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Original article

Synthesis, biological and modeling studies of 1,3-di-*n*-propyl-2,4-dioxo-6-methyl-8-(substituted) 1,2,3,4-tetrahydro [1,2,4]-triazolo [3,4-f]-purines as adenosine receptor antagonists

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Abstract

A new series of potential adenosine receptor antagonists with a [1,2,4]-triazolo-[3,4-f]-purine structure bearing at the 1 and 3 position *n*-propyl groups have been synthesized, and their affinities at the four human adenosine receptor subtypes (A_1, A_{2A}, A_{2B} and A_3) have been evaluated. In this case the presence of *n*-propyl groups seems to induce potency at the A_{2A} and A_3 adenosine receptor subtypes as opposed to our previously reported series bearing methyl substituents at the 1 and 3 positions. In particular the non-acylated derivative **17** showed affinity at these two receptor subtypes in the micromolar range. Indeed, preliminary molecular modeling investigations according to the experimental binding data indicate a modest steric and electrostatic antagonist-receptor complementarity.

Keywords: Adenosine receptors; Antagonists; Xanthine; G-protein-coupled receptors; Cycloaddition

1. Introduction

Adenosine is a widely distributed modulator, which regulates many physiological functions [1,2]. The action of adenosine occurs through the stimulation of P1 purinergic receptors, which are subdivided into A_1, A_{2A}, A_{2B} and A_3 adenosine receptors subtypes [1,2]. All these receptors are coupled to G-proteins and while A_1 and A_3 stimulation induces adenylyl cyclase inhibition and consequently reduced cAMP levels, the activation of A_{2A} and A_{2B} produces the opposite effect [3–6]. All these receptors are widely distributed and they are strongly involved in several patho-physiological functions and/or disorders as extensively reported [1].

For this reason great efforts have been dedicated to the search for potent and selective agonists and antagonists over the last decades. Such compounds could be used as pharmacological tools for receptor characterization as well as potential drug candidates [7,8].

In the field of antagonists, naturally occurring xanthines like caffeine or theophylline are adenosine receptor ligands with micromolar affinity. These compounds represent an ideal starting point for the development of potent and selective adenosine receptor antagonists.

Several substitutions at the 1-, 3-, and 8-positions led to the discovery of potent and selective antagonists for the different adenosine receptor subtypes. (Fig. 1) In particular, substitution at the 8-position with a cyclopentyl group led to DPCPX **1** [9] which is one of the most potent and selective A_1 adenosine receptor antagonists. The introduction at the same position of styryl (**2**) [10] or bulky aryl substituents (**3**) [11,12] permitted to obtain A_{2A} and A_{2B} adenosine receptor antagonists (**2, 3**). Recently it has been demonstrated that analogs constrained between 7- and 8- (**4**) [13] or 3- and 4- positions (**5**) [14] possess A_3 affinity and selectivity (Fig. 1).

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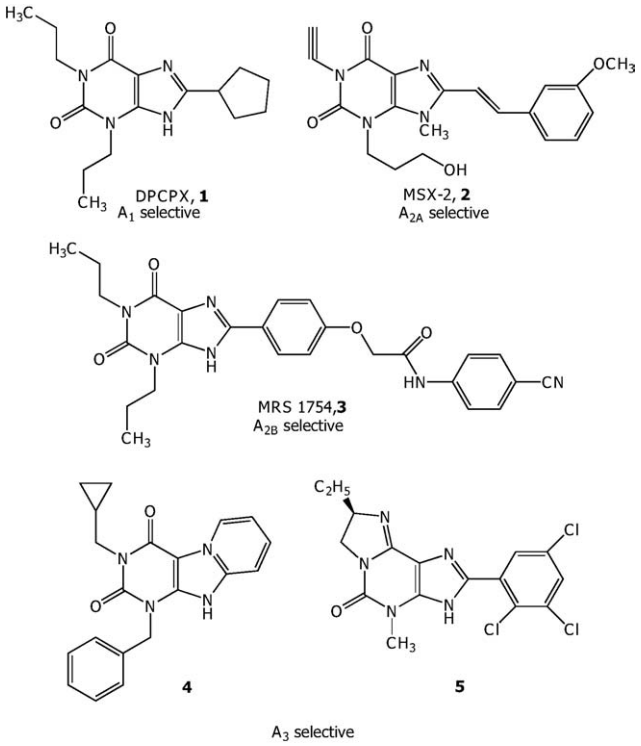


Fig. 1. Structures of xanthine derivatives as potent and selective adenosine receptor antagonists.

Taking into account these experimental observations we have recently reported a new series of 1,3-dimethyl-2,4-dioxo-6-methyl-8-(substituted) 1,2,3,4-tetrahydro [1,2,4]-triazolo [3,4-*f*]-purines of general formula **6** as potential adenosine receptor antagonists. (Fig. 2) Unfortunately, all the synthesized compounds resulted to be totally inactive at all four adenosine receptor subtypes [15].

A molecular modeling study clearly indicated that a poor steric and electrostatic complementarity for the hA_3 binding site. In particular, this inactivity could be attributed to the absence of *n*-propyl or bulkier groups at the 1 and 3 positions (see reference compounds **1**–**5**), which on these bases could be considered an ideal structural requirement for having binding affinity at the adenosine receptor subtypes.

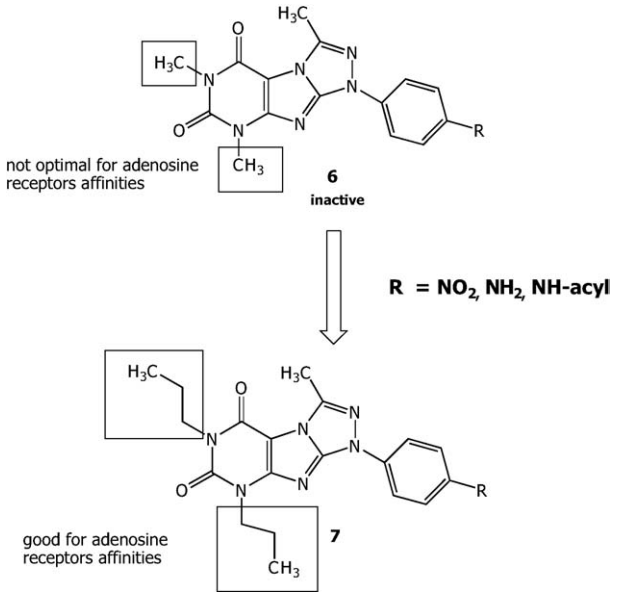


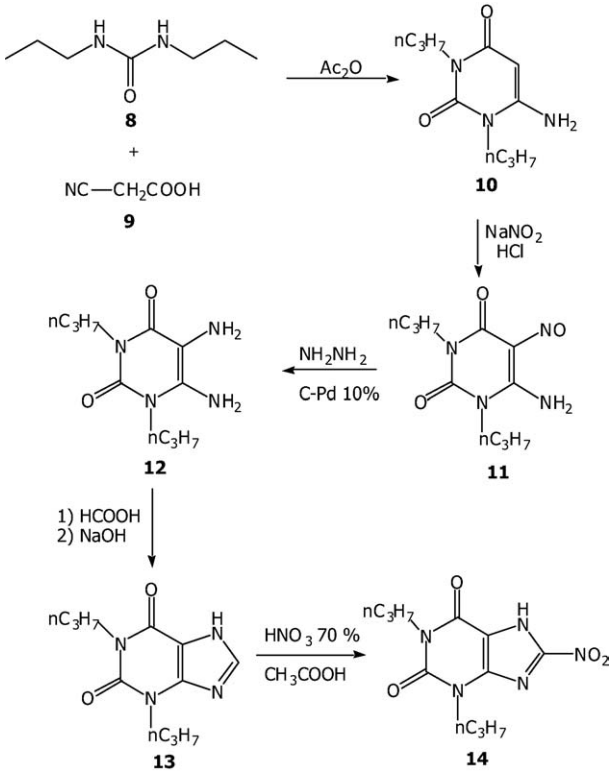
Fig. 2. Rational design of newly synthesized derivatives.

For this reasons we designed a new series of derivatives in which the methyl groups at the 1 and 3 positions were replaced by *n*-propyl groups that are characteristics of adenosine receptor antagonists with the xanthine nucleus (Fig. 2).

2. Chemistry

The designed compounds (**7a**–**j**) have been prepared by one-pot cycloaddition reaction between the 8-substituted xanthine with an appropriate hydrazonoyl halide [16,17] as previously reported. The appropriate 8-substituted *di*propyl xanthine was prepared following a standard procedure and briefly reported in Scheme 1.

Reacting *N*-*N*′-dipropyl urea **8** with cyanacetic acid **9** in presence of acetic anhydride afforded the 5-aminouracil **10**, which by treatment with sodium nitrite in acidic conditions gave the nitroso derivative **11**. The latter was then reduced with hydrazine and C–Pd 10% to afford the diaminouracil **12** [18] which was then cyclized to dipropylxanthine **13** by treatment with formic acid and then with NaOH [19,20]. Reaction of **13** with HNO_3 70% yield to the desired 8-nitro-dipropyl xanthine **14** [21–23]. Finally, reacting at reflux (10 h) in presence of triethylamine, compound **14** with the *N*-(2-bromo-4-nitrophenyl) acetohydrazonoyl bromide (**15**) [24] the corresponding cycloadduct **16** was obtained in a good yield



Scheme 1.