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Discovery of potent, selective, and orally bioavailable PDE5 inhibitor: Methyl-4-(3-chloro-4-methoxybenzylamino)-8-(2-hydroxyethyl)-7-methoxyquinazolin-6-ylmethylcarbamate (CKD 533)

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ABSTRACT

In a continuing effort to discover novel PDE5 inhibitors, we have successfully found quinazolines with 4-benzylamino substitution as potent and selective PDE5 inhibitors. Initial lead compound (**1**) was found to be easily metabolized when incubated with human liver microsomes mainly through C6 amide hydrolysis. Blocking of this metabolic hot spot led to discovery of **10** (CKD533) which is highly potent, selective and orally efficacious in conscious rabbit model for erectile dysfunction and now is undergoing preclinical toxicology study.

Male erectile dysfunction (MED), now assumed to affect >500 million in the world, was a largely unmet medical need before the introduction of sildenafil (Viagra®) in 1998. Although it was discovered serendipitously,[1] the launch of Viagra marked the new age in drug discovery by affecting one's quality-of-life. 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] PDE5 inhibition blocks cGMP degradation, thus increases the cGMP concentration, enhancing the erection.[3] To date, 11 families (PDE1–PDE11) of phosphodiesterase were identified, and PDE5 is abundant in smooth muscle, lung and platelets.[4]

Although successful commercially, sildenafil use is associated with many adverse effects such as headache, nausea, flushing, and visual disturbances, which are the result of low PDE1 and PDE6 (PDE6 is the sole cGMP PDE in rod and cone cells within the eye) selectivity,[5] and similar side effects (indigestion, back pain, etc.) were seen with vardenafil (Levitra®)[6] and Tadalafil (Cialis®).[7]

The physiological significance of PDE11 is not clearly understood, but several lines of evidence suggested the PDE11 inhibition might have negative effect in male reproduction and muscle tissue, since PDE11 is predominantly present in muscle, prostate and testes.[8] Since tadalafil cross reacts with PDE11 at sub μM range (merely 10-fold selectivity ratio relative to PDE5), there is a growing concern over PDE11 selectivity. So, it is highly desirable to maintain high PDE11 selectivity when developing PDE5 inhibitors. Thus, there have been numerous efforts toward the discovery of more isozyme selective PDE5 inhibitors,[9] which is of great medicinal and commercial interest, although with limited success.

In this Letter, we detail our continuing efforts in PDE5 inhibitor, which have yielded a very potent inhibitor with IC_{50} value of picomolar range and highly selective against other PDE isozymes as well as orally efficacious.

Previously, we reported the discovery of 6,7,8-substituted quinazolines as potent and selective PDE5 inhibitors with in vivo efficacy.[10] Optimization of PDE5 activity, isozyme selectivity and physicochemical properties led us to identification of **1** (Fig. 1) as a potent and selective PDE5 inhibitor (PDE5 IC_{50} = 1 nM, selectivity ratio for PDE6 >470, and PDE11 >8600). Compound **1** demonstrated excellent efficacy in conscious rabbit model when dosed

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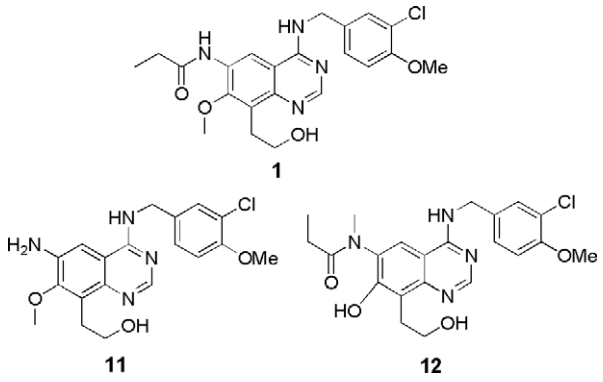
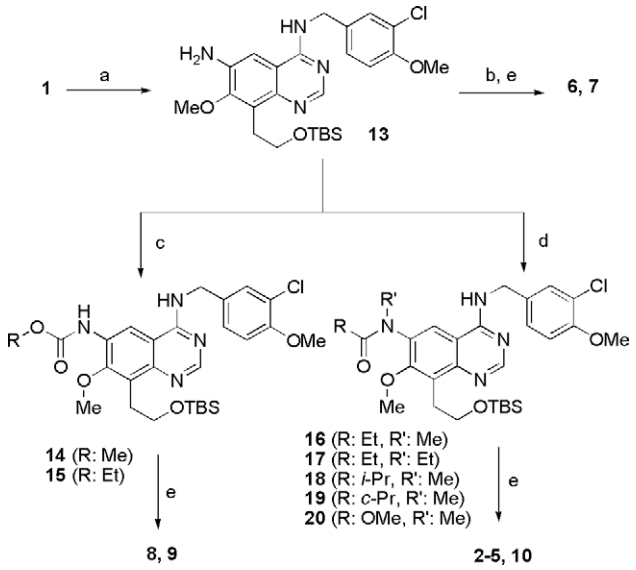


Figure 1. Structures of **1** and metabolites (**11**, **12**).

orally. However, disappointingly it was later found to be highly unstable with just 3% of parent compound left when incubated in human liver microsomes (HLM).[11] This was in sharp contrast to the results from rat and rabbit liver microsomes where 96% and 85% of **1** remained, respectively. A LC/MS analysis of incubation mixture with human liver microsomes (1.25 mg/ml) revealed that **1** was rapidly converted to **11** (ca. 90% of all metabolites determined from peak areas), indicating amide hydrolysis was a major culprit of instability (Fig. 1). Interestingly, negligible amount of **11** was detected from incubation mixtures with rat and rabbit liver microsomes, and this may be attributed to different type and/or content of proteases across species.[12a] It was clear that more stable compound might be obtained if the rapid amide hydrolysis at C6 in **1** were blocked. To this end, several analogs that have a variation at C6 position of **1** were prepared according to Scheme 1.

We utilized the already synthesized compound **1**[10] to facilitate synthesis, that is, compound **1** was hydrolyzed under acidic condition followed by TBS protection to provide common intermediate **13**. Compounds **6** and **7** were prepared by reaction of appropriate sulfonyl chloride or sufamoyl chloride followed by acidic desilylation. Alternatively, secondary carbamate was installed by reaction of methyl- and ethyl chloroformate to provide **8** and **9**. The suitable

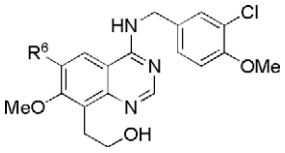


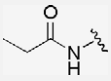
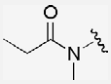
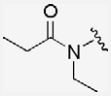
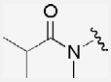
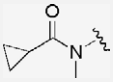
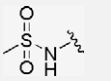
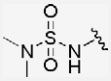
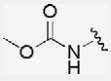
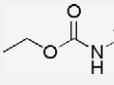
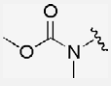
Scheme 1. Reagents and conditions: (a) (i) 2 M HCl, MeOH, reflux, 15 h; (ii) imidazole, TBSCl, DCM, rt, 2 h; (b) MsCl (for **6**) or dimethyl sulfamoyl chloride (for **7**), pyridine, DCM, 0 °C to rt; (c) $ClCO_2Me$ or $ClCO_2Et$, pyridine, DCM, rt, 2 h; (d) (i) amide formation or $ClCO_2Me$, rt, 2 h; (ii) MeI or EtI, NaH, THF, rt, 6 h; (e) 1 M HCl, 1,4-dioxane, rt, 1 h.

amides prepared from **13** were further manipulated to provide tertiary amides (NaH, MeI or EtI, THF), which afforded compounds **2**–**5** after desilylation. Finally, N-methylation of **14** followed by desilylation afforded tertiary carbamate **10**.[13] In the case of **10**, another efficient procedure was developed in which up to 1 Kg of **10** was synthesized for preliminary toxicology study, and its synthesis will be reported elsewhere.

Initially, it was assumed that incorporation of tertiary amide would render **1** more resistant toward hydrolysis by various amidases in HLM. As shown in Table 1, *N*-methyl analog (**2**) had com-

Table 1
PDE5 activity and isozyme selectivity of 4-(3-chloro-4-methoxy)-benzylamino-7-methoxy-8-hydroxyethyl quinazoline derivatives



Compound	R^6	PDE5[a]	PDE6[a]	PDE11[a]
1		0.001	0.46	10.5
2		0.001	0.32	5.2
3		0.001	ND	ND
4		0.003	1.1	ND
5		0.001	0.34	ND
6		0.011	ND	ND
7		0.012	7.95	ND
8		0.003	0.71	5.7
9		0.017	0.6	5.0
10		0.0006	0.14	12.0
Tadalafil		0.012	3.0	0.29
Sildenafil		0.01	0.14	3.0

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

parable PDE5 inhibitory activity[14] and PDE6, PDE11 selectivity with compound **1**. As anticipated, metabolic stability of **2** in HLM was enhanced (16% of **2** left after 1 h incubation) relative to **1** (3%) while metabolic stability in rat and rabbit liver microsomes remains excellent (>90%) as in the case of **1**.[15] It is interesting to note that while hydrolyzed metabolite (**11**) was not detected, a large amount of C7 de-methylated metabolite (**12**) was formed in liver microsomes from all three species (Fig. 1). Although PDE5 inhibitory activities and isozyme selectivity of *N*-ethyl analog (**3**) and *N*-methyl analog with different acyl groups (**4**, **5**) were retained, metabolic stability in HLM (5.5%, 4.8%, and 25% remained, respectively) had not increased dramatically compared with **2**. The effect of other functional groups was explored in **6** and **7** and there was a 10-fold loss in PDE5 inhibition potency. Moreover, both compounds were found to be unstable in HLM presumably due to hydrolysis at C6 and/or de-methylation at C7, although metabolic study was not undertaken in this case. Low PDE5 inhibition potency combined with unfavorable physicochemical property such as high protein binding that is close to 100% precluded these analogs from further profiling.

Based on high PDE5 activity and isozyme selectivity together with preferable physicochemical properties such as good Caco-2 permeability[12b] (17×10^{-6} cm/sec) and moderate protein binding (95% vs 97% for sildenafil), excellent in vivo efficacy of **2** was expected. Indeed, **2** demonstrated equal efficacy relative to tadalafil at 3 mg/kg when dosed orally in conscious rabbit model[16] (data not shown). Although **2** is effective in rabbit model, we felt that metabolic stability in HLM was not satisfactory and might be a potential liability in further development in human clinical trial. Thus, we sought further modification to enhance metabolic stability, by incorporating functional group that may be highly resistant to hydrolysis from various proteases in human plasma. In this regard, several carbamate analogs were prepared (**8**–**10**) since various carbamate containing drugs are being used successfully.[17]

Gratifyingly, the simplest analog (**8**) was found to have potent PDE5 inhibitory activity while isozyme selectivity remained excellent. There was a slight loss of PDE5 inhibitory activity with ethyl carbamate (**9**) with isozyme selectivity remained at the same level as **8**. A tertiary carbamate (**10**) which was prepared to further increase metabolic stability was found to have highly potent PDE5 inhibitory activity in pM range. Compound **10** is not only 20-fold more potent than tadalafil and sildenafil, it is significantly more selective against other PDE isozymes as indicated in Table 1 (selectivity ratio for PDE6 >230, and PDE11 >20,000). Compound **10** exhibited even greater selectivity against PDE1, PDE2, PDE3, and PDE4 (IC_{50} = 12 μM, 32 μM, 43 μM, and 84 μM, respectively).[18] Most importantly, when incubated in HLM (1.25 mg/ml) both **8** and **10** were highly stable with 72% and 85% of parent compound remained after 1 h incubation, indicating robustness of carbamate relative to amide series against various proteases present in liver microsomes.

To understand the underlying mechanism of the high selectivity toward PDE11, the docking modes of **10** and tadalafil were compared in the active sites of PDE5 and PDE11 (Fig. 2). HOMOLOGY and AFFINITY modules of INSIGHT2000 were used for the modeling and docking studies.[19] Overall, the compositions of amino acids in the active sites are highly conserved except that Phe820 in PDE5 was replaced with Trp298 in PDE11. Tadalafil makes favorable π–π stacking interaction with Trp298 in PDE11 and with Phe820 in PDE5, which explains high affinity toward both PDE5 and PDE11. In contrast, **10** snugly fits into the active site of PDE5, with favorable interaction with Phe820, however, Trp298 in PDE11 kept **10** from tightly binding due to the steric hindrance with C8 hydroxyethyl group, thus illustrating very low affinity.

Tertiary carbamate **10** possessed preferable physicochemical properties such as good Caco-2 permeability (35×10^{-6} cm/sec),

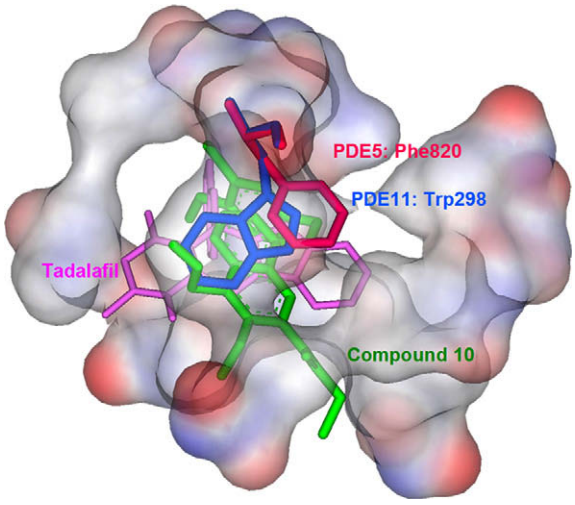


Figure 2. Docking model of **10** and tadalafil in PDE5 and PDE11 active site.

moderate protein binding (same as sildenafil with 97%) and high aqueous solubility (>2 mg/ml as methanesulfonate salt). Moreover, **10** was found to have negligible cell cytotoxicity (CC_{50} = 85 μM) in L-5178Y (murine leukemia) cells and did not block hERG channel (<20% inhibition at 10 μM). Oral exposure study in rat and rabbit was carried out to estimate intestinal absorption and in vivo efficacy (Table 2). In rat, **10** (as HCl salt) was absorbed very rapidly with T_{max} of 0.2 h, and its moderate half-life (2.2 h) would be suitable for once-a day dosing in human. In contrast, a more sustained exposure was achieved in rabbit with longer half-life >4 h.

In vivo efficacy of **10** in inducing penile erection using conscious rabbit was then evaluated (Fig. 3).[16] 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

Table 2
Selected PK Parameters for compound **10** after oral dosing[a]

Species[a]	T_{max} (h)	C_{max} (ng/ml)	$t_{1/2}$ (h)	AUC_{last} (ng h/ml)
Rat	0.2 ± 0.08	420 ± 85	2.25 ± 1.8	540 ± 146
Rabbit	2.4 ± 0.55	870 ± 490	4.2 ± 3.2	2760 ± 1540

[a] Dose: 10 mg/kg (0.5% Tween 80), n = 4.

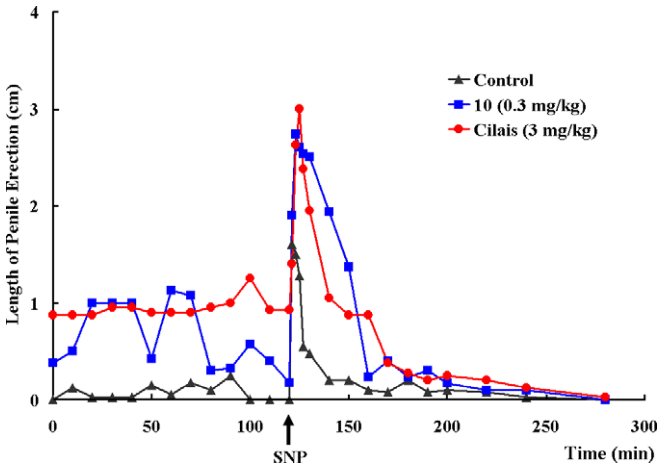


Figure 3. Effect of **10** on the length of penis in conscious rabbit model. The length of penis was measured after oral administration of **10**, followed by a sodium nitroprusside (SNP, 0.1 mg/kg) injection after 2 h (n = 4). See Ref. 16 for details.

that is used as a sexual stimulant. Compound **10** demonstrated equal efficacy at a dose (0.3 mg/kg) of one-tenth of tadalafil (3 mg/kg) when dosed orally, which is in agreement with potent PDE5 inhibitory activity. Compound **10** was advanced into preclinical safety studies from these encouraging results together with favorable physicochemical and pharmacokinetic profiles across species (rat and rabbit).

In conclusion, we have discovered a potent and selective PDE5 inhibitor possessing novel quinazoline scaffold. An analysis of previously identified potent PDE5 inhibitor (**1**) posed a metabolic instability issue in HLM, mainly through C6 amide hydrolysis. This was circumvented by switching to tertiary carbamate, resulting in substantial increase in metabolic stability and ultimately led to identification of **10**. Compound **10** is not only more potent (20-fold) PDE5 inhibitor than tadalafil, but highly selective against other isozymes including PDE6 and PDE11. Based on favorable profiles of this molecule, **10** (CKD533) was selected for preclinical safety evaluations as a potential candidate for the treatment of male erectile dysfunction.

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13. *Selected data for* **2**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.63 (t, J = 5.9 Hz, 1H), 8.49 (s, 1H), 8.17 (s, 1H), 7.41 (s, 1H), 7.29 (d, J = 8.5 Hz, 1H), 7.08 (d, J = 8.5 Hz, 1H), 4.74 (br, 1H), 4.60–4.69 (m, 2H), 3.80 (s, 3H), 3.74 (s, 3H), 3.59 (br, 2H), 3.20–3.30 (m, 2H), 3.19 (s, 3H), 1.97–2.12 (m, 2H), 0.91 (t, J = 7.3 Hz, 3H); MS (ESI) m/z 459 (M^++1). For **8**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.09 (s, 1H), 8.65 (t, J = 5.8 Hz, 1H), 8.41 (s, 1H), 8.30 (s, 1H), 7.39 (d, J = 2.0 Hz, 1H), 7.28 (dd, J = 6.4, 2.0 Hz, 1H), 7.06 (d, J = 8.5 Hz, 1H), 4.79 (br, 1H), 4.65 (d, J = 5.8 Hz, 2H), 3.79 (s, 3H), 3.76 (s, 3H), 3.70 (s, 3H), 3.59 (br, 2H), 3.20 (t, J = 7.7 Hz, 2H); MS (ESI) m/z 447 (M^++1). For **10**: ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.64 (t, J = 4.8 Hz, 1H), 8.47 (s, 1H), 8.11 (s, 1H), 7.41 (d, J = 1.9 Hz, 1H), 7.29 (dd, J = 8.5, 1.9 Hz, 1H), 7.06 (d, J = 8.8 Hz, 1H), 4.80 (t, J = 7.8 Hz, 1H), 4.65 (d, J = 5.9 Hz, 2H), 3.80 (s, 3H), 3.75 (s, 3H), 3.56–3.59 (m, 5H), 3.22 (m, 2H), 3.19 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.9, 156.1, 155.3, 153.9, 148.9, 133.0, 129.7, 129.4, 127.9, 122.1, 121.2, 113.0, 111.8, 61.6, 61.1, 56.4, 53.2, 43.1, 37.9, 29.2; MS (ESI) m/z 461 (M^++1); Anal. Calcd for $C_{22}H_{25}ClN_4O_5$: C, 57.33; H, 5.47; N, 12.16. Found: C, 57.29; H, 5,43; N, 12.36.
14. PDE5 assays were carried out as described in: Turko, I. V.; Ballard, S. A.; Francis, S. H.; Corbin, J. D. *Mol. Pharmacol.* **1999**, *56*, 124. assays for other isozymes were conducted in a similar manner.
15. Human, rat, and rabbit liver microsomes were purchased from BD Sciences, Inc. To a mixture of compound (5 μM) and liver microsomes in phosphate buffer (pH 7.4) was added a solution of NADPH-regenerating system (2.5 mM $MgCl_2$, 2.5 mM glucose-6-phosphate, 1 mM NADP, 1 U/ml glucose-6-phosphate dehydrogenase) and incubated at 37 °C for 1 h. Samples were withdrawn, reaction terminated by adding acetonitrile, then extracted and analyzed by LC/MS.
16. (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 2 h, and the length of penis (covered + mucosa area) was measured with sliding calipers, recorded in cm for total 2 h after administration of test compounds.
17. Ray, S.; Pathak, S. R.; Chaturvedi, D. *Drugs Future* **2005**, *30*, 161.
18. Enzyme sources: PDE1: bovine heart, PDE2: bovine heart, PDE3: bovine platelet, PDE4: human recombinant, PDE5: bovine platelet, PDE6: bovine retina, and PDE11: human recombinant.
19. INSIGHT 2000, Accelrys Inc., San Diego, CA.