Naunyn-Schmiedeberg's Archives of Pharmacology
https://doi.org/10.1007/s00210-019-01689-0

ORIGINAL ARTICLE

Stylosin and some of its synthetic derivatives induce apoptosis in prostate cancer cells as 15-lipoxygenase enzyme inhibitors

Seyed Navid Goftari[1] · Hamid Sadeghian[2] · Ahmad Reza Bahrami[1,3] · Fatemeh Maleki[1] · Maryam M. Matin[1,4,5]

Received: 6 April 2019 / Accepted: 28 June 2019

Abstract

Overexpression of 15-lipoxygenase-1 (15-LOX-1) enzyme has been reported in prostate tumors, and its expression levels are associated with the degree of cancer malignancy. The aim of this study was to investigate inhibitory effects of stylosin and some similar synthetic monoterpenoids on 15-LOX and also their cytotoxic and anti-cancer activities on prostate cancer cells. Cytotoxicity of compounds was evaluated on prostate cancer cell line "PC-3" and normal human fibroblast "HFF3" cells using AlamarBlue reduction test. The inhibitory effects of the compounds against soybean 15-LOX, a commercially available enzyme, were also assessed. Finally, mechanism of cell death was investigated by flow cytometry. Some of these terpenoids had cytotoxic effects on PC-3 cells, and strong positive correlation was observed between the 15-LOX-1 inhibition potential and the cytotoxicity of the compounds. Moreover, flow cytometry results indicated that apoptosis was the predominant mechanism of induced cell death, which emphasizes the potential of these compounds in prostate cancer therapy. Among studied terpenoids, "fenchyl ferulate" exhibited about three times more cytotoxicity than cisplatin. Strong positive correlation observed between 15-LOX inhibition potential and cytotoxicity of the compounds indicates selective anti-cancer properties of the compounds might be exerted via inhibition of 15-LOX-1 in PC-3 cells. Furthermore, observed cytotoxicity is mediated through apoptosis, which is probably triggered via 15-LOX-1 inhibition.

Keywords Prostate cancer · Stylosin · 15-LOX-1 · Monoterpenoids · Cytotoxicity

Introduction

Cancer is a major cause of death all around the world, and prostate cancer (PC) is the most commonly diagnosed malignancy in men and the second leading cause of cancer-related deaths in the USA (Siegel et al. 2016). The high incidence rate of PC indicates that treatment of this type of malignancy is still not efficient, and the search for finding novel therapeutic approaches should be continued and more researches need to be done to attain more efficient and targeted therapies/compounds. Use of plant-derived compounds in traditional medicine has made them a valuable source with reputable therapeutic potential in cancer treatment. Despite working on herbal medications for more than 2000 years, plants still remain a precious source for discovery of novel drugs (Atanasov et al. 2015; David et al. 2015). More than 500 pharmaceutical

✉ Maryam M. Matin
matin@um.ac.ir

Seyed Navid Goftari
navid.goftari.se@mail.um.ac.ir

Hamid Sadeghian
sadeghianh@mums.ac.ir

Ahmad Reza Bahrami
ar-bahrami@um.ac.ir

Fatemeh Maleki
fateme.maleki@mail.um.ac.ir

[1] Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

[2] Neurogenic Inflammation Research Center, Department of Laboratory Sciences, School of Paramedical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran

[3] Industrial Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran

[4] Novel Diagnostics and Therapeutics Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran

[5] Stem Cell and Regenerative Medicine Research Group, Iranian Academic Center for Education, Culture and Research (ACECR), Khorasan Razavi Branch, Mashhad, Iran

Published online: 11 July 2019

natural-based small molecules have been approved between 1981 and 2014 (Newman and Cragg 2012, 2016), and the search still continues. However, using natural products has some limitations like biodiversity diminishing due to overharvesting of such plants (Cordell 2011; Vines 2004), cost of cultivation, extraction, and purification of specific agents and supply limitation (the well-known example for this is "taxol supply crisis") (Cragg et al. 1993; Kingston 2010). Moreover, natural compounds are finite in number, but synthetic compounds can be considered as an infinite source. So finding effective natural compounds and synthesizing their derivatives to improve pharmacologic specifications such as solubility and drug delivery characteristics can be an appropriate practice to reach efficient drugs eventually.

PC incidence rates indicate more than 25-fold variation worldwide. This variation is due in part to differences in dietary fat intake (Allott et al. 2017; Zadra et al. 2013). Among different dietary fats, two types of polyunsaturated fatty acids (PUFAs), ω-6 PUFAs and ω-3 PUFAs, have the highest impact on prostate malignancies with opposite effects, as ω-3 PUFAs have protective effects while ω-6 PUFAs have cancer-promoting effects in both human and animal models (Akinsete et al. 2011; Berquin et al. 2007; Kobayashi et al. 2006; Larsson et al. 2004; Norrish et al. 1999; Rose 1997; Rose and Connolly 1991; Terry et al. 2001, 2003). PUFAs exert their effects on PC cells in part via 15-lipoxygenase (15-LOX) enzyme pathways. There are two isoforms of 15-LOX in humans, 15-LOX-1 and 15-LOX-2, which have opposite roles in PC development. 15-LOX-1 is highly expressed in malignant prostate tissues and cells, e.g., PC-3 cell line, whereas 15-LOX-2 expression decreases during transformation. It has been shown that overexpression of 15-LOX-1 increased prostate cell proliferation while its downregulation caused cell senescence and apoptosis (Hu et al. 2010, 2012; O'flaherty et al. 2012; Suraneni et al. 2010). 15-LOX-1 preferentially converts linoleic acid (18:2 ω-6 PUFA) into metabolites such as 13-S-hydroxyoctadecadienoic acid (13-HODE) by which exerts its pro-carcinogenic effects. Thus, downregulation/inhibition of 15-LOX-1 can slow down or even inhibit the PC development via induction of apoptosis in PC cells, so it can be considered as a suitable target for PC treatment.

Terpenes are a large and diverse family of natural organic compounds which have been shown to possess a wide range of pharmacological activities such as cytotoxic and anti-cancer properties (Arghiani et al. 2014; Chen et al. 2007; Choi and Lee 2009; Jagetia et al. 2005; Mansoor et al. 2009; Matin et al. 2014; Paterna et al. 2015; Shang-Gao et al. 2012). Many terpenes have been reported to inhibit 15-LOX enzymes (Chung et al. 2009; Gutierrez-Lugo et al. 2004; Rai et al. 2010). Stylosin is a terpene which has been isolated from *Ferula stylosa*, *Fraxinus stylosa*, and *Ferula ovina* species and shown to have cytotoxic and proapoptotic effects on 5637 (transitional cell carcinoma), CH1 (ovary cancer), SK-MEL-28 (melanoma), and A549 (lung cancer) cells (Bagirov et al. 1980; Guo and Zhang 1983; Rassouli et al. 2011; Valiahdi et al. 2013). We have synthesized some stylosin derivatives (Fig. 1) in order to reach more potent compounds on prostate cancer cells, and in this research, the cytotoxicity and anti-cancer effects of stylosin and its synthetic derivatives were evaluated on PC-3 cell line. Moreover, cisplatin, a common chemotherapeutic drug in clinical practice, was also used for comparison purposes in all experiments. 4-Methyl-2-(4-

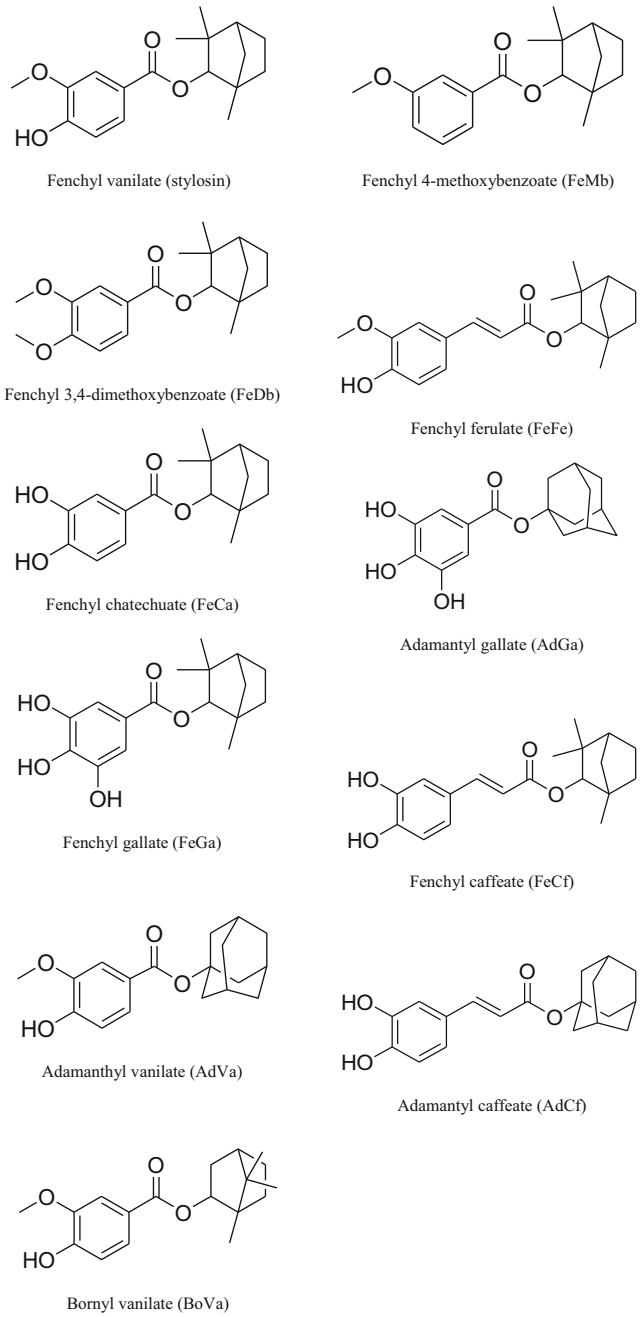


Fig. 1 Chemical structures of tested compounds (reproduced from Sadeghian et al. 2018)

methylpiperazinyl) pyrimido [4,5-b] benzothiazine (4-MMPB), a potent 15-LOX-1 inhibitor (Bakavoli et al. 2007), was also considered as a positive control.

Materials and methods

Synthesis of monoterpenoid compounds

Stylosin and its mentioned derivatives were synthesized through the esterification of corresponding alcohols and acids as described previously (Sadeghian et al. 2018).

Cell culture

Human prostate cancer cell line, PC-3, was obtained from Pasteur Institute, Tehran, Iran, and in order to determine the toxicity of the compounds on normal cells, experiments were also carried out on a nonneoplastic human foreskin fibroblast cell line, HFF3, kindly provided by Royan Institute, Tehran, Iran.

PC-3 cells were cultured in Roswell Park Memorial Institute, RPMI 1640 medium (Gibco), supplemented with 10% fetal bovine serum (Biosera) and 2 g/L $NaHCO_3$. HFF3 cells were kept in Dulbecco's modified Eagle's high glucose (4500 mg/L) medium, DMEM-HG (Gibco), supplemented

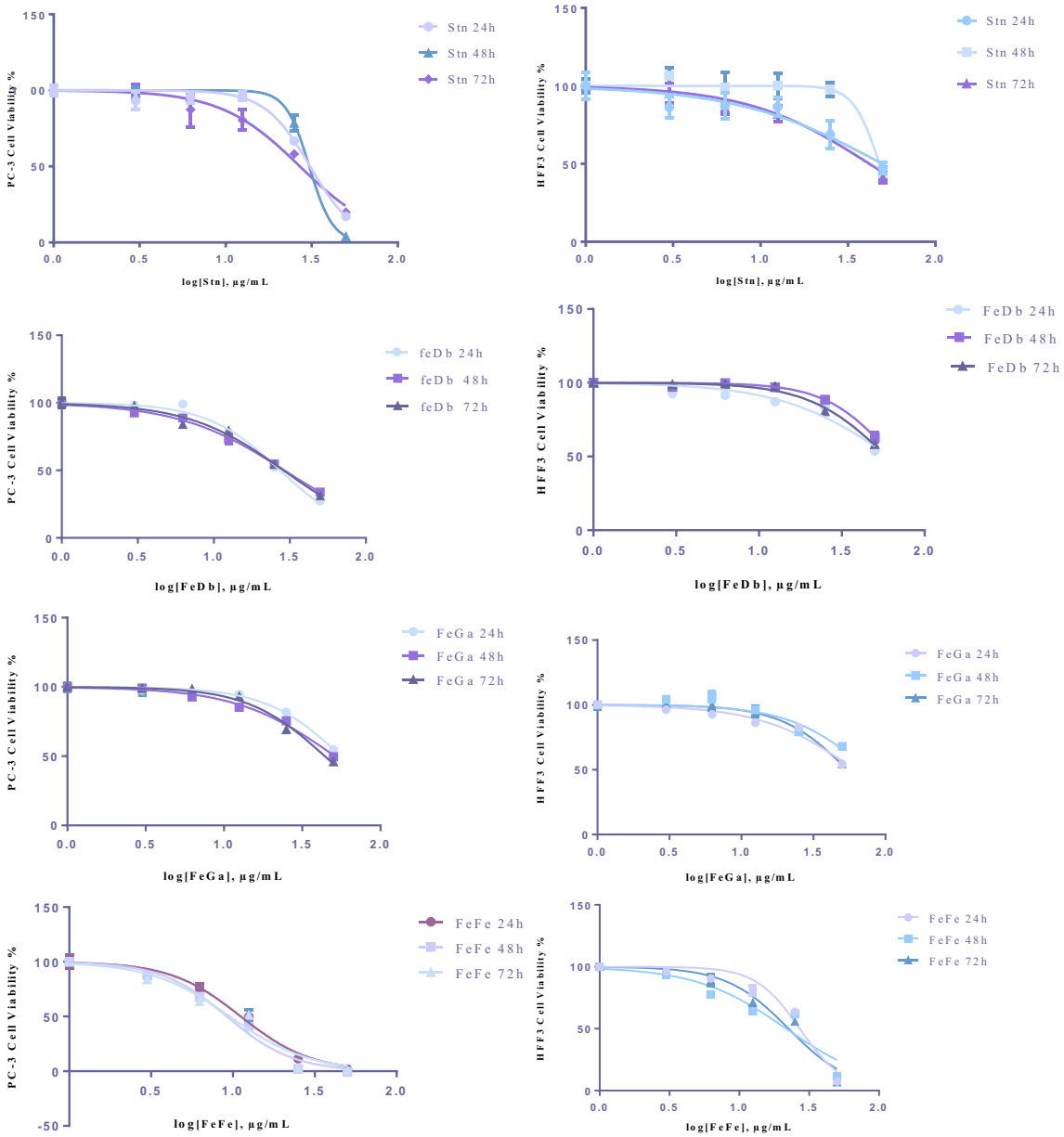


Fig. 2 Dose-response curves indicating the effects of different concentrations of tested compounds on PC-3 and HFF3 cell lines viability, during 24, 48, and 72 h time intervals. Data are expressed as mean ± SEM, $n = 3$

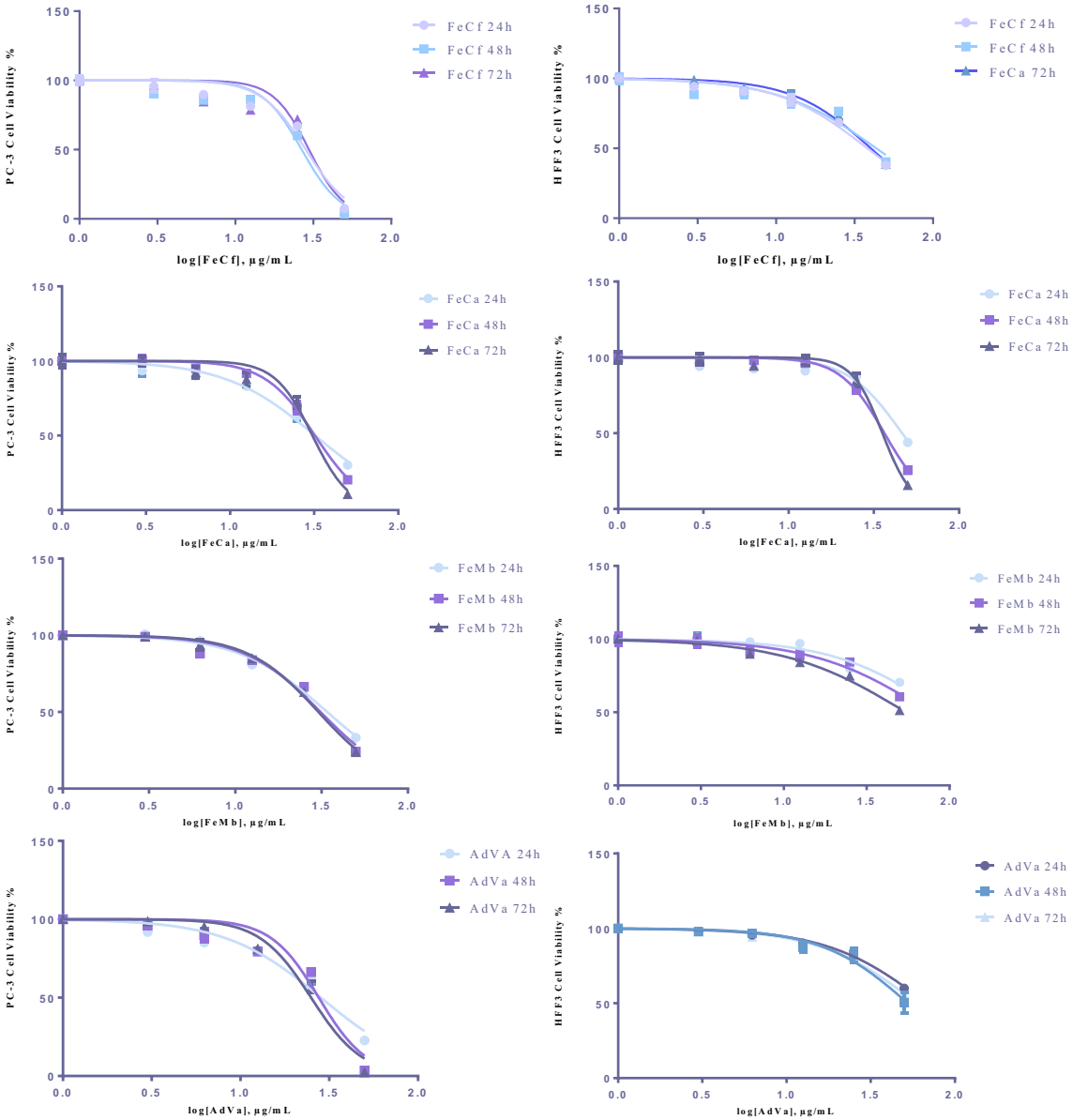


Fig. 2 (continued)

with 15% fetal bovine serum and 3.7 g/L $NaHCO_3$. Both cell lines were maintained at 37 °C under 5 and 10% CO_2, respectively, and subcultured twice a week.

Solution preparation

Two milligrams of each tested compound was dissolved in 100 µL of dimethyl sulfoxide (DMSO) to make a stock solution of 20,000 µg/mL. Different concentrations were then prepared by four times 1:1 *v/v* serial dilution from the stock solution, and then 2.5 µL of each solution was diluted to 1 mL with culture medium. Thus, the concentrations of the obtained solutions were 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, and 3.125 µg/mL and the final amount of DMSO in all solutions was 0.25% *v/v*. To prepare 4-MMPB solutions, 2 mg of 4-MMPB was dissolved in 100 µL 0.3 M hydrochloric acid (HCl) and further dilutions were prepared as mentioned above. For cisplatin preparation, aqueous solution of cisplatin (Mylan) with 1 mg/mL concentration was used as primary stock and 50 µg/mL, 25 µg/mL, 12.5 µg/mL, 6.25 µg/mL, and 3.125 µg/mL concentrations were obtained in culture media.

Cytotoxicity assay

Cytotoxic properties of compounds were assessed using a fluorescent dye AlamarBlue (resazurin, Sigma-Aldrich) reduction assay, following the manufacturer's protocol. To do so, PC-3 and HFF3 cells were seeded at densities of 10,000 and 8000 cells/well, in 96-well flat-bottom microplates, respectively. After 24 h, the media were replaced with 200 µL of prepared

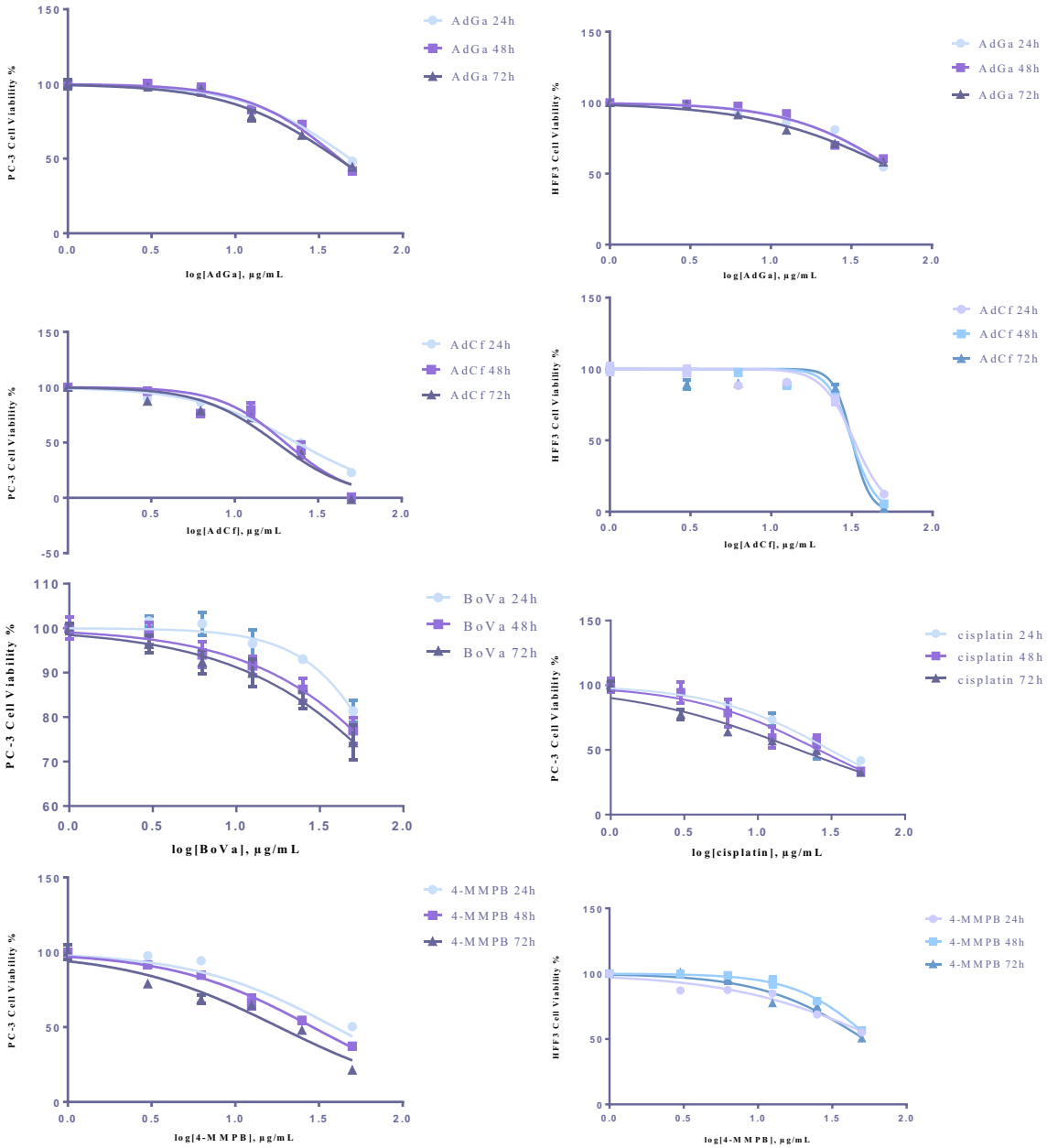


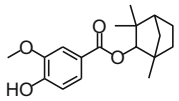
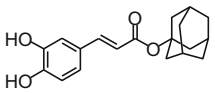
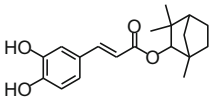
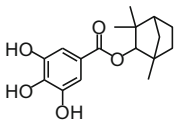
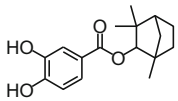
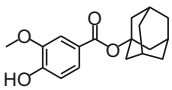
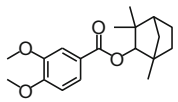
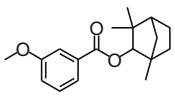
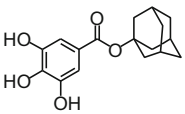
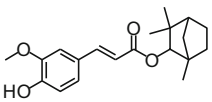
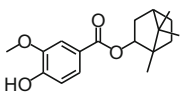
Fig. 2 (continued)

solutions in each well and incubated for 24, 48, and 72 h at 37 °C. After that, 20 µL AlamarBlue solution was added and incubated for 3–6 h at 37 °C in the dark. The spectrophotometrical emission of the samples was then measured using Biotek Synergy H4 microplate reader (USA) with the 530–560 nm and 590 nm wavelengths of excitation and emission, respectively (Khalifa et al. 2013; Rampersad 2012). Experiments were performed in triplicate for each compound tested.

Enzyme activity assay

The inhibitory activity of compounds against soybean 15-LOX (L1; type I-B; EC 1,13,11,12) was evaluated using modified DMAB-MBTH method as described by Iranshahi and his colleagues (Iranshahi et al. 2012), and 4-MMPB, a potent 15-LOX inhibitor, was used as a positive control. The method is based on oxidative coupling of 3-methyl-2-benzothiazolinone hydrazone (MBTH) with 3-(dimethylamino) benzoic acid (DMAB) in a hemoglobin catalyzed reaction. The reaction is triggered in the presence of lipoxygenase catalytic product and linoleic acid hydroperoxide and results in formation of a blue color with a peak absorption at 598 nm. Linoleic acid and two stock solutions (A and B) were prepared in advance. Solution A was composed of 50 mM DMAB and l00 mM phosphate buffer (pH = 7.0) while solution B was prepared by mixing 3 mL

Table 1 In vitro cytotoxic activity of tested compounds on human cell lines during 24, 48, and 72 h of treatments

compound	structure	IC_{50} (μM) ± SEM					
		PC-3			HFF3		
		24 h	48 h	72 h	24 h	48 h	72 h
stn		101.75 ± 7.33	100.63 ± 5.19	88.80 ± 10.22	163.49 ± 15.16	157.58 ± 6.81	137.78 ± 13.31
AdCf		74.71 ± 5.35	63.85 ± 9.52	56.44 ± 11.33	103.96 ± 9.04	100.49 ± 8.72	100.84 ± 16.11
FeCf		89.60 ± 11.26	84.28 ± 11.35	93.87 ± 14.33	120.79 ± 9.93	137.74 ± 15.02	127.12 ± 8.73
FeGa		183.21 ± 12.93	167.11 ± 14.05	144.05 ± 11.56	201.37 ± 30.61	246.97 53.21	177.95 ± 10.03
FeCa		110.36 ± 8.48	110.22 ± 4.79	106.43 ± 10.17	159.71 ± 19.23	125.73 ± 4.65	122.42 ± 6.13
AdVa		93.83 ± 12.94	91.38 ± 11.85	82.71 ± 9.04	225.22 ± 19.46	174.09 ± 18.07	196.52 ± 14.03
FeDb		88.58 ± 7.43	94.49 ± 4.83	93.39 ± 9.60	201.64 ± 24.47	208.29 ± 13.43	192.03 ± 16.45
FeMb		118.33 ± 8.64	108.20 ± 12.15	105.25 ± 4.48	314.85 ± 46.57	262.55 ± 42.37	189.40 ± 24.99
AdGa		159.37 ± 11.84	137.87 ± 13.61	135.21 ± 15.22	200.80 ± 26.14	210.83 ± 32.68	218.03 ± 28.01
FeFe		33.86 ± 3.54	27.83 ± 3.51	28.98 ± 5.66	82.02 ± 9.09	65.78 ± 9.96	68.24 ± 10.69
BoVa		401.75 ± 81.24	652.27 ± 93.07	636.49 ± 84.79	–	–	–
4-MMPB		124.65 ± 44.24	92.24 ± 9.51	58.08 ± 15.43	211.74 ± 53.18	183.62 ± 25.10	165.71 ± 27.74
cisplatin		103.36 ± 13.93	86.53 ± 15.10	63.73 ± 15.60	–	–	–

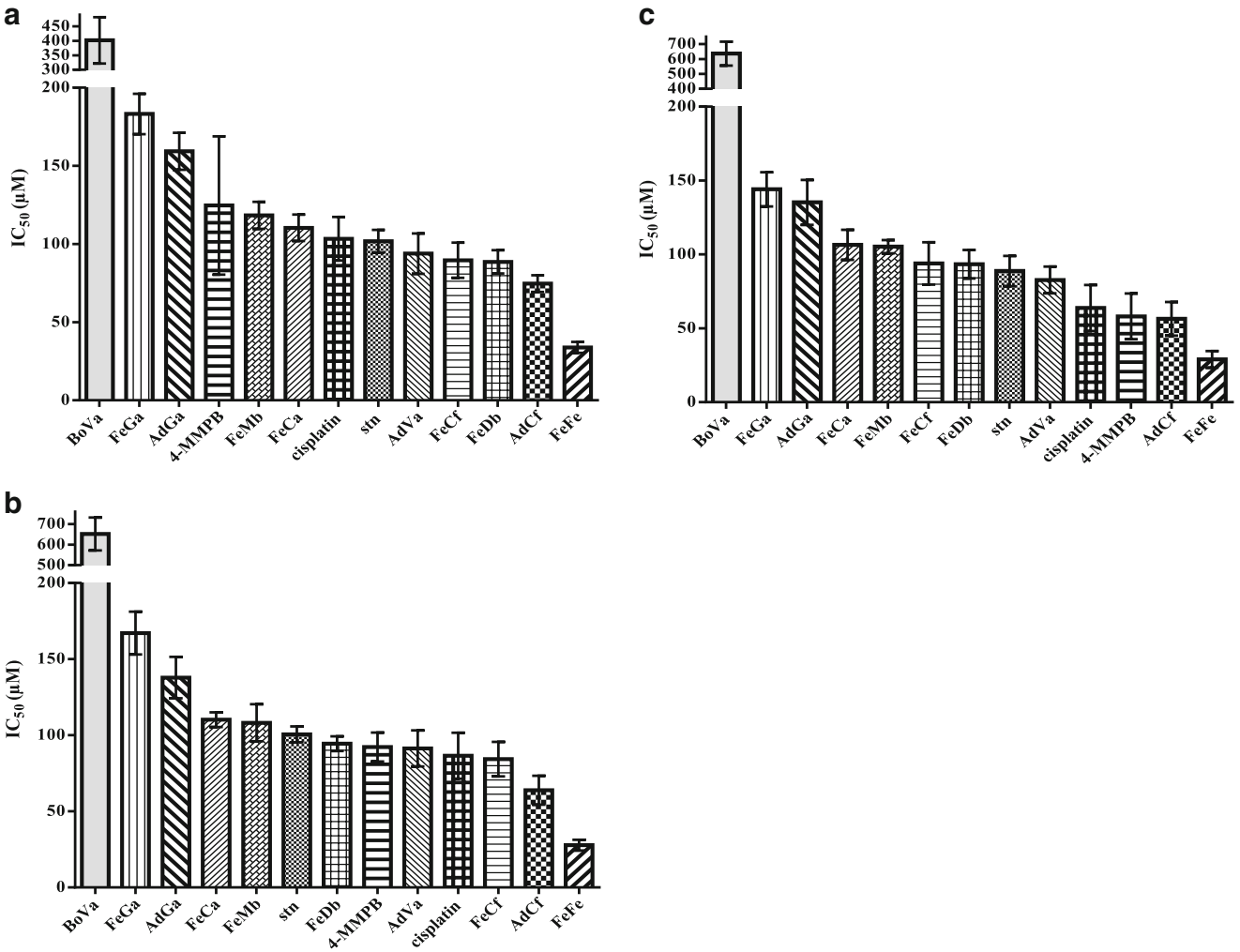


Fig. 3 Comparison of the cytotoxic potential of compounds on PC-3 cells, after **a** 24 h, **b** 48 h, and **c** 72 h of their administration in vitro. Data are expressed as mean ± SEM, $n = 3$

MBTH (10 mM) and hemoglobin (5 mg/mL, 3 mL) in 50 mM phosphate buffer at pH 5.0 (25 mL). The linoleic acid solution was prepared by adding 5 mg linoleic acid to 0.5 mL ethanol and diluting the resulted solution with KOH (100 mM) to a final volume of 5 mL. Different concentrations (0.5, 5, 50, and 500 μM) of stn, FeCf, AdCf, BoVa, and 4-MMPB were dissolved separately in 25 μL ethanol and mixed in a test tube with soybean 15-LOX (4000 units/mL, prepared in 25 μL phosphate buffer pH = 7.0, 50 mM) and 900 μL phosphate buffer (50 mM, pH = 7) and preincubated for 5 min at room temperature. Fifty microliters of linoleic acid was then added to start the peroxidation reaction. After 7 min, solution A (270 μL) and solution B (130 μL) were added to the tube. Five minutes later, 200 μL of sodium dodecyl sulfate (SDS) solution (2%) was added to terminate the reaction. The absorbance at 598 nm was measured and compared with the control reaction, which had ethanol without any compound. All tests were carried out in triplicate.

Apoptosis evaluation

Apoptosis was quantified using flow cytometry with "FITC Annexin V Apoptosis Detection Kit with PI" (Biolegend) to measure the levels of detectable phosphatidylserine on the surface of apoptotic cells. PC-3 cells were seeded in 6-well plates (3×10^5 cells/well) and incubated with the compounds solutions. The concentration of each compound was equal to its corresponding IC_{50} value on PC-3 cells. After 24 h, cells were harvested and washed twice with phosphate-buffered saline (PBS) followed by centrifugation (130×*g* for 5 min) and re-suspended in cell staining buffer at 4 °C. Cells were then centrifuged and re-suspended in binding buffer at a concentration of 7×10^6 cells/mL. One hundred microliters of each cell suspension was added to a flow cytometry tube. Five microliters of FITC Annexin V and 10 μL of 20 mg/mL propidium iodide (PI) were further added to the tube,

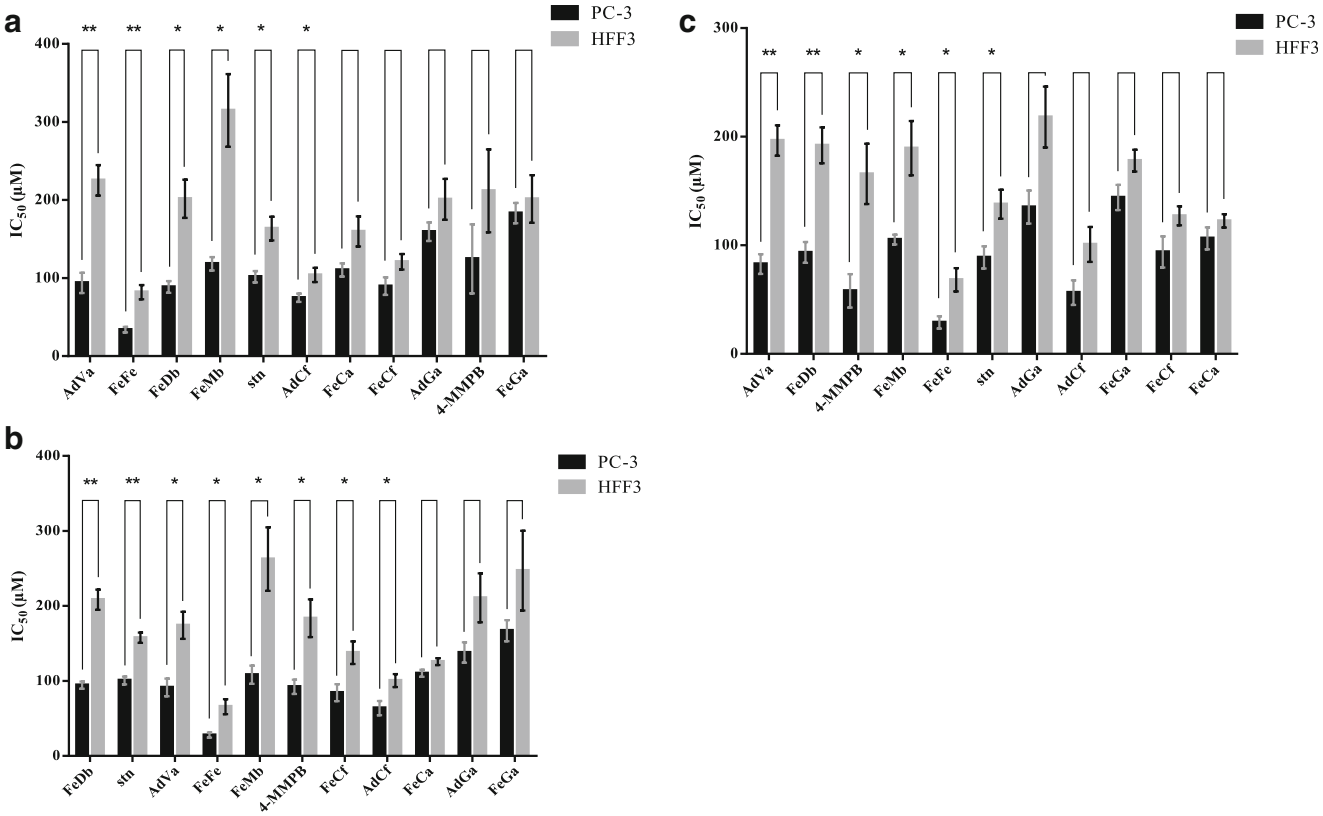


Fig. 4 Comparing the IC_{50} values of each compound on PC-3 and HFF3 cells after **a** 24 h, **b** 48 h, and **c** 72 h of their administration in vitro. Data are expressed as mean ± SEM, $n = 3$. (Compounds are arranged based on P value increment, demonstrating reduction in selective anti-cancer effects.)

followed by 15-min incubation in the dark. Quantitative analysis of apoptotic cells was performed using a Flow Cytometer (BD Accuri C6), and data analysis was performed using the flowJo 7.6 software.

Calculations and statistics

Cytotoxicity tests were performed and emissions data were standardized following the method described by Al-Nasiry and his colleagues (Al-Nasiry et al. 2007). Emission values were compared with baseline (cells treated with solvent), and results were expressed as percentage of cell survival. Dose-response curves were developed for various concentrations of each tested compound and corresponding IC_{50} values were calculated using a nonlinear regression model curve fitting based on sigmoidal dose-response curve (variable slope) which was computed using GraphPad Prism 6.0.1. Significant differences between groups were assessed by Student's t test using 95% confidence interval, and P value of < 0.05 was considered significant.

To assess the correlation between 15-LOX inhibition potential and tumor cell death caused by tested compounds, the data with the skewness between - 2 and 2 were considered as distributed normally and Pearson's correlation coefficient was calculated.

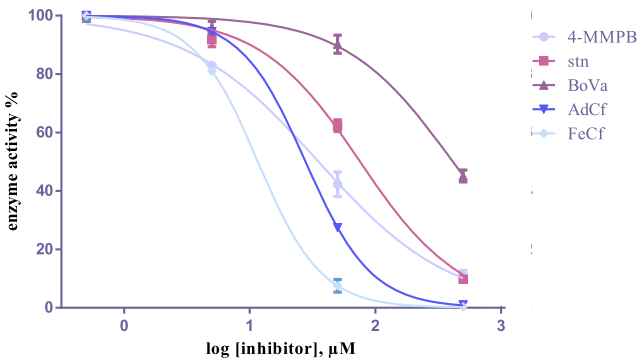


Fig. 5 Dose-response curves indicating the effects of different tested compounds on soybean 15-LOX enzyme activity. Data are expressed as mean ± SEM, $n = 3$

Table 2 Enzyme inhibitory assessment data of the compounds against soybean 15-LOX in vitro

Compound	IC_{50} (μM)	SEM
4-MMPB	17.1	5.48
stn	66.8	17.6
BoVa	399	72.2
AdCf	26.1	1.45
FeCf	11.0	0.72

Table 3 Flow cytometry analysis of PC-3 cells stained with FITC Annexin V Apoptosis Detection Kit with PI, after 24 h incubation with corresponding IC_{50} values for each compound

Compound	Annexin (+) PI (−) (%)	Annexin (+) PI (+) (%)
Untreated	0.06	0.04
DMSO	2.20	3.49
stn	30.6	5.64
FeDb	30.5	6.67
AdCf	31.1	5.23
AdVa	33.1	5.24
FeCf	28.0	14.1
FeFe	34.0	5.17
HCl	6.47	2.56
Cisplatin	28.6	17.3
4-MMPB	30.4	33.8

Results

Cytotoxicity assay

The cytotoxic effects of stylosin and some of its synthetic derivatives on PC-3 and HFF3 cell lines were investigated. Both cell lines were treated with various concentrations (3.125–50 μg/mL) of the compounds. After 24, 48, and 72 h of treatments, the percentage of live cells was measured by AlamarBlue assay and cell viabilities were calculated. Dose-response curves are presented in Fig. 2. PC-3 cells exhibited different sensitivities to various monoterpenoid compounds. The IC_{50} values of tested agents on both cell lines are shown in Table 1. BoVa and FeFe exhibited the lowest and highest cytotoxic potential among the tested compounds, respectively. Gallic acid derivatives showed relatively weak cytotoxic effects. Others had only moderate cytotoxicity (Fig. 3). To investigate the anti-cancer properties of these monoterpenoids, the IC_{50} value of each compound on HFF3 cell line was also determined and compared with the corresponding value on PC-3 cells (Fig. 4).

Enzyme activity assay

Inhibitory properties of synthesized compounds were examined against soybean 15-LOX (L1; type I-B; EC 1,13,11,12) enzyme and dose-response curves were plotted (Fig. 5). IC_{50} values were calculated and compared with 4-MMPB as a reference compound (Table 2). In general, most compounds demonstrated 15-LOX inhibitory potential. BoVa displayed the lowest inhibitory activity in this series while FeCf had the best 15-LOX inhibitory potential ($IC_{50} = 11.0$ μM) even more than the reference compound, 4-MMPB, ($IC_{50} = 17.1$ μM).

Apoptosis evaluation

Many natural and synthetic compounds can inhibit tumor cell proliferation and result to cell death, but not all of them can trigger apoptosis. To determine whether apoptosis was induced by the compounds, flow cytometric analysis was performed using FITC Annexin V Apoptosis Detection Kit with PI. Apoptotic cells can be identified by attachment of FITC Annexin V to phosphatidylserines on the outer membrane of the cells at early stages of apoptosis. Late apoptotic/necrotic cells are also distinguished by their ability to take up PI. The results indicated that the predominant mechanism of cell death induced by all tested compounds was apoptosis (Table 3). Figure 6 illustrates the comparison of apoptosis/necrosis induction between different studied compounds.

Discussion

Cancer is the result of misregulation in multiple cell signaling pathways which lead to uncontrolled cell proliferation and differentiation. Thus, one approach to treat cancer is to modulate these dysregulated pathways or to activate death promoting signals in such cells. Natural products and their derivatives represent potent sources for cancer treatment causing such modifications (Anand et al. 2008). 15-LOX-1 is one of these signaling mediators, and its overexpression specifically in the

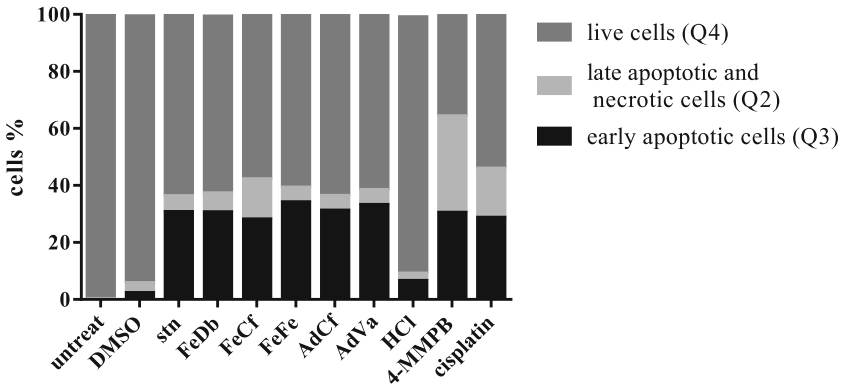


Fig. 6 Comparison of apoptosis induced by different compounds tested. Data are expressed as percentage of cells placed in each quadrant (Q2-Q4)

normal prostate epithelium could be one of the contributory factors facilitating the progression to high-grade neoplasia. Since many studies have reported high levels of 15-LOX-1 expression in PC-3 cell line (Hosseinymehr et al. 2016; Kelavkar et al. 2000, 2007; Orafaie et al. 2017; Spindler et al. 1997), these cells were used as a model to investigate the inhibitory effects of selected monoterpenes on its activity. 15-LOX-1 regulates the expression and activation of insulin-like growth factor-1 receptor (IGF-1R) and thereby affects the proliferation of PC cells. Blocking 15-LOX-1 inhibited the IGF-1/MAP (mitogen-activated protein) kinase signaling and proliferation of PC-3 cells (Kelavkar and Cohen 2004). In this study, the cytotoxic effects of stylosin and some of its synthesized derivatives were evaluated against PC-3 tumor cell line. BoVa exhibited very poor anti-proliferative properties while some compounds had remarkable cytotoxicity, as stn, AdVa, FeCf, FeDb, and AdVa indicated equal or slightly more anti-proliferation potential than cisplatin, a conventional chemotherapy drug, and FeFe indicated more than 3-fold cytotoxic effects in comparison to cisplatin, against PC-3 cells after 24 h of administration. Results revealed that almost all compounds act in a time- and dose-dependent manner, but overall comparison of corresponding data indicated that gallate derivatives were the weakest growth inhibitors; caffeate, vanillate and chatechuate derivatives had almost similar effects and ranked in second place, while ferulate derivative showed the most cytotoxic potential on PC-3 cells, among them. In the case of alcoholic part of compounds, adamantyl was similar to fenchyl and exerted growth inhibition effects far more than bornyl. In order to determine their selective anti-cancer effects, fast growing human HFF3 normal cells were also used and corresponding IC_{50} values against the two cancerous and normal cell lines were compared. AdVa, FeDb, and FeFe exhibited the most selective anti-cancer effects among the others, having higher cytotoxicity in cancerous cells during 24, 48, and 72 h, of administration. However, further in vitro investigations on wider range of prostate cancer cells and also using animal studies are still needed to confirm the potential use of these agents as anti-cancer drugs. The exact mechanism of action for these compounds is still not clearly understood. In a specific tumor, one pathway may play a more important role than the others but the cytotoxic effects may not be due to a single mechanism, and it might be due to operation of several pathways in concert with each other. To discover the eventual mechanism of action of the compounds on tumor cells, their inhibitory potencies against soybean 15-LOX were further assessed. The linear correlation coefficient for 15-LOX inhibition and tumor cell death potential indicated a strong positive correlation ($R = 0.983$, $R = 0.993$, and $R = 0.991$ for 24, 48, and 72 h respectively), which suggests that 15-LOX pathway may have great impact on inhibition of PC-3 cell proliferation. This hypothesis gets strengthened by the observation of apoptosis, as tumor cell death mechanism, since 15-LOX inhibition will trigger cascades which lead to apoptosis eventually. However, other pathways may in part have been responsible for the observed anti-proliferative effects.

Conclusion

We evaluated the inhibitory effects of some synthesized stylosin derivatives on proliferation of human fibroblast and PC-3 cancer cells. We showed that some of these compounds exerted cytotoxic and anti-cancer effects on PC-3 prostate cancer cells. Our results suggest that the observed anti-proliferative effects are mediated through apoptosis, which is probably triggered via 15-LOX-1 inhibition. However, further investigations are needed to confirm the potential of these compounds as anti-cancer drugs and to discover their exact chemotherapeutic mechanism of action.

Acknowledgments The authors are grateful to Prof. K. Abnous and Dr. P. Lavaee from Mashhad University of Medical Sciences, and Dr. R. Jalal from Ferdowsi University of Mashhad (FUM) for their kind technical support. We would like to thank M. Mirahmadi for his technical assistance on flow cytometry.

Author contributions SNG conducted the methodology and inferences, carried out the statistical analyses, and wrote the manuscript. HS carried out the chemicals synthesis and had discussions about the content. ARB designed the study and had discussions about the content. FM had technical assistance. MMM conceived the original idea and designed the study, analyzed the results, and wrote the manuscript.

Funding information This study was funded by FUM under grant no. 31788.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

References

Akinsete JA, Ion G, Witte TR, Hardman WE (2011) Consumption of high ω-3 fatty acid diet suppressed prostate tumorigenesis in C3 (1) Tag mice. Carcinogenesis 33:140–148. https://doi.org/10.1093/carcin/bgr238

Allott EH, Arab L, Su LJ, Farnan L, Fontham ETH, Mohler JL, Bensen JT, Steck SE (2017) Saturated fat intake and prostate cancer aggressiveness: results from the population-based North Carolina-Louisiana Prostate Cancer Project. Prostate Cancer Prostatic Dis 20:48–54. https://doi.org/10.1038/pcan.2016.39

Al-Nasiry S, Geusens N, Hanssens M, Luyten C, Pijnenborg R (2007) The use of Alamar Blue assay for quantitative analysis of viability, migration and invasion of choriocarcinoma cells. Hum Reprod 22:1304–1309. https://doi.org/10.1093/humrep/dem011

Anand P, Sundaram C, Jhurani S, Kunnumakkara AB, Aggarwal BB (2008) Curcumin and cancer: an "old-age" disease with an "age-old" solution. Cancer Lett 267:133–164. https://doi.org/10.1016/j.canlet.2008.03.025

Arghiani N, Matin MM, Bahrami AR, Iranshahi M, Sazgarnia A, Rassouli FB (2014) Investigating anticancer properties of the sesquiterpene ferutinin on colon carcinoma cells, *in vitro* and *in vivo*. Life Sci 109:87–94. https://doi.org/10.1016/j.lfs.2014.06.006

Atanasov AG, Waltenberger B, Pferschy-Wenzig EM, Linder T, Wawrosch C, Uhrin P, Temml V, Wang L, Schwaiger S, Heiss EH, Rollinger JM, Schuster D, Breuss JM, Bochkov V, Mihovilovic MD, Kopp B, Bauer R, Dirsch VM, Stuppner H (2015) Discovery and resupply of pharmacologically active plant-derived natural products: a review. Biotechnol Adv 33:1582–1614. https://doi.org/10.1016/j.biotechadv.2015.08.001

Bagirov VY, Sheichenko V, Aliev G, Pimenov M (1980) Esters from *Ferula stylosa*. Chem Nat Compd 16:562–563. https://doi.org/10.1007/BF00564858

Bakavoli M, Nikpour M, Rahimizadeh M, Saberi M, Sadeghian H (2007) Design and synthesis of pyrimido [4, 5-b][1, 4] benzothiazine derivatives, as potent 15-lipoxygenase inhibitors. Bioorg Med Chem 15:2120–2126. https://doi.org/10.1016/j.bmc.2006.12.022

Berquin IM, Min Y, Wu R, Wu J, Perry D, Cline JM, Thomas MJ, Thornburg T, Kulik G, Smith A, Edwards IJ, D'Agostino R Jr, Zhang H, Wu H, Kang JX, Chen YQ (2007) Modulation of prostate cancer genetic risk by omega-3 and omega-6 fatty acids. J Clin Investig 117:1866–1875. https://doi.org/10.1172/JCI31494

Chen C-N, Huang HH, Wu CL, Lin CPC, Hsu JTA, Hsieh HP, Chuang SE, Lai GM (2007) Isocostunolide, a sesquiterpene lactone, induces mitochondrial membrane depolarization and caspase-dependent apoptosis in human melanoma cells. Cancer Lett 246:237–252. https://doi.org/10.1016/j.canlet.2006.03.004

Choi J-H, Lee K-T (2009) Costunolide-induced apoptosis in human leukemia cells: involvement of c-Jun N-terminal kinase activation. Biol Pharm Bull 32:1803–1808

Chung LY, Soo WK, Chan KY, Mustafa MR, Goh SH, Imiyabir Z (2009) Lipoxygenase inhibiting activity of some Malaysian plants. Pharm Biol 47:1142–1148

Cordell GA (2011) Sustainable medicines and global health care. Planta Med 77:1129–1138. https://doi.org/10.1055/s-0030-1270731

Cragg GM, Schepartz SA, Suffness M, Grever MR (1993) The taxol supply crisis. New NCI policies for handling the large-scale production of novel natural product anticancer and anti-HIV agents. J Nat Prod 56:1657–1668

David B, Wolfender J-L, Dias DA (2015) The pharmaceutical industry and natural products: historical status and new trends. Phytochem Rev 14:299–315. https://doi.org/10.1007/s11101-014-9367-z

Guo X-S, Zhang Y-Z (1983) Chemical studies on the Chinese drug Qin pi, the bark of *Fraxinus stylosa*. Acta Pharm Sin 6:006

Gutierrez-Lugo M-T, Deschamps JD, Holman TR, Suarez E, Timmermann BN (2004) Lipoxygenase inhibition by anadanthoflavone, a new flavonoid from the aerial parts of *Anadenanthera colubrina*. Planta Med 70:263–265. https://doi.org/10.1055/s-2004-818920

Hosseinymehr M, Matin MM, Sadeghian H, Bahrami AR, Kaseb-Mojaver N (2016) 8-Farnesyloxycoumarin induces apoptosis in PC-3 prostate cancer cells by inhibition of 15-lipoxygenase-1 enzymatic activity. Anti-Cancer Drugs 27:854–862. https://doi.org/10.1097/CAD.0000000000000399

Hu Y, Sun H, Owens RT, Gu Z, Wu J, Chen YQ, O'Flaherty JT, Edwards IJ (2010) Syndecan-1-dependent suppression of PDK1/Akt/bad signaling by docosahexaenoic acid induces apoptosis in prostate cancer. Neoplasia 12:826–836

Hu Y, Sun H, O'flaherty JT, Edwards IJ (2012) 15-Lipoxygenase-1-mediated metabolism of docosahexaenoic acid is required for syndecan-1 signaling and apoptosis in prostate cancer cells. Carcinogenesis 34:176–182. https://doi.org/10.1093/carcin/bgs324

Iranshahi M, Jabbari A, Orafaie A, Mehri R, Zeraatkar S, Ahmadi T, Alimardani M, Sadeghian H (2012) Synthesis and SAR studies of mono O-prenylated coumarins as potent 15-lipoxygenase inhibitors. Eur J Med Chem 57:134–142. https://doi.org/10.1016/j.ejmech.2012.09.006

Jagetia GC, Baliga MS, Venkatesh P, Ulloor JN, Mantena SK, Genebriera J, Mathuram V (2005) Evaluation of the cytotoxic effect of the monoterpene indole alkaloid echitamine *in vitro* and in tumour-bearing mice. J Pharm Pharmacol 57:1213–1219. https://doi.org/10.1211/jpp.57.9.0017

Kelavkar UP, Cohen C (2004) 15-Lipoxygenase-1 expression upregulates and activates insulin-like growth factor-1 receptor in prostate cancer cells. Neoplasia 6:41–52

Kelavkar U, Cohen C, Kamitani H, Eling TE, Badr KF (2000) Concordant induction of 15-lipoxygenase-1 and mutant p53 expression in human prostate adenocarcinoma: correlation with Gleason staging. Carcinogenesis 21:1777–1787

Kelavkar U et al (2007) DNA methylation paradigm shift: 15-lipoxygenase-1 upregulation in prostatic intraepithelial neoplasia and prostate cancer by atypical promoter hypermethylation. Prostaglandins Other Lipid Mediat 82:185–197. https://doi.org/10.1016/j.prostaglandins.2006.05.015

Khalifa RA, Nasser MS, Gomaa AA, Osman NM, Salem HM (2013) Resazurin microtiter assay plate method for detection of susceptibility of multidrug resistant Mycobacterium tuberculosis to second-line anti-tuberculous drugs. Egypt J Chest Dis Tuberc 62:241–247. https://doi.org/10.1016/j.ejcdt.2013.05.008

Kingston DG (2010) Modern natural products drug discovery and its relevance to biodiversity conservation. J Nat Prod 74:496–511. https://doi.org/10.1021/np100550t

Kobayashi N, Barnard RJ, Henning SM, Elashoff D, Reddy ST, Cohen P, Leung P, Hong-Gonzalez J, Freedland SJ, Said J, Gui D, Seeram NP, Popoviciu LM, Bagga D, Heber D, Glaspy JA, Aronson WJ (2006) Effect of altering dietary ω-6/ω-3 fatty acid ratios on prostate cancer membrane composition, cyclooxygenase-2, and prostaglandin E2. Clin Cancer Res 12:4662–4670. https://doi.org/10.1158/1078-0432.CCR-06-0459

Larsson SC, Kumlin M, Ingelman-Sundberg M, Wolk A (2004) Dietary long-chain n-3 fatty acids for the prevention of cancer: a review of potential mechanisms. Am J Clin Nutr 79:935–945. https://doi.org/10.1093/ajcn/79.6.935

Mansoor TA, Ramalho RM, Mulhovo S, Rodrigues CM, Ferreira MJU (2009) Induction of apoptosis in HuH-7 cancer cells by monoterpene and β-carboline indole alkaloids isolated from the leaves of *Tabernaemontana elegans*. Bioorg Med Chem Lett 19:4255–4258. https://doi.org/10.1016/j.bmcl.2009.05.104

Matin MM, Nakhaeizadeh H, Bahrami AR, Iranshahi M, Arghiani N, Rassouli FB (2014) Ferutinin, an apoptosis inducing terpenoid from *Ferula ovina*. Asian Pac J Cancer Prev 15:2123–2128

Newman DJ, Cragg GM (2012) Natural products as sources of new drugs over the 30 years from 1981 to 2010. J Nat Prod 75:311–335. https://doi.org/10.1021/np200906s

Newman DJ, Cragg GM (2016) Natural products as sources of new drugs from 1981 to 2014. J Nat Prod 79:629–661. https://doi.org/10.1021/acs.jnatprod.5b01055

Norrish A, Skeaff C, Arribas G, Sharpe S, Jackson R (1999) Prostate cancer risk and consumption of fish oils: a dietary biomarker-based case-control study. Br J Cancer 81:1238–1242. https://doi.org/10.1038/sj.bjc.6690835

O'flaherty JT et al (2012) 15-lipoxygenase metabolites of docosahexaenoic acid inhibit prostate cancer cell proliferation and survival. PLoS One 7:e45480. https://doi.org/10.1371/journal.pone.0045480

Orafaie A, Sadeghian H, Bahrami AR, Saboormaleki S, Matin MM (2017) 5-farnesyloxycoumarin: a potent 15-LOX-1 inhibitor, prevents prostate cancer cell growth. Med Chem Res 26:227–234. https://doi.org/10.1007/s00044-016-1737-1

Paterna A, Borralho PM, Gomes SE, Mulhovo S, Rodrigues CM, Ferreira M-JU (2015) Monoterpene indole alkaloid hydrazone derivatives with apoptosis inducing activity in human HCT116 colon and HepG2 liver carcinoma cells. Bioorg Med Chem Lett 25:3556–3559. https://doi.org/10.1016/j.bmcl.2015.06.084

Rai G, Kenyon V, Jadhav A, Schultz L, Armstrong M, Jameson JB II, Hoobler E, Leister W, Simeonov A, Holman TR, Maloney DJ (2010) Discovery of potent and selective inhibitors of human reticulocyte 15-lipoxygenase-1. J Med Chem 53:7392–7404. https://doi.org/10.1021/jm1008852

Rampersad SN (2012) Multiple applications of Alamar Blue as an indicator of metabolic function and cellular health in cell viability bioassays. Sensors 12:12347–12360. https://doi.org/10.3390/s120912347

Rassouli FB, Matin MM, Iranshahi M, Bahrami AR (2011) Investigating the cytotoxic and apoptosis inducing effects of monoterpenoid stylosin *in vitro*. Fitoterapia 82:742–749. https://doi.org/10.1016/j.fitote.2011.03.005

Rose DP (1997) Dietary fatty acids and prevention of hormone-responsive cancer. Proc Soc Exp Biol Med 216:224–233

Rose DP, Connolly JM (1991) Effects of fatty acids and eicosanoid synthesis inhibitors on the growth of two human prostate cancer cell lines. Prostate 18:243–254

Sadeghian H, Seyedi SM, Jafari Z (2018) Design and synthesis of new esters of terpenoid alcohols as 15-lipoxygenase inhibitors. Iran J Basic Med Sci 21:738. https://doi.org/10.22038/IJBMS.2018.27910.6794

Shang-Gao L et al (2012) Cytotoxic sesquiterpene lactones from *Vernonia bockiana*. Chin J Nat Med 10:230–233. https://doi.org/10.3724/SP.J.1009.2012.00230

Siegel RL, Miller KD, Jemal A (2016) Cancer statistics, 2016. CA Cancer J Clin 66:7–30. https://doi.org/10.3322/caac.21442

Spindler SA, Sarkar FH, Sakr WA, Blackburn ML, Bull AW, Lagattuta M, Reddy RG (1997) Production of 13-hydroxyoctadecadienoic acid (13-HODE) by prostate tumors and cell lines. Biochem Biophys Res Commun 239:775–781. https://doi.org/10.1006/bbrc.1997.7471

Suraneni M et al (2010) Transgenic expression of 15-lipoxygenase 2 (15-LOX-2) in mouse prostate leads to hyperplasia and cell senescence. Oncogene 29:4261–4275. https://doi.org/10.1038/onc.2010.197

Terry P, Lichtenstein P, Feychting M, Ahlbom A, Wolk A (2001) Fatty fish consumption and risk of prostate cancer. Lancet 357:1764–1766. https://doi.org/10.1016/S0140-6736(00)04889-3

Terry PD, Rohan TE, Wolk A (2003) Intakes of fish and marine fatty acids and the risks of cancers of the breast and prostate and of other hormone-related cancers: a review of the epidemiologic evidence. Am J Clin Nutr 77:532–543. https://doi.org/10.1093/ajcn/77.3.532

Valiahdi SM, Iranshahi M, Sahebkar A (2013) Cytotoxic activities of phytochemicals from *Ferula* species. DARU J Pharm Sci 21:39. https://doi.org/10.1186/2008-2231-21-39

Vines G (2004) Herbal harvests with a future: towards sustainable sources for medicinal plants. Plantlife International

Zadra G, Photopoulos C, Loda M (2013) The fat side of prostate cancer. Biochim Biophys Acta (BBA)-Mol Cell Biol Lipids 1831:1518–1532. https://doi.org/10.1016/j.bbalip.2013.03.010