# Design, Synthesis, and Biological Evaluation of New 8-Heterocyclic Xanthine Derivatives as Highly Potent and Selective Human $A_{2B}$ Adenosine Receptor Antagonists

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Here we report the synthesis of 8-heterocycle-substituted xanthines as potent and selective $A_{2B}$ adenosine receptor antagonists. The structure−activity relationships (SAR) of the xanthines synthesized in binding to recombinant human $A_{2B}$ adenosine receptors (ARs) in HEK-293 cells (HEK-$A_{2B}$) and at other AR subtypes were explored. The synthesized compounds showed $A_{2B}$ adenosine receptor affinity in the nanomolar range and good levels of selectivity evaluated in radioligand binding assays at human (h) $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$ ARs. We introduced several heterocycles, such as pyrazole, isoxazole, pyridine, and pyridazine, at the 8-position of the xanthine nucleus and we have also investigated different spacers (substituted acetamide, oxyacetamide, and urea moieties) on the heterocycle introduced. Various groups at the 3- and 4-positions of phenylacetamide moiety were studied. This study allowed us to identify the derivatives 2-(3,4-dimethoxyphenyl)-*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 (**29b**, MRE2028F20) [$K_i$(h$A_{2B}$) = 38 nM, $K_i$(h$A_1$,h$A_{2A}$,-h$A_3$) >1000 nM], *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 (**62b**, MRE2029F20) [$K_i$(h$A_{2B}$) = 5.5 nM, $K_i$(h$A_1$,h$A_{2A}$,h$A_3$) > 1000 nM], and *N*-(3,4-dimethoxyphenyl)-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 (**72b**, MRE2030F20) [$K_i$(h$A_{2B}$ = 12 nM, $K_i$(h$A_1$,h$A_{2A}$, h$A_3$) > 1000 nM], which showed high affinity at the $A_{2B}$ receptor subtype and very good selectivity vs the other ARs. Substitution of the acetamide with an urea moiety afforded bioisosteric xanthines with good affinity and selectivity comparable to the acetamide derivatives. Substitution at the para-position of a 4-benzyloxy group of the phenylacetamido chain enhanced affinity at the $A_{2B}$ receptor [compound **30b** ($K_i$(h$A_{2B}$) = 13 nM) vs compound **21b** ($K_i$(h$A_{2B}$ = 56 nM)] but did not favor selectivity. The derivatives with higher affinity at human $A_{2B}$ AR proved to be antagonists, in the cyclic AMP assay, capable of inhibiting the stimulatory effect of NECA (100 nM) with $IC_{50}$ values in the nanomolar range, a trend similar to that observed in the binding assay (**62b**, $IC_{50}$ = 38 nM; **72b**, $IC_{50}$ = 46 nM). In conclusion, the 8-pyrazolo-1,3-dipropyl-1*H*-purine-2,6-dione derivatives described herein represent a new family of selective antagonists for the adenosine $A_{2B}$ receptor.

## Introduction

In the past few years, significant advancement has been made in the understanding of the molecular pharmacology and physiology of $A_{2B}$ adenosine receptors, but due to the lack of highly potent and selective ligands for this subtype, many questions about the pathophysiological role of $A_{2B}$ receptors have not yet been answered.[1,2] The biological activity of adenosine occurs through the activation of specific receptors located on cell membranes and belonging to the extensive family of G-protein coupled receptors.[1,2] Adenosine activates four subtypes of receptors (ARs): $A_1$, $A_{2A}$, $A_3$, and $A_{2B}$.[1,3]

The adenosine receptors are associated with different messenger systems: $A_1$ and $A_3$ mediate adenylate cyclase inhibition, whereas $A_{2A}$ and $A_{2B}$ stimulate the adenylate cyclase activity controlling intracellular cyclic AMP levels.[4] Both $A_{2A}$ and $A_{2B}$ ARs are positively coupled to adenylate cyclase via $G_s$. However, coupling to phospholipase C via $G_q$, resulting in mobilization of intracellular calcium and direct coupling to calcium channels, has also been described.[1,2]

Functional $A_{2B}$ receptors have been found in mast cells.[5] Its expression and activation is associated with control of gene expression,[6] cell growth,[7] intestinal function,[8] neurosecretion,[9] vascular tone,[10] and asthma.[2,11,12] The alkylxanthine theophylline, **I** (Figure 1), is a weak,

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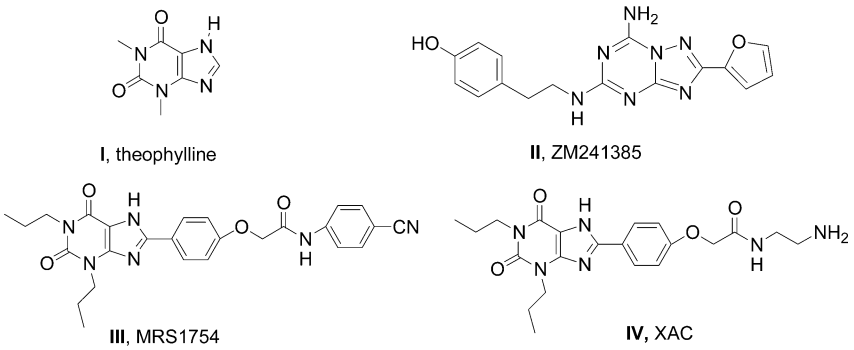



**Figure 1.** Representative structures of nonxanthine and xanthines that act as antagonists at $A_{2B}$ receptors.

nonselective AR antagonist used therapeutically for the treatment of asthma.[13,14] The use of theophylline has been associated with unpleasant side effects, such as insomnia and diuresis. While the $A_1$, $A_{2A}$, and $A_3$ adenosine receptors have been pharmacologically characterized through the use of highly potent and selective agonists and/or antagonists, the study of the $A_{2B}$ receptor has been precluded due to the lack of selective ligands[15,16] and the absence of an appropriate radioligand binding assay. Only recently, [$^3$H]ZM241385, **II**, has been proposed as a useful radioligand for studying the $A_{2B}$ adenosine receptor subtype.[17] In this field of research, Jacobson and co-workers have reported some xanthine derivatives endowed with good affinity but limited significant selectivity for the human $A_{2B}$ adenosine receptor subtype.[18,19]

An evolution of this study led to the synthesis of 8-phenylxanthinecarboxylic acid congeners, which proved to be potent and selective $A_{2B}$ antagonists. In particular, the derivative named MRS-1754, **III**[20] ([*N*-(4-cyanophenyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purin-8-yl)phenoxy]acetamide], proved to be the most potent and selective $A_{2B}$ adenosine receptor antagonist ever reported. This result led to the synthesis and characterization of the tritium-labeled form of MRS-1754 as the first radioligand for the $A_{2B}$ receptor.[21] Different nonxanthine structures displaying affinity for the $A_{2B}$ subtype in binding assays were recently reported.[22,23]

In recent years, we have investigated a series of pyrazolo[4,3-*e*]1,2,4-triazolo-[1,5-*c*]pyrimidine derivatives, modifying the chain at either the $N^7$ or $N^8$ pyrazole nitrogen and introducing different chains at the amino function in the 5-position,[24−27] with the aim of studying the affinity of this class of compounds for the human adenosine receptor subtypes. In particular, the derivatives with the free amino group at the 5-position and the compound with a $\beta$-phenylethyl chain at the N-8 pyrazole nitrogen showed good affinity for $A_{2B}$ adenosine receptors.[28]

Müller and co-workers have synthesized 1,8-disubstituted xanthine derivatives bearing polar substituents to obtain water-soluble derivatives that appeared to be less potent than MRS-1754.[29] More recently, Jacobson and co-workers have reported xanthine derivatives substituted at the 1-, 3-, 7-, and 8-positions.[30]

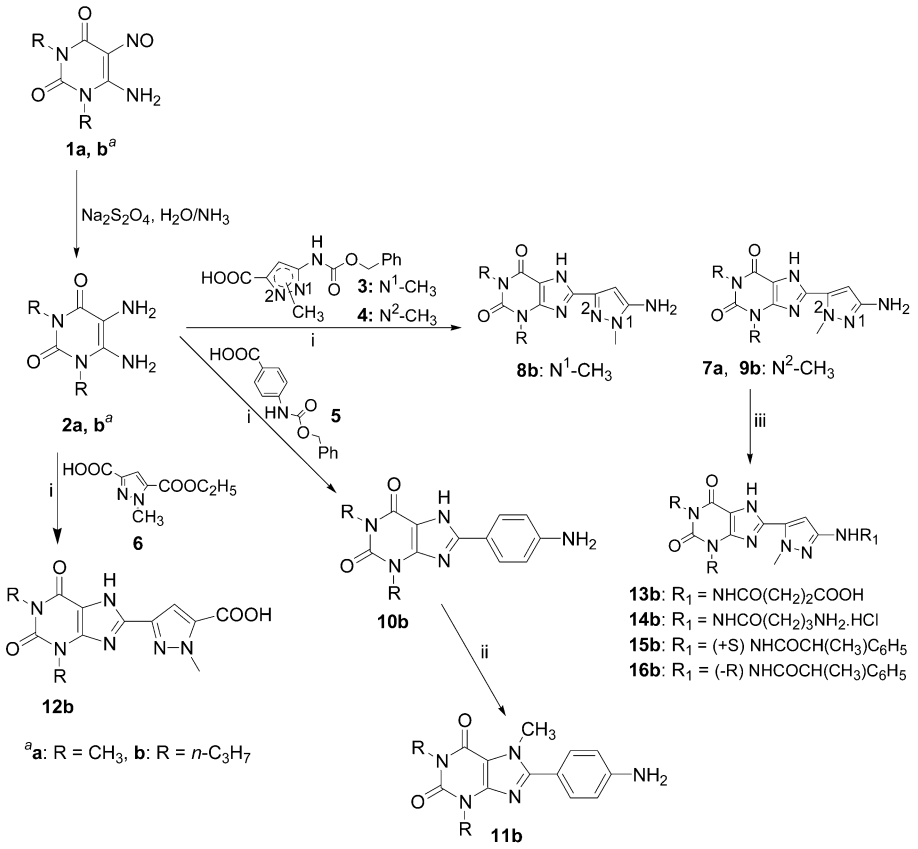
Suzuki and co-workers evaluated previously the 8-polycycloalkyl-1,3-dipropylxanthines as potent and selective antagonists for the $A_1$ adenosine receptors.[31] Recently, Volpini et al. have reported the para-substituted 1,3-dialkyl-8-phenylxanthines as classical antagonists for adenosine receptors.[32] As demonstrated by Jacobson and co-workers, the introduction at the 8-position of the xanthine nucleus of a phenyl group functionalized in the para-position with oxymethylene chains is associated with increased affinity at $A_{2B}$ receptors. In our initial attempts, we prepared several different compounds maintaining the xanthine nucleus and introducing at the 8-position a differently substituted pyrazole ring. Subsequently, we synthesized and evaluated a series of 8-pyrazolylxanthine derivatives, linking a substituted phenylacetamido moiety on the pyrazole 5-amino function. We also prepared several bioisosteric xanthines containing phenyl-substituted urea moieties. In some cases, we have prepared the corresponding 8-phenylxanthine derivatives for comparison of biological data. From these initial studies we have identified a new class of xanthine derivatives with high affinity and selectivity at human $A_{2B}$ receptors. Compound **29b** [2-(3,4-dimethoxyphenyl)-*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] in this series showed high affinity (38 nM) for human $A_{2B}$ adenosine receptors and good selectivity vs human $A_1$, $A_{2A}$, and $A_3$ adenosine subtypes.

To further understand the structure−activity relationships of the most potent $A_{2B}$ antagonists discovered in our initial search and to explore structural modifications that might lead to enhanced affinity and selectivity, we designed a new series of 8-heterocyclic xanthine derivatives. In this series we have maintained the 8-pyrazole xanthine nucleus and, as recently reported by Jacobson and co-workers in the XAC **IV**[20] (Figure 1) series, we have introduced an oxyacetamide chain on the 3-position of the pyrazole ring. Furthermore, we replaced the pyrazole nucleus with different heterocycles, such as pyridine, pyridazine, and isoxazole, in order to verify the importance of the nature of the heterocycle at this position. To obtain water-soluble compounds, we synthesized derivatives with a piperazine nucleus on the lateral chain. The synthesized compounds have been evaluated using binding experiments to CHO cells transfected with $A_1$, $A_{2A}$, and $A_3$ human adenosine receptor subtypes. Moreover, competition experiments were performed to study the affinity ($K_i$) of the new compounds to the $A_{2B}$ adenosine receptor subtype using [$^3$H]DPCPX as radioligand.[33]

## Chemistry

Synthesis of the intermediates 8-aminopyrazolo- and 8-aminophenylxanthines **7a**, **8b**−**10b** was performed by the classical method starting from 1,3-disubsituted-5,6-

**Scheme 1**




Reagents: i: (1) methanol, EDAC, 4-5 hrs; (2) methanol, NaOH 2.5 N, 70 °C, 12 hrs. ii: $K_2CO_3$, DMF, $CH_3I$, iii: ( **13b** ) Dioxane, 90°C, 5 hrs, Dihydro-furan-2,5-dione, ( **14b** ): 1) DMF, TEA, EDAC, DMAP, t.a, 4 hrs; 2 ehtylacetate/HCl, t.a. (**15, 16b**): $SOCl_2$, TEA, $CH_2Cl_2$, (+S), (-R)phenyl-propionic acid

diaminouracils.[34,35] The preparation of the target compounds was achieved by a two-step sequence involving reaction between 1,3-disubsituted-5,6-diaminouracils **2a**,**b** and the corresponding pyrazole carboxylic acids **3** and **4**[36] or phenyl carboxylic acid **5** in methanol solution using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDAC) as condensing agent, followed by ring closure in the presence of sodium hydroxide at 70 °C. The diamino uracils **2a,b** were obtained by reduction of the nitroso uracils **1a,b** using sodium dithionite (see Scheme 1).

5-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-2-methyl-2*H*-pyrazole-3-carboxylic acid, **12b**, was synthesized by condensation of **2b** and 1-methyl-1*H*-pyrazole-3,5-dicarboxylic acid 5-ethyl ester, **6**,[36] under basic conditions. The methylation of 8-(aminophenyl)-xanthine **10b** in the 7-position led to xanthine **11b**.in good yields. Derivatives **13b**−**16b** were prepared starting from **9b**, by condensation with appropriate carboxylic acids.

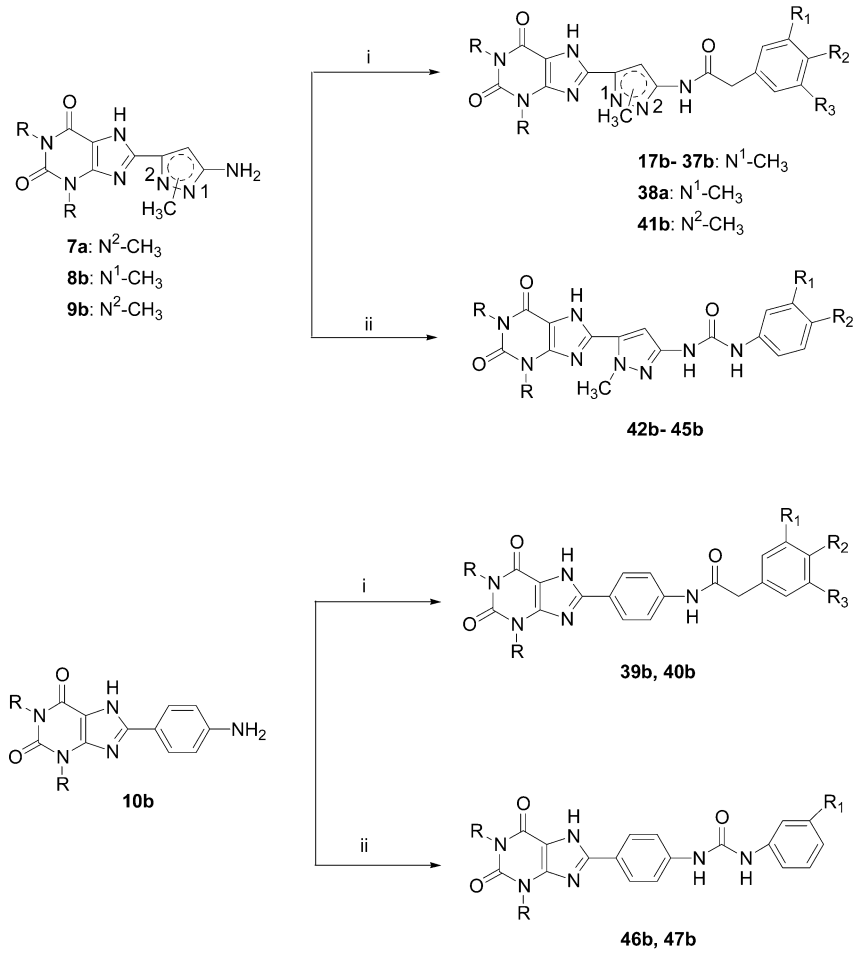
Xanthine amide derivatives **17b**−**37b**, **38a**, and **39b**−**41b** were prepared by a coupling reaction among the amine derivatives **7a**, **8b**−**10b**, and the appropriate phenylacetic acid chloride derivatives obtained, in turn, by reaction of the corresponding phenylacetic acid with thionyl chloride and triethylamine in dichloromethane. (Scheme 2).

The urea derivatives **42b**−**47b** were synthesized by condensation of the intermediates **9b** and **10b** with the appropriate isocyanates at room temperature in anhydrous dioxane (see Scheme 2).

Oxyacetamide xanthine derivatives (**60b**−**73b**, **74c**, **75c**, **80b**−**83b**, **86d**, **76b**, **77b**, **78c**, **79c**, **88b**−**91b**) were synthesized through the condensation of 5,6-diaminouracils, **2b**−**d**, with the appropriate carboxylic acid intermediates (**48**, **49**, or **52**), to yield 8-substituted xanthines containing the oxyacetic acid chain (**50b**−**d**, **51b**,**c**, **87b**). Subsequently, condensation with meta- or para-substituted anilines or *N*-substituted piperazine in the presence of EDAC and HOBt (1-hydroxybenzotrizole) in DMF at room temperature yielded the target compounds. The piperazinyl amides **80b** and **82b** were transformed into the corresponding hydrochlorides **84b** and **85b** by treatment with a saturated methanolic HCl solution. 8-(6-Hydroxypyridin-3-yl)-1,3-dipropyl-3,7-dihydropurine-2,6-dione and 8-(6-hydroxypyridazin-3-yl)-1,3-dipropyl-3,7-dihydropurine-2,6-dione (**55b**, **56b**) were prepared by condensation of **2b** with 6-hydroxypyridine-3-carboxylic acid or 6-hydroxypyridazine-3-carboxylic acid (**53**),[37] respectively (Scheme 3). These intermediates were alkylated with α-bromo-4-iodoacetanilide (**57**)[38] in the presence of TEA and DMF at room temperature, to yield the final compounds **58b** and **59b**.

The preparation of intermediate derivatives **48**, **49**, **52** is described in Scheme 4. Alkylation of hydroxypyrazolecarboxylic acid methyl esters **92** and **93**[39,40] and hydroxyisoxazolecarboxylic acid methyl esters **100**[41,42] with ethyl bromoacetate and subsequent alkaline hydrolysis afforded the corresponding dicarboxylic acids derivatives **96**, **97**, and **102**. These were transformed into derivatives **48**, **49**, and **52** by a selective esterifi-

**Scheme 2**




Reagents: i) substituted phenylacetc acids, $SOCl_2$, TEA, $CH_2Cl_2$, ii) 3(4)substituted isocyanate, dioxane, r.t.

cation of the aliphatic carboxylic acids in the presence of toluene-4-sulfonic acid at room temperature.

The chemical characterization of all the final compounds are reported in Tables 1−7.

## Results

Affinities of xanthine derivatives in radioligand binding assays at $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$ receptors are reported in Table 8. Receptor binding assays were performed using Chinese hamster ovary cells (CHO) transfected with $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$ adenosine receptors and human embryonic kidney cells (HEK-293) stably transfected with $A_{2B}$ adenosine receptors. The derivatives with higher affinity at human $A_{2B}$ adenosine receptors were antagonists capable of inhibiting the cAMP production induced by 100 nM NECA in CHO cells transfected with human (h) $A_{2B}$ adenosine receptor. Binding parameters ($K_i$, nM) and functional data ($IC_{50}$, nM) are listed in Table 9.

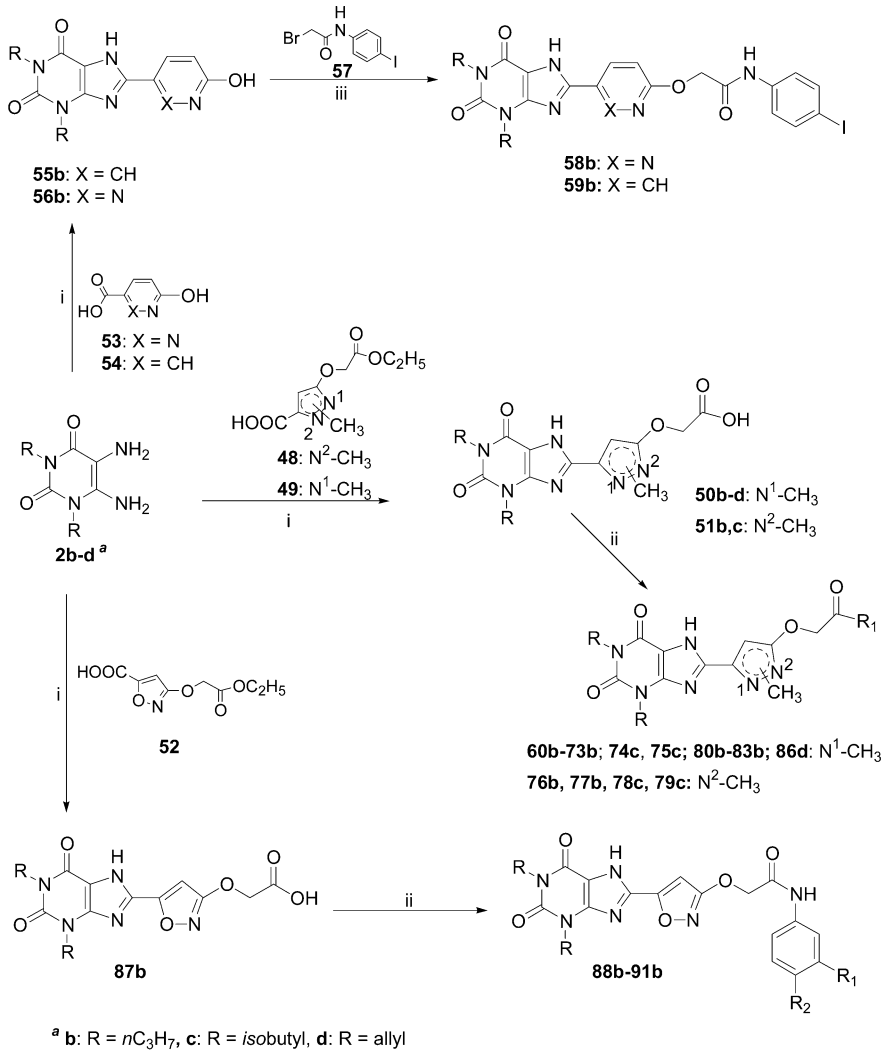
From our preliminary studies, we have inferred that the introduction of an $N^1$- or $N^2$-methylpyrazole-3-yl ring in place of the phenyl ring in MRS1754 gave compounds **7a**, **8b**, and **9b**, showing moderate $A_{2B}$ adenosine receptor affinity. Comparison of biological data for compound **8b** vs compound **9b** suggests that increased affinity and selectivity for $A_{2B}$ should be achieved with the substitution pattern of compound **8b**. On the contrary, the binding parameters of compounds **17b** and **41b** show that the affinities for $A_{2B}$ are essentially identical, while the greater selectivity is with compound **17b** and not for compound **41b**.

Comparison of compound **7a** vs compound **9b** suggests that smaller substitution (methyl vs *n*-propyl) at N-1 and N-3 of the purine nucleus leads to enhanced affinity and greatly enhanced selectivity. But comparison of compounds **17b** and **38a** shows that it is not the case when the amine has been acylated with phenylacetic acid. This observation prompted us to prepare the series of compounds **18b**−**37b**.

The enhanced affinity of compound **29b** ($K_i(hA_{2B})$ = 38 nM) and compound **17b** ($K_i(hA_{2B})$ = 35 nM) with respect to compound **40b** ($K_i(hA_{2B})$ = 95 nM) and compound **39b** ($K_i(hA_{2B})$ = 649 nM) confirms the importance of replacing the phenyl ring by the pyrazolo nucleus.

Comparison of **8b** and **9b** vs **12b** suggests the importance of an amine function (positive charge) on the pyrazole, rather than a negatively charged carboxylate group. Trying to enhance this interaction by acylation with 4-aminobutyric acid to provide compound **14b** decreased the affinity for both $A_1$ and $A_{2B}$ receptors and decreased selectivity for $A_{2B}$ receptors. Comparison of compounds **13b** and **14b**, designed following Jacobson's findings,[27] further confirms the need for a posi-

**Scheme 3**




Reagents: (i): 1)EDAC, $CH_3OH$, r.t 2) NaOH, methanol; (ii): amines in DMF, HOBt, EDAC; (iii): DMF, TEA

tively charged group rather than a negatively charged group, as suggested by the comparison of **9b** vs **12b**. Further substitution (*o*-$CF_3$, *m*-$CF_3$, *p*-$NO_2$) of the 4-benzyloxy group at the para-position of compound **21b** produced compounds **30b**, **31b**, **32b**. Compound **30b** is more potent ($K_i$(h$A_{2B}$) = 13 nM) than **21b** ($K_i$(h$A_{2B}$) = 56 nM), but less selective versus the $A_1$ adenosine receptor.

Bulky substituents, such as a 3,4-dimethoxy group, at the 3- and 4-positions on the phenyl ring of the phenylacetamide chain led to a potent and selective $A_{2B}$ adenosine receptor antagonist (compound **29b**, MRE-2028F20), whereas 3,4,5-trimethoxy substitution (compound **35b**) showed no affinity.

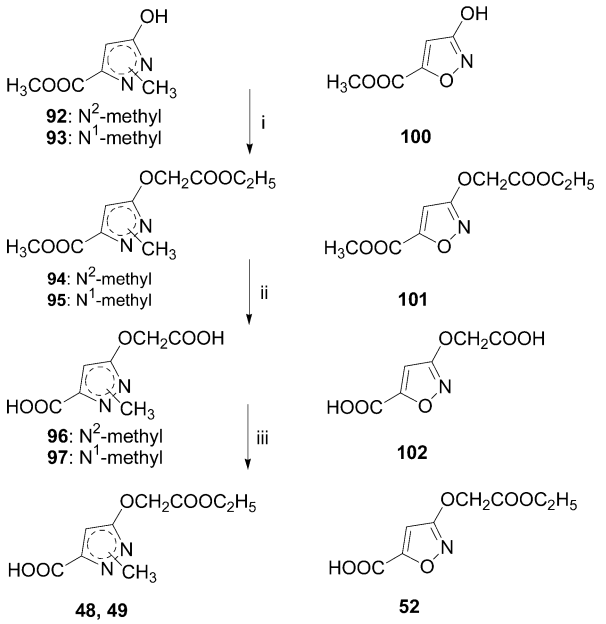
All of the urea derivatives show good affinity and rather high selectivity as $A_{2B}$ adenosine receptor antagonists. In some cases, compounds **42b** and **43b**, the introduction of the bioisosteric urea moiety in place of the acetamido chain (compounds **27b** and **24b**) increases the affinity at the $A_{2B}$ adenosine receptor.

The derivatives **17b**, **21b**, **26b**, **30b** ($K_i$ = 35, 56, 50, 13 nM, respectively) are antagonists at the human $A_{2B}$ adenosine receptor, capable of inhibiting cAMP production induced by 100 nM NECA, with $IC_{50}$ values in the nanomolar range (93−185 nM) (Figure 2). Interestingly, in the cyclic AMP assay the compounds examined exhibited a rank order of potency very close to that observed in binding experiments (Figure 3). The Spearman's rank correlation coefficient between $IC_{50}$ values in the cyclic AMP assay and receptor affinity values ($K_i$) showed a highly significant positive correlation.

From these initial results, it is evident that introduction of the pyrazole nucleus at the 8-position of xanthine is fundamental for the $A_{2B}$ subtype in binding assays. Identifying a new lead (MRE2028F20) for this receptor, we wished to explore the structural modifications that might lead to enhanced activity and/or selectivity. The purpose of our investigations was to synthesize a new series of 8-substituted xanthine derivatives in which the xanthine nucleus was linked to a various substituted oxyacetamide chains by a heterocyclic spacer (pyrazole, isoxazole, pyridine, pyridazine) and to determinate the effects of such substitutions on the affinity and selectivity of these compounds as $A_{2B}$ adenosine receptor antagonists. MRE2028F20 was the reference compound for the biological data comparison.

The results reported provide interesting information about the effect of substitution at the 8-position of the xanthine nucleus. Many of the tested compounds appeared to be potent $A_{2B}$ receptor ligands ($K_i$ in the

**Scheme 4**




i = $K_2CO_3$, $(CH_3)_2CO$, $BrCH_2COOC_2H_5$, 70 °C
ii = $CH_3OH$, $H_2O$, NaOH, 100 °C
iii = $C_2H_5OH$, toluen-4-sulfonic acid, t.a.

nanomolar range) with different degrees of selectivity. Among them, several derivatives also showed good potency as $A_{2B}$ antagonists in terms of $IC_{50}$ in the functional cAMP assay. Derivatives **68b**, **69b**, **73b**, **81b**, **88b**, **89b**, and **91b** were comparable to MRE2028F20 as $A_{2B}$ ligands, whereas several compounds appeared to have more $A_{2B}$ receptor affinity than the reference compound (**60b**, **62b**, **70b**−**72b**, **80b**, **84b**).

In particular, the presence of a pyrazole ring at the 8-position of the xanthine moiety was confirmed to be an important structural requirement for $A_{2B}$ adenosine receptor binding. In fact, all compounds containing such a heterocyclic ring (**60b**−**73b**, **76b**, **77b**, **80b**−**85b**, **74c**, **75c**, **77c**, **78c**, **86d**) showed high affinity toward $A_{2B}$ receptors. Among them, derivatives **76b**, **77b**, **78c**, and **79c**, bearing the $N^2$-methylpyrazol-3-yl isomer, exhibited lower affinity than the corresponding $N^1$-methylpyrazol-3-yl analogues. In addition, it was observed that $N^2$-methyl derivatives demonstrated a global increase of affinity toward $A_1$ receptor, with consequent loss of selectivity.

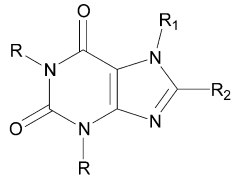
Compounds **88b**−**91b**, bearing the isoxazole nucleus at the 8-position, showed lower affinity at the $A_{2B}$ receptor than the corresponding 8-pyrazole derivatives. However, replacing the pyrazole ring with an isoxazole enhanced selectivity versus the $A_1$ adenosine receptor. Another result that supported the fundamental importance of the pyrazole is the complete loss of affinity produced by substitution of the pyrazole with pyridine or pyridazine rings (**58b**, **59b**). Therefore, it seemed that replacing the five-membered heterocycle with a six-members heterocycle determined the loss of important interactions between the examined molecules and the$A_{2B}$ adenosine receptor. Although pyridine and pyridazine were shown to be detrimental in terms of affinity, the pyridine derivative seemed to interact with the $A_{2B}$ receptor better than the pyridazine derivative. This suggests that the electronic and lipophilic nature of this heterocyclic spacer ring, in addition to steric factors, plays a fundamental role in influencing $A_{2B}$ binding parameters.

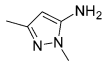
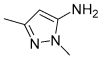
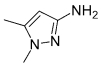
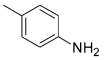
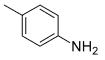
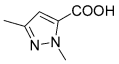
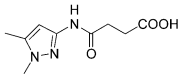
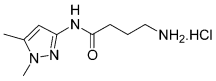
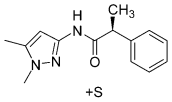
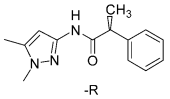
Further important information was furnished by the introduction of different moieties on the side chain of the heterocyclic spacer. In particular, we investigated the effect of several chemically different substitutions at the 3- and/or 4-position of the phenyl ring of the phenylcarbamoyl moiety (**60b**−**73b**, **76b**, **77b**, **74c**, **75c**, **78c**, **79c**, **88b**−**91b**). The modifications performed (4-F, 4-Br, 3,4-methylenedioxy, 4-*sec*-butyl, 4-*N*-morpholine, 3,4-dimethoxy, 3,4-dimethyl) differed in electronic, steric, and lipophilic features. Small hydrophobic residues in the 3- and/or 4-position of the phenyl ring seemed to increase affinity toward the $A_{2B}$ receptor (**60b**, **61b**, **62b**, **66b**, **70b**−**72b**). In particular, the presence of electron-donating groups, such as methoxy or 3,4-methylenedioxy (**72b**, **62b**), increased affinity. An additional aspect that appeared important was the capability of these moieties (−OR) to form a hydrogen bond with the $A_{2B}$ adenosine receptor interaction site. Bulky lipophilic groups, such as 4-*sec*-butyl chain, led to a loss of affinity (**65b**). Introduction of substituents containing a carbonyl function (**64b**, **68b**, **69b**) yielded a slight loss of affinity but an improvement of selectivity versus other adenosine receptors subtypes, in particular versus the $A_1$ adenosine receptors. One of the most interesting compounds in terms of affinity was **62b** (MRE2029F20, $K_i(hA_{2B}) = 5.5$ nM), containing the 3,4-methylenedioxy function on the phenyl ring, while the corresponding 3,4-dimethoxy derivative **72b** (MRE2030F20, $K_i(hA_{2B}) = 12$ nM) retained good affinity with improved selectivity for the $A_{2B}$ receptor. In compounds **63b** and **73b**, the phenyl ring of the anilide moiety was replaced by a pyridine ring. This modification led to a substantial loss of affinity toward the $A_{2B}$ adenosine receptors, especially when a small lipophilic function such as $-CH_3$ was introduced on the heterocycle (**63b**).

To obtain water-soluble compounds, we replaced the phenyl ring in the side chain with a 4-substituted piperazine moiety (**80b**−**83b**, **86d**) and prepared the corresponding hydrochloride derivatives (**84b**, **85b**). Introducing different kinds of substituents at the N-4 position of piperazine, we obtained the series of compounds shown in Table 5. The choice of a 4-fluorophenyl ring appeared to be the most interesting modification in terms of both affinity and selectivity (**81b**). In particular, the presence of the fluorine moiety in the para-position seemed to confer selectivity toward $A_{2B}$ versus $A_1$ adenosine receptors (see compounds **80b** and **81b**). 8-{2-Methyl-5-[2-oxo-2-(4-phenylpiperazin-1-yl)-ethoxy]-2*H*-pyrazol-3-yl}-1,3-dipropyl-3,7-dihydropurine-2,6-dione (**80b**) and its hydrochloride salt (**84b**) showed high affinity and good selectivity for the $A_{2B}$ receptor (respectively $K_i(hA_{2B}) = 15$ nM, $K_i(hA_{2B}) = 12$ nM). For the same reason, we synthesized compound **69b** bearing a free carboxylic function at the para-position of the anilide moiety ($K_i(hA_{2B}) = 36$ nM).

In our SAR study, we have also investigated the importance of the alkyl substitution at the 1,3-positions of the xanthine structure. Introduction of an isobutyl chain produced a marked decrease of affinity toward $A_{2B}$ adenosine receptors (compare **60b** and **61b** versus **74c** and **75c**). Another interesting finding was that replace-

**Table 1.** Chemical Characterization of 8-Substituted Xanthine Derivatives **7a** and **8b**−**16b**



| Compd. | R | $R_1$ | $R_2$ | Formula | M.P.(°C) | MW | Analysis |
|---|---|---|---|---|---|---|---|
| **7a** | $CH_3$ | H |  | $C_{11}H_{13}N_7O_2$ | >300 | 275.27 | C, H, N |
| **8b** | *n*-$C_3H_7$ | H |  | $C_{15}H_{21}N_7O_2$ | 249-250 | 331.37 | C, H, N |
| **9b** | *n*-$C_3H_7$ | H |  | $C_{15}H_{21}N_7O_2$ | 285-288 | 331.37 | C, H, N |
| **10b** | *n*-$C_3H_7$ | H |  | $C_{17}H_{21}N_5O_2$ | >300 | 327.38 | C, H, N |
| **11b** | *n*-$C_3H_7$ | $CH_3$ |  | $C_{18}H_{23}N_5O_2$ | 175-176 | 341.41 | C, H, N |
| **12b** | *n*-$C_3H_7$ | H |  | $C_{16}H_{20}N_6O_4$ | >300 | 360.37 | C, H, N |
| **13b** | *n*-$C_3H_7$ | H |  | $C_{19}H_{25}N_7O_5$ | 265-266 | 431.45 | C, H, N |
| **14b** | *n*-$C_3H_7$ | H |  | $C_{19}H_{29}ClN_8O_3$ | 251-252 | 452.94 | C, H, N |
| **15b** | *n*-$C_3H_7$ | H |  +S | $C_{24}H_{29}N_7O_3$ | 125-126 | 463.53 | C, H, N |
| **16b** | *n*-$C_3H_7$ | H |  -R | $C_{24}H_{29}N_7O_3$ | 125-126 | 463.53 | C, H, N |

ment of the propyl groups at the 1- and 3-positions of the xanthine nucleus with allyl chains produced an increase of selectivity (versus $A_1$ receptor) with a slight decrease of affinity at $A_{2B}$ adenosine receptors (**80b** vs **86d**). The most interesting compounds of this series in binding assays were evaluated in the cAMP assay on CHO cells. The $IC_{50}$ values thus obtained are reported in Table 9. The most potent derivatives showed an $IC_{50}$ value of about 40 nM (**62b**, **70b**, **72b**). Very interestingly, the compounds that show an high $A_{2B}$ affinity were also the most potent in functional assays.

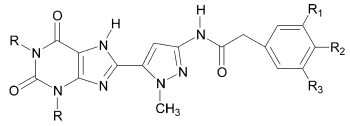
## Conclusions

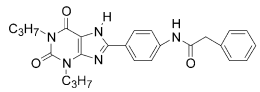
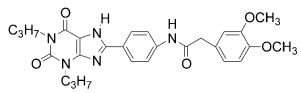
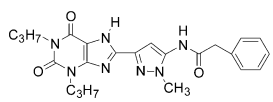
From our study on this new class of 8-substitued xanthine derivatives emerged a series of important considerations. The most significant results were obtained by introducing *n*-propyl chains on the 1- and 3-positions of the xanthine nucleus and an $N^1$-methylpyrazole spacer on the 8-position linked to an oxyacetamide moiety in the side chain bearing small lipophilic electron-releasing functions in the meta- and/or para-position of an aromatic ring (**62b**, **72b**). Replacement of the pyrazole spacer with other heterocycles led, in some cases (**88b**−**91b**), to an increase of selectivity toward $A_{2B}$ receptors. The introduction of a piperazine moiety between the oxyacetyl and aryl groups allowed us to improve the water solubility of derivative compounds, especially for hydrochloride salts (**84b**, **85b**), while receptor affinity was maintained.

## Experimental Section

**Chemistry. General.** Reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel (precoated

**Table 2.** Chemical Characterization of Phenyl and Pyrazolo Acetamide Xanthine Derivatives **17b**−**37b**, **38a**, and **39b**−**41b**



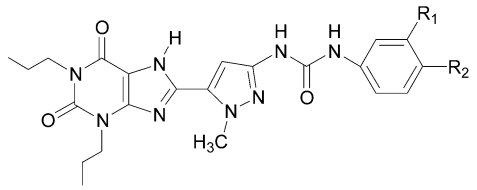
| Compd | R | $R_1$ | $R_2$ | $R_3$ | M.p(°C) | MW | Formula | Analysis |
|---|---|---|---|---|---|---|---|---|
| **17b** | *n*-$C_3H_7$ | H | H | H | 139-140 | 449.51 | $C_{23}H_{27}N_7O_3$ | C, H, N |
| **18b** | *n*-$C_3H_7$ | $OCH_3$ | H | H | 158-159 | 479.53 | $C_{24}H_{29}N_7O_4$ | C, H, N |
| **19b** | *n*-$C_3H_7$ | H | $CH_2CH(CH_3)_2$ | H | 124-126 | 505.61 | $C_{27}H_{35}N_7O_3$ | C, H, N |
| **20b** | *n*-$C_3H_7$ | H | $NO_2$ | H | 285-287 | 494.50 | $C_{23}H_{26}N_8O_5$ | C, H, N |
| **21b** | *n*-$C_3H_7$ | H | $OCH_2C_6H_5$ | H | 184-185 | 555.63 | $C_{30}H_{33}N_7O_4$ | C, H, N |
| **22b** | *n*-$C_3H_7$ | H | OH | H | 145-150 | 465.51 | $C_{23}H_{27}N_7O_4$ | C, H, N |
| **23b** | *n*-$C_3H_7$ | H | F | H | 176 | 467.50 | $C_{23}H_{26}FN_7O_3$ | C, H, N |
| **24b** | *n*-$C_3H_7$ | H | $OCH_3$ | H | 125-126 | 479.53 | $C_{24}H_{29}N_7O_4$ | C, H, N |
| **25b** | *n*-$C_3H_7$ | Cl | H | H | 143-145 | 483.95 | $C_{23}H_{26}ClN_7O_3$ | C, H, N |
| **26b** | *n*-$C_3H_7$ | F | H | H | 148-150 | 467.50 | $C_{23}H_{26}FN_7O_3$ | C, H, N |
| **27b** | *n*-$C_3H_7$ | H | $N(CH_3)_2$ | H | 215 | 492.57 | $C_{25}H_{32}N_8O_3$ | C, H, N |
| **28b** | *n*-$C_3H_7$ | H | Cl | H | 159-161 | 483.95 | $C_{23}H_{26}ClN_7O_3$ | C, H, N |
| **29b** | *n*-$C_3H_7$ | $OCH_3$ | $OCH_3$ | H | 140-142 | 509.56 | $C_{25}H_{31}N_7O_5$ | C, H, N |
| **30b** | *n*-$C_3H_7$ | H | $OCH_2$-*o*-$CF_3$-$C_6H_5$ | H | 184-186 | 623.63 | $C_{31}H_{32}F_3N_7O_4$ | C, H, N |
| **31b** | *n*-$C_3H_7$ | H | $OCH_2$-*m*-$CF_3$-$C_6H_5$ | H | 218-220 | 623.63 | $C_{31}H_{32}F_3N_7O_4$ | C, H, N |
| **32b** | *n*-$C_3H_7$ | H | $OCH_2$-*p*-$NO_2$-$C_6H_5$ | H | 136-139 | 600.63 | $C_{30}H_{32}N_8O_6$ | C, H, N |
| **33b** | *n*-$C_3H_7$ | H | $CF_3$ | H | 240 | 517.50 | $C_{24}H_{26}F_3N_7O_3$ | C, H, N |
| **34b** | *n*-$C_3H_7$ | F | F | H | 250-252 | 481.49 | $C_{23}H_{25}F_2N_7O_3$ | C, H, N |
| **35b** | *n*-$C_3H_7$ | $OCH_3$ | $OCH_3$ | $OCH_3$ | 298-300 | 555.63 | $C_{25}H_{33}N_7O_5$ | C, H, N |
| **36b** | *n*-$C_3H_7$ | $OCH_2O$ | | H | 138-140 | 493.52 | $C_{24}H_{27}N_7O_5$ | C, H, N |
| **37b** | *n*-$C_3H_7$ | $OCH_3$ | OH | H | 185 | 495.53 | $C_{24}H_{29}N_7O_5$ | C, H, N |
| **38a** | $CH_3$ | H | H | H | >300 | 393.40 | $C_{19}H_{19}N_7O_3$ | C, H, N |
| **39b** |  | | | | >300 | 445.51 | $C_{25}H_{27}N_5O_3$ | C, H, N |
| **40b** |  | | | | 270-271 | 505.27 | $C_{27}H_{31}N_5O_5$ | C, H, N |
| **41b** |  | | | | 279-281 | 449.51 | $C_{23}H_{27}N_7O_3$ | C, H, N |

$F_{245}$ Merck plates). Products were visualized with iodine or potassium permanganate solution. $^1$H NMR spectra were determined in $CDCl_3$ or DMSO-$d_6$ solutions with a Bruker AC 200 spectrometer. Peaks positions are given in parts per million ($\delta$) downfield from tetramethylsilane as internal standard, and *J* values are given in Hz. Light petroleum ether refers to the fractions boiling at 40−60 °C. Melting points were determined on a Buchi-Tottoli instrument and are uncorrected. Chromatography was performed using Merck 60−200 mesh silica gel. All products reported showed $^1$H NMR spectra in agreement with the assigned structures. Organic solutions were dried over anhydrous magnesium sulfate. Elemental analyses were performed by the microanalytical laboratory of Dipartimento di Chimica, University of Ferrara, and were within ±0.4% of the theoretical values for C, H, and N.

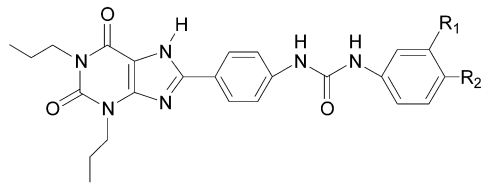
**General Procedure for the Preparation of Aminopyrazolo Derivatives 7a**, **8b**, **9b**, **Aminophenyl Derivative 10b**, **and Pyrazolo Carboxylic Acid Derivative 12b.**

To a solution of 1,3-disubstituted-5,6-diaminouracil **2a** or **2b** (2.2 mmol), in methanol (10 mL), was added an equimolar amount of the appropriate carboxylic acid (**3**−**6**) (protected as carbamic acid benzyl ester on the amino group for compounds **7a**, **8b**, **9b**, **10b** and as ethyl ester on carboxylic function for compound **12b**) and EDAC (2.21 mmol). The reaction mixture was stirred at room temperature for 4−5 h with being monitored by TLC. The solvent was concentrated in vacuo and

**Table 3.** Chemical Characterization of Urea Xanthine Derivatives (**42b**−**47b**)



**42b-45b**



**46b, 47b**

| compd | $R_1$ | $R_2$ | mp (°C) | MW | formula | anal |
|---|---|---|---|---|---|---|
| **42b** | H | $N(CH_3)_2$ | 266-268 dec | 509.60 | $C_{24}H_{31}N_9O_3$ | C, H, N |
| **43b** | Cl | H | >300 | 500.98 | $C_{22}H_{25}ClN_8O_3$ | C, H, N |
| **44b** | $OCH_3$ | H | >300 | 496.56 | $C_{23}H_{28}N_8O_4$ | C, H, N |
| **45b** | H | $OCH_3$ | >300 | 496.56 | $C_{23}H_{28}N_8O_4$ | C, H, N |
| **46b** | Cl | H | >300 | 480.95 | $C_{24}H_{25}ClN_6O_3$ | C, H, N |
| **47b** | $OCH_3$ | H | >300 | 476.53 | $C_{25}H_{28}N_6O_4$ | C, H, N |

the amidic intermediate derivative was precipitated by the addition of water. After filtration, the solid was dissolved in methanol (10 mL) and NaOH (2.5 N, 15 mL) and stirred at 70−80 °C for 12 h**.** The methanol was distilled off, and the residue was taken up in $H_2O$ and acidified with HCl to pH 4−5. The precipitate was filtered off, washed with water, and purified by flash chromatography by elution with different mixtures of ethyl acetate−petroleum ether.

**Preparation of 8-(4-Aminophenyl)-7-methyl-1,3-dipropyl-3,7-dihydropurine-2,6-dione (11b).** To a solution of compound **10b** (0.73 mmol) in anhydrous DMF (10 mL) were added $K_2CO_3$ (0.88 mmol) and dimethyl sulfate (0.88 mmol, 0.08 mL). The reaction mixture was stirred at room temperature for 1 h, DMF was removed in vacuo. To the residue was added water and the mixture was extracted with ethyl acetate (30 mL). After evaporation in vacuo, the residual solid was purified by silica gel column chromatography by elution with petroleum ether−ethyl acetate (1:1).

**Preparation of *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]succinamic Acid (13b).** To a solution of compound **9b** (60 mg, 0.18 mmol) in dioxane (20 mL) was added succinic anhydride (18 mg, 0.18 mmol). The mixture was stirred at 80−90 °C for 5 h. The solvent was evaporated to obtain **13b** as a crude solid, which was suspended in water, collected by filtration, and crystallized from ethanol.

**Preparation of 4-Amino-*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]butyramide (Hydrochloride) (14b).** A solution of compound **9b** (50 mg, 0.15 mmol), 4-*tert*-butoxycarbonylamino butyric acid (0.9 mmol), EDAC (0.9 mmol), TEA (0.3 mL, 2.25 mmol), and a catalytic amount of DMAP in anhydrous DMF (7 mL) was stirred at room temperature for 4 h. The mixture was evaporated to dryness under reduced pressure, and the residue was purified by column chromatography on silica gel eluting with ethyl acetate−petroleum ether (3:2) to yield 40 mg of *N*-Boc derivative as a white solid (mp 215−216 °C), which was stirred in a saturated solution of ethyl acetate/HCl-(g) (5 mL) for 1 h at room temperature. The product was filtered off and the white solid was crystallized by methanol−diethyl ether.

**General Procedure for the Preparation of Pyrazolo Propionamide Derivatives 15b and 16b and Pyrazolo (Phenyl) Acetamide Xanthines Derivatives 17b−37b, 38a, and 39b−41b.** A solution of the appropriate carboxylic acid (0.45 mmol) in 10 mL of thionyl chloride was refluxed for 1 h. The excess of thionyl chloride was removed by evaporation. The residue was dissolved in $CH_2Cl_2$ (5 mL) and added to a solution of the desired amine derivative (0.30 mmol) and triethylamine (0.08 mL) in $CH_2Cl_2$. The reaction mixture was stirred at room temperature for 4 h and subsequently evaporated to dryness under reduced pressure.The residue was taken up in $H_2O$ (10 mL) and the aqueous layer was extracted with EtOAc (2 × 20 mL). The organic phase was dried on anhydrous $MgSO_4$, filtered, and concentrated at reduced pressure to afford the amide derivatives. The purification of compounds was achieved by flash chromatography eluting with a suitable mixture of solvents (ethyl acetate−hexane).

**Gerneral Procedure for the Preparation of Urea Derivatives 42b−47b.** A solution of amino derivatives **9b** and **10b** (3 mmol) and the appropriate isocyanate derivatives (3.2 mmol) in 10 mL of anhydrous dioxane was stirred at room temperature for 2 h with monitoring by TLC. The reaction mixture was concentrated under reduced pressure and the residue was purified by flash chromatography on silica gel eluting with a suitable mixture of solvents to afford the desired products.

**General Procedure for the Preparation of Pyrazolo Oxyacetic Acid Xanthines 50b−d and 51b,c, Isoxazole Oxyacetic acid Xanthine 87b, and Hydroxypyridin/pyridazine Xanthines 55b and 56b.** To a solution of 1,3-disubstituted-5,6-diaminouracil **2b**−**d** (2.2 mmol), in methanol, was added an equimolar amount of the appropriate carboxylic acid derivative, and then a slightly excessive amount of EDAC (*N*-(3-(dimethylamino)propyl)-*N*′-ethylcarbodiimide hydrochloride) was added. The reaction mixture was stirred at room temperature for 4−5 h with monitoring by TLC. Addition of water caused precipitation, and the solid was filtered off. The solid was taken up with 20 mL of 10% aqueous NaOH solution and stirred at 70 °C for 0.5 h. The reaction mixture was allowed to cool and acidified with 10% HCl to pH 5, and the product was collected by filtration.

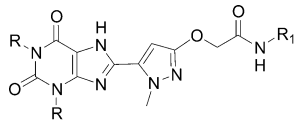
**General Procedure for the Preparation of 2-[6-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-pyridazin-3-yloxy]-*N*-(4-iodophenyl)acetamide (58b) and 2-[5-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)pyridin-2-yloxy]-*N*-(4-iodophenyl)acetamide (59b).** To a solution of hydroxypyridazine xanthine derivative **56b** or 8-hydroxypyridine **55b** (0.20 mmol) in anhydrous DMF (10 mL) was added an equimolar amount of triethylamine (TEA), and the reaction mixture was stirred at room temperature for 10 min. 2-Bromo-*N*-(4-iodophenyl)acetamide **57** (0.2 mmol) was added, and the mixture was stirred at room temperature for 10 h. The solvent was distilled off, the residue was taken up in cold water, and the precipitate was filtered off. The product was purified by column chromatography on silica gel eluting with a suitable mixture of $CH_2Cl_2$–$CH_3OH$.

**General Procedure for the Preparation of 5-[(Ethoxycarbonyl)methoxy)]-2-methyl-2*H*-pyrazole-3-carboxylic Acid (48), 5-[(Ethoxycarbonyl)methoxy]-1-methyl-1*H*-pyrazole-3-carboxylic Acid (49), and 3-[(Ethoxycarbonyl)methoxy]isoxazole-5-carboxylic Acid (52).** The acid derivative **96**, **97**, or **102** (4 mmol) was dissolved in ethanol (50 mL), and to the solution was added a catalytic amount of toluene-4-sulfonic acid (100 mg). The reaction mixture was stirred overnight at room temperature, ethanol was distilled off, and the residue was crystallized by ethanol and water.

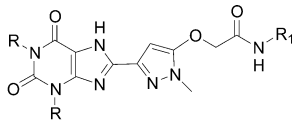
**General Procedure for the Preparation of 5-(Carboxymethoxy)-2-methyl-2*H*-pyrazole-3-carboxylic Acid (96), 5-(Carboxymethoxy)-1-methyl-1*H*-pyrazole-3-carboxylic Acid (97), 3-(Carboxymethoxy)isoxazole-5-carboxylic Acid (102). 94**, **95**, or **101** (4.0 mmol) was refluxed in a mixture of methanol (60 mL) and 5% aq NaOH solution (20 mL) for 1 h at 100 °C. Methanol was distilled off, and the residue was taken up in water and acidified with HCl to pH 4. The precipitate was filtered off and washed with cold water.

**General Procedure for the Preparation of 5-[(Ethoxycarbonyl)methoxy]-2-methyl-2*H*-pyrazole-3-carboxylic Acid Methyl Ester (94), 5-[(Ethoxycarbonyl)methoxy]-**

**Table 4.** Analytical Data of $N^1/N^2$-Methylpyrazole Oxyacetamide Derivatives



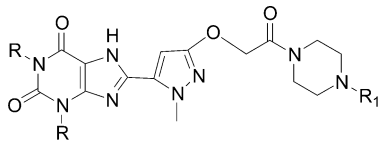
60b-73b, 74c, 75c



76b, 77b, 78c, 79c

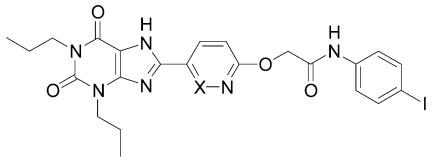
| compd | R | $R_1$ | mp (°C) | MW | formula | anal. |
|---|---|---|---|---|---|---|
| **60b** | *n*-$C_3H_7$ | 4-F-phenyl | 264 | 483.50 | $C_{23}H_{26}FN_7O_4$ | C, H, N |
| **61b** | *n*-$C_3H_7$ | 4-Br-phenyl | 280 | 544.40 | $C_{23}H_{26}BrN_7O_4$ | C, H, N |
| **62b** | *n*-$C_3H_7$ | 3,4-(methylenedioxy)phenyl | 272−273 | 509.51 | $C_{24}H_{27}N_7O_6$ | C, H, N |
| **63b** | *n*-$C_3H_7$ | 5-methylpyridin-2-yl | 278−280 | 480.52 | $C_{23}H_{28}N_8O_4$ | C, H, N |
| **64b** | *n*-$C_3H_7$ | 4-acethylphenyl | 273−275 | 507.54 | $C_{25}H_{29}N_7O_5$ | C, H, N |
| **65b** | *n*-$C_3H_7$ | 4-*s*-Bu-phenyl | 240−242 | 521.61 | $C_{27}H_{35}N_7O_4$ | C, H, N |
| **66b** | *n*-$C_3H_7$ | 4-tolyl | 256−257 | 479.53 | $C_{24}H_{29}N_7O_4$ | C, H, N |
| **67b** | *n*-$C_3H_7$ | 4-*N*-morpholinylphenyl | 296−298 | 550,61 | $C_{27}H_{34}N_8O_5$ | C, H, N |
| **68b** | *n*-$C_3H_7$ | 4-(ethoxycarbonyl)phenyl | 294 | 537.57 | $C_{26}H_{31}N_7O_6$ | C, H, N |
| **69b** | *n*-$C_3H_7$ | 4-carboxyphenyl | >300 | 509.51 | $C_{24}H_{27}N_7O_6$ | C, H, N |
| **70b** | *n*-$C_3H_7$ | 3,4-dimethylphenyl | 264−265 | 493.56 | $C_{25}H_{31}N_7O_4$ | C, H, N |
| **71b** | *n*-$C_3H_7$ | 3,4-dichlorophenyl | 258 | 534.39 | $C_{23}H_{25}Cl_2N_7O_4$ | C, H, N |
| **72b** | *n*-$C_3H_7$ | 3,4-dimethoxyphenyl | 291−293 | 525.56 | $C_{25}H_{31}N_7O_6$ | C, H, N |
| **73b** | *n*-$C_3H_7$ | pyridin-4-yl | 248−251 | 466.49 | $C_{22}H_{26}N_8O_4$ | C, H, N |
| **74c** | *i*-$C_4H_9$ | 4-F-phenyl | 227−230 | 511.55 | $C_{25}H_{30}FN_7O_4$ | C, H, N |
| **75c** | *i*-$C_4H_9$ | 4-Br-phenyl | 258−260 | 572.45 | $C_{25}H_{30}BrN_7O_4$ | C, H, N |
| **76b** | *n*-$C_3H_7$ | 4-Br-phenyl | 242 | 544.40 | $C_{23}H_{26}BrN_7O_4$ | C, H, N |
| **77b** | *n*-$C_3H_7$ | 4-F-phenyl | 262 | 483.50 | $C_{23}H_{26}FN_7O_4$ | C, H, N |
| **78c** | *i*-$C_4H_9$ | 4-Br-phenyl | 234 | 572.45 | $C_{25}H_{30}BrN_7O_4$ | C, H, N |
| **79c** | *i*-$C_4H_9$ | 4-F-phenyl | 251−252 | 511.55 | $C_{25}H_{30}FN_7O_4$ | C, H, N |

**Table 5.** Analytical Data of Pyrazolo Piperazinamide Derivatives **80b**−**85b** and **86d**



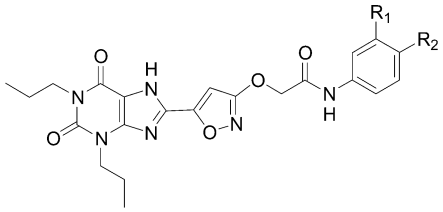
| compd | R | $R_1$ | mp (°C) | MW | formula | anal. |
|---|---|---|---|---|---|---|
| **80b** | *n*-$C_3H_7$ | phenyl | 273−274 | 507.24 | $C_{27}H_{34}N_8O_4$ | C, H, N |
| **81b** | *n*-$C_3H_7$ | 4-F-phenyl | 235−237 | 552.60 | $C_{27}H_{33}FN_8O_4$ | C, H, N |
| **82b** | *n*-$C_3H_7$ | $CH_3$ | 192−194 | 472.54 | $C_{22}H_{32}N_8O_4$ | C, H, N |
| **83b** | *n*-$C_3H_7$ | benzyl | 236−237 | 548.64 | $C_{28}H_{36}N_8O_4$ | C, H, N |
| **84b** | *n*-$C_3H_7$ | phenyl | 290 | 534.61 | $C_{27}H_{34}N_8O_4$·HCl | C, H, N |
| **85b** | *n*-$C_3H_7$ | $CH_3$ | 273 | 509.24 | $C_{22}H_{32}N_8O_4$·HCl | C, H, N |
| **86d** | $CH_2CH{=}CH_2$ | phenyl | 289−290 | 530.58 | $C_{27}H_{30}N_8O_4$ | C, H, N |

**Table 6.** Analytical Data of Isoxazole Oxyacetmide Derivatives **88b**−**91b**



| compd | $R_1$ | $R_2$ | mp (°C) | MW | formula | anal. |
|---|---|---|---|---|---|---|
| **88b** | $OCH_2O$ | | 278−280 | 496.47 | $C_{23}H_{24}N_6O_7$ | C, H, N |
| **89b** | $OCH_3$ | $OCH_3$ | 253−255 | 512.52 | $C_{24}H_{28}N_6O_7$ | C, H, N |
| **90b** | H | F | 287 | 470.45 | $C_{22}H_{23}FN_6O_5$ | C, H, N |
| **91b** | H | $OCH_3$ | 285−287 | 482.49 | $C_{23}H_{26}N_6O_6$ | C, H, N |

**Table 7.** Analytical Data of Pyridine/Pyridazine Oxyacetamide Derivatives **58b** and **59b**



| compd | X | mp (°C) | MW | formula | anal. |
|---|---|---|---|---|---|
| **58b** | N | 310 | 589.09 | $C_{23}H_{24}IN_7O_4$ | C, H, N |
| **59b** | CH | 293 | 588.10 | $C_{24}H_{25}IN_6O_4$ | C, H, N |

**1-methyl-1*H*-pyrazole-3-carboxylic Acid Methyl Ester (95), 3-[(Ethoxycarbonyl)methoxy]-isoxazole-5-carboxylic Acid Methyl Ester (101).** To a solution of **92**, **93**, or **100** (7.14 mmol) in anhydrous acetone (30 mL) was added 1.20 g (8.6 mmol) of $K_2CO_3$ and an equimolar amount of ethyl bromoacetate. The reaction mixture was refluxed for 2 h with monitoring by TLC. Acetone was removed in vacuo, and the residue was taken up with water and extracted with ethyl acetate (50 mL). The organic layer was anhydrified on $MgSO_4$ and evaporated, affording the desired alkylated derivatives **94**, **95**, **101**.

**Preparation of 2-Bromo-*N*-(4-iodophenyl)acetamide (57).** To a solution of 4-iodoaniline (14.5 mmol) in anhydrous $CH_2Cl_2$ (30 mL) were added α-bromoacethyl bromide (1.4 mL) and TEA (15.0 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 1 h. Then the solvent was removed and the residue was treated with 5% HCl (30 mL). The aqueous layer was extracted with ethyl acetate. The organic extract was washed with brine, dried with $Na_2SO_4$, and evaporated. The solid residue was purified by crystallization from ethyl acetate.

**General Procedure for the Preparation of Oxyacetamide Derivatives 60b−73b, 74c, 75c, 76b, 77b, 78c, 79c, 80b−83b, 86d, and 88b−91b.** To a solution of the carboxylic acid derivatives **50b**−**d**, **51b**, **51c**, or **87b** (1.80 mmol) in 30 mL of anhydrous DMF (dimethylformamide) was added EDAC

**Table 8.** Adenosine Receptor Affinities and $A_{2B}$ Selectivities of Synthesized Xanthine Derivatives

| | $K_i$ (nM) | | | | | | |
|---|---|---|---|---|---|---|---|
| compd | $hA_1$[a] | $hA_{2A}$[b] | $hA_{2B}$[c] | $h_{A3}$[d] | $h_{A1}/hA_{2B}$ | $hA_1/hA_{2B}$ | $hA_1/hA_{2B}$ |
| **7a** | >1000 | >1000 | 175 (134−229) | >1000 | >6 | >6 | >6 |
| **8b** | 140 (123−159) | >1000 | 58 (45−74) | >1000 | 2.4 | >18 | >18 |
| **9b** | 201 (172−236) | >1000 | 235 (209−264) | >1000 | 0.8 | >4 | >4 |
| **10b** | 65 (56−75) | >1000 | 9 (7−12) | >1000 | 7.2 | >100 | >100 |
| **11b** | >1000 | >1000 | 516 (455−585) | >1000 | >2 | >2 | 2 |
| **12b** | >1000 | >1000 | >1000 | >1000 | − | − | − |
| **13b** | >1000 | >1000 | >1000 | >1000 | − | − | − |
| **14b** | 548 (464−648) | >1000 | 2065 (1866−2284) | >1000 | 0.3 | >0.5 | >0.5 |
| **15b** | >1000 | >1000 | >1000 | >1000 | − | − | − |
| **16b** | >1000 | >1000 | >1000 | >1000 | − | − | − |
| **17b** | 900 (811−996) | >1000 | 35 (27−45) | >1000 | 26 | >29 | >29 |
| **18b** | >1000 | >1000 | 96 (80−114) | >1000 | >11 | >11 | >11 |
| **19b** | >1000 | >1000 | >1000 | >1000 | − | − | − |
| **20b** | >1000 | >1000 | 78 (63−96) | >1000 | >13 | >13 | >13 |
| **21b** | >1000 | >1000 | 56 (42−77) | >1000 | >18 | >18 | >18 |
| **22b** | >1000 | >1000 | 103 (79−136) | >1000 | >10 | >10 | >10 |
| **23b** | 200 | >1000 | 88 (84−92) | >1000 | 2.3 | >12 | >12 |
| **24b** | 850 (762−946) | >1000 | 100 (83−120) | >1000 | 8.5 | >10 | >10 |
| **25b** | 4481 (3650−5501) | >1000 | 160 (142−179) | >1000 | 28 | >6 | >6 |
| **26b** | 3227 (2799−3720) | >1000 | 50 (41−60) | >1000 | 65 | >20 | >20 |
| **27b** | >1000 | >1000 | 1628 (1374−1930) | >1000 | >0.6 | >0.6 | >0.6 |
| **28b** | 520 (484−558) | >1000 | 28 (23−33) | >1000 | 19 | >36 | >36 |
| **29b** | >1000 | >1000 | 38 (33−43) | >1000 | >27 | >27 | >27 |
| **30b** | 56 (47−67) | >1000 | 13 (11−16) | >1000 | 4.3 | >77 | >77 |
| **31b** | 100 (83−120) | >1000 | 90 (73−110) | >1000 | 1.1 | >11 | >11 |
| **32b** | 163 (137−193) | >1000 | 111 (100−124) | >1000 | 1.5 | >9 | >9 |
| **33b** | 746 (659−843) | >1000 | 130 (113−150) | >1000 | 3.9 | >6 | >6 |
| **34b** | 1898 (1723−2091) | >1000 | 78 (63−96) | >1000 | 15 | >8 | >8 |
| **35b** | >1000 | >1000 | >1000 | >1000 | − | − | − |
| **36b** | 566 (516−621) | >1000 | 18 (12−27) | >1000 | 31 | 69 | >56 |
| **37b** | >1000 | >1000 | 342 (274−426) | >1000 | >3 | 5.1 | >3 |
| **38a** | >1000 | >1000 | 569 (506−640) | >1000 | >2 | >2 | >2 |
| **39b** | >1000 | >1000 | 649 (563−747) | >1000 | >2 | >2 | >2 |
| **40b** | 1725 (1374−2165) | >1000 | 95 (86−105) | >1000 | 18 | >11 | >11 |
| **41b** | 55 (46−65) | >1000 | 34 (26−46) | >1000 | 1.6 | >30 | >30 |
| **42b** | 2410 (1760−3301 ) | >1000 | 59 (44−81) | >1000 | 41 | >17 | >17 |
| **43b** | 448 (365−550) | >1000 | 39 (33−46) | >1000 | 11 | >26 | >26 |
| **44b** | 1993 (1658−2397) | >1000 | 90 (73−110) | >1000 | 22 | >11 | >11 |
| **45b** | 1440 (1250−2211) | >1000 | 81 (70−110) | >1000 | 18 | >13 | >13 |
| **46b** | 1361 (1103−1679) | >1000 | 110 (93−130) | >1000 | 12.3 | >9 | >9 |
| **47b** | 1175 (1103−1679) | >1000 | 58 (53−64) | >1000 | 20 | >17 | >17 |
| **58b** | >1000 | >1000 | >1000 | >1000 | − | − | − |
| **59b** | >1000 | >1000 | 108 (75−155) | >1000 | >10 | >10 | >10 |
| **60b** | 65 (48−86) | >1000 | 12 (7−21) | >1000 | 5.4 | >84 | >84 |
| **61b** | 150 (132−170) | >1000 | 20 (16−25) | >1000 | 7.5 | >50 | >50 |
| **62b** | 200 (180−226) | >1000 | 5.5 (4.6−6.5) | >1000 | 40 | >182 | >182 |
| **63b** | >1000 | >1000 | 1012 (819−1250) | >1000 | >1 | >1 | >1 |
| **64b** | >1000 | >1000 | 86 (77−96) | >1000 | >12 | >12 | >12 |
| **65b** | 1005 (916−1103) | >1000 | 74 (67−81) | >1000 | 14 | >14 | >14 |
| **66b** | 79 (72−86) | >1000 | 19 (12−29) | >1000 | 4 | >53 | >53 |
| **67b** | >1000 | >1000 | 86 (78−93) | >1000 | >12 | >12 | >12 |
| **68b** | >1000 | >1000 | 32 (22−45) | >1000 | >32 | >32 | >32 |
| **69b** | >1000 | >1000 | 36 (27−47) | >1000 | >28 | >28 | >28 |
| **70b** | 700 (650−760) | >1000 | 10 (8−13) | >1000 | 70 | >100 | >100 |
| **71b** | 300 (240−380) | >1000 | 16 (12−20) | >1000 | 19 | >63 | >63 |
| **72b** | >1000 | >1000 | 12 (8−17) | >1000 | >84 | >84 | >84 |
| **73b** | 955 (896−1017) | >1000 | 41 (35−48) | >1000 | 23 | >25 | >25 |
| **74c** | 467 (400−546) | >1000 | 303 (260−352) | >1000 | 1.5 | >4 | >4 |
| **75c** | 2427 (2067−2850) | >1000 | 132 (98−178) | >1000 | 18 | >8 | >8 |
| **76b** | 168 (140−201) | >1000 | 93 (82−105) | >1000 | 1.8 | >11 | >11 |
| **77b** | 181 (127−258) | >1000 | 185 (163−210) | >1000 | 1 | >6 | >6 |
| **78c** | 49 (34−72) | >1000 | 66 (38−116) | >1000 | 0.7 | >16 | >16 |
| **79c** | 72 (45−114) | >1000 | 207 (162−265) | >1000 | 0.4 | >5 | >5 |
| **80b** | 250 (181−348) | >1000 | 15 (10−21) | >1000 | 17 | >67 | >67 |
| **81b** | >1000 | >1000 | 55 (46−65) | >1000 | >19 | >19 | >19 |
| **82b** | >1000 | >1000 | 122 (108−136) | >1000 | >9 | >9 | >9 |
| **83b** | 810 (763−859) | >1000 | 85 (66−95) | >1000 | 9.5 | >12 | >12 |
| **84b** | 260 (232−287) | >1000 | 12 (7−19) | >1000 | 22 | >84 | >84 |
| **85b** | >1000 | >1000 | 76 (67−86) | >1000 | >14 | >14 | >14 |
| **86d** | >1000 | >1000 | 24 (18−32) | >1000 | >42 | >42 | >42 |
| **88b** | >1000 | >1000 | 47 (43−52) | >1000 | >22 | >22 | >22 |
| **89b** | >1000 | >1000 | 51 (44−58) | >1000 | >20 | >20 | >20 |
| **90b** | >1000 | >1000 | 70 (61−80) | >1000 | >15 | >15 | >15 |
| **91b** | >1000 | >1000 | 53 (40−69) | >1000 | >19 | >19 | >19 |

[a] Displacement of specific [$^3$H]DPCPX binding at human $A_1$ receptors expressed in CHO cells ($n$ = 3−6). [b] Displacement of specific [$^3$H]ZM241385 binding at human $A_{2A}$ receptors expressed in CHO cells ($n$ = 3−6). [c] Displacement of specific [$^3$H] DPCPX binding at human $A_{2B}$ receptors expressed in HEK293 cells ($n$ = 3−6). [d] Displacement of specific [$^3$H]MRE3008-F20 binding at human $A_3$ receptors expressed in CHO cells ($n$ = 3−6). Data are expressed as geometric means with 95% confidence limits.

**Table 9.** Binding and Functional Data of Selected $A_{2B}$ Adenosine Compounds[a]

| compd | [$^3$H]DPCPX binding in HEK-293 membranes expressing human $A_{2B}$ adenosine receptors $K_i$ (nM) | cAMP assay in CHO cells expressing human $A_{2B}$ adenosine receptors $IC_{50}$ (nM) |
|---|---|---|
| **17b** | 35 (27−45) | 103 (92−115) |
| **21b** | 56 (42−77) | 185 (154−198) |
| **26b** | 50 (41−60) | 160 (146−188) |
| **28b** | 28 (23−33) | 128 (114−144) |
| **29b**[b] | 38 (33−43) | 120 (103−140) |
| **30b** | 13 (11−16) | 93 (84−102) |
| **36b** | 18 (12−27) | 95 (90−101) |
| **41b** | 34 (26−46) | 152 (136−170) |
| **59b** | 108 (75−155) | 477 (420−510) |
| **60b** | 12 (7−21) | 88 (82−95) |
| **61b** | 20 (16−25) | 108 (91−129) |
| **62b**[c] | 5.5 (4.6−6.5) | 38 (29−51) |
| **66b** | 19 (12−29) | 75 (66−89) |
| **68b** | 32 (22−45) | 120 (103−140) |
| **69b** | 36 (27−47) | 115 (74−89) |
| **70b** | 10 (8−13) | 40 (32−50) |
| **71b** | 16 (12−20) | 60 (52−69) |
| **72b**[d] | 12 (8−17) | 46 (35−61) |
| **80b** | 15 (10−21) | 65 (52−78) |
| **84b** | 12 (7−19) | 70 (64−78) |
| **85b** | 76 (67−86) | 322 (280−348) |
| **86d** | 24 (18−32) | 81 (74−89) |
| **88b** | 47 (43−52) | 140 (124−165) |

[a] Each value is the geometric mean (with 95% confidence limits in parentheses) of at least three experiments performed in duplicate. [b] MRE2028F20. [c] MRE2029F20 [d] MRE2030F20.

(2.16 mmol), (HOBt) 1-hydroxy-benzotrizole (2.05 mmol), and at last the appropriate amine derivative (1.80 mmol). The reaction mixture was stirred at room temperature for 4−5 h with monitoring by TLC. The solvent was removed by evaporation under reduced pressure, and the residue was chromatographed on silica gel (ethyl acetate:petroleum ether).

**Biology Experiments. CHO Membranes Preparation.** The human $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$ receptors were transfected in CHO cells according with the method described by Klotz et al.[43] 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 $\mu$g/mL), L-glutamine (2 mM), and Geneticin (G418, 0,2 mg/mL) at 37 °C in 5% $CO_2$/95% air. CHO cells were split two or three times weekly at a ratio between 1:5 and 1:20. For membrane preparation, the culture medium was removed and the cells were washed with PBS and scraped off T75 flasks in ice-cold hypotonic buffer (5 mM Tris HCl, 2 mM EDTA, pH 7.4). The cell suspension was homogenized with a Polytron and the homogenate was spun for 10 min at 1000*g*. The supernatant was then centrifuged for 30 min at 100 000*g*. The membrane pellet was suspended in 50 mM Tris HCl buffer, pH 7.4 (for $A_3$ adenosine receptors, 50 mM Tris HCl, 10 mM $MgCl_2$, 1 mM EDTA) and incubated with 3 UI/mL of adenosine deaminase for 30 min at 37 °C. Then the suspension was frozen at −80 °C. HEK 293 cells transfected with the human recombinant $A_{2B}$ adenosine receptor were obtained from Receptor Biology, Inc. (Beltsville, MD).

**Human Cloned $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$ Adenosine Receptor Binding Assay.** All synthesized compounds have been tested for their affinity at 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 receptor were performed for 120 min at 25 °C in 0.2 mL of 50 mM Tris HCl buffer, pH 7.4, containing 1 nM [$^3$H]DPCPX, diluted membranes (50 $\mu$g of protein/assay), and at least six to eight different concentrations of antagonists studied. Nonspecific binding was determined in the presence of 10 $\mu$M of CHA and this was always ≤10% of the total binding.

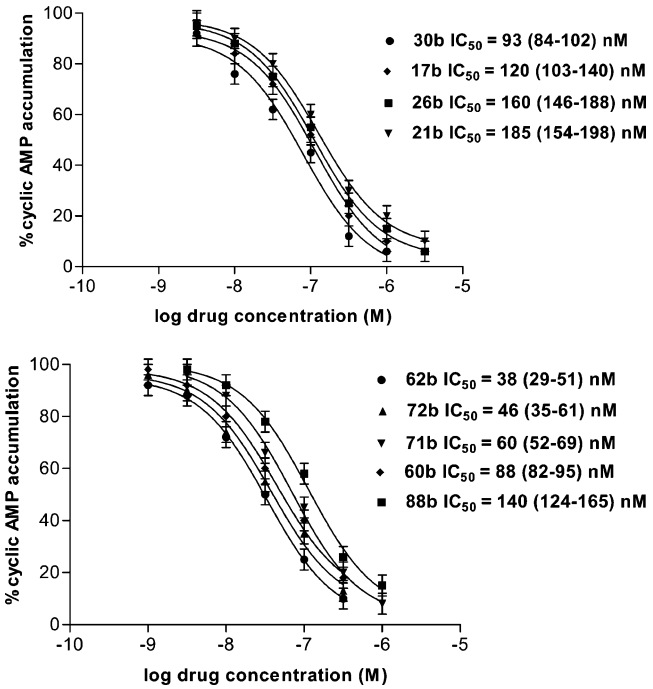



**Figure 2.** Inhibition curves representing the capability of the antagonists to block the effect of 100 nM NECA on adenylyl cyclase assays of human $A_{2B}$ adenosine receptors. Values are the means and vertical lines show the SE of the mean. Formation of cyclic AMP was detected in the absence of stimuli (basal levels = 15 ± 3 pmol cAMP/$10^6$ cells) and upon stimulation of 100 nM NECA (stimulated levels = 90 ± 10 pmol cAMP/$10^6$ cells).

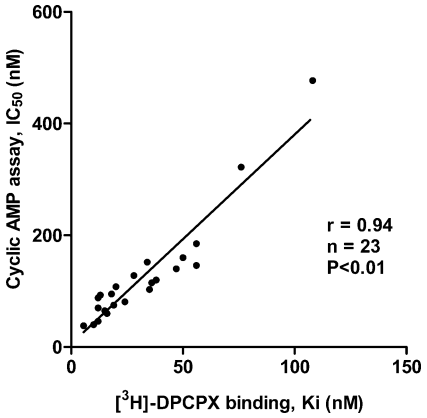



**Figure 3.** Correlation of 23 compounds studied for affinity and potency versus human $A_{2B}$ adenosine receptors (Table 9). Comparison between affinity values ($K_i$) of [$^3$H]DPCPX binding and $IC_{50}$ obtained in cAMP assays of human $A_{2B}$ adenosine receptors.

Binding of [$^3$H]ZM 241385 to CHO cells transfected with the human recombinant $A_{2A}$ adenosine receptors (50 $\mu$g of protein/assay) was performed using 0.2 mL of 50 mM Tris HCl buffer, 10 mM $MgCl_2$, pH 7.4, and at least six to eight different concentrations of antagonists studied for an incubation time of 60 min at 4 °C. Nonspecific binding was determined in the presence of 1 $\mu$M ZM 241385 and was about 20% of total binding.

Competition experiments of [$^3$H]DPCPX to HEK-293 cells transfected with the human recombinant $A_{2B}$ adenosine receptor were performed for 60 min at 25 °C in 0.1 mL of 50 mM Tris HCl buffer, 10 mM $MgCl_2$, 1 mM EDTA, 0.1 mM benzamidine, pH 7.4, 2 IU/mL adenosine deaminase containing 40 nM [$^3$H]DPCPX, diluted membranes (20 $\mu$g of protein/assay), and at least six to eight different concentrations of selected compounds. Nonspecific binding was determined in

the presence of 100 $\mu$M of NECA and was always ≤30% of the total binding.

Binding of [$^3$H]MRE 3008F20 to CHO cells transfected with the human recombinant $A_3$ adenosine receptors was previously performed.[33] Competition experiments were carried out in duplicate in a final volume of 250 $\mu$L in test tubes containing 1 nM [$^3$H]MRE 3008F20, 50 mM Tris HCl buffer, 10 mM $MgCl_2$, 1 mM EDTA, pH 7.4, 100 $\mu$L of diluted membranes (50 $\mu$g of protein/assay), and at least six to eight different concentrations of examined ligands for 120 min at 4 °C. Nonspecific binding was defined as binding in the presence of 1 $\mu$M 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.

**Adenylate Cyclase Assay. 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 and diluted tripsine and centrifuged for 10 min at 200*g*. The pellet containing the CHO cells ($1 \times 10^6$ cells/assay) was suspended in 0.5 mL of incubation mixture [(mM) NaCl 15, KCl 0.27, $NaH_2PO_4$ 0.037, $MgSO_4$ 0.1, $CaCl_2$ 0.1, Hepes 0.01, $MgCl_2$ 1, glucose 0.5, pH 7.4 at 37 °C], 2 IU/mL adenosine deaminase, and 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724) as phosphodiesterase inhibitor and preincubated for 10 min in a shaking bath at 37 °C. 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 pmol) were added to each test tube containing the incubation buffer (0.1 M trizma base, 8.0 mM aminophylline, 6.0 mM 2 mercaptoethanol, 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, samples were centrifuged at 2000*g* for 10 min. The clear supernatant was counted in a Beckman scintillation counter.

**Data Analysis.** The filter-bound radioactivity was counted on Top Count Microplate Scintillation Counter (efficiency 57%) with Micro-Scint 20. The protein concentration was determined according to a Bio-Rad method[44] with bovine albumin as a standard reference. Inhibitory binding constant, $K_i$, values were calculated from $IC_{50}$ values according to the Cheng−Prusoff equation,[45] $K_i = IC_{50}/(1 + [C^*]/K_D^*)$, where [C*] is the concentration of the radioligand and $K_D$* is its dissociation constant. A weighted nonlinear least-squares curve-fitting program LIGAND[46] was used for computer analysis of saturation and inhibition experiments. Data are expressed as the geometric mean, with 95% or 99% confidence limits in parentheses.

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**Supporting Information Available:** Selected $^1$H NMR data of synthesized compounds. This material is available free of charge via Internet at http://pubs.acs.org.

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