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New pyrazolo[1′,5′:1,6]pyrimido[4,5-*d*]pyridazin-4(3*H*)-ones as potent and selective PDE5 inhibitors

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Abstract—A series of potent PDE5 inhibitors with high selectivity versus PDE6 isoenzymes was identified.

1. Introduction

Phosphodiesterases 5 (PDE5) are enzymes responsible for the hydrolysis of cyclic guanosine-3′,5′-phosphate (cGMP) and are mainly distributed in kidney, spleen, lung and smooth muscle tissue.[1] PDE5 inhibitors, elevating cGMP levels, represent very attractive agents for the treatment of cardiovascular pathologies and erectile dysfunction (ED).[2,3] This latter is a common problem in men over 40, and in the United States alone more than 30 million men suffer from ED.[4–6] Penile smooth muscle relaxation is mainly mediated by nitric oxide (NO) released from vascular endothelium and parasympatic nerve ending. NO stimulates the soluble guanilate cyclase to increase cGMP levels which, acting as an intracellular second messenger, induces the relaxation of smooth muscle cells of corpus cavernosus.[7] PDE5 inhibitors, stopping the process of hydrolysis of cGMP, enhance these effects and related erectile function.

The first PDE5 inhibitor used for treating male erectile dysfunction (MED) was Sildenafil (Viagra™) **1**[8–10] in 1998 (see Fig. 1). This drug, despite its potency, showed many notable side effects such as nausea, headache, cutaneous flushing and retinal effects (bluish haze, or increased light sensitivity) due to inhibition of PDE6 isoform.[11,12] The research in this field, aimed at discovering more selective PDE5 inhibitors, led to very potent compounds with reduced secondary effects such as Vardenafil **2**[13] and Tadalafil **3**.[14] The latter was launched (Cialis™) for treatment of MED at the beginning of 2003 showing a longer half-life (>17 h) with respect to Sildenafil and the absence of colour vision disturbances and cardiovascular effects.[15,16] Very promising results are coming from Bristol–Myers research which has identified some substituted pyrazolopyridopyridazines of type **4** as potent (IC_{50} = 0.03–0.3 nM) and selective PDE5 inhibitors, orally bioavailable, potentially useful in the treatment of ED.[17]

Our studies in the field of PDE inhibitors[18–21] led us to design and develop a new series of pyrazolopyrimidopyridazinones (compounds **10**–**42**), structurally related to compound **4**, showing a good inhibitory activity on PDE5 and a fair selectivity towards PDE6.

2. Chemistry

Compounds **10**–**42** were prepared by following the general procedure reported in Scheme 1. Isoxazolo[3,4-*d*]-pyridazin-7(6*H*)-one **6** was obtained by treatment with hydrazine of isoxazole **5**, following the synthetic procedure reported in the literature.[22] Intermediate **6** was condensed with the opportune arylaldehydes (or pivalic aldehyde) to give the vinyl derivatives **7**.[23] Treatment of compounds **7** with hydrazine in ethanol afforded, in very good yields, the pyrazole intermediates **8** by opening the isoxazole ring and at the same time closure to pyrazole.[24] Compounds **9** were obtained by treatment of 4-amino-5-(1*H*-pyrazol-5-yl)pyridazin-3(2*H*)-ones **8** with

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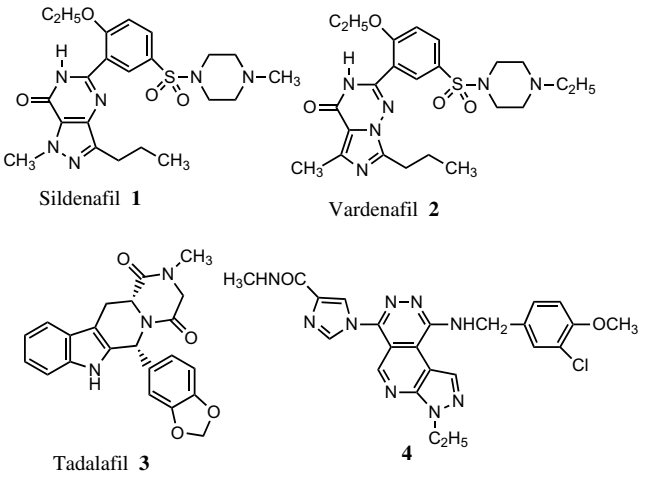
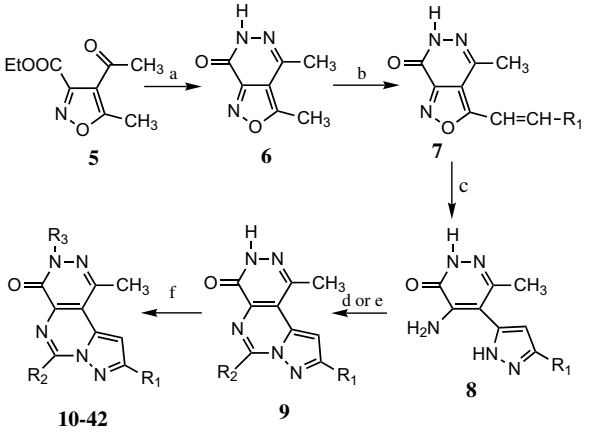


Figure 1.



Scheme 1. Reagents and conditions: (a) hydrazine hydrate, EtOH, rt; (b) R_1CHO, MeONa, abs MeOH, reflux; (c) hydrazine hydrate, EtOH, rt; (d) $(R_2CO)_2O$, reflux; (e) R_2COOH, dichloromethane, anhydrous DMF, DMAP, EDC, reflux; (f) R_3X, NaH, DMSO, rt.

the opportune anhydride under refluxing conditions without solvent. When acids were used, the condensation was performed at room temperature using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride as coupling agent and the resulting amides were then thermally cyclized. Finally, alkylation of the pyrazolopyrimidopyridazinones **9** with a variety of halides in standard conditions afforded the final compounds **10**–**42**.

3. Results and discussion

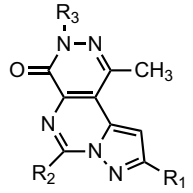
All compounds were evaluated for their ability to inhibit PDE5 and PDE6. PDE5 was purified from human platelets as described by Gristwood et al.[25] PDE6 was purified from bovine retinas as described by Beavo et al.[26] Cyclic nucleotide phosphodiesterase activities were measured using a two-step procedure according to Thompson and Strada.[27] Sildenafil was used as reference drug.

The first term of this series was compound **10** (R_1 = Ph, R_2 = Me, R_3 = Bn), for which an appreciable activity and selectivity was found (IC_{50} = 160 nM for PDE5 and 25% inhibition at 2 μM for PDE6). Keeping the same tricyclic skeleton, structural modifications were performed at R_1, R_2 and R_3 and related results are summarized in Table 1.

As regards substituents R_1 at position 9 of the tricyclic system, aryl, substituted phenyl as well as *t*-butyl groups were inserted. Data showed a good activity associated with the presence of a 2-pyridyl ring (compounds **18**, IC_{50} = 270 nM) comparable to **10**. Introduction of an amino group in para position of the phenyl (compound **20**) reduced the activity (IC_{50} = 920 nM), but increased the selectivity towards PDE6 isoenzymes (4.5% inhibition at 2 μM) with respect to the more active compounds **10** and **18**. On the other hand, a complete loss of potency was found for the 3,4-methylenedioxy and the 4-nitrophenyl derivatives **16** and **19**, indicating that steric and electronic factors may limit the tolerance in this position. Finally, also the *t*-butyl derivative resulted completely inactive, suggesting that the aromatic system in this part of the molecule could play a significant role in determining PDE5 inhibitory activity.

Modifications of substituent R_2 at position 6 gave interesting results, achieving for some compounds IC_{50}s in the 34–81 nM range. Starting from the analysis of aromatic and aralkyl substituents, it seems that exact requirements were necessary. In fact, when a pyridyl system was inserted at 6, different results were obtained depending on the linked position. The 2-pyridyl derivative (compound **24**) is the most interesting compound, showing a very good balance of potency and selectivity versus PDE5 (IC_{50} = 81 nM for PDE5 and 21.3% at 2 μM for PDE6). The potency, together with the selectivity, decreased when a 3-pyridyl ring was inserted (compound **25**), but a complete loss of activity was observed for the 4-pyridyl derivative (**26**), suggesting that the nitrogen could be involved in a specific bond. On the other hand, activity and selectivity were restored by inserting a methylenic spacer (compound **22**). The 6-phenyl derivative **21** showed an analogous potency (IC_{50} = 81 nM) to compound **24**, but lower selectivity. Introduction of an alkyl spacer between the phenyl ring and the tricyclic system led to different results: the benzyl derivative **23** was found completely devoid of activity, whereas the insertion of a phenylethyl residue partially restored the inhibitory activity (compound **27**, IC_{50} = 980 nM). A good level of potency and selectivity was found for compound **29**, in which a *m*-tolyl was inserted; on the contrary, the isomer *o*-tolyl led to an inactive product indicating that steric factors could play an important role for interaction with the biological target. Similarly, compounds **30** and **31**, in which a cyclohexylmethyl and a cyclopentylmethyl residue respectively were inserted, are completely inactive. Very interesting results were obtained by the introduction of alkyl chains and functionalized alkyl chains. For this type of substituent a higher tolerance seems to be allowed since the IC_{50}s of all tested compounds are in the 430–434 nM range.

Table 1. PDE5 and PDE6 inhibitory activity of compounds **10**–**42**



10-42

Compd	R_1	R_2	R_3	PDE5 IC_{50} or % inhibition (μM)[b]	PDE6 IC_{50} or % inhibition (μM)[b]
10	Ph	Me	Bn	0.16	25(2)
11	Ph	Me	Cyclohexylmethyl	3.1	11
12	Ph	Me	*p*-Nitrobenzyl	65.9(20)	10.4(2)
13	Ph	Me	*p*-Aminobenzyl	1.1	2.0
14	Ph	Me	Ph	11.9(0.2)	3.7(0.2)
15	Ph	Me	*m*-(H_2PO_4)benzyl	0.23	28.6(0.2)
16	A[a]	Me	Bn	13.0	78
17	*t*-Butyl	Me	Bn	31.0(2)	−20.9(2)
18	2-Pyridyl	Me	Bn	0.27	10
19	4-Nitrophenyl	Me	Bn	65.5(20)	14.4(0.2)
20	4-Aminophenyl	Me	Bn	0.92	4.5(2)
21	Ph	Ph	Bn	0.081	2.8
22	Ph	4-Pyridylmethyl	Bn	0.45	20.4(2)
23	Ph	Bn	Bn	22.5(2)	13.6(2)
24	Ph	2-Pyridyl	Bn	0.081	21.3(2)
25	Ph	3-Pyridyl	Bn	0.18	17.7(2)
26	Ph	4-Pyridyl	Bn	69.0(2)	−4.8(2)
27	Ph	Phenylethyl	Bn	0.98	30
28	Ph	*o*-Tolyl	Bn	55.1(2)	−16.6(2)
29	Ph	*m*-Tolyl	Bn	0.075	11.5(2)
30	Ph	Cyclohexylmethyl	Bn	33.6(2)	19.2(2)
31	Ph	Cyclopentylmethyl	Bn	18.2(2)	4.2(2)
32	Ph	CH_2OCH_3	Bn	0.1	53.3(2)
33	Ph	$CH_2OCH_2CH_3$	Bn	0.35	45(2)
34	Ph	CH_2SCH_3	Bn	0.1	38.1(2)
35	Ph	CH_2CH_2COOH	Bn	0.43	0.59
36	Ph	CH=CHCOOH	Bn	0.32	0.58
37	Ph	$(CH_2)_3COOH$	Bn	0.03	0.11
38	Ph	$(CH_2)_4COOH$	Bn	0.09	0.063
39	Ph	$CH(C_2H_5)_2$	Bn	0.067	37.4(2)
40	Ph	$CH(CH_3)CH_2CH_3$	Bn	0.034	42.5(2)
41	Ph	$CH(CH_3)CH_2CH_3$	*m*-(H_2PO_4)benzyl	0.059	66.9(2)
42	2-Pyridyl	$CH(CH_3)CH_2CH_3$	Bn	0.039	34.6(2)
Sildenafil				0.020	0.040

[a] A = 3,4-methylendioxyphenyl.
[b] The IC_{50} were obtained from dose–response curves at three or four different concentrations (n = 2–3).

Ether derivatives (**32** and **33**) and the corresponding thioether **34**, as well as the carboxylic acids **35** and **36**, showed a good level of activity and an appreciable selectivity with the exception of compounds **35** and **36** which demonstrated the same affinity for PDE5 and PDE6 isoenzymes. Homologation of carboxylic acid **35** (compounds **37** and **38**) resulted in an increase of potency, but, as the above compounds, in the complete absence of selectivity (IC_{50} = 30, 90 nM for PDE5 and IC_{50} = 110, 63 nM for PDE6). The best results were associated with the introduction of branched chains (compounds **39** and **40**) which showed a significant improvement of the potency with respect to the corresponding 6-methyl derivative **10**. These compounds appeared particularly interesting because of their high activity towards PDE5 associated to very good selectivity (IC_{50} = 67 and 34 nM for PDE5; 37.4% and 42.5% inhibition at 2 μM for PDE6).

Finally, some modifications were performed at position 3 by inserting substituents on the benzyl group as well as by removing the methylenic spacer with the introduction of a phenyl ring. The best result is related to the introduction of a phosphate group at meta position of the benzyl (compound **15**), the activity being comparable, but not improved with respect to the 3-unsubstituted benzyl derivative **10.**

The best substituents selected for position 9, 6 and 3 (2-pyridyl, $CH(CH_3)CH_2CH_3$ and *m*-(H_2PO_4)-benzyl, respectively) were finally combined in compounds **41** and **42** in order to verify if hybridization is allowed. In

both cases activity and selectivity are very good, but no improvement of potency was found, suggesting that the combining approach is not effective.

4. Conclusion

In conclusion we have identified a novel series of potent and selective PDE5 inhibitors potentially useful as peripheral vasodilators. The most interesting compounds showed values of PDE5 inhibitory activity in the same nanomolar range of Sildenafil but with a much better selectivity versus PDE6; this last aspect could make them good candidates to be used as MED treating drugs devoid of some well-known unwanted effects.

Further modifications at positions 9, 6, 3 and 1 are in progress to completely define structure–activity relationships and in order to improve the potency and selectivity profile of the present series.

Acknowledgements

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