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1,7- and 2,7-Naphthyridine Derivatives as Potent and Highly Specific PDE5 Inhibitors

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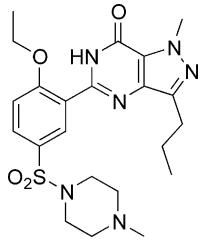
Abstract—Novel 1,7- and 2,7-naphthyridine derivatives, designed by the introduction of nitrogen atom into the phenyl ring of previously reported 4-aryl-1-isoquinolinone derivatives, were disclosed as a new structural class of potent and specific PDE5 inhibitors. Among them, 2,7-naphthyridine **4c** showed potent PDE5 inhibition ($IC_{50} = 0.23$ nM) and one of the best PDE5 specificities against PDEs1–4,6 (>100,000-fold selective versus PDE1–4, 240-fold selective vs PDE6). This compound showed more potent relaxant effects on isolated rabbit corpus cavernosum ($EC_{30} = 5.0$ nM) than Sildenafil ($EC_{30} = 8.7$ nM). The compound **4c** (T-0156) was selected for further biological and pharmacological evaluation of erectile dysfunction.

Introduction

Cyclic nucleotide phosphodiesterases (PDEs) regulate physiological processes by the hydrolysis of intracellular second messengers, cyclic guanosine monophosphate (cGMP) and cyclic adenosine monophosphate (cAMP) to their corresponding 5′-nucleoside monophosphates. PDEs have been classified into at least 11 identified families to date,[1] based on their amino acid sequences, substrate specificities, endogeneous and exogeneous regulators, and pharmacological properties. PDE5, cGMP-binding cGMP-specific PDE, is the primary enzyme that responsible tor the degradation of cGMP in human corpus cavernosum, and inhibition of this enzyme cause penile erection via relaxation of the vascular smooth muscle and corpus cavernosum tissue.[2,3]

Sildenafil (**1**, viagra®) is a first efficacious and orally active PDE5 inhibitor, which is used for the treatment of male erectile dysfunction.[4] Despite its effectiveness, **1** shows clinically significant adverse effects such as headache, nausea, cutaneous flushing, and visual disturbances.[5] It has been postulated that some of these side effects may be ascribed to the modest selectivities toward the other PDE isoforms, notably PDE1 and PDE6. Thus the discovery of new PDE5 inhibitors with improved selectivities against these PDE isoforms and greater potency for PDE5 inhibition have recently attracted considerable interest.[6]

We have reported a series of 1-isoquinolinone derivatives as a new structural class of PDE5 inhibitors, and selected compound **2** (T-1032) for further biological and pharmacological evaluation.[7]



1 (Sildenafil)

In order to obtain more potent and specific PDE5 inhibitors with better pharmacokinetic properties as backup compounds, we have done further synthetic studies on compounds structurally relating to **2**. In previous paper,[7] we expected that isoquinolinone ring in **2** mimicked the purine nucleus of cGMP and that the pendant aryl group at the 4-position of **2** filled a space occupied by the cyclic

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phosphate group of cGMP. Beavo et al. reported that N7-nitrogen of cGMP pointed out by an arrow in Figure 1 contributes to binding at the catalytic site of PDE5 as a hydrogen acceptor.[8] Thus, we envisioned that the additional introduction of nitrogen atom to a suitable position of isoquinolinone ring for the favorable interaction with PDE5 might enhance PDE5 inhibition and improve selectivities over the other PDE isoforms. Futhermore, we expected that this modification might lead to improvement of PK profiles of compounds based on lowering the log *P* value (clog *P* value: **2**, 3.611; **3a**, 2.652).[9]

This paper describes the synthesis and structure–activity relationships (SARs) of 1,7- and 2,7-naphthyridine derivatives (**3** and **4**) obtained by the introduction of nitrogen atom into the phenyl ring of isoquinolinone in **2**.

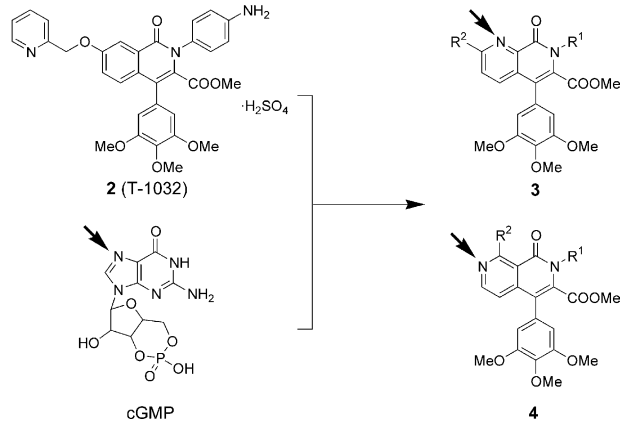
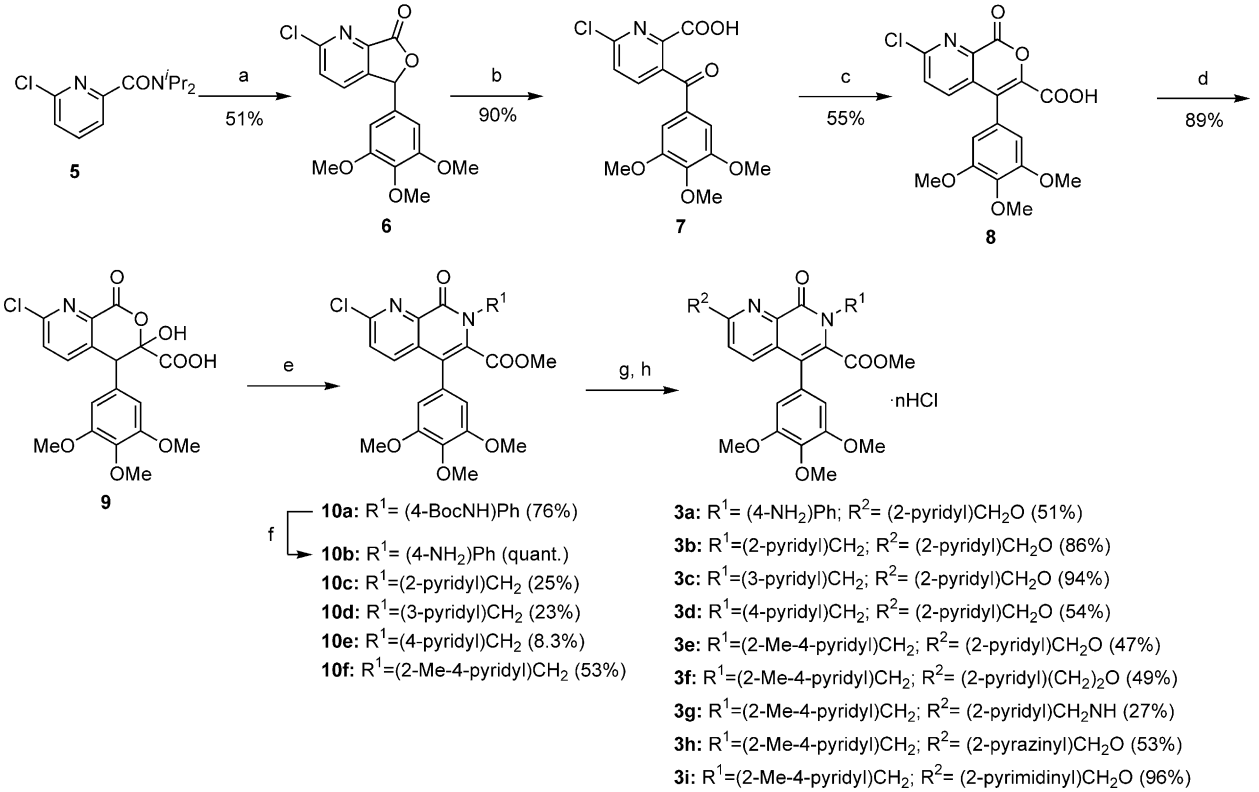


Figure 1.

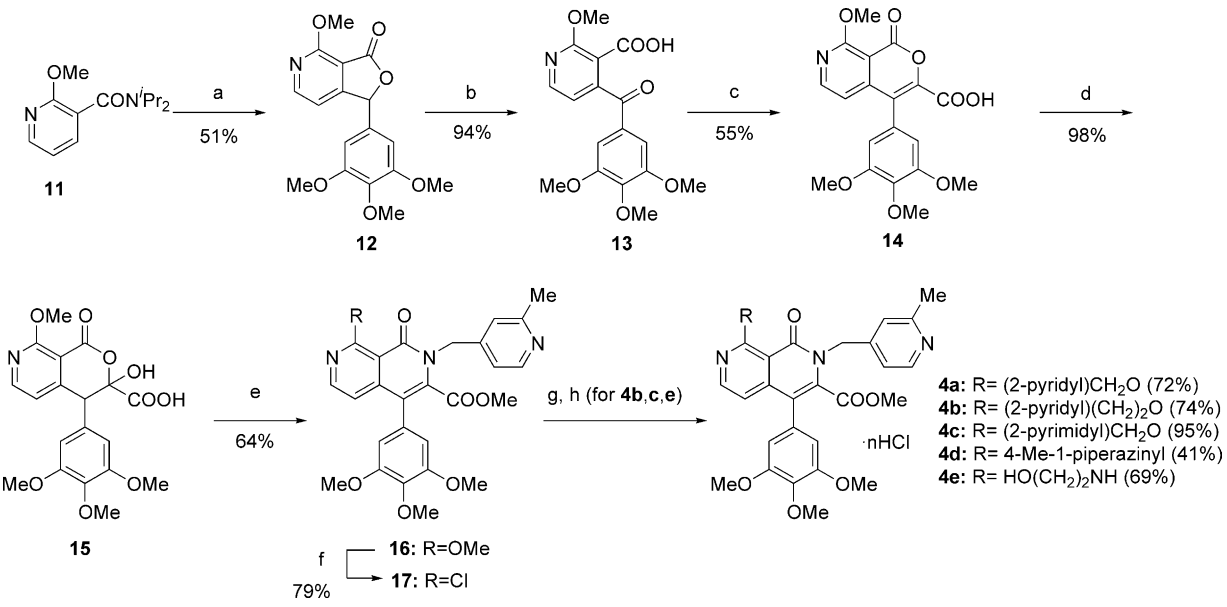
Chemistry

The synthetic methods of 1,7-naphthyridine derivatives **3** are outlined in Scheme 1. Lithiation of pyridine-2-carboxamide **5** with *n*-BuLi followed by the trapping with 3,4,5-trimethoxybenzaldehyde, and subsequent cyclization under acidic condition gave **6** in 51% yield. The oxidation of **6** with $KMnO_4$ in aqueous KOH afforded keto acid **7** in a good yield. Alkylation of **7** with di-*tert*-butyl bromomalonate followed by the DBU-mediated cyclization, treatment with HCl/AcOEt solution, and the heating in dioxane gave a key intermediate **8** in 55% yield. The reaction of **9**, which is obtained by the hydrolysis of **8**, with amines followed by the esterification afforded 1,7-naphthyridine-3-carboxylate derivatives **10**. The nucleophilic substitution reactions of **10** with appropriate alkoxides or amine gave **3a–i**.

The syntheses of 2,7-naphthyridines **4** are summarized in Scheme 2. 8-Methoxy-2,7-naphthyridine **16** was prepared from *N,N*-diisopropyl-2-methoxynicotinamide **11** according to the analogous procedures for **10** as described above. The treatment of **16** with $POCl_3$ afforded 8-chloro compound **17** in 79% yield. The addition-elimination reaction of **17** with various types of nucleophiles such as alkoxides or amines gave **4a–e**.



Scheme 1. Reagents and conditions: (a) (1) *n*-BuLi, 3,4,5-trimethoxybenzaldehyde, THF, (2) concd HCl-dioxane; (b) $KMnO_4$, pyridine, 2N NaOHaq; (c) (1) $BrCH(COOt\text{-}Bu)_2$, $KHCO_3$, DMF, (2) DBU, toluene, (3) 4N HCl/AcOEt, (4) dioxane, reflux; (d) 2N NaOHaq, DMF; (e) (1) R^1NH_2, DMI or THF, (2) MeI, K_2CO_3, DMF or $TMSCH_2N_2$, MeOH, THF; (f) 4N HCl/AcOEt–$CHCl_3$; (g) For **3a–f,h,i**: R^2H, NaH, THF or DMF. For **3g**: R^2H, DMF; (h) 4N HCl/AcOEt–$CHCl_3$.



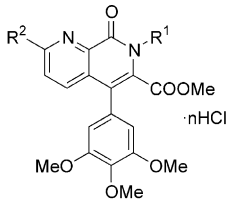
Scheme 2. Reagents and conditions: (a) (1) *n*-BuLi, TMEDA, 3,4,5-trimethoxybenzaldehyde, THF, (2) AcOH-dioxane; (b) $KMnO_4$, KOHaq; (c) (1) $BrCH(COOt\text{-}Bu)_2$, $KHCO_3$, DMF, (2) DBU, toluene, (3) AcOH, reflux; (d) 2N NaOHaq, MeOH; (e) (1) 2-methyl-4-picolylamine, THF, (2) MeOH, DEAD, PPh_3, CH_2Cl_2; (f) $POCl_3$, DMF, $CHCl_3$; (g) For **4a–c**: RH, NaH, THF or CH_2Cl_2. For **4d,e**: RH, dioxane; (h) 4N HCl/AcOEt–$CHCl_3$.

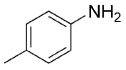
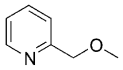
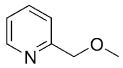
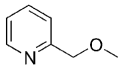
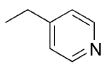
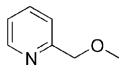
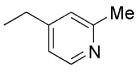
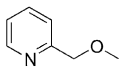
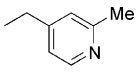
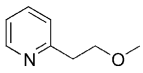
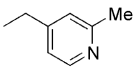
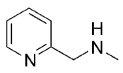
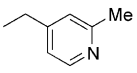
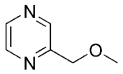
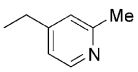
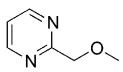
Biological Results and Discussion

The compounds reported in this paper were firstly evaluated for the inhibitory activities against the three different isoforms of PDEs isolated from canine lung (PDE1 and PDE5), and bovine retina (PDE6) shown in Tables 1 and 2. Compounds selected on the basis of the PDE5 inhibitory activities were next evaluated the relaxant effects on rabbit corpus cavernosum, and investigated for the three additional different isoforms of PDEs isolated from bovine adrenal gland (PDE2), canine heart (PDE3), and canine lung (PDE4) shown in Table 3.

Table 1 summarizes the SARs of 1,7-naphthyridine derivatives **3a–i**. In comparison to the isoquinolinone, T-1032 (**2**, Table 3), 1,7-naphthyridine **3a** showed increased PDE5 inhibitory activity and improved selectivity over PDE1. As a result of the modification of the substituent at the 7-position, we found that 7-picolyloxy derivatives **3b–d** exhibited potent PDE5 inhibitory activities. The comparison of compounds **3b–d** showed that the order of potencies toward PDE5 was 4-picolyl (**3d**) > 3-picolyl (**3c**) > 2-picolyl (**3b**). These results indicate that the spatial disposition of basic nitrogen atom at the 7-position might be important to exert high PDE5 inhibition. Introduction of methyl group in the ortho position to the pyridine nitrogen of picolyl group of **3d**, which was expected to reduce the inhibitory potency toward P450,[10] did not affect PDE5 inhibitory activity (**3e**, $IC_{50} = 0.22$ nM). Next we examined the effect of substituents at the 2-position of **3**. One carbon elongation of the 2-picolyloxy group of **3e** resulted in the loss of activity (**3f**, $IC_{50} = 1.2$ nM). The replacement etheral oxygen of **3e** at the 2-position with N–H group resulted in the decrease of activity (**3g**, $IC_{50} = 1.8$ nM). 2-Pyrazinyloxy compound **3h** exhibited almost the same potency as **3e**, although 2-pyrimidinyloxy compound **3i** had lower activity than **3e** (**3h**, $IC_{50} = 0.29$ nM; **3i**, $IC_{50} = 1.9$ nM).

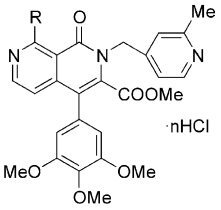
Table 1. Structures and PDE inhibitions of 1,7-naphthyridine derivatives

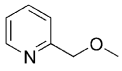
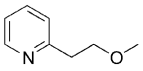
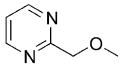
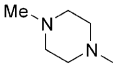
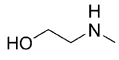


Compd	R^1	R^2	*n*	PDE inhibition, IC_{50}, nM[a]		
				PDE1	PDE5	PDE6
3a			2	40,000	0.51	22
3b			2	20,000	11	230
3c			2	12,000	2.3	38
3d			2	45,000	0.38	11
3e			2	33,000	0.22	8.6
3f			2	nd[b]	1.2	nd[b]
3g			2	41,000	1.8	22
3h			1	22,000	0.29	7.7
3i			1	> 100,000	1.9	42

[a] IC_{50} values were determined from the logarithmic concentration–inhibition curve (at least four points). The value is given as the mean of at least two duplicate experiments.
[b] nd = not determined.

Table 2. Structures and PDE inhibitions of 2,7-naphthyridine derivatives



Compd	R	*n*	PDE inhibition, IC_{50}, nM[a]		
			PDE1	PDE5	PDE6
4a		0	25,000	0.31	4.4
4b		2	20,000	0.52	72
4c		1	>100,000	0.23	56
4d		0	>100,000	94	1400
4e		2	19,000	2.6	280

[a]IC_{50} values were determined from the logarithmic concentration–inhibition curve (at least four points). The value is given as the mean of at least two duplicate experiments.

As shown in Table 2, 2,7-naphthyridine **4a** exhibited almost the same PDE5 inhibitory activity and isozyme selectivities over PDE1 and PDE6 as 1,7-naphthyridine **3e**. One carbon elongation of the substituent at the 8-position improved the selectivity over PDE6 without decreasing PDE5 potency (**4b**: IC_{50} = 0.52 nM, PDE6/PDE5 = 140), in contrast to 1,7-naphthyridine **3f**. These results suggest that appropriate modification of 2,7-naphthyridines at the 8-position may be expected to lead more selective compounds with high potency. Among the compounds we synthesized, 8-pyrimidinyloxy compound **4c** displayed enhanced PDE5 potency (IC_{50} = 0.23 nM) and improved selectivities over PDE1 and PDE6 (PDE5/PDE1 >100,000, PDE5/PDE6 = 240). In comparison with the corresponding 1,7-naphthyridine **3i** (IC_{50} = 1.9 nM, PDE5/PDE6 = 22), 2,7-naphthyridine **4c** showed more potent PDE5 inhibitory activity and improved PDE5/PDE6 selectivity. These results may imply that the location of the hydrogen bond acceptor atoms (nitrogen and carbonyl oxygen) of 2,7-naphthyridine **4** is more suitable than that of 1,7-naphthyridine **3** for the favorable interaction with PDE5. Althouth the introduction of secondary amine (**4d**) resulted in marked loss of PDE activities, incorporation of primary amine (**4e**) maintained the modest PDE5 inhibition.

Next we selected six compounds (**3e**,**i** and **4b**,**c**,**e**) on the basis of PDE5 potencies and isozyme selectivities over PDE1 and PDE6 for further evaluation of three additional PDEs (PDE2, PDE3, and PDE4) inhibitory activities and relaxant effects on isolated rabbit corpus cavernosum, as shown in Table 3. These compounds showed modest to good selectivities for PDE5 against PDEs1-4. It should be noted that PDE5 selectivities of 2,7-naphthyridine **4b**,**c**,**e** versus PDE6 have been greatly enhanced (IC_{50} ratio >100), since the visual disturbances associated with Sildenafil **1** are believed to be the results of its low selectivity over PDE6 (IC_{50} ratio≈8). To our knowledge, compound **4c** is one of the most potent and specific PDE5 inhibitors disclosed to date. These compounds also possessed the relaxant effects on rabbit corpus cavernosum, and compound **4c** showed better efficacy than **1** (**4c**, EC_{30} = 5.0 nM ; **1**, EC_{30} = 8.7 nM).

Conclusion

Novel 1,7- and 2,7-naphthyridine derivatives, designed by the introduction of nitrogen atom into the phenyl ring of previously reported 4-aryl-1-isoquinolinone derivatives, were disclosed as a new structural class of potent and specific PDE5 inhibitors. Among them, 2,7-naphthyridine **4c** was apparently more potent (IC_{50} = 0.23 nM) and specific (>100,000-fold selective vs PDE1-4, 240-fold selective vs PDE6) than Sildenafil

Table 3. PDE Inhibitions and relaxant effects on isolated rabbit corpus cavernosun

Compd	PDE inhibition, IC_{50}, nM[a]						Relaxant effect
	PDE1	PDE2	PDE3	PDE4	PDE5	PDE6	EC_{30}, nM[b]
3e	33,000	40,000	>100,000	30,000	0.22	8.6	53
3i	>100,000	25,000	>100,000	19,000	1.9	42	37
4b	20,000	>100,000	>100,000	50,000	0.52	72	22
4c	>100,000	>100,000	>100,000	67,000	0.23	56	5.0
4e	19,000	78,000	5300	450	2.6	280	14
1 (Sildenafil)	270	43,000	>100,000	11,000	3.6	29	8.7
2 (T-1032)	3000	9700	>100,000	3300	1.0	28	7.9

[a]IC_{50} values were determined from the logarithmic concentration–inhibition curve (at least four points). The value is given as the mean of at least two duplicate experiments.
[b]EC_{30} values were determined from the logarithmic concentration–inhibition curve. The value is given as the average of at least two experiments.

1. **4c** also showed more potent relaxant effects on isolated rabbit corpus cavernosum (**4c**, $EC_{30} = 5.0$ nM; **1**, $EC_{30} = 8.7$ nM) than **1**. **4c** (T-0156) was selected for further biological and pharmacological evaluation of erectile dysfunction.

References and Notes

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