Design and Synthesis of Novel Chalcone-Phenylpyranone derivatives as Estrogen Receptor Modulators

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Abstract:

Selective estrogen receptor modulators (SERMs) are a class of drugs that act on the estrogen receptor (ER). SERMs are used for treatment and reduction of risk of breast cancer. Herewith we had designed, synthesized and evaluated chalcone-phenylpyran-2-one derivatives bearing N,N-dimethyl ethylamine side chain for their anti-breast cancer activity on MCF-7 and Zr-75-1 cell lines in-vitro. The pharmacological data indicated that most of tested compounds showed moderate to significant cytotoxicity and high selectivity toward estrogen receptor. The SAR analyses indicated that compounds **5f** with 2,6-dichloro substitution was more effective. Docking study was performed to predict binding orientation towards the estrogen receptor-α.

Keywords: SERM, breast cancer, chalcone, phenylpyranone, MCF-7, Docking

1. Introduction

Breast cancer mortality has declined by 24%from 1990 to 2000, likely due to increases in the use of both mammography screening (followed by surgery) and adjuvant therapy, including chemotherapy and antihormonal therapy. Worldwide, it is anticipated that in the coming decade, 5 million women will be affected by breast cancer. Clearly, further advances in the development of treatments, particularly ones with fewer undesirable side-effects, are necessary.[1,2]

Endocrine therapy alone, most notably Tamoxifen, a selective estrogen receptor modulator (SERM) that blocks estrogen action in breast cancer, is estimated to account for a 9.8% (median value) decrease in breast cancer mortality.[3]

Phytoestrogens are plant derived substances that are structurally and functionally similar to estrogens and are found in many foods. Mainly there are three classes of phytoestrogens - isoflavones, coumestans, and lignans.

Epidemiological data indicates that Asiatic societies which consume phytoestrogen-rich diet have a lower risk of so called "Western diseases" such as breast and prostate cancer, osteoporosis, and cardiovascular diseases.[1,3,4]

Chalcones (1,3-diaryl-2-propen-1-ones) are a class of compounds consisting of two aryl rings linked by an a,b-unsaturated ketone moiety. Chalcone moieties are common substructures in numerous natural products belonging to the flavonoid family. Derivatives of chalcone are versatile as pharmaceutically active compounds, and have been shown to display many desirable properties for human diseases, including anticancer, anti-HIV, antimalarial, antioxidant, anti-inflammatory, and anti-allergic activities. Published data showed that chalcone compounds possess strongantiproliferative activities against both primary cells and established cell lines.

We synthesized 6heteroarylchalcone compounds, and examined their antiproliferative activity against breast cancer cell lines. We show here for the first time that heteroaryl chalcones can be effective therapeutics against human breast cancer with relatively low side effects on non-cancer cells.[4,5]

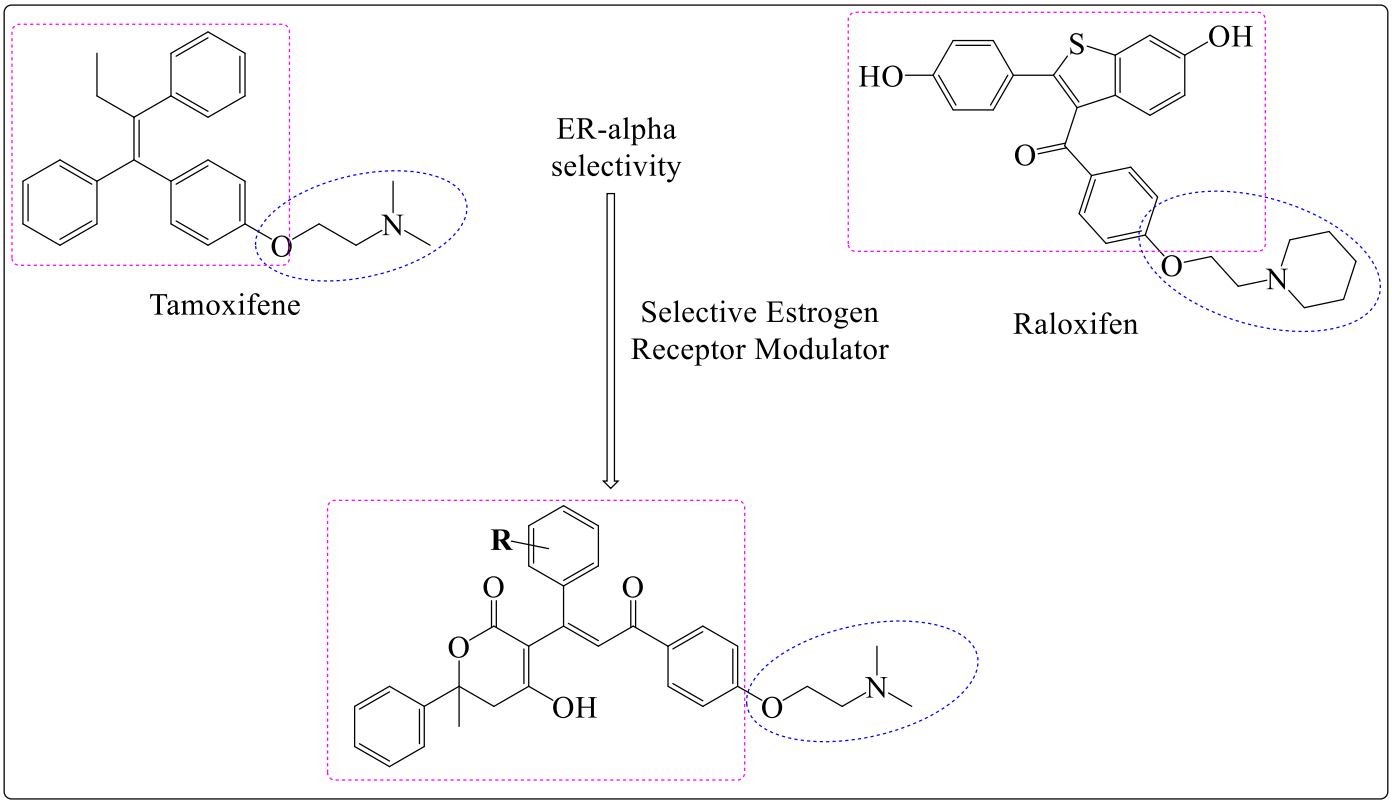
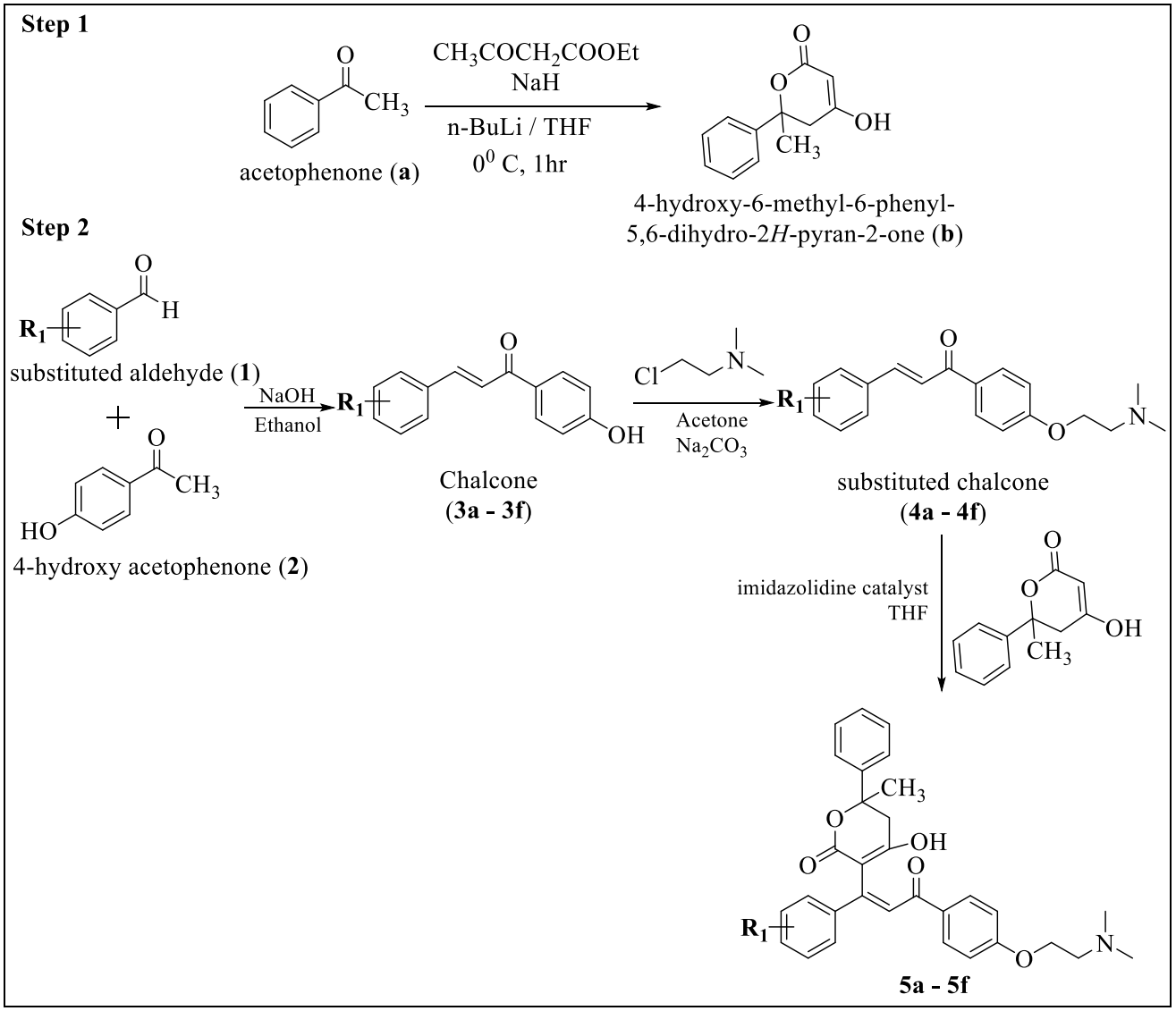


Figure 1: Pharmacophore of designed compounds

2. MATERIAL AND METHODS

2.1. Chemistry

Melting points were recorded in open capillaries with electrical melting point apparatus and were uncorrected. IR spectra of all synthesized compound in KBr were recorded utilizing a (JASCO FT-IR 4000) spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on BrukerAvance (400 MHz) Spectrometer in $CDCl_3$ solutions, with TMS as an internal reference. Mass spectra were recorded on a Varian Inc, 410 Prostar Binary LC with 500 MS IT PDA Detectors. All the reagents and solvents used were of analytical grade.



Scheme 1: Synthesis of designed compounds (**5a – 5f**)

2.1.1. Synthesis of 4-hydroxy-6-methyl-6-phenyl-5,6-dihydro-2H-pyran-2-one (b)

As shown in step 1 (scheme 1), a mixture of acetophenone (a) and ethyl 3-oxobutanoate, NaH and n-BuLi was stir for overnight at 0^0C. The resultant solution were workup into ice, wash with water. The precipitate was recrystallized from absolute ethanol.

2.1.2. Synthesis of substituted p-hydroxyChalcone (3)

An equimolar mixture of 4-hydroxy acetophenone, substituted benzaldehydes and KOH (2mmol) was stirred in PEG-400 (15 ml) at 40°C for 2-3 hr. After the completion of the reaction (monitored by TLC), the crude mixture was worked up in ice-cold water (100 ml). The resultant product was separated out and recrystallized from absolute ethanol. [5,6]

2.1.3. Synthesis of substituted [(4-(2-(dimethylamino)ethoxy)] chalcone

A mixture of substituted chalcone (0.625mmol), anhydrous K_2CO_3 (3.12mmol), 2-chloro-N,N-dimethylethanamine (0.93mmol), and dry acetone (10mL) was refluxed for 24 hr. K_2CO_3 was filtered off and acetone was distilled out. The residue was diluted with water and extracted with ethyl acetate. The organic layer was washed with water, brine, and dried over anhydrous Na_2SO_4. The precipitate was recrystallized from absolute ethanol.[6,7]

2.1.4. Synthesis of target compounds (5a-5f)

A mixture of substituted [(4-(2-(dimethylamino)ethoxy)] chalcone (0.497mmol), compound **b** (0.204mmol), imidazolidine catalyst (10 mmol) & THF (10mL) was stirred for 36 hr. The residue was diluted with water and extracted with ethyl acetate. The organic layer was washed with water, brine, and dried over anhydrous Na_2SO_4. The precipitate was recrystallized from absolute ethanol.[7,8]

2.1.4.1. 3-(3-(4-(2-(dimethylamino)ethoxy)phenyl)-3-oxo-1-phenylprop-1-en-1-yl)-4-hydroxy-6-methyl-6-phenyl-5,6-dihydro-2H-pyran-2-one (5a)

% Yield: 66.24; MW: 497.59; MF: $C_{31}H_{31}NO_5$; MP: 142-144°C; IR (KBr): 677 (Ar-H), 1191 (C-O), 1339 (C-N), 1538 (C=C), 1722 (C=O) cm^{-1}; ^1H NMR (DMSO, 400MHz): δ= 8.4 (s, 1H, OH), 7.8-7.4 (m, 14H, Ar-H), 7.1 (s, 1H, CH), 4.1 (t, 2H, CH_2), 2.9 (t, 2H, CH_2), 2.3, 2.5 (s, 2H, CH_2 of pyranone), 2.2 (s, 6H, CH_3 of diamine), 1.8 (s, 3H, CH_3 of pyranone); ^{13}C NMR (DMSO, 100MHz): δ= 186.8 (C=O), 179.2 (OH), 164.5 (Ar-C-O), 162.1 (C=O), 154.7 (Phenyl-pyranone), 142.4, 140.6 (Ar-propene), 130.2, 129.8, 129.7, 129.2, 128.4, 127.8-127.4 (Ar-C), 68.9 ($C_{pyranone}$), 57.3 (CH_2-N), 51.4 (CH_2-O), 48.9, 48.8 (CH_3-N), 31.3 (CH_3); MS: *m/z* = 498.4 [M+1].

2.1.4.2. 3-(1-(4-chlorophenyl)-3-(4-(2-(dimethylamino)ethoxy)phenyl)-3-oxoprop-1-en-1-yl)-4-hydroxy-6-methyl-6-phenyl-5,6-dihydro-2H-pyran-2-one (5b)

% Yield: 71.30; MW: 532.03; MF: $C_{31}H_{30}ClNO_5$; MP: 170-172°C; IR (KBr): 681, 678 (Ar-H), 1194 (C-O), 1329 (C-N), 1529 (C=C), 1725 (C=O) cm^{-1}; ^1H NMR (DMSO, 400MHz): δ= 8.7 (s, 1H, OH), 7.9-7.4 (m, 13H, Ar-H), 7.0 (s, 1H, CH), 4.2 (t, 2H, CH_2), 2.9 (t, 2H, CH_2), 2.4, 2.5 (s, 2H, CH_2 of pyranone), 2.1 (s, 6H, CH_3 of diamine), 1.9 (s, 3H, CH_3 of pyranone); ^{13}C NMR (DMSO, 100MHz): δ= 188.9 (C=O), 179.4 (OH), 168.6 (Ar-C-O), 163.0

(C=O), 157.1 (Phenyl-pyranone), 143.5, 142.8 (Ar-propene), 130.4, 130.2, 129.8, 129.5, 129.1, 128.3, 127.7-127.2 (Ar-C), 68.7 ($C_{pyranone}$), 57.2 (CH_2-N), 51.7 (CH_2-O), 48.8, 48.7 (CH_3-N), 32.4 (CH_3); MS: *m/z* = 534.2 [M+2].

2.1.4.3. 3-(3-(4-(2-(dimethylamino)ethoxy)phenyl)-3-oxo-1-(p-tolyl)prop-1-en-1-yl)-4-hydroxy-6-methyl-6-phenyl-5,6-dihydro-2H-pyran-2-one (5c)

% Yield: 54.82; MW: 511.62; MF: $C_{32}H_{33}NO_5$; MP: 158-160°C; IR (KBr): 668 (Ar-H), 1190 (C-O), 1333 (C-N), 1540 (C=C), 1723 (C=O) cm^{-1}; ^{1}H NMR (DMSO, 400MHz): δ= 8.1 (s, 1H, OH), 7.8-7.3 (m, 13H, Ar-H), 7.1 (s, 1H, CH), 4.2 (t, 2H, CH_2), 2.9 (t, 2H, CH_2), 2.5 (s, 3H, CH_3), 2.3, 2.5 (s, 2H, CH_2 of pyranone), 2.2 (s, 6H, CH_3 of diamine), 1.8 (s, 3H, CH_3 of pyranone); ^{13}C NMR (DMSO, 100MHz): δ= 182.5 (C=O), 175.1 (OH), 166.6 (Ar-C-O), 163.2 (C=O), 151.8 (Phenyl-pyranone), 143.0, 141.6 (Ar-propene), 131.3, 130.2, 129.8, 129.5, 128.8, 127.8-127.5 (Ar-C), 61.5 ($C_{pyranone}$), 57.8 (CH_2-N), 52.1 (CH_2-O), 48.8, 48.7 (CH_3-N), 33.5, 31.6 (CH_3); MS: *m/z* = 512.6 [M+1].

2.1.4.4. 3-(3-(4-(2-(dimethylamino)ethoxy)phenyl)-1-(4-methoxyphenyl)-3-oxoprop-1-en-1-yl)-4-hydroxy-6-methyl-6-phenyl-5,6-dihydro-2H-pyran-2-one (5d)

% Yield: 50.14; MW: 527.23; MF: $C_{32}H_{33}NO_6$; MP: 180-182°C; IR (KBr): 680 (Ar-H), 1190 (C-O), 1335 (C-N), 1539 (C=C), 1724 (C=O) cm^{-1}; ^{1}H NMR (DMSO, 400MHz): δ= 8.1 (s, 1H, OH), 7.8-7.4 (m, 13H, Ar-H), 7.2 (s, 1H, CH), 4.2 (t, 2H, CH_2), 3.3 (s, 3H, CH_3-O), 2.9 (t, 2H, CH_2), 2.3, 2.4 (s, 2H, CH_2 of pyranone), 2.1 (s, 6H, CH_3 of diamine), 1.8 (s, 3H, CH_3 of pyranone); ^{13}C NMR (DMSO, 100MHz): δ= 180.6 (C=O), 177.3 (OH), 164.7 (Ar-C-O), 162.2 (C=O), 159.7 (Ar-OCH_3), 153.5 (Phenyl-pyranone), 142.1, 140.8 (Ar-propene), 131.5, 130.3, 129.9, 129.7, 129.2, 128.5, 127.8-127.4 (Ar-C), 66.8 ($C_{pyranone}$), 57.7 (CH_2-N), 55.1 (OCH_3), 52.0 (CH_2-O), 49.1, 48.9 (CH_3-N), 31.2 (CH_3); MS: *m/z* = 528.2 [M+1].

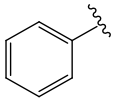
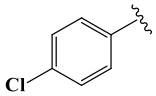
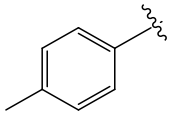
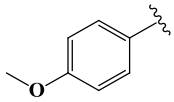
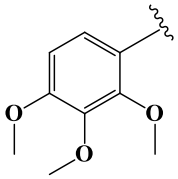
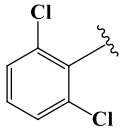
2.1.4.5. 3-(3-(4-(2-(dimethylamino)ethoxy)phenyl)-3-oxo-1-(2,3,4-trimethoxyphenyl)prop-1-en-1-yl)-4-hydroxy-6-methyl-6-phenyl-5,6-dihydro-2H-pyran-2-one (5e)

% Yield: 68.62; MW: 587.67; MF: $C_{34}H_{37}NO_8$; MP: 194-196°C; IR (KBr): 671 (Ar-H), 1188 (C-O), 1341 (C-N), 1540 (C=C), 1726 (C=O) cm^{-1}; ^{1}H NMR (DMSO, 400MHz): δ= 8.3 (s, 1H, OH), 7.9-7.4 (m, 11H, Ar-H), 7.0 (s, 1H, CH), 4.2 (t, 2H, CH_2), 3.8-3.7 (s, 9H, OCH_3), 3.0 (t, 2H, CH_2), 2.5, 2.4 (s, 2H, CH_2 of pyranone), 2.1 (s, 6H, CH_3 of diamine), 1.8 (s, 3H, CH_3 of pyranone); ^{13}C NMR (DMSO, 100MHz): δ= 182.1 (C=O), 177.4 (OH), 164.4 (Ar-C-O), 162.8 (C=O), 161.1, 159.7 (Ar-OCH_3), 154.7 (Phenyl-pyranone), 142.4, 140.6 (Ar-propene), 130.2, 129.8, 129.7, 129.2, 128.4, 127.8-127.4 (Ar-C), 68.9 ($C_{pyranone}$), 61.1, 60.3 (OCH_3), 57.3 (CH_2-N), 51.4 (CH_2-O), 48.9, 48.8 (CH_3-N), 31.3 (CH_3); MS: *m/z* = 588.6 [M+1].

2.1.4.6. 3-(1-(2,6-dichlorophenyl)-3-(4-(2-(dimethylamino)ethoxy)phenyl)-3-oxoprop-1-en-1-yl)-4-hydroxy-6-methyl-6-phenyl-5,6-dihydro-2H-pyran-2-one (5f)

% Yield: 59.72; MW: 566.48; MF: $C_{31}H_{29}Cl_2NO_5$; MP: 170-172°C; IR (KBr): 682 (Ar-H), 1190 (C-O), 1327 (C-N), 1529 (C=C), 1725 (C=O) cm^{-1}; ^1H NMR (DMSO, 400MHz): δ= 8.6 (s, 1H, OH), 7.9-7.4 (m, 12H, Ar-H), 7.1 (s, 1H, CH), 4.3 (t, 2H, CH_2), 2.9 (t, 2H, CH_2), 2.5, 2.4 (s, 2H, CH_2 of pyranone), 2.1 (s, 6H, CH_3 of diamine), 1.9 (s, 3H, CH_3 of pyranone); ^{13}C NMR (DMSO, 100MHz): δ= 187.5 (C=O), 179.8 (OH), 168.8 (Ar-C-O), 164.1 (C=O), 157.3 (Phenyl-pyranone), 143.8, 142.4 (Ar-propene), 131.1, 130.8, 129.8, 129.4, 129.1, 128.4, 127.8-127.3 (Ar-C), 66.9 ($C_{pyranone}$), 57.3 (CH_2-N), 51.8 (CH_2-O), 48.7, 48.6 (CH_3-N), 33.5 (CH_3); MS: *m/z* = 568.5 [M+2].

Table 1 *In-vitro* anticancer activity (µg/ml) of synthesized compounds (5a-5f)

Sr. No.	R	MCF-7			ZR-75-1		
		LC_{50}[a]	**TGI[b]**	**GI_{50}[c]**	**LC_{50}**	**TGI**	**GI_{50}**
5a		>100	>100	88.5	>100	>100	78.6
5b		>100	>100	74.6	>100	>100	91.2
5c		>100	>100	52.2	>100	>100	>100
5d		>100	>100	36.8	>100	>100	89.4
5e		>100	>100	68.3	>100	>100	71.9
5f		84.4	61.3	**28.2**	>100	>100	58.2
ADR	-	87.8	25.6	<0.1	>100	>100	<0.1
TAM	-	39.5	16.3	<10	>100	>100	<0.1

Most potent compounds shown by bold text as compare to standard
[TAM]tamoxifen, [ADR]adriamycin

[a] Compound concentration that produces 50 % cytocidal effect

[b] Compound concentration that produces total growth inhibition

[c] Compound concentration that produces 50 % growth inhibition

2.2. *In-vitro*antitumor activity

In-vitro testing done using SRB assay protocols [14], each drug is tested at 4 dose levels ($1x10^{-7}$M, $1x10^{-6}$M, $1x10^{-5}$M, $1x10^{-4}$M, or 10, 20, 40, 80 µg/ml). Appropriate positive controls are run in each experiment and each experiment is repeated thrice. Results are given in terms of GI_{50}, TGI and LC_{50} values. The compounds were tested for their cytotoxic assay using MCF-7and ZR-75-1 breast cancer cell lines.[8,9]

3. RESULT AND DISCUSSION

3.1. Chemistry

All the compounds were synthesized according to steps depicted in scheme 1 and their structures were verified by IR, ^{1}H-NMR, ^{13}C-NMR, and LC-MS spectroscopy. The first step in the synthetic route consisted of the cyclisation of acetophenone with ethyl 3-oxobutanoate in presence of n-BuLi and THF as solvent at 0^{0}C to give 4-hydroxy-6-methyl-6-phenyl-5,6-dihydro-2H-pyran-2-one (compound **b**) (**Scheme 1**). The second step consisted of the synthesis of chalcone (**3a-3f**). The etherification of chalcone done at hydroxyl group by amino side chain i.e. 2-chloro-N,N-dimethylethan-1-amine. The target compounds were synthesized by addition of compound **b** to **4a-4f**compounds are shown in **Table 1**.

3.2. *In-vitro*Cytotoxic Assay

The target compounds were evaluated for anticancer activity against estrogen receptor alpha positive (ER+) human breast cancer cell lines i.e. MCF-7 and ZR-75-1. The *in-vitro* activity profile was shown in **Table 1**. The GI_{50} concentration for each compound was calculated with reference to a control sample, which represents the concentration that results in a 50% decrease in cell growth/proliferation after 48h incubation in the presence of drug. The total growth inhibition (TGI) is the concentration of test drug which signifies a cytostatic effect. The LC_{50} is concentration of compound that produces 50% cytotoxic effect. Tamoxifen and Adriamycin were used as reference.

The compound **5f** showed most prominent cytotoxic activity against MCF-7 breast cancer cell line. The structure activity indicate that the presence of hydrophobic group at 2 and 6 position at substituted phenyl ring increases the activity while substitution at para position increases activity. The trimethoxy substitution also showed decrease in activity. The synthesized derivatives have the half potency to that of standard tamoxifen.

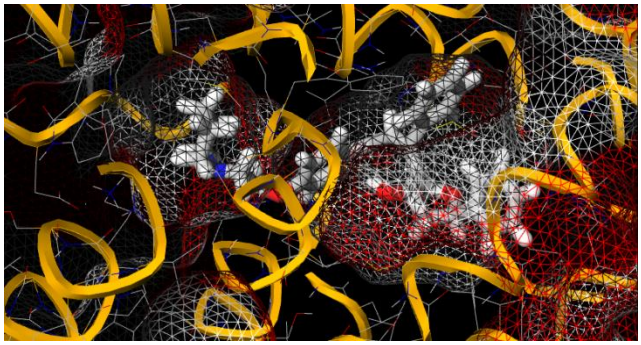


Figure 2: docking pose of compound 5a.

3.3. Docking analysis

The docking study was performed online at Mcule.com using 1 Click docking on estrogen receptor alpha (PDB code: 1L2I). Binding

orientation showed that tertiary amine is important for hydrogen binding with Asp351 amino acid while substitution with hydrophobic group at phenyl ring increases docking score as shown in **Fig 2**.

4. References

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