PYRAZOLOPYRIMIDINES AS ANTICANCER AGENTS; SYNTHESES AND MODE OF ACTION (REVIEW ARTICLE)

Wafaa Zaghary[1], Tamer Nasr[1] , Rania H. Abd El-Hameed[2], Tamer M Sakr[3], Dina Adel Osman[4], Khaled El-Adl[5,6,*]

[1] Pharmaceutical Chemistry Department, Faculty of Pharmacy, Helwan University, Helwan, Cairo, Egypt.

[2] Pharmaceutical Organic Chemistry Department, Faculty of Pharmacy, Helwan University, Helwan, Cairo, Egypt.

[3] Radioactive Isotopes and Generator Department, Hot Labs Center, Egyptian Atomic Energy Authority, 13759, Cairo, Egypt

[4] Pharmaceutical Chemistry Department, Faculty of Pharmacy, October University of Modern Sciences and Arts (MSA), Giza, Egypt.

[5] Pharmaceutical Chemistry Department, Faculty of Pharmacy, Heliopolis University for Sustainable Development, Cairo, Egypt

[6] Pharmaceutical Chemistry Department, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt

[*] **Corresponding author: Email** : khaled.eladl@hu.edu.eg

Abstract

Pyrazolopyrimidines are composed of a pyrazole ring fused with pyrimidine moiety, unlike the imidazole moiety in purines; they are initially reported as adenosine receptor antagonists. Different methods of synthesis of substituted pyrazolo[3,4-*d*]pyrimidines and pyrazolo[1,5-*a*]pyrimidines have been reported with a survey of their biological activities especially anti-cancer activity. During the last two decades, pyrazolopyrimidines have gained great attention as biologically active compounds; they are reported to have anti-cancer activity, anti-microbial, anti-inflammatory, anti-hyperuricemia, anti-viral and anti-hypertensive activities. Researchers paid great attention to pyrazolopyrimidines due to their high anti-cancer activity, so they tried to prepare new derivatives and examine their anti-cancer activity. In this review we are going to focus on the different methods used for the synthesis of pyrazolopyrimidines, their anticancer activities as well as their reported mode of action.

Keywords: Pyrazolopyrimidine, Anti-cancer, Synthesis, Mode of Action

1. Introduction

Cancer is a disease in which a group of cells displays uncontrolled growth, sometimes invasion which destroys adjacent tissues and sometimes metastasizes to lymph nodes or other body sites via the lymphatic system or through the bloodstream. These properties differentiate malignant tumors from benign cancer (**Mandour *et al*., 2022; Upadhyay, 2021**).

Pyrimidines and fused pyrimidines, being an integral part of DNA and RNA, play an essential role in several biological processes and have significant chemical and pharmacological prominence **(Ali, 2004; Fatahala *et al*., 2017; Fatahala *et al*., 2018; Mohamed *et al*., 2015; Mohamed *et al*., 2017; Nasr *et al*., 2007; Nasr *et al*., 2009; Nasr *et al*., 2012; Pramanik *et al*., 2023; Abbas *et al*., 2021)**.

Similarly, the pyrazole nucleus is a highly multipurpose drug-like template that is widely used in the design of anticancer therapies **(Abd El-Karim *et al*., 2015; Ameziane El Hassani *et al*., 2023; Bondock *et al*., 2013; Elgemeie *et al*., 2005; Nasr *et al*., 2014; Nitulescu *et al*., 2015; Yadav *et al*., 2022)**. The synthesis and study of pyrazole-fused compounds have emerged as an important heterocyclic system due to their wide range of biological activities **(Akbar *et al*., 2024; Elgemeie *et al*., 2009; Nasr *et al*., 2017; Shi *et al*., 2015)**.

As there is no big difference in the basic structures of pyrazolopyrimidines and purine structure **(Elbakry *et al*., 2024; Hassaballah *et al*., 2024; Holla *et al*., 2006)**, pyrazolopyrimidine is an interesting bioactive core used for developing molecules of biological interest **(Abbas *et al*., 2015; Kurban *et al*., 2023)** and plays a critical role in treating numerous disease conditions (**Chauhan *et al*., 2013)**. Pyrazolo[3,4-d]pyrimidines have different biological activities (**Laamari *et al*., 2024)** like, anti-inflammatory (**Yewale *et al*., 2012)**, antimicrobial **(Abdallah *et al*., 2018; Mohamed *et al*., 2018; Rostamizadeh *et al*., 2013)**, xanthine oxidase inhibition **(Gupta *et al*., 2008; Khadri *et al*., 2023)** and antiviral **(Rashad *et al*., 2009; Youssef *et al*., 2023)**. Also, they could exhibit anticancer activity by inhibiting different types of enzymes and receptors **(Chauhan *et al*., 2013)** such as cyclin-dependent kinase **(Azmy *et al*., 2023; Kim *et al*., 2003)** Src and Abl tyrosine kinase **(Ismail *et al*., 2016; Schenone *et al*., 2008)**, glycogen synthase kinase-3 **(Dessalew *et al*., 2007; Peat *et al*., 2004)**, insulin-like growth factor receptor protein tyrosine kinase **(Hubbard *et al*., 2007; Nitulescu *et al*., 2023)** and they are an important starting material for many heterocyclic systems **(Jismy *et al*., 2020)**.

Both pyrazolo[1,5-*a*]pyrimidines and pyrazolo[3,4-*d*]pyrimidines are of considerable chemical and pharmacological importance as purine analogs, and many derivatives have been reported to exhibit cytotoxic activity **(Abd El Hamid *et al*., 2012; Akbar *et al*., 2024; Hassan *et al*., 2015; Metwally *et al*., 2016)**, In most purine biochemical reactions, a number of these ring structure derivatives are thought to be significant as antimetabolic agents **(Asati *et al*., 2021; Hebishy *et al*., 2020)**. Many researchers noted that many pyrazolo[3,4-d]pyrimidines possessed cytotoxic activity as Src kinase inhibitors against human ovarian adenocarcinoma (SK-Ov-3) **(Halim *et al*., 2023)**, breast carcinoma (MDA-MB-361) **(Alharthy *et al*., 2023)**, liver carcinoma cell

line (Hep-G2) and colon adenocarcinoma (HT-29) **(Kumar *et al*., 2013; Poonia *et al*., 2016)**. This review is going to focus on the different methods used for the synthesis of pyrazolopyrimidines, their anticancer activities as well as their reported mode of action.

2. Synthesis of pyrazolo[3,4-*d*]pyrimidine as anticancer agents

2.1. Starting with 5-aminopyrazole-4-carboxylate

Schmidt *et al*. reacted ethyl-5-aminopyrazole-4-carboxylate derivative **1** with formamide to give the 4-hydroxy-pyrazolo[3,4-*d*]pyrimidine derivative **2 (Schmidt and Druey, 1956)**. While, Holla *et al.* have synthesized the pyrazolo[3,4-*d*]pyrimidine-4,6-(5*H*,7*H*)-dione derivative **3** and 6,7-dihydro-6-thioxo-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-onederivative **5** by reaction of **1** with urea and thiourea respectively **(Holla *et al*., 2006)**. Alternatively, derivative **3** could be synthesized by the reaction of compound **1** with chlorosulfonyl isocyanate in the presence of potassium hydroxide **(Wamhoff, 1985)** (**Fig. 1**). Alternatively, compound **5** could be prepared by refluxing compound **1** with potassium thiocyanate and benzoyl chloride in acetone to obtain the benzoylthioureido substituted pyrazole carboxylate derivative **4** which then was cyclized with methanolic sodium hydroxide to produce compound **5 (Kasibhatla *et al*., 2008) (Fig. 1)**.

Wang *et al.* obtained the substituted 6-(phenylamino)-pyrazolo[3,4-*d*]pyrimidin-4(7*H*)-one **7** *via* reaction of compound **1** with triphenylphosphine and bromine to afford the iminophosphorane intermediate **6** which was reacted with phenylisocyanate and alkylamine **(Wang *et al*., 2004)**. Also, compound **1** was reacted under reflux with acetic anhydride, and then reacted with ammonium hydroxide to give the 5-acetamido-4-carboxylate-pyrazole derivative **8**. Compound **8** was then condensed with the appropriate amine in the presence of phosphorus oxychloride to afford the corresponding pyrazolopyrimidinone derivatives **9 (Wang *et al*., 2007)**.

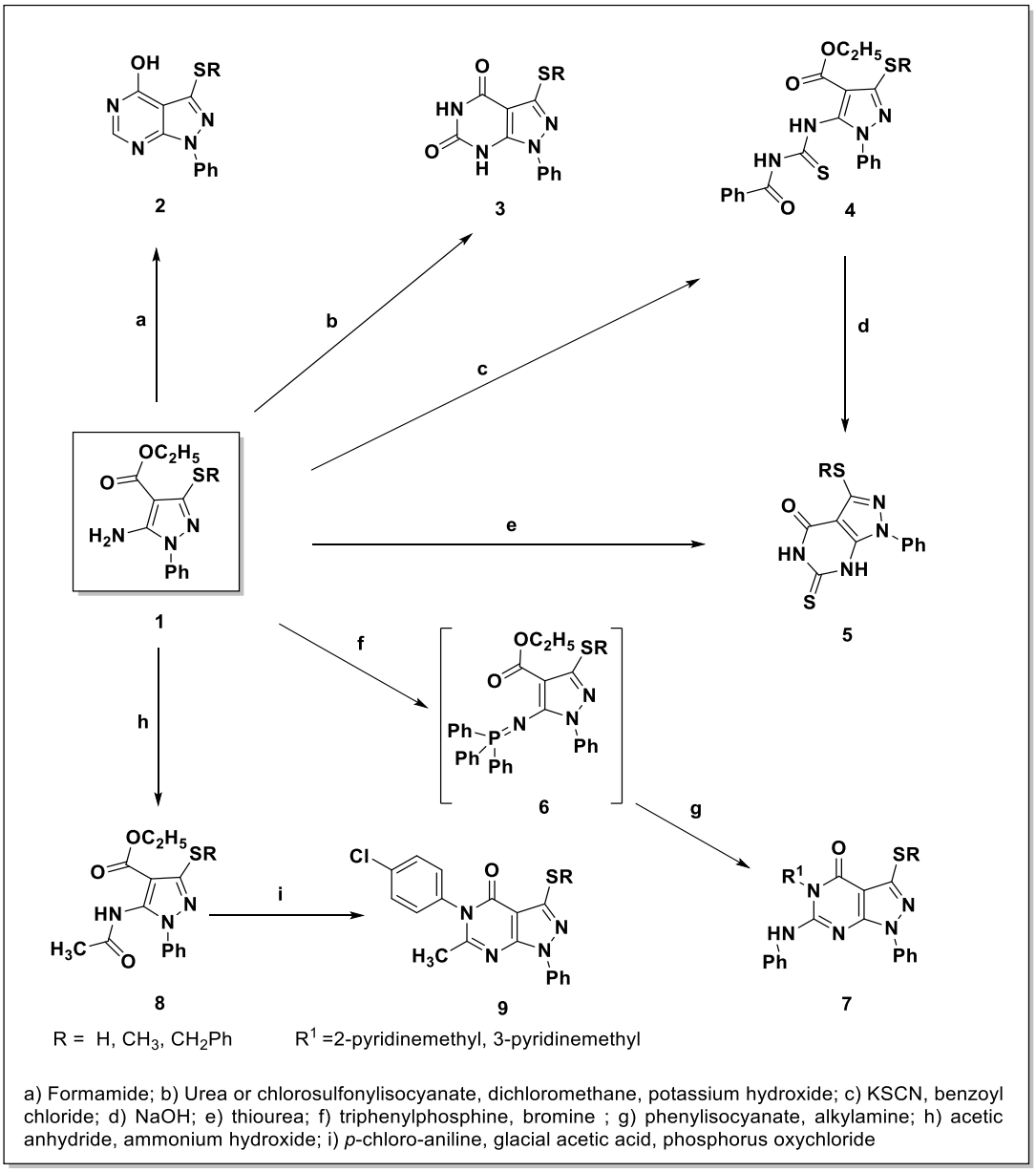


Fig. 1: General procedures for the synthesis of various pyrazolo[3,4-*d*]pyrimidines starting with 5-aminopyrazole-4-carboxylate

2.2. Starting with 5-amino-1-substituted-1*H*-pyrazole-4-carbonitrile derivatives

Monosubstituted hydrazine derivatives were refluxed with ethoxymethylene malononitrile **10** to afford 5-amino-1-substituted-1*H*-pyrazole-4-carbonitrile derivatives **11** which are used as starting materials for the synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives (**Fig**. **2**). Pyrazole derivatives **11** were then reacted with formic acid, formamide, urea or thiourea to obtain the target 1-substituted-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one derivatives **12**, 4-amino-pyrazolo[3,4-*d*]pyrimidine derivatives **13**, 4-amino-1-substituted-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-ol **14** and 4-amino-1-substituted-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-thiol **15**; respectively **(Abdelazeem *et al*., 2014)**.

Moreover, Gupta *et. al*. have synthesized different derivatives of the target compounds **13** by reacting **11** with triethyl orthoformate in acetic anhydride to give intermediates which were then cyclized with ammonia in methanol **(Gupta *et al*., 2008)**. Also, Quintela *et. al*. have synthesized the chloroamidine derivatives **16** by the reaction of compound **11** with phosogeneiminium chloride. The latter derivatives were then cyclized by hydrochloric acid gas at room temperature in 1,2-dichloroethane to afford 4-chloro-*N,N*-dimethyl-substituted-1*H*- pyrazolo[3,4-*d*]pyrimidine-6-amine derivatives **17** **(Quintela *et al*., 2001)**. Finally, 6-methyl-1-substituted-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one derivatives **18** were easily synthesized by refluxing the starting material **11** in acetic anhydride and acetic acid **(Ali *et al*., 2009)**.

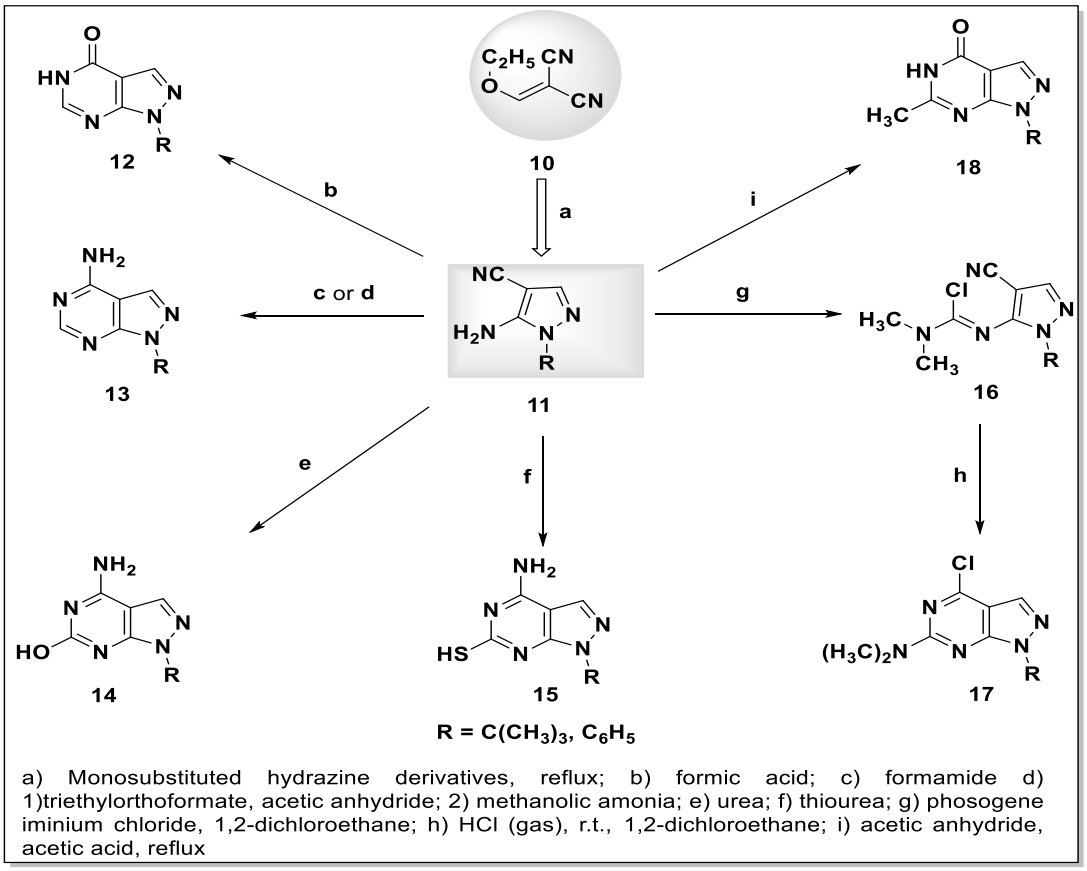


Fig. 2: General procedure for the synthesis of different substituted pyrazolo[3,4-*d*]pyrimidine starting with 5-amino-s1-substituted-1*H*-pyrazole-4-carbonitrile

2.3. Starting with 5-aminopyrazole-4-carbohydrazide derivatives

Wamhoff synthesized the target compound 1-substituted-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-dione derivatives **21b** (**Fig. 3**) by the reaction of 5-aminopyrazole-4-carbohydrazide derivatives **19** with urea **(Wamhoff, 1985)**. Furthermore, Baviskar *et al*. reacted compounds **19** under reflux with different benzaldehyde derivatives in methanol to afford the intermediate compounds **20** which were then reacted with triethylorthoformate under reflux for 4 hours to obtain the

targeted 5-(benzylideneamino)-1-substituted-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one **21b (Baviskar *et al*., 2013)**.

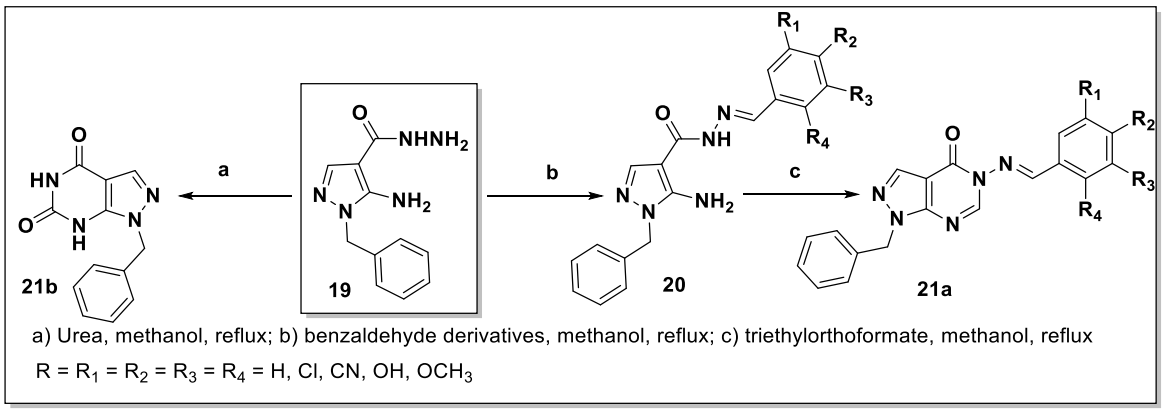


Fig. 3: General procedure for the synthesis of substituted different pyrazolo[3,4-*d*]pyrimidine derivatives starting with 5-aminopyrazole-4-carbohydrazide derivatives**.**

2.4. Starting with 5-aminopyrazole derivatives

In 2012, Huang *et al*. have synthesized 1-substitued-1*H*-pyrazolo[3,4-*d*]pyrimidine **23** in a good yield by the reaction of compound **22** with formamide and phosphorous bromide **(Huang *et al*., 2012)** or phosphorous oxychloride **(Chang *et al*., 2013)**. Furthermore, in 2014 Ryabukhin *et al*. prepared the urea derivatives **24** by the reaction of compound **22** with the corresponding isocyanate derivatives. The compounds **24** were allowed to undergo cyclocondensation reaction with different aldehydes in the presence of chlorotrimethylsilane to yield the corresponding pyrazolo[3,4-*d*]-4,5-dihydropyrimidin-6-ones **25**. While the reaction between isatin and the urea derivatives **24** in the presence of chlorotrimethylsilane gave the target compounds 5',7'-dihydrospiroindoline-3,4'-pyrazolo[3,4-*d*]pyrimidine-2,6'-diones derivatives **26** (**Fig. 4**) in good yields **(Ryabukhin *et al*., 2014)**.

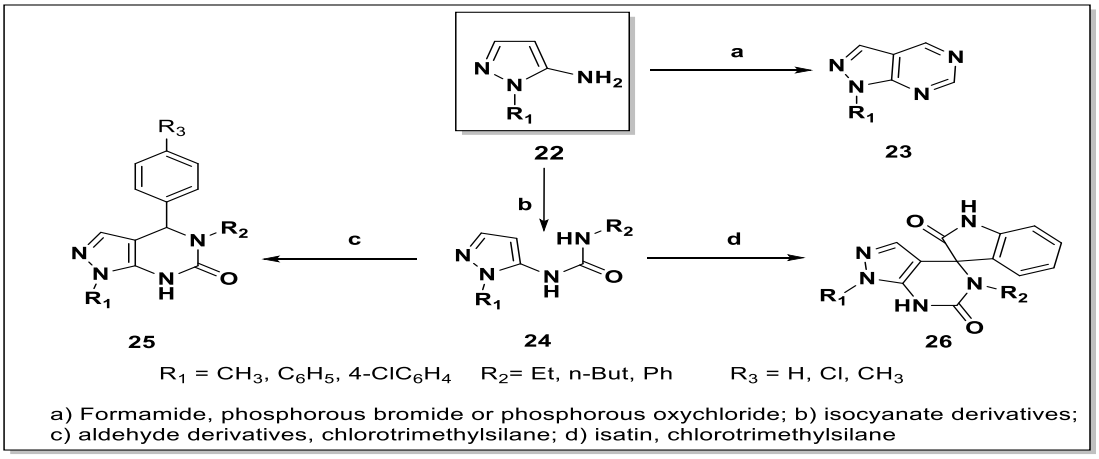


Fig. 4: General procedure for the synthesis of substituted pyrazolo[3,4-*d*]pyrimidine derivatives from 5-amino-pyrazole derivatives

2.5. Starting with 5-amino-4-cyano-pyrazole derivatives

In 2016, Liu *et al*. synthesized 1-(2,4-dichlorophenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**28**) *via* the reaction of 5-amino-1-(2,4-dichlorophenyl)-1*H*-pyrazole-4-carbonitrile (**27**) with triethylorthoformate in acetic anhydride followed by treating the formed intermediate pyrazol-5-yl-formimidate derivative with ammonium hydroxide solution (**Fig. 5**) **(Liu *et al*., 2016)**.

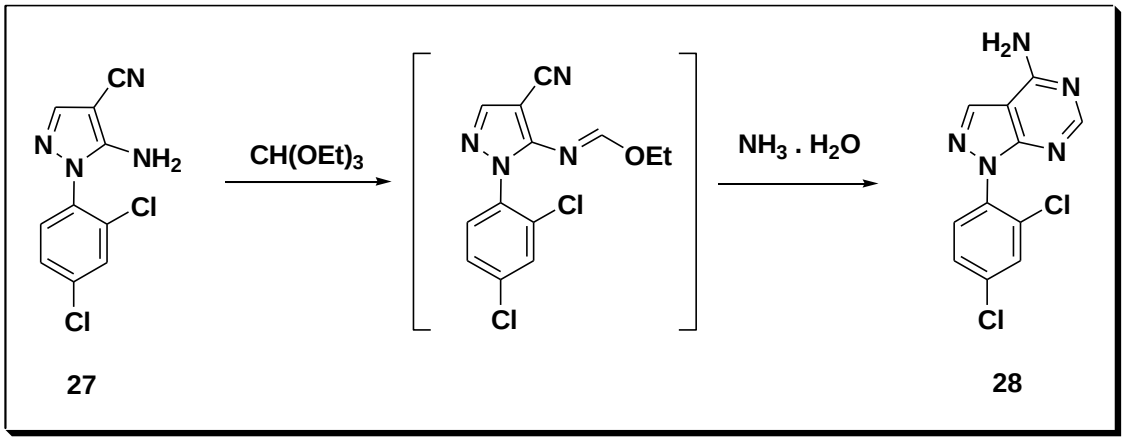


Fig. 5: Synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives from 5-amino-4-cyano-pyrazole derivatives

2.6. Starting with pyrimidine derivatives

In 2013, Rostamizadeh *et al*. prepared 3-amino-4-aryl-6-phenyl-pyrazolo[3,4-*d*]pyrimidine derivatives **30** by reacting 4-amino-6-aryl-2-phenyl-pyrimidine-5-carbonitrile derivatives **29** and hydrazine hydrate in ethanol under reflux **(Rostamizadeh *et al*., 2013)**. In a promising procedure, Rostamizadeh *et al*. debuted a one-pot (**Fig. 6**) procedure for the synthesis of compounds 30, through the reaction of different aldehydes **31**, 2-hydroxysuccinonitrile **32**, benzamidine hydrochloride **33** and hydrazine hydrate in the presence of basic alumina-supported sodium acetate ($AcONa/Al_2O_3$) under refluxing conditions. This protocol offers several advantages over the previously reported methods such as excellent yields of the products, simple workup, straightforward synthesis of pyrazolo[3,4-*d*]pyrimidine derivatives *via* available starting materials **(Rostamizadeh *et al*., 2013)**.

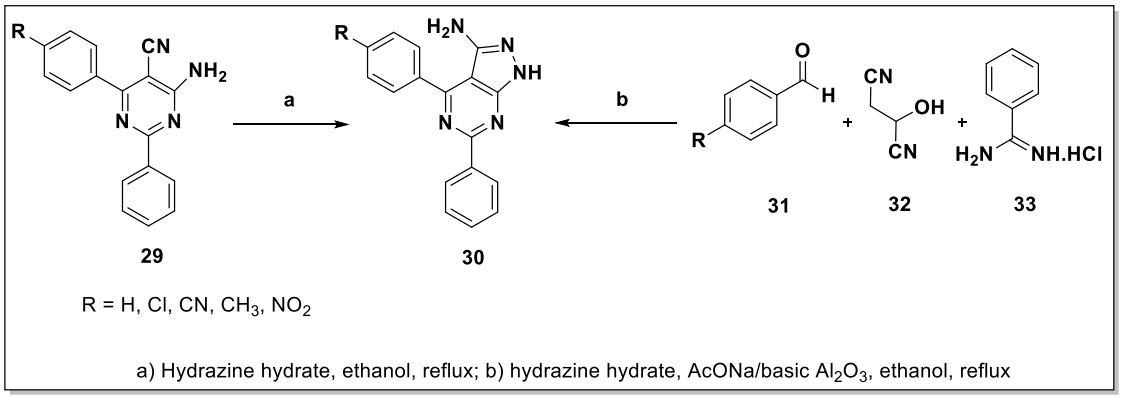


Fig. 6: Different methods for the synthesis of 3-amino-4-aryl-6-phenyl-pyrazolo[3,4-*d*]pyrimidine derivatives**.**

Finally, Soth *et al*. carried out the reaction of tert-butyl carbazate with 4-chloro-5-cyano-pyrimidine **34** in ethylamine to afford the target *N*-substituted pyrazolo[3,4-*d*]pyrimidine derivative **35 (Soth *et al*., 2011) (Fig. 7)**.

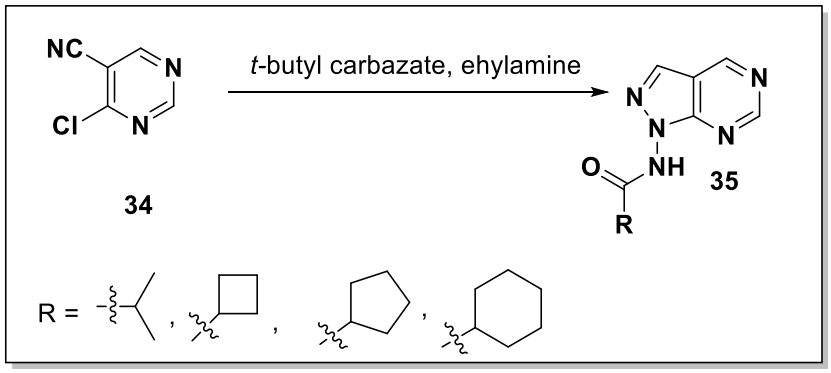


Fig. 7: Synthesis of pyrazolo[3,4-*d*]pyrimidine derivativesusing4-chloro-5-cyano-pyrimidine derivatives as a starting compound

3. Anticancer activities of pyrazolo[3,4-*d*]pyrimidine derivatives

Baviskar *et al*. showed that the 5-(benzylideneamino)-6,7-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one derivatives **36-38** are potent antitumor agents against prostate cancer, breast cancer and liver cancer **(Baviskar *et al*., 2013) (Fig. 8)**. In 2011, Abd El-Razik *et al*. showed that 7-(4-chlorophenyl)-*N*-(4-fluorophenyl)-7*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[4,3-*c*]pyrimidin-3-amine (**39**) is a potent inhibitor for hepatocellular carcinoma Hep-G2 and cervical carcinoma Hela-S3 cell lines **(Abd El Razik *et al*., 2011)**. Ghorab *et al.* have synthesized the pyrazolo[3,4-*d*]pyrimidine derivative **40** which showed potent activity against Ehrlich Ascites carcinoma (EAC) cell line in comparison to the referenced drug doxorubicin **(Ghorab *et al*., 2010)**. In 2013, Kandeel *et al*. showed that 2-[3-methyl-4-oxo-1-phenyl-5-(p-tolylcarbamoylmethyl)-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-ylmethylsulphanyl]-*N*-p-tolyl-acetamide (**41**) has high activity against breast cancer cell line MCF7. Moreover, Kandeel *et al.* have synthesized pyrazolopyrimidine derivatives

42-44 which showed promising *in vitro* cytotoxic activity against different cancer cell lines in comparison to the referenced drug olomoucine **(Kandeel *et al*., 2013)**.

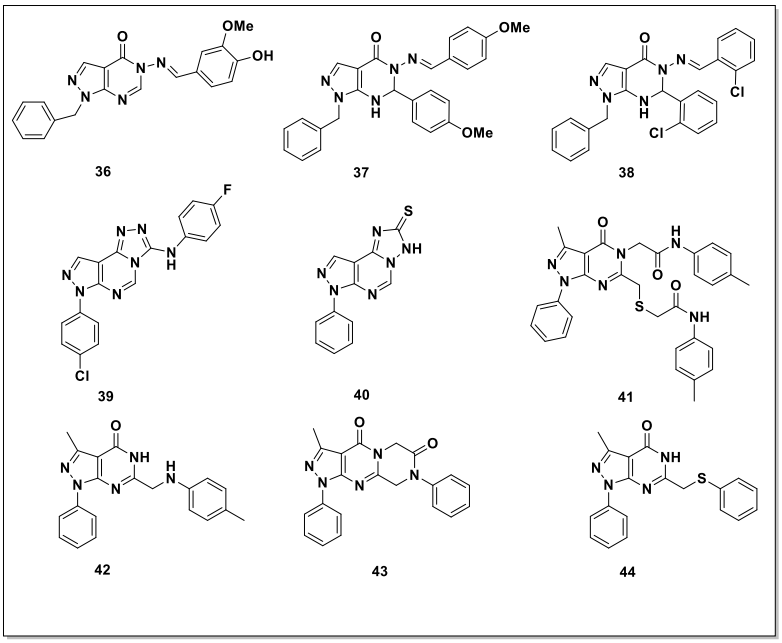


Fig. 8: Examples of different pyrazolo[3,4-*d*]pyrimidine derivatives producing anticancer activity.

Additionally, 1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine derivatives **45-47** tagged on position 4 with the 5-cyano-4-(4-substitutedphenyl)-6-oxopyrimidine moiety through a sulfanyl linker emerged promising anticancer activity. Interestingly, compound **45** was the most active **(Abbas *et al*., 2015)** with IC_{50} less than 0.08 µM/mL (**Fig. 9)**. In 2012, Abd El Hamid *et al*. have synthesized 4-[2-(4-fluorobenzylidene)hydrazinyl]-3-(methylsulphanyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **48** which demonstrated high anticancer activity against human breast carcinoma cell line MCF **(Abd El Hamid *et al*., 2012)**. In 2016, Hassan *et al*. have synthesized a pyrazolo[3,4-*d*]pyrimidine derivative **49** fused to 1,2,4-triazole ring which displayed potent anticancer activity against HCT-116, PC-3 and Hep-G2 human cancer cell lines **(Hassan *et al*., 2017)**. Pyrazolotriazolopyrimidine derivatives **50** and pyrazolopyrimidin-hydrazono-butyric acid ethyl ester **51** were synthesized by Kandeel *et al*. and were screened for their anticancer activity against MCF-7 cell line. They showed very potent in-vitro activity with IC_{50} = 0.013 µM **(Kandeel *et al*., 2012)**. Moreover, Rashad *et al*. reported that 6-(2,2-dihydroxyethylthio)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**52**) was very potent against Hep-G2 human cancer cell line and its activity was similar to that of doxorubicin against the same cell line **(Rashad *et al*., 2015)**. In 2014, Lamie has synthesized 3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-ones **53**, **54** linked at position 6 to arylidene-1-methyl-hydrazine moiety and their corresponding tricyclic derivative **55** and the three compounds displayed very potent in-vitro activity against human breast adenocarcinoma cell line (MCF7) with IC_{50} values in the range 0.032 µM-0.035 µM **(Phoebe, 2014)**. Two years ago, Rahmouni *et. al*. had synthesized 3-methyl-5-(5-methyl-2-oxohexyl)-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one **56** and its corresponding 2-oxoheptyl analogue **57** which possessed excellent and promising *in-*

vitro anticancer activity against HCT-116 cancer cell line. Finally, the percent inhibition of MCF-7 cancer cell line by (*Z*)-3,6-dimethyl-1-phenyl-5-((1-(1-p-tolyl-1*H*-1,2,3-triazol-4(5*H*)-ylidene)methyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one (**58**) and its chloro-analogue (**59**) were 67.3% and 72.0%, respectively **(Rahmouni *et al*., 2016)**.

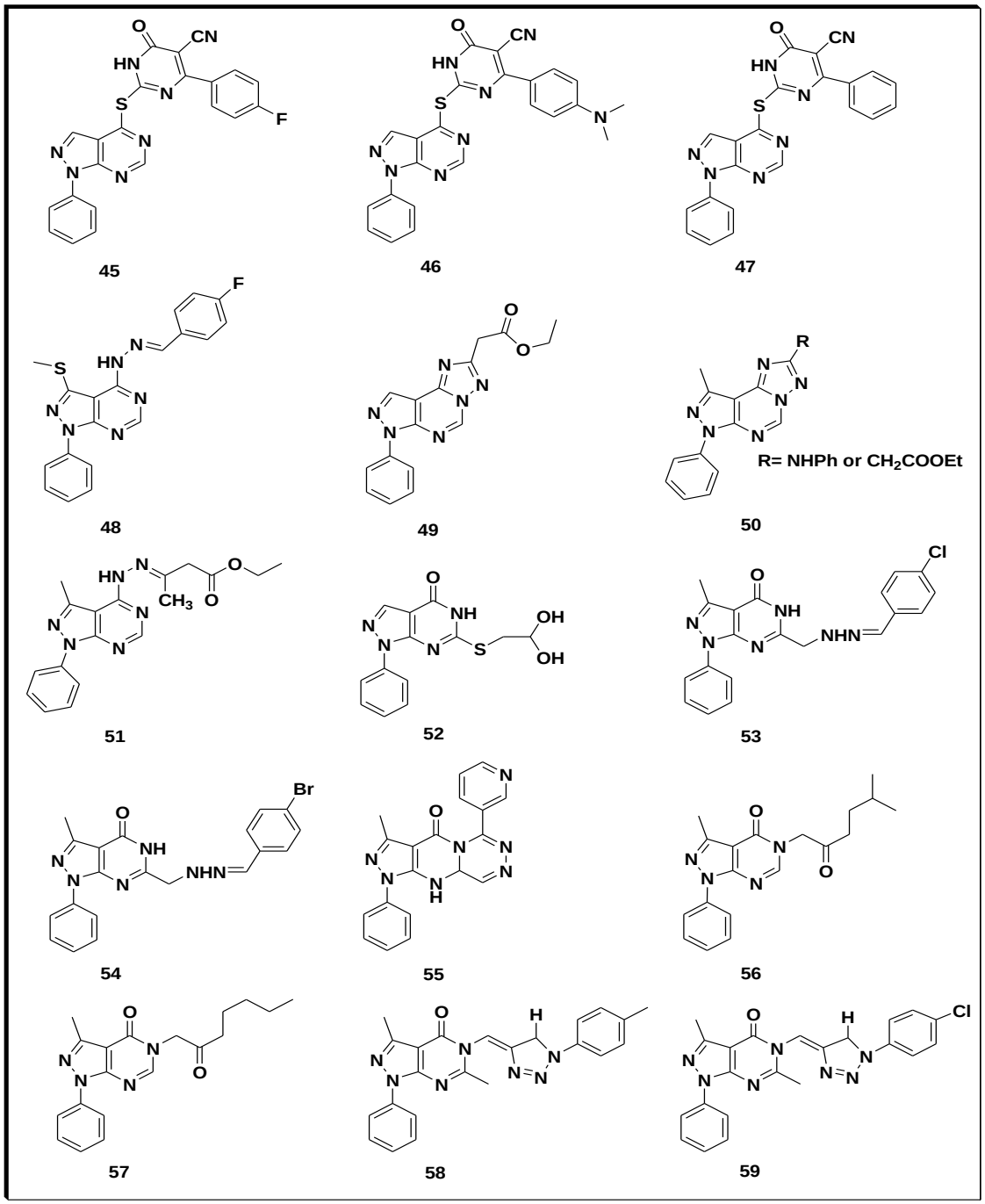


Fig. 9: Different examples for pyrazolo[3,4-*d*]pyrimidine derivatives exhibiting anticancer activity.

4. Reported anticancer mode of action for pyrazolo[3,4-*d*]pyrimidines

4.1. Tyrosine kinase inhibition

The tyrosine kinase enzymes (TK) regulate several physiological mechanisms, including cell proliferation, differentiation, migration and metabolism by transferring the ATP terminal phosphate to the tyrosine residues of protein substrates **(Carmi *et al*., 2012; Ebrahimi *et al*., 2023; Roskoski, 2023)**.

4.1.1. Src tyrosine kinase inhibition

The Src family of tyrosine kinases (SFKs) is constituted of nine members: Src, Lck, Fyn, Yes, Hck, Blk, Fgr, Lyn and Yrk. SFKs play an important role in signalling pathways that control a diverse spectrum of biological activities, such as cell division, growth factor signalling, differentiation, survival, adhesion, migration, invasion and so on **(Ghossoub *et al*., 2023; Dang *et al*., 2024; Bußmann *et al*., 2021)**.

Manetti *et al.* explored the antiproliferative and proapoptopic activities of pyrazolo[3,4-*d*]pyrimidines as Src kinase inhibitors in human osteosarcoma cells **(Manetti *et al*., 2007)**. In 2007, Tatton *et al.* showed that 1-tert-butyl-3-p-tolyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**60**) is a selective and potent inhibitor for Src family tyrosine kinases. It inhibited two cell lines RET/PTC oncoproteins in mouse embryonic fibroblasts (NIH3T3) leading to suppression of papillary thyroid carcinoma. Interestingly, its corresponding chloro-derivative **61** was a selective inhibitor for Src kinase family Lck and Fyn and played an essential role for suppression of human glioma **(Tatton *et al*., 2003)**. In 2013, Kumar *et al*. have synthesized different pyrazolo[3,4-*d*]pyrimidin-4-amines as **62**, **63** and **64** evaluated their activity against Src kinase enzymes. The compounds exhibited promising Src kinase inhibition **(Kumar *et al*., 2013) (Fig. 10).**

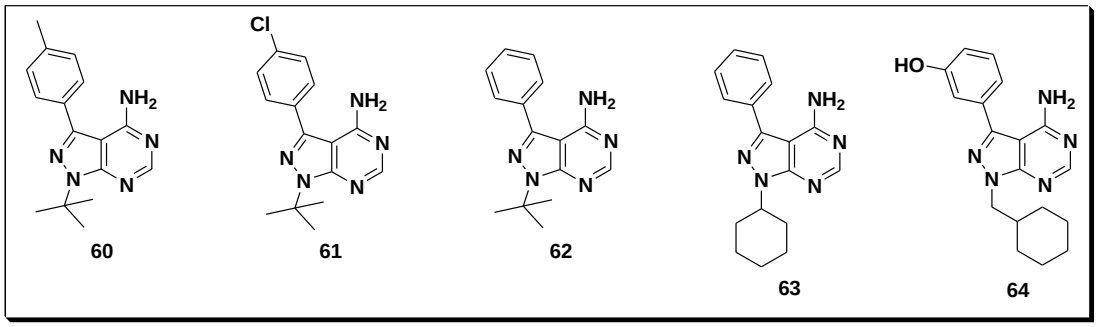


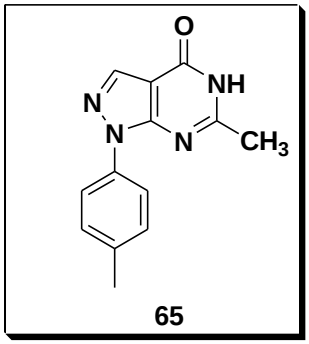
Fig. 10: Different examples for pyrazolo[3,4-*d*]pyrimidine-4-amine derivatives having Src tyrosine kinase inhibitory activity**.**

4.1.2. Inhibition of urokinase plasminogen activator (uPA)

Urokinase plasminogen activator (uPA) is a serine protease enzyme that functions in the conversion of the circulating plasminogen to the active, broad-spectrum serine protease, plasmin **(Chu *et al*., 2024; Kumar *et al*., 2022)**.

uPA is secreted as an inactive single-chain proenzyme by many different cell types and exists in a soluble or cell associated forms by binding to a specific membrane uPA receptor (uPAR) **(Lin *et al*., 2013)**. Elevated expression levels of urokinase is implicated in a large number of malignancies e.g. Lung cancer **(Chu *et al*., 2014)**, prostate **(Vyas and Singh, 2014)** and thyroid carcinoma **(Horvatic Herceg *et al*., 2013)**. Different mechanisms account for the cytotoxic effect of this class of compounds; they have been reported to act as vascular endothelial growth factor receptor inhibitors **(Manley *et al*., 2003)** and cyclin dependent kinase inhibitors **(Dwyer *et al*., 2007)**.

It has been reported that the 6-methyl-1-*p*-tolyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-4(5*H*)-one **65** was synthesized and evaluated in-vitro for its anticancer activity against MCF-7 breast cancer and Hep-G2 liver cancer cell lines **(Fig. 11)**. It showed 77% inhibition for MCF-7 breast cancer cell line and 63% inhibition for Hep-G2 liver cancer cell line relative to 91% ,88% produced by Doxorubicin, respectively, this antiproliferative activity was considered due to uPA inhibition **(Shamroukh *et al*., 2014)**.



65

Fig. 11: Pyrazolo[3,4-*d*]pyrimidine derivative as uPA inhibitor

4.1.3. ATP competitive inhibition of tyrosine kinases

Antonelli *et al*. have synthesized 1-phenethyl-*N*-(1-phenylethyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**66**) and its corresponding *N*-phenyl-derivative (**67**) and showed that they were potent candidates in suppression of papillary thyroid cancer (PTC) (**Fig. 12**). Compounds **66** and **67** exerted their effects through ATP competitive inhibition of the tyrosine kinases RET, VEGFR-1, VEGFR-2 and VEGFR-3 and antiangiogenic activity **(Antonelli *et al*., 2011)**. Also, it was reported that the naphthyl compound **68** is an ATP competitive inhibitor with high selectivity for protein kinase D (PKD) with little or no inhibitory activity for protein kinase C (PKC) and Ca^{2+}/calmodulin-dependent protein kinase class of enzymes (CAMK). It showed inhibition of prostate cancer cell proliferation as it induces G2/M arrest leading to cell death **(Kulkarni *et al*., 2014)**.

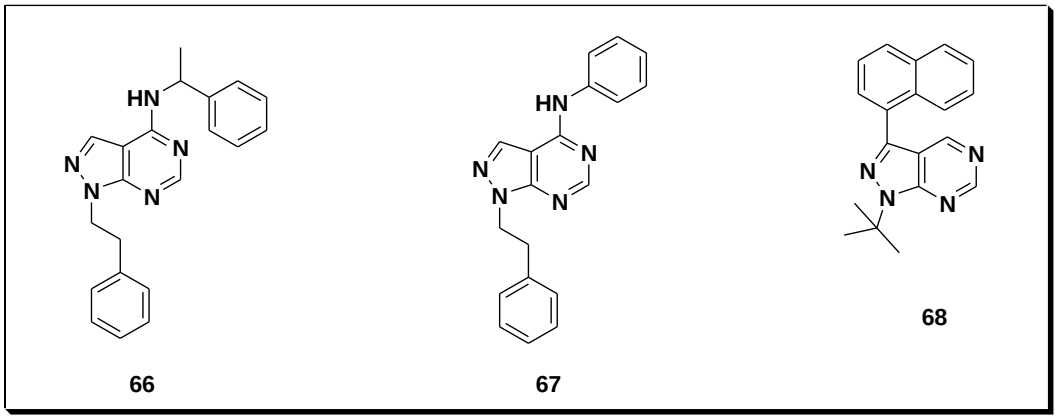


Fig. 12: Pyrazolo[3,4-*d*]pyrimidine derivatives as ATP competitive inhibitors of tyrosine kinases

4.1.4. Merkinase inhibition

It was reported by Zhang *et al*. that 4-(6-(butylamino)-3-(4-((4-methylpiperazin-1-yl)phenyl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-1-yl)cyclohexanol **69a** could be used selectively and effectively to supress bone marrow leukaemia (**Fig. 13)**. It acts as a dual inhibitor for both Mer and Flt3 tyrosine kinase enzymes. It has the advantages of good oral bioavailability, improved solubility, drug metabolism and pharmacokinetics **(Zhang *et al*., 2014)**. In 2016, Myers *et al*. showed that both **69 (b,c)** were potent and active AXL and Mer kinase family inhibitors leading to suppression of leukaemia **(Myers *et al*., 2016)**.

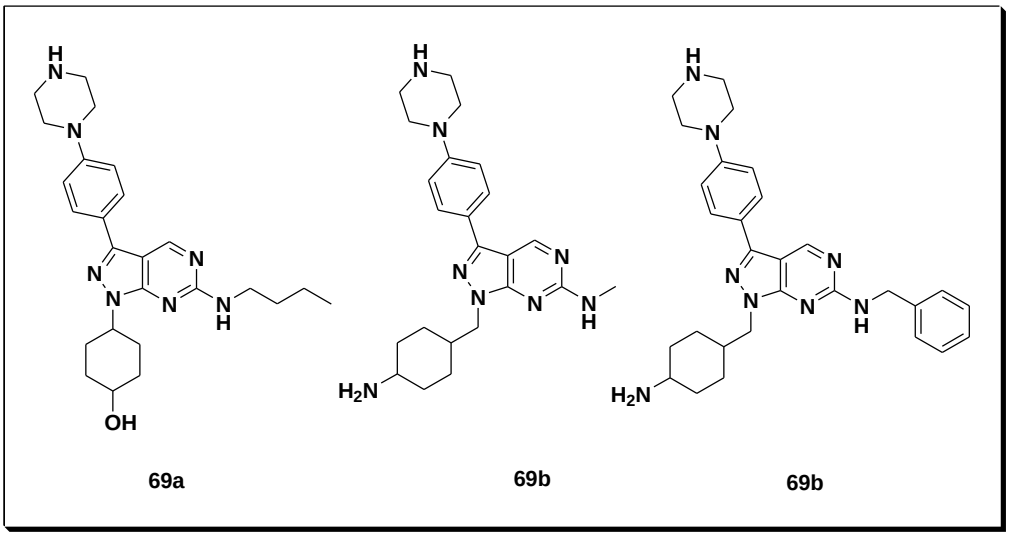


Fig. 13: Pyrazolo[3,4-*d*]pyrimidine derivatives as Mer kinase inhibitors

4.1.5. Glycogen Synthase Kinase (GSK-3) inhibition

Peat *et al*. reported the synthesis and biological evaluation of the pyrazolo[3,4-*d*]pyrimidin-4-yl-aryl hydrazine **70** as GSK-3 inhibitor **(Fig. 14)**. It was proved that compound **70** could bind in a competitive manner with ATP **(Peat *et al*., 2004)**.

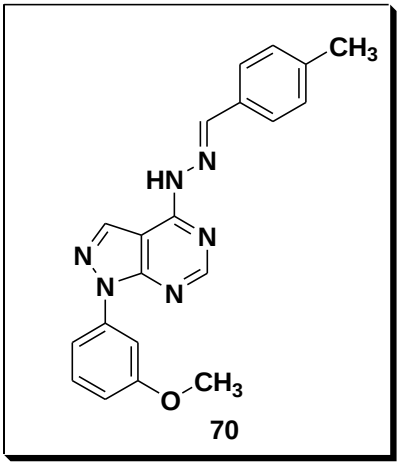


Fig. 14: Pyrazolo[3,4-*d*]pyrimidine derivative acting as glycogen synthase kinase (GSK-3) inhibitor

4.1.6. EGFR-TK inhibition

The derivative 4-(4-fluorophenyl)-6-oxo-2-((1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-yl)thio)-1,6-dihydropyrimidine-5-carbonitrile (**71**) was synthesized and screened for its antiproliferative activity against breast (MCF-7) and lung (A-549) cancer cell lines (**Fig. 15**). This compound was able to inhibit epidermal growth factor receptor-tyrosine kinase enzyme (EGFR-TK) **(Abbas *et al*., 2015)**.

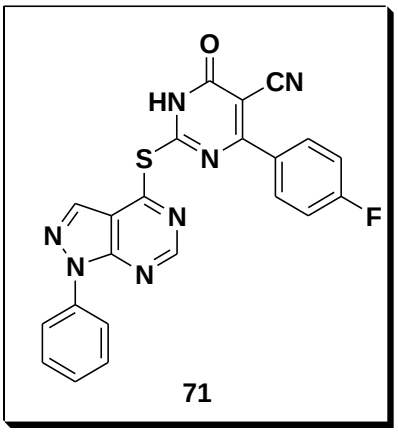


Fig. 15: Pyrazolo[3,4-*d*]pyrimidine compound acting as EFGR-TK inhibitor

4.1.7. Akt/p70S6K inhibition

In 2012, Rice *et al*. have evaluated the role of different pyrazolo[3,4-*d*]pyrimidine derivatives as dual Akt/p70S6K inhibitors **(Fig. 16)**. Compounds having bromine on C-3 position as compound **72** exhibited high inhibitory activity against this target. Furthermore, the inhibition of the serine/threonine protein kinases ribosomal protein S6 Kinase (p70S6K) and Akt (protein kinase B) was enhanced by the presence of larger lipophilic groups at position-5 which resulted in potent and metabolically stable candidates with good pharmacokinetic profiles **(Rice *et al*., 2012)**.

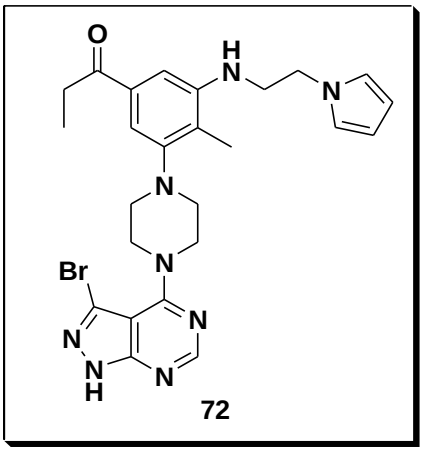


Fig. 16: Pyrazolo[3,4-*d*]pyrimidine derivativeacting asAkt/p70S6K inhibitor

4.1.8. Mammalian target of rapamycin (mTOR) inhibition

It has reported that compound **73** is a potent and selective mTOR inhibitor leading to inhibition of leukaemia **(Hayman *et al*., 2013)** (**Fig. 17).** Similarly, Yu *et al*. showed that compound **74** is an ATP competitive inhibitor producing inhibition of mTOR kinase family. The later compound can also inhibit the phosphorylation of p70SK1 and Akt and induces apoptosis **(Yu *et al*., 2009)**.

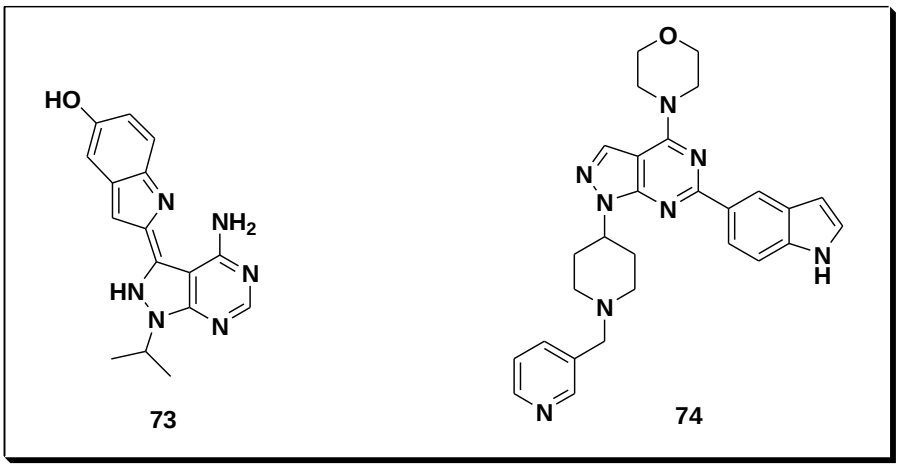
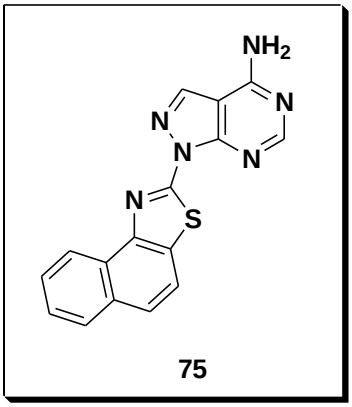


Fig. 17: Pyrazolo[3,4-*d*]pyrimidine derivatives as mTOR inhibitors

4.2. Androgen receptor antagonist:

In 2014, Bahashwan *et al*. showed that 1-(naphtho[1,2-*d*]thiazol-2-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**75**) is an androgen receptor antagonist which could suppress prostate cancer disease **(Bahashwan *et al*., 2014) (Fig. 18).**

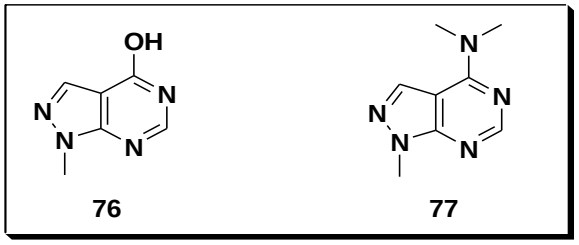


75

Fig. 18: Pyrazolo[3,4-*d*]pyrimidine acting as an androgen receptor antagonist

4.3. Purine antagonism

In 1956, Cheng and Robins have synthesized different pyrazolo[3,4-*d*]pyrimidines **76-77** which showed activity as purine antagonists (**Fig. 19)**. The *N*,*N*,1-trimethyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-amine (**77**) displayed potent antitumor activity against adenocarcinoma and leukaemia cell lines **(Cheng and Robins, 1956)**[109].



76 77

Fig. 19: Pyrazolo[3,4-*d*]pyrimidine derivatives acting as purines antagonists

5. Synthesis of pyrazolo[1,5-*a*]pyrimidine derivatives as anticancer agents

5.1. Starting with 5-amino-1*H*-pyrazole derivatives

Literature is enriched with bidentate reagents reaction with 5-amino-1*H*-pyrazoles; as the most common starting agents to afford pyrazolo[1,5-*a*]pyrimidine.

In 2016, Fadda *et al*. have synthesized pyrazolo[1,5-*a*]pyrimidine derivatives, by the reaction of 5-amino-1*H*-pyrazole derivatives **78** with either acetylacetone or acetoacetanilide in boiling acetic acid to afford compounds **79** and **80**; respectively

(Fadda *et al.*, 2017). Similarly, Soliman *et al.*, have synthesized pyrazolo[1,5-*a*]pyrimidine derivatives **79-81** by refluxing the 5-amino-1*H*-pyrazole derivative **78** with different dicarbonyl reagents, namely; acetyl acetone, ethyl acetoacetate, diethymalonate or ethyl 3-oxo-3-phenylpropanoate (**Fig. 20) (Soliman *et al.*, 2014)**.

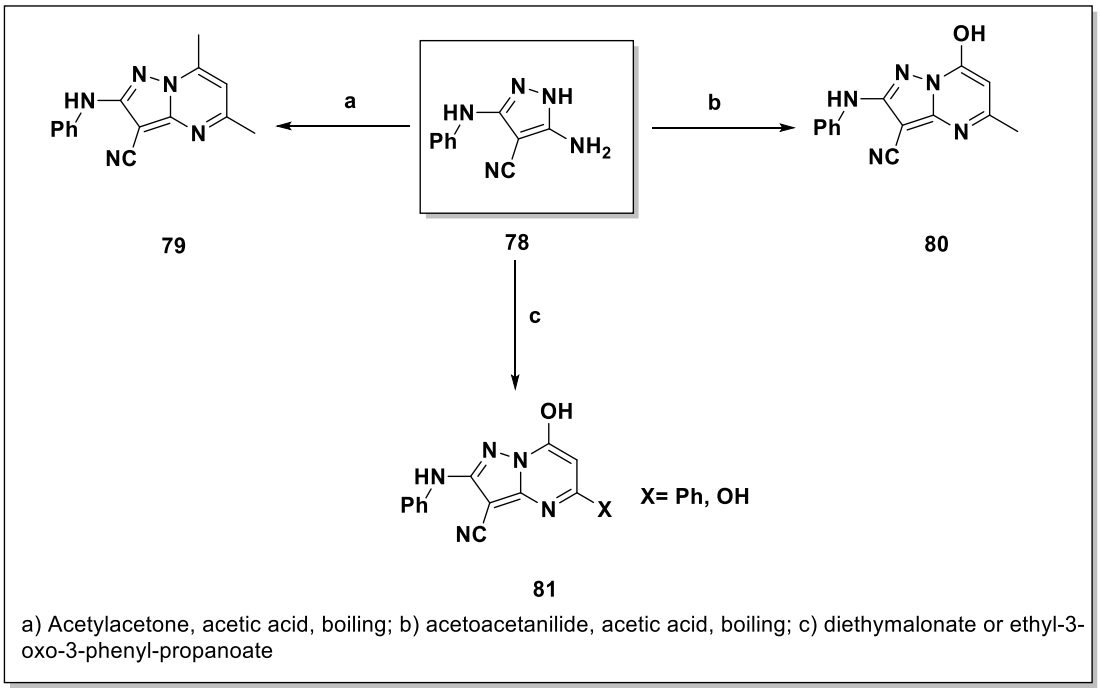


Fig. 20: Synthesis of pyrazolo[1,5-*a*]pyrimidine derivatives **79-81** starting with 5-amino-1*H*-pyrazoles.

Literature survey also showed that cyclization reaction of 5-amino-1*H*-pyrazole derivatives **78** with diethyl ethoxymethylene malonate in the presence of acetic acid afforded the pyrazolo[1,5-*a*]pyrimidine derivatives **82** in good yield as reported by Tabrizi *et al.* in 2013 (**Fig. 21**) **(Aghazadeh Tabrizi *et al.*, 2013)**. In 2003, Selleri *et al.*, obtained the pyrazolo[1,5-*a*]pyrimidine derivatives **83** by the one pot reaction of 5-amino-1*H*-pyrazole derivatives **78** with ethyl-3-hydroxy-2-(thiophen-3-yl)acrylate **(Selleri *et al.*, 2003)**. Moreover, the microwave aided condensation reaction of 5-amino-1*H*-pyrazole derivatives 78 and2-(4-methoxyphenyl) malonaldehyde afforded the pyrazolo[1,5-*a*]pyrimidine derivatives **84 (Engers *et al.*, 2013)**. In 2005, Selleri *et al.*, have synthesized pyrazolo[1,5-*a*]pyrimidine derivatives **85** by condensation reaction of the starting compounds **78** with 4,4-dimethoxy-2-butanone in ethanol **(Selleri *et al.*, 2005)**.

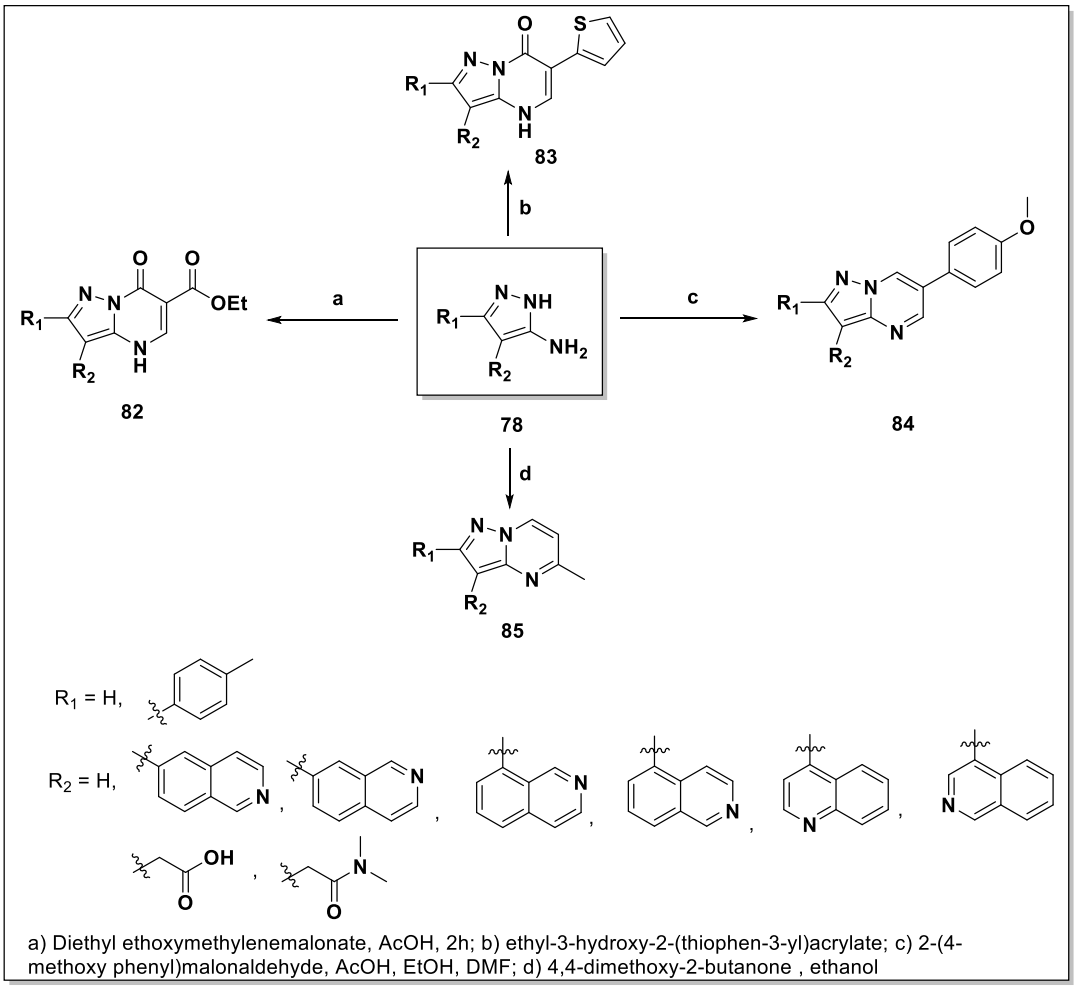


Fig.21: Synthesis of various pyrazolo[1,5-*a*]pyrimidine derivatives **82-85** starting with 5-amino-1*H*-pyrazoles

In 2009, Wang *et. al.* have synthesized the pyrazolo[1,5-*a*]pyrimidine derivatives **86** (**Fig. 22)** by refluxing 5-amino-1*H*-pyrazole derivatives **78** with methyl (*E*)-3(3(dimethylamino)-acryloyl)benzoate in the presence of acetic acid **(Wang *et al*., 2009)**. Also, Fraley *et al*. have synthesized pyrazolo[1,5-*a*]pyrimidine derivatives **87** by refluxing 5-amino-1*H*-pyrazole derivatives **78** with 3-hydroxy-2-phenylacrylaldehyde in ethanol and a catalytic amount of acetic acid **(Fraley *et al*., 2002)**.

Interestingly, Aggarwal *et al*. have synthesized the bi(pyrazolo[1,5-*a*]pyrimidinyl)-7-ones **88** by refluxing 5-amino-1*H*-pyrazole derivatives **78** with 3-acetyl-4-hydroxy-6-methyl-2*H*-pyran-2-one in ethanol in the presence of the catalytic amount of acetic acid **(Aggarwal *et al*., 2014)**. While the pyrazolo[1,5-*a*]pyrimidine derivatives **89** have been synthesized by refluxing 5-amino-1*H*-pyrazole derivatives **78** with 3-(dimethylamino)-2-(4-nitrophenyl)acrylonitrile in the presence of catalytic amount of hydrochloric acid **(Gommermann *et al*., 2010)**.

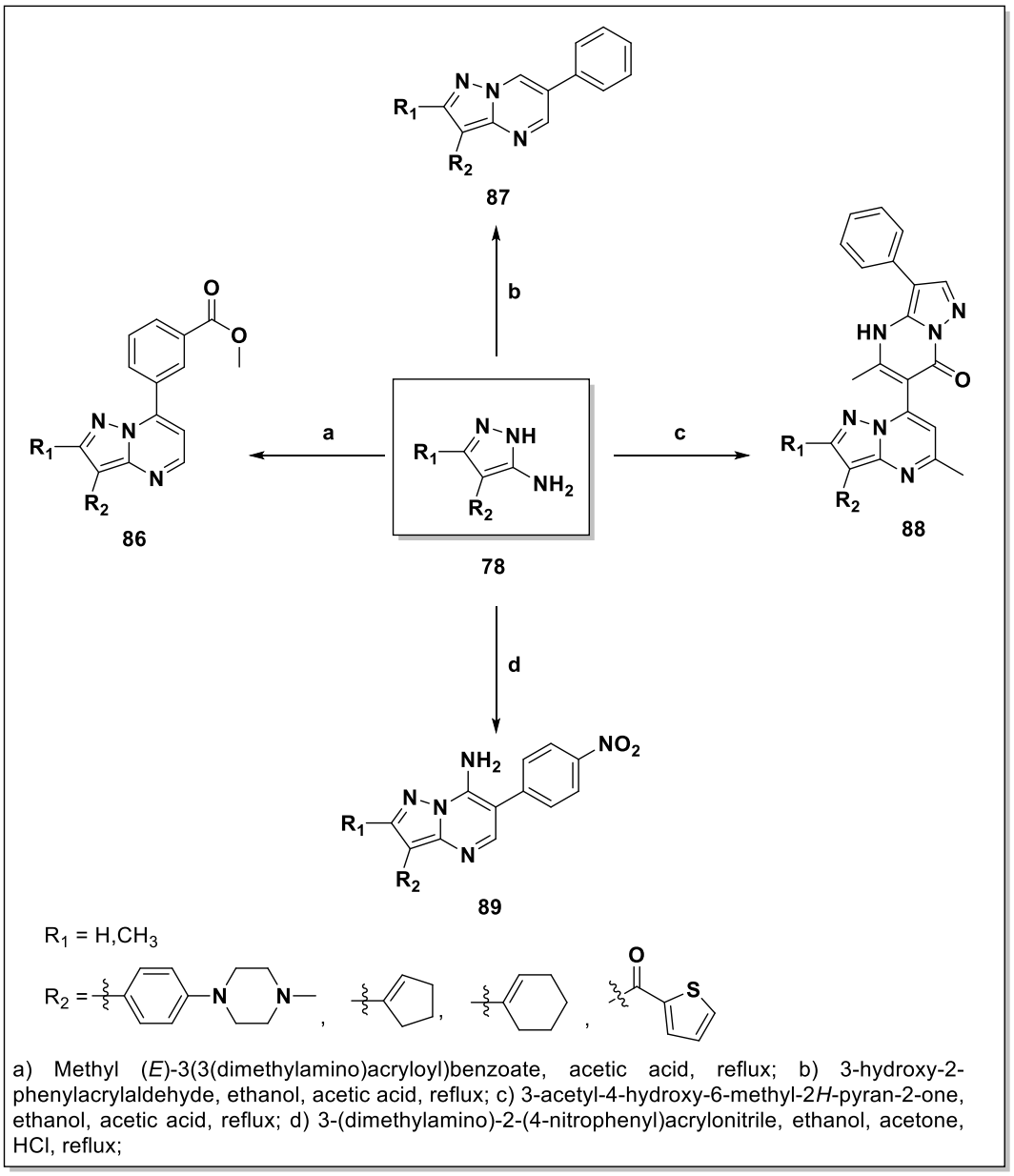


Fig.22: Synthesis of pyrazolo[1,5-*a*]pyrimidine derivatives **86-89** from 5-amino-1*H*-pyrazoles

Kosugi *et al*. have synthesized the pyrazolo[1,5-*a*]pyrimidine derivatives **90** (**Fig. 23**) by condensation reaction of 5-amino-1*H*-pyrazole derivatives **78** with two substituted malonic acid diester in ethanolic sodium ethoxide under reflux conditions **(Kosugi *et al*., 2012)**. Xu et al. have synthesized pyrazolo[1,5-*a*]pyrimidine derivatives **91** by refluxing *N*-methyluracilwith5-amino-1*H*-pyrazole derivatives **78** in the presence of sodium ethoxide for 3 hours **(Xu *et al*., 2015)**. On other hand Dwyer *et al*. have synthesized pyrazolo[1,5-*a*]pyrimidine derivatives **92** by condensation reaction of 5-amino-1*H*-pyrazole derivatives **78** with 1,3-dimethyluracil in the presence of sodium methoxide followed by reaction with phosphorous oxychloridethen *N*-iodo-succinimide (NIS) **(Dwyer *et al*., 2013)**. El-Enany *et al.* have made reaction between 5-amino-1*H*-pyrazole derivatives **78** and malanonitrile, arylidene malononitrile or ethylcyano acetate

in ethanol and catalytic amount of triethylamine to afford pyrazolo[1,5-*a*]pyrimidine derivatives **93 (El-Enany *et al*., 2011)**. Paruch *et al*. have obtained pyrazolo[1,5-*a*]pyrimidine derivatives **94** through the reaction of methyl-3-oxobutanoate in toluene followed by reaction with phosphorus oxychloride in *N*,*N* dimethylaniline **(Paruch *et al*., 2007)**. Pyrazolo[1,5-*a*]pyrimidine derivatives **95** have been synthesized by the reaction of 5-amino-1*H*-pyrazole derivatives **78** andethyl-4-chloroacetoacetate in acetone and few drops of acetic acid under reflux **(Li *et al*., 2006)**.

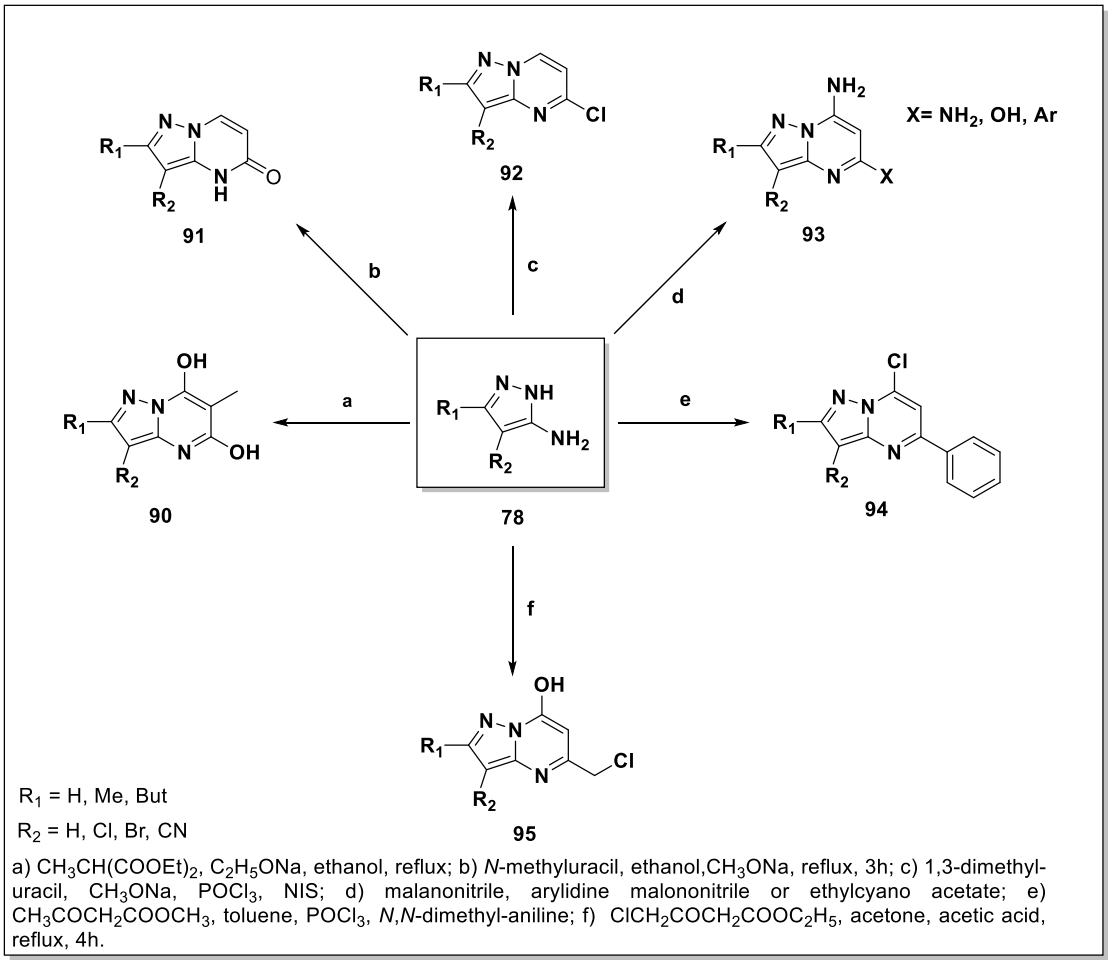


Fig.23: Synthesis of pyrazolo[1,5-*a*]pyrimidine derivatives **90-95** from 5-amino-1*H*-pyrazoles**.**

Elsaman *et al*. have synthesized pyrazolo[1,5-*a*]pyrimidine derivatives **97**, **99-100** (**Fig. 24**). A mixture of the aminopyrazole derivative **96** and ethyl cyanoacetate in ethanol was refluxed for 15 min to afford pyrazolo[1,5-*a*]pyrimidine derivative **97**. Also, upon reaction of the same starting material **96** with ethyl acetoacetate or acetylacetone in glacial acetic acid to afford the intermediate products **98** which without separation were cyclized under reflux to afford the target pyrido[2,3:3,4]pyrazolo[1,5-*a*]pyrimidines **99** and **100**, respectively **(Elsaman *et al*., 2013)**.

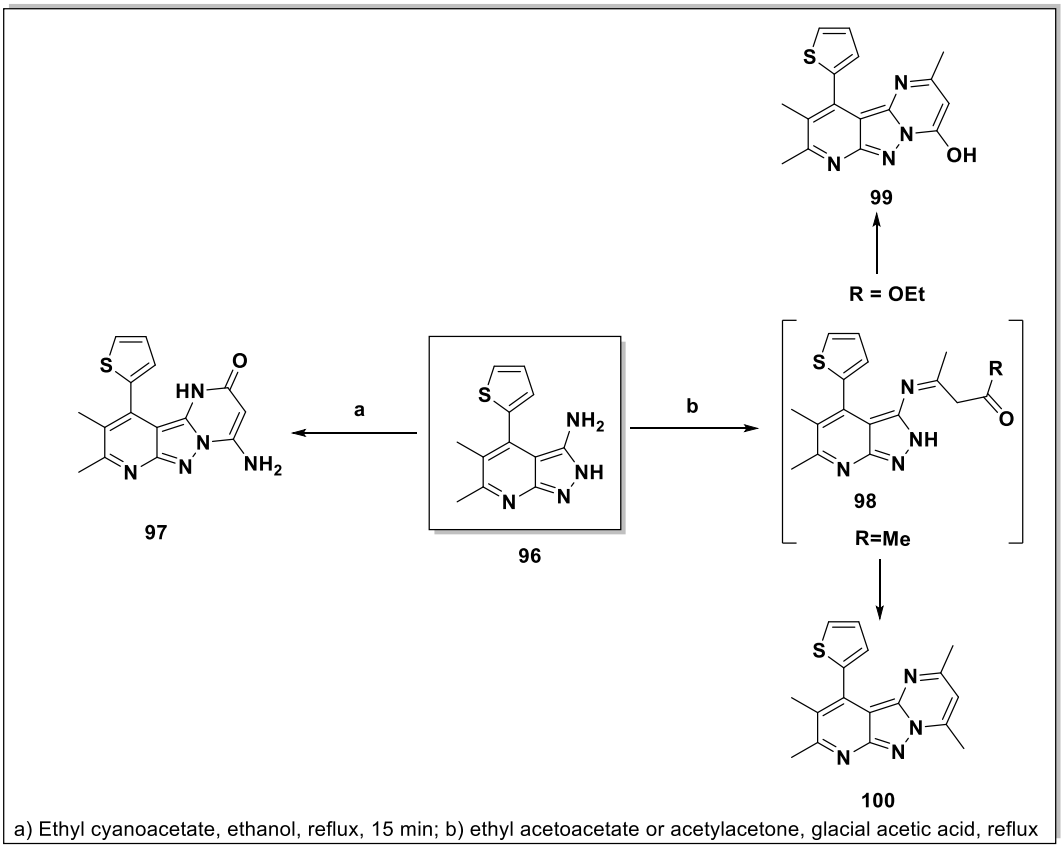


Fig. 24: Synthesis of pyrido[2,3:3,4]pyrazolo[1,5-*a*]pyrimidine derivatives.

In 2012, Chobe *et al*. have synthesized the tricyclic structure containing pyrazolo[1,5-*a*]pyrimidine unit **103** by reacting 4-(2-(4-chlorophenyl)diazenyl)-1*H*-pyrazole-3,5-diamine **102** with 4-substituted benzylidene-3-methyl-1*H*-pyrazol-5(4*H*)-one **101** and sodium hydroxide in polyethylene glycol (PEG-400) as green reaction solvent (**Fig. 25**) **(Chobe *et al*., 2012)**.

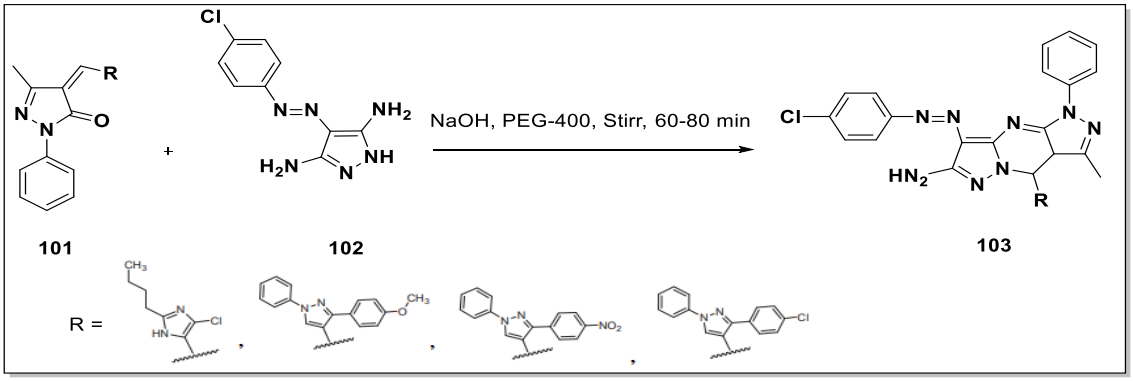


Fig. 25: Synthesis of dipyrazolo[1,5-a:3',4'-*d*]pyrimidine derivative using polyethylene glycol (PEG-400) as a green reaction solvent

In 2014, Shekarrao *et al*. reported that steroidal β-bromovinyl aldehydes can be reacted with pyrazoloamines to afford steroidal D-ring fused pyrazolo[1,5-*a*]pyrimidines (**106-109**) *via* a microwave mediated reaction using palladium (II) catalyst **(Shekarrao *et al*., 2014)** (**Fig. 26**).

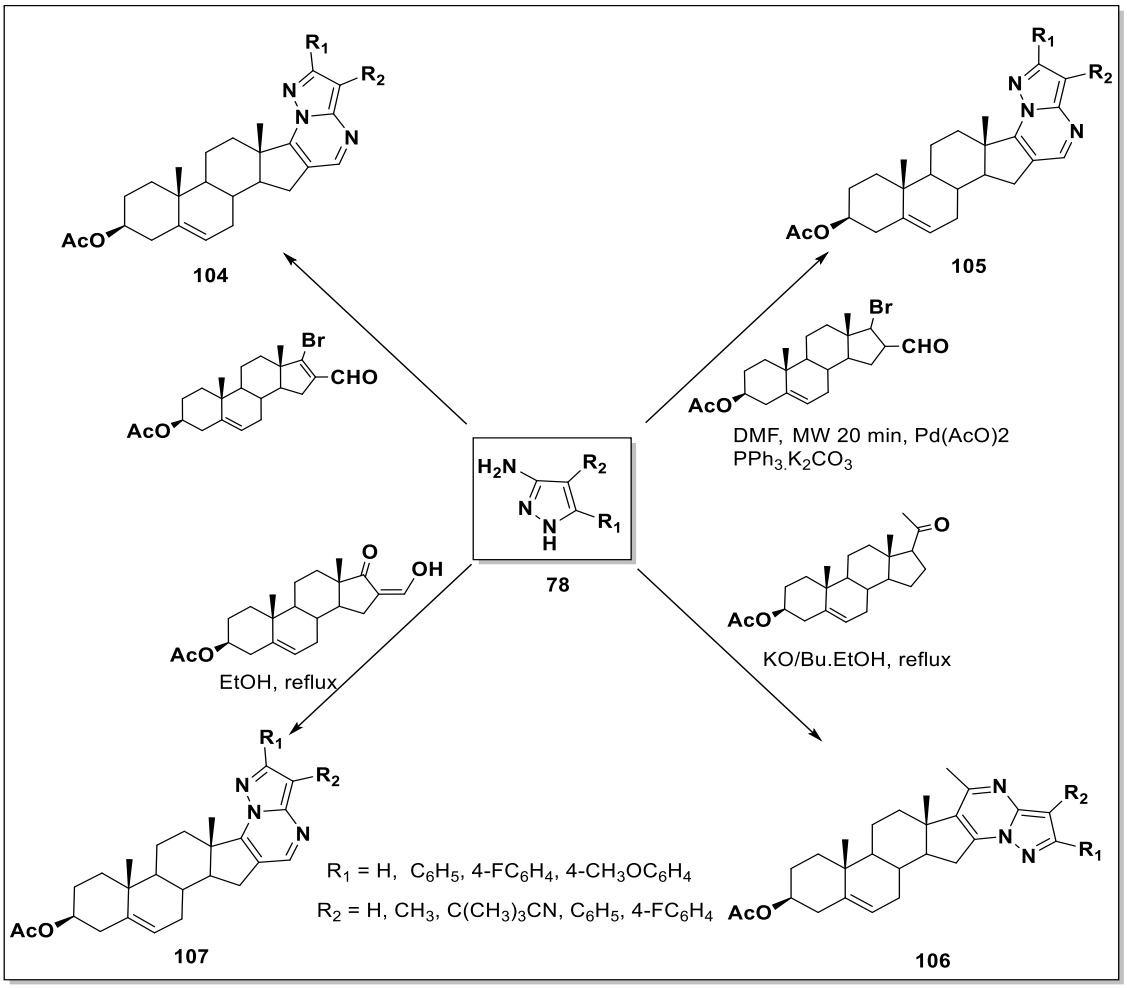


Fig 26: Synthesis of steroidal pyrazolo[1,5-*a*]pyrimidine derivatives under microwave mediated reaction

5.2. Anticancer activity of pyrazolo[1,5-*a*]pyrimidine

Pyrazolo[1,5-*a*]pyrimidine derivatives are well known for their anticancer activity **(Fig. 27)**. For example, Abdelall *et al.* showed that compound 108was able to inhibit human breast adenocarcinoma (MCF-7) and BT474 cell lines as well as the non-small lung cancer cell line (A549) with IC_{50} = 1.98, 2.20 and 2.61 μM, respectively **(Abdelall *et al*., 2016)**.

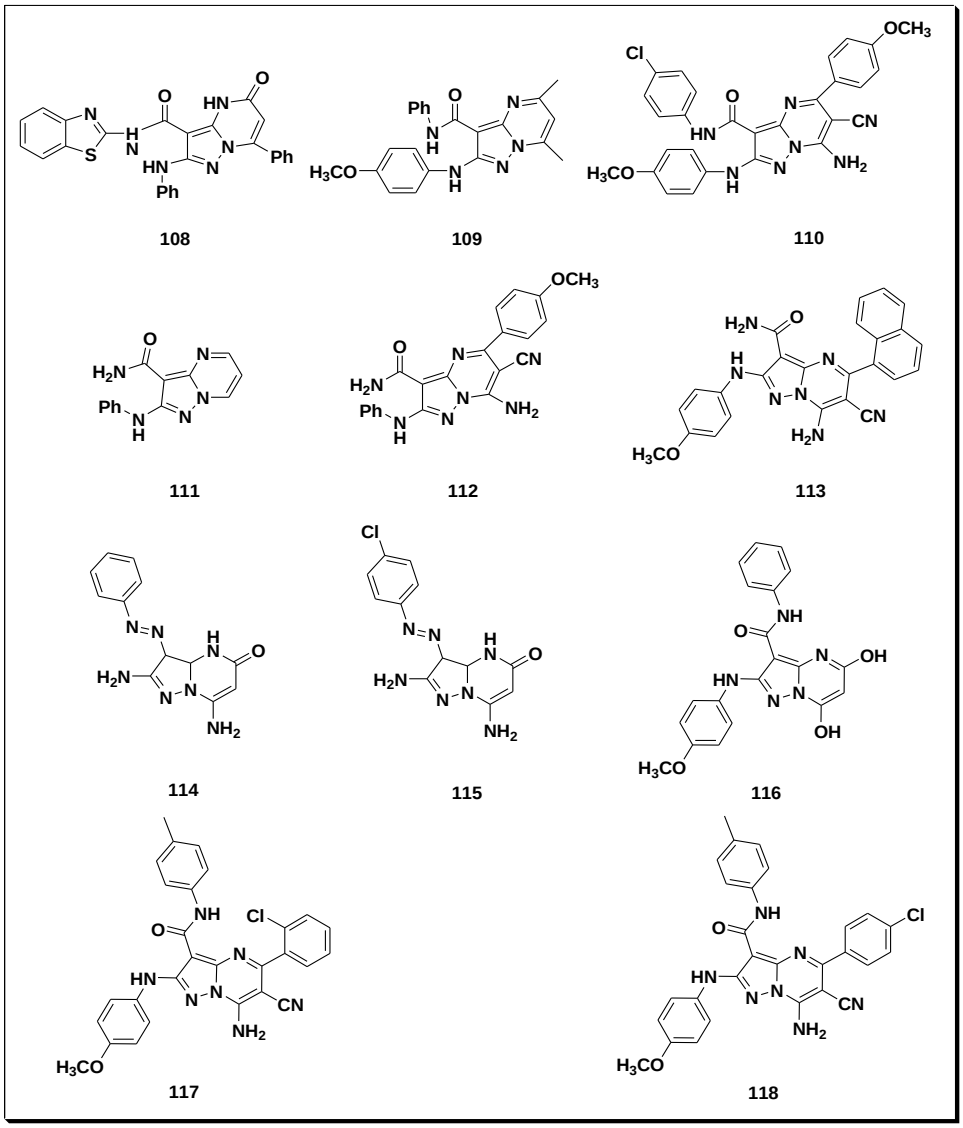


Fig. 27: Examples for pyrazolo[1,5-*a*]pyrimidine derivatives with anticancer activity**.**

Furthermore, Hassan *et al.* showed that compounds **109-110** were highly potent against Ehrlich Ascites Carcinoma (EAC) cells with IC_{50} values 10, 25 µg/ml respectively **(Hassan *et al*., 2015)**. Moreover, Hafez *et al.* reported that compounds **111-113** inhibited the growth activity of breast cancer (MCF-7), ovarian carcinoma (SKOV-3), leukemia (K562) and cervical HeLa human cancer cell lines. The pyrazolo[1,5-*a*]pyrimidine derivative **113** displayed higher antiproliferative activity against MCF-7 cell line than doxorubicin (IC_{50} =0.085 µMvs 96.41 µM; respectively) **(Hafez *et al*., 2013)**. In 2016, Metwally *et al.* have synthesized pyrazolo[1,5-*a*]pyrimidine derivatives which showed promising antitumor activity against human Hep-G2 and MCF-6 cell lines. Their results showed that compound **114** was very potent against Hep-G2 (IC_{50} 3.53 µM), while, compound **115** was the most active candidate against MCF-7 cell line (IC_{50} 4.5 µM) **(Metwally *et al*., 2016)**.

Also, Hassan *et al*. have synthesized some pyrazolo[1,5-*a*]pyrimidine derivatives with different methods and screened them for their anti-cancer activity. The

results showed that compounds **116-118** showed promising antitumor activity against HCT-135 11 6 carcinoma cells (IC_{50} values; 58.44, 59.18 and 62.11 µg/ml compared to that of doxorubicin 73.50 µg/ml) **(Hassan *et al*., 2017)**[79].

6. Reported anticancer mode of actions for pyrazolo[1,5-*a*]pyrimidine

6.1. Kinase inhibition

6.1.1. Kinase inserts domain-containing receptor tyrosine kinase (KDR) inhibitor

In 2002, Fraley *et al*. have synthesized and evaluated the 3,6-disubstitutedpyrazolo[1,5-*a*]pyrimidine derivatives **119-120** (**Fig. 28)** as a class of KDR kinase inhibitors. The IC_{50} for compound **119** as KDR inhibitor was 224 nM. Upon substitution of the parent pyrazolo[1,5-*a*]pyrimidine core at the 3 and 6 positions with 3-thienyl and 4-methoxy phenyl moieties, compound **120**, greatly increased the potency (IC_{50} = 19 nM) **(Bußmann *et al*., 2021)**. Later on, Frey *et al.* succeeded to synthesize compound **121** which was proofed to be a potent inhibitor of KDR (IC_{50}= 0.7nM) **(Frey *et al*., 2008)**.

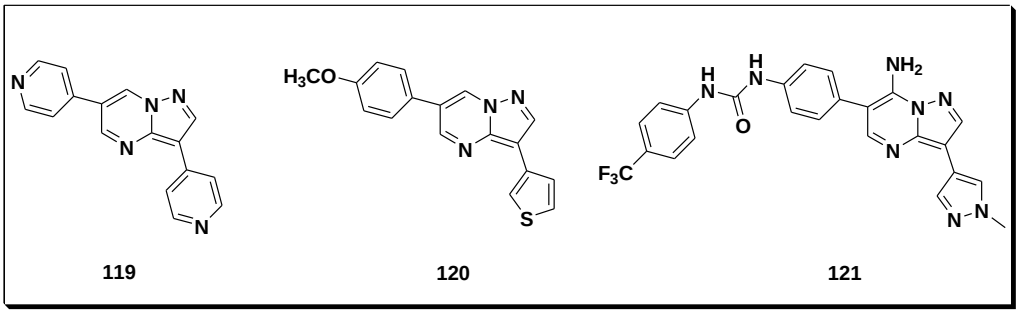


Fig. 28: Reported pyrazolo[1,5-*a*]pyrimidine derivatives acting as KDR inhibitors**.**

6.1.2. Mitogen-Activated Protein Kinase-Activated Protein-Kinase 2 (MAPKAP-K2) inhibitors

In 2014, Kosugi *et al*. have evaluated the anti-cancer activity of the pyrazolo[1,5-*a*]pyrimidine derivatives **122-123** (**Fig. 29)**. These compounds inhibited both MAPKAP-K2 (IC_{50} = 0.13 µM and 0.054 µM; respectively) and CDK2 (IC_{50} = 23 µM and 25 µM; respectively) **(Kosugi *et al*., 2012)**.

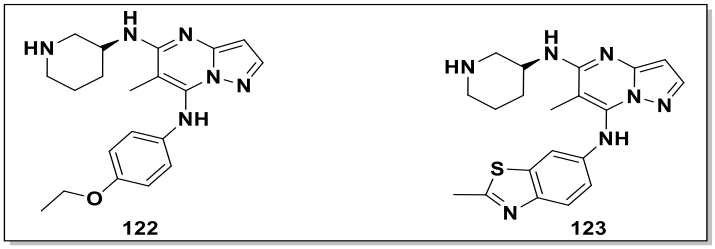


Fig. 29: Reported pyrazolo[1,5-*a*]pyrimidine derivatives acting as MAPKAP-K2 inhibitors**.**

6.1.3. Cyclin-dependent kinase (CDK) inhibitor

During the last two decades, there are many promising drugs available for the treatment of cancer diseases. For example, Dinaciclib **124** was synthesized by Paruch *et al*. (**Fig. 30)** as a potent and selective cyclin-dependent kinase (CDK) inhibitor that currently undergoing clinical evaluation **(Paruch *et al*., 2007)**.

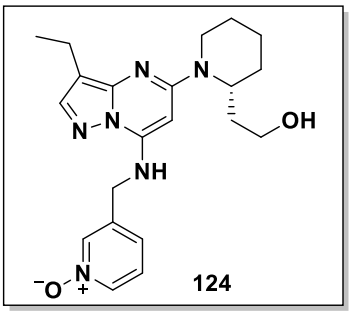


Fig. 30: The chemical structure of Dinaciclib

Furthermore, in 2015, Phillipson *et al.* have synthesized some pyrazolo[1,5-*a*]pyrimidine derivatives and evaluated them as CDK9 inhibitors (**Fig. 31)** which is a target of emerging interest for the development of anti-cancer drugs. The results showed that the ethylamino compounds **125** and **126** represented significant activities. Both compounds displayed promising CDK9 affinity (IC_{50} about 200 nM). Moreover, compound 126 possessed excellent selectivity to CDK9 over PI3Ka and CDK7. There are multiple lines of evidence linking CDK9 activity to cancer, the results showed that compound 126 is the most potent one as it showed IC_{50} = 203 nM for CDK9 **(Phillipson *et al*., 2015)**.

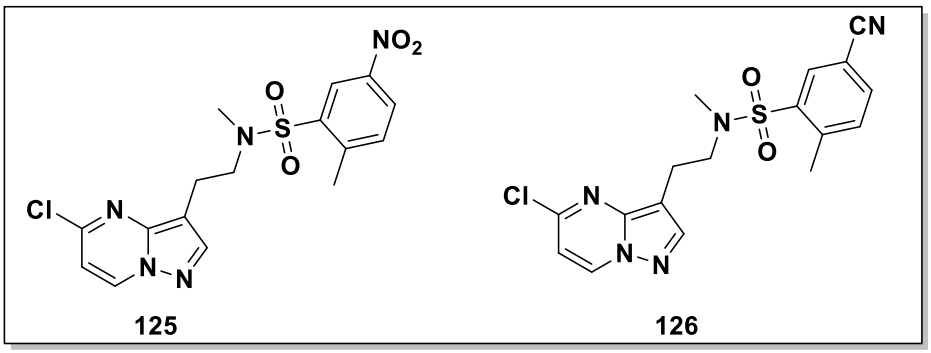


Fig. 31: Reported pyrazolo[1,5-*a*]pyrimidine derivatives acting as CDK9 inhibitors**.**

In 2007, Paruch *et al.* have synthesized the pyrazolo[1,5-*a*]pyrimidine derivative **127** which was potent and selective CDK2 inhibitor (**Fig. 32**) (IC_{50} = 0.013 μM) **(Paruch *et al*., 2007)**. Moreover, it was also reported that the anticancer activity of compound **128** was due to the inhibition of CDK 2 (IC_{50} = 3 nmol/L) **(Heathcote *et al*., 2010)**.

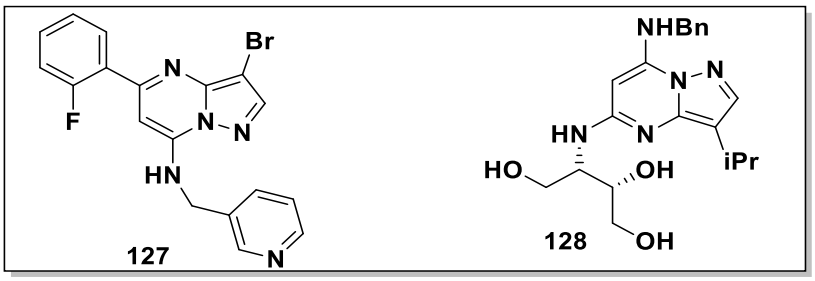


Fig. 32: Reported pyrazolo[1,5-*a*]pyrimidine derivatives as CDK 2 inhibitors**.**

6.1.4. Lymphocyte-specific kinase (Lck) inhibitors

Gommermann *et al.* have synthesized different pyrazolopyrimidine derivatives **129** from a series of pyrazolo[1,5-*a*]pyrimidines and were optimized to target lymphocyte-specific kinase (Lck) (**Fig. 33)**. These derivatives exhibited significant potency against LCK cell line with IC_{50} =7 nM. Compound **129a** showed very potent inhibition of Lck and displayed very good selectivity against cSrc, Hck and KDR, while its acylation with glycine led to the highly active compound **129b** that demonstrated a low clearance and long half-life **(Gommermann *et al*., 2010)**.

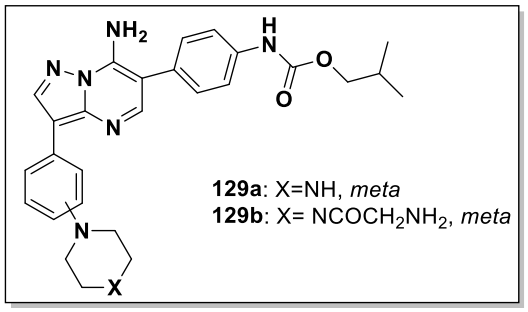


Fig. 33: Reported pyrazolo[1,5-*a*]pyrimidine derivatives as Lck inhibitor.

6.1.5. B-Raf kinase inhibitors

In 2009, Gopalsamy *et al*. have synthesized the lead compound **130** which produced enhanced inhibition of a number of B-Raf kinases with good selectivity with IC_{50}= 0.032 **(Gopalsamy *et al*., 2009)** (**Fig. 34**).

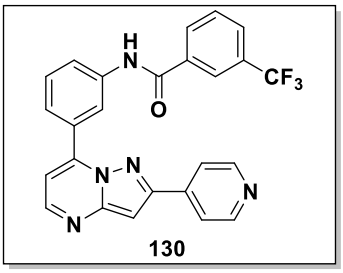


Fig. 34: Reported pyrazolo[1,5-*a*]pyrimidine derivative as B-Raf kinase inhibitor**.**

6.2. Selective bone morphogenetic protein receptor (BMP) inhibitor

In 2013, Engers *et al*. have synthesized the pyrazolo[1,5-*a*]pyrimidine derivative **131** which showed most potent derivative with IC_{50} less than 1nMfor Bone Morphogenetic Protein 4 human cell line (BMP-4) (**Fig. 35) (Engers *et al*., 2013)**.

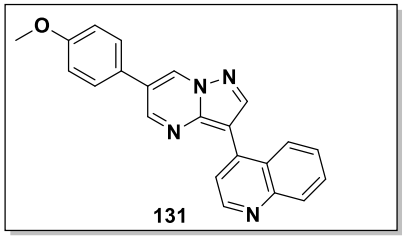


Fig. 35: Reported pyrazolo[1,5-*a*]pyrimidine derivative as BMP inhibitor**.**

6.3. Allosteric agonists for the high affinity nicotinic acid receptor GPR109A

Shen *et al*. have synthesized pyrazolopyrimidine derivatives and they were discovered as the first class of allosteric agonists for the high affinity nicotinic acid receptor GPR109A. In addition to its intrinsic activity, compound **132** significantly enhances nicotinic acid binding to the receptor, thereby potentiating the functional efficacy of nicotinic acid (**Fig. 36) (Shen *et al*., 2008)**.

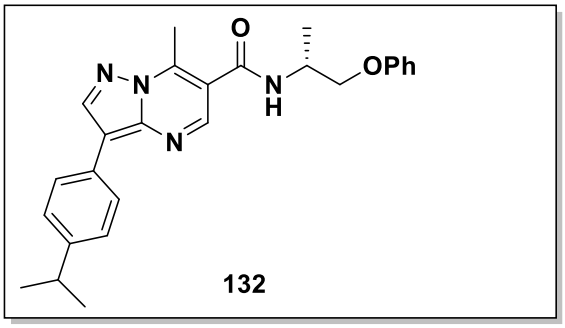


Fig. 36: Reported pyrazolo [1,5-*a*]pyrimidine derivative as an allosteric agonist for the high affinity nicotinic acid receptor GPR109A

6.4. HT6 receptor blocker

In 2012, Ivachtchenko *et al*. have synthesized a series of 3-(phenylsulfonyl)pyrazolo[1,5-*a*]pyrido[4,3-*d*]pyrimidine derivatives which were highly potent and selective blockers of 5-HT6 receptors. Compound **133** was considered most agent producing 5-HT2B blocking activity (IC_{50} = 6.16 nM as compared with IC_{50} = 1.8 nM for 5-HT6 receptors) and very low hERG potassium channel blocking potency (IC_{50} = 54.2 nM) **(Ivachtchenko *et al*., 2012)** (**Fig. 37**).

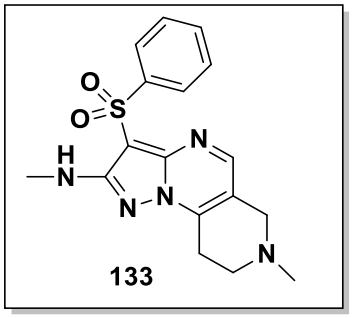


Fig. 37: Reported pyrazolo[1,5-*a*]pyrimidine derivative as HT6 receptor blocker

6.5. 18 kDatranslocator protein (TSPO)

In 2017, Werry *et al*. have synthesized pyrazolopyrimidine derivatives and screened them for TSPO as it is a target for human glioblastoma. The results showed that compound **134** was the most potent derivative with IC_{50} = 4.3 nM for TSPO cell line **(Werry *et al*., 2017)** (**Fig. 38**).

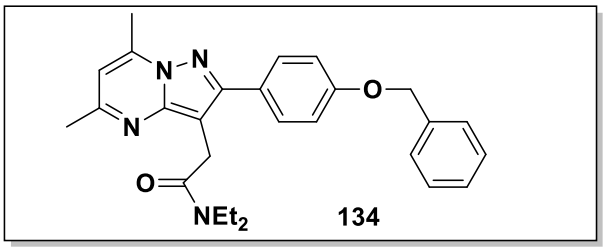


Fig. 38: Reported pyrazolo[1,5-*a*]pyrimidine derivative targeting the TSPO protein

6.6. Histone lysine demethylase 5 (KDM5) inhibitors

In 2016, Horton *et al*. have synthesized pyrazolopyrimidine derivatives which have histone lysine demethylase inhibitory effect. The results showed that compound **135** exhibited the highest potency (IC_{50} values of ~1 μM for KDM5A and KDM5B) **(Horton *et al*., 2016)** (**Fig. 39**).

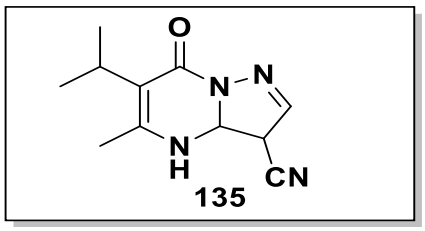


Fig. 39: Reported pyrazolo[1,5-*a*]pyrimidine derivative as KDM5D inhibitor

7. Interaction between the pyrazolopyrimidine nucleus and the corresponding anticancer target proteins

Literature is rich with several studies which illustrate the importance of the heterocyclic pyrazolopyrimidine ring systems in interaction with the corresponding anti-cancer target proteins.

7.1. Binding modes of pyrazolo[1,5-*a*]pyrimidines with different anticancer target enzymes

We have found many molecular docking studies and x-ray co-crystal structures of different pyrazolo[1,5-*a*]pyrimidine derivatives with their corresponding anticancer target enzymes **(Dass, 2022)**.

Below are some examples which demonstrates the importance of the pyrazolo[1,5-*a*]pyrimidine ring system in the development of anticancer agents.

In 2019, Wood *et al.* reported that Dinaciclib **136** interacts with the ATP binding pocket of CDK1-Cks2 through a series of hydrogen bonds and hydrophobic interactions. The N1 of the pyrazole ring acts as a hydrogen bond acceptor and involved in a hydrogen bond interaction with the leu 83 **(Wood *et al.*, 2019) (Fig. 40).**

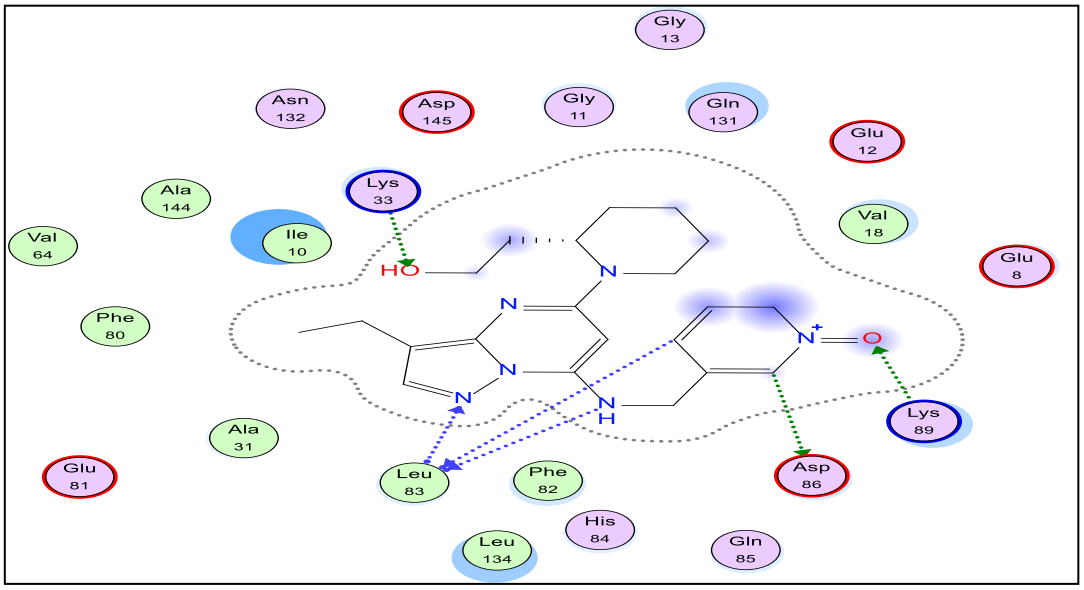


Fig. 40: 2D interaction between Dinaciclib **136** and the target protein CDK1-Cks2 retrieved from reference **(Engers *et al.*, 2013)**.

In 2017, McCoull *et al*. identified a pyrazolo[1,5-*a*]pyrimidine series as selective B-cell lymphoma 6 (BCL6) inhibitors from which compound **137** showed the best binding affinity. The N1 of the pyrazole ring acts as a hydrogen bond acceptor and forms a hydrogen bond with the amino acid val116 **(McCoull *et al.*, 2017) (Fig. 41).**

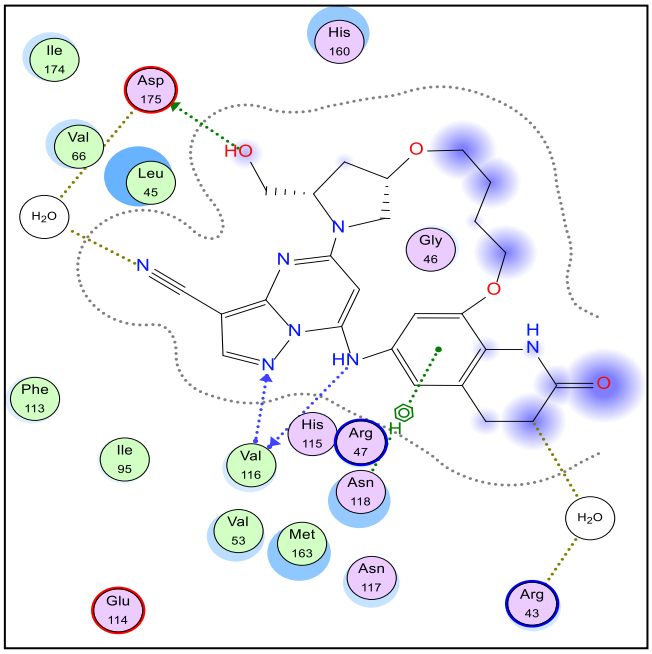


Fig. 41: 2D interaction between compound **137** and the target protein BCL6 (PDB 5N1Z) retrieved from reference **(Selleri *et al.*, 2005)**.

In 2016, Sack *et al.* reported the molecular modeling study of pyrazolopyrimidine derivative **138** with ATP-binding site of MARK4. They showed that the N1 of the pyrazole ring is able to form a hydrogen bond with the amino acid Ala135 in the hinge region of the target enzyme **(Sack *et al.*, 2016) (Fig. 42).**

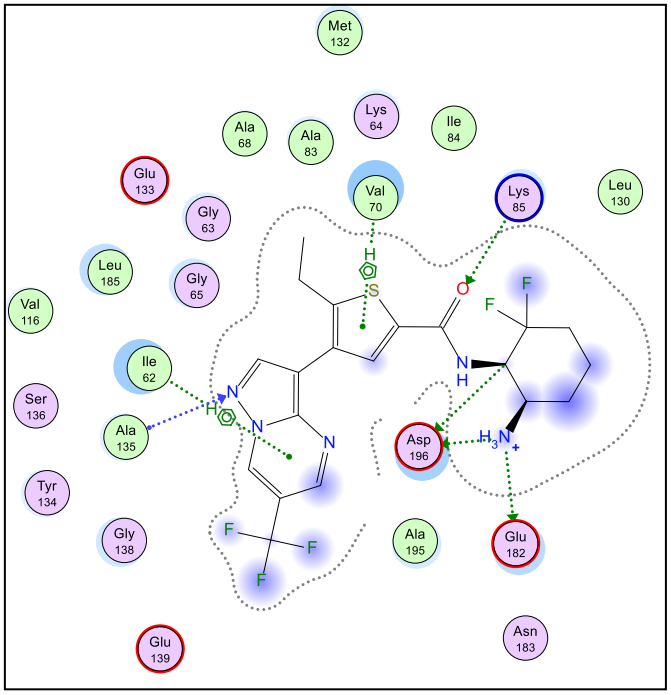


Fig. 42: 2D interaction between compound **138** and the ATP-binding site of MARK4 retrieved from reference **(Wang *et al.*, 2009)**.

Dwyer *et al.* reported the binding mode of pyrazolopyrimidine **139** with the proto-oncogene serine/threonine-protein kinase (Pim1). The N1 of the pyrazolo[1,5-*a*]pyrimidine acts as a hydrogen bond acceptor for Lys67A with the C3 napthyl group oriented parallel to the hinge region of the protein. In addition, the primary amine on the cyclohexyl ring makes a hydrogen bond with the oxygen of Glu171A **(Dwyer *et al*., 2013) (Fig. 43).**

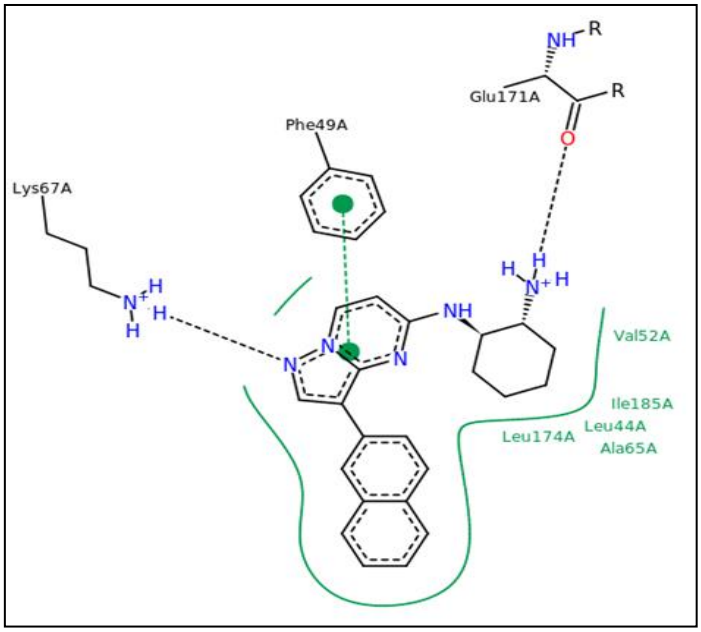


Fig. 43: 2D interaction between compound **139** and Pim1 (PDB: 4MBL)

7.2. Binding modes of pyrazolo[3,4-*d*]pyrimidines with different anticancer target enzymes

Literature survey is rich with the molecular docking studies as well as x-ray co-crystal structures of different pyrazolo[3,4-d]pyrimidine derivatives with their corresponding target proteins. Below are some examples which demonstrates the importance of the pyrazolo[3,4-d]pyrimidine ring system in the development of anticancer agents.

In 2017, Wang *et al*. discovered pyrazolo[3,4-*d*]pyrimidine **140** as epidermal growth factor receptor (EGFR) inhibitor which displayed strong anti-proliferative effect against EGFR mutant-driven non-small cell lung cancer (NSCLC) cell lines such as H1975, PC9, HCC827, and H3255. Its molecular docking study revealed that the 4-amino-pyrazolo[3,4-*d*]pyrimidine moiety formed two hydrogen bonds with Met793 and Gln791 in the hinge area, respectively. Additionally, the N2 and the N7 of the pyrazolo[3,4-*d*]pyrimidine moiety were involved in another two hydrogen bonds with Lys 745 and Gly 796, respectively through water bridges. Moreover, the pyrazole part of the pyrazolo[3,4-*d*]pyrimidine moiety was involved in a hydrophobic interaction with

the Val726. As expected, the acrylamide formed a covalent bond with the Cys797 residue **(Wang *et al*., 2017)** (**Fig. 44**).

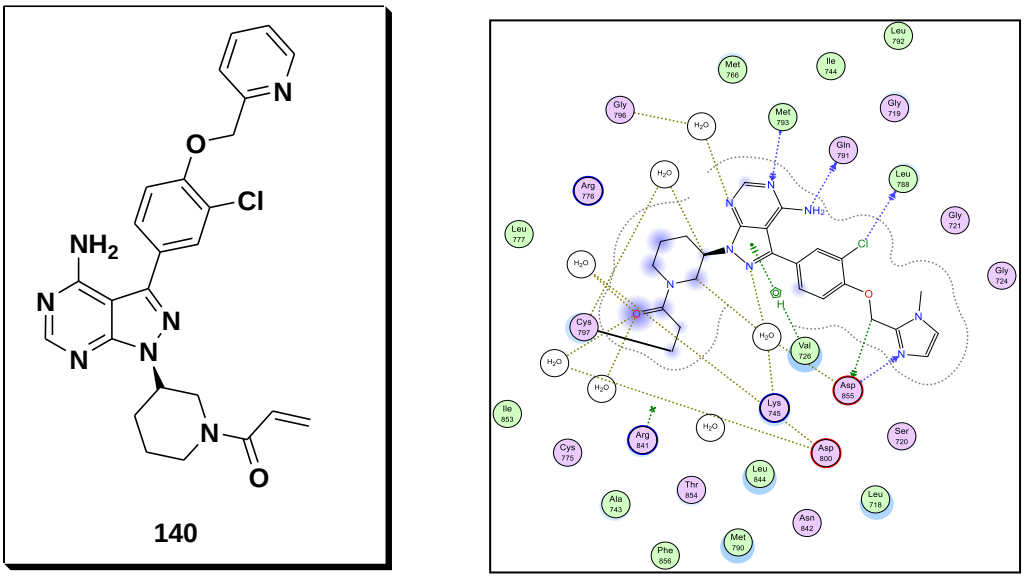


Fig. 44: 2D interaction between compound **140** and EFGR retrieved from (**Fraley *et al*., 2002)**

Tintori *et al.* reported the x-ray co-crystal structure of the 4-amino-pyrazolo[3,4-*d*]pyrimidine derivative **141** with the Src enzyme [PDB: 4O2P]. Within the Src binding pocket, the pyrazolo[3,4-*d*]pyrimidine nucleus accommodated the adenine region. The C4 substituent was located in hydrophobic region I, while hydrophobic region II hosted the N1 side chain. Two hydrogen bond interactions were also found, one involving the C4 amino group and the side chain of Thr338A and the other between the N2 of the pyrazolo[3,4-*d*]pyrimidine nucleus and the NH-backbone of Met341A (**Fig. 45**) **(Tintori *et al*., 2015)**.

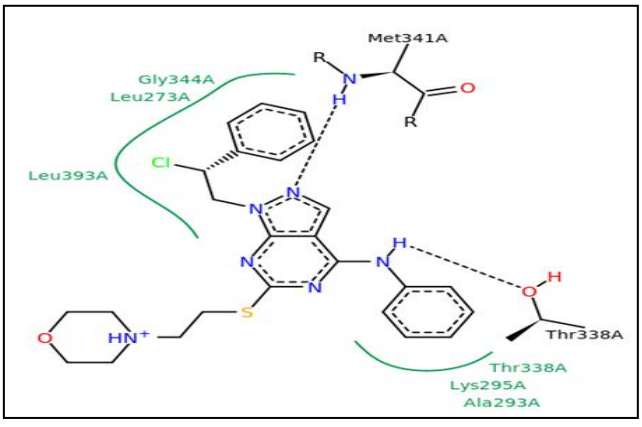


Fig. 45: 2D binding mode of compound **141** within the ATP pocket of Src retrieved from [PDB: 4O2P]

Yang *et al.* reported co-crystal structures of a complex of truncated mammalian target of rapamycin

(mTOR) and Torkinib (PP242, **142**). Torkinib consists of the adenine-mimetic pyrazolopyrimidine scaffold common to tyrosine kinase inhibitors, with a hydroxyindole substituent at a position that often points to the inner hydrophobic pocket. The 4-amino group was involved in hydrogen bond with the Gly2238A. Additionally, the N5 of the pyrazolo[3,4-*d*]pyrimidine nucleus acts as hydrogen bond acceptor and formed a hydrogen bond with Val2240A. Moreover the pyrazolo[3,4-*d*]pyrimidine nucleus showed a hydrophobic interaction with Trp2239A (**Fig. 46**). These interactions explain the obtained potent anticancer activity of Torkinib **(Yang *et al.*, 2013)**.

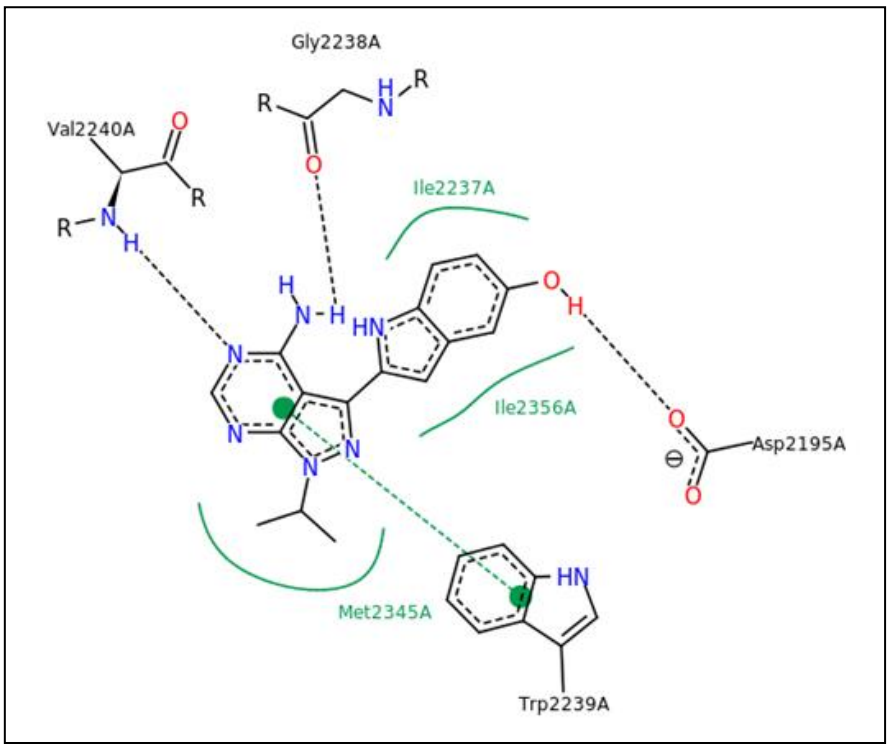


Fig. 46: 2D binding mode of Torkinib (PP242) **142** within the mTOR kinase enzyme retrieved from [PDB: 4JT5]

8. Conclusion

This review article seeks to provide an up-to-date overview of the latest advances in the development of the synthetic approaches to construct pyrazolo[1,5-*a*]pyrimidine and pyrazolo[3,4-*d*]pyrimidine skeletons which have always been of a paramount chemical significance in pharmaceutical and synthetic chemistry field. This review presented a broad range of simple and efficient synthetic methods to access a wide range of functionalized pyrazolo[1,5-*a*]pyrimidine and pyrazolo[3,4-*d*]pyrimidine scaffolds through several strategies. Also, this article clearly showed that these ring systems play an important role in medicinal chemistry being evaluated against numerous biological targets. Many studies revealed that different pyrazolopyrimidine derivatives possess extensive potential applications as antitumor agents and kinase

inhibitors and have been successfully developed, marketed, and extensively used in the clinic in treating various types of cancer diseases with low toxicity, high bioavailability, and good curative effects.

Much effort contributed to structural modification of clinical pyrazolopyrimidine drugs are needed to retain the advantages of these drugs and overcome their shortcomings. One significant strategy is to employ some functionality that are helpful for improving the physicochemical properties and affinity with the target sites to modify clinical drugs. This intention is to increase their biological activities and overcome drug resistances.

REFERENCES

Abbas, N., et al., (2021) Fused and substituted pyrimidine derivatives as profound anti-cancer agents. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents),. 21(7): p. 861-893.

Abbas, S.E.S., et al., (2015) 4-Substituted-1-phenyl-1H-pyrazolo [3, 4-d] pyrimidine derivatives: design, synthesis, antitumor and EGFR tyrosine kinase inhibitory activity. Chemical Biology & Drug Design,. 85(5): p. 608-622.

Abd El Hamid, M.K., M.D. Mihovilovic, and H.B. El-Nassan, (2012) Synthesis of novel pyrazolo [3, 4-d] pyrimidine derivatives as potential anti-breast cancer agents. European journal of medicinal chemistry,. 57: p. 323-328.

Abd El Razik, H.A. and A.E. Abdel Wahab, (2011) Synthesis and biological evaluation of some novel fused pyrazolopyrimidines as potential anticancer and antimicrobial agents. Archiv der Pharmazie,. 344(3): p. 184-196.

Abd El-Karim, S.S., et al., (2015) Design, synthesis, biological evaluation and molecular docking studies of novel benzofuran–pyrazole derivatives as anticancer agents. Bioorganic chemistry,. 63: p. 1-12.

Abdallah, A.E. and G.H. Elgemeie, (2018) Design, synthesis, docking, and antimicrobial evaluation of some novel pyrazolo [1, 5-a] pyrimidines and their corresponding cycloalkane ring-fused derivatives as purine analogs. drug design, development and therapy,: p. 1785-1798.

Abdelall, E.K. and J.N. Philoppes, (2016) Synthesis and cytotoxic activity of new pyrazolo [1, 5-a] pyrimidines and determination of pyrimidine regiospecific ring formation with 2D NMR.: Ann Arbor, MI: Michigan Publishing, University of Michigan Library.

Abdelazeem, A.H., et al., (2014) Novel pyrazolopyrimidine derivatives targeting COXs and iNOS enzymes; design, synthesis and biological evaluation as potential anti-inflammatory agents. European Journal of Pharmaceutical Sciences,. 62: p. 197-211.

Aggarwal, R., et al., (2014) Synthesis of new bi (pyrazolo [1, 5-a] pyrimidinyl)-7-one derivatives from dehydroacetic acid and its analogues as antibacterial agents. Arkivoc, 2014.: p. 120-134.

Aghazadeh Tabrizi, M., et al., (2013) Discovery of 7-oxopyrazolo [1, 5-a] pyrimidine-6-carboxamides as potent and selective CB2 cannabinoid receptor inverse agonists. Journal of Medicinal Chemistry,. 56(11): p. 4482-4496.

Akbar, I., et al., (2024) Vitex Negundo–Fe 3 O 4–CuO green nanocatalyst (VN–Fe 3 O 4–CuO): synthesis of pyrazolo[3, 4-c]pyrazole derivatives via the cyclization of isoniazid with pyrazole and their antimicrobial activity, cytotoxicity, and molecular docking studies. RSC advances,. 14(1): p. 677-688.

Alharthy, R.D., et al., (2023) Pyrazole derivatives of pyridine and naphthyridine as proapoptotic agents in cervical and breast cancer cells. Scientific Reports,. 13(1): p. 5370.

Ali, T.E.-S., (2009) Synthesis of some novel pyrazolo [3, 4-b] pyridine and pyrazolo [3, 4-d] pyrimidine derivatives bearing 5, 6-diphenyl-1, 2, 4-triazine moiety as potential antimicrobial agents. European journal of medicinal chemistry,. 44(11): p. 4385-4392.

Aly, A., (2004) A convenient synthesis and pharmacological activity of novel annelated pyrimidine derivatives. Journal of the Chinese Chemical Society,. 51(6): p. 1381-1388.

Ameziane El Hassani, I., et al., (2023) Novel Pyrazole-Based Benzofuran Derivatives as Anticancer Agents: Synthesis, Biological Evaluation, and Molecular Docking Investigations. Chem Biodivers,. 20(11): p. e202301145.

Antonelli, A., et al., (2011) Novel pyrazolopyrimidine derivatives as tyrosine kinase inhibitors with antitumoral activity in vitro and in vivo in papillary dedifferentiated thyroid cancer. The Journal of Clinical Endocrinology & Metabolism,. 96(2): p. E288-E296.

Asati, V., et al., (2021) Pyrazolopyrimidines as anticancer agents: A review on structural and target-based approaches. European Journal of Medicinal Chemistry,. 225: p. 113781.

Azmy, E.M., et al., (2023) Development of pyrolo[2,3-c]pyrazole, pyrolo[2,3-d]pyrimidine and their bioisosteres as novel CDK2 inhibitors with potent in vitro apoptotic anti-proliferative activity: Synthesis, biological evaluation and molecular dynamics investigations. Bioorganic Chemistry,. 139: p. 106729.

Bahashwan, S.A., et al., (2014) Androgen receptor antagonists and anti-prostate cancer activities of some newly synthesized substituted fused pyrazolo-, triazolo-and thiazolo-pyrimidine derivatives. International Journal of Molecular Sciences,. 15(11): p. 21587-21602.

Baviskar, A.T., et al., (2013) Synthesis of imine-pyrazolopyrimidinones and their mechanistic interventions on anticancer activity. Bioorganic & medicinal chemistry,. 21(18): p. 5782-5793.

Bondock, S., T. Naser, and Y.A. Ammar, (2013) Synthesis of some new 2-(3-pyridyl)-4, 5-disubstituted thiazoles as potent antimicrobial agents. European Journal of Medicinal Chemistry,. 62: p. 270-279.

Bußmann, L., et al., (2021) Analyzing tyrosine kinase activity in head and neck cancer by functional kinomics: Identification of hyperactivated Src family kinases as prognostic markers and potential targets. International Journal of Cancer,. 149(5): p. 1166-1180.

Carmi, C., et al., (2012) Clinical perspectives for irreversible tyrosine kinase inhibitors in cancer. Biochemical pharmacology,. 84(11): p. 1388-1399.

Chang, C.-H., et al., (2013) Selective synthesis of pyrazolo [3, 4-d] pyrimidine, N-(1H-pyrazol-5-yl) formamide, or N-(1H-pyrazol-5-yl) formamidine derivatives from N-1-substituted-5-aminopyrazoles with new Vilsmeier-type reagents. Tetrahedron,. 69(4): p. 1378-1386.

Chauhan, M. and R. Kumar, (2013) Medicinal attributes of pyrazolo [3, 4-d] pyrimidines: a review. Bioorganic & medicinal chemistry,. 21(18): p. 5657-5668.

Cheng, C. and R.K. Robins, (1956) Potential purine antagonists. VI. Synthesis of 1-alkyl-and 1-aryl-4-substituted pyrazolo [3, 4-d] pyrimidines1, 2. The Journal of Organic Chemistry,. 21(11): p. 1240-1256.

Chobe, S.S., et al., (2012) An ecofriendly synthesis and DNA binding interaction study of some pyrazolo [1, 5-a] pyrimidines derivatives. Bioorganic & medicinal chemistry letters,. 22(24): p. 7566-7572.

Chu, J., et al., (2024) Soluble urokinase plasminogen activator receptor and cardiotoxicity in doxorubicin-treated breast cancer patients: a prospective exploratory study. Cardiooncology,. 10(1): p. 3.

Chu, S.-C., et al., (2014) Berberine reverses epithelial-to-mesenchymal transition and inhibits metastasis and tumor-induced angiogenesis in human cervical cancer cells. Molecular pharmacology,. 86(6): p. 609-623.

Dang, X.-W., et al., (2024) Recent advances of small-molecule c-Src inhibitors for potential therapeutic utilities. Bioorganic Chemistry,. 142: p. 106934.

Dass, R., (2022) Synthesis and Anticancer Evaluation of Novel Pyrazolo [1, 5-a] Pyrimidines: Discovery of a Novel Lead Compound with Selective Activities Against VPS34 and JAK1-JH2 Pseudokinase., Brigham Young University.

Dessalew, N., D.S. Patel, and P.V. Bharatam, (2007) 3D-QSAR and molecular docking studies on pyrazolopyrimidine derivatives as glycogen synthase kinase-3β inhibitors. Journal of Molecular Graphics and Modelling,. 25(6): p. 885-895.

Dwyer, M.P., et al., (2013) Discovery of pyrazolo [1, 5-a] pyrimidine-based Pim inhibitors: A template-based approach. Bioorganic & medicinal chemistry letters,. 23(22): p. 6178-6182.

Dwyer, M.P., et al., (2007) Versatile templates for the development of novel kinase inhibitors: Discovery of novel CDK inhibitors. Bioorganic & medicinal chemistry letters,. 17(22): p. 6216-6219.

Ebrahimi, N., et al., (2023) Receptor tyrosine kinase inhibitors in cancer. Cellular and Molecular Life Sciences,. 80(4): p. 104.

Elbakry, O.M., et al., (2024) Development of pyrazolo [1, 5-a] pyrimidine derivatives: Synthesis, anticancer activity and docking study. Azhar International Journal of Pharmaceutical and Medical Sciences,. 4(1): p. 76-90.

El-Enany, M.M., et al., (2011) Synthesis and antitumor activity of novel pyrazolo [1, 5-a] pyrimidine derivatives. European Journal of Chemistry,. 2(3): p. 331-336.

Elgemeie, G.H., et al., (2009) First synthesis of thiophene thioglycosides. Journal of Carbohydrate Chemistry,. 28(3): p. 161-178.

Elgemeie, G.H., et al., (2005) New trends in synthesis of pyrazole nucleosides as new antimetabolites. Nucleosides, Nucleotides, and Nucleic Acids,. 24(8): p. 1227-1247.

Elsaman, T., et al., (2013) A Facile Synthesis of Pyrido[: 3, 4]pyrazolo[1, 5-a]pyrimidine and Pyrido[: 3, 4]pyrazolo[5, 1-c][1, 2, 4]triazine Bearing a Thiophene Moiety. Journal of Chemistry, 2013.

Engers, D.W., et al., (2013) Synthesis and structure–activity relationships of a novel and selective bone morphogenetic protein receptor (BMP) inhibitor derived from the pyrazolo [1.5-a] pyrimidine scaffold of Dorsomorphin: the discovery of ML347 as an ALK2 versus ALK3 selective MLPCN probe. Bioorganic & medicinal chemistry letters,. 23(11): p. 3248-3252.

Fadda, A.A., R. Rabie, and H.A. Etman, (2017) Synthesis of Thiazolinone, Aminopyrazole, Pyrazolopyrimidine, and Pyrazolotriazine Derivatives Starting from 1-Naphthyl-2-Cyanoacetamide. Journal of Heterocyclic Chemistry,. 54(2): p. 1015-1023.

Fatahala, S.S., et al., (2017) Synthesis and evaluation of cytotoxic activity of some pyrroles and fused pyrroles. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents),. 17(7): p. 1014-1025.

Fatahala, S.S., et al., (2018) Synthesis and evaluation of novel spiro derivatives for pyrrolopyrimidines as anti-hyperglycemia promising compounds. Journal of Enzyme Inhibition and Medicinal Chemistry,. 33(1): p. 809-817.

Fraley, M.E., et al., (2002) Synthesis and initial SAR studies of 3, 6-disubstituted pyrazolo [1, 5-a] pyrimidines: a new class of KDR kinase inhibitors. Bioorganic & Medicinal Chemistry Letters,. 12(19): p. 2767-2770.

Frey, R.R., et al., (2008) 7-Aminopyrazolo [1, 5-a] pyrimidines as potent multitargeted receptor tyrosine kinase inhibitors. Journal of medicinal chemistry,. 51(13): p. 3777-3787.

Ghorab, M.M., et al., (2010) Synthesis of some new pyrazolo [3, 4-d] pyrimidine derivatives of expected anticancer and radioprotective activity. European journal of medicinal chemistry,. 45(1): p. 171-178.

Ghossoub, R., G. David, and P. Zimmermann, (2023) Intercellular Communication: What Syndecan-Syntenin May Teach us About the Molecular Mechanisms That Support Vesicular Exchanges, in Encyclopedia of Cell Biology (Second Edition), R.A. Bradshaw, G.W. Hart, and P.D. Stahl, Editors., Academic Press: Oxford. p. 401-407.

Gommermann, N., et al., (2010) New pyrazolo [1, 5a] pyrimidines as orally active inhibitors of Lck. Bioorganic & medicinal chemistry letters,. 20(12): p. 3628-3631.

Gopalsamy, A., et al., (2009) Hit to lead optimization of pyrazolo [1, 5-a] pyrimidines as B-Raf kinase inhibitors. Bioorganic & medicinal chemistry letters,. 19(24): p. 6890-6892.

Gupta, S., et al., (2008) Synthesis of N-aryl-5-amino-4-cyanopyrazole derivatives as potent xanthine oxidase inhibitors. European journal of medicinal chemistry,. 43(4): p. 771-780.

Hafez, T.S., et al., (2013) Synthesis, structural elucidation, and in vitro antitumor activities of some pyrazolopyrimidines and Schiff bases derived from 5-amino-3-(arylamino)-1H-pyrazole-4-carboxamides. Scientia pharmaceutica,. 81(2): p. 339-358.

Halim, P.A., S.M.Z. Sharkawi, and M.B. Labib, (2023) Novel pyrazole-based COX-2 inhibitors as potential anticancer agents: Design, synthesis, cytotoxic effect against resistant cancer cells, cell cycle arrest, apoptosis induction and dual EGFR/Topo-1 inhibition. Bioorganic Chemistry,. 131: p. 106273.

Hassaballah, A.I., et al., (2024) New pyrazolo [3, 4-d] pyrimidine derivatives as EGFR-TK inhibitors: design, green synthesis, potential anti-proliferative activity and P-glycoprotein inhibition. RSC advances,. 14(3): p. 1995-2015.

Hassan, A.S., et al., (2017) Synthesis and antitumor activity of some new pyrazolo [1, 5-a] pyrimidines. Chinese Chemical Letters,. 28(2): p. 388-393.

Hassan, A.S., T.S. Hafez, and S.A. Osman, (2015) Synthesis, characterization, and cytotoxicity of some new 5-aminopyrazole and pyrazolo [1, 5-a] pyrimidine derivatives. Scientia Pharmaceutica,. 83(1): p. 27-39.

Hayman, T.J., et al., (2013) Competitive but not allosteric mTOR kinase inhibition enhances tumor cell radiosensitivity. Translational oncology,. 6(3): p. 355-362.

Heathcote, D.A., et al., (2010) A novel pyrazolo [1, 5-a] pyrimidine is a potent inhibitor of cyclin-dependent protein kinases 1, 2, and 9, which demonstrates antitumor effects in human tumor xenografts following oral administration. Journal of medicinal chemistry,. 53(24): p. 8508-8522.

Hebishy, A.M., H.T. Salama, and G.H. Elgemeie, (2020) New route to the synthesis of Benzamide-Based 5-aminopyrazoles and their fused heterocycles showing remarkable antiavian influenza virus activity. ACS omega,. 5(39): p. 25104-25112.

Holla, B.S., et al., (2006) Synthesis of some novel pyrazolo [3, 4-d] pyrimidine derivatives as potential antimicrobial agents. Bioorganic & medicinal chemistry,. 14(6): p. 2040-2047.

Horton, J.R., et al., (2016) Structural basis for KDM5A histone lysine demethylase inhibition by diverse compounds. Cell chemical biology,. 23(7): p. 769-781.

Horvatic Herceg, G., et al., (2013) Urokinase plasminogen activator and its inhibitor type-1 as prognostic factors in differentiated thyroid carcinoma patients. Otolaryngology--Head and Neck Surgery,. 149(4): p. 533-540.

Huang, Y.-Y., et al., (2012) One-pot synthesis and antiproliferative evaluation of pyrazolo [3, 4-d] pyrimidine derivatives. Tetrahedron,. 68(47): p. 9658-9664.

Hubbard, R.D., et al., (2007) Pyrazolo [3, 4-d] pyrimidines as potent inhibitors of the insulin-like growth factor receptor (IGF-IR). Bioorganic & medicinal chemistry letters,. 17(19): p. 5406-5409.

Ismail, N.S., et al., (2016) Pyrazolo [3, 4-d] pyrimidine based scaffold derivatives targeting kinases as anticancer agents. Future Journal of Pharmaceutical Sciences,. 2(1): p. 20-30.

Ivachtchenko, A.V., et al., (2012) Antagonists of 5-HT6 receptors. Substituted 3-(phenylsulfonyl) pyrazolo [1, 5-a] pyrido [3, 4-e] pyrimidines and 3-(phenylsulfonyl) pyrazolo [1, 5-a] pyrido [4, 3-d] pyrimidines—Synthesis and 'structure–activity'relationship. Bioorganic & medicinal chemistry letters,. 22(13): p. 4273-4280.

Jismy, B., et al., (2020) Efficient Access to 3, 5-Disubstituted 7-(Trifluoromethyl) pyrazolo [1, 5-a] pyrimidines Involving SN Ar and Suzuki Cross-Coupling Reactions. Molecules,. 25(9): p. 2062.

Kandeel, M.M., et al., (2013) Design, synthesis and cytotoxic activity of some novel compounds containing pyrazolo [3, 4-d] pyrimidines nucleus. Journal of Chemical Sciences,. 125: p. 1029-1043.

Kandeel, M.M., et al., (2012) Synthesis and antitumor activity of novel pyrazolo [3, 4-d] pyrimidines and related heterocycles. Der Pharma Chemica,. 4: p. 1704-1715.

Kasibhatla SR, et al., (2008) Modulators of mitotic kinases. U.S. patent CA2676665 A1..

Khadri M J, N., et al., (2023) Synthesis and docking studies of pyrazole-benzamide-benzothiazole conjugates as xanthine oxidase inhibitor candidates. Journal of Molecular Structure,. 1290: p. 135937.

Kim, D.C., et al., (2003) Synthesis and biological evaluations of pyrazolo[3,4-d]pyrimidines as cyclin-dependent kinase 2 inhibitors. Eur J Med Chem,. 38(5): p. 525-32.

Kosugi, T., et al., (2012) Mitogen-activated protein kinase-activated protein kinase 2 (MAPKAP-K2) as an antiinflammatory target: discovery and in vivo activity of selective pyrazolo [1, 5-a] pyrimidine inhibitors using a focused library and structure-based optimization approach. Journal of Medicinal Chemistry,. 55(15): p. 6700-6715.

Kulkarni, A., et al., (2014) 1, 3-disubstituted-4-aminopyrazolo [3, 4-d] pyrimidines, a new class of potent inhibitors for Phospholipase D. Chemical Biology & Drug Design,. 84(3): p. 270-281.

Kumar, A., et al., (2013) Synthesis of Pyrazolo [3, 4-d] pyrimidine Derivatives and Evaluation of their Src Kinase Inhibitory Activities..

Kumar, A.A., B.J. Buckley, and M. Ranson, (2022) The Urokinase Plasminogen Activation System in Pancreatic Cancer: Prospective Diagnostic and Therapeutic Targets. Biomolecules,. 12(2): p. 152.

Kurban, B., et al., (2023) Synthesis and Anticancer Activities of Pyrazole–Thiadiazole-Based EGFR Inhibitors. ACS Omega,. 8(34): p. 31500-31509.

Laamari, Y., et al., (2024) Synthesis, characterization and cytotoxic activity of pyrazole derivatives based on thymol. Journal of Molecular Structure,. 1297: p. 136864.

Li, J., et al., (2006) Synthesis and Anti-tumor Activities of Novel Pyrazolo [1, 5-a] pyrimidines. Archiv der Pharmazie: An International Journal Pharmaceutical and Medicinal Chemistry,. 339(11): p. 593-597.

Lin, Y., et al., (2013) Herbal compound triptolide synergistically enhanced antitumor activity of amino-terminal fragment of urokinase. Molecular cancer,. 12: p. 1-16.

Liu, J., et al., (2016) Synthesis of several new 1-arylpyrazolo [3, 4-d] pyrimidines containing an amide moiety. Journal of Chemical Research,. 40(2): p. 92-94.

Mandour, A.A., et al., (2022) Synthesis, biological evaluation, and in silico studies of new CDK2 inhibitors based on pyrazolo[3,4-d]pyrimidine and pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidine scaffold with apoptotic activity. Journal of Enzyme Inhibition and Medicinal Chemistry,. 37(1): p. 1957-1973.

Manetti, F., et al., (2007) Identification of a novel pyrazolo [3, 4-d] pyrimidine able to inhibit cell proliferation of a human osteogenic sarcoma in vitro and in a xenograft model in mice. Journal of medicinal chemistry,. 50(23): p. 5579-5588.

Manley, P.J., et al., (2003) 2, 4-Disubstituted pyrimidines: A novel class of KDR kinase inhibitors. Bioorganic & medicinal chemistry letters,. 13(10): p. 1673-1677.

McCoull, W., et al., (2017) Discovery of pyrazolo [1, 5-a] pyrimidine B-cell lymphoma 6 (BCL6) binders and optimization to high affinity macrocyclic inhibitors. Journal of Medicinal Chemistry,. 60(10): p. 4386-4402.

Metwally, N.H., F.M. Abdelrazek, and S.M. Eldaly, (2016) Synthesis and anticancer activity of some new heterocyclic compounds based on 1-cyanoacetyl-3, 5-dimethylpyrazole. Research on Chemical Intermediates,. 42: p. 1071-1089.

Mohamed, H.S., et al., (2018) Synthesis, characterization and antibacterial activities of novel thieno, pyrazol pyridines and pyrazolopyrimidine derivatives. Der Pharma Chem,. 10(5): p. 121-127.

Mohamed, M., A. El-Hameed, and A. Sayed, (2017) Synthesis strategies and biological value of pyrrole and pyrrolopyrimidine. Journal of advanced pharmacy research,. 1(1): p. 1-24.

Mohamed, M.S., et al., (2015) Novel antiviral compounds against gastroenteric viral infections. Archiv der Pharmazie,. 348(3): p. 194-205.

Myers, S.H., V.G. Brunton, and A. (Unciti-Broceta, (2016) AXL Inhibitors in Cancer: A Medicinal Chemistry Perspective: Miniperspective. Journal of medicinal chemistry,. 59(8): p. 3593-3608.

Nasr, T., et al., (2012) Properties of oligonucleotide with phenyl-substituted carbocyclic nucleoside analogs for the formation of duplex and triplex DNA. Nucleosides, Nucleotides and Nucleic Acids,. 31(12): p. 841-860.

Nasr, T., et al., (2009) Selective fluorescence quenching of the 8-oxoG-clamp by 8-oxodeoxyguanosine in ODN. Bioorganic & medicinal chemistry letters,. 19(3): p. 727-730.

Nasr, T., et al., (2017) Synthesis, antitumor evaluation and microarray study of some new pyrazolo [3, 4-d][1, 2, 3] triazine derivatives. European Journal of Medicinal Chemistry,. 141: p. 603-614.

Nasr, T., S. Bondock, and S. Eid, (2014) Design, synthesis, antimicrobial evaluation and molecular docking studies of some new thiophene, pyrazole and pyridone derivatives bearing sulfisoxazole moiety. Eur J Med Chem,. 84: p. 491-504.

Nasr, T., Y. Taniguchi, and S. Sasaki, (2007) Synthesis of 1'-phenyl substituted nucleoside analogs. Heterocycles,. 71(12): p. 2659-2668.

Nitulescu, G.M., et al., (2015) Synthesis and apoptotic activity of new pyrazole derivatives in cancer cell lines. Bioorganic & Medicinal Chemistry,. 23(17): p. 5799-5808.

Nitulescu, G.M., et al., (2023) The Importance of the Pyrazole Scaffold in the Design of Protein Kinases Inhibitors as Targeted Anticancer Therapies. Molecules,. 28(14).

Paruch, K., et al., (2007) Pyrazolo [1, 5-a] pyrimidines as orally available inhibitors of cyclin-dependent kinase 2. Bioorganic & medicinal chemistry letters,. 17(22): p. 6220-6223.

Peat, A.J., et al., (2004) Novel pyrazolopyrimidine derivatives as GSK-3 inhibitors. Bioorganic & medicinal chemistry letters,. 14(9): p. 2121-2125.

Phillipson, L.J., et al., (2015) Discovery and SAR of novel pyrazolo [1, 5-a] pyrimidines as inhibitors of CDK9. Bioorganic & Medicinal Chemistry,. 23(19): p. 6280-6296.

Phoebe, F.L., (2014) Synthesis and antitumor activity of novel pyrazolo [3, 4-D] pyrimidine and their tricyclic derivatives. Int J Pharm Sci Rev Res,. 24(2): p. 208-214.

Poonia, K., et al., (2016) In vitro anticancer activities of Schiff base and its lanthanum complex. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,. 155: p. 146-154.

Pramanik, C., et al., (2023) Recent synthetic journey on pyrrole-fused compounds. New Journal of Chemistry,. 47(14): p. 6476-6527.

Quintela, J.M., et al., (2001) Pyrazolopyrimidines: synthesis, effect on histamine release from rat peritoneal mast cells and cytotoxic activity. European journal of medicinal chemistry,. 36(4): p. 321-332.

Rahmouni, A., et al., (2016) Synthesis and biological evaluation of novel pyrazolopyrimidines derivatives as anticancer and anti-5-lipoxygenase agents. Bioorganic Chemistry,. 66: p. 160-168.

Rashad, A.E., et al., (2015) Synthesis and anticancer evaluation of some fused pyrazolopyrimidines and their S-acyclic nucleosides. Pharma Chem,. 7.

Rashad, A.E., et al., (2009) Synthesis and anti-HSV-1 evaluation of some pyrazoles and fused pyrazolopyrimidines. Eur J Med Chem,. 44(8): p. 3285-92.

Rice, K.D., et al., (2012) Pyrazolopyrimidines as dual Akt/p70S6K inhibitors. Bioorganic & medicinal chemistry letters,. 22(8): p. 2693-2697.

Roskoski, R., (2023) Properties of FDA-approved small molecule protein kinase inhibitors: A 2023 update. Pharmacological Research,. 187: p. 106552.

Rostamizadeh, S., et al., (2013) A Facile Synthesis of New Pyrazolo [3, 4-d] pyrimidine Derivatives via a One-Pot Four-Component Reaction with Sodium Acetate Supported on Basic Alumina as Promoter. Helvetica Chimica Acta,. 96(12): p. 2267-2275.

Ryabukhin, S.V., et al., (2014) Synthesis of pyrazolo[3,4-d]-4,5-dihydropyrimidin-6-ones. Tetrahedron Letters,. 55(10): p. 1846-1847.

Sack, J.S., et al., (2016) Crystal structure of microtubule affinity-regulating kinase 4 catalytic domain in complex with a pyrazolopyrimidine inhibitor. Acta Crystallographica Section F: Structural Biology Communications,. 72(2): p. 129-134.

Schenone, S., et al., (2008) Synthesis, biological evaluation and docking studies of 4-amino substituted 1H-pyrazolo [3, 4-d] pyrimidines. European journal of medicinal chemistry,. 43(12): p. 2665-2676.

Schmidt, P. and J. Druey, (1956) Heilmittelchemische Untersuchungen in der heterocyclischen Reihe. Mitteilung. Pyrazolo-(3,4-d)-pyrimidine. Helvetica Chimica Acta,. 39(3): p. 986-991.

Selleri, S., et al., (2005) A novel selective GABAA α1 receptor agonist displaying sedative and anxiolytic-like properties in rodents. Journal of medicinal chemistry,. 48(21): p. 6756-6760.

Selleri, S., et al., (2003) Synthesis and benzodiazepine receptor affinity of pyrazolo [1, 5-a] pyrimidine derivatives. 3. New 6-(3-thienyl) series as α1 selective ligands. Journal of medicinal chemistry,. 46(2): p. 310-313.

Shamroukh, A.H., et al., (2014) Some Pyrazole and Pyrazolo [3, 4-d] pyrimidine Derivatives: Synthesis and Anticancer Evaluation. Archiv der Pharmazie,. 347(8): p. 559-565.

Shekarrao, K., et al., (2014) A facile synthesis of steroidal D-ring fused pyrazolo [1, 5-a] pyrimidines. Tetrahedron Letters,. 55(38): p. 5251-5255.

Shen, H.C., et al., (2008) Discovery of pyrazolopyrimidines as the first class of allosteric agonists for the high affinity nicotinic acid receptor GPR109A. Bioorganic & medicinal chemistry letters,. 18(18): p. 4948-4951.

Shi, J.B., et al., (2015) Novel pyrazole-5-carboxamide and pyrazole–pyrimidine derivatives: Synthesis and anticancer activity. European journal of medicinal chemistry,. 90: p. 889-896.

Soliman, A.M., et al., (2014) Synthesis of some novel imidazopyrazole and pyrazolopyrimidine derivatives. Journal of Heterocyclic Chemistry,. 51(5): p. 1476-1481.

Soth, M., et al., (2011) 3-Amino-pyrazolo [3, 4-d] pyrimidines as p38α kinase inhibitors: design and development to a highly selective lead. Bioorganic & medicinal chemistry letters,. 21(11): p. 3452-3456.

Tatton, L., et al., (2003) The Src-selective kinase inhibitor PP1 also inhibits Kit and Bcr-Abl tyrosine kinases. Journal of Biological Chemistry,. 278(7): p. 4847-4853.

Tintori, C., et al., (2015) Combining X-ray crystallography and molecular modeling toward the optimization of pyrazolo [3, 4-d] pyrimidines as potent c-Src inhibitors active in vivo against neuroblastoma. Journal of Medicinal Chemistry,. 58(1): p. 347-361.

Upadhyay, A., (2021) Cancer: An unknown territory; rethinking before going ahead. Genes & Diseases,. 8(5): p. 655-661.

Vyas, A.R. and S.V. Singh, (2014) Functional relevance of D, L-sulforaphane-mediated induction of vimentin and plasminogen activator inhibitor-1 in human prostate cancer cells. European journal of nutrition,. 53: p. 843-852.

Wamhoff, H., (1985) Heterocyclic β-Enamino Esters, Versatile Synthons in Heterocyclic Synthesis, in Advances in Heterocyclic Chemistry, A.R. Katritzky, Editor., Academic Press. p. 299-368.

Wang, A., et al., (2017) Discovery of (R)-1-(3-(4-amino-3-(3-chloro-4-(pyridin-2-ylmethoxy) phenyl)-1 H-pyrazolo [3, 4-d] pyrimidin-1-yl) piperidin-1-yl) prop-2-en-1-one (CHMFL-EGFR-202) as a novel irreversible EGFR mutant kinase inhibitor with a distinct binding mode. Journal of medicinal chemistry,. 60(7): p. 2944-2962.

Wang, H.-Q., Z.-J. Liu, and M.-W. Ding, (2004) Versatile synthesis and fungicidal activities of 6-amino-3- alkylthio-1,5-dihydro-1-phenyl-pyrazolo[3,4-d]pyrimidin-4-one derivatives. Phosphorus, Sulfur, and Silicon and the Related Elements,. 179(10): p. 2039-2050.

Wang, X., et al., (2007) Rapid hit to lead evaluation of pyrazolo [3, 4-d] pyrimidin-4-one as selective and orally bioavailable mGluR1 antagonists. Bioorganic & medicinal chemistry letters,. 17(15): p. 4303-4307.

Wang, Y.D., et al., (2009) Synthesis, SAR study and biological evaluation of novel pyrazolo [1, 5-a] pyrimidin-7-yl phenyl amides as anti-proliferative agents. Bioorganic & medicinal chemistry,. 17(5): p. 2091-2100.

Werry, E.L., et al., (2017) Derivatives of the pyrazolo [1, 5-a] pyrimidine acetamide DPA-713 as translocator protein (TSPO) ligands and pro-apoptotic agents in human glioblastoma. European Journal of Pharmaceutical Sciences,. 96: p. 186-192.

Wood, D.J., et al., (2019) Differences in the conformational energy landscape of CDK1 and CDK2 suggest a mechanism for achieving selective CDK inhibition. Cell chemical biology,. 26(1): p. 121-130. e5.

Xu, Y., et al., (2015) Synthesis and biological evaluation of pyrazolo [1, 5-a] pyrimidine compounds as potent and selective Pim-1 inhibitors. ACS medicinal chemistry letters,. 6(1): p. 63-67.

Yadav, T.T., et al., (2022) A Review on Fused Pyrimidine Systems as EGFR Inhibitors and Their Structure-Activity Relationship. Front Chem,. 10: p. 861288.

Yang, H., et al., (2013) mTOR kinase structure, mechanism and regulation. Nature,. 497(7448): p. 217-223.

Yewale, S.B., et al., (2012) Novel 3-substituted-1-aryl-5-phenyl-6-anilinopyrazolo [3, 4-d] pyrimidin-4-ones: Docking, synthesis and pharmacological evaluation as a potential anti-inflammatory agents. Bioorganic & medicinal chemistry letters,. 22(21): p. 6616-6620.

Youssef, Y.M., et al., (2023) Synthesis and antioxidant, antimicrobial, and antiviral activity of some pyrazole-based heterocycles using a 2(3H)-furanone derivative. Journal of the Iranian Chemical Society,. 20(9): p. 2203-2216.

Yu, K., et al., (2009) Biochemical, cellular, and in vivo activity of novel ATP-competitive and selective inhibitors of the mammalian target of rapamycin. Cancer research,. 69(15): p. 6232-6240.

Zhang, W., et al., (2014) UNC2025, a Potent and Orally Bioavailable MER/FLT3 Dual Inhibitor. Journal of Medicinal Chemistry,. 57(16): p. 7031-7041.

بيرازولوبيريميدين كعوامل مضادة للسرطان: التشييد وطريقة العمل (مقالة المراجعة)

وفاء زخاري[١]، تامر نصر[١]، رانيا عبد الحميد[٢]، تامر صقر[٣]، دينا عادل عثمان[٤]، خالد العدل[٥،٦]*

[١]قسم الكيمياء الصيدلية، كلية الصيدلة، جامعة حلوان، حلوان، القاهرة، مصر.

[٢] قسم الكيمياء العضوية الصيدلية، كلية الصيدلة، جامعة حلوان، حلوان، القاهرة، مصر.

[٣] قسم النظائر المشعة والمولدات، مركز المعامل الساخنة، هيئة الطاقة الذرية المصرية، ١٣٧٥٩، القاهرة، مصر.

[٤] قسم الكيمياء الصيدلانية، كلية الصيدلة، جامعة أكتوبر للعلوم الحديثة والآداب (MSA)، الجيزة، مصر.

[٥] قسم الكيمياء الصيدلية، كلية الصيدلة، جامعة هليوبوليس للتنمية المستدامة، القاهرة، مصر

[٦] قسم الكيمياء الصيدلية، كلية الصيدلة، جامعة الأزهر، القاهرة، مصر

البريد الالكتروني للباحث الرئيسي : khaled.eladl@hu.edu.eg

تسعى المقالة المرجعية هذه إلى تقديم نظرة عامة محدثة عن أحدث التطورات في تطوير الأساليب الكيميائية لتشييد هياكل بيرازولو[١،٥-أ]بيريميدين وبيرازولو[٣،٤-د]بيريميدين والتي كانت دائمًا ذات أهمية كيميائية قصوى في مجال الكيمياء الصيدلانية والتخليقية. قدمت هذه المرجعية مجموعة واسعة من الطرق الكيميائية الجديدة والبسيطة والفعالة للوصول إلى مجموعة واسعة من مشتقات بيرازولو [١،٥-أ] بيريميدين وبيرازولو [٣،٤-د] بيريميدين من خلال عدة استراتيجيات. كما أظهرت هذه المقالة بوضوح أن هذه الأنظمة الحلقية تلعب دورًا مهمًا في الكيمياء الطبية التي يتم تقييمها مقابل العديد من الأهداف البيولوجية. كشفت العديد من الدراسات أن مشتقات البيرازولوبيريميدين المختلفة تمتلك تطبيقات محتملة واسعة النطاق كعوامل مضادة للأورام ومثبطات للبروتين كيناز وقد تم تطويرها وتسويقها واستخدامها على نطاق واسع بنجاح في علاج أنواع مختلفة من أمراض السرطان و كانت ذات سمية منخفضة وتوافر حيوي مرتفع وتأثيرات علاجية جيدة.

هناك حاجة إلى الكثير من الجهود التي ساهمت في التعديل الهيكلي لأدوية البيرازولوبيريميدين السريرية للاحتفاظ بمزايا هذه الأدوية والتغلب على عيوبها. تتمثل إحدى الإستراتيجيات المهمة في استخدام بعض المجموعات الوظيفية التي تساعد في تحسين الخواص الفيزيائية والكيميائية والتقارب مع المواقع المستهدفة لتعديل الأدوية السريرية. وتهدف هذه النية إلى زيادة أنشطتها البيولوجية والتغلب على مقاومة الأدوية.

الكلمات المفتاحية : بيرازولوبيريميدين، مضاد للسرطان، تشييد، طريقة التأثير