Novel Heterocyclic-Fused Pyridazinones as Potent and Selective Phosphodiesterase IV Inhibitors

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A series of 6-aryl-4,5-heterocyclic-fused pyridazinones were designed and synthesized as selective phosphodiesterase (PDE) IV inhibitors. Biological evaluation of these compounds demonstrated a good selectivity profile toward the PDE IV family and greatly attenuated affinity for the Rolipram high-affinity binding site that seems to be responsible for undesiderable side effects. Structure−activity relationships (SARs) studies showed that the presence of an ethyl group at pyridazine N-2 is associated with the best potency and selectivity profile.

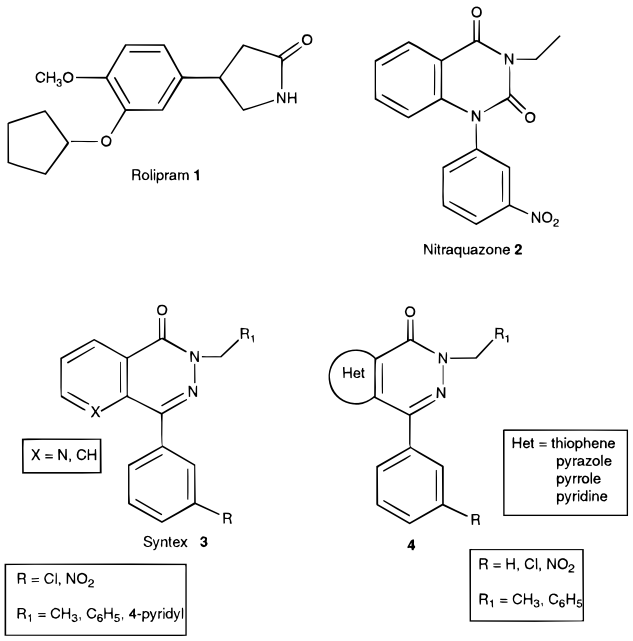
Introduction

Phosphodiesterases (PDEs) are enzymes responsible for the hydrolysis of cyclic adenosine monophosphate (c-AMP) and cyclic guanosine monophosphate (c-GMP), which are second messengers involved in the regulation of important cell functions, such as secretion, contraction, metabolism, and growth.[1] At least seven different families of PDEs are known at present, characterized by different tissue distribution as well as by different functional roles.[2,3]

Presently the interest in therapeutic utility of PDEs inhibitors (PDEIs) is mostly focused on new agents selectively inhibiting the PDE IV family,[4] one of the c-AMP specific PDEs. These inhibitors are promising drugs in the treatment of asthma, inflammation, and several CNS pathologies: in fact PDE IV isoenzyme is particulary abundant in brain[5] and immunocompetent cells such as neutrophiles,[6] T-lymphocytes,[7] macrophages,[8] and eosinophiles,[9] where PDE IV inhibitors reduce the synthesis and release of proinflammatory mediators, cytokines, and active oxygen species.[2] Moreover they modulate "in vitro" the tone of bronchial smooth muscle tissue and have "in vivo" bronchodilatory effects.[10] Since inflammation is generally accepted as an important component in the pathogenesis of asthma,[11] these data seem to confirm the therapeutic utility of PDE IV inhibitors in the bifunctional treatment of asthma.

Rolipram (**1**), which is the best known PDE IV inhibitor, was clinically tested as an antidepressant several years before the discovery of its potent and selective PDE IV inhibitory activity.[12] Rolipram and its analogs,[13,14] as well as Rolipram structurally unrelated PDE IV inhibitors, like Nitraquazone (**2**)[15,16] and congeners **3**,[17] exhibit Rolipram binding site affinity in the nanomolar range for preparation of animal and human brain tissue homogenate. Very recently the high-affinity Rolipram binding site has been associated with emesis and nausea, which are common side effects of PDE IV inhibitors. Thus it appears particularly important to dissociate affinity for this binding site from PDE IV inhibitory activity in order to obtain more selective agents potentially useful as antiasthmatics. This goal was recently reached with several Rolipram analogs.[18,19]

Chart 1



In connection with our studies on the chemistry and pharmacology of pyridazine derivatives,[20−22] we synthesized a group of heterocyclic-fused pyridazinones **4** structurally related to **2** and **3**. Compounds **4** still retain the pyridazione ring, present in **3**, from which they significantly differ in the condensed system, which was modified with the aim to possibly have potent and selective PDE IV inhibitors with attenuated affinity for Rolipram binding site.

Chemistry

Compounds **5a**−**c** were synthesized by 1,3-dipolar cycloaddition between the appropriate nitriloxide[23] and the suitable 1,3-diketone.[24] Ring closure of **5** with hydrazine, followed by treatment with the appropriate alkyl bromide, afforded **7a**−**d**. Following a procedure previously reported by us,[25] oxidative cleavage of the isoxazole ring with cerium ammonium nitrate (CAN) gave the key intermediates **8a**−**d** (Scheme 1).

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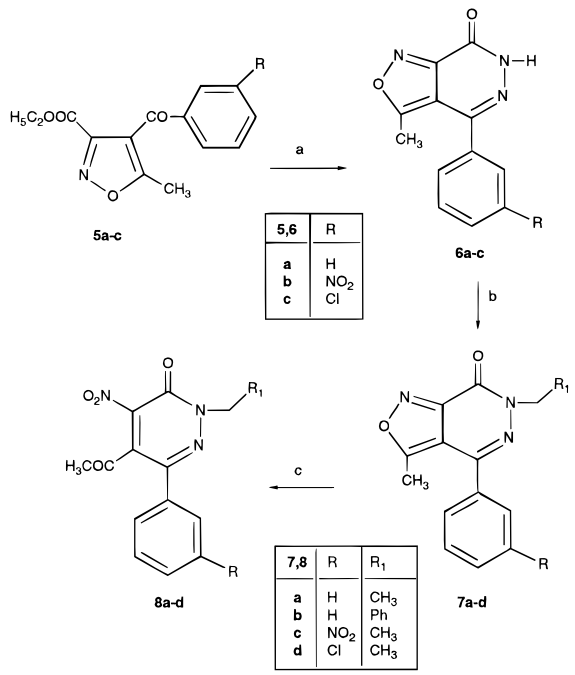
Scheme 1[a]



[a] (a) NH_2NH_2; (b) alkyl bromide; (c) CAN.

Compounds **5a**, **6a**, **7b**, and **8b** were previously described.[26−28]

Treatment of **8a**−**d** with sodium ethyl thioglycolate in absolute ethanol at room temperature afforded the thienopyridazinones **9a**−**d** in moderate yields. Following similar reaction conditions pyrazolopyridazinones **10a**−**d** were synthesized using hydrazine or methylhydrazine. Finally, the synthesis of pyrrolopyridazinones **11a**,**b** and pyridopyridazinone **12** was performed, generally in good yield, in two steps: treatment with sarcosine ethyl ester and *N*-methyl-*β*-alaninenitrile, respectively, in ethanol at room temperature, followed by short heating with sodium ethoxide in ethanol.

Biological Results and Discussion

All of the final compounds were tested as PDE III and PDE IV inhibitors and evaluated for their ability to displace [^{3}H]Rolipram from its binding site. Rolipram, Milrinone,[29] and Syntex compound **3** (X = N, R = Cl, R_1 = 4-pyridyl)[17] were tested as reference substances (see Table 1).

Unlike compounds **3**, all compounds substituted at pyridazine N-2 with an ethyl group, with the exception of only **10c**, were 12−50-fold more potent as PDE IV inhibitors with respect to the corresponding benzyl analogs, which also showed lower selectivity. In the series of pyrazolopyridazinones, replacement of hydrogen in compound **10a** with a methyl group (**10b**) brought about a 2.5-fold reduction of activity. The same structural modification performed on **10c** led to a 60-fold reduction of potency (**10d**).

In the thienopyridazinones series, compound **9a** displayed high potency but low selectivity; introduction at the meta position of the phenyl ring of a nitro (**9c**) or a chlorine substituent (**9d**) left the potency versus PDE IV unchanged, but strongly enhanced the selectivity.

The pyrrolopyridazinone **11a** was the most interesting compound, having a IC_{50} for PDE IV in the same submicromolar range of Rolipram and showing a good selectivity profile (PDE III/PDE IV > 30). Moreover this compound, as well as **9c**,**d**, **10a**,**c**, and **12**, which also emerged for their good balance of potency and selectivity versus PDE IV, displayed an affinity for Rolipram binding site by 2 orders of magnitude lower than that of compound **3** (X = N, R = Cl, R_1 = 4-pyridyl) and Rolipram. It should be highlighted that for the majority of our compounds a remarkable better balance between potency and selectivity was evidenced, with respect to the reference drugs, as clearly indicated by low value of PDE IV/[^{3}H]Rolipram ratio.

Due to their structural resemblance with xanthines and analogs, the synthesized compounds were also preliminarly evaluated for their affinity toward A_1 and A_2 adenosine receptors. The absence of significant affinity (data not shown) confirmed the interesting profile of these derivatives as potential antiasthmatic and anti-inflammatory agents, devoided of CNS and cardiovascular side effects.

In conclusion these data seem to demonstrate that for compounds structurally related to **2** and **3** it is possible to dissociate the affinity for [^{3}H]Rolipram binding site and PDE IV inhibitory activity.

Further structure−activity relationships studies as well as the in vivo evaluation of some selected compounds are in progress.

Experimental Section

Chemistry. All melting points were determined with Buchi 510 melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded as Nujol mulls with a Perkin-Elmer spectrometer. Nuclear magnetic resonance (^{1}H-NMR) spectra were obtained using Gemini 200 spectrometer in $CDCl_3$. Chemical shift values are reported in ppm (δ). Analyses indicated by the symbols of the elements or function were within ±0.4% of the theoretical values. Analytical TLC using E. Merck F-254 commercial plates was used to follow the course of reaction. Silica gel 60 (Merck 70−230 mesh) was used for column chromatography. Extracts were dried over sodium sulfate, and solvents were removed under reduced pressure.

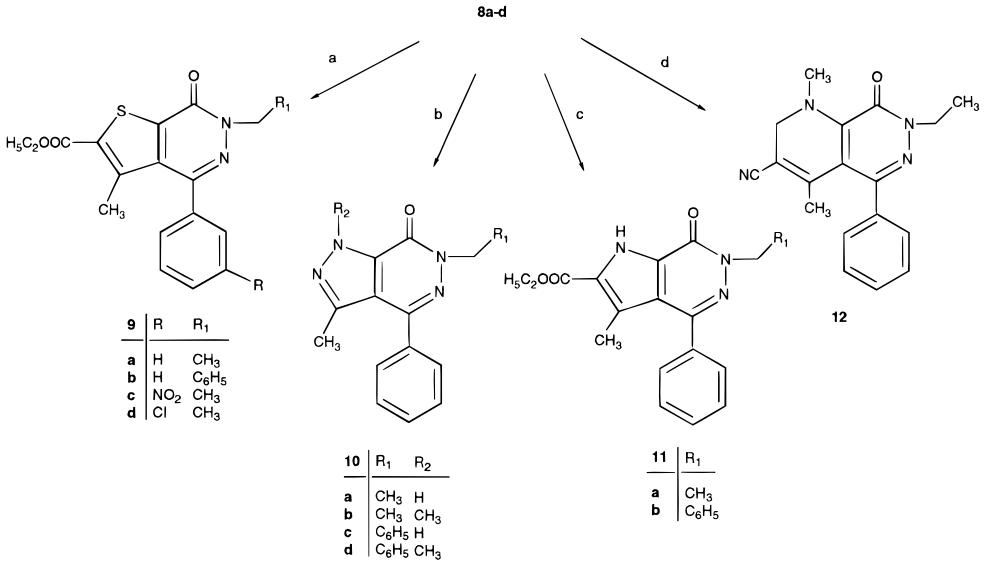
N,*N*-Dimethylformamide (DMF) was purchased from Aldrich Chemical Co. and dried over 4 Å molecular sives before use.

Ethyl 5-Methyl-4-(3-nitrobenzoyl)isoxazole-3-carboxylate (5b). To a cooled and stirred solution of sodium ethoxide (0.3 g, 13 mmol) in anhydrous EtOH (20 mL) was added a solution of 1-(3-nitrophenyl)butane-1,3-dione[24] (2.8 g, 13 mmol) in the same solvent (70 mL). Then a solution of ethyl chloro(hydroximino)acetate[25] (2.0 g, 13.3 mmol) in anhydrous EtOH (10 mL) was added dropwise over 1 h period. After solvent evaporation, the residue was washed with cold 0.5 N NaOH and water and recovered by suction: 67% yield; mp 105−106 °C (EtOH); IR (cm^{-1}) 1740 (CO), 1670 (CO), 1350 (NO_2); ^{1}H-NMR 1.15 (t, 3H, *J* = 7.2 Hz, CH_2CH_3), 2.60 (s, 3H, CH_3), 4.15 (q, 2H, *J* = 7.2 Hz, CH_2CH_3), 7.70 (t, 1H, *J* = 8.1 Hz, Ar), 8.10 (d, 1H, *J* = 8.0 Hz) Ar), 8.40−8.55 (m, 2H, Ar). Anal. ($C_{14}H_{12}N_2O_6$) C, H, N.

Ethyl 4-(3-Chlorobenzoyl)-5-methylisoxazole-3-carboxylate (5c). Compound **5c** was synthesized following the procedure reported for **5b**, using the same molar ratio: 51% yield; mp 67−68 °C (EtOH); IR (cm^{-1}) 1740 (CO), 1670 (CO); ^{1}H-NMR 1.15 (t, 3H, *J* = 7.2 Hz, CH_2CH_3), 2.60 (s, 3H, CH_3), 4.20 (q, 2H, *J* = 7.2 Hz, CH_2CH_3), 7.40−7.65 (m, 3H, Ar), 7.75 (s, 1H, Ar). Anal. ($C_{14}H_{12}ClNO_4$) C, H, N.

3-Methyl-4-(3-nitrophenyl)isoxazolo[3,4-*d*]pyridazin-7(6*H*)-one (6b). The isoxazole **5b** (0.15 g, 0.5 mmol) was dissolved in EtOH (5 mL), and then 0.15 mL of hydrazine hydrate was added. After 1 h the crude product was collected by suction from the cooled mixture: 87% yield; mp 255−256 °C (EtOH); IR (cm^{-1}) 3280 (NH), 1670 (CO), 1530 (NO_2); ^{1}H-

Scheme 2[a]



[a] (a) $NaSCH_2COOC_2H_5$; (b) NH_2NH_2 or CH_3NHNH_2; (c) (1) $NH_2CH_2COOC_2H_5$; (2) EtONa; (d) (1) $CH_3NH(CH_2)_2CN$; (2) EtONa.

Table 1. Effects of Heterocyclic-Fused Pyridazinones **9**−**12** on PDE III and PDE IV Isoenzymes and Displacement of [^{3}H]Rolipram from Its Binding Site

compd	PDE III[a,b]	PDE IV[a,b]	[^{3}H]ROL[a,c]	PDE IV/[^{3}H]ROL
9a	5.9 ± 1.4	0.9 ± 0.2	1.8 ± 0.2	0.50
9b	20.0 ± 2.8 (20 μM)	11.0 ± 1.0	22.0 ± 2.0	0.50
9c	25.0 ± 1.0 (20 μM)	1.3 ± 0.3	0.4 ± 0.1	3.25
9d	56.0 ± 8.0 (20 μM)	0.8 ± 0.4	1.6 ± 0.2	0.50
10a	35.0 ± 2.0 (20 μM)	2.0 ± 0.3	1.0 ± 0.2	2.00
10b	35.0 ± 4.0 (20 μM)	7.4 ± 0.7	46.0% ± 3.0 (10 μM)	−
10c	60.0 ± 26.0 (200 μM)	3.1 ± 0.4	54.0% ± 7.0 (10 μM)	−
10d	30.0 ± 6.0 (200 μM)	45.0 ± 9.0 (200 μM)	11.0% ± 6.0 (10 μM)	−
11a	30.0 ± 3.0 (20 μM)	0.6 ± 0.1	2.0 ± 1.0	0.30
11b	33.0 ± 9.0 (20 μM)	31.0 ± 9.0	28.0% ± 2.0 (10 μM)	−
12	51.0 ± 0.3 (20 μM)	1.1 ± 0.4	1.0 ± 0.4	1.10
milrinone	0.73 ± 0.03			
rolipram	242 ± 11.0	0.32 ± 0.09	0.006 ± 0.004	53.33
3[d]	5.1 ± 2.0	0.056 ± 0.01	0.0048 ± 0.001	11.67

[a] Data are indicated as IC_{50} (μM) ± SEM or inhibition percentage ± SEM at indicated concentration (μM) ($n = 3$). [b] PDE III and PDE IV were obtained from guinea pig ventricular tissue[30] and dosed following the procedure of Thompson et al. (ref 31). [c] [^{3}H]Rolipram tests were performed using brain membranes according to ref 13. [d] X = N, R = Cl, R_1 = 4-pyridyl (ref 17).

NMR (DMSO-d_6) 2.55 (s, 3H, CH_3), 7.85 (t, 1H, J = 8.3 Hz, Ar), 8.15 (d, 1H, J = 8.3 Hz, Ar), 8.35−8.50 (m, 2H, Ar). Anal. ($C_{12}H_8N_4O_4$) C, H, N.

4-(3-Chlorophenyl)-3-methylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one (6c). Compound **6c** was synthesized following the same procedure reported for **6b**: 85% yield; mp 252−253 °C (EtOH); IR (cm^{-1}) 3290 (NH), 1670 (CO); ^{1}H-NMR (DMSO-d_6) 2.50 (s, 3H, CH_3), 7.55−7.70 (m, 4H, Ar). Anal. ($C_{12}H_8ClN_3O_2$) C, H, N.

6-Ethyl-3-methyl-4-phenylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one (7a). A mixture of isoxazolopyridazinone **6a**[27] (0.5 g, 2.2 mmol), anhydrous K_2CO_3 (1.8 g, 13 mmol) and ethyl bromide (1.9 g, 15 mmol) in anhydrous DMF (6 mL) was heated at 60 °C with stirring for 30 min. After dilution with cold water (80−100 mL), the suspension was extracted with ethyl acetate (3 × 60 mL). Removal of the solvent afforded compound **7a**: 59% yield; mp 132−135 °C (EtOH); IR (cm^{-1}) 1680 (CO); ^{1}H-NMR 1.40 (t, 3H, J = 7.0 Hz, CH_2CH_3), 2.50 (s, 3H, CH_3), 4.25 (q, 2H, J = 7.0 Hz, CH_2CH_3), 7.55 (s, 5H, Ar). Anal. ($C_{14}H_{13}N_3O_2$) C, H, N.

6-Ethyl-3-methyl-4-(3-nitrophenyl)isoxazolo[3,4-*d*]pyridazin-7(6*H*)-one (7c). Compound **7c** was synthesized following the procedure reported for **7a**, using the same molar ratio: 73% yield; mp 132−134 °C (EtOH); IR (cm^{-1}) 1660 (CO), 1350 (NO_2); ^{1}H-NMR 1.40 (t, 3H, J = 7.0 Hz, CH_2CH_3), 2.60 (s, 3H, CH_3), 4.30 (q, 2H, J = 7.0 Hz, CH_2CH_3), 7.80 (s, 2H, Ar), 8.50 (s, 2H, Ar). Anal. ($C_{14}H_{12}N_4O_4$) C, H, N.

4-(3-Chlorophenyl)-6-ethyl-3-methylisoxazolo[3,4-*d*]-pyridazin-7(6*H*)-one (7d). Compound **7d** was synthesized following the procedure reported for **7a**, using the same molar ratio: 84% yield; mp 138−140 °C (EtOH); IR (cm^{-1}) 1665 (CO); ^{1}H-NMR 1.40 (t, 3H, J = 6.9 Hz, CH_2CH_3), 2.55 (s, 3H, CH_3), 4.25 (q, 2H, J = 6.9 Hz, CH_2CH_3), 7.40−7.60 (m, 4H, Ar). Anal. ($C_{14}H_{12}ClN_3O_2$) C, H, N.

5-Acetyl-2-ethyl-4-nitro-6-phenyl-3(2*H*)-pyridazinone (8a). To a stirred suspension of isoxazolo[3,4-*d*]pyridazinone **7a** (0.3 g, 1.2 mmol) in a mixture of 50% AcOH (10 mL) and 14.4 M HNO_3 (2 mL) was added portionwise cerium ammonium nitrate (CAN) (4.5 g, 8 mmol) at 55 °C during 30 min. Addition of ice−water (50 mL) furnished a precipitate which was recovered by suction: 45% yield; mp 107−108 °C (EtOH); IR (cm^{-1}) 1720 (CO), 1680 (CO), 1550 and 1350 (NO_2); ^{1}H-NMR 1.50 (t, 3H, J = 6.9 Hz, CH_2CH_3), 2.15 (s, 3H, CH_3), 4.40 (q, 2H, J = 6.9 Hz, CH_2CH_3), 7.40−7.55 (m, 4H, Ar). Anal. ($C_{14}H_{13}N_3O_4$) C, H, N.

5-Acetyl-2-ethyl-4-nitro-6-(3-nitrophenyl)-3(2*H*)-pyridazinone (8c). Compound **8c** was synthesized following the procedure reported for **8a**, using the same molar ratio: 60% yield; mp 118−119 °C (EtOH); IR (cm^{-1}) 1710 (CO), 1680 (CO), 1530 and 1350 (NO_2); ^{1}H-NMR 1.50 (t, 3H, J = 7.0 Hz, CH_2CH_3), 2.25 (s, 3H, CH_3), 4.45 (q, 2H, J = 7.0 Hz, CH_2CH_3), 7.65−7.75 (m, 2H, Ar), 8.30−8.45 (m, 2H, Ar). Anal. ($C_{14}H_{12}N_4O_6$) C, H, N.

5-Acetyl-6-(3-chlorophenyl)-2-ethyl-4-nitro-3(2*H*)-pyridazinone (8d). Compound **8d** was synthesized following the procedure reported for **8a**, using the same molar ratio: 42% yield; mp 104−105 °C (EtOH); IR (cm^{-1}) 1710 (CO), 1680 (CO), 1540 (NO_2); ^{1}H-NMR 1.50 (t, 3H, J = 7.0 Hz, CH_2CH_3), 2.20

(s, 3H, CH_3), 4.40 (q, 2H, $J = 7.0$ Hz, CH_2CH_3), 7.40−7.60 (m, 4H, Ar). Anal. ($C_{14}H_{12}ClN_3O_4$) C, H, N.

Ethyl 6,7-Dihydro-6-ethyl-3-methyl-7-oxo-4-phenylthieno[2,3-*d*]pyridazine-2-carboxylate (9a). To a solution of sodium ethyl thioglycolate, prepared from EtONa (0.35 g, 5 mmol) and ethyl thioglycolate (0.6 g, 5 mmol) in anhydrous EtOH (10 mL), was added pyridazinone **8a** (0.24 g, 0.8 mmol) dissolved in the same solvent (2 mL). The mixture was stirred at room temperature for 30 min, after it was diluted with ice−water and the crude precipitate recovered by suction: 46% yield; mp 151−152 °C (EtOH); IR (cm^{-1}) 1730 (CO), 1660 (CO); ^{1}H-NMR 1.35−1.55 (m, 6H, 2 × CH_2CH_3), 2.15 (s, 3H, CH_3), 4.30−4.45 (m, 4H, 2 × CH_2CH_3), 7.35−7.60 (m, 5H, Ar). Anal. ($C_{18}H_{18}N_2O_3S$) C, H, N.

Ethyl 6-Benzyl-6,7-dihydro-3-methyl-7-oxo-4-phenylthieno[2,3-*d*]pyridazine-2-carboxylate (9b). Compound **9b** was synthesized following the procedure reported for **9a**, using the same molar ratio: 40% yield; mp 141 °C (EtOH); IR (cm^{-1}) 1700 (CO), 1650 (CO); ^{1}H-NMR 1.40 (t, 3H, $J = 7.0$ Hz, CH_2CH_3), 2.15 (s, 3H, CH_3), 4.40 (q, 2H, $J = 7.0$ Hz, CH_2CH_3), 5.45 (s, 2H, CH_2), 7.25−7.55 (m, 10H, 2Ar). Anal. ($C_{23}H_{20}N_2O_3S$) C, H, N.

Ethyl 6,7-Dihydro-6-ethyl-3-methyl-4-(3-nitrophenyl)-7-oxothieno[2,3-*d*]pyridazine-2-carboxylate (9c). Compound **9c** was synthesized following the procedure reported for **9a**, using the same molar ratio: 61% yield; mp 183−184 °C (EtOH); IR (cm^{-1}) 1720 (CO), 1660 (CO); ^{1}H-NMR 1.35−1.55 (m, 6H, 2 × CH_2CH_3), 2.15 (s, 3H, CH_3), 4.30−4.45 (m, 4H, 2 × CH_2CH_3), 7.35−7.60 (m, 4H, Ar). Anal. ($C_{18}H_{17}N_3O_5S$) C, H, N.

Ethyl 4-(3-Chlorophenyl)-6,7-dihydro-6-ethyl-3-methyl-7-oxothieno[2,3-*d*]pyridazine-2-carboxylate (9d). Compound **9d** was synthesized following the procedure reported for **9a**, using the same molar ratio: 38% yield; mp 158−159 °C (EtOH); IR (cm^{-1}) 1720 (CO), 1660 (CO); ^{1}H-NMR 1.35−1.55 (m, 6H, 2 × CH_2CH_3), 2.20 (s, 3H, CH_3), 4.20−4.45 (m, 4H, 2 × CH_2CH_3), 7.25−7.55 (m, 4H, Ar). Anal. ($C_{18}H_{17}ClN_2O_3S$) C, H, N.

6-Ethyl-3-methyl-4-phenylpyrazolo[3,4-*d*]pyridazin-7(6*H*)-one (10a). To a suspension of nitro derivative **8a** (0.1 g, 0.3 mmol) in EtOH (5 mL) was added an excess of hydrate hydrazine (1.5 mmol), and the mixture was stirred at room temperature for 10 min. Compound **10a** was directly recovered by suction: 57% yield; mp 233−234 °C (EtOH); IR (cm^{-1}) 3150 (NH), 1650 (CO); ^{1}H-NMR 1.50 (t, 3H, $J = 7.0$ Hz, CH_2CH_3), 2.25 (s, 3H, CH_3), 4.45 (q, 2H, $J = 7.0$ Hz, CH_2CH_3), 7.45−7.65 (m, 5H, Ar). Anal. ($C_{14}H_{14}N_4O$) C, H, N.

1,3-Dimethyl-6-ethyl-4-phenylpyrazolo[3,4-*d*]pyridazin-7(6*H*)-one (10b). Compound **10b** was synthesized following the procedure reported for **10a** using methylhydrazine in the same molar ratio of hydrazine. In this case the mixture was diluted with ice−water and the product recovered by suction: 60% yield; mp 147−148 °C (EtOH); IR (cm^{-1}) 1670 (CO); ^{1}H-NMR 1.45 (t, 3H, $J = 7.1$ Hz, CH_2CH_3), 2.15 (s, 3H, CCH_3), 4.30 (q, 2H, $J = 7.1$ Hz, CH_2CH_3), 4.40 (s, 3H, NCH_3), 7.50 (s, 5H, Ar). Anal. ($C_{15}H_{16}N_4O$) C, H, N.

6-Benzyl-3-methyl-4-phenylpyrazolo[3,4-*d*]pyridazin-7(6*H*)-one (10c). Compound **10c** was synthesized following the procedure reported for **10a** starting from **8b** and using the same molar ratio. In this case the reaction mixture was diluted with water and extracted with ethyl acetate. The crude product was purified by column chromatography (eluent: cyclohexane/ethyl acetate, 1:2): 85% yield; mp 167−168 °C (EtOH); IR (cm^{-1}) 3140 (NH), 1650 (CO); ^{1}H-NMR 2.30 (s, 3H, CH_3), 5.55 (s, 2H, CH_2), 7.25−7.40 (m, 3H, Ar), 7.45−7.60 (m, 7H, Ar). Anal. ($C_{19}H_{16}N_4O$) C, H, N.

6-Benzyl-1,3-dimethyl-4-phenylpyrazolo[3,4-*d*]pyridazin-7(6*H*)-one (10d). Compound **10d** was synthesized from **8b** and methylhydrazine following the procedure reported for **10c** and using the same molar ratio. The crude product was purified by column chromatography (eluent: cyclohexane/ethyl acetate, 1:1): 88% yield; mp 166 °C (EtOH); IR (cm^{-1}) 1670 (CO); ^{1}H-NMR 2.15 (s, 3H, CCH_3), 4.35 (s, 3H, NCH_3), 5.45 (s, 2H, CH_2), 7.25−7.40 (m, 3H, Ar), 7.45−7.60 (m, 7H, Ar). Anal. ($C_{20}H_{18}N_4O$) C, H, N.

Ethyl 6,7-Dihydro-6-ethyl-3-methyl-4-phenyl-1*H*-pyrrolo[2,3-*d*]pyridazine-2-carboxylate (11a). A mixture of **8a** (0.1 g, 0.3 mmol) and sarcosine ethyl ester (0.07 g, 0.6 mmol) in EtOH (3−4 mL) was stirred at room temperature. for 20 min. The suspension was diluted with ice−water and 4-*N*-ethylglycinate recovered by suction. The crude product was then added to EtONa prepared from Na (0.06 g, 2.6 mmol) and absolute EtOH (4 mL), and the mixture was heated at 50 °C for 30 min. After dilution with water, the mixture was acidified with 6 N HCl and the final product was isolated by suction: 85% yield; mp 184−186 °C (EtOH); IR (cm^{-1}) 3200 (NH), 1720 (CO), 1660 (CO); ^{1}H-NMR 1.35−1.50 (m, 6H, 2 × CH_2CH_3), 2.10 (s, 3H, CH_3), 4.30−4.50 (m, 4H, 2 × CH_2CH_3), 7.50 (s, 5H, Ar). Anal. ($C_{18}H_{19}N_3O_3$) C, H, N.

Ethyl 6-Benzyl-6,7-dihydro-3-methyl-4-phenyl-1*H*-pyrrolo[2,3-*d*]pyridazine-2-carboxylate (11b). Compound **11b** was synthesized following the procedure reported for **11a**, using the same molar ratio: 84% yield; mp 153−154 °C (EtOH); IR (cm^{-1}) 3180 (NH), 1720 (CO), 1660 (CO); ^{1}H-NMR 1.40 (t, 3H, $J = 7.1$ Hz, CH_2CH_3), 2.15 (s, 3H, CCH_3), 4.40 (q, 2H, $J = 7.1$ Hz, CH_2CH_3), 5.50 (s, 2H, CH_2), 7.20−7.55 (m, 10H, 2Ar). Anal. ($C_{23}H_{21}N_3O_3$) C, H, N.

3-Cyano-1,4-dimethyl-7-ethyl-8-oxo-5-phenyl-1,2,7,8-tetrahydropyrido[2,3-*d*]pyridazine (12). A mixture of **8a** (0.15 g, 0.5 mmol) and *N*-methyl-β-alaninenitrile (0.1 g, 1.2 mmol) in EtOH (5 mL) was stirred at room temperature for 20 min. The suspension was diluted with water (40 mL) and extracted with CH_2Cl_2 (3 × 20 mL). Evaporation of the solvent afforded a brown oil which was dissolved in EtOH (3 mL) and added to a solution of EtONa prepared from Na (0.11 g, 2.5 mmol) and absolute EtOH (5 mL). The mixture was heated at 50−60 °C for 90 min. Then it was diluted with ice−water, and the final product was recovered by suction: 45% yield; mp 119−121 °C (EtOH); IR (cm^{-1}) 2200 (CN), 1640 (CO); ^{1}H-NMR 1.40 (t, 3H, $J = 7.0$ Hz, CH_2CH_3), 1.65 (s, 3H, CCH_3), 3.75 (s, 3H, NCH_3), 3.90 (s, 2H, CH_2), 4.20 (q, 2H, $J = 7.0$ Hz, CH_2CH_3), 7.40 (s, 5H, 2Ar). Anal. ($C_{18}H_{18}N_4O$) C, H, N.

Biology. Drugs and Reagents. [8-^{3}H]Adenosine 3′:5′ cyclic monophosphate was from Amersham (Bucks, UK). Benzamidine, cAMP, calmodulin, and PMSF (phenyl methanesulfonyl fluoride) were obtained from Sigma-Aldrich Quimica S.A. (Madrid, Spain). Milrinone was obtained from IMPEX Quimica (Barcelona, Spain).

Rolipram was synthesized in the Department of Chemical Synthesis, Laboratorios Almirall, S.A., Spain.

Purification of Phosphodiesterase Isoenzymes. Cyclic nucleotide phosphodiesterases III and IV were obtained from guinea pig ventricular tissue following the procedure described by Gristwood et al.[30] Briefly, the tissue was homogenized in 20 mM Bis-Tris pH 6.5 buffer, containing 50 mM sodium acetate, 2 mM benzamidine, 2 mM EDTA, 5 mM β-mercaptoethanol, and 50 μM PMSF using an Ultraturrax homogenizer. The sample was centrifuged at 40000*g* for 20 min, and the supernatant was filtered through a 0.22 μm filter. The clean sample was chromatographed on a 1 mL ion-exchange MONO-Q column equilibrated with the same buffer using an FPLC system. The column was developed at a flow rate of 1 mL/min using a linear gradient of sodium acetate from 50 to 1000 mM in a total volume of 25 mL. Fractions of 500 μL were collected.

The isoenzymes were characterized prior to use in terms of substrate selectivity and affinity and by the effect of calcium ions (10 μM) plus calmodulin (1.2 μM) and the selective inhibitors Rolipram, SK&F 94120. Active fractions were pooled and kept frozen at −20 °C in presence of 1 g/L bovine serum albumin until used.

Phosphodiesterase Assay. Cyclic nucleotide phosphodiesterases were assayed following the procedure of Thompson and Strada (1984).[31] Inhibition assays were run in duplicate at a substrate concentration of 0.25 μM. Substrate was cAMP for PDE III and IV. IC_{50} values were obtained by nonlinear regression using the program InPlot from GraphPad Software. Drugs were dissolved in DMSO, and the effects of this solvent were taken into consideration in the calculations.

[^{3}H]Rolipram Displacement. The binding of [^{3}H]Rolipram to rat brain membranes was performed according to

Schneider et al. [13] At least six drug concentrations were assayed in duplicate to generate individual displacement curves. IC_{50} values were calculated for those curves by nonlinear regression using the program Inplot, from GraphPad Sofware. The effect of drug vehicle was taken into account in the calculation.

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