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Synthesis, Molecular Modeling and Biological Evaluation of Novel 4H-Chromene Derivatives as Potential Cytotoxic Agents

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Sathish Kumar Mittapalli*[1], Samreen Sultana, Sanaf Summaiya, Shaik Abdul Subhan, Mohd. Moosa[2]

[1]Supervisor, Associate Professor, Department of Pharmaceutical Chemistry, Deccan School of Pharmacy, Nampally, Hydearabad-500001 Telangana, India

[2]Research Scholar, Department of Pharmaceutical Chemistry, Deccan School of Pharmacy, Nampally, Hydearabad-500001 Telangana, India

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ABSTRACT

Coumarins are organic compounds that have been associated with beneficial effects on human health, such as anticancer, antidiabetics and cardiovascular diseases. These are proven as radical scavenging effect, due to their antioxidant activities. Coumarins are an entity which is being constructed and synthesized in many ways and the moeity is major source of interest for many of medicinal chemist to explore its various pharmacological potentials especially anticoagulant activity. Here, the research has been attempted designing the various chromene analogs and screening through various computational approaches like molecular modeling and molinspiration software is used by hundreds of cheminformatics experts in industry and academia to produce high-quality scientific results we review recent derivatives of coumarin that are synthesized with their pharmacological activities like antioxidant and anticancer activities. We employed the MTT assay to test the cytotoxicity of synthesized compounds against MCF-7(breast carcinoma) and HeLa (cervical carcinoma) cell lines. The anticancer study found that the compound 7 and 6 Series has a significant potential to destroy tumor cells. De novo modeling of novel chemicals to treat cancer is aided by molecular modelling research. Overall, the study found that the majority of the chemicals synthesised, particularly those in the Chromene Series with the electron-donating system at para position like amino, and hydroxyl group at ethylene Bridge had the potential to be cytotoxic. In the future, I'd want to focus on the respective prospective molecular series in terms of developing a big compound library and doing QSAR studies.

INTRODUCTION:

Antioxidants in food play an important role as a health-protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Plant-sourced food antioxidants like vitamin C, vitamin E, carotenes, phenolic acids, phytate and phytoestrogens have been recognized as having the potential to reduce disease risk. Most of the antioxidant compounds in a typical diet are derived from plant sources and belongs to various classes of compounds with a wide variety of physical and chemical properties. Some compounds, such as gallates have strong antioxidant activity, while others, such as the mono-phenols are weak antioxidants. Coumarins represent one of the most active classes of compounds possessing a wide spectrum of biological activity. (1,2)

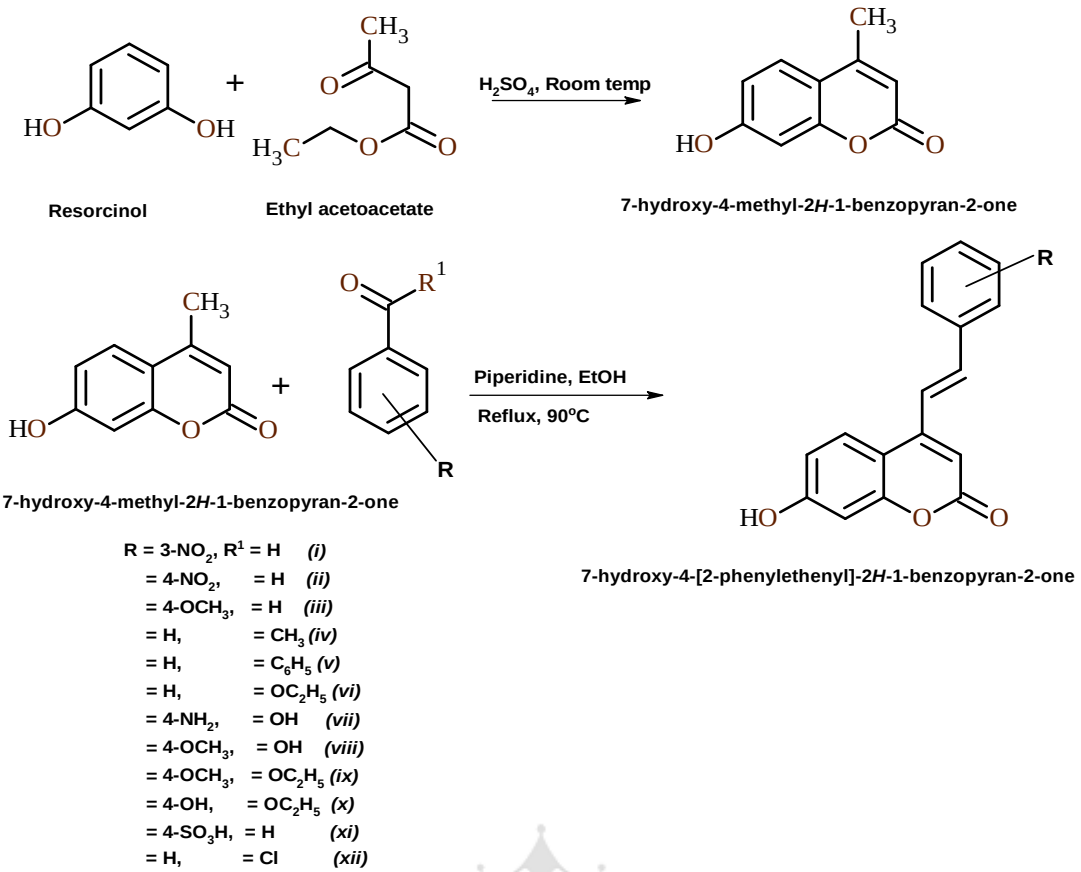
Many of these compounds have proven to be active as antibacterial,(3,4) antifungal,(5) anti-inflammatory,(6) anticoagulant,(7–9) anti-Viral(10) and anticancer agents(11–14). Coumarins are widely used as additives in food, perfumes, cosmetics,(15) pharmaceuticals and optical brighteners(16) and would dispersed fluorescent and laser dyes.(17) Coumarins also have the super thermal stability and outstanding optical properties including extended spectral response, high quantum yields and superior photo stability. Optical applications of these compounds, such as laser dyes, nonlinear optical chromophores, fluorescent whiteners, fluorescent probes, polymer science, optical recording and solar energy collectors have been widely investigated.(18,19) Classical routes to coumarins incorporate Pechmann, Knoevenagel, Perkin, Reformat sky, and Wittig condensation reactions.(20–22)

Antioxidant Activity Singh OM et al. developed a facile, convenient and high-yielding synthesis of a combinatorial library of 3-alkanoyl/aroyl/heteroaryl-2Hchromene-2-thiones (23). The assessment of radical scavenging capacity of the compounds towards the stable free radical 2,2-diphenyl-1- picrylhydrazyl (DPPH) was measured and these compounds were found to scavenge DPPH free radicals efficiently. The newly synthesized compounds exhibited profound antioxidant activity. Five selected compounds were able to protect curcumin from the attack of sulfur-free radical generated by radiolysis of glutathione (GSH).(24)Breast cancer is one of the most common types of malignancies in women worldwide. Breast carcinogenesis is unrecognized because of a variety of risk factors in context to bio-molecular dynamics. The risk of breast cancer has increased since the past 50

years and accounts for 23% of all cancer deaths in Asia according to the statistical reports of WHO.(25)

Docking studies of antioxidant activity Before the simulations, all bound ligands, cofactors, and water molecules were removed from the proteins. The macromolecule was checked for polar hydrogen, and torsion bonds of the inhibitors were selected and defined. Gasteiger charges were computed, and the Auto Dock atom types were defined using Auto Dock version 4.2, the graphical user interface of Auto Dock supplied by MGL Tools. The Lamarckian genetic algorithm (LGA), which is considered one of the best docking methods available in Auto Dock, was employed. (26,27)

Finally, Auto Dock was used to calculate the binding free energy of a given inhibitor in the macromolecular structure. Finally, Auto Dock was used to calculating the binding free energy of a given inhibitor conformation in the macromolecular structure. It evaluates how small molecule (substrate, inhibitor, drug or drug candidate) and the target macromolecule (receptor, enzyme or nucleic acid) fit together. This can be useful for developing better drug candidates and also for understanding the nature of the binding. Therefore molecular docking studies were carried out in order to explain in silico antioxidant studies, a specific protein tyrosine kinase (2HCK) and peroxiredoxin 5 (1HD2) were identified as the target for antioxidant compounds. Their PDB file was obtained from the protein data bank and used after removal of all bound water, ligands and cofactors. To investigate the ability for an anti-oxidant agent, the molecular docking was first conducted with α-Tocopherol used as reference ligand. (28)



SCHEME 1: Synthetic Scheme for the synthesis of 4H-Chromene Derivatives

The ultimate aim and objective of the research is that design some novel chromene derivatives and screen of the designed molecules through the online *in silico* tools and synthesis of the selected molecules and characterization by Spectral methods like FT-IR, ^{1}HNMR, ^{13}CNMR Techniques and evaluation for a biological activity like antioxidant activities by DPPH method, Anticancer activities by utilizing Cell lines.

Table-1: Molecular Docking studies with different biochemical targets in the carcinogenesis

S.No	Compound Code	Tyrosine Kinase (2HCK)	Peroxiredoxin-5 (1HD2)
1	7-hydroxy-4-[2-(3-nitrophenyl)ethenyl]-2*H*-1-benzopyran-2-one**(compd 1)**	-8.0	-6.3
2	7-hydroxy-4-[2-(4-nitrophenyl)ethenyl]-2*H*-1-	-7.4	-5.3

	benzopyran-2-one**(compd 2)**		
3	7-hydroxy-4-[2-(4-methoxy-phenyl)ethenyl]-2*H*-1-benzopyran-2-one**(compd 3)**	-9.4	-7.6
4	4-[2-(4-aminophenyl)-2-hydroxyethenyl]-7-hydroxy-2*H*-1-benzopyran-2-one**(compd 4)**	-7.6	-8.3
5	7-hydroxy-4-[2-phenoxy-2-phenylethenyl]-2*H*-1-benzopyran-2-one**(compd 5)**	-8.4	-8.4
6	4-[2-ethoxy-2-phenylethenyl]-7-hydroxy-2*H*-1-benzopyran-2-one**(compd 6)**	-8.2	-8.8
7	4-[2-(4-aminophenyl)-2-hydroxyethenyl]-7-hydroxy-2*H*-1-benzopyran-2-one**(compd 7)**	-8.1	-9.7
8	7-hydroxy-4-[2-hydroxy-2-(4-methoxyphenyl)ethenyl]-2*H*-1-benzopyran-2-one**(compd 8)**	-8.3	-8.2
9	4-[2-ethoxy-2-(4-methoxyphenyl)ethenyl]-7-hydroxy-2*H*-1-benzopyran-2-one**(compd 9)**	-9.3	-8.7
10	7-hydroxy-4-[2-(4-hydroxyphenyl)-2-phenoxyethenyl]-2*H*-1-benzopyran-2-one**(compd 10)**	-6.3	-6.3
11	4-[(2-(7-hydroxy-2-oxo-2*H*-1-benzopyran-4-yl)ethenyl]benzene-1-sulfonic acid**(compd 11)**	-5.2	-5.3
12	4-[2-chloro-2-phenylethenyl]-7-hydroxy-2*H*-1-benzopyran-2-one**(compd 12)**	-6.9	-7.2

From the molecular docking simulations and in silico screening parameters, some of the molecules were selected based on the binding score. The selected compounds like were synthesized and evaluated for antioxidant and anticancer activity studies (Table 1).

MATERIALS AND METHODS:

Synthetic part

In the present work all synthetic reactions were monitored by TLC. All the synthesized compounds were characterized by analytical and spectroscopic methods. Melting points were determined on Vecego melting point apparatus, model no-MPI by open capillary method and are uncorrected. The FTIR spectra were recorded on Jasco FTIR instrument model no-5300, using KBr pellets. ^{1}HNMR spectra (Table 2) and ^{13}CNMR spectra (Table 3) were recorded on BRUKER AVANCE II 400 NMR spectrometer at 400 MHz, for which $CDCl_3$ was used as solvent and TMS as internal standard.

Synthesis of 7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one by a conventional method with conc. Sulphuric acid as catalyst:

A solution of 1.1 gram of resorcinol and 1.2 gram of EAA was added drop wise with stirring to 10 ml of conc.H_2SO_4. So that the temperature of reaction mixture did not raise above the 10°C the reaction on complete addition mixture was kept at ambient temperature for 18 hours and then poured with vigorous stirring to reaction mixture of ice and water. The precipitate was filtered off and washes with cold water then dried under reduced pressure offering the crude solid mass. On recrystallized from aq. Alcohol gives final compound.

Synthesis of 7-hydroxy-4-[2-phenylethenyl]-2*H*-1-benzopyran-2-one:

A mixture of 7-Hydroxy-4-methyl-coumarin (1eq.) and corresponding Substituted Aromatic aldehydes and ketones (acetophenone, benzophenone, ethyl benzoate and p-amino benzoic acid)(1.2eq.) in EtOH was stirred with a few drops of piperidine under reflux during 4-8 hours. Mixture was cooled and the resulting solid was filtered and purified by recrystallization. Purification of compounds was made by recrystallization in MeOH.

Spectral Data of the synthesized compounds

4-[2-(4-aminophenyl)-2-hydroxyethenyl]-7-hydroxy-2*H*-1-benzopyran-2-one (compd 4)

MP: 242°C with 75% yield.IR (KBr, cm^{-1}) ῡ: 2925 (stretch, C-H, aromatic), 1698(stretch, C=O), 917, 844, 778, 659; ^{1}H NMR (300MHz, DMSO-d6): δ (ppm)= 2.02 (3H, s), 5.88 (1H, s), 6.59-6.72 (2H, 6.65 (d, *J* = 8.1, 2.1 Hz), 6.65 (d, *J* = 2.1, 0.4 Hz), 7.09 (1H, s, *J* = 7.9, 1.4, 1.2, 0.5 Hz), 7.36-7.50 (3H, 7.43 (d, *J* = 7.9, 7.2, 2.0, 0.5 Hz), 7.43 (t, *J* = 7.2, 1.4 Hz)), 8.09 (1H, d, *J* = 8.1, 0.4 Hz). 7.30 (2H, d, *J* = 7.9, 1.4, 1.2, 0.5 Hz), 7.36-7.50 (3H, 7.43 (d, *J* = 7.9, 7.2, 2.0, 0.5 Hz), 7.43 (t, *J* = 7.2, 1.4 Hz), 8.09 (1H, d, *J* = 8.1, 0.4 Hz**);** ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 99.1 (1C, s), 114.8 (1C, s), 122.2 (1C, s), 124.5 (1C, s), 124.6 (1C, s), 125.1 (1C, s), 125.9 (1C, s), 126.8 (1C, s), 127.3 (1C, s), 127.5 (2C, s), 127.8 (1C, s), 128.0 (1C, s), 128.4 (2C, s), 133.0 (1C, s), 138.9 (1C, s), 155.6 (1C, s), 156.3 (1C, s), 156.6 (1C, s), 159.4 (1C, s).

7-hydroxy-4-[2-phenoxy-2-phenylethenyl]-2*H*-1-benzopyran-2-one (compd 5)

MP: 266°C with 64% yield.IR (KBr, cm^{-1}) ῡ: (stretch, C-H, aromatic), 1698 (stretch, C=O), 828, 743;^{1}H NMR (300MHz, DMSO-d6): δ (ppm) =1.32 (3H, t, *J* = 7.0 Hz), 4.33 (2H, *J* = 7.0 Hz), 5.91 (1H, s), 6.60 (1H, d, *J* = 0.5 Hz), 6.86 (1H, s), 7.26-7.53 (6H, 7.32 (d, *J* = 8.2, 1.5, 0.5 Hz), 7.44 (*J* = 7.5, 1.5 Hz), 7.45 (d, *J* = 8.3, 1.4 Hz), 7.46 (d, *J* = 8.2, 7.5, 2.0, 0.5 Hz), 7.63 (1H, d, *J* = 8.9, 8.3 Hz), 7.85 (1H, d, *J* = 8.9, 1.4, 0.5 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) =99.1 (1C, s), 114.8 (1C, s), 122.2 (1C, s), 124.5 (1C, s), 124.6 (1C, s), 125.1 (1C, s), 125.9 (1C, s), 126.8 (1C, s), 127.3 (1C, s), 127.5 (2C, s), 127.8 (1C, s), 128.0 (1C, s), 128.4 (2C, s), 133.0 (1C, s), 138.9 (1C, s), 155.6 (1C, s), 156.3 (1C, s), 156.6 (1C, s), 159.4 (1C, s).

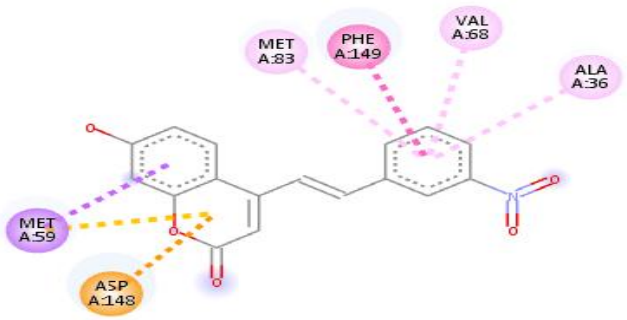
4-[2-ethoxy-2-phenylethenyl]-7-hydroxy-2*H*-1-benzopyran-2-one (compd 6)

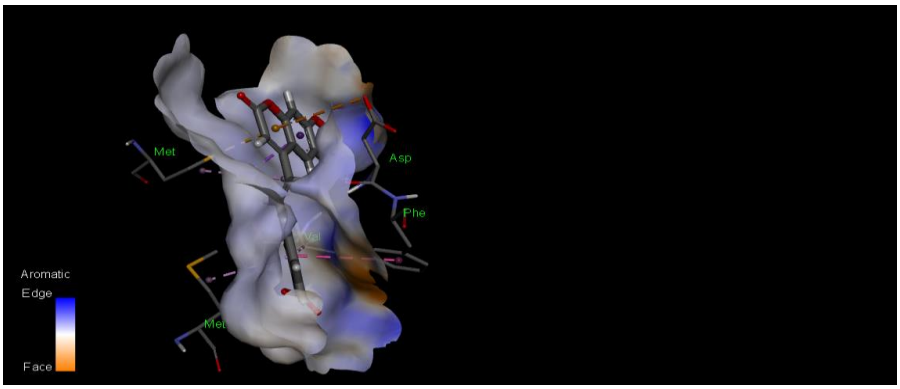
MP: 245°C with 64% yield.IR (KBr, cm^{-1}) ῡ: 3027(stretch, C-H, aromatic), 2814(stretch, C-O), 2674 (stretch, C-H, aliphatic), 928, 748; ^{1}H NMR (300MHz, DMSO-*d6*): δ (ppm) = 21.32 (3H, t, *J* = 7.0 Hz), 4.33 (2H, q, *J* = 7.0 Hz), 5.76 (1H, s), 6.55-6.71 (2H, 6.60 (d, *J* = 2.1, 0.4 Hz), 6.64 (d, *J* = 8.1, 2.1 Hz)), 6.78 (1H, s), 7.38-7.60 (3H, 7.44 (t, *J* = 7.3, 1.4 Hz), 7.53 (d, *J* = 7.6, 7.3, 1.6, 0.4 Hz), 8.06 (1H, d, *J* = 8.1, 0.4 Hz), 8.21 (2H, d, *J* = 7.6, 1.4, 0.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 14.2 (1C, s), 66.2 (1C, s), 95.8 (1C, s), 105.9 (1C, s), 114.8 (1C, s), 115.7 (1C, s), 116.4 (1C, s), 125.7 (2C, s), 127.3 (1C, s), 127.8 (1C, s),

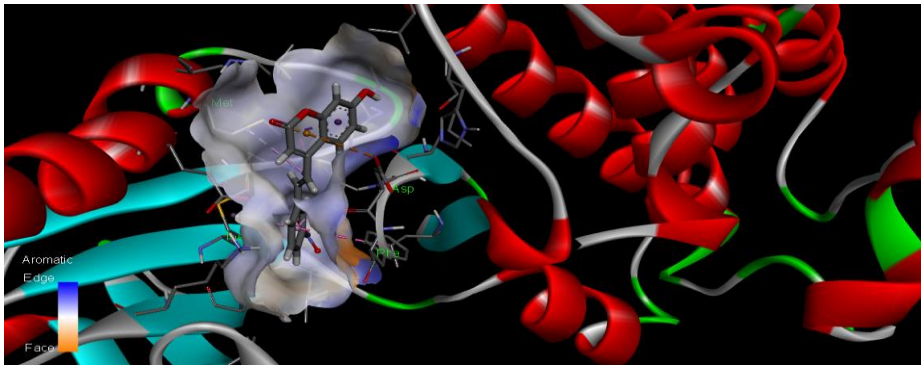
128.4 (2C, s), 129.3 (1C, s), 130.3 (1C, s), 152.6 (1C, s), 159.4 (1C, s), 160.1 (1C, s), 162.6 (1C, s).

4-[2-(4-aminophenyl)-2-hydroxyethenyl]-7-hydroxy-2*H*-1-benzopyran-2-one (compd 7)

MP: 235°C with 58% yield.IR (KBr, cm^{-1}) ῡ: 3381(stretch, N-H), 3194(stretch, C-H, aromatic), 1616 (bend, N-H), 820,697;^{1}H NMR (300MHz, DMSO-*d6*): δ (ppm) =5.46 (1H, s), 6.45 (1H, s), 6.54-6.70 (2H, 6.59 (d, *J* = 2.2, 0.4 Hz), 6.64 (d, *J* = 8.1, 2.2 Hz)), 6.89 (2H, d, *J* = 8.5, 1.1, 0.5 Hz), 7.64 (2H, d, *J* = 8.5, 1.8, 0.5 Hz), 8.05 (1H, d, *J* = 8.1, 0.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$): δ (ppm) = 95.8 (1C, s), 105.9 (1C, s), 114.3 (2C, s), 114.8 (1C, s), 115.7 (1C, s), 116.4 (1C, s), 126.0 (2C, s), 127.3 (1C, s), 129.3 (1C, s), 134.4 (1C, s), 148.4 (1C, s), 152.6 (1C, s), 159.4 (1C, s), 160.1 (1C, s), 183.5 (1C, s).



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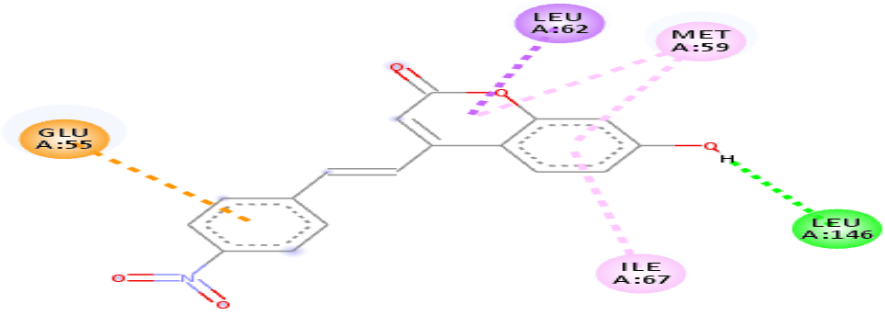
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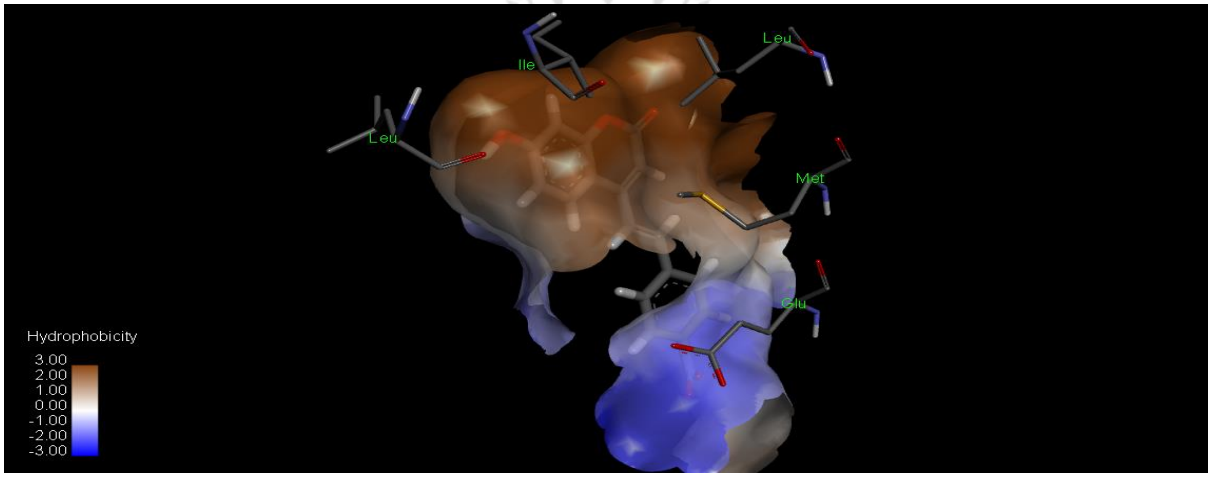
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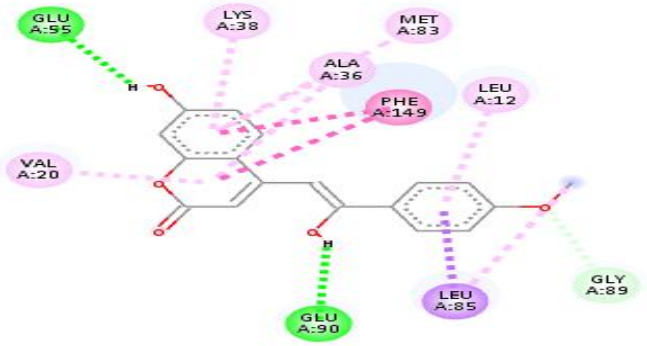
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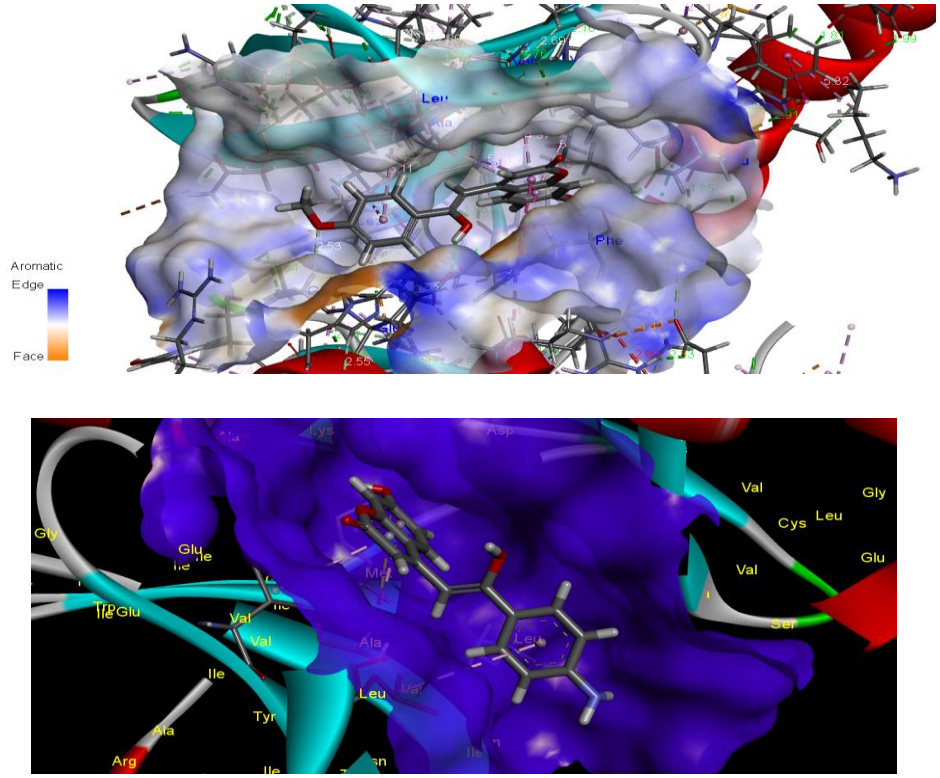

Figure 1: Images of Chromenes interacting with targets

MATERIALS AND METHODS

Requirements for docking studies

Choosing the right molecule is always best way in the process of drug design and development. “Virtual screening” is a one of best methodologies, a successful approach to find out HIT, lead compounds and optimization. Molecular modeling and docking of a large library of small enzymes), thereby identifying the potentiality of small compounds.In recent, protein-ligand docking studies became popular suggested by Grosdidier (29), although there are several approaches available for “high throughput screening”. This method is accurate,

reliable and computes the binding free energy of the ligand and intermolecular forces between targets and ligands. Compounds (ligands) or micro molecules against targets or macromolecules.

Evaluation Study

Antioxidant activity by DPPH method(30–32)

The antioxidant behavior of these imidazole derivatives is measured in vitro by the inhibition of generated stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. Methods vary greatly as to the generated radical, the reproducibility of the generation process, and the end point that is used for the determination. The DPPH solution was prepared by dissolving accurately weighed 22 mg of DPPH in 100ml of ethanol. From this stock solution, 18 ml was diluted to 100 ml with ethanol to obtain 100 µM DPPH solutions. The sample solution was prepared by accurately weighed 2.1 mg of each of the compounds and dissolved in 1 ml of freshly distilled DMSO separately to obtain solutions of 2.1 mg/ml concentration and the standard solution of was prepared by accurately weighing 10.5 mg of α-Tocopherol and dissolved in 1 ml of freshly distilled DMSO to get 10.5 mg/ml concentration. A solution of test compound in ethanol (500 µl) was added to the ethanolic solution of DPPH radical. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm against the corresponding blank solution. The final concentration of the samples and standard α-Tocopherol solutions used is 100µg/ml. The percentage scavenging DPPH radical inhibitions were calculated by using the following formula:

DPPH radical scavenging activity (%) = [(Abscontrol-Abs sample)/(Abscontrol)]×100 Where, Abscontrol was the absorbance of DPPH radical and ethanol, Abssample was the absorbance of DPPH radical and sample/standard. The scavenging activity was expressed in terms of IC_{50}, the concentration of the samples required to give a 50% reduction in the intensity of the signal of the DPPH radical. The results were done at least in triplicate. The structure of DPPH and its reduction by an antioxidant are shown in Scheme 2. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in color. The molar absorptivity of the DPPH radical at 517 nm decreases when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolorization is stoichiometric concerning number of electrons captured.

DPPH Assay

1. Preparation of reagents

DPPH Solution: Weighed accurately about 3.96mg of DPPH and dissolved in 50ml of methanol.

2. Preparation of test and standard solutions

In buffered methanol, 1000 g/ml stock solutions of synthesized compounds and ascorbic acid were generated by mixing 40 ml of 0.1 M acetate buffer (pH 5.5) with 60 ml methanol. To obtain the desired concentrations (5g/ml to 100g/ml), these produced solutions were diluted with methanol solvent.

3. Preparation of stock solution

1 mg of test material was diluted in 10 ml of buffered methanol to get 100 µg/ml solutions, and then repeated dilutions were performed to get the required concentrated solutions.(33)

4. Procedure

This assay-performed after modification of the method established by "Brand-Williams" (34) and the antioxidant nature of the molecules and standard reference was analyzed by DPPH-scavenging ability. Label eight test tubes with one blank, one standard, and six samples with varied concen's (5, 10, 25, 50, 75 & 100 µg/ml) for each evaluation. Prepare samples at various concentrations, such as 5, 10, 25, 50, 75, and 100 µg/ml. A 0.5 ml DPPH solution (0.1mM) was added to each test tube except the blank. A 100 l or 0.1 ml ascorbic acid standard solution was added to the standard.0.5 ml of 5, 10, 25, 50, 75, and 100 µg/ml solutions were added to all of the sample test tubes. Fill all of the test tubes with buffered methanol to a volume of 5 ml. Wrap all of the test tubes in aluminium foil and incubate for 30 minutes at 37°C in the dark. Using a UV/Vis spectrophotometer, the amount of absorbance of the resultant solution measured at 517 nm. The IC_{50} was computed, which is the concentration required to capture 50% of the free radical DPPH. The activity is calculated according to the following equation:

$$\% \text{ of Radical Scavenging Activity} = \frac{Ac - At}{Ac} \times 100$$

DPPH scavenging activity (%) = [(Ac–At)/Ac]×100 where, Ac is the "abs of the control" (contain all reagents except the molecule to be tested) and At is the "abs of the test sample". The % of inhibitions was plotted against concentration and from the graph IC_{50} was calculated.

Cytotoxicity Studies(35–37)

1. Maintenance of Cell Line

The cell lines were procured from NCCS, Pune and preserved in Dulbecco's Modified Eagle's Medium (DMEMD) supplemented with 10% FBS cultivated using trypsin and antibiotic substances penicillin or streptomycin (0.5 ml-1) in a 5 percent CO_2 at 95 percent air at 37ºC atmosphere.

2. Preparation of Test sample and MTT Solvent

Each of the test substances was carefully weighed and dissolved in Dimethyl sulphoxide (DMSO) or Dimethy formamide solvent to a concentration of 1mg/ml, then diluted to 100 µg/ml treated with a series of concentrations ranging from 10 to 100µg/ml. MTT is a water-soluble substance (10mg/ml), alcohol (20mg/ml), buffered salt solutions, and culture medium(5mg/ml), and we recommend using 4millimolar HCl and 0.1 percent isopropanol as a solvent system for MTT.

3. MTT assay-Procedure

Three independent experiments with six doses of chemicals in triplicates were used to assess cell viability. The cells were trypsinized, and the tryphan blue assay has been used to determine the cell viability in suspension. The cells were counted using a "hemocytometer" and planted at a density of 5.0 X 10^3 cells per well. The cells were trypsinized separately on DMEM-prepared cell suspensions before discarding the media. To counteract disaggregation in the flask, DMEM with 10% FBS was added. In each well, 50µl of serum-free media and 100µl of MTT solutions were added and incubated for 24 hours at 37^0C. After the incubation period, fill each well with 150 µl of MTT solution. Precipitates are generated as a result of the reduction of the MTT salt to chromophore formazan crystals by cells with metabolically active mitochondria by wrapping the plate in aluminium foil and shaking it occasionally with an orbital shaker for 15 minutes. On a microplate reader, the absorption spectrum of solubilized crystals in DMSO was determined at 570 nm.

The following formula was used to compute the percentage of growth inhibition.

$$\% \text{ of inhibition} = 100 - \frac{\text{Mean.OD of Individual Test Group}}{\text{Mean.OD of Control Group}} \times 100$$

The IC_{50} value was determined by using "linear-regression-equation i.e. Y = mx+C". Here,

Y = 50, M and C values were derived from the "viability graph".

Table 2: Antioxidant activity of Compounds by DPPH Assay

Compound		**Control**	**5**	**10**	**25**	**50**	**75**	**100**	**IC_{50}**
Compound 4	OD	0.32	0.25	0.23	0.21	0.19	0.16	0.15	88.39
	%	0	3.10	12.24	17.65	29.21	42.28	56.12	
Compound 5	OD	0.32	0.32	0.50	0.51	0.48	0.42	0.38	17.54
	%	0	0	5.36	12.64	15.85	27.54	41.68	
Compound 6	OD	0.32	0.46	0.45	0.41	0.38	0.36	0.32	22.47
	%	0	12.36	22.57	34.35	56.28	59.67	65.08	
Compound 7	OD	0.32	0.35	0.31	0.28	0.26	0.21	0.21	61.54
	%	0	6.84	8.25	17.84	24.84	48.38	51.08	
Ascorbic acid	OD	0.32	0.44	0.41	0.35	0.32	0.30	0.28	11.62
	%	0	41.25	65.08	73.0	84.47	87.85	91.65	

Table 3: MTT Assay results for HeLa Cell lines

Compound		**Blank**	**Control**	**5**	**10**	**25**	**50**	**100**	**R^2**	**IC_{50}**
Compound 4	OD	0	0.524	0.355	0.322	0.312	0.284	0.254	0.654	125.36
	% I	0	0	20.35	26.35	25.36	41.38	45.37		
Compound 5	OD	0	0.454	0.395	0.314	0.265	0.233	0.454	0.554	82.36
	% I	0	24.36	35.36	48.21	50.37	55.36	24.36		
Compound 6	OD	0	0.524	0.454	0.395	0.314	0.265	0.233	0.651	46.64
	% I	0	0	24.36	35.36	48.21	50.37	55.36		
Compound 7	OD	0	0.524	0.242	0.201	0.152	0.126	0.144	0.477	36.66
	% I	0	0	48.72	57.41	68.22	73.3	69.49		
5-FU (5-Fluro Uracil)	OD	0	0.524	0.277	0.248	0.212	0.188	0.156	0.745	9.36
	% I	0	0	21.36	27.2	36.35	42.25	46.25		

Table 4: MTT Assay results with MCF-7 Cell lines

Compound		Blank	Control	5	10	25	50	100	R^2	IC_{50}
Compound 4	OD	0	0.472	0.241	0.225	0.184	0.175	0.142	0.356	65.52
	% I	0	0	48.69	57.41	68.22	73.3	69.59		
Compound 5	OD	0	0.472	0.279	0.258	0.241	0.186	0.152	0.94	69.35
	% I	0	0	35.96	39.86	43.53	46.85	53.62		
Compound 6	OD	0	0.472	0.324	0.294	0.251	0.217	0.185	0.801	36.63
	% I	0	0	22.56	34.76	55.76	64.88	75.68		
Compound 7	OD	0	0.472	0.322	0.298	0.248	0.185	0.322	0.713	19.28
	% I	0	0	23.65	47.64	48.37	61.65	48.32		
5-FU (5-Fluro Uracil)	OD	0	0.472	0.524	0.285	0.247	0.21	0.208	0.776	4.08
	% I	0	0	31.2	41.7	53.7	65.5	67		

With the current novel tactics in targets such CDKs, Topoisomerases, Check point kinases, Cdc phosphates, and ABC transporters, the idea of cell cycle mechanisms and biochemical pathways in the etiology of cancer is rising. With these developments, cancer medication development went from being a low-stakes, multibillion-dollar Endeavour to a high-stakes, multibillion-dollar enterprise.

Because molecule development is a costly and time-consuming procedure that can also lead to failure in many cases, novel, safe, and efficacious molecules to ease the pangs of humans suffering from cancer have yet to be investigated. From molecule design by SBDD or LBDD to synthesis, preclinical to clinical testing from molecular or cellular to global effects, the production of molecules requires many issues. In recent years, Computer-Aided Drug Design-CADD and reverse pharmacology have become attractive methods for developing medications that can specifically target the proteins or DNA responsible for cancer.

The antioxidant ability the produced agents were tested using the "DPPH" method. All produced compounds were tested for cytotoxicity activity using the MTT, which is "3-(4,5-dimethyl-thiazol-2-yl)-2,5-di-ph-tetrazolium bromide" assay method against MCF-7 and Hela cell-lines, and the results were used to assist screen the best chemical capable of killing cancer cells. As a result, two graphs were plotted to examine the results: a percentage of inhibition graph and a percentage of viability graph.

The cytotoxic response of the used cell lines against the different compounds was evaluated through *in vitro* high-throughput cell-based assay which is the MTT assay a standard

colorimetric assay (measure of color intensity) for measuring the cell proliferation or cell growth. The method also has advantages like rapidity, quantization, sample management and cell number required for assay.

The antioxidant-activity by DPPH-assay-method reveals that Chromene derivatives with hydroxy groups and para amino on phenyl ring (Compound 7) followed by ethoxy at ethylene bridge reported better activity profile was represented in Table-3 and 4.

The MTT detects both cell respiration and the proportion of "formazan" generated, which is relative to the number of live cells in the culture media. A change in the amount of formazan generated in response to a decrease or rise in the number of cells indicates the degree of cytotoxicity caused by the substance. The IC_{50} value, which is the concentration of a manufactured molecule that will kill 50% of cells, can be used to anticipate cytotoxic effects. The IC_{50} number indicates how cytotoxic a chemical is. The study was designed with different cell lines including MCF-7(Breast carcinoma) and HeLa (cervical carcinoma) cell lines against synthesized-Chromenes in different concentrations. The concentrations were used by DPPH assay and MTT assay like 5, 10, 25, 50, and 100µg/ml used to evaluate the cytotoxicity by dose-dependent manner. Ascorbic acid was taken as standard drugs for these studies. The consolidated data of "Cytotoxicity" of all the Series compounds and based on observations found that Compound Compound 7 successfully able to kill MCF-7 cell with percentage of inhibition at the concentration was found to be 19.28µg/ml with R^2 value 0.713 followed by Compound 6 with percentage of inhibition at the concentration was found to be 36.63µg/ml with R^2 value 0.801 represented in table-4.

The consolidated data of "Cytotoxicity" of all the Series compounds and based on observations found that Compound Compound 7 successfully able to kill MCF-7 cell with percentage of inhibition at the concentration was found to be 46.64µg/ml with R^2 value 0.651followed by Compound 6 with the percentage of inhibition at the concentration was found to be 36.63µg/ml with R^2 value 0.801 represented in table-3. The standard compound 5-FU reported with the IC_{50} value 4.08µg/ml and 9.36µg/ml for MCF 7 and Hela Cell lines.

SUMMARY AND CONCLUSION

I have chosen Chromes, as ligands for anticancer action targeting different enzymes or cellular kinases. Many analogues were created based on various schemes and screened using chemoinformatics and molecular docking studies using Auto-Dock and Patch-Dock Server,

with selective cellular kinase targets Thymidylate synthase, tyrosine kinase, cell division protein kinase, and cyclin-dependent kinase 2 as targets.

The Chromene-Series class of chemicals has the best interactions and may have powerful inhibitory effects against the targeting thymidylate synthase and tyrosine kinases as anticancer activities. All of the substances were produced using various traditional synthetic processes; with reaction progress monitored using chromatographic techniques such as TLC. FT-IR, ^{1}H-NMR, and ^{13}C-NMR Spectrophotometric methods used to structurally characterize all of the synthesized molecules. DPPH method was used to investigate the antioxidant potential of all the molecules using standard reference chemicals ascorbic acid. We employed the MTT assay to test the cytotoxicity of manufactured fused pyrimidine ligands against MCF-7(breast carcinoma) and HeLa(cervical carcinoma) cell lines against generated ligands in various doses.The anticancer study found that the chemical Compound 7 and 6 Series has a significant potential to destroy tumor cells, confirming the earlier theory.

De novo modeling of novel chemicals to treat cancer is aided by molecular modelling research. Overall, the study found that the majority of the chemicals synthesized, particularly those in the chromene Series with electron-donating system at para position like amino, and hydroxyl group at Ethylene Bridge had the potential to be cytotoxic. In the future, I'd want to focus on the respective prospective molecular series in terms of developing a big compound library and doing QSAR studies to improve physicochemical parameters and cytotoxic enormous potential.

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