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Review

Medicinal attributes of pyrazolo[3,4-*d*]pyrimidines: A review

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ABSTRACT

Pyrazolopyrimidines are the fused heterocyclic ring systems which structurally resemble purines which prompted biological investigations to assess their potential therapeutic significance. They are known to play a crucial role in numerous disease conditions. The advent of their first bioactivity as adenosine antagonistic property divulged their medicinal potential. Radioactivity test on mice cells, morphometric and serological tests on rat hepatocytes, antitumor testing against L1210 and P388 leukemias in mice threw light on their biophysical aspects of significance. Biochemical properties were explored via xanthine oxidase assay, antioxidant enzyme assays, Western blot analysis, mRNA expression of apoptopic genes, receptor binding assays, and tryptan blue exclusion cytotoxicity evaluation. The collective results of biochemical and biophysical properties foregrounded their medicinal significance in central nervous system, cardiovascular system, cancer, inflammation etc. The present manuscript to the best of our knowledge is the first compilation on synthesis and medicinal aspects including structure–activity relationships of pyrazolo[3,4-*d*]pyrimidines reported to date.

Contents

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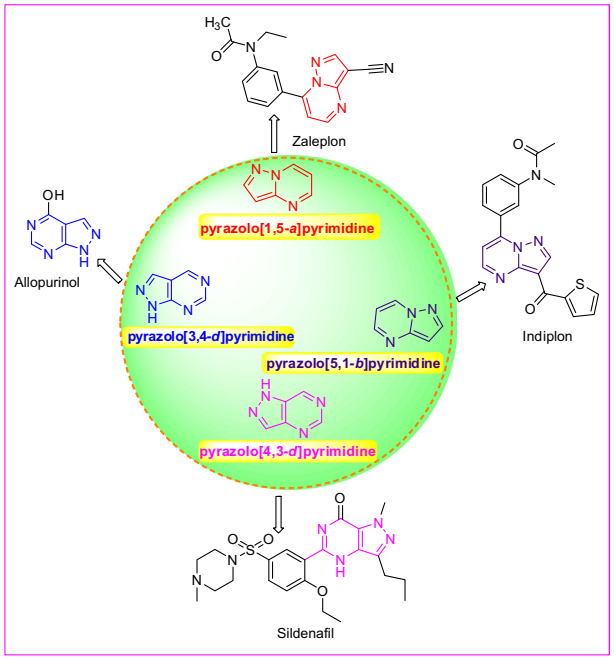


Figure 1. Pyrazolopyrimidine containing drugs.

1. Introduction

The pyrazolopyrimidines comprise of a pyrazole ring fused with the pyrimidine moiety unlike the imidazole moiety in purines.[1–7] Historically, pyrazolopyrimidines were initially reported as adenosine receptor antagonists.[8–15] A number of chemical compounds consisting of pyrazolopyrimidines as central core were synthesized which demoed encouraging activity such as the pyrazolopyrimidine antibiotics that represent a class of modified nucleosides containing the unusual C-riboside link.[16] Recently Mahajan and Mahajan wrote on ACK1 tyrosine kinase targeted inhibition of cancer cells including derivatives of pyrazolo[3,4-*d*]pyrimidines.[17] Pyrazolopyrimidins consist of various isomeric forms like pyrazolo[3,4-*d*]pyrimidines, pyrazolo[4,3-*d*]pyrimidines,[18–21] pyrazolo[5,1-*b*]pyrimidines[22,23] and pyrazolo[1,5-*a*]pyrimidines[24–28] which exemplify some important classes of drugs as shown in Figure 1.

Pyrazolopyrimidines and pyrazolo[3,4-*d*]pyrimidines are reported to encompass pharmacological potential as antiviral,[29–32] anticoccidials,[33,34] antimicrobial,[35–43] antitumor,[31,44–47] herbicidal, antileukemic,[48–50] pesticides,[51] CNS agents,[52–54] tuberculostatic,[55–57] antileishmanial,[58–63] radioprotectant,[64] anti-inflammatory[3,65] and cardiovascular activities.[66–68] The present manuscript to the best of our knowledge is the first review including synthetic strategies, medicinal aspects and structure activity

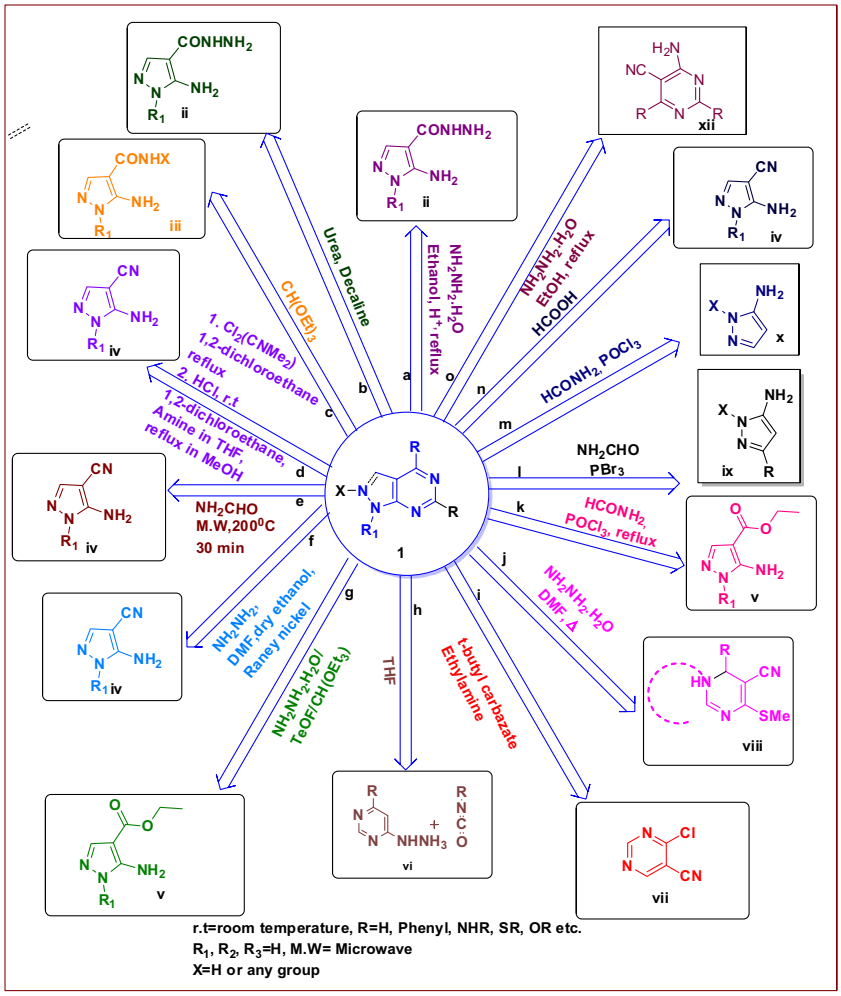
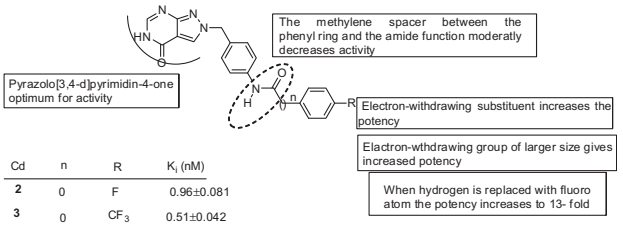
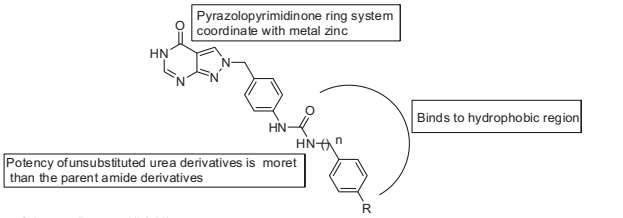


Figure 2. Retro-synthetic approaches for pyrazolo[3,4-*d*]pyrimidines.



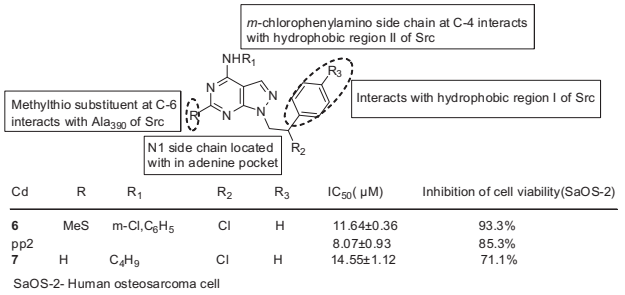
Cd	n	R	K_i (nM)
2	0	F	0.96±0.081
3	0	CF_3	0.51±0.042

Figure 3. SAR of carboxamide derivatives of adenosine deaminase inhibitors.



Cd	n	R	Ki (nM)
4	0	F	1.15±0.10
5	0	CF_3	0.16 ± 0.010

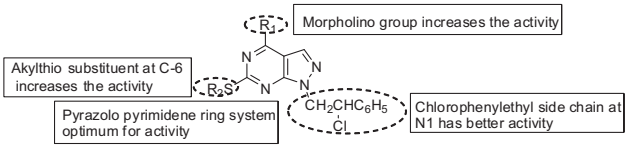
Figure 4. SAR of urea derivatives as adenosine deaminase inhibitors.



Cd	R	R_1	R_2	R_3	IC_{50}(μM)	Inhibition of cell viability(SaOS-2)
6	MeS	m-Cl,C_6H_5	Cl	H	11.64±0.36	93.3%
pp2					8.07±0.93	85.3%
7	H	C_4H_9	Cl	H	14.55±1.12	71.1%

SaOS-2- Human osteosarcoma cell

Figure 5. SAR and cell viability results of potent Lck, Src, Kdr and Tie-2 inhibitors.



Cd	R_1	R_2	IC_{50} (μM)
8	$NH(CH_2)_2C_6H_5$	Me	31.2±0.5
9	NHC_4H_9	Et	38.8±0.5
Pp2			61.8± 4.4

Figure 6. Effect of substitution on A431and 8701-breact cancer cells.

relationships (SAR) of pyrazolo[3,4-*d*]pyrimidines that is covered from the year 2006 to present.

2. Synthetic strategies

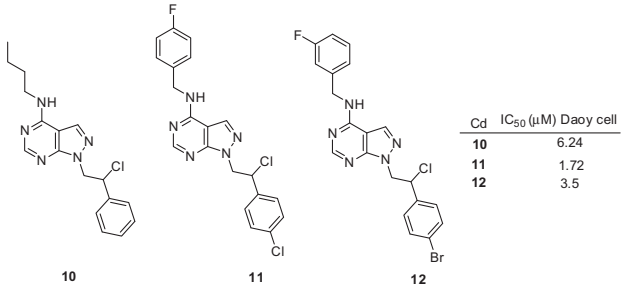
Research and development in the past years have effectively accomplished the purpose of introduction of various synthetic strategies. Numerous synthetic strategies (Fig. 2) have been outlined for the synthesis of pyrazolo[3,4-*d*]pyrimidines (**1**). Todorovic et al. treated ii with formamide and hydrazine hydrate (route a) under microwave irradiation to afford **1**.[69] 5-Amino-4-cyanopyrazole has been used in the number of reactions to attain the synthesis of desired compound **1** through different routes. Ghorab et al. carried out the reaction of (ii) with urea and decaline (route b)[64] as well as treated (v) with hydrazine hydrate (route g) and triethylorthoformate to afford **1**.[64] Diadine and co-workers synthesized **1** by allowing iii to react (route c) with triethylorthoformate.[70] Quintela et al. accomplished the synthesis of the final compound by (route d) cyclization of chloroamidines of (iv), that is, *N*-substituted *o*-aminocyanopyrazoles.[71] Song et al. reported cyclo-condensation of amidines of iv with the suitable 2-amino-5-subsitituted-1,3,4-thiadiazoles or their hydrochloride in acetic acid yielding **1** (route e) under microwave irradiation.[72] Mukkanti et al. produced **1** by allowing the 5-amino-1-(4-cyanophenyl)-1*H*-pyrazole-4-carbonitrile (iv) to react with formamide, potassium borohydride, (route f) Raney Ni and dry ethanol.[73] Bhuyan et al. presented one-pot synthesis of **1**(route h) using isocyanates.[74] Soth et al. carried out the reaction of *tert*-butyl carbazate (route i) with 4-chloro-5-cyano pyrimidine (vii) in ethyl amine to afford **1**.[75] Khobragade et al. blended 3-cyano-2-methylthio-4-oxo-4*H*-6(substitutedphenyl)thiazolo[3,2-*a*]pyrimidine and hydrazine hydrate (route j) in dry DMF to give **1**.[76] Carraro et al. obtained **1** by reacting v with formamide (route k) and $POCl_3$.[77] Huang et al. offered one pot synthesis of **1** (route l) employing reaction of ix with phosphorous tribromide.[78] La Motta et al. boiled (route n) 1-alkyl-3-amino-4-pyrazolecarbonitrile (iv) in formic acid to acquire **1**.[79] Chang et al. obtained **1** via treating iv with (route m) formamide and $POCl_3$ (novel Vilsmeier agents).[80] Rostamizadeh et al. synthesized **1** by reacting 4-amino-6-aryl-2-phenyl pyrimidine-5-carbonitrile derivatives and hydrazine hydrate in EtOH under reflux (route o).

3. Biological activities

The biological investigations of pyrazolo[3,4-*d*]pyrimidines involve various mechanisms like oxidative stress, enzymatic action, receptor mediated mechanism etc. The biological investigations have revealed that substitution of various groups on the ring imparts different activity.

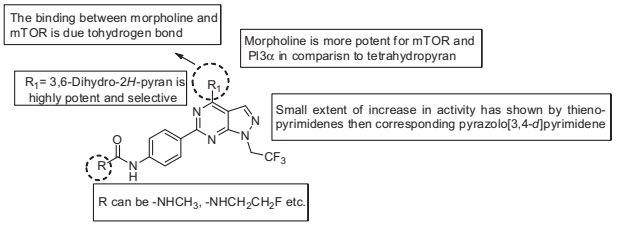
3.1. Adenosine deaminase Inhibitors

In 2009, Motta et al.[79] demonstrated the pyrazolo[3,4-*d*]pyrimidin-4-one ring system as a potential source for potent adenosine deaminase inhibitors. The position-2 of the pyrazolo[3,4-*d*]pyrimidin-4-one nucleus was substituted with various alkyl and arylalkyl groups. A series of compounds were synthesized and studied for their SAR. The compound **2** was evaluated in animal models of experimental colitis. The results revealed amelioration of both systemic and intestinal inflammatory alterations. Urea derivatives (Fig. 3) with the same substitution as carboxamide derivatives (Fig. 4) were found to have less activity with the exception of R = triflouromethyl group. Triflouromethyl group in the *para* position of the distal phenyl ring disclosed three-fold increase in potency as compared to the corresponding carboxamide derivative. Some of the important SAR features are summarized in Figures 3 and 4.



Cd	IC_{50} (μM) Daoy cell
10	6.24
11	1.72
12	3.5

Figure 7. SAR of pyrazolo[3,4-*d*]pyrimidines and effect as Src kinase inhibitors.



Cd	R	R1	mTOR Average IC_{50} (nM)	PI3Kα Average IC_{50} (nM)	PI3KαIC_{50}/ mTOR/IC_{50} IC_{50} (nM)	LNCap Average IC_{50} (nM)	Nude mouse $T_{1/2}$ (min)
13	$-NHCH_3$	DHP	2	200	100	26	13
		Morpholine	1	194	194	2300	16

mTOR- Mammalian target of rapamycin
LNCap- Androgen-sensitive human prostate adenocarcinoma cells
PI3Kα– Phosphoinositide 3-kinase
DHP- Dihydropyran

Figure 8. SAR of pyrazolo[3,4-*d*]pyrimidines as ATP-competitive inhibitors.

3.2. Anticancer activity

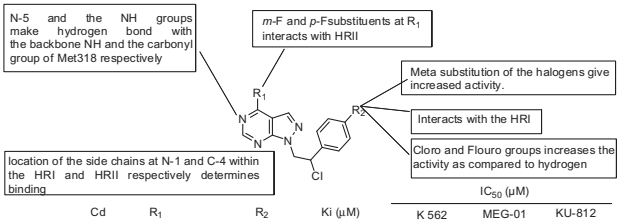
The pyrazolo[3,4-*d*]pyrimidines and their derivatives exhibit anticancer activity via interaction with different enzymes and receptors.

3.2.1. Lck, Src, Kdr and Tie-2 inhibitors

Spreafico et al.[46] explored the antiproliferative and proapoptopic activities of pyrazolo[3,4-*d*]pyrimidines as Src kinase inhibitors in human osteosarcoma cells. They concluded that pyrazolo[3,4-*d*]pyrimidines are involved in the stimulation of programmed cell death and decrease the Src phosphorylation. Cell viability assay revealed that the inhibitory activity of the compounds is dose dependent. The compounds block the various phases of the cell cycle. PP2 (4-amino-5-(4-chlorophenyl)-7-(*t*-butyl)pyrazolo[3,4-*d*]pyrimidine) was taken as the lead compound having Src kinase inhibitory activity. Compound **6** was found to act via DNA damage whereas pp2 and **7** act via increasing apoptosis. The SAR and results of cell viability assay are laid out in Figure 5.

In 2006, Carraro et al.[77] reported pyrazolo[3,4-*d*]pyrimidines as potent antiproliferative and proapoptotic agents against A431and 8701-BC cells in culture acting via inhibition of *c*-Src phosphorylation in a cell free assay. The antiproliferative activity was attributed to substituents at position-4 of the heterocyclic moiety. The chlorophenyl ethyl side chain at N-1 and a 6-methylthio group was reported to be imperative for optimum activity. The compounds as presented in Figure 6 are shown to have maximum inhibitory action on phosphorylation of Src as compared to the reference pp2.

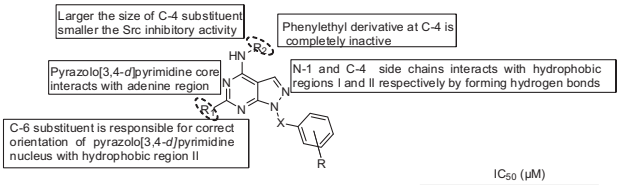
In 2010, Rossi et al.[81] synthesized new pyrazolo[3,4-*d*]pyrimidine derivative as Src kinase inhibitors leading to cell cycle arrest at G2/M phase of the cell cycle and tumor growth reduction of human medulloblastoma cells. After evaluating a series of compounds using Western blot, daoy xenograft, MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulf



Cd	R_1	R_2	Ki (μM)	IC_{50} (μM) K 562	MEG-01	KU-812
14	$NHCH_2$(o-F-Ph)	F	0.02±0.01	36±2	15±1	-
15	$NHCH_2$(p-F-Ph)	Cl	0.08±0.04	9±1	5.5±1.4	8±1
pp2			0.50 ± 0.20	25±3	17±1	45±3

K 562, MEG-01, KU-812- Human leukemia cell lines

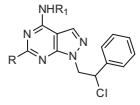
Figure 9. SAR and K_i values as Abl Inhibitors and antiproliferative agents.



Cd	R	R_1	R_2	X	IC_{50} (μM) c-Src	c-Abl
Reference					0.8±0.1	0.08±0.01
16	H	$-(CH_2)_2S-$	Bn	CH_2CHCl	2.9±0.3	0.09±0.01
17	H	$-(CH_2)_2S-$	Ph	CH_2CHCl	0.21±0.02	0.15±0.02
18	4-Cl	H	$-CH_2-$	CH_2CHCl	3.9±0.3	0.66±0.07

c-Src, c-Abl- Human leukemia cells

Figure 10. SAR and activity of pyrazolo[3,4-*d*]pyrimidines as Bcr-Abl kinase inhibitors.



Cd	R	R_1	S_{Drug}(mol L^{-1})	S_{Drug}-HPβCD (mol L^{-1})	Solubility enhancement factor	IC_{50}(μM) K-562	KU-812
19	MeS	o-F-$C_6H_4CH_2$	7.24 $X10^{-7}$	6.05 $x10^{-4}$	8.36 x 10^3	4.55±0.13	4.83±0.09
20	MeS	m-Cl-C_6H_4	2.95 $X10^{-7}$	3.14 x 10^{-4}	1.06 x 10^3	2.00±0.03	14.97±0.43

K-562, KU-812 - Leukemia cell lines

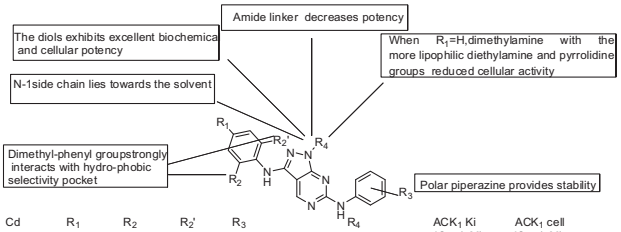
Figure 11. Solubility and cytotoxicity of pyrazolo[3,4-*d*]pyrimidines.

ophenyl)-2*H*-tetrazolium) assay, three most active compounds **10** (S7), **11** (S29), **12** (SI163) (as delineated in Fig. 7) having antiproliferative action were obtained. The kinetic differences were revealed due to their alteration in affinity for the target site.

In 2010, Kaplan et al.[82] discovered 6-aryl-1*H*-pyrazolo[3,4-*d*]pyrimidines and 2-arylthieno[3,2-*d*]pyrimidines where replacement of morpholine with 3,6-dihydro-2*H*-pyran (DHP) at position-4 resulted in enhancement of ATP-competitive inhibitory activity of the mammalian target of rapamycin (mTOR; Fig. 8). Further, it was proposed that higher mTOR inhibitory activity of DHP substituted compounds could be due to their coplanar, minimum energy conformations in the binding site which was not the case with tetrahydropyran (THP) substituted compounds.

3.2.2. Abl inhibitors

In 2008, Manetti et al.[83] optimized the pyrazolo[3,4-*d*]pyrimidines as Abl inhibitors and antiproliferative agents against human leukemia cell line. Molecular modeling studies revealed the effect



Cd	R_1	R_2	R_2'	R_3	R_4	ACK_1 Ki IC_{50} (μM)	ACK_1 cell IC_{50} (μM)
21	H	Me	Me	4-Piperazin-1-yl	OMe	0.002	0.01
22	H	Cl	Cl	4-Piperazin-1-yl	OMe	0.012	0.01
23	…	Me	Me	……	OMe	0.003	0.02
24	…	Cl	Cl	……	OH OH	0.01	0.01
25	…	Cl	Cl	……	NMe_2	0.01	0.01

ACK1=Activated Cdc42Hs-associated Kinase 1is a nonreceptor tyrosine kinase.

Figure 12. SAR and effect of substitution on activated Cdc42Hs-associated kinase 1 inhibitors.

of substitution of various groups like halogens and the hydrophobic regions of the ATP binding play a decisive role in determining affinity toward Abl. The halogen substitution caused the additional contacts via orientation within the ATP binding pocket of Abl. The C-4 substituents were also deemed important for activity. The orientation of R_1 in hydrophobic region II depends on the C-4 substitution. Some of the important SAR features are represented in Figure 9.

Radi et al.[84] synthesized pyrazolo[3,4-*d*]pyrimidines having inhibitory activity in hypoxic human leukemia cells and reported the in vitro ADME properties and metabolic activities. These compounds were observed to act via inhibition of Bcr-Abl kinase activity, increased caspase-3 activity and escalated cleavage of poly-ADP-ribose-polymerase. Molecular simulation studies featured the binding modes and structural requirements in the ATP pocket for dual Src/Abl inhibitors. C-4 amino group forms hydrogen bond with Met318. The synthesized compounds were found to suffer from pharmacokinetic issues due to polar group substitution on C-6. A beneficial equilibrium was exhibited by compounds with respect to different ADME properties and biological activity in leukemia cells. Effect of substitution on inhibitory activities has been summarized in Figure 10.

Dreassi and co-workers generalized that 2-hydroxypropyl-β-cyclodextrin strongly improves water solubility by 100 to 1000-folds and antiproliferative activity of pyrazolo[3,4-*d*]pyrimidines as Src–Abl dual inhibitors. The phase solubility study of a series of compounds complexed with HPβCD was performed and further activity was checked in leukemia and SaoS cell lines (Fig. 11). Cyclodextrin forms noncovalent inclusion complexes and noninclusion based complexes.

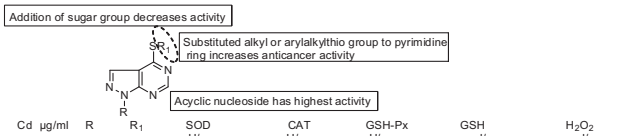
3.2.3. Activated Cdc42Hs-associated kinase 1 inhibitors

In 2008, Kopecky et al.[85] identified and optimized *N*3,*N*6-diaryl-1*H*-pyrazolo[3,4-*d*]pyrimidine-3,6-diamines as a novel class of activated Cdc42Hs-associated kinase 1 inhibitors. In silico data suggested that the compounds having hydrogen bonding with Thr205 possess high potency. Piperazine group interacts with solvent exposed region. Further, derivatives of noncyclic amino group linked to two to three carbon and *N*,*N*-dimethylaminopropyl substituted at R_4 were more potent than *N*,*N*-dimethylaminoethyl derivatives. Cyclic amines at R_4 without amide linkage were potent. The C-4 polar group was not found to be critical for inhibitory activity. SAR studies are portrayed as represented in Figure 12.

3.2.4. Generation of reactive oxygen species

In 2011, Rashad et al.[86] reported the synthesis and anticancer effects of some novel pyrazolo[3,4-*d*]pyrimidine derivatives by generating reactive oxygen species (ROS) in human breast adenocarcinoma cells. Higher levels of ROS in cancer cells than normal cells make tumor cells more sensitive to the additional oxidative stress generated by anticancer agents. This oxidative stress as a result caused injury to all the vital cellular components like proteins, DNA and membrane lipids which led to cell death. Antitumor effect of these novel pyrazolo[3,4-*d*]pyrimidine compounds is partly by production of H_2O_2 and the H_2O_2 produced should be rapidly removed through the activation of catalase, and glutathione peroxidase. Compound **27** was found to be the most potent antitumor compound (Fig. 13).

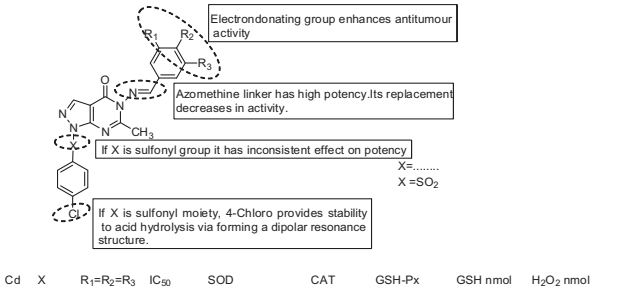
In 2011, Hassan et al.[87] noted that pyrazolo[3,4-*d*]pyrimidines possessed in vitro cytotoxic activity toward breast adenocarcinoma. The mechanism of action was an enhanced production of hydrogen peroxide and other free radicals causing oxidative distress. The potency of pyrazole in place of pyrazolo[3,4-*d*]pyrimidines was less. In the presence of sulfonyl group between pyrazolo[3,4-*d*]pyrimidine and 4-chlorophenyl moiety, anticancer activity was increased. The azomethine proton is optimum for cytotoxic activity and replacement with amide led to decrease in activity. Tribenzylidene moiety proved to improve the activity (Fig. 14).



Cd	µg/ml	R	R_1	SOD U/mg protein	CAT U/mg protein	GSH-Px U/mg protein	GSH nmol/mg protein	H_2O_2 nmol/mg protein
26	2			131.22 ± 15.60	2.80 ± 0.30	4.40 ± 0.42	18. 30 ± 2.00	38.00 ± 4.11
	5	Cisplatin		160.00 ± 17.00	2.00 ± 0.25	3.00 ± 0.35	16.20 ± 1.60	58.80 ± 6.80
	10			390.50 ± 40.80	1.40 ± 0.15	2.11 ± 0.45	13.50 ± 1.80	77.00 ± 8.00
	20			470.60 ± 55.00	1.20 ± 0.11	0.181±0.25	11.20 ± 1.30	84.80 ± 9.6
27	2	I	CH_2CH_3	125.00 ± 14.00	3.60 ± 0.30	5.60 ± 0.60	20.11 ± 2.25	35.00 ± 3.70
	5			150.25 ± 16.50	2.90 ± 0.35	4.25 ± 0.50	19.16 ± 2.20	55.00 ± 5.50
	10			360.00 ± 37.50	1.76 ± 0.19	3.00 ± 0.37	17.00 ± 1.80	60.50 ± 7.00
	20			85.00 ± 39.00	1.55 ± 0.20	2.20 ± 0.24	14.80 ± 1.80	80.00 ± 8.60

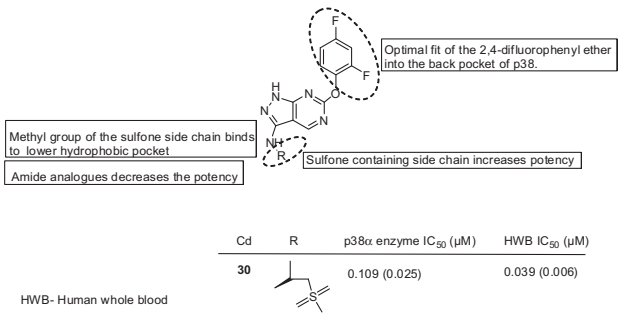
2, 5,10 and 20 mg/ml-different concentrations ,superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione (GSH) hydrogen peroxide (H_2O_2)

Figure 13. Pyrazolo[3,4-*d*]pyrimidines and their effects on GSH, SOD, CAT, GSH-Px on MCF-7 cell lines.



Cd	X	R_1=R_2=R_3	IC_{50} (µM)	SOD U/ mg protein	CAT U/mg protein	GSH-Px mg protein	GSH nmol mg protein	H_2O_2 nmol mg protein
28	.....	OCH_3	7.60± 0.71	130.80± 15.65	2.96± 0.22	4.40± 0.40	21.60± 2.40	47.50± 5.70
29	Cisplatin		13.29	110.00± 12.90	2.20± 0.24	4.85± 0.62	17.80± 0.20	53.30± 4.80

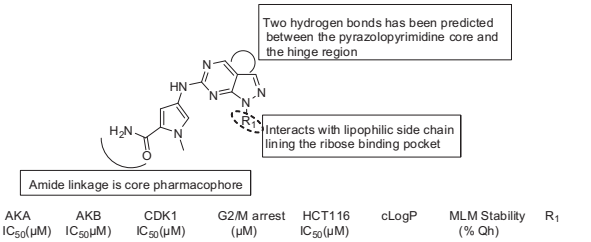
Figure 14. SAR of pyrazolo[3,4-*d*]pyrimidines having in vitro cytotoxic activity and effect on MCF-7 treated cells.



Cd	R	p38α enzyme IC_{50} (µM)	HWB IC_{50} (µM)
30		0.109 (0.025)	0.039 (0.006)

HWB- Human whole blood

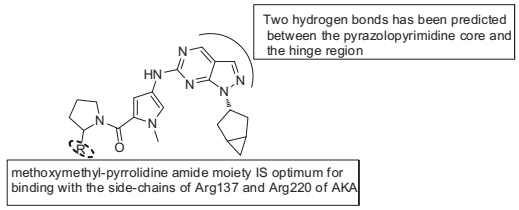
Figure 15. SAR and effect of pyrazolo[3,4-*d*]pyrimidines as p38α kinase inhibitors.



Cd	AKA IC_{50}(µM)	AKB IC_{50}µM)	CDK1 IC_{50}(µM)	G2/M arrest (µM)	HCT116 IC_{50}(µM)	cLogP	MLM Stability (% Qh)	R_1
31	0.017	0.004	0.024	0.1	0.079	1.6	69	
32	0.23	0.038	0.16	1	1	1.2	27	

HCT- colon adenocarcinoma cell line, CDK- cyclin dependent kinase,AKB- Aurora B kinases,AKA- Aurora A-kinases

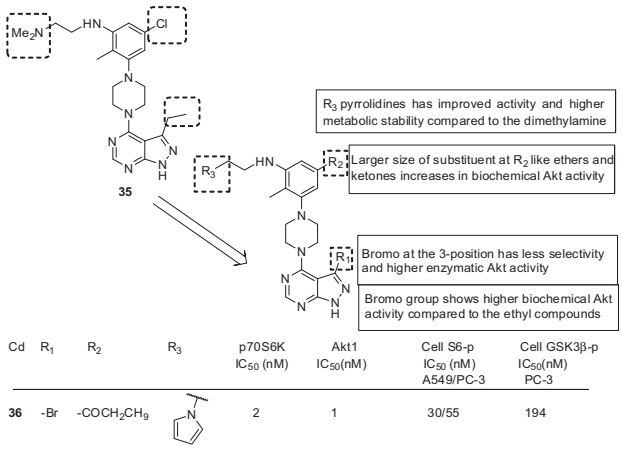
Figure 16. SAR of pyrazolo[3,4-*d*]pyrimidines as dual inhibitors of aurora kinases, CDK1, cell cycle arrest at G2/M phase and HCT116.



Cd	AKA IC_{50} (I μM)	AKB IC_{50} (I μM)	CDK1 IC_{50} (I μM)	G2/M 100% (I μM)	HCT116 IC_{50} (I μM)	R
33	0.004	0.005	0.022	0.03	0.01	(S)CH2OMe
34	0.016	0.006	0.15	0.065	0.06	(R)CH2OMe

HCT- colon adenocarcinoma cell line, CDK- cyclin dependent kinase,AKB- Aurora B kinases,AKA- Aurora A-kinases

Figure 17. SAR and effect of pyrazolo[3,4-*d*]pyrimidines as dual inhibitors of aurora kinases and CDK1.



Cd	R_1	R_2	R_3	p70S6K IC_{50} (nM)	Akt1 IC_{50}(nM)	Cell S6-p IC_{50} (nM) A549/PC-3	Cell GSK3β-p IC_{50}(nM) PC-3
36	-Br	$-COCH_2CH_3$		2	1	30/55	194

p70S6k- serine/threonine kinase, Akt1- serine/threonine-protein kinase,PC-3- human prostate cancer cell line, GSK3β–p- Glycogen synthase kinase 3 beta

Figure 18. SAR of pyrazolo[3,4-*d*]pyrimidines as dual Akt/p70S6K inhibitors.

3.2.5. p38α Kinase inhibitors

In 2011, Soth et al.[75] designed and established 3-amino-pyrazolo[3,4-*d*]pyrimidines as p38α kinase inhibitors. The enzymatic assay revealed that the amide functionality has a mild effect on potency owing to the fact that it is away from the binding site whereas amine moiety showed high potency due to its tight binding to the active site. Sulfonamide series was found to be nearly impermeable to cells. The human whole blood (HWB) assay was carried out and branched sulfone containing compound showed good solubility as well as stability towards microsomes. Compound **30** was highly selective as a p38α kinase inhibitor (Fig. 15). Pyrazolo[3,4-*d*]pyrimidine core having sulfone in front pocket side chain was found to bind better as compared to the corresponding pyrimidinopyridone and further increase in potency by adding methyl group was seen due to its binding to Ala157.

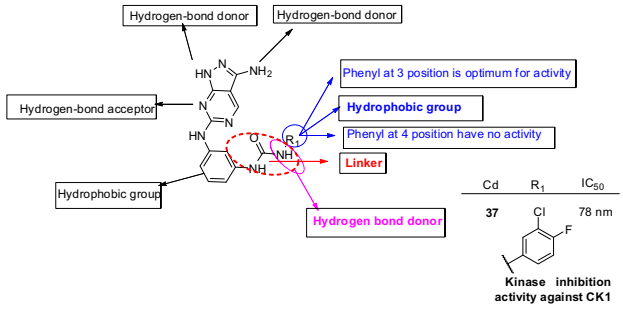


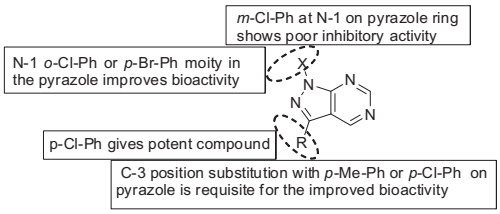
Figure 19. SAR of lead compound containing pyrazolo[3,4-*d*]pyrimidine as CK1 inhibitors.

3.2.6. Aurora kinases and CDK1 inhibitors

In 2012, Brazidec et al.[88] synthesized and reported SAR of 1,6-disubstituted-1*H*-pyrazolo[3,4-*d*]pyrimidines as dual inhibitors of Aurora kinases and CDK1. With the objective of designing biochemically potent and controlled *c*log*P* value they envisioned that R_1 is significant for activity since it interacts with binding site of CDK1. The activity of various compounds with SAR is as given in Figure 16. It was observed that spirobicyclic, fused tricyclic, monocyclic rings were metabolically unstable and possessed high potency. *S*-Methoxymethyl (spirobicylic) displayed higher abilityto inhibit AKB phosphorylation (Fig. 17). The pharmacokinetics study revealed that compound **33** was highly potent with low distribution volumes, high clearance rate, good ADME properties, neutropenic index of **32** and was a good antitumor agent.

3.2.7. 7Akt/p70S6K inhibitors

In 2012, Rice et al.[89] evaluated the role of pyrazolopyrimidines as dual Akt/p70S6K inhibitors. The underlying objective was to convert the highly potent and selective compound **35** into dual inhibitors of Akt/p70S6K. The compound having bromine at C-3 position (**36**) exhibited high inhibitory activity against Akt and in

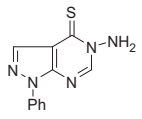


Cd	X (N-1)	R (C-3)	NCI-H226 μM	NPC-TW01 GI50 (μM)	Jurkat GI50 (μM)
38	Ph	*p*-Cl-Ph	18	23	36
39	2-Quinolinyl	*p*-Me-Ph	29	30	54
40	2-Quinolinyl	i-Cl-Ph	39	35	69
41	2-Quinolinyl	*p*-OMe-Ph	37	36	>100
42	Ph	*p*-Cl-Ph	18	23	36

NCI-H226- lung carcinoma cell line, NPC-TW01- human nasopharyngeal cell line Jukrat- CD4(+) T-cell leukemia cell line

Figure 20. SAR of pyrazolo[3,4-*d*]pyrimidines as antiproliferative agents.

Table 1
Effect of compound on GSH, SOD, LPx of normal and irradiated mice



Cd	GSH (mg/dl)	SOD (U/ml)	LPx (μmol/ml)	Survival (%)
Control	47.28 ± 3.02	2.4 ± 0.16	120.88 ± 1.37	100
CMC	45.42 ± 0.97	2.25 ± 0.170	113.66 ± 3.38	100
Rad	33.56 ± 2.60	1.69 ± 0.12	140.82 ± 7.38	80
43	49.63 ± 2.31	2.35 ± 0.03	127.32 ± 11.77	100
43+Rad	51.84 ± 0.97	2.18 ± 0.18	113.40 ± 5.05	100

Rad, radiation; CMC, carboxymethylcellulose.

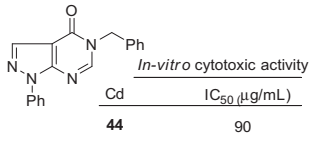


Figure 21. In-vitro cytotoxic activity of pyrazolo[3,4-*d*]pyrimidine derivative.

PC-3 cell lines. Further, Akt activity was enhanced by the presence of larger lipophilic groups at position-5 which resulted in a potent, metabolically stable potential candidate **36** (Fig. 18) exhibiting good pharmacokinetic profile.

3.2.8. CK 1 inhibitors

In 2012, Yang et al.[78] highlighted the role of *N*6-phenyl-1*H*-pyrazolo[3,4-*d*]pyrimidine-3,6-diamine derivatives as novel CK1 inhibitors. The steps taken for hit-to-lead optimization established that (a) the shift of group from position-3 at R_1 to position-4 leads to decrease in the activity and (b) the molecule possessing one hydrogen-bond acceptor, two hydrogen-bond donors, and three hydrophobic portions are suitable inhibitors. Compound **37** showed the highest activity with perfect fit into the active site of CK1 (Fig. 19).

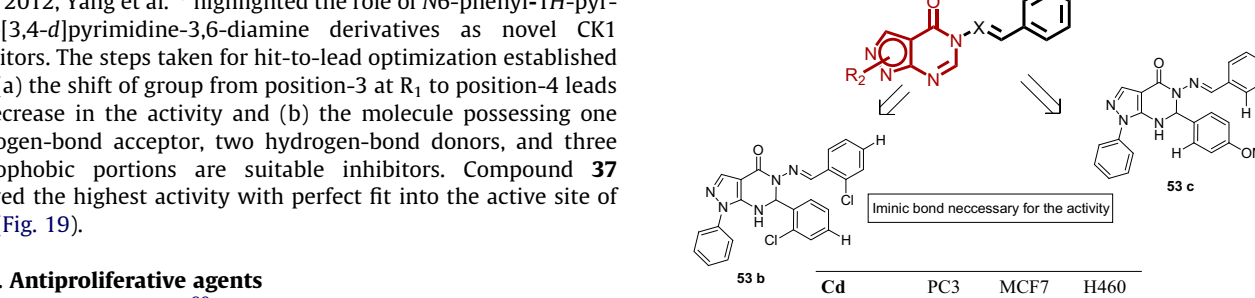
3.2.9. Antiproliferative agents

In 2012, Huang et al.[90] carried out one-pot synthesis and evaluated antiproliferative activity of pyrazolo[3,4-*d*]pyrimidines against lung carcinoma (NCI-H226), nasopharyngeal (NPC-TW01), and T-cell leukemia (Jurkat) cells. As anticipated, the solubility of the various compounds was directly related to bioactivity. Some important SAR features have been displayed in Figure 20.

3.2.10. Radioprotective and anticancer agents

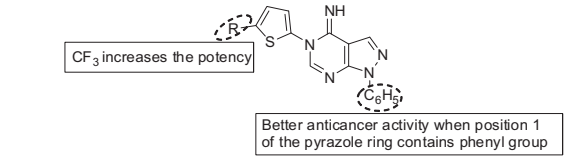
In 2010, Ghorab et al.[64] projected pyrazolo[3,4-*d*]pyrimidine derivatives to have anticancer and radioprotective activities. The studies emphasized the effects of the compounds on inhibition of toxicity of γ-rays. 5-Amino-1-phenyl-1,5-dihydropyrazolo[3,4-*d*]pyrimidine was found to be active against blood glutathione, superoxide dismutase and malondialdehyde decreasing lipid peroxidation as shown in Table 1 (see Fig. 21).

In 2011, Song et al.[72] carried out microwave-assisted synthesis of some novel fluorinated pyrazolo[3,4-*d*]pyrimidine derivatives containing 1,3,4-thiadiazole as potential antitumor agents exploiting the fact that flouro and triflouromethyl containing compounds lead to improvement of ADME profile, physicochemical potency and biological activity. They evaluated the compounds against human leukemia cancer cells (HL-60) by MTT assay and concluded that the compound with a phenyl group at N-1 and CF_3 at R emerged to be a better antitumor candidate (Fig. 22).



Cd	PC3 Prostate	MCF7 Breast	H460 Liver
	IC_{50} (μM)		
53 a	10.1	29.7	34.3
53 b	17.6	18.1	35.7
53 c	18.1	20.1	39.8
Etoposide	18.2	20.9	<30

Figure 24. Structure and SAR of imine-pyrazolopyrimidinones.

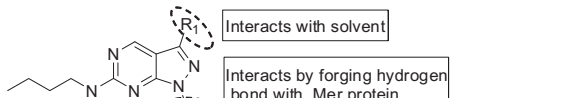


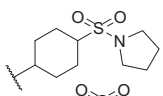
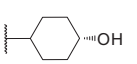
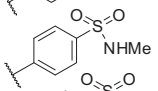
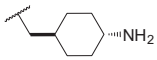
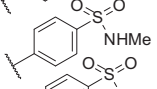
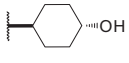
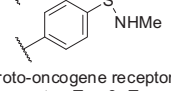
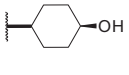
Cd	R	Formula	IC_{50} (μm/L)
45	CF_3	$C_{14}H_8F_3N_7S$	0.08
46	4-FC_6H_4	$C_{19}H_{12}FN_7S$	1.01
47	2-FC_6H_4	$C_{19}H_{12}FN_7S$	0.90
48	4-$CF_3C_6H_4$	$C_{20}H_{12}F_3N_7S$	0.21
49	Doxorubicin		0.55

Figure 22. SAR of pyrazolo[3,4-*d*]pyrimidine as antitumor agents.

3.2.11. Proto-oncogene tyrosine-protein kinase Mer inhibitors

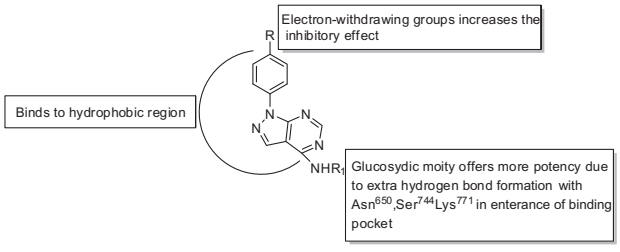
In 2013, Liu et al. persuaded the study of UNC1062 (**50**) and other sulfonamide collimates as small molecule Mer inhibitor.[91] The increase in linker length between pyrazole and cyclohexyl moiety or open chain adaptation of cyclohexyl moiety or only



Cd	R_1	R_2	Mer	Axl	Tyro3
			IC_{50} (nM)		
50			1.1	85	60
51			3.5	59	27
52			2.4	74	47
53			4.4	70	44

Mer- proto-oncogene receptor tyrosine-protein kinase, Axl- tyrosine kinase receptor, Tyro3- Tyrosine-protein kinase receptor TYRO3 enzyme

Figure 23. Effect of substitution and SAR of small molecules as Mer inhibitors.



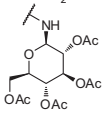
Cd	R	R_1	IC_{50} (μM)
54	Me	NH_2	17.35±1.19
55	OMe	NH_2	19.58±0.20
56	COOH	NH_2	80.97±4.64
57	CN	NH_2	0.40±0.01
58	NO_2	NH_2	2.20±0.05
59	CF_3	NH_2	0.18±0.02
60	$CONHCH_2CO_2CH_3$	NH_2	0.08±0.01
61	COOH		18.44±2.59

Figure 25. SAR and effect of substitution on xanthine oxidase inhibition.

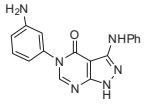
cyclohexyl ring or hydroxyl group at R_2 position resulted in diminishing of the activity whereas *trans*-4-hydroxycyclohexyl on the same position or decreased linker length intensified the activity. At R_1 position cycloalkyl moiety depicted enhanced activity; propyl or isopropyl afforded equal activity whereas activity subsided with *para*-fluorophenyl group substitution. Inhibitory concentrations of the potent compounds are represented in Figure 23.

3.2.12. Topoisomerase II inhibitors

Recently our research group has reported[92] synthesis and mechanism of imine-pyrazolopyrimidinones as anticancer agents through multiple stress pathways in the cancer cells including elevated ROS levels, thus causing DNA damage and topoisomerase II inhibition. We also presented some notions about SAR and performed molecular modeling studies in order to understand its binding interactions with topoisomerase II. Some important SAR and the anticancer activity of the best compounds **53a–c** have been depicted in Figure 24.

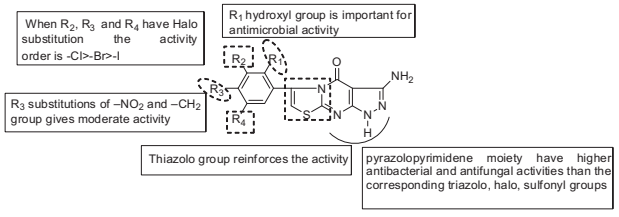
3.3. Xanthine oxidase inhibitors

Allopurinol (1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one) initially screened as anticancer agent[93] was the first US Food and Drug Administration approved inhibitor of xanthine oxidase[94] in 1966 for the treatment of gout and hyperuricemia. Gupta et al.[95] synthesized and evaluated *N*-aryl-5-amino-4-cyanopyrazole derivatives for their potential as xanthine oxidase inhibitors (Fig. 25). The results revealed that some compounds possessed good xanthine oxidase inhibitory activity.



Cd	Inhibition zone in mm			
	Bacteria		Fungi	
	Gram positive bacteria	Gram negative bacteria		
	B. thuringiensis	*K. pneumonia*	*B. fabae*	*F. oxysporum*
62	28	29	27	25
Reference compounds				
Ampicillin	18	19	17	15
Chloramphenicol	23	20	16	15
Fluconazole	-	-	22	16

Figure 26. Antimicrobial activity of pyrazolo[3,4-*d*]pyrimidine derivatives.



Cd	Bacteria (MIC 50 μg/mL)												
	EC	PA	PV	SA	KN	BS	BM	SM	AN	TV	PC	AF	CA
63	-	-	±	-	15	10	-	21	12	20	26	23	13
64	-	±	9	31	13	10	12	18	16	27	25	11	12
65	14	±	-	18	-	-	-	-	10	27	15	10	10
66	12	±	-	-	±	-	-	-	9	27	32	12	17
67	12	±	±	-	±	-	-	-	11	22	23	18	10
68	-	25	14	30	11	12	-	17	16	26	29	21	-
69	-	±	±	16	±	14	-	-	-	21	14	-	-
TC	-	32	20	25	17	20	27	15	-	-	-	-	-
NY	-	-	-	-	-	-	-	-	14	18	17	14	17
Control	-	-	±	-	±	±	-	±	-	±	±	±	-

TC- Tetracycline, NY- Nystatin, EC- *Escherichia coli*, PA- *Proteus vulgaris*, PA- *Pseudomonas aeruginosa* KN- *Klebsiella Pneumoniae*, SA- *Staphylococcus aureus*, BS- *Bacillus subtilis*, SM- *Serratia marcescens* BM- *Bacillus megaterium*, AN- *Aspergillus niger*, AF- *Aspergillus flavus*, PC- *Penicillium chrysogenum*, TV- *Trichoderma viridae*, CA- *Candida albicans*

Figure 27. SAR of pyrazolo[3,4-*d*]pyrimidine as antimicrobial agents.

The SAR studies highlighted that the lipophilic region adjacent to the active site of xanthine oxidase could complex with the aromatic systems. A complex is formed with lipophilic region when the pyrazole ring is substituted with aryl groups such as the pyrimidine ring which further increases the activity in the presence of an electron withdrawing group. The higher potency of compound **60** as compared to compound **59** is owing to the amide bond. The glucosydic moiety in compound **61** was found to be involved in the formation of extra hydrogen bonds (Fig. 25).

3.4. Antimicrobial activity

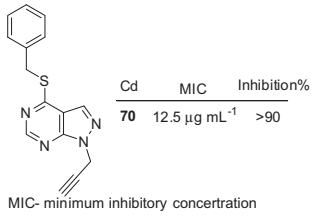
3.4.1. Antibacterial and antifungal activity

In 2008, Bondock et al.[36] synthesized compounds with antipyrine moiety as antimicrobial agents. Agar diffusion method was employed for evaluating the antimicrobial properties against *Bacillus thuringiensis*, *Fusarium oxyysporum*, *Botrytis fabae* and *Klebsiella pneumonia*. The compound **62** exhibited commendable antimicrobial activity as shown in Figure 26.

In 2010, Khobragade et al.[76] prepared and reported antimicrobial activity of novel pyrazolo[3,4-*d*]pyrimidine derivatives. Agar diffusion method was opted for the evaluation of antimicrobial properties against various bacteria and fungi using tetracycline (antibacterial) and nystatin (antifungal) as reference compounds. Compounds **63** and **64** were the potent antibacterial agents and displayed a zone of inhibition of 10–31 mm whereas compounds **65**–**69** showed 100% antifungal activity as presented in Figure 27.

3.4.2. Antitubercular activity

Chafiq and his research group[96] synthesized acyclonucleosides comprising of alkylating chain of acyclovir (Fig. 28). They evaluated the synthesized compounds against HIV-1 and HIV-2 in MT-4 cells



Cd	MIC	Inhibition%
70	12.5 μg mL^{-1}	>90

Figure 28. Antitubercular compound **70**.

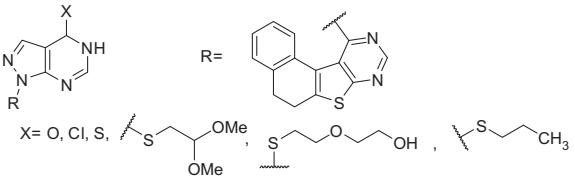
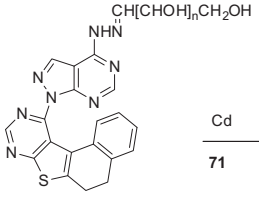
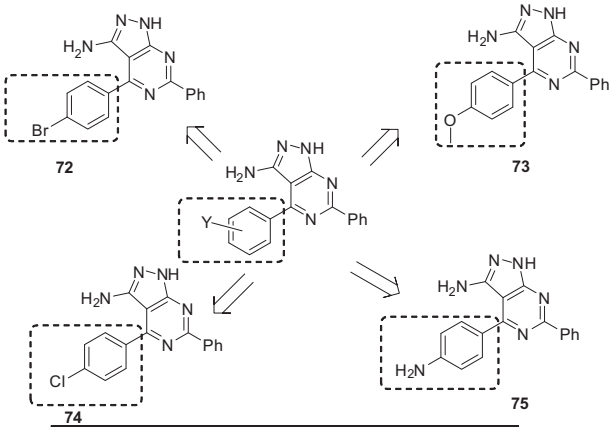


Figure 29. Pyrazolo[3,4-*d*]pyrimidine as antiviral agents.



Cd	% of HSV-1 reduction at 10 μg/10^5 cells	% of HSV-1 reduction at 20 μg/10^5 cells
71	43	99

Figure 30. Anti-HSV-1 activity of fused pyrazolo[3,4-*d*]pyrimidines.



Cd	MIC (μmol/L)	
	Enterococcus raffinosus	*Staphylococcus aureus*
72	12.3	3.8
73	14.2	4.2
74	>300	190.3
75	82.8	34.5

Figure 31. Pyrazolo[3,4-*d*]pyrimidine as antibacterial agents.

as well as for their antitumor and antitubercular activities. However, only one compound from a series emerged as antitubercular agent.

3.4.3. Antiviral activities

In 2008, Rashad et al.[97] disclosed synthesis and antiviral evaluation of some new pyrazole and fused pyrazolopyrimidine derivatives (Fig. 29). Antiviral assay was performed using different concentrations of the series of compounds synthesized which consequently revealed their anti-HCV potential. *S*-acyclic nucleoside derivatives at 20 μg/105 cells showed lesser potency than fused pyrazolo[3,4-*d*]pyrimidine.

Further the same research group in 2009 synthesized and evaluated anti-HSV-1 activity of some pyrazoles and fused pyrazolopyrimidines.[98] They tested the series of synthesized pyrazolo[3,4-*d*]pyrimidine derivatives for plaque infectivity assay. The design

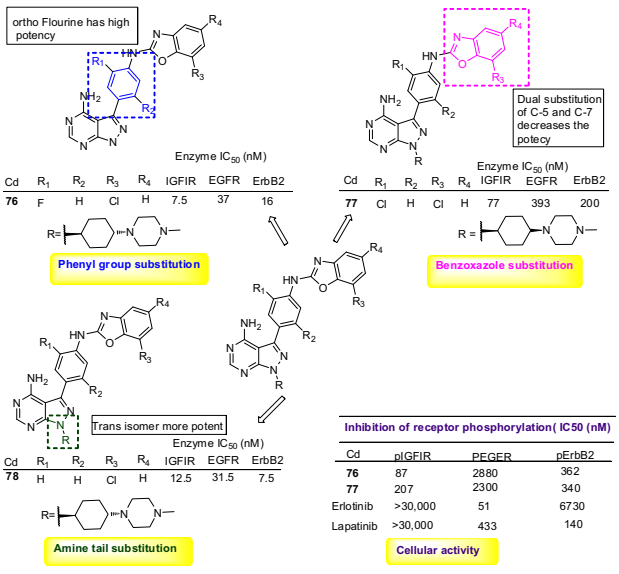
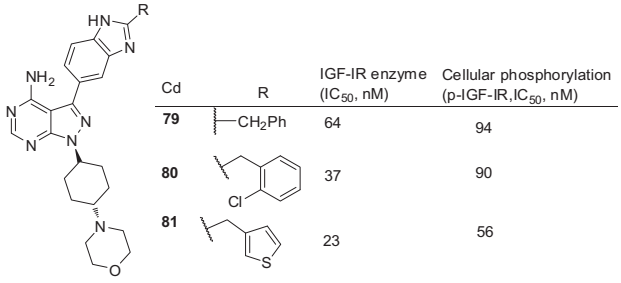


Figure 32. SAR and activity against IGF1R, EGFR and ErbB kinase inhibitors.



Cd	R	IGF-IR enzyme (IC_{50}, nM)	Cellular phosphorylation (p-IGF-IR,IC_{50}, nM)
79	CH_2Ph	64	94
80	Cl	37	90
81	S	23	56

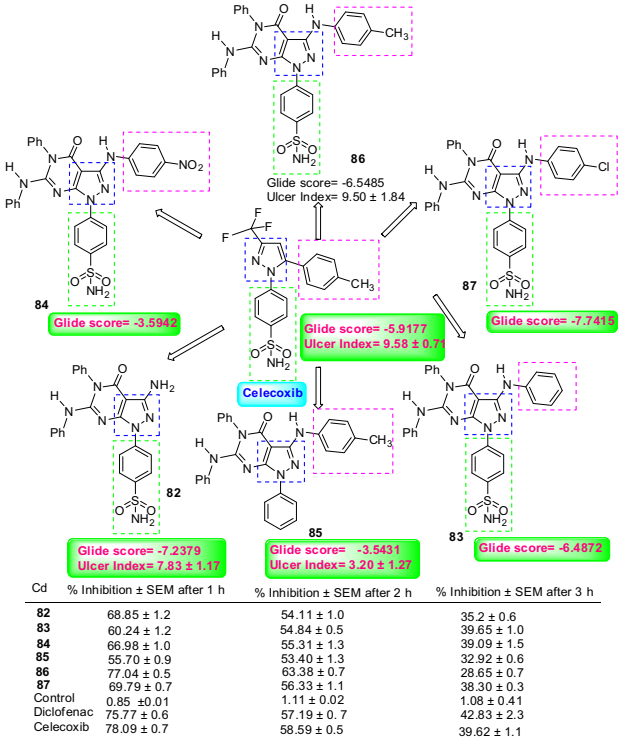
Figure 33. SAR and effect on IGF1R, ErbB2 and EGFR.

was based on 5-amino-1-substituted-1*H*-pyrazole-4-carbonitrile. Anti-HSV-1 result is shown in Figure 30.

In 2013, Rostamizadeh et al. generated a library of pyrazolo[3,4-*d*]pyrimidine derivatives and elaborated their anti-bacterial activity. They studied the activity on *Enterococcus raffinosus* and *Staphylococcus aureus*. The pyrazolo[3,4-*d*]pyrimidines having different substitutions and their minimum inhibitory concentrations are enlisted in Figure 31.

3.5. Insulin-like growth factor-1 receptor (IGF1R) and ErbB-family receptor kinases

In 2010, Wang et al.[99] evaluated and assessed the substituted 4-amino-1*H*-pyrazolo[3,4-*d*]pyrimidines as multi-targeted inhibitors of insulin-like growth factor-1 receptor (IGF1R) and members of ErbB-family receptor kinase. They optimized the SAR of benzoxazole group, N-1 tail moiety, polar capping groups and benzene ring substitution. *Trans* cyclohexyl was more active towards EGFR and ErbB2. C-7 chlorine steps us IGFIR and EGFR potency and the phenyl group have optimum potency. The cellular assay on Mia-Paca and BT474 cell lines was executed on the compounds. Further ADME profile for the potent compounds was calculated. Figure 32



Cd	% Inhibition ± SEM after 1 h	% Inhibition ± SEM after 2 h	% Inhibition ± SEM after 3 h
82	68.85 ± 1.2	54.11 ± 1.0	35.2 ± 0.6
83	60.24 ± 1.2	54.84 ± 0.5	39.65 ± 1.0
84	66.98 ± 1.0	55.31 ± 1.3	39.09 ± 1.5
85	55.70 ± 0.9	53.40 ± 1.3	32.92 ± 0.6
86	77.04 ± 0.5	63.38 ± 0.7	28.65 ± 0.7
87	69.79 ± 0.7	56.33 ± 1.1	38.30 ± 0.3
Control	0.85 ±0.01	1.11 ± 0.02	1.08 ± 0.41
Diclofenac	75.77 ± 0.6	57.19 ± 0. 7	42.83 ± 2.3
Celecoxib	78.09 ± 0.7	58.59 ± 0.5	39.62 ± 1.1

Figure 34. SAR of pyrazolo[3,4-*d*]pyrimidines as anti-inflammatory agents and ulcer index of anti-inflammatory agents.

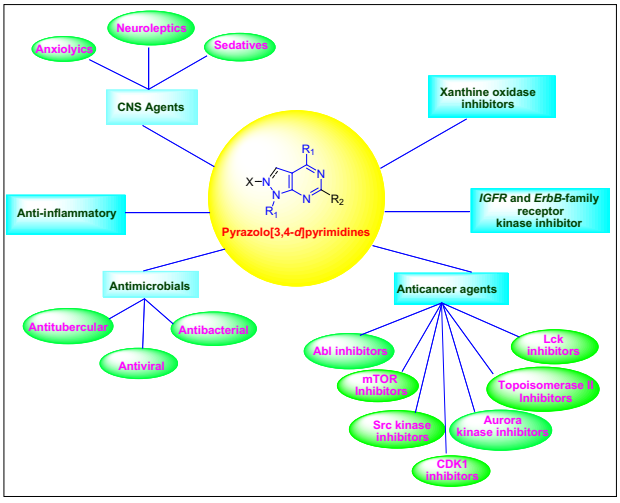


Figure 35. Biologial activities of pyrazolo[3,4-*d*]pyrimidines.

represents the SAR of compounds and their potency toward IGFIR, EGFR and ErbB2 as well as effect on receptor phosphorylation.

Hubbard et al.[100] synthesized and also investigated the potential of pyrazolo[3,4-*d*]pyrimidines to inhibit the insulin-like growth factor receptor (IGF-IR). They assessed the compounds with significant potency for cellular and enzymatic activity (Fig. 33). The SAR divulged that the benzimidazole series showed promising cellular and enzymatic inhibitory activity but the activity decreased with the substitution at R with halogens, methyl and methoxy groups. Further, 2-chloro benzyl and benzyl at R was found to balance the cellular and enzymatic amendments. Substitution at R with methylenethiophene increased the cellular activity. Further, in vitro result showed by ADME and pharmacodynamic profile

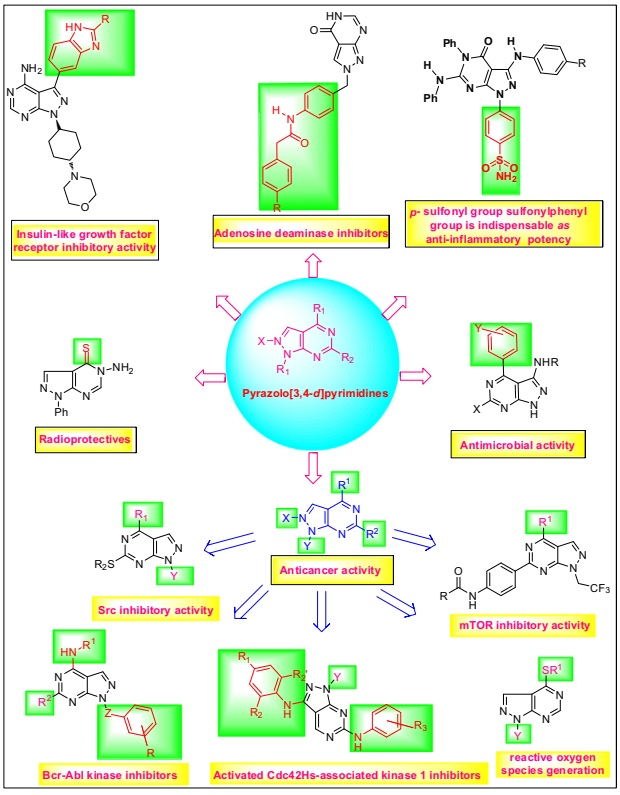


Figure 36. Lucid summary of potential held by different positions at pyrazolo[3,4-*d*]pyrimidines to influence the activity.

was congruous with the in vivo results. Recently a review article by Negi et al. had covered pyrazolo[3,4-*d*]pyrimidines as small molecules against tyrosine kinase of IGF-1R inhibitors as anticancer agents.[101]

3.6. Anti-inflammatory activity

In 2012, Yewale et al.[102] synthesized, evaluated and reported novel 3-substituted-1-aryl-5-phenyl-6-anilinopyrazolo[3,4-*d*]pyrimidin-4-ones as potential anti-inflammatory agents. With the intended goal to synthesize the novel series of antimicrobial agents, the compounds were designed, synthesized and their activity was ascertained by various assays and the docking studies performed to confirm the conformational requirements. These studies highlighted *N*-1 *para* sulfonylphenyl group as indispensable for potency. Celecoxib and diclofenac were taken as reference compounds for selective and non-selective COX-2 inhibitory activity, respectively. Docking studies showed amino and oxygen of sulfonamide interacting with valine and histidine via hydrogen bond. The ulcer index, glide score and inhibition results of various compounds is represented in Figure 34.

4. Conclusion

Pyrazolo[3,4-*d*]pyrimidines are paradigm acting via various mechanisms that appears to be addictive in several diseases. Pyrazolo[3,4-*d*]pyrimidines revolutionized the chemistry of fused purines and pyrimidines by their diverse biological activities which make them a beneficial scaffold. Their antagonistic nature towards the natural purines makes them potential candidates for the synthesis of various potent and efficacious molecules. A number of drugs like allopurinol, containing pyrazolo[3,4-*d*]pyrimidines are already discovered and successfully used in the treatment of multiple ailments. Pyrazolo[3,4-*d*]pyrimidines have been explored for their activity towards adenosine receptor, antimicrobials, anticancer and CNS agents (Fig. 35). Several applications have been devised based on their biophysical, biochemical and medicinal aspects and much more is yet to be explored. A number of researchers explored their SAR, as well as conformation and orientation requirements for binding site through simulation and QSAR studies. A C-4 site was found to be vital for antimicrobial activity whereas *N*-1, *N*-2, C-3 and C-4 were imperative in anticancer activities, *N*-1 and C-4 been most influential. Further, anti-inflammatory property and insulin-like growth factor receptor inhibitory activity have been attributed to the *N*-1 *p*-sulfonyl moiety and *N*-1, C-3 positions respectively (Fig. 36). A better considerate of crystal structure and chemical properties of pyrazolo[3,4-*d*]pyrimidines may be beacon to explore the moiety further as multiple disease inhibitor. In addition, recognition of a rational picture towards the substitutions responsible for its potency and toxicity may be a future framework so that the toxicity problems associated with the pyrazolo[3,4-*d*]pyrimidines can be identified and adjudicated.

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