Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 2376–2379
Bioorganic & Medicinal Chemistry Letters

Potent and selective xanthine-based inhibitors of phosphodiesterase 5

Nichola J. Arnold,[a] Ruth Arnold,[a] David Beer,[a] Gurdip Bhalay,[a] Stephen P. Collingwood,[a] Sarah Craig,[a] Nicholas Devereux,[a] Mark Dodds,[a] Andrew R. Dunstan,[a] Robin A. Fairhurst,[a] David Farr,[a] Joseph D. Fullerton,[a] Angela Glen,[a] Sylvie Gomez,[a] Sandra Haberthuer,[a] Julia D. I. Hatto,[a] Colin Howes,[a] Darryl Jones,[a] Thomas H. Keller,[a] Beate Leuenberger,[b] Heinz E. Moser,[a] Irene Muller,[b] Reto Naef,[b] Paul A. Nicklin,[a] David A. Sandham,[a,*] Katharine L. Turner,[a] Morris F. Tweed,[a] Simon J. Watson[a] and Mauro Zurini[b]

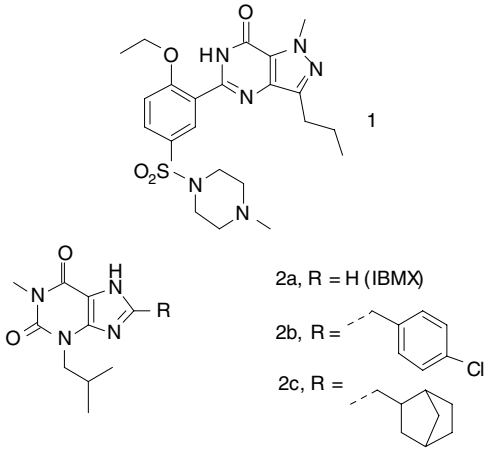
[a]*Novartis Institutes of Biomedical Research, Horsham Research Centre, Wimblehurst Road, Horsham, West Sussex RH12 5AB, UK*
[b]*Novartis Institutes of Biomedical Research, Lichtstrasse 35, CH-4056 Basel, Switzerland*

Received 18 October 2006; revised 4 November 2006; accepted 6 November 2006
Available online 10 November 2006

Abstract—Inhibitors of PDE5 are useful therapeutic agents for treatment of erectile dysfunction. A series of novel xanthine derivatives has been identified as potent inhibitors of PDE5, with good levels of selectivity against other PDE isoforms, including PDE6. Studies in the dog indicate excellent oral bioavailability for compound **21**.

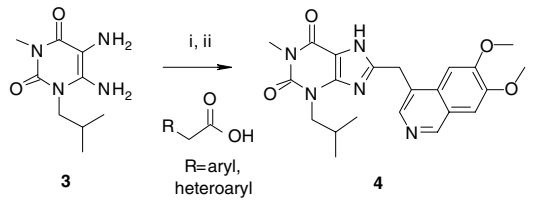
The cGMP metabolising enzyme phosphodiesterase 5 (PDE5) plays a pivotal role in regulating male erectile function.[1] Sexual stimulation results in release of NO from the cavernosal nerve, activating guanylate cyclase, which leads to an increase in intracellular cGMP levels. Relaxation of smooth muscle is thus facilitated, leading to increased blood influx into the corpus cavernosum and hence penile erection. Inhibition of PDE5 is a clinically proven concept for treatment of male erectile dysfunction (MED), with sildenafil **1** the first to market of three currently approved PDE5 inhibitors.[1,2]

Notwithstanding the utility of **1**, adverse effects such as headaches, flushing and visual disturbance have been noted in a small number of MED patients taking sildenafil,[3] some of which could be associated with cross reactivity on other PDE isoforms. Competitive inhibition of PDE6, which controls function of rod and cone cells within the eye, may be responsible for some of the ocular side effects observed.[4] In this communication, we disclose the discovery of novel, xanthine-based PDE5 inhibitors which exhibit good levels of in vitro potency and selectivity over other PDE isoforms, including PDE6, as well as an acceptable in vivo pharmacokinetic profile in the dog. 3-Isobutyl-1-methylxanthine (IBMX) **2a** has been long known as a general, non-selective PDE



Keywords: Phosphodiesterase 5; Xanthine; Erectile dysfunction.
* Corresponding author. Fax: +44 1403 323837; e-mail: david.sandham@novartis.com

0960-894X/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2006.11.019



Scheme 1. Parallel synthesis approach to xanthines. Reagents and conditions: (i) acid, HATU or EDC; DIPEA, DMF, RT; (ii) NaOH, MeOH, H_2O, 60 °C.

inhibitor.[5] Corbin[6] described a series of 8-substituted derivatives of IBMX such as **2b** and **2c**, which exhibited low nM potency against PDE5 and modest selectivity against PDE1. As no selectivity data against PDE6 had been reported, we were interested in re-evaluating this class of compounds as a potential source of selective PDE5 inhibitors.

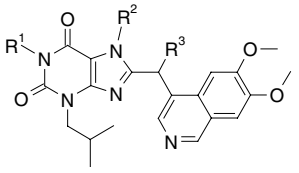
We have communicated a parallel synthetic method to construct a library of C-8-substituted xanthines, reacting the diaminouracil **3** with a range of aryl- and heteroarylacetic acids (Scheme 1),[7] which were either commercially available or privileged structures obtained from the Novartis Compound Archive. We were gratified to identify the 4-isoquinolylmethyl derivative **4**[8] with an IC_{50} of 9 nM for inhibition of human platelet PDE5. We then set about a systematic investigation of the structure–activity and selectivity relationships around the xanthine–isoquinoline moiety in **4**.

Diaminouracils analogous to **3** were either known compounds or prepared using similar methodology.[9] Isoquinoline-4-acetic acids were prepared by the three methods outlined in Scheme 2. Method A utilised the procedure of Bobbitt,[10] for compounds with an activating 7-alkoxy group, method B was used for direct functionalisation of isoquinolines lacking the 7-alkoxy substituent, via formation of the 1,2-dihydroisoquinoline, which was either isolated or reacted in situ.[11] Method C employed a variant of the Bischler–Napieralski procedure to assemble the 1-substituted isoquinoline ring with the 4-acetate ester motif already installed.[12]

We established the initial structural requirements for PDE5 activity[13] (Table 1), with a small alkyl group at N-1 of the xanthine being optimal (**4** vs **7**). Substitution at N-7 in **5** resulted in a dramatic loss of activity, while substitution of the methylene linker in **6** was also detrimental.

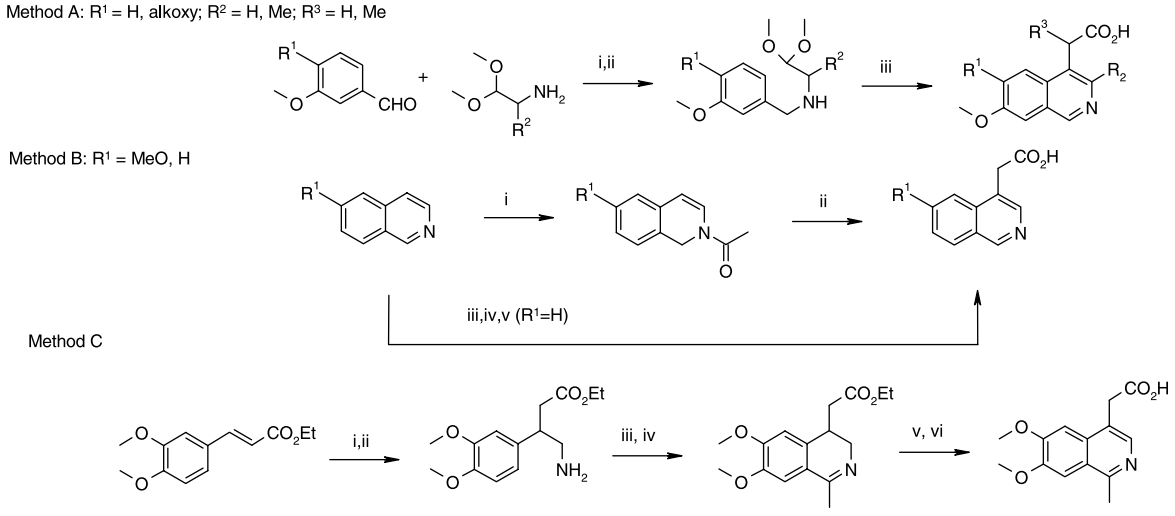
We now probed the effect of variation of the xanthine N-3 substituent on PDE5 activity, with selectivity profiling on selected compounds against bovine retinal PDE6, human platelet PDE3 and bovine heart PDE1, as indicated in Table 2. For comparison purposes, we also screened the xanthine **2c** reported by Corbin[6] and were encouraged by the increased level of PDE6 selectivity exhibited by **4**. Within the series of N-3 alkyl substituents **8**–**10**,

Table 1. PDE5 activity of **4** and derivatives **5-7**



Compound	R^1	R^2	R^3	hPDE5
4	Me	H	H	0.009
5	Me	Me	H	0.553
6	Me	H	Me	0.094
7	*i*-Bu	H	H	0.462

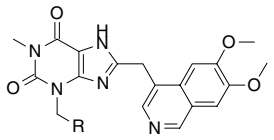
IC_{50} values are reported in μM.

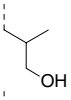
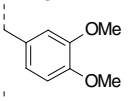
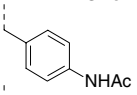
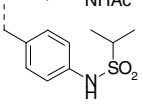


Scheme 2. Synthesis of isoquinoline-4-acetic acids. Method A. Reagents and conditions: (i) toluene, reflux; (ii) $NaBH_4$, EtOH, RT; (iii) glyoxylic (R_3 = H) or pyruvic (R_3 = Me) acid, 6 N HCl, 100 °C. Method B reagents and conditions: (i) $NaBH_4$, Ac_2O–AcOH, 60 °C; (ii) glyoxylic acid, 6 N HCl, 100 °C; (iii) $NaEt_3BH$, THF–toluene, RT; (iv) ethyl glyoxalate, RT; (v) NaOH–H_2O_2, RT. Method C reagents and conditions: (i) CH_3NO_2, tetramethylguanidine, 70 °C; (ii) $SnCl_2{\cdot}H_2O$, EtOH, reflux; (iii) AcCl, Et_3N, CH_2Cl_2, 0 °C; (iv) $POCl_3$, MeCN, reflux; (v) Pd/C, decalin, reflux; (v) LiOH, THF–H_2O, RT.

Table 2. PDE5 activity and isoform selectivity: effect of xanthine N-3 variation

4, 8-15



Compound	R	hPDE5	bPDE6	bPDE1	hPDE3
2c		0.025	0.065	—	—
4		0.009	0.389	0.417	0.299
8		0.030	0.333	—	—
9		0.020	0.823	1.32	1.64
10		0.028	1.13	—	—
11		0.008	0.294	2.79	1.06
12 (racemic)		0.006	0.363	0.670	2.71
13		0.033	1.82	3.91	—
14		0.042	0.471	—	—
15		0.005	0.399	3.44	3.13

IC_{50} values are reported in μM.

there was a slight loss of PDE5 potency, although the level of selectivity against PDE6 was similar to **4**. An increase in steric bulk in **11** furnished superior selectivity over PDEs 1 and 3, while carbinol derivative **12** (prepared from **8** by treatment with 9-BBN–H_2O_2) gave slightly superior PDE5 potency, as well as significantly enhanced selectivity against PDE3. The effect of larger polar functionality was explored in **13**–**15** with the latter compound being prepared by NaOH hydrolysis of the amide in **14** followed by treatment with isopropylsulfonyl chloride. Although the sulfonamide functionality in **15** provided the highest levels of potency and selectivity hitherto observed, this substituent did confer rather low solubility on the molecule compared with the simpler N-3 alkyl derivatives.

The isoquinoline moiety in **4** offered a plethora of substitution possibilities, some of which we next examined in Table 3.

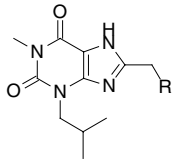
At this stage, for reasons of synthetic convenience and greater solubility (vide supra), we chose to retain the N-3 isobutyl group. 1-Substitution (**16**) gave excellent PDE5 potency and selectivity over PDE6, however, there was a significant loss of selectivity over PDEs 1 and 3. Conversely, there was a 10-fold loss in potency with the 3-methyl derivative **17**. Conformationally restricted dioxole **18** delivered a dramatic loss of potency, suggesting the importance of spatial orientation of at least one of the substituents, although unsubstituted isoquinoline **19** did retain reasonable potency. We ultimately established the 6-substituent (**20** vs **21**) as being critical to providing optimal potency and selectivity.

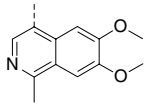
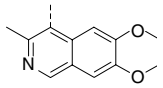
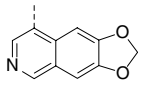
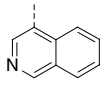
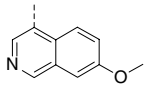
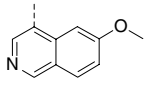
Caco-2 permeabilities[14], together with calculated PSA[15], were then assessed for a selection of compounds (Table 4). Good permeability was observed with the mono- and dimethoxy isoquinoline derivatives **4**, **11** and **21**, although further substitution on the isoquinoline in **16** appeared deleterious. The absence of permeability for **12** and **15** appeared to correlate with their higher PSA values.[16]

The hydrochloride salt of compound **21**[17] was selected for further profiling. Against a panel of additional PDE isoforms, human recombinant PDE4B, PDE4D and PDE7A all gave IC_{50} values >10 μM, while human

Table 3. PDE5 activity and isoform selectivity: effect of isoquinoline substitution

16-21



Compound	R	hPDE5	bPDE6	bPDE1	hPDE3
16		0.002	0.226	0.072	0.042
17		0.026	0.525	—	—
18		10	—	—	—
19		0.034	0.857	7.29	1.85
20		0.051	0.247	—	—
21		0.002	0.180	2.85	2.22

IC_{50} values are reported in μM

Table 4. Caco-2 permeability and calculated PSA

Compound	Caco-2 $P_{app}/10^{-5}$cm s^{-1} Net flux A–B	Calculated PSA/Å^2
4	3.18	88.37
11	2.34	85.61
12	0	109.37
15	0	130.49
16	0.09	87.73
21	1.38	79.35

Table 5. Microsomal clearance of **21**

Rat clint (μL/min/mg)	Human clint (μL/min/mg)	Dog clint (μL/min/mg)
162	4	6

Table 6. In vivo pharmacokinetics of **21**

	iv *T*1/2 (min)	iv Cl (ml/min/kg)	V_{ss} (L/kg)	% *F*
Rat[a]	21.0 ± 0.9	118.4 ± 16.9	2.9 ± 0.4	~1[c]
Dog[b]	63.4 ± 12.0	28.7 ± 9.2	2.1 ± 0.3	103 ± 20[d]

Data are means ± SEM.
[a] $n = 5$.
[b] $n = 3$.
[c] Dosed intraduodenally.
[d] Dosed orally.

platelet PDE2 gave an IC_{50} of 4.6 μM. In addition, **21** was profiled in the MDS Pharma Services Spectrum Screen™ consisting of 111 receptors, where the only activity observed was at the adenosine A1 receptor (K_i 2.3 μM) and the adenosine transporter (K_i 2.8 μM).

In vitro microsomal clearance experiments showed a much higher lability in rat compared with dog and human microsomes (Table 5).

In vivo, (Table 6) a short half-life and high clearance were observed in the rat after 0.8 mg/kg iv dosing. When dosed intraduodenally at 3.3 mg/kg, very low plasma levels of **21** were observed, suggesting a high first-pass metabolism. Contrastingly, excellent oral bioavailability was exhibited in the dog after dosing at 2.5 mg/kg, along with an improved half-life after 0.2 mg/mg iv dosing. These properties were considered appropriate for further evaluation in the MED indication, where a relatively short duration of action in man was desirable.

In conclusion, we have shown that xanthines containing an 8-(4-isoquinolylmethyl) substituent can function as potent inhibitors of PDE5. Optimal activity is achieved with a methyl group at N-1 of the xanthine. At the N-3 position, a variety of simple alkyl and polar substituents are tolerated, although Caco-2 permeability is favoured only in alkyl derivatives. It is also essential for N-7 to be unsubstituted. On the isoquinoline ring, the 6-position has been identified as critical to delivering maximal potency and selectivity against other PDE isoforms. Furthermore, compound **21** with optimal potency and selectivity shows a favourable in vivo pharmacokinetic profile in the dog.

References and notes

1. (a) Stamford, A. W. In *Annual Reports in Medicinal Chemistry*; Doherty, A. M., Ed.; Academic Press: New York, 2002; p 53; (b) Doggrell, S. A. *Exp. Opin. Pharmacother.* **2005**, *6*, 75.
2. (a) Terrett, N. K.; Bell, A. S.; Brown, D.; Ellis, P. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1819; (b) Eardley, I. *Br. J. Urol.* **1998**, *81*, 122.
3. Osterloh, I. H.; Riley, A. *Br. J. Clin. Pharmacol.* **2002**, *53*, 219.
4. Laties, A. M.; Zrenner, E. *Prog. Retin. Eye Res.* **2002**, *21*, 485.
5. Beavo, J. A.; Rogers, N. L.; Crofford, O. B.; Hardman, J. G.; Sutherland, E. W.; Newman, E. V. *Mol. Pharmacol.* **1970**, *6*, 597.
6. Sekhar, K. R.; Grondin, P.; Francos, S. H.; Corbin, J. D. In *Phosphodiesterase Inhibitors*; Academic Press: New York, 1996; p 137.
7. (a) Beer, D.; Bhalay, G.; Dunstan, A.; Glen, A.; Haberthuer, S.; Moser, H. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 1973; (b) Arnold, R.; Beer, D.; Bhalay, G.; Baettig, U.; Collingwood, S. P.; Craig, S.; Devereux, N.; Dunstan, A.; Glen, A.; Gomez, S.; Haberthuer, S.; Howe, T.; Jelfs, S.; Moser, H.; Naef, R.; Nicklin, P.; Sandham, D.; Stringer, R.; Turner, K.; Watson, S.; Zurini, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2587.
8. Selected data for **4**. NMR (400 MHz, DMSO-d_6 + TFA-d_1) δ_H 0.93 (3H d *J* 6), 2.30 (m 1H), 3.32 (3H s), 3.91 (2H d *J* 8), 4.10 (3H s), 4.16 (3H s), 4.81 (2H s), 7.79 (1H s), 8.01 (1H s), 8.60 (1H s), 9.59 (1H s). HRMS: $C_{22}H_{25}N_5O_4$ requires 423.1907; found 423.1889.
9. Merlos, M.; Gomez, L.; Vericat, M. L.; Bartroli, J.; Garcia-Rafanell, J.; Forn, J. *Eur. J. Med. Chem.* **1990**, *25*, 653.
10. (a) Bobbitt, J. M.; Winter, D. P.; Kiely, J. M. *J. Org. Chem.* **1965**, *30*, 2459; (b) Dyke, S. F.; Sainsbury, M.; Moon, B. J. *Tetrahedron* **1968**, *24*, 1467.
11. (a) Katayama, H.; Ohkoshi, M.; Yasue, M. *Chem. Pharm. Bull.* **1980**, *28*, 2226; (b) Minter, D. E.; Re, M. A. *J. Org. Chem.* **1988**, *53*, 2653.
12. Marivet, M. C.; Bourguignon, J.-J.; Lugnier, C.; Mann, A.; Stoclet, J.-C.; Wermuth, C. G. *J. Med. Chem.* **1989**, *32*, 1450.
13. Inhibition of PDE5 enzyme activity was determined with a [^{3}H]cGMP scintillation proximity assay using yttrium silicate beads (Amersham Pharmacia Biotech). Assays for other PDE isoforms were conducted similarly. Data generally represent the means of at least two experiments and individual IC_{50} values were generally within 2-fold of each other.
14. Wang, Z.; Hop, C. E. C. A.; Leung, K. H.; Pang, J. *J. Mass Spectrom.* **2000**, *35*, 71.
15. Ertl, P.; Rohde, B.; Selzer, P. *J. Med. Chem.* **2000**, *43*, 3714.
16. Artursson, P.; Bergstroem, C. A. S. *Methods Princ. Med. Chem.* **2003**, *18*, 341.
17. Selected data for **21**. NMR (400 MHz, DMSO-d_6) δ 0.95 (3H d *J* 7), 2.30 (1H m), 3.34 (3H s), 3.91 (2H d *J* 8), 4.07 (3H s), 4.59 (3H s), 7.45 (1H d *J* 9), 7.66 (1H s), 8.19 (1H d *J* 9), 8.51 (1H s), 9.22 (1H s). HRMS: $C_{21}H_{23}N_5O_3$ requires 393.1801; found 393.1801.