Synthesis and Biological Evaluation of New Imidazole, Pyrimidine, and Purine Derivatives and Analogs as Inhibitors of Xanthine Oxidase

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Several derivatives of 4,5-disubstituted imidazole, 2,4,5-trisubstituted pyrimidine, 2-substituted purine, thiazolo[3,2-*a*]purine, [1,3]thiazino[3,2-*a*]purine, thiazolo[2,3-*i*]purine, [1,3]thiazino-[2,3-*i*]purine, and 6-substituted pyrazolo[3,4-*d*]pyrimidine were synthesized and tested as inhibitors of the xanthine oxidase enzyme. Of those, some 4-(acylamino)-5-carbamoylimidazoles and 2-thioalkyl-substituted purines exhibited very good inhibitory activity, being at least 500 times more effective than allopurinol. The ineffectiveness of 6-*n*-alkylpyrazolo[3,4-*d*]pyrimidines is imputable to the alkyl chain which could hinder the coordination with molybdenum according to the known mechanism for the binding of the inhibitor allopurinol; the effectiveness of imidazole derivatives, by contrast with the ineffectiveness of 4,5-diamino-2-(thioalkyl)-6-hydroxypyrimidines, indicates the relative importance of the five-membered ring in the interaction with the enzyme. Moreover, the marked effectiveness of the angularly-cyclized [1,3]thiazino[2,3-*i*]purinones, which constitute an interesting new class of inhibitors, together with the weak activity of linearly-cyclized derivatives, allowed us to characterize more precisely the lipophilic region of the enzyme facing the N(1)−C(2) positions of the substrate hypoxanthine.

Introduction

The enzyme xanthine oxidase (XO) catalyzes the hydroxylation of hypoxanthine at position 2 and of xanthine at position 8, in the presence of molecular oxygen as electron acceptor, to yield uric acid and superoxide anions.[1,2] XO-derived superoxide anions have been linked to postischemic tissue injury and edema[3] as well as to changes in vascular permeability.[4] XO can also oxidize synthetic purine drugs, thus neutralizing their biological activity. For example, antileukemic 6-mercaptopurine is converted into inactive 6-thiouric acid.[5] The inhibition of this enzyme is therefore useful in the treatment of several diseases, such as gout, and in ischemia-reperfusion processes, in that it prevents damage caused by free radicals.

Some potent inhibitors of this enzyme, such as allopurinol, have been known for a long time. Allopurinol is transformed by the enzyme into alloxanthine, 6-hydroxy-1*H*-pyrazolo[3,4-*d*]pyrimidin-4-one, which forms a very stable complex with the reduced enzyme;[6] however, given its side effects and its inability to prevent the enzyme-forming oxygen free radicals,[7] the search for new inhibitors is a matter of some urgency.

In 1985, a comprehensive study of XO inhibitors with a purine-like heterocyclic ring system was reported.[6] In that work, a detailed mechanism of XO oxidation of hypoxanthine and xanthine was proposed. Two types of binding of the two substrates, called type-II binding and type-I binding, respectively, were described; accordingly, the inhibitors were classified on the basis of the type of binding they can perform. Examination of the structures of the most active compounds led to some general observations: The heterocyclic structures always consist of two fused rings with five and six nuclear atoms; they contain from three to five nitrogen atoms; an oxygen or sulfur atom is bound to the carbon corresponding to C(6) of purines; one or two couples of heteroatoms are often present in positions corresponding to C(6)═O, N(7), and N(3) or N(9) of hypoxanthine; these couples of heteroatoms could be able to coordinate molybdenum; unsubstituted compounds may improve their activity when a lipophilic group, e.g., a phenyl, is introduced. There was a 100-fold reduction in the activity of a few compounds with two lipophilic groups with respect to the unsubstituted ones. The positions which more frequently bore the lipophilic group may correspond to the 2, 8, and 9 positions of hypoxanthine.

Some information about the size of the lipophilic region in the active site of the enzyme can be deduced. The type-II region should be larger than the type-I region as only *lin*-naphthohypoxanthine can be oxidized by the enzyme, while *lin*-naphthoxanthine is not metabolized.[8] Recently, in an attempt to define the role of a lipophilic substituent, on the basis of these observations, we reported the positive effect of a linear alkyl chain of suitable length on the binding with the enzyme.[9] This effect was recorded on both 2- and 8-*n*-alkylhypoxanthines. Since 2-*n*-pentylhypoxanthine was a feeble inhibitor and 8-*n*-pentylhypoxanthine a substrate, the observation of the negative interaction of 2,8-di-*n*-pentylhypoxanthine prompted us to hypothesize that there is only one narrow lipophilic pocket, capable of accepting an alkyl chain, in the XO active site.

In our earlier studies, we observed that the *n*-alkyl chain enhances its positive effect when a bridge group, containing an oxygen atom, binds the chain to the heterocyclic system. The effectiveness of some 4-amino-5-carbamoyl-1*H*-1,2,3-triazoles, bearing a linear acyl substituent on N(4), showed that the five−six hetero-

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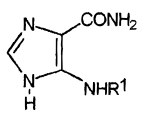
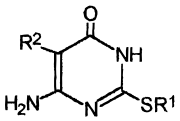
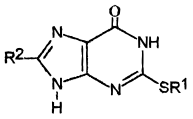
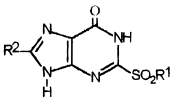
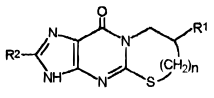
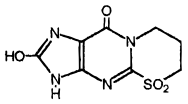
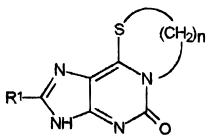
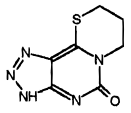
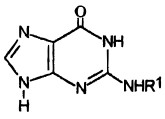
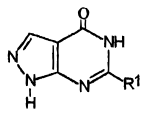
Table 1. Synthesized and Tested Compounds

	Comp.	R^1	R^2	n
 **1a - d**	**1a**	$CO(CH_2)_4CH_3$		
	1b	$COCOO(CH_2)_3CH_3$		
	1c	$COCOO(CH_2)_4CH_3$		
	1d	$COCOO(CH_2)_5CH_3$		
 **2a - f**	**2a**	$(CH_2)_3CH_3$	H	
	2b	$(CH_2)_4CH_3$	H	
	2c	$(CH_2)_5CH_3$	H	
	2d	$(CH_2)_3CH_3$	NHCHO	
	2e	$(CH_2)_4CH_3$	NHCHO	
	2f	$(CH_2)_5CH_3$	NHCHO	
 **3a - i**	**3a**	$(CH_2)_3CH_3$	H	
	3b	$(CH_2)_4CH_3$	H	
	3c	$(CH_2)_5CH_3$	H	
	3d[15]	$CH_2C_6H_5$	OH	
	3f	$(CH_2)_5CH_3$	OH	
	3g	H	C_6H_5	
	3h	CH_3	C_6H_5	
	3i	$(CH_2)_5CH_3$	C_6H_5	
	**3e**	$CH_2C_6H_5$	OH	
	4a[16]	H	SH	0
	4b[16]	H	SH	1
	4c[16]	CH_3	SH	1
 **4a - h**	**4d**[16]	H	OH	0
	4e[16]	H	OH	1
	4f[16]	CH_3	OH	1
	4h	H	C_6H_5	1
	**4g**			
 **5a - e**	**5a**[17]	SH		2
	5b[17]	SH		3
	5c[17]	OH		2
	5d[17]	OH		3
	5e	C_6H_5		3
	**6a**[17]			
 **7a - f**	**7a**	$CO(CH_2)_2CH_3$		
	7b	$CO(CH_2)_3CH_3$		
	7c	$CO(CH_2)_4CH_3$		
	7d	$(CH_2)_3CH_3$		
	7e	$(CH_2)_4CH_3$		
	7f	$(CH_2)_5CH_3$		
 **8a - d**	**8a**	CH_2CH_3		
	8b	$(CH_2)_2CH_3$		
	8c	$(CH_2)_3CH_3$		
	8d	$(CH_2)_4CH_3$		

cyclic system is not essential to ensure binding with the enzyme.[10] Accordingly, we synthesized some imidazoles **1** and some suitable pyrimidines **2** to ascertain the relative importance of the two rings in active purine-like compounds. Further, in an attempt to define the best chemical structure for the bridge between the heterocyclic nucleus and the alkyl chain, we synthesized 2-thioxanthine derivatives **3** and some guanine analogs **7**, bearing an alkyl chain on the exocyclic heteroatom.

We also synthesized several thiazolo[3,2-*a*]purine and [1,3]thiazino[3,2-*a*]purine derivatives **4** and thiazolo[2,3-*i*]purine and [1,3]thiazino[2,3-*i*]purine derivatives **5**, bearing an ethylenic and propylenic bridge between N(1) and S(2) or N(1) and S(6), respectively. The activity of these compounds **4** and **5**, together with that of the 2-linearly-substituted derivatives **3**,[11] allowed us to define the lipophilic wall of the active site facing these positions. Finally, as it was known that the length of a C(2) *n*-alkyl chain could enhance the inhibitory activity of hypoxanthines and 8-azahypoxanthines,[9,10] we prepared some 6-*n*-alkylpyrazolo[3,4-*d*]pyrimidin-4-ones **8** to verify their potential inhibitory properties. The structures of the studied compounds are reported in Table 1.

Chemistry

All the synthesized compounds are listed in Table 1. Compound **1a** was obtained from 4-amino-5-imidazolecarboxamide and hexanoic anhydride, while compounds **1b**−**d** were synthesized from the same starting compound, and the reagent was obtained *in situ* by reacting the appropriate alcohol with oxalyl chloride.[10,12]

Compounds **2a**−**f** and **3a**−**c** were obtained by alkylation of the corresponding mercapto derivatives with the appropriate alkyl bromide in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU);[13] starting material 9-hydro-2-mercapto-1*H*-purin-6-one was prepared according to the literature.[14] Compound **3d** was synthesized according to ref 15 and **3f** by alkylation of 2-thioxo-1,7-dihydro-9*H*-purine-6,8-dione. This was obtained by heating a mixture of 4,5-diamino-6-hydroxy-

Table 2. Physical and Biological Properties of Compounds **1**−**8**

compd	formula	anal.	mp (°C)	yield (%)	MS (M^+)	IC_{50}	K_i
1a	$C_{10}H_{16}N_4O_2$	C,H,N	142−145	55	224	12.6	2.56
1b	$C_{10}H_{14}N_4O_4$	C,H,N	198−200	15	254	4.29	1.06
1c	$C_{11}H_{16}N_4O_4$	C,H,N	213−215	10	268	1.92	1.04
1d	$C_{12}H_{18}N_4O_4$	C,H,N	218−220	10	282	1.11	0.26
2a	$C_8H_{13}N_3OS$	C,H,N	175	75	199	inactive	
2b	$C_9H_{15}N_3OS$	C,H,N	183	75	213	inactive	
2c	$C_{10}H_{17}N_3OS$	C,H,N	195	75	227	inactive	
2d	$C_9H_{14}N_4O_2S$	C,H,N	187	43	242	>100	
2e	$C_{10}H_{16}N_4O_2S$	C,H,N	189	48	256	>100	
2f	$C_{11}H_{18}N_4O_2S$	C,H,N	193	54	270	>100	
3a	$C_9H_{12}N_4OS$	C,H,N	262−264	84	224	3.88	0.176
3b	$C_{10}H_{14}N_4OS$	C,H,N	265−266	85	238	2.85	0.155
3c	$C_{11}H_{16}N_4OS$	C,H,N	278−279	89	252	0.115	0.00 98
3d[15]			>300 (>300[15])	90			4.49
3e	$C_{12}H_{10}N_4O_4S$	C,H,N	>300	67			77.84
3f	$C_{11}H_{16}N_4O_2S$	C,H,N	265−267	59			0.16
3g	$C_{11}H_8N_4OS$	C,H,N	>300	83	244		2.07
3h	$C_{12}H_{10}N_4OS$	C,H,N	>300	73			5.70
3i	$C_{17}H_{20}N_4OS$	C,H,N	>300	22			0.48
4a[16]							24.39
4b[16]							131.0
4c[16]							>100
4d[16]							40.25
4e[16]							66.80
4f[16]							>100
4g	$C_8H_8N_4O_4S$	C,H,N	>300	25		inactive	
4h	$C_{14}H_{12}N_4OS$	C,H,N	>300	88			
							36.52
5a[17]							1.86
5b[17]							0.89
5c[17]							1.26
5d[17]							0.33
5e	$C_{14}H_{12}N_4OS$	C,H,N	>300	58			93
6a[17]							>100
7a	$C_9H_{11}N_5O_2$	C,H,N	>300	23	221	>100	
7b	$C_{10}H_{13}N_5O_2$	C,H,N	>300	30	235	inactive	
7c	$C_{11}H_{15}N_5O_2$	C,H,N	>300	50	249	inactive	
7d[24]	$C_9H_{13}N_5O$	C,H,N	275 (286−288,[24a] 268−270[24b])	15	207	25.5	17.7
7e	$C_{10}H_{15}N_5O$	C,H,N	278	23	221	28.3	25.7
7f[24a]	$C_{11}H_{17}N_5O$	C,H,N	280 (287−289[24a])	28	235	30.5	32.1
8a[25]			>300 (>300[25])	63		>100	
8b	$C_8H_{10}N_4O$	C,H,N	287	36		>100	
8c	$C_9H_{12}N_4O$	C,H,N	266	25		>100	
8d	$C_{10}H_{14}N_4O$	C,H,N	255	43		>100	
allopurinol						4.2	7.0[21]

2-mercaptopyrimidine, urea, and sodium acetate. Oxidation with hydrogen peroxide of **3d** and **4e** gave **3e** and **4g**, respectively. Melting 4,5-diamino-6-hydroxy-2-mercaptopyrimidine and benzamidine afforded 8-phenyl-2-thioxanthine (**3g**), which was converted into **3h**,**i** by alkylation.

Compounds **4h** and **5e** were prepared from 7,8-diamino-3,4-dihydro-2*H*-pyrimido[2,1-*b*][1,3]thiazin-6-one[16] and 8,9-diamino-3,4-dihydro-2*H*,6*H*-pyrimido[6,1-*b*][1,3]thiazin-6-one,[17] respectively, by reaction with benzamidine. Compounds **7a**,**b** were obtained by reaction of guanine and the appropriate anhydride, whereas **7c** was obtained by reaction with hexanoyl chloride. Reduction by lithium aluminum hydride of these compounds gave **7d**−**f**. The remaining compounds, **4a**−**f**,[16] **5a**−**d**, and **6a**,[17] were obtained according to reported procedures. The preparation of compounds **8a**−**d** followed the described method using 3-(acylamino)-4-pyrazolecarbonitriles as intermediates.[18]

Biological Results and Discussion

The biological results are summarized in Table 2. As expected, 4-(acylamino)-5-carbamoylimidazoles **1** exhibited good affinity very similar to that of the corresponding 1*H*-1,2,3-triazoles.[10] The *n*-alkyl chain in both series appeared to play the same role, probably interacting with the same lipophilic site.

It is worth noting that the derivative **1a** (R′ = CO-*n*-alkyl) was less effective than oxalyl monoesters **1b**−**d** (R′ = CO-COO-*n*-alkyl). The imidazole compounds **1b**−**d** and the 1*H*-1,2,3-triazole derivatives previously reported[10] represent one of the first examples of efficient monocyclic inhibitors of XO.[19] As regards the pyrimidine derivatives **2**, the activity remained very weak, in spite of the presence of the promising 2-alkylthio substituent.[11]

The monosubstituted 2-(thioalkyl)purines **3a**−**c** were very active, in the order **3a** < **3b** < **3c**, which follows increasing in the length of the alkyl linear chain. The same effect is found in compounds of general formula **1**. Compound **3c** displayed the lowest K_i value (K_i 0.0098 μM) of all the compounds synthesized and was 700 times more potent than allopurinol. We experimented with the possible oxidation of **3c** into **3f** by xanthine oxidase; the failure of these enzymatic reactions allowed us to exclude type-I binding in the case of compounds **1** and **3** and to possibly ensure type-II binding. Moreover, the isosteric substitution of sulfur (compounds **3a**−**c**) with an NH group (compounds **7d**−

f) caused at least a 10-fold loss of activity. The substituted purine **3d** displayed an affinity (K_i 4.5 μM) comparable to that of allopurinol (K_i 7 μM),[20] whereas the analog with a sulfone function, **3e**, was much less active (K_i 77.8 μM). A type-II binding might similarly be hypothesized with compounds **3a**−**c**; the reduction of activity would be ascribable to the presence of the sulfonic group which could render the nitrogen atom incapable of coordinating molybdenum. This would indicate that complexation with the enzyme was negatively affected by the electron-withdrawing group or, alternatively, that a steric hindrance by the sulfone group was involved. Similarly, in compound **4e**, which was only weakly active, oxidation to sulfone **4g** led to a complete lack of activity.

In agreement with the hypothesis of a single lipophilic pocket, the introduction of a phenyl ring in position 8 of compound **3c** led to the less active compound **3i**, which is in agreement with the results obtained in a previous paper.[9] In fact, 2-*n*-pentylhypoxanthine and 8-*n*-pentylhypoxanthine interacted with the enzyme, whereas 2,8-di-*n*-pentylhypoxanthine was inactive.

Another interesting class of compounds (**4a**−**g**, **5a**−**e**, and **6a**) was structurally characterized by inserting a thioethylene or thiopropylene bridging group on pyrimidine to obtain linearly- or angularly-cyclized derivatives. Of these, compounds **5** were the most active, those with three methylene units being more active than those with two (**5b**,**a** and **5d**,**c**). As in the case of compounds **3**, introducing a substituent in position 8 of **5d** produced a less active compound (**5e**). This confirms the presence of only one lipophilic pocket in this region of the enzyme.

The low affinity of compounds **4**, together with the high affinity of compounds **5**, might signify that the location of the lipophilic wall portion of the enzyme facing the methylene units is such that it cannot receive the linearly-cyclized derivatives. Further, the reported XO oxidation of *lin*-naphthohypoxanthine to nonoxidizable *lin*-naphthoxanthine[8] showed that these molecules can interact with the enzyme only according to type-II binding.[5] These findings led us to conclude that products **5a**−**e** and the less active **4**, which lacks a methyl group, could afford a similar binding. In the case of compounds **5**, steric hindrance may also contribute to prevent type-I binding. The loss of potency in **6a**, in contrast with the activity exerted by **5b**,**d**, is worthy of note and may be chiefly ascribed to the presence of the l*H*-1,2,3-triazole ring.

Finally, compounds **8** proved completely inactive. This result may be in agreement with the proposed mechanism of action of allopurinol, which underwent oxidation in position 6 through a type-II binding to the more active alloxanthine which, in turn, gave a type-III binding.[6] Therefore, it appeared that type-II binding, in the case of compounds **8**, might be the only way of binding with the enzyme. The poor inclination shown by 2-*n*-alkylhypoxanthines to link the enzyme[9] may agree with the biological behavior of compounds **8**, which were unable to establish a type-III binding because of the steric hindrance due to the presence of the linear alkyl chain on C(6).

Conclusions

The imidazole ring proved to be more important than the pyrimidine ring with a view to ensuring good inhibitory activity. The presence of a cyclized or linear alkyl chain of suitable length attached to C(2) improved binding to the enzyme as in compounds **5** and **3**, respectively. The C(2) sulfur atom afforded the best bridge between the heterocyclic system and the *n*-alkyl chain as is evident from comparison between compounds **3** and **7**. The C(8) oxygen atom slightly lowered the inhibitory activity in purine derivatives.

Experimental Section

Chemistry. 4,5-Diamino-6-hydroxy-2-mercaptopyrimidine, 4-amino-6-hydroxy-2-mercaptopyrimidine, 5(4)-aminoimidazole-4(5)-carboxamide hydrochloride, and 2-amino-6-hydroxypurine (guanine) were purchased from Sigma (Sigma-Aldrich s.r.l.). 3-Amino-4-pyrazolecarbonitrile was purchased from Aldrich (Sigma-Aldrich s.r.l.). Melting points were determined on a Büchi 510 apparatus and are uncorrected. IR spectra were recorded in Nujol suspension on a Perkin-Elmer Model 681 spectrophotometer. The ^{1}H-NMR spectra were recorded in the solvent indicated on a Brücker AMX 400 (400 MHz) or a Brücker AC 200 (200 MHz) spectrometer. Chemical shifts are reported in ppm relative to tetramethylsilane as an internal standard. Microanalyses (C, H, N) were carried out on a Carlo Erba elemental analyser (Model 1106) and were within ±0.4% of the theoretical values. TLC was performed on precoated silica gel F_{254} plates (Merck). Flash column chromatographies were performed using Merk Kieselgel 60 (230−400 mesh).

4(5)-Carbamoyl-5(4)-(*n*-pentanoylamino)imidazole (1a). A mixture of 4-amino-5-imidazolecarboxamide hydrochloride (50 mg, 0.31 mmol), hexanoic anhydride (0.366 g, 1.71 mmol), and triethylamine (0.039 g, 0.39 mmol) was heated at 80 °C for 1 h. After cooling, aqueous methanol was added, and the mixture was evaporated to obtain a viscous oil which was treated with petroleum ether to precipitate triethylamine salt. After filtration, further petroleum ether was added to the filtrate to give **1a** as a crystallized solid: ^{1}H NMR [$(CD_3)_2SO$] δ 7.76 (s, 1H, ArH), 6.76 and 6.50 (2br, 2H, NH), 2.97 (t, 2H, OCH_2), 1.67−1.20 (m, 6H, $(CH_2)_3CH_3$), 0.90 (t, 3H, CH_3).

4(5)-Carbamoyl-5(4)-[[(*n*-alkyloxy)oxalyl]amino]imidazoles 1b−d. A solution of oxalyl chloride (0.065g, 0.52 mmol) and the appropriate alcohol (*n*-butanol for **1b**, *n*-pentanol for **1c**, and *n*-hexanol for **1d**; 0.52 mmol) was stirred at 0 °C for 1 h, after which a solution of 5(4)-aminoimidazole-4(5)-carboxamide hydrochloride (100 mg, 0.62 mmol) and triethylamine (0.25 g, 2.48 mmol) in anhydrous DMF (5 mL) was added. The mixture was stirred for 8 h at 0 °C and then concentrated *in vacuo*, diluted with water, and extracted with $CHCl_3$. Evaporation of the organic layer gave a solid which was crystallized from absolute EtOH. **1b**: ^{1}H NMR [$(CD_3)_2SO$] δ 7.37 (m, 3H, ArH + 2NH), 4.20 (t, 2H, OCH_2), 1.61−1.20 (m, 4H, $(CH_2)_2CH_3$), 0.92 (t, 3H, CH_3). **1c**: ^{1}H NMR [$(CD_3)_2SO$] δ 7.38 (s, 1H, ArH), 7.35 (br, 1H, NH), 4.20 (t, 2H, OCH_2), 1.72−1.19 (m, 6H, $(CH_2)_3CH_3$), 0.87 (t, 3H, CH_3). **1d**: ^{1}H NMR [$(CD_3)_2SO$] δ 7.41 (s, 1H, ArH), 7.35 (br, 1H, NH), 4.25 (t, 2H, OCH_2), 1.72−1.19 (m, 8H, $(CH_2)_4CH_3$), 0.88 (t, 3H, CH_3).

2-(Alkylthio)-4-amino-6-hydroxypyrimidines 2a−c.[21] To a solution of 4-amino-6-hydroxy-2-mercaptopyrimidine (1.43 g, 0.01 mol) in the minimum amount of DMF were added the appropriate alkyl bromide (*n*-butyl bromide for **2a**, *n*-pentyl bromide for **2b**, and *n*-hexyl bromide for **2c**; 0.01 mol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.01 mol). The mixture was stirred at room temperature for 2 h. The pure product that precipitated was filtered and washed with petroleum ether. **2a**: ^{1}H NMR [$(CD_3)_2SO$ + $CDCl_3$] δ 6.19 (br, 3H, D_2O exchangeable), 4.92 (s, 1H, ArH), 3.06 (t, 2H, SCH_2), 1.44−1.63 (m, 4H, $(CH_2)_2CH_3$), 0.91 (t, 3H, CH_3). **2b**: ^{1}H NMR [$(CD_3)_2SO$ + $CDCl_3$] δ 6.23 (br, 3H, D_2O exchangeable), 4.92 (s, 1H, ArH), 3.04 (t, 2H, SCH_2), 1.24−1.54 (m, 6H, $(CH_2)_3CH_3$), 0.88 (t, 3H, CH_3). **2c**: ^{1}H NMR [$(CD_3)_2SO$ + $CDCl_3$] δ 6.24 (br, 3H, D_2O exchangeable), 4.95 (s, 1H, ArH), 3.04 (t, 2H, SCH_2), 1.29−1.73 (m, 8H, $(CH_2)_4CH_3$), 0.88 (t, 3H, CH_3).

2-(Alkylthio)-4-amino-5-(formylamino)-6-hydroxypyrimidines 2d−f. To 4-amino-5-(formylamino)-6-hydroxy-2-mercaptopyrimidine, obtained from 4,5-diamino-6-hydroxy-2-mercaptopyrimidine by reflux with formic acid[22] (0.20 g, 1.07 mmol), and dissolved in the minimum amount of DMF, were added the appropriate bromide (butyl bromide for **2d**, propyl bromide for **2e**, and hexyl bromide for **2f**; 1.07 mmol) and DBU (1.07 mmol); the mixture was stirred for 2 days at room temperature. After evaporation *in vacuo*, water was added to precipitate the product which was then flash-chromatographed on silica gel (eluent: $CHCl_3$−MeOH, 92:8). **2d**: ^{1}H NMR [$(CD_3)_2SO$] δ 11.82, 8.75, and 6.00 (3br, 3H, D_2O exchangeable), 8.04 (s, 1H, COH), 3.08 (t, 2H, SCH_2), 1.70−1.19 (m, 4H, $(CH_2)_2CH_3$), 0.91 (t, 3H, CH_3). **2e**: ^{1}H-NMR [$(CD_3)_2SO$] δ 11.82, 8.74, and 6.13 (3br, 3H, D_2O exchangeable), 8.03 (s, 1H, COH), 3.07 (t, 2H, SCH_2), 1.85−1.27 (m, 6H, $(CH_2)_3CH_3$), 0.87 (t, 3H, CH_3). **2f**: ^{1}H-NMR [$(CD_3)_2SO$] δ 11.82, 8.77, and 5.89 (3br, 3H, D_2O exchangeable), 8.05 (s, 1H, COH), 3.08 (t, 2H, SCH_2), 1.79−1.30 (m, 8H, $(CH_2)_4CH_3$), 0.88 (t, 3H, CH_3).

9-Hydro-2-(alkylthio)-1*H*-purin-6-ones 3a−c. To a solution of 2-thio-9-hydro-1*H*-purin-6-one (1.68 g, 0.01 mol) in the minimum amount of DMF were added 0.01 mol of the appropriate alkyl bromide (*n*-butyl bromide for **3a**, *n*-pentyl bromide for **3b**, and *n*-hexyl bromide for **3c**) and 0.01 mol of DBU. The mixture was stirred at room temperature for 2.5 h. The pure product was precipitated by adding a little water to the mixture and then filtered and dried. **3a**: ^{1}H NMR ($CDCl_3$) δ 6.43 and 5.56 (2br, 2H, D_2O exchangeable), 3.17 (t, 2H, SCH_2), 1.30−1.68 (m, 4H, $(CH_2)_2CH_3$), 0.92 (t, 3H, CH_3). **3b**: ^{1}H NMR ($CDCl_3$) δ 6.43 and 5.60 (2br, 2H, D_2O exchangeable), 3.17 (t, 2H, SCH_2), 1.30−1.68 (m, 6H, $(CH_2)_3CH_3$), 0.89 (t, 3H, CH_3). **3c**: ^{1}H NMR ($CDCl_3$) δ 8.97 (br, 1H, D_2O exchangeable), 7.78 (s, 1H, ArH), 3.21 (t, 2H, SCH_2), 1.30−1.73 (m, 8H, $(CH_2)_4CH_3$), 0.88 (t, 3H, CH_3).

7,9-Dihydro-2-thioxo-1*H*-purine-6,8-dione. The mixture, consisting of 4,5-diamino-6-hydroxy-2-mercaptopyrimidine (4.00 g, 25.3 mmol), urea (6.0 g, 0.10 mol), and anhydrous sodium acetate (4.76 g, 58.02 mmol), was heated at 220 °C for 45 min. The product was washed with water and treated with 2% NaOH. The solution was saturated with CO_2 to yield the title compound: yield 3.80 g (78%); mp >300 °C. Anal. ($C_5H_4N_4O_2S$) C, H, N.

7,9-Dihydro-2-[(phenylmethyl)thio]-1*H*-purine-6,8-dione (3d). To a suspension of 7,9-dihydro-2-thioxo-1*H*-purine-6,8-dione (1.00 g, 5.4 mmol) in DMF (10 mL) were added K_2CO_3 (0.10 g) and benzyl chloride (0.62 mL, 5.4 mmol, dropwise). The mixture was stirred at room temperature for 3 days. The insoluble was filtered, and the crude proved to be chromatographically-pure **3d**: ^{1}H NMR [$(CD_3)_2SO$] δ 4.48 (s, 2H, SCH_2), 7.25−7.39 (m, 3H, ArH), 7.45−7.49 (m, 2H, ArH), 10.95, 11.50, and 12.75 (3br, 3H, D_2O exchangeable).

7,9-Dihydro-2-[(phenylmethyl)sulfonyl]-1*H*-purine-6,8-dione (3e). Hydrogen peroxide (35%, 4.00 mL) was added to a suspension of **3d** (0.50 g, 1.8 mmol) in glacial acetic acid (5.00 mL). The reaction mixture was heated under reflux for 40 min. The insoluble product was filtered off to yield **3e**: ^{1}H NMR [$(CD_3)_2SO$] δ 4.95 (s, 2H, SCH_2), 7.20−7.40 (m, 5H, ArH), 11.50 and 12.00 (2d, 2H, D_2O exchangeable), 13.50 (br, 1H, D_2O exchangeable).

7,9-Dihydro-2-(hexylthio)-1*H*-purine-6,8-dione (3f). To a suspension of 7,9-dihydro-2-thioxo-1*H*-purine-6,8-dione (1.00 g, 5.4 mmol) in DMF (10 mL) were added K_2CO_3 (0.10 g) and iodohexane (0.80 mL, 5.4 mmol, dropwise). The reaction mixture was mantained at room temperature for 3 days, and the crude compound was then filtered off: ^{1}H NMR [$(CD_3)_2SO$] δ 0.96 (m, 3H, CH_3), 1.33 (m, 6H, CH_2), 1.60 (m, 2H, CH_2), 3.22 (m, 2H, SCH_2), 10.82, 11.39, and 12.60 (3br, 3H, D_2O exchangeable).

9-Hydro-2-mercapto-8-phenyl-1*H*-purin-6-one (3g). A mixture of 4,5-diamino-6-hydroxy-2-mercaptopyrimidine (1.00 g, 6.32 mmol), benzamidine hydrochloride (4.00 g, 25.54 mmol), and finely powdered anhydrous sodium acetate (1.20 g, 14.63 mmol) was heated at 200−210 °C for 1 h. The solid thus obtained was disaggregated with water to yield **3g** in a practically-pure form: ^{1}H NMR [$(CD_3)_2SO$] δ 7.53−7.90 (m, 3H, ArH, SH), 8.20 (m, 2H, ArH), 11.92 and 12.30 (2br, 2H, D_2O exchangeable).

9-Hydro-2-(methylthio)-8-phenyl-1*H*-purin-6-one (3h). Methyl iodide (0.13 mL, 2.1 mmol) was added to a solution of **3g** (0.50 g, 2.05 mmol) in DMF (10 mL), and the reaction mixture was mantained at room temperature for 2 days and then poured on ice. The solid that precipitated was filtered, washed with cyclohexane, and then crystallized from acetone/petroleum ether: ^{1}H NMR [$(CD_3)_2SO$] δ 2.66 (s, 3H, CH_3), 7.54−7.62 (m, 3H, ArH), 8.19−8.21 (m, 2H, ArH), 12.58 and 13.70 (2br, 2H, D_2O exchangeable).

9-Hydro-2-(hexylthio)-8-phenyl-1*H*-purin-6-one (3i). To a solution of **3g** (0.45 g, 1.84 mmol) in a mixture of $CHCl_3$−water (1:1, v/v, 20 mL) were added 1 N NaOH (1.84 mL), tetrabutylammonium bromide (200 mg), and *n*-iodohexane (0.27 mL, 1.84 mmol). The resulting reaction mixture was stirred vigorously at room temperature for 3 days. The two phases were then separated, and from the organic layer, treated with 1 N HCl, compound **3i** separated out as a precipitate. It was washed with water and proved to be practically pure: ^{1}H NMR [$(CD_3)_2SO$] δ 0.97 (t, 3H, CH_3), 1.39−1.41 (m, 4H, $2CH_2$), 1.51 (m, 2H, CH_2), 1.79 (m, 2H, CH_2), 3.28 (t, 2H, CH_2), 7.56−7.63 (m, 3H, ArH), 8.21−8.23 (m, 2H, ArH), 12.54 (br, 1H, NH).

5,5-Dioxo-1,3,5,6,7,8-hexahydro-5-thio-1,3,4,8a-tetraazacyclopenta[*b*]naphthalene-2,9-dione (4g). Hydrogen peroxide (35%, 2.50 mL) was added to a suspension of 2*H*,10*H*-7,8-dihydro-[1,3]thiazin[3,2-*a*]purine-2,10-dione (**4e**)[16] (0.50 g, 2.2 mmol) in acetic acid. The reaction proceeded as described for **3e**. The insoluble compound proved to be chromatographically-pure **4g**: ^{1}H NMR [$(CD_3)_2SO$] δ 2.59 (m, 2H, CH_2), 3.89 (t, 2H, CH_2), 4.20 (t, 2H, CH_2), 11.51 and 11.91 (2br, 2H, D_2O exchangeable).

2-Phenyl-7,8-dihydro-3*H*,6*H*-5-thia-1,3,4,8a-tetraazacyclopenta[*b*]naphthalen-9-one (4h). A mixture of 7,8-diamino-3,4-dihydro-2*H*-pyrimido[2,1-*b*][1,3]thiazin-6-one[17] (0.50 g, 2.52 mmol), benzamidine hydrochloride (2.50 g, 16.0 mmol), and anhydrous sodium acetate (0.75 g, 9.1 mmol) was heated at 220 °C for 45 min. The product obtained was washed with water and proved to be chromatographically-pure: ^{1}H NMR [$(CD_3)_2SO$] δ 2.28 (m, 2H, CH_2), 3.33 (m, 2H, SCH_2), 4.21 (m, 2H, NCH_2), 7.59−7.63 (m, 3H, ArH), 8.14−8.27 (m, 2H, ArH), 13.48 (br, 1H, NH).

2-Phenyl-7,8-dihydro-3*H*,6*H*-9-thiatetraazacyclopenta-[*a*]naphthalen-5-one (5e). 8,9-Diamino-3,4-dihydro-2*H*,6*H*-pyrimido[6,1-*b*][1,3]thiazin-6-one[16] (0.66 g, 3.3 mmol), benzamidine hydrochloride (2.08 g, 13.3 mmol), and anhydrous sodium acetate (0.62 g, 7.6 mmol) were heated at 220 °C for 45 min. The solid thus obtained was washed with water and then with acetone and purified by flash-chromatography ($CHCl_3$−MeOH−cyclohexane, 4.5:0.5:5): ^{1}H NMR [$(CD_3)_2SO$] δ 2.35 (m, 2H, CH_2), 3.34 (m, 2H, SCH_2), 4.11 (m, 2H, NCH_2), 7.58−7.63 (m, 3H, ArH), 8.15−8.52 (2H, m, ArH), 13.07 (br, 1H, NH).

2-(Butanoylamino)-1*H*-purin-6-one (7a). A suspension of 2-amino-6-hydroxypurine (guanine) (0.5 g, 3.31 mmol) in butyric anhydride (2.7 mL, 16.5 mmol) was heated for 5 h at 120 °C. After cooling, the mixture was diluted with petroleum ether and filtered and the solid washed with hot toluene. Compound **7a** precipitated from the collected filtering and washing liquids and was recrystallized from toluene: ^{1}H NMR [$(CD_3)_2SO$] δ 13.15, 12.09, and 11.42 (3br, 3H, D_2O exchangeable), 7.95 (s, 1H, ArH), 2.46 (t, 2H, $COCH_2$), 1.63 (m, 2H, CH_2-CH_3), 0.91 (t, 3H, CH_3).

2-(Pentanoylamino)-1*H*-purin-6-one (7b). A suspension of guanine (0.5 g, 3.31 mmol) in pentanoic anhydride (3.26 mL, 16.55 mmol) was heated for 5 h at 160 °C. The mixture was treated like **7a**: ^{1}H NMR [$(CD_3)_2SO$] δ 13.57, 12.01, and 11.45 (3br, 3H, D_2O exchangeable), 7.93 (s, 1H, ArH), 2.44 (t, 2H, $COCH_2$), 1.70−1.17 (m, 4H, $(CH_2)_2CH_3$), 0.91 (t, 3H, CH_3).

2-(Hexanoylamino)-1*H*-purin-6-one (7c).[23] A suspension of guanine (0.2 g, 1.32 mmol), anhydrous pyridine (6.6 mL), and hexanoyl chloride (0.55 mL, 3.96 mmol) was heated to 130 °C for 2 h. The mixture was then evaporated *in vacuo* and the residue diluted with 50 mL of ethanol. This mixture was refluxed for 1 h and then filtered to yield a solid which, on

washing with hot ethanol, proved to be pure: ^{1}H NMR [$(CD_3)_2$SO] δ 13.78, 12.31, and 11.45 (3br, 3H, D_2O exchangeable), 7.95 (s, 1H, ArH), 2.44 (t, 2H, $COCH_2$), 1.68−1.17 (m, 6H, $(CH_2)_3CH_3$), 0.92 (t, 3H, CH_3).

2-(Alkylamino)-1*H*-purin-6-ones 7d−f.[24] $LiAlH_4$ (0.5 g) was added to 1.5 mmol of **7a** (or **7b** or **7c**) in 100 mL of anhydrous THF, and the mixture was heated to 80 °C for 48 h. After cooling to 0 °C, the excess of $LiAlH_4$ was destroyed by MeOH, and the mixture was evaporated *in vacuo*. The solid residue was powdered, and 5% NH_3 (75 mL) was added. This mixture was stirred continuously at 60 °C for 1 h and then filtered and the filtrate concentrated to 30 mL. HCl (2 N) was then added to bring the mixture to pH = 8. The solid which slowly precipitated was flash-chromatographed (eluent: $CHCl_3$−MeOH, 92:8) to obtain the pure product. **7d**: ^{1}H NMR [$(CD_3)_2$SO + $CDCl_3$] δ 7.75, 6.40, and 6.03 (3br, 3H, D_2O exchangeable), 7.50 (s, 1H, ArH), 3.25 (m, 2H, $NHCH_2$), 1.50−1.21 (m, 4H, $(CH_2)_2CH_3$), 0.90 (t, 3H, CH_3). **7e**: ^{1}H NMR [$(CD_3)_2$SO] δ 11.32, 10.22, and 6.25 (3br, 3H, D_2O exchangeable), 7.52 (s, 1H, ArH), 3.27 (m, 2H, $NHCH_2$), 1.50−1.23 (m, 6H, $(CH_2)_3CH_3$), 0.88 (t, 3H, CH_3). **7f**: ^{1}H NMR [$(CD_3)_2$SO] δ 11.41, 10.30, and 6.25 (3br, 3H, D_2O exchangeable), 7.55 (s, 1H, ArH), 3.26 (m, 2H, $NHCH_2$), 1.50−1.20 (m, 8H, $(CH_2)_4CH_3$), 0.89 (t, 3H, CH_3).

3-(Acylamino)-4-pyrazolecarbonitriles. These compounds (acyl = propanoyl, butanoyl, pentanoyl, hexanoyl) were prepared by the method of Cheng and Robins.[25] Acyl = propanoyl, mp 220−221 °C; butanoyl, mp 195−196 °C; pentanoyl, mp 180−182 °C; hexanoyl, mp 181−183 °C.

4-Hydroxy-6-alkylpyrazolo[3,4-*d*]pyrimidines 8a,[25]b−d.[26] The appropriate 3-(acylamino)-4-pyrazolecarbonitrile was treated according to a known procedure[25] to obtain products **8**. **8a**: crystallization solvent EtOH; ^{1}H NMR [$(CD_3)_2$SO] δ 7.97 (s, 1H, ArH), 2.63 (q, 2H, CH_2), 1.21 (t, 3H, CH_3). **8b**: crystallization solvent EtOH; ^{1}H NMR [$(CD_3)_2$SO] δ 8.03 (s, 1H, ArH), 2.67 (t, 2H, α-CH_2), 1.79 (m, 2H, CH_2CH_3), 0.98 (t, 3H, CH_3). **8c**: crystallization solvent MeOH; ^{1}H NMR [$(CD_3)_2$SO] δ 7.90 (s, 1H, ArH), 2.49 (t, 2H, α-CH_2), 1.61 (m, 2H, β-CH_2), 1.25 (m, 2H, CH_2CH_3), 0.85 (t, 3H, CH_3). **8d**: crystallization solvent MeOH; ^{1}H NMR [$(CD_3)_2$SO] δ 7.89 (s, 1H, ArH), 2.47 (t, 2H, α-CH_2), 1.62 (m, 2H, β-CH_2), 1.23 (m, 4H, $CH_2CH_2CH_3$), 0.81 (t, 3H, CH_3).

Xanthine Oxidase Inhibitory Assay. Xanthine was puchased from Fluka (Sigma-Aldrich s.r.l.) and XO (from buttermilk, 1.36 U/mg) from Boehringer (Boehringer Mannheim Italia s.p.a.).

XO activity was assayed spectrophotometrically in air-saturated phosphate buffer, pH = 7.6, I = 0.1 at 25 ± 0.2 °C, using a Perkin-Elmer UV/vis λ16 or Beckman DU 50 spectrophotometer with a thermostated cuvette holder. The increase in uric acid (product of enzymatic reaction) concentration was evaluated at 295 nm with xanthine as substrate (8 μM) and XO sufficient to obtain an average reaction rate for the control reaction of 0.100 ± 0.005 absorbance units/min. The inhibitors in question were dissolved in DMSO. DMSO concentration in all the assays was kept at 3% (v/v), which has no effect on XO activity. IC_{50} values (the concentration required to produce 50% inhibition of the enzyme-catalyzed reaction) were determined from least-squares analysis of the linear portion of log dose−inhibition curves. Each curve was generated using at least three concentrations of inhibitor producing an inhibition of between 20% and 80%, with three replicates at each concentration.

Kinetic studies were performed with at least three different concentrations of inhibitor in the presence of variable concentrations of xanthine (2−20 μM). K_i values (the dissociation constants of the enzyme−inhibitor complex) were determined from the slopes in double-reciprocal plots.[27]

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