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Synthesis, Docking Study of 2-(3-Substituted)-7-hydroxyl-4H-1-benzopyran-4-one Derivatives as Anti-Cancer agents

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ABSTRACT

Flavonoids are the natural phyto constituents widely distributed in plants originate in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine. Flavonoids have been recognized as secondary metabolites of plant, with marked biological significance such as Anti-inflammatory, Antioxidant, Anticancer and Antimicrobial activity. Hence, flavonoids are considered as an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications with versatile health benefits. Recent researches on flavonoids received an added impulse with the discovery on anticancer by several mechanisms, but the most important mechanism is the inhibition of aromatase generating enzyme. In this research, attempt has been made to synthesize a novel series of synthetic flavones after molecular docking studies of compounds a-y. Novel flavones are synthesizing using 2, 4-dihydroxyacetophenone as starting material through Claisen-Schmidt Condensation Reaction. All the synthesized compounds were confirmed by their physicochemical properties. Novel flavone derivatives were assessing for anticancer activity by using SRB assay on MCF-7 cell line. Novel flavone derivatives were performed to establish correlation between biological activity and molecular properties. Among the synthesized compounds (a, b, c, k, l) showed good anticancer activity comparable to the reference drug Fadrazole. Thus, the conclusion can be made that the flavone moiety can exhibit a good anticancer activity.

Keywords: Flavonoid; Synthetic flavones; Claisen-Schmidt condensation reaction; Molecular docking; Anticancer; MCF-7 cell line

INTRODUCTION

Aromatase enzyme is an essential in estrogen biosynthesis converting the aliphatic androgens testosterone and androstenedione to the aromaticestrogens, estradiol and estrone, respectively. Estrogens play a key role in normal cell proliferation by binding to the nuclear Estrogen Receptor (ER) and triggering a sequence of reactions leading to cell division [1]. Estrogens are also a key factor in hormone-dependent (ER-

positive) tumor development [2]. One approach to treat and/or prevent hormone-dependent tumor development is to decrease the level of circulating estrogens and local tumor estrogen production by inhibiting estrogen producing enzymes [3]. Flavonoids are the natural phytoconstituents broadly spread in plants originate in fruits, vegetables, grains, bark, roots, stems, flowers, tea and wine [4,5]. Flavonoids have been recognized as secondary metabolites of plant, with marked biological significance such as antiinflammatory, antioxidant, anticancer and antimicrobial activity. Hence, flavonoids are considered as an important component in a variety of nutraceuticals, pharmaceuticals, medicinal and cosmetic applications with versatile health benefits. It is observed that the Flavone moiety possess specific pharmacophore pattern which is necessary for binding to aromatase enzyme and their by its inhibition.

In this study, non-steroidal aromatase inhibitors possess aromatic/aliphatic amines at side chain for suitable position bound with MET 374 present in energetic site of aromatase enzyme and acts as H bond acceptor. Basic nucleus plays role as hydrophobic spacer moiety which maintained distance between heme coordinating group and hydrogen bond acceptor moiety. In this research molecular docking we provide an atomic level explanation for the binding of novel flavone derivative to the aromatase active site. In these study to report the binding mode of phytoestrogens to the aromatase enzyme using Molecular Dynamics Simulations (MDS) and ligand-protein docking (Table 1).

MATERIALS AND METHODS

Melting points were determined using a VEEGO make microprocessor based melting point apparatus having silicone oil bath and are uncorrected. IR spectra (wave numbers in cm^{-1}) were recorded on a BRUKER ALPHA FT-IR spectrophotometer using potassium bromide discs [6]. The progress of all reactions was monitored by TLC on 2 cm × 5 cm pre-coated silica gel 60 F254 (Merck) plates of thickness of 0.25 mm. The chromatograms were visualized under UV 254 nm and/or exposure to iodine vapors [7-9]. All reagents/chemicals used were of analytical grade, obtained from LOBA chemicals, SDFCL and spectrochem (Figure 1). Chemicals and solvents were purified by general laboratory techniques before use. All moisture free operations were performed in oven dried glass wares and under nitrogen atmosphere (Table 1).

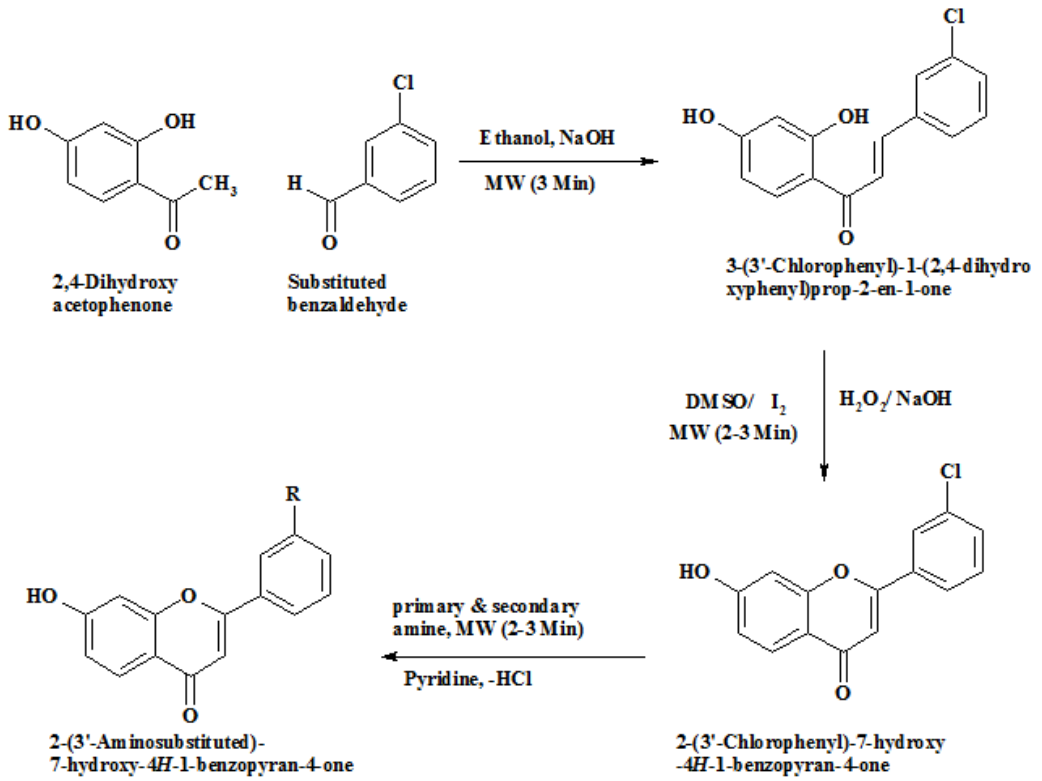

Figure 1: Scheme for the synthesis of 2-(3-Substituted)-7-hydroxyl-4H-1-benzopyran-4-one (a-z).

Table 1: Derivatization of flavone.

Sr. No.	Compound	-R/Ar	Sr. No.	Compound	-R/Ar
1	a	Methylamino	5	l	Ethylmethylamino
2	b	Ethylamino	6	t	p-Methylaniline
3	c	Propylamino	7	v	p-Chloroaniline
4	k	Diethylamino	8	w	p-Nitroaniline

Preparation of 3-(3'-chlorophenyl)-1-(2,4-dihydroxyphenyl)-prop-2-en-1-one

Accurately weighed 1.52 g (0.01 mole) of 2,4-dihydroxyacetophenone and 1.40 g (0.01) mole of 3-chloro benzaldehyde was taken in 250 ml Round Bottom Flask (R.B.F.) and dissolved in ethanol (5 ml) then add 10% NaOH (5 ml) solution with stirring, by keeping the flask in ice bath, then resulting mixture was irradiated in microwave oven at level 5, after confirmation by TLC, the reaction mixture was poured into crushed ice and acidify by 5% HCI, the crude chalcone was obtained [10]. Crude chalcone was dried and then recrystallized from methanol. Yield 84.8%, m.p.140-158ºC, Rf 0.7 Ethyl acetate: N Hexane (2: 8), IR (KBr, cm^{-1}) 3286.70, 1670.35, 1490.97, 1161.15, 766.50.

Preparation of 2-(3'-chlorophenyl)-7-hydroxyl-4h-1-benzopyran-4-one

3-(3'-chlorophenyl)-1-(2,4-dihydroxyphenyl)-prop-2-en-1-one was dissolved in 5 ml of Dimethyl sulfoxide (DMSO) and 2-3 crystals of Iodine was added and reaction mixture was irradiated for 2-3 minute in microwave at level 5, and reaction monitored by TLC. After the completion of reaction, reaction mixture poured into the crushed ice crude flavone was obtained. Crude flavone was dried and then recrystallized from methanol [11]. Yield 78.8%, m.p.158-160ºC, Rf 0.82 Ethyl acetate: N Hexane (2: 8), IR (KBr, cm^{-1}) 3286.70, 1670.35, 1490.97, 1161.15, 766.50.

Preparation of 7-hydroxy-2-[3-(methylamino)-phenyl]-4h-1-benzopyran-4-one

Weigh accurately 2.72 g (0.01 moles) of 2-(3-chlorophenyl)-7-hydroxyl-4H-1-benzopyran-4-one and 0.31 g (0.01 mole) of methylamine were taken in 250 mL of RBF. In above mixture add pyridine (5 mL) dropwise followed by rapid stirring, by keeping the RBF in ice bath, then resulting mixture was irradiated in microwave oven for 2-3 min at level 5. Completion of reaction was monitored by TLC, after completion of reaction the residue was wash with water to remove pyridine and crystalized above mixture by using methanol [12]. Crude product was then re-crystallized from methanol and further purified by column chromatography using silica gel 60-120 mesh eluted with Ethyl acetate: N Hexane (2: 8). Yield 63.12%, m.p.178-180ºC, Rf 0.6 Ethyl acetate: N Hexane (2: 8), IR (KBr, cm^{-1}) 1151.68, 1741.49, 1583.64, 3365, 766.50.

Preparation of 2-[3-(ethylamino)-phenyl]-7-hydroxy-4h-1-benzopyran-4-one

Weigh accurately 2.72 g (0.01 mole) of 2-(3'-chlorophenyl)-7-hydroxyl-4H-1-benzopyran-4-one and 0.45 g (0.01 mole) of ethylamine were taken in 250 mL of RBF. In above mixture add pyridine (5 mL) dropwise followed by rapid stirring, by keeping the RBF in ice bath, then resulting mixture was irradiated in microwave oven for 2-3 min at level 5. Completion of reaction was monitored by TLC, after completion of reaction the residue was wash with water to remove pyridine and crystalized above mixture by using methanol [13]. Crude product was then re-crystallized from methanol and further purified by column chromatography using silica gel 60-120 mesh eluted with Ethyl acetate: N Hexane (2: 8). Yield 72.00%, m.p.184-185ºC, Rf 0.82 Ethyl acetate: N Hexane (2: 8), IR (KBr, cm^{-1}) 1149.41, 1649.50, 1402.10, 3372, 705.61.

Preparation of 7-hydroxy-2-[3-(propylamino)-phenyl]-4h-1-benzopyran-4-one

Weigh accurately 2.72 g (0.01 mole) of 2-(3'-chlorophenyl)-7-hydroxyl-4H-1-benzopyran-4-one and 0.59 g (0.01 mole) of propylamine were taken in 250 mL of RBF. In above mixture add pyridine (5 mL) dropwise followed by rapid stirring, by keeping the RBF in ice bath, then resulting mixture was irradiated in microwave oven for 2-3 min at level 5. Completion of reaction was monitored by TLC, after completion of reaction the residue was wash with water to remove pyridine and crystalized above mixture by using methanol. Crude product was then re-crystallized from methanol and further purified by column chromatography using silica gel 60-120 mesh eluted with Ethyl acetate: N Hexane (2: 8). Yield 57.86%, m.p.187-190ºC, Rf 0.84 Ethyl acetate: N Hexane (2: 8), IR (KBr, cm^{-1}) 1140.44, 1630.96, 1542.80, 3255.50, 712.45.

Preparation of 2-[3-(diethylamino)-phenyl]-7-hydroxy-4h-1-benzopyran-4-one

Weigh accurately 2.72 g (0.01 mole) of 2-(3'-chlorophenyl)-7-hydroxyl-4H-1-benzopyran-4-one and 0.73 g (0.01 mole) of diethylamine were taken in 250 mL of RBF. In above mixture add pyridine (5 mL) dropwise followed by rapid stirring, by keeping the RBF in ice bath, then resulting mixture was irradiated in microwave oven for 2-3 min at level 5. Completion of reaction was monitored by TLC, after completion of reaction the residue was wash with water to remove pyridine and crystalized above mixture by using methanol. Crude product was then re-crystallized from methanol and further purified by column chromatography using silica gel 60-120 mesh eluted with Ethyl acetate: N Hexane (2: 8). Yield 61.21%, m.p.179-182ºC, Rf 0.62 Ethyl acetate: N Hexane (2: 8), IR (KBr, cm^{-1}) 1145.18, 1741.49, 1480.80, 3227, 727.15.

Preparation of 2-{3-[ethylmethylamino]-phenyl}-7-hydroxy-4h-1-benzopyran-4-one

Weigh accurately 2.72 g (0.01 mole) of 2-(3-chlorophenyl)-7-hydroxyl-4H-1-benzopyran-4-one and 0.59 g (0.01 mole) of ethylmethylamine were taken in 250 mL of RBF. In above mixture add pyridine (5 mL) dropwise followed by rapid stirring, by keeping the RBF in ice bath, then resulting mixture was irradiated in microwave oven for 2-3 min at level 5. Completion of reaction was monitored by TLC, after completion of reaction the residue was wash with water to remove pyridine and crystalized above mixture by using methanol [14,15]. Crude product was then re-crystallized from methanol and further purified by column chromatography using silica gel 60-120 mesh eluted with ethyl acetate: N Hexane (2: 8). Yield 59.09%, m.p.181-183ºC, Rf 0.47 Ethyl acetate: N Hexane (2: 8), IR (KBr, cm^{-1}) 1125.18, 1631.49, 1427.80, 3286.70, 703.15.

RESULTS AND DISCUSSION

Molecular docking

Schrodinger software was used to perform all docking simulations. A set of new 2-(3'-Substituted)-7-hydroxyl-4H-1-benzopyran-4-one derivatives were subjected to docking with Crystal structure of human placental aromatase inhibitors (PDB ID:3EQM) from the Protein Data Bank (RCSB). To carry out in docking studies, the 2D structures of the synthesized ligands (a-z) were drawn and converted to energy minimized 3D, By removing the hetero atoms, water molecule and cofactors, the target protein file was prepared by leaving the associated residue with protein by using auto dock 4.2 (MGL tools-1.5.6). Preparation of target protein file auto dock 4.2 (MGL tools-1.5.6) tools has been done, which involves the assign of Gasteiger charges for all the atoms of molecules converting into AD4 type (Table 2). Docking simulations for the compounds (a-z) were performed in Figures 2 and 3.

Table 2: Result of the docking study.

Code	Docking Score	Glide Score	H-Bond	Code	Docking Score	Glide Score	H-Bond
a	-8.253	-8.253	1	i	-6.419	-6.431	1
b	-9.077	-9.089	2	k	-8.331	-8.443	1
c	-8.06	-8.06	1	Fadrazole	-7.564	-7.725	1

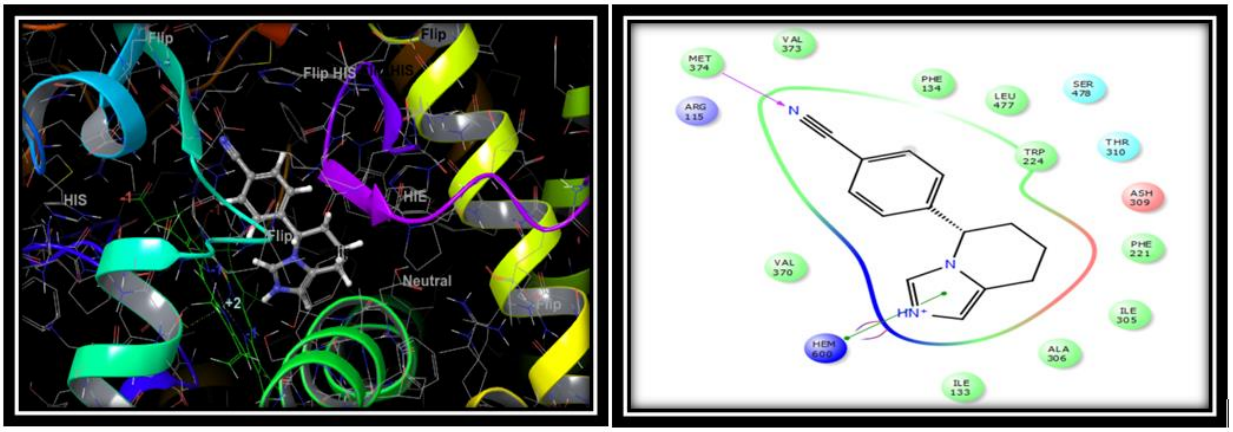

Figure 2: Docking and 2D interactions of fadrazole with the active amino acids 3EQM.

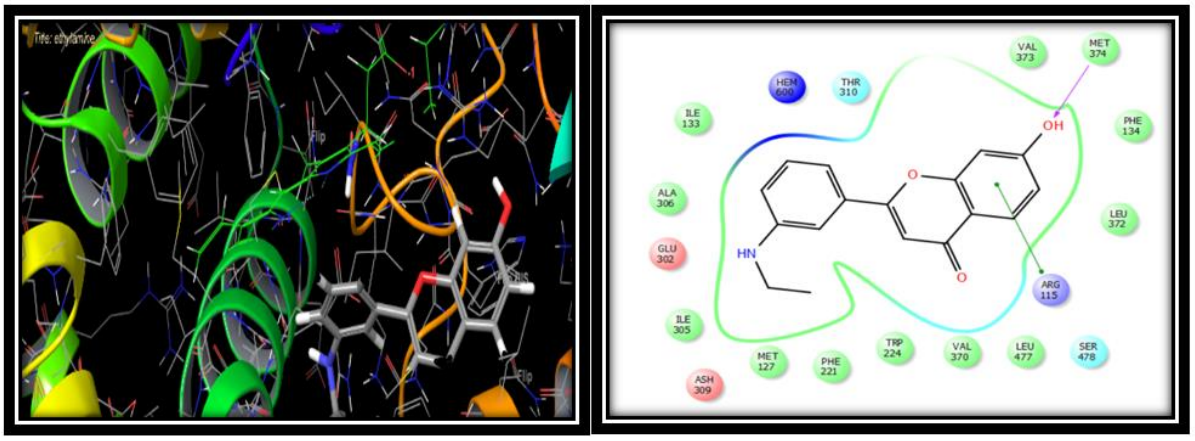

Figure 3: Docking and 2D interactions of compound with the active amino acids 3EQM.

Cell culture

MCF-7 is a breast cancer cell line is the acronym of Michigan Cancer Foundation-7. MCF-7, it is not possible for cancer researchers to obtain a mammary cell line that was capable of living longer than a few months [16]. The cell lines were grown in RPMI (Rosswell Park Memorial Institute) 1640 medium containing 10% fetal bovine serum and 2 mM L-glutamine.

SRB assay

For present screening experiment, cells were inoculated into 96 well microtiter plates in 90 µL at plating densities as shown in the study details above, depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37°C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs [17]. After 24 h, one plate of each cell line was fixed in situ with TCA, to represent a measurement of the cell population for each cell line at the time of drug addiction (Tz). Experimental drugs were solubilized in 0.1% DMSO at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to 10 times the desired final maximum test concentration with complete medium containing test article at a concentration [18-21]. Additional three, 10-fold serial dilutions were made to provide a total of four drug concentrations plus control (Table 3). Aliquots of 10 µl of these different drug dilutions were added to the appropriate micro-titer wells already containing 90 µl of medium, resulting in the required final drug concentrations. After compound addition, plates were incubated at standard conditions for 48 hours and assay was terminated by the addition of cold TCA. Cells were fixed in situ by the gentle addition of 50 µl of cold 30% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 minutes at 4°C. The supernatant was discarded; the plates were washed five times with tap water and air dried. Sulforhodamine-B (SRB) solution (50 µl) at 0.4% (w/v) in 1% acetic acid was added to each of

the wells, and plates were incubated for 20 minutes at room temperatsure [22]. After staining, unbound dye was recovered and the residual dye was removed by washing five times with 1% acetic acid. The plates were air dried. Bound stain was subsequently eluted with 10 m Mtrizma base, and the absorbance was read on an Elisa plate reader at a wavelength of 540 nm with 690 nm reference wavelength. Percent growth was calculated on a plate-by-Plate basis for test wells relative to control wells [23-25]. Percent growth was expressed as the ratio of average absorbance of the test well to the average absorbance of the control wells 100. Using the six absorbance measurements Time zero (Tz), Control growth (C), and test growth in the presence of drug at the four concentration levels (Ti), the percentage growth was calculated at each of the drug concentration levels (Figures 4 and 5).

Percentage growth inhibition was calculated as:

[(Ti-Tz)/(C-Tz)] × 100 for concentrations for which Ti>/=Tz (Ti-Tz) positive or zero

[(Ti-Tz)/Tz] × 100 for concentrations for which Ti<Tz. (Ti-Tz) negative

The IC50 value is determine by graph pad prism software.

Table 3: % cell growth inhibition data on MCF-7 cell line.

Conc. (µg/ml)	Log Conc.	%Inhibition of Cell Growth					
		Fadrazole	a	b	c	k	l
0.05	-1.29	-27.26	-32.61	-29.06	-30.06	-28.39	-29.71
0.15	-0.82	-22.69	-28.02	-26.89	-28.32	-26.02	-27.45
0.46	-0.34	-16.88	-22.08	-23.33	-24	-21.36	-24.15
1.37	0.14	-10.8	-15.88	-18.18	-17.08	-13.19	-18.02
4.12	0.61	-4.89	-7.1	-11.24	-14.82	-9.08	-10.56
12.35	1.09	3.34	9.91	3.59	1.59	8.87	6.06
37.04	1.57	10.61	15.07	5.66	3.14	19.21	13.88
111.11	2.05	17.84	22.62	11.06	7.63	24.81	22.06
333.33	2.52	24.29	32.81	15.08	10.21	34.02	30.87
1000	3	32.02	46.29	21.22	17.87	49.88	41.96

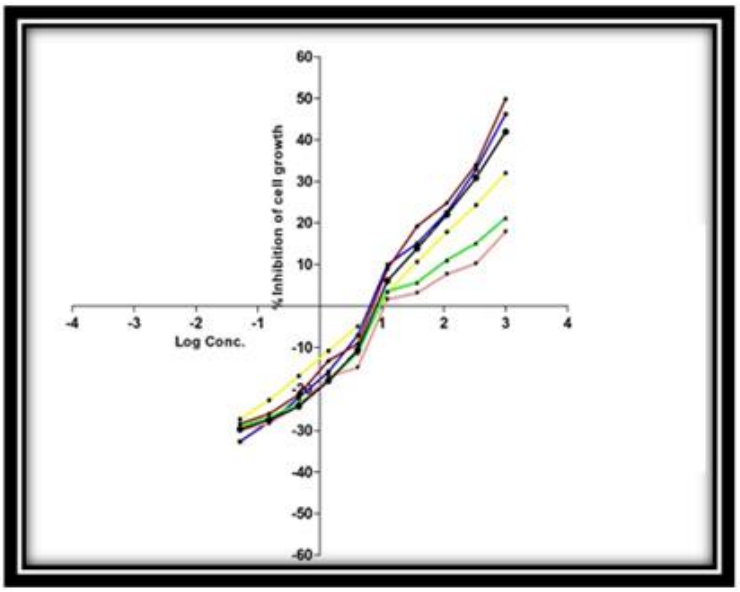

Figure 4: Line chart expressing anti-proliferative effect of synthesized novel compounds (a, b, c, k, l) and standard drug on MCF-7 cell line.

Note: : Std; : a; : b; : c; : k; : L

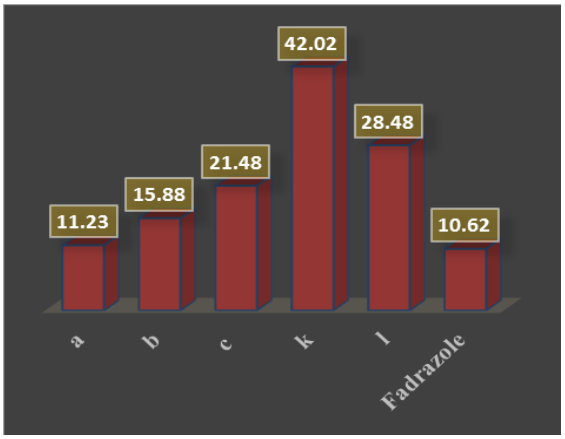

Figure 5: Graphical representation of comparison of cell growth inhibition (IC50) of synthesized compounds.

The IC50 values of standard drug (Fadrazole) and test compounds (a, b, c, k, l) are shown in following Table 4. From the above observation found that compounds (a) has highest anticancer activity whereas compound (b, c) has moderate activity as compared to standard [26].

Table 4: IC50 values.

Compound	IC50 µM
a	11.23
b	15.88
c	21.48
k	42.02
l	28.48
Fadrazole	10.62

All the derivatives were synthesized as per the designed scheme using 2, 4-dihydroxyacetophenone as starting material through Claisen-Schmidt condensation reaction after getting a docking score and gliding score [27]. Molecular docking was performed by Schrodinger software. All the designed compounds and standard drug fadrazole were docked against crystal structure of human placental aromatase inhibitors (PDB ID 3EQM) compound (b) has shown the highest docking score (-9.077) and glide score (-9.089) and compound (a, c, k) shown moderate docking score (-8.253, -8.060, -8.331) and glide score (-8.253, -8.060, -8.443) compare with standard drug Fadrazole having docking score (-7.564) and glide score (-7.725) respectively. All the compounds were synthesized with satisfied yield, and characterized by IR, MASS.

CONCLUSION

Anti-cancer activity of the entire compound was taken by SRB assay, at Advanced Centre for Treatment Research and Education in Cancer (ACTREC), Khargarh, Mumbai in collaboration with Dr. Arti S. Juvekar, Head, Screening department. In present work both docking studies and biological studies revealed that all the compounds (a, b, c, k and l) shows good hydrogen bond with 3EQM protein and also good anti-cancer activity when compared with standard fadrazole. From the analysis of IC50 values we may conclude that the compound having aliphatic amino substituted derivatives may exhibits potent anticancer activity. Thus, flavone derivatives may be utilized as promising anticancer agents in drug development process. Analysis of results of both docking studies and biological studies revealed that all the compounds (a, b, c, k and l) shows good hydrogen bond with 3EQM protein and also good anti-cancer activity when compared with standard Fadrazole. From the analysis of IC50 values we may conclude that the compound having aliphatic amino substituted derivatives may exhibits potent anticancer activity compare to aromatic amino substituted derivatives. Thus, flavone derivatives may be utilized as promising anticancer agents in drug development process.

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