Supplemental Information

for

3-Hydroxyquinolin-2(1*H*)-ones as Inhibitors ofInfluenza A Endonuclease

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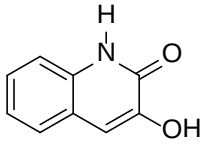
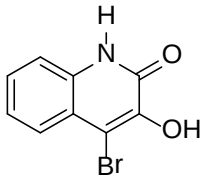
Chemistry: General Methods.

All reactions, unless otherwise stated, were done under nitrogen atmosphere. Reaction monitoring and follow-up were done using aluminum backed Silica G TLC plates with UV254 (Sorbent Technologies), visualizing with ultraviolet light. Flash column chromatography was done on a Combi Flash Rf Teledyne ISCO using hexane, ethyl acetate, dichloromethane, and methanol. The ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were done in $CDCl_3$, Methanol-d_4, and DMSO-d_6 and recorded on a Bruker Avance III (400 MHz) Multinuclear NMR Spectrometer. Data is expressed in parts per million relative to the residual nondeuterated solvent signals, spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublets), t (triplet), dt (doublet of triplets), q (quartet), m (multiplet), and bs (broad singlet), and coupling constants (*J*) are reported in Hertz. Melting points were determined using Mel-temp II apparatus and are uncorrected. HRMS experiments were conducted by Washington University Resource for Biomedical and Bioorganic Mass Spectrometry Department of Chemistry.



3-Hydroxyquinolin-2(1*H*)-one (1)

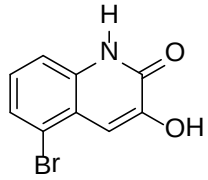
1*H*-Indole-2,3-dione (147.03 mg, 1 mmol), (trimethylsilyl)diazomethane (0.5 mL of 2M in toluene, 1 mmol), and triethylamine (0.14 mL, 2 mmol) were dissolved in ethanol (5 mL) and placed under argon. It was stirred at room temperature for 15 hours. A yellow suspension was formed. Then, the reaction mixture was filtered and the solid was collected and dried under vacuum to reveal 3-hydroxyquinoli-2(1*H*)-one as a beige solid (89.2 mg, 55%); mp = 248-250 ^{o}C; ^{1}H NMR (400MHz, DMSO-d_6) δ 11.98 (s, 1H), 9.42 (s, 1H), 7.48 (d, *J* = 8 Hz, 1H), 7.31-7.24 (m, 2H), 7.12 (t, *J* = 8 Hz, 1H) 7.08 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.5, 146.2, 133.5, 126.2, 125.8, 122.0, 120.7, 114.7, 112.4



4-Bromo-3-hydroxyquinolin-2(1*H*)-one (2)

3-Hydroxyquinolin-2(1*H*)-one (120.6 mg, 0.748 mmol) and N-bromosuccinimide (139.7 mg, 0.785 mmol) were dissolved in DMF (5 mL) and placed under argon. It was stirred at room temperature for 15 hours, and it became an orange solution. Then, DMF was removed via Kugelrohr distillation and the resulting residue was suspended in DCM. It was filtered and the solid was washed with methanol. The solid was collected and dried under vacuum to reveal 4-

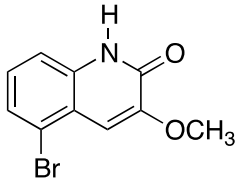
bromo-3-hydroxyquinolin-2(1*H*)-one as an orange solid (100 mg, 56%); mp = 243-245 ^{o}C; ^{1}H NMR (400MHz, DMSO-d_6) δ 12.31 (s, 1H), 10.40 (s, 1H), 7.73 (d, *J* = 8 Hz, 1H), 7.41 (t, *J* = 8 Hz, 1H), 7.33 (d, *J* = 8 Hz, 1H), 7.28 (t, *J* = 8 Hz, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.6, 145.0, 132.5, 127.4, 125.1, 122.9, 119.7, 115.3, 109.2



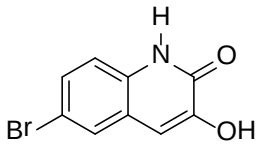
5-Bromo-3-hydroxyquinolin-2(1H)-one (3)

5-Bromo-3-methoxyquinolin-2(1*H*)-one (70 mg, 0.276 mmol) was dissolved in anhydrous DCM (5 mL) and placed under argon. The reaction mixture was cooled to 0 ^{o}C and the 1M in DCM BBr_3 (3 mL, 3 mmol) was added. It was then allowed to warm to room temperature and stirred for 36 hours. Then, the solvent was removed under reduced pressure. The resulting residue was suspended in DCM and it was filtered. The solid was washed with methanol. The solid was collected and dried under vacuum to reveal 5-bromo-3-hydroxyquinolin-2(1*H*)-one as a white solid (42.9 mg, 65%); mp = 308-310 ^{o}C ^{1}H NMR (400MHz, DMSO-d_6) δ 12.21 (s, 1H), 10.04 (s, 1H), 7.44 (d, *J* = 8 Hz, 1H), 7.29 (d, *J* = 8 Hz, 1H), 7.24 – 7.20 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.1, 147.9, 134.5, 127.2, 125.8, 119.6, 119.1, 114.7, 110.7 HRMS (ESI) calculated for $C_9H_5BrNO_2$ $(M-H)^-$ 239.9498, found 239.9507.

5-Bromo-3-methoxyquinolin-2(1H)-one

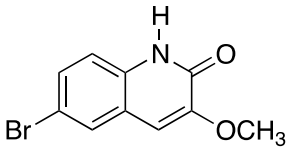


1*H*-4-Bromoindole-2,3-dione (452.06 mg, 2 mmol), (trimethylsilyl)diazomethane (2 mL of 2M in toluene, 4 mmol), and triethylamine (0.56 mL, 4 mmol) were dissolved in ethanol (10 mL) and placed under argon. It was stirred at room temperature for 18 hours. The resulting suspension was filtered and the solid was collected and dried under vacuum to reveal 5-bromo-3-methoxyquinolin-2(1*H*)-one as a white solid (267 mg, 53%); mp = 286 - 288 ^{o}C; ^{1}H NMR (400MHz, DMSO-d_6) δ 11.97 (s, 1H), 7.31 (dd, *J* = 8 Hz, , *J* = 2 Hz, 1H), 7.15-7.09 (m, 2H), 7.02 (s, 1H), 3.73 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.9, 150.0, 135.0, 128.0, 125.8, 119.7, 118.6, 114.6, 109.0, 55.7 HRMS (ESI) calculated for $C_{10}H_9BrNO_2$ $(M+H)^+$ 253.9811, found 253.9811.



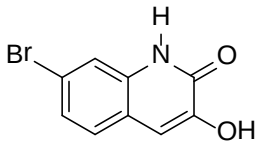
6-Bromo-3-hydroxyquinolin-2(1*H*)-one (4)

6-Bromo-3-methoxyquinolin-2(1*H*)-one (70 mg, 0.276 mmol) was dissolved in anhydrous DCM (5 mL) and placed under argon. The reaction mixture was cooled to 0^{o}C and the 1M in DCM BBr_3 (3 mL, 3 mmol) was added. It was then allowed to warm to room temperature and stirred for 42 hours. Then, the solvent was removed under reduced pressure. The resulting residue was suspended in DCM and it was filtered. The solid was washed with methanol. The solid was collected and dried under vacuum to reveal 6-bromo-3-hydroxyquinolin-2(1*H*)-one as a beige solid (38.2 mg, 58%); mp 272 = 274 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.11 (s, 1H), 9.73 (s, 1H), 7.75 (d, *J* = 2 Hz, 1H), 7.43 (dd, *J* = 8 Hz, *J* = 2 Hz, 1H), 7.20 (d, *J* = 8 Hz, 1H), 7.08 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.3, 147.1, 132.6, 128.7, 127.6, 122.8, 116.7, 113.8, 111.3 HRMS (ESI) calculated for $C_9H_5BrNO_2$ $(M-H)^-$ 237.9498, found 237.9505.



6-Bromo-3-methoxyquinolin-2(1*H*)-one

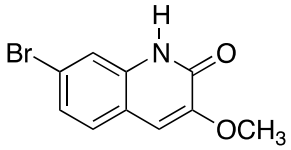
1*H*-5-Bromoindole-2,3-dione (452.06 mg, 2 mmol), (trimethylsilyl)diazomethane (2 mL of 2M in toluene, 4 mmol), and triethylamine (0.56 mL, 4 mmol) were dissolved in ethanol (10 mL) and placed under argon. It was stirred at room temperature for 18 hours. The resulting suspension was filtered and the solid was collected and dried under vacuum to reveal 6-bromo-3-methoxyquinolin-2(1*H*)-one as a white solid (273.6 mg, 54%); mp = 265- 267 ^{o}C; ^{1}H NMR (400MHz, DMSO-d_6) δ 11.92 (s, 1H), 7.73 (s, 1H), 7.40 (d, *J* = 8 Hz, 1H), 7.15-7.12 (m, 2H), 3.74 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.0, 149.5, 133.0, 129.4, 128.1, 121.8, 116.6, 113.6, 110.1, 55.7 HRMS (ESI) calculated for $C_{10}H_9BrNO_2$ $(M+H)^+$ 253.9811, found 253.9811.



7-Bromo-3-hydroxyquinolin-2(1*H*)-one (5)

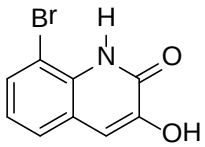
7-Bromo-3-methoxyquinolin-2(1*H*)-one (53 mg, 0.208 mmol) was dissolved in anhydrous DCM (5 mL) and placed under argon. The reaction mixture was cooled to 0 ^{o}C and the 1M in DCM

BBr_3 (2 mL, 2 mmol) was added. It was then allowed to warm to room temperature and stirred for 24 hours. Then, the solvent was removed under reduced pressure. The resulting residue was suspended in DCM and it was filtered. The solid was washed with methanol. The solid was collected and dried under vacuum to reveal 7-bromo-3-hydroxyquinolin-2(1*H*)-one as a gray solid (24.9 mg, 50%); mp = 273 - 275 ^{o}C; ^{1}H NMR (40MHz, DMSO-d_6) δ 12.07 (s, 1H), 7.46 (d, *J* = 8 Hz, 1H), 7.43 (d, *J* = 2 Hz, 1 H), 7.28 (dd, *J* = 8 Hz, *J* = 2 Hz, 1H), 7.10 (s, 1 Hz); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.3, 146.6, 134.6, 127.6, 124.8, 119.9, 118.6, 116.9, 112.0; HRMS (ESI) calculated for $C_9H_5BrNO_2$ $(M-H)^-$ 237.9498, found 237.9507.



7-bromo-3-methoxyquinolin-2(1*H*)-one

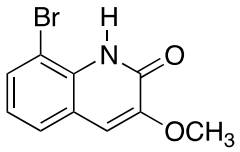
1*H*-6-Bromoindole-2,3-dione (904.12 mg, 4 mmol), (trimethylsilyl)diazomethane (4 mL of 2M in toluene, 8 mmol), and triethylamine (1.12 mL, 8 mmol) were dissolved in ethanol (15 mL) and placed under argon. It was stirred at room temperature for 33 hours. The resulting suspension was filtered and the solid was collected and dried under vacuum to afford a beige solid (553 mg). Then, the filtrate was concentrated under reduced pressure and the resulting residue was flash chromatographed on SiO_2 eluting with 0 to 100% EtOAc/hexane. This afforded the product as beige solid (52.9 mg). The solids were combined to give 7-bromo-3-methoxyquinolin-2(1*H*)-one as a beige solid (606 mg, 60%); mp = 265- 267 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.80 (s, 1H), 7.39 (d, *J* = 8 Hz, 1H), 7.29 (d, *J* = 2 Hz, 1 H), 7.16 (dd, *J* = 8 Hz, *J* = 2 Hz, 1H), 7.09 (s, 1 Hz), 3.67 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.0, 149.0, 135.0, 128.1, 124.7, 119.4, 119.0, 116.8, 110.7, 55.6; HRMS (ESI) calculated for $C_{10}H_9BrNO_2$ $(M+H)^+$ 253.9811, found 253.9811.



8-Bromo-3-hydroxyquinolin-2(1*H*)-one (6)

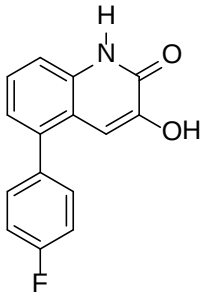
8-Bromo-3-methoxyquinolin-2(1*H*)-one (47.1 mg, 0.185 mmol) was dissolved in anhydrous DCM (5 mL) and placed under argon. The reaction mixture was cooled to 0 degree and the 1M in DCM BBr_3 (3.7 mL, 3.7 mmol) was added. It was then allowed to warm to room temperature

and stirred for 48 hours. Then, the solvent was removed under reduced pressure. The resulting residue was treated with 3N HCl solution for facilitating crystallization. It was filtered and basified with sat. $NaHCO_3$ and then washed with DCM followed by methanol. The solid was collected and dried under vacuum to reveal 8-bromo-3-hydroxyquinolin-2(1*H*)-one as a white solid (34.5 mg, 77%); mp = 210-212 ^oC; ^1H NMR (400MHz, DMSO-d_6) δ 7.49 (d, *J* = 8 Hz, 1H), 7.44 (d, *J* = 8 Hz, 1H), 7.02 (t, *J* = 8 Hz, 1H), 6.97 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 160.2, 149.1, 130.7, 128.3, 124.9, 123.7, 122.9, 110.8, 108.1; HRMS (ESI) calculated for $C_9H_5BrNO_2$ $(M-H)^-$ 237.9498, found 237.9493.



8-Bromo-3-methoxyquinolin-2(1*H*)-one

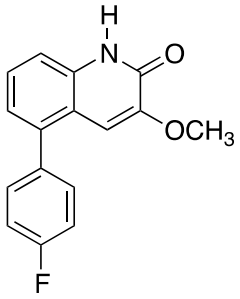
1*H*-7-Bromoindole-2,3-dione (226.03 mg, 1 mmol), (trimethylsilyl)diazomethane (1 mL of 2M in toluene, 2 mmol), and triethylamine (0.28 mL, 2 mmol) were dissolved in ethanol (5 mL) and placed under argon. It was stirred at room temperature for 27 hours. The solvent was removed under reduced pressure. The resulting residue was flash chromatographed on silica gel eluting with 50 to 100% EtOAc/hexane. 8-Bromo-3-methoxyquinolin-2(1*H*)-one was obtained as a white solid (95.8 mg, 41%); mp = 175 – 177 ^oC; ^1H NMR (400 MHz, $CDCl_3$) δ 9.16 (s, 1H), 7.58 (dd, *J* = 8 Hz, *J* = 2 Hz, *1H*), 7.45 (d, *J* = 8 Hz, 1H), 7.09 (t, *J* = 8 Hz, 1H), 6.90 (s, 1H), 3.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.9, 149.0, 130.9, 130.5, 126.0, 123.7, 121.6, 110.9, 106.7, 56.3; HRMS



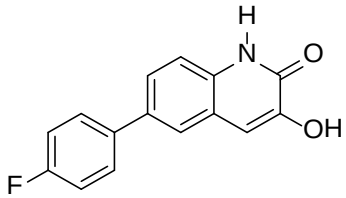
5-(4-Fluorophenyl)-3-hydroxyquinolin-2(1*H*)-one (7)

5-(4-Fluorophenyl)-3-methoxyquinolin-2(1*H*)-one (65.6 mg, 0.244 mmol) was dissolved in anhydrous DCM (5 mL) and placed under argon. The reaction mixture was cooled to 0 ^oC and the 1M in DCM BBr_3 (3 mL, 3 mmol) was added. It was then allowed to warm to room temperature and stirred for 28 hours. Then, additional 1M in DCM BBr_3 (3 mL, 3 mmol) was added and stirred for additional 44 hours at room temperature. Then, the solvent was removed

under reduced pressure. The resulting residue was suspended in DCM and it was filtered. The solid was washed with methanol. The solid was collected and dried under vacuum to reveal 5-(4-fluorophenyl)-3-hydroxyquinolin-2(1*H*)-one as a white solid (30.1 mg, 48%); mp = 278 - 280 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.11 (s, 1H), 9.58 (s, 1H), 7.46 – 7.43 (m, 2H), 7.38 – 7.31 (m, 4H), 7.06 (d, J = 8 Hz, 1H), 6.89 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.6 ($J_{C,F}$ = 243 Hz), 158.1, 146.6, 137.0, 135.7, 134.1, 131.3 ($J_{C,F}$ = 8 Hz), 126.1, 123.2, 118.2, 115.4 ($J_{C,F}$ = 22 Hz), 114.4, 109.9; ^{19}F NMR (400 MHz, DMSO-d_6) δ -115.0; HRMS (ESI) calculated for $C_{15}H_9FNO_2$ $(M-H)^-$ 254.0612, found 254.0622.



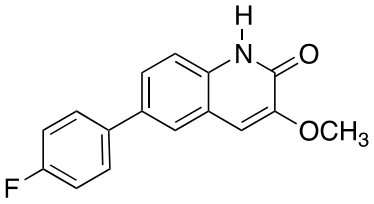
5-(4-Fluorophenyl)-3-methoxyquinolin-2(1*H*)-one 5-Bromo-3-methoxyquinolin-2(1*H*)-one (100 mg, 0.394 mmol), (4-fluorophenyl)boronic acid (83 mg, 0.591 mmol), $Pd(PPh_3)_4$ (46 mg, 0.040 mmol) and Na_2CO_3(125 mg, 1.182 mmol) were dissolved in a mixture of dioxane (6 mL) and water (3 mL). The air was evacuated and replaced with N_2. Then, the reaction mixture was refluxed for 8 hours. The reaction mixture was diluted with EtOAc and washed with sat. $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure and the resulting residue was flash chromatographed on silica gel eluting with 0 to 100% EtOAc/hexane. 5-(4-Fluorophenyl)-3-methoxyquinolin-2(1*H*)-one was obtained as a white solid (70.3 mg, 66%); mp = 233 – 235 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.03 (s, 1H), 7.54 – 7.50 (m, 2H), 7.43 – 7.31 (m, 4H), 7.09 (dd, J = 7 Hz, J = 1 Hz, 1H), 6.92 (s, 1H), 3.66 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.7 ($J_{C,F}$ = 243 Hz), 156.9, 148.8, 137.5, 135.5 ($J_{C,F}$ = 3 Hz), 134.6, 131.4 ($J_{C,F}$ = 8 Hz), 126.9, 123.3, 117.1, 115.5 ($J_{C,F}$ = 21 Hz) 114.3, 108.4, 55.2; ^{19}F NMR (376 MHz, DMSO-d_6) δ -114. 8; HRMS (ESI) calculated for $C_{16}H_{13}FNO_2$ $(M+H)^+$ 270.0925, found 270.0925.



6-(4-Fluorophenyl)-3-hydroxyquinolin-2(1*H*)-one (8)

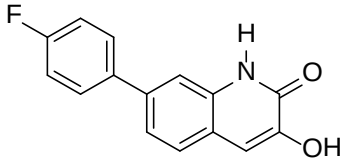
6-(4-Fluorophenyl)-3-methoxyquinolin-2(1*H*)-one (81 mg, 0.300 mmol) was dissolved in anhydrous DCM (5 mL) and placed under argon. The reaction mixture was cooled to 0 ^{o}C and the 1M in DCM BBr_3 (3 mL, 3 mmol) was added. It was then allowed to warm to room

temperature and stirred for 16 hours. Then, the solvent was removed under reduced pressure. The resulting residue was suspended in DCM and it was filtered. The solid was washed with methanol. The solid was collected and dried under vacuum to reveal 6-(4-fluorophenyl)-3-hydroxyquinolin-2(1*H*)-one as a white solid (46.7 mg, 60%); mp = 291 - 293 ^{o}C; ^{1}H NMR (400MHz, DMSO-d_6) δ 12.08 (s, 1H), 9.56 (s, 1H) 7.80 (d, J = 2 Hz, 1H), 7.73 – 7.70 (m, 2H), 7.60 (dd, J = 8 Hz, J = 2 Hz, 1H), 7.35 – 7.27 (m, 3H), 7.17 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.6 ($J_{C,F}$ = 243 Hz), 158.5, 146.5, 136.3 ($J_{C,F}$ = 3 Hz), 133.0, 132.9, 128.4 (8 Hz), 124.9, 123.6, 121.1, 115.6 ($J_{C,F}$ = 21 Hz), 115.3, 112.5; ^{19}F NMR (376 MHz, DMSO-d_6) δ -116.1; HRMS (ESI) calculated for $C_{15}H_9FNO_2$ $(M-H)^-$ 254.0612, found 254.0622



6-(4-Fluorophenyl)-3-methoxyquinolin-2(1*H*)-one

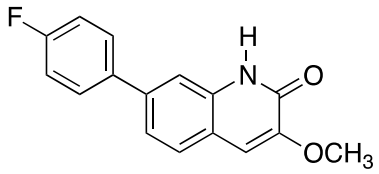
6-Bromo-3-methoxyquinolin-2(1*H*)-one (100 mg, 0.394 mmol), (4-fluorophenyl)boronic acid (83 mg, 0.591 mmol), $Pd(PPh_3)_4$ (46 mg, 0.040 mmol) and Na_2CO_3(125 mg, 1.182 mmol) were dissolved in a mixture of dioxane (6 mL) and water (3 mL). The air was evacuated and replaced with N_2. Then, the reaction mixture was refluxed for 8 hours. The reaction mixture was diluted with EtOAc and washed with sat. $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure and the resulting residue was flash chromatographed on silica gel eluting with 0 to 100% EtOAc/hexane. 6-(4-Fluorophenyl)-3-methoxyquinolin-2(1*H*)-one was obtained as a beige solid (89.6 mg, 85%); mp = 240 - 242 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.94 (s, 1H), 7.88 (d, J = 2 Hz, 1H), 7.74 – 7.70 (m, 2H), 7.64 (dd, J = 8 Hz, J = 2 Hz, 1H), 7.35 – 7.28 (m, 4H), 3.84 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.6 ($J_{C,F}$ = 242 Hz), 157.2, 149.0, 136.2 ($J_{C,F}$ = 3 Hz), 133.4, 132.9, 128.3 (8 Hz), 125.6, 124.1, 121.2, 115.7 ($J_{C,F}$ = 22 Hz), 115.1, 111.3, 55.6; ^{19}F NMR (376 MHz, DMSO-d_6) δ – 116.0; HRMS (ESI) calculated for $C_{16}H_{13}FNO_2$ $(M+H)^+$ 270.0925, found 270.0925.



7-(4-Fluorophenyl)-3-hydroxyquinolin-2(1*H*)-one (9)

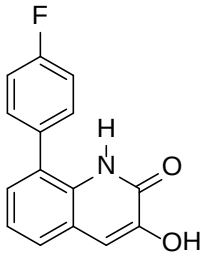
7-(4-Fluorophenyl)-3-methoxyquinolin-2(1*H*)-one (106.3 mg, 0.395 mmol) was dissolved in anhydrous DCM (5 mL) and placed under argon. The reaction mixture was cooled to 0 ^{o}C and the 1M in DCM BBr_3 (4 mL, 4 mmol) was added. It was then allowed to warm to room

temperature and stirred for 18 hours. Then, the solvent was removed under reduced pressure. The resulting residue was suspended in DCM and it was filtered. The solid was washed with methanol. The solid was collected and dried under vacuum to reveal 7-(4-fluorophenyl)-3-hydroxyquinolin-2(1*H*)-one as a white solid (68.1 mg, 68%): mp = 283- 285 ^{o}C; ^{1}H NMR (400MHz, DMSO-d_6) δ 12.06 (s, 1H), 9.54 (s, 1H), 7.70 – 7.66 (m, 2H), 7.59 (d, *J* = 8Hz, 1H), 7.48 (d, *J* = 2Hz, 1H), 7.42 (dd, *J* = 8Hz, *J* = 2Hz, 1H), 7.36 – 7. 30 (m, 2H), 7.13 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.8 ($J_{C,F}$ = 243 Hz), 159.0, 147.3, 136.7, 136.4, 133.8, 128.5 ($J_{C,F}$ = 8 Hz), 126.1, 120.7, 120.4, 115.8 ($J_{C,F}$ = 21 Hz), 112.3, 111.7; ^{19}F NMR (400 MHz, DMSO-d_6) δ -115.3; HRMS (ESI) calculated for $C_{15}H_9FNO_2$ $(M-H)^-$ 254.0612, found 254.0620.



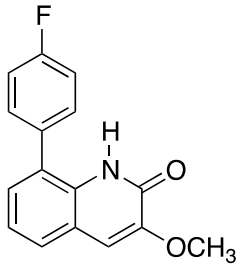
7-(4-Fluorophenyl)-3-methoxyquinolin-2(1H)-one

7-Bromo-3-methoxyquinolin-2(1H)-one (200 mg, 0.790 mmol), (4-fluorophenyl)boronic acid (166 mg, 1.185 mmol), $Pd(PPh_3)_4$ (91 mg, 0.079 mmol) and Na_2CO_3(251 mg, 2.370 mmol) were dissolved in a mixture of dioxane (6 mL) and water (3 mL). The air was evacuated and replaced with N_2. Then, the reaction mixture was refluxed for 8 hours. The reaction mixture was diluted with EtOAc and washed with sat. $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure and the resulting residue was flash chromatographed on silica gel eluting with 0 to 100% EtOAc/Hexane. 7-(4-Fluorophenyl)-3-methoxyquinolin-2(1*H*)-one was obtained as a beige solid (107.8 mg, 51%): mp = 252- 254 ^{o}C**;** ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.76 (s, 1H), 7.55 – 7.49 (m, 3H), 7.32 (s, 1H), 7.28 (dd, *J* = 8 Hz, *J* = 2 Hz, 1H), 7.19 – 7.15 (m, 2H), 7.11 (s, 1H), 3.68 (s, 3H)**;** ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.0 ($J_{C,F}$ = 244 Hz), 157.3, 148.8, 137.8, 136.2 ($J_{C,F}$ = 3 Hz), 134.4, 128.6 (8 Hz), 126.9, 120.7, 119.1, 115.9 ($J_{C,F}$ = 21 Hz), 112.2, 110.9, 55.6**;** ^{19}F NMR (376 MHz, DMSO-d_6) δ -115.1; HRMS (ESI) calculated for $C_{16}H_{13}FNO_2$ $(M+H)^+$ 270.0925, found 270.0925.



8-(4-Fluorophenyl)-3-hydroxyquinolin-2(1*H*)-one (10)

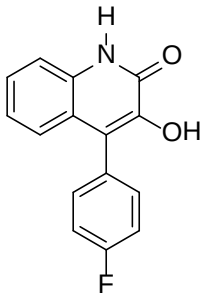
8-(4-Fluorophenyl)-3-methoxyquinolin-2(1*H*)-one (43 mg, 0.159 mmol) was dissolved in anhydrous DCM (5 mL) and placed under argon. The reaction mixture was cooled to 0 ^{o}C and the 1M in DCM BBr_3 (1.60 mL, 1.60 mmol) was added. It was then allowed to warm to room temperature and stirred for 54 hours. Then, the solvent was removed under reduced pressure. The resulting residue was treated with 3N HCl solution for facilitating crystallization. It was filtered and basified with sat. $NaHCO_3$ and then washed with DCM followed by methanol. The solid was collected and dried under vacuum to reveal 8-(4-fluorophenyl)-3-hydroxyquinolin-2(1*H*)-one as a white solid (18.5 mg, 46%); mp = 209 - 211 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.45 (dd, *J* = 8 Hz, *J* = 2 Hz, 1H), 7.41 – 7.37 (m, 2H), 7.28 – 7.23 (m, 2H), 7.15 – 7.10 (m, 2H), 7.08 (s, 1H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.0 ($J_{C,F}$ = 243 Hz), 158.7, 146.5, 133.5 ($J_{C,F}$ = 3 Hz), 131.3 ($J_{C,F}$ = 8 Hz), 130.4, 127.6, 127.2, 125.5, 122.1, 121.5, 115.8 ($J_{C,F}$ = 21 Hz) 112.7; ^{19}F NMR (376 MHz, DMSO-d_6) δ -114.9; HRMS (ESI) calculated for $C_{15}H_9FNO_2$ $(M-H)^-$ 254.0612, found 254.0621.



8-(4-Fluorophenyl)-3-methoxyquinolin-2(1*H*)-one

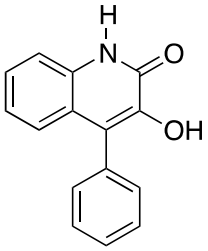
8-Bromo-3-methoxyquinolin-2(1*H*)-one (55 mg, 0.216 mmol), (4-fluorophenyl)boronic acid (39.5 mg, 0.282 mmol), $Pd(PPh_3)_4$ (25 mg, 0.022 mmol) and Na_2CO_3(92 mg, 0.868 mmol) were dissolved in a mixture of dioxane (6 mL) and water (3 mL). The air was evacuated and replaced with N_2. Then, the reaction mixture was refluxed for 4 hours. The reaction mixture was diluted with EtOAc and washed with sat. $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure and the resulting residue was flash chromatographed on silica gel eluting with 0 to 100% EtOAc/hexane. 8-(4-Fluorophenyl)-3-methoxyquinolin-2(1*H*)-one was obtained as a white solid (43.2 mg, 75%); mp = 183 – 185 ^{o}C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (br s, 1H), 7.52 - 7.50 (m, 1H), 7.39 – 7.36 (m, 2H), 7.27 – 7.19 (m, 4H), 7.00 (s, 1H) ,3.95 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.7 ($J_{C,F}$ = 247 Hz), 157.9, 149.0, 132.2 ($J_{C,F}$ = 3 Hz), 130.6 ($J_{C,F}$ = 8 Hz), 130.5, 128.5, 127.2, 126.2, 122.8, 120.6, 116.6 ($J_{C,F}$ = 21 Hz) 111.6, 56.03; ^{19}F NMR (376 MHz, $CDCl_3$) δ -112.7; HRMS (ESI) calculated for $C_{16}H_{13}FNO_2$ $(M+H)^+$ 270.0925, found 270.0925.

(ESI) calculated for $C_{10}H_9BrNO_2$ $(M+H)^+$ 253.9811, found 253.9812.



4-(4-Fluorophenyl)-3-hydroxyquinolin-2(1*H*)-one (11)

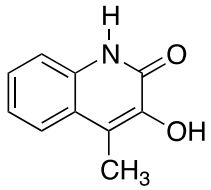
4-Bromo-3-hydroxyquinolin-2(1*H*)-one (60.9 mg, 0.254 mmol), (4-fluorophenyl)boronic acid (53.3 mg, 0.381 mmol), $Pd(PPh_3)_4$ (29.4 mg, 0.025 mmol) and Na_2CO_3(92 mg, 0.868 mmol) were dissolved in a mixture of dioxane (6 mL) and water (3 mL). The air was evacuated and replaced with N_2. Then, the reaction mixture was refluxed for 4 hours. The reaction mixture was diluted with EtOAc and washed with sat. $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure and the resulting residue was flash chromatographed on silica gel eluting with 0 to 100% EtOAc/hexane with 1% acetic acid. This afforded the product as a light brown solid. The acetic acid from flash chromatography was removed by Kugelrohr distillation. 4-(4-fluorophenyl)-3-methoxyquinolin-2(1*H*)-one was obtained as a light brown solid (36.5 mg, 56%): mp = 236 – 238 ^oC; ^1H NMR (400 MHz, DMSO-d_6) 12.23 (s, 1H), 9.26 (s, 1H), 7.41 – 7.32 (m, 6H), 7.11 – 7.05 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 161.6 ($J_{C,F}$ = 244 Hz), 158.2, 142.7, 133.2, 132.0 ($J_{C,F}$ = 8 Hz), 129.4, 126.5, 124.1, 122.9, 122.2, 120.8, 115.3 ($J_{C,F}$ = 21 Hz), 115.2; ^{19}F NMR (376 MHz, DMSO-d_6) δ – 114.4; HRMS (ESI) calculated for $C_{15}H_9FNO_2$ $(M-H)^-$ 254.0612, found 254.0614.



4-Phenyl-3-hydroxyquinolin-2(1*H*)-one (12)

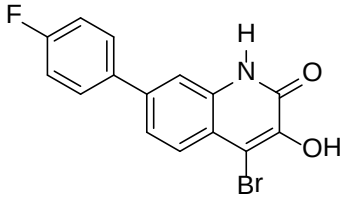
4-Bromo-3-hydroxyquinolin-2(1*H*)-one (100 mg, 0.417 mmol), phenylboronic acid (76 mg, 0.625 mmol), $Pd(PPh_3)_4$ (49 mg, 0.042 mmol) and Na_2CO_3(133 mg, 1.250 mmol) were dissolved in a mixture of dioxane (6 mL) and water (3 mL). The air was evacuated and replaced with N_2. Then, the reaction mixture was refluxed for 4 hours. The reaction mixture was diluted with EtOAc and washed with 2N HCl (pH 2). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure and the resulting residue was flash chromatographed on silica gel eluting with 0 to 5% MeOH/DCM. 4-Phenyl-3-hydroxyquinolin-2(1*H*)-one was obtained as a white solid (42.1 mg, 43%); mp = 239 - 241 ^oC; ^1H NMR (400 MHz, DMSO-d_6)

12.24 (s, 1H), 9.22 (s, 1H), 7.52 (t, *J* = 7 Hz, 2H), (t, *J* = 7 Hz, 1H), 7.37 – 7. 42 (m, 4H), 7.12 – 7. 02 (m, 2H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 158.3, 142.4, 133.7, 133.2, 129.8, 128.3, 127.6, 126.4, 124.3, 123.9, 122.1, 120.9, 115.2



4-Methyl-3-hydroxyquinolin-2(1*H*)-one (13)

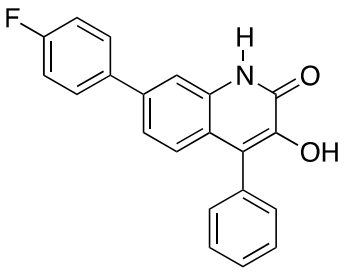
4-Bromo-3-hydroxyquinolin-2(1*H*)-one (50 mg, 0.208 mmol), TMSCl (0.08 mL, 0.633 mmol) and triethylamine (0.06 mL, 0.433 mmol) were dissolved in toluene (5 mL). The reaction mixture was stirred for 4 hours at room temperature. After starting material was gone, it was evaporated under reduced pressure revealing white solid. Along with the resulting residue, trimethylboroxine (0.05 mL, 0.358 mmol), $Pd(PPh_3)_4$ (24 mg, 0.021 mmol) and Na_2CO_3(66 mg, 0.624 mmol) were dissolved in a mixture of dioxane (6 mL) and water (3 mL). The air was evacuated and replaced with N_2. Then, the reaction mixture was refluxed for 10 hours. 3N HCl (5 mL) was added and stirred for 15 minutes, and then it was diluted with EtOAc. (pH 2). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure and the resulting residue was flash chromatographed on silica gel eluting with 0 to 80 % EtOAc/Hexane with 1% acetic acid. This afforded the product as a white solid. The acetic acid from flash chromatography was removed by Kugelrohr distillation. 4-Methyl-3-hydroxyquinolin-2(1*H*)-one was obtained as a white solid (12.9 mg, 36 %); mp = 234 - 236 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.98 (s, 1H), 9.09 (s, 1H), 7.58 (d, *J* = 8 Hz, 1H), 7.34 – 7. 26 (m, 2H), 7.18 (t, *J* = 7 Hz, 1H), 2.27 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 157.8, 142.8, 133.0, 126.3, 123.1, 122.0, 121.3, 119.3, 115.1, 10.5



4-Bromo-7-(4-fluorophenyl)-3-hydroxyquinolin-2(1*H*)-one (14)

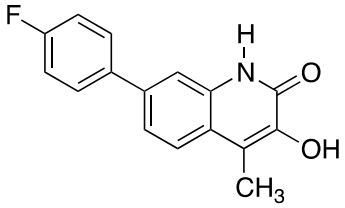
7-(4-Fluorophenyl)-3-hydroxyquinolin-2(1*H*)-one (67.5 mg, 0.264 mmol) and N-bromosuccinimide (49.3 mg, 0.277 mmol) were dissolved in anhydrous DMF and placed under argon. It was stirred at room temperature for 18 hours, and the solution turned orange. Then, DMF was removed via Kugelrohr distillation and the resulting residue was suspended in DCM.

It was filtered and the solid was washed with methanol. The solid was collected and dried under vacuum to reveal 4-bromo-7-(4-fluorophenyl)-3-hydroxyquinolin-2(1*H*)-one as a beige solid (58.6 mg, 66%): mp = 263 – 265 ^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.39 (s, 1H), 10.53 (s, 1H), 7.80 (d, *J* = 8 Hz, 1H), 7.73 – 7.69 (m, 2H), 7.58 (dd, *J* = 8 Hz, *J* = 2 Hz, 1H), 7.54 (d, *J* = 2 Hz, 1H), 7.38 – 7.33 (m, 2H);^{13}C NMR (100 MHz, DMSO-d_6) δ 162.1 ($J_{C,F}$ = 243 Hz), 156.7, 145.0, 138.2, 135.6 ($J_{C,F}$ = 3 Hz), 132.9, 128.7 ($J_{C,F}$ = 8 Hz), 125.9, 121.7, 119.1, 116.0 ($J_{C,F}$ = 22 Hz), 112.9, 109.0; ^{19}F NMR (376 MHz, DMSO-d_6) δ -114.7; HRMS (ESI) calculated for $C_{15}H_8BrFNO_2$ $(M-H)^-$ 331.9717, found 331.9712.



4-Phenyl-7-(4-fluorophenyl)-3-hydroxyquinolin-2(1*H*)-one (15)

4-Bromo-7-(4-fluorophenyl)-3-hydroxyquinolin-2(1*H*)-one (41.7 mg, 0.125 mmol), (4-fluorophenyl)boronic acid (22.9 mg, 0.188 mmol), $Pd(PPh_3)_4$ (15.0 mg, 0.013mmol) and Na_2CO_3(39.7 mg, 0.375 mmol) were dissolved in a mixture of dioxane (6 mL) and water (3 mL). The air was evacuated and replaced with N_2. Then, the reaction mixture was refluxed for 16 hours. The reaction mixture was diluted with EtOAc and washed with sat. $NaHCO_3$. The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure and the resulting residue was flash chromatographed on silica gel eluting with 0 to 100% EtOAc/hexane with 1% acetic acid. This afforded the product as a white solid. The acetic acid from flash chromatography was removed by Kugelrohr distillation. 4-Phenyl-7-(4-fluorophenyl)-3-hydroxyquinolin-2(1*H*)-one was obtained as a white solid (10.5 mg, 27%): mp = 234 - 236 ^{o}C; ^{1}H NMR 12.29 (s, 1H), 9.31 (br s, 1H), 7.69 – 7.65 (m, 2H), 7.57 - 7.52 (m, 3H), 7.46 (t, 1H, *J* = 7 Hz), 7.39 – 7.31 (m, 5H), 7.13 (d, 1H, *J* = 8 Hz; (400MHz, DMSO-d_6); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.0 ($J_{C,F}$ = 243 Hz), 158.4, 142.6, 137.2, 136.03 ($J_{C,F}$ = 3 Hz), 133.7, 133.6, 129.9, 128.6 ($J_{C,F}$ = 8 Hz), 128.4, 127.7, 125.0, 123.7, 120.9, 120.3, 115.9 ($J_{C,F}$ = 21 Hz), 112.9; ^{19}F NMR (376 MHz, DMSO-d_6) δ – 115.1; HRMS (ESI) calculated for $C_{21}H_{15}FNO2$ $(M+H)^+$ 332.1081, found 332.1082



4-Methyl-7-(4-fluorophenyl)-3-hydroxyquinolin-2(1*H*)-one (16)

4-Bromo-7-(4-fluorophenyl)-3-hydroxyquinolin-2(1*H*)-one (51.3 mg, 0.154 mmol), TMSCl (0.06 mL, 0.475 mmol) and triethylamine (0.04 mL, 0.289 mmol) were dissolved in toluene (5 mL). The reaction mixture was stirred for 4 hours at room temperature. Then, additional TMSCl (0.10 mL, 0.792 mmol) was added and stirred for 2 hours at room temperature. After starting material was gone, it was evaporated under reduced pressure revealing white solid. Along with the resulting residue, trimethylboroxine (0.03 mL, 0.215 mmol), $Pd(PPh_3)_4$ (17 mg, 0.015 mmol) and Na_2CO_3(49 mg, 0.462 mmol) were dissolved in a mixture of dioxane (6 mL) and water (3 mL). The air was evacuated and replaced with N_2. Then, the reaction mixture was refluxed for 10 hours. 3N HCl (5 mL) was added and stirred for 15 minutes, and then it was diluted with EtOAc. (pH 2). The organic layer was dried over Na_2SO_4 and concentrated under reduced pressure and the resulting residue was flash chromatographed on silica gel eluting with 0 to 40 % EtOAc/Hexane with 1% acetic acid. This afforded the product as a white solid. The acetic acid from flash chromatography was removed by Kugelrohr distillation. 4-Methyl-3-hydroxyquinolin-2(1*H*)-one was obtained as a white solid (11.7 mg, 29 %); mp = 162 - 164^{o}C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.04 (s, 1H), 9.19 (s, 1H), 7.70 – 7.67 (m, 3H), 7.49 – 7.47 (m, 2H), 7. 35 – 7. 31 (m, 2H), 2.30 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) 161.9 ($J_{C,F}$ = 244 Hz), 157.9, 142.9, 137.1, 136.1, 133.4, 128.5 ($J_{C,F}$ = 8 Hz), 123.9, 120.8, 120.7, 119.2, 115.9 ($J_{C,F}$ = 21 Hz), 112.7, 10.5; ^{19}F NMR (376 MHz, DMSO-d_6) δ -115.2; HRMS (ESI) calculated for $C_{16}H_{13}FNO_2$ $(M+H)^+$ 270.0930, found 270.0922

Expression, Purification, and Crystallization

Pandemic H1N1 endonuclease (residues 1-204) was expressed in BL21 (RIL) cells (Stratagene). The BL21 cells were grown to an OD_{600} of 0.8 and induced with 0.15 mM IPTG at 17 degrees Celsius for 17 hours. Cells were harvested by centrifugation and purified on Ni-NTA (Qiagen) according to manufacturers recommendations. The dual hexahis tag was then removed by HRV14 3C protease cleavage. S2C was further purified by size exclusion chromatography using HiLoad 26/60 Superdex 75 (GE Healthcare). The buffer used for size exclusion and the final buffer for storage of the protein was 100 mM NaCl and 20 mM Tris pH 8.0. The protein was concentrated to 5 mg/ml using a Ultrafree 10K (Millipore), aliquoted and stored at -80˚ C.

Crystals are formed by mixing in a 1:1 ratio endonuclease (5 mg/ml) with crystallization buffer containing 200 mM MES pH 6.7, 27% PEG8k, 200 mM ammonium sulfate, 1 mM manganese chloride, 10 mM magnesium acetate, 10 mM taurine, and 50 mM sodium fluoride. Trays are stored at 20 degrees Celsius and crystals form within a few hours and grow to maximum size in one to two weeks.

Endonuclease Assay

The Influenza A PA_N domain has been shown to cleave ssRNA as well as ssDNA. To demonstrate the inhibition of endonuclease cleavage by PA_N, a high throughput assay was developed (U.S. Patent Application Serial Number 13/554,709). A TaqMan-like oligonucleotide

was used containing a 6-carboxy-fluorescein (FAM) fluorophore at the 5'-end followed by 19 nucleotides and a minor groove binding non-fluorescent quencher (MGBNFQ, Applied Biosystems) at the 3'-end. When excited by light at a wavelength of 488 nm, MGBNFQ quenches the fluorescence of FAM via fluorescence resonance energy transfer. If the endonuclease cleaves the oligonucleotide, the quencher is no longer coupled to the fluorophore, and therefore, FAM fluoresces. This assay can be performed in a high-throughput (e.g. 96 well plate) format. The assay can be used to evaluate the inhibitory characteristics of compounds that are found to bind PA_N and to screen libraries of drug-like compounds. The assay uses the probe 6FAM-TGGCAATATCAGCTCCACA-MGBNFQ.

The assay can be performed in a 40 μl reaction volume with 50 mM Tris pH 7.5, 50 mM NaCl, 1 mM $MgSO_4$, 0.05 mM $MnSO_4$, 1 mM DTT, 0.75 mM CHAPS, 50 nM probe, and 25 nM endonuclease. The reaction mixture is set up as a master mix with the buffer, probe, and protein on ice. The inhibitor is then added to a maximum DMSO concentration of 2.5% (v/v) and serial dilutions are made on ice. Varioskan Fluorometer (Thermo Scientific), set to an excitation of 488 nm and emission of 518 nm, is used to measure the fluorescence of the samples at 37 degrees Celsius. Fluorescence is measured at various time points (5, 120, and 240 minutes) during the 37 degrees Celsius incubation. Activity/inhibition is calculated based on the change in fluorescence over time using Prism Graphpad non-linear regression analysis.

Compound soaking, data collection, and processing

Crystal structure of compound **9** was determined in complex with influenza A 2009 H1N1 influenza A endonuclease enzyme. The soak of **9** was performed by taking crystals and by step-wise gradient shifting the surrounding crystallization solution to 1 mM manganese sulfate, 200 mM HEPES pH 7.7, 25% (w/v) PEG 8000, 50 mM ammonium sulfate, 5 mM magnesium acetate, and 10% (v/v) ethylene glycol. 80-100 mM L-arginine was included to improve solubility of the compounds. Crystals were then soaked with the ligand for 2-17 hours at 20 degrees Celsius before placing into liquid nitrogen for storage. X-ray diffraction data collection was performed at the Cornell High Energy Synchrotron Source (CHESS) F1 beamline. The diffraction data were indexed, processed, scaled and merged using *HKL2000* (Otwinowski, Z.; Minor, W. Processing of X-ray Diffraction Data Collected in Oscillation Mode. *Methods in Enzymology* **1997,** 276, 307-326). The structure was solved and refined using the software PHENIX (Adams, P. D.; Afonine, P. V.; Bunkoczi, G.; Chen, V. B.; Davis, I. W.; Echols, N.; Headd, J. J.; Hung, L. W.; Kapral, G. J.; Grosse-Kunstleve, R. W.; McCoy, A. J.; Moriarty, N. W.; Oeffner, R.; Read, R. J.; Richardson, D. C.; Richardson, J. S.; Terwilliger, T. C.; Zwart, P. H. PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr* 66, 213-21).

X-ray crystal structures of **9** in complex with 2009 H1N1 influenza A endonuclease enzyme revealed a novel mode of chelation of the compounds to two metal ions (Mg^{2+}or Mn^{2+}at the positions A and B) at the active site.

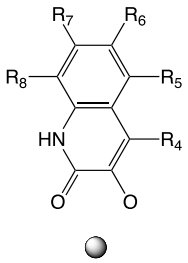


Table 1: X-ray data and refinement statistics

Protein Data Bank (PDB) accession code	**4KIL**
Compound	**10**
X-ray source	CHESS F1
Wavelength (Å)	0.917
Space group	$C222_1$
Cell constants (a, b, c in Å)	88.70, 100.87, 66.44
Resolution range (last shell) (Å)	50.00-1.75 (1.78-1.75)
Completeness (last shell) (%)	99.2 (97.9)
R_{merge} (last shell)	0.075 (0.628)
Average I/σ (*I*) (last shell)	22.38 (1.35)
Sigma cut-off (I)	$\|I\| < -3.0\sigma$
Refinement Statistics	
Total no. of atoms (solvent atoms)	3415 (117)
Resolution (Å)	1.75
No. of reflections (R_{free} set)	30,340 (1,525)
Completeness (R_{free} set)	99.78 (5.03)
R_{work}	0.179
R_{free}	0.203
Ramachandran statistics (% of residues in favored/disallowed regions)	97.47/0.00
RMSD bond length (Å)	0.013
RMSD bond angles (°)	1.344