Synthesis of kinase inhibitors as an anti-cancer agents

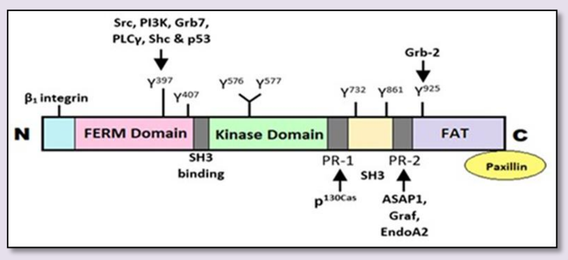
Akshita Chauhan [1]*, Tabassum Khan [2]
[1] Department of Quality Assurance, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, Mumbai, Maharashtra, India
[2] Department of Pharmaceutical Chemistry and Quality Assurance, SVKM's Dr. Bhanuben Nanavati College of Pharmacy, Mumbai, Maharashtra, India

Introduction

- Cancer is a category of diseases involving irregular cell growth that have the ability to invade or spread to other areas of the body and it's the second most deadly in terms of morbidity and mortality.
- The various molecular targets explored in cancer therapeutics includes tyrosine kinase, proteasome, histone deacetylase (HDAC), thymidine phosphorylase, cyclin-dependent kinase (CDK), aurora kinase, mTOR, STAT proteins, ETS proteins, PLK-1 and SCF protein.
- **Tyrosine kinases** are mainly classified as receptor tyrosine kinase (RTK) e.g. EGFR, PDGFR, FGFR, IR, ALK and non-receptor tyrosine kinase (NRTK) e.g. SRC, ABL, **FAK**, and Janus kinase.
- There are currently only 12 small molecule FAK inhibitors in different phases of clinical trials, with no marketed molecule till date and there is scope to increase this armamentarium of anti-cancer drugs by identifying promising leads.

Focal adhesion kinase

- FAK is a non-receptor tyrosine kinase that resides at the sites of focal adhesions.
- FAK is an important mediator of cell adhesion, growth, proliferation, survival, angiogenesis and migration, all of which are often disrupted in cancer cells.
- Normal tissues have low expression of FAK, while primary and metastatic tumors overexpress this protein.



Aim and Objectives

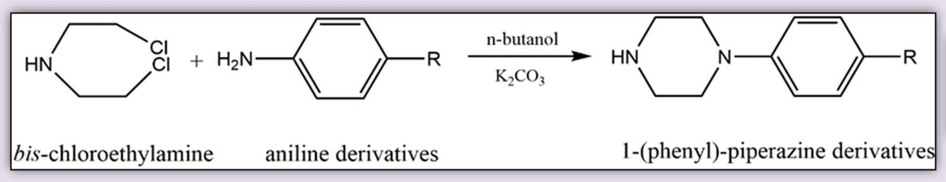
Aim: To develop and screen molecules as potential FAK inhibitor in cancer.

Objectives:

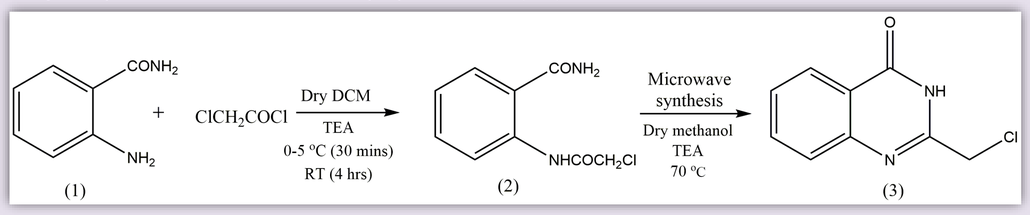
- To develop and synthesize molecules by conventional and microwave methods.
- To screen molecules as FAK inhibitors using computational studies
- To conduct in vitro cytotoxicity assays and FAK inhibition studies on MCF-7 and MDA-MB-231 cancer cell lines.

Experimental

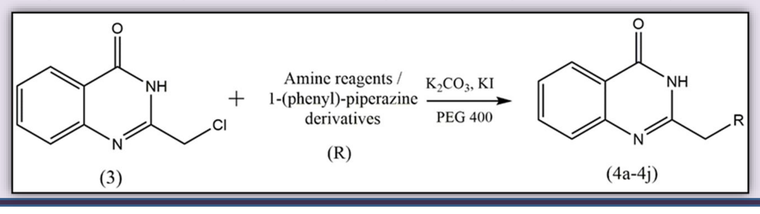
Step 1: Synthesis of 1-(phenyl)-piperazine derivatives [Intermediate I]



Step 2: Synthesis of 2-chloromethyl quinazolin-4(3H)-one



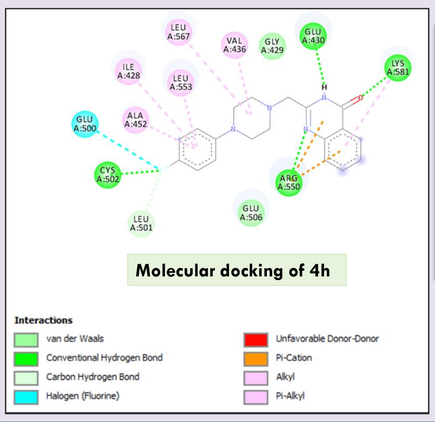
Step 3: General procedure for the synthesis of quinazolin-4(3H)-one – amine analogues (4a-4j)

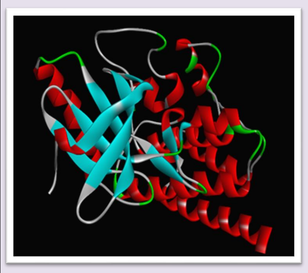


Molecular docking

- 3D structure of FAK (PDB ID: 6I8Z) was used for docking of 10 synthesized molecules and compared with pharmacophore model of 8 known FAK inhibitors under clinical trials to identify a novel inhibitor.
- Autodock tool (Autodock 1.5.6) was used for molecular docking, and the docked complex molecules were visualised and interpreted using Biovia discovery studio 2020 client.

Code	Binding Affinity (kcal/mol)
4a	-7.3
4b	-7.4
4c	-7.6
4d	-7.7
4e	-7.3
4f	-7.2
4g	-7.0
4h	**-8.9**
4i	**-8.9**
4j	-8.6





FAK protein (PDB ID: 6I8Z)

Biological evaluation

The synthesized molecules were evaluation on human cancer cell lines: MCF-7 and MDA-MB-231 (breast cancer). The study was carried out using MTT assay.

Cell lines	Sample Code	Concentration	% cytotoxicity
MCF-7	4a	40 ppm	28.57 ± 11.92
	4b	40 ppm	55.74 ± 54.72
	4c	40 ppm	23.51 ± 9.29
	4d	40 ppm	12.95 ± 10.73
	4h	40 ppm	41.28 ± 45.4
MDA-MB-231	4a	40 ppm	28.57 ± 11.98
	4b	40 ppm	25.37 ± 3.26
	4c	40 ppm	60.22 ± 2.47
	4d	40 ppm	82.05 ± 7.68
	4h	40 ppm	48.08 ± 7.15

ADME Prediction

Code	Mol Wt	Log P	HBA	HBD	CNS	Human oral absorption	% Human oral absorption	Rule of Five
Standard	**<500**	**≤ 5**	**≤ 10**	**≤ 5**	**-2 to +2**	**1-low 2-medium 3-high**	**>80% is high <25% is low**	**Max 4**
4a	243.31	1.479	5.5	1	1	3	82.986	0
4b	245.28	0.526	7.2	1	1	3	77.297	0
4c	229.28	0.405	6.5	1	0	2	74.792	0
4d	244.30	-0.046	7.0	2	1	2	57.925	0
4e	258.33	0.323	7.5	1	1	2	64.044	0
4f	272.35	0.689	7.5	1	1	2	66.838	0
4g	288.35	-0.182	9.2	2	1	2	53.866	0
4h	338.39	2.811	6.5	1	1	3	90.260	0

Results and Conclusion

- A series of quinazolinone-amine hybrid molecules were designed and synthesized using conventional and microwave method and evaluated as a potential anticancer agent. The synthesized molecules (4a-4j) were characterised using IR, NMR and MS spectroscopy.
- FAK inhibitors in clinical trials as mainly contains fluoro (-F) that augments the cytotoxic activity. Molecular docking studies indicated **4h** and **4i** has maximum binding affinity of **-8.9 kcal/mol** that is attributes to –F and –NO_2 group respectively.
- In quinazolinone-amine series, **4h** emerged as an attractive lead for development for potent FAK inhibitors in cancer therapeutics.

References

- Golubovskaya V.M.; Focal Adhesion Kinase as a Cancer Therapy Target. Anticancer Agents Med Chem. 2010 Dec; 10(10): 735–741.
- Swamy Murahari M, Prakash JR, Kar SS, Kumar GT, Raj VP, et al. Synthesis, characterization and in vitro anti-cancer activity of quinazolinone derivatives. J pharm res. 2012;55:2743–6.
- Acharyulu PVR, Dubey PK, Prasada Reddy PVV, Suresh T. Synthesis of new 4(3H)-quinazolinone derivatives under solvent-free conditions using PEG-400. Arkivoc. 2008;2008:104–11.

The 7th International Electronic Conference on Medicinal Chemistry
01–30 NOVEMBER 2021 | ONLINE