Acyl-Hydrazide Derivatives of a Xanthine Carboxylic Congener (XCC) as Selective Antagonists at Human $A_{2B}$ Adenosine Receptors

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Abstract

The structure–activity relationships (SAR) of 8-phenyl-1,3-dipropylxanthine derivatives in binding to recombinant human $A_{2B}$ adenosine receptors were explored, in order to identify selective antagonists. Based on the finding of receptor selectivity in MRS 1204, containing an N-hydroxysuccinimide ester attached through the $p$-position of the 8-phenyl substituent [Jacobson et al. (1999): Drug Dev. Res., 47:45–53], a hydrazide and its more stable imide derivatives were synthesized. The hydrazide of XCC (8-[4-[[carboxy]methyl]oxy]phenyl]-1,3-dipropylxanthine) was acylated with a variety of mono- and dicarboxylic acids. $K_i$ values were determined in the adenosine receptor binding assays. At recombinant human $A_{2B}$ receptors expressed in membranes of HEK-293 cells, antagonist radioligands used were the xanthine $125^I$-ABOPX ($125^I$-3-(4-amino-3-iodobenzyl)-8-oxyacetate-1-propyl-xanthine) and the nonxanthine antagonist $^3H$ZM 241385 ($^3H$4-(2-[7-amino-2-{furyl}{1,2,4}triazolo{2,3-a}{1,3,5}triazin-5-ylamino-ethyl)phenol). The initial screening utilized rat $A_1/A_2A$ receptors and human $A_3$ receptors, and selected compounds were examined at the human $A_1/A_2A$ subtypes. A 1,2-dimethylmaleimide derivative, 14 (MRS 1595), bound to human $A_{2B}$ receptors with a $K_i$ of 19 nM and proved to be selective vs. human $A_1/A_2A/A_3$ receptors by 160-, 100-, and 35-fold, respectively. Enprofylline (3-propylxanthine) is slightly selective for $A_{2B}$ receptors, suggesting removal of the 1-propyl group; however, combination of the 1-H-3-Pr and 8-phenyl substituents eliminated the selectivity. Other potent and moderately selective $A_{2B}$ antagonists were a tetrahydrophthaloyl derivative 18b (MRS 1614, $K_i$ value 10 nM) and amino acid conjugates of the XCC-hydrazide, i.e., the glutarimide 24b (MRS 1626, $K_i$ value 13 nM), and protected dipeptide 27 (MRS 1615, $K_i$ value 11 nM). Drug Dev. Res. 47:178–188, 1999.

Graphical Abstract

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INTRODUCTION

Adenosine receptors [Linden and Jacobson, 1998] constitute four members of the G protein-coupled receptor superfamily, have structure–function homology to the biogenic amine receptors [Jiang et al., 1997], and are widely distributed in the body. Adenosine is a local modulator in the cardiovascular, renal, and immune systems and in the central nervous system. The A_{2B} adenosine receptor [Daly et al., 1983; see review by Feoktistov and Biaggioni, 1997] is involved in the control of cell growth and gene expression [Neary et al., 1996], vasodilation [Martin et al., 1993], and fluid secretion from intestinal epithelia [Strohmeier et al., 1995].

A selective A_{2B} receptor antagonist may have potential use as an antiasthmatic agent [Feoktistov and Biaggioni, 1997]. A possible role for A_{2B}ARs in asthma is consistent with the therapeutic efficacy of enprofylline, 1, and theophylline, 2, in treating asthma. In radioligand binding assays, both of these xanthines were confirmed to be effective, although not very potent, antagonists of human A_{2B}ARs in the therapeutic dose range [Jacobson et al., 1999]. Furthermore, enprofylline, with a K_i value of 7 μM, even appears to be somewhat selective for human A_{2B}ARs [Robeva et al., 1996b]. A_{2B}ARs are expressed in some mast cells, such as canine BR mastocytoma cells, in which they appear to be responsible for triggering acute Ca^{2+} mobilization and degranulation [Auchampach et al., 1997]. A_{2B}ARs also participate in a delayed IL8 release from human HMC-1 mast cells [Feoktistov et al., 1999]. The A_3AR may also play a role in asthma, since it mediates the degranulation of rat RBL mast-like cells [Ramkumar et al., 1993] and is present in high density in human blood eosinophils [Kohno et al., 1996].

Although adenosine receptor subtype-selective probes are available for the A_1, A_{2A}, and A_3 adenosine receptors [Jacobson and van Rhee, 1997], very few selective antagonists and agonists are known for the A_{2B} receptor, in part because the absence of radioligand binding assays has precluded a detailed investigation of the SAR at this subtype. MRS 1224, 7b, a derivative of the triazoloquinazoline, CGS15943, 7a, was highly potent at the A_{2B} receptor [Kim et al., 1998]. Although selective for the A_{2A} receptor, the triazolotriazine ZM 241385 was also shown to be a potent antagonist at the A_{2B} receptor and useful as a radioligand in cells expressing the recombinant A_{2B} receptor [Ji and Jacobson, 1999]. Alloxazine, 6, [Brackett and Daly, 1994] has been reported to be approximately one order of magnitude selective as antagonists at the A_{2B} receptor vs. other subtypes. Among xanthines, an 8-
phenyl group is associated with increased affinity at A\textsubscript{2B} receptors. The 8-phenyl analog, 3, of theophylline, 2, displayed a 22-fold enhancement of affinity at A\textsubscript{2B} receptors [Jacobson et al., 1999]. A lead for achieving moderate selectivity (at least 20-fold vs. A\textsubscript{1}, A\textsubscript{2A}, and A\textsubscript{3} adenosine receptors) have been found in the category of complex 8-phenylxanthine derivatives. 8-[4-[[Carboxy]methyl]oxy]phenyl]-1,3-dipropylxanthine (XCC), 4a, and its ethyl ester, 4b, displayed high affinity for the A\textsubscript{2B} receptor. Moreover, MRS 1204 (N-hydroxysuccinimide ester of XCC), 4d, displayed moderate selectivity (at approximately 20-fold for human A\textsubscript{2B} receptors [Jacobson et al., 1999] vs. A\textsubscript{1}, A\textsubscript{2A}, and A\textsubscript{3} adenosine receptors).

As an approach to finding selective antagonists for the A\textsubscript{2B} receptor, we synthesized novel 8-phenyl-1,3-dialkylxanthines related structurally to 4d, in most of which the active ester bond has been replaced by a more stable acyl-hydrazide bond, and screened them for receptor affinity and selectivity in binding to the recombinant human A\textsubscript{2B} receptor and other adenosine receptor subtypes. In order to identify potent adenosine receptor subtype-selective antagonists, in this study we utilized radioligand binding assays based on the use of membranes derived from HEK-293 cells that overexpress recombinant human A\textsubscript{2B}ARs.

**MATERIALS AND METHODS**

**Materials**

The starting compounds, 4c and 4b, were prepared according to Jacobson et al. [1985]. NECA, XAC, and 2-chloroadenosine were purchased from Research Biochemicals International (Natick, MA). All reagents were obtained from Aldrich (Milwaukee, WI) and Sigma (St. Louis, MO).

**Synthesis**

Proton nuclear magnetic resonance spectroscopy was performed on a Varian GEMINI-300 spectrometer and spectra were taken in DMSO-\textsubscript{d}\textsubscript{6} or CDCl\textsubscript{3}. Unless noted, chemical shifts are expressed as ppm downfield from tetramethylsilane, or relative ppm from DMSO (2.5 ppm). Chemical-ionization (CI) mass spectrometry was performed with a Finnigan 4600 mass spectrometer, and Electron-impact (EI) mass spectrometry with a VG7070F mass spectrometer at 6 kV. FAB (fast atom bombardment) mass spectrometry was performed with a JEOL SX102 spectrometer using 6-kV Xe atoms. All xanthine derivatives tested in binding assays were shown to be homogeneous by TLC (MK6F silica, 0.25 mm, glass-backed; Whatman Inc., Clifton, NJ). NMR and mass spectra were shown to be consistent with the assigned structure.

**General Procedure for the Preparation of Xanthine Hydrazide Derivatives**

**Carboxyalkyl amide derivatives**—A mixture of 4c (10 mg, 0.025 mmol) and two equivalents of anhydride were stirred in 1 mL of DMF for 6–24 h. The reaction mixture was concentrated to dryness and the residue was purified on preparative TLC (CHCl\textsubscript{3}: MeOH = 10:1) to give the corresponding carboxyalkylamide derivative as a white solid with 40–70% yield (compounds 4e, 9, 18a, 19a and 20a).
Cyclic imide derivatives—A mixture of 4c (10 mg, 0.025 mmol), 1.5–2.0 equivalents of anhydride, and one equivalent of DIPEA were stirred in 1 mL of DMF at room temperature. When the starting material 4c disappeared as judged by TLC, a mixture of 2–3 equivalents of HOBt, EDAC, and DIPEA dissolved in 0.5 mL of DMF was added and the mixture was stirred at room temperature or at 50°C for 6–24 h. The reaction mixture was concentrated to dryness and the residue was purified on preparative TLC (CHCl₃: MeOH = 10:1) to give the cyclic imide derivative as a white solid, 40–70% yield (compounds 10, 11, 12, 13, 14, 15, 16, 17, 18b, 19b, 20b, 21, 22, 23).

Coupling with activated N-protected amino acids—A mixture of 4c (10 mg, 0.025 mmol), 1.5–2.0 equivalents of activated (hydroxy-succinimide or 4-nitrophenyl ester) N-protected amino acid and one equivalent of DIPEA and DMAP was stirred in 1 mL of DMF at 25–50°C for 8–24 h. The reaction mixture was concentrated to dryness and the residue was purified on preparative TLC (CHCl₃: MeOH = 10:1) to give the product as a white solid, 40–70% yield (compounds 25, 26, and 27).

8-[4-[(Carboxymethyl)oxy]phenyl]-1,3-di-(n-propyl)xanthine N-Acetylhydrazide (4e): ^1H NMR (DMSO-d₆). 0.89 (2t, 6H, J = 7.8 Hz, 2x-CH₃), 1.58 and 1.74 (2m, 4H, 2x-CH₂-), 1.88 (s, 3H, CH₃CO-), 3.87 and 4.02 (2t, 4H, J = 6.8 Hz, 2x-NCH₂-), 4.68 (s, 2H, -OCH₂-), 7.11 (d, 2H, J = 8.8 Hz, Ar), 8.08 (d, 2H, J = 8.8 Hz, Ar); MS-FAB (M + H⁺) 443.

8-[4-[(Carboxymethyl)oxy]phenyl]-1,3-di-(n-propyl)xanthine N,N'-Succinylhydrazide (10): ^1H NMR (DMSO-d₆). 0.89 (2t, 6H, J = 7.8 Hz, 2x-CH₃), 1.59 and 1.73 (2m, 4H, 2x-CH₂-), 2.81 (s, 4H, CH₂CH₂), 3.87 and 4.03 (2t, 4H, J = 6.8 Hz, 2x-NCH₂-), 4.85 (s, 2H, -OCH₂-), 7.15 (d, 2H, J = 8.8 Hz, Ar), 8.10 (d, 2H, J = 8.8 Hz, Ar); MS-FAB (M + H⁺) 483.

8-[4-[(Carboxymethyl)oxy]phenyl]-1,3-di-(n-propyl)xanthine N,N'-[(2S)-Trifluoroacetamido]- succinyl]hydrazide (11): ^1H NMR (DMSO-d₆). 0.89 (2t, 6H, J = 7.8 Hz, 2x-CH₃), 1.58 and 1.74 (2m, 4H, 2x-CH₂-), 2.70–2.90 (m, 2H, -CH₂-), 2.3–2.5 and 2.8–3.1 (m, 5H, -CH- and 2x-CH₂-), 4.04 and 4.12 (2t, 4H, J = 6.8 Hz, 2x-NCH₂-), 4.69 (s, 2H, -OCH₂-), 4.95 (s, 1H, -CH-), 7.15 (d, 2H, J = 8.8 Hz, Ar), 8.10 (d, 2H, J = 8.8 Hz, Ar); MS-FAB (M + H⁺) 594.

8-[4-[(Carboxymethyl)oxy]phenyl]-1,3-di-(n-propyl)xanthine N,N'-[(2-Phenyl)glutaryl]hydrazide (12): ^1H NMR (CDCl₃). 1.05 (2t, 6H, J = 7.8 Hz, 2x-CH₃), 1.75 and 1.90 (2m, 4H, 2x-CH₂-), 2.3–2.5 and 2.8–3.1 (m, 5H, -CH- and 2x-CH₂-), 4.04 and 4.12 (2t, 4H, J = 6.8 Hz, 2x-NCH₂-), 4.70–4.90 (m, 2H, -OCH₂-), 6.6 (d, 2H, J = 8.8 Hz, Ar), 7.08 (m, 2H, -Ph), 7.43 (m, 5H, -Ph and Ar); MS-FAB (M + H⁺) 573.

8-[4-[(Carboxymethyl)oxy]phenyl]-1,3-di-(n-propyl)xanthine N,N'-Citraconylhydrazide (13): ^1H NMR (DMSO-d₆). 0.89 (2t, 6H, J = 7.8 Hz, 2x-CH₃), 1.59 and 1.73 (2m, 4H,
2x-CH$_2$), 2.07 (s, 3H, CH$_3$), 3.87 and 4.03 (2t, 4H, J = 6.8 Hz, 2x-NCH$_2$), 4.86 (s, 2H, -OCH$_2$), 6.83 (s, 1H =CH-), 7.15 (d, 2H, J = 8.8 Hz, Ar), 8.10 (d, 2H, J = 8.8 Hz, Ar); MS-FAB (M + H$^+$) 495.

8-[4-[Carboxy(methyl)oxy]phenyl]-1,3-di-($n$-propyl)xanthine N,N-{[(1,2-Dimethyl)maleyl]hydrazide (14):;} $^1$H NMR (DMSO-d$_6$). 0.89 (2t, 6H, J = 7.8 Hz, 2x-CH$_3$), 1.58 and 1.74 (2m, 4H, 2x-CH$_2$), 1.97 (s, 6H, 2x-CH$_3$), 3.87 and 4.03 (2t, 4H, J = 6.8 Hz, 2x-NCH$_2$), 4.86 (s, 2H, -OCH$_2$), 7.14 (d, 2H, J = 8.8 Hz, Ar), 8.10 (d, 2H, J = 8.8 Hz, Ar); MS-FAB (M + H$^+$) 509.

8-[4-[Carboxy(methyl)oxy]phenyl]-1H-3-($n$-propyl)xanthine N,N-{[(1,2-Dimethyl)maleyl]hydrazide (15):;} $^1$H NMR (DMSO-d$_6$). 0.91 (t, 3H, J = 7.8 Hz, 2x-CH$_3$), 1.73 (m, 2H, 2x-CH$_2$), 1.97 (s, 6H, 2x-CH$_3$), 3.96 (t, 2H, J = 6.8 Hz, 2x-NCH$_2$), 4.85 (s, 2H, -OCH$_2$), 7.14 (d, 2H, J = 8.8 Hz, Ar), 8.09 (d, 2H, J = 8.8 Hz, Ar); MS-EI (M$^+$) 509, calculated for C$_{22}$H$_{22}$N$_6$O$_6$ 466.1601; found 466.1580.

8-[4-[Carboxy(methyl)oxy]phenyl]-1,3-di-($n$-propyl)xanthine N,N-{[(2-Phenyl)maleyl]hydrazide (16):;} $^1$H NMR (DMSO-d$_6$). 0.89 (2t, 6H, J = 7.8 Hz, 2x-CH$_3$), 1.59 and 1.73 (2m, 4H, 2x-CH$_2$), 3.87 and 4.03 (2t, 4H, J = 6.8 Hz, 2x-NCH$_2$), 4.91 (s, 2H, -OCH$_2$), 7.15 (d, 2H, J = 8.8 Hz, Ar), 7.51 (s, 1H =CH-), 7.55–7.57 (m, 3H, -Ph), 8.04–8.06 (m, 2H, -Ph), 8.11 (d, 2H, J = 8.8 Hz, Ar); MS-FAB (M + H$^+$) 557.

8-[4-[Carboxy(methyl)oxy]phenyl]-1,3-di-($n$-propyl)xanthine N,N-{[(1,2-Diphenyl)maleyl]hydrazide (17):;} $^1$H NMR (DMSO-d$_6$). 0.89 (2t, 6H, J = 7.8 Hz, 2x-CH$_3$), 1.59 and 1.73 (2m, 4H, 2x-CH$_2$), 2.30–2.50 (m, 4H, 2x-CH$_2$), 2.80–2.95 (m, 2H, 2x-CH), 3.83 and 3.90 (2t, 4H, J = 6.8 Hz, 2x-NCH$_2$), 4.66 (s, 2H, -OCH$_2$), 5.63 (s, 2H, 2x =CH-), 7.09 (d, 2H, J = 8.8 Hz, Ar), 8.06 (d, 2H, J = 8.8 Hz, Ar); MS-FAB (M + H$^+$) 633.

8-[4-[Carboxy(methyl)oxy]phenyl]-1,3-di-($n$-propyl)xanthine N-[2-((1-Carboxy)-cis-4-cyclohexene)-carbonyl]hydrazide (18a):;} $^1$H NMR (DMSO-d$_6$). 0.89 (2t, 6H, J = 7.8 Hz, 2x-CH$_3$), 1.87 (m, 2H, -CH$_2$), 2.70 (m, 4H, 2x-CH$_2$), 3.56 (m, 2H, 2x-CH), 3.83 and 3.90 (2t, 4H, J = 6.8 Hz, 2x-NCH$_2$), 4.66 (s, 2H, -OCH$_2$), 5.89 (s, 2H, 2x =CH-), 7.09 (d, 2H, J = 8.8 Hz, Ar), 8.06 (d, 2H, J = 8.8 Hz, Ar); MS-FAB (M + H$^+$) 535.

8-[4-[Carboxy(methyl)oxy]phenyl]-1,3-di-($n$-propyl)xanthine N-[2-((1-Carboxy)-1-cyclopentene)-carbonyl]hydrazide (19a):;} $^1$H NMR (DMSO-d$_6$). 0.89 (2t, 6H, J = 7.8 Hz, 2x-CH$_3$), 1.87 (m, 2H, -CH$_2$), 2.70 (m, 4H, 2x-CH$_2$), 3.56 (m, 2H, 2x-CH), 3.83 and 3.90 (2t, 4H, J = 6.8 Hz, 2x-NCH$_2$), 4.71 (s, 2H, -OCH$_2$), 7.09 (d, 2H, J = 8.8 Hz, Ar), 8.06 (d, 2H, J = 8.8 Hz, Ar); MS-FAB (M + H$^+$) 539.
8-[4-[(Carboxymethyl)oxy]phenyl]-1,3-di-(n-propyl)xanthine N,N-(1-Cyclopentene-1,2-dicarbonyl)-hydrazide (19b): 1H NMR (DMSO-d6). 0.89 (2t, 6H, J = 7.8 Hz, 2x-CH\textsubscript{3}), 1.58 and 1.74 (2m, 4H, 2x-CH\textsubscript{2}-), 2.40 (m, 2H, -CH\textsubscript{2}-), 2.67 (4H, m, 2x-CH\textsubscript{2}-), 3.81 and 3.98 (2t, 4H, J = 6.8 Hz, 2x-NCH\textsubscript{2}-), 4.85 (s, 2H, -OCH\textsubscript{2}-), 7.15 (d, 2H, J = 8.8 Hz, Ar), 8.1 (d, 2H, J = 8.8 Hz, Ar); MS-FAB (M + H\textsuperscript{+}) 521.

8-[4-[(Carboxymethyl)oxy]phenyl]-1,3-di-(n-propyl)xanthine N-[2-((1-Carboxy)-1-cyclohexene)-carbonyl]hydrazide (20a): 1H NMR (DMSO-d6). 0.89 (2t, 6H, J = 7.8 Hz, 2x-CH\textsubscript{3}), 1.59 (m, 6H, 3x-CH\textsubscript{2}-), 1.74 (m, 2H, -CH\textsubscript{2}-), 2.27 (m, 4H, 2x-CH\textsubscript{2}-), 3.87 and 4.02 (2t, 4H, J = 6.8 Hz, 2x-NCH\textsubscript{2}-), 4.68 (s, 2H, -OCH\textsubscript{2}-), 7.09 (d, 2H, J = 8.8 Hz, Ar), 8.06 (d, 2H, J = 8.8 Hz, Ar); MS-FAB (M + H\textsuperscript{+}) 553.

8-[4-[(Carboxymethyl)oxy]phenyl]-1,3-di-(n-propyl)xanthine N,N-(3,4,5,6-Tetrahydrophthaloyl)-hydrazide (20b): 1H NMR (DMSO-d6). 0.89 (2t, 6H, J = 7.8 Hz, 2x-CH\textsubscript{3}), 1.58 (m, 2H, -CH\textsubscript{2}-), 1.72 (m, 6H, 3x-CH\textsubscript{2}-), 2.30 (m, 4H, 2x-CH\textsubscript{2}-), 3.83 and 3.90 (2t, 4H, J = 6.8 Hz, 2x-NCH\textsubscript{2}-), 4.86 (s, 2H, -OCH\textsubscript{2}-), 7.15 (d, 2H, J = 8.8 Hz, Ar), 8.12 (d, 2H, J = 8.8 Hz, Ar); MS-FAB (M + H\textsuperscript{+}) 535.

8-[4-[(Carboxymethyl)oxy]phenyl]-1,3-di-(n-propyl)xanthine N,N-Phthaloylhydrazide (21): 1H NMR (DMSO-d6). 0.89 (2t, 6H, J = 7.8 Hz, 2x-CH\textsubscript{3}), 1.58 and 1.74 (2m, 4H, 2x-CH\textsubscript{2}-), 3.87 and 4.02 (2t, 4H, J = 6.8 Hz, 2x-NCH\textsubscript{2}-), 4.75 (s, 2H, -OCH\textsubscript{2}-), 7.14 (d, 2H, J = 8.8 Hz, Ar), 7.57 (m, 4H, Ar), 8.09 (d, 2H, J = 8.8 Hz, Ar); MS-FAB (M + H\textsuperscript{+}) 531.

8-[4-[(Carboxymethyl)oxy]phenyl]-1,3-di-(n-propyl)xanthine N,N-Glutarylhydrazide (22): 1H NMR (CDCl\textsubscript{3}). 1.05 (2t, 6H, J = 7.8 Hz, 2x-CH\textsubscript{3}), 1.75 and 1.90 (2m, 4H, 2x-CH\textsubscript{2}-), 2.10–2.30 (m, 2H, -CH\textsubscript{2}-), 2.80–3.10 (m, 4H, 2x-CH\textsubscript{2}-), 4.05 and 4.16 (2t, 4H, J = 6.8 Hz, 2x-NCH\textsubscript{2}-), 4.80 (s, 2H, -OCH\textsubscript{2}-), 6.75 (d, 2H, J = 8.8 Hz, Ar), 7.70 (d, 2H, J = 8.8 Hz, Ar); MS-FAB (M + H\textsuperscript{+}) 497.

8-[4-[(Carboxymethyl)oxy]phenyl]-1,3-di-(n-propyl)xanthine N,N-(3-Hydroxy)glutarylhydrazide (23): 1H NMR (DMSO-d6). 0.89 (2t, 6H, J = 7.8 Hz, 2x-CH\textsubscript{3}), 1.59 and 1.73 (2m, 4H, 2x-CH\textsubscript{2}-), 2.70–3.10 (m, 4H, 2x-CH\textsubscript{2}-), 3.87 and 4.03 (2t, 4H, J = 6.8 Hz, -NCH\textsubscript{2}-), 4.21 (bs, 1H, -CHOH-), 4.77 (s, 2H, -OCH\textsubscript{2}-), 7.15 (d, 2H, J = 8.8 Hz, Ar), 8.1 (d, 2H, J = 8.8 Hz, Ar); MS-FAB (M + H\textsuperscript{+}) 513.

8-[4-[(Carboxymethyl)oxy]phenyl]-1,3-di-(n-propyl)xanthine N-(4-Carboxy-(2S)-Trifluoroacetamido)-n-butanoil)hydrazide (24a): A mixture of 4c (10 mg, 0.025 mmol), 7.6 mg of L-N-Boc-glutamic acid 5-tert-butyl ester (0.025 mmole), 7 mg of HOBT (0.05 mmole), 19 mg of DIPEA (0.15 mmole), and 15 mg of EDAC (0.078 mmole) in 1 mL of dry DMF was stirred for 8 h at 25°C. DMF was removed by nitrogen stream and the residue was washed with 1 mL of 1 M NaHCO\textsubscript{3} solution and dried overnight. The crude product was suspended in 0.5 mL of CHCl\textsubscript{3} and 0.5 mL of TFA was added. After 30 min stirring at 25°C, the mixture was concentrated to dryness and dried under high vacuum. The residue was dissolved in 0.5 mL of TFAA and the solution was stirred for 30 min at 25°C. The reaction mixture was concentrated to dryness and the residue was purified on preparative TLC (CHCl\textsubscript{3}: MeOH = 10:1) to give 6 mg of 24a as a white solid (yield 40%). 1H NMR
(DMSO-d$_6$). 0.89 (2t, 6H, $J = 7.8$ Hz, 2x-CH$_3$), 1.59 and 1.73 (2m, 4H, -CH$_2$-), 1.90–2.30 (m, 4H, 2x-CH$_2$), 3.87 and 4.02 (2t, 4H, $J = 6.8$ Hz, 2x-NCH$_2$-), 4.12 (m, 1H, -CH), 4.68 (s, 2H, -OCH$_2$-), 7.08 (d, 2H, $J = 8.8$ Hz, Ar), 8.06 (d, 2H, $J = 8.8$ Hz, Ar); MS-FAB (M+H$^+$) 626.

**8-[4-[[Carboxymethyl]oxy]phenyl]-1,3-di-(n-propyl)xanthine N,N-((2S)-Trifluoroacetamido)-glutaryl]hydrazide (24b).** A mixture of 24a (10 mg, 0.016 mmol), 7 mg of HOBt (0.05 mmole), 19 mg of DIPEA (0.15 mmole), and 15 mg of EDAC (0.078 mmole) in 1 mL of dry DMF was stirred overnight at 25°C. The reaction mixture was concentrated to dryness and the residue was purified on preparative TLC (CHCl$_3$:MeOH=10:1) to give 5 mg of 24b as a white solid (yield 53%).

**1H NMR (DMSO-d$_6$).** 0.89 (2t, 6H, $J = 7.8$ Hz, 2x-CH$_3$), 1.59 and 1.73 (2m, 4H, 2x-CH$_2$-), 1.90–2.30 (m, 4H, 2x-CH$_2$-), 3.87 and 4.02 (2t, 4H, $J = 6.8$ Hz, 2x-NCH$_2$-), 4.81 (s, 2H, -OCH$_2$-), 4.18 (m, 1H, -CH), 7.15 (d, 2H, $J = 8.8$ Hz, Ar), 8.1 (d, 2H, $J = 8.8$ Hz, Ar); MS-FAB (M+H$^+$) 608.

**8-[4-[[Carboxymethyl]oxy]phenyl]-1,3-di-(n-propyl)xanthine N,N-([N-tert-Butoxycarbonyl-L-leucinyl]-hydrazide (25).**

**1H NMR (DMSO-d$_6$).** 0.89 (m, 13H, 2x-CH$_3$ and (CH$_3$)$_2$CH-), 1.35 (s, 9H, Boc), 1.42 (m, 2H, -CH$_2$-), 1.58 and 1.74 (2m, 4H, 2x-CH$_2$-), 3.85 and 4.0 (2t, 4H, $J = 6.8$ Hz, 2x-NCH$_2$-), 4.12 (m, 1H, -CH), 4.64 (s, 2H, -OCH$_2$-), 7.06 (d, 2H, $J = 8.8$ Hz, Ar), 8.05 (d, 2H, $J = 8.8$ Hz, Ar); MS-FAB (M+H$^+$) 614.

**8-[4-[[Carboxymethyl]oxy]phenyl]-1,3-di-(n-propyl)xanthine N,N-([N-tert-Butoxycarbonyl-L-methionyl]-hydrazide (26).**

**1H NMR (DMSO-d$_6$).** 0.89 (2t, 6H, $J = 7.8$ Hz, 2x-CH$_3$), 1.25 (m, 2H, -CH$_2$-), 1.37 (s, 9H, Boc), 1.58 and 1.74 (2m, 4H, 2x-CH$_2$-), 1.88 (m, 2H, -CH$_2$-), 2.03 (s, 3H, -SCH$_3$), 3.81 and 3.98 (2t, 4H, $J = 6.8$ Hz, 2x-NCH$_2$-), 4.15 (m, 1H, -CH), 4.68 (s, 2H, -OCH$_2$-), 7.03 (d, 2H, $J = 8.8$ Hz, Ar), 8.03 (d, 2H, $J = 8.8$ Hz, Ar); MS-FAB (M+H$^+$) 632.

**8-[4-[[Carboxymethyl]oxy]phenyl]-1,3-di-(n-propyl)xanthine N,N-([N-Benzylxocarbonyl-glycylglycinyl]hydrazide (27).**

**1H NMR (DMSO-d$_6$).** 0.89 (2t, 6H, $J = 7.8$ Hz, 2x-CH$_3$), 1.58 and 1.74 (2m, 4H, 2x-CH$_2$-), 3.67 (m, 1H, -CH$_2$- in glycine), 3.81 (m, 3H, -NCH$_2$- and -CH$_2$- in glycine), 3.98 (t, 2H, $J = 6.8$ Hz, -NCH$_2$-), 4.64 (s, 2H, -OCH$_2$-), 5.03 (s, 2H, -OCH$_2$-Ph), 7.03 (d, 2H, $J = 8.8$ Hz, Ar), 7.3–7.5 (m, 5H, -Ph), 8.03 (d, 2H, $J = 8.8$ Hz, Ar); MS-FAB (M+H$^+$) 649.

**8-[4-[[Carboxymethyl]oxy]phenyl]-1H-3-(n-propyl)xanthine methyl ester (36).** To a suspension of 3.2 g of 32 [Papesch and Schroeder, 1951] (18.9 mmole), 1.5 mL of glacial acetic acid and 3.4 mL of 6 N HCl in 50 mL of water was added dropwise to a solution of 1.38 g of sodium nitrite (20 mmole) in 5 mL of water at 0°C. The mixture was stirred for 1 h and the pink precipitate was collected by filtration to give 3.17 g of 33 (yield 78%).

**1H NMR (DMSO-d$_6$).** 0.87 (t, 3H, $J = 7.8$ Hz, -CH$_3$), 1.51 (m, 2H, -CH$_2$-), 3.72 (t, 2H, $J = 6.8$ Hz, -NCH$_2$-), 9.12 (s, 1H, -NH$_2$), 0.086 g of 33 (0.4 mmole) was hydrogenated with 10% Pd/C in 5 mL of MeOH under H$_2$ atmosphere (1 atm) at 25°C until the pink color disappeared (30 min). After the removal of the balloon of H$_2$, 5 mL of DMF was added and the mixture was stirred for 10 min and filtered through a Celite bed. To the solution
of crude 34 was added 0.078 g of methyl 4-formylphenyloxyacetate (0.4 mmole) and 0.5 mL of acetic acid. The mixture was heated at 50°C for 30 min, evaporated under reduced pressure, and suspended with 20 mL of ether. The yellow precipitate (mixture of 35 and 36) was collected by filtration, dissolved in 5 mL of DMF, and treated with 1 mL of aqueous solution of 0.085 g of sodium periodate (0.4 mmole) for 2 h. After evaporation, the product was purified by crystallization in MeOH/H2O to give 0.048 g of 36 (yield 34%). 1H NMR (DMSO-d6). 0.90 (t, 3H, J = 7.8 Hz, -CH3), 1.72 (m, 2H, -CH2-), 3.71 (s, 3H, -OCH3), 3.95 (t, 2H, J = 6.8 Hz, -NCH2-), 4.89 (s, 2H, -OCH2-), 7.08 (d, 2H, J = 8.8 Hz, Ar), 8.05 (d, 2H, J = 8.8 Hz, Ar), 11.07 (s, 1H, -NH); MS-EI (M+) 358, calculated for C17H18N4O5 358.1277; found 358.1269.

8-[4-[(Carboxymethyl)oxy]phenyl]-1H-3-(n-propyl)xanthine Hydrazide (37).: A solution of 0.05 g of 36 (0.14 mmole) and 0.5 mL of hydrazine anhydrous in 2 mL of dry DMF was heated overnight at 50°C. After evaporation, the residue was suspended in MeOH and the white precipitate was collected by filtration to give 0.025 g of 37 (yield 50%). m.p. = 267°C; 1H NMR (DMSO-d6). 0.90 (t, 3H, J = 7.8 Hz, -CH3), 1.72 (m, 2H, -CH2-), 3.71 (s, 3H, -OCH3), 3.95 (t, 2H, J = 6.8 Hz, -NCH2-), 4.34 (bs, 2H, NH2), 4.56 (s, 2H, -OCH2-), 7.08 (d, 2H, J = 8.8 Hz, Ar), 8.05 (d, 2H, J = 8.8 Hz, Ar), 9.39 (s, 1H, -NH); MS-EI (M+) 358, calculated for C16H18N6O4 358.1389; found 358.1389.

Pharmacology

The human A2B receptor cDNA was subcloned into the expression plasmid pDoubleTrouble [Robeva et al., 1996a]. The plasmid was amplified in competent JM109 cells and plasmid DNA isolated using Wizard Megaprep columns (Promega Corp., Madison, WI). A2B adenosine receptors were introduced into HEK-293 cells by means of Lipofectin [Felgner et al., 1987].

Cell culture—Transfected HEK cells were grown under 5% CO2/95% O2 humidified atmosphere at a temperature of 37°C. Colonies were selected by growth of cells in 0.6 mg/mL G418. Transfected cells were maintained in DMEM supplemented with Hams F12 nutrient mixture (1/1), 10% newborn calf serum, 2 mM glutamine, and containing 50 IU/mL penicillin, 50 μg/mL streptomycin, and 0.2 mg/mL Geneticin (G418, Boehringer Mannheim, Indianapolis, IN). Cells were cultured in 10 cm diameter round plates and subcultured when grown confluent (approximately after 72 h).

Radioligand binding studies—Confluent monolayers of HEK-A2B cells were washed with PBS followed by ice-cold Buffer A (10 mM HEPES, 10 mM EDTA, pH 7.4) with protease inhibitors (10 mg/mL benzamidine, 100 mM phenylmethanesulfonyl fluoride, and 2 mg/mL of each aprotinin, pepstatin, and leupeptin). The cells were homogenized in a Polytron (Brinkmann) for 20 sec, centrifuged at 30,000g, and the pellets washed twice with buffer HE (10 mM HEPES, 1 mM EDTA, pH 7.4 with protease inhibitors). The final pellet was resuspended in buffer HE, supplemented with 10% sucrose and frozen in aliquots at −80°C. For binding assays, membranes were thawed and diluted 5–10-fold with HE to a final protein concentration of approximately 1 mg/mL. To determine protein concentrations, membranes, and bovine serum albumin standards were dissolved in 0.2%
NaOH/0.01% SDS and protein determined using fluorescamine fluorescence [Stowell et al., 1978]. Saturation binding assays for human A2B adenosine receptors were performed with [125I]-ABOPX (2,200 Ci/mmol). To prepare [125I]-ABOPX, 10 mL of 1 mM ABOPX in methanol/1 M NaOH (20:1) was added to 50 mL of 100 mM phosphate buffer, pH 7.3. One or 2 mCi of Na125I was added, followed by 10 mL of 1 mg/mL chloramine-T in water. After incubating for 20 min at room temperature, 50 mL of 10 mg/mL Na-metabisulfite in water was added the quench the reaction. The reaction products were applied to a C18 HPLC column using 4 mM phosphate, pH 6.0/methanol. After 5 min in 35% methanol, the methanol concentration was ramped to 100% over 15 min. Unreacted ABOPX eluted in 11–12 min; [125I]-ABOPX eluted at 18–19 min in a yield of 50–60% of the initial 125I. In equilibrium binding assays the ratio of [127I]/[125I]-ABOPX was 10–20/1. Radioligand binding experiments were performed in triplicate with 20–25 μg membrane protein in a total volume of 0.1 mL HE buffer supplemented with 1 U/mL adenosine deaminase and 5 mM MgCl2. The incubation time was 3 h at 21°C. Nonspecific binding was measured in the presence of 100 mM NECA. Competition experiments were carried out using 0.6 nM 125I-ABOPX. Membranes were filtered on Whatman GF/C filters using a Brandell cell harvester (Gaithersburg, MD) and washed three times over 15–20 sec with ice-cold buffer (10 mM Tris, 1 mM MgCl2, pH 7.4). Bmax and KD values were calculated by Marquardt’s nonlinear least squares interpolation for single site binding models [Marquardt, 1963]. K values for different compounds were derived from IC50 values as described previously [Linden, 1982]. Data from replicate experiments are tabulated as means ± SEM.

[3H]CPX, 125I-ZM 241385 and 125I-ABA were utilized in radioligand binding assays to membranes derived from HEK-293 cells expressing recombinant human A1, A2A, and A3 adenosine receptors, respectively. Binding of [3H]N6-phenylisopropyladenosine ([3H]R-PIA; Amersham, Chicago, IL) to A1 receptors from rat cerebral cortical membranes and of [3H]CGS 21680 (NEN Life Sciences, Boston, MA) to A2A receptors from rat striatal membranes was performed as described previously [Schwabe and Trost, 1980; Jarvis et al., 1989]. Adenosine deaminase (3 units/mL) was present during the preparation of the brain membranes in a preincubation of 30 min at 30°C and during the incubation with the radioligands. All nonradioactive compounds were initially dissolved in DMSO and diluted with buffer to the final concentration, where the amount of DMSO never exceeded 2%. Incubations were terminated by rapid filtration over Whatman GF/B filters using a Brandell cell harvester. The tubes were rinsed three times with 3 mL buffer each.

At least six different concentrations of competitor, spanning 3 orders of magnitude adjusted appropriately for the IC50 value of each compound, were used. IC50 values, calculated with the nonlinear regression method implemented in Graph-Pad Prism (San Diego, CA) were converted to apparent Ki values as described by Linden [1982]. Hill coefficients of the tested compounds were in the range of 0.8–1.1.

RESULTS AND DISCUSSION

The structures of the xanthine derivatives, 4, 9–27, tested for affinity in radioligand binding assays at adenosine receptors, are shown in Table 1. Most of the xanthisnes are derivatives of XCC [Jacobson et al., 1985], in which an acyl-hydrazide group is present. This group was
included based on the high potency in the A$_{2B}$ receptor binding assay (K$_i$ value of 9.75 nM [Jacobson et al., 1999]) of an N-hydroxsuccinimide ester of XCC, 4d. The hydrazide of XCC, 4c, was acylated with a variety of mono- and dicarboxylic acids. Cyclization reactions were carried out for dicarboxylic acids, in two steps using the anhydride, 28, for acylation, leading to imide (5- or 6-membered ring) derivatives (Fig. 2). The final step of ring-closure of 29a to 29b was effected at 50 °C, using excess carbodiimide and 1-hydroxybenzotriazole as catalyst. In some cases, where symmetric dicarboxylic acids were used, it was possible to isolate both the open structure, 29a, and the cyclized imide form, 29b. Pairs of open and cyclized derivatives of symmetric dicarboxylic acids prepared include compounds 18–20. Also, the glutamic acid derivative 24a was prepared using orthogonal protecting and the corresponding imide, 24b. An 8-phenyl analog, 15, of enprofylline was synthesized by standard methods from the asymmetric urea, 30 (Fig. 3).

At A$_{2B}$ receptors, two radioligand binding assays (Table 1) were used. K$_i$ values of xanthine derivatives were determined in displacement of binding of the non-selective radioligands [³H]ZM 241385, 8 (4-(2-[7-amino-2-{furyl}{1,2,4}triazolo{2,3-a} {1,3,5}tr nzyl]-8-phenyloxacetate-1-propyl-xanthine), at human A$_{2B}$ receptors expressed in HEK-293 cell membranes [Linden and Jacobson, 1998]. In order to evaluate selectivity, selected derivatives were subjected to standard binding assays at A$_1$, A$_{2A}$, and A$_3$ receptors. The initial screening utilized rat brain A$_1$/A$_{2A}$ receptors (with radioligands [³H]R-PIA and [³H]CGS-21680), and selected compounds were examined at the recombinant human subtypes (Table 1), using [³H]CPX ([³H]8-cyclopentyl-1,3-dipropylxanthine) and ¹²⁵I-ZM 241385, ¹²⁵I-4-(2-[7-amino-2-[2-furyl][1,2,4]triazolo[2,3-a] [1,3,5]triazin-5-yl-amino]ethyl)phenol) [Palmer et al., 1996]. Affinity at cloned human A$_3$ receptors expressed in HEK-293 cells was determined using ¹²⁵I-ABA (N$^6$-(4-amino-3-[¹²⁵I]iodobenzyl)-adenosine) and ¹²⁵I-AB-MECA (N$^6$-(4-amino-3-iodobenzyl)-adenosine-5'-N-methyluronamide).

The initial screening utilized rat A$_1$/A$_{2A}$ receptors, and selected compounds were examined at the human subtypes. Selectivities for the human A$_{2B}$ vs. rat A$_1$/A$_{2A}$ receptors were generally small (3–4-fold at best), while comparisons within the same species (human) generally lead to greater selectivities. A 1,2-dimethylmaleimide derivative, 14, bound to human A$_{2B}$ receptors with a K$_i$ of 19 nM and proved to be selective vs. human A$_1$/A$_{2A}$/A$_3$ receptors by 160-, 100-, and 35-fold, respectively.

Enprofylline (3-propylxanthine) is slightly selective for A$_{2B}$ receptors; however, combination of the 1-H-3-Pr and 8-phenyl substituents eliminated the selectivity (cf. 14 and 15).

Other potent and moderately selective A$_{2B}$ antagonists were a tetrahydrophthaloyl derivative 18b (K$_i$ value 10 nM) and amino acid conjugates of the XCC-hydrazide, i.e. the glutarimide 24b (K$_i$ value 13 nM) and protected dipeptide 27 (K$_i$ value 11 nM). Compound 20a displayed a K$_i$ value of 17 nM. Other derivatives displaying selectivity for A$_{2B}$ receptors, but with less potency (K$_i$ values in nM in parentheses) were: 11 (30), 16 (67), 17 (28), 24a (25), 25 (48), and 26 (40). A direct comparison of either shows increased (18b or 19b) or decreased (20b) A$_{2B}$ receptor affinity upon cyclization.
The identification of 14 (MRS 1595) as an adenosine antagonist which is potent and selective for human $A_{2B}$ receptors and should be hydrolytically stable will provide an opportunity to test the hypothesis that this subtype is involved in asthma. Further SAR studies are in progress to enhance the pharmacological profile of these xanthine derivatives as $A_{2B}$ receptor antagonists.

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Abbreviations:

| Abbreviation | Definition |
|--------------|------------|
| CGS          | 21680 2-[4-[(2-carboxyethyl)phenyl]ethylamino]-5′-N-ethylcarbamoyl adenosine |
| CHA          | $A^6$-cyclohexyladenosine |
| CHO          | Chinese hamster ovary cells |
| CPX          | 8-cyclopentyl-1,3-dipropylxanthine |
| DIPEA        | diisopropylethylamine |
| DMAP         | 4-dimethylaminopyridine |
| DMF          | N,N-dimethylformamide |
| DMSO         | dimethylsulfoxide |
| EDTA         | ethylenediaminetetraacetate |
| HEK cells    | human embryonic kidney cells |
| HOBl         | 1-hydroxybenzotriazole |
| $[^{125}I]ABA$ | $[^{125}I]N^6$-(4-aminobenzyl)-adenosine |
| $[^{125}I]AB-MECA$ | $[^{125}I]N^6$-(4-amino-3-iodobenzyl)-adenosine-5′-N-methyluronamide |
| $^{125}$I-ABOPX | $^{125}$I-3-(4-amino-3-iodobenzyl)-8-oxacetate-1-propylxanthine |
| $K_i$        | equilibrium inhibition constant |
| NECA         | 5′-(N-ethylcarbamoyl)adenosine |
| NHS          | N-hydroxysuccinimide ester |
| R-PIA        | R-$A^6$-phenylisopropyladenosine |
| SAR          | structure–activity relationship |
| TFA          | trifluoroacetic acid |
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Fig. 1.
Structures of xanthines and nonxanthines previously identified as antagonists at A_{2B} receptors.
Fig. 2.
Derivatization of a xanthine containing a hydrazide group attached through the $p$-position of an 8-phenyl substituent [Jacobson et al., 1985]. The hydrazide, 4c, was acylated with the anhydride, 28, of a variety of dicarboxylic acids, followed by ring closure leading to stable imide derivatives, 29b.
Fig. 3. Synthesis of xanthine derivatives containing both 8-phenyl substituents and the 1-H-3-propyl substitution present in enprofylline, 1, as potentially selective A<sub>2B</sub> receptor antagonists.
TABLE 1.

Affinities of Xanthine Derivatives in Radioligand Binding Assays at Rat A₁, A₂B, Human A₂B, and Human A₃ receptors, unless noted.

| Compund | R       | R²  | rA₁   | rA₂B⁻ | hA₂B⁻ | hA₃⁻ | rA₁/hA₂B⁻ |
|---------|---------|-----|-------|--------|--------|--------|------------|
| 4b      | —       | Pr  | 51.6 ± 8.0, 203 ± 59(h) | 128 ± 15, 342 ± 10(h) | 18.7 ± 0.5, 34.5 ± 6.3 | 48.5 ± 0.8 | 2.8 |
| 4c      | NH₂     | Pr  | 16.0 ± 0.5 | 63.8 ± 21.3 | 13.2 ± 5.9 | 498 ± 139 | 1.2 |
| 4e      | NH-COCH₃| Pr  | 6.51 ± 1.24, 125 ± 14(h) | 227 ± 64, 186 ± 9(h) | 65.4 ± 6.5, 33.8 ± 13.7 | 30.9 ± 8.2 | 0.10 |
| 9       | —       | Pr  | 73.3 ± 22.0, 219 ± 3(h) | 174 ± 32, 795 ± 98(h) | 116 ± 10, 97.8 ± 3.3 | 173 ± 27 | 1.6 |
| 10      | —       | Pr  | 55.9 ± 25.1, 75.2 ± 5.5(h) | 805 ± 44, 27.2 ± 8.6 (h) | 18.6 ± 6.1 | 766 ± 176 | 3.0 |

Drug Dev Res. Author manuscript; available in PMC 2022 June 14.
| Compound | R          | R' | $r_A^a$ | $r_A^{b \Lambda}$ | $h_A^{b \Lambda}$ | $h_A^c$ | $r_A^c$/$h_A^{b \Lambda}$ |
|----------|------------|----|--------|------------------|------------------|-------|------------------|
| 11       | Pr         |    | 74.3 ± 6.6 | 139 ± 32 | 30.2 ± 0.5 | 1,560 | 2.5              |
| 12       | Pr         |    | 3.87 ± 1.20 | 21.4 ± 6.1 | 3.86 ± 0.7 | 151 ± 99 | 1.0              |
| 13       | Pr         |    | 203 ± 41   | 1,230 ± 270 | 144 ± 11 | 551 ± 106 | 1.4              |
| 14d      | Pr         |    | 11.1 ± 2.4, 3.030 ± 1110 (h) | 126 ± 41, 1.970 ± 550 (h) | 19.4 ± 6.2, 33.8 ± 1.9 | 670 ± 154 | 0.57             |

**Ki (nM) or % displacement**
| Compound | R | R^* | Ki (nM) or % displacement |
|----------|---|-----|--------------------------|
| 15 |  | H | 3.590 ±920, 8,080 \( \pm 1720 \text{ (h)} \)^c | 36 ± 4% (10^-4) | 1,800 ± 0, 1,900 \( \pm 280 \text{ (h)} \)^c | 14,200 ± 11,500 \( \pm 20 \text{ (h)} \)^c | 2.0 |
| 16 | Pr |  | 225 ± 76 | 1,540 ± 280 | 66.7 ± 37.0 | 748 ± 234 | 3.4 |
| 17 | Pr |  | 95.8 ± 25.1 | 2,100 ± 630 | 27.9 ± 8.5 | 3,450 ± 1,470 | 3.4 |
| 18a | Pr | NH | 134 ± 19 | 813 ± 299 | 51.0 ± 7.0 | 1,060 ± 150 | 2.6 |
| 18b | Pr |  | 36.4 ± 6.2 129 ± 20 \( \pm 3 \text{ (h)} \)^c | 689 ± 477 301 ± 31 \( \pm 3 \text{ (h)} \)^c | 10.0 ± 3.0 | 370 ± 190 | 3.6 |
| Compound | R             | R"          | RA_{1}^{a}  | RA_{2A}^{b} | hA_{2B}^{b} | hA_{3}^{c} | RA_{2A}/hA_{2B} |
|----------|---------------|-------------|-------------|-------------|-------------|-------------|-----------------|
| 19a      | Pr            |             | 81.7 ± 31.2 | 708 ± 169   | 78.5 ± 20.5 | 1,180 ± 700 | 1.0             |
| 19b      | Pr            |             | 41.3 ± 6.4  | 1,160 ± 337 | 21.5 ± 1.5  | 308 ± 88    | 1.9             |
| 20a      | Pr            | (S)         | 47.2 ± 6.8  | 422 ± 136   | 17.3 ± 6.3  | 438 ± 109   | 2.7             |
| 20b      | Pr            |             | 61.9 ± 11.3 | 415 ± 157   | 35.8 ± 0.7  | 245 ± 45    | 1.7             |

Ki (nM) or % displacement.
| Compound | R   | R*  | $r_{A1}$ | $r_{A2A}$ | $h_{A2B}$ | $h_{A3}$ | $r_{A3}/h_{A2B}$ |
|----------|-----|-----|----------|-----------|-----------|----------|-----------------|
| 21       | Pr  | O   | 26.3 ± 2.3 | 392 ± 117, 359 ± 21 | 64.4 ± 0.8, 46.4 ± 14.5 | 147 ± 21 | 0.41            |
| 22       | Pr  | O   | 14.0 ± 2.3 | 135 ± 39 | 22.0 ± 5.5 | 200 ± 45 | 0.6            |
| 23       | Pr  | O   | 41.2 ± 16.6 | 164 ± 61 | 25.7 ± 5.5 | 290 ± 88 | 1.6            |
| 24a      | Pr  | O   | 70.8 ± 30.9 | 872 ± 412 | 24.8 ± 7.3 | 430 ± 44 | 2.9            |

Ki (nM) or % displacement

*Ki values are given in nM where applicable.*

$Pr$ values are provided as well.
\begin{table}[h]
\centering
\begin{tabular}{lcccccc}
\hline
\textbf{Compound} & \textbf{R} & \textbf{R''} & \textbf{Ki (nM)} & \textbf{Compounds} & \textbf{Ki (nM)} & \textbf{Ki (nM)} \\
\hline
24b & TFA-L-Glu-NH & NHCOCF$_3$ & 53.5 ± 6.5 & 149 ± 6 & 149 ± 6 & \text{(h)\textsuperscript{e}} \\
 & & & 440 ± 106 & 178 ± 20 & & \text{(h)\textsuperscript{e}} \\
 & Pr & & 13.0 ± 3.5 & & & 726 ± 245 \\
 & \text{(S)} & & & & & 4.1 \\
25 & NH & CH$_2$CH$_2$OH$_2$ & 197 ± 67 & 2,750 ± 950 & & 4.1 \\
 & (S) & & & & & \\
 & t-Boc-L-Leu-NH & & & & & \\
26 & NH & CH$_2$SH$_2$O$_3$ & 113 ± 27 & 524 ± 285 & & 4.1 \\
 & (S) & & & & & \\
 & t-Boc-L-Met-NH & & & & & \\
27 & Cbz-Gly$_2$-NH & Pr & 36.0 ± 6.6 & 200 ± 22 & 609 ± 95 & 830 ± 84 & 10.8 ± 5.0 & 323 ± 47 & 3.3 \\
 & & & \text{(h)\textsuperscript{e}} & & \text{(h)\textsuperscript{e}} & & & & & \\
\hline
\end{tabular}
\end{table}

\textbf{a} Displacement of specific $[^3]$H-$R$-PIA binding to A$_1$ receptors in rat brain membranes, expressed as Ki ± S.E.M. (n = 3–5), unless noted.

\textbf{b} Displacement of specific $[^3]$H-CGS 21680 binding to A$_2A$ receptors in rat striatal membranes, expressed as Ki ± S.E.M. (n = 3–6), and at A$_2B$ receptors expressed in HEK-293 cells vs $[^3]$H-ZM241385, unless noted.

\textbf{c} Displacement of specific $[^125]$I-AB-MECA binding at human A$_3$ receptors expressed in HEK cells, in membranes, expressed as Ki ± S.E.M. (n = 3–4), unless noted.

\textbf{d} MRS 1595.

\textbf{e} Ki values were determined in radioligand binding assays at recombinant human A$_1$ and A$_2A$ receptors expressed in HEK-293 cells vs $[^3]$H-CPX and $[^125]$I-ZM241385, respectively. Affinity of xanthine derivatives at human A$_2B$ receptors expressed in HEK-293 cells was determined using $[^125]$I-ABOPX. Affinity at recombinant human A$_3$ receptors expressed in HEK-293 cells was determined using $[^125]$I-ABA.