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Quinazolines as potent and highly selective PDE5 inhibitors as potential therapeutics for male erectile dysfunction

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ABSTRACT

In an effort to minimize side effects associated with low selectivity against PDE isozymes, we have successfully identified a series of 6,7,8-substituted quinzaolines as potent inhibitors of PDE5 with high level of isozyme selectivity, especially against PDE6 and PDE11. PDE5 potency and isozyme selectivity of quinazolines were greatly improved with substitutions both at 6- and 8-position. The synthesis, structure–activity relationships and in vivo efficacy of this novel series of potent PDE5 inhibitors are described.

Male erectile dysfunction (MED) was a largely unmet medical need before the introduction of sildenafil (Viagra®) in 1998. As a potent inhibitors of phosphodiesterase 5 (PDE5) in the *corpus cavernosum* of the penis, it was originally studied for the treatment of angina before its effectiveness in treating MED was found in clinical trials serendipitously.[1] Increased levels of cGMP leads to decreased intracellular calcium in the cells of *corpus cavernosum*, resulting in vasorelaxation, inflow of arterial blood, and ultimately an erection.[2] As PDE5 is a member of phosphodiesterase family of enzymes metabolizing cGMP in penis, inhibition of PDE5 increases the cGMP concentration, enhancing the erection.[3]

The launch of Viagra® and vardenafil (Levitra®)[4] marked the new age in drug discovery by affecting one's quality-of-life, however, despite their success, side effects such as headache, nausea, flushing, and visual disturbances have been noted, which are associated with low selectivity against other PDE isozymes, most notably PDE1 and PDE6.[5] Although tadalafil (Cialis®) was shown to have a better PDE1 and PDE6 selectivity, it is not free from side effects such as headache, indigestion, and back pain.[6] The visual disturbances associated with use of PDE5 inhibitors can be linked to inhibition of PDE6, which controls function of rod and cone cells within the eye.[5] The function of PDE11 remains largely unknown,

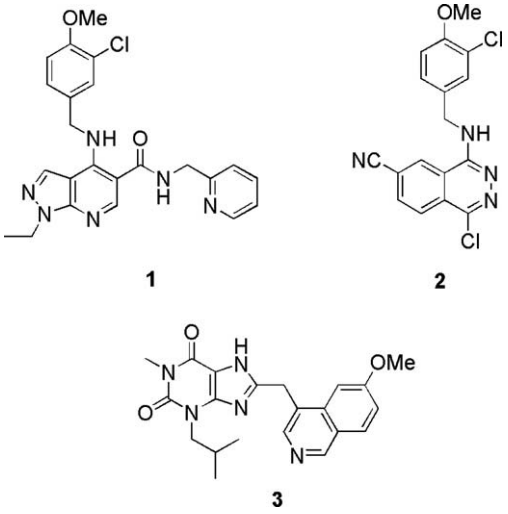

Figure 1. Some representative heterocyclic PDE5 inhibitors.

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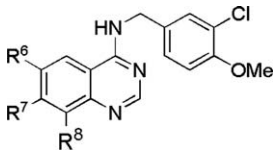
Table 1
PDE5 activity of quinazoline derivatives



Compound	R^6	R^7	R^8	PDE5[a]
6	No_2	Cl	H	0.008
8	No_2		H	0.57
9	No_2		H	0.071
10	No_2	OMe	H	0.032
12	No_2	OH		0.012
13	No_2	OMe		0.012
14	NH_2	OMe		1.1
Tadalafil				0.012
Sildenafil				0.01

[a] IC_{50} values are reported in μM (values are mean of >2 determinations).

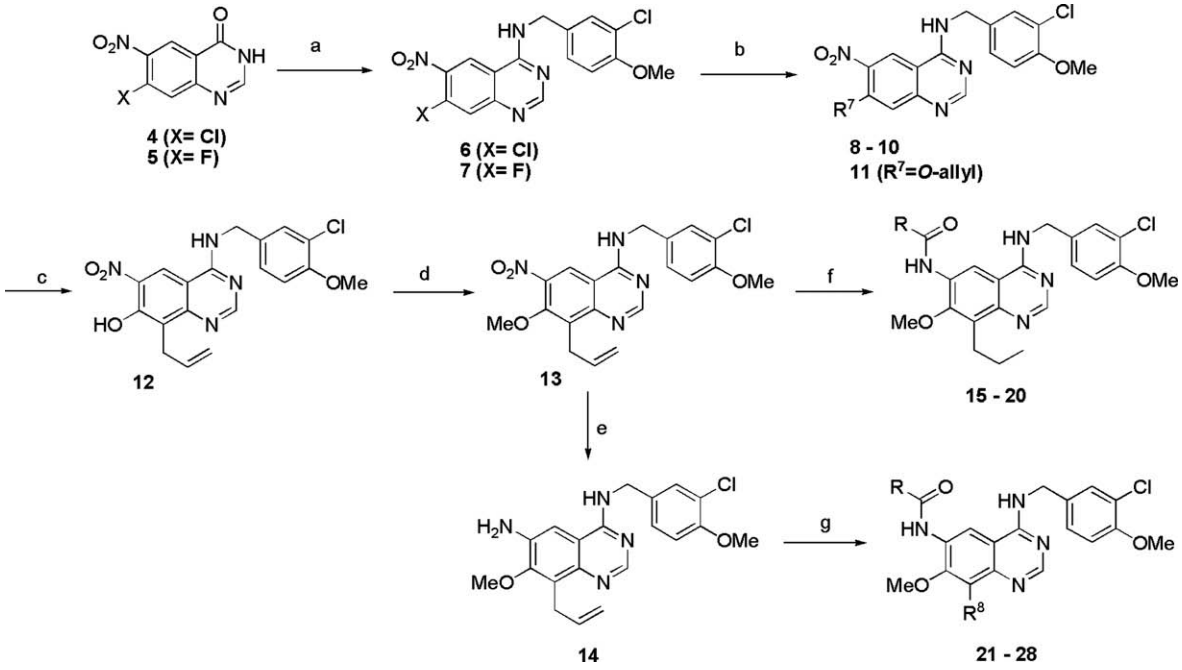
but potential role in male reproduction was recently suggested from several lines of evidences.[7] Since tadalafil cross reacts with PDE11 at sub μM range, there is a growing concern over PDE11 selectivity. Therefore, the identification of highly selective, especially against PDE1, PDE6 and PDE11 as well as more potent PDE5 inhibitors is of great medicinal and commercial interest. Thus, there have been numerous efforts toward the discovery of more isozyme selective PDE5 inhibitors[8] and some representative examples (**1**[9], **2**[10], and **3**[11]) are shown in Figure 1.

In this communication, we disclose the discovery of novel, 6,7,8-substituted quinazoline-based PDE5 inhibitors which exhibit good potency and high levels of selectivity over other PDE isoforms especially PDE6 and PDE11 as well as potent in vivo efficacy in inducing penile erection in conscious rabbit model.

Screening of focused in-house library yielded a 4-(3-chloro-4-methoxy)benzylamino substituted quinazoline hit (**6**) for PDE5, which is equipotent to tadalafil (Table 1). Initially, there was a concern about this quinazoline scaffold because of its prevalence in protein kinase inhibitors, most of which showed potent anticancer activity.[12] However, it was found that 4-aniline or 4-phenol substitution is required to possess anticancer activity, whereas our PDE5 inhibitors contain 4-benzylamino group, which is devoid of cytotoxicity. Quinazoline with simple substitution at 6-, 7-, or 8-position was described[13] as potent inhibitors of several PDEs', but no selectivity data against PDE6 and PDE11 had been reported let alone the in vivo efficacy for erectile dysfunction.

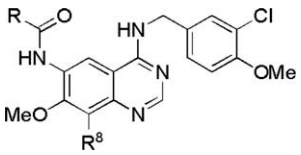
The quinazolines in this study were obtained in a straightforward manner and their synthesis is shown in Scheme 1. The C-4 carbonyl group of **4** or **5**[13] was chlorinated ($SOCl_2$), followed by reaction with 3-chloro-4-methoxybenzyl amine to give **6** or **7** in good yield. With appropriate amines or sodium methoxide, compound **6** (or **7**) was converted to **8**, **9** and **10** (see Table 1 for substitution pattern), while reaction with allyl alcohol gave intermediate **11**. Allyl group was incorporated at C8 by Claisen rearrangement (xylene, 150 °C in sealed tube) of **12** and C7 hydroxyl group was capped with methyl to give **13** for further modification. Selective reduction of C6 ($SnCl_2$, EtOH) afforded **14,** which was used for further allyl group modification (vide infra).

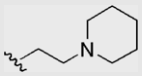
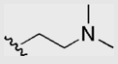
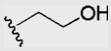
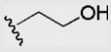
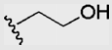
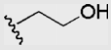
The effect of modification with simple R6, R7, and R8 is shown in Table 1. Tadalafil was used as a positive reference together with sildenafil.[14] By varying the amine substituent at C4 of the quinazoline, we identified 3-chloro-4-methoxybenzylamine as the optimal amine for PDE5 inhibitory potency, which was also observed in several heterocyclic PDE5 inhibitors.[15] Introduction of longer ethylenediamine group (**8**) at C7 resulted in a dramatic loss of activity, while shorter dimethylamino linker (**9**) was also detri-



Scheme 1. Reagents and conditions: (a) 1—DMF (cat), $SOCl_2$, 2—3-chloro-4-methoxybenzylamine, Et_3N, isopropyl alcohol; (b) amine, DIPEA, DMF or NaOMe, MeOH or allyl alcohol, NaH, DMF; (c) xylene, 150 °C, sealed tube; (d) CH_3I, K_2CO_3, acetone, reflux; (e) $SnCl_2$, EtOH, reflux; (f) 1—H_2, PtO_2, MeOH; 2—amide formation; (g) 1—amide formation, 2—OsO_4/$NaIO_4$, then reductive amination ($NaCNBH_3$, AcOH) or $NaBH_4$, MeOH, 0 °C.

Table 2
PDE5 activity and isozyme selectivity of 4-(3-chloro-4-methoxy)-benzylamino-7-methoxy quinazoline derivatives



Compound	R	R^8	PDE5[a]	PDE6[a]	PDE11[a]
15	CF_3		0.004	0.44	7.1
16	H_2NCH2-		0.029	ND	ND
17	CH_3		0.018	4.5	22.7
18	*c*-Propyl		0.019	0.82	14.7
19	Ethyl		0.005	0.42	7.7
20	*tert*-Butyl		0.014	3.7	9.5
21	CF_3		0.019	14.1	39.3
22	CF_3		0.014	ND	ND
23	Ethyl		0.018	0.79	7.8
24	*n*-Propyl		0.032	ND	ND
25	CH_3		0.01	5.9	24.3
26	2-Pyridyl		1.6	ND	ND
27	*i*-Propyl		0.021	2.4	7.7
28	Ethyl		0.001	0.46	10.5
Tadalafil			0.012	18.1	0.29
Sildenafil			0.01	0.14	3.0

[a] Enzyme sources: see Ref. [18]. IC_{50} values are reported in μM (values are mean of >2 determinations). ND, not determined.

mental. No other bulky functional groups gave better activity than simple methoxy group (data not shown), and for reasons of synthetic convenience and molecular weight increase, we decided to maintain methoxy group at C7 position. Regarding C8 position, we assumed that small alkyl groups would impart better activity and moreover overall property required to be efficacious in animal model could be controlled by slight modification of this alkyl chains, which proved to be so.

Compound **12** and methoxy analog (**13**) showed better activity than des-allyl analog (**10**) by 3-fold, supporting our rationale. C7 hydroxyl analog (**12**) was equipotent to methoxy analog, however, due to a concern of metabolic instability, we decided to cap free hydroxyl group by methylation and most of derivatives maintain methoxy at C7 position. Interestingly, the activity was abolished in the case of **14** in which C6 nitro group was reduced to give aniline. Although it was not clear why the activity dropped sharply, we envisioned that this amino group in **14** would serve as another handle for further modification and the chemistry to provide analogs with C8 variation is shown in Scheme 1. The nitro and allyl group in **13** were reduced simultaneously (H_2/PtO_2), followed by amide formation to provide **15**–**20** with *n*-propyl group at C8. Conversely, starting from **14** various amide groups were first introduced at C6 position with suitable acid or anhydride, followed by $OsO_4/NaIO_4$ oxidation to give aldehyde intermediates, from which **21** and **22** were obtained by reductive amination or **25**–**28**[16] were obtained by reduction ($NaBH_4$/MeOH). Simple amide formation of **14** without changing C8 position gave compounds **23** and **24**.

Then, we probed the effect of substitution both at C6 and C8 position, with selectivity profiling against PDE6 and PDE11. Since many derivatives can be prepared rapidly by amide formation reaction of C6 amine with a range of alkyl- and aryl-acetic acids, we initially synthesized focused library with C6 amide linkage. This effort afforded many potent and selective PDE5 inhibitors as indicated in Table 2. We were delighted to observe that trifluoroacetamido derivative **15** significantly improved PDE5 activity by about 300-fold compared to **14** with excellent selectivity against PDE6 and PDE11. As mentioned before, many side effects of currently marketed drugs especially visual disturbances are ascribed to PDE6 inhibition because PDE6 is the sole cGMP PDE in the retina. In addition, tadalafil, a more selective against PDE6 than sildenafil, suffered from poor PDE11 selectivity as described earlier.[6] In an effort to discover novel, potent PDE5 inhibitors with excellent PDE6 and PDE11 selectivity, most of derivatives were routinely screened against these isozymes.

Changing trifluoromethyl group to various alkyl groups showed clear SAR that PDE5 activity of analogs **16**, **17**, **18**, and **20** (R = aminomethyl, Me, cyclopropyl, and *tert*-butyl, respectively) were very similar and slightly lower than tadalafil while isozyme selectivity remains good in all cases (Table 2). The inhibitory activity was improved with ethyl analog (**19**) and the ethyl appears to be optimal within this small set of series. This trend was also observed in the case of C8 allyl analogs (**23** vs **24**) although the PDE5 potency was slightly inferior to C8 *n*-propyl analogs (**19** vs **23**). The effect of larger polar functionality at C8 was explored in **21** and **22**, and there

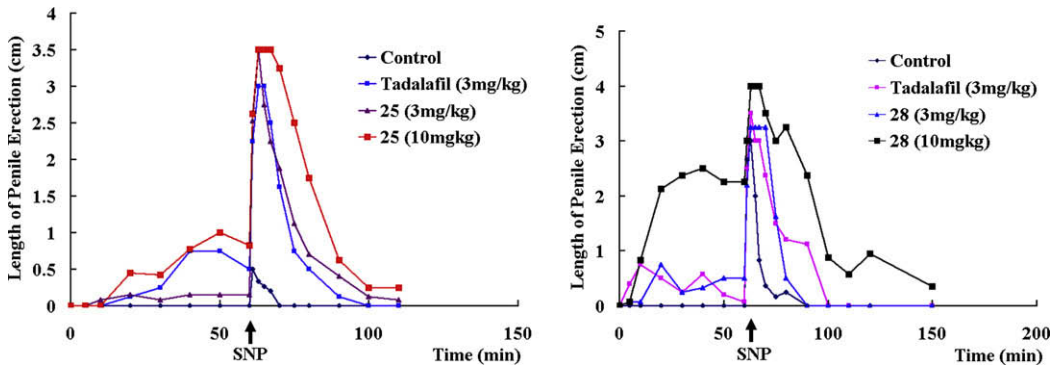


Figure 2. Effects of **25** (a), and **28** (b) on the length of penis in conscious rabbit model. The length of penis was measured after oral administration of **25** and **28**, followed by a sodium nitroprusside (SNP, 0.1 mg/kg) injection after 60 min ($n = 4$). See Ref. 17 for details.

was a slight loss of PDE5 activity when compared with **15**, although the selectivity has been increased.

In order to evaluate the oral efficacy of a compound in inducing the penile erection, conscious rabbits were used in this study.[17] Initially, **15** was evaluated in this model, but despite excellent PDE5 potency, **15** was shown to be far less efficacious than tadalafil (data not shown), and we hypothesized that low membrane permeability and/or high protein binding (>99%) was attributed to low efficacy. Therefore, we turned our attention to the preparation of derivatives that have high membrane permeability as well as relatively lower protein binding, and we believe this could be achieved by introduction of polar functional groups instead of simple alkyl groups at C8 position. As depicted in Scheme 1, oxidation with $OsO_4/NaIO_4$ followed by reduction gave C8 hydroxyethyl analogs (Table 2). Other analogs including carboxylic acid, amino, and 1,2-diol functionality were also prepared and screened as PDE5 inhibitors; however, C8 hydroxyethyl analogs were consistently the best moiety in terms of activity and synthetic convenience as well as PDE5 potency. Gratifyingly, acetamido analog with C8 hydroxyethyl group (**25**) improved PDE5 activity by 2-fold relative to **17**, while isozyme selectivity remains the same. Incorporation of aromatic group in **26** resulted in dramatic loss of activity suggesting that there might be an optimal steric requirement at C8 position. With isopropyl group in **27** restored activity, although it is 2-fold inferior to methyl analog **25**. Of special note is the propionamide analog **28** which was 10-fold more potent than sildenafil and tadalafil and represent the most potent in our series. Compound **28** is not only 10-fold more potent than tadalafil and sildenafil, it is significantly selective against other PDE isozymes as indicated in Table 2 (selectivity for PDE6 > 470 and PDE11 > 8700) and this ratios were also found to be very large against PDE1, PDE2, and PDE3 (IC_{50} = 2.4 μM, 10.4 μM, and 25.5 μM, respectively).[18]

As mentioned before, we expected that incorporation of polar functionality would lead to compounds that possess more preferable physicochemical properties such as solubility, membrane permeability, and protein binding. With highly potent derivatives in our hands, selected compounds (**25** and **28**) were further assessed for animal study. In contrast to **15** which has very low permeability and high protein binding, both **25** and **28** were found to have excellent Caco-2 permeability[19] (17.3×10^{-6} cm s^{-1} and 46.9×10^{-6} cm s^{-1}, respectively) and moderate protein binding relative to sildenafil (95% each, and 98% for sildenafil). Metabolic stability issue was excluded since both **15** and **28** had fairly good microsomal stabilities (>80% of parent compounds were remained after incubation for 1 h in rat and rabbit liver microsomes).

Encouraged by these results, both compounds (as hydrochloride salts) were evaluated in conscious rabbit model as described earlier[17] (Fig. 2). Both compounds demonstrated equal efficacy compared to tadalafil (3 mg/kg) when dosed orally and this effect was shown to be dose-dependent (3 and 10 mg/kg) for both **25** and **28**. No penile erection was observed in vehicle-treated animals, while the erectogenic effect was potentiated by SNP injection (0.1 mg/kg), a nitric oxide donor that was used as a sexual stimulant. When dosed alone at 3 mg/kg, negligible erection was observed in both analogs; however, a significant penile erection was potentiated at 10 mg/kg even in the absence of SNP injection. It is interesting to note that Caco-2 permeability and protein binding were major predictors for oral in vivo efficacy of our quinazoline series as clearly demonstrated by **15** vs **25** and **28**.

In summary, starting from screening hit, we have identified a series of potent PDE5 inhibitors based on 4-benzylaminoquinazoline scaffold. By systematic variation of C6, C7, and C8 positions of quinazoline scaffold through unique and efficient chemistry, we were able to obtain potent and highly selective analogs against PDE6 and PDE11. Initial lead compound (**15**), albeit potent in vitro lacks in vivo efficacy in conscious rabbit model when dosed orally, which was ascribed to low membrane permeability. When polar hydroxyethyl group was incorporated at C8 (**25** and **28**), physicochemical properties such as Caco-2 permeability and protein binding were substantially improved. Compound **28** is not only more potent (10-fold) than tadalafil and sildenafil for PDE5, but highly selective against many PDE isozymes (selectivity ratios for PDE6 > 470, PDE11 > 8600, PDE1 > 2000, PDE2 > 8600, and PDE3 > 20,000). Moreover, both **25** and **28**, were orally effective in conscious rabbit model, demonstrating equal efficacy compared to tadalafil. Thus, because of its improved PDE isozyme selectivity profile compared with sildenafil and tadalafil, compounds **25** and **28** might be expected have fewer side effects if used for the treatment of MED.

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16. Selected data for **15**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.53 (s, 1H), 8.47 (s, 1H), 8.17 (m, 1H), 7.48 (d, J = 2.1 Hz, 1H), 7.37 (dd, J = 8.5, 2.1 Hz, 1H), 7.05 (d, J = 8.5 Hz, 1H), 4.83 (m, 2H), 3.90 (s, 3H), 3.86 (s, 3H), 3.10 (m, 2H), 1.68 (m, 2H), 0.98 (t, J = 3.8 Hz, 3H); MS (ESI) m/z 483 (M+H). For **25**: ^{1}H NMR (400 MHz, CD_3OD) δ 8.51 (s, 1H), 8.43 (s, 1H), 7.40 (d, J = 2.1 Hz, 1H), 7.10 (dd, J = 8.5, 2.1 Hz, 1H), 7.01 (d, J = 8.5 Hz, 1H), 4.75 (s, 2H), 3.88–3.82 (m, 8H), 3.36 (m, 2H), 2.27 (s, 3H); MS (ESI) m/z 431 (M + H). For **28**: ^{1}H NMR (400 MHz, CD_3OD) δ 8.50 (s, 1H), 8.41 (s, 1H), 7.38 (d, J = 2.0 Hz, 1H), 7.28 (dd, J = 8.5, 2.0 Hz, 1H), 6.98 (d, J = 8.5 Hz, 1H), 4.73 (s, 2H), 3.89–3.81 (m, 8H), 3.35 (t, J = 6.9 Hz, 2H), 2.54 (q, J = 7.6 Hz, 2H), 1.25 (t, J = 7.6 Hz, 3H); MS (ESI) m/z 445 (M+H).
17. (a) Bischoff, E.; Niewoehner, U.; Haning, H.; Es Sayed, M.; Schenke, T.; Schlemmer, K. H. *J. Urol.* **2001**, *165*, 1315; b Kang, K. K.; Ahn, G. J.; Ahn, B. O.; Yoo, M.; Kim, W. B. *Eur. Urol.* **2003**, *43*, 689. In brief, New Zealand white rabbits weighing 3–4 kg (n = 4) were orally dosed (dissolved in distilled water) with compounds, followed by injection of SNP (0.1 mg/0.1 ml/kg, dissolved in saline) after 60 min, and the length of penis (covered + mucosa area) was measured with sliding calipers, recorded in cm for total 2 or 2.5 h after administration of test compounds.
18. Enzyme sources: PDE1, bovine heart; PDE2, bovine heart; PDE3, bovine platelet; PDE5, bovine platelet; PDE6, bovine retina; and PDE11, human recombinant.
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