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Synthesis and pharmacological characterization of novel xanthine carboxylate amides as A_{2A} adenosine receptor ligands exhibiting bronchospasmolytic activity

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ABSTRACT

The carboxylate amides of 8-phenyl-1,3-dimethylxanthine described herein represent a new series of selective ligands of the adenosine A_{2A} receptors exhibiting bronchospasmolytic activity. The effects of location of 8-phenyl substitutions on the adenosine receptor (AR) binding affinities of the newly synthesized xanthines have also been studied. The compounds displayed moderate to potent binding affinities toward various adenosine receptor subtypes when evaluated through radioligand binding studies. However, most of the compounds showed the maximum affinity for the A_{2A} subtype, some with high selectivity *versus* all other subtypes. Xanthine carboxylate amide **13b** with a diethylaminoethylamino moiety at the *para*-position of the 8-phenylxanthine scaffold was identified as the most potent A_{2A} adenosine receptor ligand with K_i = 0.06 μM. Similarly potent and highly A_{2A}-selective are the isovanillin derivatives **16a** and **16d**. In addition, the newly synthesized xanthine derivatives showed good *in vivo* bronchospasmolytic activity when tested in guinea pigs.

1. Introduction

The development of potent and selective ligands of adenosine receptors (ARs) has been the subject of medicinal chemistry research for more than three decades. The expression and activation of the four adenosine receptor subtypes A_1, A_{2A}, A_{2B}, and A_3 are associated with the control of gene expression, cell growth, intestinal function, neurosecretion, vascular tone and asthma. Therefore all AR subtypes have been considered as potential targets for therapies of neurodegenerative, cardiac, immune and inflammatory disorders [1,2]. Although several adenosine receptor-specific compounds have entered clinical trials and an A_{2A} AR selective agonist Regadenosan® has gained approval from US Food and Drug Administration (FDA), in practice this goal remains elusive even today [3,4]. The possibility of side effects due to the ubiquitous nature of ARs suggests subtype selectivity to be of significance for numerous therapeutic applications [5].

A_{2A} receptor subtype represents a fascinating target for the development of small molecules as antiasthmatic agents as these receptors are expressed in lungs and in inflammatory cells involved in asthma. Therefore selective ligands of this subtype are being widely explored by a large number of research bodies to generate novel therapies for asthma and chronic obstructive pulmonary disease (COPD) [6,7].

Substituted xanthines represent the first potent class of adenosine receptor antagonists reported till date [8]. It has been established that appropriate substitutions as well as location of 8-phenyl substituents affects the potency and selectivity of xanthines toward ARs and thus their pharmacological effects [9,10]. The progressive studies on alkylxanthines led to the development of 8-phenylxanthine carboxylic acid congeners as potent and selective adenosine A_2 receptor antagonists. In particular, the derivative named MRS-1754 (**1**) has emerged as a potent and selective xanthine based A_{2B} adenosine receptor antagonist [11,12] (Fig. 1). Jacobson and co-workers demonstrated the increased affinity of xanthine amides at A_2 receptors with the introduction of an 8-phenyl group functionalized in the *para*-position with a carboxymethoxy chain [13]. However the effect of this moiety on other positions of the 8-phenyl group remains relatively unexplored. In continuation of our earlier investigations [14–16] directed toward finding therapeutically useful molecules through the preparation of substituted xanthines, we considered it worthwhile to synthesize and study additional amide derivatives of xanthine carboxylates as represented by structure **2** in Fig. 1.

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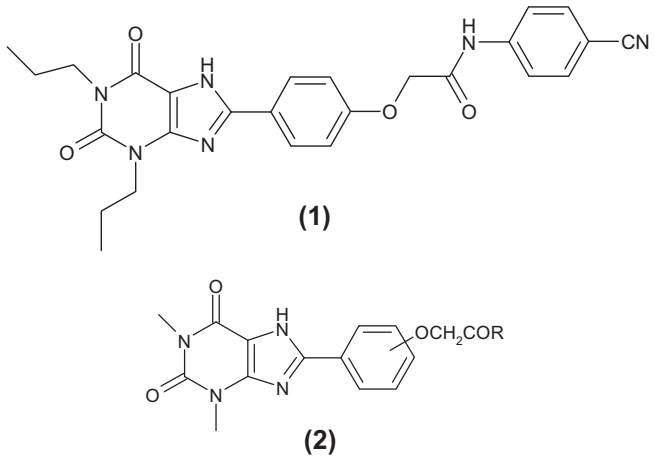

Fig. 1. Structures of potent xanthine based A_{2B} adenosine receptor antagonist MRS-1754 (**1**) and general structure (**2**) of proposed xanthine derivatives.

Taking into consideration the related interesting literature reports, an acetyloxy group linked through a nitrogen atom to a variety of cyclic and acyclic substituents was introduced on the 8-phenyl ring in this series of xanthine derivatives. Previous studies have indicated that suitable selection and positioning of aryl substituents lead to the development of potent and selective xanthine based AR ligands [14]. Shifting of *para* substituents to *meta* position of 8-phenyl ring resulted into xanthines with almost equal affinity for A_1 and A_{2A} subtypes while the former were more selective for A_{2A} receptors. Therefore, the effects of varying the location of 8-phenyl substituent in the current series of xanthine carboxylate amides on the biological properties were also examined. It is also anticipated that coupling of a carboxylic group with various polar groups such as amines may improve the water solubility of xanthines, so one of the series was also selected randomly to evaluate the hydrophobicity of the synthesized compounds [17,18].

The synthesized compounds have been biologically evaluated using radioligand binding to membranes prepared from CHO cells transfected with human A_1, A_{2A}, A_{2B} and A_3 adenosine receptor subtypes [19,20]. *In vivo* experiment using a histamine chamber was also performed to study the bronchospasmolytic activity of the new compounds using theophylline as a standard drug [21].

2. Results and discussion

2.1. Chemistry

5,6-Diaminouracil (**4**), the intermediate used for the preparation of new xanthine derivatives was prepared according to the reported method [22,23]. Synthesis of substituted aldehydes **5–8**, Schiff bases **9–12** and xanthine carboxylate esters **13–16** is presented in Scheme 1. The starting aldehydes such as 4-hydroxybenzaldehyde, 3-hydroxybenzaldehyde, vanillin and isovanillin, possessing a vulnerable *para* or *meta* hydroxy group on the 8-phenyl ring for derivatization, were selected to study the effect of such substitution pattern on affinity and selectivity of xanthine derivatives for various adenosine receptor subtypes. This choice is not only characterized by ease of substitution but also helped to evaluate the impact of various substituting groups in *para* or *meta* positions of the 8-phenyl ring, with or without an *ortho* methoxy group, on adenosine binding affinity and selectivity.

Condensation of various substituted aldehydes **5–8** with 5,6-diaminouracil (**4**) in the presence of MeOH:AcOH (4:1) at room temperature afforded Schiff bases **9–12**, oxidative cyclization of which in refluxing thionyl chloride formed xanthine carboxylates **13–16** as shown in Scheme 1. A singlet integrating for one proton appeared at ~δ 9.7 ppm for N=C*H* in ^{1}H NMR all the benzylidene derivatives **9–12**.

Amide derivatives **13a-i–16a-i** of parent xanthine carboxylate esters **13–16** were prepared according to Scheme 1 by fusing them with appropriate acyclic and cyclic amines. A prominent singlet for —OCH_2— ranging from δ 4.5 to 4.8 and presence of two *N*-methyl of xanthine ring at ~3.40 and ~3.50 ppm were observed in the nuclear magnetic resonance spectra of all the amide derivatives **13a-i–16a-i**. Three *N*-methylenes of —CH_2—N(CH_2CH_3)$_2$ moiety appeared together as a multiplet at δ 2.5–2.6 for diethylamino derivatives **13b–16b**, whereas protons of —CH_2—N of dimethylamino derivatives **13a–16a** resonated separately at ~2.5 ppm. Two *N*-methylenes of the heterocyclic ring for pyrrolidino xanthines **13c–16c** were found a little upfield than those of piperidino derivatives **13d–16d**. The compounds **13–16** were also fused with homoveratrylamine to observe the effect of such functionality on the pharmacological profile of xanthines since this type of moiety form an integral part of a potent and selective A_{2B} adenosine receptor antagonist, MRE-2028F20 [18].

2.2. Partition coefficient

The partition coefficient values of xanthine carboxylate ester **14** and its amidic congeners **14a-i** belonging to 3-hydroxybenzaldehyde series were measured using the shake flask method and an *in silico* (ChemDraw Ultra 8.0.3 and Biotage Path Finder) method [18]. Partition coefficient data (Log PC) of various xanthine derivatives have been compiled in Table 1. In general, the compounds displayed good hydrophobicity except the compounds **14a-b** and **14h**.

There is also a significant correlation between chloroform/phosphate buffer and *n*-octanol/phosphate buffer partition coefficient values of these derivatives ($r^2 = 0.64$; $p < 0.05$) as shown in Fig. 2. The regression line (solid line) was calculated by the least-squares method. The dotted lines indicate 95% confidence limits for the regression line. The three xanthine amides **14c-e**, substituted with 5-, 6-, and 7-membered heterocyclic ring, respectively, exhibited higher partition coefficient values, which indicate their preferential distribution to hydrophobic compartments such as the lipid bilayers of cells. The ideal not too hydrophobic nor too hydrophilic character of the new xanthines predicts their good bioavailability. It is assumed that these compounds might produce good bronchodilating effects as the potency of relaxant effects of xanthines depends on the cell membrane permeability based on their hydrophobic property. The hydrophobicity may also be the major factor for the molecules to bind to the receptors.

2.3. Radioligand binding assays

Table 2 summarizes the observed binding affinities of newly synthesized 8-(carboxymethyloxyphenyl)xanthine derivatives toward the four human adenosine receptor subtypes (A_1, A_{2A}, A_{2B} and A_3). In general, the 8-phenylsubstituted xanthine carboxylate esters **13–16** and their amidic congeners **13a-i–16a-i** displayed moderate to potent binding affinities toward various adenosine receptor subtypes, however, augmented affinity and thus resulting in selectivity was markedly present for the A_{2A} subtype in the majority of the cases. Overall the binding selectivity for A_{2A} is somewhat more pronounced *versus* A_3 receptors (up to >400 fold) as compared to the A_1 (maximally about 60-fold) and A_{2B} (up to >130 fold) subtypes. Monosubstituted 8-phenylxanthine carboxylate esters **13** and **14** with a polar side chain present at *para* or *meta* positions of the 8-phenyl ring, respectively, displayed higher binding affinity for all AR subtypes and more notably for A_{2A} receptors in comparison to disubstituted analogues **15** and **16**, which possess