Supporting Information

Exploiting Drug-Resistant Enzymes as Tools to Identify Thienopyrimidinone Inhibitors of Human Immunodeficiency Virus Reverse Transcriptase-Associated Ribonuclease H.

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Methods

Characterization of compounds

2-amino-4,5-dimethylthiophene-3-carboxamide (**40**). Dark yellow solid (m.p. 178°C). Yield % = 20. IR (KBr): 3500, 3300, 3150, 2900, 1640, 1560, 1460 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 6.64 (s, 2H), 4.02 (s, 2H), 2.07 (s, 6H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 172.10, 163.15, 143.25, 123.51, 116.30, 111.20, 19.78 ppm. MS: calculated for $C_7H_{10}N_2OS$ M.W. 170, observed (APCI +) $[M]^+$ 170, (APCI -) $[M-H]^-$ 169 *m/z*.

2-amino-5-methyl-4-propylthiophene-3-carboxamide (**41**). Beige solid (m.p. 164°C). Yield (%) 32. IR (KBr): 3480, 3300, 3180, 2850, 1640, 1554 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 6.74 (s, 2H), 6.26 (s, 2H), 2.08 (s, 3H), 1.34 (m, 2H), 1.07 (t, J = 7.5 Hz, 1H), 0.97 (t, J = 7.5 Hz, 1H), 0.81 (t, J = 7.5 Hz, 1H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 168.12, 163.34, 114.22, 133.50, 129.14, 23.89, 20.12, 14.01, 13.36 ppm. MS: calculated for $C_9H_{14}N_2OS$ M.W. 198, observed (APCI +) $[M]^+$ 198, (APCI -) $[M-H]^-$ 197 *m/z*.

2-amino-4-butyl-5-methylthiophene-3-carboxamide (**42**). Light brown solid. (m.p.= 110 °C).Yield (%) 47. IR (KBr): 3460, 3310, 3200, 2850, 1660 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 6.74 (s, 2H), 6.24 (s, 2H), 2.53 (t, J = 7.3 Hz, 2H), 2.08 (s, 3H), 1.35 (m, 2H), 1.30 (m, 2H), 0.89 (t, J = 8.0 Hz, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 169.56, 162.99, 115.17, 133.54, 129.12, 23.92, 20.20, 14.08, 13.37 ppm. MS: calculated for $C_{10}H_{16}N_2OS$ M.W. 212, observed (APCI +) $[M-H_20]^+$ 195 *m/z*.

2-amino-5-ethyl-4-propylthiophene-3-carboxamide (**43**). Light brown solid. (m.p.= 103 °C).Yield (%) 42. IR (KBr): 3480, 3300, 3180, 2850, 1640, 1554 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 6.76 (s, 2H), 6.23 (s, 2H), 2.52 (t, J = 7.5 Hz, 2H), 1.77 (dd, J = 8.0 Hz, 2H), 1.39 (m, 2H), 1.08 (t, J = 8.0 Hz, 1H), 0.83 (t, J = 8.0 Hz, 2H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 169.24, 163.14, 115.27, 133.50, 129.09, 23.90, 20.17, 14.11, 13.39 ppm. MS: calculated for $C_{10}H_{16}N_2OS$ M.W. 212, observed (APCI +) $[M-H_20]^+$ 195 *m/z*.

2-amino-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carboxamide (**44**). Beige solid. (m.p.= 170 °C).Yield (%) 67. IR (KBr): 3430, 3300, 3200, 2850, 1666 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 6.68 (s, 2H), 6.17 (s, 2H), 2.77 (t, J = 7.3 Hz,, 2H), 2.66 (t, J = 7.3 Hz, 2H), 2.28 (m, 2H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 168.23, 163.15, 139.27, 125.18, 116.72, 33.27, 25.11, 23.18 ppm. MS: calculated for $C_8H_{10}N_2OS$ M.W. 182, observed (ESI +) $[M+H]^+$ 183 *m/z*.

2-amino-4,5,6,7-tetrahydrobenzo[b]thiophene-3-carboxamide (**45**). Beige solid. (m.p.= 152 °C).Yield (%) 61. IR (KBr): 3790, 3482, 3206, 2854, 1645 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 6.89 (s, 2H), 6.52 (s, 2H), 2.79 (t, J = 7.5 Hz, 2H), 2.59 (t, J = 7.5 Hz, 2H), 2.51 (m, 2H), 1.89 (M, 2H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 170.03, 163.22, 139.29, 125.07, 116.99, 33.56, 26.41, 22.00, 21.78 ppm. MS: calculated for $C_9H_{12}N_2OS$ M.W. 196, observed (ESI +) $[M+H]^+$ 197.2 *m/z*.

2-amino-5,6,7,8-tetrahydro-4H-cyclohepta[b]thiophene-3-carboxamide (**46**). Beige solid (m.p. 150°C). Yield (%) 80. IR (KBr): 3450, 3260, 3160, 2850, 1640, 1554 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 6.78 (s, 2H), 6.01 (s, 2H), 2.68 (t, *J* = 5.5Hz, 2H), 2.51 (t, *J* = 5.5Hz, 2H), 1.73 (m, 2H), 1.61(m, 4H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 165.14, 161.23, 139.5, 123.09, 115.71, 32.00, 28.71, 26.15, 21.17, 19.89 ppm. MS: calculated for $C_{10}H_{14}N_2OS$ M.W. 210, observed (APCI +) $[M+H]^+$ 211, (APCI -) $[M-H]^-$ 209 *m/z*.

2-amino-4,5,6,7,8,9-hexahydrocycloocta[b]thiophene-3-carboxamide (**47**). Beige solid. (m.p.= 202 °C).Yield (%) 52. IR (KBr): 3650, 3450, 3190, 2860, 1650 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 6.71 (s, 2H), 6.54 (s, 2H), 2.78 (t, *J* = 7.3 Hz, 2H), 2.66 (t, *J* = 7.3 Hz, 2H), 2.02 (m, 4H), 1.77 (m, 4 H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 167.81, 156.90, 133.18, 119.37, 109.42, 32.07, 29.91, 26.12, 24.33, 24.15. ppm. MS: calculated for $C_{11}H_{16}N_2OS$ M.W. 224, observed(APCI -) $[M-H]^-$ 223 *m/z*.

5,6-dimethyl-2-phenylthieno[2,3-d]pyrimidin-4(3H)-one (**1**) White solid. (m.p.= 298 °C).Yield (%) 71. IR (KBr): 3097, 1660 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.48 (s, 1H), 8.12 (d, *J* = 7.0 Hz, 2H), 7.53 (m, 3H), 2.43 (s, 3H), 2.38 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 169.67, 165.34, 159.30, 134.99, 127.12, 124.33, 122.56, 122.11, 119.03, 117.00, 14.21, 14.03 ppm. MS: calculated for $C_{14}H_{12}N_2OS$ M.W. 256, observed (APCI+) $[M+H]^+$ 257 *m/z*.

2-(4-hydroxyphenyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (**2**). Light yellow solid (m.p. >360°C). Yield (%) = 67. IR (KBr): 3550, 3350, 2980, 1660 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.20 (s, 1H), 10.07 (s, 1H), 8.03 (d, *J* = 8.0 Hz, 2H), 6.87 (d, *J* = 8.0 Hz, 2H), 2.41 (s, 3H), 2.35 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 162.40, 159.98, 157.43, 155.65, 137.12, 130.43, 127.15, 119.00, 19.98, 18.09 ppm. MS: calculated for $C_{14}H_{12}N_2O_2S$ M.W. 272, observed (APCI +) $[M+H]^+$ 273, (APCI -) $[M-H]^-$ 271 *m/z*.

2-(4-(trifluoromethyl)phenyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (**3**). Pale yellow solid (m.p. 330°C). Yield (%) = 82. IR (KBr): 3250, 2965, 1710, 1640 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.69 (s, 1H), 8.43 (d, *J* = 7.9 Hz, 2H), 7.88 (d, *J* = 7.9 Hz, 2H), 2.48 (s, 3H), 2.43 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 164.80, 161.00, 155.89, 135.66, 133.22, 131.12, 129.16, 126.78, 125.34, 121.00, 20.01, 19.99 ppm. HRMS calculated for $C_{15}H_{11}F_3N_2OS$ 324.0544, found 324.0548.

4-(3,4-dihydro-5,6-dimethyl-4-oxothieno[2,3-d]pyrimidin-2-yl)benzoic acid (**4**). Yellow solid (m.p. > 360°C). Yield (%) = 70. IR (KBr): 3500, 2980, 1690, 1620 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.63 (s, 1H), 8.31(d, *J* = 8.1 Hz, 2H), 8.10 (d, *J* = 8.1 Hz, 2H), 2.48 (s, 3H), 2.42 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 171.12, 165.21, 161.00, 156.32, 134.23, 132.00, 129.76, 127.66, 117.99, 19.78, 18.76 ppm. MS: calculated for $C_{15}H_{12}N_2O_3S$ M.W. 300, observed (APCI +) $[M]^+$ 300 *m/z*.

5,6-dimethyl-2-phenylthieno[2,3-d]pyrimidin-4(3H)-one (**5**) Light yellow solid. (m.p.= 321 °C).Yield (%) 69. IR (KBr): 3120, 1660 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.36 (s, 1H), 8.35 (d, *J* = 7.3 Hz, 2H), 7.48. (d, *J* = 7.3 Hz, 2H), 3.67 (s, 3H), 2.39 (s, 3H), 2.12

(s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 169.79, 165.42, 158.99, 135.00, 127.45, 124.13, 122.66, 122.58, 119.63, 117.00, 14.00, 13.89 ppm. MS: calculated for $C_{15}H_{14}N_2O_2S$ M.W. 286, observed (APCI+) $[M+H]^+$ 287 *m/z*.

5,6-dimethyl-2-(3-nitrophenyl)thieno[2,3-d]pyrimidin-4(3H)-one (**6**). Light yellow solid (m.p. 332°C). Yield (%) = 75. IR (KBr): 3350, 2980, 1660, 1450 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.80 (s, 1H), 8.95 (s, 1H), 8.54 (d, *J* = 8.5 Hz, 1H), 8.38 (d, *J* =8.5 Hz, 1H), 7.81 (t, *J* =8.5 Hz, 1H), 2.43 (s, 3H), 2.39 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 163.78, 160.0, 155.89, 153.65, 135.23, 132.43, 129.99, 129.23, 126.71, 118.00, 18.98, 17.87 ppm. MS: calculated for $C_{14}H_{11}N_3O_3S$ M.W. 301, observed (APCI +) $[M+H]^+$ 302, (APCI -) $[M-H]^-$ 300 *m/z*.

2-(3-hydroxyphenyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (**7**). White solid (m.p. 318°C). Yield (%) = 65. IR (KBr): 3520, 2940, 1740, 1630 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.28 (s, 1H), 9.74 (s, 1H), 7.55 (s, 1H), 7.53 (d, *J* = 7.5 Hz, 1H), 7.28 (t, *J* = 7.5 Hz, 1H), 6.94 (d, *J* = 7.5 Hz, 1H), 2.41 (s, 3H), 2.36 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 162.70, 158.98, 156.13, 153.77, 137.12, 130.23, 127.00, 120.00, 19.98, 18.09 ppm. MS: calculated for $C_{14}H_{12}N_2O_2S$ M.W. 272, observed (APCI +) $[M+H]^+$ 273, (APCI -) $[M-H]^-$ 271 *m/z*.

2-(3-(trifluoromethyl)phenyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (**8**). Light yellow solid (m.p. 295°C). Yield (%) = 68. ^{1}H NMR (500 MHz, DMSO): δ 12.72 (s, 1H), 8.47 (s, 1H), 8.42 (d, *J* = 8.0 Hz, 1H), 7.92 (d, *J* = 8.0 Hz, 1H), 7.77 (t, *J* = 8.0 Hz, 1H), 2.42 (s. 3H), 2.38 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 163.18, 160.80, 153.09, 135.69, 133.29, 131.45, 129.26, 126.88, 124.44, 121.50. 20.16, 19.87 ppm. MS: calculated for $C_{15}H_{11}F_3N_2OS$ M.W. 324, observed (APCI +) $[M+H]^+$ 325, (APCI -) $[M-H]^-$ 323 *m/z*.

2-(3,4-dihydroxyphenyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (**9**). Yellow solid (m.p. 337°C). Yield (%) = 71. IR (KBr): 3500, 2980, 1670 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.27 (s, 1H), 7.57 (s, 1H), 7.48 (dd, *J* = 7.1 Hz, 1H), 6.80 (d, *J* = 7.1 Hz, 1H), 2.39 (s, 3H), 2.34 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 165.88, 158.76, 159.00, 145.98, 143.73, 135.22, 132.44, 122.11, 117.88, 113. 30, 19.33, 18.99 ppm. HRMS calculated for $C_{14}H_{12}N_2O_3S$ 288.0569, found 288.0574.

2-(4-chloro-3-nitrophenyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (**10**). Pale green solid (m.p. >360°C). Yield (%) = 68. IR (KBr): 3350, 2940, 1665 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.78 (s, 1H), 8.79 (s, 1H), 8.41 (d, *J* = 8.5 Hz, 1H), 7.94 (d, *J* = 8.5 Hz, 1H), 2.42 (s, 3H), 2.38 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 165.88, 159.77, 155.80, 149.88, 140.10, 134.34, 133.66, 130.00, 122.24, 118.00, 19.47, 18.99 ppm. HRMS calculated for $C_{14}H_{10}ClN_3O_3S$ 335.0131, found 355.0131.

2-(3,4-difluorophenyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (**11**). Grey solid (m.p. 345°C). Yield (%) = 75. IR (KBr): 3220, 2940, 1740, 1630 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.55 (s, 1H), 8.18 (d, *J* = 9.0 Hz, 1H), 8.03 (s, 1H), 7.58 (d, *J* = 9.0 Hz, 1H), 2.41 (s, 3H), 2.37 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 162.00, 159.89,

156.80, 152.00, 149.67, 134.67, 132.44, 126.55, 123.33, 119.88, 115.42, 19.67, 18.99 ppm. HRMS calculated for $C_{14}H_{10}F_2N_2OS$ 293.0482, found 292.0486.

2-(3,5-bis(trifluoromethyl)phenyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (**12**). White solid (m.p. 308°C). Yield (%) = 74. ^{1}H NMR (500 MHz, DMSO): δ 12.27 (s, 1H), 8.68 (s, 1H), 8.04 (s, 1H), 7.26 (s, 1H), 2.38 (s, 3H), 2.30 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 162.33, 159.98, 157.77, 135.01, 132.11, 131.00, 124.55, 122.17, 118.00, 19.87, 19.33 ppm. HRMS calculated for $C_{16}H_{10}F_6N_2OS$ 392.0418, found 392.0423.

2-(3-fluoro-4-hydroxyphenyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (**13**). Green solid (m.p. >360°C). Yield (%) = 75. ^{1}H NMR (500 MHz, DMSO): δ 12.28 (s, 1H), 7.96 (d, *J* = 12.5 Hz, 1H), 7.85 (d, *J* = 8.5 Hz, 1H), 7.05 (t, *J* = 85. Hz, 1H), 2.39 (s, 3H), 2.34 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 161.99, 159.90, 155.60, 153.83, 149.10, 133.55, 132.88, 123.21, 117.00, 112.08, 19.99, 19.64 ppm. HRMS calculated for $C_{14}H_{11}FN_2O_2S$ 290.0525, found 290.0526.

2-(3,4-dimethoxyphenyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (**14**). Yellow solid (m.p. 290°C). Yield (%) = 70. ^{1}H NMR (500 MHz, DMSO): δ 12.34 (s, 1H), 7.79 (d, *J* = 8.5 Hz, 1H), 7.72 (s, 1H), 7.07 (d, *J* = 8.5 Hz, 1H), 3.85 (s, 3H), 3.82 (s, 3H), 2.40 (s, 3H), 2.35 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 161.80, 159.07, 157.77, 151.32, 149.90, 135.66, 133.12, 122.00, 119.34, 117.99, 111.32, 56.24, 19.98, 19.67 ppm. MS: calculated for $C_{16}H_{16}N_2O_3S$ M.W. 316, observed (APCI +) $[M+H]^+$ 317, (APCI -) $[M-H]^-$ 315 *m/z*.

2-(4-hydroxy-3-methoxyphenyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (**15**). Yellow solid (m.p. 350°C). Yield (%) = 67. ^{1}H NMR (500 MHz, DMSO): δ 12.25 (s, 1H), 9.72 (bs, 1H), 7.72 (s, 1H), 7.66 (d, *J* = 8.0 Hz, 1H), 6.87 (d, *J* = 8.0 Hz, 1H), 3.86 (s, 1H), 2.40 (s, 1H), 2.35 (s, 1H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 162.50, 159.00, 157.14, 151.28, 147.10, 138.66, 131.19, 122.10, 118.38, 118.01, 111.39, 57.14, 19.99, 19.70 ppm. MS: calculated for $C_{15}H_{14}N_2O_3S$ M.W. 302, observed (APCI +) $[M+H]^+$ 303, (APCI -) $[M-H]^-$ 301 *m/z*.

5,6-dimethyl-2-(pyridin-3-yl)thieno[2,3-d]pyrimidin-4(3H)-one (**16**). Dark grey solid. (m.p. >360 °C). Yield (%) = 73. IR (KBr): 3180, 2940, 1740, 1640 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.68 (s, 1H), 9.26 (s, 1H), 8.71 (d, *J* = 6.0 Hz, 1H), 8.43 (d, *J* = 8.0 Hz, 1H), 7.54 (m, 1H), 2.42 (s, 3H), 2.38 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 164,89, 162.01, 156.00, 154.85, 136.09, 135.23, 132.68, 125.35, 1191, 19.22, 18.20 ppm. MS: calculated for $C_{13}H_{11}N_3OS$ M.W. 257, observed (APCI +) $[M+H]^+$ 258 (APCI -) $[M-H]^-$ 256 *m/z*.

5,6-dimethyl-2-(pyridin-2-yl)thieno[2,3-d]pyrimidin-4(3H)-one (**17**). Pale green solid. (m.p. >360°C). Yield (%) = 61. IR (KBr): 3210, 2950, 1740, 1640 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 11.62 (s, 1H), 8.74 (d, *J* = 4.5 Hz, 1H), 8.34 (d, *J* = 7.5 Hz, 1H), 8.04 (t, *J* = 7.5 Hz, 1H), 7.63 (t, *J* = 4.5Hz, 1H), 2.43 (s, 3H), 2.39 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 163.99, 160.08, 155.00, 154.55, 136.13, 134.22, 132.67, 125.16,

118.02, 18.22, 17.98 ppm. MS: calculated for $C_{13}H_{11}N_3OS$ M.W. 257, observed (APCI +) $[M]^+$ 257.1 *m/z*.

2-(1H-indol-2-yl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (**18**). Brown solid (m.p. >360°C). Yield (%) = 64. IR (KBr): 3350, 3230, 2960, 2750, 1665 cm^{-1}. ^1H NMR (500 MHz, DMSO): 12.06 (s, 1H), 11.84 (s, 1H), 8.50 (s, 1H), 8.43 (d, *J* = 7.2 Hz, 1H), 7.46 (d, *J* = 7.2 Hz, 1H), 7.22-7.16 (m, 2H), 2.40 (s, 3H), 2.35 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 169.00, 163.89, 160.11, 155.66, 138.24, 133.56, 132.16, 130. 87, 126.11, 122.34, 120.56, 111.22, 19.96, 18.09 ppm. HRMS calculated for $C_{16}H_{13}N_3OS$ 295.0779, found 295.0781.

5,6-dimethyl-2-(5-nitrofuran-2-yl)thieno[2,3-d]pyrimidin-4(3H)-one (**19**). Dark orange solid (m.p. >360°C). Yield (%) = 70. IR (KBr): 3350, 3000, 1670 cm^{-1}. ^1H NMR (500 MHz, DMSO): δ 12.96 (s, 1H), 7.85 (d, *J* = 4 Hz, 1H), 7.80 (d, *J* = 4 Hz, 1H), 2.41 (s, 3H), 2.39 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 162.99, 160.77, 156.71, 154.00, 148.11, 135.13, 134.00, 117.09, 112.99, 20.00, 19.79 ppm. MS: calculated for $C_{12}H_9N_3O_4S$ M.W. 291, observed (APCI +) $[M+H]^+$ 292, (APCI -) $[M-H]^-$ 290 *m/z*.

2-phenyl-3,5,6,7,8,9-hexahydro-4H-cyclohepta[4,5]thieno[2,3-d]pyrimidin-4-one (**20**). Beige solid (m.p. 307°C). Yield (%) = 77. ^1H NMR (500 MHz, DMSO): δ 12.47 (s, 1H), 8.11 (d, *J* = 7.5 Hz, 2H), 7.55 (dd, *J* = 7.5 Hz, 3H), 3.30 (t, *J* = 5.5 Hz, 2H), 2.85 (t, *J* = 5.5 Hz, 2H), 1.86 (m, 2H), 1.65 (m, 4H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 161.12, 156.57, 153.78, 149.90, 144.27, 138.00, 126.78, 121.48, 120.05, 118.67, 115,10, 112.24, 33.03, 29.89, 28.45, 20.00 ppm. MS: calculated for $C_{17}H_{16}N_2OS$ M.W. 296, observed (APCI +) $[M+H]^+$ 297, (APCI -) $[M-H]^-$ 295 *m/z*.

4-(4-oxo-3,5,6,7,8,9-hexahydro-4H-cyclohepta[4,5]thieno[2,3-d]pyrimidin-2-yl)benzoic acid (**21**). Yellow solid (m.p. >360°C). Yield (%) = 65. ^1H NMR (500 MHz, DMSO): δ 13.14 (bs, 1H), 12.61 (s, 1H), 8.21 (d, *J* = 8.0 Hz, 2H), 8.04 (d, *J* = 8.0 Hz, 2H), 3.29 (t, *J* = 5.5Hz, 2H), 2.86 (t, *J* = 5.5Hz, 2H), 1.86 (m, 2H), 1.65 (m, 4H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 169.88, 163.21, 160.00, 155.19, 134.23, 133.07, 129.11, 127.16, 118.02, 33.57, 29.98, 28.63, 20.03 ppm. MS: calculated for $C_{18}H_{16}N_2O_3S$ M.W. 340, observed (APCI +) $[M+H]^+$ 341, (APCI -) $[M-H]^-$ 339 *m/z*.

2-[4-(trifluoromethyl)phenyl]-3,5,6,7,8,9-hexahydro-4H-cyclohepta[4,5]thieno[2,3-d]pyrimidin-4-one (**22**). Beige solid (m.p. 320°C). Yield (%) = 62. ^1H NMR (500 M Hz, DMSO): δ 12.70 (s, 1H), 8.31 (d, *J* = 8.0 Hz, 2H), 7.87 (d, *J* = 8.0 Hz, 2H), 3.29 (t, *J* = 5.5Hz, 2H), 2.86 (t, *J* = 5.5Hz, 2H), 1.85 (m, 2H), 1.65 (m, 4H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 163.80, 161.26, 155.78, 135.44, 133.00, 132.12, 128.86, 126.78, 124.22, 120.90, 33.17, 29.98, 28.68, 20.04 ppm. HRMS calculated for $C_{18}H_{15}F_3N_2OS$ 364.0857, found 364.0860.

2-(4-hydroxyphenyl)-3,5,6,7,8,9-hexahydro-4H-cyclohepta[4,5]thieno[2,3-d]pyrimidin-4-one (**23**). Light yellow solid (m.p. 311°C). Yield (%) = 75. ^1H NMR (500 MHz, DMSO): δ 12.20 (s, 1H), 10.11 (s, 1H), 7.99 (d, *J* = 8.5Hz, 2H), 6.86 (d, *J* = 8.5Hz, 2H), 3.26 (t, *J* = 5.5Hz, 2H), 2.83 (t, *J* = 5.5Hz, 2H), 1.84 (m, 2H), 1.63 (m, 4H) ppm. ^{13}C

NMR (500 MHz, DMSO): δ 162.22, 158.89, 155.67, 149.90, 148,09, 144.27, 139.22, 121.86, 119.96, 118.43, 115,12, 112.00, 33.07, 29.41, 28.55, 19.98 ppm. MS: calculated for $C_{17}H_{16}N_2O_2S$ M.W. 312, observed (APCI +) $[M+H]^+$ 313, (APCI -) $[M-H]^-$ 311 *m/z*.

2-(3,4-dihydroxyphenyl)-6-methyl-5-propylthieno[2,3-d]pyrimidin-4(3H)-one (**24**). Grey solid (m.p. 280°C). Yield (%) = 71. ^{1}H NMR (500 MHz, DMSO): δ11.99 (s, 1H), 9.40 (bs, 2H), 7.57 (s, 1H), 7.49 (d, *J* = 8.5 Hz, 2H), 6.82 (d, *J* = 8.5 Hz, 2H), 2.83 (m, 2H), 2.36 (s, 3H), 1.54 (q, 2H), 0.88 (t, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 163.58, 157.89, 158.90, 145.96, 142.73, 135.77, 131.44, 122.00, 118.18, 113. 30, 23.95, 21.56, 15.08, 13.44 ppm. HRMS calculated for $C_{16}H_{16}N_2O_3S$ 316.0882, found 316.0884.

5-butyl-2-(3,4-dihydroxyphenyl)-6-methylthieno[2,3-d]pyrimidin-4(3H)-one (**25**). Grey solid (m.p. 285 °C). Yield (%) = 70. IR (KBr): 3500, 2980, 1670, 1553 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.14 (s, 1H), 9.62 (s, 2H), 9.23 (s, 1H), 7.57 (s, 1H), 7.49 (d, *J* = 8.0 Hz, 1H), 2.37 (s, 3H), 1.51 (t, *J* = 7.0 Hz, 2H), 1.31 (m, 2H), 0.95 (m, 2H), 0.89 (t, *J* = 7.5 Hz, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 163.88, 158.89, 157.70, 145.45, 142.13, 136.77, 132.24, 122.20, 118.19, 113. 30, 24.01, 21.66, 15.08, 13.44, 13.26 ppm. HRMS calculated for $C_{17}H_{18}N_2O_3S$ 330.1038, found 330.1039.

2-(3,4-dihydroxyphenyl)-6-ethyl-5-propylthieno[2,3-d]pyrimidin-4(3H)-one (**26**). Beige solid. (m.p. >360 °C). Yield (%) = 65. IR (KBr): 3550, 2940, 1660, 1550 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.11 (s, 1H), 9.62 (s, 2H), 9.24 (s, 1H), 7.58 (s, 1H), 7.49 (d, *J* = 8.5 Hz, 1H), 6.81 (d, *J* = 8.5 Hz, 1H), 2.85 (m, 4 H), 1.56 (m, 2H) 1.23 (t, *J* = 7 Hz, 3H), 0.91 (t, *J* = 7 Hz, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 163.45, 157.89, 157.00, 146.01, 142.89, 135.75, 133.44, 121.10, 119.18, 113. 30, 24.14, 21.56, 15.01, 13.46, 13.05 ppm. HRMS calculated for $C_{17}H_{18}N_2O_3S$ 330.1038, found 330.1042.

2-(3,4-dihydroxyphenyl)-3,5,6,7-tetrahydro-4H-cyclopenta[4,5]thieno[2,3-d]pyrimidin-4-one (**27**). Dark grey solid (m.p. >360°C). Yield (%) = 75. IR (KBr): 3550, 2940, 1660, 1550 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.23 (s, 1H), 9.64 (s, 1H), 9.24 (s, 1H), 7.59 (s, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 6.81 (d, *J* = 8.0 Hz, 1H), 2.91 (dd, *J* = 7.5 Hz, 4H), 2.38 (t, *J* = 7.5 Hz, 2H), ppm. ^{13}C NMR (500 MHz, DMSO): δ 163.00, 159.88, 155.24, 148.87, 147,53, 146.67 140.59, 122.89, 121.07, 119.09, 114,18, 112.40, 30.11, 28.10 ppm. HRMS calculated for $C_{15}H_{12}N_2O_3S$ 300.0569, found 300.0566.

2-(3,4-dihydroxyphenyl)-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4(3H)-one (**28**). Light brown solid (m.p. >360°C). Yield (%) = 75. IR (KBr): 3550, 2940, 1660, 1550 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.14 (s, 1H), 9.63 (s, 1H), 9.22 (s, 1H), 7.59 (s, 1H), 7.51 (d, *J* = 8.0 Hz, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 2.88 (t, *J* = 5.5 Hz, 2H), 2.82 (t, *J* = 5.5 Hz, 2H), 1.81 (m, 2H), 1.80 (m, 2H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 162.53, 159.79, 155.22, 148.80, 147,57, 145.27, 140.58, 122.89, 120.00, 119.00, 114,15, 112.00, 32.11, 29.00, 20.03 ppm. HRMS calculated for $C_{16}H_{14}N_2O_3S$ 314.0725, found 314.0728.

2-(3,4-dihydroxyphenyl)-3,5,6,7,8,9-hexahydro-4H-cyclohepta[4,5]thieno[2,3-d]pyrimidin-4-one (**29**). Grey solid (m.p. >360°C). Yield (%) = 78. ^{1}H NMR (500 MHz, DMSO): δ 12.12 (s, 1H), 9.60 (s, 1H), 9.21 (s, 1H), 7.56 (s, 1H), 7.49 (d, *J* = 8.5 Hz, 1H), 6.81 (d, *J* = 8.5Hz, 1H), 3.25 (t, *J* = 5.5Hz, 2H), 2.81 (t, *J* = 5.5Hz, 2H), 1.84 (m, 2H), 1.63 (m, 4H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 162.55, 159.88, 155.20, 148.90, 147,74, 144.27, 139.56, 121.89, 120.16, 118.99, 114,15, 112.00, 32.11, 29.00, 28.55, 19.67 ppm. HRMS calculated for $C_{17}H_{16}N_2O_3S$ 328.0882, found 328.0882.

2-(3,4-dihydroxyphenyl)-5,6,7,8,9,10-hexahydrocycloocta[4,5]thieno[2,3-d]pyrimidin-4(3H)-one (**30**). Beige solid (m.p. >360°C). Yield (%) = 65. IR (KBr): 3570, 2940, 1640, 1550 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.14 (s, 1H), 9.62 (s, 1H), 9.23 (s, 1H), 7.58 (s, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 6.82 (d, *J* = 8.0 Hz, 1H), 3.05 (t, *J* = 6.0 Hz, 2H), 2.84 (t, *J* = 6.0 Hz, 2H), 1.61 (m, 4H), 1.41 (m, 2H), 1.28 (m, 2H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 162.99, 160.65, 154.22, 149.70, 146,59, 145.28, 140.61, 122.89, 120.10, 119.30, 114,01, 112.036, 32.15, 29.13, 20.03, 19.65 ppm. HRMS calculated for $C_{18}H_{18}N_2O_3S$ 342.1038, found 342.1042.

2-(2,3-dihydroxyphenyl)-6-methyl-5-propylthieno[2,3-d]pyrimidin-4(3H)-one (**31**). Beige solid. (m.p. >360 °C). Yield (%) = 70. IR (KBr): 3500, 2980, 1670, 1553 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.22 (s, 1H), 8.57 (bs, 2H), 7.57 (d, *J* = 8 Hz, 1H), 6.95 (d, *J* = 8 Hz, 1H), 6.76 (t, *J* = 8 Hz, 1H), 2.85 (t, *J* = 7 Hz, 2H), 2.40 (s, 3H) 1.57 (m, 2H), 0.90 (t, *J* = 7 Hz, 3H), ppm. ^{13}C NMR (500 MHz, DMSO): δ 163.78, 162.82, 155.00, 154.26, 137.45, 133.16, 132.76, 126.14, 119.00, 20.01, 19.87, 17.21, 15.68 ppm. HRMS calculated for $C_{16}H_{16}N_2O_3S$ M.W. 316, found (APCI +) $[M+H]^+$ 317.5 *m/z*.

2-(2,3-dihydroxyphenyl)-3,5,6,7,8,9-hexahydro-4H-cyclohepta[4,5]thieno[2,3-d]pyrimidin-4-one (**32**). Beige solid. (m.p. >360 °C). Yield (%) = 68. IR (KBr): 3500, 2980, 1650, 1554 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.12 (s, 1H), 9.50 (bs, 2H), 7.59 (d, *J* = 8.0 Hz, 1H), 6.97 (d, *J* = 8.0 Hz, 1H), 6.77 (t, *J* = 8.0 Hz, 1H), 3.27 (t, *J* = 5.0 Hz, 2H), 2.85 (t, *J* = 5.0 Hz, 2H), 1.85 (m, 2H), 1.64 (m, 2H), 1.60 (m, 2H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 165.01, 162.12, 155.10, 153.24, 137.17, 134.12, 132.69, 125.16, 118.21, 33.84, 31.15, 29.64, 22.20 ppm. HRMS calculated for $C_{17}H_{16}N_2O_3S$ 328.0882, found 328.0881.

2-(2,4-dihydroxyphenyl)-3,5,6,7,8,9-hexahydro-4H-cyclohepta[4,5]thieno[2,3-d]pyrimidin-4-one (**33**). Beige solid (m.p. >360°C). Yield (%) = 72. IR (KBr): 3570, 2940, 1640, 1560 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 12.27 (s, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.74 (s, 1H), 7.44 (d, *J* = 8.0 Hz, 1H), 3.26 (t, *J* = 5.0 Hz, 2H), 2.83 (t, *J* = 5.0 Hz, 2H), 1.85 (m, 2H), 1.66 (m, 2H), 1.60 (m, 2H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 166.51, 162.16, 155.19, 153.43, 137.19, 134.32, 132.68, 125.08, 118.71, 33.64, 31.10, 28.45, 21.90 ppm. HRMS calculated for $C_{17}H_{16}N_2O_3S$ 328.0882, found 328.0881.

2-(2,5-dihydroxyphenyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (**34**). Yellow solid (m.p. > 360 °C). Yield (%) = 75. IR (KBr): 3500, 2980, 1670 cm^{-1}. ^{1}H NMR (500 MHz, DMSO): δ 11.96 (s, 1H), 9.09 (bs, 2H), 7.58 (s, 1H), 6.87 (d, *J* = 5.0 Hz, 2H), 2.41 (s, 3H), 2.37 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 165.88, 158.76, 159.00,

145.98, 143.73, 135.22, 132.44, 122.11, 117.88, 113. 30, 19.33, 18.99 ppm. HRMS calculated for $C_{14}H_{12}N_2O_3S$ 288.0569, found 288.0567.

2-(2,5-dihydroxyphenyl)-3,5,6,7,8,9-hexahydro-4H-cyclohepta[4,5]thieno[2,3-d]pyrimidin-4-one (**35**). Yellow solid. (m.p. > 360°C). Yield (%) = 61. IR (KBr): 3500, 2980, 1650, 1554 cm^{-1}. ^1H NMR (500 MHz, DMSO): δ 11.94 (s, 1H), 11.16 (bs, 1H), 9.08 (bs, 1H), 7.56 (s, 1H), 6.85 (d, *J* = 4.5 Hz, 2H), 3.33 (t, *J* = 5.1 Hz, 2H), 2.84 (t, *J* = 5.1 Hz, 2H), 1.85 (m, 2H), 1.64 (m, 2H), 1.59 (m, 2H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 165.33, 163.00, 155.56, 152.99, 137.14, 134.44, 133.00, 125.19, 118.24, 33.88, 31.27, 29.69, 22.14 ppm. HRMS calculated for $C_{17}H_{16}N_2O_3S$ 328.0882, found 328.0883.

2-(3,5-dihydroxyphenyl)-5,6-dimethylthieno[2,3-d]pyrimidin-4(3H)-one (**36**). Dark grey solid (m.p. > 360 °C). Yield (%) = 72. IR (KBr): 3600, 2930, 1650 cm^{-1}. ^1H NMR (500 MHz, DMSO): δ 12.25 (s, 1H), 9.54 (s, 2H), 6.95 (s, 2H), 6.40 (s, 1H), 2.50 (s, 3H), 2.40 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 165.47, 159.16, 157.00, 146.00, 143.69, 135.42, 132.21, 122.17, 117.98, 113. 60, 20.03, 18.99 ppm. HRMS calculated for $C_{14}H_{12}N_2O_3S$ 288.0569, found 288.0568.

2-(3,5-dihydroxyphenyl)-3,5,6,7,8,9-hexahydro-4H-cyclohepta[4,5]thieno[2,3-d]pyrimidin-4-one (**37**). Dark grey solid. (m.p. > 360°C). Yield (%) = 68. IR (KBr): 3550, 2960, 1650, 1557 cm^{-1}. ^1H NMR (500 MHz, DMSO): δ 12.25 (s, 1H), 9.53 (bs, 2H), 6.50 (s, 2H), 6.40 (s, 1H), 3.26 (t, *J* = 5.0 Hz, 2H), 2.82 (t, *J* = 5.0 Hz, 2H), 1.85 (m, 2H), 1.64 (m, 2H), 1.59 (m, 2H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 165.33, 163.00, 155.56, 152.99, 137.14, 134.44, 133.00, 125.19, 118.24, 33.88, 31.27, 29.69, 22.14 ppm. HRMS calculated for $C_{17}H_{16}N_2O_3S$ 328.0882, found 328.0883.

5,6-dimethyl-2-(3,4,5-trihydroxyphenyl)thieno[2,3-d]pyrimidin-4(3H)-one (**38**). Brown solid (m.p. > 360 °C). Yield (%) = 75. IR (KBr): 3600, 2930, 1650 cm^{-1}. ^1H NMR (500 MHz, DMSO): δ 12.07 (s, 1H), 9.13 (s, 2H), 8.34 (s, 1H), 7.13 (s, 2H), 2.50 (s, 3H), 2.38 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 165.68, 160.08, 158.00, 146.50, 143.89, 135.63, 132.61, 123.20, 118.13, 113.80, 20.03, 19.03 ppm. HRMS calculated for $C_{14}H_{12}N_2O_4S$ 377.0831, found 377.0831.

5,6-dimethyl-2-(2,4,5-trihydroxyphenyl)thieno[2,3-d]pyrimidin-4(3H)-one one (**39**). Light brown solid (m.p. > 360 °C). Yield (%) = 65. IR (KBr): 3600, 2930, 1650 cm^{-1}. ^1H NMR (500 MHz, DMSO): δ 11.81 (s, 1H), 9.98 (s, 2H), 8.70 (s, 1H), 7.64 (s, 1H), 6.44 (s, 1H), 2.39 (s, 3H), 2.35 (s, 3H) ppm. ^{13}C NMR (500 MHz, DMSO): δ 166.13, 160.63, 159.01, 147.00, 143.99, 135.61, 132.71, 123,12, 118.17, 113.60, 21.30, 20.92 ppm. HRMS calculated for $C_{14}H_{12}N_2O_4S$ 304.0518, found 304.0518.

Table

Table S1. Effect of RT p51 thumb domain mutations on compound **9**

p51 mutants	IC_{50} (μM)
WT	0.26 ± 0.01
K275A	0.31 ± 0.01
V276A	0.39 ± 0.04
R277A	0.26 ± 0.002
Q278A	0.30 ± 0.01
L279A	0.42 ± 0.03
C280A	0.32 ± 0.01
K281A	0.33 ± 0.01
L282A	0.38 ± 0.01
L283A	0.61 ± 0.02
R284A	0.30 ± 0.01
G285A	0.27 ± 0.01
T286A	0.28 ± 0.04

Figure

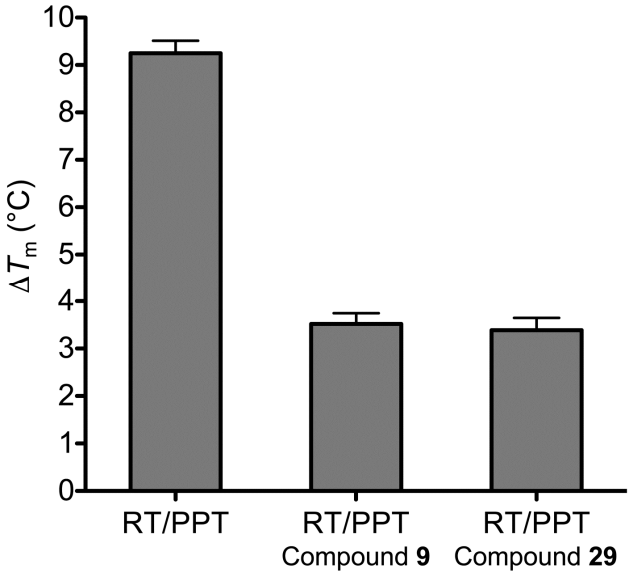

Figure S1. Effect of thienopyrimidinone RNase H inhibitors on the thermal stability of p66/p51 HIV-1 RT in the presence of a PPT-containing RNA/DNA hybrid. T_m values are the average of triplicate analysis.