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Synthesis and Biological Activities of Novel β-Carbolines as PDE5 Inhibitors

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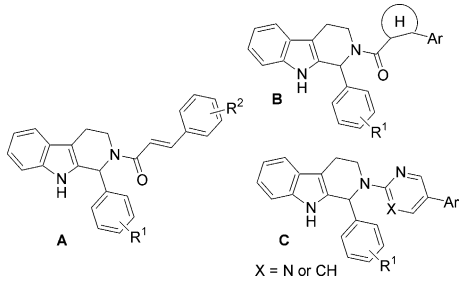
Abstract—A series of N^2-furoyl and N^2pyrimidinyl β-carbolines was discovered to possess potent inhibitory activity against PDE5. During the synthesis we developed a tandem resin quenching protocol, which allowed us to synthesize large number of target compounds in a rapid fashion. Representative compounds exhibit superior selectivity to sildenafil versus other isozymes of PDEs, and demonstrated in vivo efficacy in increasing introcavernosal pressure in dogs.

Cyclic nucleotides (cAMP and cGMP) are important secondary messengers which control many physiological processes. The levels of intracellular cyclic nucleotides are determined by the activities of cyclases that synthesize them and phosphodiesterases (PDEs) that degrade them. PDEs play critical roles in modulating the levels of cyclic nucleotide, their duration of action and ultimately the physiological outcome. To date, the 21 mammalian PDE genes which have been cloned are classified into 11 families according to sequence homology and biochemical properties.[1–3] These families are: PDE1, Ca^{2+}/calmodulin dependent; PDE2, cGMP-stimulated; PDE3, cGMP-inhibited; PDE4, cAMP-specific and rolipram-sensitive; PDE5, cGMP-specific; PDE6, photoreceptor cGMP-specific; PDE7, cAMP-specific and rolipram-insensitive; PDE8, cAMP-specific and IBMX-insensitive; PDE9, cGMP-specific; PDE10 and PDE11, hydrolyzing both cAMP and cGMP. Among the 11 families, PDE5, initially discovered in lung tissues, was the first recognized cGMP specific subclass. Subsequent studies revealed that it is the major isozyme of PDEs in *Corpus cavernosum* in penis and plays an important role in penile erection.[4,5] Many potent PDE5 inhibitors have been reported in the literature as vasodialators.[6] The introduction of sildenafil,[7] a PDE5 inhibitor, as an agent for the treatment of male erectile dysfunction (MED) in 1998 has raised the public awareness of MED, and generated significant interest in the discovery of more selective PDE5 inhibitors. The results of this increased interest have begun to emerge in the literature.[8–12] As part of our on-going efforts to identify novel PDE5 inhibitors that have improved selectivity versus PDE1 and PDE6,[13–15] we became interested in compounds structurally distinct from sildenafil. Among the many structures reported in the literature, β-carbolines such as structure **A** drew our attention.[16] β-Carbolines are readily accessible and well suited for parallel synthesis. To avoid the potential metabolic liability of the olefins in **A**, we decided to replace the alkene or the acrylate moieties in structure **A** with a series of heterocycles as indicated in structures **B** and **C**. We envisioned that the nitrogen atom in the pyrimidine and pyridine of **C** would mimic the amide moiety in structure **A**.



The β-carboline precursors **3** were synthesized through Pictet–Spengler reactions of tryptamine **1** and

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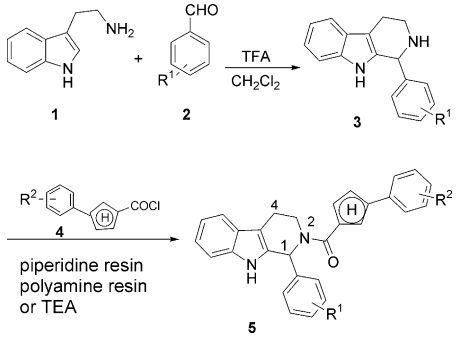
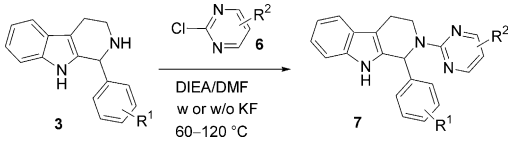
benzaldehydes **2** in accord with the known procedures as shown in Scheme 1. The acyl analogues **5** were synthesized through *N*-acylation of **3** with appropriate acid chlorides **4** either by conventional solution reactions or by a tandem resin quenching protocol in a parallel fashion with dichloromethane as solvent. In the parallel synthesis, piperidinomethyl polystyrene resin was used as an acid scavenger and excess acid chloride was used during the acylation. The reaction mixture was then treated with a primary amine resin, tris-(2-aminoethyl)-amine polystyrene to react with any excess acid chloride. Simple filtration removed most of the impurities, and recrystallization where necessary gave products in high purity. This allowed us to synthesize target compounds of high purity in a rapid fashion without chromatographic purification.

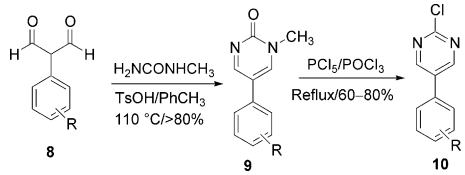
The pyrimidine analogues were synthesized by reacting 2-chloropyrimdines **6** with **3** in the presence of Hünig's base in DMF at 120 °C. When employing temperature-sensitive substrates, the reaction temperature can be lowered to 60 °C by pre-heating the chloropyrimidine with KF (Scheme 2). This modification was especially useful in the synthesis of the pure enantiomers. 2-Chloropyrimidines were synthesized from substituted malondialdehydes **8** by treatment with methylurea, followed by reaction with chlorinating reagents such as phosphorous pentachloride and phosphorus oxychloride as shown in Scheme 3. When R^2 substituent is basic (e.g., pyridine and imidazole) or contains a basic functional group, the chlorination reaction is sluggish due to the precipitation of the hydrochloride salt. In these cases, the target compounds were synthesized by palladium-mediated couplings of a bromopyrimidinyl precursor with a corresponding boronic acid or stannane under standard Suzuki or Stille conditions (Scheme 4). All compounds were characterized by conventional analytical methods such as MS, NMR and elemental analysis.



Scheme 1.



Scheme 2.



Scheme 3.

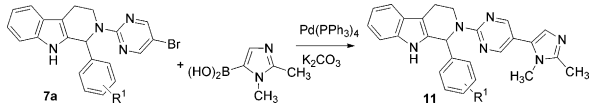
We first studied the acyl analogues where we utilized the cluster analysis concept and knowledge of carboline PDE5 inhibitor SAR to select the following 10 different substituents for the initial set of R^1: 4-NO_2, 4-OCH_3, 4-CN, 4-CH_3, 3,4-Cl_2, 3,4-$(OCH_3)_2$, 3,4-$(CH_3)_2$, 3,5-$(CH_3)_2$, 4-Cl-3-CF_3 and 3,4-OCH_2O-. We then selected a set of *N*-acyl groups with a wide variety of structures resembling the acyl moiety described in structure **B** (Fig. 1). The initial selection of the acyl groups was primarily based on the commercial availability.

A large number of analogues were rapidly synthesized by a combination of conventional methods and the parallel protocol previously described. A quick survey of the SAR of these analogues in a PDE5 inhibition study revealed that a wide range of acyl groups are tolerated. Interestingly, among the R^1 selected here, only compounds bearing a 3,4-methylenedioxy were active against PDE5 in the inhibition assays. We decided to conduct more in-depth SAR studies on structures with methylenedioxy as R^1 and **a1**, the 5-arylfuroyl, as the acyl moiety.

As illustrated in Figure 2, replacing NH in the indole moiety with S abolished the activity (**12b** vs **13**), indicating that the indole proton is essential to the biological activity. Eliminating the carbonyl group decreased the activity (**12a** vs **14**), indicating the importance of the amide structure.

We then decided to choose *N*-phenylfuroyl β-carboline as pharmacophore for further studies. Table 1 summarizes the SAR study on the phenyl ring in the phenylfuroyl moiety of structure **12** where the heterocycle is a 2,5-disubstituted furan.

Biological studies showed that both electron-donating and electron-withdrawing groups are tolerated for PDE5 inhibition. The positions of the substituents do not significantly influence the potency. We took advantage of this observation to introduce polar functional groups to improve the solubility of this series. Indeed,



Scheme 4.

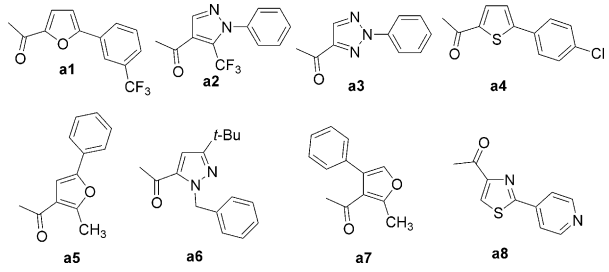


Figure 1. Representative *N*-acyl groups.

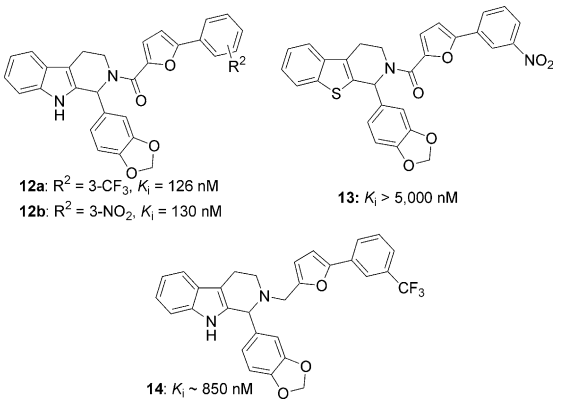


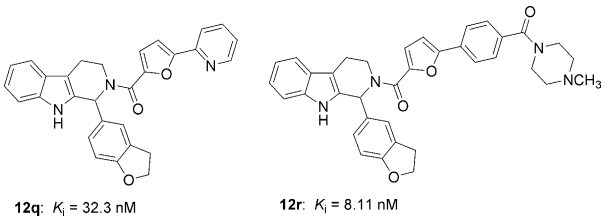
Figure 2. Pharmacophore identification.

Table 1. PDE5 Inhibition by phenyl-furoyl β-carbolines[17]

Compd	R^2	K_i, nM (SD)[a]
12a	3-CF_3	126 (±73)
12b	3-NO_2	130 (±30)
12c	4-NO_2	77.5 (±16)
12d	2-NO_2	219 (±30)
12e	H	~750
12f	3-NH_2	179 (±52)
12g	4-NH_2	123 (±18)
12h	4-Cl	145 (±34)
12i	4-MeCONH–	64.2 (±8.6)
12j	3-MeCONH–	50.6 (±22)
12k	3-$HOOC(CH_2)_3CONH$–	~95
12l	4-$HOOC(CH_2)_3CONH$–	~100
Sildenafil		1.98 (±0.3)

[a]Values are means of three experiments except **12e**, **12k**, and **12k** where IC_{50} was determined instead of K_i.

analogues with acidic functional groups such as **12k** and **12l** showed potency comparable to the aniline precursors (**12f** and **12g**). Furthermore, replacing methylenedioxyphenyl with dihydrobenzofuran at R^1 position does not significantly decrease the potency against PDE5. When we introduced basic functional groups in R^2 region of the molecule to improve solubility, we identified more potent analogues such as **12q** and **12r.**



In the pyrimidine series, similar SAR was observed in the R^1 region. Among many substituents tested, only bicyclic moieties such as 3,4-methylenedioxy showed activity in the PDE5 inhibition assay. Neither monosubstituted nor simple disubstituted phenyl groups, including electron donating, electron withdrawing, lipopholic and functional groups with hydrogen bonding abilities, showed any inhibition. For example, when R^1 is dimethoxy (**16**) in place of methylenedioxy (**15a**) the activity is abolished. Interestingly, **15f** showed potency similar to **15a**, indicating that bicyclic structure is required in this region (Fig. 3).

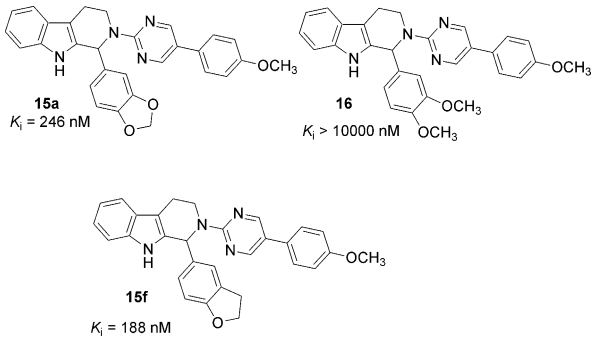


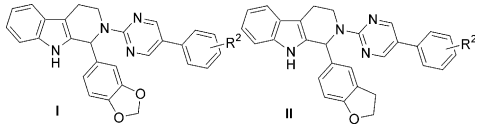
Figure 3. Examples of R^1 contributions.

Table 2 summarizes the SAR of phenylpyrimidinyl moiety. Even though both electron-donating groups such as methoxy and hydroxy and electron-withdrawing groups such as nitro are tolerated, lipophilic groups such as methyl seem to be disfavored. In addition, an attempt to increase the aqueous solubility through the introduction of a basic appendage (**15g**) was not successful.

In an effort to improve the aqueous solubility without increasing the size of the molecules, we synthesized pyridine and imidazole analogues such as **17** and **18**. To our delight, these modifications not only increased the solubility to facilitate the in vivo studies, but also increased the potency. As shown in Figure 4, replacing phenyl with dimethylimidazole significantly increased the potency. Replacing both the phenyl and pyrimidinyl with pyridinyl also significantly increased the potency against PDE5. The potency of **18** is comparable to that of sildenafil.

During SAR studies, we discovered that alkyl substitution on the indole nitrogen with alkyl groups abolished the activity (not shown). On the other hand, introduction

Table 2. PDE5 inhibition by phenylpyrimidyl-β-carbolines



Compd	Structure	R^2	K_i, nM (SD)[a]
8a	I	4-OMe	246 (±41)
8b	I	3,4-$(OMe)_2$	62.8 (±8.2)
8c	I	2-NO_2, 4-SO_2Me	132 (±18)
8d	I	4-Me	543 (±10)
8e	I	4-Cl	126 (±38)
8f	II	4-OMe	189 (±94)
8g	II	4-$O(CH_2)_2$pyrrolidine	>1000
8h	II	4-OH	154 (±17)
Sildenafil			1.87

[a]Values are means of three experiments.

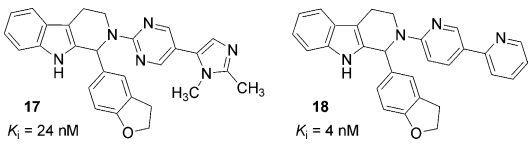


Figure 4. More soluble analogues.

of a carbonyl group at 4-position of the β-carboline significantly improved the potency against PDE5 (Fig. 5). Compound **19** has a K_i comparable to that of sildenafil versus PDE5. The carbonyl group can serve as a hydrogen-bonding acceptor as well as increase the acidity of the indole nitrogen. If the carbonyl group contribution to the potency increase is due to hydrogen-bonding, one would expect that the hydroxy analogue **20** should possess at least comparable potency to the parent compound **15b** since hydroxy group can serve as both a hydrogen-bond acceptor and donor. The fact that **20** (tested as mixture of diastereomers) did not show potent activity confirms our earlier observation that the ability of the indole proton as hydrogen-bonding donor may play a critical role. It is unclear to us at this point why the unsubstituted analogue **15b** is more potent than the hydroxy analogue **20**.

To determine the selectivity of the potent analogues, the inhibition of a panel of PDEs by our compounds was studied in comparison with sildenafil (Table 3). Compound **12r** has a comparable potency to sildenafil but much better selectivity versus other PDE isozymes, especially versus PDE1, which might contribute to the cardiovascular side effects of sidenafil, and PDE6 which is responsible for the visual disturbance experienced by patients using sildenafil. Compound **17** also demonstrated better selectivity than sildenafil versus PDE1-6.

To determine the influence of the chiral center on the biological activity, compound **12m** was separated with chiral HPLC into the two enantiomers, **12n** (*R*) and **12p** (S). The stereochemistry was proved independently by N-acylation of the 2-position-unsubstituted enantiopure β-carboline. As shown in Figure 6, only the *R*-enantiomer is active while the *S*-enantiomer is inactive. This confirmed the importance of the chiral center to PDE5 inhibition. In line with this observation, in the pyrimidine series, the *R*-enantiomer of **15b** is also more potent than the racemate.

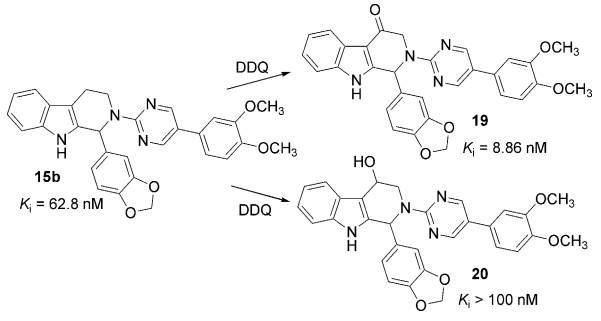


Figure 5. Importance of the indole proton.

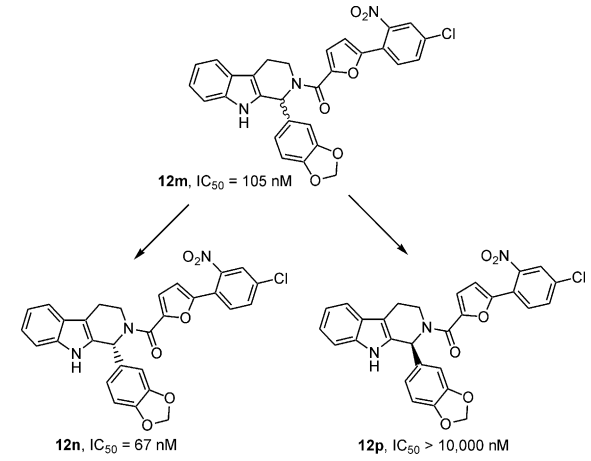


Figure 6. Influence of the chiral center.

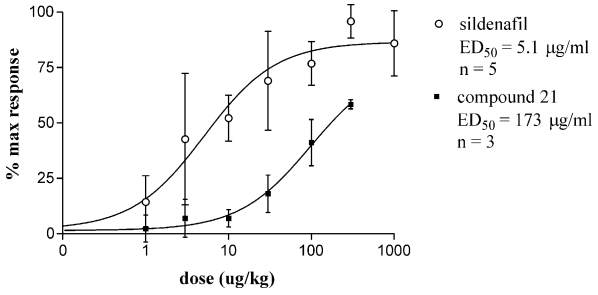


Figure 7. Efficacy in increasing intracavernosal pressure in dogs by the iv route.[18]

During the in vivo studies in dogs (Fig. 7), a compound **21** (K_i = 18 nM, 4-methoxy in place of 3,4-dimethoxy in **19**) showed efficacy with an EC_{50} of 173 μg/mL by the intravenous route. Sildenafil has an EC_{50} of 5.1 μg/mL. The efficacy difference reflects in part the potency difference of the two compounds. More potent analogues such as the *R*-enantiomer of **18** may demonstrate better efficacy in this model.

In summary, we have identified a series of novel β-carboline PDE5 inhibitors by parallel synthesis utilizing a tandem resin quenching protocol and intuitive design. Representative compounds demonstrated better selectivity versus other isozymes of PDEs (PDE1–6). Systematic SAR studies identified the essential structural features for PDE5 inhibition. This information was crucial for the identification of more potent PDE5 inhibitors. Representative compounds showed in vivo efficacy in dogs by the intravenous route.

Table 3. Selectivity studies versus other isozymes of PDEs[a]

Compd	PDE5, K_i (nM)	PDE1/PDE5	PDE2/PDE5	PDE3/PDE5	PDE4/PDE5	PDE6/PDE5
12q	32.3	>3000	308	>3000	180	32
12r	8.11	2963	>10,000	>10,000	>10,000	119
17	24	>4000	>4000	>4000	>4000	187
19	8.86	674	23,000	>24,000	161	4.0
Sildenafil	1.87	120	4000	7000	3,000	4.0

[a]Values are means of three experiments; Source of enzymes are from the following human tissues: PDE1, heart; PDE2, *corpus cavernosum*; PDE3, platelets; PDE4, skeleton muscle; PDE5, *corpus cavernosum*; PDE6, retina cone (PDE6 from retina rod showed slightly higher ratio vs PDE5).

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17. Cyclic Nucleotide Phosphodiesterase (PDE) Assay: PDE5 was isolated from human corpus cavernosum or platelet according to the protocol described by: Boolell, M.; Allen, M. J.; Ballard, S. A.; Geo-Attee, S.; Muirhead, G. J.; Naylor, A. M.; Osterloh, I. H.; Gingell, C. *Intl. J. Impotence Res*. **1996**, *8*, 47, with minor modifications. The PDE5 activity was determined in a two-step assay described by Thompson and Appleman in *Biochemistry* **1971**, *10*, 311, with adaptation. The K_i values for the compounds were determined as follows. Stock solution of the compounds were prepared in 100% DMSO, diluted in 100% DMSO to the appropriate concentrations, and added to the assay buffer to give a final concentration of 2% DMSO. The amount of enzyme used in each reaction was such that the hydrolysis of substrates did not exceeded 15% so that the amount of product increased linearly with time. A 30 nM substrate concentration ($[S] \ll K_m$) was used such that IC_{50} values approximate the K_i values.
18. This study was adapted from Carter, A. J.; Ballard, S. A.; Naylor, A. M. *J. Urol*. **1998**, *160*, 212, with modifications. Male beagles were anesthetized and the anesthesia was maintained throughout the course of the experiment by pentobarbital infusion. Arterial blood pressure was monitored. The pelvic nerve was exposed and hooked to a bipolar electrode. Penis was denuded and a 19-gauge needle attached to a pressure transducer via a catheter was inserted into the corpus cavernosum to record intracavernosal pressure (ICP). Control ICP responses were generated by electrical stimulation to the pelvic nerve at appropriate current output and frequency setting that increased the ICP to about 30% of systolic pressure. After the control responses were established, ascending concentrations of test compound were given intravenously at 40-min intervals. The effect on ICP was evaluated at 15 min after each dosing by electrical stimulation to the pelvic nerve at the same setting. To determine the effect of the compound, the area under the curve (AUC) for ICP at each stimulation was computed and the control response AUC was subtracted. At the end of each experiment, 300 μg/kg sildenafil was given intravenously. This dose was shown to induce a maximal response in our hands and thus designated as 100%.