Tricyclic Imidazoline Derivatives as Potent and Selective Adenosine A_1 Receptor Antagonists

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Novel tricyclic imidazoline antagonists of the adenosine A_1 receptor are described. For key compounds, the selectivity level over other adenosine receptor subtypes is examined along with their in vivo effects in a rat diuresis model. Compound **14**, the (*R*)-isomer of 7,8-dihydro-8-ethyl-2-(4-bicyclo[2.2.2]octan-1-ol)-4-propyl-1*H*-imidazo[2,1-*i*]purin-5(4*H*)-one, is a particularly potent adenosine A_1 receptor antagonist with good selectivity over the other three adenosine receptor subtypes: A_1 (human) $K_i = 22$ nM; A_{2A} (human) $K_i = 4400$ nM; A_{2B} (human) $K_i = 580$ nM; A_3 (human) $K_i \geq 10\,000$ nM. Imidazoline **14** is a competitive adenosine A_1 receptor antagonist with a pA_2 value of 8.88 and is highly soluble in water (>100 mg/mL). In addition, it has an oral bioavailability of 84% and an oral half-life of 3.8 h in rats. When orally administered in a rat diuresis model, compound **14** promoted sodium excretion ($ED_{50} = 0.01$ mg/kg). This level of efficacy is comparable to that of BG9928, a selective adenosine A_1 receptor antagonist that is currently in clinical trials as a treatment for congestive heart failure. Additional modifications to **14** also showed that the bridgehead hydroxyl group could be replaced with a propionic acid (compound **36**) without a significant loss in binding affinity or in vivo activity.

Introduction

The biochemical messenger adenosine has the ability to bind to and activate seven-transmembrane-spanning G-protein-coupled receptors (GPCRs) and thereby initiate a variety of immunological, cardiovascular, renal, and neurological responses.[1] Among the four adenosine receptors that have been extensively characterized (A_1, A_{2A}, A_{2B}, and A_3),[2] the adenosine A_1 receptors hold particular promise in the treatment of kidney disorders. These receptors have been identified in the proximal tubule as well as the afferent arteriole of the kidney.[3] They have been shown to be mediators for tubuloglomerular feedback and are thus important elements in the control of fluid balance.[4,5] The adenosine A_1 receptors' role in mediating sodium transport in the proximal tubule has now been demonstrated in a number of animal studies[6] as well as in recent human clinical trials.[7] Adenosine A_1 receptor antagonists have been shown to cause immediate diuresis (water excretion) and natriuresis (sodium excretion) without affecting the potassium excretion.[5] In rats, selective adenosine A_1 receptor antagonists have also been shown to possess protective effects against glycerol-, cisplatin-, gentamycin-, or cephaloridine-induced acute renal failure.[6] In congestive heart failure (CHF) patients, renal insufficiency usually leads to excess fluid retention, which necessitates the use of a diuretic such as furosemide.[3] Extended usage of furosemide, however, leads to a decrease in glomerular filtration rate (GFR) and further deterioration of renal function. In recent clinical trials, administration with the selective adenosine A_1 receptor antagonist BG9719 to CHF patients has been shown to induce the desired diuretic and natriuretic effects without lowering the GFR.[7]

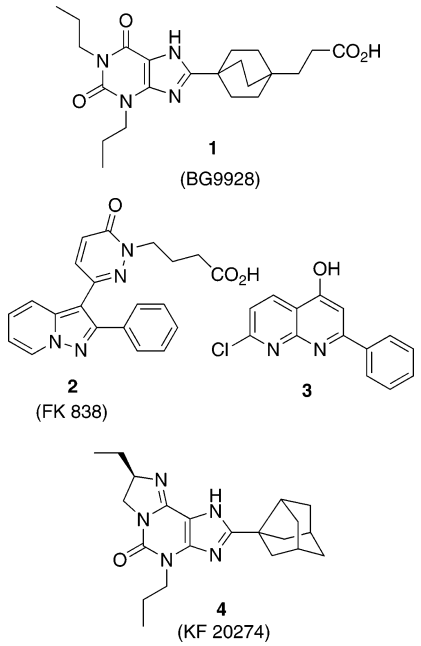

Figure 1. Structurally diverse adenosine A_1 receptor antagonists.

Previous work from this laboratory disclosed the discovery of the xanthine derivative **1** (BG9928) as a potent and selective adenosine A_1 receptor antagonist.[8] This compound is currently being evaluated in human clinical trials for the treatment of congestive heart failure in both the acute and chronic settings. In connection with this ongoing clinical work, we became interested in identifying other classes of adenosine A_1 receptor antagonists that could serve as potential backup candidates. To date, a number of interesting non-xanthine adenosine A_1 receptor antagonists have been identified,[9] and three of these are shown in Figure 1. The pyrazolopyridine **2** (FK 838) lacks the desired selectivity over the adenosine A_{2a} receptor, but it is a potent diuretic with good water solubility.[10] More recently, the novel 1,8-naphthyridine series, as exemplified by **3**, was reported to

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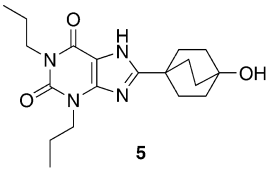
contain highly potent and selective adenosine A_1 receptor antagonists.[11] However, the in vivo properties of these 1,8-naphthyridines have not yet been disclosed. We were particularly interested in the reports regarding tricyclic imidazoline derivatives,[12,13] for example, compound **4** (KF 20274). These tricyclic imidazolines are essentially derivatives of xanthines. However, the additional basic site significantly increased their water solubility relative to xanthines without a substantial loss in A_1 binding affinity.[12] We set out to determine if this modification would afford similar improvements to a series of compounds based on xanthine derivative **5**. Against the rat A_1 receptor, **5** had a K_i of 0.6 nM. Against cloned human receptors, **5** displayed high A_1 selectivity and the K_i for the various adenosine receptor subtypes are as follows: A_1 K_i = 2 nM, A_{2A} K_i = 550 nM, A_{2B} K_i = 430 nM, A_3 K_i > 1 μM. Xanthine **5** was also highly potent in a rat diuresis model with an ED_{50} of 0.01 mg/kg when administered orally. One of the pharmaceutical product weaknesses of compound **5** was its low water solubility (0.1 mg/mL), which could limit its use in the acute clinical setting where iv administration would be desirable. Conversion of **5** to the corresponding imidazolines of the general structure **6** would provide access to a new series of compounds and may improve water solubility while retaining most of the potency and selectivity.



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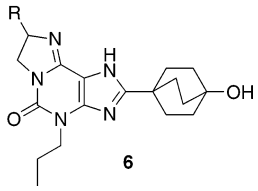

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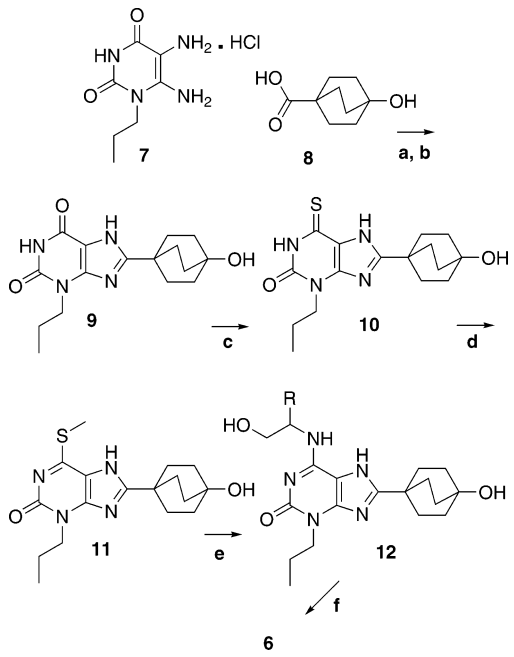
Results and Discussion

Imidazolines of the general structure **6** were prepared using the synthetic sequence outlined in Scheme 1. The monopropyldiaminouracil **7** was prepared by a variety of known methods.[14] However, in the free base form, **7** was highly insoluble in most organic solvents, including DMF, and therefore was difficult to manipulate. In our hands, the hydrochloride salt of **7** was significantly more soluble in DMF. Thus, **7** was coupled conveniently with the acid derivative **8**[15] using HATU/Et_3N/DMF and then treated with ethanolic NaOH to form the xanthine derivative **9**. Thioamide **10** was obtained by treating **9** with an excess of phosphorus pentasulfide in pyridine at reflux. When **10** was alkylated with MeI in ethanolic NaOH, the thioether **11** was obtained. This sulfide was easily displaced with a variety of amino alcohols in DMSO at 150 °C in order to obtain **12**. Upon treatment with $SOCl_2$, cyclization took place to afford the desired imidazolines. Table 1 lists the imidazoline derivatives that have been prepared using this procedure.

The binding affinities of the antagonists were determined first in rat A_1 (rA_1) and A_{2A} (rA_{2A}) receptors by methods previously described.[8] Compounds that displayed sufficient rA_1 activity were then screened against the available human adenosine receptors (hA_1, hA_{2A}, hA_{2B}, hA_3).[8] These results are summarized in Tables 1 and 2. When R = H, as in compound **13**, a significant loss of rA_1 binding affinity was observed (more than 100-fold) when compared to xanthine **5** (rA_1 K_i = 0.6 nM).

Scheme 1[a]

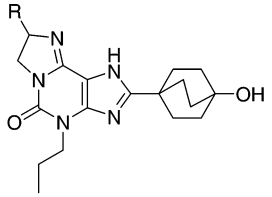


[a] Reagents and conditions: (a) HATU/Et_3N/DMF, room temp; (b) NaOH, EtOH/H_2O, reflux, acidification to pH 2 with concentrated HCl; (c) P_4S_{10}, pyridine, reflux; (d) MeI, NaOH, EtOH/H_2O, room temp; (e) $HOCH_2CH(R)NH_2$, DMSO, 150 °C; (f) $SOCl_2$, reflux.

When R = alkyl, the rA_1 binding affinity was greatly improved but only for alkyl substituents in the (*R*)-configuration. This result is in agreement with an earlier report of tricyclic imidazoline analogues (**4**).[10] In that report, the (*R*)-configuration showed substantially better rA_1 binding affinity than the (*S*)-configuration. In our series **6**, the same trend favoring the (*R*)-configuration was observed. This is clearly illustrated with compounds **14** and **15**. Compound **14**, with the (*R*)-configuration, was significantly more potent against the rA_1 receptor (>10-fold) than the corresponding (*S*)-isomer **15**. Most of the imidazolines displayed in Table 1 also possessed low affinities for the rA_{2A} receptor. Shortening the ethyl side chain of **14** resulted in slight loss of rA_1 binding affinity (**16**, rA_1 K_i = 10 nM). Lengthening the ethyl side chain, as in **17**, resulted in a larger decrease of rA_1 activity. Addition of a methyl group to form an isobutyl group, as in **18**, resulted in a further decrease in rA_1 binding affinity. However, movement of the methyl to the adjacent carbon, as in the *sec*-butyl derivative **19**, improved the rA_1 activity slightly. The rA_1 binding affinity could be restored more effectively by using the isopropyl group, as in compound **20**. With larger R substitution, as in *tert*-butyl, phenyl, or benzyl (as in **21**, **22**, and **23**), a significant loss in rA_1 binding affinity was observed. The six-membered-ring homologues **24** and **25** were synthesized, but both compounds were significantly less active against the rA_1 receptor than the corresponding imidazolines **14** and **16**. Interestingly for regioisomers **26** and **27**, the stereochemistry was no longer critical but both compounds were less active against the rA_1 receptor than compound **16**. In previously described xanthine series, significant selectivity over hA_{2A} receptors has been achieved while control over hA_{2B} receptor affinity has been difficult.[8−10] Imidazoline **14** showed significant selectivity over hA_{2A} but not over hA_{2B} receptors (Table 2). In addition, the selectivity level over hA_{2B} was improved for imidazoline **20**.

Compounds **14** and **20** were tested in a rat model of diuresis.[16] In this model, rats were placed in metabolic cages to allow for urine collection. Test compounds, formulated as hydrochloride

Table 1. Binding Data for Tricyclic Imidazolines



		K_i[a] (nM) or % of specific radioligand binding[b]	
Cmpds	**R**	**rA_1**	**rA_{2A}**
13	H	>100	(77%)
14		6	2700
15		>100	(70%)
16		10	(32%)
17		43	(90%)
18		170	(97%)
19		14	(91%)
20		4	1100
21		155	NT[c]
22		68	(79%)
23		710	(84%)

[a] For the rA_1 receptor, membranes were prepared from rat cerebral cortex and the radioligand binding assay was performed using [^{3}H]DPCPX. For the rA_{2A} receptor, membranes were prepared from rat striatum and the radioligand binding assay was performed using [^{3}H]ZM241385. All K_i values were calculated from binding curves generated from the mean of three determinations per concentration (seven antagonist concentrations), with the variation in individual values being <15%. [b] Values in parentheses indicate the percent of specific radioligand binding in the presence or 10 μM antagonist relative to control (no antagonist). [c] Not tested.

Table 2. Summary of Binding Data Against the Various Rat[a] and Human[b] Adenosine Receptor Subtypes

	K_i (nM)					selectivity ratios	
compd	rA_1	hA_1	hA_{2A}	hA_{2B}	hA_3	hA_{2A}/hA_1	hA_{2B}/hA_1
1	1	7	6400	90	>10000	914	13
5	0.6	2	550	430	>1000	275	215
14	6	22	4400	580	>10000	200	26
16	10	60	NT[c]	2200	NT[c]	NT[c]	37
20	4	35	1600	2000	>10000	46	57
35	8	35	>10000	1900	>10000	>280	54
36	10	45	18000	3100	>10000	400	69

[a] Refer to Table 1 for additional details regarding the rat radioligand binding assay. All K_i values were calculated from binding curves generated from the mean of three determinations per concentration (seven antagonist concentrations), with the variation in individual values being <15%. [b] See ref 8 for details regarding human adenosine receptor binding assay. [c] Not tested

salts, were dosed orally in 0.5% cmc suspension. Urines were collected over a period of 4 h, and the sodium excretion levels (UNaV) were determined. The dose-response curve for **14** is shown in Figure 2 along with that of **1**, the current clinical candidate for treatment of congestive heart failure. From this experiment, the ED_{50} for **14** was determined to 0.01 mg/kg po, comparable to that of **1**. In a similar fashion, the ED_{50} for **20** was determined to be 0.01 mg/kg po (dose-response curve not shown). Pharmacokinetic (PK) experiments were carried out using standard protocols where rats were dosed both orally and

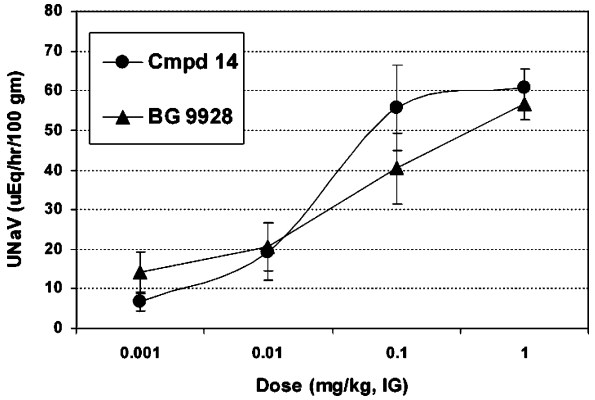
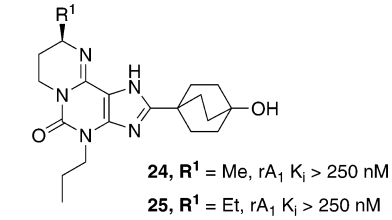
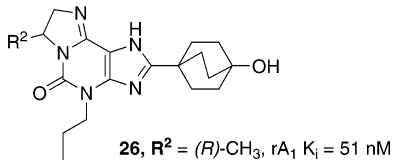


Figure 2. Natriuresis dose-response of compound **14** compared to BG9928. Rats are placed in metabolic cages to allow for urine collection. The compounds are dosed at the indicated doses, by gavage in 0.5% cmc suspension. Urines are collected for 4 h, and sodium excretion (UNaV) is determined.





iv at 1 mg/kg. Both **14** and **20** were formulated as their hydrochloride salts and dissolved in saline before dosing. Plasma samples were collected at nine time points over a period of 24 h and analyzed by extraction and LC/MS. In rats, **20** had an oral bioavailability of 54% and an oral half-life of 2.9 h (1 mg/kg po and iv administration: oral AUC_{last} = 810 ng·h·mL^{-1}; oral CL = 20.8 mL·min^{-1}·kg^{-1}; V_{dss} = 1.7 L·kg^{-1}). In contrast, **14** had a higher oral bioavailability of 84% and a longer oral half-life of 3.8 h (1 mg·kg^{-1} po and iv administration: oral AUC_{last} = 800 ng·h·mL^{-1}; oral CL = 17.8 mL·min^{-1}·kg^{-1}; V_{dss} = 4.1 L·kg^{-1}). Table 3 summarizes the efficacy data for these three compounds along with their PK properties.

In order to determine the intrinsic potency of imidazoline **14** against the A_1 receptor, the pA_2 was determined by an experiment involving blockade of agonism in isolated, beating rat atria (Figure 3). With this type of experiment, spontaneously beating rat atria are hung in tissue baths.[17] Either vehicle or antagonist **14** is added at three different concentrations (3, 30, and 300 nM **14**). An increasing concentration of the selective A_1 receptor agonist CPA (Figure 3, *X*-axis) is then added to reduce the beating rate (Figure 3, *Y*-axis). The EC_{50} values for the agonist CPA are then calculated, and the pA_2 value (which is the −log of the value required to double the agonist's IC_{50}) is determined. As shown in Figure 3, the pA_2 value for compound **14** is 8.88. The characteristic shift to the right with varying concentration of **14** also indicated that this compound was a full competitive antagonist of the adenosine A_1 receptor. This level of intrinsic potency compares favorably with that of compound **1**,[8] a full competitive adenosine A_1 receptor antago-

Table 3. Summary of in Vivo and Pharmacokinetic Data[a]

		pharmacokinetic (PK) properties				
compd	rat diuresis model, po dosing ED_{50} ($mg \cdot kg^{-1}$)	F (%)	oral $t_{1/2}$ (h)	oral AUC_{last} ($ng \cdot h \cdot mL^{-1}$)	oral CL ($mL \cdot min^{-1} \cdot kg^{-1}$)	V_{dss} ($L \cdot kg^{-1}$)
1	0.01	99	3.1	10000	1.6	0.3
14	0.01	84	3.8	800	18	4.1
20	0.01	54	2.9	810	21	1.7
36	0.01	77	3.1	2600	22	0.9

[a] Details regarding the rat diuresis model can be found in ref 8. Rat PK experiments were carried out using standard protocols. Compounds were dosed both iv and orally at 1 $mg \cdot kg^{-1}$. Test compounds were formulated as the hydrochloride salt and dissolved in saline. Plasma samples were taken at nine time points over a period of 24 h. F refers to the % oral bioavailability; V_{dss} refers to the volume of distribution following oral administration.

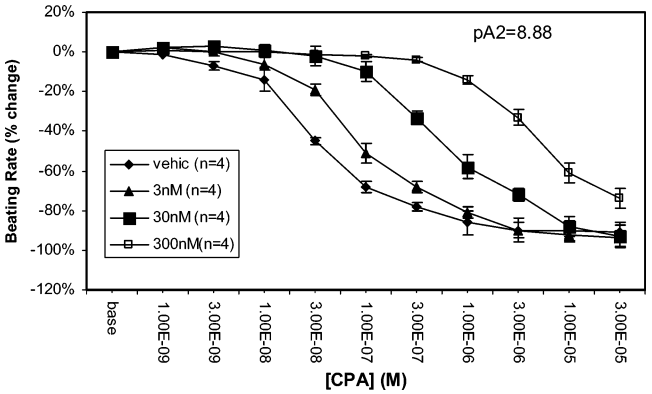


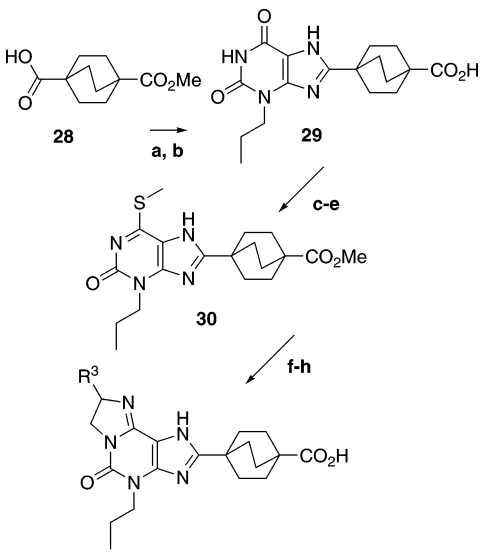
Figure 3. Determination of the pA_2 value for compound **14**. The pA_2 determination was carried out using the blockade of agonism in isolated, beating rat atria. Spontaneous beating rat atria are hung in tissue baths. Either vehicle or antagonist is added at the concentration shown. Increasing concentration of the adenosine A_1 receptor agonist CPA (*X*-axis of the above graph) is then added to reduce beating rate (*Y*-axis). CPA's EC_{50} values are calculated, and the pA_2 (i.e., the $-\log$ of the value required to double the agonist's EC_{50}) of the antagonist **14** is determined.

nist with a pA_2 value of 9.8. When evaluated against cloned human receptors, **14** showed a high level of selectivity across the four receptor subtypes: hA_1 $K_i = 22$ nM; hA_{2A} $K_i = 4400$ nM; hA_{2B} $K_i = 580$ nM; hA_3 $K_i \geq 10\,000$ nM. These data are summarized in Table 3. For comparison purposes, the binding data for compounds **1** and **5** are also included. In terms of physical characteristics, compound **14** was completely soluble in water at 100 mg/mL, when formulated as the hydrochloride salt. It was also quite stable and showed no sign of decomposition after heating in boiling water for 10 days.

Compound **20** was evaluated in the same isolated, beating rat atria experiment described above. Like **14**, **20** was also a full competitive adenosine A_1 receptor antagonist, and its pA_2 was determined to be 9.9 (curves not shown). When tested against the cloned human adenosine receptors, compound **20** also exhibited a good level of selectivity (Table 2, hA_1 $K_i = 35$ nM, hA_{2A} $K_i = 1600$ nM, hA_{2B} $K_i = 2000$ nM, and hA_3 $K_i > 10$ μM). In terms of water solubility and physical stability, compound **20** was essentially indistinguishable from **14**.

After the optimization of the imidazoline series **6**, we were interested in modifying the bicyclo[2.2.2]octyl ring system. In our next modification, we replaced the bridgehead hydroxyl group with a carboxyl group as in compounds **31** and **32**. Scheme 2 illustrates how these compounds were prepared from the hemiester **28**.[8] This acid was first coupled with the uracil **4** and then cyclized in the same manner as described previously in order to obtain the monopropylxanthine **29**. Esterification and construction of the imidazoline gave **31** and **32**. Compound **31**, the (*R*)-isomer, had a K_i of 40 nM against the rA_1 receptor; compound **32**, the (*S*)-isomer, was substantially less active with an rA_1 $K_i > 250$ nM. This dependence on the stereochemistry

Scheme 2[a]



31, R^3 = (*R*)-Et, rA_1 K_i = 40 nM; rA_{2A} = 48% at 10 uM

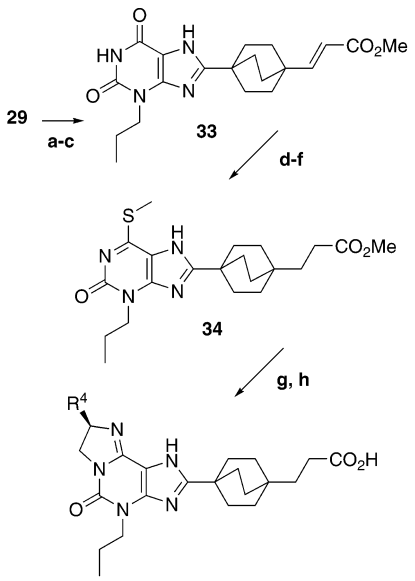
32, R^3 = (*S*)-Et, rA_1 K_i > 250 nM; rA_{2A} = 80% at 10 uM

[a] Reagents and conditions: (a) **4**, HATU/Et_3N/DMF, room temp; (b) NaOH. (c) MeOH, H_2SO_4, reflux; (d) P_4S_{10}, pyridine, reflux; (e) MeI, NaOH, EtOH/H_2O; (f) For the preparation of **31**, (*R*)-$HOCH_2CH(Et)NH_2$, DMSO, 150 °C; for the preparation of **32**, (*S*)-$HOCH_2CH(Et)NH_2$, DMSO, 150 °C; (j) $SOCl_2$, reflux, NaOH.

at the imidazoline ring has already been encountered with tricyclic series **6**. However, the successful conversion of compound **14** to **31** without a significant drop in rA_1 binding affinity suggested that the dipropylxanthine **1** could also accommodate the imidazoline ring.

Scheme 3 illustrates how compounds **35** and **36** were prepared from acid **29**. Reduction to the alcohol using BH_3·THF followed by oxidation gave the corresponding aldehyde. The acrylate of **33** was then introduced by a Wittig coupling and then hydrogenated over Pd/C. Propionate **34** was converted to the imidazolines **35** and **36** using the same reaction sequence as described in Scheme 1. Against the rA_1 receptor, both **35** and **36** were still very potent with a K_i of 8 and 10 nM, respectively. When evaluated against the human receptors, both **35** and **36** exhibited an excellent level of selectivity across all four subtypes (both rat and human data are summarized in Table 3). It is clear that the level of selectivity over the hA_{2B} receptor of the dipropylxanthine **1** can be improved quite significantly by conversion to the corresponding imidazoline derivative. On the basis of the slightly improved selectivity profile, compound **36** was selected for additional profiling in pharmacological assays. Compound **36** was dosed po and iv in rats at 1 mg/kg in order to determine its PK properties. The oral bioavailability for **36** was 77%, surprisingly high for a zwitterionic species (oral half-life of 3.1 h; oral $AUC_{last} = 2600$ $ng \cdot h \cdot mL^{-1}$; oral CL = 1.3 $L \cdot h^{-1} \cdot kg^{-1}$; $V_{dss} = 0.7$ $L \cdot kg^{-1}$). Most significantly, in terms of

Scheme 3[a]



[a] Reagents and conditions: (a) BH_3−THF, room temp; (b) pyridine.SO_3, DMSO, room temp; (c) THF, PPh_3CHCO_2Me, reflux; (d) H_2, 10%Pd/C, THF; (e) P_4S_{10}, pyridine, reflux; (f) MeI, NaOH, EtOH/H_2O; (g) for the preparation of **35**, (*R*)-$HOCH_2CH(Et)NH_2$, DMSO, 150 °C; for the preparation of **36**, (*R*)-$HOCH_2CH(CH_3)_2NH_2$, DMSO, 150 °C; (h) $SOCl_2$, reflux, NaOH.

in vivo efficacy, compound **36**, with an ED_{50} of 0.01 mg/kg po, was just as effective as **1** when administered orally in the rat diuresis model (Table 3).

In summary, we have demonstrated how a tricyclic imidazoline ring system can be used to improve the water solubility relative to dipropylxanthines such as **2**. In addition, we have shown how the dipropylxanthine **1** could be converted to the corresponding imidazoline ring system without a significant loss in adenosine A_1 binding affinity or in vivo activity. Three compounds (**14**, **20**, and **36**) appeared to have many of the desirable attributes of the clinical candidate BG9928. More complete profiling of these compounds is still underway, and these results will be disclosed in due course.

Experimental Section

General Information. All proton nuclear magnetic resonance spectra were determined in the indicated solvent using a Bruker 400 MHz with the appropriate internal standard. Low-resolution MS experiments were performed on a Micromass single quadrupole electrospray platform. High-resolution MS experiments were performed on a MALDI-TOF MS (Voyager-DE STR, Perseptive Biosystems) in the reflector mode with delayed extraction and an accelerating voltage of 20 kV. Each spectrum was an average of 100 laser shots, and the experimental monoisotopic $M^+ + H$ value was calculated by averaging five spectra. Elemental analyses were carried out at Quantitative Technologies Inc. (QTI, Whitehouse, NJ). Unless indicated otherwise, reagent-grade chemicals and solvents were purchased from Aldrich (Milwaukee, WI), Lancaster (Ward Hill, MA), Fisher (Geel, Belgium), or Maybridge (Cornwall, U.K.). Analytical HPLC analysis was carried out using a HP 1100 series, with a 100 mm × 4.6 mm i.d. YMC column with S-5 μm packing (catalog no. AM-301). Preparative HPLC was carried out using a Gilson platform equipped with UV/visible detector and an automatic fraction collector. The preparative HPLC column was a 50 mm × 20 mm i.d. YMC column with S-5 μm packing. HPLC solvents (H_2O and CH_3CN) were buffered with 0.1% TFA.

General Procedure for the Preparation of Compounds of the General Structure 6 (Compounds 13−23 and 24−27). Step 1. 5,6-Diamino-1-propyl-1*H*-pyrimidine-2,4-dione Hydrochloride Salt (7). The starting material, 6-amino-1-propyl-1*H*-pyrimidine-2,4-dione, was prepared according to a known literature procedure.[12] This material (8.5 g, 50 mmol) was dissolved in 250 mL of aqueous acetic acid and then cooled in an ice bath. Sodium nitrite (4.14 g, 1.2 equiv) was added as a solution in 10 mL of water over a period of about 15 min. After about 10 min, a light-red solid began to precipitate out of the reaction mixture. The solids were collected by filtration and dried under vacuum overnight to afford 8.0 g of the nitroso intermediate.

The nitroso intermediate (6.0 g, 30 mmol) was suspended in 100 mL of water and heated to 80−85 °C. Sodium dithionite (15.8 g, 3.0 equiv) was added fairly rapidly over a period of about 5 min. After about 5 min, the heating source was removed and the light-green reaction mixture was cooled to room temperature and then in an ice bath. The solids were collected by filtration and dried under vacuum to afford the diaminouracil. This was then converted to the hydrochloride salt by dissolving in 10 mL of H_2O containing 1.5 equiv of HCl and then lyophilized. ^{1}H NMR (DMSO-d_6) δ 11.1 (s, 1 H), 9.2 (br s, 3 H), 7.6 (s, 2 H), 3.6 (t, J = 7.0 Hz, 2 H), 1.3 (m, 2 H), 0.8 (t, J = 7.0 Hz, 3 H) ppm.

Step 2. 8-(4-Hydroxybicyclo[2.2.2]oct-1-yl)-3-propyl-3,7-dihydropurine-2,6-dione (9). 5,6-Diamino-1-propyl-1*H*-pyrimidine-2,4-dione hydrochloride salt (3.4 g) was dissolved in 80 mL of DMF along with 4-hydroxybicyclo[2.2.2]octane-1-carboxylic acid (2.5 g, 15 mmol).[15] HATU (5.9 g, 1.05 equiv) was added, followed by Et_3N (8.30 mL, 4.05 equiv). The reaction mixture was stirred at room temperature overnight. The following morning, the reaction mixture was filtered to remove some precipitate and the resulting filtrate was concentrated under reduced pressure. The residue was dissolved in 60 mL of H_2O containing 10 equiv of NaOH (5.9 g), stirred under reflux for 1 h, then cooled to room temperature and acidified to pH 2 with concentrated HCl. The resulting precipitate was collected by filtration and dried to afford 1.85 g of the xanthine derivative **9**. ^{1}H NMR (DMSO-d_6) δ 11.1 (s, 1 H), 3.6 (t, J = 7.0 Hz, 2 H), 1.9−1.3 (m, 14 H), 0.9 (t, J = 7.0 Hz, 3 H) ppm. MS m/z 317 amu ($M^+ - H$).

Step 3. 8-(4-Hydroxybicyclo[2.2.2]oct-1-yl)-3-propyl-6-thioxo-1,3,6,7-tetrahydropurin-2-one (10). 8-(4-Hydroxybicyclo[2.2.2]-oct-1-yl)-3-propyl-3,7-dihydropurine-2,6-dione (**9**, 500 mg, 1.57 mmol) was dissolved in 10 mL of pyridine. P_4S_{10} (1.05 g, 1.5 equiv) was added, and the reaction mixture was stirred under reflux for 6 h. The reaction mixture was then cooled to room temperature and quenched slowly with 5 mL of H_2O. It was next acidified at 0 °C to pH 5 with 6 N HCl. The aqueous layer was extracted with EtOAc. The combined organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Purification by preparative HPLC afforded 100 mg of the titled compound. ^{1}H NMR (DMSO-d_6) δ 3.6 (t, J = 7.0 Hz, 2 H), 1.9−1.3 (m, 14 H), 0.9 (t, J = 7.0 Hz, 3 H) ppm. MS m/z 333 amu ($M^+ - H$).

Step 4. 8-(4-Hydroxybicyclo[2.2.2]oct-1-yl)-6-methylsulfanyl-3-propyl-3,7-dihydropurin-2-one (11). 8-(4-Hydroxybicyclo[2.2.2]-oct-1-yl)-3-propyl-6-thioxo-1,3,6,7-tetrahydropurin-2-one (**10**, 120 mg, 0.36 mmol) was suspended in 3 mL of H_2O and 1.5 mL of EtOH. NaOH was added as a solution in 0.4 mL of H_2O, followed by 2 molar equiv of MeI. The reaction mixture was stirred at room temperature for 1 h. It was then neutralized with 0.1 N HCl and extracted with $CHCl_3$. The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to afford an essentially quantitative yield of the title compound. ^{1}H NMR (DMSO-d_6) δ 3.6 (t, J = 7.0 Hz, 2 H), 3.3 (s, 3 H), 1.9−1.3 (m, 14 H), 0.9 (t, J = 7.0 Hz, 3 H) ppm. MS m/z 349 amu ($M^+ + H$).

Step 5. Intermediate 12. In order to prepare compound **12**, (*R*)-(−)-2-amino-1-butanol was used. 8-(4-Hydroxybicyclo[2.2.2]oct-1-yl)-6-methylsulfanyl-3-propyl-3,7-dihydropurin-2-one (**11**, 125 mg, 0.36 mmol) was dissolved in 3 mL of DMSO along with an excess of (*R*)-(−)-2-amino-1-butanol (0.24 mL, 7 equiv). The resulting reaction mixture was stirred at 150 °C for 3 h. It was then cooled to room temperature and purified by preparative HPLC to afford 110 mg of the intermediate **12** (where R = (*R*)-Et): ^{1}H NMR (DMSO-d_6) δ 3.8 (m, 2 H), 3.6 (m, 1 H), 3.4 (m, 1 H), 2.0−

1.7 (m, 15 H), 1.0−0.8 (m, 10 H) ppm. MS *m*/*z* = 388 amu (M^+ + H). HPLC retention time = 2.4 min (Agilent HP1100 series equipped with a 100 mm × 4.6 mm i.d. YMC column with S-5 μm packing; gradient elution at a rate of 2 mL/min, 5% aqueous CH_3CN to 95% aqueous CH_3CN in 10 min).

Step 6. Imidazoline of the General Structure 6. For the preparation of compound **14**, the intermediate **12** (where R = (*R*)-Et) prepared in step 5 above was used. 8-(4-Hydroxybicyclo[2.2.2]-oct-1-yl)-6-(1-hydroxymethylpropylamino)-3-propyl-3,7-dihydropurin-2-one (110 mg) was dissolved in 3 mL of $SOCl_2$ and stirred under reflux for 20 min. It was then cooled to room temperature and concentrated. The residue was quenched with saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$. The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. Purification by preparative HPLC afforded 50 mg of compound **14** as the TFA salt: ^{1}H NMR (DMSO-d_6) δ 4.2 (m, 1 H), 3.8 (m, 2 H), 3.7 (m, 1 H), 2.0−1.7 (m, 15 H), 1.0−0.8 (m, 10 H) ppm. ^{13}C NMR (DMSO-d_6) δ 162.1, 147.2, 146.5, 97.8, 67.0, 62.3, 48.1, 44.5, 33.5, 33.1, 32.2, 31.4, 25.4, 22.3, 17.5, 9.5 ppm. MS *m*/*z* = 372 amu (M^+ + H). HPLC t_R= 2.8 min (Agilent HP1100 series equipped with a 100 mm × 4.6 mm i.d. YMC column with S-5 μm packing; gradient elution at a rate of 2 mL/min, 5% aqueous CH_3CN to 95% aqueous CH_3CN in 10 min). Anal. Calcd for $C_{20}H_{29}N_5O_8$·HCl: C, 58.92; H, 7.42; N, 17.18. Found: C, 58.15; H, 7.09; N, 16.98.

In order to prepare compounds **13**, **15**−**23**, and **24**−**27**, the appropriate amino alcohol was used in step 5 of the above general procedure. For instance, in the preparation of compound **20**, (*R*)-2-amino-3-methyl-1-butanol was used as the amino alcohol component in step 5. Spectroscopic data for **20**: ^{1}H NMR (DMSO-d_6) δ 4.2 (m, 1 H), 3.8 (m, 2 H), 3.7 (m, 1 H), 2.0−1.7 (m, 15 H), 1.0−0.8 (m, 12 H) ppm. ^{13}C NMR (DMSO-d_6) δ 162.5, 147.8, 147.6, 97.8, 67.0, 62.8, 48.9, 44.5, 33.3, 32.9, 32.0, 31.1, 25.4, 20.6, 18.0, 17.8, 10.9 ppm. MS *m*/*z* = 386 amu (M^+ + H). HPLC t_R = 3.1 min (Agilent HP1100 series equipped with a 100 mm × 4.6 mm i.d. YMC column with S-5 μm packing; gradient elution at a rate of 2 mL/min, 5% aqueous CH_3CN to 95% aqueous CH_3CN in 10 min). High-resolution MS (M^+ + H) calcd for $C_{21}H_{32}N_5O_2$ 386.2556; found 386.2527.

General Procedure To Prepare Compounds 31 and 32. Step 1. Preparation of Intermediate 29. 5,6-Diamino-1-propyl-1*H*-pyrimidine-2,4-dione hydrochloride salt (570 mg) was dissolved in 20 mL of DMF along with bicyclo[2.2.2]octane-1,4-dicarboxylic acid monomethyl ester (520 mg, 2.45 mmol). HATU (980 mg, 1.05 equiv) was added, followed by Et_3N (1.40 mL, 4.05 equiv). The reaction mixture was stirred at room temperature overnight. The following morning, the reaction mixture was filtered to remove some precipitate, and the clear filtrate was concentrated under reduced pressure. The resulting residue was dissolved in 10 mL of H_2O containing 10 equiv of NaOH (980 mg). The reaction mixture was stirred under reflux for 2 h. It was then cooled to room temperature and acidified to pH 2 with concentrated HCl. The resulting precipitate was collected by filtration and dried to afford 680 mg of **29** as a white solid (80% yield). ^{1}H NMR (DMSO-d_6) δ 3.6 (t, *J* = 7.0 Hz, 2 H), 1.9−1.3 (m, 14 H), 0.9 (t, *J* = 7.0 Hz, 3 H) ppm. MS *m*/*z* 345 amu (M^+ − H). HPLC t_R = 1.8 min (Agilent HP1100 series equipped with a 100 mm × 4.6 mm i.d. YMC column with S-5 μm packing; gradient elution at a rate of 2 mL/min, 5% aqueous CH_3CN to 95% aqueous CH_3CN in 10 min).

Step 2. Preparation of Intermediate 30. The intermediate **29** above (1.4 g) was suspended in 50 mL of MeOH, and 5 drops of concentrated sulfuric acid was added. The reaction mixture was stirred under reflux for 18 h. It was then cooled to room temperature and concentrated under reduced pressure. The resulting residue was diluted with CH_2Cl_2 and washed with aqueous $NaHCO_3$ and brine, dried (Na_2SO_4), and concentrated to afford 1.2 g of the methyl ester. This material (1.2 g, 3.33 mmol) was dissolved in 20 mL of pyridine. P_4S_{10} (2.22 g, 1.5 equiv) was added, and the reaction mixture was stirred under reflux for 3 h. It was then cooled to 0 °C and carefully quenched with water. Enough 6 N HCl was added to bring the pH to 5, and the reaction mixture was extracted with CH_2Cl_2. The organic layer was dried (Na_2SO_4) and concentrated to afford 860 mg of the thioamide. MS *m*/*z* 375 amu (M^+ + H). This material (860 mg, 2.29 mmol) was dissolved in 5 mL of EtOH and 5 mL of H_2O. NaOH (183 mg, 2 equiv) was added as a solution in 2 mL of H_2O, followed by MeI (213 μL, 1.5 equiv). The resulting reaction mixture was stirred at room temperature for 30 min. It was then extracted with EtOAc. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to afford 800 mg of **30**. ^{1}H NMR (DMSO-d_6) δ 3.6 (t, *J* = 7.0 Hz, 2 H), 3.3 (s, 3 H), 2.4 (s, 3 H), 1.9−1.3 (m, 14 H), 0.9 (t, *J* = 7.0 Hz, 3 H) ppm. MS *m*/*z* 391 amu (M^+ + H).

Step 3. Preparation of 31. Intermediate **30** (50 mg) was dissolved in 1−2 mL of DMSO along with 7 equiv of (*R*)-(−)-2-amino-1-butanol. The reaction mixture was stirred at 150 °C for 3 h. It was then cooled to room temperature and purified by preparative HPLC. The resulting compound (30 mg) was dissolved in 1 mL of $SOCl_2$ and stirred under reflux for 15 min. The reaction mixture was then cooled to room temperature and concentrated under reduced pressure. The resulting residue was dissolved in a solution containing 1 mL of water, 0.5 mL of MeOH, and 0.1 mL of 10% aqueous NaOH. The reaction mixture was stirred at room temperature for 30 min. It was then acidified to pH 2 with dilute 1 N HCl and concentrated. The resulting crude product was purified by preparative HPLC to afford **31**: ^{1}H NMR (DMSO-d_6) δ 4.2 (m, 1 H), 3.8 (m, 2 H), 3.7 (m, 1 H), 2.0−1.7 (m, 15 H), 1.0−0.8 (m, 10 H) ppm. ^{13}C NMR (DMSO-d_6) 181.1, 162.3, 147.2, 146.5, 98.7, 67.7, 48.1, 44.5, 35.6, 33.5, 33.1, 32.2, 29.4, 25.4, 22.3, 17.1, 9.8 ppm. MS *m*/*z* = 400 amu (M^+ + H). HPLC t_R = 2.2 min (Agilent HP1100 series equipped with a 100 mm × 4.6 mm i.d. YMC column with S-5 μm packing; gradient elution at a rate of 2 mL/min, 5% aqueous CH_3CN to 95% aqueous CH_3CN in 10 min).

Compound **32** was prepared using the same procedure outlined above but using (*S*)-(−)-2-amino-1-butanol.

General Procedure for Preparing Compounds 35 and 36. Step 1. Preparation of Intermediate 33. The acid **29** (3.2 g, 9.25 mmol) was dissolved in 100 mL of anhydrous THF and cooled to 0 °C. Borane−THF (1.0 M in THF, 18.5 mL, 2 equiv) was added, and the reaction mixture was stirred at 0 °C for 10 min. It was then warmed to room temperature and stirred for 48 h. The reaction mixture was then carefully quenched with 10 mL of MeOH and then concentrated under reduced pressure. The resulting residue was dissolved in 20 mL of MeOH and concentrated under reduced pressure. This treatment was repeated four more times to afford the desired alcohol. MS *m*/*z* 333 amu (M^+ + H). ^{1}H NMR (DMSO-d_6) δ 3.6 (t, *J* = 7.0 Hz, 2 H), 3.2 (br s, 2 H), 1.9−1.3 (m, 14 H), 0.9 (t, *J* = 7.0 Hz, 3 H) ppm. This alcohol (2.70 g, 8.13 mmol) was dissolved in 40 mL of DMSO. Pyridine−SO_3 (3.88 g, 3 equiv) was added, followed by Et_3N (7.4 mL, 7 equiv) at room temperature. The resulting reaction mixture was stirred at room temperature for 18 h. It was then diluted with EtOAc and washed with 5% aqueous citric acid, H_2O, and brine, dried (Na_2SO_4), and concentrated under reduced pressure to afford 900 mg of the desired aldehyde. MS *m*/*z* 329 amu (M^+ − H). ^{1}H NMR (DMSO-d_6) δ 9.1 (s, 1 H), 3.6 (t, *J* = 7.0 Hz, 2 H), 1.9−1.3 (m, 14 H), 0.9 (t, *J* = 7.0 Hz, 3 H) ppm. This material (900 mg, 2.73 mmol) was dissolved in 25 mL of THF, and methyl (triphenylphosphoranylidene)acetate (1.83 g, 2 equiv) was added. The resulting reaction mixture was stirred under reflux for 18 h. It was then cooled to room temperature and purified by preparative HPLC using a mixture of aqueous acetonitrile to afford 300 mg of intermediate **33**. ^{1}H NMR (DMSO-d_6) δ 7.0 (d, *J* = 12 Hz, 1 H), 5.8 (d, *J* = 12 Hz, 1 H), 3.8 (s, 3 H), 3.6 (t, *J* = 7.0 Hz, 2 H), 1.9−1.3 (m, 14 H), 0.9 (t, *J* = 7.0 Hz, 3 H) ppm. MS *m*/*z* 387 amu (M^+ + H). HPLC t_R = 3.4 min (Agilent HP1100 series equipped with a 100 mm × 4.6 mm i.d. YMC column with S-5 μm packing; gradient elution at a rate of 2 mL/min, 5% aqueous CH_3CN to 95% aqueous CH_3CN in 10 min).

Step 2. Preparation of Intermediate 34. Intermediate **33** (300 mg) was dissolved in 20 mL of THF. Then 10% Pd on C (25 mg) was added and the resulting reaction mixture was hydrogenated under 50 psi of H_2 at room temperature for 6 h. The reaction mixture was filtered through Celite, and the filtrate was concentrated under

reduced pressure to afford 280 mg of the propionate. MS *m*/*z* 389 ($M^+ + H$). ^{1}H NMR (DMSO-d_6) δ 3.8 (s, 3 H), 3.6 (t, $J = 7.0$ Hz, 2 H), 2.2−1.3 (m, 18 H), 0.9 (t, $J = 7.0$ Hz, 3 H) ppm. This material (250 mg, 0.64 mmol) was dissolved in 8 mL of pyridine. P_4S_{10} (430 mg, 1.5 equiv) was added, and the reaction mixture was stirred under reflux for 3 h. It was then cooled to room temperature and quenched with 3 mL of H_2O and then with enough 6 N HCl to bring the pH to 3. The resulting reaction mixture was extracted with $CHCl_3$. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was purified by preparative HPLC to afford 100 mg of the thioamide. MS *m*/*z* 405 ($M^+ + H$). This fairly labile thioamide (100 mg) was dissolved in 2 mL of EtOH and 1 mL of H_2O. NaOH (20 mg) was added as a solution in 1 mL of H_2O, followed by MeI (23 μL, 1.5 equiv). The resulting reaction mixture was stirred at room temperature for 30 min. It was then extracted with EtOAc. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to afford 105 mg of intermediate **34**. ^{1}H NMR (DMSO-d_6) δ 3.8 (s, 3 H), 3.6 (t, $J = 7.0$ Hz, 2 H), 3.2 (s, 3 H), 2.2−1.3 (m, 18 H), 0.9 (t, $J = 7.0$ Hz, 3 H) ppm. MS *m*/*z* 417 ($M^+ - H$). HPLC $t_R = 4.0$ min (Agilent HP1100 series equipped with a 100 mm × 4.6 mm i.d. YMC column with S-5 μm packing; gradient elution at a rate of 2 mL/min, 5% aqueous CH_3CN to 95% aqueous CH_3CN in 10 min).

Step 3. Preparation of 35. The methyl ester derivative **35** (105 mg) was dissolved in 2 mL of DMSO along with 160 μL of (*R*)-2-amino-1-butanol. The reaction mixture was stirred at 150 °C for 3 h. It was then cooled to room temperature and purified by preparative HPLC to afford 50 mg of the desired amino alcohol. This material (30 mg) was dissolved in 1 mL of $SOCl_2$ and stirred under reflux for 15 min. The reaction mixture was then cooled to room temperature and concentrated under reduced pressure. The resulting residue was dissolved in a solution containing 1 mL of water, 0.5 mL of MeOH, and 0.1 mL of 10% aqueous NaOH. The reaction mixture was stirred at room temperature for 30 min. It was then acidified to pH 2 with dilute 1 N HCl and concentrated. The resulting crude product was purified by preparative HPLC to afford **35**: ^{1}H NMR (DMSO-d_6) δ 4.2 (m, 2 H), 3.8 (m, 2 H), 3.7 (m, 1 H), 2.2 (m, 2 H), 2.0−1.7 (m, 18 H), 1.0−0.8 (m, 6 H) ppm. ^{13}C NMR (DMSO-d_6) δ 177.7, 162.6, 147.7, 146.9, 99.2, 67.7, 48.1, 44.5, 35.6, 33.4, 32.9, 32.2, 31.6, 29.4, 25.4, 22.3, 20.3, 17.1, 9.8 ppm. MS *m*/*z* = 428 amu ($M^+ + H$). HPLC $t_R = 4.5$ min (Agilent HP1100 series equipped with a 100 mm × 4.6 mm i.d. YMC column with S-5 μm packing; gradient elution at a rate of 2 mL/min, 5% aqueous CH_3CN to 95% aqueous CH_3CN in 10 min). Anal. Calcd for $C_{23}H_{33}N_5O_8 \cdot 2H_2O$: C, 59.59; H, 8.04; N, 15.11. Found: C, 58.97; H, 7.21; N, 14.57.

Compound **36** was prepared using the same procedure outlined above, but using (*R*)-2-amino-3-methyl-1-butanol.

Biological Assays. Rat A_1 Receptor. The membranes were prepared from rat cerebral cortex isolated from freshly euthanized rats. The tissues were homogenized in buffer A (10 mM EDTA, 10 mM Na-HEPES, pH 7.4) supplemented with protease inhibitors (10 μg/mL benzamidine, 100 μM PMSF, and 2 μg/mL each of aprotinin, pepstatin, and leupeptin) and centrifuged at 20000*g* for 20 min. The pellets were resuspended and washed twice with buffer HE (10 mM Na-HEPES, 1 mM EDTA, pH 7.4, plus protease inhibitors). The final pellets were resuspended in buffer HE, supplemented with 10% (w/v) sucrose and protease inhibitors, and frozen in aliquots at −80 °C. The protein concentrations were measured using the BCA protein assay kit (Pierce).

Rat A_{2A} Receptor. The membranes were prepared from rat striatum tissues purchased from Pel-Freez. The tissues were homogenized in buffer A (10 mM EDTA, 10 mM Na-HEPES, pH 7.4) supplemented with protease inhibitors (10 μg/mL benzamidine, 100 μM PMSF, and 2 μg/mL each of aprotinin, pepstatin, and leupeptin) and centrifuged at 20000*g* for 20 min. The pellets were resuspended and washed twice with buffer HE (10 mM Na-HEPES, 1 mM EDTA, pH 7.4, plus protease inhibitors). The final pellets were resuspended in buffer HE, supplemented with 10% (w/v) sucrose and protease inhibitors, and frozen in aliquots at −80 °C. Protein concentrations were measured using the BCA protein assay kit (Pierce).

Radioligand Binding Assays. Membranes (40−70 μg of membrane protein), radioligands, and varying concentrations of competing ligands were incubated in triplicate in 0.1 mL of buffer HE plus 2 units/mL adenosine deaminase for 2.5 h at 21 °C. Radioligand [^{3}H]DPCPX (2 nM) was used for competition binding assays on A_1 receptors, and [^{3}H]ZM241385 (0.5−1.2 nM) was used for A_{2A} adenosine receptors. Nonspecific binding was measured in the presence of 10 μM NECA for A_1 receptors or 10 μM XAC for A_{2A} receptors. The binding assays were terminated by filtration over Whatman GF/C glass fiber filters using a BRANDEL cell harvester. The filters were rinsed three times with 3−4 mL of ice-cold 10 mM Tris-HCl, pH 7.4, and 5 mM $MgCl_2$ at 4 °C and were counted in a Wallac β-counter.

Analysis of Binding Data. For K_i determinations, competition binding data were fit to a single-site binding model and plotted using GraphPad Prism (GraphPad Software, San Diego, CA). The Cheng−Prusoff equation $K_i = IC_{50}/(1 + [I]/K_d)$ was used to calculate K_i values from IC_{50} values, where K_i is the affinity constant for the competing ligand, [I] is the concentration of the free radioligand, and K_d is the dissociation constant for the radioligand.

Human adenosine receptor binding assays, pA_2 determinations, and the rat oral efficacy screens were performed as outlined in the preceding article.[8a]

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Supporting Information Available: Results 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.

References

(1) DeNino, M. P. Adenosine. *Annu. Rep. Med. Chem.* **1998**, *33*, 111−121.

(2) (a) Müller, C. E.; Scior, T. Adenosine Receptors and Their Modulators. *Pharm. Acta Helv.* **1993**, *68*, 77−111. (b) Fredholm, B. B.; Abbraccio, M. P.; Burnstock, G.; Daly, J. W.; Harden, T. K.; Jacobson, K. A.; Leff, P.; Williams, M. Nomenclature and Classification of Purinoceptors. *Pharmacol. Rev.* **1994**, *46*, 143−156.

(3) Welch, W. J. Adenosine A_1 Receptor Antagonists in the Kidney: Effects in Fluid-Retaining Disorders. *Curr. Opin. Pharmacol.* **2002**, *2*, 165−170.

(4) Osswald, H. The Role of Adenosine in the Regulation of Glomerular Filtration Rate and Renin Secretion. *Trends Pharmacol. Sci.* **1984**, *5*, 94−97.

(5) (a) Mizumoto, H.; Karasawa, A.; Kubo, K. Diuretic and Renal Protective Effects of 8-(Noradamantan-3-yl)-1,3-dipropylxanthine (KW-3902), a Novel Adenosine A_1 Receptor Antagonist, via Pertussis Toxin Insensitive Mechanism. *J. Pharmacol. Exp. Ther.* **1993**, *266*, 200−206. (b) Wilcox, C. S.; Welch, W. J.; Schreiner, G. F.; Belardinelli, L. Natriuretic and Diuretic Actions of a Highly Selective Adenosine A_1 Receptor Antagonist. *J. Am. Soc. Nephrol.* **1999**, *10*, 714−720.

(6) (a) Mizumoto, H.; Kobayashi, T.; Karasawa, A.; Nonaka, H.; Ishii, A.; Kubo, K.; Shimada, J.; Suzuki, F. Renal Protective Effects of a Novel Adenosine A_1 Receptor Antagonist, KW-3902. *Jpn J. Pharmacol.* **1992**, *58* (Suppl. I), 194P. (b) Yao, K.; Kusaka, H.; Sato, K.; Karasawa, A. Protective Effects of KW-3902, a Novel Adenosine A_1 Receptor Antagonist, against Gentamycin-Induced Acute Renal Failure in Rats. *Jpn. J. Pharmacol.* **1994**, *65*, 167−170. (c) Yao, K.; Kusaka, H.; Sano, J.; Sato, K.; Karasawa, A. Diuretic Effects of KW-3902, a Novel Adenosine A_1 Receptor Antagonist, in Various Models of Acute Renal Failure in Rats. *Jpn. J. Pharmacol.* **1994**, *65*, 281−288. (d) Nagashima, K.; Kusaka, H.; Sato, K.; Karasawa, A. Effects of KW-3902, a Novel Adenosine A_1 Receptor Antagonist, on Cephaloridine-Induced Acute Renal Failure in Rats. *Jpn J. Pharmacol.* **1994**, *64*, 9−17. (e) Nagashima, K.; Kusaka, H.; Karasawa, A. Protective Effects of KW-3092, an Adenosine A_1 Receptor

Antagonist, against Cisplatin-Induced Acute Renal Failure in Rats. *Jpn. J. Pharmacol.* **1995**, *67*, 349−357. (f) Uehara, Y.; Numabe, A.; Hirawa, N.; Kawabata, Y.; Nagoshi, H.; Kaneko, H.; Gomi, T.; Goto, A.; Toyo-oka, T.; Omata, M. A New Adenosine Subtype-1 Receptor Antagonist, FK-838, Attenuates Salt-Induced Hypertension in Dahl Salt-Sensitive Rats. *Am. J. Hypertens.* **1995**, *8*, 1189−1199.

(7) (a) Gellai, M.; Schreiner, G. F.; Ruffolo, R. R., Jr.; Fetcher, T.; Dewolf, R.; Brooks, D. P. CVT-124, a Novel Adenosine A_1 Receptor Antagonist with Unique Diuretic Activity. *J. Pharmacol. Exp. Ther.* **1998**, *286*, 1191−1196. (b) Gottlieb, S. S.; Skettino, S. L.; Wolff, A.; Beckman, E.; Fisher, M. L.; Freudenberger, R.; Gladwell, T.; Marshall, J.; Cines, M.; Bennett, D.; Littschwager, E. B. Effects of BG9719 (CVT-124), an A_1-Adenosine Receptor Antagonist, and Furosemide on Glomerular Filtration Rate and Natriuresis in Patients with Congestive Heart Failure. *J. Am. Coll. Cardiol.* **2000**, *35*, 56−69.

(8) (a) Kiesman, W. F.; Zhao, J.; Conlon, P. R.; Dowling, J. E.; Petter, R. C.; Lutterodt, F.; Jin, X.; Smits, G.; Fure, M.; Jayaraj, A.; Kim, J.; Sullivan, G.; Linden, J. Potent and Orally Bioavailable 8-Bicyclo-[2.2.2]octylxanthines as Adenosine A_1 Receptor Antagonists. *J. Med. Chem.* **2006**, *49*, 7119−7131. (b) Petter, R. C.; Kiesman, W. F.; Conlon, P. R.; Kumaravel, G.; Ensinger, C. L.; Dowling, J.; Peng, B.; Smits, G.; Jin, X.; Lutterodt, F. A.; Fu, K.; LePage, D.; Jayaraj, A.; Gill, A.; Costa, D.; Wortham, K.; Porter, K.; Linden, J.; Sullivan, G. *Abstracts of Papers*, 224th National Meeting of the American Chemical Society, Boston, MA, 2002; American Chemical Society: Washington, DC, 2002; MEDI-417. (c) Ticho, B.; Whalley, E.; Gill, A.; Lutterodt, F.; Jin, X.; Auchampach, J.; Smits, G. Renal effects of BG9928, an A_1 Adenosine Receptor Antagonist, in Rats and Nonhuman Primates. *Drug Dev. Res.* **2003**, *58*, 486−492.

(9) For reviews on adenosine A_1 receptor antagonists, see the following. (a) Müller, C. E.; Stein, B. Adenosine Receptor Antagonists: Structures and Potential Therapeutic Applications. *Curr. Pharm. Des.* **1996**, *2*, 501−530. (b) Müller, C. E. A_1-Adenosine Receptor Antagonists. *Expert Opin. Ther. Pat.* **1997**, *7*, 419−440. (c) Jacobson, K. A.; Gao, Z. G. Adenosine receptors as therapeutic targets. *Nat. Rev. Drug Discovery* **2006**, *5*, 247−264. (d) Novellino, E.; Abignente, E.; Cosimelli, B.; Greco, G.; Iadanza, M.; Laneri, S.; Lavecchia, A.; Rimoli, M. G.; Da Settimo, F.; Primofiore, G.; Tuscano, D.; Trincavelli, L.; Martini, C. Design, Synthesis and Biological Evaluation of Novel *N*-Alkyl- and *N*-Acyl-(7-substituted-2-phenylimidazo-[1,2-*a*][1,3,5]triazin-4-yl)amines (ITAs) as Novel A_1 Adenosine Receptor Antagonists. *J. Med. Chem.* **2002**, *45*, 5030−5036.

(10) Akahane, A.; Katayama, H.; Mitsunaga, T.; Kato, T.; Kinoshita, T.; Kita, Y.; Kusunoki, T.; Terai, T.; Yoshida, K.; Shiokawa, Y. Discovery of 6-Oxo-3-(2-phenylpyrazolo[1,5-*a*]pyridin-3-yl-1(6*H*)-pyridazinebutanoic Acid (FK 838): A Novel Non-Xanthine Adenosine A_1 Receptor Antagonist with Potent Diuretic Activity. *J. Med. Chem.* **1999**, *42*, 779−783.

(11) Ferrarini, P. L.; Mori, C.; Manera, C.; Martinelli, A.; Mori, F.; Saccomanni, G.; Barili, P. L.; Betti, L.; Giannaccini, G.; Trincavelli, L.; Lacacchini, A. A Novel Class of Highly Potent and Selective A_1 Adenosine Antagonists: Structure−Affinity Profile of a Series of 1,8-Naphthyridine Derivatives. *J. Med. Chem.* **2000**, *43*, 2814−2823.

(12) (a) Suzuki, F.; Shimada, J.; Nonaka, H.; Ishii, A.; Shiozaki, S.; Ichikawa, S.; Ono, E. 7,8-Dihydro-8-ethyl-2-(3-noradamantyl)-4-propyl-1*H*-imidazo[2,1-*i*]purin-5(4*H*)-one: A Potent and Water-Soluble Adenosine A_1 Antagonist. *J. Med. Chem.* **1992**, *35*, 3581−3583. (b) Suzuki, F.; Shimada, J.; Shiozaki, S.; Ichikawa, S.; Ishii, A.; Nakamura, J.; Nonaka, H.; Kobayashi, H.; Fuse, E. Adenosine A_1 Antagonists. 3. Structure−Activity Relationships on Amelioration against Scopolamine- or N^6-((*R*)-Phenylisopropyl)adenosine-Induced Cognitive Disturbance. *J. Med. Chem.* **1993**, *36*, 2508−2518.

(13) Müller, C. E.; Thorand, M.; Qurishi, R.; Diekmann, M.; Jacobson, K. A.; Padgett, W. L.; Daly, J. W. Imidazo[2,1-*i*]purin-5-ones and Related Tricyclic Water-Soluble Purine Derivatives: Potent A_{2a}- and A_3-Adenosine Receptor Antagonists. *J. Med. Chem.* **2002**, *45*, 3440−3450.

(14) (a) Papesch, V.; Schroeder, E. F. Synthesis of 1-Mono- and 1,3-Di-Substituted 6-Amino-Uracils. Diuretic Activity. *J. Org. Chem.* **1951**, *16*, 1879−1890. (b) Shamin, M. T.; Ukena, D.; Padgett, W. L.; Daly, J. W. Effects of 8-Phenyl and 8-Cycloalkyl Substituents on the Activity of Mono-, Di-, and Trisubstituted Alkylxanthines with Substitution at the 1-, 3-, and 7-Positions. *J. Med. Chem.* **1989**, *32*, 1231−1237. (c) Cottam, H. B.; Shih, H.; Tehrani, L. R.; Wasson, D. B.; Carson, D. A. Substituted Xanthines, Pteridinediones, and Related Compounds as Potential Antiinflammatory Agents. Synthesis and Biological Evaluation of Inhibitors of Tumor Necrosis Factor α. *J. Med. Chem.* **1996**, *39*, 2−9.

(15) Roberts, J. D.; Moreland, W. T., Jr.; Frazer, W. Syntheses of Some 4-Substituted Bicyclo[2.2.2]octane-1-carboxylic Acids. *J. Am. Chem. Soc.* **1953**, *75*, 637−640.

(16) Additional details regarding the rat diuresis model can be found in ref 8.

(17) Additional details regarding the determination of the pA_2 values can be found in ref 8.

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