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Original Article

Cytotoxic activity of abietane diterpenoids from roots of *Salvia sahendica* by HPLC-based activity profiling

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ABSTRACT

Screening of medicinal plants from Iranian flora against human cancer cell-lines have shown that an *hexane* extract from roots of *Salvia sahendica* Boiss. & Buhse, Lamiaceae, is active against human cervical cancer (HeLa) and colorectal adenocarcinoma (Caco-2) cell-lines at the test concentration of 100 μg/ml (100% inhibition). Cytotoxicity of the extract was localized with the aid of HPLC-time-based activity profiling adapted to the tetrazolium colorimetric bioassay. Four abietane-type diterpenoids in active time-windows were identified as cytotoxic compounds namely: sahandone (**1**), sahandol (**2**), 12-deoxy-salvipisone (**3**) and sahandinone (**4**). Compound **1** showed the highest toxicity against HeLa cells (IC_{50} = 5.6 ± 0.1 μg/ml), which was comparable with betulinic acid (IC_{50} = 4.3 ± 1.2 μg/ml), the positive control. Compound **2** was active against the HeLa cells (IC_{50} = 8.9 ± 0.7 μg/ml) but not the Caco-2 cell-line. Compounds **1**, **3** and **4** exhibited moderate activity (IC_{50} = 22.9–41.4 μg/ml) against the Caco-2 cells. This study reveals that the HeLa cells are more sensitive to all tested compounds than the Caco-2 cells. *In silico* molecular docking study showed a rigid binding of the compounds to tyrosine kinase pp60src, and proved their cytotoxic activity.

Introduction

Unregulated proliferation of cells due to genetic alterations in the body generates cancer. Each year, more than 10 million people worldwide are diagnosed with cancer while half of the patients are not successfully treated with the existing anticancer medications (Saeidnia, 2015). The restrictions and side effects of the current anti-cancer drugs create a constant demand for discovery of new effective agents.

Salvia as the most prevalent genus of the Lamiaceae family represents more than 58 species in Iran (Mozaffarian, 2010). Arial parts of the plant are aromatic, and have been used in Iranian folk medicine as an anti-inflammatory remedy, and also for treatment of dyspepsia and gastric disorders (Esmaeili et al., 2009). *Salvia sahendica* Boiss. & Buhse, an endemic plant of Iran, grows widely in northwest of the country, at the feet of the Sahand Mountains (Rechinger, 1982). Previous phytochemical studies have revealed the presence of sesterterpenes, nor-sesterterpenes and nor-diterpenes in the aerial parts of *S. sahendica* (Moghaddam et al., 1995). However, roots of the plant were reported to be rich in abietane type diterpenoids (Hadavand Mirzaei et al., 2017; Jassbi et al., 2006; Salehi et al., 2007; Moradi-Afrapoli et al., 2013). Continuing our investigations on Iranian medicinal plants, in the current study, we report on cytotoxic activities of roots of *S. sahendica* against human cancer cell-lines. We have implemented HPLC-time-based activity profiling as a miniaturized and rapid approach for tracking cytotoxic constituents in the plant extract. In recent years this approach has been successfully applied for discovery of new anti-protozoal substances, positive $GABA_A$ receptor modulators and anti-HIV compounds (Kim et al., 2008; Adams et al., 2009; Moradi-Afrapoli et al., 2013; Potterat and Hamburger, 2014). In this article, we report novel

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cytotoxic compounds obtained from a hexane extract of *S. sahendica*'s roots, and evaluate their activity against human cervical cancer (HeLa) and colorectal adenocarcinoma (Caco-2) cell-lines with combination of *in silico* methods for evaluating cytotoxic potential.

Material and methods

General materials

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and Bovine serum albumin (BSA) were provided by Sigma–Aldrich (Germany). Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) "Gold" were from PAA (Germany). Trypsin-EDTA was purchased from Boehringer Ingelheim (Germany). HeLa cell line was provided by Pasteur Institute of Iran. Caco-2 cells were gifted by Professor Georgios Imanidis at University of Basel. HPLC separations were performed on an Agilent 1100 series HPLC system consisting of a quaternary low-pressure mixing pump with degasser module, PDA detector, and an autosampler. For MPLC, a Buchi Sepacore system consisting of a control unit C-620, a fraction collector C-660, and two pump modules C-605 was used. Waters SunFire C18 (3.5 μm, 3.0 × 150 mm) and SunFire® Prep C18 (5 μm, 30 × 150 mm) columns were used for analytical HPLC and preparative RP-HPLC, respectively. The detection wavelength was 280 nm. HPLC solvents contained 0.1% formic acid for separations. NP column chromatography was carried out on silica gel (Merck, 40–63 μm; 460 × 50 mm). Pre-coated silica gel F_{254} (20 × 20 cm) plates for TLC (Merck) was used for monitoring fractions. Solvents used for extraction, open column chromatography, and MPLC were of technical grade. NMR spectra were recorded on a Bruker AVANCE III spectrometer operating at 500.13 MHz for ^{1}H NMR and 125.77 MHz for ^{13}C NMR. A 1 mm TXI microprobe with *z*-gradient was used for ^{1}H-detecting experiments. ^{13}C NMR spectra were recorded with a 5 mm BBO probe with *z*-gradient. Spectra were analyzed using Bruker TopSpin 3.0 software.

Plant material

Roots of *Salvia sahendica* Boiss. & Buhse, Lamiaceae, were collected in September 2009 from Sardrood, East-Azerbaijan province of Iran. The plant was identified by Dr. Ali Sonboli, and a voucher specimen (MPH-848) has been deposited at the Herbarium of the Department of Biology, Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran.

Preparation of extract

The plant material was shade-dried and ground with a Retsch ZM1 mill. The powdered roots were percolated with hexane, ethylacetate and methanol, successively. Extraction with each of the solvents lasted for 48 h at room temperature. The extracts were concentrated under reduced pressure and finally dried with a freeze dryer.

Microfractionation for activity profiling

Aliquots of 360 μg extract in DMSO (36 μl of 10 mg/ml solution) were separated by analytical HPLC using a C18 column (5 μl, 10 × 150 mm, Interchim). The mobile phase was a mixture of water–acetonitrile containing 0.1% formic acid. The gradient started from 25% acetonitrile all the way to 100% in 30 min, and stayed at 100% for another 5 min. The flow rate was 0.4 ml/min. Micro-fractions of the extract were collected in a 96-well deep well plate within 1-min intervals (1-min fractions). The plate was dried by a nitrogen evaporator set at 40 °C (Evaporex EVX-96, Apricots Designs Inc., USA).

Cell culture

The Caco-2 cells (human colorectal adenocarcinoma cell line) were grown in DMEM culture medium supplemented with FBS (10%), L-glutamate (200 mM), and nonessential amino acids (1%). The HeLa cells (human cervical cancer cell line) were grown in IPMI medium. The cell culture flasks were maintained in a humidified incubator at 37 °C with 5% CO_2. The media were changed every alternate day. The flasks with 80% confluence were used in the experiments.

MTT cytotoxicity assay: cytotoxicity of extracts and pure compounds

Cytotoxicity against the HeLa and Caco-2 cell-lines was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay in 96-well format (Gerlier and Thomasset, 1986; Tofighi et al., 2014). A suspension of each cell-line was seeded on a 96-well flat-bottomed tissue culture plate at the density of 5×10^3 cells/well and incubated in a humidified incubator (37 °C, 5% CO_2) for 48 h. The cells were treated with 200 μl test samples dissolved in culture medium at the final concentration of 1–100 μg/ml, and incubated for the next 48 h. Afterwards, 20 μl MTT reagent (5 mg/ml) in PBS was added to each well. The plate was maintained on an orbital shaker at 100 rpm and 37 °C for 4 h to allow the MTT reaction by the cellular mitochondrial dehydrogenases. Finally, the medium was replaced with 150 μl DMSO and the cells were agitated on an orbital shaker for 15 min to dissolve the formazan crystals formed by the live cells. The UV/Vis absorbance of each well was read by a microplate reader at 570 nm with a reference filter of 620 nm. A set of wells with no test sample, treated with the highest concentration of DMSO (1%), was considered as control. The naturally occurring triterpenoid, betulinic acid, was used as positive control. Cell-viability and cytotoxicity were calculated according to the following equations:

$$\text{Cell viability}(\%) = (\text{Absorbance of test well}/\text{Absorbance of control}) \times 100$$

$$\text{Cytotoxicity}\ \ (\%) = 100 - \text{cell viability}$$

Cytotoxicity of microfractions

Each of the dried micro-fractions in the 96-well plate was dissolved with 5 μl solution of DMSO in PBS. Afterwards, culture medium was added to each well up to the final volume of 450 μl. The volumes of the medium being added to the wells were calculated to approach a concentration of the active constituents in the micro-fractions proportional to that found in 100 μg/ml of the original extract. The calculation was based on the assumption that each active constituent may be eluted in one or two 1-min fractions, and also the consideration of a 50% loss that may occur during chromatography and re-dissolution (Potterat and Hamburger, 2014). The plate was shaken by an orbital shaker at 100 rpm for 30 min. The micro-fractions were submitted to MTT bioassay. The final dilutions (2 fold) occurred in the cell-culture wells. The experiments were performed in duplicates. The final content of DMSO in each well did not exceed 1%.

Isolation of active constituents

Phytochemical constituents of *S. sahendica* were purified as described previously (Ebrahimi et al., 2013).

Spectroscopic data of the constituents

Sahandone (**1**): Colorless; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.08 (1H, d, J = 7.6 Hz, H-6), 6.95 (1H, d, J = 7.3 Hz, H-7), 6.87 (1H, s, H-12), 5.48 (1H, dq, J = 8.5, 1.1 H-3), 5.01 (ddd, J = 13.1, 8.9, 4.4 Hz, H-2), 3.24 (s, OCH_3), 2.95 (sep, J = 7.0 Hz, H-15), 2.68 (dd, J = 17.0, 4.3 Hz, Hβ-1), 2.53 (dd, J = 17.1, 11.1 Hz, Hα-1), 2.19 (s, H-20), 1.80 (d, J = 1.1 Hz, CH_3-18), 1.78 (d, J = 1.1 Hz, CH_3-19), 1.15 (d, J = 7.0 Hz, CH_3-16), 1.14 (d, J = 7.0 Hz, CH_3-17). ^{13}C NMR (125 MHz, $CDCl_3$) δ 30.9 (CH_2-1), 65.4 (CH-2), 125.0 (CH-3), 136.2 (C-4), 136.7 (C-5), 130.5 (CH-6), 126.4 (CH-7), 128.4 (C-8), 132.3 (C-9), 133.5 (C-10), 93.6 (C-11), 135.7 (CH-12), 140.2 (C-13), 195.5 (C-14), 27.2 (CH-15), 22.1 (CH_3-16), 21.4 (CH_3-17), 25.9 (CH_3-18), 18.4 (CH_3-19), 18.9 (CH_3-20), 50.3 (O-CH_3).

Sahandol (**2**): Colorless; $C_{20}H_{24}O_2$; ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.53 (1H, d, J = 8.4 Hz, H-7), 7.27 (1H, s, H-14), 7.12 (1H, d, J = 8.4 Hz, H-6), 5.80 (1H, OH12), 5.53 (1H, brd, J = 8.5, H-3), 4.93 (1H, ddd, J = 10.8, 8.5, 3.1, H-2), 3.44 (1H, heptet, J = 6.9, H-15), 3.12 (dd, J = 16.4, 3.1 Hz, Hβ-1), 2.97 (dd, J = 16.4, 10.8 Hz, Hα-1), 2.37 (3H, s, H-20), 1.86 (3H, d, J = 1.5, H-19), 1.81 (3H, d, J = 1.5, H-18), 1.37 (3H, d, J = 6.6 Hz, H-16), 1.35 (3H, d, J = 6.6 Hz, H-17); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 138.0 (C-11), 138.2 (C-12), 136.2 (C-13), 136.2 (C-4), 129.2 (C-5), 126.5 (C-7), 125.3 (C-10), 125.1 (C-6), 124.5 (C-8), 124.4 (C-3), 120.2 (C-9), 115.9 (C-14), 73.2 (C-2), 32.5 (C-1), 27.9 (C-15), 25.8 (C-19), 22.8 (C-17), 22.7 (C-16), 18.5 (C-18), 18.4 (C-20).

Sahandinone (**3**): Red crystals; ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.35 (1H, d, J = 7.8 Hz, H-6), 7.07 (1H, s, H-12), 7.03 (1H, d, J = 7.8 Hz, H-7), 5.29 (1H, t, J = 7.1, H-3), 3.08 (2H, m, H-1), 3.01 (1H, heptet, J = 6.9, H-15), 2.39 (3H, s, H-20), 2.19 (2H, ddd, J = 6.8, 8.7, 7.8 Hz, H-2), 1.70 (3H, s, H-18), 1.60 (3H, 3, H-19), 1.17 (3H, d, J = 6.9 Hz, H-16), 1.19 (3H, d, J = 6.9 Hz, H-17); ^{13}C NMR ($CDCl_3$,100.6 MHz): δ = 182.5 (C-11), 181.5 (C-14), 148.1 (C-10), 144.5 (C-13), 140.3 (C-12), 140.2 (C-5), 136.6 (C-6), 134.9 (C-9), 132.8 (C-4), 128.5 (C-8), 128.0 (C-7), 123.8 (C-3), 30.2 (C-1), 27.5 (C-2), 26.9 (C-15), 25.7 (C-18), 21.34 (C-16), 21.34 (C-17), 19.7 (C-20), 17.4 (C-19).

12-Deoxy-salvipisone (**4**): Red crystals; ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 7.35 (1H, d, J = 7.8 Hz, H-6), 7.07 (1H, s, H-12), 7.03 (1H, d, J = 7.8 Hz, H-7), 4.64 (2H, brs, H-18), 3.08 (2H, m, H-1), 3.01 (1H, heptet, J = 6.9, H-15), 2.39 (3H, s, H-20), 2.23 (2H, m, H-3), 2.19 (2H, ddd, J = 6.8, 8.7, 7.8 Hz, H-2), 1.60 (3H, 3, H-19), 1.17 (3H, d, J = 6.9 Hz, H-16), 1.19 (3H, d, J = 6.9 Hz, H-17); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ = 182.5 (C-11), 181.5 (C-14), 148.1 (C-10), 144.5 (C-13), 140.3 (C-12), 140.2 (C-5), 136.6 (C-6), 134.9 (C-9), 148.4 (C-4), 128.5 (C-8), 128.0 (C-7), 38.4 (C-3), 30.2 (C-1), 27.5 (C-2), 26.9 (C-15), 110.1 (C-18), 21.34 (C-16), 21.34 (C-17), 19.7 (C-20), 22.4 (C-19).

Protein preparation

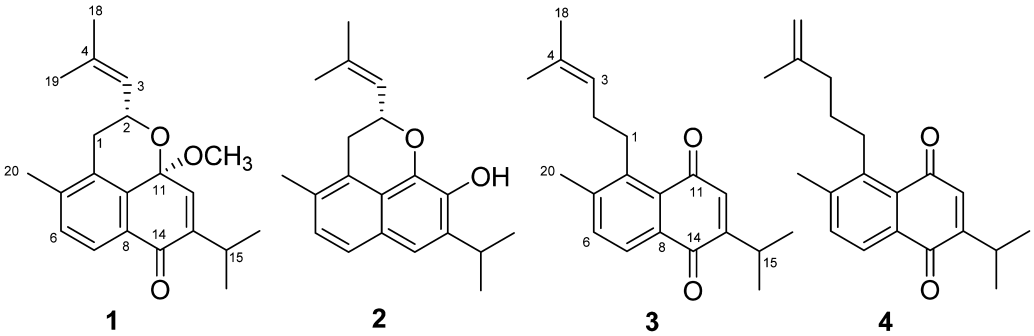
The enzyme for *in silico* studies was selected based on previously published data (Tintori et al., 2009; Paul and Mukhopadhyay, 2004). The Protein Data Bank PDB file of kinase ATP kinase in complex with AP23451 downloaded from the RCSB (2BDF) and applied as a target for molecular modeling studies El-Karim et al. (2015). Subsequently, all water molecules were deleted and hydrogens were added. In the next step, Gasteiger charges were calculated and the created pdbqt files were saved using AutoDock tools. The modified PDB file was saved as pdbqt file format.

Molecular docking

Molecular docking was done for all ligands in the workspace of AutoDock vina software (Trott and Olson, 2010). The active site ATP kinase was considered using crystallographic ligand position. Binding mechanism and related interactions of four ligands were investigated in Ligplot+ (Laskowski and Swindells, 2011), Discovery Studio Visualizer v4.5.

Results and discussion

Screening of the extracts from selected Iranian medicinal plants against human cervical cancer (HeLa) and colorectal adenocarcinoma (Caco-2) cell-lines revealed significant cytotoxicity of an hexane extract from *S. sahendica*'s roots at the test concentration of 100 μg/ml (100% inhibition). Cytotoxicity of the extract was localized with the aid of HPLC-time-based activity profiling. Activity profile of the 1-min micro-fractions was correlated with the corresponding analytical HPLC chromatogram as shown in Fig. 1. Fractions 7 (*Rt* 6–7 min) and 14 (*Rt* 13–14 min) showed the highest cytotoxicity in both cell-lines (>50%). Based on the high-resolution ESI-TOF-MS data, the molecular formula of the compounds in the active time windows were similar to the diterpenoids which were isolated from this plant, previously (Ebrahimi et al., 2013). In order to fully characterize the active compounds and assess their activity quantitatively, their isolation at a larger scale was carried out. The phytochemicals in the active time-windows were purified using column chromatography. The structures were established with HR-ESIMS, 1D and 2D NMR spectroscopy, and by comparison with the published data (Ebrahimi et al., 2013). Four abietane-type diterpenoids, sahandone (**1**), sahandol (**2**), 12-deoxy-salvipisone (**3**) and sahandinone (**4**) were finally identified.



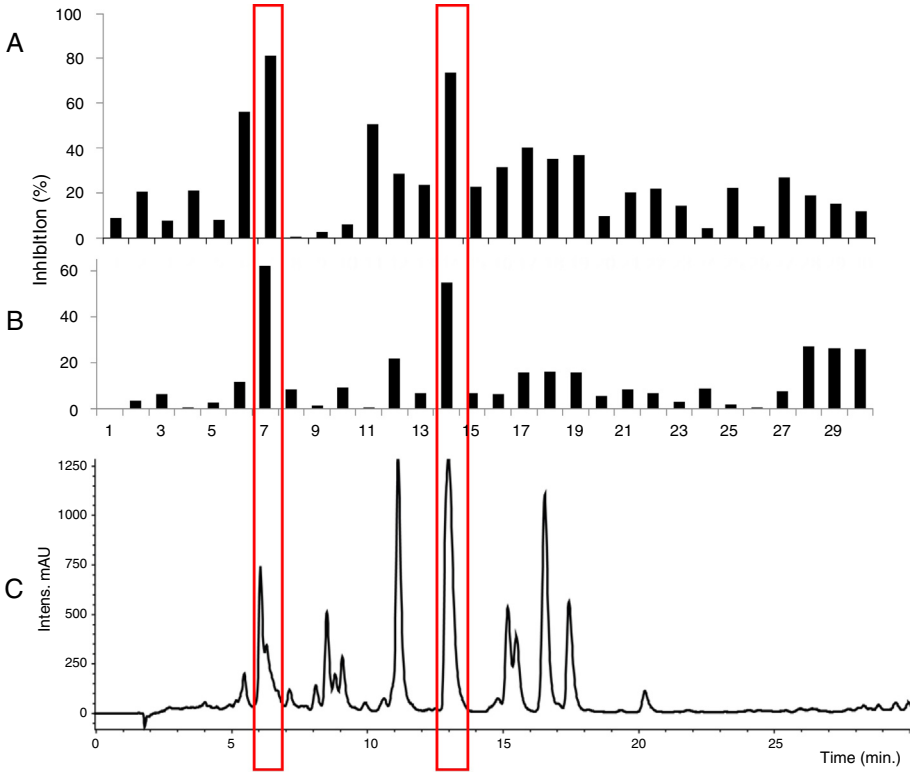


Fig. 1. HPLC-based activity profiling of an hexane extract from roots of *Salvia sahendica*; the bar graph represents the cytotoxic activity (in %) of the 1-min micro-fractions against HeLa (A) and Caco-2 (B) cells, correlated with the HPLC-UV (280 nm) chromatogram of the extract (C).

Table 1
Cytotoxic activities of compounds **1–4** against HeLa and Caco-2 cell lines.

Compound	$IC_{50} \pm SEM^a$ (μg/ml)	
	HeLa	Caco-2
Sahandone (**1**)	5.6 ± 0.1	41.4 ± 0.3
Sahandol (**2**)	8.9 ± 0.7	>100
12-Deoxy-salvipisone (**3**)	19.2 ± 6.6	29.6 ± 2.0
Sahandinone (**4**)	14.2 ± 6.9	22.9 ± 0.6
Betulinic acid	4.3 ± 1.2	9.2 ± 1.2

[a] Standard error of the mean.

Cytotoxicity of the purified compounds **1–4** was subsequently assessed against HeLa and Caco-2 cell-lines (Table 1). The Hela cells were more sensitive to the tested compounds than the Caco-2 cells. Compound **1** showed the highest toxicity against Hela cells ($IC_{50} = 5.6 \pm 0.1$), comparable with that of betulinic acid ($IC_{50} = 4.3 \pm 1.2$). Compound **2** showed active against the HeLa cells but not the Caco-2 cell-line. Compounds **1**, **3** and **4** exhibited moderate activity against the Caco-2 cells.

All isolated compounds belong to abietane diterpenes. Despite the fact that abietane diterpenes are popular phytochemicals in the mint family (Lamiaceae) (Chang et al., 1990; Ikeshiro et al., 1991; Topçu and Gören, 2007; Wang et al., 2007), compounds **1–3** have not been reported from any other members of the family rather than *S. sahendica*, so far (Ebrahimi et al., 2013; Jassbi et al., 2006). However, compound **4** had been identified in roots of *Zhumeria majdae* (Rustaiyan et al., 1995). Compounds **1–4** were previously introduced as antiplasmodial and antitrypanosomal principles in roots of *S. sahendica* (Ebrahimi et al., 2013). Sahandinone (**4**) has also shown activity against human pancreatic tumor cells (MIAPaCa-2) (Fronza et al., 2011). 12-Deoxy-salvipisone (**3**) and sahandinone (**4**) were reported active against a zygomycetes fungi, (+) *Blakeslea trispora* (Jassbi et al., 2006).

Molecular docking

The drug-likeness, molecular properties and toxicity are essential assessments for selection of drug candidates in early stages of drug discovery. Based on Lipinski's rule of 5 (Ro5), a drug molecule should have a molecular weight of 500 Da or less, maximum 5 H-donor bonds or less, equal to or less than 10 H-acceptor bonds and log P (octanol/water partition coefficient) not greater than 5. If a compound has more than two violations of these criteria, it would not probably be orally active (Lipinski et al., 1997, 2012). In this study rotatable bond count (ROTB) and topological polar surface area (tPSA) are also analyzed on top of Ro5 requisites. The results of our analysis showed that the tested compounds satisfy the drug-likeness criteria for a molecular weight below 500 Da, acceptable tPSA, and H donor/acceptor capabilities.

The molecular docking is a valuable computational method in drug discovery, providing complementary information on mode of interaction of the bioactive compounds. The possible mechanisms of cytotoxicity of *S. sahendica* components investigated by *in silico* molecular docking approach on the kinase/ATP binding site of Src tyrosine kinase. This class of enzymes regulates growth, differentiation, relocation and apoptosis in mammalian cells by catalyzing terminal ATP phosphate bind to specific tyrosine residues present on the target substrate (Tintori et al., 2009; Paul and Mukhopadhyay, 2004). Drug-like properties and docking scores of compounds **1–4** with Src kinase are shown in Table 2. Among these, compound **1** possess greater negative binding energy (−9.0 kcal/mol) in comparison with rest of the compounds. These computational results were in compliance with the experimental values, and persuaded investigation of interactions between compound **1** and kinase in detail.

Several amino acids take part in hydrophobic interactions between compound **1** and Src kinase such as Tyr 340, Leu 273, Leu 293, Val 281, Met 341, Ala 293, Thr 338, Ala 403, Val 323

Table 2
Drug Likeness of the **1–4** and docking energy.

Compound	*c*Log *P*[a]	H-donor	H-acceptor	tPSA[b]	MW[c]	Docking energy (kJ/mol)
Sahandone (**1**)	4.78	0.0	3	35.53	326.188	−9.0
Sahandol (**2**)	6.30	1.0	2.0	29.46	296.17	−8.9
Sahandinone (**3**)	6.35	0.0	2.0	34.14	298.15	−8.0
12-Deoxy-salvipisone (**4**)	6.35	0.0	2.0	34.14	298.15	−8.7
Betulinic acid	8.47	2.0	2.0	57.53	456.36	−8.1

[a] Logarithm of compound partition coefficient between *n*-octanol and water.
[b] Topological polar surface area.
[c] Molecular weight.

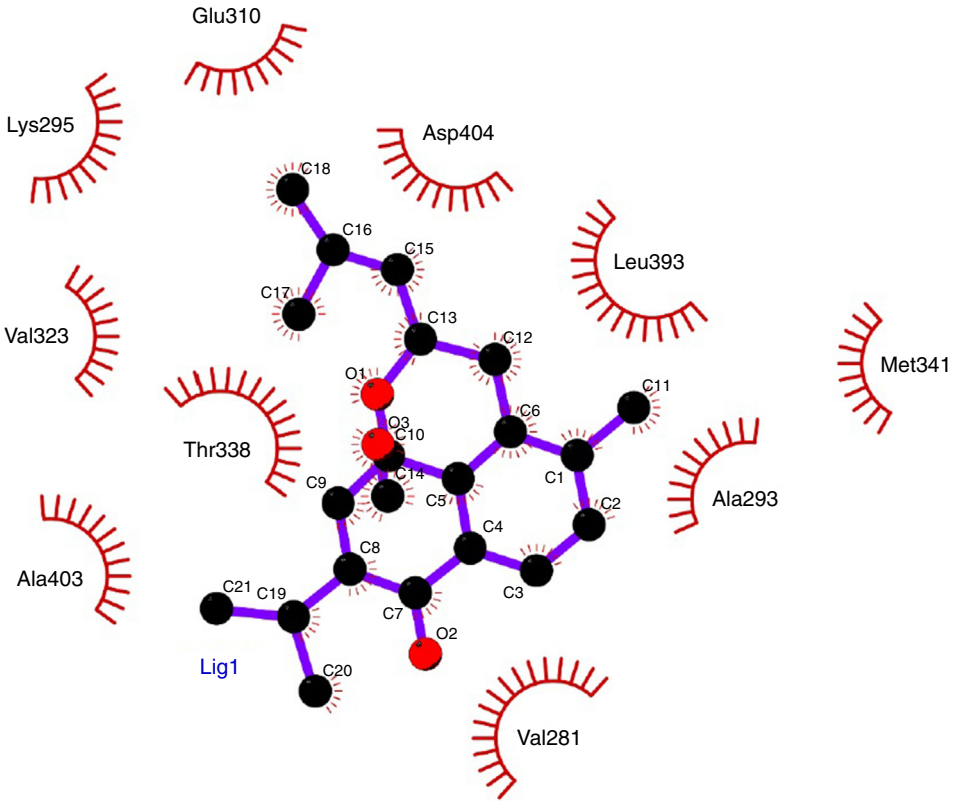


Fig. 2. Docking model structure of compounds **1** into the Src tyrosine kinase protein binding pocket.

and Met 314. These residues play vital roles in setting the binding pocket on this protein (Fig. 2). The close vicinity of amino acids such as Leu 393, Val 281 and Ala 293 to benzyl group of compound **1** proposes existence of hydrophobic interactions between those. In addition, Met 341, Leu 393 and Ala 293 attribute in hydrophobic interactions with the CH_3 connected to benzyl moiety of the ligand. It is remarkable that Val 281 is a versatile residue that comprises several hydrophobic interactions with different moieties (Fig. 2). Thus, it has been resolved that compound **1** can locate in hydrophobic cavity of the active site on Src kinase (Fig. 3). These results are in agreement with those observed from the native ligand AP23451 (co-crystallized ligand in RCSB Protein Data Bank) with common residues of Leu 393, Thr 338, Ala 293, Met 341 and Lys 295 (Fig. 3). The number of hydrophobic interactions with residues present in the active site of Src kinase could be a possible reason for the potent cytotoxic activity of compound **1**.

It is crucial to predict toxicity of drug-like molecules in early investigation stages. Prediction of toxicity of drug-like candidates can help to anticipate potential "off-target" activities. Recent advances in computer sciences and bioinformatics made it possible to obtain an estimated toxic potential (TP). Virtual ToxLab™, is a powerful open access platform for estimation of TP of endocrine and/or metabolic disruption, carcinogenicity, and cardiotoxicity (Vedani et al., 2015). It calculates the toxic potential (TP) and the binding affinity (binding constant K) of any molecule to sixteen proteins: 10 receptors (androgen, estrogen α, estrogen β, glucocorticoid, liver X, mineralocorticoid, progesterone, peroxisome proliferator-activated receptor c (PPARc), thyroid a and thyroid b), four members of the cytochrome P450 enzyme family (1A2, 2C9, 2D6 and 3A4), one transcription factor (aryl hydrocarbon receptor) and one potassium ion channel (hERG). The isolated components and standard compounds in this study were evaluated toward 16 target proteins. The toxic potential (TP) of compounds were calculated by normalized binding affinities and the values ranging from 0 (none) to 1 (extreme). The calculated TP values for **1**–**4** was between 0.45 and 0.59, showing a moderate to high risk of binding to the receptors (Table 3). The main target for **1**–**4** was the PPARγ receptor with binding affinity values 663, 338, 25 and 136 nM, respectively, where all compounds showed no binding to cytochrome receptors. The predictions showed these compounds might also bind to GR, AR and TRβ receptors.

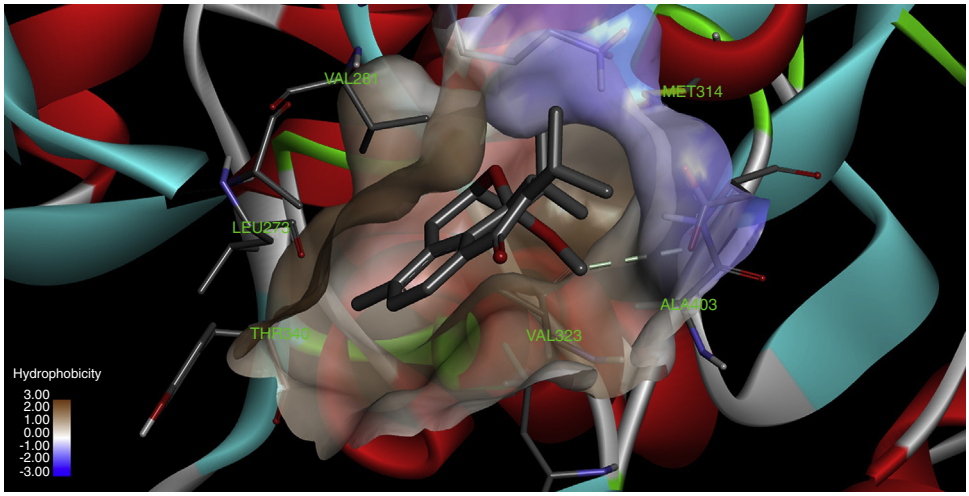


Fig. 3. Binding model of **1** into active pocket of Src tyrosine kinase.

Table 3
VirtualToxLab™. Binding affinity profile (binding constant *K*) and estimated toxic potential (TP) of BPA, BPF, BPS, Pergafast® 201 and D-8. The lower the concentration, the stronger the binding affinity to the target protein. Binding affinity Q4 >100 μM are considered not binding.

Receptor	Sahandone (**1**)	Sahandol (**2**)	Sahandinone (**3**)	12-Deoxy-salvipisone (**4**)	Betulinic acid
Androgen receptor	0.89 μM	3.65 μM	0.92 μM	0.07 μM	1.74 μM
Aryl hydrocarbon receptor	0.29 μM	5.57 μM	0.51 μM	0.43 μM	23.20 μM
CYP450 1A2	NB	NB	6.89 μM	23.90 μM	90.6 μM
CYP450 2C9	NB	NB	41.00 μM	NB	44.40 μM
CYP450 2D6	33.20 μM	6.43 μM	2.54 μM	25.80 μM	0.06 μM
CYP450 3A4	63.7 μM	NB	8.82 μM	6.73 μM	0.06 μM
Estrogen receptor α	1.42 μM	3.69 μM	2.07 μM	2.52 μM	0.04 μM
Estrogen receptor β	1.68 μM	1.28 μM	1.30 μM	1.43 μM	0.02 μM
Glucocorticoid receptor	0.45 μM	3.7 μM	0.06 μM	0.13 μM	1.09 μM
hERG	2.67 μM	0.30 μM	0.73 μM	0.36 μM	0.03 μM
Liver X receptor	1.62 μM	10.30 μM	2.23 μM	0.66 μM	0.62 μM
Mineralocorticoid receptor	1.62 μM	3.33 μM	1.40 μM	0.21 μM	0.03 μM
PPAR γ	0.66 μM	0.34 μM	0.03 μM	0.14 μM	0.02 μM
Progesterone	0.90 μM	0.26 μM	0.74 μM	0.20 μM	0.29 μM
Thyroid receptor α	6.14 μM	32.6 μM	0.36 μM	0.16 μM	1.97 μM
Thyroid receptor γ	1.10 μM	0.06 μM	1.01 μM	0.61 μM	13.7 μM
Toxic potential	**0.45**	**0.55**	**0.59**	**0.58**	**0.60**

TP 6 0.3 (low), 0.3 < TP 6 0.6 (moderate), and TP > 0.6 (high).

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Authors' contributions

FMF, MS, FM and GGB participated in the evaluation of cytotoxicity activity, acquisition and interpretation of data. FMS and SNE carried out phytochemical procedures and analysis. RZ carried out molecular modeling part. All the authors have contributed to critical reading of the final manuscript and approved its submission.

Conflicts of interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bjp.2017.11.007.

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