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Molecular Docking, Synthesis & Biological Evaluation of Benzopyran Derivatives

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Abstract: *A series of 2-(3'-aminesubstituted)-7- hydroxyl-4H-1-benzopyran-4-one derivatives has been designed and synthesized through Claisen-Schmidt condensation reaction by condensation of 2,4-Dihydroxyacetophene (1.40 ml) and 3-Chlorobenzaldehyde (0.01 mole). After molecular simulation study of 25 benzopyran derivatives we select 10 molecular for synthesis having good docking score. Recent researches on Benzopyran derivatives received an added impulse with the discovery on Anticancer by several mechanisms, but the most important mechanism is the inhibition of aromatase generating enzyme.*

All the synthesized compounds was confirmed by their physicochemical properties and spectral studies. Novel benzopyran derivatives were assess for anticancer activity by using SRB assay on MCF-7 cell line. Novel benzopyran derivatives were performed to establish correlation between biological activity & molecular properties. Among the synthesized compounds (a, b, c, k, l) showed good anticancer activity; whereas compounds (t, v, w, k) shows good anti- inflammatory activity comparable to the reference drug Fadrazole and Celecoxib respectively. Thus, the conclusion can be made that the benzopyran moiety can exhibit a good anticancer as well as good anti-inflammatory activity..

Keywords: Benzopyran, Synthetic benzopyran derivatives, Claisen-Schmidt Condensation Reaction, Molecular docking, Anticancer

I. INTRODUCTION

Benzopyran derivatives, commonly referred to as flavonoids, are naturally occurring polyphenolic compounds abundantly present in fruits, vegetables, grains, tea, wine, and various medicinal plants. Structurally, they are classified into flavones, flavanols, isoflavones, flavonols, flavanones, and flavanonols, depending on the saturation and substitution pattern of the central pyran ring. Human dietary intake of flavonoids is estimated at several hundred milligrams per day, and their use in traditional medicine, particularly in Chinese herbal formulations, highlights their therapeutic relevance. Notably, flavones such as apigenin, luteolin, baicalein, and tangeretin exhibit diverse pharmacological properties including anticancer, anti-inflammatory, antimicrobial, antioxidant, gastroprotective, neuroprotective, and anti-malarial activities.

Due to structural and functional similarity with endogenous estrogens, flavonoids display potential in modulating estrogen receptor (ER)-dependent breast cancer. Aromatase, a key enzyme in estrogen biosynthesis, catalyzes the conversion of androgens (testosterone and androstenedione) into estrogens (estradiol and estrone). Overexpression of aromatase and subsequent elevation of estrogen levels contribute significantly to ER-positive breast cancer development and progression. Consequently, inhibition of aromatase represents an effective therapeutic strategy to reduce circulating and local estrogen levels in hormone-dependent tumors.

Non-steroidal aromatase inhibitors typically possess structural pharmacophores including hydrogen bond acceptors, hydrophobic spacers, and aromatic moieties, facilitating interactions with critical residues such as MET374 within the active site. Flavone derivatives, by virtue of their pharmacophoric features, are promising scaffolds for the design of novel aromatase inhibitors.

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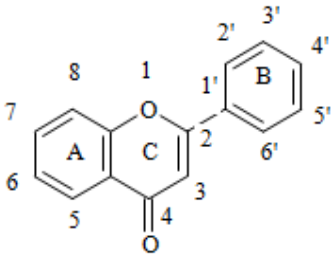
The present study employs molecular docking and molecular dynamics simulations (MDS) to investigate the binding interactions of novel flavone derivatives with aromatase at the atomic level. This approach provides mechanistic insights into ligand–protein interactions and supports the rational design of potent non-steroidal aromatase inhibitors with potential application in breast cancer therapy.

Flavones (flavus = yellow), are a class of flavonoids based on the backbone of 2- phenylchromen-4-one (2-phenyl-1-benzopyran-4-one). Naturally occurring flavones include Apigenin, Luteolin, Tangeritin, Baicalein, Scutellarein. Flavone shows wide variety of biological activities such as anti-cancer, anti-inflammatory, anti-microbial, Alzheimer's Disease, anti-malarial, antioxidant, gastro-protection and α1-adrenoceptor (α1-AR) antagonists. Due to their structural and functional similarities with endogenous estrogen, flavonoids have potential role in ER-dependent breast cancer.[24] From the structure activity relationship and pharmacophore pattern of aromatase inhibitors, it has been felt worthwhile to take up the present investigation involving hydroxy flavones with hope to achieve novel compounds as possible antineoplastic agents.



Aromatase enzyme is an essential in estrogen biosynthesis converting the aliphatic androgens testosterone and androstenedione to the aromatic estrogens 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 widely distributed 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 Anti-inflammatory[6,], Antioxidant[7], Anticancer[8,9] and Antimicrobial activity.[10-13] Hence, flavonoids are considered as an essential 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 **Figures 1** which is necessary for binding to aromatase enzyme and their by its inhibition. In these study, non-steroidal aromatase inhibitors[14] possess aromatic/aliphatic amines at side chain for suitable position acts as H bond acceptor which bound with MET374present in active site of aromatase enzyme. Basic nucleus plays role as hydrophobic spacer moiety which maintained distance between Heme coordinating group and hydrogen bond acceptor moiety.[15]

In organic chemistry several methods exist for the synthesis of benzopyran derivatives:

- Allan–Robinson reaction
- Auwers synthesis
- Baker–Venkataraman rearrangement
- Algar–Flynn–Oyamada reaction

Flavone can be synthesized by dehydrative cyclization of certain 1, 3-diaryl diketones.[10]

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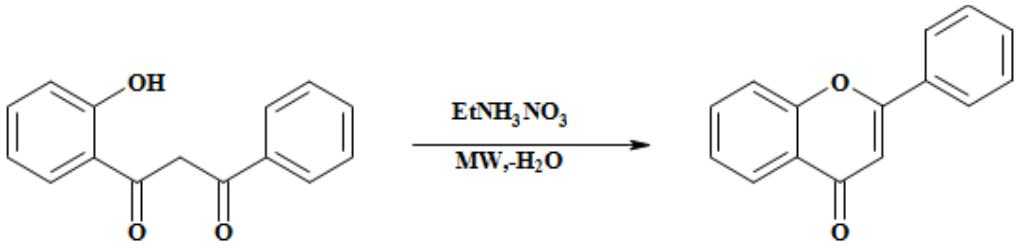
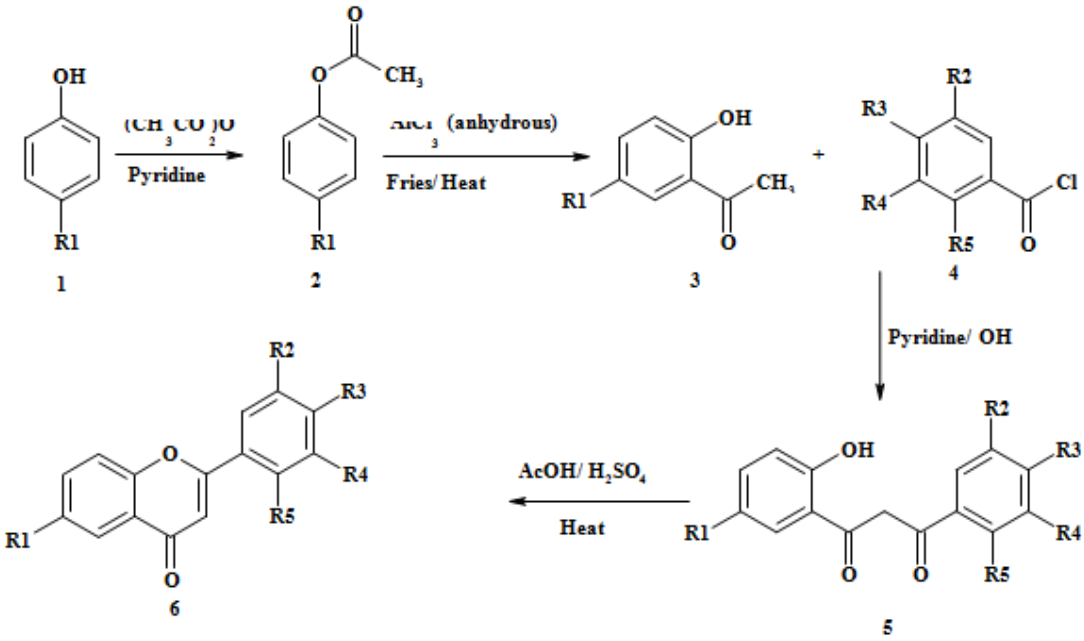


Such types of flavones are synthesized by using ionic liquid and microwave irradiation method.

Baker–Venkataraman rearrangement



Scheme I

Flavone were synthesized using the Baker–Venkataraman rearrangement.[2,] as shown in Scheme I. Acetylation of substituted/unsubstituted phenols (1) was carried out in the presence of acetic anhydride to yield intermediates (2) which on refluxing in the presence of anhydrous aluminum chloride underwent Fries rearrangement to afford (3) in good yields (80%).

Substituted/unsubstituted 2-hydroxyacetophenones (3) were further allowed to react with substituted/unsubstituted benzoyl chlorides in the presence of anhydrous pyridine, and subsequent heating the mixture in the presence of KOH yielded substituted/unsubstituted-2- hydroxydibenzoyl methane (5). Intermediates (5) were further cyclized in the presence of glacial acetic acid and conc. H_2SO_4 at 100^0C to afford the desired flavone (6) again in good yield 80%.

II. MATERIAL AND METHOD

All chemicals were obtained from S.D. Fine chemicals, Loba chemicals and Merck Pvt. Chemicals and solvents were purified by general laboratory techniques before use. All moisture free operations were performed in oven dried glassware's and under nitrogen atmosphere. Melting points were determined by using open capillary tube method and are uncorrected. Ultraviolet spectra were obtained on Shimadzu 1700 UV-Visible spectrophotometer using methanol as a solvent. IR spectra (wave numbers in cm^{-1}) were recorded on a Shimadzu FT- IR spectrophotometer using potassium bromide discs. NMR spectra were recorded on BRUKER AVANCE II 400 MHz instrument in DMSO with TMS as

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internal standard for ^{1}H- NMR. Chemical shift values are mentioned in δ ppm. Chromatographic separations were performed on columns using silica gel 100-200 mesh. The progress of all the reactions were monitored by TLC on 2 cm x 5 cm pre-coated silica gel 60 F254 (Merck) plates of thickness of
0.25 mm. The chromatograms were visualized under UV 200-400 nm and/or exposure to iodine vapours.

III. EXPERIMENTAL WORK & RESULTS

The molecular docking study of amino substituted novel flavone derivatives was carried out using Maestro 11.5 Schrodinger software .The ligands were docked on the aromatase enzyme with PDB ID **(3EQM)** taken from the Protein Data Bank (www.rscb.org).
Following steps are taken in to consideration for molecular docking study

- Ligand preparation
- Protein preparation and its refinement
- Receptor grid generation
- Protein ligand docking

Ligand preparation

The Schrödinger ligand preparation was done by using LigPrep panel application which consists of series of steps that perform conversion of 2D structures to 3D structure, apply correction to the structure by minimizing the proper bond angles and distances and optimize the structure by minimizing its energy through force-field OPLS3.

Protein Preparation and its Refinement

The crystal structure of human placental microsomal aromatase with its bound natural substrate androstenedione was taken from the Protein Data Bank (PDB ID: 3EQM) for protein preparation. The multistep Schrodinger's protein preparation wizard tool (PPrep) has been used for protein preparation, which was minimized using OPLS-3 force field with polack-ribiere conjugate gradient (PRCG) algorithm. As protein is the essential component for molecular docking study it is necessary to minimize the energy of protein molecule prior to docking studies with ligands. These protein for ligand docking study was prepared by using protein preparation wizard tool in which was used to import proteins for the protein data bank (PDB). Proteins obtained from the PDB, vendors and other sources frequently have missing hydrogen, partial charges, side chain and whole loops region. So, to overcome all these barriers in docking study the proteins to undergone through pre-processing and it was done by selecting following parameters,

- Add hydrogen
- Create zero order bonds to metals
- Create disulfide bonds
- Filling missing side chains using prime
- Fill in missing loops using prime
- Delete water beyond 5.00 Å From het group
- Generate het state using Epik: PH 7.0+/- 2.0

Receptor grid generation

Grid generation must be performed in order to run a virtual screen with glide. The shape and properties of the receptor are represented in a grid by field that provides progressively more accurate scoring of the ligand poses. For receptors that adopt more than one conformation on binding, Glide prepares grids for each conformation, to ensure that possible actives are not missed
To open the Receptor Grid Generation panel, Receptor Grid Generation sub-menu of Glide was selected from the Application menu. The Receptor Grid Generation panel has three tabs, which are used to specify settings for the receptor grid generation job.

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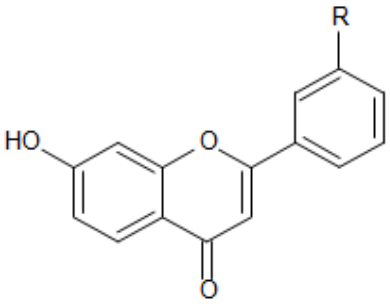
Protein ligand docking

For the ligand protein molecular docking twenty six **(a-z)** amino substituted flavones derivative were designed and docked on of aromatase enzyme with **(PDB: 3EQM)**.

The ligand docking process helps to predict ligand conformation and orientation (posing) within a targeted binding site and thus helps to interpret interactions of ligand atoms with amino acids of proteins, and to understand the binding affinity. The ligand-protein docking was carried out in the extra precision (XP) mode.[17-22]

Table 1. Compound consider for Molecular docking

Compound consider for molecular docking



Compound Code	-R	Compound Code	-R
a	Methylamine	**n**	Anilineamine
b	Ethylamine	**o**	Benzalamine
c	n-propylamine	**p**	*o*-chloroaniline
d	iso-propylamine	**q**	*o*-nitroaniline
e	n-butylamine	**r**	3,4-dimethylaniline
f	iso-butylamine	**s**	*p*-bromoaniline
g	n- pentylamine	**t**	*p*-methylaniline
h	iso-pentylamine	**u**	*p*-aminophenol
i	neo-pentylamine	**v**	*p*-chloroaniline
j	di-methylamine	**w**	*p*-nitroaniline
k	di-ethylamine	**x**	*2-chloro,4-bromoaniline*
l	Ethylmethylamine	**y**	*4-methoxyaniline*
m	Ethylpropylamine	**z**	*Fadrazole (standard)*

Table 2. Compound show Docking Score, Glide score and number of H-bonds for the novel derivatives and Fadrazole

Sr. No.	Code	-R	Docking Score (Kcal/mole)	Glide Score (Kcal/mole)	H-Bonds	Amino acids involved in interaction with ligands
1	a	Methylamine	-6.757	-6.770	1	MET 374, ARG 115
2	b	Ethylamine	-7.173	-7.186	1	ARG 115, MET 374
3	c	n-propylamine	**-8.286**	**-8.286**	1	MET 374, ARG 115
4	d	iso-propylamine	-6.827	-6.850	2	ASH 309, HEM 600, ARG 115, LEU 477
5	e	n-butylamine	-7.200	-7.213	1	ASH 309, PHE 134, ARG 115
6	f	iso-butylamine	**-7.923**	**-7.943**	2	ARG 115, ASH 309,

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						HEM 600, LEU 477
7	g	n- pentylamine	-7.498	-7.511	1	MET 374, ARG 115
8	h	iso-pentylamine	**-8.056**	**-8.078**	2	ALA 306, ARG 115, LEU 477
9	i	neo-pentylamine	-6.419	-6.431	1	LEU 477
10	j	di-methylamine	-6.819	-6.832	0	---
11	k	di-ethylamine	**-8.000**	**-8.112**	1	ARG 115, MET 374
12	l	Ethylmethylamine	-6.491	-6.511	1	MET 374, HEM 600
13	m	Ethylpropylamine	-6.096	-6.223	0	---
14	n	Anilineamine	-7.748	-7.761	1	LEU 372, ARG 115, TRP 224
15	o	Benzalamine	-5.817	-5.830	1	MET 374
16	p	*o*-chloroaniline	-5.739	-5.752	1	LEU 477, ARG 192, HIE 480
17	q	*o*-nitroaniline	-5.316	-5.329	0	PHE 221, TRP 224, ARG 115, HEM 600
18	r	3,4-dimethylaniline	-6.391	-6.404	1	LEU 477, ARG 192, HIE 480
19	s	*p*-bromoaniline	-6.524	-6.537	0	TRP 224, HIE 480, ARG 192
20	t	*p-methylaniline*	-6.658	-6.671	1	ARG 192, HIE 480, TRP 224, LEU 477
21	u	*p-aminophenol*	-7.168	-7.181	1	LEU 477, ARG 192, HIE 480
22	v	*p-chloroaniline*	-4.464	-6.476	0	ARG 192, HIE 480, TRP 224
23	w	*p-nitroaniline*	-7.684	-7.696	1	GLU 480, LEU 477, HIE 480, ARG 192, ASP 222
24	x	*2-chloro,4-bromoaniline*	-2.871	-2.884	1	---
25	y	*4-methoxyaniline*	-5.152	-5.165	1	LEU 477
26	z	*Fadrazole (standard)*	-7.564	-7.725	1	HEM 600, MET 374

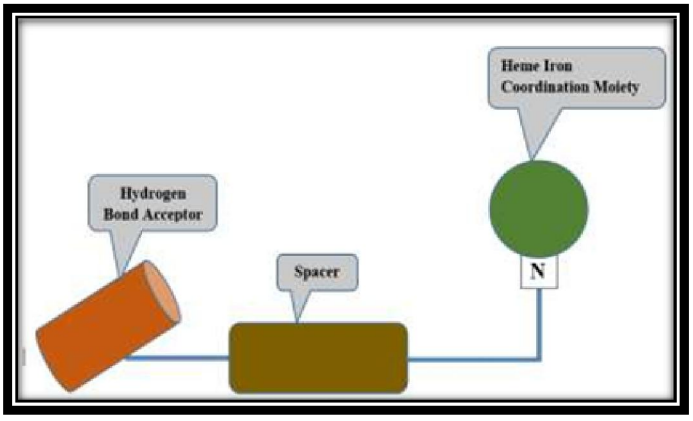

Figure 1. Pharmacophore pattern of molecule

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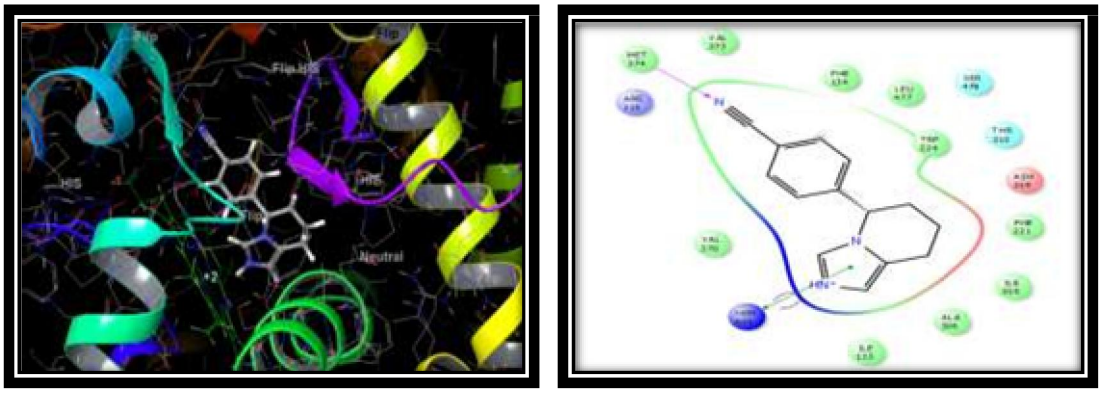


Figure 2. Ribbon structure and Amino acid interaction of 3EQM with Fadrazole

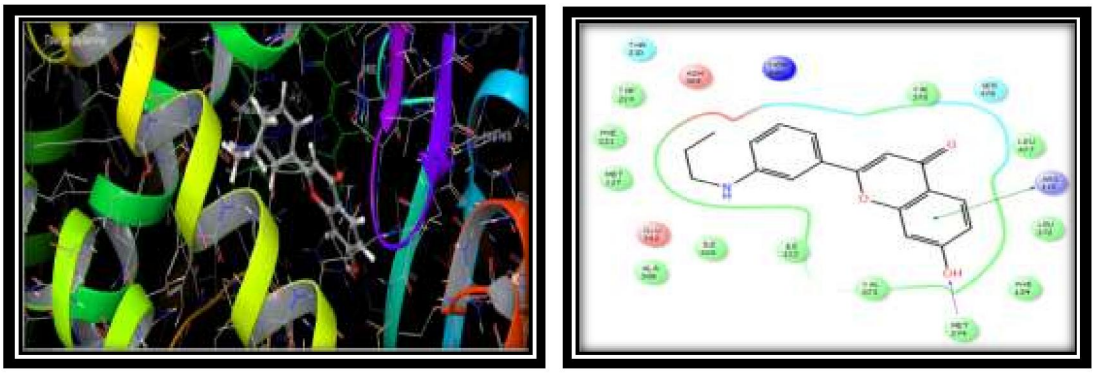
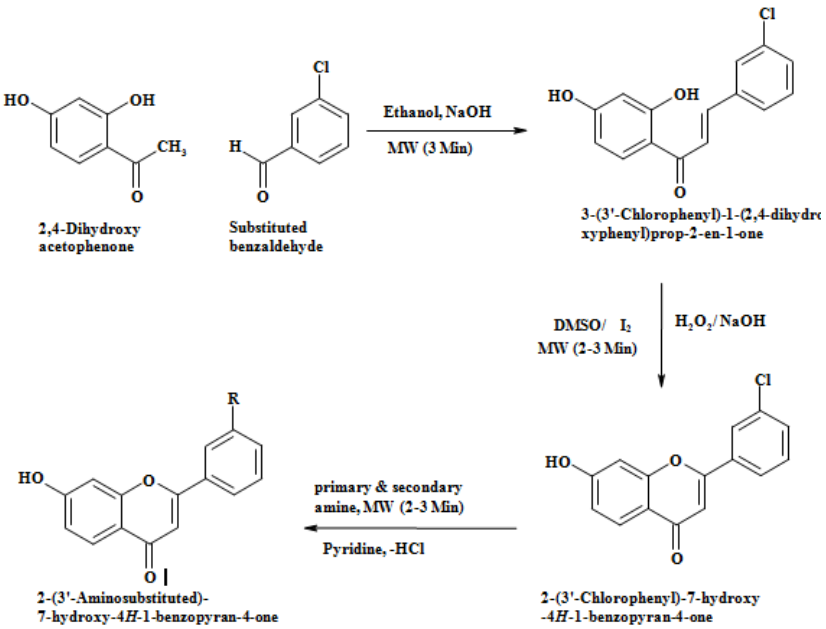


Figure 3. Ribbon structure and Amino acid interaction of 3EQM with (c)

SYNTHETIC WORK



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Derivatization of Flavone

Table 3: Novel Benzopyran Derivatives.

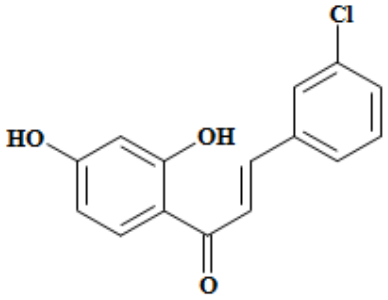
Sr. No.	Compound	-R/Ar
1	a	Methylamino
2	b	Ethylamino
3	c	Propylamino
4	k	Diethylamino
5	l	Ethylmethylamino
6	t	p-Methylaniline, ,
7	v	p-Chloroaniline
8	w	p-Nitroaniline

Synthetic procedure

Pharmacophore Synthesis

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

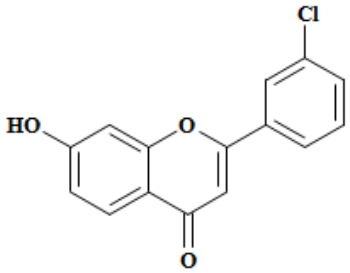
Accurately weighed 1.52 g (0.01 mole) of 2,4-dihydroxyacetophenone and 1.40 ml (0.01mole) of 3-Chlorobenzaldehyde was taken in 250 ml Round Bottom Flask (R.B.F.) and dissolved in ethanol (5 ml) then add 10 % NaOH (5 ml) solution with stirring, by keeping the flask in ice bath, then resulting mixture 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. Crude chalcone was dried and then recrystallized from methanol



Pharmacophore Cyclization

Preparation of 2-(3'-Chlorophenyl)-7- hydroxyl-4*H*-1-benzopyran-4-one:

3-(3'-chlorophenyl)-1-(2,4-dihydroxyphenyl)-prop-2-en-1-one was dissolved in 5ml 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



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Derivatization of Novel Benzopyran Derivatives

Preparation of 7-Hydroxy-2-[3-(methylamino)-phenyl]-4*H*-1-benzopyran-4-one: (a)

Accurately weighed 2.72 g (0.01 mole) of 2-(3'-Chlorophenyl)-7- hydroxyl-4*H*-1-benzopyran- 4-one and 0.31 g (0.01 mole) of amine derivatives were taken in 250 ml of RBF. In above mixture pyridine (5 ml) was added 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).

Table 4: Physicochemical data of synthesized compounds.

Sr. No.	Code	Molecular Formula	Mol. Weight (g/mol)	Yield %	Melting Point o(C)	Rf- Value	U.V. λmax (nm)
1	a	$C_{16}H_{13}NO_3$	267	63.12	178-180	0.6	241.60
2	b	$C_{17}H_{14}NO_3$	280	72.00	184-185	0.82	309.20
3	c	$C_{18}H_{17}NO_3$	295	57.86	187-190	0.84	297.00
4	k	$C_{19}H_{19}NO_3$	309	66.21	179-182	0.62	298.00
5	l	$C_{18}H_{17}NO_3$	295	59.09	181-183	0.47	319.00
6	t	$C_{22}H_{18}NO_3$	365	54.25	190-195	0.7	340.00
7	v	$C_{21}H_{15}NO_3CI$	375	53.24	192-196	0.36	290.00
8	w	$C_{21}H_{15}N_2O_5$	344	48.16	190-194	0.6	318.00

Table 5: The representative IR spectra of synthesized compounds.

Sr. No.	Compound code	IR (cm^{-1})
1	a	1151.68 cm^{-1}(C-O-C str. of ether), 1741.49 cm^{-1}(-C=O str. of ketone), 1583.64 cm^{-1}(Ar C=C str.), 3365 cm^{-1}(-NH-) and 766.50 cm^{-1}(monosubstituted benzene)
2	b	1149.41 cm^{-1}(C-O-C str. of ether), 1649.50 cm^{-1}(-C=O str. of ketone), 1402.10 cm^{-1}(Ar C=C str.), 3372 cm^{-1}(-NH-) and 705.61 cm^{-1}(monosubstituted benzene)
3	c	1140.44 cm^{-1}(C-O-C str. of ether), 1630.96 cm^{-1}(-C=O str. of ketone), 1542.80 cm^{-1}(Ar C=C str.), 3255.50 cm^{-1}(-NH-) and 712.45 cm^{-1}(monosubstituted benzene)
4	k	1145.18 cm^{-1}(C-O-C str. of ether), 1741.49 cm^{-1}(-C=O str. of ketone), 1480.80 cm^{-1}(Ar C=C str.), 3227 cm^{-1}(-NH-) and 727.15 cm^{-1}(monosubstituted benzene)
5	l	1125.18 cm^{-1}(C-O-C str. of ether), 1631.49 cm^{-1}(-C=O str. of ketone), 1427.80 cm^{-1}(Ar C=C str.), 3286.70 cm^{-1}(-NH-) and 703.15 cm^{-1}(monosubstituted benzene)
6	t	1216.18 cm^{-1}(C-O-C str. of ether), 1601.49 cm^{-1}(-C=O str. of ketone), 1470.80 cm^{-1}(Ar C=C str.), 3307 cm^{-1}(-NH-) and 724.15 cm^{-1}(monosubstituted benzene)
7	v	1135.18 cm^{-1}(C-O-C str. of ether), 1752.49 cm^{-1}(-C=O str. of ketone), 1520.80 cm^{-1}(Ar C=C str.), 3312 cm^{-1}(-NH-) and 703.64 cm^{-1}(monosubstituted benzene)
8	w	1133.18 cm^{-1}(C-O-C str. of ether), 1721.25 cm^{-1}(-C=O str. of ketone), 1472.80 cm^{-1}(Ar C=C str.), 3243 cm^{-1}(-NH-) and 727.15 cm^{-1}(monosubstituted benzene)

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Table 6: The representative ^{1}H- NMR spectra of synthesized compounds.

Compound code	^{1}H- NMR (δ ppm)
a	2.60 (3H, s), 6.55 (1H, s), 6.59 (1H, dd), 6.84 (1H, dd), 7.19 (1H, ddd), 7.31 (1H, td), 7.38 (1H, ddd), 7.59 (1H, ddd), 8.11 (1H, dd).
b	1.16 (3H, t), 3.04 (2H, q), 6.55 (1H, s), 6.59 (1H, dd), 6.84 (1H, dd), 7.20 (1H, dt), 7.31 (1H, td), 7.38 (1H, ddd), 7.60 (1H, ddd), 8.11 (1H, dd).
c	0.89 (3H, t), 1.59 (2H, tq), 3.10 (2H, t), 6.55 (1H, s), 6.84 (1H, dd), 7.20 (1H, dt), 7.31 (1H, td), 7.38 (1H, ddd), 7.59 (1H, ddd), 8.11 (1H, dd).
k	1.04 (6H, t), 3.17 (4H, q), 6.55 (1H, s), 6.59 (1H, dd), 6.84 (1H, dd), 7.07 (1H, ddd), 7.26-7.36 (2H, ddd), 7.31 (tt), 7.59 (1H, ddd), 8.11 (1H, dd).
t	2.21 (3H, s), 6.27 (2H, ddd), 6.55 (1H, s), 6.59 (1H, dd), 6.84 (1H, dd), 7.06 (2H, ddd), 7.26-7.39 (3H, ddd), 7.34 (ddd), 7.45 (1H, ddd), 8.11 (1H, dd).
v	6.55 (1H, s), 6.59 (1H, dd), 6.84 (1H, dd), 7.27-7.39 (5H, ddd), 7.34 (ddd), 7.32 (ddd), 7.28 (1ddd), 7.42-7.49 (3H, ddd), 7.45 (ddd), 8.11 (1H, dd).
w	6.55 (1H, s), 6.59 (1H, dd), 6.84 (1H, dd), 7.27-7.39 (5H, ddd), 7.34 (ddd), 7.32 (ddd), 7.28 (1ddd), 7.42-7.49 (3H, ddd), 7.45 (ddd), 8.11 (1H, dd).

Anticancer activity (Aromatase inhibitor) Sulforhodamine-B (SRB) Assay

Table 7: % 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	-24.00	-21.36	-24.15
1.37	0.14	-10.80	-15.88	-18.18	-17.08	-13.19	-18.02
4.12	0.61	-4.89	-07.1	-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
1000.00	3.00	32.02	46.29	21.22	17.87	49.88	41.96

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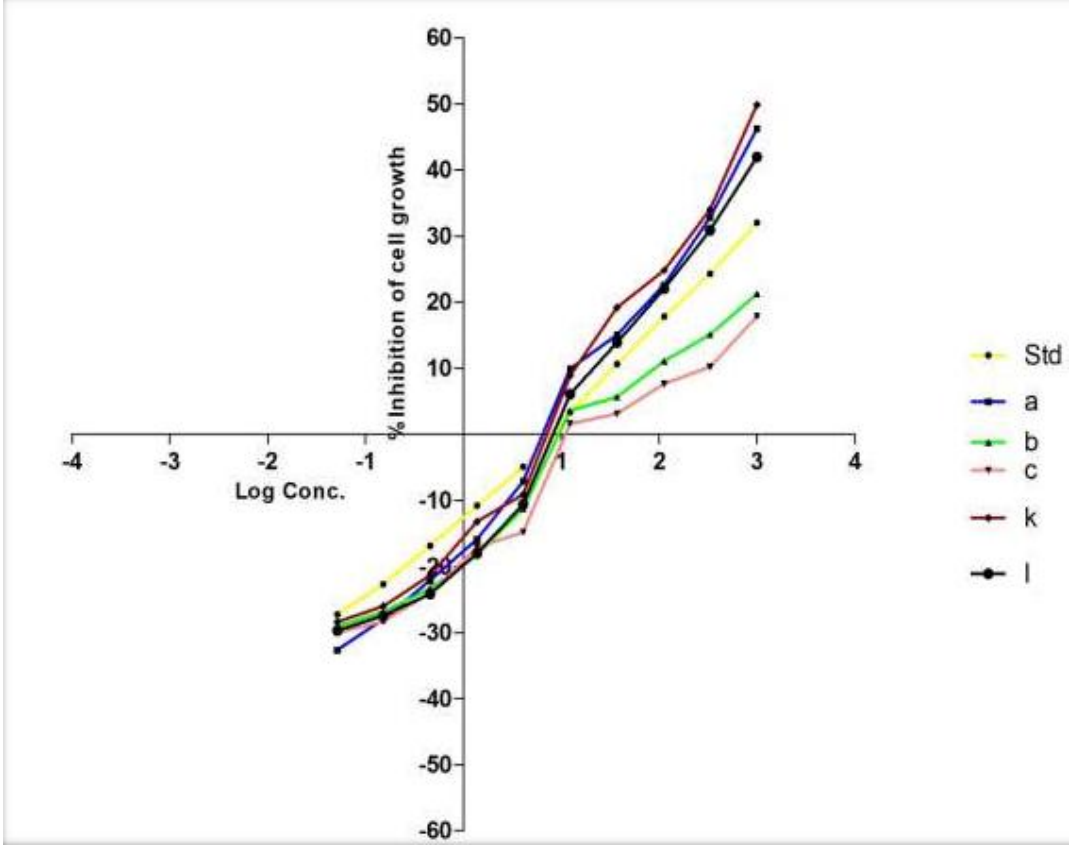

Fig. 4: Line chart expressing anti-proliferative effect of synthesized novel compounds (a, b, c, k, l) and Standard drug on MCF-7 cell line.

The IC_{50} values of standard drug (Fadrazole) and test compounds (a, b, c, k, l) are shown in following table.

Table 8: IC_{50} values

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

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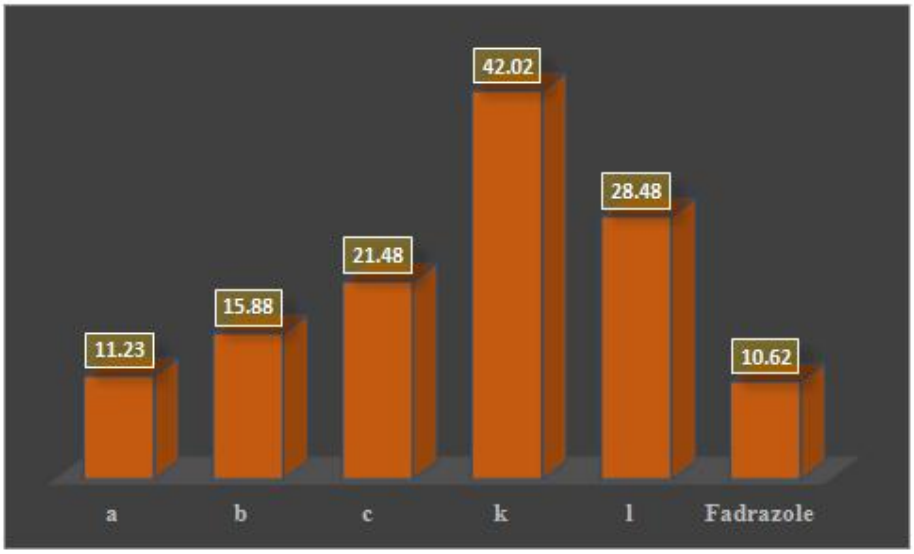

Fig. 5: Graphical representation of comparison of cell growth inhibition (IC_{50}) of synthesized compounds.

From the above observation found that compounds (a) has highest anticancer activity where as compound (b, c) has moderate activity as compared to standard.

IV. CONCLUSION

The compounds **a, b, c, k, l, t, v, w** have been synthesized as per scheme. The 2,4- dihydroxyacetophenone was reacts with 3-chlorobenzaldehyde in presence of ethanol and NaOH via Claisen-schimdt condensation to form 3-(3′-chlorophenyl)-1-(2,4- dihydroxyphenyl)-prop-2-en-1-one after cyclized by DMSO/I2 or H_2O_2/ NaOH to form 2- (3′-chlorophenyl)-7- hydroxyl-4*H*-1-benzopyran-4-one. In last step, various primary and secondary amine reaction with 2-(3′-chlorophenyl)-7- hydroxyl-4*H*-1-benzopyran-4-one to achieve target derivatives. The compounds thus obtained were purified and characterized by their physical and spectral interpretation. Anti-inflammatory and anti-cancer activity of all synthesized compound was evaluated for *in-vitro* and *in-vivo* measurement by respective methods.

All the synthesized compounds were characterized by physicochemical parameters and spectral interpretations such as UV, FT-IR and ^{1}H-NMR spectroscopy. All synthesized compounds were evaluated for anti-inflammatory activity by Inhibition of protein (albumin) denaturation as well as carrageenan induced rat paw edema method and anti-cancer activity by using MCF-7 cell line through SRB assay.

The result illustrates that, The compound also shows good hydrogen bonding and glide score. The compounds substituted with p-methylaniline (v), p-nitroaniline (w), diethylamine (k) and ethylmethylamine (l) showed moderate activity, compound (a, b, c) which shows less glide score and hydrogen bonding compared to standard celecoxib. The above result indicates that compound which are having good hydrogen bonding and glide score with protein molecule may act as strong anti-inflammatory agent, and can be take in to consideration as a good candidature for further study. The above result also indicates that compound which are aromatic amine substituted flavone having good anti-inflammatory activity as compare to aliphatic substituted amine.

Anticancer activity (Aromatase inhibitors) using MCF-7 cell line through SRB assay

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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