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Synthesis and molecular docking studies of few novel Pyrimidine derivatives

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ABSTRACT

This study was designed to synthesize some novel pyrimidine derivatives by using chalcone and thiourea. 10 different compounds thus formed were further reacted with ethylchloroacetate and then with hydrazine hydrochloride. The synthesized pyrimidinethioester hydrazide were subjected to docking studies against protein Human Cyclin Dependent Kinase 2 Complexed with the CDK4 Inhibitor (1GII) using Argus Lab software. The result indicates that the synthesized derivatives shows ligand bind pose energy varies from -8.35388 kcal/mol to -10.7195 kcal/mol. Of the 10 compounds, compound IV shows ligand bind pose energy -10.0084 kcal/mol with 4 hydrogen bond having best anticancer activity. This study also suggested that all the synthesized pyrimidine derivatives show potent anticancer activity than the standard drug 6-mercaptopurine.

Key Words: Pyrimidine, 6-mercaptopurine, 1GII, Argus Lab.

INTRODUCTION:

Pyrimidines are the most important six membered heterocyclic containing 2 nitrogen atoms in 1 and 3 positions. Pyrimidine was isolated by Gabriel and Colman in 1899. Pyrimidines are the most important member of all the diazines as this ring system occurs widely in living organisms[1]. Pyrimidines have considerable biological importance because of their relation to the nucleic acids and forms the building blocks of DNA and RNA[2]. pyrimidine derivatives are used as anticancer agents, nucleic acid analogues, drugs used for hyperthyroidism, antimalarial agents, antibiotics, antitubercular agents, antihypertensive and diuretic agents[3].

Pyrimidine derivatives are widely used as anticancer drug as they are cell cycle specific which kills only actively dividing cells as their toxicity is expressed in S phase of cell cycle. e.g. cytarabine, 5-fluorouracil. It is used alone or in combination therapy for the treatment of cancer e.g. COAP[4].

Neoplasia literally means “new uncontrolled growth”. Cancers account for 20-25% of deaths in clinical practices. The antimetabolite, pyrimidine and purine, most commonly stop the de novo purine synthesis of DNA by inhibiting the formation of nucleotides[5].

Cyclin-dependent kinase 2 also known as cell division protein kinase 2 is an enzyme in humans encoded by CDK2 gene. Cyclin-dependent kinase 2 is a catalytic subunit of cyclin-dependent kinase complex, whose activity is restricted to G1-S phase of the cell cycle. This protein associates with and is regulated by the regulatory subunits of the complex including cyclin E or A which binds to G1 phase. Cyclin-dependent kinase 2 is required for the transition from G1 to S phase. Docking is performed against the human protein Human Cyclin Dependent Kinase 2 Complexed with the CDK4 Inhibitor (1GII)[6].

Here various pyrimidine derivatives were synthesized from different chalcones and those compounds were subjected for docking studies (flexible docking). Docking is a method which predicts the preferred orientation of one molecule to another when bound to each other to form a stable complex[7]. For pyrimidine derivatives the docking was performed against the human protein Human Cyclin Dependent Kinase 2 Complexed with the CDK4 Inhibitor (1GII) using Argus Lab software.

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MATERIALS AND METHODS:

10% sodium hydroxide, ethanol, 1N hydrochloric acid, ethylchloroacetate, anhydrous potassium carbonate, hydrazine hydrochloride, ammonia solution, mechanical shaker, magnetic stirrer.

The melting points of the synthesized compounds were determined by open capillary tube method and uncorrected. A representative compound belonging to the series was taken for spectral studies and the rest were compared with the IR spectra and the structures were assigned on the basis of physical and chemical data (MP, R_f value etc.).

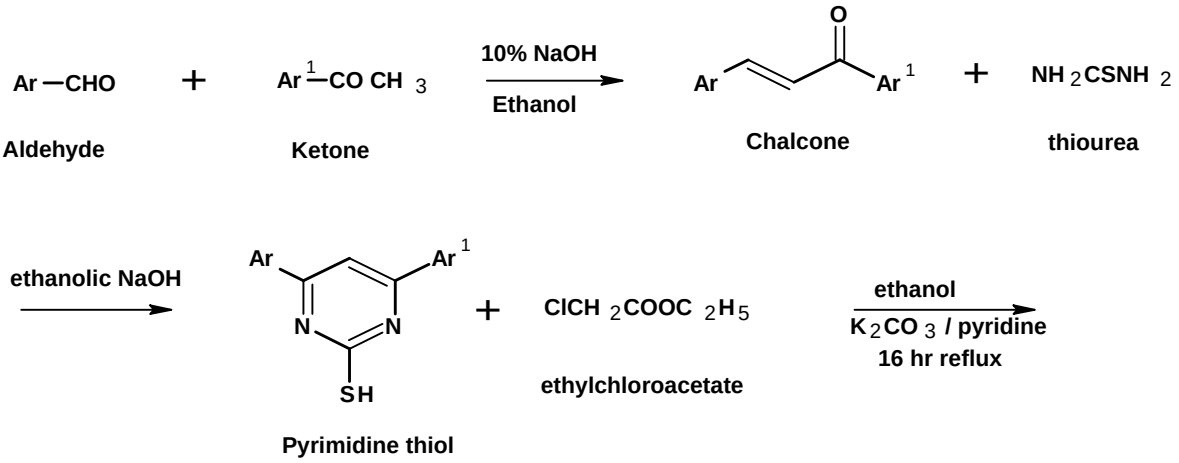
Procedure for the synthesis:

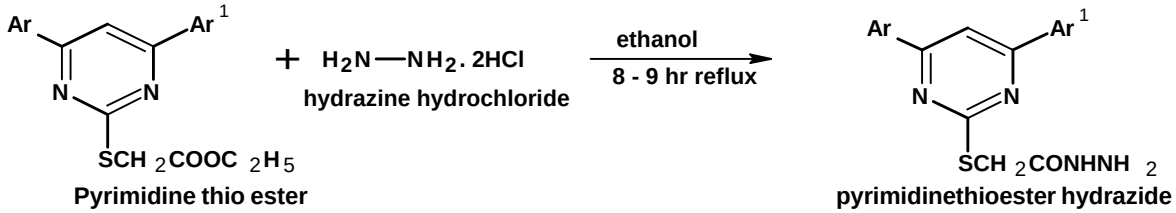
Step I: synthesis of different chalcones[8, 9, 10, 11]:

Placed 10mL 10% sodium hydroxide and 6.1 mL of rectified spirit in a conical flask. It was then kept on an ice bath and to this added equimolar

quantity (0.02M) of aldehyde and ketone with continuous stirring. After addition the conical flask was placed on mechanical shaker for 2-3hours to form a thick mass and then kept overnight under refrigeration. If the product was not formed, added sufficient amount of 1N hydrochloric acid to make it acidic and hence precipitated the chalcone. It was then filtered under suction on a Buchner funnel and washed it with cold ethanol and recrystallised from ethanol-water mixture (80%).

Step II: Synthesis of pyrimidine thiol from chalcone[9, 10, 12]:
Equimolar quantity (0.02M) of chalcone and thiourea were dissolved in 15ml of 10% ethanolic sodium hydroxide. It was then stirred with a magnetic stirrer for 2-3 hours and then poured in to 400mL of cold water with continuous stirring for 1 hour and then kept for refrigeration over night. The precipitate was filtered and recrystallised from ethanol-water mixture (70%).





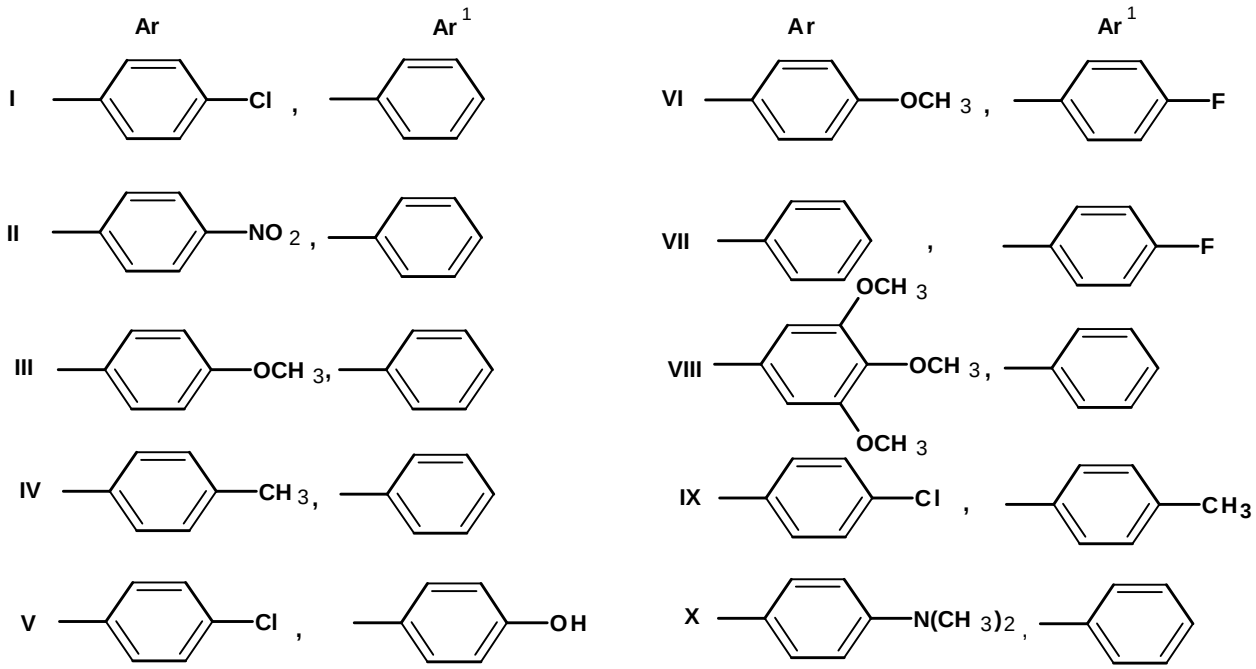
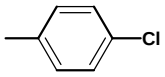
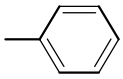
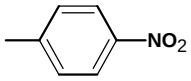
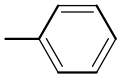
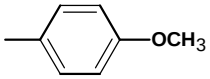
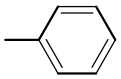
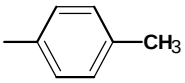
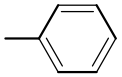
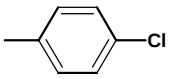
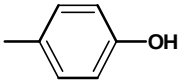
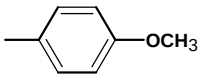
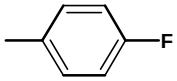
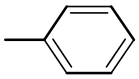
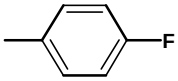
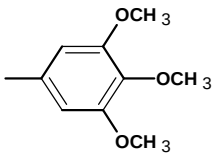
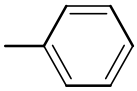
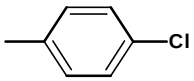
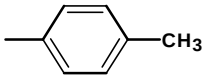
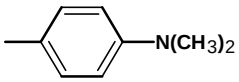
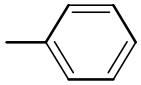


Table 1: physical data of the synthesized pyrimidine derivatives

Compound	Ar	Ar[1]	Molecular formula	Mol.wt	M.P^{0}C	% yield	R_f^*
I			$C_{18}H_{15}N_4OSCl$	370	83-86	78	0.43
II			$C_{18}H_{15}N_5O_3S$	387	118-121	69	0.74
III			$C_{19}H_{18}N_4O_2S$	368	70-73	65	0.69
IV			$C_{19}H_{18}N_4OS$	352	65-68	62	0.72
V			$C_{18}H_{15}N_4O_2SCl$	386	167-170	72	0.47
VI			$C_{19}H_{17}N_4O_2SF$	384	88-90	67	0.75
VII			$C_{18}H_{15}N_4OSF$	354	115-117	60	0.40
VIII			$C_{21}H_{22}N_4O_4S$	426	75-77	76	0.50
IX			$C_{19}H_{17}N_4OSCl$	381	60-63	69	0.47
X			$C_{20}H_{21}N_5OS$	379	69-72	74	0.49

solvent system: Chloroform: benzene (7:3)

Table 2: Summary of binding energy of all the synthesized compounds against the target protein 1GII

Sl. No	Compound code	No. of conformation	Binding energy Kcal/mol	No. of hydrogen bonds
1	I	140	-8.97021	1
2	II	140	-10.2068	2
3	III	140	-9.65656	Nil
4	IV	140	-10.0084	4
5	V	140	-8.35388	1
6	VI	140	-8.66031	1
7	VII	140	-9.67939	2
8	VIII	140	-8.81078	1
9	IX	140	-8.97164	1
10	X	140	-10.3229	Nil
11	6-mercaptopurine	140	-7.02586	1

Step III: Synthesis of pyrimidine thioester from pyrimidine thiol[13]:
Equimolar quantity (0.02M) of pyrimidine thiol and ethylchloroacetate were dissolved in rectified spirit and refluxed with 0.02M potassium carbonate for 14-16 hour. The mixture was filtered after reflux and the solvent was evaporated to collect the crude product. It was then recrystallised from ethanol-water mixture (70%).

Step IV: Synthesis of pyrimidinethioester hydrazide from pyrimidine thioester:
To 0.02M pyrimidine thioester added 0.025M hydrazine hydrochloride neutralized with ammonia solution and dissolved in rectified spirit. It was then refluxed for 8-10 hours. The solvent was then evaporated on a water bath to get the pyrimidinethioester hydrazide and recrystallized from ethanol-water mixture (70%).

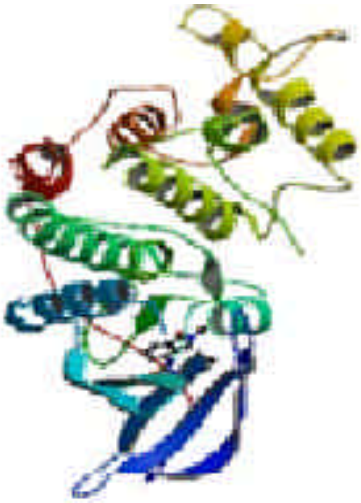


Fig 1: Structure of PDB ID: 1GII

Fig 2: Binding site of PDB ID: 1GII from CASTp

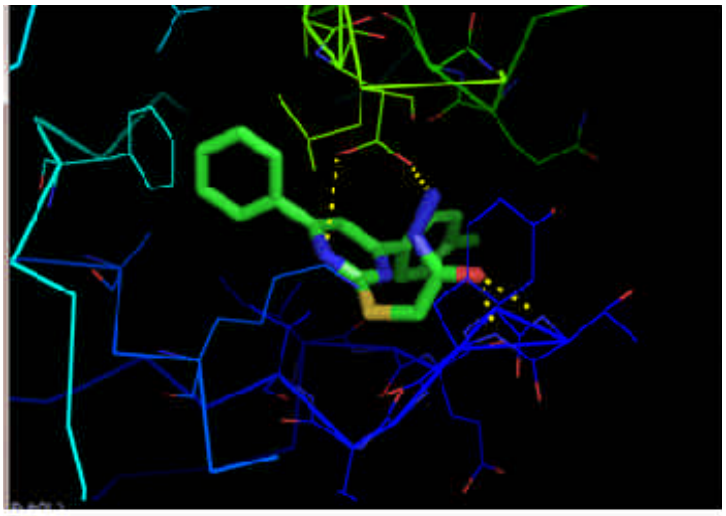


Fig 3. Dock complex of PDB ID : 1GII with compound IV

Molecular docking studies[14, 15, 16, 17]:

The structure of the protein Human Cyclin Dependent Kinase 2 Complexed with the CDK4 Inhibitor having PDB ID: 1GII was retrieved from Protein Data Bank. After obtaining the structure from Protein Data Bank, the possible binding sites of the protein were searched using Computed Atlas of Surface Topography of Proteins (CASTp). The synthesized molecules (inhibitor) and target protein were geometrically optimized and docked using the docking engine Argus Dock. Argus Lab is an electronic structure program that is based on the quantum mechanics. It predicts the potential energies, molecular structures, geometry optimization of structure, vibration frequencies of co-ordinates of atoms, bond length, bond angle and reaction pathway.

RESULTS AND DISCUSSION:

In this present work, totally 10 novel pyrimidine derivatives were synthesized in a single scheme. Step 1 involves the synthesis of chalcone and step 2 involves the formation of pyrimidine thiol from chalcone and thiourea. Step 3 is the esterification of pyrimidine thiol with ethylchloroacetate and step 4 is the formation of thioester hydrzide from thioester. The physical data of the synthesized compounds are given in **Table 1**. Molecular docking study was carried out for the synthesized 10 pyrimidine derivatives (I-X). **Fig 1** shows structure of protein Human Cyclin Dependent Kinase 2 Complexed with the CDK4 Inhibitor (1GII). The potential active site amino acids of 1GII complex were predicted using CASTp. The **Fig 2** shows the active site of the target protein. The target protein and inhibitors were geometrically optimized. All the 10 synthesized pyrimidine derivatives were docked against active site of target protein using Argus lab. Out of the 10 inhibitors analyzed **IV** has showed binding energy of -10.0064 kcal/mol with 4 hydrogen bond against the target protein. The binding energy and hydrogen bonds of all the inhibitors was shown in **Table: 2**. **Fig 3** shows the docked complex of the compound **IV** with the target protein.

CONCLUSION:

In this study, overall 10 pyrimidine derivatives were synthesized from different chalcones. All the synthesized compounds were subjected to docking against Human Cyclin Dependent Kinase 2 Complexed with the CDK4 Inhibitor (1GII) from humans using Argus lab software. The best drug was selected, depending upon the binding energy and hydrogen bond formed. Out of the 10 derivatives compound IV shows highest affinity towards the protein 1GII compared with the standard drug 6-mercaptopurine. Thus compound **IV** may act as a better and efficient anticancer drug.

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