Journal of Enzyme Inhibition and Medicinal Chemistry, 2002 Vol. 17 (4), pp. 227–233

Synthesis and Evaluation of Some Pyrazolo[3,4-d]pyridazinones and Analogues as PDE 5 Inhibitors Potentially Useful as Peripheral Vasodilator Agents

VITTORIO DAL PIAZ[a,*], MARIA CARLA CASTELLANA[a], CLAUDIA VERGELLI[a], MARIA PAOLA GIOVANNONI[a], AMADEU GAVALDÀ[b], VICTOR SEGARRA[b], JORGE BELETA[b], HAMISH RYDER[b] and JOSÈ MARIA PALACIOS[b]

[a]*Dipartimento di Scienze Farmaceutiche, Via G. Capponi 9, 50121 Firenze, Italy;* [b]*Almirall Prodesfarma Research Center, Cardener 68-74, 08024, Barcelona, Spain*

(Received 15 May 2002)

A series of pyrazolo[3,4-d]pyridazinones and analogues, potentially useful as peripheral vasodilators, were synthesized and evaluated as inhibitors of PDE5 extracted from human platelets. Several of them showed IC_{50} values in the range 0.14–1.4 μM. A good activity and selectivity profile versus PDE6 was found for compound 11e (6-benzyl-3-methyl-1-isopropyl-4-phenylpyrazolo[3,4-d] pyridazin-7(6H)-one). Structure–activity relationship studies demonstrated the essential role played by the benzyl group at position-6 of the pyrazolopyridazine system. Other types of pyridazinones fused with five and six membered heterocycles (pyrrole, isoxazole, pyridine and dihydropyridine), as well as some open models were prepared and evaluated. Besides the pyrazole, the best fused systems proved to be isoxazole and pyridine.

Keywords: Pyrazolopyridazinones; PDE5; Inhibitors

INTRODUCTION

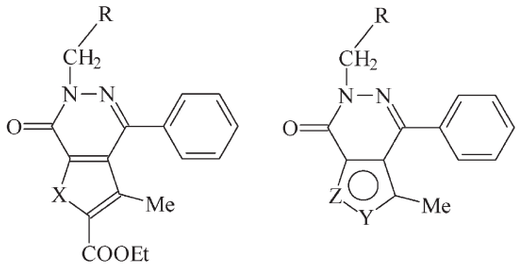
Phosphodiesterases (PDE) are enzymes responsible for the hydrolysis of cyclic adenosine (c-AMP) and guanosine monophosphate (c-GMP) which are important second messengers playing a central role in regulating many relevant cell functions. Among the eleven different PDE families which have been identified and characterized until now PDE 4 and PDE 5 are today the main target for small molecules of inhibitors which are promising candidates to be developed as antiinflammatory/immunosoppressive and peripheral vasodilatory agents, respectively.[1,2]

We previously reported the synthesis of some series of 4,5-heterocyclic-fused-3(2H)-pyridazinones **1a–d** and **2a–f** (Fig. 1), whose evaluation on different PDE families allowed us to identify some potent and selective PDE 4 inhibitors with low affinity for the high affinity Rolipram binding site.[3] Affinity for this site is related to side-effects such as nausea, vomiting and headache which until now hampered development of PDE 4 inhibitors as drugs.[4] During these studies we were able to define the structural requirements which address the activity to the PDE 4 isoenzyme family. Thus the presence of an ethyl group at pyridazine N-2 is connected with submicromolar and selective (versus PDE 3) inhibitory activity, while a benzyl group in the same position brings about a significant weaker activity at PDE 4.[3]

Evaluation of the benzyl derivatives **2b** and **2e** on PDE 5 isoenzyme revealed a potent ($IC_{50} = 1.4$ and 0.14 μM respectively) and selective (versus PDE 3 and PDE 4) inhibitory activity, which led us to undertake a novel investigation on these molecules with the aim of verifying if the pyrazolo[3,4-d] pyridazinone system is an appropriate substrate for PDE 5 inhibitors, potentially useful as peripheral vasodilators. Indeed in the structures **2** can be recognised some structural features common both to the oldest non-selective PDE 5 inhibitor Zaprinast **3**,[5] which was originally developed as an anti-allergic, and to the recent more potent Bristol Meyers **4** (Fig. 2).[6] The marketing of Sildenafil **5** (Viagra®), a

*Corresponding author. Tel.: +39-55-2757290. Fax: +39-55-240776. E-mail: vittorio.dalpiaz@unifi.it

ISSN 1475-6366 print/ISSN 1475-6374 online © 2002 Taylor & Francis Ltd
DOI: 10.1080/1475636021000008494



1	R	X
a	Me	NH
b	Ph	NH
c	Me	S
d	Ph	S

2	R	Y	Z
a	Me	N	NH
b	Ph	N	NH
c	Me	O	N
d	Ph	O	N
e	Ph	N	NMe
f	Me	N	NMe

FIGURE 1 Pyridazinones as lead compounds.

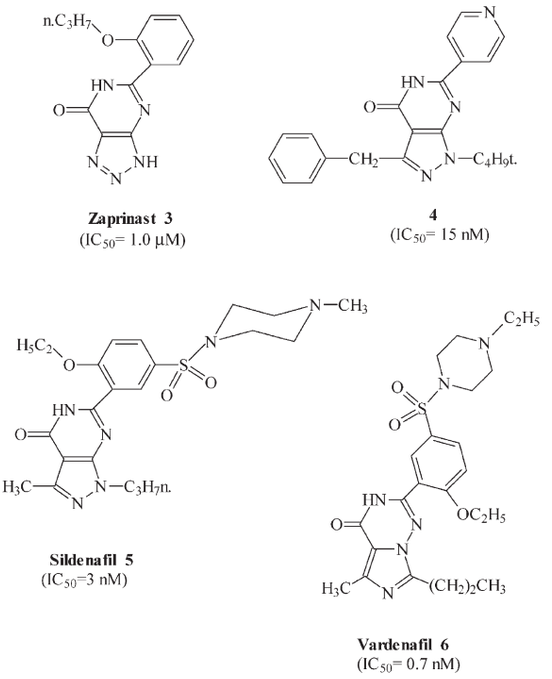


FIGURE 2 Some PDE 5 Inhibitors.

nanomolar PDE 5 inhibitor characterized by a pyrazolo[3,4-e]pyrimidine structure, useful for the pharmacological treatment of male erectile dysfunction (MED), which is a common problem in men over 40 years old, represented a breakthrough in this area and stimulated a lot of studies.[7] A new drug, Vardenafil **6**, showing fewer side effects with respect to Sildenafil is now in phase III clinical trials and it was found to be much more selective (42-fold) towards PDE6 with respect to Sildenafil,[8] affinity for PDE6 being responsible for retinal effects such as bluish haze and increased light sensitivity.

Thus we report here the results of the evaluation on the PDE 5 family of some previously described compounds,[3] as well as of a group of novel pyrazolo[3,4-d]pyridazinones **10a**,**b**, **11a**–**h**, **13**–**16** and **20a**,**b** structurally related to **2b** and **2e**.

MATERIALS AND METHODS

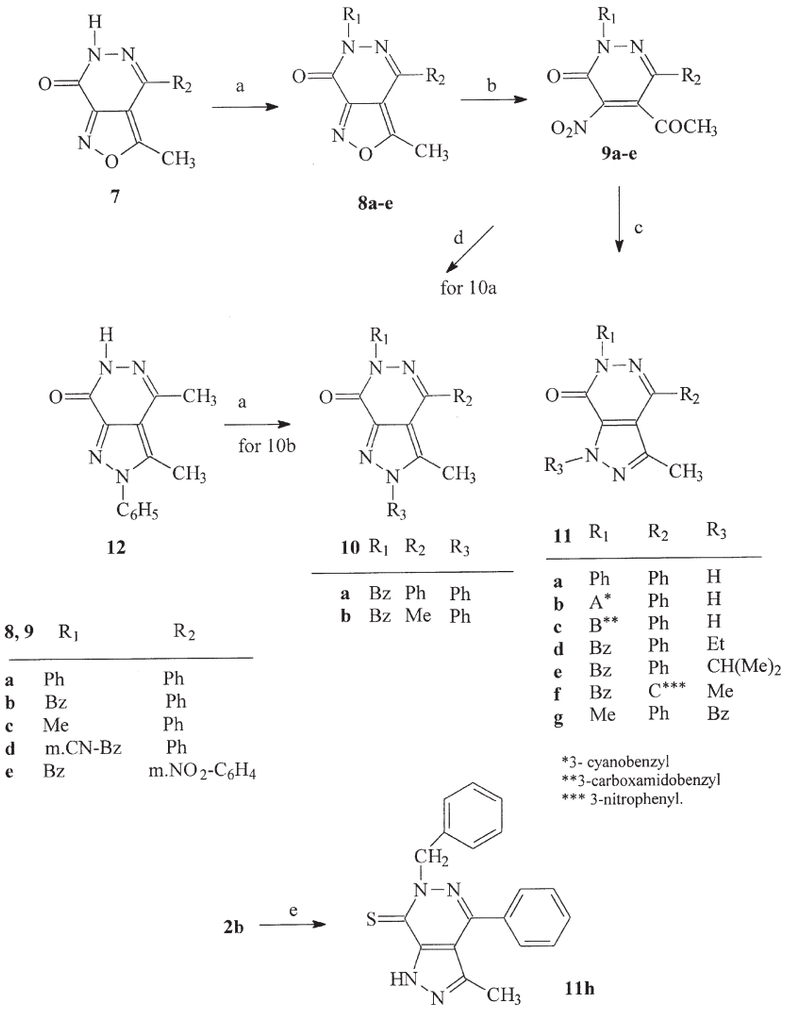
All melting points were determined on a Buchi apparatus and are uncorrected. ^{1}H-NMR spectra were recorded with Varian Gemini 200 instruments. Chemical shifts are reported in ppm, using the solvent as internal standard. Extracts were dried over Na_2SO_4 and the solvents were removed under reduced pressure.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

Chemistry

The synthesis of the pyrazolo[3,4-d]pyridazine derivatives **10a**,**b** and **11a**–**h** is depicted in Scheme 1. 4-Nitro-5-acetylpyridazinones of type **9** are the key intermediates for many of the final compounds and were prepared starting from **7** which was treated with arylalkyl halide to afford compounds **8** and then with CAN. Most of the above intermediates **7**–**9** have been previously described (**7**,[3,9,10] **8a**–**c**,[9,11] **9a**–**c**[3,11,12]). Compounds **11a**–**g** were smoothly obtained by briefly stirring the suitable **9** with hydrazine, alkylhydrazines or benzylhydrazine in ethanol at room temperature. When phenylhydrazine was used under the same reaction conditions, compound **10a** was obtained, while the corresponding 4-methylderivative **10b** was isolated by alkylation of **12**[13] with benzylchloride.

Structures **10a**,**b** and **11a**–**g** were unequivocally assigned on the basis of our previous studies.[14] Finally, the thioderivative **11h** was synthesized by reacting **2b** with Lawesson's reagent in toluene.

The synthesis of the open model **14** and of heterocyclic-fused pyridazinones derivatives **15**, **16** and **20a**,**b** is shown in Scheme 2. **14** Resulted from the treatment of **13**[15] with benzylchloride under the above conditions. The pyridopyridazinone **16** was prepared by reacting **9b** with N-methyl-β-alanine nitrile in ethanol, followed by heating shortly with sodium ethoxide in absolute ethanol. Finally **20a**,**b** were synthesized following this procedure: the 1,3-dipolar cycloaddition between the appropriate nitriloxide, generated *in situ* from ethylchloro-oximido acetate **17** and heteroaryl-butane-1,3-diones **18**[16,17] afforded the isoxazoles **19**,[11] which, in turn, were transformed to the final **20** by cyclocondensation



SCHEME 1 Reagents and conditions: (a) R_1X, DMF K_2CO_3, 50–80°C 2–6 h, 75–90%; (b) CAN, AcOH 50%, HNO_3, 50–60°C, 45–90 min, 40–60%; (c) hydrazine or alkylhydrazines or benzylhydrazine, EtOH, rt, 10–30 min 65–80° (d) phenyhydrazine, rt, 15 min 75%; (e) Lawesson's reagent, toluene, reflux, 90 min 70%.

with hydrazine followed by alkylation with benzylchloride.

All new compounds were fully characterized by means of ^{1}H-NMR, melting points and elemental analysis data, which confirmed the proposed structures. General procedures and data for several representative compounds are shown below.

General Procedure for Compounds 10a and 11a–g

The appropriate 5-acyl-4-nitropyridazinone **9** (0.4 mmol) was suspended in EtOH (5 mL) and the required (substituted)hydrazine (2.0 mmol) was added. The mixture was stirred at rt for 20–30 min (3 h for **11g**) and the precipitate was recovered by suction and recrystallized from ethanol (with the only exception of **11f** which was purified by column chromatography using cyclohexane/ethyl acetate 2:1 as eluent).

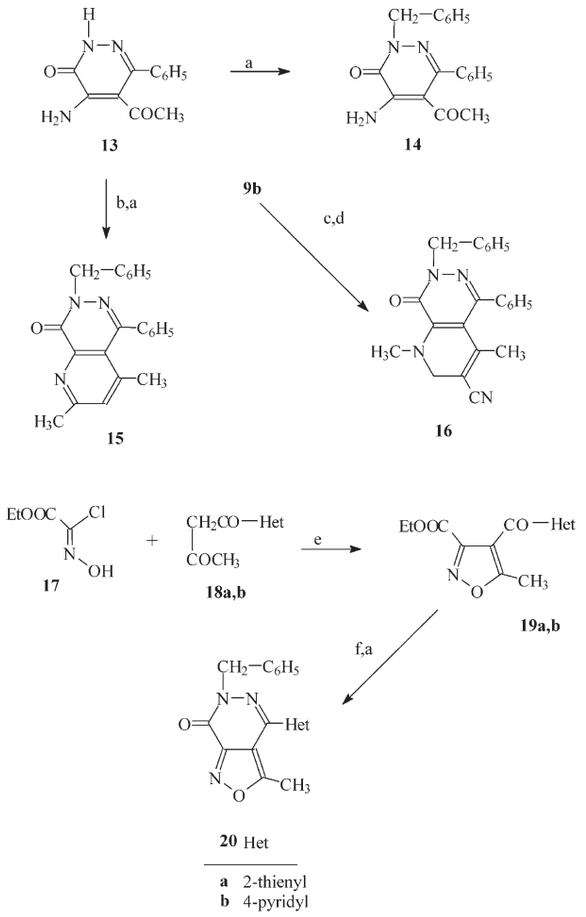
Compound **11c** was synthesized by treatment of **11b** (0.5 mmol) with conc. H_2SO_4 (1 mL) at rt for 6 h. After dilution with ice water, the crude precipitate was isolated by suction.

6-Benzyl-2,4-diphenyl-3-methylpyrazolo[3,4-d] pyridazin-7(6H)-one **10a**

Mp = 183–185°C; crystallization solvent = EtOH. ^{1}H-NMR ($CDCl_3$), δ, ppm: 2.20 (s, 3H, CH_3), 5.45 (s, 2H, CH_2), 7.40 (m, 15H, 3Ar); Anal. Found: C, 76.28; H, 5.13; N, 14.24. $C_{25}H_{20}N_4O$ requires: C, 76.50; H, 5.15; N, 14.28%.

6-(3-Cyanobenzyl)-3-methyl-phenylpyrazolo[3,4-d]pyridazin-7(6H)-one **11b**

Mp = 203–205°C; crystallization solvent = EtOH. ^{1}H-NMR ($CDCl_3$), δ, ppm: 2.30 (s, 3H, CH_3), 5.55 (s, 2H, CH_2), 7.50 (m, 9H, 2Ar). Anal. Found: C, 70.43; H, 4.45; N, 20.59. $C_{20}H_{15}N_5O$ requires: C, 70.36; H, 4.44; N, 20.52%.



SCHEME 2 Reagents and conditions: (a) $C_6H_5CH_2Cl$, DMF, K_2CO_3, 90°C, 1 h, 50–80%; (b) acetone, abs. EtOH, EtONa, 100°C, 4 days, 60%; (c) $H_3CNH(CH_2)_2CN$, EtOH, rt, 30 min 65%; (d) abs EtOH, EtONa, 50–60°C, 2 h, 70%; (e) abs. EtOH, EtONa, 50–60°C, 2 h 70%; (e) abs. EtOH, EtONa, −5°C, 1 h, 75%; (f) hydrazine, EtOH, rt, 20 min, 90%.

6-Benzyl-1-ethyl-3-methyl-4-phenylpyrazolo[3,4-d]pyridazin-7(6H)-one **11d**

Mp = 118–120°C; crystallization solvent = EtOH. ^{1}H-NMR ($CDCl_3$), δ, ppm: 1.50 (t, 3H, CH_2CH_3), 2.20 (s, 3H, C–C*H*$_3$), 4.80 (q, 2H, CH_2CH_3), 5.40 (s, 2H, CH_2Ar), 7.40 (m, 10H, 2Ar). Anal. Found: C, 72.98; H, 5.88; N, 16.22. $C_{21}H_{20}N_4O$ requires: C, 73.22; H, 5.86; N, 16.27%.

6-Benzyl-1-isopropyl-3-methyl-4-phenylpyrazolo[3,4-d]pyridazin-7(6H)-one **11e**

Mp = 177–179°C; crystallization solvent = EtOH. ^{1}H-NMR ($CDCl_3$), δ, ppm: 1.60 (d, 6H, $(CH_3)_2CH$), 2.20 (s, 3H, C–CH_3), 4.65 (m, 1H, $(CH_3)_2CH$), 5.45 (s, 2H, CH_2), 7.40 (m, 10H, 2Ar). Anal. Found: C, 77.20; H, 6.51; N, 16.31. $C_{22}H_{22}N_4O$ requires: C, 77.15; H, 6.49; N, 16.36%.

6-Benzyl-3-methyl-4-phenylpyrazolo[3,4-d]pyridazin-7(6H)-thione **11h**

Compound **11h** was synthesized by suspending **2b** (0.4 mmol) in toluene (4 mL) and added Lawesson's reagent (10 mmol). The mixture was refluxed for 90 min and, after cooling, the residue was filtered off. The organic layer was evaporated *in vacuo* and the residue oil treated with cold ethanol to afford **11h**. Mp = 145–147°C; crystallization solvent = EtOH. ^{1}H-NMR ($CDCl_3$), δ, ppm: 2.30 (s, 3H, CH_3), 6.10 (s, 2H, CH_2), 7.50 (m, 10H, 2Ar). Anal. Found: C, 68.80; H, 4.87; N, 16.91. $C_{19}H_{16}N_4S$ requires: C, 68.64; H, 4.86; N, 16.86%.

5-Acetyl-4-amino-2-benzyl-6-phenylpyridazin-3(2H)-one **14**

A suspension of **13** (0.6 mmol), potassium carbonate (1.8 mmol), benzyl chloride (1.0 mmol) in anhydrous DMF (5.0 mL) was heated at 90°C for 1 h. After cooling, water (20 mL) was added and the crude **14** was recovered by suction. Mp = 114–116°C; solvent crystallization = EtOH. ^{1}H-NMR ($CDCl_3$), δ, ppm: 1.75 (s, 3H, CH_3), 5.45 (s, 2H, CH_2), 7.45 (m, 10H, 2Ar). Anal. Found: C, 71.25; H, 5.40; N, 13.13. $C_{19}H_{17}N_3O_2$ requires: C, 71.45; H, 5.38; N, 13.16%.

7-Benzyl-7,8-dihydro-2,4-dimethyl-5-phenyl-8-oxopyrido[2,3-d]pyridazine **15**

A suspension of **13** (0.5 mmol) and EtONa (0.10 g Na in 2.5 mL EtOH) in anhydrous acetone was stirred at 100°C for 4 days in a sealed tube. After cooling, the solvent was evaporated *in vacuo* and the residue treated with water (10 mL) and extracted with CH_2Cl_2 (3 × 15 mL). After evaporation of the solvent, the crude product was alkylated to afford **15** using standard conditions (benzyl chloride (0.8 mmol), anhydrous K_2CO_3 (1.6 mmol) in anhydrous DMF (3 mL) at 90°C for 1 h). Mp = 181–184°C; crystallization solvent = EtOH. ^{1}H-NMR ($CDCl_3$), δ, ppm: 1.95 (s, 3H, C–C–CH_3), 2.80 (s, 3H, N–C–CH_3), 5.40 (s, 2H, CH_2), 7.40 (m, 10H, 2Ar). Anal. Found: C, 77.08; H, 5.60; N, 12.33. $C_{22}H_{19}N_3O$ requires: C, 77.38; H, 5.62; N, 12.31%.

7-Benzyl-3-cyano-1,4-dimethyl-5-phenyl-8-oxo-1,2,7,8-tetrahydropyrido[2,3-d]pyridazine **16**

A solution of **9b** (0.5 mmol), N-methyl-β-alanine nitrile (1.2 mmol) in EtOH (2 mL) was stirred at rt for 30 min. The reaction was diluted with water (10 mL), extracted with CH_2Cl_2 (3 × 20 mL) and the solvent mixture evaporated *in vacuo*. The residue was dissolved in absolute EtOH (3 mL), EtONa (0.11 g Na in 2.5 mL EtOH) was added and the mixture was stirred at 50–60°C for 2 h. After dilution the crude precipitate was recovered by suction. Mp = 239–240°C; crystallization solvent = EtOH. ^{1}H-NMR ($CDCl_3$), δ, ppm: 1.60 (s, 3H, C–CH_3), 3.75 (s, 3H, N–CH_3), 3.90 (s, 2H, CH_2–C–CN), 5.30

(s, 2H, CH_2Ar), 7.40 (m, 10H, 2Ar). Anal. Found: C, 75.12; H, 5.49; N, 15.26. $C_{23}H_{20}N_4O$ requires: C, 74.97; H, 5.48; N, 15.21%.

General Procedure for Compounds 20a,b

The synthesis of isoxazoles **19a**,**b** was performed following the procedure reported for **19b** in reference 11. Cyclocondensation of **19a**,**b** (0.5 mmol) with hydrazine hydrate (0.6 mmol) in EtOH (2 mL) at rt afforded the 6-unsubstituted derivatives which, in turn, after filtration, were converted to the benzyl-derivatives **20a**,**b** using the standard conditions described for **15**.

6-Benzyl-3-methyl-4-(2-thienyl)-isoxazolo[3,4-d] pyridazin-7(6H)-one **20a**

Mp = 172–174°C; crystallization solvent = EtOH. ^{1}H-NMR ($CDCl_3$), δ, ppm: 2.75 (s, 3H, CH_3), 5.35 (s, 2H, CH_2), 7.35 (m, 8H, 2Ar). Anal. Found: C, 63.30; H, 4.05; N, 12.96. $C_{17}H_{13}N_3O_2S$ requires: C, 63.13; H, 4.06; N, 13.00%.

Pharmacology

Purification of Phosphodiesterase 5

PDE5 was purified from human platelets as described by Gristwood *et al.*[18] Briefly, the supernatant of the cell lysate from 10^9 platelets was chromatographed using a Mono-Q ion exchange column attached to a Pharmacia FPLC system. PDE5 was characterised in front other PDE isoenzymes, according to Beavo *et al.*[19] 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 F-94836 and Zaprinast. PDE5 was kept frozen at −80°C in the presence of 1 g/l bovine serum albumin until used.

Phosphodiesterase Assay

Cyclic nucleotide phosphodiesterase activities were measured using a two step procedure according to Thompson and Strada.[20] PDE5 (activated by 250 μg/ml trypsin) was assayed using 0.25 μM ^{3}H-cGMP as substrate. IC_{50} values were obtained by nonlinear regression using the Prism programme by GraphPad Software. The reported values are the average of at least three independent assays. Sildenafil and Zaprinast were used as reference substances.

RESULTS AND DISCUSSION

The previously described compounds **1a**–**d** and **2a**–**f**[3] were tested for their ability to inhibit PDE 5. The obtained data (Table 1) clearly indicate that, with the only exception of the pyrrolopyridazinones **1a**–**b**, in all the series the best substituent at the pyridazine N-2 is a benzyl group, whose presence is associated with considerable higher potency with respect to that of the corresponding ethyl derivative. In particular compound **2b** showed an IC_{50} value in the low micromolar range (1.4 μM), whereas the ethyl analogue **2a** did not show any inhibitory activity at 2 μM concentration. The nitrogen alkylated pyrazole **2e** was the most potent (IC_{50} = 140 nM); again the corresponding ethyl derivative **2f** was much less potent. An interesting level of activity was also displayed by the isoxazolopyridazinone **2d** (IC_{50} = 3.1 μM).

The data related to the novel compounds are reported in Table 2. We carried out SAR studies with reference to the leads **2b**, **2d** and **2e**. Structural modifications of **2b** afforded activity improvement only in the case of compound **10a** (IC_{50} = 0.7 μM), where the presence of a phenyl group on the pyrazole was associated with a twofold increase in potency with respect to **2b**. PDE5 inhibitory activity in the low micromolar range was seen for the thio derivative **11h**. Replacement of the phenyl group at the pyridazine 6-carbon with a methyl (**10b**) reduced the activity. A more detrimental effect on potency was associated with the introduction of an carboxamide group in the meta position of the benzyl group (**11c**). The corresponding cyano derivative **11b** also proved to be less potent than **2b**. Compound **11g** in which the benzyl group was moved to the pyrazole was significantly less potent that **2b**. Finally, elimination of the methylenic spacer (**11a**) led a reduction of activity. The open model **14** was three fold less potent with respect to the prototype **2b**. Replacement of pyrazole in the reference compound **2b** with a functionalized dihydropyridine (**16**) was detrimental, whereas a fully aromatic pyridine system (**15**) left the activity (IC_{50} = 2.3 μM) almost unchanged.

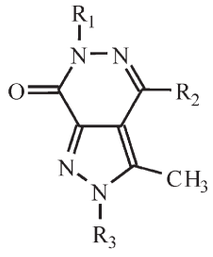
Replacement of the phenyl system appended at position 4 in **2d** with isosteric groups afforded

TABLE 1 PDE 5 inhibitory activity of **1a**–**d** and **2a**–**f**

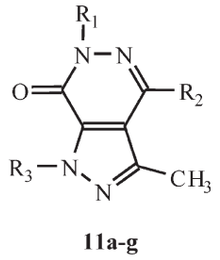
Comp.[a]	IC_{50} (μM) or % inhibition[b,c]
1a	14%
1b	14%
1c	2%
1d	13%
2a	0%
2b	1.4
2c	20%
2d	3.1
2e	0.14
2f	13%
Zaprinast	1.0
Sildenafil	0.003

[a]All compounds have been previously described.[3] [b]Human platelets. [c]Inhibition at 2 μM.

TABLE 2 PDE 5 inhibitory activity of **10a**,**b**, **11a**–**h**, **14**–**16** and **20a**,**b**



10a-c



11a-g

Comp	R1	R2	R3	M.p[a] (°C)	Yield	IC_{50} (μM) or % inhibition[b,c]
10a	Bz	Ph	Ph	183–185	89	0.70
10b	Bz	Me	Ph	180–182	72	29%
11a	Ph	Ph	H	238–239	73	1.40
11b	A[d]	Ph	H	203–205	62	20%
11c	B[e]	Ph	H	161–163	47	7%
11d	Bz	Ph	Et	118–120	51	0.63
11e	Bz	Ph	$CHMe_2$	177–179	60	0.18
11f	Bz	C[f]	Me	135–138	77	0.24
11g	Me	Ph	Bz	119–121	47	33%
11h				145–147	78	1.90
14				114–116	73	4.20
15				182–184	85	2.30
16				239–240	50	4.00
20a				172–174	87	1.40
20b				158–160	74	5.80
2b						1.40
2d						3.10
2e						0.14
Zaprinast						1.0
Sildenafil						0.005

[a]All compounds were crystallized from ethanol with the exception of **11g** which was purified by column chromatography using cyclohexane/ethyl acetate 2:1 as eluent; [b]PDE5 from human platelets; [c]inhibition at 2 μM; [d]3-cyanobenzyl; [e]3-carboxamidobenzyl; [f]3-nitrophenyl.

different results: the 2-thienyl analogue **20a** (IC_{50} = 1.4 mM) was slightly more potent than **2d**, whereas the 4-pyridyl analogue **20b** proved to be fourfold less active with respect to **2d**.

Structural modifications performed on **2e** led to compounds with a comparable level of potency: thus homologation of the methyl group on the pyrazole (**11d**) led to a slight reduction of activity whereas introduction of a branched alkyl chain (**11e**) left the activity (IC_{50} = 0.18 μM) unchanged but enhanced selectivity versus PDE6. Likewise introduction of a nitro at the meta position of the phenyl ring (**11f**) led to a compound with similar PDE5 inhibitory activity with respect to **2e**.

Taken together these data seem to indicate that the structure **2b** has limited tolerance for substitution: thus the phenyl group opposite to the carbonyl dipole is an essential requirement. Moreover the benzyl group either cannot be modified or moved from the pyridazine to pyrazole.

As regards the heterocyclic system fused with the pyridazinone moiety, among the five different heterocyclic systems examined, the best results were obtained with the pyrazole backbone (**2b**, **2e** and **11d**–**f**) which is an important feature of compounds **4** and **5**. The isoxazole (**20a**) and the fully aromatic pyridine system (**15**) afforded weaker activity, whereas the pyrrole ring was tolerated worst. Finally dissection of the five membered system (**14**) was associated with a considerable reduction in activity. In conclusion these studies allowed us to identify some compounds (**2e** and **11d**–**f**) endowed with an interesting level of PDE5 inhibitory activity. Regarding the selectivity issue, **2b** showed high selectivity versus PDE3 (60% inhibition at 200 μM), but was not selective versus PDE4 (IC50 = 3.1 μM).[3] Interestingly **2e** showed a good selectivity both versus PDE3 and PDE4 (30% and 45% inhibition at 200 μM respectively[3]).Our compounds although clearly less potent that Sildenafil and Vardenafil favourably compete with Zaprinast as regards PDE5 inhibitory potency.

In conclusion the obtained data suggest that in the present series the ethyl group at the pyridazine 2-nitrogen selectively addresses the activity towards

the PDE 4 family, whereas the presence of the benzyl in the same position is associated with potent and selective inhibitory activity versus the PDE 5 isoenzyme.

References

[1] William Harvey Research Conferences: "PDE inhibitors: drugs with an expanding range of therapeutic uses". Nice, December 1–3, 1999.
[2] Dal Piaz, V. and Giovannoni, M.P. (2000) *Eur. J. Med. Chem.* **35**, 463.
[3] Dal Piaz, V., Giovannoni, M.P., Castellana, M.C., Palacios, J.M., Beleta, J., Doménech, T. and Segarra, V. (1997) *J. Med. Chem.* **40**, 1417.
[4] Barnette, M.S., Christensen, S.B., Essayan, D.M., Grous, M., Prabhakar, V., Rush, J.A., Kagey-Sobota, A. and Torphy, T.J. (1998) *J. Pharmacol. Exper. Therap.* (284), 105.
[5] Coates, W.J., Connolly, B., Dhanak, D., Flynn, S.T. and Worby, A. (1993) *J. Med. Chem.* **36**, 1387.
[6] Czarniecki, M., Ahn, H.S. and Sybertz, J. (1996) In: Doherty, A.M., ed, *Annual Reports in Medicinal Chemistry* (Academic Press, San Diego) **31**, p. 61.
[7] Terret, N.K., Bell, A.S., Brown, D. and Ellis, P. (1996) *Bioorgan. Med. Chem. Lett.* **6**, 1819.
[8] Vardenafil (2001) *Drugs of the Future* **26**, 141.
[9] Renzi, G. and Dal Piaz, V. (1965) *Gazz. Chim. Ital.* **95**, 1478.
[10] Renzi, G. and Pinzauti, S. (1969) *Il Farm. Ediz. Scientif.* **24**, 885.
[11] Dal Piaz, V., Ciciani, G., Turco, G., Giovannoni, M.P., Miceli, M., Pirisino, R. and Perretti, M. (1991) *J. Pharm. Sci.* **80**, 341.
[12] Dal Piaz, V., Ciciani, G. and Turco, G. (1989) *Synthesis*, 213.
[13] Hassan, A.A. (1996) *J. Chem. Res.* **7**, 1757.
[14] Dal Piaz, V., Ciciani, G., Turco, G. and Giovannoni, M.P. (1989) *Heterocycles* **29**, 1595.
[15] Dal Piaz, V., Ciciani, G. and Giovannoni, M.P. (1991) *Heterocycles* **32**, 1173.
[16] Harris, S.R. and Levine, R. (1948) *J. Am. Chem. Soc.* **70**, 3360.
[17] Sing, B., Lesher, G.Y., Pluncket, K.C., Pagani, E.D., Bode, D.C., Bentley, R.S., Connell, M.J., Hamel, L.T. and Silver, P.J. (1992) *J. Med. Chem.* **35**, 4858.
[18] Gristwood, R.W., Beleta, J., Bou, J., Cardelus, I., Fernandez, A.G., Llenas, J. and Berga, P. (1992) *Br. J. Pharmacol.* **105**, 985.
[19] Beavo, J.A., Conti, M. and Heaslip, R. (1994) *J. Mol. Pharmacol.* **46**, 399.
[20] Thompson, W.J. and Strada, S.J. (1984) In: Bergmayer, H.U., ed, *Methods of Enzymatic Analysis* (John Wiley and Sons, Germany) **Vol. IV**, p. 127.