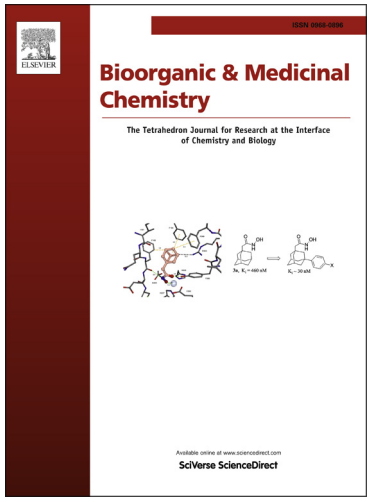

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Phenyl substituted 3-hydroxypyridin(1*H*)-2-ones: Inhibitors of influenza A endonuclease

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Keywords: Antiviral; influenza A, pyridinones, 3-hydroxypyridin-2-ones, endonuclease

Abstract: Inhibition of the endonuclease activity of influenza RNA-dependent RNA polymerase is recognized as an attractive target for the development of new agents for the treatment of influenza infection. Our earlier study employing small molecule fragment screening using a high-resolution crystal form of pandemic 2009 H1N1 influenza A endonuclease domain ($PA_{N)}$ resulted in the identification of 5-chloro-3-hydroxypyridin-2(1*H*)-one as a bimetal chelating ligand at the active site of the enzyme. In the present study, several phenyl substituted 3-hydroxypyridin-2(1*H*)-one compounds were synthesized and evaluated for their ability to inhibit the endonuclease activity as measured by a high-throughput fluorescence assay. Two of the more potent compounds in this series, **16** and **18,** had IC_{50} values of 11 and 23 nM in the enzymatic assay, respectively. Crystal structures revealed that these compounds had a superimposable binding mode that chelates the two active site metal ions (M1 and M2) using only two chelating groups. The SAR and the binding mode of these 3-hydroxypyridin-2-ones provide a basis for developing a new class of anti-influenza drugs.

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Antivirals play a major role as a prophylactic measure and for treatment of seasonal and pandemic influenza infections. There are two current classes of antivirals available for the treatment of influenza. These include, adamantanes, which target the M2 ion-channel protein, as well as zanamivir and oseltamivir, which target neuraminidase (NA). However, adamantanes are not currently recommended in treating influenza due to widespread drug resistance. Oseltamivir (Tamiflu) is the most widely used oral anti-influenza drug; however, oseltamivir-resistant seasonal influenza A strains are in circulation.[1] When accompanied by compensatory mutations, these mutant viruses exhibit fitness comparable to wild-type influenza A and remain resistant to oseltamivir.[2] With the emergence of oseltamivir resistant strains of influenza A, H1N1,[3] H5N1[4] and H7N9[5], there is a pressing need for new antiviral therapies.

The heterotrimeric influenza RNA-dependent RNA polymerase (RdRP) is an attractive target for anti-influenza therapy. RdRP, which is composed of polymerase acidic (PA), polymerase basic (PB1) and polymerase basic 2 (PB2) polypeptide chains, is responsible for the replication and transcription of viral genes into viral mRNA. This requires hijacking of 5'-capped RNA fragment from host pre-mRNAs in a process called "cap-snatching" in which the 5'-cap is bound at the PB2 subunit and the mRNA is cleaved 10-13 nucleotides downstream by the endonuclease activity of the PA subunit ([6] for review). The cleaved end of the capped RNA fragment is then used to prime the transcription of the viral mRNA by RdRP.[7] Cap-snatching is an attractive target for drug therapy since it is essential for replication of influenza A, B, and C viruses, and the host cell has no analogous activity.

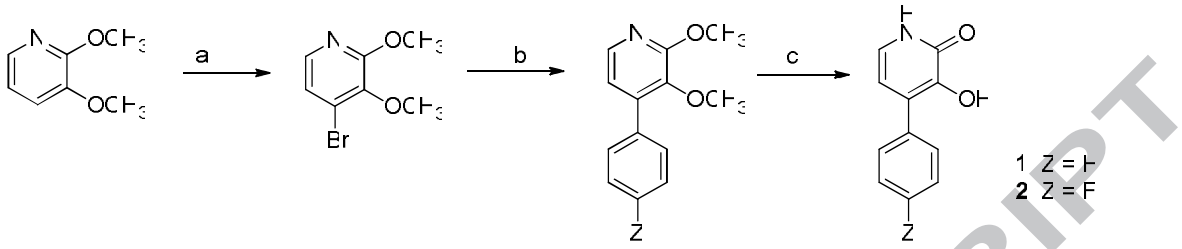
Inhibition of the endonuclease activity, which resides at the N-terminal domain of the PA subunit, PA_N (residues 1-197), by small molecules has been the focus of several studies. Structural characterization of PA_N and complexes with various inhibitors has provided opportunities for structure-based design of effective endonuclease inhibitors as potential influenza drugs.[8-11] Inhibitors of PAN include 2,4-dioxobutanoic acid derivatives,[12,13] flutimide and its derivatives,[14,15] 5-hydroxy-1,6-dihydropyrimidine-4-carboxylic acid derivatives,[16] and tetramic acid derivatives.[17] Crystal structures of many of these inhibitors have revealed a two metal binding mode using three chelating groups.

We have recently conducted a fragment screening campaign using a new crystal form of pandemic H1N1 PA_N to identify novel chemical scaffolds inhibiting the endonuclease activity (Sagong et al., 2013; Bauman et al., submitted).[18,19] Here, we report the development of a 3-hydroxypyridin-2(1*H*)-one series of compounds with strong inhibitory activity in the enzymatic assay. Structural biology in combination with efficient chemistry allowed for rapid exploration of the hydroxypridinone scaffold to identify key ligand-protein interactions necessary for higher potency.

1. Chemistry

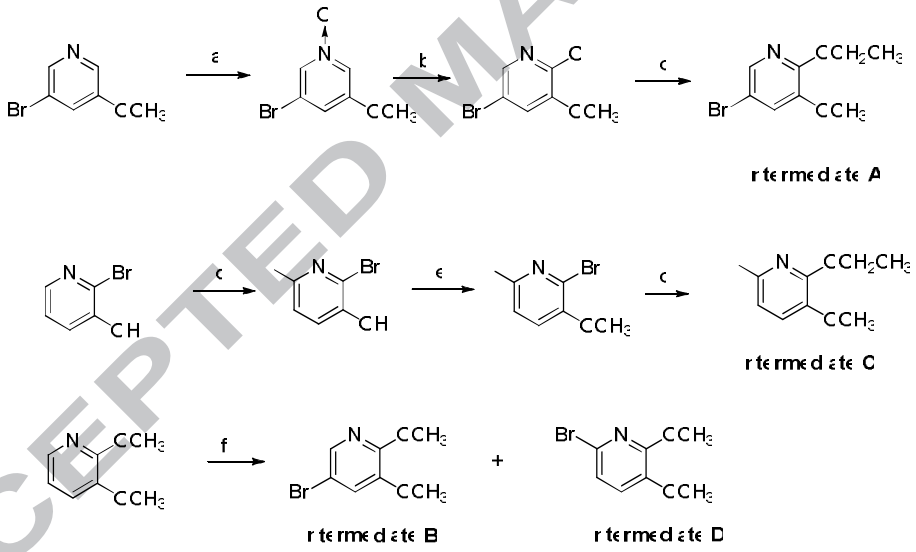
4-Phenyl- and 4-(*p*-fluorophenyl)-3-hydroxypyridin(1*H*)-2-one, **1** and **2**, were prepared from 4-bromo-2,3-dimethoxypyridine as outlined in Scheme 1. Commercially available, 2,3-dimethoxypyridine was converted as previously described to 4-bromo-2,3-dimethoxypyridine,[20] which was then treated with either phenylboronic acid or *p*-fluorophenylboronic acid under Suzuki-coupling conditions to provide

either 4-phenyl- or 4(*p*-fluorophenyl)-2,3-dimethoxypyridine in good yield. Treatment of these dimethoxypyridine derivatives with excess BBr_3 provided **1** and **2**.



Scheme 1. Preparation of 4-phenyl-3-hydroxypyridin(1*H*)-2-one and 4-(*p*-fluorophenyl)-3-hydroxypyridin(1*H*)-2-one. Reagents and conditions: (a) n-BuLi, THF, Br_2, - 78 ºC, then rt, (b) *p*-Z-Phenylboronic acid $Pd(PPh_3)_4$, K_2CO_3, Dioxane:H_2O (3:1), 100 ºC; (c) BBr_3, CH_2Cl_2, rt.

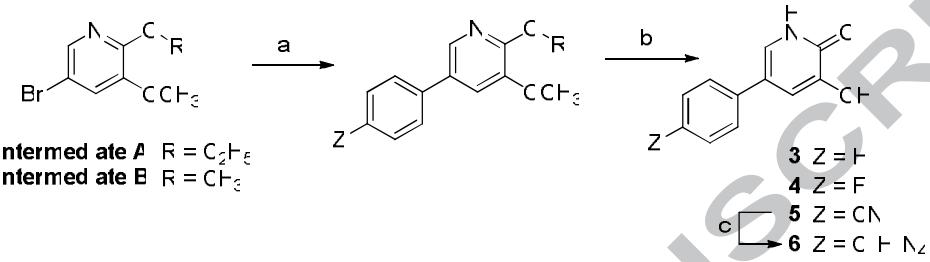
The requisite halogenated intermediates that were used for the preparation of 5- and 6-phenyl substituted 3-hydroxypyridin(1*H*)-2-ones were synthesized as outlined in Scheme 2. Intermediate A was prepared as previously described.[21] Alternatively, 5-bromo-2,3-dimethoxypyridine, Intermediate B could be



Scheme 2. Methods for the preparation of 5- and 6-bromo 3-hydroxypyridin(1*H*)-2-ones. Reagents and conditions: (a) m-CPBA, CH_2Cl_2, rt; (b) $POCl_3$, CH_2Cl_2, rt; (c) EtONa, EtOH, 80 ºC; (d) I_2, H_2O, K_2CO_3, rt; (e) CH_3I, K_2CO_3, THF, 0-20 ºC; (f) Br_2 (0.5 equiv.), AcOH, NaOAc, -32-0 ºC.

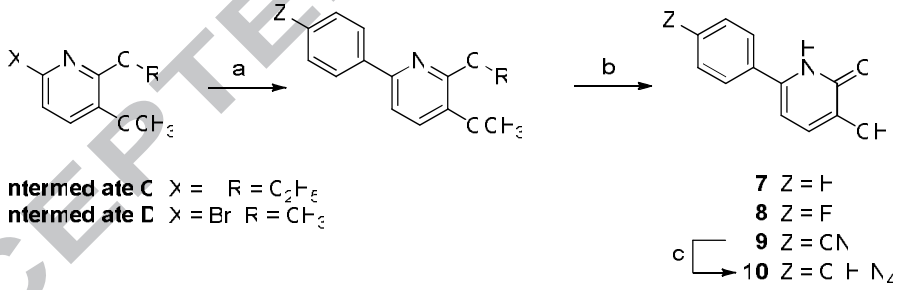
formed together with 6-bromo-2,3-dimethoxypyridine (intermediate D) by bromination of 2,3-dimethoxypyridine and these two monobrominated products were separated by chromatography.[22] We also prepared 6-iodo-2,3-dimethoxypyridine, intermediate C, by an initial iodination of 2-bromo-3-hydroxypyridine, followed by formation of its O-methyl ether and subsequent selective displacement of its 2-bromo substituent with sodium ethoxide as described in the literature.[23]

Using either intermediate A or B, Suzuki-coupling with phenylboric acid, (*p*-fluorophenyl)boronic acid, or (*p*-cyanophenylboronic acid) as outlined in Scheme 3 provided good yields of their respective phenyl dialkoxypyridines. Treatment of these phenyl dialkoxypyridines with excess BBr_3 provided the 3-hydroxypyridin-2-ones, **3-5.** In the presence of NaN_3 in acetic acid/dimethylformamide, **5** was converted to the 3-hydroxy-5-[4-(tetrazoly-5-yl)phenyl]pyridin(1*H*)-2-one, **6**.



Scheme 3. Preparation of substituted 5-phenyl-3-hydroxypyridin(1*H*)-2-ones. Reagents and conditions: (a) *p*-Z-Phenylboronic acid $Pd(PPh_3)_4$, K_2CO_3, Dioxane:H_2O (3:1), 100 ºC; (b) BBr_3, CH_2Cl_2, rt; (c) NaN_3, AcOH, DMF, sealed tube, 120 ºC.

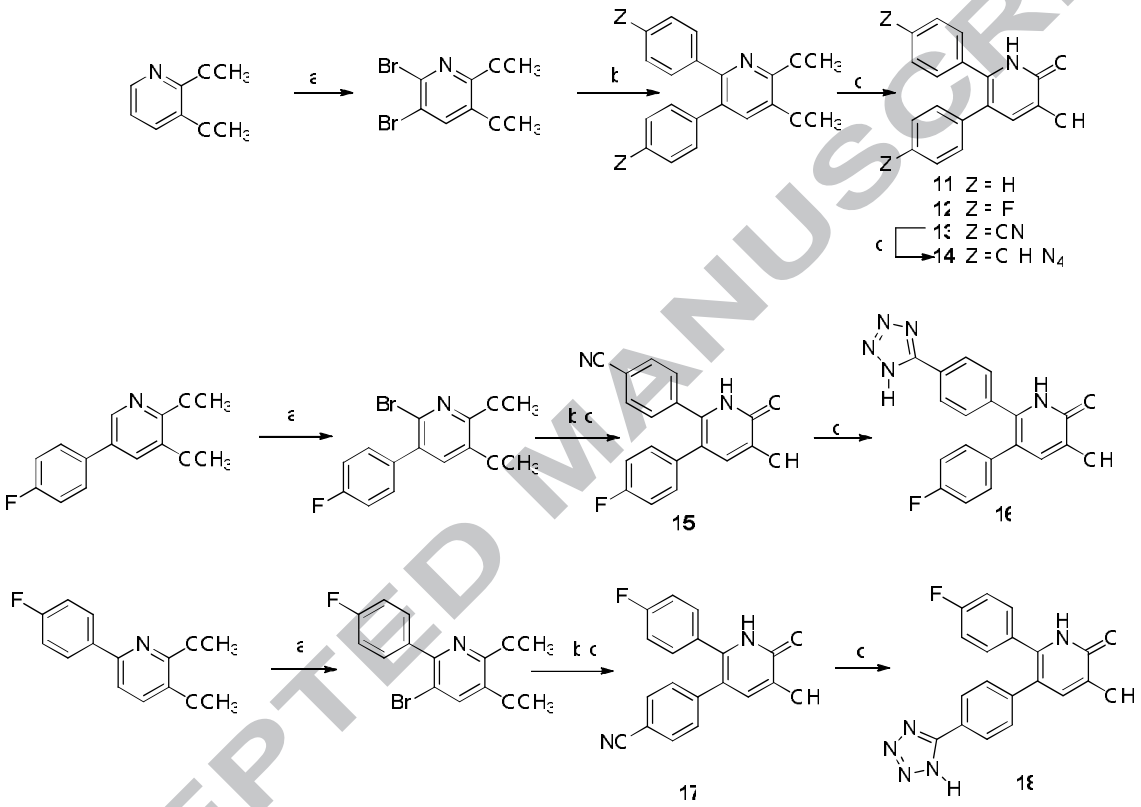
The synthesis of 6-phenyl substituted 3-hydroxypyridin(1*H*)-2-ones was similarly accomplished as outlined in Scheme 4. Using either intermediate C or D, Suzuki-coupling was performed to provide the desired 6-phenyl 2,3-dialkoxypyridine derivatives. Treatment of these 2,3-dialkoxypyridine derivatives with excess BBr_3 provided **7-9**. In the presence of NaN_3 in acetic acid/dimethylformamide, **9** was converted to the 3-hydroxy-5-[4-(tetrazoly-5-yl)phenyl]pyridin(1*H*)-2-one, **10**.



Scheme 4. Preparation of substituted 6-phenyl-3-hydroxypyridin(1*H*)-2-ones. Reagents and conditions: (a) *p*-Z-Phenylboronic acid $Pd(PPh_3)_4$, K_2CO_3, Dioxane:H_2O (3:1), 100 ºC; (b) BBr_3, CH_2Cl_2, rt; (c) NaN_3, AcOH, DMF, sealed tube, 120 ºC.

The synthesis of 5,6-diphenylpyridin(1*H*)-2-ones wherein the phenyl substituents had identical substituents could be prepared from 5,6-dibromo-2,3-dimethoxypyridine as outlined in Scheme 5. Suzuki coupling using 5,6-dibromo-2,3-dimethoxypyridine, which was prepared as previously described[23], gave the diphenyldimethoxypyridine intermediates, which in the presence of excess BBr_3 provided **11-13**. Treatment of **13** with NaN_3 in acetic acid and dimethylformamide in a sealed tube at 120 ºC provided 5,6-bis-[(tetrazoly-5-yl)phenyl]-3-hydroxypyridin(1*H*)-2-one, **14.**

The synthesis of 5-(*p*-fluorophenyl)-6-(*p*-cyanophenyl)-2,3-dimethoxypyridine was accomplished as illustrated in Scheme 5. Bromination of 5-(*p*-fluorophenyl)-2,3-dimethoxypyridine provided the 6-bromo derivative, which upon Suzuki-coupling with *p*-cyanophenylboronic acid gave 5-(*p*-fluorophenyl)-6-(*p*-cyanophenyl)-2,3-dimethoxypyridine. Treatment of this dimethoxypyridine with BBr_3 gave **15**, which was converted in the presence of azide to **16**. In a similar manner as illustrated in Scheme 5, 6-(*p*-fluorophenyl)-5-(*p*-cyanophenyl)-2,3-dimethoxypyridine was prepared and converted to **17**. Treatment of **17** with NaN_3 in acetic acid and dimethylformamide gave 5-[(4-tetrazol-5-yl)phenyl]-6-(*p*-fluorophenyl-3-hydroxypyridin(1*H*)-2-one, **18**.



Scheme 5. Methods for the preparation of substituted 5,6-diphenyl-3-hydroxypyridin(1*H*)-2-ones. Reagents and conditions: (a) Br_2 (2 equiv.), AcOH, NaOAc, 0 ºC; (b) *p*-Z-Phenylboronic acid for **9-11** and *p*-fluorophenylboronic acid for **13** and **15**, $Pd(PPh_3)_4$, K_2CO_3, Dioxane:H_2O (3:1), 100 ºC; (c) BBr_3, CH_2Cl_2, rt; (d) NaN_3, AcOH, DMF, sealed tube, 120 ºC

2. Pharmacology and Structure-Activity Relationships

Each phenyl substituted or diphenyl substituted 3-hydroxypyridin-2-one was evaluated for its capacity to inhibit influenza A endonuclease. Using a high-throughput 96-well plate based assay as previously employed in the evaluation of a series of 3-hydroxyquinolin-2-ones,[18] the relative potential to block endonuclease cleavage by PA_N was determined. A TaqMan-like oligonucleotide 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 was employed in these

studies. When excited, MGBNFQ quenches the fluorescence of FAM via fluorescence resonance energy transfer. Upon cleavage of the oligonucleotide, the quencher is no longer coupled to the fluorophore, and therefore, FAM fluoresces. This assay was used to determine the inhibitory IC_{50} values for these 3-hydroxypyridin-2-ones.

The results of these assays are summarized in Table 1. These data indicate that the presence of either a 4-fluorophenyl or 4-(1*H*-tetrazol-5-yl)phenyl at the 5-position of 3-hydroxypyridin-2(1*H*)-one, as in **4**

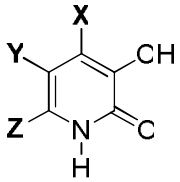


Table 1. Inhibition assay of influenza A endonuclease by phenyl substituted 3-hydroxypyridin(1*H*)-2-ones. IC_{50} is the mean of triplicate measurements ± standard error of the mean (SEM).

Compd	IC_{50} (μM)	X	Y	Z
1	35 ± 1.76	Phenyl	H	H
2	>200	4-Fluorophenyl	H	H
3	2.1 ± 0.107	H	Phenyl	H
4	0.730 ± 0.047	H	4-Fluorophenyl	H
5	3.83 ± 0.288	H	4-Cyanophenyl	H
6	0.368 ± 0.041	H	4-(Tetrazol-5-yl)phenyl	H
7	0.87 ± 0.040	H	H	Phenyl
8	0.430 ± 0.010	H	H	4-Fluorophenyl
9	0.802 ± 0.044	H	H	4-Cyanophenyl
10	0.085 ± 0.008	H	H	4-(Tetrazol-5-yl)phenyl
11	0.047 ± 0.005	H	Phenyl	Phenyl
12	0.041 ± 0.003	H	4-Fluorophenyl	4-Fluorophenyl
13	0.103 ± 0.009	H	4-Cyanophenyl	4-Cyanophenyl
14	0.285 ± 0.027	H	4-(Tetrazol-5-yl)phenyl	4-(Tetrazol-5-yl)phenyl
15	0.136±0.013	H	4-Cyanophenyl	4-Fluorophenyl
16	0.011 ± 0.0005	H	4-(Tetrazol-5-yl)phenyl	4-Fluorophenyl
17	0.054 ± 0.003	H	4-Fluorophenyl	4-Cyanophenyl
18	0.023 ± 0.001	H	4-Fluorophenyl	4-(Tetrazol-5-yl)phenyl

and **6**, is associated significant enzyme inhibition (IC_{50} < 1.0 μM). There was little difference in relative enzyme inhibition among 6-phenyl-3-hydroxypyridin(1*H*)-2-ones wherein the phenyl moiety was unsusbstituted, **7,** or had a 4-cyano substituent, **9**. However, there was a slight increase in activity observed with the *p*-fluorophenyl derivative, **8**, and a significant increase in potency with a *p*- (tetrazol-5-yl) moiety, **10**. The substitution of either a 4- phenyl or 4-fluorophenyl substituent at the 4-position of 3-hydroxypyridin-2(1*H*)-one, **1** and **2**, is clearly associated with a dramatic loss in potency as an enzyme inhibitor in comparison to the isomers where these substituents are at either the 5- or 6-position.

All of the 5,6-diphenyl substituted 3-hydroxypyridin(1*H*)-2-ones exhibited significant activity as inhibitors of influenza A endonuclease. The 5,6-bisphenyl and 5,6-bis(4-fluorophenyl) derivatives **11**

and **12** are much more potent than the monophenyl substituted derivatives **3** and **7** or their analogous 4-fluorophenyl derivatives **4** and **8**. 5,6-bis(4-Tetrazol-5-yl)phenyl-3-hydroxypyridin(1*H*)-2-one, **14**, was the least active of these 5,6-diphenyl substituted derivatives with an IC_{50} of 0.285 μM. A single 4-(tetrazol-5-yl)phenyl together at either the 5- or 6-position combined with a 4-fluorophenyl moiety at the adjacent 5- or 6-position, was associated with the greatest inhibitory activity with IC_{50} values of 11 and 23 nM for **16** and **18**, respectively. These studies indicate that significant enhancement in enzyme inhibition can be achieved by the placement of phenyl groups at both the 5- and 6-position. In addition, the selection of substituents on these phenyl moieties can have a profound impact on potency.

Cytotoxicity studies were performed using Madin-Darby Canine Epithelial (MDCK) Cells and human embryonic kidney (A293) cells. There was no correlation between the potency of inhibition of influenza endonuclease activity and relative cytotoxic activity. In MDCK cells, **13** and **17** had IC_{50} values of 40 μM, **15** and **16** had IC_{50} values of 50 μM. In this same cell line, **3** and **7** had IC_{50} values of 80 and 60 μM, the remaining twelve compounds have IC_{50} values >100 μM. In the human A293 cell line, compounds **3**, **7**, **9-11**, **13** and **15-17** have IC_{50} values that are ≥28 μM, while **6** has an IC_{50} value of 80 μM and **1**, **2**, **4**, **5**, **8**, **12**, **14** and **18** have IC_{50} values >100 μM. While at an early stage of development, these data do not indicate any major concern that would interfere in future studies directed toward the establishment of ex-vivo antiviral data for this series of compounds.

Crystal structures of PA_N in complex with compounds of this series were routinely determined as part of a structure-based drug design effort.[18] In brief, the pyridinone ring chelates to the active site metals, M1 and M2, in a mode that allows for π-stacking with His41. The pyridinone nitrogen is within hydrogen-bonding distance (2.9 Å for both) to a coordinating water of M2 and to the catalytic residue Glu80. During the course of fragment screening a third metal was discovered to exist in the active site (M3), which can make cation-π interactions with a phenyl ring substituted at the 6-position of the pyridinone. Crystal structures of the two most potent compounds, **16** and **18**, bound to the endonuclease active site are described here. Soaking of **18** into apo crystals of PA_N revealed the compound bound to the active site metal in a flipped orientation compared to what was detected previously (Figure 1-A). The pyridinone ring nitrogen makes an electrostatic interaction with Tyr130 (4.1 Å). The *p*-fluorophenyl forms a cation-π interaction with M3 and makes hydrophobic interactions with the side chains of Ala20, Tyr24, and Ile38. The electron density for Tyr24 is weak, indicating that the residue is flexible and further optimization at the 6-position may strengthen this interaction and thereby will improve the potency. The 4-(tetrazol-5-yl)phenyl forms hydrophobic interactions with the side chains of Lys34, Ala37, and Ile38. The tetrazolyl group displaces an ethylene glycol molecule, previously detected in other crystal structures of PA_N, to form bidentate hydrogen bonds to Arg84. When the 4-(tetrazol-5-yl)phenyl was substituted at the 5-position, compound **16** (Figure 1-B) retains the standard orientation seen in previous structures of compounds in this series.[18] The greater potency of **16** in comparison to **18** can be attributed to the optimal position of the pyridinone nitrogen and its favorable interactions with metal chelating atoms.

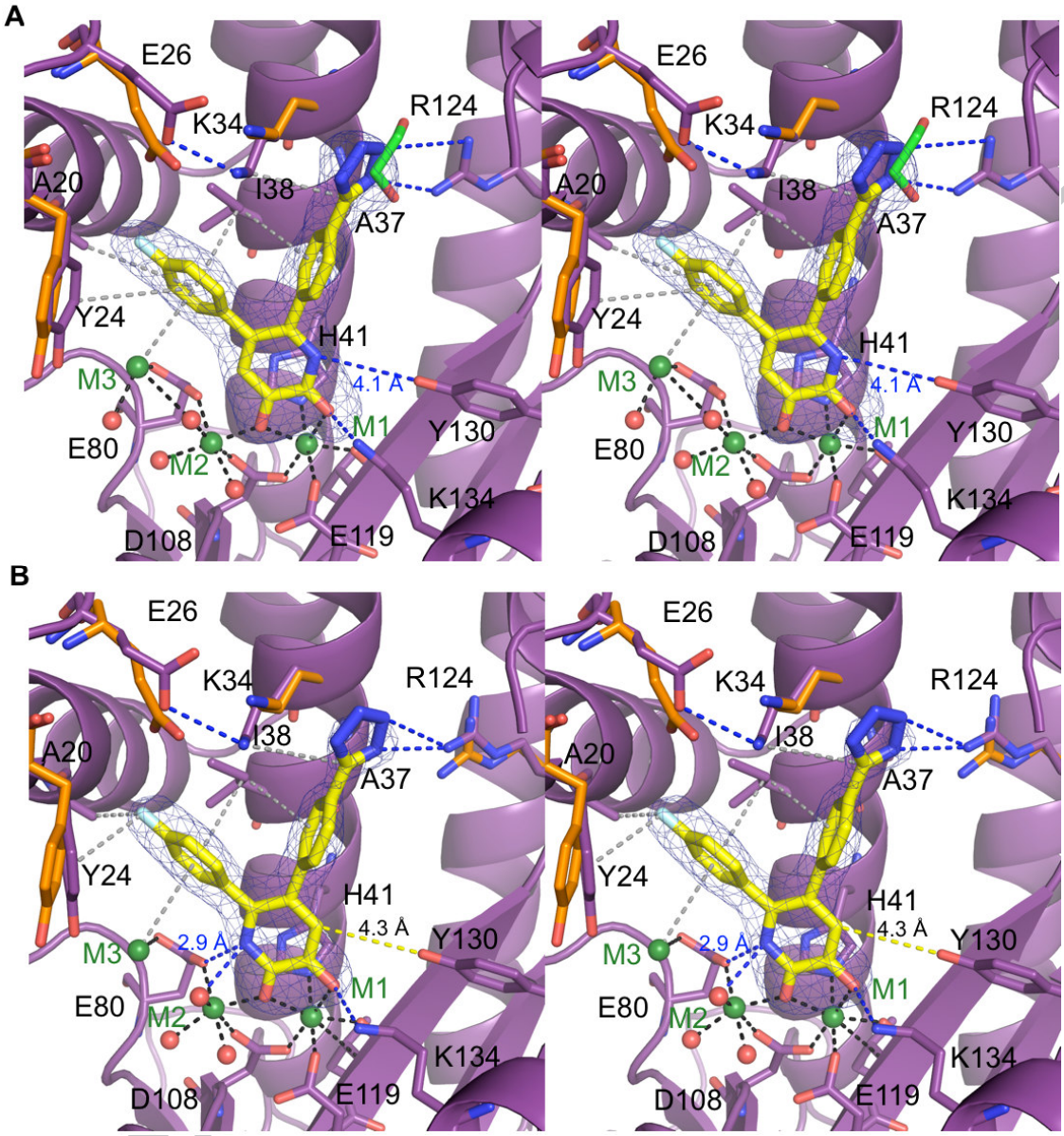


Figure 1. Stereoview images of crystal structures of **16** and **18** bound to PA_N. Metal-coordinating bonds are depicted as black dashed lines, hydrophobic and cation-π interactions are grey, and hydrogen or electrostatic bonds as blue dashed lines. Residues with significant structural changes upon binding of a ligand are depicted with the apo structure shown in orange. Electron density calculated from an omit map is contoured at 4.0σ. (A) Crystal structure of **18** bound to PA_N. Ethylene glycol, as detected in other PA_N crystal structures, is shown in light green. (B) Crystal structure of **16** bound to PA_N.

3. Conclusion

Several 5- and 6-phenyl substituted 3-hydroxypyridin(1*H*)-2-ones have significant activity as inhibitors of influenza A endonuclease. The presence of phenyl substituents at both the 5- and 6-positions of 3-hydroxypyridin(1*H*)-2-one resulted in a further enhancement in their potency as inhibitors. Among the

diphenyl 3-hydroxypyridin(1*H*)-2-ones evaluated, the two more potent analogs, **16** and **18**, had a tetrazo-5-yl moiety at the *para*-position of either the 5- or 6-phenyl substituent, respectively. The binding mode of each of these compounds as determined by X-ray crystallography differed dramatically and was influenced by the interaction of this tetrazole within the active site of the enzyme. This observation is of major importance in understanding the structure-activity of similarly substituted 3-hydroxypyridin(1*H*)-2-ones. It also provides critical insights that could prove useful in efforts to further the development of 3-hydroxypyridin(1*H*)-2-ones as potent inhibitors of influenza A endonuclease.

4. Chemistry Experimental

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 (300 MHz) and ^{13}C (75 MHz) NMR spectra were done in $CDCl_3$, Methanol-d_4, and DMSO-d_6 and recorded on a Varian Unity Inova (300 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.

4.1.1 4-Phenyl-3-hydroxypyridin-2(1*H*)-one (1)

To a solution of (80 mg, 0.37 mmol) in CH_2Cl_2 (3.0 ml) under nitrogen, boron tribromide (1.0 ml of a 1.0 M solution in CH_2Cl_2) was added. After addition was completed, the reaction mixture was stirred for 16 hours at room temperature. Dichloromethane was removed from the reaction mixture followed by addition of HCl (3N). The resulting solid was filtered. The solid was redissolved in CH_2Cl_2 and washed with $NaHCO_3$ and brine. The organic phase was then dried and evaporated under reduced pressure to afford the crude product which was purified by ISCO flash chromatography using 10% MeOH in DCM to furnish the pure product (25 mg) as white solid, yield: 36%. mp 186-188 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.62 (d, J = 7.5 Hz, 2H), 7.41 (m, 2H), 7.33 (m, 1H), 6.92 (d, J = 6.3 Hz, 1H), 6.25 (d, J = 6.3 Hz, 1H). ^{13}C NMR (75 MHz, DMSO d_6) δ: 172.5, 159.4, 143.9, 136.7, 129.3, 128.9, 128.4, 123.9, 107.6. HRMS Calcd for $C_{11}H_9NO_2$ $(M + H)^+$ 188.0706. Found 188.0706.

4.1.2 2,3-Dimethoxy-4-phenylpyridine

A mixture of 4-bromo-2,3-dimethoxypyridine (100 mg, 0.46 mmol) , phenylboronic acid (84 mg, 0.69 mmol), $Pd(PPh_3)_4$ (58 mg, 0.05 mmol) and K_2CO_3 (125 mg, 0.92 mmol) in 1,4- dioxane (3.0 ml) and H_2O (1.0 ml) was degassed for 30 min. This mixture was heated to 100 °C and stirred for 16 hours. The reaction mixture was cooled to room temperature and partitioned between $NaHCO_3$ and EtOAc (3x), and washed with NaCl (1x). The organic phase was dried over Na_2SO_4 and was concentrated. The

resulting residue was purified by ISCO flash chromatography using 10% EtOAc in hexane to give 80 mg (81%) of the desired product. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.92 (d, *J* = 5.1 Hz, 1H), 7.58 (d, *J* = 7.2 Hz, 2H), 7.41 (m, 3H), 6.88 (d, *J* = 5.1Hz, 1H), 4.05 (s, 3H), 3.63 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 176.1, 158.4, 142.3, 140.7, 135.9, 128.9, 128.4, 128.2, 118.3, 60.3, 53.7.

4.1.3 4-Bromo-2,3-dimethoxypyridine

To a solution of 2,3-dimethoxypyridine (1.59 g, 10.77 mmol) in THF (30 ml) at -75 °C was added n-BuLi (10.6 ml, 1.6 M solution in hexane) dropwise. The reaction mixture was stirred at 0 °C for 1 h and cooled again to -70 °C before the addition of Br_2 (0.6 ml, 10.77 mmol). The reaction mixture was stirred at -70 °C for 2 h and then was allowed to warm to room temperature. The reaction was then quenched with water, and was diluted with ethyl acetate. The layers were separated and the organic layer was washed with sodium thiosulphate and water. The organic layer was dried with Na_2SO_4, concentrated under reduced pressure to afford a brown oil. Purification in ISCO using 10% EtOAc in hexane furnished the product (1.2 g, 51% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.66 (d, *J* = 5.4 Hz, 1H), 7.01 (d, *J* = 5.4 Hz, 1H), 3.96 (s, 3H), 3.84 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 172.3, 158.4, 141.1, 126,1, 121.9, 60.4, 53.9.

4.2.1 4-(*p*-Fluorophenyl)-3-hydroxypyridin-2(1*H*)-one (2)

To a solution of 2,3-dimethoxy-4-(*p*-fluorophenyl)pyridine (190 mg, 0.81 mmol) in CH_2Cl_2 (5.0 ml) under nitrogen, boron tribromide (1.5 ml of 1.0 M solution in CH_2Cl_2) was added. After addition was completed, the reaction mixture was stirred for 16 h at room temperature. Dichloromethane was removed from the reaction mixture followed by addition of HCl (3N). The resulting solid was filtered. The solid was redissolved in CH_2Cl_2 and washed with $NaHCO_3$ and brine. The organic phase was then dried and evaporated under reduced pressure to afford the crude product which was purified in ISCO using 10% MeOH in DCM to furnish the pure product (100 mg) as white solid, yield: 60%. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.83 (bs, 1H), 7.71 (m, 1H), 7.50 (m, 1H), 7.30-7.19 (m, 2H), 6.96 (d, *J* = 4.8 Hz, 1H), 6.30 (m, 1H). HRMS Calcd for $C_{11}H_8FNO_2$ $(M + H)^+$ 206.0612. Found 206.0609.

4.2.2 2,3-Dimethoxy-4-(4-fluorophenyl)pyridine

A mixture of 4-bromo-2,3-dimethoxypyridine (100 mg, 0.46 mmol), 4-fluorophenylboronic acid (84 mg, 0.6 mmol), $Pd(PPh_3)_4$ (58 mg, 0.05 mmol) and K_2CO_3 (125 mg, 0.92 mmol) in 1,4- dioxane (3.0 ml) and H_2O (1.0 ml) was degassed for 30 min. This mixture was heated to 100 °C and stirred for 16 hours. The reaction mixture was cooled to room temperature and partitioned between $NaHCO_3$ and EtOAc (3x), and washed with NaCl (1x). The organic phase was dried over Na_2SO_4 and was concentrated. The resulting residue was purified by ISCO flash chromatography using 20% EtOAc in hexane to give 95.5 mg (89%) of the desired product. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.89 (d, *J* = 5.1 Hz, 1H), 7.57-7.53 (m, 2H), 7.13-7.07 (m, 2H), 6.83 (d, *J* = 5.4 Hz, 1H), 4.02 (s, 3H), 3.61 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 176.2, 164.4, 161.1, 158.5, 141.2, 140.8, 130.8, 130.7, 118.1, 115.5, 115.2, 60.3, 53.7.

4.3.1 5-Phenyl-3-hydroxypyridin-2(1*H*)-one (3)

To a solution of 2,3-dimethoxy-5-phenylpyridine (100 mg, 0.465 mmol) in CH_2Cl_2 (3.0 ml) under nitrogen, boron tribromide was added (1.0 ml of 1.0 M solution in CH_2Cl_2). After addition was completed, the reaction mixture was stirred for 16 h at room temperature. Dichloromethane was removed and the reaction mixture was diluted with ethyl acetate. The solution was washed with $NaHCO_3$ and brine. The organic phase was then dried and evaporated under reduced pressure to afford the crude product which was purified in ISCO using 10% MeOH in DCM to furnish the pure product (21 mg) as white solid, yield: 24%. mp 175-177 ^oC; ^1H NMR (300 MHz, DMSO-d_6) δ: 9.33 (bs, 1H), 7.62-7.38 (m, 5H), 7.30 (s, 1H), 7.20 (s, 1H). ^{13}C NMR (75 MHz, DMSO d_6) δ: 172.5, 158.3, 147.9, 137.3, 129.6, 126.0, 121.9, 115.6, 107.7. HRMS Calcd for $C_{11}H_9NO_2$ $(M + H)^+$ 188.0706. Found 188.0708.

4.3.2. 2,3-Dimethoxy-5-phenylpyridine

A mixture of 5-bromo-2,3-dimethoxypyridine (100 mg, 0.46 mmol) , phenylboronic acid (84 mg, 0.69 mmol), $Pd(PPh_3)_4$ (58 mg, 0.05 mmol) and K_2CO_3 (125 mg, 0.92 mmol) in 1,4- dioxane (3.0 ml) and H_2O (1.0 ml) was degassed for 30 min. This mixture was heated to 100 ^0C and stirred for 16 h. The reaction mixture was cooled to room temperature and partitioned between $NaHCO_3$ and EtOAc (3x), and washed with NaCl (1x). The organic phase was dried over Na_2SO_4 and was concentrated. The resulting residue was purified by ISCO flash chromatography using DCM to give 100 mg (99.9%) desired product. ^1H NMR (300 MHz, $CDCl_3$) δ: 7.97 (s, 1H), 7.53-7.36 (m, 5H), 7,24 (s, 1H), 4.06 (s, 3H), 3.92 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 176.1, 153.8, 144.0, 138.0, 134.9, 130.8, 129.6, 128.9, 127.4, 126.8, 116.4, 56.2, 54.2.

4.3.3. 5-Bromo-2,3-dimethoxypyridine

To a 25 ml flask containing (63.8 mg, 1.2 mmol) of sodium ethoxide was added 3 ml dry ethanol. The mixture was stirred for 30 minutes. 2-Chloro-3-methoxy-5-bromopyridine (230 mg, 1.04 mmol) was added to the mixture and heated to 60 ^oC for 16 h. The reaction was allowed to cool and the solvent was removed. Ethyl acetate (50 ml) was added to the residue. The ethyl acetate solution was washed with water and then brine. The organic solvent was dried and concentrated under reduced pressure, and purified by ISCO flash chromatography using 10% ethyl acetate in hexane to give 220 mg (97% yield) of the desired product. ^1H NMR (300 MHz, $CDCl_3$) δ: 7.71 (s, 1H), 7.09 (s, 1H), 4.37 (qt, 2H), 3.82 (s, 3H), 1.38 (t, 3H).

4.3.4. 2-Chloro-3-methoxy-5-bromopyridine

Phosphoryl chloride ($POCl_3$) (4.8 ml, 52.9 mmol) was added to a solution of 5-bromo-3-methoxypyridine oxide (540 mg, 2.6 mmol) in 15 ml DCM. The reaction mixture was stirred at room temperature overnight. The reaction mixture was washed with saturated $NaHCO_3$and brine. The organic layer was dried, concentrated under reduced pressure and purified using ISCO flash chromatography using 50% ethyl acetate in hexane to provide 369 mg product (62% yield). ^1H NMR (300 MHz, $CDCl_3$) δ: 8.05 (s, 1H), 7.34 (s, 1H), 3.93 (s, 3H).

4.3.5. 5-Bromo-3-methoxypyridine oxide

A solution of 3-(methoxy)-5-bromopyridine (2.0 g, 10.6 mmol.) and *meta*-chloroperbenzoic acid (mCPBA) (2.5 g, 14.8 mmol).) in dichloromethane (50 ml) was stirred at room temperature for 3 h. The reaction mixture was washed with 15 ml 2N KOH followed by brine. The organic layers were dried over anhydrous $MgSO_4$ and concentrated under reduced pressure to give product as white solid (1.06 g, 51%). mp 201 ^oC, ^1H NMR (300 MHz, $CDCl_3$) δ: 8.01 (s, 1H), 7.91 (s, 1H), 7.04 (s, 1H), 3.85 (s, 3H).

4.4.1. 5-(4-Fluorophenyl-3-hydroxypyridin-2(1*H*)-one (4)

To a solution of 2,3-dimethoxy-5(4-fluorophenyl)pyridine (60 mg, 0.257 mmol) in CH_2Cl_2 (3.0 ml) under nitrogen, boron tribromide (1.0 ml, 1.0 M solution in CH_2Cl_2) was added. After addition was completed, the reaction mixture was stirred for 16 h at room temperature. Dichloromethane was removed and the reaction mixture was diluted with ethyl acetate. The solution was washed with $NaHCO_3$, water, and brine. The organic phase was then dried and evaporated under reduced pressure to afford the crude product which was purified in ISCO using 10 % MeOH in DCM to furnish the pure product (28 mg) as off-white solid, yield: 53%. mp 136-138 ^oC; ^1H NMR (300 MHz, DMSO-d_6) δ: 9.33 (bs, 1H), 7.66 (m, 2H), 7.30 (m, 3H), 7.17 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 172.5, 158.3, 148.0, 133.9, 128.1, 128.0, 121.8, 116.4, 116.1, 115.5, 107.6. HRMS Calcd for $C_{11}H_8FNO_2$ $(M + H)^+$ 206.0612. Found 206.0614.

4.4.2. 2,3-Dimethoxy-5-(4-fluorophenyl)pyridine

A mixture of 5-bromo- 2,3-dimethoxypyridine (651 mg, 3.0 mmol) ,4-fluorophenylboronic acid (630 mg, 4.5 mmol), $Pd(PPh_3)_4$ (404 mg, 0.35 mmol) and K_2CO_3 (828 mg, 6.0 mmol) in 1,4- dioxane (6.0 ml) and H_2O (2 .0 ml) was degassed for 30 min. This mixture was heated to 100 °C and stirred for 24 h. The reaction mixture was cooled to room temperature and partitioned between $NaHCO_3$ and EtOAc (3x), and washed with NaCl (1x). The organic phase was dried over Na_2SO_4 and was concentrated. The resulting residue was purified by ISCO flash chromatography using 30-70% EtOAc in hexane to give 665 mg (95% yield) desired product. ^1H NMR (300 MHz, $CDCl_3$) δ: 7.90 (s, 1H), 7.51-7.45 (m, 2H), 7.19-7.11 (m, 3H), 4.06 (s, 3H), 3.94 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 176.3, 164.2, 160.9, 144.2, 134.9, 129.9, 128.6, 128.5, 116.4, 116.1, 115.8, 55.8, 53.8.

4.5.1. 5-(*p*-Cyanophenyl-3-hydroxypyridin-2(1*H*)-one (5)

To a solution of 2,3-dimethoxy-5(4-cyanophenyl)pyridine (100 mg, 0.417 mmol) in toluene (3.0 ml) under nitrogen, boron tribromide (1.2 ml of 1.0 M solution in CH_2Cl_2) was added. After addition was completed, the reaction mixture was heated to 100 ^oC in a sealed tube for 2 h and then at room temperature overnight. The solvent was removed from the reaction mixture and the resulting solid was purified in ISCO using 10 % MeOH in DCM to furnish the pure product (86 mg) yield: 60%. ^1H NMR (300 MHz, Acetone d_6) δ: 7.67 (s, 4H), 7.36 (s, 1H), 7.10 (s, 1H). HRMS Calcd for $C_{12}H_8N_2O_2$ $(M + H)^+$ 212.0580. Found 212.0578.

4.5.2. 2,3-Dimethoxy-5-(4-cyanophenyl)pyridine

A mixture of 5-bromo-2,3-dimethoxypyridine (570 mg, 2.63 mmol) ,4-cyanophenylboronic acid (578 mg, 3.94 mmol), $Pd(PPh_3)_4$ (311 mg, 0.27 mmol) and K_2CO_3 (717 mg, 5.2 mmol) in 1,4- dioxane (6.0 ml) and H_2O (1.5 ml) was degassed for 30 min. This mixture was heated to 100 °C and stirred for 16 h. The reaction mixture was cooled to room temperature and partitioned between $NaHCO_3$ and EtOAc (3x), and washed with NaCl (1x). The organic phase was dried over Na_2SO_4 and was concentrated. The resulting residue was purified by ISCO flash chromatography using 20% EtOAc in hexane to give 495 mg (78% yield) desired product. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.74 (s, 1H), 7.51 (d, *J* = 8.4 Hz, 2H), 7.41 (d, *J* = 8.4 Hz, 2H), 7.0 (s, 1H), 3.84 (s, 3H), 3.73 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 176.2, 154.7, 144.3, 142.6, 135.4, 132.7, 128.7, 127.3, 118.8, 115.7, 110.9, 55.8, 54.0.

4.6.1. 5-[4-(1*H*-tetrazol-5-yl)phenyl]-3-hydroxypyridin-2(1*H*)-one (6)

To a sealed tube equipped with a small stirring bar was added 4-(5,6-dihydroxypyridin-3-yl)benzonitrile (86 mg, 0.40 mmol), DMF (3.0 ml), NaN_3 (104 mg, 1.6 mmol) followed by AcOH (1.0 ml). The resulting mixture was heated to 120 °C overnight. After the completion of the reaction, the solvent was removed under vacuum. Addition of 1N HCl and stirring produced a solid which was filtered to give the pure product (35 mg, 34% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.96 (m, 3H), 7.53 (d, *J* = 9.0 Hz, 2H), 7.22 (s, 1H), 7.16 (s, 1H). HRMS Calcd for $C_{12}H_9N_5O_2$ $(M + H)^+$ 256.0929. Found 256.0827.

4.7.1. 6-Phenyl-3-hydroxypyridin-2(1*H*)-one (7)

To a solution of 2-ethoxy-3-methoxy-6-phenylpyridine (150 mg, 0.65 mmol) in CH_2Cl_2 (2.0 ml) under nitrogen, was added boron tribromide (1.0 ml of a 1.0 M solution in CH_2Cl_2). After addition was completed, the reaction mixture was stirred for 16 h at room temperature. Dichloromethane was removed from the reaction mixture followed by addition of HCl (3N). The resulting solid was filtered. The solid was redissolved in CH_2Cl_2 and washed with $NaHCO_3$ and brine. The organic phase was then dried and evaporated under reduced pressure to afford the crude product which was purified in ISCO using 10% MeOH in DCM to furnish the pure product (41 mg) as white solid, yield: 34%. mp 167-169 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.8 (bs, 1H), 9.19 (s, 1H), 7.66 (d, *J* = 7.8 Hz, 2H), 7.26-7.37 (m, 3H), 6.80 (d, *J* = 7.5 Hz, 1H), 6.45 (d, *J* = 7.5 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 172.5, 159.5, 147.1, 136.2, 134.4, 129.4, 129.0, 126.7, 116.7. HRMS Calcd for $C_{11}H_9NO_2$ $(M + H)^+$ 188.0706. Found 188.0703.

4.7.2. 2-Ethoxy-3-methoxy-6-phenylpyridine

A mixture of 6-iodo-2-ethoxy-3-methoxypyridine (200 mg, 0.717 mmol), phenylboronic acid (131 mg, 1.07 mmol), $Pd(PPh_3)_4$ (127 mg, 0.11 mmol) and K_2CO_3 (195 mg, 1.43 mmol) in 1,4- dioxane (3.0 ml) and H_2O (1.0 ml) was degassed for 30 min. This mixture was heated to 100 °C and stirred for 16 h. The reaction mixture was cooled to room temperature and partitioned between $NaHCO_3$ and EtOAc (3x), and washed with NaCl (1x). The organic phase was dried over Na_2SO_4 and was concentrated. The resulting residue was purified by ISCO flash chromatography using 15% EtOAc in hexane to give 150

mg (91%) of the desired product. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.97 (d, *J* = 5.4 Hz, 2H), 7.44-7.39 (m, 2H), 7.34-7.26 (m, 2H), 7.06 (d, *J* = 8.1 Hz, 1H), 4.60 (qt, 2H), 3.88 (s, 3H), 1.50 (t, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 176.2, 153.2, 145.1, 143.2, 138.9, 128.5, 127.8, 126.0, 117.9, 112.5, 61.8, 55.1, 14.2.

4.7.3. 6-Iodo-2-ethoxy-3-methoxypyridine

To a solution of 6-iodo-2-bromo-3-methoxypyridine (1.5 g, 4.8 mmol) in EtOH (100 ml), NaOEt (487.6 mg) in EtOH (20 ml) was added for 30 min. This reaction was heated to 100 °C for 2 h. After cooling to room temperature, ethanol was removed under reduced pressure and diluted with 50 ml water. The resulting residue was extracted with CH_2Cl_2 (3x), washed with brine, dried over Na_2SO_4 and evaporated under reduced pressure to give a crude oil, which was purified by ISCO flash chromatography using 20% EtOAc in hexanes, to provide recovered starting material (400 mg) and 6-iodo-2-ethoxyl-3-methoxylpyridine: 600 mg (yield: 45%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.22 (d, *J* = 7.8 Hz, 1H), 7.78 (d, *J* = 7.8 Hz, 1H), 4.45 (qt, *J* = 7.5 Hz, 2H), 3.86 (s, 3H), 1.45 (t, *J* = 4.5 Hz, 3H).

4.7.4. 6-iodo-2-bromo-3-methoxypyridine

To a solution of 6-iodo-2-bromopyridin 3-ol (1.7 g, 5.9 mmol) in THF (50 ml), K_2CO_3 (1.22 g, 8.85 mmol) was added, and the mixture was stirred for 10 min at 0 °C in an ice bath. To this mixture CH_3I (1.0 g, 7.0 mmol) was then added slowly. The reaction mixture was stirred for 16 h at room temperature. The reaction mixture was poured into ice water (100 ml), then extracted with EtOAc (3x), dried in Na_2SO_4 and evaporated under reduced pressure to afford a white solid 1.5 g (yield: 80.96%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.50 (d, *J* = 8.1 Hz, 1H), 6.74 (d, *J* = 8.4 Hz, 1H), 3.88 (s, 3H).

4.7.5. 6-Iodo-2-bromopyridin 3-ol

To a solution of 2-bromopyridin 3-ol (2.4 g, 13.79 mmol) in H_2O (34 ml) was added potassium carbonate (2.85 g, 20.7 mmol) followed by iodine (3.8 g, 15.2 mmol) and this mixture was stirred at room temperature overnight. This mixture was cooled to 0 °C, then slowly quenched by 2N HCl to pH 6. The resulting precipitate was collected by filtration, washed with water and dried to give 2-bromo-6-iodopyridine-3-ol (3 g, 73% yield). LC/MS: 300 (M +H).

4.8.1. 6-(4-Fluorophenyl-3-hydroxypyridin-2(1*H*)-one (8)

To a solution of 2-ethoxy-3-methoxy-6-(4-fluorophenyl)pyridine (57 mg, 0.245 mmol) in CH_2Cl_2 (3.0 ml) under nitrogen, was added boron tribromide (1.0 ml of a 1.0 M solution in CH_2Cl_2). After addition was completed, the reaction mixture was stirred for 16 h at room temperature. Dichloromethane was removed from the reaction mixture followed by addition of HCl (3N). The resulting solid was filtered. The solid was redissolved in CH_2Cl_2 and washed with $NaHCO_3$ and brine. The organic phase was then dried and evaporated under reduced pressure to afford the crude product which was purified in ISCO using 5% MeOH in DCM to furnish the pure product (35 mg) as white solid, yield: 70%. mp 159-165 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.57-7.53 (m, 2H), 7.12 (t, *J* = 8.7 Hz, 2H), 6.84 (d, *J* = 7.5 Hz, 1H), 6.27 (d, *J* = 6.9 Hz, 1H). HRMS Calcd for $C_{11}H_8FNO_2$ $(M + H)^+$ 206.0612. Found 206.0608. .

4.8.2. 2-Ethoxy-3-methoxy-6-fluorophenylpyridine

A mixture of 6-iodo-2-ethoxy-3-methoxypyridine (530 mg, 2.44 mmol), 4-fluorophenylboronic acid (512 mg, 3.67 mmol), $Pd(PPh_3)_4$ (416 mg, 0.36 mmol) and K_2CO_3 (673 mg, 4.88 mmol) in 1,4-dioxane (6.0 ml) and H_2O (2.0 ml) was degassed for 30 min. This mixture was heated to 100 °C and stirred for 16 h. The reaction mixture was cooled to room temperature and partitioned between $NaHCO_3$ and EtOAc (3x), and washed with NaCl (1x). The organic phase was dried over Na_2SO_4 and was concentrated. The resulting residue was purified by ISCO flash chromatography using 20% EtOAc in hexane to give 551 mg (97%) of the desired product. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.95 (m, 2H), 7.27-7.05 (m, 4H), 4.59 (qt, 2H), 3.91 (s, 3H), 1.47 (t, 3H).

4.9.1. 6-(4-Cyanophenyl-3-hydroxypyridin-2(1*H*)-one (9)

To a solution of 2-ethoxy-3-methoxy-6-(4-cyanophenyl)pyridine (150 mg, 0.59 mmol) in CH_2Cl_2 (5.0 ml) under nitrogen, was added boron tribromide (2.0 ml, 1.0 M solution in CH_2Cl_2). After addition was completed, the reaction mixture was stirred for 16 h at room temperature. Dichloromethane was removed from the reaction mixture followed by addition of HCl (3N). The resulting solid was filtered. The solid was redissolved in CH_2Cl_2 and washed with $NaHCO_3$ and brine. The organic phase was then dried and evaporated under reduced pressure to afford the crude product which was purified in ISCO using 10 % MeOH in DCM to furnish the pure product (90 mg) as tan solid, yield: 72%. mp 161-163 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.88 (s, 4H), 6.85 (d, J = 7.5 Hz, 1H), 6.68 (d, J = 7.5 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 172.5, 159.1, 148.0, 138.8, 134.6, 133.3, 127.3, 119.4, 116.9, 111.1, 107.6. HRMS Calcd for $C_{12}8_9N_2O_2$ $(M + H)^+$ 213.0659. Found 213.0651. .

4.9.2. 2-Ethoxy-3-methoxy-6-(4-cyanophenyl)pyridine

A mixture of 6-iodo-2-ethoxy-3-methoxypyridine (150 mg, 0.894 mmol) , 4-cyanophenylboronic acid (197 mg, 1.34mmol), $Pd(PPh_3)_4$ (155 mg, 0.136 mmol) and K_2CO_3 (276 mg, 2.0 mmol) in 1,4-dioxane (3.0 ml) and H_2O (1.0 ml) was degassed for 30 min. This mixture was heated to 100 °C and stirred for 16 h. The reaction mixture was cooled to room temperature and partitioned between $NaHCO_3$ and EtOAc (3x), and washed with NaCl (1x). The organic phase was dried over Na_2SO_4 and was concentrated. The resulting residue was purified by ISCO flash chromatography using 20% EtOAc in hexane to give 185 mg (82% yield) desired product. ^{1}H NMR (300 MHz, $CDCl_3$) δ:8.07 (d, J = 8.4 Hz, 2H), 7.70 (d, J = 8.1 Hz, 2H), 7.37 (d, J = 8.4 Hz, 1H), 7.12 (d, J = 8.1 Hz, 1H), 4.59 (qt, 2H), 3.93 (s, 3H), 1.51 (t, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 172.2, 153.5, 144.5, 143.0, 142.5, 132.4, 126.3, 119.1, 117.5, 113.7, 110.9, 62.1, 55.9, 14.6.

4.10.1. 6-[4-(1*H*-tetrazol-5-yl)phenyl]-3-hydroxypyridin-2(1*H*)-one (10)

To a sealed tube equipped with a small stirring bar was added 6-(4-cyanophenyl-3-hydroxypyridin-2(1*H*)-one (50 mg, 0.235 mmol), DMF (2.0 ml), NaN_3 (61 mg, 0.94 mmol) followed by AcOH (1.0 ml). The resulting mixture was heated to 120 °C overnight. After the completion of the reaction, the solvent was removed under vacuum. Addition of 1N HCl and stirring produced a solid which was filtered to give the pure product (51 mg, 85% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.07 (d, 2H), 7.83 (d, J = 8.1 Hz, 2H), 7.76 (d, J = 8.4 Hz, 1H), 6.84 (d, J = 7.5 Hz, 1H), 6.59 (d, J = 7.5 Hz, 1H). ^{13}C NMR (75

MHz, DMSO-d_6) δ: 172.5, 159.4, 135.5, 127.6, 127.2, 114.3, 107.6. HRMS Calcd for $C_{12}H_9N_5O_2$ $(M + H)^+$ 256.0829. Found 256.0820.

4.11.1. 5,6-Diphenyl-3-hydroxypyridin-2(1*H*)-one (11)

To a solution of 2,3-dimethoxy-5,6-diphenylpyridine (130 mg, 0.45 mmol) in CH_2Cl_2 (6.0 ml) under nitrogen, was added boron tribromide (2.0 ml of a 1.0 M solution in CH_2Cl_2). After addition was completed, the reaction mixture was stirred for 16 h at room temperature. Dichloromethane was removed from the reaction mixture followed by addition of HCl (3N). The resulting solid was filtered. The solid was redissolved in CH_2Cl_2 and washed with $NaHCO_3$ and brine. The organic phase was then dried and evaporated under reduced pressure to afford a solid (60 mg), yield: 55%. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 11.89, 9.31, 7.58 (m, 6H), 7.23 (t, 1H), 7.15 (m, 1H), 7.07 (t, 1H), 6.7 (d, J = 7.2 Hz, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 172.5, 158.7, 146.9, 139.2, 134.7, 130.6, 130.1, 128.8, 128.7, 127.1, 119.3, 107.7. HRMS Calcd for $C_{17}H_{13}NO_2$ $(M + H)^+$ 264.1019. Found 264.1020. mp 249-255 °C.

4.11.2. 2,3-Dimethoxy-5,6-diphenylpyridine

A mixture of 5,6-dibromo-2,3-dimethoxypyridine (150 mg, 0. 5 mmol, phenylboronic acid (242 mg, 2.0 mmol), $Pd(PPh_3)_4$ (231 mg, 0.2 mmol) and K_2CO_3 (276 mg, 2.0 mmol) in 1,4- dioxane (5.0 ml) and H_2O (1.5 ml) was degassed for 30 min. This mixture was heated to 100 °C and stirred for 16 h. The reaction mixture was cooled to room temperature and partitioned between $NaHCO_3$ and EtOAc (3x), and washed with NaCl (1x). The organic phase was dried over Na_2SO_4 and was concentrated. The resulting residue was purified by ISCO flash chromatography using 10% EtOAc in hexane to give 130 mg of the desired product, yield: 89%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.4 (m, 2H), 7.29 (m, 3H), 7.24-7.21 (m, 5H), 7.12 (s, 1H), 4.15 (s, 3H), 3.96 (s, 3H). LC/MS: 292.23 (M + H).

4.11.3. 5,6-Dibromo-2,3-dimethoxypyridine

To a solution of commercially available 2,3-dimethoxypyridine (2.0 g, 14.7 mmol) and NaOAc (5.4 g, 44 mmol) in AcOH (25 ml) at 0 °C was added a solution of bromine (2.0 ml, 36.6 mmol) in AcOH (0.5 ml). The cooling bath was removed and the reaction was then stirred at room temperature for 16 h. The mixture was poured into crushed ice followed by neutralization with 25% aqueous NaOH solution; the aqueous phase was extracted with CH_2Cl_2 (3x). The combined organic phases were dried over Na_2SO_4 and concentrated. Purification by ISCO flash chromatography using a gradient of 10-15% EtOAc in hexanes provided the desired product 3.86 g, tan solid (89%): ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.18 (s, 1H), 3.98 (s, 3H), 3.84 (s, 3H).

4.12.1. 5,6-bis(4-Fluorophenyl)-3-hydroxypyridin-2(1*H*)-one (12)

To a solution of 2,3-dimethoxy-5,6-bis(4-fluorophenyl)pyridine (100 mg, 0.3 mmol) in CH_2Cl_2 (3.0 ml) under nitrogen, was added boron tribromide (1.0 M solution in CH_2Cl_2) (1.5 ml). After addition was completed, the reaction mixture was stirred for 16 h at room temperature. Dichloromethane was removed from the reaction mixture followed by addition of HCl (3N). The resulting solid was filtered, which was redissolved in CH_2Cl_2 and washed with $NaHCO_3$ and brine, dried using anhydrous sodium sulfate and evaporated under reduced pressure to afford a solid (80 mg), yield: 87%. mp 152-157 °C; ^{1}H

NMR (300 MHz, DMSO-d_6) δ: 7.66 (m, 2H), 7.6 (m, 2H), 7.46 (m, 2H), 7.20 (d, *J* = 6.0 Hz, 2H), 7.08 (m, 1H), 6.7 (d, 1H), 6.28 (d, 1H). HRMS Calcd for $C_{17}H_{11}F_2NO_2$ $(M + H)^+$ 300.0831. Found 300.0830.

4.12.2. 2,3-bis(4-Fluorophenyl)-5,6-dimethoxypyridine

The mixture of 5,6-dibromo-2,3-dimethoxypyridine (260 mg, 0.87 mmol), 4-fluorophenylboronic acid (367 mg, 3.0 mmol), $Pd(PPh_3)_4$ (303 mg, 0.26 mmol) and K_2CO_3 (414 mg, 3.0 mmol) in 1,4- dioxane (4.0 ml) and H_2O (1.0 ml) was degassed for 30 min. This mixture was heated to 100 °C and stirred for 16 h. The reaction mixture was cooled to room temperature and partitioned between $NaHCO_3$ and EtOAc (3x), and washed with NaCl (1x). The organic phase was dried over Na_2SO_4 and was concentrated. The resulting residue was purified by ISCO flash chromatography using 10% EtOAc in hexane to give 210 mg of the desired product, yield: 73%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.35 (m, 2H), 7.20 (m, 2H), 7.20 (m, 2H), 7.17 (m, 2H), 7.04-6.98 (m, 4H), 4.18 (s, 3H), 3.01 (s, 3H). LC/MS: 328.205(M + H).

4.13.1. 5,6-bis(4-Cyanophenyl)-3-hydroxypyridin-2(1*H*)-one (13)

To a solution of 2,3-dimethoxy-5,6-bis(4-cyanophenyl)pyridine (50 mg, 0.146 mmol) in CH_2Cl_2 (3.0 ml) under nitrogen, was added boron tribromide (1.0 M solution in CH_2Cl_2) (1.5 ml). After addition was completed, the reaction mixture was stirred for 16 h at room temperature. Dichloromethane was removed from the reaction mixture followed by addition of HCl (3N). The resulting solid was filtered, which was redissolved in CH_2Cl_2 and washed with $NaHCO_3$ and brine, dried using anhydrous sodium sulfate and evaporated under reduced pressure to afford a solid (15 mg), yield: 33%. mp 159-162 °C; ^{1}H NMR (300 MHz, MeOD-d_4) δ: 7.62 (m 4H), 7.3 (m, 4H), 7.03 (s, 1H). HRMS Calcd for $C_{19}H_{11}N_3O_2$ $(M + H)^+$ 314.0924. Found 314.0920.

4.13.2. 2,3-bis(4-Cyanophenyl)-5,6-dimethoxypyridine

The mixture of 5,6-dibromo- 2,3-dimethoxypyridine (100 mg, 0.338 mmol), 4-cyanophenylboronic acid (102 mg, 0.7 mmol), $Pd(PPh_3)_4$ (58 mg, 0.05 mmol) and K_2CO_3 (136 mg, 1.0 mmol) in 1,4-dioxane (3.0 ml) and H_2O (1.0 ml) was degassed for 30 min. This mixture was heated to 100 °C and stirred for 16 h. The reaction mixture was cooled to room temperature and partitioned between $NaHCO_3$ and EtOAc (3x), and washed with NaCl (1x). The organic phase was dried over Na_2SO_4 and was concentrated. The resulting residue was purified by ISCO flash chromatography using 30% EtOAc in hexane to give 56 mg of the desired product, yield: 49%. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.56 (d, *J* = 8.1 Hz, 2H), 7.47 (d, *J* = 9.0 Hz, 2H), 7.36 (d, *J* = 8.4 Hz, 2H), 7.25 (d, *J* = 8.1 Hz, 2H), 7.0 (s, 1H), 4.05 (s, 3H), 3.91 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ:172.3, 153.7, 144.3, 144.0, 143.6, 142.0, 132.4, 131.7, 130.5, 130.4, 128.1, 119.6, 118.7, 118.5, 111.2, 111.1, 58.2, 56.2.

4.14.1. 5,6-bis[4-(1*H*-tetrazol-5-yl)phenyl]-3-hydroxypyridin-2(1*H*)-one (14)

To a sealed tube equipped with a small stirring bar was added 5,6-bis(4-cyanophenyl)-3-hydroxypyridin-2(1*H*)-one (100 mg, 0.319 mmol), DMF (2.0 ml), NaN_3 (61 mg, 0.94 mmol) followed by AcOH (1.0 ml). The resulting mixture was heated to 120 °C overnight. After completion of the reaction, the solvent was removed under vacuum. Addition of 1N HCl and stirring produced a solid which was filtered to give the pure product (45 mg, 35% yield). mp 180-182 °C; ^{1}H NMR (300 MHz,

DMSO-d_6) δ: 7.96 (bs, 2H), 7.85-7.77 (m, 4H), 7.18 (d, *J* = 8.4 Hz, 2H), 7.07 (d, *J* = 8.4 Hz, 2H), 6.84 (s, 1H). HRMS Calcd for $C_{19}H_{13}N_9O_2$ $(M + H)^+$ 400.1265. Found 400.1262. .

4.15.1. 5-(4-Cyanophenyl)-6-(4-fluorophenyl)-3-hydroxypyridin-2(1*H*)-one (15)

To a solution of 5-(4-cyanophenyl)-6-(4-fluorophenyl)-2,3-dimethoxypyridine (150 mg, 0.45 mmol) in CH_2Cl_2 (4.0 ml) under nitrogen, was added boron tribromide (2.0 ml of a 1.0 M solution in CH_2Cl_2). After addition was completed, the reaction mixture was stirred for 20 h at room temperature. Dichloromethane was removed from the reaction mixture followed by addition of HCl (3N). The resulting solid was filtered. The solid was redissolved in CH_2Cl_2 and washed with $NaHCO_3$ and brine. The organic phase was then dried and evaporated under reduced pressure to afford the crude product which was purified in ISCO using 5% MeOH in DCM to furnish the pure product (85 mg) as white solid, yield: 62%. mp 255-257 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.39 (bs, 1H), 7.60 (d, *J* = 8.1 Hz, 2H), 7.14-7.03 (m, 6H), 6.77 (s, 1H). HRMS Calcd for $C_{18}H_{11}FN_2O_2$ $(M + H)^+$ 307.0877. Found 307.0870.

4.15.2. 5-(4-Cyanophenyl)-6-(4-fluorophenyl)-2,3-dimethoxypyridine

A mixture of 6-bromo-5-(4-cyanophenyl)-2,3-dimethoxypyridine (150 mg, 0.47 mmol) , 4-fluorophenylboronic acid (99 mg, 0.70 mmol), $Pd(PPh_3)_4$ (70 mg, 0.06 mmol) and K_2CO_3 (129 mg, 0.94 mmol) in 1,4-dioxane (3.0 ml) and H_2O (1.0 ml) was degassed for 30 min. This mixture was heated to 100 ^{0}C and stirred for 16 h. The reaction mixture was cooled to room temperature and partitioned between $NaHCO_3$ and EtOAc (3x), and washed with NaCl (1x). The organic phase was dried over Na_2SO_4 and was concentrated. The resulting residue was purified by ISCO flash chromatography using 25% EtOAc in hexane to give 150 mg (95% yield) desired product. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.51 (m, 2H), 7.35-7.33 (m, 2H), 7.27-7.22 (m, 2H), 6.99 (s, 1H), 6.89 (t, *J* = 8.7 Hz, 2H), 4.08 (s, 3H), 3.92 (s, 3H).

4.15.3. 6-Bromo-5-(4-cyanophenyl)-2,3-dimethoxypyridine

To a solution of 2,3-dimethoxy-5-(4-cyanophenyl)pyridine (350 mg, 1.46 mmol) in acetic acid (8.0 ml) was added sodium acetate (402 mg, 2.9 mmol). The reaction mixture was cooled to 0 °C and Br_2 (238 mg) in acetic acid (1.0 ml) was added dropwise. The reaction mixture was stirred at room temperature for 16 h. To this mixture 25% NaOH was added at 0 °C until pH 6 and then extracted with dichloromethane three times. Organic layer wad dried, concentrated under reduced pressure and purified by ISCO using 100% DCM to afford pure product 310 mg (66 % yield) as an oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.70 (d, *J* = 8.1 Hz, 2H), 7.54 (d, *J* = 8.1 Hz, 2H), 6.99 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 172.5, 153.4, 143.9, 132.3, 130.7, 120.7, 118.8, 111.9, 56.5, 54.4.

4.16.1 5-[4-(1*H*-tetrazol-5-yl)phenyl]-6-(4-fluorophenyl)-3-hydroxypyridin-2(1*H*)-one (16)

To a sealed tube equipped with a small stirring bar was added 5-(4-cyanophenyl)-6-(4-fluorophenyl)-3-hydroxypyridin-2(1*H*)-one (45 mg, 0.141 mmol), DMF (2.0 ml), NaN_3 (38 mg, 0.56 mmol) followed by

AcOH (0.1 ml). The resulting mixture was heated to 120°C for 4 h. After the completion of the reaction, the solvent was removed under reduced pressure. Addition of 3N HCl and stirring produced a solid which was filtered to give the pure product (15 mg, 31% yield). mp 185-188 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 9.4 (bs, 1H), 7.83 (d, *J* = 8.1 Hz, 2H), 7.20 (d, *J* = 7.8 Hz, 4H), 7.10 (m, 2H), 6.86 (s, 1H). HRMS Calcd for $C_{18}H_{12}FN_5O_2$ $(M + H)^+$ 350.1048. Found 350.1057.

4.17.1. 5-(4-Fluorophenyl)-6-(4-cyanophenyl)-3-hydroxypyridin-2(1*H*)-one (17)

To a solution of 5-(4-fluorophenyl)-6-(4-cyanophenyl)-2,3-dimethoxypyridine (240 mg, 0.72 mmol) in CH_2Cl_2 (3.0 ml) under nitrogen, was added boron tribromide (2.0 ml of a 1.0 M solution in CH_2Cl_2). After addition was completed, the reaction mixture was stirred for 16 h at room temperature. Dichloromethane was removed from the reaction mixture followed by addition of HCl (3N). The resulting solid was filtered. The solid was redissolved in CH_2Cl_2 and washed with $NaHCO_3$ and brine. The organic phase was then dried and evaporated under reduced pressure to afford the crude product which was purified in ISCO using 5% MeOH in DCM to furnish the pure product (183 mg) as off-white solid, yield: 83%. mp 255-258 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ: ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.75 (d, *J* = 8.1 Hz, 2H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.07 (d, *J* = 7.2 Hz, 4H), 6.82 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6) δ: 172.5, 147.0, 132.7, 132.5, 132.2, 132.1, 131.6, 119.2, 116.1, 115.7, 107.6. HRMS Calcd for $C_{18}H_{11}FN_2O_2$ $(M + H)^+$ 307.0877. Found 307.0844.

4.17.2. 5-(4-Fluorophenyl)-6-(4-cyanophenyl)-2,3-dimethoxypyridine

A mixture of 5-bromo-6-(*p*-cyanophenyl)-2,3-dimethoxypyridine (360 mg, 1.13 mmol) , 4-fluorophenylboronic acid (237 mg, 1.69 mmol), $Pd(PPh_3)_4$ (173 mg, 0.15 mmol) and K_2CO_3 (307 mg, 2.26 mmol) in 1,4- dioxane (3 ml) and H_2O (1.5 ml) was degassed for 30 min. This mixture was heated to 100 °C and stirred for 16 h. The reaction mixture was cooled to room temperature and partitioned between $NaHCO_3$ and EtOAc (3x), and washed with NaCl (1x). The organic phase was dried over Na_2SO_4 and was concentrated. The resulting residue was purified by ISCO flash chromatography using 25% EtOAc in hexane to give 340 mg (90% yield) of the desired product. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.49-7.40 (m, 4H), 7.13-7.09 (m, 2H), 7.02-6.95 (m, 3H), 4.07 (s, 3H), 3.92 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ: 163.8, 160.5, 153.1, 144.17, 143.76, 141.56, 135.47, 135.42, 132.86, 131.54, 131.31, 131.20, 130.50, 129.05, 127.94, 120.16, 118.96, 115.96, 115.57, 110.58, 56.01, 53.88.

4.17.3. 6-(4-Cyanophenyl)-5-bromo-2,3-dimethoxypyridine

To a mixture of 2,3-dimethoxy-6-(4-cyanophenyl)pyridine (210 mg, 0.875 mmol) in acetic acid (3.0 ml) was added sodium acetate (217 mg, 1.6 mmol). The reaction mixture was cooled to 0 °C and Br_2 (0.045 ml) in acetic acid (1.0 ml) was added dropwise. The reaction mixture was stirred at room temperature for 12 h. To this mixture 25% NaOH was added at 0 °C until pH 6 and then extracted with DCM three times. Organic layer wad dried, concentrated under reduced pressure and purified by ISCO using 20% EtOAC in hexane to afford pure product 360 mg (91% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 7.51 (d, *J* = 8.7 Hz, 2H), 7.55 (d, *J* = 8.4 Hz, 2H), 6.97 (s, 1H), 4.07 (s, 3H), 3.89 (s, 3H).

4.17.4 2,3-Dimethoxy-6-(4-cyanophenyl)pyridine

A mixture of 6-bromo-2,3-dimethoxypyridine (434 mg, 2.0 mmol) , 4-cyanophenylboronic acid (440 mg, 3.0 mmol), $Pd(PPh_3)_4$ (346 mg, 0.3 mmol) and K_2CO_3 (552 mg, 4.0 mmol) in 1,4-dioxane (3 ml) and H_2O (1.0 ml) was degassed for 30 min. This mixture was heated to 100 °C and stirred for 16 h. The reaction mixture was cooled to room temperature and partitioned between $NaHCO_3$ and EtOAc (3x), and washed with NaCl (1x). The organic phase was dried over Na_2SO_4 and was concentrated. The resulting residue was purified by ISCO flash chromatography using 25% EtOAc in hexane to give 200 mg desired product. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.04 (d, *J* = 8.7 Hz, 2H), 7.6 (d, *J* = 8.4 Hz, 2H), 7.36 (d, *J* = 8.1 Hz, 1H), 7.09 (d, *J* = 8.1 Hz, 1H), 4.09 (s, 3H), 3.90 (s, 3H).

4.17.5 6-Bromo-2,3-dimethoxypyridine

To a solution of 2,3-dimethoxypyridine (5.0 g, 36 mmol) in AcOH (10.0 ml), NaOAC (9.78 g, 71.94 mmol) was added and the mixture was cooled to -32 °C. After 30 minutes, Br_2 (1.0 ml) in AcOH (5 ml) was added dropwise for 30 minutes. After the addition the reaction mixture was stirred for 2 h at -20 °C. To this mixture 25% NaOH was added at 0 °C until pH 6 and then extracted with DCM three times. Organic layer wad dried, concentrated under reduced pressure and purified by ISCO using 10% EtOAC in hexane to afford a mixture of 5- and 6-bromo intermediates along with starting material which can be separated to afford the 6-bromo-2,3-dimethoxypyridine (1.4 g). mp 172-175 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 6.95 (d, *J* = 8.1 Hz, 1H), 6.89 (d, *J* = 8.1 Hz, 1H), 3.96 (s, 3H), 3.83 (s, 3H).

4.18.1 5-(4-Fluorophenyl)-6-[4-(1*H*-tetrazol-5-yl)phenyl]- -3-hydroxypyridin-2(1*H*)-one (18)

To a sealed tube equipped with a small stirring bar was added 5-(4-fluorophenyl)-6-(4-cyanophenyl)-3-hydroxypyridin-2(1*H*)-one (50 mg, 0.16 mmol), DMF (2.0 ml), NaN_3 (42 mg, 0.64 mmol) followed by AcOH (1.0 ml). The resulting mixture was heated to 120 °C overnight. After completion of the reaction, the solvent was removed under vacuum. Addition of 1N HCl and stirring produced a solid which was filtered to give the pure product (30 mg, 54% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 7.83 (d, *J* = 8.1 Hz, 2H), 7.14 (d, *J* = 8.1 Hz, 2H), 7.09-7.00 (m, 4H), 6.81 (s, 1H). HRMS Calcd for $C_{18}H_{12}FN_5O_2$ $(M + H)^+$ 350.1048. Found 350.1058.

5. 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 °C for 17 h. 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.

6. 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 °C. Fluorescence is measured at various time points (5, 120, and 240 minutes) during the 37 °C incubation. Activity/inhibition is calculated based on the change in fluorescence over time using Prism Graphpad non-linear regression analysis.

7. Compound Soaking, Data Collection and Processing

Crystal structures of compounds **16** and **18** were determined in complex with influenza A 2009 H1N1 influenza A endonuclease enzyme as previously described.[18] The soaks of **16** and **18** were performed by taking crystals and by stepwise 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 °C 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.*[24] The structure was solved and refined using the software PHENIX.[25]

8. Cytotoxicity Assays. Cytotoxicity was determined using the MTT-microtiter plate tetrazolinium cytotoxicity assay (MTA). The human embryonic kidney 293 (HEK293) cell line was provided by Dr. Zue-Hung Hsu (formerly at Columbia University, presently at Beijing National academy). The Madin-Darby Canine Kidney (MDCK) epithelial cells were obtained from Prof. Patrick Sinko (Rutgers

University). The cytotoxicity assay was performed using 96-well microtiter plates. Cells were grown in suspension at 37 °C in 5% CO_2 and maintained by regular passage in DMEM media. For determination of IC_{50}, cells were exposed continuously for four days to varying concentrations of drug in triplicate wells, each seeded with 3,000 cells. Each assay was performed with a control that did not contain any drug. The MTT assays were performed at the end of the fourth day.

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Supplementary data

Representative kinetic curves associated with the inhibition of endonuclease actitivy for six compounds is provided in Figure 1S. X-ray data and refinement statistics for the analysis performed with **18**. The parameters associated with the analysis of the X-ray data for **16** are reported elsewhere.[19]

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Phenyl substituted 3-hydroxypyridin(1*H*)-2-ones: Potential inhibitors of influenza A endonuclease.

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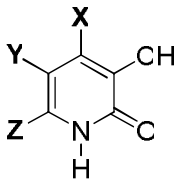

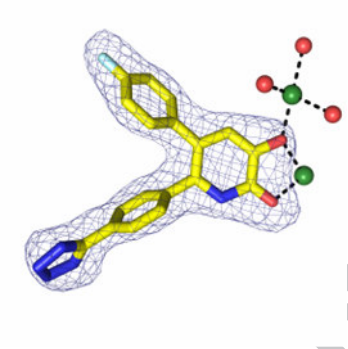

X = phenyl or *p*-fluoropheny, Y = Z = HX = Z = H; Y = phenyl, *p*-fluorophenyl, *p*-cyanophenyl or *p*-(tetrazol-5-yl)phenylX = Y = H; Z = phenyl, *p*-fluorophenyl, *p*-cyanophenyl or *p*-(tetrazol-5-yl)phenylX = H; Y = Z = phenyl, *p*-fluorophenyl, *p*-cyanophenyl or *p*-(tetrazol-5-yl)phenylX = H; Y = *p*-cyanophenyl or *p*-(tetrazol-5-yl)phenyl; Z = *p*-fluorophenylX = H; Y = *p*-fluorophenyl; Z = *p*-cyanophenyl or *p*-(tetrazol-5-yl)phenyl