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# An Overview of Pyrazolopyrimidine Hybrids with Their Biological Activities

**Dolphy Anto[1], Menna Chandran[2], Ashly George[3], Jismy Francis[4], Vishnu P V[5], Silvipriya K S[6], K Krishna Kumar[7]**

[1,2,3,4,5,6,7]Department of Pharmaceutical Chemistry, St. James' College of Pharmaceutical Sciences and St. James' Hospital Trust Pharmaceutical Research Centre (DSIR Recognized), Kerala, India, Pin code-680307

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## ABSTRACT

**A pyrazole's fused N-heterocyclic systems are known as pyrazolopyrimidines. Which have many important applications, such as medicinal, pharmaceuticals, pesticides, dyes, and pigments and they are regarded as a key structural motif. Pyrazolopyrimidines are fused heterocyclic ring systems that are structurally equivalent to adenine, a nucleotide essential for all aspects of cellular life. In recent decades, their synthetic routes have increased significantly. The current review is an examination of various pyrazolopyrimidines with their recent applications. Synthesis of fused pyrimidine compounds was first reported in mid-1950. Since then, thousands of derivatives have been synthesized by applying numerous synthetic strategies and analyzed for their pharmacological properties. It was initially created and tested as adenosine nucleoside analogues for antiviral and anticancer treatments. The pyrazolopyrimidine nucleus used as a pharmacophore in the development of pharmaceuticals, promotes a variety of biological actions.**

**Keywords: Anti-cancer, Purine, Pyrimidine, Pyrazoline.**

## INTRODUCTION

In a cell's typical life cycle, heterocyclic compounds with a nitrogen atom play a crucial role. Pyrazolopyrimidines are a special category of fused heterocyclic compounds that contain nitrogen have a significant portion role in medicinal chemistry. Since pyrazolopyrimidine is similar to the adenine base in DNA, it always leaves a distinct pharmacophore thumbprint. As a purine isostere, the pyrazolo-pyrimidine ring system has received a lot of attention (Poulsen and Quinn, 1996).

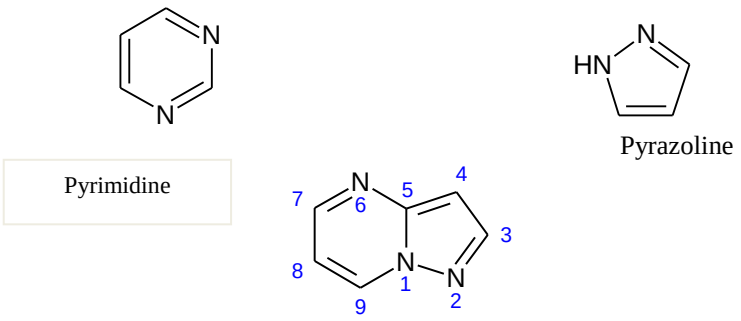


**Fig 1: Structure of Pyrimidine, Pyrazoline And Pyrazolopyrimidine**

It has been documented in the literature that pyrazolopyrimidines have a wide range of pharmacological properties, including those of antimicrobial [1] [2], anticancer [7] [8], anti-inflammatory [4] [5] [6], anti-malarial [9] [10], antidiabetic [11]. Through the inhibition of various targets, various biological investigations and recent developments of compounds related to the pyrazolopyrimidine ring have led to the development of potentially active compounds as anti-neoplastic agents. However, we were able to demonstrate the various biological functions used by the pyrazolopyrimidine ring system in this review of literature.

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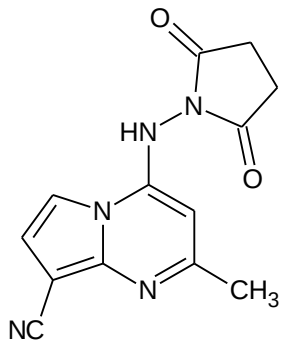
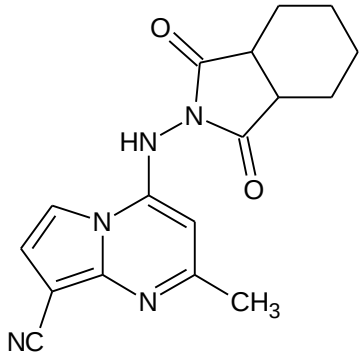
## BIOLOGICAL ACTIVITY

### Antimicrobial

**Someshwar Deshmukh *et al*., (2016)** were synthesized appropriately functionalized pyrazolo[1,5-a] pyrimidines by using the agar cup plate method to assess the synthetic compounds' antimicrobial properties. The antibacterial and antifungal assays were carried out in the appropriate Czapek Dox and Muller-Hinton broths. While *Candida albicans* MTCC 277, *Candida tropicalis* MTCC 184, *Aspergillus niger* MCIM 545, and *Aspergillus clavatus* MTCC 1323 were used as standard fungal strains, the antibacterial activity of tested samples was investigated against one Gram positive *Bacillus subtilis* NCIM 2250 and Gram-negative *Escherichia Coli* ATCC 25922 bacteria. Compound 7-(2,5-Dioxopyrrolidin-1-ylamino)-5-methylpyrazolo [1, 5-a] pyrimidine-3-carbonitrile was discovered to be a potent antibacterial agent, and compound 7-(Hexahydro-1, 3-dioxo-1H-isoindol-2(3H)-yl amino)- 5-methylpyrazolo [1, 5-a] pyrimidine-3-carbonitrile to be a superb antifungal agent. [1]



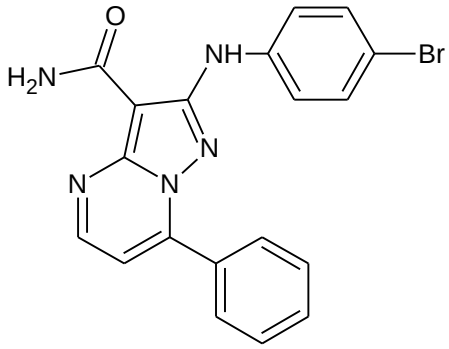

7-(2,5-Dioxopyrrolidin-1-ylamino)-5-methylpyrazolo [1, 5-a] pyrimidine-3-carbonitrile



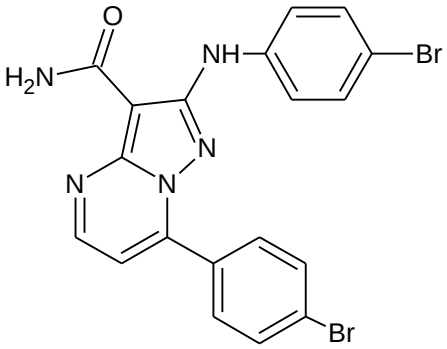

7-(Hexahydro-1, 3-dioxo-1H-isoindol-2(3H)-ylamino)- 5-methylpyrazolo [1, 5-a] pyrimidine-3-carbonitrile

**Fig 2: Anti-microbial activity of 5-methylpyrazolo [1, 5-a] pyrimidine-3-carbonitrile derivatives**

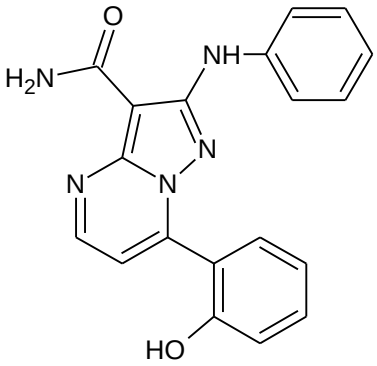
**Amira EM Abdallah *et al*., (2018)** have mentioned about twenty-two novel pyrazolo[1,5-a] pyrimidine derivatives and their corresponding cycloalkane ring-fused derivatives were prepared through the reaction of 5-aminopyrazoles with different sodium salts of (hydroxymethylene) cycloalkanones and sodium salts of unsaturated ketones. The study used to assess each newly synthesized product's reactivity to various bacterial and fungal species. Bactericidal activity is present in pyrazolopyrimidine compounds against MurC enzymes for both Gram-positive and Gram-negative bacteria. The most effective compounds against Gram-positive and Gram-negative bacterial species were discovered to be compounds 2-((4-Bromophenyl) amino)-7-phenylpyrazolo[1,5-a] pyrimidine-3-carboxamide, 2-((4-Bromophenyl) amino)-7-phenylpyrazolo[1,5-a] pyrimidine-3-carboxamide, and 7-(2-Hydroxyphenyl)-2-(phenylamino) pyrazolo[1,5-a] pyrimidine-3-carboxamide.[2]




2-((4-Bromophenyl) amino)-7-phenylpyrazolo[1,5-a] pyrimidine-3-carboxamide




2-((4-Bromophenyl) amino)-7-phenylpyrazolo[1,5-a] pyrimidine-3-carboxamide

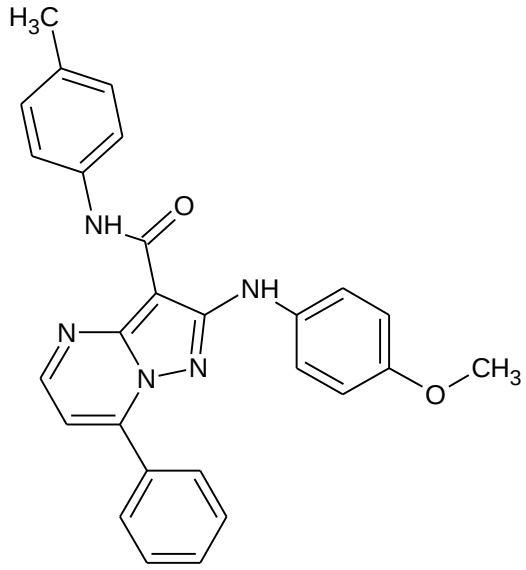


7-(2-Hydroxyphenyl)-2-(phenylamino) pyrazolo[1,5-a] pyrimidine-3-carboxamide

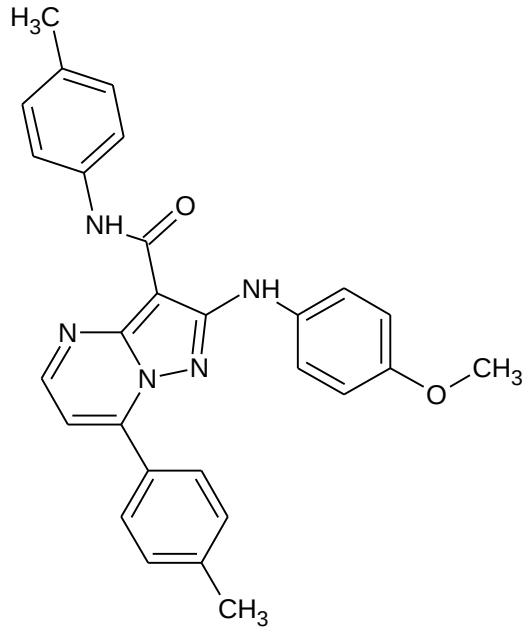
**Fig 3: Anti-Microbial Activity of 7-Phenylpyrazolo [1,5-A] Pyrimidine-3-Carboxamide Derivatives**

**Immunomodulatory**

**Ahmed M Naglah *et al*., (2020)** had done the biological evaluation and molecular docking with *in-silico* physicochemical, pharmacokinetic and toxicity prediction of pyrazolo[1,5-a] pyrimidines. An *in-vitro* test was used to look into the immunomodulatory potential of the active pyrazolopyrimidine compounds. From the antimicrobial results, the strongest compounds were selected to test their immunomodulatory activity because it was thought that these substances might serve more than one purpose. For many different infections, neutrophils serve as the main effecting or killer cell. The nitro blue tetrazolium (NBT) reduction test was used to evaluate the active pyrazolopyrimidine compounds 5,7-dimethylpyrazolo[1,5-a] pyrimidines derivative and 5,7-dihydroxypyrazolo[1,5-a]pyrimidines derivative had the highest immunostimulatory actions, with respective values of 136.5 0.3 and 129.8 0.47.[3]



5,7-dimethylpyrazolo[1,5-a] pyrimidines



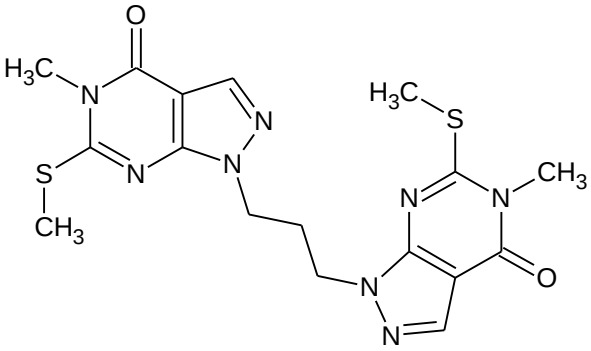
5,7-dihydroxypyrazolo[1,5-a] pyrimidines derivative

Fig 4: Immunomodulatory activity of pyrazolo[1,5-a] pyrimidines derivatives

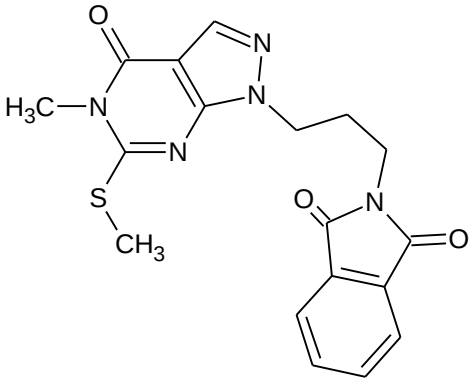
**Anticoagulation and Anti Inflammatory**

**Umesh Yadava *et al*., (2013)** discussed on molecular docking studies on pyrazolo[3,4-d] pyrimidines as inhibitor of phospholipase A2's anti-coagulation and anti-inflammatory activities. Using the AUTODOCK and GLIDE (Standard precision and Extra precision) modules, nine pyrazolo[3,4-d] pyrimidine molecules were in-silico docked with the X-ray

crystal structure of Russell's viper PLA2 (PDB ID: 3H1X) to predict the binding affinity, molecular recognition, and to explicate the binding modes. It is clear from the docking results obtained using each technique that pyrazolo[3,4-d] pyrimidine molecules with a trimethylene linker can bind to the PLA2 enzymatic and anti-coagulation regions. Based on these calculations, it has been determined that pyrazolo[3,4-d] pyrimidine 3 and that pyrazolo[3,4-d] pyrimidine 7 have greater potential than indomethacin for inhibiting vPLA2's anti-coagulation and inflammatory activities.[4]



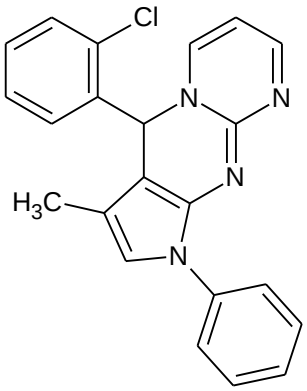
Pyrazolo[3,4-d] pyrimidine derivative 3



Pyrazolo[3,4-d] pyrimidine derivative7

**Fig 5: Anti-Coagulant and Anti-Inflammatory Activity of Pyrazolo[3,4-D] Pyrimidine Derivatives**

**Priyanka T Patil *et al*., (2017)** studied on one-pot novel synthesis of pyrazolo[3,4-b] [1,8] naphthyridine and pyrazolo[3,4-d] pyrimido[1,2-a] pyrimidine derivatives as anti-inflammatory agent. Inflammatory-reduction capacity using a carrageenan-induced paw edema assay in rats, the newly synthesized compounds were tested for their *in vivo* anti-inflammatory activity. Diclofenac sodium served as the reference compound. Carrageenan was used to cause paw edema, and the outcomes were compared to those of diclofenac, the reference medication. Rats pretreated with the tested compounds had the thickness of their edema measured every hour up to five hours after the induction of inflammation. The best performance (86%) was achieved by the compound 4-(2-chlorophenyl)-3-methyl-1-phenyl-1,4-dihydropyrazolo[3,4-d] pyrimido[1,2-a] pyrimidine. The key molecule in this study acting as an anti-inflammatory agent has been discovered to be pyrimidine derivative. [5]

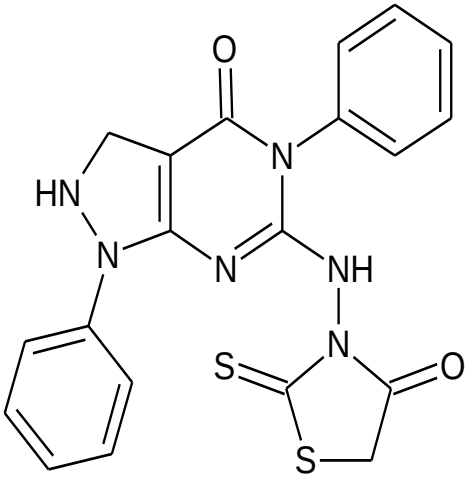


4-(2-chlorophenyl)-3-methyl-1-phenyl-1,4-dihydropyrazolo[3,4-d] pyrimido[1,2- a]pyrimidine

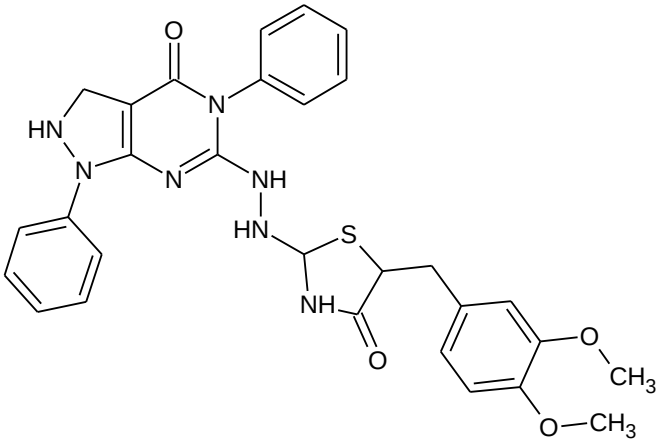
**Fig 6: Anti-inflammatory activity of 4-(2-chlorophenyl -1, 4-dihydropyrazolo[3,4-d]pyrimido[1,2a]pyrimidine**

**Tageldin et al., (2018)** Studied on synthesis and evaluation of some pyrazolo[3,4-d] pyrimidine derivatives bearing thiazolidinone moiety as anti-inflammatory agents**.** The COX-1 and COX-2 inhibitory assay of the newly synthesized compounds was assessed. Following *in- vivo* anti-inflammatory screening using formalin-induced paw edema (acute model) and cotton-pellet-induced granuloma (chronic model) assays with celecoxib and diclofenac sodium as reference drugs, compounds that demonstrated promising COX-2 selectivity were tested. Thiazolidinone derivatives 6-(4-Oxo-2-thioxo-1,3-thiazolidin-3-yl)amino-1,5-diphenyl-1H-pyrazolo[3,4- d]pyrimidin-4(5H)-one, 6-{2-(5-(3,4-

Dimethoxybenzylidene)-4-oxo-1,3-thiazolidin-2-ylidene)}hydrazinyl-1,5- diphenyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-onebearing pyrazolo[3,4-d]pyrimidine were created, and their *in-vitro* (COX1 and COX2) inhibitory assay was assessed. Using the formalin-induced paw edema model, compounds 6-(4-Oxo-2-thioxo-1,3-thiazolidin-3-yl) amino-1,5-diphenyl-1H-pyrazolo[3,4-d] pyrimidin-4(5H)-one,6-{2-(5-(3,4-Dimethoxybenzylidene)-4-oxo-1,3-thiazolidin-2-ylidene)} hydrazinyl-1,5-diphenyl-1H-pyrazolo[3,4-d] pyrimidin-4(5H)-one showed greater anti-inflammatory activity than the diclofenac reference drug. [6]




6-(4-Oxo-2-thioxo-1,3-thiazolidin-3-yl) amino-1,5-diphenyl-1H-pyrazolo [3,4- d] pyrimidin-4(5H)-one



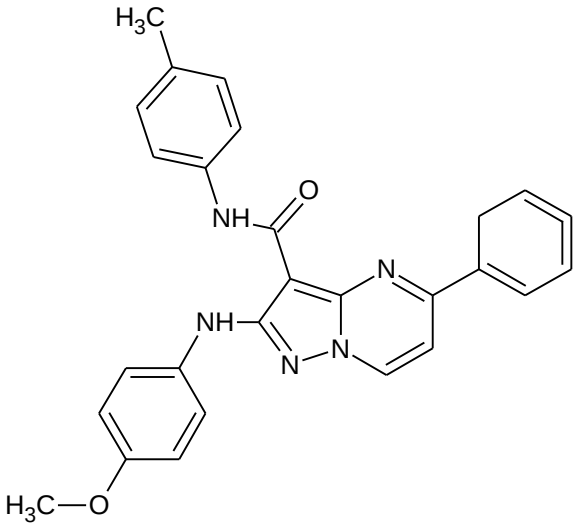

6-{2-(5-(3,4-Dimethoxybenzylidene)-4-oxo-1,3-thiazolidin-2-ylidene)} hydrazinyl-1,5- diphenyl-1H-pyrazolo[3,4-d] pyrimidin-4(5H)-one

**Fig 7: Anti-inflammatory activity of 1, 3-thiazolidine 1, 5- diphenyl-1H-pyrazolo[3,4-d] pyrimidin-4(5H)-one derivatives**

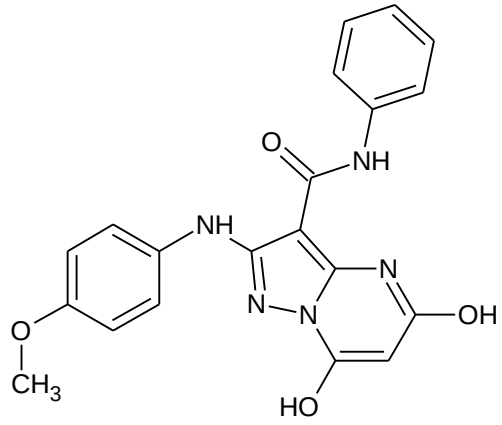
**Anticancer**

**Eman M Oath *et al*., (2021)** discussed on Design, Synthesis, and Anticancer Screening for Repurposed Pyrazolo[3,4-d] pyrimidine derivatives on four mammalian cancer cell lines**.** Using MTT assays, the viability of A549 and Caco-2 cells was evaluated. The compounds of interest were incubated with cells in triplicates for 48 hours. The MTT assay demonstrates how the drug affects the viability and growth of various cancer cell lines. Crystal violet staining was used to measure the cytotoxic effect at 595 nm. Each plate contained a triplicate of cells treated with DMSO, the compound used to calculate 100% viable cells, in order to normalize cell viability values. In order to demonstrate maximum cell death and 0% viability,

a triplicate of cells were simulated with a cytotoxic combination (200 ng/mL TNF, 200 ng/mL CD95L, 200 ng/mL TRAIL, 25 g/mL CHX, and 1% (w/v) sodium azide). The averages of these triplicates were used to normalize all other viability values, which were then assessed using the Graph Pad Prism 5 program (La Jolla, California, USA). The goal of the study was to synthesize and assess some new pyrazolo[3,4-d] pyrimidines' anti-cancer properties. The obtained results unmistakably demonstrated that compounds 2-(4-Methoxybenzylidene)-1-(1-phenyl-1H-pyrazolo[3,4-d] pyrimidin-4-yl) hydrazine and 2-(3,4,5-Trimethoxybenzylidene)-1-(1-phenyl-1H-pyrazolo[3,4-d] pyrimidin4yl) hydrazine are promising anti-cancer agents against cancer cell lines, and as a result, they represent a useful matrix deserving of further study and derivatization. [7]




2-(4-Methoxybenzylidene)-1-(1-phenyl-1H-pyrazolo[3,4-d]




2-(3,4,5-Trimethoxybenzylidene)-1-(1-phenyl-1H-pyrazolo[3,4-d] pyrimidin4yl) hydrazine

**Fig 8: Anti-cancer activity of 1-phenyl-1H-pyrazolo[3,4-d] pyrimidin-4-yl) hydrazine derivatives**

**Kim *et al.*, (2002)** discussed about development of pyrazolo[3,4-D] pyrimidine-6-amine-based TRAP1 inhibitors that demonstrate *in-vivo* cancer activity in mouse xenograft models. A mitochondrial paralog of Hsp90 known as TNF Receptor Associated Protein 1 (TRAP1) is linked to the promotion of tumorigenesis in a number of cancers by preserving mitochondrial integrity, lowering the production of reactive oxygen species, and reprogramming cellular metabolism. As a result, Hsp90 and TRAP1 have been chosen as targets for the creation of cancer therapeutics. In this article they present a group of mitochondria-permeable TRAP1 inhibitors called pyrazolo[3,4-d] pyrimidine derivatives. The mitochondrial permeability of pyrazolo[3,4-d] pyrimidine-6-amine was reported, and its inhibition activity against TNF Receptor Associated Protein 1 (TRAP1), which causes abnormal mutations in various cell lines, was also clarified. Additionally, they demonstrated that the above compound possesses higher metabolic and plasma stability also.[8]

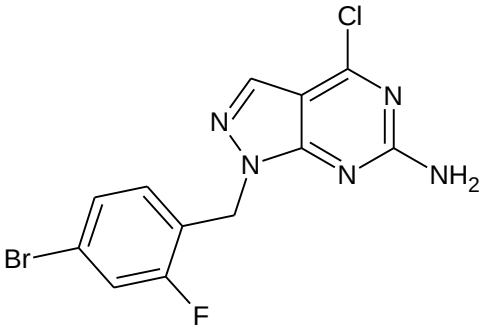
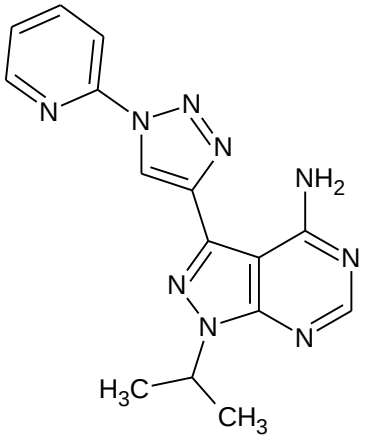


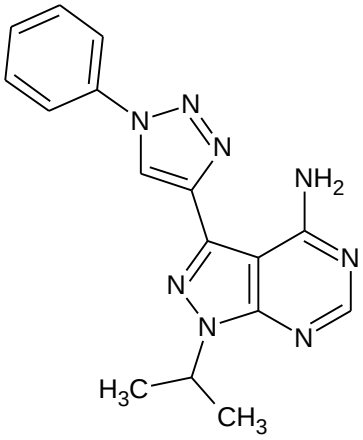

**Fig 9: Anti-Cancer Activity Of Pyrazolo[3,4-D] Pyrimidine-6-Amine**

**Anti Malarial**

**Klein *et al.*, (2009)** Synthesized of 3-(1,2,3-triazol-1-yl)- and 3-(1,2,3-triazol-4-yl)-substituted pyrazolo[3,4-d] pyrimidin-4-amines are potential inhibitors of the *Plasmodium falciparum* PfPK7 protein kinase. The pyrazolo[3,4-d] pyrimidine nucleus was discovered and shows anti-malarial activity. Based on their ability to inhibit the activity of the *plasmodium falciparum* protein kinase (PfPK7), the 3-(1,2,3-triazol-4-yl)-substituted pyrazolo[3,4-d] pyrimidin-4-amines were assessed as anti-malarial. The two substances 4-Amino-1-isopropyl-3-(1-phenyl-1H-[1,2,3] triazol-4-yl)-1Hpyrazolo[3,4-d]pyrimidine, 4-Amino-3-(4-benzyl-1H-1,2,3-triazol-1-yl)-1-isopropyl-1Hpyrazolo[3,4-d]pyrimidine (b demonstrated a preference for inhibiting PfPK7. [9]



4-Amino-1-isopropyl-3-(1-phenyl-1H- [1, 2, 3] triazol-4-yl)-1Hpyrazolo[3,4-d] pyrimidine



4-Amino-3-(4-benzyl-1H-1, 2, 3-triazol-1-yl)-1-isopropyl-1Hpyrazolo[3,4-d] pyrimidine

**Fig 10: Anti-Malarial Activity of -Isopropyl-1 Hpyrazolo[3,4-D] Pyrimidine Derivatives**

***Silveira et al., (2018)*** have synthesized novel pyrazolopyrimidines for the evaluation of anti-*plasmodium falciparum*. Additionally, the pyrazolo[3,4-d] pyrimidine derivatives bearing a flexible butyl amino side chain and a benzene sulfonamide moiety were tested for their ability to treat *plasmodium falciparum* malaria. The findings showed that the designed compound 1-phenyl-1*H*-pyrazolo[3,4-*d*] pyrimidine derivatives had *in-vitro* growth inhibitory activity against chloroquine-resistant *P. falciparum* clones, with IC50 values of 5.13.[10]

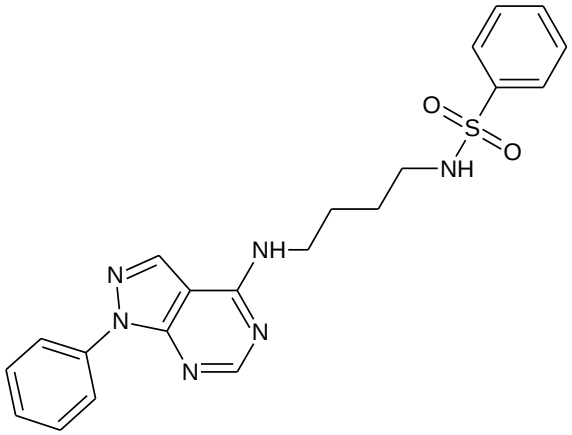
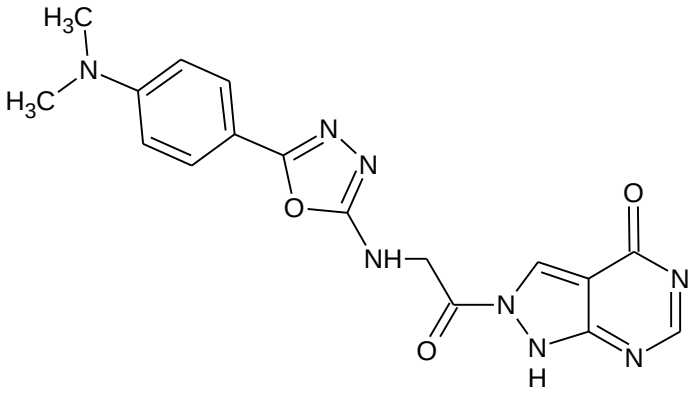


**Fig 11: Anti-Malarial Activity of 1-Phenyl-1H-Pyrazolo [3,4-D] Pyrimidine Derivative**

**2.6 Anti-Diabetic**

**Reddy *et al.*, (2019)** studied on novel pyrazolo[3,4-D] pyrimidine containing amide derivatives. The most effective -amylase inhibitors 8 d and 8 k were also screened for their *in- vivo* antidiabetic activity against alloxan induced diabetic rat model at the dose of 25 and 50 mg/kg. These tested substances were administered orally, and in a dose-dependent manner, they significantly lowered the fasting blood glucose levels. Additionally, biological activities and binding energies derived from docking studies with the -amylase enzyme (PDB ID: 1HNY) show that compounds with a nitro moiety on the phenyl

group made a significant contribution to the antidiabetic activity. Further, the most potent α-amylase inhibitors N, N-Dimethyl derivative of pyrazolo[3,4-D]pyrimidine containing amide and P-Nitro derivative of pyrazolo[3,4-D]pyrimidine containing amide were also screened for their *in- vivo* antidiabetic activity against alloxan induced diabetic rat model at the dose of 25 and 50 mg/kg. [11]



N, N-Dimethyl derivative of pyrazolo[3,4-D]pyrimidine containing amide

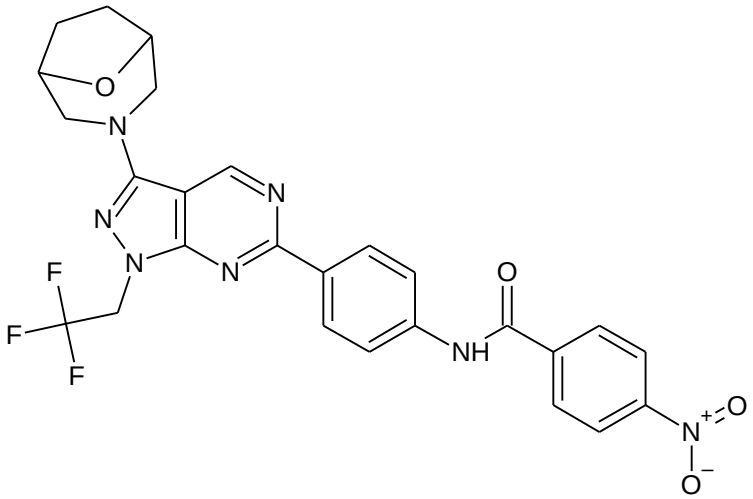


**Fig 12: Anti-Diabetic Activity of Pyrazolo[3,4-D] Pyrimidine Containing Amide Derivatives**

## CONCLUSION

In summary, this review has outlines widespread distribution and numerous bioactivities of pyrazolopyramidines as anti-microbial, anti-inflammatory, anti-cancer, and anti-diabetic, anti-malarial. The current survey of works carried out in pyrazolopyramidines revealed that these moieties have attracted a great deal of of interest of medicinal chemists and biochemists and rendered them like a lead molecule for designing potential bioactive agents. We can conclude that many other derivatives of pyrazolopyramidine can be synthesized which will be expected to show potent pharmacological activities.

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