8-Azapurine Nucleus: A Versatile Scaffold for Different Targets

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Abstract: 8-Azapurine nucleus is a bioisoster of the purine nucleus. Variously substituted 8-azapurines have been synthesised and studied for their interactions with many enzymes and receptors and for their antitumor and antiviral activity. In this paper the main results of the studies made in these last years on this topic are reported.

Key Words: 8-Azapurines, 1,2,3-triazolo[4,5-*d*]pyrimidines, adenosine deaminase inhibitors, xanthine oxidase inhibitors, adenosine receptor ligands, antitumor and antiviral agents.

INTRODUCTION

In the search for new compounds of therapeutic interest, purine derivatives are very important for their possible interaction with many biological targets. Among the modifications of the purine nucleus, the replacement of the carbon atom in the 8 position with a nitrogen gives 1,2,3-triazolo [4,5-*d*]pyrimidines named also 8-azapurines. The numbering systems for purines and triazolopyrimidines are shown in Fig. (**1**). 8-Azapurines possess a broad spectrum of biological activity and are studied for their interactions with many enzymes and receptors and for their antitumor and antiviral activity. After the comprehensive review by Albert [1], biological properties of 1,2,3-triazolo[4,5-*d*]pyrimidines prepared in the following years have not been summarized up to now in a review paper. The chemistry of 8-azapurines and, in brief, the structure-activity relationships on some targets are described in a more recent review by El Ashry and Rashed [2].

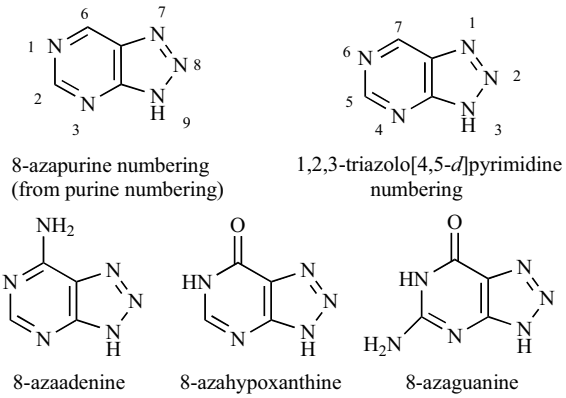

Fig. (1).

Here some recent results on synthesis and biological activity of 8-azapurines are described.

SYNTHETIC METHODS

All methods to synthesize 8-azapurines can be included in two main routes. The first one needs the synthesis of a 4,5-diaminopyrimidine ring and then the formation of the triazolo ring. This method allows one to obtain either 8-azaadenines (by reaction of pyrimidine with nitrous acid) or adenines (by reaction with triethylorthoformate) starting from the same substrate, but to obtain the conducive pyrimidine can be a multi-step process that may imply the use of problematic initial reactants (Scheme (**1**), Route a) [1]. The second one, developed by us, is a "one-pot synthesis" which allows us, using relatively simple initial materials, to obtain variously substituted 8-azahypoxanthines [3,4] or 8-azaadenine [4,5]: 2,9-substituted 8-azahypoxanthines can be obtained by a 1,3 dipolar addition reaction of an azide and cyanoacetamide; the obtained intermediate 4-carbamoyl-5-amino-1H-1,2,3-triazole, which is not isolated, gives 8-azahypoxanthines by annulation reaction with an ester.

The prepared 8-azahypoxanthines can be converted to 8-azaadenines by reaction with an amine and hexamethyldisilazane or through the corresponding 6-chloro-8-azapurines, obtained by reaction with phosphorus oxychloride, by nucleophilic displacement of the chlorine atom by the suitable amine (Scheme (**1**), Route b) [6].

2,9-Substituted 8-azaadenines can also be directly obtained by the reaction of malononitrile and an azide, affording the intermediates 4-cyano-5-amino-triazoles which are converted to 8-azaadenines by treatment, in the same flask, with a nitrile (Scheme (**1**), Route c) [3].

Some authors have studied these approaches to obtain small libraries of variously substituted 1,2,3-triazolo[4,5-d] pyrimidines and the results are principally published in organic chemistry journals [7-14]. Among the cited references it is worthwhile mentioning, because of the mild reaction conditions, the efficient synthesis of some 5-substituted-3,6-dihydro-7*H*-1,2,3-triazolo[4,5-*d*]pyrimidin-7-ones (Scheme (**1**), Route d) [13,14].

In most of the articles in the literature on 8-azapurines, synthetic methods aim at obtaining new pharmacologically active compounds and in the related papers also the biological assays are reported. In the next paragraphs the recent findings concerning these molecules are described, divided by biological activity.

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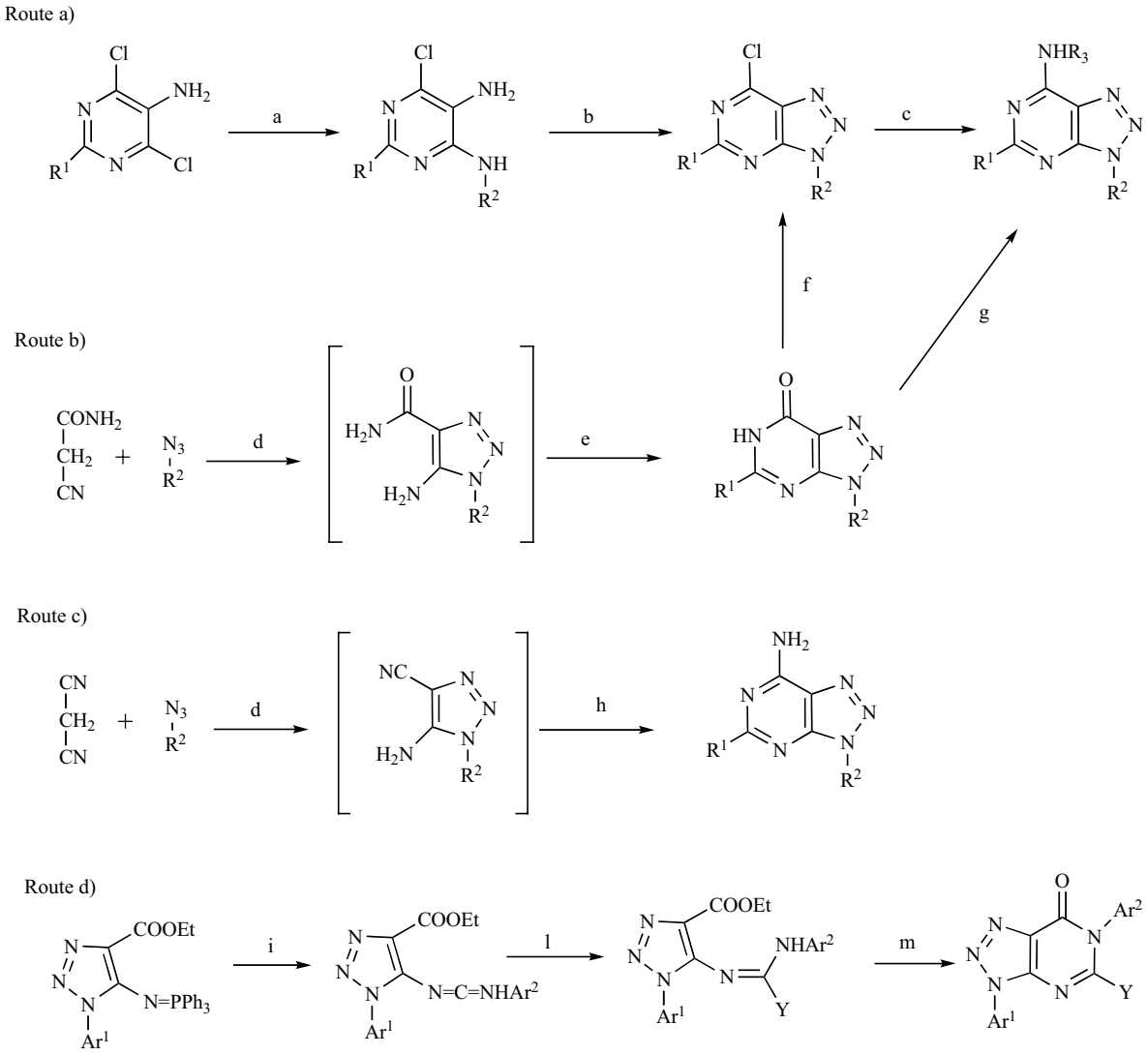


Scheme 1.

8-AZAPURINES AS XANTHINE OXIDASE INHIBITORS

Xanthine oxidase (XO), a flavoprotein, catalyzes the oxidation of hypoxanthine to uric acid playing an important role in the catabolism of purines. Allopurinol, an XO inhibitor, is used in the treatment of gout. A positive correlation between antitumor activity and inhibitors of XO was also suggested [15]. Recently, inhibition of XO has been proposed as being beneficial to patients with congestive heart failure [16].

Unsubstituted 8-azaadenine and 8-azaguanine demonstrated good activity as XO inhibitors (K_i = 0.66 μM and 10.3 μM respectively), whereas 8-azahypoxanthine were inactive [15].

An *n*-pentyl chain substituted on C(2) of the 8-azahypoxanthine, compound **1**, Fig. (**2**), conferred weak activity, and 2-*n*-pentyloxycarbonyl-8-azahypoxanthine, compound **2**, Fig. (**2**), was a good inhibitor (K_i = 2.87 μM) [17] . The corresponding "open-ring" compounds **3** (R= n-alkyl) and **4** (R= n-alkoxycarbonyl group) [17], showed better inhibitory activity. It is worth noting that activity was dependent on the length of the *n*-alkyl chain with a maximum with the C(5) or C(6) chain. These findings allowed us to prepare successively other compounds as XO inhibitors. Whereas 8-azapurines bearing on the C(2) a 1-amino-n-alkyl group, compounds **5**, Fig. (**2**), and their precursors 4(5)-(2-amino-alkanoylamino)-1*H*-[1,2,3]triazole-4(5)-carboxylic acid amides, compounds **6**, Fig. (**2**), did not show any activity [18]; on the contrary, a series of new 2-alkylthio-hypoxanthines was found very active, especially compound **7**, Fig. (**2**), with K_i = 9.8 nM [19], 700 times more active than allopurinol. Also some very active 2-alkyloxyalkylthiohypoxanthines were studied [20].

8-AZAPURINES AS ADENOSINE DEAMINASE INHIBITORS

Adenosine deaminase (ADA) is a crucial enzyme of the purine metabolic pathway that catalyzes deamination of adenosine and of other exogenous substrates. In 1970 8-

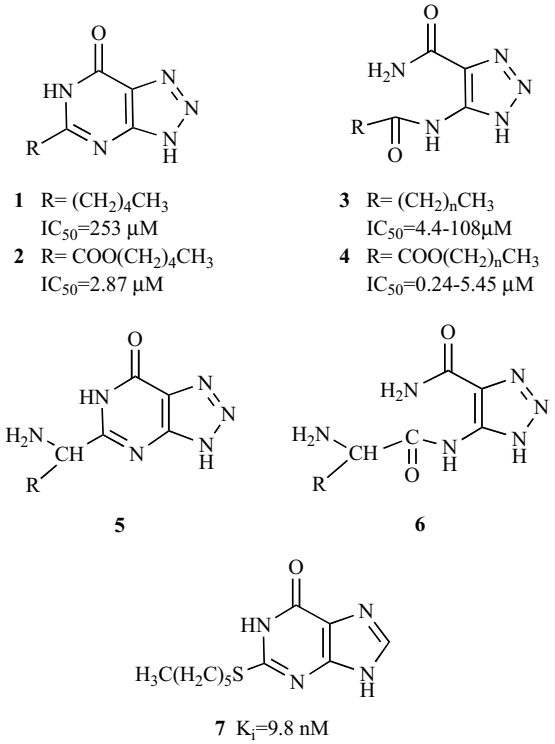


Fig. (2). Xanthine Oxidase inhibitors.

azaadenosine was found to be a substrate for the enzyme and also a competitive inhibitor of the deamination of adenosine [21].

Although some 2-substituted 9-(2',3'-dihydroxypropyl)-8-hypoxanthines and 8-azaadenines [22] showed poor affinity and inhibitory activity towards the ADA enzyme and also 2-fluoro-8-azaadenosine showed itself to be a poor substrate compared with adenosine [23], some 2-aryl-8-azaadenosines were more active [24]. Among the latter, that having the 2-(*m*-tolyl)- substituent was the most potent, K_i 8.5 μM, compound **8**, Fig. (**3**); whereas 2-phenyl and 2-(*p*-tolyl)-8-adenosine was less active (Ki 28.7 and 49.3 μM, respectively). Furthermore the 2-aryl substituent on 8-azaa-denines improved activity, compared with the 2-unsubsti-tuted molecules, in the presence of an *n*-alkyl or 2-hydroxy-3-*n*-alkyl chain on N(9) [25], and the activity was found to be related to the length of the chain itself and to the configuration of the chiral carbon bound to the hydroxy group as in *erythro* N(9)-hydroxynonyladenine, EHNA, a very potent inhibitor of ADA [26]. In fact, 2-phenyl-EHNA (*erythro*-2-phenyl-9-(2-hydroxy-3-nonyl)-adenine), compound **9**, Fig. (**3**), and its 8-aza-analogue, compound **10**, Fig. (**3**), showed very high inhibitory activity towards ADA, with K_i 0.55 and 1.67 nM respectively, better than *threo* isomers with K_i = 1.30 and 14.4 nM respectively [27].

The lower potency of the 2-unsubstituted compounds, EHNA and *threo* N(9)-hydroxynonyladenine (THNA) with K_i = 4 and 100 nM respectively [28] and the same enantioselectivity as the 2-phenyl substituted compounds are worth noting. Therefore it was possible to hypothesize that the new compounds would interact with the same site of the enzyme as EHNA, with strong interactions with the alkyl chain and with the aryl group on C(2). The 8-azaderivatives were slightly less active than adenines, demonstrating a substantial bioisosterism between the adenine and the 8-azaadenine nucleus. Furthermore, in the literature it is reported that 8-azaadenosine was hydrolyzed from ADA twice as fast as adenosine [21]. It was hypothesized that this deamination involves an unstable hydrated intermediate engendered by the addition of water across the purine N(1)-C(6) double bond; the presence of an additional heteroatom on the het-

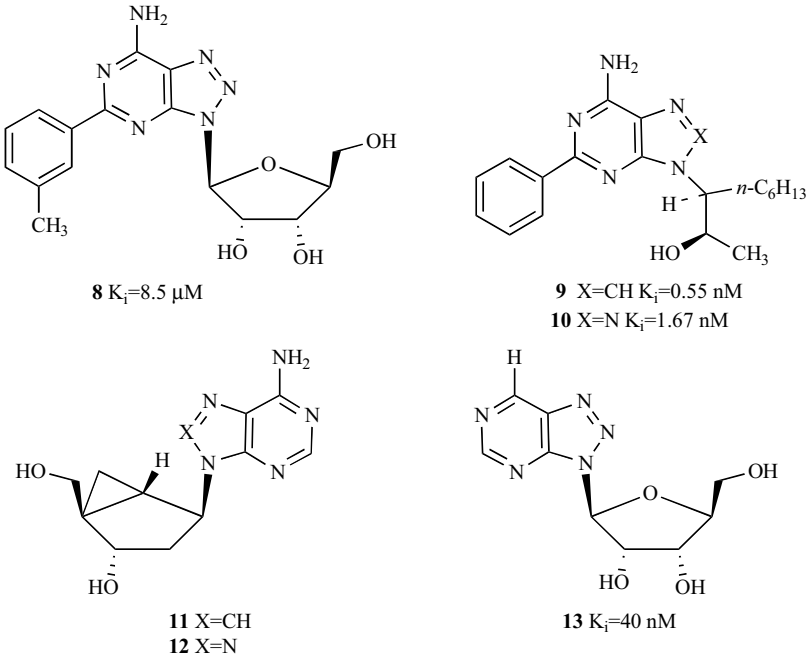


Fig. (3). Adenosine deaminase inhibitors.

erocyclic nucleus appears to facilitate the process [29]. In carbocyclic compounds, on the contrary, the corresponding purine analogous **11**, Fig. (**3**), was hydrolyzed by ADA at approximately double the rate than the 8-azaderivative **12**, Fig. (**3**) [27]. These results argue in favour of an important role for the oxygen atom in assisting hydrolysis.

More 8-azapurine nucleosides were considered interesting for their activity as inhibitors of ADA. For example 8-azanebularine, compound **13**, Fig. (**3**), a derivative of 8-azaadenosine with hydrogen substituted for the C-6 amino group, is a potent ADA inhibitor with K_i = 40 nM, a value 400-times lower than that for nebularine (K_i = 16 μM) [30].

Recently 8-azanebularine [31] and its C-6 substituted analogues [32] were considered also as potent inhibitors of ADARs (Adenosine Deaminase that Act on RNA), adenosine deaminases that act on duplex RNA substrates and are responsible for mRNA editing by adenosine deamination.

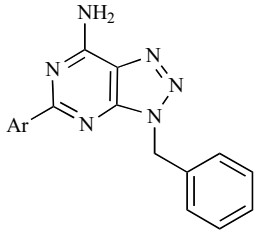
8-AZAPURINES AS ADENOSINE RECEPTOR LIGANDS

Adenosine modulates a wide variety of physiological functions on the central nervous, cardiovascular, immune, and hormonal systems. This ubiquitous molecule also inhibits lipolysis, platelet aggregation, neurotransmitter release from nerve endings and potentiates histamine release from mast cells. The most important results of these actions are tissue protection and repair. Adenosine modulates its physiological functions mainly through four specific G-protein-coupled transmembrane receptors, classified as A_1, A_{2A}, A_{2B}, and A_3. These receptors are present practically in each organ and tissue but subtype distribution and density differ greatly. Many ligands for these receptors have been developed but none is used in therapy. Only adenosine itself has been used in the treatment of acute supraventricular tachycardia.

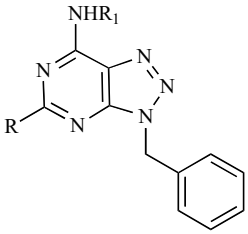
Generally, the substitution of C-H with N at the position 8 in 1,3-dialkylxanthines reduced affinity with respect to the corresponding xanthine derivatives [33]. Whereas our group demonstrated that N(6)-unsubstituted 8-azaadenines, having lipophilic groups on N(9) and on C(2), showed promising affinity towards A_1 receptors. The most active compounds were 9-benzyl-2-phenyl , 2-(*p*-tolyl)- and 2-(*m*-tolyl)-8-azaadenines, compounds **14** Fig. (**4**), [34]. In the same paper 2-aryl-8-azaadenosines were also synthesized and assayed and showed poorer ability to bind both A_1 and A_2 receptors with slightly more preference for A_1. Further analysis on the biological data by the comparison of selectivity ratios in both series and K_i value ratios, calculated for each receptor, when the C(2)-substituent was the same, led authors to conclude that the 9-benzyl-8-azaadenines were characterized by the most important differences in arrangements inside the A_1 receptor.

Starting from these interesting results, during the last fifteen years, our group has dedicated much effort to study 2,6,9-substituted 8-azapurines as potential active and selective ligands for adenosine receptors; many derivatives have proved to be good ligands for A_1 subtype and some for A_{2A} and A_3 ones.

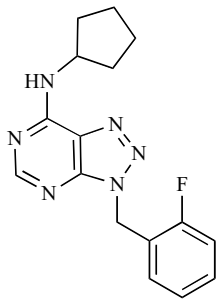
Several C(2),N^6-substituted compounds, **15** and **16**, Fig. (**4**), have been prepared and assayed [35, 36]. The comparison between 2-*n*-butyl and 2-phenyl-9-benzylazaadenines



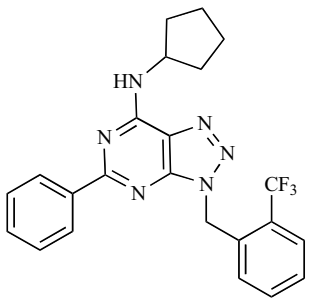
14 Ar=phenyl (K_i=40 nM), p-tolyl (K_i=50nM), m-tolyl (K_i 100nM)



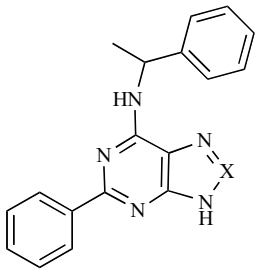
15 R=n-butyl; R_1=alkyl, cycloalkyl (K_i=0.3-83 μM)
16 R=phenyl; R_1=alkyl, cycloalkyl (K_i=11-353 nM)



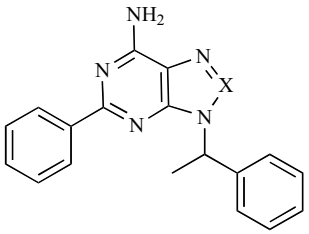
17 K_i=10.2 nM



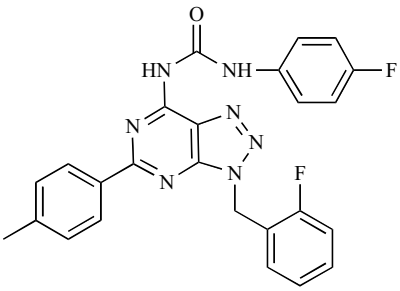
18 K_i=0.6 nM



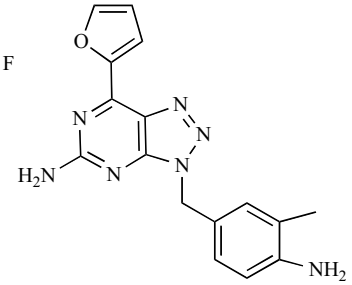
***R*-19** X=CH K_i=12.8 nM
S-19 X=CH K_i=291 nM
***R*-20** X=N K_i=354 nM
***S*-20** X=N K_i=4620 nM



***R*-21** X=CH K_i=48.5 nM
S-21 X=CH K_i=216 nM
***R*-22** X=N K_i=34.7 nM
***S*-22** X=N K_i=401 nM



23 K_i=6 nM



24 K_i=1.3 nM

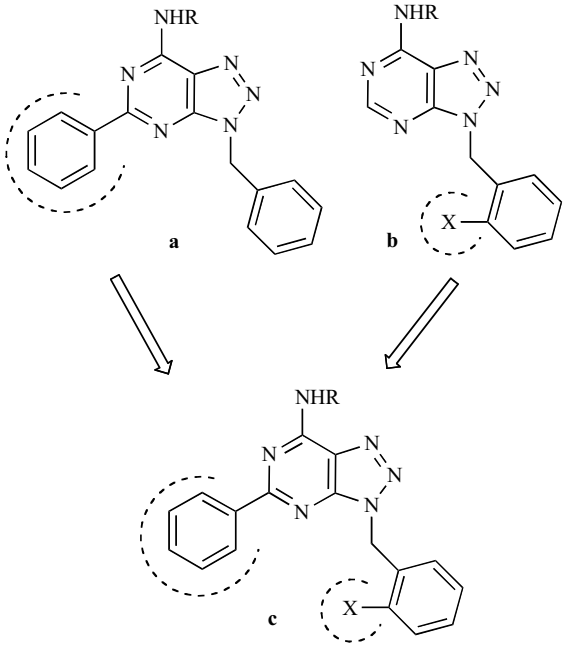
Fig. (4). Adenosine receptor ligands: A_1 ligands (compounds **14-22**); A_3 ligands (compound **23**); A_{2A} ligands (compound **24**).

bearing on N^6 the same substituent, i.e. n-alkyl, cycloalkyl, phenyl, benzyl group, proved that the phenyl group could give the better contribution to the binding towards A_1 receptors compared to the n-butyl one. Therefore, a third lipophilic pocket, able to receive the substituent on N(9) and placed in a receptor region close to that which interacts with ribose moiety of adenosine has been hypothesized. Furthermore, in that paper a double possible disposition of 2,6,9-trisubstituted adenines and 8-azaadenines in the binding site of A_1 receptors by a 180° rotation around C(2)-N(7) axis of 8-azapurine nucleus has been hypothesized. This hypothesis has been supported by comparing the K_is of 2-phenyl-8-azaadenosines and 2-phenyl-8-azaadenines, bearing on N^6 the same substituent, towards A_1 and A_2 subtypes [37]. Successively, some N^6 or N(9) substituted 2-phenyl-8-azaadenines [38] with the same substituents alternatively in the two positions have been prepared and the A_1 activities of the two series have been compared. In some cases, the biological results indicated the ability of the receptor to accept these exogenous molecules in at least two different arrangements, sterically related by a rotation around an ideal C(2)-N(7) axis, as seen above. Furthermore, pharmacological results have also shown the antagonistic behaviour of the tested compounds.

A 3D model of the A_1 adenosine receptor and several complexes between the model and the same previously synthesized 8-azaadenines were built [39]. In this model, based on the crystal structure of Rodhopsin, it was noted that His 278, giving H-bonds with sugar hydroxyl groups at position 2',3' and 5' when the agonist adenosine is arranged in the receptor, gives instead a stacking interaction with the benzyl substituent at position 9 of the studied antagonists; other significant stacking interactions in the case of the cited compounds could exist between Phe 86, Phe 275 and the 2-phenyl substituent. The binding site was not seen as a sum of rigid subsite pockets, each capable of binding a particular chemical function of a ligand; instead the pocket itself was hypothesized to be capable of varying in the position and orientation of aminoacid residues to allow them the best interactions with different ligands. This idea, that can be seen as the rationale for receptor selectivity, is in accordance with that reported by Jaakola and coll. in a recent paper about the A_{2A} receptor, on the basis of determined crystal structure of the human A_{2A} adenosine receptor in complex with a selective antagonist [40].

Another paper [41] describes the preparation of 3-aralkyl-7-(amino-substituted)-1,2,3-triazolo[4,5-*d*]pyrimidines with high affinity toward A_1 adenosine receptors. The most interesting series had the 2-chlorobenzyl group on N(3); in this series, 6 compounds, out of 15, showed K_i values < 120 nM. This study was continued [42] with the preparation of similar compounds with new substituents on N(3), as 4-chlorobenzyl, 2- and 4-fluorobenzyl, and 2-thiophenemethyl groups. The results were all good (K_is < 100 nM), especially for the 2-fluorobenzyl series that contained several active compounds such as the 7-cyclopentylamino derivative (K_i 10.5 nM), compound **17**, Fig. (**4**).

Recently [43], we have described the synthesis and biological activity of new 8-azaadenines (**c**, Scheme (**2**)) bearing both a phenyl group on C(2) (as in the previously reported [27, 39] compounds **a**, Scheme (**2**)) and a 9-benzyl group substituted in the *ortho* position with a Cl or a F atom or a CF_3 group (as in the previously reported [41,42] compounds **b**, Scheme (**2**)), to verify the synergistic effect of a combination of these substitution patterns on binding with A_1 adenosine receptors. In position N^6 aliphatic and cycloaliphatic substituents were chosen which had been shown to bind well with A_1 receptors. The compounds obtained generally showed very good affinity and selectivity to A_1 receptors. Some of the compounds showed K_i in the nanomolar range, one even in the subnanomolar range (0.6 nM), compound **18**, Fig. (**4**). Further, molecular docking analysis suggested also for these ligands different binding modes towards A_1 receptors, obtained by a 180° rotation through C(2)-N(7) axis of 8-azapurine nucleus as previously hypothesised [38,39]. The compounds synthesised in the paper of ref.[43] were also used for a QSAR study aimed at obtaining the identification of relevant features for the interaction of this kind of ligands with A_1 receptors [44].



Scheme 2.

Another important contribution to the understanding of binding modes to A_1 adenosine receptors of effective N^6- and N(9)-substituted 2-phenyladenines and 8-azaanalogues has been published [45]. Bioisosterism of the adenine and 8-azaadenine nuclei has again been demonstrated by the comparison of A_1 adenosine receptor binding affinity of 2-phenyl N^6-substituted adenines and the corresponding 8-azaadenines. That work also describes the synthesis and A_1 adenosine receptor binding assay of the enantiomers of some 2-phenyladenines and 8-azaadenines substituted with a 1-phenylethyl chiral group in N^6, compounds **19** and **20**, Fig. (**4**), or N(9) position, compounds **21** and **22**, Fig. (**4**). Biological results, showing the same stereoselectivity for all the couples of enantiomers, and theoretical studies, based on an improved A_1 adenosine receptor model, supported the above hypothesis of a possible double arrangement of 2-phenylsubstituted adenines and 8-azaadenines inside A_1 adenosine receptors.

Many of the described 8-azaadenines substituted with lipophilic substituents are nearly water-insoluble, sometimes causing problems in binding assays and making the compounds unsuitable for *in vivo* studies. So, synthesis of some potent and selective A_1 ligands more water-soluble was obtained introducing a hydroxy group on the substituent in position N^6 or N(9) of effective 8-azaadenines and adenines previously described [46]. Biological results showed that the presence of the OH function did not lower affinity for A_1 receptors, so these compounds could represent a useful tool for pharmacological and biological studies.

In a successive paper [47] we have demonstrated that the introduction of a phenylureido function on C(6) of substituted-9-benzyl-8-azaadenines turns affinity towards the A_3 subtype. Some of the compounds synthesized have shown very good affinity and selectivity for the A_3 subtype, (see for example compound **23**, Fig. (**4**), K_iA_3 6 nM) revealing the first class of A_3 adenosine receptor selective antagonists with a bicyclic structure strictly correlated to the adenine nucleus.

Only a few other researchers have studied 8-azaadenine derivatives as adenosine receptor ligands. In 2003 a patent regarding derivatives of adenine, 8-azaadenine and pyrazolopyrimidine was published [48]. Some 2-phenyl-8-azaadenine derivatives have shown good affinity and selectivity towards A_1 receptors, but lower than the corresponding purine compounds.

Very recently important efforts have been made to obtain A_{2A} antagonists as a new therapeutical approach in neurodegenerative disorders. Two recent patents [49,50] claim synthesis of 2-amino-9-substituted benzyl-6-(α-furyl)-triazolo [4,5-d]pyrimidines having very interesting activity and selectivity towards A_{2A} receptors. Lately a paper [51] describes the optimization of these compounds for obtaining an 8-azaadenine derivative, compound **24**, Fig. (**4**), with high A_{2A} potency (K_i 1.3 nM) and selectivity and with pharmacokinetic properties useful for oral administration. This compound has successfully completed phase I clinical studies.

8-AZAPURINES AS ANTITUMOR AND ANTIVIRAL AGENTS

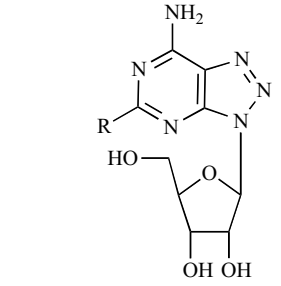
Starting from the first studies about the antitumor activity of 8-azainosine, 8-azaadenosine [52-54], and 8-azaguanosine [55], a number of their derivatives were synthesized and exhibited antiviral and antitumor activities. Among them 2-amino, compound **25**, Fig. (**5**), and 2-fluoro-8-azaadenosine, compound **26**, Fig. (**5**), [56] showed a marked cytotoxicity in H.Ep.-2 cell culture screen. As several carbocyclic nucleosides exhibited antitumor and antiviral properties [57], the Vince group synthesized their 8-azaderivatives, and some of them, i.e. compounds **27**, Fig. (**5**), demonstrating citotoxicity and antileukemic activity in P388 cells and in mice inoculated with the same cells [58-60]. On the contrary, whereas some purine derivatives, for example compound **28**, Fig. (**5**), showed anti-HIV [60] activity, the replacement of C(8) in the same carbocyclic nucleosides with a nitrogen and/or saturation of carbocyclic sugar moiety abolished activity as in compounds **29**, Fig. (**5**) [60]. The same isosteric substitution was detrimental also on 2'3'-dideoxy-3'-oxoadenosine, a nucleoside with good anti-HIV potency: in fact compound **30**, Fig. (**5**), was not active as an anti-HIV agent [61]. This fact was attributed to the poor affinity of the 8-aza carbocyclic nucleosides to cellular kinases [61]. To better analyse this hypothesis, i.e. that a number of nucleosides is not converted to its monophospate derivative by kinases causing lack of antitumor or antiviral activity, a study was published in 2002 [62]. So, 8-aza-2'-deoxyadenosine, a poor substrate for mammalian kinase, was converted to its 5'-bis(pivaloyloxymethyl)phosphate prodrug, compound **31**, Fig. (**5**). The results showed that this compound possesses significantly enhanced cytotoxicity in CEM cells when compared with the corresponding parent nucleosides [62].

In recent years, also De Clercq and coll. have been interested in synthesizing and assaying carbocyclic nucleosides, especially with guanine, 8-azaguanine, adenine and 8-azaadenine nucleus, as antiviral agents [63]. Among synthesized carbocyclic compounds, containing a cyclobutyl ring, compounds **32** and **33**, Fig. (**5**), showed some activity against vaccinia and herpes simplex virus. Similar carbonucleotides containing a cyclopentyl ring such as compounds **34**, Fig. (**5**), did not have appreciable antiviral activity [64], but appreciable antiviral and/or antitumor activity were demonstrated by the 2,6-diamino- or 2-amino-6-chloro-purines or 8-azapurines derivatives **35**, Fig. (**5**) [65]. In a review of 1999 were reported, in structure-activity relationships of carbocyclic nucleosides, that the replacement of the C-8 of the purine ring with nitrogen eliminates activity [66]. But more recently 1,2-disubstituted carbonucleosides have been described [67] and the 8-aza compound **36**, Fig. (**5**), showed anti-HIV activity comparable to that of Abacavir, a drug recently approved by FDA in the treatment of AIDS [68].

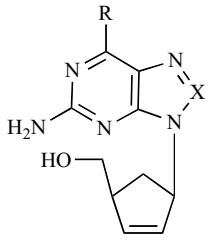
Synthesis of nucleoside analogues in which the cyclic carbohydrate moiety is replaced by an acyclic side chain has led to the discovery of the potent antiherpetic drug Acyclovir [69]. A lot of modifications of this molecule have been made, including many modifications of the heterocyclic nucleus [70]. Among monocyclic (isocytosine, triazole, imidazole), bicyclic (8-azapurine, pyrrolo[2,3-d]pyrimidine, pyrazolo[3, 4-d]pyrimidine] and tricyclic (linear benzoguanine) congeners, only the 8-azapurine analogue **37**, Fig. (**5**), showed some activity against HSV-1 (herpes simplex virus-1).

During the 1990s, acyclic nucleoside phosphonates were discovered as potent antiviral agents: in fact several phosphonomethoxyalkyl derivatives of purines and pyrimidines have high activity against the DNA virus and retrovirus and some acyclic nucleoside phosphonates have acquired a prominent therapeutic position [71,72]. Among them, very interesting were the broad spectrum antiviral agent **38** [73] and the anti-HIV agent **39**, Fig. (**5**) [74]: the substitution of the purine with an 8-azapurine ring, compounds **40** and **41**, Fig. (**5**), maintained anti-HIV activity and remarkably reduced cytotoxicity [75,76]. In 1996 a paper from Holy and coll. [77] reported synthesis and antiviral activity of a number of acyclic nucleotide analogues derived from 8-azapurines that confirmed antiviral and cytoprotective activity of some of them, like compound **42**, Fig. (**5**).

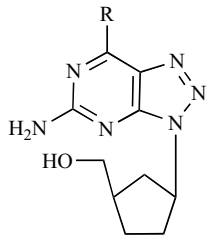
In 2005 Hassen and coll. described some 8-azapurine compounds as potent anti-hepatitis C virus agents [78]. These compounds, **43-46**, Fig. (**5**), were more potent than the



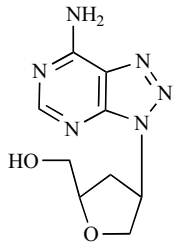
25 R=NH_2 [56]
26 R=F [56]



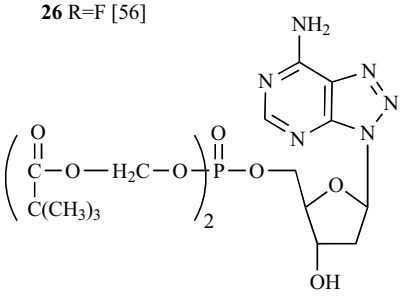
27 R=Cl, OH; X=N [57]
28 R=NH_2; X=CH [60]



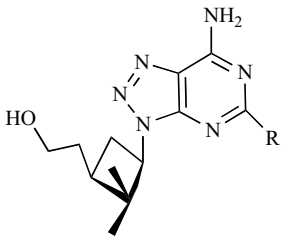
29 R=Cl, OH [60]



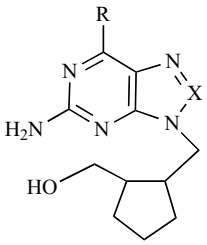
30 [61]



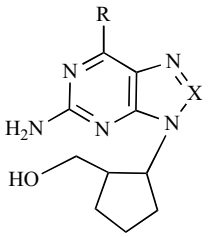
31 [62]



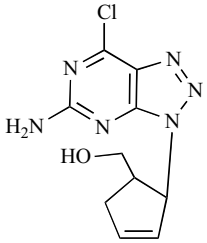
32 R=NH_2 [63]
33 R=H [63]



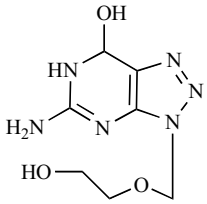
34 X=Cl, OH, NH_2; X=N, CH [64]



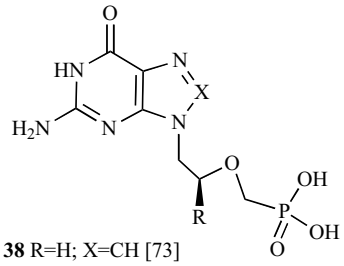
35 R=NH_2, Cl; X=N, CH [65]



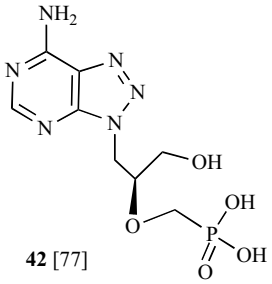
36 [67]



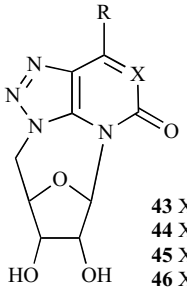
37 [70]



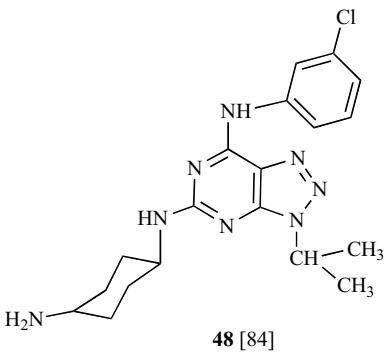
38 R=H; X=CH [73]
39 R=CH_3; X=CH [74]
40 R=H X=N [75]
41 R=CH_3 X=N [76]



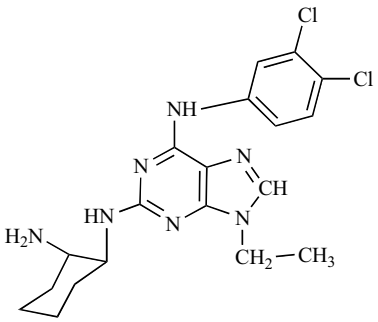
42 [77]



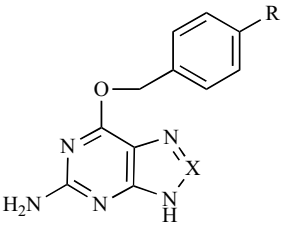
43 X=N R=NH_2 [78]
44 X=N R=$NHCH_3$ [78]
45 X=N R=SH [78]
46 X=N R=OH [78]
47 X=C R=H [79]



48 [84]



49 [85]



50 X=CH R=H [89,90]
51 X=CH R=Cl [89,90]
52 X=CH R=CH_3 [89,90]
53 X=N R=H [91]

Fig. (5). Antiviral e/o antitumor compounds.

analogous *v*-triazolo[4,5-*b*]pyridine-2-one compound, **47**, Fig. (**5**), previously described [79], in antiviral activity but showed increased cytotoxicity.

A program of synthesis of fused pyrimidine derivatives for biological evaluation of a number of 2-phenyl-8-azaadenines, 8-aryl-8-azaxanthines and their 6-thioderivatives was recently reported [80]. Some of the 8-azaadenines showed antiviral activity against herpes virus type 1 and 2 at a concentration >4μg/ml. A sulphur atom in position 6 did not improve the very weak activity of the 8-azaxanthine derivatives.

Recently it was clarified that human tumor development is associated with the alteration of cyclin-dipendent kinase (CDK) and their regulators, and CDK inhibitors are considered as potential anticancer drugs [81,82]. As 2,6,9 trisubstituted purines were proposed as a new class of CDK and cell proliferation inhibitors [83], in 2005 Havlicek and coll. prepared 8-azaanalogues of those molecules [84]. This modification of the purine scaffold led to a lowering of CDK2 inhibitory activity in comparison with model trisubstituted purines, whereas antiproliferative properties remained high for some compounds. In particular, compound **48**, Fig. (**5**), a 9-isopropyl-2-(*trans*-4-aminocyclohexyl)amino-6-(3-chloro-phenyl)amino-8-azapurine, derived from compound CGP74514 (N^2-(2-amino-cyclohexyl)-N^6-(3,4-dichloro-phenyl)-9-ethyl-9*H*-purine-2,6-diamine), compound **49**, Fig. (**5**) [85], a potent purine-based CDK inhibitor, showed interesting cytotoxic activity on cell lines from various tumor tissues [84]. Another interesting 9-isopropyl-purine derivative with antitumor activity is myoseverin (9-isopropyl-2,6-(*p*-methoxy-benzylamino)purine), a microtubule interfering agent [86]. Antiproliferative activity of its 8-aza derivative was found to be weaker, and neither myoseverin nor 8-azamyoseverin resulted to be CDK inhibitors. The pyrazolo[4,3-*d*]pyrimidine analogues, on the contrary, displayed inhibitory activity towards both tubulin polymerization and the activity of some CDKs [87].

Mammalian O^6-alkylguanine-DNA alkyltransferase (AGT) repairs alkylation damage to the O^6-position of DNA guanine residues [88]. O^6-benzylguanine, **50**, Fig. (**5**), O^6-(p-chlorobenzy1)guanine, **51**, Fig. (**5**), and O^6-(*p*-methyl-benzy1)guanine, **52**, Fig. (**5**), as alternative substrates for this protein, produce dramatic and rapid depletion of AGT in human tumor cell extracts and in intact tumor cells and consequently significant enhancement in the cytotoxic response to a number of chloroethylating and methylating antitumor drugs [89, 90]. Chae *et al.* [91] demonstrated in 1995 that 8-substituted-O^6-benzylguanine derivatives bearing electron-withdrawing substituents at the 8 position, like 8-aza-O^6-benzylguanine, compound **53**, Fig. (**5**), are more efficient than O^6-benzylguanine in enhancing the therapeutic effectiveness of antitumor agents for which the mechanism of action involves modification of the O^6-position of DNA guanine residues. More 8-aza-O^6-benzylguanine derivatives are described in some patents [92,93].

OTHER TARGETS

Corticotropin releasing factor (CRF), a 41-amino acid peptide, is the prime regulator of the hypothalamic-pituitary-adrenal (HPA) stress–response. CRF exerts its biological functions through activation of its receptors CRF-1 and CRF-2, both of which belong to the class B subfamily of G-protein coupled receptors. Some small molecules have been synthesized as ligands of these receptors having a nitrogen-rich bicyclic nucleus. Among them some 1,2,3-triazolo[4,5-*d*]pyrimidines demonstrated high affinity. In fact, in a paper of 1999 [94] were presented a number of 2-methyl-purine and 2-methyl-8-azapurine derivatives variously substituted in the 6- and 9-positions and their SAR was discussed. Compound **54**, Fig. (**6**), was selected for further pharmacological studies due to its high affinity and good oral bioavailability. Two years later some more modifications of compound **54** were reported either on the bicyclic nucleus or on the 6- and 9-substituents [95]. In this case 1,2,3-triazolopyrimidines were not the best compounds obtained.

Some arylsubstituted purine derivatives, among them 8-azaadenines, were recently patented as ligands towards capsaicin VR1 receptors. These compounds are claimed to be useful in the treatment of pain or other pathological associated modulation of VR1, a major ion channel expressed in nociceptive primary afferent neurons [96].

Recently compound VAS28270, **55**, Fig. (**6**), 3-benzy-7-(2-benzoxazolyl)thio-1,2,3-triazolo[4,5-d]pyrimidine, was patented as a novel non-peptide NAD(P)H oxidase (Nox) inhibitor [97]. This enzyme is an important source of superoxide anions in endothelial cells which, reducing NO availability, can induce the oxidation of lipoprotein with the possible development of atherosclerosis. In 2006 this compound was investigated for its ability to reduce oxidative stress induced by oxidized low-density lipoprotein, in human endothelial cells and was proposed as a new therapeutic tool for the treatment of cardiovascular diseases [98].

Interesting antimalarian activity was discovered in 2006 by Keough *et al*. [99] They demonstrated that 8-azaguanine and 8-azahypoxanthine were effective substrates for *Plasmodium falciparum* hypoxanthine-guanina-xanthine phosphoribosyltransferase, an essential enzyme for DNA and RNA synthesis. Moreover, 8-azaguanine and 8-azahypoxanthine were very poor substrates for the human enzyme with high specificity ratios (80 and 336 respectively), so these compounds were considered as antimalarial lead compounds, being effective in inhibiting the growth of the parasite *in vitro*.

In a patent of 2004 [100] novel 2-substitutedphenyl-8-azahypoxanthine derivatives were synthesized and exhibited selective efficacy as smooth muscle phosphodiesterase 5 inhibitors and could be used for treating hypertension, pulmonary hypertension, renal disease or male sexual impotence without potentiating an anti-aggregating treatment. An example is compound **56**, Fig. (**6**), but biological data were not reported.

5-Pyridin-4-yl-3,6-dihydro-[1,2,3]triazolo[4,5-*d*]pyrimidin-7-one **57**, Fig. (**6**), was studied as a competitive AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor antagonist [101]. The promising activity of the compound was not more closely examined in later papers.

Platelet aggregation is the central process in the pathophysiology of acute coronary syndromes; adenosine diphos-

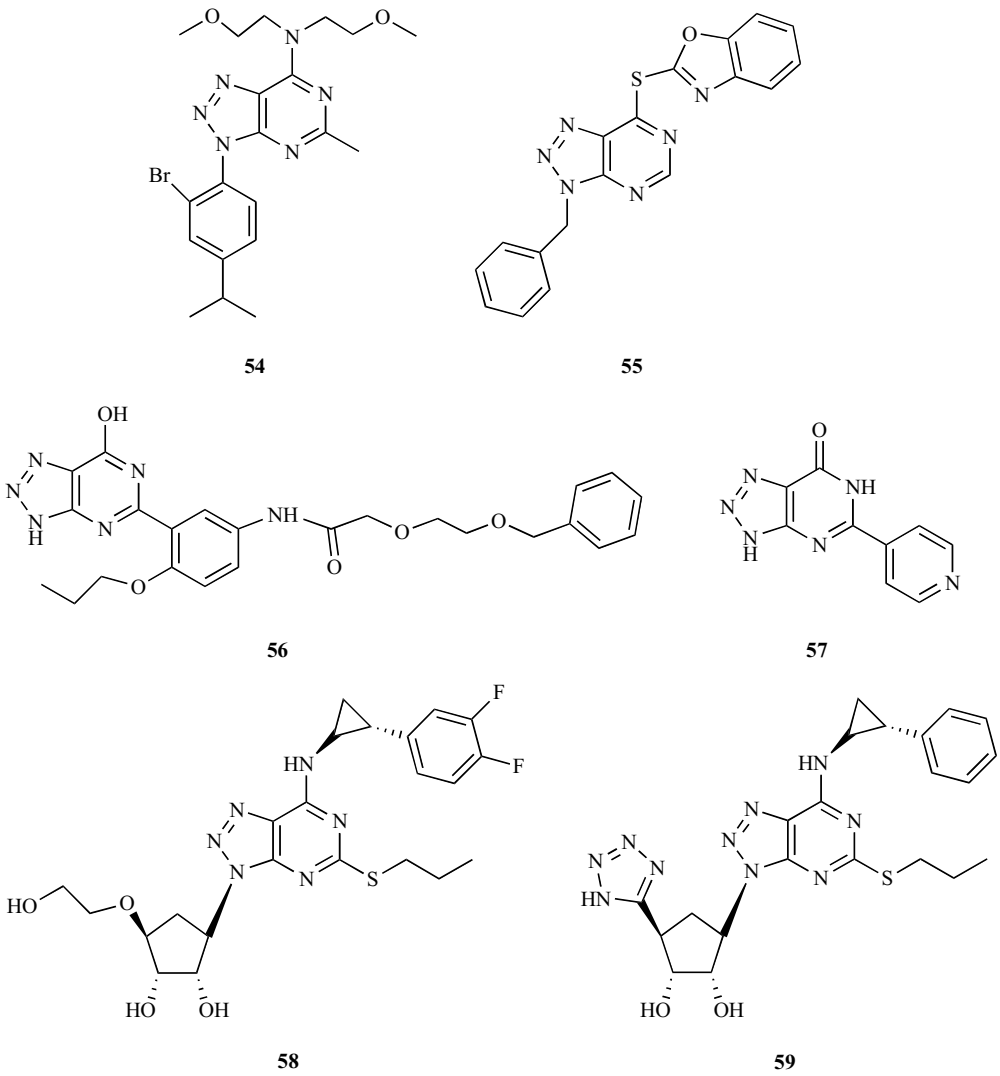


Fig. (6). Compounds active towards other targets.

phate (ADP) is an important platelet agonist and ADP released from platelet dense granules amplifies responses to other agonists. There are three known subtypes of ADP receptor on platelets: $P2X_1$, $P2Y_1$ and $P2Y_{12}$ receptors. Starting from 1994 a number of patents claim synthesis and use of 1,2,3-triazolopyrimidine derivatives as P_{2T} receptor ligands [102].

Starting from the structure of AR-C69931MX ([(2R, 3S,4R,5R)-3,4-dihydroxy-5-[6-(2-methylsulfanylethylamino)-2-(3,3,3-trifluoropropylsulfanyl)purin-9-yl]oxolan-2-yl]methyl dihydrogen phosphate) [103], a selective P_{2T} receptor antagonist and novel antithrombotic agent, a new orally active P_{2T} receptor antagonist having a 8-azaadenine scaffold was discovered and studied [104]. Changing the core purine to triazolopyrimidine an over 100-fold increased affinity was reached with compound **58**, Fig. (**6**), which is now in phase III clinical trials in acute coronary syndromes. A recent patent [105] describes other similar compounds with a modification of the substituent in the position 9 of the 8-azaadenine nucleus having potent activity as inhibitors of platelet aggregation, for example compound **59**, Fig. (**6**), IC_{50} 2 nM.

CONCLUSIONS

Examination of the literature and our experience in this matter allow us to identify the 8-azapurine nucleus, and in particular 1*H*-1,2,3-triazolo[4,5-*d*]pyrimidine, as a good scaffold that, with suitable substituents added, can give a number of active compounds for different biological targets. Furthermore, in some cases, the relatively easy synthetic methods to obtain them and their bioisosterism with the corresponding purines made these compounds useful as a preliminary step in studies on the interactions of purine derivatives with enzymes and/or receptors. An example of bioisosterism in the activity towards XO are 2-n-pentyl-hypoxanthine [106] and 2-n-pentyl-8-azahypoxanthine [17] that showed similar IC_{50} values (372 μM and 243 μM, respectively); another example is the similar activity towards ADA of 2-phenyl-EHNA and 2-phenyl-8-aza-EHNA (K_i = 0.55 nM and 1.67 nM, respectively) [27]. As examples of bioisosterism towards A_1 adenosine receptors of purines and the corresponding 8-azaderivatives, N^6-cyclohexyl-9-benzyl-2-phenyladenine (K_i = 9.4 nM and N^6-cyclohexyl-9-benzyl-2-phenyl-8-azaadenine (K_i = 1.6 nM) or N^6-cyclopentyl-9-

benzyl-2-phenyladenine (K_i = 12.2 nM and N^6-cyclopentyl-9-benzyl-2-phenyl-8-azaadenine (K_i = 11 nM) can be reported [45].

With regard to antiviral and antitumor activity, bioisosterism between 8-azapurine and purine compounds cannot be proved because very often different biological results in the same assays are obtained from the two classes of molecules. These results probably can be attributed to more complex biological phenomena involving the molecules in the tests used to verify antiviral and antitumor activity compared to the assays for activity towards enzymes or receptors.

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Received: 04 June, 2009 **Revised: 28 August, 2009** **Accepted: 28 August, 2009**