistacia integerrima* precisely attached to a region where co-crystallized ligand for receptor was previously existing. In-silico screening predicts that lesser the free energy (local energy minimization) more effective is the docking score and higher is the activity of compound (Sliwoski et al., 2014).

Cell efflux-pump, including ATP-binding cassette transporters, may be considered as enzyme having varied

substrate specificity. Inhibition of ATP-binding cassette transporters may be regarded as a potential opportunity in order to overcome multidrug resistance (MDR). MDR cancer cells may be modulated by administering conventional chemo-therapeutics along with resistance modifiers. For this purpose, numerous natural bioactive biomolecules and synthetic metabolites have been investigated to inhibit the efflux-pump activity (Barath et al., 2006). In this study, FAR (fluorescence activity ratio) value was determined to assess the modulating potential of 3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyrin on ABCB1 transporter. In flow cytometry, FSC and SSC value enhanced revealing that compound 1 increased the granulation of cytoplasm. Results of FAR value showed that 3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyrin was an effective MDR-modulator. As shown in Tab. 2, compound 1 exhibited significantly modulated the efflux pump activity (FAR: 125, 4  $\mu$ g/ml). Similar results were obtained in study conducted on Pistagremic acid (PA), a triterpenoid, present in *Pistacia integerrima* on reversal of MDR (Rauf et al., 2016c). They concluded that there might be certain important chemical features of Pistagremic acid responsible for its inhibitory potential of P-glycoprotein (P-gp). Likewise, reversal of MDR mediated by P-gp in mouse Lymphoma Cells due to crude extract and two (dihydrokaempferol & naringenin) isolated compounds from *Pistacia integerrima* has also been experimented by Rauf et al. (2016b). They demonstrated that crude extract, dihydrokaempferol & naringenin were promising modulators of efflux pump activity (FAR = 64.02, 1.58, & 1.79; 4  $\mu$ g/ml).

The octanol/water partition coefficient, or best known as cLogP, is calculated by the molecular properties mining and quality assurance software package Molinspiration and Osiris (Open Source Independent Review and Interpretation System Background), respectively, as a sum of fragment contributions & correction factors (Husain et al., 2016; Jarrahpour et al., 2010). This procedure can be practically



**FIGURE 5.** 2-D (left) and 3-D (right) interaction of 3-oxo-6  $\beta$ -hydroxy-  $\beta$ -amyrin in the binding site of P-glycoprotein.

applied to all organic and organometallic based molecules. In addition, the method published by Ertl *et al.* (2000) is employed in the calculation of the total molecular polar surface area (TPSA). This quantity is simply the sum of fragment contributions; O- and N- centered polar fragments. Furthermore, TPSA characterizes intestinal drug absorption, permeability of Caco-2, bioavailability and penetration of blood-brain barrier.

The rule of five (Ro5) is based on the physicochemical profiles (distribution, absorption, excretion and metabolism) of synthetic drugs, which among other criteria, deals with target and ligand alignment, bioavailability, etc. (Murugan *et al.*, 2015). Natural products do not generally follow the Ro5, due to their intra-molecular hydrogen bonding and easy metabolizability (Zhang and Wilkinson, 2007).

## Conclusions

In summary, findings from this investigation suggest that bark of *P. integerrima* contain 3-oxo-6 $\beta$ -hydroxy- $\beta$ -amyrin; which exhibits anticancer activities ranging from cancer chemoprevention to reversal of multidrug resistance of cancer cells. The findings may explain the medicinal use of *P. integerrima* as an medicinal plant with anticancer effects. However, more detailed research are needed to establish the safety and efficacy of the isolated compound.

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