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Design and Synthesis of New A_{2B} Adenosine Receptor Antagonists

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Introduction

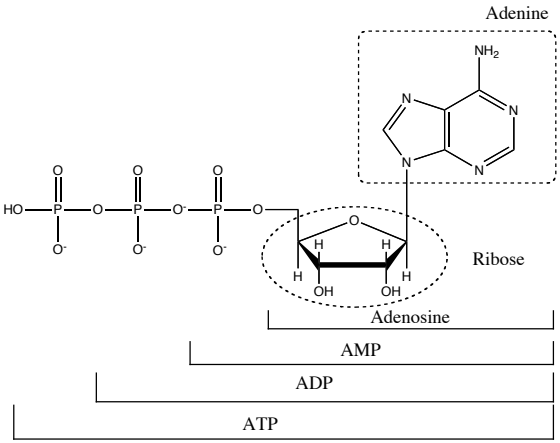
Adenosine is an endogenous nucleoside and has several proprieties in common with classical neurotransmitters:

- it exerts its action via receptors
- adenosine-producing enzymes are present in synapses
- its actions can be blocked by specific antagonists
- its actions are termined by an efficient reuptake system and a metabolising system.

Adenosine is usually designated as a neuromodulator because there is no evidence that it is stored in or released by specific purinergic nerves.

Adenosine is involved in important biochemical processes, such as energy transfer (ATP and ADP) and signal transduction (cyclic adenosine monophosphate, cAMP), and it is a building block for some biologically significant molecules such as NAD (nicotinamide-adenine-dinucleotide), FAD (flavin-adenine-dinucleotide), SAM (S-adenosyl-L-methionine), DNA and RNA.

Figure 1. *The structure of ATP and derivatives*.



The endogenous purine nucleoside (Figure 1) is ubiquitous in mammalian cell types and, in view of its function in regulating a wide number of physio-pathological events (as cytoprotective, anti-inflammatory, CNS neurotransmitters regulator, pain transmission and metabolism modulator agent),[1-9] a great interest in the understanding of its molecular pharmacology and physiology is widely spread in the scientific community.

Adenosine and ATP have been shown to induce signalling via P1 and P2 receptors, respectively. P1 (adenosine receptors, ARs) receptors are subdivided into four subtypes, all belonging to the family of cell membrane G protein-coupled receptors and named A_1, A_{2A}, A_{2B} and A_3, which have been cloned so far from many mammalian and some non-mammalian species.[10-13] In particular A_1 and A_3 inhibit, through G_i proteins, adenylyl cyclase activity, whereas A_{2A} and A_{2B} stimulate, via G_s proteins, this enzyme (see Table 1). Collectively, these receptors are widespread on virtually every organ and tissue and represent promising drug targets for the pharmacological intervention in many pathophysiological conditions that are believed to be associated with changes of adenosine levels such asthma, neurodegeneratives disorders, chronic inflammatory diseases and cancer. [14-15]

Table 1. *G-protein coupling and signal trasduction mechanism of Adenosine receptor subtypes*

	A_1	**A_{2A}**	**A_{2B}**	**A_3**
G-protein	G_i/G_0	G_s/G_{olf}	$G_s/G_{q/11}$	$Gi/G_{q/11}$
Signal trasduction	↓cAMP	↑cAMP	↑cAMP	↓cAMP
	↑IP_3/DAG		↑IP_3/DAG	↑IP_3/DAG
Affinity for adenosine	High	High	Low	Low

It has been suggest that adenosine receptors act as “sensor” and that extracellular adenosine acts as a “reporter” of metabolic changes in the local

tissue environment.[16,17] Therefore, under normal conditions adenosine, which is continuously produces intracellularly and extracellularly and maintained at low intracellular levels (about 100 nM) by adenosine kinase and adenosine deaminase, interacts with the high-affinity A_1 and A_{2A} receptor subtypes. In hypoxic, ischemic or inflamed conditions the intracellular production of adenosine is increased to very high micromolar concentrations and transported across cell membranes by specific agents finally leading to the stepwise activation of all four adenosine receptors, including the low affinity A_{2B} and A_3 subtypes (Table 1).[18]

Chapter 1

The A_{2B} Adenosine Receptor

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 differs 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. Different signal transduction mechanisms have been identified for each subtype. In particolar, activation of A_{2B} AR is associated with stimlation of adenylate cyclase and activation of phospholipase C through the coupling to Gs and Gq/11 proteins, respectively (Figure 2).

A_{2B}ARs are known as the most poorly characterised of the adenosine P1 receptors from a pharmacological point of view, since their general low affinity toward prototypic ligands exerting specific high affinity and potency in activating each of the remaining AR subtypes. Especially the scarceness of medicinal chemistry acquirements about the structural requirements necessary for a potent and selective activation of A_{2B}AR subtype has determined a wide-ranging difficulty in detecting the physiological effects mediated by a direct and selective A_{2B} AR stimulation.

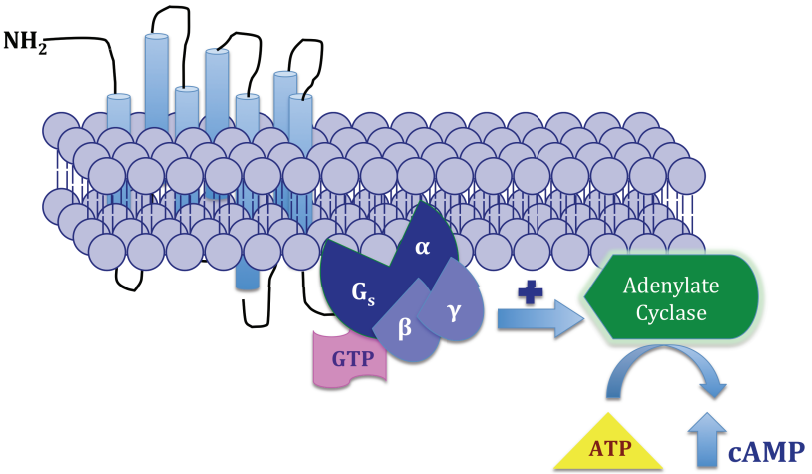


Figure 2. *A_{2B} Adenosine Receptor and its signal trasduction mechanism.*

Despite these limitations, growing and promising information in the understanding of the physiological meaning of these receptors have arisen from the exploitation of potency and selectivity of ligands for A_1, A_{2A} and/or A_3ARs employing a strategy of exclusion in model in which more AR subtypes are co-expressed. The A_{2A} selective agonist CGS 21680 has been reported, for example, as a useful tool for differentiation between A_{2A} and A_{2B}ARs.[19,20] Moreover some potent and selective antagonists of the A_{2B}AR

have been employed to distinguish A_{2B}-mediated effects. The characterization of A_{2B} receptors, through radioligand binding studies, has been performed, till a few years ago, by using low-affinity and non-selective antagonists like [^{3}H]DPCPX, [^{3}H]ZM241385, [^{125}I]ABOPX.[21] A helpful advancement in the pharmacological characterization of A_{2B} ARs is supposed to be increased by the recent identification of the tritiated form of some new A_{2B} antagonists with improved potency and selectivity such as [^{3}H]MRS1754,[22] [^{3}H]OSIP339391[23] and [^{3}H]MRE2029F20.[24]

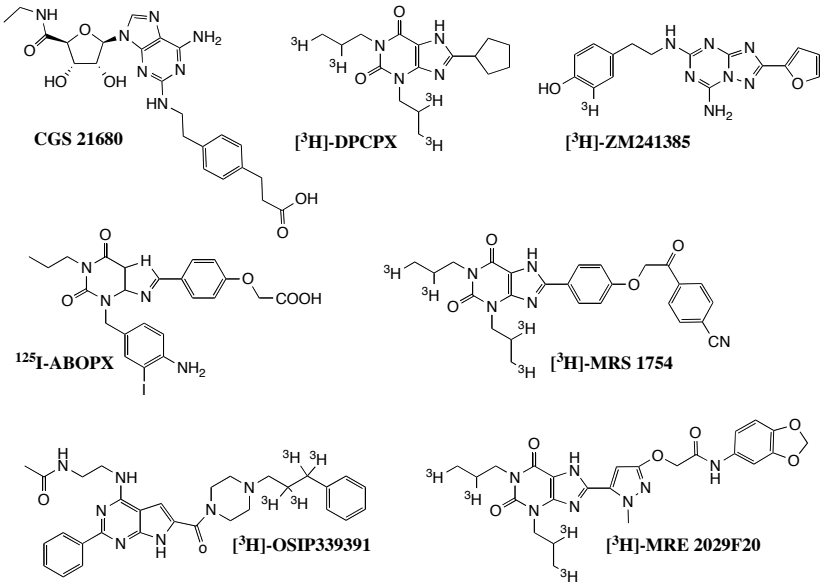


Figure 3. *Chemical structure of non-selective and selective radioligands for biological assays at Adenosine Receptors.*

The contribution of genetic engineering and manipulation (for example generation of A_{2B}AR knockout mice and transgenic mice over expressing this receptor), especially if combined with classical pharmacological investigations, have determined relevant progress in establishing the therapeutic po-

tential of A_{2B}ARs ligands and, generally, the role of ARs in a variety of deseases.[25-28]

A_{2B} Adenosine Receptors Physiology and Pharmacology

The A_{2B}AR subtype has been recognized to regulate a wide range of physiopathological events. However it results mainly involved in the modulation of the cardiovascular functions and in the genesis of inflammation processes.

A role for A_{2B}AR in regulation of vascular tone, cardiac myocyte contractility, neurosecretion and neurotrasmission, cell growth, gene expression, intestinal tone and secretion, and mast cells function has been suggested.[19] Activation of the A_{2B}AR is known to induce angiogenesis,[29,30] reduce vascular permeabilization,[31] increase release of inflammatory mediators from human and canine mast cells[29] and modulate neurohypophysial hormone output.[32]

Adenosine, through A_{2B} receptors, can exert long-term control over glycogen levels in primary cultures of mouse cortical astrocytes and might therefore play a significant role in patho-physiological processes involving long-term modulation of brain energy metabolism.[26] Evidences of a probable involvement of A_{2B} adenosine receptors in some tumors growth and development have been as well furnished and A_{2B}ARs have been proposed as targets to control cell growth and proliferation in a human breast cancer cell line.[33]

ARs play a significant role in regulating ions transport in epithelial tissues through a variety of intracellular signalling pathways. Each of the four P1 receptors has distinctive roles in different epithelial tissue types. The A_{2B} receptor has been identified on both the mucosal and basolateral aspect of colonic epithelial cells. Activation at either site results in Cl- secretion, via

direct activation of the cAMP-activated Cl^- channel CFTR (Cystic Fibrosis Transmembrane Conductance Regulator).[34] An analogous process of adenosine-mediated activation of Cl- secretion has been located at the lung epithelium.[35] The stimulated secretory response has been identified to be the result of A_{2B} receptor activation and was lost in a cell line derived from a cystic fibrosis patient with a defect in ion transport at CFTR, implicating this ion channel as the one responsible for the A_{2B}-mediated Cl- secretion.[36]

Therapeutic potential of A_{2B} adenosine receptor antagonists

There are growing findings supporting that adenosine plays a role in asthma and chronic obstructive pulmonary disease (COPD),[37] moreover adenosine stimulates production of IL-4 and IL-13 in mast cells via A_{2B} AR activation.[38] Treatment of asthma with selective A_{2B} adenosine receptor antagonists has been, so far, one of the most significant therapeutic ambition among ARs ligands.[39-43]

A_{2B}AR antagonists are directed toward clinical use for treating diabetes since these seem to antagonize the adenosine-induced hepatic glucose production determining reduction of blood glucose levels after oral administration.[44] A_{2B}AR has been likewise reported to be involved in stimulation of proliferation, differentiation and migration of retinal endothelial cells, thus A_{2B} antagonists may offer a way to inhibit retinal angiogenesis providing a novel therapeutic approach for treatment of diseases associated with aberrant neovascularization, such as diabetic retinopathy.[45]

The opioid and adenosine systems seem to cooperate to some extent in the modulation of pain signalling, in particular, a participation of A_{2B} receptors in the analgesic effects mediated by caffeine in an acute animal model of nociception (hot plate test) has been documented.[46] These findings sup-

ported the potential therapeutic employment of specific A_{2B} antagonists as valuable adjuvant drugs for opioid analgesia with minimal side effects.

The purinergic regulation of epithelial transports and, above all, the involvement of the A_{2B}AR subtype in determining secretion stimulation, suggest the possibility to employ A_{2B} specific ligands as potential modulators of the ions transport and the parallel flux of water which can be considered a natural defense system working to "wash away" injuries in the setting of cellular damage or inflammation. Selective A_{2B}AR ligands are under investigation for the treatment of diarrhea and cystic fibrosis.[47-49]

A_{2B} adenosine receptor and asthma: Clinical development and patents

Asthma is a chronic inflammatory disease of the airway that involves many inflammatory and structural cell types such as mast cells, eosinophils, basophils and lymphocytes. Activation of this cells result in the release of various inflammatory mediators that contribute to the typical pathopysiological processes that lead to the manifestation of asthma.[50] Mast cells, in particular, play an essential role in this system.[50,51] These cells possess high-affinity IgE receptors (FceRI) on their surface, and in the acute phase of the disease, the cross linking of mast cell-bound IgE by allergians induces their activation and degranulation, whereby they release proinflammatory, plasma exudation and mucus hypersecretion. In the chronic phase of asthma, mast cell also secrete a wide array of cytokines and growth factors including IL-1, IL-2, IL-3, IL-4, IL-5, IL-8, IL-9, IL-13, IFN-γ, TNF-α and GM-CSF.[52] These secretions contribute to the chronic inflammatory changes that take place, including fibrosis, hypertrophy and hyperplasia of mucus-secreting cells and angiogenesis. Such changes may be irreversible, leading to permanent narrowing of the airways.[53]

There is much evidence to suggest that adenosine plays a central role in the asthmatic process being the activation of mast cells a key element.[54] In human lung mast cells, adenosine was shown to potentiate mediator release.[55] Similary in bronchoalveolar fluid (BALF) cells obtained from asthmatic patients after allergen challenge, histamine was shown to be released after exposure to adenosine.[56] The result of in vivo studies are futher supported by in vivo finding: different studies have shown that inhalated adenosine, or AMP, provoke strong broncoconstriction in ashmatic patients, but not in healthy subjects,[57] and elevated concentrations of adenosine were observed in BALF of asthmatic patients.[58]

There is increasing evidence to suggest that A_{2B}AR are responsible for the degranulation of mast cells.[55,57,59,60-63] In the human mast cell line HMC-1, adenosine A_{2B} receptor are also linked to the pathogenesis of asthma, partly by the so called "enpropylline paradox". Enpropylline has a potent anti-asthmatic action that has been attributed to its ability to block A_{2B}AR, but with low affinity and selectivity.[19,59,64] It has also been shown that the release of some inflammatory cytokines such as IL-8 and IL-6 from different cells is mediated by the activation of A_{2B} subtypes.[41,59,63] The lack of selective A_{2B}AR ligands justifies the development of an effective and selevtive antagonists in order to validate this receptor as therapeutic target in asthma.

The A_{2B}AR antagonists that have being investigated for the treatment of asthma are now in Phase I and II trials.

CVT-6883 (compound **32,** see Table 2 and 3, Chapter 2) is a selective, potent and orally available A_{2B}AR antagonist. CV Therapeutics has initiated a clinical program for CVT-6883 intended to treat asthma with once a day dosing. A randomized, double-blind, placebo-controlled, single-dose phase I study of oral CVT-6883 in 24 healthy adults is assessing the safety and tolerability of a range of doses of CVT-6883. Blockade of the A_{2B}AR may

limit or prevent mast cell degranulation, which can lead to bronchoconstriction and the inflammatory process associated with asthma and cardiopulmonary disease. In a recent study, mice deficient in Adenosine deaminase (ADA) developed severe pulmonary inflammation and injury, which could be attenuated by CVT-6883 treatment. This compound was also able to decrease elevated proinflammatory cytokine lung levels in ADA-deficient mice. CVT-6883 also suppressed the increased expression of matrix metalloproteinases and their inhibitors, as well as pulmonary fibrosis, developed by mutant mice. ADA deficiency was also related with enhanced lung expression of adenosine and A_{2B}AR, the levels of which tended to be lower in the presence of CVT-6883. In a mouse model of bleomycin-induced pulmonary fibrosis, lung injury was successfully reduced by CVT6883. CVT6883 also attenuated the airway reactivity induced by 5'-*N*-ethyl-carboxamide adenosine (NECA), AMP, or allergen in an allergic mouse model of asthma.[65] Together, these results suggest that antagonizing A_{2B}AR may be beneficial in the management of chronic pulmonary diseases. Results from a randomized, double-blind, placebo-controlled, single ascending dose phase I clinical study of CVT-6883 indicated that it was well tolerated with no serious adverse events reported.[66] CVT-6883 has been patented for asthma and COPD.[67] Moreover A_{2B}AR antagonists have been claimed for use in promoting the healing of wounds caused by mechanical, chemical or thermal activity, for example for diabetic ulcer.[68] MRE2029F20 (compound **16**, see Table 2 and 3, Chapter 2) is one of several high-affinity antagonists of King Pharmaceuticals's in preclinical phase. This compound has been reported to be a potent and selective A_{2B} antagonist able to counteract stimulation of cAMP levels from in vitro studies performed in HEK293 cells and in inflammatory cells relevant for asthma like neutrophils, lymphocytes and HMC1 cells.[69] Several A_{2B} antagonists have been patented for asthma and

eye disorders.[70] Other interesting A_{2B} antagonists in preclinical phase include MRS1754,[71] MRS1706,[71] MRS1668,[71] PSB-1115[72] developed at National Institutes of Health (NIH) and PSB 53,[72] from the University of Bonn.

Therapeutic potential of A_{2B} adenosine receptor agonists

Ischemic preconditioning (IPC) is a cardioprotective mechanism according which brief and repeated episodes of sub-lethal ischemia and reperfusion, before myocardial infarction, cause the heart to become resistant to infarction and result in attenuation of infarct size. Activation of cardiac A_{2B} receptors at reperfusion showed to be protective in the rabbit but, because of the very low affinity of the receptors, endogenous cardiac adenosine is unable to elicit their signalling. Protein kinase C physiologically increases the heart's sensitivity to adenosine so that endogenous adenosine can activate A_{2B}-dependent signalling. BAY 60-6583, a highly selective A_{2B} agonist,[73] limited infarct size when given to rabbit ischemic hearts at reperfusion.[74] Postconditioning protects the heart with multiple brief reperfusion/ischemia cycles immediately following the ischemic insult. In rabbit hearts, binding of endogenous adenosine to A_{2B} receptors in early reperfusion is a requirement for both IPC and postconditioning to limit infarction.[75]

A pharmacological and gene-targeting approach, performed with mice models to study the contributions of adenosine receptor signalling to ischemic preconditioning cardioprotection, gave evidences that selective A_{2B}AR agonists may offer important advantages in comparison with classical therapies of acute myocardial ischemia.[76] Intravenous administration of the non-selective adenosine is associated with side effects (bradycardia, hypotension, rapid receptor desensitization) which could be circumvented by the use of specific A_{2B}AR agonists.[28]

Deletion of the gene encoding the A_{2B}AR in the mouse (A_{2B}AR-knockout mouse model) recently showed to result in increased production of proinflammatory cytokines, altered response to endotoxin exposure, increased leukocyte adhesion and increased leukocyte rolling on blood vessels.[77] Activation of A_{2B}AR subtype would moreover increase production of the anti-inflammatory cytokine IL-10.[78]

The described association of A_{2B}AR with pre/postconditioning cardioprotection along with the documented strong antinflammatory role of A_{2B}AR signalling,[77] suggest that A_{2B}AR agonists may represent a new group of therapeutics for patients suffering from coronary artery disease. Several reports contribute to strengthen this perspective, highlighting essential A_{2B}-mediated cardiovascular effects. A NECA-mediated coronary vasodilation via the A_{2B}AR subtype in isolated hearts from young (1-2 months) and mature (12-18 months) Wistar rats, has been documented.[79] Adenosine-mediated vasorelaxation in mouse aorta is partially dependent on A_{2B}AR,[80] besides, A_{2B} ARs mediate relaxation in human small resistance-like coronary arteries which is independent of NO but partly coupled to K^+ channels function.[81]

Human aortic smooth muscle cells (SMCs) synthesize adenosine which seems to protect against vasoocclusive disorders by inhibiting SMCs proliferation and collagen synthesis via activation of A_{2B} receptors.[82] Apoptosis of arterial smooth muscle cells (ASMCs) could play an important role in the pathogenesis of atherosclerosis and restenosis. Recent results indicate that adenosine-induced apoptosis of cultured human ASMCs is essentially mediated via A_{2B}-adenosine receptor and involves a cAMP-dependent pathway.[83] These studies speculated that adenosine could play a dual role in the evolution of intimal thickening. Its action can be considered beneficial for what concerning the control of intimal hyperplasia and thickening forma-

tion. In contrast, the same authors indicated that adenosine could contribute to the formation of the necrotic core in advanced atherosclerotic lesions promoting, along with other concurrent factors, the plaques rupture. These opposing effects suggest different therapeutic strategies based on the role of A_{2B}ARs stimulation-mediated effects in the pathogenesis of atherosclerosis and restenosis. Glomerular mesangial cells (GMCs) growth is inhibited by activation of A_{2B} receptors coupled to inhibition of mitogen-activated protein kinase (MAPK) activity.[84] A_{2B}AR function may therefore largely affect glomerular remodeling associated with GMC proliferation. Identification of pharmacological agents able to specifically activate A_{2B} receptors has been supposed of therapeutic importance in protecting against glomerular remodeling associated with glomerulosclerosis, renal disease, and abnormal GMC growth associated with hypertension and diabetes.

Studies performed by Shiseido Research group would state that adenosine, via A_{2B}AR, might stimulate hair growth through fibroblast growth factor-7 gene expression upregulation in dermal papilla cells.[85]

In the research field concerning the vasculogenic erectile dysfunctions emerged a key importance of purinergic transmission for initiation and maintenance of penile erection.[86] Endothelial dysfunction of human corpus cavernosum may be correlated with the loss of adenosine A_{2B} receptors activity indicating a possible employment of specific A_{2B}AR agonists as new therapeutic approach to manage severe vasculogenic impotence resistant to common vasodilators.[87] Since interaction of adenosine with A_{2B} receptors inhibits production of the pro-inflammatory cytokine Tumor Necrosis Factor (TNFa) by lipopolysaccharide activated monocytes,[77,88] A_{2B} agonists have been proposed for treatment of septic shock, confirming the broad anti-inflammatory potential of AR agonists in treatment of inflammatory disorders.[89]

Chapter 2

Medicinal Chemistry of Xanthine-based A_{2B} Adenosine Receptor Antagonists

The pharmacological evidence of the therapeutical applications of A_{2B}AR antagonists has stimulated the research for the development of potent and selective ligands for this subtype. Among the different potential therapeutic applications postulated for the A_{2B}AR antagonists treatment of asthma seems the most interesting and promising therapeutic goal.

Xanthine derivatives represent most of the high affinity Adenosine receptor antagonists.

Due to its clinical relevance, much research is devoted to the development of A_{2B} AR antagonists.

On the bases of chemical structure and biological activity of xanthine-based A_{2B} antagonists the new series of 8-heterocycles xanthine were designed and will be discussed in this PhD thesis. An overview of previous A_{2B} antagonists is described in this chapter.

Table 2 and 3 present the most important xanthine derivatives as A_{2B} antagonists and relative biological data respectively reported in literature.

Research on the adenosine A_1, A_{2A}, and A_3 receptors indicated that xanthines contain a promising core structure, modification of which led to the identification of selective antagonists for these three receptor subtypes. Initial efforts to develop antagonists selective for adenosine A_{2B} receptor focused therefore on this class of compound.

The natural compound caffeine (1,3,7-trimethylxanthine, **1**) and theophylline (1,3-dimethylxanthine, **2**) are examples of compounds with a xanthine core strucure and both of them have micromolar affinities for all adenosine receptors (see tables 2, 3).[90]

Theophylline is used therapeutically for the treatment of asthma and even if it has been in clinical use for decades, the mechanism of action through the adenosine A_{2B} receptor has just recently been identified.[90] The application of theophylline in the treatment of asthma is, however, accompanied with unpleasant side-effects such as insomnia and diuresis. Moreover, theophylline has a low therapeutic index due to its interaction with phosphodiesterase enzyme (PDE) and side effects in the Central Nervous System (CNS). Identification of selective adenosine A_{2B} receptor antagonist is therefore desirable. Initial attempts to obtain selective antagonists for the adenosine A_{2B} receptor yielded the xanthine analog enprofylline (3-propylxanthine, **3**). The affinity of this compound for the human adenosine A_{2B} receptor ranged

from 4.7 to 23 μM. Like theophylline, the treatment of asthma with enprofylline is based on its interaction with the adenosine A_{2B} receptor.[90]

Several research groups have synthesized 8-phenyl substituted xanthines that have shown high A_{2B} AR affinity and selectivity against the other AR subtypes.

The phenyl ring at the 8-position of xanthine core was introduced by Muller and co-workers.[91] 1,3-disubstituted-8-(*p*-substituted-phenyl)-xanthine derivatives were reported as A_{2B} adenosine antagonists (compounds **4**-**7**, table 2). In order to increase the water solubility polar groups, basic or acid functions, were introduced into the *para*-position of the phenyl ring.[72]

Jacobson and co-workers have been also demonstrated that the introduction of a *para*-substituted phenyl derivative at the 8-position of the xanthine core increases the A_{2B}AR affinity and selectivity against the other AR as illustrated in table 2 and 3.[92] In particular, two different series of compounds, with a substituted phenyl moiety at the 8 position of xanthine nucleus, have been developed by Jacobson et al.: the 8-phenylxanthine carboxylic congener (XCC) reported as a selective A_{2B} antagonists (table 2 and 3) and the series of the corresponding xanthine amino congener (XAC).

The most selective antagonist among a large series of XAC and XCC derivatives was the analog MRS1204 (**10**). It possessed a low nanomolar affinity for the A_{2B} receptor and at least 20-fold selectivity over the other AR subtype. The structural analogs MRS1706 (**11**) and MRS1754 (**12**) had an even higher affinity for the human adenosine A_{2B} receptor than MRS1204 (**10**), 1.4 and 2.0 nM *versus* 9.8 nM as it is shown in Tables 2 and 3.

Zablocki et al. used MRS1754 as a reference compound to synthesize a series of metabolically more stable analogs by replacing the anilide moiety.[93]

In particular the authors prepared the amide surrogates analogs of MRE1754 such as 8-(4-((3-phenyl-1,2,4-oxadiazol-3-yl)methoxy)phenyl)-xanthine and 8-(4-((5-phenyl-1,2,4-oxadiazol-3-yl)methoxy)phenyl)-xanthine analogs.

The oxadiazole is a constrained amide surrogate that led to a different SAR result than the anilide series previously described by Jacobson and co-worker. In direct contrast with the 3-phenyl-1,2,4-oxadiazole series, that prefer electron-withdrawing groups (EWG) substituents on the phenyl ring, the 5-phenyl-1,2,4-oxadiazole series favors electron-donating (EDG) groups in relation to affinity for the A_{2B}AR. It was identified a clear trend with respect to phenyl substitution and affinity for the A_{2B} AR within the 5-phenyl-1,2,4-oxadiazole series: MeO > Me > F = CF_3 > Cl. Among this series, CVT 5440 (**13**) had the highest selectivity and an affinity of 50 nM for the adenosine A_{2B} receptor. The analog of CVT 5440, conpound **14**, showed a loss of affinity for A_{2B}AR (hA_{2B} K_i = 1680 nM).[93]

In this field of research, Baraldi et al. published a series of 8-heterocycle-substituted xanthines as A_{2B} adenosine receptor antagonists.[79,94]

Several heterocycles, such as pyrazole, isoxazole, pyridine, and pyridazine at the 8-position of the xanthine nucleus, were studied. The synthesized compounds showed A_{2B}AR affinity in the nanomolar range and good levels of selectivity evaluated in radioligand binding assays at hA_1, hA_{2A}, hA_{2B}, and hA_3 ARs. These studies allowed to identify the derivatives 2-(3,4-dimethoxy-phenyl)-*N*-[5-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-1-methyl-1*H*-pyrazol-3-yl]-acetamide (MRE2028F20, **15**), *N*-benzo[1,3]dioxol-5-yl-2-[5-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-1-methyl-1*H*-pyrazol-3-yloxy]-acetamide (MRE2029F20, **16**), and *N*-(3,4-dimethoxy-phenyl)-2-[5-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-1-methyl-1*H*-pyrazol-3-yloxy]-acetamide (MRE2030F20, **17** Tables 2, 3), which showed high affinity to the A_{2B}AR

subtype and very good selectivity *vs* the other AR subtypes. Substitution of the acetamide with an urea moiety afforded bioisosteric xanthines with good affinity and selectivity comparable to the acetamide derivatives. The 8-heterocycles-substituted xanthine with higher affinity to hA_{2B}AR proved to be antagonists, in the cAMP assay, capable of inhibiting the stimulatory effect of NECA (100 nM) with IC_{50} values in the nanomolar range and a trend significantly related to that observed in the binding assay. Consequently, Baraldi and co-workers synthesized the *N*-benzo[1,3]dioxol-5-yl-2-[5-(1,3-diallyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-1-methyl-1*H*-pyrazol-3-yloxy]-acetamide that led to prepare the tritium labelled form [^{3}H]MRE2029F20, (K_D value of 1.65±0.10 nM in Chinese Hamster Ovary (CHO) cells expressing hA_{2B} receptors)**.** This compound was found to be a selective, high-affinity radioligand useful for characterizing recombinant hA_{2B} receptors.[24]

Starting from the discovery of *N*-(2-chlorophenyl)-2-(4-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-1*H*-pyrazol-1-yl)-acetamide (**18)** a new series of 1,3-dipropyl-8-(1-heteroarylmethyl-1H-pyrazol-4-yl)-xanthine derivatives have been synthesized by Zablocki and co-worker.[95]

These new compounds possess a *N*-substituted pyrazole at the 8 position of the xanthine nucleus. Also in these series the amide bonds have been replaced with a wide variety of structural moieties in attempts to achieve metabolic stability and oral bioavailability. Heterocyclic 5-membered ring such as 1,2,4-oxadiazoles, oxazoles and isoxazoles have been extensively used as amide bond bioisosteres.[96] Taking into account, they replaced the amide bond in the compound **19** with different oxadiazoles and isoxazoles. In this study three different series of compound have been synthesized and evaluated *vs* the four AR subtypes (**19**-**23**).

The replacement of the amide bond compound **18** (hA_1 K_i = 102 nM, hA_{2A} K_i = 1500 nM, hA_{2B} K_i = 22 nM, hA_3 K_i = 1200 nM) with 1,2,4-oxadiazole resulted in compound **19** (hA_1 K_i = 2500 nM, hA_{2A} K_i = 2500 nM, hA_{2B} K_i = 19 nM, hA_3 K_i = 490 nM) that displayed similar binding affinity for the A_{2B} AR compared to compound **18**. The authors observed that, in the new series of compounds, derivatives that incorporated EWD groups in the phenyl ring displayed higher affinity and selectivity for the A_{2B} AR relative to those having EDG substituents. The unsubstituted phenyl analog **20** exhibited very high affinity (hA_{2B} K_i = 1 nM) and selectivity for the A_{2B} AR and suggests that in the 3-phenyl-1,2,4-oxadiazole class of compounds, unsubstituted phenyl ring is optimal for both A_{2B} AR binding affinity and selectivity.

Among 5-phenyl-1,2,4-oxadiazole series, the regio isomer of compound **20**, derivative **21** showed a decrease of selectivity and affinity for A_{2B} AR but a strong EWD in the 3-position of the phenyl ring (3-CF_3) provided the most active and selective analog **22** (hA_1 K_i > 6000 nM, hA_{2A} K_i > 5000 nM, hA_{2B} K_i = 21 nM, hA_3 K_i = 1300 nM). The remaining compounds of the series displayed a slight decrease of activity respect of previous series derivatives. One could envision the 5-phenylisoxazole series as a direct analog of the 5-phenyl-1,2,4-oxadiazole series where the 4-nitrogen atom in the isoxazole ring is isosterically replaced with a carbon atom such as in compound **23**. In general this third series was less active and selective for A_{2B} AR in comparison to the other oxadiazole classes of compounds as it is shown in tables 2 and 3.[95]

Another series of 8-pyrazole xanthine derivative was patented by Kalla et al. as A_{2B} AR antagonists. The new series of compounds displayed good affinity and selectivity for the A_{2B} subtype and confirming the relevance of a pyrazole ring at 8-position of xanthine nucleus.[97] Kalla and co-worker analyzed the substitution of the pyrazole ring at the 8-position of xanthine core

and synthesized three different series of compounds: 8-(*N*-1-pyrazolyl) xanthine, 8-(C5-pyrazolyl) xanthine and 8-(4-pyrazolyl) xanthine.

The 8-(*N*-1-pyrazolyl) xanthine derivatives, **24**-**26** demonstrated low affinity for the A_{2B}AR. This suggests that this mode of pyrazole attachment is not conductive for binding to A_{2B}AR. Also 8-(C5-pyrazolyl) xanthine derivatives **27**-**29** showed weak binding affinities (in the range of 1-3 μM) for the A_{2B}AR.[97]

Instead the incorporation of C4-pyrazole ring at the 8-position of the xanthine nucleus resulted in high affinity and low selectivity for A_{2B}AR subtypes. The two options for increasing the binding selectivity were to change the substitution either at the *N*-1 position of the pyrazole or at the *N*-1 and *N*-3 positions of the xanthine core. Starting from the compound **30** of the 8-(4-pyrazolyl) xanthine series, characterized by a phenyl ring at the *N*-1 position of pyrazole group (hA_{2B} K_i = 310 nM), the importance of the spacer between the phenyl and the pyrazole ring was investigated by Kalla et al. The introduction of a benzyl group at the *N*-1 position of pyrazole ring resulted in compound **31** which displayed slightly enhanced selectivity over A_1 AR. The effect of introducing of EDG and EDG groups on the phenyl ring was investigated with the goal to enhancing its A_{2B} affinity and selectivity. The best compound of this series was CVT6883, **32** (hA_{2B} K_i = 14 nM).[97]

Increasing the distance between the phenyl group and the *N*-1 position of pyrazole ring from one carbon atom to two carbon atoms, as in compounds **33** and **34**, resulted in a decrease in affinity and selectivity for the A_{2B}AR relative to **31**. After this study, the effect of varying the dialkyl substitution of the xanthine core *N*-1 and *N*-3 on A_{2B}AR affinity and selectivity was evaluated and they confirmed that 1,3-diproyl xanthine derivatives showed the best results in terms of both affinity and selectivity.

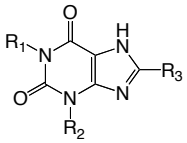
Continuing the research of 8-pyrazole xanthine derivatives, Baraldi and co-worker developed and characterized a new series of compounds **35**-**41** obtained by replacement of the side chain from the 3-position of the pyrazole ring of the MRE series to the 1-position.[98] As it is shown in Tables 2 and 3, the synthesis of this new class of 8-(pyrazole-3-yl) xanthine derivatives led to the identification of several A_{2B}AR antagonists with high affinity and very good selectivity for the adenosine receptor subtypes. Different positions in phenyl ring of the xanthine-based structure were investigated in order to design a SAR profile. In particular were prepared derivatives to study the effect of substituting the 3-and/or 4-position of phenyl ring with EDG and EWG groups. The substitution at the *para*-position of the phenyl ring with EWG groups, such as halogen atoms, resulted in an increase of selectivity while retaining affinity at the A_{2B} receptor (**35**-**37** derivatives). The 4-chloro-phenyl derivative **35** was found to be the most potent (K_i hA_{2B} = 7 nM) and selective compound within the series (A_1, A_{2A}, A_3/A_{2B} > 140). The substitution at the *para*-position of the phenyl ring with EDG moiety, (**38, 39)** also showed good affinity at the A_{2B}, retaining selectivity at AR subtypes while bulky lipophilic groups led to a loss of affinity. Shifting of substituents from the *para*-position to the *meta*-position of the phenyl ring resulted in compounds with lower binding affinity at the A_{2B} receptor compared to the corresponding *para*-position derivatives. Disubstituted phenyl analogues which contain a 3,4-substituent were found to have lower A_{2B} AR affinity than mono-substituted analogues. Moreover the effect of substitution of methyl group at the 5-position of pyrazole was investigated. The presence of ethyl moiety at the 5-position (**40)** led to a loss of affinity and the derivative with hydrogen atom at the same position (**41)** displayed a 3-fold decrease of affinity and selectivity compared to the reference compound **35**. In this study was also explored the effect of 4-substituted pyra-

zoles on A_{2B} AR affinity with the 4-halo-derivatives that resulted in a loss of affinity compared to unsubstituted derivative **35**.[98]

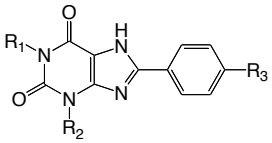
9-Deaza Xanthine-based Derivatives

Another subclass of xanthine core, the 8-substituted-9-deazaxanthines also yielded derivatives with nano molare affinity for the adenosine A_{2B} receptor. The most potent antagonist, C8-4-aminophenyl, 1,3-dipropyl-9-deazaxanthine (**43)** was, however, poorly selective (7-fold) with respect to the adenosine A_{2A} receptor.[99] Moreover this compound was not tested on the human adenosine A_1 and A_3 receptors. The unsubstituted compound (**42**) was tested on all four human adenosine receptors and had the highest affinity for the A_1 adenosine receptor. Kalla et al.[100] also described a series of 8-substituted-9-deazaxanthines as selective A_{2B}AR antagonists. The structure of two of the more potent compounds was revealed (**44**, **45**, Tables 2, 3) and their affinities provided. The 3-fluorobenzyl substituted compound (**44**) had a slightly higher hA_{2B} K_i value, 13 nM, *versus* 9 nM for the 3-trifluoromethylbenzyl substituted compound (**45**) as it is shown in Table 2 and 3.

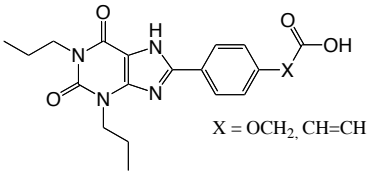
Table 2. *Xanthine-based derivatives as A_{2B} Adenosine Antagonists*



1, Caffeine $R_1 = R_2 = R_3 = CH_3$
2, Theophillyne $R_1 = R_2 = CH_3$, $R_3 = H$
3, Enprophylline $R_1 = H$, $R_2 = C_3H_7$, $R_3 = H$

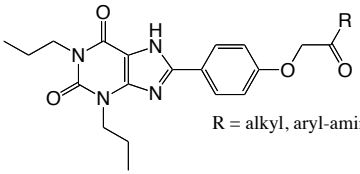


4, $R_1 = R_2 = R_3 = H$
5, $R_1 = R_2 = C_3H_7$, $R_3 = 4$-Br
6, $R_1 = C_4H_9$, $R_2 = H$, $R_3 = 4$-COOH
7, $R_1 = C_4H_9$, $R_2 = H$ $R_3 = 4$-COOMe



X = OCH_2, CH=CH

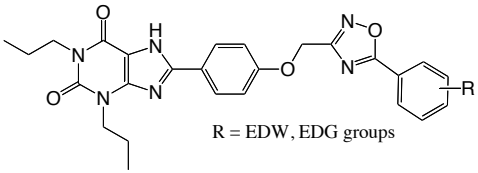
XCC Series
9, X = OCH_2



R = alkyl, aryl-amine

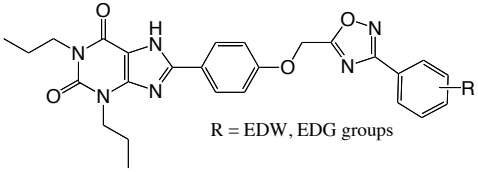
XAC Series
8, R = $NH(CH_2)_2NH_2$

MRS Series
10, MRS1204 R = 
11, MRS1704 R = $NHPhCOCH_3$
12, MRS1754 R =NHPh4-CN



R = EDW, EDG groups

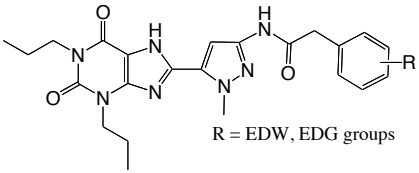
5-phenyl-1,2,4-oxadiazole Series
13, CVT5440 R = 3-OCH_3



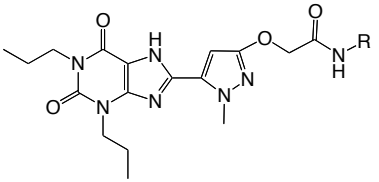
R = EDW, EDG groups

3-phenyl-1,2,4-oxadiazole Series
14, R = 3-OCH_3

Table 2. *Continued…*

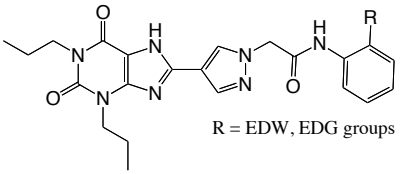


15, MRE2028F20 R = 2, 3-OMe

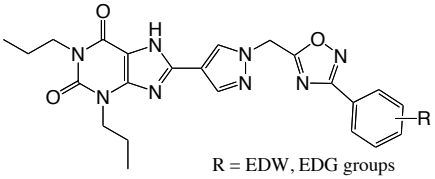


16, MRE2029F20 R = 

17, MRE2030F20 R = 

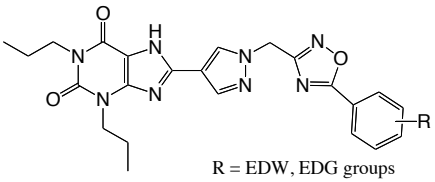


18, R = Cl



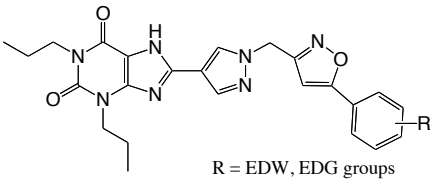
3-phenyl-1,2,4-oxadiazole Series

19, R = 2-Cl
20, R = H



5-phenyl-1,2,4-oxadiazole Series

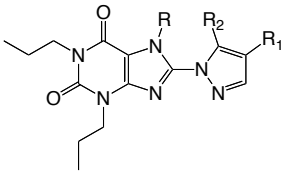
21, R = H
22, R = 4-CF_3



5-phenyl-isoxazole Series

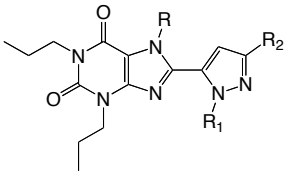
23, R = 4-CF_3

Table 2. *Continued…*



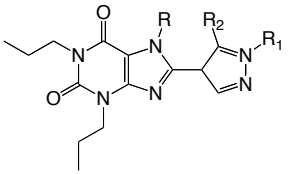
8-(N-1-pyrazolyl)-xanthine Series

24, R = Bn, $R_1 = R_2 = H$
25, R = Bn, $R_1 = I$, $R_2 = H$
26, $R = R_1 = R_2 = H$



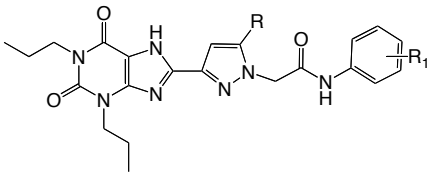
8-(5-pyrazolyl)-xanthine Series

27, $R = H$, $R_1 = R_2 = CH_3$
28, R = H, R_1 = Et, R_2 = CH
29, R = H, R_1 = Ph, R_3 = H



8-(4-pyrazolyl)-xanthine Series

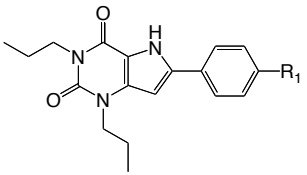
30, R = H, R_1 = Ph, R_2 = H
31, R = H, R_1 = Bn, R_2 = H
32, CVT6883 R = H, R_1 = 3-CF_3Ph, R_2 = H
33, R = H, $R_1 = CH_2CH_2Ph$, R_2 = H
34, R = H, R1 = $CH_2CH_2CH_2Ph$, R_2 = H



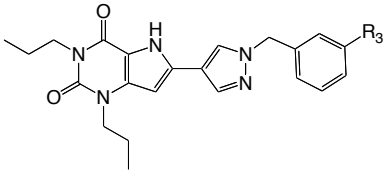
8-(3-pyrazolyl)-xanthine Series

35, $R = CH_3$, R_1 = 4-Cl
36, $R = CH_3$, R_1 = 4-Br
37, $R = CH_3$, R_1 = 4-I
38, $R = R_1 = CH_3$
39, $R = CH_3$, R_1 = 4-OCH_3
40, R = CH_2CH_3, R_1 = 4-Cl
41, R = H, R_1 = 4-Cl

9-Deaza Xanthine derivatives A_{2B} Adenosine Antagonists



42, R = H
43, R = NH_2



44, R_1 = H, R_2 = F
45, R_1 = OH, R_2 = CF_3

***Table 3.** Biological affinity of the A_{2B}AR antagonists.*

Compound	Ki nM			
	hA_1	**hA_{2A}**	**hA_{2B}**	**hA_3**
1[90]	10700	9560	10400	-
2[90]	6800	1700	6700	86000
3[90]	156000	32000	7000	65000
4[91]	-	-	810	
5[91]	-	-	3.8	--
6[72]	-	-	24	-
7[72]	-	-	15.1	-
8[92]	6.8	18	7.8	26
9[92]	175	595	13.6	3,910
10[92]	153	127	9.8	227
11[92]	157	112	1.4	230
12[92]	403	503	2.0	570
13[93]	> 10000	> 5000	50	> 8350
14[93]	> 6000	> 5000	1680	-
15[94]	> 1000	> 1000	38	> 1000
16[94]	200	> 1000	5.5	> 1000
17[94]	> 1000	> 1000	41	> 1000
18[95]	102	1500	22	1200
19[96]	2500	2500	19	490
20[96]	370	1100	1	480
21[96]	1000	1800	21	630
22[96]	> 6000	> 5000	21	1300
23[96]	2300	1700	48	1200
24[97]	-	-	> 5800	-
25[97]	-	-	> 5800	-
26[97]	-	-	> 5800	-
27[97]	-	-	3700	-
28[97]	-	-	2100	-
29[97]	-	-	1100	-
30[97]	-	-	310	-
31[97]	76	290	11	-
32[97]	170	400	14	-
33[97]	17	570	74	-
34[97]	77	220	100	-
35[98]	> 1000	> 1000	7	> 1000
36[98]	> 1000	> 1000	35	> 1000
37[98]	> 1000	> 1000	28	> 1000
38[98]	> 1000	> 1000	56	> 1000
39[98]	> 1000	> 1000	12	> 1000
40[98]	> 1000	> 1000	> 1000	> 1000
41[98]	140	> 1000	20	> 1000
42[99]	34	646	229	95
43[99]	-	112	17	-
44[100]	-	-	13	-
45[100]	-	-	9	-

**Experimental conditions for biological assays are reported in the cited references.*

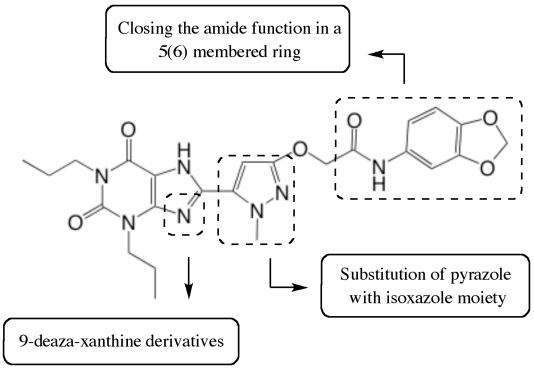
Chapter 3

Design and synthesis of new A_{2B} Adenosine Antagonists

The new A_{2B}AR antagonists were designed and synthesized on the basis of previouse chemical structure of our 8-pyrazolyl-xanthines. This chapter describes the synthesis and the pharmacological properties of a set of new 8-heterocycle-xanthines that confirm the A_{2B} selectivity of this xanthine class of compounds.

Starting from chemical structure of MRE2029F20,[94] a new series of A_{2B} antagonists has been designed and synthesized. As shown in Figure 4, various structural modifications were realized using the previously reported chemical structure of 8-[(pyrazol-3-yloxy)-acetanilide)] xanthine[94] as a template.

Figure 4.*Structure modifications considered MRE2029F20 as a template*.

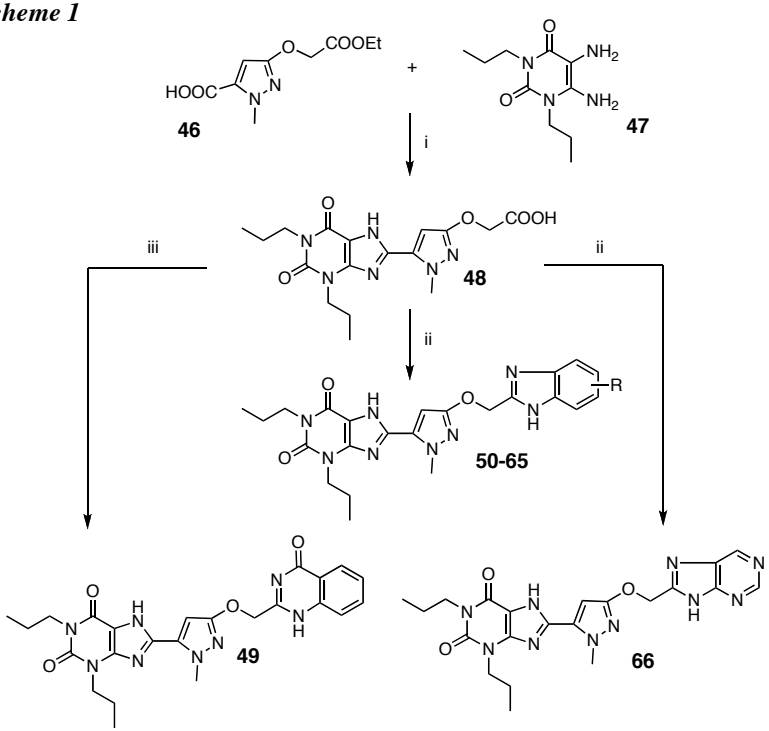


The bioisosteric replacement of the anilide moiety with benzimidazole or quinazoline rings, the effect of the substitution of pyrazole with isoxazole moiety was investigated. Amide bond has been also replaced with the 5-phenyl-1,2,4-oxadiazole nucleus on the basis of other adenosine pharmacophores such as those reported previously by Zablocki et al.[93]

In this context the effect of the nitrogen at the 9-position has been also studied preparing four 9-deaza direct analogs of 8-pyrazol-xanthine compounds to compare affinity and selectivity at A_{2B} adenosine receptor.

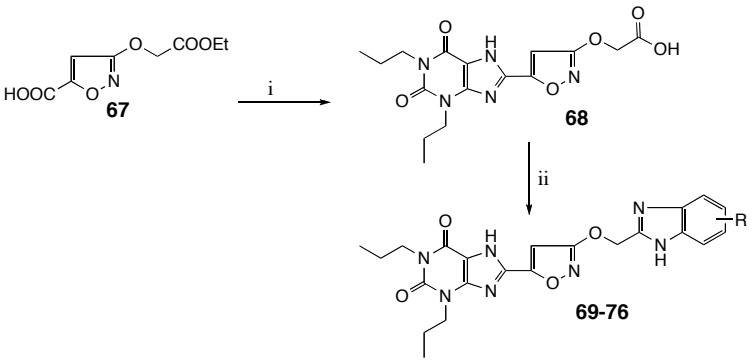
The compounds described in this report are shown in Tables 4-7, Chapter 4 and their syntheses are outlined in Schemes 1-4**.** The carboxylic acid [94] derivatives **48** or **68,** in anhydrous dimethylformamide (DMF), were condensed with phenylendiamines (2-amino-benzamide for compound **66**) using 1-[3-(dimethyl-amino)-propyl]-3-ethylcarbodiimide hydrochloride (EDC) to furnish the amides that were subsequently cyclized in the presence of acetic acid at 80-100 °C to provide the 8-heteroaryl derivatives **49**-**66** and **69-76** (Scheme 1 and 2).

Scheme 1



Reagents: *i: a. 1,3-dipropyl-5,6-diamino-uracil (**47**), EDC, methanol, rt; b. NaOH, 80 °C; ii: a. 2-amino-benzamide, EDC, DMF, rt, b. CH_3CO_2H, 80 °C; iii: a. appropiate diamine, EDC, DMF, rt; b. CH_3CO_2H, 80-100 °C.*

Scheme 2

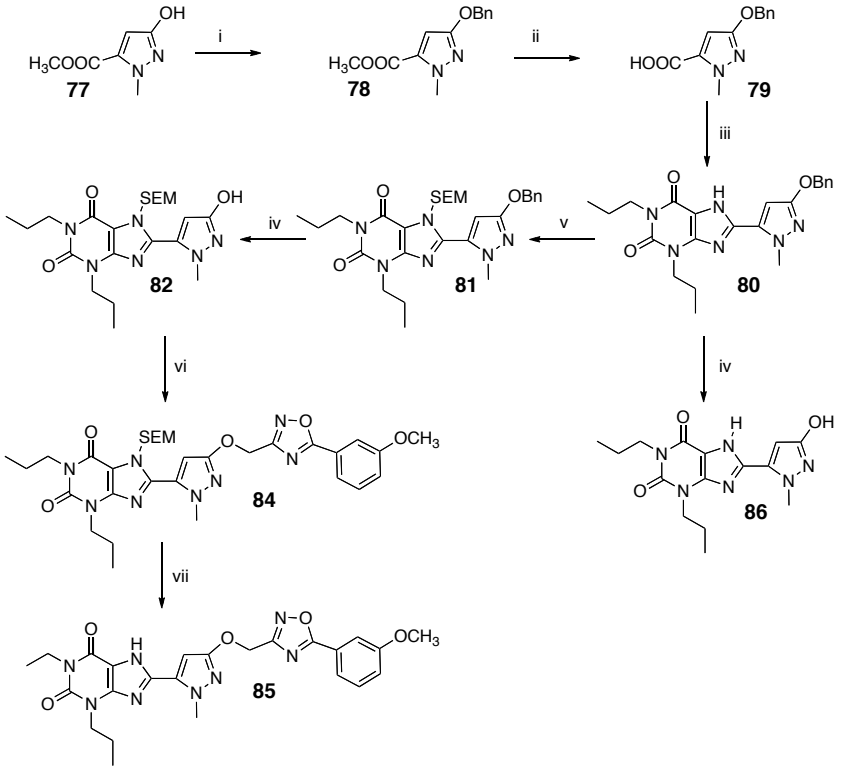


Reagents: *i: a. 1,3-dipropyl-5,6-diamino-uracil (**47**), EDC, methanol, rt; b. NaOH, 80 °C; ii: a. appropiate diamine, EDC, DMF, rt; b. CH_3CO_2H, 80-100 °C.*

The target xanthine derivatives **85**, **86** were prepared as shown in Scheme 3. 3-Benzyloxy-1-methyl-1*H*-pyrazole-5-carboxylic acid **79** was prepared by direct alkylation of ethyl 1*H*-pyrazole-4-carboxylate[94] **77** with benzyl chloride in acetone using K_2CO_3 as a base, followed by ester hydrolysis. Coupling of **79** with 1,3-dipropyl-5,6-diaminouracil[101] using EDC afforded the amidic intermediate, which was cyclized in NaOH to afford compound **80**. Debenzylation with ammonium formate and palladium on carbon yielded the target compound **86**.

Treatment of **80** with 2-(trimethylsilyl)-ethoxymethyl chloride (SEM-Cl) and K_2CO_3 introduced the SEM protection group at *N*-7. Debenzylation yielded our key intermediate **82**. This Compound was then alkylated with 3-chloromethyl-5-(3-methoxy-phenyl)-[1,2,4]oxadiazole (**83**)[95] to yield **84.** SEM deprotection with 3 N HCl in ethanol at 80 °C afforded the target compound **85**.

Scheme 3



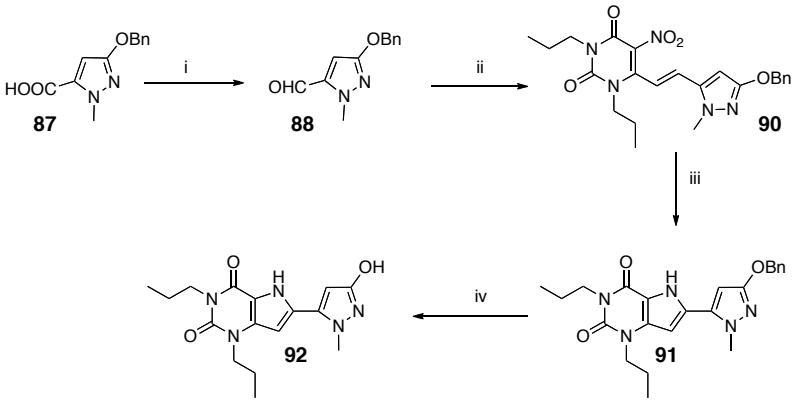
Reagents: *i: benzyl bromide, K_2CO_3, acetone, rt; ii: KOH, dioxane, rt; iii: a. 1,3-dipropyl-5,6-diamino-uracil (**47**), EDC, methanol, rt; b. NaOH, 80 °C; iv: Pd/C, HCO_2NH_4, methanol, rfx; v: SEM-Cl, K_2CO_3, DMF, 70 °C; vi: 3-chloromethyl-5-(3-methoxy-phenyl)-[1,2,4]oxadiazole (**83**), K_2CO_3, acetone; vii: HCl, ethanol,90 °C.*

The synthesis of 9-deaza-xanthines **92, 98-100** analogs was performed in knowledge to published procedures[102,103] with some modifications to reflect the change from benzaldehyde to the pyrazolo aldehyde (Schemes 4, 5).

Pyrazolo aldehyde **88** was prepared by conversion of **87** into a mixed anhydride with ethyl chloroformate, then it was reduced with sodium borohy-

dride to yield the corresponding alcohol, which was reacted with the pyridinium chlorocromate (PCC) (Scheme 4). Condensation of the 1,3-dipropyl-6-methyl-5-nitrouracil[104] (**89)** with the 3-benzyloxy-1-methyl-1*H*-pyrazole-5-carbaldehyde **88** in dry benzene and catalytic morpholine allowed us to obtain the corresponding (*E*)-6-styryl derivative **90** in satisfactory yields. The ring closure of **90** to intermediate **91** was performed by reductive cyclization using iron in acetic acid under reflux. Debenzylation of **91** in a manner similar to that for the synthesis of **86,** yielded the deaza derivative **92** (Scheme 4).

Scheme 4

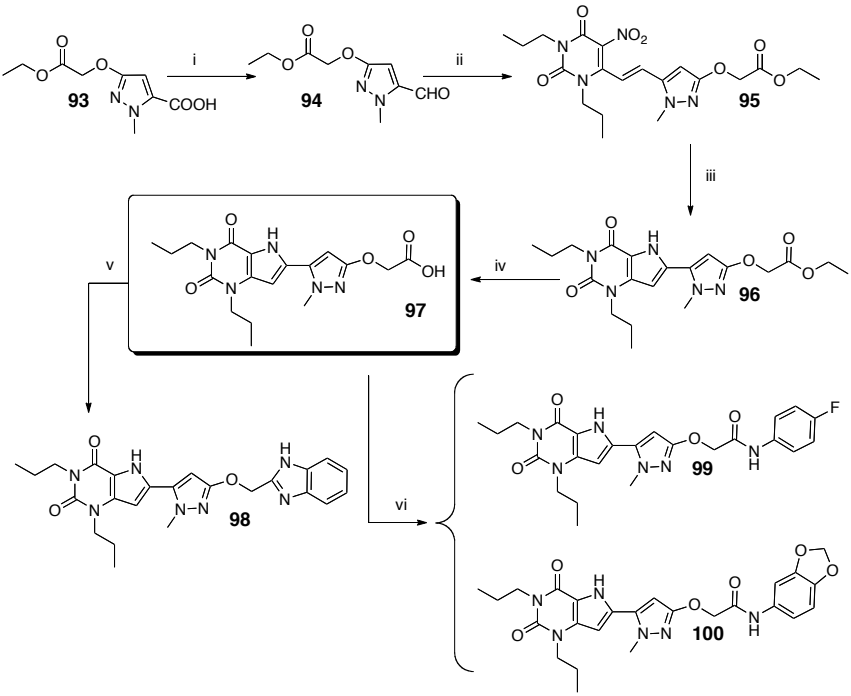


Reagents: *i: a. $ClCO_2Et$, TEA, $NaBH_4$, THF, -10°C; b. PCC, CH_2Cl_2, rt; ii: 1,3-dipropyl-6-methyl-5-nitro-uracil (**89**), morpholine, pTsOH, toluene, rfx; iii: Fe, CH_3O_2H, ethanol, rfx; iv: HCO_2NH_4, Pd/C, methanol, rfx.*

The target 9-deaza derivatives **98, 99, 100** were prepared by the same synthetic route reported for compound **92** starting from the pyrazolo carboxylic acid **93**[94] to afford the intermediate pyrrolo[3,2-*d*]pyrimidin-6-yl)-pyrazol-3-yloxy]-acetic acid **97** (Scheme 5). Target compound **98** was obtained as the same procedure used for analogs **50-65**, while direct condensation of car-

boxylic acid **97** and the suitable anilines in DMF in the presence of 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDC) as coupling reagent yielded **99** and **100**.

Scheme 5



Reagents: *i: a. $ClCO_2Et$, TEA, $NaBH_4$, THF, -10 °C; b. PCC, CH_2Cl_2, rt; ii: 1,3-dipropyl-6-methyl-5-nitro-uracil (**89**), morpholine, pTsOH, toluene, rfx; iii: Fe, CH_3CO_2H, ethanol, rfx; iv: NaOH 10%, methanol; v: a. 1,2-phenylendiamine, EDC, DMF, rt; b. CH_3CO_2H, 60 °C; vi: aniline, EDC, DMF, rt.*

Chapter 4

Results and Conclusions

In this chapter are reported the biological data of compound have been investigated. All the 8-heterocycle-xanthines were evaluated in radioligand binding assays to determine their affinities for human A_1, A_{2A}, A_{2B} and A_3 adenosine receptors. It has been used [^{3}H]-DPCPX (1,3-[3H]-dipropyl-8-cyclopentylxanthine) for A_1 and A_{2B}, [^{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), and [^{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) for A_{2A} and A_3 respectively, as radioligands. Efficacy of the compounds versus hA_{2B}AR was also investigated evaluating their capability to inhibit (100 nM) NECA stimulated cAMP production (Table 4-7).

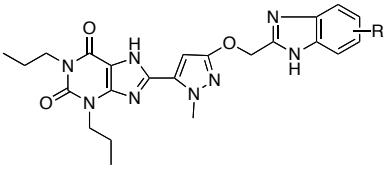
Binding affinities of the synthesised compounds for the, A_1, A_{2A}, A_3 and A_{2B}ARs are presented in Tables 4-7. The SAR of the benzimidazol-methoxy-pyrazole and benzimidazol-methoxy-isoxazole analogues with respect to the A_{2B}AR affinity are shown in Table 4, 5. A general comparison of similarly substituted phenyl analogues between the pyrazole and isoxazole series (e.g. **52** vs **70**, **55** vs **72**, etc) favours the pyrazole series with respect to the selectivity *versus* the A_1 receptor.

As shown in Table 4, the parent unsubstituted phenyl analog **50** was highly potent at the human A_{2B} receptor, with a K_i value of 18 nM, although not selective for A_{2B} *vs* A_1 receptors (hA_1/hA_{2B} = 12). The 5-substituted-phenyl analogs **51-60** showed high affinity for the A_{2B}AR and the best compound was 5-Cl derivative **52** (hA_{2B} K_i = 5 nM). Introducing a stronger electron-withdrawing group (Br or CF_3) in place of the 5-Cl group, as in compounds **54** and **55** resulted in 2-3-fold loss in binding affinity (**54**, hA_{2B} K_i = 15.9 nM; **55**, hA_{2B} K_i =8.1). However, compound **55** improved selectivity for the A_2AR versus the A_1AR. The 5-F derivative **51** exhibited lower affinity and selectivity in comparison to 5-Cl **54**.

Introduction of substituents containing carbonyl function such as 5-carboxylic acid ethyl ester **59** produced good affinity and selectivity for A_{2B}AR (hA_{2B} K_i = 12 nM A_1/A_{2B} = 42). The carboxylic acid derivative **60** exhibited less affinity at both A_1 and A_{2B} receptors (hA_{2B} K_i = 88, A_1/A_{2B} = 52). The 5-nitro analog **58** showed 3-fold lower affinity although comparable selectivity for the A_{2B}AR relative to the 5-Cl analog **54**.

Substitution of the phenyl ring with the naphthyl nucleus produced compound **57** that had similar binding affinity (hA_{2B} K_i = 20 nM) to the phenyl analog **50**, while it showed nearly 10-fold more selectivity for the A_{2B}AR *versus* A_1, relative to **50** suggesting an increased binding domain in the A_{2B} receptor *versus* the A_1AR.

Table 4. *Chemical structure and biological activity at ARs of 1,3 dipropyl-8-(3-benzoimidazol-yl-methoxy-1-methyl-pyrazol-5yl) xanthine derivatives.*



Comp.	R	hA_1 [b]	hA_{2A} [c]	hA_{2B} [d]	hA_3 [e]	cAMP hA_{2B}
			K_i (nM)[a]			IC_{50} (nM)
50	H	222 (181-273)	> 1000	17.9 (12.6-25.6)	> 1000	65 (56-75)
51	5-F	645 (560-744)	> 1000	30 (24-39)	> 1000	95 (86-105)
52	5-Cl	150 (107-210)	> 1000	5.0 (3.6-7.0)	> 1000	15 (11-21)
53	5-OCH_3	752 (665-851)	> 1000	14.2 (7.8-25.7)	> 1000	54 (39-76)
54	5-Br	280 (245-319)	> 1000	15.9 (14.5-17.3)	> 1000	65 (56-75)
55	5-CF_3	576 (532-625)	> 1000	8.1 (4.6-14.1)	> 1000	38 (31-52)
56	5-CH_3	736 (666-814)	> 1000	10.2 (5.0-20.5)	> 1000	45 (37-55)
57	5,6-C_4H_4	> 1000	> 1000	20 (13-30)	> 1000	80 (63-100)
58	5-NO_2	155 (133-180)	> 1000	14.8 (10.2-21.5)	> 1000	62 (54-72)
59	5-CO_2Et	503 (476-532)	> 1000	12 (11.4-12.5)	> 1000	35 (29-42)
60	5-CO_2OH	4558 (3953-5255)	> 1000	88 (75-104)	> 1000	220 (183-266)
61	4-CH_3	685 (593-792)	> 1000	14.1 (9.3-21.3)	> 1000	60 (51-70)
62	4-Cl, 6-CF_3	2530 (2267-2824)	> 1000	9.4 (8.8-9.9)	> 1000	26 (17-380
63	4,6-CF_3	4462 (4020-4952)	> 1000	25 (20-31)	> 1000	70 (59-84)
64	5-Cl, 6-F	210 (192-229)	> 1000	19 (15-24)	> 1000	32 (24-42)
65	5,6-CH_3	734 (613-879)	> 1000	22 (16-31)	> 1000	53 (37-75)

[a]The data are expressed as the geometric mean with 95% confidence limits in parentheses and derived from inhibition binding experiments and cAMP assays. [b]Displacement of specific [^{3}H]DPCPX binding at human A_1 receptors expressed in CHO cells (n = 3-6). [c] Displacement of specific [^{3}H]ZM241385 binding at human A_{2A} receptors expressed in CHO cells (n = 3-6). [d] Displacement of specific [^{3}H] DPCPX binding at human A_{2B} receptors expressed in HEK293 cells (n) 3-6). [e] Displacement of specific [^{3}H]MRE3008-F20 binding at human A_3 receptors expressed in CHO cells (n = 3-6).

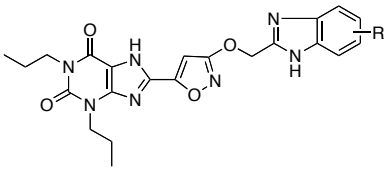
The 5-methoxy-phenyl analog **53** and the 5-methyl analog **56** had favorable binding affinities for the $A_{2B}AR$ (hA_{2B} K_i = 14.2 nM, hA_{2B} K_i = 10.2 nM respectively). They also showed more selectivity for the $A_{2B}AR$ versus A_1, relative to **52**. Shifting the methyl group from the 5- to the 4-position in analog **61** had no significant effect on affinity or selectivity for the $A_{2B}AR$ versus A_1. The 5,6-dimethyl analog **65** resulted slightly less potent at $A_{2B}AR$ (hA_{2B} K_i = 22 nM) with also lower selectivity versus A_1 receptor compared with 5-methyl analog **56**.

The 4,6-disubstituted analog **62** showed high affinity and selectivity at $A_{2B}AR$. In fact, compound **62** (4-Cl, 6-CF_3) is our most active and selective analog among the two classes of compounds we synthesized (hA_{2B} K_i = 9.4 nM A_1/A_{2B} = 269). Introducing a stronger electron-withdrawing group (CF_3) in place of the 4-Cl group such as in compound **63** resulted in 3-fold loss in binding affinity (hA_{2B} K_i =25 nM). The 5-Cl, 6-F analog **64** displayed similar affinity, with 5,6-disubstituted analog **65**, even though the 5,6-dimethyl substitution produced 3-fold increasing of A_1 selectivity.

Compounds **69-76** were prepared by replacing the pyrazole ring with isoxazole nucleus. In general, the benzimidazol-isoxazole series, was less selective for the $A_{2B}AR$ *versus* the A_1AR when compared to the benzimidazole-pyrazole class of compounds. This data is in contrast with the pyrazole-oxyacetamide derivatives synthesised in our previous work[94] that were more selective when compared to the pyrazole parent compounds. A similar trend was observed when comparing the isoxazole analog **69** to the pyrazole analog **50.** The 5-halogen analogs **70-72** displayed similar affinities at the $A_{2B}AR$ compared to that of the 5-halogen-substituted pyrazole analogs, but they were slightly less selective versus A_1. A noticeable exception was the 5-bromo analog **71** that was 4-fold more active at $A_{2B}AR$ in comparison to analog **54**. Further substitution of the 5-position of the phenyl ring by a

methoxy group produced compound **73** that was less potent (hA_{2B} K_i =35 nM) than **53** (hA_{2B} K_i =14.2 nM), and also less selective versus the A_1AR.

Table 5. *Chemical structure and biological activity at ARs of 1,3 dipropyl-8-(3-benzoimidazol-yl-methoxy-isoxazol-5yl) xanthine derivatives.*



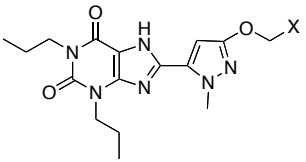
Comp.	R	hA_1[b]	hA_{2A}[c]	hA_{2B}[d]	hA_3[e]	cAMP hA_{2B}
		K_i (nM)[a]				IC_{50} (nM)
69	H	277 (227-338)	> 1000	23 (17-30)	> 1000	74 (65-84)
70	5-Cl	66 (57-76)	> 1000	3.5 (2.9-4.2)	> 1000	6.4 (4.9-8.3)
71	5-Br	89 (77-104)	> 1000	4.1 (2.9-5.7)	> 1000	11 (7-17)
72	5-CF_3	175 (134-229)	> 1000	10.0 (7.5-13.1)	> 1000	51 (38-68)
73	5-OCH_3	304 (257-361)	> 1000	35 (27-47)	> 1000	114 (86-151)
74	5-Cl, 6-F	65 (55-78)	> 1000	5.5 (4.8-6.3)	> 1000	8.0 (7.2-8.9)
75	4-Cl, 6-CF_3	> 1000	> 1000	22 (16-31)	> 1000	64 (50-81)
76	4,6-CF_3	> 1000	> 1000	76 (65-87)	> 1000	197 (136-287)

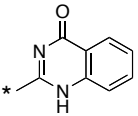
[a]The data are expressed as the geometric mean with 95% confidence limits in parentheses and derived from inhibition binding experiments and cAMP assays. [b]Displacement of specific [^{3}H]DPCPX binding at human A_1 receptors expressed in CHO cells (n = 3-6). [c] Displacement of specific [^{3}H]ZM241385 binding at human A_{2A} receptors expressed in CHO cells (n = 3-6). [d] Displacement of specific [^{3}H] DPCPX binding at human A_{2B} receptors expressed in HEK293 cells (n) 3-6). [e] Displacement of specific [^{3}H]MRE3008-F20 binding at human A_3 receptors expressed in CHO cells (n = 3-6).

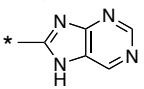
The introduction of fluorine in the *ortho*-position of 5-chloro analog **70** produced non-significant effects on affinity and selectivity at A_{2B}, as shown by analog **74**. Also in the isoxazolo series, as with the pyrazole analogues, the best compound was 4-Cl, 6-CF_3-disubstituted phenyl analog **75** (hA_{2B} K_i = 22 nM, hA_1/hA_{2B} > 50) in relation to affinity at A_{2B} and selectivity versus A_1ARs. Introduction of trifluoromethyl at the 4 and 6 positions produced a

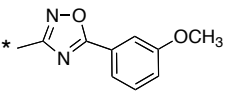
decrease of affinity and selectivity such as compound **76** in comparison with analog **63**. The enlargement of benzimidazole nucleus of analog **50** did not improve affinity at the A_{2B}AR as demonstrated by 4-oxo-3,4-dihydro-quinazoline **49**, but did increase the selectivity 3-fold *versus* A_1AR. Replacement of the phenyl ring with pyrimidine, such as in compound **66** produced a complete loss of affinity at the A_{2B}R. The benzimidazole nucleus was also replaced by 5-*m*-methoxyphenyl-1,2,4-oxadiazole to afford analog **85** (hA_{2B} *Ki* = 70 nM). This analog displayed 5-fold lower affinity compared to 5-methoxy-benzimidazole analog **53.**

Table 6. *Chemical structure and biological activity at ARs of 1,3 dipropyl-8-(3-heterocycl-yl- methoxy-1-methyl-pyrazol-3yl) xanthine derivatives.*



49: X = 

66: X = 

85: X = 

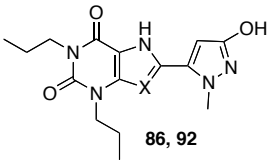
Comp.	hA_1[b]	hA_{2A}[c]	hA_{2B}[d]	hA_3[e]	cAMP hA_{2B}
			K_i (nM)[a]		IC_{50} (nM)
49	> 1000	> 1000	49 (39-62)	> 1000	180 (153-212)
66	> 1000	> 1000	> 1000	> 1000	> 1000
85	> 1000	> 1000	61 (49-77)	> 1000	219 (186-259)

[a]The data are expressed as the geometric mean with 95% confidence limits in parentheses and derived from inhibition binding experiments and cAMP assays. [b]Displacement of specific [^{3}H]DPCPX binding at human A_1 receptors expressed in CHO cells (n = 3-6). [c] Displacement of specific [^{3}H]ZM241385 binding at human A_{2A} receptors expressed in CHO cells (n = 3-6). [d] Displacement of specific [^{3}H] DPCPX binding at human A_{2B} receptors expressed in HEK293 cells (n) 3-6). [e] Displacement of specific [^{3}H]MRE3008-F20 binding at human A_3 receptors expressed in CHO cells (n = 3-6).

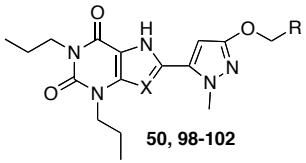
The 8-(3-hydroxy-1-methyl-1*H*-pyrazol-5-yl)-xanthine **86** was synthesised as a simplified chemical structure of pyrazole-xanthine series. This com-

pound displayed very high affinity at A_{2B} receptors with good selectivity over A_1. (Table 7, hA_{2B} K_i = 4.0 nM, hA_1/hA_{2B} = 183). The 9-deaza xanthines **92**, **98-100** differing from the absence of a nitrogen atom at the 9-position of heterocyclic system of xanthines were synthesised based on their noted antagonistic activity at the A_{2B} receptor.[103]

Table 7. *Chemical structure and binding activity at ARs of various sets of Xanthine and 9-deaza Xanthine derivatives.*



86, 92



50, 98-102

Comp.	X	R	hA_1[b]	hA_{2A}[c]	hA_{2B}[d]	hA_3[e]	cAMP hA_{2B}
			K_i (nM)[a]				IC_{50} (nM)
86	N	-	733 (661-812)	> 1000	4.0 (2.5-6.4)	> 1000	20 (14-29)
92	C	-	140 (123-159)	960 (956-992)	7.5 (7.1-8.0)	> 1000	9 (7.3-11)
50	N		222 (181-273)	> 1000	17.9 (12.6-25.6)	> 1000	65 (56-75)
98	C		34 (27-42)	> 1000	11.4 (11-11.9)	> 1000	27 (22-34)
99	C		45 (37-55)	> 1000	13.4 (12.5-14.4)	> 1000	43 (37-50)
100	C		55 (46-65)	> 1000	87 (75-101)	> 1000	236 (176-317)
101	N		65 (48-96)	> 1000	12 (7.0-21)	> 1000	88 (82-95)
102	N		200 (180-226)	> 1000	5.5 (4.6-6.5)	> 1000	38 (29-51)

[a]The data are expressed as the geometric mean with 95% confidence limits in parentheses and derived from inhibition binding experiments and cAMP assays. [b]Displacement of specific [^{3}H]DPCPX binding at human A_1 receptors expressed in CHO cells (n = 3-6). [c] Displacement of specific [^{3}H]ZM241385 binding at humanA_{2A} receptors expressed in CHO cells (n = 3-6). [d] Displacement of specific [^{3}H] DPCPX binding at human A_{2B} receptors expressed in HEK293 cells (n) 3-6).[e] Displacement of specific [^{3}H]MRE3008-F20 binding at human A_3 receptors expressed in CHO cells (n = 3-6).

The ARs binding affinities of deaza analogs in comparison with xanthines are reported in Table 7. The analogs **99** and **100** were prepared comparing to those recently published 8-pyrazolyl oxyacetamide derivatives **101** and **102**. In general, the new deaza analogs showed less selectivity over A_1 even though displayed high affinity at A_{2B}AR.

The deaza analog **92** compared to **86** displaying 2-fold less affinity at A_{2B} and 10-fold less selectivity versus A_1AR. The deaza analoge **98** exhibited 2-fold increased of affinity compared to its xanthine derivative **50** , while was 4-fold less selective versus A_1. The deaza oxyacetamides **99, 100** showed less affinity and selectivity at A_{2B}AR in respect to their xanthine analogs **101** and **102**.

Conclusions

We have identified new leads for the design of xanthine derivatives as selective antagonists of the A_{2B}AR. From our study on this new class of 8-substituted xanthine derivatives emerged a series of important considerations. The most significant result was obtained by bioisosteric replacement of the anilide moiety with benzimidazole, achieving antagonists with high affinity and selectivity toward the A_{2B}AR. In particular compounds **62, 63**, **52** and **70** showed the best biological data.

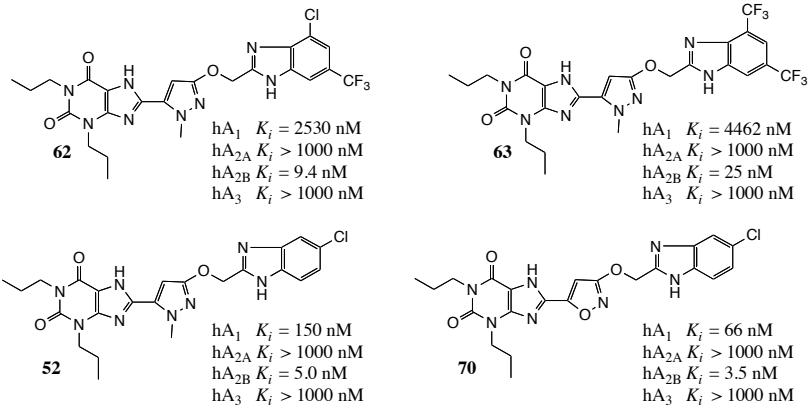


Figure 5. *Chemical structure of the most potent A_{2B} adenosine antagonists.*

Moreover the effect of the substitution of pyrazole at 8-position of xanthine core with isoxazole was investigated and in general, the benzimidazol-isoxazole series, was less selective for the A_{2B}AR *versus* the A_1AR when compared to the benzimidazole-pyrazole analogs.

The bioisosteric substitution of the nitrogen at the 9-position of the xanthine nucleus with a carbon atom displayed a decrease of selectivity for the A_{2B}AR while the affinity was maintained. Such selective compounds will aid in the elucidation of the physiological role of this receptor and possibily lead to therapeutilally useful agents for treating asthma, diabetes and other diseases.

Chapter 5

Experimental Section

Chemical Materials and Methods

Reagent grade solvents were dried according to standard techniques. Sodium sulfate was used as a drying agent for water containing organic phases. Reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel (precoated F245 Merck plates). Chromatographic spots were visualized by UV light. Purification of crude compounds and separation of reaction mixtures were carried out by column chromatography on silica gel 60 (230–400 mesh from Merck) using appropriate eluents. Melting points (uncorrected) were determined in a 240 Buchi-Tottoli melting point apparatus. Chemical shifts (d) are reported in parts per million (ppm) relative to the solvent central peak. 1H NMR spectra were recorded at 200 MHz on a Bruker AC 200 spectrometer. Electrospray Ionization Mass Spectrometry (ESI/MS) was performed with an Agilent 1100 Series LC/MSD model in positive scan mode. The molecular weights from the MS spectra (reported only for final compounds) were in full agreement with the proposed chemical structures of target compounds.

General Procedure for the Preparation of 8-[3-(1H-Benzoimidazol-2-ylmethoxy)-1-methyl-1H-pyrazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione 50-66 and 8-[3-(1H-Benzoimidazol-2-ylmethoxy)-isoxazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione 69-76.

(Except compound 60 that was prepared from 59)

To a mixture of the carboxylic acid (**48** or **68**, see Scheme 1) (0.25 mmol), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (0.25 mmol), and anhydrous Dimethylformamide (DMF) (5 mL) was added the appropriate phenylenediamine (0.25 mmol) and the mixture was stirred at room temperature overnight under argon atmosphere. The resulting solution was evaporated under reduced pressure, and the residue was dissolved in acetic acid (5 mL) and heated under stirring at 60-70 °C for 1 h. The resulting solution was cooled to room temperature and poured into water. The precipitate was collected by filtration and washed with water . The resulting crude was purified by flash column chromatography on silica gel or crystallized when necessary.

8-[3-(1H-Benzoimidazol-2-ylmethoxy)-1-methyl-1H-pyrazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (50).

Purification by crystallization from dioxane. Yield: 96%, mp: 290°C

^{1}H NMR (DMSO-d_6): δ 0.86 (m, 6H); 1.53 (q, 2H); 1.70 (q, 2H); 3.84 (t, 2H); 3.98 (t, 2H); 4.08 (s, 3H); 5.35 (s, 2H); 6.53 (s, 1H); 7.16-7.75 (m, 4H); 12.63 (s, 1H); 14.00 (bs, 1H); MS *m/z* 443 (M H^+).

8-[3-(5-Fluoro-1H-benzoimidazol-2-ylmethoxy)-1-methyl-1H-pyrazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (51).

Purification by column chromatography on silical gel (AcOEt). Yield: 48%, mp: 280°C.

^{1}H NMR (DMSO-d_6): δ 0.88 (m, 6H); 1.57 (q, 2H); 1.72 (q, 2H); 3.86 (t, 2H); 4.01 (t, 2H); 4.15 (s, 3H); 5.36 (s, 2H); 6.54 (s, 1H); 7.00-7.68 (m, 3H); 12.77 (s, 1H); 13.97 (bs, 1H); MS *m/z* 481 (M H$^+$).

8-[3-(5-Chloro-1H-benzoimidazol-2-ylmethoxy)-1-methyl-1H-pyrazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (52).

Purification by crystallization from dioxane/Et_2O. Yield: 52%, mp: 285-286°C.

^{1}H NMR (DMSO-d_6): δ 0.88 (m, 6H); 1.57 (q, 2H); 1.73 (q, 2H); 3.86 (t, 2H); 4.01 (t, 2H); 4.12 (s, 3H); 5.38 (s, 2H); 6.53 (s, 1H); 7.21-7.67 (m, 3H); 12.92 (s, 1H); 13.97 (bs, 1H).

8-[3-(5-Methoxy-1H-benzoimidazol-2-ylmethoxy)-1-methyl-1H-pyrazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (53).

Purification by crystallization from dioxane/water. Yield: 55%, mp: 271-272°C.

^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H); 1.57 (q, 2H); 1.73 (q, 2H); 3.77 (s, 3H); 3.86 (t, 2H); 4.00 (t, 2H); 4.10 (s, 3H); 5.31(s, 2H); 6.53 (s, 1H); 6.79-7.48 (m, 3H); 12.48 (s, 1H); 13.97 (bs, 1H); MS *m/z* 493 (M H$^+$).

8-[3-(5-Bromo-1H-benzoimidazol-2-ylmethoxy)-1-methyl-1H-pyrazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (54).

Purification by crystallization from dioxane/water. Yield: 52%, mp: 299 °C

^{1}H NMR (DMSO-d_6): δ 0.88 (m, 6H); 1.58 (q, 2H); 1.75 (q, 2H); 3.85 (t, 2H); 4.00 (t, 2H); 4.09 (s, 3H); 5.37(s, 2H); 6.66 (s, 1H); 7.22-7.80 (m, 3H); 12.95 (bs, 1H); 12.99 (bs, 1H); MS *m/z* 542 (M H$^+$).

8-[1-Methyl-3-(5-trifluoromethyl-1H-benzoimidazol-2-ylmethoxy)-1H-pyrazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (55).

Purification by column chromatography on silical gel (AcOEt). Yield: 50%, mp: 301 °C.

^{1}H NMR (DMSO-d_6): δ 0.86 (m, 6H); 1.57 (q, 2H); 1.71 (q, 2H); 3.82 (t, 2H); 3.89(t, 2H); 4.09 (s, 3H); 5.44(s, 2H); 6.55 (s, 1H); 7.50-8.00 (m, 3H); 13.01 (bs, 1H); 13.99 (bs, 1H); MS *m/z* 531 (M H$^+$).

8-[1-Methyl-3-(5-methyl-1H-benzoimidazol-2-ylmethoxy)-1H-pyrazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (56).

Purification by column chromatography on silical gel (AcOEt). Yield: 86%, mp: 276 °C.

^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H); 1.57 (q, 2H); 1.73 (q, 2H); 2.4 (s, 3H); 3.86 (t, 2H); 4.00(t, 2H); 4.10 (s, 3H); 5.33(s, 2H); 6.48 (s, 1H); 6.93-7.49 (m, 3H); 12.5 (bs, 1H); 13.96 (bs, 1H); MS *m/z* 477 (M H$^+$).

8-[1-Methyl-3-(1H-naphtho[2,3-d]imidazol-2-ylmethoxy)-1H-pyrazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (57).

Purification by crystallization from DMF/Et_2O. Yield: 85%, mp: 297 °C.

^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H); 1.55 (q, 2H); 1.71 (q, 2H); 3.86 (t, 2H); 4.00 (t, 2H); 4.14 (s, 3H); 5.47 (s, 2H); 6.58 (s, 1H); 7.37 (m, 2H); 8.02 (m, 4H); 12.73 (s, 1H); 13.98 (bs, 1H);); MS *m/z* 513 (M H$^+$).

8-[3-(5-Nitro-1H-benzoimidazol-2-ylmethoxy)-1-methyl-1H-pyrazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (58).

Purification by crystallization from dioxane. Yield: 48 %, mp: 263 °C.

^{1}H NMR (DMSO-d_6): δ 0.88 (m, 6H); 1.58 (q, 2H); 1.72 (q, 2H); 3.86 (t, 2H); 3.99 (t, 2H); 4.01 (s, 3H); 5.49(s, 2H); 6.57 (s, 1H); 7.74-8.16 (m, 2H); 8.47 (s, 1H); 12.95 (bs, 1H); 13.99 (bs, 1H); MS *m/z* 508 (M H$^+$).

2-[5-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1H-purin-8-yl)-1-methyl-1H-pyrazol-3-yloxymethyl]-1H-benzoimidazole-5-carboxylic acid ethyl ester (59).

Purification by crystallization from methanol. Yield: 60 %, mp > 300 °C.

^{1}H NMR (DMSO-d_6): δ 0.89 (m, 6H); 1.32 (t, 3H);1.62 (q, 2H); 1.78 (q, 2H); 3.86 (t, 2H); 4.01 (t, 2H); 4.10 (s, 3H); 4.34 (q, 2H); 5.42(s, 2H); 6.53 (s, 1H); 7.76-8.22 (m, 3H); 13.00 (bd, 1H); 13.99 (bs, 1H); MS *m/z* 535 (M H^+).

2-[5-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1H-purin-8-yl)-1-methyl-1H-pyrazol-3-yloxymethyl]-1H-benzoimidazole-5-carboxylic acid (60).

Aqueous NaOH 10% (6 mL) was added to a suspension of the ester **59** (0.15 mmol) in dioxane (10 mL) and the mixture was heated at 50ºC for 3 hours. Most of the solvent was evaporated under vacuum, the remaining aqueous mixture cooled to room temperature and HCl 10% was added until an acidic pH was reached. The resulting precipitate was collected by filtration, washed with water and dried to yield the corresponding carboxylic acid **60**, that was purified by crystallization (dioxane). Yield: 55 %, mp: > 300 °C.

^{1}H NMR (DMSO-d_6): δ 0.89 (m, 6H);1.66 (q, 2H); 1.77 (q, 2H); 4.01 (t, 2H); 4.05 (t, 2H); 4.10 (s, 3H); 5.41 (s, 2H); 6.66 (s, 1H); 7.67-8.24 (m, 3H); 12.99 (bs, 1H); 13.00 (bs, 1H); 13.99 (bs, 1H); MS *m/z* 507 (M H^+).

8-[1-Methyl-3-(4-methyl-1H-benzoimidazol-2-ylmethoxy)-1H-pyrazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (61).

Purification by column chromatography on silical gel (AcOEt/MeOH 9.5/0.5). Yield: 86%, mp: 276-277°C.

^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H); 1.57 (q, 2H); 1.73 (q, 2H); 2.41 (s, 3H); 3.86 (t, 2H); 4.00 (t, 2H); 4.12 (s, 3H); 5.33 (s, 2H); 6.48 (s, 1H); 6.93-7.49 (m, 3H); 12.58 (s, 1H); 13.96 (s, 1H); MS *m/z* 477 (M H^+).

8-[3-(4-Chloro-6-trifluoromethyl-1H-benzoimidazol-2-ylmethoxy)-1-methyl-1H-pyrazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (62).

Purification by crystallization from methanol. Yield: 48 %, mp: 267 °C. ^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H); 1.59 (q, 2H); 1.78 (q, 2H); 3.83 (t, 2H); 3.96 (t, 2H); 4.09 (s, 3H); 5.48 (s, 2H); 6.55 (s, 1H); 7.80 (s, 1H); 7.98 (s, 1H); 13.50 (bs, 1H); 14.00 (bs, 1H); MS *m*/*z* 566 (M H$^+$).

8-[3-(4,6-Bis-trifluoromethyl-1H-benzoimidazol-2-ylmethoxy)-1-methyl-1H-pyrazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (63).

Purification by crystallization from methanol. Yield: 55 %, mp: 280-281 °C. ^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H); 1.59 (q, 2H); 1.78 (q, 2H); 3.93 (t, 2H); 4.09 (t, 2H); 4.10 (s, 3H); 5.51 (s, 2H); 6.551 (s, 1H); 7.80 (s, 1H); 8.20 (s, 1H); 13.55 (bs, 1H); 14.00 (bs, 1H); MS *m*/*z* 599 (M H$^+$).

8-[3-(5-Chloro-6-fluoro-1H-benzoimidazol-2-ylmethoxy)-1-methyl-1H-pyrazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (64).

Purification by crystallization from dioxane. Yield: 55 %, mp: 288 °C. ^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H); 1.55 (q, 2H); 1.78 (q, 2H); 3.84 (t, 2H); 3.99 (t, 2H); 4.07 (s, 3H); 5.35 (s, 2H); 6.50 (s, 1H); 7.50-7.88 (m, 2H); 13.00 (bd, 1H); 14.00 (bs, 1H);); MS *m*/*z* 516 (M H$^+$).

8-[3-(5,6-Dimethyl-1H-benzoimidazol-2-ylmethoxy)-1-methyl-1H-pyrazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (65).

Purification by crystallization from methanol. Yield: 75 %, mp: 281 °C. ^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H); 1.55 (q, 2H); 1.70 (q, 2H); 2.29 (s, 6H); 3.84 (t, 2H); 4.00 (t, 2H); 4.10 (s, 3H); 5.31 (s, 2H); 6.53 (s, 1H); 7.30 (s, 2H); 12.45 (bs, 1H); 14.00 (bs, 1H); MS *m*/*z* 491 (M H$^+$).

8-[3-(1H-Benzoimidazol-2-ylmethoxy)-isoxazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (69).

Purification by crystallization from methanol. Yield: 70 %, mp: 282 °C. ^{1}H NMR (DMSO-d_6): δ 0.88 (m, 6H); 1.58 (q, 2H); 1.80 (q, 2H); 3.98 (t, 2H); 4.10 (t, 2H); 5.53 (s, 2H); 6.97 (s, 1H); 7.00-7.59 (m, 4H); 12.76 (bs, 1H); 14.59 (bs, 1H); MS *m*/*z* 450 (M H$^+$).

8-[3-(5-Chloro-1H-benzoimidazol-2-ylmethoxy)-isoxazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (70).

Purification by crystallization from methanol. Yield: 68 %, mp: 260 °C. ^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H); 1.55 (q, 2H); 1.70 (q, 2H); 3.86 (t, 2H); 3.94 (t, 2H); 5.54 (s, 2H); 6.99 (s, 1H); 7.20-7.77 (m, 3H); 13.00 (bs, 1H); 14.55 (bs, 1H)**;** MS *m*/*z* 476 (M H$^+$).

8-[3-(5-Bromo-1H-benzoimidazol-2-ylmethoxy)-isoxazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (71).

Purification by crystallization from methanol. Yield: 70 %, mp: 230 °C ^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H); 1.58 (q, 2H); 1.72(q, 2H); 3.89 (t, 2H); 3.98 (t, 2H); 5.54 (s, 2H); 6.98 (s, 1H); 7.36-7.80 (m, 3H); 13.00 (bs, 1H); 14.60 (bs, 1H). MS *m*/*z* 529 (M H$^+$).

8-[3-(5-Trifluoromethyl-1H-benzoimidazol-2-ylmethoxy)-isoxazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (72).

Purification by crystallization from methanol. Yield: 65 %, mp: 268 °C ^{1}H NMR (DMSO-d_6): δ 0.88 (m, 6H); 1.55 (q, 2H); 1.69 (q, 2H); 3.86 (t, 2H); 3.98 (t, 2H); 5.51 (s, 2H); 7.00 (s, 1H); 7.50-8.00 (m, 3H); 13.20 (bs, 1H); 14.70 (bs, 1H); MS *m*/*z* 518 (M H$^+$).

8-[3-(5-Methoxy-1H-benzoimidazol-2-ylmethoxy)-isoxazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (73).

Purification by crystallization from methanol. Yield: 75%, mp: 199 °C. ^{1}H NMR (DMSO-d_6): δ 0.88 (m, 6H); 1.58 (q, 2H); 1.69 (q, 2H); 3.78 (s, 3H); 3.86 (t, 2H); 3.98 (t, 2H); 5.48 (s, 2H); 6.86 (s, 1H); 6.97-7.46 (m, 3H); 13.01 (bs, 1H); 14.30 (bs, 1H); MS *m*/*z* 480 (M H$^+$).

8-[3-(5-Chloro-6-fluoro-1H-benzoimidazol-2-ylmethoxy)-isoxazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (74).

Purification by crystallization from methanol. Yield: 55 %, mp: 249 °C. ^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H); 1.62 (q, 2H); 1.69 (q, 2H); 3.86 (t, 2H); 3.97 (t, 2H); 5.54 (s, 2H); 6.98 (s, 1H); 7.50-7.88 (m, 2H); 13.06 (bs, 1H); 14.61(bs, 1H); MS *m*/*z* 502 (M H$^+$).

8-[3-(4-Chloro-6-trifluoromethyl-1H-benzoimidazol-2-ylmethoxy)-isoxazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (75).

Purification by crystallization from methanol. Yield: 50%, mp: 251 °C. ^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H); 1.59 (q, 2H); 1.69 (q, 2H); 3.86 (t, 2H); 3.97 (t, 2H); 5.96 (s, 2H); 6.95 (s, 1H); 7.48 (s, 1H); 7.98 (s, 1H); 12.99 (bs, 1H); 14.60(bs, 1H); MS *m*/*z* 552 (M H$^+$).

8-[3-(4,6-Bis-trifluoromethyl-1H-benzoimidazol-2-ylmethoxy)-isoxazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (76).

Purification by crystallization from methanol. Yield: 60%, mp: 233 °C. ^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H); 1.52 (q, 2H); 1.56 (q, 2H); 3.86 (t, 2H); 3.94 (t, 2H); 5.64 (s, 2H); 6.68 (s, 1H); 7.84 (s, 1H); 8.26 (s, 1H); 13.06 (bs, 1H); 14.00(bs, 1H). MS *m*/*z* 586 (M H$^+$).

8-[1-Methyl-3-(4-oxo-1,4-dihydro-quinazolin-2-ylmethoxy)-1H-pyrazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (49).

To a solution of **46** (100 mg, 0.25 mmol) and EDC (60 mg, 0.30 mmol) in DMF (5 mL), was added 2-aminobenzamide (35 mg, 0.25 mmol). The reaction mixture was stirred at room temperature for 2h. The solvent was evaporated under vacuo and the residue poured in water. The amidic intermediate was filtered and reacted with NaOH (2.5 N, 6 mL) at 80 °C for 2h. After cooling to 0 °C, the reaction was acidified to pH 2–3, the resultant solid was filtered, and washed with water. The titled compound was purified by flash chromatography (ethyl acetate/methanol 9/1). Yield: 60%, mp: > 300 °C.

^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H); 1.56 (q, 2H); 1.72 (q, 2H); 3.85 (t, 2H); 4.03 (t, 2H); 4.07 (s, 3H); 5.08 (s, 2H); 6.50 (s, 1H); 7.52-8.14 (m, 4H);12.55 (s, 1H), 13.88 (s, 1H); MS *m/z* 491 (M H^+).

8-[1-Methyl-3-(7H-purin-8-ylmethoxy)-1H-pyrazol-5-yl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (66).

The *same procedure* as reported for the preparation of compounds 50-65, *expect the temperature of 100 °C necessary for the ring closure of the amide intermediate*.

Purification by crystallization from DMF/water. Yield: 60%, mp: 293-294°C.

^{1}H NMR (DMSO-d_6): δ 0.84 (m, 6H); 1.54 (q, 2H); 1.70 (q, 2H); 3.83 (t, 2H); 3.97 (t, 2H); 4.05 (s, 3H); 5.44 (s, 2H); 6.50 (s, 1H); 8.87 (s, 1H); 9.03 (s, 1H); 13.77 (bs, 2H); MS *m/z* 465 (M H^+).

Methyl 3-(benzyloxy)-1-methyl-1H-pyrazole-5-carboxylate (78).

K_2CO_3 (15 mmol) was added to a solution of the methyl 3-hydroxy-1-methyl-1*H*-pyrazole-5-carboxylate 77 (2g, 12 mmol) in anhydrous acetone (100 mL) at 0°C and the mixture was stirred for 30 min. Benzyl bromide

(1.8 mL 15 mmol) was added and the mixture was stirred for 20 h at room temperature. The solvent was evaporated, the residue taken up with water and the solution was extracted with AcOEt. The organic phase was dried over Na_2SO_4 and evaporated to give an oil compound that was used in the next reaction without purification. Yield 85%.

^{1}H NMR (DMSO-d_6): δ 3.93 (s, 3H); 4.10 (s. 3H); 5.13 (s, 2H); 6.25 (s, 1H); 7.36-7,43 (m, 5H).

3-(Benzyloxy)-1-methyl-1H-pyrazole-5-carboxylic acid (79).

Aqueous KOH 2N (10 mL) was added to a suspension of the ester **78** (2 g, 8.1 mmol) in dioxane (30 mL) and the mixture was stirred at room temperature for 6 hours. Most of the solvent was evaporated under vacuum, the remaining aqueous mixture cooled to room temperature and HCl 10% was added until an acidic pH was reached. The resulting precipitate was collected by filtration, washed with water and dried to yield the corresponding carboxylic acid **79**, that was purified by crystallization (ethanol: water). Yield: 98%, mp: 137-138 °C.

^{1}H NMR (DMSO-d_6): δ 3.93 (s, 3H); 5.13 (s, 2H); 6.25 (s, 1H); 7.36-7.43 (m, 5H); 13.20 (s, 1H).

8-(3-Benzyloxy-1-methyl-1H-pyrazol-5-yl)-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (80).

To a mixture of the 3-(benzyloxy)-1-methyl-1*H*-pyrazole-5-carboxylic acid **79** (700 mg, 3.0 mmol), *EDC* (560 mg, 3.0 mmol), and methanol (20 mL) was added 5,6-diamino-1,3-dipropyl-1,3-dihydropyrimidine-2,4-dione (680 mg, 3.0 mmol) and the mixture was stirred at room temperature overnight under argon atmosphere. The resulting solution was evaporated under reduced pressure, and the residue was dissolved in NaOH 2 N (10 mL) and heated at 80 °C for 2 hours. After cooling to 0 °C, the reaction was acidified

to pH 2–3, the resultant solid was filtered, and washed with water. The titled compound was purified by crystallization from DMF. Yield: 65%, mp: 257-258 °C.

^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H); 1.56 (q, 2H); 1.73 (q, 2H); 3.86 (t, 2H); 4.00 (t, 2H); 4.08 (s, 3H); 5.16 (s, 2H); 6.48 (s, 1H); 7.37-7.46 (m, 5H); 13.97 (s, 1H).

8-(3-Benzyloxy-1-methyl-1H-pyrazol-5-yl)-1,3-dipropyl-7-(2-trimethylsilanyl-ethoxymethyl)-3,7-dihydro-purine-2,6-dione (81).

A mixture of **80** (600 mg, 1.42 mmol), potassium carbonate (1.0 g, 7.1 mmol) in DMF (20 mL) with SEM-Cl (0.5 mL, 2.84 mmol) was stirred at 70 °C for 72 h. After concentration under vacuum, the residue was extracted with ethyl acetate and washed with brine. The organic layer was dried on Na_2SO_4 and evaporated to afford the N-7 SEM protected **81** as an oil. Yield: 90%.

^{1}H NMR ($CDCl_3$): δ 0.017 (s, 9H); 0.95 (m, 6H); 1.65 (m, 2H); 1.83 (m, 4H); 3.76 (t, 2H); 3.99 (t, 2H); 4.02 (s, 3H); 4.11 (t, 2H); 5.23 (s, 2H); 5.72 (s, 2H); 6.45 (s, 1H); 7.37-7.45 (m, 5H).

8-(3-Hydroxy-1-methyl-1H-pyrazol-5-yl)-1,3-dipropyl-7-(2-trimethylsilanyl-ethoxymethyl)-3,7-dihydro-purine-2,6-dione (82).

The N-7-SEM protected **81** (600 mg, 1.08 mmol), palladium on carbon (10%, 600 mg), and ammonium formate (340 mg, 5.4 mmol) in methanol (100 mL) was refluxed for 6 h. The resultant suspension was filtered through Celite, washed with methanol to afford **82** after concentration under vacuum. The solid was purified by crystallization from Et_2O. Yield: 95%, mp: 170-171 °C.

^{1}H NMR (DMSO-d_6): δ 0.095 (s, 9H); 0.86 (m, 6H); 1.58 (q, 2H); 1.72 (q, 2H); 3.64 (t, 2H); 3.83 (s, 3H); 3.86 (t, 2H); 4.01 (t, 2H); 5.67 (s, 2H); 6.16 (s, 1H); 10.20 (bs, 1H).

8-{3-[5-(3-Methoxy-phenyl)-[1,2,4]oxadiazol-3-ylmethoxy]-1-methyl-1H-pyrazol-5-yl}-1,3-dipropyl-7-(2-trimethylsilanyl-ethoxymethyl)-3,7-dihydro-purine-2,6-dione (84).

The hydroxyl pyrazolo **82** (50 mg, 0.1 mmol) and potassium carbonate (40 mg, 0.3 mmol) in anhydrous acetone (10 mL) was reacted with 5-[(3-methoxy)phenyl]-3-chloromethyl-1,2,4-oxadiazole **83** (0.1 mmol) at 60 °C for 24 h. The mixture was cooled, diluted with water and extracted with AcOEt. The organic layer was dried over Na_2SO_4 and evaporated under vacuum to give a solid residue that was purified by column chromatography on silica gel (ethyl acetate–hexane 2/8) to afford **82**. Yield 98%, mp: 68-69 °C.

^{1}H NMR (DMSO-d_6): δ 0.10 (s, 9H); 0.85 (m, 10H); 1.59 (q, 2H); 1.74 (q, 2H); 3.62 (t, 2H); 3.86 (s, 3H); 3.88 (s, 3H); 4.02 (t, 2H); 5.43 (s, 2H); 5.70 (s, 2H); 6.46 (s, 1H); 7.24-7.78 (m, 4H).

8-{3-[5-(3-Methoxy-phenyl)-[1,2,4]oxadiazol-3-ylmethoxy]-1-methyl-1H-pyrazol-5-yl}-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (85).

The SEM protected 84 (50 mg, 0.07 mmol) was dissolved in ethanol (2 mL) and treated with HCl 1N (0.5 ml) for 4 h at 85 °C. After cooling to room temperature, the solid obtained was filtered, washed with ethanol to afford pure 85. Yield: 85 %, mp: 242 °C.

^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H); 1.55 (q, 2H); 1.73 (q, 2H); 3.87 (s, 3H); 3.89 (t, 2H); 4.01 (t, 2H); 4.09 (s, 3H); 5.40 (s, 2H); 6.53 (s, 1H); 7.31-7.74 (m, 4H); 13.98 (s, 1H); MS *m/z* 521 (M H$^+$).

8-(3-Hydroxy-1-methyl-1H-pyrazol-5-yl)-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (86).

The *same procedure* as reported for the preparation of compound 82, *expect used* 80. Yield: 80%, mp: > 300 °C.

^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H); 1.55 (q, 2H); 1.75 (q, 2H); 3.86 (t, 2H); 3.89 (t, 2H); 4.04 (s, 3H); 6.25 (s, 1H); 9.95 (s, 1H); 13.80 (bs, 1H); MS *m*/*z* 333 (M H^+).

3-Benzyloxy-1-methyl-1H-pyrazole-5-carbaldehyde (87).

Ethyl chloroformate (2 mmol) was added at -10 °C to a solution of 5-pyrazole carboxylic acid **87** (440 mg, 1.9 mmol), anhydrous tetrahydrofurane (THF) (15 mL), and triethylamine (0.2 mL, 2 mmol). After stirring for 30 min at 0°C the white precipitate was filtered and washed with THF The filtrate was added slowly to a suspension of $NaBH_4$ (5 mmol) in water (5 mL) at 0°C. The stirring was continued for 1 h at room temperature. The resultant suspension was acidified with HCl 5N and extracted with ethyl acetate. The organic layer was dried (Na_2SO_4) and evaporated to afford the corresponding alcohol as an oil that used for the next step without further purification. The alcohol (400 mg. 1.8 mmol) was dissolved in CH_2Cl_2 (5mL) and added dropwise to a suspension of pyridinium chlorochromate (5.5 mmol in 10 mL CH_2Cl_2) under gentle stirring. The reaction mixture was allowed at room temperature for 20 minutes. The solvent layer was decanted from the black residue, and rinsed the residue twice with about 5 mL of methylene chloride. Evaporated the combined methylene chloride solutions on a rotary evaporator. The residual material was diluted with 50 mL of diethyl ether and filter through cotton plug to remove the insoluble chromium salts. The ether layer was dried with anhydrous Na_2SO_4. The solvent was evaporated in vacuo and the residue was purified by flash chro-

matography (ethyl acetate–hexane 2/8) to afford aldehyde 88 as a colorless oil. Yield 85%.

^{1}H NMR ($CDCl_3$-*d*): δ -4.04 (s, 3H); 5.21 (s, 2H); 6.24 (s, 1H); 7.35-7.43 (m, 5H); 9.73 (s, 1H).

6-[2-(3-Benzyloxy-1-methyl-1H-pyrazol-5-yl)-vinyl]-5-nitro-1,3-dipropyl-1H-pyrimidine-2,4-dione (90).

A solution of 6-methyl-5-nitro-1,3-dipropyl-1H-pyrimidine-2,4-dione **89** (200 mg, 0.78 mmol), morpholine (0.07 mL, 0.86 mmol), and *p*-toluensulphonic acid (catalytic amount) in dry toluene (5 mL) was refluxed for 2 h, under argon atmosphere. The pyrazolo aldehyde **88** (168 mg, 0.78 mmol) was added, the reaction mixture was allowed at reflux overnight. The resulting solution was diluted with ethyl acetate, and washed with HCl 5 % and brine. The solvent was dried with anhydrous Na_2SO_4 and evaporated in vacuum; the residue was purified by crystallization. (diethyl ether–hexane) to yield the expected nitrostyryl derivatives **90** as a pale yellow solid. Yield 65%; mp: 120-121 °C.

^{1}H NMR (DMSO-d_6): δ 0.85 (m, 6H); 1.58 (m, 4H); 3.66 (s, 3H); 3.81 (m, 4H); 5.13 (s, 2H); 6.36 (s, 1H); 6.98 (d, 1H, J = 16.1 Hz); 7.05 (d, 1H, J = 16.1 Hz); 7.38 (m, 5H).

6-(3-Benzyloxy-1-methyl-1H-pyrazol-5-yl)-1,3-dipropyl-1,5-dihydro-pyrrolo[3,2-d]pyrimidine-2,4-dione (91).

To a solution of 5-nitro-6-styryluracil **90** (100 mg, 0.22 mmol) in ethanol (4 mL), and acetic acid 20% (0.8 mL) was added iron (160 mg), and the mixture was refluxed overnight. The resulting suspension was cooled to room temperature. After concentration under vacuum, residual material was diluted with 50 mL of ethyl acetate and 30 mL water and filtered through Celite. The organic layer was separated, washed with brine, and dried with

anhydrous Na_2SO_4. The solvent was evaporated in vacuum and the residue was purified by crystallization (diethyl ether / hexane) to yield the expected. 1*H*-pyrrolo[3,2-*d*]-pyrimidine-2,4(3*H*,5*H*)-dione **91** as a white solid. Yield 92%; mp: 158-159 °C

^{1}H NMR (DMSO-d_6): δ 0.95 (m, 6H); 1.66 (q, 2H); 1.81 (q, 2H); 3.90 (s, 3H); 4.01(m, 4H); 5.23 (s, 2H); 6.04 (s, 1H); 6.07 (s, 1H)); 7.43 (m, 5H); 11.02 (bs, 1H).

6-(3-Hydroxy-1-methyl-1H-pyrazol-5-yl)-1,3-dipropyl-1,5-dihydro-pyrrolo[3,2-d]pyrimidine-2,4-dione (92).

Same procedure as for compound 82 expect used 91. Yield 80 %; mp: 282°C

^{1}H NMR (DMSO-d_6): δ 0.87 (m, 6H); 1.62 (m, 2H); 3.76 (s, 3H); 3.85 (m, 4H); 5.96 (s, 2H); 6.51 (s, 1H); 9.77 (bs, 1H); 12.3 (bs, 1H); MS *m*/*z* 332 (M H^+).

(5-Formyl-1-methyl-1H-pyrazol-3-yloxy)-acetic acid ethyl ester (94).

The same procedure as for compound 88 except used 93. Colourless oil, yield: 85%

^{1}H NMR ($CDCl_3$-*d*): δ 1.29 (t, 3H); 4.00 (s, 3H); 4.28 (q, 2H); 4.77(s, 2H); 6.30 (s, 1H); 9.73 (s, 1H).

{1-Methyl-5-[2-(5-nitro-2,6-dioxo-1,3-dipropyl-1,2,3,6-tetrahydro-pyrimidin-4-yl)-vinyl]-1H-pyrazol-3-yloxy}-acetic acid ethyl ester (95).

The same procedure as for compound 90 except used 94.

Yellow oil**,** yield: 55%

^{1}H NMR ($CDCl_3$-*d*): δ 0.97 (m, 6H); 1.29 (t, 3H); 1.69 (m, 4H); 3.70 (s, 3H); 3.94 (m, 4H); 4.28 (q, 2H); 4.75 (s, 2H); 5.99 (s, 1H); 6.45 (d, 1H, J = 16.2 Hz); 6.87 (d, 1H, J = 16.2 Hz).

[5-(2,4-Dioxo-1,3-dipropyl-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]-pyrimidin-6-yl)-1-methyl-1H-pyrazol-3-yloxy]-acetic acid ethyl ester (96).

The same procedure as for compound 91 except used 95. Yield: 60%, mp: 186-187 °C**.**

^{1}H NMR (DMSO-d_6): δ 0.90 (m, 6H); 1.17 (t, 3H); 1.71 (m, 4H); 3.81 (s, 3H); 3.87 (m, 4H); 4.15 (q, 2H); 4.74 (s, 2H); 6.19 (s, 1H); 6.56 (s, 1H); 12.38 (bs, 1H).

[5-(2,4-Dioxo-1,3-dipropyl-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-6-yl)-1-methyl-1H-pyrazol-3-yloxy]-acetic acid (97).

Pyrazolo ethyl estere **96** (300 mg, 0.71 mmol) was dissolved in a solution of aqueous NaOH 1 N (4 mL) and methanol (10 mL), followed by stirring at room temperature for 2 h. Water (15 mL) and a 1N aqueous solution of HCl was added to the mixture up to an acidic pH to afford **97** as a white crude precipitate that was filtered, dried and used for the next step without further purification. Yield : 77%, mp: 223-224 °C.

^{1}H NMR (DMSO-d_6): δ 0.86 (m, 6H); 1.62 (m, 4H); 3.81 (s, 3H); 3.86 (m, 4H);4.63 (s, 2H); 6.18 (s, 1H); 6.57 (s, 1H); 12.43 (bs, 1H); 13.00 (bs, 1H).

6-[3-(1H-Benzoimidazol-2-ylmethoxy)-1-methyl-1H-pyrazol-5-yl]-1,3-dipropyl-1,5-dihydro-pyrrolo[3,2-d]pyrimidine-2,4-dione (98).

The same procedure as for compound 50 except used 97. Yield: 85%, mp: 224°C.

^{1}H NMR (DMSO-d_6): δ 0.86 (m, 6H); 1.75 (m, 4H); 3.86 (s, 3H); 3.99 (m, 4H); 5.34 (s, 2H); 6.26 (s, 1H); 6.55 (s, 1H); 7.20-7.62 (m, 4H); 12.50 (bs, 1H); 12.65 (bs, 1H); MS *m/z* 462 (M H$^+$).

Preparation of 2-[5-(2,4-Dioxo-1,3-dipropyl-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-6-yl)-1-methyl-1H-pyrazol-3-yloxy]-N-(4-fluoro-phenyl)-acetamide (99) and N-Benzo[1,3]dioxol-5-yl-2-[5-(2,4-dioxo-1,3-dipropyl-2,3,4,5-tetrahydro-1H-pyrrolo[3,2-d]pyrimidin-6-yl)-1-methyl-1H-pyrazol-3-yloxy]-acetamide (100).

To a mixture of the carboxylic acid **97** (0.15 mmol), *N*-(3-dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (0.15 mmol), and anhydrous DMF (5 mL) was added the appropriate aniline (0.15 mmol) and the mixture was stirred at room temperature 5 hours under argon atmosphere. The resulting solution was evaporated under reduced pressure, and at the residue was added water and the precipitate was collected by filtration and washed with water . The resulting crude was purified by flash column chromatography on silica gel (AcOEt-methanol 9/1). Yield: 65%.

99: mp: 215 °C. ^{1}H NMR (DMSO-d_6): δ 0.86 (m, 6H); 1.62 (m, 4H); 3.82 (s, 3H); 3.86 (m, 4H);4.73 (s, 2H); 6.24 (s, 1H); 6.57 (s, 1H); 7.15 (m, 2H); 7.62 (m, 2H); 10.15 (bs, 1H); 12.40 (bs, 1H); MS *m*/*z* 483 (M H$^+$).

100: mp: 226 °C. ^{1}H NMR (DMSO-d_6): δ 0.86 (m, 6H); 1.62 (m, 4H); 3.82 (s, 3H); 3.86 (m, 4H);4.70 (s, 2H); 5.98 (s, 2H); 6.23 (s, 1H); 6.56 (s, 1H); 6.83-7.02 (m, 2H); 7.32 (s, 1H); 9.99 (bs, 1H); 12.44 (bs, 1H), MS *m*/*z* 509 (M H$^+$).

Determination of affinity (K_i) and potency (IC_{50}) values of the novel adenosine compounds.

Biological Esperiments were performed by Prof. Borea et al., Dipartimento di Medicina Clinica e Sperimentale-Sezione di Farmacologia, Università di Ferrara.

Cell membranes preparation.

The human adenosine receptors have been transfected in CHO or HEK293 cells according with the method previously described.[105,106] Briefly, the cells were grown adherently and maintained in Dulbecco's Modified Eagles Medium with nutrient mixture F12 (DMEM/F12) without nucleosides, containing 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 mM) and Geneticin (G418, 0,2 mg/ml) at 37°C in 5% CO_2/95% air. For membrane preparation the culture medium was removed and the cells were washed with phosphate-buffered saline and scraped off T75 flasks in ice-cold hypo tonic buffer (5 mM Tris HCl, 1 mM EDTA, pH 7.4). The cell suspension was homogenized with Polytron, the homogenate was spun for 10 min at 1000 x g and the supernatant was then centrifuged for 30 min at 100,000 x g. The membrane pellet was suspended in 50 mM Tris HCl buffer (pH 7.4) for A_1 adenosine receptors, in 50 mM Tris HCl, 10 mM $MgCl_2$ (pH 7.4) for A_{2A} adenosine receptors, in 50 mM Tris HCl, 10 mM $MgCl_2$, 1 mM EDTA (pH 7.4) for A_{2B} and A_3 adenosine receptors.

Human cloned A_1, A_{2A}, A_{2B} and A_3 Adenosine Receptor Binding Assay.

All new synthesized compounds have been tested to evaluate their affinity to human A_1, A_{2A}, A_{2B} and A_3 adenosine receptors. Displacement experiments of [^{3}H]-DPCPX to CHO cells transfected with the human recombinant A_1 adenosine receptors were carried out for 120 min at 25°C incubating diluted membranes (50 μg of protein/assay) and at least 6-8 different con-

centrations of examined antagonists.[107] Non specific binding was determined in the presence of DPCPX 10 μM and this was always ≤ 10% of the total binding. Binding of [^{3}H]-ZM 241385 to CHO cells transfected with the human recombinant A_{2A} adenosine receptors was performed by using a suspension of membranes (50 μg of protein/assay) and at least 6-8 different concentrations of studied antagonists for an incubation time of 60 min at 4°C.[108] Non specific binding was determined in the presence of 1 μM ZM 241385 and was about 20% of total binding. Competition binding experiments of [^{3}H]-MRE 2029F20 to HEK293 cells transfected with the human recombinant A_{2B} adenosine receptors were carried out incubating for 120 min at 4°C diluted membranes (50 μg of protein/assay) and at least 6-8 different concentrations of examined compounds.[109] Non-specific binding was defined as binding in the presence of 1 μM MRE 3029F20 and was about 25% of total binding. Inhibition binding assays of [^{3}H]-MRE 3008F20 in CHO cells transfected with the human recombinant A_3 adenosine receptors were performed incubating for 120 min at 4°C diluted membranes (50 μg of protein/assay) and at least 6-8 different concentrations of examined ligands.[69] Non-specific binding was defined as binding in the presence of 1 μM MRE 3008F20 and was about 25% of total binding. Bound and free radioactivity were separated by filtering the assay mixture through Whatman GF/B glass fiber filters using a Micro-Mate 196 cell harvester (Packard Instrument Co.). The filter bound radioactivity was counted on a Top Count (efficiency 57%) with Micro-Scint 20.

Measurement of cyclic AMP levels in CHO cells transfected with human A_{2B} adenosine receptors.

CHO cells transfected with human A_{2B} adenosine receptors were washed with phosphate-buffered saline, diluted tripsine and centrifuged for 10 min

at 200 g. The pellet containing the CHO cells ($1x10^6$ cells /assay) was suspended in 0.5 ml of incubation mixture: NaCl 150 mM, KCl 2.7 mM, NaH_2PO_4 0.37 mM, $MgSO_4$ 1 mM, $CaCl_2$ 1 mM, Hepes 5 mM, $MgCl_2$ 10 mM, glucose 5 mM, pH 7.4 at 37°C. Then 2.0 IU/ml adenosine deaminase and 0.5 mM 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724) as phosphodiesterase inhibitor were added and preincubated for 10 min in a shaking bath at 37°C (6- Varani et al., 2005). The potencies of antagonists studied were determined by antagonism of NECA (100 nM)-induced stimulation of cyclic AMP levels. The reaction was terminated by the addition of cold 6% thrichloroacetic acid (TCA). The TCA suspension was centrifuged at 2000 g for 10 min at 4°C and the supernatant was extracted four times with water saturated diethyl ether. The final aqueous solution was tested for cyclic AMP levels by a competition protein binding assay. Samples of cyclic AMP standard (0-10 pmoles) were added to each test tube containing the incubation buffer (trizma base 0.1 M, aminophylline 8.0 mM, 2 mercaptoethanol 6.0 mM, pH 7.4) and [^{3}H] cyclic AMP in a total volume of 0.5 ml. The binding protein previously prepared from beef adrenals, was added to the samples previously incubated at 4°C for 150 min, and after the addition of charcoal were centrifuged at 2000 g for 10 min. The clear supernatant was counted with 4 ml of Atomlight liquid scintillator and counted in a Tri Carb Packard 2500 TR scintillation counter.

Data Analysis. The protein concentration was determined according to a Bio-Rad method[110] with bovine albumin as a standard reference. Inhibitory binding constant, K_i, values were calculated from those of IC_{50} according to Cheng and Prusoff equation $K_i = IC_{50}/(1+[C^*]/K_D^*)$, where [C*] is the concentration of the radioligand and K_D* its dissociation constant.[111] In addition, a weighted non linear least-squares curve fitting program LIGAND

was used for computer analysis of the inhibition binding experiments.[112] The IC_{50} values obtained in cyclic AMP assays were calculated by nonlinear regression analysis using the equation for a sigmoidal concentration response curves (Graph Pad Prism, San Diego, USA). All experimental data are expressed as the geometric mean with 95% confidence limits in parentheses of three or four independent experiments performed in duplicate.

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Experience at

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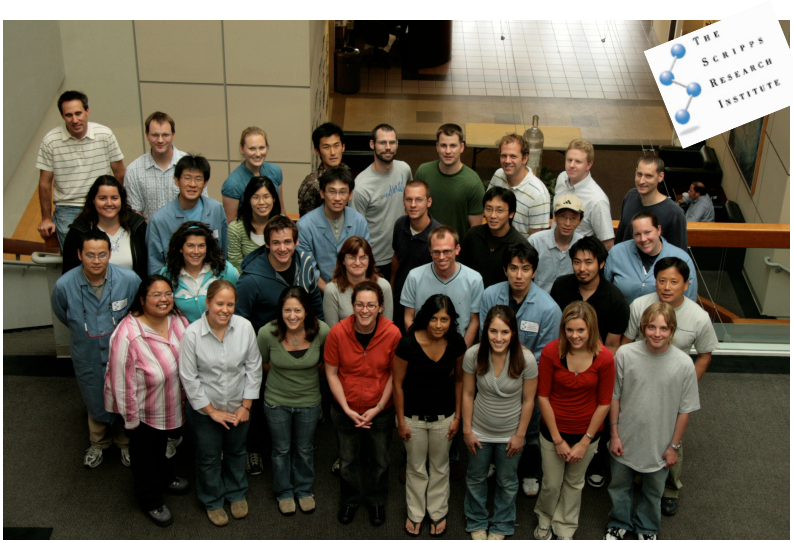
Inhibitors of

Fatty Acid Amide Hydrolase

During my PhD I spent a period of six months to *The Scripps Research Institute (TSRI), Department of Chemistry and The Skaggs Institute for Chemical Biology, La Jolla, California* in the Professor Boger Lab.

It has been a pleasure and I have learned much from my short time in his lab. I'm greatly appreciative for the time and guidance that Professor Boger provided me and for giving me the chance to be a part of his group.



Reasearch group of Professor Boger, 2007-2008

During my stage as External Graduate Student, I've completely changed the field of my research. I was involved in their Inhibitors of Fatty Acid Amide Hydrolase (FAAH) Project. My work included the scale up synthesis of key α-ketoheterocycle inhibitors of FAAH for *in vivo* analysis.

For patent and publication reasons I'm not able to present the experimental section about the compound that were investigated.

I will report in the following chapter a short overview on the research aim I have performed at TSRI.

Inhibitors of Fatty acid Amide Hydrolase

The medicinal use of cannabis dates back to early recorded history. As early as 2600 B.C., ancient Chinese texts record the use of cannabis for the relief of numerous conditions including rheumatic and menstrual pain.[1] Modern scientific investigations into the biological mechanisms surrounding the effects of cannabis have resulted in the discovery of its principle active components cannabidiol and Δ^9-tetrahydrocannabinol (THC),[2,3] as well as the receptors at which they act (CB1 and CB2).[4,5] More recently in the USA, cannabis-derived extracts or synthetic CB1 agonists have been approved by regulatory agencies for refractory chemotherapy-induced nausea and vomiting (nabilone, dronabinol), appetite stimulation in AIDS patients (dronabinol), neuropathic pain of multiple sclerosis, and adjunctive treatment of advanced cancer pain (Sativex).

However, a major limitation to the utility of direct cannabinoid agonists as therapeutic agents is the undesirable profile of side effects, which includes dysphoria, dizziness, and effects on motor coordination and memory. In particular, the cognitive effects of these agents appear to be sufficiently aversive to markedly limit their use.[6,7]

Figure 6. *Leaves of a Cannabis plant.*

The discovery of the CB1 and CB2 receptors instigated a search for endogenous agonists of these receptors and subsequently the enzymes that control their synthesis and degradation. Anandamide (AEA) was the first endocannabinoid signaling molecule to be recognized as such.[8] This discovery was followed by the identification of 2-arachidonyl

glycerol (2-AG) as a second endocanabinoid.[9,10] Today it is recognized that a wide variety of fatty acid amide signaling molecules exist, and while their historic origin resulted in the term "endocannabinoids", it is now clear that the actions of these signaling lipids are much more diverse. Further investigations revealed that the actions of these signaling lipids are terminated by the hydrolytic activity of a number of enzymes.

Although the existence of an ethanolamide degrading activity was known since 1966,[11] it was only in 1996 when researchers at Scripps identified fatty acid amide hydrolase (FAAH) as the enzyme responsible for rat oleamide hydrolysis that the first enzyme degrading these signaling lipids was cloned and characterized.[12]

Its distribution is consistent with its role in degrading and regulating such neuromodulating and signaling fatty acid amides at their sites of action.[13] Although it is a member of the amidase signature family of serine hydrolases, for which there are a number of prokaryotic enzymes, it is currently the only characterized mammalian enzyme bearing the family's unusual Ser–Ser–Lys catalytic triad.[12-18]

One attractive approach to elicit the desirable effects of cannabinoid activation, while avoiding the negative effects of global CB1 stimulation, is to manipulate endogenous cannabinoid signaling through the inhibition of FAAH and related enzymes. Resting concentrations of AEA in the CNS are very low because of its rapid hydrolysis by FAAH.[19,20] In addition, FAAH controls the levels of other lipid mediators with anti-inflammatory and analgesic properties, including PEA, which exerts its analgesic and antiinflammatory effects via non-cannabinoid pathways. Inhibition of FAAH would be expected to elevate the endogenous concentrations of all of its substrates and consequently prolong and potentiate their biological effects.

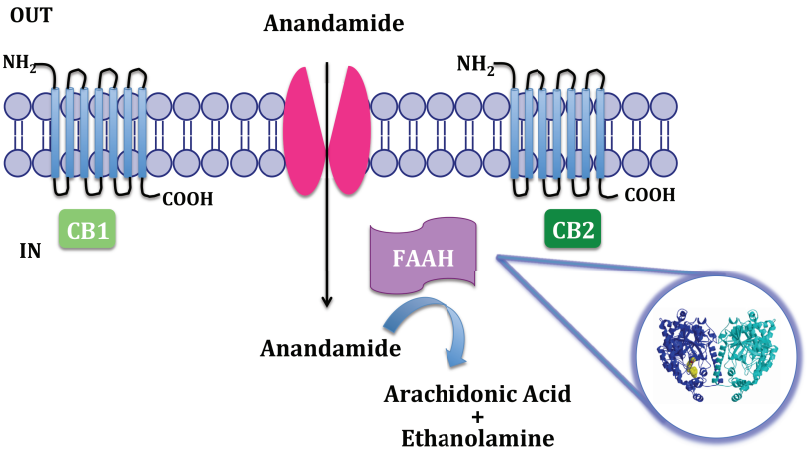


Figure 7. *The enzyme regulation of FAAH signaling and its structure determinated by X-ray crystallography. Cartoon model showing FAAH-catalyzed hydrolysis of anandamide to arachidonic acid, leading to the inactivation of this endocannabinoid signaling molecule.*

Such potentiation, acting preferentially on active pathways, might be expected to have a reduced risk of psychotropic effects compared with global activation of cannabinoid receptors by exogenously applied agonists. Direct evidence for such up regulation of synthesis in a context relevant to nociception was shown by the demonstration that intraplantar injection of formalin led to release of AEA selectively in the periaqueductal gray region of the brain, which is associated with nociceptive processing.[21] Anandamide synthesis is also up-regulated following physiological stress, and it has been proposed that an increased endocannabinoid tone under stressful physiological situations may represent an endogenous neuroprotective mechanism.[22-24]

As it is reported below, preliminary evidence suggested that the limited pharmacological activity of anandamide may be due to its rapid catabolism

in vivo, as this lipid is hydrolyzed to arachidonic acid within minutes of exogenous administration.[25] Nonetheless, the relative contribution made by FAAH to the hydrolysis of anandamide in vivo remained unclear until a mouse model was generated in which this enzyme was genetically disrupted (FAAH knockout (KO) mice).[20] Is reported that tissues from these FAAH-KO mice displayed a 50–100- fold reduction in hydrolysis rates for anandamide and related FAAs. In contrast to wild-type mice, in which administered anandamide failed to produce significant behavioral effects, FAAH-KO mice exhibited robust responses to this FAA, becoming hypomotile, analgesic, cataleptic and hypothermic. Notably, all of the behavioural effects of anandamide in FAAH-KO mice were blocked by pre-treatment with the CB1 receptor antagonist SR141716A, indicating that anandamide acted as a potent and selective CB1 ligand in these animals. Consistent with this notion, the apparent binding affinity of anandamide for the CB1 receptor increased approximately 15-fold in brain homogenates from FAAH-KO mice.[26] Neurochemical studies revealed that endogenous levels of anandamide and other NAEs were elevated 10–15 fold in several brain regions of FAAH-KO mice, including hippocampus, cortex and cerebellum.[20,27] Interestingly, these augmented central nervous system levels of FAAs correlated with a CB1-dependent reduction in pain sensation in FAAH-KO mice.[20] Collectively, these findings indicate that FAAH is a key enzyme involved in FAA catabolism in vivo and suggest that pain pathways are under the influence of a FAAH-regulated endocannabinoid one.

Notably, however, FAAH-KO mice exhibited normal motility, body weight and body temperature, indicating that several other neurobehaviors affected by exogenously applied CB1 agonists were not under tonic control of anadamide in these animals. (Table 8)

Table 8. *A comparison of the behavioral effects produced by CB1 agonists versus the genetic (KO) or chemical (inhibitor) inactivation of FAAH.*

	Potential therapeutic effects (application)				
	Analgesia (pain)	Anxiolysis (anxiety)	Anti-spasticity (multiple sclerosis)	Anti-emesis (nausea)	Decreases IOP^{++} (glaucoma)
CB1 agonist*	Yes	Yes/No	Yes	Yes	Yes
FAAH-KO mice	Yes	Unknown	Unknown	Unknown	No
FAAH inhibitor	Yes	Yes	Unknown	Unknown	No

	Side Effects		
	Hypomotility	Hypothermia	Catalepsy
CB1 agonist*	Yes	Yes	Yes
FAAH-KO mice	No	No	No
FAAH inhibitor	No	No	No

Due to the therapeutic potential of inhibiting FAAH[14,28-30], there has been an increasing interest in the development of selective and potent inhibitors of the enzyme.[27] Early studies shortly following the initial characterization of the enzyme led to the discovery that the endogenous sleep-inducing molecule 2-octyl α-bromoacetoacetate is an effective FAAH inhibitor,[31] the disclosure of a series of nonselective reversible inhibitors bearing an electrophilic ketone (e.g., trifluoromethyl ketone-based inhibitors),[32-35] and the reports of a set of irreversible inhibitors[36-41] (e.g., fluorophosphonates and sulfonyl fluorides). To date, only two classes of inhibitors have been disclosed that provide opportunities for the development of inhibitors with therapeutic potential. One class is the reactive aryl carbamates and ureas[42-60] that irreversibly acylate a FAAH active site serine[39] and that have been shown to exhibit anxiolytic activity[42] and produce analgesic effects.[61] To date and

with some exceptions, the selectivity of such inhibitors has often been low[35,46,47,60-64] further complicating the development of inhibitors that irreversibly and covalently modify the target enzyme. A second class is the α-ketoheterocycle-based inhibitors[65-73] that bind to FAAH via reversible hemiketal formation with an active site serine. Many of these competitive inhibitors are not only potent and extraordinarily selective for FAAH versus other mammalian serine hydrolases, but members of this class have been shown to be efficacious analgesics in vivo.[72,73]

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List of Abbreviations

^{125}I-ABOPX: ^{125}I-3-(3,4-aminobenzyl)-8-(4-oxyacetate)phenyl-1-propyl-xanthine

ADA: Adenosine Deamnase

ADP: Adenosine Diphosphate

AEA: Anandamide

2-AG: 2-arachidonyl glycerol

ARs: Adenosine Receptors

ASMCs: Arterial Smooth Muscle Cells

ATP: Adenosine Triphosphate

BALF: bronchoalveolar fluid

BAY 60-6583: 2-[6-amino-3,5-dicyano-4-(4-hydroxyphenyl)pyridin-2-ylsulfanyl]acetamide

cAMP: cyclic Adenosine Monophosphate

CB: Cannabinoid

CFTR: Cystic Fibrosis Transmembrane Conductance Regulator

CGS21680: 2-([4-(2-carboxyethyl)phenylethyl]amino)-5'-*N*-ethylcarboxamido - adenosine

CHO cells: Chinese Hamster Ovary cells

CNS: Central Nervous System

COPD: Chronic Obstructive Pulmonary Disease

DAG: Diacylglycerol

DMF: Dimethylformamide

DMSO: Dimethylsulfoxide

DNA: Deoxyribonucleic acid

[^{3}H]-DPCPX: [^{3}H]-1,3-dipropyl-8-cyclopentyl-xanthine

EDC: 1-[3-(dimethyl-amino)-propyl]-3-ethylcarbodiimide hydrochloride

EDG: Elettron Donating Group

EWG: Elettron Withdrawing Group

FAA: Fatty Acid Amide

FAAH: Fatty Acid Amide Hydrolase

FAD: Flavin Adenine Dinucleotide

GMCs: Glomerular Mesangial Cells

HEK293 cells: Human Embryonic Kidney cells

HMC-1: Human Mast Cells-1

IC_{50}: Inhibitory Concentration(50%)

IL: Interleukin

IP_3: Inosytoltriphospate

IPC: Ischemic Preconditioning

K_i: Equilibrium inhibition costant

KO: Knockout

Lys: Lysine

MAPK: Mitogen-Activated Protein Kinase

[^{3}H]-MRE 2029-F20: [^{3}H]-N-benzo[1,3]dioxol-5-yl-2-[5-(1,3-dipropyl-2,6-dioxo-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-1-methyl-1*H*-pyrazol-3-yloxy]-acetamide]

[^{3}H]-MRS 1754: [^{3}H]-[*N*-(4-cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)-phenoxy]acetamide

MS: Mass Spettrometry

NAD: Nicotinamide Adenine Dinucleotide

NECA: 5'-*N*-ethylcarboxamidoadenosine

NMR: Nuclear Magnetic Resonance

NO: Nitric Oxide

[^{3}H]-OSIP339391: [^{3}H]-N-(2-(2-Phenyl-6-[4-(2,2,3,3-tetratritrio-3-phenylpropyl)-piperazine-1-carbonyl]-7*H*pyrrolo[2,3-d]pyrimidin-4-ylamino)-ethyl)-Acetamide

PCC: Pyridinium chlorocromate

PDE: Phosphodiesterase Enzyme

PEA: Palmitoylethanolamide

RNA: Ribonucleic acid

RT: Room Temperature

SAM: S-Adenosyl-L-Methionine

SAR: Structure Activity Relationship

SEM-Cl: 2-(trimethylsilyl)-ethoxymethyl choride

Ser: Serine

TEA: Triethylamine

THC: Δ^9-tetrahydrocannabinol

THF: Tetrahydrofuran

TNFα: Tumor Necrosis Factor α

XAC: Xanthine Amino Congener

XCC: Xanthine Carboxilic Congener

[^{3}H]-ZM 241385: [^{3}H]-(4-(2-[7-amino-2-(2-furyl)-[1,2,4]triazolo-[2,3-a][1,3,5]triazin-5-ylamino] ethyl)-phenol