Design, Synthesis and Apoptosis Inducing Activity of Non-steroidal Flavone- methanesulfonate Derivatives on MCF-7 cell line as Potential Sulfatase Inhibitor

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Abstract

In recent years, focusing on new potent anticancer agents with selective activity is one of the greatest challenges in cancer therapy. Breast cancer is the most common cancer and the main cause of cancer deaths in women. The sulfatase enzyme plays an important role in converting the sulfated steroids into non-sulfate steroid hormones, which increases the growth and development of many hormone-dependent cancers, such as breast cancer. In this regard, structure-based optimization was conducted to design novel flavone-sulfonates pharmacophore as a new steroid sulfatase inhibitor. In the present work, the conventional methods for the synthesis of 4-oxo-2-phenyl-*4H*-chromen-7-yl methanesulfonate derivatives were reported. Their cytotoxicity was evaluated with MTT assay against a breast cancer cell line (MCF-7). The apoptosis inducing activity of the most cytotoxic compound **3c** with an IC_{50} value of 0.615 μM was evaluated in comparison to docetaxel in the presence of estradiol which is a crucial growth factor to survive the cancerous cells. The results of double staining Annexin V-FITC/PI analysis suggested that the cytotoxic activity of this compound **3c** in MCF-7 cells occurs *via* apoptosis. Molecular docking studies were conducted to clarify the inhibition mode of the most promising compound (**3c**) over the sulfatase (**1P49**) binding site. The analysis revealed the role of hydrogen bond interaction with Gly181 and hydrophobic interactions through the **1P49** active site in the ligand-receptor complex as significant descriptors to rationalize the potential inhibition activity.

1. Introduction

Cancer is known as a chronic and non-communicable disease [1]. In more than a hundred different kinds of cancers, breast cancer is the most common cancer and the main cause of cancer deaths in women [2]. The World Health Organization (WHO) reports biologically active hormones, including estrogens, as one of the most important factors to develop breast cancer. It was reported that the steroid levels, including estrone (E1), estradiol (E2), estrone sulfate (E1S), and estradiol sulfate (E2S), are enhanced in breast tumors compared to levels in the plasma and breast normal tissue [3]. The importance of estrogen production in the pathogenesis of breast carcinoma is supported by numerous studies [4, 5]. Modern targeted cancer therapy has now focused on decreasing estrogen levels [6]

Sulfatase is widely distributed throughout the body, and it acts in physiological processes and pathological conditions [7]. Steroid sulfatase (STS) is known as a sulfatase enzyme that catalyzes the conversion of sulfated steroid (hormone precursors) to free steroid (active form) that stimulates the growth of tumors in various tissues especially the breast. STS has a mushroom-like shape, with two hydrophobic anti-parallel α-helices structures [8]. STS has been found in the membranes of the endoplasmic reticulum which anchoring the functional domain on the surface. The active site of STS is located deep in a cavity of the rounded part of the STS and rests near the membrane surface which forms four potential and two functional glycosylation sites [9]. The inhibition of STS is a novel therapeutic strategy for the treatment of estrogen-dependent tumors. Some STS inhibitors have entered clinical trials and their efficacy is under investigation in postmenopausal women with breast cancer [10, 11].

Therefore, inhibition of this enzyme decreases the level of the active hormone, which responsible strategy to target the breast, endometrial, prostate, and other hormone-sensitive cancers [12].

2. Results And Discussion

2.1 The inhibitor design

The STS inhibitors can be classified into four main categories; including steroid sulfamate based inhibitors, non-steroidal sulfamate inhibitors, steroid non-sulfamate based inhibitors and non-steroid non-sulfamate based inhibitors. The first steroidal STS inhibitors were designed based on the similarity with the substrate parental structure. Hallmark in this regard back to 1994 in which the replacement of OH of the sulfate group (A, Fig. 1) by an NH_2, generated estrone-3-O-sulfamate known as EMATE (C, Fig. 1,) as an irreversible steroidal inhibitor. This compound performed a great activity in MCF-7 cells, with an IC_{50} value of 65 pM [13]. The overall designing strategic was based on the substitution at the steroidal 3-phenolic position with the resistance group to hydrolyze by the enzyme [14]. Therefore, the core aryl O-sulfamate pharmacophore was developed over the past few years [15]. Other steroidal inhibitors of STS containing different functional groups, for example, phosphonothioate (D), thiophosphate (E) and phosphate (F) have been developed over time [16,17]. Unexpectedly, high estrogenicity was observed with estrogenic inhibitors and thus unsuitable effects were seen as anticancer agents. These results stimulate an intense search for orally active and non-estrogenic STS inhibitors [18].

The coumarin derivatives (containing two-ring coumarin aryl sulphamate) exhibits high activity against STS such as 4-methylcoumarin-7-O-sulfamate (G, COUMATE) with an IC_{50} value of 380 nM against placental microsomes. Compound H demonstrated 71% STS inhibition in rat liver after 24 h single oral dose [19]. 667-COUMATE (I) as coumarin tricyclic derivatives showed an IC_{50} value of 8 nM without significant estrogenic side effects. Also, compound J (Flavone-based agents) efficiently inhibited the purified human STS with IC_{50}= 0.026 μM and K_i= 0.19 μM without estrogenic side effects in MCF-7 cells and good profile for the treatment of breast cancer [20,21].

As a result, in this study, flavon structure as potent and irreversible STS inhibitors with time and concentration-dependent effects was selected as the backbone [22,23]. The sulphamate group attached to the aryl ring seems to blocks STS activity *via* providing nonbreaking bound. Besides the structural derivatization of target compounds mainly focused on the substitution on the methanesulfonate-flavone at the various position of the B aryl ring.

2.2 Synthesis of inhibitors

Initially, for the synthesis of desired compounds, the benzoyl chloride derivatives (1a-i) were prepared, then reacted with 2,4-dihydroxyacetophenone, after baker-vankatarman rearrangement and neutralized with HCl. The 7-hydroxy-2-phenyl-*4H*-chromen-4-one derivatives (2a-i) were synthesized in the presence of

K_2CO_3 in acetone under reflux conditions for 8 h. Finally, the reaction of 2a-i with methane sulfonyl chloride in the presence of triethylamine gave the corresponding products 3a-i.

2.3 Cytotoxic evaluation

MCF-7 as a breast cancer cell line with the expression of STS were selected for further study. The IC_{50}s of all compounds are shown in table 2 comparing with docetaxel as a reference drug. The most active compound was 3c (R = 4-OCH_3) with an IC_{50} value of 0.61 μM.

- The unsubstituted derivatives (3a) showed relatively good cytotoxicity with an IC_{50} value of 1.59 μM.
- The presence of one methoxy as a bulk-electron donating group led to an improvement in the cytotoxicity (3c, IC_{50} = 0.61 μM) comparing with the unsubstituted one. While the methyl moiety as small electron-donating without heteroatom getting compound 3b (IC_{50}= 2.0 μM) in which the cytotoxic potency significantly decreased.
- Similarly, the introduction of the electron-withdrawing group into 3a, resulting in 3d (R = *para*-Cl) and 3e (R = *para*-Br) which led to increasing the cytotoxic activity compared to the unsubstituted derivative. However, the smaller group (Cl) recorded better activity in comparison with Br counterparts.
- For the multi-substituted compound possessing MeO, the reduction of anticancer activity was afforded for two substituted groups. The activity of these analogs changes are in the following order: 2,3-diOMe (IC_{50} = 2.60 μM) > 2,4-diOMe (IC_{50} = 3.21 μM) > 3,4-diOMe (IC_{50} = 5.67 μM).
- However, improvement in cytotoxicity was seen in compound 3i containing 2,3,4-triOMe with an IC_{50} value of 1.02 μM.

2.4 Inhibition of STS in a cell-based assay

Based on the results, compound 3c with the least IC_{50} value (IC_{50}= 0.615± 0.077 μM) was selected to measure STS inhibitory activity. In this regard, proliferation assay was done for two concentrations of the selected compound alone and in the presence of 100 nM estradiol. In these experiments, the well-known chemotherapy agent docetaxel (Doc) was used as the toxicity positive control. Based on data indicated in Fig. 2. The control group demonstrated around 100% of viability. As can be expected exposure to estradiol increases viability to more than 100% (p-value <0.05). The results of the assay defined that the 3c was able to significantly decrease cell viability in two tested concentrations to around 58.2 and 68.3%, respectively. Also, the group which was exposed to the co-treatment of 3c and estradiol indicated more cell viability in comparison with the 3c confirming the decreased number of active steroids and the inhibition of STS. The cell viability in cell receives 500 nM of docetaxel were significantly decreased compared to the control group. However, cells treated with Doc + estradiol showed approximately the same % viability compare to cells exposed to Doc alone proposing the other mechanism of anticancer

activity, not STS inhibition. In other words, estradiol treatment diminished the toxicity of 3c but not that of docetaxel in MCF-7 cells. This is based on the experiment in which, estradiol could compensate the 3c toxicity but not Doc toxicity. This could be explained by different inhibitory mechanisms of 3c and Doc in cell proliferation. Doc induced anticancer activity *via* effects by tubulin polymerization inhibition while 3c seems to work as an STS inhibitor.

2.5 Cell apoptosis on MCF-7 cells

Analysis of the flow cytometry double staining Annexin V-FITC/PI revealed that the synthetic compounds 3c reduced cell viability and induced apoptosis in human breast cancer cells. The flow cytometry analysis was used as a quantitative method of determining early and late apoptosis in treated cancer cells. Fig. 3. summarizes the data and showed an increase in the apoptotic index in MCF-7 cells treated with synthetic compound compared with negative control. The results of flow cytometric analysis showed that exposure of the MCF-7 cell line to the IC_{50} concentration of compound 3c induced early apoptosis in 16.3% of cells and late apoptosis in 10% of treated cells. According to Fig. 3., it was revealed that 13.5% of MCF-7 cells treated with positive control were at the early stage of apoptosis and 8.11% of the cells were at the late stage of apoptosis after 24 h treatment. The results confirmed that the cytotoxic activity of 3c breast cancer cells occurs via apoptosis.

2.6 Docking study of STS

Molecular docking was performed using smina in the Linux platform. The most potent ligand 3c was subjected to dock with the 3D structure of STS, 1P49. The interactions of the best-docked confirmation of 3c with the active site residues of STS are depicted in Fig. 4. The methanesulfonate group is well oriented near the entrance to the active site with conventional hydrogen bond interaction with Gly181 (distance: 2.61 Å) and two carbon-hydrogen bonds with Thr180 and Gly181. Pi-pi T-shaped interaction was also observed between the flavone backbone of 3c and the Phe178 residue (distance: 1.94 Å). The *para*-methoxy pendant was surrounded with several lipophilic amino acids and demonstrated two pi-alkyl interactions with Leu185 and Val186 as well as two carbon-hydrogen bonds with Ile226 and Phe230 with 3.43 Å and 3.06 Å distance.

3. Conclusion

4-oxo-2-phenyl-4H-chromen-7-yl methanesulfonate derivatives were designed, synthesized, and evaluated for their inhibitory activity toward STS. This led to the identification of the most potent cytotoxic agent of the series, compound **3c**, with an IC_{50} value of 0.615 μM. Compound **3c** showed a remarkable potency as anticancer agents on breast cancerous cell line (MCF-7) in the presence of estradiol *via* inhibiting cell proliferation through the proposed mechanism (blocking sulfatase enzyme). **3c** significantly decreased the number of active estrogens by blocking STS, which can be counterbalanced by estradiol treatment.

This mechanism is further authenticated by the lack of estradiol potential in decreasing Doc toxicity that exerts its effects by tubulin polymerization inhibition. The results of double staining Annexin V-FITC/PI analysis suggested that the cytotoxic activity of compound **3c** in MCF-7 cells occurs via apoptosis. Molecular modeling studies showed compound **3c** accommodated well in the STS active site *via* forming interaction with important residues.

4. Material And Method

4.1 Synthesis of benzoyl chloride derivatives (1a-i)

To the benzoic acid derivatives (1 mmol), thionyl chloride (4 mmol, 0.28 mL) was added dropwise at room temperature and the resulting mixture was heated under reflux for 2 hours. The extra thionyl chloride was evaporated under vacuum and benzene was added 3 times to remove the remaining of thionyl chloride.

4.2 Synthesis of 7-hydroxy-2-phenyl-*4H*-chromen-4-one derivatives (2a-i)

Initially, 2,4-dihydroxyacetophenone (152 mg, 1 mmol) and potassium carbonate (553 mg, 4 mmol) were dissolved in dry acetone. Then the appropriate benzoyl chloride derivative (2 mmol) was added dropwise to the reflux solution for 5 minutes, and the resulting mixture was refluxed for 8 hours. After the completion of the reaction, the solvent was evaporated under vacuum. Next, 5 ml of water and methanol (1:1) were added and the mixture was refluxed for 2 hours. The progression of the reaction was monitored by TLC. Then, the mixture was cooled to room temperature and was poured into ice and then neutralized with 5% HCl solution. The precipitates were filtered and purified by preparative TLC (hexane: EtOAc, 1:1).

7-Hydroxy-2-phenyl-*4H*-chromen-4-one (2a)

White crystal, yield: 23%, mp: >250˚C, IR (KBr, cm^{-1}): 3178 (OH), 1630; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.81 (s, 1H, OH), 8.07 (d, J=8.7 Hz ,2H, $H_{2',6'}$), 7.89 (d, J=8.7 Hz, 1H, H_5), 7.55-7.62 (m, 3H, $H_{3',4',5'}$), 7.01 (d, J=2.1 Hz, 1H, H_8), 6.93 (dd, J=8.7, 2.1 Hz, 1H, H_6), 6.91 (s, 1H, H_3(. Anal. calcd. for $C_{15}H_{10}O_3$: C, 76.62; H, 4.23; O, 20.15. Found: C, 75.98; H, 4.54; O, 20.01.

7-Hydroxy-2-(4-methylphenyl)-*4H*-chromen-4-one (2b)

White crystal, yield: 21%, mp: >250˚C, IR (KBr, cm^{-1}): 3069 (OH), 1628; ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.78 (s, 1H, OH), 7.96 (d, J=8.7 Hz, 2H, $H_{2',6'}$), 7.88 (d, J=8.7 Hz, 1H, H_5), 7.38 (d, J=8.7 Hz, 2H, $H_{3',5'}$), 7.0

(d, J=2.1 Hz, 1H, H_8), 6.92 (dd, J=8.7, 2.1 Hz, 1H, H_6), 6.85 (s, 1H, H_3), 2.36 (s, 3H, CH_3). Anal. calcd. for $C_{16}H_{12}O_3$: C, 76.18; H, 4.79; O, 19.03. Found: C, 76.33; H, 4.52; O, 18.79.

7-Hydroxy-2-(4-methoxyphenyl)-*4H*-chromen-4-one (2c)

White crystal, yield: 32%, mp: >250˚C, IR (KBr, cm^{-1}): 2981 (OH), 1685; ^1H NMR (500 MHz, DMSO-d_6): δ 10.93 (s, 1H, OH), 8.03 (d, J=8.7 Hz ,2H, $H_{2',6'}$), 7.87 (d, J=8.7 Hz, 1H, H_5), 7.11 (d, J=8.7 Hz, 2H, $H_{3',5'}$), 7.0 (d, J=2.1 Hz, 1H, H_8), 6.92 (dd, J=8.7, 2.1 Hz, 1H, H_6), 6.80 (s, 1H, H_3), 3.86 (s, 3H, OCH_3). Anal. calcd. for $C_{16}H_{12}O_4$: C, 71.64; H, 4.51; O, 23.86. Found: C, 71.22; H, 4.82; O, 24.01.

2-(4-chlorophenyl)-7-Hydroxy-*4H*-chromen-4-one (2d)

White crystal, yield:12%, mp: >250˚C, IR (KBr, cm^{-1}): 3278 (OH), 1645; ^1H NMR (500 MHz, DMSO-d_6): δ 10.79 (s, 1H, OH), 8.10 (d, J=8.7 Hz, 2H, $H_{2',6'}$), 7.89 (d, J=8.7 Hz, 1H, H_5), 7.64 (d, J=8.7 Hz, 2H, $H_{3',5'}$), 7.01 (d, J=2.1 Hz, 1H, H_8), 6.95 (s, 1H, H_3), 6.93 (d, J=2.1 Hz, 1H, H_6). Anal. calcd. for $C_{15}H_9ClO_3$: C, 66.07; H, 3.33; O, 17.60. Found: C, 65.89; H, 3.62; O, 17.33.

2-(4-bromophenyl)-7-Hydroxy-*4H*-chromen-4-one (2e)

White crystal, yield:16%, mp: >250˚C, IR (KBr, cm^{-1}): 3301 (OH), 1607; ^1H NMR (500 MHz, DMSO-d_6): δ 10.69 (s, 1H, OH), 8.02 (d, J=8.7 Hz, 2H, $H_{2',6'}$), 7.88 (d, J=8.7 Hz, 1H, H_5), 7.78 (d, J=8.7 Hz, 2H, $H_{3',5'}$), 6.99 (d, J=2.1 Hz, 1H, H_8), 6.94 (s, 1H, H_3), 6.92 (dd, J=8.7, 2.1 Hz, 1H, H_6). Anal. calcd. for $C_{15}H_9BrO_3$: C, 56.81; H, 2.86; O, 15.13. Found: C, 56.63; H, 3.07; O, 15.43.

2-(2,3-dimethoxyphenyl)-7-Hydroxy-*4H*-chromen-4-one (2f)

White crystal, yield:13%, mp: >250˚C, IR (KBr, cm^{-1}): 3005 (OH), 1627; ^1H NMR (500 MHz, DMSO-d_6): δ 10.42 (s, 1H, OH), 7.90 (d, J=8.7 Hz ,2H, H_5), 7.32 (dd, J=8.7, 2.1 Hz, 1H, H_6), 7.19 (dd, J=8.7, 2.1 Hz, 1H, $H_{4'}$), 6.94 (d, J=2.1 Hz, 1H, H_8), 6.91-6.93 (m, 2H, $H_{5',6'}$), 6.61 (s, 1H, H_3), 3.88 (s, 1H, OCH_3), 3.82 (s, 1H, OCH_3). Anal. calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73; O, 26.82. Found: C, 68.18; H, 5.01; O, 26.65.

2-(2,4-dimethoxyphenyl)-7-Hydroxy-*4H*-chromen-4-one (2g)

White crystal, yield:15%, mp: >250˚C, IR (KBr, cm^{-1}): 2998 (OH), 1638; ^1H NMR (500 MHz, DMSO-d_6): δ 10.35 (s, 1H, OH), 8.11 (d, J=8.7 Hz ,1H, H_5), 7.84 (d, J=8.7 Hz, 1H, $H_{6'}$), 7.08 (s, 1H, H_3), 7.01 (d, J=8.7 Hz,

1H, H_6), 6.97 (s, 2H, $H_{3'}$), 6.60 (d, *J*=8.7 Hz, 1H, $H_{5'}$), 6.52 (s, 1H, H_8), 3.89 (s, 1H, OCH_3), 3.86 (s, 1H, OCH_3). Anal. calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73; O, 26.82. Found: C, 68.76; H, 4.41; O, 27.12.

2-(3,4-dimethoxyphenyl)-7-Hydroxy-*4H*-chromen-4-one (2h)

White crystal, yield:17%, mp: >250˚C, IR (KBr, cm^{-1}): 3013 (OH), 1653; ^1H NMR (500 MHz, DMSO-d_6): δ 10.38 (s, 1H, OH), 7.94 (d, *J*=8.7 Hz ,1H, H_5), 7.70 (dd, *J*=8.7, 2.1 Hz, 1H, $H_{6'}$), 7.59 (s, 1H, H_3), 7.37 (dd, *J*=8.7, 2.1 Hz, 1H, H_6), 7.11-7.14 (m, 2H, $H_{2',5'}$), 6.97 (s, 1H, H_8), 3.89 (s, 1H, OCH_3), 3.85 (s, 1H, OCH_3). Anal. calcd. for $C_{17}H_{14}O_5$: C, 68.45; H, 4.73; O, 26.82. Found: C, 68.09; H, 4.98; O, 26.86.

7-Hydroxy-2-(2,3,4-trimethoxy phenyl)-*4H*-chromen-4-one (2i)

White crystal, yield:26%, mp: >250˚C, IR (KBr, cm^{-1}): 3014 (OH), 1629; ^1H NMR (500 MHz, DMSO-d_6): δ 10.80 (s, 1H, OH), 8.21 (d, *J*=8.7 Hz ,1H, H_5), 7.87 (d, *J*=8.7 Hz, 1H, $H_{6'}$), 7.56 (d, *J*=8.7 Hz, 1H, $H_{5'}$), 7.23 (s, 1H, H_3), 7.12-7.16 (m, 1H, H_6), 6.78 (s, 1H, H_8), 4.19 (s, 1H, OCH_3), 4.11 (s, 1H, OCH_3), 3.91 (s, 1H, OCH_3). Anal. calcd. for $C_{18}H_{16}O_6$: C, 65.85; H, 4.91; O, 29.24. Found: C, 65.30; H, 5.12; O, 29.40.

Synthesis of 4-oxo-2-phenyl-4*H*-chromen-7-yl methanesulfonate (3a-i)

To a solution of 2a-i (1 mmol) in dry tetrahydrofuran (5 mL) methanesulfonyl chloride (1.5 mmol) and triethylamine (2 mmol) was added dropwise in an ice bath and stirred at room temperature overnight. Then the volatiles were evaporated under vacuum. To this residue water was added and it was extracted with ethyl acetate. The organic phase was dried with sodium sulfate and the solvent was evaporated under vacuum. The product was purified by preparative TLC (hexane: EtOAc, 3:2).

4-oxo-2-phenyl-4*H*-chromen-7-yl methanesulfonate (3a)

White crystal, yield:67%, mp:120-121 ˚C, IR (KBr, cm^{-1}): 1638, 1335 (S=O), 1199; ^1H NMR (500 MHz, DMSO-d_6): δ 8.17 (d, *J*=8.4 Hz, 1H, H_5), 8.15 (d, *J*=8.4 Hz, 2H, $H_{2',6'}$), 7.91 (d, *J*=1.9 Hz, 1H, H_8), 7.67-7.59 (m, 3H, $H_{3',4',5'}$), 7.50 (dd, *J*=8.4, 1.9 Hz, 1H, H_6), 7.13 (s, 1H, H_3), 3.55 (s, 3H, CH_3SO_2). Anal. calcd. for $C_{16}H_{12}O_5S$: C, 60.75; H, 3.82; O, 25.29. Found: C, 60.81; H, 4.02; O, 25.53.

2-(4-methyl phenyl)-4-oxo-4*H*-chromen-7-yl methanesulfonate (3b)

White crystal, yield:69%, mp:184-185 ˚C, IR (KBr, cm^{-1}): 1642, 1342 (S=O), 1177; ^1H NMR (500 MHz, DMSO-d_6): δ 8.15 (d, J=8.7 Hz, 1H, H_5), 8.06 (d, J=8.7 Hz, 2H, $H_{2',6'}$), 7.91 (d, J=2.2 Hz, 1H, H_8), 7.48 (dd, J=8.7, 2.2 Hz, 1H, H_6), 7.42 (dd, J=8.7, 2.2 Hz, 1H, $H_{3',5'}$), 7.08 (s, 1H, H_3), 3.55 (s, 3H, CH_3SO_2), 2.83 (s, 3H, CH_3), ^{13}C NMR (125 MHz, DMSO-d_6): 177.0 C_4, 163.9 C_2, 156.6 C_{8a}, 152.9 C_7, 142.88 $C_{4'}$, 130.2 $C_{3',5'}$, 128.1 C_5, 127.5 $C_{1'}$, 126.8 $C_{2',6'}$, 122.5 C_{4a}, 120.4 C_6, 112.6 C_8, 106.7 C_3, 38.2 C-S, 21.4 CH3. Anal. calcd. for $C_{17}H_{14}O_5S$: C, 61.81; H, 4.27; O, 24.22. Found: C, 61.48; H, 4.43; O, 24.18.

2-(4-methoxy phenyl)-4-oxo-4*H*-chromen-7-yl methanesulfonate (3c)

White crystal, yield:78%, mp:208-212 ˚C, IR (KBr, cm^{-1}): 1637, 1363 (S=O), 1179; ^1H NMR (500 MHz, DMSO-d_6): δ 8.13 (d, J=8.7 Hz, 1H, H_5), 8.09 (d, J=8.9 Hz, 2H, $H_{3',5'}$), 7.85 (d, J=2.2 Hz, 1H, H_8), 7.46 (d, J=8.7 Hz, 1H, H_6), 7.13 (d, J=8.9 Hz, 2H, $H_{2',6'}$), 6.98 (s, 1H, H_3), 3.87 (s, 3H, OCH_3), 3.52 (s, 3H, CH_3SO_2), ^{13}C NMR (125 MHz, DMSO-d_6): 176.7 C_4, 163.6 C_2, 163 $C_{4'}$, 156.6 C_{8a}, 153 C_7, 128.7 $C_{2',6'}$, 127.5 C_5, 123.3 $C_{1'}$, 122.8 C_{4a}, 120.5 C_6, 115.1 $C_{3',5'}$, 112.7 C_8, 106.0 C_3, 56.0 C-O, 37.4 C-S. Anal. calcd. for $C_{17}H_{14}O_6S$: C, 58.95; H, 4.07; O, 27.72. Found: C, 59.22; H, 4.33; O, 28.10.

2-(4-chloro phenyl)-4-oxo-4*H*-chromen-7-yl methanesulfonate (3d)

White crystal, yield:71%, mp:201-203 ˚C, IR (KBr, cm^{-1}): 1636, 1340 (S=O), 1179; ^1H NMR (500 MHz, DMSO-d_6): δ 8.19 (d, J=8.7 Hz, 2H, $H_{2',6'}$), 8.16 (d, J=8.7 Hz, 1H, H_5), 7.92 (d, J=2.2 Hz, 1H, H_8), 7.69 (d, J=8.7 Hz, 2H, $H_{3',5'}$), 7.50 (dd, J=8.7, 2.2 Hz, 1H, H_6), 7.16 (s, 1H, H_3), 3.55 (s, 3H, CH_3SO_2); MS: m/z 350 [M+1]. Anal. calcd. for $C_{16}H_{11}ClO_5S$: C, 54.78; H, 3.16; O, 22.81. Found: C, 54.42; H, 3.33; O, 23.01.

2-(4-bromo phenyl)-4-oxo-4*H*-chromen-7-yl methanesulfonate (3e)

White crystal, yield:65%, mp:229-230 ˚C, IR (KBr, cm^{-1}): 1640, 1340 (S=O), 1178; ^1H NMR (500 MHz, DMSO-d_6): δ 8.16 (d, J=8.7 Hz, 1H, H_5), 8.11 (d, J=8.7 Hz, 1H, $H_{2',6'}$), 7.92 (d, J=2.2 Hz, 1H, H_8), 7.83 (d, J=8.7 Hz, 2H, $H_{3',5'}$), 7.50 (dd, J=8.7, 2.2 Hz, 1H, H_6), 7.17 (s, 1H, H_3), 3.55 (s, 3H, CH_3SO_2); MS: m/z 395 [M+1]. Anal. calcd. for $C_{16}H_{11}BrO_5S$: C, 48.62; H, 2.81; O, 22.24. Found: C, 48.43; H, 3.13; O, 22.56.

2-(2,3-dimethoxy phenyl)-4-oxo-4*H*-chromen-7-yl methanesulfonate (3f)

White crystal, yield:78%, mp:208-212 ˚C, IR (KBr, cm^{-1}): 1648, 1368 (S=O), 1149;^{1}H NMR (500 MHz, DMSO-d_6): δ 8.17 (d, J=8.7 Hz, 1H, H_5), 7.82 (d, J=2.2 Hz, 1H, H_8), 7.50 (dd, J=8.9, 2.2 Hz, 1H, H_6), 7.42 (d, J=2.2 Hz, 1H, $H_{4'}$), 7.26-7.33 (m, 2H, $H_{5',6'}$), 6.83 (s, 1H, H_3), 3.89 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.53 (s, 3H, CH_3SO_2);); ^{13}C NMR (125 MHz, DMSO-d_6): 177.2 C_4, 163.9 C_2, 161.9 C_{8a}, 161.0 C_7, 157.0 $C_{3'}$, 155.9 $C_{2'}$, 131.1 C_5, 120.8 $C_{1'}$, 120.6 $C_{5'}$, 117.7 $C_{6'}$, 114.9 C_{4a}, 112.5 $C_{4'}$, 110.9 C_3, 110.0 C_6, 105.1 C_8, 59.5 C-$O_{2'}$, 58.5 C-$O_{3'}$, 42.0 C-S. Anal. calcd. for $C_{18}H_{16}O_7S$: C, 57.44; H, 4.28; O, 29.76. Found: C, 57.73; H, 4.56; O, 30.03.

2-(2,4-dimethoxy phenyl)-4-oxo-4*H*-chromen-7-yl methanesulfonate (3g)

White crystal, yield:78%, mp:208-212 ˚C, IR (KBr, cm^{-1}): 1659, 1349 (S=O), 1133; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.06 (d, J=8.7 Hz, 1H, H_5), 7.94 (dd, J=8.9, 2.2 Hz, 1H, $H_{6'}$), 7.62 (d, J=2.2, 1H, H_8), 7.56 (d, J=2.2 Hz, 1H, $H_{3'}$), 7.51 (dd, J=8.9, 2.2 Hz, 1H, H_6), 7.27 (dd, J=8.9, 2.2 Hz, 1H, $H_{5'}$), 4.02 (s, 3H, OCH_3), 3.95 (s, 3H, OCH_3), 3.50 (s, 3H, CH_3SO_2);); ^{13}C NMR (125 MHz, DMSO-d_6): 178.3 C_4, 163.0 C_2, 162.8 $C_{4'}$, 160.4 $C_{2'}$, 159.4 C_{8a}, 157.9 C_7, 130.2 $C_{6'}$, 128.7 C_5, 117.7 C_{4a}, 114.4 $C_{1'}$, 113.4 C_3, 111.0 C_6, 106.1 $C_{5'}$, 105.1 C_8, 101.2 $C_{3'}$, 55.5 C-$O_{2'}$, 55.4 C-$O_{4'}$, 40.3 C-S. Anal. calcd. for $C_{18}H_{16}O_7S$: C, 57.44; H, 4.28; O, 29.76. Found: C, 57.61; H, 4.55; O, 29.98.

2-(3,4-dimethoxy phenyl)-4-oxo-4*H*-chromen-7-yl methanesulfonate (3h)

White crystal, yield:78%, mp:208-212 ˚C, IR (KBr, cm^{-1}): 1655, 1329 (S=O), 1169; ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.94 (d, J=8.7 Hz, 1H, H_5), 7.70 (d, J=8.9 Hz, 1H, $H_{6'}$), 7.59 (s, 1H, H_8), 7.37 (d, J=8.7 Hz, 1H, H_6), 7.11-7.15 (m, 2H, $H_{2',5'}$), 6.98 (s, 1H, H_3), 4.01 (s, 3H, OCH_3), 3.89 (s, 3H, OCH_3), 3.85 (s, 3H, CH_3SO_2); ^{13}C NMR (125 MHz, DMSO-d_6): 178.9 C_4, 165 C_2, 160.0 C_{8a}, 157.6 C_7, 154.2 $C_{3'}$, 149.3 $C_{4'}$, 130.0 C_5, 128.0 $C_{1'}$, 127.5 $C_{6'}$, 126.8 C_{4a}, 112.5 $C_{5'}$, 108 C_6, 105.2 $C_{2'}$, 104.5 C_8, 103.9 C_3, 56.9 C-$O_{3'}$, 57.4 C-$O_{4'}$, 42.4 C-S. Anal. calcd. for $C_{18}H_{16}O_7S$: C, 57.44; H, 4.28; O, 29.76. Found: C, 57.09; H, 4.46; O, 29.88.

4-oxo-2-(2,3,4-trimethoxy phenyl)-4*H*-chromen-7-yl methanesulfonate (3i)

White crystal, yield:78%, mp:208-212 ˚C, IR (KBr, cm^{-1}): 1680, 1328 (S=O), 1145; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.15 (d, J=8.7 Hz, 1H, H_5), 7.81 (s, 1H, H_8), 7.67 (d, J=8.7 Hz, 1H, H_6), 7.47 (d, J=8.9 Hz, 1H, $H_{6'}$), 7.03 (d, J=8.9 Hz, 1H, $H_{5'}$), 6.84 (s, 1H, H_3), 3.89 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.53 (s, 3H, CH_3SO_2); ^{13}C NMR (125 MHz, DMSO-d_6): 178.8 C_4, 168.5 C_2, 157.5 C_{8a}, 157 C_7, 154.6 $C_{4'}$, 152.9 $C_{2'}$, 141.9 $C_{3'}$, 129.0 C_5, 122.7 $C_{6'}$, 119.5 $C_{1'}$, 118.9 C_{4a}, 110.3 C_3, 109.0 C_6, 107.5 $C_{5'}$, 103.0 C_8, 56.6

C-$O_{2'}$, 54.9 C-$O_{3'}$, 54.0 C-$O_{4'}$, 38.4 C-S. Anal. calcd. for $C_{19}H_{18}O_8S$: C, 56.15; H, 4.46; O, 31.49. Found: C, 56.55; H, 4.87; O, 31.73.

4.3 Cell culture

A human breast cancer cell line, MCF-7, was obtained from the Iranian Biological Resource Center (*Tehran, Iran*) and maintained in RPMI 1640 medium (*Biowest*). The medium was supplemented with 10% fetal bovine serum (FBS) (*Gibco, Carlsbad, CA, USA*) and 1% antibiotics (Penicillin and Streptomycin). The cells were incubated at 37 °C under the standard condition of 95% humidity and 5% CO_2 to reach 70% cell confluency [24].

4.4 Cell proliferation assay

The cytotoxicity of compounds was assessed by MTT assay which measures the percentage of viable cells. Cells were seeded in a 96-well cell culture plate at 7×10^3 cells/well and incubated for 48 h. Then, cells were exposed to fresh medium containing different concentrations of compounds. Subsequently, the medium was replaced with tetrazolium salt (5 mg/ml of PBS) (MTT, (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) (*Sigma, St.Louis, MO, USA*) followed by additional 4 h incubation at 37 °C. The formed formazan crystals were dissolved in 100 µl of Dimethyl sulfoxide (DMSO) (Merck) and measured for absorbance at 570 nm using a plate reader (*BioRad, Model 680*). Also finally, IC_{50} values were defined as the half-maximal inhibitory concentration.

4.5 Flow cytometry analysis of cellular apoptosis

The Annexin V-FITC/PI (Propidium iodide) dual staining assay (BD Pharmingen™ kit) was used to determine the induction of apoptosis by the most potent compound 3c, in comparison with commercial anticancer drug docetaxel. The breast cancer cell line MCF-7 (3×10^5 cells/well) were seeded into 6-well plates and incubated overnight at 37 °C under 5% CO_2 overnight. After treatment the cells with IC_{50} concentrations of compound 3c, docetaxel as positive control, and 1% DMSO as negative control, incubated with for 24h. Then, the treated cells were trypsinized, harvested and washed with PBS (pH 7.4). The cell suspension was centrifuged at 1200 and resuspended in 500 µl of 1× annexin V binding buffer containing 1.4 M NaCl, 25 mM $CaCl_2$, 0.1 M HEPES/ NaOH (pH 7.4). The cells were double stained with 5 µL of Annexin V-PE and 5 µL of PI solution and incubated at room temperature in dark for 15 mins. After adding 400 µL of 1× annexin binding buffer to the vials, the cells were analyzed by flow cytometry using FITC signal detector (FL1) and PI staining by the phycoerythrin emission signal detector (FL2) [25].

4.6 Docking analysis

To determine the possible binding modes of the compound, docking analysis was carried out against the STS enzyme using the smina molecular docking The X-ray crystal structures of STS enzyme (PID: 1P49) were extracted from the PBD site and were prepared by removing solvent molecules and the co-crystallized ligands. Polar hydrogen atoms were added to the enzymes and the Kollmann charges were assigned. The compound (3c) was drawn using Marvin Sketch and subjected to energy minimization using the steepest descent algorithm and then Gasteiger charges were calculated by Open Babel. Binding sites were determined automatically using the coordinates of native co-crystallized ligands of enzymes [26].

Declarations

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This is to acknowledge any financial interest or benefit that has arisen from the direct applications of this research.

Conflicts of interest/Competing interests

The authors declare that they have no competing interests.

Availability of data and material

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

M.H.J., contributed to the synthesis and characterization of compounds; A.I., contributed to the designing and preparation of the manuscript; M.S., contributed to the apoptosis inducing activity evaluation of compounds; H.M., contributed to the designing and cytotoxicity evaluation of compounds and preparation of the related part in manuscript; P.T., contributed to the cytotoxicity evaluation of compounds; S.E. contributed to the cytotoxicity evaluation of compounds; L.N. supervised all phases of the study; S.S.M. supervised all phases of the study.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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Tables

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Figures

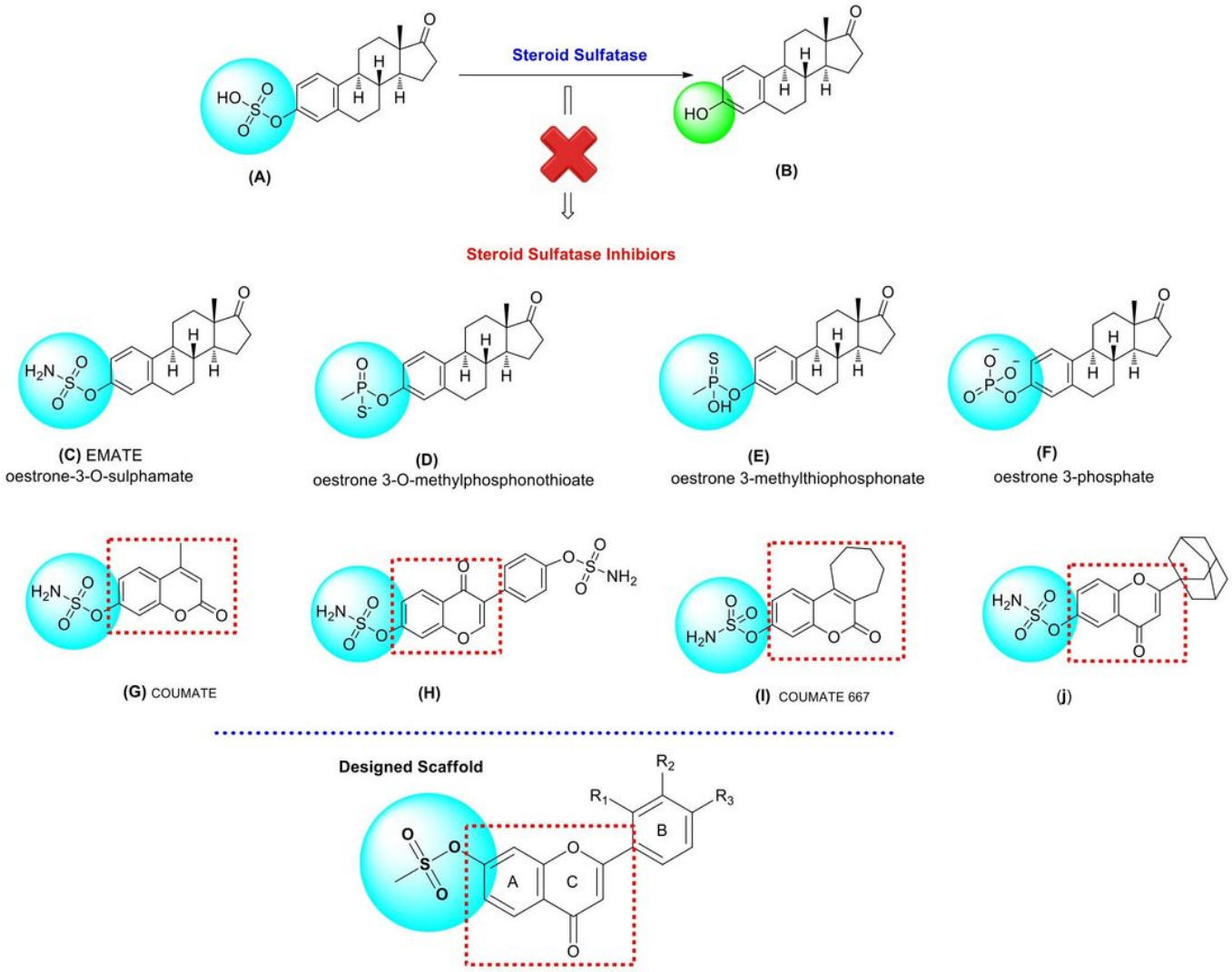

Figure 1

Structures of most potent STS inhibitors and designed compound

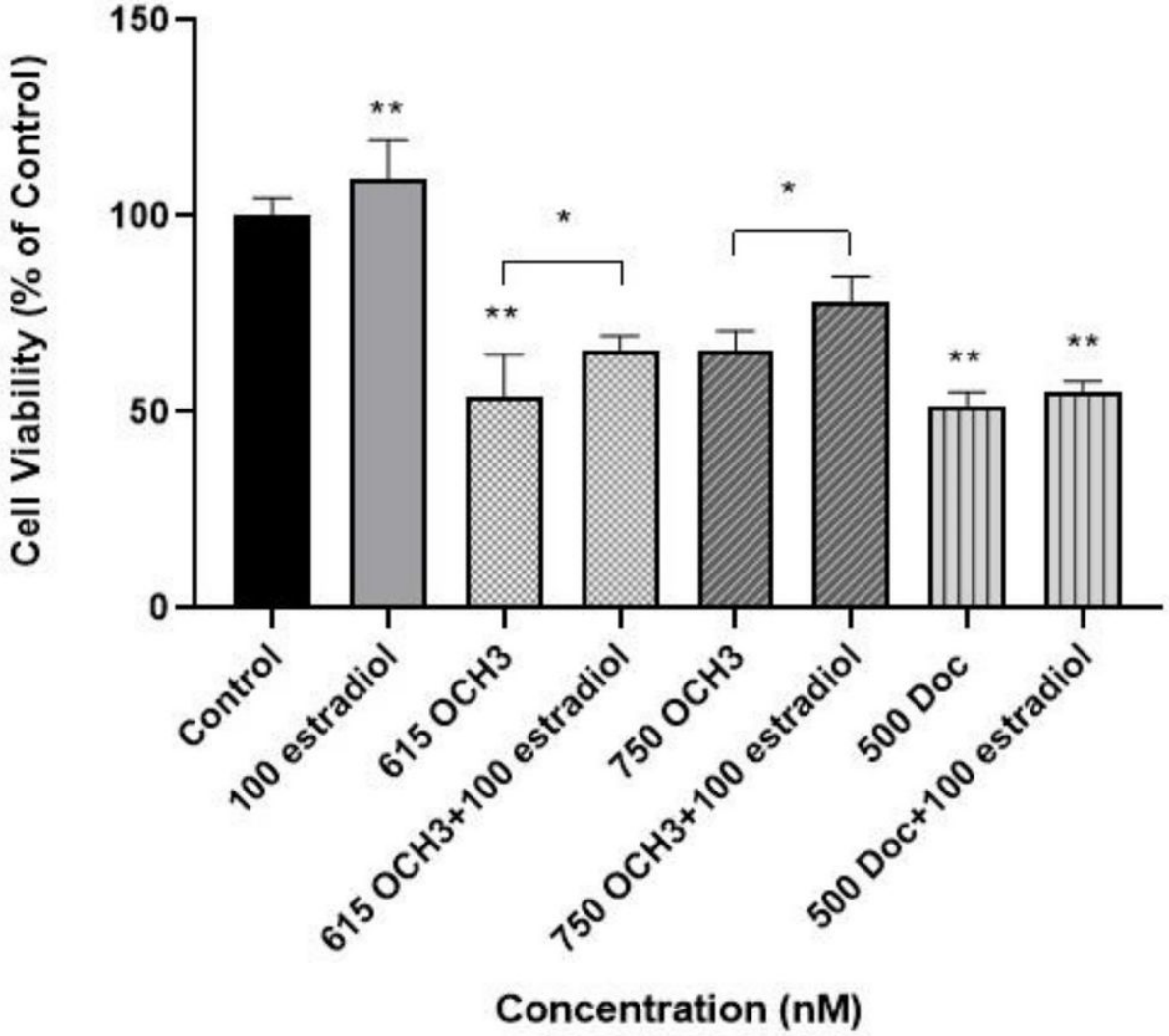

Figure 2

Viability of MCF-7 cells treated with estradiol, OCH3 (3c) and Doc alone and in combination for 48 h. Absorbance values were normalized to the control group (Mean ± SE, n≥3). *p<0.05, **p<0.01.

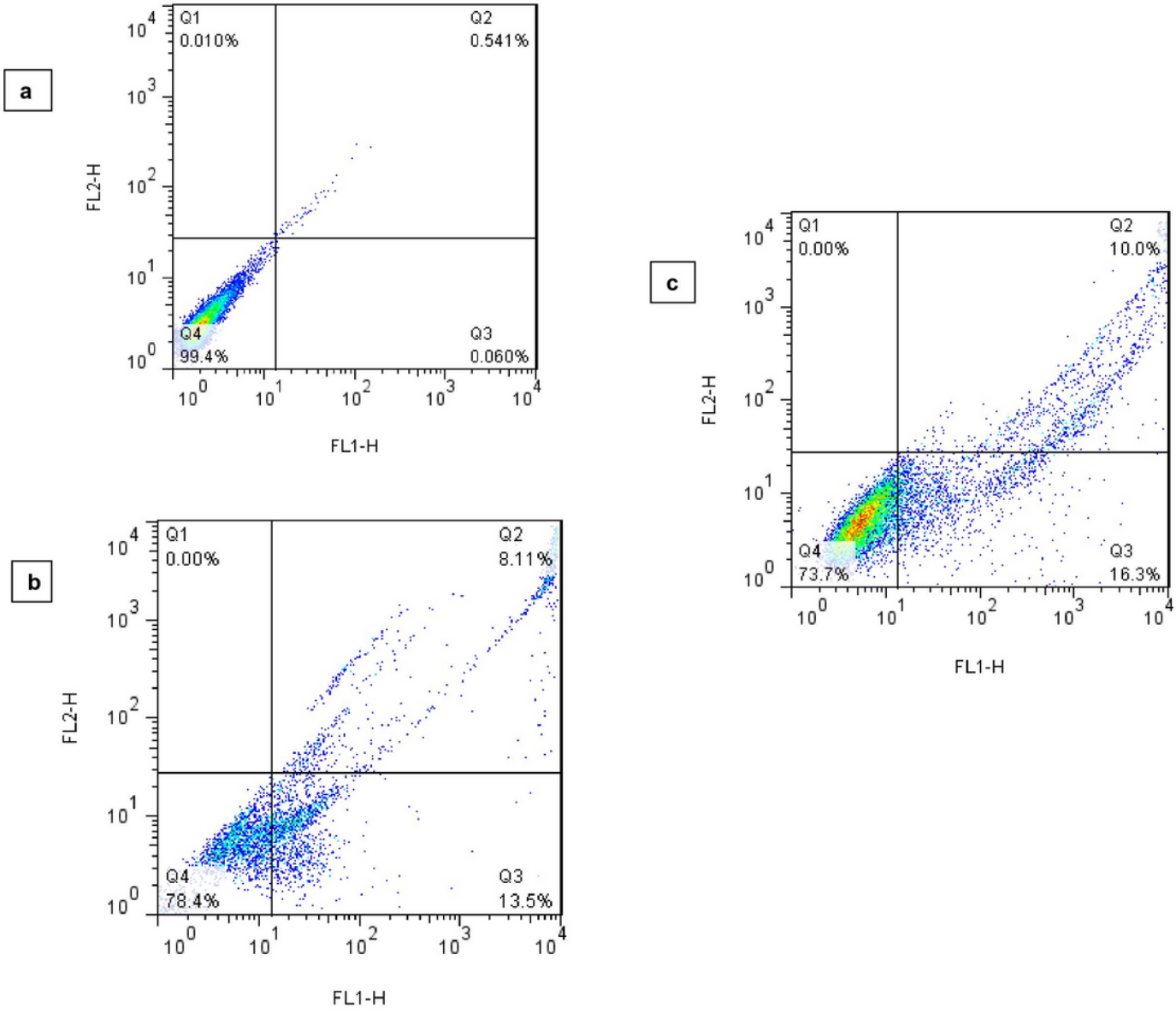

Figure 3

Flow cytometric analysis of Annexin V-FITC/PI stained Mcf-7 cell line treated with compound 3c. The cells treated with a) DMSO 1% (negative control); b) IC50 concentration of Doc; C) IC50 concentration of compound 3c.

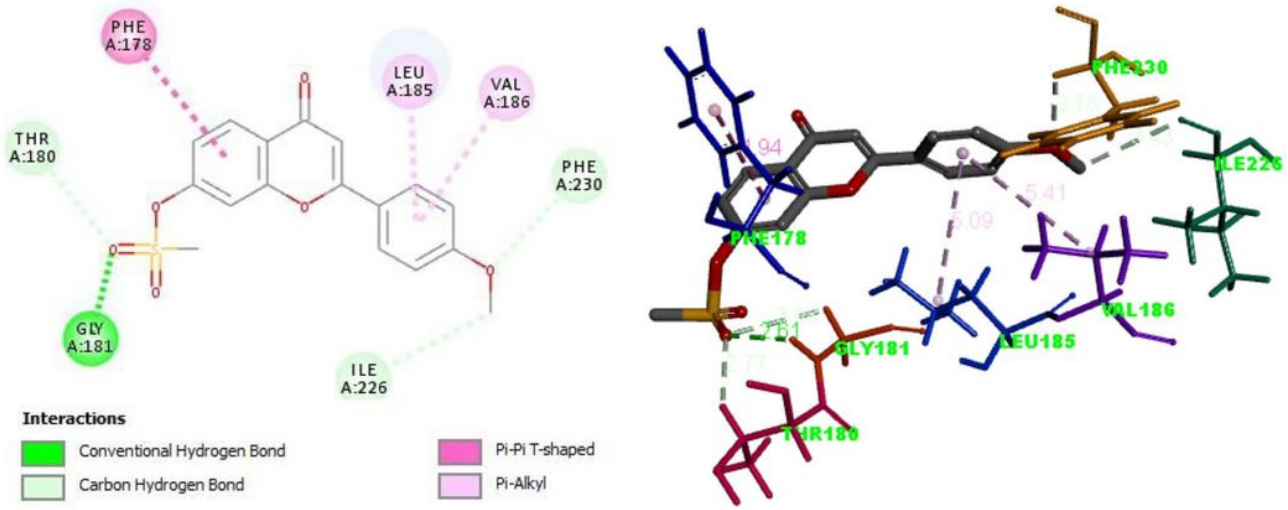

Figure 4

Compound 3c was docked to the binding pocket of the STS (PDB: 1P49).

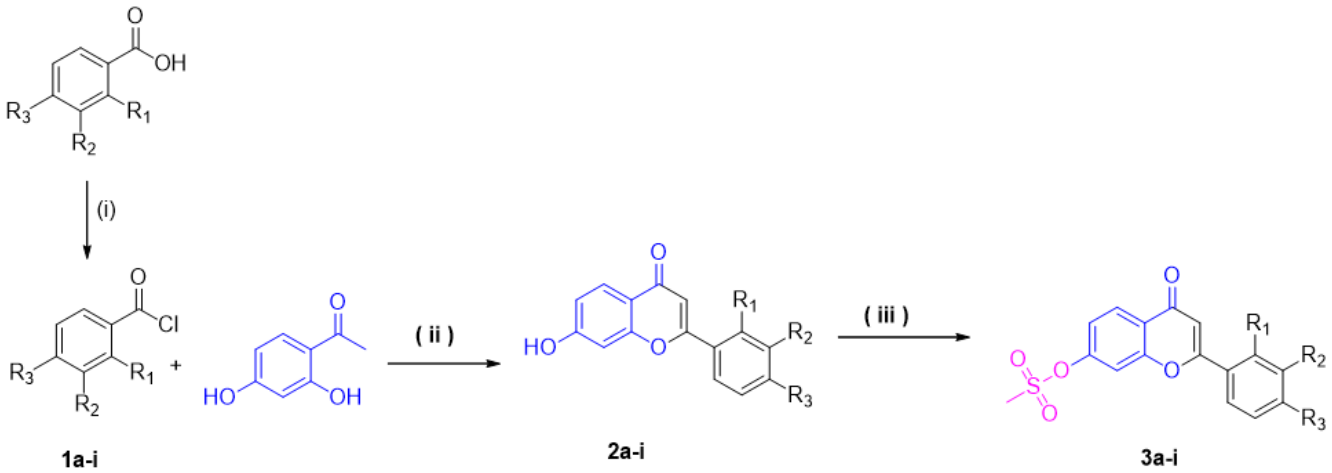

Figure 5

Scheme 1. Synthesis of 2-phenyl-4-oxo-4H-chromen-7-yl methanesulfonate, Reagents and conditions: (i) Thionyl chloride, Reflux, 2h; (ii) 1: K2CO3, Acetone, Reflux, 8h; 2: H2O and Methanol (1:1), Reflux, 2h; (iii) THF, MSC, Triethylamine, RT, 24h

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