RESEARCH ARTICLE

**Editorial Process:** Submission:04/08/2022 Acceptance:12/13/2022

# Selected Statins as Dual Antiproliferative-Antiinflammatory Compounds

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## Abstract

**Background:** We hypothesized that superlative dual cytotoxicity-antiinflammtion bioefficacies of 9 selected lipophilic statins correlate to their chelation effect of 3,5-dihydroxyheptanoic acid. **Methodology:** Lipophilic-acid chelating statins have been screened for in vitro duality of proliferation inhibition and NO-radical scavenging capacities. Results: Their spectrum of selectivity indices for safety in PDL fibroblasts -based 72h incubations was reported. Surprisingly despite its lack on macrophages LPS-triggered inflammation over 5-200 μM and unlike the 8 statins; cerivastatin had growth inhibition $IC_{50}$ values of 40nM (SW620), 110nM (HT29), 2.9 μM (HCT116), 6μM (SW480), and most notably 38μM (<50 μM, in $Caco_2$). Exclusively cerivastatin exerted antitumorigenesis $IC_{50}$ values <50 μM in all T47D, MCF7 and PANC1 72h cultures. In statins with greater antiinflammation affinity than indomethacin's; lovastatin had cytotoxicity $IC_{50}$ values <20 μM in SW620<HT29<ACT116<SW480 and >100 μM in $Caco_2$. Atorvastatin was found of viability reduction $IC_{50}$ value <20 μM in HCT116<SW620. Simvastatin exerted growth inhibition $IC_{50}$ values <20 μM in HT29< SW620<SW480 and MCF7. Rosuvastatin, pitavastatin and fluvastatin proved equipotency to indomethacin but cytotoxicity $IC_{50}$ values >50μM in T47D, MCF7 and PANC1. Rosuvastatin had antineoplastic $IC_{50}$ values (<50 μM) in SW620<SW480<MCF7. Pitavastatin was ascribed cytotoxicity $IC_{50}$ values (<50μM) in HT29<SW620<HCT116<SW480. Fluvastatin had antiproliferation $IC_{50}$ values (<50μM) in SW620< HT29<SW480<HCT116, and the rest were >50 μM in remaining colorectal, breast and pancreatic cancer cell lines. In statins with appreciable antiinflammation but reasonably lower affinity than indomethacin's and cytotoxicity $IC_{50}$ values >50μM in T47D, MCF7 and PANC1; pravastatin had viability reduction $IC_{50}$ values <50μM in HT29<HCT116. Mevastatin was reported for growth inhibition $IC_{50}$ values <50μM in HT29<SW620<HCT112<SW480. Antitumorigenesis $IC_{50}$ values>50 μM were for statins in remaining colorectal cancer cell lines, breast cancer and pancreatic cancer cell lines.Conclusion: Among the rest, cerivastatin warrants further novel scaffold development to maximize efficacy and optimal molecular action mechanisms of chemotherapy/prevention.

**Keywords:** Statins- inflammation-sulforhodamine B- cisplatin- cancer and chelation

*Asian Pac J Cancer Prev,* **23 (12)**, 4047-4062

## Introduction

Statins are family of cholesterol lowering drugs, 3-hydroxy-3-methylglutaryl coenzyme (HMG-CoA) reductase Inhibitors. They potently inhibit the conversion of HMG-CoA to mevalonic acid (MA) which is the cornerstone of cholesterol synthesis (Istvan and Deisenhofer, 2001). From a physicochemical view; statins are classified into two major groups; Lipophilic and hydrophilic statins and each group has its line in absorption and metabolism. Lipophilic group; which includes fluvastatin, pitavastatin, lovastatin, simvastatin, atorvastatin and cerivastatin; have the ability to cross cell membrane to directly approach HMG-CoA reductase and they are widely distributed. This property gives them the priority to be used in disease therapy (Kunutsor and Laukkanen, 2020). The other main group is hydrophilic group; including pravastatin and rosuvastatin; which need transmembrane peptide transporter (OATP1B transporter) to cross cell membrane, and because OATP1B transporters do largely exist in the liver; hydrophilic statins are minimally distributed and are concentrated in the liver and this may contribute to their lower pleiotropic effects than lipophilic ones (Ahmadi et al., 2020). Statins are cost-effective drugs, with potent role duality in the treatment of atherosclerosis due to both their immune-modulating and lipid-lowering effects. They are FDA approved for the treatment of Heterozygous (or Homozygous) familial hypercholesterolemia. Since the last decade, they have been used in the primary and secondary prevention of coronary heart disease (Pitts, 2015) and in some off-label use in post heart or post kidney

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transplantation; fatal- and nonfatal-MI, revascularization and a composite of fatal and non-fatal strokes (De Denus and Spinler, 2002; Ray and Cannon, 2005; Mills et al., 2011; Ahmad, 2020).

*Statins repurposing in chemotherapy of cancer*

In cancer field, the slow responsiveness of the approved anticancer agents and increasing resistance of tumor cells to these agents led to massive spread of cancer worldwide to become one of the 10 top leading causes of death accounting for 1.7 million deaths by 2016 according to Word Health Organization, and the 4th leading cause of death in the upper-middle-income countries by 2019; it is expected by 2030 to grow to 21.7 million new cancer cases and 13 million cancer deaths (Siegel et al., 2019; WHO, 2019). On top, discovering a new anticancer drug is extremely expensive and far from universal reach. Repurposing existing safe therapeutics may offer an alternative approach to known agents; can be known as "Therapeutic Switching".Repurposed medications can likewise minimize a great percentage of the early expense and time expected to put up a medication for sale to the public, thereby cutting short linkage between research bench work and treatment at bedside (Papapetropoulos and Szabo, 2018). Thus, because the drugs used are FDA-approved , there is no need for performing phase I and phase IIa clinical trials and so risk of therapeutic failure because severe side effects are lower (Oprea and Mestres, 2012; Hanusova, 2015). This approach grows in many fields in pharmacologies, one of these fields is anticancer therapy.Increasing evidence from both in vitro and in vivo studies suggests that statins exert pleiotropic effects as they performe antiproliferative, antiangiogenic, and antimetastatic properties in addition to their cholesterol lowering effect (Hindler et al., 2006). Since they work potentially by new mechanism against cancer, the potential resistance does not exist. Statins have a well-established safety profile with cheap prices compared to chemotherapy.In a meta-analysis with 15 studies included, demonstrated the anitinflammatoy effect of statins (atorvastatin and rosuvastatin were used) in rheumatoid arthritis (RA) autoimmune inflammation (Lv et al., 2015). Moreover, Simvastatin, as a member of statin class, showed an effective decrease in mortality rate in a population-based cohort study done involving 3,653 Prostate Cancer (PC) patients with hyperlipidemia (Chen et al., 2018).

Besides, statins can perform as antiinflammatory agents in atherogenesis via molecular action mechanisms including: Inhibition of isoprenoids production; geranyl-geranyl pyrophosphate (GG-PP) and farnesyl pyrophosphate (FPP) in vascular cells which are responsible of inflammatory activation and in turn inhibiting nuclear transcription factors as nuclear factor-kappa B (NF-kB) (Ray and Cannon, 2005), inhibiting production of L-mevalonate- and GGPP that leads to overall antiatherogenic effects (Antonopoulos et al., 2012), up-regulation of dimethylarginine-dimethylaminohydrolase gene transcription by statins and thus inflammation-induced endothelial dysfunction reduction (Serban et al., 2015) and decreasing the level of serum C - reactive protein (CRP) (Weitz-Schmidt, 2002).

This work is a continuation of a recent research line on re-purposing drugs toward new activities or diseases. Importantly a significant high antiproliferative activity of atorvastatin on two cell lines A375.2S cell line and T47D cell line with $IC_{50}$ of 0.0156 μM and 34.59 μM respectively were revealed (AlKhalil et al., 2020). These data were further validated with other cell lines including colorectal cancer (CRC); fortifying the fact that atorvastatin has an apoptotic effect on cancer cells, particularly colorectal (CRC) and BC (in SW620 cells with $IC_{50}$ value accounting to 0.0018 ±0000 μM) and accordingly high safety (Mamdooh et al., 2019). This foundation furnished for this work basically constructed for elucidation of possible molecular antineoplastic action mechanism via antiinflammation (Hallaq et al., 2022; Khaleel et al., 2022; Qashou et al., 2022; Salih et al., 2022).

## Materials and Methods

*In vitro antiproliferation assay*

The cytotoxicity measurements were determined using Sulforhodamine B (SRB; Santa Cruz Biotechnology, Inc. Texas, USA) colorimetric assay (using Spectro Scan 80D UV-VIS spectrophotometer (Sedico Ltd., Nicosia, Cyprus). For proliferation inhibition screening, Breast cancer cell lines MCF7 (ATCC® HTB-22) and T47D (ATCC® HTB-133), PANC1 pancreatic cell line (ATCC® CRL-1469), and colorectal cancer cell lines namely; HT-29 (ATCC® HTB-38), HCT116 (ATCC® CCL-247), SW620 (ATCC® CCL-227), SW480 (ATCC® CCL-228) and CACO2 (ATCC® HTB-37) cell lines were procured (Kaur and Dufour, 2012). Periodontal ligament fibroblasts (PDL) were used for determinations of selective cytotoxicity. The cell lines were cultured in high glucose DMEM (Bio Whittaker, Verviers, Belgium) containing 10% FBS, HEPES Buffer (10 mM), L-glutamine (2 mM), gentamicin (50 μg/mL), penicillin (100 U/mL), and streptomycin sulfate (100 mg/mL). The cells were incubated with test compounds at different concentrations (5-200 μg/mL, except for cerivastatin of range: 0.0001-10 μg/mL). The mechanism of reduction of cell viability was adopted as described previously (Vichai and Kirtikara, 2006). As a robust and classical antineoplastic apoptogenic reference agent (El-Hamoly et al., 2017), cisplatin (1-200 μM) was recruited for comparison purposes (Alabsi et al., 2018; Mamdooh et al., 2019; AlKhalil et al., 2020; Hallaq et al., 2022; Khaleel et al., 2022; Qashou et al., 2022; Salih et al., 2022). Dose–response curves were plotted and values were expressed as percentage of control optical density and $IC_{50}$ values 50% inhibitory concentration were estimated by regression analysis (Papazisis et al., 1997). Selectivity index (SI) is the term that describes the safety of tested drugs. It is calculated by dividing $IC_{50}$ value of tested compound on fibroblasts by the least $IC_{50}$ value of the same compound on any specific pathological cell line (Hoffmann, et al., 2011; Hallaq et al., 2022; Khaleel et al., 2022; Qashou et al., 2022; Salih et al., 2022).

*Antiinflammation Determination in Vitro*

RAW 264.7 mouse macrophage cell line (ATCC®

TIB-71) were cultured in high glucose DMEM supplemented with 10% (FBS), penicillin (100 U/mL), streptomycin (100 µg/mL), and L-glutamate (100 µg/mL) in a 37°C humidified atmosphere with 95% air and 5% $CO_2$. Confluent macrophages (2 x $10^5$ /well) were incubated with macrophage prompting lipopolysaccharide (LPS; 20 µg/mL; Sigma, St. Luis, MO, USA) added simultaneously with indomethacin (25-200 µg/mL) as the positive control (Ghimeray et al., 2015; Assanga et al., 2017; Arabiyat et al., 2019; Hallaq et al., 2022; Khaleel et al., 2022; Qashou et al., 2022; Salih et al., 2022) and test compounds at different concentrations (5-200 µg/mL, except for cerivastatin of range: 0.0001-10 µg/mL), for 24 hour incubations. A 100 µLGriess reagent (50 µL of 1 % Sulfanilamide in 5 % phosphoric acid and 50 µL of 0.1 % napthylehtyllenediamine-HCL) were mixed with aliquots of 100 µL of cell culture media and incubated at R.T. for 10 minutes. Absorbance at 550 nm was determined using microplate reader (Biotekmultiwell plate reader MQX200, USA). The concentration of nitrite was determined by comparison with sodium nitrite standard curve. SRB cytotoxity protocol was performed for evaluation of the effect of studied test compounds on RAW 264.7 viability (Huang et al., 2016; AbdulFattah et al., 2019; Hallaq et al., 2022; Khaleel et al., 2022; Qashou et al., 2022; Salih et al., 2022).

*DPPH Free Radical Scavenger Assay*

This method depends on the reduction of the radicals resulting in a color change from oxidized purple to reduced yellow. Principally Diphenyl-2-picryl-hydrazyl (DPPH) undergoes reduction in methanol (MeOH) solution, in the presence of a hydrogen-donating compound due to the formation of the non-radical form DPPH-H. This change in color can be quantitatively measured using a spectrophotometer at 515–520 nm. In contrast to other radical scavenging assays, a DPPH radical is stable and can provide reproducible spectroscopic values (Sharma and Bhat, 2009; Marinova and Batchvarov, 2011; Shalaby and Shanab, 2013; Hidayat and Kuswandi, 2017; Haida, 2019; Hallaq et al., 2022; Khaleel et al., 2022; Qashou et al., 2022; Salih et al., 2022). A DPPH solution (0.2 mM) was diluted with MeOH and then mixed with test compounds as well as ascorbic acid with a DPPH solution in a concentration ratio of 1:1 using a 96-well plate (so that a final concentration range 6.25-200 µg/mL was obtained for test agents); the treated solution was incubated one hour isolated from light. Finally, a change in absorbance at 517 nm wavelength was measured using microplate reader (Bio-Tek Instrument, USA). Ascorbic acid was the robust and classical standard radical scavenging reference agent for comparison purposes. The calculation of the DPPH radical scavenging activity inhibition was determined by the following equation where A represents photometric absorbance: in % = (A control – A sample) / A control x 100% (Litwinienko and Ingold, 2004; Sharma and Bhat, 2009; Marinova and Batchvarov, 2011; Shalaby and Shanab, 2013; Karahan et al., 2015; Hidayat, et al., 2017; Haida, 2019; Paulpriya et al., 2015; Shen et al., 2010; Hallaq et al., 2022; Khaleel et al., 2022; Qashou et al., 2022; Salih et al., 2022).

*Statistical analysis*

The values were presented as mean ± standard deviation (SD) of 3-4 independent experiments. Statistical differences between reference agent and different treatment drugs were determined using GraphPad Prism software unpaired t-test [version 5.01 for Windows; GraphPad software, San Diego, CA, USA]. Values were considered significantly different if $P< 0.05$ and highly significantly different if $P<0.001$.

## Results

Using SRB bioassay; Cisplatin exerted marked dose-dependent viability reduction of colorectal (3.4-7 <50 µM), pancreatic and breast (540-590 > 50 µM) cancer cell lines. Most notably statins exerted to a greater extent nanomolar-micromolar affinities of antiproliferation potencies <50µM (Table 1a,b).

Lovastatin had an $IC_{50}$ value of 80 nM in SW620, with $IC_{50}$ values <50 µM in HT29, HCT116 and SW480 and >50 µM in $Caco_2$. Atorvastatin was found in HCT116 and SW620 of cytotoxicity $IC_{50}$ value of 6 µM (<50 µM).

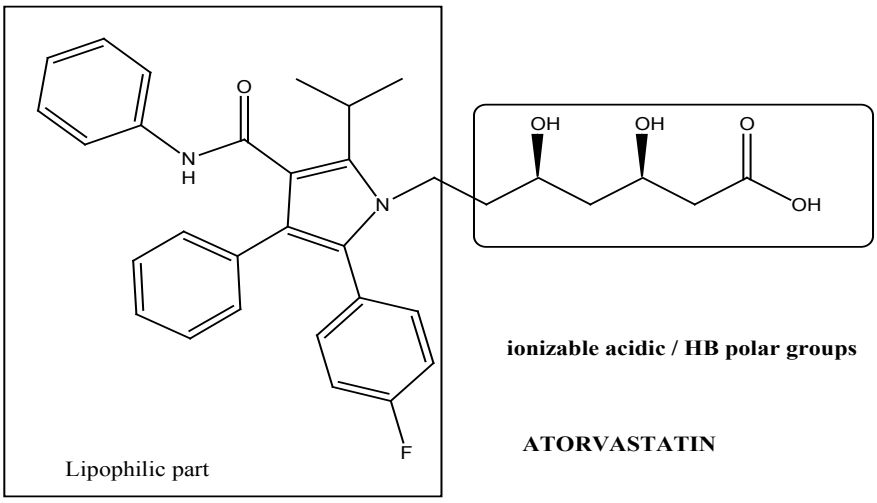
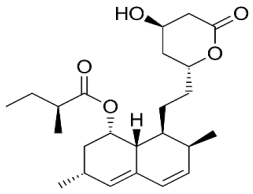
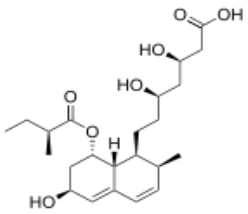
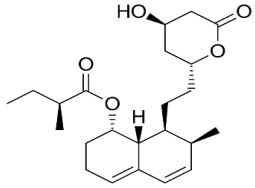
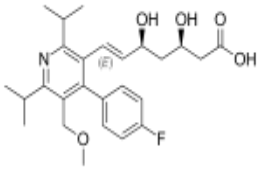
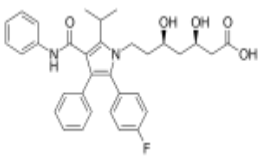
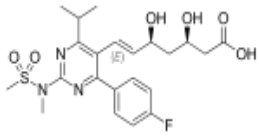
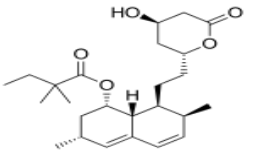
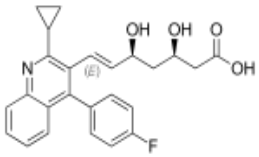
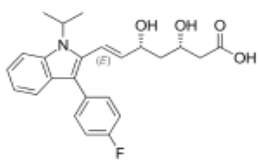



Figure 1. Proposed Structural Functionalities Required for Antiproliferative Effect in Atorvastatin.

Table 1A. Cytotoxicity (as of %Control) $IC_{50}$ value in µM of the selected statins series vs. Cisplatin

| Cytotoxicity (as of %Control) IC50 value µM (µg/mL) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Treatment | | Chemical structure | HT29 | HCT116 | SW620 | CACO2 | SW480 | SI |
| 1 | Lovastatin (prodrug) |  | 17.94± 0.85*<br>(7.26± 0.34) | 16.78± 0.60*<br>(6.79± 0.24) | 0.08±0.00*<br>(0.03±0.00) | 105.47± 5.7*<br>(42.67± 2.30) | 21.87± 1.20*<br>(8.85± 0.48) | 31. 25<br>(SW620) |
| 2 | Pravastatin (hydrophilic) |  | 0.36± 0.01*<br>(0.16± 0.01) | 3.52± 0.13*<br>(1.57± 0.03) | 425.31± 22.79*<br>(189.91± 22.79) | 281.01± 13.02*<br>(125.47± 5.82) | 1231.52± 161.00*<br>(549.89± 71.89) | 1,024.61<br>(HT29) |
| 3 | Mevastatin |  | 0.18± 0.02*<br>(0.07± 0.01) | 12.00± 0.37*<br>(4.57± 0.23) | 1.22± 0.14*<br>(0.48± 0.06) | 305.73± 51.49*<br>(119.39± 20.11) | 35.56± 5.92*<br>(13.89± 2.31) | 19.277<br>(HT29) |
| 4 | Cerivastatin |  | 0.11± 0.02*<br>(0.05± 0.01) | 2.88± 0.53*<br>(1.39± 0.26) | 0.04± 0.01*<br>(0.02±0.00) | 37.94± 3.39*<br>(18.27± 1.63) | 6.05± 1.20*<br>(2.91±0.58) | 60<br>(SW620) |
| 5 | Atorvastatin |  | 50.54±1.74*<br>(28.23±0.97) | 6.66± 0.18*<br>(3.79±0.12) | 6.10± 0.78*<br>(3.41±0.44) | 124.32± 12.88*<br>(69.45±7.19) | 61.77± 9.55*<br>(34.51±5.43) | 4.085<br>(SW620) |
| 6 | Rosuvastatin (hydrophilic) |  | 68.14± 12.70*<br>(32.81±6.12) | 103.79± 2.35*<br>(49.98±1.13) | 14.94± 0.85*<br>(7.19±0.41) | 163.19± 17.85*<br>(78.58±8.59) | 22.67± 2.52*<br>(10.91± 1.21) | 22<br>(MCF7) |
| 7 | Simvastatin (prodrug) |  | 2.80± 0.36*<br>(1.17± 0.15) | 11.14± 1.58*<br>(4.66± 0.66) | 1.44± 0.16*<br>(0.60± 0.07) | 28.40± 4.04*<br>(11.89± 1.69) | 6.71± 0.95*<br>(2.81± 0.40) | 8.04<br>(MCF7) |
| 8 | Pitavastatin |  | 0.2± 0.04*<br>(0.09±0.02) | 16.93± 2.32*<br>(7.46±1.02) | 1.22± 0.14*<br>(0.54±0.06) | 5.98± 0.59*<br>(2.63± 0.26) | 73.42± 10.97*<br>(32.34± 4.83) | 4.65<br>(HT29) |
| 9 | Fluvastatin |  | 3.69± 0.52*<br>(1.67± 0.24) | 15.63± 2.39*<br>(7.06± 1.08) | 1.08± 0.19*<br>(0.49±0.09) | 247.93± 38.54*<br>(111.93±17.40) | 9.38± 0.57*<br>(4.24±0.26) | 0.04<br>(SW620) |
| 10 | Cisplatin | | 6.38± 0.60<br>(1.91± 0.18) | 4.69± 0.40<br>(1.41± 0.12) | 4.37± 0.03<br>(1.31±0.01) | 7.05± 0.40<br>(2.11± 0.12) | 3.37± 0.41<br>(1.01±0.12) | |

Results are mean ± SD (n = 3 independent replicates). $IC_{50}$ values (concentration at which 50% inhibition of cell proliferation took place in comparison to non-induced basal 72 h incubations) were calculated within 0.1-200 µg/mL range. NI is lack of cytotoxicity within the tested 0.1-200 µg/mL concentration range. P-value calculated by unpaired t-test between test compound $IC_{50}$ values and cisplatin's (µM) using GraphPad Prism software version 5.0.1* When P<0.05, NS, not significantly different from reference agent

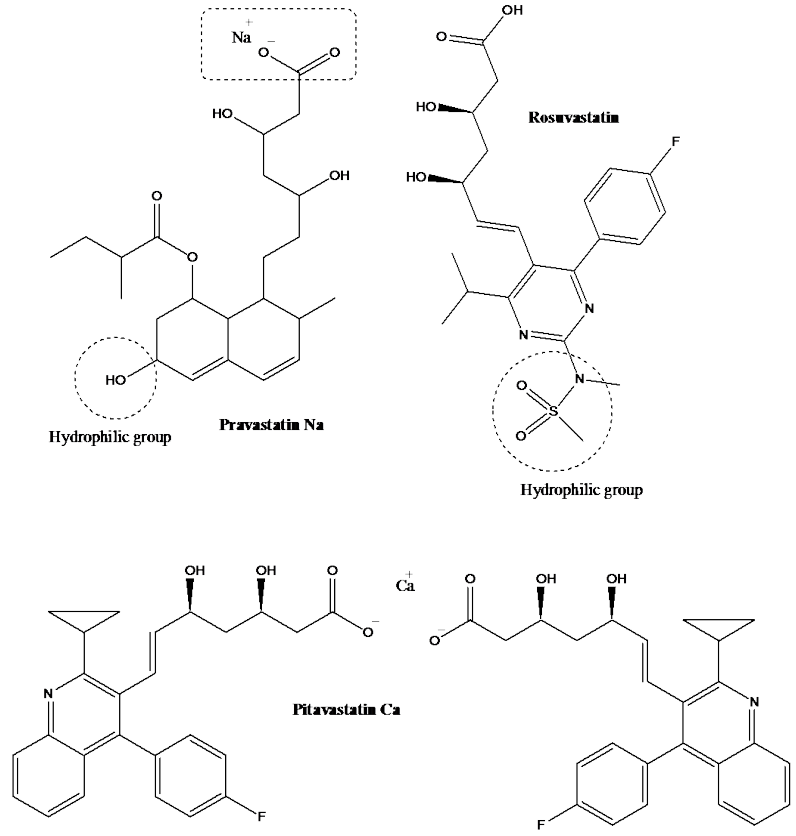



Figure 2.Structure of Pitavastatin Ca, Pravastatin and Rosuvastatin.

Simvastatin exerted growth inhibition $IC_{50}$ values <20 μM in HT29, SW620 and SW480 and MCF7 (1.1μM). Antitumorigenesis $IC_{50}$ values>50 μM were for lovastatin, atorvastatin and simvastatin in remaining colorectal cancer cell lines, breast cancer and pancreatic cancer cell lines (Table 1a,b).

Rosuvastatin, pitavastatin and fluvastatin exhibited cytotoxicity $IC_{50}$ values >50μM in T47D, MCF7 and PANC1; rosuvastatin had antineoplastic $IC_{50}$ values (<50 μM) of 15 μM (SW620), 23 μM (SW480) and 1.5 μM (MCF7) and the rest were >50 μM in remaining colorectal, breast and pancreatic cancer cell lines. Pitavastatin exhibited cytotoxicity $IC_{50}$ values (<50μM) of 200nM (HT29), 1.2 μM (SW620), 12 μM (HCT116) and 36 μM (SW480). Fluvastatin had antiproliferation $IC_{50}$ values (<50μM) of 1.1 μM (SW620), 4 μM (HT29), 9.4 μM (SW480) and 16 μM (HCT116) (Table 1a,b).

Pravastatin and mevastatin exhibited cytotoxicity $IC_{50}$ values >50μM in T47D, MCF7 and PANC1; pravastatin had viability reduction $IC_{50}$ values (<50μM) of 360nM (HT29) and 3.5 μM (HCT116) with $IC_{50}$ values >50μM in remaining cells monolayers. Mevastatin was reported for growth inhibition $IC_{50}$ values (<50μM) of 180 nM (HT29), 1.2 μM (SW620), 12 μM (HCT116) and 36 μM (SW480) (Table 1a,b).

Surprisingly despite its lack on macrophages LPS-triggered inflammation; cerivastatin had growth inhibition $IC_{50}$ values of 40nM (SW620), 110nM (HT29), 2.9 μM (HCT116), 6μM (SW480), and most notably 38μM (<50 μM, in $Caco_2$; the most aggressive and resistant colon cancer cells) unlike the aforementioned 8 statins. Exclusively cerivastatin exerted antitumorigenesis $IC_{50}$ values <50 μM in T47D, MCF7 and PANC (Table 1a,b).

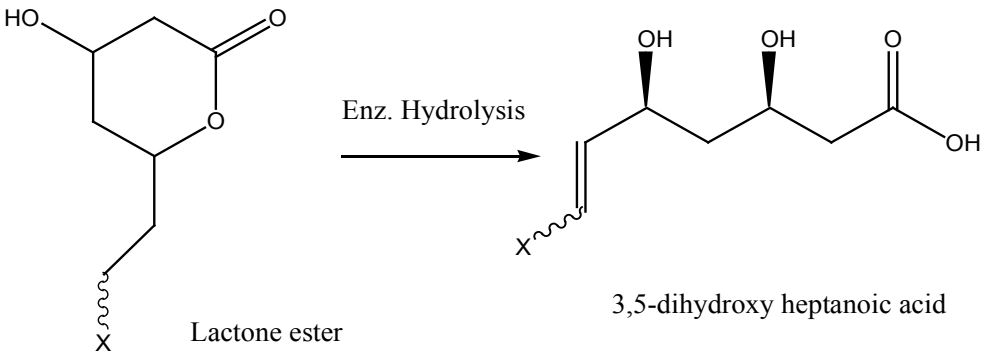
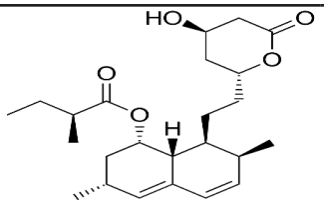
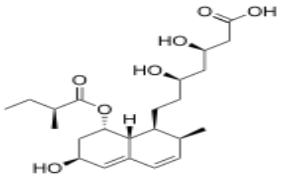
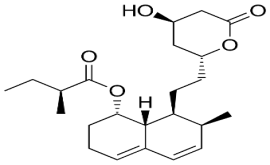
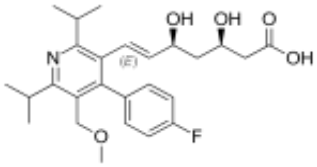
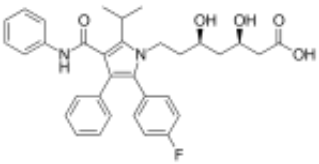
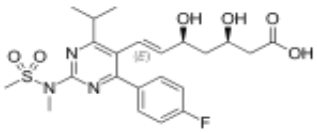
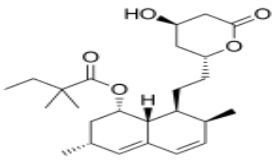
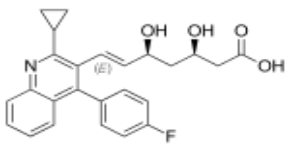
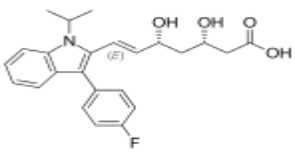



Figure 3. Potential Enzymatic Hydrolysis of the Lactone into Active Free Acid

Table 1B. Cytotoxicity (as of %Control) $IC_{50}$ Value in μM of the Selected Statins Series vs. Cisplatin

| | Treatment | Chemical structure | T47D | MCF7 | PANC 1 | PDL Fibroblasts | SI |
|---|---|---|---|---|---|---|---|
| 1 | Lovastatin (prodrug) |  | 90.62± 5.08*<br>(36.66±2.05) | 63.16± 3.32*<br>(25.55±1.34) | 48.78± 4.66*<br>(19.73±1.89) | 2.53± 0.02*<br>(1.02±0.01) | 31. 25<br>(SW620) |
| 2 | Pravastatin (hydrophilic) |  | 298.46± 11.23*<br>(133.26±5.02) | 219.59± 35.36*<br>(98.05±15.79) | 471.29± 70.90*<br>(210.44±31.66) | 368.86± 20.56 NS<br>(164.70±9.18) | 1,024.61<br>(HT29) |
| 3 | Mevastatin |  | 93.28± 8.89*<br>(36.43±3.47) | 111.52± 11.44*<br>(43.55±4.47) | 54.94± 1.65*<br>(21.45±0.65) | 3.47± 0.15*<br>(1.35±0.06) | 19.277<br>(HT29) |
| 4 | Cerivastatin |  | 16.23± 1.29*<br>(7.81±0.62) | 44.54± 2.47*<br>(21.45±1.19) | 35.33± 1.72*<br>(17.01±0.83) | 2.40± 0.31*<br>(1.16±0.15) | 60<br>(SW620) |
| 5 | Atorvastatin |  | 44.36± 1.31*<br>(24.78±0.73) | 105.71± 4.95*<br>(59.05±2.77) | 140.41± 8.82*<br>(82.65±5.19) | 24.92± 2.82*<br>(13.92±1.58) | 4.085<br>(SW620) |
| 6 | Rosuvastatin (hydrophilic) |  | 425.0± 60.11*<br>(204.64±28.94) | 1.49± 0.08*<br>(0.72±0.04) | 478.63± 63.49*<br>(230.46±30.57) | 32.56± 2.74 NS<br>(15.68±1.32) | 22<br>(MCF7) |
| 7 | Simvastatin (prodrug) |  | 299.36± 32.68*<br>(125.30±13.68) | 1.12± 0.19*<br>(0.47±0.08) | 60.21± 5.39*<br>(25.20±2.26) | 8.89± 0.97*<br>(3.72±0.41) | 8.04<br>(MCF7) |
| 8 | Pitavastatin |  | 137.84±17.38*<br>(60.72±7.66) | 480.22± 14.95*<br>(211.54±6.59) | 271.03± 34.91*<br>(119.39±15.38) | 0.93± 0.03*<br>(0.41±0.01) | 4.65<br>(HT29) |
| 9 | Fluvastatin |  | 171.60± 11.56*<br>(77.47±5.22) | 442.74± 40.89*<br>(199.88±18.46) | 76.98± 9.48*<br>(34.76±4.28) | 0.04±0.01*<br>(0.02±0.00) | 0.04<br>(SW620) |
| | Cisplatin | | 588.21± 80.74<br>(176.49±24.22) | 540.13± 39.51<br>(162.07±11.86) | 550.76± 80.01<br>(165.25±24.01) | 456.94± 23.06<br>(137.10±6.92) | 134.4<br>(SW480) |

Results are mean ± SD (n = 3 independent replicates). $IC_{50}$ values (concentration at which 50% inhibition of cell proliferation took place in comparison to non-induced basal 72 h incubations) were calculated within 0.0001-200 μg/mL range. NI is lack of cytotoxicity within the tested 0.0001-200 μg/mL concentration range. P-value calculated by unpaired t-test between test compound $IC_{50}$ values and cisplatin's (μM) using GraphPad Prism software version 5.0.1.* When P<0.05, NS, not significantly different from reference agent.

Table 2. $IC_{50}$ Values (μM; μg/mL) of *in vitro* DPPH-radical and RAW264.7 cell line NO- radical scavenging properties of selected Statins vs. respective reference agents

| Drug | | DPPH radical scavenging $IC_{50}$ value (μg/mL)+ | NOS- $IC_{50}$ value μM (μg/mL)++ |
|---|---|---|---|
| 1 | Lovastatin | NI | **41.61± 4.34*** |
| | | | (16.83± 1.76) |
| 2 | Pravastatin | NI | 204.33± 14.15* |
| | | | (91.23± 6.32) |
| 3 | Mevastatin | NI | 141.52± 17.16* |
| | | | (55.27± 6.70) |
| 4 | Cerivastatin | NI | NI |
| 5 | Atorvastatin | NI | **42.78± 7.17*** |
| | | | (23.90± 4.00) |
| 6 | Rosuvastatin | NI | 74.21± 13.07NS |
| | | | (35.25± 6.29) |
| 7 | Simvastatin | NI | **42.47± 6.41*** |
| | | | (17.78± 2.68) |
| 8 | Pitavastatin | NI | 98.67± 1.87 NS |
| | | | (43.48± 1.01) |
| 9 | Fluvastatin | NI | 110.14± 20.58 NS |
| | | | (53.13± 7.73) |
| Reference Drug | | Ascorbic acid | Indomethacin |
| | | 16.33± 0.21 (2.88± 0.04) | **86.45± 10.52** (30.93± 3.76) |

Results are mean ± SD (n = 3 independent replicates); + $IC_{50}$ values (concentration at which 50% inhibition of DPPH in comparison to non-induced basal 30 minutes incubations or cell proliferation in comparison to non-induced basal incubations) were calculated within testing dose range; ++ The $IC_{50}$ value is the concentration at which 50% inhibition of Nitric oxide synthase took place in comparison to non-induced basal 24h incubations, P-value is calculated by unpaired t-test between test compound $IC_{50}$ values μM and ascorbic acid $IC_{50}$ values μM (DPPH) or Indomethacin $IC_{50}$ values μM (NOS) using GraphPad Prism software version 8.0.1 * When $P<0.05$ and *, NS: not significantly different from reference agent. Bolded numerals stand out as the least $IC_{50}$ values (most active) among others enlisted in the same tested cell line. NI: Non-Inhibitory.

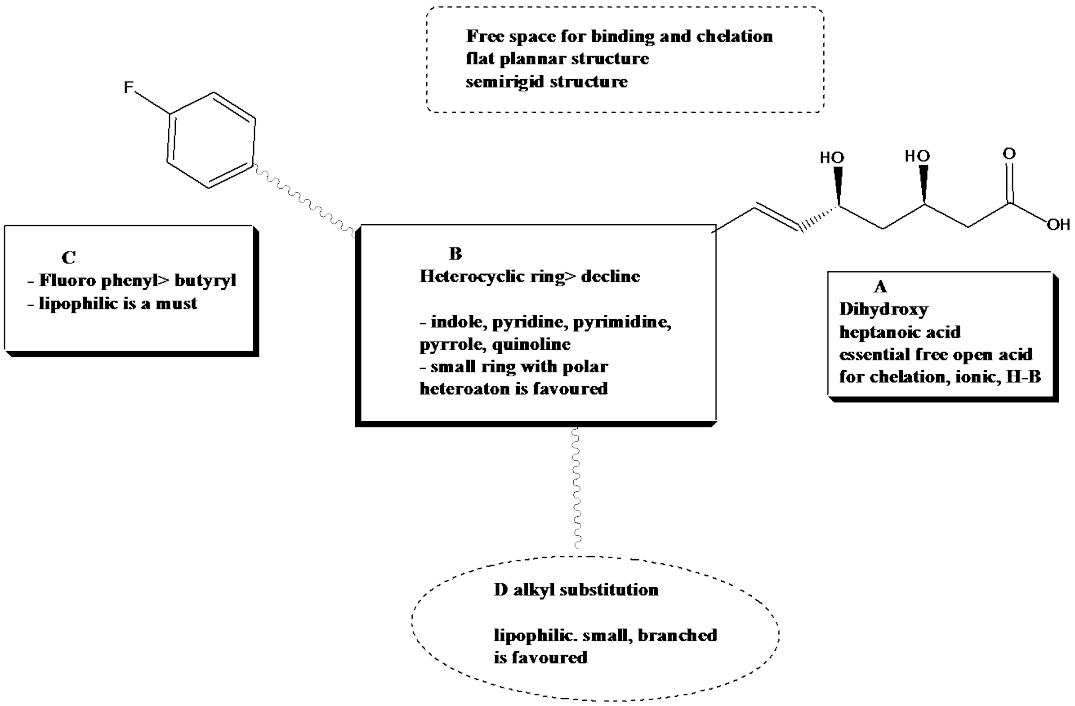



Figure 4. SARS of Antiproliferative Lipophilic Statins: acidic, lipohilic and H-B chelators are the essential features

Table 3. The Arrangement of Statins' Cytotoxicity in Comparison with Cisplatin against the 5 CRC Cell Line (HT29, HCT116, SW620, $CACO_2$ and SW480) from Strongest to Weakest.

| HT29 (C1) | HCT116 (C2) | SW620 (C3) | $CACO_2$ (C4) | SW480 (C5) |
|---|---|---|---|---|
| Cerivastatin | Cerivastatin | Cerivastatin | Pitavastatin | Cisplatin |
| Mevastatin | Pravastatin (hydrophilic) | Lovastatin (prodrug) | Cisplatin | Cerivastatin |
| Pitavastatin | Cisplatin | Fluvastatin | Simvastatin (prodrug) | Simvastatin (prodrug) |
| Pravastatin (hydrophilic) | Atorvastatin | Mevastatin | Cerivastatin | Fluvastatin |
| Simvastatin (prodrug) | Simvastatin (prodrug) | Pitavastatin | Lovastatin (prodrug) | Lovastatin (prodrug) |
| Fluvastatin | Mevastatin | Simvastatin (prodrug) | Atorvastatin | Rosuvastatin (hydrophilic) |
| Cisplatin | Fluvastatin | Cisplatin | Rosuvastatin (hydrophilic) | Mevastatin |
| Lovastatin (prodrug) | Lovastatin (prodrug) | Atorvastatin | Fluvastatin | Atorvastatin |
| Atorvastatin | Pitavastatin | Rosuvastatin (hydrophilic) | Pravastatin (hydrophilic) | Pitavastatin |
| Rosuvastatin (hydrophilic) | Rosuvastatin (hydrophilic) | Pravastatin (hydrophilic) | Mevastatin | Pravastatin (hydrophilic) |

Shaded blocks represent drugs below 50μM); Red highlights ($IC_{50}$ values [μM] below 10 μM.)

As for safety profile of tested market statins in comparison to cisplatin (SI= 134.4); Selective cytotoxicity against PDL fibroblasts was investigated and statins' SI in ascending order was (Table 1a,b): Fluvastatin (0.04) < atorvastatin (4.1) < pitavastatin (4.7) < simvastatin (8) < mevastatin (19) < rosuvastatin (22) < lovastatin (31) < cerivastatin (60) < pravastatin (1,025) (Table 1a,b).

While the selected tested statins exhibited negligible radical scavenging activity versus ascorbic acid (Table 5); in testing immunomodulatory effectiveness of statins in LPS-triggered in vitro inflammation in RAW 267.4 macrophages; lovastatin, atorvastatin and simvastatin with comparable $IC_{50}$ values of 42 μM were substantially more potent than indomethacin's ($IC_{50}$ value of 87 μM). Rosuvastatin, pitavastatin and fluvastatin proved equipotency to indomethacin while cerivastatin found

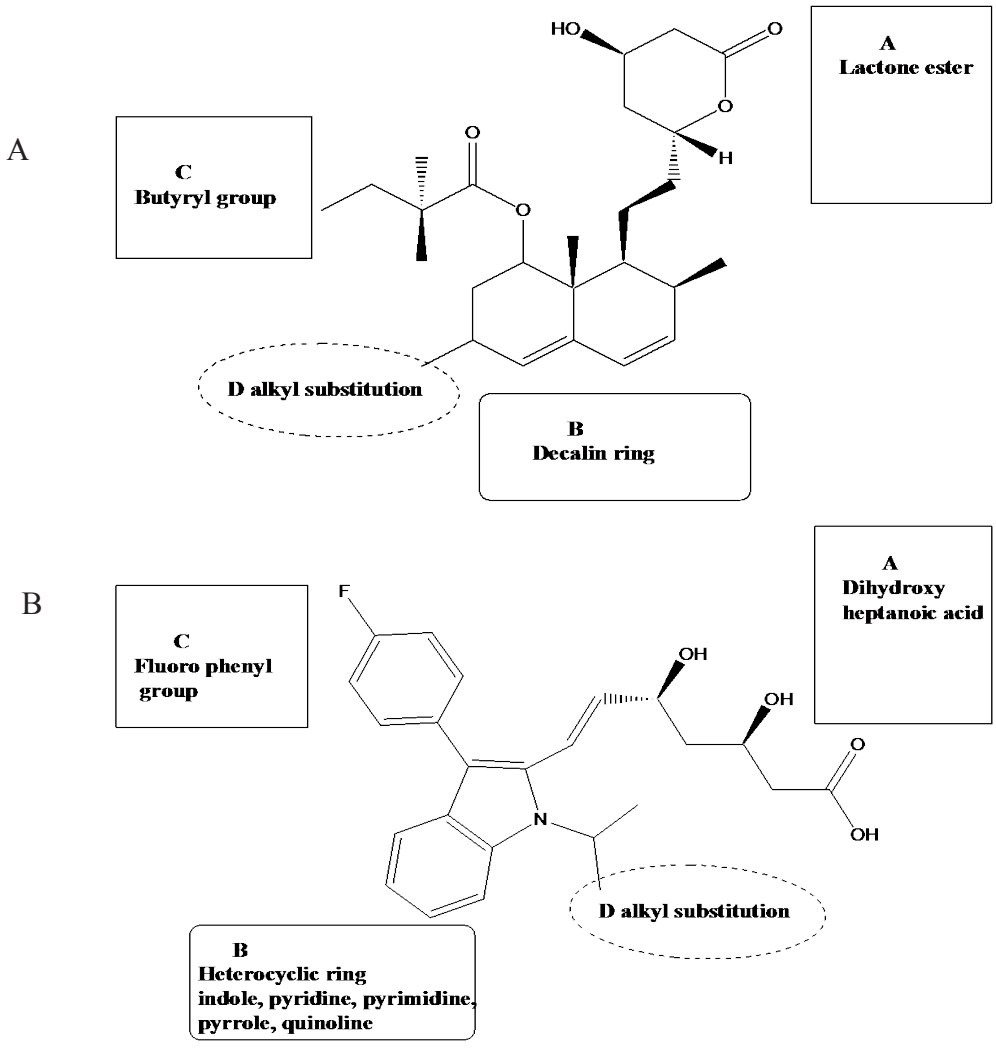



Figure 5. A, Type 1 statins. B, Type 2 Statins

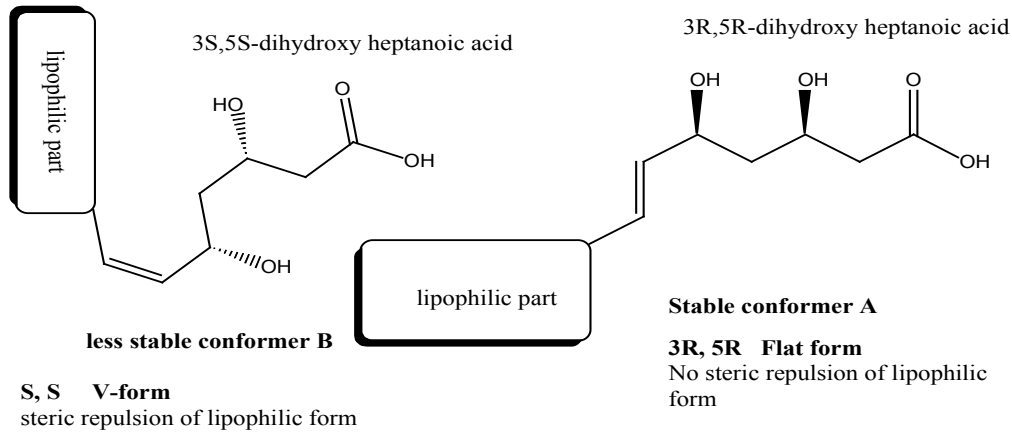



Figure 6. Dihydroxyheptanoic Acid Stero- Effect on Target Binding

surprisingly noninhibitory. In contrast; pravastatin and mevastatin, ascribed inferior antiinflammation potency to indomethacin (Table 2).

## Discussion

Statins were reported to exhibit antiproliferative/ anticancer activity in particular against CRC cell lines. Although many biochemical mechanisms and parameter were introduced to explain their antiproliferative effect, one main mechanism has drawn our attention. The proposed mechanism addresses the cells' response for ionizing radiation-induced cell death which are existing in late G1 and G2-M phases of the cell cycle (Chan et al., 2003). It was alleged that statins can cause cancer cell death by inhibiting the transition of G1-S in the cell cycle (Alexandrova et al., 2019; Chen et al., 2020). This finding was supported by treating colon cancer cell lines

Table 4.The Arrangement of Statins' Cytotoxicity in Comparison with Cisplatin against the Cell Lines (T47D, MCF7 and PANC-1) from Strongest to Weakest

| T47D | MCF7 | PANC-1 |
|---|---|---|
| Cerivastatin (active drug) 16.23 | Simvastatin (prodrug) 1.12 | Cerivastatin (active drug) 35.33 |
| Atorvastatin (active drug) 44.36 | Rosuvastatin (active drug) | Lovastatin (prodrug) |
| | (hydrophilic) 1.49 | 48.78 |
| | Cerivastatin (active drug) 44.54 | |

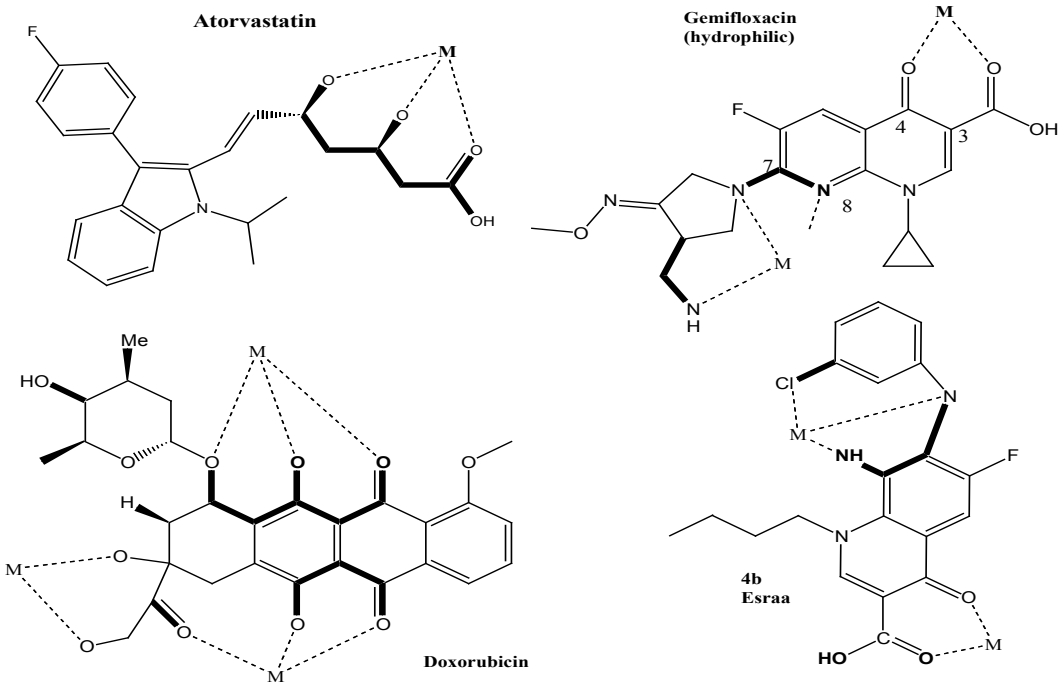



Figure 7A. Tridentate-Chelation Groups Functionalities Shared by All Antiproliferating CRC: Statins, FQs, Doxorubicin.

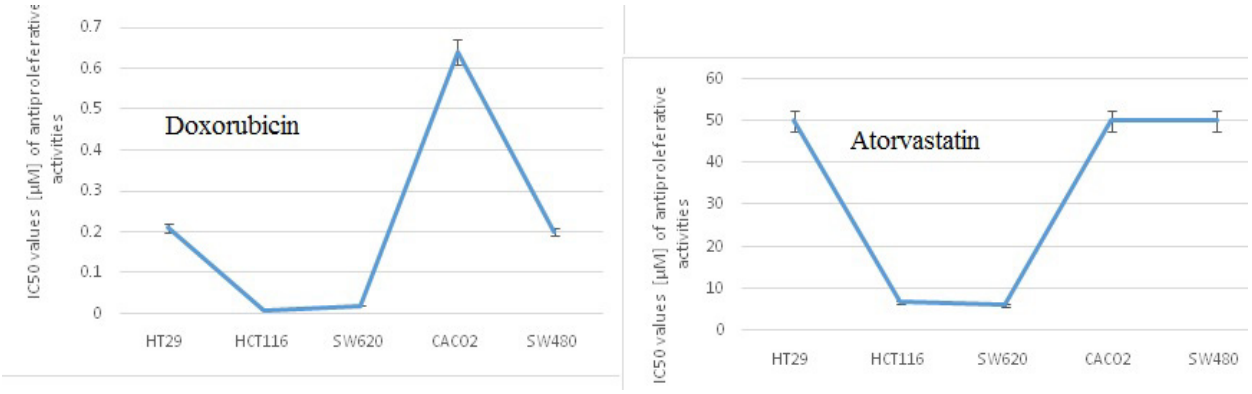



Figure 7B. Most Potent Antiproliferative Activity of Doxorubicin vs. atorvastatin on 5 CRC cell lines ($IC_{50}$ values below 50 µM for both treatments on HCT116 and SW620)

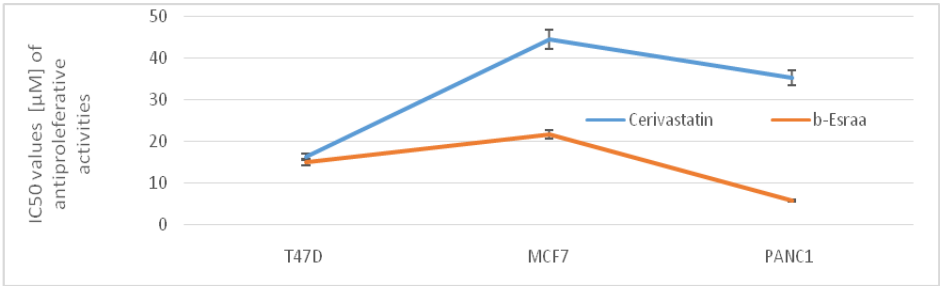



Figure 7C.Cerivastatin Cytotoxicity against Cell Lines (T47D, MCF7 and PANC-1) in Comparison with FQ (4b; 3-chloro aniline FQ), $IC_{50}$ values below 50µM

HCT116 and HT29 with atorvastatin showing a marked antiproliferation on HCT116 cell line (Xiao et al., 2008). Furthermore cerivastatin had inhibitive effect in treating some selected human breast cancer cell lines (Kozar et al., 2004). Although no one have linked the proposed mechanism to the structure, we have noticed that this is

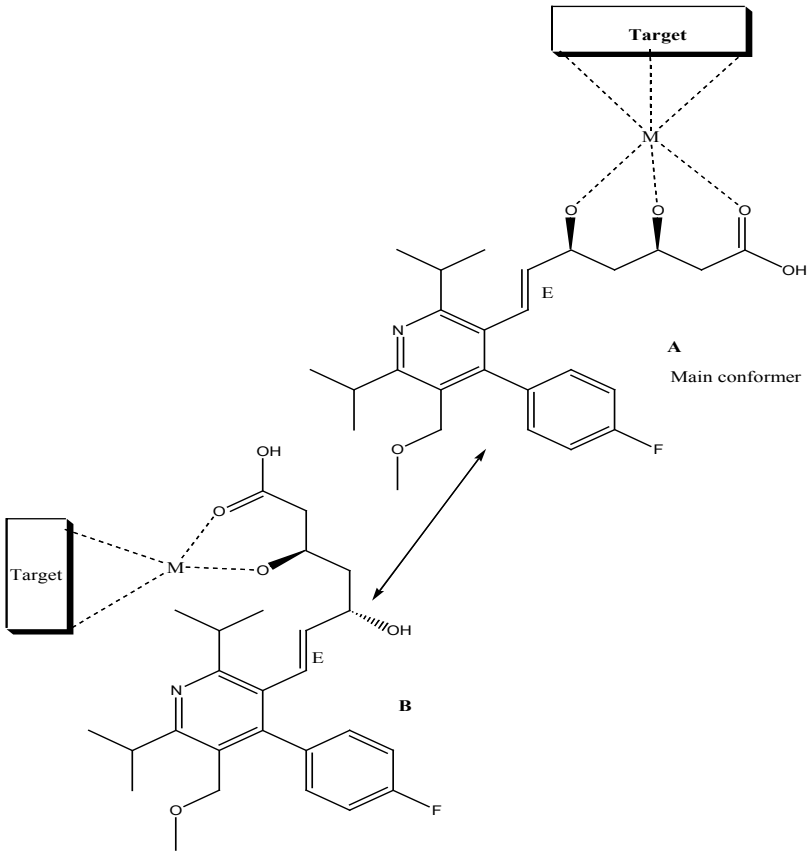



Figure 8. Statins 3, 5-dihydroxyheptanoic Acid as Chelator Group in Cerivastatin

the same mechanism reported for both fluoroquinolones (FQs) and doxorubicin anticancer drugs (Mizutani et al., 2005; Abbas and Stuart, 2012). In a previous research by our group, we have found that drug candidates with significant antiproliferative effect against CRC and BC cells share the lipophilic character and increased number of hydrogen bonds (Mamdooh et al., 2019; AlKhalil et al., 2020). Since all three Vosaroxin (FQs), doxorubicin and atorvastatin showed similar pattern against CRC cancer cell lines tested, we assumed that the activity is related to similar or isosteric functional group on their basic scaffold regardless lipophilicity. The main properties is to be lipophilic and having polar acidic groups. Acidic group was also a common functionality in all 3 drugs tested. Statins have 3, 5-dihydroxyheptanoic acid scaffold that achieves the first requirement. Similar to our finding with atorvastatin, most proposed statins in this work have lipophilic structure facilitating cancer cell penetration. Therefore, it is justified to screen all statins as anticancer since almost all are acidic having 3, 5-dihydroxyheptanoic acid polar group.

We have assumed that statins with the following properties might have antiproliferative effects.

1- Contain strong acidic groups (heptanoic acid) that forms ionic bonds with cationic amino acids involved in cancer cell target.

2- They have high lipophilic structure that facilitates the cancer cell entry (cell membrane).

3- Prodrug statins (Lactone cyclic ester) might be hydrolyzed to the free acidic form which might aid anticancer properties.

4- Statins have high number of hydrogen bonding polar groups (3, 5-dihydroxy groups) that might increase interaction with different targets involved in cancer.

5- We suggest that statins have also chelation groups which put forward metal chelation involvement in their activity.

*SAR for statins Antiproliferative activity against Colorectal Cancer (CRC)*

*Statins' Lipophilicity vs. hydrophilicity*

Tables 3 and 4 reveal that lipophilic statin exhibited the highest activity, whereas hydrophilic statins pravastatin and rosuvastatin displayed much weaker activity against almost all CRC and BC cell lines with favored activity of rosuvastatin. The extra hydroxyl and sulfonamide groups' functionalities imposed hydrophilic properties decreasing their activity (Figure 1). These data proposed clearly that lipophilic statins are essential for antiproliferative activity since lipophilic compounds can easily penetrate cancer cell membrane. It seems also that since pravastatin is Na salts, it has lost the contribution of free COOH group in activity explaining its weaker activity and pattern to rosuvastatin. Moreover, the Na salt has also decreased lipophilic character of the molecule. It is worth mentioning that pravastatin has also shown different and weaker activity pattern from other statins in general, this might be due to the extra OH H-B donor group on decalin ring that allowed different target interaction. This functionality was lost from rosuvastatin and replaced with H-B acceptor sulfonamide that retained interaction to the same target of other statins, meaning it works on the same target, with higher activity and similar pattern (Figure 1).

*The role of free acidic group in lipophilic statins: (acidic statins) as Compared to metal salts*

Further support to the role of free COOH groups comes from the lipophilic Pitavastatin Ca salt which has different pattern and activity per cell from the rest lipophilic acidic statins (Figure 2). Furthermore, the total polarization of the molecule in its dimer form has increased hydrophilic character potentially explain different pattern and weaker activity. It is well documented that ionic salts are more soluble in water since they increased polarization effect.

*Free 3, 5-dihydroxyheptanoic acid vs. lactone ester*

Apparently there was no clear difference in antiproliferative activity between cyclic lactones and free acidic statins. The order of activity was different per cell. Nanomolar activity was presented by both ester and acid on same and different cells. Thorough investigation in literature has been cited with antihyperlipidemic activity. It is evident that esterase enzymes are found inside cancer biological cells and can hydrolyze lactones into free acidic form (Figure 3) (Wells and Grandis, 2003; Niu et al., 2012). Cerivastatin was the odd case since it was most active on almost all cells, indicating that other factors might contribute to final activity in addition to lipophilicity. It is not clear yet if the lactone or the free acid interact with their target by exact structural formula or in their final hydrolyzed form.

This needs further investigation in the future. However, we propose that it is most likely to interact with their targets through the free acidic form indicating same functional group did bind to the same target revealing similar mechanism. Further support to stronger acid form relies on the fact that free acidic cerivastatin was the most active. These finding indicate the role of free dihydroxy heptanoic acid group in antiproliferative activity.

*The number of H-B polar groups group in lipophilic statins*

It was evident that the free 3,5-dihydroxy heptanoic acid has a major role in activity (Figure 3). The salt form (pitavastatin Ca) of the drugs has decreased activity since the total number of H-B group was decreased in addition to lower lipophilicity. Pitavastatin has a distinctive pattern of activity different from all statins, indicating different target or mechanism due to the lost 2 OH donor H from COOH acidic group. Furthermore, it is logical to say that dimer form has led in steric interaction with the main target shared by all statin, changing the pattern to alternative target. The findings that no difference between lactones and free acids again aid that the free acid is the one which is involved in activity not the lactone form since all lipophilic statins showed similar pattern. Since all lipophilic statins showed similar pattern of inhibitory activity and different one from cisplatin and other hydrophilic statins, it is most likely that they have a common mechanism at same target imposed by a similar functional group shared by all which is dihydroxy heptanoic acid. This support the statement that free 3,5-dihydroxy heptanoic acid is essential for any antiproliferative activity in statins. Losing activity in salt

form illuminates that the free COOH has a bigger role in activity go beyond HB interaction, we propose chelation role for this functionality mediated by metals in front of the vicinity of the target. The weak activity of hydrophilic statins (Figure 2) with extra hydrogen polar groups (rosuvastatin and pravastatin; OH and sulfonamides) indicates that the antiproliferative activity is related to the type of the polar group not the count. Again, this finding put forward the role of the dihydroxy heptanoic acid in front. Although both drugs have extra polar side groups in hydrophilic structure, they were much weaker in activity, meaning they are not involved in antiproliferative activity, rather it is the dihydroxy heptanoic acid.

*Chelation effect of 3, 5-dihydroxy heptanoic acid*

This research propose and potentiate for the first time the chelation effect of 3,5-dihydroxyheptanoic acid as a potential antiproliferative mechanism.

*A pharmacophore vs. antiproliferative effectiveness*
*The statin pharmacophore / SARS*

The essential structural components of all statins as antihyperlipidemic drugs are a 3, 5-dihydroxyheptanoic acid unit (A) and a ring system (B) with different substituents (C) and alkyl substitution on the main ring (D) (Figure 4). It has also been shown that the HMGR is stereoselective and as a result all statins need to have the required 3R, 5R stereochemistry or at least rigid structure through an E isomer-double bond. Since we test same statins, it is plausible to have same SARS and pharmacophore as anticancer.

*Differences in statin structure as anticancer pharmacophore /SARS*

The statins differ with respect to their ring structure (B) and substituents (C), (Figures 5a and 5b). These differences in structure affect the pharmacological properties of the statins. They are classified into 2 types based on their structure (Figures 5a and 5b); this classification and structural features applies to anticancer pharmacophore since we use the same drugs:

Type 1: statins have substituted decalin-ring structure (B, Figure 5a) that resemble the first statin ever discovered, and the butyryl ester group (C). The lactone (A) was the dominant pharmacophore in this type. They include lovastatin, pravastatin, simvastatin, and mevastatin.

Type 2: statins that are fully synthetic and have larger groups linked to the HMG-like moiety. They are often referred to as type 2 statins. Type 2 statins are dominated by dihydroxy heptanoic acid (A, Figure 5b) in most derivatives. The butyryl group (C) of type 1 statins was replaced with the fluorophenyl group. This group is responsible for additional polar interactions that causes tighter binding to the HMGR enzyme through polarizable fluorine atom and increased the total lipophilic properties of this class. The main decalin ring (B) was also replaced with heterocyclic ring including indole,pyrole, pyrimidine, pyridine, and quinolone. Type 2 statins that belong to this group are: fluvastatin, atorvastatin, rosuvastatin and cerivastatin.

*Statins SARS as cytotoxicity compounds: based on our data*

Most statins which showed good antiproliferative activity need to have the required 3R, 5R stereochemistry since they seem to exhibit a conformational flexibility and stability of their target that causes statins to exploit and to accommodate their hydrophobic moieties in a flat form (Figure 6).

The open flat form imposed by this 3R, 5R stable stereochemistry allows the flat statin to interact freely with the vicinity of the target (Figure 6), since both OH are directed on the same plane and direction. The lipophilic part (C) of the statin is far away from the heptanoic acid and separated by a bridge (ring B) leading to best fit with target with no steric repulsion of both groups. However, other steroforms (Figure 6) make the lipophilic part to come closer in front of heptanoic acid chain forming an intramolecular HB with each other or interfere with each other in front of the target enzyme. Such steric repulsion hindered the interaction of both group with the receptor, decreasing activity. It is worth mentioning that the E-isomer imposed by the double bond in heptanoic acid moiety has also aided the flat form interaction as in fluvastatin. This phenomenon supports the need of free vicinity in front of the dihydroxy group to generate the chelation effect in such big space. The fact that both OH groups and carbonyl directed in same direction aid this chelation effect.

Polar and ionic interactions formed between the dihydroxy heptanoic acid (part A) residues and the enzyme were essential feature in all statins, aiding the proposition that the dihydroxy acidic group is essential in anticancer activity; again possibly through chelation. This also is supported by weaker activity and different mode of salt form as in pitavastatin. Polar interaction was also a common feature to target interaction of ring C from both butyryl ester and Fluorine atom in both types of statins. Hydrophobic and Van der Waals interactions are provided by decalin, Hetero ring and fluorobenzene indicating that lipophilicity is essential for anticancer activity.

It was clear that more lipophilic characters of statin have increased activity. This was distintive with the most lipophilic cerivastatin with nanomolar activity on almost all CRC cells and showed strongest activity on all BC cells. It was reported that lipophilic compounds diffuse passively and non-selectively to cancer cell membrane whereas hydrophilic ones need active transport process. Lipophilicity of the statins is considered to be quite important as the hepato-selectivity of the statins is related to their lipophilicity (Kunutsor and Laukkanen, 2020). The more lipophilic statins tend to achieve higher levels of exposure in non-hepatic tissues, while the hydrophilic statins tend to be more hepatoselective. This might explain the weaker activity of both rosuvastatin and pravastatin and justify testing them for hepatic cancer in the future.

The lipophilic alkyl substituents (D, Figures 3 and 4) on main ring (B) have increased the activity by increasing lipophilicity. Small side chains, branched groups, less steric and far from both A and C ring, have increased activity the most. Strongest antiproliferative statins have small one or two methyls; isopropyl and cyclopropyl

substitution on main ring B, pointing backward from the vicinity of A and C. The bulk groups such as benzene and amides (atorvastatin) have decreased activity possibly due to steric effect. Evidently; the front side must be avoided from any substitution and most substitutions occurred on the back side of ring B. This finding and flat form requirement also illuminate that such big space involves chelation rather than simple bonds.

Hydrophilic polar groups on main ring B such as OH and sulfone amide have showed the weakest anticancer activity as in both pravastatin and rosuvastatin. H-B donor such as OH group that significantly decreased activity and changed the pattern of activity (pravastatin).

It was apparent that smaller rings (main ring B) with polar N-heteroatom as in type 2 have better activity mainly on HT29, whereas decalin were more active on SW620. This change in pattern was possibly due to small vicinity with the lipophilic part of the target between the 2 types of cell lines.

The most important point is the chelation binding that possibly mediates statin interaction in cancer cells. The dihydroxyl groups were situated in a correct distance from the COOH group in the heptanoic acid moiety allowing excellent chelation potency with di and trivalent metals. Although few hints are cited toward the role of chelation in statin antihyperlipidemic therapy, few researchers have illuminated this statin mechanism as of antiinflammatory effect (Ali et al., 2007; Kell, 2009).

This work concludes SAR for active anticancer statins with the following requirements (Figure 4):

1. Strong free acidic groups such as free aliphatic COOH or any strong isoster (A).

2. Strong acidic chelators such as 3, 5-dihydroxy heptanoic acid (A).

3. Free ionisable acidic groups that provide enough number of HB acceptors and donors (A).

4. Lipophilic characters of the drug with high log p. (fluorophenyl (C) and alkyl substitution (D)

5. Large size exceeding 350 dalton.

6. Flat rigid structure (middle main hetero ring B) that provide rigid stero-selective binding.

*Statins pharmacophore vs. Fluoroquinolones and Doxorubicin (Hypothesis: Chelation with trivalent metals (iron) as potential anticancer mechanism of all compounds against colorectal cancer cells)*

It was noticed from our data in Tables 1a and 1b that all lipophilic statins regardless of their $IC_{50}$ values expressed similar pattern of activity. This suggests that these drugs possibly share at least one related target or mechanism as part of probably multiple targets. This finding necessitates at least one similar functional group shared by all statins. Furthermore, previous work by our group has revealed that gemifloxacin and atorvastatin shared a similar pattern against CRC cell lines (Al-Khalil et al., 2020). Similarly, three master students have also detailed their synthetic chelator Fluoroquinolones (FQs) sharing a similar pattern to the drug doxorubicin on CRC cell lines (Hallaq et al., 2022; Khaleel et al., 2022; Qashou et al., 2022; Salih et al., 2022 ). We hypothesized that chelation group as a structural group shared by all on these structural scaffolds is responsible of their similar activity (Figure 7a).Figure 7a shows that all 4 compounds have a divalent and trivalent chelator group. To validate our hypothesis, we compared the pattern of activity of statins with the mentioned compounds in Figure 7b which demonstrates the pattern similarity (similar order and close activity) between 5 lipophilic statins vs. FQ 4b-Esraa; (Qashou et al., 2022) and Doxorubicin against 5 CRC cell line (Figures 7b and 7c). The activity of FQ 4b (3-chloro aniline FQ) showed a very similar pattern on almost 4 cells with weaker activity than doxorubicin (Doxorubicin was in nanomolar) (Figures 7a,b,c). Such close pattern and order suggest that both have same target and both have similar functional groups responsible for activity. In fact, the pattern of activity of doxorubicin and atorvastatin was a match against all CRC cells (Figures 7a,b,c). This highlights the importance of the trivalent groups rather than divalent. Figure 7 shows that trivalent gemifloxacin (Mamdooh et al., 2019; Qashou et al., 2022) was active against CRC similar to statin; whereas ciprofloxacin of divalent group did not show any activity against CRC (Mamdooh, et al., 2019; AlKhalil et al., 2020; Hallaq et al., 2022; Khaleel et al., 2022; Qashou et al., 2022; Salih et al., 2022 ). This points out the role of trivalent chelators in anticancer activity against CRC cells, and clearly indicates that iron metal is the target one. Reduced 3-chloro aniline, gemifloxacin, and statins share one trivalent group including the ethylene diamine and chlorine atom in reduced 3-chloro aniline, methylene diamine and side chain amine in gemifloxacin and 3, 5-dihydroxy heptanoic acid in statins. This might explain the weaker activity compared to doxorubicin. Doxorubicin has displayed much lower $IC_{50}$ attaining low nanomolar lever, possibly due to exhibiting extra trivalent group yielding flexibility and potency of fitting many targets in CRC thus exhibiting slight difference in pattern and higher potency (Figures 7a,b,c). Likewise, Figures 7a,b,c show similarity in pattern and $IC_{50}$ values of Cerivastatin and FQ4b. Although inconclusive at this stage; such finding gives also a clue that it might share same mechanism and target even in BC cell lines. However, we do like to highlight the superior activity of our cerivastatin compared to cisplatin in some CRC cells. This phenomenon can be explained by the stronger double chelator groups in statins (Figure 8). The resonance in statins allows the extremely strong trivalent chelator group (Figure 8) 3, 5-dihydroxy-1-COOH (rotamer A) to resonate to another conformer structure (rotamer B) producing 3, 5-dihydroxy ionisable divalent chelator. Such dual chelators in addition to acidic properties, fluorophenyl and high lipophilicity substitutions contribute to the significant antiproliferative activity of cerivastatin. From a biochemical point of view, shared mechanisms reported for all agents can associate chelation as potential shared one. Many statins including atorvastatin and cerivastatin induce apoptosis as one potential anticancer mechanism leading to cell cycle arrest of cancer cells at the G2/M checkpoint (Zhang et al., 2018).This mechanism is also shared by many anticancer agents either natural or synthetic such as topoisomerase inhibitors; doxorubicin (You and Gao, 2019) and fluoroquinolones (Hawtin et al., 2010; Sharma,

et al., 2020) and many anthracyclines (Gewirtz, 1999). Remarkably, many vital cellular processes such as energy metabolism and DNA synthesis consist of reactions that require catalysis by di- and tri-valent metals such as iron and zinc-containing proteins. These proteins include cytochromes and ribonucleotide reductase (RR). The latter is more significant in the context of cellular proliferation due to its role in catalyzing the rate-limiting step of DNA synthesis.

Ultimately, the importance of these metals; particularly iron is highlighted by the fact that iron-deprivation leads to G1/S cell cycle arrest and apoptosis (Buss et al., 2003; Dayani et al., 2004). Cancer cells in particular, have a higher iron requirement because of their rapid rate of proliferation. In order to satisfy their iron requirement, some cancer cells have altered iron metabolism. In addition, iron chelators also demonstrate the ability to inhibit growth of aggressive tumors such as neuroblastoma. For these reasons, iron-deprivation through iron chelation is seen as an exploitable therapeutic strategy.

Quite recently, the antibacterial fluoroquinolone were spotted to have shown potent in vitro antiproliferative activity (Azéma et al., 2009). Vosaroxin (Voreloxin) is the only anticancer quinolone agent that inhibits topoisomerase-II leading to cell cycle arrest and apoptosis (Abbas and Stuart, 2012). Even doxorubicin has been reported to cause cardiac problems through iron chelation although no body links that to cancer (Buzdar et al., 1985). Our group has revealed many FQs compounds with anticancer potential. Recently, new anticancer FQs were revealed by our group as topoisomerase II inhibitors, target for anticancer (Swellmeen et al., 2017; Arabiyat et al., 2017; Kasabri et al., 2020; Hallaq et al., 2022; Khaleel et al., 2022; Qashou et al., 2022; Salih et al., 2022). It was established that topoisomerase II inhibitors including FQs and doxorubicin involveG1/S cell cycle arrest and apoptosis. These were achieved in preciously iron chelator agent. Therefore, it can be concluded that iron chelation might be a possible explanation for the antiproliferative mechanism of statins since they are reported to induce G1/S cell cycle arrest and apoptosis.We could have observed the biochemical similarity between the mechanism of action of statins and other chelator drugs based on functionalities. The proposed mechanism links statins with metal chelators in cancer therapy rather than specific enzymes and compares their biochemical processes. Iron chelation might have significant anticancer effect and this possibly applies to statins (Habel et al., 2013).

*Conclusion and Future work*

The work divulges more than 6 lipophilic statins with potentially excellent antiproliferative properties. Candidly, cerivastatin is proposed as new anticancer drug that needs further clinical evaluation since it has good safety profile and excellent efficiency. Similar wise, this work reveals 5 potential antiinflammatory statins and validates that inflammation could reduce cancer risk and can to some extent explain the anticancer mechanism of our drugs since most anticancer statins showed antiinflammatory effect. This work highlights iron mediated trivalent chelation as potential anticancer approach, exemplified by statins. It also confirms the role of phenolic free radicals in antioxidant chelators as well.

*We propose the following points for future work*

- To screen and test more statins for another cancer cell lines.
- Optimization and modification of lipophilic statin to eliminate side effects and exploring more potent hits, with special focus on cerivastatin scaffold.
- Explore more lipophilic chelator drugs against cancer.
- Explore hydrophilic statin for hepatic cancer cells.
- Investigate chelation as potential mechanism parallel to exploring biochemical mechanisms.
- Investigate stating binding to special cancer targets such as HDAC, TOP II, PIK3, and Pim-1 kinase inhibitor, Estrogen receptor and GSK-3β.

## Author Contribution Statement

All authors contributed equally towards rationale conceptualization, experimental design, data collection and analyses, manuscript write up and proofreading.

## Acknowledgments

*Funding statement*

We are grateful for the Deanship of Scientific Research at the University of Jordan for research funds (project 171/2018-2019, dated 28/8/2019, No. 2280). We also wish to thank the University of Jordan and Al-Zaytoonah Private University for providing research facilities and funds. This research was conducted during the sabbatical year granted to Professor. Yusuf Al-Hiari by the University of Jordan in the academic year 2021/2022 at Al-Zaytoonah Private University.

Ethical committees that approved the research: Scientific Committee/School of Pharmacy and Deanship of Research at University of Jordan approved the research.

*Availability of data (if apply to your research)*

Data can be made available upon furthering requests to authors

*Conflicts of interests*

Authors declare no conflict of interest.

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