Articles

Pyrido[2,1-*f*]purine-2,4-dione Derivatives as a Novel Class of Highly Potent Human A_3 Adenosine Receptor Antagonists

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1*H*,3*H*-Pyrido[2,1-*f*]purine-2,4-diones, which can be described as fused xanthine structures, have been synthesized by a novel synthetic procedure, and their affinities for the human adenosine A_1, A_{2A}, and A_3 receptors have been evaluated in radioligand binding studies. The synthetic procedure employed was developed in our laboratory and involved a two-step one-pot reaction that consists of the treatment of 6-aminouracil derivatives with *N*-bromosuccinimide to generate a 5,5-dibromo-6-imino intermediate that reacts "in situ" with pyridine, 4-methoxypyridine, 4-*tert*-butylpyridine, or 4-phenylpyridine to afford the corresponding 1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-diones (**2**−**5**). Functionalization at the N^3 position in compounds **2**−**5** was performed by reaction with DBU and different alkyl, alkenyl, alkynyl, or benzyl halides. Binding studies at human adenosine A_1, A_{2A}, and A_3 receptors revealed significant antagonist effects in the low nanomolar range, in particular against the A_3 receptor. Thus, the 1-benzyl-3-propyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione derivative **6**, which can be considered a lead compound in this series, exhibited a K_i value of 4.0 ± 0.3 nM against the hA_3 receptor. Because xanthine derivatives have traditionally been considered poor A_3 antagonists, the described pyrido[2,1-*f*]purine-2,4-dione derivatives represent a new family of adenosine receptor antagonists which deserves further exploration.

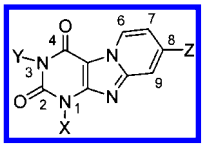
Introduction

Adenosine exerts physiological effects by the activation of specific cell membrane-bound receptors. To date, four different adenosine receptor subtypes have been identified, named A_1, A_{2A}, A_{2B}, and A_3. While activation of A_1 and A_3 receptor subtypes leads to inhibition of the enzyme adenylate cyclase, A_{2A} and A_{2B} subtypes stimulate cAMP production through this enzyme.[1,2]

The xanthine core structure has served as the basis for numerous selective antagonists for adenosine A_1, A_{2A}, and A_{2B} receptors, while at the level of A_3 receptors, xanthines are much less potent. Therefore, the search for A_3 receptor antagonists has relied on library screening, and this explains the structural diversity among A_3 receptor antagonists which include triazolonaphthopyridines,[3] thiazolopyrimidines,[3] pyridines and 1,4-dihydropyridines,[4−8] triazoloquinazolines,[9,10] flavonoids,[11] triazolopyrimidines,[12,13] isoquinolines,[14−16] and thiazoles and thiadiazoles.[17]

We have recently reported a new and simple synthesis of pyrido[2,1-*f*]purine-2,4-dione derivatives,[18] the general formula of which is represented in Chart 1. Such structures can also be described as fused xanthine derivatives, and therefore, we were interested in evaluating these compounds with the adenosine receptors.

Chart 1. General Formula and Numbering of 1*H*,3*H*-Pyrido[2,1-*f*]purine-2,4-diones



In this study, we describe the synthesis of an extended series of such pyridopurinedione derivatives and their affinities for the human adenosine A_1, A_{2A}, and A_3 receptors, as evaluated in radioligand binding studies. We learned that most compounds show moderate antagonist effects at the level of A_1 receptors, low or negligible activity at the level of A_{2A} receptors, and substantial affinity at the A_3 adenosine receptor.

Results and Discussion

Chemistry. In the course of our research program on the synthesis of 6-aminouracil derivatives,[19,20] we recently reported that treatment of 6-amino-1-benzyluracil with excess *N*-bromosuccinimide (NBS) in pyridine afforded, besides the expected 6-amino-5-bromo derivative, a second strong UV absorbing product that was identified as 1-benzyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione.[18] A careful examination of the reaction conditions led us to propose that the reaction pathway involves a 5,5-dibromination of the 6-aminouracil derivative that further reacts with the pyridine present in the reaction

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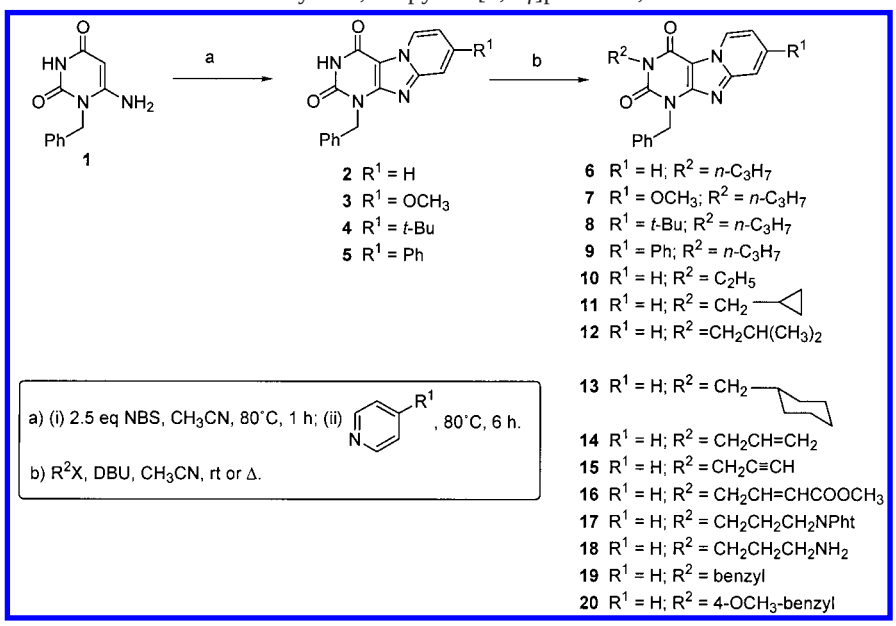
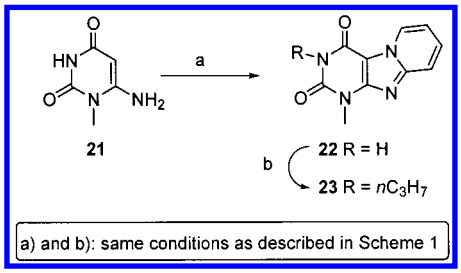
Scheme 1. Synthesis of 3-Substituted 1-Benzyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione Derivatives



medium to generate the tricyclic structure. This represents a novel synthetic pathway for obtaining such 1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione skeletons. Previous records on such structures are scarce and involve a two-step synthesis by reaction of 6-chloro-1,3-dialkyluracils with 2-aminopyridines in the presence of NaH, followed by heating with thionyl chloride.[21,23] Our optimized synthetic procedure for obtaining these 1*H*,3*H*-pyrido-[2,1-*f*]purine-2,4-diones is a two-step one-pot reaction that consists of treatment of the 6-aminouracil derivative with 2.5 equiv of NBS in acetonitrile to generate the intermediate 5,5-dibromo-6-imino derivative which is not isolated but reacted "in situ" with different pyridines to afford the target compounds.[18] Therefore, according to this approach, treatment of 6-amino-1-benzyluracil (**1**)[23] (Scheme 1) with 2.5 equiv of NBS in acetonitrile at 80 °C followed by the addition of pyridine, 4-methoxypyridine, 4-*tert*-butylpyridine, or 4-phenylpyridine afforded the corresponding 1-benzyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-diones (**2**−**5**) in 60, 74, 58, and 59% yields, respectively. In all cases, a variable amount (15−25%) of 6-amino-1-benzyl-5-bromouracil[18,23] was also obtained. The structures of **2**−**5** were unequivocally determined by ^{1}H and ^{13}C NMR, HMQC, and HMBC experiments, and elemental composition was established by mass spectrometry and combustion analysis.

Attempts to alkylate the N^3 position of **2** by reaction with K_2CO_3 and propyl iodide were unsuccessful. However, reacting **2** with DBU and propyl iodide in dry CH_3CN at room temperature afforded the 3-propyl derivative **6** in 65% yield (Scheme 1). Under similar reaction conditions, derivatives **3**, **4**, and **5** were transformed into their corresponding 3-propyl analogues **7**, **8**, and **9**, in 82, 75, and 93% yields, respectively. The smoothness of these reaction conditions allowed the introduction of different alkyl, alkenyl, alkynyl, or benzyl substituents at position 3 of the core structure **2**; their chemical structures (**10**−**20**) are represented in Scheme 1. In general, the yields obtained were from good to excellent, and the only other product detected on TLC was unreacted starting material. It should also be mentioned

Scheme 2. Synthesis of 1-Methyl-3-propyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (**23**)



that, based on spectroscopic data, alkylation occurs exclusively at the N^3 position.

To explore the importance of the substituent at position 1 while keeping a propyl at position 3 (see the Binding Studies and Structure−Affinity Relationships), two synthetic approaches were followed. The first approach involved a reaction of the corresponding N^1-substituted 6-aminouracil derivative with NBS followed by treatment with pyridine, as exemplified for the 6-amino-1-methyluracil[24] (**21**) in Scheme 2. This procedure afforded the 1-methyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (**22**) which was not easy to isolate and, therefore, was further alkylated at position 3 by a reaction with propyl iodide. In this way, 1-methyl-3-propyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (**23**) was prepared. The second approach (Scheme 3) involved debenzylation of the lead compound **6** to afford the NH free compound **24** which could be further modified at position 1. Debenzylation was performed by treatment of **6** with $AlCl_3$ in dry toluene,[25] affording **24** in 80% yield. Then, a reaction of **24** with 4-methoxybenzyl bromide or 3-methylbenzyl bromide in the presence of K_2CO_3 yielded the N^1-substituted benzyl derivatives **25** and **26** in 89 and 83% yields, respectively.

Binding Studies and Structure−Affinity Relationships. All synthesized compounds were tested in radioligand binding assays to determine their affinities for the adenosine A_1, A_{2A}, and A_3 receptors. The affinities at human adenosine A_1 receptors were determined

Scheme 3. Synthesis of 1-Substituted 3-Propyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione Derivatives

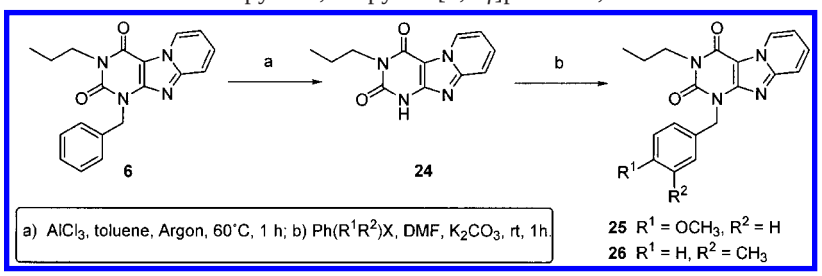
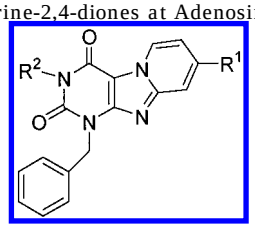


Table 1. Affinities of 1-Benzyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-diones at Adenosine hA_1, hA_{2A}, and hA_3 Receptors



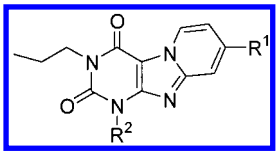
			K_i (nM) or % displacement[a]		
compd	R^1	R^2	hA_1[b]	hA_{2A}[c]	hA_3[d]
2	H	H	47%	32%	370 ± 40
3	OCH_3	H	49%	10%	210 ± 219
4	*t*-Bu	H	53%	23%	555 ± 65
5	Ph	H	11%	0%	200 ± 67
6	H	$CH_2CH_2CH_3$	50 ± 17	119 ± 23	4.0 ± 0.3
7	OCH_3	$CH_2CH_2CH_3$	179 ± 34	44%	10.0 ± 0.6
8	*t*-Bu	$CH_2CH_2CH_3$	1460 ± 650	385 ± 38	950 ± 130
9	Ph	$CH_2CH_2CH_3$	25%	8%	35 ± 13
10	H	CH_2CH_3	325 ± 77	584 ± 82	36 ± 7
11	H	$CH_2C_3H_5$	40.9%	242 ± 73	4.2 ± 1.1
12	H	$CH_2CH(CH_3)_2$	28 ± 15	40.0%	6.3 ± 2.2
13	H	$CH_2C_6H_{10}$	21%	22%	114 ± 60
14	H	$CH_2CH{=}CH_2$	42 ± 12	161 ± 104	5.0 ± 1.8
15	H	$CH_2C{\equiv}CH$	124 ± 39	96 ± 23	14 ± 9
16	H	$CH_2CH{=}CHCOOCH_3$	1%	12%	1125 ± 293
18	H	$CH_2CH_2CH_2NH_2$	7%	11%	6%
19	H	benzyl	20%	25,800/28,100	77 ± 32
20	H	4-OCH_3-benzyl	25%	16%	213 ± 61

[a] Percent displacement at [10 μM] ($n = 2$, average) or K_i ± SEM (nM, $n = 3$, unless otherwise stated). [b] Displacement of [^{3}H]DPCPX from CHO cell membranes expressing the human adenosine A_1 receptor. [c] Displacement of [^{3}H]ZM241385 from CHO cell membranes expressing the human adenosine A_{2A} receptor. [d] Displacement of [^{125}I]AB-MECA from HEK 293 cell membranes stably expressing the human adenosine A_3 receptor.

on membranes of CHO cells stably expressing this receptor, using [^{3}H]DPCPX as the radioligand. Affinities at human adenosine A_{2A} receptors were measured in a similar preparation with [^{3}H]ZM241385 as the radioligand. The affinity at adenosine A_3 receptors was determined on membranes from HEK 293 cells stably expressing the human A_3 receptor, using [^{125}I]AB-MECA as the radioligand. The results are shown in Tables 1 and 2.

The initially synthesized fused xanthine structures (**2**−**5**) exhibited significant affinity at the human adenosine A_3 receptor in the low micromolar range (0.2−0.6 μM), while they exhibited negligible affinity at adenosine A_1 and A_{2A} receptors (<50% ligand displacement at 10 μM, Table 1). These data were crucial in our research because, traditionally, xanthine derivatives have been considered good leads for A_1 and A_{2A} antagonists, but their affinity for the A_3 receptor was always weaker.[26] To the best of our knowledge, compounds **2**−**5** represent the first example of xanthine derivatives that show selectivity for the A_3 receptor. These findings urged us to explore this new family of compounds as adenosine antagonists by introducing modifications at positions 1, 3, and 8 of the core structure of 1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione.

Table 2. Affinities of 3-Propyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-diones at Adenosine hA_1, hA_{2A}, and hA_3 Receptors



			K_i (nM) or % displacement[a]		
compd	R^1	R^2	hA_1[b]	hA_{2A}[c]	hA_3[d]
6	H	benzyl	50 ± 17	119 ± 23	4.0 ± 0.3
23	H	CH_3	2100 ± 900	49%	3020 ± 250
25	H	4-OCH_3-benzyl	83 ± 20	40%	8.3 ± 2.8
26	H	3-CH_3-benzyl	44%	35%	68 ± 34

[a] Percent displacement at 10 μM ($n = 2$, average) or K_i ± SEM (nM, $n = 3$, unless otherwise stated). [b] Displacement of [^{3}H]DPCPX from CHO cell membranes expressing the human adenosine A_1 receptor. [c] Displacement of [^{3}H]ZM241385 from CHO cell membranes expressing the human adenosine A_{2A} receptor. [d] Displacement of [^{125}I]AB-MECA from HEK 293 cell membranes stably expressing the human adenosine A_3 receptor.

Introduction of a propyl moiety at position N^3 in compounds **2**−**5** (Table 1) led to a marked increase in affinity for all three adenosine receptors, in particular

for the A_3 receptor. Thus, the N^3-propyl derivative **6** exhibited almost an 100-fold increased affinity at the human adenosine A_3 receptor compared to the unsubstituted compound **2**, with a K_i value of 4.0 ± 0.3 nM. Compound **6** also exhibited significant affinity for the A_1 and A_{2A} receptors, but the selectivity for the A_3 receptor was clear (ratio of hA_1:hA_3 around 13 and ratio of hA_{2A}:hA_3 close to 30). Also, a marked increase in the A_3 affinity was observed for compounds **7** and **9** when compared to their parent compounds **3** and **5**, respectively. Compound **9** may also be the most selective in the series, displaying at least 300-fold selectivity versus human A_1 and A_{2A} receptors. Only the bulky *tert*-butyl derivative **8** was considerably less active against the three receptor subtypes. It was concluded that the introduction of a propyl moiety at the N^3 position increases the affinity for all three receptors while retaining the selectivity for the human A_3 receptors. Therefore, these pyrido[2,1-*f*]purine-2,4-dione derivatives can be considered as a new family of potent antagonists for the human A_3 receptor.

Because the introduction of a propyl moiety at position N^3 of the core structure **2** had such an impact on the affinity for the adenosine receptors, the influence of different alkyl, alkenyl, alkynyl, and benzyl substituents at position 3 of compound **2** was explored (Table 1). Among the alkyl substituents, the ethyl derivative **10** was almost 1 order of magnitude less potent on all three receptors than the propyl analogue **6**, while the methylcyclopropyl and isobutyl derivatives (compounds **11** and **12**, respectively) were as potent as **6** on the human A_3 receptors, with K_i values in the low nanomolar range and only differing in the degree of selectivity versus the other receptor subtypes. Only the more bulky methylcyclohexyl derivative **13** was considerably less potent (30-fold) at the A_3 receptor than the propyl derivative **6**. The rigidity of the chain at position N^3 was investigated by introducing unsaturated chains, such as allyl (**14**) or propargyl (**15**). These results indicate that such conformational restriction did not markedly affect affinity or selectivity in all three receptor subtypes. However, functionalization of the alkyl or alkenyl chain, as exemplified by the methyl ester **16** or the amine **18**, has a detrimental effect on all three adenosine receptors. Finally, introduction of a benzyl (**19**) or 4-methoxybenzyl (**20**) substituent at position 3 of the core structure **2** abolishes the affinity for the A_1 and A_{2A} receptors, while the affinity for A_3 receptors is reduced 20- and 50-fold, respectively, when compared to the affinity of propyl derivative **6**. These data indicate that steric demands at this position are more stringent at the level of A_1 and A_{2A} than at the A_3 receptor.

In a final series of modifications, the propyl substituent was kept at position N^3 of the pyrido[2,1-*f*]purine-2,4-dione structure while modifications were incorporated at N^1 (Table 2). A dramatic reduction of affinity and selectivity was observed by the replacement of the benzyl substituent in **6** by a methyl moiety, as shown for compound **23**. This N^1-methyl derivative had affinity for the A_1 and A_3 receptors in only the micromolar range, being 1000-fold less active than the benzyl derivative **6**. Next, substitutions were performed on the benzyl moiety at position N^1. Thus, the 4-methoxybenzyl derivative (**25**) almost kept the affinity of the benzyl derivative **6** for A_1 and A_3 receptors but lost its affinity for the hA_{2A} receptor, so selectivity was increased. More pronouncedly, the 3-methylbenzyl derivative (**26**) did not show significant affinity for the A_1 and A_2 receptors at 10 μM, while affinity for the A_3 receptor was reduced 15-fold when compared to that of the benzyl derivative **6**. Therefore, these data point to the importance of the benzyl substituent at position N^1 with regard to selectivity versus the different receptor subtypes among this new family of adenosine receptor antagonists.

Conclusions

1*H*,3*H*-Pyrido[2,1-*f*]purine-2,4-diones, a novel class of fused xanthine structures, represent a new family of potent A_3 receptor antagonists with affinities in the low nanomolar range. These compounds were prepared by a new synthetic procedure in a two-step one-pot reaction which consists of treatment of 6-aminouracil derivatives with NBS to generate a 5,5-dibromo-6-imino intermediate that reacts in situ with different pyridines to afford the 1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-diones. Functionalization at the N^3 position was performed smoothly, allowing for the introduction of different alkyl, alkenyl, alkynyl, or benzyl substituents. Structure−affinity relationships at the three adenosine receptors (A_1, A_{2A}, and A_3) established the basic requirements in this family of compounds for affinity versus the different adenosine receptors. Their potency, in particular against the A_3 receptor and exemplified by 1-benzyl-3-propyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione derivative **6** with a K_i value of 4.0 ± 0.3 nM, supports their potential value as a new family of adenosine receptor antagonists which deserves further exploration.

Experimental Section

Chemical Procedures. Melting points were obtained on a Reichert-Jung Kofler apparatus and are uncorrected. Microanalyses were obtained with a Heraeus CHN-O-RAPID instrument. Electrospray mass spectra were measured on a quadrupole mass spectrometer equipped with an electrospray source (Hewlett-Packard, LC−MS HP 1100). ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini spectrometer operating at 200 (^{1}H) and 50 MHz (^{13}C), respectively, a Varian INNOVA 300 spectrometer operating at 299 (^{1}H) and 75 MHz (^{13}C), respectively, and a Varian INNOVA 400 spectrometer operating at 399 (^{1}H) and 99 MHz (^{13}C), respectively. Monodimensional ^{1}H and ^{13}C spectra were obtained using standard conditions. Two-dimensional inverse proton-detected heteronuclear one-bond shift correlation spectra were obtained using the Pulsed Field Gradient HSQC pulse sequence. Data were collected in a 2048 × 512 matrix with a spectral width of 3460 Hz in the proton domain and a 2048 × 1024 matrix with a spectral width of 22 500 Hz in the carbon domain. The experiment was optimized for a one-bond heteronuclear coupling constant of 150 Hz. Two-dimensional inverse proton-detected heteronuclear long-range shift correlation spectra were obtained using the Pulsed Field Gradient HMBC pulse sequence. The HMBC experiment was carried out in the same conditions as the HSQC experiment and optimized for long-range coupling constants of 7 Hz.

Analytical TLC was performed on silica gel 60 F_{254} (Merck) precoated plates (0.2 mm). Spots were detected under UV light (254 nm) and/or by charring with phosphomolybdic acid and/or ninhydrin. Separations on silica gel were performed by preparative centrifugal circular thin-layer chromatography (CCTLC) on a Chromatotron instrument (Kiesegel 60 PF_{254} gipshaltig, Merck), with a layer thickness of 1 or 2 mm and a flow rate of 4 or 8 mL/min, respectively. Flash column

chromatography was performed with silica gel 60 (230−400 mesh, Merck).

All experiments involving water-sensitive compounds were conducted under scrupulously dry conditions. Acetonitrile and toluene were dried by refluxing over calcium hydride. Anhydrous *N*,*N*′-dimethylformamide was purchased from Aldrich.

General Procedure for the Synthesis of 1-Benzyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-diones. *N*-Bromosuccinimide (NBS) (445 mg, 2.5 mmol) was added into a suspension of 6-amino-1-benzyluracil (**1**) (217 mg, 1.0 mmol) in dry CH_3CN (8 mL), and the mixture was heated at 80 °C for 1 h. After the mixture was cooled to room temperature, the corresponding pyridine (5−10 mmol) was added, and the resulting mixture was heated at 80 °C for 6 h. The resulting precipitate, which contains the target compound, was collected by filtration and washed with ethyl ether. Evaporation of the filtrate, washing of the solid obtained with CH_2Cl_2 (2 × 5 mL), and purification of this solid by CCTLC on the Chromatotron instrument (CH_2Cl_2/MeOH mixtures) afforded a second portion of the target compound followed by varying amounts (15−25%) of 6-amino-1-benzyl-5-bromouracil.

1-Benzyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (2). Global yield: 60%. Mp (CH_2Cl_2/MeOH): 298−299 °C. MS (EI): *m*/*z* 292 (M^+, 59). ^{1}H NMR (DMSO-d_6) δ: 5.22 (s, 2H, CH_2Ph), 7.10−7.50 (m, 6H, H-7, Ph), 7.66 (m, J = 7.1, 1.2 Hz, H-8), 7.75 (d, J = 9.1 Hz, 1H, H-9), 8.94 (d, J = 6.6 Hz, H-6), 11.35 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 44.92 (CH_2Ph), 101.95 (C-4a), 114.51 (C-7), 116.08 (C-9), 127.11, 127.20, 128.32, 136.71 (C-6, Ph), 130.34 (C-8), 147.08 (C-9a), 150.85 (C-10a), 151.60 (C-2), 154.53 (C-4). Anal. ($C_{16}H_{12}N_4O_2$) C, H, N.

1-Benzyl-8-methoxy-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (3). Global yield: 74%. Mp (CH_2Cl_2/MeOH): 277−278 °C. MS (EI): *m*/*z* 322 (M^+, 100). ^{1}H NMR (DMSO-d_6) δ: 3.88 (s, 3H, OCH_3), 5.18 (s, 2H, CH_2Ph), 7.01 (dd, J = 7.5, 2.6 Hz, H-7), 7.10−7.50 (m, 6H, H-9, Ph), 8.70 (d, J = 7.5 Hz, 1H, H-6), 11.30 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 44.95 (CH_2Ph), 56.23 (OCH_3), 95.74 (C-9), 100.38 (C-4a), 107.72 (C-7), 127.20, 127.70, 128.32, 136.92 (C-6, Ph), 149.05 (C-9a), 150.92 (C-10a), 152.41 (C-2), 154.12 (C-4), 161.12 (C-8). Anal. ($C_{17}H_{14}N_4O_3$) C, H, N.

1-Benzyl-8-*tert*-butyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (4). Global yield: 58%. Mp (EtOAc): 258−259 °C. MS (EI): *m*/*z* 348 (M^+, 100). ^{1}H NMR ($CDCl_3$) δ: 1.33 [s, 9H, $(CH_3)_3C$], 5.31 (s, 2H, CH_2Ph), 7.16 (dd, J = 7.1, 1.8 Hz, H-7), 7.20−7.65 (m, 5H, Ph), 7.67 (d, J = 1.8 Hz, H-9), 8.90 (d, J = 7.1 Hz, 1H, H-6), 9.20 (br s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ: 30.47 [(CH_3)$_3$C], 35.46 [(CH_3)$_3$*C*], 46.01 (CH_2Ph), 100.05 (C-4a), 111.95 (C-9), 113.22 (C-7), 126.61 (C-6), 127.79, 128.49, 128.65, 136.27 (Ph), 148.60 (C-9a), 151.13 (C10a), 152.80 (C-2), 154.41 (C-4), 155.17 (C-8). Anal. ($C_{20}H_{20}N_4O_2$) C, H, N.

1-Benzyl-8-phenyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (5). Global yield: 59%. Mp (EtOAc/MeOH): 282−284 °C. MS (ES, positive mode): *m*/*z* 369 [(M + 1)$^+$], 391 [(M + Na)$^+$]. ^{1}H NMR (DMSO-d_6) δ: 5.23 (s, 2H, CH_2Ph), 7.24−7.90 (m, 11H, Ph, H-7), 8.10 (s, 1H, H-9), 8.95 (d, J = 7.0 Hz, 1H, H-6), 11.14 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 44.99 (CH_2Ph), 101.11 (C-4a), 112.31 (C-9), 113.34 (C-7), 126.82, 126.98, 127.19, 128.28, 129.08, 136.73 (C-6, Ph), 141.53, 147.53 (C-8, C-9a), 150.85, 152.19 (C-10a, C-2), 154.45 (C-4). Anal. ($C_{22}H_{16}N_4O_2$) C, H, N.

General Procedure for the Preparation of N^3-Substituted 1-Benzyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-diones. A solution of the corresponding pyridopurinedione (**2**−**5**) (0.30 mmol) in dry CH_3CN (3 mL) was reacted with DBU (0.05 mL, 0.33 mmol) and the corresponding alkyl, alkenyl, alkynyl, or benzyl halide (0.45 mmol) at room temperature or at 80 °C. After 4−6 h, volatiles were removed. The residue was taken up in CH_2Cl_2 (50 mL) and washed with 1N HCl (20 mL), water (20 mL), and brine (20 mL). The organic phase was dried on anhydrous Na_2SO_4, filtered, and evaporated. The residue was purified by CCTLC on the Chromatotron instrument (2:1 ratio of hexane/EtOAc) except where specified.

1-Benzyl-3-propyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (6). Compound **6** was obtained by the reaction of **2** with propyl iodide. Yield: 65%. Mp (CH_2Cl_2/MeOH): 163−165 °C. MS (EI): *m*/*z* 334 (M^+, 71). ^{1}H NMR ($CDCl_3$) δ: 0.95 (t, J = 7.5 Hz, 3H, CH_3), 1.69 (m, J = 7.5 Hz, 2H, CH_2CH$_3$), 4.01 (pt, J = 7.3 Hz, 2H, NCH_2), 5.37 (s, 2H, CH_2Ph), 7.06 (pt, J = 6.1 Hz, H-7), 7.25−7.52 (m, 5H, Ph), 7.52 (pt, J = 7.0 Hz, H-8), 7.65 (d, J = 7.0 Hz, 1H, H-9), 9.04 (d, J = 6.7 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 11.32 (CH_3CH_2), 21.38 (CH_3CH_2), 42.83 (NCH_2), 46.67 (CH_2Ph), 101.90 (C-4a), 113.90 (C-7), 116.41 (C-9), 127.48 (C-6), 127.79, 128.51, 128.66, 136.37 (Ph), 129.87 (C-8), 147.63 (C-9a), 150.69 (C-10a), 151.39 (C-2), 155.01 (C-4). Anal. ($C_{19}H_{18}N_4O_2$) C, H, N.

1-Benzyl-8-methoxy-3-propyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (7). Compound **7** was obtained by the reaction of **3** with propyl iodide. Yield: 82%. Mp (CH_2Cl_2/MeOH): 188−190 °C. MS (EI): *m*/*z* 364 (M^+, 100). ^{1}H NMR ($CDCl_3$) δ: 0.97 (t, J = 7.5 Hz, 3H, CH_3), 1.69 (m, J = 7.7 Hz, 2H, CH_2CH$_3$), 3.93 (s, 3H, OCH_3), 4.01 (pt, J = 7.5 Hz, 2H, NCH_2), 5.36 (s, 2H, CH_2Ph), 6.75 (dd, J = 7.2, 2.4 Hz, H-7), 6.99 (d, J = 2.3 Hz, H-9), 7.25−7.53 (m, 5H, Ph), 8.83 (d, J = 7.5 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 11.30 (CH_3CH_2), 21.44 (CH_3CH_2), 42.78 (NCH_2), 46.66 (CH_2Ph), 55.84 (OCH_3), 95.39 (C-9), 101.06 (C-4a), 107.54 (C-7), 127.72, 127.90, 128.49, 128.60, 136.62 (C-6, Ph), 150.01, 151.49 (C-2, C-9a, C-10a), 154.67 (C-4), 161.56 (C-8). Anal. ($C_{20}H_{20}N_4O_3$) C, H, N.

1-Benzyl-8-*tert*-butyl-3-propyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (8). Compound **8** was obtained by the reaction of **4** with propyl iodide and was purified by CCTLC on the Chromatotron instrument (30:1 ratio of CH_2Cl_2/MeOH). Yield: 75%. Mp (CH_2Cl_2/MeOH): 142−144 °C. MS (EI): *m*/*z* 390 (M^+, 100). ^{1}H NMR ($CDCl_3$) δ: 0.97 (t, J = 7.3 Hz, 3H, CH_3), 1.38 [s, 9H, $(CH_3)_3C$], 1.70 (m, J = 7.6 Hz, 2H, CH_2CH$_3$), 4.02 (pt, J = 7.5 Hz, 2H, NCH_2), 5.38 (s, 2H, CH_2Ph), 7.14 (dd, J = 7.1, 1.8 Hz, H-7), 7.26−7.54 (m, 5H, Ph), 7.63 (dd, J = 1.8, 0.9 Hz, H-9), 8.92 (dd, J = 7.1, 0.9 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 11.28 (CH_3CH_2), 21.43 (CH_3CH_2), 30.51 [(CH_3)$_3$C], 35.43 [(CH_3)$_3$*C*], 42.80 (NCH_2), 46.67 (CH_2Ph), 101.47 (C-4a), 111.76, 112.90 (C-9, C-7), 126.64, 127.71, 128.49, 128.60, 136.61 (C-6, Ph), 148.23 (C-9a), 151.11, 151.53 (C-10a, C-2), 154.76, 154.92 (C-4, C-8). Anal. ($C_{23}H_{26}N_4O_2$) C, H, N.

1-Benzyl-8-phenyl-3-propyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (9). Compound **9** was obtained by the reaction of **5** with propyl iodide and was purified by CCTLC on the Chromatotron instrument (40:1 ratio of CH_2Cl_2/MeOH). Yield: 93%. Mp (CH_2Cl_2/MeOH): 202−204 °C. MS (ES, positive mode): *m*/*z* 411 [(M + 1)$^+$], 433 [(M + Na)$^+$]. ^{1}H NMR ($CDCl_3$) δ: 0.99 (t, J = 7.5 Hz, 3H, CH_3), 1.74 (m, J = 7.5 Hz, 2H, CH_2CH$_3$), 4.05 (t, J = 7.5 Hz, 2H, NCH_2), 5.42 (s, 2H, CH_2Ph), 7.26−7.73 (m, 11H, Ph, H-7), 7.89 (s, 1H, H-9), 9.08 (d, J = 7.0 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 11.32 (CH_2CH_3), 21.41 (CH_2CH_3), 42.86 (NCH_2), 46.72 (CH_2Ph), 101.62 (C-4a), 113.19 (C-9), 113.42 (C-7), 126.97, 127.21, 127.80, 128.52, 128.70, 129.19, 129.28, 136.46, 137.73 (C-6, Ph), 142.94, 148.14 (C-9a, C-8), 151.25, 151.42 (C-10a, C-2), 154.91 (C-4). Anal. ($C_{25}H_{22}N_4O_2$) C, H, N.

1-Benzyl-3-ethyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (10). Compound **10** was obtained by the reaction of **2** with ethyl iodide and was purified by CCTLC on the Chromatotron instrument (40:1 ratio of CH_2Cl/MeOH). Yield: 34%. Mp (CH_2Cl_2/MeOH): 118−120 °C. MS (ES, positive mode): *m*/*z* 321 [(M + 1)$^+$], 343 [(M + Na)$^+$]. ^{1}H NMR ($CDCl_3$) δ: 1.28 (t, J = 7.1 Hz, 3H, CH_3), 4.15 (q, J = 7.1 Hz, 2H, NCH_2CH$_3$), 5.40 (s, 2H, CH_2Ph), 7.10 (m, J = 6.9, 1.2 Hz, H-7), 7.23−7.39 (m, 4H, Ph), 7.55 (t, J = 7.2 Hz, 2H, Ph, H-8), 7.70 (m, J = 9.0, 1.2 Hz, 1H, H-9), 9.08 (m, J = 6.8, 1.2 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 13.37 (CH_3), 36.43 (NCH_2), 46.65 (CH_2Ph), 101.85 (C-4a), 113.87 (C-7), 116.41 (C-9), 127.47 (C-6), 127.78, 128.49, 128.69, 136.38 (Ph), 129.82 (C-8), 147.61 (C-9a), 150.70, 151.20 (C-10a, C-2), 154.81 (C-4). Anal. ($C_{18}H_{16}N_4O_2$) C, H, N.

1-Benzyl-3-cyclopropylmethyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (11). Compound **11** was obtained by the reaction of **2** with (bromomethyl)cyclopropane. Yield: 50%. Mp (CH_2Cl_2/MeOH): 151−152 °C. MS (ES, positive mode): *m*/*z* 347 [(M + 1)$^+$], 369 [(M + Na)$^+$]. ^{1}H NMR ($CDCl_3$) δ: 0.46−0.50 (m, 4H, CH_2), 1.33 (m, 1H, CH), 3.98 (d, J = 7.1 Hz, 2H, NCH_2), 5.42 (s, 2H, CH_2Ph), 7.09 (m, J = 6.8, 1.2 Hz, H-7), 7.26−7.56 (m, 6H, Ph, H-8), 7.70 (m, J = 9.0, 1.2 Hz, 1H, H-9),

9.08 (m, J = 6.8, 1.2 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 3.82 (CH_2), 10.14 (CH), 45.59 (NCH_2), 46.65 (CH_2Ph), 113.84 (C-7), 116.38 (C-9), 127.48 (C-6), 127.77, 128.47, 128.66, 138.39 (Ph), 129.82 (C-8), 147.62 (C-9a), 150.73, 151.64 (C-10a, C-2), 155.14 (C-4). Anal. ($C_{20}H_{18}N_4O_2$) C, H, N.

1-Benzyl-3-(2-methylpropyl)-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (12). Compound **12** was obtained by the reaction of **2** with 1-bromo-2-methylpropane. Yield: 92%. Mp (CH_2Cl_2/MeOH): 188−190 °C. MS (ES, positive mode): m/z 349 [(M + 1)$^+$], 371 [(M + Na)$^+$]. ^{1}H NMR ($CDCl_3$) δ: 0.96 (d, J = 6.8 Hz, 6H, CH_3), 2.21 (m, J = 6.6 Hz, 1H, CH), 3.92 (d, J = 7.2 Hz, 2H, 3-NCH_2), 5.41 (s, 2H, CH_2Ph), 7.09 (t, J = 6.8 Hz, H-7), 7.27−7.59 (m, 6H, Ph, H-8), 7.70 (d, J = 9.0 Hz, 1H, H-9), 9.08 (d, J = 6.2 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 20.11 (CH_3), 27.27 (CH), 46.68 (NCH_2), 48.03 (CH_2Ph), 101.76 (C-4a), 113.86 (C-7), 116.41 (C-9), 127.50 (C-6), 127.75, 128.48, 128.56, 136.41 (Ph), 129.84 (C-8), 147.66 (C-9a), 150.71, 151.64 (C-10a, C-2), 155.26 (C-4). Anal. ($C_{20}H_{20}N_4O_2$) C, H, N.

1-Benzyl-3-cyclohexylmethyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (13). Compound **13** was obtained by the reaction of **2** with (bromomethyl)cyclohexane. Yield: 93%. Mp (CH_2Cl_2/MeOH): 184−186 °C. MS (ES, positive mode): m/z 389 [(M + 1)$^+$], 411 [(M + Na)$^+$]. ^{1}H NMR ($CDCl_3$) δ: 1.02−1.24 (m, 6H, CH_2), 1.65 (m, 2H, CH_2), 1.85 (m, 1H, CH), 3.92 (d, J = 7.3 Hz, 2H, NCH_2), 5.38 (s, 2H, CH_2Ph), 7.05 (m, J = 6.8, 1.1 Hz, H-7), 7.21−7.49 (m, 6H, Ph, H-8), 7.65 (m, J = 9.2, 1.1 Hz, 1H, H-9), 9.05 (m, J = 6.6, 1.1 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 25.82, 26.35, 30.79 (CH_2), 36.61 (CH), 46.72 (NCH_2), 47.01 (CH_2Ph), 101.79 (C-4a), 113.82 (C-7), 116.41 (C-9), 127.52 (C-6), 127.75, 128.48, 128.61, 136.45 (Ph), 129.80 (C-8), 147.67 (C-9a), 150.73, 151.68 (C-10a, C-2), 155.31 (C-4). Anal. ($C_{23}H_{24}N_4O_2$) C, H, N.

3-Allyl-1-benzyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (14). Compound **14** was obtained by the reaction of **2** with allyl bromide. Yield: 98%. Mp (CH_2Cl_2/MeOH): 152−154 °C. MS (ES, positive mode): m/z 333 [(M + 1)$^+$], 355 [(M + Na)$^+$]. ^{1}H NMR ($CDCl_3$) δ: 4.67 (m, J = 5.7, 1.5 Hz, 2H, NCH_2), 5.16−5.29 [qAB, 2H, CH=CH_{2A} 5.17 (dq, J = 10.2, 1.3 Hz), CH=CH_{2B} 5.26 (dq, J = 17.1, 1.3 Hz)], 5.38 (s, 2H, CH_2Ph), 5.94 (m, 1H, CH=CH_2), 7.07 (m, J = 6.9, 1.3 Hz, H-7), 7.23−7.55 (m, 6H, Ph, H-8), 7.6 (m, J = 9.0, 1.3 Hz, 1H, H-9), 9.04 (m, J = 6.6, 1.3 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 43.18 (NCH_2), 46.71 (CH_2Ph), 101.74 (C-4a), 113.97 (C-7), 116.46 (C-9), 117.48 (CH_2=CH), 127.52 (C-6), 127.82, 128.51, 128.66, 136.28 (Ph), 130.00 (C-8), 132.23 (CH_2=CH), 147.71 (C-9a), 150.83 (C-10a), 151.19 (C-2), 154.63 (C-4). Anal. ($C_{19}H_{16}N_4O_2$) C, H, N.

1-Benzyl-3-propargyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (15). Compound **15** was obtained by the reaction of **2** with propargyl bromide (80% in toluene) and was purified by CCTLC on the Chromatotron instrument (40:1 ratio of CH_2Cl_2/MeOH). Yield: 63%. Mp (CH_2Cl_2/MeOH): 148−150 °C. MS (ES, positive mode): m/z 331 [(M + 1)$^+$], 353 [(M + Na)$^+$]. ^{1}H NMR ($CDCl_3$) δ: 2.20 (t, J = 2.6 Hz, 1H, C≡CH), 4.86 (d, J = 2.4 Hz, 2H, NCH_2), 5.42 (s, 2H, CH_2Ph), 7.12 (m, J = 6.7, 1.2 Hz, H-7), 7.27−7.58 (m, 6H, Ph, H-8), 7.71 (m, J = 9.0, 1.2 Hz, 1H, H-9), 9.06 (m, J = 6.6, 1.2 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 30.34 (NCH_2), 46.85 (CH_2Ph), 70.61 (C≡CH), 78.55 (C≡CH), 101.63 (C-4a), 114.16 (C-7), 116.54 (C-9), 127.58 (C-6), 127.93, 128.51, 128.87, 136.07 (Ph), 130.24 (C-8), 147.84 (C-9a), 150.80, 150.95 (C-10a, C-2), 153.91 (C-4). Anal. ($C_{19}H_{14}N_4O_2$) C, H, N.

1-Benzyl-3-[(*E*)-3-methoxycarbonyl-2-propenyl]-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (16). Compound **16** was obtained by the reaction of **2** with methyl-4-bromocrotonate. Yield: 90%. Mp (CH_2Cl_2/MeOH): 179−181 °C. MS (ES, positive mode): m/z 391 [(M + 1)$^+$], 413 [(M + Na)$^+$]. ^{1}H NMR ($CDCl_3$) δ: 3.70 (s, 3H, OCH_3), 4.84 (d, J = 5.3 Hz, 2H, NCH_2), 5.40 (s, 2H, CH_2Ph), 5.93 (d, J = 15.7 Hz, 1H, CH_2CH=CH), 6.94−7.15 (m, 2H, CH_2CH=CH, H-7), 7.27−7.67 (m, 6H, Ph, H-8), 7.71 (d, J = 9.0 Hz, 1H, H-9), 9.03 (d, J = 6.6 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 41.37 (NCH_2), 46.83 (CH_2Ph), 51.56 (OCH_3), 101.57 (C-4a), 114.16 (C-7), 116.57 (C-9), 122.29 (CH_2-CH=CH), 127.53 (C-6), 127.91, 128.56, 128.69, 136.09 (Ph), 130.24 (C-8), 142.21 (CH_2CH=CH), 147.87 (C-9a), 151.02 (C-10a, C-2), 154.54 (C-4), 166.25 (CO_2CH_3). Anal. ($C_{21}H_{18}N_4O_4$) C, H, N.

1-Benzyl-3-(3-phthalimidopropyl)-1*H*,3*H*-pyrido[2,1-*f*]-purine-2,4-dione (17). Compound **17** was obtained by the reaction of **2** with *N*-(3-bromopropyl)phthalimide and was purified by CCTLC on the Chromatotron instrument (30:1 ratio of CH_2Cl_2/MeOH). Yield: 81%. Mp (CH_2Cl_2/MeOH): 243−245 °C. MS (ES, positive mode): m/z 480 [(M + 1)$^+$], 502 [(M + Na)$^+$]. ^{1}H NMR ($CDCl_3$) δ: 2.13 (m, J = 7.3 Hz, 2H, CH_2), 3.82 (t, J = 7.1 Hz, 2H, 3-NCH_2), 4.18 (t, J = 7.0 Hz, 2H, NPht-CH_2), 5.38 (s, 2H, CH_2Ph), 7.08 (m, J = 7.0, 1.3 Hz, H-7), 7.27−7.85 (m, 11H, Ph, H-8, H-9), 9.01 (m, J = 6.6, 1.1 Hz, 1H, H-6). Anal. ($C_{27}H_{21}N_5O_4$) C, H, N.

1-Benzyl-3-(3-aminopropyl)-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (18). To a solution of **17** (200 mg, 0.42 mmol) in EtOH (14 mL) was added H_2N−NH_2·H_2O (0.2 mL, 4.2 mmol), and the mixture was stirred at room temperature for 18 h. It was filtered, and the filtrate was evaporated. The residue was purified by CCTLC on the Chromatotron instrument (15:1:0.16 ratio of CH_2Cl_2/MeOH/NH_4OH) to afford 101 mg (69% yield) of **18**. Mp (CH_2Cl_2/MeOH): 127−129 °C. MS (ES, positive mode): m/z 350 [(M + 1)$^+$], 699.4 [(2M + 1)$^+$]. ^{1}H NMR (DMSO-d_6) δ: 1.66 (m, J = 7.0 Hz, 2H, CH_2), 2.53 (t, J = 6.8 Hz, 2H, CH_2−NH_2), 3.99 (d, J = 7.1 Hz, 2H, NCH_2), 5.28 (s, 2H, CH_2Ph), 7.24−7.82 (m, 8H, Ph, H-7, H-8, H-9), 9.01 (m, J = 6.6, 1.1 Hz, 1H, H-6). ^{13}C NMR (DMSO-d_6) δ: 31.56 (CH_2), 38.47 (CH_2−NH_2), 39.03 (NCH_2), 45.96 (CH_2Ph), 101.02 (C-4a), 114.65 (C-7), 116.07 (C-9), 127.19 (C-6), 127.29, 127.36, 128.39, 136.67 (Ph), 130.66 (C-8), 147.02 (C-9a), 150.19, 150.90 (C-10a, C-2), 154.24 (C-4). Anal. ($C_{19}H_{19}N_5O_2$) C, H, N.

1,3-Dibenzyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (19). Compound **19** was obtained by the reaction of **2** with benzyl bromide and was purified by CCTLC on the Chromatotron instrument (40:1 ratio of CH_2Cl_2/MeOH). Yield: 94%. Mp (CH_2Cl_2/MeOH): 176−178 °C. MS (ES, positive mode): m/z 383 [(M + 1)$^+$], 405 [(M + Na)$^+$]. ^{1}H NMR ($CDCl_3$) δ: 5.25 (s, 2H, 3-NCH_2Ph), 5.38 (s, 2H, 1-NCH_2Ph), 7.07 (m, J = 6.8, 1.1 Hz, H-7), 7.23−7.55 (m, 11H, Ph, H-8), 7.66 (m, J = 9.0, 1.2 Hz, 1H, H-9), 9.06 (m, J = 6.6, 1.2 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 44.34 (3-NCH_2Ph), 46.76 (1-NCH_2Ph), 101.81 (C-4a), 113.95 (C-7), 116.47 (C-9), 127.50 (C-6), 127.82, 128.41, 128.51, 128.62, 128.69, 136.30, 137.27 (Ph), 129.99 (C-8), 147.76 (C-9a), 150.83, 151.55 (C-10a, C-2), 154.89 (C-4). Anal. ($C_{23}H_{18}N_4O_2$) C, H, N.

1-Benzyl-3-(4-methoxybenzyl)-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (20). Compound **20** was obtained by the reaction of **2** with 4-methoxybenzyl chloride. Yield: 90%. Mp (CH_2Cl_2/MeOH): 203−205 °C. MS (ES, positive mode): m/z 413 [(M + 1)$^+$], 435 [(M + Na)$^+$]. ^{1}H NMR ($CDCl_3$) δ: 3.78 (s, 3H, OCH_3), 5.20 (s, 2H, 3-NCH_2), 5.39 (s, 2H, 1-NCH_2), 6.84 (d, J = 8.8 Hz, 2H, Ph), 7.08 (t, J = 7.0 Hz, H-7), 7.26−7.57 (m, 8H, Ph, H-8), 7.68 (d, J = 7.9 Hz, 1H, H-9), 9.08 (d, J = 6.6 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 43.77 (3-NCH_2), 46.71 (CH_2Ph), 55.19 (OCH_3), 113.91 (C-7), 116.44 (C-9), 127.53, 127.80, 128.51, 128.58, 129.52, 129.95, 130.38, 136.29 (C-7, C-6, C-8, Ph) 147.70 (C-9a), 150.75, 151.53 (C-10a, C-2), 158.98 (C-4). Anal. ($C_{24}H_{20}N_4O_3$) C, H, N.

1-Methyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (22). 6-Amino-1-methyluracil (**21**) (200 mg, 1.41 mmol) was suspended in dry CH_3CN (12 mL) and was reacted with NBS (627 mg, 3.52 mmol) at 80 °C for 2 h. The mixture was cooled to room temperature; pyridine (1.14 mL, 14.10 mmol) was added, and heating to 80 °C was continued for 6 h. The mixture was allowed to reach room temperature, and then it was diluted with EtOAc (10 mL). The precipitate obtained was filtered. The collected solid (220 mg) contained **22** and was used in the next step without further purification.

1-Methyl-3-propyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (23). Crude **22** (200 mg) was suspended in dry CH_3CN (8 mL), and DBU (0.14 mL, 0.92 mmol) and propyl iodide (0.13 mL, 1.38 mmol) were added. The mixture was heated at 80 °C for 5 h. Then, volatiles were removed, and the residue was taken up in MeOH (30 mL) and filtered through Celite. The filtrate was purified twice by CCTLC on the Chromatotron

instrument (first, CH_2CL_2/MeOH, 10:1; then, Hex/EtOAc, 1:1) to afford 70 mg (19% yield from **21**) of **23** as a white solid. Mp: 132−134 °C. MS (EI): *m/z* 258 (M^+, 49). ^{1}H NMR ($CDCl_3$) δ: 0.99 (t, J = 7.5 Hz, 3H, CH_3), 1.71 (m, J = 7.5 Hz, 2H, CH_2-CH_3), 3.69 (s, 2H, NCH_3), 4.04 (pt, J = 7.3 Hz, 2H, NCH_2), 7.10 (pt, J = 7.0, 1.5 Hz, 1H, H-7), 7.56 (pt, J = 7.0, 1.5 Hz, 1H, H-8), 7.67 (d, J = 9.0 Hz, 1H, H-9), 9.06 (d, J = 6.6 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 11.45 (CH_3CH_2), 21.51 (CH_3CH_2), 30.00 (CH_3N), 42.91 (NCH_2), 46.71 (CH_2Ph), 101.10 (C-4a), 114.07 (C-7), 116.35 (C-9), 127.62 (C-6), 130.09 (C-8), 147.76 (C-9a), 151.03, 151.79 (C-10a, C-2), 155.13 (C-4). Anal. ($C_{13}H_{14}N_4O_2$) C, H, N.

3-Propyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (24). To a stirred solution of **6** (156 mg, 0.47 mmol) in toluene (5 mL, freshly distilled) under an argon atmosphere was added dry $AlCl_3$ (311 mg, 2.34 mmol), and the mixture was heated at 60 °C for 1 h. After the mixture was cooled to room temperature, iced water (10 mL) and EtOAc (20 mL) were added, and stirring was continued for 30 min. The aqueous phase was further extracted with EtOAc (3 × 20 mL). The combined organic phases were dried ($MgSO_4$), filtered, and evaporated. The residue was purified by flash column chromatography (40:1 ratio of CH_2Cl_2/MeOH) to yield 91 mg (80%) of **24**. Mp (CH_2Cl_2/MeOH): 274−276 °C. MS (ES, positive mode): *m/z* 245 [$(M + 1)^+$], 267 [$M + Na)^+$]. ^{1}H NMR (DMSO-d_6) δ: 0.87 (t, J = 7.5 Hz, 3H, CH_3), 1.59 (m, J = 7.3 Hz, 2H, CH_2CH_3), 3.83 (t, J = 6.1 Hz, 2H, NCH_2), 7.22 (t, J = 6.8 Hz, H-7), 7.61−7.70 (m, 2H, H-8, H-9), 8.95 (d, J = 6.6 Hz, 1H, H-6), 12.13 (br s, 1H, NH). ^{13}C NMR (DMSO-d_6) δ: 11.21 (CH_3CH_2), 20.92 (CH_3CH_2), 41.11 (NCH_2), 100.91 (C-4a), 114.21 (C-7), 115.91 (C-9), 127.00 (C-6), 130.39 (C-8), 147.35 (C-9a), 149.78, 151.13 (C-10a, C-2), 154.99 (C-4). Anal. ($C_{12}H_{12}N_4O_2$) C, H, N.

1-(4-Methoxybenzyl)-3-propyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (25). To a stirred solution of **24** (66 mg, 0.27 mmol) in anhydrous DMF (4.2 mL) was added K_2CO_3 (56 mg, 0.40 mmol), and the mixture was stirred at room temperature for 1 h. Then, 4-methoxybenzyl chloride (42 μl, 0.31 mmol) was added, and the reaction mixture was heated at 40 °C for 3 h. After the mixture had cooled to room temperature, volatiles were removed, and the residue was taken up in EtOAc (50 mL) and washed with a saturated $NaHCO_3$ solution (20 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated. The residue was purified by CCTLC on the Chromatotron instrument using hexane/EtOAc (2:1) as eluent to yield 88 mg (89%) of **25**. Mp (CH_2Cl_2/MeOH): 165−167 °C. MS (ES, positive mode): *m/z* 365 [$(M + 1)^+$], 387 [$(M + Na)^+$]. ^{1}H NMR ($CDCl_3$) δ: 0.97 (t, J = 7.5 Hz, 3H, CH_3), 1.70 (m, J = 7.6 Hz, 2H, CH_2CH_3), 3.76 (s, 3H, OCH_3), 4.02 (t, J = 7.6 Hz, 2H, NCH_2), 5.32 (s, 2H, CH_2Ph), 6.84 (d, J = 8.8 Hz, 2H, Ph), 7.08 (m, J = 6.7, 1.2 Hz, H-7), 7.54−7.57 (m, 3H, Ph, H-8), 7.69 (m, J = 8.9, 1.2 Hz, 1H, H-9), 9.05 (m, J = 6.6, 1.2 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 11.32 (CH_3CH_2), 21.37 (CH_3CH_2), 42.78 (NCH_2), 46.14 (CH_2Ph), 55.18 (OCH_3), 113.82 (C-7), 116.38 (C-9), 127.48 (C-6), 113.78, 128.63, 130.37 (Ph), 129.80 (C-8), 147.61 (C-9a), 150.65, 151.36 (C-10a, C-2), 155.00 (C-4). Anal. ($C_{20}H_{20}N_4O_3$) C, H, N.

1-(3-Methylbenzyl)-3-propyl-1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-dione (26). A procedure analogous to that described for the synthesis of **25** was followed, and compound **26** was obtained by the reaction of **24** with (3-methyl)benzyl bromide. Yield: 83%. Mp (CH_2Cl_2/MeOH): 173−175 °C. MS (ES, positive mode): *m/z* 349 [$(M + 1)^+$], 371 [$(M + Na)^+$]. ^{1}H NMR ($CDCl_3$) δ: 1.05 (t, J = 7.5 Hz, 3H, CH_3CH_2), 1.79 (m, J = 7.5 Hz, 2H, CH_2CH_3), 2.39 (s, 3H, CH_3), 4.11 (t, J = 7.5 Hz, 2H, NCH_2), 5.43 (s, 2H, CH_2Ph), 7.16 (t, J = 7.0 Hz, 2H, Ph, H-7), 7.25−7.42 (m, 3H, Ph), 7.62 (m, J = 8.1, 1.3 Hz, H-8), 7.76 (m, J = 9.0, 1.1 Hz, 1H, H-9), 9.14 (m, J = 6.6, 1.3 Hz, 1H, H-6). ^{13}C NMR ($CDCl_3$) δ: 11.32 (CH_3CH_2), 21.36 (CH_3CH_2, CH_3), 42.79 (NCH_2), 46.64 (CH_2Ph), 101.79 (C-4a), 113.83 (C-7), 116.39 (C-9), 125.57, 127.44, 128.52, 129.23, 129.45, 136.27, 138.13 (C-6, C-8, Ph), 147.61 (C-9a), 150.70, 151.38 (C-10a, C-2), 156.00 (C-4). Anal. ($C_{20}H_{20}N_4O_2$) C, H, N.

Radioligand Binding Studies. Radioligand binding studies were performed on stably transfected cell lines expressing human adenosine receptors. CHO cells expressing the human adenosine A_1 receptor were obtained from Dr. A. Townsend-Nicholson. These cells were cultured at 37 °C in a 5% CO_2 atmosphere in a 1:1 mixture of DMEM/F12, 2 mM Glutamax (a stable analogue of glutamine), 10% newborn calf serum with 50 IU/mL penicillin, and 50 mg/mL streptomycin. Dr. S. Rees kindly provided CHO cells expressing the human A_{2A} receptor. These cells were cultured at 37 °C in a 5% CO_2 atmosphere in a 1:1 mixture of DMEM/F12, 2 mM Glutamax, 10% newborn calf serum, 1 mg/mL G418 with 50 IU/mL penicillin, and 50 mg/mL streptomycin. HEK 293 cells expressing human adenosine A_3 receptors were from Dr. K.-N. Klotz. These cells were cultured at 37 °C in a 7% CO_2 atmosphere in a mixture of DMEM, 2 mM Glutamax, 10% newborn calf serum, 0.5 mg/mL G418 with 50 IU/mL penicillin, and 50 mg/mL streptomycin. Confluent cells expressing the human A_1 or A_{2A} receptor or semiconfluent cells expressing the human A_3 adenosine receptor were trypsinized and centrifuged for 10 min at 1000 rpm. The cell pellets were resuspended in 50 mM Tris/HCl (pH 7.4) at room temperature and homogenized on ice for 5 s at position 8 with an Ystral homogenizer. The homogenate was centrifuged for 45 min at 12 700 rpm in an SW-30 rotor at 4 °C. The resulting pellet was resuspended in 50 mM Tris/HCl (pH 7.4) at room temperature. Adenosine deaminase, 2 IU/mL, was added, and aliquots were stored at −80 °C.

Stock solutions of ligands were made in DMSO. The final concentration of DMSO in the assay did not exceed 1%.

[^{3}H]DPCPX and [^{125}I]AB-MECA were obtained from Amersham, and [^{3}H]ZM241385 was obtained from Tocris Cookson, Ltd. (Northpoint, U.K.).

Adenosine A_1 Receptor. Membranes containing 40 mg of protein were incubated in a total volume of 400 mL of 50 mM Tris/HCl (pH 7.4) and [^{3}H]DPCPX (final concentration, 1.6 nM) for 1 h at 25 °C in a shaking water bath. Nonspecific binding was determined in the presence of 10 μM CPA. The incubation was terminated by filtration over Whatman GF/B filters under reduced pressure with a Brandell harvester. Filters were washed three times with ice cold buffer and placed in scintillation vials. Emulsifier Safe (3.5 mL) was added, and after 2 h, radioactivity was counted in an LKB rack β scintillation counter.

Adenosine A_{2A} Receptor. Membranes containing 40 mg of protein were incubated in a total volume of 400 mL of 50 mM Tris/HCl (pH 7.4) and [^{3}H]ZM241385 (final concentration, 2.0 nM) for 2 h at 25 °C in a shaking water bath. Nonspecific binding was determined in the presence of 100 μM CPA. The incubation was terminated by filtration over Whatman GF/B filters under reduced pressure with a Brandell harvester. Filters were washed four times with ice cold buffer and placed in scintillation vials. Emulsifier Safe (3.5 mL) was added, and after 2 h, radioactivity was counted in an LKB rack β scintillation counter.

Adenosine A_3 Receptor. Membranes containing 20−40 mg of protein were incubated in a total volume of 100 mL of 50 mM Tris/HCl, 10 mM $MgCl_2$, 1 mM EDTA, 0.01% CHAPS (pH 7.4), and [^{125}I]AB-MECA (final concentration, 0.10 nM) for 1 h at 37 °C in a shaking water bath. Nonspecific binding was determined in the presence of 100 μM R-PIA. The incubation was terminated by filtration over Whatman GF/B filters under reduced pressure with a Brandell harvester. Filters were washed three times with ice cold buffer and placed in vials. Radioactivity was counted by a γ counter.

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