Discovery of a Novel A_{2B} Adenosine Receptor Antagonist as a Clinical Candidate for Chronic Inflammatory Airway Diseases

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Received November 28, 2007

Recently, we have reported a series of new 1,3-symmetrically ($R_1 = R_3$) substituted xanthines (**3** and **4**) which have high affinity and selectivity for the human adenosine A_{2B} receptors (hA_{2B}-AdoR). Unfortunately, this class of compounds had poor pharmacokinetic properties. This prompted us to investigate the effect of differential alkyl substitution at the *N*-1 and *N*-3 positions (N_1-R ≠ N_3-R) on A_{2B}-AdoR affinity and selectivity; we had the dual objectives of enhancing affinity and selectivity for the A_{2B}−AdoR, as well as improving oral bioavailability. This effort has led to the discovery of compound **62**, that displayed high affinity and selectivity for the hA_{2B}-AdoR ($K_i = 22$ nM). In addition, compound **62** showed high functional potency in inhibiting the accumulation of cyclic adenosine monophosphate induced by 5′-*N*-ethylcarboxamidoadenosine in HEK-A_{2B}-AdoR and NIH3T3 cells with K_B values of 6 and 2 nM, respectively. In a single ascending-dose phase I clinical study, compound **62** had no serious adverse events and was well tolerated.

Introduction

Adenosine is an endogenous purine nucleoside present in every cell of the human body. Four distinct adenosine receptor subtypes have been identified to date, A_1, A_{2A}, A_{2B}, and A_3, all of which belong to the family of G-protein-coupled receptors characterized by 7-transmembrane-spanning helical domains.[1] Interaction of adenosine with its receptors initiates signal transduction pathways, including the classical adenylate cyclase effector system that utilizes cyclic adenosine monophosphate (cAMP)[a] as a second messenger. Activation of the A_1 and A_3 adenosine receptors (A_1-AdoR and A_3-AdoR) inhibits adenylate cyclase activity through activation of pertussis-sensitive G_i proteins and results in a decrease in intracellular levels of cAMP. On the other hand, activation of the A_{2A} and A_{2B} adenosine receptors (A_{2A}-AdoR and A_{2B}-AdoR) stimulates adenylate cyclase via activation of G_s proteins and leads to intracellular accumulation of cAMP. Coupling of adenosine receptors to other second messenger systems has also been described, for example, stimulation of phospholipase C (A_1-, A_{2B}-, and A_3-AdoR's), activation of potassium and inhibition of calcium channels in cardiac muscles and neurons (A_1-AdoR), mobilization of intracellular calcium (A_3-AdoR), and coupling to mitogen-activated protein kinase (all four receptors).[2–4]

Adenosine receptors have been recognized to play an important role in chronic inflammatory airway conditions such as asthma, chronic obstructive pulmonary disease (COPD), and fibrosis.[5–7] The elevation of adenosine in bronchoalveolar lavage fluid of asthmatics combined with its bronchoconstriction effect on airways in asthmatics has led to increased research into the contribution of adenosine in the pathology of inflammation and asthma.[8,9] There is ample evidence now to provide support for the key role that adenosine and its A_{2B} receptors play in asthma.[10–15] Theophylline (**1**), which has a well-established role in the therapy of asthma, selectively blocks the AMP-induced bronchoconstriction in asthmatics. The bronchodilating effect of theophylline and its structural analogue enprofylline (**2**) has been attributed to a selective antagonism of the A_{2B}-AdoR.[16] In addition, the recent discovery that A_{2B}-AdoR's are functionally active on both human airway smooth muscle cells (where they enhance cytokine and chemokine release) and lung fibroblast cells (where they promote differentiation to a myofibroblast phenotype) provides further support to the role of A_{2B}-AdoR in inflammation and asthma.[14,17] Therefore, antagonists at the A_{2B}-AdoR would provide a novel approach to the management and treatment of asthma and COPD.

Even though a number of high-affinity A_{2B}-AdoR antagonists have been reported, only a few have shown high affinity and selectivity for the A_{2B}-AdoR relative to the A_1-, A_{2A}-, and A_3-AdoR's.[18–21] Recently, we have reported a series of new 1,3-symmetrically ($R_1 = R_3$) substituted xanthines (Figure 1, compounds **3** and **4**), which have high affinity and selectivity for the A_{2B}-AdoR.[22] Unfortunately, this class of compounds showed poor pharmacokinetic (PK) properties. The 1,3-diethyl analogue **3a** showed greater affinity and selectivity for the A_{2B}-AdoR in comparison to the 1,3-dimethyl and dipropyl analogues **3b** and **3c** (Figure 1). However, when the 3-fluro substituent in the phenyl ring was replaced with a 3-trifluromethyl group, the 1,3-dimethyl analogue **4b** displayed increased affinity and selectivity for the A_{2B}-AdoR ($K_i = 1$ nM) relative to the 1,3-diethyl and dipropyl analogues **4a** and **4c**. This prompted us to further investigate the effect of differential alkyl substitution at the *N*-1 and *N*-3 positions (1,3-nonsymmetrical alkyl substitution, *N*-R_1 ≠ *N*-R_3) on A_{2B}-AdoR affinity and selectivity, our overall objective being to expand upon the structure−activity relationship (SAR) of our 8-pyrazolyl xanthine scaffold, enhanc-

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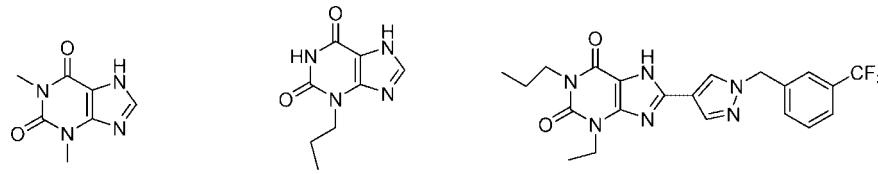
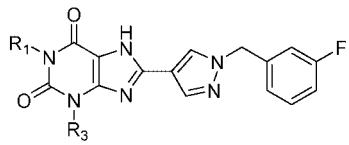
[a] Abbreviations: NECA, 5′-*N*-ethylcarboxamidoadenosine; HEK, human embryonic kidney; 3T3 cells, 3-day transfer inoculum 3×10^5 cells; cAMP, cyclic adenosine monophosphate; COPD, chronic obstructive pulmonary disease; PK, pharmacokinetic; SAR, structure−activity relationship; HEPES, 4-(2-hydroxyl)piperazine-1-ethanesulfonic acid; DMF, dimethyl formamide; DMA, dimethyl acetal; dAUC, dose-adjusted area under curve; CHO, chinese hamster ovary; CPX, 1,3-dipropyl-8-cyclopentylxanthine; I-AB-MECA, N^6-(3-iodo-4-aminobenzyl)adenosine-5′-*N*-methyluronamide.

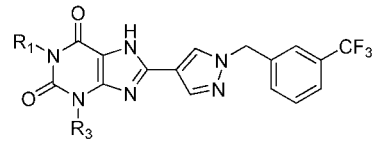
10.1021/jm7014815 CCC: $40.75
Published on Web 03/06/2008



1 Theophylline **2** Enprofylline **62**



3a $R_1 = R_3 = Et$
$K_i(hA_{2B}) = 5$ nM; $A_1/A_{2B} = 76$; $A_{2A}/A_{2B} = 58$; $A_3/A_{2B} = 91$
3b $R_1 = R_3 = Me$
$K_i(hA_{2B}) = 27$ nM; $A_1/A_{2B} = 17$; $A_{2A}/A_{2B} = 7$
3c $R_1 = R_3 =$ propyl
$K_i(hA_{2B}) = 14$ nM; $A_1/A_{2B} = 13$; $A_{2A}/A_{2B} = 17$; $A_3/A_{2B} = 4$



4a $R_1 = R_3 = Et$
$K_i(hA_{2B}) = 13$ nM; $A_1/A_{2B} = 44$; $A_{2A}/A_{2B} = 34$; $A_3/A_{2B} = 34$
4b $R_1 = R_3 = Me$
$K_i(hA_{2B}) = 1$nM; $A_1/A_{2B} = 992$; $A_{2A}/A_{2B} = 686$; $A_3/A_{2B} = 1000$
4c $R_1 = R_3 =$ propyl
$K_i(hA_{2B}) = 14$ nM; $A_1/A_{2B} = 12$; $A_{2A}/A_{2B} = 28$; $A_3/A_{2B} = 10$

Figure 1. A_{2B}-AdoR antagonists.

ing affinity and selectivity for the A_{2B}-AdoR, as well as improving oral bioavailability. This effort has led to the discovery of compound **62**, which in a separate study inhibited pulmonary inflammation and injury in the lung of adenosine deaminase-deficient mice and in a model of bleomycin-induced lung injury.[23] In addition, compound **62** attenuated the airway reactivity induced by 5′-*N*-ethylcarboxamidoadenosine (NECA), AMP, or allergen in sensitized mice.[24] To our knowledge, this represented the first in vivo preclinical evidence that A_{2B}-AdoR antagonism may be of significant therapeutic value to the management of chronic lung diseases such as asthma and COPD. Herein, we describe the synthesis and SAR that led to the discovery of compound **62**.

In these new analogues, the substituent at the *N*-3 position was fixed to either a methyl or an ethyl group, whereas the substituent at the *N*-1 position was varied to include methyl, ethyl, propyl, isobutyl, and cyclopropyl methyl groups. Furthermore, the nature and position of substituents in the phenyl ring were limited to the 3-F and 3-CF_3, because in previous studies, these substituents bestowed the highest A_{2B}-AdoR binding affinity and selectivity.[22] In our discovery paradigm, the compounds were first screened to determine their A_{2B}-AdoR binding affinities. In general, compounds that displayed K_i values <100 nM were further screened for their A_1- and A_{2A}-AdoR's binding affinities, and compounds that showed >10-fold selectivity for the A_{2B}-AdoR over A_1- and A_{2A}-AdoR's were subsequently evaluated for their A_3-AdoR binding affinity. Our ultimate goal was to identify analogues that fulfill our targeted affinity and selectivity profile of $K_{iA2B} < 50$ nM and >25-fold selectivity over A_1-, A_{2A}-, and A_3-AdoR's. Finally, analogues that met the targeted profile of affinity and selectivity were further evaluated for their functional activity and selectivity, as well as for their PK properties.

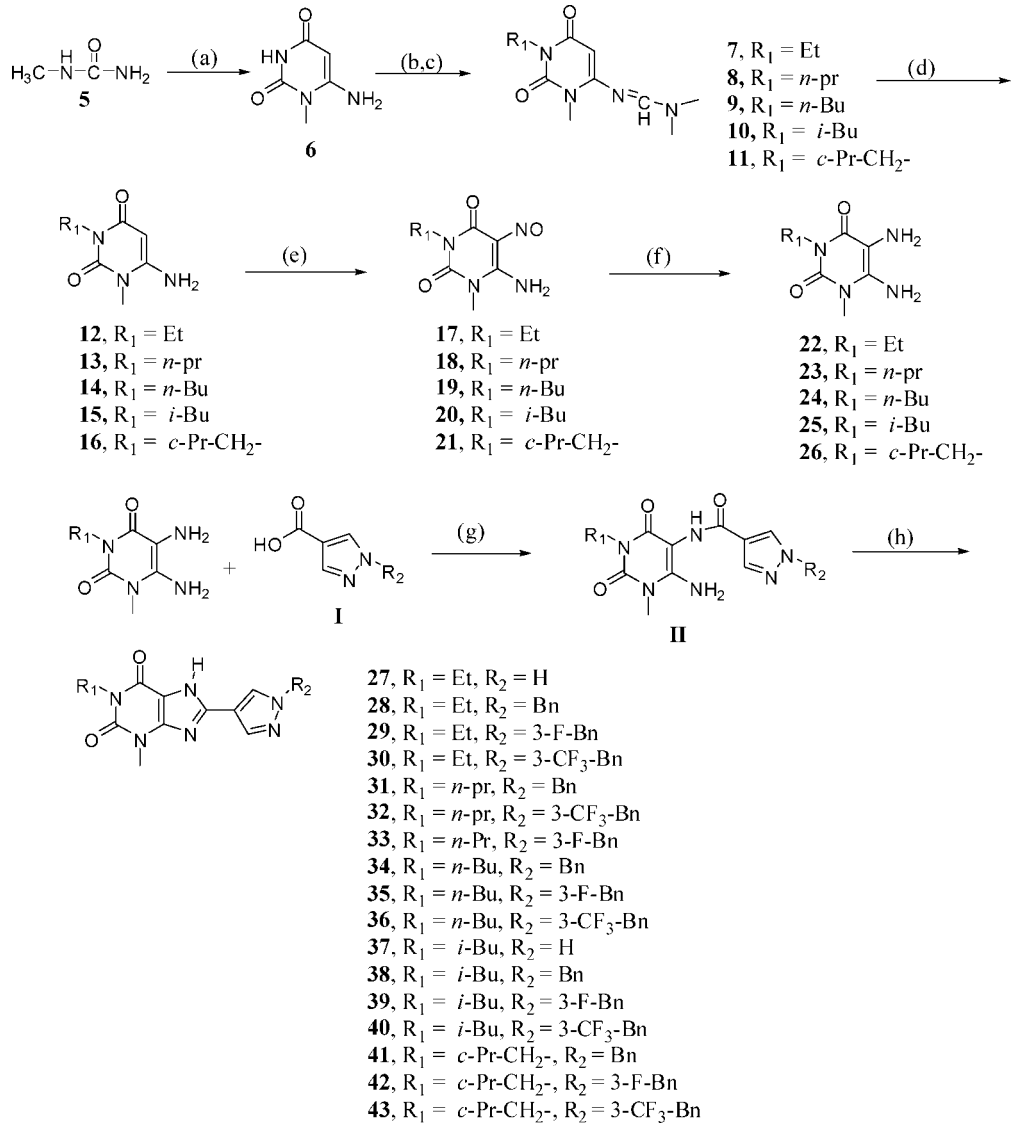
Chemistry

The synthesis of the target compounds is outlined in Schemes 1−3. Condensation of commercially available 1-methyl or 1-ethylurea with ethyl-2-cyanoacetate in the presence of NaOEt afforded 6-amino-1-methyluracil (**6**) or 6-amino-1-ethyluracil (**45**), respectively.[25] Intermediates **7**−**11** were generated in situ by using dimethyl formamide dimethyl acetal (DMF/DMA) in DMF at 40 °C. This step was followed by *N*-1-alkylation of the resulting formamidines with alkyl iodide and K_2CO_3 at 90 °C. Deprotection of the *N*,*N*-dimethylaminomethylene moiety (formamidino) was efficiently achieved by using aqueous ammonia in methanol.[26] Transformation of the key intermediates 1,3-dialkyl-6-aminouracils (**12**−**16** and **47**−**50**) to the target compounds was achieved by following our previously reported literature procedures.[22]

Results and Discussion

The A_{2B}-AdoR binding affinity and selectivity of the *N*-3-methyl-substituted analogues are presented in Table 1. Compound **27** incorporated an unsubstituted 8-pyrazole ring and displayed modest binding affinity for the A_{2B}-AdoR ($K_i = 120$ nM). However, introducing a benzylic group at the *N*-1-pyrazole nitrogen, as in compound **28**, resulted in a 60-fold increase in A_{2B}-AdoR binding affinity ($K_i = 2$ nM) relative to **27**. In addition, compound **28** showed 130-, 36-, and 46-fold greater selectivity for the A_{2B}-AdoR over the A_1-, A_{2A}-, and A_3-AdoR's, respectively. The 3-F and 3-CF_3 benzyl-substituted analogues **29** and **30** exhibited comparable affinities ($K_i = 6$ nM) and selectivities for the A_{2B}-AdoR. Therefore, introducing 3-F or 3-CF_3 substituent in the phenyl ring had no profound effect on affinity and selectivity in comparison to those of the unsubstituted analogue **28**. Expanding the *N*-1-ethyl group in compound **28** to propyl, as in **31**, resulted in a 10-fold loss in A_{2B}-AdoR binding affinity ($K_i = 19$ nM). Compound **31** also showed decreased selectivity for the A_{2B}-AdoR over A_1-, A_{2A}-, and A_3-AdoR's relative to **28**. Similarly, increasing the length of the N_1-alkyl chain in compound **29** from ethyl to propyl group, as in **33**, adversely affected both A_{2B}-AdoR affinity and selectivity. However, the *N*-1-propyl analogue of **30**, compound **32**, showed acceptable A_{2B}-AdoR binding affinity and selectivity that fit the target profile of affinity and selectivity. Further expanding the *N*-1-ethyl group in compounds **28** and **29** to *n*-butyl group resulted in a profound loss in A_{2B}-AdoR binding affinity and selectivity (**34** and **35**). When the same structural modifications were applied to compound **30** that incorporated 3-CF_3 group, A_{2B}-AdoR binding affinity and selectivity were not compromised (**30** vs **36**). This trend is similar to the one that was observed earlier when comparing compound **30** to compound **32**. This suggested that the 3-CF_3 group is the favored substituent as the size of the *N*-1-alkyl chain is extended from two to three carbons. Introducing sterically demanding substituents (isobutyl or cyclopropyl methyl) at the *N*-1-position yielded analogues with decreased A_{2B}-AdoR affinity and selectivity relative to the *N*-1-ethyl analogues (**37**−**43**). In this series of *N*-3-methyl-

Scheme 1[a]



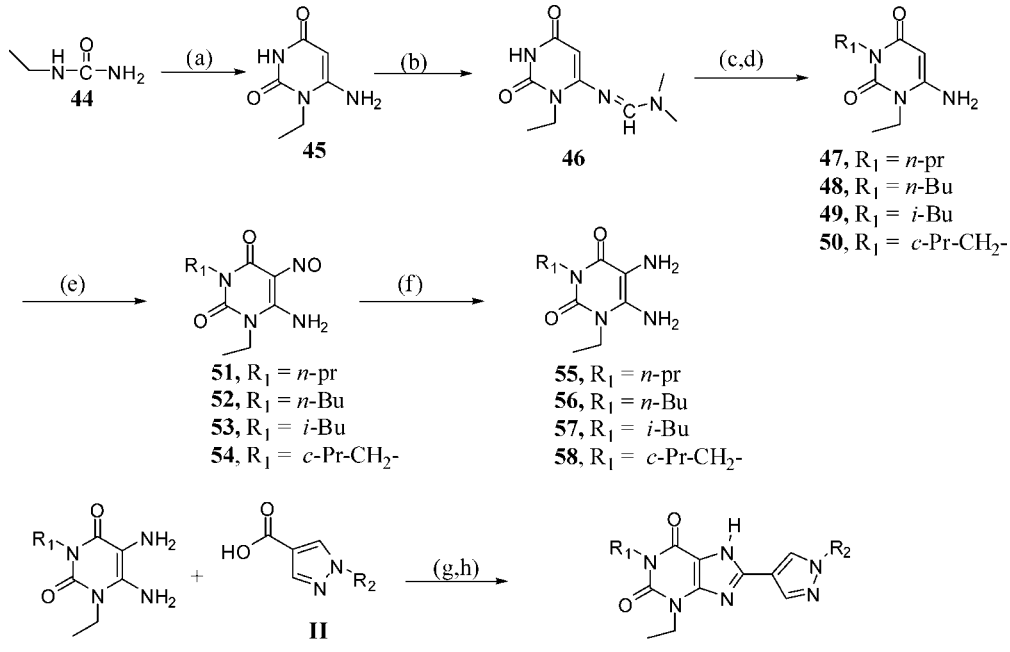
[a] Reagents and conditions: (a) ethyl 2-cyanoacetate, NaOEt, EtOH, reflux; (b) DMF/DMA, DMF, 40 °C; (c) alkyl iodide, K_2CO_3, DMF; (d) MeOH/28% NH_4OH, rt; (e) 50% aq AcOH, $NaNO_2$, 70 °C; (f) 13% aq NH_4OH, $Na_2S_2O_4$, 70 °C; (g) EDCI, MeOH; (h) 10% NaOH, MeOH, reflux.

substituted analogues, it appears that the SAR around the *N*-1-position is relatively well defined. In general, having an ethyl group at the *N*-1-position confers an enhanced A_{2B}-AdoR binding affinity and selectivity, whereas larger or branched alkyl substituents are detrimental to A_{2B}-AdoR binding affinity and selectivity.

Replacing the *N*-3 methyl group in compound **28** with an ethyl group yielded compound **60** (Table 2) that showed 15-fold less A_{2B}-AdoR binding affinity (K_i = 31 nM) compared to **28**. Furthermore, compound **60** showed <10-fold selectivity for the A_{2B}-AdoR over A_1- and A_{2A}-AdoR's. However, when 3-CF_3 substituent was introduced in the phenyl ring (**62**, K_i = 22 nM), significant enhancement in selectivity for the A_{2B}-AdoR over the A_1-, A_{2A}-, and A_3-AdoR's was achieved relative to the unsubstituted analogue **60**. Compound **62** displayed A_{2B}-AdoR selectivity ratios of 88, 149, and 48 over A_1-, A_{2A}-, and A_3-AdoR's, respectively. The 3-F analogue **61** showed less A_{2B}-AdoR binding affinity and selectivity compared to **62**. With the exception of compound **68** (i-butyl), replacing the *N*-1-propyl group in compound **62** with sterically demanding groups (isobutyl and cyclopropyl methyl) resulted in a significant loss in A_{2B}-AdoR selectivity over other AdoR subtypes, which is consistent with the early findings that steric factors at the *N*-1-position may play a crucial role in determining the binding affinity and selectivity for the A_{2B}-AdoR.

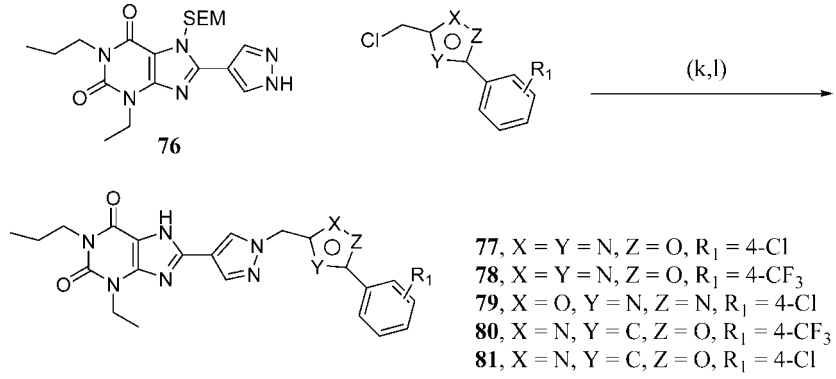
At this juncture, among the **27** compounds we synthesized, eight compounds (**28**−**30**, **32**, **36**, **61**, **62**, and **68**) showed the targeted A_{2B}-AdoR affinity (K_i < 50 nM) and selectivity profile and were therefore screened for their drug-like properties. During the course of the study, we observed that compounds that incorporated *N*-3 methyl group (**28**−**30**, **32**, and **36**) had a less-favorable PK profile than the corresponding *N*-3-ethyl analogues (**61**, **62**, and **68**) when dosed orally to rats. Furthermore, within the *N*-3-ethyl class of compounds, analogue **62** showed a better selectivity profile than compounds **61** and **68**, and hence, compound **62** was selected for further structural optimization in order to enhance its PK properties. We chose to replace the 3-CF_3 phenyl ring in compound **62** with a basic functionality such as 2-pyridyl and 3-pyridyl groups (**69** and **70**, Table 3), and this, in turn, resulted in significant compromise to selectivity for the A_{2B}-AdoR. However, the 3-pyridyl analogue (**70**, K_i = 22 nM) showed better A_{2B}-AdoR affinity and selectivity profile than the 2-pyridyl analogue (**69,** K_i = 46 nM) and was subjected to additional structural modifications.

Scheme 2[a]



[a] Reagents and conditions: (a) ethyl 2-cyanoacetate, NaOEt, EtOH, reflux; (b) DMF/DMA, DMF, 40 °C; (c) alkyl iodide, K_2CO_3, DMF; (d) MeOH/28% NH_4OH, rt; (e) 50% aq AcOH, $NaNO_2$, 70 °C; (f) 13% aq NH_4OH, 10% Pd/C, 30 psi; (g) EDCI, MeOH; (h) 10% NaOH, MeOH, reflux.

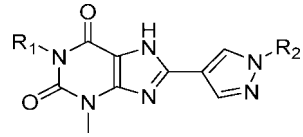
Scheme 3[a]



[a] Reagents and conditions: (k) DMF, K_2CO_3, 80 °C, 18 h; (l) 3 N HCl, EtOH, 80 °C, 18 h.

Introducing a CH_3 group into the 6-position of the pyridyl ring of compound **70** had no effect on A_{2B}-AdoR binding affinity (**71**, $K_i = 45$ nM). However, introducing an electron-withdrawing group at the 6-position of the pyridyl ring of **70** yielded substantial increase in both A_{2B}-AdoR binding affinity and selectivity relative to **70** (**72**, $K_i = 18$ nM). Exchanging the *N*-1-propyl group in compound **72** with isobutyl or cyclopropyl methyl groups (**73** and **74**) had no appreciable effect on A_{2B}-AdoR binding affinity. Not surprisingly, all attempts to prepare salt forms of the analogues that fulfilled the targeted profile of affinity and selectivity (**72** and **74**) were unsuccessful, and this can be attributed to the strong electron-withdrawing ability of

Table 1. Binding Affinities of *N*-3-Methyl-xanthine Derivatives for the A_{2B}-, A_1-, A_{2A}-, and A_3-AdoR's



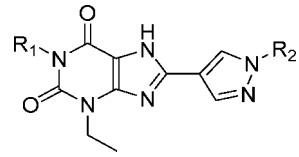
Compd	R_1	R_2	K_i nM[a]				A_1/A_{2B}	A_{2A}/A_{2B}	A_3/A_{2B}
			(hA_{2B})[b]	(hA_1)[c]	(hA_{2A})[d]	(hA_3)[c]			
27	CH_3-CH_2-	H	120	NT	NT	NT	NT	NT	NT
28	CH_3-CH_2-	Bn	2	260	73	92	130	36	46
29	CH_3-CH_2-	3-F-Bn	6	350	231	282	58	38	47
30	CH_3-CH_2-	3-CF_3-Bn	6	274	234	262	45	39	43
31	CH_3-$(CH_2)_2$-	Bn	19	582	125	NT	30	6	NT
32	CH_3-$(CH_2)_2$-	3-CF_3-Bn	14	441	450	408	31	32	29
33	CH_3-$(CH_2)_2$-	3-F-Bn	13	290	139	95	22	10	7
34	CH_3-$(CH_2)_3$-	Bn	30	416	247	NT	13	8	NT
35	CH_3-$(CH_2)_3$-	3-F-Bn	26	305	217	NT	11	8	NT
36	CH_3-$(CH_2)_3$-	3-CF_3-Bn	11	427	481	453	38	43	41
37		H	42	376	1390	NT	9	33	NT
38		Bn	21	347	408	204	16	19	9
39		3-F-Bn	60	352	301	107	6	5	2
40		3-CF_3-Bn	199	617	633	NT	3	3	NT
41		Bn	11	154	149	91	14	13	8
42		3-F-Bn	7	185	73	35	26	10	5
43		3-CF_3-Bn	21	167	312	160	8	15	7

[a] NT, not tested. 95% confidence limits were generally ±15% of the mean value. [b] Binding affinity for hA_{2B}-AdoR was determined by using HEK-A_{2B} cells with 4-(2-(7-amino-2-(furan-2-yl)-[1,2,4] triazolo[1,5-a][1,3,5]triazin-5-ylamino)ethyl)phenol (**82**, ^{3}HZM241385)[32] as the radioligand. [c] Binding affinity for h_{A1}-AdoR was determined by using CHO-A_1 cells with ^{3}H-CPX as the radioligand. [d] Binding affinity for hA_{2A}-AdoR was determined by using HEK-A_{2A} cells with compound **82** as the radioligand. [e] Binding affinity for hA_3-AdoR was determined by using CHO-A_3 cells with ^{I}125-AB-MECA as the radioligand.

the CF_3 group, which in turn decreases the basicity of the pyridine nitrogen. Oxidation of a pyridyl nitrogen to an *N*-oxide can have various effects on drug-like behavior,[27] often producing drugs with good oral bioavailability, PK, solubility, and metabolic stability.[28,29] Accordingly, we synthesized the *N*-oxide analogue **75** that showed much diminished A_{2B}-AdoR binding affinity.

Encouraged by the success of replacing the phenyl moiety with a six-membered heteroaryl (pyridine), we sought to assess the effect of introducing five-membered heteroaryls in place of the phenyl or the pyridine ring in order to further expand our SAR. This strategy has served us well in the past without compromising A_{2B}-AdoR affinity or selectivity.[30] We prepared analogues that contained either 5-phenyl-1,2,4-oxadiazole (**77** and **78**, Table 4), 3-phenyl-1,2,4-oxadiazole (**79**), or 5-phenyl-isoxazole (**80** and **81**). In addition, 4-Cl or 4-CF_3 substituents were introduced in the phenyl ring, because they were found to be conducive to high A_{2B}-AdoR affinity and selectivity in similar classes of compounds.[30] The 5-(4-Cl-phenyl)-1,2,4-oxadiazole analogue showed weak binding affinity for the A_{2B}-AdoR (**77**, K_i = 335 nM); however, replacing the 4-Cl substituent with a 4-CF_3 group brought about >13-fold improvement in binding affinity and selectivity for the A_{2B}-AdoR (**78**, K_i = 24 nM). In addition, compound **78** displayed >200-fold selectivity for the A_{2B}-AdoR relative to the A_1-, A_{2A}-, and A_3-AdoR's. Remarkably, compound **79** (K_i = 16 nM) showed 20-fold greater affinity and much improved selectivity than its regioisomer **77**. The 4-Cl isoxazole analogue **81** could be envisioned as a direct analogue of the oxadiazole **77**, where the 4-nitrogen atom in the 5-phenyl-1,2,4-oxadiazole ring was isosterically replaced with a carbon atom. Although compound **81** showed poor A_{2B}-AdoR selectivity, similarly to **77**, the 4-CF_3 analogue **80** exhibited good affinity and selectivity for the A_{2B}-AdoR, a trend similar to that observed in the 3-phenyl-1,2,4-oxadiazole series when the 4-Cl group was replaced with a 4-CF_3 group (**77** vs **78**). Unfortunately, compounds that incorporated oxadiazole or isoxazoles

Table 2. Binding Affinities of *N*-3-Ethyl-xanthine Derivatives for the A_{2B}-, A_1-, A_{2A}-, and A_3-AdoR's (**59**−**68**)



Compd	R_1	R_2	K_i nM[a]				A_1/A_{2B}	A_{2A}/A_{2B}	A_3/A_{2B}
			(hA_{2B})[b]	(hA_1)[c]	(hA_{2A})[d]	(hA_3)[c]			
59	CH_3-$(CH_2)_2$-	H	49	178	496	NT	4	10	NT
60	CH_3-$(CH_2)_2$-	Bn	31	275	261	NT	9	8	NT
61	CH_3-$(CH_2)_2$-	3-F-Bn	35	1200	2500	950	34	71	27
62	CH_3-$(CH_2)_2$-	3-CF_3-Bn	22	1940	3280	1070	88	149	48
63		H	38	76	234	NT	2	6	NT
64		Bn	27	314	181	NT	11	6	NT
65		3-CF_3-Bn	22	218	272	326	10	12	15
66		H	141	348	1220	NT	2	9	NT
67		3-F-Bn	31	1480	3850	324	47	124	10
68		3-CF_3-Bn	11	631	1090	250	57	99	23

[a] NT, not tested. 95% confidence limits were generally ±15% of the mean value. [b] Binding affinity for hA_{2B}-AdoR was determined by using HEK-A_{2B} cells with compound **82** as the radioligand. [c] Binding affinity for hA_1-AdoR was determined by using CHO-A_1 cells with ^{3}H-CPX as the radioligand. [d] Binding affinity for hA_{2A}-AdoR was determined by using HEK-A_{2A} cells with compound **82** as the radioligand. [e] Binding affinity for hA_3-AdoR was determined by using CHO-A_3 cells with ^{1}125-AB-MECA as the radioligand.

were not further pursued because of their low oral bioavailability in rats (see Supporting Information).

Among all the compounds that displayed the targeted A_{2B}-AdoR affinity and selectivity, compound **62** had the best PK profile in rats. When dosed orally to rats at 2 mg/kg, **62** had excellent systemic exposure with $t_{1/2}$ of 4 h, C_{max} and dose-adjusted area under curve (dAUC) >1100 ng/mL and 6500 ng·h/mL, respectively (Table 5). The functional potency of **62** for human and mouse A_{2B}-AdoR was also determined by using cAMP assay in HEK-A_{A2B} (human) and NIH3T3 (mouse) cells.[23] In HEK-A_{2B} cells, **62** was found to be a potent antagonist for the NECA-induced increase in cAMP mediated by the human A_{2B}-AdoR with a K_B value of 6 nM. Compound **62** also antagonized the NECA-induced cAMP accumulation in NIH-3T3 cells with a K_B value of 2 nM. Furthermore, compound **62** showed no significant inhibition (10 μM) when screened against other receptors, ion channels, transporters, and enzymes in a broad Cerep screening panel that consisted of 68 receptors and 16 enzymes.[31] In addition to compound **62**, the pyridyl analogue **72** also fulfilled the targeted affinity, selectivity, and PK profile. When dosed orally (2 mg/Kg) to rats, **72** displayed C_{max} of 5300 ng·h/mL and dAUC > 670 ng/mL (Table 5). However, compound **72** showed 15-fold less potency than **62** in inhibiting accumulation of NECA-induced cAMP in NIH-3T3 cells with a K_B value of 36 nM (Figure 2).

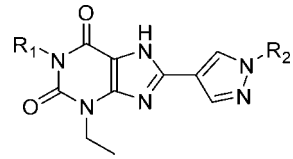
Recent studies have shown that compound **62** inhibited the airway inflammation and airway reactivity induced by allergen or AMP in mouse model of asthma. In addition, compound **62** attenuated pulmonary inflammation and lung injury of adenosine deaminase-deficient mice and in a model of bleomycin-induced lung injury.[23,24] These findings provided further support for the hypothesis that A_{2B}-AdoR signaling contributes to the proinflammatory and profibrotic activities associated with chronic lung diseases.

In summary, we have synthesized a series of novel potent A_{2B}-AdoR antagonists with high affinity and selectivity. Compound **62** displayed high affinity (K_i = 22 nM) and selectivity for the A_{2B}-AdoR relative to the A_1-, A_{2A}-, and A_3-AdoR's and good PK profile in rats. In addition, compound **62** showed high potency in inhibiting the accumulation of NECA-induced cAMP in HEK-A_{2B}-AdoR and NIH3T3 cells, with K_B values of 6 and 2 nM, respectively. To our knowledge, compound **62** is the first A_{2B}-AdoR antagonist to demonstrate in vivo preclinical evidence that A_{2B}-AdoR antagonism may be of significant therapeutic value to the management of chronic lung diseases such as asthma, COPD, and pulmonary fibrosis. In a single ascending-dose phase I clinical study, compound **62** had no serious adverse events and was well tolerated, and we may soon have a definitive answer regarding the effect of A_{2B}-AdoR antagonism on chronic inflammatory lung diseases.

Experimental Section

Commercial chemicals and solvents were of reagent grade and were used without further purification. All reported yields are of isolated products and are not optimized. The following abbreviations are used for reagents and solvents: Whatman silica gel (60 Å, 230−400 mesh) was used for column chromatography. Analtech thin-layer chromatography plates (20 × 20 cm, 2000 μm) were used for preparative thin-layer chromatography. Proton NMR (^{1}H NMR) spectra were recorded on a Varian Gemini-400 spectrometer

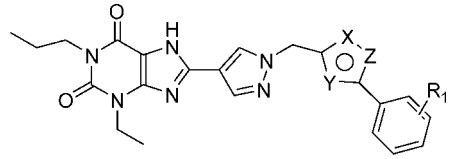
Table 3. Binding Affinities of *N*-3-Ethyl-xanthine Derivatives for the A_{2B}-, A_1-, A_{2A}-, and A_3-AdoR's (**69**−**75**)



Compd	R_1	R_2	K_i nM[a]				A_1/A_{2B}	A_{2A}/A_{2B}	A_3/A_{2B}
			$(hA_{2B})^b$	$(hA_1)^c$	$(hA_{2A})^d$	$(hA_3)^c$			
69	CH_3-$(CH_2)_2$-	2-pyridyl	46	410	411	NT	9	9	NT
70	CH_3-$(CH_2)_2$-	3-pyridyl	22	522	312	NT	23	14	NT
71	CH_3-$(CH_2)_2$-	3-(6-CH_3)pyridyl	45	1520	670	290	33	15	6
72	CH_3-$(CH_2)_2$-	3-(6-CF_3)pyridyl	18	2500	2250	440	139	125	24
73		3-(6-CF_3)pyridyl	20	3590	5410	350	180	270	17
74		3-(6-CF_3)pyridyl	16	1280	2280	400	80	142	25
75	CH_3-$(CH_2)_2$-	3-pyridyl- N-oxide	506	1120	1950	NT	2	4	NT

[a] NT, not tested. 95% confidence limits were generally ±15% of the mean value. [b] Binding affinity for hA_{2B}-AdoR was determined by using HEK-A_{2B} cells with compound **82** as the radioligand. [c] Binding affinity for hA_1-AdoR was determined by using CHO-A_1 cells with ^{3}H-CPX as the radioligand. [d] Binding affinity for hA_{2A}-AdoR was determined by using HEK-A_{2A} cells with compound **82** as the radioligand. [e] Binding affinity for hA_3-AdoR was determined by using CHO-A_3 cells with ^{1}125-AB-MECA as the radioligand.

Table 4. Binding Affinities of *N*-3-Ethyl-xanthine Derivatives for the A_{2B}-, A_1-, A_{2A}-, and A_3-AdoR's (**77**−**81**)



compd	R_1	X	Y	Z	K_i, nM[a]				hA_1/A_{2B}	A_{2A}/A_{2B}	A_3/A_{2B}
					$(hA_{2B})^b$	$(hA_1)^c$	$(hA_{2A})^d$	$(hA_3)^c$			
77	4-Cl	N	N	O	335	>6000	>5000	NT	>18	>15	N
78	4-CF_3	N	N	O	24	>6000	>5000	>9000	>250	>208	>375
79	4-Cl	O	N	N	16	910	3780	413	56	236	25
80	4-CF_3	N	C	O	25	2190	2370	3810	87	94	152
81	4-Cl	N	C	O	42	200	781	NT	5	18	NT

[a] NT, not tested. 95% confidence limits were generally ±15% of the mean value. [b] Binding affinity for hA_{2B}-AdoR was determined by using HEK-A_{2B} cells with compound **82** as the radioligand. [c] Binding affinity for hA_1-AdoR was determined by using CHO-A_1 cells with ^{3}H-CPX as the radioligand. [d] Binding affinity for hA_{2A}-AdoR was determined by using HEK-A_{2A} cells with compound **82** as the radioligand. [e] Binding affinity for hA_3-AdoR was determined by using CHO-A_3 cells with ^{125}I-AB-MECA as the radioligand.

Table 5. PK Properties of Compounds **62** and **72** in Sprague−Dawley Rats[a]

compd	oral dose (mg/kg)	dAUC (ng·h/mL)[b]	C_{max} (ng/mL)[b]	$t_{1/2}$ (h)[b]
62	2.0	6500	1110	4.25
72	2.0	5300	671	9.7

[a] PO formulation: DMSO/ethanol/PEG300/0.1% *N*-methylglucamine (2.5/10/20/67.5, v/v/v/v). [b] Values are average of $n = 3$.

(400 MHz). Chemical shifts are reported in δ units (parts per million, ppm) downfield from tetramethylsilane and are assigned as singlet (s), doublets (d), doublet of doublets (dd), triplets (t), quartet (q), multiplets (m), and broad (br). Coupling constants (*J*) are reported in hertz (Hz). Mass spectra (MS) were recorded on a Micromass LCZ instrument (electron spray ionization, ESI). Elemental analysis data for final compounds were obtained from Desert Analytics and were within ±0.4% of the theoretical values for formulas given.

6-Amino-1-methylpyrimidine-2,4(1*H*,3*H*)-dione (6). Sodium (10 g, 434 mmol) was dissolved in anhydrous ethanol (300 mL). To the solution was added ethyl 2-cyanoacetate (24.5 g, 217 mmol) and then 1-methylurea (16 g, 217 mmol), and the mixture was stirred at reflux for 24 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in 50 mL of water. The pH of the solution was adjusted to 7 by using concentrated HCl. The yellow solid formed was collected by filtration, washed with water, and dried under vacuum to afford 28.5 g of **6** (95% yield). ^{1}H NMR (CD_3OD): δ 3.32 (s, 3H), 4.82 (s, 1H); MS *m*/*z* 142 $(M + H)^+$.

***N*′-(1-Alkyl-1,2,3,6-tetrahydro-3-methyl-2,6-dioxopyrimidin-4-yl)-*N*,*N*-dimethylformamidine (7−11). General Procedure.** To a suspension of **6** (5 g, 35 mmol) in DMF (170 mL) was added DMF/DMA (17 mL). The mixture was heated at 40 °C for 1 h. After disappearance of the starting material (TLC, DCM/MeOH 10:1), K_2CO_3 (7.2 g, 52 mmol) was added. Then, alkyl iodide (52 mmol) was added, and the mixture was further heated at 90 °C for

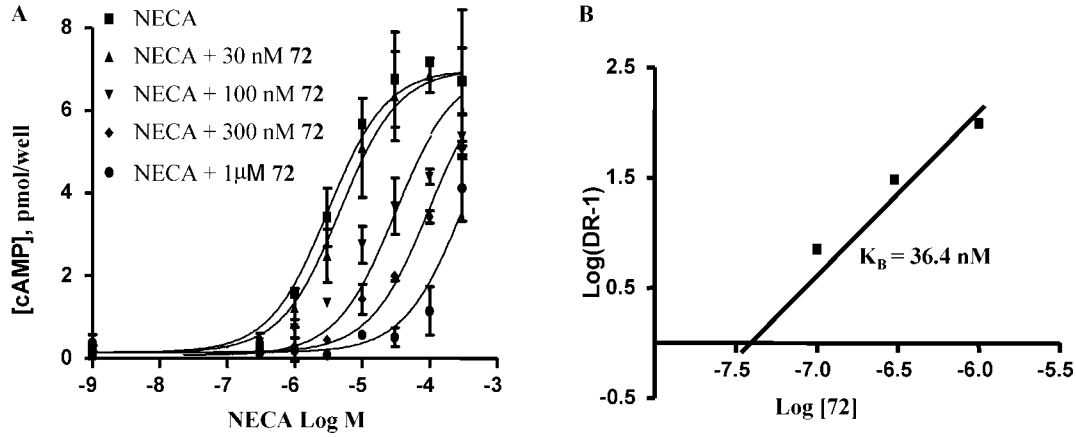


Figure 2. (A) Concentration−response curves of NECA-induced increases in cAMP in the absence or presence of increasing concentrations of **72**. Mouse NIH/3T3 cells were treated with NECA (1 nM to 100 μM) in the absence or presence of increasing concentrations of compound **72**, and cAMP accumulation was measured. Data are mean ± SEM from three experiments performed in duplicate. (B) Schild plot. DR = 1, concentration ratio = 1.

18 h. The solvent was removed, and the residue was treated with 50 mL of water. The resulting precipitate was filtered, washed with water, and air-dried to afford the products in 69−77% yields.

***N′*-(1-Ethyl-1,2,3,6-tetrahydro-3-methyl-2,6-dioxopyrimidin-4-yl)-*N*,*N*-dimethylformamidine (7).** Yield 6.0 g (77%, over 2 steps); MS *m*/*z* 245 $(M + H)^+$.

***N′*-(1,2,3,6-Tetrahydro-3-methyl-2,6-dioxo-1-propylpyrimidin-4-yl)-*N*,*N*-dimethylformamidine (8).** Yield 6.2 g (75%, over 2 steps); MS *m*/*z* 239 $(M + H)^+$.

***N′*-(1-Butyl-1,2,3,6-tetrahydro-3-methyl-2,6-dioxopyrimidin-4-yl)-*N*,*N*-dimethylformamidine (9).** Yield 6.0 g (69%, over 2 steps); MS *m*/*z* 253 $(M + H)^+$.

***N′*-(1,2,3,6-Tetrahydro-1-isobutyl-3-methyl-2,6-dioxopyrimidin-4-yl)-*N*,*N*-dimethylformamidine (10).** Yield 6.1 g (73%, over 2 steps); MS *m*/*z* 253 $(M + H)^+$.

***N′*-(1-(Cyclopropylmethyl)-1,2,3,6-tetrahydro-3-methyl-2,6-dioxopyrimidin-4-yl)-*N*,*N*-dimethylformamidine (11).** Yield 6.1 g (70%, over 2 steps); MS *m*/*z* 251 $(M + H)^+$.

1-Methyl-3-alkyl-6-aminouracils (12−16). General Procedure. A solution of the formamidino derivatives (**7**–**11**, 20 mmol) in MeOH (70 mL) was treated with 120 mL ammonium hydroxide (28%), and the mixture was stirred at ambient temperature for 48 h. The solvent was evaporated, and the residue was purified by using column chromatography (DCM/MeOH 15:1) to yield the products in 66−70% yields.

6-Amino-3-ethyl-1-methylpyrimidine-2,4(1*H*,3*H*)-dione (12). Yield 2.2 g (66%); ^{1}H NMR (CD_3OD): δ 1.11 (t, 3H), 3.36 (s, 3H), 3.72 (q, 2H), 4.82 (s, 1H); MS *m*/*z* 170 $(M + H)^+$.

6-Amino-1-methyl-3-propylpyrimidine-2,4(1*H*,3*H*)-dione (13). Yield 2.5 g (69%); MS *m*/*z* 184 $(M + H)^+$.

6-Amino-3-butyl-1-methylpyrimidine-2,4(1*H*,3*H*)-dione (14). Yield 2.6 g (66%); MS *m*/*z* 198 $(M + H)^+$.

6-Amino-3-isobutyl-1-methylpyrimidine-2,4(1*H*,3*H*)-dione (15). Yield 2.7 g (70%); MS *m*/*z* 198 $(M + H)^+$.

6-Amino-3-(cyclopropylmethyl)-1-methylpyrimidine-2,4(1*H*,3*H*)-dione (16). Yield 2.6 g (67%); MS *m*/*z* 196 $(M+H)^+$.

6-Amino-1-methyl-5-nitroso-3-alkylyrimidine-2,4(1*H*,3*H*)-dione (17−21). General Procedure. A solution of 1-methyl-3-alkyl-6-aminouracils (**12**−**16**, 10 mmol) in 50% aqueous AcOH (50 mL) was prepared by heating the mixture at 70 °C. To the solution was added $NaNO_2$ (2 g, 30 mmol) in portions, and the mixture was stirred at 70 °C for 1.5 h. The mixture was concentrated, and the dark-orange solid formed was collected by filtration and washed with water to afford compounds **17**−**21** that were used without further purification.

6-Amino-3-ethyl-1-methyl-5-nitrosopyrimidine-2,4(1*H*,3*H*)-dione (17). Yield 1.7 g (86%); MS *m*/*z* 199 $(M + H)^+$.

6-Amino-1-methyl-5-nitroso-3-propylpyrimidine-2,4(1*H*,3*H*)-dione (18). Yield 1.9 g (92%); MS *m*/*z* 213 $(M + H)^+$.

6-Amino-3-butyl-1-methyl-5-nitrosopyrimidine-2,4(1*H*,3*H*)-dione (19). Yield 2.0 g (88%); MS *m*/*z* 227 $(M + H)^+$.

6-Amino-3-isobutyl-1-methyl-5-nitrosopyrimidine-2,4(1*H*,3*H*)-dione (20). Yield 1.9 g (86%); MS *m*/*z* 227 $(M + H)^+$.

6-Amino-3-(cyclopropylmethyl)-1-methyl-5-nitrosopyrimidine-2,4(1*H*,3*H*)-dione (21). Yield 2.1 g (95%); MS *m*/*z* 225 $(M + H)^+$.

5,6-Diamino-3-alkyl-1-methylpyrimidine-2,4(1 *H*,3 *H*)-dione (22−26). The synthesis of compounds **22**−**26** was acomplished by using on of the following methods.

Method A. A solution of 6-amino-1-methyl-5-nitrosouracils (**17**−**21**, 8 mmol) in 13% aqueous ammonium hydroxide was prepared by heating the mixture to 70 °C. To the solution was added $Na_2S_2O_4$ (2.8 g, 24 mmol) in small portions at 70 °C over a period of 20 min, while the mixture was vigorously stirred. The volume of the solution was reduced under vacuum until the product started to crystallize. The mixture was cooled to room temperature, and the precipitate was collected by filtration and washed with cold water to afford the diamines **22**−**26**. The products were sensitive to moisture and hence were carried immediately to the next step.

Method B. A solution of 6-amino-1-methyl-5-nitrosouracils (**17**−**21**, 8 mmol) in 20 mL MeOH was prepared by heating the mixture to 70 °C. To the solution was added 10% Pd/C, and the mixture was hydrogenated at 25 psi for 1.5 h. The mixture was filtered through celite and washed with MeOH. The solvent was evaporated, and the products were taken immediately to the next step.

8-(1-Benzyl-1 *H*-pyrazol-4-yl)-3-methyl-1-alkyl-1 *H*-purine-2,6(3*H*,7*H*)-dione (27−43). General Procedure. 5,6-Diamino-3-alkyl-1-methylpyrimidine-2,4(1*H*,3*H*)-dione (**22**−**26**, 1.0 mmol) were suspended in 15 mL of MeOH. To the suspension was added 1-substituted-1 *H*-pyrazole-4-carboxylic acid (**I**, 1.3 mmol) and then EDCI (1.3 mmol, 0.25 g). The mixture was stirred at room temperature for 24 h. TLC (DCM/MeOH 10:1) showed no starting material. The solvent was evaporated, and the residue was purified by using Biotage (DCM/MeOH 20:1) to afford the *N*-(6-amino-3-alkyl-1,2,3,4-tetrahydro-1-benzyl-2,4-dioxopyrimidin-5-yl)-1-methyl-1*H*-pyrazole-4-carboxamides **II**.

The carboxamides (**II**, 0.3 mmol) were dissolved in a mixture of 1:2 MeOH/10% NaOH. The solution was stirred at 100 °C for 3−5 h. The MeOH was evaporated, and the aqueous solution was acidified to pH 4−5 by using concentrated HCl. The precipitate formed was collected by filtration, washed with water and ether, and air-dried under vacuum to afford compounds **27**−**43**.

1-Ethyl-3-methyl-8-(1*H*-pyrazol-4-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (27). Yield 0.06 g, 76%. ^{1}H NMR (DMSO-d_6): δ 1.13 (t, J = 8.0 Hz, 3H), 3.47 (s, 3H), 3.93 (q, J = 8.0 Hz,2H), 8.21 (s, 1H), 13.48 (s, 1H); MS *m*/*z* 261 $(M + H)^+$ Anal. ($C_{11}H_{12}N_6O_2.H_2O$) C, H, N.

8-(1-Benzyl-1*H*-pyrazol-4-yl)-1-ethyl-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (28). Yield 0.08 g, 80%. ^{1}H NMR (DMSO-d_6): δ 1.12 (t, J = 8.0 Hz, 3H), 3.45 (s, 3H), 3.92 (d, J = 8.0 Hz, 2H), 5.40 (s, 2H), 7.29−7.39 (m, 5H), 8.09 (s, 1H), 8.45 (s, 1H), 13.53 (s, 1H); MS m/z 351 $(M + H)^+$; Anal. ($C_{18}H_{18}N_6O_2$) C, H, N.

8-(1-(3-Fluorobenzyl)-1*H*-pyrazol-4-yl)-1-ethyl-3-methyl-1*H*-purine-2,6(3*H*, *H*)-dione (29). Yield 0.09 g, 80%. ^{1}H NMR (DMSO-d_6): δ 1.12 (t, J = 8.0 Hz, 3H), 3.35 (s, 3H), 3.84 (q, J = 8.0 Hz, 2H), 5.43 (s, 2H),7.12−7.18 (m, 3H), 7.39−7.44 (m, 1H), 8.11 (s, 1H), 8.49 (s, 1H), 13.55 (s, 1H); MS m/z 369 $(M + H)^+$; Anal. ($C_{18}H_{17}FN_6O_2$) C, H, N.

8-(1-(3-(Trifluoromethyl)benzyl)-1*H*-pyrazol-4-yl)-1-ethyl-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (30). Yield 0.11 g, 84%. ^{1}H NMR (DMSO-d_6): δ 1.13 (t, J = 8.0 Hz, 3H), 3.46 (s, 3H), 3.92 (q, J = 8.0 Hz, 2H), 5.52 (s, 2H), 7.58−7.70 (m, 4H), 8.11 (s, 1H), 8.52 (s, 1H), 13.54 (s, 1H); MS m/z 419 $(M + H)^+$; Anal. ($C_{19}H_{17}F_3N_6O_2$) C, H, N.

8-(1-Benzyl-1*H*-pyrazol-4-yl)-3-methyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (31). Yield 0.09 g, 79%. ^{1}H NMR (DMSO-d_6): δ 0.87 (t, J = 8.0 Hz, 3H), 1.60−1.50 (m, 2H), 3.45 (s, 3H), 3.84 (d, J = 4.0 Hz, 2H), 5.39 (s, 2H), 7.40−7.20 (m, 5H), 8.09 (s, 1H), 8.45 (s, 1H), 13.53 (s, 1H); MS m/z 363.16 $(M - H)^+$; Anal. ($C_{19}H_{20}N_6O_2.H_2O$) C, H, N.

8-(1-(3-(Trifluoromethyl)benzyl)-1*H*-pyrazol-4-yl)-3-methyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (32). Yield 0.11 g, 85%. ^{1}H NMR (DMSO-d_6): δ 0.86 (t, J = 8.0 Hz, 3H), 1.65−1.45(m, 2H), 3.43 (s, 3H), 3.82 (d, J = 8.0 Hz, 2H), 5.48 (s, 2H), 7.80−7.50 (m, 4H), 7.98 (s, 1H), 8.34 (s, 1H), 13.50 (s, 1H); MS m/z 433 $(M + H)^+$; Anal. ($C_{20}H_{19}F_3N_6O_2$) C, H, N.

8-(1-(3-Fluorobenzyl)-1*H*-pyrazol-4-yl)-3-methyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (33). Yield 0.09 g, 87%. ^{1}H NMR (DMSO-d_6): δ 0.87 (t, J = 8.0 Hz, 3H), 1.60−1.50 (m, 2H), 3.45 (s, 3H), 3.84 (d, J = 8.0 Hz, 2H), 5.42 (s, 2H), 7.20−7.00 (m, 3H), 7.50−7.36 (m, 1H), 8.06 (s, 1H), 8.43 (s, 1H), 13.54 (s, 1H); MS m/z 383 $(M + H)^+$; Anal. ($C_{19}H_{19}FN_6O_2$) C, H, N.

8-(1-Benzyl-1*H*-pyrazol-4-yl)-1-butyl-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (34). Yield 0.09 g, 79%. ^{1}H NMR (DMSO-d_6): δ 0.90 (t, J = 8.0 Hz, 3H), 1.40−1.20 (m, 2H), 1.60–1.50 (m, 2H), 3.44 (s, 3H), 3.87 (t, J = 8.0 Hz, 2H), 5.39 (s, 2H), 7.45−7.25 (m, 5H), 8.05 (s, 1H), 8.40 (s, 1H); MS m/z 379 $(M + H)^+$; Anal. ($C_{20}H_{22}N_6O_2$) C, H, N.

8-(1-(3-Fluorobenzyl)-1*H*-pyrazol-4-yl)-1-butyl-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (35). Yield 0.10 g, 88%. ^{1}H NMR (DMSO-d_6): δ 0.90 (t, J = 8.0 Hz, 3H), 1.40−1.20 (m, 2H), 1.60−1.50 (m, 2H), 3.46 (s, 3H), 3.90 (t, J = 8.0 Hz, 2H), 5.44 (s, 2H), 7.25−7.10 (m, 3H), 7.50−7.35 (m, 1H), 8.12 (s, 1H), 8.50 (s, 1H), 13.55 (s, 1H); MS m/z 397 $(M + H)^+$; Anal. ($C_{20}H_{21}FN_6O_20.5H_2O$) C, H, N.

8-(1-(3-(Trifluoromethyl)benzyl)-1*H*-pyrazol-4-yl)-1-butyl-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione(36). Yield 0.11 g, 82%. ^{1}H NMR (DMSO-d_6): δ 0.90 (t, J = 8.0 Hz, 3H), 1.40−1.20 (m, 2H), 1.60−1.50(m, 2H), 3.46 (s, 3H), 3.88 (t, J = 8.0 Hz, 2H), 5.53 (s, 2H), 7.80−7.50 (m, 4H), 8.12 (s, 1H), 8.53 (1H), 13.50 (s, 1H); MS m/z 447 $(M + H)^+$; Anal. ($C_{21}H_{21}F_3N_6O_2$) C, H, N.

1-Isobutyl-3-methyl-8-(1*H*-pyrazol-4-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (37). Yield 0.06 g, 73%. ^{1}H NMR (DMSO-d_6): δ 0.82 (d, J = 8.0 Hz, 6H), 2.02−2.09 (m, 1H), 3.45 (s, 3H), 3.72 (d, J = 8.0 Hz, 2H), 8.19 (s, 2H); MS m/z 289 $(M + H)^+$; Anal. ($C_{13}H_{16}N_6O_2.HCl$) C, H, N.

8-(1-Benzyl-1*H*-pyrazol-4-yl)-1-isobutyl-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (38). Yield 0.09 g, 83%. ^{1}H NMR (DMSO-d_6): δ0.84 (d, J = 8.0 Hz, 6H), 2.02−2.09 (m, 1H), 3.45 (s, 3H), 3.74 (d, J = 8.0 Hz, 2H), 5.40 (s, 2H), 7.29−7.39 (m, 5H), 8.09 (s, 1H), 8.46 (s, 1H), 13.52 (s, 1H); MS m/z 379 $(M + H)^+$; Anal. ($C_{20}H_{22}N_6O_2.H_2O$) C, H, N.

8-(1-(3-Fluorobenzyl)-1*H*-pyrazol-4-yl)-1-isobutyl-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (39). Yield 0.09 g, 88%.^{1}H NMR (DMSO-d_6): δ 0.84 (d, J = 8.0 Hz, 6H), 2.05−2.08 (m, 1H), 3.46 (s, 3H), 3.74 (m, J = 8.0 Hz, 2H), 5.43 (s, 2H), 7.12−7.16 (m, 3H), 7.41−7.42 (m, 1H) 8.11 (s, 1H), 8.50 (s, 1H), 13.52 (s, 1H); MS m/z 397 $(M + H)^+$; Anal. ($C_{20}H_{21}FN_6O_20.5H_2O$) C, H, N.

8-(1-(3-(Trifluoromethyl)benzyl)-1*H*-pyrazol-4-yl)-1-isobutyl-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (40). Yield 0.11 g, 80%. ^{1}H NMR (DMSO-d_6): δ 0.83 (d, J = 8.0 Hz, 6H), 2.02−2.09 (m, 1H), 3.45 (s, 3H), 3.71 (d, J = 8.0 Hz, 2H), 5.52 (s, 2H), 7.57−7.70 (m, 4H), 8.12 (s, 1H), 8.53 (s, 1H), 13.54 (s, 1H); MS m/z 447 $(M + H)^+$; Anal. ($C_{21}H_{21}F_3N_6O_2.H_2O$) C, H, N.

8-(1-Benzyl-1*H*-pyrazol-4-yl)-1-(cyclopropylmethyl)-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (41). Yield 0.09 g, 80%. ^{1}H NMR (DMSO-d_6): δ 0.50−0.20 (m, 4H), 1.30-.10 (m, 1H), 3.47 (s, 3H), 3.77 (d, J = 4.0 Hz, 2H), 5.40 (s, 2H), 7.50−7.15 (m, 5H), 8.08 (s, 1H), 8.44 (s, 1H), 13.52 (s, 1H); MS m/z 377 $(M + H)^+$; Anal. ($C_{20}H_{20}N_6O_2$) C, H, N.

8-(1-(3-Fluorobenzyl)-1*H*-pyrazol-4-yl)-1-(cyclopropylmethyl)-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (42). Yield 0.08 g, 77%. ^{1}H NMR (DMSO-d_6): δ 0.45−0.25 (m, 4H), 1.30−1.15 (m, 1H), 3.45 (s, 3H), 3.76 (d, J = 4.0 Hz, 2H), 5.41 (2H), 7.25−7.00 (m, 3H), 7.50−7.35 (m, 1H), 8.02 (s, 1H), 8.38 (s, 1H), 13.53 (s, 1H); MS m/z 395 $(M + H)^+$; Anal. ($C_{20}H_{19}FN_6O_2.HCl$) C, H, N.

8-(1-(3-(Trifluoromethyl)benzyl)-1*H*-pyrazol-4-yl)-1-(cyclopropylmethyl)-3-methyl-1*H*-purine-2,6(3*H*,7*H*)-dione (43). Yield 0.11 g, 81%. ^{1}H NMR (DMSO-d_6): δ 0.50−0.25 (4H), 1.30–1.10 (m, 1H), 3.47 (s, 3H), 3.78 (s, 2H), 5.53 (s, 2H), 7.75−7.55 (m, 4H), 8.13 (s, 1H), 8.54 (s, 1H), 13.52 (s, 1H); MS m/z 445 $(M + H)^+$; Anal. ($C_{21}H_{19}F_3N_6O_2.2HCl$) C, H, N.

6-Amino-1-ethylpyrimidine-2,4(1*H*,3*H*)-dione (45). Sodium (5.2 g, 226 mmol) was dissolved in anhydrous ethanol (150 mL). To the solution was added ethyl 2-cyanoacetate (12.8 g, 113 mmol) and then 1-ethylurea (10 g, 113 mmol), and the mixture was stirred at reflux for 24 h. The solvent was evaporated under reduced pressure, and the residue was dissolved in 50 mL water. The pH of the solution was adjusted to 7 by using concentrated HCl. The yellow solid formed was collected by filtration, washed with water, and dried under vacuum to afford 16.7 g of **45** (95% yield). ^{1}H NMR (DMSO-d_6): δ 1.16 (t, 3H), 3.95 (q, 2H), 4.50 (s, 1H), 6.78 (br s, 2H), 10.36 (s, 1H); MS m/z 156 $(M + H)^+$.

***N*′-(3-Ethyl-1,2,3,6-tetrahydro-2,6-dioxopyrimidin-4-yl)-*N*,*N*-dimethylformamidine (46).** To a suspension of **45** (15 g, 96 mmol) in DMF (300 mL) was added DMF/DMA (60 mL). The mixture was heated at 40 °C untill disappearance of starting material was observed (1−3 h, TLC, DCM/MeOH 10:1). The solvent was removed under reduced pressure, and the residue was treated with 40 mL EtOH. The solid formed was collected by filtration and washed with EtOH and then with ether to afford 16.5 g of **46** (82% yield) that was used without further purification. MS m/z 211 $(M + H)^+$.

6-Amino-1-ethyl-3-alkylpyrimidine-2,4(1*H*,3*H*)-dione (47−50). General procedure. The formamidine **46** (2.0 g, 9.5 mmol) was dissolved in 40 mL DMF. To the solution was added the corresponding alkyl iodide (10.45 mmol) and then K_2CO_3 (1.7 g, 12.3 mmol), and the mixture was heated at 40 °C for 5 h. The solvent was evaporated, and the residue was dissolved in a 40 mL solution containing a 1:2 mixture of MeOH and 28% ammonium hydroxide. The mixture was stirred at ambient temperature for 48 h. The solvent was evaporated, and the residue was purified by using column chromatography (DCM/MeOH 15:1) to yield the compounds **47−50** in 68−73% yields over two steps.

6-Amino-1-ethyl-3-propylpyrimidine-2,4(1*H*,3*H*)-dione (47). Yield 1.3 g, 68%; MS m/z 198 $(M + H)^+$.

6-Amino-3-butyl-1-ethylpyrimidine-2,4(1*H*,3*H*)-dione (48). Yield 1.2 g, 63%; MS m/z 212 $(M + H)^+$.

6-Amino-1-ethyl-3-isobutylpyrimidine-2,4(1*H*,3*H*)-dione (49). Yield 1.3 g, 64%; MS m/z 212 $(M + H)^+$.

6-Amino-3-(cyclopropylmethyl)-1-ethylpyrimidine-2,4(1*H*,3*H*)-dione (50). Yield 1.5 g, 73%; MS m/z 210 $(M + H)^+$.

6-Amino-3-alkyl-1-ethyl-5-nitrosopyrimidine-2,4(1*H*,3*H*)-dione (51−54). General Procedure. A solution of 1-ethyl-3-alkyl-6-aminouracils (**47−50,** 5 mmol) in 50% aqueous AcOH (25 mL) was prepared by heating the mixture at 70 °C. To the solution was added $NaNO_2$ (1 g, 15 mmol) in portions, and the mixture was stirred at 70 °C for 1.5 h. The mixture was concentrated, and the-dark orange solid formed was collected by filtration and washed

with water to afford compounds **51**−**54** (88–93% yield) that were used without further purification.

6-Amino-1-ethyl-5-nitroso-3-propylpyrimidine-2,4(1*H*,3*H*)-dione (51). Yield 1.1 g, 92%; MS *m*/*z* 227 $(M + H)^+$.

6-Amino-3-butyl-1-ethyl-5-nitrosopyrimidine-2,4(1*H*,3*H*)-dione (52). Yield 1.0 g, 88%; MS *m*/*z* 241 $(M + H)^+$.

6-Amino-1-ethyl-3-isobutyl-5-nitrosopyrimidine-2,4(1*H*,3*H*)-dione (53). Yield 1.0 g, 88%; MS *m*/*z* 241 $(M + H)^+$.

6-Amino-3-(cyclopropylmethyl)-1-ethyl-5-nitrosopyrimidine-2,4(1*H*,3*H*)-dione (54). Yield 1.1 g, 93%; MS *m*/*z* 239 $(M + H)^+$.

5,6-Diamino-1-ethyl-3-alkyl-pyrimidine-2,4(1*H*,3*H*)-dione (55−**58).** A solution of 6-amino-1-ethyl-3-alkyl-5-nitrosouracils (**51**−**54**, 3 mmol) in 15 mL MeOH was prepared by heating the mixture at 70 °C. To the solution was added 10% Pd/C, and the mixture was hydrogenated at 25 psi for 1.5 h. The mixture was filtered through celite and washed with MeOH. The solvent was evaporated, and the products were taken immediately to the next step without further purification.

8-(1-Benzyl-1*H*-pyrazol-4-yl)-3-ethyl-1-alkyl-1*H*-purine-2,6(3*H*,7*H*)-dione (59−**75). General Procedure.** 5,6-Diamino-3-alkyl-1-ethylpyrimidine-2,4(1*H*,3*H*)-dione (**55**−**58**, 1.0 mmol) were suspended in 15 mL of MeOH. To the suspension was added 1-substituted-1*H*-pyrazole-4-carboxylic acid (**I**, 1.3 mmol) and then EDCI (1.3 mmol, 0.25 g). The mixture was stirred at rom temperature for 24 h. TLC (DCM/MeOH 10:1) showed no starting material. The solvent was evaporated, and the residue was purified by using Biotage (DMC/MeOH 20:1) to afford the *N*-(6-amino-3-alkyl-1,2,3,4-tetrahydro-1-benzyl-2,4-dioxopyrimidin-5-yl)-1-methyl-1*H*-pyrazole-4-carboxamides **II**.

The carboxamides (**II**, 0.3 mmol) were dissolved in a mixture of 1:2 MeOH/10% NaOH. The solution was stirred at 100 °C for 3−5 h. The MeOH was evaporated, and the aqueous solution was acidified to pH 4−5 by using concentrated HCl. The precipitate formed was collected by filtration, washed with water and ether, and air-dried under vacuum to afford compounds **59**-**75** in 65–85%.

3-Ethyl-1-propyl-8-(1*H*-pyrazol-4-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (59). Yield 0.06 g, 70%. ^{1}H NMR (DMSO-d$_6$): δ 0.87 (t, J = 8.0 Hz, 3H), 1.24 (t, J = 8.0 Hz, 3H), 1.52−1.61 (m, 2H), 3.82–3.86 (m, 2H), 4.05 (q, J = 8.0 Hz, 2H), 8.14 (s, 1H), 8.35 (s, 1H), 13.32 (s, 1H), 13.52 (s, 1H); MS *m*/*z* 289 $(M + H)^+$; Anal. ($C_{13}H_{16}N_6O_2$) C, H, N.

8-(1-Benzyl-1*H*-pyrazol-4-yl)-3-ethyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (60). Yield 0.09 g, 80%. ^{1}H NMR (DMSO-d$_6$): δ 0.87 (t, J = 8.0 Hz, 3H), 1.24 (t, J = 8.0 Hz, 3H), 1.52–1.61 (m, 2H), 3.82–3.86 (m, 2H), 4.05 (q, J = 8.0 Hz, 2H), 5.40 (s, 2H), 7.21−7.33 (m, 5H), 8.13 (s, 1H), 8.47 (s, 1H), 13.55 (s, 1H); MS *m*/*z* 379 $(M + H)^+$; Anal. ($C_{20}H_{22}N_6O_2.0.5H_2O$) C, H, N.

8-(1-(3-Fluorobenzyl)-1*H*-pyrazol-4-yl)-3-ethyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (61). Yield 0.10 g, 81%. ^{1}H NMR (DMSO-d_6): δ 0.87 (t, J = 8.0 Hz, 3H), 1.24 (t, J = 8.0 Hz, 3H), 1.52−1.61 (m, 2H), 3.82–3.86 (m, 2H), 4.05 (q, J = 8.0 Hz, 2H), 5.40 (s, 2H), 7.12−7.18 (m, 3H), 7.40–7.46 (m, 1H), 8.12 (s, 1H), 8.52 (s, 1H), 13.55 (s, 1H); MS *m*/*z* 397 $(M + H)^+$; Anal. ($C_{20}H_{21}FN_6O_2$) C, H, N.

8-(1-(3-(Trifluoromethyl)benzyl)-1*H*-pyrazol-4-yl)-3-ethyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (62). Yield 0.11 g, 82%. ^{1}H NMR (DMSO-d_6): δ 0.87 (t, J = 8.0 Hz, 3H), 1.24 (t, J = 8.0 Hz, 3H), 1.52−1.61 (m, 2H), 3.82–3.86 (m, 2H), 4.05 (q, J = 8.0 Hz, 2H), 5.52 (s, 2H), 7.58−7.64 (m, 2H), 7.68−7.72; MS *m*/*z* 447 $(M + H)^+$; Anal. ($C_{21}H_{21}F_3N_6O_2$) C, H, N.

1-(Cyclopropylmethyl)-3-ethyl-8-(1*H*-pyrazol-4-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (63). Yield 0.06 g, 71%. ^{1}H NMR (DMSO-d_6): δ 0.35−0.48 (m, 4H), 1.20−1.28 (m, 4H), 3.80 (d, J = 8.0 Hz, 2H), 4.10 (q, J = 8.0 Hz, 2H), 8.18 (s, 1H), 8.36 (s, 1H), 13.32 (s, 1H), 13.50 (s, 1H); MS *m*/*z* 301 $(M + H)^+$; Anal. ($C_{14}H_{16}N_6O_2$) C, H, N.

8-(1-Benzyl-1*H*-pyrazol-4-yl)-1-(cyclopropylmethyl)-3-ethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (64). Yield 0.10 g, 84%. ^{1}H NMR (DMSO-d_6): δ 0.35−0.48 (m, 4H), 1.20−1.26 (m, 4H), 3.80 (d, J = 8.0 Hz, 2H), 4.06 (q, J = 8.0 Hz, 2H), 5.48 (s, 2H), 7.12−7.19 (m, 2H), 7.50−7.57 (m, 2H), 8.12 (s, 1H), 8.52 (s, 1H), 13.55 (s, 1H); MS *m*/*z* 409 $(M + H)^+$; Anal. ($C_{21}H_{21}FN_6O_20.5H_2O$) C, H, N.

8-(1-(3-(Trifluoromethyl)benzyl)-1-pyrazol-4-yl)-1-(cyclopropylmethyl)-3-ethyl-1*H*-purine-2,6(3*H*,7*H*)-dione (65). Yield 0.11 g, 82%. ^{1}H NMR (DMSO-d_6): δ 0.35−0.48 (m, 4H), 1.20−1.26 (m, 4H), 3.80 (d, J = 8.0 Hz, 2H), 4.06 (q, J = 8.0 Hz, 2H), 5.52 (s, 2H), 7.58−7.64 (m, 2H), 7.68−7.72 (m, 2H), 8.13 (s, 1H), 8.55 (s, 1H), 13.56 (s, 1H); MS *m*/*z* 459 $(M + H)^+$; Anal. ($C_{22}H_{21}F_3N_6O_2$) C, H, N.

3-Ethyl-1-isobutyl-8-(1*H*-pyrazol-4-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (66). Yield 0.06 g, 71%. ^{1}H NMR (DMSO-d_6): δ 0.88 (d, J = 8.0 Hz, 6H), 1.26 (t, J = 8.0 Hz, 3H), 2.06−2.12 (m, 1H), 3.76 (d, J = 8.0 Hz, 2H), 4.08 (q, J = 8.0 Hz, 2H), 8.12 (s, 1H), 8.40 (s, 1H), 13.42 (s, 1H), 13.49 (s, 1H); MS *m*/*z* 303 $(M + H)^+$; Anal. ($C_{14}H_{18}N_6O_2$) C, H, N.

8-(1-(3-Fluorobenzyl)-1*H*-pyrazol-4-yl)-3-ethyl-1-isobutyl-1*H*-purine-2,6(3*H*,7*H*)-dione (67). Yield 0.10 g, 83%. ^{1}H NMR (DMSO-d_6): δ 0.86 (d, J = 8.0 Hz, 6H), 1.26 (t, J = 8.0 Hz, 3H), 2.04−2.10 (m, 1H), 3.75 (d, J = 8.0 Hz, 2H), 4.05 (q, J = 8.0 Hz, 2H), 5.45 (s, 2H), 7.12−7.18 (m, 3H), 7.40−7.46 (m, 1H), 8.12 (s, 1H), 8.52 (s, 1H), 13.55 (s, 1H); MS *m*/*z* 411 $(M + H)^+$; Anal. ($C_{21}H_{23}FN_6O_2$) C, H, N.

8-(1-(3-(Trifluoromethyl)benzyl)-1*H*-pyrazol-4-yl)-3-ethyl-1-isobutyl-1*H*-purine-2,6(3*H*,7*H*)-dione (68). Yield 0.11 g, 81%. ^{1}H NMR (DMSO-d_6): δ 0.86 (d, J = 8.0 Hz, 6H), 1.26 (t, J = 8.0 Hz, 3H), 2.04−2.10 (m, 1H), 3.75 (d, J = 8.0 Hz, 2H), 4.05 (q, J = 8.0 Hz, 2H), 5.55 (s, 2H), 7.61−7.64 (m, 2H), 7.68−7.71 (m, 2H), 8.12 (s, 1H), 8.58 (s, 1H), 13.57 (s, 1H); MS *m*/*z* 461 $(M + H)^+$; Anal. ($C_{22}H_{23}F_3N_6O_2$) C, H, N.

3-Ethyl-1-propyl-8-(1-((pyridin-2-yl)methyl)-1*H*-pyrazol-4-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (69). Yield 0.08 g, 73%. ^{1}H NMR (DMSO-d_6): δ 0.87 (t, J = 8.0 Hz, 3H), 1.24 (t, J = 8.0 Hz, 3H), 1.52−1.61 (m, 2H), 3.82–3.86 (m, 2H), 4.05 (q, J = 8.0 Hz, 2H), 5.65 (s, 2H), 7.35−7.39 (m, 1H), 7.54−7.58 (m, 1H), 8.03–8.07 (m, 1H), 8.15 (s, 1H), 8.55 (s, 1H), 8.68−8.70 (m, 1H); MS *m*/*z* 379 $(M + H)^+$; Anal. ($C_{19}H_{21}N_7O_2$) C, H, N.

3-Ethyl-1-propyl-8-(1-((pyridin-3-yl)methyl)-1*H*-pyrazol-4-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (70). Yield 0.08 g, 73%. ^{1}H NMR (DMSO-d_6): δ 0.87 (t, J = 8.0 Hz, 3H), 1.24 (t, J = 8.0 Hz, 3H), 1.52−1.61 (m, 2H), 3.82–3.86 (m, 2H), 4.05 (q, J = 8.0 Hz, 2H), 5.65 (s, 2H), 7.25−7.29 (m, 1H), 7.44−7.48 (m, 3H), 8.50–8.53 (m, 2H); MS *m*/*z* 379 $(M + H)^+$; Anal. ($C_{19}H_{21}N_7O_2$) C, H, N.

3-Ethyl-8-(1-((6-methylpyridin-3-yl)methyl)-1*H*-pyrazol-4-yl)-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (71). Yield 0.08 g, 65%. ^{1}H NMR (DMSO-d_6): δ 0.87 (t, J = 8.0 Hz, 3H), 1.24 (m, J = 8.0 Hz, 3H), 1.55−1.60 (m, 2H), 2.68 (s, 3H), 3.82–3.86 (m, 2H), 4.05 (q, J = 8.0 Hz, 2H), 5.58 (s, 2H), 7.78 (d, J = 0.72 Hz, 1H), 8.18 (s, 1H), 8.20 (d, J = 0.72 Hz, 1H), 8.59 (s, 1H), 8.77 (s, 1H), 13.62 (s, 1H); MS *m*/*z* 394 $(M + H)^+$; Anal. ($C_{20}H_{23}N_7O_2$.HCl) C, H, N.

3-Ethyl-8-(1-((6-(trifluoromethyl)pyridin-3-yl)methyl)-1*H*-pyrazol-4-yl)-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (72). Yield 0.09 g, 65%. ^{1}H NMR (DMSO-d_6): δ 0.87 (t, J = 8.0 Hz, 3H), 1.24 (m, 3H, J = 8.0 Hz), 1.55−1.60 (m, 2H), 3.82−3.86 (m, 2H), 4.05 (q, J = 8.0 Hz, 2H), 5.59 (s, 2H), 7.91−7.95 (m, 2H), 8.14 (s, 1H), 8.59 (s, 1H), 8.76 (s, 1H); MS *m*/*z* 448 $(M + H)^+$; Anal. ($C_{20}H_{20}F_3N_7O_2$) C, H, N.

3-Ethyl-8-(1-((6-(trifluoromethyl)pyridin-3-yl)methyl)-1*H*-pyrazol-4-yl)-1-isobutyl-1*H*-purine-2,6(3*H*,7*H*)-dione (73). Yield 0.09 g, 66%. ^{1}H NMR (DMSO-d_6): δ 0.84 (d, J = 8.0 Hz, 6H), 1.26 (t, J = 8.0 Hz, 3H), 2.04−2.10 (m, 1H), 3.75 (d, J = 8.0 Hz, 2H), 4.05 (q, J = 8.0 Hz, 2H), 5.58 (s, 2H), 7.88−7.91 (m, 2H), 8.10 (s, 1H), 8.57 (s, 1H), 8.74 (s, 1H); MS *m*/*z* 462 $(M + H)^+$; Anal. ($C_{21}H_{22}F_3N_7O_2$.HCl) C, H, N.

1-(Cyclopropylmethyl)-3-ethyl-8-(1-((6-(trifluoromethyl)pyridin-3-yl)methyl)-1*H*-pyrazol-4-yl)-1*H*-purine-2,6(3*H*,7*H*)-dione (74). Yield 0.09 g, 66%. ^{1}H NMR (DMSO-d_6): δ 0.35−0.48 (m, 4H), 1.20−1.26 (m, 4H), 3.80 (d, J = 8.0 Hz, 2H), 4.06 (q, J = 8.0 Hz, 2H), 5.61 (s, 2H), 7.91−7.98 (m, 2H), 8.18 (s, 1H),

8.58 (s, 1H), 8.77 (s, 1H); MS *m/z* 460 $(M + H)^+$; ($C_{21}H_{20}F_3N_7O_2$.HCl) C, H, N.

3-((4-(3-Ethyl-2,6-dioxo-1-propyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-1*H*-pyrazol-1-yl)methyl)pyridine-1-oxide (75). Yield 0.09 g, 71%. ^{1}H NMR (DMSO-d_6): δ 0.87 (t, J = 8.0 Hz, 3H), 1.24 (t, J = 8.0 Hz, 3H), 1.54−1.60 (m, 2H), 3.83−3.86 (m, 2H), 4.05 (q, J = 8.0 Hz, 2H), 5.41 (s, 2H), 7.23 (d, J = 8.0 Hz, 1H), 7.39−7.42 (m, 1H), 8.14 (s, 1H), 8.18 (d, J = 8.0 Hz, 1H), 8.22 (s, 1H), 8.56 (s, 1H), 13.59 (s, 1H); MS *m/z* 395 $(M + H)^+$; Anal. ($C_{19}H_{21}N_7O_2$) C, H, N.

3-Ethyl-8-(heteroaryl)methyl)-1*H*-pyrazol-4-yl)-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (77−81). General Procedure. 3-Ethyl-1-propyl-8-(1*H*-pyrazol-4-yl)-7-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-purine-2,6(3*H*,7*H*)-dione **76** (0.4 mmol, 0.17 g)[22] was dissolved in 10 mL of anhydrous DMF. To the solution was added the compound **III** (0.48 mmol) and K_2CO_3 (0.06 g, 0.48 mmol). The mixture was heated at 80 °C for 18 h. The mixture was filtered to remove K_2CO_3, and the solvent was evaporated under reduced pressure. The residue was purified by using preparative TLC (DCM/MeOH 10:1) to afford the SEM-protected derivatives of compounds **78**−**82**. Deprotection of the SEM group was achieved in 3N HCl to afford the target compounds in 65−73% yield.[22]

8-(1-((5-(4-Chlorophenyl)-1,2,4-oxadiazol-3-yl)methyl)-1*H*-pyrazol-4-yl)-3-ethyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (77). Yield 0.13 g, 67%. ^{1}H NMR (DMSO-d_6): δ 0.87 (t, J = 8.0 Hz, 3H), 1.24 (t, J = 8.0 Hz, 3H), 1.55−1.60 (m, 2H), 3.83−3.86 (m, 2H), 4.05 (q, J = 8.0 Hz, 2H), 5.74 (s, 2H), 7.69 (d, J = 8.0 Hz, 2H), 8.09 (d, J = 8.0 Hz, 2H), 8.13 (s, 1H), 8.58 (s, 1H), 13.62 (s, 1H); MS *m/z* 481 $(M + H)^+$; Anal. ($C_{22}H_{21}ClN_8O_3$) C, H, N.

3-Ethyl-8-(1-((5-(4-(trifluoromethyl)phenyl)-1,2,4-oxadiazol-3-yl)methyl)-1*H*-pyrazol-4-yl)-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (78). Yield 0.15 g, 73%. ^{1}H NMR (DMSO-d_6): δ 0.87 (t, J = 8.0 Hz, 3H), 1.24 (t, J = 8.0 Hz, 3H), 1.52−1.61 (m, 2H), 3.82–3.86 (m, 2H), 4.05 (q, J = 8.0 Hz, 2H), 5.80 (s, 2H), 8.00 (d, J = 8.0 Hz, 2H), 8.13 (s, 1H), 8.21 (d, J = 8.0 Hz, 2H), 8.58 (s, 1H), 13.57 (s, 1H); MS *m/z* 515 $(M + H)^+$; Anal. ($C_{23}H_{21}F_3N_8O_3.0.1H_2O$) C, H, N.

8-(1-((3-(4-Chlorophenyl)-1,2,4-oxadiazol-5-yl)methyl)-1*H*-pyrazol-4-yl)-3-ethyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (79). Yield 0.13 g, 68%. ^{1}H NMR (DMSO-d_6): δ 0.87 (t, J = 8.0 Hz, 3H), 1.24 (t, J = 8.0 Hz, 3H), 1.55−1.60 (m, 2H), 3.83−3.87 (m, 2H), 4.03−4.08 (m, 2H), 6.00 (s, 2H), 7.62−7.65 (m, 2H), 7.97−8.00 (m, 2H), 8.18 (s, 1H), 8.64 (s, 1H); MS *m/z* 481 $(M + H)^+$; Anal. ($C_{22}H_{21}ClN_8O_3$) C, H, N.

3-Ethyl-8-(1-((5-(4-(trifluoromethyl)phenyl)isoxazol-3-yl)-methyl)-1*H*-pyrazol-4-yl)-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (80). Yield 0.13 g, 65%. ^{1}H NMR (DMSO-d_6): δ 0.87 (t, J = 8.0 Hz, 3H), 1.24 (t, J = 8.0 Hz, 3H), 1.52−1.61 (m, 2H), 3.82−3.86 (m, 2H), 4.05 (q, J = 8.0 Hz, 2H), 5.65 (s, 2H), 7.20 (s, 1H), 7.92 (d, J = 8.0 Hz, 2H), 8.12 (d, J = 8.0 Hz, 2H), 8.18 (s, 1H), 8.60 (s, 1H); MS *m/z* 514 $(M + H)^+$; Anal. ($C_{24}H_{22}F_3N_7O_3$.) C, H, N.

8-(1-((5-(4-Chlorophenyl)isoxazol-3-yl)methyl)-1*H*-pyrazol-4-yl)-3-ethyl-1-propyl-1*H*-purine-2,6(3*H*,7*H*)-dione (81). Yield 0.13 g, 66%. ^{1}H NMR (DMSO-d_6): δ 0.87 (t, J = 8.0 Hz, 3H), 1.24 (t, J = 8.0 Hz, 3H), 1.55−1.60 (m, 2H), 3.83−3.86 (m, 2H), 4.05 (q, J = 8.0 Hz, 2H), 5.60 (s, 2H), 7.03 (s, 1H), 7.59 (d, J = 8.0 Hz, 2H), 7.90 (d, J = 8.0 Hz, 2H), 8.14 (s, 1H), 8.55 (s, 1H), 13.61 (s, 1H); MS *m/z* 479 $(M + H)^+$; Anal. ($C_{23}H_{22}ClN_7O_3$.) C, H, N.

Radioligand Binding for A_{2B} Adenosine Receptor.[22] Human A_{2B} adenosine receptor cDNA was stably transfected into HEK-293 cells (referred to as HEK-A_{2B} cells). Monolayer of HEK-A_{2B} cells were washed with PBS once and harvested in a buffer containing 10 mM HEPES (pH 7.4), 10 mM EDTA, and protease inhibitors. These cells were homogenized in polytron for 1 min at setting 4 and centrifuged at 29000*g* for 15 min at 4 °C. The cell pellets were washed once with a buffer containing 10 mM HEPES (pH 7.4), 1 mM EDTA, and protease inhibitors and were resuspended in the same buffer supplemented with 10% sucrose. Frozen aliquots were kept at −80 °C. Competition assays were started by mixing 10 nM compound **82** (Tocris Cookson) with various concentrations of test compounds and 50 μg membrane proteins in TE buffer (50 mM Tris and 1 mM EDTA) supplemented with 1 U/mL adenosine deaminase. The assays were incubated at 25 °C for 90 min under gentle agitation, stopped by filtration by using Packard Harvester, and washed four times with ice-cold TM buffer (10 mM Tris, 1 mM $MgCl_2$, pH 7.4). Nonspecific binding was determined in the presence of 10 μM cold **82**. The affinities of compounds (i.e., K_i values) were calculated by using GraphPad software.

Radioligand Binding for A_1, A_{2A}, and A_3 Adenosine Receptors.[22] Human A_1, A_{2A}, and A_3 adenosine receptor cDNAs were stably transfected into either CHO or HEK-293 cells (referred to as CHO-A_1, HEK-A_{2A}, and CHO-A_3). Membranes were prepared from these cells by using the same protocol as described above. Competition assays were started by mixing 0.5 nM ^{3}H-CPX (for CHO-A_1) and 2 nM compound **82** (HEK-A_{2A}) or 0.1 nM ^{125}I-AB-MECA (CHO-A_3) with various concentrations of test compounds, and the respective membranes in TE buffer (50 mM Tris and 1 mM EDTA fo CHO-A_1 and HEK-A_{2A}) or TEM buffer (50 mM Tris, 1 mM EDTA and 10 mM $MgCl_2$ for CHO-A_3) were supplemented with 1 Unit/mL adenosine deaminase. The assays were incubated at 25 °C for 90 min under gentle agitation, stopped by filtration by using Packard Harvester, and washed four times with ice-cold TM buffer (10 mM Tris, 1 mM $MgCl_2$, pH 7.4). Nonspecific binding was determined in the presence of 1 μM CPX (CHO-A_1), 1 μM cold **82** (HEK-A_{2A}), and 1 μM IB-MECA (CHO-A_3). The affinities of compounds (i.e., K_i values) were calculated by using GraphPad software.

Supporting Information Available: Table of elemental analysis and PK data. This material is available free of charge via the Internet at http://pubs.acs.org.

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JM7014815