Potent and Orally Bioavailable 8-Bicyclo[2.2.2]octylxanthines as Adenosine A_1 Receptor Antagonists

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In the search for a selective adenosine A_1 receptor antagonist with greater aqueous solubility than the compounds currently in clinical trials as diuretics, a series of 1,4-substituted 8-cyclohexyl and 8-bicyclo-[2.2.2]octylxanthines were investigated. The binding affinities of a variety of cyclohexyl and bicyclo[2.2.2]-octylxanthines for the rat and human adenosine A_1, A_{2A}, A_{2B}, and A_3 receptors are presented. Bicyclo[2.2.2]octylxanthine **16** exhibited good pharmaceutical properties and in vivo activity in a rat diuresis model (ED_{50} = 0.3 mg/kg po). Optimization of the bridgehead substituent led to propionic acid **29** (BG9928), which retained high potency (hA_1, K_i = 7 nM) and selectivity for the adenosine A_1 receptor (915-fold versus adenosine A_{2A} receptor; 12-fold versus adenosine A_{2B} receptor) with improved oral efficacy in the rat diuresis model (ED_{50} = 0.01 mg/kg) as well as high oral bioavailability in rat, dog, and cynomolgus monkey.

Introduction

Adenosine, a metabolite of ATP with a variety of intra- and extracellular signaling functions, is released from cells under ischemic or hypoxic conditions.[1] Although a transient signaling molecule with a plasma half-life under a few seconds,[2] adenosine exerts a plethora of pharmacologic effects via four G-protein coupled adenosine receptors: A_1, A_{2A}, A_{2B}, A_3. The adenosine receptor subtypes belong to a family of rhodopsin-like receptors that contain seven transmembrane helical domains linked by three intracellular and three extracellular loops.[3] The alpha-helices of the adenosine A_1 receptor designated HI through HVII form a ligand binding pocket.[4] Site-directed mutagenesis studies have postulated direct interaction of adenosine with transmembrane domains, while the third intracellular loop and the carboxyl terminus interact with G_i proteins.[5] The adenosine A_1 receptors in nervous tissues, heart, and kidney modulate neurotransmitter release, heart rate, and renal hemodynamics, respectively.[6] Antagonists have been examined clinically as renal protective agents and also as possible treatments for congestive heart failure.[7]

There are many examples of potent adenosine A_1 antagonists that contain bulky lipophilic substitution at the 8-position of 1,3-dipropylxanthines (Figure 1).[8,9] The highest affinity xanthine-based molecules pictured in Figure 1 lack appreciably polar substituents. The utility of most of these compounds for intravenous administration in the treatment of acutely decompensated congestive heart failure patients in the clinic, however, may be limited because of their low water solubility. An exception to the general property of low solubility among potent A_1 antagonists is the 8-aryl-substituted xanthine amine congener (XAC) first described by Jacobson et al.[10a] XAC has long been used in the elucidation of the pharmacologic actions of adenosine A_1 receptors in living systems and possesses moderate aqueous solubility (90 μM in 0.1 M sodium phosphate at pH 7.2; Figure 2). Numerous reports describe a variety of XAC derivatives with linear substitution patterns (i.e., 1,4-disubstitution on the aryl ring attached to the 8-position of the xanthine) and their effects on the binding affinities and selectivities.[11] Olah et al. suggested

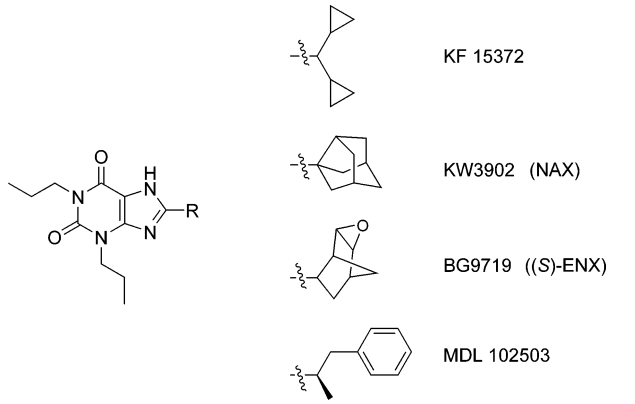

Figure 1. Adenosine A_1 receptor antagonists containing bulky lipophilic substitution at the 8-position of 1,3-dipropylxanthine.

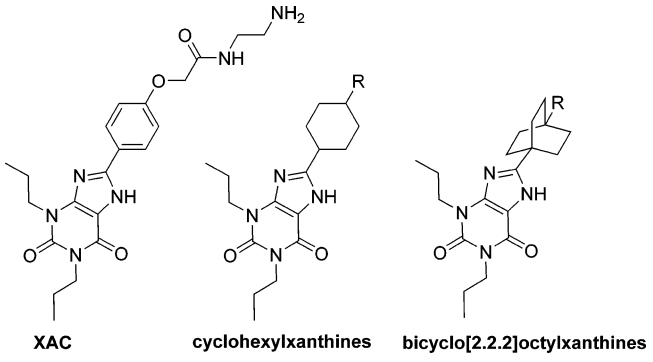

Figure 2. Xanthine amine congener (XAC), cyclohexylxanthine-, and bicyclo[2.2.2]octylxanthine targets with linear substitution patterns.

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that amino acids in an 11-residue segment of the second extracellular loop of the adenosine A_1 receptor may directly interact with antagonist ligands and lead to the high binding affinities.[5d] In addition, covalent attachment of XAC-related compounds to the receptor through reactive functional groups[10d,12] and a number of modeling studies[13] suggested that the xanthine portion of the antagonist binds deep within the receptor transmembrane binding cleft and that the 8-position on the xanthine ring system is oriented outward toward the membrane surface. This binding mode suggests that tethered polar substituents might be introduced without greatly affecting binding affinity. Despite this promising lead in the 8-aryl series, relatively little work has been done to examine in a systematic way the effects of linear substitution on saturated carbocycles at the xanthine 8-position. One notable exception is the examination of binding affinities and adenylate cyclase activity of a small group of cyclopentyl- and cyclohexyl-substituted xanthines by Wells and colleagues.[14] We have expanded the examination of this class of ligands and herein describe the SAR of 8-cyclohexyl and 8-bicyclo[2.2.2]octylxanthines that contain linear substitution patterns (Figure 2). Also presented are data regarding in vivo efficacy and bioavailability of some of the most potent of these adenosine A_1 receptor antagonists.

Chemistry

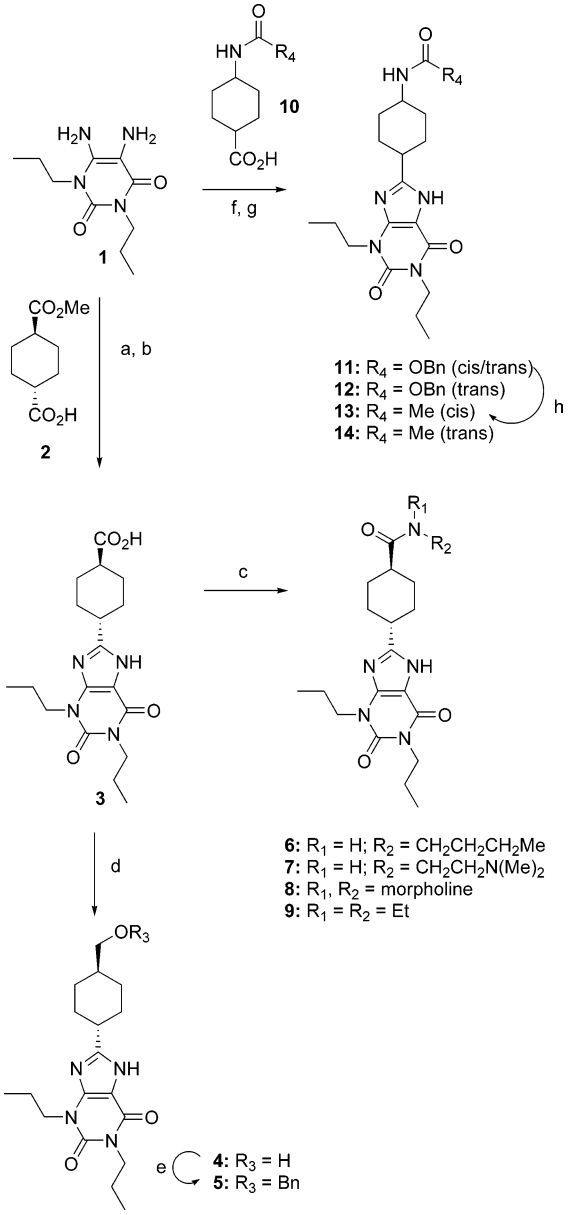
The targeted 8-cyclohexyl-substituted xanthines were prepared in the classical 2-step procedure outlined in Scheme 1. *trans*-Cyclohexane-1,4-dicarboxylic acid monomethyl ester (**2**)[15] was coupled with 5,6-diamino-1,3-dipropyl-1*H*-pyrimidine-2,4-dione (**1**)[16] via a HATU-mediated (*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate) amidation. Subsequent ring closure and dehydration to form xanthine **3** occurred in hot 1 N KOH/isopropyl alcohol. Yields for the 1,3-dipropylxanthines ranged from 40 to 95% overall for the two-step process. Acid **3** was subjected to a second coupling reaction to produce amides **6**−**9**. Reduction of **3** with BH_3−THF gave alcohol **4**, which was then capped by reaction with benzyl bromide under basic conditions to give benzyl ether **5**. A similar coupling−cyclization sequence with the protected cyclohexyl amino acid **10** and **1** was followed to produce compounds **11** and **12** as a mixture of *cis* and *trans* isomers. Hydrogenation of **11** with in situ trapping with acetic anhydride and chromatographic separation gave acetyl derivatives **13** and **14**.

The analogous 8-bicyclo[2.2.2]octyl-substituted xanthine **16** was prepared in the same manner to the cyclohexyl acid **3** (Scheme 2.). Bicyclo[2.2.2]octane-1,4-dicarboxylic acid monomethyl ester (**15**) was obtained commercially and also synthesized by literature procedures.[17] Again, a subsequent coupling reaction of **16** with a variety of amines gave amides **17**−**22**. Coupling of **1** with the pentyl-substituted acid **23** and base-induced cyclization gave compound **24**. Esterification of acid **16** with acidic MeOH gave ester **25**, which underwent a clean $LiBH_4$ reduction to alcohol **26** (Scheme 3). Treatment of **26** with Dess−Martin periodinane (DMPI) produced aldehyde **27**, which served as a common starting material for a series of homologs of acid **16**. Wittig-type olefinations with a series of phosphonates added one, two, or three carbon atoms between the bridgehead position and the carboxylic acid in compounds **28**, **29**, **31**, and **33**.

Results and Discussion

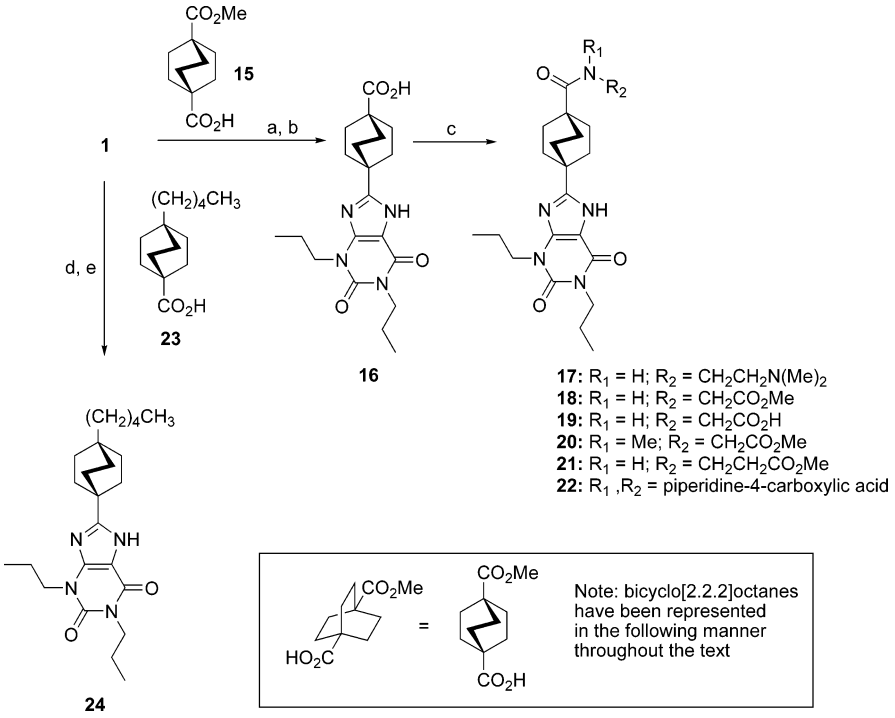
Our attention was initially drawn to the 8-cyclohexyl derivatives by an article published by Wells and co-workers.[14b] His work described a series of 1,4-substituted cyclohexanes that showed some promise as moderately selective adenosine A_1 antagonists and employed binding affinity determinations with a mix of rat-derived A_1 receptors and human platelet-derived A_{2A} receptors, which were available at the time. We chose to examine the binding affinities of the target compounds with the four available cloned human adenosine receptors (hA_1, hA_{2A}, hA_{2B}, and hA_3). The biological activities of the antagonists were evaluated by the following procedures. The primary screen 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 one of the four human adenosine receptor subtypes (hA_1, hA_{2A}, hA_{2B}, and hA_3).[18] Membranes were incubated at room temperature for 2 h with ^{125}I-labeled radioligands, competing antagonists, and 1 U/mL adenosine deaminase, filtered over glass fiber filters, and retained radioactivity counted in a γ-counter. Nonspecific binding was measured in the presence of 50 μM XAC or 10 μM BW-1433 (hA_3). Data are presented as percent (%) of

Scheme 1. Synthesis of 8-Cyclohexylxanthines[a]



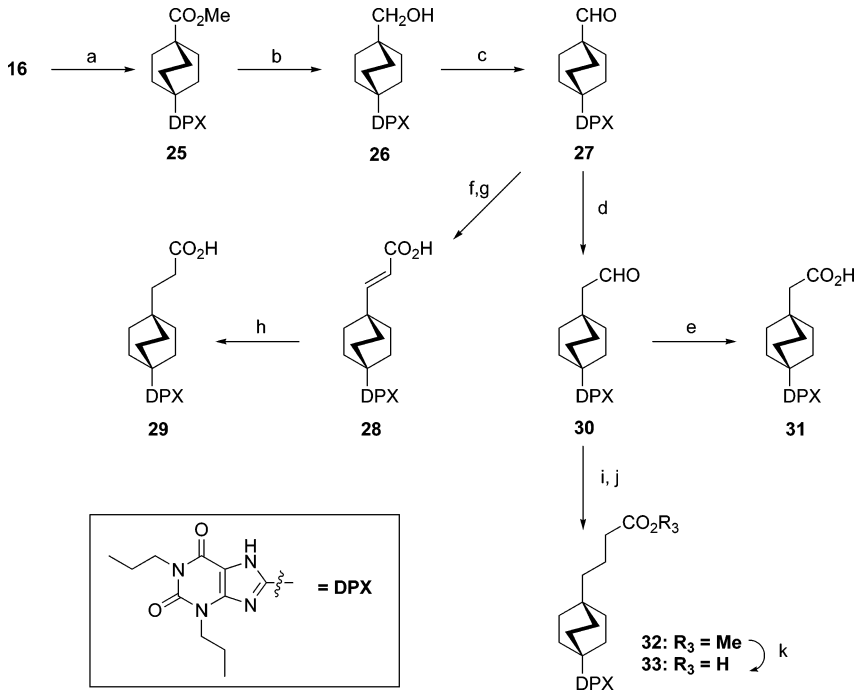
[a] Reagents and conditions: (a) acid **2**, NEt_3, HATU, CH_3CN; (b) 1 N KOH, *i*-PrOH/H_2O (1:1), reflux; (c) amine, NEt_3, HATU, CH_3CN; (d) 1.0 M BH_3-THF, 40 °C, THF; (e) 1.0 M *t*-BuOK, THF, BnBr; (f) acid **10**, NEt_3, HATU, CH_3CN; (g) 1 N KOH, *i*-PrOH:H_2O (1:1), reflux; (h) H_2, 5% Pd/C, MeOH, Ac_2O (isomers separated by prep HPLC).

Scheme 2. Synthesis of Bicyclo[2.2.2]octylxanthines[a]



[a] Reagents and conditions: (a) acid **15**, NEt_3, HATU, CH_3CN, 25 °C; (b) 1 N KOH, *i*-PrOH/H_2O (1:1), reflux; (c) amine, NEt_3, HATU, CH_3CN, 25 °C; (d) acid **23**, NEt_3, HATU, CH_3CN, 25 °C; (e) 1 N KOH, *i*-PrOH/H_2O (1:1), reflux.

Scheme 3. Synthesis of Higher Homologs of Carboxylic Acid **16**[a]

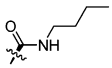
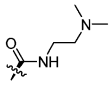


[a] Reagents and conditions: (a) H_2SO_4, MeOH, 25 °C; (b) $LiBH_4$, MeOH, THF, reflux; (c) DMPI, CH_2Cl_2, 25 °C; (d) methoxymethyl triphenylphosphonium chloride, KHMDS, toluene/THF, −78 °C, then hydrolysis with 1 N HCl at 25 °C; (e) *t*-BuOH, 2-methyl-2-butene, $NaClO_2$, 0 °C to 25 °C; (f) trimethyl phosphonoacetate, KHMDS, toluene, 0 °C; (g) KOH, MeOH/H_2O, reflux; (h) H_2, Pd/C, MeOH/H_2O; (i) methoxymethyl triphenylphosphonium chloride, KHMDS, toluene/THF, −78 °C; (j) H_2, Pd/C, EtOH; (k) 1 M LiOH, THF, 25 °C.

radioligand bound in the presence of target compound relative to control. Compounds that displayed good hA_1 binding activity in the single-point assays were further evaluated to determine IC_{50} values and inhibition constants (K_i values).[19] Duplicate full binding curves were derived from antagonist concentrations that ranged from 10^{-11}−10^{-5} M. The binding affinities for rat A_1 (rA_1) and A_{2A} (rA_{2A}) receptors were also determined for specific compounds that exhibited high human adenosine A_1 receptor binding affinity. Compounds were incubated with either ^{3}H-labeled radioligand (DPX or ZM241385) and aliquots of crude membrane suspensions 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 specific radioligands.[18]

Our testing of the *trans*-4-carboxylic acid **3** gave a low binding affinity (31% in the single-point assay; estimated K_i >

Table 1. Adenosine Receptor Binding Affinities for Cyclohexyl-Substituted Derivatives of 1,3-Dipropylxanthine

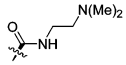
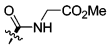
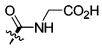
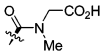
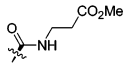
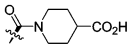
			K_i (nM)[a] or % of specific radioligand binding[b]				
Compd	R	1,4-stereochem	hA_1	hA_{2A}	hA_{2B}	hA_3	hA_{2A}/hA_1
3		*trans*	(31%)	(75%)	(69%)	(88%)	--
4		*trans*	41	313	(18%)	(77%)	8
5		*trans*	171	2720	(28%)	(95%)	16
6		*trans*	46	2260	(11%)	(93%)	49
7		*trans*	12	168	(16%)	(91%)	14
8		*trans*	(60%)	(99%)	(48%)	(88%)	--
9		*trans*	(46%)	(70%)	(39%)	(100%)	--
11		*cis/trans*	(23%)	(69%)	(30%)	(100%)	--
12		*trans*	109	2960	(24%)	(86%)	27
13		*cis*	(60%)	(63%)	(46%)	(100%)	--
14		*trans*	(45%)	(92%)	(38%)	(100%)	--

[a] All K_i values were calculated from binding curves generated from the mean of four determinations per concentration (seven antagonist concentrations), with the variation in individual values of <15%. [b] Data are presented as percent (%) of radioligand bound in the presence of target compound relative to control.

500 nM) relative to the result reported by Wells and co-workers (rA_1 = 59 nM). This result was not surprising because it has been our experience that, in general, the same antagonist can have a 10-fold higher affinity for the rA_1 receptor versus the hA_1 receptor. Jacobson et al., in examining a series of 8-arylxanthines, also observed a loss in hA_1 activity with the introduction of a 4-carboxyl group.[10a] Alcohol **4** had increased hA_1 and hA_{2A} affinity when compared to the acid (Table 1). Capping with a benzyl group decreased hA_1 affinity by about 4-fold but had a greater negative effect on hA_{2A} affinity (8-fold loss). This result suggested that the hA_{2A} receptor was less able to accommodate the nonpolar benzyl group in the outer region of the receptor (i.e., near the membrane surface). Examples of tertiary amides in the 4-position, compounds **8** and **9**, showed poor binding. The *n*-butyl amide **6**, with an N−H in the analogous position to the O−H in **4**, had about the same affinity for the hA_1 receptor as alcohol **4** but was more selective versus hA_{2A} (hA_{2A}/hA_1 ratio = 49 vs 8). Substitution with the basic *N*,*N*-dimethylethylenediamine gave **7**, the most potent hA_1 antagonist of this series, with a hA_1 K_i = 12 nM and significant activity against the hA_{2A} receptor (K_i = 168 nM). This observation mirrors Wells' results in the cyclohexyl series and indicated that amino substitution at the terminus was well received by both hA_1 and hA_{2A} receptors. Acylamino substitution on the cyclohexyl ring (with *cis* or *trans* stereochemistry) produced compounds with low affinities (**13**, **14**). The *trans* benzylcarbamate **12** had modest hA_1 activity (109 nM) and selectivity against all of the human receptors similar to that of benzyl ether **5** (see Table 1).

Addition of a two-carbon bridge linking the 1- and 4-positions across the cyclohexane ring gave bicyclo[2.2.2]octane derivatives with added steric bulk at the 8-position and no stereochemical complexity (Figure 2). The first example, **16**, despite bearing a bridgehead carboxylic acid, demonstrated surprisingly

Table 2. Adenosine Receptor Binding Affinities for Bicyclo[2.2.2]octyl-Substituted Derivatives of 1,3-Dipropylxanthine

		K_i (nM)[a] or % of specific radioligand binding[b]					
Compd	R	hA_1	hA_{2A}	hA_{2B}	hA_3	hA_{2A}/hA_1	hA_{2B}/hA_1
16		33	1070	(48%)	(100%)	32	--
17		6	132	(3%)	(79%)	22	--
18		8	681	207	6700	85	26
19		49	7880	(53%)	(70%)	161	--
20		112	>10000	296	(88%)	>89	3
21		22	1400	505	>10000	63	23
22		96	7820	(41%)	(100%)	81	--
24		(25%)	(79%)	(100%)	(100%)	--	--
25		9	912	(19%)	(44%)	101	--
26		16	414	(27%)	(73%)	26	--
27		15	799	(14%)	(76%)	53	--

[a] All K_i values were calculated from binding curves generated from the mean of four determinations per concentration (seven antagonist concentrations), with the variation in individual values of <15%. [b] Data are presented as percent (%) of radioligand bound in the presence of target compound relative to control.

good hA_1 affinity (33 nM; Table 2), a marked improvement over its cyclohexyl congener **3** (estimated K_i > 500 nM), and was in direct contrast to the negative effects of acid substitution noted with 8-arylxanthines.[10a] To probe the area beyond the bicycle for polar binding interactions that would differentiate between the hA_1 and the hA_{2A} receptors, a series of amides were prepared that tethered amines, acids, and esters with a variety of methylene spacers. The *N*,*N*-dimethylethylenediamine amide **17** had a hA_1 binding affinity similar to the cyclohexyl variant **7** and also remarkably similar hA_{2A} affinity (132 nM vs 168 nM). The terminal amine substitution offered no selectivity enhancement, so a series of carboxylic acids were then examined. The glycine methyl ester analog **18** maintained hA_1 affinity (8 nM) and had a hA_{2A}/hA_1 selectivity ratio = 85, similar to that of the bridgehead methyl ester **25**; hA_{2A}/hA_1 = 100. Methylation of the amide nitrogen (**20**) or installation of piperidine-4-carboxylate (**22**) led to >10-fold hA_1 affinity losses. Insertion of a methylene spacer gave **21** and led to a 3-fold loss in hA_1 potency and had a smaller effect on hA_{2A} binding affinity. The glycine-free-acid analog (**19**) on the other hand gave the most hA_1-selective example with the hA_{2A}/hA_1 selectivity ratio of 160. It appeared that proper placement of the carboxylate in 8-bicyclo[2.2.2]octyl xanthines markedly decreased hA_{2A} affinities and generally maintained the hA_1 binding properties. Other less-polar examples, alcohol **26** and aldehyde **27**, had better hA_1 affinities, but had selectivity ratios similar to acid **16**. Replacement of the carboxylic acid with a pentyl chain (**24**) dramatically diminished the hA_1 binding affinity (>500 nM). This result suggested that there were either polar pockets within the adenosine receptors in regions between the xanthine binding domain and the cell surface or that acid **16** had a significantly different binding mode.

Bicyclo[2.2.2]octyl structures (sans amide linkages) with carboxylic acids of various lengths attached to the bridgehead position were investigated (Table 3). The addition of a methylene spacer between the bridgehead position and the carboxylic acid in **16** led to a 3-fold loss in rA_1 affinity (**31**). Insertion of a *trans*-double bond, compound **28**, increased A_1 affinity to single-digit nanomolar and imparted a 10-fold increase in hA_1 selectivity over hA_{2A}: ratio = 333. The saturated propionic acid analog **29** had similar hA_1 affinity (7.4 nM) but extraordinary selectivity (915-fold) over the hA_{2A} receptor. Evidently, the hA_{2A} receptor was unable to accommodate the modest increase in bulk of the alkyl linker. Further elongation of the chain with an additional methylene spacer gave butyrate **33** that had binding affinities and selectivities similar to acid **16**. All of the acids

Table 3. Carboxylic Acid Substitution at the Bridgehead Position of Bicyclo[2.2.2]octylxanthines

		K_i (nM)[a] or % of specific radioligand binding[b]					
Compd	R	hA_1	hA_{2A}	hA_{2B}	hA_3	hA_{2A}/hA_1	hA_{2B}/hA_1
16		33 *(7.8) rat*[c]	1070	(48%)	(100%)	32	--
28		9.6	3330	100	(100%)	333	10
29		7.4 *(1.3) rat*[c]	6410 *(2440) rat*[c]	90	>10000	915	12
31		*(22.5) rat*[c]	*(8960) rat*[c]	--	--	--	--
33		29 *(4.0) rat*[c]	*(50%) rat*[c]	127	(26%)	--	4
(*S*)-ENX	--	12 *(0.7) rat*[d]	1660 *(1250) rat*[d]	611	4810	138	51
NAX	--	8.0	673	296	4390	84	37

[a] All K_i values were calculated from binding curves generated from the mean of four determinations per concentration (seven antagonist concentrations), with the variation in individual values of <15%. [b] Data are presented as percent (%) of radioligand bound in the presence of target compound relative to control. [c] 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}). [d] (*S*)-ENX: (1,3-dipropyl-8-[2-(5,6-exo-epoxy-(1*S*,2*S*)-norborn-2-yl)]-xanthine) rat values from ref 9. [e] NAX : 3-noradamantyl-1,3-dipropylxanthine.

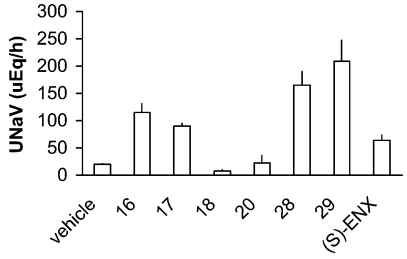


Figure 3. Rat oral efficacy screen: measurement of UNaV in μEq/h (mean ± SEM), in a 4-hour period, of a 0.3 mg/kg oral dose of antagonist in a 0.5% CMC suspension.

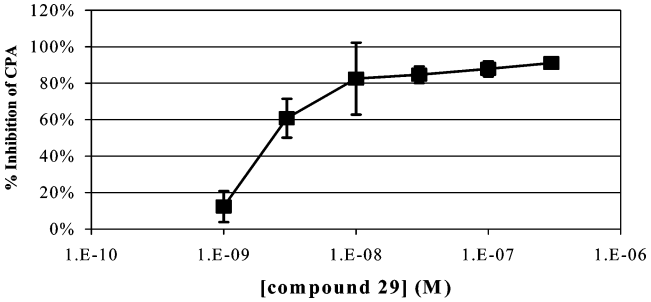


Figure 4. Reversal effect (inhibition) of increasing concentrations of compound **29** on CPA (130 nM) suppression of the isoproterenol-stimulated (30 nM) heart rate in beating, isolated rat atria. (5 atria/dose group).

exhibited virtually no adenosine hA_3 receptor binding at concentrations up to 1 μM. The most potent hA_1 antagonists, **28**, **29**, and **33**, all had some cross activity (~100 nM) against the hA_{2B} receptor. Antagonism of this ubiquitously expressed low-affinity adenosine receptor is thought to play a beneficial role in ischemic preconditioning of the heart under hypoxic conditions and modulation of mast cell degranulation in asthmatics.[20] Propionic acid **29** had similar hA_1 affinity when compared to previous clinical compounds, (*S*)-ENX and NAX, but better hA_{2A} selectivity (>7-fold higher).

Biological evaluations of the most potent hA_1 antagonists in both the cyclohexyl and bicyclo[2.2.2]octyl series were performed. Oral activity was assessed in a rat diuresis model at a fixed dose of 0.3 mg/kg. The test article was delivered by gavage as a 0.5% carboxymethylcellulose (CMC) suspension to rats housed in metabolic cages. Over a 4-hour period, urine was collected and Na and K excretions (determined as microequivalents) were measured by FIS. The results for selected compounds appear in Figure 3. Despite a 10-fold difference in rA_1 binding affinity between the bridgehead carboxylate **16** and the (*S*)-ENX, the rat urinary sodium excretion (UNaV) values were similar. The *N*,*N*-dimethylethylenediamine amide **17** also showed good in vivo activity, in contrast to the amides that possessed a terminal carboxylic acid or ester (**18**, **20**). It is noteworthy that the bridgehead carboxylate **16**, some 5-fold less-active in vitro than amine **17**, exhibited better in vivo efficacy. Propi-

Table 4. Pharmacokinetic Parameters Following a Single Oral Dose of Selected Adenosine A_1 Receptor Antagonists[a]

compd	species	F (%)	$t_{1/2}$ (h)	CL (mL/min/kg)	V_{ds} (L/kg)
16	rat (2 mg dose)	97	2.14 ± 0.87	2.26 ± 0.41	0.57 ± 0.03
29	rat (1 mg/kg)	99	3.14 ± 0.14	1.56 ± 0.26	0.32 ± 0.02
	dog (1 mg/kg)	78	6.40 ± 4.0	11.8 ± 0.6	2.64 ± 1.29
	cyno (1 mg/kg)	94	11.1 ± 4.2	5.82 ± 0.45	4.25 ± 0.70
33	rat (1 mg/kg)	48	2.04 ± 0.65	7.10 ± 2.58	1.16 ± 0.20

[a] $n = 3$ male rats, 4 male dogs, and 4 male cynomolgus monkeys.

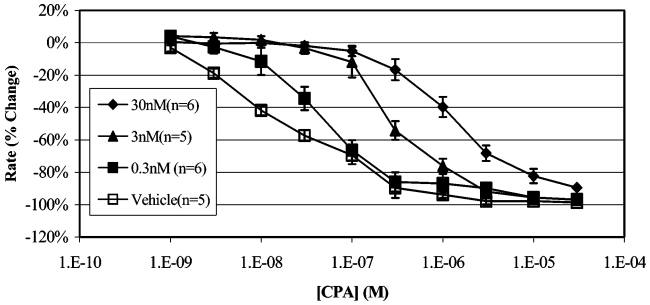


Figure 5. Blockade by 0.3, 3.0, or 30.0 nM compound **29** or vehicle control (DMSO) of the inhibitory effect of increasing concentrations of CPA on isoproterenol-stimulated rat atria in vitro (5−6 atria/group). In Schild analysis: slope = −0.865; intercept = 8.49; and $pA_2 = 9.8$. See Supporting Information for plot.

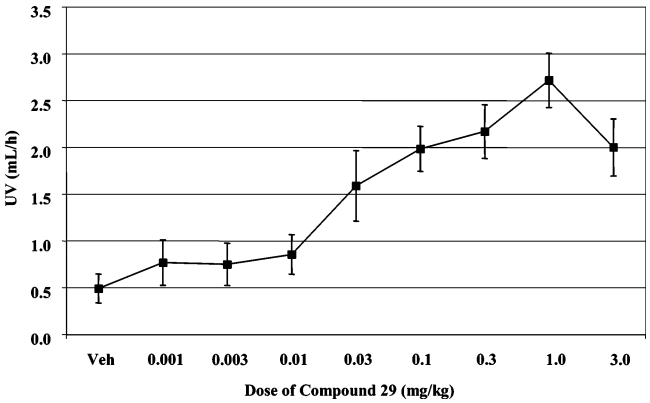


Figure 6. Dose response for urine volume in mL (mean ± SEM) over 4 h, following single oral doses of vehicle ($n = 3$) or compound **29** ranging from 0.001 to 3 mg/kg in rats (0.001 mg/kg, 0.003 mg/kg, 0.01 mg/kg, each $n = 4$; 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, each $n = 5$; 1.0 mg/kg, 3.0 mg/kg, each $n = 3$).

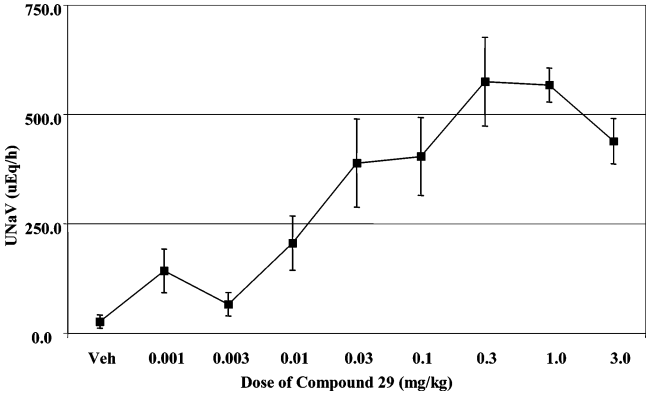


Figure 7. Dose response for UNaV in μEq/h (mean ± SEM) over 4 h, following single oral doses of vehicle ($n = 3$) or compound **29**, ranging from 0.001 to 3 mg/kg in rats (0.001 mg/kg, 0.003 mg/kg, 0.01 mg/kg, each $n = 4$; 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, each $n = 5$; 1.0 mg/kg, 3.0 mg/kg, each $n = 3$).

onates **28** and **29** demonstrated superiority over the other compounds, with sodium output almost twice that of any of the other compounds tested.

The pharmacokinetic parameters of **16**, **29**, and **33**, administered as a single oral dose to male Sprague−Dawley rats, beagle dogs, and cynomolgus monkeys, are presented in Table 4. In the rat, **16** and **29** had excellent bioavailability, 97 and 99%, respectively, and exhibited relatively low clearance and volume of distribution. The half-life of **29** was also quite good in the rat (>3 h). In contrast, the higher homolog **33** had about half the bioavailability of the other acids, higher clearance, and

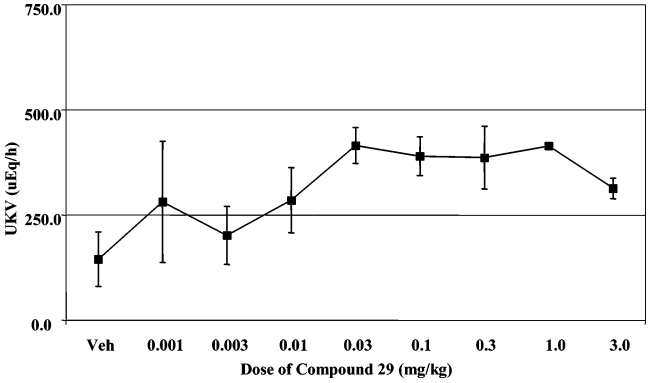


Figure 8. Dose response for UKV in μEq/h (mean ± SEM) over 4 h, following single oral doses of vehicle ($n = 3$) or compound **29**, ranging from 0.001 to 3 mg/kg in rats (0.001 mg/kg, 0.003 mg/kg, 0.01 mg/kg, each $n = 4$; 0.03 mg/kg, 0.1 mg/kg, 0.3 mg/kg, each $n = 5$; 1.0 mg/kg, 3.0 mg/kg, each $n = 3$).

Table 5. Solubility Profiles of Adenosine A_1 Antagonists in Various Solvent Systems[a]

compd	WFI[b]	0.9% saline	D5W[c]	EtOH	octanol
16	18.1	29.8	63.0	51.0	60.0
29	24.4	25.4	12.4		
33	0.5				
(*S*)-ENX[d]	0.46	0.43	0.25	85.3	4.6
NAX[e]	<0.01			24.0	82.3

[a] All solubility values were measured in units of mg/mL and calculated from duplicate determinations in each solvent, with the variation in individual values of <5%. [b] Sterile water for injection. [c] Dextrose 5% in water. [d] 1,3-Dipropyl-8-[2-(5,6-exo-epoxy-(1*S*,2*S*)-norborn-2-yl)]-xanthine. [e] 3-Noradamantyl-1,3-dipropylxanthine.

a larger volume of distribution. The pharmacokinetics of **29** was further evaluated in the dog and cynomolgus monkey and demonstrated rapid absorption and widespread distribution, followed by bi-phasic disposition. Bioavailability was nearly complete in the cynomolgus monkey and slightly lower in the dog. Exposure was highest in the rat, followed by the monkey and dog, with correspondingly increased clearance with increasing body weight. Elimination half-lives following a 1 mg/kg dose are relatively similar in the rat and dog at 3 and 6 h, respectively, and longer in the monkey at approximately 11 h.

Further work explored the functional activity of compound **29**. The sinoatrial (S-A) node is a small crescent strip of specialized muscle located in the posterior wall of the right atrium immediately beneath and medial to the opening of the superior vena cava. Any action potential that begins in the S-A node spreads immediately to the atrium, providing the atrium with automatic rhythmicity that allows it to beat independently when isolated from the heart. Decreases or increases in heart rate, as assessed in rat atria, have been used to quantify responses mediated by the adenosine A_1 receptor. Pharmacological potency of a series of reference adenosine analogues possessing selectivity for the adenosine A_1 receptor has been used to define the profile for A_1 adenosine receptor antagonists.[21] The following study was designed to evaluate the ability of **29** to block the negative chronotropic effects of activation of A_1 receptors by N^6-cyclopentyl adenosine (CPA) in the isolated rat atrium and, thus, to assess its potency as an adenosine A_1 receptor antagonist. After atrial beat rate stabilization, 130 nM CPA was added to baths to cause a 75% reduction in atrial beating rate (zero point). Increasing concentrations of **29** were then added until the rate was restored to maximum (Figure 4). The mean EC_{50} of **29** in the CPA dose reversal experiment was 16.1 ± 7.7 nM. The next set of experiments was used to determine the affinity of **29** for its receptor. Atrial rate was recorded in the

presence of 30 nM isoproterenol and compound **29** (0, 0.3, 3.0, and 30 nM). In the continued presence of isoproterenol and **29**, increasing concentrations of CPA from 1 nM to 30 μM were added cumulatively until the atrial rate was lowered to zero. The EC_{50} was determined for the vehicle control and each of the antagonist concentrations. Schild analysis was used to calculate the affinity of **29**, the competitive antagonist, for its receptor (pA_2). Parallel rightward shifts in CPA inhibition curves were seen with increasing concentrations of compound **29**, indicative of competitive antagonism (Figure 5). The pA_2 for compound **29** was calculated to be 9.8.

The oral activity of **29** was investigated in a series of experiments that examined, in a dose-related fashion, diuresis (Figure 6), natriuresis (Figure 7), and kaliuresis (Figure 8). Compound **29** caused a dose-related diuretic and natriuretic effect, which reached a maximum for both with a dose between 0.3 and 1 mg/kg. The half-maximal effect (ED_{50}) was approximately 15 μg/kg. The diuretic and natriuretic effects were associated with a neutral effect on potassium excretion (UKV), that is, UKV increased in proportion to volume. Potassium concentration in urine remained constant or slightly decreased across the dose ranges (Figure 8).

As the product was targeted for delivery to acutely decompensated congestive heart failure patients in the hospital setting, the solubilities of **16**, **29**, and **33** were evaluated in comparison to the earlier clinical candidate, (*S*)-ENX, and another adenosine A_1 antagonist, NAX, in prototype solutions suitable for intravenous administration. These results are shown in Table 5. Acids **16** and **29** had acceptable solubilities in most of the preformulation solutions tested and were significantly better than the other clinical candidates.

Compound **29** was designated a clinical development candidate, given the product code BG9928, and put into a series of preclinical toxicity studies where it was well tolerated when administered intravenously or orally to rats and cynomolgus monkeys for periods up to 3 months. The clinical candidate has been examined in IV and oral studies in healthy volunteers and stable congestive heart failure patients and is the subject of ongoing longer term human clinical trials.[22]

Conclusion

In summary, we have prepared a novel series of xanthine-based adenosine A_1 receptor antagonists. Bicyclo[2.2.2]octyl substitution at the 8-position produced antagonists with high potencies toward the adenosine A_1 receptor. Optimization of the lead molecule, **16**, by the linear extension of an acidic bridgehead side chain gave **29**, which possessed remarkable hA_1/hA_{2A} selectivity, 915-fold, and moderate selectivity over the hA_{2B} receptor (K_i = 90 nM, 12-fold versus hA_1). The in vivo activity (ED_{50} = 0.01 mg/kg-rat) and pharmacokinetics of **29** in animal studies support its use either as a potential once-daily oral therapy or in an IV formulation for acute use. Single-dose and multiple-dose studies in healthy volunteers and congestive heart failure patients are ongoing and will be reported in due time.

Experimental Section

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. The purity of all compounds listed were >95A% at 254 nm. Reversed-phase HPLC was also used for preparative purposes (LiChroprep C-18, 310 × 25 mm). ^{1}H and ^{13}C NMR spectra were obtained using Bruker 300, 400, and 500 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 chemicals and reagents were supplied by Aldrich Chemical Co., Inc, Milwaukee, WI, unless otherwise indicated.

4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-cyclohexanecarboxylic acid, 3. To a stirred mixture of 2.00 g (10.7 mmol) of *trans*-cyclohexane-1,4-dicarboxylic acid monomethyl ester (**2**),[15] 2.82 g (10.7 mmol) of 5,6-diamino-1,3-dipropyl-1*H*-pyrimidine-2,4-dione hydrochloride (**1**),[16] 4.52 mL (32.2 mmol) of NEt_3, and 50 mL anhydrous acetonitrile were added 4.25 g (11.2 mmol) of HATU. The reaction solution was stirred at rt for 30 min. The reaction mixture was concentrated in vacuo and combined with 40 mL of EtOAc and 40 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 concentrated in vacuo. The resultant oil was combined, in a 200-mL round-bottom flask equipped with a condenser, with a mixture of 30 mL of *i*-PrOH and 32.2 mL of 1 N KOH (32.2 mmol) and heated to reflux. After heating for 1 h, the reaction solution was concentrated in vacuo, taken up in 40 mL of water, chilled in an ice bath, and acidified with concentrated HCl. The resultant precipitate was collected by suction filtration, washed with water, and dried to give 2.44 g (63% yield) of an off-white solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (m, 6H), 1.64 (m, 8H), 1.96 (m, 4H), 2.29 (dt, 1H), 2.71 (dt, 1H), 3.81 (dd, 2H), 3.91 (dd, 2H), 8.98 (s, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 11.0, 11.1, 20.8, 28.1, 30.0, 37.0, 41.6, 41.9, 44.2, 106.0, 147.5, 150.6, 153.9, 157.7, 176.5; HRMS m/z = 363.20279 (MH^+), calcd = 363.20268; t_R = 3.97 min.

8-(4-Hydroxymethyl-cyclohexyl)-1,3-dipropyl-3,7-dihydro-purine-2,6-dione, 4. To a stirred suspension of **3** (0.200 g, 0.55 mmol) in 10 mL THF at 40 °C was added 1.50 mL of 1.0 M BH_3−THF. The mixture resolved into a clear solution over 1 h, which was then treated with 2.00 mL of 50% glacial acetic acid and stirred for an additional 3 h. The solution was concentrated in vacuo, and the residue was washed with water and dried to give 0.160 g of a white solid (83% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (m, 6H), 1.11 (m, 2H), 1.65 (m, 8H), 1.98 (m, 2H), 2.09 (m, 2H), 2.80 (dt, 1H), 3.47 (d, 2H), 4.03 (m, 4H); MS (MH^+) = 349.25; ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.2, 11.2, 11.5, 21.4, 21.4, 28.7, 29.0, 30.9, 38.7, 40.0, 43.3, 45.4, 68.1, 106.6, 148.6, 151.0, 155.6, 158.9; HRMS m/z = 349.22357 (MH^+), calcd = 349.22342; t_R = 3.90 min.

8-(4-Benzyloxymethyl-cyclohexyl)-1,3-dipropyl-3,7-dihydro-purine-2,6-dione, 5. To a stirred solution of **4** (0.040 g, 0.11 mmol) in 6 mL of THF was added 0.13 mL of 1.0 M *t*-BuOK in THF. After 30 min, 0.014 mL (0.12 mmol) of benzyl bromide was added. The solution was kept under reflux for another 3 h and then concentrated in vacuo. The residue was purified by flash chromatography (ethyl acetate/hexane, 4:1) to give 0.044 g (87%) of a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (m, 6H), 1.10 (m, 2H), 1.70 (m, 8H), 1.95 (m, 2H), 2.07 (m, 2H), 2.80 (dt, 1H), 3.35 (d, 2H), 4.00 (m, 4H), 4.45 (s, 2H), 7.33 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.3, 11.4, 21.4, 28.4, 30.0, 30.8, 31.3, 35.8, 37.3, 42.7, 44.7, 73.0, 75.7, 107.1, 127.6, 127.8, 138.6, 148.0, 151.2, 155.5, 157.6; HRMS m/z = 439.2706 (MH^+), calcd = 439.27037; t_R = 6.94 min.

8-[4-(Morpholine-4-carbonyl)-cyclohexyl]-1,3-dipropyl-3,7-dihydro-purine-2,6-dione, 8. To a stirred solution of 0.050 g (0.138 mmol) of **3** in 1 mL of acetonitrile and 58 μL (0.414 mmol) of NEt_3 and morpholine (12 μL; 0.138 mmol) was added 0.055 g (0.144 mmol) of HATU. The reaction solution was stirred at rt for 30 min. The reaction mixture was concentrated in vacuo and purified by preparative HPLC (acetonitrile/water gradient 10−90%; C18 stationary phase, over 30 min) to give a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.00 (m, 6H), 1.80 (m, 8H), 2.00 (m, 2H),

2.10 (m, 1H), 2.30 (m, 2H), 2.65 (m, 1H), 3.00 (m, 1H), 3.65 (s, 2H), 3.80 (m, 4H), 4.10 (m, 4H); MS m/z = 432.25 (MH^+); t_R = 3.99 min.

The following compounds were made in an analogous manner.

4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-cyclohexanecarboxylic Acid Butylamide, 6. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (m, 9H), 1.30 (m, 2H), 1.43 (m, 2H), 1.63 (m, 8H), 2.09 (m, 4H), 2.74 (m, 1H), 2.94 (s, 1H), 3.19 (dt, 2H), 4.00 (m, 4H), 5.37 (t, 1H); MS (MH^+ = 418.31); ^{13}C NMR (125 MHz, DMSO-d_6) δ 11.1, 11.2, 13.7, 19.5, 20.9, 21.0, 28.9, 30.7, 31.3, 37.9, 42.0, 43.4, 44.2, 148.2, 150.8, 154.9, 159.2, 163.3, 165.3, 174.8; HRMS m/z = 418.28143 (MH^+), calcd = 418.28127; t_R = 4.78 min.

4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-cyclohexanecarboxylic Acid (2-Dimethylamino-ethyl)-amide, 7. ^{1}H NMR (300 MHz, DMSO-d_6) δ 0.85 (m, 6H), 1.53 (m, 6H), 1.67 (dt, 2H), 1.81 (m, 2H), 1.94 (m, 3H), 2.14 (s, 6H), 2.26 (t, 2H), 2.70 (m, 1H), 3.13 (q, 2H), 3.82 (dd, 2H), 3.92 (dd, 2H), 7.70 (t, 1H); MS (MH^+ = 433.25); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.2, 11.5, 21.4, 21.4, 29.1, 30.5, 36.5, 37.9, 43.3, 44.6, 45.1, 45.3, 57.7, 106.6, 149.1, 151.0, 155.7, 158.9, 175.4; HRMS m/z = 433.29218 (MH^+), calcd = 433.29217; t_R = 3.04 min.

4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-cyclohexanecarboxylic Acid Diethylamide, 9. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (t, 6H), 1.05 (t, 3H), 1.15 (t, 3H), 1.75 (m, 10H), 2.15 (m, 2H), 2.50 (m, 1H), 2.95 (m, 1H), 3.30 (dd, 2H), 3.35 (dd, 2H), 3.90 (dd, 2H), 4.05 (dd, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.2, 11.5, 13.2, 15.1, 21.4, 29.2, 30.5, 37.8, 39.7, 40.5, 41.9, 43.2, 45.4, 106.5, 151.1, 155.4, 158.5, 174.8; MS m/z = 418.90 (MH^+); t_R = 4.97 min.

[4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-cyclohexyl]-carbamic Acid Benzyl Ester, 11. See the procedure to make compound **3**, starting material *cis*−*trans* mixture of 4-benzyloxycarbonylamino−cyclohexanecarboxylic acid (**10**) and gave a white solid (68% yield). HRMS m/z = 468.26067 (MH^+), calcd = 468.26053; t_R = 5.62 min (*cis*) and 5.74 min (*trans*).

***trans*-[4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-cyclohexyl]-carbamic Acid Benzyl Ester, 12.** See procedure to make compound **3**, starting material all-*trans* 4-benzyloxycarbonylamino-cyclohexanecarboxylic acid and gave a white solid. ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.2, 11.5, 21.4, 21.4, 30.1, 32.9, 37.8, 43.3, 45.3, 49.5, 66.7, 106.7, 127.8, 128.2, 128.6, 136.5, 149.0, 151.0, 155.7, 156.4, 158.5; Anal. ($C_{25}H_{33}N_5O_4$) C, H, N; HRMS m/z = 468.26067 (MH^+), calcd = 468.26053.

***trans*-*N*-[4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-cyclohexyl]-acetamide, 14.** Palladium on carbon (10%; 0.015 g) was added to a solution of **11** (0.120 g, 0.257 mmol) in 3 mL of MeOH and 48 μL of Ac_2O. The vessel was flushed with nitrogen and charged with hydrogen (∼15 psi). The mixture was stirred for 3 h and concentrated in vacuo, and the residue was redissolved in 10 mL of EtOAc, filtered, and washed with 10-mL portions of satd $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4 and concd to give 0.059 g (60% yield) of a mixture of *cis* and *trans* isomers. The isomers were separated by preparative HPLC (acetonitrile/water, 30 min gradient, 10−90% ACN; C18 stationary phase) to give a white solid. ^{1}H NMR (300 MHz, MeOD) δ 0.85 (m, 6H), 1.20 (m, 2H), 1.25 (m, 1H), 1.50−1.70 (m, 7H), 1.85 (s, 3H), 1.95 (m, 4H), 2.95 (m, 1H), 3.85 (dd, 2H), 3.95 (dd, 2H); HRMS m/z = 376.23445 (MH^+), calcd = 376.23432; t_R = 3.64 min.

***cis*-*N*-[4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-cyclohexyl]-acetamide, 13.** Prepared according to the procedure for **14**. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (m, 6H), 1.60−2.00 (m, 9H), 2.05 (s, 3H), 1.95 (m, 4H), 3.05 (m, 1H), 4.00 (dd, 2H), 4.10 (dd, 2H), 6.40 (d, 1H); MS m/z = 376.25 (MH^+); t_R = 3.71 min.

4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2.2]octane-1-carboxylic Acid, 16. To a stirred mixture of 2.00 g (8.84 mmol) of bicyclo[2.2.2]octane-1,4-dicarboxylic acid monomethyl ester (**15**),[17] 2.60 g (9.89 mmol) of 5,6-diamino-1,3-dipropyl-1*H*-pyrimidine-2,4-dione hydrochloride (**1**), 5.32 mL (38.1 mmol) of NEt_3, and 30 mL of anhydrous acetonitrile was added 3.76 g (9.89 mmol) of HATU. The reaction solution was stirred at rt for 1 h. The reaction mixture was concentrated in vacuo and combined with 40 mL of EtOAc and 40 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 concentrated in vacuo. The resultant solid was combined, in a 200-mL round-bottom flask equipped with a condenser, with a mixture of 35 mL of *i*-PrOH and 35 mL of 1 N KOH (35 mmol) and heated to reflux. After heating for 1 h, the reaction solution was concentrated 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 concentrated HCl, and the resultant precipitate was collected by suction filtration to give 3.00 g (87% yield) of an off-white solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.95 (two triplets partially obscured, 6H), 1.69 (q, 2H), 1.80 (q, 2H), 2.05 (m, 12 H), 4.00 (q, 2H), 4.11 (q, 2H), 12.70 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.5, 11.6, 21.6, 21.7, 28.5, 30.2, 34.2, 39.0, 43.7, 45.7, 106.9, 149.7, 151.3, 156.8, 161.8, 182.6; HRMS m/z = 389.21850 (MH^+), calcd = 389.21833; t_R = 4.62 min.

The following compounds were made in an analogous manner.

8-(4-Pentyl-bicyclo[2.2.2]oct-1-yl)-1,3-dipropyl-3,7-dihydro-purine-2,6-dione, 24. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (m, 9H), 1.10 (m, 2H), 1.20 (m, 4H), 1.30 (m, 2H), 1.40 (m, 6H), 1.50 (dq, 2H), 1.75 (dq, 2H), 1.85 (m, 6H), 3.85 (dd, 2H), 3.95 (dd, 2H), 12.80 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.6, 11.9, 14.5, 21.7, 23.0, 23.8, 31.0, 31.1, 31.17, 33.2, 34.1, 41.9, 43.5, 45.5, 107.2, 149.3, 151.5, 155.7, 162.7; Anal. ($C_{24}H_{38}N_4O_2$) C, H, N; HRMS m/z = 415.30689 (MH^+), calcd = 415.30675; t_R = 8.52 min.

4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2.2]octane-1-carboxylic Acid Methyl Ester, 25. Acid **16** (1.50 g, 3.86 mmol) was combined with 60 mL of MeOH and 10 drops of concentrated H_2SO_4. The reaction solution was brought to reflux until consumption of starting material ceased. Saturated $NaHCO_3$ was then added until neutral pH, and the reaction mixture was concentrated in vacuo. The residue was taken up in EtOAc and washed with satd $NaHCO_3$ and brine and dried over Na_2SO_4. The EtOAc solution was concentrated in vacuo to give 1.51 g (97% yield) of a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (m, 6H), 1.58 −1.80 (m, 4H), 1.90 (m, 6H), 1.98 (m, 6H), 3.6 (s, 3H), 4.00 (m, 4H), 12.00 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.6, 11.8, 21.7, 28.1, 28.5, 30.2, 34.1, 39.2, 52.2, 107.3, 149.3, 151.5, 155.9, 161.6, 178.1; MS m/z = 403.13 (MH^+); t_R = 5.33 min.

8-(4-Hydroxymethyl-bicyclo[2.2.2]oct-1-yl)-1,3-dipropyl-3,7-dihydro-purine-2,6-dione, 26. Ester **25** (1.40 g, 3.48 mmol) was combined with $LiBH_4$ (0.379 g, 17.4 mmol), MeOH (0.141 mL, 3.48 mmol), and 100 mL of THF, and the resultant mixture was brought to reflux for 18 h. After cooling to rt, 50 mL of 1 M HCl were added, and the mixture was concentrated in vacuo. The residue was dissolved in EtOAc and washed with 1 M HCl, satd $NaHCO_3$, and brine and dried over Na_2SO_4. The EtOAc solution was concentrated in vacuo to give 1.15 g (88% yield) of a white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (m, 6H), 1.50 (m, 6H), 1.55−1.80 (m, 4H), 1.93 (m, 6H), 3.28 (s, 2H), 3.95 (dd, 4H), 4.05 (dd, 4H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.2, 11.5, 21.4, 27.7, 30.2, 30.3, 34.3, 39.2, 43.2, 45.2, 71.2, 106.8, 148.8, 151.1, 155.4, 161.8; Anal. ($C_{20}H_{30}N_4O_3$) C, H, N; HRMS m/z = 375.23916 (MH^+), calcd = 375.23907; t_R = 4.34 min.

4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2.2]octane-1-carbaldehyde, 27. To a solution of 0.092 g (0.246 mmol) of **26** in 5 mL of CH_2Cl_2 was added 0.125 g (0.295 mmol) Dess−Martin periodinane. The reaction mixture was stirred at rt until the oxidation was complete. The reaction solution was filtered through a plug of basic alumina, washed with satd $NaHCO_3$, and brine and dried over Na_2SO_4. The CH_2Cl_2 solution was concentrated in vacuo to give 0.057 g (62% yield) of an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (m, 6H), 1.60 −1.80 (m, 10H), 2.05 (m, 6H), 4.00 (m, 4H), 9.50 (s, 1H), 12.00 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 10.2, 10.5, 20.3, 23.7, 24.4, 28.4,

33.5, 42.6, 105.9, 147.9, 150.1, 154.5, 159.8, 204.1; HRMS m/z = 373.22358 (MH^+), calcd = 373.22342; t_R = 4.86 min.

2-((4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)bicyclo[2.2.2]octan-1-yl)(methyl)amino)acetic Acid, 20. To a stirred mixture of 0.100 g (0.257 mmol) of **16**, 0.039 g (0.257 mmol) of sarcosine hydrochloride, 0.143 mL (1.03 mmol) of NEt_3, and 2 mL of anhydrous acetonitrile was added 0.103 g (0.270 mmol) of HATU. The reaction solution was stirred at rt for 16 h. The reaction mixture was concentrated in vacuo and combined with 10 mL of EtOAc and 10 mL of 10% citric acid. The aqueous layer was separated and washed twice with 10-mL portions of EtOAc. The combined organic fractions were washed with 10-mL portions of satd $NaHCO_3$ and brine and concentrated in vacuo. The resultant solid was dissolved in a mixture of 5 mL of MeOH and 5 mL of 1 N NaOH and stirred for 16 h. The reaction solution was concentrated in vacuo, taken up in 10 mL of water, and washed twice with 10-mL portions of CH_2Cl_2. The aqueous layer was acidified with concentrated HCl, and the resultant precipitate was collected by suction filtration to give 0.094 g (77% yield) of an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.90 (m, 6H), 1.65 (m, 4H), 1.75 (dt, 2H), 1.95 (s, 3H), 2.00 (m, 12H), 3.20 (s, 3H), 3.95 (dd, 2H), 4.00 (dd, 2H), 4.10 (s, 2H), 12.05 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 2.3, 11.6, 11.7, 28.2, 30.2, 34.2, 38.9, 40.1, 151.1, 173.9, 176.9; MS m/z = 460.18 (MH^+); t_R = 3.91 min.

The following compounds were made in an analogous manner.

4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2.2]octane-1-carboxylic Acid (2-Dimethylamino-ethyl)-amide, 17. ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.95 (m, 6H), 1.68 (dt, 2H), 1.75 (dt, 2H), 1.90 (m, 6H), 2.00 (m, 6H), 2.95 (s, 6H), 3.30 (m, 2H), 3.65 (m, 2H), 3.98 (dd, 2H), 4.08 (dd, 2H), 10.40 (s, 1H); MS m/z = 459.17 (MH^+); t_R = 3.41 min.

{**[4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2.2]octane-1-carbonyl]-amino**}**-acetic Acid Methyl Ester, 18.** ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.97 (m, 6H), 1.68−1.84 (m, 4H), 1.98 (m, 6H), 2.06 (m, 6H), 3.78 (s, 3H), 4.06 (s, 6H), 6.25 (t, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 11.0, 11.2, 20.8, 20.8, 27.7, 29.6, 33.2, 37.9, 41.9, 44.2, 60.0, 72.2, 106.4, 147.3, 150.6, 153.9, 160.3, 171.3, 176.5; MS m/z = 460.30 (MH^+); t_R = 4.26 min.

{**[4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2.2]octane-1-carbonyl]-amino**}**-acetic Acid, 19.** ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.87 (t, 3H), 0.90 (t, 3H partially obscured), 1.59 (q, 2H), 1.72 (q, 2H), 1.92 (m, 6H), 1.99 (m, 6H), 3.94 (t, 2H), 4.03 (t, 2H partially obscured), 4.07 (m, 2H), 6.06 (s, 1H), 12.18 (s, 1H), 13.55 (br s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.2, 11.3, 21.2, 21.3, 28.4, 29.8, 33.8, 38.9, 40.9, 43.5, 45.5, 106.1, 149.5, 150.6, 156.3, 161.4, 174.5, 177.0; MS m/z = 446.06 (MH^+).

3-{**[4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2.2]octane-1-carbonyl]-amino**}**-propionic Acid Methyl Ester, 21.** ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.96 (m, 6H), 1.63−1.83 (m, 4H), 1.83−2.07 (m, 12H), 2.56 (t, 2H), 3.55 (dt, 2H), 3.72 (s, 3H), 4.02 (dt, 2H), 4.10 (dt, 2H), 6.53 (t, 1H); MS m/z = 474.40 (MH^+); t_R = 4.32 min.

1-[4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2.2]octane-1-carbonyl]-piperidine-4-carboxylic Acid, 22. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.84 (t, 3H), 0.085 (t, 3H), 1.50−1.68 (m, 6H), 1.84−1.92 (m, 14H), 2.44 (m, 1H), 2.86 (m, 2H), 3.78 (t, 2H), 3.91 (t, 2H), 4.15 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 11.9, 12.0, 22.8, 22.8, 29.6, 30.1, 31.3, 35.4, 41.5, 42.4, 46.5, 79.2, 79.6, 79.8, 80.0, 122.6, 130.4, 149.9, 152.6, 153.3, 156.5, 162.6, 177.5, 178.4; t_R = 4.20 min.

[4-(2,6-Dioxo-1,3-dipropyl-2,3,6,9-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2.2]oct-1-yl]-acetaldehyde, 30. To a stirred suspension of methoxymethyl triphenylphosphonium chloride (1.1 g, 3.2 mmol) in THF (60 mL) at −78 °C was added a solution of KHMDS (0.5 M in toluene, 10 mL, 5 mmol). The resulting yellow mixture was stirred at this temperature for 1.5 h, and a solution of **27** (372 mg, 1.0 mmol) in THF (12 mL) was added over a period of 20 min. The mixture was held at −78 °C for 6 h and allowed to reach ambient temperature overnight (12 h). The reaction mixture was partitioned between satd aqueous NH_4Cl (100 mL) and EtOAc (100 mL), and the aqueous phase was extracted with EtOAc (50 mL). The combined organic extracts were washed with satd aqueous NaCl (100 mL), concentrated in vacuo, redissolved in THF, and concentrated to a volume of approximately 20 mL. To the solution was added an equal volume of 1 N HCl, and the mixture was stirred overnight. The mixture was diluted with EtOAc (20 mL), and the aqueous phase was separated and extracted with EtOAc (10 mL). The combined organic phases were then washed with saturated aqueous NaCl (2 × 25 mL), dried ($MgSO_4$), filtered, and concentrated in vacuo. The resulting orange oil was purified in batches by radial chromatography (2 mm plate) using 3% MeOH and 3% THF in CH_2Cl_2 as eluent. Product-containing fractions were combined and concentrated to afford 290 mg (75%) of a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (t, 3H), 0.93 (t, 3H), 1.63 (m, 2H), 1.77 (m, 2H, partially obscured), 1.82 (m, 6H), 2.01 (m, 6H), 2.32 (s, 2H), 3.95 (m, 2H), 4.07 (m, 2H), 12.74 (s, 1H); MS m/z = 387.37 (MH^+); t_R = 7.46 min.

[4-(2,6-Dioxo-1,3-dipropyl-2,3,6,9-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2.2]oct-1-yl]-acetic Acid, 31. To a solution of **30** (170 mg, 0.440 mmol) in *t*-BuOH (10 mL) and 2-methyl-2-butene (10 equiv, 4.4 mmol, 470 μL), cooled with the aid of an ice bath, was added $NaClO_2$ (1.5 equiv, 0.66 mmol). The resulting yellow solution was allowed to reach ambient temperature over a period of 14 h and then concentrated in vacuo. The resulting oily residue was partitioned between water (10 mL) and CH_2Cl_2 (10 mL). The aqueous phase was acidified by the dropwise addition of concentrated HCl, and the resulting precipitate was collected, washed with water, and dried to afford 105 mg (59%) as a white powder. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.91 (t, 3H), 0.93 (t, 3H), 1.63 (m, 2H), 1.77 (m, 2H, partially obscured), 1.82 (m, 6H), 2.01 (m, 6H), 2.32 (s, 2H), 3.95 (m, 2H), 4.07 (m, 2H), 12.74 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.2, 11.3, 21.3, 21.3, 30.1, 30.6, 33.6, 43.0, 43.1, 43.3, 45.3, 107.1, 148.8, 151.0, 156.3, 161.9, 176.5; HRMS m/z = 403.23414 (MH^+), calcd = 403.23398.

(*E*)-3-[4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2.2]oct-1-yl]-acrylic Acid, 28. Trimethylphosphono acetate (0.161 g, 0.886 mmol) was dissolved in 12 mL of toluene and cooled to between 0 and 5 °C. KHMDS (0.5 M in toluene; 3.54 mL) was added dropwise while stirring over a period of 5 min. After an additional 30 min at 0−5 °C, 0.300 g (0.805 mmol) of **27** was added, and the reaction was allowed to warm to rt and stirred for 16 h. The reaction mixture was concentrated in vacuo. To the dissolved crude material in 25 mL of MeOH and 10 mL of water was added 0.150 g LiOH, and the mixture was stirred at rt overnight, concentrated in vacuo, and redissolved in 15 mL of water. The water layer was extracted thrice with 20-mL portions of EtOAc and acidified with concentrated HCl, and the precipitate was collected by suction filtration to give 0.190 g (57% yield) of the *trans*-acrylic acid product. ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.78 (2 t partially obscured, 6H), 1.50 (m, 2H), 1.52 (m, 6H), 1.88 (m, 6H), 3.83 (dd, 1H), 3.93 (dd, 2H), 5.67 (d, 1H), 6.85 (d, 1H), 12.27 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.2, 11.4, 21.3, 21.3, 30.0, 30.2, 34.0, 34.2, 43.4, 45.3, 106.5, 118.2, 149.2, 150.9, 156.2, 158.1, 161.7, 170.6; Anal. ($C_{22}H_{30}N_4O_4$) C, H, N; HRMS m/z = 415.23414 (MH^+), calcd = 415.23398; t_R = 4.80 min.

3-[4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2.2]oct-1-yl]-propionic Acid, 29. Acrylic acid **28** (0.050 g) was dissolved in 5 mL of MeOH and combined with 0.005 g of 10% Pd/C. The reaction vessel was purged three times with N_2 and then placed under a balloon of H_2 gas. After 2 h, the reaction mixture was filtered and concd to give 0.037 g (74% yield) of a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.598 (t, 3H), 0.604 (t, 3H), 1.14 (m, 8H), 1.28 (tq, 2H), 1.41 (tq, 2H), 1.59 (m, 6H), 1.86 (dd, 2H), 3.57 (t, 2H), 3.67 (t, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 11.4, 11.5, 21.2, 29.0, 30.2, 30.2, 30.4, 33.6, 36.0, 42.3, 44.5, 106.7, 147.7, 151.0, 154.3, 161.0, 175.3; mp 278 °C; Anal. ($C_{22}H_{32}N_4O_4$) C, H, N; HRMS m/z = 417.24976 (MH^+), calcd = 417.24963; t_R = 4.90 min.

4-[4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2.2]oct-1-yl]-butyric Acid Methyl Ester, 32. A

solution of **30** (233 mg, 0.604 mmol) and (triphenyl-phosphanylidene)-acetic acid methyl ester (242 mg, 0.725 mmol) in THF (25 mL) was heated at 75 °C for 6 h. The reaction mixture was allowed to cool to rt and concentrated in vacuo to afford an oil that was purified by radial chromatography (2 mm plate) using 2−5% MeOH in CH_2Cl_2 as eluent. The resulting mixture of *cis*/*trans*-olefins was dissolved in EtOH (6 mL) and hydrogenated using Pd on carbon (10 mol %) and a balloon of hydrogen affixed to a 3-way stopcock/ground glass adapter. After stirring overnight, the mixture was degassed, filtered through Celite, and concentrated in vacuo to give a brittle foam (140 mg, 54%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.93 (m, 6H), 1.11−1.15 (m, 2H), 1.47−1.68 (m, 10H), 1.74 (dd, 2H), 1.92−1.96 (m, 6H), 2.65 (dd, 2H), 3.64 (s, 3H), 3.99 (dd, 2H), 4.06 (dd, 2H), 11.55 (s, 1H); Anal. ($C_{24}H_{36}N_4O_4$) C, H, N; HRMS m/z = 445.28102 (MH^+), calcd = 445.28093; t_R = 8.93 min.

4-[4-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-bicyclo[2.2.2]oct-1-yl]-butyric Acid, 33. A solution of ester **32** (45 mg, 100 μmol) in THF (4 mL) was treated with 1 M LiOH (2 mL), and the resulting turbid solution was stirred at rt overnight. The solution was concentrated in vacuo, diluted with water (2 mL), and acidified by the dropwise addition of concentrated HCl. The resulting precipitate was collected, washed with water, and dried to afford a white powder (35 mg, 81%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.96 (t, 3H), 1.00 (t, 3H), 1.28 (dd, 2H), 1.60 (m, 8H), 1.68 (q, 2H), 1.82 (q, 2H), 2.05 (m, 6H), 2.48 (t, 2H), 4.01 (t, 2H), 4.19 (t, 2H), 12.59 (s, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.1, 11.3, 18.1, 21.2, 21.4, 30.5, 30.6, 32.8, 34.1, 40.6, 43.5, 45.7, 106.2, 148.8, 150.7, 156.1, 162.1, 178.2; HRMS m/z = 431.26540 (MH^+), calcd = 431.26528; t_R = 7.52 min.

Human Adenosine Receptor Screening: Initial screening was of a solution of the antagonist (1 μM) 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 except hA_3 at a final concentration of 5%. Radioligands consisted of the following: hA_1, 0.3 nM ^{125}I-aminobenzyladenosine (^{125}I-ABA); hA_{2A}, 0.7 nM ^{125}I-ZM241385; hA_{2B}, 0.5 nM ^{125}I-3-(4-aminobenzyl)-8-phenyloxyacetate-1-propyl-xanthine; and hA_3, 0.6 nM ^{125}I-ABA. Nonspecific binding was measured in the presence of 50 μM xanthine amine congener or 10 μM BW-1433 (hA_3).

Rat Adenosine Receptor Screening: Compounds were incubated at room temperature for 90 min with radioligand (2 nM ^{3}H-CPX for rA_1; 0.5−1.2 nM ^{3}H-ZM241385 for rA_{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 rA_1) or rat brain striatum (for rA_{2A}). Incubations were terminated by the addition of ice-cold 50 mM Tris−HCl buffer and the collection of membranes was done on 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).

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.

Male Sprague−Dawley rats were purchased from Charles River Laboratories (Raleigh, NC) and housed in the Biogen virus−free laboratory animal facility in ventilated isolator cage racks. Animals were allowed to acclimatize for 4 days prior to the beginning of the study. Rats had ad libitum access to irradiated standard chow (LabDiet Prolab 5P75 Isopro RMH 3000) and sterile water throughout the acclimatization and experimental period.

Isolation of Atria From Rat Heart. Hearts were removed from the rats and placed in petri dishes containing Krebs Henseleit (Krebs) buffer prewarmed 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 preload tension of 2 grams (g) was applied using a precalibrated Gould recorder. Hung tissue was washed with warm, oxygenated Krebs buffer, while maintaining 2 g of tension. Baseline atrial beat rate was measured on Ponemah software from Gould Instruments (Valley View, Ohio).

Determination of EC_{50} of 29 Using the CPA Dose Reversal Paradigm. Isoproterenol (30 nM) was added to all baths containing atria to increase the baseline atrial 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 **27** 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. The effects of compound **29** were fully reversible (in the presence of CPA) after washout of the compound from the isolated atria.

Blockade Paradigm: Schild Plot (pA_2) Analysis. Isoproterenol (30 nM) was added to all baths containing atria to increase the baseline atrial rate to between 350 and 400 bpm. Varying concentrations of **29** (0.3 nM, 3.0 nM, and 30.0 nM; or vehicle control (dimethylsulfoxide [DMSO])) were then added to isolated tissue baths with beating atria, and 5 min was allowed to ensure stabilization (control 0). Increasing concentrations of CPA from 1 nM to 30 μM were added cumulatively until the atrial rate was lowered to zero. The EC_{50} was determined for the vehicle control and each of the **29** concentrations. Schild analysis was used to calculate the affinity of **29**, the competitive antagonist, for its receptor (pA_2). Five or six atria were used for each **29** concentration and the vehicle control.

Statistical Analysis. In the dose reversal experiment, the mean and standard error of the mean (SEM) of the atrial beating rate (bpm) were calculated at baseline and following the addition of isoproterenol, CPA, and each dose of **29**. The mean (±SEM) percent change from baseline for the control (0) was calculated as (CPA bpm at baseline − CPA bpm at baseline)/(isoproterenol bpm at baseline − CPA bpm at baseline) × 100. The mean (±SEM) percent change from baseline for each **29** dose was calculated as (**29** dose bpm − CPA baseline bpm)/(isoproterenol bpm at baseline − CPA bpm at baseline) × 100. The EC_{50} was determined as the effective concentration of **29** at which a 50% response was obtained. In the blockade experiment, atrial rates were recorded at baseline and following addition of isoproterenol, **29**, or vehicle control (control 0) and each CPA dose. The percent change was calculated as (CPA dose bpm − **29** bpm [control])/(**29** bpm [control] bpm) × 100. Using the data from the blockade experiment, a Schild analysis was performed to determine the affinity of **29**, the antagonist, for its receptor with CPA as the agonist (pA_2).

Rat Oral Efficacy Screen: Rats were placed into metabolic cages and dosed by gavage with various doses of **29**. The doses and group sizes were: vehicle (0.5% CMC; n = 3); **29**, 0.001 mg/kg (n = 4), 0.003 mg/kg (n = 4), 0.01 mg/kg (n = 4), 0.03 mg/kg (n = 5), 0.1 mg/kg (n = 5), 0.3 mg/kg (n = 5), 1.0 mg/kg (n = 3), and 3.0 mg/kg (n = 3). Urine was collected for 4 h after dosing.

Urine volume was measured gravimetrically, and sodium and potassium concentrations were determined by flame photometry. Urine flow, UNaV, and UKV were calculated and are shown as units per hour as an average for the 4-hour collection period.

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Supporting Information Available: Experimental details (details of HPLC, MS, and elemental analyses of compounds) and

data for pA_2 determination and statistical analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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