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Design and Synthesis of New A_{2A} and A_3 Adenosine Receptors Antagonists

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Introduction

1. Introduction

The purine nucleoside adenosine is consensually identified as a major local regulator of tissue function especially when energy supply fails to meet cellular energy demand. Due to its ability to equalize energy intake to metabolic demand in the 1980s it earned the reputation of a “retaliatory metabolite”.[1]

Adenosine is omnipresent, released from almost all cells, and generated in the extracellular space by breakdown of ATP through a series of ectoenzymes, including apyrase (CD39) and 5′-nucleotidase (CD73).[2] The latter dephosphorylates extracellular AMP to adenosine, regulating the limiting step for its formation. Extracellularly, adenosine concentration is kept in equilibrium by reuptake mechanisms operated through the action of specific transporters. Then inside the cell it is phosphorylated to AMP by adenosine kinase or degraded to inosine by adenosine deaminase (ADA). Intracellularly, adenosine formation is dependent upon the hydrolysis of AMP by an intracellular 5-nucleotidase or hydrolysis of *S*-adenosylhomocysteine. It is estimated that the levels of adenosine in the interstitial fluid are in the range 30-300 nM.[3]

Adenosine concentrations increase under metabolically unfavorable conditions. Tissue hypoxia, for example, leads to an enhanced breakdown of ATP and the increased generation of adenosine. In addition to this route, the release of adenosine might be potentiated by hypoxia-dependent inhibition of the salvage enzyme adenosine kinase which rephosphorylates the nucleoside to AMP.[4]

As adenosine is unstable and its half-life is limited by deamination or cellular reuptake, hypoxia-induced increase typically affects only local adenosine receptor signaling. As adenosine is not released in a

transmitter or hormone-like fashion, it is likely to belong to the group of autacoids.

Adenosine mediates its effects through activation of a family of four G-protein-coupled adenosine receptors (ARs), named A_1, A_{2A}, A_{2B} and A_3. These receptors differ in their affinity for adenosine, in the type of G proteins that they recruit, and finally in the downstream signaling pathways that are activated in the target cells. A_1 and A_3 ARs display high and low affinity for adenosine, respectively, and are inhibitory toward regulation of adenylyl cyclase activity. By contrast, activation of high-affinity A_{2A} and low-affinity A_{2B} subtypes stimulates adenylyl cyclase leading to an increase of cyclic AMP (cAMP) levels. Early pharmacological evidence for the existence of ARs has been provided by specific antagonism by methylxanthines, caffeine, and theophylline of adenosine-induced effects in the heart and brain.[5] These receptors are widely distributed through the body, and their presence on basically every cell makes them an interesting target for the pharmacological intervention in many pathophysiological situations linked to an increase of adenosine levels.

The first recorded report describing evidence for an ARs originates from 1976. Now, 30 years later, advances in understanding the role of adenosine and its receptors in physiology and pathophysiology as well as new developments in medicinal chemistry of these receptors have enabled researchers to identify potential therapeutic areas for drug development.

With the combination of pharmacological data, using selective ligands and genetically modified mice, important progress has been made toward an understanding of the role of ARs in a variety of diseases, such as inflammatory conditions, sepsis, heart attack, ische-

mia-reperfusion injury, vascular injury, spinal cord injury, chronic obstructive pulmonary disease (COPD), asthma, diabetes, obesity, inflammatory bowel disease, retinopathy, and Parkinson's Disease (PD). Nonselective AR antagonists are used to maintain wakefulness (caffeine) and, less commonly at present, treat bronchospasm (theophylline, aminophylline, enprofylline). Currently a number of new selective AR agonists and antagonists are in testing for a variety of new indications.

1.1. A_{2A} Adenosine Receptor

1.1.1. Pharmacology

The gene for the A_{2A} AR has been cloned from several species including dog,[6] rat,[7,8] human,[9] guinea pig,[10] and mouse[11] and demonstrated a high degree of homology among human, mouse, and rat.[12] The A_{2A} AR stimulates adenylyl cyclase activity through the coupling with G_s proteins leading to activation of cAMP-dependent protein kinase A. This in turn phosphorylates and activates various receptors, ion channels, phosphodiesterases, and phosphoproteins like CREB and DARPP-32.[13-15] Activation of protein kinase C has been also reported in PC12 cells.[16] In brain striatum the A_{2A} subtype stimulates G_{olf}, another member of the G_s subfamily of G proteins.[17] In addition A_{2A} AR can interact with different types of Ca^{2+} channels to either increase intracellular Ca^{2+} or decrease Ca^{2+} influx[18,19] and is involved like the other adenosine subtypes in the modulation of ERKs activity.[20]

Due to a long carboxy terminal domain, the A_{2A} AR shows a greater molecular weight (45 kDa) in comparison to the other subtypes (36-37 kDa). The A_{2A} AR C terminus has been defined as a crowded

place where different accessory proteins may interact such as D_2-dopamine receptors,[21] R-actinin,[22] ADP-ribosylation factor nucleotide site opener (ARNO),[23] ubiquitin-specific protease (USP4),[24] and translin-associated protein X (TRAX).[25] The lack or presence of such different partners may explain conflicting results deriving by A_{2A} ARs activation, e.g., neuroprotection versus neurotoxicity.[26]

Within the brain A_{2A} ARs are richly expressed in the striatum, nucleus accumbens, and olfactory tubercle. A coexpression of A_{2A} with D_2 dopamine receptors has been reported in the GABAergic striatopallidal neurons where adenosine and dopamine agonists exert antagonistic effects in the regulation of locomotor activity. Activation of A_{2A} ARs in striatopallidal neurons decreases the affinity of D_2 receptors for dopamine, antagonizing the effects of D_2 receptors (Fig.1). The negative interaction between A_{2A} and D_2 receptors is at the basis of the use of A_{2A} antagonists as a novel therapeutic approach in the treatment of PD.[27] In addition, A_{2A} ARs may have an important role in the neurodegenerative process. Accordingly, a neuroprotective effect was demonstrated after caffeine intake or A_{2A} AR inactivation against dopaminergic neurodegeneration in a neurotoxin model of PD.[28] Concomitantly, two large prospective epidemiological studies have strongly associated caffeine consumption to a reduced risk of developing PD.[29,30] Last, the recent discovery that the A_{2A} can form functional heteromeric receptor complexes with other Gprotein- coupled receptors such as D_2 and the mGlu5 receptors has also suggested new opportunities for the potential of A_{2A} antagonists in PD.[21] In the future development of bivalent ligands, able to activate D_2 and block A_{2A} ARs or antagonize both A_{2A} and mGlu5 subtypes, would be a

promising strategy for the treatment of this neurodegenerative disease.[31-33]

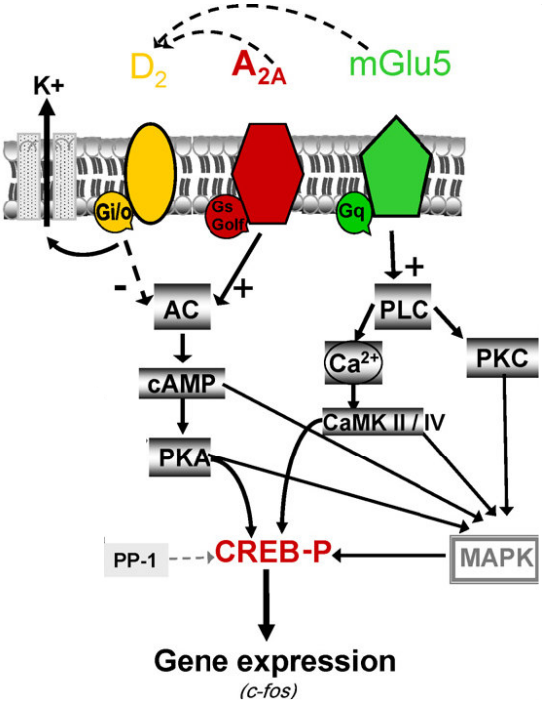


Figure 1. Functional interactions between dopamine D_2, adenosine A_{2A} and metabotropic glutamate 5 receptors in striatopallidal neurons.[34]

In addition to the protection against striatal and nigral neuron loss by A_{2A} antagonists, there are data also supporting their protective role outside the basal ganglia.[35] Local injection of an A_{2A} antagonist prevents glutamate-dependent death of neurons in hippocampal cortex[36] and also reduced cortical damage in a variety of ischemic stroke models. In A_{2A} knockout (KO) mice transient focal ischemia caused less neuronal damage in comparison to their wild-type (WT) littermates.[37] Therefore, it seems that tonic activation of A_{2A} ARs may be responsible for dangerous signal during injury, in contrast to the

neuroprotective effects induced by endogenous A_1 activation. Recently, selective inactivation or reconstitution of A_{2A} ARs in bone-marrow cells revealed their contribution to the development of ischemic brain injury.[38]

The involvement of A_{2A} ARs in neuroprotection is likely to be complex as stimulation of this subtype also diminishes brain damage after excitotoxic and traumatic injury.[39,40]

A_{2A}-mediated protection has been reported against ischemia in the myocardia, kidney, and liver and in ischemia-reperfusion injury in the spinal cord.[41-44]

High expression of A_{2A} ARs has been found in platelets, leukocytes, vascular smooth muscle and endothelial cells with important implications in the regulation of inflammatory responses. It is now well established that stimulation of the A_{2A} AR in immune cells induces anti-inflammatory effects, mostly due to its ability to increase cAMP levels, which has strong immunosuppressive effects.[45] Stimulation of A_{2A} ARs inhibits neutrophil adherence to the endothelium, degranulation of activated neutrophils and monocytes, plus superoxide anion generation. A_{2A} ARs have been recently defined as sensors and terminators of proinflammatory activities. The strongest evidence for the key role of A_{2A} in inflammation derived by the elegant study of Ohta et al.[46] using mice deficient in A_{2A} ARs. In this model the lack of A_{2A} subtype leads to increased tissue inflammation and damage, thus suggesting a negative and nonredundant regulatory role for the A_{2A} AR. This model permits one to appreciate that adenosinergic regulation of immune cells is fundamental in normal physiological control of inflammation in vivo in spite of the fact that other G_s-protein-coupled receptors and cAMP elevating ligands are present such as catheco-

lamines, prostaglandins, dopamine, and histamine.[45] Interestingly, the A_{2A} AR has been demonstrated to be involved in promotion of wound healing and angiogenesis in healing wounds.[47,48]

Moreover, it plays an active role in the pathogenesis of dermal fibrosis, suggesting a role for antagonists as novel therapeutic approach in the treatment and prevention of dermal fibrosis in diseases such as scleroderma.[49]

1.1.2. A_{2A} Adenosine Receptor Antagonists

The discovery and development of potent and selective A_{2A} AR antagonists became, in the last 10 years, an attractive field of research to the discovery of new drugs for the treatment of neurodegenerative disorders, such as PD.

Different compounds have been deeply investigated as A_{2A} AR antagonists, which could be classified in two great families: nitrogen polyheterocyclic systems and styrylxanthine derivatives. Table 1 summarizes the examples of A_{2A} AR antagonists reported in this section.

Table 1. Affinity of AR antagonists at the A_1, A_{2A}, A_{2B} and A_3 ARs.

A_{2A} antagonists	°K_i values for ARs (nM)			
	A_1	A_{2A}	A_{2B}	A_3
1, CGS-15943	3.5[a]	0.15[b]	71[c]	50.8[d]
2, 8FBPTP	*3.3[51]	*1.2[51]	*ND	*ND
3, SCH-58261	549[55]	1.1[55]	>10000[55]	>10000[55]
4, SCH-63390	350[55]	1.2[55]	>10000[55]	>10000[55]
5, SCH-442416	1,111[54]	0.048[54]	>10000[54]	>10000[54]
7	253[53]	1.5[53]	ND[53]	>10000[53]
8	4,927[55]	4.63[55]	>10000[55]	>10000[55]
9	139[55]	140[55]	>10000[55]	>10000[55]
10	2,160[55]	0.22[55]	>10000[55]	>10000[55]
11, SCH-BT2	369[55]	3.8[55]	>10000[55]	>10000[55]
12	ND	0.94[59]	ND	ND
13, SCH-420814	ND	1.1[60]	ND	ND
14, KF-17837	>10000[62]	71[62]	ND	2500[62]
15, CSC	*28000[63]	*54[63]	ND	>10000[63]
16, BS-DMPX	*1200	*8.2	ND	ND
17, KW-6002	2830[66]	36[66]	1800[66]	>3000[66]
18, ST-1535	72	6.6	352	>1000

°Binding experiments at recombinant hA_1, A_{2A}, A_{2B} and A_3 ARs, unless noted; *Binding experiments at rat brain (A_1) and striatum (A_{2A}) ARs; ND not determined. [a] Ongini, E.; Dionisotti, S.; Gessi, S.; Irenius, E.; Fredholm, B. B. *Naunyn Schmiedebergs Arch. Phamacol.* **1999**, *359*, 7. [b] Varani, K.; Gessi, S.; Dionisotti, S.; Ongini, E.; Borea, P. A. *Br. J. Pharmacol.* **1998**, *123*, 1723. [c] de Zwart, M.; Vollinga, R.; Beukers, M. W.; Sleegers, D. F.; von Frijtag Drabbe Kuenzel, J. K.; de Groote, M.; Ijzerman, A. P. *Dru. Dev Res.* **1999**, *48*, 95. [d] Klotz, K.-N.; Hessling, J.; Hegler, J.; Owaman, C.; Kull, B.; Fredholm, B. B.; Lohse, M.J. *Naunyn Schmiedebergs Arch. Phamacol.* **1998**, *357*, 1.

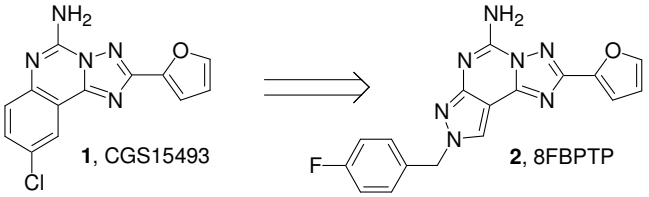
1.1.3. Medicinal Chemistry

Pyrazolo[4,3-e][1,2,4]triazolo[1,5-c]pyrimidines (PTPs)

9-Chloro-2-furan-2-yl-[1,2,4]triazolo[1,5-*c*]quinazolin-5-ylamine named CGS-15943 (**1**, Figure 2) represented the first potent but poorly selective antagonist for the A_{2A} AR subtype.[50] Bioisosteric replacement of the phenyl ring of CGS-15943 with an N^7-substituted pyrazole led to the first example of an adenosine antagonist displaying the pyrazolo-triazolo-pyrimidine (PTP) core named 8FBPTP (**2**,

8-(4-fluorobenzyl)-2-(2-furyl)-8*H*-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidin-5-amine, Figure 2).[51] Some structural features of this compound highlighted the essential requirements for the A_{2A} affinity, i.e., the furyl moiety and the free amino group at the 5- position. Starting from these observations Baraldi et al.[52,53] focused their interest on the pattern of substitution on the pyrazolo preserving the other structural elements.

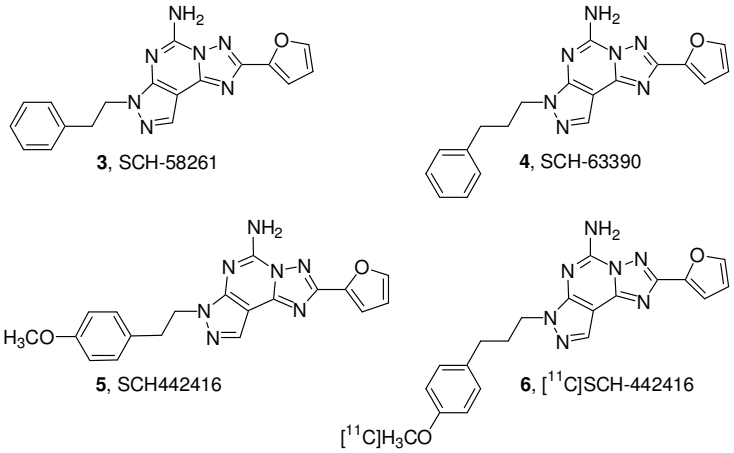
Figure 2. Structural relationships between CGS15943 and 8FBPTP (the first A_{2A} AR antagonist)



Several alkyl, aryl, and phenylalkyl substituents have been introduced at both the N^7 and the N^8 positions. The biological data derived from the molecules obtained, indicated that the best radicals were phenylalkyl chains and among these it was possible to discern the length of the spacer introduced between the phenyl ring and the pyrazolo nitrogen that was optimized in two or three carbon atoms. Two selected compounds of this family named SCH-58261 (**3**, Figure 3, 5-amino- 7-(*β*-phenylethyl)2-(2-furyl)-pyrazolo[4,3-*e*][1,2,4]triazolo-[1,5-*c*]pyrimidine) and SCH- 63390 (**4**, Figure 3, 5-amino-7-(3-phenylpropyl)2-(2-furyl)-pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine)[52,53] proved to be potent and selective A_{2A} AR antagonists both in rat and human models. It was also noted that the N^7 derivatives were more selective for the A_{2A} AR than the corresponding N^8 derivatives. [53]

From the family of SCH compounds, 5-amino-7-[3-(4-methoxyphenyl)propyl]-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine (SCH-442416, **5**, Figure 3) was selected for the development of a new positron emission tomography (PET) ligand, whose chemical structure allows an easy introduction of a methyl group by direct *O*-alkylation of the phenolic function with [^{11}C]CH_3I under alkaline conditions.[54] The aim of this study was to use [^{11}C]SCH 442416, (**6**, Figure 3) as a new ligand for the in vivo imaging of A_{2A} ARs using PET. The in vitro binding in the brain and periphery, the good signal-to-noise ratio observed between 5 and 15 min after injection, and the low occurrence of radioactive metabolites all suggested that [^{11}C]SCH-442416 was applicable as the first non-xanthine ligand suitable for the in vivo imaging of A_{2A} ARs using PET. In addition, the data obtained from the binding experiments showed a higher affinity of the title compound for hA_{2A} *vs* rat ARs (0.048 vs 0.5 nM).[54]

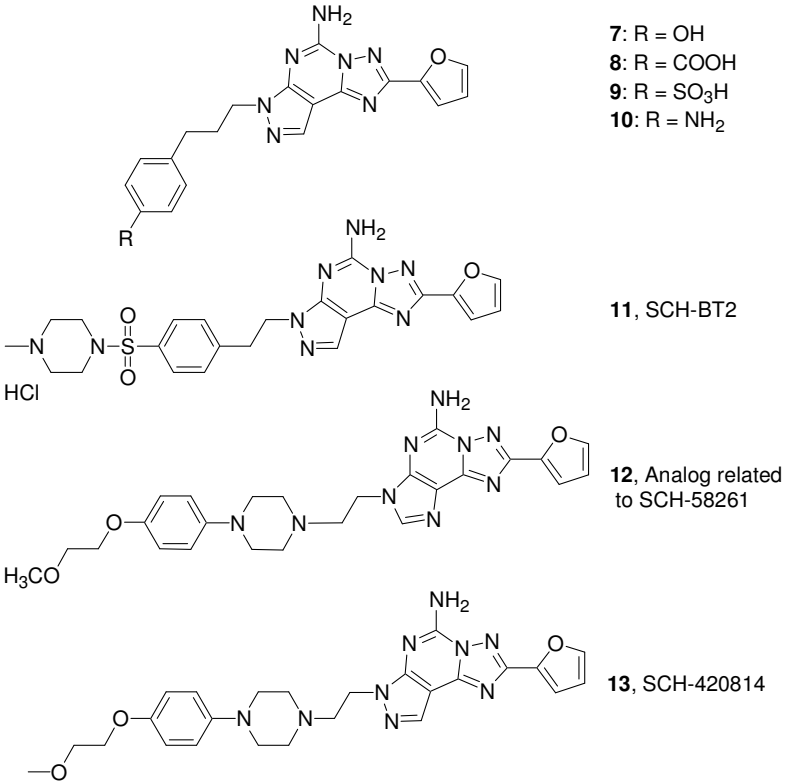
Figure 3. A_{2A} AR anatgonists (Pyrazolo-triazolo-pyrimidines).



Water-Soluble A_{2A} Adenosine Receptor Antagonists

The major restriction of the tricyclic adenosine antagonists was the low solubility in aqueous media that limited the pharmacological screening. Starting from this limit Baraldi et al.[53-55] reported a second generation of pyrazolo-triazolo-pyrimidines bearing oxygenated substituents on the phenylalkyl chains at the 7-position (compounds **7**-**10**). The most interesting compounds are depicted in Figure 4. Compound **7** displayed the best value of A_{2A} AR affinity indicating that the 4-hydroxy group positively influenced the receptor interaction but was not enough for reaching a good profile of water solubility.

Figure 4. Water-soluble A_{2A} AR antagonists.



A water-soluble analogue of SCH-58261, named SCH-BT2 (**11**, Figure 4), was prepared by introduction of a 4-methyl-piperazine-1-sulfonyl moiety at the para position of the phenyl ring. SCH-BT2 altered neither motor behaviour nor produced postural asymmetry by itself. However, when infused concomitantly with levodopa (L-DOPA, capable of inducing modest controlateral rotational behavior), SCH-BT2 significantly potentiated the number of contraversive rotations.[56-58]

Very recently, a novel series of 3-substituted 8-furyl-[1,2,4]-triazolo[1,5-*i*]purin-5-amine analogs related to SCH-58261 was reported as A_{2A} AR antagonists.[59] Most of the N^3- substituted aryl piperazine and piperidine analogs demonstrated in vivo A_{2A} receptor binding affinity and A_1 receptor selectivity profiles superior to those of SCH-58261. In these series compound **12**, Figure 4, displayed both superior in vitro and promising in vivo profiles.

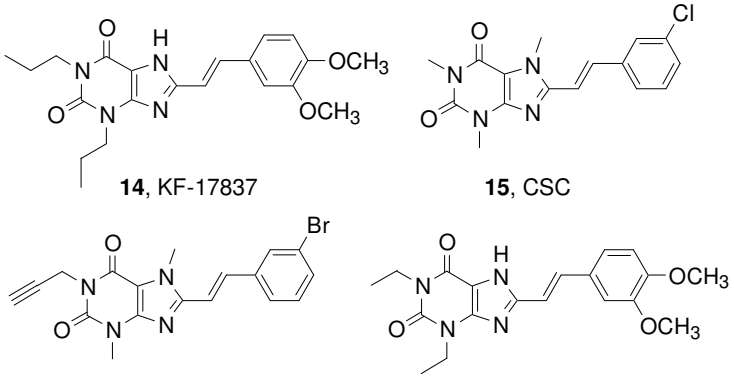
Neustadt et al.[60] recently reported the arylpiperazine derivatives of pyrazolo[4,3-*e*]triazolo[1,5-*c*]pyrimidines with antagonist activity on the A_{2A} AR. Among these derivatives, SCH-420814 (**13**, Figure 4) demonstrated potent antagonist activity at the A_{2A} AR. Structure-activity relationship studies revealed additional compounds incorporating an aryl-piperazine side chain that also showed potent oral activity in the haloperidol-induced catalepsy model in rats.

Styrylxanthines

1,3-Dipropyl-7-methyl-8-(3,4-dimethoxystyryl)-xanthine (**14**, KF-17837, Figure 5) was the first A_{2A} AR antagonist in this chemical class of compounds.[61,62] The 3-chlorostyrylcaffeine **15** (CSC, Figure 5) was identified as being less potent than KF17837 but with an increased selectivity vs A_1 AR subtype.[63,64]

Introduction of a propargyl at the 1- position in combination with the 8-styryl group in compound **16** (BS-DMPX, Figure 5) increased affinity to the A_{2A} AR with retention of selectivity.[65] 1,3-Diethyl-7-methyl-8-(3,4-dimethoxystyryl)- xanthine **17** (KW-6002, also named istradefylline, Figure 5) is an 8-styrylxanthine with high affinity for the rat striatal A_{2A} AR.[66] Due to its high affinity and selectivity, a radiolabeled derivative, [^{11}C]-KW-6002 labeled at the aromatic *O*-methyl position, was developed to be used in pharmacological testing to trace the A_{2A} ARs in vivo.[67,68]

Figure 5. A_{2A} AR antagonists (styrylxantine).



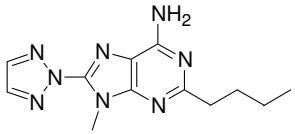
14, KF-17837 **15**, CSC

16, BS-DMPX **17**, KW-6002

9H-Purine derivatives

Minetti et al., on the basis of the molecular modeling of a number of potent AR antagonists, designed and synthesized a number of 2-alkyl-substituted purine derivatives as A_{2A} AR antagonists.[69] From them ST-1535 (2-*n*-butyl-9-methyl- 8-[1,2,3]triazol-2-yl-9*H*-purin-6-ylamine **18**, Figure 6), was the most interesting.

Figure 6. *9H*-Purine derivative



18, ST-1535

1.1.4. Clinical Development and Patents

PD is a progressive, incurable disorder with no definite preventive treatment, although drugs are available to alleviate the symptoms and/or slow down the progress of the disease. Current therapy is based on dopamine replacement therapy, the most common drug treatments being dopaminomimetic agents, including L-DOPA, a dopamine precursor, as well as direct or indirect dopamine receptor agonists. L-DOPA is the mainstay in the treatment of PD but, because of tolerance problems and a wide range of adverse reactions, including involuntary movements and vomiting, a strong demand for new therapies exists. Among the various strategies, A_{2A} AR blockers are considered a potential approach to treatment of the disease.[27, 70] KW-6002, an adenosine A_{2A} antagonist, is currently undergoing phase III clinical trials at Kyowa Hakko for the oral treatment of PD. As monotherapy or combination therapy with L-DOPA or dopamine agonists, it has been shown to improve the symptoms of the disease in a parkinsonian monkey model without increasing the incidence or severity of dopaminergic-related side effects or inducing or worsening dyskinesia. The company had been developing the drug for the treatment of depression, but phase II studies were discontinued. In mice and rats, KW-6002, like other A_{2A} AR antagonists, dose-dependently prevented reserpine and haloperidol-induced catalepsy, suggesting that it modulates dopaminergic neurotransmission.[71,72]

On the other hand, in D_2 receptor knockout mice, which are a model of motor impairment that resembles PD, blockade of A_{2A} ARs with KW-6002 rescued the behavioral parameters and reestablished altered enkephalin and substance P expression, suggesting a non-dopaminergic mechanism for the antiparkinsonian activity of KW-6002.[73] KW-6002 improved motor disability in experimental nonhuman primate parkinsonian models. Coadministration of KW-6002 and L-DOPA/benserazide potentiated the motor effects of levodopa (30%) without increasing the dyskinetic response.[74,75] Recently low doses of KW-6002 coadministered with low doses of L-DOPA attenuated the development of L-DOPA-induced dyskinesia as well as rotational responses to repeated L-DOPA in hemiparkinsonian mice. These results encourage consideration of future A_{2A} antagonist trials in PD that are aimed at reducing the development rather than the expression of dyskinesia.[76] Kyowa Hakko Kogyo has completed three phase III studies of KW-6002 in development for the treatment of PD (registration number [clinicaltrial.gov] 6002- EU-007, 6002-US-013, or 6002-US-018). KW-6002 has a specific antagonistic effect on the A_{2A} AR in the brain. The studies were conducted in PD patients with wearing-off phenomenon on treatment with L-DOPA alone or L-DOPA administered concomitantly with other PD medications. Two studies were conducted in North America and one study was conducted in 14 countries of the European Union and other regions. KW-6002 was administered for 12-16 weeks. The primary endpoint was the reduction in the percentage of awake time spent in the “off” state, which served as an indicator of the improvement in the wearing-off phenomenon. One of the North American studies revealed a statistically significant reduction in the percentage of awake time

spent in the off state. The other North American study and the trial conducted in the European Union/other regions did not demonstrate a significant reduction in percentage of awake time per day spent in the off state compared with placebo patients but showed a significant improvement or a trend toward improvement in one of the secondary endpoints, the motor function score, assessed using the Unified Parkinson's Disease Rating Scale subscore III. Kyowa Hakko intended to submit a new drug application to the Food and Drug Administration in the latter half of 2006. The long-term safety of KW-6002 in patients who have completed 6002-EU-007, 6002-US-013, or 6002-US-018 studies has been assessed in an extension phase III study started in October 2004 (registration number [clinicaltrial.gov] 6002-INT-001). Other open-label phase III studies of the continued safety of KW- 6002 for patients who completed the prior double-blind study 6002-INT-001 started in March 2005 (registration number [clinicaltrial.gov] 13711A) and in October 2005 (registration number [clinicaltrial.gov] 6002-US-025) and are currently recruiting patients. Phase II trials are also under way by the company for the treatment of restless legs syndrome (RLS).

KW-6002 has been patented as a therapeutic agent for behavioral disorders,[77] anxiety[78] and higher brain dysfunction,[79] in medicinal composition with dopaminergic agents, monoamine oxidase-B (MAO-B) inhibitors, or catechol-*O*-methyltransferase (COMT) inhibitors for PD, RLS, and attention deficit hyperactivity disorder,[80] in medicinal composition with antidepressant agent such as the serotonin and/or norepinephrine reuptake inhibitors for depression[81] and for disease accompanied by chronic muscle/skeleton pain[82] and drug dependence.[83]

SCH-420814 is a selective, orally active A_{2A} AR antagonist discovered by scientists at Schering-Plough and currently under phase II investigation for PD.[60] It reversed haloperidol-induced catalepsy in rats and potentiated L-DOPA induced turning behaviour in neurotoxin 6-hydroxydopamine (6-OHDA)-lesioned rats. Also, it was effective in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) monkey model of PD and several rodent models of depression. Pharmacokinetic profiling revealed oral availability of 57%, 41% and 4% in rats, dogs, and cynomolgus monkeys, respectively. SCH-420814 and SCH-412348 were tested in vivo in rats treated with the A_{2A} agonist CGS-21680, which reduces locomotion. At doses ranging from 0.1 to 1 mg/kg, both compounds dose-dependently reversed the effects of the A_{2A} agonist 2-*p*-(2-carboxyethyl)phenethylamino-5'-*N*-ethylcarboxamidoadenosine (CGS 21680). They also potentiated L-DOPA-induced turning behaviour in 6-OHDA-lesioned rats at the same dose ranges. These results suggest these agents may have potential in PD as well as in other conditions associated with reduced dopaminergic activity.[60]

Both SCH-420814 and SCH-412348 have been patented for PD[84] and other involuntary movement disorders.[85] Moreover, SCH 420814 has been patented as a method for treating anxiety disorders including panic disorder, agoraphobia, obsessive-compulsive disorders, social phobia, and posttraumatic stress disorder.[78]

ST-1535 is an A_{2A} AR antagonist in preclinical phase at Sigma-Tau. The compound displayed A_{2A} AR antagonist activity in vivo as it increased spontaneous motor activity in mice and was able to antagonize haloperidol-induced catalepsy at a dose of 10 mg/kg. It also exhibited antidepressant activity in the mouse forced swim test. Poten-

tially useful for the treatment of PD and other motor disorders, it was selected for in vivo characterization in animal models.[86] ST- 1535 (10, 20 and 40 mg/kg, per os (po)) when administered alone to MPTP-treated common marmosets produced a dose-related increase in locomotor activity and tended to reverse motor disability. Treatment with a threshold dose of L-DOPA (2.5 mg/kg, po) produced an increase in locomotor activity and again tended to reverse motor disability.[87,88] ST-1535, at oral doses of 5 and 10 mg/kg, antagonizes catalepsy induced by intracerebroventricular administration of CGS 21680 in mice. Oral ST-1535, at 1.25 and 2.5 mg/kg, potentiates L-DOPA effects in reducing haloperidol-induced catalepsy.[89] ST-1535 potentiates the effects of a threshold dose of L-DOPA in unilaterally 6-OHDA-lesioned rats.[88]

Subchronic (18 days, twice a day) ST-1535 (20 mg/kg ip) + L-DOPA (3 mg/kg ip) did not induce sensitization to turning behavior or abnormal involuntary movements during the course of treatment, indicating a low dyskinetic potential of the drug; acute administration of ST-1535 (20 mg/kg ip) proved capable of reducing jaw tremors in a tacrine model of Parkinson's disease tremor, thus representing a potential new compound, with long-lasting activity, for the treatment of PD.[90] ST-1535 has been patented for the treatment of PD and other motor disorders, Alzheimer's disease, Huntington's disease, Wilson's disease, and neurodegenerative conditions including cerebral ischemia.[91]

1.2. A_3 Adenosine Receptor

1.2.1. Pharmacology

The A_3 AR is the only adenosine subtype cloned before its pharmacologic identification.[92] It was originally isolated as an orphan receptor from rat testis, having 40% sequence homology with canine A_1 and A_{2A} subtypes.[93] Homologs of the rat striatal A_3 AR have been cloned from sheep and human. Interspecies differences in A_3 AR structure are large, showing the rat A_3 AR only 74% sequence homology with sheep and human.

Table 2 . Distribution and therapeutical potential of A_3 AR

Organ and Tissue [101]	A_3 Agonist	A_3 Antagonist
Heart	Protection from myocardic ischemia, ischemic preconditioning.	
Brain	Cerebrovascular protection (chronic treatment), stroke prvenction.	Cerebro-protective (acute treatment).
Eyes		Therapy of glaucoma
Inflammatory cells	Antiasthmatic, antiinflammatory, immunosuppressive	Antiasthmatic, antialergic.
Tumor cells	Combination therapy with classical chemoterapy agents	Anticancer[102]

A_3 ARs activation inhibits adenylyl cyclase activity by coupling with G_i proteins.[94] In the rat mast cell line RBL- 2H3 and rat brain, A_3 ARs stimulation activate phospholipase C through G_q proteins.[95,96] The A_3

AR is widely distributed with its mRNA expressed in testis, lung, kidneys, placenta, heart, brain, spleen, liver, uterus, bladder, jejunum, proximal colon and eye of rat, sheep and humans (Table 2).[92,97-100]

A dual role of A_3 ARs has been reported in the brain. In particular, it seems that chronic preischemic administration of the agonist IB-MECA induces a significant neuronal protection and reduction of the subsequent mortality, while acute administration of the drug results in a pronounced worsening of neuronal damage and postischemic mortality.

Mice with functional deletions of the A_3 AR (A_3 AR-/-) reveal a number of CNS functions where the A_3 ARs play a role, including nociception, locomotion, behavioral depression and neuroprotection. Consistent with previous reports of the neuroprotective actions of A_3 AR agonists, A_3 AR-/- mice show an increase in neurodegeneration in response to repeated episodes of hypoxia suggesting the possible use of A_3 agonists in the treatment of ischemic, degenerative conditions of the CNS.[103] To date, much evidence supports that activation of A_3 ARs is crucial for cardioprotection during and following ischemiareperfusion and it is likely that a consistent part of the cardioprotective effects exerted by adenosine, once largely attributed to the A_1 AR, may now in part be ascribed to A_3 AR activation.[104,105] The molecular mechanism of A_3 AR cardioprotection has been attributed to regulation of ATPsensitive potassium channels. The cardioprotective effects of A_3 ARs were also detected in mice overexpressing low levels of A_3 ARs without detectable adverse effects, while higher levels of A_3 expression lead to the development of a dilated cardiomyopathy.[106] Similar data were observed in the case of A_1 ARs overexpression.[107]

In addition to reducing injury in myocardial and vascular tissues, other beneficial actions at the inflammatory level have been attributed to the A_3 subtype. For example, A_3 ARs are expressed in human neutrophils where they are involved together with A_{2A} in the reduction of superoxide anion generation[108] and have been implicated in suppression of tumor necrosis factor alpha (TNFR) release induced by endotoxin from human monocytes.[109] Moreover, A_3 activation seems to inhibit degranulation and superoxide anion production in human eosinophils.[110] Transcript levels for the A_3 subtype are elevated in the lungs of asthma and COPD patients, where expression is localized to eosinophilic infiltrates. Similar evidence was observed in the lungs of ADA-deficient mice that exhibited adenosine-mediated lung disease. Treatment of ADA-deficient mice with MRS 1523, a selective A_3 antagonist, prevented airway eosinophilia and mucus production. These results are in contrast to experiments performed in human eosinophils ex vivo, where chemotaxis was reduced by A_3 AR activation, suggesting that significant differences exist between the impact of A_3 signaling on eosinophil migration ex vivo and in the whole animal.[111] The functional role of the A_3 subtype in the pathogenesis of asthma remains controversial and differences in the pharmacology of A_3 subtype from different species render it difficult to understand whether an A_3 AR agonist or antagonist is better for use in antiasthmatic therapies. A very interesting area of application of A_3 ligands concerns cancer therapies. The possibility that A_3 AR plays a role in the development of cancer has aroused considerable interest in recent years.[112] A_3 subtype has been described in the regulation of the cell cycle and both pro- and antiapoptotic effects have been reported depending on the level of receptor activation.[113-116]

A_3 activation has been demonstrated to be involved in inhibition of tumor growth both in vitro and in vivo, leading to the development of A_3 agonists in clinical trials for colon carcinoma. The molecular mechanisms involved in the anticancer effects induced by A_3 agonists included regulation of the WNT pathway.[117] On the other hand, it has been reported that adenosine upregulates HIF-1R protein expression and vascular endothelial growth factor (VEGF) protein accumulation by activating A_3 AR subtype in tumoral cells, suggesting a role for A_3 subtype in the regulation of angiogenesis.[118] Overexpression of the A_3 subtype has been demonstrated in colon cancer tissues obtained from patients undergoing surgery in comparison to normal mucosa. Overexpression in tissues was also reflected at the level of peripheral blood cells, rendering this adenosine subtype a possible marker for cancer detection.[119] Similar data were also found in the case of arthritis, where A_3 activation shows beneficial effects by suppression of TNFR production.[120,121] Adenosine receptors have been implicated in many ocular and systemic ischemic diseases (e.g., retinal ischemia). The A_3 KO mouse showed lower intracellular pressure, suggesting a role for A_3 antagonists in the therapy of glaucoma.[122,123]

1.2.2. A_3 Adenosine Receptor Antagonists

A_3-selective AR antagonists have been postulated as novel anti-inflammatory and antiallergic agents; recent studies also indicated a possible employment of these derivatives as antitumor agents. In recent years many efforts have been made to search for potent and selective hA_3 AR antagonists (Table 3).

Table 3

A_3 antagonists	K_i[a] values for ARs (nM)			
	A_1	A_{2A}	A_{2B}	A_3
19, PSB-10[126]	1700[b]	2700[b]	ND	0.43
20, KF-26777[127]	1800	470	620	0.20
21[128]	>1000	>1000	>1000	0.80
22, OT-7999[129]	°°>10000	°°>10000	°°>10000	0.95
23, MRS1097[133]	5930[c]	4770[c]	ND[e]	108
24, MRS1191[133]	40100[c]	<10%[c]	ND[e]	31.4
25, MRS1334[134]	>100[c]	>100[c]	ND[e]	2.69
26, MRS1523[135]	15600[c]	2050[c]	ND[e]	18.9
27, MRE-3008-F20[141]	1200	141	2100	0.82
28, MRE-3005-F20[143]	250	60	200	0.04
31, VUF-5574[148]	>10000[c]	>10000[c]	ND[e]	4.0

[a] Binding experiments at recombinant hA_1, A_{2A}, A_{2B} and A_3 ARs, unless noted; [b] Binding experiments at human cortex (A_1), striatum (A_{2A}) ARs.; [c] Binding experiments at rat cortex (A_1), striatum (A_{2A}) ARs.; [d] IC_{50} values; [e] ND = not determined.

1.2.3. Medicinal Chemistry

Xanthines

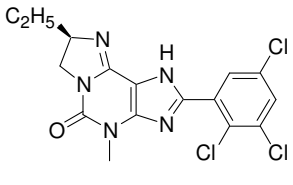
Natural antagonists for ARs, such as caffeine and theophylline, show in general low affinity for the A_3 AR subtype.[124] Different positions of the xanthine core have been modified with the aim of improving A_3 AR affinity. A series of tricyclic imidazo[2,1-*i*]purinones and ring-enlarged analogues derived from xanthine derivatives has been prepared as AR antagonists. In comparison with xanthines, the tricyclic compounds exhibit increased water solubility due to a basic nitrogen atom, which can be protonated under physiological conditions.[125] Among this series PSB-10, 8(*R*)-ethyl-4-methyl-2-(2,3,5-trichlorophenyl)-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-*i*]purin-5-one (**19**, Figure 7), is a high-affinity ligand for A_3 ARs (hA_3K_i = 0.43 nM) with high selectivity over hA_1 and hA_{2A} ARs (K_i = 1700 and 2700 nM, respectively). The compound showed inverse agonist activity in binding

studies in CHO cells expressing recombinant hA_3 ARs (IC_{50} = 4 nM).[126] Another similar compound is 2-(4-bromophenyl)-7,8-dihydro-4-propyl-1*H*-imidazo[2,1-*i*]purin-5(4*H*) one, also named KF-26777 (**20**, Figure 7), endowed with subnanomolar affinity to hA_3 ARs (K_i = 0.20 nM) and high selectivity over A_1, A_{2A}, and A_{2B} ARs (9000-, 23500- and 31000-fold, respectively). It concentration-dependently inhibited 2-chloro-N^6-(3-iodobenzyl)-*N*-methyl-5'-carbamoyl-adenosine (Cl-IB-MECA) -induced [^{35}S]guanosine 5'-*O*-(3-thiotriphosphate) ([^{35}S]-GTPγS) binding to human embryonic kidney 293 cells (HEK293) (IC_{50} = 270 nM) and enhanced intracellular Ca^{2+} concentration in human promyelocytic cells (K_B = 0.42 nM). This agent was indicated for potential interest for treatment of brain ischemia and inflammatory diseases such as asthma.[127]

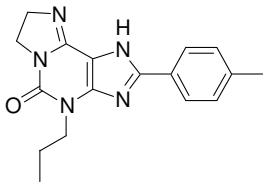
The discovery of 1-benzyl-3-propyl-1*H*,8*H*-imidazo[2,1-*f*]purine-2,4-diones by cyclization between the 7- and 8- positions of the xanthine core lead to **21** (Figure 7), a highly potent and selective A_3 adenosine receptor antagonist.[128] This compound shows a subnanomolar affinity (hA_3K_i = 0.8 nM) toward the desired receptor target with a noteworthy selectivity versus the other adenosine receptors subtypes.

In this field of research the triazolopurine derivatives in which the xanthine structure is extended are also reported. One example is OT-7999 (**22**, Figure 7), which proved to be a potent and selective hA_3 AR ligand. In receptor binding assays, OT-7999 displayed high affinity for the A_3 AR (K_i = 0.95 nM) and >10500-fold selectivity relative to other AR subtypes. Significant reductions in intraocular pressure were obtained in cynomolgus monkeys at 2-4 h following topical application to the eye of OT-7999 (500 mcg).[129,130]

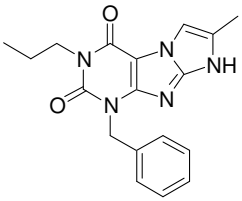
Figure 7. A_3 AR antagonists (xanthines).



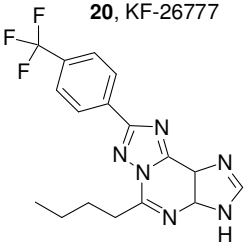
19, PSB-10



20, KF-26777



21

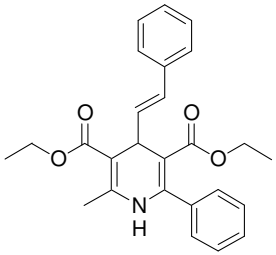


22, OT-7999

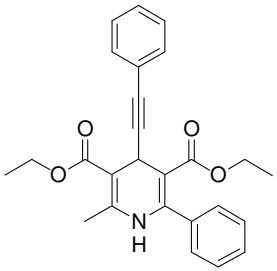
1,4-Dihydropyridine and Pyridines

Starting from the experimental observations that 1,4- dihydropyridines bind A_1 adenosine receptors in the rat brain,[131,132] Jacobson et al. used the 1,4-dihydropyridine nucleus as a template for probing the SAR profile at the A_3 AR subtype.[133] SAR studies of adenosine receptor antagonists indicated that sterically bulky groups are well tolerated at the 4-, 5-, and 6-positions. The combination of substitutions led to the discovery of MRS 1097 (2-methyl-6-phenyl-4-styryl-1,4-dihydro-pyridine-3,5-dicarboxylic acid diethyl ester, **23**, Figure 8), MRS 1191, (2-methyl-6-phenyl-4-phenylethynyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid 5-benzyl ester, **24**, Figure 8), and MRS 1334 (2-methyl-6-phenyl-4-phenylethynyl-1,4-dihydro-pyridine-3,5-dicarboxylic acid 3-ethyl ester 5-(4-nitro-benzyl) ester, **25**, Figure 8) as the first A_3 antagonists related to 1,4-dihyropyridines.

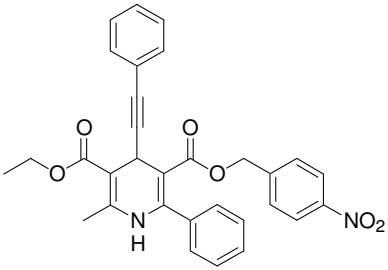
Figure 8. Dihydropyridine and Pyridine derivatives as A_3 AR antagonists



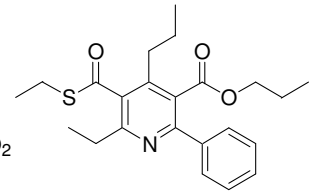
23, MRS 1097



24, MRS 1191



25, MRS 1334



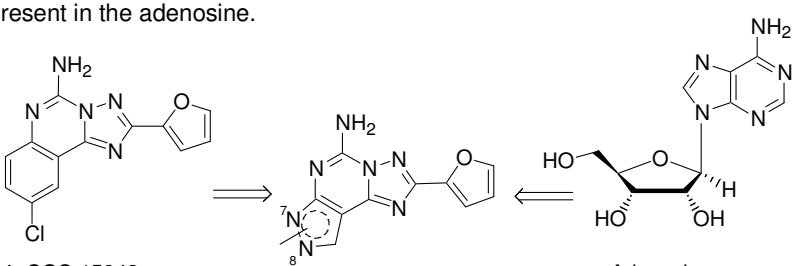
26, MRS 1523

In this study, they also synthesized pyridine derivatives[133,134] through oxidation of the corresponding 1,4-dihydropyridine. In this class of compounds, small groups at the 4-position were found to be essential such as in MRS 1523 (6-ethyl-5-ethylsulfanylcarbonyl-2-phenyl-4-propyl-nicotinic acid propyl ester, **26**, Figure 8), which showed favourable affinity at the hA_3 AR subtype. Comparing the structural requirements for the two related classes of compounds indicated that bulky substituents at the 4- position and a 5-benzyl ester, which are affinity enhancing in dihydropyridines, are not well tolerated in the pyridine series for A_3 receptor binding. At other positions, structural parallels occur between corresponding dihydropyridine and pyridine analogues.[135]

Pyrazolo-triazolo-pyrimidines (PTPs)

The pyrazolo-triazolo-pyrimidine nucleus, due to its strong structural correlation with the nonselective antagonists CGS-15943, **1**, and the adenine nucleus present in the endogenous modulator adenosine (Figure 9), has been strongly investigated in the past decade as a prototypical template for adenosine antagonists.

Figure 9. Structural correlation with CGS-15943 and the adenine nucleus present in the adenosine.



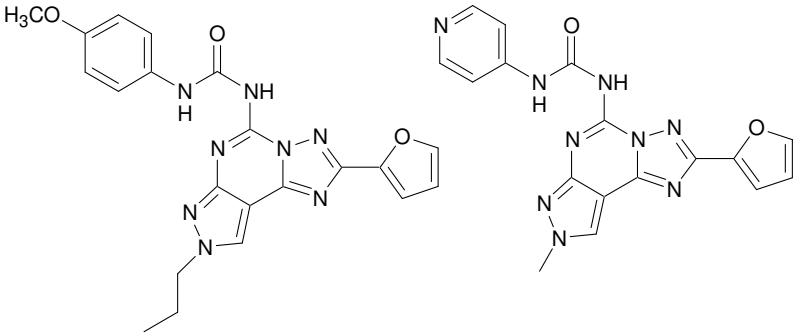
1, CGS-15943 Adenosine

The triazolo-quinazoline derivative CGS-15943 represented the starting point for searching for new potent and selective hA_3 adenosine receptor antagonists.

MRS-1220, a 5-*N*-phenylacetyl derivative of CGS-15943, in receptor binding studies displayed K_i values of 305 ± 51, 52.0 ± 8.8 and 0.65 ± 0.25 nM for rat A_1, A_{2A}, and hA_3 receptors, respectively, being 470- and 80-fold selective for hA_3 ARs vs rat A_1 and A_{2A} ARs, respectively. MRS-1220 also antagonized the effects of an A_3 agonist in functional assays.[136,137]

An innovative series of tricyclic compounds (MRE series) reported by Baraldi's group represented new selective A_3 AR antagonists. In this class attention was focused on the N^8 patterns of substitution due to the quite complete inactivity of the N^7-substituted derivatives at the hA_3 subtype (e.g., SCH-58261).

Figure 10. A_3 AR antagonists (pyrazolo-triazolo-pyrimidines).



27, MRE-3008-F20 **28**, MRE-3005-F20

MRE-3008-F20 (**27**, Figure 10), one of several high affinity antagonists, is an A_3 AR ligand (K_i = 0.29 nM against 4-aminobenzyl-5'-*N*-methylcarboxamidoadenosine ([^{125}I]-AB-MECA) binding to human receptors expressed in HEK293 cells) with high selectivity over rat A_1 and A_{2A} ARs (K_i > 10000 and 1993 nM, respectively) as well as hA_1 and hA_{2A} ARs (K_i = 1197 and 141 nM, respectively).[138] The compound showed antagonist activity in a functional assay being capable of blocking the effect of IB-MECA on cAMP production in CHO cells (IC_{50} = 4.5 nM).[139-141] The tritium-labeled compound was able to bind hA_3 ARs expressed in CHO cells with a K_D value of 0.82 nM and a B_{max} value of 297 fmol/mg protein and represents the first high-affinity, selective radiolabeled antagonist for this subtype resulting in a useful tool for characterization of A_3 ARs in both normal and pathological conditions.[142] The isosteric replacement of the phenyl with a 4-pyridyl moiety provided higher hydrosolubility and led to the first water-soluble hA_3 antagonist (MRE-3005-F20, **28**, Figure 10) which is an ideal candidate for the pharmacological and clinical investigations of the hA_3 AR subtype.[143]

In molecular modeling studies reported by Moro et al. on pyrazolo-triazolo-pyrimidines, a combined target-based and ligand-based drug design has been carried out to define a novel pharmacophore model for the hA_3R antagonists. A high-throughput docking strategy has been applied on the pyrazolo-triazolo-pyrimidine series. All low-energy docked conformations have been superimposed and used to characterize the common features crucial to the recognition process. A novel target-based pharmacophore model has been proposed for human A_3 AR antagonists. A CoMFA (comparative molecular field analysis) approach has been used as an alternative scoring function for prediction of ligand receptor binding affinity. The new target-based pharmacophore model was coherent with the structure-activity relationships collected on the pyrazolo-triazolo-pyrimidine analogues.[144,145]

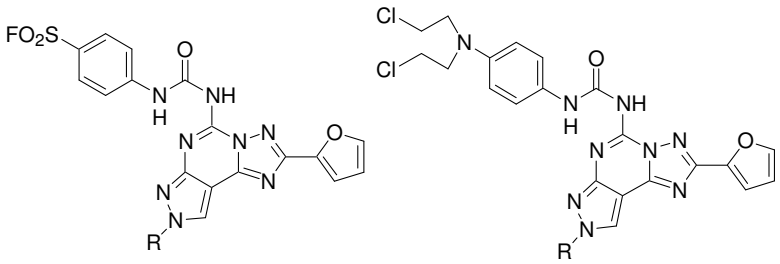
Moreover, very recently Botta, Martinelli and Baraldi et al. performed a pharmacophoric study using the software Catalyst, which yielded three different common feature hypotheses for antagonists of the hA_3R. The three pharmacophores referred to a recurring scheme consisting of three hydrophobic interactions lying at the vertexes of a triangle. They seemed particularly good in handling pyrazolo-triazolo-pyrimidine derivatives.[146] These results confirm the importance of this tricycle as the most potent class of A_3 AR antagonists.

Fluorosulfonyl- and Bis(β-chloroethyl)amino-phenylamino-pyrazolo[4,3-e]1,2,4-triazolo[1,5-c]pyrimidines

Synthesis of irreversible A_3 antagonists was realized to provide useful tools for structure-activity studies. Electrophilic groups, specifically sulfonyl fluoride and nitrogen mustard (bis-(β-chloroethyl)amino)

moieties, have been incorporated at the 4-position of the aryl urea group (compunds **29** and **30**, Figure 11).[147]

Figure 11. A_3 irreversible antagonists.



29 **30**

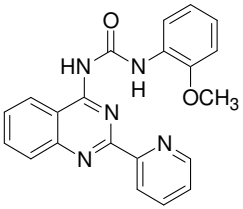
Compounds containing a fluorosulfonyl moiety proved to be irreversible antagonists at the hA_3 AR (at 100 nM, 79% of inhibition), while the corresponding nitrogen mustard derivatives were unable to covalently bind this receptor subtype. This difference in the receptor interaction between the **29** and **30** series has been explained on the basis of chemical reactivity of the two different groups: the $-SO_2F$ group is highly reactive versus all nucleophilic functions, while the nitrogen mustard reacts only with amino functions.

Isoquinoline and Quinazoline Urea Analogues as Antagonists for the Human Adenosine A_3 Receptor

A structure-affinity analysis reported by IJzerman et al.[148] indicated that at the 2- position of the quinazoline ring or the equivalent 3-position of the isoquinoline ring a phenyl or heteroaryl substituent increased the A_3 AR affinity in comparison to unsubstituted or aliphatic derivatives. Combination of the optimal substituents in the two series led to the potent hA_3 AR antagonist *N*-(2-methoxyphenyl)-*N'*-[2-(3-

pyridyl)quinazolin-4-yl]urea (VUF5574, **31**, Figure 12) with a K_i value of 4 nM and a selectivity of at least 2500- fold vs A_1 and A_{2A} ARs. In an in vitro functional assay the compound competitively antagonized the inhibition of cAMP production induced by the adenosine agonist NECA in CHO cells expressing hA_3 ARs with a pA_2 value of 8.1.[148]

Figure 12. Quinazoline urea derivative



31, VUF-5574

1.2.4. Clinical Development and Patents

At the moment there are not A_3 antagonists in clinical phases. However, in light of the plethora of biological effects attributed to A_3 ARs, substantial efforts in medicinal chemistry have been addressed to develop antagonists for the A_3 subtype.[149] As a result a number of molecules are in biological testing as therapeutic agents for asthma and COPD, glaucoma, cancer and stroke.

Use of A_3 antagonists has been patented for inhibition of tumor growth.[150] The pre- or coadministration of pharmaceutical compositions comprising high-affinity adenosine A_3 receptor antagonists, such as MRE-3008-F20, has been patented for synergistically accentuating the response to chemotherapy consisting of taxane (e.g., paclitaxel), vinca alkaloid (e.g., vincristine), camptothecin (e.g., irinotecan), or antibiotic (e.g., doxorubicin) treatment.[151] The claim further embodies the prevention of multidrug resistance (MDR) and tar-

geted tumors include those expressing MDRassociated protein (MRP), A_3 ARs, or P-glycoprotein, as found in leukemia, melanoma, and carcinoma of the pancreas, ovary, and lung. Moreover, MRE-3008-F20 has been also patented for the treatment of cardiac hypoxia, allergic diseases, cerebral ischemia, and cancers with high concentrations of A_3 ARs.[152]

Other patents of A_3 antagonists also concern their use for cognitive disorders, multiple sclerosis, neurodegeneration, PD, stroke, traumatic brain injury,[153] asthma and COPD,[154-157] glaucoma[158] and arthritis.[159]

Chapter 2

Design and Synthesis

2. Design and Synthesis

2.1. First Project: *Pyrrolo[3,4-e][1,2,4]triazolo[1,5-c]pyrimidines*

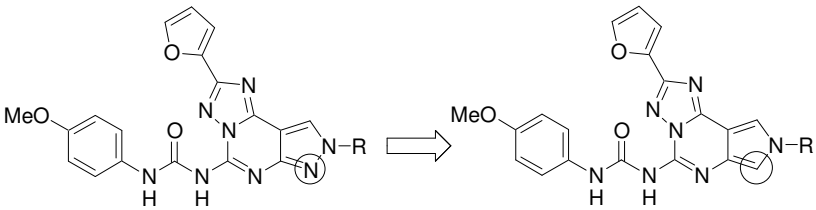
In the last 10 years the pyrazolo-triazolo-pyrimidine (PTP) nucleus has distinguished as an attractive key intermediate for obtaining adenosine receptor antagonists due to its strong structural correlation with the non-selective AR antagonist CGS15943 (**1**, Fig. 2). A wide number of compounds originated from the structure-activity optimization work based on the systematic substitution of the N^5, N^7, N^8, C^2 or C^9.[160]

According to the literature results, a structure-activity relationship (SAR) profile of the pyrazolo-triazolo-pyrimidines could be delineated.

The furan ring at the 2- position of the nucleus is fundamental for the affinity toward all four adenosine receptor subtypes.

The presence of the free amino group at the 5- position and an arylalkyl chain at the N^7 position of the PTPs are essential for both affinity and selectivity at the A_{2A} AR, whereas the concurrent presence of the 4-methoxy-phenyl carbamoyl moiety and small alkyl chain (such as methyl or ethyl) at the 5- and 8- position, respectively, play an important role in determining potency and selectivity at human A_3 AR.

Figure 13.

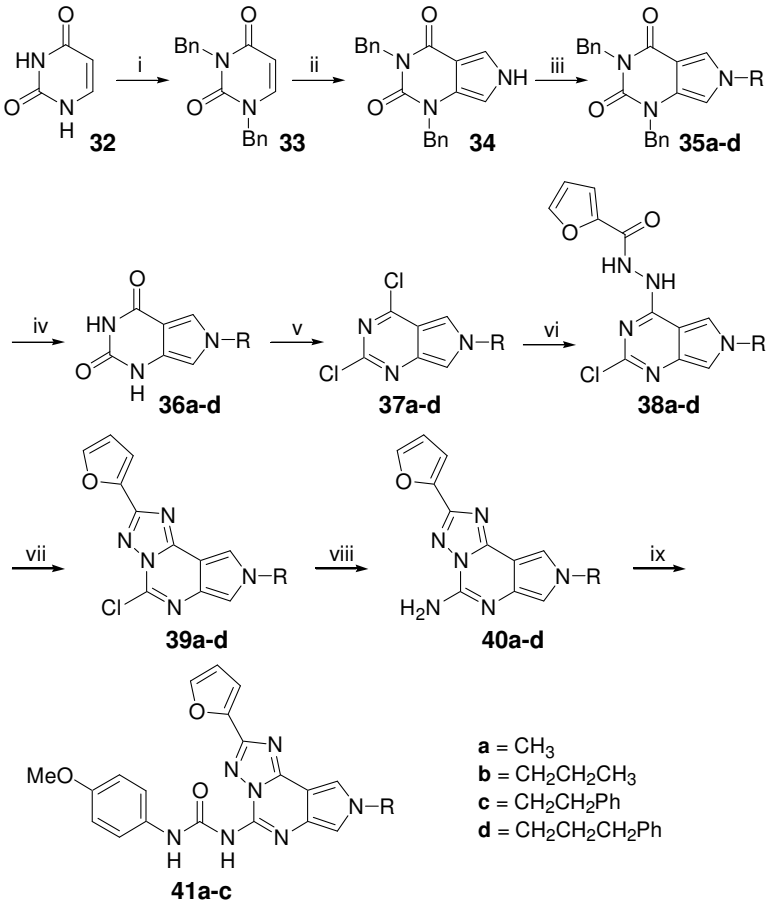


Previous series New project

In order to identify a new series of A_3 AR antagonists and with the aim to better investigate the role of the nitrogen at the 7- position on the interaction with ARs, we performed a synthetic strategy for the preparation of the pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine nucleus which can be considered the 7-deaza-analogue of the pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine core (Fig. 13).

As depicted in scheme **1**, commercially available uracil (**32**) has been employed as starting material. The 1,3-dibenzyl-1*H*-pyrimidine-2,4-dione **33** was obtained via a bis-alkylation with benzylbromide.[161] Treatment of **33** with *p*-toluenesulphonylmethyl isocyanide (TosMIC) in presence of 60% NaH gave the desired 1,3-dibenzyl-1,6-dihydropyrrolo[3,4-*d*]pyrimidine-2,4-dione **34** which was then alkylated with the appropriate alkyl halide to furnish 6-alkyl-derivatives (**35a-d**). The debenzylation at the 1- and 3- positions with $AlCl_3$ in anhydrous toluene provided derivatives **36a-d**. The 2,4-dichloro-6-alkyl-6*H*-pyrrolo[3,4-*d*]pyrimidines **37a-d** were obtained by treatment of **36a-d** with $POCl_3$ and DBU. Selective substitution of the chlorine atom at the 4-position with furoic acid hydrazide followed by a Dimroth rearrangement led to the desired pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine nucleus (**39a-d**). Compounds **40a-d** were obtained treating derivatives **39a-d** with a solution of ethanol saturated with ammonia. These were converted into the corresponding 4-methoxyphenyl urea derivatives **41a-c** by reaction with 4-methoxyphenylisocyanate.

Scheme 1

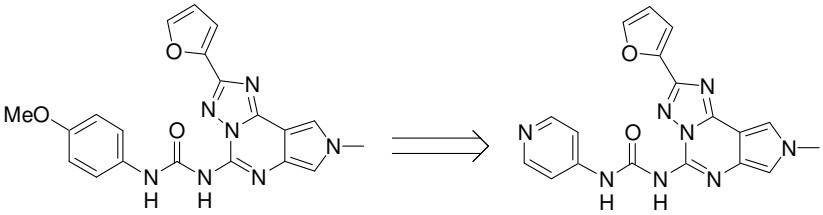


REAGENTS: i) NaOH 10%, tetrabutylammonium bromide, benzylbromide, CH_2Cl_2, 80 °C, 18h; ii) NaH, TosMIC, Et_2O, DMSO, rt, 5h; iii) K_2CO_3, RX, DMF, 40-80 °C, 4h; iv) $AlCl_3$, toluene, 40 °C, 1h; v) $POCl_3$, DBU, 50 °C, 4h; vi) 2-Furoic acid hydrazide, TEA, 1,4-dioxane, rfx, 5h; vii) HMDS, BSA, 120 °C, 18h; viii) EtOH sat. ammonia sol., 60 °C, 18h; ix) 4-OCH_3-phenyl isocyanate, THF, 50 °C, 18h.

A relevant problem of the pyrazolo-triazolo-pyrimidines was the typically low water-solubility which could limit their employment as pharmacological and diagnostic tools.

Compound 5-{[(4-methoxy-phenyl)carbamoyl]amino}-(2-furan-2-yl)-8-methyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (**41a**, hA_1K_i = 800 nm, $hA_{2A}K_i$ = 500 nm, $hA_{2B}IC_{50}$ = 838 nm and hA_3K_i = 15 nm) is characterized by good binding data but, unfortunately, by low water-solubility, so we tried to improve the hydrophicity of this compound by introducing 4-pyridil moiety on the side chain at the 5- position (Fig.14), accordingly to a similar efficient strategy previously reported.[143]

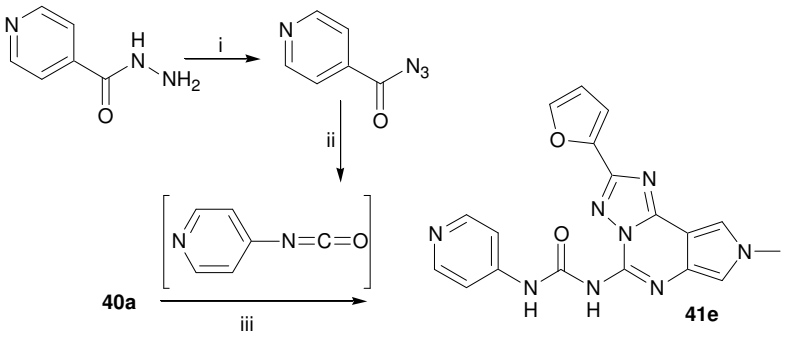
Figure 14.



41a **41e**

Because of the reactivity and instability of the 4-pyridil isocyanate, this intermediate was prepared as depicted in scheme 2 , starting from the commercially available nicotinic acid hydrazide, which after reaction with sodium nitrite under acid conditions afforded the corresponding acyl azide. The latter was heated at reflux for 2 hours in dry toluene to give the isocyanate upon Curtius rearrangement. The crude isocyanate was heated for 5 hours in dry toluene with compound **40a** to give the desired urea derivative **41e**.[143]

Scheme 2

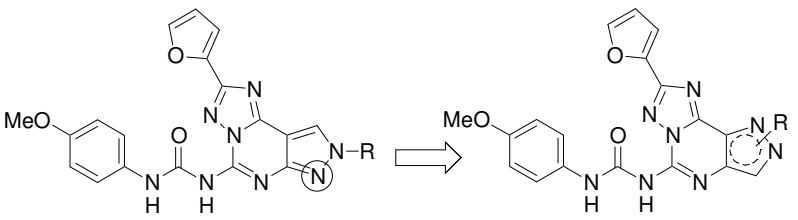


REAGENTS: i) $NaNO_2$, HCl acq., 1h, 0 °C; ii) Toluene, 2h, rfx; iii) Toluene, 5h, 100 °C.

Pyrazolo[3,4-e][1,2,4]triazolo[1,5-c]pyrimidines

In order to complete the SAR studies on this class of compounds, we decided to synthesis a novel series of pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives which can be considered the structural isomers of the parent pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine derivatives (Fig. 15).

Figure 15



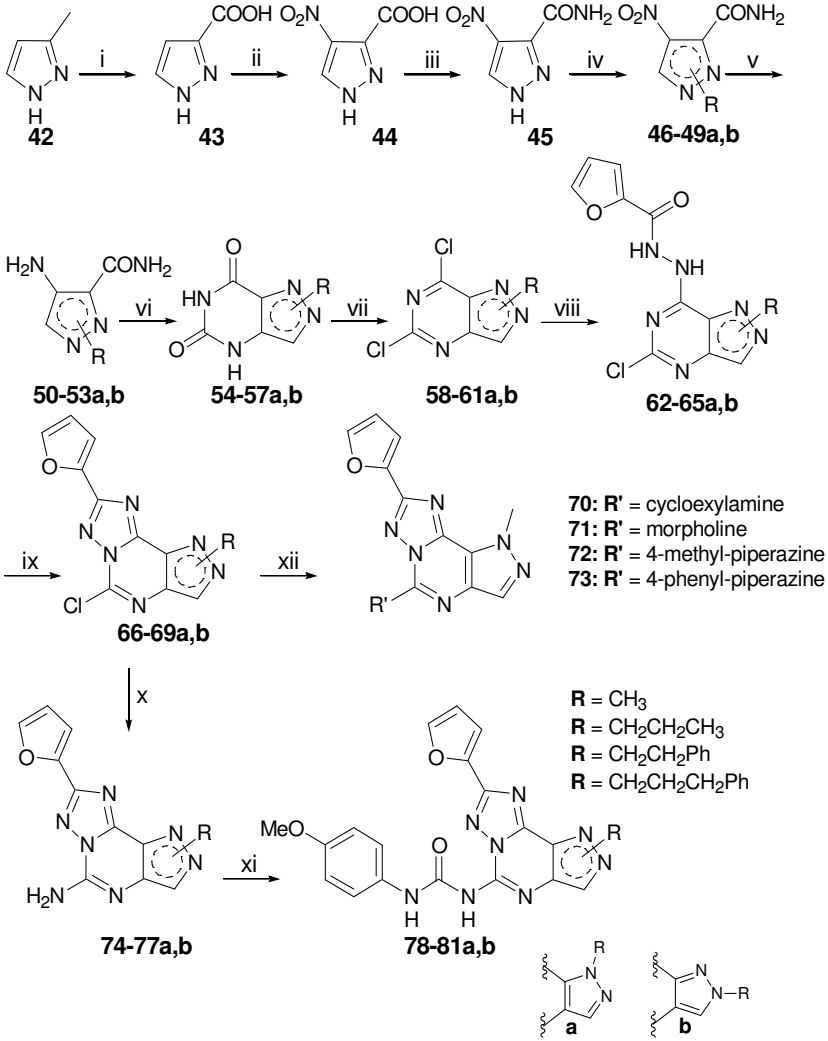
Starting from the data obtained from the previous series of pyrazolo-[4,3-*e*]triazolo-pyrimidine, we introduced at the N^8 or N^9 positions, small alkyl chain, such as methyl or propyl, and arylalkyl chain, such as phenylethyl or phenylpropyl. These modifications allowed us to explore the interaction of this side of the molecule with the adenosine

receptors. In addition to the substitution at the pyrazole ring, we studied the 5- position of the PTP structure introducing a free amino group, the 4-methoxy-phenyl moiety, a chlorine atom, morpholine and substituted piperazine rings.

For the synthesis of these new compounds we followed the synthetic strategy depicted in scheme 3. The 3-methyl-pyrazole (**42**) has been oxidized with $KMnO_4$ and then nitrated at the 4 position with HNO_3 and H_2SO_4. The carboxylic function was converted into the corresponding carboxamide *via* esterification and subsequent treatment with a solution of NH_4OH.[162] 4-Nitro-1*H*-pyrazole-3-carboxylic acid amide **45** was alkylated with the appropriate alkyl halide and K_2CO_3 in DMF to give an approximately 1:1 mixture of the two isomers **a** and **b** which were efficiently separated *via* column chromatography. The nitro group was then reduced with hydrogen in presence of a catalytic amount of C/Pd 10% and intermediates **50-53a,b** were converted into the corresponding 1/2-methyl-1,4-dihydro-pyrazolo[4,3-*d*]pyrimidine-5,7-dione **54-57a,b** by heating with an excess of urea.

The 5,7-dichloro-1/2-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidines **58-61a,b** were obtained by treatment of **54-57a,b** with $POCl_3$ and DBU. Selective substitution of the chlorine atom at the 7- position with furoic acid hydrazide followed by a Dimroth rearrangement led to the desired pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine nucleus (**66-69a,b**). Compounds **74-77a,b** were obtained treating derivatives **66-69a,b** with a solution of ethanol saturated with ammonia. These were converted into the corresponding 4-methoxy-phenyl urea derivatives **78-81a,b** by reaction with 4-methoxy-phenylisocyanate. Compound **66a** was also reacted with different primary and secondary amines to give final derivatives **70-73**.

Scheme 3



REAGENTS: i) $KMnO_4$, rfx, 4hrs; ii) HNO_3, H_2SO_4, 100 °C, 4hrs; iii) a:H_2SO_4, EtOH, rfx, 10hrs; b:NH_4OH 30%, 100 °C, 4hrs; iv) alkyl halide, K_2CO_3, DMF, rt, 10hrs; v) H_2, C/Pd 10%; vi) Urea, 250 °C; vii) $POCl_3$, DBU, 80 °C, 8hrs; viii) 2-Furoic acid hydrazide, TEA, 1,4-dioxane, rfx, 5h; ix) HMDS, BSA, 120 °C, 18h; x) EtOH sat. ammonia sol., 60 °C, 18h; xi) 4-OCH_3-phenyl isocyanate, THF, 50 °C, 18h; xii) Amines, 2-methoxyethanol, 100 °C, 3hrs.

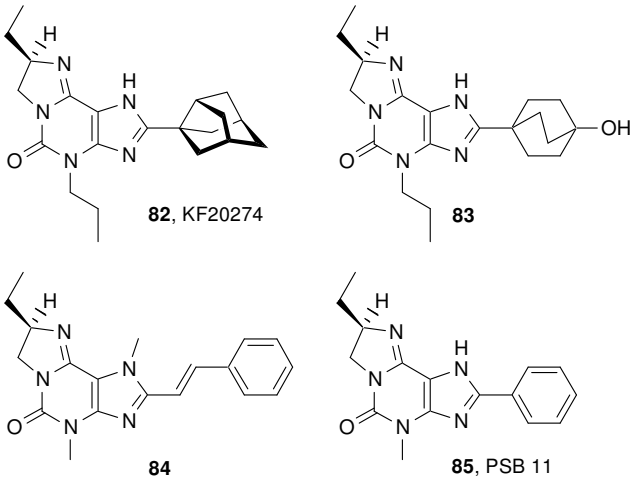
2.2. Second Project: *Imidazo[2,1-i]purin-5-ones as A_3 adenosine receptor antagonists with improved water solubility*

The aim of the second project of this PhD thesis was to obtain A_3 adenosine receptor antagonists with high selectivity and affinity along with increased water-solubility.

We focused our attention on the imidazo[2,1-*i*]purin-5-one scaffold,[126,127,163-166] obtained by the fusion of a third imidazoline ring on the xanthine bicycle, as an interesting tricyclic structure useful for the development of ARs ligands. A particular attention has been in past played to the substitution at the 2- position of the imidazo[2,1-*i*]purinone nucleus, which theoretically corresponds to the 8-position of the original xanthine core. The crucial role played by the 2-substituent in the subtype selectivity of the AR antagonists so far reported has been established. Compound KF20274 (**82**, Fig. 16),[163] substituted at the 2- position with a 3-noradamantyl moiety can be structurally associated to the xanthine labelled as KW3902, (1,3-dipropyl-8-(3-noradamantyl)xanthine). The 3-noradamantyl function showed to be able to induce A_1 selective antagonist activity in both the tricycle KF20274 and the xanthine analogue KW3902. The main advantage claimed for the annelation approach of the xanthine core into the imidazo[2,1-*i*]purinone scaffold, is the enhancement of water solubility awarded by the imidazoline basic nitrogen which has been reported to be subject to protonation at physiological pH. Compound **83**, (Fig. 16) (*R*)-7,8-dihydro-8-ethyl-2-(4-bicyclo[2.2.2]octan-1-ol)-4-propyl-1*H*-imidazo[2,1-i]purin-5(4*H*)-one,[164] is a particularly potent A_1 AR antagonist with good selectivity over the other three AR subtypes and high water solubility (>100 mg/mL) and showed a good *in vivo* profile after oral administration in a rat diuresis model. Müller and co-

worker explored the imidazopurinone nucleus introducing at the 2-position substituents previously known to promote A_{2A} or A_3 AR activity in the corresponding 8-substituted xanthine analogues.[165] Compound **84** (Fig. 16) has been conceived as water soluble A_{2A} AR antagonist as tricyclic congener of 8-styrylxanthines, while, derivative PSB11, (**85**, Fig. 16), (R)-4-methyl-8-ethyl-2-phenyl-4,5,7,8-tetrahydro-1*H*-imidazo[2,1-i]purin-5-one, exhibited a K_i value of 2.3 nM for A_3 receptor and good selectivity *vs* all other adenosine receptor subtypes. The radiolabelled derivative of this compound ([^{3}H]PSB-11) exhibited a K_D value of 4.9 nM and a B_{max} value of 3500 fmol/mg of protein.[166] The 2-(2,3,5-trichlorophenyl) substituted analogue, PSB10, showed inverse agonist activity in binding studies in CHO cells expressing recombinant hA_3 ARs (IC_{50}= 4 nM).[126]

Figure 16



In a recent study performed in Baraldi's laboratories, a wide series of 8-heterocyclyl-substituted xanthine derivatives has been identified as

very potent and selective human A_{2B} AR antagonists.[167] With this series, whose design based on the structure of the 8-phenyl-xanthine derivative MRS1754 (*N*-(4-cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)phenoxy]acetamide),[168] it was demonstrated that the phenyl and the pyrazole rings may occasionally behave as bioisosters.

Given these findings, in the present study we evaluated the effect of the replacement of the 2-phenyl ring of PSB11 and congeners with differently substituted 5-membered heterocycles, in particular 1,3- and 1,5- disubstituted pyrazoles or a 3- substituted isoxazole. At the 4- position an allyl or a benzyl group have been introduced whereas, the 8- position has been functionalized with a methyl or an ethyl, as the efficacy of such substituents was suggested by previous SAR studies on different series of xanthine-related ARs antagonists. Moreover, with the aim to verify a possible enantioselective interaction between the newly reported series of imidazo[2,1-*i*]purinones and ARs, for a selected number of compounds the pharmacological properties of the optically pure enantiomers have been compared to those of the corresponding racemates.

The synthesis of the 2-heterocyclyl tricyclic purinone derivatives has been performed, in analogy to described procedures, as depicted in scheme 7.[126,163,165,169]

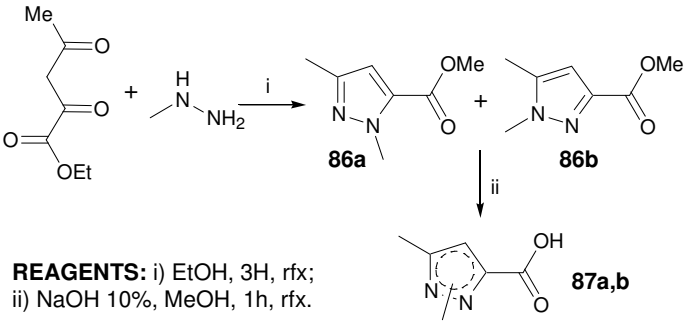
1-Subsituted-5,6-diaminouracils **95a**,**b**[170] and the appropriate pyrazole/isoxazole carboxylic acids were reacted in DMF solution in presence of 1-ethyl-3-[3 (dimethylamino)propyl]carbodiimide hydrochloride (EDAC) as condensing agent, followed by ring closure with sodium hydroxide at reflux to afford the desired 3-allyl/benzyl-8-

[(substituted)isoxazol/pyrazol-3/5-yl]-1*H*-purine-2,6(3H,7H)-dione derivatives (**96a-j**).[167]

The diamino uracils **95a,b** were obtained by reduction of the corresponding nitroso uracils using sodium dithionite.[167]

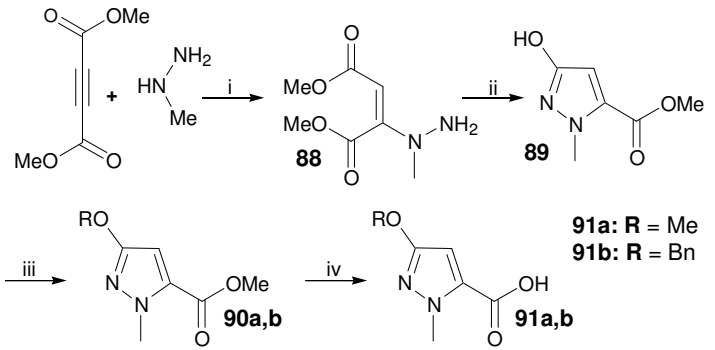
The substituted pyrazole carboxylic acids (**87a,b** and **91a,b**) were prepared according to procedures reported in literature (Scheme 4 and 5).[151]

Scheme 4



REAGENTS: i) EtOH, 3H, rfx; ii) NaOH 10%, MeOH, 1h, rfx.

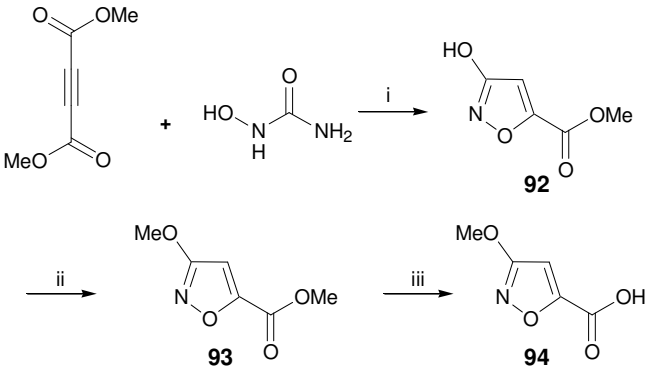
Scheme 5



REAGENTS: i) Et_2O, 1h, 0 °C; ii) benzene/ CH_3COOH 1:1, 1h, rfx; iii) CH_3I/ benzyl bromide, K_2CO_3, dry acetone, 2h, rt; iv) NaOH 10%, MeOH, 1h, rfx.

The 3-methoxy-isoxazole-5-carboxylic acid methyl ester (**94**, Scheme 6), obtained from dimethyl acetylenedicarboxylate[172] was *O*-alkylated with methyliodide in presence of K_2CO_3, followed by classical saponification.

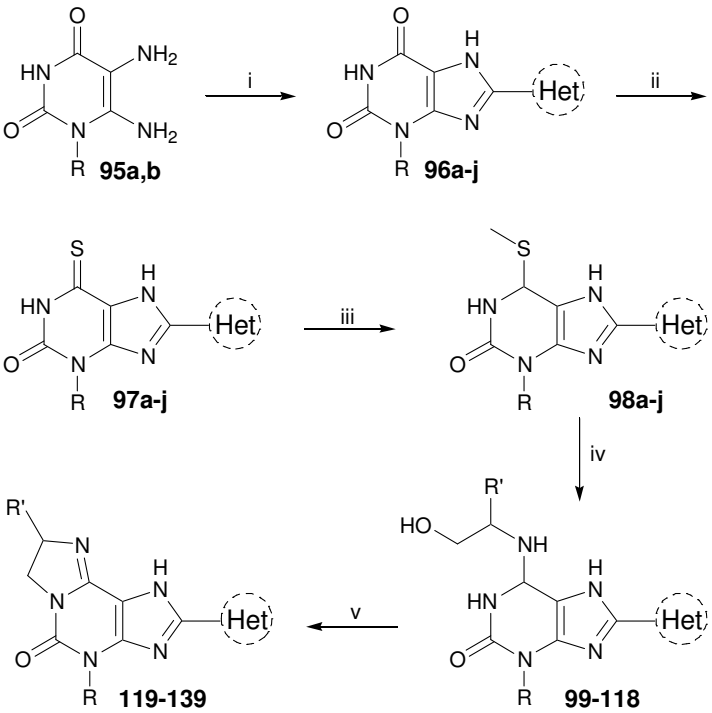
Scheme 6



REAGENTS: i) DBU, MeOH, 30', rt; ii) CH_3I, K_2CO_3, DMF, 6h, 60 °C; iii) NaOH 10%, MeOH, 1h, rfx.

The 8-heterocyclyl-xanthine derivatives **96a-j** were treated with phosphorous pentasulfide in dry pyridine at reflux to give the corresponding 6-thioxanthine **97a-j**. The subsequent reaction with methyliodide in presence of sodium hydroxide furnished the *S*-methyl-derivatives **98a-j** with good yields. Compounds **98a-j** were then treated with the appropriate amino alchol (2-amino-butan/propan-1-ol purchased both as racemic mixtures and optically active reagents), in anhydrous DMSO at 150 °C for 1.5 h. Final cyclizations have been performed in dichloromethane solution by treatment with thionyl chloride heating at reflux for 18 h. This kind of cyclization is known to yield final compounds **119-139** with retained stereochemistry.

Scheme 7



REAGENTS: i) a: (substituted)pyrazole/isoxazole carboxilic acid, EDC, HOBt, DMF, 12h, rt; b: NaOH 10%, MeOH, 1-2h, rfx ; ii) P_2S_5, Pyr, 5-6 h, 140 °C; iii) CH_3I, NaOH 0.5N, MeOH, 3h, rt; iv) (R/S, R, S)-2 -amino -butan/propan-1-ol, DMSO, 1.5h, 150 °C; v) $SOCl_2$, CH_2Cl_2, 18h, rfx.

Chapter 3

Results and Conclusions

3. Results and Conclusions

3.1. First Project: *Pyrrolo/pyrazolo-triazolo pyrimidines*

All the synthesized compounds were evaluated in radioligand binding assays to determine their affinities at the human A_1, A_{2A} and A_3 adenosine receptors. Potency of the compounds versus hA_{2B} adenosine receptors were studied evaluating their capability to inhibit (100 nM) NECA stimulated cAMP production. Affinity data for A_1, A_{2A} and A_3 receptors, expressed as K_i values, and IC_{50} values derived from the cAMP assay carried out for hA_{2B} subtypes, are listed in Table 4 and 5.

The biological experiments was performed by Prof. Borea and co-workers, Dipartimento di Medicina Clinica e Sperimentale – Sezione di Farmacologia, Università di Ferrara.

Derivatives **40a-d** bearing a free amino group at the5- position , were substituted at the N^8 with, respectively, methyl, propyl, phenylethyl and phenylpropyl chains. These compounds can be considered quite potent (low nanomolar range) but non selective ligands for the four adenosine receptors. In particular, the affinity *vs* A_3 receptor decreased with the increase of the steric hindrance in fact derivative with phenylpropyl chain was 5-fold less potent then the compound with the methyl group. Derivative **40c** displayed high affinity toward the A_1 and A_{2A} receptors (hA_1K_i = 4.3 nM and $hA_{2A}K_i$ = 3.9 nM) while a lower affinity has been detected at the remaining subtypes. A similar results were found with the compound bearing a phenylpropyl moiety **40d**. Both methyl group (**40a**) and propyl chain (**40b**) showed a good K_i values for A_{2A} and A_3 but they also bind other two subtypes in the nanomolar range.

As previously observed, when the phenylcarbamoyl moiety is present at the N^6 position all the synthesised derivatives **41a-c** show affinities in the nanomolar range toward A_3 receptor with different degree of selectivity versus the other receptor subtypes. From the biological data, it is evident that methyl group at the 8 position of the pyrrolo triazolo pyrimidine urea (**41a**) produce the best compound in term of both affinity (hA_3K_i = 15nM) and selectivity.

The propyl derivative (**41b**) showed a high affinity for the human A_3 but it showed a 2- fold increase of A_{2A} affinity with respect to the methyl derivative. Growing of steric hindrance at the 8- position with a phenyl-ethyl chain (**41c**) generated a remarkable loss of selectivity *vs* A_1 and A_2, however maintaining a good potency for A_3 subtype.

The pyridine urea derivative (**41e**) has been synthesised to improve water-solubility but unfortunately it resulted 10-fold less potent then the corresponding phenylcarbamoyl compound **41a** toward A_3 receptor.

With the aim to better investigate the role of the nitrogen at the 7- position on affinity toward A_3 ARs, we synthesised a series of structural isomers of the pyrazolo[4,3-*e*]triazolo pyrimidine, obtaining a new class of pyrazolo[3,4-*e*]triazolo pyrimidine substituted at the 8- or 9- positions.

The N^8 alkylated amino derivatives (**74-77b**) showed less affinity compared with the corresponding N^8 pyrrolo-triazolo pyrimidine (**40a-d)** at the A_3 receptor (125nM < K_i < 526nM) whereas they displayed a good affinity toward A_{2A} (8.1nM < K_i < 17nM) and a low selectivity *versus* hA_1 and hA_{2B} ARs.

Introduction of a methyl or propyl chain at the N^8 position in combination with the 4-methoxy-phenyl-urea moiety at the 5- position yielded

respectively compounds **78b** and **79b**. The biological assays showed that the propyl compound **79b** (hA_3K_i = 50 nM) exerted 2-fold higher affinity at A_3 AR compared to the methyl derivative **78b** (hA_3K_i = 110 nM).

The synthesis of compounds **74-81a** allowed us to evaluate the effect of the substitution at the N^9.

The increase of the steric hindrance of the substituents at the 9- position doesn't seems to affect in the same way of the pyrrolic series the binding profile of the ligands. Infact, compound with methyl group (**74a**, hA_3K_i = 749nM) was 10-fold less active compared to the propyl derivative (**75a**, hA_3K_i = 83nM).

The free amino derivatives (**74-77a**) confirmed the previous data exibiting a good affinity toward A_{2A} AR but, unfortunately, the ligands interacted also with A_1 and A_{2B} subtypes in the same range of concentration. Except for the propyl derivative **75a** (hA_3K_i = 83 nM), the affinity of the free amino compounds toward A_3 AR resulted significantly lower.

It is quite difficult to delineate a SAR profile for these class of compounds because the same substitution seems able to exert opposite effects; in fact, for example, N^8 alkylated (**74b**, hA_3K_i = 125 nM) revealed a lower K_i value at the hA_3 than the corresponding N^9 alkylated (**74a**, hA_3K_i = 749 nM) instead, analyzing the derivatives bearing the propyl chain at the 9- (**75a**, hA_3K_i = 83 nM) or 8- (**75b**, hA_3K_i = 432 nM) position of the nucleus, the binding profile resulted opposed.

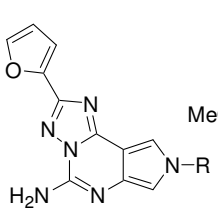
Surprisingly the conversion of the free amino group into the corresponding 4-OCH_3-phenyleurea derivatives (**78-81a**) did not provide the desired effect on the binding profile of the designed compounds. Compounds **78-81a** show a slight improvement of the

affinity at the A_3 adenosine receptor in comparison with the corresponding amino derivatives **74-77a** but the selectivity versus A_1, A_{2A} and A_{2B} subtypes resulted quite poor.

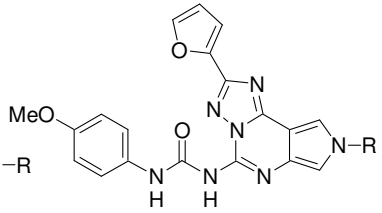
Compound **66a**, bearing a chlorine atom and a methyl function at the 5- and 9- positions respectively, showed to be completely unable to bind the four investigated receptors. Among compounds **70-73**, in which the chlorine atom has been substituted with cyclic amines such as cyclohexyl-amine, morpholine, methyl-piperazine and phenyl-piperazine, only the ciclohexyl-amino derivative (**70**) showed some affinity versus ARs, in particular for A_1 receptor.

These results would confirm previously reported studies indicating the importance of the amino function at the 5- position to establish a hydrogen bond with the adenosine receptors.

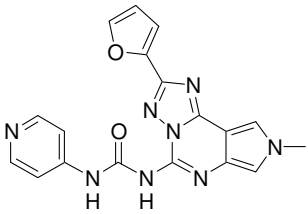
Interestingly compound **70**, bearing the cyclohexyl ring which is a typical N^6 substitution of NECA (5'-*N*-ethylcarboxamidoadenosine)-related A_1 AR agonists, resulted a potent A_1 antagonist (hA_1K_i = 12 nM) with 12- ,75- and 42- fold selectivity *vs* A_{2A}, A_{2B} and A_3 ARs respectively.



40a-d



41a-c

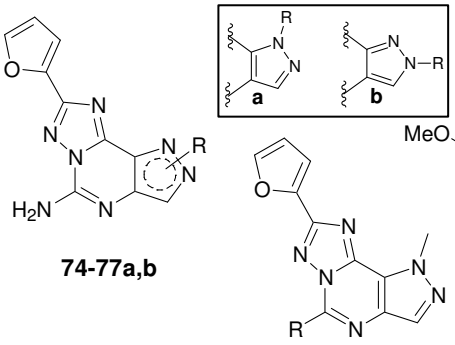


41e

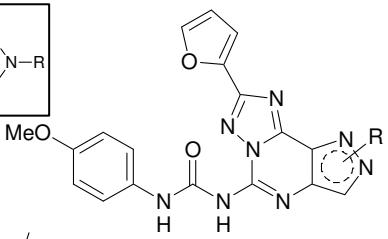
Table 4. Binding and Functional Data of derivatives **40a-41e**.

Compd	R	[^{3}H] DPCPX binding hA$_1$ CHO cells *Ki* (nM)	[^{3}H] ZM 241385 binding hA$_{2A}$CHO cells *Ki* (nM)	cAMP assay in hA$_{2B}$CHO cells IC$_{50}$ (nM)	[^{3}H] MRE 3008F20 binding hA$_3$CHO cells *Ki* (nM)
40a	Me	100 (83-120)	20 (12-31)	42 (31-57)	50 (41-60)
40b	Propyl	35 (27-45)	30 (23-38)	90 (81-99)	55 (46-65)
40c	2-Phenylethyl	4.3 (3.1-6.0)	3.9 (2.5-6.3)	46 (37-56)	124 (96-161)
40d	3-Phenylpropyl	18 (13-23)	50 (41-60)	251 (205-306)	241 (176-330)
41a	Me	800 (701-913)	500 (420-595)	838(713-984)	15 (10-21)
41b	Propyl	743 (671-821)	200 (166-240)	906 (852-964)	10 (6-17)
41c	2-Phenylethyl	178 (148-213)	148 (126-173)	740 (722-759)	12 (10-16)
41e	-	355 (289-437)	>1000 (13%)	>1000 (8%)	111 (74-167)

[a] Displacement of specific [^{3}H]DPCPX binding at human A$_1$ receptors expressed in CHO cells.
[b] Displacement of specific [^{3}H]ZM241385 binding at human A$_{2A}$ receptors expressed in CHO cells.
[c] Potency (IC$_{50}$) of examined compounds to inhibit 100 nM NECA stimulation cAMP levels in hA$_{2B}$ CHO cells. In parentheses are reported the % of inhibition to hA$_1$, A$_{2A}$, A$_{2B}$ and A$_3$ CHO cells. [d] Displacement of specific [^{3}H]MRE3008F20 binding at human A$_3$ receprors expressed in CHO cells.



74-77a,b



78-81a,b

66a, 70-73

Table 5. Binding and Functional Data of derivatives **66a, 70-81a**.

Compd	R	[^{3}H] DPCPX binding hA$_1$ CHO cells *Ki* (nM)	[^{3}H] ZM241385 binding hA$_{2A}$ CHO cells *Ki* (nM)	cAMP assay in hA$_{2B}$CHO cells IC$_{50}$ (nM)	[^{3}H] MRE3008F20 binding hA$_3$CHO cells *Ki* (nM)
66a	Cl	>1000 (28%)	>1000 (40%)	>1000 (10%)	>1000 (9%)
70	Cyclohexylamine	12 (8-18)	119 (87-162)	915 (786-1064)	507 (443-582)
71	Morpholine	>1000 (6%)	>1000 (4%)	>1000 (4%)	>1000 (1%)
72	4-Me-Piperazine	>1000 (5%)	>1000 (2%)	>1000 (2%)	>1000 (1%)
73	4-Ph-Piperazine	>1000 (8%)	>1000 (9%)	>1000 (5%)	>1000 (1%)
74a	Me	10 (8-13)	3.6 (2.5-5.3)	31 (25-38)	749 (662-847)
74b	Me	30 (24-38)	8.1 (6.9-9.7)	33 (28-37)	125 (85-182)
75a	Propyl	14 (11-20)	12 (8-18)	40 (35-46)	83 (70-99)
75b	Propyl	22 (18-27)	17 (12-24)	45 (40-51)	432 (363-514)
76a	2-Phenylethyl	33 (28-38)	42 (37-47)	319 (275-371)	603 (542-670)
76b	2-Phenylethyl	4.9 (3.4-7.2)	9.2 (7.9-10.6)	27 (23-32)	315 (255-390)
77a	3-Phenylpropyl	85 (68-105)	122 (86-172)	595 (502-706)	802 (719-895)
77b	3-Phenylpropyl	7.1 (5.7-8.9)	11 (9-13)	32 (28-37)	526 (482-575)
78a	Me	129 (97-172)	68 (55-84)	122 (84-177)	61 (42-88)
78b	Me	923 (878-970)	222 (181-273)	580 (453-742)	110 (93-130)
79a	Propyl	61 (44-85)	20 (14-29)	32 (26-45)	161 (132-196)
79b	Propyl	240 (222-259)	208 (177-245)	863 (801-930)	50 (45-56)
80a	2-Phenylethyl	524 (421-652)	626 (546-717)	772 (660-902)	208 (153-283)
81a	3-Phenylpropyl	>1000 (38%)	>1000 (32%)	>1000 (32%)	>1000 (25%)

[a] Displacement of specific [^{3}H]DPCPX binding at human A$_1$ receptors expressed in CHO cells.
[b] Displacement of specific [^{3}H]ZM241385 binding at human A$_{2A}$ receptors expressed in CHO cells.
[c] Potency (IC$_{50}$) of examined compounds to inhibit 100 nM NECA stimulation cAMP levels in hA$_{2B}$ CHO cells. In parentheses are reported the % of inhibition to hA$_1$, A$_{2A}$, A$_{2B}$ and A$_3$ CHO cells. [d] Displacement of specific [^{3}H]MRE3008F20 binding at human A$_3$ receprors expressed in CHO cells.

3.2. Second Project: *Imidazo[2,1-i]purin-5-ones*

All the 2-heterocyclyl-imidazo[2,1-*i*]purin-5-one derivatives **119-138** (Table 6) were evaluated in radioligand binding assays to determine their affinities for human A_1, A_{2A}, and A_3 adenosine receptors using [^{3}H]-DPCPX (1,3-[^{3}*H*]-dipropyl-8-cyclopentylxanthine), [^{3}H]-ZM 241385 (4-(2-[7-amino-2-(2-furyl)[1,2,4]-triazolo[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol), [^{3}H]-MRE3008F20 (5-*N*-(4-methoxyphenylcarbamoyl)amino-8-propyl-2-(2-furyl)pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidine), respectively, as radioligands. Efficacy of the compounds versus hA_{2B} AR was investigated evaluating their capability to inhibit (100 nM) NECA stimulated cAMP production. Antagonism of selected ligands versus hA_3AR was also assessed through cAMP experiments performed evaluating their capability to block the inhibitory effect mediated by Cl-IB-MECA. Affinity data for A_1, A_{2A} and A_3 receptors (expressed as K_i values), and IC_{50} values for hA_{2B} subtypes, derived from the cAMP, are listed in Tables 6, 7.

The biological experiments was performed by Prof. Borea and co-workers, Dipartimento di Medicina Clinica e Sperimentale – Sezione di Farmacologia, Università di Ferrara.

Structure-Activity Relationships Analysis: **hA_3AR Affinity.** All the synthesised molecules exhibited high affinity at the hA_3AR subtype with K_i values ranging from 1.46 (compound *R*-**124**) to 44.8 nM (compound **129**). The different kind of heterocycle introduced at the 2- position gave various contributions to the binding profile of the examined imidazo[2,1-*i*]purin-5-one derivatives. The affinity at hA_3AR of 4-allyl-2-(1′,5′-dimethyl-pyrazole) derivatives (**119**, **120**) showed to be about 3/4-fold higher then that of the corresponding 4-allyl-2-(1′,3′-

dimethyl-pyrazole) isomers (**131**, **132** respectively), whereas, 4-benzyl-2-(1′,5′-dimethyl-pyrazole) derivatives (**121**, **122**) show hA_3 AR K_i binding values similar to those of the corresponding 4-benzyl-2-(1′,3′-dimethyl-pyrazole) derivatives **133** and **134**. This would indicate that 1′,5′-disubstitution of the 2-pyrazole ring is equivalent or slightly detrimental, with regard to hA_3 binding affinity, if compared with 1′,3′-disubstitution. The comparison between hA_3 affinities of 2-(1′-methyl-3′-methoxy-pyrazole) derivatives **123**-**126** and 2-(3′-methoxy-isoxazole) derivatives **135-138** indicated a substantial bioisosterism of these heterocycles as regards the interaction with hA_3 AR of the examined ligands. As indicated by the affinity values of compounds **119**-**130**, the type of substitutions at the 3′-position of the 2-pyrazole ring appreciably affects the affinity for the hA_3 AR. The order of efficacy OMe (compounds **123**-**126**) > Me (**119**-**122**) > OBn (**127**-**130**) is maintained in the whole subset of molecules. For example the 3′-OCH_3-derivative **123** (hA_3K_i = 2.36 nM) appeared 2- and 10-fold more potent in binding hA_3 subtype than the analogously substituted 3′-CH_3-derivative **119** (hA_3K_i = 4.56 nM) and 3′-OBn-derivative **127** (hA_3K_i = 24.7 nM), respectively. Similarly, the 3′-OCH_3-derivative **124** (hA_3K_i = 1.96 nM) resulted 1.5- and 11-fold more potent then the corresponding 3′-CH_3-derivative **120** (hA_3K_i = 3.01 nM) and 3′-OBn-derivative **128** (hA_3K_i = 21.5 nM), respectively, at the hA_3AR. This trend led us to synthesise 3′-OCH_3-isoxazole derivative **135**-**138** which confirmed the efficacy of the introduction of a methoxy group at the 3′- position of the 2- heterocycle. From the set of molecules we analysed, it emerged that the kind of substitutions of the 5-membered heterocycle at the 2- position of the imidazo[2,1-*i*]purin-5-one tricycle, is particularly important for hA_3 AR binding af-

finity. A steric control seems to take place around the 3′-position of the 2-heterocycle, as suggested by the decrease of affinities of the 3′-OBn derivatives **127**-**130** (hA_3K_i ranging from 21.5 nM to 44.8 nM). Moreover the angularity component of the 3′-OCH_3, in association with the possibility of this function to establish a hydrogen bond, could contribute to favour particular ligand-receptor interactions.

A benzyl or an allyl substituent have been introduced at the 4-position of the imidazo[2,1-*i*]purin-5-one core. The pairwise comparison between the K_i (hA_3) values of 4-allyl and 4-benzyl derivatives resulted in most cases in a fairly prevalent affinity of the allyl-substituted compounds (i.e. compounds **121, 122, 126** were 3.8-, 1.6- and 2-fold less potent then the correspondent 4-allyl derivatives **119**, **120**, **124** as hA_3 ligands). Nevertheless some exceptions have been observed (see compounds **133**, **134**, **137** *versus* **131**, **132**, **135**, respectively).

The substitution of the 8- position with a methyl or an ethyl seems to be essentially equivalent in terms of hA_3 affinity, as it can be deduced from the binding equipotency between 8-methyl and 8-ethyl derivatives. For example, compounds **119** exhibited a binding affinity similar to that of the correspondent 8-ethyl derivative **120**. The same can be observed, for example, for compounds **127**, **129**, **131** versus **128**, **130** and **131**, respectively.

The monosubstitution of the C^8 generated an asymmetric carbon. For selected compounds (**119**, **122**, **124**, **125**, **132**) both racemic mixtures and optically active derivatives have been prepared in order to determine a stereoselective criterion for the interaction with hA_3 AR. The affinities of racemates appeared generally comparable to those of the respective *R* and *S* pure isomers. Only a very weak pref-

erence for the *R* isomer can be noted (*R*-**119** compared to *S*-**119** or *R*-**124** compared to *S*-**124**) with the exception of compound **132**. The data related to the 8- position seem to suggest that this side of the molecule could not be involved in determining interactions with the hA_3 receptor binding site. In fact, neither the kind of substitution nor the stereoisomery of the C^8 seem to particularly affect the affinity of the ligands for the hA_3 AR.

Structure-Activity Relationships Analysis: **hA_3AR *vs* hA_{2A} and hA_1 ARs Selectivity.** Very high level of hA_3 *vs* hA_{2A} selectivity has been obtained with all the compounds. Indeed, with the exception of derivatives **129** ($hA_{2A}K_i$ = 2010 nM), **130** ($hA_{2A}K_i$ = 2130 nM) and **136** ($hA_{2A}K_i$ = 1520 nM), the K_i values calculated in the binging assay at the hA_{2A} AR were higher then 5 μM. Whereas, the affinity at the hA_1 AR subtype resulted occasionally relevant (lower then 1 μM for compounds **120**, **122**, **126**, **138**). Comparing the type of heterocycle at the two position, the 1,5-disubstituted pyrazole seems to assure a low affinity at the hA_1 AR, however, derivatives **131**-**134** (hA_1K_i > 5 μM), displaying from about 330- to 540-fold selectivity for the hA_3 AR over the hA_1 AR, are not the most selective of the series because of the concomitant small reduction of hA_3 affinity. The most selective derivatives have been obtained with the introduction of the 2-(1′-methyl-3′-methoxy-pyrazole) or the 2-(3′-methoxy-isoxazole) functions (derivatives **123**-**126** and **135**-**138** respectively), in particular compounds **123** (4-allyl-7,8-dihydro-2-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-8-methyl-1*H*-imidazo[2,1-*i*]purin-5(4*H*)-one) and **137** (4-benzyl-8-methyl-7,8-dihydro-2-(3-methoxyisoxazol-5-yl)-1*H*-imidazo[2,1-*i*]purin-5(4H)-one) appeared the most selective of the series of the 2-heterocyclyl-imidazo[2,1-*i*]purin-5-ones herein de-

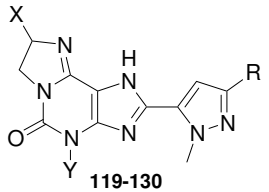
scribed, displaying more than 2100- and 1800-fold selectivity, respectively, for the hA_3 AR over the other ARs subtypes. The kind of substituent at the 3′-position of the 2-(1′,3′-disubstituted-pyrazole) moiety seems somewhat to affect the selectivity of the ligands for the hA_3 *vs* hA_1 AR, in fact the related affinities ratios (hA_1K_i / hA_3K_i) clearly suggested that the selectivity follows the sequence 3′-OMe > 3′-Me > 3′-OBn, for example compound **123** (3′-OMe), **129** (3′-Me) and **127** (3′-OBn) were 2100-, 450- and 202-fold selective for hA_3 *vs* hA_1, respectively. The same can be noticed in the comparison between compounds **124** (3′-OMe, hA_1K_i / hA_3K_i = 780), **120** (3′-Me, hA_1K_i / hA_3K_i = 170), **128** (3′-OBn, hA_1K_i / hA_3K_i = 56) as well as between compounds **125** (hA_1K_i / hA_3K_i = 610), **121** (hA_1K_i / hA_3K_i = 291), **129** (hA_1K_i / hA_3K_i = 57) and derivatives **126** (hA_1K_i / hA_3K_i = 105), **122** (hA_1K_i / hA_3K_i = 60), **130** (hA_1 K_i/ hA_3K_i = 42).

In most of the evaluated examples the 4-allyl substitution seems quite more favourable for hA_1K_i / hA_3K_i selectivity in comparison with 4-benzyl substitution. For example the 4-allyl derivative **119** (hA_1K_i / hA_3K_i = 450) is 1.5-fold more selective for hA_3 *vs* hA_1 then the corresponding 4-benzyl derivative **121** (hA_1K_i / hA_3K_i = 290). The same behaviour can be observed for compounds **123** (hA_1K_i / hA_3K_i = 2119) which is 3.5-fold more selective then the corresponding 4-benzyl substituted **125** (hA_1K_i / hA_3K_i = 610), et cetera. The only exceptions to this leaning are compounds **131** (hA_1K_i / hA_3K_i = 359) compared to **133** (hA_1K_i / hA_3K_i = 400), in which the 4-substitution with a benzyl or an allyl functions seems practically equivalent, and compounds **135** (hA_1K_i / hA_3K_i = 1397) compared to **137** (hA_1K_i / hA_3K_i = 1866), in which the effect on hA_3 *vs* hA_1 selectivity of the two substituents at the 4- position appeared reversed.

While practically equivalent for the effect on hA_3 affinity, the introduction of a methyl or an ethyl at the 8- position appears to sensibly influence the affinity of the examined molecules towards hA_1 AR subtype. The choice of the substituent at this position resulted therefore important for the optimization of hA_3 over hA_1 selectivity. The general selectivity pattern would quite strongly suggest a preference by A_1 adenosine receptor for the 8-ethyl substitution. This effect is fairly evident if the 8-methyl derivative **137** (hA_1K_i = > 50000, hA_1K_i / hA K_i = 1866) was compared to the 8-ethyl analog **138** (hA_1K_i = 485, hA_1K_i / hA_3K_i = 179). With the exception of compound **134**, the molecules exerting higher affinity at the hA_1 subtype were concomitantly substituted at the 4- and 8- positions with a benzyl and an ethyl moieties, respectively, indicating a synergistic detrimental effect of the two substituents on hA_3 selectivity. On the contrary, the 4-allyl-8-methyl-imidazo[2,1-*i*]purin-5-one derivatives displayed the best hA_1K_i / hA_3K_i ratios of the series.

A slight level of enantioselectivity seems to concern the interaction of the described ligands with the hA_1AR subtype. The affinities of *S* isomers of compounds **119**, **122**, **124** and **125**, resulted from 1.3- to 2.3-fold higher then those of the corresponding *R* isomers, thus resulting in a higher hA_3 *vs* hA_1 selectivity of the *R* isomers with reference to the corresponding *S*-configured molecules. This data resulted in agreement with the marks previously described by Müller and co-workers[165] and appeared to give evidence for a direct involvement of the additional imidazolidine ring in critical interactions between the 2-heterocyclyl-imidazo[2,1-*i*]purinones and the hA_1 AR binding pocket. This could be the reason for the responsiveness of hA_1 AR binding affinities to the kind of substitution and/or the configuration of the C^8.

Compound *R*-**124** showed to be the most potent hA_3 AR ligands of the 2-heterocyclyl-imidazo[2,1-*i*]purin-5-one derivatives herein described. It also confirmed to have very high selectivity versus A_{2A}, A_{2B} and also at A_1 AR with one of the heist value of hA_1K_i / hA_3K_i ratio of the series (1,729).

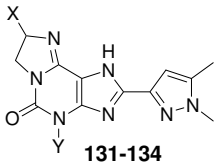


119-130

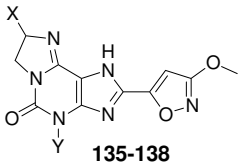
Table 6. Binding and Functional Data of derivatives **119-130**.

Compd	X	Y	R	A_1 K_i (nM)[a]	A_{2A} K_i (nM)[b]	A_{2B} IC_{50} (nM)[c]	A_3 K_i (nM)[d]
119	Me	Allyl	Me	2040 (1710-2450)	>5000 (7%)	>5000 (30%)	4.56 (3.44-6.05)
***R*-119**	Me	Allyl	Me	2510 (2050-3060)	>5000 (1%)	>5000 (10%)	5.04 (5.14-6.01)
***S*-119**	Me	Allyl	Me	1960 (1540-2480)	>5000 (4%)	>5000 (12%)	6.03 (5.64-6.38)
120	Et	Allyl	Me	515 (437-608)	>5000 (8%)	>5000 (20%)	3.01 (2.29-3.96)
121	Me	Bn	Me	>5000 (20%)	>5000 (1%)	>5000 (8%)	17.2 (12.2-24.0)
122	Et	Bn	Me	615 (536-707)	>5000 (5%)	>5000 (20%)	10.3 (7.9-13.4)
***R*-122**	Et	Bn	Me	803 (654-986)	>5000 (3%)	>5000 (10%)	12.6 (9.4-16.9)
***S*-122**	Et	Bn	Me	545 (468-635)	>5000 (4%)	>5000 (13%)	13.2 (9.7-17.9)
123	Me	Allyl	OMe	>5000 (30%)	>5000 (1%)	>5000 (13%)	2.36 (1.60-3.49)
124	Et	Allyl	OMe	1530 (1280-1830)	>5000 (1%)	>5000 (16%)	1.96 (1.22-3.13)
***R*-124**	Et	Allyl	OMe	2525 (2106-3027)	>5000 (13%)	>5000 (21%)	1.46 (0.88-2.42)
***S*-124**	Et	Allyl	OMe	1078 (868-1340)	>5000 (15%)	>5000 (14%)	2.37 (1.48-3.83)
125	Me	Bn	OMe	1900 (1720-2090)	>5000 (1%)	>5000 (11%)	3.11 (2.01-4.80)
***R*-125**	Me	Bn	OMe	2550 (2060-3170)	>5000 (11%)	>5000 (19%)	2.38 (1.79-3.17)
***S*-125**	Me	Bn	OMe	1730 (1470-2030)	>5000 (1%)	>5000 (22%)	2.52 (1.64-3.88)
126	Et	Bn	OMe	405 (338-486)	>5000 (9%)	>5000 (22%)	3.85 (3.14-4.72)
127	Me	Allyl	OBn	>5000 (47%)	>5000 (25%)	>5000 (17%)	24.7 (17.0-35.8)
128	Et	Allyl	OBn	1200 (1030-1400)	>5000 (5%)	>5000 (14%)	21.5 (17.0-27.3)
129	Me	Bn	OBn	2560 (2300-2860)	2010 (1720-2360)	>5000 (13%)	44.8 (36.5-55.0)
130	Et	Bn	OBn	1680 (1380-2040)	2130 (1870-2430)	>5000 (12%)	39.8 (31.6-50.1)

[a] Displacement of specific [^{3}H]-DPCPX binding to human A_1 receptors expressed in CHO cells (K_i nM); [b] Displacement of specific [^{3}H]-ZM 241385 binding to human A_{2A} receptors expressed in CHO cells (K_i nM); [c] cAMP assay in CHO cells expressing hA_{2B} receptors (IC_{50} nM); [d] Displacement of specific [^{3}H]-MRE3008F20 binding to human A_3 receptors expressed in CHO cells (K_i nM).



131-134



135-138

Table 7. Binding and Functional Data of derivatives **131-138**.

Compd	X	Y	A_1 K_i (nM)[a]	A_{2A} K_i (nM)[b]	A_{2B} IC_{50} (nM)[c]	A_3 K_i (nM)[d]
131	Me	Allyl	>5000 (13%)	>5000 (1%)	>5000 (9%)	13.9 (10.7-18.1)
132	Et	Allyl	>5000 (20%)	>5000 (1%)	>5000 (16%)	12.5 (8.6-18.2)
***R*-132**	Et	Allyl	>5000 (12%)	>5000 (3%)	>5000 (14%)	12.2 (8.4-17.7)
***S*-132**	Et	Allyl	>5000 (29%)	>5000 (1%)	>5000 (8%)	9.20 (7.61-11.12)
133	Me	Bn	>5000 (22%)	>5000 (9%)	>5000 (24%)	12.2 (8.4-17.7)
134	Et	Bn	>5000 (28%)	>5000 (13%)	>5000 (27%)	15.0 (13.3-16.9)
135	Me	Allyl	>5000 (26%)	>5000 (11%)	>5000 (13%)	3.58 (2.84-4.51)
136	Et	Allyl	3023 (2388-3827)	1520 (1126-2051)	>5000 (25%)	1.93 (1.28-2.93)
137	Me	Bn	>5000 (35%)	>5000 (5%)	>5000 (11%)	2.68 (2.11-3.41)
138	Et	Bn	485 (407-578)	>5000 (12%)	>5000 (18%)	2.71 (2.20-3.33)

[a] Displacement of specific [^{3}H]-DPCPX binding to human A_1 receptors expressed in CHO cells (K_i nM); [b] Displacement of specific [^{3}H]-ZM 241385 binding to human A_{2A} receptors expressed in CHO cells (K_i nM); [c] cAMP assay in CHO cells expressing hA_{2B} receptors (IC_{50} nM); [d] Displacement of specific [^{3}H]-MRE3008F20 binding to human A_3 receptors expressed in CHO cells (K_i nM).

Table 8. Functional Data.

Compd	cAMP hA_3 IC_{50} (nM)	Compd	cAMP hA_3 IC_{50} (nM)
119	34.2 (27.4-42.6)	**125**	27.6 (23.3-32.7)
(*R*)119	35.9 (29.9-43.0)	**(R)125**	15.9 (12.6-20.1
(*S*)119	40.9 (34.9-47.9)	**(*S*)125**	17.5 (13.4-22.9)
123	14.5 (10.5-20.1)	**135**	24.2 (19.1-30.7)
124	12.2 (8.4-17.7)	**136**	14.6 (12.0-17.8)

As shown in Table 8, some compounds were tested in a specific functional model where the inhibition of cAMP generation by IB-MECA was measured in CHO cells stably transfected with the hu-

man A_3 receptor. All the tested derivatives proved to be competitive antagonists. Interestingly, a notable concordance between binding and functional experiments has been revealed. Among the examined compounds, the molecules showing some of the best affinities for the A_3 AR have also proved to have very high potency in functional assays. In particular, derivative **124** can be consider the most potent compound, exhibiting an IC_{50} value of 12.2 nM.

3.3. Conclusions

First Project: *Pyrrolo/Pyrazolo[3,4-e][1,2,4]triazolo[1,5-c]pyrimidines*

Herein we evaluated the importance of the nitrogen at the 7- position of the pyrazolo[4,3-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine nucleus. From the biological data obtained, we can assert that N^7 is fundamental for the selectivity of these A_{2A}/A_3 ligands *versus* the remaining ARs subtype.

Furthermore, the substitution of the carbon atom at the 9- position with a nitrogen is detrimental for both affinity and selectivity, probably cause from a negative interaction with the receptor.

Our results confirmed the importance of the presence of the NH at the 5- position of the PTPs nuclus, this could be due to the formation of an essential ligand-receptor hydrogen bond.

Second Project: *Imidazo[2,1-i]purin-5-ones*

We analyzed the effect of the introduction of different disubstituted five-membered heterocycles at the 2- position in a new series of imidazo[2,1-*i*]-purinones. In particular, we introduced 1,3-/1,5- disubstituted pyrazoles and 3-substituted isoxazoles. All the synthesised molecules exhibited high affinity at the hA_3AR subtype with K_i values ranging from 1.46 (compound *R*-**124**) to 44.8 nM (compound **129**).

The best results were obtained with 1,3-disubstituted heterocycles. In particular, the concomitant presence of a methyl group at the 1-position and a methoxy function at the 3- position of the five membered ring afforded the most potent compounds.

The presence of an allyl moiety at the 4- position of the imidazo[2,1-*i*]purin-5-one resulted to be more effective than the benzyl group for the affinity toward A_3 AR.

The binding affinity of the optical isomers highlighted that the compounds did not bind stereo-selectively to the A_3 receptor.

The substitution of the 8- position with a methyl or an ethyl seems to be essentially equivalent in terms of hA_3 affinity.

Chapter 4

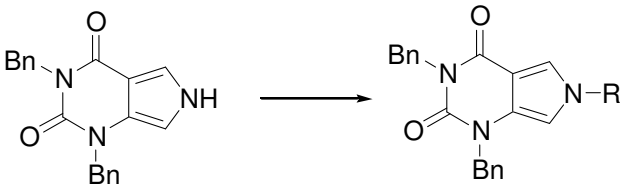
Experimental Section

Experimental section of pyrrolo/pyrazolo-triazolo-pyrimidines

Chemical Materials and Methods. Starting materials were purchased and used without any purification. All reactions were carried out under in inert atmosphere of dry nitrogen unless otherwise described. Standard hypodermic syringe (glass/metal Luer) techniques were applied for transferring dry solvents. Reaction progress and product mixtures were monitored by thin-layer chromatography (TLC) on silica gel (precoated F_{254} Macherey-Nagel plates) and visualized with aqueous potassium permanganate. ^{1}H NMR data were determined in $CDCl_3$ or DMSO-d_6 solutions with a Varian VXR 200 spectrometer or a Varian Mercury Plus 400 spectrometer. Peak positions are given in parts per million (δ) downfield from tetramethylsilane as internal standard, and *J* values are given in hertz. All products reported showed ^{1}H NMR spectra in agreement with the assigned structures. Light petroleum refers to the fractions boiling at 40-60 °C. Melting points (m.p.) were determined on a Buchi-Tottoli instrument and are uncorrected. Electrospray Ionization Mass Spectrometry (ESI/MS) was performed with an Agilent 1100 Series LC/MSD model in positive scan mode using direct injection of the purified compound solution (MH^+). Chromatography was performed on Merck 230-400 mesh silica gel. Organic solutions were dried over anhydrous sodium sulfate.

1,3-Dibenzyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (**34**) was synthesized according to known procedures.[161]

General procedure for preparation of 1,3-dibenzyl-6-alkyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-diones (35a-d).



1,3-Dibenzyl-6-methyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (35a) To a solution of 1,3-dibenzyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (**34**, 1.5 mmol) in anhydrous DMF (5 mL) was added K_2CO_3 (4.5 mmol) and the resulting mixture was stirred at 40 °C for 10'. After cooling at room temperature, CH_3I (4.5 mmol) was added and the reaction heated at 40 °C for further 2 h. The solvents were removed *in vacuo* and the residue was suspended with water and extracted with EtOAc. The organic phase was dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure to obtain a crude solid which was purified by crystallization from Et_2O/ Petroleum ether. White solid; 97% yield; mp 164-166 °C; ^{1}H NMR (200MHz, $CDCl_3$) δ ppm 7.52-7.22 (m, 10H), 7.18 (d, *J* = 2.2 Hz, 1H), 6.13 (d, *J* = 2.4 Hz, 1H), 5.24 (s, 2H), 5.01 (s, 2H), 3.66 (s, 3H). MS (ESI): $[MH]^+$ = 346.4.

1,3-Dibenzyl-6-propyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (35b) To a solution of 1,3-dibenzyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (**34**, 1.5 mmol) in anhydrous DMF (5 mL) was added K_2CO_3 (4.5 mmol) and the resulting mixture was stirred at 40 °C for 10'. After cooling at room temperature, $CH_3CH_2CH_2Br$ (4.5 mmol) was added and the reaction heated at 40 °C for further 4 h. The solvents were removed *in vacuo* and the residue was sus-

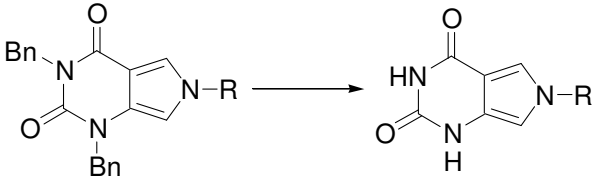
pended with water and extracted with EtOAc. The organic phase was dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure to obtain a crude solid which was purified by crystallization from Et_2O/ Petroleum ether. White solid; 89% yield; mp 122 °C; ^{1}H NMR (200MHz, $CDCl_3$) δ ppm 7.51-7.48 (m, 2H), 7.29-7.22 (m, 9H), 6.15 (d, *J* = 2.4 Hz, 1H), 5.24 (s, 2H), 5.01 (s, 2H), 3.80 (t, *J* = 7.2 Hz, 2H), 1.77-1.73 (m, *J* = 7.2 Hz, 2H), 0.86 (t, *J* = 7.4 Hz, 3H).

1,3-Dibenzyl-6-phenethyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (35c) To a solution of 1,3-dibenzyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (**34**, 1.5 mmol) in anhydrous DMF (5 mL) was added K_2CO_3 (4.5 mmol) and the resulting mixture was stirred at 40 °C for 10'. After cooling at room temperature, $PhCH_2CH_2Cl$ (4.5 mmol) was added and the reaction heated at 80 °C for further 4 h. The solvents were removed *in vacuo* and the residue was suspended with water and extracted with EtOAc. The organic phase was dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure to obtain a crude solid which was purified by crystallization from Et_2O/ Petroleum ether. White solid; 76% yield; mp 159-160 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 7.46 (d, *J* = 2 Hz, 1H), 7.29-7.13 (m, 15H), 6.83 (d, *J* = 2 Hz, 1H), 5.04 (s, 2H), 4.93 (s, 2H), 4.18 (t, *J* = 7.6 Hz, 2H), 3.02 (t, *J* = 7.2 Hz, 2H). MS (ESI): $[MH]^+$ = 436.8.

1,3-Dibenzyl-6-(3-phenyl-propyl)-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (35d) To a solution of 1,3-dibenzyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (**34**, 1.5 mmol) in anhydrous DMF (5 mL) was added K_2CO_3 (4.5 mmol) and the resulting mixture was stirred at 40 °C for 10'. After cooling at room tempera-

ture, $Ph(CH_2)_3Cl$ (4.5 mmol) was added and the reaction heated at 80 °C for further 4 h. The solvents were removed *in vacuo* and the residue was suspended with water and extracted with EtOAc. The organic phase was dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure to obtain a crude solid which was purified by column chromatography eluting with a mixture EtOAc/ Petroleum ether 1:4. White solid; 82% yield; mp 129-130 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 7.57 (d, *J* = 2 Hz, 1H), 7.33-7.12 (m, 15H), 6.86 (d, *J* = 2 Hz, 1H), 5.08 (s, 2H), 4.99 (s, 2H), 3.97 (t, *J* = 7.2 Hz, 2H), 2.51-2.44 (m, 2H), 2.03 (m, 2H).

General procedure for preparation of 6-alkyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-diones (36a-d).



To a solution of the appropriate dibenzyl derivative **35a-d** (4 mmol) in anhydrous toluene (50 mL) was added $AlCl_3$ (28 mmol) and the resulting suspension was stirred at room temperature for 1,5-3h. The solvent was concentrated *in vacuo* and the residue treated with crashed ice

6-Methyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (36a) White solid; 82% yield; mp >300 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.36 (bs, 1H), 10.28 (bs, 1H), 7.29 (s, 1H), 6.44 (s, 1H), 3.67 (s, 3H). MS (ESI): $[MH]^+$ = 166.1.

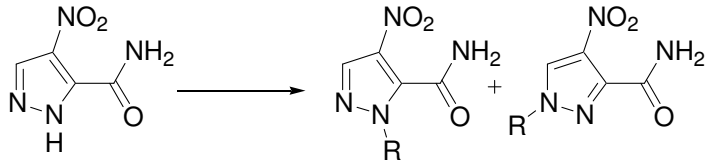
6-Propyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (36b) Pale yellow solid; 87% yield; mp dec. 270 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.37 (bs, 1H), 10.27 (bs, 1H), 7.35 (d, *J* = 2.2 Hz, 1H), 6.47 (d, *J* = 2.2 Hz, 1H), 3.89 (t, *J* = 7 Hz, 2H), 1.77-1.66 (m, 2H), 0.79 (t, *J* = 7.4 Hz, 3H).

6-Phenethyl-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (36c) White solid; 81% yield; mp dec. 263 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.38 (bs, 1H), 10.28 (bs, 1H), 7.30-7.10 (m, 6H), 6.49 (d, *J* = 2.4 Hz, 1H), 4.19 (t, *J* = 7.6 Hz, 2H), 3.03 (t, *J* = 7.2 Hz, 2H). MS (ESI): $[MH]^+$ = 256.3.

6-(3-Phenyl-propyl)-1,6-dihydro-pyrrolo[3,4-*d*]pyrimidine-2,4-dione (36d) White solid; 80% yield; mp dec. 225 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.40 (bs, 1H), 10.30 (bs, 1H), 7.38 (d, *J* = 2 Hz, 1H), 7.30-7.17 (m,5H), 6.51 (d, *J* = 2 Hz, 1H), 3.96 (t, *J* = 6.8 Hz, 2H), 2.52-2.48 (m, 2H), 2.051-2.00 (m, 2H).

4-nitro-1*H*-pyrazole3-carboxylic acid amide (45) was synthesised according to the known procedure.[162]

General procedure for preparation of 1-alkyl-4-nitro-1*H*-pyrazole-5-carboxamide and 1-alkyl-4-nitro-1*H*-pyrazole-3-carboxamide (46-49a,b)



To a solution of nitroamide (**45**, 1.2 mmol) in anhydrous DMF (36 mL) was added K_2CO_3 (2.3 mmol) and the resulting mixture was

stirred for 10'. Then, the iodomethane (1.4 mmol) was added and the mixture was stirred for 6 h. The solvent was removed *in vacuo* and the residue was suspended with water and extracted with EtOAc. The organic phase was dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure to obtain a crude solid which was purified *via* column chromatography (gradient from EtOAc/ EtP 1:1 to EtOAc) to obtain the desired products.

1-Methyl-4-nitro-1*H*-pyrazole-5-carboxamide (46a) White solid; 40% yield; mp 167 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.49 (bs, 1H), 8.34 (bs, 1H), 8.27 (s, 1H), 3.86 (s, 3H).

1-Methyl-4-nitro-1*H*-pyrazole-3-carboxamide (46b) White solid; 38% yield; mp 166 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.83 (s, 1H), 8.03 (bs, 1H), 7.79 (bs, 1H), 3.90 (s, 3H).

To a solution of nitroamide (**45**, 1.2 mmol) in anhydrous DMF (36 mL) was added K_2CO_3 (2.3 mmol) and the resulting mixture was stirred for 10'. Then, the bromopropane (1.4 mmol) was added and the mixture was stirred for 6 h. The solvent was removed *in vacuo* and the residue was suspended with water and extracted with EtOAc. The organic phase was dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure to obtain a crude solid which was purified *via* column chromatography (gradient from Etp/ EtOAc 7:3 to EtOAc) to obtain the desired products.

1-Propyl-4-nitro-1*H*-pyrazole-5-carboxamide (47a) White solid; 48% yield; mp 98-100 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.48 (bs, 1H), 8.28-8.27 (m, 2H), 4.08 (t, J = 6.8 Hz, 2H), 1.81-1.76 (m, 2H), 0.813 (t, J = 7.6 Hz, 3H).

1-Propyl-4-nitro-1*H*-pyrazole-3-carboxamide (47b) White solid; 43% yield; mp 115-116 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.87 (s, 1H), 7.98 (bs, 1H), 7.73 (bs, 1H), 4.10 (t, J = 6.8 Hz, 2H), 1.83-1.78 (m, 2H), 0.83 (t, J = 7.2 Hz, 3H).

To a solution of nitroamide (**45**, 1.2 mmol) in anhydrous DMF (36 mL) was added K_2CO_3 (2.3 mmol) and the resulting mixture was stirred for 10'. Then, $PhCH_2CH_2Cl$ (1.4 mmol) was added and the mixture was heated at 60 °C for 6 h. The solvent was removed *in vacuo* and the residue was suspended with water and extracted with EtOAc. The organic phase was dried over anhydrous Na_2SO_4 and the solvents removed under reduced pressure to obtain a crude solid which was purified *via* column chromatography (gradient from Etp/ EtOAc 4:1 to EtOAc) to obtain the desired products.

4-Nitro-1-(2-phenylethyl)-1*H*-pyrazole-5-carboxamide (48a) White solid; 25% yield; mp 133 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.45 (bs, 1H), 8.31 (bs, 1H), 8.25 (s, 1H), 7.29-7.16 (m, 5H), 4.38-4.35 (m, 2H), 3.09 (t, J = 7.6 Hz, 2H).

4-Nitro-1-(2-phenylethyl)-1*H*-pyrazole-3-carboxamide (48b) White solid; 50% yield; mp 158-160 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.74 (s, 1H), 8.00 (bs, 1H), 7.76 (bs, 1H), 7.30-7.18 (m,5H), 4.40 (t, J = 7.2 Hz, 2H), 3.13 (t, J = 7.6 Hz, 2H).

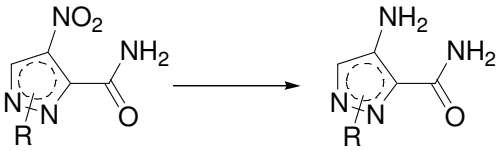
To a solution of nitroamide (**45**, 1.2 mmol) in anhydrous DMF (36 mL) was added K_2CO_3 (2.3 mmol) and the resulting mixture was stirred for 10'. Then, $Ph(CH_2)_3Cl$ (1.4 mmol) was added and the mixture was heated at 80 °C for 5 h. The solvent was removed *in vacuo* and the residue was suspended with water and extracted with EtOAc. The organic phase was dried over anhydrous Na_2SO_4 and

the solvents removed under reduced pressure to obtain a crude solid which was purified *via* column chromatography (gradient from Etp/ EtOAc 4:1 to EtOAc) to obtain the desired products.

4-Nitro-1-(3-phenylpropyl)-1*H*-pyrazole-5-carboxamide (49a)
White solid; 34% yield; mp 93-95 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.53 (bs, 1H), 8.33-8.32 (m, 2H), 7.30-7.19 (m, 5H), 4.16 (t, J = 6.8 Hz, 2H), 2.57 (t, J = 7.2 Hz, 2H), 2.11-2.08 (m, 2H).

4-Nitro-1-(3-phenylpropyl)-1*H*-pyrazole-3-carboxamide (49b)
White solid; 57% yield; mp 111-113 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.90 (s, 1H), 8.00 (bs, 1H), 7.77 (bs, 1H), 7.31-7.19 (m, 5H), 4.18 (t, J = 7.2 Hz, 2H), 2.59 (t, J =7.2 Hz, 2H), 2.15-2.12 (m, 2H).

General procedure for preparation of 4-amino-1-alkyl-1*H*-pyrazole-5-carboxamide and 4-amino-1-alkyl-1*H*-pyrazole-3-carboxamide (50-53a,b)



1.3 g of nitroamide (**46-49a,b**) were dissolved in EtOH (200 mL), 0.15 g of Pd on activated charcoal was added and the mixture was hydrogenated at 50 psi for 5 h at room temperature. The mixture was filtered on celite and then the solvent was removed under reduced pressure to obtain the crude product which was purified by crystallization EtOAc/ Et_2O/ Petroleum ether.

4-Amino-1-methyl-1*H* pyrazole-5-carboxamide (50a) White solid; 60% yield; mp 174-175 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 7.37 (bs, 2H), 7.01 (s, 1H); 4,39 (bs, 2H), 3.89 (s, 3H).

4-Amino-1-methyl-1*H* pyrazole-3-carboxamide (50b) White solid; 60% yield; mp 171-172 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 7.10 (bs, 1H), 7.06 (s, 1H), 6.95 (bs, 1H), 4.63 (bs, 2H), 3.72 (s, 3H).

4-Amino-1-propyl-1*H* pyrazole-5-carboxamide (**51a**) White solid; 65% yield; mp 91-92 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 7.40 (bs, 2H), 7.04 (s, 1H), 4.33-4.24 (bm, 4H), 1.69 (m, 2H), 0.75 (t, J = 7.6 Hz, 3H).

4-Amino-1-propyl-1*H* pyrazole-3-carboxamide (51b) White solid; 91% yield; mp 115 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 7.10 (s, 1H), 7.07 (bs, 1H), 6.95 (bs, 1H), 4.62 (bs, 2H), 3.92 (t, J = 6.8 Hz, 2H), 1.79-1.69 (m, 2H), 0.82 (t, J = 7.2 Hz, 3H).

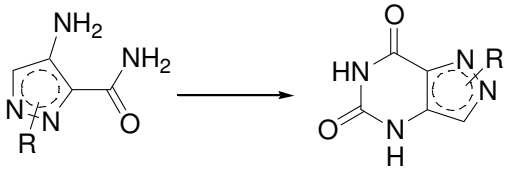
4-Amino-1-(2-phenylethyl)-1*H*-pyrazole-5-carboxamide (52a) White solid; 68% yield; mp 87-88 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 7.40 (bs, 2H), 7.27-7.15 (m, 5H), 7.05 (s, 1H), 4.53 (t, J = 7.6 Hz, 2H), 4.37 (bs, 2H), 2.94 (t, J = 7.8 Hz, 2H).

4-Amino-1-(2-phenylethyl)-1*H*-pyrazole-3-carboxamide (52b) White solid; 87% yield; mp 82-83 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 7.29-7.17 (m, 5H), 7.11 (bs, 1H), 7.04 (s, 1H), 6.97 (bs, 1H), 4.61 (bs, 2H), 4.22 (t, J =7 Hz, 2H), 3.07 (t, J = 7.6 Hz, 2H).

4-Amino-1-(3-phenylpropyl)-1*H*-pyrazole-5-carboxamide (53a) White solid; 87% yield; mp 78 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 7.42 (bs, 2H), 7.26-7.15 (m, 5H), 7.07 (s, 1H), 4.38-4.31 (m, 4H), 2.51-2.43 (m, 2H), 1.97-1.93 (m, 2H).

4-Amino-1-(3-phenylpropyl)-1*H*-pyrazole-3-carboxamide (53b)
White solid; 90% yield; mp 111-112 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 7.32-7.11 (m, 7H), 6.97 (bs, 1H), 4.65 (bs, 2H), 3.98 (t, J = 6.8 Hz, 2H), 2.58-2.49 (m, 2H), 2.08-2.00 (m, 2H).

General procedure for preparation of 1-alkyl-1*H*-pyrazolo[4,3-d]pyrimidine 5,7 (4*H*, 6*H*)-dione and 2-alkyl-2*H*-pyrazolo[4,3-d]pyrimidine 5,7 (4*H*, 6*H*)-dione (54-57a,b)



The urea (33.3 mmol) was added to aminoamide (**50-53a,b**, 7.1 mmol) and the mixture was heated at 200 °C for 2 h. The crude product was purified by crystallization NaOH 10%/ Acetic acid.

1-Methyl-1*H*-pyrazolo[4,3-*d*]pyrimidine 5,7 (4*H*, 6*H*)-dione (54a)
White solid; quantitative yield; mp >300 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 11.15 (bs, 1H), 10.96 (bs, 1H), 8.16 (s, 1H), 4.04 (s, 3H).

2-Methyl-2*H*-pyrazolo[4,3-*d*]pyrimidine 5,7 (4*H*, 6*H*)-dione (54b)
White solid; 81% yield; mp >300 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.87 (bs, 1H), 10.71 (bs, 1H), 7.64 (s, 1H), 3.94 (s, 3H). MS (ESI): $[MH]^+$ = 167.1.

1-Propyl-1*H*-pyrazolo[4,3-*d*]pyrimidine 5,7 (4*H*, 6*H*)-dione (55a)
White solid; quantitative yield; mp >300 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 9.34 (bs, 2H), 7.37 (s, 1H), 4.35 (t, J = 6.8 Hz, 2H), 1.81-1.70 (m, 2H), 0.78 (t, J = 7.2 Hz, 3H).

2-Propyl-2*H*-pyrazolo[4,3-*d*]pyrimidine 5,7 (4*H*, 6*H*)-dione (55b) White solid; 74% yield; mp >300 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.48 (bs, 2H), 7.68 (s, 1H), 4.14 (t, *J* = 6.8 Hz, 2H), 1.85-1.74 (m, 2H), 0.80 (t, *J* = 7.4 Hz, 3H).

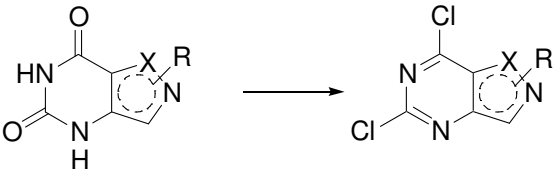
1-(2-Phenylethyl)-1*H*-pyrazolo[4,3-*d*]pyrimidine 5,7 (4*H*, 6*H*)-dione (56a) White solid; quantitative yield; mp >300 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 11.08 (bs, 2H), 7.34 (s, 1H), 7.27-7.10 (m, 5H), 4.62 (t, *J* = 7.2 Hz, 2H), 3.07 (t, *J* = 7.4 Hz, 2H).

2-(2-Phenylethyl)-2*H*-pyrazolo[4,3-*d*]pyrimidine 5,7 (4*H*, 6*H*)-dione (56b) White solid; 70% yield; mp >300 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.91 (bs, 1H), 10.38 (bs, 1H), 7.58 (s, 1H), 7.32-7.17 (m, 5H), 4.45 (t, *J* = 7.4 Hz, 2H), 3.14 (t *J* = 7.2 Hz, 2H).

1-(3-Phenylpropyl)-1*H*-pyrazolo[4,3-*d*]pyrimidine 5,7 (4*H*, 6*H*)-dione (57a) White solid; 63% yield; mp 280 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 11.19 (bs, 1H), 10.02 (bs, 1H), 7.38 (s, 1H), 7.26-7.154 (m, 5H), 4.43 (t, *J* = 6.8 Hz, 2H), 2.57-2.50 (m, 2H), 2.11-2.04 (m, 2H).

2-(3-Phenylpropyl)-2*H*-pyrazolo[4,3-*d*]pyrimidine 5,7 (4*H*, 6*H*)-dione (57b) White solid; 55% yield; mp >300 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.51 (bs, 2H), 7.71 (s, 1H), 7.28-7.17 (m, 5H), 4.22 (t, *J* =6.8 Hz, 2H), 2.58-2.48 (m, 2H), 2.15-2.11 (m, 2H).

General procedure for preparation of 2,4-dichloro-6-alkyl-6*H*-pyrrolo[3,4-*d*]pyrimidines (37a-d), 5,7-dichloro-1/2-alkyl-1/2*H*-pyrazole[4,3-*d*]pyrimidines (58-61a,b)



A mixture of pyrimidine-2,4-dione (**37a-d** and **58-61a,b**, 6.1 mmol) and phosphoroxy chloride (60 mmol) was heated at 50 °C under argon atmosphere and DBU (36.6 mmol) was added dropwise under vigorous stirring then the reaction was heated for further 8 h. After cooling at room temperature, the reaction mixture was slowly poured in cold water and treated with a 50% aqueous solution of NaOH until pH 4. The solution was extracted with Et_2O, the organic phase was dried over anhydrous Na_2SO_4 and the solvent was removed under reduced pressure to obtain the desired intermediate. Because these compounds are unstable, they were used for the next reaction step without further purification. The NMR analysis were performed on the crude product.

2,4-Dichloro-6-methyl-6*H*-pyrrolo[3,4-*d*]pyrimidine (37a) Pale yellow solid; 75% yield; crude product; ^{1}H NMR (400MHz, $CDCl_3$) δ ppm 7.32 (s, 1H), 7.24 (s, 1H), 4.07 (s, 3H). MS (ESI): $[MH]^+$ = 202.1.

2,4-Dichloro-6-propyl-6*H*-pyrrolo[3,4-*d*]pyrimidine (37b) Pale yellow solid; 63% yield; crude product; ^{1}H NMR (200MHz, $CDCl_3$) δ ppm 7.34 (d, *J* = 2.2 Hz, 1H), 7.25 (s, 1H), 4.20 (t, *J* = 7 Hz, 2H), 2.02-1.92 (m, 2H), 0.96 (t, *J* = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 230.1.

2,4-Dichloro-6-phenethyl-6*H*-pyrrolo[3,4-*d*]pyrimidine (37c) Pale yellow solid; 73% yield; crude product; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 7.88 (d, *J* = 2 Hz, 1H), 7.72 (d, *J* = 2 Hz, 1H), 7.29-7.19 (m, 5H), 4.59 (t, *J* = 7.2 Hz, 2H), 3.21 (t, *J* = 7.6 Hz, 2H).

2,4-Dichloro-6-(3-phenyl-propyl)-6*H*-pyrrolo[3,4-*d*]pyrimidine (37d) Pale yellow oil; 81% yield; crude product; ^{1}H NMR (400MHz, $CDCl_3$) δ ppm 7.33-7.14 (m, 7H), 4.23 (t, *J* = 7.2 Hz, 2H), 2.65 (t, *J* = 6.8 Hz, 2H), 2.31- 2.28 (m, 2H).

5,7-Dichloro-1-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidine (58a) Pale yellow solid; 63% yield; crude product; ^{1}H NMR (400MHz, $CDCl_3$) δ ppm 8.17 (s, 1H), 4.41 (s, 3H).

5,7-Dichloro-2-methyl-2*H*-pyrazolo[4,3-*d*]pyrimidine (58b) Pale yellow solid; 63% yield; crude product; ^{1}H NMR (400MHz, $CDCl_3$) δ ppm 8.17 (s, 1H), 4.41 (s, 3H).

5,7-Dichloro-1-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidine (59a) Pale yellow oil; 57% yield; crude product; ^{1}H NMR (200MHz, $CDCl_3$) δ ppm 8.20 (s, 1H), 4.69 (t, *J* = 7 Hz, 2H), 2.04-1.92 (m, 2H), 0.96 (t, *J* = 7.2 Hz, 3H).

5,7-Dichloro-2-propyl-2*H*-pyrazolo[4,3-*d*]pyrimidine (59b) Pale yellow solid; 55% yield; crude product; ^{1}H NMR (200MHz, $CDCl_3$) δ ppm 8.16 (s, 1H), 4.49 (t, *J* =7.2 Hz, 2H), 2.11-2.04 (m, 2H), 0.99 (t, *J* = 7.6 Hz, 3H).

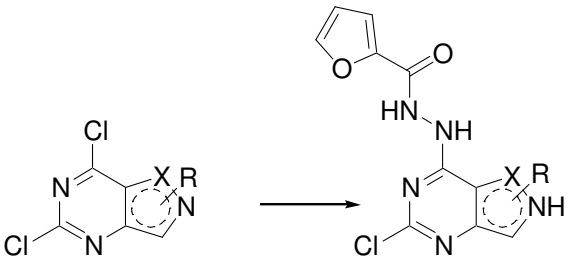
5,7-Dichloro-1-phenethyl-1*H*-pyrazolo[4,3-*d*]pyrimidine (60a) Pale yellow solid; 58% yield; crude product; ^{1}H NMR (200MHz, $CDCl_3$) δ ppm 8.22 (s, 1H), 7.26-7.23 (m, 3H), 7.10-7.07 (m, 2H), 4.94 (t, *J* = 7.4 Hz, 2H), 3.23 (t, *J* = 7.6 Hz, 2H).

5,7-Dichloro-2-phenethyl-2*H*-pyrazolo[4,3-*d*]pyrimidine (60b) Pale yellow solid; 50% yield; crude product; ^{1}H NMR (200MHz, $CDCl_3$) δ ppm 7.83 (s, 1H), 7.29-7.26 (m, 3H), 7.08-7.04 (m, 2H), 4.75 (t, *J* = 7.2 Hz, 2H), 3.34 (t, *J* = 7 Hz, 2H).

5,7-Dichloro-1-(3-phenyl-propyl)-1*H*-pyrazolo[4,3-*d*]pyrimidine (61a) Pale yellow solid; 58% yield; crude product; ^{1}H NMR (200MHz, $CDCl_3$) δ ppm 8.19 (s, 1H), 7.27-7.14 (m, 5H), 4.73 (t, *J* = 7 Hz, 2H), 2.71 (t, *J* = 7.4 Hz, 2H), 2.33-2.62 (m, 2H).

5,7-Dichloro-2-(3-phenyl-propyl)-2*H*-pyrazolo[4,3-*d*]pyrimidine (61b) Pale yellow solid; 62% yield; crude product; ^{1}H NMR (200MHz, $CDCl_3$) δ ppm 7.77 (s, 1H), 7.30-7.14 (m, 5H), 4.55-4.35 (m, 2H), 2.65 (t, *J* = 7.4 Hz, 2H), 2.45-2.30 (m, 2H).

General procedure for preparation of furan-2-carboxylic acid *N'*-(2-chloro-6-alkyl-6*H*-pyrrolo[3,4-*d*]pyrimidin-4-yl)-hydrazides (38a-d) and furan-2-carboxylic acid *N'*-(5-chloro-1/2-alkyl-1/2*H*-pyrazolo[4,3-*d*]pyrimidin-7-yl)-hydrazide (62-65a,b)



To a solution of dichloro derivative (**37a-d** and **58-61a,b**, 0.5 mmol) in anhydrous THF (4 mL) were added TEA (50 mmol) and furan-2-carboxylic acid hydrazide (69 mmol). The reaction was refluxed for 24 h. The solvent was removed under reduced pressure to obtain a

crude solid which was purified *via* column chromatography eluting with a mixture CH_2Cl_2/ CH_3OH 9:1.

Furan-2-carboxylic acid *N'*-(2-chloro-6-methyl-6*H*-pyrrolo[3,4-*d*]pyrimidin-4-yl)-hydrazide (38a) Pale yellow solid; 65% yield; mp 250-251 °C dec.; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 11.45 (bs, 1H), 10.40 (bs, 1H), 7.89 (s, 1H), 7.77 (t, *J* = 4.8 Hz, 1H), 7.40 (bs, 1H), 7.23 (d, *J* = 2.8 Hz, 1H), 6.66-6.65 (m, 1H), 3.45 (s, 3H). MS (ESI): $[MH]^+$ = 292.2.

Furan-2-carboxylic acid *N'*-(2-chloro-6-propyl-6*H*-pyrrolo[3,4-*d*]pyrimidin-4-yl)-hydrazide (38b) Pale yellow solid; 72% yield; mp 202-204 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.60 (bs, 1H), 9.91 (bs, 1H), 7.96 (s, 1H), 7.52 (s, 1H), 7.31-7.28 (m, 1H), 7.18 (bs,1H), 6.72-6.71 (m, 1H), 4.17 (m, 2H), 1.81 (m, 2H), 0.88-0.80 (m, 3H). MS (ESI): $[MH]^+$ = 320.2.

Furan-2-carboxylic acid *N'*-(2-chloro-6-phenethyl-6*H*-pyrrolo[3,4-*d*]pyrimidin-4-yl)-hydrazide (38c) Pale yellow solid; 48% yield; mp 209-212 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.58 (bs, 1H), 10.17 (bs, 1H), 7.95 (s, 1H), 7.45 (s, 1H), 7.27-7.12 (m, 8H), 6.71 (s, 1H), 4.47-4.41 (m, 2H), 3.15-3.12 (m, 1H). MS (ESI): $[MH]^+$ = 382.3.

Furan-2-carboxylic acid *N'*-[2-chloro-6-(3-phenyl-propyl)-6*H*-pyrrolo[3,4-*d*]pyrimidin-4-yl]-hydrazide (38d) Pale yellow solid; 56% yield; mp 204-206 °C dec.; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.63 (bs, 1H), 9.97 (bs, 1H), 7.96 (s, 1H), 7.55 (s, 1H), 7.32-6.99 (m, 7H), 6.71 (s, 1H), 4.22 (t, *J* = 6.8 Hz, 2H), 2.56 (t, *J* = 7.2 Hz, 2H), 2.16- 2.12 (m, 2H).

Furan-2-carboxylic acid *N'*-(5-chloro-1-methyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-7-yl)-hydrazide (62a) Pale yellow solid; 82% yield; mp

205 ℃ dec.; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.76 (bs, 1H), 9.98 (bs, 1H), 8.07 (s, 1H), 7.98 (d, *J* = 1.2 Hz, 1H), 7.33 (d, *J* = 3.2 Hz, 1H), 6.73-6.72 (m, 1H), 4.29 (s, 3H).

Furan-2-carboxylic acid *N'*-(5-chloro-2-methyl-2*H*-pyrazolo[4,3-*d*]pyrimidin-7-yl)-hydrazide (62b) White solid; 40% yield; mp 158 ℃ dec.; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.67 (bs, 1H), 10.61 (bs, 1H), 8.42 (s, 1H), 7.96 (d, *J* =1.2 Hz, 1H), 7.29 (d, *J* = 3.2 Hz, 1H), 6.72-6.70 (m, 1H), 4.19 (s, 3H). MS (ESI): $[MH]^+$ = 293.3.

Furan-2-carboxylic acid *N'*-(5-chloro-1-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-7-yl)-hydrazide (63a) White solid; 70% yield; mp 217 ℃ dec.; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.77 (bs, 1H), 9.86 (bs, 1H), 8.11 (s, 1H), 7.98-7.97 (m, 1H), 7.32 (d, *J* = 3.4 Hz, 1H), 6.74-6.71 (m, 1H), 4.61 (t, *J* = 7Hz, 2H), 1.85-1.75 (m, 2H), 0.80 (t, *J* = 7.4 Hz, 3H).

Furan-2-carboxylic acid *N'*-(5-chloro-2-propyl-2*H*-pyrazolo[4,3-*d*]pyrimidin-7-yl)-hydrazide (63b) White solid; 50% yield; mp 164-166 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.66 (bs, 2H), 8,48 (s, 1H), 7.96 (s, 1H), 7.29 (d, *J* = 3.6 Hz, 1H), 6.72-6.70 (m, 1H), 4.41 (t, *J* = 7.2 Hz, 2H), 1.97-1.90 (m, 2H), 0.87 (t, *J* = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 321.0.

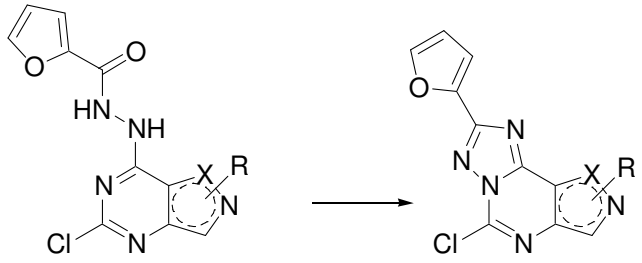
Furan-2-carboxylic acid *N'*-(5-chloro-1-phenethyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-7-yl)-hydrazide (64b) White solid; 60% yield; mp 197-199 ℃ dec.; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.78 (bs, 1H), 10.04 (bs, 1H), 8.03 (s, 1H), 7.98 (s, 1H), 7.33 (d, *J* = 3.4 Hz, 1H), 7.24-7.20 (m, 5H), 6.74-6.72 (m, 1H), 4.94-4.87 (m, 2H), 3.16-3.09 (m, 2H). MS (ESI): $[MH]^+$ = 383.0.

Furan-2-carboxylic acid *N'*-(5-chloro-2-phenethyl-2*H*-pyrazolo[4,3-*d*]pyrimidin-7-yl)-hydrazide (64b) White solid; 42% yield; mp 137-139 ℃ dec.; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.66 (bs, 2H), 8.37 (s, 1H),7.96 (s, 1H), 7.32-7.17 (m, 6H), 6.72-6.70 (m, 1H), 4.71 (t, *J* = 6.8 Hz, 2H), 3.36-3.24 (m, 2H). MS (ESI): $[MH]^+$ = 383.0.

Furan-2-carboxylic acid *N'*-[5-chloro-1-(3-phenyl-propyl)-1*H*-pyrazolo[4,3-*d*]pyrimidin-7-yl]-hydrazide (65a) Pale yellow solid; 50% yield; mp 115-116 ℃ dec.; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.79 (bs, 1H), 9.98 (bs, 1H), 8.10 (s, 1H), 7.99-7.98 (m, 1H), 7.34-7.16 (m, 6H), 6.74-6.71 (m, 1H), 4.70 (t, *J* = 7 Hz, 2H), 2.59-2.49 (m, 2H), 2.13-2.05 (m, 2H). MS (ESI): $[MH]^+$ = 397.0.

Furan-2-carboxylic acid *N'*-[5-chloro-2-(3-phenyl-propyl)-2*H*-pyrazolo[4,3-*d*]pyrimidin-7-yl]-hydrazide (65b) Pale yellow solid; 56% yield; mp 169-171 ℃ dec.; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.67 (bs, 2H), 8.51 (s, 1H), 7.97-7.96 (m, 1H), 7.30-7.19 (m, 6H), 6.72-6.70 (m, 1H), 4.49-4.43 (m, 2H), 2.62-2.49 (m, 2H), 2.29-2.21 (m, 2H). MS (ESI): $[MH]^+$ = 397.0.

General procedure for preparation of 5-chloro-2-furan-2-yl-8-alkyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines (39a-d) and 5-chloro-2-furan-2-yl-8/9-alkyl-8/9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidines (66-69a,b)



The pyrrolo/pyrazolepyrimidine (**38a-d**, **62-65a,b**, 0.2 mmol) was suspended in a mixture of HMDS (0.5 mL) and BSA (0.5 mL) and the reaction was heated at 120 °C for 15 h. The excess of reagents was removed under reduced pressure and the residue was purified via column chromatography eluting with a mixture CH_2Cl_2/ CH_3OH 9.5:0.5.

5-Chloro-2-furan-2-yl-8-methyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (39a) White solid; 75% yield; mp 275 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.02- 8.01 (m, 1H), 7.86 (d, *J* = 2Hz, 1H), 7.62 (d, *J* = 2 Hz, 1H), 7.04- 7.03 (m, 1H), 6.76- 6.74 (m, 1H), 3.96 (s, 3H). MS (ESI): $[MH]^+$ = 274.2.

5-Chloro-2-furan-2-yl-8-propyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (39b) White solid; 63% yield; mp 81-82 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 8.02 (d, *J* = 1 Hz, 1H), 7.93 (d, *J* = 2 Hz, 1H), 7.68 (d, *J* = 2 Hz, 1H), 7.02-7.01 (m, 1H), 6.76-6.75 (m, 1H), 4.18 (t, *J* = 7.4 Hz, 2H), 1.91-1.79 (m, 2H), 0.85 (t, *J* = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 302.1.

5-Chloro-2-furan-2-yl-8-phenethyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (39c) White solid; 60% yield; mp 182-184 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.02-8.01 (m, 1H), 7.87 (d, *J* = 2 Hz, 1H), 7.63 (d, *J* = 1.6 Hz, 1H), 7.29-7.20 (m, 5H), 7.02-7.01 (m, 1H), 6.75-6.74 (m, 1H), 4.49 (t, *J* = 7.2 Hz, 2H), 3.19 (t, *J* = 7.2 Hz, 2H). MS (ESI): $[MH]^+$ = 364.0.

5-Chloro-2-furan-2-yl-8-(3-phenyl-propyl)-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (39d) White solid; 65% yield; mp 138 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.02 (t, *J* = 0.8 Hz, 1H), 7.97 (d, *J* = 2 Hz, 1H), 7.72 (d, *J* = 2 Hz, 1H), 7.29-7.19 (m, 5H), 7.03

(d, *J* = 3.2 Hz, 1H), 6.76-6.74 (m, 1H), 4.26 (t, *J* = 6.8 Hz, 2H), 2.55 (t, *J* = 8.4 Hz, 2H, 2.18 (m, 2H). MS (ESI): $[MH]^+$ = 379.2.

5-Chloro-2-(2-furyl)-9-methyl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (66a) White solid; 43% yield; mp 221-223 ℃; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.31 (s, 1H), 8.08-8.07 (m, 1H), 7.10-7.09 (m, 1H), 6.80-6.79 (m, 1H), 4.42 (s, 3H). MS (ESI): $[MH]^+$ = 275.2.

5-Chloro-2-(2-furyl)-8-methyl-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (66b) White solid; 52% yield; mp 238 ℃; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.70 (s, 1H), 8.06-8.05 (m, 1H), 7.07-7.06 (m, 1H), 6.78-6.77 (m, 1H), 4.20 (s, 3H). MS (ESI): $[MH]^+$ = 275.2.

5-Chloro-2-(2-furyl)-9-propyl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (67a) White solid; 76% yield; mp 104-105 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 8.32 (s, 1H), 8.08-8.06 (m, 1H), 7.10-7.07 (m, 1H), 6.80-6.78 (m, 1H), 4.73 (t, *J* = 7 Hz, 2H), 2.02-1.99 (m, 2H), 0.88 (t, *J* = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 303.0.

5-Chloro-2-(2-furyl)-8-propyl-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (67b) White solid; 46% yield; mp 155 ℃; ^{1}H NMR (200MHz, $CDCl_3$) δ ppm 8.04 (s, 1H), 7.71-7.70 (m, 1H), 7.00-6.98 (m, 1H), 6.64-6.62 (m, 1H), 4.41 (t, *J* = 7.2 Hz, 2H), 2.10-2.06 (m, 2H), 0.97 (t, *J* = 7.6 Hz, 3H). MS (ESI): $[MH]^+$ = 303.0.

5-Chloro-2-(2-furyl)-9-(2-phenylethyl)-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (68a) White solid; 89% yield; mp 178 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 8.30 (s, 1H), 8.08-8.07

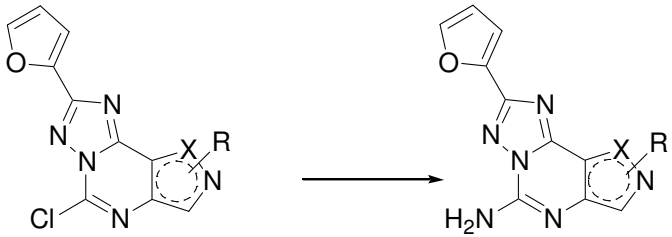
(m, 1H), 7.26-7.21 (m, 5H), 7.11-7.09 (m, 1H), 6.81-6.78 (m, 1H), 4.99 (t, *J* = 7.2 Hz, 2H), 3.33 (t, *J* = 7 Hz, 2H).

5-Chloro-2-(2-furyl)-8-(2-phenylethyl)-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (68b) White solid; 57% yield; mp 186 ℃; ^{1}H NMR (200MHz, $CDCl_3$) δ ppm 7.71-7.70 (m, 2H), 7.25-7.24 (m, 3H), 7.11-7.02 (m, 2H), 6.99 (m, 1H), 6.61 (m, 1H), 4.66 (t, *J* = 7.2 Hz, 2H), 3.35 (t, *J* = 7 Hz, 2H).

5-Chloro-2-(2-furyl)-9-(3-phenylpropyl)-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (69a) White solid; 76% yield; mp 155-157 ℃; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.34 (s, 1H), 8.08-8.07 (m, 1H), 7.25-7.15 (m, 5H), 7.10-7.09 (m, 1H), 6.80-6.79 (m, 1H), 4.81 (t, *J* = 6.8 Hz, 2H), 2.63 (t, *J* = 7.2 Hz, 2H), 2.33-2.29 (m, 2H). MS (ESI): $[MH]^+$ = 378.9.

5-Chloro-2-(2-furyl)-8-(3-phenylpropyl)-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (69b) White solid; 62% yield; mp 173 ℃; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.09 (s, 1H), 7.89-7.81 (m, 1H), 7.35-7.22 (m, 5H), 7.01-6.99 (m, 1H), 6.75-6.67 (m, 1H), 4.32 (t, *J* = 6.8 Hz, 2H), 2.54 (t, *J* = 7.2 Hz, 2H), 2.25-2.19 (m, 2H). MS (ESI): $[MH]^+$ = 378.9.

General procedure for preparation of 5-amino-2-furan-2-yl-8-alkyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (40a-d) and 5-amino-2-furan-2-yl-8/9-alkyl-8/9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (74-77a,b)



130 mg of opportune pyrrolo/pyrazolo-triazolo pyrimidine (**39a-d**,**66-69a,b**) were dissolved in 20 mL of EtOH previously saturated at 0 °C with ammonia. The mixture was heated in a steel bomb at 60 °C for 24 h. The solvent was removed under reduced pressure and the residue was purified *via* column chromatography eluting with a mixture CH_2Cl_2/ CH_3OH 9.5:0.5.

5-Amino-2-furan-2-yl-8-methyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (40a) White solid; 80% yield; mp 273 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 7.92 (d, J = 1 Hz, 1H), 7.55 (d, J = 2.2 Hz, 1H), 7.16 (t, J = 3.4 Hz, 1H), 7.01-7.00 (m, 3H), 6.72 (m, 1H), 3.87 (s, 3H). MS (ESI): $[MH]^+$ = 255.2.

5-Amino-2-furan-2-yl-8-propyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (40b) White solid; 75% yield; mp 176 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 7.92-7.90 (m, 1H), 7.61 (d, J = 2.2 Hz, 1H), 7.31 (bs, 1H), 7.17-7.15 (m, 1H), 7.06 (d, J = 2.2 Hz, 1H), 6.98 (s, 1H), 6.72-6.70 (m, 1H), 4.08 (t, J = 6.8 Hz, 2H), 1.84-1.77 (m, 1H), 0.84 (t, J = 7.4 Hz,3H). MS (ESI): $[MH]^+$ = 283.1.

5-Amino-2-furan-2-yl-8-phenethyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (40c) White solid; 77% yield; mp 148-150 ℃; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 7.91-7.90 (m, 1H), 7.53 (d, *J* = 2Hz, 1H), 7.29-7.20 (m, 5H), 7.16-7.15 (m, 1H), 7.05 (d, *J* = 2 Hz, 1H), 6.99 (bs, 2H), 6.71-6.70 (m, 1H), 4.38 (t, *J* = 7.2 Hz, 2H), 3.15 (t, *J* = 7.2 Hz, 2H). MS (ESI): $[MH]^+$ = 345.4.

5-Amino-2-furan-2-yl-8-(3-phenyl-propyl)-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (40d) White solid; 77% yield; mp 155-157 ℃;^{1}H NMR (400MHz, $CDCl_3$) δ ppm 7.62 (bs, 1H), 7.44 (bs, 1H), 7.30-7.15 (bm, 6H), 6.97 (bs, 1H), 6.59 (bs, 1H), 6.19 (bs, 2H), 4.08 (bm, 2H), 2.61 (bm, 2H), 2.23 (bm, 2H). MS (ESI): $[MH]^+$ = 359.2.

5-Amino-2-(2-furyl)-9-methyl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (74a) White solid; 72% yield; mp 270 ℃; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 7.98-7.97 (m, 1H), 7.85 (s, 1H), 7.47 (bs, 2H), 7.27-7.26 (m, 1H), 6.76-6.75 (m, 1H), 4.28 (s, 3H).

5-Amino-2-(2-furyl)-8-methyl-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (74b) White solid; 77% yield; mp 295 ℃; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.14 (s, 1H), 7.96 (d, *J* = 1.2 Hz, 1H), 7.37 (bs, 2H), 7.24 (d, *J* = 3.6 Hz, 1H), 6.75-6.74 (m, 1H), 4.11 (s, 3H).

5-Amino-2-(2-furyl)-9-propyl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (75a) White solid; 82% yield; mp 229 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 7.98 (s, 1H), 7.88 (s, 1H), 7.47 (bs, 2H), 7.26 (d, *J* =3.2 Hz, 1H), 6.77-6.74 (m, 1H), 4.58 (t, *J* = 7 Hz, 2H), 1.99-1.95 (m, 2H), 0.85 (t, *J* = 7.6 Hz, 3H).

5-Amino-2-(2-furyl)-8-propyl-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (75b) White solid; 78% yield; mp 198 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 8.18 (s, 1H), 7.96-7.95 (m, 1H), 7.36 (bs, 2H), 7.25-7.23 (m, 1H), 6.76-6.73 (m, 1H), 4.32 (t, *J* = 7 Hz, 2H), 1.94-1.90 (m, 2H), 0.86 (t, *J* = 7.4 Hz, 3H).

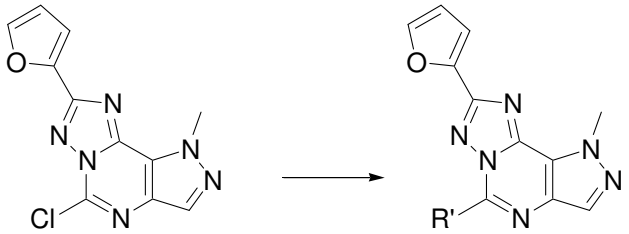
5-Amino-2-(2-furyl)-9-(2-phenylethyl)-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (76a) White solid; 85% yield; mp 239 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 7.99 (d, *J* = 1.2 Hz, 1H), 7.85 (s, 1H), 7.47 (bs, 2H), 7.28-7.14 (m, 6H), 6.78-6.76 (m, 1H), 4.84 (t, *J* = 6.8 Hz, 2H), 3.33-3.24 (m, 2H).

5-Amine-2-(2-furyl)-8-(2-phenylethyl)-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (76b) White solid; 82% yield; mp 218 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 8.07 (s, 1H), 7.96 (d, *J* = 1Hz, 1H), 7.37 (bs, 2H), 7.27-7.18 (m, 6H), 6.76-6.74 (m, 1H), 4.61 (t, *J* = 7 Hz, 2H), 3.35-3.25 (m, 2H).

5-Amino-2-(2-furyl)-9-(3-phenylpropyl)-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (77a) White solid; 78% yield; mp 208 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 7.99-7.98 (m, 1H), 7.89 (s, 1H), 7.48 (bs, 2H), 7.25-7.15 (m, 6H), 6.78-6.75 (m, 1H), 4.63 (t, *J* = 7 Hz, 2H), 2.61 (t, *J* = 7.2 Hz, 2H), 2.32-2.24 (m, 2H).

5-Amino-2-(2-furyl)-8-(3-phenylpropyl)-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (77b) White solid; 84% yield; mp 161-163 ℃; ^{1}H NMR (200MHz, $CDCl_3$) δ ppm 7.78 (s, 1H), 7.65-7.63 (m, 1H), 7.37-7.16 (m, 6H), 6.62-6.60 (m, 1H), 6.21 (bs, 2H), 4.37 (t, *J* = 7.2 Hz, 2H), 2.66 (t, *J* = 7.8 Hz, 2H), 2.41-2.37 (m, 2H).

General procedure for preparation of 5-alkyl-2-(2-furyl)-9-methyl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (70-73)



50 mg of pyrazolo-triazolo pyrimidine (**66a**) were dissolved in 2 mL of 2-methoxyethanol and 1 mL of the opportune amine was added to the solution. The mixture was heated in a steel bomb at 100 °C for 4-5 h. The solvent was removed under reduced pressure and the residue was purified via column chromatography eluting with a mixture EtP/ EtOAc 1:4.

***N*-cyclohexyl-2-(2-furyl)-9-methyl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (70)** White solid; 76% yield; mp 175 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 7.98 (m, 1H), 7.94 (s, 1H), 7.37 (d, J = 8 Hz, 1H), 7.29-7.28 (m, 1H), 6.76-6.75 (m, 1H), 4.28 (s, 3H), 4.12-3.82 (m, 1H), 1.95-1.13 (m, 10H).

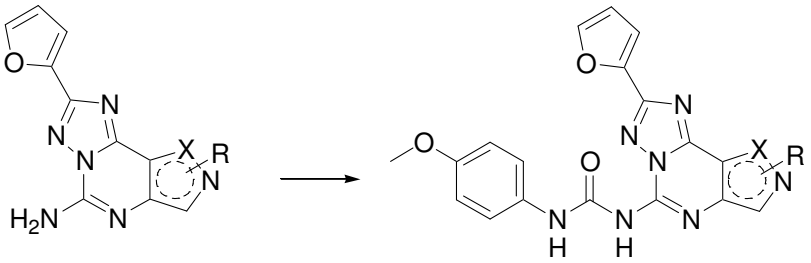
2-(2-furyl)-9-methyl-5-morpholin-4-yl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (71) White solid; 68% yield; mp 193 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.15 (s, 1H), 8.08 (m, 1H), 7.14-7.13 (m, 1H), 6.83-6.82 (m, 1H), 4.38 (s, 3H), 3.33 (m, 4H), 2.98 (m, 4H).

2-(2-furyl)-9-methyl-5-(4-methylpiperazin-1-yl)-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (72) White solid; 73% yield; mp 159-160 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.13 (s, 1H), 8.05-

8.04 (m, 1H), 7.11-7.10 (m, 1H), 6.81-6.80 (m, 1H), 4.37 (s, 3H), 3.33 (m, 4H), 3.01 (m, 4H), 2.10 (s, 3H).

2-(2-furyl)-9-methyl-5-(4-phenylpiperazin-1-yl)-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (73) White solid; 67% yield; mp 105-106 ℃; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 8.16 (s, 1H),8.03-8.02 (m, 1H), 7.23-7.19 (m, 2H), 7.15-7.14 (m, 1H), 6.91-6.89 (m, 2H), 6.80-6.77 (m, 2H), 4.39 (s, 3H), 3.58 (m, 4H), 3.15 (m, 4H).

General procedure for preparation of 5-{[(4-methoxy-phenyl)carbamoyl]amino}-8-alkyl-(2-furan-2-yl)-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (41a-c) and 5-{[(4-methoxy-phenyl)carbamoyl]amino}-8/9-alkyl-(2-furan-2-yl)-8/9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (78-81a, 78-79b)



To a solution of the free amino derivative (**40a-c, 78-81a, 78-79b,** 0.27 mmol) in anhydrous THF (5 mL) was added 4-methoxy-phenyl-isocyanate (53.4 mmol) and the mixture was heated at 50 ℃ for 12h. The solvent was removed under reduced pressure and the residue was purified via column chromatography eluting with CH_2Cl_2. The crude solid was purified by crystallization from CH_3OH.

5-{[(4-methoxy-phenyl)carbamoyl]amino}-(2-furan-2-yl)-8-methyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (41a)

White solid; 37% yield; mp 214-216 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.68 (bs, 1H), 9.18 (bs, 1H), 7.96-7.95 (m, 1H), 7.74 (d, *J* = 2Hz, 1H), 7.52 (dd, *J* = 9.2 Hz, 2H), 7.45 (bs, 1H), 7.26 (d, *J* = 3.2 Hz, 1H), 6.81 (dd, *J* = 9 Hz, 2H), 6.75-6.73 (m, 1H), 3.95 (s, 3H), 3.76 (s, 3H). MS (ESI): $[MH]^+$ = 404.3.

5-{[(4-methoxy-phenyl)carbamoyl]amino}-(2-furan-2-yl)-8-propyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (41b) White solid; 40% yield; mp 198-199℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.72 (bs, 1H), 9.16 (bs, 1H), 7.97-7.96 (m, 1H), 7.55-7.50 (m, 3H), 7.26 (d, *J* = 3.4 Hz, 1H), 6.95 (d, *J* = 9.2 Hz, 2H), 6.76-6.73 (m, 1H), 4.18 (t, *J* = 7.4 Hz, 2H), 3.75 (s, 3H), 1.89-1.85 (m, 2H), 0.86 (t, *J* = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 432.1.

5-{[(4-methoxy-phenyl)carbamoyl]amino}-(2-furan-2-yl)-8-phenylethyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (41c) White solid; 35% yield; mp 181-183 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.66 (bs, 1H), 9.14 (bs, 1H), 7.96 (s, 1H), 7.74 (d, *J* = 2Hz, 1H), 7.54 (d, *J* = 2Hz, 1H), 7.51 (dd, *J* = 9Hz, 2H), 7.28-7.21 (m, 6H), 6.95 (dd, *J* = 9Hz, 2H), 6.76-6.73 (m, 1H), 4.49 (t, *J* = 7.2 Hz, 2H), 3.76 (s, 3H), 3.20 (t, *J* = 7.2 Hz, 2H). MS (ESI): $[MH]^+$ = 494.2.

5-{[(4-pyridinyl)-carbamoyl]amino}-2-(2-furyl)-8-methyl-8*H*-pyrrolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (41e) White solid; 45% yield; mp 205-207 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.8 (bs, 1H), 9.69 (bs, 1H),8.45-8.44 (m, 2H), 7.96-7.95 (m, 1H), 7.75 (m, 1H), 7.60-7.58 (m, 2H), 7.48 (m, 1H), 7.25-7.24 (m, 1H), 6.75-6.73 (m, 1H), 3.96 (s, 3H).

5-{[(4-methoxy-phenyl)carbamoyl]amino}-2-(2-furyl)-9-methyl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5*c*]pyrimidine (78a) White solid; 55% yield; mp 223 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.35 (bs, 1H), 9.62 (bs, 1H), 8.24 (s, 1H), 8.03-8.02 (m, 1H), 7.51 (dd, *J* = 8.8 Hz, 2H), 7.37 (d, *J* = 3.2 Hz, 1H), 6.95 (dd, *J* = 8.8 Hz, 2H), 6.78-6.78 (m, 1H), 4.36 (s, 3H), 3.75 (s, 3H). MS (ESI): $[MH]^+$ = 405.0.

5-{[(4-methoxy-phenyl)carbamoyl]amino}-2-(2-furyl)-8-methyl-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (78b) White solid; 61% yield; mp 253-255 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.38 (bs, 1H), 9.50 (bs, 1H), 8.56 (s, 1H), 8.01-8.00 (m, 1H), 7.50 (dd, *J* = 9.2 Hz, 2H), 7.34 (d, *J* = 2.8 Hz, 1H), 6.95 (dd, *J* = 9.2 Hz, 2H), 6.78-6.77 (m, 1H), 4.20 (s, 3H), 3.75 (s, 3H). MS (ESI): $[MH]^+$ = 405.0.

5-{[(4-methoxy-phenyl)carbamoyl]amino}-2-(2-furyl)-9-propyl-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (79a) White solid; 50% yield; mp 202 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.37 (bs, 1H), 9.61 (bs, 1H), 8.26 (s, 1H), 8.02 (d, *J* =1 Hz, 1H),7.52 (dd, *J* = 8.8 Hz, 2H), 7.36 (d, *J* = 2.6 Hz, 1H), 6.95 (d, *J* = 9Hz, 2H), 6.78-6.77 (m, 1H), 4.66 (t, *J* = 6.6 Hz, 2H), 3.75 (s, 3H), 2.08-1.99 (m, 2H), 0.87 (t, *J* = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 433.0.

5-{[(4-methoxy-phenyl)carbamoyl]amino}-2-(2-furyl)-8-propyl-8*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (79b) White solid; 40% yield; mp 238 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.43 (bs, 1H), 9.58 (bs, 1H), 8.60 (s, 1H), 8.01 (s, 1H), 7.51 (dd, *J* = 8.8 Hz, 2H), 7.33 (d, *J* =3.4 Hz, 1H), 6.95 (dd, *J* = 9 Hz, 2H), 6.79-

6.76 (m, 1H), 4.41 (t, *J* =6.8 Hz, 2H), 3.75 (s, 3H), 1.98-1.94 (m, 2H), 0.88 (t, *J* = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 433.1.

5-{[(4-methoxy-phenyl)carbamoyl]amino}-2-(2-furyl)-9-(2-phenylethyl)-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (80a) White solid; 27% yield; mp 199 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.39 (bs, 1H), 9.55 (bs, 1H), 8.23 (s, 1H), 8.04 (s, 1H), 7.51 (dd, *J* =8.8 Hz, 2H), 7.37 (d, *J* = 3.6 Hz, 1H), 7.24-7.23 (m, 5H), 6.95 (dd, *J* = 8.8 Hz, 2H), 6.80 (d, *J* = 1.2 Hz, 1H), 4.92 (t, *J* = 7 Hz, 2H), 3.75 (s, 3H), 3.34-3.27 (m, 2H).

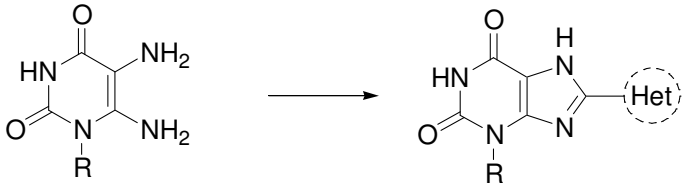
5-{[(4-methoxy-phenyl)carbamoyl]amino}-2-(2-furyl)-9-(3-phenylpropyl)-9*H*-pyrazolo[3,4-*e*][1,2,4]triazolo[1,5-*c*]pyrimidine (81a) White solid; 32% yield; mp 205-207 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.39 (bs, 1H), 9.68 (bs, 1H), 8.26 (s, 1H), 8.03-8.02 (m, 1H), 7.52 (dd, *J* =9 Hz, 2H), 7.33-7.31 (m, 1H), 7.22-7.19 (m, 5H), 6.95 (dd, *J* = 9 Hz, 2H), 6.81-6.78 (m, 1H), 4.72 (t, *J* = 7 Hz, 2H), 3.75 (s, 3H), 2.62 (t, *J* = 7.2 Hz, 2H), 2.39-2.21 (m, 2H).

Experimental section of imidazo[2,1-*i*]purin-5-one derivatives

Chemical Material and Methods Reaction progress and product mixtures were monitored by thin-layer chromatography (TLC) on silica gel (precoated F254 Merck plates) and visualized with aqueous potassium permanganate. ^{1}H NMR data were determined in $CDCl_3$ or DMSO-d_6 solutions with a Varian VXR 200 spectrometer or a Varian Mercury Plus 400 spectrometer. Peak positions are given in parts per million (δ) downfield from tetramethylsilane as internal standard, and *J* values are given in hertz. Light petroleum refers to the fractions boiling at 40-60 °C. Melting points were determined on a Buchi-Tottoli instrument and are uncorrected. Chromatography was performed on Merck 230-400 mesh silica gel. Organic solutions were dried over anhydrous sodium sulfate. Chiral amino alcohols were purchased from Alfa Aesar or Aldrich in the highest available purity grade. The mass spectra were obtained on a ESI Micromass ZMD 2000 mass spectrometer.

All final compounds revealed a purity of not less then 95%.

General procedure for the synthesis of 3-allyl/benzyl-8-[(substituted)isoxazol/pyrazol-3/5-yl]-1*H*-purine-2,6(3*H*,7*H*)-dione derivatives (96a-j).



To a solution of the appropriately substituted isoxazol/pyrazol-carboxylic acid (**87a,b, 91a,b, 94**, 2.5 mmol) in DMF (7 mL),

EDC·HCl (2.5 mmol, *N*-Ethyl-*N'*-(3-dimethylamino-propyl)-carbodiimide hydrochloride) and HOBt (2.5 mmol, hydroxybenzotriazole) were added. The mixture was stirred at room temperature for 10' then, 5,6-diamino-1-allyl/benzyl-1H-pyrimidine-2,4-dione (**95a,b**, 2.5 mmol) was added. The mixture was stirred for further 24 h then the solvent was evaporated and the residue was suspended with H_2O (30 mL) to favor the precipitation of a solid which was subsequently filtrated and washed with cold H_2O. The solid was suspended with a 10% aqueous solution of NaOH and the mixture was refluxed for 1.5 h. The reaction was cooled at 0°C and acidified with a 10% aqueous solution of HCl to obtain a precipitate which was filtered, washed with cold water and finally crystallized from DMF-H_2O.

3-Allyl-8-(1,3-dimethyl-1*H*-pyrazol-5-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (96a) Pale white solid; 65% yield; mp >300 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.83 (bs, 1H), 11.24 (bs, 1H), 6.77 (s,1H), 5.97-5.88 (m, 1H), 5.22-5.14 (m, 2H), 4.56 (m, 2H), 4.11 (s, 3H), 2.18 (s, 3H).

3-Benzyl-8-(1,3-dimethyl-1*H*-pyrazol-5-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (96b) White solid; 57% yield; mp >300 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.90 (bs, 1H), 11.25 (s, 1H), 7.32 (m, 5H), 6.75 (s, 1H), 5.15 (s, 2H), 4.10 (s, 3H), 2.17 (s, 3H).

3-Allyl-8-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (96c) White solid; 81% yield; mp >300 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.93 (bs, 1H), 11.27 (s, 1H), 6.45 (s, 1H), 6.11-5.92 (m, 1H), 5.22-5.13 (m, 2H), 4.57 (d, J = 5 Hz, 2H), 4.06 (s, 3H), 3.79 (s, 3H).

3-Benzyl-8-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (96d) White solid; 60% yield; mp >300 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.89 (bs, 1H), 11.32 (s, 1H), 7.43-7.22 (m, 5H), 6.41 (s, 1H), 5.15 (s, 2H), 4.05 (s, 3H), 3.79 (s, 3H).

3-Allyl-8-(3-(benzyloxy)-1-methyl-1*H*-pyrazol-5-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (96e) White solid; 57% yield; mp 296 ℃ dec.; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.87 (bs, 1H), 11.27 (s, 1H), 7.43-7.36 (m, 5H), 6.46 (s, 1H), 6.02-5.87 (m, 1H), 5.16-5.13 (m, 4H), 4.57 (d, J = 5 Hz, 2H), 4.07 (s, 3H).

3-Benzyl-8-(3-(benzyloxy)-1-methyl-1*H*-pyrazol-5-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (96f) White solid; 58% yield; mp >300 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.94 (bs, 1H), 11.32 (s, 1H), 7.43-7.29 (m, 10H), 6.45 (s, 1H), 5.16 (s, 4H), 4.06 (s, 3H).

3-Allyl-8-(1,5-dimethyl-1H-pyrazol-3-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione(96g) White solid; 66% yield; mp >300 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.61 (bs, 1H), 11.08 (s, 1H), 6.65 (s, 1H), 5.87 (m, 1H), 5.15-5.04 (m, 2H), 4.56 (m, 2H), 3.80 (s, 3H), 2.29 (s, 3H).

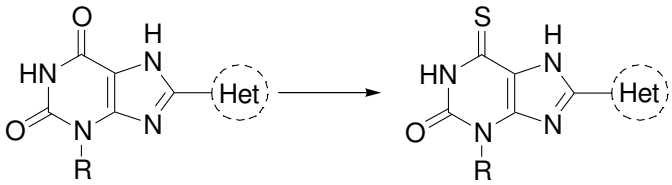
3-Benzyl-8-(1,5-dimethyl-1*H*-pyrazol-3-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (96h) White solid; 77% yield; mp >300 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.45 (bs, 1H), 11.14 (s, 1H), 7.33-7.10 (m, 5H), 6.65 (s, 1H), 5.15 (s, 2H), 3.79 (s, 3H), 2.29 (s, 3H).

3-Allyl-8-(3-methoxyisoxazol-5-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (96i) Pale white solid; 45% yield; mp 297 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 14.55 (bs, 1H), 11.37 (s, 1H), 6.84 (s, 1H), 6.01-5.87 (m, 1H), 5.16-5.06 (m, 2H), 4.56 (m, 2H), 3.96 (s, 3H).

3-Benzyl-8-(3-methoxyisoxazol-5-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (96j) Pale yellow solid; 40% yield; mp 284 ℃ dec.; ^{1}H NMR

(200MHz, DMSO-d_6) δ ppm 14.45 (bs, 1H),11.41 (bs, 1H), 7.34-7.22 (m, 5H), 6.84 (s, 1H), 5.15 (s, 2H), 3.96 (s, 3H).

General procedure for the synthesis of 3-allyl/benzyl-1,6-dihydro-8-[(substituted)isoxazol/pyrazol-3/5-yl]-6-thioxo-3*H*-purin-2(7*H*)-one derivatives (97a-j)[165]



The appropriate 3-allyl/benzyl-8-[(substituted)isoxazol/pyrazol-3/5-yl]-1*H*-purine-2,6(3H,7H)-dione derivative (**96a-j**, 1.5 mmol) was dissolved with pyridine (10 mL) and 2.55 mmol of P_2S_5 were added. The reaction was vigorously stirred at 140°C for 5h then cooled at 0°C. After the addition of water (30 mL) a pale green solid precipitated which was filtered, washed with cold water and crystallized from DMF/H_2O.

3-Allyl-1,6-dihydro-8-(1,3-dimethyl-1*H*-pyrazol-5-yl)-6-thioxo-3*H*-purin-2(7*H*)-one (97a) Pale yellow solid; 95% yield; mp 272 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.80 (bs, 1H), 12.40 (s, 1H), 7.01 (s, 1H), 6.10-5.80 (m, 1H), 5.25-5.16 (m, 2H), 4.59 (d, 2H, J = 5.8), 4.12 (s, 3H), 2.19 (s, 3H).

3-Benzyl-1,6-dihydro-8-(1,3-dimethyl-1*H*-pyrazol-5-yl)-6-thioxo-3*H*-purin-2(7*H*)-one (97b) Yellow solid; 87% yield; mp 265 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.66 (bs, 1H), 12.28 (bs, 1H), 7.32 (m, 5H), 6.86 (s, 1H), 5.17 (s, 2H), 3.81 (s, 3H), 2.30 (s, 3H).

3-Allyl-1,6-dihydro-8-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-6-thioxo-3*H*-purin-2(7*H*)-one (97c) Pale yellow; 82% yield; mp 272 °C dec; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.81 (bs, 1H), 12.43 (bs, 1H), 6.68 (s, 1H), 6.11-5.97 (m, 1H), 5.25-5.16 (m, 2H), 4.60 (d, J = 4.6 Hz, 2 H), 4.07 (s, 3H), 3.80 (s, 3H).

3-Benzyl-1,6-dihydro-8-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-6-thioxo-3*H*-purin-2(7*H*)-one (97d) Pale yellow solid; 84% yield; mp 293-295 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.92 (bs, 1H), 12.48 (bs, 1H), 7.40-7.30 (m, 5H), 6.68 (s, 1H), 5.18 (s, 2H), 4.06 (s, 3H), 3.80 (s, 3H).

3-Allyl-8-(3-(benzyloxy)-1-methyl-1*H*-pyrazol-5-yl)-1,6-dihydro-6-thioxo-3*H*-purin-2(7*H*)-one (97e) Pale yellow solid; 71% yield; mp 241 °C dec.; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.82 (bs, 1H), 12.43 (bs, 1H), 7.44-7.36 (m, 5H), 6.71 (s, 1H), 6.15-5.82 (m, 1H), 5.25-5.16 (m, 2H), 4.60 (d, J = 4 Hz, 2H), 4.08 (s, 3H).

3-Benzyl-8-(3-(benzyloxy)-1-methyl-1*H*-pyrazol-5-yl)-1,6-dihydro-6-thioxo-3*H*-purin-2(7*H*)-one (97f) Pale yellow solid; 96% yield; mp 244 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.89 (bs, 1H), 12.50 (s, 1H), 7.47-7.30 (m, 10H), 6.72 (s, 1H), 5.19 (s, 2H), 5.17 (s, 2H), 4.08 (s, 3H).

3-Allyl-1,6-dihydro-8-(1,5-dimethyl-1*H*-pyrazol-3-yl)-6-thioxo-3*H*-purin-2(7*H*)-one (97g) Yellow solid; yield 83%; mp 280-282 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.60 (bs, 1H), 12.23 (bs, 1H), 6.86 (s, 1H), 5.97-5.89 (m, 1H), 5.18-5.06 (m, 2H), 4.58 (d, J = 4.6 Hz, 2H), 3.82 (s, 3H), 2.31 (s, 3H).

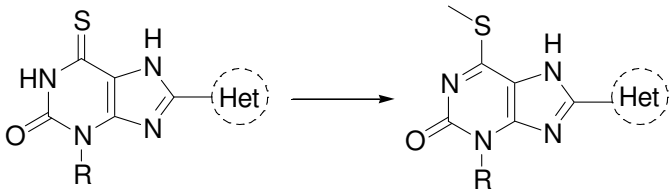
3-Benzyl-1,6-dihydro-8-(1,5-dimethyl-1*H*-pyrazol-3-yl)-6-thioxo-3*H*-purin-2(7*H*)-one (97h) Yellow solid; yield 87 %; mp 288 °C; ^{1}H

NMR (200MHz, DMSO-d_6) δ ppm 13.63 (bs, 1H), 12.26 (bs, 1H), 7.30 (m, 5H), 6.84 (s, 1H), 5.15 (s, 2H), 3.78 (s, 3H), 2.28 (s, 3H).

3-Allyl-1,6-dihydro-8-(3-methoxyisoxazol-5-yl)-6-thioxo-3*H*-purin-2(7*H*)-one (97i) Pale yellow solid; 75% yield; mp 254 °C dec.; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 14.51 (bs, 1H), 12.61 (bs, 1H), 8.63 (s, 1H), 6.12-5.82 (m, 1H), 5.21 (m, 2H), 4.62 (m, 2H), 4.02 (s, 3H).

3-Benzyl-1,6-dihydro-8-(3-methoxyisoxazol-5-yl)-6-thioxo-3*H*-purin-2(7*H*)-one (97j) Pale green solid; 96 % yield; mp 205 °C dec. ; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 14.45 (bs, 1H), 12.62 (bs, 1H), 8.59 (s, 1H), 7.43-7.19 (m, 5H), 5.17 (s, 2H), 3.97 (s, 3H).

General procedure for the synthesis of 3-allyl/benzyl-8-[(substituted)isoxazol/pyrazol-3/5-yl]-6-(methylthio)-3*H*-purin-2(7*H*)-one derivatives (98a-j)



The appropriate 3-allyl/benzyl-1,6-dihydro-8-[(substituted)isoxazol/pyrazol-3/5-yl]-6-thioxo-3*H*-purin-2(7*H*)-one derivative (**97a-j**, 1 mmol) was dissolved in 10 mL of NaOH 0.5N and EtOH (5 mL). After cooling at 0°C, CH_3I (1.5 mmol) was added and the reaction was stirred at room temperature for 3h. The mixture was acidified with HCl 5% to obtain a precipitate which was collected by filtration, washed with cold H_2O and dried under vacuum.

3-Allyl-8-(1,3-dimethyl-1*H*-pyrazol-5-yl)-6-(methylthio)-3*H*-purin-2(7*H*)-one (98a) Pale white solid; 68% yield; mp 250-252 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.63 (bs, 1H), 6.82 (s 1H), 6.17-5.85 (m, 1H), 5.19 (m, 2H), 4.68 (d, J =5.2 Hz, 2H), 4.14 (s, 3H), 2.63 (s, 3H), 2.21 (s, 3H).

3-Benzyl-8-(1,3-dimethyl-1*H*-pyrazol-5-yl)-6-(methylthio)-3*H*-purin-2(7*H*)-one (98b) Yellow solid; 93% yield; mp 163-165 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.76 (bs, 1H), 7.30-7.26 (m, 5H), 6.69 (s, 1H), 5.25 (s, 2H), 3.83 (s, 3H), 2.56 (s, 3H), 2.32 (s, 3H).

3-Allyl-8-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-6-(methylthio)-3*H*-purin-2(7*H*)-one (98c) Pale yellow solid; 78% yield; mp 243 °C dec. ; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.63 (bs, 1H), 6.39 (s, 1H), 6.15-5.86 (m, 1H), 5.17-5.11 (m, 2H), 4.66 (d, J = 4.8 Hz, 2H), 4.07 (s, 3H), 3.80 (s, 3H), 2.62 (s, 3H).

3-Benzyl-8-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-6-(methylthio)-3*H*-purin-2(7*H*)-one (98d) White solid; 48% yield; mp 268-270 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.73 (bs, 1H), 7.39-7.27 (m, 5H), 6.46 (s, 1H), 5.25 (s, 2H), 4.08 (s, 3H), 3.81 (s, 3H), 2.63 (s, 3H).

3-Allyl-8-(3-(benzyloxy)-1-methyl-1*H*-pyrazol-5-yl)-6-(methylthio)-3*H*-purin-2(7*H*)-one (98e) Pale yellow solid; 85% yield; mp 224 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.79 (bs, 1H), 7.4-7.34 (m, 5H), 6.53 (s, 1H), 6.15-5.87 (m, 1H), 5.19-5.11 (m, 4H), 4.68 (d, J = 4.4 Hz, 2H), 4.09 (s, 3H), 2.62 (s, 3H).

3-Benzyl-8-(3-(benzyloxy)-1-methyl-1*H*-pyrazol-5-yl)-6-(methylthio)-3*H*-purin-2(7*H*)-one (98f) White solid; 87% yield; mp 228 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.79 (bs, 1H), 7.43-

7.27 (m, 5H), 6.47 (s, 1H), 5.26 (s, 2H), 5.18 (s, 2H), 4.10 (s, 3H), 2.63 (s, 3H).

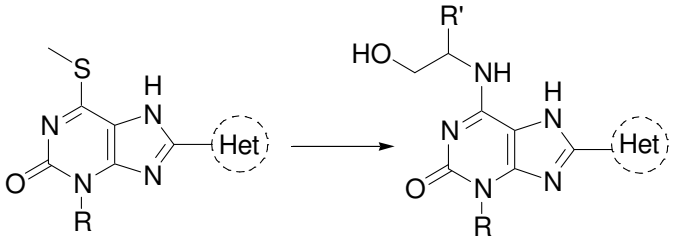
3-Allyl-8-(1,5-dimethyl-1H-pyrazol-3-yl)-6-(methylthio)-3*H*-purin-2(7*H*)-one (98fg) White solid; 95% yield; mp 242-244 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.99 (bs, 1H), 6.71 (s, 1H), 6.11-5.85 (m, 1H), 5.16-5.03 (m, 2H), 4.67 (d, J = 4.6 Hz, 2H), 3.84 (s, 3H), 2.61 (s, 3H), 2.32 (s, 3H).

3-Allyl-8-(1,5-dimethyl-1*H*-pyrazol-3-yl)-6-(methylthio)-3*H*-purin-2(7*H*)-one (98h) White solid; 82% yield; mp 183 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 13.80 (bs, 1H), 7.30 (m, 5H), 6.69 (s, 1H), 5.25 (s, 2H), 3.83 (s, 3H), 2.56 (s, 3H), 2.31 (s, 3H).

3-Allyl-8-(3-methoxyisoxazol-5-yl)-6-(methylthio)-3*H*-purin-2(7*H*)-one (98i) Pale yellow solid; 73% yield; mp 271 ℃ dec.; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 14.38 (bs, 1H), 6.90 (s, 1H), 6.09-5.80 (m, 1H), 5.11 (m, 2H), 4.65 (m, 2H), 3.97 (s, 3H), 2.67 (s, 3H).

3-Benzyl-8-(3-methoxyisoxazol-5-yl)-6-(methylthio)-3*H*-purin-2(7*H*)-one (98j) Pale yellow solid; 75% yield; mp 249-251 ℃ dec.; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 14.62 (bs, 1H),7.35-7.28 (m, 5H), 6.91 (s, 1H), 5.25 (s, 2H), 3.97 (s, 3H), 2.68 (s, 3H).

General procedure for the synthesis of 6-(1-hydroxybutan/propan-2-ylamino)-3-allyl/benzyl-8-[(substituted)isoxazol/pyrazol-3/5-yl]-3*H*-purin-2(7*H*)-one derivatives (99-118)



The appropriate 3-allyl/benzyl-8-[(substituted)isoxazol/pyrazol-3/5-yl]-6-(methylthio)-3*H*-purin-2(7*H*)-one derivative (**98a-j**, 0.3 mmol) was suspended in anhydrous DMSO (0.3 mL) and, after cooling at 0°C, the opportune (R/S/R,S)-2-amino-butan/propan-1-ol (1.5 mmol) was added. The reaction was heated at 150 °C for 1h. The solvent was evaporated under vacuum and the product was precipitated from CH_2Cl_2-Et_2O, filtered, washed with Et_2O and finally purified *via* column chromatography on silica gel eluting with EtOAc/CH_3OH 9.5:0.5.

(*R,S*)-3-allyl-8-(1,3-dimethyl-1*H*-pyrazol-5-yl)-6-[(2-hydroxy-1-methylethyl)amino]-3,7-dihydro-2*H*-purin-2-one (99) White solid; 56 % yield; mp 255-256 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 12.98 (bs, 1H), 7.43 (bs, 1H), 6.45 (s, 1H), 6.18-5.83 (m, 1H), 5.18-5.03 (m, 3H), 4.59 (d, J = 3 Hz, 2H), 4.22 (m, 1H), 4.13 (s, 3H), 3.58 (m, 2H), 2.18 (s, 3H), 1.22 (m, 3H); MS (ESI): $[MH]^+$ = 344.3.

(*R*)-3-allyl-8-(1,3-dimethyl-1*H*-pyrazol-5-yl)-6-[(2-hydroxy-1-methylethyl)amino]-3,7-dihydro-2*H*-purin-2-one ((*R*)99) White solid; 66% yield; mp 253-254 °C, dec.; ^{1}H NMR (200MHz, DMSO-d_6)

δ ppm 12.38 (bs, 1H), 7.43 (bs, 1H), 6.45 (s, 1H), 6.03-5.84 (m, 1H), 5.14-5.08 (m, 3H), 4.57 (d, *J* = 4.8 Hz, 2H), 4.21 (m, 1H), 4.11 (s, 3H), 3.51 (m, 2H), 2.17 (s, 3H), 1.22-1.16 (m, 3H). MS (ESI): $[MH]^+$ = 344.3.

(*S*)-3-allyl-8-(1,3-dimethyl-1*H*-pyrazol-5-yl)-6-[(2-hydroxy-1-methylethyl)amino]-3,7-dihydro-2*H*-purin-2-one ((*S*)99) White solid; 52% yield; mp 253-254 ℃ dec.; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 12.80 (bs, 1H), 7.42 (bs, 1H), 6.48 (s, 1H), 6.05-5.82 (m, 1H), 5.15-4.92 (m, 3H), 4.58 (d, *J* = 5.0 Hz, 2H), 4.22 (m, 1H), 4.12 (s, 3H), 3.51 (m, 2H), 2.19 (s, 3H), 1.22-1.17 (m, 3H). MS (ESI): $[MH]^+$ = 344.3.

(*R,S*)-3-allyl-8-(1,3-dimethyl-1*H*-pyrazol-5-yl)-6-{[1-(hydroxymethyl)propyl]amino}-3,7-dihydro-2*H*-purin-2-one (100) White solid; 62% yield; mp 253-255 ℃ dec.; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 12.47 (bs, 1H), 7.52 (bs, 1H), 6.45 (s, 1H), 6.10-5.85 (m, 1H), 5.20-5.10 (m, 2H), 5.00-4.80 (bs, 1H), 4.57 (d, *J* = 4.6 Hz, 2H), 4.12 (m, 4H), 4.75-4.67 (m, 2H), 2.16 (m, 3H), 1.80-1.60 (m, 2H), 1.12-0.80 (m, 3H). MS (ESI): $[MH]^+$ = 357.5.

(*R,S)*-3-benzyl-8-(1,3-dimethyl-1*H*-pyrazol-5-yl)-6-[(2-hydroxy-1-methylethyl)amino]-3,7-dihydro-2*H*-purin-2-one (101) White solid; 46% yield; mp 230-233 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 12.70 (bs, 1H), 7.31-7.20 (m, 6H), 6.55 (s, 1H), 5.14 (s, 2H), 4.95 (bs, 1H), 4.19 (m, 1H), 3.82 (s, 3H), 3.47 (m, 2H), 2.30 (s, 3H), 1.18 (d, 3H, *J* = 6.6 Hz). MS (ESI): $[MH]^+$ = 394.5.

(*R,S*)-3-benzyl-8-(1,3-dimethyl-1*H*-pyrazol-5-yl)-6-{[1-(hydroxymethyl)propyl]amino}-3,7-dihydro-2*H*-purin-2-one (102) White solid; 72% yield; mp 171-172 ℃; ^{1}H NMR (200MHz, DMSO-

d_6) δ ppm 12.65 (bs, 1H), 7.40-7.26 (m, 6H), 6.56 (s, 1H), 5.16 (s, 2H), 4.89-4.81 (m, 1H), 4.12 (m, 4H), 3.60-3.40 (m, 2H), 2.19-2.15 (m, 3H), 1.80-1.67 (m, 2H), 0.87 (t, *J* = 7.2 Hz, 3H). MS (ESI): $[MH]^+$ = 408.2.

(*R*)-3-benzyl-8-(1,3-dimethyl-1*H*-pyrazol-5-yl)-6-{[1-(hydroxymethyl)propyl]amino}-3,7-dihydro-2*H*-purin-2-one ((*R*)102) White solid; 45% yield; mp 170-172 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 12.70 (bs, 1H), 7.31-7.26 (m, 6H), 6.56 (s, 1H), 5.16 (s, 2H), 4.88-4.79 (m, 1H), 4.06-4.04 (m, 1H), 3.82 (s, 3H), 3.532-3.43 (m, 1H), 2.31 (s, 3H), 1.69-1.67 (m, 1H), 1.52-1.49 (m, 1H), 0.92 (t, *J* = 7.2 Hz, 3H). MS (ESI): $[MH]^+$ = 408.2.

(*S*)-3-benzyl-8-(1,3-dimethyl-1*H*-pyrazol-5-yl)-6-{[1-(hydroxymethyl)propyl]amino}-3,7-dihydro-2*H*-purin-2-one ((*S*)102) White solid; 48% yield; mp 174 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 12.61 (bs, 1H), 7.28-7.19 (m, 6H), 6.53 (s, 1H), 5.11 (s, 2H), 4.83 (m, 1H), 4.20-4.10 (m, 1H), 3.79 (s, 3H), 3.47 (m, 2H), 2.28 (s, 3H), 1.81-1.42 (m, 2H), 0.90 (t, *J* = 7.2 Hz, 3H). MS (ESI): $[MH]^+$ = 408.2.

(*R,S*)-3-allyl-6-[(2-hydroxy-1-methylethyl)amino]-8-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-3,7-dihydro-2*H*-purin-2-one (103) White solid; 58% yield; mp 225 °C dec.; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 12.72 (bs, 1H), 7.53 (bs, 1H), 6.12-5.83 (m, 2H), 5.19-5.02 (m, 3H), 4.62.4.55 (m, 2H), 4.21 (m, 1H), 4.07 (s, 3H), 3.78 (s, 3H), 3.56-3.44 (m, 2H), 1.35-1.19 (m, 3H); MS (ESI): $[MH]^+$ = 360.4

(*R,S*)-3-allyl-6-{[1-(hydroxymethyl)propyl]amino}-8-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-3,7-dihydro-2*H*-purin-2-one (104) White solid; 52% yield; mp 231 °C dec.; ^{1}H NMR (200MHz, DMSO-d_6) δ

ppm 12.89 (bs, 1H), 7.51 (bs, 1H), 6.11-5.82 (m, 2H), 5.19-5.02 (m, 2H), 4.99-4.80 (m, 1H), 4.59 (m, 2H), 4.07 (m, 4H), 3.77 (s, 3H), 3.62-3.41 (m, 2H),1.79-1.52 (m, 2H), 0.98-0.82 (m, 3H). MS (ESI): $[MH]^+$ = 374.4

(*R*)-3-allyl-6-{[1-(hydroxymethyl)propyl]amino}-8-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-3,7-dihydro-2*H*-purin-2-one ((*R*)104) White solid; 55% yield; mp 230 °C dec.; ^{1}H NMR (200MHz, $CDCl_3$) δ ppm 12.12 (bs, 1H), 7.53 (bs, 1H), 6.14 (s, 1H), 6.07-5.95 (m, 1H), 5.26-5.14 (m, 2H), 4.81-4.79 (m, 2H), 4.15 (s, 3H), 3.87 (s, 3H), 3.87-3.75 (m, 1H), 3.44 (m, 2H), 1.40-1.20 (m, 2H), 0.69-0.65 (m, 3H). MS (ESI): $[MH]^+$ = 374.4

(*S*)-3-allyl-6-{[1-(hydroxymethyl)propyl]amino}-8-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-3,7-dihydro-2*H*-purin-2-one ((*S*)104 White solid; 43% yield; mp 230 °C dec.; ^{1}H NMR (200MHz, $CDCl_3$) δ ppm 12.13 (bs, 1H), 7.55 (bs, 1H), 6.13 (s, 1H), 6.07-5.85 (m, 1H), 5.26-5.14 (m, 2H), 4.81-4.79 (m, 2H), 4.15 (m, 3H), 3.87 (s, 3H), 3.87-3.75 (m, 1H), 3.41 (m, 2H), 1.40-1.20 (m, 2H), 0.69-0.65 (m, 3H). MS (ESI): $[MH]^+$ = 374.4

(*R,S*)-3-benzyl-6-[(2-hydroxy-1-methylethyl)amino]-8-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-3,7-dihydro-2*H*-purin-2-one (105) White solid; 53% yield; mp 258-260 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 13.88 (bs, 1H), 7.51 (bs, 1H), 7.39-7.25 (m, 5H), 6.02 (s, 1H), 5.16 (s, 2H), 5.08 (s, 1H), 4.24 (m, 1H), 4.07 (s, 3H), 3.78 (s, 3H), 3.49 (m, 2H), 1.26-1.17 (m, 3H). MS (ESI): $[MH]^+$ = 410.3.

(*R*)-3-benzyl-6-[(2-hydroxy-1-methylethyl)amino]-8-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-3,7-dihydro-2*H*-purin-2-one ((*R*)105) White solid; 57% yield; mp 257-259 °C; ^{1}H NMR (400MHz, DMSO-

d_6) δ ppm 12.83 (bs, 1H), 7.56 (bs, 1H), 7.39-7.25 (m, 5H), 6.02 (s, 1H), 5.16 (s, 2H), 5.06 (bs, 1H), 4.23 (m, 1H), 4.07 (s, 3H), 3.77 (s, 3H), 3.50 (m, 2H), 1.26-1.19 (m, 3H). MS (ESI): $[MH]^+$ = 410.3.

(*S*)-3-benzyl-6-[(2-hydroxy-1-methylethyl)amino]-8-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-3,7-dihydro-2*H*-purin-2-one ((*S*)105) White solid; 71% yield; mp 259 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 12.93 (bs, 1H), 7.56 (bs, 1H), 7.39-7.25 (m, 5H), 6.02 (s, 1H), 5.16 (s, 2H), 5.09 (bs, 1H), 4.23 (m, 1H), 4.07 (s, 3H), 3.78 (s, 3H), 3.50 (m, 2H), 1.26-1.19 (m, 3H). MS (ESI): $[MH]^+$ = 410.3.

(*R,S*)-3-benzyl-6-{[1-(hydroxymethyl)propyl]amino}-8-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-3,7-dihydro-2*H*-purin-2-one (106) White solid; 60% yield; mp 259-260 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 12.85 (bs, 1H), 7.67 (bs, 1H), 7.40-7.30 (m, 5H), 6.02 (s, 1H), 5.17 (s, 2H), 5.02 (bs, 1H), 4.07 (m, 4H), 3.77 (s, 3H), 3.61 (m, 2H), 1.79-1.45 (m, 2H), 0.93 (m, 3H). MS (ESI): $[MH]^+$ = 424.3.

(*R,S*)-3-allyl-8-[3-(benzyloxy)-1-methyl-1*H*-pyrazol-5-yl]-6-[(2-hydroxy-1-methylethyl)amino]-3,7-dihydro-2*H*-purin-2-one (107) White solid; 56% yield; mp 180-182 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 12.89 (bs, 1H), 7.43-7.35 (m, 6H), 6.02-5.87 (m, 2H), 5.16-5.10 (m, 5H), 4.58 (d, J = 4.4 Hz, 2H), 4.22 (m, 1H), 4.08 (s, 3H), 3.56-3.43 (m, 2H), 1.23 (m, 3H). MS (ESI): $[MH]^+$ = 436.5.

(*R,S*)-3-allyl-8-[3-(benzyloxy)-1-methyl-1*H*-pyrazol-5-yl]-6-{[1-(hydroxymethyl)propyl]amino}-3,7-dihydro-2*H*-purin-2-one (108) White solid; 54% yield; mp 224-225 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 12.91 (bs, 1H), 7.43-7.36 (m, 6H), 6.19-5.76 (m, 2H), 5.16-5.00 (m, 4H), 4.92 (bs, 1H), 4.59 (m, 2H), 4.08 (m, 4H), 3.51-3.42

(m, 2H), 1.79-1.46 (m, 2H), 1.08-0.86 (m, 3H). MS (ESI): $[MH]^+$ = 450.5.

(*R,S*)-3-benzyl-8-[3-(benzyloxy)-1-methyl-1*H*-pyrazol-5-yl]-6-[(2-hydroxy-1-methylethyl)amino]-3,7-dihydro-2*H*-purin-2-one (109) White solid; 63% yield; mp 121 ℃; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 12.83 (bs, 1H), 7.61 (bs, 1H), 7.45-7.25 (m, 10H), 6.10 (s, 1H), 5.16 (s, 4H), 5.01 (bs, 1H), 4.22 (m, 1H), 4.08 (s, 3H), 3.50 (m, 2H), 1.26-1.19 (m, 3H). MS (ESI): $[MH]^+$ = 486.3.

(*R,S*)-3-benzyl-8-[3-(benzyloxy)-1-methyl-1*H*-pyrazol-5-yl]-6-{[1-(hydroxymethyl)propyl]amino}-3,7-dihydro-2*H*-purin-2-one (110) White solid; 61% yield; mp 248 ℃; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 12.98 (bs, 1H), 7.56 (bs, 1H), 7.44-7.30 (m, 10H), 6.08 (s, 1H), 5.17 (s, 2H), 5.14 (s, 2H), 5.02 (m, 1H), 4.86 (m, 1H), 4.08 (s, 3H), 3.61-3.45 (m, 2H), 1.79-1.47 (m, 2H), 0.95-9.91 (m, 3H). MS (ESI): $[MH]^+$ = 500.4.

(*R,S*)-3-allyl-8-(1,5-dimethyl-1*H*-pyrazol-3-yl)-6-[(2-hydroxy-1-methylethyl)amino]-3,7-dihydro-2*H*-purin-2-one (111) Pale yellow solid; 57% yield; mp 177-180 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 12.53 (bs, 1H), 7.26 (d, J = 7.4 Hz, 1H), 6.55 (s, 1H), 5.93-5.85 (m, 1H), 5.09-4.97 (m, 3H), 4.54 (d, J = 4.8 Hz, 2H), 4.19 (m, 1H), 3.82 (s, 3H), 3.47 (m, 2H), 2.31 (s, 3H), 1.18 (d, J = 6.8 Hz, 3H). MS (ESI): $[MH]^+$ = 344.4.

(*R,S*)-3-allyl-8-(1,5-dimethyl-1*H*-pyrazol-3-yl)-6-{[1-(hydroxymethyl)propyl]amino}-3,7-dihydro-2*H*-purin-2-one. (112) White solid; 52% yield; mp 169-170 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 12.61 (bs, 1H), 7.25 (d, J = 8 Hz, 1H), 6.56 (s, 1H), 6.02-5.81 (m, 1H), 5.10-4.98 (m, 3H), 4.54 (d, J = 4.8 Hz, 2H), 4.17-3.99

(m, 1H), 3.82 (s, 3H), 3.50- 3.48 (m, 2H), 2.31 (s, 3H), 2.79-2.61 (m, 2H), 0.92 (t, *J* = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 358.4.

(*R*)-3-allyl-8-(1,5-dimethyl-1*H*-pyrazol-3-yl)-6-{[1-(hydroxymethyl)propyl]amino}-3,7-dihydro-2*H*-purin-2-one ((*R*)112) White solid; 62% yield; mp 168-170 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 12.66 (bs, 1H), 7.21 (bs, 1H), 6.55 (s, 1H), 5.95-5.88 (m, 1H), 5.08-5.05 (m, 2H), 4.89 (bs, 1H), 4.54 (d, *J* = 4.8 Hz, 2H), 4.05-4.04 (m, 1H), 3.82 (s, 3H), 3.51 (m, 2H), 2.31 (s, 3H), 1.72-1.66 (m, 1H), 1.54-1.47 (m, 1H), 0.92 (t, 7.6 Hz, 3H). MS (ESI): $[MH]^+$ = 358.6.

(*S*)-3-allyl-8-(1,5-dimethyl-1*H*-pyrazol-3-yl)-6-{[1-(hydroxymethyl)propyl]amino}-3,7-dihydro-2*H*-purin-2-one ((S)112) White solid; 59% yield; mp 167-169 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 12.63 (bs, 1H), 7.21-7.18 (bd, *J* = 7.4, Hz 1H), 6.54 (s, 1H), 5.98-5.84 (m, 1H), 5.14-4.82 (m, 3H), 4.54 (d, 2H), 4.15-3.93 (m, 1H), 3.80 (s, 3H), 3.49 (m, 2H), 2.92 (s, 3H), 1.79-1.43 (m, 2H), 0.9 (t, *J* = 7 Hz, 3H). MS (ESI): $[MH]^+$ = 358.6.

(*R,S*)-3-benzyl-8-(1,5-dimethyl-1*H*-pyrazol-3-yl)-6-[(2-hydroxy-1-methylethyl)amino]-3,7-dihydro-2*H*-purin-2-one (113) White solid; 56% yield; mp 212 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 12.84 (bs, 1H), 7.30-7.22 (m, 6H), 6.55 (s, 1H), 5.13 (s, 2H), 5.00 (bs, 1H), 4.20 (m, 1H), 3.81 (s, 3H), 3.45 (m, 2H), 2.30 (s, 3H), 1.18 (d, 3H, *J* = 6.6). MS (ESI): $[MH]^+$ = 394.7.

(*R,S*)-3-benzyl-8-(1,5-dimethyl-1*H*-pyrazol-3-yl)-6-{[1-(hydroxymethyl)propyl]amino}-3,7-dihydro-2*H*-purin-2-one (114) White solid; 47% yield; mp 199-201 °C dec.; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 12.78 (bs, 1H), 7.31-7.24 (m, 6H), 6.56 (s, 1H),

5.14 (s, 2H), 4.90 (bs, 1H), 4.10 (m, 1H), 3.82 (s, 3H), 3.45 (m, 2H), 2.30 (s, 3H), 1.8-1.6 (m, 2H), 0.92 (t, 3H, *J* = 7.6); MS (ESI): $[MH]^+$ = 408.7.

(*R,S*)-3-allyl-6-[(2-hydroxy-1-methylethyl)amino]-8-(3-methoxyisoxazol-5-yl)-3,7-dihydro-2*H*-purin-2-one (115) White solid; 64% yield; mp 289-290 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 8.52 (bs, 1H), 7.78 (bs, 1H), 6.44 (s, 1H), 6.03-5.84 (m, 1H), 5.15-5.10 (m, 3H), 4.58 (d, *J* = 4.8 Hz, 2H), 4.22 (m, 1H), 3.92 (s, 3H), 3.50 (m, 2H), 1.24-1.19 (m, 3H); MS (ESI): $[MH]^+$ = 347.2.

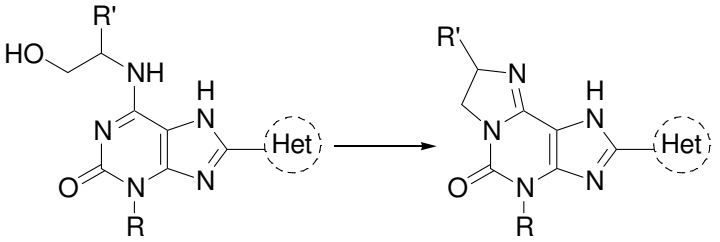
(*R,S*)-3-allyl-6-{[1-(hydroxymethyl)propyl]amino}-8-(3-methoxyisoxazol-5-yl)-3,7-dihydro-2*H*-purin-2-one (116) White solid; 54% yield; mp 270-271 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 8.58 (bs, 1H), 7.71 (bs, 1H), 6.42 (s, 1H), 6.02-5.88 (m, 1H), 5.16-4.91 (m, 3H), 4.58 (d, *J* = 4.6 Hz, 2H), 4.11 (m, 1H), 3.93 (s, 3H), 3.61-3.52 (m, 2H), 1.62-1.59 (m, 2H), 0.97-0.86 (m, 3H). MS (ESI): $[MH]^+$ = 361.5.

(*R,S*)-3-benzyl-6-[(2-hydroxy-1-methylethyl)amino]-8-(3-methoxyisoxazol-5-yl)-3,7-dihydro-2*H*-purin-2-one (117) White solid; 40% yield; mp 292-293 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 8.68 (bs, 1H), 7.78 (bs, 1H), 7.31 (m, 5H), 6.43 (s, 1H), 5.17 (s, 2H), 5.16-5.00 (m, 1H), 4.22 (m, 1H), 3.92 (s, 3H), (m, 2H), 1.35-1.24 (m, 3H). MS (ESI): $[MH]^+$ = 397.5.

(*R,S*)-3-benzyl-6-{[1-(hydroxymethyl)propyl]amino}-8-(3-methoxyisoxazol-5-yl)-3,7-dihydro-2*H*-purin-2-one (118) White solid; 67% yield; mp 284-286 °C dec.; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 12.91 (bs, 1H), 7.80 (bs, 1H), 7.31 (m, 5H), 6.44 (s, 1H), 5.15 (s, 2H), 4.90-5.00 (s, 1H), 4.22 (m, 1H), 3.20 (s, 3H), 3.60-3.50 (m,

2H), 1.75-1.55 (m, 2H), 0.91 (t, 3H, J = 7.6). MS (ESI): $[MH]^+$ = 411.5.

General procedure for the preparation of 4-allyl/benzyl-7,8-dihydro-8-methyl/ethyl-2-[(substituted)isoxazol/pyrazol-3/5-yl]-1*H*-imidazo[2,1-*i*]purin-5(4*H*)-one derivatives (119-138)



The opportune 6-(1-hydroxybutan/propan-2-ylamino)-3-allyl/benzyl-8-[(substituted)isoxazol/pyrazol-3/5-yl]-3*H*-purin-2(7*H*)-one derivative (**99-118**, 0.2 mmol) was dissolved in freshly distilled CH_2Cl_2 (7 mL) and, after cooling at 0°C, 0.3 mL of $SOCl_2$ were added to the mixture. The reaction was stirred for further 10' at room temperature, then heated at reflux for 18h. The solvent and the excess of $SOCl_2$ were removed under vacuum and the residue was purified *via* column chromatography on silica gel eluting with EtOAc/CH_3OH 9:1.

(*R,S*)-4-allyl-2-(1,3-dimethyl-1*H*-pyrazol-5-yl)-8-methyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (119) White solid; 64% yield; mp 232 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.17 (bs, 1H), 6.40 (s, 1H), 6.05-5.83 (m, 1H), 5.22-5.13 (m, 2H), 4.62 (d, J = 5 Hz, 2H), 4.32 (m, 2H), 4.15 (s, 3H), 3.78 (m, 1H), 2.14 (s, 3H), 1.34 (d, J = 5.6 Hz, 3H). MS (ESI): $[MH]^+$ = 326.3.

(*R*)-4-allyl-2-(1,3-dimethyl-1*H*-pyrazol-5-yl)-8-methyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one ((*R*)119) White solid; 64%

yield; mp 233 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.17 (bs, 1H), 6.40 (s, 1H), 5.97-5.88 (m, 1H), 5.23-5.13 (m, 2H), 4.62 (d, *J* = 5.2 Hz, 2H), 4.37-4.22 (m, 2H), 4.15 (s, 3H), 3.75-3.67 (m, 1H), 2.14 (s, 3H), 1.34 (d, *J* = 5.8 Hz, 3H). MS (ESI): $[MH]^+$ = 325.8.

(*S*)-4-allyl-2-(1,3-dimethyl-1*H*-pyrazol-5-yl)-8-methyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one ((*S*)119) White solid; 48% yield; mp 233 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.17 (bs, 1H), 6.40 (s, 1H), 6.02-5.88 (m, 1H), 5.22-5.13 (m, 2H), 4.62 (d, *J* = 5.2 Hz, 2H), 4.39-4.22 (m, 1H), 4.15 (s, 3H), 3.75-3.67 (m, 1H), 2.14 (s, 3H), 1.34 (d, *J* = 5.8 Hz, 3H). MS (ESI): $[MH]^+$ = 325.8.

(*R,S*)-4-allyl-2-(1,3-dimethyl-1*H*-pyrazol-5-yl)-8-ethyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (120) White solid; 51% yield; mp 188 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.17 (bs, 1H), 6.40 (s, 1H), 6.02-5.88 (m, 1H), 5.22-5.13 (m, 2H), 4.62 (d, *J* = 5.2 Hz, 2H), 4.25-4.22 (m, 2H), 4.15 (s, 3H), 3.75-3.67 (m, 1H), 2.14 (s, 3H), 1.68 (m, 2H), 0.94 (t, *J* = 7.4 Hz, 3H). MS (ESI): $[MH]^+$ = 340.8.

(*R,S*)-4-benzyl-2-(1,3-dimethyl-1*H*-pyrazol-5-yl)-8-methyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (121) White solid; 57% yield; mp 244 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 1.07 (bs, 1H), 7.38-7.24 (m, 5H), 6.41 (s, 1H), 5.19 (s, 2H), 4.38-4.17 (m, 2H), 3.73 (s, 3H), 3.69-3.60 (m, 1H), 2.26 (s, 3H), 1.32 (d, *J* = 6.4 Hz, 3H). MS (ESI): $[MH]^+$ = 376.6.

(*R,S*)-4-benzyl-2-(1,3-dimethyl-1*H*-pyrazol-5-yl)-8-ethyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (122) White solid; 48% yield; mp 295 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.12 (bs, 1H), 7.42-7.28 (m, 5H), 6.68 (s, 1H), 5.19 (s, 2H), 4.20-4.00 (m, 5H),

3.80 (m, 1H), 2.15 (s, 3H), 1.65 (m, 2H), 0.95 (t, *J* = 7.2 Hz, 3H). MS (ESI): $[MH]^+$ = 390.2.

(*R*)-4-benzyl-2-(1,3-dimethyl-1*H*-pyrazol-5-yl)-8-ethyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one ((*R*)122) White solid; 48% yield; mp 294 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.12 (bs, 1H), 7.39-7.27 (m, 5H), 6.42 (s, 1H), 5.20 (s, 2H), 4.20-4.18 (m, 2H), 3.73 (m, 4H), 2.26 (s, 3H), 1.65 (m, 2H), 0.94 (t, *J* = 7.2 Hz, 3H). MS (ESI): $[MH]^+$ = 390.2.

(*S*)-4-benzyl-2-(1,3-dimethyl-1*H*-pyrazol-5-yl)-8-ethyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one ((*S*)122) White solid; 43% yield; mp 295 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.21 (bs, 1H), 7.35-7.27 (m, 5H), 6.42 (s, 1H), 5.20 (s, 2H), 4.17 (m, 2H), 3.73 (m, 4H), 2.26 (s, 3H), 1.79-1.62 (m, 2H), 0.94 (t, *J* = 7.2 Hz, 3H). MS (ESI): $[MH]^+$ = 390.2.

(*R,S*)-4-allyl-2-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-8-methyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (123) White solid; 55% yield; mp 121 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.17 (bs, 1H), 5.99 (s, 1H), 5.95-5.91 (m, 1H), 5.20-5.14 (m, 2H), 4.62 (d, *J* = 5.2 Hz, 2H), 4.4.38-4.25 (m, 2H), 4.10 (s, 3H), 3.77 (s, 3H), 3.75-3.70 (m, 1H), 1.34 (d, *J* = 6 Hz, 3H). MS (ESI): $[MH]^+$ = 342.4.

(*R,S*)-4-allyl-2-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-8-ethyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (124) White solid; 47% yield; mp 133 °C ; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.23 (bs, 1H), 6.00 (s, 1H), 5.98-5.89 (m, 1H), 5.20-5.14 (m, 2H), 4.62 (d, *J* = 5.2 Hz, 2H), 4.26-4.24 (m, 2H), 4.10 (s, 3H), 3.83-3.78 (m, 1H), 3.77 (s, 3H), 1.68 (m, 2H), 0.95 (t, *J* = 7.2 Hz, 3H). MS (ESI): $[MH]^+$ = 356.2.

(*R*)-4-allyl-8-ethyl-2-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one ((*R*)124) White solid; 54% yield; mp 133 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.23 (bs, 1H), 6.00 (s, 1H), 5.98-5.89 (m, 1H), 5.22-5.13 (m, 2H), 4.62 (d, *J* = 5.2 Hz, 2H), 4.26-4.24 (m, 2H), 4.10 (s, 3H), 3.83-3.78 (m, 1H), 3.77 (s, 3H), 1.68 (m, 2H), 0.95 (t, *J* = 7.2 Hz, 3H). MS (ESI): $[MH]^+$ = 356.2.

(*S*)-4-allyl-8-ethyl-2-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one ((*S*)124) White solid; 51% yield; mp 133 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.23 (bs, 1H), 6.02 (s, 1H), 5.98-5.89 (m, 1H), 5.18-5.13 (m, 2H), 4.62 (d, *J* = 5.2 Hz, 2H), 4.26-4.24 (m, 2H), 4.10 (s, 3H), 3.83-3.78 (m, 1H), 3.77 (s, 3H), 1.68 (m, 2H), 0.95 (t, *J* = 7.2 Hz, 3H). MS (ESI): $[MH]^+$ = 356.2.

(*R,S*)-4-benzyl-2-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-8-methyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (125) White solid; 57% yield; mp 243 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.21 (bs, 1H), 7.44-7.25 (m, 5H), 5.98 (s, 1H), 5.19 (s, 2H), 4.35-4.22 (m, 2H), 4.10 (s, 3H), 3.76 (s, 3H), 3.70-3.66 (m, 1H), 1.32 (d, *J* = 6.4 Hz, 3H). MS (ESI): $[MH]^+$ = 392.2.

(*R*)-4-benzyl-2-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-8-methyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one ((*R*)125) White solid; 67% yield; mp 242 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.19 (bs, 1H), 7.42-7.23 (m, 5H), 5.98 (s, 1H), 5.18 (s, 2H), 4.35-4.24 (m, 2H), 4.08 (s, 3H), 3.75 (s, 3H), 3.73-3.68 (m, 1H), 1.32 (d, *J* = 6 Hz, 3H). MS (ESI): $[MH]^+$ = 392.2.

(*S*)-4-benzyl-2-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-8-methyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one ((*S*)125) White solid; 60% yield; mp 244 ℃; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.23 (bs, 1H), 7.43-7.24 (m, 5H), 6.06 (s, 1H), 5.20 (s, 2H), 4.39-4.26 (m, 2H), 4.08 (s, 3H), 3.76 (s, 3H), 3.73-3.71 (m, 1H), 1.34 (d, *J* = 6.4 Hz, 3H). MS (ESI): $[MH]^+$ = 392.2.

(*R,S*)-4-benzyl-8-ethyl-2-(3-methoxy-1-methyl-1*H*-pyrazol-5-yl)-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (126) White solid; 53% yield; mp 140 ℃; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.38 (bs, 1H), 7.43-7.25 (m, 5H), 6.08 (s, 1H), 5.21 (s, 2H), 4.27-4.23 (m, 2H), 4.08 (s, 3H), 3.81-3.80 (m, 1H), 3.76 (s, 3H), 1.70-1.66 (m, 2H), 0.93 (t, *J* = 7.2 Hz, 3H). MS (ESI): $[MH]^+$ = 406.2.

(*R,S*)-4-allyl-2-[3-(benzyloxy)-1-methyl-1*H*-pyrazol-5-yl]-8-methyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (127) White solid; 59% yield; mp 214 ℃; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.18 (bs, 1H), 7.45-7.38 (m, 5H), 6.03 (s, 1H), 5.98-5.84 (m, 1H), 5.20-5.14 (m, 4H), 4.62 (d, *J* = 5.2 Hz, 2H), 4.37-4.25 (m, 2H), 4.11 (s, 3H), 3.74-3.70 (m, 1H), 1.34 (d, *J* = 6.4 Hz, 3H). MS (ESI): $[MH]^+$ = 418.2.

(*R,S*)-4-allyl-2-[3-(benzyloxy)-1-methyl-1*H*-pyrazol-5-yl]-8-ethyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (128) White solid; 60% yield; mp 223 ℃; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.25 (bs, 1H), 7.43-7.38 (m, 5H), 6.03 (s, 1H), 5.98-5.91 (m, 1H), 5-20-5.14 (m, 4H), 4.62 (d, *J* = 5.6 Hz, 2H), 4.28-4.21 (m, 2H), 4.11 (s, 3H), 3.81-3.78 (m, 1H), 1.70-1.66 (m, 2H), 0.94 (t, *J* = 7.2 HZ, 3H). MS (ESI): $[MH]^+$ = 432.2.

(*R,S*)-4-benzyl-2-[3-(benzyloxy)-1-methyl-1*H*-pyrazol-5-yl]-8-methyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (129) White solid; 42% yield; mp 143 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.22 (bs, 1H), 7.45-7.31 (m, 10H), 6.05 (s, 1H), 5.20 (s, 2H), 5.14 (s, 2H), 4.37-4.26 (m, 2H); 4.11 (s, 3H), 3.75-3.71 (m, 1H), 1.34 (d, J = 6 Hz, 3H). MS (ESI): $[MH]^+$ = 468.2.

(*R,S*)-4-benzyl-2-[3-(benzyloxy)-1-methyl-1*H*-pyrazol-5-yl]-8-ethyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (130) White solid; 49% yield; mp 120 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.22 (bs, 1H), 7.45-7.29 (m, 10H), 6.05 (s, 1H), 5.20 (s, 2H), 5.14 (s, 2H), 4.26-4.20 (m, 2H), 3.82-3.78 (m, 2H), 4.11 (s, 1H), 3.82-3.78 (m,1H), 1.70-1.66 (m, 2H), 0.94 (t, J = 7.2 Hz, 3H). MS (ESI): $[MH]^+$ = 482.2.

(*R,S*)-4-allyl-2-(1,5-dimethyl-1*H*-pyrazol-3-yl)-8-methyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (131) White solid; 49% yield; mp 214 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.43 (bs, 1H), 6.41 (s, 1H), 5.98-5.91 (m, 1H), 5.14-5.11 (m, 2H), 4.60 (d, J = 5.2 Hz, 2H), 4.39-4.18 (m, 2H), 3.74 (s, 3H), 3.67-3.62 (m, 1H), 2.26 (s, 3H), 1.31 (d, J = 6 Hz, 3H). MS (ESI): $[MH]^+$ = 326.2.

(*R,S*)-4-allyl-2-(1,5-dimethyl-1*H*-pyrazol-3-yl)-8-ethyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (132) White solid; 46% yield; mp 249 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.43 (bs, 1H), 6.45 (s, 1H), 5.98-5.91 (m, 1H), 5.15-5.11 (m, 2H), 4.61 (d, J = 5.2 Hz, 2H), 4.22-4.20 (m, 2H), 3.76 (m, 4H), 2.27 (s, 3H), 1.68-1.65 (m, 2H), 0.95 (t, J = 7.2 Hz, 3H). MS (ESI): $[MH]^+$ = 340.2.

(*R*)-4-allyl-2-(1,5-dimethyl-1*H*-pyrazol-3-yl)-8-ethyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one ((*R*)132) White solid; 48%

yield; mp 249 °C; ^{1}H NMR (400MHz, DMSO-d_6) δ ppm 10.11 (bs, 1H), 6.41 (s, 1H), 5.99-5.87 (m, 1H), 5.14-5.09 (m, 2H), 4.60 (d, *J* = 4.8 Hz, 2H), 4.17-4.60 (m, 2H), 3.80-3.70 (m, 4H), 2.26 (s, 3H), 1.65 (m, 2H), 0.94 (t, *J* = 7.2 Hz, 3H). MS (ESI): $[MH]^+$ = 339.9.

(*S*)-4-allyl-2-(1,5-dimethyl-1*H*-pyrazol-3-yl)-8-ethyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one ((*S*)132) White solid; 47% yield; mp 249 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.08 (bs, 1H), 6.41 (s, 1H), 6.00-5.88 (m, 1H), 5.16-5.07 (m, 2H), 4.60 (d, *J* = 4.8 Hz, 2H), 4.20-4.17 (m, 2H), 3.74 (s, 3H), 3.70 (m, 1H), 2.26 (s, 3H), 1.68-1.62 (m, 2H), 0.94 (t, *J* = 7.2 Hz, 3H). MS (ESI): $[MH]^+$ = 340.2.

(*R,S*)-4-benzyl-2-(1,5-dimethyl-1*H*-pyrazol-3-yl)-8-methyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (133) White solid; 65% yield; mp 245 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.20 (bs, 1H), 7.35-7.27 (m, 5H), 6.42 (s, 1H), 5.20 (s, 2H), 4.40-4.20 (m, 2H), 3.80-3.60 (m, 4H), 2.26 (s, 3H), 1.31 (d, *J* = 5.8 Hz, 3H). MS (ESI): $[MH]^+$ = 376.9.

(*R,S*)-4-benzyl-2-(1,5-dimethyl-1*H*-pyrazol-3-yl)-8-ethyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (134) White solid; 61% yield; mp 275 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.20 (bs, 1H), 7.35-7.27 (m, 5H), 6.42 (s, 1H), 5.20 (s, 2H), 4.20-4.10 (m, 2H), 3.80-3.60 (m, 4H), 2.26 (s, 3H), 1.70-1.60 (m, 2H), 0.94 (t, *J* = 7.2 Hz, 3H). MS (ESI): $[MH]^+$ = 390.9.

(*R,S*)-4-allyl-2-(3-methoxyisoxazol-5-yl)-8-methyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (135) White solid; 57% yield; mp 169 °C; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.74 (bs, 1H), 6.53 (s, 1H), 5.93-5.88 (m, 1H), 5.21-5.13 (m, 2H), 4.63 (d, *J* =

4.8 Hz, 2H), 4.45-4.26 (m, 2H), 3.94 (s, 3H), 3.80-3.72 (m, 1H), 1.36 (d, *J* = 6 Hz, 3H). MS (ESI): $[MH]^+$ = 329.2.

(*R,S*)-4-allyl-8-ethyl-2-(3-methoxyisoxazol-5-yl)-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (136) White solid; 51% yield; mp 171 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.79 (bs, 1H), 6.50 (s, 1H), 6.02-5.88 (m, 1H), 5.20-5.12 (m, 2H), 4.63 (d, *J* = 4.8 Hz, 2H), 4.30-4.27 (m, 2H), 3.94 (s, 3H), 3.84-3.82 (m, 1H), 1.74-1.67 (m, 2H), 0.95 (t, *J* = 7.2 Hz, 3H). MS (ESI): $[MH]^+$ = 343.3.

(*R,S*)-4-benzyl-2-(3-methoxyisoxazol-5-yl)-8-methyl-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (137) White solid; 38% yield; mp 111 ℃; ^{1}H NMR (200MHz, DMSO-d_6) δ ppm 10.64 (bs, 1H), 7.41-7.21 (m, 5H), 6.47 (s, 1H), 5.22 (s, 2H), 4.43-4.26 (m, 2H), 3.94 (m, 4H), 1.36 (d, *J* = 5.6 Hz, 3H). MS (ESI): $[MH]^+$ = 379.1.

(*R,S*)-4-benzyl-8-ethyl-2-(3-methoxyisoxazol-5-yl)-1,4,7,8-tetrahydro-5*H*-imidazo[2,1-*i*]purin-5-one (138) White solid; 39% yield; mp 157 ℃; ^{1}H NMR (200MHz, $CDCl_3$) δ ppm 10.64 (bs, 1H), 7.60-7.56 (m, 2H), 7.32-7.28 (m, 3H), 6.46 (s, 1H), 5.35 (s, 2H), 4.63-4.31 (m, 2H), 4.01 (s, 3H), 3.90-3.85 (m, 1H), 1.60-1.40 (m, 2H), 0.74 (t, *J* = 7.6 Hz, 3H). MS (ESI): $[MH]^+$ = 393.1.

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