Synthesis, In silico and In vitro Studies of 7-methoxy-3-((4-phenylpiperazin-1-yl)methyl)-2H-chromen-2-one Analogues as Derivatives as Anti-prostate Cancer Agents

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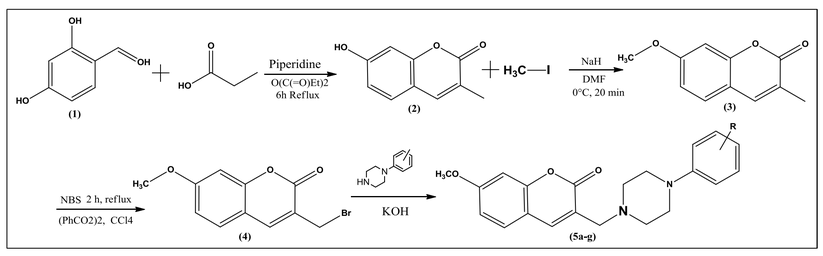
INTRODUCTION

- One of the most common diseases found among men in recent days is prostate cancer (PCa). The growth of cancer is generally due to the activation of the androgen receptor by androgens.
- Structural modification and molecular docking approaches were done with the protein (PDB ID: 3V49) to identify the novel 7-methoxy-3-((4-phenylpiperazin-1-yl)methyl)-2H-chromen-2-one derivatives.
- The compounds (5a-g) was synthesized and characterized well by IR, NMR, and LC-MS spectral techniques. The compound 5a and 5b were reconfirmed by single crystal XRD.
- The in vitro anticancer studies were carried out for the compounds (5a-g) against LNCaP, PC3and 3T3 cell line. Among them 5b showed highest cytotoxicity against LNCaP (10.35 ± 1.22) µM, PC3 (34.65±1.46) µM and reduced cell viability.
- For the compound 5b, simulations of molecular dynamics are conducted to test protein-ligand interactions. Drug similarity and pharmacokinetic properties for all compounds were anticipated. The outcome of these results may give vital information in further development.

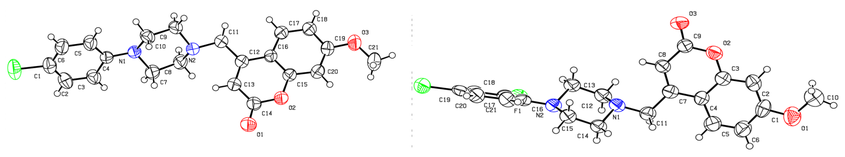
OBJECTIVE OF THE WORK

- Propose novel small molecule inhibitors for prostate cancers, using a structure-based drug design technique, and proceed with wet-lab synthesis.
- Using **SchrÖdinger** drug discovery software to expose the 2D structures of novel compounds to investigate the biological properties in silico like ADME.
- Investigate potential interactions and binding affinity through molecular docking studies between selected estrogen receptors.
- Using FT-IR, Mass, NMR, and single crystal X-ray diffraction studies to elucidate the structure of the synthesized compounds.
- It is evaluated in vitro cytotoxicity and anticancer activity against prostate cancer cell lines of all novel compounds.
- Molecular dynamics simulations were carried out for a hit molecule to study the stability and interaction of the protein-ligand complex.

Synthesis of 1,3,4-oxadiazole derivatives



Single Crystal X-ray Diffraction Study



ACKNOWLEDGEMENT

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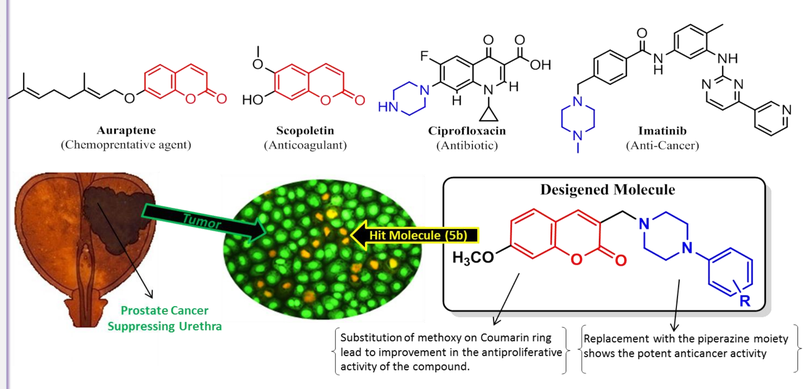
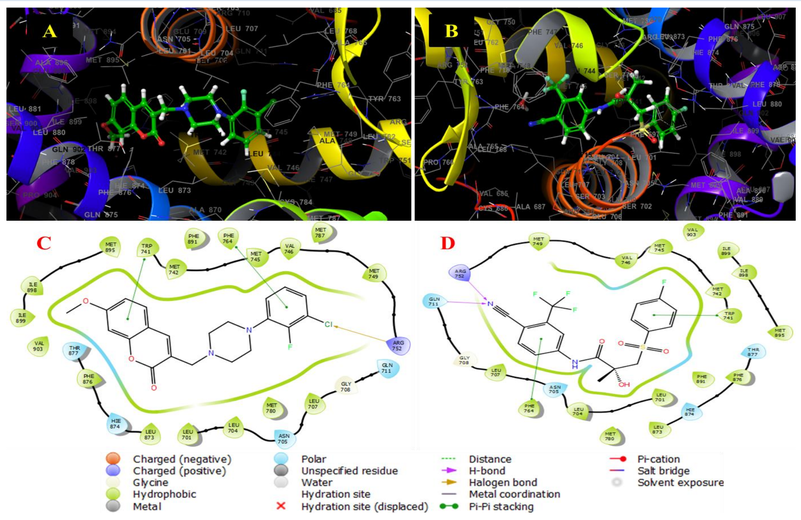
GRAPHICAL ABSTRACT



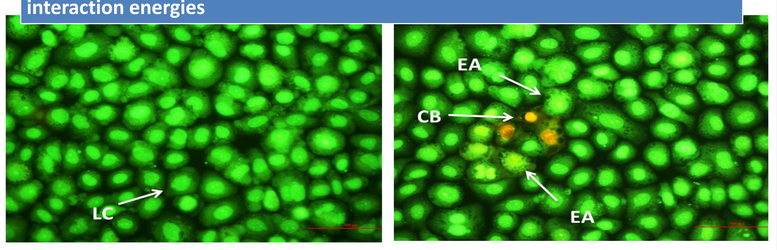
Molecular Docking Studies



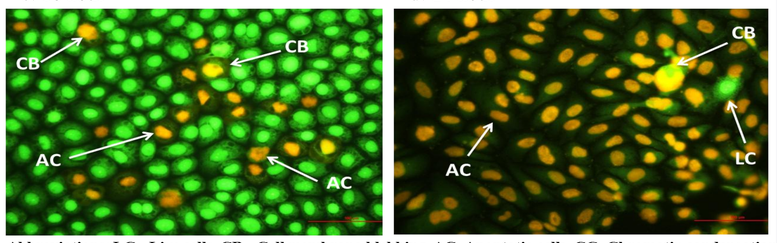
Molecular docking studies of compounds (5a-g) with 3V49

Comp.	glide g score	glide evdw	glide ecoul	glide energy	Interacting Residues
5a	-7.101	-21.625	0.868	-20.757	TRP741, PHE764
5b	-9.274	-21.917	-2.491	-34.408	TRP741, PHE764, ARG752
5c	-8.331	-25.777	1.125	-24.652	TRP741, PHE764
5d	-6.582	-30.532	0.757	-29.775	TRP741, PHE764
5e	-9.560	-27.528	0.138	-27.39	TRP741, PHE764
5f	-8.586	-33.015	1.386	-31.629	TRP741, PHE764
5g	-6.775	-20.139	-0.3	-20.439	TRP741, PHE764
Bicalutamide	-11.064	-42.986	-1.726	-44.712	ARG752, TRP741

glide evdw = van der Waals interaction energies, glide ecoul = Coulomb interaction energies



c: 16 hour d: 24 hour



Abbreviations: LC - Live cells, CB - Cell membrane blebbing, AC- Apoptotic cells, CC- Chromatin condensation

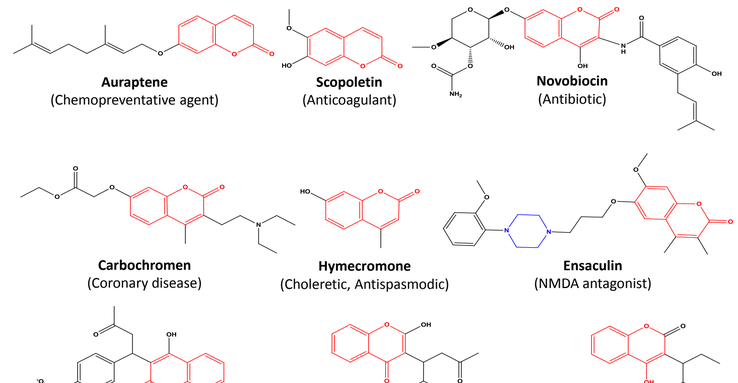
The 5b treated in LNCaP cells at different time points. Live cells appear green, late apoptotic cells are appearing light orange, and necrotic cells are colored orange.

Analysis of (5a-g) molecules and standard drug for anticancer studies

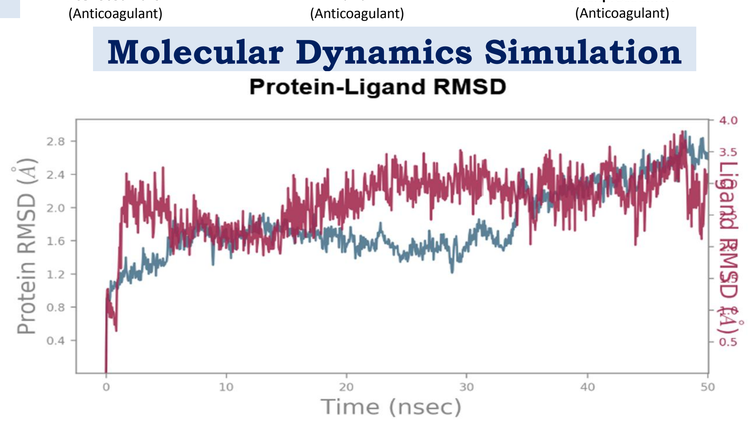
Ligand	LNCaP (µM)*,[a] (T877A AR)	PC3 (µM)[a] (AR-)	3T3 (µM)[a] (non-cancer)
5a	28.32 ± 1.42	48.20 ± 2.87	54.21 ± 1.68
5b	10.35 ± 1.22	34.65 ±1.46	>100
5c	21.76 ± 1.86	43.74 ± 1.32	>100
5d	37.57± 1.79	74.14± 1.78	>100
5e	>100	>100	>100
5f	44.57± 1.69	>100	82.47 ± 1.64
5g	17.56 ± 1.42	58.82 ±1.32	62.32± 1.64
Bicalutamide[b]	16.3 ± 0.07	28.26 ±1.86	92.55 ± 0.32

*IC_{50} of the compounds stimulated by 1nM DHT. [a]The values are the mean ± standard deviation (SD) of three independent experiments performed in triplicate. [b]Positive control

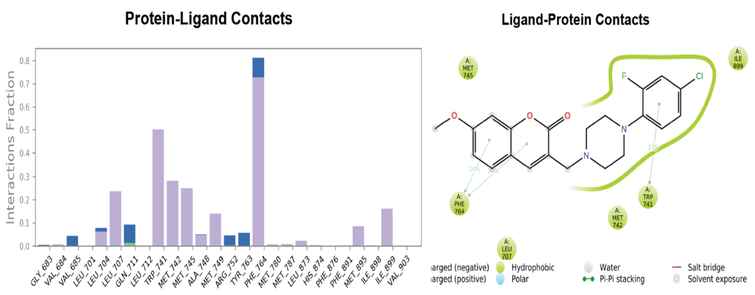
Some Clinical Coumarin Drugs



Molecular Dynamics Simulation



The compounds of the 5B & 3V49 complex for RMSD were obtained



The Histogram and the number of Molecular Dynamic simulations interactions.

RESULTS AND DISCUSSIONS

- Molecular docking studies of compounds (5a-g) are conducted using 3D-coordinated for androgen receptors. The amino acids **ARG752, TRP741** and **PHE764** play a role in androgen receptor binding. The glide energy of the compounds with 3ERT ranges from -44.712 to -20.439 kcal/mol.
- Compounds (5a-g) have not shown any violations under Lipinski's rule five.
- Further the Compounds (5a-g) were synthesized and evaluated for their anticancer activity. FT-IR, NMR, and mass spectrometry techniques were used to characterize the synthesized compounds.
- Single-crystal XRD analysis further reconfirms the structure of 5a & 5b.
- The anticancer activity of the novel compounds (5a-g) was tested against the human breast cancer cell line. The Compound **5b** showed (10.35 ± 1.22) µM potent activity against LNCaP and (34.65 ±1.46) µM against PC3 cell lines.
- The analysis showed that the synthetic compound **5b** decreased cell viability and stimulated apoptosis in chromatin condensation of LNCaP cell lines.
- In order to study the stability and interaction of the protein-ligand complex, molecular dynamic simulations of hit molecule **5b** have been performed. Potential interactions in the active site of an estrogen receptor protein have been studied.

REFERENCES

1. Schrödinger, Maestro, 2016 LLC, New York, NY.
2. G. M. Sheldrick, 'Crystal structure refinement with SHELXL', Acta Crystallography. 2015, C 71, 3..
3. Schrödinger, QikProp 2019-3, LLC, New York, NY.

[1]H NMR spectrum of compound **4c**

[13]C NMR spectrum of compound **4c**