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RATIONAL DESIGN AND MULTIDIMENSIONAL EVALUATION OF BENZOPYRAN-2-ONE DERIVATIVES AS PROMISING ANTICANCER CANDIDATES

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ABSTRACT

A novel series of benzopyran-2-one derivatives was synthesized and structurally validated through ^{1}H-NMR, ^{13}C-NMR (acquired at −15 °C), and mass spectrometry. ADMET and drug-likeness analyses revealed that all 20 compounds possess favorable pharmacokinetic properties, demonstrating good oral bioavailability, minimal predicted toxicity, and adequate absorption while complying with Lipinski's rule of five (rule of five). Notably, compounds **FC-H** and **FC-P** exhibited the most promising profiles, making them strong candidates for further optimization and biological evaluation.

Keywords: ADMET Studies, *insilico* studies, Benzopyran-2-ones, Anticancer, Druglikeness, ^{1}H and ^{13}C NMR Spectrum.

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INTRODUCTION

As secondary metabolites, benzopyran-2-ones, often known as coumarins, have undergone significant investigation over the past decade. Numerous anticancer drugs currently on the market have drawbacks like poor pharmacokinetic profiles, non-specific toxicity, and drug resistance, despite tremendous advancements in cancer chemotherapy. This has prompted ongoing research into novel chemotypes with improved safety and effectiveness. The anticancer potential of benzopyran-2-one (coumarin) derivatives has lately drawn attention. These compounds are well-known for their wide range of biological actions, which include anticoagulant, anti-inflammatory, antibacterial, anti-cancer[1,2], anti-oxidant[3,4], neuroprotective[5,6], anti-diabetic[7,8], anti-microbial[9,10], anti-hypertensive[11,12], anti-epileptic[13,14,] and anti-HIV[15,16]. By means of kinase targeting, topoisomerase regulation, and tubulin inhibition, a number of coumarin-based compounds have shown cytotoxicity. However, the systematic design and optimization of benzopyran-2-one frameworks specifically tailored for cancer-related targets remain underexplored. Furthermore, a comprehensive *in silico*-to-*in vitro* evaluation, including molecular docking, ADMET profiling, and structure–activity correlations, is often lacking in published reports.

EXPERIMENTAL

Computational Studies

The docking studies were conducted with utmost thoroughness to assess the binding affinities between ligand and receptors and analyze the various modes of bimolecular interactions. These critical investigations used advanced software programs, including PyMOL, PyRX, Discovery Studio 2020, Biovia, Vina, and AutoDock. The targeted proteins for this study were the crystal structure protein binding/ inhibitor (PDB ID: 4DHR). This cutting-edge technology facilitated the efficient execution of docking studies, yielding valuable insights into essential bimolecular processes.[17]

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Protein Preparation
The crystal structure of the target protein for this study (PDB ID: 4DHR) was retrieved from the RCSB Protein Data Bank. The protein was then prepared by removing extraneous ligands using Swiss PDB Viewer. The processed protein was saved in PDB format for further analysis.[18,19]

Ligand Preparation
ChemSketch generated the 3D ligand models, which were then uploaded to BIOVIA Discovery Studio Visualizer 2020. The ligands underwent further optimization through a minimization process, and the optimized structures were saved as a clustered SDF file.[20]

Docking Studies
These studies were conducted using the PyRX (virtual screening tool) to ensure accuracy and precision. The ligand was converted to PDBQT format within PyRX and selected for use in the Vina wizard. The prepared proteins were also loaded into PyRX to serve as the macromolecule. This setup facilitated the calculation of interaction energy between the ligand and receptor, as well as the identification of specific amino acids involved in binding. Docking is a critical step in minimizing false positives and determining the optimal orientation of the ligand within the protein's active site. Through these studies, we aim to identify potential compounds with high binding affinity for targeted applications.[20]

Drug Likeliness Studies
The proposed derivatives were imported into DruLiTO in SDF format to perform a drug-likeness assessment. This assessment is a crucial step in drug development, as it helps to predict the pharmacokinetic and safety profiles of the compounds, thereby guiding further research and development.[21]

ADME/T Studies
The SMILES representations of the proposed derivatives were uploaded to Swiss ADME/T, and their ADME/T properties were recorded.[22, 23]

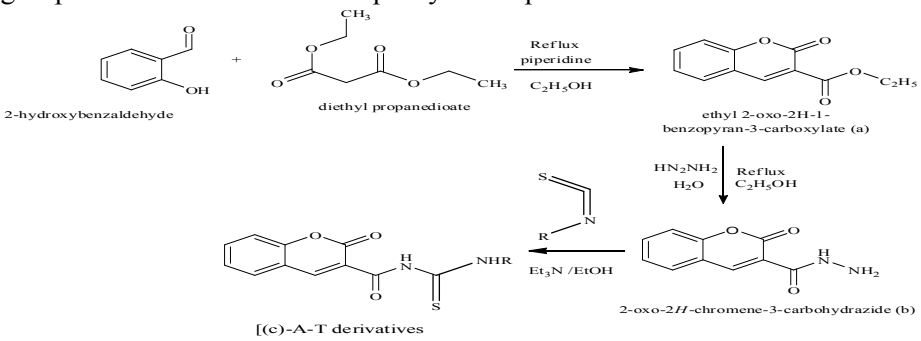
Synthesis
Here, we meticulously designed novel flavonoid derivatives, 3-substituted benzopyran-2-one derivatives [(c)-A-O)] derivatives as potential anticancer drug candidates. Scheme 1 lists the synthetic approaches for the target and intermediate compounds. FT-IR, ^{1}H-NMR, ^{13}C-NMR, and LC-MS spectroscopy were used to confirm the compounds' structures.

Step I: Procedure for the synthesis of ethyl 2-oxo-2H-1-benzopyran-3-carboxylate (a)
The Knoevenagel condensation, a key step in our synthesis process, involves the reaction of 2-hydroxybenzaldehyde (1.5 mol) with diethyl propanedioate (2.25 mol), catalyzed with piperidine in alcohol (20 ml). Thin-layer chromatography (TLC) was used to monitor this reaction, and after completion, the solid was purified by re-crystallization in ethanol. A single spot-on TLC confirmed the purity of the product.

Step-II: Procedure for the synthesis of 2-oxo-2*H*-1-benzopyran-3-carbohydrazide (b)
At room temperature, a solution of the molecule (a) (0.0562 mol) in 100 ml of ethanol was mixed with hydrazine hydrate (0.1686 mol) and swirled for 2 h. We quenched the white solid with water, filtered it, and washed it with approximately 50 ml of water. Finally, the solid material was recrystallized using ethanol and vacuum-dried to obtain the 2-oxo-2H-1-benzopyran-3-carbohydrazide molecule. A single spot-on TLC plate confirmed the purity of the product.

Step-III: Procedure for the Synthesis of Derivatives of 3-substituted benzopyran-2-one Derivatives [(c)-A-T)]
A mixture of 3 g of compound (b) and 20 ml of triethylamine in alcohol was prepared. To this, various substituted isothiocyanates (0.1 mol) were added, and refluxed for 12 hours. The reaction yielded a series of 3-substituted benzopyran-2-one derivatives, designated as [(c)-A–T]. TLC was used to monitor this

reaction, and after completion, the solid was washed with water and finally purified by recrystallization in ethanol. A single spot-on TLC confirmed the purity of the product.



Scheme-1: Schematic Representation of Synthesis of 3-substituted Benzopyran-2-one Derivatives [(c)-A-T)]

RESULTS AND DISCUSSION

In the present study, *in-silico* docking was performed on the proposed derivatives, and their efficacy was compared to that of the standard drugs 5-fluorouracil and doxorubicin against the crystal structure of 4DHR. All compounds exhibited significant docking scores (Table-1). The 2D and 3D interactions involving the standard drug and the synthesized compounds with the best binding energies are presented in the following Fig.-1 to 6.

Table-1: Molecular Docking Scores of Proposed Compounds with (PDB ID: 4DHR)

Compound Code	Docking Score	Compound Code	Docking Score
5-Flurouracil	-3.8	FC-J	-2.1
Doxorubicin	-4.2	FC-K	-2.1
FC-A	-3.6	FC-L	-2.5
FC-B	-3.8	FC-M	-2.7
FC-C	-2.5	FC-N	-3.0
FC-D	-2.9	FC-O	-2.5
FC-E	-3.4	FC-P	-4.5
FC-F	-2.2	FC-Q	-2.8
FC-G	-1.8	FC-R	-2.4
FC-H	-4.3	FC-S	-1.9
FC-I	-2.5	FC-T	-2.1

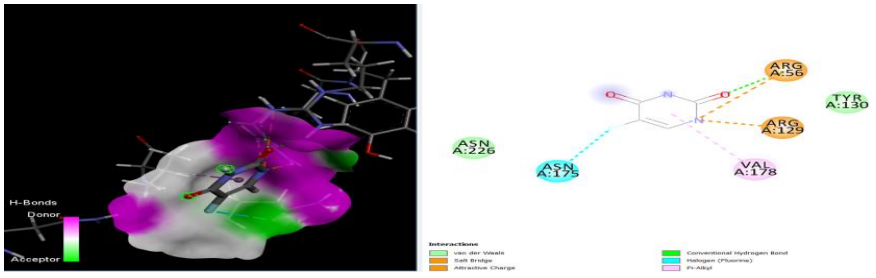


Fig.-1: Study of 5-Fluorouracil Interactions with the 4DHR Crystal Structure using 2D and 3D Binding Analysis

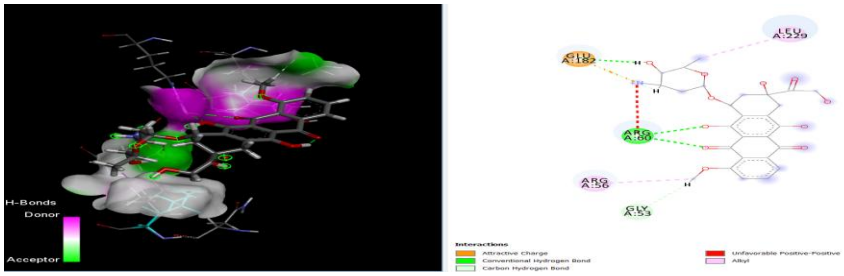


Fig.-2: Study of Doxorubicin Interactions with the 4DHR Crystal Structure using 2D and 3D Binding Analysis

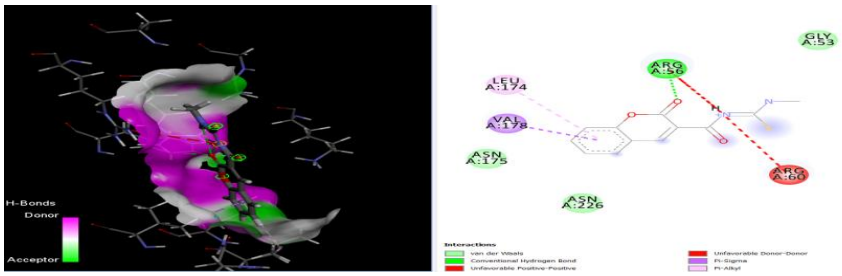


Fig.-3: Study of FC-A Interactions with the 4DHR crystal structure using 2D and 3D Binding Analysis

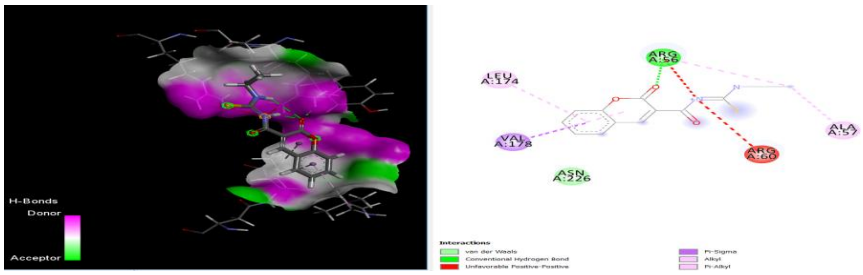


Fig.-4: Study of FC-B Interactions with the 4DHR Crystal Structure using 2D and 3D Binding Analysis

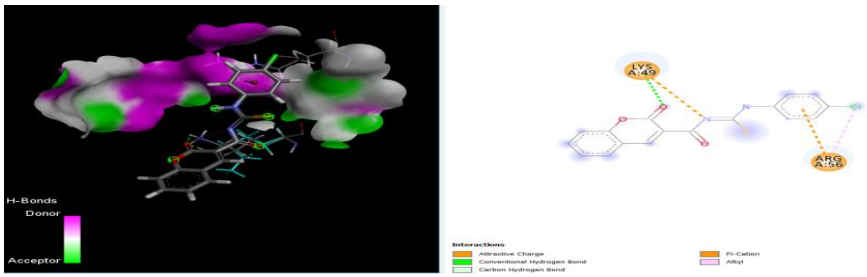


Fig.-5: Study of FC-H Interactions with the 4DHR Crystal Structure using 2D and 3D Binding Analysis

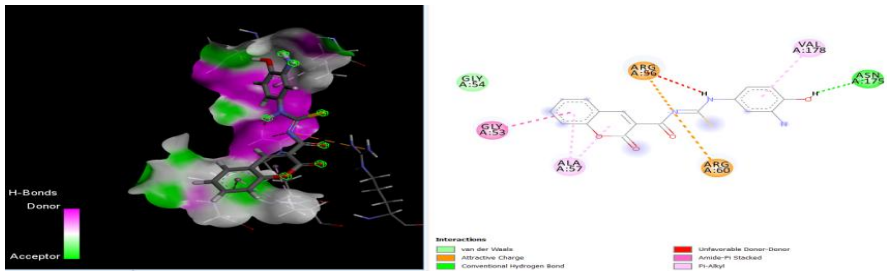


Fig.-6: Study of FC-P Interactions with the 4DHR Crystal Structure using 2D and 3D Binding Analysis

Drug Likeness Studies

In silico Drug likeness studies were performed using DruliTO software. The *in-silico* Drug Likelihood (Table-2) prediction of the molecules showed that all compounds adhere to the Lipinski rule of five, suggesting that these compounds fulfilled all the requirements that were crucial for anticancer activity.

Table-2: Drug Likeness Studies

Compound Codes	Mol Weight	Log P	Rotatable Bonds	Acceptors	Donors	Lipinski's Violations
FC-A	262.29	1.0271	1	4	2	0
FC-B	276.317	1.4172	2	4	2	0
FC-C	324.361	2.9198	2	4	2	0
FC-D	340.36	2.6254	2	5	3	0
FC-E	354.387	2.93382	2	5	3	0
FC-F	338.388	3.22822	2	4	2	0

FC-G	372.833	3.88162	2	4	2	0
FC-H	358.806	3.5732	2	4	2	0
FC-I	374.805	3.2788	2	5	3	0
FC-J	366.398	3.1224	3	5	2	0
FC-K	445.294	3.8849	3	5	2	0
FC-L	403.257	3.6823	2	4	2	0
FC-M	350.399	3.5628	3	4	2	0
FC-N	404.425	2.1665	3	6	3	0
FC-O	339.376	2.502	2	5	3	0
FC-P	355.375	2.2076	2	6	4	0
FC-Q	353.403	2.81042	2	5	3	0
FC-R	373.821	3.1554	2	5	3	0
FC-S	403.803	3.4814	3	6	2	0
FC-T	369.358	2.828	3	6	2	0

Pharmacokinetic Studies

In silico ADMET studies were performed by using Swiss ADMET. The *in silico* ADMET studies (Table-3) prediction of the molecules showed good pharmacokinetic properties, having high gastrointestinal absorption, oral bioavailability, and less toxicity.

Table-3: The Predicted ADME/T Properties for Derivatives FC-A, FC-B, FC-H and FC-P

ADME/T Characteristics	FC-A	FC-B	FC-H	FC-P
Absorption				
Water solubility	-2.874	-3.079	-4.113	-3.139
Caco2 permeability	0.619	0.67	0.927	0.459
Intestinal absorption	74.77	94.353	90.616	79.314
Skin Permeability	-3.106	-3.093	-2.777	-2.736
Distribution				
CNS Permeability	-2.447	-2.455	-1.828	-2.381
BBB Permeability	-0.306	-0.313	-0.128	-1.201
Fraction unbound	0.315	0.296	0.069	0.193
VDss	-0.501	-0.423	-0.52	-0.459
Metabolism				
CYP3A4 inhibitor	No	No	Yes	No
CYP2C9 inhibitor	No	No	Yes	Yes
CYP2C19 inhibitor	No	No	Yes	Yes
CYP1A2 inhibitor	Yes	Yes	Yes	Yes
Substrate for CYP3A4	Yes	Yes	Yes	No
Substrate for CYP2D6	No	No	No	No
Excretion				
Renal OCT2 transporter	No	No	No	Yes
Overall Systemic Clearance	-0.237	-0.287	-0.543	-0.502
Toxicity				
hERG inhibitor (type-I)	No	No	No	No
hERG inhibitor (type-II)	No	No	Yes	Yes
AMES toxicity (for mutagenic potential)	Yes	Yes	No	Yes
Max. tolerated dose	0.026	0	0.022	0.524
Minnow toxicity	1.415	1.249	-0.254	2.297

T. Pyriformis toxicity	0.96	1.062	0.95	0.305
Hepatotoxicity	No	Yes	Yes	Yes
Skin Sensitization risk	No	No	No	No
Oral Chronic Toxicity (Rats)	1.302	1.164	1.137	1.842
Oral Acute Toxicity (Rats)	2.494	2.547	2.699	2.613

Characterization

The physicochemical properties of all the synthesized compounds are depicted in Table-4, and these synthesized compounds were analyzed for FT-IR, ^{1}H NMR, and LC-MS (depicted in Table-5).

Table-4: Physicochemical Properties of Synthesized Compounds (FC-A, FC-B, FC-H and FC-P)

Compound	Mol. Formula	Mol. Wt.	% yield	Melting point	R_f Value
FC-A	$C_{12}H_{10}N_2O_3S$	262.28	71.33%	92-94^0 °C	0.80
FC-B	$C_{13}H_{12}N_2O_3S$	276.31	78.33%	96-98^0 °C	0.87
FC-H	$C_{17}H_{11}N_2O_3SCl$	358.79	69.33%	110-112^0 C	0.85
FC-P	$C_{17}H_{13}N_3O_4S$	355.36	70.00%	112-114^0 C	0.89

Table-5: Spectroscopic Data of Synthesized Compounds (FC-A, FC-B, FC-H and FC-P)

Compound	FT-IR		^{1}H NMR		^{13}C NMR		LC-MS	
FC-B	Wave number (cm^{-1})	Functional group assigned	δ Value (ppm)	Peak assigned (Multiplicity)	Carbon's	δ Value (ppm)	M^+ Peaks (m/z)	Base Peak
	3000-3100	-N-H Stretch of 2^0 amine	4.110 4.113	-NH (2H)	1C –CH_3	23.14	276.31	243.2
	2900-3000	Aromatic C-H Stretch	0.876 1.209 1.262 2.490 3.313	-CH_3, CH_2(5H)	1C CH_2	57.10		
	2800-2900	Aliphatic C-H Stretch	7.0-8.5	Ar-H (multiplate)	8C from the Aromatic ring	90-135		
	1490-1500	C = O Stretch			2C from C=O	156.10 157.00		
	800-830	CH_3, CH_2Stretch			1C from C=S	159.66		
	1100	-C=S stretch						
FC-p	2900-3000	-N-H stretch 1°, 2° or 3° amine	2.490 3.313 4.111 4.133	-$(NH)_2$, -NH_2 (4H)	14C from Aromatic ring	90-135	355.36	337.1
	2400-2500	Aliphatic -C-H Stretch	7.05-8.5	Ar-H(multiplate)	3C from C=Oand C=S	148.26 155.10 155.17		
	1105	-C=S Stretching						
	1400-1410	C = O Stretch						
	2800-2900	Aromatic -C-H Stretch	13.130	-OH (1H)				

CONCLUSION

In this study, selected flavonoid derivatives were computationally evaluated for their binding affinity against the cancer target (PDB ID: 4DHR) to identify promising lead candidates. ADME/T and drug-likeness analysis revealed that all 20 compounds possess favorable pharmacokinetic properties, including oral bioavailability, low toxicity, and compliance with Lipinski's rule of five. These findings highlight their potential as anticancer agents and provide a valuable framework for further structural optimization. To validate these results, subsequent *in vitro* and *in vivo* studies are recommended.

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CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

The present work is related to *SDG-3: Good Health and Well Being*. All the authors contributed significantly to this manuscript, participated in reviewing/editing, and approved the final draft for publication. The research profile of the authors can be verified from their ORCID IDs, given below:

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