

## Pyrazolo[1,5-*a*][1,3,5]triazine based scaffold as purine analogues with diverse biological activity

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**Abstract:** Purine can be considered as the most ubiquitous and functional N-heterocyclic compounds in nature. Structural modifications of natural purines, particularly using isosteric ring systems, have been in the focus of many drug discovery programs. Due to the structural similarity between the pyrazolo[1,5-*a*] [1,3,5] triazine scaffold and the purine system, modifications of this scaffold have given rise to a lot of bioactive agents which could interact with targets of biogenic purines. The present review to the best of our knowledge about synthesis of pyrazolo[1,5-*a*] [1,3,5] triazine scaffold as enzyme inhibitors with therapeutic value.

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**Keywords:** Bioisostere, Purine, Pyrazolo [1,5-*a*][1,3,5]triazine, Synthetic strategy.

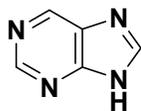
### 1. Introduction

Purine, a nitrogen containing heterocycle, is found abundantly in nature. It is the core structure of adenine and guanine in RNA and DNA [1]. From a biological perspective, attention to purines is mainly driven by the fact that purine nucleotides (ATP, GTP, cAMP, cGMP, NAD, FAD) act as co-factors, substrates, or mediators in the functioning of many proteins [1,2]. These proteins are estimated to include half of the most druggable targets, primarily enzymes and receptors. For example, phosphorylation of ATP is carried out by protein kinases [3] While hydrolysis of cAMP and cGMP is associated with cyclic nucleotide phosphodiesterases [4]. Purine catabolism involves the key enzyme xanthine oxidase [5] whereas the ubiquitous purine nucleoside phosphorylase [6] also plays a vital role in the purine salvage pathway. Adenosine receptors constitute a very promising group of G-protein coupled receptors (GPCRs) [7].

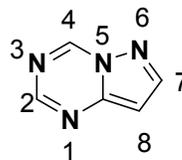
The pyrazolo[1,5-*a*][1,3,5]triazine heterocyclic system [8] is a 5-aza-9-deaza- isostere of purine,

which is one of the most common and functional natural N-heterocycles [9]. The close structural resemblance of pyrazolo[1,5-*a*] [1,3,5] triazines to biogenic purines allows for targeting purinergic signaling receptors and enzymes. Being the focus of active research as bioactive molecules with promising therapeutic potential, this heterocyclic system has been established as a privileged scaffold in medicinal chemistry [10]. Well defined targets for pyrazolo[1,5-*a*][1,3,5]triazines are enzymes, particularly cyclin-dependent kinases (CDK) [11], phosphodiesterases (PDE) [12], xanthine oxidase(XO) [13] and thymidine phosphorylase (TP) [14], and receptors such as cannabinoid (CB) [15] and corticotrophin-releasing factor (CRF)[16].

In the current work we will put highlights on the most important aspect of pyrazolo [1,5-*a*][1,3,5]triazine, which an analogue of purine, and this scaffold has been investigated particularly in the area of nucleoside chemistry for developing biologically active agents [17].



Purine



Pyrazolo[1,5-*a*][1,3,5]triazine

### 2. Synthetic strategies of pyrazolo [1,5-*a*][1,3,5]triazine

Pyrazolo [1,5-*a*][1,3,5]triazine was synthesized for the first time by Checchi and Ridi [17], in 1957. Since that time, several approaches have been developed for the preparation of compounds with the pyrazolo [1,5-*a*][1,3,5]triazine scaffold.

#### 2.1. Synthesis of pyrazolo [1,5-*a*][1,3,5]triazine from pyrazole scaffold

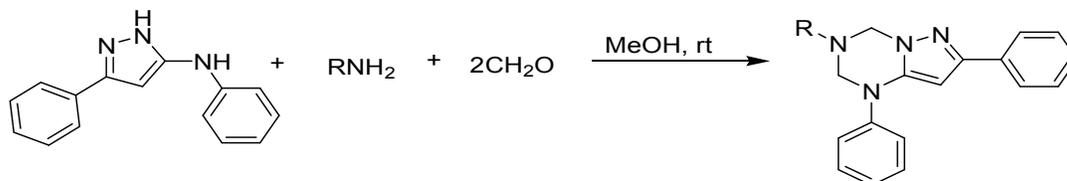
Among these four common synthetic approaches, the most frequently utilized approach to construct the pyrazolo [1,5-*a*][1,3,5]triazine heterocyclic system is the 1,3,5-triazine ring annulation method. Suitable

pyrazoles can be cyclized to form pyrazolo [1,5-*a*][1,3,5]triazine according to the following methods:

- I. Four-bond formation through cyclization of 3-aminopyrazoles with two carbon and one nitrogen atoms.

It has been reported that 1,2,3,4-tetrahydropyrazolo [1,5-*a*][1,3,5]triazine could be prepared by the condensation of 3-phenylaminopyrazoles with two equivalents of formaldehyde and one equivalent of

primary amines (**Scheme 1**) [18]. In particular, 5-phenyl-3-phenylaminopyrazole provided two of the three triazine nitrogens while the third was derived from appropriately selected amine as a reagent. The remaining two carbons of the triazine came from two moles of formaldehyde. The reaction could be carried out at room temperature in methanol with the yields ranging (83-95%) [18].



**Scheme 1.** Synthesis of 1,2,3,4-tetrahydropyrazolo[1,5-*a*][1,3,5]triazine through four-bond cyclization.

- II. Three-bond formation through cyclization of 3-aminopyrazoles with reagents introducing a C-N fragment and one carbon atom.

4-Aryloxy-2,2-dimethyl-2,3-dihydropyrazolo[1,5-*a*][1,3,5]triazines could be synthesized by the reaction between 3-aminopyrazole and aryl cyanates in acetone (**Scheme 2**) [19]. In this synthetic scheme, 3-amino pyrazole provided two of the three

triazine nitrogens, and the third nitrogen as well as one carbon were derived from appropriately selected cyanate. The remaining one carbon of the triazine came from acetone which also served as the solvent of the reaction mixture. Reactions could be run without heating to a high temperature, but yields were below (50%) [19].

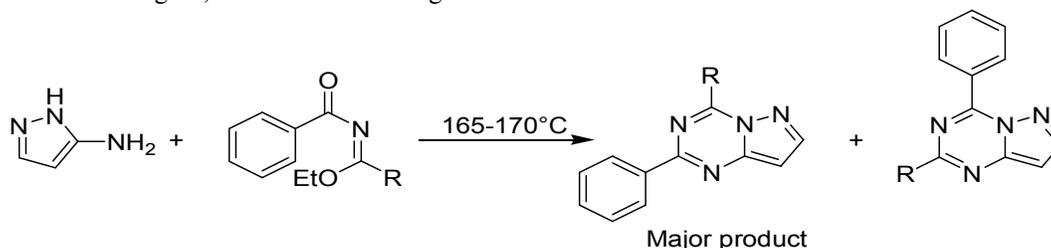


**Scheme 2.** Synthesis of 4-aryloxy-2,2-dimethyl-2,3-dihydropyrazolo[1,5-*a*][1,3,5]triazines through three-bond cyclization.

- III. Two-bond formation through cyclization of 3-aminopyrazoles with reagents introducing a C-N-C fragment.

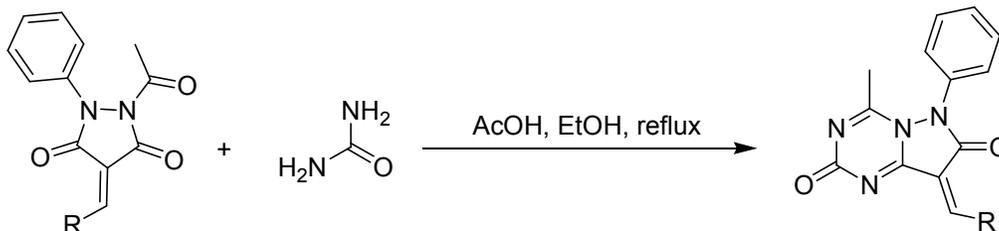
2,4-Diarylpyrazolo[1,5-*a*][1,3,5]triazines could be generated by the reaction between 3-aminopyrazole and ethyl *N*-benzoylimidates (**Scheme 3**) [20]. In this type of reaction, 3-aminopyrazole provided two of the three triazine nitrogens, and the third nitrogen as

well as two carbons were derived from appropriately selected ethyl *N*-benzoylimidates. Although the nucleophilicity of exocyclic nitrogen atom of 3-aminopyrazole was higher, the major product turned out to be the one formed by the nucleophilic attack of endocyclic nitrogen atom with yields above (80%) [20].



**Scheme 3.** Synthesis of 2,4-Diarylpyrazolo[1,5-*a*][1,3,5]triazines through two-bond cyclization.

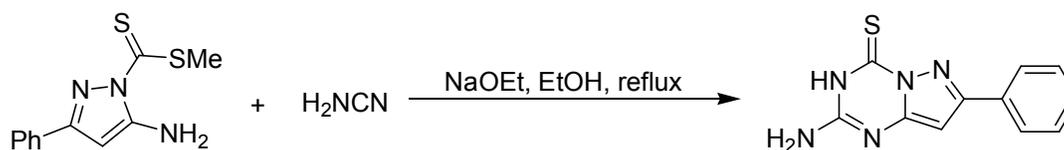
- IV. Two-bond formation through cyclization of *N*-acylpyrazolidindiones with reagents introducing an N-C-N fragment. Refluxing a mixture of *N*-acylpyrazolidindiones and urea in ethanol containing small amounts of acetic acid could afford target pyrazolo[1,5-*a*][1,3,5]triazines with yield around (50%) (**Scheme 4**) [21].



**Scheme 4.** Synthesis of pyrazolo[1,5-*a*][1,3,5]triazines through two-bond cyclization

Appropriately selected *N*-acylpyrazolidindiones would provide one of the three triazine nitrogens and two carbons. The other two nitrogens as well as one carbon were derived from urea. The cyclization was fulfilled by the nucleophilic attack of nitrogen atoms of urea.

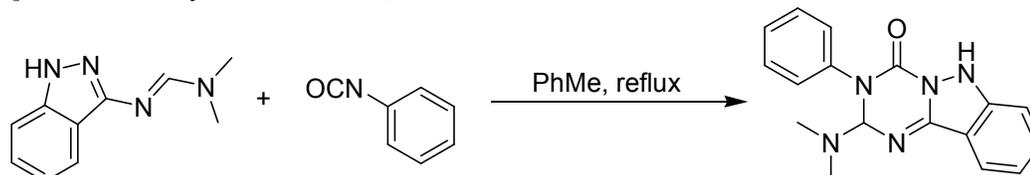
- V. Two-bond formation through cyclization of 5-aminopyrazoles having carbon atom at N1 with reagents introducing a C-N fragment. 2-Amino-4-thioxopyrazolo [1,5-*a*][1,3,5]triazines could be prepared *via* refluxing cyanamide and methyl 5-amino-3-arylpyrazole-1-carbodithioates in ethanol in



**Scheme 6.** Synthesis of 2-Amino-4-thioxopyrazolo [1,5-*a*][1,3,5]triazines through two-bond cyclization.

the presence of sodium ethoxide (**Scheme 5**), giving yields around (60%) [22]. In this case, 5-amino-3-phenylpyrazole-1-carbodithioate provided two of the three triazine nitrogens and two of the three triazine carbons. The remaining one carbon and one nitrogen of the triazine were derived from cyanamide.

- VI. Two-bond formation through cyclization of pyrazoles having N-C appendage at C3 with reagents introducing a C-N fragment. It has been discovered that the formation of fused tricyclic benzopyrazolo [1,5-*a*][1,3,5]triazine ring system could be achieved by refluxing of phenyl isocyanate and 3-azomethine substituted indazole in toluene, giving a yield of (47%) (**Scheme 6**) [23]. In this synthetic scheme, 3-



**Scheme 6.** Synthesis of fused tricyclic benzopyrazolo [1,5-*a*][1,3,5]triazine ring system through two-bond cyclization.

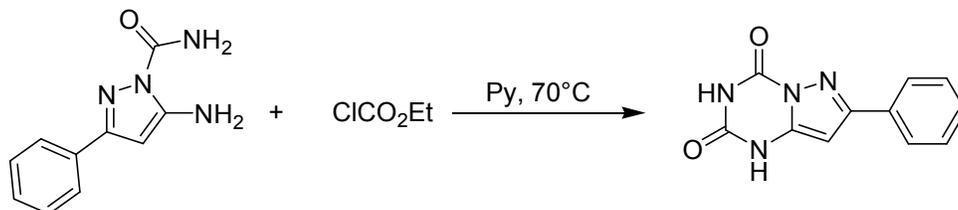
azomethine substituted indazole provided two of the three triazine nitrogens and two of the three triazine carbons. The remaining one carbon and one nitrogen of the triazine were derived from phenyl isocyanate. The carbon atom in the isocyanate moiety served as a good electrophilic center which could be easily attacked by the nitrogen atom located on the indazole to form the product.

- VII. Two-bond formation through cyclization of 5-aminopyrazoles having a C-N appendage at N1 with one carbon atom.

2,4-Dioxo-7-phenylpyrazolo [1,5-*a*][1,3,5]triazine could be obtained by the reaction between 5-amino-1-carbamoyl-3-

phenylpyrazole and ethyl chloroformate in the presence of pyridine with a yield of (34%) (**Scheme 7**)[22]. In particular, 5-amino-1-carbamoyl-3-phenylpyrazole provided all the three triazine nitrogens and

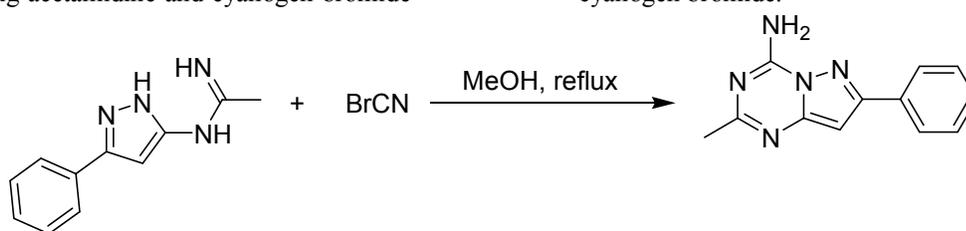
two of the three triazine carbons. The remaining one carbon was derived from ethyl chloroformate. The cyclization was fulfilled via the nucleophilic attack of two amino groups on the phenylpyrazole.



**Scheme 7.** Synthesis of 2,4-Dioxo-7-phenylpyrazolo [1,5-*a*][1,3,5]triazine through two-bond cyclization.

VIII. Two-bond formation through cyclization of pyrazoles having an N-C-N appendage at C3(5) with one carbon atom. Synthesis of 4-amino-2-methyl-7-phenylpyrazolo [1,5-*a*][1,3,5]triazine by refluxing acetamidine and cyanogen bromide

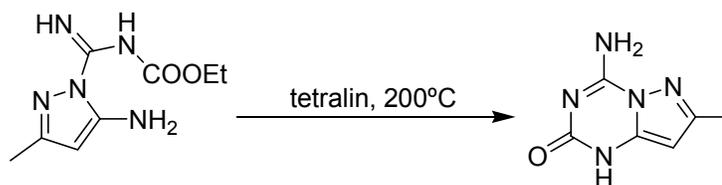
in methanol has been reported, giving a yield of (42%) (**Scheme 8**)[24]. In this reaction, acetamidine provided three triazine nitrogens and two of the three triazine carbons. The remaining one carbon was derived from cyanogen bromide.



**Scheme 8.** Synthesis of 4-amino-2-methyl-7-phenylpyrazolo [1,5-*a*][1,3,5]triazine through two-bond cyclization.

IX. One-bond formation through intramolecular cyclization of 5-aminopyrazoles having a C-N-C appendage at N1. 4-Amino-7-methyl-2-oxopyrazolo [1,5-*a*][1,3,5]triazine could be prepared by a thermal intermolecular ring closure reaction conducted at 200°C using [(5-amino-3-methyl-pyrazol-1-yl)-imino-methyl]-carbamic acid ethyl ester as the starting

material with yield of (96%) (**Scheme 9**)[25]. In this example, all three triazine nitrogens and three triazine carbons came from [(5-amino-3-methyl-pyrazol-1-yl)-imino-methyl]-carbamic acid ethyl ester. The nitrogen atom of the primary amino group attacked the electrophilic carbon atom of the carbonyl group to form the triazine ring in the target compound.



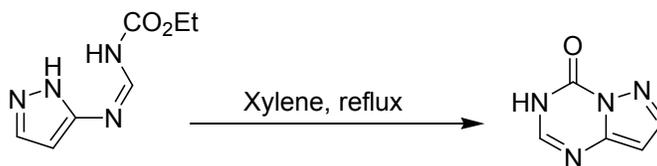
**Scheme 9.** Synthesis of 4-Amino-7-methyl-2-oxo pyrazolo [1,5-*a*][1,3,5]triazine through one-bond intramolecular cyclization.

X. One-bond formation through intermolecular cyclization of pyrazoles having an N-C-N-C appendage at C3(5). It has been reported that 5-aza-9-deazahypoxanthine could be generated by

refluxing *N*-carboethoxy-*N'*-(3(5)-pyrazoly)formamidine in xylene, giving a yield around (80%) (**Scheme 10**)[26]. In this synthetic scheme, [(2H-pyrazol-3-ylimino)-methyl]-carbamic acid ethyl ester provided

all three triazine nitrogens as well as three triazine carbons. The product was formed by the nucleophilic attack of the nitrogen of the

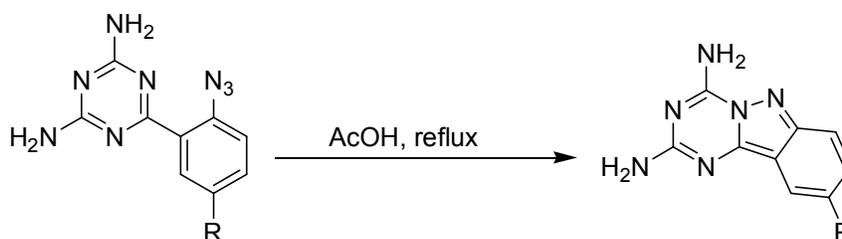
secondary amine group located at the pyrazole ring.



**Scheme 10.** Synthesis of 5-aza-9-deazahypoxanthine through one-bond intermolecular cyclization.

## 2.2. Synthesis of pyrazolo [1,5-*a*][1,3,5]triazine from 1,3,5-triazine scaffold

Annulation of the pyrazole ring onto a 1,3,5-triazine scaffold is an alternative approach to generate pyrazolo [1,5-*a*][1,3,5]triazine scaffold. However, unlike using pyrazoles as starting materials, in this case only one type of cyclization was reported which was a one-bond formation through intramolecular cyclization of 1,3,5-triazines possessing C-C-N fragment [27-29].

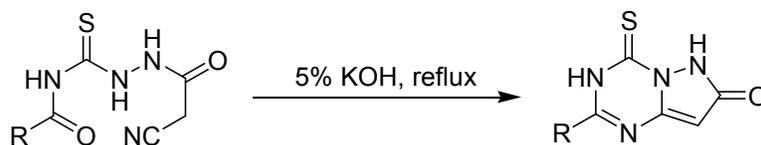


**Scheme 11.** Synthesis of 2,4-diamino-1,3,5-triazino[1,2-*b*]indazoles.

## 2.3. Synthesis of pyrazolo [1,5-*a*][1,3,5]triazines by concurrent formation of both the 1,3,5-triazine and pyrazole rings

Another approach for synthesizing pyrazolo [1,5-*a*][1,3,5] triazines is by forming both the 1,3,5-triazine ring as well as the pyrazole ring at the same time, which could be fulfilled *via* a two-bond formation through intramolecular cyclization of open-chain structures consisting of five carbon and four nitrogen atoms.

In the literature, there are reports that described 2,4-diamino-1,3,5-triazino[1,2-*b*] indazoles could be prepared by the decomposition of relative azides in the refluxing acetic acid with yields ranging (80-90%) (**Scheme 11**)[28]. In this type of reaction, the starting azides provided all atoms of the pyrazole ring in the target compounds. Target compounds were formed *via* the evolution of nitrogen at the starting azides.



**Scheme 12.** Synthesis of 7-oxo-4-thioxopyrazolo[1,5-*a*][1,3,5]triazines.

## 2.4. Synthesis of pyrazolo [1,5-*a*][1,3,5]triazines by ring transformation reactions

This approach is represented by a category of reactions in which the fused bicyclic ring system has already presented in the starting materials and will be

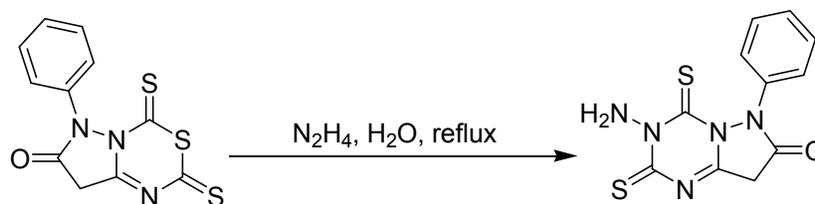
One example was the synthesis of 7-oxo-4-thioxopyrazolo[1,5-*a*][1,3,5]triazines, which could be generated by refluxing *N*-acyl-2-cyanoacetylthiosemicarbaside in potassium hydroxide solution, giving yields in range (55-92%) (**Scheme 12**)[30-32]. Both rings of the pyrazolo [1,5-*a*][1,3,5]triazine scaffold were constructed at the same time through two intramolecular nucleophilic reactions involving nitrogen atoms and carbon atoms, resulting in target compounds with different substituents at position 2.

later converted into the pyrazolo [1,5-*a*][1,3,5]triazine scaffold.

According to literature, a target the pyrazolo [1,5-*a*][1,3,5]triazine was successfully prepared by the transformation of the 1,3,5-thiadiazine ring of the

starting material into the 1,3,5-triazine ring, giving a yield of (63%) (**Scheme 13**)[33]. The transformation was fulfilled by the nucleophilic attack of one

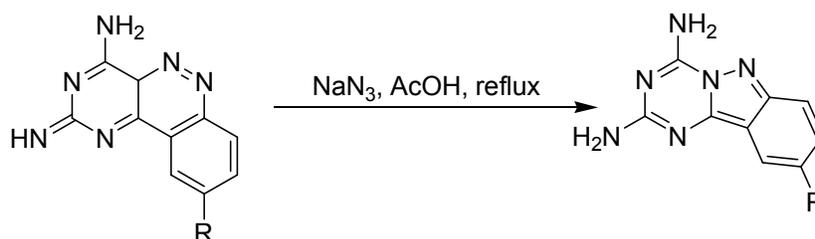
nitrogen atom in the hydrazine molecule and the subsequent release of one molecule hydrogen sulfide.



**Scheme 13.** Pyrazolo [1,5-*a*][1,3,5]triazine scaffold generated by transformation of the 1,3,5-thiadiazine ring.

Besides, transformation of the 1,2,3-triazine ring into the pyrazole ring could also result in the formation of pyrazolo [1,5-*a*][1,3,5]triazine with reported yields around (70-80%) (**Scheme 14**)[28]. During the reaction process, an azide intermediate

would be formed first *via* the cleavage of N-N single bond in the middle ring, target compound was then generated by the subsequent evolution of one molecule nitrogen.



**Scheme 14.** Pyrazolo [1,5-*a*][1,3,5]triazine scaffold generated by transformation of the 1,2,3-triazine ring.

### 3. Biological activity of pyrazolo [1,5-*a*][1,3,5]triazines

There are extensive and ongoing research and development activities around purine isosteres and one of the promising directions has a focus on using 1,3,5-triazine-based isosteres of purine. This group of azolo[1,3,5]triazine systems has a nitrogen atom in the position 5 of the purine ring and therefore can be generally categorized as 5-aza-isosteres of purine. The present review focuses on the scope of biological activities displayed by pyrazolo [1,5-*a*][1,3,5]triazines and their potential applications as therapeutic agents.

#### 3.1. Enzymes inhibitors

Almost half of all marketed drugs specifically target enzymes, therefore demonstrating a huge potential for development of new therapeutic agents modulating enzyme activity [34]. The compounds constructed using pyrazolo [1,5-*a*][1,3,5]triazines-based isosteres of purine affect enzymes of different groups as discussed below.

##### 3.1.1. Kinase inhibitors

Kinases are one of the most promising groups of enzymes in the field of drug discovery. Protein kinases represent about 20% of the druggable genome. They have been in the focus of intensive investigations resulting in 20 kinase-targeting drugs approved for clinical use over the past decade and hundreds of drug

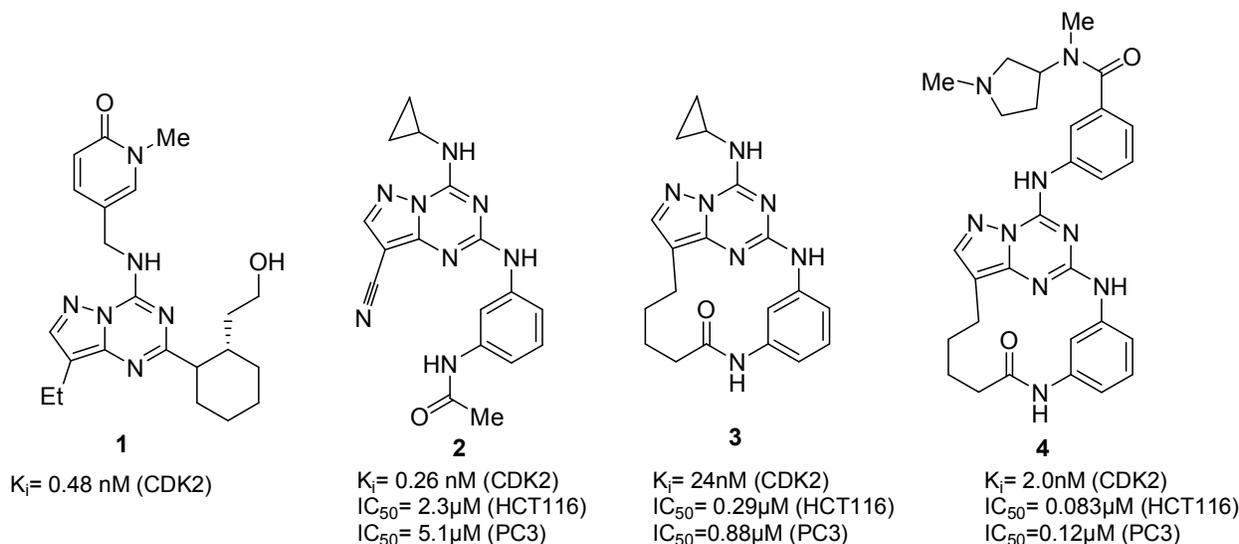
candidates undergoing clinical trials [35]. It is estimated that protein kinase inhibitors are main targets in 50-70% of current cancer drug discovery programs [35].

Cyclin-dependent kinases (CDKs) are a family of enzymes playing a key role in cell cycle regulation. Over expression of CDKs in cancer cells makes them an attractive drug target in the fight against oncological diseases [36-40]. Development of CDK inhibitors appears to be a promising strategy in the search for new effective anticancer agents. A number of CDK inhibitors are currently at different stages of clinical trials.

Isosteric to purine system, pyrazolo[1,5-*a*][1,3,5]triazine scaffold was used as a template for new potent CDK2 inhibitors. Various decorated 2,4-diamino substituted pyrazolo[1,5-*a*][1,3,5]triazines (**1-4**) demonstrated high CDK2 inhibitory activity (**Fig. 1**) [41-43]. The CDK inhibitory activity was translated into *in vitro* antiproliferative activity against prostate (PC3) and colon cancer (HCT116) cell lines [43]. Even though there was a decrease of about a hundred times in the potency of CDK2 inhibition between compounds **2** and **3**, macrocyclic compound **3** showed almost ten times improvement in the anticancer activity [43]. The macrocyclic lactam ring provided a less planar and more three-dimensional structure of

overall molecule resulting in an increase in membrane permeability ultimately improving cellular activity. Enhancement of CDK2 inhibitory potency was successfully achieved by replacing the cyclopropylamino group with a more complex

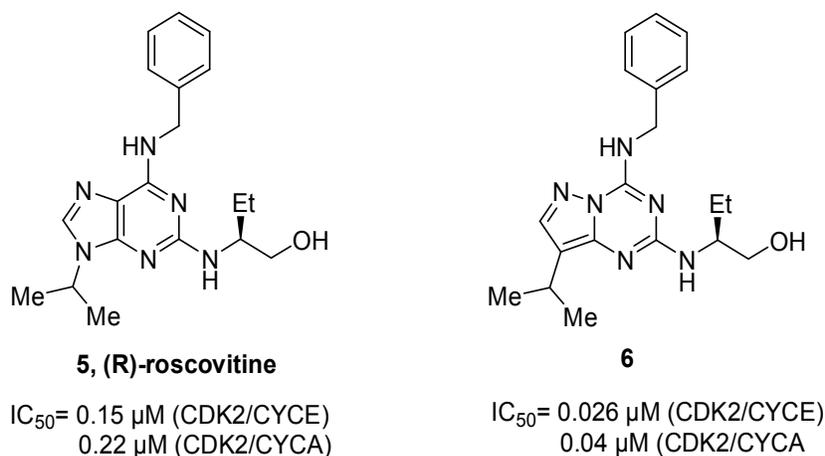
substituted arylamino moiety (compound **4**) [43]. This modification of the structure also improved aqueous solubility of **4** which led to an increase in cellular activity against both human prostate and colon cancer cell lines in *in vitro* experiments [43].



**Fig. 1.** CDK inhibitors.

A purine based CDK inhibitor (R)-roscovitine (**5**) (**Fig. 2**) is a drug candidate currently undergoing clinical trials as an anticancer therapeutic agent [44-46]. This molecule has been serving as a lead for the development of new potent compounds targeting CDKs. An isosteric replacement of purine system of roscovitine (**5**) with the pyrazolo[1,5-*a*][1,3,5]triazine core led to the development of a potent CDK2 inhibitor **6** [45]. This isosteric modification provided more than 5 times improvement in the inhibitory activity. Roscovitine (**5**) and pyrazolo[1,5-*a*][1,3,5]triazine **6** demonstrated similar

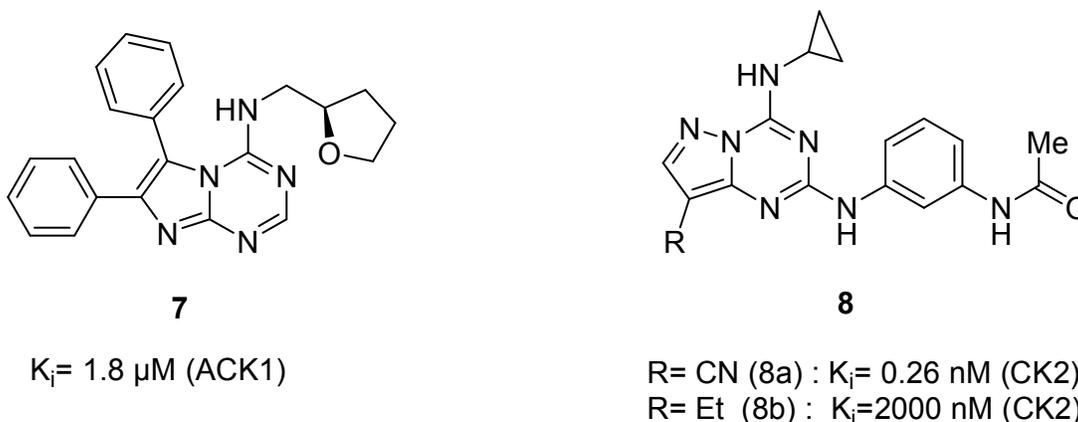
conformations and binding modes to CDK2 [45]. A similarity in the pharmacokinetic profile of **6** and roscovitine (**5**) was also observed [45]. Tested against the National Cancer Institute panel of 60 tumor cell lines, **6** was about 14 times more potent than roscovitine (**5**) with no preference towards any specific form of tumor [46]. An improved *in vitro* biological activity of compound **6** was also proven in the Ewing's sarcoma xenograft mouse model system. Both roscovitine (**5**) and **6** showed more than 70% of tumor inhibition, but dose of roscovitine was twice higher (50 mg/kg) than that of **6** (25 mg/kg) [46].



**Fig. 2.** Compounds 5 (roscovitine) and 6 as CDK2 inhibitor.

Activated Cdc42-associated tyrosine kinase 1 (ACK1) is a non receptor tyrosine kinase, which has been recently explored as a target for cancer therapy [47]. Amplification of the ACK1 gene in primary tumors leads to poor prognosis. Hence, using ACK1 inhibitors was suggested for the anti-cancer treatment. In the search for new inhibitors of ACK1, Jiao *et al.* identified imidazo[1,2-*a*] [1,3,5]triazine **7** exhibiting inhibition of ACK1 in micromolar concentration when evaluated in biochemical autophosphorylation assays (**Fig. 3**) [48]. Casein Kinase II (CK2) is a ubiquitous and highly conserved protein serine/threonine kinase involved in cell proliferation, transformation,

senescence and apoptosis [49,50]. Experimental evidence confirming the association of an increase expression of CK2 in human cancers underlines the importance of CK2 as an excellent target for anti-cancer therapy [49,50]. Hu *et al.* [49] reported that substituted 2,4-diaminopyrazolo[1,5-*a*][1,3,5]triazine, such as **8a, b** effectively targeted CK2 (**Fig. 3**). Cyano group in position 8 of the pyrazolo[1,5-*a*][1,3,5]triazine system seems to play a critical role in the activity of the compounds. Even small alteration by changing the cyano group to an ethyl caused almost a 4-order drop in the inhibitory potency of the compounds.



**Fig. 3.** Other kinase inhibitors.

### 3.1.2. Phosphodiesterase inhibitors

Cyclic nucleotide phosphodiesterases (PDEs) represent a group of enzymes responsible for the hydrolysis of secondary messengers cAMP and cGMP. Uneven distribution of specific PDE types in various tissues and cell types prompted the search of novel PDE inhibitors with focus on the selective inhibition of enzyme isoforms. Using purine and its isosteres as scaffolds in the PDE inhibitor design has been proved a fruitful avenue with several launched blockbuster drugs [51-58]. Variations in isosteric scaffolds and manipulation with substitution pattern allowed tuning the selectivity of compounds towards specific PDE types.

Some PDE inhibitory compounds with pyrazolo[1,5-*a*][1,3,5]triazine scaffold were identified at the beginning of the PDE era (**Fig. 4**). These compounds, such as **9**, were hundreds time more potent than reference of that time theophylline [59]. A wide range of substituents at the pyrazolo[1,5-*a*][1,3,5]triazine system was tolerated with changing selectivity profile towards various PDE. Compound **10** [60,61] exhibited selective inhibition of cAMP PDE

isolated from the lung, while **11** [60,62,63] demonstrated high level of selectivity on cAMP PDE obtained from the brain. Another interesting pyrazolo[1,5-*a*][1,3,5]triazine derivative revealed at that time was 4-azido-7-phenylpyrazolo[1,5-*a*][1,3,5] triazine (**12**) [64]. This compound (**12**) demonstrated strong irreversible inhibition of PDE1. Detail analysis of selectivity for this compound has not been reported, but earlier data [59] suggested some selectivity in the inhibitory activity of **12** to PDE1. Development of PDE4 inhibitors as potential candidates for the treatment of autoimmune and inflammatory diseases led to the discovery of very potent pyrazolo[1,5-*a*][1,3,5]triazines, e.g. **13a, b** [61]. The selectivity profile of **13a, b** towards PDE4 was analyzed by comparison with PDE1, PDE2, PDE3 and PDE5. The results showed a high level of their selectivity towards the PDE4 isoform [65]. Pyrazolo[1,5-*a*][1,3,5]triazine **14** demonstrated subnanomolar inhibition of PDE10 and should be mentioned among recently claimed inhibitors of this enzyme [66].

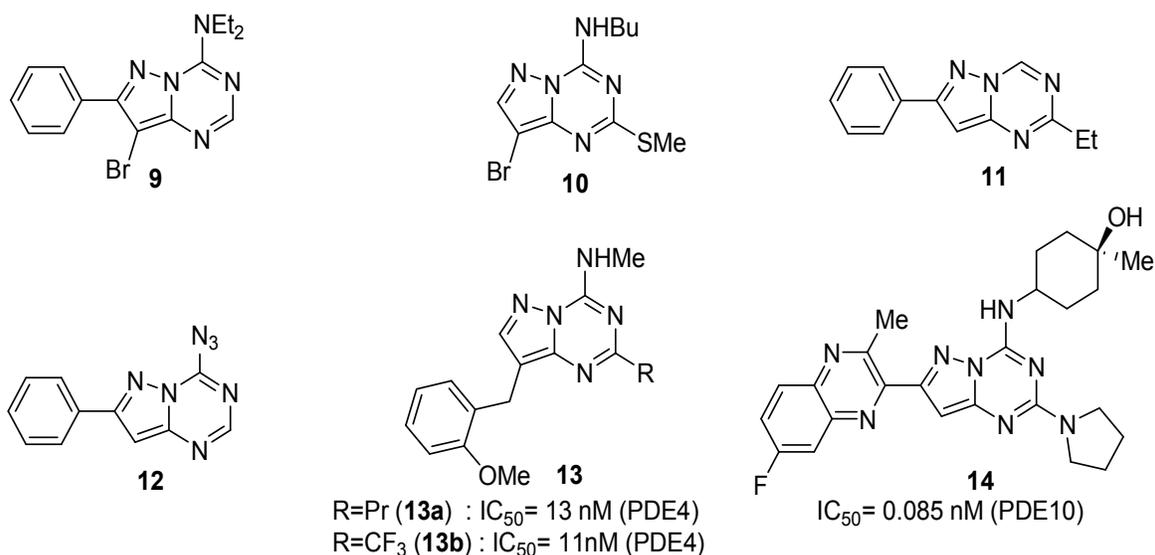


Fig. 4. PDE inhibitors.

### 3.1.3. Xanthine oxidase inhibitors

Xanthine oxidase (XO) is a key enzyme of the purine catabolism involved in the oxidation of hypoxanthine to xanthine and finally to uric acid. This enzyme is a well-recognized target in therapy of hyperuricemia and chronic gout [67-69].

A variety of compounds based on isosteric to guanine, hypoxanthine and xanthine scaffolds were extensively investigated by Robins *et al.* (Fig. 5)[70]. 5-Aza-hypoxanthine (**15**), which is also an isostere of the standard anti-gout drug allopurinol, demonstrated relatively low XO inhibitory activity. 5-Azaxanthine (**16a**), isosteric to oxypurinol, and its thio-analogue **16b** were also marginally active. S-Methylation of **16b** yielded compound **17b**, which was 70 times more active than the parent molecule (**16b**) and was also more effective than allopurinol. O-Methylation of 5-azaxanthine(**16a**) had an opposite effect on the activity (**17a** was even less active than parent structure **16a**). An isostere of guanine with imidazo[1,2-*a*][1,3,5]triazine scaffold (**18**) also demonstrated some XO inhibitory effect [70]. The most interesting results were obtained when pyrazolo[1,5-*a*][1,3,5]triazine skeleton was used for the construction of XO inhibitors. The isostere of hypoxanthine and allopurinol based on this skeleton (**19a**) possessed low activity, similar to that of its structural analogue with 1,2,4-triazolo[1,5-*a*][1,3,5]triazine scaffold (**15**) [70]. Interestingly, introduction of phenyl group to position 7 of the pyrazolo[1,5-*a*][1,3,5]triazine system led to

dramatic increase in the XO inhibitory effect. Compound **19b** was identified as a very potent XO inhibitor with more than three order greater activity in comparison to unsubstituted 5-aza-9-deazahypoxanthine (**19a**). It was demonstrated that XO inhibitory activity could not tolerate substitutions at position 2 of **19b**. Even minor structural modification at this position (e.g. **19c**) led to significant loss of the activity.

A very potent XO inhibitor, BOF 4272 (**20**) was synthesized by a Japanese research group [71-73]. Promising results from *in vitro* and *in vivo* studies [74-76]together with experimental data on healthy human volunteers [77]demonstrated great potential of BOF 4272. A significant reduction of the XO activitywas achieved bytargeting the liver and small intestine, which are main organs for uric acid production in human. Cell necrosis could also be prevented by BOF 4272 through the decrease in concentration of free radicals generated by XO [78]. Detailed study of the mechanism of XO inhibition [74,75]by BOF 4272 showed that the activity ofBOF 4272 was governed by the stereochemistry of the molecule. Both enantiomers were determined to be mixed type inhibitors with the (S)-(-)-enantiomer having much higher potency than the(R)-(b)-enantiomer (Fig. 5) [74,75]. The stereoselectivity also determined pharmacokinetic parameters and biotransformation of BOF 4272 [79-83]. Therefore, further studies were conducted on the asymmetrical (S)-(-)-BOF 4272 [84-86].

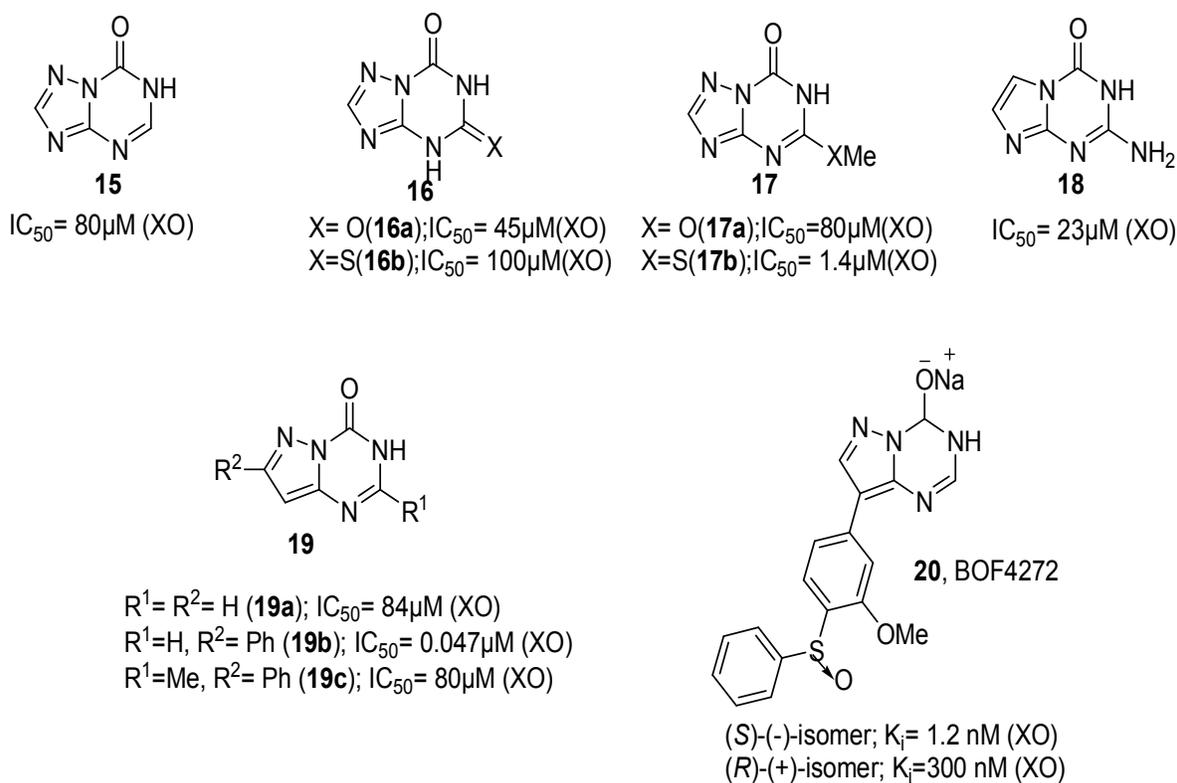


Fig. 5. XO inhibitors.

### 3.1.4. Thymidine phosphorylase inhibitors

Thymidine phosphorylase (TP), also known as gliostatin or platelet-derived endothelial cell growth factor, is a key enzyme in the pyrimidine nucleoside salvage pathway. TP is over expressed in various solid tumors where it is involved in regulation of cell apoptosis, proliferation and angiogenesis which makes this enzyme a valid target for anticancer therapy [87,88].

Many substitutions were tested at position 2 of the triazolo[1,5-*a*][1,3,5]triazine system without any loss of activity (Fig. 6). Particular improvement was observed with introduction of an aromatic group in this position (e.g. compound **21**) [89-91]. One of the most active compound identified in this series was triazolo[1,5-*a*][1,3,5]triazine **22**, which inhibited TP *via* a competitive mixed-type (with respect to thymidine) mechanism with a K<sub>i</sub> value of 20.1 μM. The TP inhibitory potency was further improved by introducing a methylenespacer between dichlorophenyl moiety and the triazolo[1,5-*a*][1,3,5] triazine core. The resulted compound **23** was also found to be a competitive mixed-type TP inhibitor (K<sub>i</sub> ¼ 19.6 μM). Compounds **22** and **23** inhibited

expression of some angiogenesis markers, namely matrix metalloproteinase 9 (MMP-9) and vascular endothelial growth factor (VEGF) in MDA-MB-231 cells [90]. Further exploration of structure activity relationship revealed that changes of the substitution pattern at the 1,3,5-triazine ring cannot be tolerated. The alkylation of a thiocarbonyl group or swapping positions of 5-thiocarbonyl and 7-carbonyl groups resulted in significant loss of TP inhibitory activity [90-92]. Similar observations were reported for related molecules based on the pyrazolo[1,5-*a*][1,3,5]triazine core [93]. Phenyl substitution at C-7 of the pyrazolo[1,5-*a*][1,3,5]triazine system also increased TP inhibitory activity (**24a**), which were further improved by introducing lipophilic substituents to the phenyl ring. The most active compound in this series was **24b**. Changing position of the aryl substituent at the pyrazole ring significantly improved the TP inhibitory activity (**25a**). Interestingly, introduction of a pentafluorosulfonyl group (compound **25b**) to the phenyl ring led to dramatic increase of the activity. Among pyrazolo[1,5-*a*][1,3,5]triazines, this compound (**25b**) was the most potent, inhibiting TP in the non-competitive manner [93].

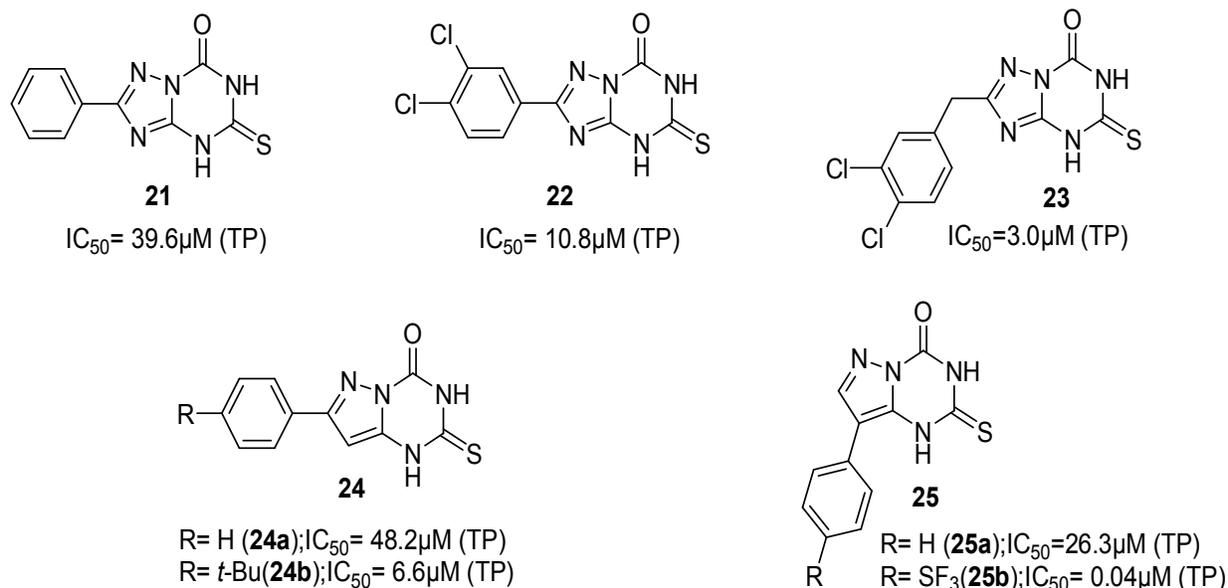


Fig. 6. TP inhibitors.

### 3.1.5. Miscellaneous enzyme inhibitors

Dipeptidyl peptidase IV (DPP-IV), also known as adenosine deaminase complexing protein 2, is a type II membrane protein used as a target in the treatment of some metabolic disorders, most notably type 2 diabetes [94]. Compounds with various isosteric to purine scaffolds, such as **26**, **27** and **28** demonstrated selective inhibition of DPP-IV (**Fig. 7**) [95]. They were claimed to inhibit DPP-IV at the concentrations 50 times lower than those required for inhibition of similar enzyme, fibroblast activation protein  $\alpha$  (FAP $\alpha$ ).

Compounds constructed using the pyrazolo[1,5-*a*][1,3,5]triazine scaffold were reported to have inhibitory activity against various enzymes. Compounds **29** and **30** (**Fig. 7**), with the basic alkylamino moiety linked to position 4 of the pyrazolo[1,5-*a*][1,3,5]triazine skeleton via long alkyl chain, were tested in form of hydroiodides and demonstrated inhibition of protein methyl transferase 1 (PRMT1) [96]. Some pyrazolo[1,5-*a*][1,3,5]triazines were able to inhibit bacterial enzyme, DNA gyrase, a target for the antibacterial therapy [97]. Compound **31**

demonstrated similar values (0.5 mg/mL) for the maximal non-effective concentration (MNEC) towards DNA gyrase and the minimal inhibitory concentration against *Staphylococcus pyogenes* [97]. Similarly to purine-based antiviral agent (S)-9-(2,3-dihydroxypropyl)adenine (S-DHPA), its pyrazolo[1,5-*a*][1,3,5]triazine analogue **32** was reported [98] to inhibit S-adenosylhomocysteinase, though being less active than S-DHPA. The 1,2,4-triazolo[4,3-*a*][1,3,5]triazine ring system was used in the design of **33**, which selectively inhibited migration of COS-1 cells expressing MMP-9 and did not affect migration of MMP-2 and MMP-14 expressing cells (**Fig. 7**) [99]. This compound (**33**) also inhibited migration and invasion processes of human invasive cancer cell lines expressing high endogenous level of MMP-9 (HT-1080 and MBD-MB-435). At the same time, **33** did not affect MMP-9 expression and demonstrated no proteolytic activity or cytotoxicity providing therefore solid evidence of MMP-9 inhibition as an explanation of its effects. It should also be noted that the same compound (**33**) was also claimed to be a PDE4 inhibitor [100].

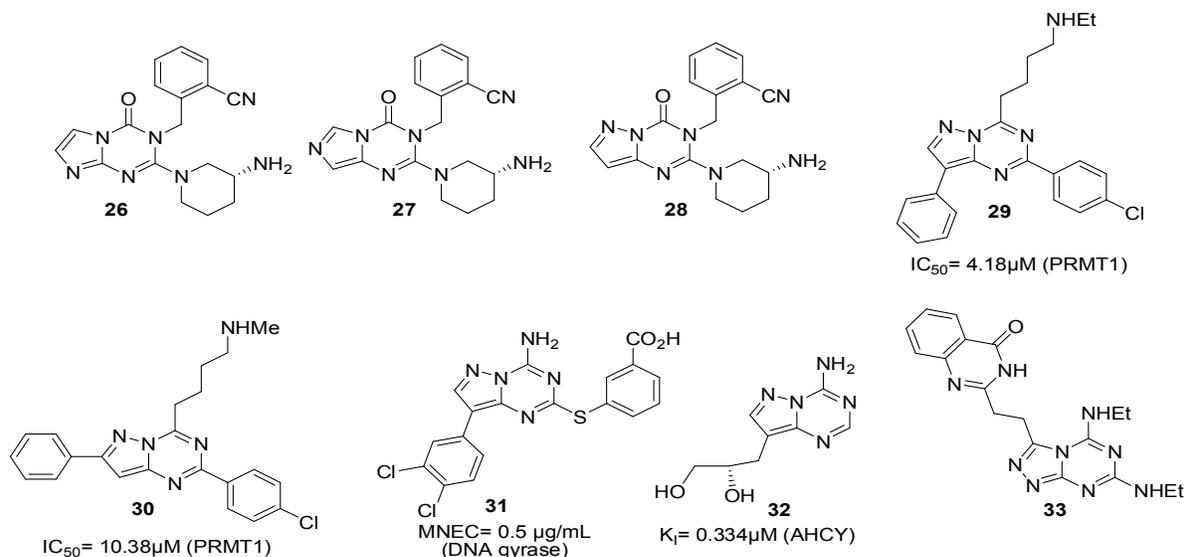


Fig. 7. Miscellaneous enzyme inhibitors.

### 3.2. Receptor ligands

Receptors are responsive to a plethora of endogenous ligands and stimuli and hence responsible for the regulation of diverse cellular responses. An estimate of 40% clinically approved drugs utilizes the receptor-ligand signaling pathway making them attractive targets for drug discovery [101,102]. The compounds constructed using pyrazolo[1,5-*a*][1,3,5]triazines-based isosteres of purine affect different types of receptors as discussed below.

#### 3.2.1. Adenosine receptor ligands

Adenosine is an endogenous nucleoside ubiquitous in mammalian cells. It mediates many physiological effects *via* widely distributed specific cell membrane G-protein coupled receptors (GPCR) of four known subtypes viz. A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub> and A<sub>3</sub> adenosine receptors [103,104]. The A<sub>2A</sub> adenosine receptors are widely expressed in the central nervous system and are involved in the regulation of many physiological and pathological processes.

The pyrazolo[1,5-*a*][1,3,5]triazine scaffold was successfully used in the design of A<sub>2A</sub> receptor antagonists. Compound **34** was synthesized (Fig. 8)[105]. This compound (**34**) was found to possess high affinity to adenosine receptors. A number of structural variations were explored for compounds **35** with the imidazo[1,2-*a*][1,3,5]triazine scaffold (Fig. 8)[106,107]. The phenyl moiety was preferred at position 2, while position 7 could tolerate various groups (including phenyl, e.g. **35a**), but small alkyl groups were preferred. Modifications of the amino group at position 4 were used to tune selectivity to adenosine receptor subtypes. Thus, introduction of acetyl moiety to the amino group at position 4 led to a very potent and highly selective A<sub>1</sub> adenosine receptor ligand **35b**[107]. Similarly to 5-azapurines and other similar structures, acylation of the primary amino group increase affinity to A<sub>3</sub> adenosine receptors and decrease affinity to A<sub>2A</sub> receptors as exemplified by very potent A<sub>3</sub> receptor ligands **35c** and **35d**[106].

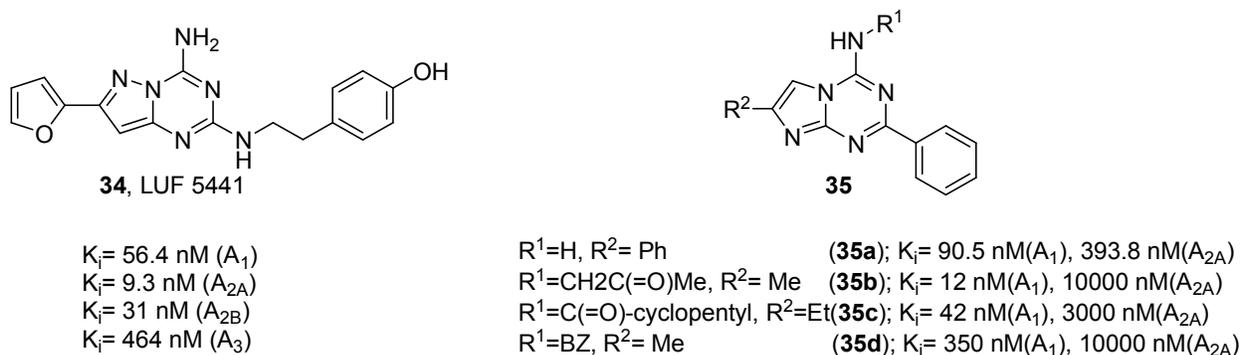


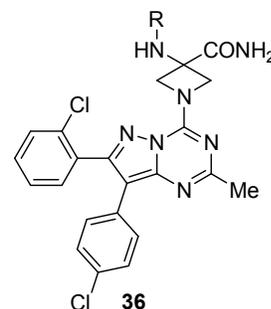
Fig. 8. Adenosine receptor ligands

### 3.2.2. Cannabinoid receptor ligands

Cannabinoid (CB) receptors are known to control downstream signaling pathways regulating energy metabolism and processes in the CNS and immune system. To date, two subtypes of the receptors have been identified: CB1 receptors, localized predominantly on presynaptic terminals, and CB2 receptors, widely distributed in peripheral tissues, primarily in immune cells [108]. Other receptors (GPR55 and GPR119) are also sometimes claimed to be CB receptors [109].

The CB1 receptors are connoted to be targets for the treatment of CNS disorders, inflammation and metabolic diseases [108-110]. It was reported that pyrazolo[1,5-*a*][1,3,5]triazine **36a** (CE 178253), used as benzenesulfonate salt, possessed high affinity and selectivity towards CB1 receptors (**Fig. 9**) [111]. Antagonism of compound **36a** towards CB1 receptors and its synergism with antiparkinsonian action of levodopa was demonstrated using a model of Parkinsonism in rhesus monkeys thus providing a new drug combination for treatment of Parkinson's disease [111,112]. It was demonstrated that administration of CE 178253 (**36a**) led to sustained weight loss in diet induced obese rats and mice suggesting the use of CB1 receptor antagonists for the treatment of obesity [112]. CE 178253 (**36a**) showed not only substantial anorectic activity in rodents relative to its CB1-binding affinity but also an increase in energy expenditure. Another investigation was carried out using obese cynomolgus monkeys to explore the relative contribution of CB1 antagonism on weight loss. Treatment using CB1 antagonist PF 095453 (**36b**) showed significant reduction in food consumption, body weight and body fat in addition to higher adiponectin and lower leptin concentrations [113]. Adiponectin and leptin are both involve in the regulation of fat metabolism and metabolic processes. Improvement in inflammation, insulin sensitivity and vascular reactivity due to increased adiponectin levels by CB1 antagonists are yet to be confirmed.

Even though CB2 receptors are more predominant in immune cells, CB1 antagonists were also found to exhibit anti-inflammatory properties. Thus, administration of CE 178253 (**36a**) using an *in vivo* model of LPS induced inflammation led to significant decrease in the plasma TNF- $\alpha$  level [114]. Prophylactic treatment with **36a** effectively decreased inflammation in the mouse model of collagen induced arthritis [114].



R= Et (**36a**, CE 178253);  $K_i$ = 0.33nM (CB<sub>1</sub>), 10,000nM (CB<sub>2</sub>)  
R= Me (**36b**, PF095453);  $K_i$ = 0.48nM (CB<sub>1</sub>), 11,000nM (CB<sub>2</sub>)

**Fig. 9.** CB receptor ligands

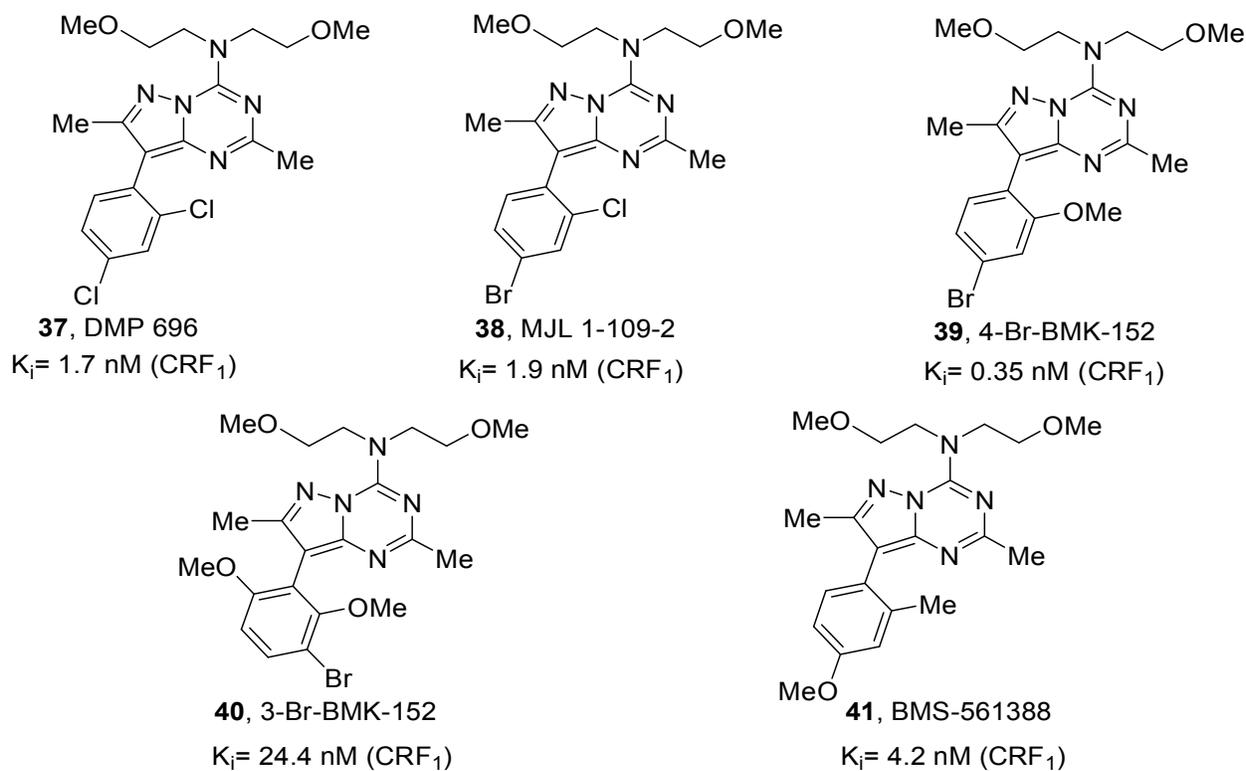
### 3.2.3. Corticotrophin-releasing factor receptor ligands

Corticotrophin-releasing factor (CRF) is a neurotransmitter and hormone that regulates stress responses through the endocrine and autonomic nervous system. Receptors for CRF are members of G-protein coupled receptors divided into type 1 and type 2. CRF1 receptor antagonists are believed to be potential candidates for the treatment of anxiety and mood disorders [115].

DMP-696 (**37**) was found to exhibit non-competitive antagonism for CRF1 receptor with high affinity and selectivity (**Fig. 10**) [116,117]. Additionally, this compound (**37**) was shown to have significant oral bioavailability and desirable pharmacokinetic properties with high anxiolytic and antidepressant activities. DMP-696 (**37**) has been extensively used as a reference compound for further investigations in the role of CRF and its receptors [116,117]. Another compound with CRF1 receptor antagonism (**38**) was synthesized and used in the radiolabeled form [<sup>76</sup>Br] MJL 1-109-2 for the autoradiography visualization of CRF1 receptor. To increase penetration through the blood brain barrier, compound **38** was modified by the addition of methoxy groups into the phenyl moiety yielding therefore compounds **39** and **40** [118]. From the results of the *in vitro* autoradiography saturation studies, compound **39** demonstrated high subnanomolar affinity binding to both rat and monkey frontal cortex:  $K_d$   $\frac{1}{4}$  0.2 nM (rat prefrontal cortex), 0.3 nM (monkey prefrontal cortex). These results for **39** were consistent with the CRF1 receptor regional distribution while  $K_d$  of **40** could not be determined due to non-specific binding [118,119]. The *in vivo* studies in rats and mice showed the ability of compounds **39** and **40** to penetrate the blood brain barrier and remain mostly metabolized in the rat's brain in 1 h post injection [118]. In the studies conducted with P-glycoprotein (P-gp) knockout mice, data showed that compounds **39** and **40** are not P-gp substrates [118].

From a study of 8-(4-methoxyphenyl)pyrazolo[1,5-*a*][1,3,5]triazines, BMS-561388 (**41**) [120] was found to exhibit potent antagonism with CRF1 receptor. It showed good pharmacokinetic profile in dogs in addition to being

orally effective when tested in two rat models of anxiety. On the basis of its favorable pharmacokinetic profile, compound **41** was advanced to clinical studies in humans.



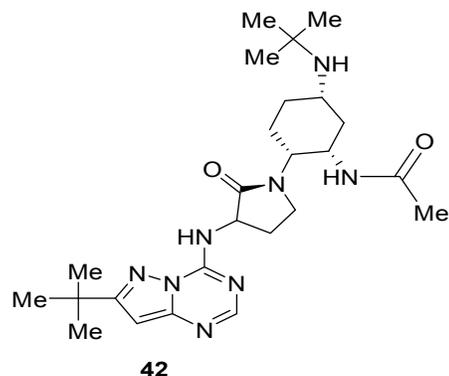
**Fig.10.** CRF receptor ligands

### 3.2.4. Chemokine receptor ligands

Chemokine receptors are responsible for the regulation through GPCRs a variety of biological processes, predominantly cell migration and inflammation [121,122]. Involvement of chemokine receptors in angiogenesis, oncogenesis, hematopoiesis and immune modulation makes them feasible targets in the therapy of inflammatory diseases, cancers and HIV-1 infections.

Pyrazolo[1,5-*a*][1,3,5]triazine **42** exhibited dual antagonist activity against CeC chemokine receptor type 2 and C-C chemokine receptor type 5 together with good bioavailability (**Fig. 11**) [123]. *In vivo* study of **42** was conducted in a murine model to explore its effects on thioglycollate-induced peritonitis over the duration of two days. Significant inhibition of cellular

infiltration (71%) was achieved at a dose of 200 mg/kg [123].



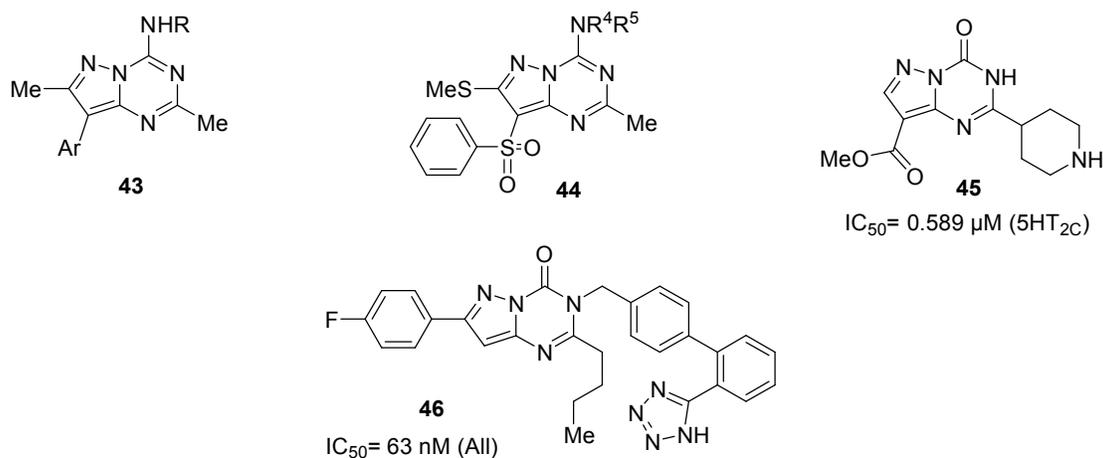
**Fig. 11.** Chemokine receptor ligand.

### 3.2.5. Miscellaneous receptor ligands

A number of active compounds with affinity to different receptors were prepared varying the substitution pattern of 5-aza-9-deaza-isosteres of adenine and hypoxanthine. Involvement of the neuropeptide Y receptor signaling system in regulation of a number of psychological responses such as feeding and fat storage, anxiolytic behaviors and regulation of the coronary tone makes this system an attractive therapeutic target for the treatment of metabolic disorders, CNS disorders and cardiovascular diseases [124]. Pyrazolo[1,5-*a*][1,3,5]triazines with general structure **43** were prepared and claimed to be modulators of neuropeptide Y1 receptors (**Fig. 12**) [125,126].

5-Hydroxytryptamine (5-HT) receptors are highly diverse. They include 14 types and subtypes involved in the regulation of many important physiological

processes in the CNS and other systems [127]. Pyrazolo[1,5-*a*][1,3,5]triazines **44** were claimed to be effective ligands for 5-HT<sub>6</sub> receptors [128], which are the suitable target for the treatment of cognitive dysfunction associated with Alzheimer's disease, obesity, depression and anxiety [129]. Another subtype of 5-HT receptors linked with the potential for the CNS disorder treatment, namely 5-HT<sub>2c</sub> receptors, was effectively targeted by pyrazolo[1,5-*a*][1,3,5]triazine **45**, which was identified in the virtual screening and showed submicromolar affinity to 5-HT<sub>2c</sub> receptors *in vitro* [130]. Pyrazolo[1,5-*a*][1,3,5]triazine skeleton was used for the construction of angiotensin II (AII) receptor antagonists, e.g. **46** [131]. This compound (**46**) showed high affinity to AII receptors *in vitro* and demonstrated significant decrease in AII hypertensive response *in vivo*.



**Fig. 12.** Miscellaneous receptor ligands.

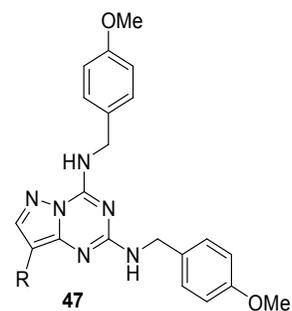
### 3.3. Biological activity with undefined molecular target

For the compounds based on purine isosteres with pyrazolo[1,5-*a*][1,3,5]triazine as a part of the molecule skeleton, exact biological targets were not always identified and mechanisms of their biological activity remain unknown or unclear. This situation is often observed for the compounds with antiproliferative and antiviral activities.

#### 3.3.1. Antiproliferative activity

Anticancer evaluation of pyrazolo[1,5-*a*][1,3,5]triazines yielded compounds **47** (**Fig. 13**) [132]. In antiproliferative screening, **47b** displayed the activity against colorectal cancer cells (HCT116, SW48, SW480) and CEM lymphoma cell line. This compound (**47b**) also exhibited excellent cell cycle arrest at the G<sub>2</sub>/M phase after 24 h treatment of colorectal cancer cells. In *in vitro* testing using bovine brain purified tubulin, both compounds **47a** and **47b**

were equally potent in inhibition of tubulin polymerization.



R = H (**47a**):  $IC_{50} = 13 \mu M$  (CEM),  $6.2 \mu M$  (HCT116),  $8.1 \mu M$  (SW480)  
 R = *i*-Pr (**47b**):  $IC_{50} = 5.4 \mu M$  (CEM),  $4.6 \mu M$  (HCT116),  $5.5 \mu M$  (SW480)

**Fig. 13.** Compounds with antiproliferative activity.

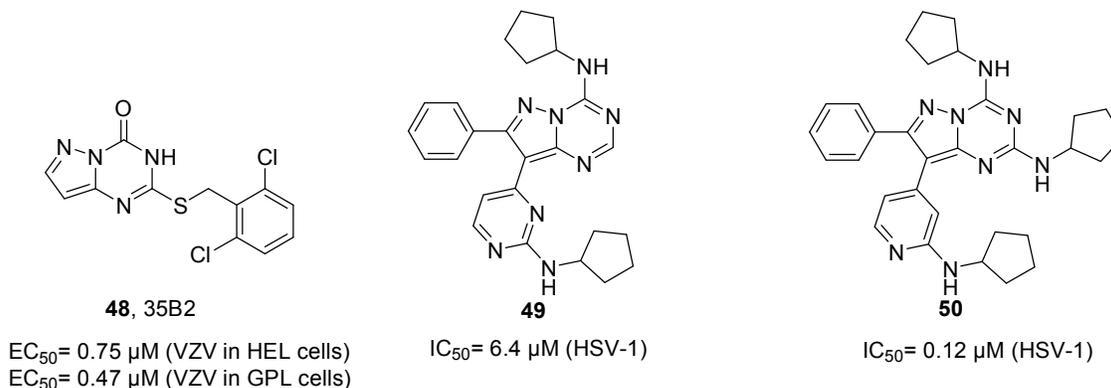
#### 3.3.2. Antiviral activity

Viruses are the leading cause of human diseases worldwide. Hence, there is an active ongoing search

for novel antiviral agents, which may provide highly effective and safe treatment of human virus infections. The antiviral properties of purines and their derivatives have been well recognized resulting in a number of potent drugs in the market. Therefore, interest to the purine isosteres with pyrazolo[1,5-*a*][1,3,5]triazines scaffold in this area of medicinal chemistry is logical. Results of the research in this direction are outlined below.

Compound 35B2 (**48**), a rather simple derivative of pyrazolo[1,5-*a*][1,3,5]triazin-4-one, was identified

as an active varicella-zoster virus (VZV) inhibitor (**Fig. 14**) [133]. Antiviral activity of 35B2 (**48**) against VZV (V-Oka) was determined using plaque reduction assay in GPL and HEL cells. Analysis indicated that 35B2 (**48**) targeted ORF40, VZV major capsid protein (MCP) by inducing MCP aggregation and inhibiting capsid assembly [133]. More decorated pyrazolo[1,5-*a*][1,3,5]triazines **49** and **50** showed antiviral activity against herpes simplex virus-1 (HSV-1) with low cytotoxicity [134].

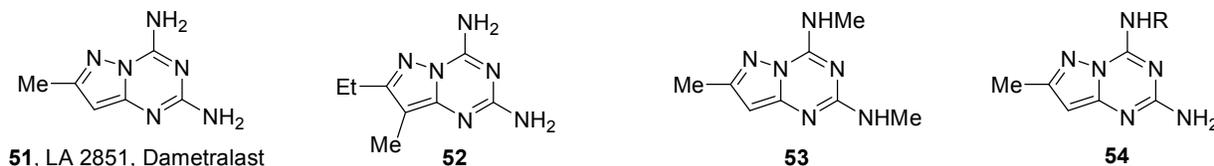


**Fig. 14.** Compounds with antiviral activity.

### 3.3.3. Miscellaneous biological activities

The spectrum of biological effects exhibited by the pyrazolo[1,5-*a*][1,3,5]triazines-based isosteres of purines is very wide ranges as anti-inflammatory and antiallergic properties. 2,4-Diaminopyrazolo[1,5-*a*][1,3,5]triazines were studied as potential anti-asthmatic agents [135-139]. Dametralast (LA 2851, **51**) (**Fig. 15**) was found to be a potential anti-asthmatic agent exhibiting pronounced bronchodilatory [135,136]. and antiallergic [135-137] activities together with low toxicity. Dametralast (**51**) also possessed

anti-inflammatory properties not related to cyclooxygenase inhibition or mediator release [138]. The lipoxygenase pathway was postulated to be responsible for the pharmacological action of dametralast (**51**). The inhibition of PDE to a certain extent was also mentioned as the secondary activity of dametralast (**51**). The synthesis and further evaluation of anti-asthmatic properties using a variety of animal models together with clinical testing in humans were conducted with dametralast (**51**), its analogues (**52** and **53**) and derivatives (**54**) [139].



**Fig. 15.** Compounds with miscellaneous biological activities

## 4. Conclusion

Continuous inspiration by successes in purine chemistry and pharmacology led to extensive exploration of the bioisostere heterocyclic systems. Interest towards purine-like heterocycles comprising pyrazolo[1,5-*a*][1,3,5]triazine scaffold. These heterocyclic systems demonstrated a great potential in the development of inhibitors of various kinases, PDE, XO, and TP, antagonists of adenosine, CB and CRF

receptors, anticancer and antiviral agents, therefore becoming privileged scaffolds in medicinal chemistry.

The success in the development for the synthesis of differently substituted pyrazolo[1,5-*a*][1,3,5]triazines scaffold, with various substituents, is considered as a good template for developing new chemical entities with different biological activities and physicochemical properties. Generally, most substitutions were made at position 2, 4, 7 and 8. In

particular, since many fused bicyclic ring systems were formed by nucleophilic reactions between amino groups and electrophilic carbon atoms attaching to oxygen, sulfur, or nitrogen, the substituents at position 2 or 4 were usually OH, SH, or NH<sub>2</sub>. However, substituents at position 7 and 8 could vary, which are usually determined by the corresponding starting materials. Eventually, the selection of a specific method for preparing target compounds should depend on the position of substituents to be introduced on the target compounds.

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