Synthesis of some pyrazolo[3,4-*d*]pyrimidine derivatives of biological effect

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To everyone who made this work possible, I am indeed very thankful.

To my family

List of abbreviations

A548	lung cancer cell line
Asp	Aspartic acid
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
BCL2	B-cell lymphoma 2
CALD	Computer aided ligand design
CDK2	Cyclin dependent kinase 2
CML	Chronic myeloid leukemia
DMF-DMA	Dimethylformamide dimethylacetal
EAC	Ehrlich ascites carcinoma
EGFR	Epidermal growth factor receptor
ELISA	Enzyme linked immunosorbent assay
FBS	Fetal bovine serum
FCS	Fetal calf serum
Gln	Glutamine
Glu	Glutamic acid
GSK-3	Glycogen synthase kinase-3
HCT116	Human colon tumor cell line
IC_{50}	Half maximal inhibitory activity
IIe	Isoleucine
Leu	Leucine
Lys	Lysine
MAPK	Mitogen-activated protein kinase
MCF-7	Human breast adenocarcinoma cell line
Min	Minute
MOE	Molecular Operating Environment

O.D	Optical density
PBS	Phosphate-buffered saline
PDB	Protein data bank
p38α MAP	p38α Mitogen-activated protein
ppm	Part per million
RCSB	Research Collaboration for Structural Bioinformatics
Rmsd	Root mean standard deviation
Rpm	Revolutions per minute
SOD	superoxide dimutase
SRB	Sulphorhodamine-B
Src	Proto-oncogene tyrosine protein kinase Src
TCA	Trichloroacetic acid

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Abstract

Abstract

This thesis comprises four chapters. The first one is an introduction which comprises a brief survey on the different methods to synthesize pyrazolo[3,4-*d*]-pyrimidine compounds and their anticancer activity.

The second chapter deals with the aim of the work and schemes that have been carried out to obtain the new required pyrazolo[3,4-*d*]pyrimidine derivatives.

The third chapter clarifies the discussion of the experimental work for the preparation of the starting materials **I** and **II** and the newly synthesized compounds. Compound **II** was hydrolyzed to afford pyrazole-4-carboxylic acid **III** which was cyclized through the reaction with acetic anhydride to afford pyrazolo[3,4-*d*][1,3]oxazin-4-one **IV**. In addition, compounds **V**, **VI**, **VIIa-e** and **VIIIa-c** were synthesized from the reaction of pyrazolo[1,3]oxazin-4-one **IV** with phenylhydrazine, hydroxylamine hydrochloride, different aromatic amines and the appropriate amide or thioamide, sequentially.

Reacting the oxazinone derivative **IV** with hydrazine hydrate afforded 5-amino derivative **IX** which was condensed with some aromatic aldehydes, certain acid anhydrides, pyrazolo[3,4-*d*][1,3]oxazin-4-one derivative **IV** and urea or thiourea to afford compounds **Xa-e**, **XIa,b**, **XII** and **XIIIa,b**, respectively. On the other hand, reacting **IX** with chloroacetyl chloride gave **XIV**. Cyclization of **XIV** with ammonium acetate and ammonium thiocyanate yielded pyrazolopyrimidotriazine **XV** and thiazolidinone **XVI**, respectively. Compound **XIV** was subjected to nucleophilic substitution reactions with different aromatic amines and two pyrimidothiones **XVIIIa,b** to afford compounds **XVIIa-c** and **XIXa,b**, respectively.

Also, reacting pyrazolo[3,4-*d*][1,3]oxazin-4-one **IV** with formamide furnished pyrazolo[3,4-*d*]pyrimidin-4-one derivative **XX** which upon reaction with phosphorous oxychloride yielded the chloro derivative **XXI**. Additionally, a series of 4-substituted aminopyrazolo[3,4-*d*]pyrimidines **XXIIa-e** was obtained from the reaction of **XXI** with different aromatic amines.

Compound **XXIIb** was subjected to Claisen-Schmidt condensation reaction to give chalcones **XXIIIa-e** which subsequently reacted with hydrazine hydrate to afford pyrazoline derivatives **XXIVa-e**.

The structure elucidation of the new compounds was confirmed by elemental analysis, IR, ^{1}H NMR, ^{13}C NMR in addition to mass spectral data. Additionally, a brief account on the docking study was explained through the binding conformations in comparison with the cytotoxic results.

The fourth chapter consists of the experimental part of this work which contains the detailed procedures used for the synthesis of the starting materials **I** and **II**, the intermediates **IV**, **IX**, **XIV, XX**, **XXI** and **XXIIIa-e** and the new pyrazolo[3,4-*d*]pyrimidine compounds **V-VIIIa-c, Xa-e-XIIIa,b, XV-XVIIa-c, XIXa,b, XXIIa-e** and **XXIVa-e**. In addition, data obtained from the elemental and spectral analyses as well as their physical properties are given in this chapter. It also sheds the light on the anticancer activity of twenty-eight compounds of newly synthesized derivatives compared with doxorubicin as a standard cytotoxic agent. This chapter also clarifies the correlation between the results of molecular docking and the anticancer activity.

1. Introduction

1.1. Synthesis of some pyrazolo[3,4-*d*]pyrimidines

1.1.1. From pyrazole derivatives

1.1.2. From pyrimidine derivatives

1.2. Cancer and chemotherapy

1.2.1. Cancer

1.2.1.1. Cell cycle

1.2.1.2. Control of the cell cycle

1.2.2. Classification of chemotherapeutic agents

1.2.2.1. Hormone based therapy

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1.2.2.3. Antimetabolites

1.2.2.4. Intercalating agents

1.2.2.5. Spindle poisons

1.2.2.6. Topoisomerase inhibitors

1.2.2.7. Protein kinase inhibitors

1.3. Pyrazolo[3,4-*d*]pyrimidines as anticancer agents

1.4. Pyrazolo[3,4-*d*]pyrimidines as protein kinase inhibitors

1. Introduction

Pyrazolo[3,4-*d*]pyrimidines are of considerable chemical and pharmacological importance as purine isosteres.[1,2]

Various compounds with related structures were found to exhibit a diverse of pharmacological activities as anticancer[3-5], antiviral[6-9], antimicrobial[10,11], antimycobacterial[12,13], antifungal[14], antidepressant[15] and anti-inflammatory.[16-19]

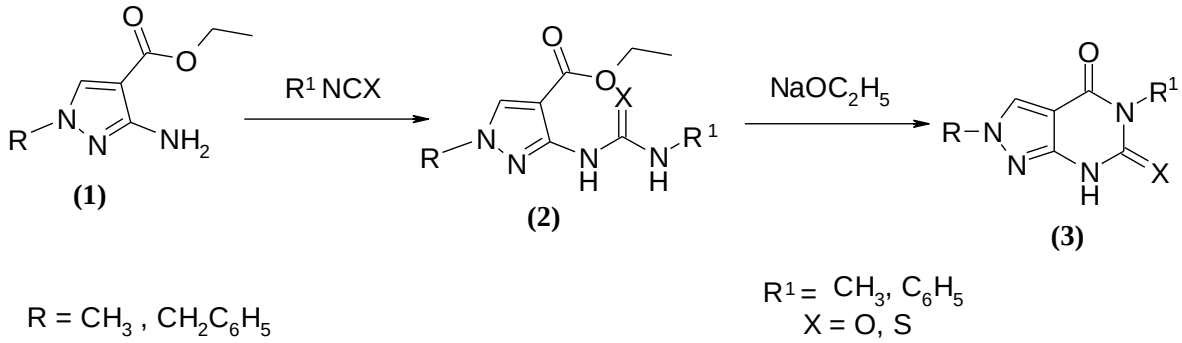
Based on the above findings, a brief survey is given below covering the synthetic pathways and biological importance of certain pyrazolo[3,4-*d*]pyrimidine derivatives as anticancer agents.

1.1. Synthesis of some pyrazolo[3,4-*d*]pyrimidines

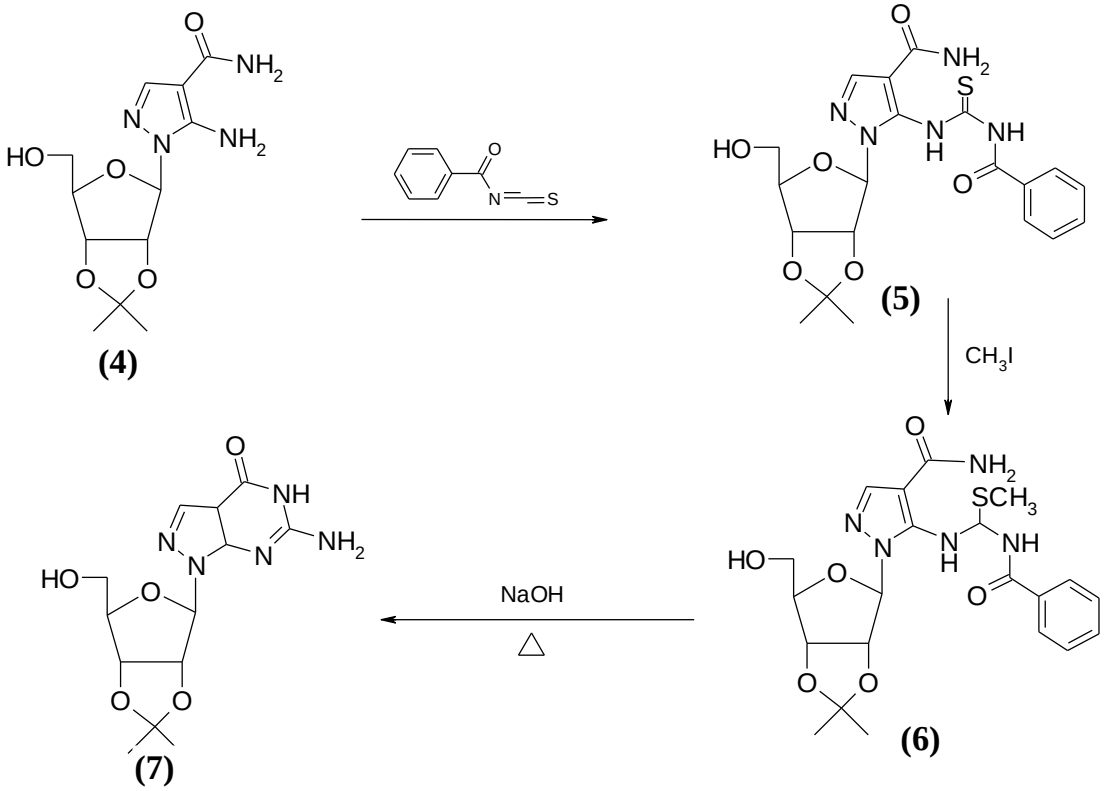
There are several methods for pyrazolo[3,4-*d*]pyrimidine synthesis depending on the starting nuclei. These methods include:

1.1.1. From pyrazole derivatives:

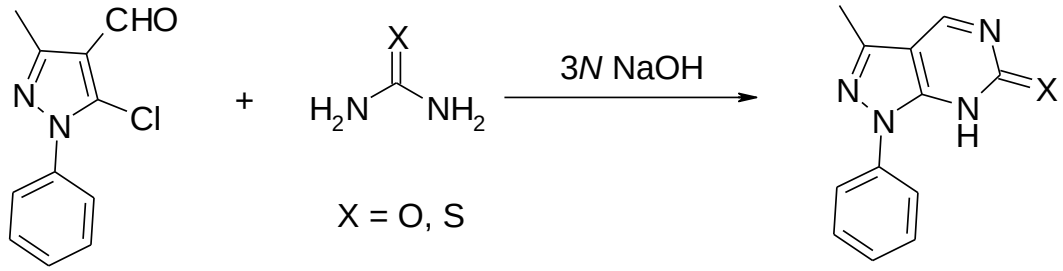
It was reported that some 2,5-disubstituted pyrazolo[3,4-*d*]pyrimidine derivatives **3** were obtained *via* condensation of ethyl 3-amino-1-substituted-*1H*-pyrazole-4-carboxylates (**1**) with isocyanates or isothiocyanate to yield certain pyrazolylurea derivatives **2** which were cyclized to **3** upon heating with sodium ethoxide in ethanol.[20]



Treatment of 5-amino-1-(2,3-*o*-isopropylidene-*β*-D-ribofuranosyl)-pyrazole-4-carboxamide (**4**) with benzoyl isothiocyanate gave compound **5** which was methylated to furnish methylthio derivative **6**. The latter was heated with 2*N* sodium hydroxide to give pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one **7**.[21]

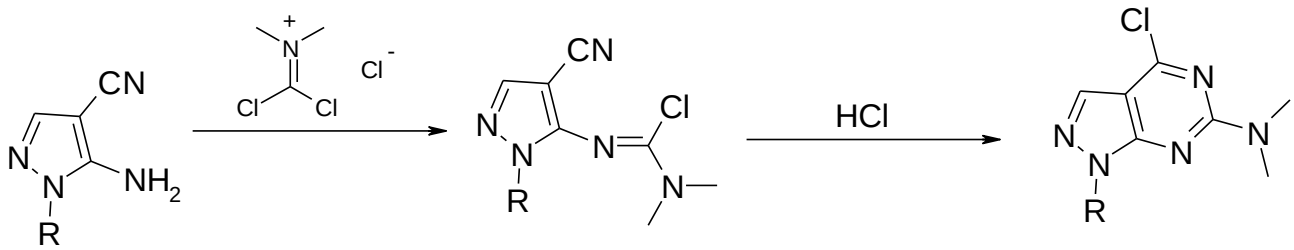


The formation of pyrazolo[3,4-*d*]pyrimidine derivatives **9** was achieved by heating the aldehydic derivative **8** with urea or thiourea in presence of 3*N* sodium hydroxide.[22]



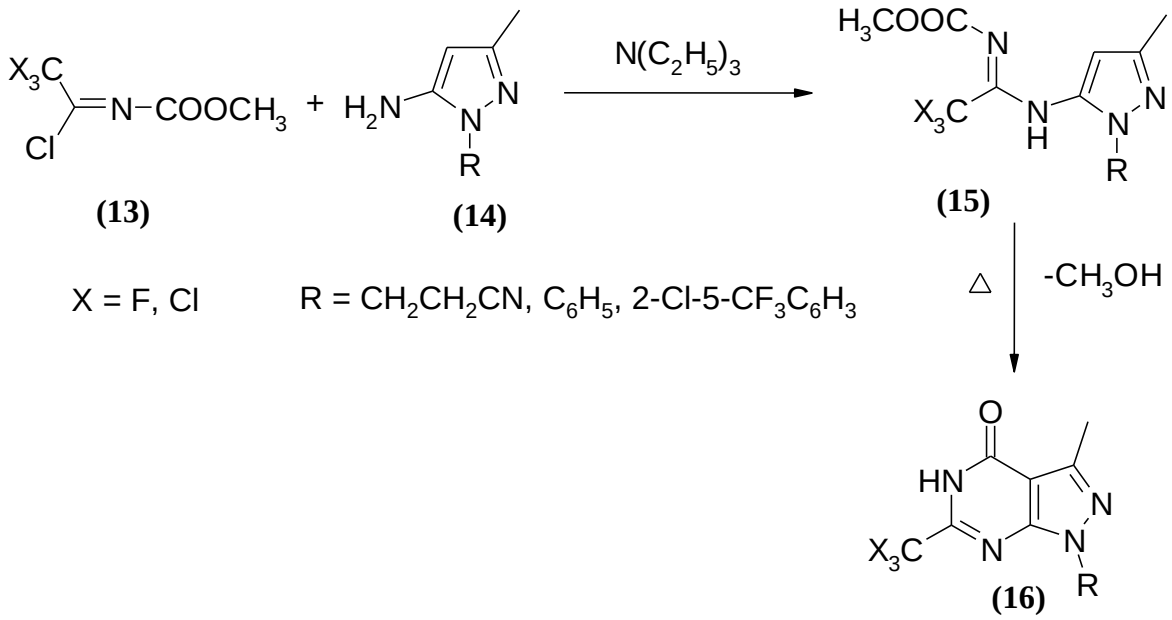
(8) **(9)**

In 2001, Quintela *et al*[23] illustrated a new strategy for the preparation of 4-chloro-6-dimethylaminopyrazolo[3,4-*d*]pyrimidine derivatives **12** *via* reaction of 5-aminocyanopyrazoles **10** with phosgene iminium chloride to yield the corresponding chloroamidines **11** which subjected to a stream of dry hydrogen chloride giving the target pyrazolopyrimidine derivatives **12**.

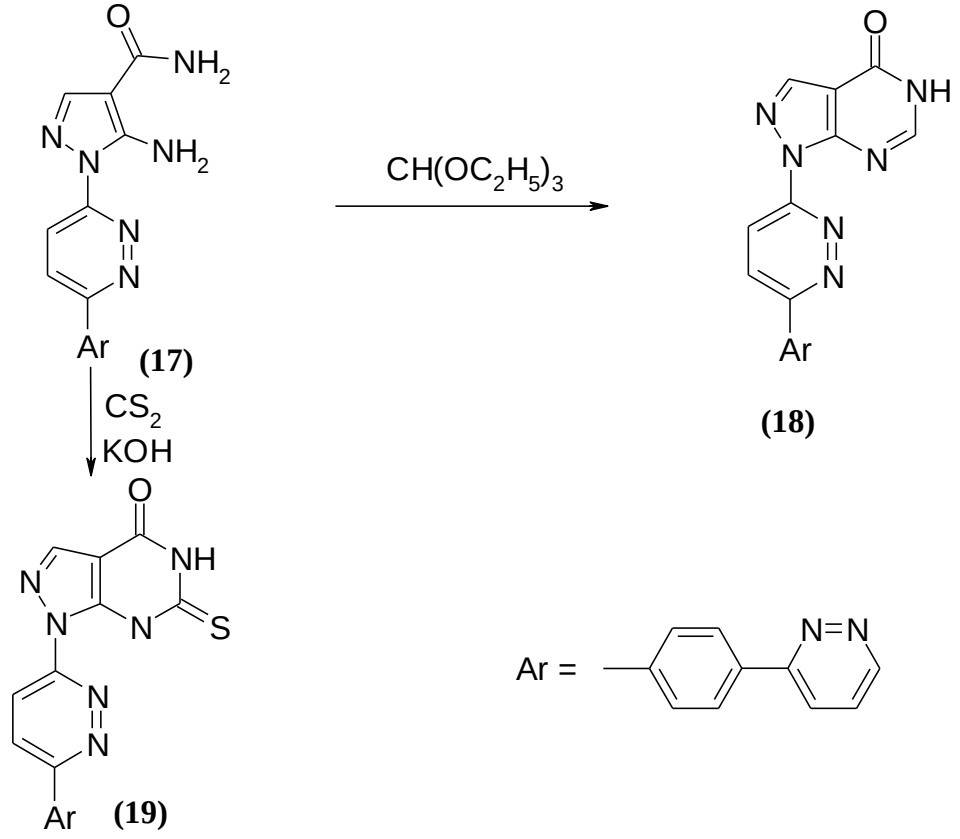


(10) R = C_6H_5, $C(CH_3)_3$ **(11)** **(12)**

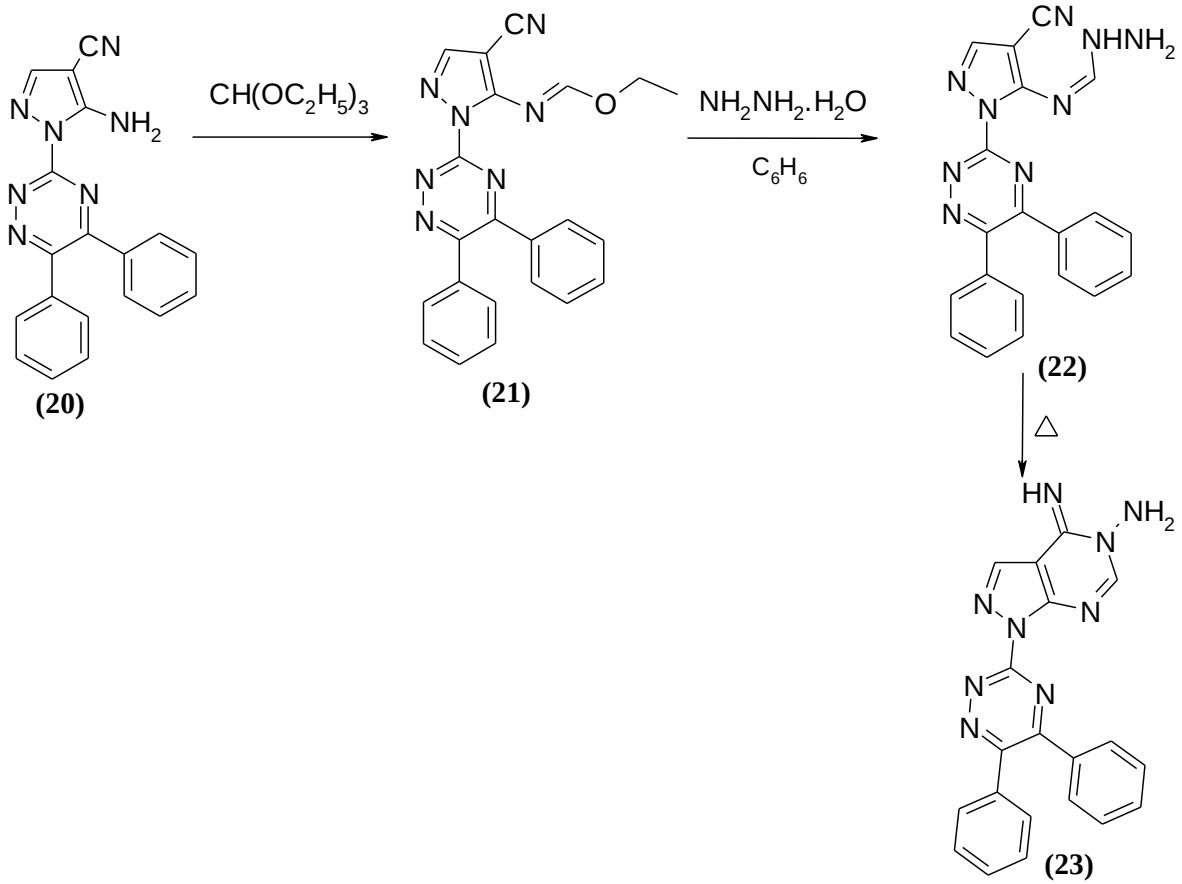
A new synthetic approach for the synthesis of 6-trihalomethyl-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-ones **16** was achieved by the reaction of *N*-(methoxycarbonyl)trihaloacetimidoyl chloride **13** with 5-aminopyrazoles **14** in benzene at room temperature in the presence of triethylamine to afford compounds **15**, which further cyclocondensed to compounds **16** through a thermal removal of one mole of methanol.[24]



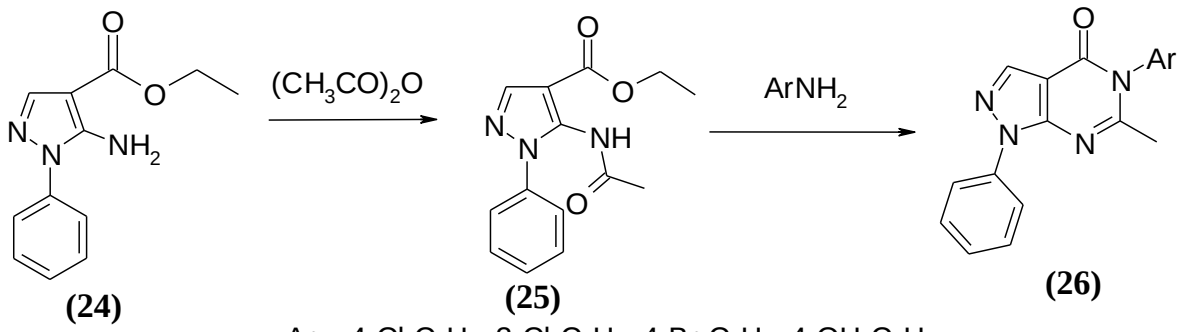
Later on, a new methodology for pyrazolopyrimidine synthesis was achieved by heating the *o*-amino amide **17** either with triethyl orthoformate or carbon disulphide that resulted in pyrazolo[3,4-*d*]pyrimidin-4-one derivatives **18** or **19**, respectively.[25]



Moreover, ethyl-4-cyano-1-[5,6-diphenyl-1,2,4-triazin-3-yl]-1*H*-pyrazol-5-ylimidoformate (**21**) was obtained *via* treatment of compound **20** with triethyl orthoformate in acetic anhydride. Heating compound **21** with hydrazine hydrate resulted in compound **22**, which further transformed into iminopyrazolo[3,4-*d*]pyrimidin-5-amine derivative **23** by heating in dry benzene.[26]

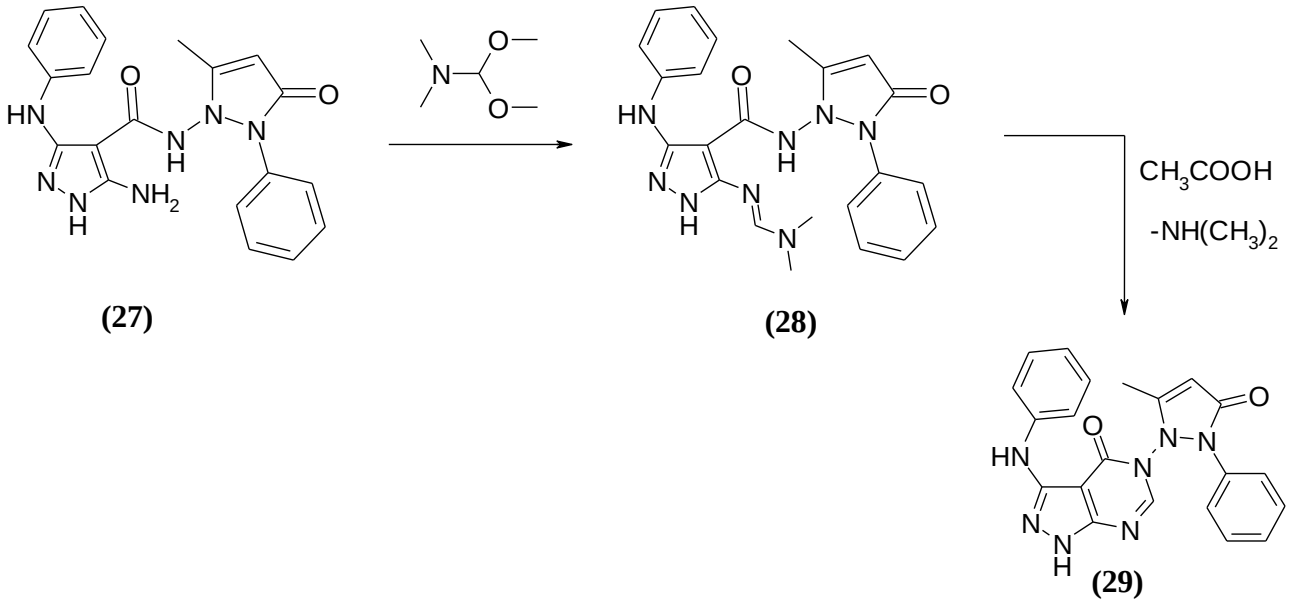


A facile pathway for synthesis of pyrazolo[3,4-*d*]pyrimidin-4-ones **26** was demonstrated by reaction of the *o*-amino ester derivative **24** with acetic anhydride to give *N*-acetyl derivative **25**. The latter was cyclized by heating with different aromatic amines in presence of phosphorous oxychloride to yield the target pyrazolo[3,4-*d*]pyrimidin-4-ones **26**.[27]

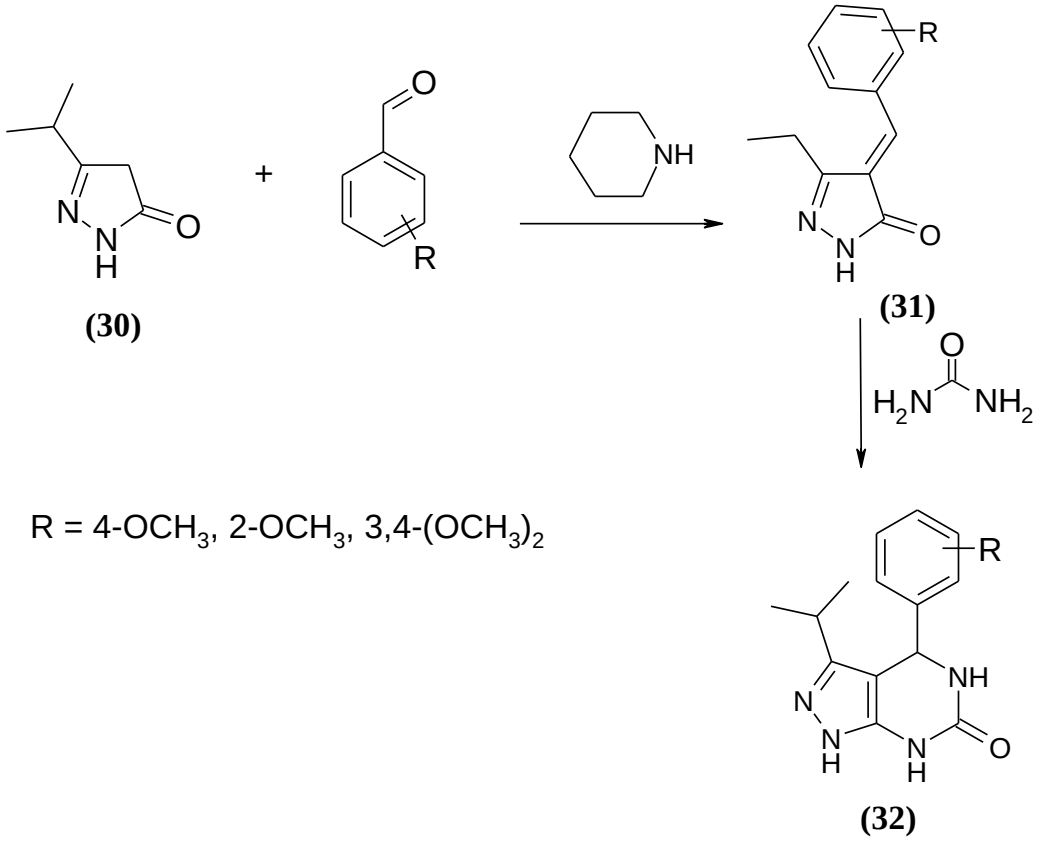


Ar = 4-Cl-C_6H_4, 3-Cl-C_6H_4, 4-Br-C_6H_4, 4-OH-C_6H_4

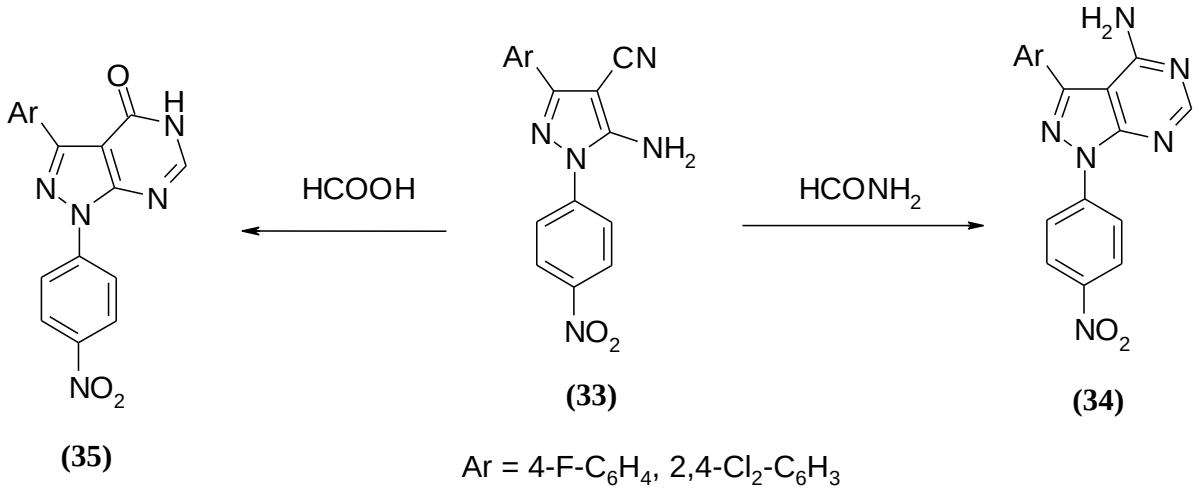
Furthermore, the reaction of *o*-amino amide derivative **27** with DMF-DMA in dry dioxane afforded *N*,*N*-dimethylaminomethyleneaminopyrazole derivative **28** which could be cyclized to pyrazolo[3,4-*d*]pyrimidine derivative **29** upon heating in glacial acetic acid *via* losing a mole of dimethylamine.[28]



In 2008, Akbari *et al*[29] synthesized a new series of pyrazolo[3,4-*d*]pyrimidin-6-one derivatives **32** in two steps. First, by knovenagel condensation of 5-isopropyl-2,4-dihydro-3-pyrazolone **(30)** with different aromatic aldehydes yielding 4-benzylidenepyrazolone derivatives **31**. Second, the latter was reacted with urea to yield the desired compounds **32**.

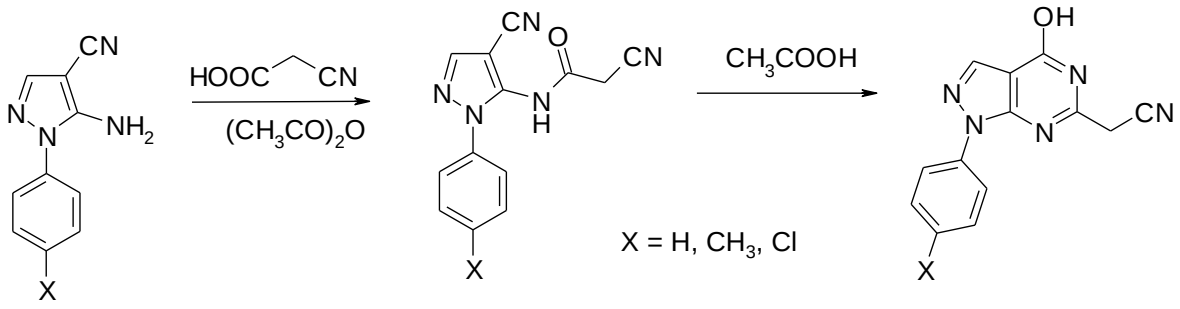


Additionally, cyclization of pyrazole derivatives **33** with excess formamide or formic acid gave the corresponding pyrazolo[3,4-*d*]pyrimidine derivatives **3**4 or **35**, respectively.[30]



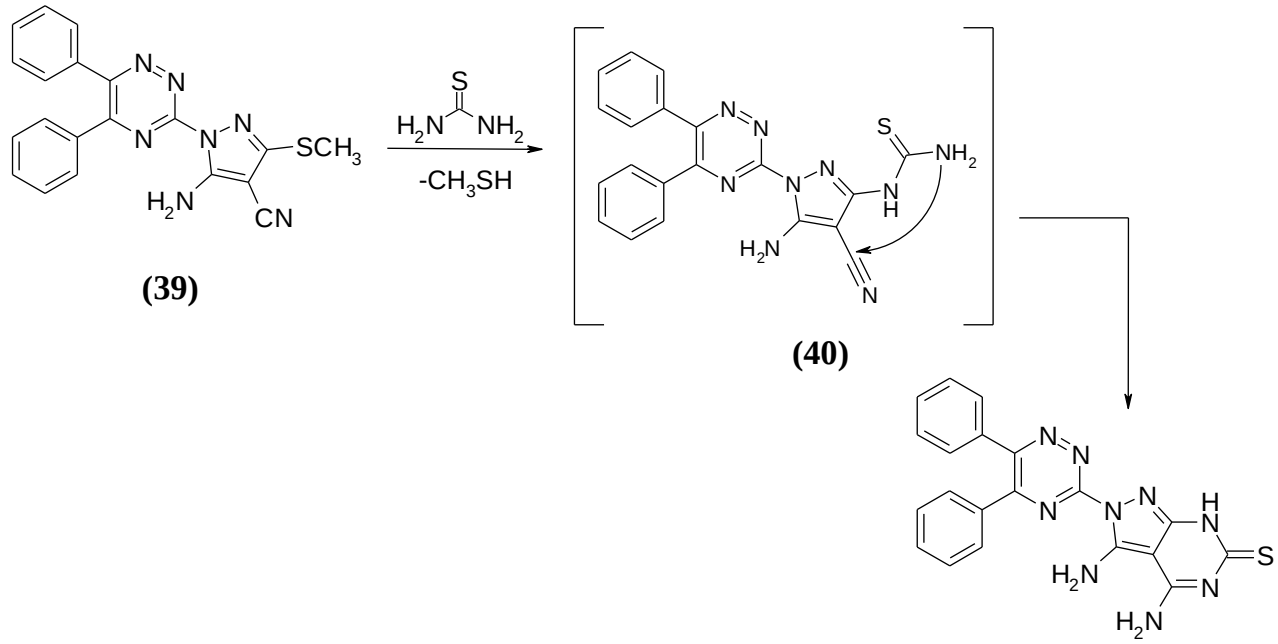
It was also reported that cyanoacetylation of *N*-substituted-5-amino-4-cyanopyrazoles (**36**) with a mixture of cyanoacetic acid and acetic anhydride

gave *N*-substituted-2-cyanoacetamide derivatives **37**. The latter was cyclized in glacial acetic acid affording 4-hydroxypyrazolo[3,4-*d*]pyrimidines **38**.[31]



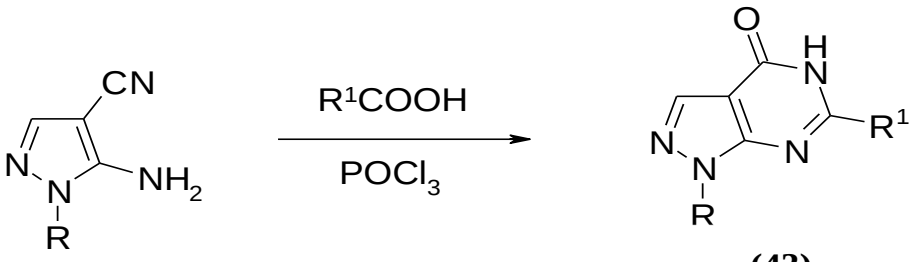
(36) **(37)** **(38)**

Furthermore, the reaction of compound **39** with thiourea in ethanolic sodium ethoxide furnished 3,4-diamino-2-(5,6-diphenyl-1,2,4-triazin-3-yl)-2,7-dihydro-6*H*-pyrazolo[3,4-*d*]pyrimidin-6-thione **(41)**.[32]



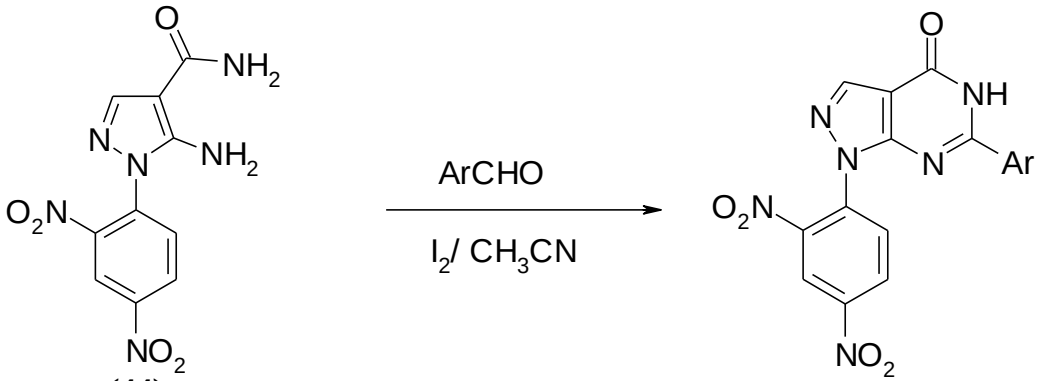
(39) **(40)** **(41)**

A facile and efficient one pot synthesis of 1,6-disubstituted pyrazolo[3,4-*d*]pyrimidin-4-ones **43** was developed *via* the reaction of 5-amino-*N*-substituted-1*H*-pyrazole-4-carbonitrile (**42**) with some different lower aliphatic acids in the presence of phosphorous oxychloride.[33]



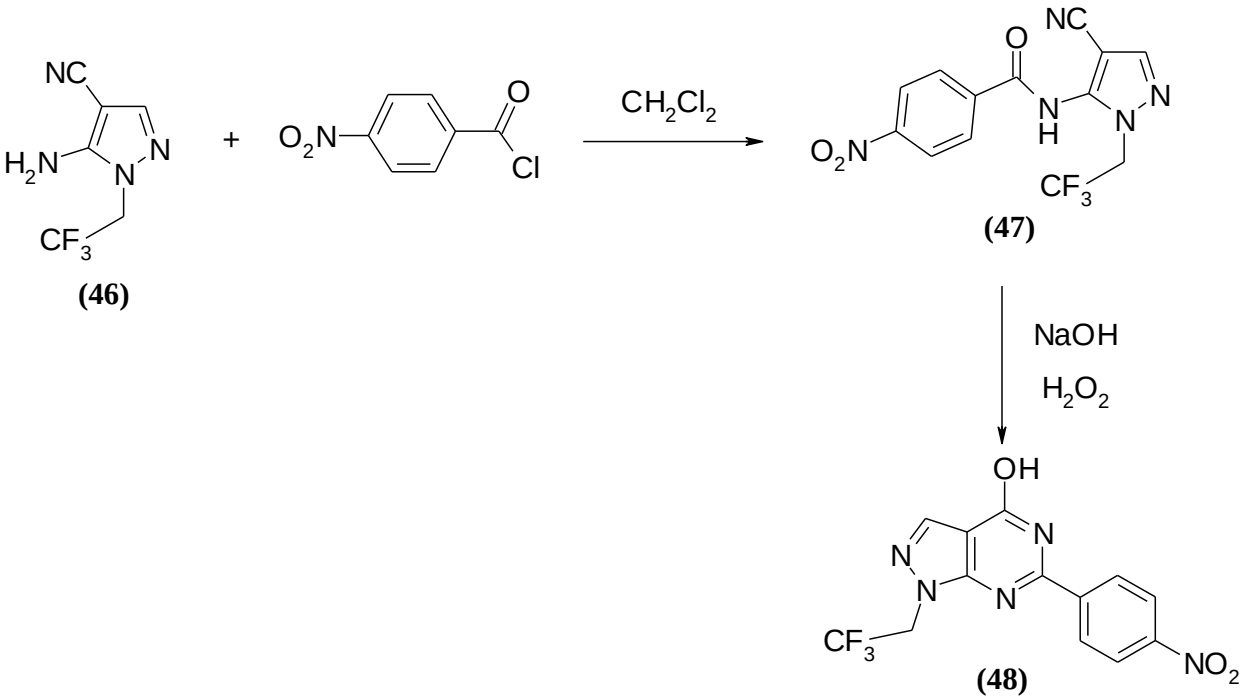
(42) R = 2,6-Cl_2-4-CF_3-C_6H_2, 4-CH_3O-C_6H_4 **(43)**
R^1 = H, CH_3, C_2H_5, CCl_3

Moreover, oxidative cyclization of 5-amino-1-(2,4-dinitrophenyl)-1*H*-4-pyrazolcarboxamide (**44**) with various substituted aromatic aldehydes in the presence of equimolar molecular iodine as a mild Lewis acid in boiling acetonitrile gave a new series of pyrazolo[3,4-*d*]pyrimidine derivatives **45** in good to excellent yields.[34]



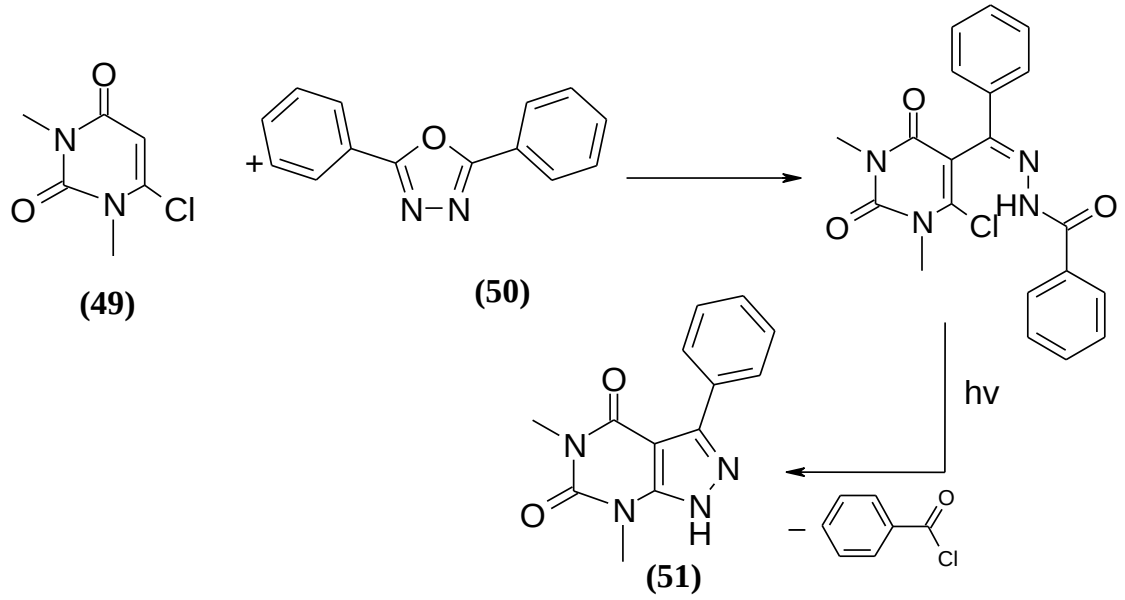
(44) Ar = 4-CH_3-C_6H_4, 4-CH_3O-C_6H_4, 4-Br-C_6H_4 **(45)**

Furthermore, benzoylation of 5-aminocyanopyrazole derivative **46** with *p*-nitrobenzoyl chloride in methylene chloride afforded the pyrazole derivative **47** which upon treatment with peroxide under reflux conditions provided pyrazolo[3,4-*d*]pyrimidine derivative **48**.[35]

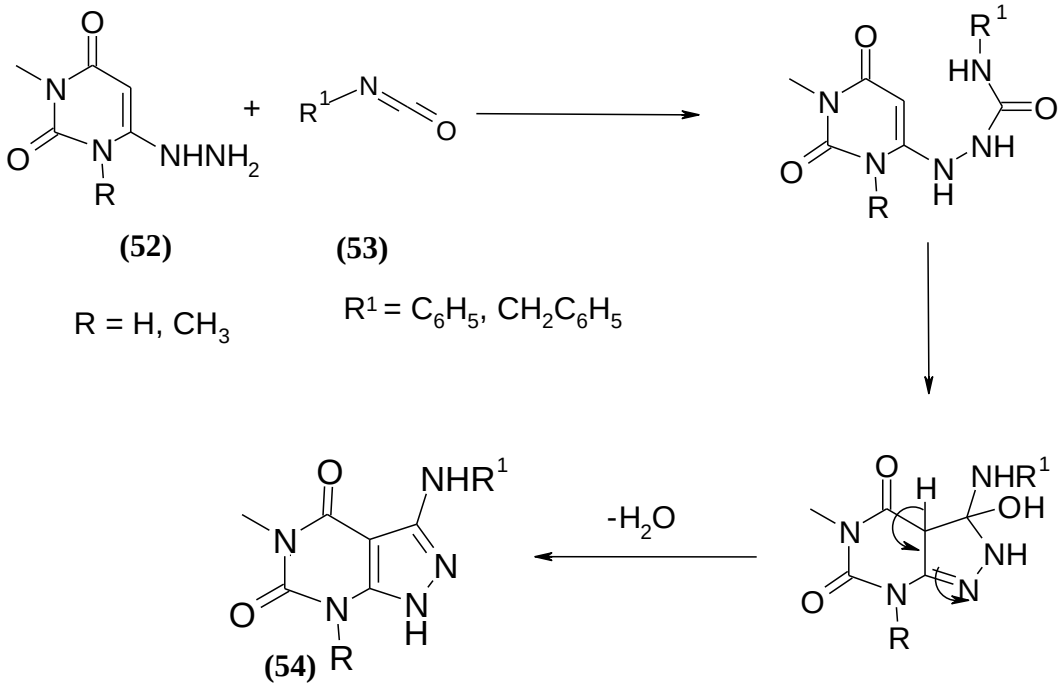


1.1.2. From pyrimidine derivatives:

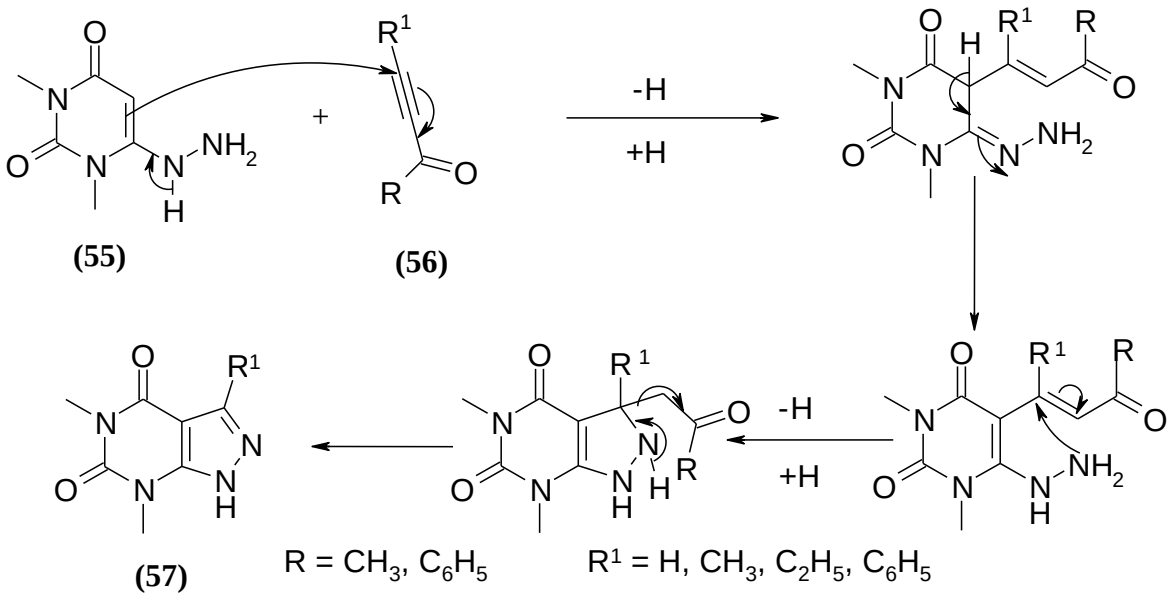
One method from pyrimidine derivative include photolysis of 6-chloro-1,3-dimethyluracil (**49**) with 2,5-diphenyl-1,3,4-oxadiazole (**50**) affording a pyrazolo[3,4-*d*]pyrimidine **51** with elimination of a mole of benzoyl chloride.[36]



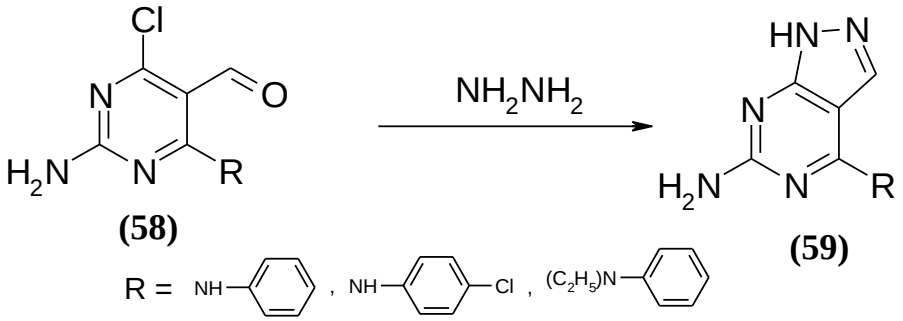
Another synthetic strategy utilizing 6-hydrazinouracils **52** with isocyanates **53** afforded unprecedented one-pot synthesis of pyrazolo[3,4-*d*]pyrimidines **54** in excellent yields. The reaction occurs *via* a nucleophilic attack of the hydrazine group onto the carbon atom of isocyanate which tautomerized to imine which might be formed during the reaction. The imine suffers another nucleophilic attack by C-5 of the uracil and followed by elimination of one mole of water to get the target compounds **54**.[37]



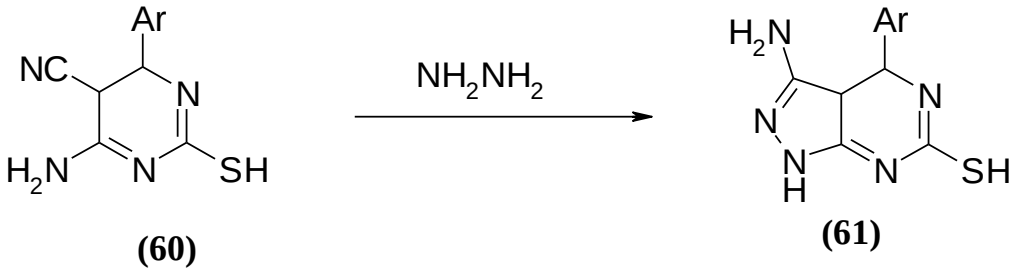
Prajapati *et al*[38] reported the preparation of pyrazolo[3,4-*d*]pyrimidine derivatives **57** *via* Michael addition of α-ketoalkynes **56** to 1,3-dimethyl-6-hydrazinouracil (**55**) followed by cyclization to pyrazole ring.



In 2008, a novel series of N^4-substituted-4,6-diaminopyrazolo[3.4-d]pyrimidines **59** was synthesized in good yield *via* reacting N^4-substituted-2,4-diamino-6-chloropyrimidine-5-aldehydes (**58**) with hydrazine hydrate.[39]



The literature survey showed that pyrazolo[3,4-*d*]pyrimidines **61** were prepared *via* one pot reaction of 4-amino-6-aryl-5-cyano-2-mercapto-5,6-dihydropyrimidines (**60**) with hydrazine hydrate in ethanol.[40]



1.2. Cancer and chemotherapy

1.2.1. Cancer:

Cancer is a disease in which the control of growth is lost in one or more cells leading to a solid mass of cells known as a tumor.[41] However, death is most often caused by spread of the primary tumor to one or more sites in the body (metastasis) to establish secondary cancerous growths.[42] Cancer cells acquire aberrations that favor their growth in the complex environments of living tissues; this includes their ability to recruit blood vessels into tumor masses, that is the process of angiogenesis.[43]

Cancer treatment often encompasses more than one approach, and the strategy adopted is largely dependent on the nature of the cancer and how far it has progressed. The main treatments are surgery, radiotherapy and chemotherapy.[43]

In chemotherapy, the anticancer chemotherapeutic agents interfere with cell growth and division in several different ways. Accordingly, knowledge of cell cycle is important to appreciate the mechanism of action of cancer chemotherapy.

1.2.1.1. Cell cycle:

The cell cycle is divided into a number of phases, normal nondividing cells are in resting phase (G0). When actively recruited into the cell cycle they then pass through four phases (Fig. 1):[44]

1- G1 (Gap 1): the growth phase in which the cell increases in size and prepares to copy its DNA.

2- S (synthesis): which allows doubling of the chromosomal material.

3- G2 (Gap 2): a further growth phase before cell division.

4- M (mitosis): where the chromosomes separate and the cell divides.

At the end of a cycle the daughter cells can either continue through the cycle, leave and enter the resting phase (G0) or become terminally differentiated.

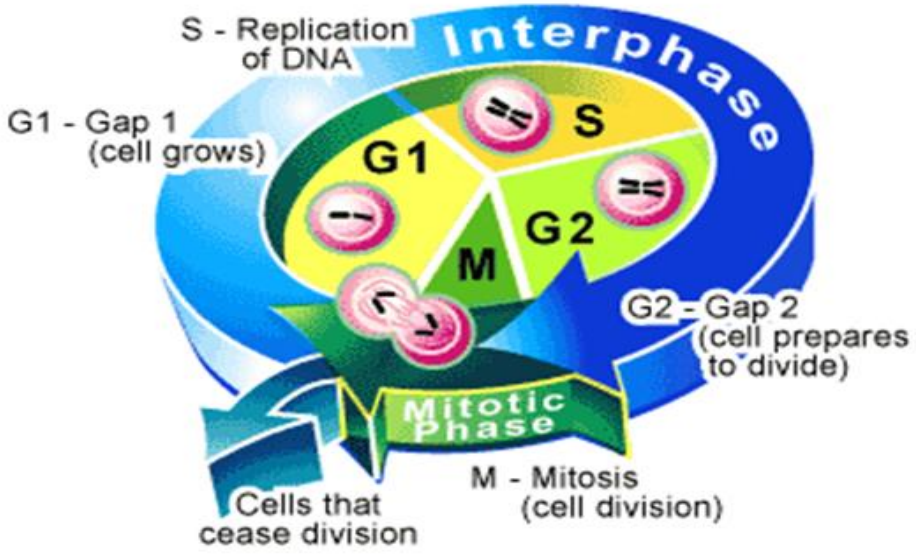


Fig.1. The phases of cell cycle.

1.2.1.2. Control of the cell cycle:

Protein kinases are known to regulate the cell cycle, among them cyclin dependent kinases (CDKs), Aurora, and Checkpoint kinases.[45] CDKs are serine/threonine kinases that must bind to cyclin proteins to become active. They were originally identified as essential regulators of cell cycle progression. They are required for the G1-to-S phase cell cycle transition, initiation of DNA replication, the G2-to-M phase cell cycle transition, and initiation of multiple mitotic events (Fig.2).[46]

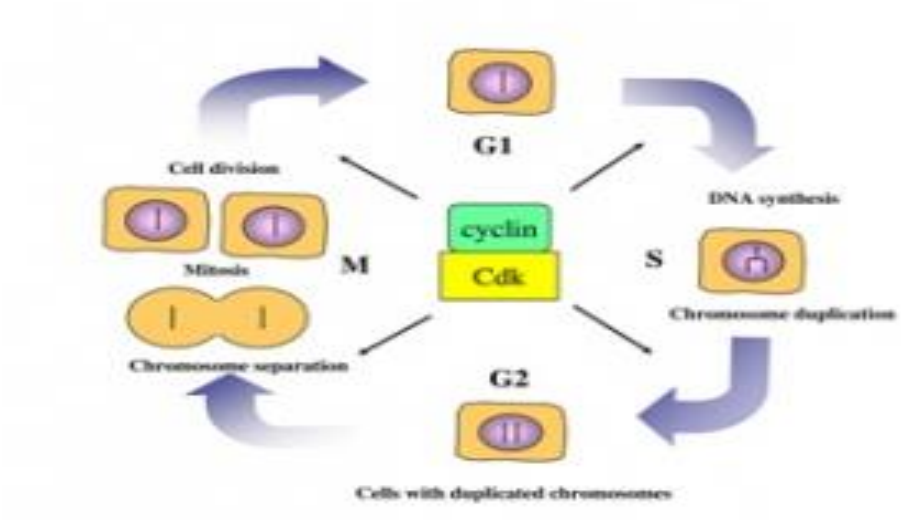


Fig.2. Cell cycle control by CDK-cyclin complex.

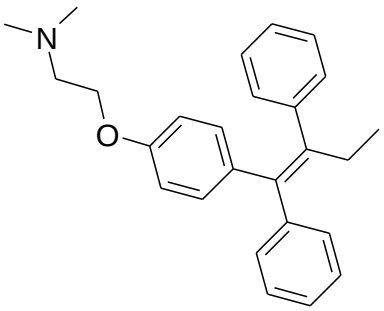
1.2.2. Classification of chemotherapeutic agents:

The anticancer chemotherapeutic agents can be classified into the following different classes:

1.2.2.1. Hormone based therapy:

Hormone based therapy is used for cancers which are hormone dependent. If the cancer cell requires a specific hormone, then a hormone can be administrated which has an opposing effect. Alternatively, hormone antagonists can be used to block the action of required hormone.[47]

Examples of this class include; antiestrogens e.g. tamoxifen (**62**)[48], antiandrogens e.g. flutamide (**63**)[49] and estrogens e.g. diethylstilbestrol (**64**).[50]



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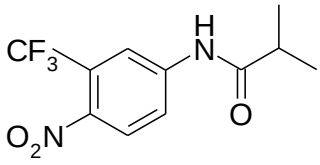

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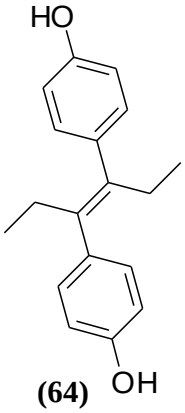
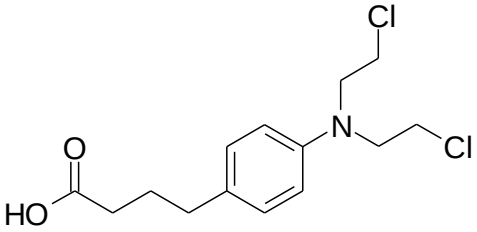


(64)

1.2.2.2. Alkylating agents:

Alkylating agents are highly reactive small molecules that bind covalently to electron-rich nucleophilic moieties in nucleic acids or proteins.[51] Examples include nitrogen mustards e.g. chlorambucil (**65**)[51], aziridines e.g. thiotepa (**66**)[52], nitrosoureas e.g. carmustine (**67**)[52] and alkyl sulphonates e.g. busulphan (**68**).[53]



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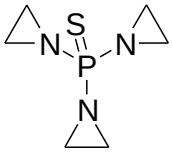

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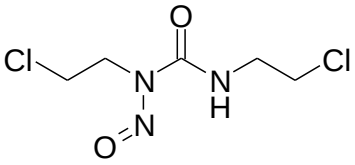

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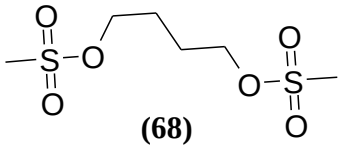
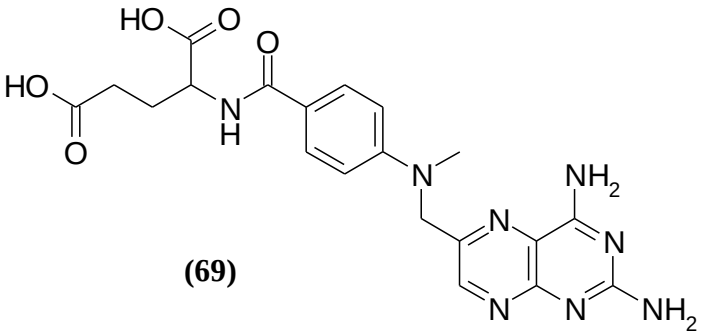


(68)

1.2.2.3. Antimetabolites:

Antimetabolite drugs work by inhibiting essential biosynthetic processes or by being incorporated into macromolecules such as DNA and RNA, and inhibiting their normal function.[54]

There are three main classes of antimetabolites: folate antagonists e.g. methotrexate (**69**)[55], pyrimidine antagonists e.g. 5-fluorouracil (**70**)[55] and purine antagonists e.g. 6-mercaptopurine (**71**).[56]



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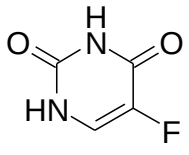

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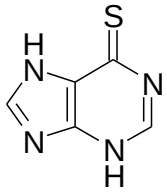
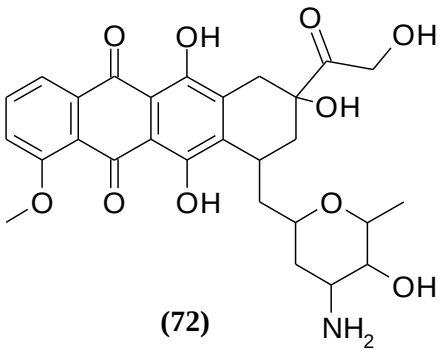


(71)

1.2.2.4. Intercalating agents:

The DNA intercalating agents are one of the most widely used families of antitumor agents. They are flat in shape, consisting of three or four fused aromatic rings. Their mechanism of action involves insertion between the base pairs of DNA, peripendicular to the axis of the helix. Once in position, they are held in place by interactions including hydrogen bonding and van der waals forces.[57] The largest family of intercalating agents in clinical use is the anthracyclines e.g. doxorubicin (**72**) and daunorubicin (**73**).[58]



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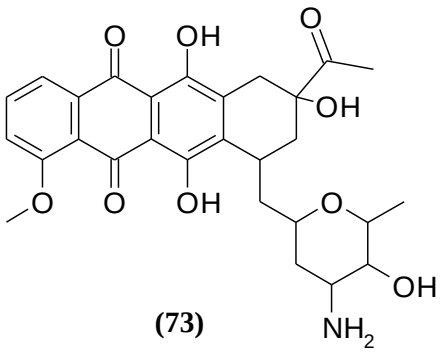
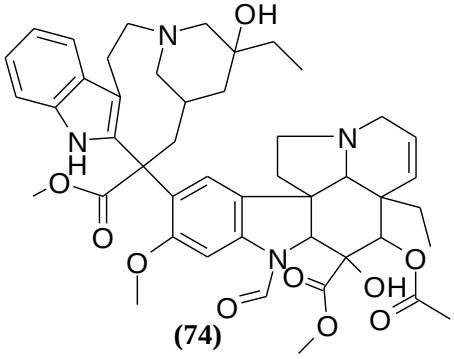


(73)

1.2.2.5. Spindle poisons:

Spindle poisons act by binding to tubulin, the building block of microtubules. This inhibits further assembly of the spindle during metaphase, thus inhibiting mitosis.[44]

Vinca alkaloids, including vincristine (**74**) and vinblastine (**75**) are examples of spindle poisons that are widely used in cancer treatment.[59,60]



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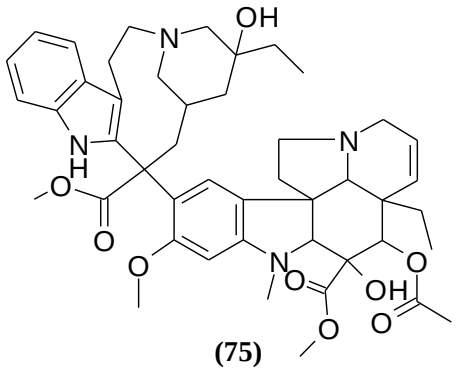
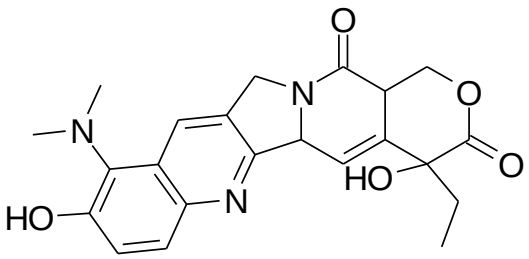


(75)

1.2.2.6. Topoisomerase inhibitors:

Topoisomerases are responsible for altering the 3D structure of DNA by a cleaving/unwinding/rejoining reaction. They are involved in DNA replication, chromatid segregation and transcription.[44]

There are two broad classes: topoisomerase I inhibitors e.g. topotecan (**76**)[61] and topoisomerase II inhibitors e.g. podophyllotoxin (**77**).[62]



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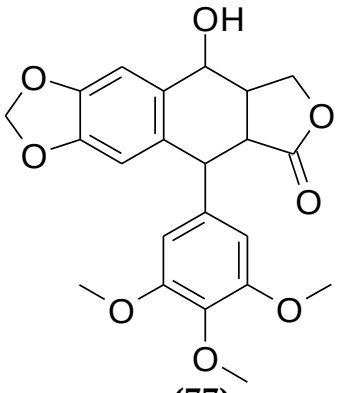


(77)

There are number of problems with the safety profile and efficacy of chemotherapeutic agents. Cytotoxics predominantly affect rapidly dividing cells so do not specifically target cancer cells. They also only influence a cell's ability to divide and have little effect on other aspects of tumour progression such as tissue invasion, metastases or progressive loss of differentiation. Finally, cytotoxics are associated with a high incidence of adverse effects. The most notable examples include bone marrow suppression, alopecia, mucositis, nausea and vomiting.[44]

1.2.2.7. Protein kinase inhibitors:

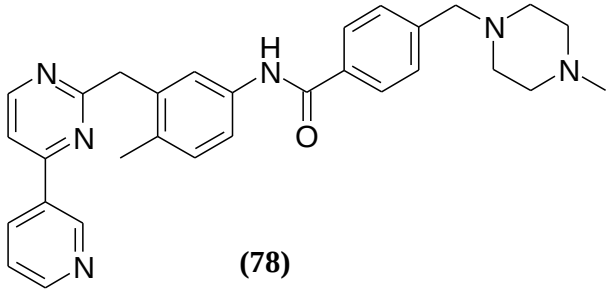
In an attempt to circumvent these side effects, new therapies of cancer known as molecular targeted therapies have been developed. These treatments inhibit specific molecules that have a role in tumor growth or progression and are frequently altered in tumors but not in normal cells so

being more specific toward tumor cells.[63] Protein kinases form a family of over 100 enzymes that all participate in signal transduction pathways regulating cell cycle progression, activation and differentiation. They catalyse the phosphorylation of protein residues by transfer of phosphate group from ATP.[64]

Catalytic activity of many protein kinases *via* mutation or overexpression plays an important role in numerous pathological conditions including cancer.[65]

Protein kinase inhibitors have been widely used to probe the role of protein phosphorylation in cellular signaling and therefore they are representing the best characterized targeted treatment to date.[66]

pharmaceutical companies started extensive research projects on these targets and now there are more than 30 kinase inhibitors in clinical trials. Imatinib mesylate (Gleevec) (**78**) is the first kinase inhibitor to be approved for the treatment of Chronic Myeloid Leukemia (CML). Also, gefitinib (Iressa) (**79**) was approved for the chemotherapeutic treatment of patients with advanced non small lung cancer.[67]



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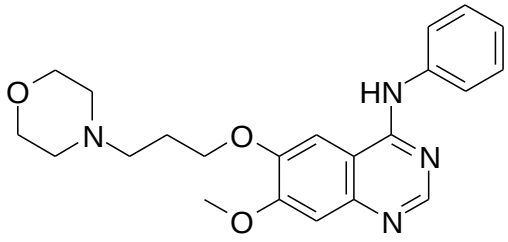
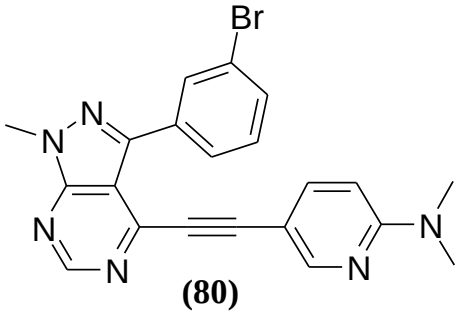


(79)

1.3. Pyrazolo[3,4-*d*]pyrimidines as anticancer agents

Gomtsyan *et al*[68] prepared a series of pyrazolo[3,4-*d*]pyrimidines which was evaluated as adenosine kinase inhibitors. Compound **80** with pyrazolo[3,4-*d*]pyrimidine core (IC_{50} = 7.5 nM) was superior to its open chain alkynylpyrimidine analogue **81** (IC_{50} = 22 nM).



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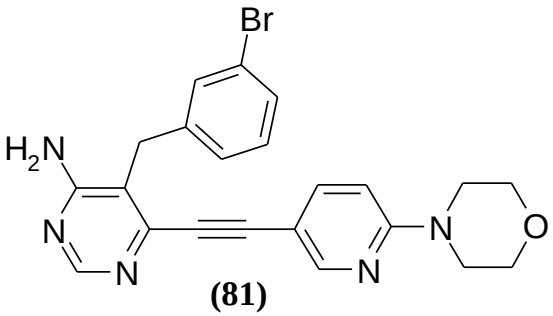
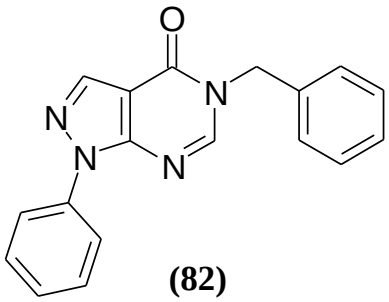
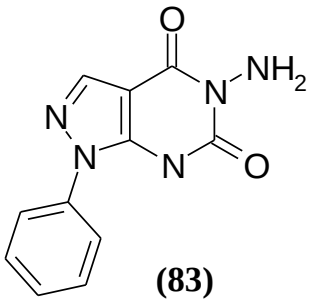


(81)

Moreover, compounds **82** and **83** are active against Ehrlich ascites carcinoma (EAC) cell line with IC_{50} = 90 and 100 µg/ml, respectively.[69]

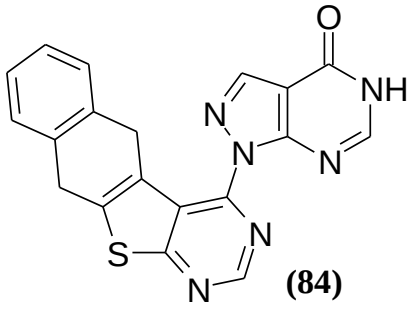


(82)



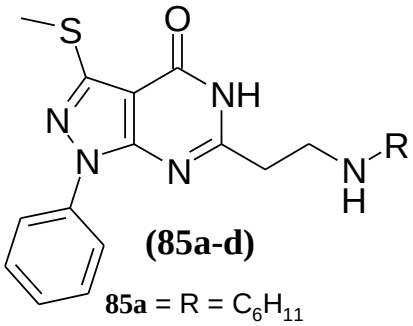
(83)

In 2010, many pyrazolo[3,4-*d*]pyrimidine derivatives were synthesized and evaluated *in vivo* for their antiproliferative activity. In ascites tumor model, compound **84** exhibited an 73% increase in life span of the animals compared with cisplatin which showed 86% increase.[70]



(84)

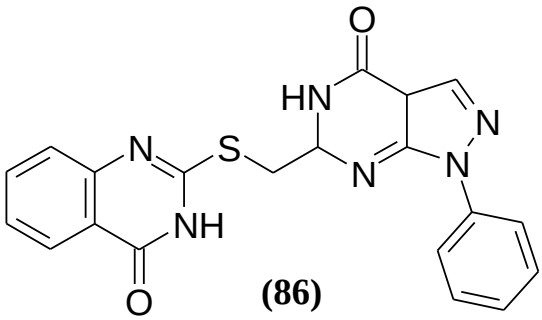
Also, a new series of 6-alkyl or aryl pyrazolo[3,4-*d*]pyrimidin-4-ones derivatives **85a-d** was synthesized and tested *in vitro* on human colon tumor cell line (HCT116). Most of the test compounds displayed potent antitumor activity, especially compound **85a** which displayed the highest activity with IC_{50} = 0.47 µg/ml.[71]



(85a-d)

85a = R = C_6H_{11}
85b = R = C_6H_5
85c = R = 4-CH_3O-C_6H_4
85d = R = 4-NH_2SO_2-C_6H_4

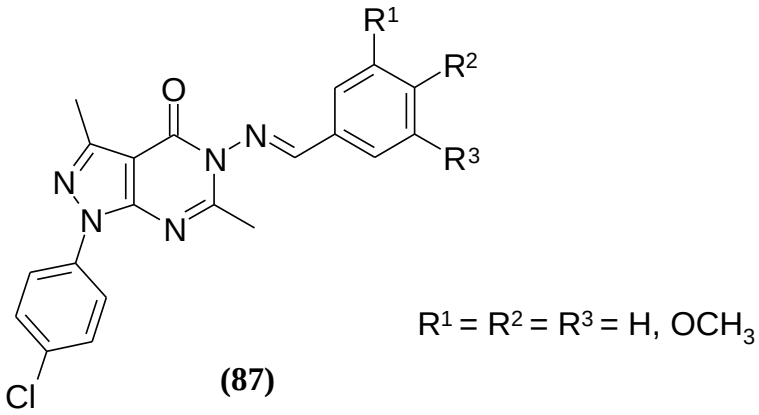
Compound **86** bearing a pyrazolo[3,4-*d*]pyrimidin-4-one scaffold exhibited high anticancer activity *in vitro*, especially for the lung cancer cell line (A548) with an IC_{50} value of 2.24 µM. Moreover, flow cytometric analysis revealed that this compound could significantly induce apoptosis in lung cancer cell line A548 cells *in vitro* at low micromolar concentrations.[72]



(86)

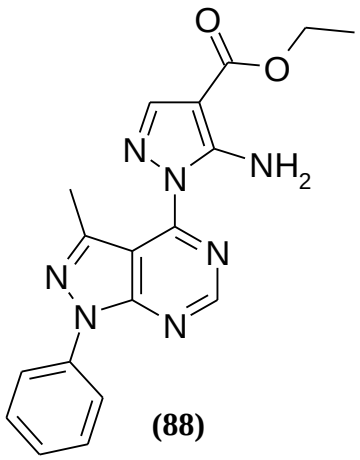
Moreover, new derivatives of pyrazolo[3,4-*d*]pyrimidine **87** endowed with the structural elements of hydrazones were synthesized and evaluated for their antiproliferative activity against human breast adenocarcinoma cell line (MCF-7). They exerted their antitumor activities by modulating free radicals production through increasing the activity of superoxide dimutase (SOD) and

depletion of intracellular glutathione level, accompanied with high production of hydrogen peroxide, nitric oxide and other free radicals causing tumor cells death, as monitored by reduction in the synthesis of protein and nucleic acids.[73]



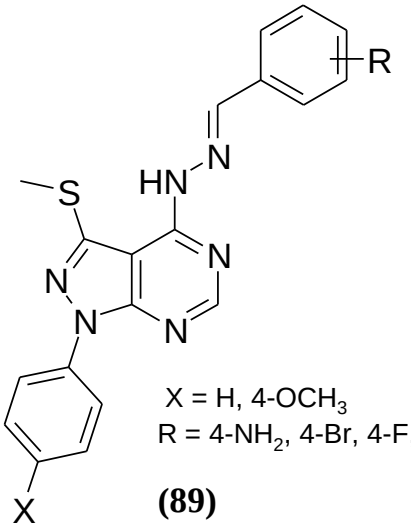
(87)

Ethyl 5-amino-1-(3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)-1*H*-pyrazole-4-carboxylate (**88**) showed potent activity against MCF-7 cell line with IC_{50} value of 0.018 µM.[74]



(88)

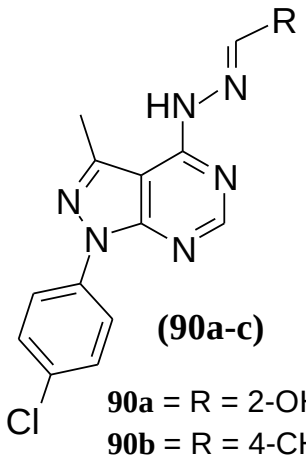
Moreover, a series of new 1-aryl-4-benzylidenehydrazinyl-3-methylsulphanyl pyrazolo[3,4-*d*]pyrimidine derivatives (**89**) was synthesized. The cytotoxic activity of newly synthesized compounds against MCF-7 cell line was investigated. It was found that the optimal activity (IC_{50} = 7.5 nM) was achieved through introducing phenyl moiety at N1 and *p*-fluorobenzylidene hydrazinyl group at position 4.[75]



X = H, 4-OCH_3
R = 4-NH_2, 4-Br, 4-F, 4-OH, 2-OH, 3-OH

(89)

A new series of pyrazolo[3,4-*d*]pyrimidines **90a-c** was synthesized by Kandeel *et al*.[76] The new compounds were tested for their antitumor activity on 60 different cell lines, and some of the tested compounds were found to have potent antitumor activity. particularly, compound **90a** was found to be the most effective one, showing IC_{50} values of 0.326 to 4.31 µM on 57 different cell lines.

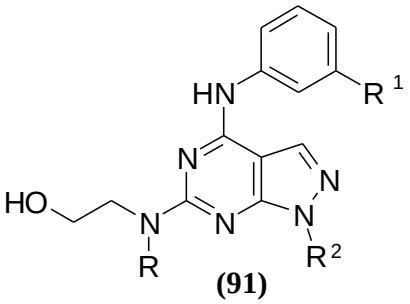


(90a-c)

90a = R = 2-OH-C_6H_4
90b = R = 4-CH_3O-C_6H_4
90c = R = 3-NO_2-C_6H_4

1.4. Pyrazolo[3,4-*d*]pyrimidines as protein kinase inhibitors

A series of 1,4,6-trisubstitutedpyrazolo[3,4-*d*]pyrimidines **91** capable of selectively inhibiting cyclin dependent kinase 2 (CDK2) was synthesized. Compounds having a 3-fluoroaniline group at C4 showed superior CDK2 inhibitory activity to those of olomoucine (**92)** and roscovitine **(93)** as reference compounds.[77]

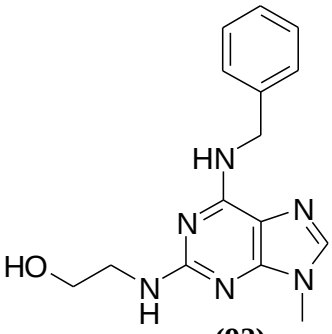


(91)

R = H, $(CH_2)_2OH$

R^1 = H, F, OCH_3, Br

R^2 = H, CH_3, $CH(CH_3)_2$



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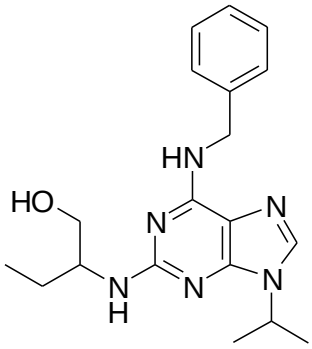
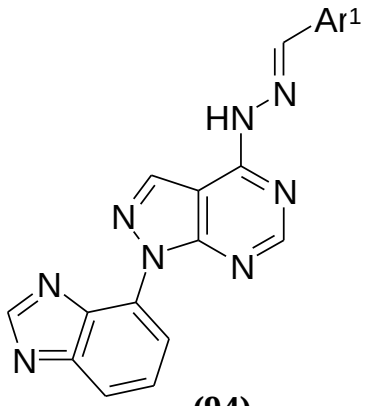


(93)

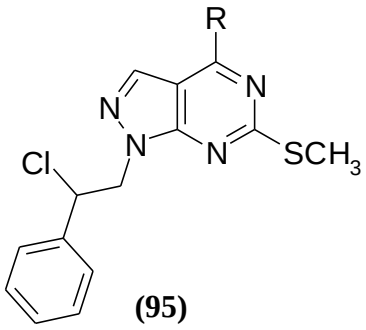
Moreover, a novel series of [1-(1*H*-benzimidazol-7-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl]arylhydrazones (**94**) was prepared and evaluated as glycogen synthase kinase-3 (GSK-3) by Peat *et al*.[78] The benzimidazole substituent formed an intramolecular hydrogen bond to N-2 nitrogen of pyrazole core, resulting in a coplanar geometry that allowed enhanced binding to the kinase active site.



(94)

Ar^1 = 4-C_5H_4N, 4-F-C_6H_4, 4-COOH-C_6H_4

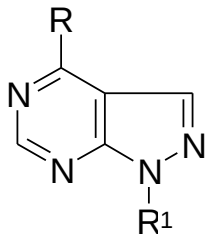
Schenone and coworkers[79] registered the synthesis of 4-substituted amino-6-methylthio-1*H*-pyrazolo[3,4-*d*]pyrimidines **95** bearing 2-chloro-2-phenylethyl chain at the N1. These derivatives demonstrated inhibitory activity on cell proliferation of the A431 cell line, probably acting on epidermal growth factor receptor (EGFR) catalytic domain.



(95)

R = NH—  , NHC_3H_7, NHC_4H_9, $N(C_2H_5)_2$

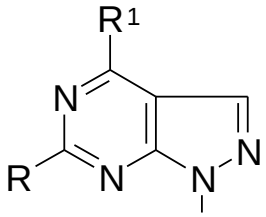
Moreover, a series of 1-aryl-4-substituted amino-1*H*-pyrazolo[3,4-*d*]pyrimidine derivatives **96** was prepared and evaluated as Src and MAPK inhibitors by Schenone *et al*.[80]



(96)

R = $NH(CH_2)_2C_6H_5$, NHC_4H_9, $NHCH_2C_6H_5$
R^1 = $CH_2CHClC_6H_5$, $CH{=}CHC_6H_5$

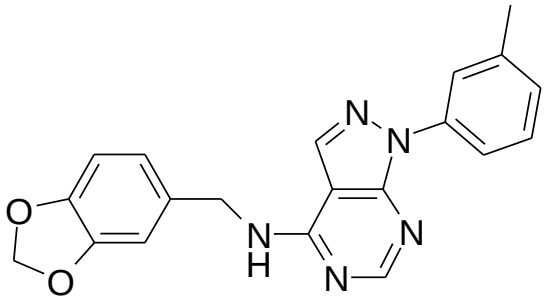
Carraro *et al*[81] reported the synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives **97** as potent antiproliferative agents toward A431 and 8701-BC cells. Such compounds block the growth of cancer cells by interfering with the phosphorylation of Src and they act as proapoptotic agents through the inhibition of the antiapoptotic gene BCL2.



(97)

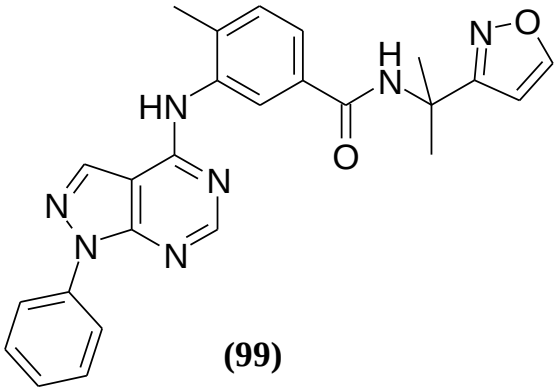
R = H, SCH_3, SC_2H_5
R^1 = $NH(CH_2)_2C_6H_5$, NHC_4H_9, $NH(C_2H_5)_2$
R^2 = $CH_2CHClC_6H_5$, $CH{=}CHC_6H_5$

N-(1,3-Benzodioxol-5-ylmethyl)-1-(3-methylphenyl)-1*H*-pyrazolo-[3,4-*d*]pyrimidin-4-amine (**98**) was identified as a low-micromolar inhibitor of EGFR tyrosine kinase activity with antiproliferative effect against cancer cells.[82]



(98)

The synthesis and structure activity relationships (SAR) of p38α MAP kinase inhibitors based on pyrazolopyrimidine scaffold were described. These studies led to the identification of compound **99** as a potent and selective inhibitor of p38α MAP kinase.[83]



(99)

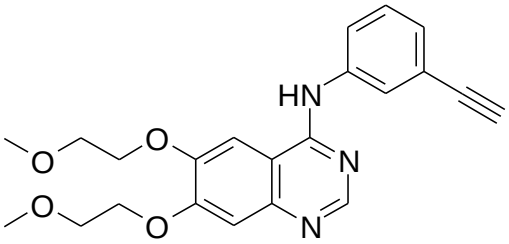
2. Research Objectives

Conventional chemotherapy is the most common type of anticancer treatment, But this type of therapy does not discriminate between dividing normal and tumor cells which leads to severe side effects.[63] In the last decade, the use of molecular targeted therapy (new generation of selective anticancer drugs which interfere with specific receptors and signaling pathways that promote tumor cell growth) had made more specific treatment.[84]

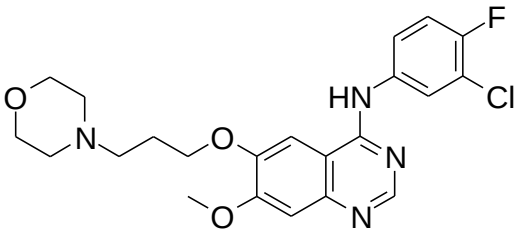
In recent years, protein kinases had been recognized as central players and regulators of cancer cell proliferation, apoptosis and angiogensis and therefore are considered suitable potential targets for anticancer therapies.[63]

However, deregulation of protein kinase function has been implicated in other disorders, including immunological, neurological, metabolic and infectious disease. This has generated considerable interest in the development of small molecule kinase inhibitors for the treatment of these disorders.[85]

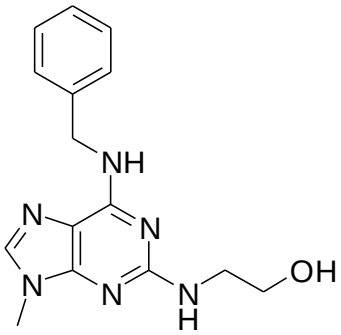
Most kinase inhibitors share common properties, e.g. low molecular weight (small molecules), hydrophobic heterocycles and act by competing with ATP for binding in kinase ATP binding site.[85] From these inhibitors, numbers of small molecule EGFR kinase inhibitors have been evaluated in cancer clinical trials. For example, anilinoquinazoline-containing compounds erlotinib **(100)**[86] and gefitinib **(79)**[87] had been approved for the chemotherapeutic treatment of patients with advanced non small lung cancer. Also, purine derivatives as olomucine (**92**)[88] and roscovitine (**93**)[89] exhibited good selectivity for cyclin dependent kinase 2 (CDK2).



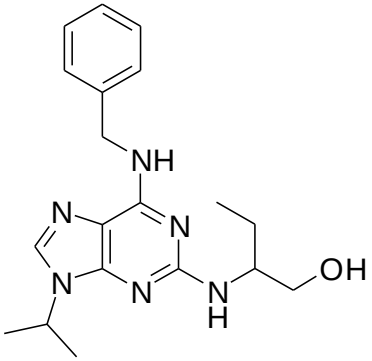
(100)



(79)



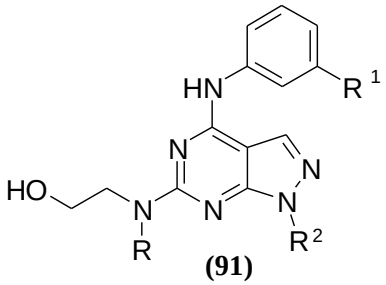
(92)



(93)

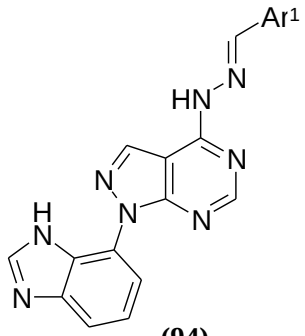
The chemistry of pyrazolo[3,4-*d*]pyrimidine derivatives received great attention due to their structural similarity with purines and hence several pyrazolo[3,4-*d*]pyrimidine derivatives exhibited promising anticancer activity.[68-76]

In the recent years, several pyrazolo[3,4-*d*]pyrimidine derivatives were found to be selective ligands with antagonist activity for protein kinases. Compounds **91**, **94**, **95** and **96** had been reported to act as cyclin dependent kinase (CDK) inhibitors[77], glycogen synthase kinase-3 (GSK-3) inhibitors[78], epidermal growth factor receptor (EGFR) inhibitors[79] and src kinase inhibitors[80,81], respectively.



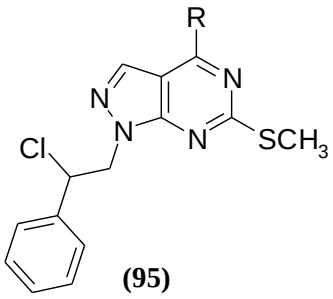
(91)

R^1 = H, F, OCH_3, Br
R^2 = H, CH_3, $CH(CH_3)_2$

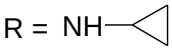


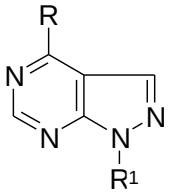
(94)

Ar^1 =  . 4-F-C_6H_4, 4-COOH-C_6H_4



(95)

R =  , NHC_3H_7, NHC_4H_9, $N(C_2H_5)_2$



(96)

R = $NH(CH_2)_2C_6H_5$, NHC_4H_9, $NHCH_2C_6H_5$
R^1 = $CH_2CHClC_6H_5$, $CH{=}CHC_6H_5$

In the view of the aforementioned facts, we thought of preparing new compounds containing pyrazolo[3,4-*d*]pyrimidine scaffold as anticancer agents.

The rational for the design of the target compounds was based upon some structural modifications on the general features of purine containing compounds olomucine (**92**) and roscovitine (**93**) (Fig.3). These modifications comprise the replacement of purine imidazole ring with pyrazole one since several analogues containing the pyrazolo[3,4-*d*]pyrimidine core showed superior CDK2 inhibitory activity to those of olomucine (**92)** and roscovitine **(93)** as reference compounds[(77)] and led to new models of CDK2 kinase inhibitors.

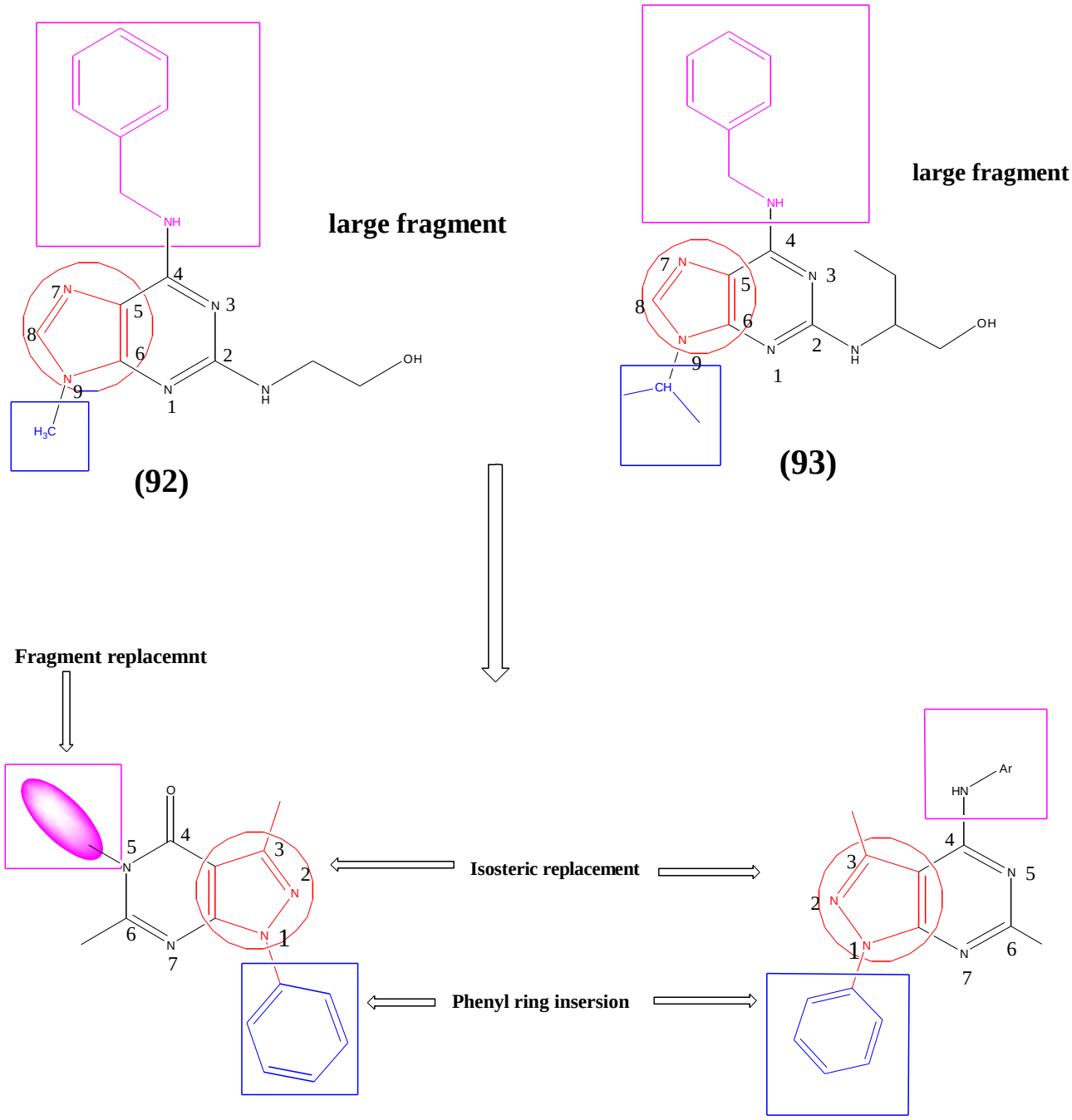


Fig.3. Planned design of new pyrazolo[3,4-*d*]pyrimidine derivatives for cytotoxic activity.

Also, the methyl or isopropyl groups at N9 of potent CDK2 inhibitors **92** and **93** were replaced with phenyl group at N1 of pyrazolopyrimidine ring in order to increase the hydrophobic interactions. Additional modification at N5 of pyrazolopyrimidine ring was added through several side chains incorporating hydrogen bond donor-acceptor pairs and finally, fusion at site

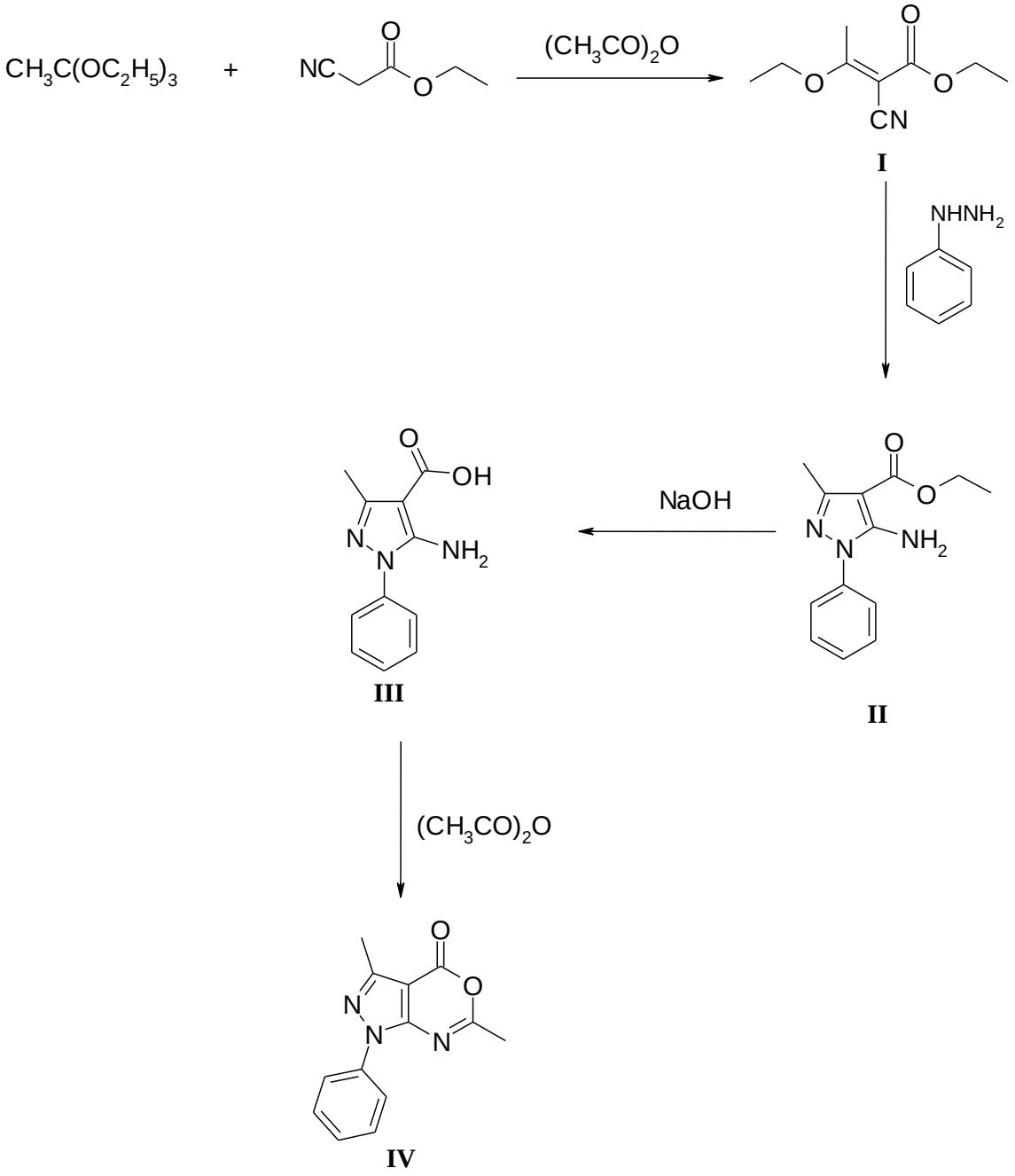
C4-N5 of pyrazolopyrimidine either with triazole or triazine moieties was done to examine the effect on the cytotoxic activity.

All the target compounds were subjected to molecular modeling study to show their affinity and binding mode to CDK2.

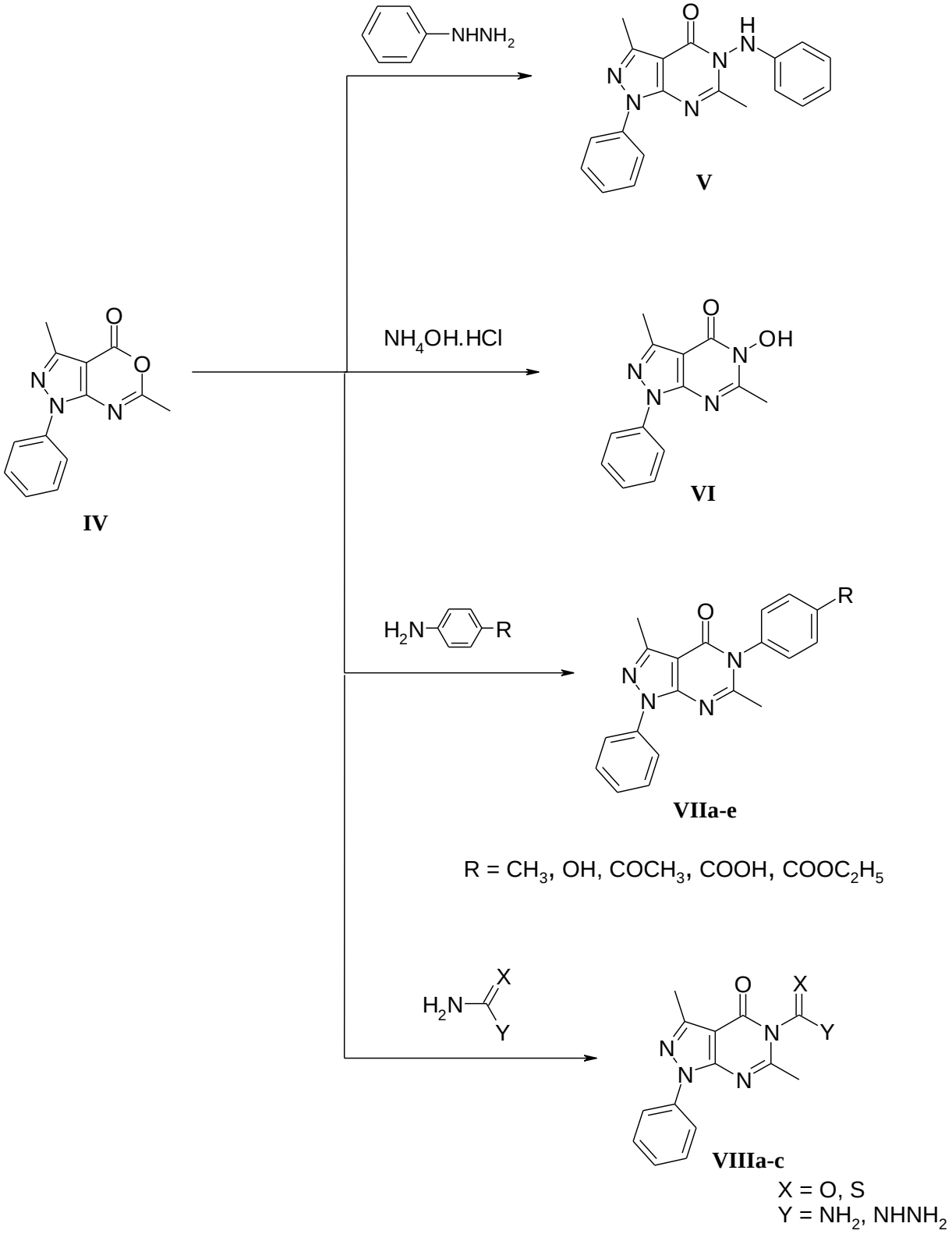
Most of the target compounds were evaluated for their *in vitro* cytotoxic activity against human breast adenocarcinoma cell line (MCF-7).

The synthetic routes adopted in this work for constructing the target compounds are illustrated in the following schemes,

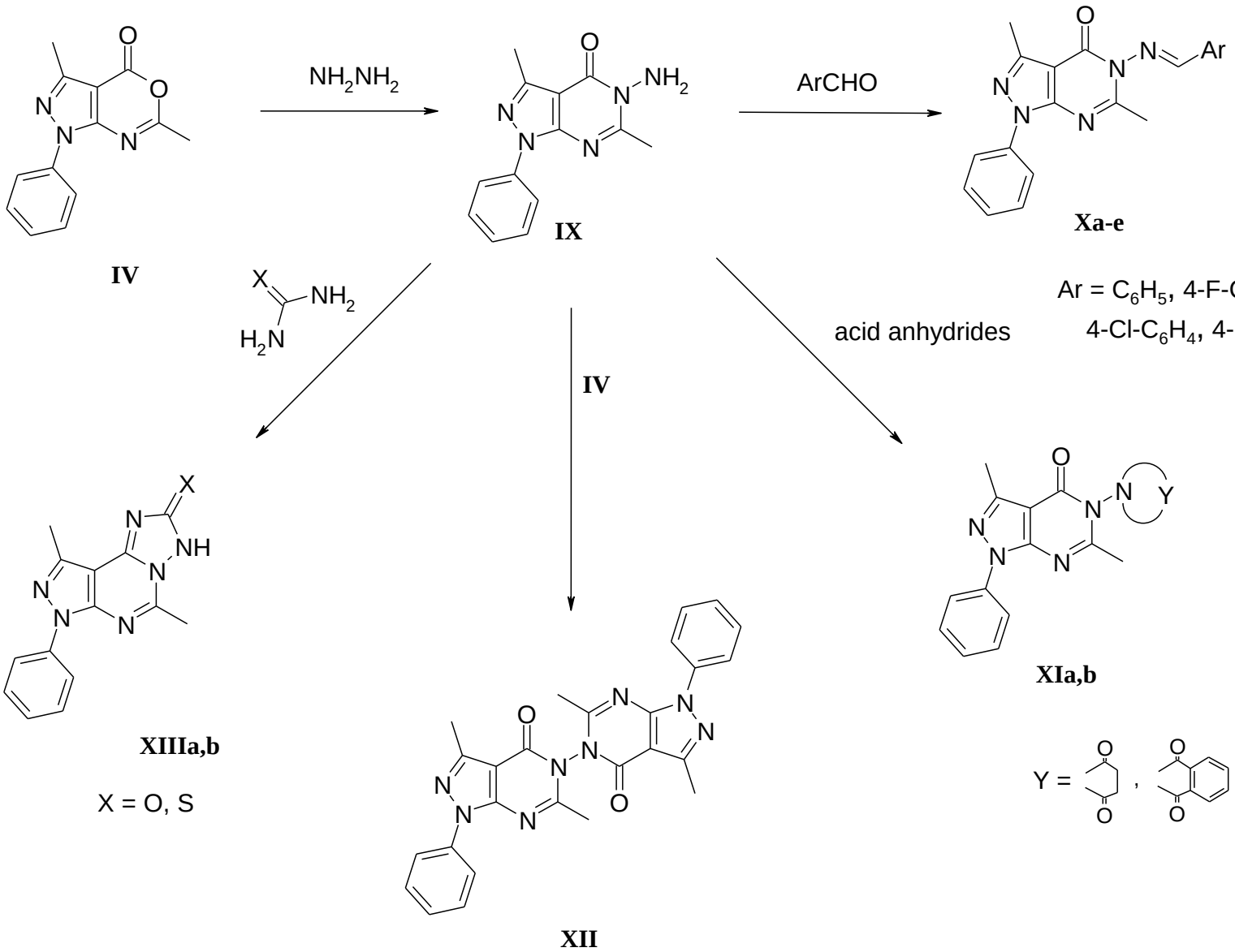
Scheme 1:


CH3C(OC2H5)3
+
NC
O
O
(CH3CO)2O
O
O
O
CN
I
NHNH2
O
OH
N
N
NH2
III
NaOH
O
O
N
N
NH2
II
(CH3CO)2O
O
O
N
N
N
IV

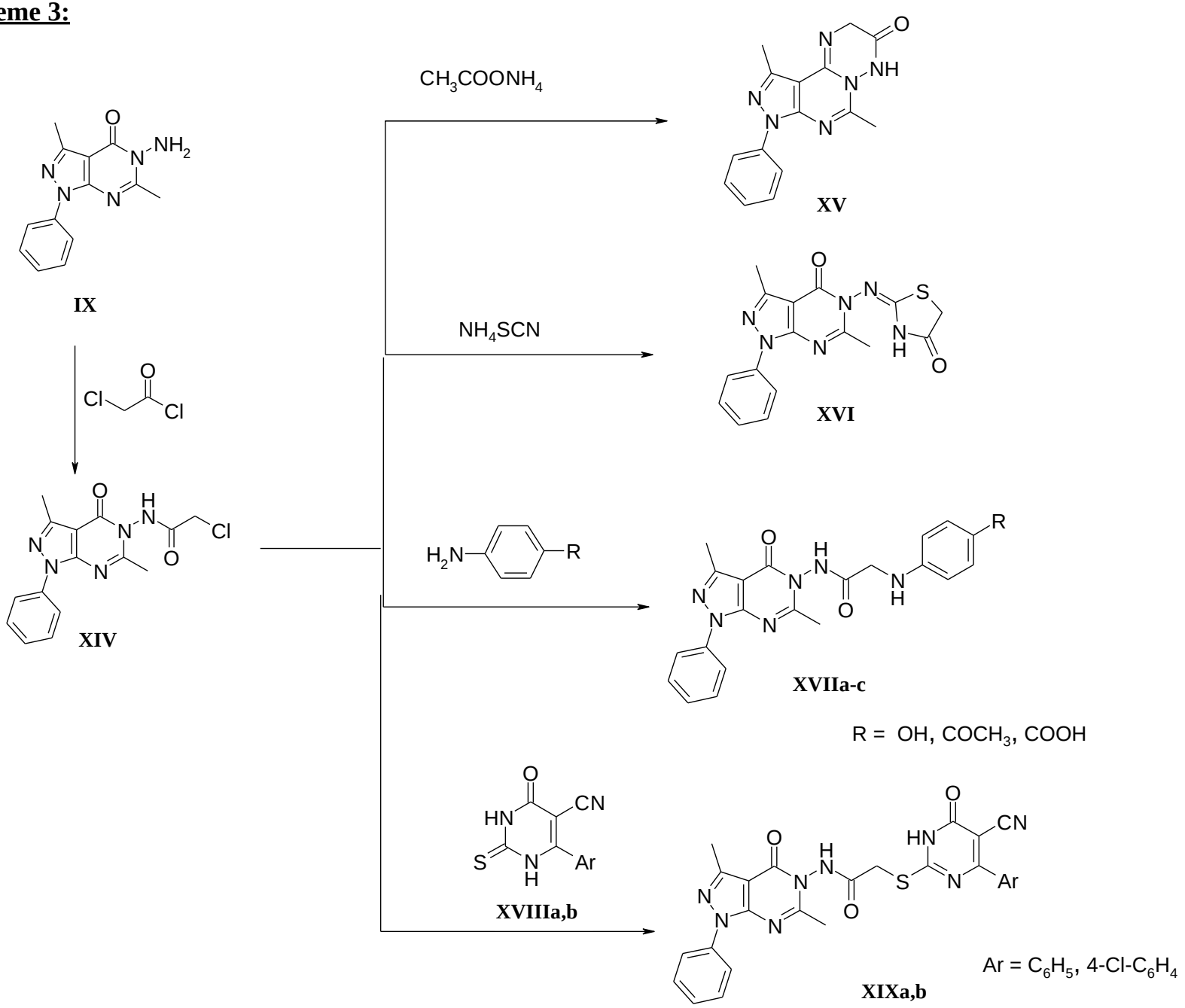
Scheme 1 (cont.):



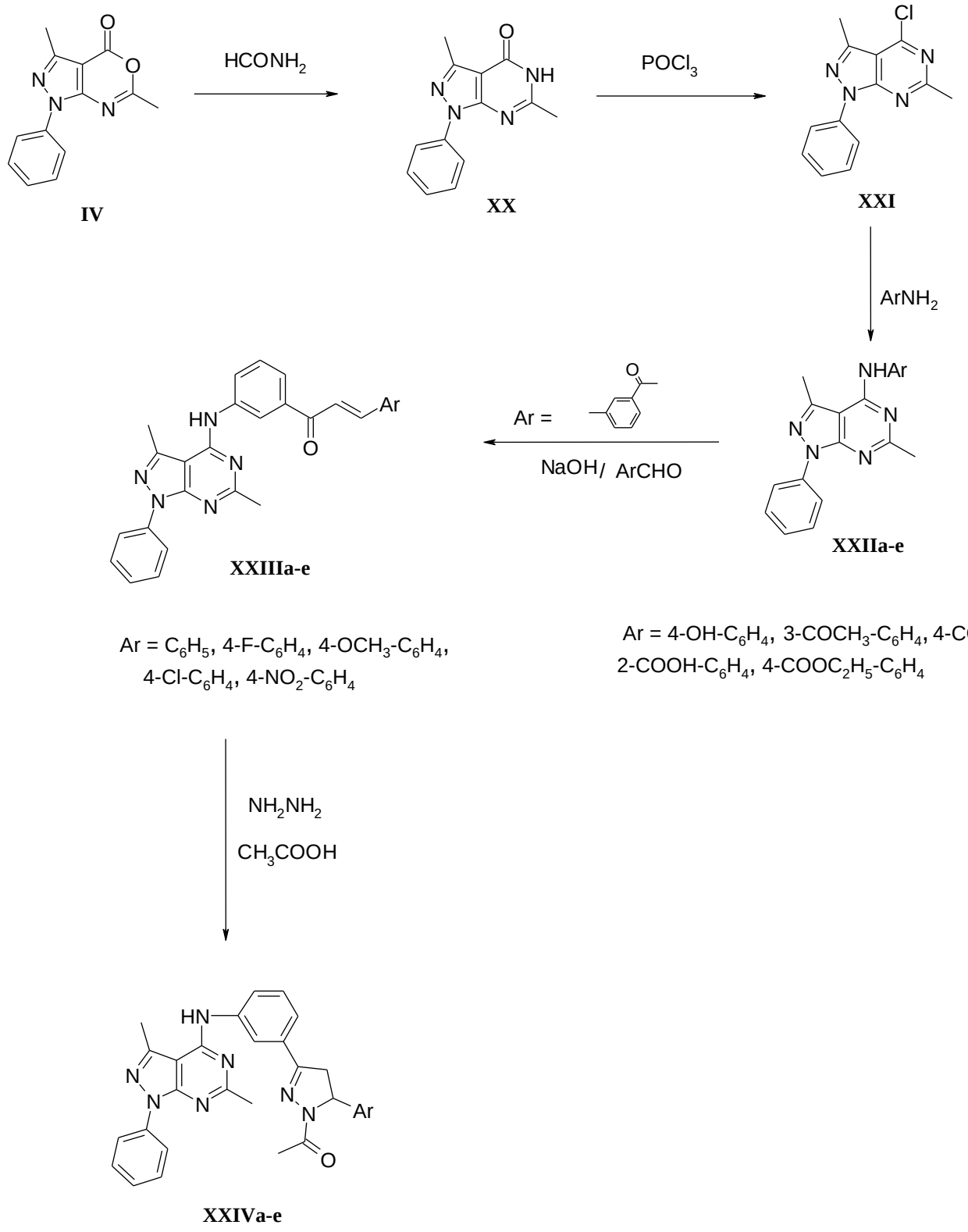
Scheme 2:


NH2NH2
ArCHO
Ar
IV
IX
Xa-e
Ar = C6H5, 4-F-C6H4, 4-CH3O-C6H4,
4-Cl-C6H4, 4-NO2-C6H4
acid anhydrides
IV
XIa,b
Y =
XIIIa,b
X = O, S
XII

Scheme 3:


CH3COONH4
NH4SCN
IX
XIV
XV
XVI
XVIIa-c
R = OH, COCH3, COOH
XVIIIa,b
XIXa,b
Ar = C6H5, 4-Cl-C6H4

Scheme 4:

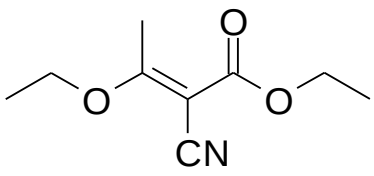

HCONH2
POCl3
ArNH2
NaOH/ ArCHO
Ar =
NH2NH2
CH3COOH
IV
XX
XXI
XXIIa-e
XXIIIa-e
XXIVa-e
Ar = C6H5, 4-F-C6H4, 4-OCH3-C6H4, 4-Cl-C6H4, 4-NO2-C6H4
Ar = 4-OH-C6H4, 3-COCH3-C6H4, 4-COOH-C6H4, 2-COOH-C6H4, 4-COOC2H5-C6H4

3. Discussion

3.1. Theoretical discussion of experimental

Scheme 1:

Ethyl 2-cyano-3-ethoxy-2-butenoate (I)

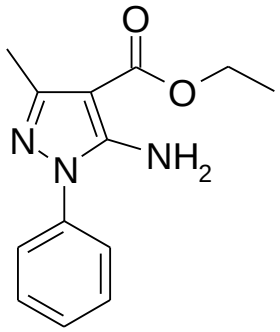


Literature survey revealed that reaction of orthoesters with ethyl cyanoacetate in absence[(90)] or presence[(91)] of acetic anhydride gave the corresponding ethyl 2-cyano-3-ethoxycarboxylate in different yields.

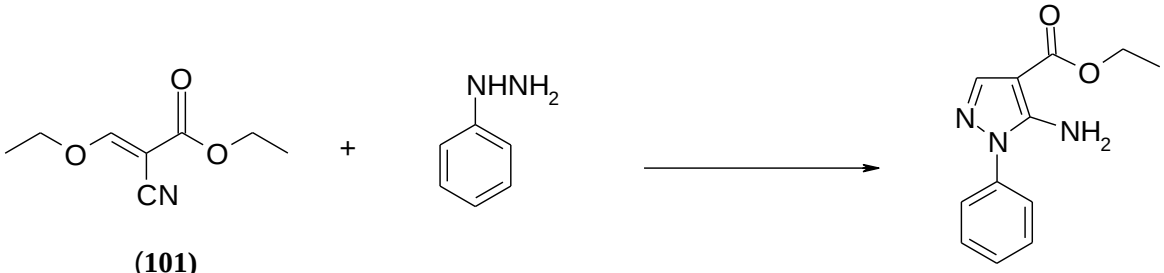
In 2006, Iranian research workers[(92)] prepared compound **I** by mixing ethyl cyanoacetate with triethyl orthoacetate in *N*,*N*-dimethylacetamide in a microwave oven for 10 min in 60% yield.

In this work, compound **I** was prepared in a pure form *via* the reaction of ethyl cyanoacetate with triethyl orthoacetate in acetic anhydride adopting conditions of analogous method.[(91)]

Ethyl 5-amino-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylate (II)

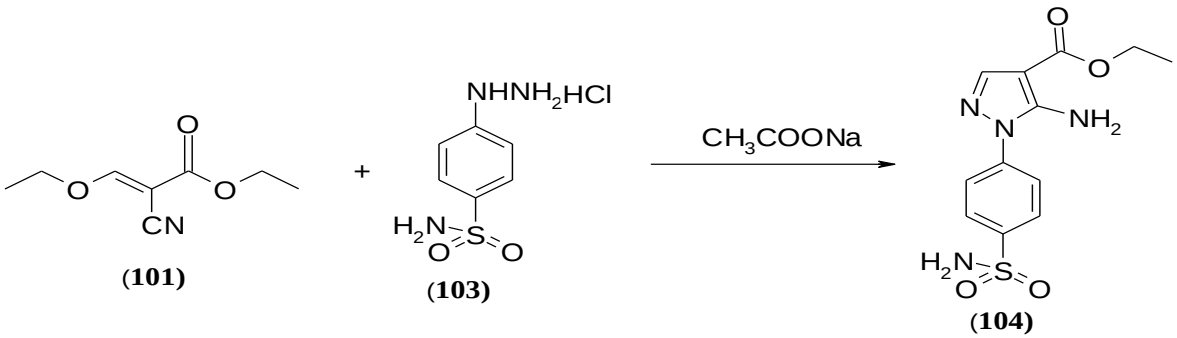


Peet *et al*[93] synthesized *o*-amino ester pyrazole derivative **102** by reacting ethyl ethoxymethylenecyanoacetate (**101**) with phenylhydrazine in ethanol for 15 h.



(**101**) (**102**)

literature survey revealed that ethyl 5-amino-1-(4-sulphamoylphenyl)- 1*H*-pyrazole-4-carboxylate (**104**) was prepared by heating under reflux ethyl ethoxymethylenecyanoacetate (**101**) and *p*-sulphamoylphenylhydrazine hydrochloride (**103**) in the presence of sodium acetate in ethanol for 3 h.[94]

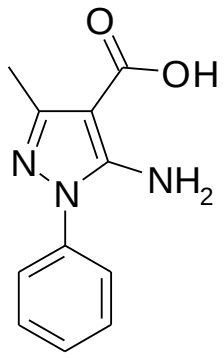


(**101**) (**103**) (**104**)

In 2006, Heravi *et al*[92] reported the synthesis of amino ester **II** by reacting ethyl 2-cyano-3-ethoxy-2-butenoate (**I**) with phenylhydrazine in *N*,*N*-dimethylacetamide using a microwave irradiation method**.**

In this thesis, compound **II** was obtained from the reaction of ethyl 2-cyano-3-ethoxy-2-butenoate (**I**) with phenylhydrazine in ethanol adopting conditions of analogue method.[93]

5-Amino-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid (III)



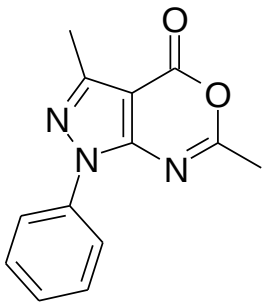
The carboxylic acid **III** was prepared from the corresponding amino ester **II** through hydrolysis. Such hydrolysis was operated by using methanolic sodium hydroxide according to reported precautions for a similar ester.[95]

IR spectrum of compound **III** showed a strong absorption broad band at 3389-3204 cm^{-1} corresponding to OH and NH_2 groups, in addition to an absorption band at 1651 cm^{-1} corresponding to C=O group.

^1H NMR spectrum revealed the presence of two exchangeable singlet signals at δ 6.30 ppm and at δ 12.07 ppm corresponding to NH_2 protons and OH proton, respectively.

Additionally, the mass spectrum of compound **III** demonstrated a molecular ion peak at *m/z* 217 ($M^{+\cdot}$) and a base peak at *m/z* 80.

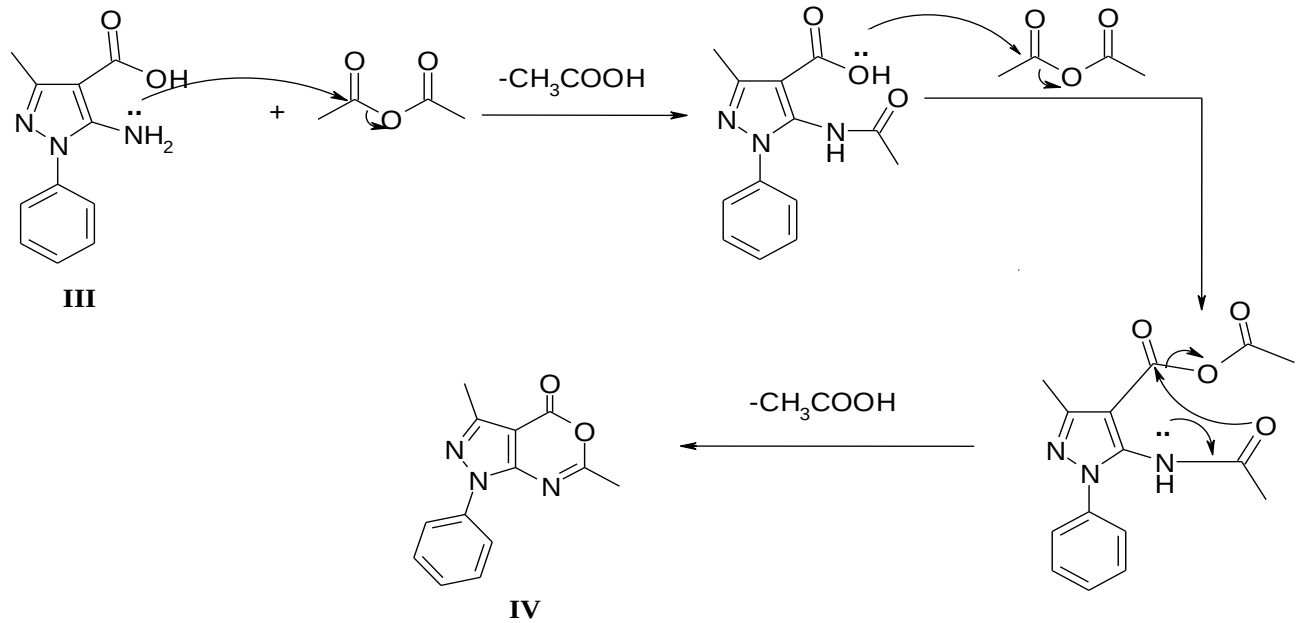
3,6-Dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*][1,3]oxazin-4-one (IV)



Literature survey revealed that 2-substituted[1,3]oxazin-4-ones were generally prepared either *via* reacting *o*-amino acid derivatives with different acyl chlorides in pyridine at room temperature[96-99] or by heating the suitable *o*-amino acid with the appropriate acid anhydride.[100-104]

In this work, the key intermediate **IV** was obtained by reacting the corresponding *o*-amino acid **III** with excess acetic anhydride.

A suggested mechanism for the formation of **IV** might be illustrated as follow:[105]



III **IV**

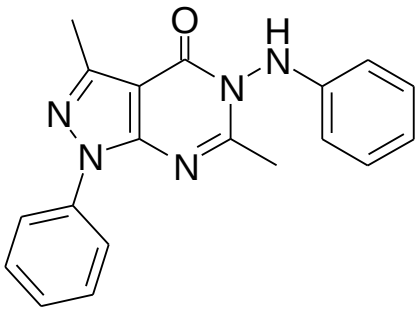
The structure of the product was confirmed by elemental analysis, IR, ^{1}H NMR and mass spectrum.

IR spectrum of compound **IV** lacked the presence of OH and NH_2 groups at 3389-3204 cm^{-1}. Also the presence of C=O group at 1764 cm^{-1} confirmed the structure.

Also, ^{1}H NMR spectrum revealed the appearance of a singlet signal at δ 2.49 ppm corresponding to CH_3 protons of oxazine. In addition to, the disappearance of D_2O exchangeable signals of NH_2 and OH protons of the precursor compound **III**.

Moreover, mass spectrum of **IV** demonstrated a molecular ion peak at *m/z* 241 ($M^{+\cdot}$) as a base peak.

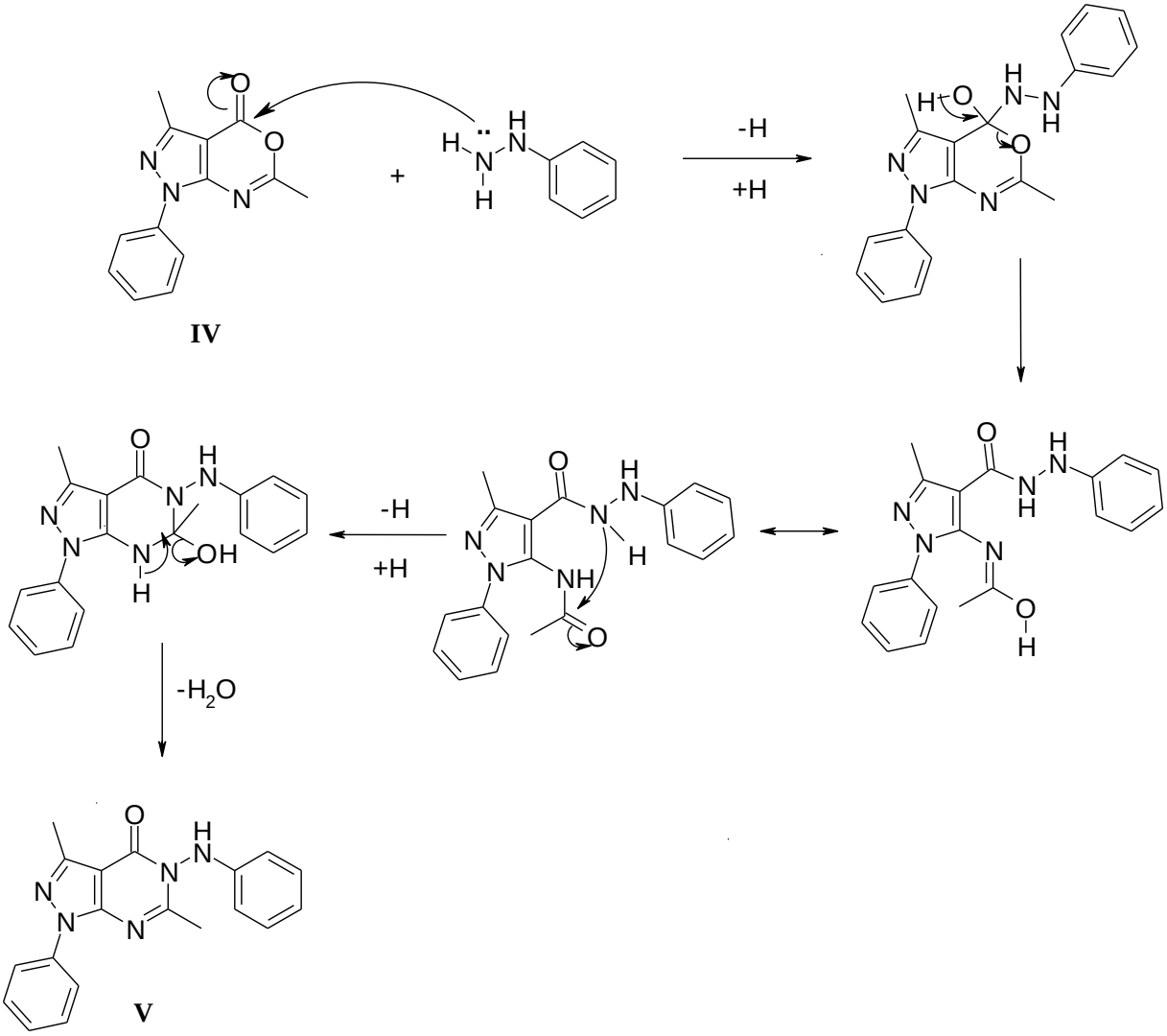
3,6-Dimethyl-1-phenyl-5-phenylamino-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (V)



Consulting the literature pointed out that phenylaminopyrimidine derivatives were prepared *via* the interaction between the appropriate [1,3]oxazin-4-one and phenylhydrazine in ethanol, either by stirring at room temperature[106] or heating under reflux.[105]

In the present study, heating compound **IV** and phenylhydrazine in ethanol afforded compound **V**.

A suggested mechanism for the formation of the title compound **V** might be illustrated as follows:[(105)]



The structure of the target compound **V** was substantiated by elemental analysis, IR, ^{1}H NMR and mass spectrum.

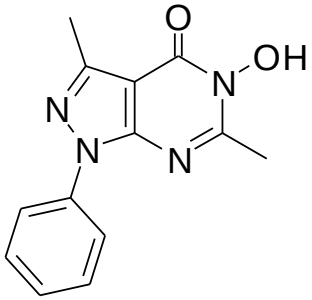
The IR spectrum of **V** displayed the presence of NH absorption band at 3250 cm^{-1} and an absorption band at 1685 cm^{-1} corresponding to C=O group.

The ^{1}H NMR spectrum confirmed **V** by the appearance of a singlet signal exchangeable with D_2O at δ 9.09 ppm corresponding to NH proton. In addition

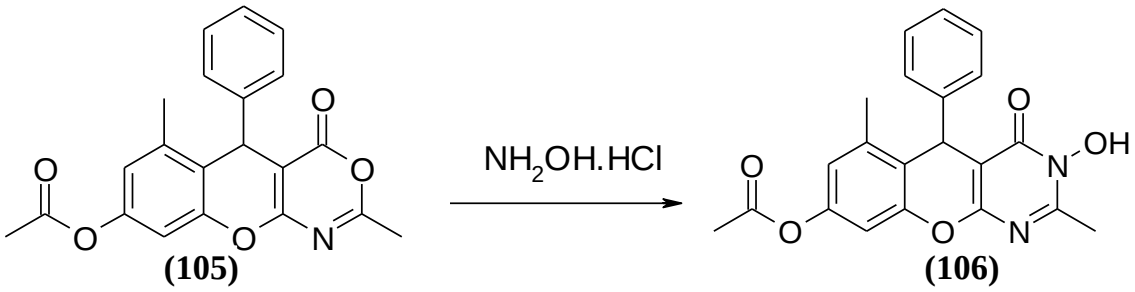
to the appearance of five additional aromatic protons corresponding to phenylamino part at δ 6.68-7.23 ppm.

Moreover, mass spectrum of **V** demonstrated a molecular ion peak at *m/z* 331 ($M^{+\cdot}$) as a base peak.

5-Hydroxy-3,6-dimethyl-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (VI)



Literature survey showed that 3-hydroxypyrimidine derivative **106** was prepared by heating the corresponding [1,3]oxazin-4-one **105** with hydroxylamine hydrochloride in pyridine.[107]



(105) **(106)**

In this investigation, the synthesis of **VI** was carried out by reacting **IV** with hydroxylamine hydrochloride in pyridine using the same precautions of similar reaction.[107]

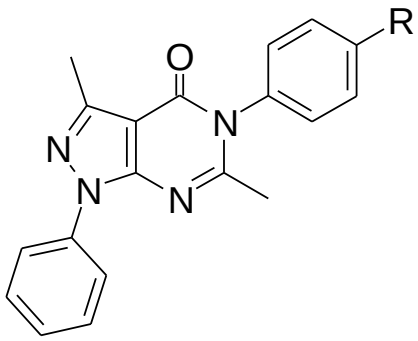
IR spectrum was used to confirm the structure of **VI** and showed a new absorption band corresponding to OH group at 3426 cm^{-1}.

^{1}H NMR spectrum of **VI** revealed the presence of a D_2O exchangeable singlet signal at δ 11.51 ppm corresponding to OH proton.

Moreover, ^{13}C NMR spectrum of **VI** showed two peaks of aliphatic carbons at δ 13.71, 21.38 corresponding to 2 CH_3.

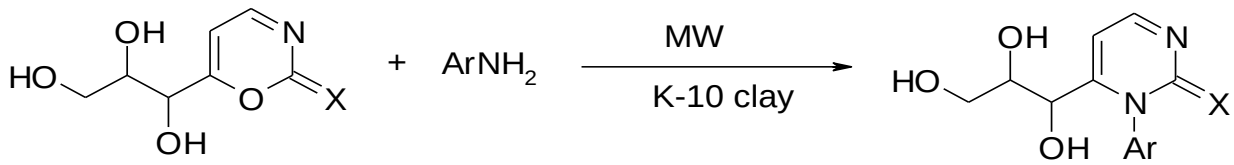
Meanwhile, the mass spectrum of **VI** displayed a molecular ion peak at *m/z* 256 ($M^{+\cdot}$) and a base peak at *m/z* 77.

3,6-Dimethyl-1-phenyl-5-(4-substituted phenyl)-1,5-dihydro pyrazolo[3,4-*d*]pyrimidin-4-ones (VIIa-e)



R = CH_3, OH, $COCH_3$, COOH, $COOC_2H_5$

In 2008, Yadav *et al*[108] reported that pyrimidine derivatives **108** were obtained in 83-94% yields by solvent-free microwave irradiation of mixture of 1,3-oxazin-2-ones or (thiones) **107** and aromatic amines in the presence of K-10 clay.

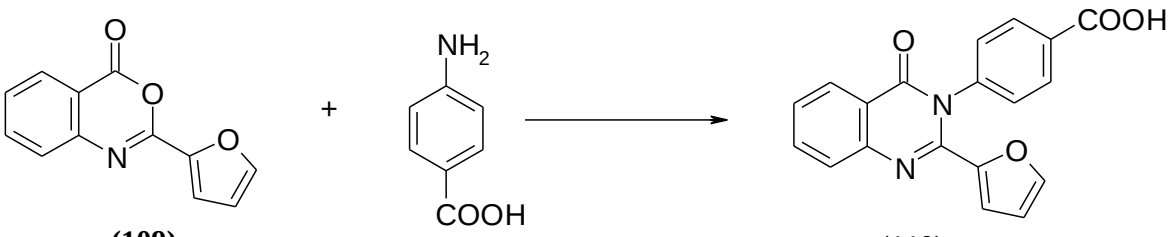


(107)
X = O, S

Ar = 4-Cl-C_6H_4, 4-CH_3O-C_6H_4

(108)

More recently in 2011, the fusion of 2-(furan-2-yl)-4*H*-3,1-benzoxazin-4-one (**109**) with 4-amiobenzoic acid afforded the corresponding quinazolin-4-one derivative **110**.[105]



(109) **(110)**

Recently, synthesis of pyrimidine derivatives was achieved by heating equimolar amounts of [1,3]oxazin-4-one derivatives with different aromatic amines either in ethanol[109], toluene[97], acetonitrile[110] or pyridine.[111]

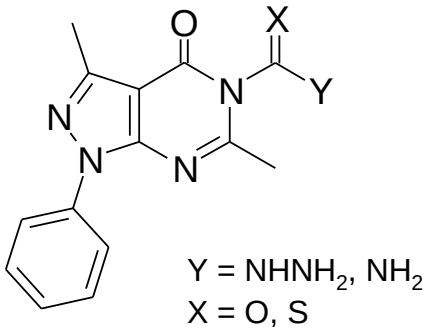
In the course of this study, compounds **VIIa-e** were prepared by the reaction of oxazine derivative **IV** with different aromatic amines in pyridine.

The structure of the target compounds **VIIa-e** was confirmed on the basis of elemental analysis and spectral data. IR spectrum of **VIIe** revealed the presence of a strong broad absorption band at 1709 cm^{-1} due to 2C=O groups.

The ^{1}H NMR cofirmed compound **VIIe** by the appearance of four additional aromatic protons corresponding to the ethylcarboxyphenyl part at δ 7.53-8.15 ppm. Also, the presence of a triplet signal at δ 1.35 ppm and a quartet signal at δ 4.37 ppm with *J* value 7.2 Hz corresponding to CH_3 and CH_2 protons of ester group, confirmed the structure.

Moreover, the mass spectrum of compound **VIIe** showed a molecular ion peak at *m/z* 388 ($M^{+\cdot}$) and a base peak at *m/z* 77.

3,6-Dimethyl-1-phenyl-5-substituted-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-ones (VIIIa-c)



The newly formed compounds **VIIIa-c** were prepared *via* fusion of the oxazin-4-one derivative **IV** with urea, thiourea and / or thiosemicarbazide at high temperature adopting the conditions of analogue method.[105]

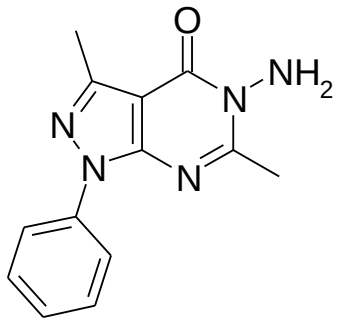
The IR spectra of **VIIIa-c** indicated the presence of strong absorption bands at the range of 3432-3188 cm^{-1} for NH and NH_2 groups. In addition, C=O groups appeared at 1694-1671 cm^{-1}.

Also, the ^1H NMR spectra of **VIIIa-c** revealed the presence of D_2O exchangeable signals at δ 11.14-12.24 ppm corresponding to NH_2 protons. In addition to an exchangeable singlet signal at δ 12.19 ppm corresponding to NH proton in **VIIIc**.

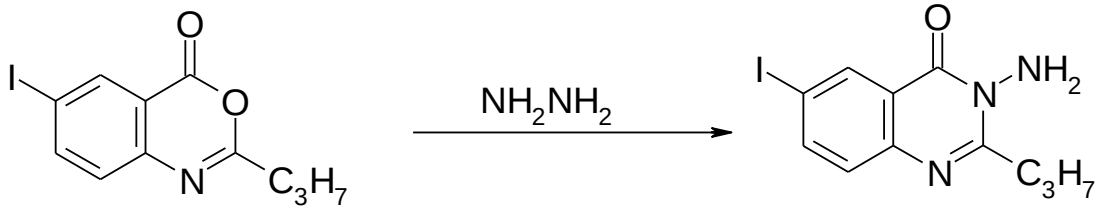
In addition, the mass spectra of **VIIIa** and **VIIIc** displayed molecular ion peaks at m/z 283 and 314 attributed to ($M^{+\cdot}$), seqentially.

Scheme 2:

5-Amino-3,6-dimethyl-1-phenyl-1,5-dihydropyrazolo[3,4-*d*] pyrimidin-4-one (IX)



literature review showed that 3-aminoquinazolin-4-one derivative **112** was prepared by heating the corresponding benzoxazin-4-one derivative **111** with excess hydrazine hydrate.[112]



(111) **(112)**

In addition, it had been reported that the formation of pyrimidine ring bearing amino group from oxazine derivatives could be brought by the treatment with hydrazine hydrate in dry benzene[113], pyridine[114], ethanol[115,116] or butanol.[73]

In the present work, the reaction of oxazin-4-one derivative **IV** with hydrazine hydrate in butanol afforded the title compound **IX**.

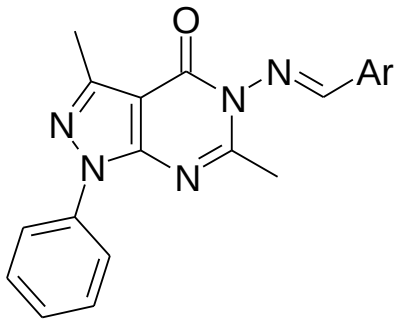
The data obtained from elemental analysis, IR, ^{1}H NMR and mass spectra supported the postulated structure **IX**.

IR showed the appearance of the forked bands of NH_2 at 3322, 3262 cm^1.

Moreover, ^{1}H NMR spectrum of **IX** revealed the appearance of an exchngeable singlet signal of NH_2 protons at δ 5.71 ppm.

In addition, the mass spectrum of **IX** displayed a molecular ion peak at *m/z* 255 ($M^{+\cdot}$) as a base peak.

5-(Arylideneamino)-3,6-dimethyl-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-ones (Xa-e)



Ar = C_6H_5, 4-F-C_6H_4, 4-CH_3O-C_6H_4, 4-Cl-C_6H_4, 4-NO_2-C_6H_4

Literature survey revealed that arylidene derivatives were prepared by heating equimolar amounts of the amine and the appropriate aromatic aldehyde or ketone in different solvents such as acetonitrile[117], glacial acetic acid[118] or ethanol.[119,120] In addition, ethanol containing catalytic amount of acetic acid[121-123] or drops of piperidine[124] could be used.

In the present work, **Xa-e** were obtained by condensing the 5-aminopyrazolo[3,4-*d*]pyrimidine derivative **IX** with the appropriate aromatic aldehyde in glacial acetic acid.

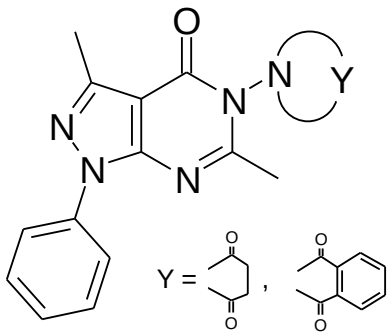
The structure of the target compounds was substantiated by elemental analysis, IR, ^{1}H NMR and mass spectra.

The ^{1}H NMR spectra of these compounds showed azomethine proton (N=CH) peak at δ 8.85-10.94 ppm.

The ^{13}C NMR spectrum of **Xd** demonstrated the appearance of four additional aromatic carbons, in addition to the presence of azomethine carbon at 168.91 ppm.

Further, the mass spectrum of **Xe** demonstrated a molecular ion peak at *m/z* 388 ($M^{+\cdot}$) and a base peak at *m/z* 240.

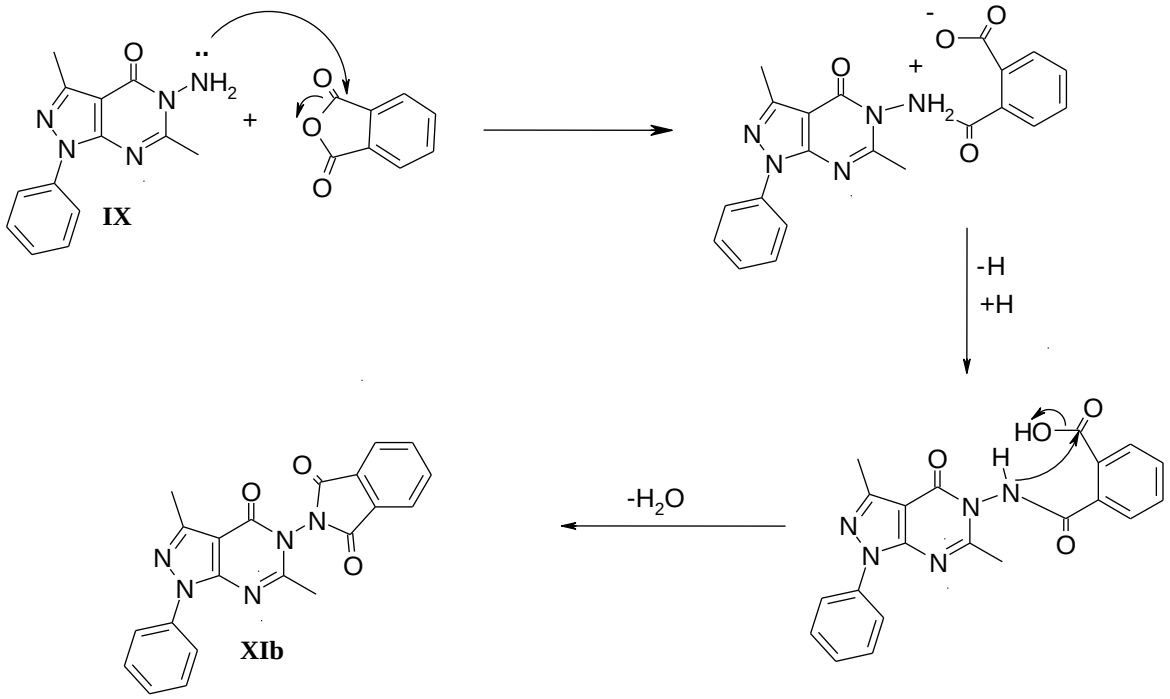
3,6-Dimethyl-1-phenyl-5-substituted-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-ones (XIa,b)



Literature survey revealed that imide derivatives were generally prepared by condensation of equimolar amounts of an amine with different acid anhydrides. It is worth to mention that different methods were used for such reaction as fusion at high temperature[125,126], eco-friendly microwave irradiation method where montmorillonite-KSF was used as reusable clay catalyst[127], stirring at room temperature using chloroform as a solvent[128], or heating under reflux temperature either in glacial acetic acid[129], dimethylformamide with P_2O_5 and few drops of concentrated sulphuric acid[130], ionic liquid such as [bmim][BF_4][131] or choline chloride.2$ZnCl_2$[132] or 10 mol % of sulphamic acid.[133]

In the present work, **XIa,b** were obtained by reacting 5-aminopyrazolo[3,4-*d*]pyrimidine derivative **IX** with the appropriate acid anhydride in glacial acetic acid.

A postulated mechanism for the reaction of **IX** with phthalic anhydride might be illustrated as follows:[127]



The structure assigned to the title compounds **XIa,b** was supported by elemental analysis and spectral data.

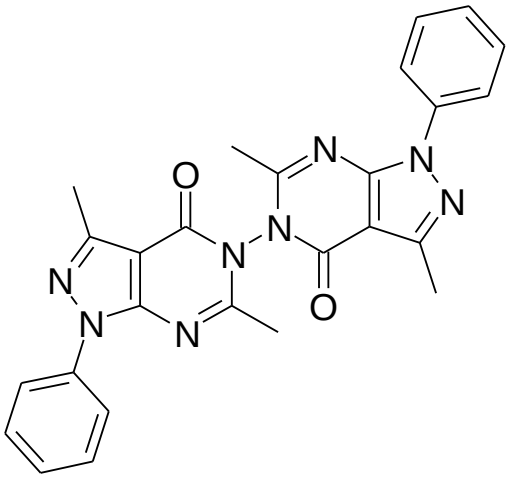
The IR spectra of compounds **XIa,b** showed broad absorption band at 1745 and 1742 cm^{-1} attributed to two C=O groups, and another broad absorption band at 1717 and 1715 cm^{-1} corresponding to C=O group of pyrimidinone ring.

The ^{1}H NMR spectra of **XIa,b** revealed the disappearance of the exchangeable singlet signal due to NH_2 protons in the precursor **IX**, in addition to the existance of multiplet signal at δ 2.98-3.13 ppm attributed to $2CH_2$ of succinimido moiety in compound **XIa**. Also in compound **XIb**, the appearance of four additional aromatic protons of phenyl part of phthalimido moiety at δ 7.88-8.04 ppm revealed its structure.

In addition, inspection of ^{13}C NMR spectrum of **XIa** indicated the appearance of CH_2 peak at δ 27.32 ppm.

Furthermore, mass spectrum of compound **XIb** showed a molecular ion peak at *m/z* 385 ($M^{+\cdot}$) and a base peak at *m/z* 303.

3,6,3\',6\'-Tetramethyl-1,1\'-diphenyl-1*H*,1\'*H*-[5,5\']bi[pyrazolo[3,4-*d*]pyrimidinyl]-4,4\'-dione (XII)



The desired bipyrazolo[3,4-*d*]pyrimidine derivative **XII** was synthesized *via* fusion of the oxazin-4-one derivative **IV** with 5-amino-3,6-dimethyl-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (**IX**).

The structure assigned to the title compound **XII** was supported by elemental analysis and spectral data.

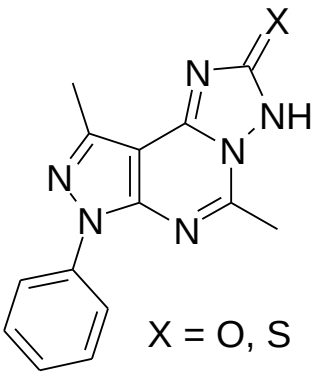
IR spectrum showed the disappearance of the forked bands of NH_2 at 3322-3262 cm^{-1}, in addition to the presence of a broad absorption band at 1715 cm^{-1} corresponding to 2C=O groups.

Inspection of The ^{1}H NMR spectrum of **XII** revealed the disappearance of the exchangeable singlet signal due to NH_2 protons in the precursor **IX** at δ 5.71

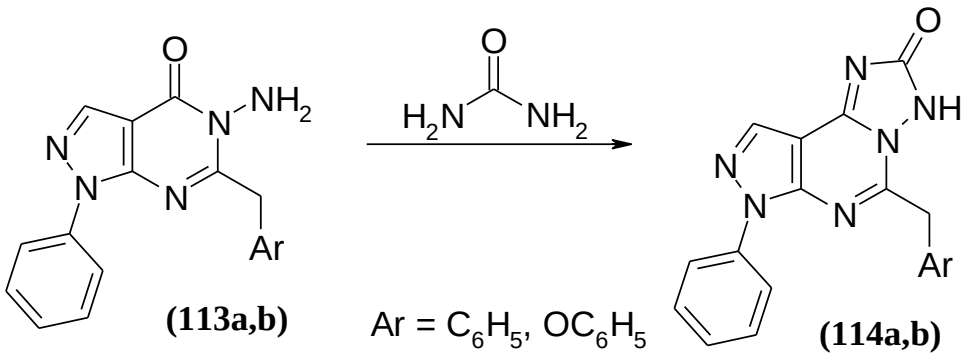
ppm. In additon to, the presence of $2CH_3$ protons of both pyrazoles and pyrimidines at δ 2.52 and 2.63 ppm, respectively. Also, the existance of ten aromatic protons at δ 7.37-7.96 ppm confirmed the structure of the title compound.

Also, the mass spectrum of **XII** revealed a molecular ion peak at *m/z* 478 for ($M^{+\cdot}$), and at m/z 80 for a base peak.

5,9-Dimethyl-7-phenyl-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-2-one or (2-thione) (XIIIa,b)

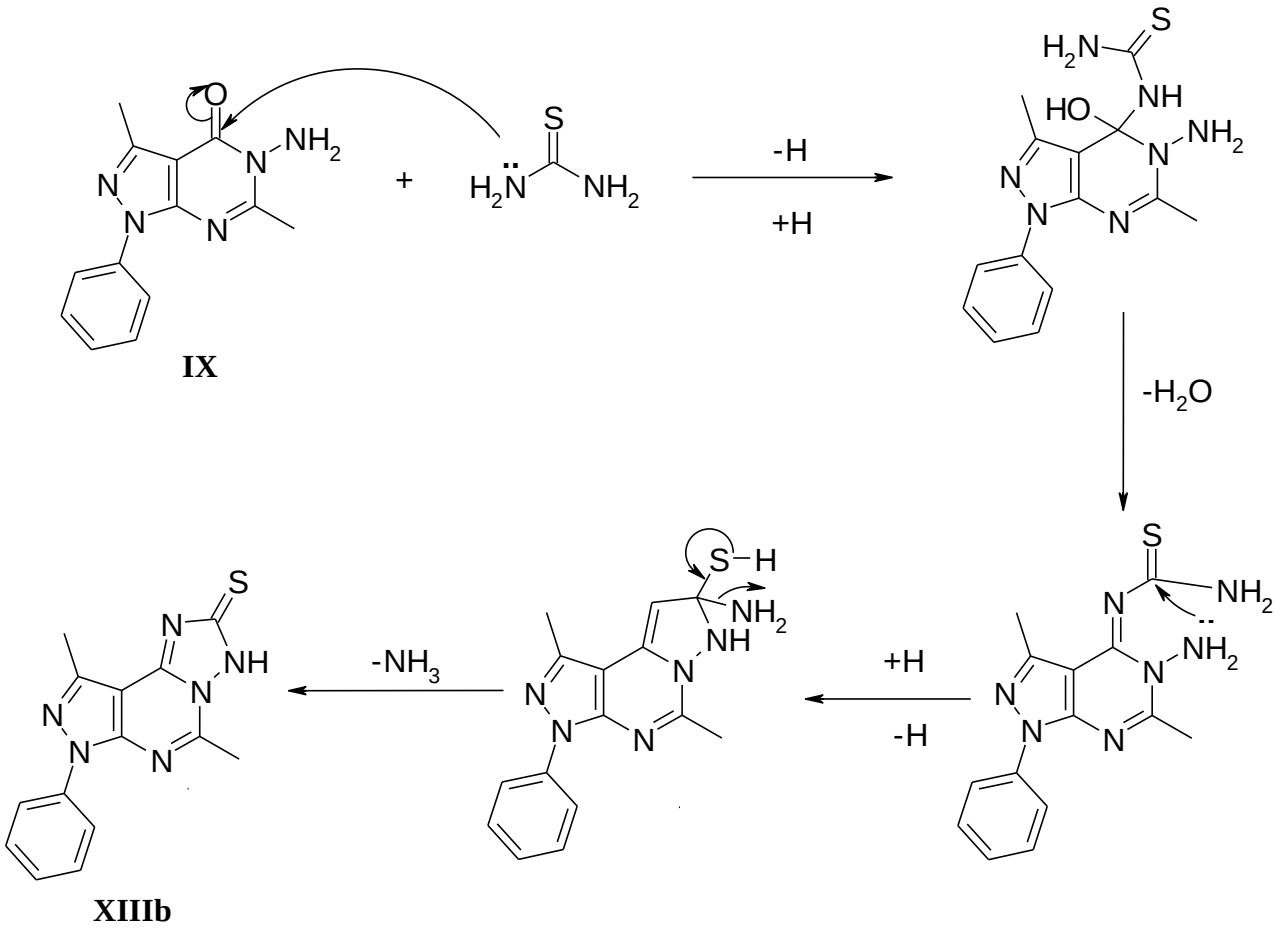


In 2011, Hussein *et al*[134] reported the preparation of similar 1,2,4-triazol derivatives **114a,b** *via* fusion of the corresponding pyrazolopyrimidine derivatives **113a,b** with urea at 220 °C.



In the present study, compounds **XIIIa,b** were prepared through the fusion of 5-amino-3,6-dimethyl-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (**IX**) with urea or thiourea at 220 °C.

A suggested mechanism for the formation of the title compound **XIIIb** could be illustrated as follows:



The structure of compounds **XIIIa,b** was established on the basis of microanalytical and spectral data.

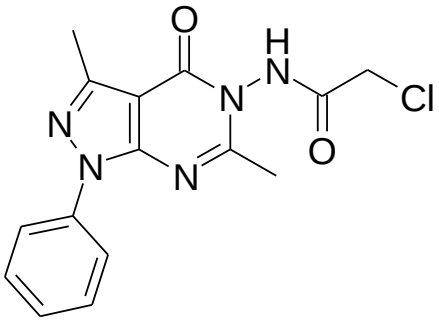
IR spectrum of **XIIIb** demonstrated the appearance of an absorption band at 3344 cm^{-1} related to NH group. In addition to an absorption band at 1121 cm^{-1} corresponding to C=S group.

The ^{1}H NMR spectrum of **XIIIb** revealed the presence of the an exchangeable singlet signal due to NH proton appeared at δ 9.83 ppm.

Moreover, the mass spectrum of **XIIIb** displayed a molecular ion peak at *m/z* 296 correspoding to ($M^{+\cdot}$).

Scheme 3:

2-Chloro-*N*-(3,6-dimethyl-4-oxo-1-phenyl-1,4-dihydropyrazolo[3,4-*d*]pyrimidin-5-yl)acetamide (XIV)



Reported studies showed that chloroacetamido derivatives were prepared *via* the treatment of chloroacetyl chloride with the appropriate amine derivative in different suitable solvent only such as benzene[135], dioxane[136], dimethylformamide[137] or acetone[138] or in presence of bases as potassium carbonate[139-142] or triethylamine.[143]

In the present study, compound **XIV** was prepared by the reaction of compound **IX** with chloroacetyl chloride in dimethylformamide in presence of potassium carbonate.

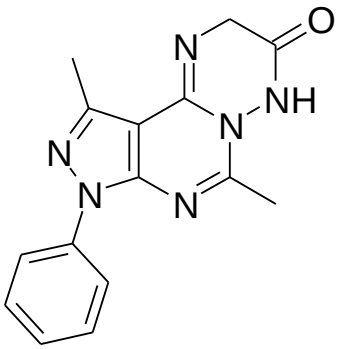
The IR spectrum of compound **XIV** demonstrated the presence of a broad absorption band at 1698 cm^{-1} corresponding to broad band of two C=O groups.

Also, ^{1}H NMR spectrum of compound **XIV** revealed the presence of two doublet signals at δ 4.26 and 4.33 ppm with *J* value 13.5 Hz corresponding to

CH_2 and the appearance of D_2O exchangeable signal at δ 11.39 ppm corresponding to NH proton.

Additionally, the mass spectrum of compound **XIV** revealed molecular ion peak at *m/z* 331 ($M^{+\cdot}$) and 333 ($M+2^{+\cdot}$) in a ratio 3:1 (Cl pattern).

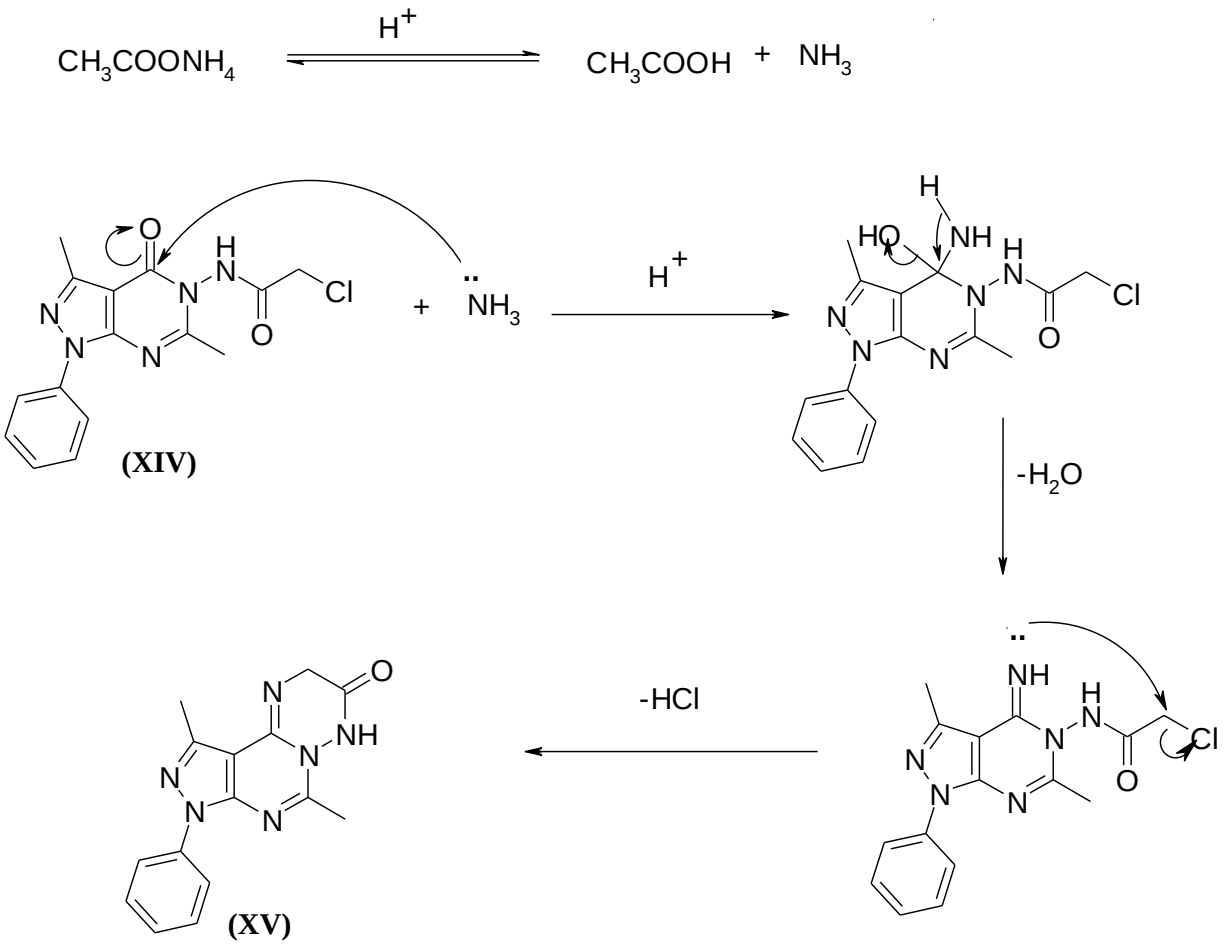
6,10-Dimethyl-8-phenyl-2,4-dihydro-8*H*-pyrazolo[3\',4\':4,5] pyrimido[1,6-*b*][1,2,4]triazin-3-one (XV)



It was reported that the formation of a triazine ring starting with pyrimidinone vicinal to chloroacetamido side chain could be achieved *via* condensation with ammonia followed by intramolecular cycloaddition giving the target compound.[144]

In this thesis, compound **XV** was obtained *via* reacting the chloroacetamido derivative **XIV** with ammonium acetate adopting a reported method.[144]

A suggested mechanism for the formation of the title compound **XV** might be illustrated as follows:

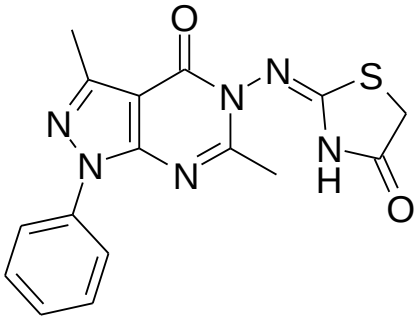


The structure assigned to the title compound **XV** was supported by elemental analysis and spectral data.

The IR spectrum of compound **XV** displayed the appearence of an absorption band at 1693 cm^{-1} corresponding to C=O group.

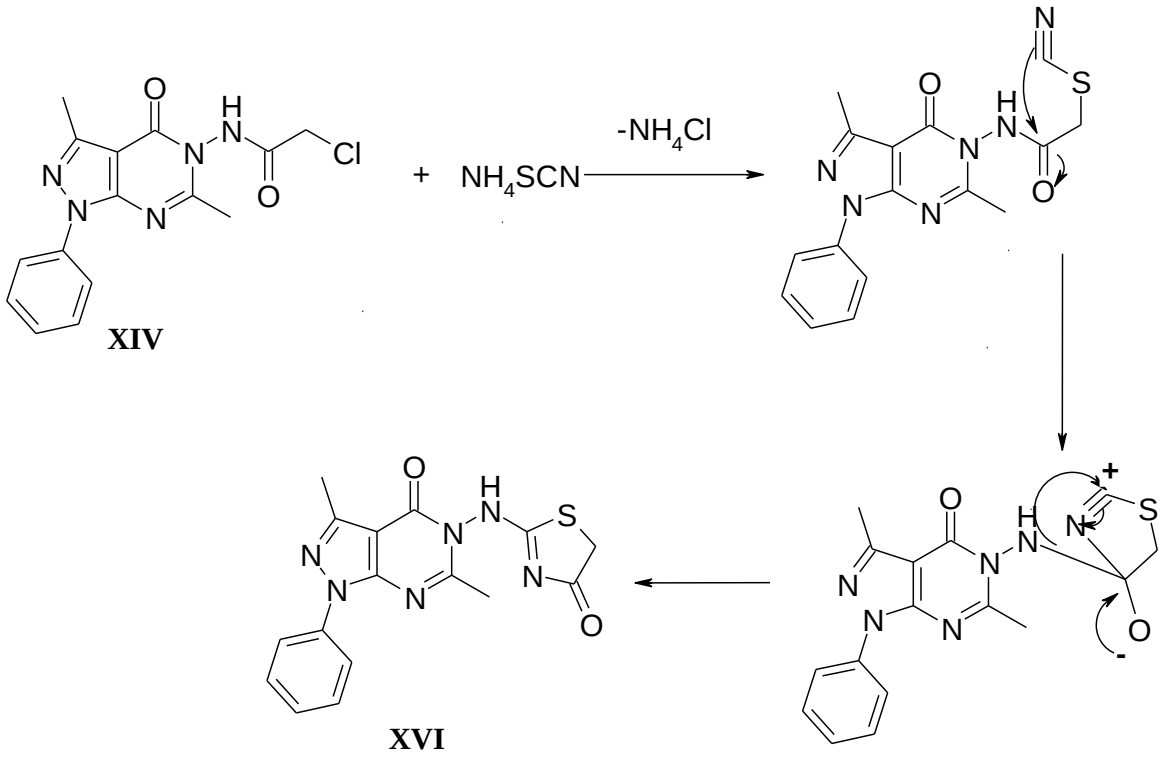
The ^{1}H NMR spectrum confirmed **XV** by the appearance of a singlet signal at δ 2.41 ppm corresponding to CH_2 protons. In addition to, the appearance of a singlet signal exchangeable with D_2O at δ 10.94 ppm corresponding to NH proton.

3,6-Dimethyl-5-(4-oxothiazolidin-2-ylideneamino)-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (XVI)



The newly formed imino-4-thiazolidinone derivative **XVI** was prepared from the reaction of chloroacetamido derivative **XIV** with ammonium thiocyanate in ethanol following the precautions of similar reactions.[145-149]

A suggested mechanism for the formation of the title compound **XVI** might be illustrated as follows:[145]



XIV

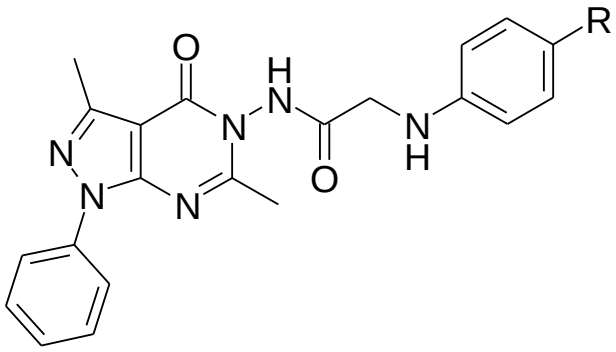
XVI

The structure of compound **XVI** was established on the basis of microanalytical and spectral data. IR spectrum revealed the appearance of absorption band at 1737 and 1673 corresponding to two C=O groups.

The ^{1}H NMR spectrum confirmed the structure of **XVI** by the appearance of a singlet signal corresponding to CH_2 protons of thiazolidinone moiety at δ 4.06 ppm.

Moreover, the mass spectrum of **XVI** demonstrated molecular ion peak at *m/z* 354 indicating ($M^{+\cdot}$) and base peak at *m/z* 307.

***N*-(3,6-Dimethyl-4-oxo-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-5-yl)-2-(4-substituted phenylamino)acetamides (XVIIa-c)**



R = OH, $COCH_3$, COOH

Literature survey revealed that the reaction of chloroacetamido derivatives and different primary amines could be proceed in different solvents such as ethanol using equimolar amount of sodium bicarbonate[(150)], tetrahydrofuran containing anhydrous potassium carbonate[(151)], dioxane containing triethyl amine[(152)] and glacial acetic acid.[(153)]

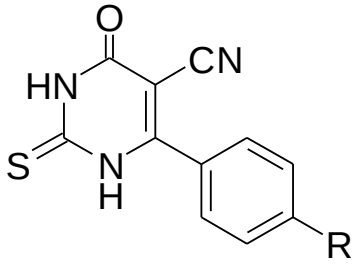
In the present study, compounds **XVIIa-c** were obtained by the reaction of the chloroacetamido **XIV** with the appropriate primary aromatic amine in ethanol containing anhydrous potassium carbonate.

Microanalytical and spectral data were in accordance with the expected structures, hence, the IR spectra of **XVIIa-c** showd the presence of absorption bands at 3380-3260 cm^{-1} corresponding to two NH groups.

The ^{1}H NMR spectra of **XVIIa-c** revealed the appearance of four additional aromatic protons corresponding to the amine part. Moreover, D_2O exchangeable signals appeared at δ 11.11 corresponding to carboxylic OH proton in **XVIIc**.

Furthermore, mass spectrum of compound **XVIIb** showed a molecular ion peak at *m/z* 430 ($M^{+\cdot}$) and a base peak at *m/z* 77.

6-Aryl-4-oxo-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carbonitrile (XVIIIa,b)

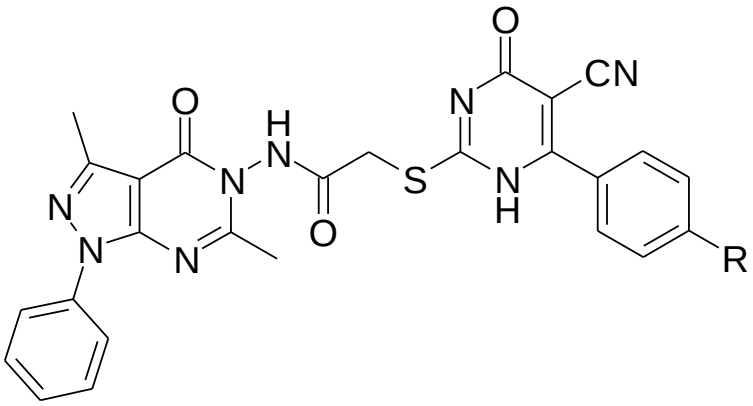


R = H, Cl

The title compounds were reported to be synthesized by Kambe′s *et al*[154] *via* treating equimolar amounts of ethyl cyanoacetate, the appropriate aromatic aldehyde and thiourea in presence of potassium carbonate.

In this investigation, the synthesis of **XVIIIa,b** was carried out by adopting Kambe′s *et al* method.[154]

2-[5-Cyano-6-aryl-4-oxo-1,4-dihydropyrimidin-2-ylsulfanyl]-*N*-(3,6-dimethyl-4-oxo-1-phenyl-1,4-dihydropyrazolo[3,4-*d*]pyrimidin-5-yl)acetamides (XIXa,b)



R = H, Cl

It has been reported that S-alkylation reaction could be achieved by heating the thiol compound with alkyl or aryl halide in dry acetone containing potassium carbonate[155] or triethyl amine[156], methanol containing triethyl amine[157], ethanol containing sodium acetate[158], dry pyridine[159] or dry benzene containing potassium carbonate.[160]

In the present study, compounds **XIXa,b** were obtained by the reaction of the chloroacetamido **XIV** with the appropriate 4-oxo-6-(4-substituted phenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile **(XVIIIa,b)** in acetone containing potassium carbonate.

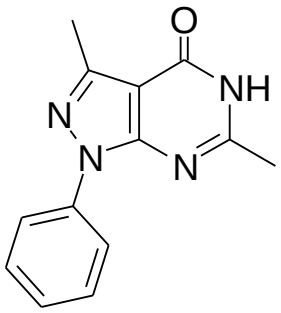
Microanalytical and spectral data were in accordance with the expected structures, hence, the IR spectra of **XIXa,b** showd the presence of absorption bands at 2204-2203 cm^{-1} corresponding to C≡N group.

The ^{1}H NMR cofirmed **XIXa,b** by the appearance of new additional aromatic protons.

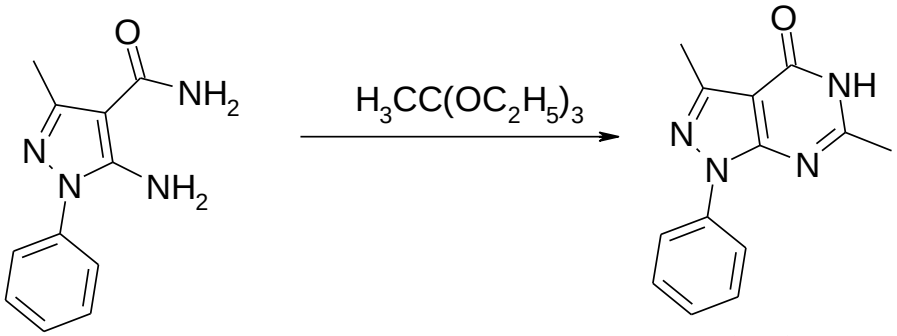
Furthermore, mass spectrum of compound **XIXa** showed a molecular ion peak at *m/z* 524 ($M^{+\cdot}$) and a base peak at *m/z* 105.

Scheme 4:

3,6-Dimethyl-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (XX)

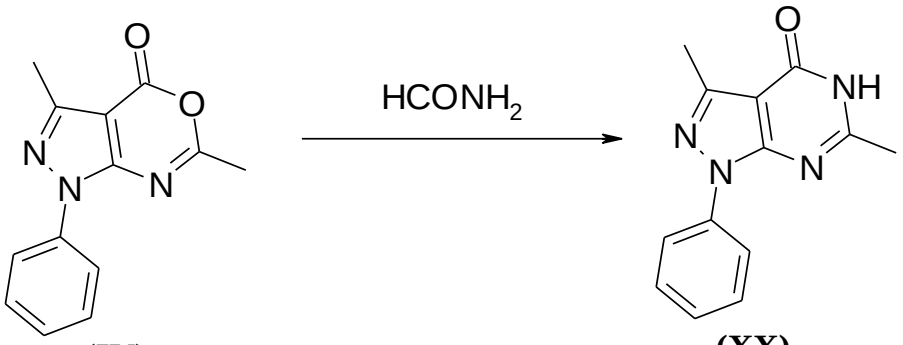


In 2006, Davoodnia *et al*[161] reported the synthesis of **XX** *via* microwave promoted solvent-free cyclization of 5-aminopyrazole-4-carboxamide derivative **115** with triethyl orthoacetate in the presence of silicagel.



(115) **(XX)**

In the present study, a new method for synthesis of the reported compound **XX** was prepared from the reaction of pyrazoloxazin-4-one derivative **IV** with excess formamide under reflux.

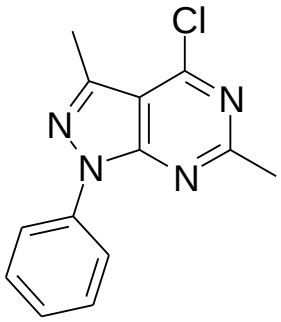


(IV) **(XX)**

The structure of compound **XX** was confirmed by elemental analysis and spectral data. The IR spectrum of **XX** showed absorption band 3420 cm^{-1} due to NH group.

The ^{1}H NMR proved the structure, as it showed one exchangeable singlet signal at δ 12.24 ppm. Also, the mass spectrum of **XX** displayed a molecular ion peak at m/z 240 ($M^{+\cdot}$) as a base peak.

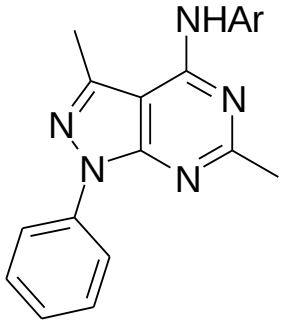
4-Chloro-3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (XXI)



Davoodnia *et al*[162] revealed that the reaction of 3,6-dimethyl-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (**XX**) with phosphorous oxychloride gave 4-chloro-1*H*-pyrazolo[3,4-*d*]pyrimidine derivative **XXI**.

In this investigation, the title compound **XXI** was prepared adopting Davoodnia *et al* method.[162]

3,6-Dimethyl-1-phenyl-4-substituted amino-1*H*-pyrazolo[3,4-*d*]pyrimidines (XXIIa-e)



Ar = 4-OH-C_6H_4, 3-$COCH_3$-C_6H_4, 4-COOH-C_6H_4, 2-COOH-C_6H_4, 4-$COOC_2H_5$-C_6H_4

Consulting the literature pointed out that 4-substituted amino derivatives were prepared *via* reacting the chloro derivative with the appropriate aromatic amine in a suitable solvent such as methanol[163], dimethylformamide[164], isopropyl alcohol[165] and ethanol containing triethyl amine.[76]

In this work, **XXIIa-e** were obtained by reacting 4-chloro-3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (**XXI**) with the selected amines in isopropyl alcohol.

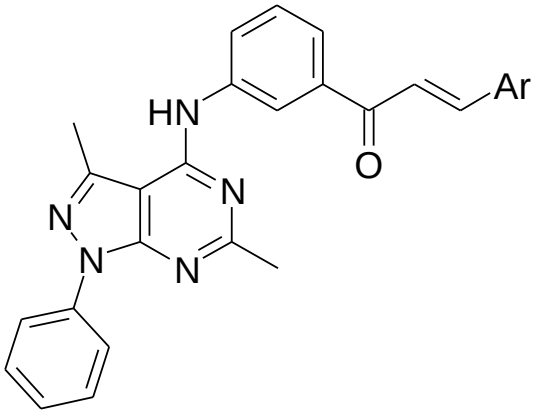
The structure of the title compounds was substantiated by elemental analysis, IR, ^{1}H NMR and mass spectra.

The IR spectrum of **XXIIe** showed the presence of an absorption band at 3310 cm^{-1} corresponding to NH group. In addition to an absorption band at 1710 cm^{-1} due to C=O group.

The ^{1}H NMR spectrum of compound **XXIIe** displayed the appearance of a triplet signal at δ 1.42 ppm and a quartet signal at δ 4.40 ppm with *J* value 6.9 Hz corresponding to CH_3 and CH_2 protons of ester part. Also, the presence of two doublet signals at δ 7.87 and 8.11 ppm with *J* value 8.4 Hz due to four additional aromatic protons of ethylcarboxyphenyl part confirmed the structure of **XXIIe**.

In addition, the mass spectrum of **XXIIe** displayed a molecular ion peak at *m/z* 387 ($M^{+\cdot}$) as a base peak.

(*E*) 3-Aryl-1-[3-(3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)phenyl]prop-2-en-1-one (XXIIIa-e)



Ar = C_6H_5, 4-F-C_6H_4, 4-CH_3O-C_6H_4, 4-Cl-C_6H_4, 4-NO_2-C_6H_4

It was reported that chalcones were synthesized by base catalysed condensation reaction of the suitable acetophenone and the appropriate aromatic aldehyde.[166]

The chalcone derivatives **XXIIIa-e** were obtained by condensation of 4-(3-acetylphenyl)amino-3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **(XXIIb)** with the appropriate aromatic aldehyde in presence of (10%) aqueous sodium hydroxide solution in ethanol.

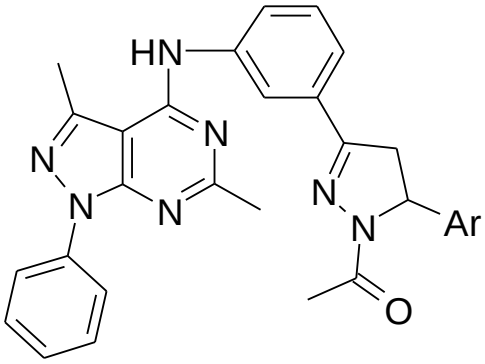
The IR spectra of **XXIIIa-e** showed the presence of an absorption band at 3438-3423 cm^{-1} corresponding to NH group. In addition to an absorption band at 1681-1655 cm^{-1} due to C=O group.

The ^1H NMR spectra of **XXIIIa-e** revealed the disappearance of the singlet signal at δ 2.79 due to CH_3 protons in the precursor **XXIIb** and showed signals at

δ 7.32-7.95 ppm for CH=CH protons. Also, the presence of a singlet signal at δ 3.83 ppm of OCH_3 confimed the structure of **XXIIIc**.

In addition, mass spectrum of **XXIIId** revealed molecular ion peak at *m/z* 479 ($M^{+\cdot}$) and 481 ($M+2^{+\cdot}$) in a ratio 3:1 (Cl pattern).

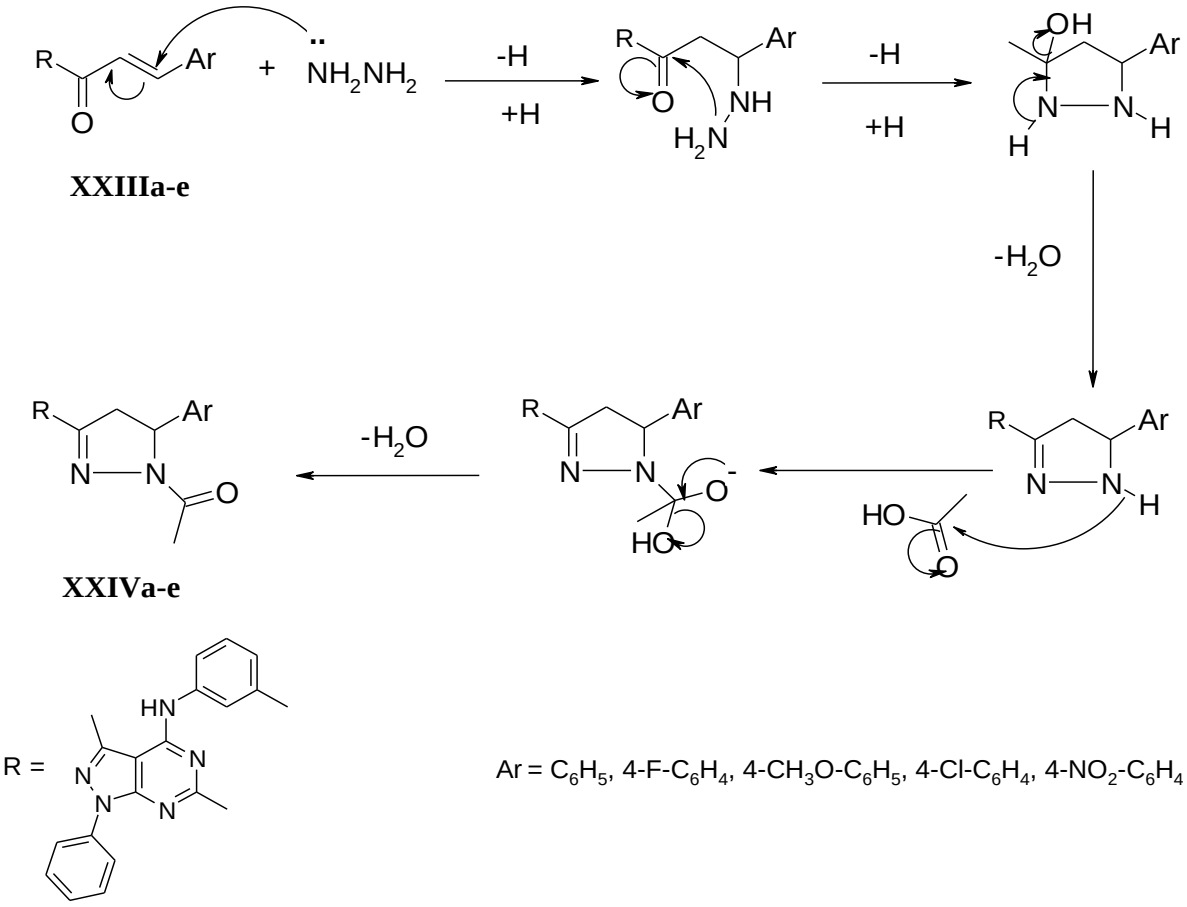
4-[3-(1-Acetyl-5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)phenylamino]-3,6-dimethyl-1-phenyl-1*H*-pyazolo[3,4-*d*]pyrimidines (XXIVa-e)



Ar^1 = C_6H_5, 4-F-C_6H_4, 4-CH_3O-C_6H_4, 4-Cl-C_6H_4, 4-NO_2-C_6H_4

The desired compounds **XXIVa-e** were prepared by cyclization of the chalcone derivatives **XXIIIa-e** with hydrazine hydrate in glacial acetic acid adopting conditions of analogue method.[167]

A suggested mechanism for the formation of the title compound **XXIVa-e** might be illustrated as follows:[168]



XXIIIa-e

XXIVa-e

The ^{1}H NMR spectra showed two protons of pyrazoline CH_2 protons as two doublet of doublet signals at δ 3.03-3.20 ppm and 3.74-4.01 ppm, in addition to the appearance of doublet of doublet signal at 5.56-5.74 ppm for pyrazoline H-5 proton. Also, the presence of a singlet signal at δ 2.70-2.80 ppm for O=C-CH_3 protons cofirmed the formation of acetylpyrazolines **XXIVa-e**.

Additionally, mass spectrum of **XXIVd** demonstrated molecular ion peaks at *m/z* 535 and 537 corresponding to ($M^{+\cdot}$) and ($M+2^{+\cdot}$) in a ratio of 3:1 (Cl pattern).

3.2. Docking study

Molecular modeling is a study for the development of new drugs using computer aided ligand design (CALD). The CALD technology deals with 3-dimensional (3D) structures of both the target (receptor or enzyme) and its ligand. CALD study is currently in use in order to develope a high affinity ligand. CALD is a first step beside other as bioavailability, toxicity and metabolism in the way of discovering more sucessfully profile and efficient drugs. New kinase inhibitors are primarily developed with a combination of methods including high-throughput screening using biochemical or cellular assays, analogue synthesis, structure-guided design and fragment-based assembly strategies.[85]

Protein kinases play critical roles in cellular signaling networks, and many proteins in this class are established targets for pharmaceutical intervention. Small molecule kinase inhibitors have generated much interest, as both potential therapeutics and experimental tools for understanding the physiological roles of these enzymes. Various small molecule target-selective inhibitors of disease-relevant protein kinases are currently in different stages of clinical testing, and the first representatives of this class have already received Food and Drug Administration (FDA) approval.[169]

Historically, many type ATP competitive inhibitors were discovered by performing high throughput screening of compound collections. Unfortunately, this approach is becoming less effective, as it has now identified most of scaffolds that are capable of serving as ATP competitive ligands. However, this method is still of use when a kinase with an unusual active site is screened or when allosteric inhibitors are specifically being sought. New ATP site targeted ligands are primarily developed using a combination of methods including analogue synthesis, structure-informed design and fragment-based assembly strategies.[85]

Activation of the kinases is achieved by ligand-binding to the extracellular domain, which induces homo/hetero-dimerization of the receptor. Activated receptors phosphorylate protein residues outside their catalytic domain *via* cross-phosphorylation. This phosphorylation stabilizes the receptor conformation in an active state and creates signal transduction within the cell.(63) In cancer, this mechanism of ligand-dependent activation can be by passed *via* either over expression of kinases which increases the dynamics of receptor homo/hetero-dimerization in the absence of ligands or mutation which stabilizes the receptor active conformation.(63) Therefore, small molecule inhibitors targeting ATP binding pocket of protein kinases has become the focus of development of new therapies for cancer.(170)

Structure of the ATP binding site in protein kinases

The kinase catalytic domain is a single polypeptide chain which folds into two lobes joined by a segment referred as hinge lobe (Fig. 4). The N-terminal lobe consists of mostly antiparallel β-sheets and a conserved α-helix, while the C-terminal lobe is mainly helical. The adenosine moiety of ATP binds in a cavity between the two lobes.(170)

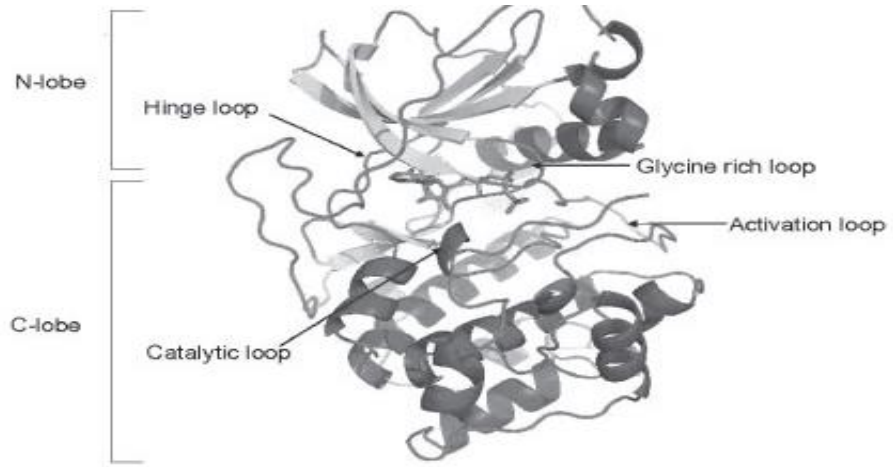


Fig.4. X-ray crystal structure of ATP bound to protein kinase

Five regions are distinguished in the ATP binding site:[45]

1- The adenine region: it is a hydrophobic region that forms the two key hydrogen bonds formed by the interaction of N1 and N6 amino groups of the adenine ring and with the NH and carbonyl groups of the adenine anchoring hinge region of the protein kinase. Many protein kinase inhibitors use at least one of these hydrogen bonds.

2- The hydrophobic pocket (or selective pocket): it is not used by ATP, but is exploited by most of kinase inhibitors and this plays an important role in the inhibitor selectivity.

3- The hydrophobic channel on the N-terminal lobe is induced by the ordered αC helix. As it is not used by ATP, it can be exploited to gain binding affinity.

4- The sugar region: it is hydrophilic in most of protein kinases.

5- The phosphate-binding region: in this region the triphosphate group of ATP is constrained by a glycine-rich loop and is bound by an array of basic amino acid residues, which are involved in the catalytic process.

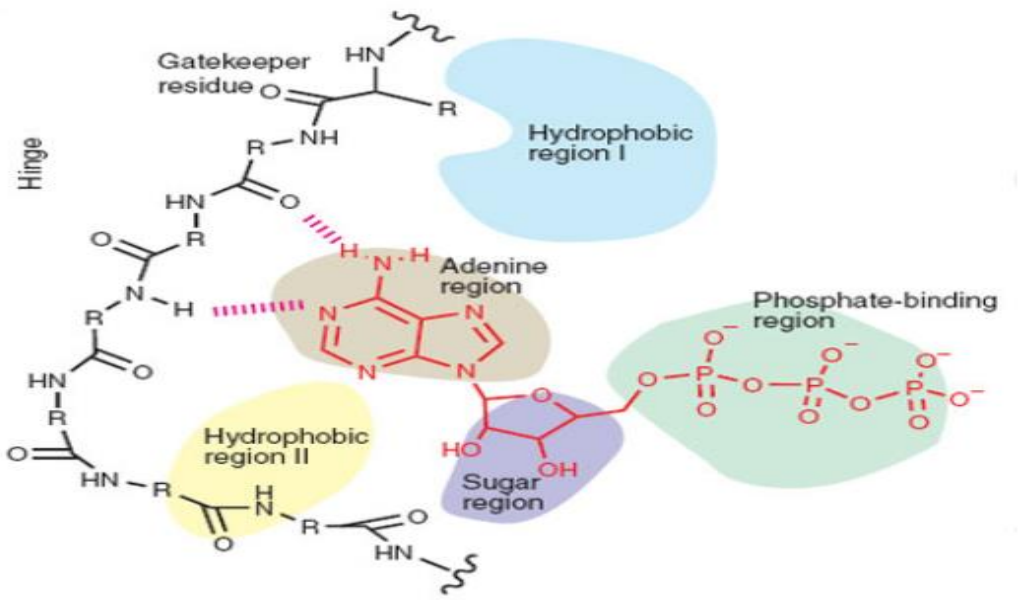


Fig.5. Schematic representation of ATP binding site and its five pockets.

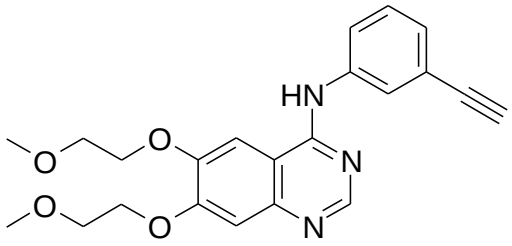
Small molecule protein kinase inhibitors

Protein kinase inhibitors are chemically diverse, low molecular weight (small molecules), hydrophobic heterocycles and most PKIs compete with the ATP for binding in kinase ATP binding site.[85]

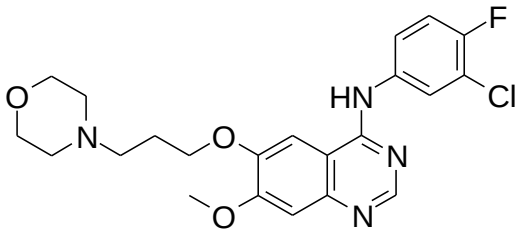
Kinase inhibitors should contain the following attributes to gain selectivity and potency :[85]

1- A portion that closely mimics ATP molecule.

2- One to three hydrogen bonds to the amino acids located in the hinge region of the target kinases, e.g. erlotinib (**100**) and gefitinib (**79**).[85]

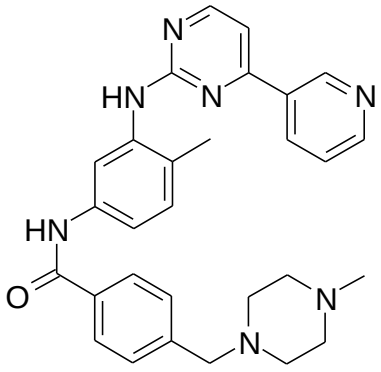


(100)

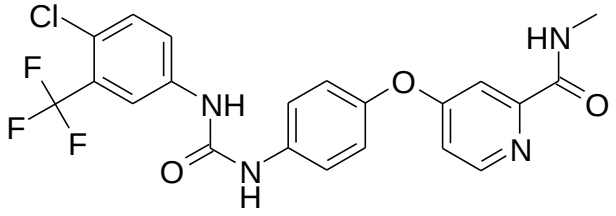


(79)

3- An additional hydrophobic binding site which is directly adjacent to the ATP binding site (allosteric site), e.g. imatinib mesylate (**78**)[171] and sorafenib (**116**).[172]



(78)



(116)

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However, other mechanisms of achieving may be possible as well, for instance, this could be achieved through binding outside the ATP- binding site at an allosteric site[173] and by forming irreversible covalent bond to the kinase active site.[85]

CDK2 kinase is a target for a remarkable variety of antitumor drugs, such as olomucine.[88]

The 3D structure of CDK2 protein kinase complexed with olomucine was obtained from Protein Data Bank (PDB entry: 1W0X) at Research Collaboration for Structural Bioinformatics (RCSB) protein database Fig.6.[174]

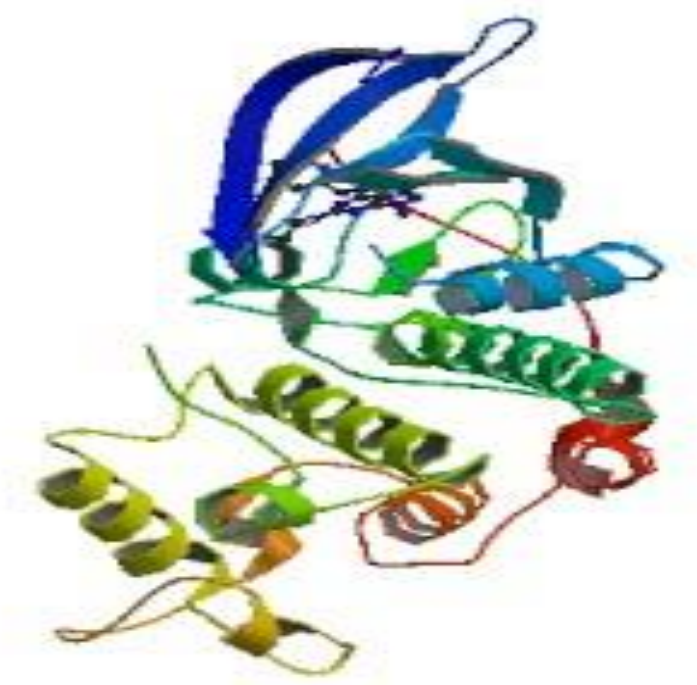


Fig.6. 3D structure of CDK2 with olomucine as antagonist.

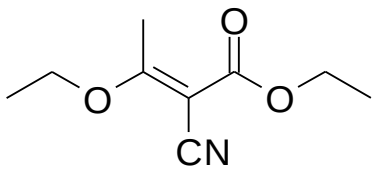
In the present work, the prepared compounds were docked manually using (MOE) version 2008.10. The data obtained for the prepared compounds from the docking study were explained in the experimental section 4.3.

4. Experimental

4.1. Practical work

- Melting points were determined on a Griffin apparatus and are uncorrected.
- IR spectra were determined as KBr discs on Shimadzu IR 435 spectrophotometer, Microanalytical Center, Cairo University and values were represented in cm^{-1}.
- ^{1}H NMR spectra were carried out on Varian Gemini 300 MHZ spectrometer, at nuclear magnetic resonance, Cairo University, or Brucker 400 MHZ spectrometer, at Faculty of pharmacy, Beni Suef University, using TMS as internal standard and chemical shifts were recorded in ppm on δ scale.
- ^{13}C NMR spectra were carried out on Brucker 400 MHZ spectrometer, at Faculty of pharmacy, Beni Suef University.
- Mass spectra were run at 70 ev on EI Shimadzu QP-2010 plus spectrometer, at the Microanalytical center, Cairo University.
- Element analyses were carried out at the Microanalytical Center, Cairo University.
- Progress of the reaction was monitored by TLC using aluminium sheets precoated with UV fluorescent silica gel (MERCK 60 F 254) that was visualized using UV lamp. The used solvent system was chloroform and methanol.
- The docking was performed using Molecular Operating Environment (MOE) software.

Ethyl 2-cyano-3-ethoxy-2-butenoate (I)[92]

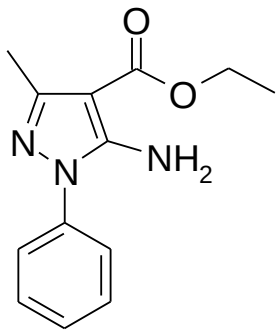


A mixture of ethyl cyanoacetate (5.65 gm, 0.05 mol), triethyl orthoacetate (8.1 gm, 0.05 mol) and acetic anhydride (9.4 mL) was heated under reflux for 5 h. The reaction mixture was concentrated under reduced pressure and left overnight in refrigerator. The separated solid was filtered and crystallized from ethanol to yield **I**.

m.p. 74 ^{o}C (as reported).[92]

Yield: 7.35 gm, 60%

Ethyl 5-amino-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylate (II)[92]



A mixture of ethyl 2-cyano-3-ethoxy-2-butenoate (**I**) (9.95 gm, o.055 mol) and phenylhydrazine (5.4 gm, o.05 mol) in absolute ethanol (60 mL) was heated under reflux for 15 h. The reaction mixture was filtered and the filterate was concentrated to its half volume and cooled. The resulting crystalline solid was filtered, washed with ethanol and dried to yield **II**.

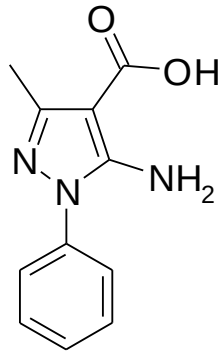
m.p. 215 ^{o}C (as reported).[92]

Yield: 8.35 gm, 62%,

IR cm^{-1}:

3433, 3314 (NH_2), 3069 (CH aromatic), 2929 (CH aliphatic), 1658 (C=O), 1615 (C=N).

5-Amino-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid (III)



A mixture of ethyl 5-amino-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylate (**II**) (12.25 gm, 0.05 mol) and sodium hydroxide (4.20 gm, 0.1 mol) in methanol (60 mL) was heated under reflux for 5h. The reaction mixture was cooled and poured into ice-cold water, then adjusted pH of the mixture to 4 using concentrated hydrochloric acid. The obtained solid was filtered, dried and crystallized from ethanol/water mixture (3:1) to yield **III**.

m.p. 156-157 ^{o}C.

Yield: 6.10 gm, 56.2%.

Analysis for $C_{11}H_{11}N_3O_2$ (217.23):

Element	C%	H %	N%
Calcd.	60.82	5.10	19.34
Found	60.69	5.20	19.67

IR cm^{-1}:

3389-3204 (OH & NH_2), 3009 (CH aromatic), 2927 (CH aliphatic), 1651 (C=O), 1613 (C=N).

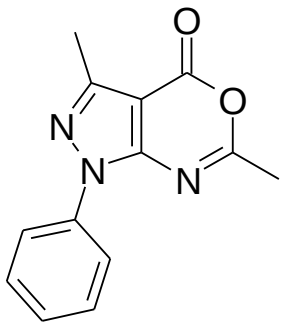
^{1}H NMR (DMSO-*d_6*, δ-ppm):

δ 2.24 (s, 3H, CH_3), 6.30 (s, 2H, NH_2, D_2O exchangeable), 7.36-7.54 (m, 5H, ArH), 12.07 (s,1H, OH, D_2O exchangeable).

EIMS (m/z) (relative abundance %):

217 ($M^{+\cdot}$, 27.65), 199 ($C_{11}H_9N_3O^{+\cdot}$, 68.16), 173 ($C_{10}H_{11}N_3^{+\cdot}$, 26.73), 91 ($C_6H_5N^{+\cdot}$, 63.29), 80 ($C_4H_4N_2^{+\cdot}$, 100).

3,6-Dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*][1,3]oxazin-4-one (IV)



A mixture of 5-amino-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylic acid (**III**) (2.17 gm, 0.01 mol) and acetic anhydride (5 mL) was heated under reflux for 5 h. After cooling, the formed solid was filtered, dried and crystallized from methanol to yield **IV**.

m.p. 129-130 ^{o}C.

Yield: 1.20 gm, 49.79%.

Analysis for $C_{13}H_{11}N_3O_2$ (241.25):

Element	C%	H %	N%
Calcd.	64.72	4.60	17.42
Found	65.01	4.73	17.19

IR cm^{-1}:

3078 (CH aromatic), 2923 (CH aliphatic), 1764 (C=O), 1599 (C=N).

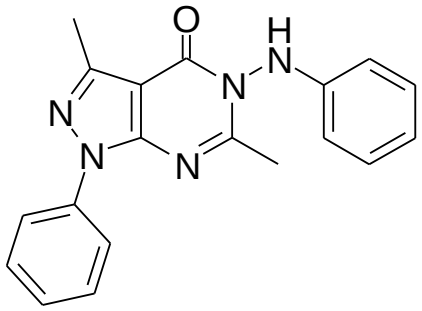
^{1}H NMR (DMSO-d_6, δ-ppm):

δ 2.45 (s, 3H, pyrazole CH_3), 2.49 (s, 3H, oxazine CH_3), 7.39-7.45 (m, 1H, phenyl H-4), 7.53-7.59 (m, 2H, phenyl H-3, H-5), 7.90 (d, *J* = 8.4 Hz, 2H, phenyl H-2, H-6).

EIMS (m/z) (relative abundance %):

241 ($M^{+\cdot}$, 100), 226 ($C_{12}H_8N_3O_2^{+\cdot}$, 75.72), 91 ($C_6H_5N^{+\cdot}$, 19.98), 77 ($C_6H_5^{+\cdot}$, 36.72).

3,6-Dimethyl-1-phenyl-5-phenylamino-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (V)



A mixture of 3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*][1,3]oxazin-4-one (**IV**) (2.41 gm, 0.01mol) and phenylhydrazine (1.08 gm, 0.01 mol) in ethanol (20 mL) was heated under reflux for 6 h. The formed precipitate was filtered while hot, dried and crystallized from butanol to yield **V**.

m.p. 265-266 °C.

Yield: 1.80 gm, 54.38%.

Analysis for $C_{19}H_{17}N_5O$ (331.38):

Element	C%	H %	N%
Calcd.	68.87	5.17	21.13
Found	68.68	5.09	21.05

IR cm^{-1}:

3250 (NH), 3115-3011 (CH aromatic), 2930 (CH aliphatic), 1685 (C=O).

^{1}H NMR (DMSO-d_6, δ-ppm):

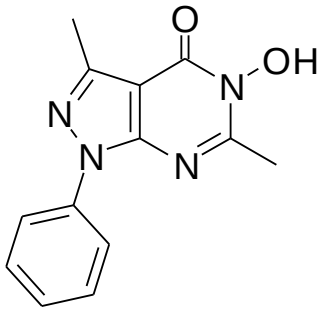
δ 2.49 (s, 3H, pyrazole CH_3), 2.52 (s, 3H, pyrimidine CH_3), 6.68 (d, *J* = 7.8 Hz, 2H, phenylamino H-2, H-6), 6.82-6.87 (m, 1H, phenylamino H-4), 7.18-7.23 (m, 2H, phenylamino H-3, H-5), 7.36-7.41 (m, 1H, phenyl H-4),

7.53-7.58 (m, 2H, phenyl H-3, H-5), 8.05 (d, *J* = 7.8 Hz, 2H, phenyl H-2, H-6), 9.09 (s, 1H, NH, D_2O exchangeable).

EIMS (m/z) (relative abundance %):

331 ($M^{+\cdot}$, 100), 198 ($C_{11}H_8N_3O^{+\cdot}$, 25.97), 91 ($C_6H_5N^{+\cdot}$, 79.86), 77 ($C_6H_5^{+\cdot}$, 79.06).

5-Hydroxy-3,6-dimethyl-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (VI)



A mixture of 3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*][1,3]oxazin-4-one (**IV**) (2.41 gm, 0.01mol) and hydroxylamine hydrochloride (0.69 gm, 0.01 mol) in dry pyridine (30 mL) was heated under reflux for 8 h. The reaction mixture was concentrated to its half volume and the separated solid was filtered, washed with water, dried and crystallized from dioxane to give **VI**.

m.p. 180-181 ^{o}C.

Yield: 1.90 gm, 74%.

Analysis for $C_{13}H_{12}N_4O_2$ (256.27):

Element	C%	H %	N%
Calcd.	60.93	4.72	21.86
Found	60.81	5.00	21.66

IR cm^{-1}:

3426 (OH), 3050 (CH aromatic), 2926 (CH aliphatic), 1680 (C=O), 1568 (C=N).

^{1}H NMR (DMSO-d_6, δ-ppm):

δ 2.48 (s, 3H, pyrazole CH_3), 2.53 (s, 3H, pyrimidine CH_3), 7.32-7.37 (m, 1H, phenyl H-4), 7.50-7.56 (m, 2H, phenyl H-3, H-5), 8.03 (d, *J* = 7.5 Hz, 2H, phenyl H-2, H-6), 11.51 (s, 1H, OH, D_2O exchangeable).

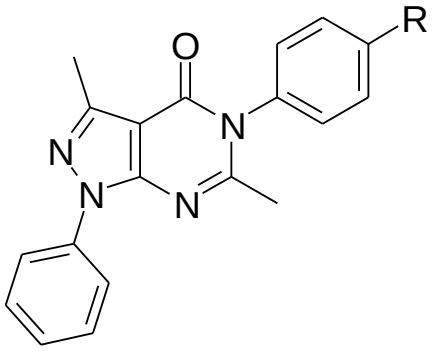
^{13}C NMR (DMSO-d_6, δ-ppm):

δ 13.71, 21.38, 105.07, 121.58, 126.92, 129.59, 138.75, 145.98, 149.94, 155.24, 157.86.

EIMS (m/z) (relative abundance %):

256 ($M^{+\cdot}$, 89.42), 239 ($C_{13}H_{11}N_4O^{+\cdot}$, 14.82), 91 ($C_6H_5N^{+\cdot}$, 29.69), 77 ($C_6H_5^{+\cdot}$, 100).

3,6-Dimethyl-1-phenyl-5-(4-substituted phenyl)-1,5-dihydro pyrazolo[3,4-*d*]pyrimidin-4-ones (VIIa-e)



A mixture of 3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*][1,3]oxazin-4-one (**IV**) (2.41 gm, 0.01mol) and the appropriate aromatic amine (0.01 mol) in dry pyridine (30 mL) was heated under reflux for 6 h. The reaction mixture was cooled, poured into cold water and acidified with concentrated hydrochloric acid till neutral to litmus paper. The separated product was filtered, washed with water, dried and crystallized from the appropriate solvent to give **VIIa-e**, (Table 1 and 2).

Table 1: Physical and analytical data of compounds VIIa-e

VII	R	Yield %	m.p.(°C)/ Solvent of crystallization	Mol. Formula (M.Wt)	Analysis %		
					Element	Calcd.	Found
a	$-CH_3$	50	170-171 Methanol	$C_{20}H_{18}N_4O$ (330.39)	C H N	72.71 5.49 16.96	72.77 5.20 16.64
b	-OH	76	250-251 Acetone	$C_{19}H_{16}N_4O_2$ (332.36)	C H N	68.66 4.85 16.86	68.30 4.50 16.98
c	$-COCH_3$	66	186-187 Ethanol	$C_{21}H_{18}N_4O_2$ (358.40)	C H N	70.38 5.06 15.63	70.31 4.90 15.94
d	-COOH	59	200-201 Acetic acid	$C_{20}H_{16}N_4O_3$ (360.38)	C H N	66.66 4.48 15.55	66.90 4.71 15.78
e	$-COOC_2H_5$	85	190-191 Ethanol	$C_{22}H_{20}N_4O_3$ (388.43)	C H N	68.03 5.19 14.42	68.21 5.51 14.66

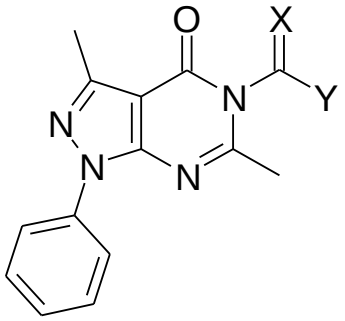
Table 2: Spectral data of compounds VIIa-e

VII	IR (cm^{-1}), ^1H NMR (DMSO-d_6, δ-ppm), EIMS (m/z) (relative abundance %)	
a	**IR**	3049 (CH aromatic), 2923 (CH aliphatic), 1693 (C=O), 1568 (C=N).
	^1H NMR	δ 2.16 (s, 3H, pyrazole CH_3), 2.51 (s, 3H, pyrimidine CH_3), 2.65 (s, 3H, CH_3), 7.38-7.40 (m, 1H, phenyl H-4), 7.53-7.62 (m, 4H, phenyl H-3, H-5, *p*-tolyl H-3, H-5), 8.06-8.15 (m, 4H, phenyl H-2, H-6, *p*-tolyl H-2, H-6).
	MS	330 ($M^{+\cdot}$, 100), 315 ($C_{19}H_{15}N_4O_2^{+\cdot}$, 47.68), 91 ($C_6H_5N^{+\cdot}$, 77.11), 77 ($C_6H_5^{+\cdot}$, 75.52).
b	**IR**	3439 (OH), 3065 (CH aromatic), 2930 (CH aliphatic), 1694 (C=O), 1566 (C=N).
	^1H NMR	δ 2.16 (s, 3H, pyrazole CH_3), 2.51 (s, 3H, pyrimidine CH_3), 6.89 (d, *J* = 8.7 Hz, 2H, hydroxyphenyl H-3, H-5), 7.17 (d, *J* = 8.7 Hz, 2H, hydroxyphenyl H-2, H-6), 7.26-7.29 (m, 1H, phenyl H-4), 7.52-7.57 (m, 2H, phenyl H-3, H-5), 8.07 (d, *J*= 7.8 Hz, 2H, phenyl H-2, H-6), 9.80 (s, 1H, OH, D_2O exchangeable).
	MS	332 ($M^{+\cdot}$, 100), 317 ($C_{18}H_{13}N_4O_2^{+\cdot}$, 61.26), 303 ($C_{17}H_{11}N_4O_2^{+\cdot}$, 14.05), 77 ($C_6H_5^{+\cdot}$, 57.48).

c	**IR**	2924 (CH aliphatic), 1699 (broad band of 2C=O), 1591 (C=N).
	^{1}H NMR	δ 2.15 (s, 3H, pyrazole CH_3), 2.44 (s, 3H, pyrimidine CH_3), 2.50 (s, 3H, O=C-CH_3), 7.23 (d, *J* = 7.8 Hz, 2H, acetylphenyl H-2, H-6), 7.26 (d, *J* = 7.8 Hz, 2H, acetylphenyl H-3, H-5), 7.52-7.64 (m, 3H, phenyl H-3, H-4, H-5), 8.08 (d, *J* = 7.8 Hz, 2H, phenyl H-2, H-6).
	MS	358 ($M^{+\cdot}$, 100), 343 ($C_{20}H_{15}N_4O_2^{+\cdot}$, 44.06), 91 ($C_6H_5N^{+\cdot}$, 20.03), 77 ($C_6H_5^{+\cdot}$, 29.07).
d	**IR**	3450 (OH), 3068 (CH aromatic), 2929 (CH aliphatic), 1713 (C=O), 1678 (pyrimidinone C=O), 1558 (C=N).
	^{1}H NMR	δ 2.16 (s, 3H, pyrazole CH_3), 2.51 (s, 3H, pyrimidine CH_3), 7.35-7.40 (m, 1H, phenyl H-4); 7.54-7.65 (m, 4H, phenyl H-3, H-5, benzoyl H-2, H-6), 8.07-8.13 (m, 4H, phenyl H-2, H-6, benzoyl H-3, H-5), 13.14 (s, 1H, OH, D_2O exchangeable).
	MS	360 ($M^{+\cdot}$, 79.41), 345 ($C_{19}H_{13}N_4O_3^{+\cdot}$, 27.92), 316 ($C_{19}H_{16}N_4O^{+\cdot}$, 16.73), 238 ($C_{13}H_{10}N_4O^{+\cdot}$, 14.46), 173 ($C_{10}H_{11}N_3^{+\cdot}$, 100), 120 ($C_7H_4O_2^{+\cdot}$, 73.74).
e	**IR**	3070 (CH aromatic), 2925 (CH aliphatic), 1709 (broad band of 2C=O), 1555 (C=N).

	^{1}H NMR	δ 1.35 (t, *J* = 7.2 Hz, 3H, $CH_2C\underline{H}_3$), 2.15 (s, 3H, pyrazole CH_3), 2.50 (s, 3H, pyrimidine CH_3), 4.37 (q, *J* = 7.2 Hz, 2H, $C\underline{H}_2CH_3$), 7.37-7.40 (m, 1H, phenyl H-4), 7.53-7.62 (m, 4H, phenyl H-3, H-5, ethylcarboxyphenyl H-2, H-6), 8.07-8.15 (m, 4H, phenyl H-2, H-6, ethylcarboxyphenyl H-3, H-5).
	MS	388 ($M^{+\cdot}$, 93.03), 373 ($C_{21}H_{17}N_4O_3^{+\cdot}$, 19.95), 359 ($C_{20}H_{15}N_4O_3^{+\cdot}$, 5.57), 77 ($C_6H_5^{+\cdot}$, 100).

3,6-Dimethyl-1-phenyl-5-substituted-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-ones (VIIIa-c)



A mixture of 3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*][1,3]oxazin-4-one (**IV**) (2.41 gm, 0.01 mol) and the appropriate amide or thioamide (0.01 mol) was fused at 200 ^{o}C for 1h. The mixture was cooled and methanol (20 Ml) was added to the mixture. The separated product was filtered, washed with methanol, dried and crystallized from the appropriate solvent to give **VIIIa-c**, (Table 3 and 4).

Table 3: Physical and analytical data of compounds VIIIa-c

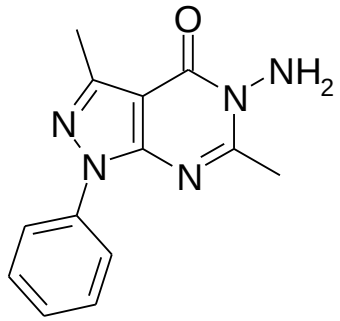
VIII	X	Y	Yield %	m.p.(°C)/ Solvent of crystallization	Mol. Formula (M.Wt)	Analysis %		
						Element	Calcd.	Found
a	O	$-NH_2$	65	> 300 Ethanol	$C_{14}H_{13}N_5O_2$ (283.29)	C H N	59.36 4.63 24.72	59.07 4.69 24.40
b	S	$-NH_2$	45	> 300 Ethanol	$C_{14}H_{13}N_5OS$ (299.36)	C H N	56.17 4.38 23.39	56.01 4.34 23.64
c	S	$-NHNH_2$	63	250-251 Acetone	$C_{14}H_{14}N_6OS$ (314.37)	C H N	53.49 4.49 26.73	53.28 4.47 27.01

Table 4: Spectral data of compounds VIIIa-c

VIII	IR (cm^{-1}), ^1H NMR (DMSO-d_6, δ-ppm), ^{13}C NMR (DMSO-d_6, δ-ppm), EIMS (m/z) (relative abundance %)	
a	**IR**	3432-3344 (NH_2), 3073 (CH aromatic), 2926 (CH aliphatic), 1671 (broad band of 2C=O), 1592 (C=N).
	^1H NMR	δ 2.37 (s, 3H, pyrazole CH_3), 2.49 (s, 3H, pyrimidine CH_3), 7.30-7.35 (m, 1H, phenyl H-4), 7.48-7.54 (m, 2H, phenyl H-3, H-5), 8.02 (d, *J* = 7.8 Hz, 2H, phenyl H-2, H-6), 12.20 (s, 2H, NH_2, D_2O exchangeable).
	^{13}C NMR	13.79, 21.96, 104.18, 121.76, 126.90, 129.55, 138.88, 146.16, 150.74, 153.37, 155.40, 159.09.
	MS	283 ($M^{+\cdot}$, 12.81), 240 ($C_{13}H_{12}N_4O^{+\cdot}$, 41.63), 55 ($C_2H_3N_2^{+\cdot}$, 100).
b	**IR**	3425 (NH_2), 2923 (CH aliphatic), 1675 (C=O), 1596 (C=N), 1118 (C=S).
	^1H NMR	δ 2.38 (s, 3H, pyrazole CH_3), 2.50 (s, 3H, pyrimidine CH_3), 7.33-7.36 (m, 1H, phenyl H-4), 7.49-7.54 (m, 2H, phenyl H-3, H-5), 8.02 (d, *J* = 7.8 Hz, 2H, phenyl H-2, H-6), 12.24 (s, 2H, NH_2, D_2O exchangeable).

c	**IR**	3321-3188 (NH & NH_2), 3064 (CH aromatic), 2920 (CH aliphatic), 1694 (C=O), 1590 (C=N), 1105 (C=S).
	^{1}H NMR	δ 2.37 (s, 3H, pyrazole CH_3), 2.49 (s, 3H, pyrimidine CH_3), 7.33-7.36 (m, 1H, phenyl H-4), 7.49-7.53 (m, 2H, phenyl H-3, H-5), 8.01 (d, *J* = 7.2 Hz, 2H, phenyl H-2, H-6), 11.14 (s, 2H, NH_2, D_2O exchangeable), 12.19 (s, 1H, NH, D_2O exchangeable).
	MS	314 ($M^{+\cdot}$, 7.02), 69 ($C_2HN_2O^{+\cdot}$, 100).

5-Amino-3,6-dimethyl-1-phenyl-1,5-dihydropyrazolo[3,4-*d*] pyrimidin-4-one (IX)



A mixture of 3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*][1,3]oxazin-4-one (**IV**) (2.41 gm, 0.01 mol) and hydrazine hydrate (99.9%) (0.5 mL, 0.01 mol) in butanol (20 mL) was heated under reflux for 6 h. After cooling, the separated solid was filtered, dried and crystallized from ethanol to yield **IX**.

m.p. 129-130 °C

Yield: 1.20 gm, 47.06%.

Analysis for $C_{13}H_{13}N_5O$ (255.28):

Element	C%	H %	N%
Calcd.	61.17	5.13	27.43
Found	61.53	5.03	27.23

IR cm^{-1}:

3322, 3262 (forked, NH_2), 3065 (CH aromatic), 2924 (CH aliphatic), 1692 (C=O).

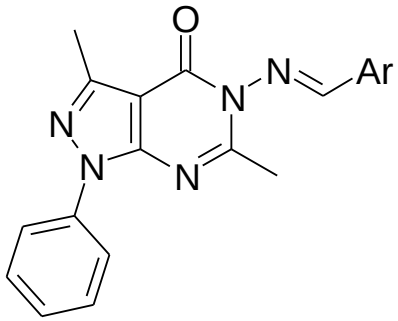
^{1}H NMR (DMSO-d_6, δ-ppm):

δ 2.50 (s, 3H, pyrazole CH_3), 2.59 (s, 3H, pyrimidine CH_3), 5.71 (s, 2H, NH_2, D_2O exchangeable), 7.30-7.35 (m, 1H, phenyl H-4), 7.49-7.54 (m, 2H, phenyl H-3, H-5), 8.05 (d, *J* = 7.5 Hz, 2H, phenyl H-2, H-6).

EIMS (m/z) (relative abundance %):

255 ($M^{+\cdot}$, 100), 122 ($C_5H_4N_3O^{+\cdot}$, 51.25).

5-(Arylideneamino)-3,6-dimethyl-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-ones (Xa-e)



A mixture of 5-amino-3,6-dimethyl-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (**IX**) (2.55 gm, 0.01 mol) and the appropriate aromatic aldehyde (0.012 mol) in glacial acetic acid (15 mL) was heated under reflux for 20 h. The reaction mixture was concentrated under reduced pressure and then cooled. The obtained solid was filtered, washed with water, dried and crystallized from the appropriate solvent to give **Xa-e**, (Table 5 and 6).

Table 5: Physical and analytical data of compounds Xa-e

X	Ar	Yield %	m.p.(°C)/ Solvent of Crystallization	Mol. Formula (M.Wt)	Analysis %		
					Element	Calcd.	Found
a	C_6H_5-	70	250-251 Ethanol	$C_{20}H_{17}N_5O$ (343.39)	C H N	69.96 4.99 20.39	69.77 5.20 20.54
b	4-F-C_6H_4-	68	160-161 Methanol	$C_{20}H_{16}FN_5O$ (361.38)	C H N	66.47 4.46 19.38	66.75 4.80 19.00
c	4-CH_3O-C_6H_4-	55	255-256 Toluene	$C_{21}H_{19}N_5O_2$ (373.42)	C H N	67.55 5.13 18.75	67.10 5.01 18.47
d	4-Cl-C_6H_4-	60	> 300 Ethanol	$C_{20}H_{16}ClN_5O$ (377.84)	C H N	63.58 4.27 18.54	63.28 4.04 18.59
e	4-NO_2-C_6H_4-	95	> 300 Acetic acid	$C_{20}H_{16}N_6O_3$ (388.39)	C H N	61.85 4.15 21.64	62.15 4.44 21.75

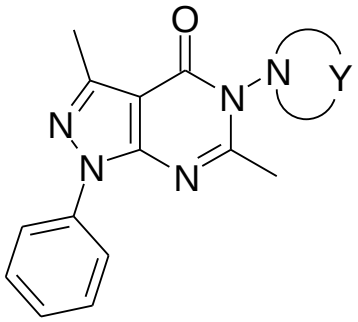
Table 6: Spectral data of compounds Xa-e

X	IR (cm^{-1}), ^1H NMR (DMSO-d_6, δppm), ^{13}C NMR (DMSO-d_6, δ-ppm), EIMS (m/z) (relative abundance %)	
a	**IR**	3055 (CH aromatic), 2925 (CH aliphatic), 1702 (C=O), 1555 (C=N).
	^1H NMR	δ 2.50 (s, 3H, pyrazole CH_3), 2.55 (s, 3H, pyrimidine CH_3), 7.37-7.39 (m, 1H, phenyl H-4), 7.53-7.65 (m, 5H, phenyl H-3, H-5, benzylidene H-3, H-4, H-5), 7.98 (d, *J* = 7.2 Hz, 2H, phenyl H-2, H-6), 8.08 (d, *J* = 8.1 Hz, 2H, benzylidene H-2, H-6), 8.89 (s, 1H, N=CH).
	MS	343 ($M^{+\cdot}$, 10.13), 240 ($C_{13}H_{12}N_4O^{+\cdot}$, 100), 103 ($C_7H_5N^{+\cdot}$, 26.01), 77 ($C_6H_5^{+\cdot}$, 77.14).
b	**IR**	3067 (CH aromatic), 2928 (CH aliphatic), 1709 (C=O), 1596 (C=N).
	^1H NMR	δ 2.10 (s, 3H, pyrazole CH_3), 2.41 (s, 3H, pyrimidine CH_3), 7.38-7.40 (m, 1H, phenyl H-4), 7.52-7.57 (m, 4H, phenyl H-3, H-5, fluorobenzylidene H-3, H-5), 7.99-8.01 (m, 4H, phenyl H-2, H-6, fluorobenzylidene H-2, H-6), 10.94 (s, 1H, N=CH).
c	**IR**	3072 (CH aromatic), 2925 (CH aliphatic), 1671 (C=O), 1594 (C=N).
	^1H NMR	δ 2.49 (s, 3H, pyrazole CH_3), 2.50 (s, 3H, pyrimidine CH_3), 3.84 (s, 3H, OCH_3), 7.20-7.23 (m, 1H, phenyl H-4), 7.37-7.39 (m, 2H, phenyl H-3, H-5), 7.49-7.57 (m, 4H, methoxybenzylidene H-3, H-5, phenyl H-2, H-6), 8.07 (d, *J* = 8.7 Hz, 2H, methoxybenzylidene H-2, H-6), 8.85 (s, 1H, N=CH).

	MS	373 ($M^{+\cdot}$, 5.71), 240 ($C_{13}H_{12}N_4O^{+\cdot}$, 100), 77 ($C_6H_5^{+\cdot}$, 84.20).
d	**IR**	3064 (CH aromatic), 2925 (CH aliphatic), 1698 (C=O); 1596 (C=N).
	^{1}H NMR	δ 2.09 (s, 3H, pyrazole CH_3), 2.54 (s, 3H, pyrimidine CH_3), 7.35-7.38 (m, 1H, phenyl H-4), 7.53-7.58 (m, 2H, phenyl H-3, H-5), 7.66 (d, *J* = 8.4 Hz, 2H, chlorobenzylidene H-3, H-5), 7.99 (d, *J* = 8.4 Hz, 2H, phenyl H-2, H-6), 8.32 (d, *J* = 8.1 Hz, 2H, chlorobenzylidene H-2, H-6), 8.92 (s, 1H, N=CH).
	^{13}C NMR	13.72, 23.32, 104.40, 121.72, 127.13, 129.68, 129.83, 130.93, 131.63, 137.96, 138.66, 146.86, 150.30, 154.86, 157.74, 168.91.
	MS	377 ($M^{+\cdot}$, 11.12), 240 ($C_{13}H_{12}N_4O^{+\cdot}$, 100), 139 ($C_7H_6NCl^{+\cdot}$, 58.47), 77 ($C_6H_5^{+\cdot}$, 53.03).
e	**IR**	3057 (CH aromatic), 2925 (CH aliphatic), 1690 (C=O), 1597 (C=N).
	^{1}H NMR	δ 2.59 (s, 3H, pyrazole CH_3), 2.61 (s, 3H, pyrimidine CH_3), 7.37-7.39 (m, 1H, phenyl H-4), 7.54-7.59 (m, 2H, phenyl H-3, H-5), 8.07 (d, *J* = 7.8 Hz, 2H, phenyl H-2, H-6), 8.25 (d, *J* = 9 Hz, 2H, nitrobenzylidene H-2, H-6), 8.42 (d, *J* = 9 Hz, 2H, nitrobenzylidene H-3, H-5), 9.16 (s, 1H, N=CH).

MS	388 ($M^{+\cdot}$, 18.10), 240 ($C_{13}H_{12}N_4O^{+\cdot}$, 100).

3,6-Dimethyl-1-phenyl-5-substituted-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-ones (XIa,b)



A mixture of 5-amino-3,6-dimethyl-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (**IX**) (2.55 gm, 0.01 mol) and the appropriate acid anhydride (0.01 mol) in glacial acetic acid (10 mL) was heated under reflux for 5 h. After cooling, the reaction mixture was cooled and poured into ice-cold water while stirring. The formed precipitate was filtered, dried and crystallized from methanol to give **XIa,b**, (Table 7 and 8).

<u>Table 7:</u> Physical and analytical data of compounds XIa,b

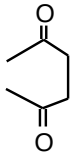
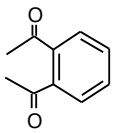
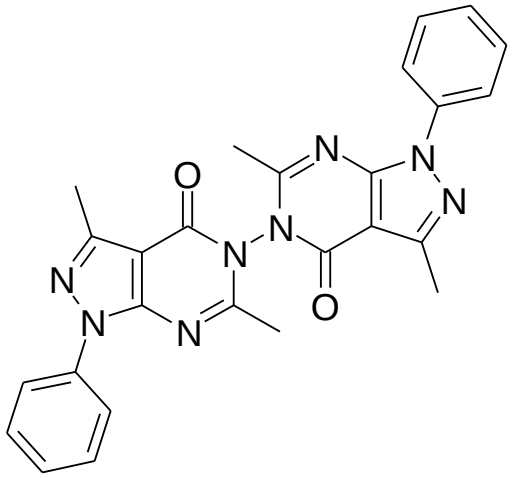
XI	Y	Yield %	m.p. (°C)	Mol. Formula (M.Wt)	Analysis %		
					Element	Calcd.	Found
a		55	194-195	$C_{17}H_{15}N_5O_3$ (337.34)	C H N	60.53 4.48 20.76	60.36 4.19 20.78
b		65	> 300	$C_{21}H_{15}N_5O_3$ (385.39)	C H N	65.45 3.92 18.17	65.28 4.17 18.00

Table 8: Spectral data of compounds XIa,b

XI	IR (cm^{-1}), ^1H NMR (DMSO-d_6, δ-ppm), ^{13}C NMR (DMSO-d_6, δ-ppm), EIMS (m/z) (relative abundance %)	
a	**IR**	3058 (CH aromatic), 2940 (CH aliphatic), 1742 (broad band of 2C=O), 1717 (pyrimidinone C=O), 1578 (C=N).
	^1H NMR	δ 2.41 (s, 3H, pyrazole CH_3), 2.51 (s, 3H, pyrimidine CH_3), 2.98-3.13 (m, 4H, $2CH_2$), 7.40-7.45 (m, 1H, phenyl H-4), 7.54- 7.59 (m, 2H, phenyl H-3, H-5), 7.95-7.99 (m, 2H, phenyl H-2, H-6).
	^{13}C NMR	13.54, 21.40, 27.32, 103.31, 122.81, 127.91, 129.70, 138.07, 147.04, 150.21, 153.63, 159.20, 173.53.
	MS	337 ($M^{+\cdot}$, 100), 239 ($C_{13}H_{11}N_4O^{+\cdot}$, 44.04), 77 ($C_6H_5^{+\cdot}$, 60.00).
b	**IR**	3061 (CH aromatic), 2923 (CH aliphatic), 1745 (broad band of 2C=O), 1715 (pyrimidinone C=O), 1575 (C=N).
	^1H NMR ($CDCl_3$)	δ 2.52 (s, 3H, pyrazole CH_3), 2.61 (s, 3H, pyrimidine CH_3), 7.37-7.39 (m, 1H, phenyl H-4), 7.49-7.54 (m, 2H, phenyl H-3, H-5), 7.88-7.91 (m, 2H, phthalimido H-4, H-5), 8.01-8.04 (m, 4H, phthalimido H-3, H-6, phenyl H-2, H-6).
	MS	385 ($M^{+\cdot}$, 8.00), 303 ($C_{15}H_5N_5O_3^{+\cdot}$, 100), 274 ($C_{14}H_4N_5O_2^{+\cdot}$, 13.20).

3,6,3\',6\'-Tetramethyl-1,1\'-diphenyl-1*H*,*1*\'*H*-[5,5\']bi[pyrazolo[3,4-*d*]pyrimidinyl]-4,4\'-dione (XII)



A mixture of 5-aminopyrazolo[3,4-*d*]pyrimidin-4-one derivative **IX** (2.55 gm, 0.01 mol) and 3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*][1,3]oxazin-4-one (**IV**) (2.41 gm, 0.01 mol) was fused at 200 ^{o}C for 2h. The reaction mixture was cooled and methanol (20 mL) was added to the mixture. The separated product was filtered, washed with methanol, dried and crystallized from methanol to give **XII**.

M.p. 160-162 ^{o}C.

Yield: 2.00 gm, 41.84%.

Analysis for $C_{26}H_{22}N_8O_2$ (478.52):

Element	C%	H %	N%
Calcd.	65.26	4.63	23.42
Found	64.98	4.93	23.35

IR cm^{-1}:

3064 (CH aromatic), 2924 (CH aliphatic), 1715 (2C=O).

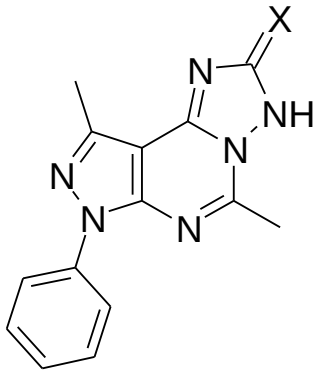
^{1}H NMR ($CDCl_3$, δ-ppm):

δ 2.52 (s, 6H, pyrazoles $2CH_3$), 2.63 (s, 6H, pyrimidines $2CH_3$), 7.37-7.41 (m, 2H, phenyl H-4, H-4\), 7.50-7.54 (m, 4H, phenyl H-3, H-3\, H-5, H-5\), 7.96 (d, *J* = 7.6 Hz, 4H, phenyl H-2, H-2\, H-6, H-6\).

EIMS (m/z) (relative abundance %):

478 ($M^{+\cdot}$, 5.24), 463 ($C_{25}H_{19}N_8O_2^{+\cdot}$, 10.41), 241 ($C_{13}H_{13}N_4O^{+\cdot}$, 53.67), 240 ($C_{13}H_{12}N_4O^{+\cdot}$, 66.59), 226 ($C_{12}H_{10}N_4O^{+\cdot}$, 35.05), 80 ($C_4H_4N_2^{+\cdot}$, 100).

5,9-Dimethyl-7-phenyl-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine-2-one or (2-thione) (XIIIa,b)



A mixture of 5-amino-3,6-dimethyl-1-phenyl-1,5-dihydro pyrazolo[3,4-*d*]pyrimidin-4-one (**IX**) (2.55 gm, 0.01 mol) and urea or thiourea (0.01 mol) was fused at 220 ^{o}C for 30 min. The mixture was cooled and methanol (20 mL) was added to the mixture. The separated product was filtered, washed with methanol, dried and crystallized from ethanol to give **XIIIa,b**, (Table 9 and 10).

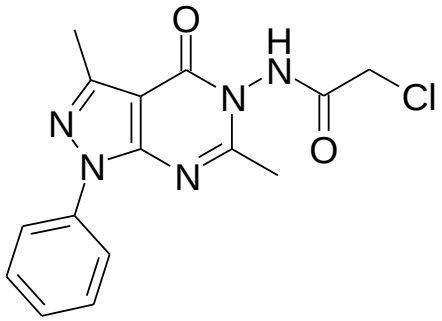
Table 9: Physical and analytical data of compounds XIIIa,b

XIII	X	Yield %	m.p. (^{o}C)	Mol. Formula (M.Wt)	Analysis %		
					Element	Calcd.	Found
a	O	55	198-199	$C_{14}H_{12}N_6O$ (280.29)	C H N	59.99 4.32 29.98	60.15 3.99 29.68
b	S	65	203-204	$C_{14}H_{12}N_6S$ (296.36)	C H N	56.74 4.08 28.36	56.62 4.34 28.62

Table 10: Spectral data of compounds XIIIa,b

XIII	IR (cm^{-1}), ^1H NMR (DMSO-d_6, δ-ppm), EIMS (m/z) (relative abundance %)	
a	**IR**	3424 (NH), 3052 (CH aromatic), 2918 (CH aliphatic), 1693 (C=O), 1567 (C=N).
	^1H NMR	δ 2.33 (s, 3H, pyrazole CH_3), 2.49 (s, 3H, pyrimidine CH_3), 7.32-7.35 (m, 1H, phenyl H-4), 7.50- 7.53 (m, 2H, phenyl H-3, H-5), 8.02 (d, *J* = 7.6 Hz, 2H, phenyl H-2, H-6), 12.23 (s, 1H, NH, D_2O exchangeable).
b	**IR**	3344 (NH), 2924 (CH aliphatic), 1594 (C=N), 1121 (C=S).
	^1H NMR	δ 2.08 (s, 3H, pyrazole CH_3), 2.49 (s, 3H, pyrimidine CH_3), 6.90-6.96 (m, 1H, phenyl H-4), 7.27-7.32 (m, 2H, phenyl H-3, H-5), 7.55 (d, *J* = 7.8 Hz, 2H, phenyl H-2, H-6), 9.83 (s, 1H, NH, D_2O exchangeable).
	MS	296 ($M^{+\cdot}$, 1.94), 241 ($C_{11}H_7N_5S^{+\cdot}$, 53.67), 224 ($C_{13}H_{12}N_4^{+\cdot}$, 37.79).

2-Chloro-*N*-(3,6-dimethyl-4-oxo-1-phenyl-1,4-dihydropyrazolo[3,4-*d*]pyrimidin-5-yl)acetamide (XIV)



A mixture of 5-amino-3,6-dimethyl-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (**IX**) (2.55 gm, 0.01 mol) (10 mL), chloroacetyl chloride (0.8 mL, 0.01 mol) and anhydrous potassium carbonate (1.38 gm, 0.01 mol) in dry dimethylformamide was stirred at room temperature for 24 h. The reaction mixture was poured into ice-cold water and the separated product was filtered, dried and crystallized from benzene to give **XIV**.

m.p. 290-291

Yield: 1.5 gm, 50.67%.

Analysis for $C_{15}H_{14}ClN_5O_2$(331.76):

Element	C%	H %	N%
Calcd.	54.31	4.25	21.11
Found	54.00	4.04	20.75

IR cm^{-1}:

3334 (NH), 3072 (CH aromatic), 2921 (CH aliphatic), 1698 (broad band of 2C=O).

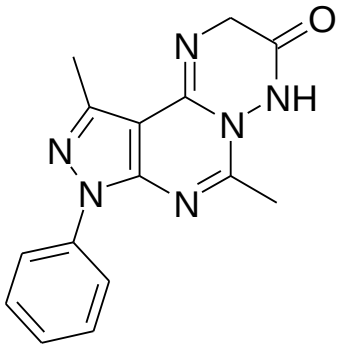
^{1}H NMR (DMSO-d_6, δ-ppm):

δ 2.42 (s, 3H, pyrazole CH_3), 2.51 (s, 3H, pyrimidine CH_3), 4.26 (d, *J* = 13.5 Hz, 1H, acetamido H), 4.33 (d, *J* = 13.5 Hz, 1H, acetamido H), 7.36-7.41 (m, 1H, phenyl H-4), 7.52-7.57 (m, 2H, phenyl H-3, H-5), 7.99 (d, *J* = 7.8 Hz, 2H, phenyl H-2, H-6), 11.39 (s, 1H, NH, D_2O exchangeable).

EIMS (m/z) (relative abundance %):

333 ($M+2^{+\cdot}$, 35.49), 331 ($M^{+\cdot}$, 100), 282 ($C_{14}H_{12}N_5O_2^{+\cdot}$, 82.44), 255 ($C_{13}H_{13}N_5O^{+\cdot}$, 68.18), 240 ($C_{13}H_{12}N_4O^{+\cdot}$, 19.75).

6,10-Dimethyl-8-phenyl-2,4-dihydro-8*H*-pyrazolo[3\',4\':4,5] pyrimido[1,6-*b*][1,2,4]triazin-3-one (XV)



A mixture of 2-chloro-*N*-(3,6-dimethyl-4-oxo-1-phenyl-1,4-dihydropyrazolo[3,4-*d*]pyrimidin-5-yl)acetamide (**XIV**) (3.31 gm, 0.01 mol) and anhydrous ammonium acetate (0.77 gm, 0.01 mol) in glacial acetic acid (10 mL) was heated under reflux for 6 h. After cooling, the solution was poured into ice-cold water while stirring. The precipitate formed was filtered, dried and crystallized from benzene to give **XV**.

m.p. 294-295 ^{o}C

Yield: 2.10 gm, 71.43%.

Analysis for $C_{15}H_{14}N_6O$ (294.32):

Element	C%	H %	N%
Calcd.	61.22	4.79	28.55
Found	60.97	4.78	28.72

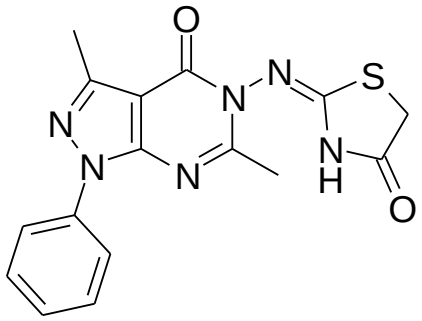
IR cm^{-1}:

3438 (NH), 3034 (CH aromatic), 2932 (CH aliphatic), 1693 (C=O), 1568 (C=N).

^{1}H NMR (DMSO-d_6, δ-ppm):

δ 2.10 (s, 3H, pyrazole CH_3), 2.41(s, 2H, CH_2), 2.51 (s, 3H, pyrimidine CH_3), 7.38-7.40 (m, 1H, phenyl H-4), 7.51-7.57 (m, 2H, phenyl H-3, H-5), 8.00 (d, *J* = 7.2 Hz, 2H, phenyl H-2, H-6), 10.94 (s, 1H, NH, D_2O exchangeable).

3,6-Dimethyl-5-(4-oxothiazolidin-2-ylideneamino)-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (XVI)



A mixture of 2-chloro-*N*-(3,6-dimethyl-4-oxo-1-phenyl-1,4-dihydropyrazolo[3,4-*d*]pyrimidin-5-yl)acetamide (**XIV**) (3.31 gm, 0.01 mol) and ammonium thiocyanate (0.15 gm, 0.02 mol) in absolute ethanol (20 mL) was heated under reflux for 10 h. The reaction mixture was concentrated to its half volume and the separated solid was filtered, washed with water, dried and crystallized from dioxane to give **XVI**.

m.p. 267-268 ^{o}C

Yield: 2.50 gm, 70.62%.

Analysis for $C_{16}H_{14}N_6O_2S$ (354.39):

Element	C%	H %	N%
Calcd.	54.23	3.98	23.71
Found	54.50	4.20	23.59

IR cm^{-1}:

3431 (NH), 3061 (CH aromatic), 2928 (CH aliphatic), 1737 (thiazolidinone C=O), 1673 (pyrimidinone C=O), 1550 (C=N).

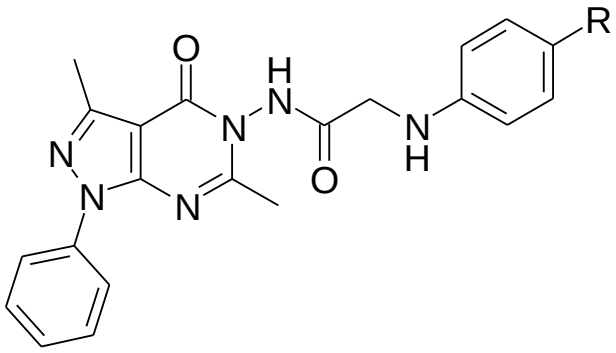
^{1}H NMR (DMSO-*d_6*, δ-ppm):

δ 2.42 (s, 3H, pyrazole CH_3), 2.52 (s, 3H, pyrimidine CH_3), 4.06 (s, 2H, CH_2), 7.33-7.38 (m, 1H, phenyl H-4), 7.51-7.56 (m, 2H, phenyl H-3, H-5), 8.05 (d, *J* = 7.5 Hz, 2H, phenyl H-2, H-6), 12.49 (s, 1H, NH, D_2O exchangeable).

EIMS (m/z) (relative abundance %):

354 ($M^{+\cdot}$, 49.85), 307 ($C_{15}H_{11}N_6O_2^{+\cdot}$, 100), 281 ($C_{14}H_{13}N_6O^{+\cdot}$, 14.55), 240 ($C_{13}H_{14}N_5^{+\cdot}$, 10.50), 77 ($C_6H_5^{+\cdot}$, 55.19).

N-(3,6-Dimethyl-4-oxo-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-5-yl)-2-(4-substituted phenylamino)acetamides (XVIIa-c)



A mixture of 2-chloro-*N*-(3,6-dimethyl-4-oxo-1-phenyl-1,4-dihydropyrazolo[3,4-*d*]pyrimidin-5-yl)acetamide (**XIV**) (3.31 gm, 0.01 mol), the appropriate aromatic amine (0.01 mol) and anhydrous potassium carbonate (1.38 gm, 0.01 mol) in absolute ethanol (30 mL) was heated under reflux for 12 h. After cooling, the formed precipitate was filtered, washed with ethanol and crystallized from ethanol to give **XVIIa-c**, (Table 11 and 12).

Table 11: Physical and analytical data of compounds XVIIa-c

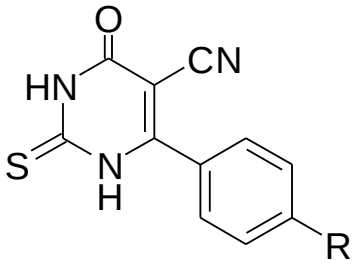
XVII	R	Yield %	m.p. (°C)	Mol. Formula (M.Wt)	Analysis %		
						Calcd.	Found
a	-OH	63	240-241	$C_{21}H_{20}N_6O_3$ (404.43)	C H N	62.37 4.98 20.78	62.00 4.60 20.48
b	$-COCH_3$	56	196-197	$C_{23}H_{22}N_6O_3$ (430.47)	C H N	64.18 5.15 19.52	63.92 5.03 19.50
c	-COOH	44	203-204	$C_{22}H_{20}N_6O_4$ (432.44)	C H N	61.11 4.66 19.43	60.71 4.78 19.40

Table 12: Spectral data of compounds XVIIa-c

XVII	IR (cm^{-1}), ^1H NMR (DMSO-d_6, δ-ppm), EIMS (m/z) (relative abundance %)	
a	**IR**	3279 (broad band of 2NH & OH), 1693 (broad band of 2C=O), 1574 (C=N).
	^1H NMR	δ 2.32 (s, 3H, pyrazole CH_3), 2.50 (s, 3H, pyrimidine CH_3), 3.84 (d, *J* = 4.8 Hz, 1H, acetamido H), 3.88 (d, *J* = 4.8 Hz, 1H, acetamido H), 5.72 (s, 1H, NH, D_2O exchangeable), 6.54 (d, *J* = 7.2 Hz, 2H, hydroxyphenyl H-2, H-6), 6.60 (d, *J* = 7.2 Hz, 2H, hydroxyphenyl H-3, H-5), 7.34-7.38 (m, 1H, phenyl H-4), 7.53-7.56 (m, 2H, phenyl H-3, H-5), 7.99 (d, *J* = 8.4 Hz, 2H, phenyl

		H-2, H-6), 8.56 (s, 1H, NH, D_2O exchangeable), 10.37 (s, 1H, OH, D_2O exchangeable).
	MS	404 ($M^{+\cdot}$, 60.64), 386 ($C_{21}H_{18}N_6O_2^{+\cdot}$, 67.02), 107 ($C_6H_5NO^{+}$, 100).
b	**IR**	3380, 3260 (2NH), 3054 (CH aromatic), 2992 (CH aliphatic), 1711-1670 (broad band of 3C=O), 1600 (C=N).
	^{1}H NMR	δ 2.11 (s, 3H, O=C-CH_3), 2.40 (s, 3H, pyrazole CH_3), 2.43 (s, 3H, pyrimidine CH_3), 4.10 (d, *J* = 9.2 Hz, 1H, acetamido H), 4.13 (d, *J* = 9.2 Hz, 1H, acetamido H), 6.71 (d, *J* = 6.8 Hz, 2H, acetylphenyl H-2, H-6), 7.38-7.41 (m, 1H, phenyl H-4), 7.52-7.55 (m, 2H, phenyl H-3, H-5), 7.66 (s, 1H, NH, D_2O exchangeable), 7.78 (d, *J* = 6.8 Hz, 2H, acetylphenyl H-3, H-5), 7.99 (d, *J* = 9 Hz, 2H, phenyl H-2, H-6), 11.17 (s, 1H, NH, D_2O exchangeable).
	MS	430 ($M^{+\cdot}$, 16.45), 240 ($C_{13}H_{14}N_5^{+\cdot}$, 51.95), 77 ($C_6H_5^{+\cdot}$, 100).
c	**IR**	3368 (broad band of 2NH & OH), 2923 (CH aliphatic), 1699 (broad band of 3C=O), 1601 (C=N).
	^{1}H NMR	δ 2.38 (s, 3H, pyrazole CH_3), 2.51 (s, 3H, pyrimidine CH_3), 3.50 (s, 2H, NH, D_2O exchangeable), 4.06 (d, *J* = 5.6 Hz, 1H, acetamido H), 4.10 (d, *J* = 5.6 Hz, 1H, acetamido H), 6.71 (d, *J* = 8.7 Hz, 2H, benzoyl H-2, H-6), 7.51-7.54 (m, 3H, phenyl H-3, H-4, H-5), 7.75 (d, *J* = 8.7 Hz, 2H, benzoyl H-3, H-5), 7.99 (d, *J* = 8.7 Hz, 2H, phenyl H-2, H-6), 11.11 (s, 1H, OH, D_2O exchangeable).

6-Aryl-4-oxo-2-thioxo-1,2,3,4-tetrahydro-pyrimidine-5-carbonitrile (XVIIIa,b)

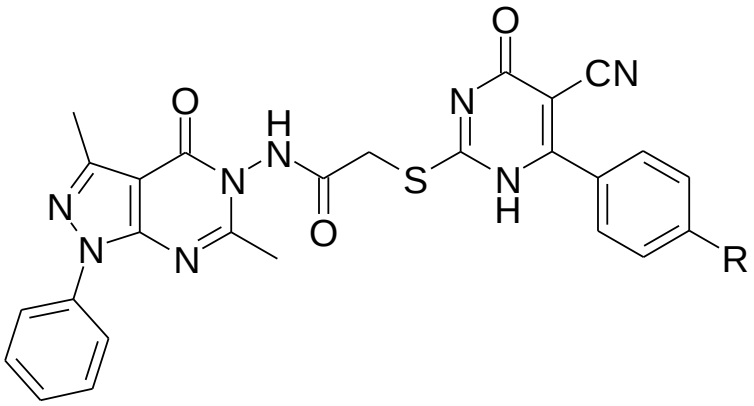


A mixture of ethyl cyanoacetate (1.07 ml, 0.01 mol), the appropriate aromatic aldehyde (0.01 mol), thiourea (0.76 gm, 0.01 mol) and anhydrous potassium carbonate (1.38 gm. 0.01 mol) in absolute ethanol (30 mL) was heated under reflux for 7 h. The reaction mixture was cooled, poured onto ice and neutralized with glacial acetic acid. The formed precipitate was filtered, dried and crystallized from methanol to give **XVIIIa,b**, (Table 13).

Table 13: Physical data of compounds XVIIIa,b.

XVIII	R	m.p. (^{o}C)	Yield %
a	H	300 (as reported).[154]	94
b	Cl	270 (as reported).[154]	89

2-[5-Cyano-6-aryl-4-oxo-1,4-dihydropyrimidin-2-ylsulfanyl]-*N*-(3,6-dimethyl-4-oxo-1-phenyl-1,4-dihydro-pyrazolo[3,4-*d*]pyrimidin-5-yl)acetamides (XIXa,b)



A mixture of the appropriate 4-oxo-6-(4-substituted phenyl)-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carbonitrile (**XVIIIa,b**) (0.01 mol) and 2-chloro-*N*-(3,6-dimethyl-4-oxo-1-phenyl-1,4-dihydropyrazolo[3,4-*d*]pyrimidin-5-yl)acetamide (**XIV**) (3.31 gm, 0.01 mol) and anhydrous potassium carbonate (1.38 gm, 0.01 mol) in acetone (30 mL) was heated under reflux for 6 h. The formed precipitate was filtered while hot, dried and crystallized from acetic acid to yield **XIXa,b**, (Table 14 and 15).

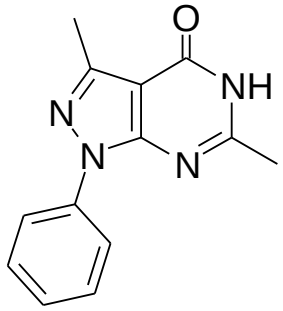
Table 14: Physical and analytical data of compounds XIXa,b

XIX	R	Yield %	m.p. (°C)	Mol. Formula (M.Wt)	Analysis %		
					Element	Calcd.	Found
a	H	45	256-258	$C_{26}H_{20}N_8O_3S$ (524.57)	C H N	59.53 3.84 21.36	59.21 3.52 21.41
b	Cl	68	> 300	$C_{26}H_{19}ClN_8O_3S$ (559.01)	C H N	55.86 3.43 20.05	55.49 3.22 20.12

Table 15: Spectral data of compounds XIXa,b

XIX	IR (cm^{-1}), ^1H NMR (DMSO-d_6, δ-ppm), EIMS (m/z) (relative abundance %)	
a	**IR**	3408, 3255 (2NH), 3048 (CH aromatic), 2932 (CH aliphatic), 2203 (C≡N), 1715-1693 (broad band of 3C=O), 1555 (C=N).
	^{1}H NMR	δ 1.96 (s, 3H, pyrazole CH_3), 2.50 (s, 3H, pyrimidine CH_3), 4.30 (d, *J* = 16 Hz, 1H, acetamido H), 4.41 (d, *J* = 4.8 Hz, 1H, acetamido H), 7.35-7.38 (m, 1H, phenyl H-4), 7.38- 7.64 (m, 5H, phenyl H-3, H-3\', H-4\', H-5, H-5\'), 7.95 (d, *J* = 8 Hz, 2H, phenyl H-2, H-6), 8.00 (d, *J* = 7.6 Hz, 2H, phenyl H-2\', H-6\'), 11.36 (s, 1H, NH, D_2O exchangeable).
	MS	524 ($M^{+\cdot}$, 66.13), 509 ($C_{25}H_{17}N_8O_3S^{+\cdot}$, 59.68), 431 ($C_{19}H_{11}N_8O_3S^{+\cdot}$, 61.29), 264 ($C_{13}H_6N_5O_2^{+\cdot}$, 79.03), 105 ($C_6H_5N_2^{+\cdot}$, 100).
b	**IR**	3418, 3252 (2NH), 3061 (CH aromatic), 2929 (CH aliphatic), 2204 (C≡N), 1714-1693 (broad band of 3C=O), 1573 (C=N).
	^{1}H NMR	δ 2.01 (s, 3H, pyrazole CH_3), 2.50 (s, 3H, pyrimidine CH_3), 4.29 (d, *J* = 16 Hz, 1H, acetamido H), 4.40 (d, *J* = 16 Hz, 1H, acetamido H), 7.34-7.38 (m, 1H, phenyl H-4), 7.50-7.54 (m, 2H, phenyl H-3, H-5), 7.65 (d, *J* = 8.4 Hz, 2H, chlorophenyl H-3, H-5), 7.95 (d, *J* = 7.6 Hz, 2H, phenyl H-2, H-6), 8.03 (d, *J* = 8.4 Hz, 2H, chlorophenyl H-2, H-6), 11.36 (s, 1H, NH, D_2O exchangeable).

3,6-Dimethyl-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one (XX)



A mixture of the 3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*][1,3] oxazin-4-one (**IV**) (2.41 gm, 0.01mol) and formamide (40 mL) was heated under reflux for 6 h. On cooling, the separated solid was filtered, washed with water and crystallized from ethanol to give **XX**.

m.p. 298-300 ^{o}C (as reported).[161]

Yield: 1.30 gm, 60.19%.

Analysis for $C_{13}H_{12}N_4O$ (240.27):

Element	C %	H %	N%
Calcd.	64.99	5.03	23.32
Found	65.20	5.13	23.02

IR cm^{-1}:

3420 (NH), 3056 (CH aromatic), 2923 (CH aliphatic), 1676 (C=O), 1595 (C=N).

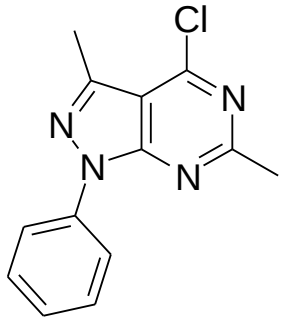
^{1}H NMR (DMSO-d_6, δ-ppm):

δ 2.37 (s, 3H, pyrazole CH_3), 2.49 (s, 3H, pyrimidine CH_3), 7.31-7.35 (m, 1H, phenyl H-4), 7.48-7.54 (m, 2H, phenyl H-3, H-5), 8.02 (d, *J* = 7.5 Hz, 2H, phenyl H-2, H-6), 12.24 (s,1H, NH, D_2O exchangeable).

EIMS (m/z) (relative abundance%):

240 ($M^{+\cdot}$, 100).

4-Chloro-3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine (XXI)[162]

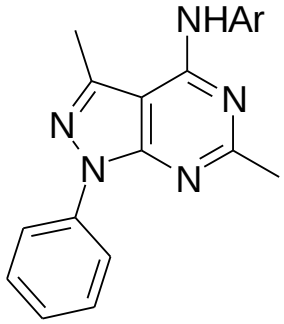


A mixture of 3,6-dimethyl-1-phenyl-1,5-dihydropyrazolo[3,4-*d*] pyrimidin-4-one **(XIX)** (12 gm, 0.05 mol) and phosphorous oxychloride (30 mL) was heated under reflux for 5 h. Excess phosphorus oxychloride was distilled under reduced pressure and the residual syrup was poured onto crushed ice. The aqueous suspension was extracted with chloroform (3×30 mL). Drying the extract overnight over anhydrous sodium sulfate (10 gm) and chloroform was distilled to yield a slightly yellow coloured product. This crude product was crystallized from *n*-heptane to give of compound **XXI**.

M.p. 85-87 ^{o}C (as reported).[162]

Yield: 8.40 gm, 70%.

3,6-Dimethyl-1-phenyl-4-substituted amino-1*H*-pyrazolo[3,4-*d*]pyrimidines (XXIIa-e)



A mixture of 4-chloro-3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*] pyrimidine (**XXI**) (2.58 gm, 0.01 mol), the appropriate aromatic amine (0.01 mol) and sodium iodide (0.2 gm) in isopropyl alcohol (20 mL) was heated under reflux for 4h. The reaction mixture was cooled and neutralized with sodium carbonate solution (20%). The formed precipitate was collected by filtration, washed with water and crystallized from ethanol to give **XXIIa-e**, (Table 16 and 17).

Table 16: Physical and analytical data of compounds XXIIa-e

XXII	Ar	Yield %	m.p. (^oC)	Mol.Formula (M.Wt.)	Analysis %		
					Element	Calcd.	Found
a	4-OH-C_6H_4	60	268-269	$C_{19}H_{17}N_5O$ (331.38)	C H N	68.87 5.17 21.13	69.10 4.88 21.40
b	3-$COCH_3$-C_6H_4	54	180-181	$C_{21}H_{19}N_5O$ (357.42)	C H N	70.57 5.36 19.59	70.62 5.33 20.01
c	4-COOH-C_6H_4	82	> 300	$C_{20}H_{17}N_5O_2$ (359.39)	C H N	66.84 4.77 19.49	66.83 4.67 19.86
d	2-COOH-C_6H_4	69	250-251	$C_{20}H_{17}N_5O_2$ (359.39)	C H N	66.84 4.77 19.49	66.45 4.54 19.27
e	4-$COOC_2H_5$-C_6H_4	83	229-230	$C_{22}H_{21}N_5O_2$ (387.44)	C H N	68.20 5.46 18.08	68.44 5.53 18.18

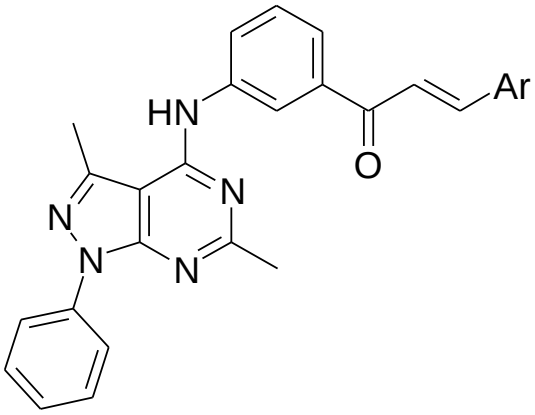
Table 17: Spectral data of compounds XXIIa-e

XXII	IR (cm^{-1}), ^{1}H NMR (DMSO-d_6, δ-ppm), EIMS (m/z) (relative abundance %)	
a	**IR**	3367 (OH), 3204 (NH), 3050 (CH aromatic), 2926 (CH aliphatic), 1517 (C=N).
	^{1}H NMR	δ 2.42 (s, 3H, pyrazole CH_3), 2.65 (s, 3H, pyrimidine CH_3), 6.77 (d, *J* = 6.6 Hz, 2H, hydroxyphenyl H-2, H-6), 7.25-7.31 (m, 1H, phenyl H-4), 7.42 (d, *J* = 6.6 Hz, 2H, hydroxyphenyl H-3, H-5), 7.48-7.53 (m, 2H, phenyl H-3, H-5), 8.16 (d, *J* = 7.2 Hz, 2H, phenyl H-2, H-6), 8.47 (s, 1H, NH, D_2O exchangeable), 9.38 (s, 1H, OH, D_2O exchangeable).
	MS	332 ($M+1^{\cdot+}$, 23.10), 331 ($M^{\cdot+}$, 100), 77 ($C_6H_5^{\cdot+}$, 36.95).
b	**IR**	3435 (NH), 3056 (CH aromatic), 2920 (CH aliphatic), 1682 (C=O), 1564 (C=N).
	^{1}H NMR ($CDCl_3$)	δ 2.66 (s, 3H, pyrazole CH_3), 2.70 (s, 3H, pyrimidine CH_3), 2.79 (s, 3H, $COCH_3$), 7.29-7.32 (m, 1H, phenyl H-4), 7.48-7.54 (m, 4H, phenyl H-3, H-5, acetylphenyl H-6, 1NH, D_2O exchangeable), 7.73 (d, *J*= 6.9 Hz, 1H, acetylphenyl H-5), 8.14-8.22 (m, 3H, phenyl H-2, H-6, acetylphenyl H-4), 8.34 (s, 1H, acetylphenyl H-2).
	MS	358 ($M+1^{\cdot+}$, 25.19), 357 ($M^{\cdot+}$, 100), 314 ($C_{19}H_{16}N_5^{\cdot+}$, 14.82), 77 ($C_6H_5^{\cdot+}$, 30.03).

c	**IR**	3443-2917 (NH & OH), 3054 (CH aromatic), 2917 (CH aliphatic), 1681 (C=O), 1603 (C=N).
	^{1}H NMR	δ 2.51 (s, 3H, pyrazole CH_3), 2.57 (s, 3H, pyrimidine CH_3), 7.30-7.34 (m, 1H, phenyl H-4), 7.51-7.57 (m, 2H, phenyl H-3, H-5), 7.95-7.98 (m, 4H, phenyl H-2, H-6, benzoyl H-2, H-6), 8.19 (d, *J* = 9 Hz, 2H, benzoyl H-3, H-5), 8.89 (s, 1H, NH, D_2O exchangeable), 12.66 (s, 1H, OH, D_2O exchangeable).
	MS	360 ($M+1^{\cdot+}$, 21.51), 359 ($M^{\cdot+}$, 100), 344 ($C_{19}H_{14}N_5O_2^{\cdot+}$, 6.42), 314 ($C_{19}H_{16}N_5^{\cdot+}$, 8.73), 77 ($C_6H_5^{\cdot+}$, 89.15).
d	**IR**	3432 (NH), 3182 (OH), 3098 (CH aromatic), 2926 (CH aliphatic), 1684 (C=O), 1605 (C=N).
	^{1}H NMR	δ 2.53 (s, 3H, pyrazole CH_3), 2.71 (s, 3H, pyrimidine CH_3), 7.08-7.28 (m, 2H, phenyl H-4, benzoyl H-6), 7.49-7.58 (m, 3H, phenyl H-3, H-5, benzoyl H-5), 7.99-8.13 (m, 4H, phenyl H-2, H-6, benzoyl H-3, H-4), 9.16 (s, 1H, NH, D_2O exchangeable), 11.52 (s, 1H, OH, D_2O exchangeable).
	MS	359 ($M^{\cdot+}$, 18.46), 340 ($C_{20}H_{14}N_5O^{\cdot+}$, 71.13), 314 ($C_{19}H_{16}N_5^{\cdot+}$, 64.76), 77 ($C_6H_5^{\cdot+}$, 100).
e	**IR**	3310 (NH), 3057 (CH aromatic), 2987 (CH aliphatic), 1710 (C=O), 1632 (C=N).
		δ 1.42 (t, *J* = 6.9 Hz, 3H, CH_2-C<u>H</u>$_3$), 2.70 (s, 3H, pyrazole CH_3), 2.73 (s, 3H, pyrimidine CH_3), 4.40 (q, *J* = 6.9 Hz, 2H, C<u>H</u>$_2$$CH_3$), 7.26-7.34 (m, 2H, phenyl H-4, 1NH, D_2O exchangeable), 7.48-7.53(m, 2H, phenyl H-3, H-5 of phenyl ring), 7.87 (d, *J* = 8.4 Hz, 2H, ethylcarboxyphenyl H-2, H-6), 8.11 (d, *J* = 8.4 Hz, 2H,

	^{1}H NMR ($CDCl_3$)	ethylcarboxyphenyl H-3, H-5), 8.19 (d, *J*= 7.5 Hz, 2H, phenyl H-2, H-6).
	MS	388 ($M+1^{\cdot+}$, 24.90), 387 ($M^{\cdot+}$, 100), 358 ($C_{20}H_{16}N_5O_2^{\cdot+}$, 32.17), 314 ($C_{19}H_{16}N_5^{\cdot+}$, 10.05), 77 ($C_6H_5^{\cdot+}$, 41.49).

(*E*) 3-Aryl-1-[3-(3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-ylamino)phenyl]prop-2-en-1-one (XXIIIa-e)



A mixture of compound 4-(3-acetylphenyl)amino-3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **(XXIIb)** (0.36 gm, 0.001 mol) and an aqueous solution of sodium hydroxide (10%) (1 mL) was dissolved in ethanol (10 mL). After cooling in ice bath, the appropriate aromatic aldehyde (0.001 mol) was added while stirring and the temperature was not exceeded 20^{o}C. The reaction mixture was stirred at room temperature for 12 h. The obtained solid was filtered, washed with water and crystallized from the appropriate solvent to give **XXIIIa-e**, (Table 18 and 19).

Table 18: Physical and analytical data of compounds XXIIIa-e

XXIII	Ar	Yield %	m.p.(°C)/ Solvent of Crystallization	Mol. Formula (M.Wt)	Analysis %		
					Element	Calcd.	Found
a	C_6H_5-	75	> 300 Acetic acid	$C_{28}H_{23}N_5O$ (445.53)	C H N	75.49 5.20 15.72	75.51 5.37 15.94
b	4-F-C_6H_4-	65	202-203 Isopropyl alcohol	$C_{28}H_{22}FN_5O$ (463.52)	C H N	72.56 4.78 15.11	72.82 4.75 15.01
c	4-CH_3O-C_6H_4	55	255-256 Acetic acid	$C_{29}H_{25}N_5O_2$ (475.55)	C H N	73.25 5.30 14.73	73.55 5.40 15.00
d	4-Cl-C_6H_4-	81	212-213 Isopropyl Alcohol	$C_{28}H_{22}ClN_5O$ (479.97)	C H N	70.07 4.62 14.59	70.37 4.90 14.38
e	4-NO_2-C_6H_4-	80	220-221 Isopropyl Alcohol	$C_{28}H_{22}N_6O_3$ (490.53)	C H N	68.56 4.52 17.13	68.29 4.70 17.00

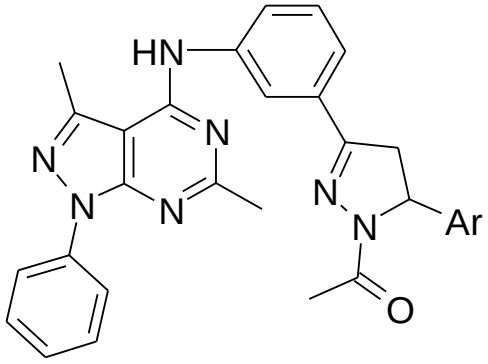
Table 19: Spectral data of compounds XXIIIa-e

XXIII	IR (cm^{-1}), ^1H NMR (DMSO-d_6, δ-ppm), EIMS (m/z) (relative abundance %)	
a	**IR**	3429 (NH), 3046 (CH aromatic), 2920 (CH aliphatic), 1655 (C=O), 1601 (C=N).
	^1H NMR ($CDCl_3$)	δ 2.72 (s, 3H, pyrazole CH_3), 2.80 (s, 3H, pyrimidine CH_3), 7.32-7.35 (m, 1H, phenyl H-4), 7.45-7.53 (m, 4H, phenyl H-3, H-5 phenylamino H-6, O=C-CH=C<u>H</u>), 7.54-7.59 (m, 5H, phenyl H-3\', H-4\', H-5\' , O=C-C<u>H</u>=CH, NH, D_2O exchangeable), 7.61-7.70 (m, 3H, phenyl H-2\', H-6\', phenylamino H-5), 7.87-8.17 (m, 3H, phenyl H-2, H-6, phenylamino H-4), 8.19 (s, 1H, aminophenyl H-2).
	MS	446 ($M+1^{\cdot+}$, 31.92), 445 ($M^{\cdot+}$, 100), 444 ($M-1^{\cdot+}$, 33.38), 416 ($C_{26}H_{18}N_5O^{\cdot+}$, 43.82), 315 ($C_{19}H_{17}N_5^{\cdot+}$, 19.30), 77 ($C_6H_5^{\cdot+}$, 64.95).
b	**IR**	3434 (NH), 3030 (CH aromatic), 2924 (CH aliphatic), 1681 (C=O), 1594 (C=N).
	^1H NMR	δ 2.50 (s, 3H, pyrazole CH_3), 2.83 (s, 3H, pyrimidine CH_3), 7.33-7.35 (m, 1H, phenyl H-4), 7.52-7.88 (m, 4H, phenyl H-3, H-5, CH=CH), 8.03 (d, *J* = 7.5 Hz, 2H, fluorophenyl H-2, H-6), 8.08-8.18 (m, 5H, fluorophenyl H-3, H-5, phenylmino H-4, H-5, H-6), 8.29 (d, *J* = 8.10 Hz, 2H, phenyl H-2, H-6), 8.47 (s, 1H, phenylamino H-2), 9.03 (s, 1H, NH, D_2O exchangeable).

c	**IR**	3433 (NH), 3051 (CH aromatic), 2924 (CH aliphatic), 1655 (C=O), 1602 (C=N).
	^{1}H NMR	δ 2.48 (s, 3H, pyrazole CH_3), 2.79 (s, 3H, pyrimidine CH_3), 3.83 (s, 3H, OCH_3), 7.32-7.60 (m, 8H, phenyl H-3, H-4, H-5, methoxyphenyl H-2, H-3, H-5, H-6, O=C-CH=C<u>H</u>), 7.78-7.95 (m, 3H, phenylamino H-5, H-6, O=C-C<u>H</u>=CH), 8.18-8.21 (m, 3H, phenyl H-2, H-6, phenylamino H-4), 8.48 (s, 1H, phenylamino H-2), 8.87 (s, 1H, NH, D_2O exchangeable).
	MS	476 ($M+1^{+\cdot}$, 29.47), 475 ($M^{+\cdot}$, 88.81), 474 ($M-1^{+\cdot}$, 32.76), 315 ($C_{19}H_{17}N_5^{+\cdot}$, 22.98), 77 ($C_6H_5^{+\cdot}$, 39.54).
d	**IR**	3438 (NH), 3049 (CH aromatic), 2917 (CH aliphatic), 1657 (C=O), 1595 (C=N).
	^{1}H NMR	δ 2.48 (s, 3H, pyrazole CH_3), 2.79 (s, 3H, pyrimidine CH_3), 7.31-7.34 (m, 1H, phenyl H-4), 7.51-7.62 (m, 5H, phenyl H-3, H-5, chlorophenyl H-2, H-6, O=C-CH=C<u>H</u>), 7.76 (d, *J* = 15.6 Hz, 1H, O=C-C<u>H</u>=CH), 7.91-7.97 (m, 4H, chlorophenyl H-3, H-5, phenylamino H-5, H-6), 8.13-8.21 (m, 3H, phenyl H-2, H-6, phenylamino H-4), 8.47-8.48 (s, 1H, phenylamino H-2), 8.86 (s, 1H, NH, D_2O exchangeable).
	MS	481 ($M+2^{+\cdot}$, 37.07), 480 ($M+1^{+\cdot}$, 40.93), 479 ($M^{+\cdot}$, 100), 478 ($M-1^{+\cdot}$, 33.07), 450 ($C_{26}H_{17}ClN_5O^{+\cdot}$, 50.86), 315 ($C_{19}H_{17}N_5^{+\cdot}$, 55.61),77 ($C_6H_5^{+\cdot}$, 57.27).
e	**IR**	3423 (NH), 3073 (CH aromatic), 2922 (CH aliphatic), 1665 (C=O), 1598 (C=N).

	^{1}H NMR	δ 2.72 (s, 3H, pyrazole CH_3), 2.82 (s, 3H, pyrimidine CH_3), 7.50-7.54 (m, 2H, phenyl H-4, phenylamino H-6), 7.81-7.86 (m, 8H, phenyl H-3, H-5, nitrophenyl H-2, H-6, phenylamino H-5, CH=CH &NH, D_2O exchangeable), 8.20 (d, *J*= 7.6 Hz, 2H, nitrophenyl H-3, H-5), 8.31 (m, 3H, phenyl H-2, H-6, phenylamino H-4), 8.49 (s, 1H, aminophenyl H-2).
	MS	491 ($M+1^{\cdot+}$, 32.71), 490 ($M^{\cdot+}$, 100), 489 ($M-1^{\cdot+}$, 35.79), 461 ($C_{26}H_{17}N_6O_3^{\cdot+}$, 34.44), 315 ($C_{19}H_{17}N_5^{\cdot+}$, 31.98), 77 ($C_6H_5^{\cdot+}$, 42.30).

4-[3-(1-Acetyl-5-aryl-4,5-dihydro-1*H*-pyrazol-3-yl)phenylamino]-3,6-dimethyl-1-phenyl-1*H*-pyazolo[3,4-*d*]pyrimidines (XXIVa-e)



A mixture of hydrazine hydrate (99.9%, 0.1 mL, 0.002 mol) and the appropriate chalcone **XXIIIa-e** (0.001 mol) in glacial acetic acid (5 mL) was heated under reflux for 5 h. After cooling, the reaction mixture was poured into ice-cold water. The obtained solid product was collected by filteration, washed with water and crystallized from ethanol to yield **XXIVa-e** (Table 20 and 21).

Table 20: Physical and analytical data of compounds XXIVa-e

XXIV	Ar	Yield %	m.p. (°C)	Mol. Formula (M.Wt)	Analysis %		
					Element	Calcd.	Found
a	C_6H_5-	60	> 300	$C_{30}H_{27}N_7O$ (501.60)	C H N	71.84 5.43 19.55	71.69 5.31 19.37
b	4-F-C_6H_4-	68	160-161	$C_{30}H_{26}FN_7O$ (519.59)	C H N	69.35 5.04 18.87	69.08 4.79 18.94
c	4-CH_3O-C_6H_4	50	270-271	$C_{31}H_{29}N_7O_2$ (531.62)	C H N	70.04 5.50 18.44	70.30 5.70 18.13
d	4-Cl-C_6H_4-	45	220-221	$C_{30}H_{26}ClN_7O$ (536.04)	C H N	67.22 4.89 18.29	66.85 4.94 18.00
e	4-NO_2-C_6H_4-	66	262-263	$C_{30}H_{26}N_8O_3$ (546.59)	C H N	65.92 4.79 20.50	65.79 4.90 20.54

Table 21: Spectral data of compounds XXIVa-e

XXIV	IR (cm^{-1}), ^1H NMR (DMSO-d_6, δ-ppm), EIMS (m/z) (relative abundance %)	
a	**IR**	3320 (NH), 3057 (CH aromatic), 2925 (CH aliphatic), 1690 (C=O), 1597 (C=N).
	^1H NMR	δ 2.34 (s, 3H, pyrazole CH_3), 2.53 (s, 3H, pyrimidine CH_3), 2.76 (s, 3H, O=C-CH_3), 3.13 (dd, 1H, J_1 = 5.2 Hz, J_2 = 18 Hz, pyrazoline H-4), 3.90 (dd, J_1 = 10.4 Hz, J_2 = 18 Hz, 1H, pyrazoline H-4), 5.58 (dd, J_1 = 5.2 Hz, J_2 = 10.4 Hz,1H, pyrazoline H-5), 7.23-7.25 (m, 3H, phenyl H-3\', H-4\', H-5\'), 7.32-7.35 (m, 3H, phenyl H-3, H-4, H-5), 7.40-7.57 (m, 4H, aminophenyl H-5, H-6, phenyl H-2\', H-6\'), 7.86-7.91 (m, 2H, aminophenyl H-2, H-4), 8.14 (d, *J* = 7.2 Hz, 2H, phenyl H-2, H-6), 8.39 (s, 1H, NH, D_2O exchangeable).
b	**IR**	3417 (NH), 3058 (CH aromatic), 2923 (CH aliphatic), 1678 (C=O), 1542 (C=N).
	^1H NMR	δ 2.37 (s, 3H, pyrazole CH_3), 2.50 (s, 3H, pyrimidine CH_3), 2.80 (s, 3H, O=C-CH_3), 3.03 (dd, 1H, J_1 = 4.5 Hz, J_2 = 17.4 Hz, pyrazoline H-4), 4.01 (dd, J_1 = 12 Hz, J_2 = 17.4 Hz, 1H, pyrazoline H-4), 5.74 (dd, J_1 = 4.5, J_2 = 12 Hz, 1H, pyrazoline H-5), 7.04 (d, *J*= 7.8 Hz, 2H, fluorophenyl H-2, H-6), 7.24 (d, *J*= 7.8 Hz, 2H, fluorophenyl H-3, H-5), 7.33-7.35 (m, 2H, phenyl H-4, aminophenyl H-6), 7.45-7.54 (m, 2H, phenyl H-3, H-5), 7.67 (m, 1H, aminophenyl H-5), 7.89 (d, *J* = 7.8 Hz, 1H, aminophenyl H-4),8.17 (d, *J* = 8.1 Hz, 2H, phenyl H-2, H-6), 8.44 (s, 1H, aminophenyl H-2), 8.91 (s, 1H, NH, D_2O exchangeable).

c	**IR**	3433 (NH), 3055 (CH aromatic), 2929 (CH aliphatic), 1653 (C=O), 1563 (C=N).
	^{1}H NMR ($CDCl_3$)	δ 2.48 (s, 3H, pyrazole CH_3), 2.70 (s, 6H, pyrimidine CH_3 & O=C-CH_3), 3.20 (dd, J_1 = 4 Hz, J_2 = 14.8 Hz, 1H, pyrazoline H-4), 3.74-3.86 (m, 4H, OCH_3 & pyrazoline H-4), 5.60 (dd, J_1 = 4 Hz, J_2 = 11.6 Hz, 1H, pyrazoline H-5), 6.81-6.85 (m, 2H, phenyl H-4, aminophenyl H-6), 7.25-7.31 (m, 3H, phenyl H-3, H-5, aminophenyl H-5), 7.46-7.76 (m, 6H, methoxyphenyl H-2, H-3, H-5, H-6, aminophenyl H-4, NH, D_2O exchangeable), 8.20 (d, J = 7.2 Hz, 2H, phenyl H-2, H-6), 8.37 (s, 1H, aminophenyl H-2).
	MS	532 ($M+1^{+\cdot}$, 30.80), 531 ($M^{+\cdot}$, 84.43), 530 ($M-1^{+\cdot}$, 12.84), 489 ($C_{29}H_{27}N_7O^{+\cdot}$, 36.99), 460 ($C_{27}H_{22}N_7O^{+\cdot}$, 67.84), 412 ($C_{23}H_{22}N_7O^{+\cdot}$, 37.56), 77 ($C_6H_5^{+\cdot}$, 100).
d	**IR**	3436 (NH), 3056 (CH aromatic), 2921 (CH aliphatic), 1657 (C=O), 1591 (C=N).
	^{1}H NMR ($CDCl_3$)	δ 2.33 (s, 3H, pyrazole CH_3), 2.50 (s, 3H, pyrimidine CH_3), 2.77 (s, 3H, O=C-CH_3), 3.11 (dd, J_1 = 4.5 Hz, J_2 = 17.5 Hz, 1H, pyrazoline H-4), 3.88 (dd, J_1 = 10.2 Hz, J_2 = 17.5 Hz, 1H, pyrazoline H-4), 5.56 (dd, J_1 = 4.5 Hz, J_2 = 10.2 Hz, 1H, pyrazoline H-5), 7.23 (d, J = 6.6 Hz, 2H, chlorophenyl H-2, H-6),7.25-7.34 (m, 1H, phenyl H-4), 7.39 (d, J = 6.6 Hz, 2H, chlorophenyl H-3, H-5), 7.41-7.56 (m, 4H, phenyl H-3, H-5, aminophenyl H-5, H-6), 7.88 (d, J = 6.6 Hz, 1H, aminophenyl H-4), 8.19 (d, J = 9.6 Hz, 2H, phenyl H-2, H-6),8.39 (s, 1H, aminophenyl H-2), 8.75

		(s, 1H, NH, D_2O exchangeable).
	MS	537 (M+2$^{+\cdot}$, 38.23), 535 (M$^{+\cdot}$, 99.60), 534 (M-1$^{+\cdot}$, 12.20), 493 ($C_{28}H_{23}ClN_7^{+\cdot}$, 52.35), 464 ($C_{26}H_{18}ClN_7^{+\cdot}$, 100), 77 ($C_6H_5^{+\cdot}$, 31.28).
e	**IR**	3438 (NH), 3055 (CH aromatic), 2926 (CH aliphatic), 1656 (C=O), 1590 (C=N).
	^{1}H NMR	δ 2.36 (s, 3H, pyrazole CH_3), 2.50 (s, 3H, pyrimidine CH_3), 2.77 (s, 3H, O=C-CH_3), 3.19 (dd, J_1 = 4.8 Hz, J_2 = 17.6 Hz, 1H, pyrazoline H-4), 3.98 (dd, J_1 = 11.2 Hz, J_2 = 17.6 Hz, 1H, pyrazoline H-4), 5.72 (dd, J_1 = 4.8 Hz, J_2 = 11.2 Hz, 1H, pyrazoline H-5), 7.30-7.34 (m, 1H, phenyl H-4), 7.48-7.56 (m, 6H, phenyl H-3, H-5, aminophenyl H-5, H-6, nitrophenyl H-2, H-6), 8.15-8.22 (m, 5H, phenyl H-2, H-6, aminophenyl H-4, nitrophenyl H-3, H-5), 8.41 (s, 1H, aminophenyl H-2).

4.2. Docking steps:

In this investigation, the docking study was done using Molecular Operating Evironment (MOE program; Chemical Computing Group, Canada) according to the following steps:

1-**Enzyme downloading:**

Enzyme cyclin dependent kinase 2 (CDK2) was downloaded from the protein data bank (PDB: ID 1W0X).[174]

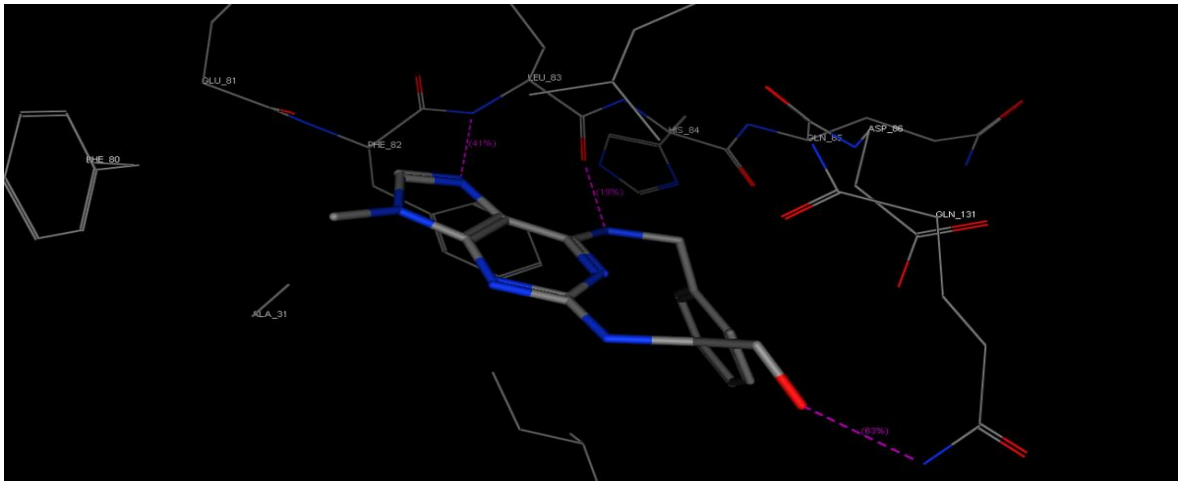
2- Docking procedure for the ligand:

- Acting on one chain of amino acids containing one molecule of ligand.
- 3D protonation for the amino acid side chain and ligand hydrogen atoms were added on the protein because all the enzymes downloaded from PDB are always shown without hydrogen atoms attached to different atoms inside the enzyme.
- Deleting all water of crystallization away from the active site.
- Surfaces and maps adjusting: all the possible H-bonding, hydrophobic and polar regions of the enzyme were shown in this step. Also, the color and the appearance of binding site could be changed by converting its surface to molcad or slab surface, which helps to see the active site in details.
- Studing the interaction of the ligand with the amino acids of the active site.

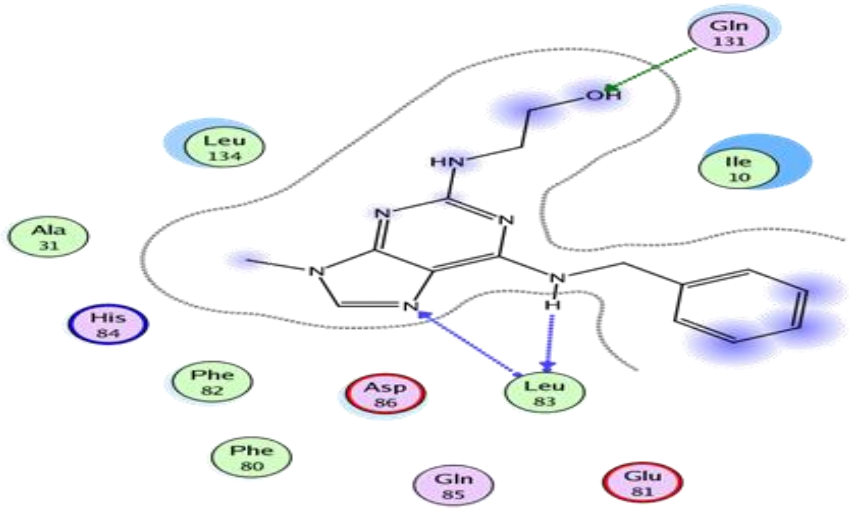
Validation of the docking reliability: the ligand of the enzyme was docked into the binding site, and the docking conformation corresponding to the

lowest energy score value (-17.39 Kcal/mol) was selected as the most propable binding conformation with root mean standard deviation (rmsd) of 0.67A was determined to ensure the validity of the docking steps.

All the above procedures were and the docking result of the internal ligand of (CDC2-olomucine) and the amino acids of the active site is shown in the following: (Fig.7)



a



b

Fig.7. (a) Represents olomucine in the binding site of CDK2, the dotted lines represent H-bonding interactions, (b) 2D interactions of olomucine with **Leu**83 and **Gln**131 acid residues.

3- Docking procedure for the target compounds:

All the compounds to be docked were prepared *via* their 3D structure built by MOE. Certain procedure should be taken before docking which includes:

- 3D protonation of the structures.
- Energy minimization of tested compounds inside the active site using MMFF94x force field to produce the lowest energy conformers.
- Compounds were grouped in databases and docked applying the same docking protocol used with the ligand.
- Filteration of the results: for each docked compound, one pose was selected based on the number of binding interactions, amino acids residues forming H-bonds, superposition with the original ligand and docking score.

4.3. Docking results:

The results obtained from the docking study (amino acid interactions, hydrogen bond lengths and binding energy scores) and cytotoxic activity for the newly synthesized compounds were summarized in the following table (Table 22).

<u>Table 22:</u> Docking study data and cytotoxic activity (IC_{50}) for olomucine and newly synthesized compounds:

Compound number	No. of H-Bonds	Atoms of compound forming H-bonds	Amino acid Residues forming-bonds (H-bond length in A^o)	Binding Energy Score Kcal/mol	IC50 (µg/ml)	IC_{50} (µM)
Olomucine	3	Imidazole N7 C6-NH OH	**Leu**83 (2.96), **Leu**83 (1.82), **Gln**131 (2.86)	-17.39	_	7[88]
V	2	C=O NH	**Gln**131 (1.94), **Gln**131 (2.72)	-16.50	4.88	14.74
VI	1	OH	**Gln**131 (1.93)	-11.34	12.6	49.21
VIIa	1	C=O	**Gln**131 (2.65)	-15.55	12.1	33.70
VIIb	2	C=O OH	**Gln**131 (2.87), **Leu**83 (2.96)	-16.23	6.53	25.51
VIIc	1	C=O	**Gln**131 (2.60)	-17.01	10.4	26.80
VIId	2	C=O OH	**Gln**131 (2.71), **Leu**83 (2.29)	-16.24	9	25
VIIe	2	C=O Pyrazole N2	**Gln**131 (2.94), **Leu**83(3.02)	-16.56	10.7	27.57
VIIIa	1	NH_2	**Asp**86 (1.73)	-15.59	14.8	52.29
VIIIb	1	NH_2	**Asp**86 (1.73)	-16.95	11.5	38.46
VIIIc	1	NH_2	**Gln**81 (1.84)	-10.43	16.4	52.23

Compound number	No. of H-Bonds	Atoms of compound forming H-bonds	Amino acid Residues forming-bonds (H-bond length in A^o)	Binding Energy Score Kcal/mol	IC50 (µg/ml)	IC50 (µM)
Xa	1	C=O	**Gln**131 (2.85)	-20.78	6.15	17.93
Xb	1	C=O	**Gln**131 (2.93)	-22.62	4.65	12.88
Xc	2	Pyrazole N2 OCH_3	**Gln**131 (2.69), **Lys**20 (2.98)	-19.34	6.9	18.49
Xd	1	C=O	**Gln**131 (2.84)	-21.41	4.80	12.73
Xe	1	C=O	**Gln**131 (2.76)	-23.98	4.58	11.80
XIa	2	Pyrimidinone C=O, SuccinimidoC=O	**Gln**131 (2.67), **Leu**83 (2.89)	-15.20	–	–
XIb	2	Pyrimidinone C=O, phthalimidoC=O	**Gln**131 (2.61), **Leu**83 (2.64)	-20.07	1.80	4.67
XII	2	Pyrazole N2, C=O	**Gln**131 (2.61), **Lys**89 (2.64)	-16.35	8.35	17.46
XIIIa	2	C=O NH	**Leu**83 (2.93), **Leu**83 (2.20)	-15.19	–	–
XIIIb	2	C=O NH	**Leu**83(2.61), **Leu**83 (2.93)	-19.95	2.53	8.54
XV	0	–	**–**	-10.11	18.39	62.55
XVI	2	Thiazolidinone C=O, Thiazolidinone C=O	**Gln**131 (2.79), **Lys**33 (2.64)	-18.05	3.08	8.7
XVIIa	2	Pyrimidinone C=O, NH	**Lys**89 (2.93), **Leu**83 (1.37)	-17.98	1.10	2.74

Compound number	No. of H-bonds	Atoms of compound forming H-bonds	Amino acid Residues forming-bonds (H-bond length in A^o)	Binding Energy Score Kcal/mol	IC50 (µg/ml)	IC50 (µM)
XVIIb	2	Pyrimidinone C=O, NH	**Gln**131 (2.93), **Asp**86 (1.37)	-16.11	_	_
XVIIc	3	Pyrimidinone C=O, OH, NH	**Lys**89 **(**2.27), **Lys**20 (2.92), **Leu**83 (2.57)	-18.55	0.943	2.18
XVIIIa	2	NH, CN	**Leu**83 (1.92), **Lys**20 (3.11)	-17.38	_	_
XVIIIb	2	NH, CN	**Leu**83 (2.20), **Lys**20 (2.95)	-16.20	_	_
XXIIa	1	OH	**Glu**81 (2.93), **Leu**83 (1.37)	-20.13	0.99	2.99
XXIIc	2	OH, NH	**Asp**831 (1.24), **Lys**721 (2.61)	-18.04	2.02	5.62
XXIId	2	OH, NH	**Asp**831 (1.50), **Lys**721 (2.52)	-14.00	_	_
XXIIe	2	Pyrazole N2 C=O	**Gln**131 (2.73), **Lys**20 (2.15)	-23.51	0.65	1.67
XXIVa	1	Pyrazole N2	**Gln131** (2.73)	-13.35	_	_
XXIVb	2	Pyrimidine N7 C=O	**Gln**131 (3.24), **Gln**85 (2.62)	-23.50	0.835	1.60
XXIVc	2	Pyrazole N2 OCH_3	**Gln**131 (3.24), **Lys**20 (2.95)	-19.58	1.37	2.58
XXIVd	1	Pyrazole N2	**Gln**131 (2.88)	-14.45	_	_
XXIVe	2	Pyrazole N2 NH	**Gln131** (3.24), **IIe10** (2.95)	-18.59	1.45	2.65

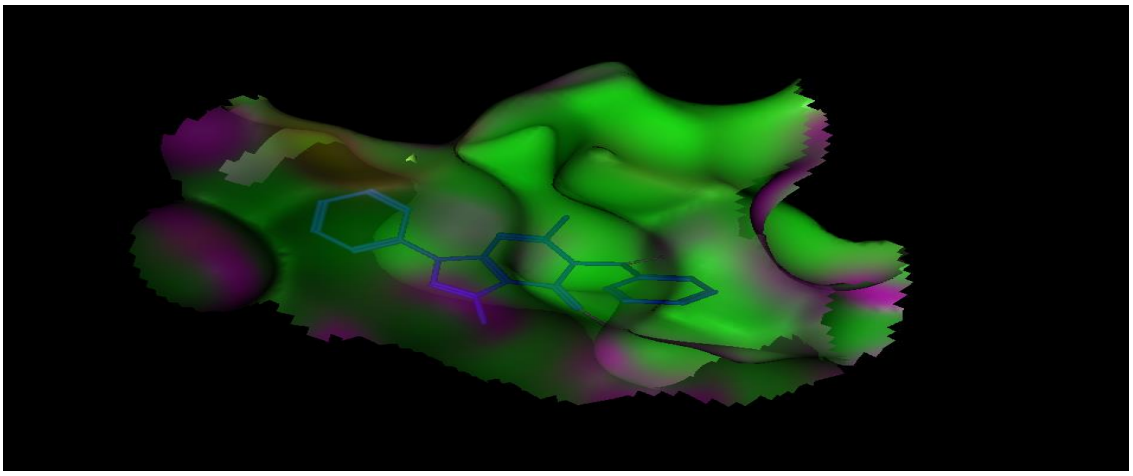
- Binding Energy Score (Kcal/mol): energy of interaction of the ligand in the active site.
- IC_{50} (μM): the concentration required to produce 50% inhibition of cell growth.

Analysing results:

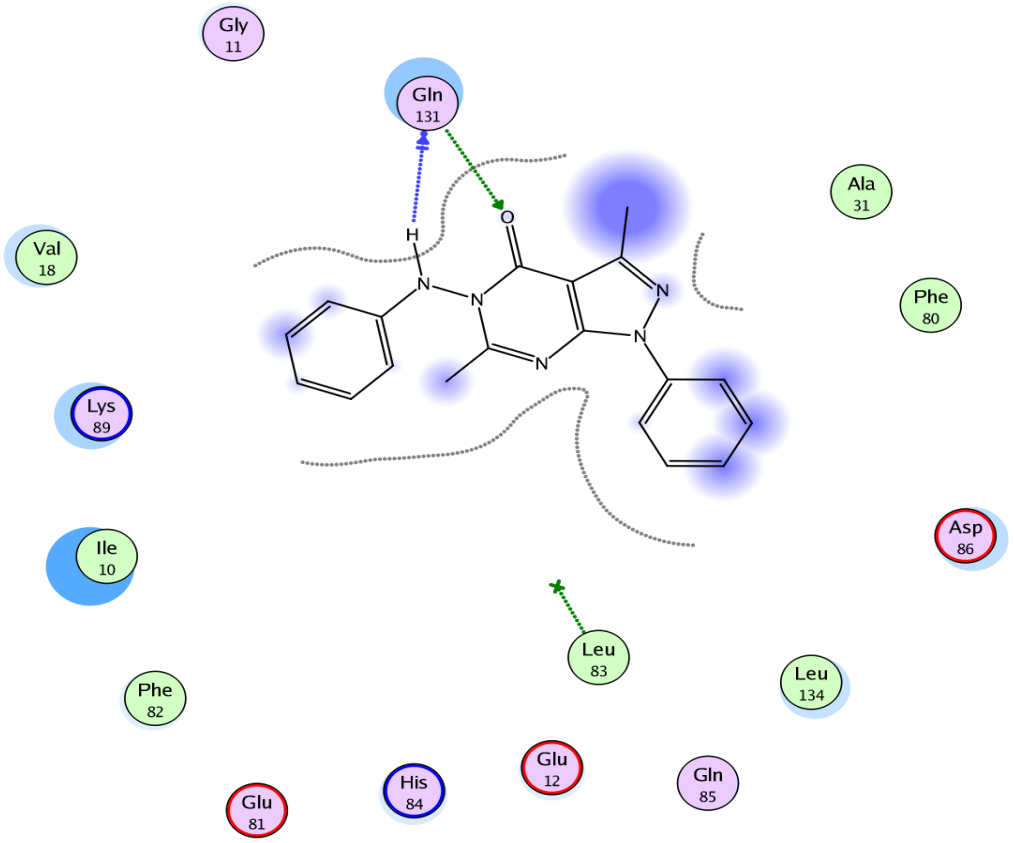
1- All the compounds subjected to docking protocol showed good binding scores and fitting well to the active sites inside the pocket.

2- Most of the docked compounds exhibit good interactions with amino acids of the active site.

3- Figures (8-12) show the interactions between some of the docked compounds and CDK enzyme .

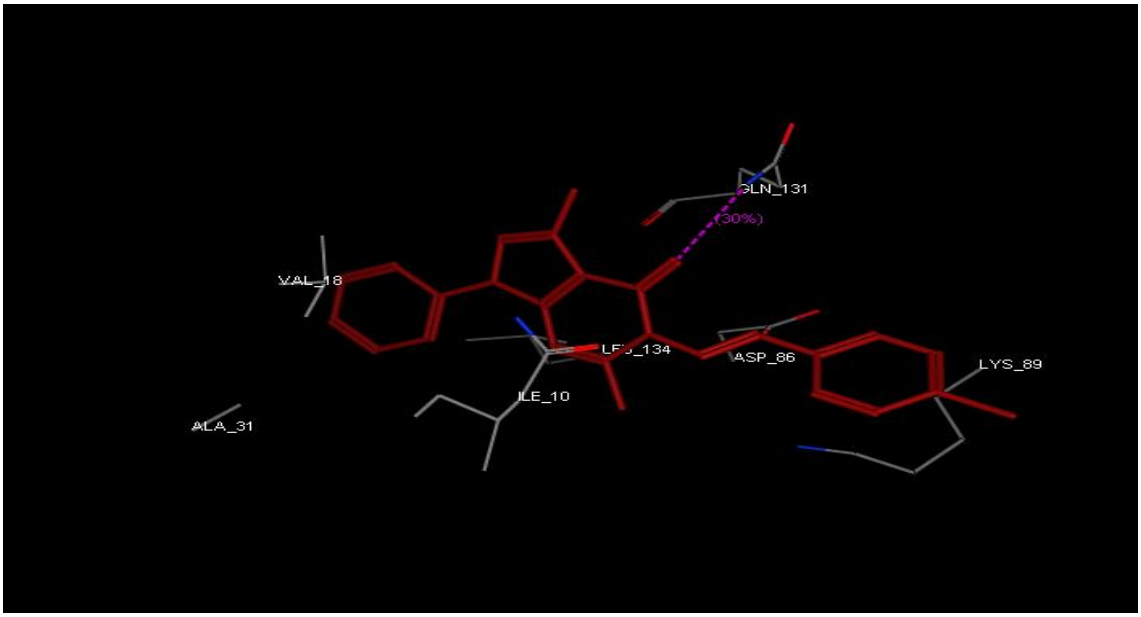


a



b

Fig.8. (a) Represents **V** in the binding site of CDK2, the dotted lines represent H-bonding interactions, **(b)** 2D interaction of V with **Gln131** acid residue.



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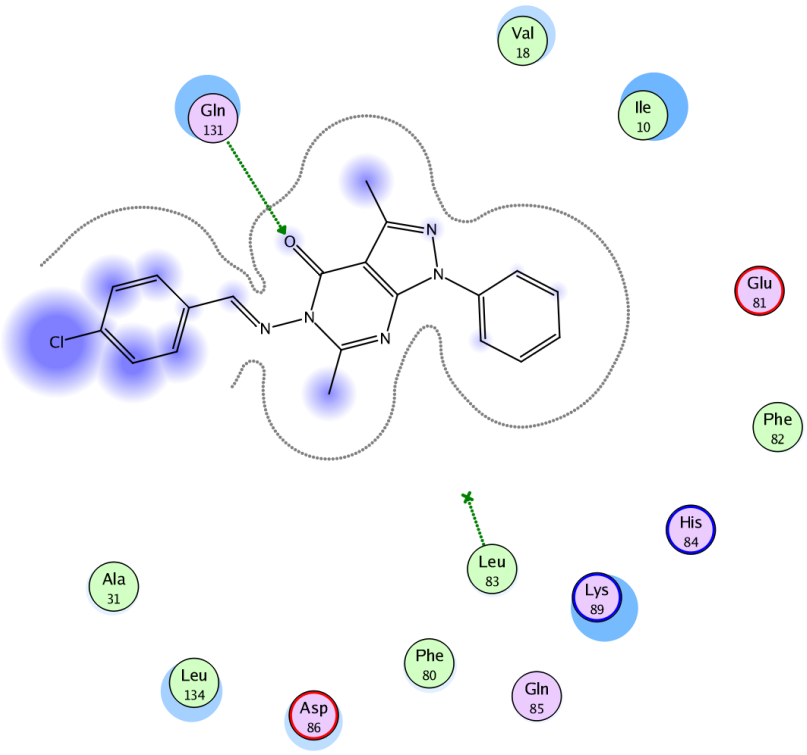
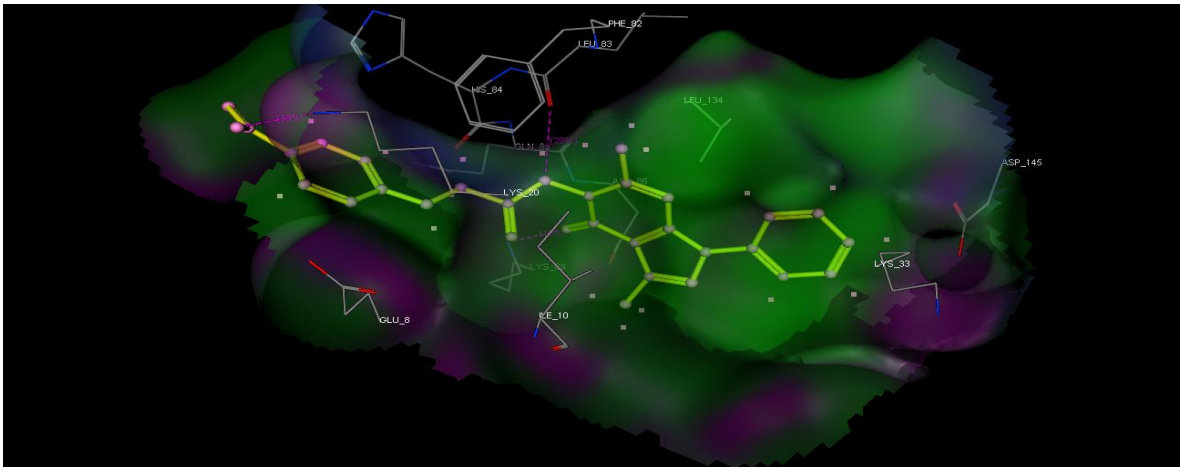


b

Fig.9. (a) Represents **Xd** in the binding site of CDK2, the dotted lines represent H-bonding interactions, (b) 2D interaction of **Xd** with **Gln**131 acid residue.



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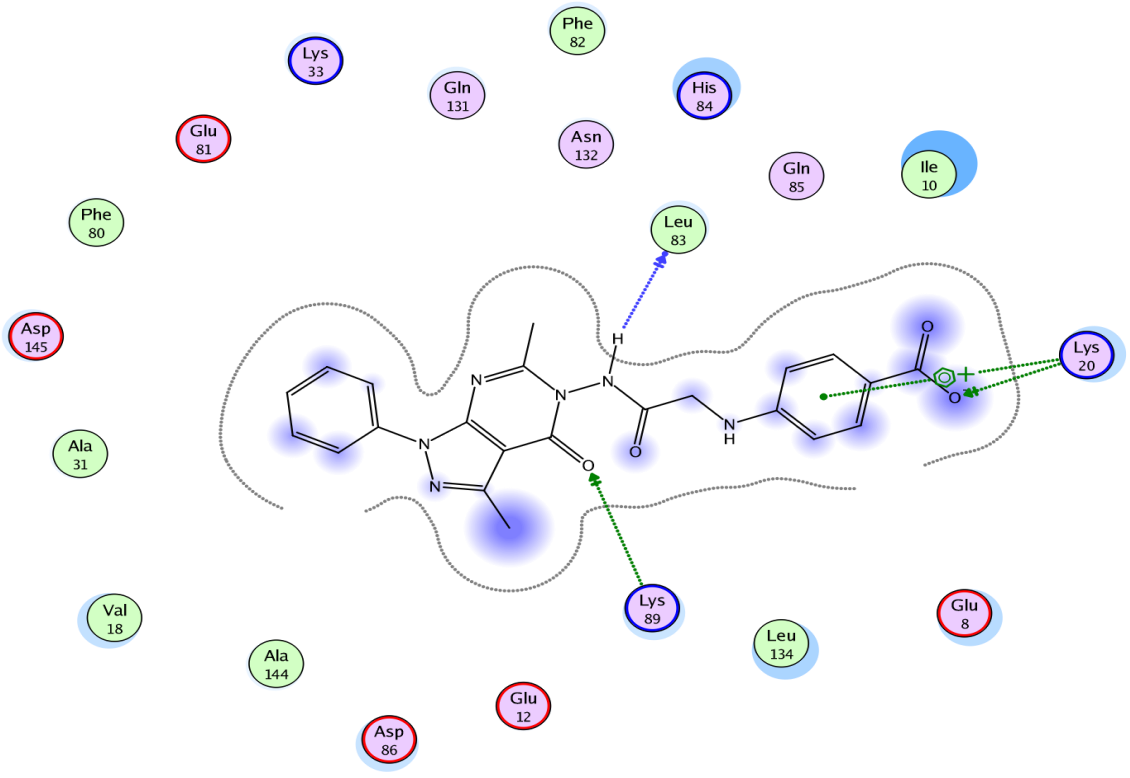
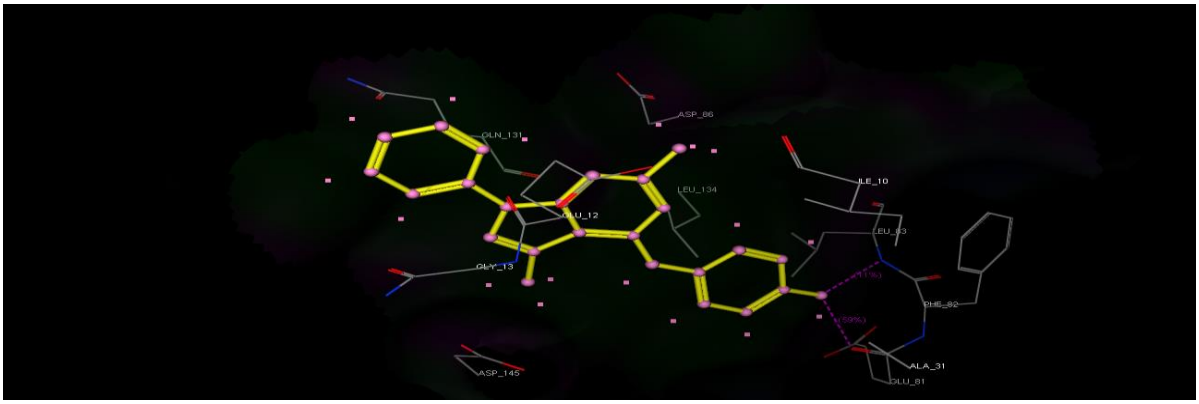


b

Fig.10. (a) Represents **XVIIc** in the binding site of CDK2, the dotted lines represent H-bonding interactions, **(b)** 2D interactions of **XVIIc** with **Lys**89**, Lys**20 and **Leu**83 acid residues.



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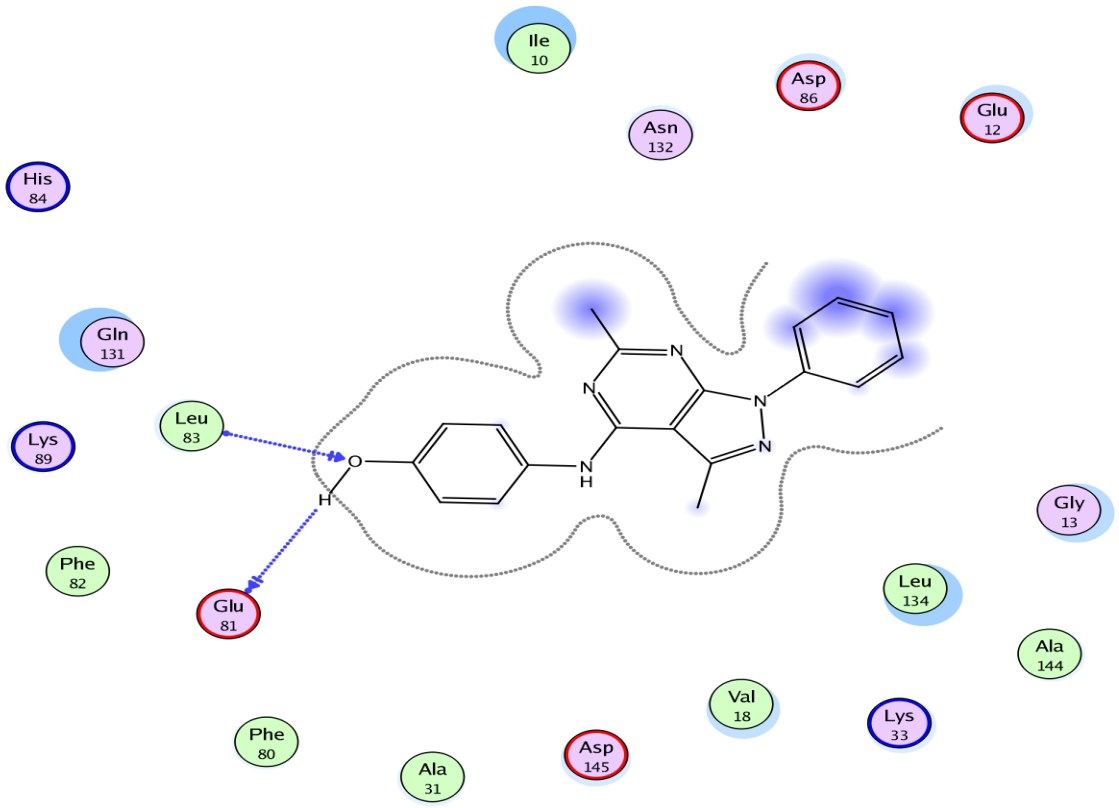
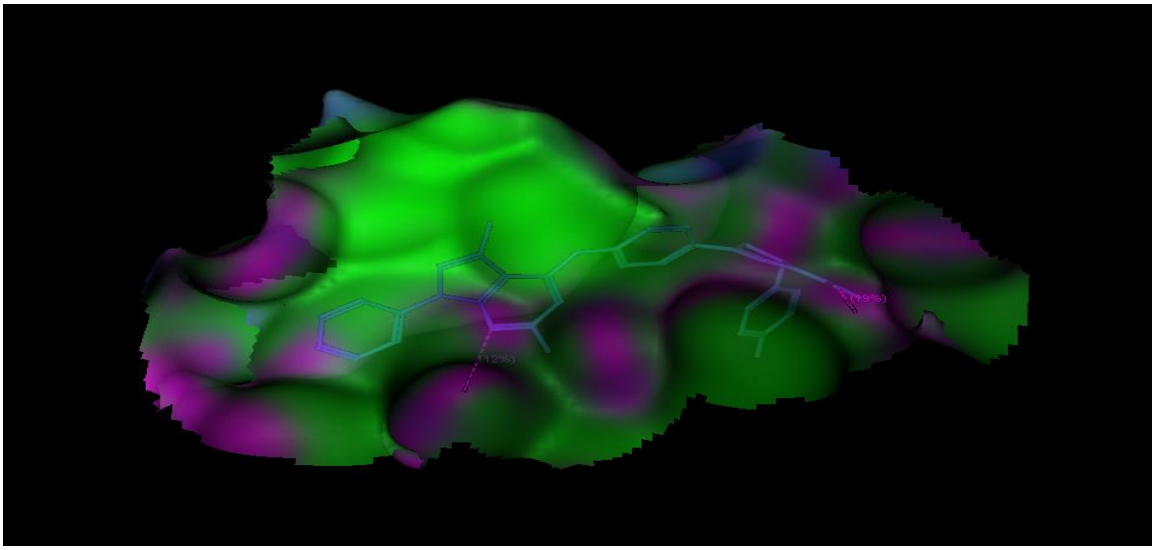


b

Fig.11. (a) Represents **XXIIa** in the binding site of CDK2, the dotted lines represent H-bonding interactions, (b) 2D interactions of **XXIIa** with **Leu**83 and **Glu**81 acid residue.



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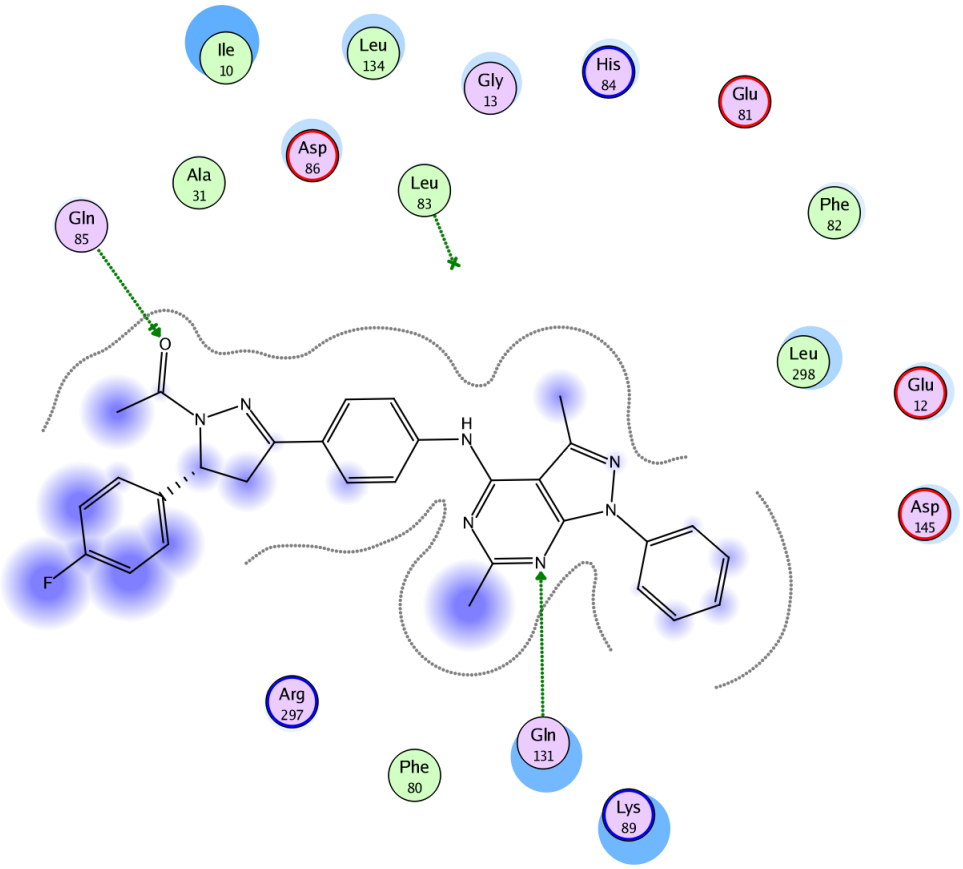


b

Fig.12. (a) Represents **XXIVb** in the binding site of CDK2, the dotted lines represent H-bonding interactions, (b) 2D interactions of **XXIVb** with **Gln85** and **Gln131** acid residue.

4.4. Cytotoxic activity:

In this study, twenty-eight of the newly synthesized compounds were tested for their anticancer activity using human breast carcinoma cell line (MCF-7).

Materials and methods:

1-Drug:

Doxorubicin was used in this experiment as a positive control. The title compounds were dissolved in 20% dimethylsulfoxide (DMSO) in concentration 1 mg/ml.

Serial dilution were made reaching final concentration of the compounds to 5, 12.5, 25, 50 μg/ml.

2-Chemicals:

All chemicals used in this study are of high analytical grade. They are either obtained from (Sigma-Alderich or Biorad).

3-Human tumor cell lines:

The breast carcinoma cell line was obtained frozen in liquid nitrogen (-180 ^{o}C) from the American Type Culture Collection (ATCC) and was maintained at National Cancer Institute, Cairo, Egypt, by serial subculturing.

4-Cells and cell culture conditions:

MCF-7 was grown as monolayer culture in RPM 11640 medium supplemented with 10% fetal bovine serum (FBS) and 1% Penicillin/Streptomycin. The cell line was incubated at 37 ^{o}C 5% CO_2 95% air

and high humidity atmosphere in the water jacketed incubator (Revco, GS laboratory equipment, RCO 3000 TVBB, U.S.A). The cell line was regularly subcultured to be maintained in the exponential growth phase. The sterile conditions were strictly attained by working under the equipped laminar flow (Microflow Laminar flow cabinet, Hamsphire SP 105aa, U.K.).

Maintenance of the human tumor cell line:

1. A cryotube containing frozen cells was taken out of the nitrogen container and then thawed in a water bath at 37 ^{o}C.

2. The cryotube was opened under strict aseptic conditions and its contents were transferred into sterile 50 ml disposable falcon tube supplemented by 5 ml medium drop by drop.

3. The tube was incubated for 2h then its contents were centrifuged at 1200 rpm for 10 min.

4. The supernatant was discarded and the cell pellet was suspended and seeded in 5 ml supplemented medium in T25 Nunclon sterile tissue culture flasks.

5. The cell suspension was incubated and followed up daily with replacing the supplemented medium every 2-3 days.

6. Incubation was continued until a confluent growth was achieved and the cells were freshly subcultured before each experiment to be in the exponential phase of growth.

Collection of cells by trypsinization:

1. The medium was discarded.

2. The cell monolayer was washed with 10 ml phosphate-buffered saline (PBS).

3. All the adherent cells were dispersed from their monolayer by the addition of (1 ml) trypsin solution (0.025% trypsin w/v).

4. The flask was left in incubator till complete detachment of the cells and checked with the inverted microscope (Olympus 1×70, Tokyo, Japan).

5. Trypsin was inactivated by the addition of (5 ml) of the supplemented medium containing fetal calf serum (FCS). The trypsin content was discarded by centrifugation at 1200 rpm for 10 min. Cells were separated in a single suspension by gentle dispersion several times.

Determination and counting of viable cells:

1. 100 µl of 0.05% trypan blue solution was added to 100 µl of the single cell suspension.

2. The cells were examined under the inverted microscope using the haemocytometer.

3. Non stained (viable) cells were counted and the following equation was used to calculate the cell count /ml of cell suspension.

4. Viable cells/ml = [Number of cells in 4 quarters x 2 (dilution factor) $\times 10^4$]/4.

5. The cells were then diluted to give the required concentration of single cell suspension.

Cytotoxicity of the test compounds using Sulphorhodamine-B (SRB) assay:

The principle: The cytotoxicity of the newly synthesized compounds were tested on the MCF-7 cell lin using SRB assay.[175]

SRB is a bright pink aminoxanthene dye with two sulfonic groups. It is a protein stain that binds to the amino groups of intracellular protein under mild acidic conditions to provide a sensitive index of cellular protein content.

Procedure:

1. The breast cancer cells were seeded in 96-well microlitre plates and left to attach for 24 h.

2. Cells were incubated with the tested compounds at concentration range from 0, 5, 12.5, 25 and 50 μg/ml) and incubation was continued for 48 h.

3. After 48h treatment, the cells were fixed with 50 μl cold 50% trichloroacetic acid (TCA) for 1h at 4^{o}C.

4. Wells were then washed 5 times with water and stained for 30 min at room temperature with 50 μl of 0.4% SRB dissolved in 1% acetic acid.

5. The wells were then washed 4 times with 1% acetic acid.

6. The plates were air dried and the dye was solubilized with 100 μl/well of 10Mm tris base (pH 10.5) for 5 min on a shaker (Orbital Shaker OS, Boeco, Germany) at 1600 rpm.

7. The optical density (O.D) of each well was measured spectrophotometrically at 564 nm with enzyme linked immunosorbent assay

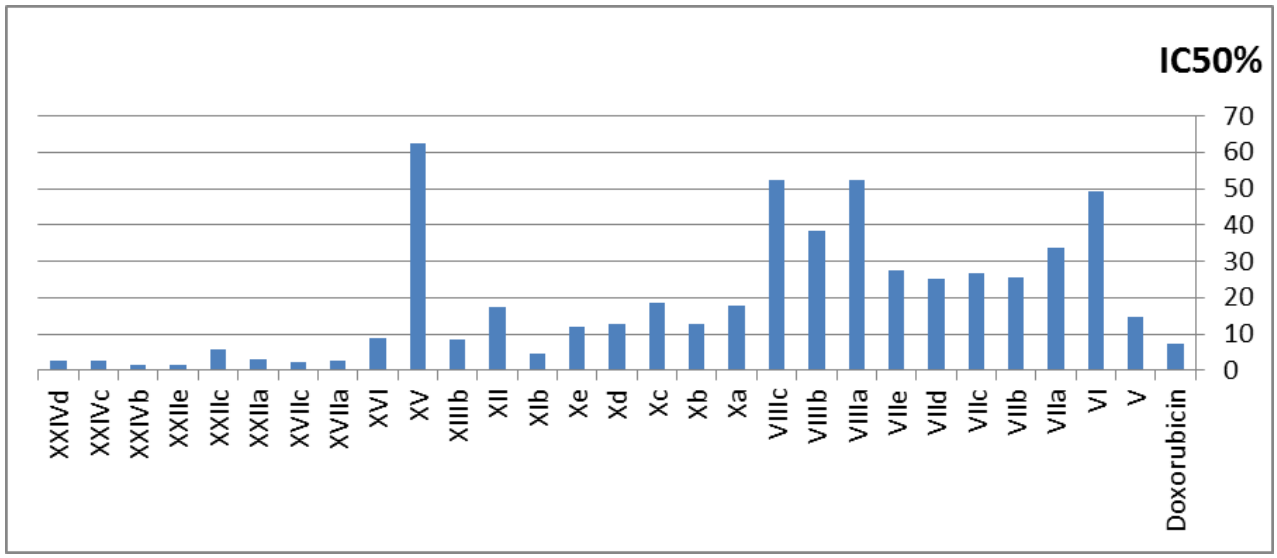
(ELISA) micraplate reader (Tecan Sunrise, Austria). The mean values for each drug concentration was calculated.

Results:

The percentage of cell survival was calculated as follows:

Survival fraction= O.D. (treated cells)/O.D. (control cells)

The IC_{50} values were calculated using sigmodial dose response curve fitting models (GraphPad, Prizm software incorporated). Each concentration was repeated 3 times and data represented in Table 22.



Conclusion:

In this study comparison between the docking scores (in negative terms) and *in vitro* biological cytotoxic activity expressed by IC_{50} (µM) showed that:

- Compounds **XIb, XIIIb, XVI, XVIIa, XVIIc, XXIIa, XXIIc, XXIIe, XXIVb, XXIVc** and **XXIVe** exhibited high binding energy scores (-20.07, -19.95, -18.05, -17.98, -18.55, -20.13,

-18.04, -23.51, -23.50, -19.58 and -18.59, respectively) and made one or more hydrogen bonds with different amino acid residues showed the highest *in vitro* cytotoxic activity (1.60-8.7 μM).

- Compounds **V, VIIa-e, VIIIb, Xa-e** and **XII** showed energy scores (-23.98: -15.55) and made one or two hydrogen bonds with different amino acids showed moderate *in vitro* cytotoxic activity (11.80-38.46 μM).

- The docking study of compounds **VI, VIIIa** and **VIIIc** showed binding energy scores (-15.59: -10.43) and forming one hydrogen bond. They showed weak *in vitro* cytotoxic activity (49.21-52.29 μM).

- On the other hand, compound **XV** is the least active compound with IC_{50} = 62.55 μM and showed binding energy score -10.11 forming no hydrogen bonds.

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الملخص العربى

تتضمن الرسالة أربعة أجزاء:

*الجزء الأول: هو عبارة عن مقدمة تحتوى على عرض مختصر للطرق المختلفة لتشييد مركبات محتوية على البيرازولو[٣,٤-د]بيريميدين كما يحتوى على نبذة عن مرض السرطان وطرق علاجه المختلفة بما فيها استخدامات مركبات البيرازولو[٣,٤-د]بيريميدين كمضادات للأورام السرطانية.

*الجزء الثاني: ويتناول الهدف من البحث و عرض المخططات التي توضح الطرق العملية للوصول إلى تحضير هذه المركبات الجديدة.

*الجزء الثالث: يوضح المناقشة النظرية للجزء العملى فى تحضير المركبات الأولية **I** و **II**. فعند تحلل المركب **II** نتج عنه مشتق حمض البيرازول-٤-كربوكسيليك **III** الذى تم تفاعله مع الأسيتيك أنهيدريد معطيا مشتق البيرازولو[٣,٤-د][٣,١] أوكسازين-٤-أون **IV**.

و قد تم الحصول على المركبات **V** ، **VI**، **VIIa-e** ، **VIIIa-c** من تفاعل البيرازولو[٣,١] أوكسازين-٤-أون **IV** مع الفينيل هيدرازين، هيدروكسيل أمين هيدروكلوريد ، الأمينات الأروماتية المختلفة والأميد أو الثيوأميد المناسبة، بالتتابع.

بالاضافة الى ذلك، تم تفاعل **IV** مع الهيدرازين هيدرات ليعطى مشتق ٥-امينوبيرازولو[٣,٤-د]بيريميدين-٤-أون **IX** الذى تم تفاعله مع الألدهيدات الأروماتية المختلفة ، انهيدريد الأحماض، البيرازولو[٣,٤-د][٣,١] أوكسازين-٤-أون **و** اليوريا أو الثيويوريا ليعطى المركبات **Xa-e** ، **XIa,b**، **XII** و **XIIIa,b**، بالتتابع. و من ناحية أخرى فقد تم تفاعل **IX** مع كلوروأستيل كلوريد لينتج **XIV** ثم تم تفاعل **XIV** مع الأمونيوم أسيتات و أمونيوم ثيوسيانات لينتج المركبات **XV** و **XVI**، بالتتابع. مركب **XIV** أخضع لتفاعلات الاستبدال النيوكليوفيلية مع الأمينات الأروماتية المختلفة و اثنين من البيريميدوثيون **XVIIIa,b** للحصول على المركبات **XVIIa-c** و **XIXa,b** ، بالتتابع.

أيضا تم تفاعل تكثيف البيرازولو[٣,٤-د] [٣,١] أوكسازين-٤-أون مع **IV** الفورماميد لينتج المركب **XX** الذى تم تفاعله مع الفوسفورس أوكسى كلورايد ليعطى **XXI**. كذلك قد تم تحضير المركبات

XXIIa-e من تفاعل **XXI** مع الأمينات الأروماتية المختلفة وقد أخضع المركب **XXIIb** لتفاعل التكثيف ليعطى الشالكونات **XXIIIa-e** التى تم تفاعلها مع الهيدرازين هيدرات لينتج مشتقات البيرازولين **XXIVa-e**. و قد تم تدعيم اثبات المركبات المشيدة عن طريق التحليل العنصرى الدقيق و استعمال الأشعة تحت الحمراء و الرنين النووى المغناطيسى و طيف الكتلة. بالاضافة الى ذلك، تم شرح وصفا موجزا عن دراسة الارساء الجزيئى من خلال مقارنة الشكل المتحد المناسب مع النتائج العملية.

*الجزء الرابع: : يحتوى على الأجزاء العملية التي اتبعت لتشييد المشتقات و الطرق المفصلة لتحضير المركبات الأولية **I** و **II** والمركبات الوسيطة **IV**، **IX**، **XIV**، **XX**، **XXI**، **XXIIIa-e.** بالاضافة الى مركبات البيرازولوبيريميدين الجديدة **V-VIIIa-c** ، **Xa-e -XIIIa,b**، **XV-** **XVIIa-c**، **XIXa,b** ، **XXIIa-e** و **XXIVa-e**. بالاضافة الى الخواص الفيزيائية و النتائج المفصلة للتحليل العنصرى للمركبات. كما انه تم القاء الضوء على نشاط ثمانية و عشرون مركب من المركبات الجديدة كمضادات للأورام مقارنة بالدوكسوروبسين. ويوضح هذا الجزء أيضا الارتباط بين نتائج الإرساء الجزيئي و تقييم المركبات كمضادات للأورام.

تشييد بعض مشتقات البيرازولو [٣ ,٤ -د] بيريميدين و التي لها تأثير بيولوجي

رسالة مقدمة من

الماجستير/ رانيا بدوى بكر محمد

ماجستير العلوم الصيدلية (كيمياء عضوية صيدلية)

للحصول على درجة الدكتوراة الفلسفية فى العلوم الصيدلية

تحت إشراف

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2014