Synthesis, Biological Evaluation of SPF-32629A-Based 2- and 4-Pyridone Analogs as Chymase Inhibitors

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Chymase (EC 3.4.21.39) is a chymotrypsin-like serine protease present in the secretory granules of mast cells, located mainly in the heart, blood vessels, and skin of many mammalian species. Its physiological substrate is angiotensin I, which upon cleavage by chymase yields angiotensin II in steps of the renin-angiotensin system. Therefore chymase is regarded as a potential therapeutic target for cardiovascular diseases as well as allergic/inflammatory diseases.

SPF-32629A (1) and B (2) were isolated from the cultured broth of Penicillium sp. SPF-32629 and have the inhibitory activities against human chymase with the IC₅₀ of 0.25 and 0.42 μg/mL, respectively, as selective inhibitors. They have a 4-hydroxy-2-pyridone skeleton on phenylmethanol (Figure 1). Mukkanti group reported first synthesis and structural determination of 1 and 2, however there were not the analogs developed for chymase inhibition so far. In this work, 2- and 4-pyridone analogs of SPF-32629A were synthesized via short and efficient routes and evaluated as chymase inhibitors in order to establish SAR such as the necessity of 4-hydroxy-2-pyridone and the effects on C4’ modification of phenyl group (a) with p-fluorophenyl (b), p-chlorophenyl (c), p-tolyl (d) and p-methoxyphenyl (e).

Our synthetic route for 2-pyridones is shown in Scheme 1. First, aryl(2-pyridinyl)methanols (3a-e) were synthesized by Grignard reagents addition to the starting material 6-methoxy-2-pyridinecarboxaldehyde. The treatment of 3 with TMSI afforded the 2-pyridones (4a-e), which were converted into the isovalerates (6a-e) along with bis-isovalerates (6a-e) under EDCI-mediated condition. The isovalerate (7a-3) was also prepared from 4 in a similar manner.

4-Pyridone analogs of SPF-32629A were synthesized from the known nitrile (8) (Scheme 2). The ketones (9a-e) were given by Grignard reagents addition to 8, followed by acid treatment. With 9 in hands, NaNH₄ reduction and subsequent hydrogenolysis of the alcohol (10a-e) afforded the 4-pyridones (11a-e). Finally, 11 were converted into the isovalerates (12a-e). The diesters (13a-e) were easily hydrolyzed to afford the corresponding 12.

The inhibitory effect on chymase of SPF-32629A analogs at 100 μM is shown in Figure 2. The IC₅₀ of selected analogs was tested as shown as Table 1. The introduction of 2-methoxyxypyrindine (3 and 7) and 4-benzyloxypyrindine (9 and 10) instead of 2- and 4-pyridone resulted in a loss of activity.

Scheme 1. Synthesis of 2-pyridone analogs (5a-5e) of SPF-32629A (a for R=H, b for F, c for Cl, d for CH₃, e for OCH₃). Reagents and conditions: a) p-R-C₂H₅MgBr, THF, –40 °C; b) TMSI, CHCl₃, 60 °C; c) isovaleric acid, EDCI, DMAP, CH₂Cl₂.

Scheme 2. Synthesis of 4-pyridone analogs (12a-12e) of SPF-32629A (a for R=H, b for F, c for Cl, d for CH₃, e for OCH₃). Reagents and conditions: a) ArMgBr, THF, –40 °C then 6 N HCl; b) NaBH₄, MeOH, –78 °C; c) H₂, Pd/C, MeOH; d) isovaleric acid, EDCI, DMAP, CH₂Cl₂; e) LiOH·H₂O, THF/H₂O.
Table 1. Percent (%) inhibition at 100 μM and IC₅₀ value of selected analogs against chymase and α-chymotrypsin

<table>
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<th>Chymase</th>
<th>α-Chymotrypsin</th>
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<tr>
<td></td>
<td>IC₅₀ (μM)</td>
<td>%I C₅₀ (μM)</td>
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<tr>
<td>chymostatin</td>
<td>–</td>
<td>(0.005 μg/mL)</td>
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<tr>
<td>1</td>
<td>– (0.25 μg/mL)</td>
<td>–</td>
</tr>
<tr>
<td>5a</td>
<td>24 &gt;100</td>
<td>31 ND</td>
</tr>
<tr>
<td>5b</td>
<td>45 &gt;100</td>
<td>ND</td>
</tr>
<tr>
<td>5d</td>
<td>87 11.6 ± 2.8</td>
<td>54 ND</td>
</tr>
<tr>
<td>12a</td>
<td>0 ND³</td>
<td>0 ND</td>
</tr>
<tr>
<td>13b</td>
<td>42 &gt;100</td>
<td>ND</td>
</tr>
<tr>
<td>13d</td>
<td>39 &gt;100</td>
<td>ND</td>
</tr>
</tbody>
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*In parentheses, the IC₅₀ values reported on ref 5. *ND: not determined

Analogs (4 and 11) not having isovaleroyl moiety was not tolerated. Interestingly, 2-pyridone analogs (5b and 5d) into which the substituent such as F and CH₃ was incorporated on C4' exhibited considerable activities against human chymase. Whereas 4-pyridones 12 completely lost activity, 13b and 13d inhibit chymase slightly. As our results, 4-hydroxy-2-pyridone skeleton is essential to inhibit chymase and C4'-substitution offer a chance to increase the biological activity.

In conclusion, 2- and 4-pyridine analogs of SPF-32629A were synthesized and evaluated for chymase inhibition. SAR studies revealed that 4-hydroxy-2-pyridone skeleton is critical to inhibition activity to human chymase and C4'-modification is expected to improve activity. These findings provide the information on the structural features that influence the biological activities within this class of compounds and need attention for further investigation.

Experimental

General Procedures for Synthesis of 3a-e. To a solution of 6-methoxy-2-pyridinecarboxaldehyde (1.0 eq) in THF (0.05 M) was added dropwise ArMgBr (1.0 M in Et₂O, 1.2 eq) at −78 °C. After stirring at −78 °C for 1 h, the reaction mixture was quenched by 1 N HCl and extracted with EtOAc. A combined organic layer was washed with brine, dried over MgSO₄ and concentrated in vacuo. The residue was purified by flash column chromatography (EtOAc/hexanes).

(6-Methoxypyridin-2-yl)(phenyl)methanol (3a): 1H-NMR (600 MHz, CDCl₃) δ 7.48 (m, 1H, 7.44 (d, 2H, J = 7.2 Hz), 7.35 (m, 2H), 7.29 (m, 1H), 6.73 (d, 1H, J = 7.2 Hz), 6.63 (d, 1H, J = 7.8 Hz), 5.72 (s, 1H), 5.09 (s, 1H), 3.98 (s, 3H); 13C-NMR (150 MHz, CDCl₃) δ 163.2, 158.9, 143.2, 139.5, 128.5, 127.8, 127.0, 113.7, 109.2, 74.8, 53.4. LRMS (ESI⁺) m/z 216.0 (M+H⁺).

(4-Fluorophenyl)(6-methoxypyridin-2-yl)methanol (3b): Yield 73%. 1H-NMR (600 MHz, CDCl₃) δ 7.42 (t, 1H, J = 7.8 Hz), 7.36-7.34 (m, 2H), 6.98-6.96 (m, 2H), 6.7 (d, 1H, J = 7.2 Hz), 6.58 (d, 1H, J = 8.4 Hz), 5.64 (d, 1H, J = 4.8 Hz), 5.08 (d, 1H, J = 4.8 Hz), 4.00 (s, 3H); 13C-NMR (150 MHz, CDCl₃) δ 163.2, 161.4, 158.9, 139.5, 139.1, 128.6, 115.2, 113.4, 109.2, 74.3, 53.3, LRMS (ESI⁺) m/z 232.3 (M+H⁺).

(4-Chloro(phenyl)(6-methoxypyridin-2-yl)methanol (3c): Yield 80%. 1H-NMR (600 MHz, CDCl₃) δ 7.45 (d, 1H, J = 4.8 Hz), 7.30 (2H, J = 8.4 Hz), 7.06 (2H, J = 8.4 Hz), 6.67 (d, 1H, J = 7.2 Hz), 6.60 (s, 1H), 5.62 (s, 1H), 5.03 (s, 1H), 3.92 (s, 3H); 13C-NMR (150 MHz, CDCl₃) δ 163.2, 158.4, 141.7, 139.6, 133.4, 128.5, 128.3, 116.7, 113.4, 119.4, 74.2, 60.5, 53.4, 21.0, 14.1; LRMS (ESI⁺) m/z 250.0 (M+H⁺).

(4-Methoxy(phenyl)(p-tolyl)methanol (3d): Yield 80%. 1H-NMR (600 MHz, CDCl₃) δ 7.48 (t, 1H, J = 7.8 Hz), 7.32 (2H, J = 7.8 Hz), 7.17 (2H, J = 7.8 Hz), 6.73 (d, 1H, J = 7.2 Hz), 6.63 (d, 1H, J = 7.8 Hz), 5.68 (d, 1H, J = 3.6 Hz), 4.98 (d, 1H, J = 4.2 Hz), 4.00 (s, 3H), 2.35 (s, 3H); 13C-NMR (150 MHz, CDCl₃) δ 163.2, 158.4, 159.2, 140.4, 139.5, 137.3, 129.2, 126.9, 113.6, 109.1, 74.7, 53.4, 21.1, 14.1; LRMS (ESI⁺) m/z 230.1 (M+H⁺).

General Procedures for Synthesis of 4a-e. To a CHCl₃ solution of the 3 (1.0 eq) iodotrimethylsilane (3.0 eq) was added at 60 °C. After stirring for 1 h, the reaction mixture was quenched withaq NaI, and extracted with CHCl₃. The organic phase was washed with brine, dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (CH₂Cl₂/MeOH).

(6-Hydroxy(phenyl)methyl)pyridin-2(1H)-one (4a): Yield 76%. 1H-NMR (600 MHz, CDCl₃) δ 7.52 (dd, 1H, J = 9.0 Hz), 7.41-7.40 (m, 2H), 7.36-7.33 (m, 2H), 7.30-7.29 (m, 1H), 6.39 (dd, 1H, J = 9.0, 0.6 Hz), 6.32 (d, 1H, J = 7.2 Hz), 5.62 (s, 1H); 13C-NMR (150 MHz, CDCl₃) δ 164.5, 151.0, 142.2, 141.2, 128.2, 127.9, 126.4, 117.3, 104.3, 71.7; LRMS (ESI⁺) m/z 202.1 (M+H⁺).

(4-(4-Fluorophenyl)(hydroxy)methyl)pyridin-2(1H)-one (4b): Yield 74%. 1H-NMR (600 MHz, CDCl₃) δ 7.50 (dd, 1H, J = 9.0, 7.2 Hz), 7.44-7.41 (m, 2H), 7.05 (t, 2H, J = 8.7 Hz), 6.41 (d, 1H, J = 9.0 Hz), 6.32 (d, 1H, J = 7.2 Hz), 5.68 (s, 1H); 13C-NMR (150 MHz, CDCl₃) δ 164.4, 150.9, 142.3, 137.3, 128.6, 128.5, 117.4, 104.6, 71.0. LRMS (ESI⁺) m/z 220.1 (M+H⁺).

(4-(Chlorophenyl)(hydroxy)methyl)pyridin-2(1H)-one (4c): Yield 70%. 1H-NMR (600 MHz, CDCl₃) δ 7.52 (dd, 1H, J = 7.2, 9.0 Hz), 7.41-7.39 (m, 2H), 7.36-7.34 (m, 2H), 6.40 (d, 1H, J = 9.0 Hz), 6.33 (d, 1H, J = 7.2 Hz), 5.62 (s, 1H); 13C-NMR (150 MHz, CDCl₃) δ 164.5, 150.6, 142.2, 140.0, 133.6, 128.3, 128.0, 117.5, 104.4, 70.9; LRMS (ESI⁺)
m/z 216.2 (M+H+).

6-(Hydroxy(p-tolyl)methyl)pyridin-2(1H)-one (4d): Yield 77%. 1H-NMR (600 MHz, CDCl3) δ 7.52 (t, 1H, J = 7.2, 9.0 Hz), 7.27 (d, 2H, J = 7.8 Hz), 7.17 (d, 2H, J = 7.8 Hz), 6.38 (d, 1H, J = 9.0 Hz), 6.31 (d, 1H, J = 7.2 Hz), 5.58 (s, 1H), 2.31 (s, 3H); 13C-NMR (150 MHz, CDCl3) δ 164.4, 151.1, 142.2, 138.7, 128.5, 126.4, 117.1, 104.2, 71.5, 19.75. LRMS (ESI+) m/z 216.2 (M+H+).

General Procedures for Synthesis of 5a-e and 6a-e. To a solution of the 2-pyridone 4 (1.0 eq) in CH2Cl2 was added EDCI (1.2 eq), DMAP (0.3 eq) and isovaleric acid (1.2 eq) at 0 °C. After stirring at ambient temperature for 1 h, the reaction mixture was washed with saturatedaq. NaHCO3 and extracted with EtOAc. A combined organic layer was dried over MgSO4 and concentrated in vacuo. The residue was purified by column chromatography (CH2Cl2/MeOH) to afford the monooester 5 and diester 6.

(6-Oxo-1,6-dihydropyridin-2-yl)(phenyl)methyl isovalerolactone (5a): 1H-NMR (600 MHz, CDCl3) δ 11.85 (br s, 1H), 7.43-7.45 (m, 2H), 7.31-7.37 (m, 4H), 6.64 (s, 1H), 6.45 (dd, 1H, J = 0.6, 9.0 Hz), 6.09 (dt, 1H, J = 1.2, 6.6 Hz), 2.33 (d, 2H, J = 7.2 Hz), 2.13 (m, 1H), 0.91 (d, 6H, 6.6 Hz); 13C-NMR (150 MHz, CDCl3) δ 171.6, 164.4, 146.4, 140.9, 136.7, 128.9, 128.8, 127.2, 119.8, 104.4, 73.1, 43.2, 25.6, 22.4, 22.3; LRMS (ESI+) m/z 286.0 (M+H+).

(4-Fluorophenyl)(6-oxo-1,6-dihydropyridin-2-yl)methyl isovalerolactone (5b): Yield 22%. 1H-NMR (600 MHz, CDCl3) δ 12.96 (s, 1H), 7.47-7.45 (m, 2H), 7.35 (dd, 1H, J = 7.2, 9 Hz), 7.01 (t, 2H, J = 8.4 Hz), 6.62 (s, 1H), 6.11 (d, 2H, J = 6.6 Hz), 6.44 (d, 1H, J = 8.4 Hz), 6.11 (d, 1H, J = 6.6 Hz), 2.32 (dd, 2H, J = 1.8, 7.8 Hz), 2.13-2.09 (m, 1H), 0.89 (d, 6H, J = 6.6 Hz); 13C-NMR (150 MHz, CDCl3) δ 171.6, 165.0, 163.6, 162.0, 146.9, 141.0, 132.9, 129.4, 129.9, 119.6, 115.8, 115.6, 104.0, 77.3, 77.1, 76.9, 72.6, 43.2, 25.6, 22.4, 22.4; LRMS (ESI+) m/z 304.1 (M+H+).

General Procedures for Synthesis of 1a-d. To a solution of the alcohol 10 in MeOH was treated with 10% Pd/C and hydrogenated for 1 h. The reaction mixture was filtered through Celite® and the solvent was removed in vacuo. The residue was purified by flash column chromatography (CH2Cl2/MeOH to afford the 4-pyridone 11.

(4-Benzoxylpyridin-2-yl)(phenyl)methanone (11a): Yield 80%. 1H-NMR (600 MHz, CDCl3) δ 8.53 (d, 1H, J = 5.4 Hz), 8.08 (dd, 2H, J = 1.2, 8.4 Hz), 7.65 (d, 1H, J = 2.4 Hz), 7.59-7.56 (m, 1H), 7.49-7.45 (m, 2H), 7.45-7.40 (m, 4H), 7.34-7.35 (m, 1H), 7.05 (dd, 1H, J = 2.4, 6.0 Hz), 5.17 (s, 2H); 13C-NMR (150 MHz, CDCl3) δ 193.7, 165.6, 156.8, 149.9, 136.3, 135.3, 132.9, 131.0, 128.8, 128.5, 128.1, 127.6, 113.3, 110.8, 70.2; LRMS (ESI+) m/z 290.1 (M+H+).

(4-(Benzyloxy)pyridin-2-yl)(phenyl)methanone (9a): Yield 80%. 1H-NMR (600 MHz, CDCl3) δ 8.53 (d, 1H, J = 5.4Hz), 8.07 (dd, 2H, J = 1.2, 8.4 Hz), 7.65 (d, 1H, J = 2.4 Hz), 7.59-7.56 (m, 1H), 7.49-7.45 (m, 2H), 7.45-7.40 (m, 4H), 7.34-7.35 (m, 1H), 7.05 (dd, 1H, J = 2.4, 6.0 Hz), 5.17 (s, 2H); 13C-NMR (150 MHz, CDCl3) δ 193.7, 165.6, 156.8, 149.9, 136.3, 135.3, 132.9, 131.0, 128.8, 128.5, 128.1, 127.6, 113.3, 110.8, 70.2; LRMS (ESI+) m/z 290.1 (M+H+).

General Procedures for Synthesis of 9a-d. To a solution of the 2-pyridone 4 (1.0 eq) in CH2Cl2 was added EDCI (1.2 eq), DMAP (0.3 eq) and isovaleric acid (1.2 eq) at 0 °C. After stirring for 1 h, the reaction mixture was washed with saturatedaq. NaHCO3 and filtered through Celite® and the solvent was removed in vacuo. The residue was purified by column chromatography (CH2Cl2/MeOH) to afford the ketone 9.

(4-Oxo-1,4-dihydropyridin-2-yl)(phenyl)methyl isovalerato (12a): Yield 70%. 1H-NMR (600 MHz, CDCl3) δ 7.55 (d, 1H, J = 7.8 Hz), 7.54-7.36 (m, 2H), 7.31-7.29 (m, 3H), 6.71 (s, 1H), 6.60 (s, 1H), 6.37 (dd, 1H, J = 2.4, 8.4 Hz), 5.31 (s, 1H), 3.47 (s, 1H), 2.31 (s, 3H), 2.30 (s, 3H), 2.28 (d, 2H, J = 8.4 Hz), 0.90 (dd, 6H, J = 3.6, 7.8 Hz); 13C-NMR (150 MHz, CDCl3) δ 177.5, 171.7, 152.5, 140.4, 134.7, 128.9, 128.8, 127.4, 113.9, 113.8, 73.9, 53.4, 50.5, 43.1, 29.7, 25.6, 22.3; LRMS (ESI+) m/z 286.1 (M+H+).

(4-Fluorophenyl)(4-oxo-1,4-dihydropyridin-2-yl)methyl isovalerato (12b): Yield 21%. 1H-NMR (600 MHz, CDCl3) δ 7.61 (d, 1H, J = 7.8 Hz), 7.37 (dd, 2H, J = 6.6, 10.2 Hz), 6.98 (t, 2H, J = 10.2 Hz), 6.72 (s, 1H), 6.61 (d, 1H, J = 3 Hz), 6.41-6.40 (m, 1H), 5.29 (s, 1H), 2.27 (d, 2H, J = 9 Hz), 0.97 (d, 1H, J = 7.8), 0.89 (dd, 6H, J = 3, 8.4 Hz); 13C-NMR (125 MHz, CDCl3) δ 177.8, 177.1, 171.5, 163.8, 161.8, 152.5, 140.4, 133, 129.4, 129.3, 115.6, 115.7, 115.4, 113.5, 72.9, 53.4, 43.1, 25.7, 25.6, 22.5, 22.3; LRMS (ESI+) m/z 301.2 (M+H+).

(4-Oxo-1,4-dihydropyridin-2-yl)(p-tolyl)methyl isovalerato (12d): Yield 21%. 1H-NMR (600 MHz, CDCl3) δ 7.56 (d, 1H, J = 8.4 Hz), 7.28 (d, 1H, J = 12 Hz), 7.11 (d, 1H, J = 9.6 Hz), 6.69 (s, 1H), 6.61 (d, 1H, J = 3 Hz), 6.39 (dd, 1H, J = 8.4 Hz), 5.31 (s, 1H), 2.31 (s, 3H), 2.27 (d, 2H, J = 8.4 Hz), 0.99 (d, 1H, J = 7.8 Hz), 0.90 (dd, 6H, J = 3, 8.4 Hz); 13C-NMR (125 MHz, CDCl3) δ 177.9, 171.6, 152.5, 140.1, 138.8, 134.0, 129.5, 127.4, 115.4, 113.7, 73.6, 43.1, 25.7, 25.6, 22.5, 22.4, 22.3, 21.1; LRMS (ESI+) m/z 300.2 (M+H+).

(4-(4-Fluorophenyl)(isovalerloyloxy)methyl)pyridin-4-yl isovalerato (13b): 1H-NMR (600 MHz, CDCl3) δ 8.60 (d, 1H, J = 6.6 Hz), 7.42 (dd, 2H, J = 6.6, 10.2 Hz), 7.28 (d, 1H, J = 2.4 Hz), 7.07-7.01 (m, 3H), 6.90 (s, 1H), 2.47 (d, 1H, J = 8.4 Hz), 2.47 (d, 1H, J = 8.4 Hz), 2.36 (d, 2H, J = 8.4 Hz), 1.07 (d, 6H, J = 7.8 Hz), 1.00 (d, 4H, J = 7.8 Hz), 0.96 (d, 6H, J = 7.8 Hz); LRMS (ESI+) m/z 388.1 (M+H+).

(2-(Isovaleryloxy)(p-tolyl)methyl)pyridin-4-yl isovalerato (13d): Yield 90%. 1H-NMR (600 MHz, CDCl3) δ 8.56 (d, 1H, J = 6.6 Hz), 7.35 (d, 2H, J = 9.6 Hz), 7.28 (d, 1H, J = 3 Hz), 7.17 (d, 2H, J = 9.6 Hz), 7.04 (dd, 1H, J = 2.4, 6.6 Hz), 6.91 (s, 1H), 2.48 (d, 2H, J = 8.4 Hz), 2.36 (t, 2H, J = 8.4, 14.4 Hz), 1.09 (d, 6H, J = 7.8 Hz), 0.98 (dd, 6H, J = 1.2, 7.8 Hz); LRMS (ESI+) m/z 384.2 (M+H+).

Chymase Inhibition Assay. Chymase assay was performed according to the manufacturer’s protocol. Briefly, test compounds dissolved in DMSO were incubated with 0.3 mg of chymase (Cat. Number C8118) in assay buffer (Cat. number A9606). The reaction was initiated by addition of substrate A (N-succinyl-Ala-Ala-Pro-Phe-p-nitroanilide, Cat. Number S0448), and enzymatic activity was monitored by measuring absorbance at 405 nm for 20 min using Vector X3 (Perkin-Elmer) microplate reader. Percent inhibition and IC50 of each sample were calculated by Prism (GraphPad). Chymostatin was used as positive control.

α-Chymotrypsin Inhibition Assay. α-Chymotrypsin assay was performed in 38 mM Tris–HCl buffer (pH 7.8) with 53 mM CaCl2. α-Chymotrypsin (2-5 units/mL prepared in buffer mentioned above) with various concentrations (1, 10, 100 μM) of test compounds prepared in DW was incubated at 30 °C or 10 min. The reaction was started by the addition of the substrate, p-NA. All the reactions were performed in triplicate in a final volume of 180 μL, by using a plate reader. The plate reader absorbance wavelength was settled at 405 nm. Aprotinin was used as positive control.

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Supporting Information. The 1H- and 13C-NMR spectral data of synthesized analogs are available as Supporting Information.

References