Cathepsin B, an abundant expressed cysteine peptidase, plays a key role in cancer cell proliferation, tumor metastasis, apoptosis, angiogenesis, invasion and migration. Therefore, development of cathepsin B inhibitors to treat cancer is of great significance. In this study, dihydronaphthalenone chalconoid derivatives containing different benzyliden moieties were synthesized via an efficient route in microwave condition that resulted in the desired compounds in high yields compared to acid- or base-catalyzed refluxing conditions. Cytotoxicity of the compounds was evaluated against K562, HT-29 and MCF-7 human cancer cell lines by MTT assay. P1, P3 and P9 (containing 4-OCH$_3$, 3-NO$_2$ and 4-CN moieties on phenyl ring, respectively) exhibited good cytotoxic activity with an IC$_{50}$ range of 7.1–28.9 μM. Molecular docking analysis was carried out to investigate the possible interactions and binding modes of all compounds with cathepsin B. The most promising compounds, P1, P3 and P9 were well accommodated within the active site and had the least estimated free binding energies. It was concluded from both MTT assay and docking studies that some dihydronaphthalenone chalconoid derivatives could be suggested as effective cytotoxic agents and potential cathepsin B inhibitors.

Keywords: Aldol reaction. Benzylidene-dihydronaphthalenone. Cancer. Chalcone. Cysteine proteases. Microwave assisted synthesis.

INTRODUCTION

Cancer is a major cause of mortality and morbidity worldwide, and it is estimated that the number of annual cancer-related deaths will increase to 11.4 million by 2030 (Mathers, Loncar, 2005). Despite the availability of various therapeutic strategies, cancer management is still a challenge, and scientist intend to discover new, effective and less toxic agents (Bavadi et al., 2017; Ranjarb et al., 2017; Ranjabar et al., 2018b).

Cathepsin B belongs to cysteine proteases family and is a lysosomal proteolytic enzyme. It is significantly expressed in malignant cells and plays a role in cancer cell proliferation and tumor metastasis (Lim et al., 2004), apoptosis (Foghsaard et al., 2001), angiogenesis, invasion (Joyce et al., 2004) and migration (Nalla et al., 2010). Hence, cathepsin B can be a potential therapeutic target in cancer (Aggarwal, Sloane, 2014; Kramer et al., 2017), and development of small synthetic molecules that effectively inhibit this enzyme is of great therapeutic significance (Eatemadi et al., 2017; Sosić et al., 2018).

Chalcones are known to possess a wide range of interesting biological activities (Reddy et al., 2017; Cao et al., 2018; Kocyigit et al., 2018; Ranjarbar et al., 2018a), and have been investigated for their anticancer properties (Dimmock et al., 1999; Lawrence et al., 2003; Ducki et al., 2009; Katsori, Hadjipavlou-Litina, 2009; Prakasham et al., 2012; Drutovic et al., 2014;
Moreover, chalcones have been reported to exhibit significant inhibitory activity on cathepsin B enzyme (Kim et al., 2013; Ramalho et al., 2013; Raghav, Singh, 2014b; Garg, Raghav, 2015; Raghav, Kaur, 2015; Ravish, Raghav, 2015; Soliman et al., 2017). The structures of some chalcones reported as cathepsin B inhibitors are depicted in Figure 1. It was reported that some natural cyclohexyl chalcones such as panduratin A and nicolaioidesin C showed cytotoxic activity against prostate cancer cells and also in vitro cathepsin inhibitory activity (Majumdar et al., 2011). Raghav and Singh reported that o-hydroxychalcones and chalcone phenyl hydrazones with nitro substitution showed promising cathepsin B inhibition (Raghav, Garg, 2014; Raghav, Singh, 2014b). Moreover, it was shown that some 2,6-bis(benzylidene) cyclohexanones possessed significant cytotoxic activity against different human cancer cell lines (Nakhjiri et al., 2012). Later, bis(benzylidene) cyclohexanone derivatives had shown to have efficient inhibitory activity on cathepsin B enzyme (Raghav, Singh, 2014a).

In this study, on the basis of chalcone and 2,6-bis(benzylidene) cyclohexanone structures, benzylidene-3,4-dihyronaphthalenone chalcon-like backbone was designed and after the synthesis in high yields, compounds were evaluated for their cytotoxicity against three human cancer cell lines. Furthermore, molecular docking study was carried out to gain insight about the binding modes and interactions of these compounds in the active site of cathepsin B.

![Figure 1](image-url)  
**FIGURE 1** – Structures of some reported natural and synthetic chalconoids as cathepsin B inhibitors and the designed benzylidene-3,4-dihyronaphthalenone chalconoids.
MATERIAL AND METHODS

Apparatus

Melting points were determined using a hot stage apparatus (Electrothermal, Essex, UK) and were uncorrected. Mass spectra were recorded on an Agilent spectrometer (Agilent technologies 9575c inert MSD, USA). NMR spectra were done on a Burker-Advance DPX-300 MHz in CDCl$_3$. All spectra affirmed the structure of the synthesized compounds. Elemental analysis was performed by Microanalytical Department, Central Laboratories for Research, Shiraz University of Medical Sciences and was within 0.4% of the calculated value.

Chemicals and reagents

All reagents and solvents were purchased from commercial suppliers and were used without further purification. Sodium hydroxide (NaOH), α-tetralone and p-toluenesulfonic acid (PTSA) were purchased from Merck. Analytical thin layer chromatography (TLC) was performed on MERCK precoated silica gel 60-F254 (0.5 mm) aluminum plates. RPMI 1640, Dulbecco’s phosphate buffered saline, trypsin and penicillin-G/streptomycin were from Biosera, Ringmer, UK and FBS (Fetal Bovine serum) was product of Invitrogen, San Diego, CA, USA. Thiazolyl blue tetrazolium bromide (MTT) was purchased from Sigma-Aldrich, Saint louis, MO. Doxorubicin and cisplatin were obtained from Ebewe Pharma, Unterach, Austria.

PTSA catalyzed synthesis of (E)-2-benzylidene-3,4-dihydronaphthalen-1(2H)-one under reflux condition

The α-tetralone (1 mmol) and corresponding aldehyde (1 mmol) were added to a stirred solution of PTSA (1 mmol) in ethanol (8 mL) and the resulting reaction mixture was refluxed for 24 h. After completion of reaction, the solvent evaporated and the resulting reaction mixture was acidified with HCl. The mixture was dried over anhydrous Na$_2$SO$_4$ and after being concentrated under reduced pressure precipitates were formed, filtered, recrystallized in ethanol and washed with diethyl ether, petroleum ether and cool ethanol.

NaOH catalyzed synthesis of (E)-2-benzylidene-3,4-dihydronaphthalen-1(2H)-one under reflux conditions

The α-tetralone (1 mmol) and corresponding aldehyde (1 mmol) were added to a stirred solution of NaOH (1 mmol) in ethanol (8 mL) and the resulting reaction mixture was refluxed for 24 h. After the completion of reaction, the solvent evaporated and the resulting reaction mixture was acidified with HCl. The mixture was dried over anhydrous Na$_2$SO$_4$ and after being concentrated under reduced pressure precipitates were formed, filtered, recrystallized in ethanol and washed with diethyl ether, petroleum ether and cool ethanol.

PTSA catalyzed synthesis of (E)-2-benzylidene-3,4-dihydronaphthalen-1(2H)-one derivatives under microwave condition

A mixture of α-tetralone (1 mmol), PTSA (1 mmol) and corresponding benzaldehyde (1 mmol) were mixed together without any solvent in a flask capped with a glass funnel and irradiated at 300 W, 120 ºC for 1.5 min. Reaction completion was checked by thin layer chromatography (TLC). Then, the reaction mixture was cooled to room temperature and treated with cold water. The solid was filtered, washed with water and recrystallized from ethanol to give the pure products P1-P10.

(E)-2-(4-methoxybenzylidene)-3,4-dihydronaphthalen-1(2H)-one (P1)

Yellow solid; M.P: 108-111 ºC. $^1$H NMR (300 MHz, CDCl$_3$, 25 ºC, TMS): δ = 2.94-2.97 (m, 2H, CH$_2$), 3.14-3.18 (m, 2H, CH$_2$), 3.87 (s, 3H, OCH$_3$), 6.97 (d, 2H, H-3',5', J=8.7 Hz), 7.27 (d, 1H, H-5, J=8.4 Hz), 7.38 (t, 1H, H-7, J=7.5 Hz), 7.44-7.52 (m, 3H, H-6, 2',6'), 7.87 (s, 1H, -C=C-Ph), 8.14 (d, 1H, H-8, J=7.8 Hz). $^{13}$C NMR (125 MHz, CDCl$_3$, 25 ºC, TMS): δ = 27.23, 28.80, 55.35 (OCH$_3$), 113.99, 126.98, 128.09, 128.16, 131.76, 131.10, 133.55, 133.67, 134.08, 159.98 (C$_6$), 187.85 (C=O). MS (EI, 70 eV): m/z(%) = 263 (M$^+$, 100). Anal. Calculated for C$_{18}$H$_{16}$O$_2$: C, 81.79; H, 6.10; Found: C, 81.61; H, 6.23.

(E)-2-(4-methoxybenzylidene)-3,4-dihydronaphthalen-1(2H)-one (P1)

Yellow solid; M.P: 108-111 ºC. $^1$H NMR (300 MHz, CDCl$_3$, 25 ºC, TMS): δ = 2.94-2.97 (m, 2H, CH$_2$), 3.14-3.18 (m, 2H, CH$_2$), 3.87 (s, 3H, OCH$_3$), 6.97 (d, 2H, H-3',5', J=8.7 Hz), 7.27 (d, 1H, H-5, J=8.4 Hz), 7.38 (t, 1H, H-7, J=7.5 Hz), 7.44-7.52 (m, 3H, H-6, 2',6'), 7.87 (s, 1H, -C=C-Ph), 8.14 (d, 1H, H-8, J=7.8 Hz). $^{13}$C NMR (125 MHz, CDCl$_3$, 25 ºC, TMS): δ = 27.23, 28.80, 55.35 (OCH$_3$), 113.99, 126.98, 128.09, 128.16, 131.76, 133.10, 133.55, 133.67, 134.08, 159.98 (C$_6$), 187.85 (C=O). MS (EI, 70 eV): m/z(%) = 263 (M$^+$, 100). Anal. Calculated for C$_{18}$H$_{16}$O$_2$: C, 81.79; H, 6.10; Found: C, 81.61; H, 6.23.

(E)-2-(4-nitrobenzylidene)-3,4-dihydronaphthalen-1(2H)-one (P2)

Yellow solid; M.P: 184-186 ºC. $^1$H NMR (300 MHz, CDCl$_3$, 25 ºC, TMS): δ = 2.99-3.03 (m, 2H, CH$_2$), 7.29 (d, 1H, H-5, J=8.7 Hz), 7.41 (t, 1H, H-7, J=7.5 Hz), 7.52-7.60 (m, 3H, H-6, 2',6'), 7.86 (s, 1H, -C=C-Ph), 8.15 (d, 1H, H-8, J=7.8 Hz), 8.29 (d, 1H, H-3',5', J=8.7 Hz). $^{13}$C NMR (125 MHz, CDCl$_3$, 25 ºC, TMS): δ = 27.23, 28.80, 55.35 (OCH$_3$), 113.99, 126.98, 128.09, 128.16, 131.76, 133.10, 133.55, 133.67, 134.08, 159.98 (C$_6$), 187.85 (C=O). MS (EI, 70 eV): m/z(%) = 263 (M$^+$, 100). Anal. Calculated for C$_{18}$H$_{16}$O$_2$: C, 81.79; H, 6.10; Found: C, 81.61; H, 6.23.
Yellow solid; M.P: 147-149 ºC. 1H NMR (300 MHz, CDCl₃, 25 ºC, TMS): δ = 2.99-3.03 (m, 2H, CH₂), 3.12-3.17 (m, 2H, CH₂), 7.29 (d, 1H, H-5, J=8.7 Hz), 7.41 (t, 1H, H-7, J=7.5 Hz), 7.52 (td, 1H, H-6, J=7.5/1.2 Hz), 7.63 (t, 1H, H-5', J=7.8 Hz), 7.76 (d, 1H, H-8, J=7.8 Hz), 7.86 (s, 1H, -C=CH-Ph), 8.15 (dd, 1H, H-6, J=7.8/0.9 Hz), 8.23 (d, 1H, H-4', J=8.1 Hz), 8.30 (s, 1H, H-2'). 13C NMR (125 MHz, CDCl₃, 25 ºC, TMS): δ = 27.15, 28.68, 123.07, 124.13, 127.25, 128.33, 128.36, 129.54, 133.10, 133.48, 133.70, 135.68, 137.52, 137.92, 143.10, 143.38 (C₅), 187.21 (C=O). MS (EI, 70 eV): m/z(%) = 278 (M⁺, 100), 262 (31.6), 232 (52.6). Anal. Calculated for C₁₇H₁₃NO₂: C, 73.11; H, 4.69; N, 5.02; Found: C, 73.25; H, 4.76; N, 5.12.

Yellow solid; M.P: 138-142 ºC. 1H NMR (300 MHz, CDCl₃, 25 ºC, TMS): δ = 2.96-3.01 (m, 2H, CH₂), 3.11-3.16 (m, 2H, CH₂), 6.90 (td, 1H, H-2', J=8.4/2.1 Hz), 7.15 (d, 1H, H-4', J=5.9 Hz), 7.22-7.30 (m, 2H, H-5, 6'), 7.37-7.45 (m, 2H, H-5, 6), 7.51 (td, 1H, H-6, J=7.5/1.2 Hz), 7.83 (s, 1H, -C=CH-Ph), 8.15 (d, 1H, H-8, J=7.8 Hz). 13C NMR (125 MHz, CDCl₃, 25 ºC, TMS): δ = 27.15, 28.41, 113.14, 114.72, 127.57, 127.95, 129.18, 130.93, 131.87, 132.01, 132.70, 132.99, 134.32, 136.49 (C₅), 161.88, 186.90 (C=O). MS (EI, 70 eV): m/z(%) = 251 (M⁺, 100), 233 (15.8). Anal. Calculated for C₁₇H₁₃F₃O: C, 80.93; H, 5.19; Found: C, 81.99; H, 5.00.

Yellow solid; M.P: 136-138 ºC. 1H NMR (300 MHz, CDCl₃, 25 ºC, TMS): δ = 2.86-2.90 (m, 2H, CH₂), 2.99-3.04 (m, 2H, CH₂), 7.18 (d, 1H, H-5, overlapped with chloroform), 7.23 (d, 2H, H-3, 5', J=8.1 Hz), 7.29 (t, 1H, H-7, J=7.5 Hz), 7.40-7.49 (m, 3H, H-6, 2',6'), 7.70 (s, 1H, -C=CH-Ph), 8.05 (dd, 1H, H-8, J=7.5/1.2 Hz). 13C NMR (125 MHz, CDCl₃, 25 ºC, TMS): δ = 27.24, 28.65, 126.99, 128.05, 128.32, 128.96, 133.10, 133.59, 134.27, 135.37, 138.31, 142.64, 143.43, 144.22 (C₅), 187.64 (C=O). MS (EI, 70 eV): m/z(%) = 302 (M⁺, 3), 267 (80.0), 232 (100). Anal. Calculated for C₁₇H₁₂Cl₂O: C, 67.35; H, 3.99; Found: C, 67.69; H, 4.05.
Brown solid; M.P: 161-163 °C. 1H NMR (300 MHz, CDCl, 25 °C, TMS): δ = 2.97-3.01 (m, 2H, CH2), 3.08-3.13 (m, 2H, CH2), 7.29 (d, 2H, H-5, J=6.6 Hz), 7.40 (d, 2H, H-3’, J=8.1 Hz), 7.82 (s, 1H, -C=CH-Phe), 8.15 (d, 1H, H-8, J=7.5 Hz). 13C NMR (125 MHz, CDCl, 25 °C, TMS): δ = 27.24, 28.71, 111.82, 118.62 (CN), 127.26, 128.33, 128.36, 130.23, 132.21, 133.09, 133.71, 134.10, 138.10, 140.53, 143.12 (C6), 187.29 (C=O). MS (EI, 70 eV): m/z(%) = 258 (M+, 100). Anal. Calculated for C18H13NO: C, 83.37; H, 5.05; N, 5.40; Found: C, 83.59; H, 4.92; N, 5.49.

Pale yellow solid; M.P: 102-105 °C. 1H NMR (300 MHz, CDCl, 25 °C, TMS): δ = 2.95-3.01 (m, 4H, CH2), 7.26-7.55 (m, 8H, H-Ar), 7.84 (s, 1H, -C=CH-Phe), 8.17 (dd, 1H, H-8, J=7.8/1.2 Hz). MS (EI, 70 eV): m/z(%) = 233 (M+, 100). Anal. Calculated for C17H14O: C, 87.15; H, 6.02; Found: C, 88.01; H, 5.95.

**Cells and Cell cultures**

K562 (human chronic myelogenous leukemia), HT-29 (Human Colorectal Adenocarcinoma) and MCF-7 (Human Breast Adenocarcinoma) cells were obtained from the National Cell Bank of Iran, Pasteur Institute, Tehran, Iran. All cell lines were maintained in RPMI 1640 supplemented with 10% FBS, and 100 U/mL penicillin-G and 100 U/mL streptomycin. K562 cells were cultured in suspension while, HT-29 and MCF-7 cells were grown in monolayer culture, at 37 °C in humidified air containing 5% CO2.

**MTT Assay**

Cytotoxicity of the synthesized compounds was estimated using the MTT reduction assay against three human cancer cell lines including; K562 (myelogenous leukemia), HT-29 (colorectal adenocarcinoma) and MCF-7 (breast adenocarcinoma). Cells were seeded into 96-well microplates at a density of 5×10⁴ cells/mL (100 μL per well). Control wells contained no drugs and blank wells contained only growth medium for background correction. After overnight incubation at 37 °C, 50 μL of the growth medium was removed and 50 μL of medium containing different concentrations of synthetic compounds were added. Compounds were all first dissolved in DMSO, and then diluted in medium so that the maximum concentration of DMSO in the wells was 0.5%. All compounds were tested at the final concentration in the range of 1–100 μM. Plates with suspension cells were centrifuged before this procedure. After 72 h of incubation, the medium was removed and MTT was added to each well at a final concentration of 0.5 mg/mL. Afterwards, plates were incubated for another 4 h at 37 °C to allow the formazan crystals to be formed and then crystals were solubilized in 200 μL DMSO. Absorbance was measured at 570 nm with background correction at 655 nm using a Bio-Rad microplate reader (Model 680). The percentage of inhibition of viability compared to control wells was assessed for each concentration and IC50 values were calculated with CurveExpert software version 1.34 for Windows. Each experiment was repeated 3-5 times.

**Molecular docking analysis**

To expose the binding mode of studied dihydronaphthalene chalconoids in the active site of cysteine protease cathepsin B *in silico* docking study was performed. All docking studies were performed using AutoDock 4.2 and AutoDock Tools 1.5.4. The X-ray crystallographic structure of cathepsin B containing 2-pyridinethiol as the innate ligand was retrieved from Protein Data Bank as 2IPP. Before docking, 2-pyridinethiol and water molecules was omitted from 2IPP, hydrogens were added and non-polar hydrogens were merged. Finally, Gasteiger charges were calculated for the protein. The ligand structures were sketched and minimized by molecular mechanics and semi empirical methods. All the aforementioned procedures were carried out by HYPERCHEM 7.0 software. PDBQT formats of the ligands were constructed by adding Gasteiger charges and setting the degree of torsions. The grid maps were prepared by AutoGride and grid box dimensions were set to 40×40×40 with 0.375 Å grid spacing. The active site including Cys29 was selected for docking and the grids’ center were placed on the 2-pyridinethiol’s binding site. In order to determine the docking parameter file, rigid macromolecule was chosen. Lamarckian genetic search algorithm was applied and the number of GA runs was set at 100. Validity of the docking procedure was tested using co-crystallized
inhibitor as ligand and the above-mentioned protocol (self-docking).

RESULTS AND DISCUSSION

Synthesis

Ten benzylidene-3,4-dihydronaphthalen-1-one derivatives were synthesized by aldol condensation of α-tetralone and different benzaldehydes (Figure 2). The structures were confirmed by $^1$H NMR, $^{13}$C NMR, MS and elemental analysis. Structures of synthesized compounds are shown in Table I. The impact of reagent and condition on the yield of tetralone-based chalcones was studied (Table I). For this purpose, the tetralone was allowed to condense with various aldehydes applying different reagents/conditions; including refluxing ethanol condition in the presence of stoichiometric amounts of NaOH or PTSA and microwave conditions in the presence of stoichiometric amounts of PTSA. The base-catalyzed reaction under ethanol refluxing condition gave the lowest yields for all derivatives. This might be due to overall reduction in the active concentration of aldehydes as a result of aldehydes oxidation to their corresponding carboxylic acids in the presence of the base. The synthesis under microwave condition led to the highest yields of the products.

![Figure 2](image-url)

**FIGURE 2** – Synthesis of 3,4-dihydronaphthalenone chalconoids under different reaction conditions.

Biological evaluation

The cytotoxicity of the synthesized compounds was evaluated against K562, HT-29 and MCF-7 cell lines, using MTT assay and the results are exhibited in Table II. Most of the derivatives showed considerable cytotoxic activity against cancer cells. **P1** (R: 4- OCH$_3$), **P2** (R: 3-NO$_2$) and **P9** (R: 4-CN) were the most active compounds against all there cell lines. **P1** with IC$_{50}$ value of 7.1 ± 0.5 μM showed better cytotoxicity against K562 cells compared to the positive control, cisplatin with an IC$_{50}$ value of 9.1 ± 1.7 μM. The cytotoxic effect of **P3** (IC$_{50}$ = 11.2 ± 1.1 μM) and **P9** (IC$_{50}$ = 9.2 ± 0.2 μM) were comparable to that of cisplatin in these cells. Moreover, **P1**, **P3** and **P9** showed lower IC$_{50}$s than cisplatin in HT-29 and MCF-7 cell lines.

Generally, the cytotoxicity of benzylidene-dihydronaphthalenone derivatives is affected by the
TABLE I – Comparison of the aldol condensation reaction yield using different reagents and conditions

| Compound | R          | Yield (%) | NaOH/Refluxing ethanol | PTSA/Refluxing ethanol | PTSA/Microwave |
|----------|------------|-----------|------------------------|------------------------|----------------|
| P1       | 4- OCH₃    | 79        | 81                     | 94                     |
| P2       | 4- NO₂     | 53        | 68                     | 80                     |
| P3       | 3- NO₂     | 18        | 39                     | 78                     |
| P4       | 3- F       | 45        | 63                     | 90                     |
| P5       | 4- Cl      | 66        | 75                     | 86                     |
| P6       | 4- Br      | 79        | 78                     | 94                     |
| P7       | 2,3- Cl    | 42        | 69                     | 85                     |
| P8       | 2,4- Cl    | 31        | 72                     | 96                     |
| P9       | 4- CN      | 48        | 57                     | 93                     |
| P10      | H          | 26        | 44                     | 81                     |

nature of substitution on the phenyl ring. Considering the IC₅₀ values in Table II, it can be stated that inserting methoxy, nitro, fluor and cyanide functions on benzylidene moiety, as in P1, P2, P3, P4 and P9, leads to a noticeable increase in the cytotoxicity as compared to P10. Compounds bearing a nitro group at the meta position of benzylidene residue, (P3), display greater cytotoxic activity, compared to P2, which has a nitro moiety at the para position. In the case of halogen containing compounds, P4 with a 3-fluoro substitution exhibit moderate cytotoxic activity against the three cell lines. Replacing the 3-fluoro with a 4-chloro substituent, as in P5, reduce the activity and introducing 4-bromo on phenyl ring causing P6 to become inactive. The IC₅₀ values for P5 and P10 in MCF-7 cell line were reported to be more than 30 μM by Huber et al., (2015) which is in agreement with the obtained results in this study. A summary of cytotoxic activity profile of the dihydronaphthalenone derivatives against the three tested cancer cell lines is presented in Figure 3.

**Molecular docking experiment**

In order to elucidate the binding mode of the synthesized compounds in the active site of cathepsin B enzyme, molecular docking analysis was performed.
Validation of molecular docking was done by re-docking the innate ligand into the receptor (Figure 4). The root mean square deviation (RMSD) between the best pose of co-crystallized ligand docked into the active site of tyrosinase and the one in the crystal structure was 1.98 Å. Details of docking outcomes are listed in Table III. Three dimensional representations of the best docked pose for the most active compounds, P1, P3, P9 and P6, as an inactive derivative, are depicted in Figure 5, Figure 6, Figure 7 and Figure 8, respectively. Generally, docking results were in good agreement with the cytotoxicity evaluation. The most cytotoxic compounds (P1, P3 and P9) formed more stable drug-receptor complex as they possess the least estimated binding free energies. Compound P6 with no considerable cytotoxicity on the three tested cell lines, showed the highest estimated binding free energy (-5.97 kcal/mol). The molecular docking analysis of the derivatives indicated that the active site comprising of catalytic dyad, Cys29 and His199 and amino acid residues Cys26, Trp30, Gly74, Ala200 and Gln23 was found to interact with the compounds

| Compound | R     | IC₅₀ (μM) ± SE |
|----------|-------|--------------|
|          | K 562 | HT-29        | MCF-7        |
| P1       | 4-OCH₃| 7.1 ± 0.5    | 10.5 ± 0.9   | 28.9 ± 5.1 |
| P2       | 4-NO₂ | >100         | 19.3 ± 2.3   | 30.7 ± 3.1 |
| P3       | 3-NO₂ | 11.2 ± 1.1   | 8.0 ± 0.4    | 15.6 ± 1.8 |
| P4       | 3-F   | 26.4 ± 1.8   | 20.2 ± 2.3   | 43.7 ± 4.1 |
| P5       | 4-Cl  | 78.0 ± 12.6  | 24.5 ± 2.7   | 62.7 ± 6.0 |
| P6       | 4-Br  | >100         | >100         | 85.1 ± 3.8 |
| P7       | 2,3-Cl| 63.2 ± 3.0   | 27.0 ± 4.8   | >100       |
| P8       | 2,4-Cl| 53.4 ± 5.5   | 33.8 ± 4.5   | 76.7 ± 10.5|
| P9       | 4-CN  | 9.2 ± 0.2    | 15.2 ± 1.2   | 21.7 ± 2.5 |
| P10      | H     | 37.9 ± 4.5   | 32.6 ± 1.6   | 54.7 ± 2.5 |
| Cisplatin|       | 9.1 ± 1.7    | 16.4 ± 2.0   | 39.8 ± 7.7 |
| Doxorubicin|     | 0.041 ± 0.009| 0.353 ± 0.052| 0.211 ± 0.019|

Values represent mean ± S.E.M. of 3-7 independent experiments.
Dihydronaphthalenone chalconoid derivatives as potential cathepsin B inhibitors; design, synthesis, cytotoxicity evaluation and docking analysis

FIGURE 3 – Summary of cytotoxicity profile of different dihydronaphthalenone chalconoid derivatives on K562, HT-29 and MCF-7 cancer cell lines.

under consideration. Compound **P1**, having 4-methoxy substitution on benzylidene moiety, showed the lowest estimated binding free energy (-6.74 kcal/mol) and estimated inhibition constant (11.30 μM) (Table III). **P1** is well accumulated in the active site of cathepsin B by hydrogen bonds and Pi interactions. Oxygen atom of carbonyl group on dihydronaphthalen core exhibited two hydrogen bonds with Cys29 and His199, while the 4-methoxyphenyl ring established Pi-H and Pi-alkyl interactions with Gly74 and Ala200 residues, respectively (Figure 5). Compounds **P9** and **P3**, bearing electron withdrawing 4-CN and 3-NO$_2$ substitutions, exhibited the second and the third best estimated binding free energies (-6.68 and -6.67 kcal/mol, respectively) (Table III). Replacing the electron donating methoxy group with electron withdrawing 4-CN and 3-NO$_2$ substitutions, provided the formation of additional hydrogen bonds with Ala200 (in the case of **P9** and Gly74, Trp30, Cys29 (in the case of **P3**) residues and reduced the potency of the phenyl ring for participating in Pi interactions with Cys29 and Ala200 residues (Figure 6 and Figure 7). As it is depicted in Figure 8, in the case of compound **P6**, oxygen atom of carbonyl group on dihydronaphthalen core was involved in the formation of a weak hydrogen bond with Cys29. Moreover, dihydronaphthalen moiety and phenyl ring exhibited Pi interactions with Cys26 and Cys29, respectively.

FIGURE 4 – 3D representation of the co-crystallized inhibitor (cyan) docked into the active site of cathepsin B and superimposed on co-crystallized inhibitor (red) in the crystal structure of the enzyme (PDB ID: 2IPP).
TABLE III – Docking results of the tested compounds and the co-crystallized ligand into the binding site of cathepsin B (PDB code 2IPP)

| Compound | ΔG (kcal/mol) | Ki (μM) | Interactions | Atom of ligand | Amino acid | Distance (Å) |
|----------|---------------|---------|--------------|----------------|------------|--------------|
| P1       | -6.74         | 11.38   | H-bonding    | Carbonyl       | Cys29      | 3.19         |
|          |               |         | H-bonding    | Carbonyl       | His299     | 2.67         |
|          | H-bonding     | 11.38   | Phenyl       | Cys29         | Ala200     | 5.19         |
|          | Pi-H          | 11.38   | Phenyl       | Gly74         | Gly74      | 2.01         |
|          | Pi-alkyl      | 11.38   | Dihydronaphthalen | Gly74      | Gly74      | 2.01         |
|          | Amid-Pi stacked | 11.38  | Dihydronaphthalen | Cys26      | Cys29      | 4.54         |
| P2       | -6.12         | 32.74   | H-bonding    | Nitro         | Trp30      | 1.94         |
|          |               |         | H-bonding    | Nitro         | Trp30      | 1.98         |
|          | H-bonding     | 32.74   | Nitro        | Cys29        | Cys29      | 3.16         |
|          | Pi-alkyl      | 32.74   | Dihydronaphthalen | Val176     | Val176     | 4.77         |
|          | Pi-alkyl      | 32.74   | Dihydronaphthalen | Met196     | Met196     | 4.31         |
| P3       | -6.67         | 12.38   | H-bonding    | Carbonyl      | Cys29      | 3.54         |
|          |               |         | H-bonding    | Carbonyl      | His199     | 2.00         |
|          | H-bonding     | 12.38   | Phenyl       | Cys29        | Met196     | 4.67         |
|          | Pi-alkyl      | 12.38   | Dihydronaphthalen | Val176     | Val176     | 4.17         |
|          | Pi-Pi         | 12.38   | Dihydronaphthalen | Trp221     | Trp221     | 4.99         |
| P4       | -6.36         | 21.69   | H-bonding    | Carbonyl      | Gln23      | 1.72         |
|          |               |         | H-bonding    | Fluorine      | Cys26      | 2.48         |
|          | H-bonding     | 21.69   | Fluorine     | Gly121       | Gly121     | 2.70         |
|          | Halogen       | 21.69   | Dihydronaphthalen | Cys29      | Cys29      | 3.73         |
| P5       | -6.06         | 43.98   | H-bonding    | Carbonyl      | Cys29      | 5.06         |
|          |               |         | H-bonding    | Carbonyl      | Cys29      | 5.14         |
|          | Pi-H          | 43.98   | Phenyl       | Ala200       | Ala200     | 5.41         |
|          | Pi-alkyl      | 43.98   | Dihydronaphthalen | Cys29      | Cys29      | 4.48         |
| P6       | -5.97         | 42.01   | H-bonding    | Carbonyl      | Cys29      | 4.50         |
|          |               |         | H-bonding    | Carbonyl      | Cys29      | 4.50         |
|          | Pi-H          | 42.01   | Phenyl       | Cys29        | Cys29      | 4.47         |
|          | Amid-Pi stacked | 42.01  | Dihydronaphthalen | Cys29      | Cys29      | 4.47         |
| P7       | -6.62         | 14.14   | H-bonding    | Carbonyl      | Gln23      | 1.98         |
|          |               |         | H-bonding    | Carbonyl      | Cys29      | 3.26         |
|          | H-bonding     | 14.14   | Phenyl       | Cys29        | Cys29      | 4.66         |
|          | Pi-alkyl      | 14.14   | Dihydronaphthalen | Trp221     | Trp221     | 4.85         |

(continuing)
**TABLE III** – Docking results of the tested compounds and the co-crystallized ligand into the binding site of cathepsin B (PDB code 2IPP)

| Compound | ΔG (kcal/mol) | Ki (μM) | Interactions | Atom of ligand | Amino acid | Distance (Å) |
|----------|--------------|---------|--------------|----------------|------------|--------------|
| P8       | -6.50        | 14.71   | H-bonding    | Carbonyl       | Cys29      | 3.59         |
|          |              |         | Pi-H         | Phenyl         | Cys29      | 3.59         |
|          |              |         | Amid-Pi stacked | Dihydronaphthalen | Cys26      | 4.40         |
| P9       | -6.68        | 12.61   | H-bonding    | Carbonyl       | Cys29      | 2.31         |
|          |              |         | H-bonding    | Cyanide        | Ala200     | 3.45         |
|          |              |         | Pi-H         | Phenyl         | Cys29      | 4.22         |
|          |              |         | Amid-Pi stacked | Dihydronaphthalen | Cys26      | 4.84         |
| P10      | -6.14        | 31.53   | H-bonding    | Carbonyl       | His199     | 2.43         |
|          |              |         | Pt-alkyl     | Phenyl         | Ala200     | 5.11         |
|          |              |         | Pi-H         | Phenyl         | Cys29      | 3.06         |
|          |              |         | Amid-Pi stacked | Dihydronaphthalen | Cys26      | 4.60         |
| 2-pyridinethiol | -3.53 | 2570 | H-bonding | SH | Cys29 | 2.95 |

**FIGURE 5** – Dock pose of compound P1 in the active site of cathepsin B.

**FIGURE 6** – Dock pose of compound P3 in the active site of cathepsin B.
CONCLUSION

In this study, ten 2-benzylidene-3,4-dihydronaphthalenone derivatives were synthesized in high yields (78\%-96\%) using microwave assisted synthesis method and were evaluated for their cytotoxic activity against three human cancer cell lines. P1, P3 and P9 were the most potent cytotoxic derivatives. Molecular docking analysis results revealed that substitutions on benzylidene moiety played an important role in drug-receptor interaction and the presence of 4-OCH3, 3-NO2 and 4-CN functions on phenyl ring, as in P1, P3 and P9, could lead to the most favorable interactions and the best orientations. Therefore, it can be suggested that P1, P3 and P9 analogs to be introduced as cytotoxic agents and potential cathepsin B inhibitors. Further development of such compounds might be of interest; however, complementary biological evaluations will be the subject of future studies to confirm our findings.

FIGURE 7 – Dock pose of compound P9 in the active site of cathepsin B.

FIGURE 8 – Dock pose of compound P6 in the active site of cathepsin B.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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