Novel Pyrazolopyrimidopyridazinones with Potent and Selective Phosphodiesterase 5 (PDE5) Inhibitory Activity as Potential Agents for Treatment of Erectile Dysfunction

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Pyrazolo[1′,5′:1,6]pyrimido[4,5-*d*]pyridazin-4(3*H*)-ones and their analogues, potentially useful for the treatment of erectile dysfunction, were synthesized and evaluated as inhibitors of phosphodiesterase 5 (PDE5). Several compounds showed IC_{50} values in the low nanomolar range, and in particular, compound **5r**, displaying high potency toward PDE5 (IC_{50} = 8.3 nM) and high selectivity versus PDE6 (240-fold) appeared to be a very promising new lead both in comparison with the potent but not selective sildenafil and in comparison with some analogues previously reported by us. SAR studies in this triheterocyclic scaffold led us to conclude that the best arranged groups are a methyl in position 1, a benzyl in position 3, a phenyl in position 9, and a linear four-carbon chain in position 6.

Introduction

Erectile dysfunction (ED) is a very common pathology that affects millions of men worldwide, and it appears to be directly proportional to aging.[1,2] The most important epidemiological studies in this field from the Massachusetts Male Aging Study (MMAS) demonstrated that about one-third of men over 40 suffer from moderate to complete ED.[3] Recent studies suggest that ED, rather than being an inevitable consequence of aging, depends not only on a series of elements such as heart disease, hypertension, diabetes, and psychogenic causes such as anxiety and depression but also on education.[4,5] Finally, it was found that some drugs induced ED as an undesired effect, in particular antipsychotic drugs and also products belonging to other classes such as antihypertensives, diuretics, antilipidemics, and narcotics.[5,6]

The therapy for erectile dysfunction includes α-adrenoceptor antagonists such as moxisylyte (Thymoxamine),[7] synthetic prostaglandin E1 (Alprostadil),[8] and opioids such as papaverine[9] (which are all used for intracavernosal, intraurethral, or topical administration) and apomorphine, a dopamine D1 and D2 receptor agonist, which has been recently approved for marketing in Europe.[10] Its use (sublingual via) for moderate ED is limited by notable side effects such as nausea, orthostatic hypertension, and vomiting. However,the most widely prescribed drugs for erectile dysfunction are the orally active and remarkably potent phosphodiesterase 5 (PDE5) inhibitors.[11]

PDE5 belongs to a superfamily of enzymes that catalyzes the hydrolysis of cyclic nucleotides cAMP and cGMP to the corresponding 5-nucleoside monophosphate. Currently, 11 different isoforms of phosphodiesterases (PDE1−PDE11) and their subtypes are known, distinguished by substrate specificities and tissue concentration.[12,13] In particular, PDE5 is localized in cells of the corpus cavernosum, in vascular smooth muscle, and in platelets.

Currently, three PDE5 inhibitors are commercially available with excellent expected results and somewhat unique profiles: the archetypal sildenafil, **A**[14,15] (Viagra), which was launched in 1998, showed a lot of side effects such as increased light sensitivity, nausea, and headache due to its low selectivity toward PDE6 isoform; tadalafil, **B**[16,17] (Cialis), which presents small secondary effects and a longer half-life with respect to sildenafil; vardenafil, **C**[18,19] (Levitra), which shows a positive efficacy−security profile and was launched by Bayer Pharmaceutical and GlaxoSmithKline at the beginning of 2004 (Figure 1).

The therapeutic effect of PDE5 inhibitors is related to the increase of cyclic guanosine monophosphate (cGMP) levels, which are responsible for the relaxation of smooth muscle cells and vasodilation in the corpus cavernosum, thereby potentiating penile erection.[20] At present, the primary objective of academics and pharmaceutical companies in this field is to identify new potent compounds with a very significant selectivity profile, in particular toward isoform 6, which is responsible for the most common side effects. The efforts expended in this area have led to excellent products with different chemical structures showing an activity in the (sub)nanomolar range and a very attractive specificity toward PDE5 isoenzyme (to 600-fold).[21−24]

Our previous studies and experience in the field of PDE inhibitors[25−28] together with the interesting results coming from Bristol-Myers on the identification of potent, selective, and orally bioavailable pyrazolopyridopyridazines **D**[29] led us to synthesize new pyrazolopyrimidopyridazinones of type **E**, which showed good inhibitory activity on PDE5 and a fair selectivity toward PDE6[30] (Figure 1).

We report here the development of the above project with the synthesis and biological evaluation of a wide series of novel pyrazolopyrimidopyridazinones as PDE5 inhibitors.

Chemistry

The final compounds were prepared following the synthetic routes reported in Schemes 1−5.

We previously described [30,31] the synthesis of the tricyclic system that is based on three common steps: (a) condensation of the starting isoxazolo[3,4-*d*]pyridazinones with the appropriate arylaldehydes to give the vinyl derivatives (**3a**−**n**, **15**, **17**, **28a**,**b**, **33a**,**b**); (b) treatment of the vinyl derivatives with hydrazine to obtain the pyrazolylpyridazinone intermediates by isoxazole ring opening (**4a**−**n**, **16**, **18**, **29a**,**b**, **34a**,**b**); (c) ring

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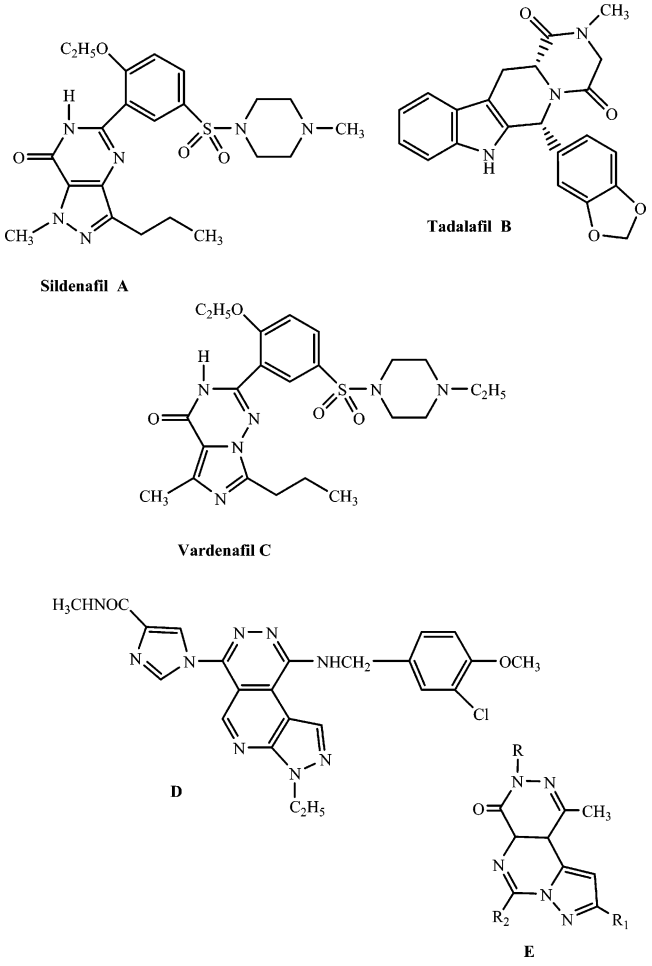


Figure 1. PDE5 inhibitors.

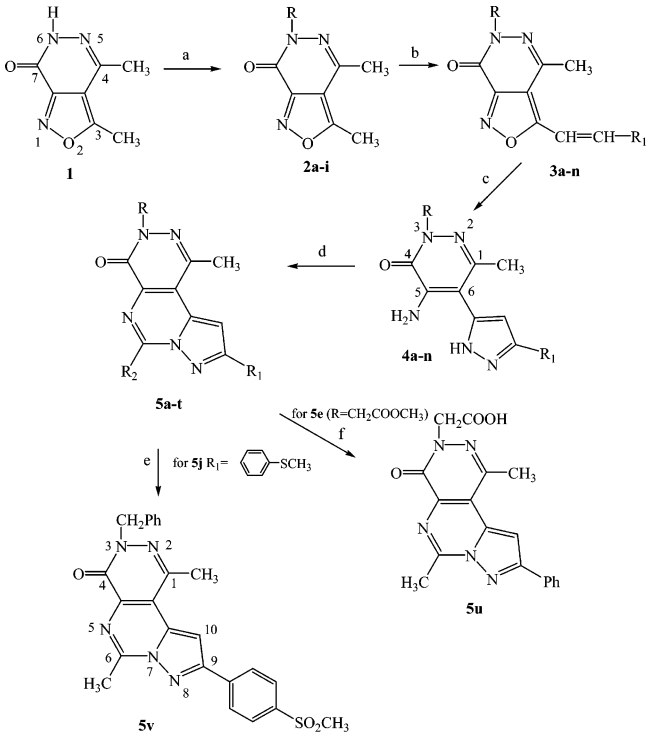
closure to pyrazolo[1′,5′:1,6]pyrimido[4,5-*d*]pyridazin-4(3*H*)-ones with the appropriate anhydride under refluxing or with the opportune carboxylic acid at room temperature in the presence of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (**5a**−**t**, **19**, **20a**,**b**, **30a**,**b**, **35a**,**b**). The alkylation under standard conditions with the appropriate halides, which allowed us to obtain a variety of 3-substituted final compounds, was carried out on the isoxazolo[3,4-*d*]pyridazinones or on the tricyclic system.

Scheme 1 shows the alkylation performed on the precursor **1**[32] to give the corresponding *N*-alkyl derivatives. In this synthetic pathway the tricyclic compounds **5e** and **5j** were further elaborated through an alkaline hydrolysis and through an oxidative reaction with H_2O_2 in acetic acid, respectively, to afford compounds **5u** and **5v**.

Scheme 2 depicts the synthesis of the final compounds **7a**−**d**, which was performed by alkylation of pyrazolopyrimidopyridazin-4(3*H*)-ones **6a**,**b**.[30,31] Moreover, compound **6b**, treated with $POCl_3$ followed by the appropriate amine, furnished the corresponding pyrazolopyrimido[4,5-*d*]pyridazines **9a**,**b**.

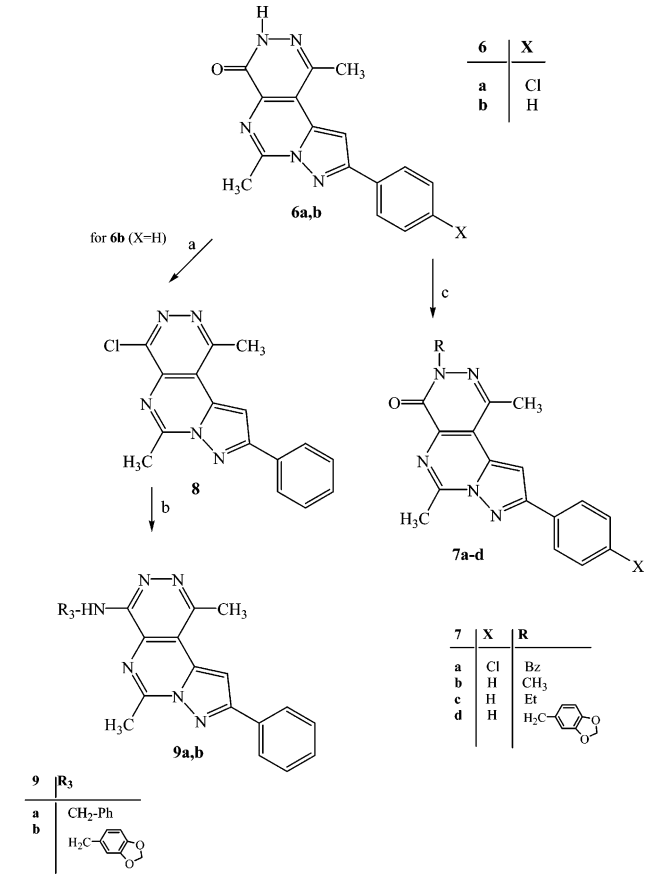
Schemes 3−5 report the synthetic routes for the introduction of different substituents at position 1 of the tricyclic system. The difunctionalized isoxazoles **12a**,**b**, **22**, and **23** were prepared from the appropriate diketones **10a**,**b** and **21a**,**b**[33−36] and the ethyl (chlorohydroximino)acetate **11**, which is commercially available. 1,3-Dipolar cycloaddition is not a regioselective reaction in the presence of the above diketones and led to a mixture of the two isomers (4-$COCH_3$ and 4-COR_4 derivatives), which were separated by column chromatography (Scheme 3)

Scheme 1[a]



[a] (a) RX, DMF, K_2CO_3; (b) R_1CHO, CH_3ONa, CH_3OH; (c) NH_2NH_2, C_2H_5OH; (d) $(R_2CO)_2O$ or R_2COOH, DMF, CH_2Cl_2, DMAP, EDC; (e) H_2O_2, CH_3COOH; (f) $NaOH/C_2H_5OH$.

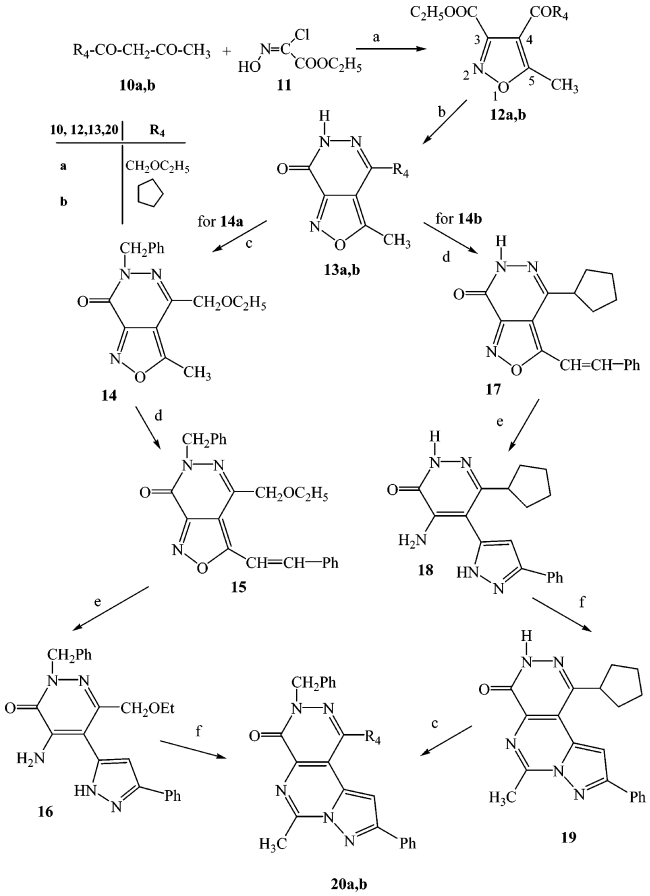
Scheme 2[a]



[a] (a) $POCl_3$; (b) R_3NH_2, C_2H_5OH; (c) RX, DMF, K_2CO_3.

or subjected as a mixture to the usual train of reactions (Scheme 4). In fact, when the mixture of **26** and **27** was treated with

Scheme 3[a]



[a] (a) C_2H_5ONa/C_2H_5OH; (b) NH_2NH_2, C_2H_5OH; (c) $PhCH_2Br$, DMF, K_2CO_3; (d) PhCHO, CH_3ONa, CH_3OH; (e) NH_2NH_2, C_2H_5OH; (f) $(CH_3CO)_2O$.

benzaldehyde in the presence of CH_3ONa, only the isomer **26** reacted[37] affording the 3-arylvinylderivatives **28a**,**b**, which were collected as precipitates from the reaction mixtures. Compounds **28a**,**b** were converted into the final **30a**,**b** by using the same two-step procedure.

Finally, compounds **35a**,**b** were prepared starting from precursor **14**, which, when treated with HBr in acetic acid, afforded the corresponding 4-bromomethyl derivatives as good substrates for a nucleophilic displacement (Scheme 5).

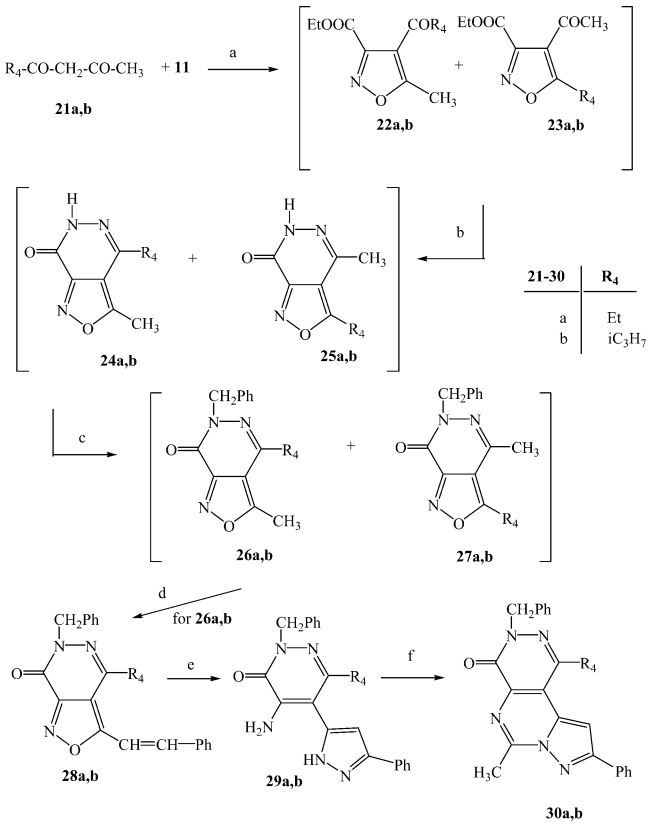
Physical and chemical data of compounds **2a**−**i**, **3a**−**n**, **4a**−**n**, and **5a**−**v** are reported in Tables 1−4.

Results and Discussion

All the final compounds were evaluated for their PDE5 and PDE6 inhibitory activity.

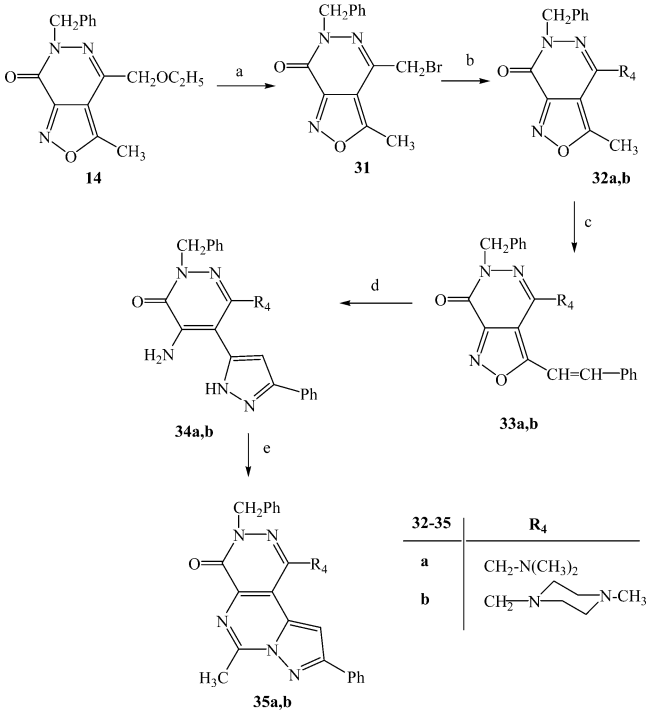
First, we extensively explored the role played by the substituent at position 3, keeping methyl groups at positions 1 and 6 and a phenyl at position 9 (Table 5). Indeed, the presence of linear (**5a**, **5c**, and **7c**) or branched alkyls (**5b**) is generally associated with an almost complete absence of activity (PDE5 inhibition of ≤54.6% at 2 μM). Compounds **5d** and **5u**, bearing a propargyl and an acetic acid residue, respectively, were tested at 0.2 μM, showing low inhibitory activity. For the methyl derivative **7b**, a dose response curve was determined and an $IC_{50} = 1.5$ μM was found, but this compound was completely devoid of selectivity. It is interesting to observe that all the derivatives bearing linear or branched alkyls at position 3 were demonstrated to have very similar levels of activity, ranging from 50% at 1.5 μM for **7b** to 44.7% at 2 μM for **5b**. Thus, the

Scheme 4[a]



[a] (a) C_2H_5ONa/C_2H_5OH; (b) NH_2NH_2, C_2H_5OH; (c) $PhCH_2Br$, DMF, K_2CO_3; (d) PhCHO, CH_3ONa, CH_3OH; (e) NH_2NH_2, C_2H_5OH; (f) $(CH_3CO)_2O$.

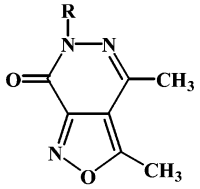
Scheme 5[a]



[a] (a) HBr, CH_3COOH; (b) dimethylamine or 4-methylpiperazine, C_2H_5OH; (c) PhCHO, CH_3ONa; (d) NH_2NH_2, C_2H_5OH; (e) $(CH_3CO)_2O$.

length and the steric hindrance of the carbon chain do not play any role in determining PDE5 inhibitory activity. This finding lends strong support to the hypothesis that these groups do not

Table 1. Physical and Chemical Data of Compounds **2a**−**i**

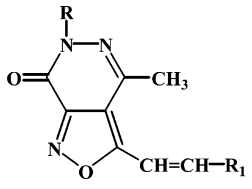


2a-i

compd	R	yield (%)	mp (°C)[a]
2a	Bn	89	143−146
2b	*n*-C_3H_7	88	83−85
2c	*i*-C_3H_7	40	110−113
2d	*n*-C_4H_9	50	54−56
2e	$CH_2C{\equiv}CH$	74	121−123
2f	CH_2COOMe	72	110−112
2g	3-OCH_3−Bn	90	126−129
2h	CH_2−4-pyridyl	64	oil[b]
2i	$(CH_2)_2$−4-piperidyl	70	84−87

[a] All solid compounds were purified by crystallization from EtOH. [b] Purified by column chromatography using 1:2 cyclohexane/ethyl acetate.

Table 2. Physical and Chemical Data of Compounds **3a**−**n**



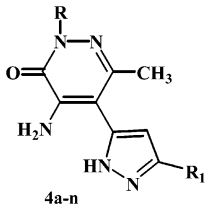
3a-n

compd	R	R_1	yield (%)	mp (°C)	cryst solvent
3a	Bn	Ph	80	215−217	MeOH
3b	*n*-C_3H_7	Ph	78	181−183	MeOH
3c	*i*-C_3H_7	Ph	86	225−226	MeOH
3d	*n*-C_4H_9	Ph	75	173−175	MeOH
3e	$CH_2C{\equiv}CH$	Ph	80	238−240	MeOH
3f	CH_2COOCH_3	Ph	49	204−207	EtOH
3g	3-OCH_3−Bn	Ph	70	188−190	EtOH
3h	CH_2−4-pyridyl	Ph	90	193−196	EtOH
3i	$(CH_2)_2$−4-piperidyl	Ph	61	216, dec	EtOH
3j	Bn	4-F−Ph	95	231−233	EtOH
3k	Bn	4-S−CH_3−Ph	85	257−259	MeOH
3l	Bn	3-pyridyl	63	205−207	EtOH
3m	Bn	3-thienyl	66	209−212	EtOH
3n	Bn	3-furyl	98	197−199 dec	EtOH

interact with any amino acidic residue of the protein, suggesting that in these derivatives the carbon chain is probably directed outside the catalytic pocket. In contrast, we found that an aryl group separated from the tricyclic core by a methylenic spacer is an important feature. In agreement with the result obtained with the previously reported 3-benzyl derivative,[30] the analogues **5f**, **5g**, and **7d** showed submicromolar activity coupled with a certain degree of selectivity versus PDE6. The best compound proved to be the 3-methoxybenzyl derivative **5f**, which showed good potency at PDE5 (IC_{50} = 100 nM) and at least 20-fold selectivity versus PDE6.

By examining the results obtained from keeping a methyl at position 1 and a benzyl at position 3 and from varying R_1 and R_2 (Table 6), we observe that several compounds are endowed with affinity versus PDE5 in the same order of magnitude as the corresponding 9-phenyl derivative.[30] Thus, with CH_3 at R_2, the introduction of a chlorine in the para position of the phenyl (compound **7a**) or its replacement with different heterocyclic systems (3-furyl, 3-thienyl, 3-pyridyl) gave compounds with IC_{50} in the range 0.05−0.47 μM for PDE5 inhibition, but only the

Table 3. Physical and Chemical Data of Compounds **4a**−**n**

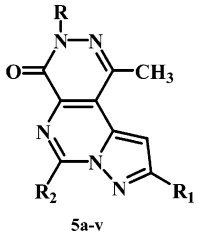


4a-n

compd	R	R_1	yield (%)	mp (°C)[a]
4a	Bn	Ph	86	192−193
4b	*n*-C_3H_7	Ph	60	182−183
4c	*i*-C_3H_7	Ph	47	188−190
4d	*n*-C_4H_9	Ph	85	142−145
4e	$CH_2C{\equiv}CH$	Ph	50	192−194
4f	CH_2COOCH_3	Ph	43	204−208
4g	3-OCH_3−Bn	Ph	53	138−141
4h	CH_2−4-pyridyl	Ph	20	143−146
4i	$(CH_2)_2$−4-piperidyl	Ph	81	177−180
4j	Bn	4-F−Ph	71	258−259
4k	Bn	4-S−CH_3−Ph	96	200−203
4l	Bn	3-pyridyl	40	209−210
4m	Bn	3-thienyl	60	239−241
4n	Bn	3-furyl	58	220−222

[a] All compounds were purified by crystallization from EtOH.

Table 4. Physical and Chemical Data of Compounds **5a**−**v**



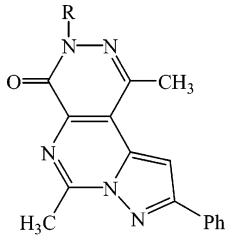
5a-v

compd	R	R_1	R_2	yield (%)	mp (°C)[a]
5a	*n*-C_3H_7	Ph	CH_3	63	189−190
5b	*i*-C_3H_7	Ph	CH_3	53	196−198
5c	*n*-C_4H_9	Ph	CH_3	44	183−185
5d	$CH_2C{\equiv}CH$	Ph	CH_3	45	255−258
5e	CH_2COOCH_3	Ph	CH_3	41	>300
5f	3-OCH_3−Bn	Ph	CH_3	35	187−190
5g	CH_2−4-pyridyl	Ph	CH_3	66	231−234
5h	$(CH_2)_2$−4-piperidyl	Ph	CH_3	46	143−145
5i	Bn	4-F−Ph	CH_3	47	280−284
5j	Bn	4-SCH_3−Ph	CH_3	48	270−273
5k	Bn	3-pyridyl	CH_3	54	235−237
5l	Bn	3-thienyl	CH_3	92	230−232
5m	Bn	3-furyl	CH_3	78	233, dec
5n	Bn	Ph	H	64	267−269
5o	Bn	Ph	C_2H_5	89	220−222
5p	Bn	Ph	*n*-C_3H_7	72	191−192
5q	Bn	Ph	*i*-C_3H_7	45	234−237
5r	Bn	Ph	$(CH_2)_2COCH_3$	33	225−227
5s	Bn	Ph	$(CH_2)_2COOCH_3$	42	215−218
5t	Bn	Ph	$(CH_2)_4COOCH_3$	39	205−207
5u	CH_2COOH	Ph	CH_3	63	>300
5v	Bn	4-SO_2CH_3Ph	CH_3	36	248−251

[a] All compounds were purified by crystallization from EtOH.

3-thienyl derivative **5l** retained an appreciable level of selectivity (16) versus PDE6. In contrast, the introduction of a fluorine (compound **5i**) or a methylsulfonyl (compound **5v**) in the para position of the phenyl was very detrimental, since a complete loss of activity was observed. This finding strongly suggests that both steric and electronic features play an important role in this part of the molecule because the presence of the small and strongly electronegative F (π = 0.14, σ_p = 0.06, MR = 0.092)[38] and the large electron-withdrawing CH_3SO_2 group (π = −1.63, σ_p = 0.72, MR = 1.35) is associated with absence of

Table 5. PDE5 and PDE6 Inhibitory Activity of Pyrazolopyrimidopyridazinones

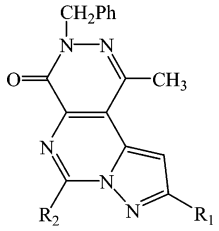


5a-d, 5f-h, 5u, 7b-d

compd	R	PDE5[c]	PDE6[c]
5a	*n*-C_3H_7	44.9 (2 μM)	6.9 (2 μM)
5b	*i*-C_3H_7	44.7 (2 μM)	1.2 (2 μM)
5c	*n*-C_4H_9	54.6 (2 μM)	32.5 (2 μM)
5d	$CH_2C{\equiv}CH$	19.7 (0.2 μM)	0.3 (0.2 μM)
5f	3-OCH_3−Bn	0.1	3.8 (2 μM)
5g	CH_2−4-pyridyl	0.24	39.4 (2 μM)
5h	$(CH_2)_2$−4-piperidyl	67.7 (2 μM)	26.6 (2 μM)
5u	CH_2COOH	7.6 (0.2 μM)	11.2 (0.2 μM)
7b	CH_3	1.5	2.6
7c	C_2H_5	49.3 (2 μM)	53.4 (0.2 μM)
7d	A[b]	0.39	7 (2 μM)
E[a]	Bn	0.16	25 (2 μM)
sildenafil		0.020	0.040

[a] See ref 30. [b] A = 3,4-methylendioxyphenylmethyl. [c] Data are indicated as IC_{50} (μM) or inhibition percentage at indicated concentration (μM). The IC_{50} values were obtained from dose response curves at three or four different concentrations (n = 2−3).

Table 6. PDE5 and PDE6 Inhibitory Activity of Pyrazolopyrimidopyridazinones



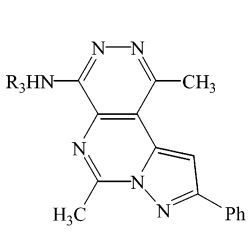
5i, 5k, 5l-t, 5v, 7a

compd	R_1	R_2	PDE5[b]	PDE6[b]
5i	4-F−Ph	CH_3	38.8 (2 μM)	4.5 (2 μM)
5k	3-pyridyl	CH_3	0.47	0.28
5l	3-thienyl	CH_3	0.14	2.2
5m	3-furyl	CH_3	0.05	0.33
5n	Ph	H	0.18	0.86
5o	Ph	C_2H_5	0.038	0.55
5p	Ph	*n*-C_3H_7	0.04	0.61
5q	Ph	*i*-C_3H_7	0.023	0.062
5r	Ph	$(CH_2)_2COCH_3$	0.0083	22.8 (2 μM)
5s	Ph	$(CH_2)_2COOCH_3$	0.079	16.9 (2 μM)
5t	Ph	$(CH_2)_4COOCH_3$	0.095	1.1 (2 μM)
5v	4-SO_2CH_3−Ph	CH_3	3.8 (2 μM)	2.8 (2 μM)
7a	4-Cl−Ph	CH_3	0.37	4.7
E[a]	Ph	CH_3	0.16	25 (2 μM)
sildenafil			0.020	0.040

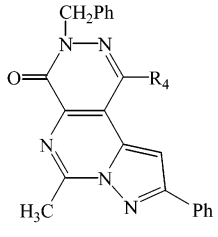
[a] See ref 30. [b] Data are indicated as IC_{50} (μM) or inhibition percentage at indicated concentration (μM). The IC_{50} values were obtained from dose response curves at three or four different concentrations (n = 2−3).

activity in comparison with the active chloro derivative **7a**, where the substituent is characterized by an electronegative effect and limited steric hindrance (π = 0.71, σ_p = 0.23, MR = 0.603). Studying the effect due to the modification of the substituent at position 6, with R_1 = Ph, we affirm that, moving from H (compound **5n**) to low alkyls (**5o**−**p**), the activity is significantly improved (at least 5-fold); moreover, the presence of a branched chain (**5q**) was associated with the best result in this subseries of compounds. The best balance of activity and selectivity was that of the *n*-propyl derivative **5p** (PDE5 IC_{50} = 40 nM, PDE6/PDE5 = 15). When position 6 is decorated with an oxygenated carbon chain, both the activity and the selectivity were dramatically improved. Thus, compound **5r**, bearing the 2-oxobutyryl group in this position, was characterized by high PDE5 affinity (IC_{50} = 8.3 nM) and very high selectivity (>240). Compared with sildenafil, compound **5r** was 2.4-fold more potent and at least 200-fold more selective. When the oxygenated carbon chain of **5r** was replaced by a methyl butyrate residue (**5s**), an approximately 10-fold reduction of activity was observed, without modification of the selectivity. Elongation of the methylenic carbon chain of **5s** gave compound **5t**, which showed the same activity and selectivity profile. Compounds **5s** and **5t** showed potency at the same level as the analogues with ethyl, *n*-propyl, and isopropyl at R_2, but in terms of the selectivity issue, the presence of a carbonyl dipole in the carbon chain seems to be an essential requirement. Taken together, these results suggest that the presence of a hydrogen bond acceptor in the carbon chain at R_2 plays an important role in the interaction with the biological target, also allowing us to nicely discriminate between PDE5 and PDE6. Moreover, the position of the carbonyl group inside the carbon chain and/or the nature of the functional group plays a very critical role in the affinity at PDE5.

Table 7. PDE5 and PDE6 Inhibitory Activity of Pyrazolopyrimidopyridazinones



9a,b



20a,b, 30a,b, 35a,b

compd	R_3	R_4	PDE5[e]	PDE6[e]
9a	Bn		52.4 (2 μM)	NT[d]
9b	A[b]		56.8 (2 μM)	NT[d]
20a		$CH_2OC_2H_5$	0.071	0.33
20b		cyclopentyl	14.3 (2 μM)	12.3 (2 μM)
30a		C_2H_5	0.22	21.8 (2 μM)
30b		*i*-C_3H_7	0.1	59.8 (2 μM)
35a		CH_2−$N(CH_3)_2$	1.4	1.0
35b		B[c]	23.0 (0.2 μM)	30.0 (0.2 μM)
E[a]		CH_3	0.16	25 (2 μM)
sildenafil			0.020	0.040

[a] See ref 30. [b] A = 3,4-methylendioxyphenylmethyl. [c] B = *N*-methylpiperazinylmethyl. [d] NT = not tested. [e] Data are indicated as IC_{50} (μM) or inhibition percentage at indicated concentration (μM). The IC_{50} values were obtained from dose response curves at three or four different concentrations (n = 2−3).

We also evaluated a series of analogues modified at position 1 (Table 7); thus, the methyl group of our previous prototype (compound **E**, R_4 = CH_3)[30] was elongated, branched, functionalized, and replaced by cycloalkyl groups. All these manipulations did not improve the activity. In this subseries the best compound proved to be **20a**, bearing an ethoxymethyl group (PDE5 IC_{50} = 71 nM), but a complete absence of selectivity was found for this molecule. Low linear and branched alkyls (compounds **30a**,**b**) were better than cyclopentyl (**20b**); amino and cycloalkylamino groups linked to the tricyclic system through a methylenic spacer (compounds **35a**,**b**) proved to be almost devoid of activity.

Finally we synthesized some examples of compounds in which the (substituted) benzyl group was shifted from position 3 to position 4 through aromatization of the pyridazinone system. Both compounds **9a** and **9b**, bearing a (alkoxy)benzylamino fragment similar to that of Bristol-Myers compound **D**, proved to be inactive. This result seems to indicate that the presence of the carbonyl dipole together with the benzyl group at the adjacent nitrogen in our series is an essential feature for the interaction with the protein. This finding is in agreement with the SAR found for compounds such as sildenafil and vardenafil targeting PDE5 and also with agents targeting PDE3 and PDE4 isoenzymes, which show a remarkable degree of homology in the catalytic site with PDE5.[39] Compound **D** probably interacts with this site with a different orientation with respect to both the above drugs and our compounds.

In conclusion, exploration of the space around the pyrazolopyrimidopyridazinone system allowed us to identify compound **5r** displaying PDE5 inhibitory activity in the low nanomolar range and 240-fold selectivity versus PDE6. This in vitro profile suggests **5r** as a very promising new lead both in comparison with the potent but not selective sildenafil and in comparison with previously reported analogues.[30] In fact, optimization of these compounds allowed us to improve potency at PDE5 by more than 1 order of magnitude; moreover, selectivity versus PDE6 was increased from 66- to 240-fold.

SAR studies in this triheterocyclic scaffold led us to conclude that the best arranged groups are a methyl at R_4, benzyl at R, and a phenyl at R_1. At R_2 the best substituent was a linear four-carbon chain bearing a carbonyl dipole.

Taking into account these findings, further modifications are in progress to improve the in vitro profile of the present series. In the meantime, compound **5r** is under evaluation in some experimental models of penile dysfunction.

Experimental Section

Chemistry. All melting points were determined on a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were recorded with Varian Gemini 200 instruments. Chemical shifts are reported in ppm, using the solvent as internal standard. Extracts were dried over Na_2SO_4, and the solvents were removed under reduced pressure. Merck F-254 commercial plates were used for analytical TLC to follow the course of reaction. Silica gel 60 (Merck 70−230 mesh) was used for column chromatography. Reagent **11** is commercially available.

General Procedure for Compounds 2a−i. A mixture of isoxazolopyridazinone **1**[32] (2.2 mmol), K_2CO_3 (4.4 mmol), and the appropriate alkyl halide (3.0−4.5 mmol) in anhydrous DMF (6 mL) was stirred at 70−110 °C for 0.5−3 h. For compound **2h** the reaction was carried out at 110 °C for 16 h. After cooling, the mixture was diluted with cold water, and the precipitate was recovered by suction. For compounds **2e**,**f**,**h**, after dilution with water, the suspension was extracted with CH_2Cl_2 (3 × 15 mL). Then the solvent was evaporated in vacuo to afford a crude precipitate.

Compound **2i** was synthesized starting from 6-(2-bromoethyl)-3,4-dimethylisoxazole[3,4-*d*]pyridazin-7(6*H*)-one[40] (0.73 mmol), which was suspended in anhydrous DMF (4 mL). K_2CO_3 (1.16 mmol) and piperidine (1.2 mmol) were added, and the mixture was stirred at 70 °C for 1 h and 30 min. After the mixture was cooled, cold water was added and the suspension was extracted with CH_2Cl_2 (3 × 20 mL). Evaporation of the solvent afforded **2i.**

6-Benzyl-3,4-dimethylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one, 2a. Yield = 89%; mp = 143−146 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.50 (s, 3H, 4-C*CH*$_3$), 2.80 (s, 3H, 3-C*CH*$_3$), 5.25 (s, 2H, *CH*$_2$Ar), 7.20−7.50 (m, 5H, Ar).

3,4-Dimethyl-6-isopropylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one, 2c. Yield = 40%; mp = 110−113 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.30 (d, 6H, CH(*CH*$_3$)$_2$), 2.50 (s, 3H, 4-C*CH*$_3$), 2.85 (s, 3H, 3-C*CH*$_3$), 5.25−5.35 (m, 1H, *CH*(CH$_3$)$_2$).

General Procedure for Compounds 3a−n. To a suspension of isoxazolopyridazinone **2a−i** (1 mmol) and the appropriate arylaldehyde (2−3 mmol) in anhydrous methanol, CH_3ONa (1−2.5 mmol) was added. The mixture was refluxed under stirring for 1−45 min. After the mixture was cooled, the solid was recovered by suction. For compound **3h** the mixture was concentrated and diluted with ice−water (10 mL) and the precipitate was isolated by filtration.

6-Benzyl-4-methyl-3-styrylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one, 3a. Yield = 80%; mp = 215−217 °C (MeOH); ^{1}H NMR ($CDCl_3$) δ 2.60 (s, 3H, 4-C*CH*$_3$), 5.30 (s, 2H, *CH*$_2$Ar), 7.20 (d, 1H, *CH*═), 7.30−7.60 (m, 10H, Ar), 7.80 (d, 1H, ═*CH*).

6-Isopropyl-4-methyl-3-styrylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one, 3c. Yield = 86%; mp = 225−226 °C (MeOH); ^{1}H NMR ($CDCl_3$) δ 1.35 (d, 6H, CH(*CH*$_3$)$_2$), 2.65 (s, 3H, 4-C*CH*$_3$), 5.30−5.40 (m, 1H, *CH*(CH$_3$)$_2$), 7.25 (d, 1H, *CH*═), 7.45−7.65 (m, 5H, Ar), 7.80 (d, 1H, ═*CH*).

General Procedure for Compounds 4a−n. A suspension of compounds **3a−n** (0.3 mmol) in ethanol (2−3 mL) and hydrazine hydrate (2−9 mmol) was stirred at room temperature for 0.5−5 h (compounds **4d** and **4k** for 0.5−1 h at 60 °C). Then the mixture was concentrated in vacuo and cooled for 1−2 h and the precipitate was recovered by suction. For compound **4h**, after concentration, the mixture was diluted with water (5 mL) and after the mixture was cooled, the solid was filtered off.

4-Amino-2-benzyl-6-methyl-5-(5′-phenyl-1*H*-pyrazol-3-yl)pyridazin-3(2*H*)-one, 4a. Yield = 86%; mp = 192−193 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, 6-C*CH*$_3$), 5.30 (s, 2H, *CH*$_2$Ar), 6.00 (exch br s, 2H, *NH*$_2$), 6.70 (s, 1H, Ar), 7.30−7.70 (m, 10H, Ar).

4-Amino-2-isopropyl-6-methyl-5-(5′-phenyl-1*H*-pyrazol-3-yl)-pyridazin-3(2*H*)-one, 4c. Yield = 47%; mp = 188−190 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.30 (d, 6H, CH(*CH*$_3$)$_2$), 2.20 (s, 3H, 6-C*CH*$_3$), 5.20−5.30 (m, 1H, *CH*(CH$_3$)$_2$), 6.60 (s, 1H, Ar), 7.35−7.80 (m, 5H, Ar).

General Procedure for Compounds 5a−m,o−q. A mixture of 4-amino-5-pyrazolyl derivatives **4a−n** (0.2−0.3 mmol) and the suitable anhydride (10−15 mmol) was refluxed under stirring for 5 min to 1 h. After the mixture was cooled, the solid that separated was filtered and washed with water. For compound **5h**, the mixture was diluted with cold water (15 mL) and neutralized with 6 N NaOH and the precipitate was recovered by suction. Compound **5k** was recovered by suction after dilution of the reaction mixture with ice−water (10 mL).

1,6-Dimethyl-3-isopropyl-9-phenylpyrazolo[1′,5′:1,6]pyrimido-[4,5-*d*]pyridazin-4(3*H*)-one, 5b. Yield = 53%; mp = 196−198 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.40 (d, 6H, CH(*CH*$_3$)$_2$), 2.80 (s, 3H, 1-C*CH*$_3$), 3.20 (s, 3H, 6-C*CH*$_3$), 5.45−5.55 (m, 1H, *CH*(CH$_3$)$_2$), 7.35−7.60 (m, 4H Ar), 8.10 (d, 2H, Ar).

3-Benzyl-1-methyl-9-phenylpyrazolo[1′,5′:1,6]pyrimido[4,5-*d*]pyridazin-4(3*H*)-one, 5n. A mixture of **4a** (0.17 mmol), triethylorthoformate (12 mmol), anhydrous DMF (4 mL), and a catalytic amount of concentrated sulfuric acid was stirred at room temperature for 30 min. The mixture was diluted with cold water (10 mL), and the precipitate was recovered by suction. Yield = 64%; mp = 267−269 °C (EtOH); ^{1}H NMR (CDC_{l3}) δ 2.80 (s, 3H, 1-C*CH*$_3$), 5.40 (s, 2H, *CH*$_2$Ar), 7.25−7.60 (m, 9H, Ar), 8.05 (m, 2H, Ar), 9.45 (s, 1H, Ar).

General Procedure for Compounds 5r−t. A suspension of **4a** (0.42 mmol) in anhydrous CH_2Cl_2 (13 mL), anhydrous DMF (1−1.5 mL), 0.52 mmol of suitable acid or ester (levulinic acid, acetoxypropionic acid, methyladipate), 0.51 mmol of 4-(dimethylamino)pyridine, and 0.6 mmol of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride was stirred at room temperature for 6 h and then refluxed for 20 h. After the mixture was cooled, the solid was filtered off and dissolved in CH_2Cl_2. The organic layer was washed with 2 N HCl and with 2 N NaOH solution sequentially and then evaporated in vacuo to afford a crude precipitate.

3-Benzyl-1-methyl-6-(3-oxobutyl)-9-phenylpyrazolo[1′,5′:1,6]-pyrimido[4,5-*d*]pyridazin-4(3*H*)-one, 5r. Yield = 33%; mp = 225−227 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ 2.30 (s, 3H, COCH_3), 2.80 (s, 3H, 1-CCH_3), 3.20 (m, 2H, $COCH_2CH_2$), 3.65 (m, 2H, $COCH_2CH_2$), 5.35 (s, 2H, CH_2Ar), 7.35−8.20 (m, 11H, Ar).

Methyl 3-Benzyl-1-methyl-4-oxo-9-phenyl-3,4-dihydropyrazolo[1′,5′:1,6]pyrimido[4,5-*d*]pyridazin-6-yl)propanoate, 5s. Yield = 42%; mp = 215−218 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ 2.80 (s, 3H, 1-CCH_3), 3.00 (m, 2H, CH_2CH_2COO), 3.60 (s, 3H, OCH_3), 3.65−3.75 (m, 2H, CH_2CH_2COO), 5.40 (s, 2H, CH_2Ar), 7.30−8.20 (m, 11H, Ar).

1,6-Dimethyl-4-oxo-9-phenyl-3,4-diidropyrazolo[1′,5′:1,6]pyrimido[4,5-*d*]pyridazin-3-acetic Acid, 5u. A mixture of **5e** (0.22 mmol), ethanol (1−1.5 mL), and 6 N NaOH (2 mL) was stirred at room temperature for 1 h. After acidification with 6 N HCl, the final compound **5u** was recovered by suction. Yield = 63%; mp > 300 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ 2.80 (s, 3H, 1-CCH_3), 3.10 (s, 3H, 6-CCH_3), 4.85 (s, 2H, CH_2COO), 7.55 (m, 3H, Ar), 7.80 (s, 1H, Ar), 8.20 (m, 2H, Ph).

3-Benzyl-1,6-dimethyl-9-(4-methylsulfonylphenyl)pyrazolo-[1′,5′:1,6]pyrimido[4,5-*d*]pyridazin-4(3*H*)-one, 5v. A suspension of compound **5j** (0.19 mmol) in anhydrous acetic acid (2 mL) and H_2O_2 (30%, 2.5 mL) was stirred at 90 °C for 1 h and 30 min. After the mixture was cooled, the precipitate was recovered by suction. Yield = 36%; mp = 248−251 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.10 (s, 3H, CH_3-SO_2), 2.80 (s, 3H, 1-CCH_3), 3.15 (s, 3H, 6-CCH_3), 5.50 (s, 2H, CH_2Ar), 7.30−7.60 (m, 6H, Ar), 8.10 (d, 2H, Ar), 8.30 (d, 2H, Ar).

General Procedure for Compounds 7a−d. Compounds **7a−d** were obtained starting from **6a**,**b** following the general procedure described for **2a−i**. For compound **7c** the reaction was carried out at 60 °C for 5h. The residue was purified by column chromatography using 9:1 $CHCl_3$/MeOH as eluent.

3-Benzyl-9-(4-chlorophenyl)-1,6-dimethylpyrazolo[1′,5′:1,6]-pyrimido[4,5-*d*]pyridazin-4(3*H*)-one, 7a. Yield = 59%; mp = 266−269 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.80 (s, 3H, 1-CCH_3), 3.20 (s, 3H, 6-CCH_3), 5.45 (s, 2H, CH_2Ar), 7.30−7.65 (m, 8H, Ar), 8.00 (d, 2H, Ar).

1,6-Dimethyl-3-ethyl-9-phenylpyrazolo[1′,5′:1,6]pyrimido[4,5-*d*]pyridazin-4(3*H*)-one, 7c. Yield = 65%; mp = 218−220 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.45 (t, 3H, CH_2CH_3), 2.80 (s, 3H, 1-CCH_3), 3.20 (s, 3H, 6-CCH_3), 4.35 (q, 2H, CH_2CH_3), 7.30−7.60 (m, 4H, Ar), 8.15 (d, 2H, Ar).

4-Chloro-1,6-dimethyl-9-phenylpyrazolo[1′,5′:1,6]pyrimido-[4,5-*d*]pyridazine, 8. A suspension of **6b** (0.52 mmol) in $POCl_3$ (1.5 mL, 9.5 mmol) was stirred at 130 °C for 2 h. After cooling, the mixture was treated with cold water (10−15 mL) and the precipitate was recovered by suction and washed with water. Yield = 87%; mp = 253−256 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ 2.45 (s, 3H, 1-CCH_3), 3.10 (s, 3H, 6-CCH_3), 7.60 (m, 3H, Ar), 8.10 (s, 1H, Ar), 8.30 (m, 2H, Ar).

General Procedure for Compounds 9a,b. A suspension of **8** (0.2 mmol), the appropriate amine (benzylamine, methylendioxybenzylamine, 10−20 mmol), anhydrous ethanol (1 mL), and triethylamine (0.05 mL) was stirred at 140 °C for 6 h in a sealed tube. After cooling, the mixture was diluted with cold water and compound **9a** was recovered by suction. For compound **9b**, the mixture was evaporated and the residue was treated with hot cyclohexane (3 × 10 mL). The final compound **9b** was recrystallized with ethanol.

4-Benzyl-1,6-dimethyl-9-phenylpyrazolo[1′,5′:1,6]pyrimido-[4,5-*d*]pyridazin-4-amine, 9a. Yield = 96%; mp = 231−233 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.70 (s, 3H, 1-CCH_3), 3.25 (s, 3H, 6-CCH_3), 5.60 (s, 2H, CH_2Ar), 7.20−7.53 (m, 10H, Ar), 7.95 (m, 1H, Ar).

4-(3,4-Methylendioxybenzyl)-1,6-dimethyl-2-phenylpyrazolo-[1′,5′:1,6]pyrimido[4,5-*d*]pyridazin-4-amine, 9b. Yield = 56%; mp = 250−253 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.70 (s, 3H, 1-CCH_3), 3.40 (s, 3H, 6-CCH_3), 5.50 (s, 2H, CH_2Ar), 5.95 (s, 2H, O-CH_2−O), 6.75−6.85 (m, 3H, Ar), 7.20−7.65 (m, 4H, Ar), 7.90 (d, 2H, Ar).

General Procedure for Compounds 12a,b. To a cooled (0 °C) and stirred solution of sodium ethoxide (15 mmol) and anhydrous ethanol (20 mL), the appropriate diketone (8 mmol) **10a**,**b**[33,34] dissolved in the same solvent (10 mL) was slowly added. After the mixture was cooled at −5 °C, a solution of ethyl (chlorohydroximino)acetate **11** (10 mmol) in anhydrous ethanol (10 mL) was added dropwise. The mixture, neutralized with 6 N HCl, was evaporated, and cold water was added. The aqueous layer was extracted with CH_2Cl_2 (3 × 20 mL), and evaporation of the solvent afforded compounds **12a**,**b**, which were purified by column chromatography using 2:1 cyclohexane/ethyl acetate as eluent for compound **12a** and 1:2 cyclohexane/ethyl acetate for compound **12b**.

Ethyl 4-Ethoxymethylcarbonyl-5-methylisoxazole-3-carboxylate, 12a. Yield = 68.7%; oil; ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H, CH_3CH_2OCO), 1.40 (t, 3H, CH_3CH_2O), 2.65 (s, 3H, C-CH_3), 3.60 (q, 2H, CH_3CH_2O), 4.30 (s, 2H, CO−CH_2−O), 4.40 (q, 2H, CH_3CH_2OCO).

Ethyl 4-Cyclopentancarbonyl-5-methylisoxazole-3-carboxylate, 12b. Yield = 52%; oil; ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H, CH_3CH_2OCO), 1.60−1.75 (m, 9H, cC_5H_9), 2.65 (s, 3H, C−CH_3), 4.40 (q, 2H, CH_3CH_2OCO).

General Procedure for Compounds 13a,b. To a solution of the isoxazole derivative **12a**,**b** (2 mmol) in EtOH (2 mL), hydrazine hydrate (3−4 mmol) was added. The mixture was stirred at room temprature for 1−3 h. The mixture was concentrated in vacuo, diluted with water (10−15 mL), and extracted with CH_2Cl_2 (3 × 15 mL). Evaporation of the solvent afforded the **13a**,**b**.

4-Ethoxymethyl-3-methylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one, 13a. Yield = 73%; mp = 155−157 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, CH_3CH_2O), 2.85 (s, 3H, C−CH_3), 3.60 (q, 2H, CH_3CH_2O), 4.60 (s, 2H, CH_2OEt), 9.80 (exch br s, 1H, *NH*).

4-Cyclopentyl-3-methylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one, 13b. Yield = 30%; mp = 154−157 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.75−1.90 (m, 9H, cC_5H_9), 2.90 (s, 3H, C−CH_3).

6-Benzyl-4-ethoxymethyl-3-methylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one, 14. 14 was obtained from **13a** following the reported general procedure to obtain **2a−i**. The mixture was diluted with cold water and was extracted with CH_2Cl_2 (3 × 15 mL). After evaporation of solvent, a crude precipitate was obtained. Yield = 48%; oil; ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, CH_3CH_2O), 2.80 (s, 3H, C−CH_3), 3.60 (q, 2H, CH_3CH_2O), 4.55 (s, 2H, CH_2OEt), 5.20 (s, 2H, CH_2Ar), 7.30−7.40 (m, 5H, Ar).

6-Benzyl-4-ethoxymethyl-3-styrylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one, 15. 15 was obtained from compound **14** following the general procedure described for **3a−n**. Yield = 53%; mp = 127−130 °C (MeOH); ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3H, CH_3CH_2O), 3.60 (q, 2H, CH_3CH_2O), 4.80 (s, 2H, CH_2OEt), 5.30 (s, 2H, CH_2Ar), 7.20−7.60 (m, 11H, 10H Ar and 1H *CH*═CH), 7.80 (d, 1H, CH═*CH*).

4-Amino-2-benzyl-6-ethoxymethyl-5-(5′-phenyl-1*H*-pyrazol-3-yl)pyridazin-3(2*H*)-one, 16. Compound **16** was obtained from compound **15** following the general procedure described for **4a−n**. Yield = 77%; mp = 197−199 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, CH_3CH_2O), 3.45 (q, 2H, CH_3CH_2O), 4.30 (s, 2H, CH_2OEt), 5.30 (s, 2H, CH_2Ar), 7.00 (s, 1H, Ar), 7.20−7.60 (m, 8H, Ar), 8.00 (m, 2H, Ar).

4-Cyclopentyl-3-styrylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one, 17. 17 was obtained from **13b** following the reported general procedure to obtain **3a−n**. Yield = 71%; mp = 280−282 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.65−2.20 (m, 9H, cC_5H_9), 7.20 (d, 1H, CH═*CH*), 7.45−7.65 (m, 5H, Ar), 7.85 (d, 1H, *CH*═CH).

4-Amino-6-cyclopentyl-5-(5′-phenyl-1*H*-pyrazol-3-yl)pyridazin-3(2*H*)-one, 18. 18 was obtained from **17** following the general procedure to obtain **4a−n**. Yield = 67%; mp = 154−157 °C, dec (EtOH); ^{1}H NMR (DMSO-d_6) δ 1.45−1.90 (m, 9H, cC_5H_9), 6.90 (s, 1H, Ar), 7.25−7.75 (m, 7H, Ar), 7.50 (exch br s, 2H, NH_2).

1-Cyclopentyl-6-methyl-9-phenylpyrazolo[1′,5′:1,6]pyrimido-[4,5-*d*]pyridazin-4(3*H*)-one, 19. Compound **19** was obtained from compound **18** following the general procedure described for **5a−m**. Yield = 46%; mp = 216−220 °C (EtOH); ^{1}H NMR

($CDCl_3$) δ 1.80−2.20 (m, 9H, cC_5H_9), 3.20 (s, 3H, 6-CCH_3), 7.40 (s, 1H, Ar), 7.45−7.60 (m, 3H, Ar), 8.05 (m, 2H, Ar).

3-Benzyl-1-ethoxymethyl-6-methyl-9-phenylpyrazolo[1′,5′:1,6]-pyrimido[4,5-*d*]pyridazin-4(3*H*)-one, 20a. Compound **20a** was obtained from compound **16** following the general procedure described for **5a**−**m**. Yield = 47%; mp = 154−157 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, CH_3CH_2O), 3.20 (s, 3H, 6-CCH_3), 3.65 (q, 2H, CH_3CH_2O), 4.80 (s, 2H, CH_2OEt), 5.50 (s, 2H, CH_2Ar), 7.20−7.60 (m, 9H, Ar), 8.10 (m, 2H, Ar).

3-Benzyl-1-cyclopentyl-6-methyl-2-phenylpyrazolo[1′,5′:1,6]-pyrimido[4,5-*d*]pyridazin-4(3*H*)-one, 20b. Compound **20b** was obtained from compound **19** following the general procedure described for **2a**−**i**. In this case the mixture was stirred at 60 °C for 5 h. Yield = 43%; mp = 205−207 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.80−2.20 (m, 9H, cC_5H_9), 3.20 (s, 3H, 6-CCH_3), 5.25−7.60 (s, 2H, CH_2Ar), 7.40 (m, 9H, Ar), 8.10 (m, 2H, Ar).

General Procedure for Compounds 28a,b. 28a,**b** were obtained following the procedure described for compound **15**, starting from 1,3-diketones **21a**,**b**[35,36] and compound **11**. The mixture of the isoxazole intermediates **22a**,**b** and **23a**,**b** as well as the isoxazolopyridazinones **24a**,**b**−**27a**,**b** were not separated until the final condensation with benzaldehyde. From this reaction the pure **28a**,**b** were isolated as precipitates.

6-Benzyl-4-ethyl-3-styrylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one, 28a. Yield = 25%; mp = 176−178 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.35 (t, 3H, CH_3CH_2), 2.90 (q, 2H, CH_3CH_2), 5.30 (s, 2H, CH_2Ar), 7.20 (d, 1H, *CH*═CH), 7.25−7.60 (m, 10H, Ar), 7.80 (d, 1H, CH═*CH*).

6-Benzyl-4-isopropyl-3-styrylisoxazolo[3,4-*d*]pyridazin-7(6H)-one, 28b. Yield = 24%; mp = 163−164 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.40 (d, 6H, $(CH_3)_2$CH), 3.15−3.25 (m, 1H, *CH*$(CH_3)_2$), 5.30 (s, 2H, CH_2Ar), 7.20 (d, 1H, *CH*═CH), 7.25−7.65 (m, 10H, Ar), 7.80 (d, 1H, CH═*CH*).

4-Amino-2-benzyl-6-ethyl-5-(5′-phenyl-1*H*-pyrazol-3-yl)pyridazin-3(2*H*)-one, 29a. Compound **29a** was obtained from compound **28a** following the general procedure described for **4a**−**n**. Yield = 73%; mp = 189−190 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, CH_3CH_2), 2.65 (q, 2H, CH_3CH_2), 5.40 (s, 2H, CH_2Ar), 6.70 (s, 1H, Ar), 7.25−7.80 (m, 10H, Ar).

4-Amino-2-benzyl-6-isopropyl-5-(5′-phenyl-1*H*-pyrazol-3-yl)-pyridazin-3(2*H*)-one, 29b. Compound **29b** was obtained from compound **28b** following the general procedure described for **4a**−**n**. After dilution with water (10 mL), the mixture was extracted with CH_2Cl_2 (3 × 15 mL). Evaporation of the solvent afforded compound **29b**. Yield = 84%; mp = 151−154 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.10 (d, 6H, $(CH_3)_2$CH), 3.05−3.15 (m, 1H, *CH*$(CH_3)_2$), 5.40 (s, 2H, CH_2Ar), 6.70 (s, 1H, Ar), 7.20−7.70 (m, 10H, Ar).

3-Benzyl-1-ethyl-6-methyl-9-phenylpyrazolo[1′,5′:1,6]pyrimido-[4,5-*d*]pyridazin-4(3*H*)-one, 30a. 30a was obtained from **29a** following the general procedure to obtain **5a**−**m** and **5o**−**q**. Yield = 38%; mp > 300 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.40 (t, 3H, CH_3CH_2), 3.10−3.25 (m, 5H, 2H CH_3CH_2 and 3H 6-CCH_3), 5.55 (s, 2H, CH_2Ar), 7.25−7.60 (m, 9H, Ar), 8.10 (m, 2H, Ar).

3-Benzyl-1-isopropyl-6-methyl-9-phenylpyrazolo[1′,5′:1,6]pyrimido[4,5-*d*]pyridazin-4(3*H*)-one, 30b. 30b was obtained from **29b** following the general procedure to obtain **5a**−**m** and **5o**−**q**. Yield = 57%; mp = 202−204 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.40 (d, 6H, $(CH_3)_2$CH), 3.20 (s, 3H, 6-CCH_3), 3.55−3.65 (m, 1H, *CH*$(CH_3)_2$), 5.40 (s, 2H, CH_2Ar), 7.20−7.55 (m, 9H, Ar), 8.10 (m, 2H, Ar).

6-Benzyl-4-bromomethyl-3-methylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one, 31. A mixture of **14** (0.7 mmol) and HBr (1 mmol) in acetic acid was stirred at 140 °C for 8 h in a sealed tube. After the mixture was cooled and diluted with cold water (10 mL), a crude precipitate was recovered by suction and purified by column chromatography using 8:2 toluene/ethyl acetate as eluent. Yield = 60%; mp = 185−188 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.90 (s, 3H, 3-CCH_3), 4.65 (s, 2H, CH_2Br), 5.30 (s, 2H, CH_2Ar), 7.20−7.58 (m, 5H, Ar).

General Procedure for Compounds 32a,b. A mixture of compound **31** (0.5 mmol), K_2CO_3 (1.1 mmol), anhydrous DMF (3 mL), and the appropriate amine (0.5 mmol) (dimethylamine or methylpiperazine) was stirred at room temperature for 2 h. For compound **32a**, after dilution with water, the solution was acidified with 2 N HCl and extracted with CH_2Cl_2. The aqueous layer was then was made alkaline with 6 N NaOH and extracted again with CH_2Cl_2. Evaporation of the solvent afforded final compound **32a**. Compound **32b** was isolated by dilution of the reaction mixture with water, extraction with CH_2Cl_2, and evaporation of the solvent.

6-Benzyl-4-[(dimethylamino)methyl]-3-methylisoxazolo[3,4-*d*]pyridazin-7(6*H*)-one, 32a. Yield = 57%; mp = 185−188 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 6H, $(CH_3)_2$N), 2.85 (s, 3H, 3-CCH_3), 3.60 (s, 2H, CH_2N), 5.25 (s, 2H, CH_2Ar), 7.20−7.45 (m, 5H, Ar).

6-Benzyl-4-[(4-methylpiperazin-1-yl)methyl]-3-methylisoxazolo-[3,4-*d*]pyridazin-7(6*H*)-one, 32b. Yield = 62%; mp = 153−154 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.60 (s, 3H, CH_3N), 2.70−2.85 (m, 8H, piperazine), 2.85 (s, 3H, 3-CCH_3), 3.60 (s, 2H, CH_2N), 5.30 (s, 2H, CH_2Ar), 7.20−7.45 (m, 5H, Ar).

General Procedure for Compounds 33a,b. 33a,**b** were obtained following the general procedure of **3a**−**n**.

6-Benzyl-4-[(dimethylamino)methyl]-3-styrylisoxazolo[3,4-*d*]-pyridazin-7(6*H*)-one, 33a. Yield = 34%; mp > 300 °C, dec (MeOH); ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 6H, $(CH_3)_2$N), 3.60 (s, 2H, CH_2N), 5.30 (s, 2H, CH_2Ar), 7.20−7.80 (m, 12H, 10H Ar and 2H *CH*═*CH*).

6-Benzyl-4-[(4-methylpiperazin-1-yl)methyl]-3-styrylisoxazolo-[3,4-*d*]pyridazin-7(6*H*)-one, 33b. Yield = 43%; mp = 178−181 °C (MeOH); ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3H, CH_3N), 2.30−2.80 (m, 8H, piperazine), 3.65 (s, 2H, CH_2N), 5.30 (s, 2H, CH_2Ar), 7.20−7.80 (m, 12H, 10H Ar and 2H *CH*═*CH*).

General Procedure for Compounds 34a,b. 34a,**b** were obtained following the general procedure of **4a**−**n**.

4-Amino-2-benzyl-6-[(dimethylamino)methyl]-5-(5′-phenyl-1*H*-pyrazol-3-yl)pyridazin-3(2*H*)-one, 34a. Yield = 29%; mp = 235−238 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.45 (s, 6H, $(CH_3)_2$N), 3.45 (s, 2H, CH_2N), 5.40 (s, 2H, CH_2Ar), 5.60 (exch br s, 2H, NH_2), 6.80 (s, 1H, Ar), 720−7.55 (m, 8H, Ar), 7.80 (m, 2H, Ar).

4-Amino-2-benzyl-6-[(4-methylpiperazin-1-yl)methyl]-5-(5′-phenyl-1*H*-pyrazol-3-yl)pyridazin-3(2*H*)-one, 34b. Yield = 67%; mp = 186−188 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.75 (s, 3H, CH_3N), 2.80−3.20 (m, 8H, piperazine), 3.55 (s, 2H, CH_2N), 5.35 (s, 2H, CH_2Ar), 5.70 (exch br s, 2H, NH_2), 6.80 (s, 1H, Ar), 7.20−7.50 (m, 8H, Ar), 7.80 (m, 2H, Ar).

General Procedure for Compounds 35a,b. 35a,**b** were obtained following the general procedure of **5a**−**m** and **5o**−**q**.

3-Benzyl-1-[(dimethylamino)methyl]-6-methyl-9-phenylpyrazolo[1′5′:1,6]pyrimido[4,5-*d*]pyridazin-4(3*H*)-one, 35a. Yield = 52%; mp = 197−200 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.40 (s, 6H, $(CH_3)_2$N), 3.20 (s, 3H, C−CH_3), 3.80 (s, 2H, CH_2N), 5.50 (s, 2H, CH_2Ar), 7.20−7.55 (m, 9H, Ar), 8.00 (m, 2H, Ar).

3-Benzyl-1-[(4-methylpiperazin-1-yl)methyl-6-methyl-9-phenylpyrazolo[1′5′:1,6]pyrimido[4,5-*d*]pyridazin-4(3*H*)-one, 35b. Yield = 58%; mp > 300 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 2.30 (s, 3H, CH_3N), 2.40−2.80 (m, 8H, piperazine), 3.20 (s, 3H, 6-CCH_3), 3.85 (s, 2H, CH_2N), 5.45 (s, 2H, CH_2Ar), 7.25−7.60 (m, 9H, Ar), 7.95−8.05 (m, 2H, Ar).

Biological Assays. Inhibition of 3′:5′cGMP Phosphodiesterases. Purification of Phosphodiesterase Isoenzymes. PDE5 was purified from human platelets as described by Gristwood et al.[41] Briefly, the supernatant of the cell lysate from 10^9 platelets was chromatographed by a Mono-Q ion exchange column attached to a Pharmacia FPLC system.

PDE6 was purified from bovine retinas as described by Gillespie and Beavo.[42]

The isoenzymes were characterized according to Beavo et al.[43] by selectivity and affinity and by effect of calcium ions (10 μM) plus calmodulin (1.2 μM), cyclic GMP (5 μM), and the selective inhibitor sildenafil. PDE5 was kept frozen at −80 °C in the presence of 1 g/L bovine serum albumin until use.

Phosphodiesterase Assay. Cyclic nucleotide phosphodiesterase activities were measured using a two-step procedure according to

Thompson and Strada.[44] PDE5 and PDE6 (activated by 250 μg/mL trypsin) were assayed using 0.25 μM [^{3}H]cGMP as substrate. IC_{50} values were obtained by nonlinear regression using the Prism program by GraphPad Software. The reported values are the average of at least three independent assays. Sildenafil was used as the reference substance. The drugs were dissolved in DMSO at 10^{-3} M. The effect of the solvent was taken into consideration in the calculations.

Supporting Information Available: ^{1}H NMR spectral data for derivatives **2b**,**d**−**i**, **3b**,**d**−**n**, **4b**,**e**−**n**, **5a**,**c**−**q**,**t**, and **7b**,**d** and elemental analysis results for all target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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