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SCIENCE DIRECT®
Il Farmaco 60 (2005) 299–306
IL FARMACO
http://france.elsevier.com/direct/FARMAC/

Synthesis, biological studies and molecular modeling investigation of 1,3-dimethyl-2,4-dioxo-6-methyl-8-(substituted) 1,2,3,4-tetrahydro [1,2,4]-triazolo [3,4-f]-purines as potential adenosine receptor antagonists

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Received 20 November 2004; received in revised form 12 January 2005; accepted 15 January 2005

Available online 23 March 2005

Abstract

A new series of potential adenosine receptor antagonists with a [1,2,4]-triazolo-[3,4-f]-purine structure have been synthesized, and their affinities at the four adenosine receptor subtypes (A_1, A_{2A}, A_{2B} and A_3) have been evaluated. The design was based on the demonstrated approach to novel A_3 adenosine receptor antagonists of adding a third ring to the xanthine structure. Unfortunately, all the synthesized compounds were completely inactive at all four adenosine receptor subtypes independently of their substitutions. Preliminary molecular modeling investigation has demonstrated that only a low degree of steric and electrostatic complementarity has been observed for all the new synthesized triazolo-purines with respect to other structurally related A_3 receptor antagonists. This analysis yielded valuable information about structure–activity relationships and further design of potential adenosine receptor antagonists.

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

1. Introduction

Adenosine is a widely distributed modulator, which, interacting with four different receptor subtypes named A_1, A_{2A}, A_{2B} and A_3, respectively, induces a wide range of physiological effects [1,2]. All these receptor subtypes are coupled to G-protein and while activation of A_1 and A_3 produces a decrease of cAMP level, the stimulation of A_{2A} or A_{2B} adenosine receptors gives the opposite effect [3–6].

In recent decades much effort has been devoted to characterize pharmacologically these receptors through the synthesis of potent and selective agonists and/or antagonists. While this goal has been achieved for A_1, A_{2A} and A_3 receptors, due to the lack of selective ligands, the patho-physiological role of the A_{2B} adenosine receptor is still under investigation [7,8].

Xanthines (e.g., caffeine, theophylline), which are naturally occurring antagonists for adenosine receptors, have represented the starting point in the search for potent and selective antagonists for the four different adenosine receptor subtypes. In particular, an optimization of substitutions at the 1-, 3-, and 8-positions led to the discovery of potent and selective antagonists for the various adenosine receptor subtypes. (Fig. 1) In particular, while substitution at the 8-position with alkyl (**1**) [9], styryl (**2**) [10], or bulky aryl substituents (**3**) [11,12] permitted to obtain A_1, A_{2A} and A_{2B} adenosine receptor antagonists (**1-3**), while analogs constrained between the 7- and 8- (**4**) [13], or the 3- and 4-positions (**5**) [14], led to the discovery of appealing A_3 antagonists.

With the aim to identify new lead compounds as adenosine receptor antagonist, we focused our attention on a new modification of the xanthine core designing a new fused tria-

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doi:10.1016/j.farmac.2005.01.008

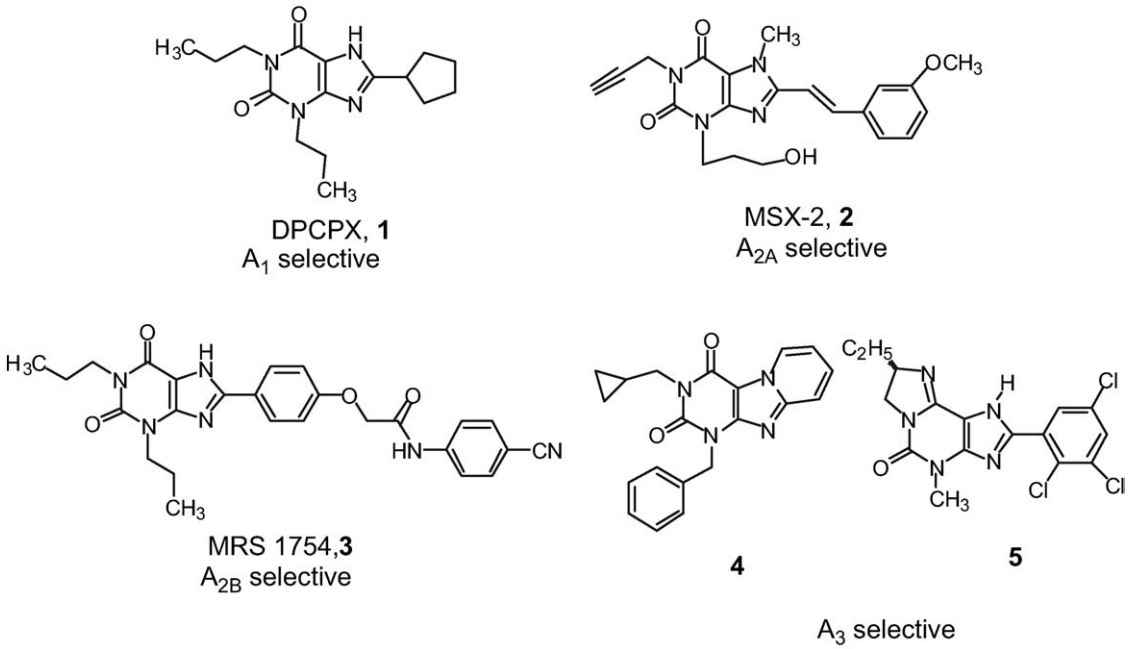
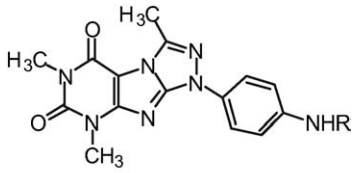


Fig. 1. Structures of xanthine derivatives as potent and selective adenosine receptor antagonists.

zolo nucleus between 7- and 8-position of theophylline structure. (**6**)



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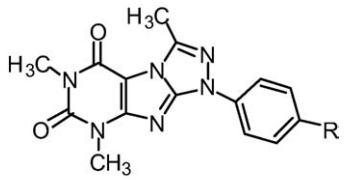
In particular, the presence at the 8-positon of a phenyl ring substituted at the *para* position with an amino function could permit to link different appendages, in fact as previously described, while small substituents could be appealing for A_{2A} and/or A_3 affinity, whereas bulky substituent could be optimal for A_{2B} activity.

2. Chemistry

The designed compounds **6a-l** have been prepared by one-pot cycloaddition reaction between 8-substituted theophylline and an appropriate hydrazonoyl halide [15,16] as reported in the literature [17]. In fact by reacting at reflux (10 h), in presence of triethylamine, the 8-nitrotheophylline **7** [18–20] with the *N*-(2-bromo-4-nitrophenyl)acetohydrazonoyl bromide **8** [21], the corresponding cycloadduct **6a** was obtained in a good yield [17]. The latter was then reduced with hydrazine in the presence of C/Pd 10% [22] to afford the corresponding amino derivative **6b**, which was reacted in dioxane at room temperature, in the presence of an equivalent amount of triethylamine, with an appropriate acyl chloride **9a-j** to yield the final compounds. (Scheme 1)

Table 1
Activity of synthesized compounds **(6a-j)** at rA_1, rA_{2A}, hA_{2B} and hA_3 adenosine receptor subtypes



6a-l

Compd.	R	rA_1 [a] % displ. (10 M)	RA_{2A} [b] % displ. (10 M)	hA_{2B} [c] EC_{50} (M)	hA_3 [d] % displ. (10 M)
6a	NO_2	<10%	<10%	>100	ND
6b	NH_2	<10%	56 ± 19%	>30	<10%
6c	$NHCOCH_2Ph$	<10%	<10%	>30	<10%
6d	$NHCOCHPh_2$	<10%	<10%	>30	ND
6e	$NHCOCH_3$	<10%	<10%	>100	ND
6f	NHCOPh	<10%	<10%	>100	ND
6g	NHCO-4-OCH_3-Ph	<10%	<10%	>30	<10%
6h	$NHCO(CH_2)_2CH_3$	<10%	<10%	>30	ND
6i	$NHCO(CH_2)_4CH_3$	<10%	<10%	>30	ND
6j	NHCOPh-4-Ph	<10%	<10%	>30	<10%
6k	NHCO-Adamantyl	<10%	<10%	>30	ND
6l	NHCO-2-Thienyl	<10%	<10%	>30	ND

ND = not determined.

[a] Displacement of specific [^{3}H]R-PIA binding (A_1) in rat brain membranes.

[b] Displacement of specific [^{3}H]CGS 21680 binding (A_{2A}) in rat striatal membranes.

[c] Measurement of adenylyl cyclase activity in CHO cells stably transfected with human recombinant A_{2B} adenosine receptor, expressed as EC_{50} (μM).

[d] Displacement of specific [^{125}I]AB-MECA binding at rat A_3 receptors expressed in HEK-293 cells.