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RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

A review on synthesis approaches and biological investigations of Pyrazolo[3,4-*d*]pyrimidine schaffold

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Abstract

Pyrazolo[3,4-*d*]pyrimidine is a bicyclic hetero organic nucleus that encompass a pyrazole ring fusion with a pyrimidine ring. Pyrazolo[3,4-*d*]pyrimidine nucleus could be synthesized using different synthetic procedures depending on either pyrazole or pyrimidine rings as starting synthones in addition to, different miscellaneous procedures. It was initially synthesized and evaluated as adenosine nucleoside analogues for cancer and viral therapy. Recently, numerous pyrazolo[3,4-*d*]pyrimidine compounds adopted anti-neoplastic activity *via* several mechanistic pathways. Also, designing drugs with pyrazolo[3,4-*d*]pyrimidine nucleus as pharmacophore encourage different biological activities such as anti-inflammatory, anti-microbial, anti-mycobacterial, anti-malarial, anti-gout and anti-diabetic. This bibliographic development presents an overview on works carried out on pyrazolo[3,4-*d*]pyrimidine derivatives during the period 2017-2021.

Keywords: Pyrazolo[3,4-*d*]pyrimidine; Synthesis; Pyrazole; Pyrimidine; Biological activity.

1. Introduction

Pyrazolo[3,4-*d*]pyrimidine ring system drawn much attention as it is considered as purine isostere **(Poulsen and Quinn, 1996)**. The pyrazolo[3,4-*d*]pyrimidine consists of a pyrazole ring fused with the pyrimidine moiety rather than imidazole moiety in purines. From this point, pyrazolo[3,4-*d*]pyrimidine was primarily reported as adenosine receptor antagonists for anticancer **(Poulsen and Quinn, 1996)** and antiviral activities **(Ettahiri et al., 2012)** through the incorporation instead of purine bases in the biological system causing inhibition in certain enzymes such as polymerase enzyme. Pyrazolo[3,4-*d*]pyrimidines were recorded in literature to exhibit many pharmacological activities as antimicrobial **(El-Sayed et al., 2009)**, antiviral **(Ettahiri et al., 2012)**, anticancer **(Poulsen and Quinn, 1996)**, antimycobacterial **(Trivedi et al., 2008)**, anti-inflammatory **(Atatreh et al., 2019)**, anti-malarial **(Silveira et al., 2018)**, antidiabetic **(Reddy et al., 2019)** and as xanthine oxidase inhibitor for treating gout **(Chu and Lynch, 1975)**. This review summarized numerous synthetic pathways for pyrazolo[3,4-*d*]pyrimidine scaffold based on its significant precursors pyrazole and pyrimidine ring derivatives beside, several unusual synthetic pathways such as One-

pot synthesis and Diel's alder reaction. In addition to, the different biological investigations and the recent developments of compounds related to pyrazolo[3,4-*d*]pyrimidine ring through the inhibition of various targets for the generation of potentially active compounds as anti-neoplastic agents. On the other hand, we proved the different biological activities adopted by pyrazolo[3,4-*d*]pyrimidine ring system in the literature.

2. Chemistry

2.1. Approaches in the synthesis of pyrazolo[3,4-*d*]pyrimidine:

There are numerous reported procedures for the synthesis of pyrazolo[3,4-*d*]pyrimidine nucleus depending on different starting materials **(Asati et al., 2021)** as illustrated in **figure 1**. Pyrazolo[3,4-*d*]pyrimidine system comprise 2 main rings; pyrazole ring and pyrimidine ring that could be used separately as starting synthones for pyrazolo[3,4-*d*]pyrimidine bicyclic system.

2.1.1. Using pyrazole ring as a starting material

2.1.1.1. From pyrazole-5-amine derivatives:

The synthesis of 3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **(2)** was succeeded through heating of 3-methyl-1-phenyl-1*H*-pyrazol-5-amine **(1)** with phosphorous tribromide (PBr_3) in presence of formamide at 60 °C **(Huang et al., 2012)**.

Moreover, reaction of 5-amino pyrazole derivative **1** with aryl isocyanate to was carried out to obtain *N*-aryl urea derivative **3** that was cyclized with p-trifluoromethylbenzaldehyde in presence of chlorotrimethyl silane (TMSCl) to afford pyrazolo[3,4-*d*]-4,5-dihydro-7*H*-pyrimidin-6(7*H*)-one compound **4**. **(Ryabukhin et al., 2014)**

2.1.1.2. From 5-amino pyrazole 4-carboxylate derivatives:

Also, it was reported that reaction of ethyl(ethoxymethylene)cyanoacetate **5** with phenyl hydrazine under microwave radiation conditions (MW) without solvent resulted in compound 5-amino-1-phenyl-pyrazole-4-ethylcarboxylate **(6)** that was cyclized with thiourea to afford thioxo pyrazolo[3,4-*d*]pyrimidinone derivative **7** **(Heravi et al., 2006)**.

Furthermore, compound 3,6-dimethyl-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidin-4-one **(11)** was prepared through basic hydrolysis of the starting material ethyl 5-amino-3-methyl-1-phenyl-1*H*-pyrazole-4-carboxylate **(8)** to obtain carboxylic acid derivative **9** . Compound **9** was cyclized by heating with acetic anhydride to give 3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*][1,3]oxazin-4-one **(10)** that was further refluxed with formamide to afford the pyrazolo[3,4-*d*]pyrimidin-4-one derivative **11**. **(Abdelgawad et al., 2016)**

Moreover, compound ethyl 2-cyano-3-ethoxy but-2-enoate **(12)** was cyclised with methyl hydrazine in ethanol to obtain 5-amino pyrazole 4-carboxylate analogue **13** that was condensed with triethyl orthoacetate (TEOA) to give a schiff's base **14** that underwent further cyclization with hydrazine hydrate in ethanol to afford pyrazolo[3,4-*d*]pyrimidinone **15**. **(Wang et al., 2018)**

In addition, The synthesis of thioxo pyrazolo[3,4-*d*]pyrimidinone derivative **16** was described by reacting aminopyrazole carboxylate **6** with phenyl isothiocyanate in pyridine. **(Aggarwal and Kumar, 2018)**

2.1.1.3. From 5-amino pyrazole-4-carboxamide derivatives:

The synthesis of pyrazolo[3,4-*d*]pyrimidin-4-one bearing benzene sulfonamide **18** was achieved *via* condensation of 5-amino-1-(4-sulfamoylphenyl)-1*H*-pyrazole-4-carboxamide **(17)** with p-chlorobenzaldehyde in presence of DMF as solvent and iodine as mild lewis acid and oxidizing agent. **(Hassan et al., 2017)**

Furthermore, synthesis of 1,6-diphenylpyrazolo[3,4-*d*]pyrimidine derivative **20** was carried out *via* cyclization of 5-amino-1-phenyl-1*H*-pyrazole-4-carboxamide **(19)** with methyl benzoate in presence of ethanolic sodium ethoxide. **(Gaber et al., 2018)**

Besides, 5-amino-1-(5,6-diphenyl-1,2,4-triazin-3-yl)-1*H*-pyrazole-4-carboxamide **(21)** was reacted with diethyl oxalate to obtain pyrazolylaminoxoacetate derivative **22**. The prepared compound **22** was cyclized with acetic acid to afford 1-(1, 2, 4-triazin-3-yl)-pyrazolo[3,4-*d*]pyrimidine-6-carboxylate derivative **23**. **(Abdellatif and Bakr, 2018)**

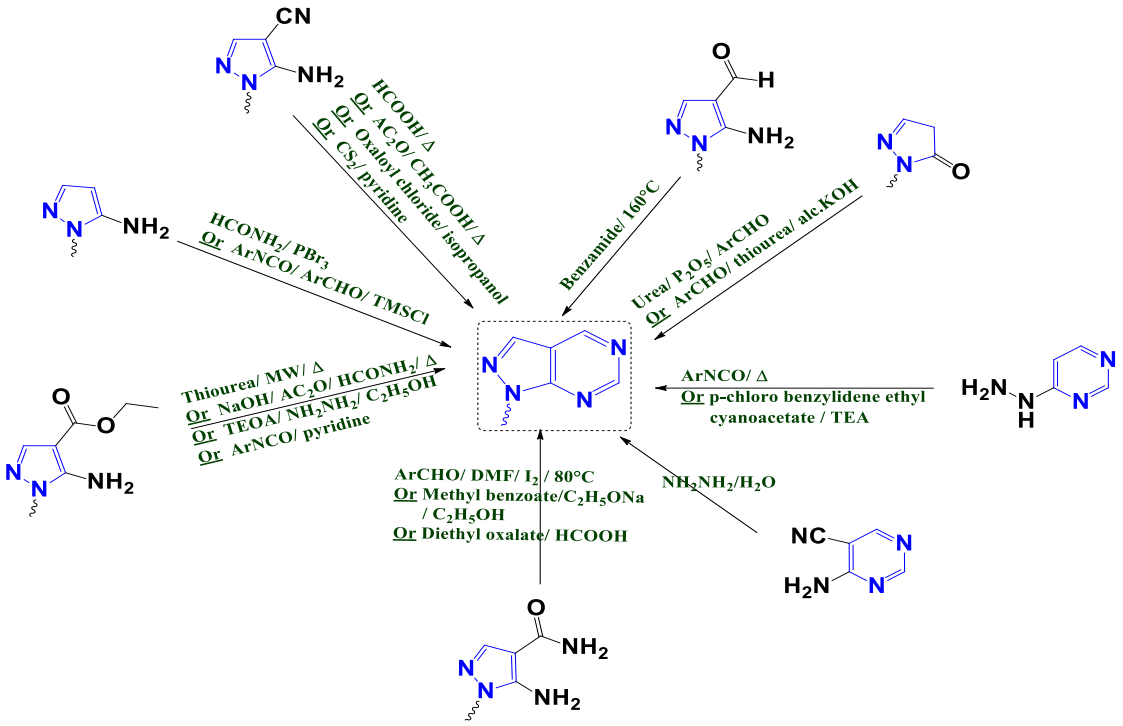
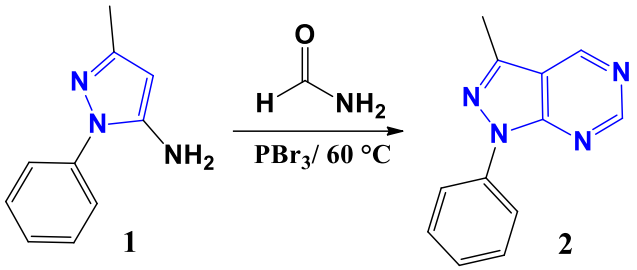
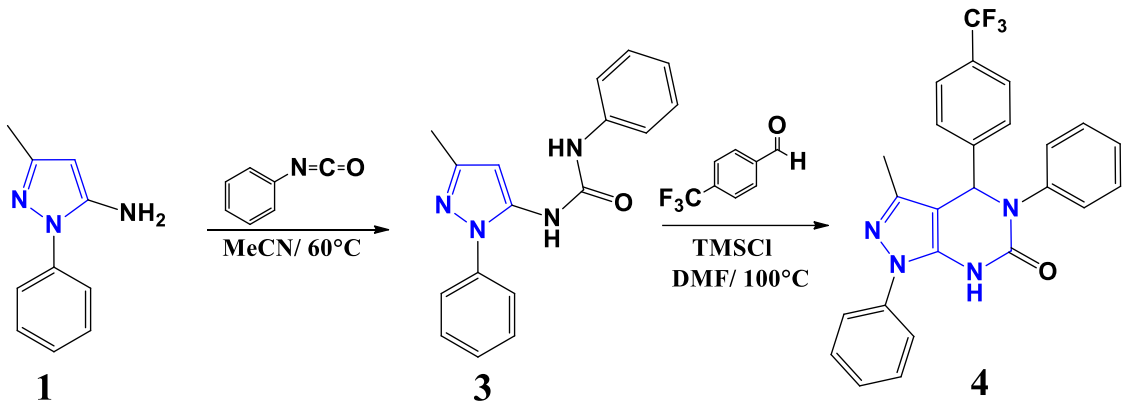


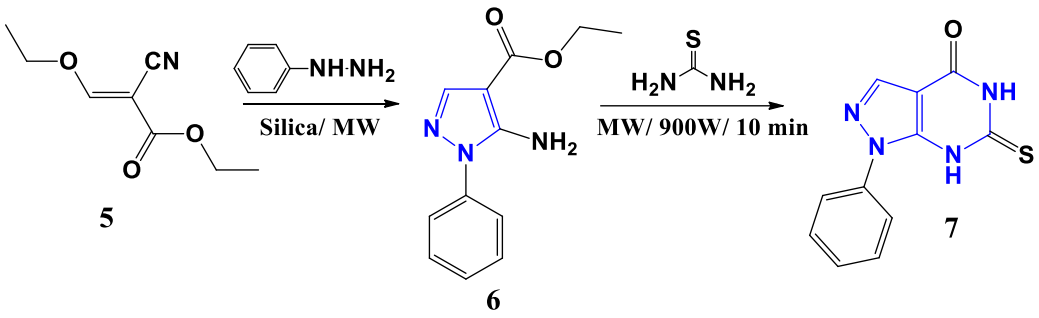
Figure 1: Different synthetic routes of pyrazolo[3,4-*d*]pyrimidine derivatives.



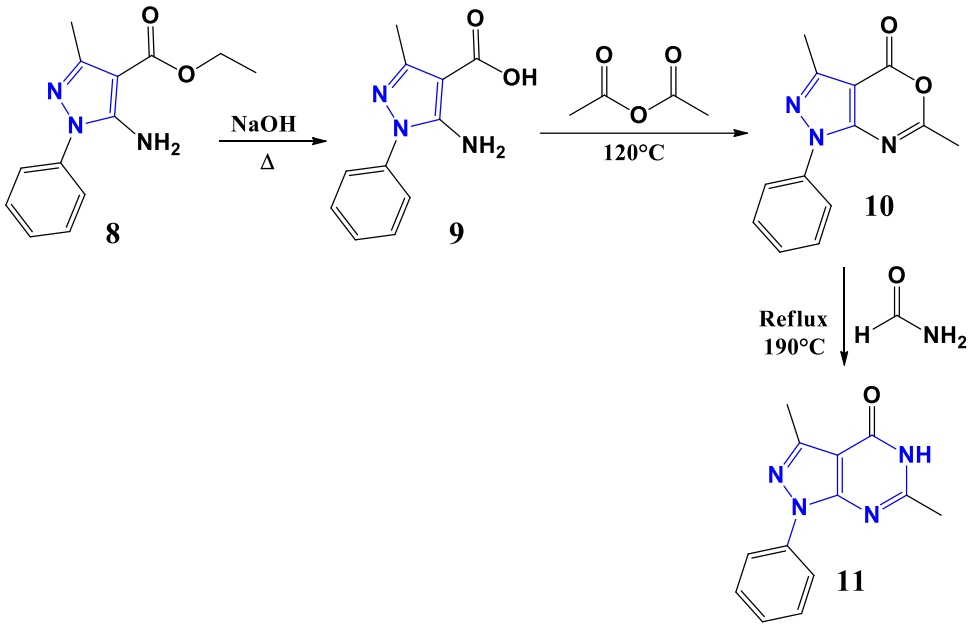
Scheme 1: Synthesis of 3-methyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **(2)**



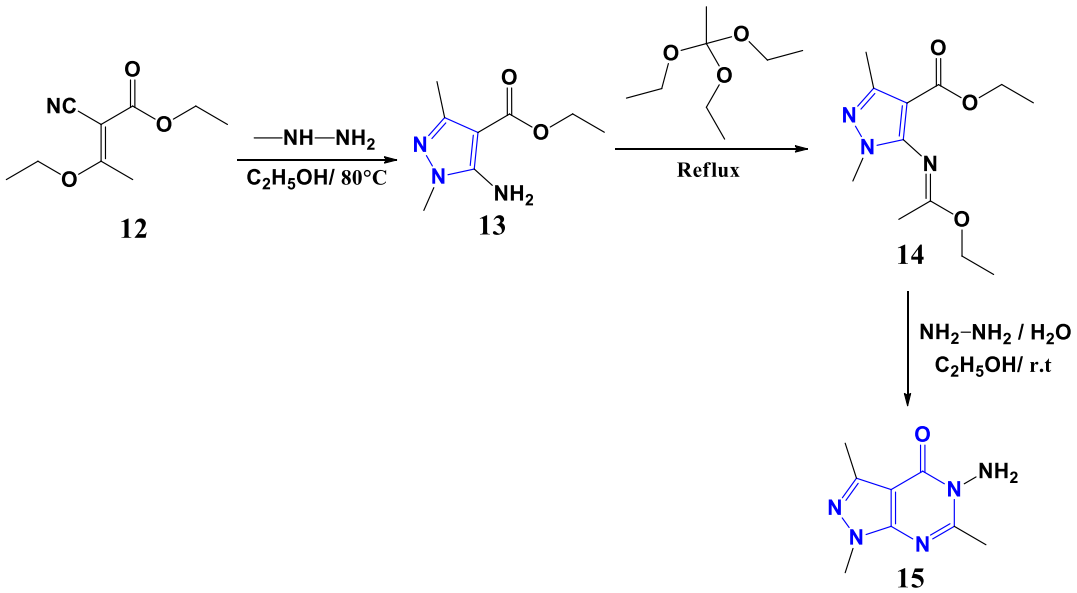
Scheme 2: Synthesis of pyrazolo[3,4-*d*]-4,5-dihydro-7*H*-pyrimidin-6(7*H*)-one **(4).**



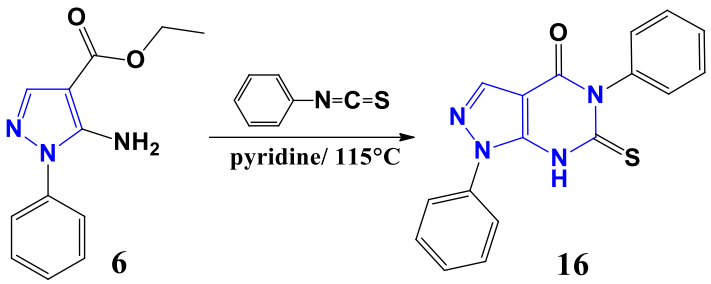
Scheme 3: Synthesis of thioxo pyrazolo[3,4-*d*]pyrimidinone derivative **7.**



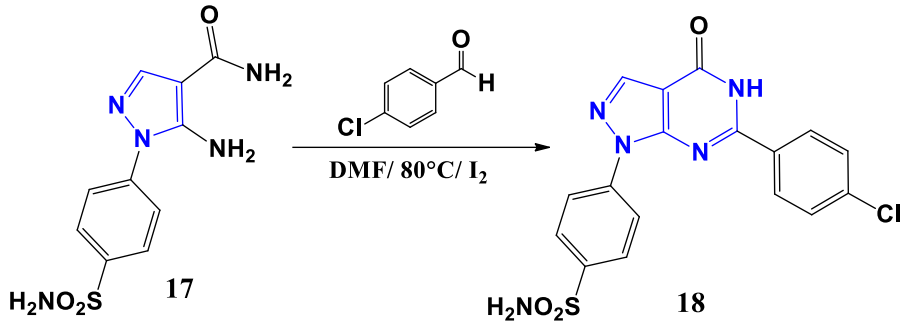
Scheme 4: Synthesis of pyrazolo[3,4-*d*]pyrimidin-4-one derivative **11**.



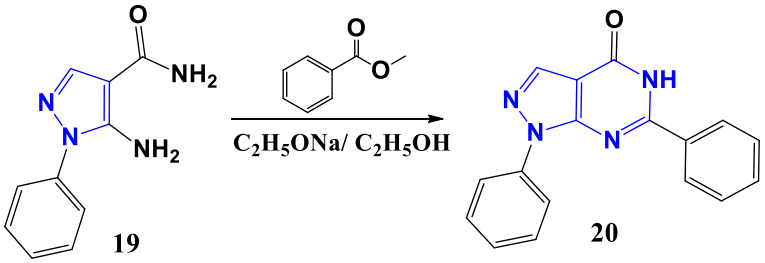
Scheme 5: Synthesis of 5-aminopyrazolo[3,4-*d*]pyrimidinone **15**.



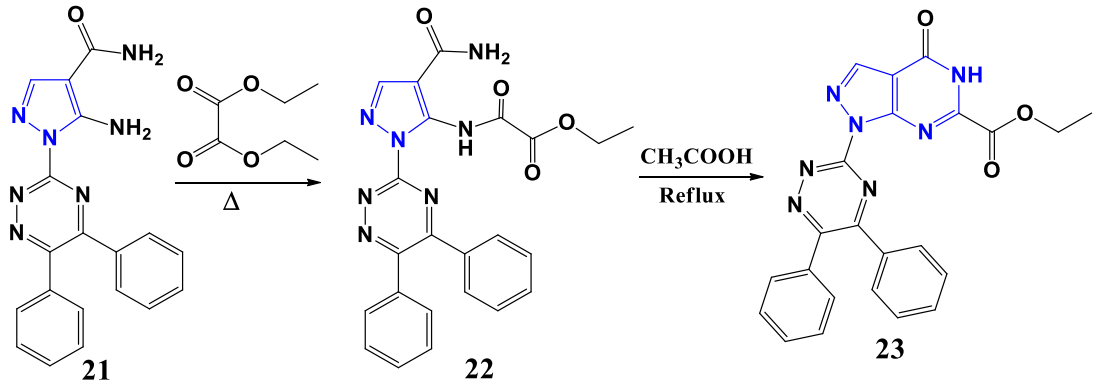
Scheme 6: Synthesis of thioxo pyrazolo[3,4-*d*]pyrimidinone derivative **16.**



Scheme 7: Synthesis of pyrazolo[3,4-*d*]pyrimidin-4-one bearing benzene sulfonamide **18.**



Scheme 8: Synthesis of 1,6-diphenylpyrazolo[3,4-*d*]pyrimidine derivative **20**



Scheme 9: Synthesis of 1-(1, 2, 4-triazin-3-yl)-pyrazolo[3,4-*d*]pyrimidine-6-carboxylate derivative **23**.

2.1.1.4. From 5-amino pyrazole-4-acid hydrazide derivatives:

Compound 5-amino-1-phenyl-1*H*-pyrazole-4-carbohydrazide **(24)** and chloroacetyl chloride were added in dry DMF to ice cooled solution to obtain chloroacetyl derivative **25**. The product **25** was cyclized with triethyl orthoformate to produce the chloroacetamidopyrazolo[3,4-*d*]pyrimidinone derivative **26**. **(Razik et al., 2016)**

2.1.1.5. From 5-amino pyrazole 4-carbonitrile derivatives:

The compound 5-amino-1-phenyl-pyrazole-4-carbonitrile **(28)** was synthesized through condensation of ethoxymethylenemalononitrile **27** with phenyl hydrazine. The produced amino cyano pyrazole derivative **28** was further cyclized through heating with formic acid to obtain 1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one **(29)**. **(Abdelazeem et al., 2014)**

Additionally, 5-amino-3-methyl-1-phenyl-1*H*-pyrazole-4-carbonitrile **(30)** was reacted with acetic anhydride in presence of acetic acid to yield 3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one **(11)**. **(Rahmouni et al., 2016)**

Furthermore, 5-amino-1-phenyl-1*H*-pyrazole-4-carbonitrile **(28)** was reacted with oxalyl chloride to produce acid chloride intermediate of pyrazolo[3,4-*d*]pyrimidine that was immediately reacted with isopropanol by nucleophilic substitution reaction to afford isopropyl 4-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-carboxylate **(31)**. **(Atatreh et al., 2019)**

Besides, 5-amino-1-tosyl-1*H*-pyrazole-3,4-dicarbonitrile **(33)** was synthesized *via* refluxing p-toluene sulfonyl hydrazide **(32)** with tetracyanoethylene in absolute ethanol. The product **33** was heated with carbon disulfide (CS_2) in presence of pyridine to give a further cyclized product 4,6-dithioxo-1-tosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine derivative **34**. **(Abdel-latif et al., 2016)**

2.1.1.6. From 5-amino pyrazole 4-carbaldehyde derivatives:

A synthesis of 3-(4-chlorophenyl)-1,6-diphenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **(36)** was reported through heating of 5-amino-3-(4-chlorophenyl)-1-phenyl-1*H*-pyrazole-4-carbaldehyde **(35)** with benzamide at 180^{o}C as a neat reaction. **(Jachak et al., 2006)**

Moreover, Amination cyclization reaction of (4-formyl-3-phenyl-1*H*-pyrazol-5-yl)-*N*,*N*-dimethyl formimidamide **(37)** with cyanamide was carried out under a series of acidic mediate solutions as dilute HCl or methane sulfonic acid to obtain 3-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **(38)**. **(Tsai et al., 2018)**

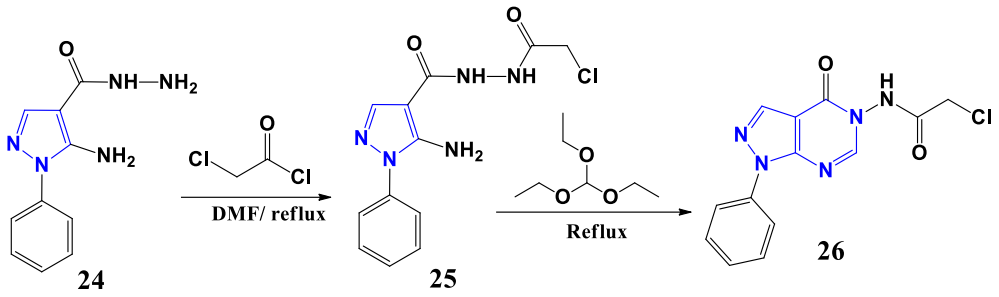
2.1.1.7. From pyrazolone derivatives:

Pyrazolo[3,4-*d*]pyrimidine derivative bearing phenothiazine nucleus **41**was synthesized through cyclization of hydrazine derivative of phenothiazine **39** with ethyl acetoacetate to obtain pyrazolone analogue **40** that was further cyclized to the desired pyrazolo[3,4-*d*]pyrimidine analogue **41** through refluxing with guanidine and p-anisaldehyde in presence of phosphorous pentaoxide (P_2O_5). **(Siddiqui et al., 2014)**

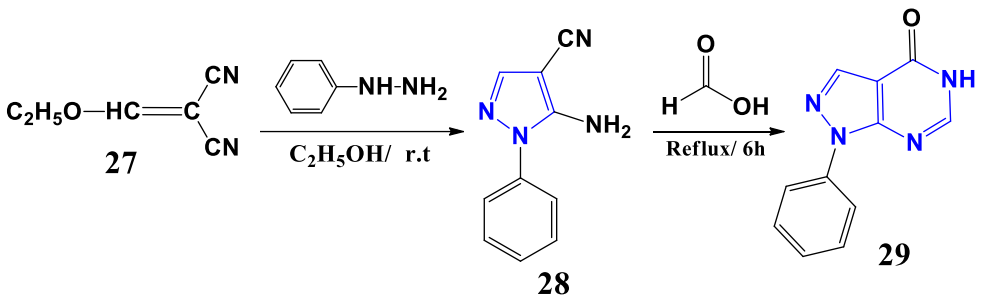
Additionally, pyrazolo[3,4-*d*]pyrimidinethione derivative **44** was synthesized starting from 1,3-diphenyl-1*H*-pyrazol-5(4*H*)-one **(42)**. The starting compound **42** was condensed with p-chlorobenzaldehyde in ethanol to give the arylidene derivative **43** which was cyclized with thiourea in presence of ethanolic potassium hydroxide to yield the required pyrazolo[3,4-*d*]pyrimidinethione derivative **44**. **(Reheim and Baker, 2017)**

2.1.2. Using pyrimidine ring as a starting material:

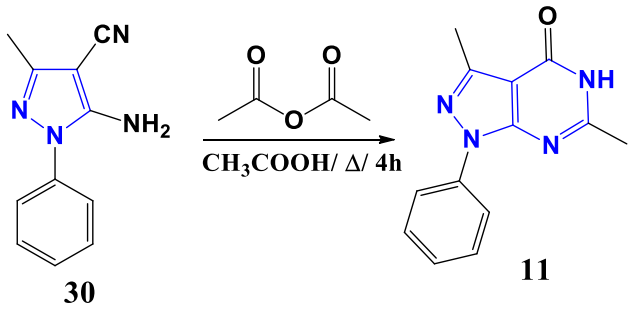
A synthesis of phenyl amino pyrazolo[3,4-*d*]pyrimidinedione derivative **46** was carried out starting from 6-hydrazino pyrimidinedione derivative **45** upon cyclization with phenyl isocyanate. **(Bhuyan et al., 2002).** Besides, starting from pyrimidine 4,6 diol **(47)** the 4,6-dichloropyrimidine-5-carbaldehyde **(48)** could be prepared through Vilsmeir reaction using phosphorous oxychloride ($POCl_3$) and dimethyl formamide (DMF). The prepared compound **48** was cyclized with methyl hydrazine using diisopropyl ethylamine (DIPEA) as a catalyst in THF. The final obtained compound was 4-chloro-1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **(49)**. **(Goshu et al., 2015)**



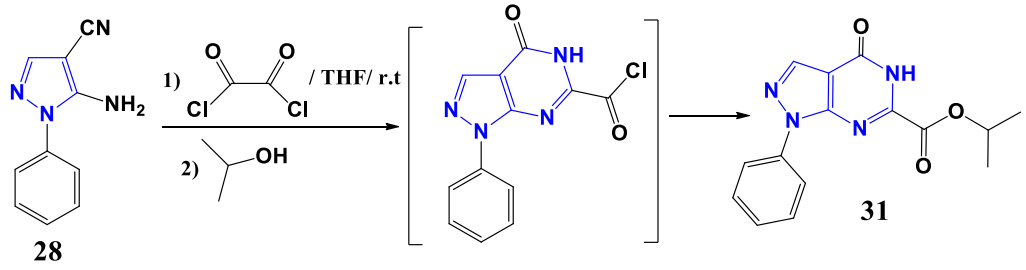
Scheme 10: Synthesis of chloroacetamidopyrazolo[3,4-*d*]pyrimidinone derivative **26**.



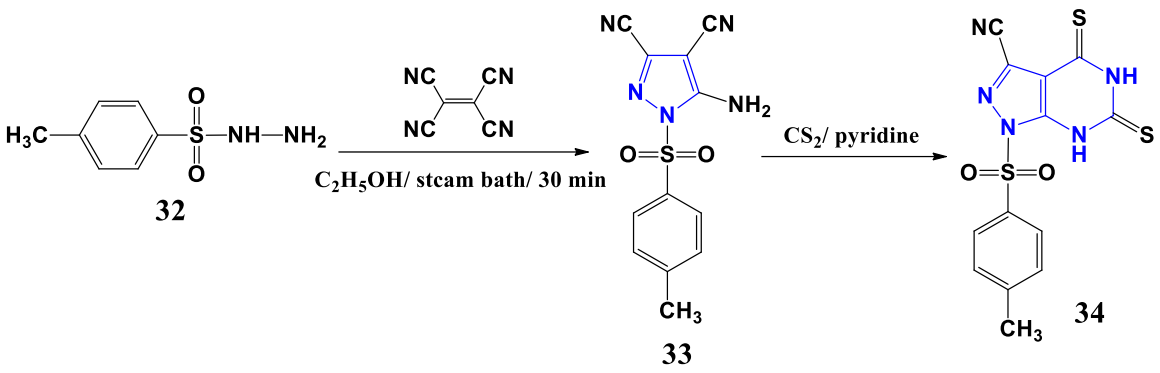
Scheme 11: Synthesis of 1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one **(29)**.



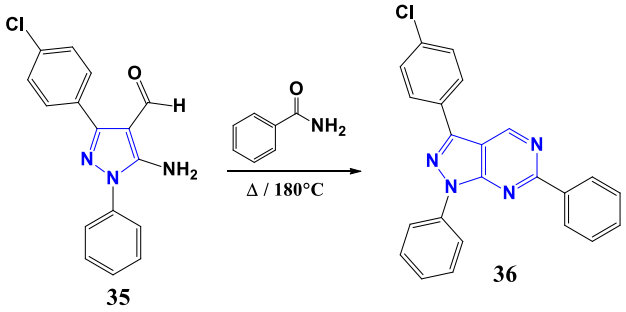
Scheme 12: Synthesis of 3,6-dimethyl-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-4(5*H*)-one **(11)**.



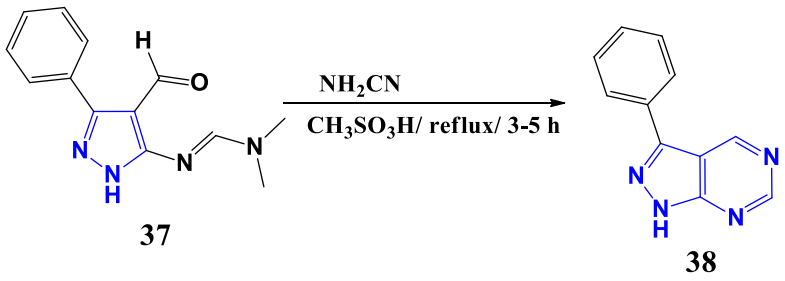
Scheme 13: Synthesis of isopropyl 4-oxo-1-phenyl-4,5-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidine-6-carboxylate **(31)**.



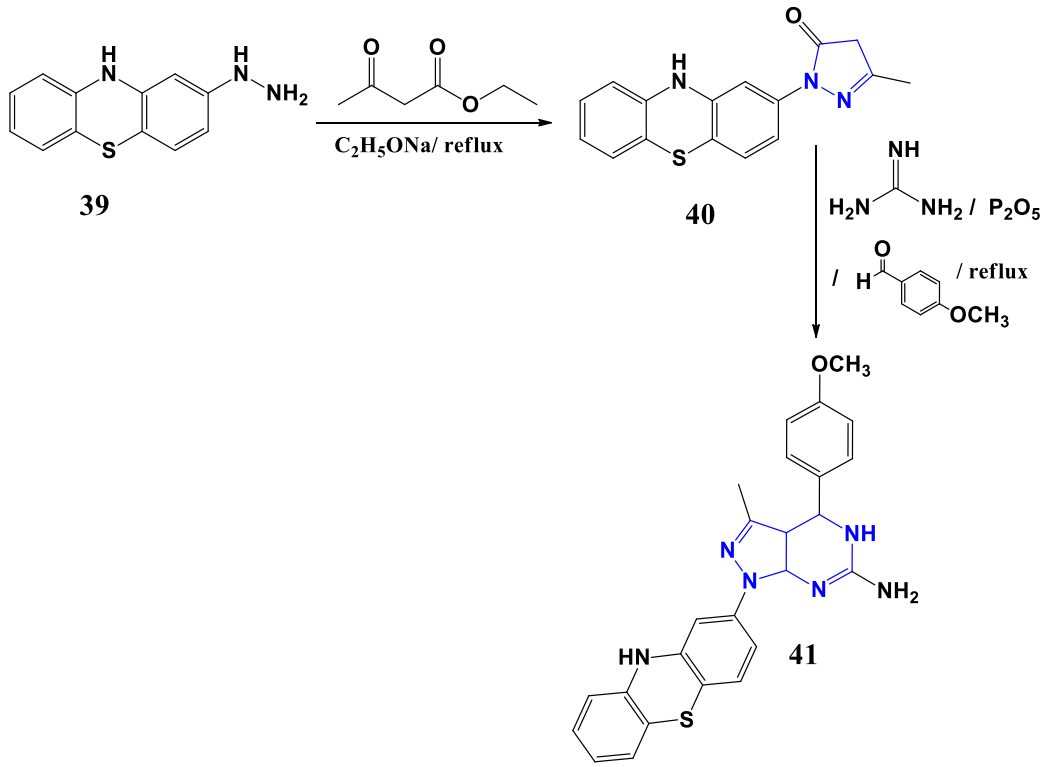
Scheme 14: Synthesis of 4,6-dithioxo-1-tosyl-1*H*-pyrazolo[3,4-*d*]pyrimidine derivative **34**.



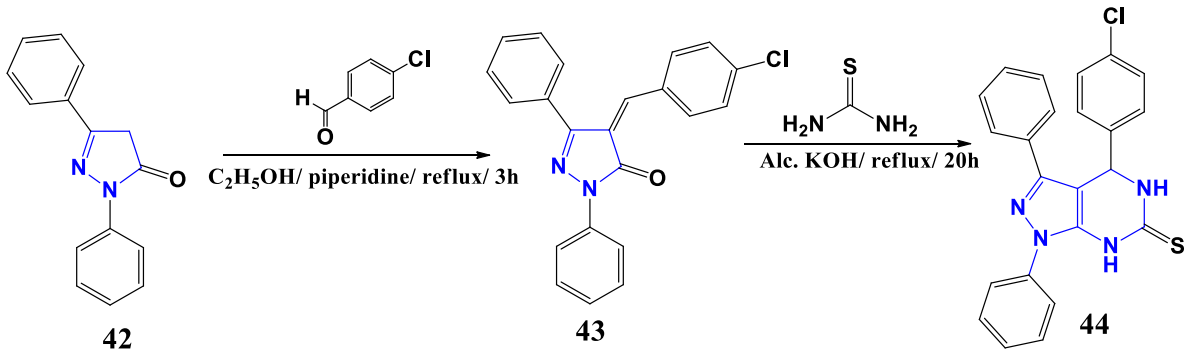
Scheme 15: Synthesis of 3-(4-chlorophenyl)-1,6-diphenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **(36)**



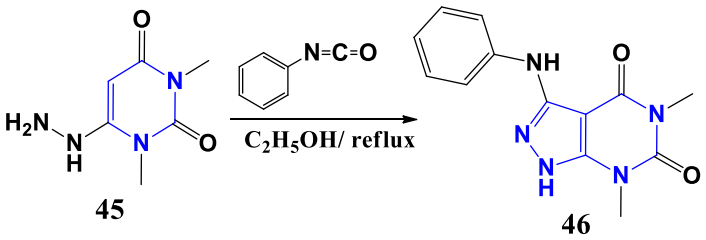
Scheme 16: Synthesis of 3-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **(38)**.



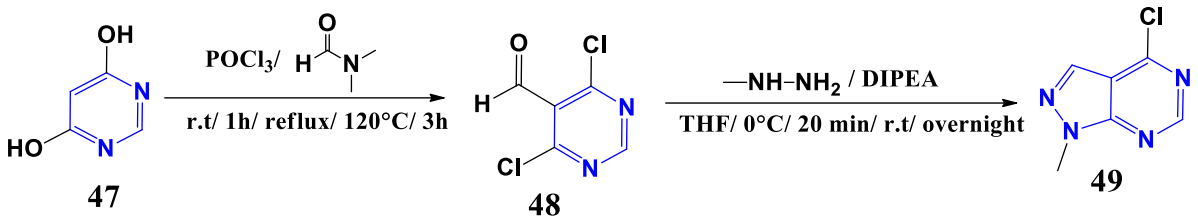
Scheme 17: Synthesis of pyrazolo[3,4-*d*]pyrimidine derivative bearing phenothiazine nucleus **41**



Scheme 18: Synthesis of pyrazolo[3,4-*d*]pyrimidinethione derivative **44.**



Scheme 19: Synthesis of phenyl amino pyrazolo[3,4-*d*]pyrimidinedione derivative **46**



Scheme 20: Synthesis of 4-chloro-1-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidine **(49)**.

In addition, 6-hydrazinyl-1-methylpyrimidine-2,4(1*H*,3*H*)-dione **(50)** was heated with p-chlorobenzylidene ethyl cyanoacetate in DMF and in presence of triethyl amine (TEA) to furnish 3-(4-chlorophenyl)-7-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-dione **(51)**. **(El-kalyoubi and Agili, 2016)**

A cycloaddition reaction of p-fluoro benzylidene malononitrile **(52)** with guanidine hydrochloride was outlined to obtain 6-amino-4-(4-fluorophenyl)-2-imino-1,2-dihydropyrimidine-5 carbonitrile **(53)**. Compound **53** was further cyclized with hydrazine hydrate to afford 4-(4-fluorophenyl)-6-imino-6,7-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-amine **(54)**. **(Khan et al., 2017)**

2.1.3. One pot synthesis of pyrazolo[3,4-*d*]pyrimidine:

There are infrequent reported miscellaneous procedures for preparation of pyrazolo[3,4-*d*]pyrimidines without accreditation on pyrazole or pyrimidine rings as starting synthones., An equi-molar amount of 4-chlorobenzaldehyde **(55)**, malononitrile **(56)** and benzaminidine hydrochloride **(57) was** heated in ethanol. After consumption of all reactants, they added basic alumina supported sodium acetate followed by addition of hydrazine hydrate to obtain 4-(4-chlorophenyl)-6-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-amine **(58)**. **(Rostamizadeh et al., 2013)**

Also, A simple and efficient one pot synthesis of 5-selenoxo-pyrazolo[3,4-*d*]pyrimidine derivative **62** was carried out *via* stirring 4-amino-1,5-dimethyl-2-phenyl-1*H*-pyrazol-3(2*H*)-one **(59)** with benzoyl chloride **(60)** in presence of potassium selenocyanate **(61)** at room temperature. **(Ehsanfar et al., 2020)**

2.1.4. Using Diel's Alder reaction:

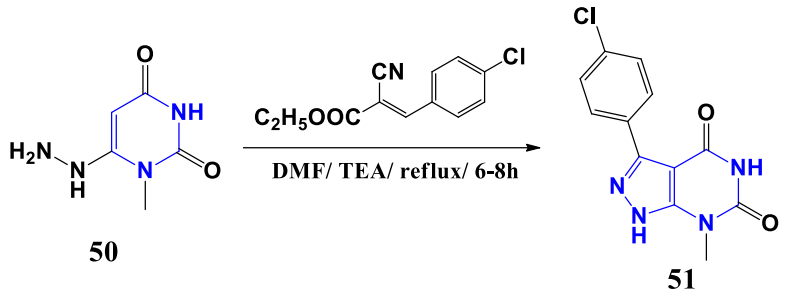
Tandem Decarboxylation/Diels-Alder (TDDA) reaction of 5-amino-1-phenyl-4-pyrazolecarboxylic acid **(63)** was reported to give 1-phenyl pyrazole-5-amine **(64)** that was cyclized with triphenyl 1,3,5-triazine **65**. The formed intermediate **66** underwent a retro Diels-Alder reaction to loss benzonitrile and form the pyrazolo[3,4-*d*]pyrimidine derivative **67** with a yield of 64%. They demonstrated that the amino phenyl pyrazole compound **64** act as dienophile then it had subsequent trapping with triphenyl 1,3,5 triazines **65** by [4+2 cycloaddition] to produce the unstable intermediate **66. (Dang et al., 2021)**

2.1.5. Synthesis of pyrazolo[3,4-*d*]pyrimidine from acyclic intermediates:

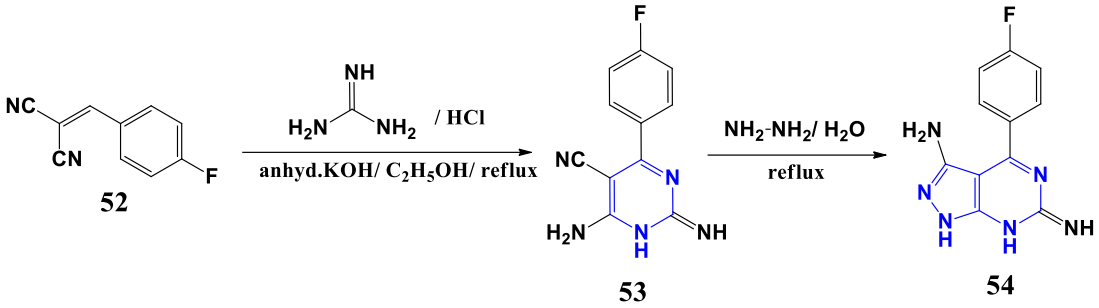
Using other miscellaneous synthetic techniques, pyraozlo[3,4-*d*]pyrimidines could be prepared from acyclic available and cheap intermediates to serve an easy and important synthesis of allopurinol with a good yield. They reacted *N*-formyl-2-cyanoacetamide **(68)** with triethyl orthoformate to produce cyanoethoxy formyl propenamide **69** that was finally treated with hydrazine hydrate to give hydrazino derivative **70** that was cyclized to obtain pyrazolo[3,4-*d*]pyrimidine derivative **71**. **(Hildick and Shaw, 1971)**

3. Biological activity:

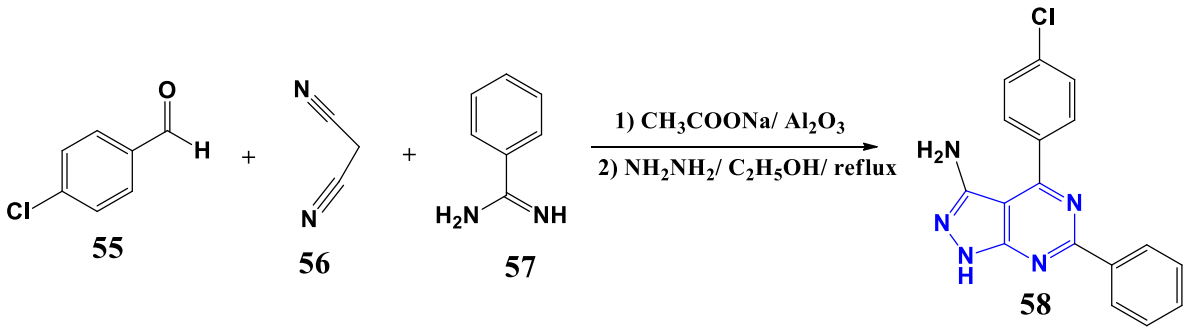
In the last two decades, many researches elucidated the pharmacological potential of pyrazolo[3,4-*d*]pyrimidine as anti-neoplastic **(Poulsen and Quinn, 1996)**, anti-inflammatory **(Atatreh et al., 2019)**, antimicrobial **(El-Sayed et al., 2009)**, anti-mycobacterial **(Trivedi et al., 2008)**, anti-malarial **(Silveira et al., 2018)**, antiviral **(Ettahiri et al., 2012)**, xanthine oxidase inhibitors **(Chu and Lynch, 1975)** and anti-diabetic agents **(Reddy et al., 2019)**.



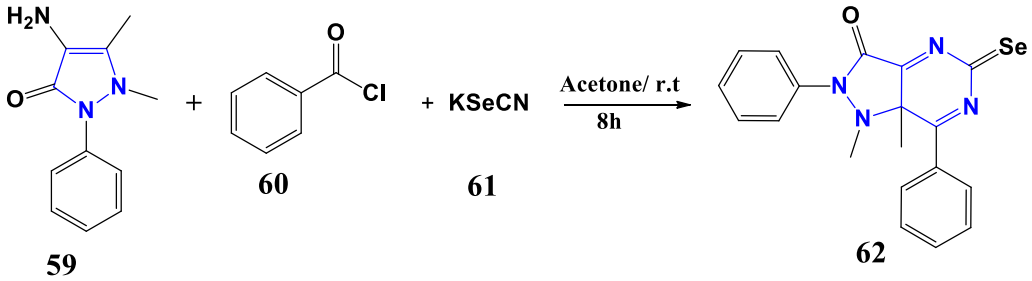
Scheme 21: Synthesis of 3-(4-chlorophenyl)-7-methyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-4,6(5*H*,7*H*)-dione **(51)**.



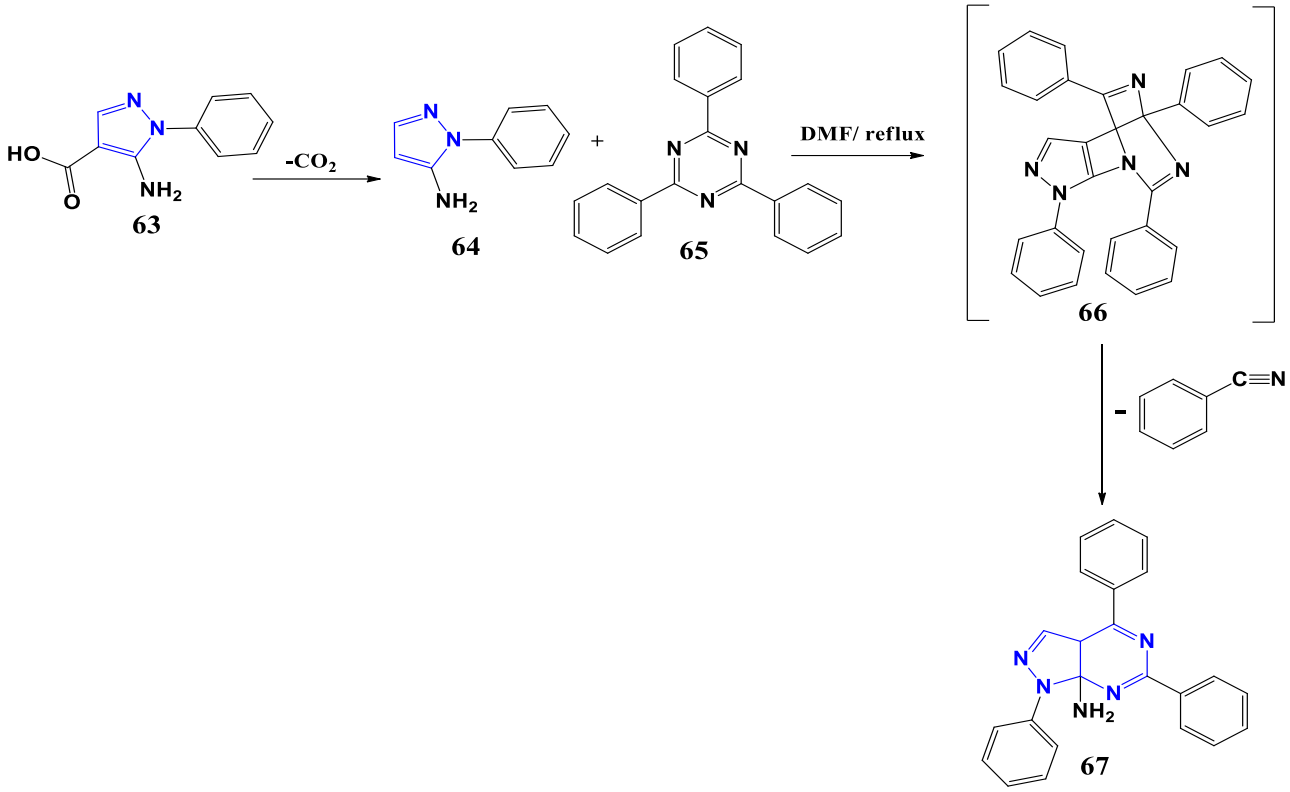
Scheme 22: Synthesis of 4-(4-fluorophenyl)-6-imino-6,7-dihydro-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-amine **(54)**.



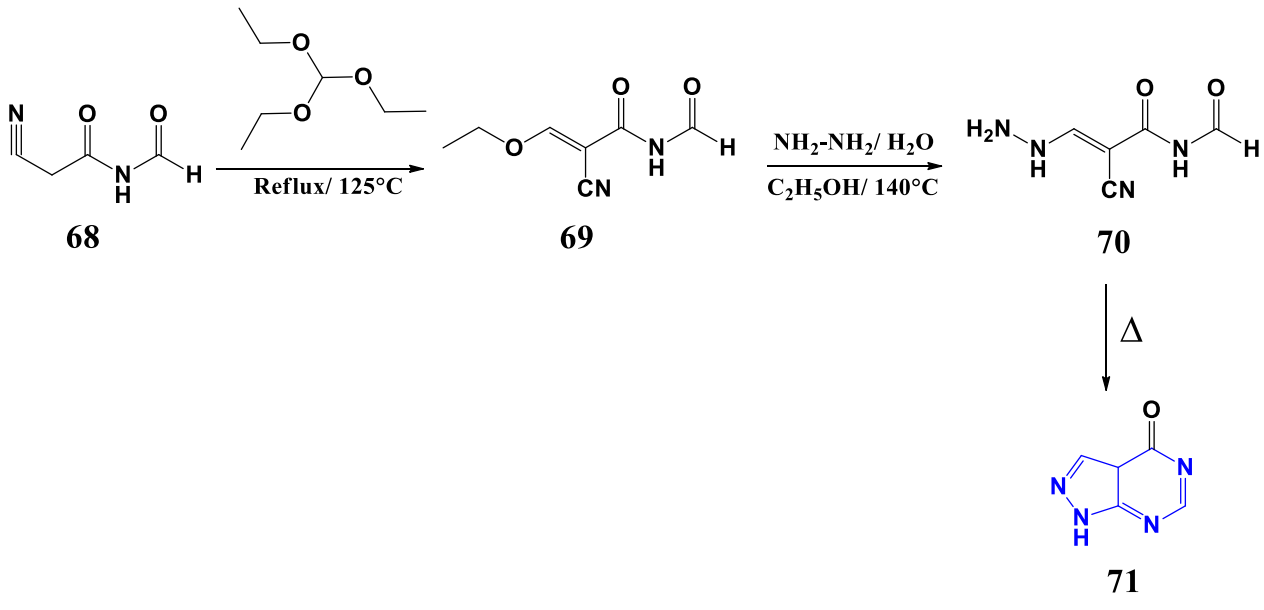
Scheme 23: Synthesis of 4-(4-chlorophenyl)-6-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidin-3-amine **(58)**.



Scheme 24: Synthesis of 5-selenoxo-pyrazolo[3,4-*d*]pyrimidine derivative **62.**



Scheme 25: Synthesis of pyrazolo[3,4-*d*]pyrimidine derivative **67.**



Scheme 26: Synthesis of pyrazolo[3,4-*d*]pyrimidine derivative **71**.

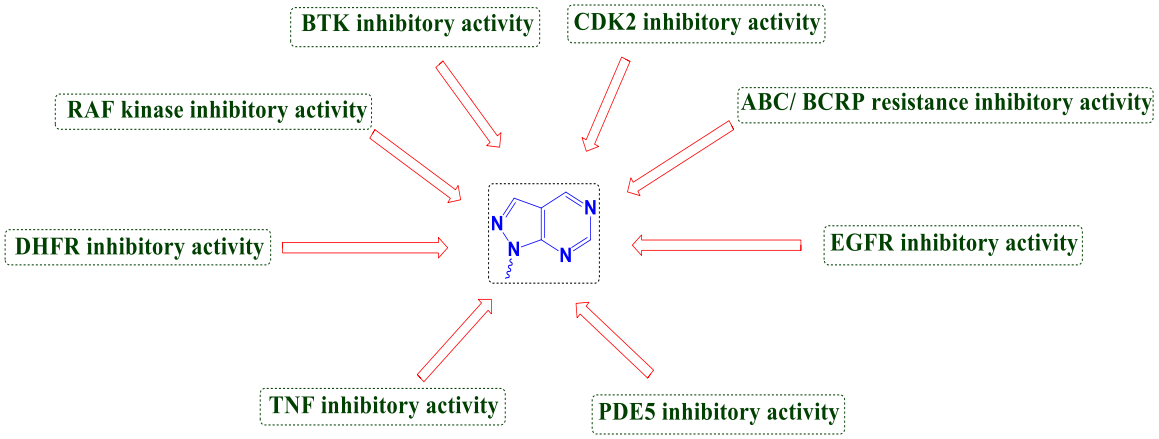
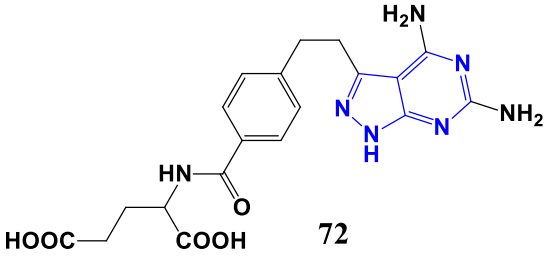


Figure 2: Different targets for pyrazolo[3,4-*d*]pyrimidine derivatives.

3.1. Antineoplastic activity:

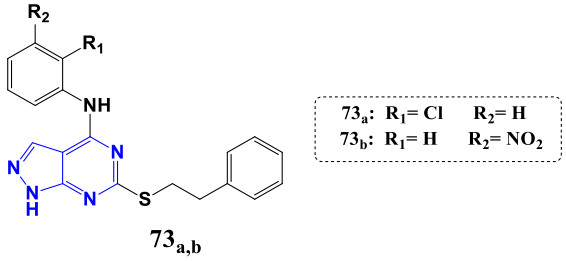
There are several considerable antitumor mechanistic pathways adopted by pyrazolo[3,4-*d*]pyrimidine nucleus **(Asati et al., 2021)** as mentioned in **figure 2**.

A synthesis of pyrazolo[3,4-*d*]pyrimidine compound **72** was carried out with structure similarity to methotrexate and its anti-proliferative activity against different human cell lines was evaluated depending on its (DHFR) inhibition activity. The prepared compound **72** demoed a considerable cytotoxic activity with IC_{50} value of 0.018µg/mL. **(Taylor and Patel, 1992)**



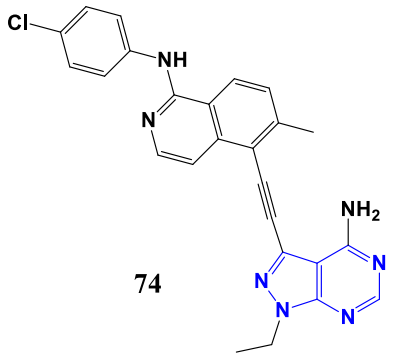
72

Besides, the synthesis of pyrazolo[3,4-*d*]pyrimidines **73$_{a,b}$** bearing various aniline derivatives at C_4-position and phenethylthio moiety at C_6-position was carried out. The synthesized compounds **73$_{a,b}$** were assayed for their *in vitro* CDK2 inhibitory activity and the results revealed their fascinating inhibitory effect with IC_{50} values of 5.1 µM for compound **73$_b$** and 7.8 µM for compound **73$_a$**. **(Cherukupalli et al., 2018)**



73$_{a,b}$

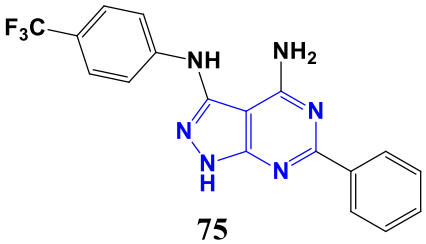
Moreover, pyrazolo[3,4-*d*]pyrimidine scaffold carrying isoquinoline moiety **74** was designed and tested as anti-propagative drug against (A375) cell line harboring the BRAF (V600E) mutant that is responsible for excessive cell signaling. The results demonstrated the ability of compound **74** to inhibit the proliferation of (A375) cells by IC_{50} value of 127 nM. **(Assadieskandar et al., 2019)**



74

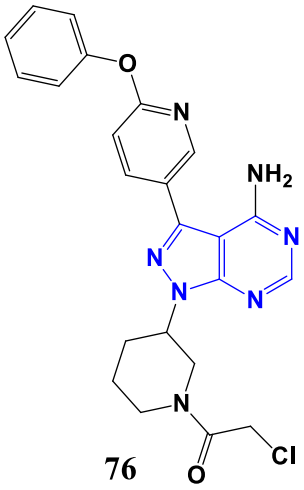
Additionally, 6-phenyl-*N*-[4-(trifluoromethyl)phenyl]-1*H*-pyrazolo[3,4-*d*]pyrimidine-3,4-diamine **(75)** was synthesized and evaluated for its anti-BCRP activity to avoid

multidrug resistance of different chemotherapeutic agents. The synthesized compound **75** restored the mitoxantrone drug sensitivity of (MDCK-II) cell lines that expressed high level of BCRP causing mitroxantrone drug resistance if used alone. **(Ambjørner et al., 2020)**



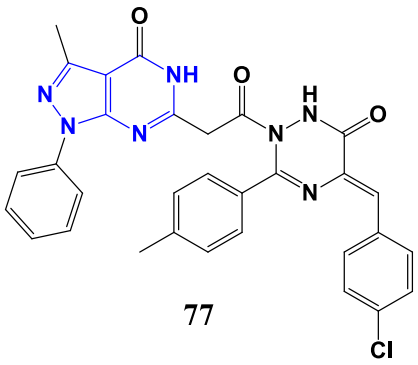
75

The compound phenoxy-3-pyridinyl pyrazolo[3,4-*d*]pyrimidine derivative **76** was synthesized and proved for its potent BTK inhibitory activity. The ability of compound **76** to inhibit the phosphorylation of BTK enzyme was explained in different (MCL) cell lines and therefore, it exhibited potent anti-proliferative activity against (MCL) cell lines with IC_{50} values lower than 1 µM. **(Ran et al., 2020)**



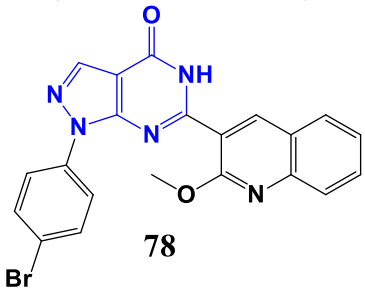
76

Also, the pyrazolo[3,4-*d*]pyrimidine/1,2,4 triazine hybrid **77** and was synthesized and its cytotoxic activity was estimated against colon (HCT-116) and breast (MCF-7)cell lines depending on its EGFR inhibitory activity. The prepared compound **77** showed cytotoxic activity against the mentioned cell lines with IC_{50} values of 4.80 nM. **(Lamie et al., 2021)**



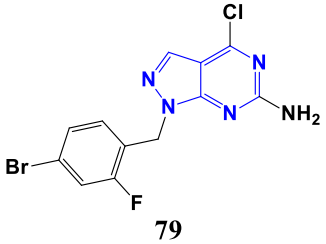
77

Furthermore, pyrazolo[3,4-*d*]pyrimidin-4-one derivative bearing quinoline scaffold **78 was** designed and evaluated as potent inhibitor of PDE-5 and inducer of apoptotic activity. The synthesized compound **78** exhibited a prominent PDE-5 inhibitory activity (with IC_{50}=1.57 nM). They demonstrated that compound **78** induced an intrinsic apoptotic pathway that was evidenced by the lower expression levels of the anti-apoptotic Bcl-2 protein. **(Ibrahim et al., 2020)**



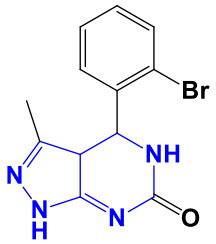
78

Besides, pyrazolo[3,4-*d*]pyrimidine-6-amine **79** was reported and its mitochondrial permeability was elucidated along with, its inhibition activity against TNF Receptor Associated Protein 1 (TRAP1) that is responsible for abnormal mutations in different cell lines. As well, they proved the higher plasma and metabolic stability of compound **79** and its CYP acceptable inhibition. **(Kim et al., 2020)**



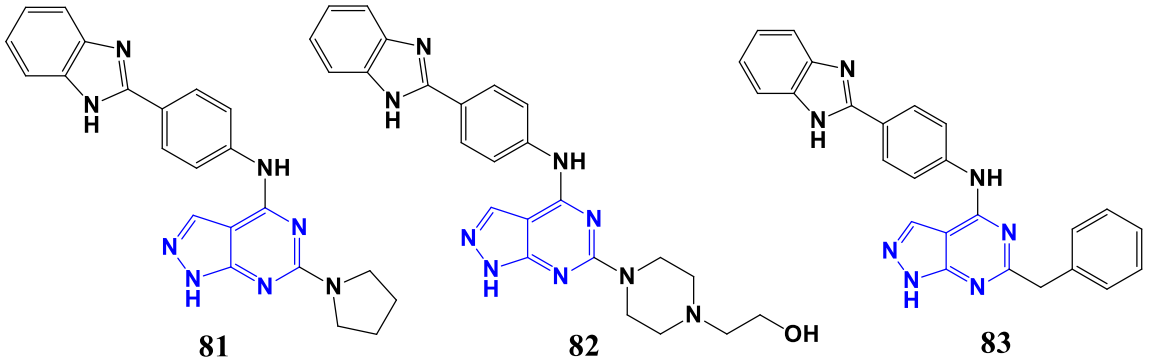
79

The synthesis of bromophenyl pyrazolo[3,4-*d*]pyrimidinone derivative **80** was carried out and its anti-EGFR activity was evaluated. The results revealed that compound **80** showed significant anti-EGFR activity with IC_{50} of 0.09µM that is 10 fold more than erlotinib reference drug (IC_{50}=10.6µM). **(Saleh et al., 2020)**



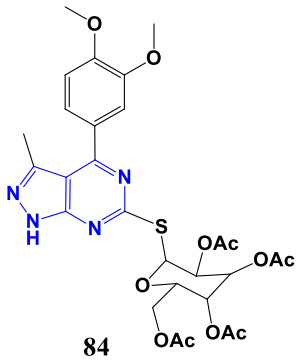
80

On the other hand, pyrazolo[3,4-*d*]pyrimidines and their derivatives are suggested to elucidate cytotoxic activity against different human cell lines with unclear mechanisms. A series of pyrazolo[3,4-*d*]pyrimidine incorporating 4-(1*H*-benzimidazol-2-yl)-phenylamine moiety at C_4 **81-83** was designed and their cytotoxic activities were evaluated against different cancer cell lines at National Cancer Institute (NCI). The synthesized compounds **81-83** proved to be the most active against different cancer cell lines with GI_{50} values of 1.30, 1.43 and 2.38 µM respectively. They deduced that the cytotoxic activity of compounds **81-83** might be due to topoisomerase-II inhibitory activity. **(Singla et al., 2016)**



81 **82** **83**

Moreover, the pyrazolo[3,4-*d*]pyrimidine derivative **84** that is S-glycosidically linked to cyclic

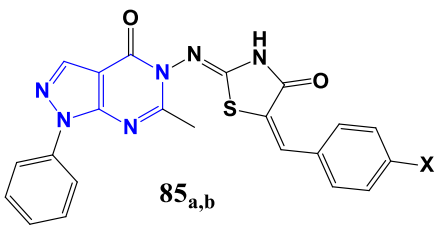


84

acetylated glucose was prepared and estimated for its anti-neoplastic activity against three tumor cell lines HEPG2 (liver), HCT116 (colon) and MCF-7 (breast). The results demonstrated that compound **84** showed a significant antitumor activity with approximate IC_{50} value of 0.39 µM comparable to doxorubicin (IC_{50}=4 µM). **(Nassar et al., 2017)**

3.2. Anti-inflammatory activity:

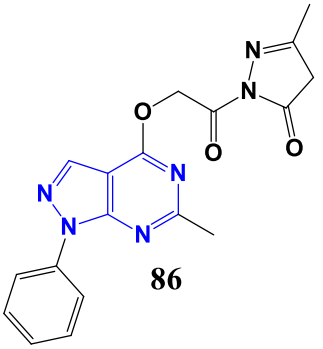
Numerous scientific researches demoed a remarkable anti-inflammatory activity of the pyrazolo[3,4-*d*]pyrimidine nucleus **(Atatreh et al., 2019)**. Pyrazolo[3,4-*d*]pyrimidine incorporating benzylidene thiazolidinone derivatives **$85_{a,b}$** was designed and it showed preferential COX-2 inhibitory activity with IC_{50}value of 0.53 µM which is more potent than meloxicam as a standard drug. **(Kadry, 2014)**



$85_{a,b}$

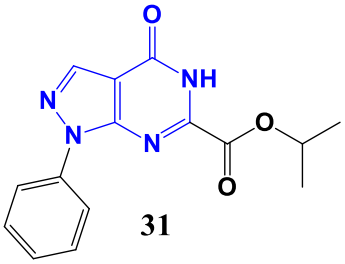
85_a: X= F
85_b: X= Cl

Moreover, A pyrazolo[3,4-*d*]pyrimidine scaffold carrying pyrazolone derivative 86 was reported and it elucidated more potential inhibition of COX-2 than COX-1. In addition, it showed higher edema inhibition percentage activities (34–68%). (Bakr et al., 2016)



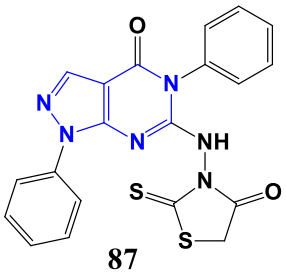
86

Additionally, a synthesis of pyrazolo[3,4-*d*]pyrimidinone analogue **31** was carried out and it was clear that it demoed a remarkable anti-inflammatory activity and a prominent COX-2 inhibition activity with IC_{50} value of 42 µM. **(Atatreh et al., 2019)**

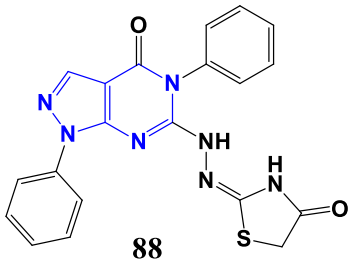


31

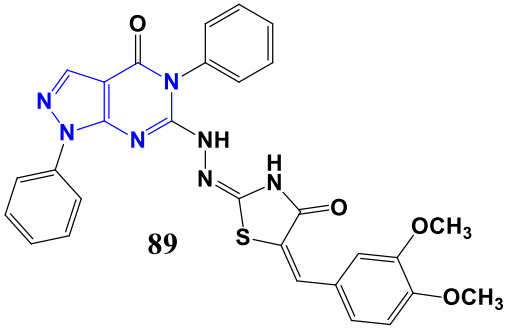
Later on, pyrazolo[3,4-*d*]pyrimidine bearing thiazolidinone derivatives 87-89 was synthesized and there *in vitro* (COX1 and COX2) inhibitory assay was evaluated. Compounds 87-89 displayed anti-inflammatory activity higher than diclofenac reference drug using the formalin induced paw edema model. (Tageldin et al., 2018)



87



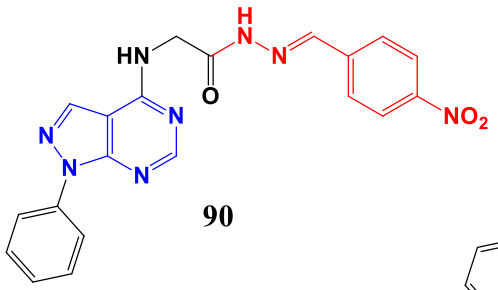
88



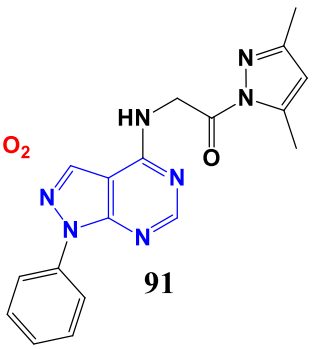
89

3.3. Antimicrobial activity:

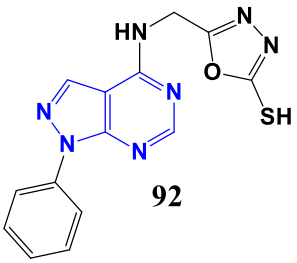
It was reported that pyrazolo[3,4-*d*]pyrimidines encompass prominent antibacterial and antifungal activity[3]. The 4-substituted-1-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine derivatives incorporating hydrazone **90**, pyrazole **91** and 1,3,4 oxadiazole **92** exhibited broad spectrum antimicrobial activity against gram positive bacteria: *Streptococcus pneumonia*, gram negative bacteria: *Pseudomonas aeruginosa* and *Escherichia coli* and fungi: *Aspergillus fumigatus* and *Candida albicans strains.* **(Samy and Mostafa, 2017)**



90

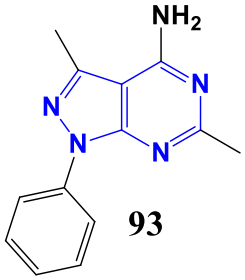


91



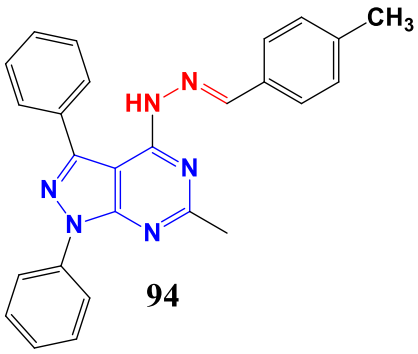
92

Furthermore, the 4-amino-pyrazolo[3,4-*d*]pyrimidine derivative **93** exhibited a significant inhibitory effect against *Streptococcus pyogenes* and *Pseudomonas aeruginosa* with MIC value of 32µg/mL. **(Beyzaei et al., 2017)**



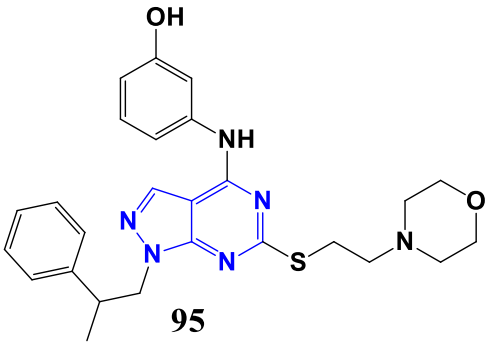
93

In addition, pyrazolo[3,4-*d*]pyrimidine compound bearing hydrazone **94** was synthesized and its antimicrobial activity was screened against *Bacillus subtilis* and *Pseudomonas aeruginosa*. It was revealed that compound **94** exerted high efficacies against the previously mentioned strains with MIC values of 40 and 60µg/mL respectively. **(Hassaneen, 2019)**

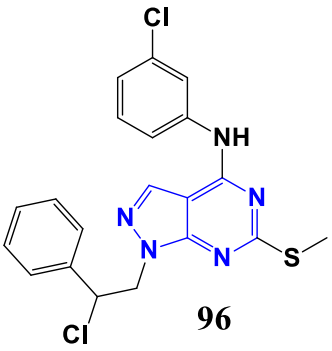


94

In addition, the pyrazolo[3,4-*d*]pyrimidine derivatives **95, 96** potential antibacterial activity was scored against *Staphylococcus aureus* and *Escherichia coli*[53]. The results showed that compound **95** was capable of almost completely inhibiting the growth of *Staphylococcus aureus* with MIC value of (200 µg/mL). However, compound **96** was able to halve *Escherichia coli* bacterial growth at MIC value of (50 µg/mL). **(Greco et al., 2020)**



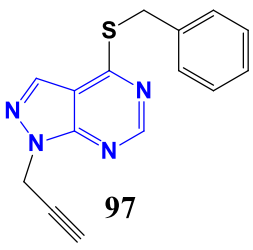
95



96

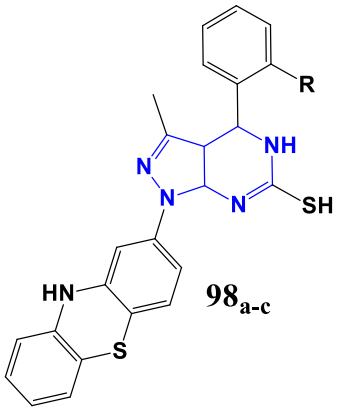
3.4. Anti-mycobacterial activity:

Synthesis benzylthio derivative of pyrazolo[3,4-*d*]pyrimidine **97** was carried out and the anti-tuberculosis evaluation was proved. Compound **97** exhibited a remarkable activity. **(Moukha et al., 2000)**



97

Additionally, a phenothiazine clubbed pyrazolo[3,4-*d*]pyrimidines **98$_{a-c}$** was developed and the ability of them to inhibit the growth of *Mycobacterium tuberculosis in vitro* was determined and the results revealed that compounds **98$_{a-c}$** showed a brilliant anti-mycobacterial activity at MIC values which were less than 6.25 µg/ml.**(Trivedi et al., 2010)**

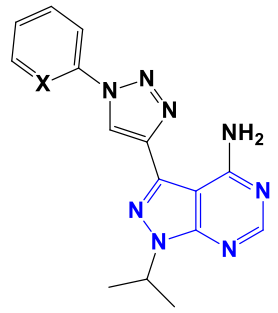


98_{a-c}

98_a: R= -OH
98_b: R= -Cl
98_c: R= -NO_2

4.5. Anti-malarial activity:

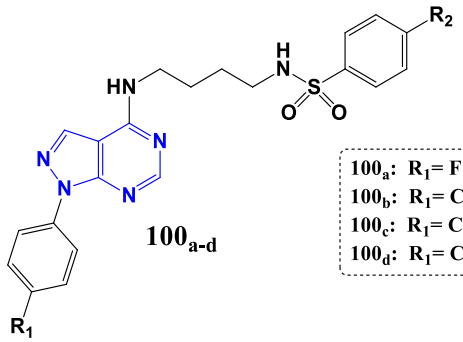
It was found that pyrazolo[3,4-*d*]pyrimidine nucleus exhibits a marked anti-malarial activity. The synthesis of 3-(1,2,3-triazol-4-yl)-substituted pyrazolo[3,4-*d*]pyrimidin-4-amines $\mathbf{99_{a,b}}$ was carried out and their anti-malarial activity against *plasmodium falciparum* was evaluated depending on their inhibition activity of *plasmodium falciparum* protein kinase (PfPK7). The two compounds $\mathbf{99_{a,b}}$ demoed a preferred inhibitory activity towards PfPK7 kinase (IC_{50}=10–20 µM). **(Klein et al., 2009)**



$99_{a,b}$

99_a: X= N
99_b: X= CH

Also, the pyrazolo[3,4-*d*]pyrimidine derivatives bearing benzene sulfonamide moiety through a flexible butyl amino side chain compounds $\mathbf{100_{a-d}}$ were screened as anti-malarial drugs against *plasmodium falciparum*. The result demonstrated that designed compounds $\mathbf{100_{a-d}}$ showed *in vitro* growth inhibitory activity against the chloroquine-resistant *P. falciparum* clones with IC_{50} values ranging from 5.13 to 43.40µM. **(Silveira et al., 2018)**

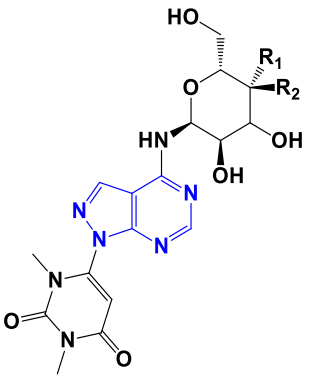


100_{a-d}

100_a: R_1= F R_2= F
100_b: R_1= Cl R_2= F
100_c: R_1= CH_3 R_2= F
100_d: R_1= CH_3 R_2= CH_3

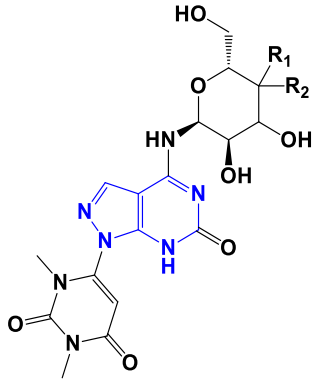
3.6. Anti-viral activity:

Pyrazolo[3,4-*d*]pyrimidines accepted a great biological importance as antiviral drugs due to their structure similarity with purine bases **(Ettahiri et al., 2012)**. It was reported that, the N_4-β-D-glycoside pyrazolo[3,4-*d*]pyrimidine derivatives $\mathbf{101_{a,b}}$ and $\mathbf{102_{a,b}}$ exhibited resplendent antiviral activity against hepatitis-B virus (HBV) with an effective MIC value of 0.2 µM. **(El-sayed et al., 2009)**



$101_{a,b}$

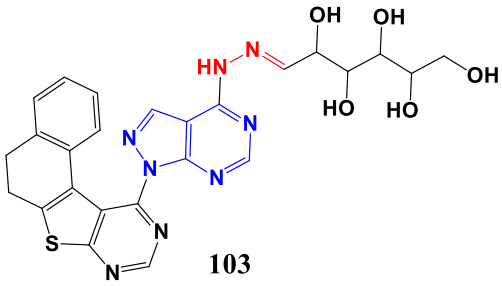
101_a: R_1= H R_2= OH
101_b: R_1= OH R_2= H



$102_{a,b}$

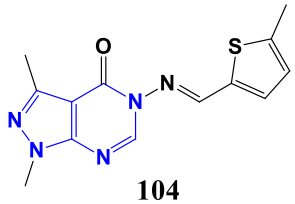
102_a: R_1= H R_2= OH
102_b: R_1= OH R_2= H

Moreover, D-glucose hydrazone derivative of pyrazolo[3,4-*d*]pyrimidine **103** was synthesized and its antiviral activity against herpes simplex virus (HSV-1) was described. The experimented results showed that compound **103** displayed an inhibition ratio of 99% at concentration of 20 µg/10^5 cells of (HSV-1) experimented cell lines. **(Rashad et al., 2009)**



103

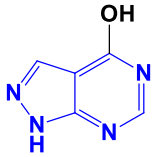
Also, pyrazolo[3,4-*d*]pyrimidine compound **104** containing schiff's base was evaluated as antiviral drug against tobacco mosaic virus (TMV). The results revealed that compound **104** displayed outstanding inactivating potential against (TMV) with half maximal effective concentration (EC_{50}) value of 53.65 µg/mL which is much better than that of ribavirin (150.45 µg/mL). **(Wang et al., 2018)**



104

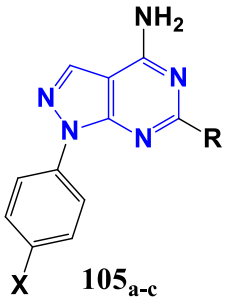
3.7. Xanthine oxidase inhibitory activity:

Pyrazolo[3,4-*d*]pyrimidine is the main nucleus of allopurinol which is the first Food and Drug Administration (FDA) approved inhibitor of xanthine oxidase enzyme for treatment of gout **(Chu and Lynch, 1975)**.



(Allopurinol)

A pyrazolo[3,4-*d*]pyrimidine derivatives **105_{a-c}** were designed and their potential xanthine oxidase inhibition activity were explained comparable with allopurinol as reference drug. **(Oliveira et al., 2008)**



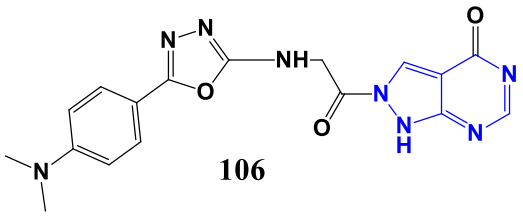
105_{a-c}

105_a: X= H R= 4-C_5H_4N
105_b: X= Br R= 4-C_5H_4N
105_c: X= OH R= -CH_2-C_6H_5

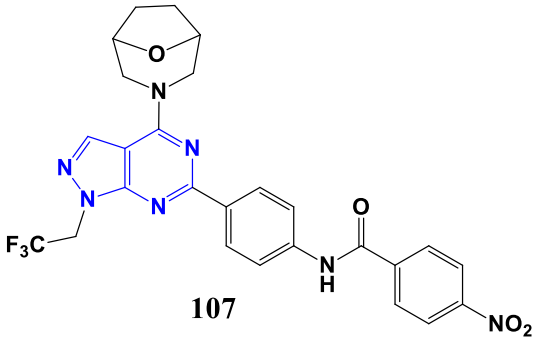
Moreover, the anti-xanthine oxidase activity of pyrazolo[3,4-*d*]pyrimidine compound incorporating a substituted 1,3,4 oxadiazole **106** was investigated and evaluated. The results revealed that compound **106** demonstrated higher xanthine oxidase inhibition activity of (IC_{50}=1.32 ± 0.05 µM) comparable to reference drug allopurinol (IC_{50}=2.61 ± 0.07 µM). **(Khammas et al., 2019)**

3.8. Anti-diabetic activity:

The pyrazolo[3,4-*d*]pyrimidine compound **107** bearing bicyclic moiety and benzamide functionality demonstrated superior anti-diabetic activity (IC_{50}=1.60± 0.48µM) over acarbose as reference drug (IC_{50}=1.73 ± 0.05 µM). **(Reddy et al., 2019)**



106



107

4. Conclusion

Pyrazolo[3,4-*d*]pyrimidine derivatives exhibited many pharmacological activities mainly as anti-inflammatory, antimycobacterial, antimicrobial, anticancer, antiviral, antidiabetic, anti-mycobacterial, antimalarial and xanthine oxidase inhibitors. This review focus on the pharmacological activity of this ring showing the newly prepared lead compounds containing pyrazolo[3,4-*d*]pyrimidine ring system with the aim at opening the way for researchers for the development of many pharmacological activity of pyrazolo[3,4-*d*]pyrimidine scaffold. Furthermore, we recorded the advanced strategies used to synthesize this ring system.

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