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REVIEW ARTICLE

A comprehensive review on bioactive fused heterocycles as purine-utilizing enzymes inhibitors

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Abstract Purine-utilizing enzymes (PUEs) are involved in the control of biological actions of nitrogen-containing bases, purines and pyrimidines, by participating in their catabolism, and this has made them a topic of considerate interest. The heterocyclics, as purine-utilizing enzyme inhibitors (PUEIs), play a vital role in a number of diseases, e.g., malaria, cancer, rheumatoid arthritis, inflammation, tissue rejection, and autoimmune disorders. The present review is first of its kind covering the literature up to 2014 on the advances in broad-spectrum medicinal activities exhibited by heterocycles as PUEIs. The drug designing of the purine and pyrimidine antimetabolites is based on the structural mimicking of the existing compounds. The basic consideration during the designing of this class is the introduction of small structural changes without the alteration of basic skeleton of pharmacophore. The balance between the existing empirical approach and rational approach is yet to be maintained during the design and synthesis of new PUEIs by combining in vivo, in vitro, and in silico methods. The data compiled in the present manuscript on SARs, IC_{50}s, K_is, K_m, in silico studies, and their reported X-ray co-crystal structures with PUEs will offer the researchers the rational approaches for the design and development of selective and specific PUEIs devoid of adverse effects.

Keywords Heterocycles · Purine-utilizing enzymes · Inhibitor · Purines · Pyrimidines

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Introduction

Purine-utilizing enzymes (PUEs) are validated purine and pyrimidine druggable targets for many diseases ranging from inflammation to cancer (Robak and Robak, 2013; Bobrovnikova-Marjon and Hurov, 2014; D'Angiolella, 2014). These are broadly categorized into six classes (Fig. 1) based on their mode of action, basic structures, selectivity, and their target sites.

They are represented as adenosine and guanine deaminases, DNA topoisomerases, xanthine oxidase, purine nucleoside phosphorylase (Guillen *et al*., 2014), and hypoxanthine–guanine phosphoribosyltransferase (Robak and Robak, 2013; Newcombe, 2013). PUEs are responsible for the transformation of some important key steps involved in the de novo purine biosynthesis of cell metabolism (Fig. 2).

The heterocycles exhibit diverse properties including electrophilic and nucleophilic action, oxidizing and reducing properties, and acidic and basic attributes which are associated with the electronic arrangements in their chemical structures (Pozharskii *et al*., 2011; Lister, 2009; Lehman, 2012; Pozharskii *et al*., 2012; DeVita and Rosenberg, 2012; Chauhan *et al*., 2005; Bertino and Johns, 1967; Pitt *et al*., 2009). Because of their similarities with the purines and pyrimidines, they act as purine-utilizing enzyme inhibitors (PUEIs) and find their applications in treatment and management of diseases such as malaria, cancer, rheumatoid arthritis, inflammation, tissue rejection, T-cell leukemia (Zavialov *et al*., 2010a; la Marca *et al*., 2014), and other autoimmune disorders (e.g., HIV) (Niedzwicki *et al*., 1991).

The present review is first of its kind detailing medicinal applications, structure−activity relationships, and conformational aspects of heterocycles as PUEIs reported till

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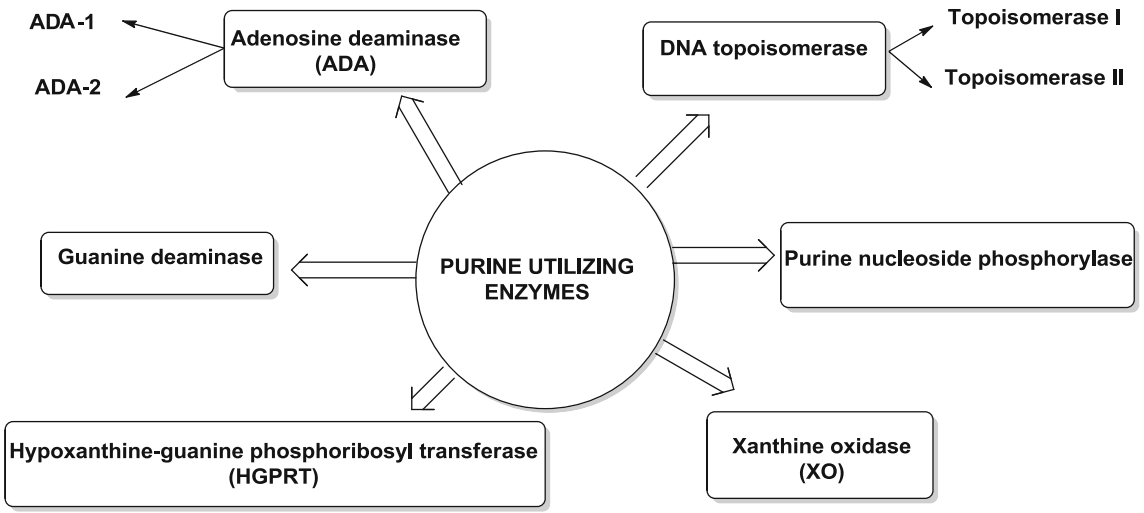


Fig. 1 Classes of purine-utilizing enzymes

date. We will discuss the various functions of PUEs, the mechanistic studies existing on several members of their superfamily, their common structural characteristics, and various PUEIs of heterocyclic skeletons at clinical and preclinical levels.

Adenosine deaminase inhibitors

Adenosine deaminase (AD; EC 3.5.4.4) is involved in metabolism of extracellular adenosine. AD catalyzes the deamination of adenosine to inosine. It binds to A1, A2A, A2B, and A3 adenosine deaminase receptors by responding through G-protein coupled receptor (Hirschhorn and Ratech, 1980; Reayi and Hosmane, 2004a). It has two kinetically distinguishable isoenzymes known as ADA1 (adenosine deaminase 1) and ADA2 (adenosine deaminase 2). ADA1 is a 30- to 40-kDa monomeric protein present in erythrocytes, kidney, lymphocytes, macrophages, liver, and intestine (Daddona and Kelley, 1977). It is present in extracellular fluid and required for the intracellular depletion of adenosine. ADA2 is a symmetrical homodimer possessing catalytic domain, disulfide bond, a signal peptide, and two ADGF/ADA2-specific domains. It regulates the level of adenosine which acts by binding to the adenosine deaminase receptors and serves as a key factor in ordinance of various cellular activities (Zavialov *et al*., 2010b). Its levels are increased in HIV infection, AIDS, autoimmune abnormalities, and tuberculosis (Andreasyan *et al*., 2005; Nosaka *et al*., 1996; Mediero and Cronstein, 2013; Kuno *et al*., 2006; Saevels *et al*., 1996; Antonioli *et al*., 2012).

Azapinomycin-based coformycin and 2-deoxycoformycin are natural, and pyrazolo[3,4-*d*]pyrimidines are synthetic adenosine deaminase inhibitors (Fig. 3) (Hong and Hosmane, 1997; Reayi and Hosmane, 2004b; Chauhan and Kumar, 2013).

In 2009, Motta *et al*. presented the pyrazolo[3,4-*d*]pyrimidin-4-one ring system as potent adenosine deaminase inhibitor. The position-2 of the pyrazolo[3,4-*d*]pyrimidin-4-one nucleus was substituted with various alkyl and aryl/alkyl groups. The series of compounds was synthesized and studied for their structure−activity relationships. The potent compound **5** of the series (Fig. 4) was evaluated in animal model of experimental colitis. The amelioration of both systemic and intestinal inflammatory alterations was seen. Urea derivatives (Fig. 5) with alike substitution as carboxamide derivatives were found to have less potency. Triflouromethyl group at the *para* position of phenyl ring disclosed threefold escalation in potency as compared to carboxamide derivative (La Motta *et al*., 2009). Electron-withdrawing substituent increases the potency and further increase in size increases the activity in carboxamide derivatives.

In 2009, Lianmei *et al*. explored the effect of nimodipine on proliferation induced by rhTGF-α and mRNA expression of RNA-dependent ADA1 in human laryngeal cancer Hep-2 cell lines. Nimodipine (**8**; Fig. 6) was found to block calcium channel of Hep-2 cell and decrease the expression of ADARI mRNA (Lianmei *et al*., 2009).

In 2010, Tite *et al*. designed and synthesized new *C*-nucleosides as potential ADA inhibitors (Fig. 7). They performed molecular docking study and predicted the high-ADA binding affinity of the compounds. The important interactions seen were hydrogen bond formation of amine group of the compounds with the glutamate carboxylate and hydrogen bonding of *N*-1 hydrogen with carboxylate of Asp296. However, the compounds were found to be poor inhibitors of ADA due to the poor covalent hydrate stability which emphasized the importance of hydration and

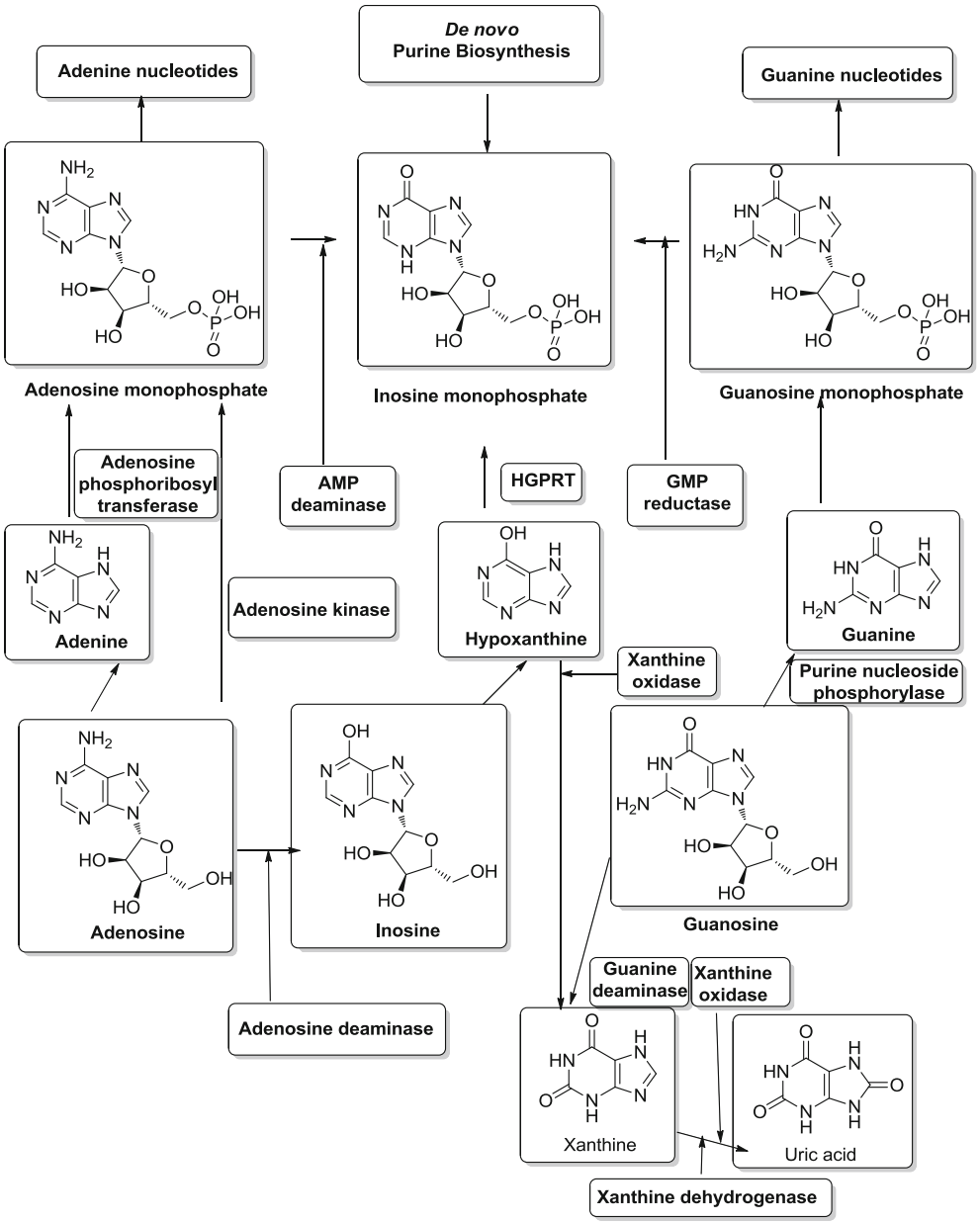


Fig. 2 Biosynthetic pathways of purine metabolism

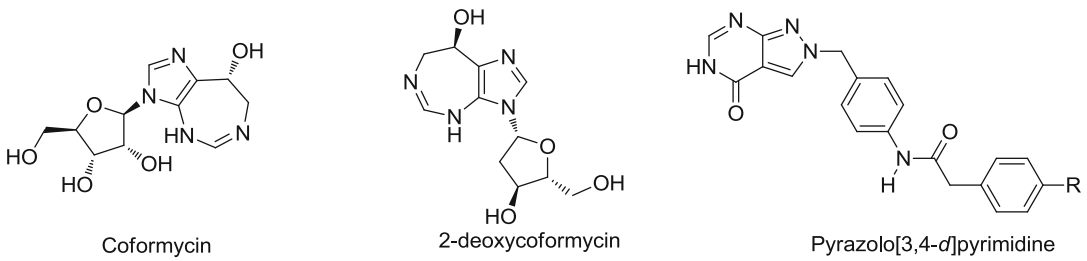


Fig. 3 Chemical structures of coformycin, 2-deoxycoformycin, and pyrazolo[3,4-*d*]pyrimidines

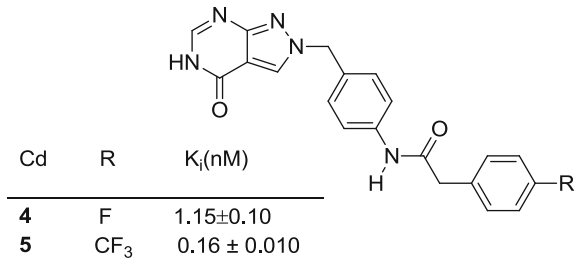


Fig. 4 Carboxamide derivatives (**4** and **5**) as adenosine deaminase inhibitors

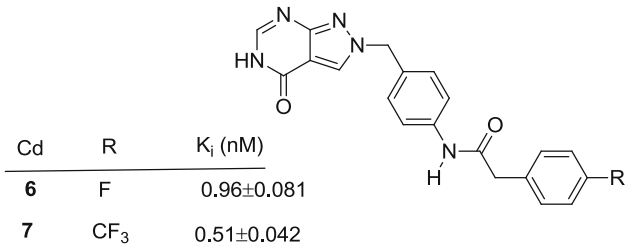
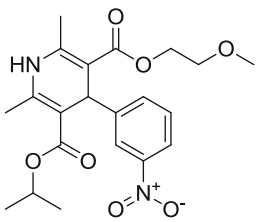


Fig. 5 Urea derivatives (**6** and **7**) as adenosine deaminase inhibitors

Fig. 6 Chemical structure of nimodipine (**8**)



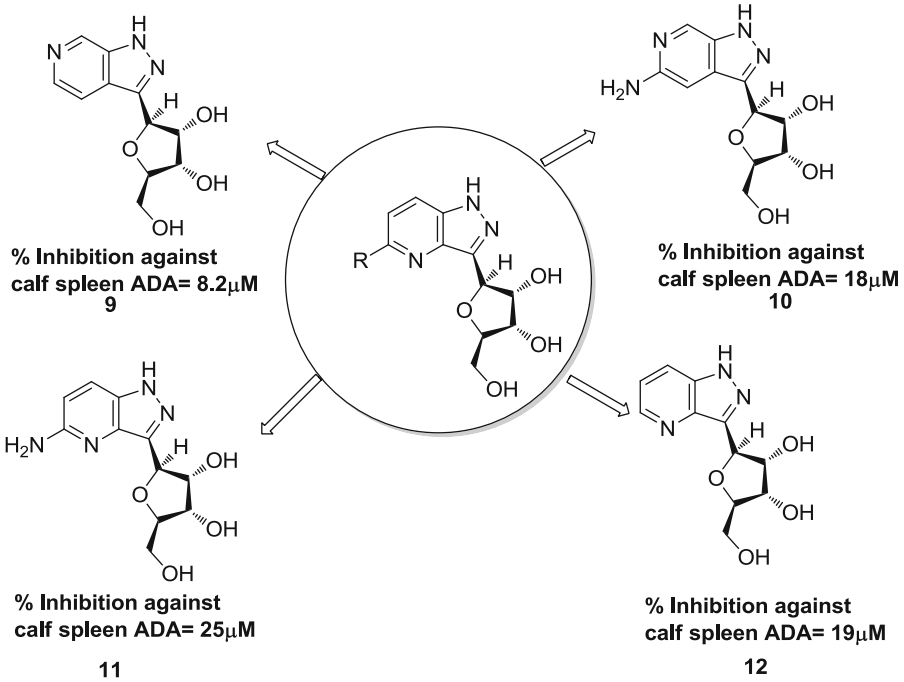
its decisive function in stability and activity against ADA (Tite *et al*., 2010).

In 2010, Zavialov *et al*. envisioned 6-hydroxy-7,8-dihydropurine nucleoside (Fig. 8) as selective inhibitor of ADA1. The team further discovered ADGF (adenosine deaminase growth factors)/ADA2-specific domains in ADA2 protein (Zavialov *et al*., 2010b).

In 2011, Gillerman and Fischer investigated the molecular recognition of several ADA inhibitors (Fig. 9). The important interactions of the compounds for the binding and stabilization with ADA were found to be hydrogen bonding of Gly184 with *N*-3 nitrogen of adenosine, hydrogen bonding between the adenosine *N*-1 and *N*-6 with Glu217, and hydrogen bond formation of Asp296 with the adenosine *N*-7. Nebularine compounds having substitution at 2-position (MeS or NH_2) showed potent inhibitory activity as compared to substitution at 8-position which were poor inhibitors of ADA exhibiting K_i values greater than 100 μM. 2-SBu-(**14**) and 2-SHex-(**16**) substituted compounds showed tight binding to the lipophilic site explaining their good potency. Further, *C*-2 substituent was found to be important for activity (Gillerman and Fischer, 2010).

In 2013, Ajlooa *et al*. studied the effect of two imidazolium-based ionic liquids (Fig. 10) on ADA activity. It was observed that the ionic liquids have a potential to inhibit the enzyme which is directly associated with their hydrophobicity (Ajloo *et al*., 2013). These act via competitive or non-competitive inhibition leading to condensed intermolecular hydrogen bond with the tertiary structure of ADA.

Fig. 7 C-nucleosides as adenosine deaminase inhibitors



DNA topoisomerase inhibitors

DNA topoisomerases have been convoluted in DNA replication, transcription, and recombination as essential enzymes in governing and aligning the topologic states of DNA (Pastor *et al*., 2012; Sperling *et al*., 2011; Gellert, 1981; Nitiss, 2009). They are classified into two major types depending on their mechanisms: DNA topoisomerase I and DNA topoisomerase II (Vos *et al*., 2014; Schneider *et al*., 1990). DNA topoisomerase I asseverates topological stress by breaking the single strand of DNA, whereas DNA topoisomerase II relaxes the supercoiled DNA by breaking both strands of phosphate backbone of the DNA and needs ATP for complete activity (Baechler *et al*., 2012). The catalytic mechanism of topoisomerases occurs via cleavage and relegation of DNA. The cleavage involves a covalent bond formation by nucleophilic attack on phosphate group of DNA phosphodiester bond by the hydroxyl group of catalytic tyrosine residue forming tyrosine phosphate (Dinh *et al*., 2014; Champoux 2001; Stewart *et al*., 1998; Corbett and Berger, 2004).The resealing of DNA strand occurs by the attack on tyrosine phosphate by deoxyribose 5′ hydroxyl group. Cancer compels speedy replication and topoisomerase inhibitors breach DNA strands, causing cell

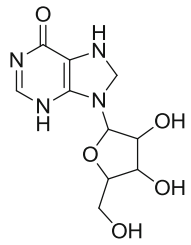


Fig. 8 Chemical structure of 6-hydroxy-7,8–dihydropurine nucleoside **(13)**

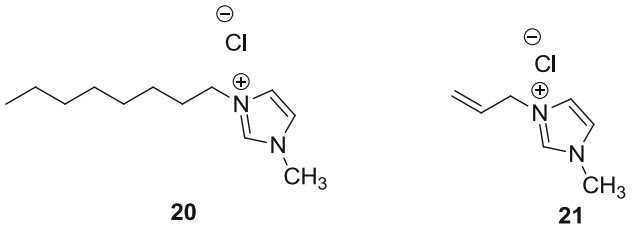


Fig. 10 Ionic liquids as adenosine deaminase inhibitors

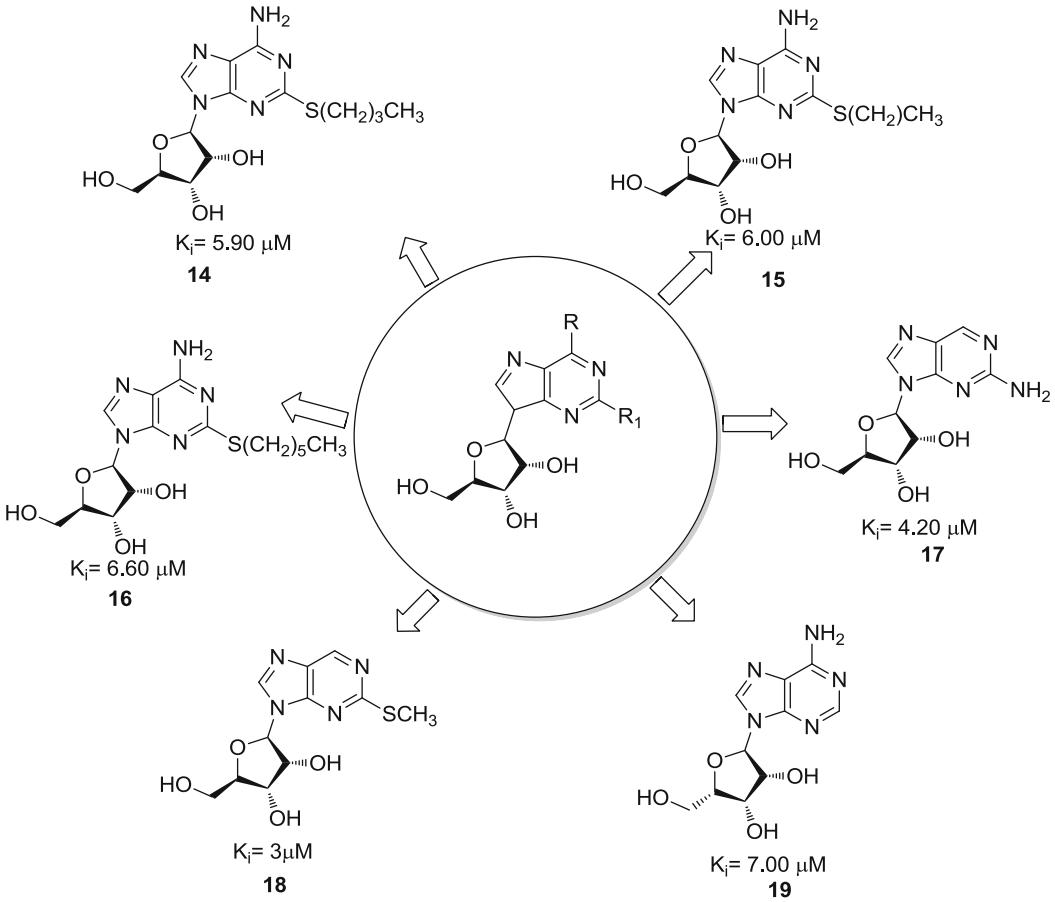


Fig. 9 Chemical structures of adenosine deaminase inhibitors

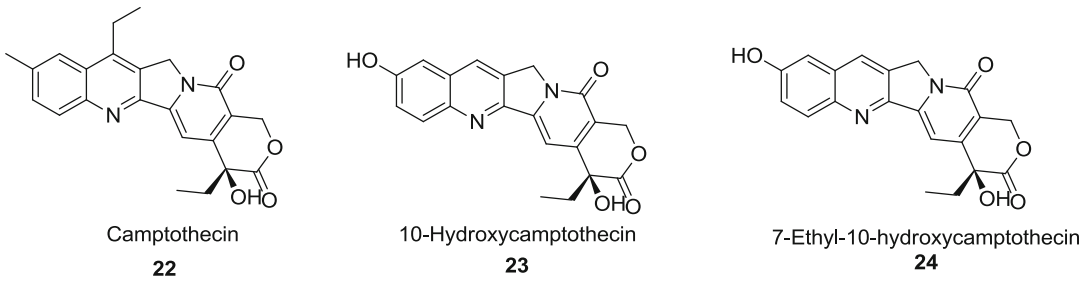
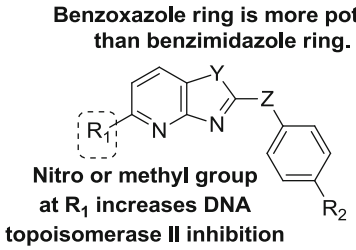


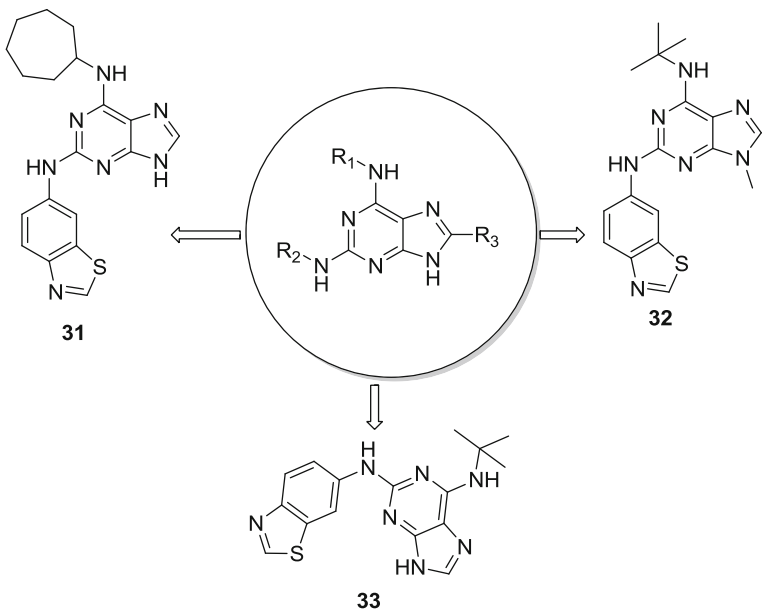
Fig. 11 Structures of camptothecin, 10-hydroxycamptothecin, and 7-ethyl-10-hydroxycamptothecin as topoisomerase I inhibitors

Fig. 12 SAR of 2,5-disubstituted benzimidazoles



Cd	R_1	R_2	Y	Z
25	Cl	H	NH	C_2H_4
26	CH_3	H	NH	C_2H_4
27	CH_3	CH_3	NH	CH_2
28	Cl	Cl	NH	CH_2O
29	H	NO_2	O	CH_2
30	NO_2	NO_2	O	CH_2

Fig. 13 Purine-based catalytic topoisomerase II inhibitors

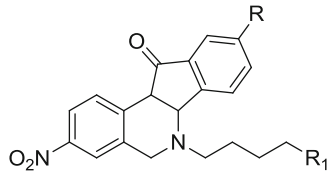


cycle arrest and thus leading to programmed cell death (Guerrant *et al*., 2013).

Camptothecin, 10-hydroxycamptothecin, and 7-ethyl-10-hydroxycamptothecin are some of the topoisomerase I inhibitors (Fig. 11). Maja and team swayed a review on explaining the topoisomerase degradation in cancer cell resistance linking to camptothecin-like topoisomerase I inhibitors (Tomicic and Kaina, 2012).

In 2008, Oksuzoglu *et al*. manifested benzoxazole and benzimidazole derivatives as potent topoisomerase I and II inhibitors (Oksuzoglu *et al*., 2008). The compound **25** was the most potent DNA topoisomerase I inhibitor having IC_{50} value of 14.1 mM. Other compounds with significant activity against topoisomerase I were 5-amino-2-(*p*-fluorophenyl)benzoxazole, 5-amino-2-(*p*-bromophenyl)benzoxazole, and 5-nitro-2-phenoxymethyl-benzimidazole having IC_{50} values of 132.3, 134.1, and 258 mM, respectively. Further 2-(*p*-nitrobenzyl)benzoxazole has shown maximum inhibitory potential against DNA Top-II having IC_{50} of 17.4 mM (Oksuzoglu *et al*., 2008). Some of the compounds

Fig. 14 Novel indenoisoquinolines as topoisomerase I inhibitors



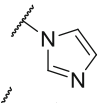
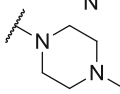
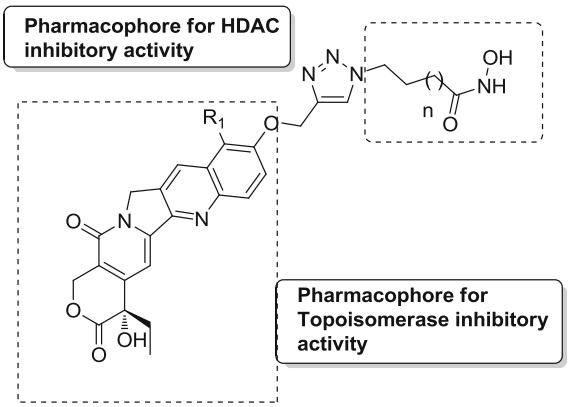
Cd	R_1	R	A549 (μM)	HepG2 (μM)	HCT-116 (μM)
34			0.023	0.16	0.0932
35			0.021	0.019	0.84

Fig. 15 Dual-acting histone deacetylase−topoisomerase I inhibitors



Cd	n	R_1	HeLa IC_{50} (nM)	HDAC 1 IC_{50} (nM)	HDAC 6 IC_{50} (nM)	HDAC 8 IC_{50} (nM)
37	1	$-CH_2CH_3$	-	-	85 ± 34	1726 ± 577
38	4	–H	56.2	37 ± 7	81 ± 26	1046 ± 316
SAHA	—	—	65.0	38 ± 2	27 ± 2	1989 ± 156

with the structure−activity relationships are presented in Fig. 12.

In 2009, Furet *et al*. designed new class of catalytic topoisomerase II inhibitors based on purine motif (Fig. 13) which competed with ATP-binding site (Furet *et al*., 2009).

In 2012, Zhang *et al*. envisioned novel indenoisoquinolines as topoisomerase I inhibitors. They evaluated the series of compounds in human cancer cell lines A549, HepG2, and HCT-116 and found out the compounds **34** and **35** to be potent against HepG2 and HCT-116 (IC_{50} of 0.019 and 0.093 μM, respectively) cancer cell lines (Fig. 14) (Zhang *et al*., 2012). The nitro group was optimum for the activity. Further methoxy and fluorine at R showed maximum potency.

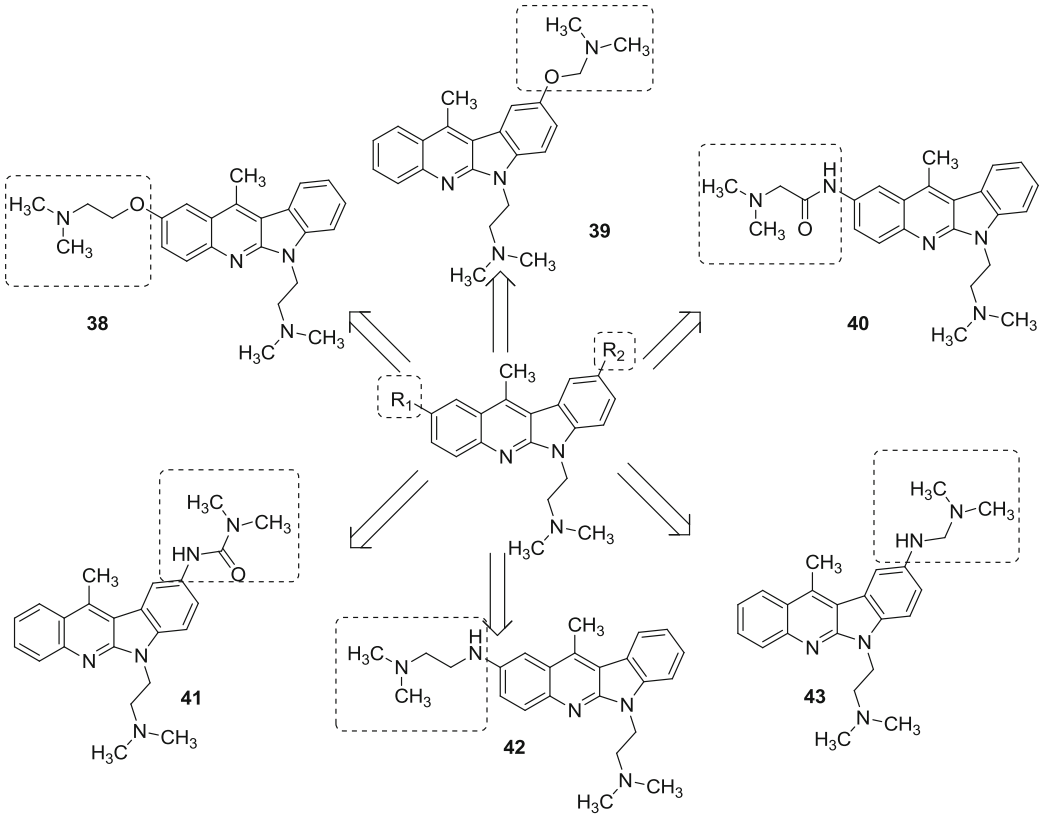
In 2013, Guerrant *et al*. synthesized dual-acting histone deacetylase−topoisomerase I inhibitors (Fig. 15) leading to cell arrest in cell-free and whole-cell studies. The compounds were designed on the camptothecin template having a triazole ring which was joined with hydroxamic moiety via a linker (Guerrant *et al*., 2013). The linker length had been concluded to be an important factor for the activity. The ethyl group at the *C*-7 of camptothecin ring system was insignificant for the inhibition of HeLa cell nuclear extract HDACs. The mechanism of topoisomerase inhibition was investigated based on hydrogen bond formation of 10-hydroxy moiety of compounds with water molecule present in Topoisomerase I, which was further supported by X-ray crystallographic data.

In 2012, Luniewski *et al*. synthesized 11-methyl-6-[2-(dimethylamino)ethyl]-6*H*-indolo[2,3-*b*]quinoline derivatives as DNA topoisomerase II inhibitors (Fig. 16). The compounds were tested on different cancer cell lines and analogs with either amine or amide linkers were found to exhibit maximum antiproliferative activity (Luniewski *et al*., 2012).

In 2013, Xu *et al*. synthesized indenoisoquinoline derivatives as topoisomerase I inhibitors that suppressed angiogenesis by affecting the HIF signaling pathway (Xu *et al*., 2013).

HGPRT inhibitors

Hypoxanthine–guanine phosphoribosyltransferase (HGPRT; EC 2.4.2.8) is an enzyme belonging to the class of



Cd	Cell line/IC_{50} ± SD (μM)				Total topoisomerase II inhibition
	MCF-7	A-549	KB	Hs294T	
38	0.66 ± 0.05	0.19 ± 0.07	0.08 ± 0.02	0.76 ± 0.14	0.025
39	0.81 ± 0.07	0.32 ± 0.03	0.31 ± 0.10	0.91 ± 0.12	0.025
40	0.38 ± 0.047	0.24 ± 0.07	0.15 ± 0.07	0.62 ± 0.07	0.05
41	0.79 ± 0.10	0.81 ± 0.10	0.15 ± 0.05	0.64 ± 0.07	0.025
42	0.47 ± 0.00	0.17 ± 0.05	0.64 ± 0.08	0.35 ± 0.05	0.025
43	0.99 ± 0.05	0.29 ± 0.05	0.36 ± 0.13	0.72 ± 0.09	0.05

Fig. 16 Chemical structures of synthesized indolo[2,3-*b*]quinoline derivatives as topoisomerase II inhibitors

phosphoribosyl transferases inscribed by *HPRT1* gene (Eads *et al*., 1994; Sculley *et al*., 1992). The enzyme is tortuous in transition of hypoxanthine and guanine to inosine monophosphate and guanine monophosphate, respectively (Fig. 17). It functions via shifting the 5-phosphoribosyl to the purine from 5-phosphoribosyl 1-pyrophosphate through salvage pathway (Finette *et al*., 2002; Torres and Puig, 2007). The transfer occurs by the binding of 5-phosphoribosyl 1-pyrophosphate with free enzyme and successive formation of bond between the C-1 of 5-phosphoribosyl 1-pyrophosphate and the *N*-9 of the purine ring of hypoxanthine or guanine (Keough *et al*., 2005). Atkinson *et al*. contemplated the HGPRT inhibitory activity of 6-mercaptopurine (Fig. 18) and structurally related compounds through its competitive inhibition.

In 2009, Keough *et al*. synthesized neutral 6-oxopurine acyclic nucleoside phosphonates, which showed inhibitory activity against recombinant PfHGXPRT (Fig. 19) leading to antimalarial effect in vitro. The docking studies revealed the formation of hydrogen bond by three oxygen atoms of phosphate/phosphonate group with main-chain amides of Gly139 and Asp137 and a water molecule, amide of Thr141, the hydroxyl atom of Thr141 and a water molecule, side-chain oxygen, and main-chain amide atom of Thr138 (Keough *et al*., 2009). Further in 2013, the same research group revealed that the lipophilic groups linked by phosphoramidate bonds of these compounds escalate the activity.

In 2012, Krecmerova *et al*. synthesized purine *N*9-[2-hydroxy-3-*O*-(phosphonomethoxy)propyl] derivatives and

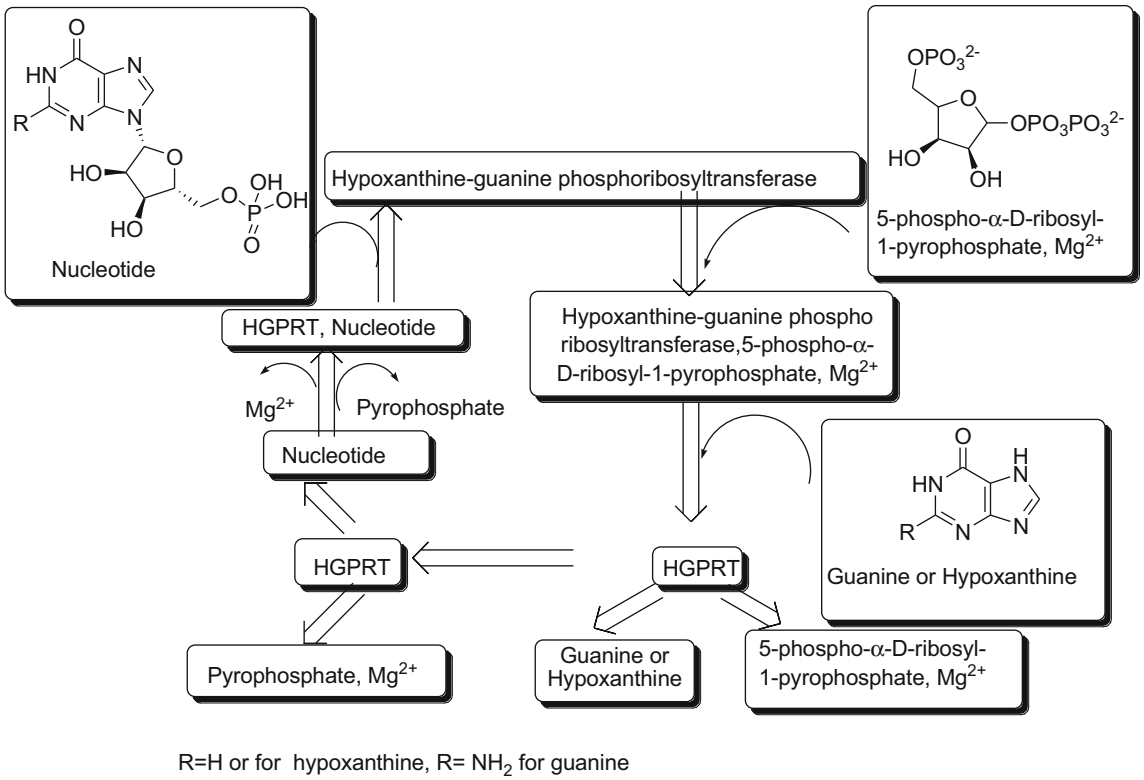
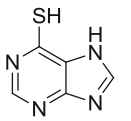


Fig. 17 Catalytic pathway for hypoxanthine–guanine phosphoribosyl transferase

Fig. 18 Chemical structure of 6-mercaptopurine (**44**)

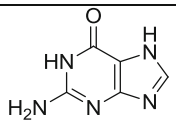
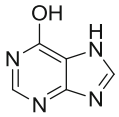


K_i = 4.5 μM

their side-chain modified analogs as potential antimalarial agents (Fig. 20). The synthesized acyclic nucleoside phosphonates exhibited very weak inhibition of human HGPRT and good inhibition of *Plasmodium falciparum* as well as *Plasmodium vivax* enzymes. The K_i values were 2 and 5 μM for *P. falciparum* HGPRT and *P. vivax* HGPRT, respectively (Krecmerova *et al.*, 2012). The docking studies revealed that guanine-based compounds were less effective.

Hockovaa *et al.* in 2012 synthesized novel *N*-branched acyclic nucleoside phosphonates (ANPs) as potent and selective inhibitors of human, *P. falciparum*, and *P. vivax* 6-oxopurine phosphoribosyl transferases (Fig. 21). Guanine emerged to be more potent base for human HGPRT and bound more tightly than hypoxanthine via formation of extra hydrogen bond between the 2-amino group and the active site amino acid groups. Further two-carbon atoms linked between the tri-substituted nitrogen and the *N*-9 atom had been found to be optimum for the HGPRT inhibitory activity. The substituents like COOMe, COOH, and OH were tolerable for inhibitory activity against

Fig. 19 Acyclic nucleoside phosphonates as a new class of HGPRT inhibitors

Cd	Base	Phosphonate tail	K_i (mM)	
			*Pf*HGXPRT	Human HGPRT
45		ribose-5'-phosphate	10 ± 2	5.8± 0.2
46		ribose-5'-phosphate	3.6 ± 1	5.4± 1.2

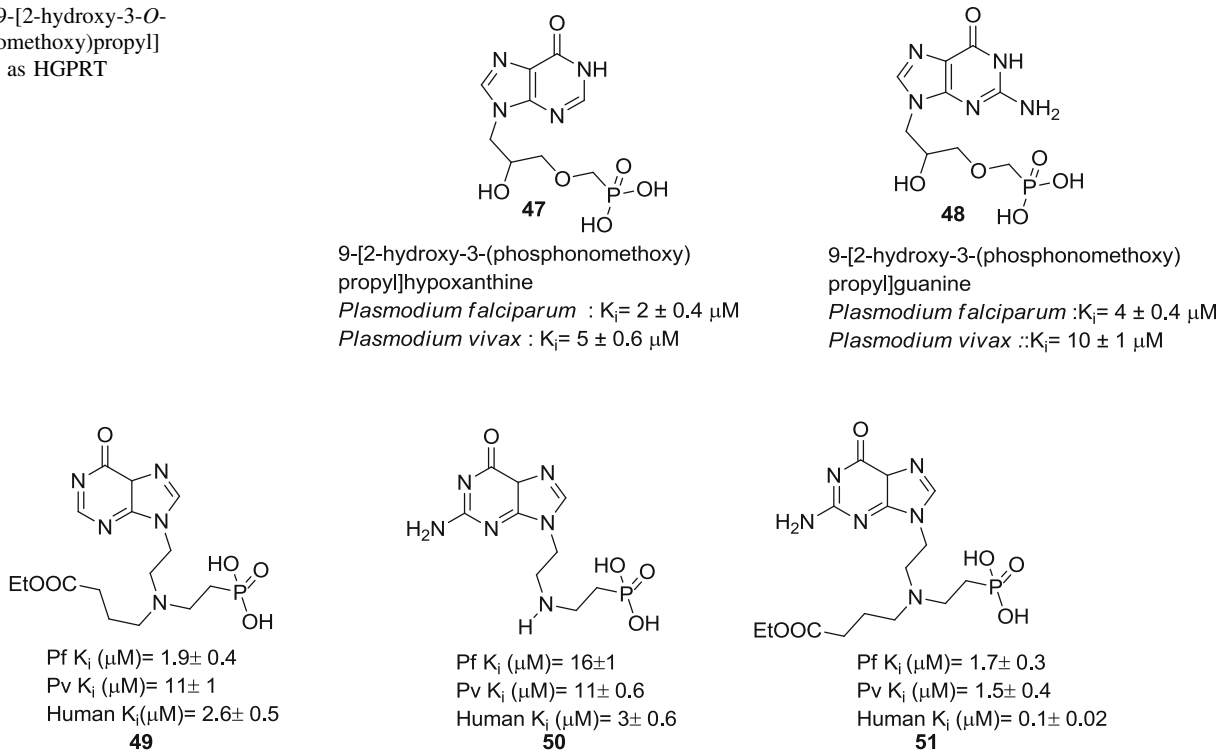


Fig. 20 *N*9-[2-hydroxy-3-*O*-(phosphonomethoxy)propyl] derivatives as HGPRT inhibitors

Fig. 21 *N*-branched acyclic nucleoside phosphonates as human and plasmodium HGPRT inhibitors

PfHGXPRT, whereas CN was preferably optimum for PvHGPRT inhibition (Hockovaa *et al*., 2012).

In 2012, Hazleton *et al.* gave acyclic immucillin phosphonates as inhibitors of *P. falciparum* Hypoxanthine–Guanine–Xanthine Phosphoribosyltransferase (Fig. 22). The phosphonate group of the acyclic immucillin phosphonates offered resistance to phosphohydrolases. The compounds were based on immucillin scaffold but having enhanced stability, specificity, and binding affinity than neutral 6-oxopurine acyclic immucillin phosphonates. The important interactions observed were hydrogen bonding with Glu144, Asp145, and water molecule coordination by Mg^{2+} ion. The cationic reaction center of these compounds had complimentary interactions leading to increased affinity for PfHGXPRT (Hazleton *et al*., 2012).

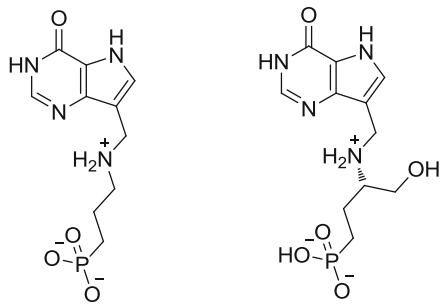
In 2012, Ansari *et al*. carried out comparative modeling of HGPRT enzyme of *L. donovani* and studied binding affinities of different analogs of GMP. Lys66, Asp74, Arg77, Asp81, Val88, Tyr182, Arg192, and Arg194 are the important amino acids involved in binding of HGPRT. It was found out that HGPRT inhibitors like acyclovir or pentamidine in combination might have the potential against HIV and visceral leishmaniasis (Ansari *et al*., 2012).

Cesnek *et al*. synthesized and evaluated 9-phosphonoalkyl and 9-phosphonoalkoxyalkyl purines as inhibitors of *P. falciparum*, *P. vivax*, and human HGPRT (Fig. 23). The linker and the purine base were crucial and found to play an important role in the binding of the ANPs to these three enzymes. The compounds with shorter linker did not show binding to the enzymes because if the base binds first, the phosphonate group is unable to reach into the 5-phosphate binding pocket or on the contrary; if the phosphonate group binds to active site first, the base is in a farther distance from its binding site (Cesnek *et al*., 2012).

In 2013, Baszczynski *et al*. demonstrated the effect of novel [3-fluoro-(2-phosphonoethoxy)propyl]purines on the inhibition of *P. falciparum*, *P. vivax*, and human HGPRT (Fig. 24). The fluorinated ANPs were highly potent as compared to non-fluorinated ANPs. Nature and location of the purine base were critical for the activity. The phosphorous and oxygen interactions with Asp137 and Thr141 amino acids were found to be indispensable and essential for the tight binding of the acyclic nucleoside phosphonates (Baszczynski *et al*., 2013).

Guanine deaminase inhibitors

Guanase or guanine deaminase (GDA; EC 3.3.4.3) is a zinc metalloenzyme involved in catalysis of first step in purine metabolism and converts guanine to xanthine by the hydrolytic deamination (Fernandez *et al*., 2010). Guanine deaminase consists of two families. The families differ in



Cd	*Pf* K_i	*Hs* K_i	*Hs* K_i/*Pf* K_i
52	10.6 ± 1.3 nM	4940± 290nM	466
53	0.65 ± 0.04 nM	385 ± 40 nM	592

Fig. 22 Chemical structures and inhibitory activities of acyclic immucillin phosphonates (**52** and **53**)

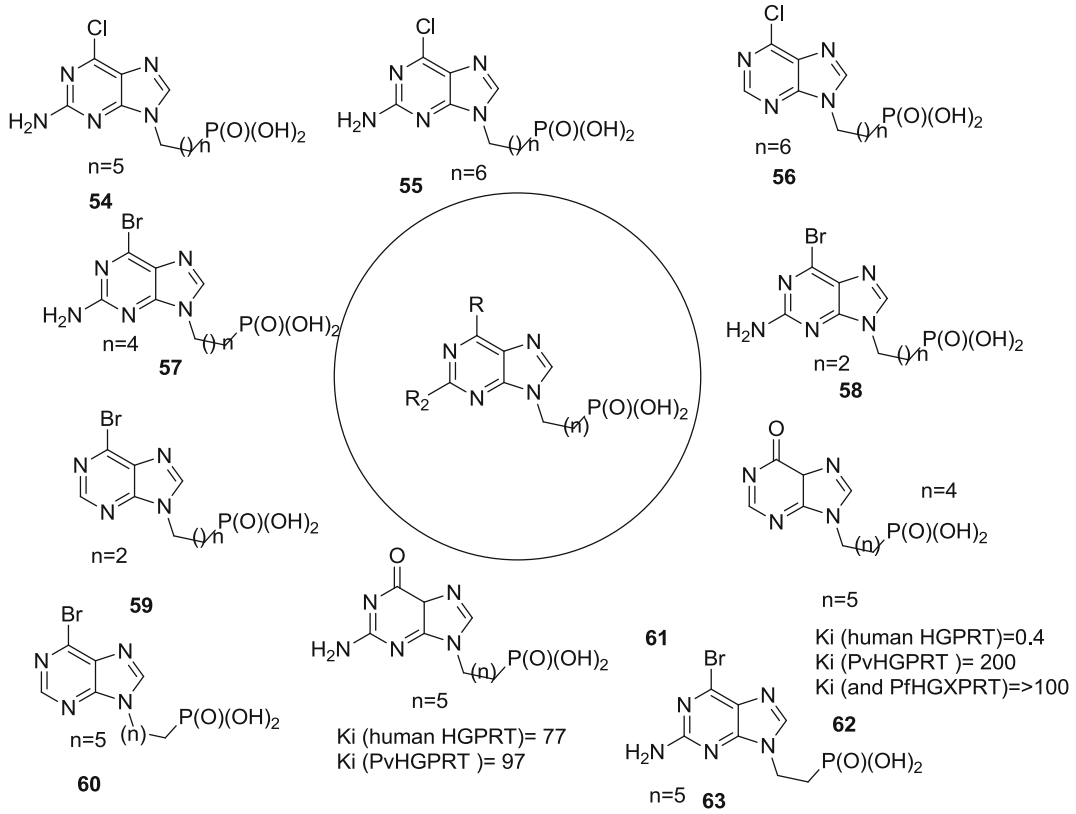


Fig. 23 Chemical structures of 9-phosphonoalkyl and 9-phosphonoalkoxyalkyl purines

number of amino acids present in them. One of them contains 160 amino acids and other consists of greater than 400 amino acids for e.g., *Bacillus subtilis* guanase and *Escherichia coli* guanase, respectively.

Wang and Hosmane reported ring-expanded acyclic nucleosides, 4,6-diamino-8*H*-1-hydroxyethoxymethyl-8-iminoimidazo[4,5-*e*][1,3]diazepines (Fig. 25) as dual inhibitors of ADA and GDA. The compounds were found to act by competent inhibition (Wang and Hosmane, 2001).

In 2011, Chakraborty *et al*. proposed the mechanism of GDA inhibitors as transition-state analog inhibitor (Fig. 26). The compound possessed geminal carbon at position-6 and a benzyl group at position-3 which provided lipophilicity in its surrounding. These groups escalated the interactions with enzyme. The 5-carbonyl group in along with 6-hydroxy group was proposed to interact with Zn^{2+} offering stability to the transition state (Chakraborty *et al*., 2011).

In 2012, the same research group synthesized and evaluated the structural analogs of azepinomycin against GDA (Fig. 27) for determining structure–activity relationships (Chakraborty *et al*., 2012). However, the compounds were not found to be as potent as natural analog.

Further, Tantravedi *et al*.. (2013) reported synthesis, biochemical screening, and structure–activity correlations of various selectively substituted imidazo[4,5-

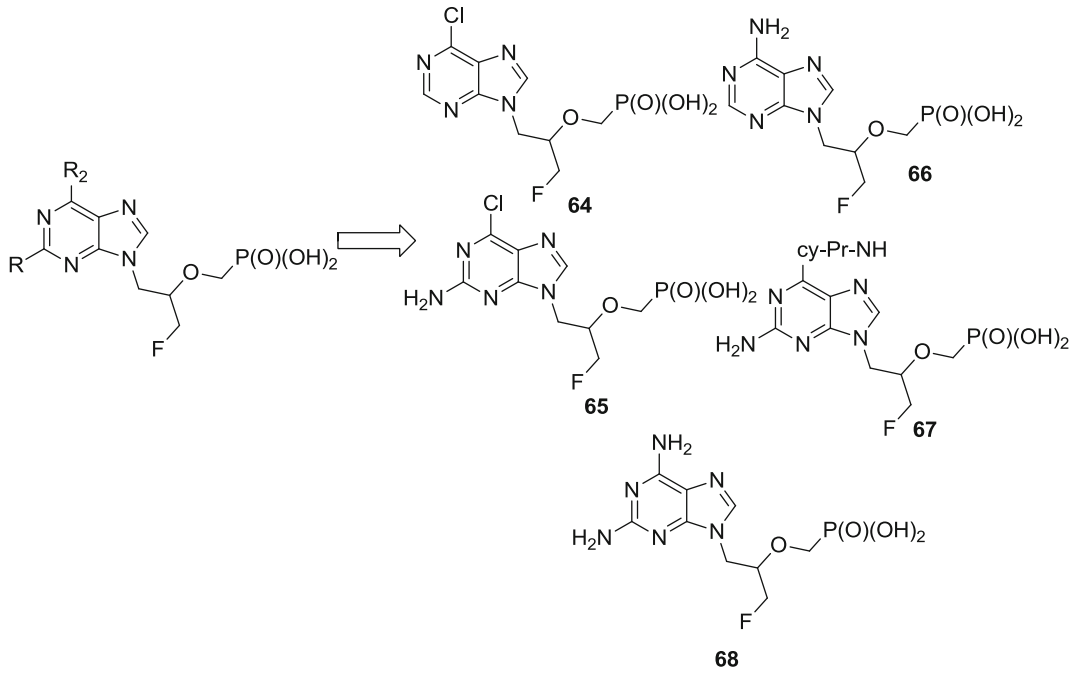


Fig. 24 [3-Fluoro-(2-phosphonoethoxy)propyl]purines as HGPRT inhibitors

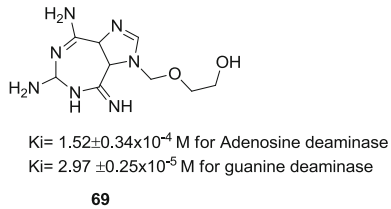


Fig. 25 4,6-Diamino-8*H*-1-hydroxyethoxymethyl-8-iminoimidazo[4,5-*e*][1,3]diazepine as dual inhibitor of adenosine and guanine deaminases

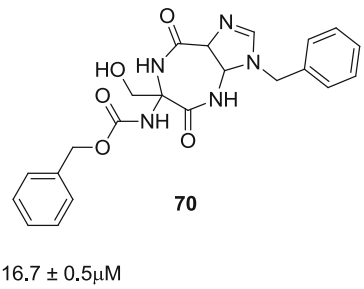


Fig. 26 Novel transition-state analog inhibitor of guanase

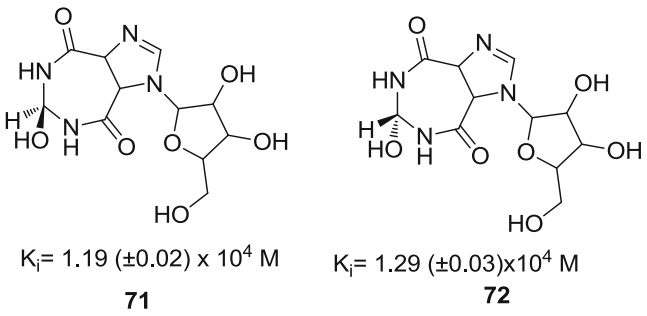
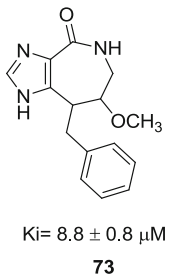


Fig. 27 Synthetic analogs of azepinomycin

Fig. 28 Potential transition-state analog inhibitor of guanase



e][1,4]diazepines as potential transition-state analog inhibitors of guanase (Fig. 28). It was observed that the lipophilicity near *O*-5 was escalated, and if the hydrophobic character of *N*-3 was shifted to *N*-1, the activity decreases. Further, *N*-3 or *N*-4 hydrophobic character was directly proportional to inhibitory activity. The *N*-3 lipophobic character was crucial for inhibitory activity. The azepinomycin exhibited K_i of 2.5 μM against GDA (Tantravedi *et al*., 2013).

Xanthine oxidase inhibitors

Xanthine oxidase (XO; EC 1.1.3.22) (Oliveira-Campos *et al*., 2008) is a metallo-flavoenzyme which is oxidized

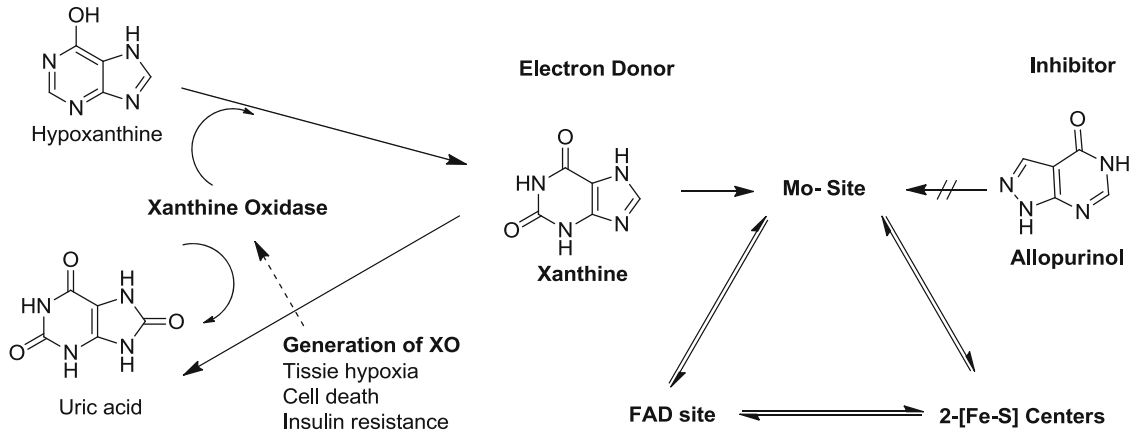
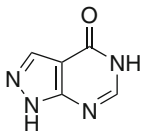


Fig. 29 Xanthine oxidase catalyzed biotransformation

Fig. 30 Chemical structure of allopurinol (**74**)



form of xanthine oxidase reductase and present in brain, kidney, heart, liver, gut, lung, etc. (Oliveira-Campos *et al*., 2008; Kumar *et al*., 2011; Nepali *et al*., 2011b). XO is also acquitted by plasma and some leukocytes for the generation of hydrogen peroxide and other forms of reactive oxygen species during inflammatory response (Kayyali *et al*., 2003; Kim *et al*., 2013). XO comprises two subunits (Nepali *et al*., 2011a) and contains two iron-sulfur centers, molybdenum-site, and flavin−adenine dinucleotide. It converts the bicyclic nitrogenous bases to uric acid i.e., hypoxanthine to xanthine and xanthine to uric acid (Fig. 29) (Bhushan *et al*., 2003). The enzyme generates superoxide anions and hydrogen peroxide which are further converted into hydroxyl radical in the presence of chelated iron (Beauchamp and Fridovich, 1970; Fridovich, 1970; Kumar *et al*., 1995).

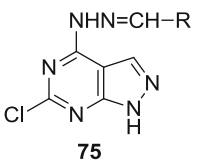
Allopurinol (Fig. 30) was the first XO inhibitor clinically approved for the treatment of gout (Borges *et al*., 2002). It is a competitive inhibitor of XO and arrests the conversion of hypoxanthine and xanthine to xanthine and uric acid, respectively (Massey *et al*., 1970).

In 2010, Ali *et al*. carried out the docking studies based on PMF scoring performances and SAR of 2-substituted pyrazolotriazolopyrimidines and 4-substituted pyrazolopyrimidines for the design of XO inhibitors (Fig. 31). The active site of the bovine milk xanthine dehydrogenase and two scoring functions AutoDock 3.05 and the CACHe 6.1.10 were used. The oxo group of pyrazolopyrimidine derivatives was found to be crucial for its activity. Further bicyclic pyrazolopyrimidine derivatives were less potent than the tricyclic derivatives. Docking score of allopurinol was −88.04 (Ali *et al*., 2010).

In 2011, our research group rationally designed and synthesized 1-acetyl-3,5-diaryl-4,5-dihydro(1*H*)pyrazoles as a new class of potential non-purine XO inhibitors (Fig. 32). 1-Naphthyl group at ring A showed good XO inhibitory activity (Fig. 32). Replacement of 1-naphthyl at ring A with 2-furyl further increased XO inhibition. 4-Pyridyl at ring B (**78**) exhibited maximum potency. Electron-withdrawing groups such as nitro amplified the activity, whereas electron-donating groups had the opposite effect. *N*-acetyl group was found out to be critical for the activity (Nepali *et al*., 2011b).

Hu *et al.* discovered novel xanthone derivatives as XO inhibitors (Fig. 33). Cyano group at *para* position showed noteworthy inhibition of XO. *Para* substituted benzyl groups exhibited more XO inhibition as compared to substitutes at *ortho* or *meta* position. The docked compounds

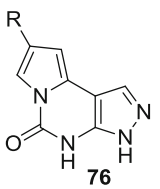
Fig. 31 Pyrazolopyrimidine and pyrazolotriazolopyrimidines as xanthine oxidase inhibitors



75

R= n-C_7H_{15}

Docking Score= -95.24



76

R=H

Docking Score= -103.37

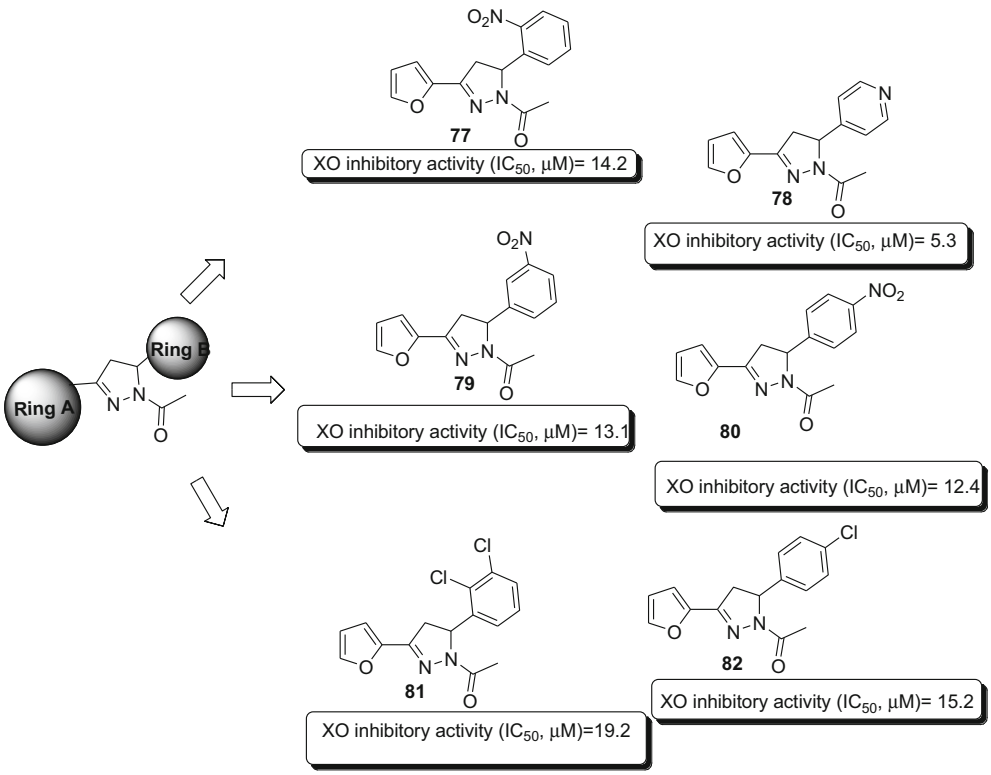


Fig. 32 1-Acetyl-3,5-diaryl-4,5-dihydro(1*H*) pyrazoles as non-purine xanthine oxidase inhibitors

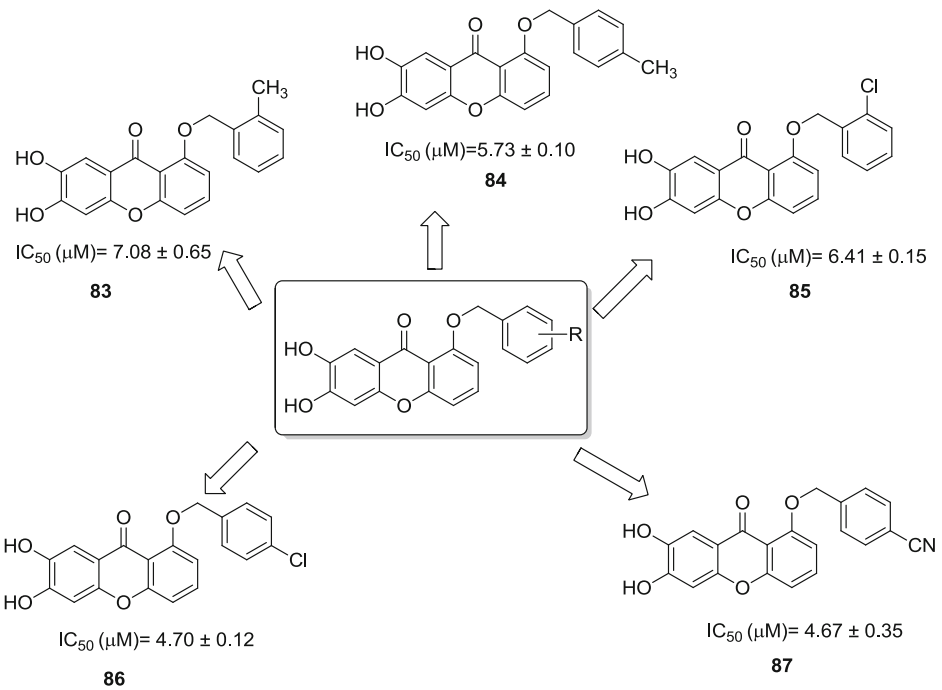


Fig. 33 Xanthone derivatives as XO inhibitors

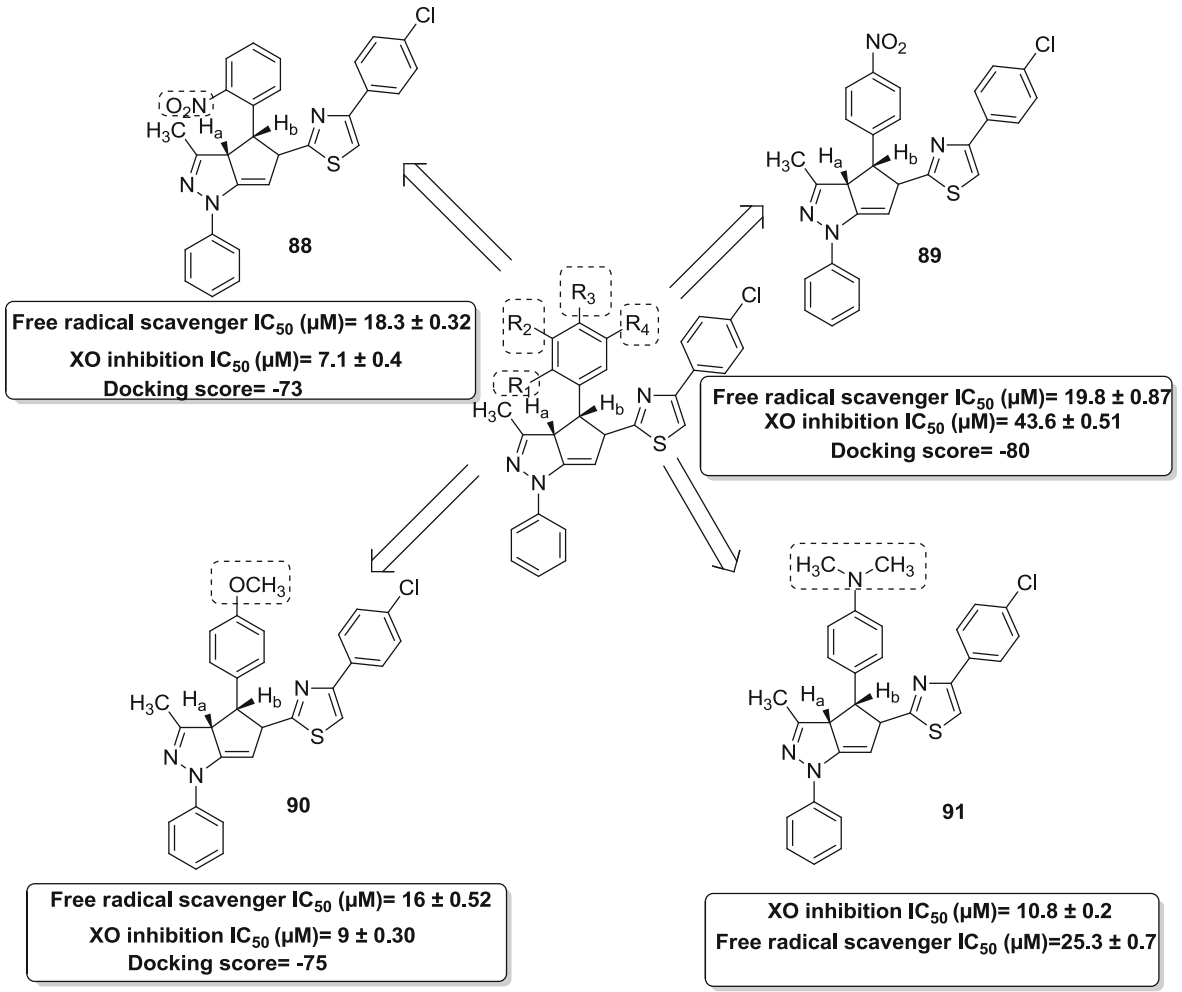


Fig. 34 Thiazolo-pyrazolyl derivatives as XO inhibitors

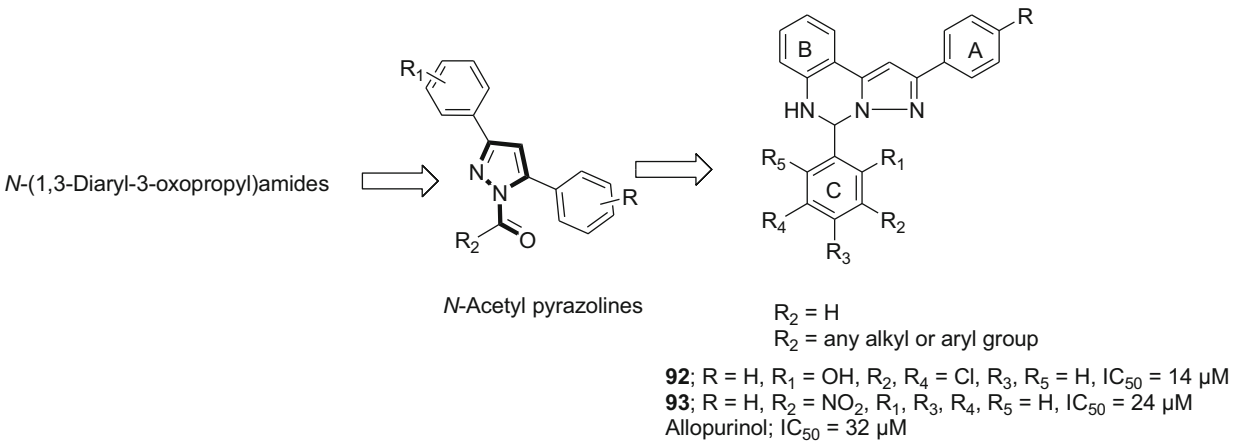


Fig. 35 5,6-Dihydropyrazolo/pyrazolo[1,5-*c*]quinazoline derivatives as xanthine oxidase inhibitors

were found to interact with molybdenum-protein active site, Phe798, Gln1194, and Gln112 (provides stabilization) and hydrogen bonding with Gln1040, Ser1082, Gln1261, Gly797, Gln767, and Cys150 (Hu *et al*., 2011).

In 2012, Beedkar *et al*. synthesized novel thiazolo-pyrazolyl derivatives and evaluated them as xanthine oxidase inhibitors and free radical scavengers (Fig. 34). In vitro XO assay and in silico study evidenced that the hydroxylation

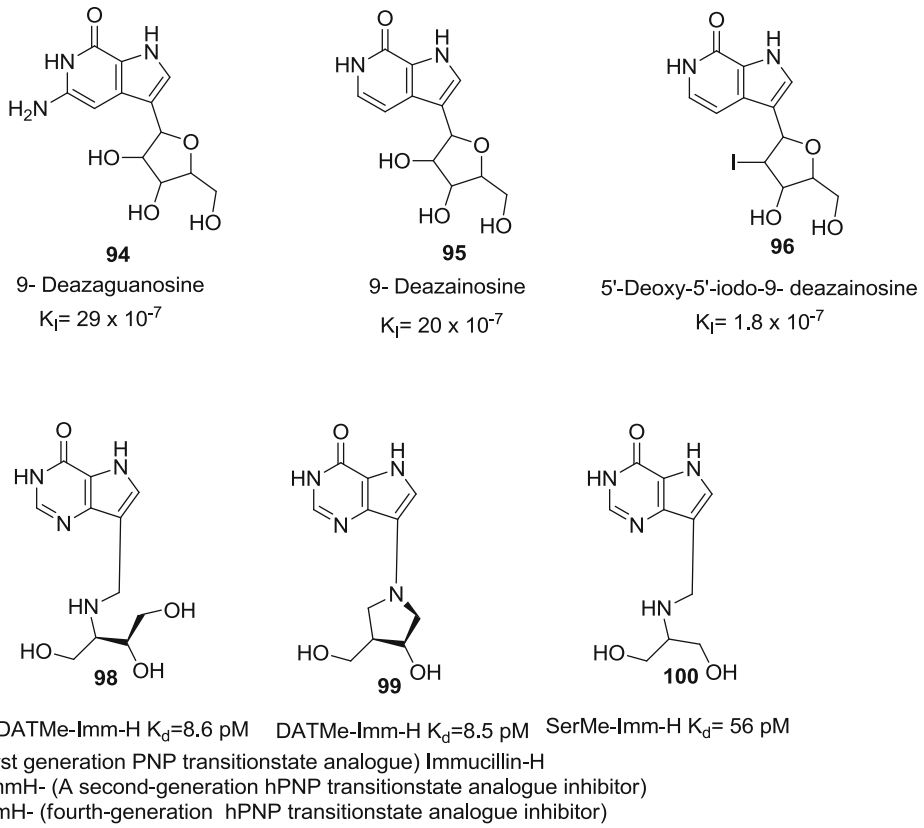


Fig. 36 9-Deazapurine ribonucleotide and its derivatives as PNPase inhibitors

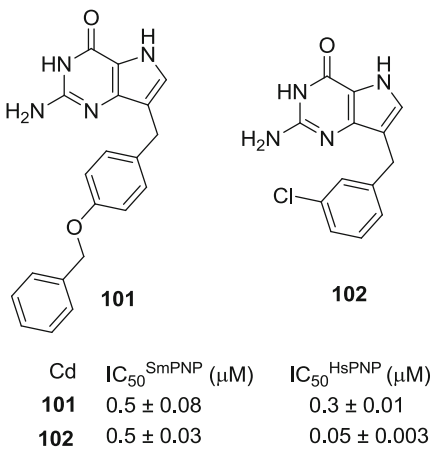
Fig. 37 Four generations of transition-state analogs as PNPase inhibitors

is furnished at the aromatic rings conjoined by a central fused pyrazole rings near molybdenum metal (Beedkar *et al*., 2012).

In 2014, our research group discovered 5,6-dihydropyrazolo/pyrazolo[1,5-*c*]quinazoline derivatives as XO inhibitors (Kumar *et al*., 2014) (Fig. 35). The compounds were also found to be free radical scavengers. The docking studies of compounds highlighted the role of important amino acids of XO in binding interactions.

PNPase inhibitors

Purine nucleoside phosphorylase (PNPase; E.C. 2.4.2.1) is an omnipresent enzyme involved in purine salvage pathway and also known as inosine phosphorylase. It catalyzes the conversion of inosine to hypoxanthine, adenosine to adenine, and guanosine into guanine (Bzowska *et al*., 2000; Kline and Schramm, 1993). PNPase has dual role in purine metabolism i.e., in synthesis as well as metabolism (Kline and Schramm, 1995). PNPase involves nucleoside segmentation resulting in a nucleobase and ribose-1 phosphate by ribose phosphorylation. T-cell immunodeficiency during PNPase deficiency led to the discovery of PNPase inhibitors against rheumatoid arthritis, tissue rejection, chemotherapy for T-cell leukemias, and other autoimmune

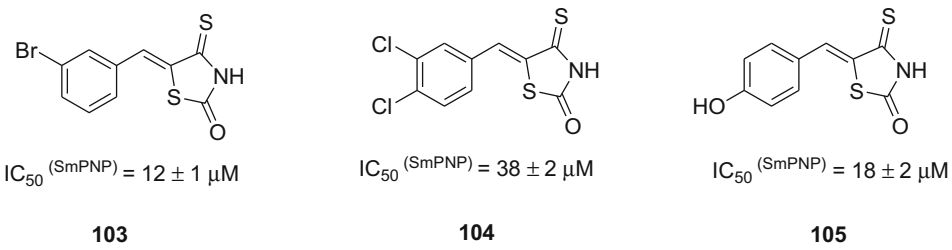


Cd	IC_{50}^{SmPNP} (μM)	IC_{50}^{HsPNP} (μM)
101	0.5 ± 0.08	0.3 ± 0.01
102	0.5 ± 0.03	0.05 ± 0.003

Fig. 38 Purine nucleoside phosphorylase inhibitors

disorders (Furihata *et al*., 2014; Giblett *et al*., 1975; Gelfand *et al*., 1978; Borgers *et al*., 1977; Somech *et al*., 2013; Crittenden and Pillinger, 2013; Stoeckler *et al*., 1986; Shewach *et al*., 1986).

Stoeckler *et al*. described 9-deazapurine ribonucleotide and its derivatives as PNPase inhibitors. These compounds were found to act via competitive inhibition of PNPase. It was observed that the 9-deazapurine ribonucleotides have



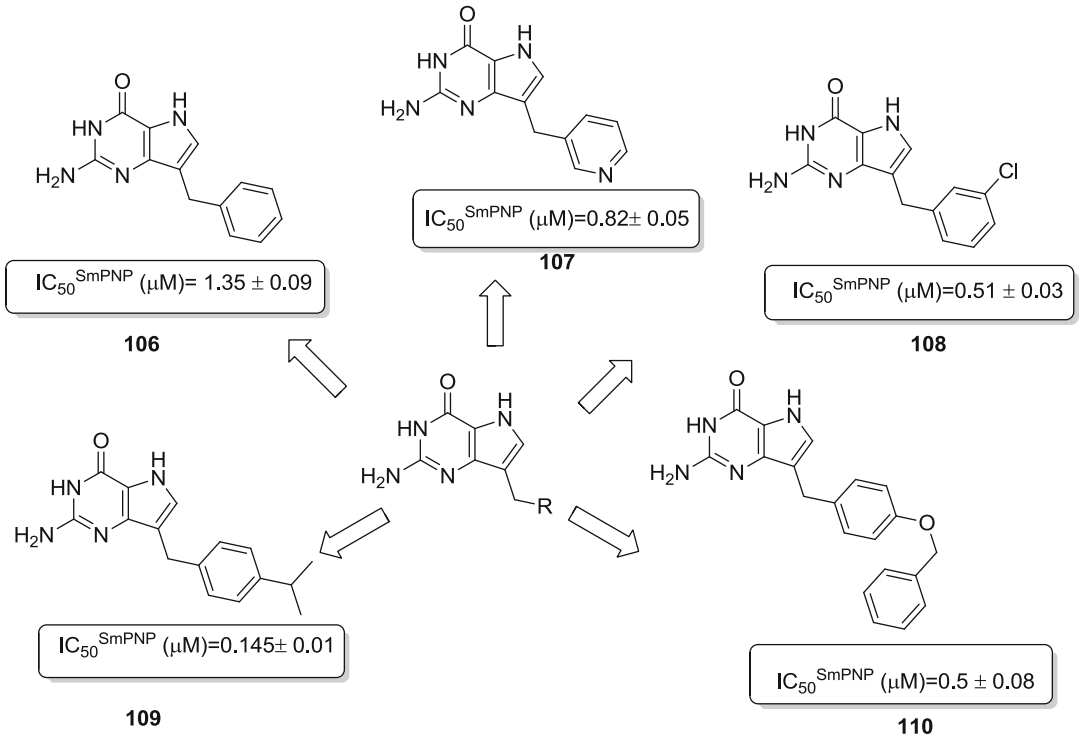


Fig. 39 Chemical structures of new inhibitors of *Schistosoma mansoni* purine nucleoside phosphorylase

Fig. 40 SmPNPase inhibitors

much higher affinity for the enzyme than corresponding formycin B series (Fig. 36) (Stoeckler *et al*., 1986).

In 2010, Ho *et al*. established and generated the four generations of transition-state analogs for human PNPase (Fig. 37).The X-ray crystal structures of human PNPase featured that there is formation of an ion-pair between bound phosphate and cation inhibitor. The phosphate group interacts with hydroxyl groups whereas leaving group interacts to *N*-1, *O*-6 and *N*-7 of 9-deazahypoxanthine. Other important interaction was of His257 with the 5′-hydroxyl group. It was concluded that third and fourth generation PNP inhibitors better occupy the binding site in comparison to first generation inhibitors (Ho *et al*., 2010).

In 2010, Castilho *et al*. studied crystallographic, kinetic, and structural bases of purine-based compounds for their selective inhibition of PNPase obtained from *Schistosoma mansoni*. Electronic, hydrogen bonding, volume, and lipophilic properties were correlated with the extent of inhibition. The *ortho* and *para* pyridine-substitutions increased the selectivity of compounds but when substituted with bulkier groups, the selectivity was lost. Further *meta*-substitutions of pyridine produced converse results. The important interaction seen was the hydrogen bonding to Tyr202 (Castilho *et al*., 2010). The compounds are represented in Fig. 38.

In 2010, Postigo *et al*. discovered new inhibitors of *S. mansoni* purine nucleoside phosphorylase (SmPNP) by pharmacophore-based virtual screening (Fig. 39). They performed the docking studies and the important interactions seen were the *meta*-bromophenyl substituent with the hydrophobic side chain of Ala118, Val262, His259, Met221, and Phe161, T-shaped π-stacking of phenyl ring with the side chain of Phe161 and Vander Waals interaction of the bromine atom with the side chain of Ala 118 and Val262. In the *para* position, the hydroxyphenyl was best suited as compared to fluorophenyl or nitrophenyl. Further *meta*-

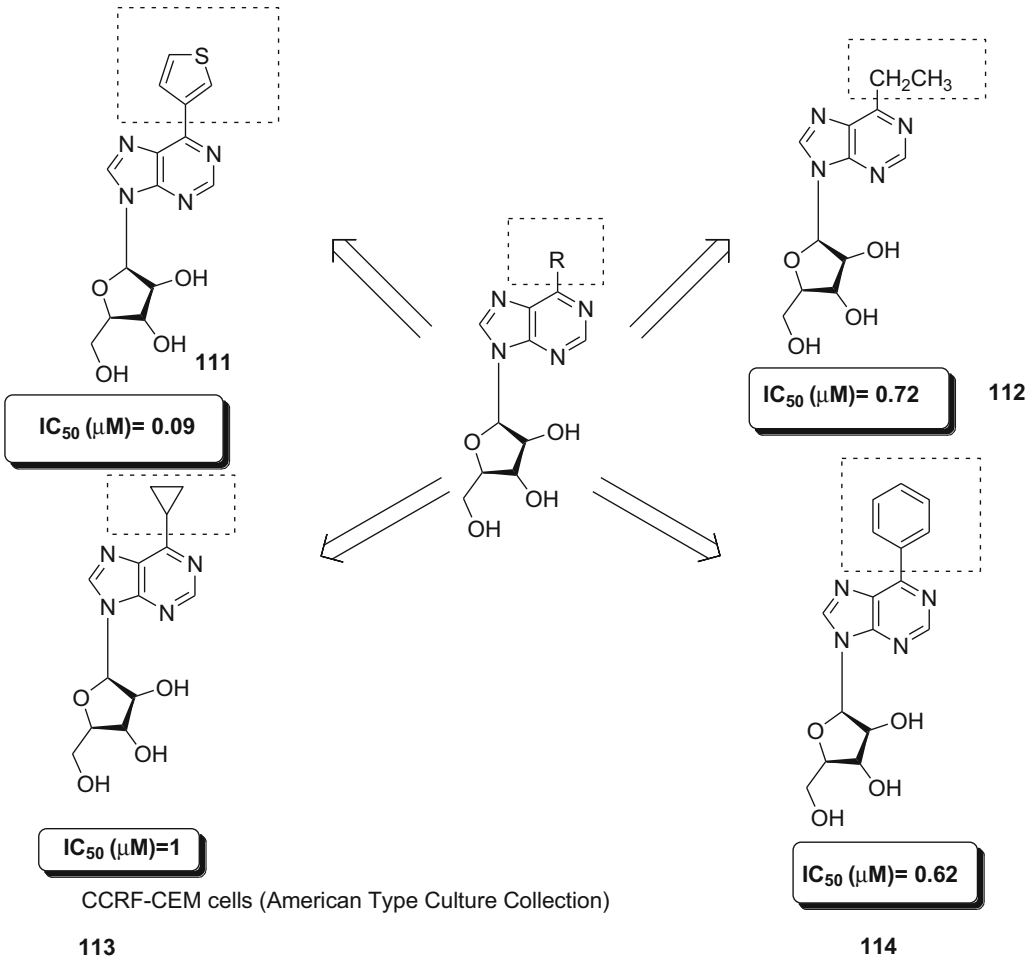
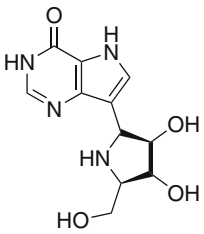


Fig. 41 Inhibition of CEM cell growth by C-6-substituted purine nucleosides

Fig. 42 Chemical Structure of immucillin-H (**115**)



substituted analogs were less potent than *para*-substituted analogs due to the steric hindrance caused by Met221 and Ala118. The mono-substituted phenyl thioxothiazolidinone derivatives were most potent followed by fair potency of disubstituted derivatives and least potent tri-substituted phenyl thioxothiazolidinone derivatives (Postigo *et al*., 2010).

In 2011, Postigo *et al*. further demonstrated enzyme kinetics, structural analysis, and molecular modeling studies on a series of SmPNP (Fig. 40). The catalytic residue of Asn245 interacts with carbonyl oxygen by donating a hydrogen bond whereas it takes a hydrogen bond from the 7′ N–H group of the purine ring system. The π–π interactions were seen between base and the side chain of Tyr202. The bulkier groups were found to play a crucial role in lipophilicity of compounds and their interactions. The compounds with aromatic ring (phenyl, pyridine, and thiophene) were less potent as compared to non-aromatic compounds. Further substitutions on phenyl ring on *ortho* or *meta* positions with hydroxyl or chlorine have shown escalation in potency. Most potent compounds were generated via substitution at *para* position (Postigo *et al*., 2011).

Hassan *et al*. synthesized C-6 alkyl, cycloalkyl, and aryl-9-(β-*d*-ribofuranosyl)purine analogs and evaluated their potential against *E. coli* PNPase (Fig. 41) (Hassan *et al*., 2012).

In 2012, Wielgus-Kutrowska *et al*. explored binding of immucillin-based compounds in the transition state of trimeric PNPase. It was concluded that one-third-of-the-sites binding does not occur for trimeric PNP (Wielgus-Kutrowska *et al*., 2012).

Table 1 Mechanisms, uses, side effects, and routes of administration of US-FDA-approved purine-utilizing enzyme inhibitors

Drug	Proprietary name	Dosage form	Route	Mechanism of action	Uses	Side effects
Febuxostat	Uloric	Injection	IV	Xanthine oxidase inhibitor (Oliveira-Campos *et al*., 2008; Becker *et al*., 2010; Takano *et al*., 2005)	Treat hyperuricemia in gout patients (Beara-Lasic *et al*., 2010)	Hepatic abnormalities, nausea, arthralgia (Becker *et al*., 2005a, b)
Pentostatin	Nipent	Injection	IV	Adenosine deaminase inhibitor	Hairy cell leukemia (Spiers *et al*., 1987; Kane *et al*., 1991)	Cough, chills, lower back pain, pain, painful urination, weakness (Poi *et al*., 2013; Dighiero, 1996)
Thioguanine	Lanvis	Tablet	Oral	Inhibition of HGPRT (Nelson *et al*., 1975)	Treatment of acute lymphoblastic leukemia in children (Nelson *et al*., 1975)	Leukopenia, thrombocytopenia, hepatic toxicity, loss of appetite etc. (Gearry *et al*., 2004)
Mercaptopurine	Purinethol	Tablet	Oral	Inhibition of HGPRT (Weinshilboum and Sladek, 1980)	Leukemia, non-hodgkin's lymphoma (Weinshilboum and Sladek, 1980)	Myelosuppression, bone marrow toxicity etc. (Weinshilboum and Sladek, 1980)
Allopurinol	Zyloprim	Tablet	Oral	Xanthine oxidase inhibitor (Moorhouse *et al*., 1987)	Treatment of joint destruction, uric acid lithiasis, leukemia, and lymphoma (Hershfield *et al*., 2013)	Sore throat, headache, peeling, redness of skin, upper stomach pain, itching, anorexia, and brown stools etc. (Hande *et al*., 1984)

Further in the same year, Deves *et al*. gave immucillin-H (Fig. 42) as transition-state analog inhibitor of PNP which arrested bone loss in rat periodontal disease models (Deves *et al*., 2013).

In 2013, Ducati *et al*. generalized that immucillin-H has no lethal effect on attenuation of growth in *Staphylococcus aureus*. Thus, the cell viability is not affected with PNPase in methicillin-resistant *S. aureus* (Stratton and Schramm, 2013).

Ducati *et al*. swayed a review on transition-state inhibitors of purine salvage and other prospective enzymes that target malaria (Ducati *et al*., 2013).

In 2014, Donaldson *et al*. determined structural characteristics of *P. falciparum* purine nucleoside phosphorylase (PfPNP) responsible for the efficiency and specificity of catalysis. Through site-directed mutagenesis, molecular simulations, and circular dichroism analysis, the significance of various residues which are vital for PfPNP activity was investigated. It was also explored that mutation in PfPNP was responsible for the loss of 5′-methylthio activity while retaining inosine activity. Tyr160 is a conventional replacement for the Phe residue present in human PNP, whereas Val66, Val73, and Tyr160 residues are responsible for the catalytic efficiency of PfPNP 5′-methylthio activity. 5′-methylthio group was used for the optimization and as the rational target against malaria (Donaldson *et al*., 2014).

Parker and Sorscher demonstrated an approach targeting wild-type *Trichomonas vaginalis* purine nucleoside phosphorylase (Tv-PNP) enzyme cancer cell progression. This involved exposure of the enzyme to a substrate cleaved by the enzyme resulting in a cytotoxic purine analog. Tailed mutant purine nucleoside phosphorylase (tm-PNP) enzymes were exploited as novel compositions for inhibition of cancer cell (Parker and Sorscher, 2014).

FDA-approved PUEIs

6-Mercaptopurine was the first approved antimetabolite followed by allopurinol in 1956 as xanthine oxidase inhibitor. Table 1 includes some of the US-FDA-approved drugs which signify the importance of purine-utilizing enzyme inhibitors in various diseases ranging from cancer to autoimmune disorders.

Summary and conclusions

In general, PUEIs require presence of a purine-like heterocyclic structure(s) as a prerequisite in their chemical architecture which is fully reflected in case of naturally occurring or synthetic heterocyclics. Most of these inhibitors act either through competitive or non-competitive inhibition and a few are allosteric inhibitors (Fig. 43). The conformational aspects of the compounds have revealed the crystal structure of the required binding site and various interactions responsible for potency. DNA topoisomerase inhibitors include isoindenoquinoline derivatives, novel benzoxazole, benzimidazole derivatives, etc. These inhibitors find their applications as anticancer, antiangiogenic, and antiproliferative agents. Novel transition-state analog

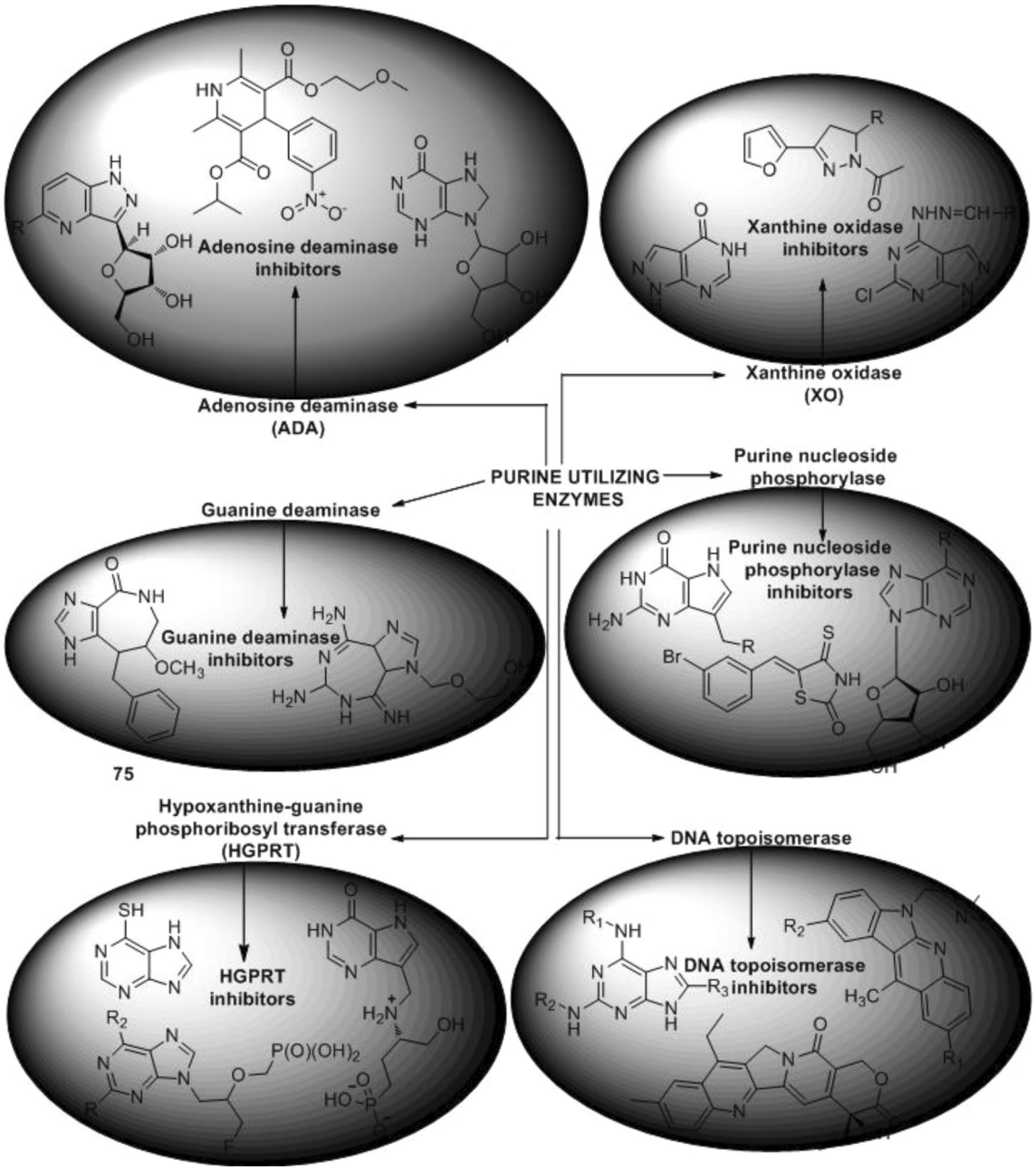


Fig. 43 Summary of purine-utilizing enzymes and their inhibitors

inhibitors of guanase were based on azepinomycin ring structure, whereas acyclic nucleoside phosphonates were found to be inhibitor of HGPRT. The inhibitors of HGPRT are mostly used as antimalarials. Non-purine analogs, thiazolo-pyrazolyl compounds, pyrazoles, substituted pyrazolotriazolopyrimidines derivatives, xanthone derivatives, and substituted pyrazolopyrimidines were established as xanthine oxidase inhibitors. These have been used as free radical scavengers and antioxidants as well. PNP inhibitors find their applications in rheumatoid arthritis, tissue rejection, chemotherapy for T-cell leukemias, and other autoimmune disorders. Recently disclosed X-ray crystal structures of PUEs with endogenous substrates or inhibitors of heterocyclic nature along with biochemical, biophysical studies, and medicinal attributes have opened up a new era of rational drug designing which will surely offer scope for the development of better and safer drugs.

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