Brief Articles

Furoyl and Benzofuroyl Pyrroloquinolones as Potent and Selective PDE5 Inhibitors for Treatment of Erectile Dysfunction

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Synthesis of furoyl and benzofuroyl pyrroloquinolones as potent and selective PDE5 inhibitors was reported. Their in vitro potencies in inhibiting PDE5 and selectivity in inhibiting other PDE isozymes (PDE1−4 and PDE6) were evaluated. Some of these compounds are more potent than sildenafil with better selectivity toward PDE1 and PDE6. Incorporation of solublizing groups resulted in bioavailable analogues. Selected compounds showed in vivo efficacy in anesthetized dog model for penile erection.

Introduction

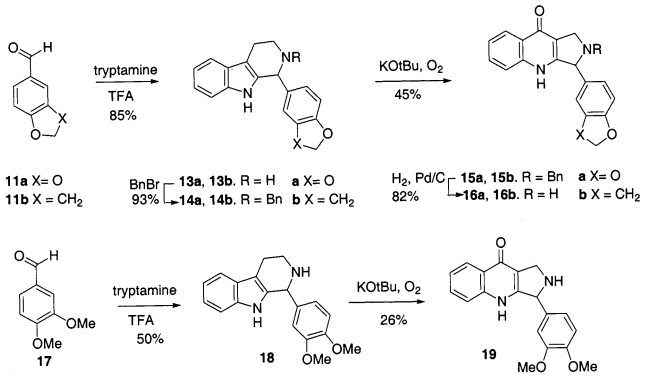
PDE5 is the major cGMP-hydrolyzing enzyme in human corpus carvernosal tissue. Upon sexual stimulation, release of nitric oxide from NANC neurons and the vascular endothelium activates soluble guanylyl cyclase in the smooth muscle cells, initiating cGMP synthesis. Inhibition of PDE5 causes further accumulation of cGMP in the penile tissue. The resulting elevated cGMP levels cause a decrease in intracellular calcium concentration, leading to relaxation of smooth muscle in the corpus cavernosum. The relaxation of this tissue permits increased arterial flow to the penis, which becomes erect when engorged with blood.[1]

The large population of people suffering from male erectile dysfunction (MED)[2,3] and the commercial success of sildenafil[4] have provided a strong stimulus for the discovery and development of second-generation PDE5 inhibitors.[5,6] One important issue facing such an agent is selectivity versus other enzymes in the phosphodiesterase superfamily. Inhibition of PDE1 and PDE6 may be associated with some of the adverse side effects of sildenafil therapy, especially visual disturbances (PDE6). Recently, we reported the discovery of both furoyl β-carbolines[7c] and pyrimidinyl pyrroloquinolones[7e] as potent and selective PDE5 inhibitors. In an effort to extend the scope of this discovery, we now report a series of furoyl and benzofuroyl pyrroloquinolones as PDE5 inhibitors with greater potency and selectivity for PDE5 versus other PDE isozymes.

Chemistry

Pyrroloquinolone core structures **16a** and **16b** were synthesized in a four-step sequence (Scheme 1). The Pictet−Spengler reaction of piperonal (**11a**) or 2,3-dihydrobenzo[*b*]furan-5-carboxaldehyde (**11b**) with tryptamine provided β-carbolines **13a** and **13b**.[8] After benzylation with benzyl bromide, compounds **14a** and **14b** were rearranged via Winterfeldt oxidation[9] to furnish protected pyrroloquinones **15a** and **15b**. Hydrogenolysis of the benzyl group provided pyrroloquinones **16a** and **16b** in good yield. Alternatively, this sequence can be reduced to two steps without the use of a protecting group. The Pictet−Spengler reaction of veratraldehyde **17** provided β-carboline **18**, which was directly oxidized to pyrroloquinolone **19**. X-ray analysis on a crystalline sample of **19** confirmed the structure of this key intermediate (Figure 1).[12] This two-step sequence was used for generating compound **16a** in similar yield and compound **16b** in lower yield. Although furoyl pyrroloquinolones were initially made by direct acylation of pyrroloquinolones **16a** and **16b** with acid chlorides (Scheme 2), coupling acids with **16a** and **16b** using standard conditions[10] was found to be more efficient. Suzuki coupling of **12a** or **12b** with aryl- and heteroarylboronic acids allowed facile introduction of diversity elements (Scheme 3).[11]

Scheme 1. Synthesis of Pyrroloquinolone **16** and **19**



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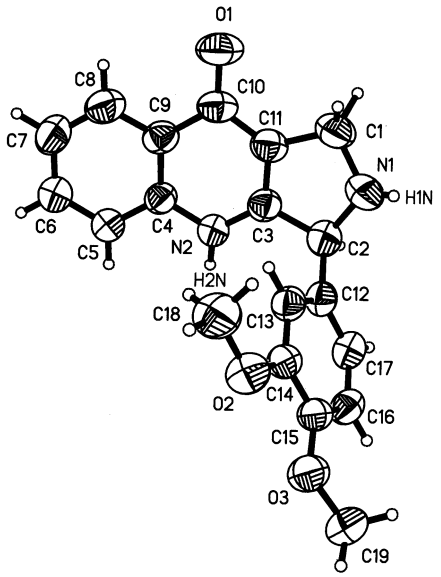
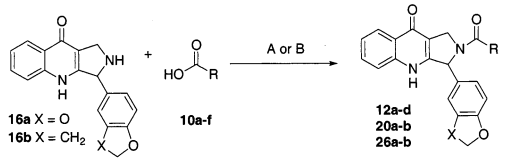
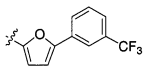
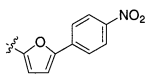
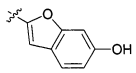


Figure 1. X-ray ORTEP picture of compound **19**.

Scheme 2. Synthesis of Furoyl and Benzofuroyl Pyrroloquinolones via Acylation Reaction



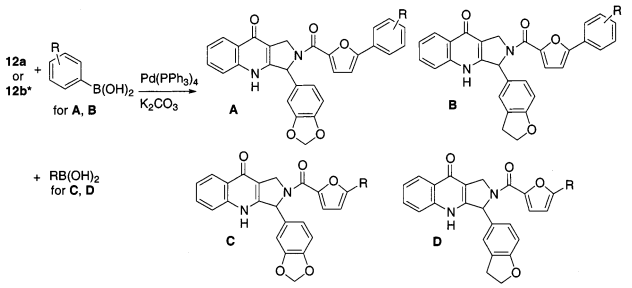
condition A: i) **10**, $(COCl)_2$, THF; ii) **16**;
condition B: PyBrOP

compd	R	X	Acid	Condition	Yield (%)
12a		O	**10a**	B	84
12b	Same as above	CH_2	**10a**	A	63
12c		O	**10b**	A	49
12d		O	**10c**	B	48
20a		O	**10d**	A	61
20b		O	**10e**	B	72
26a		O	**10f**	B	23
26b	Same as above	CH_2	**10f**	B	37

Results and Discussion

Initially, we prepared both aryl (**12c**) and heteroaryl (**12a** and **12d**) derivatives of the pyrroloquinolone core (Table 1). Although all had potencies comparable to sildenafil, for purposes of this report, we focused our attention on the furoyl series. Replacement of the bromine atom in **12a** with a phenyl ring (**20c**) was accomplished without loss of potency. The tolerance for a wide range of substituents on this phenyl ring from very electron-withdrawing (**20b**) to very electron-donating (**20n**) indicated that this area was a good place to adjust the physicochemical properties of the series.

Scheme 3. Suzuki Coupling Reaction of Furoyl Pyrroloquinolones[a]



compd*	structure	R	Yield (%)	compd*	structure	R	Yield (%)
20c	A	H	55	**20n**	A	*p*-NMe_2	15
20d	A	*p*-CH_3	69	**20o**	A	*p*-$NHSO_2CH_3$	32
20e	A	*m*-CH_2OH	28	**20p**	A		79
20f	A	*p*-CH_2OH	49	**20q**	A		67
20g	A	*m*-NO_2	16	**20r***	B		56
20h	A	*p*-CO_2H	79	**20s**	C	2-furoyl	45
20i	A	*p*-CO_2Me	11	**20t**	C	3-thienyl	72
20j	A	*p*-CHO	15	**20u**	C	4-pyridinyl	55
20k	A	*p*-CN	27	**20v**	C	3-pyridinyl	57
20l	A	*p*-OH	28	**20w***	D	3-pyridinyl	45
20m	A	*p*-OMe	12				

[a] (*) All of the compounds were synthesized from **12a** except for **20r** and **20w**. These were made from **12b**.

Table 1. Potencies toward Inhibiting PDE5

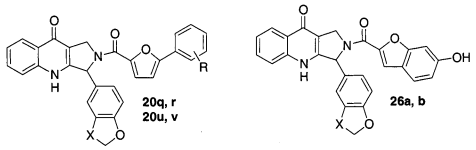
compd	K_i (SD),[a] nM	compd	K_i (SD),[a] nM
sildenafil	1.91(±0.33)	**20l**	0.73(±0.06)
12a	2.07(±0.53)	**20m**	1.71(±0.19)
12c	2.49(±1.16)	**20n**	3.74(±0.04)
12d	3.32(±1.54)	**20o**	0.24(±0.10)
20a	0.98(±0.09)	**20p**	0.76(±0.05)
20b	0.61(±0.30)	(−)-**20q**	0.20(±0.02)
20c	2.09(±0.78)	(±)-**20q**	1.82(±0.31)
20d	2.64(±0.70)	**20r**	1.78(±0.32)
20e	0.47(±0.10)	**20s**	4.66(±0.92)
20f	0.61(±0.08)	**20t**	0.71(±0.35)
20g	1.30(±0.57)	**20u**	0.31(±0.05)
20h	1.01(±0.32)	**20v**	0.15(±0.02)
20i	0.99(±0.18)	**20w**	0.53(±0.14)
20j	2.59(±0.32)	**26a**	0.35(±0.02)
20k	2.71(±1.22)	**26b**	1.74(±0.69)

[a] Values are the mean of three experiments. Standard deviations are given in parentheses.

Hydrophilic (**20e** and **20f**), basic (**20p** and **20q**), moderately acidic (**20l**), and acidic (**20h**) groups could all be introduced to produce compounds with PDE5 inhibitory activities equal to or better than sildenafil. We also investigated the replacement of the phenyl substituent in **20c** with heterocycles (**20s**−**w**). It was particularly encouraging to note that moderately basic solublizing groups (**20u** and **20v**) could be introduced with a marked increase in potency relative to sildenafil. Finally, in a bid to further reduce the molecular weight of the lead series, we prepared benzofurans **26a** and **26b** and found their potency comparable to unfused phenol **20l**.

One of the most important issues facing PDE5 inhibitors is their selectivity versus other PDE isozymes, in particular, PDE1 (found in heart) and PDE6 (primarily located in retina). All compounds tested in this series had high selectivity versus PDE1−4 (Table 2). For

Table 2. Selectivities of PDE5 over PDE1−4 and -6[a]



compd	X	R	Ratio of Ki (PDEx) / Ki (PDE5)*					
			PDE 1/5	PDE 2/5	PDE 3/5	PDE 4/5	PDE6/5 cone	PDE6/5 rod
sildenafil			136	8,900	12,858	2,555	12	8
(±)-20q	O		13,510	14,330	9,450	1,340	2	2
(-)-20q	O		72,530	56,410	221,110	7,810	13.2	13.0
20r	CH_2		11,200	8,610	18,620	3,010	12	13
20u	O	3-pyridyl	106,420	10,940	20,190	5,080	34	23
20v	CH_2	3-pyridyl	669,640	18,080	45,400	25,510	108	144
26a	O	none	289,860	289,860	289,860	12,000	29	31
26b	CH_2	none	57,470	31,780	57,470	12,380	646	291

[a] (*) Values are the mean of three experiments for K_i of PDE5 and the mean of at least two experiments for K_i of PDE1−4 and PDE6 (retina cone and rod).

PDE2, -3, and -4, these compounds showed selectivity comparable to that of sildenafil, and for PDE1, they showed selectivity a minimum of 100-fold better than sildenafil. Since our initial analogues such as **20r** had selectivity for PDE6 similar to that of sildenafil, we focused our efforts on improving the PDE6/PDE5 ratio. Replacement of methylenedioxyphenyl group (**20q** and **20u**) with dihydrobenzofuran (**20r** and **20v**, respectively) improved the PDE6/PDE5 selectivity 6- and 3-fold, respectively, without compromising the potency and the selectivity versus other isozymes. Selectivity for PDE5 over PDE6 increased even more (20-fold) when this modification was applied to benzofuroyl pyrroloquinolone (**26a** versus **26b**). This result was consistent with previous findings in the pyrimidinyl pyrroloquinolone series.[7e] We also synthesized the enantiomer (−)-**20q**, which likely has the (*R*)-configuration,[7e] by using chiral starting material (*R*)-(−)-**13a**.[8b] Biological testing confirmed that (−)-**20q** is also more potent and selective than its racemic form, (±)-**20q**.

Cell-Based Functional Assays. Further evaluation of the compounds was carried out by testing their ability to increase cGMP levels in RFL-6 cells. In comparison with sildenafil, these compounds possess similar potencies in the cell-based assays. A representative example is shown in Figure 2, where data for **20q** and (−)-**20q** are almost superimposable on that of sildenafil.

Preliminary Pharmacokinetics Studies. Next, we addressed the issue of oral bioavailability. Eight compounds bearing a range of acidic and basic functional groups were tested in a preclinical pharmacokinetics study in male rats. The sodium salt of **20l** and **26b** and the hydrochloride of **20w** showed no oral bioavailability. The hydrochloride salts of **20p**, **20u** and the sodium salt of **20h** showed only a trace of oral bioavailablity, i.e., 4%, 3%, and 0.4%, respectively. The sodium salt of **26a** showed modest bioavailabitity (17%), while the hydrochloride salt of the methylpiperazine derivative **20q** showed the best bioavailabitity (31%).

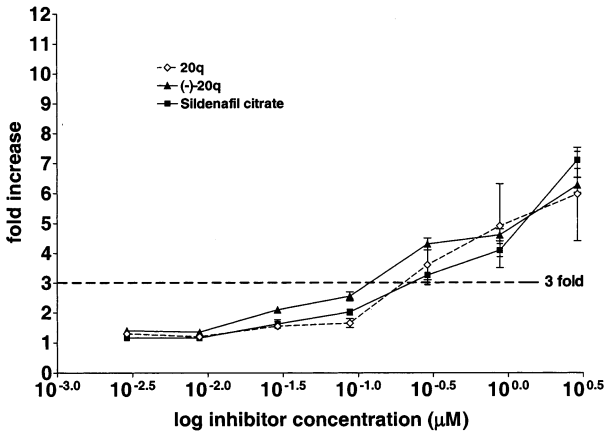


Figure 2. Results of cell-based functional assays, showing fold increase in cGMP levels above 10 μM SNP-stimulated baseline in RFL6 cells.

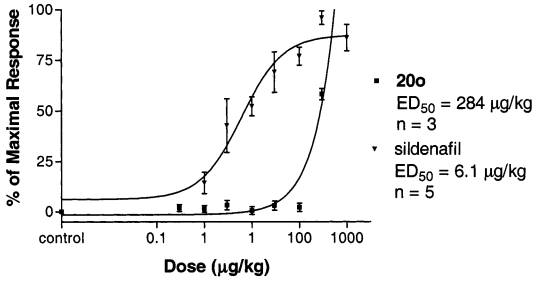


Figure 3. In vivo efficacy of **20o** in anesthetized dogs, showing ICP increase after intravenous administration.

In Vivo Efficacy. Selected analogues from this series were studied in vivo in an anesthetized dog model of erection. In these studies, drugs were administered via the intravenous route using sildenafil citrate as a positive control. Although there is no dose response at lower doses, compound **20o** significantly potentiated ICP increase at 300 μg/kg. This suggested that compound **20o** is efficacious in this model. As shown in Figure 3, the ED_{50} for compound **20o** is around 284 μg/kg.

Conclusions

In this paper, we have reported the discovery of highly selective and potent PDE5 inhibitors, furoyl and benzofuroyl pyrroloquinolones. Some of these compounds are more potent than sildenafil against the isolated PDE5 enzyme. Substituents on the phenyl group can be introduced to reach reasonable oral bioavailibility. Representative compounds showed improvement in their selectivity for PDE1 and -6 when compared to sildenafil. The SAR results in this work indicate that PDE6/PDE5 selectivity can be increased moderately when the methylenedioxyphenyl group adjacent to the chiral center was replaced with dihydrobenzofuran group. Finally, we have discovered a compound that is efficacious in an anesthetized dog model.

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Supporting Information Available: Biological assay, characterization of compounds reported (**12a**−**d**, **13a**,**b**, **14a**,**b**, **15a**,**b**, **16a**,**b**, **18**, **19**, **20a**−**w**, **26a**,**b**). This material is available

free of charge via the Internet at http://pubs.acs.org. Crystallographic data for **19** have been deposited with the Cambridge Crystallographic Data Center as supplementary publication CCDC 194662. Copies of the data can be obtained, free of charge, on application by e-mail to deposit@ccdc.cam.ac.uk.

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