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# Design, Synthesis and Biological Activity of β-Carboline-Based Type-5 Phosphodiesterase Inhibitors

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**Abstract**—The SAR of a series of β-carboline derived type 5 phosphodiesterase inhibitors has been explored and we have discovered compounds with excellent levels of PDE5 potency and selectivity over PDE6. However, the series exhibits low levels of selectivity over PDE11, a phosphodiesterase with unknown function.


Phosphodiesterases (PDEs) play critical roles in the modulation of the important secondary messenger cyclic nucleotides, adenosine cyclic 3′,5′-phosphate (cAMP) and cyclic guanasine 5′-phosphate (cGMP).[1,2] The cGMP specific PDE5 enzyme controls the levels of intracellular cGMP and plays a critical role in its modulation, duration of action and physiological function. PDE5 is the major PDE isozyme in penile *corpus cavernosum* tissue and plays a key role in the control of penile erection. Sexually stimulated penile erection is a result of *trabecular* smooth muscle relaxation.[3–5] During sexual stimulation nitric oxide (NO) is released from the non-adrenergic, non cholinergic nerves within the penis supplying the *corpus cavernosum* smooth muscle and activates guanylate cyclase to form cGMP via cyclisation of GTP. The NO diffuses into penile smooth muscle cells and binds to the heme of soluble guanylate cyclase, resulting in stimulation of cGMP synthesis, which, in turn, reduces intracellular calcium following binding to cGMP-dependant protein kinases and cGMP-dependant ion channels. Inhibitors of PDE5 amplify the neuronal NO/cGMP pathway involved in human *corpus cavernosal* relaxation and therefore enhance the normal process leading to penile erection.[6–8]

The launch of Viagra™ (sildenafil, **1**) (Fig. 1) as the first oral treatment for male erectile dysfunction revolutionised the treatment of this disease.[9] Sildenafil is a potent and selective inhibitor of phosphodiesterase type 5 (PDE5),[10] and therefore slows cGMP breakdown. This enhances the action of nitric oxide and cGMP, and facilitates penile erection in individuals suffering from erectile dysfunction (ED).[11]

As part of our continued efforts in this area we have investigated several templates other than the pyrrazolopyrimidine present in sildenafil, **1**. We have previously reported that sildenafil, **1**, tadalafil, **2**, and vardenafil, **3**, (Fig. 2) exhibit different PDE selectivity profiles, in particular in their relative affinities for PDE5 and PDE11.[12] Sildenafil, **1**, and vardenafil, **3**, show excellent PDE5 selectivity over PDE11 (760- and 1160-fold, respectively) whereas tadalafil exhibits only a modest 5-fold selectivity.

It has been suggested that PDE11 protein localization in man and mouse, coupled with the known role for cGMP and cAMP in sperm physiology and testosterone production, suggests a local role for PDE11 in testis development and/or spermatogenesis.[13] The presence in acidophils also suggests a role in modulating secretion of prolactin and/or growth hormone, hence an indirect/endocrine effect on lactation, luteal function, steroidogenesis, immunoregulation and growth, for example, as well as on the testis and spermatogenesis. The physiological significance of PDE11 inhibition is not clearly understood but could have a role in sperm function.[14] Thus, in the future PDE11 inhibitors might be explored for novel indications relating to hormonal or other functions, but when developing PDE5 inhibitors to modulate vascular function it was thought desirable to maintain high selectivity of PDE5 effects over PDE11.

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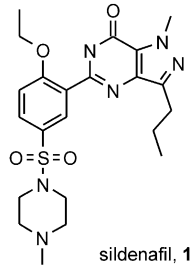



**Figure 1.** Structure of sildenafil.

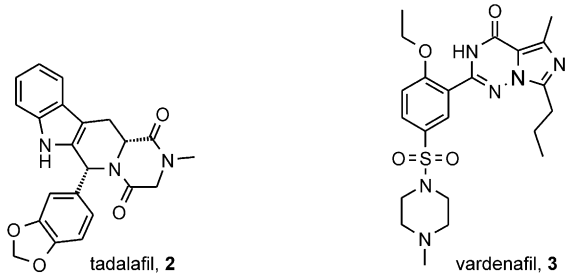
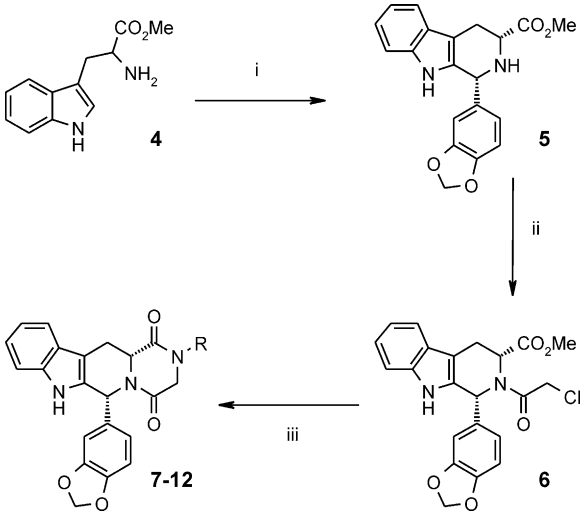



**Figure 2.** Structure of tadalafil and vardenafil.

The aims of these SAR studies were to investigate whether PDE5 selectivity, especially over PDE11 could be built into this β-carboline template. In this paper we wish to report our efforts in this regard.

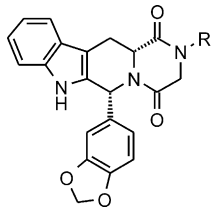
The *N*-methyl group in compound **2** was targeted for structural modification on the basis of synthetic accessibility and thus the ability to prepare a number of analogues for in vitro evaluation and rapid SAR generation. D-Tryptophan methyl ester hydrochloride **4** was treated with piperonal under Dean–Stark conditions followed by treatment with trifluoroacetic acid to give the carboline methyl carboxylate **5** as shown in Scheme 1. The key intermediate for the remainder of the synthesis **6** was prepared via the acylation of **5** with chloroacetyl chloride.

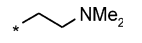
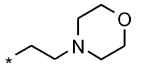
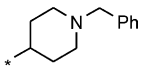
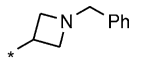
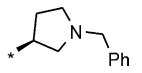
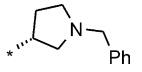



**Scheme 1.** Reagents and conditions: (i) piperonal, toluene, Dean–Stark followed by trifluoroacetic acid, (ii) chloroacetyl chloride, triethylamine, (iii) $RNH_2$, ethanol, 50 °C.

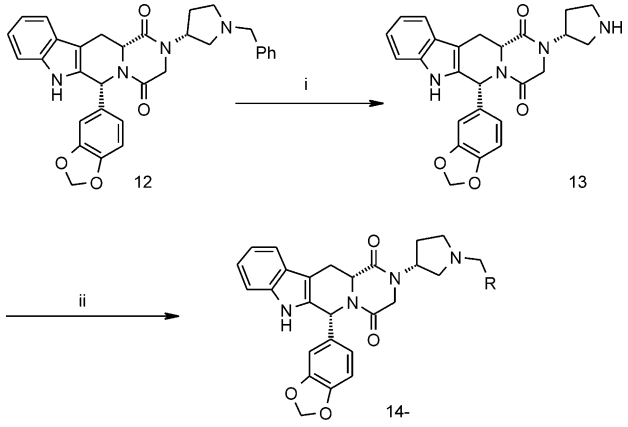
Our initial strategy was to introduce a basic nitrogen into the molecule as a means of improving aqueous solubility and to assess its impact on PDE5 potency and selectivity. To this end we found that it is possible to react compound **6** with a range of primary diamines ($RNH_2$) to afford compounds **7**–**12**, Scheme 1, Table 1.

**Table 1.** Initial SAR results for compounds **7**–**12** investigating the impact of introducing basic nitrogen[a]



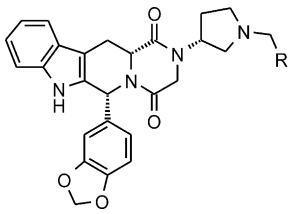
| Compd | R | $IC_{50}$ (nM) | | |
|---|---|---|---|---|
| | | PDE5 | PDE6 | PDE11 |
| **2** | $CH_3$ | 6.7 | 1260 | 37 |
| **7** |  | 134 | 8224 | 53 |
| **8** |  | 56 | 2966 | NT |
| **9** |  | 26 | 1619 | NT |
| **10** |  | 164 | NT | 950 |
| **11** |  | 17 | NT | 85 |
| **12** |  | 2.1 | 249 | 5.5 |

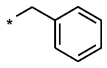
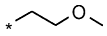
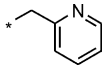
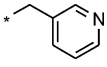
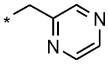
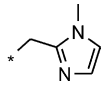
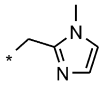
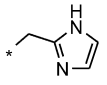
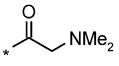
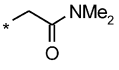
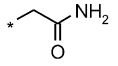
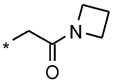
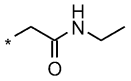
NT, Not tested.
[a]Phosphodiesterase type 5 (PDE5) was obtained from human *corpus cavernosum* tissue, PDE6 from canine or bovine retina and PDE11 from recombinant SF9 expression as previously described.[11,15,16]




**Scheme 2.** Reagents and conditions: (i) Pd/C, ammonium formate, ethanol, (ii) RCHO, sodium triacetoxyborohydride, acetic acid, THF.

**Table 2.** Optimisation of the pyrrolidine *N*-substiuent, SAR for compounds **12**–**30**

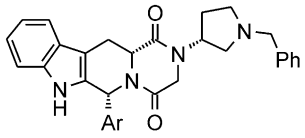


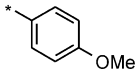
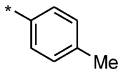
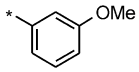
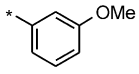
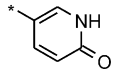
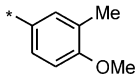
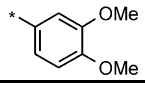
| Compd | R | $IC_{50}$ (nM) | | | | |
|---|---|---|---|---|---|---|
| | | PDE5 | PDE6 | 6/5sel | PDE11 | 11/5 sel |
| **12** |  | 2.1 | 249 | 118 | NT | |
| **15** |  | 57 | $>10^4$ | >175 | NT | |
| **16** |  | 13.2 | 1770 | 134 | 31 | 2.3 |
| **17** |  | 15.7 | 2660 | 169 | 43 | 2.7 |
| **18** |  | 4.0 | NT | | 11.8 | 3 |
| **19** |  | 7.1 | 1340 | 188 | 22 | 3 |
| **20** |  | 1.5 | 226 | 153 | 2.01 | 1.4 |
| **21** |  | 1.9 | 271 | 144 | 2.4 | 1.3 |
| **22** |  | 2.0 | 358 | 181 | 4.2 | 2.1 |
| **23** |  | 0.7 | 125 | 173 | 1.4 | 2 |
| **24** |  | 0.8 | 494 | 640 | 2.2 | 2.6 |
| **25** |  | 1.2 | 357 | 300 | 3.5 | 2.9 |
| **26** |  | 19.8 | NT | | 48 | 2.4 |
| **27** |  | 2.5 | 807 | 316 | 4.7 | 1.9 |
| **28** |  | 8.4 | 1601 | 189 | 48 | 5.7 |
| **29** |  | 4.0 | 846 | 211 | 4.7 | 1.2 |
| **30** |  | 3.6 | 620 | 172 | 4.3 | 1.2 |

NT, not tested.

Introduction of flexible linkers such as found in **7** and **8** afforded good levels of PDE5 inhibition. However, providing some degree of conformational rigidity into the tether group led to an increase of PDE5 inhibition in compounds **9**, **11** and **12**. Interestingly, the R-configuration pyrrolidine **12** showed an 8-fold increase in enzyme inhibition potency.

**Table 3.** Investigation of benzdioxolane replacements and the effect on PDE11 activity



| Compd | Ar | $IC_{50}$ (nM) | | |
|---|---|---|---|---|
| | | PDE5 | PDE11 | Selectivity |
| **27** | — | 2.6 | 3.5 | 1.4 |
| **31** |  | 2.7 | 6.7 | 2.5 |
| **32** |  | 12.8 | 6.5 | 0.5 |
| **33** |  | 15.6 | 11.8 | 0.8 |
| **34** |  | 28.6 | 44 | 1.5 |
| **35** |  | 127 | 39 | 0.3 |
| **36** |  | 132 | 71 | 0.5 |
| **37** |  | >1000 | ~1000 | — |

Compound **12** was selected for further SAR investigation through optimisation of the *N*-substituent on the pyrrolidine ring. The compound was smoothly debenzylated under transfer hydrogenation conditions over palladium-on-carbon to give **13**. This compound was then converted into a range of *N*-alkyl derivatives through straightforward reductive alkylation in the presence of a range of aldehydes and sodium triacetoxyborohydride, Scheme 2.

From Table 2 it can be seen that replacement of an sp2 hybridised carbon with an sp3 carbon in the position *beta* to the pyrrolidine nitrogen results in the loss of 6–20-fold in activity relative to compound **12** (e.g., compounds **15** and **16**). Activity is retained with the *N*-allyl derivative **18** and the SAR remains quite flat with a range of polar phenyl-group isosteres (i.e., compounds **20**–**25**).

Excellent PDE5 selectivity over PDE6 is maintained throughout the series (150–650-fold). Indeed, compound **24** combines sub-nanomolar PDE5 inhibition activity with >600-fold selectivity over PDE6.

Acylation of the pyrrolidine nitrogen as the *N*,*N*-dimethylcarbamoyl derivative **26** leads to a loss in PDE5

activity. Isomerisation of this substituent leads to **27** and the restoration of excellent PDE5 inhibition activity. Simple exploration of the amide SAR (compounds **28**–**30**) again shows a reasonably flat PDE5 SAR. However, these compounds retain good selectivity over PDE6 inhibition.

However, Table 2 also clearly shows that the series is not selective for PDE5 over PDE11 with the compounds tested showing up to a very modest 3-fold selectivity. We were interested to find out whether this was a generic limitation of the template and prepared a range of analogues containing benzdioxolane replacements, Table 3.

These compound were prepared using chemistry previously described, vide supra.

These analogues retained respectable levels of PDE5 inhibition and it is interesting to note that the PDE5 enzyme tolerates both electron withdrawing and electron donating substituents in the *para*-position of the aryl group, Table 3. However, this series of compounds was also found to be non selective over PDE11.

## Summary

Extensive SAR exploration of the β-carboline template has identified a range of compounds exhibiting excellent PDE5 potency and selectivity over PDE6. However, these compounds are not selective for PDE5 over PDE11. In addition, although the compounds showed improved aqueous solubility compared to **2**, they did not exhibit other biopharmaceutic improvements. In light of these results, and the as yet undefined role of the PDE11 enzyme especially in relation to testis development and/or spermatogenesis, we focused our efforts on exploration of new templates. The results of these studies will be the subject of further communications.

## Acknowledgements

We would like to thank Dr. N. Mount for useful discussions and Ms. E. Evrard for technical contributions. We also acknowledge technical assistance provided by the Structural and Separation Sciences group.

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16. PDE activity was measured using a Scintillation Proximity Assay (SPA)-based method as previously described.[15] The effect of PDE inhibitors was investigated by assaying a fixed amount of enzyme in the presence of varying inhibitor concentrations and low substrate, (cGMP in a 3:1 ratio unlabelled to [$^3$H]-labeled at a concn$\sim$1/3 $K_m$) such that $IC_{50} \cong K_i$. The final assay volume was made up to 100 μL with assay buffer [20 mM Tris–HCl pH 7.4, 5 mM $MgCl_2$, 1 mg/mL bovine serum albumin]. Reactions were initiated with enzyme, incubated for 30–60 min at 30 °C and terminated with 50 μL yttrium silicate SPA beads (Amersham). Plates were shaken, settled and then counted on a TopCount plate reader (Packard, Meriden, CT) Radioactivity units were converted to% activity of an uninhibited control (100%), plotted against inhibitor concentration and inhibitor $IC_{50}$ values obtained using the 'Fit Curve' Microsoft Excel extension.