Design, Synthesis, and Biological Activities of New Thieno[3,2-*d*]pyrimidines as Selective Type 4 Phosphodiesterase Inhibitors[1]

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A common pharmacophore for compounds structurally related to nitraquazone has been derived. Using this pharmacophore, new structures have been designed, synthesized, and evaluated for their inhibitory potencies against cyclic adenosine 5′-monophosphate (cAMP) specific phosphodiesterase (PDE 4). From these compounds, 4-benzylamino-2-butylthieno[3,2-*d*]-pyrimidine (**4**) was selected for optimization. The effects of changes to the lipophilic groups and the amino linkage on the PDE 4 activity have been investigated. As a result, some potent PDE 4 inhibitors, selective with respect to PDE 3, have been identified. A selected group of compounds have been further evaluated for their ability to displace [³H]rolipram from its binding site and also to potentiate isoprenaline-induced cAMP accumulation in isolated guinea pig eosinophils. Of these, 2-butyl-4-cyclohexylaminothieno[3,2-*d*]pyrimidine (**33**) has an interesting profile, with an important improvement in PDE 4/[³H]rolipram ratio with respect to reference drugs, and good activity in cAMP potentiation, consistent with efficient cell penetration.

Introduction

Interest in the potential utility of isoenzyme-selective phosphodiesterase (PDE) inhibitors has increased in recent years. The inhibition of PDE activity increases cellular levels of the key second messengers, cyclic adenosine 5′-monophosphate (cAMP) and cyclic guanosine 5′-monophosphate (cGMP), thereby activating specific protein phosphorylation cascades that elicit a variety of functional responses.[2] At the present time there are seven known PDE isoenzyme families (PDE 1–7) which share the property of hydrolyzing cyclic nucleotides to their 5-monophosphate counterparts.

For a number of reasons, interest in the potential therapeutic utility of selective PDE inhibitors has largely been focused on drugs capable of inhibiting PDE 4, one of the cAMP specific phosphodiesterases. First, the tissue distribution of PDE 4 strongly suggests that pathologies related to the central nervous[3] and immune[4] systems could be treated through the selective inhibition of PDE 4. On the other hand, the increase in intracellular cAMP concentration, the obvious biochemical consequence of PDE 4 inhibition, has been well characterized in immuno-competent cells, where it acts as a deactivating signal.[5,6] Furthermore the pathologies associated with these biological systems represent some of the more important therapeutic targets for the next century such as asthma,[5,7] atopy,[8,9] multiple sclerosis,[3,10] arthritis,[11–13] type-II diabetes,[14–16] and AIDS.[17,18]

From a structural point of view, selective PDE 4 inhibitors in the public domain can be divided into three classes:[2,19–21] structural analogues of rolipram, structural analogues of nitraquazone, and structures related to xanthines (Figure 1). The compound nitraquazone[22] is notable for its antiinflammatory and analgesic pharmacological profile. Figure 2 shows several compounds structurally related to nitraquazone. The analogue CP-77,059 has also shown interesting antiinflammatory
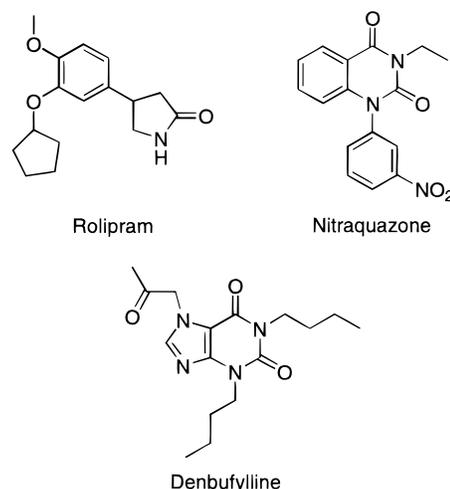

Figure 1. Compounds representative of the three chemical classes of PDE 4 inhibitors: rolipram, nitraquazone, and xanthine derivatives (denbufylline).

properties in the carrageenan-induced rat paw oedema.[23] Additionally, RS-17597[24a] and RS-14491,[24b] related to the potent PDE 4 inhibitor RS-25344,[25] have recently been described as PDE 4 inhibitors with exceptional biological activity in arachidonic acid-induced ear mouse oedema. Finally, the new PDE 4 inhibitor KF-19514[26] is related to the bronchodilator but weak PDE 4 inhibitor KF-17625[27] and KF-18280,[28] which exhibits extremely potent activity in the carrageenan-induced rat paw oedema, zymosan-induced rat paw oedema, and the reverse passive Arthus reaction-induced paw oedema in the rat, and has a glucocorticoid-like antiinflammatory profile.

However, most PDE 4 inhibitors produce nausea and emetic effects which limit their therapeutic potential.[29] [³H]Rolipram binding activity has been correlated with these side effects in a study in dogs.[30] Hence, reducing
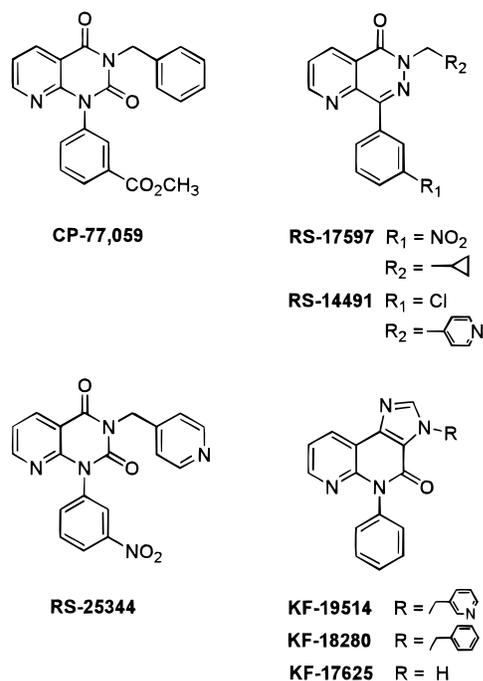
Figure 2. Compounds selected for pharmacophore evaluation and related analogues referenced in the text.

the affinity of a compound for the [³H]rolipram binding site may also reduce emetic potency.

Nitraquazone, despite its marked structural dissimilarity to rolipram, also binds with high affinity to the [³H]rolipram binding site.[31] Other series structurally related to nitraquazone such as nicotinamide ethers,[32] which may be considered as ring-opened derivatives, also had high affinity for the [³H]rolipram binding site. In series related to rolipram, several attempts have been made to reduce the [³H]rolipram binding site affinity while retaining PDE 4 inhibitory potency.[29,33−35] A recent report on a nitraquazone-related chemical series shows a great improvement in the PDE 4 inhibition/ [³H]rolipram binding site ratio.[36]

In the present work, several compounds related to the nitraquazone structure were initially studied with the aim to determine a common pharmacophore. This pharmacophore has been used as starting point to design new structures which may be potent and selective PDE 4 inhibitors but with attenuated affinity for the [³H]rolipram binding site.

Molecular Modeling Studies and Design of New Compounds

3D Pharmacophore Model. Nitraquazone (Figure 1) and its structural analogues CP-77,059, RS-17597, RS-25344, and KF-19154 (Figure 2) were selected for the pharmacophore evaluation. A rigorous systematic conformational search on the selected compounds was performed to determine their lowest energy conformations. Calculations were performed using the full molecular mechanics force field implemented in Chem-X.[37,38] Resulting geometries of the low-energy conformers were optimized by means of semiempirical quantum mechanics calculations using the MOPAC package[39,40] (method AM1).[41] Superposition of optimized structures was performed by manual geometric fitting, selecting the nitraquazone structure as a template. The align-

ment of ligands was based on their aromatic and hydrophobic moieties. Figure 3 shows the superposition of selected structures on the template molecule. The program GRID[42−46] was selected to calculate putative interaction sites of different ligands. The energy contours obtained from the GRID computation indicate favorable positions for interaction with other molecules. Figure 4 shows the interaction pattern of the five molecules studied, derived from the GRID computation using the *NH neutral flat amide probe* which identifies the areas of the molecule able to act as hydrogen bond acceptors.

The common interaction map of nitraquazone and its related compounds using the NH amide probe is depicted in Figure 5a. Three main areas of interaction are clearly identified. The distances between the points of maximal energy of interaction (HACC, Figure 5b) are 9.43, 8.06, and 10.86 Å.

In summary, in nitraquazone-related compounds, three common areas of interaction as hydrogen bond acceptors have been identified by using the program GRID. These areas of interaction, taken together with the aromatic and hydrophobic moieties discussed above, represent the 3D pharmacophore model for PDE 4 inhibitors related to the structure of nitraquazone (Figure 6).[47]

Design of New Compounds. Once the pharmacophore configuration was defined, several condensed heterocyclic systems were evaluated. The model described above requires the existence of a central planar template that comprises a heterocyclic condensed moiety, three hydrogen bond acceptor groups, and two out-of-plane regions which can be occupied by bulky aromatic or lipophilic substituents. Therefore new compounds with structural and electronic profiles compatible with the proposed pharmacophore were initially explored.

These compounds were computationally evaluated. First of all, they were built using fragments and standard geometries in Chem-X. After full conformational analysis, compounds were fitted with the aromatic and lipophilic components in the pharmacophore by using a rigid fitting procedure followed by a flexible fitting routine (FLEXIFIT). In the flexible fit step, the internal nonbonded energy of the molecules under study is minimized with respect to user-defined exocyclic bonds and the penalty functions (restraints to obtain a maximum quality fit). The geometries of resulting conformations were optimized by the AM1 method as implemented in the program MOPAC. These new structures were then studied with the program GRID (NH flat amide probe). On the basis of these considerations and taking into account synthetic feasibility, the compounds **1−8** (depicted in Figure 7) were selected as potential leads. All these compounds possess at least two areas of interaction representative of hydrogen bond acceptor groups (data not shown), whose distances correspond to those depicted in the pharmacophore model, as well as the required three aromatic lipophilic groups.

Compounds **1−8** were, therefore, synthesized and evaluated as PDE 4 inhibitors.

Chemistry

The synthetic strategies used for the preparation of the majority of the heterocyclic-fused 4-aminopyrim-

Figure 3. Superimposition of nitraquazone (purple) and related analogues: CP-77,059 (cyan), KF-19154 (red), RS-17597 (blue), and RS-25344 (green).
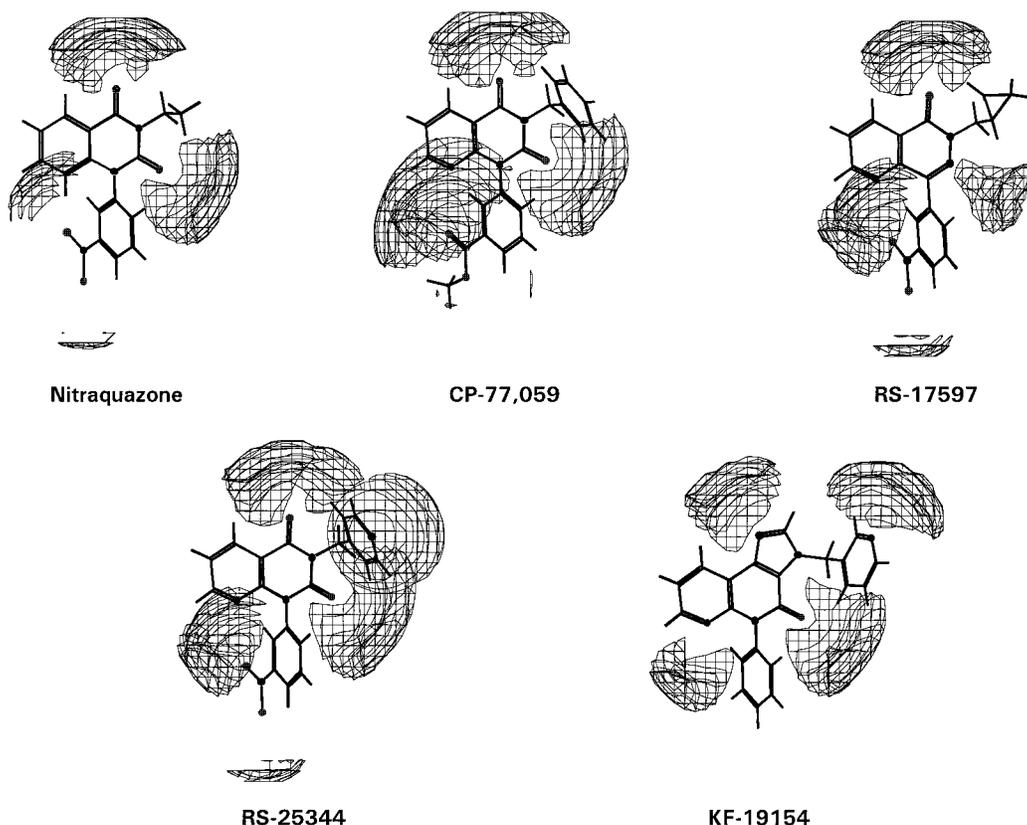

Figure 4. Interaction maps obtained with the N1 probe (NH flat amide) of GRID software. All interaction energy maps were contoured at −3 kcal/mol.

idines are shown in Scheme 1. The pyrimidinones **41**–**54** were synthesized starting from the corresponding 2-aminocarboxylic acid, ester, or carboxamide, following literature procedures.[48] Reaction of the pyrimidinones with phosphorus oxychloride or thionyl chloride gave the 4-chloropyrimidines **55**–**68**. The 4-aminopyrimidines **4**–**6**, **11**–**21**, and **23**–**39** (Tables 1, 3, and 4) were obtained by reaction of the 4-chloropyrimidines **55**–**68** and different amines, more forcing conditions being required for the less nucleophilic amines. The synthesis of compound **22** is shown in Scheme 2. Acylation of 3-amino-2-thiophenecarboxamide with 2-choroacetyl chloride gave the diamide **70**. Nucleophilic substitution, followed by basic cyclization, afforded the intermediate **72**, which was carried through a synthetic sequence

similar to that shown in Scheme 1 to give the thieno-[3,2-*d*]pyrimidine **22**. Compound **40**, in which the benzylamino group of the thieno[3,2-*d*]pyrimidine **4** has been replaced by a benzamido group, was synthesized as shown in Scheme 3.

The thioether and ether replacements for the amino linkage in **4** (compounds **9** and **10**, Table 2) were synthesized directly from benzyl bromide and the thieno-[3,2-*d*]pyrimidin-4-thione **69** (R₁ = butyl) and the 4-chlorothieno[3,2-*d*]pyrimidine **55** (R₁ = butyl), respectively (Scheme 1).

The preparation of 3-benzylpyrimidin-4-ones **1** and **2** is also illustrated in Scheme 1. Alkylation with benzyl bromide of the thieno[3,2-*d*]pyrimidinone **41** and the quinazolinone **43** (R₁ = butyl) gave **1** and **2**, respectively.
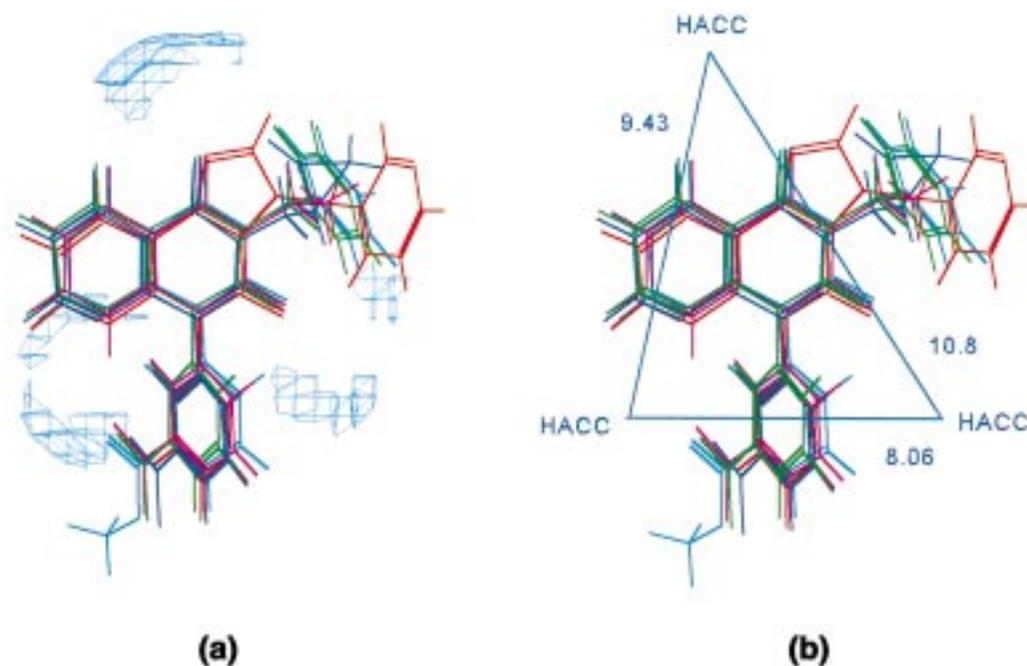
Figure 5. (a) Common interaction map using the N1 GRID probe of nitraquazone (purple), CP-77,059 (cyan), KF-19154 (red), RS-17597 (blue), and RS-25344 (green). (b) Distances between the three main interaction minima which represent the areas of the ligand for accepting hydrogen bonds (HACC) are 9.43, 8.06, and 10.86 Å.
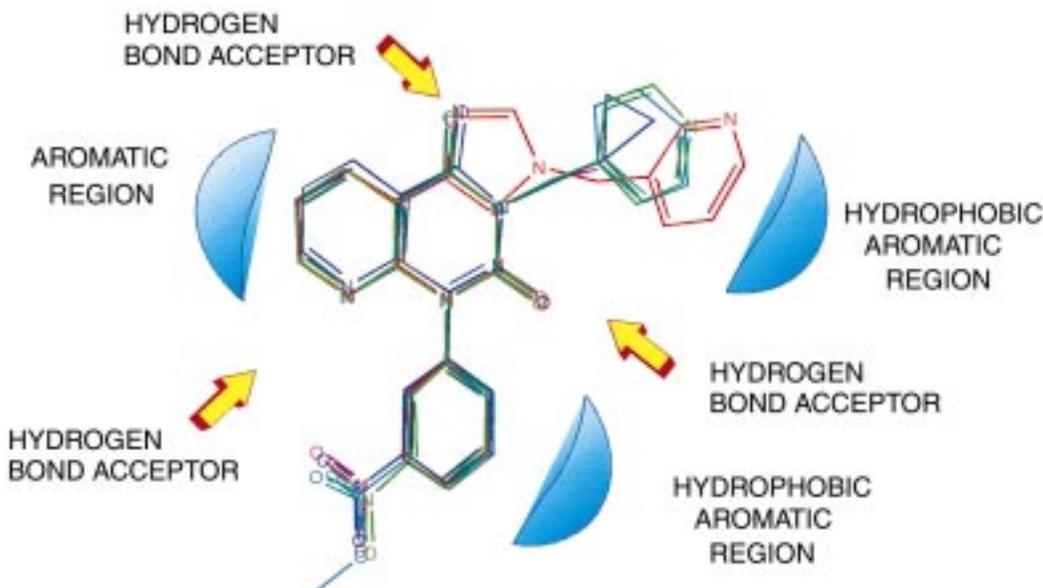

Figure 6. 3D pharmacophore model for PDE 4 inhibitors related to the nitraquazone structure.

The analogous pyridopyrimidinone **3** was synthesized in two steps starting from 3-amino-2-pyridincarboxylic acid (Scheme 4).

The syntheses[49] of pyrazinones **7** and **8** are summarized in Schemes 5 and 6, respectively. Condensation of 3-amino-2-benzylaminopyridine[50] with 2-oxopentanoic acid gave the pyrido[2,3-*b*]pyrazinone **7**. Compound **8** was prepared starting from 1-fluoro-2-nitrobenzene and D,L-norvaline. Reduction of the nitro group of the amino ester **75**, followed by oxidation with H_2O_2, yielded the intermediate quinoxaline **76**, which was converted to **8** by alkylation with benzyl bromide.

Biological Evaluation

To assess structure−activity relationships, all the final compounds were tested for their inhibitory poten-cies against isolated guinea pig ventricular PDE 4. Isoenzyme selectivity was obtained by comparing the IC_{50} values of compounds against PDE 4 with their inhibitory activity against cGMP-inhibited PDE (PDE 3) from the same source. A representative group of compounds were evaluated for their ability to displace [³H]rolipram from its binding site and also to potentiate isoprenaline-induced cAMP accumulation in isolated guinea pig eosinophils (cAMP potentiation). Rolipram, milrinone,[51] and RS-14491[24b] were used as reference substances.

Results and Discussion

Lead Finding. Table 1 shows the biological results for the set of compounds **1**−**8** suggested as possible PDE 4 inhibitors on the basis of the pharmacophore. As
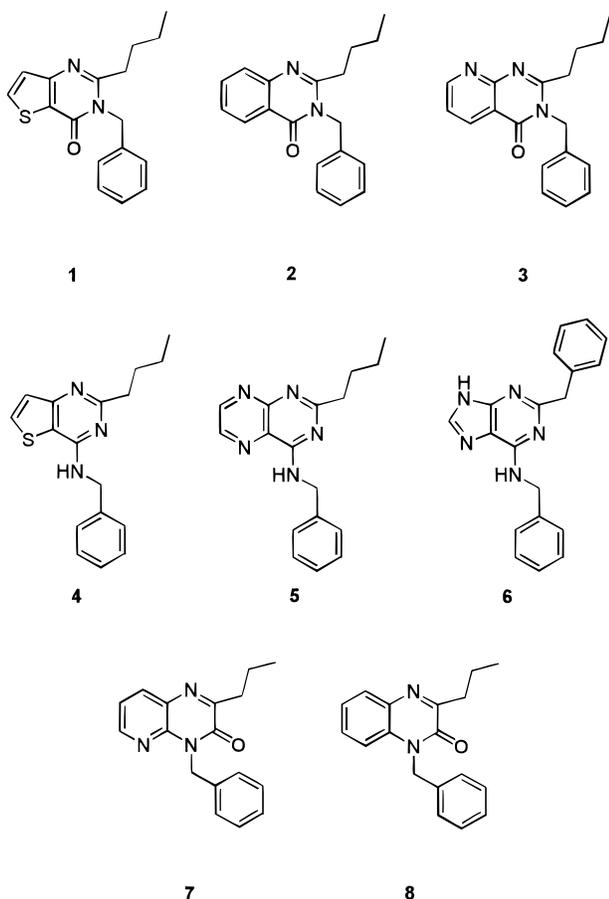
Figure 7. Chemical structures of new compounds which were designed based on PDE 4 pharmacophore configuration.

pointed out previously, these compounds fit well in the pharmacophore model, sharing at least two areas of interaction, related to hydrogen bond acceptor groups, and the three aromatic lipophilic centers. All of them were selective for PDE 4 with respect to the PDE 3 enzyme. Pyrimidinones **1−3**, in which the relative position of one of the hydrogen bond acceptor groups and one of the lipophilic groups was interchanged, showed poor PDE 4 activities. The most interesting compounds were the thienopyrimidine **4**, the pyridopyrazine **7**, and the quinoxaline **8**, with PDE 4 inhibitory activities (IC$_{50}$) of around 1 μM and more than 100-fold selectivity versus PDE 3. These results highlight the importance of a hydrogen bond acceptor in position 3 of the heterocycle ring (a nitrogen for the pirimidine and an oxo group for the pyrazinones). For compound **4**, substitution of the fused thiophene by either a pyrazine (compound **5**) or an imidazole (compound **6**) led to a reduction in PDE 4 inhibition. These data suggest that the increase of polarity in the fused aromatic ring could be detrimental for the activity. Among the more active structures, compound **4** was initially selected for further optimization.

Lead Optimization. Changes in PDE activity on modifying the linkage between the benzyl group and the thienopyrimidine were analyzed first (Table 2). The substitution of the amino group in compound **4** by an ether led to compound **10**, which is as active in PDE 4 inhibitory potency as **4** but less selective against PDE 3. The thioether **9** was less potent. In all these compounds, the PDE 4/[³H]rolipram ratio was improved

with respect to the reference drugs rolipram and RS-14491. Although PDE 4 potency and PDE 3/PDE 4 selectivity were maintained by the ether analogue **10**, for reasons of chemical stability we chose as a basis for continued structure−activity relationship exploration the amino compound **4**.

Modification of the alkyl group at C$_2$ of the thienopyrimidine was then examined (Table 3). In agreement with the proposed pharmacophore, the absence of a lipophilic group at C$_2$ yielded a less active compound (**11**). For alkyl groups at this position, the optimal volume was found to be between a butyl and a cyclopentylmethyl group, with compounds **4** and **14** being the most active and selective ones. The introduction of larger groups, such as a phenoxybutyl group (compound **18**) or even a cyclohexylmethyl group (compound **15**), reduced PDE 4 potency 4-fold. Compound **16**, with a benzyl group at C$_2$, had reasonable PDE 4 activity but was not selective against PDE 3. The introduction of a pyridyl group at the C$_2$ position led to interesting activities. PDE 4 activity was enhanced in compound **20** (R$_1$ = 3-pyridyl), although PDE 3 selectivity was reduced compared to compound **4**. Introduction of a piperidine at the C$_2$ position (compound **22**) resulted in a complete loss of activity, indicating a lack of tolerance at the receptor for a positive charge in this region of the molecule. With respect to the [³H]rolipram binding site, several compounds (**14**, **17**, **20**, and **21**) were identified with a much better PDE 4/[³H]rolipram ratio than the reference compounds.

In summary, within the synthesized compounds, the best substitution at the C$_2$ position was the 3-pyridyl group. Compound **20** was the most active with 25-fold selectivity over PDE 3 and a good PDE 4/[³H]rolipram ratio.

Table 4 shows the biological activity of analogues of 4-amino-2-butylthieno[3,2-*d*]pyrimidine. Again, in agreement with the proposed pharmacophore, a lipophilic group at C$_4$ is needed in order to obtain active compounds. As was observed previously at C$_2$, there exists an optimal volume that is exceeded by the bulky (biphenyl-4-yl)methyl group (compound **30**). The introduction of electron-donating and electron-withdrawing substituents on the benzyl group of compound **4** was studied. Compounds **23−25**, with a methoxy group at ortho, meta, and para positions showed no significant changes in PDE 4 activity with respect to compound **4**. The ortho substitution (compound **23**) maintained good PDE 3/PDE 4 selectivity while the meta and para methoxy groups (compounds **24** and **25**) exhibited better PDE 4/[³H]rolipram ratios. The introduction of a nitro group at ortho and meta positions led to compounds **26** and **27**, respectively, as active against PDE 4 as compound **4** but considerably less PDE 3 selective. Compound **28**, with a para nitro group, was less active and selective than **4**. PDE 4 potency was enhanced by a pyridylmethyl group (compound **31**) and a phenyl group (compound **32**) at the C$_4$ position, but with some reduction in selectivity. Other interesting analogues were obtained by introducing nonaromatic groups at C$_4$ (compounds **33−35**). Of particular note is compound **33**, which exhibited PDE 4 activity and PDE 3 selectivity comparable to rolipram, with the advantage of a 20-fold better PDE 4/[³H]rolipram ratio. By way of contrast,
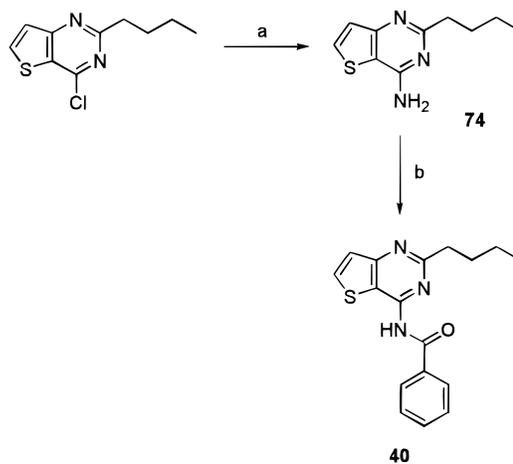
Scheme 1[a]
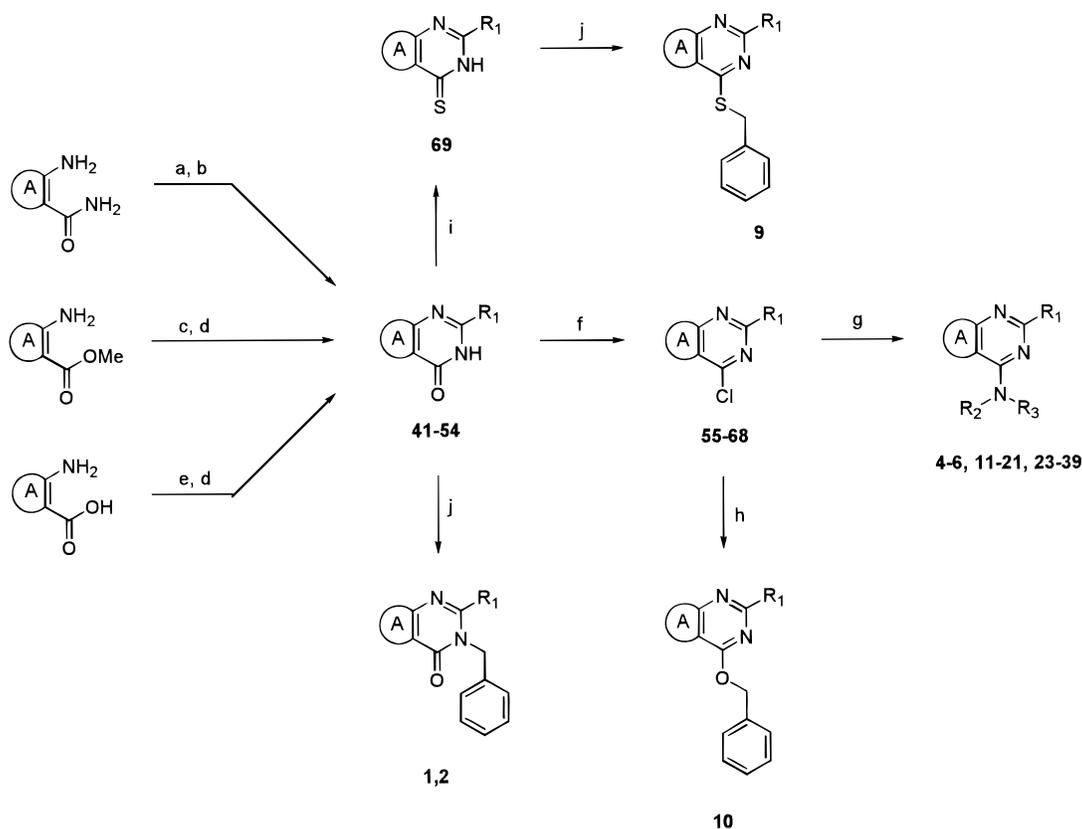
[a] Reagents: (a) (R₁CO)₂O or R₁COOH, DCC, DMAP, py, DMF; (b) 2 N NaOH; (c) R₁COCl, py, CHCl₃ or R₁COOH, DCC, DMAP, CH₂Cl₂; (d) 30% NH₃; (e) (R₁CO)₂O; (f) POCl₃ or SOCl₂ under standard conditions; (g) R₂NHR₃, Et₃N, THF or R₂NHR₃, NaOAc, HOAc, KI or R₂NHR₃, K₂CO₃, DMF; (h) PhCH₂OH, NaH, DMSO; (i) Lawesson's reagent, toluene; (j) PhCH₂Br, K₂CO₃, DMF.

Scheme 2[a]
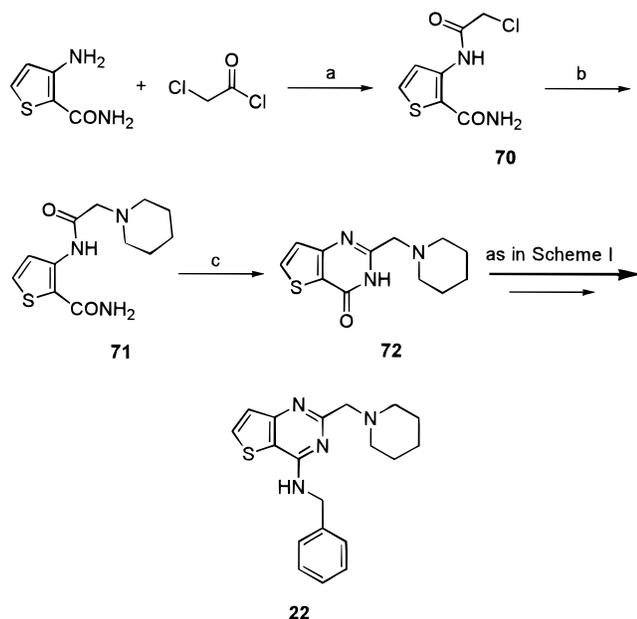
[a] Reagents: (a) Et₃N, THF; (b) piperidine, K₂CO₃, acetonitrile; (c) 2 N NaOH.

Scheme 3[a]

[a] Reagents: (a) 30% NH₃; (b) BuLi, benzoyl chloride, THF.

rolipram ratios. The introduction of an amide led to a large loss of activity (compound **40**).

cAMP Potentiation. A selected group of thieno[3,2-*d*]pyrimidines were evaluated for their ability to potentiate isoprenaline-induced cAMP accumulation in isolated guinea pig eosinophils. This cell type was chosen on account of its prominent pathological role in allergic asthma[52] and because PDE 4 appears to be the only cAMP-hydrolizing activity present in these cells.[53] The selected compounds and their activities are summarized in Table 5. In general, there is good correlation between PDE 4 inhibition and cAMP potentiation. In fact,

piperidine **35** exhibited a high [³H]rolipram affinity, in comparison with the other thienopyrimidines. The PDE 3/PDE 4 selectivity was enhanced by disubstituted amines at C₄, as was shown by compound **37** (R₂ = benzyl; R₃ = methyl). Furthermore, compound **37**, being as active as **4**, had one of the most favorable PDE 4/[³H]-

Table 1. Biological Activity (PDE 4 and PDE 3 Inhibition) of New Pharmacophore-Based Compounds

compd	PDE 4[a,b]	PDE 3[a,b]
1	5 ± 1	43 ± 3
2	16 ± 3	>200
3	10 ± 5	>200
4	0.9 ± 0.4	97 ± 5
5	6.7 ± 0.3	45 ± 7
6	29 ± 6	>200
7	1.0 ± 0.3	>200
8	0.9 ± 0.3	>200
milrinone		0.73 ± 0.03
rolipram	0.32 ± 0.09	242 ± 11

[a] Data are indicated as IC$_{50}$ (μM) $\pm$ SEM ($n = 3$). [b] PDE 3 and PDE 4 were obtained from guinea pig ventricular tissue,[59] and assays were performed following the procedure of Thomson et al.[60]

Table 2. Changes to the Linkage of Thieno[3,2-*d*]pyrimidines
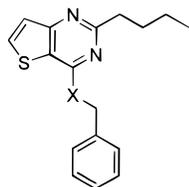
compd	X	PDE 4[a,b]	PDE 3[a,b]	^{3}H-ROL[a,c]	PDE 4/^{3}H-ROL
4	NH	0.9 ± 0.4	97 ± 5	0.23 ± 0.15	3.9
9	S	6 ± 1	>200	2.8 ± 0.8	2.1
10	O	1.1 ± 0.2	58 ± 8	1.2 ± 0.4	0.9
rolipram		0.32 ± 0.09	242 ± 11	0.006 ± 0.004	53
RS-14491		0.056 ± 0.01	5.1 ± 2.0	0.0048 ± 0.001	12

[a] Data are indicated as IC$_{50}$ (μM) $\pm$ SEM or inhibition percentage $\pm$ SEM at indicated concentration (μM) ($n = 3$). [b] PDE 3 and PDE 4 were obtained from guinea pig ventricular tissue,[59] and assays were performed following the procedure of Thomson et al.[60] [c] [^{3}H]Rolipram tests were performed using rat brain membranes.[61]
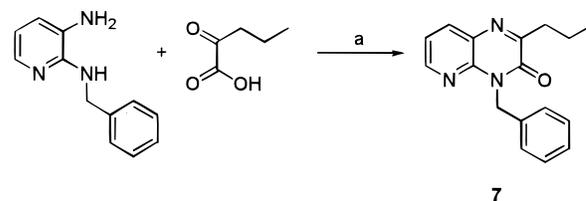
Scheme 4[a]
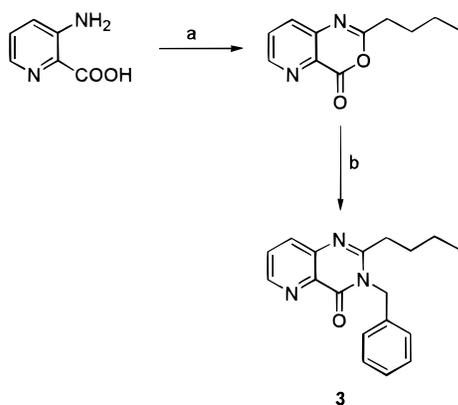
[a] Reagents: (a) (C$_4$H$_9$CO)$_2$O; (b) PhCH$_2$NH$_2$.

compounds **32**, **33**, and **35** exhibited submicromolar activities in both assays, and compounds such as **11** and **22** showed no potency in either. By contrast, pyridines **20** and **21** are less able to potentiate cAMP accumulation in isolated guinea pig eosinophils than their potency as PDE 4 inhibitors might suggest.

Conclusions

From the common pharmacophore for nitraquazone-related compounds, a series of novel heteroaromatic compounds have been designed, synthesized, and evaluated as PDE 4 inhibitors. Thienopyrimidine **4** was

Scheme 5[a]



[a] Reagents: (a) EtOH, 2 N HCl.

Scheme 6[a]
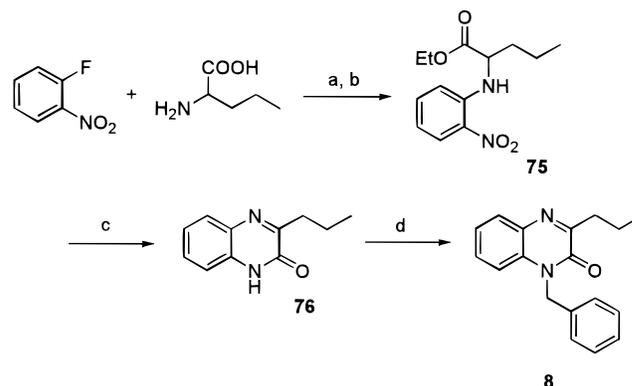
[a] Reagents: (a) NaHCO$_3$, EtOH, H$_2$O; (b) SOCl$_2$, EtOH; (c) Fe, EtOH, HCl, and then 2 N NaOH, H$_2$O$_2$; (d) benzyl bromide, K$_2$CO$_3$, DMF.

selected as a lead compound for further optimization. The biological results reported in Tables 3 and 4 confirm the importance of the presence of lipophilic and aromatic groups in both the C$_2$ and C$_4$ positions, as suggested by the pharmacophore model. In general, the compounds show a good balance of PDE 4 activity and displacement of [^{3}H]rolipram from its binding site. For the compounds evaluated, the enzyme activity correlates well with the potentiation of isoprenaline-induced cAMP accumulation in isolated guinea pig eosinophils, consistent with good cell penetration. Compounds such as **32**, **33**, and **37**, which exhibit good activity in both PDE 4 inhibition and cAMP potentiation and display an improved ratio with respect to the [^{3}H]rolipram specific binding site, are being evaluated in vivo as potential antiasthmatic agents.

Experimental Section

Molecular Modeling. All molecular modeling calculations were performed on a Digital Alpha Station 3000. Structures were built with standard bond lengths and angles by using the Chem-X molecular modeling package. All structures were initially optimized using steepest descents and conjugate gradient methods. Charge distributions were calculated after semiempirical optimization using the MOPAC program (program no. 506, version 6.0, Quantum Chemistry Program Exchange, QCPE, Bloomington, IN). The PULAY keyword and the eigenvector following (EF) routine for minimun search with GNORM = 0.01 were used in the optimization step. Electrostatic potential maps were calculated from the semiempirical charges by using the program VSS (program no. 249, QCPE) and displayed in Chem-X. The energies of interaction, determined by different chemical probes, were analyzed by GRID computations (version 14.0, Molecular Discovery, Oxford, U.K.).

Chemistry: General. Reagents, starting materials, and solvents were purchased from commercial suppliers and used as received. All organic solutions are dried over sodium sulfate. Concentration refers to evaporation under aspirator

Table 3. Biological Activity of Analogues of 4-Benzylaminothieno[3,2-*d*]pyrimidine
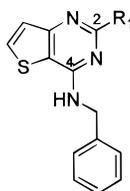
compd	R$_1$	PDE 4[a,b]	PDE 3[a,b]	^{3}H-ROL[a,c]	PDE 4/^{3}H-ROL
4	butyl	0.9 ± 0.4	97 ± 5	0.23 ± 0.15	3.9
11	H	14 ± 4	68 ± 1	nt	
12	ethyl	2.3 ± 0.6	30 ± 5	41% $\pm$ 1 at 10	
13	isobutyl	1.5 ± 0.6	58 ± 16	0.4 ± 0.2	3.7
14	cyclopentylmethyl	1.1 ± 0.3	>200	5.8 ± 2.6	0.2
15	cyclohexylmethyl	4 ± 1	>200	1.3 ± 0.1	3.1
16	benzyl	1.5 ± 0.7	5 ± 1	nt	
17	phenethyl	2.3 ± 0.6	>200	3.9 ± 0.8	0.6
18	4-phenoxybutyl	4.7 ± 0.5	>200	nt	
19	phenyl	3.0 ± 0.1	>200	8% $\pm$ 3 at 10	
20	3-pyridyl	0.6 ± 0.1	14 ± 1	1.4 ± 0.4	0.4
21	4-pyridyl	1.3 ± 0.5	>200	1.7 ± 0.5	0.8
22	piperidin-1-yl	65 ± 7	>200	nt	
rolipram		0.32 ± 0.09	242 ± 11	0.006 ± 0.004	53
RS-14491		0.056 ± 0.01	5.1 ± 2.0	0.0048 ± 0.001	12

^{a} Data are indicated as IC$_{50}$ (μM) $\pm$ SEM or inhibition percentage $\pm$ SEM at indicated concentration (μM) ($n = 3$). ^{b} PDE 3 and PDE 4 were obtained from guinea pig ventricular tissue,[59] and assays were performed following the procedure of Thomson et al.[60] ^{c} [^{3}H]Rolipram tests were performed using rat brain membranes;[61] nt = not tested.

Table 4. Biological Activity of Analogues of 4-Amino-2-butylthieno[3,2-*d*]pyrimidine
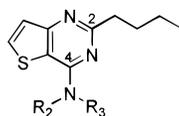
compd	R$_2$	R$_3$	PDE 4[a,b]	PDE 3[a,b]	^{3}H-ROL[a,c]	PDE 4/^{3}H-ROL
4	benzyl	H	0.9 ± 0.4	97 ± 5	0.23 ± 0.15	3.9
23	2-methoxybenzyl	H	0.7 ± 0.2	39 ± 18	0.2 ± 0.02	3.5
24	3-methoxybenzyl	H	1.4 ± 0.1	20 ± 4	2.2 ± 0.2	0.6
25	4-methoxybenzyl	H	1.5 ± 0.2	6 ± 1	1.7 ± 0.1	0.9
26	2-nitrobenzyl	H	1.0 ± 0.2	18 ± 8	0.08 ± 0.02	12.5
27	3-nitrobenzyl	H	0.84 ± 0.08	3.5 ± 0.5	1.5 ± 0.2	0.6
28	4-nitrobenzyl	H	6.3 ± 0.9	8.5 ± 0.5	nt	
29	2,6-difluorobenzyl	H	1.00 ± 0.05	64 ± 4	0.6 ± 0.3	1.7
30	(biphenyl-4-yl)methyl	H	>200	200	nt	
31	(4-pyridyl)methyl	H	0.6 ± 0.2	29 ± 17	nt	
32	phenyl	H	0.6 ± 0.2	42 ± 1	0.20 ± 0.01	3
33	cyclohexyl	H	0.4 ± 0.2	>200	0.20 ± 0.03	2
34	cyclohexylmethyl	H	1.0 ± 0.5	>200	1.1 ± 0.5	0.9
35	$-(CH_2)_4-$		0.6 ± 0.3	68 ± 20	0.03 ± 0.01	20
36	$-CH_2-C_6H_4-CH_2CH_2-$		2.7 ± 1.3	44 ± 16	2.3 ± 1	1.2
37	benzyl	methyl	0.8 ± 0.1	>200	2.5 ± 0.5	0.3
38	benzyl	propyl	2.5 ± 0.5	>200	37% $\pm$ 14 at 10	
39	benzyl	benzyl	4.0 ± 1.8	>200	37% $\pm$ 14 at 10	
40	benzoyl	H	23.0 ± 0.2	>200	nt	
rolipram			0.32 ± 0.09	242 ± 11	0.006 ± 0.004	53
RS-14491			0.056 ± 0.01	5.1 ± 2.0	0.0048 ± 0.001	12

^{a} Data are indicated as IC$_{50}$ (μM) $\pm$ SEM or inhibition percentage $\pm$ SEM at indicated concentration (μM) ($n = 3$). ^{b} PDE 3 and PDE 4 were obtained from guinea pig ventricular tissue,[59] and assays were performed following the procedure of Thomson et al.[60] ^{c} [^{3}H]Rolipram tests were performed using rat brain membranes;[61] nt = not tested.

vacuum using a Büchi rotatory evaporator. Reaction products were purified, when necessary, by flash chromatography on silica gel (40−63 μm) with the solvent system indicated. Spectroscopic data were recorded on a Varian Gemini 300 spectrometer. Melting points were recorded on a Büchi 535 apparatus. Where analyses are indicated only by symbols of the elements, results obtained were within 0.4% of the theoretical values.

General Procedures for the Synthesis of Pyrimidinones 41−54 (Methods A, B, C, and D). Method A. 2-Butyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (41). A mixture of 50 g (0.35 mol) of 3-amino-2-thiophenecarboxamide and 80 mL (0.40 mol) of valeric anhydride was heated at 90 °C for 20 min. After cooling, 600 mL of 2 N NaOH were added. The mixture was stirred under reflux for 1 h, cooled, and neutralized with 2 N HCl. The resulting white solid was filtered, washed with water, and dried to give 67.5 g (91%) of the title compound: ^{1}H NMR (CDCl$_3$) δ 12.77 (br, 1H), 7.82 (d, $J = 5$ Hz, 1H), 7.34 (d, $J = 5$ Hz, 1H), 2.86 (t, $J = 8$ Hz, 2H), 1.87 (m, 2H), 1.48 (m, 2H), 0.98 (t, $J = 8$ Hz, 3H).

2-Ethyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (42) was prepared according to method A in 71% yield starting from 3-amino-2-thiophenecarboxamide (2.5 g) and propionic anhydride: ^{1}H NMR (DMSO) δ 12.20 (br, 1H), 8.11 (d, $J = 5$ Hz, 1H), 7.32 (d, $J = 5$ Hz, 1H), 2.63 (q, $J = 8$ Hz, 2H), 1.22 (t, $J = 8$ Hz, 3H).

Table 5. cAMP Potentiation of Selected Thieno[3,2-*d*]pyrimidines

compd	PDE 4[a]	cAMP pot.[b]
4	0.9 ± 0.4	1.8 ± 0.4
11	14 ± 4	18 ± 5
14	1.1 ± 0.3	3.4 ± 1.7
15	4 ± 1	2 ± 1
20	0.6 ± 0.1	3.5 ± 0.2
21	1.3 ± 0.5	7.5 ± 3.8
22	65 ± 7	21 ± 8
24	1.5 ± 0.2	1.7 ± 0.2
27	0.84 ± 0.08	1.05 ± 0.05
32	0.6 ± 0.2	0.7 ± 0.4
33	0.4 ± 0.2	0.6 ± 0.2
34	1.0 ± 0.5	2.1 ± 1.3
35	0.6 ± 0.3	0.2 ± 0.1
37	0.8 ± 0.1	1.8 ± 0.2

[a] Data are indicated as IC_{50} (μM) $\pm$ SEM; PDE 4 was obtained from guinea pig ventricular tissue,[59] and assays were performed following the procedure of Thomson et al.[60] [b] Isoprenaline-induced cAMP accumulation in isolated guinea pig eosinophils; data are indicated as EC_{50} (μM) $\pm$ SEM.

2-Butyl-3*H*-quinazolin-4-one (43) was prepared according to method A in 89% yield starting from anthranilamide (13.6 g) and valeric anhydride: ^{1}H NMR (CDCl$_3$) δ 12.22 (br, 1H), 8.28 (d, $J = 7$ Hz, 1H), 7.80−7.70 (m, 2H), 7.46 (m, 1H), 2.83 (t, $J = 7$ Hz, 2H), 1.90 (m, 2H), 1.50 (m, 2H), 1.00 (t, $J = 7$ Hz, 3H).

Method B. 2-Benzyl-1,9-dihydropurin-6-one (44). To a solution of 1.62 g (0.01 mol) of 4-amino-5-imidazolecarboxamide hydrochloride in 25 mL of anhydrous pyridine and 50 mL of anhydrous DMF were added 1.36 g (0.01 mol) of phenylacetic acid, 2.06 g (0.01 mol) of DCC, and 1.22 g (0.01 mol) of DMAP. The mixture was stirred at 70 °C overnight. The resulting solution was concentrated, and the residue was suspended in 40 mL of 2 N NaOH. The mixture was stirred under reflux for 30 min. The suspension was cooled, the insoluble residue was filtered, and the basic solution was neutralized with 2 N HCl. The resulting white solid was filtered, washed with water, and dried to give 1.24 g (55%) of **44**: ^{1}H NMR (CDCl$_3$) δ 12.32 (br, 1H), 8.04 (br, 1H), 7.40−7.20 (m, 6H), 3.94 (s, 2H).

Method C. 2-Isobutyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (45). To a cooled solution of 4.71 g (0.03 mol) of methyl 3-amino-2-thiophenecarboxylate and 4.83 mL (0.06 mol) of pyridine in 75 mL of CHCl$_3$ was added 5.48 g (0.045 mol) of isovaleryl chloride. The mixture was stirred at room temperature overnight. The reaction mixture was washed with 2 N HCl and brine, dried, and concentrated. The resulting solid was suspended in 75 mL of 30% NH$_3$ and then was heated to 120 °C for 2 h in a pressure reactor. The mixture was cooled and neutralized. The resulting white solid was filtered, washed with water, and dried to give 2.49 g (40%) of the desired product: ^{1}H NMR (CDCl$_3$) δ 12.27 (br, 1H), 7.83 (d, $J = 5$ Hz, 1H), 7.34 (d, $J = 5$ Hz, 1H), 2.69 (d, $J = 8$ Hz, 2H), 2.30 (m, 1H), 1.05 (d, $J = 8$ Hz, 6H).

2-Cyclopentylmethyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (46) was prepared according to method C in 33% yield starting from methyl 3-amino-2-thiophenecarboxylate (3.50 g) and cyclopentylacetyl chloride: ^{1}H NMR (CDCl$_3$) δ 12.10 (br, 1H), 7.82 (d, $J = 5$ Hz, 1H), 7.33 (d, $J = 5$ Hz, 1H), 2.80 (d, $J = 8$ Hz, 2H), 2.44 (m, 1H), 1.90−1.50 (m, 6H), 1.43−1.25 (m, 2H).

2-Cyclohexylmethyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (47) was prepared according to method C in 37% yield starting from methyl 3-amino-2-thiophenecarboxylate (3.77 g) and cyclohexylacetyl chloride:[54] ^{1}H NMR (CDCl$_3$) δ 11.93 (br, 1H), 7.83 (d, $J = 5$ Hz, 1H), 7.34 (d, $J = 5$ Hz, 1H), 2.69 (d, $J = 9$ Hz, 2H), 1.94 (m, 1H), 1.82−1.60 (m, 5H), 1.32−1.04 (m, 5H).

2-Benzyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (48) was prepared according to method C in 48% yield starting from methyl 3-amino-2-thiophenecarboxylate (6.28 g) and phenylacetyl chloride: ^{1}H NMR (CDCl$_3$) δ 11.66 (br, 1H), 7.83 (d, $J = 5$ Hz, 1H), 7.45 (d, $J = 7$ Hz, 1H), 7.50−7.25 (m, 5H), 4.10 (s, 2H).

2-Phenethyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (49) was prepared according to method C in 70% yield starting from methyl 3-amino-2-thiophenecarboxylate (4.71 g) and 3-phenylpropionyl chloride: ^{1}H NMR (CDCl$_3$) δ 11.90 (br, 1H), 8.14 (d, $J = 5$ Hz, 1H), 7.37 (d, $J = 5$ Hz, 1H), 7.30−7.15 (m, 5H), 3.03 (m, 2H), 2.94 (m, 2H).

2-(4-Phenoxybutyl)-3*H*-thieno[3,2-*d*]pyrimidin-4-one (50) was prepared according to method C in 70% yield starting from methyl 3-amino-2-thiophenecarboxylate (2.84 g) and 5-phenoxyvaleryl chloride:[55] ^{1}H NMR (CDCl$_3$) δ 12.46 (br, 1H), 7.83 (d, $J = 5$ Hz, 1H), 7.34 (d, $J = 5$ Hz, 1H), 7.25 (m, 2H), 6.97−6.84 (m, 3H), 4.03 (t, $J = 6$ Hz, 2H), 2.94 (t, $J = 6$ Hz, 2H), 2.08 (m, 2H), 1.95 (m, 2H).

2-Phenyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (51) was prepared according to method C in 52% yield starting from methyl 3-amino-2-thiophenecarboxylate (4.71 g) and benzoyl chloride: ^{1}H NMR (CDCl$_3$) δ 12.66 (br, 1H), 8.15 (m, 2H), 8.11 (d, $J = 5$ Hz, 1H), 7.60−7.48 (m, 3H), 7.41 (d, $J = 5$ Hz, 1H).

Method D. 2-(3-Pyridyl)-3*H*-thieno[3,2-*d*]pyrimidin-4-one (52). To a solution of 4.50 g (0.0288 mol) of methyl 3-amino-2-thiophenecarboxylate and 3.50 g of nicotinic acid in 75 mL of CH$_2$Cl$_2$ were added 5.93 g (0.0288 mol) of DCC and 3.50 g (0.0288 mol) of DMAP. The mixture was stirred under reflux overnight. The resulting suspension was cooled and concentrated. The residue was suspended in 150 mL of 30% NH$_3$ and then heated to 120 °C for 2 h in a pressure reactor. The mixture was cooled, and the insoluble residue was filtered. The basic solution was neutralized with 2 N HCl. The resulting white solid was filtered, washed with water, and dried to give 3.60 g (55%) of **52**: ^{1}H NMR (DMSO) δ 12.93 (br, 1H), 9.26 (s, 1H), 8.77 (s, 1H), 8.46 (d, $J = 5$ Hz, 1H), 8.22 (d, $J = 7$ Hz, 1H), 7.70−7.45 (m, 2H).

2-(4-Pyridyl)-3*H*-thieno[3,2-*d*]pyrimidin-4-one (53) was prepared according to method D in 53% yield starting from methyl 3-amino-2-thiophenecarboxylate (4.50 g) and isonicotinic acid: ^{1}H NMR (DMSO) δ 12.96 (br, 1H), 8.79 (d, $J = 5$ Hz, 2H), 8.26 (d, $J = 5$ Hz, 1H), 8.06 (d, $J = 5$ Hz, 2H), 7.52 (d, $J = 5$ Hz, 1H).

2-Butyl-3*H*-pteridin-4-one (54). A mixture of 5.00 g (0.036 mol) of 3-amino-2-pyrazinecarboxylic acid and 40 mL of valeric anhydride was heated at 140 °C for 1 h. The excess of valeric anhydride was distilled. The resulting red solid was suspended in 60 mL of 30% NH$_3$ and then heated to 120 °C for 2 h in a pressure reactor. The resulting solution was cooled, neutralized with 2 N HCl, and extracted with EtOAc. The combined extracts were washed with brine, dried, and concentrated. The resulting solid was washed with pentane to give 3.06 g (42%) of the title compound: ^{1}H NMR (CDCl$_3$) δ 12.60 (br, 1H), 9.04 (s, 1H), 8.86 (s, 1H), 3.00 (t, $J = 7$ Hz, 2H), 1.96 (m, 2H), 1.50 (m, 2H), 0.97 (t, $J = 7$ Hz, 3H).

General Procedures for the Synthesis of Chloropyrimidines 55−68 (Methods E, F, and G). Method E. 2-Butyl-4-chlorothieno[3,2-*d*]pyrimidine (55). Thieno[3,2-*d*]-pyrimidinone **41** (8.15 g, 0.039 mol) was dissolved in 38 mL of POCl$_3$. The mixture was stirred under reflux for 30 min, cooled, and concentrated. The residue was poured into ice and extracted with EtOAc. The combined extracts were washed with saturated NaHCO$_3$ and brine, dried, and concentrated to give 8.56 g (96%) of **55** as a yellow oil: ^{1}H NMR (CDCl$_3$) δ 7.97 (d, $J = 6$ Hz, 1H), 7.50 (d, $J = 6$ Hz, 1H), 3.03 (t, $J = 7$ Hz, 2H), 1.86 (m, 2H), 1.44 (m, 2H), 0.96 (t, $J = 7$ Hz, 3H).

4-Chlorothieno[3,2-*d*]pyrimidine (56) was prepared according to method E in 89% yield starting from 3*H*-thieno-[3,2-*d*]pyrimidin-4-one[48b] (2.00 g): ^{1}H NMR (CDCl$_3$) δ 9.00 (s, 1H), 8.06 (d, $J = 6$ Hz, 1H), 7.62 (d, $J = 6$ Hz, 1H).

4-Chloro-2-ethylthieno[3,2-*d*]pyrimidine (57) was prepared according to method E in 65% yield starting from thieno-[3,2-*d*]pyrimidinone **42** (2.33 g): ^{1}H NMR (CDCl$_3$) δ 8.00 (d, $J = 6$ Hz, 1H), 7.52 (d, $J = 6$ Hz, 1H), 3.10 (q, $J = 8$ Hz, 2H), 1.44 (t, $J = 8$ Hz, 3H).

4-Chloro-2-isobutylthieno[3,2-*d*]pyrimidine (58) was prepared according to method E in 86% yield starting from

thieno[3,2-*d*]pyrimidinone **45** (2.48 g): ^{1}H NMR (CDCl$_3$) δ 8.02 (d, J = 6 Hz, 1H), 7.53 (d, J = 6 Hz, 1H), 2.94 (d, J = 8 Hz, 2H), 2.36 (m, 1H), 1.00 (d, J = 8 Hz, 6H).

4-Chloro-2-cyclopentylmethylthieno[3,2-*d*]pyrimidine (59) was prepared according to method E in 80% yield starting from thieno[3,2-*d*]pyrimidinone **46** (1.72 g): ^{1}H NMR (CDCl$_3$) δ 8.00 (d, J = 6 Hz, 1H), 7.53 (d, J = 6 Hz, 1H), 3.04 (d, J = 8 Hz, 2H), 2.50 (m, 1H), 1.80−1.60 (m, 7H), 1.30 (m, 1H).

4-Chloro-2-cyclohexylmethylthieno[3,2-*d*]pyrimidine (60) was prepared according to method E in 89% yield starting from thieno[3,2-*d*]pyrimidinone **47** (2.20 g): ^{1}H NMR (CDCl$_3$) δ 8.00 (d, J = 6 Hz, 1H), 7.54 (d, J = 6 Hz, 1H), 2.92 (d, J = 8 Hz, 2H), 2.00 (m, 1H), 1.75−1.60 (m, 5H), 1.32−1.02 (m, 5H).

2-Benzyl-4-chlorothieno[3,2-*d*]pyrimidine (61) was prepared according to method E in 70% yield starting from thieno-[3,2-*d*]pyrimidinone **48** (4.64 g): ^{1}H NMR (CDCl$_3$) δ 7.98 (d, J = 7 Hz, 1H), 7.54 (d, J = 7 Hz, 1H), 7.43 (m, 2H), 7.38−7.17 (m, 3H), 4.38 (s, 2H).

4-Chloro-2-phenethylthieno[3,2-*d*]pyrimidine (62) was prepared according to method E in 75% yield starting from thieno[3,2-*d*]pyrimidinone **49** (3.84 g): ^{1}H NMR (CDCl$_3$) δ 7.98 (d, J = 6 Hz, 1H), 7.52 (d, J = 6 Hz, 1H), 7.28−7.18 (m, 5H), 3.37 (m, 2H), 3.23 (m, 2H).

4-Chloro-2-(4-phenoxybutyl)thieno[3,2-*d*]pyrimidine (63) was prepared according to method E in 66% yield starting from thieno[3,2-*d*]pyrimidinone **50** (4.21 g): ^{1}H NMR (CDCl$_3$) δ 7.97 (d, J = 6 Hz, 1H), 7.50 (d, J = 6 Hz, 1H), 7.25 (m, 2H), 6.95−6.84 (m, 3H), 4.00 (t, J = 7 Hz, 2H), 3.14 (t, J = 7 Hz, 2H), 2.09 (m, 2H), 1.91 (m, 2H).

4-Chloro-2-phenylthieno[3,2-*d*]pyrimidine (64) was prepared according to method E in 80% yield starting from thieno-[3,2-*d*]pyrimidinone **51** (3.51 g): ^{1}H NMR (CDCl$_3$) δ 8.52 (m, 2H), 8.01 (d, J = 6 Hz, 1H), 7.61 (d, J = 6 Hz, 1H), 7.55−7.47 (m, 3H).

4-Chloro-2-(3-pyridyl)thieno[3,2-*d*]pyrimidine (65) was prepared according to method E in 54% yield starting from thieno[3,2-*d*]pyrimidinone **52** (3.76 g): ^{1}H NMR (CDCl$_3$) δ 9.72 (s, 1H), 8.82−8.70 (m, 2H), 8.08 (d, J = 6 Hz, 1H), 7.65 (d, J = 6 Hz, 1H), 7.44 (m, 1H).

4-Chloro-2-(4-pyridyl)thieno[3,2-*d*]pyrimidine (66) was prepared according to method E in 95% yield starting from thieno[3,2-*d*]pyrimidinone **53** (3.30 g): ^{1}H NMR (CDCl$_3$) δ 8.77 (d, J = 6 Hz, 2H), 8.35 (d, J = 6 Hz, 2H), 8.08 (d, J = 6 Hz, 1H), 7.65 (d, J = 6 Hz, 1H).

Method F. 2-Benzyl-6-chloro-9*H*-purine (67). To a suspension of 4.03 g (0.0178 mol) of **44** in 80 mL of CHCl$_3$ was added under reflux a solution of 6.50 mL (0.0893 mol) of SOCl$_2$ in 7 mL of anhydrous DMF. The mixture was stirred under reflux for 1.30 h and concentrated. The residue was dissolved in EtOAc, and the resulting solution was washed with saturated NaHCO$_3$ and brine. The organic extracts were dried and concentrated to give 2.42 g of a solid that was used in the next step without further purification (^{1}H NMR purity, 80%): yield 44%; ^{1}H NMR (CDCl$_3$) δ 13.35 (br, 1H), 8.17 (s, 1H), 7.35−7.15 (m, 5H), 4.34 (s, 2H).

Method G. 2-Butyl-4-chloropteridine (68). To a solution of 4.18 mL (0.0246 mol) of diisopropylethylamine and 1.22 mL (0.0134 mol) of POCl$_3$ in 70 mL of anhydrous toluene was added 2.74 g (0.0134 mol) of pteridinone **54**. The suspension was stirred under reflux for 2 h. The mixture was cooled and then diluted with diethyl ether. The resulting solution was washed with water, 4 N NaOH, and brine, dried, and concentrated to give 2.25 g (75%) of **68** as a brown oil: ^{1}H NMR (CDCl$_3$) δ 9.22 (s, 1H), 9.03 (s, 1H), 3.20 (t, J = 8 Hz, 2H), 1.97 (m, 2H), 1.48 (m, 2H), 1.00 (t, J = 8 Hz, 3H).

General Procedures for the Synthesis of Aminopyrimidines 4−6, 11−21, and 23−39 (Methods H, I, and J). Method H. 4-Benzylamino-2-butylthieno[3,2-*d*]pyrimidine, Hydrochloride (4). A solution of 2.00 g (8.83 mmol) of **55**, 0.94 g (9.27 mmol) of triethylamine, and 0.99 g (9.27 mmol) of benzylamine in 50 mL of anhydrous THF was stirred

under reflux for 90 h. The mixture was cooled and partitioned between EtOAc and water; the organic phase was washed with brine, dried, and concentrated. To the residue dissolved in acetone was added HCl in dioxane. The resulting solid was recrystallized from isopropyl alcohol to give 2.12 g (72%) of the title compound: mp 264−266 °C; ^{1}H NMR (DMSO) δ 10.50 (t, J = 6 Hz, 1H), 8.45 (d, J = 5 Hz, 1H), 7.52 (d, J = 5 Hz, 1H), 7.45−7.25 (m, 5H), 4.84 (d, J = 6 Hz, 2H), 2.90 (t, J = 8 Hz, 2H), 1.75 (m, 2H), 1.30 (m, 2H), 0.86 (t, J = 8 Hz, 3H). Anal. (C$_{17}$H$_{19}$N$_3$S·HCl) C, H, N, S.

4-Benzylaminothieno[3,2-*d*]pyrimidine, Hydrochloride (11) was prepared according to method H starting from **56** (2.00 g). Recrystallization from isopropyl alcohol gave 2.76 g (78%) of **11**: mp 215−217 °C; ^{1}H NMR (DMSO) δ 10.50 (br, 1H), 8.89 (br, 1H), 8.46 (d, J = 5 Hz, 1H), 7.57 (d, J = 5 Hz, 1H), 7.45−7.25 (m, 5H), 4.84 (d, J = 7 Hz, 2H). Anal. (C$_{13}$H$_{11}$N$_3$S·HCl) C, H, N, S.

4-Benzylamino-2-ethylthieno[3,2-*d*]pyrimidine, Hydrochloride (12) was prepared according to method H starting from **57** (1.65 g). Recrystallization from isopropyl alcohol gave 0.87 g (35%) of **12**: mp 247−250 °C (D); ^{1}H NMR (DMSO) δ 15.44 (br, 1H), 10.50 (br, 1H), 8.43 (d, J = 6 Hz, 1H), 7.54 (d, J = 6 Hz, 1H), 7.45−7.25 (m, 5H), 4.87 (d, J = 6 Hz, 2H), 2.93 (q, J = 8 Hz, 2H), 1.31 (t, J = 8 Hz, 3H). Anal. (C$_{15}$H$_{15}$N$_3$S·HCl) C, H, N, S.

4-Benzylamino-2-isobutylthieno[3,2-*d*]pyrimidine, Hydrochloride (13) was prepared according to method H starting from **58** (1.79 g). Recrystallization from isopropyl alcohol gave 1.19 g (46%) of **13**: mp 261−262 °C; ^{1}H NMR (DMSO) δ 15.65 (br, 1H), 10.56 (br, 1H), 8.46 (d, J = 6 Hz, 1H), 7.54 (d, J = 6 Hz, 1H), 7.40−7.25 (m, 5H), 4.84 (d, J = 5 Hz, 2H), 2.80 (d, J = 6 Hz, 2H), 2.25 (m, 1H), 0.90 (d, J = 6 Hz, 6H). Anal. (C$_{17}$H$_{19}$N$_3$S·HCl) C, H, N.

4-Benzylamino-2-cyclopentylmethylthieno[3,2-*d*]pyrimidine (14) was prepared according to method H starting from **59** (1.28 g). Recrystallization from acetonitrile gave 1.28 g (78%) of **14**: mp 160−161 °C; ^{1}H NMR (CDCl$_3$) δ 7.65 (d, J = 6 Hz, 1H), 7.45−7.25 (m, 6H), 5.12 (br, 1H), 4.87 (d, J = 6 Hz, 2H), 2.87 (d, J = 8 Hz, 2H), 2.49 (m, 1H), 1.80−1.45 (m, 6H), 1.36−1.21 (m, 2H). Anal. (C$_{19}$H$_{21}$N$_3$S) C, H, N.

4-Benzylamino-2-cyclohexylmethylthieno[3,2-*d*]pyrimidine, Hydrochloride (15) was prepared according to method H starting from **60** (2.11 g). Recrystallization from isopropyl alcohol gave 0.91 g (31%) of **15**: mp 295−297 °C (D); ^{1}H NMR (DMSO) δ 10.40 (br, 1H), 8.43 (d, J = 6 Hz, 1H), 7.50 (d, J = 6 Hz, 1H), 7.40−7.25 (m, 5H), 4.88 (d, J = 4 Hz, 2H), 2.76 (d, J = 8 Hz, 2H), 1.91 (m, 1H), 1.70−1.50 (m, 5H), 1.17−0.90 (m, 5H). Anal. (C$_{20}$H$_{23}$N$_3$S·HCl) C, H, N, S.

2-Benzyl-4-benzylaminothieno[3,2-*d*]pyrimidine (16) was prepared according to method H starting from **61** (2.15 g). Recrystallization from ethyl ether gave 0.93 mg (34%) of **16**: mp 125−126 °C; ^{1}H NMR (CDCl$_3$) δ 7.58 (d, J = 6 Hz, 1H), 7.40 (d, J = 8 Hz, 2H), 7.34 (d, J = 6 Hz, 1H), 7.30−7.15 (m, 8H), 5.74 (t, J = 6 Hz, 1H), 4.80 (d, J = 6 Hz, 2H), 4.20 (s, 2H). Anal. (C$_{20}$H$_{17}$N$_3$S) C, H, N, S.

4-Benzylamino-2-phenethylthieno[3,2-*d*]pyrimidine, Hydrochloride (17) was prepared according to method H starting from **62** (3.10 g). Recrystallization from isopropyl alcohol gave 1.98 g (46%) of **17**: mp 239−241 °C; ^{1}H NMR (DMSO) δ 15.67 (br, 1H), 10.56 (br, 1H), 8.37 (d, J = 6 Hz, 1H), 7.50 (d, J = 6 Hz, 1H), 7.40−7.10 (m, 10H), 4.84 (d, J = 6 Hz, 2H), 3.23 (m, 2H), 3.15 (m, 2H). Anal. (C$_{21}$H$_{19}$N$_3$S·HCl) C, H, N, S.

4-Benzylamino-2-(4-phenoxybutyl)thieno[3,2-*d*]pyrimidine, Hydrochloride (18) was prepared according to method H starting from **63** (2.04 g). Recrystallization from isopropyl alcohol gave 1.02 g (37%) of **18**: mp 220−222 °C; ^{1}H NMR (DMSO) δ 15.56 (br, 1H), 10.52 (br, 1H), 8.42 (d, J = 5 Hz, 1H), 7.52 (d, J = 5 Hz, 1H), 7.45−7.20 (m, 7H), 6.90−6.85 (m, 3H), 4.83 (d, J = 6 Hz, 2H), 3.93 (t, J = 6 Hz, 2H), 3.00 (t, J = 6 Hz, 2H), 1.95 (m, 2H), 1.73 (m, 2H). Anal. (C$_{23}$H$_{23}$N$_3$OS·HCl) C, H, N, S.

4-Benzylamino-2-phenylthieno[3,2-*d*]pyrimidine, Hydrochloride (19) was prepared according to method H

starting from **64** (2.56 g). Recrystallization from ethanol gave 1.36 g (37%) of **19**: mp 258 °C; ^{1}H NMR (DMSO) δ 10.44 (br, 1H), 8.55−8.35 (m, 3H), 7.82 (d, J = 6 Hz, 1H), 7.74−7.55 (m, 3H), 7.47 (d, J = 8 Hz, 2H), 7.35 (t, J = 8 Hz, 2H), 7.28 (m, 1H), 4.99 (d, J = 6 Hz, 2H). Anal. (C$_{19}$H$_{15}$N$_3$S·HCl) C, H, N, S.

4-Benzylamino-2-(3-pyridyl)thieno[3,2-d]pyrimidine, Hydrochloride (20) was prepared according to method H starting from **65** (2.20 g). Recrystallization from isopropyl alcohol gave 2.11 g (68%) of **20**: mp 227−229 °C; ^{1}H NMR (DMSO) δ 10.90 (br, 1H), 9.60 (br, 2H), 9.25 (d, J = 9 Hz, 1H), 9.00 (d, J = 5 Hz, 1H), 8.31 (d, J = 5 Hz, 1H), 8.09 (dd, J = 9 Hz, J = 5 Hz, 1H), 7.63 (d, J = 5 Hz, 1H), 7.48 (d, J = 7 Hz, 2H), 7.33 (t, J = 7 Hz, 2H), 7.23 (m, 1H), 4.90 (d, J = 5 Hz, 2H). Anal. (C$_{18}$H$_{14}$N$_4$S·1.3HCl·0.5H$_2$O) C, H, N, S.

4-Benzylamino-2-(4-pyridyl)thieno[3,2-d]pyrimidine, Hydrochloride (21) was prepared according to method H starting from **66** (3.30 g). Purification by flash chromatography, eluting with CH$_2$Cl$_2$/MeOH (95:5), gave 1.50 g (34%) of **21**: mp 270−272 °C (D); ^{1}H NMR (DMSO) δ 9.15 (br, 1H), 9.01 (d, J = 6 Hz, 2H), 8.82 (d, J = 6 Hz, 2H), 8.30 (d, J = 6 Hz, 1H), 7.60 (d, J = 6 Hz, 1H), 7.48 (d, J = 8 Hz, 2H), 7.33 (t, J = 8 Hz, 2H), 7.24 (m, 1H), 4.90 (d, J = 6 Hz, 2H). Anal. (C$_{18}$H$_{14}$N$_4$S·1.5HCl·0.4H$_2$O) C, H, N, S.

2-Butyl-4-(2-methoxybenzylamino)thieno[3,2-d]pyrimidine, Hydrochloride (23) was prepared according to method H starting from **55** (2.00 g) and 2-methoxybenzylamine. Recrystallization from acetonitrile gave 1.23 g (40%) of **23**: mp 184−186 °C; ^{1}H NMR (DMSO) δ 15.60 (br, 1H), 10.30 (br, 1H), 8.43 (d, J = 5 Hz, 1H), 7.53 (d, J = 5 Hz, 1H), 7.35−7.15 (m, 2H), 7.05 (d, J = 8 Hz, 1H), 6.90 (m, 1H), 4.80 (d, J = 5 Hz, 2H), 3.80 (s, 3H), 2.98 (t, J = 6 Hz, 2H), 1.69 (m, 2H), 1.29 (m, 2H), 0.83 (t, J = 6 Hz, 3H). Anal. (C$_{18}$H$_{21}$N$_3$OS·HCl) C, H, N.

2-Butyl-4-(3-methoxybenzylamino)thieno[3,2-d]pyrimidine, Hydrochloride (24) was prepared according to method H starting from **55** (2.00 g) and 3-methoxybenzylamine. Recrystallization from acetonitrile gave 1.94 g (52%) of **24**: mp 203−204 °C; ^{1}H NMR (DMSO) δ 15.67 (br, 1H), 10.56 (br, 1H), 8.46 (d, J = 6 Hz, 1H), 7.55 (d, J = 6 Hz, 1H), 7.24 (t, J = 9 Hz, 1H), 7.00 (s, 1H), 6.97 (d, J = 9 Hz, 1H), 6.87 (d, J = 9 Hz, 1H), 4.82 (d, J = 6 Hz, 2H), 3.73 (s, 3H), 2.93 (t, J = 8 Hz, 2H), 1.78 (m, 2H), 1.31 (m, 2H), 0.87 (t, J = 8 Hz, 3H). Anal. (C$_{18}$H$_{21}$N$_3$OS·HCl) C, H, N.

2-Butyl-4-(4-methoxybenzylamino)thieno[3,2-d]pyrimidine, Hydrochloride (25) was prepared according to method H starting from **55** (2.00 g) and 4-methoxybenzylamine. Recrystallization from acetonitrile gave 0.66 g (22%) of **25**: mp 245−246 °C; ^{1}H NMR (DMSO) δ 15.65 (br, 1H), 10.53 (br, 1H), 8.41 (d, J = 6 Hz, 1H), 7.53 (d, J = 6 Hz, 1H), 7.35 (d, J = 6 Hz, 2H), 6.90 (d, J = 6 Hz, 2H), 4.77 (d, J = 4 Hz, 2H), 3.74 (s, 3H), 2.93 (t, J = 8 Hz, 2H), 1.80 (m, 2H), 1.31 (m, 2H), 0.90 (t, J = 8 Hz, 3H). Anal. (C$_{18}$H$_{21}$N$_3$OS·HCl) C, H, N.

2-Butyl-4-(2-nitrobenzylamino)thieno[3,2-d]pyrimidine (26) was prepared according to method H starting from **55** (2.00 g) and 2-nitrobenzylamine.[56] Purification by flash chromatography, eluting with EtOAc/hexane (1:1), gave 0.57 g (19%) of **26**: mp 107−109 °C; ^{1}H NMR (CDCl$_3$) δ 8.08 (d, J = 8 Hz, 1H), 7.83 (d, J = 8 Hz, 1H), 7.65 (d, J = 6 Hz, 1H), 7.58 (t, J = 8 Hz, 1H), 7.44 (t, J = 8 Hz, 1H), 7.34 (d, J = 6 Hz, 1H), 5.80 (t, J = 8 Hz, 1H), 5.14 (d, J = 8 Hz, 2H), 2.86 (t, J = 8 Hz, 2H), 1.79 (m, 2H), 1.39 (m, 2H), 0.93 (t, J = 8 Hz, 3H). Anal. (C$_{17}$H$_{18}$N$_4$O$_2$S) C, H, N.

2-Butyl-4-(3-nitrobenzylamino)thieno[3,2-d]pyrimidine, Hydrochloride (27) was prepared according to method H starting from **55** (2.00 g) and 3-nitrobenzylamine. Recrystallization from acetonitrile gave 1.29 g (39%) of **27**: mp 205−207 °C; ^{1}H NMR (DMSO) δ 15.56 (br, 1H), 10.62 (br, 1H), 8.46 (d, J = 6 Hz, 1H), 8.30 (s, 1H), 8.15 (d, J = 8 Hz, 1H), 7.87 (d, J = 8 Hz, 1H), 7.65 (t, J = 8 Hz, 1H), 7.52 (d, J = 6 Hz, 1H), 4.95 (d, J = 4 Hz, 2H), 2.90 (t, J = 8 Hz, 2H), 1.71 (m, 2H), 1.26 (m, 2H), 0.84 (t, J = 8 Hz, 3H). Anal. (C$_{17}$H$_{18}$N$_4$O$_2$S·HCl) C, H, N.

2-Butyl-4-(4-nitrobenzylamino)thieno[3,2-d]pyrimidine (28) was prepared according to method H starting from **55** (2.00 g) and 4-nitrobenzylamine.[57] Purification by flash chromatography, eluting with EtOAc/hexane (1:1), gave 1.10 g (36%) of **28**: mp 136−138 °C; ^{1}H NMR (CDCl$_3$) δ 8.16 (d, J = 8 Hz, 2H), 7.67 (d, J = 6 Hz, 1H), 7.49 (d, J = 8 Hz, 2H), 7.33 (d, J = 6 Hz, 1H), 5.61 (br, 1H), 4.95 (d, J = 6 Hz, 2H), 2.82 (t, J = 8 Hz, 2H), 1.72 (m, 2H), 1.34 (m, 2H), 0.87 (t, J = 8 Hz, 3H). Anal. (C$_{17}$H$_{18}$N$_4$O$_2$S) C, H, N.

2-Butyl-4-(2,6-difluorobenzylamino)thieno[3,2-d]pyrimidine, Hydrochloride (29) was prepared according to method H starting from **55** (3.16 g) and 2,6-difluorobenzylamine. Recrystallization from acetonitrile gave 2.77 g (53%) of **29**: mp 225−227 °C; ^{1}H NMR (DMSO) δ 15.75 (br, 1H), 10.31 (br, 1H), 8.43 (d, J = 6 Hz, 1H), 7.52 (d, J = 6 Hz, 1H), 7.50 (m, 1H), 7.14 (m, 2H), 4.86 (d, J = 6 Hz, 2H), 2.92 (t, J = 9 Hz, 2H), 1.76 (m, 2H), 1.30 (m, 2H), 0.91 (t, J = 9 Hz, 3H). Anal. (C$_{17}$H$_{17}$F$_2$N$_3$S·HCl) C, H, N, S.

4-[(Biphenyl-4-yl)methylamino]-2-butylthieno[3,2-d]pyrimidine, Hydrochloride (30) was prepared according to method H starting from **55** (2.27 g) and (biphenyl-4-yl)-methylamine.[58] Recrystallization from ethyl alcohol gave 1.68 g (42%) of **30**: mp 274−276 °C; ^{1}H NMR (DMSO) δ 15.30 (br, 1H), 10.46 (br, 1H), 8.44 (d, J = 6 Hz, 1H), 7.65−7.62 (m, 4H), 7.55−7.40 (m, 5H), 7.35 (m, 1H), 4.87 (d, J = 5 Hz, 2H), 2.89 (t, J = 7 Hz, 2H), 1.75 (m, 2H), 1.29 (m, 2H), 0.85 (t, J = 7 Hz, 3H). Anal. (C$_{23}$H$_{23}$N$_3$S·HCl) C, H, N, S.

2-Butyl-4-[(4-pyridyl)methylamino]thieno[3,2-d]pyrimidine (31) was prepared according to method H starting from **55** (2.00 g) and (4-pyridyl)methylamine. Purification by flash chromatography, eluting with EtOAc/hexane (3:1), gave 0.24 g (9%) of **31**: mp 155−157 °C; ^{1}H NMR (CDCl$_3$) δ 8.53 (d, J = 4 Hz, 2H), 7.66 (d, J = 6 Hz, 1H), 7.38 (d, J = 6 Hz, 1H), 7.27 (d, J = 4 Hz, 2H), 5.75 (br, 1H), 4.90 (d, J = 6 Hz, 2H), 2.81 (t, J = 8 Hz, 2H), 1.73 (m, 2H), 1.35 (m, 2H), 0.91 (t, J = 8 Hz, 3H). Anal. (C$_{16}$H$_{18}$N$_4$S) C, H, S, N: calcd, 18.78; found, 18.25.

2-Butyl-4-cyclohexylaminothieno[3,2-d]pyrimidine, Hydrochloride (33) was prepared according to method H starting from **55** (1.20 g) and cyclohexylamine. Recrystallization from acetonitrile gave 0.57 g (33%) of **33**: mp 209−211 °C; ^{1}H NMR (DMSO) δ 15.36 (br, 1H), 9.67 (d, J = 8 Hz, 1H), 8.42 (d, J = 6 Hz, 1H), 7.50 (d, J = 6 Hz, 1H), 4.22 (br, 1H), 2.91 (t, J = 8 Hz, 2H), 1.95−1.65 (m, 7H), 1.50−1.10 (m, 7H), 0.92 (t, J = 8 Hz, 3H). Anal. (C$_{16}$H$_{23}$N$_3$S·HCl) C, H, N, S.

2-Butyl-4-cyclohexylmethylaminothieno[3,2-d]pyrimidine, Hydrochloride (34) was prepared according to method H starting from **55** (2.27 g) and cyclohexylmethylamine. Recrystallization from acetonitrile gave 2.11 g (62%) of **34**: mp 241−243 °C; ^{1}H NMR (DMSO) δ 15.44 (br, 1H), 9.92 (br, 1H), 8.41 (d, J = 6 Hz, 1H), 7.50 (d, J = 6 Hz, 1H), 3.47 (m, 2H), 2.90 (t, J = 8 Hz, 2H), 1.86−1.57 (m, 8H), 1.38 (m, 2H), 1.16 (m, 2H), 1.08−0.87 (m, 6H). Anal. (C$_{17}$H$_{25}$N$_3$S·HCl) C, H, N, S.

2-Butyl-4-(piperidin-1-yl)thieno[3,2-d]pyrimidine, Hydrochloride (35) was prepared according to method H starting from **55** (4.07 g) and piperidine. Purification by flash chromatography, eluting with EtOAc/hexane (1:2), gave 2.62 g (52%) of **35**: mp 218−220 °C; ^{1}H NMR (DMSO) δ 15.77 (br, 1H), 8.55 (d, J = 6 Hz, 1H), 7.62 (d, J = 6 Hz, 1H), 4.10 (br, 4H), 2.90 (t, J = 8 Hz, 2H), 1.85−1.65 (m, 8H), 1.38 (m, 2H), 0.94 (t, J = 8 Hz, 3H). Anal. (C$_{15}$H$_{21}$N$_3$S·HCl) C, H, N, S.

2-Butyl-4-(1,2,3,4-tetrahydroisoquinolin-2-yl)thieno[3,2-d]pyrimidine, Hydrochloride (36) was prepared according to method H starting from **55** (2.26 g) and tetrahydroisoquinoline. Recrystallization from acetone gave 1.99 g (56%) of **36**: mp 212−214 °C; ^{1}H NMR (DMSO) δ 15.74 (br, 1H), 8.60 (d, J = 6 Hz, 1H), 7.61 (d, J = 6 Hz, 1H), 7.36−7.24 (m, 4H), 5.25 (s, 2H), 4.30 (t, J = 6 Hz, 2H), 3.10 (t, J = 6 Hz, 2H), 2.94 (t, J = 8 Hz, 2H), 1.84 (m, 2H), 1.40 (m, 2H), 0.93 (t, J = 8 Hz, 3H). Anal. (C$_{19}$H$_{21}$N$_3$S·HCl) C, H, N, S.

4-[(N-Benzyl-N-methyl)amino]-2-butylthieno[3,2-d]pyrimidine, Hydrochloride (37) was prepared according to method H starting from **55** (2.00 g) and N-benzyl-N-methyl-

amine. Recrystallization from acetonitrile gave 1.65 g (54%) of **37**: mp 209−211 °C; ^{1}H NMR (DMSO) δ 15.70 (br, 1H), 8.35 (d, $J = 6$ Hz, 1H), 7.60 (d, $J = 6$ Hz, 1H), 7.46−7.28 (m, 5H), 4.20 (s, 2H), 3.61 (s, 3H), 2.90 (t, $J = 9$ Hz, 2H), 1.75 (m, 2H), 1.32 (m, 2H), 0.87 (t, $J = 9$ Hz, 3H). Anal. ($C_{18}H_{21}N_3S \cdot HCl$) C, H, N, S.

4-[(*N*-Benzyl-*N*-propyl)amino]-2-butylthieno[3,2-*d*]pyrimidine, Hydrochloride (38) was prepared according to method H starting from **55** (3.00 g) and *N*-benzyl-*N*-propylamine. Purification by flash chromatography, eluting with EtOAc/hexane (2:1), gave 1.85 g (37%) of **38**: mp 108−110 °C; ^{1}H NMR (DMSO) δ 15.60 (br, 1H), 8.52 (br, 1H), 7.57 (d, $J = 6$ Hz, 1H), 7.35−7.25 (m, 6H), 5.21 (s, 2H), 3.83 (t, $J = 8$ Hz, 2H), 2.88 (t, $J = 7$ Hz, 2H), 1.82−1.65 (m, 4H), 1.29 (m, 2H), 0.94 (t, $J = 8$ Hz, 3H), 0.84 (t, $J = 7$ Hz, 3H). Anal. ($C_{20}H_{25}N_3S \cdot HCl$) C, H, N, S.

4-Benzylamino-2-butylpteridine, Hydrochloride (5) was prepared according to method H starting from **68** (2.25 g) and benzylamine. Recrystallization from ethyl alcohol gave 1.15 g (35%) of **5**: mp 249−251 °C (D); ^{1}H NMR (DMSO) δ 11.11 (br, 1H), 9.15 (d, $J = 2$ Hz, 1H), 9.03 (d, $J = 2$ Hz, 1H), 7.44 (d, $J = 8$ Hz, 2H), 7.35−7.25 (m, 3H), 4.89 (d, $J = 6$ Hz, 2H), 2.91 (t, $J = 7$ Hz, 2H), 1.76 (m, 2H), 1.34 (m, 2H), 0.88 (t, $J = 7$ Hz, 3H). Anal. ($C_{17}H_{19}N_5 \cdot HCl$) C, H, N.

2-Benzyl-4-benzylamino-9*H*-purine, Hydrochloride (6) was prepared according to method H starting from **67** (1.69 g) and benzylamine. Recrystallization from isopropyl alcohol gave 0.52 g (22%) of **6**: mp 255−256 °C; ^{1}H NMR (DMSO) δ 10.24 (br, 1H), 8.55 (br, 1H), 7.40−7.20 (m, 11H), 5.60 (d, $J = 4$ Hz, 2H), 4.20 (s, 2H). Anal. ($C_{19}H_{17}N_5 \cdot HCl$) C, H, N.

Method I. 2-Butyl-4-phenylaminothieno[3,2-*d*]pyrimidine, Hydrochloride (32). To a solution of 2.00 g (8.82 mmol) of **55** and 0.80 mL (8.82 mmol) of aniline in 20 mL of acetic acid were added 0.72 g (8.82 mmol) of sodium acetate and 50 mg of potassium iodide. The mixture was stirred under reflux for 20 h, cooled, and concentrated. The residue was partitioned between EtOAc and water; the organic phase was washed with brine, dried, and concentrated. To the residue dissolved in acetone was added HCl in dioxane. The resulting solid was recrystallized from isopropyl alcohol to give 2.38 g (84%) of the title compound: mp 235−237 °C; ^{1}H NMR (DMSO) δ 11.52 (br, 1H), 8.49 (d, $J = 4$ Hz, 1H), 7.72 (d, $J = 6$ Hz, 2H), 7.60 (d, $J = 4$ Hz, 1H), 7.47 (t, $J = 6$ Hz, 2H), 7.32 (m, 1H), 2.94 (t, $J = 8$ Hz, 2H), 1.80 (m, 2H), 1.37 (m, 2H), 0.90 (t, $J = 8$ Hz, 3H). Anal. ($C_{16}H_{17}N_3S \cdot HCl$) C, H, N.

Method J. 2-Butyl-4-[(*N*,*N*-dibenzyl)amino]thieno[3,2-*d*]pyrimidine, Hydrochloride (39). To a solution of 2.00 g (8.82 mmol) of **55** and 1.84 mL (9.30 mmol) of dibenzylamine in 50 mL of DMF was added 2.43 g (17.6 mmol) of K_2CO_3. The mixture was stirred at 140 °C for 24 h. The resulting suspension was poured into water and extracted with EtOAc. The combined extracts were washed, dried, and concentrated. The residue was purified by flash chromatography, eluting with EtOAc/hexane (1:2). The resulting solid was dissolved in acetone, and HCl in dioxane was added. The crystallized hydrochloride was washed and dried to give 0.84 g (23%) of the title compound: mp 116−118 °C; ^{1}H NMR (DMSO) δ 8.46 (d, $J = 5$ Hz, 1H), 7.60 (d, $J = 5$ Hz, 1H), 7.45−7.25 (m, 11H), 5.20 (s, 4H), 2.95 (t, $J = 8$ Hz, 2H), 1.71 (m, 2H), 1.30 (m, 2H), 0.83 (t, $J = 8$ Hz, 3H). Anal. ($C_{24}H_{25}N_3S \cdot HCl \cdot 0.75H_2O$) H, S, C: calcd, 65.82; found, 65.34; N: calcd, 9.60; found, 9.15.

2-Butyl-3*H*-thieno[3,2-*d*]pyrimidin-4-thione (69). To a solution of 3.08 g (0.0127 mol) of **41** in 75 mL of toluene was added 3.84 g (9.50 mmol) of Lawesson's reagent. The mixture was stirred under reflux for 1.5 h. The solution was washed twice with 2 N NaOH, and the combined basic extracts were neutralized with 2 N HCl. The resulting yellow solid was filtered, washed, and dried to give 3.15 g (95%) of **69**: ^{1}H NMR (CDCl3) δ 12.27 (br, 1H), 7.96 (d, $J = 5$ Hz, 1H), 7.38 (d, $J = 5$ Hz, 1H), 2.89 (t, $J = 8$ Hz, 2H), 1.86 (m, 2H), 1.48 (m, 2H), 0.97 (t, $J = 8$ Hz, 3H).

General Procedure for the Alkylation of Pyrimidinones 41 and 43 and Pyrimidinthione 69. Method K. 3-Benzyl-2-butyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (1). To

a solution of 4.84 g (0.023 mol) of **41** and 5.50 mL (0.023 mol) of benzyl bromide in 50 mL of DMF was added 6.35 g (0.046 mol) of K_2CO_3. The mixture was stirred at room temperature overnight. The resulting suspension was poured into water and extracted with EtOAc. The combined extracts were washed, dried, and concentrated. Recrystallization from *n*-heptane gave 3.22 g (47%) of the title compound: mp 82−84 °C; ^{1}H NMR (CDCl3) δ 7.75 (d, $J = 6$ Hz, 1H), 7.35−7.15 (m, 6H), 5.61 (s, 2H), 2.73 (t, $J = 8$ Hz, 2H), 1.70 (m, 2H), 1.36 (m, 2H), 0.88 (t, $J = 8$ Hz, 3H). Anal. ($C_{17}H_{18}N_2OS$) C, H, N.

3-Benzyl-2-butyl-3*H*-quinazolin-4-one (2) was prepared according to method K starting from **43** (3.70 g) and benzyl bromide. Purification by flash chromatography, eluting with EtOAc/hexane (1:3), gave 2.32 g (43%) of the title compound: mp 80−81 °C; ^{1}H NMR (CDCl3) δ 8.30 (d, $J = 8$ Hz, 1H), 7.75−7.65 (m, 2H), 7.45 (t, $J = 8$ Hz, 1H), 7.35−7.22 (m, 3H), 7.18 (d, $J = 8$ Hz, 2H), 5.40 (s, 2H), 2.74 (t, $J = 8$ Hz, 2H), 1.75 (m, 2H), 1.39 (m, 2H), 0.90 (t, $J = 8$ Hz, 3H). Anal. ($C_{19}H_{20}N_2O$) C, N, H: calcd, 6.90; found, 7.34.

4-Benzylthio-2-butylthieno[3,2-*d*]pyrimidine (9) was prepared according to method K starting from **69** (3.15 g) and benzyl bromide. Crystallization from pentane at −50 °C gave 2.90 g (66%) of the title compound: mp 53−55 °C; ^{1}H NMR (CDCl3) δ 7.77 (d, $J = 6$ Hz, 1H), 7.55−7.15 (m, 6H), 4.74 (s, 2H), 3.09 (t, $J = 8$ Hz, 2H), 1.91 (m, 2H), 1.47 (m, 2H), 0.99 (t, $J = 8$ Hz, 3H). Anal. ($C_{17}H_{18}N_2S_2$) C, H, N.

4-Benzyloxy-2-butylthieno[3,2-*d*]pyrimidine (10). A suspension of 0.45 g (0.011 mol) of 60% NaH in 40 mL of anhydrous DMSO was stirred under nitrogen at 60 °C for 1 h. The mixture was cooled to room temperature prior to the addition of 1.05 mL (0.01 mol) of benzyl alcohol. After the mixture was stirred for 15 min, a solution of 2.30 g (0.01 mol) of **55** in 10 mL of DMSO was added, and the mixture was stirred overnight. The reaction mixture was partitioned between EtOAc and water; the organic phase was washed, dried, and concentrated. The residue was purified by flash chromatography, eluting with EtOAc/hexane (1:3). Crystallization of the resulting oil from pentane at −30 °C gave 1.57 g (52%) of the title compound: mp 34−36 °C; ^{1}H NMR (CDCl3) δ 7.78 (d, $J = 6$ Hz, 1H), 7.48−7.28 (m, 6H), 5.64 (s, 2H), 2.96 (t, $J = 8$ Hz, 2H), 1.86 (m, 2H), 1.42 (m, 2H), 0.96 (t, $J = 8$ Hz, 3H). Anal. ($C_{17}H_{18}N_2SO$) C, H, N.

3-(2-Chloroacetamido)-2-thiophenecarboxamide (70). To a cooled solution of 1.00 g (7 mmol) of 3-amino-2-thiophenecarboxamide and 1.01 g (10 mmol) of triethylamine in 25 mL of anhydrous THF was added a solution of 1.17 g (10 mmol) of 2-chloroacetyl chloride in 5 mL of anhydrous THF. The mixture was warmed to room temperature and stirred for 2 h. The resulting triethylamine hydrochloride was filtered, and the organic solution was concentrated. The residue was partitioned between EtOAc and water; the organic phase was washed with 2 N HCl, 2 N NaOH, and brine, dried, and concentrated. The resulting white solid was washed with diisopropyl ether to give 1.38 g (90%) of the title compound: ^{1}H NMR (CDCl3) δ 8.26 (br, 1H), 8.30 (d, $J = 5$ Hz, 1H), 7.40 (d, $J = 5$ Hz, 1H), 6.66 (br, 2H), 4.10 (s, 2H).

3-[2-(Piperidin-1-yl)acetamido]-2-thiophenecarboxamide (71). To a solution of 1.38 g (6.32 mmol) of **70** and 0.54 g (6.32 mmol) of piperidine in 30 mL of anhydrous acetonitrile was added 0.87 g (6.32 mmol) of K_2CO_3. The mixture was stirred under reflux for 7 h. The resulting suspension was partitioned between EtOAc and water; the organic phase was dried and concentrated. The resulting solid was washed with diisopropyl ether to give 1.11 g (66%) of **71**: ^{1}H NMR (CDCl3) δ 11.91 (br, 1H), 8.27 (d, $J = 6$ Hz, 1H), 7.35 (d, $J = 6$ Hz, 1H), 5.60 (br, 2H), 3.12 (s, 2H), 2.50 (t, $J = 6$ Hz, 4H), 1.72 (m, 4H), 1.50 (m, 2H).

2-(Piperidin-1-yl)methyl-3*H*-thieno[3,2-*d*]pyrimidin-4-one (72). A suspension of 1.1 g (4.1 mmol) of **71** in 20 mL of 2 N NaOH was stirred under reflux for 20 min. The resulting solution was cooled, neutralized with 2 N HCl, and extracted with EtOAc. The combined extracts were washed with brine, dried, and concentrated to give 0.85 g (83%) of **72** as a white solid: ^{1}H NMR (CDCl3) δ 7.80 (d, $J = 6$ Hz, 1H), 7.30 (d, $J = $

6 Hz, 1H), 3.54 (s, 2H), 2.51 (t, $J = 5$ Hz, 4H), 1.64 (m, 4H), 1.50 (m, 2H).

4-Chloro-2-[(piperidin-1-yl)methyl]thieno[3,2-*d*]pyrimidine (73) was prepared according to method E in 79% yield starting from thieno[3,2-*d*]pyrimidinone **72** (2.97 g): ^{1}H NMR (CDCl$_3$) δ 8.01 (d, $J = 6$ Hz, 1H), 7.61 (d, $J = 6$ Hz, 1H), 3.90 (s, 2H), 2.56 (t, $J = 5$ Hz, 4H), 1.65 (m, 4H), 1.46 (m, 2H).

4-Benzylamino-2-[(piperidin-1-yl)methyl]thieno[3,2-*d*]-pyrimidine, hydrochloride (22) was prepared according to method H starting from **73** (2.51 g). Recrystallization from isopropyl alcohol gave 1.96 g (52%) of **22**: mp 253−257 °C; ^{1}H NMR (DMSO) δ 10.72 (br, 1H), 9.97 (br, 1H), 8.37 (d, $J = 6$ Hz, 1H), 7.50 (d, $J = 6$ Hz, 1H), 7.40−7.20 (m, 5H), 4.91 (d, $J = 6$ Hz, 2H), 4.47 (s, 2H), 3.26 (br, 4H), 1.73 (br, 4H), 1.47 (br, 2H). Anal. (C$_{19}$H$_{22}$N$_4$S·1.7HCl·0.6H$_2$O) C, H, N, S.

4-Amino-2-butylthieno[3,2-*d*]pyrimidine (74). A solution of 3.00 g (0.0132 mol) of **55** in 50 mL of ethyl alcohol and 50 mL of 30% NH$_3$ was stirred at 120 °C for 4 h in a pressure reactor. The mixture was cooled and partitioned between EtOAc and water. The organic phase was washed with brine, dried, and concentrated to give 2.05 g (75%) of **74** as a brown solid: ^{1}H NMR (CDCl$_3$) δ 7.74 (d, $J = 6$ Hz, 1H), 7.35 (d, $J = 6$ Hz, 1H), 6.28 (br, 2H), 2.83 (t, $J = 8$ Hz, 2H), 1.80 (m, 2H), 1.43 (m, 2H), 0.95 (t, $J = 8$ Hz, 3H).

4-Benzamido-2-butylthieno[3,2-*d*]pyrimidine, Hydrochloride (40). To a solution of 2.00 g (9.70 mmol) of **74** in 20 mL of anhydrous THF was added 6.64 mL (0.011 mmol) of 1.6 M butyllithium at −78 °C. The mixture was stirred for 0.5 h at the same temperature, prior to the addition of a solution of 1.3 mL (0.011 mol) of benzoyl chloride in 10 mL of anhydrous THF. The resulting solution was stirred at −78 °C for 0.5 h and at room temperature overnight. The mixture was concentrated and partitioned between EtOAc and water. The organic phase was washed with brine, dried, and concentrated. The residue was purified by flash chromatography, eluting with EtOAc/hexane (1:3). To the resulting solid dissolved in ethyl ether and EtOAc was added 6 N HCl to provide 0.46 g (15%) of the title compound: mp 103−105 °C; ^{1}H NMR (DMSO) δ 11.48 (br, 1H), 8.35 (d, $J = 5$ Hz, 1H), 8.10 (d, $J = 8$ Hz, 2H), 7.66 (m, 1H), 7.55 (t, $J = 8$ Hz, 2H), 7.50 (d, $J = 5$ Hz, 1H), 2.93 (t, $J = 8$ Hz, 2H), 1.82 (m, 2H), 1.38 (m, 2H), 0.93 (t, $J = 8$ Hz, 3H). Anal. (C$_{17}$H$_{17}$N$_3$OS·HCl) C, H, N.

3-Benzyl-2-butyl-3*H*-pyrido[3,2-*d*]pyrimidin-4-one, Hydrochloride (3). A suspension of 2.50 g (0.018 mol) of 3-amino-2-pyridincarboxylic acid in 15 mL of valeric anhydride was stirred at 140 °C for 1 h. The mixture was cooled, and the excess of the valeric anhydride was distilled. The resulting solid was suspended in 1.25 mL (0.018 mol) of benzylamine and stirred at 200 °C for 1 h. The residue was purified by flash chromatography, eluting with EtOAc/hexane (2:3). To the resulting oil dissolved in acetone was added HCl in dioxane to give 0.49 g (8%) of **3**: mp 204−206 °C; ^{1}H NMR (DMSO) δ 9.07 (d, $J = 5$ Hz, 1H), 8.77 (d, $J = 8$ Hz, 1H), 7.72 (dd, $J = 5$ Hz, $J = 8$ Hz, 1H), 7.45−7.10 (m, 5H), 5.44 (s, 2H), 2.88 (t, $J = 8$ Hz, 2H), 1.67 (m, 2H), 1.31 (m, 2H), 0.81 (t, $J = 8$ Hz, 3H). Anal. (C$_{18}$H$_{19}$N$_3$O·HCl) C, H, N.

4-Benzyl-2-propyl-4*H*-pyrido[2,3-*b*]pyrazin-3-one (7). To a solution of 2.23 g (0.016 mol) of 2-oxopentanoic acid, sodium salt, in 10 mL of EtOH and 8 mL of 2 N HCl was added a solution of 3.22 g (0.016 mol) of 3-amino-2-benzylaminopyridine[50] in 35 mL of EtOH. The mixture was stirred under reflux overnight, cooled, and concentrated. The residue was extracted with EtOAc. The combined extracts were washed, dried, and concentrated. The resulting solid was purified by flash chromatography eluting with EtOAc/hexane (1:3) to provide **7** as a white solid: yield 0.86 g (19%); mp 90−91 °C; ^{1}H NMR (DMSO) δ 8.56 (d, $J = 6$ Hz, 1H), 8.43 (d, $J = 8$ Hz, 1H), 7.42 (dd, $J = 8$ Hz, $J = 6$ Hz, 1H), 7.40−7.15 (m, 5H), 5.55 (s, 2H), 2.86 (t, $J = 8$ Hz, 2H), 1.76 (m, 2H), 0.98 (t, $J = 8$ Hz, 3H). Anal. (C$_{17}$H$_{17}$N$_3$O) C, H, N.

Ethyl 2-(2-nitrophenylamino)pentanoate (75). To a solution of 4.94 g (0.035 mol) of 1-fluoro-2-nitrobenzene and 8.30 g (0.070 mol) of D,L-norvaline in 125 mL of EtOH and 30

mL of H$_2$O was added 14.1 g (0.168 mol) of NaHCO$_3$. The mixture was stirred under reflux for 66 h. The resulting red suspension was concentrated, acidified with 2 N HCl, and extracted with EtOAc. The combined extracts were washed twice with brine, dried, and concentrated. The resulting yellow solid was dissolved in 100 mL of EtOH, and the solution cooled at 0 °C. To the mixture was added 6 mL (0.0825 mol) of thionyl chloride, and the solution was stirred at room temperature overnight. The reaction mixture was concentrated and diluted with EtOAc. The resulting solution was washed twice with brine, dried, and concentrated to give 8.30 g (86%) of **75** as an orange oil: ^{1}H NMR (CDCl$_3$) δ 8.30 (d, $J = 8$ Hz, 1H), 8.17 (d, $J = 8$ Hz, 1H), 7.44 (m, 1H), 6.77−6.67 (m, 2H), 4.22 (m, 3H), 1.92 (m, 2H), 1.50 (m, 2H), 1.27 (t, $J = 8$ Hz, 3H), 0.98 (t, $J = 8$ Hz, 3H).

3-Propyl-1*H*-quinoxalin-2-one (76). To a solution of 8.30 g (0.031 mol) of ethyl pentanoate **75** in 50 mL of EtOH were added 4.0 g (0.072 mol) of iron and 30 mL of 6 N HCl in EtOH. The mixture was stirred under reflux overnight, cooled, and concentrated. The resulting residue was diluted with 100 mL of water, and the pH was adjusted to 5 with NaHCO$_3$. The suspension was extracted with EtOAc, and the combined extracts were washed with brine, dried, and concentrated. The resulting solid was suspended in 70 mL of 2 N NaOH, and the suspension warmed to 80 °C prior to the addition of 4.70 mL (0.054 mol) of 35% of H$_2$O$_2$. The mixture was stirred at 80 °C for 3 h, cooled, and neutralized with 2 N HCl. The resulting white solid was filtered, washed with water, and dried to give 4.20 g (72%) of the desired product **76**: ^{1}H NMR (CDCl$_3$) δ 12.00 (br, 1H), 7.74 (d, $J = 8$ Hz, 1H), 7.20−7.15 (m, 3H), 2.85 (t, $J = 7$ Hz, 2H), 1.92 (m, 2H), 1.04 (t, 3H).

1-Benzyl-3-propyl-1*H*-quinoxalin-2-one (8). To a solution of 4.2 g (0.022 mol) of quinoxaline **76** in 75 mL of DMF were added 3.81 g (0.022 mol) of benzyl bromide and 6.15 g (0.045 mol) of K$_2$CO$_3$. The mixture was stirred at room temperature overnight. The suspension was poured into 200 mL of water, and extracted with EtOAc. The combined extracts were washed with brine, dried, and concentrated. The solid residue was washed with pentane, filtered, and recrystallized from EtOH to give 1.2 g (20%) of the title compound as a colorless solid: mp 101−102 °C; ^{1}H NMR (CDCl$_3$) δ 7.83 (d, $J = 8$ Hz, 1H), 7.40−7.20 (m, 8H), 5.49 (s, 2H), 2.98 (t, $J = 8$ Hz, 2H), 1.87 (m, 2H), 1.07 (t, $J = 8$ Hz, 3H). Anal. (C$_{18}$H$_{18}$N$_2$O) C, H, N.

Biology. Milrinone was obtained from IMPEX Química (Barcelona, Spain). Rolipram and RS-14491 were synthesized in the Medicinal Chemistry Department of Almirall-Prodesfarma. Reagents were purchased from commercial suppliers and used as received.

Purification and characterization of cyclic nucleotide phosphodiesterases 3 and 4 and PDE 3 and PDE 4 enzyme assays have been described previously.[36,59,60] The [^{3}H]Rolipram displacement was performed according to described protocols.[36,61]

Cyclic AMP Accumulation. Preparation of Guinea Pig Eosinophils. Male Dunkin-Hartley guinea pigs (300−400 g) were injected intraperitoneally with 0.5 mL of donor horse serum once per week for 6−7 weeks. Four days after the last injection, the animals were anaesthetized with ether and killed by jugular vein bleeding. A ventral incision was made and 80 mL of HBSS (Hank's balanced salt serum) without Ca^{2+} and Mg^{2+} was poured into the abdominal cavity. The abdomen was gently massaged for 5 min and then the peritoneal exudate was aspirated and centrifuged at 250 G for 10 min at 4 °C. The supernatant was discarded, and the pellet was resuspended in 2 mL of RPMI-1640 medium containing 10% fetal bovine serum (FBS). The enriched eosinophil fraction was separated using discontinuous gradients of Percoll. For the preparation of gradients, 9 parts of Percoll were mixed with 1 part of 1.5 M NaCl, and the mixture was diluted with RPMI-1640 containing 10% FBS to obtain densities of 1.075, 1.085, and 1.105 g/mL. Starting with the most dense layer, 2 mL of decreasing densities of Percoll were carefully layered into a 15 mL conical centrifuge tube, on top of which the cells were placed in a 2 mL volume. The gradients

were centrifuged in a swing-out rotor at 425 G for 20 min at 18 °C. The eosinophil-rich cell band was collected and washed twice with HBSS containing Ca^{2+} and Mg^{2+}. Cell viability, as determined by Trypan Blue exclusion, was greater than 98%, and eosinophil purity, as determined by May-Grünwald-Giemsa staining, was greater than 95%.

Determination of Eosinophil Cyclic AMP Accumulation. Freshly prepared eosinophils (($1-2$) × 10⁶), resuspended in HBSS containing Ca^{2+} and Mg^{2+}, were incubated with the compounds at the indicated concentrations for 10 min at 37 °C. Incubations were continued for a another 2 min in the presence of isoprenaline (10 μM) and terminated by addition of two volumes of ice-cold ethanol. The samples were centrifuged (2000 G) for 15 min at 4 °C, and the supernatant was transferred to a clean tube. The samples were dried by gassing with nitrogen at 60 °C, and the pellet was resuspended in water. Cyclic AMP was quantified using an enzyme-immunoassay kit from Amersham Life Sciences (U.K.) following the protocol without acetylation. For the calculations, the cAMP levels obtained in the presence of isoprenaline were considered as 100%, and the values obtained in the presence of compounds were corrected accordingly. EC_{50} values were calculated by adjusting the data to a sigmoid curve using the program Inplot. Four independent measurements made in two different days were obtained for each drug concentration.

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