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Optimization of purine based PDE1/PDE5 inhibitors to a potent and selective PDE5 inhibitor for the treatment of male ED

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Abstract—In search of a PDE5 inhibitor for erectile dysfunction, an SAR was developed from a PDE1/PDE5 purine series of leads, which had modest PDE5 potency and poor isozyme selectivity. A compound (**41**) with PDE5 inhibition and in vivo activity similar to sildenafil was discovered from this effort. In addition, purine **41** demonstrated superior overall PDE isozyme selectivity when compared to the approved PDE5 inhibitors sildenafil, vardenafil, and tadalafil, which may result in a more favorable side-effect profile.

Phosphodiesterase type 5 (PDE5) inhibitors increase levels of cyclic guanylate cyclase (cGMP) in the nitric oxide (NO) pathway of penile erection, and have been developed for the treatment of male erectile dysfunction (ED).[1] Our objective is to develop potent and selective PDE5 inhibitors that improve upon the isozyme selectivity profile of the three marketed PDE5 inhibitors sildenafil (Viagra),[2] vardenafil (Levitra),[3] and tadalafil (Cialis)[4] (see Fig. 1). For example, the leading drug on the market, sildenafil, suffers from poor selectivity over PDE6.[5] Undesired visual side effects have been attributed to this low isozyme selectivity.[6] Our ideal candidate should improve upon the PDE6 selectivity of sildenafil and vardenafil, and improve the PDE11 selectivity over tadalafil. Increased isozyme selectivity may result in a more favorable side-effect profile.

We had synthesized initial lead compounds in a previous PDE1/PDE5 program for the treatment of hypertension.[7] The tricyclic guanine **1** was discovered to have modest PDE5 potency, but poor selectivity versus

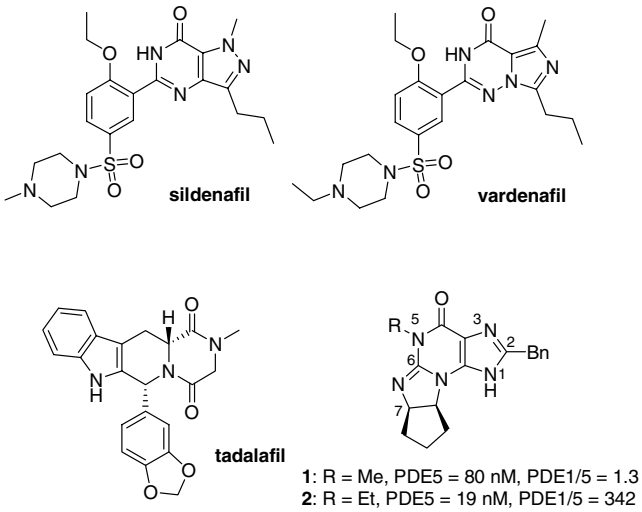

Figure 1. Lead PDE5 inhibitors.[8]

PDE1. Based on a comparison of guanine analogs **1** and **2**, we believed that the N-5 ethyl group could improve PDE5 selectivity over compounds containing the N-5 methyl group (Fig. 1).[8] Most compounds in our previous PDE1/PDE5 program had a methyl group at N-5, and due to synthetic difficulty, very little SAR

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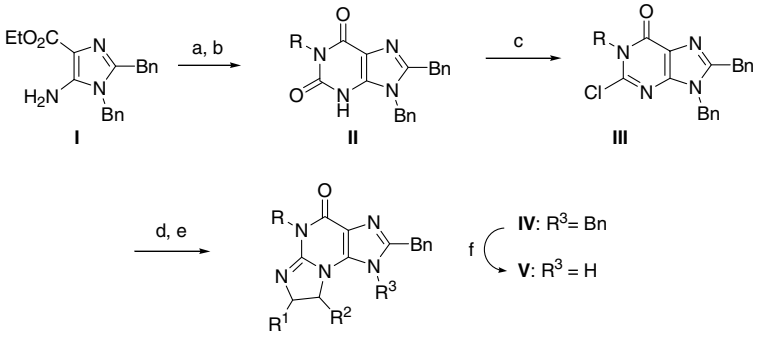


Scheme 1. Synthesis of C-7/N-5 derivatives. Reagents and conditions: (a) RNCO, Et_3N, toluene; (b) MeONa, MeOH; (c) $POCl_3$, Δ; (d) $H_2NCH(R^1)CH(R^2)OH$, DIEA, NMP, 120 °C; (e) MsCl, Et_3N; (f) $Pd(OH)_2/C$, HCO_2NH_4, MeOH, Δ.

was developed at the N-5 position. Work in the current project focused on developing SAR at C-7 and N-5, followed by exploration of the N-1, N-3, and C-2 positions to obtain selectivity versus PDE1 and PDE6 isozymes.

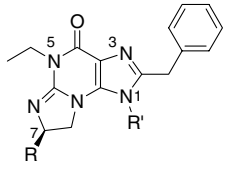
The synthesis of C-7 and N-5 derivatives is summarized in Scheme 1. Aminoimidazole **I**[9] was treated with an isocyanate and cyclized to form the purine core **II**. The C-6 chloride **III** was obtained by reaction of **II** with $POCl_3$. Condensation of the chloride **III** by an aminoalcohol followed by one-pot mesylation/cyclization afforded the tricyclic guanine **IV**. Non-substituted N-1 derivatives **V** were produced by debenzylation of **IV**.

Several C-7 analogs were synthesized in the N-1 benzyl (**IV**) and non-substituted N-1 series (**V**). In the N-1 benzyl series (Table 1), some bulk was required for PDE5 potency as the *n*-propyl, phenyl, and benzyl derivatives **4**–**6** had similar binding data, but the ethyl compound **3** was inactive. However, in the non-substituted N-1 series, the C-7 benzyl compound **10** had improved PDE5 activity and selectivity versus PDE1. Therefore, the C-7 benzyl group was used in further SAR studies at other positions in the molecule.[10]

With the C-7 benzyl group in place, a number of modifications were made at the N-5 position. As shown in Table 2, minor modifications on N-5 substituents in the N-1 benzyl series resulted in compounds with little PDE5 potency (**11**–**16**) compared with the N-5 ethyl derivative **6**. Similarly, in the N-1 non-substituted series (**17**–**22**), most non-ethyl compounds had poor PDE5 potency. Only the closely related methyl (**18**) and isopropyl (**20**) derivatives demonstrated some PDE5 activity, and as shown by methyl analog **18**, the PDE1/PDE5 selectivity was poor compared to ethyl analog **10**.

Although the N-5 ethyl and C-7 benzyl compounds **6** and **10** exhibited promising PDE5 inhibition and selectivity against PDE1, all of the derivatives displayed in Tables 1 and 2 had poor selectivity versus PDE6. To address this issue, modifications at N-1 and C-2 were made, as outlined in Scheme 2. The C-2 position was modified by halogenation of intermediate **VI**,[11] and alkynyl analogs were synthesized by a subsequent Sonagashira coupling reaction. A surprising result occurred

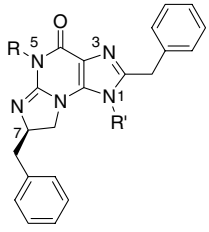
Table 1. C-7 modifications[a]



Compound	R	R′	PDE5 IC_{50} (nM)[a]	PDE1/5	PDE6/5
3	Et	Bn	4% *I*	—	—
4	*n*-Pr (racemic)	Bn	35	>286	4.9
5	Ph	Bn	40	—	1.3
6	Bn	Bn	32	>313	5.3
7	Et	H	33	>303	2.4
8	*n*-Pr (racemic)	H	60	>167	0.7
9	*i*-Pr	H	28	59	1.0
10	Bn	H	4.5	>2222	1.8

[a] Ref. 8. % *I* = % inhibition @ 100 nM.

Table 2. N-5 modifications[a]



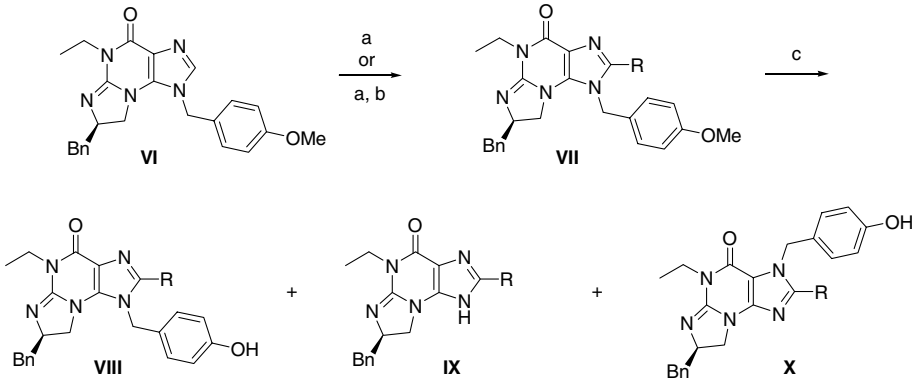
Compound	R	R′	PDE5 IC_{50} (nM)[a]	PDE1/5	PDE6/5
11	Me	Bn	6% *I*	—	—
6	Et	Bn	32	>313	5.3
12	allyl	Bn	17% *I*	—	—
13	*i*-Pr	Bn	13% *I*	—	—
14	*c*-Pr	Bn	12% *I*	—	—
15	CF_3CH_2	Bn	7% *I*	—	—
16	MeO	Bn	17% *I*	—	—
17	H	H	22% *I*	—	—
18	Me	H	100	11	2.3
10	Et	H	4.5	>2222	1.8
19	*n*-Pr	H	25% *I*	—	—
20	*i*-Pr	H	22	—	4.0
21	*c*-Pr	H	21% *I*	—	—
22	CF_3CH_2	H	34% *I*	—	—

[a] Ref. 8. % *I* = % inhibition @ 100 nM.

during the boron tribromide promoted demethylation of intermediate **VII**. The desired product **VIII** was isolated along with the debenzylated compound **IX** and the N-3 substituted regioisomer **X**. Presumably, debenzylation of **VIII** generated a benzyl bromide, which subsequently alkylated the N-3 position of **IX**. In a separate experiment, benzylation of **IX** occurred regioselectively at the N-3 position to provide product **X**.

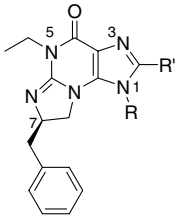
A comparison of C-2 and N-1 substituents is outlined in Table 3. The N-1 benzyl compounds **23**–**27** generally did not show any improvement in PDE5 inhibition or selectivity compared to compound **6**, but derivative **26** showed that a C-2 phenylacetylene moiety could provide improved selectivity over PDE6 while maintaining PDE5 potency. As shown by analogs **28**–**30**, the *p*-methoxybenzyl group did not improve upon the binding data of the analogous non-substituted N-1 benzyl compounds. However, the *p*-hydroxybenzyl derivatives **31**–**33** were quite interesting. While the N-1 *p*-hydroxybenzyl compound **31** did not have an affect on PDE inhibition when compared with analog **6**, both the C-2 bromo (**32**) and phenylacetylenyl (**33**) compounds had improved PDE5 potency and selectivity versus PDE1 and PDE6.

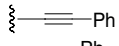
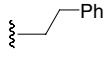
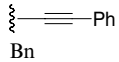
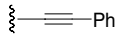
Although N-1 substituted compounds such as **32** and **33** had improved PDE6 selectivity over sildenafil, some of the N-3 substituted compounds had improved in vitro profiles, which more closely matched our project goals (Table 4). As was the case in the N-1 series, substitution with a *p*-hydroxybenzyl group generally produced an increase in PDE5 inhibition. In addition, C-2 substituents with electron withdrawing inductive effects provided



Scheme 2. Synthesis of N-1/N-3/C-2 derivatives. Reagents and conditions: (a) NBS, NCS, or NIS; (b) phenylacetylene, CuI, $PdCl_2(PPh_3)_2$; (c) BBr_3.

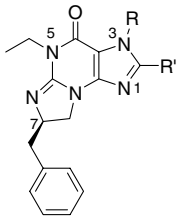
Table 3. N-1/C-2 modifications[a]

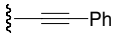
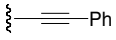
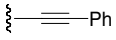


Compound	R	R′	PDE5 IC_{50} (nM)[a]	PDE1/5	PDE6/5
6	Bn	Bn	32	>313	5.3
23	Bn	H	200	—	5.0
24	Bn	Cl	60	—	7.3
25	Bn	Br	145	—	4.5
26	Bn		33	>303	15.0
27	Bn		74	—	7.4
28	*p*-MeOBn	Bn	38	—	7.5
29	*p*-MeOBn	Br	48	—	7.8
30	*p*-MeOBn		20	—	4.5
31	*p*-HOBn	Bn	28	—	7.9
32	*p*-HOBn	Br	7.8	>1587	51.3
33	*p*-HOBn		6.5	>1538	23.0

[a] Ref. 8.

Table 4. N-3/C-2 modifications[a]



Compound	R	R′	PDE5 IC_{50} (nM)[a]	PDE1/5	PDE6/5
34	Bn	Bn	23% *I*	—	—
35	Bn	I	32% *I*	—	—
36	Bn		40	—	3.6
37	*p*-MeOBn	Bn	130	—	1.8
38	*p*-MeOBn	Br	23	—	4.8
39	*p*-MeOBn		14	—	1.7
40	*p*-HOBn	Bn	29	—	7.4
41	*p*-HOBn	Br	2.5	>4000	72.0
42	*p*-HOBn		5.3	—	19.8
43	*p*-HOBn	Cl	2.3	>4348	93.5
44	*p*-HOBn	CH_3	15	—	40.0
45	*p*-HOBn	I	3.3	—	93.9

[a] Ref. 8. % *I* = % inhibition @ 100 nM.

compounds with low single digit nanomolar PDE5 potency. The greatest selectivity over PDE6 was imparted by C-2 halogen substituents, as demonstrated by analogs **41**, **43**, and **45**, all of which had PDE6 selectivity 10-fold greater than sildenafil and excellent PDE5 potency. Halogen derivatives **41** and **43** were also tested for PDE2, PDE3, and PDE4 inhibition, and both compounds had >1 μM potency for all three isozymes.[8] Furthermore, the bromo analog **41**, sildenafil, vardenafil, and tadalafil were tested against PDE isozymes 1–11.[8,12] Not only did purine **41** have good selectivity versus the PDE1 and PDE6 isozymes, but it had >200-fold selectivity versus all other PDE isozymes (Table 5). This represents an overall selectivity profile for purine **41** that is superior to the three marketed PDE5 inhibitors. Specifically, our purine **41** had superior selectivity over PDE1 and PDE6 compared with sildenafil and vardenafil, and the PDE11 selectivity of compound **41** was significantly greater than that of tadalafil.

The PDE5 inhibitor **41** was subjected to PK studies in rat and dog. As demonstrated by the AUC curve in Figure 2,[13] the oral rat PK has a similar profile to sildenafil

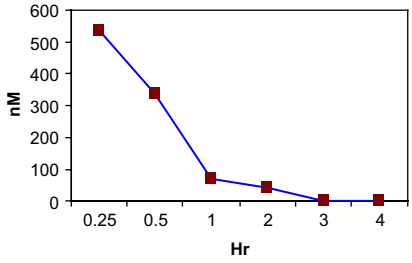


Figure 2. Rat PK of compound **41**. AUC = 351 nM·h, T_{max} = 0.25 h.[13]

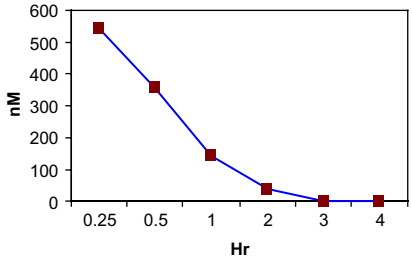


Figure 3. Rat PK of sildenafil. AUC = 413 nM·h, T_{max} = 0.25 h.[14]

(Fig. 3),[14] which features a rapid onset and fast clearance. In addition, purine **41** had similar results in an oral dog PK study, in which the AUC was 1156 nM·h, and the T_{max} was 0.75 h.[15] These results were sufficient to warrant further testing of inhibitor **41** in a dog in vivo model of efficacy.

We utilized a standard in vivo model of erectile function in which the pelvic nerve of a dog is electrically stimulated in order to mimic activation by NO, facilitating the nitric oxide/cGMP pathway of erectile function.[16] Addition of a PDE5 inhibitor such as sildenafil increases the intracavernosal pressure (ICP), which causes an erection. Administration of purine **41** in this assay (Fig. 4) produced a dose-dependent increase in ICP (reported as % of mean blood pressure). These results are similar to those observed with sildenafil in the anesthetized dog model.[16]

In conclusion, modification of several regions of the modestly potent PDE1/PDE5 inhibitors **1** and **2** resulted in the discovery of a series of compounds, which exhibited single digit nanomolar PDE5 potency and isozyme selectivity greater than the approved drugs sildenafil, vardenafil, and tadalafil. This in vitro profile may result in fewer side effects related to PDE isozyme inhibition. The PDE5 inhibitor **41** showed oral bioavailability in both rat and dog with a rapid onset and clearance

Table 5. PDE isozyme selectivity comparison[a]

Compd	PDE1	PDE2	PDE3	PDE4	PDE5	PDE6	PDE7	PDE8	PDE9	PDE10	PDE11
41	>10	>10	>10	1.4	0.0025	0.18	>10	>10	>10	2.2	0.54
Sildenafil	1.1	>10	>10	9.6	0.0035	0.025	>10	>10	3.4	3.1	2.3
Vardenafil	0.23	>10	2.1	4.5	0.0002	0.001	>10	>10	0.46	0.79	0.13
Tadalafil	>10	>10	>10	>10	0.004	0.98	>10	>10	>10	>10	0.033

[a] Refs. 8 and 12. All numbers are IC_{50} values reported as micromolar.

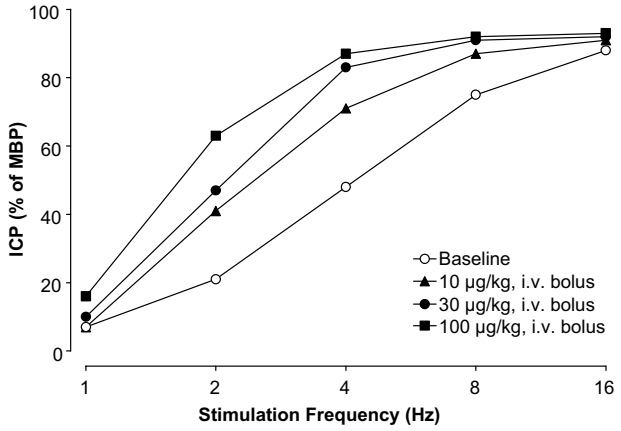


Figure 4. Effects of compound **41** on intracavernosal pressure (ICP, as a percent of mean blood pressure, MBP) following pelvic nerve stimulation in an anesthetized dog model of penile erection.

similar to sildenafil. In addition, purine **41** demonstrated oral efficacy in the anesthetized dog erectile function model. Further optimization of this series of purine PDE5 inhibitors will be disclosed in other publications.[17]

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8. See the following reference for the conditions of the PDE1–PDE6 binding assays described in this paper: Wang, Y.; Chackalamannil, S.; Hu, Z.; Boyle, C. D.; Lankin, C. M.; Xia, Y.; Xu, R.; Asberom, T.; Pissarnitski, D.; Stamford, A. W.; Greenlee, W. J.; Skell, J.; Kurowski, S.; Vemulapalli, S.; Palamanda, J.; Chintala, M.; Wu, P.; Myers, J.; Wang, P. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3149. These are assays involving human PDE, whereas the data reported in Ref. 7 are from bovine PDE assays, which explains why the IC_{50} values reported for compounds **1** and **2** are different in this paper than the published values in Ref. 7.
9. See Ref. 7 for the synthesis of imidazole **I**.
10. The (*R*)-configuration at C-7 was preferred for PDE5 inhibition. For example the (*S*)-enantiomers of compounds **6** and **10** had PDE5 inhibition of 11% and 12% @ 100 nM, respectively.
11. Imidazole **VI** was synthesized with the same chemistry used to produce imidazole **I** (Ref. 7). Triethylorthoformate was used for the formation of **VI** in place of the triethylorthoacetate employed for the synthesis of **I**.
12. The following are conditions used for the PDE7-PDE11 binding assays: for PDE7, PDE8, and PDE11 assays, Amersham's cAMP-PDE SPA Assay kit was used, with the following additions: 0.1 μM cAMP for PDE7, 0.05 μM cAMP for PDE8, and 0.0125 μM cAMP for PDE11. For PDE9 and PDE10 assays, Amersham's cGMP-PDE SPA Assay kit was used with the following additions: 0.15 μM cGMP and 100 μM $MnCl_2$ (PDE9) and 0.7 μM cGMP (PDE10).
13. Rats were orally dosed (0.4% w/v methyl cellulose) with compound **41** at 10 mg/kg. Plasma levels were sampled for 24 h ($n = 2$).
14. Sildenafil PK in rat was measured using the same conditions as Ref. 13.
15. Dogs were orally dosed (0.4% w/v methyl cellulose) with compound **41** at 5 mg/kg. Plasma levels were sampled for 4 h ($n = 3$).
16. Carter, A. J.; Ballard, S. A.; Naylor, A. M. *J. Urol.* **1998**, *160*, 242. An ICP versus stimulation frequency graph for sildenafil at doses of 1–100 μg/kg is published in this paper. The results are similar to those of compound **41** shown in Figure 4.
17. In vitro optimization of a similar series of compounds in conjunction with this work has recently been disclosed: Pissarnitski, D. A.; Asberom, T.; Boyle, C. D.; Chackalamannil, S.; Chintala, M.; Clader, J. W.; Greenlee, W. J.; Hu, Y.; Kurowski, S.; Myers, J.; Palamanda, J.; Stamford, A. W.; Vemulapalli, S.; Wang, Y.; Wang, P.; Wu, P.; Xu, R. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1291.