Letters

Pyrimidinylpyrroloquinolones as Highly Potent and Selective PDE5 Inhibitors for Treatment of Erectile Dysfunction

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Abstract: A series of N-pyrimidinylpyrroloquinolones were discovered as extremely potent and selective PDE5 inhibitors. Representative compounds demonstrated in vivo efficacy in dog erectile dysfunction models and are orally bioavailable.

Introduction. Cyclic nucleotides (adenosine cyclic 3′,5′-phosphate (cAMP) and cyclic guanosine 5′-phosphate (cGMP)) are important secondary messengers that 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. Upon extracellular stimulation, the levels of cyclic nucleotides change rapidly, producing the physiological responses. PDEs play critical roles in modulating the levels of cyclic nucleotide, their duration of action, and thus, the physiological outcome. To date, 21 mammalian PDE genes have been cloned and they are classified into 11 families according to the 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; and PDE10 and PDE11, hydrolyzing both cAMP and cGMP. Among the 11 families, PDE5 is the first recognized cGMP-binding PDE discovered initially in lung tissues. Subsequent studies revealed that it is the major isozyme of PDEs in corpus cavernosum tissue of the penis, where it plays an important role in penile erection.[4,5] Many potent PDE5 inhibitors have been reported in the literature as vasodilators.[6] The introduction of sildenafil, a PDE5 inhibitor, as an agent treating 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.[7−11] As part of our ongoing effort to identify novel PDE5 inhibitors that have improved selectivity vs PDE1 and PDE6,[12−16] we are interested in compounds that are structurally distinct from sildenafil. During the structure−activity relationship (SAR) studies of the pyrimidinyl-β-carbolines,[16] we discovered that the acidity of the indole proton correlated with potency against PDE5. Consistent with this finding, we now report a series of extremely potent PDE5 inhibitors, pyrroloquinolones, which we discovered during our work on the β-carbolines.

Scheme 1

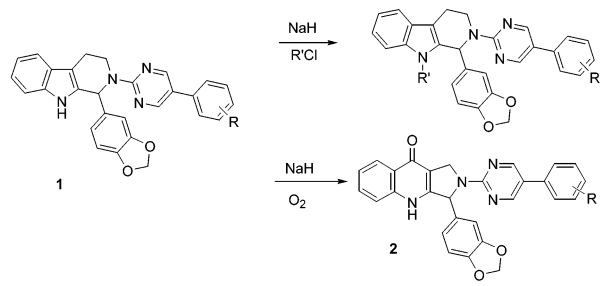
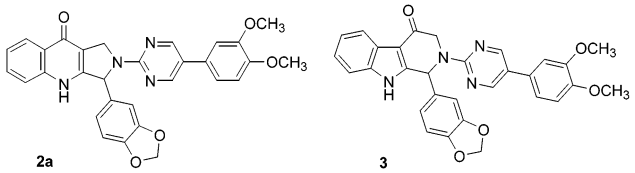


Chart 1



Results and Discussion. In the SAR studies of the β-carboline series (**1**), we wanted to determine the effect of alkylation of the indole nitrogen atom on PDE5 potency (Scheme 1). Although the N-alkylation reaction was usually high-yielding, we occasionally observed a very minor byproduct. Upon further examination, we realized that the minor product (**2**) was the result of Winterfeldt[17] oxidation of compound **1**, due to the presence of a trace amount of oxygen in the reaction mixture. Quinolones **2** are more acidic ($pK_a \sim 9$) than β-carbolines such as **3** ($pK_a \sim 14$), which according to the SAR in our previous report might provide more potent compounds against PDE5 probably due to the increased ability as a hydrogen-bonding donor (Chart 1).

SAR studies of the pyrroloquinolone series demonstrated that substituents on the phenyl ring in the pyrimidine portion of the molecule did not significantly influence the inhibitory activity against PDE5 (Table 1). In particular, the monomethoxy and dimethoxy analogues showed identical inhibition (**2a** vs **2b**). Replacing methoxy with hydroxy decreased the K_i value only 4-fold (**2c** vs **2d**). Introduction of large, basic substituents (**2e**,**f**) or electron-withdrawing groups such as 4-chloro and 4-nitro (not shown) also did not have significant effects on PDE5 potency. In fact, eliminating the entire phenyl ring resulted in only a moderate decrease in potency (**2g**,**h** in Chart 2), thereby presenting an opportunity to modify the physical properties of the series. Further studies revealed that the pyrimidine moiety can be replaced with other heterocycles or acyl groups, which will be reported in separate publications.

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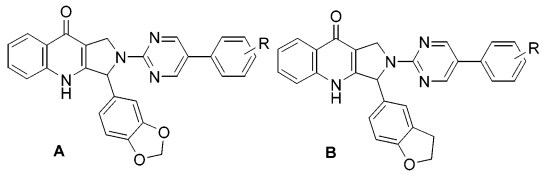
Table 1. SAR of the Phenylpyrimidine Region



compds	strain	R	K_i, nM (SD)[a]
2a	A	3,4-OMe	0.31 (±0.05)
2b	A	4-OMe	0.31 (±0.05)
2c	B	4-OMe	0.40 (±0.05)
2d	B	4-OH	1.75 (±0.05)
2e	B	4-O$(CH_2)_2$morpholino	0.29 (±0.06)
2f	B	4-O$(CH_2)_2$N$(C_2H_5)_2$	0.53 (±0.14)

[a] Means of three determinations.

Chart 2. K_i Values of Pyrimidine Analogues Against PDE5

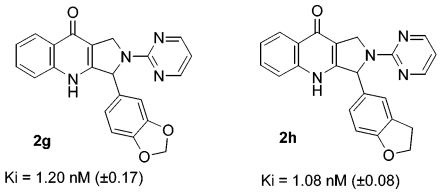
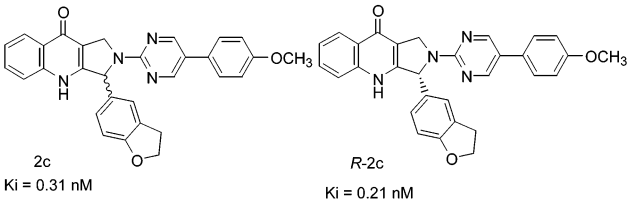
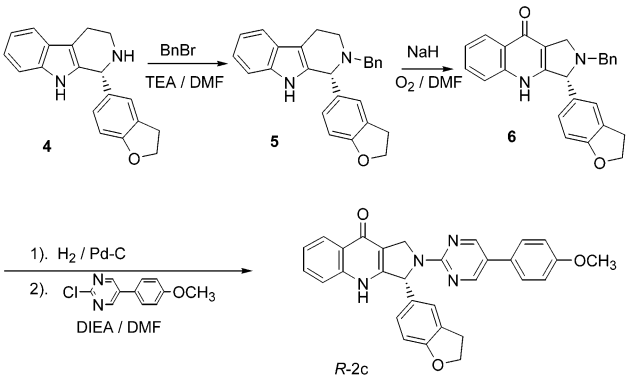


Chart 3. Influence of the Chiral Center

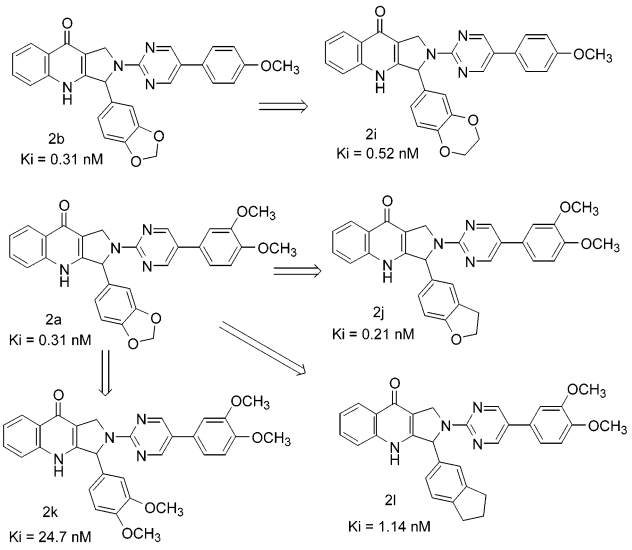


Scheme 2



The chiral center has a significant impact on PDE5 inhibition as shown in Chart 3. The *R*-enantiomer (*R*-**2c**) is the predominant active form with K_i of 0.21 nM, as compared with the racemate with K_i of 0.31 nM and the *S*-enantiomer with K_i in the micromolar range. The pure enantiomers could be obtained by chiral column chromatography or chemical resolution. Alternatively, they can be synthesized from the enantiomerically pure *β*-carboline precursor prepared by literature procedures.[18] The synthesis of the *R*-enantiomers is exemplified with *R*-**2c** (Scheme 2). Thus, the benzyl-protected *β*-carboline **5** was subjected to Winterfeldt oxidation with NaH as base in dimethylformamide to give **6**, followed by debenzylation and nucleophilic aromatic substitution reaction of the chloropyrimidine to give the *R*-enantiomer in >97% ee. No racemization was observed at any step, as demonstrated by chiral high-performance liquid chromatography.

Chart 4



The effect of the aromatic substituent attached to the chiral center was also studied as shown in Chart 4. Interestingly, replacing the methylenedioxyphenyl with other bicyclic structures, such as dihydrobenzofuran (**2j**), benzodioxane (**2i**), and indane (**2l**) did not significantly decrease the potency, while replacing the bicyclic structure with dimethoxy (**2k**) had a dramatic effect. This indicates that a bicyclic structure is preferred in this region for the binding to PDE5.

One of the most important issues for the clinical utility of PDE5 inhibitors is their selectivity vs other PDE isozymes, especially PDE1, PDE3, and PDE6. All compounds tested in this series have high selectivity vs PDE1−PDE4. However, some of our initial analogues such as **2i**,**l** have a similar selectivity vs PDE6 as sildenafil. To eliminate this potential liability to visual function, we focused our efforts on improving the PDE6/PDE5 ratio. To this end, we were able to identify compounds with high selectivity vs PDE6 by modifying the aromatic substituent attached to the chiral center. Replacement of methylenedioxyphenyl (e.g., **2b**,**g**) with dihydrobenzofuran (e.g., **2c**,**h**) improved the PDE6 selectivity without compromising the potency and the selectivity vs other isozymes. Table 2 summarizes the selectivity of representative compounds.

To further evaluate the compounds, we have tested them in RFL-6 cells for their ability to increase cGMP levels. In comparison with sildenafil, these compounds possess better efficacy in the cell-based assays. As shown in Figure 1, both **2c** and *R*-**2a** (K_i = 0.21 nM) induced a 3- and 5-fold increase in intracellular cGMP concentration at lower compound concentrations than sildenafil.

Select analogues from this series were studied in vivo in the dog model of erectile dysfunction. In these studies, drugs were dosed by the intravenous route using sildenafil citrate as a positive control. As shown in Figure 2, compound *R*-**2a** demonstrated good efficacy in this model. Similarly, *R*-**2c** had an ED_{50} of 9.6 μg/kg.

Table 2. Selectivity of Selected Compounds Against PDE Isozymes[a]

compd	PDE5, K_i (nM)	PDE1/ PDE5	PDE2/ PDE5	PDE3/ PDE5	PDE4/ PDE5	PDE6/ PDE5
R-**2a**	0.21	34 000	4320	9.690	790	0.59
2b	0.31	>50 000	>50 000	>50 000	19 380	18.5
2c	0.40	>50 000	>50 000	>50 000	20 000	55.9
R-**2c**	0.23	>434 000	110 000	42 000	22 000	27
2g	1.20	>50 000	>50 000	>50 000	1070	7.4
2h	1.08	>50 000	>50 000	>50 000	6270	310
2i	0.52	>50 000	>50 000	>50 000	1050	3.07
2l	1.14	>50 000	18 940	31 870	750	2.14
sildenafil	1.87	120	4000	7000	3000	4.0

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

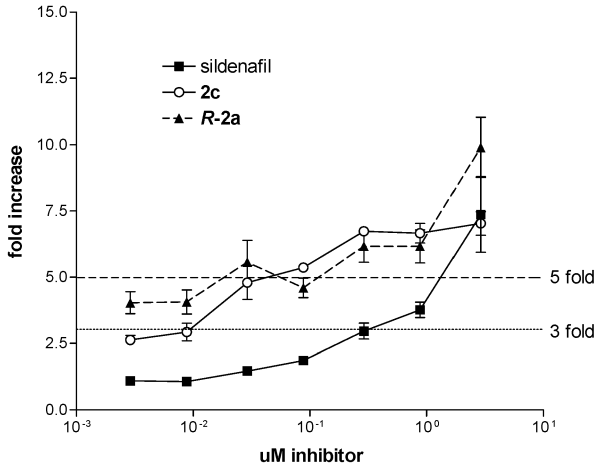


Figure 1. Cell-based functional assays.

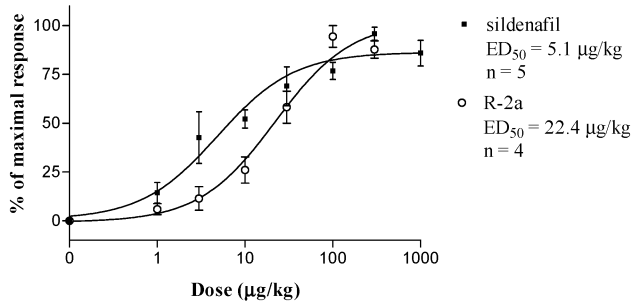


Figure 2. In vivo efficacy of *R*-**2a** in dogs.

Preliminary pharmacokinetics studies have also been conducted with select analogues. In particular, compound **2c** had an oral bioavailability of 20% in rats, as compared with 11% for sildenafil in our system. This result demonstrated the potential of these compounds as oral agents for male erectile dysfunction and female sexual disorders.

In summary, we have discovered a series of novel pyrroloquinolone analogues as extremely potent and selective PDE5 inhibitors. Representative compounds demonstrated in vivo efficacy in a dog model of erectile dysfunction. Representative compounds are also orally bioavailable in preliminary pharmacokinetics studies.

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Supporting Information Available: Experimental details of compounds reported, PDE assay protocol, and in vivo efficacy protocol. This material is available free of charge via the Internet at http://pubs.acs.org.

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