Pharmacophore-based drug design and synthesis of potential CDK2 inhibitors as anticancer entities

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INTRODUCTION

- Cancer is an irregular cell growth that uncontrollably divides with the potential for invasion or spread to other parts. Genetic or epigenetic modifications in somatic cells may ultimately be cancerous to human cells.
- Cyclin-dependent kinases (CDKs) are essential regulatory enzymes that operate all transitions in the cell cycle and are under strict control to ensure the cell division successfully. Human cells possess 20 CDKs and 29 cyclins. CDKs play a significant role including control of gene transcription, metabolism, and neuronal function.
- Cyclin-dependent Kinases (CDK2) is a Serine/Threonine protein kinase with 298 amino acid residues and a molecular weight of 34 kDa. Cdk2 is a central regulator of the cell cycle, with functions in inactivating RB1 (pRb) tumour suppressor family phosphorylation and regulating transitions between G1 / S phase.
- Implication of pyrazole scaffold have rendered it an important tool for the innovation of new pharmacological agents in multiple therapeutic classes viz. antimicrobial, anticonvulsant, anticancer, analgesic, anti-inflammatory, anti-tubercular, cardiovascular etc. ATP-competitive inhibitors from different chemical classes of CDK2 pyrazole shows a potency for the further studies to increase the enzyme inhibiting activity by using pyrazole core.

AIM & OBJECTIVES

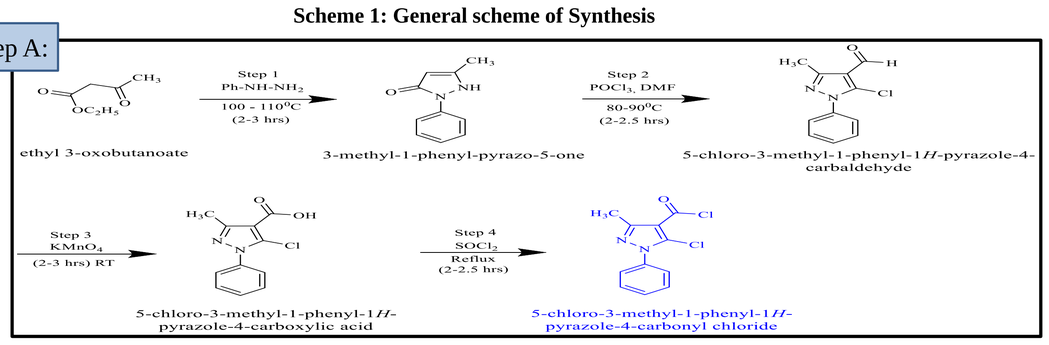
AIM: The aim of the research project is to synthesize substituted 1-Phenylpyrazole analogues and evaluate them for potential cytotoxic activity.

Objectives:

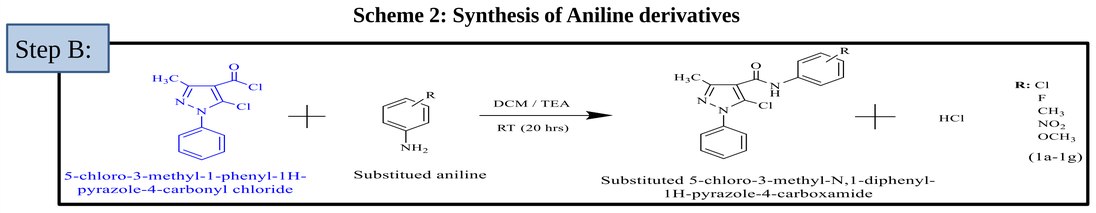
1. To design and optimize molecules as CDK2 inhibitors.
2. To synthesize substituted 1-phenylpyrazole analogues.
3. To characterize the synthesized compounds using FTIR, NMR, and MS analysis.
4. To evaluate the compounds for cytotoxic activity on MCF-7 (breast cancer) cell lines.

EXPERIMENTAL

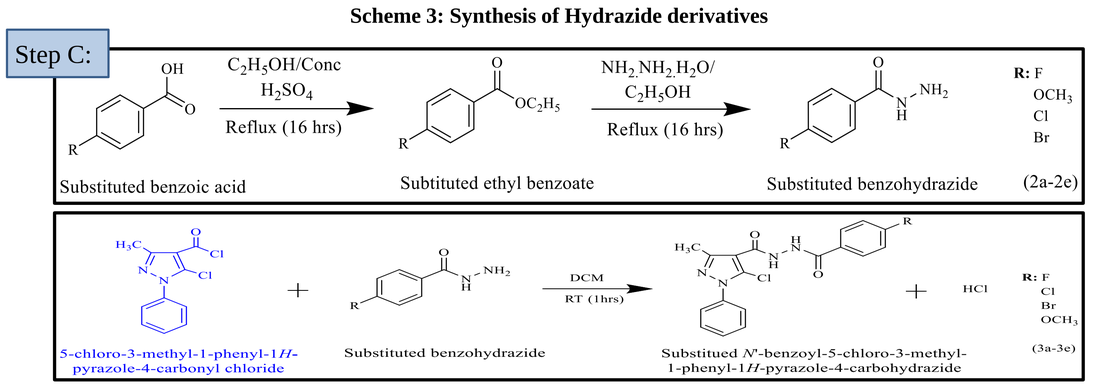
Scheme 1: General scheme of Synthesis

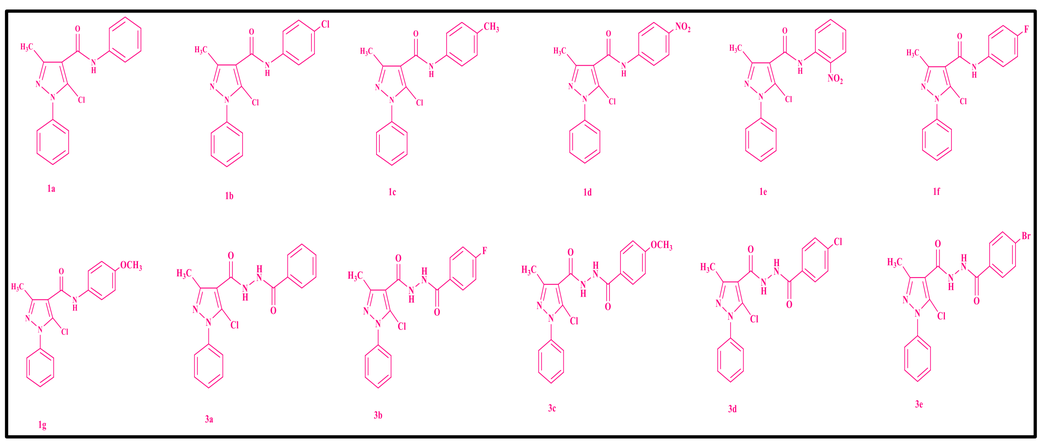


Scheme 2: Synthesis of Aniline derivatives



Scheme 3: Synthesis of Hydrazide derivatives





COMPUTATIONAL STUDIES

- The 3D structure of CDK2 (PDB ID: 1VYZ) was docked with the 12 synthesized compounds and were compared to a pharmacophore model of 12 known CDK2 inhibitors that are currently in clinical trials to identify potential inhibitor.
- The Auto Dock Tool (Auto dock 1.5.6) was used for molecular docking, and the docked complex compounds were visualized and interpreted using Schrodinger (Qikprep).
- The docking interaction of 3d with 1VYZ has the highest binding affinity of -7.6 Kcal/mol. The hydrazide group (NH) of 3d shows H-bond interaction with Asp 145. Thus, 3d can be considered to be a good lead for development of CDK2 inhibitors.

Table 1: Binding affinity of synthesized compounds

Compound Code	Binding Affinity (kcal/mol)
1a	-7.3
1b	-7.5
1c	-7.6
1d	-7.2
1e	-7.0
1f	-7.5
1g	-7.2
3a	-7.1
3b	-7.7
3c	-7.5
3d	-7.6
3e	-7.3

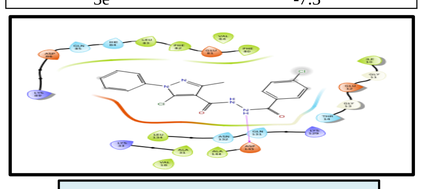


Fig 1: Docked pose of analogue 3d

BIOLOGICAL EVALUATION

- All the synthesized compounds were screened for cytotoxicity activity against breast cancer cell line MCF-7 using MTT assay . The synthesized analogues were found to exhibit good cytotoxicity against MCF-7 cell lines.
- The cytotoxicity assay indicated all compounds to exhibit good activity with 1e and 3d showing the highest % cytotoxicity in MCF-7 cell line.

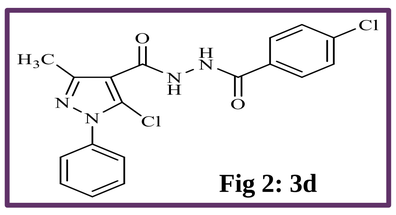


Fig 2: 3d

Table 2: Cytotoxic activity of synthesized compounds

Cell line	Sample code	Concentration	% Cytotoxicity
MCF 7	1a	40ppm	79.31 ± 20.63
	1b	40ppm	73.79 ± 35.19
	1c	40ppm	32.75 ± 9.97
	1d	40ppm	148.13 ± 11.74
	1e	40ppm	93.03 ± 35.03
	1f	40ppm	72.27 ± 42.65
	1g	40ppm	86.75 ± 16.70
MCF 7	3a	40ppm	57.03 ± 62.18
	3b	40ppm	64.48 ± 20.37
	3c	40ppm	72.13 ± 23.99
	3d	40ppm	100.34 ± 58.50
	3e	40ppm	87.03 ± 20.63

Table 3: ADME prediction

Property	Standard range	Synthesized Molecules											
		1a	1b	1c	1d	1e	1f	1g	3a	3b	3c	3d	3e
Molecular weight	<500	311.70	346.21	325.79	356.76	356.76	329.76	341.79	354.79	372.78	384.82	389.24	433.69
QP log P $^{O}/_{W}$	-2.0 to 6.5	4.39	4.88	4.70	3.68	3.78	4.61	4.49	4.27	4.51	4.36	4.77	4.84
HBA	≤10	3.5	3.5	3.5	4.5	4.5	3.5	4.2	4.5	4.5	5.2	4.5	4.5
HBD	≤5	1	1	1	1	1	1	1	0.5	0.5	0.5	0.5	0.5
CNS	-2 to +2	1	1	1	-2	-1	1	0	0	0	-1	0	0
Human oral Absorption	1-Low 2-Medium 3-High	3	1	1	3	3	3	1	1	1	1	1	1
% Human Oral Absorption	<25% poor; >80% good	100	100	100	94.92	100	100	100	100	100	100	100	100
Rule of Five	0 Best	0	0	0	0	0	0	0	0	0	0	0	0

RESULTS

- The computational studies indicated that compound 3d with electro-withdrawing chloro group showing good binding activity with the native ligand.
- The % cytotoxicity of 5-chloro-N'-(4-chlorobenzoyl)-3-methyl-1-phenyl-1H-pyrazole-4-carbohydrazide (3d) were found to be highest at concentration of 40 ppm.

CONCLUSION

- The cytotoxicity assay indicated all compounds to exhibit good activity with 3d showing the highest % cytotoxicity in MCF-7 cell line.
- The computational and cytotoxicity data indicated 3d to be the most active and it can be considered as a good lead for development of CDK2 inhibitors as anticancer agents.

REFERENCES

- Bačević K, Lossaint G, Achour TN, Georget V, Fisher D, Dulić V. Cdk2 strengthens the intra-S checkpoint and counteracts cell cycle exit induced by DNA damage. Sci Rep. 2017;7(1):1–14.
- Bennani FE, Doudach L, Karrouchi K, Ansar M, El M, Faouzi A. Overview of recent developments of pyrazole derivatives as an anticancer agent in different cell line. Bioorg Chem [Internet]. 2019;103470.
- Chohan TA, Qian H, Pan Y, Chen J-Z. Cyclin-Dependent Kinase-2 as a Target for Cancer Therapy: Progress in the Development of CDK2 Inhibitors as Anti-Cancer Agents. Curr Med Chem. 2014;22(2):237–63.