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Research paper

Structural refinement of pyrazolo[4,3-*d*]pyrimidine derivatives to obtain highly potent and selective antagonists for the human A_3 adenosine receptor

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ABSTRACT

In previous research, we identified some 7-oxo- and 7-acylamino-substituted pyrazolo[4,3-*d*]pyrimidine derivatives as potent and selective human (h) A_3 adenosine receptor (AR) antagonists.

Herein we report on the structural refinement of this class of antagonists aimed at achieving improved receptor-ligand recognition. Hence, substituents with different steric bulk, flexibility and lipophilicity (Me, Ar, heteroaryl, CH_2Ph) were introduced at the 5- and 2-positions of the bicyclic scaffold of both the 7-oxo and 7-amino derivatives, and acyl residues were appended on the 7-amino group of the latter. All the 2-phenylpyrazolo[4,3-*d*]pyrimidin-7-amines and 7-acylamines bearing a 4-methoxyphenyl- or a 2-thienyl group at the 5-position showed high hA_3 affinity and selectivity. In particular, the 2-phenyl-5-(2-thienyl)-pyrazolo[4,3-*d*]pyrimidin-7-(4-methoxybenzoyl)amine **25** ($K_i = 0.027$ nM) is one of the most potent and selective hA_3 antagonists reported so far. By using an *in silico* receptor-driven approach the obtained binding data were rationalized and the molecular bases of the observed hA_3 AR affinities were critically described.

1. Introduction

Adenosine is a neuromodulator that plays an important role in the homeostasis of the human body, both in periphery and in the central nervous system. Adenosine mediates its effects through activation of G-protein-coupled receptors classified as A_1, A_{2A}, A_{2B} and A_3 subtypes. Adenosine receptors (ARs) are coupled to G_i (A_1 and A_3) or G_s proteins (A_{2A} and A_{2B}) thus reducing or enhancing adenylate cyclase activity [1,2], but they also modulate other signaling pathways, depending on the cell type and the situation. The A_3 receptor subtype, coupled also to G_q proteins, stimulates phospholipase C activity, thus enhancing intracellular calcium levels [3]. In addition, this AR subtype modulates mitogen-activated protein kinase (MAPK) pathways, that can be both activated or inhibited, depending on the cellular model. The A_3 AR influence on MAPK activity explains the role of this receptor on cell proliferation and differentiation [3,4] and in tumor development and progression. A_3 AR is overexpressed in several types of cancer cells, and is thus considered as a possible biological marker for tumors [3].

It is well established that MAPKs are involved in tubulointerstitial fibrosis which is a common feature of kidney diseases leading to chronic renal failure [5]. In a recent study, the potent and selective A_3 AR antagonist LJ-1888 ((2R,3R,4S)-2-[2-chloro-6-(3-

Abbreviations: AR, adenosine receptor; NECA, 5′-(N-ethyl-carboxamido)adenosine; cAMP, cyclic adenosine monophosphate; Cl-IB-MECA, 2-chloro-N^6-(3-iodobenzyl)5′-(N-methylcarboxamido)adenosine; DPCPX, 8-cyclopentyl-1,3-dipropyl-xanthine; ZM-241385, 4-(2-[7-amino-2-(2-furyl)[1,2,4]-*triazolo*[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)phenol; I-AB-MECA, N^6-(4-amino-3-iodobenzyl)-5′-(N-methylcarboxamido)adenosine; IEele, electrostatic contribution to the interaction energy; IEhyd, hydrophobic contributions to the interaction energy; IEFs, interaction energy fingerprints; TM, transmembrane; EL2, second extracellular loop; PP, pyrazolo[4,3-*d*]pyrimidine; MOE, molecular operating environment.

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iodobenzylamino)-9H-purine-9-yl]tetrahydrothiophene-3,4-diol) blocked the development and attenuated the progression of renal interstitial fibrosis [5]. A_3 AR antagonists have also been shown to provide significant renal protection against acute injury, such as ischemic or myoglobinuric renal failure, a leading cause of renal injuries for patients undergoing surgery involving the kidney, liver or aorta [6,7]. These findings suggest that A_3 AR antagonists might become new therapeutic tools for the treatment of both chronic renal disease and acute renal ischemia and reperfusion injury.

A_3 AR antagonists have demonstrated efficacy in eye pathologies. In particular, nucleoside-like A_3 AR antagonists have proven to be effective in lowering intraocular pressure [8] and, recently, it has been reported that the potent A_3 AR antagonist MRS 1220 (N-[9-chloro-2-(2-furanyl)-1,2,4-triazolo[1,5-c]quinazolin-5-yl]benzeneacetamide) prevents oligodendrocyte damage and myelin loss triggered by ischemia or by activation of the A_3 receptor in the rat optic nerve [9].

Hence, blockage of the A_3AR has proven to be useful for the treatment of diverse diseases, however its role is still to be elucidated under other pathophysiological conditions, such as inflammation, cancer or pain [3,10]. Therefore, the identification of new potent and selective ligands which clarify the therapeutic potential arising from blocking or stimulating the A_3 AR remains an attractive objective [3,10,11].

Over the past two decades our research group has acquired a wide experience in the design and synthesis of AR antagonists [12–22]. These studies have led to the identification of a great number of potent and selective human (h) A_3 receptor antagonists, belonging to various heterocyclic classes. Most recently, we have developed some 7-oxo- and 7-amino-pyrazolo[4,3-*d*]pyrimidine derivatives (PP-7-oxo series and PP-7-amino series, respectively, Chart 1), bearing an aryl group at the 2-position [18,20,21]. Different structure–activity relationships (SARs) were highlighted in the two series. The 7-oxo derivatives showed, on the whole, nanomolar affinity and complete selectivity for the hA_3 AR while the 7-amino derivatives displayed a broad range of affinity for the different AR subtypes, depending on the nature of the substituents at the 5- and 7-positions of the pyrazolopyrimidine scaffold. In particular, 5-arylalkyl chains, combined with a free 7-amino group, shifted affinity toward the A_1 and A_{2A} ARs [21]. Instead, smaller groups at the 5-position, such as methyl or phenyl, combined with acyl residues (COMe, COAr, COheteroaryl) on the 7-amino group led to efficient and selective binding at the hA_3 AR [20].

Herein we report on the structural refinement of the pyrazolo[4,3-*d*]pyrimidine derivatives aimed at achieving better and selective hA_3 receptor recognition. To address this issue, substituents with different steric bulk, flexibility and lipophilicity were probed at the 5- and 2-position of the bicyclic scaffold of both the 7-oxo and 7-amino series. Further optimization of the 7-amino derivatives was pursued by introducing acyl residues on the 7-amino group. Hence, the 7-oxo- and 7-amino- substituted derivatives **1**–**10** and **11**–**34**, respectively, were synthesized and tested at hARs (Chart 2). Moreover, a small set of 2-phenyl[1,2,3]triazolo[4,5-*d*]

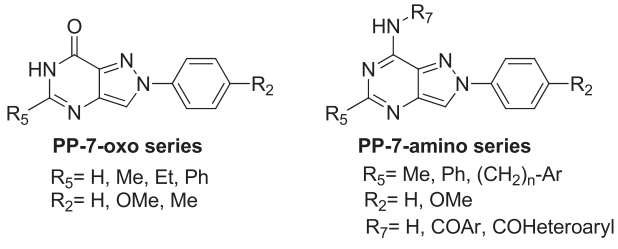


Chart 1. Previously reported Pyrazolo[4,3-*d*]pyrimidine Series.

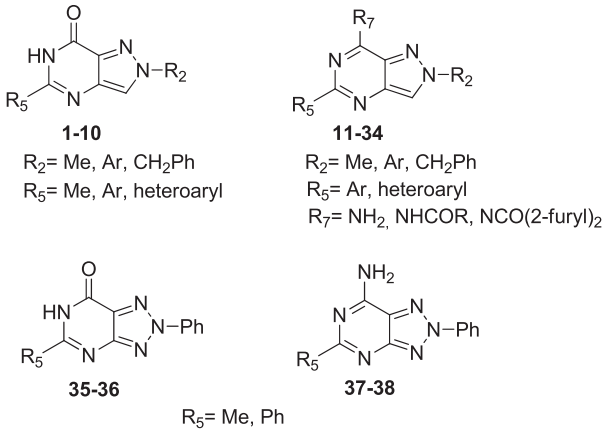


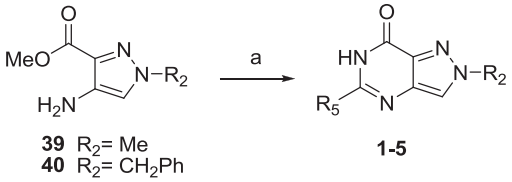
Chart 2. Herein reported Pyrazolo[4,3-*d*]pyrimidine derivatives **1**–**34** and their 3-aza-analogues 1,2,3-Triazolo[4,5-*d*]pyrimidines **35**–**38**.

pyrimidine derivatives, bearing either an oxo (**35**–**36**) or an amino (**37**–**38**) function at the 7-position (Chart 2), were synthesized. These triazolo-pyrimidine derivatives were designed as 3-aza analogues of the pyrazolo[4,3-*d*]pyrimidine series because the presence of the 3-nitrogen atom was thought to increase AR affinity, being a common feature of many potent and selective A_3 AR antagonists [10,11].

2. Chemistry

The pyrazolo[4,3-*d*]pyrimidin-7-one derivatives **1**–**10** were prepared as depicted in Schemes 1 and 2. The pyrazolopyrimidin-7-ones **1**–**5**, bearing a methyl- and a benzyl group at the 2-position, were synthesized by reacting the suitable methyl 4-aminopyrazole-3-carboxylates **39** [23] and **40** [24] with ammonium acetate and triethyl orthobenzoate (compounds **1, 4**), triethyl orthoacetate (compounds **2, 5**) or ethyl 4-methoxyphenyliminobenzoate [25] (compound **3**) (Scheme 1).

The pyrazolopyrimidin-7-ones **6**–**10**, bearing an aryl moiety at the 2-position, were obtained as shown in Scheme 2. Allowing N,N-dimethyl-2-nitroetheneamine [26] to react with suitable N_1-aryl-hydrazono-N_2-chloroacetates **41**–**43** [27–29] in chloroform, the ethyl 4-nitropyrazole-3-carboxylates **44** and **45**, **46** [18] were obtained. These compounds were reduced with cyclohexene or hydrogen and Pd/C to give the corresponding 4-amino derivatives **47** and **48**, **49** [18] which were cyclized by reaction with ammonium



	R_5	R_2
1	Ph	Me
2	Me	Me
3	C_6H_4-4-OMe	Me
4	Ph	CH_2Ph
5	Me	CH_2Ph

Scheme 1. (a) R_5-$C(OEt)_3$ or 4-$OMeC_6H_4$-$C(OEt)NH_2^+Cl^-$, NH_4OAc, mw, 130 °C.