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Similarities and differences in affinity and binding modes of tricyclic pyrimido- and pyrazinoxanthines at human and rat adenosine receptors

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ABSTRACT

A new series of 32 pyrimido- and 5 tetrahydropyrazino[2,1-*f*]purinediones was obtained and evaluated for their adenosine receptors (ARs) affinities. The 1,3-dibutyl derivative of 9-(4-(2-(dimethylamino) ethoxy)phenyl)-6,7,8,9-tetrahydropyrimido[1,2-*f*]purine-2,4(1*H*,3*H*)-dione was found to be the most potent A_1 AR antagonist of the present series, showing selectivity over the other AR subtypes. The structure–activity for the obtained purinediones was established. Docking experiments of the investigated library to homology models of the human and rat A_1 and A_{2A} ARs allowed to compare the expected binding modes for selected compounds. The detailed analysis of binding cavities within individual AR subtypes indicated small but significant structural variations that may underlie the observed differences in binding affinities of purinediones at particular subtypes and species.

1. Introduction

Methylxanthines, including caffeine, theobromine and theophylline, occur naturally in a number of species of plants belonging to 28 genera and over 17 families, but the most common sources are coffee, tea and cacao.[1] The history of the medicinal use of cacao, both as a primary remedy and as a vehicle to deliver other medicines, dates back to the Olmecs, the first elaborate pre-Columbian civilization of Mesoamerica (1200–400 BCE), where a beverage made from of *Theobroma cacao* beans 'Xocolatl' was highly valued for its stimulating effect and healing properties.[2] Nowadays methylxanthines, therapeutically used as bronchodilators and for other indications, are probably the most widely self-administered psychostimulatory drugs in the world.[1] Natural xanthines (*e.g.* caffeine **1**, Fig. 1A) and their synthetic derivatives exhibit a variety of physiological effects, such as positive inotropic and chronotropic effects on the heart, decreased airway resistance in the lung and respiratory tract, stimulation as well as significant behavioral effects on measures of locomotor activity, schedule-controlled behavior, drug self-administration, and learning and memory. Most of these effects are likely due to the non-selective inhibition of adenosine receptors.[3–5]

Adenosine, an endogenous purine nucleoside, acts as a neuromodulator in both the central and peripheral nervous systems by interacting with the P1 group of purine receptors, belonging to the class A family of G protein-coupled receptors (GPCR). Four adenosine receptor (AR) subtypes, A_1, A_{2A}, A_{2B} and A_3, are known and have been pharmacologically characterized. Activation of the A_1 and A_3 receptors inhibits the production of cyclic AMP via $G_{i/o}$ protein, while A_{2A} and A_{2B} receptor activation stimulates the activity of the adenylate cyclase via G_s protein, inducing an increase in cAMP levels.[6]

The therapeutic potential of adenosine receptors ligands depends on the diverse distribution of ARs throughout the body, both in the central nervous system (CNS) and in peripheral tissues. Thus, subtype-selective AR antagonists have been of interest as potential kidney-protective (A_1 AR), antifibrotic (A_{2A} AR), neuroprotective (A_{2A} AR), antiasthmatic (A_{2B} AR), antiglaucoma (A_3 AR), and anti-cancer (A_{2A}, A_{2B}) drugs.[3,7–10] The A_1 AR-selective

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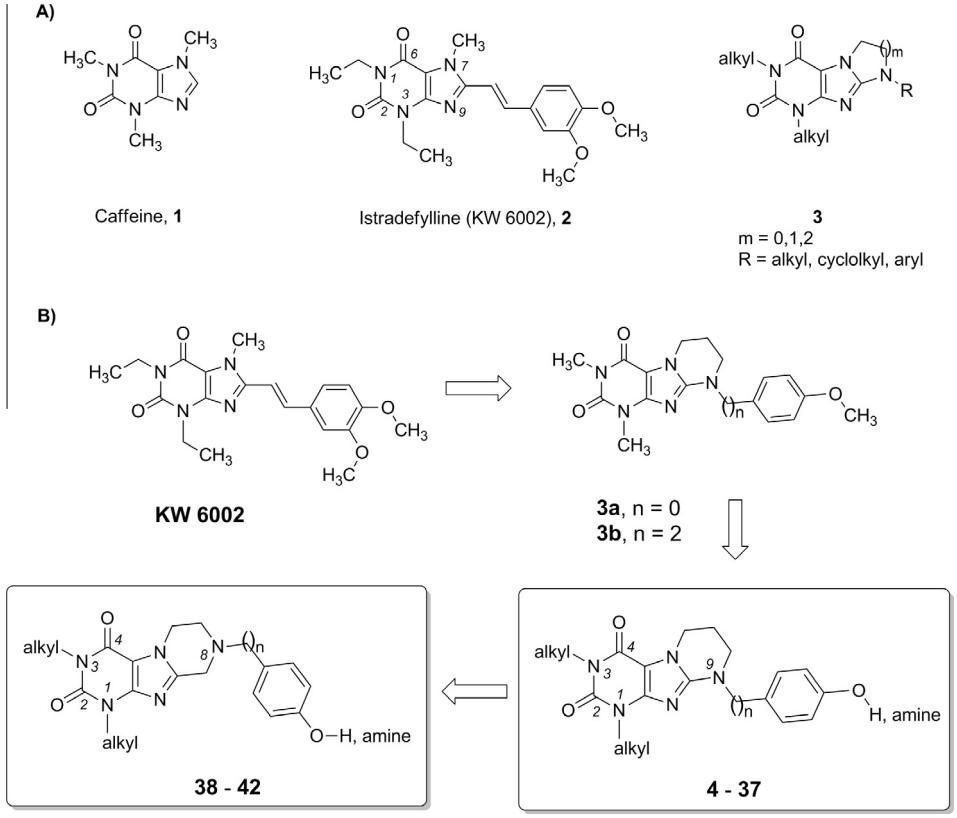

Figure 1. (A) Structures of xanthine-based antagonists of adenosine receptors. (B) Scheme of structural modifications within the current study. Please note that according to the IUPAC system the numbering of atoms for tricyclic compounds is different than for bicyclic xanthine derivatives.

xanthine-derived antagonists tonapofylline and rolofylline have been explored for clinical applications in heart failure, for improving renal function and for the treatment of acute renal failure.[11,12] Among the A_{2A} AR antagonists, the xanthine-based istradefylline (**2**, Fig. 1A) has been evaluated in clinical trials for Parkinson's disease (PD) and depression and has been recently approved in Japan as an antiparkinsonian drug.[13] The blockade of A_{2A} ARs localized in the brain has been proposed not only for the treatment of motor deficits in PD, but also for Alzheimer's disease and Huntington's disease.[14–17]

The structure–activity relationships (SAR) of xanthine-derived AR antagonists, including polycyclic fused ring systems, at various adenosine receptors subtypes has been intensively studied by our groups as well as others.[18–24] In general, modification of the xanthine scaffold at the 8-position with aryl, alkylaryl or cycloalkyl groups increases affinity at the A_1 AR or may result in high affinity for A_{2B} AR. Introducing an alkene spacer into this position connected to an aromatic ring, e.g., the styryl residue, typically leads to increased potency and selectivity for the A_{2A} AR (e.g., KW6002).[24–26] A separate class of xanthines with modified 8-position is represented by tricyclic compounds containing a third ring fused to the *f*-bond of the 2,6-purinedione system. As previously reported by our groups, tricyclic dihydroimidazo-, tetrahydropyrimido- and tetrahydrodiazepino[1,2-*f*]purinediones can act as relatively potent A_{2A} AR (or A_{2A}/A_1 AR) antagonists, in which the annelated pyrimidine ring appears to be beneficial for activity (**3**, Fig. 1A).[18–22] From a structural point of view, these compounds can be treated as sterically fixed and configurationally stable analogs of (*E*)-8-styrylxanthines, the scaffold that is contributing to the high A_{2A} AR affinity of istradefylline. Unfortunately, the drawback of this class of xanthine derivatives is their low water-solubility leading to poor bioavailability.[24]

In the present project we focused on a new series of tricyclic 9-aryl/arylethyl-tetrahydropyrimido[1,2-*f*]purine-2,4-diones. As a starting point for modifications two previously described compounds were chosen, with their constrained structure mimicking the structure of (*E*)-8-styrylxanthines previously developed as A_{2A} AR ligands (Fig. 1B): **3a** (A_{2A} AR antagonist)[18] and **3b** (dual A_{2A} and A_1 AR antagonist).[19] The primary goal of this work was to increase the affinity and selectivity for either A_{2A} or A_1 ARs and to overcome the problem of low solubility.

For these purposes the following structural changes have been introduced:

(1) In addition to the two previously reported hydroxyphenyl derivatives **4** and **8** included in this paper as reference compounds,[18,19] six new 4-hydroxyphenyl compounds were synthesized and evaluated (Tables 1–3);
(2) An aliphatic tertiary amino group which increases the basicity and solubility of the parent compound has been introduced at the alkoxy chain attached to the benzene ring. Dimethylamine, diethylamine, pyrrolidine, piperidine and morpholine derivatives were prepared (Tables 1 and 2);
(3) The tetrahydropyrimidine ring was replaced by a tetrahydropyrazine ring; this regioisomeric change shifts the *N*-arylalkylamino substituent from the 9- to the 8-position (Table 3). The *N*8 nitrogen atom is expected to exhibit increased basicity due to its increased aliphatic character, compared to the *N*9 atom.[27]

For all synthesized tricyclic xanthines their affinity to native rat A_1 (rA_1) ARs and A_{2A} (rA_{2A}) ARs as well as to human recombinant A_{2B} (hA_{2B}) ARs and A_3 (hA_3)ARs was determined in radioligand binding assays. Selected compounds were further evaluated for