8-Hydroxy-1,6-naphthyridine-7-carboxamides as Inhibitors of Human Cytomegalovirus pUL89 Endonuclease

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Reagents and conditions: a) DBU, THF, 40 °C; b) HCl (Conc.), 55 °C, 18 h, 56%, over-2-steps; c) POCl₃, MW, 130 °C, 30 min, 85%; d) R¹NH₂, AcOH, toluene, 110 °C, 18 h, 43-65%; e) RNH₂, NaOMe, DMSO, 110 °C, 43-52%; f) ArB(OH)₂, Pd(PPh₃)₄, K₂CO₃, CH₃CN, MW, 150 °C, 30 min, 37-69%; g) NH₂(CH₂)₂OR₂, 150 °C, 18 h, 39-49%.

**General synthetic procedures**

To a solution of quinolinic anhydride 10 (2 g, 13.4 mmol, 1.0 eq.) in DMF (4 mL) at room temperature under argon atmosphere was added methyl isocyanoacetate 11 (1.2 eq.) in THF (8 mL). This was stirred for 15 minutes at 40°C, followed by dropwise addition of DBU (1.5 eq.) in THF (2 mL). After 1 h, the solvents were removed under reduced pressure and the resultant crude acid product were taken up into MeOH. Concentrated HCl (12 M) was then added dropwise while the solution stirred at 55°C. This solution was stirred for 30 min and then the crude solids were collected by vacuum filtration to give compound 12. A solution of compound 12 (1 g, 4.5 mmol,
1.0 eq.) was treated with excess POCl₃ (8 mL) and the mixture was heated at 80 °C. After 3 h, the solvent was removed under reduced pressure. Following addition of ice water and NH₄OH and the crude solids were collected by vacuum filtration to afford choro compound 13. To a solution of compound 13 (1.0 eq.) in toluene was added AcOH (5.0 eq.), followed by addition of various amines, and the solution was stirred at 110 °C for 18 h. Saturated aqueous NaHCO₃ solution (30 mL) was added, and the aqueous mixture was extracted with EtOAc. The combined organic extracts were washed with brine, dried (MgSO₄) and the solvent was removed under vacuum. The crude product was purified by column chromatography to give analogs 15a-d. To a solution of compound 13 (1.0 eq.) in DMSO was added NaOMe (30% in MeOH, 5.0 eq.), followed by addition of amines, and the solution was stirred at 110 °C for 18 h. Water was added, and the aqueous mixture was extracted with EtOAc. The combined organic extracts were washed with water, brine, dried (MgSO₄) and the solvent was removed under vacuum. The crude product was purified by column chromatography to give analogs 15e-f. To a solution of a choro compound 15a-d (1.0 eq.) in CH₃CN was added 2 M K₂CO₃ (5.0 eq.), arylboronic acid (1.5 eq.) and Pd(PPh₃)₄ (0.02 eq.). The resultant mixture underwent microwave reaction at 140 °C for 30 min. The solvent was removed under vacuum and the crude product was purified by column chromatography to give analogs 16a-e. To a choro compound 15a-d (1.0 eq.) was added excess amines and then heated at 150 °C for 24 h. The solvent was removed under vacuum and the crude product was purified by column chromatography to give compounds 17a-b. Carboxylic acid 14 was prepared from ester 13 under the Suzuki reaction conditions used for synthesizing 16a-e. Complete ester hydrolysis was observed during the reaction workup.
Analytical data for tested compounds

Methyl 8-hydroxy-5-oxo-5,6-dihydro-1,6-naphthyridine-7-carboxylate (12)

![Chemical structure](image1)

$^1$H NMR (400 MHz, DMSO-$d_6$) δ 10.61 (s, 1H), 9.10 (d, $J = 4.6$ Hz, 1H), 8.62 (d, $J = 8.0$ Hz, 1H), 7.78 (dd, $J = 8.0$, 4.5 Hz, 1H), 3.89 (s, 3H); $^{13}$C NMR (101 MHz, DMSO) δ 163.31, 157.89, 153.84, 146.84, 142.26, 136.32, 124.94, 124.67, 112.53, 52.64; HRMS (ESI$^+$): m/z calcd for C$_{10}$H$_9$N$_2$O$_4$ [M+H]$^+$ 221.0557, found 221.0571.

Methyl 5-chloro-8-hydroxy-1,6-naphthyridine-7-carboxylate (13)

![Chemical structure](image2)

$^1$H NMR (600 MHz, DMSO-$d_6$) δ 9.23 (d, $J = 4.3$ Hz, 1H), 8.63 (dd, $J = 8.5$, 1.5 Hz, 1H), 7.96 (dd, $J = 8.5$, 4.2 Hz, 1H), 3.92 (s, 3H); HRMS (ESI$^+$): m/z calcd for C$_{10}$H$_6$ClN$_2$O$_3$ [M+H]$^-$ 237.0072, found 237.0080.

8-Hydroxy-5-(4-(trifluoromethyl)phenyl)-1,6-naphthyridine-7-carboxylic acid (14)

![Chemical structure](image3)
$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.98 (d, $J = 2.4$ Hz, 1H), 8.26 (dd, $J = 8.5$, 1.7 Hz, 1H), 7.87 (d, $J = 8.1$ Hz, 2H), 7.83 (d, $J = 8.1$ Hz, 2H), 7.62 (dd, $J = 8.5$, 4.1 Hz, 1H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 169.9, 161.3, 151.5, 144.8, 143.2, 141.4, 134.5, 130.5, 129.4, 127.9 (d, $J = 31.6$ Hz), 125.8, 125.1 (d, $J = 3.8$ Hz), 123.6 (d, $J = 5.5$ Hz), 123.1; HRMS (ESI$^+$): m/z calcd for C$_{16}$H$_8$F$_3$N$_2$O$_3$ [M-H]$^-$ 333.0493, found 333.0493.

5-Chloro-N-(4-fluorobenzyl)-8-hydroxy-1,6-naphthyridine-7-carboxamide (15a)

$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 13.82 (s, 1H), 9.64 (t, $J = 6.5$ Hz, 1H), 9.24 (dd, $J = 4.3$, 1.6 Hz, 1H), 8.65 (dd, $J = 8.5$, 1.6 Hz, 1H), 7.94 (dd, $J = 8.5$, 4.3 Hz, 1H), 7.27 (d, $J = 7.8$ Hz, 2H), 7.14 (d, $J = 7.8$ Hz, 2H), 4.51 (d, $J = 6.4$ Hz, 2H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 168.3, 154.8, 154.6, 143.9, 141.7, 137.7, 136.1, 135.6, 134.6, 128.9, 127.6, 126.1, 125.0, 42.1; HRMS (ESI$^+$): m/z calcd for C$_{16}$H$_{10}$ClF$_3$N$_2$O$_2$ [M-H]$^-$ 330.0451, found 330.0448.

5-Chloro-N-(3,4-dichlorobenzyl)-8-hydroxy-1,6-naphthyridine-7-carboxamide (15b)

$^1$H NMR (400 MHz, CD$_3$OD) $\delta$ 9.16 (dd, $J = 4.4$, 1.6 Hz, 1H), 8.75 (dd, $J = 8.2$, 1.6 Hz, 1H), 7.91 (dd, $J = 8.2$, 4.4 Hz, 1H), 7.58 (d, $J = 2.0$ Hz, 1H), 7.49 (d, $J = 8.2$ Hz, 1H), 7.35 (dd, $J = 8.2$, 2.0 Hz, 1H), 4.62 (s, 2H); $^{13}$C NMR (100 MHz, CD$_3$OD) $\delta$ 168.9, 154.1, 153.5, 143.7, 139.3, 138.8,
135.3, 131.5, 130.7, 129.5, 127.3, 125.9, 125.6, 125.5, 41.2; HRMS (ESI⁺): m/z calcd for C₁₆H₁₁Cl₃N₃O₂ [M+H]⁺ 381.9911, found 381.9908.

5-Chloro-N-(3-chloro-4-fluorobenzyl)-8-hydroxy-1,6-naphthyridine-7-carboxamide (15c)

1H NMR (400 MHz, CDCl₃) δ 9.23 (dd, J = 4.3, 1.7 Hz, 1H), 8.60 (dd, J = 8.5, 1.6 Hz, 1H), 8.20 (s, 1H), 7.75 (dd, J = 8.5, 4.2 Hz, 1H), 7.45 (dd, J = 6.9, 2.1 Hz, 1H), 7.15 (t, J = 8.7 Hz, 1H), 4.65 (d, J = 6.3 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 168.6, 157.9 (d, J_CF = 249.2 Hz), 154.9, 154.7, 144.8, 138.9, 135.0, 134.7 (d, J_CF = 3.9 Hz), 130.3, 127.8 (d, J_CF = 7.3 Hz), 125.8, 125.4, 124.4, 121.6 (d, J_CF = 17.4 Hz), 117.1 (d, J_CF = 21.3 Hz), 42.3; HRMS (ESI⁺): m/z calcd for C₁₆H₁₁Cl₂FN₃O₂ [M+H]⁺ 366.0207, found 366.0208.

5-Chloro-8-hydroxy-N-(4-methylbenzyl)-1,6-naphthyridine-7-carboxamide (15d)

¹H NMR (400 MHz, CD₃OD) δ 9.16 (dd, J = 4.3, 1.6 Hz, 1H), 8.73 (dd, J = 8.2, 1.6 Hz, 1H), 7.90 (dd, J = 8.2, 4.3 Hz, 1H), 7.28 (d, J = 7.8 Hz, 2H), 7.15 (d, J = 7.8 Hz, 2H), 4.59 (s, 2H), 2.31 (s, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 169.3, 155.5, 155.2, 145.2, 139.9, 138.2, 136.7, 136.6, 130.2, 128.7, 128.6, 127.0, 126.8, 43.5, 21.1; HRMS (ESI⁺): m/z calcd for C₁₇H₁₅ClN₃O₂ [M+H]⁺ 328.0847, found 328.0842.
5-chloro-N-cyclohexyl-8-hydroxy-1,6-naphthyridine-7-carboxamide (15e)

\[
\begin{align*}
\text{Cl} & \\
\text{OH} & \\
\text{N} & \\
\text{H} & \\
\text{O} & \\
\text{N} & \\
\text{H} & \\
\text{C} & \\
\end{align*}
\]

\(^1\)H NMR (400 MHz, CD\(_3\)OD) \(\delta\) 9.14 (dd, \(J = 4.4, 1.6\) Hz, 1H), 8.70 (dd, \(J = 8.2, 1.6\) Hz, 1H), 7.89 (dd, \(J = 8.2, 4.4\) Hz, 1H), 3.91 – 3.97 (m, 1H), 1.97 – 2.01 (m, 2H), 1.81 – 1.85 (m, 2H), 1.68 – 1.73 (m, 2H), 1.44 – 1.48 (m, 2H), 1.28 – 1.32 (m, 2H); \(^{13}\)C NMR (100 MHz, CD\(_3\)OD) \(\delta\) 168.9, 155.5, 155.3, 145.2, 139.7, 136.6, 126.9, 126.7, 126.0, 34.8, 33.5, 26.5, 26.2; HRMS (ESI\(^+\)): m/z calcd for C\(_{15}\)H\(_{15}\)ClN\(_3\)O\(_2\) [M-H\(^-\)] 304.0858, found 304.0857.

5-Chloro-8-hydroxy-N-(2-methoxyethyl)-1,6-naphthyridine-7-carboxamide (15f)

\[
\begin{align*}
\text{Cl} & \\
\text{OH} & \\
\text{N} & \\
\text{H} & \\
\text{O} & \\
\text{N} & \\
\text{H} & \\
\text{C} & \\
\end{align*}
\]

\(^1\)H NMR (400 MHz, CD\(_3\)OD) \(\delta\) 9.15 (dd, \(J = 4.4, 1.6\) Hz, 1H), 8.72 (dd, \(J = 8.2, 1.6\) Hz, 1H), 7.89 (dd, \(J = 8.2, 4.4\) Hz, 1H), 3.62 – 3.66 (m, 4H), 3.42 (s, 3H); \(^{13}\)C NMR (100 MHz, CD\(_3\)OD) \(\delta\) 170.0, 155.6, 155.1, 145.1, 139.9, 136.6, 133.4, 127.0, 126.0, 71.9, 59.0, 39.8; HRMS (ESI\(^+\)): m/z calcd for C\(_{12}\)H\(_{13}\)ClN\(_3\)O\(_3\) [M+H\(^+\)] 282.0640, found 282.0620.

8-Hydroxy-N-(4-methylbenzyl)-5-phenyl-1,6-naphthyridine-7-carboxamide (16a)

\[
\begin{align*}
\text{N} & \\
\text{H} & \\
\text{O} & \\
\text{N} & \\
\text{H} & \\
\text{C} & \\
\end{align*}
\]

7
$^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 9.59 (t, $J = 6.5$ Hz, 1H), 9.18 (dd, $J = 4.2$, 1.7 Hz, 1H), 8.39 (dd, $J = 8.6$, 1.7 Hz, 1H), 7.79 (dd, $J = 8.6$, 4.2 Hz, 2H), 7.73 (dd, $J = 7.8$, 1.7 Hz, 1H), 7.64 – 7.51 (m, 3H), 7.27 (d, $J = 7.8$ Hz, 2H), 7.14 (d, $J = 7.8$ Hz, 2H), 4.54 (d, $J = 4.1$ Hz, 2H), 2.27 (s, 3H); $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 169.5, 162.5, 153.6, 153.5, 149.6, 142.7, 137.5, 137.3, 135.8, 135.4, 130.1, 128.9, 128.7, 128.4, 127.5, 124.8, 124.5, 42.1, 20.7; HRMS (ESI$^+$): m/z calcd for C$_{23}$H$_{20}$N$_3$O$_2$ [M+H]$^+$ 370.1550, found 370.1532.

8-Hydroxy-5-(3-methoxyphenyl)-N-(4-methylbenzyl)-1,6-naphthyridine-7-carboxamide (16b)

![Structure](image)

$^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 13.42 (s, 1H), 9.20 (d, $J = 2.9$ Hz, 1H), 8.45 (t, $J = 6.4$ Hz, 1H), 8.42 (d, $J = 8.6$ Hz, 1H), 7.59 (dd, $J = 8.6$, 4.1 Hz, 1H), 7.45 (t, $J = 7.9$ Hz, 1H), 7.28 (d, $J = 8.0$ Hz, 2H), 7.18 – 7.11 (m, 4H), 7.05 (dd, $J = 8.2$, 2.6 Hz, 1H), 4.66 (d, $J = 6.2$ Hz, 2H), 3.87 (s, 3H), 2.34 (s, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 169.3, 159.9, 153.2, 153.0, 150.0, 142.4, 139.0, 137.6, 137.2, 134.6, 129.8, 129.6, 128.0, 125.4, 125.3, 124.2, 122.4, 115.9, 114.5, 55.6, 43.0, 21.2; HRMS (ESI$^+$): m/z calcd for C$_{24}$H$_{22}$N$_3$O$_3$ [M+H]$^+$ 400.1656, found 400.1608.

5-(2,4-dimethoxyphenyl)-8-hydroxy-N-(4-methylbenzyl)-1,6-naphthyridine-7-carboxamide (16c)

![Structure](image)
\textbf{N-(4-fluorobenzyl)-5-(2-fluorophenyl)-8-hydroxy-1,6-naphthyridine-7-carboxamide (16d)}

\begin{center}
\includegraphics[width=0.3\textwidth]{structure16d.png}
\end{center}

\textbf{1H NMR (400 MHz, CD\textsubscript{3}OD) δ 9.11 (d, J = 1.6 Hz, 1H), 8.19 (d, J = 8.6 Hz, 1H), 7.76 (dd, J = 8.6, 4.3 Hz, 1H), 7.60 (dd, J = 8.2, 2.0 Hz, 1H), 7.58 (td, J = 5.4, 2.0 Hz, 1H), 7.40 – 7.46 (m, 3H), 7.30 (ddd, J = 10.0, 6.4, 2.5 Hz, 1H), 7.08 – 7.00 (m, 2H), 4.62 (s, 2H); \textsuperscript{13}C NMR (100 MHz, CD\textsubscript{3}OD) δ 169.5, 162.2 (d, J\textsubscript{CF} = 243.8 Hz), 160.0 (d, J\textsubscript{CF} = 245.7 Hz), 153.6, 153.3, 145.5, 142.1, 136.1, 134.5 (d, J\textsubscript{CF} = 3.4 Hz), 132.1 (d, J\textsubscript{CF} = 3.1 Hz), 131.1 (d, J\textsubscript{CF} = 8.4 Hz), 129.3 (d, J\textsubscript{CF} = 8.4 Hz), 128.1, 125.7, 125.4 (d, J\textsubscript{CF} = 6.5 Hz), 124.6, 124.5 (d, J\textsubscript{CF} = 3.7 Hz), 115.4 (d, J\textsubscript{CF} = 19.4 Hz), 114.8 (d, J\textsubscript{CF} = 21.9 Hz), 41.6; HRMS (ESI\textsuperscript{+}): m/z calcd for C\textsubscript{22}H\textsubscript{16}F\textsubscript{2}N\textsubscript{2}O\textsubscript{2} [M+H]\textsuperscript{+} 392.1205, found 392.1205.

\textbf{5-(3-Chlorophenyl)-N-(4-fluorobenzyl)-8-hydroxy-1,6-naphthyridine-7-carboxamide (16e)}

\begin{center}
\includegraphics[width=0.3\textwidth]{structure16e.png}
\end{center}
**N-(4-fluorobenzyl)-8-hydroxy-5-((2-methoxyethyl)amino)-1,6-naphthyridine-7-carboxamide (17a)**

\[
\text{\includegraphics{image.png}}
\]

\[^1\text{H NMR (400 MHz, CD}_3\text{OD) \delta 8.99 (dd, } J = 4.4, 1.6 \text{ Hz, 1H), 8.60 (dd, } J = 8.2, 1.6 \text{ Hz, 1H), 7.67 (dd, } J = 8.2, 4.4 \text{ Hz, 1H), 7.40 – 7.45 (m, 2H), 7.05 – 7.10 (m, 2H), 4.63 (s, 2H), 3.76 (t, } J = 5.7 \text{ Hz, 2H), 3.65 (t, } J = 5.7 \text{ Hz, 2H), 3.35 (s, 3H); }^{13}\text{C NMR (100 MHz, CD}_3\text{OD) \delta 169.4, 162.3 (d, } J_{CF} = 244.2 \text{ Hz), 152.3, 152.2, 147.9, 144.2, 134.5 (d, } J_{CF} = 2.0 \text{ Hz), 132.3, 129.0 (d, } J_{CF} = 7.8 \text{ Hz), 125.9, 122.9, 115.0, 114.8 (d, } J_{CF} = 21.8 \text{ Hz), 70.9, 57.7, 41.5, 40.7, 29.4; HRMS (ESI\(^+\)): m/z calcd for C\(_{19}\)H\(_{20}\)FN\(_{4}\)O\(_3\) [M+H]\(^+\) 371.1514, found 371.1497.}
\]

**N-(4-fluorobenzyl)-8-hydroxy-5-((2-hydroxyethyl)amino)-1,6-naphthyridine-7-carboxamide (17b)**

\[
\text{\includegraphics{image.png}}
\]

\[^1\text{H NMR (400 MHz, CD}_3\text{OD) \delta 9.08 (dd, } J = 4.3, 1.6 \text{ Hz, 1H), 8.42 (dd, } J = 8.2, 1.6 \text{ Hz, 1H), 7.74 (dd, } J = 8.2, 4.3 \text{ Hz, 1H), 7.72 (s, } J = 1.2 \text{ Hz, 1H), 7.56 – 7.58 (m, 1H), 7.56 – 7.58 (m, 2H), 7.40 – 7.43 (m, 2H), 7.01 – 7.04 (m, 2H), 4.61 (s, 2H); }^{13}\text{C NMR (100 MHz, CD}_3\text{OD) \delta 170.9, 163.6 (d, } J_{CF} = 243.5 \text{ Hz), 154.6, 150.2, 143.9, 141.0, 137.4, 137.2, 135.9 (d, } J_{CF} = 3.1 \text{ Hz), 135.6, 131.0, 130.8, 130.6 (d, } J_{CF} = 7.7 \text{ Hz), 129.9, 129.6 126.4, 126.2, 125.9, 116.1 (d, } J_{CF} = 21.7 \text{ Hz), 43.3; HRMS (ESI\(^+\)): m/z calcd for C\(_{22}\)H\(_{14}\)ClFN\(_3\)O\(_2\) [M+H]\(^+\) 406.0764, found 406.0762.}
\]
\(^1\)H NMR (600 MHz, CD\(_3\)OD) \(\delta\) 8.98 (d, \(J = 4.3\) Hz, 1H), 8.61 (d, \(J = 8.6\) Hz, 1H), 7.67 (d, \(J = 4.6\) Hz, 1H), 7.42 (d, \(J = 8.3\) Hz, 2H), 7.06 (d, \(J = 8.5\) Hz, 2H), 4.63 (s, 2H), 3.86 – 3.67 (m, 4H); HRMS (ESI\(^+\)): m/z calcd for C\(_{18}\)H\(_{16}\)FN\(_4\)O\(_3\) [M-H]\(^-\) 355.1212, found 355.1215.
NMR spectra of tested compounds
