Available online at www.sciencedirect.com
SCIENCE DIRECT
Bioorganic & Medicinal Chemistry 14 (2006) 3654–3661
Bioorganic & Medicinal Chemistry

Norbornyllactone-substituted xanthines as adenosine A_1 receptor antagonists

William F. Kiesman,[a,*] Jin Zhao,[a] Patrick R. Conlon,[a] Russell C. Petter,[a] Xiaowei Jin,[b] Glenn Smits,[b] Frank Lutterodt,[b] Gail W. Sullivan[c] and Joel Linden[c]

[a]*Department of Medicinal Chemistry, Biogen Idec, Inc., 14 Cambridge Center, Cambridge, MA 02142, USA*
[b]*Department of Pharmacology, Biogen Idec, Inc., 14 Cambridge Center, Cambridge, MA 02142, USA*
[c]*Department of Medicine (Cardiology) and Pharmacology, University of Virginia, Charlottesville, VA 22908, USA*

Received 12 December 2005; revised 9 January 2006; accepted 10 January 2006
Available online 2 February 2006

Abstract—During the search for second-generation adenosine A_1 receptor antagonist alternatives to the clinical candidate 8-(3-oxatricyclo[3.2.1.0^{2,4}]oct-6-yl)-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (BG9719), we developed a series of novel xanthines substituted with norbornyl-lactones that possessed high binding affinities for adenosine A_1 receptors and in vivo activity.

1. Introduction

Adenosine, the ubiquitous breakdown product of ATP, modulates a variety of physiologic processes by its action on four receptor subtypes: A_1, A_{2A}, A_{2B}, and A_3.[1] The A_1 receptor has been identified as a mediator in CNS, cardiovascular, metabolic, renal, and gastro-intestinal systems.[2] Our attention has been focused upon the development of a selective adenosine A_1 receptor antagonist for the treatment of congestive heart failure and concomitant renal impairment. 8-(3-Oxa-tricyclo[3.2.1.0^{2,4}]oct-6-yl)-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (aka BG9719)[3] was found to facilitate diuresis and stabilize the renal function in CHF patients on top of standard therapy.[4] These promising clinical data prompted the initiation of a back-up program. Our investigations focused on the discovery of molecules with equivalent biological activity and enhanced pharmaceutical properties including increased solubility and thermal stability to those of the lead molecule, BG9719. One approach was to synthesize a series of norbornyl-based compounds that place an oxygen atom in a position similar to that of the epoxide oxygen in BG9719, such as the norbornyl-lactone with the xanthine ring in the *exo*-position (Fig. 1).

2. Results

The synthesis of the xanthine derivatives started from the commercially available racemic bicyclo[2.2.1]hept-5-ene-2,3-dicarboxylic acid (Fig. 2).[5] Iodolactonization followed by selective reduction of the iodide via a free-radical process gave lactone **2** in 42% yield over two steps.[6] HATU-mediated (*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate) coupling to diaminodialkyl uracils[3] produced amides **3a**–**c**, which after stepwise base-catalyzed cyclization to the xanthine and then acid-catalyzed lactonization, gave racemates **4a**–**c**. The single enantiomer, compound **4d,**

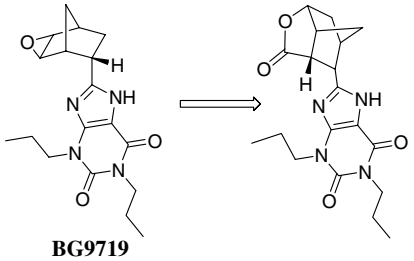

Figure 1. BG9719 and norbornyl lactone analog.

Keywords: Adenosine A_1 receptor antagonist; Xanthine; BG9719; 1,3-Dipropyl-3,7-dihydro-purine-2,6-dione.
* Corresponding author. Tel.: +1 617 679 2790; fax: +1 617 679 3635; e-mail: william.kiesman@biogenidec.com

0968-0896/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2006.01.021

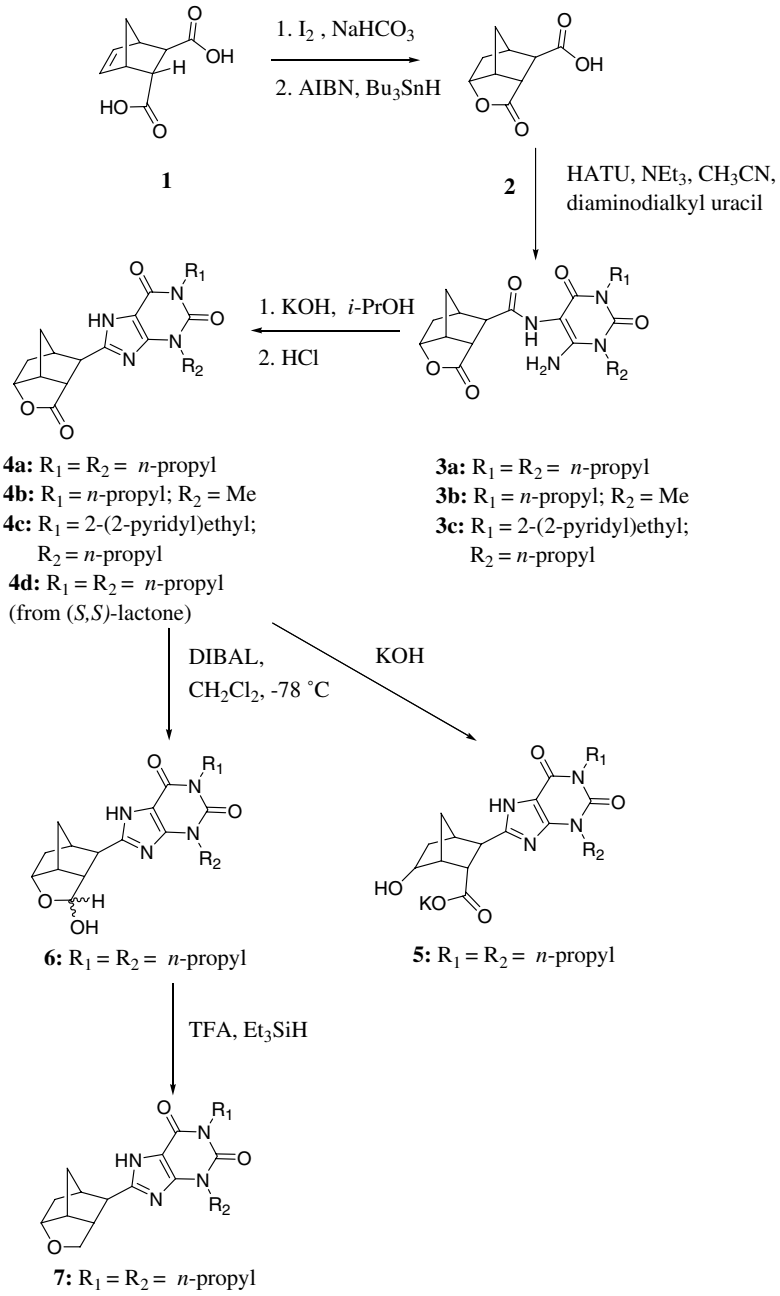

Figure 2. Synthesis of lactone-based xanthines.

was made by the same series of steps except the enantiomerically pure (*S,S*)-norbornene-5,6-dicarboxylic acid served as the starting material.[5,6]

Compound **4a** served as a starting point for further synthetic manipulation. Hydrolysis of the lactone with KOH gave the potassium salt of the hydroxy acid: **5**, which was stable at basic pH and also stable to the assay conditions used to determine binding affinities.

Selective reduction of the lactone was accomplished with DIBAL at low temperature and gave a mixture of lactols in a 3:1 ratio (*R*:*S*).[7] Further reduction of lactol **6** to ether **7** proceeded cleanly under the influence of triethylsilane and TFA.[8]

Epoxidation of **1** with *m*-CPBA gave the *exo*-epoxide, which after treatment with HCl in dioxane produced *exo*-hydroxy *endo*-lactone **8** (Fig. 3). HATU coupling of the acid with 5,6-diamino-1,3-dipropyl-1*H*-pyrimidine-2,4-dione and cyclization gave compound **10**. Iodolactonization of **1** enabled selective esterification of the *exo*-carboxylic acid followed by reductive ring opening of the iodolactone intermediate gave norbornene **11**.[5,6] Coupling to the uracil followed by base-promoted cyclization gave norbornene carboxylate **12**. Catalytic hydrogenation in *i*-PrOH produced the saturated *exo*-carboxylic acid **13**, while esterification followed by catalytic reduction gave *exo*-methyl ester **14**.

The biological activities of the antagonists were evaluated by the following procedures. The primary screen

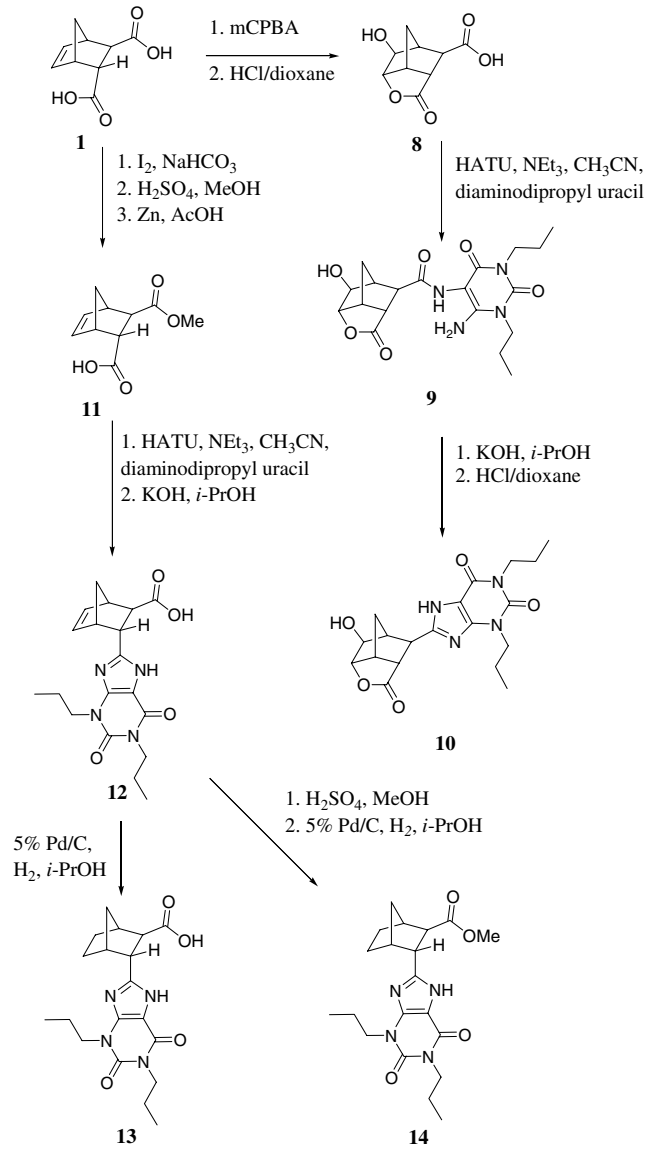

Figure 3. Hydroxylactone- and *exo*-substituted-norbornyl xanthine preparations.

consisted of a single-point assay performed in duplicate on membranes derived from stably transfected HEK (hA_{2A}, hA_{2B}, and hA_3) or CHO-K1 (hA_1 receptors) cells expressing each of the four human adenosine receptor subtypes (hA_1, hA_{2A}, hA_{2B}, and hA_3).[9a] Tubes were incubated at room temperature for 2 h, filtered, and counted in a γ-counter. The readout gave the percent (%) of specific radioligand binding; a high number denotes poor antagonist activity, while a low number indicates displacement of the radiolabel from the receptor. Compounds that displayed good A_1 binding activity in the single point assays were further evaluated to determine IC_{50} values. K_is were calculated from IC_{50} values.[10] Full binding curves used antagonist concentrations from 10^{-11} to 10^{-5} M assayed in duplicate. Table 1 lists the binding data for all of the compounds tested against all four human adenosine receptor subtypes.

The binding affinities for rat A_1 (rA_1) and A_{2A} (rA_{2A}) receptors were determined for compounds that exhibited high human adenosine A_1 receptor binding affinity. Compounds were incubated with radioligand and aliquots of crude membrane suspensions were prepared from either rat brain cortex (for rA_1) or rat brain striatum (for rA_{2A}). Values of K_i were determined from concentration–response relationships for each compound to displace binding of radioligand.[9b]

Compounds with sufficient hA_1 and rA_1 binding affinity were then tested in a rapid oral activity rat diuresis experiment. The antagonist was dosed in a 0.5% carboxymethylcellulose suspension orally. Urine volumes were collected over time and urine sodium and potassium concentrations were measured. Figures 4 and 5 display the results of the rat oral activity screen at 2 and 0.3 mg/kg, respectively.[11]

The EC_{50} values of selected antagonists were also determined in isolated rat atria in order to rank the in vivo activities.[12] All experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the protocols were approved by the Institutional Animal Care and Use Committee.

3. Discussion

The binding activity of the first member of the lactone series, compound **4a**, was 18 nM with 37-fold selectivity for A_1 versus A_{2A} receptors. This result was somewhat surprising to us in view of the relatively low A_1 affinity of the closely related trans-substituted norbornyl xanthine **13**, where the xanthine occupied the *endo*-position and the methylcarboxylate the *exo*-position. Evidently locking the carboxylate into the compact lactone ring system allowed a better fit into both the A_1 and A_{2A} receptors as affinities for both receptors increased over 4-fold. Serendipity aside, this result spurred us on to examine more closely a few readily made examples of compounds containing a norbornyl framework with *exo*-xanthine substitution. As in the case of BG9719, the chirality of the [2.2.1] system had some effect on both the binding affinity and selectivity. The single enantiomer, lactone **4d**, exhibited a 3-fold increase in binding affinity for the A_1 receptor with a marginal increase in affinity for the A_{2A} receptor. Substitution of the *n*-propyl chain in the N1 position with a 2-(2-pyridyl)ethyl group (**4c**) or truncation of the N3 propyl chain to a methyl group (**4b**) decreased A_1 and A_{2A} affinity equally. Placement of a polar carboxylic acid in either the *endo*- (**5**) or *exo*-positions (**12**,**13**) led to poor A_1 binding affinity. Even introduction of the less polar hydroxyl group in the 2-position (**10**) decreased A_1 affinity about 3-fold and A_{2A} affinity by a factor of 6. This result suggested that the pocket the norbornyl group occupies in both the A_1 and A_{2A} receptors is particularly sensitive to polar substitution.[13] Reduction of the lactone to a mixture of lactols (**6**) compromised A_1 activity, while subsequent reduction to the ether (**7**) restored A_1 binding and selectivity to levels almost identical to those for lactone **4a**. This would indicate that although there is space for the carbonyl in lactone **4a** and the CH_2 group in ether **7** the oxygen of the lactone **4a** does not contribute to

Table 1. Binding affinities for lactone-based xanthine derivatives on recombinant human adenosine receptors

Compound[c]	K_i[a] (nM) or % of specific radioligand binding[b]				hA_{2A}/hA_1
	hA_1	hA_{2A}	hA_{2B}	hA_3	
BG9719	12	1660	611	4810	138
	0.7 (rat)[d]	1250 (rat)[d]	—	—	1800 (rat)[d]
4a	18	657	802	(88%)	37
	3.0 (rat)[e]	264 (rat)[e]	—	—	105 (rat)
4b	71	2330	(69%)	(93%)	33
4c	66	3510	(82%)	(93%)	53
4d	6.0	923	1540	>10,000	154
	0.3 (rat)[e]	269 (rat)[e]	—	—	860 (rat)
5	107	4270	(70%)	(89%)	40
6	(38%)	(100%)	(81%)	(95%)	—
7	20	1510	(40%)	(89%)	76
	1.0 (rat)[e]	>10,000 (rat)[e]	—	—	>10,000 (rat)
10	62	6280	2030	>10,000	101
	13 (rat)[e]	>10,000 (rat)[e]	—	—	>800 (rat)
12	508	—	—	—	—
13	144	(94%)	(76%)	(94%)	—
14	74	3100	1080	(76%)	42

[a] K_i values are means of closely agreeing duplicate determinations (means ± 50%), each derived from seven antagonist concentrations assayed in duplicate.

[b] The readout gave the percent (%) of specific radioligand binding; a high number denotes poor antagonist activity, while a low number indicates displacement of the radiolabel from the receptor.

[c] All compounds shown are racemic mixtures, except **4d**, which was prepared from the enantiomerically pure carboxylic acid **2**.

[d] Rat receptor binding values from Ref. 3.

[e] K_i values were determined from concentration–response relationships for each compound to displace binding of radioligand to rat brain cortex (for rA_1) or rat brain striatum (for rA_{2A}).

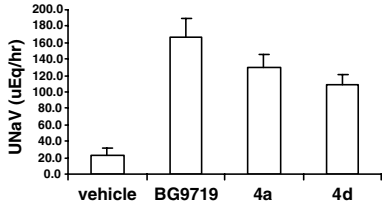

Figure 4. Rat oral efficacy screen; measurement of urinary sodium excretion per hour, over a 4 h period, caused by a 2 mg/kg oral dose of compound in a 0.5% CMC suspension (see Ref. 11 for details).

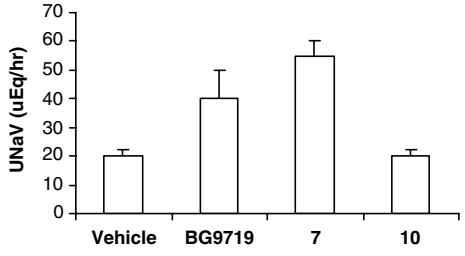

Figure 5. Rat oral efficacy screen; measurement of urinary excretion of sodium per hour, in a 4-h period, of a 0.3 mg/kg oral dose of compound in a 0.5% CMC suspension (see Ref. 11 for details).

the A_1 binding affinity or the overall selectivity versus the A_{2A} receptor.

Compounds **4a**, **4d**, **7**, and **10** were evaluated for rA_1 and rA_{2A} receptor binding affinity. For A_1 receptors, each of the compounds tested in the rat receptor assays had greater affinity for the rat versus the human receptors. About a 6- to 20-fold improvement was seen in rA_1 binding affinity. In contrast, no trend was observed between the affinities for the rA_{2A} and hA_{2A} receptors, except that the difference between the binding affinities for the species was smaller.

The in vivo activities of the most potent binders were then investigated. A fixed oral dosage of 2 mg/kg of the antagonist was administered to rats in metabolic cages. Urine output was monitored for 4 h and analyses were performed to determine the amounts of excreted sodium and potassium in the urine.

After oral dosing, there were significant increases in sodium excretion compared to vehicle in the rats receiving compounds **4a** and **4d** (Fig. 4). At this dose, the biological activity of the racemate was equivalent to that of the single enantiomer. The magnitude of sodium mobilization was also similar in both cases to that seen with an equivalent dose of BG9719. Potassium excretion was also similar between vehicle-treated animals and those treated with all three compounds. This result was not unexpected as A_1-mediated natriuresis is potassium neutral. Plasma concentrations of the compounds were also taken at the 1-h time point. The lactones **4a** and **4d** had plasma levels of 1600 and 2300 ng/mL compared to only 110 ng/mL for BG9719. This result was an early indication that the half-life of the lactone compounds in the rat may be longer than that of BG9719 and that the parent lactones could also have greater in vivo stability.

A similar but more challenging in vivo rat experiment was performed that measured the urinary sodium excretion per hour after oral administration of a lower dose of 0.3 mg/kg of the antagonist. The results of this

experiment comparing BG9719 with compounds **7** and **10** appear in Figure 5. The sodium excretion induced by ether **7** was 37% greater than that of the BG9719 standard, while the hydroxylactone **10** result was similar to vehicle.

Further work explored the functional activities of the adenosine A_1 receptor antagonists in two different isolated rat atria assays. In the first assay, isolated spontaneously beating atria were treated with BG9719 and compound **4a** at 30 nM. Atria were then treated with increasing concentration of N^6-cyclopentyladenosine (CPA) until the heart rate dropped to a minimum. The concentration response curves to both compounds showed a rightward shift with EC_{50} values of 127 nM for BG9719 and 865 nM for compound **4a**. In the second assay, isolated rat atria were treated with a solution of CPA in high enough concentration to reduce beating rate by 75%. The atria were then treated with increasing concentrations of compounds BG9719 and compound **4d,** and their effects on heart rate were determined. Both antagonists blocked the CPA-induced reduction in heart rate with EC_{50} values of 7.9 nM for BG9719 and 71 nM for compound **4d**. Despite the similarities in binding affinities for the three molecules, BG9719 demonstrated superior in vivo activity in the atria over both lactones.

Since the product was targeted for delivery to acutely decompensated congestive heart failure patients in the hospital setting, the solubility of **4a** in comparison to BG9719 in prototype solutions suitable for IV administration was determined. Both compounds exhibited uniformly poor water solubility: BG9719, 0.18 mg/mL in water; 0.16 mg/mL in 0.9% saline; 0.17 mg/mL in D5W: **4a,** 0.15 mg/mL in water; 0.09 mg/mL in 0.9% saline; 0.09 mg/mL in D5W.

The lack of water solubility coupled with the discovery of a more promising series of antagonists with increased water solubility (>100-fold increase) and better in vivo activity[14] led to the discontinuation of the investigation of this series of molecules and precluded the examination of the single enantiomers of ether **7**, but it would be reasonable to expect that the more active enantiomer would be related to the more active lactone **4d** and the in vivo activity had the potential to be greater than that of BG9719.

4. Conclusions

A series of norbornyl-substituted lactones were investigated as replacements for the epoxynorbornane in the clinical compound BG9719. These xanthine derivatives in which the xanthine occupies the *exo* position on the norbornyl ring system showed high A_1 binding affinity and selectivity over the closely related A_{2A} receptor (notably compounds **4d**: hA_1 $K_i = 6$ nM; 154-fold selectivity and **7**: hA_1 $K_i = 20$ nM; 76-fold selectivity). The lactones possessed similar if not better in vivo activity to BG9719 in the rat diuresis models.

5. Experimental

5.1. General methods

Unless otherwise stated, reactions were carried out under nitrogen in oven-dried glassware. The HPLC method used to determine purity was performed on an HP1100 system, YMC-ODS-AM C18 reversed-phase column (4.6 × 100 mm)-guard column YMC-ODS-AM S-5 120A (direct connect); 20–100% CH_3CN/H_2O gradient over 8 min, buffered with 0.1% TFA at 1.5 mL/min flow rate, detector set at dual wavelength 214 and 254 nm. Reversed-phase HPLC was also used for preparative purposes (LiChroprep C-18, 310 × 25 mm). ^1H and ^{13}C NMR spectra were obtained using Bruker 300, 400, and 600 MHz NMR spectrometers. High-resolution mass spectroscopic data were obtained on a THERMO ELECTRON LTQ FTMS. The data were acquired at the positive, FULL scan and SIM scan FT MS Mode, protonated molecular ion designated as MH^+. All the chemicals were supplied by Aldrich Chemical Co., Inc., Milwaukee, WI, USA, unless otherwise indicated.

5.2. 5-Oxo-4-oxa-tricyclo[4.2.1.0^{3,7}]nonane-9-carboxylic acid (2)

^1H NMR (300 MHz, $CDCl_3$) δ 4.76 (dd, 1H), 3.18 (s, 1H), 3.02 (s, 1H), 2.78 (s, 1H), 2.73 (s, 1H), 1.83–1.78 (m, 1H), 1.75 (d, 1H), 1.56 (m, 1H). ^{13}C NMR (600 MHz, $CDCl_3$) δ (ppm) 179.48, 177.08, 80.29, 49.89, 45.89, 42.43, 40.83, 37.89, 35.76. HRMS m/z = 183.06531 (MH^+), calcd 183.06519.

5.3. 8-(5-Oxo-4-oxa-tricyclo[4.2.1.0^{3,7}]non-9-yl)-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (4a)

To a stirred mixture of 0.500 g (2.74 mmol) of 5-oxo-4-oxa-tricyclo[4.2.1.03,7]nonane-9-carboxylic acid, 0.748 g (2.85 mmol) of 5,6-diamino-1,3-dipropyl-1*H*-pyrimidine-2,4-dione hydrochloride,[3] 1.15 mL (8.22 mmol) of NEt_3, and 10 mL anhydrous acetonitrile was added 1.08 g (2.85 mmol) of HATU.[15] The reaction solution was stirred at rt for 1 h. The reaction mixture was concd in vacuo and combined with 30 mL EtOAc and 30 mL of 10% citric acid. The aqueous layer was separated and washed twice with 40-mL portions of EtOAc. The combined organic fractions were washed with 20-mL portions of satd $NaHCO_3$ and brine, and concd in vacuo. The resultant solid was combined, in a 50-mL round-bottomed flask equipped with a condenser, with a mixture of 5 mL of *i*-PrOH and 5.5 mL of 1 N KOH (5.5 mmol) and heated to reflux. After heating for 1 h, the reaction solution was concd in vacuo, taken up in 40 mL of water, and washed twice with 30-mL portions of CH_2Cl_2. The aqueous layer was acidified with concd HCl and the resultant precipitate was collected by suction filtration to give 0.155 g (15% yield) of an off-white solid. ^1H NMR (300 MHz, $CDCl_3$) δ 4.90 (t, 1H), 4.10 (t, 2H), 4.00 (t, 2H), 3.48 (s, 1H), 3.38 (m, 2H), 2.80 (m, 1H), 2.10 (m, 1H), 1.95 (m, 1H), 1.85–1.65 (m, 5H), 1.55 (d, 1H), 0.95 (two t, 6H). ^{13}C NMR (600 MHz, $CDCl_3$) δ (ppm) 179.41, 156.11, 154.98,

150.77, 148.70, 107.25, 80.27, 46.10, 45.59, 45.41, 43.78, 43.45, 43.44, 38.52, 35.43, 21.35, 21.33, 11.21, 11.06. HRMS m/z = 373.18721 (MH^+), calcd 373.18703. HPLC retention time: 4.25 min, without YMC-ODS-AM S-5 120A (direct connect guard column).

The following compounds were made in an analogous manner.

5.4. 3-Methyl-8-(5-oxo-4-oxa-tricyclo[4.2.1.0^{3,7}]non-9-yl)-1-propyl-3,7-dihydro-purine-2,6-dione (4b)

0.058 g (15% yield) of an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.85 (m, 1H), 4.10 (t, 2H), 3.55 (s, 3H), 3.48 (s, 1H), 3.38 (m, 2H), 2.70 (m, 1H), 2.10 (m, 1H), 1.95 (m, 1H), 1.90–1.80 (m, 1H), 1.70–1.45 (m, 5H), 0.90 (t, 3H). m/z = 345.10 (MH^+). HPLC retention time: 3.18 min.

5.5. 8-(5-Oxo-4-oxa-tricyclo[4.2.1.0^{3,7}]non-9-yl)-3-propyl-1-(2-pyridin-2-yl-ethyl)-3,7-dihydro-purine-2,6-dione (4c)

0.010 g (10% yield) of a yellow oil. m/z = 435.96 (MH^+). HPLC retention time: 3.47 min.

5.6. 8-(5-Oxo-4-oxa-tricyclo[4.2.1.0^{3,7}]non-9-yl)-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (4d)

Starting from the (*S*,*S*)-norbornene-5,6-dicarboxylic acid, 0.177 g (35% yield) of an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.90 (t, 1H), 4.10 (t, 2H), 4.00 (t, 2H), 3.48 (s, 1H), 3.38 (m, 2H), 2.80 (m, 1H), 2.10 (m, 1H), 1.95 (m, 1H), 1.85–1.65 (m, 5H), 1.55 (d, 1H), 0.95 (two t, 6H). HRMS m/z = 373.18714 (MH^+), calcd 373.18703. HPLC retention time: 4.37 min.

5.7. Potassium-3-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-6-hydroxy-bicyclo[2.2.1]heptane-2-carboxylate (5)

Combined 0.113 g of **4a** (0.303 mmol) and 0.020 g of KOH (0.303 mmol) in 2 mL of water and heated to 50 °C for 24 h. Added 2 mL of CH_2Cl_2 and stirred vigorously for 2 h. Separated layers and concd water layer in vacuo to give quant. yield of an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 4.10 (m, 1H), 3.95 (t, 2H), 3.85 (t, 2H), 2.90 (d, 1H), 2.40 (m, 2H), 2.10 (dt, 1H), 1.70 (q, 2H), 1.55 (m, 3H), 1.20–1.00 (m, 2H), 0.95 (two t, 6H). HRMS m/z = 391.19770 (MH^+), calcd 391.19760. HPLC retention time: 3.54 min.

5.8. 8-(5-Hydroxy-4-oxa-tricyclo[4.2.1.0^{3,7}]non-9-yl)-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (6)

To a chilled solution (−78 °C) of **4a** (0.200 g, 0.537 mmol) in 3 mL of CH_2Cl_2 was added 1.07 mL (1.07 mmol) of a 1.0 M solution of DIBAL in CH_2Cl_2. The reaction mixture was stirred at −78 °C for 2 h and quenched by the addition of 3 mL of satd $NaHCO_3$. The reaction mixture was allowed to warm to rt, diluted with 4 mL of water and extracted with three 5-mL portions of CH_2Cl_2. The combined organic fractions were dried with Na_2SO_4 and concd in vacuo to give 0.201 g (65% yield) of an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.40 (s, 1H), 5.15 (m, 1H), 4.55 (m, 1H), 4.10–3.90 (m, 4H), 3.15–2.70 (m, 3H), 2.50–2.40 (m, 1H), 1.90 (m, 1H), 1.80–1.50 (m, 5H), 1.35 (m, 2H), 0.90 (two t, 6H). HRMS m/z = 375.20272 (MH^+), calcd 375.20268. HPLC retention time: 3.60 min.

5.9. 8-(4-Oxa-tricyclo[4.2.1.0^{3,7}]non-9-yl)-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (7)

Dissolved **6** (0.201 g, 0.537 mmol) in 2 mL TFA and added Et_3SiH (0.215 mL, 1.34 mmol) dropwise while stirring. Stirred at rt for 1 h, concd in vacuo, and purified by prep HPLC isocratic method (40% CH_3CN/60% water at 15 mL/min, 40 min) retention time 22.44 min. Obtained 0.049 g (25% yield) of an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.40 (dt, 2H), 4.05 (t, 2H), 3.95 (t, 2H), 3.85–3.75 (m, 2H), 3.07 (q, 1H), 2.70 (m, 2H), 2.40 (s, 1H), 1.90 (d, 1H), 1.80–1.60 (m, 5H), 1.43 (d, 2H), 0.90 (two t, 6H). ^{13}C NMR (600 MHz, $CDCl_3$) δ (ppm) 157.88, 157.28, 155.66, 150.69, 106.68, 78.87, 73.18, 49.46, 46.42, 45.77, 44.70, 43.45, 41.06, 40.86, 35.83, 21.43, 21.31, 11.41, 11.14. HRMS m/z = 359.20786 (MH^+), calcd 359.20777. HPLC retention time: 4.46 min.

5.10. 2-Hydroxy-5-oxo-4-oxa-tricyclo[4.2.1.0^{3,7}]nonane-9-carboxylic acid (8)[16] 8-(2-Hydroxy-5-oxo-4-oxa-tricyclo[4.2.1.0^{3,7}]non-9-yl)-1,3-dipropyl-3,7-dihydro-purine-2,6-dione (10)

Off-white solid ^{1}H NMR (400 MHz, MeOH-d_4) δ 4.40 (s, 1H), 3.90 (t, 2H), 3.85 (t, 2H), 3.65 (s, 1H), 3.15 (s, 1H), 3.05 (s, 1H), 2.70 (s, 1H), 1.90 (m, 2H), 1.70 (q, 2H), 1.60 (q, 2H), 0.85 (two t, 6H). HRMS m/z = 389.18207 (MH^+), calcd 389.18195. HPLC retention time: 3.72 min.

5.11. 3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2. 1]hept-5-ene-2-carboxylic acid (12)

To a stirred mixture of 2.50 g (12.7 mmol) of **11**, 3.33 g (12.7 mmol) of 5,6-diamino-1,3-dipropyl-1*H*-pyrimidine-2,4-dione hydrochloride,[3] 8.8 mL (50.8 mmol) of (i-Pr)$_2$NEt, and 30 mL anhydrous DMF was added 4.83 g (12.7 mmol) of HATU.[15] The reaction solution was stirred at rt for overnight. The reaction mixture was concd in vacuo to a volume of 10 mL and combined with 150 mL EtOAc. The resultant solution was washed three times with 20 mL of 1 N HCl and twice with satd brine. The organic fraction was concd in vacuo and the resultant solid was combined, in a 250-mL round-bottomed flask equipped with a condenser, with a mixture of 60 mL of i-PrOH, 4.20 g KOH, and 35 mL of water and heated to reflux. After heating for 3 h, the reaction was concd to a 30 mL volume and acidified with 1 N HCl. The mixture was extracted with three 25-mL portions of EtOAc, dried over Na_2SO_4, and concd in vacuo to give a solid which after reprecipitation from a NaOH solution with 1 N HCl gave 2.72 g (58% yield) of an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.35 (s, 1H),

6.30 (m, 1H), 6.20 (m, 1H), 4.15–3.95 (m, 3H), 3.39 (s, 1H), 3.33 (s, 1H), 2.70 (d, 1H), 1.85–1.40 (m, 7H), 0.95 (m, 6H). $m/z = 373.09$ (MH^+). HPLC retention time: 3.97 min.

5.12. 3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)- bicyclo[2.2.1]heptane-2-carboxylic acid (13)

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.10 (dd, 2H), 4.00 (dd, 2H), 3.75 (t, 1H), 3.05 (d, 1H), 2.78 (m, 2H), 1.88–1.60 (m, 5H), 1.60–1.38 (m, 4H), 1.25 (d, 3H), 0.95 (two t, 6H). HRMS $m/z = 375.20274$ (MH^+), calcd 375.20268. HPLC retention time: 4.35 min.

5.13. 3 -(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2. 1]heptane-2-carboxylic acid methyl ester (14)

Dissolved **12** (0.050 g) in 5.0 mL of anhydrous MeOH and added 3 drops concd H_2SO_4. The solution was stirred at rt for 24 h. The reaction mixture was concd in vacuo and the resultant oil was dissolved in 10 mL EtOAc. The organic layer was washed with 10 mL of satd $NaHCO_3$ and then 10 mL of brine, dried over Na_2SO_4, and concd to dryness. The solid product was then dissolved in 6.0 mL of degassed *i*-PrOH and combined with 8 mg of 5% Pd/C. The reaction mixture was flushed 3 times with hydrogen and stirred for 20 h under a balloon of hydrogen at rT. The reaction mixture was then filtered through a plug of silica and concd in vacuo to give 0.048 g (>99% yield) of a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.65 (s, 1H), 4.14 (dd, 2H), 4.04 (dd, 2H), 3.77 (t, 1H), 3.70 (s, 3H), 3.30 (d, 1H), 2.82 (m, 1H), 2.67 (m, 1H), 1.90–1.58 (m, 5H), 1.55–1.28 (m, 4H), 1.22 (d, 1H), 0.95 (two t, 6H). ^{13}C NMR (600 MHz, $CDCl_3$) δ (ppm) 166.01, 154.54, 153.89, 150.28, 150.17, 105.71, 51.18, 45.51, 45.04, 44.14, 43.09, 42.08, 41.64, 40.82, 37.40, 27.76, 20.44, 20.27, 10.32, 10.18. HRMS $m/z = 389.21845$ (MH^+), calcd 389.21833. HPLC retention time: 5.49 min.

Acknowledgment

We thank Dr. John Shryock (UFla) for determining rat receptor affinities for selected compounds, Dr. Herman van Vlijmen for molecular modeling advice, and Dr Maria Pellegrini and Dr Xiaoping Hronowski for aid in structural characterization.

References and notes

1. Fredholm, B. B.; IJzerman, A. P.; Jacobson, K. A.; Klotz, K. N.; Linden, J. *Pharmacol. Rev.* **2001**, *53*, 527–552.
2. Belardinelli, L.; Shryock, J. C.; Zhang, Y.; Scammells, P. J.; Olsson, R.; Dennis, D.; Milner, P.; Pfister, J.; Baker, S. P. *J. Pharmacol. Exp. Ther.* **1995**, *275*, 1167–1176; Dhalla, A. K.; Shryock, J. C.; Shreeniwas, R.; Belardinelli, L. *Curr. Top. Med. Chem.* **2003**, *3*, 369–385; For reviews of A_1 SAR see: Müller, C. E.; Stein, B. *Curr. Pharm. Des.* **1996**, *2*, 501–530; Jacobson, K. A.; van Galen, P. J. M.; Williams, M. *J. Med. Chem.* **1992**, *35*, 407–422; Shimada, J.; Suzuki, F.; Nonaka, H.; Ishii, A. *J. Med. Chem.* **1992**, *35*, 924–930.
3. Profiled as CVT-124 (a.k.a.1,3-dipropyl-8-[2-(5,6-epoxy)norbornyl]xanthine) in Pfister, J. R.; Belardinelli, L.; Lee, G.; Lum, R. T.; Milner, P.; Stanley, W. C.; Linden, J.; Baker, S. P.; Schreiner, G. *J. Med. Chem.* **1997**, *40*, 1773–1778.
4. Wolff, A. A.; Skettino, S. L.; Beckman, E.; Belardinelli, L. *Drug Dev. Res.* **1998**, *45*, 166–171.
5. Kiesman, W. F.; Petter, R. C. *Tetrahedron: Asymmetry* **2002**, *13*, 957, describes methods for the asymmetric synthesis of the (*R*,*R*)-norbornene-5,6-dicarboxylic acid.
6. (a) Janssen, A. J. M.; Klunder, A. J. H.; Zwanenburg, B. *Tetrahedron* **1991**, *47*, 5513; (b) Hamanaka, N.; Seko, T.; Miyazaki, T.; Naka, M. *Tetrahedron Lett.* **1989**, *9*, 2399.
7. (a) Winterfeldt, E. *Synthesis* **1975**, *7*, 617; (b) Takacs, J. M.; Helle, M. A.; Seely, F. L. *Tetrahedron Lett.* **1986**, *27*, 1257.
8. Kraus, G. A.; Molina, M. T.; Walling, J. A. J. S. *Chem. Commun.* **1986**, 1568.
9. (a) For initial screening a solution of the antagonist (1 μM) was incubated with membranes in 50 mM HEPES, pH 7.4, 1 mM EDTA, 5 mM $MgCl_2$, and 1 U/mL adenosine deaminase. DMSO was included in all assays excepting A_3 at a final concentration of 5%. Radioligands consisted of: A_1, 0.3 nM ^{125}I-aminobenzyladenosine (^{125}I-ABA); A_{2A}, 0.7 nM ^{125}I-ZM241385; A_{2B}, 0.5 nM ^{125}I-3-(4-aminobenzyl)-8-phenyloxyacetate-1-propyl-xanthine; and A_3, 0.6 nM ^{125}I-ABA. Nonspecific binding was measured in the presence of 50 μM xanthine amino congener or 10 μM BW-1433 (A_3).; (b) Compounds were incubated at room temperature for 90 min with radioligand (2 nM 3H-CPX for A_1; 0.5–1.2 nM 3H-ZM241385 for A_{2A}), 50 mM Tris–HCl buffer (pH 7.4), adenosine deaminase (2 U/mL), and 100-μL aliquots of crude membrane suspensions (10–20 μg protein) prepared from either rat brain cortex (for A_1) or rat brain striatum (for A_{2A}). Incubations were terminated by addition of ice-cold 50 mM Tris–HCl buffer and collection of membranes onto Whatman GF/C glass fiber filters by vacuum filtration. Membrane-bound radioactivity was quantified by liquid scintillation counting. Values of K_i were determined from concentration–response relationships for each compound to displace binding of radioligand, using GraphPad Prism (GraphPad, San Diego, CA).
10. Linden, J. *J. Cyclic Nucleotide Res.* **1982**, *8*, 163–172.
11. Rat oral efficacy screen: the rats were placed into metabolic cages and dosed by gavage with various doses of antagonist. The doses and group sizes were: vehicle (0.5% carboxymethylcellulose [CMC]) ($n = 3$); antagonist: 2.0 mg/kg for **4a** and **4d** and 0.3 mg/kg for **7** and **10** ($n = 3$). Urine was collected for 4 h after dosing. Urine volume was measured gravimetrically, and sodium (Na) and potassium (K) concentrations were determined by flame photometry. Urine flow (UV), sodium excretion (UNaV), and potassium excretion (UKV) were calculated and are shown as units per hour, as an average for the 4-h collection period.
12. *Isolation of atria from rat heart*. Hearts were removed from the rats, placed in petri dishes containing Krebs–Henseleit (Krebs) buffer pre-warmed to 37 °C, and bubbled with 95% O_2/5% CO_2. The composition of Krebs buffer was 118 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 25 mM $NaHCO_3$, 1.2 mM KH_2PO_4, 2.5 mM $CaCl_2$, and 11 mM glucose, pH 7.4. The right atrium was dissected and cleaned of surrounding myocardial and vascular tissue. Two lengths of thread were attached at opposite ends of the atrium. One thread anchored the tissue to a glass rod and the other was connected to an isometric force

transducer. The tissue was suspended in a water-jacketed reservoir warmed to 37 °C and bubbled with 95% O_2/5% CO_2. A pre-load tension of 2 g was applied using a pre-calibrated Gould recorder. Hung tissue was washed with warm, oxygenated Krebs buffer, while maintaining 2 g of tension. Baseline heart rate was measured on Ponemah software from Gould Instruments (Valley View, Ohio). *Determination of EC_{50} of A_1 antagonists using the CPA dose-reversal paradigm*. Isoproterenol (30 nM) was added to all baths containing atria to increase the baseline heart rate to between 350 and 400 beats per minute (bpm). Following rate stabilization, 130 nM CPA was added to baths to cause a 75% reduction in atrial beating rate (control 0). Increasing concentrations of antagonist were then added to the baths until the rate was restored to maximum, and the effective concentration at which 50% response was obtained (EC_{50}) was determined. Five atria were used in this experiment.

13. Unpublished results the subject of a forthcoming publication.
14. Petter, Russell C.; Kiesman, William F.; Conlon, Patrick R.; Kumaravel, Gnanasambandam; Ensinger, Carol L.; Dowling, James; Peng, Bo; Smits, Glenn; Jin, Xiaowei; Lutterodt, Frank A.; Fu, Kai; LePage, Doreen; Jayaraj, Andrew; Gill, Alan; Costa, Don; Wortham, Kathy; Porter, Kaela; Linden, Joel; Sullivan, Gail. *Abstracts of Papers*, 224th National Meeting of the American Chemical Society, Boston, MA, American Chemical Society: Washington, DC, 2002; MEDI-417.
15. HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate.
16. Beilstein Registry # 73036-39-2.