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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 in many mammalian species.[1] Its physiological substrate is angiotensin I, which upon cleavage by chymase yields angiotensin II in steps of the renin-angiotensin system.[2] In addition it is known to promote mast cell degranulation and plays a role in airway secretions and in atopic or allergic inflammation of the skin.[3] Therefore chymase is regarded as a potential therapeutic target for cardiovascular diseases as well as allergic/inflammatory diseases.[4]

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 IC_{50} of 0.25 and 0.42 μg/mL, respectively, as selective inhibitors.[5] 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.[6] 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 (**5a-e**) along with bis-isovalerates (**6a-e**) under EDCI-mediated condition. The isovalerate (**7a-3**) was also prepared from **4** in a similar manner.

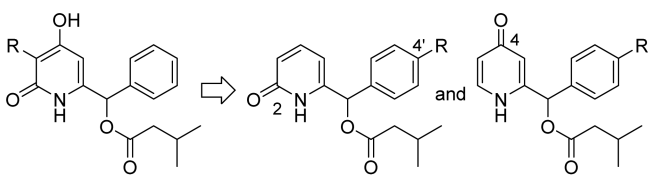
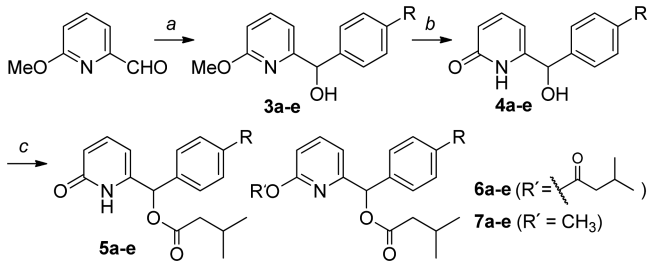


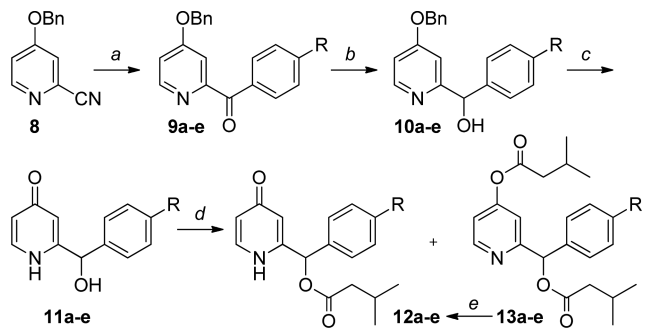
Figure 1. SPF-32629A (**1**), SPF-32629B (**2**) and the designed 2- and 4-pyridone analogs.



Scheme 1. Synthesis of 2-pyridone analogs (**5a-5e**) of SPF-32629A (**a** for R=H, **b** for F, **c** for Cl, **d** for CH_3, **e** for OCH_3. Reagents and conditions: a) *p*-R-C_6H_4MgBr, THF, –40 °C; b) TMSI, $CHCl_3$, 60 °C; c) isovaleric acid, EDCI, DMAP, CH_2Cl_2.

4-Pyridone analogs of SPF-32629A were synthesized from the known nitrile (**8**)[6] (Scheme 2). The ketones (**9a-e**) were given by Grignard reagents addition to **8**, followed by acid treatment. With **9** in hands, $NaBH_4$ 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 IC_{50} of selected analogs was tested as shown as Table 1. The introduction of 2-methoxypyridine (**3** and **7**) and 4-benzyloxypyridine (**9** and **10**) instead of 2- and 4-pyridone resulted in a loss of activity.



Scheme 2. Synthesis of 4-pyridone analogs (**12a-12e**) of SPF-32629A (**a** for R = H, **b** for F, **c** for Cl, **d** for CH_3, **e** for OCH_3. Reagents and conditions: a) ArMgBr, THF, –40 °C then 6 N HCl; b) $NaBH_4$, MeOH, –78 °C; c) H_2, Pd/C, MeOH; d) isovaleric acid, EDCI, DMAP, CH_2Cl_2; e) $LiOH{\cdot}H_2O$, THF/H_2O.

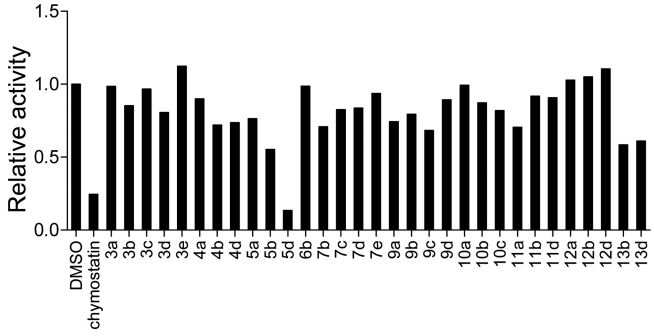


Figure 2. Inhibitory effect of SPF-32629A analogs (100 μM) on chymase.

Table 1. Percent (%) inhibition at 100 μM and IC_{50} value of selected analogs against chymase and α-chymotrypsin

	Chymase		α-Chymotrypsin	
	%	IC_{50} (μM)	%	IC_{50} (μM)
chymostatin	–	(0.005 μg/mL)[a]	–	(0.003 μg/mL)
1	–	(0.25 μg/mL)	–	(> 10 μg/mL)
5a	24	>100	31	ND
5b	45	>100	ND	ND
5d	87	11.6 ± 2.8	54	ND
12a	0	ND[b]	0	ND
13b	42	> 100	ND	ND
13d	39	> 100	ND	ND

[a]In parentheses, the IC_{50} values reported on ref 5. [b]ND: not determined

Analogs (**4** and **11**) not having isovalerate moiety was not tolerated. Interestingly, 2-pyridone analogs (**5b** and **5d**) into which the substituent such as F and CH_3 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-pyridone 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 Et_2O, 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 $MgSO_4$ and concentrated *in vacuo*. The residue was purified by flash column chromatography (EtOAc/hexanes).

(6-Methoxypyridin-2-yl)(phenyl)methanol (3a): ^{1}H-NMR (600 MHz, $CDCl_3$) 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); ^{13}C-NMR (150 MHz, $CDCl_3$) 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%. ^{1}H-NMR (600 MHz, $CDCl_3$) 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); ^{13}C-NMR (150 MHz, $CDCl_3$) 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* 233.2 ($M+H^+$).

(4-Chlorophenyl)(6-methoxypyridin-2-yl)methanol (3c): Yield 80%. ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.45 (d, 1H, *J* = 4.8 Hz), 7.30 (d, 2H, *J* = 8.4 Hz), 7.06 (d, 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); ^{13}C-NMR (150 MHz, $CDCl_3$) 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 ($M+H^+$).

(6-Methoxypyridin-2-yl)(*p*-tolyl)methanol (3d): Yield 80%. ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.48 (t, 1H, *J* = 7.8 Hz), 7.32 (d, 2H, *J* = 7.8 Hz), 7.17 (d, 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); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 163.2, 159.2, 140.4, 139.5, 137.3, 129.2, 126.9, 113.6, 109.1, 74.7, 53.4, 21.1. LRMS (ESI^+) *m/z* 230.1 ($M+H^+$).

General Procedures for Synthesis of 4a-e. To a $CHCl_3$ 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 with aq $NaHSO_3$, and extracted with CH_2Cl_2. The organic phase was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (CH_2Cl_2 /MeOH).

6-(Hydroxy(phenyl)methyl)pyridin-2(1*H*)-one (4a): Yield 76%. ^{1}H-NMR (600 MHz, CD_3OD) δ 7.52 (dd, 1H, *J* = 7.2 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); ^{13}C-NMR (150 MHz, CD_3OD) 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 ($M+H^+$).

6-((4-Fluorophenyl)(hydroxy)methyl)pyridin-2(1*H*)-one (4b): Yield 74%. ^{1}H-NMR (600 MHz, CD_3OD) 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); ^{13}C-NMR (150 MHz, CD_3OD) 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 ($M+H^+$).

6-((4-Chlorophenyl)(hydroxy)methyl)pyridin-2(1*H*)-one (4c): Yield 70%. ^{1}H-NMR (600 MHz, CD_3OD) 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); ^{13}C-NMR (150 MHz, CD_3OD) 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(1*H*)-one (4d): Yield 77%. ^{1}H-NMR (600 MHz, CD_3OD) 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); ^{13}C-NMR (150 MHz, CD_3OD) δ 164.4, 151.1, 142.2, 138.2, 137.8, 128.8, 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 CH_2Cl_2 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 saturated aq. $NaHCO_3$ and extracted with EtOAc. A combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (CH_2Cl_2/MeOH) to afford the monoester **5** and diester **6**.

(6-Oxo-1,6-dihydropyridin-2-yl)(phenyl)methyl isovaleroate (5a): ^{1}H-NMR (600 MHz, $CDCl_3$) 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 Hz), 6.09 (dt, 1H, *J* = 1.2, 6 Hz), 2.33 (d, 2H, *J* = 7.2 Hz), 2.13 (m, 1H), 0.91 (d, 6H, 6.6 Hz); ^{13}C-NMR (150 MHz, $CDCl_3$) 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 isovaleroate (5b): Yield 22%. ^{1}H-NMR (600 MHz, $CDCl_3$) 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, 1H, *J* = 6.6); ^{13}C-NMR (150 MHz, $CDCl_3$) 171.6, 165.0, 163.6, 162.0, 146.9, 141.0, 132.9, 132.9, 129.4, 129.4, 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 ($M+H^+$).

(6-Oxo-1,6-dihydropyridin-2-yl)(*p*-tolyl)methyl isovaleroate (5d): ^{1}H-NMR (600 MHz, $CDCl_3$) δ 11.52 (br s, 1H), 7.30-7.34 (m, 3H), 7.16 (d, 2H, *J* = 7.8 Hz), 6.61 (s, 1H), 6.43 (d, 1H, 9 Hz), 6.08 (d, 1H, *J* = 7.2 Hz), 2.31 (dd, 5H, *J* = 1.2, 1.8 Hz), 2.09-2.16 (m, 1H), 0.91 (d, 6H, *J* = 6.6 Hz); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 171.6, 164.4, 146.4, 140.9, 138.8, 133.6, 129.5, 127.2, 119.8, 104.2, 73.0, 43.2, 30.3, 25.6, 22.4, 22.3, 21.2; LRMS (ESI^+) *m/z* 300.1 ($M+H^+$).

6-((4-Fluorophenyl)(isovaleryloxy)methyl)pyridin-2-yl isovaleroate (6b): Yield 17%. ^{1}H-NMR (600 MHz, $CDCl_3$) δ 7.75 (t, 1H, *J* = 7.8 Hz), 7.38-7.36 (m, 2H), 7.31 (d, 1H *J* = 7.8), 7.0-6.97 (m, 3H), 5.44 (d, 2H, *J* = 7.2 Hz), 2.31 (d, 2H, *J* = 7.2 Hz), 2.25-2.18 (m, 1H), 2.17-2.10 (m, 1H), 1.03 (d, 6H, *J* = 7.2 Hz), 0.93 (d, 6H, *J* = 7.2 Hz); LRMS (ESI^+) *m/z* 388.1 ($M+H^+$).

General Procedures for Synthesis of 9a-d. To a THF solution of 4-(benzyloxy)picolinonitrile **8** (1.0 eq) was added ArMgBr (1.0 M in Et_2O, 1.0 eq) at –78 °C. After stirring for 1 h, the reaction mixture was quenched with 6 N HCl, and stirred for 12 h. The organic phase was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc/hexanes) to afford the ketone **9**.

(4-(Benzyloxy)pyridin-2-yl)(phenyl)methanone (9a): Yield 80%. ^{1}H-NMR (600 MHz, $CDCl_3$) 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); ^{13}C-NMR (150 MHz, $CDCl_3$) 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 10a-d. To a solution of **9** in MeOH was added $NaBH_4$ (1.0 eq) at –78 °C. After stirring for 30 min, the reaction mixture was quenched with MeOH and concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc/hexanes) to afford **10**.

2-(Hydroxyphenylmethyl)-4(1*H*)-pyridone (10a): Yield 71%. ^{1}H-NMR (600 MHz, CD_3OD) δ 7.74 (d, 1H *J* = 9.0 Hz), 7.45 (t, 2H, *J* = 1.2, 9.0 Hz), 7.39 (t, 2H, *J* = 9.6 Hz), 7.35-7.31 (m, 1H), 6.42-6.38 (m, 2H), 5.70 (s, 1H); ^{13}C-NMR (125 MHz, CD_3OD) δ 180.3, 155.0, 141.3, 138.2, 128.4, 128.0, 126.4, 115.4, 113.4, 72.0; LRMS (ESI^+) *m/z* 202.1 ($M+H^+$).

General Procedures for Synthesis of 11a-d. 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 (CH_2Cl_2/MeOH) to afford the 4-pyridone **11**.

(4-(Benzyloxy)pyridin-2-yl)(phenyl)methanone (11a): Yield 80%. ^{1}H-NMR (600 MHz, $CDCl_3$) 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), 5.18 (s, 2H); ^{13}C-NMR (150 MHz, $CDCl_3$) 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 ($M+H^+$).

2-((4-Fluorophenyl)(hydroxy)methyl)pyridin-4(1*H*)-one (11b): Yield 95%. ^{1}H-NMR (600 MHz, CD_3OD) δ 7.74 (d, 1H, *J* = 8.4 Hz), 7.49-7.46 (m, 2H), 7.14-7.11 (m, 2H), 6.40 (t, 2H, *J* = 3.0, 8.4 Hz), 5.71 (s, 1H); ^{13}C-NMR (150 MHz, CD_3OD) δ 163.6, 161.7, 137.4, 128.4, 115.4, 113.4, 71.2; LRMS (ESI^+) *m/z* 220.0 ($M+H^+$).

2-(Hydroxy(*p*-tolyl)methyl)pyridin-4(1*H*)-one (11d): Yield 93%. ^{1}H-NMR (600 MHz, CD_3OD) δ 7.73 (d, 1H, *J* = 9.0 Hz), 7.32 (d, 2H, *J* = 9.6 Hz), 7.21 (d, 1H, *J* = 9.6 Hz), 6.39 (t, 2H, *J* = 7.2, 3 Hz), 5.65 (s, 1H), 2.34 (s, 3H); ^{13}C-NMR (150 MHz, CD_3OD) δ 138.3, 138.0, 128.9, 126.4, 115.3, 113.3, 71.8, 19.8; LRMS (ESI^+) *m/z* 216.1 ($M+H^+$).

General Procedures for Synthesis of 12a-e and 13a-e. To a solution of **11** in CH_2Cl_2 was added EDCI (1.1 eq), DMAP (0.5 eq) and isovaleric acid (1.0 eq) at 0 °C. The reaction mixture was stirred at ambient temperature for 1 h. After the completion of the reaction, saturated aq. $NaHCO_3$ was added for quenching. The mixture was extracted with EtOAc. A combined organic layer was washed with brine, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (CH_2Cl_2/MeOH) to afford **12** and **13**.

(4-Oxo-1,4-dihydropyridin-2-yl)(phenyl)methyl isovaleroate (12a): Yield 70%. ^{1}H-NMR (600 MHz, $CDCl_3$) 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), 2.28 (d, 2H, *J* = 8.4 Hz), 0.90 (dd, 6H, *J* = 3.6, 7.8 Hz); ^{13}C-NMR (150 MHz, $CDCl_3$) δ 177.5, 171.7, 152.5, 140.4, 137.1, 128.9, 128.8, 127.4, 115.39, 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 isovaleroate (12b): Yield 28%. ^{1}H-NMR (600 MHz, $CDCl_3$) 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* = 3.6, 8.4 Hz); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 177.8, 177.1, 171..5, 163.8, 161.8, 152.5, 140.4, 133, 132.9, 129.4, 129.3, 115.9, 115.7, 115.4, 113.5, 72.9, 53.4, 44.2, 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 isovaleroate (12d): Yield 21%. ^{1}H-NMR (600 MHz, $CDCl_3$) 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* = 3, 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); ^{13}C-NMR (125 MHz, $CDCl_3$) 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 ($M+H^+$).

2-((4-Fluorophenyl)(isovaleryloxy)methyl)pyridin-4-yl isovaleroate (13b): ^{1}H-NMR (600 MHz, $CDCl_3$) 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 isovaleroate (13d): Yield 90%. ^{1}H-NMR (600 MHz, $CDCl_3$) 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 with Chymase Activity Assay kit (Sigma-Aldrich) 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 Victor X3 (Perkin-Elmer) microplate reader. Percent inhibition and IC_{50} 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 $CaCl_2$. 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 ^{1}H- and ^{13}C-NMR spectral data of synthesized analogs are available as Supporting Information.

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