The discovery of avanafil for the treatment of erectile dysfunction: A novel pyrimidine-5-carboxamide derivative as a potent and highly selective phosphodiesterase 5 inhibitor

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Several orally available phosphodiesterase 5 (PDE5) inhibitors have been developed and are prescribed as first-line therapy for the treatment of erectile dysfunction (ED).\textsuperscript{1} Since PDEs consist of a superfamily with 11 subfamilies (PDE1–11) and each of them plays important roles in various tissues,\textsuperscript{2} it is necessary to develop a PDE5 selective inhibitor in order to avoid adverse effects resulting from inhibition of the other PDEs. For example, the first launched orally active PDE5 inhibitor, sildenafil showed high efficacy, but visual side effects such as cyanopsia and visual disturbance were reported in some cases.\textsuperscript{3,4} These adverse effects are indicated to be attributed to inhibition of PDE6, which is expressed in photoreceptor cells of the retina and plays important roles for the photo signal transmission.\textsuperscript{5}

In a previous paper, we reported findings of 5-(3,4,5-trimethoxybenzoyl)-4-aminopyrimidine derivatives as a novel chemical class of highly selective PDE5 inhibitors by scaffold hopping from isoquinoline derivatives (Fig. 1).\textsuperscript{6} Although T-6932 (1) showed a potent PDE5 inhibitory activity and an improved selectivity over PDE6, its relaxant effect on isolated rabbit corpus cavernosum was moderate (EC\textsubscript{30} = 53 nM) owing to its high lipophilicity (cLogP = 4.58). Therefore, the substituents at the 2- and 5-positions of the pyrimidine ring were explored to potentiate the relaxant effect while maintaining a high PDE5 selectivity against PDE6. In this paper, we report the discovery of the novel, potent, and highly selective PDE5 inhibitor avanafil 10j. The details of synthesis and structure–activity relationships are described.

Aiming to improve its high lipophilicity of T-6932, pyrimidine-5-carboxamide derivatives were designed. In the course of the synthesis described in Scheme 1, the synthetic intermediate, ethyl pyrimidine-5-carboxylate derivative 4 was found to show single digit nanomolar activity for PDE5 (IC\textsubscript{50} = 5.5 nM) and a slightly improved relaxant effect (EC\textsubscript{30} = 37 nM) compared to that of T-6932.\textsuperscript{7} These results indicate that the 3,4,5-trimethoxybenzene moiety, which caused the high lipophilicity of T-6932, was not crucial for PDE5 inhibitory activity. Therefore, pyrimidine-5-carboxylate or carboxamide derivatives bearing more hydrophilic substituents were designed and synthesized as in Scheme 1.

4-Aminopyrimidine derivative 3 was obtained by condensation of commercially available 2 with 3-chloro-4-methoxybenzylamine. After oxidation of the methylsulfanyl group with mCPBA, the 2-position of the pyrimidine ring was substituted with 2-hydroxypyridine in the presence of sodium hydride to give 4. In this reaction, 2-hydroxypyrimidine derivative 5 was obtained in 55%
yield. In addition to this, 2-pyridylmethyl ester 6a was obtained by concomitant ester exchange in 4% yield. Hydrolysis of the ester group of 4, and subsequent condensation with various alcohols or amines gave 6b–j. The 2- and 5-positions of the pyrimidine ring were modified according to the synthetic methods summarized in Scheme 2. Hydrolysis of the ester group of 3, and the following condensation with the corresponding amines afforded 9a–d, which were led to 10a–g, 10j, 10l, and 10m after oxidation of the methylsulfanyl group. Alternatively, 10h, 10i, 10k, 10n, and 10o were synthesized via the corresponding 2-amino-substituted pyrimidine-5-carboxlates 12a–d, which were prepared from 3 or 2-hydroxy pyrimidine derivative 5.

Our investigation toward the development of a novel and optimal PDE5 inhibitor for the treatment of ED included incorporation of the following characteristics: high selectivity for PDE5, appropriately short duration of action to improve tolerability, and faster onset of action. Short duration of action should be accomplished by introduction of metabolically unstable functional groups into the molecule. Faster onset of action should be accomplished by improvement of solubility. Potentiation of the relaxant effect should be accomplished by the reduction of the lipophilicity as mentioned above. In order to satisfy these requirements, we preferentially selected the substituents bearing heteroarylmethyl moieties, amines, and primary or secondary hydroxyl groups.

PDE5 inhibitory activity of the synthesized compounds were evaluated using the enzyme isolated from canine lung, and the light-activated bovine retina PDE6 was used for evaluation of PDE6 inhibitory activity in the process of compound screening. First, the influence of the substituents on the ester or amide group for PDE5 inhibitory activity and selectivity was investigated. As shown in Table 1, incorporation of hetero aromatic rings such as pyridine and pyrimidine into the ester moiety of 4 maintained PDE5 inhibitory activity with reduced lipophilicity (6a; IC50 = 2.1 nM, cLogP = 4.18, 6b; IC50 = 4.2 nM, cLogP = 3.22). The secondary amide derivative 6c was equipotent to the corresponding ester.
derivative 6a; however, the N-methylated analogue 6e showed a significant reduction of potency (6c, \( IC_{50} = 2.7 \text{ nM} \), 6e, \( IC_{50} = 85 \text{ nM} \)). Besides the aromatic rings, incorporation of various functional groups, such as ether, amine, and alcohol maintained PDE5 inhibitory activity, but a series of 2-(2-pyridylmethoxy)pyrimidine derivatives 6a-j showed a lowered selectivity over PDE6 compared to that of T-6932 (1, \( IC_{50} \) ratio: PDE6/PDE5 = 2400).

Next, the influence of the substituent at the 2-position of the pyrimidine ring was investigated. In the previous paper, we reported that the 5-(3,4,5-trimethoxybenzoyl)-pyrimidine derivatives showed excellent selectivity over PDE6, although these analogues had various functional groups in the amide moiety. The selectivity of 10f, 10j, 10m, and 10n was over 4000-fold, which was higher than that of T-6932.

The relaxant effects on isolated rabbit corpus cavernosum of these compounds were evaluated. Most of the tested compounds showed improved relaxant effects compared to that of T-6932 as summarized in Table 3. Among them, 10j (avanafil) showed the...
Table 1
PDE5 inhibitory activity and selectivity over PDE6 of pyrimidine-5-carboxylate and carboxamide derivatives

<table>
<thead>
<tr>
<th>Compound</th>
<th>-XR</th>
<th>IC_{50} (nM)^a</th>
<th>Selectivity (PDE6/5)</th>
<th>Compound</th>
<th>-XR</th>
<th>IC_{50} (nM)^a</th>
<th>Selectivity (PDE6/5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>O</td>
<td>2.1</td>
<td>1000</td>
<td>6f</td>
<td>N</td>
<td>15</td>
<td>240</td>
</tr>
<tr>
<td>6b</td>
<td>O</td>
<td>4.2</td>
<td>640</td>
<td>6g</td>
<td>N</td>
<td>5.6</td>
<td>630</td>
</tr>
<tr>
<td>6c</td>
<td>H</td>
<td>2.7</td>
<td>480</td>
<td>6h</td>
<td>OMe</td>
<td>13</td>
<td>420</td>
</tr>
<tr>
<td>6d</td>
<td>H</td>
<td>12</td>
<td>720</td>
<td>6i</td>
<td>OH</td>
<td>12</td>
<td>480</td>
</tr>
<tr>
<td>6e</td>
<td>N</td>
<td>85</td>
<td>NT</td>
<td>6j</td>
<td>N</td>
<td>3.5</td>
<td>510</td>
</tr>
</tbody>
</table>

^a IC_{50} values of these compounds were calculated by an equation of the first degree using two data; just above and below 50% inhibition. Some of IC_{50} values were calculated using Prism®, version 3.0.

Table 2
The influence of the substituent at the 2-position of the pyrimidine ring for PDE5 inhibitory activity

<table>
<thead>
<tr>
<th>Compound</th>
<th>-NR^2R^3</th>
<th>IC_{50} (nM)^a</th>
<th>Compound</th>
<th>-NR^2R^3</th>
<th>IC_{50} (nM)^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>10a</td>
<td>Me</td>
<td>32</td>
<td>10g</td>
<td>O</td>
<td>&gt;100</td>
</tr>
<tr>
<td>10b</td>
<td>O</td>
<td>32</td>
<td>10h</td>
<td>OH</td>
<td>63</td>
</tr>
<tr>
<td>10c</td>
<td>O</td>
<td>59</td>
<td>10i</td>
<td>OH</td>
<td>25</td>
</tr>
<tr>
<td>10d</td>
<td>OH</td>
<td>54</td>
<td>10j</td>
<td>OH</td>
<td>5.2</td>
</tr>
<tr>
<td>10e</td>
<td>OH</td>
<td>6.5</td>
<td>10k</td>
<td>OH</td>
<td>36</td>
</tr>
<tr>
<td>10f</td>
<td></td>
<td>1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

^a IC_{50} values of these compounds were calculated by an equation of the first degree using two data; just above and below 50% inhibition. Some of IC_{50} values were calculated using Prism®, version 3.0.
most potent relaxant effect with an EC$_{30}$ value of 2.1 nM and 4000-fold selectivity over PDE6. These results were superior to those of sildenafil (EC$_{30}$ = 8.7 nM, PDE6/PDE5 = 500); therefore, 10j was selected for further biological and pharmacological evaluation.

PDE5, PDE6, PDE2, PDE10, and PDE11 have two GAF domains in their N-terminal half and constitute the GAF-PDE family. The overall amino acid sequence similarity in the GAF family is high compared with that of PDE family without GAF domain; therefore, improvement of the selectivity against GAF-PDE family was expected to be a critical issue in developing ideal PDE5-specific inhibitors. Nevertheless, it is very interesting that avanafil showed excellent and well-balanced selectivity against all other phosphodiesterases (PDE1–11) compared to the other marketed PDE5 inhibitors. The selectivity ratio against trypsin-activated PDE6 of avanafil was 121-fold, which was higher than those of sildenafil (16-fold) and vardenafil (21-fold). Although tadalafil showed the highest selectivity against PDE5 (550-fold) among the tested compounds, the selectivity against PDE11 was moderate (25-fold). In contrast, avanafil showed superior selectivity for PDE11 (>19231-fold) to that of tadalafil, and also showed over 1000-fold selectivity ratio to that of tadalafil, and also showed over 1000-fold selectivity ratio against all other phosphodiesterases.

In the evaluation of potentiation effect on the pelvic nerve stimulation induced tumescence in anesthetized dogs, the times to peak response for avanafil and sildenafil after intraduodenal administration were 10 and 30 min, respectively. These results indicate that avanafil has more rapid onset of action than sildenafil. In addition, avanafil exhibited relatively short duration of action correlated with the plasma drug concentration.

These profiles, that is, fast onset of action and shorter plasma half-life than the other marketed PDE5 inhibitors were observed in clinical trials. Although the (S)-2-hydroxymethyl-pyrrolidin-1-yl group was introduced in the expectation of rapid metabolism of its hydroxyl group, actual major metabolites of avanafil in human plasma were found to be oxides of the pyrrolidine ring. Thus, the (S)-2-hydroxymethyl-pyrrolidin-1-yl group at the 2-position of the pyrimidine ring plays an important role for superior profiles of avanafil.

In conclusion, we successfully found avanafil (10j) as a novel chemical class of potent and highly selective PDE5 inhibitor for the treatment of ED. The modification to reduce the lipophilicity of T-6932 led to the discovery of avanafil (cLogP = 2.36). Avanafil showed improved relaxant effect on isolated rabbit corpus cavernosum with an EC$_{30}$ value of 2.1 nM, which was about 25 more potent than that of T-6932. The key structure of avanafil was the (S)-2-hydroxymethyl-pyrrolidon-1-yl group on the pyrimidine ring, which plays an important role for superior profiles, such as high selectivity over PDE6 and short duration of action. Avanafil showed a high selectivity against all other phosphodiesterases (PDE1–11) and faster onset of action in comparison to the other marketed PDE5 inhibitors. After clinical trials, avanafil was approved by the Korea Food and Drug Administration, the Food and Drug Administration (USA), and the European Medicine Agency in 2011, 2012, and 2013, respectively.

References and notes

7. The initial resting isomeric tension of each tissue strip was adjusted to 1.5 g by gradual incremental stretching in 10 mL of organ bath chambers containing physiological salt solution (PSS) at 37 ± 0.5 °C, continuously aerated with 95% O2 and 5% CO2. The contractile response to high-KCl (120 mM) PSS was checked twice. Phenylephrine (PE) (5 × 10^{-6} M) was added into each organ bath in order to obtain a tonic contraction. After the PE contractile response was stabilized, test compound (10^{-10}–10^{-6} M) or vehicle was added to the preparation at an interval of 30 min. Papaverine hydrochloride was added into each organ bath chamber (final concentration, 10^{-4} M) to confirm the maximal relaxation of the tissue strips at the end of experiment. The composition of PSS was as follows (mM): NaCl 118, KCl 4.7, MgSO4 1.2, CaCl2 1.5, KH2PO4 1.2, NaHCO3 25.0, dextrose 11.0, EDTA-2Na 0.023 (pH 7.3 or 7.4).

8. PDE assay was done by the radiolabeled nucleotide method. PDE enzyme (100 μl) in 50 mM Tris–HCl (pH 8.0) was added to 200 μl assay buffer composed of 50 mM Tris–HCl (pH 8.0), 12.5 mM MgCl2, 10 mM 2-mercaptoethanol and 0.825 mg/ml bovine serum albumin. The enzyme reaction was started by adding 200 μl substrate solution containing [3H]cGMP plus unlabeled cGMP or in 50 mM Tris–HCl (pH8.0). Reaction mixtures were incubated at 37 °C for 30 min and then boiled for 1.5 min. Subsequently 100 μl of 1 mg/ml solution of Crotalus atrox snake venom was added and incubated at 37 °C for 30 min. The reaction was stopped by adding 500 μl methanol. Resultant solutions were applied to a Dowex (1 × 8200–400) column (volume 0.225 ml). Eluate radioactivity was measured with 5 ml aqueous scintillation cocktails. DMSO solution without the compounds served as a control. The final concentration of DMSO in the reaction mixture was 1% volume per volume.


