Brief Articles

New Pyrrolo[2,1-*f*]purine-2,4-dione and Imidazo[2,1-*f*]purine-2,4-dione Derivatives as Potent and Selective Human A_3 Adenosine Receptor Antagonists

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Compounds presenting an additional fused ring on the xanthine nucleus have been reported to exhibit antagonistic activity with various levels of affinity and selectivity toward the four adenosine receptors subtypes A_1, A_{2A}, A_{2B}, and A_3. This paper reports synthesis and biological evaluation of new 1-benzyl-3-propyl-1*H*,6*H*-pyrrolo[2,1-*f*]purine-2,4-diones and 1-benzyl-3-propyl-1*H*,8*H*-imidazo[2,1-*f*]purine-2,4-diones, among which we identified potent and selective A_3 adenosine receptors antagonists. In particular, 1-benzyl-7-methyl-3-propyl-1*H*,8*H*-imidazo-[2,1-*f*]purine-2,4-dione (**11e**) shows a K_i (hA_3) value from binding assay of 0.8 nM.

Introduction

Adenosine exerts a number of physiological functions through the activation of cell membrane G-protein coupled receptors classified into four different subtypes named A_1, A_{2A}, A_{2B}, and A_3.[1] The A_3 adenosine receptor is able to cause inhibition of forskolin-induced cAMP accumulation, to increase phosphatidylinositol-specific phospholipase C and D activity, and to elevate IP_3 levels and intracellular Ca^{2+} pools.[2] As a therapeutic target, it is the subject of intensive pharmacological characterization due to its significant involvement in several pathophysiological processes, such as inflammation, neurodegeneration,[3,4] cardiac and brain ischaemic damage,[5,6] asthma,[7] and cancer.[8]

A_3 receptor agonists appear to exert dual and opposite effects, either cytoprotective or cytotoxic, depending on the cell type and on the level of receptor activation.[9,10] A_3 receptors and their ability to regulate cell survival represent a promising therapeutic target in diseases in which excessive cell death is either undesirable, such as neurodegeneration, or desirable, such as cancer and inflammation.[11,12] Adenosine acts as a potent regulator of both normal and tumor cell growth.[13,14] Evidence of high levels of expression of A_3 adenosine receptor subtype has been provided in Jurkat cells,[15] a human leukemia cell line originating from the immune system, in the human melanoma A375 cell line,[16] and in human pancreatic, breast, prostate, colon, lung, and ovarian carcinoma cells.[17] A_3 antagonists seem to synergistically enhance cytotoxic treatment and counter P-glycoprotein efflux in multidrug resistance.[17] Furthermore, A_3 receptor antagonists may be useful in the treatment of glaucoma.[18]

In the past few years, different classes of compounds with nonxanthine structures have been reported to be A_3 adenosine receptor antagonists.[19−21] In a recent work, the approach based on the annelation of xanthine derivatives for the development of adenosine receptors antagonists has been extensively considered.[22] In particular, 1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-diones[23] and imidazo[2,1-*i*]purin-5-ones[24] have been claimed as potent A_3 adenosine receptor antagonists. Recently, we reported a series of 1,3-dipropyl-7-aryl/heteroaryl-1*H*,6*H*-pyrrolo[2,1-*f*]purine-2,4-dione derivatives which were conceived as rigid analogues of KF17837, a known A_{2A} adenosine receptor antagonist belonging to the class of styryl xanthines.[25] Unfortunately, the synthesized compounds did not show significant affinity for the investigated targets.

The report by Priego et al.[23] about the mentioned 1*H*,3*H*-pyrido[2,1-*f*]purine-2,4-diones highlighted the importance of a benzyl and a propyl moieties at the 1 and 3 positions, respectively. In light of this we thought that the lack of activity of our reported 1,3-dipropyl-pyrrolo[2,1-*f*]purine-2,4-dione derivatives might be partially due to the presence of a propyl chain, instead of the benzyl moiety at the 1 position. We therefore evaluated the effect of the introduction of a benzyl and a propyl at the 1 and 3 position, respectively, in our previous series and in a new series of fused xanthine derivatives. In particular, we performed the synthesis of 1-benzyl-3-propyl-7-aryl/alkyl-1*H*,6*H*-pyrrolo[2,1-*f*]purine-2,4-dione (**7a**−**d**, Table 1) and 1-benzyl-3-propyl-7-aryl/alkyl-1*H*,8*H*-imidazo[2,1-*f*]purine-2,4-dione (**11a**−**n**, Table 1). We report the synthesis of these new tricyclic structures and the evaluation of their affinity and activity for the human adenosine A_1, A_{2A}, A_{2B}, and

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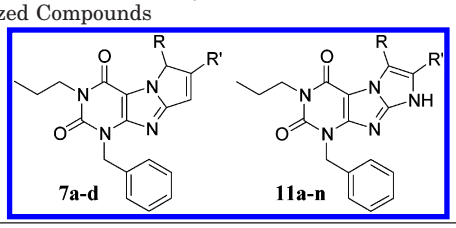
Table 1. Structures and Physiochemical Parameters of the Synthesized Compounds



compd	R	R′	mp (°C)	MW	formula	anal.
7a	H	Ph	235	398.46	$C_{24}H_{22}N_4O_2$	C, H, N
7b	H	CH_3	180	336.39	$C_{19}H_{20}N_4O_2$	C, H, N
7c	H	CH_2CH_3	148	350.41	$C_{20}H_{22}N_4O_2$	C, H, N
7d	CH_3	CH_3	114−115	350.41	$C_{20}H_{22}N_4O_2$	C, H, N
11a	H	Ph	255	399.45	$C_{23}H_{21}N_5O_2$	C, H, N
11b	H	4-OCH_3-Ph	257	429.47	$C_{24}H_{23}N_5O_3$	C, H, N
11c	H	4-Ph-Ph	272	475.54	$C_{29}H_{25}N_5O_2$	C, H, N
11d	H	4-F-Ph	250	417.44	$C_{23}H_{20}FN_5O_2$	C, H, N
11e	H	CH_3	303	337.38	$C_{18}H_{19}N_5O_2$	C, H, N
11f	H	CH_2CH_3	285	351.17	$C_{19}H_{21}N_5O_2$	C, H, N
11g	H	$CH(CH_3)_2$	128−130	365.43	$C_{20}H_{23}N_5O_2$	C, H, N
11h	H	$C(CH_3)_3$	230	379.46	$C_{21}H_{25}N_5O_2$	C, H, N
11i	H	cyclopropyl	244−245	363.41	$C_{20}H_{21}N_5O_2$	C, H, N
11l	H	cyclohexyl	130−132	405.49	$C_{23}H_{27}N_5O_2$	C, H, N
11m	CH_3	CH_3	259	351.17	$C_{19}H_{21}N_5O_2$	C, H, N
11n	CH_3	CH_2CH_3	239	365.19	$C_{20}H_{23}N_5O_2$	C, H, N

A_3 receptors through radioligand binding assays and cAMP assays.

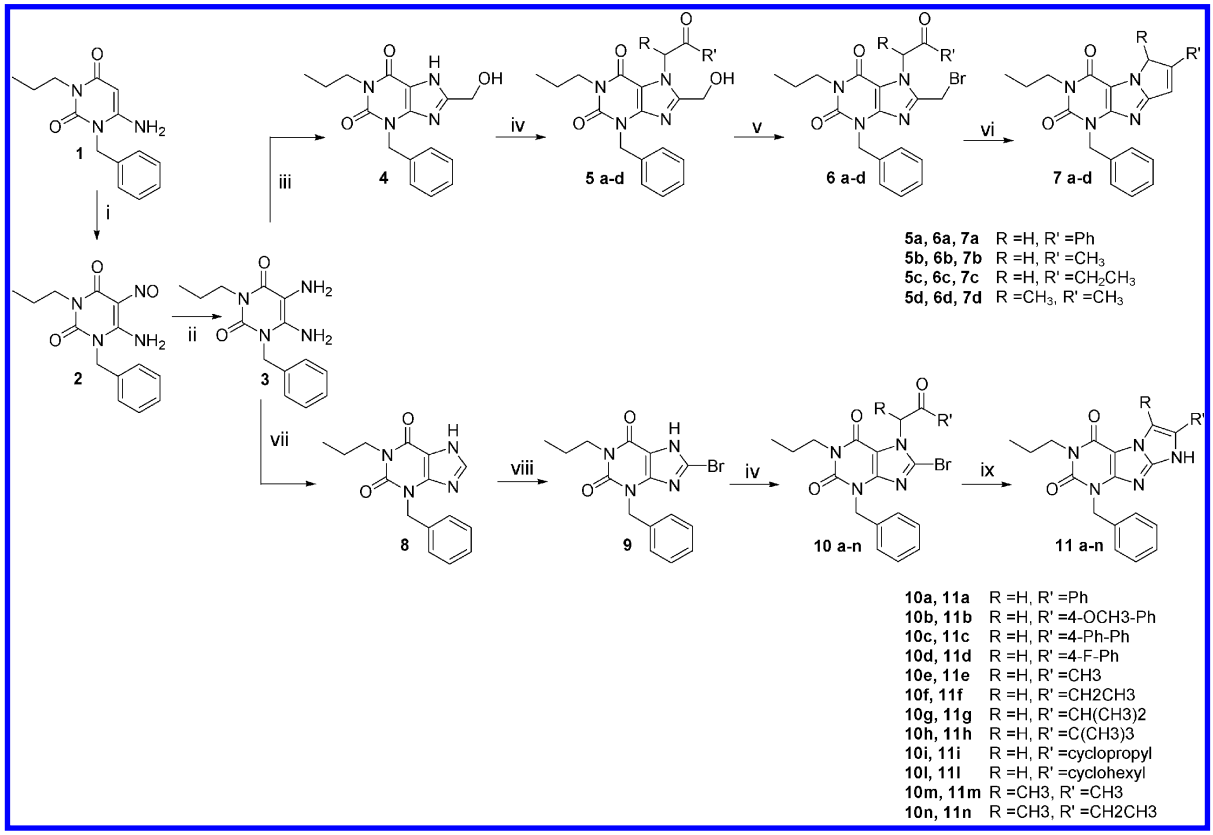
Results and Discussion

Chemistry. 1-Benzyl-3-propyl-1*H*,6*H*-pyrrolo[2,1-*f*]-purine-2,4-dione derivatives (**7a**−**d**) and 1-benzyl-3-propyl-imidazo[2,1-*f*]purine-2,4-dione derivatives (**11a**−**n**) were prepared following the general synthetic strategy depicted in Scheme 1. The 6-amino-1-benzyl-3-propyl-uracil **1** was synthesized starting from 1-benzyl-6-aminouracil according to a known procedure for the alkylation at the N^3 position via protection of the amino group at the 6-position as *N*-[(dimethylamino)methylene] derivative.[26] Subsequent nitrosation at the 5-position in acetic acid with $NaNO_2$ furnished compound **2**, and then the reduction of the nitroso group with sodium dithionite[27] gave 5,6-diamino-1-benzyl-3-propyl-uracil **3** in good yield.

The synthesis of the final 1-benzyl-3-propyl-1*H*,6*H*-pyrrolo[2,1-*f*]purine-2,4-dione derivatives **7a**−**d** required the conversion of intermediate **3** into the 3-benzyl-8-hydroxymethyl-1-propyl-3,7-dihydro-purine-2,6-dione **4** by a two-step reaction. Refluxing derivative **3** with glycolic acid, followed by cyclization of the resulting amide intermediate by heating in a solution of aqueous NaOH, afforded the desired product **4**.[25] Alkylation at the N^7-position with the appropriate α-halo-ketone using K_2CO_3 in DMF as solvent provided the 3-benzyl-8-hydroxymethyl-7-(2-oxo-alkyl)-1-propyl-3,7-dihydro-purine-2,6-dione derivatives **5a**−**d** in good yield. The obtained 7-(2-oxo-alkyl)-8-hydroxymethyl derivatives were converted into the corresponding 8-bromomethyl-purine-2,6-dione intermediates **6a**−**d** via treatment with PBr_3 in anhydrous benzene.

To obtain the cyclization which furnished the pyrrole ring condensed at the N^7−C^8 link of the purinone nucleus, we employed a strategy involving an intramo-

Scheme 1[a]



[a] Reagents: (i) $NaNO_2$, CH_3COOH, EtOH, 40 °C, 30 min; (ii) $Na_2S_2O_4$, H_2O, 85 °C, 30 min; (iii) (a) $HOCH_2CO_2H$, dioxane, 100 °C, 1 h; (b) NaOH, EtOH/H_2O, reflux, 3 h; (iv) α-halo-ketones, K_2CO_3, DMF, rt, 6−10 h; (v) PBr_3, benzene, rt, 4−6 h; (vi) (a) PPh_3, benzene, reflux, 5 h; (b) CH_3ONa, CH_3OH, 0 °C, 10′; (vii) (a) HCO_2H, reflux, 1 h; (b) NaOH, EtOH/H_2O, reflux, 1 h; (viii) Br_2, CH_3CO_2H, CH_3CO_2Na, 45 °C, 1 h; (ix) liquid ammonia, EtOH, 120 °C, ON.

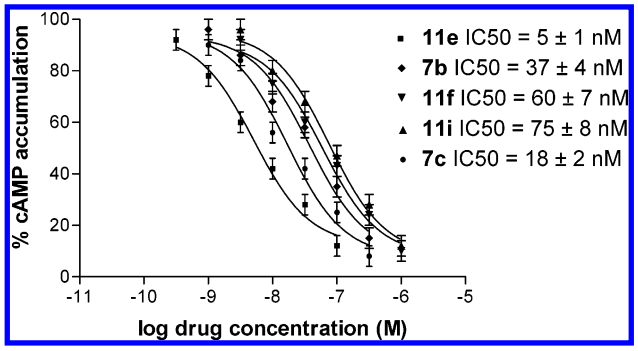


Figure 1. Inhibitory curves of cAMP accumulation in human A_3 adenosine receptors by adenosine antagonists blocking the effect of 100 nM Cl-IB-MECA.

lecular Wittig reaction between the carbonyl moiety of the introduced N^7-chain and the bromomethyl function at the 8-position. Thus, we treated the 8-bromomethyl derivatives **6a**−**d** with triphenylphosphine in benzene, heating the mixture at reflux for 5 h to allow the formation of the intermediate phosphonium salts. The crude material was easily cyclized into the corresponding pyrrolo[2,1-*f*]purine-2,4-diones **7a**−**d** by treatment with sodium methoxide.[25]

The 3-benzyl-1-propyl-3,7-dihydro-purine-2,6-dione **8** was obtained by reacting the diamino derivative **3** with formic acid[28] according to the same procedure followed for preparation of compound **4**. Bromination at the 8-position with Br_2 and sodium acetate in acetic acid at 60 °C for about 1 h led to formation of the key 8-bromo-intermediate **9** in excellent yield. Alkylation at the N^7-position with different α-halo-ketones under the same conditions employed for the synthesis of **5a**−**d** supplied 7-(2-oxo-alkyl)-1-propyl-3,7-dihydro-purine-2,6-dione derivatives **10a**−**n**. Treatment of these intermediates with liquid ammonia in a sealed tube at 120 °C overnight in ethanol effected, at first, the substitution of the bromine at the 8-position followed by the in situ cyclization of the amino group with the N^7 carbonyl function to give the desired 1-benzyl-3-propyl-1*H*,8*H*-imidazo[2,1-*f*]purine-2,4-dione derivatives **11a**−**n**.

Biological Evaluation and Structure-Affinity Relationships. All the synthesized compounds were evaluated in radioligand binding assays to determine their affinities for human A_1, A_{2A}, and A_3 adenosine receptors. Potency of the compounds versus hA_{2B} adenosine receptors were studied, evaluating their capability to inhibit (100 nM) NECA-stimulated cAMP production. Basal and NECA stimulation of cAMP levels were 15 ± 2 and 80 ± 9 pmoles cAMP/10^6 cells, respectively. NECA was able to stimulate cAMP levels in hA_{2B}CHO cells with an EC_{50} value of 145 ± 15 nM. Moreover, the compounds showing high affinity to hA_3 receptors were also studied through cAMP experiments performed in hA_3CHO cells evaluating their capability to block, in the presence of forskolin 10 μM, the inhibitory effect mediated by (100 nM)-Cl-IB-MECA (Figure 1). Basal, forskolin stimulation, and Cl-IB-MECA inhibition of cAMP levels were 14 ± 2, 75 ± 8, and 40 ± 5 pmoles cAMP/10^6 cells, respectively. Cl-IB-MECA was able to inhibit forskolin stimulated cAMP levels with an IC_{50} value of 8.7 ± 0.9 nM. Affinity data for A_1, A_{2A} and A_3 receptors, expressed as K_i values, and IC_{50} values derived from the cAMP assay carried out for hA_{2B} subtypes, are listed in Table 2.

In the reported series of compounds we evaluated the effect of different heterocycles fused on the N_7−C_8 positions of the xanthine nucleus. The fundamental feature of these molecules lies in their practically complete selectivity in binding A_3 receptor versus A_1, A_{2A} and A_3 subtypes, as reflected by the notable K_i (hA_1-hA_{2A}/hA_3) and IC_{50} (hA_{2B})/K_i (hA_3) ratios (Table 2). The K_i values related to the interaction with the adenosine A_3 receptor are strictly dependent on the nature of the substituents at the 7-position of the tricyclic structures while the ability to discriminate between the different AR subtypes is not generally affected by such structural modification. The synthesized compounds include both 7-(4-substituted-aryl)-pyrrolo/imidazo[2,1-*f*]purine-2,4-dione and 7-(cyclo)alkyl-pyrrolo/imidazo[2,1-*f*]purine-2,4-dione derivatives.

Among the examined tricycles, the imidazo[2,1-*f*]-purine-2,4-dione derivatives **11a**, **11e**, **11f**, and **11m** were 2- to 10-fold more active than the corresponding substituted-pyrrolo[2,1-*f*]purine-2,4-dione derivatives **7a**−**d** toward the adenosine A_3 receptor subtype. Both series had K_i values in the low nanomolar range (K_i = 0.8−200 nM). This indicates a possible involvement of

Table 2. Binding and Functional Parameters of Synthesized 1*H*,6*H*-Pyrrolo[2,1-*f*]purine-2,4-dione Derivatives (**7a**−**d**) and Imidazo[2,1-*f*]purine-2,4-dione Derivatives (**11a**−**n**) Toward hA_1, hA_{2A}, hA_{2B}, and hA_3 Adenosine Receptors

compd	hA_1[a]	hA_{2A}[b]	hA_{2B}[c]	hA_3[d]	hA_1/hA_3	hA_{2A}/hA_3	hA_{2B}/hA_3
7a	>1000	>1000	−	200 (134−297)	>5	>5	−
7b	>1000	>1000	400 (323−496)	8.0 (7.1−9.1)	>125	>125	50
7c	>1000	>1000	>1000	3.5 (2.7−4.4)	>290	>290	>290
7d	>1000	>1000	>1000	80 (63−100)	>13	>13	>13
11a	>1000	>1000	−	115 (89−150)	>9	>9	−
11b	>1000	>1000	−	55 (28−104)	>18	>18	−
11c	>1000	>1000	−	>1000	−	−	−
11d	>1000	>1000	−	22 (19−26)	>45	>45	−
11e	>1000	>1000	>1000	0.8 (0.6−0.9)	>1250	>1250	>1250
11f	>1000	>1000	>1000	15 (9−27)	>67	>67	>67
11g	460 (424−498)	>1000	>1000	31 (25−38)	>15	>32	>32
11h	>1000	>1000	>1000	99 (77−129)	>10	>10	>10
11i	350 (299−411)	>1000	>1000	23 (18−29)	15	>44	>44
11l	>1000	>1000	>1000	555 (467−660)	>2	>2	>2
11m	>1000	>1000	>1000	36 (31−43)	>28	>28	>28
11n	>1000	>1000	>1000	60 (53−69)	>17	>17	>17

[a] Displacement of specific [^{3}H]-DPCPX binding to human A_1 receptors expressed in CHO cells (K_i, nM). [b] Displacement of specific [^{3}H]-ZM 241385 binding to human A_{2A} receptors expressed in CHO cells (K_i, nM). [c] cAMP assay in CHO cells expressing hA_{2B} receptors (IC_{50}, nM). [d] Displacement of specific [^{3}H]-MRE3008F20 binding to human A_3 receptors expressed in CHO cells (K_i, nM).

the N^8-position in the interaction of the molecules with the receptor, suggesting an opportunity to establish a hydrogen bond.

Among the 7-aryl-substituted series, it was observed that substitution at the 4-position of the phenyl ring with a methoxy function or especially with the small electron-withdrawing fluorine atom, which is also able to form hydrogen bonds, produces an increase in affinity, while the introduction of a *p*-phenyl group leads to the total loss of affinity. This indicates that the presence of a large aromatic and lipophilic moiety, such as the biphenyl, at the 7-position of the corresponding tricyclic derivative establishes repulsive interactions with the receptor.

We then decided to evaluate the effect of replacing the phenyl ring at the 7-position of compounds **7a** and **11a**−**d** with various (cyclo)alkyl chains. Compounds **7b**−**d** and **11e**−**l** contain at the 7-position alkyl chains with different length such as -methyl (**7b** and **11e**), -ethyl (**7c** and **11f**), branched alkyl chains such as -isobutyl (**11g**), -*tert*-butyl (**11h**), and cycloalkyl chains such as -cyclopropyl (**11i**) and -cyclohexyl (**11*l***). The best results were obtained with the introduction of small linear alkyl chains, in particular a methyl group (**11e**, $K_i(hA_3) = 0.8$ nM with a surprising selectivity pattern versus the other AR subtypes). Longer chains or branching led to a loss of activity (**11g** $K_i(hA_3) = 99$ nM and **11*l*** $K_i(hA_3) = 555$ nM), supporting the observation with the 7-biphenyl derivative (**11c**), which indicates that a sterically demanding, lipophilic moiety at the 7-position would be detrimental to binding. The synthesis of compounds **7d** and **11m**,**n** permitted us to estimate the effect of the introduction of an additional methyl group at the 6-position of the tricyclic derivatives. In all the examples, this kind of structural modification decreased the affinity of the molecules for the receptor binding site, inducing a significant increase of the related $K_i(hA_3)$ values (**7d** 10-fold less active than **7b**, **11m** 45-fold less active than **11e**, **11n** 4-fold less active than **11f**). However, modification of this side of the molecule did not seem to affect the selectivity versus A_1, A_{2A}, and A_{2B} receptors.

Conclusions

In conclusion the present study can be considered an innovative contribution to the previously reported[22] approach based on annelation of xanthine derivatives. Some of the newly reported imidazo[2,1-*f*]purine-2,4-dione and pyrrolo[2,1-*f*]purine-2,4-dione derivatives represent, to the best of our knowledge, the most potent and selective hA_3 adenosine receptor antagonists containing a xanthine nucleus. In particular 1-benzyl-7-methyl-3-propyl-1*H*,8*H*-imidazo[2,1-*f*]purine-2,4-dione (**11e**) shows a subnanomolar affinity toward the desired receptor target with a noteworthy selectivity versus the other adenosine receptors subtypes ($K_i(hA_3) = 0.8$ nM, $K_i(hA_1/hA_3) = 3163$, $K_i(hA_{2A}/hA_3) > 6250$, $IC_{50}(hA_{2B})/K_i(hA_3) = 2570$). These data are even more surprising when compared with the binding profile of MRE3008F20,[29] a potent A_3 adenosine receptor antagonists belonging to the family of pyrazolo[4,3-*e*]-1,2,4-triazolo[1,5-*c*]pyrimidines (K_i (hA_3) = 0.85 nM, $K_i(hA_1/hA_3) = 1294$, $K_i(hA_{2A}/hA_3) = 165$, $K_i(hA_{2B}/hA_3) = 2471$). From the selectivity pattern, it is apparent that compound **11e** represents a significant improvement over MRE3008F20, in particular with regard to the significant increase of selectivity toward adenosine A_{2A} subtype.

Interestingly, a notable concordance between binding and functional experiments performed with the hA_3 receptor has been revealed. Among the examined compounds, the molecules showing the best affinities for the hA_3 adenosine receptor have also proved to have very high potency in functional assays (Figure 1). In particular, derivative **11e** can be considered the most potent compound, exhibiting an IC_{50} value of 5 nM.

Experimental Section

General Procedure for Preparation of 1-Benzyl-3-propyl-1*H*,6*H*-pyrrolo[2,1-*f*]purine-2,4-dione Derivatives (7a−d, Intramolecular Wittig Reaction). A solution of the corresponding bromide **6a**−**d** (0.42 mmol) and PPh_3 (0.46 mmol) in anhydrous benzene (5 mL) was refluxed for 5 h. After this time, the resulting mixture was concentrated to half-volume and the precipitates collected by filtration. The intermediate phosphonium salts (0.26 mmol) were then added to an ice-cooled and stirred solution of sodium methoxide (0.29 mmol) in anhydrous methanol (5 mL). The reaction was stirred at 0 °C for 10 min, the solvent was evaporated, and the products were purified by column chromatography on silica gel eluting with the appropriate mixture of light petroleum−EtOAc (6:4 for **7a**, 1:1 for **7b**−**d**).

General Procedure for Preparation of 1-Benzyl-3-propyl-1*H*,8*H*-imidazo[2,1-*f*]purine-2,4-dione Derivatives (11a−n). A solution of the appropriate 7-(2-oxo-alkyl)-3,7-dihydro-purine-2,6-dione derivatives **10a**−**n** (0.4 mmol) in EtOH (4 mL) was cooled at −40 °C. Liquid ammonia (3−4 mL) was then added to the mixture. The mixture was heated in a sealed tube overnight at 100−120 °C. The reaction was finally allowed to cool at room temperature, and then the solvent and the excess of ammonia were evaporated to obtain a residue that was suspended with water and extracted with EtOAc (3 × 25 mL). The organic phase was dried with anhydrous sodium sulfate, and the solvent was evaporated to give a residue, which was purified by column chromatography on silica gel, eluting with the appropriate mixture of light petroleum−EtOAc.

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Supporting Information Available: Detailed experimental procedures for the synthesis and the biological assays of the reported compounds, C, H, N analytical data, ^1H NMR data. This material is available free of charge via Internet at http://pubs.acs.org.

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