analytical chemistry

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Development of an Anti-Influenza Drug Screening Assay Targeting Nucleoproteins with Tryptophan Fluorescence Quenching

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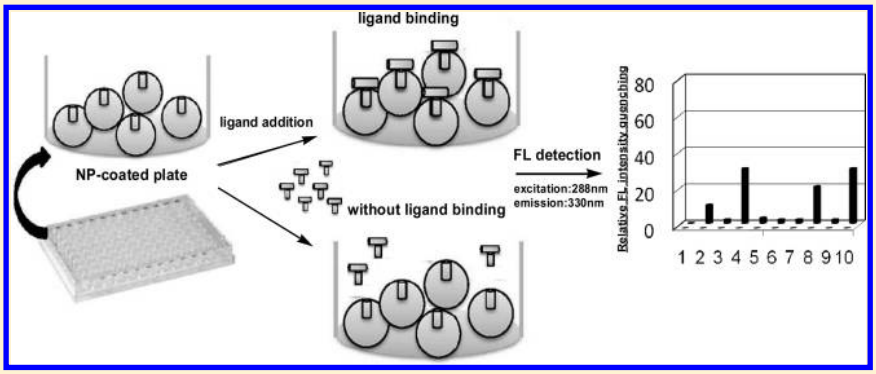
Supporting Information



ABSTRACT: Recent studies have shown that NP (nucleoprotein), which possesses multiple functions in the viral life cycle, is a new potential anti-influenza drug target. NP inhibitors reliably induce conformational changes in NPs, and these changes may confer inhibition of the influenza virus. The six conserved tryptophan residues in NP can be used as an intrinsic probe to monitor the change in fluorescence of the tryptophan residues in the protein upon binding to an NP inhibitor. In the present study, we found that the fluorescence of recombinant NP proteins was quenched following the binding of available NP inhibitors (such as nucleozin) in a concentration- and time-dependent manner, which suggests that the inhibitor induced conformational changes in the NPs. The minimal fluorescence-quenching effect and weak binding constant of nucleozin to the swine-origin influenza virus H1N1pdm09 (SOIV) NP revealed that the SOIV is resistant to nucleozin. We have used the fluorescence-quenching property of tryptophans in NPs that were bound to ligands in a 96-well-plate-based drug screen to assess the ability of promising small molecules to interact with NPs and have identified one new anti-influenza drug, CSV0C001018, with a high SI value. This convenient method for drug screening may facilitate the development of antiviral drugs that target viruses other than the influenza virus, such as HIV and HBV.

Influenza is an infectious disease of birds and mammals that is caused by influenza viruses of the *Orthomyxoviridae* family.[1] The recent, sudden swine-origin influenza virus (SOIV) H1N1v pandemic outbreak in 2009 caused 18,000 deaths.[1,3] In general, the hemeagglutinin (HA) and neuraminidase (NA) proteins are important targets for antiviral drugs, and they are also recognized by antibodies from human sera.[4] Tamiflu (oseltamivir), which is a neuraminidase inhibitor, is used to treat in patients.[5−7] However, several H1N1 influenza strains were known to be resistant to Tamiflu because of the H274Y mutation in neuraminidase.[7−10] Thus, new anti-influenza drugs and drug targets are urgently needed.

Influenza virus A nucleoproteins (NPs) are major virion structural proteins, and they have been predicted to interact with negative-strand viral RNA, which together constitute the viral nucleocapsid.[11] NPs encapsulate the viral genome for RNA

Received: August 25, 2011
Accepted: June 19, 2012
Published: June 19, 2012

   dx.doi.org/10.1021/ac2022426 | *Anal. Chem.* 2012, 84, 6391−6399

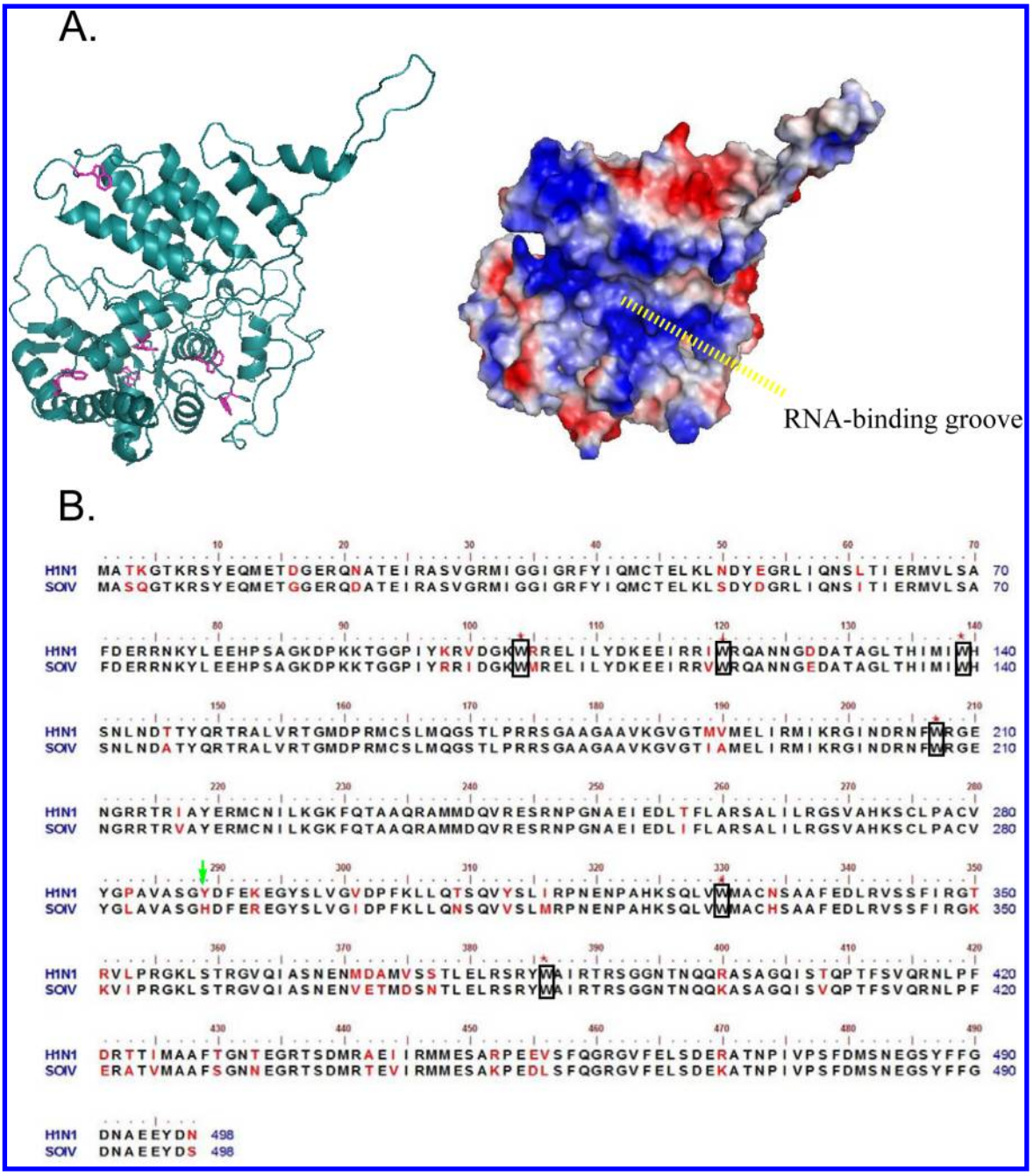


Figure 1. (A) Structural model of the influenza virus (H1N1) nucleoprotein. The six conserved tryptophan residues that contribute to the conformational change that occurs upon drug binding are marked (left). Surface representation of the homology model of the influenza virus (H1N1) nucleoprotein: electrostatic potentials are colored in blue (positive) and red (negative) (right). (B) Amino acid pairwise sequence alignment of the NPs of the H1N1 strain (A/TW/12/2001) and the SOIV strain (A/California/07/2009 (H1N1pdm09)). Tryptophan (W) and residue 289 are indicated with a box and arrow, respectively.

transcription, replication, virus packaging, and intracellular trafficking, and they also function as a key adapter molecule between viral and host cell processes.[12,13] NPs have been shown to cooperatively interact with RNA. A substantial quantity of RNA is wrapped around each NP monomer at the stoichiometric ratio of 1 NP per 20 nucleotides of RNA.[14] The NP crystal structure shows that RNAs are likely to bind to a deep groove located between the head and body domain on the exterior of the NP oligomers.[15,16] In addition to RNA, NPs interact with a wide variety of viral and cellular macromolecules, including two subunits of the viral RNA-dependent RNA polymerase, viral matrix, actin, components of the nuclear import/export apparatus, and a nuclear RNA helicase.[13] According to the protein sequence alignment, the 498-amino acid NP is highly conserved among influenza viruses. The multifunctional capabilities of NPs in the viral life cycle make NP an attractive candidate for drug development.[17] Six tryptophan residues are evenly distributed in the structure of the influenza virus NP, and these residues are not located near the predicted RNA-binding site (Figure 1A,B). Tryptophan is an important intrinsic fluorescent probe that can be used to

dx.doi.org/10.1021/ac2022426 | Anal. Chem. 2012, 84, 6391−6399

monitor microenvironmental changes around a protein's tryptophan residues.[18−20]

In this study, we used the fluorescence-quenching property of tryptophans in NPs upon drug binding to develop an in vitro drug screening assay to assess the ability of small molecules to interact with NPs. Using this method, we identified several compounds with antiviral activity. This facile method may facilitate the discovery of novel drugs that targeting influenza.

MATERIALS AND METHODS

Expression and Purification of Full-Length Recombinant Nucleoproteins. To generate full-length recombinant NPs encoded by the A/TW/12/2001 (H1N1) and swine-origin influenza virus A/California/07/2009 (SOIV, H1N1pdm09) strains, the NP genes were amplified with the polymerase chain reaction (PCR) using various primers. The PCR products were digested with *Nde*I and *Xho*I, and the DNA fragments were cloned into pET21b (Novagen) using T4 ligase (NEB). DH5α compentent cells that were transformed with the resultant plasmid were grown in culture. Protein expression was induced by supplementing the culture media with 1 mM IPTG, followed by incubation at 10 °C for 24 h. After the bacteria were harvested by centrifugation (6000 *g* for 15 min at 4 °C), the bacterial pellets were lysed with lysis buffer (50 mM Tris-buffered solution, pH 7.5, 100 mM NaCl and 15 mM imidazole). The soluble proteins were isolated from the supernatant following centrifugation (13,000 rpm for 30 min at 4 °C) to remove the precipitate. The recombinant NPs carrying an N-terminal 6 × His-tag were purified using a Ni-NTA column (Novagen) with an elution gradient that ranged from 15 to 300 mM imidazole. The pure fractions were collected and dialyzed against the buffer that lacked imidazole. Purified NPs (with greater than 95% purity) were analyzed by Coomassie blue staining (Figure 2). The protein concentrations were determined with the Bradford method using Bio-Rad protein assay reagents.

Fluorescence Spectroscopy and Compounds. The test small molecules were primarily obtained from an in-house collection of compounds and were included for screening of NP inhibitors. Nucleozin was acquired from ChemBridge Corporation (San Diego, CA). In the drug-induced fluorescence-quenching experiment, a final concentration of 4 μM NP was added to a buffer that contained various concentrations of compound, and the samples were incubated at 25 °C for various durations. The buffer consisted of 50 mM Tris (pH 7.5) and 100 mM NaCl. The tryptophan fluorescence was measured using a Hitachi F-4500 fluorescence spectrophotometer that was equipped with a cuvette of a 1 cm light path. The excitation wavelength was 288 nm, and the emission data were collected between 300 and 400 nm. For the static measurements, all of the measurements were recorded in triplicate. The relative fluorescence-quenching intensity was determined using the following equation: $(FL_{NP} - FL_{NP\text{-}drug})/FL_{NP}$, where FL_{NP} is the NP fluorescence in the absence of compound, and $FL_{NP\text{-}drug}$ is the fluorescence of the NP-compound complex.

To determine the binding constant between nucleozin and the NPs, the nucleozin-induced fluorescence changes (ΔF) from three separate experiments at 1 h after addition of test compounds were averaged and fit with the Hill equation using the GraphPad Prism software program (San Diego, CA) as follows: $\Delta F/\Delta F_{max} = 1/[1 + (K_d/X)^n]$, where ΔF_{max} is the saturating value of the fluorescence change, X is the drug concentration, K_d is the dissociation constant, and n is the Hill coefficient.

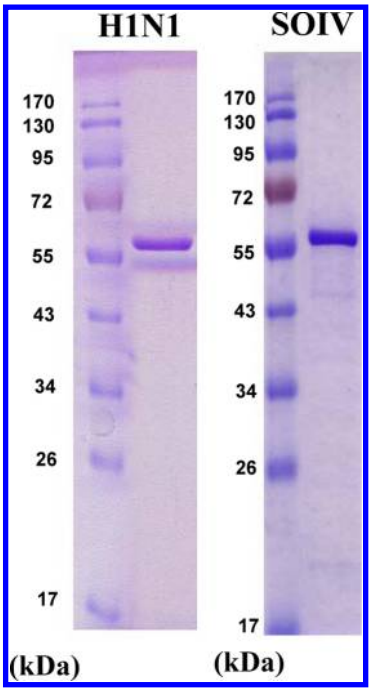


Figure 2. SDS-PAGE analysis of purified NPs of the H1N1 (left) and the SOIV strain (right).

Fluorescence Microplate Assay. In the ligand-induced fluorescence-quenching experiment, black 96-well microplates were coated with a final concentration of 4 μM NPs in a buffer containing the compounds in a concentration of 8 μM; the samples were incubated at 25 °C for 1 h. The buffer consisted of 50 mM Tris (pH 7.5) and 100 mM NaCl. The samples were detected using an Infinite M200 Pro microplate reader (Tecan Group Ltd., Mannerdorf, Switzerland). The excitation wavelength was 288 nm, and the emission wavelength was 331 nm.

Cells, Viruses, And Reagents. The cell culture conditions used in this study were similar to those described in our previous studies.[21,22] In brief, Madin−Darby canine kidney cells (MDCK cells: ATCC CCL-34) were purchased from the American Type Culture Collection (ATCC, Manassas, VA). The cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM, Gibco, USA) that was supplemented with 10% fetal bovine serum. Serum-free DMEM that contained trypsin (0.25% trypsin, Gibco, USA) was used as an infection medium for the MDCK cells. The A/WSN/1933 (H1N1) and swine-origin influenza virus A/California/07/2009 strains were utilized in this study.

Cytopathic Effect Reduction (CPE) Assay. The laboratory strain-A/WSN/1933 (H1N1) was used to determine the antiviral activity instead of A/TW/12/2001(H1N1) because the latter does not cause any apparent CPE. A comparison of the sequence similarity of the NPs from the A/TW/12/2001 (H1N1) and A/WSN/1933 (H1N1) strains showed that the NPs share 97% similarity. Therefore, the antiviral activity assays were performed using the A/WSN/1933 (H1N1) strain, which has been routinely employed in our laboratory. To determine whether the positive compounds that were identified in the fluorescence microplate assay could inhibit the cytopathic effect (CPE) of the influenza viruses on cells, 96-well tissue culture plates were seeded with 200 μL of MDCK cells (1×10^5 cells/

dx.doi.org/10.1021/ac2022426 | Anal. Chem. 2012, 84, 6391−6399

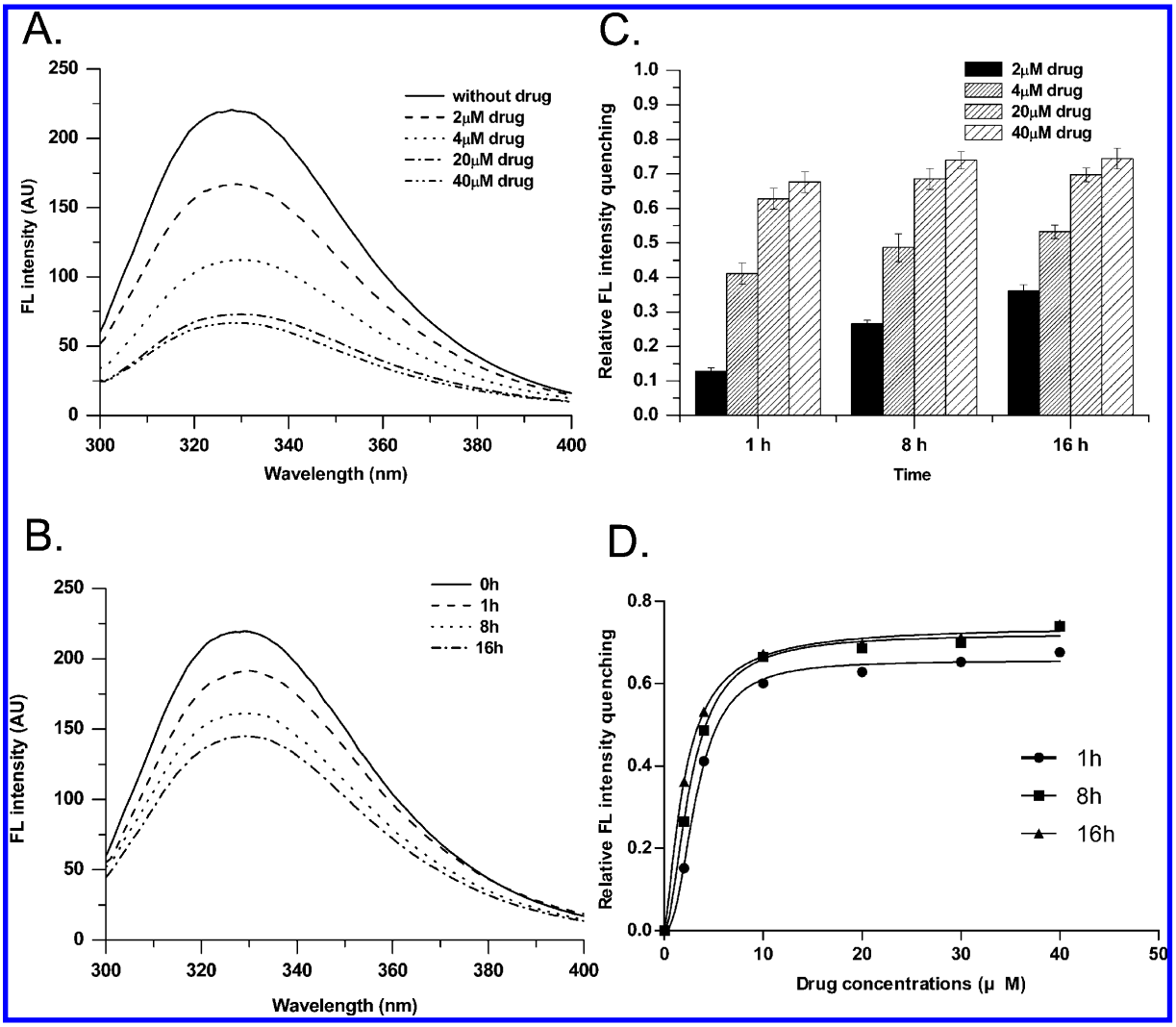


Figure 3. Changes in FL spectra of NP of the H1N1 (A/TW/12/2001) strain upon binding to nucleozin. (A) FL spectra of the NP (4 μM) incubated in the presence of nucleozin at various concentrations for 3 h; nucleozin was buffered with 20 mM Tris-HCl (pH 7.5) and 100 mM NaCl. (B) FL spectra of the NP in the presence of nucleozin at the indicated times. A drug concentration of 2 μM was used, and the drug was buffered with 20 mM Tris-HCl (pH 7.5) and 100 mM NaCl. (C) Relative FL intensity quenching of the NP upon nucleozin binding at various concentrations with time. (D) Nonlinear regression analyses of the relative fluorescence intensity quenching of the H1N1 NP in the presence of various concentrations of nucleozin at various time points.

ml) in DMEM that was supplemented with 10% FBS, and the cells were incubated for 16−20 h at 37 °C in 5% CO_2. The cells were washed once with PBS, infected with the virus at a multiplicity of infection (MOI) of 0.2 per cell in 150 μL of DMEM, and overlaid with 50 μL of DMEM that contained various concentrations of the test compounds. After the cells were incubated at 37 °C in 5% CO_2 for 48 h, they were fixed with formaldehyde (10% final concentration) and stained with 0.1% crystal violet as previously described.[21] The concentration that was required for the tested compound to reduce the viral CPE by 50% (IC_{50}, effective concentration at which 50% of the cytopathic effect was removed) was determined. Nucleozin was used as a reference control in the CPE assay.[23]

Cytoxicity Assay. The cytoxicity was evaluated using an MTS assay that was previously described by Hung et al.[21] MDCK cells were grown in 96-well plates (7,000 cells/well) for 24 h. The cell culture medium was then replaced with medium that contained serially diluted compounds, and the cells were further incubated for 48 h. The culture medium was removed, and 100 μL of the MTS and PMS assay mixture solution was added. The plate was incubated for 30 min. The optical density was measured at 490 nm on an ELISA reader plate. The CC50 values (50% Cytotoxicity Concentration) were defined as the concentration of a compound that inhibited 50% of the cell death.

Molecular Modeling. The crystal structure of the influenza A virus NP (Protein Data Bank code 2IQH) lacks a defined tertiary structure in the regions between amino acid residues 73−91, 397−401, and 429−437. The SWISS-MODEL program was used to model the complete structure of the NP. The quality of the modeled structure was tested with PROCHECK.[24] Compound CSV0C001018 was docked into the site of the H1N1 NP using the GOLD software program.[25,26] The 3D structure of CSV0C001018 was generated using the Dundee PRODRG server.[27] The binding pocket of the H1N1 virus NP was defined to include the amino acid residues (Y289) that were within a 10 Å radius sphere centered on the CSV0C001018 binding site. After the docking process, the modeled complexes between compound CSV0C001018 and influenza NP were obtained. A CHARMM force field was used

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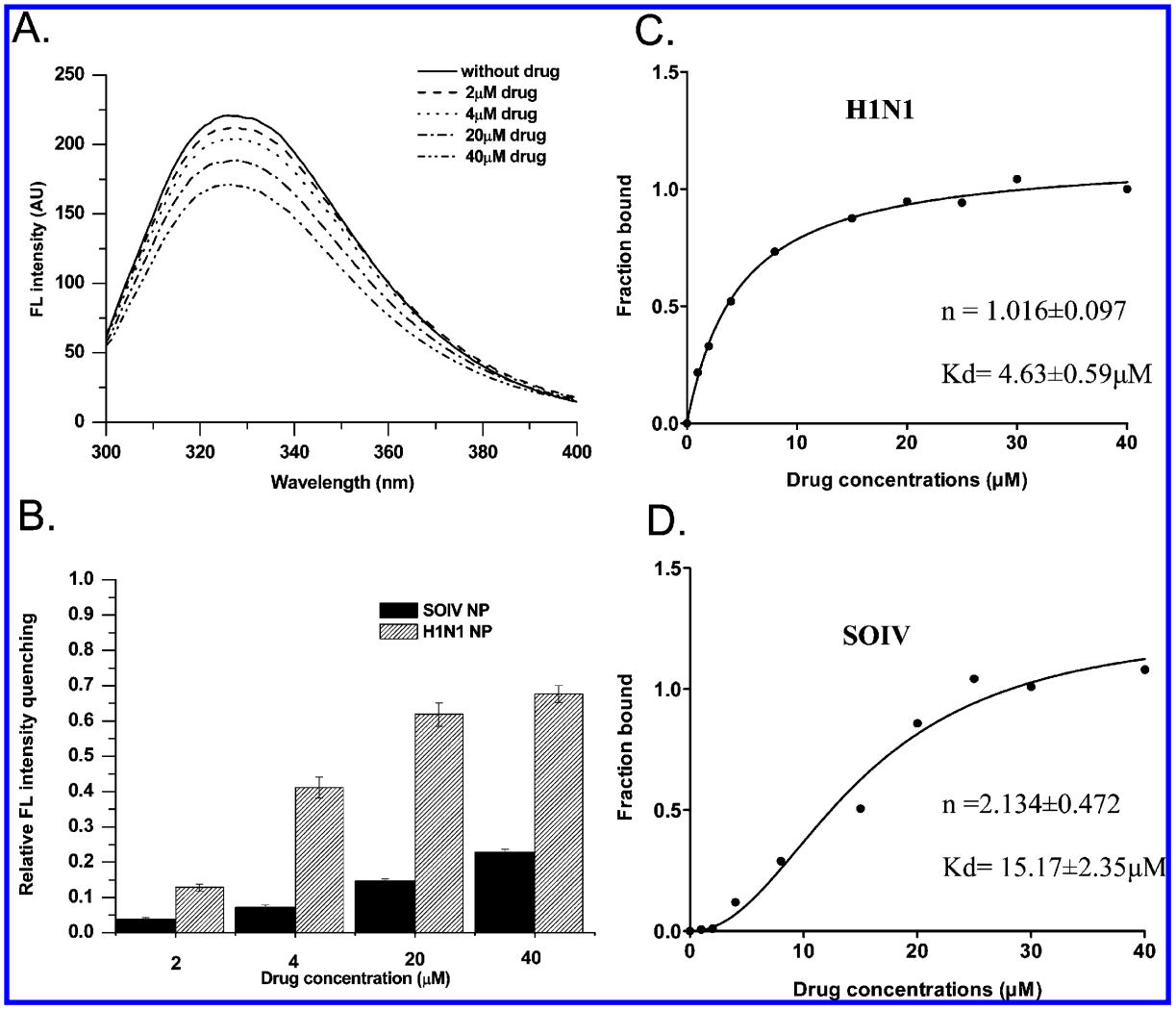

Figure 4. Characterizations of nucleozin binding to NPs of the H1N1 strain (A/TW/12/2001) and SOIV strain (A/California/07/2009 (H1N1pdm09)). (A) FL spectra of NP (4 μM) from the SOIV strain incubated in the presence of nucleozin at various concentrations for 3 h; the nucleozin was buffered with 20 mM Tris-HCl (pH 7.5) and 100 mM NaCl. (B) Relative FL intensity quenching of the two NPs upon nucleozin binding at various concentrations for 1 h. (C) Titration of the H1N1 NP with nucleozin in Tris buffer (20 mM Tris-HCl with 100 mM NaCl; pH 7.5). The average of three experiments is shown. The data are expressed as a percentage of the maximal fluorescence change as determined by a fit to the Hill equation. (D) Titration of the SOIV NP with nucleozin in Tris buffer (20 mM Tris-HCl with 100 mM NaCl; pH 7.5). The average of three experiments is shown. The data are expressed as a percentage of the maximal fluorescence change as determined by a fit to the Hill equation.

to refine the docked complexes by optimizing the drug/protein interaction.[28]

RESULTS

Studies of the Drug/NP (H1N1) Interaction Using the Fluorescence-Quenching Effect. Nucleozin, an NP inhibitor, was used to test the drug-induced fluorescence-quenching effect of the influenza virus NPs. We utilized tryptophan fluorescence to monitor the protein/drug binding because the influenza virus NP contains six tryptophan residues. The fluorescence emission spectra for the H1N1 NP showed the maximal emission wavelength at approximately 331 nm with 220 AU (Figure 3A). At drug concentrations of 2, 4, 20, and 40 μM, nucleozin decreased the fluorescence intensity of the NP at approximately 331 nm by 165, 112, 70, and 58 AU, respectively, after 3 h of drug addition. The NP fluorescence decreased with increasing drug concentrations, which suggests that this decrease reflected the interaction of NPs with nucleozin. As shown in Figure 3B, there was also a time-dependent decrease in the fluorescence of the NP that was loaded with nucleozin. This result indicates that the tryptophan fluorescence probe was spread over the NP and that nucleozin could quench the fluorescence due to conformational changes over time.

The fluorescence quenching of the NP in the presence of nucleozin at various times and concentrations was observed by monitoring the 331 nm fluorescence spectra. As shown in Figure 3C, the relative fluorescence quenching increased with time at low drug/protein ratios (0.5 and 1). At high drug/protein ratios (5 and 10), the relative fluorescence quenching was constant over time. Previous studies have shown that NPs interact at multiple drug binding sites.[23,29] Therefore, the drugs that were administered at high drug/protein ratios may have induced conformational changes in the NPs that reached saturation in a relatively short amount of time. As shown in Figure 3D, the relative fluorescence intensity quenching of the H1N1 NP in the presence of various concentrations of nucleozin at various times was analyzed with nonlinear regression analysis. The EC_{50} values of nucleozin occupying

dx.doi.org/10.1021/ac2022426 | Anal. Chem. 2012, 84, 6391−6399

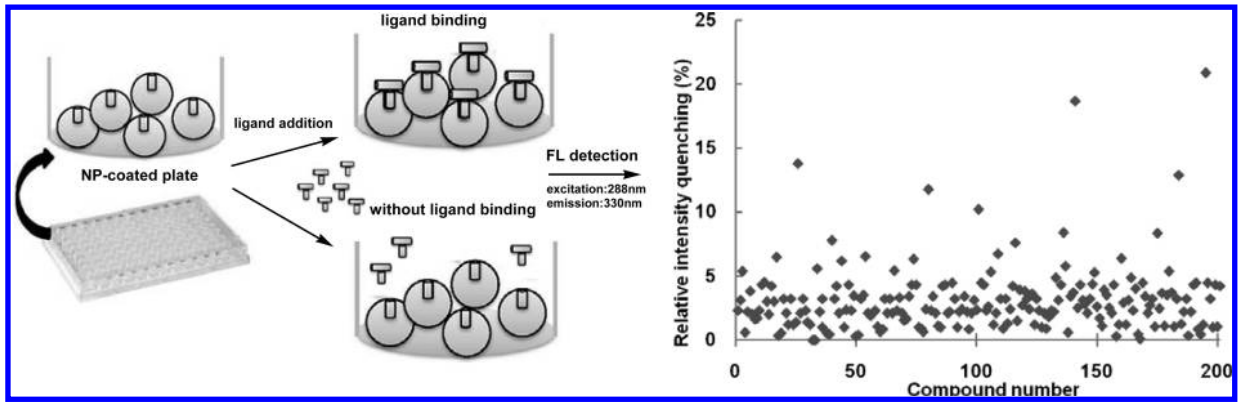

Figure 5. Schematic diagram of the recombinant NP proteins upon drug binding and the relative tryptophan FL intensity quenching.

Table 1. Anti-Influenza Virus Activity of Compounds That Decreased NP Fluorescence by More than 10%

no.	compound	relative intensity quenching (%)	IC_{50} against WSN (μM)	cytotoxicity (μM)	SI index
1	CSV0C001018	20.9	1.4 ± 0.7	>100.0	>71
2	methotrexate	13.8	>50.0	50.0 ± 2.1	–
3	amodiaquine dihydrochloride	11.8	6.3 ± 2.4	25.0 ± 3.0	4
4	dicumarol	10.2	>50.0	>50.0	–
5	N-2-,N-2−dimethyl-N∼1∼-(6-oxo-5,6-dihydrophenanthridin-2-yl) glycinamide	12.9	>50.0	>50.0	–
6	curcumin	20.8	28.8 ± 3.8	39.6 ± 1.0	1.4

half of the binding sites of H1N1 NP were calculated to be 3.2, 2.7, and 2.2 μM at 1, 8, and 16 h, respectively.

Comparisons of Drug-Induced Fluorescence Quenching Targeting the NPs from the H1N1 and SOIV Strains. We also examined the effect of nucleozin on the swine-origin strain (A/California/07/2009, H1N1pdm09) NP using the fluorescence-quenching assay. The fluorescence emission spectra revealed that the maximal emission wavelength for the SOIV NP also appeared at approximately 331 nm and 220 AU (Figure 4A). At drug concentrations of 2, 4, 20, and 40 μM, nucleozin decreased the SOIV NP fluorescence intensity at approximately 331 nm to 210, 202, 184, and 169 AU, respectively. In contrast to the strong fluorescence quenching of the H1N1 NP caused by nucleozin (Figure 3A), this compound caused only little effect on the SOIV NP as shown in Figure 4B. In the presence of nucleozin, the fluorescence of the H1N1 NP and the SOIV H1N1 NP decreased. As shown in Figure 4C and D, the fluorescence quenching of the H1N1 and the SOIV NPs by nucleozin was analyzed with the Hill plot after the addition of nucleozin. The affinity of nucleozin for the H1N1 NP (K_d of 4.63 ± 0.59 μM) was higher than that for the SOIV NP (K_d of 15.17 ± 2.35 μM). It was preciously suggested that NPs may contain an additional nucleozin binding site.[23,29] According to the n value, the binding between nucleozin and the H1N1 NP was not cooperative. However, the binding between nucleozin and the SOIV NP appeared to be cooperative.

To evaluate the antiviral activity of nucleozin, the cytopathic effect (CPE) reduction assay was used to assess the ability of nucleozin to inhibit influenza virus replicationin MDCK cells. Nucleozin was more potent for influenza A/WSN/1933-(H1N1) than for A/California/07/2009 (SOIV, H1N1pdm09) with an IC_{50} of 0.24 ± 0.05 μM and 25 ± 4.1 μM, respectively. The fluorescence-quenching results revealed in this study may be indicating that the SOIV is highly resistant to nucleozin.[23]

Identification of anti-influenza hits that target the NPs. To identify potential NP inhibitors, purified recombinant NP was treated with 202 compounds from the in-house compound collection for 1 h. The quenching of tryptophan fluorescence caused by the compound was used to measure the NP/drug interaction via a fluorescence microplate reader. To establish a cutoff point for quenching, we chose nucleozin as a reference drug. The IC_{50} of nucleozin against the swine-origin influenza virus H1N1pdm09 in the CPE reduction assay is ∼25 μM, and this result revealed that the SOIV is resistant to nucleozin, whereas ∼10% fluorescence quenching was observed when the SOIV NP was treated with nucleozin at a drug/protein molar ratio of 2. Therefore, a 10% fluorescence quenching of NP is considered as a cutoff point for inhibitor that are likely to have true biological inhibition of virus. Compounds that significantly decreased the NP fluorescence by more than 10% were selected for further characterization (Figure 5). We found six hits among the in-house collection compounds. These hits were tested for their antiviral activity in the secondary assay, and three were positive: CSV0C001018 compound, amodiaquine dihydrochloride, and curcumin (H7) (Table 1). One of the selected compounds was CSV0C001018, which is a nucleozin analogue (Figure 6A). To further confirm the screening results, a concentration−response evaluation of the effect CSV0C001018 on the fluorescence quenching of the NPs was conducted, and the results showed that CSV0C001018 decreased the NP fluorescence intensity in a concentration-dependent manner (Figure 6B). As shown in Figure 6C, the relative fluorescence intensity quenching of the H1N1 NP in the presence of various concentrations of CSV0C001018 was analyzed with nonlinear regression analysis. The EC_{50} values of CSV0C001018 occupying half of the binding sites of H1N1 NP were calculated to be 4.7, 4.0, and 3.8 μM at 1, 8, and 16 h, respectively. As a control, CSV0C001017, structurally similar to CSV0C001018 (Figure 6A), did not induce the fluorescence quenching of NPs at any

dx.doi.org/10.1021/ac2022426 | Anal. Chem. 2012, 84, 6391−6399

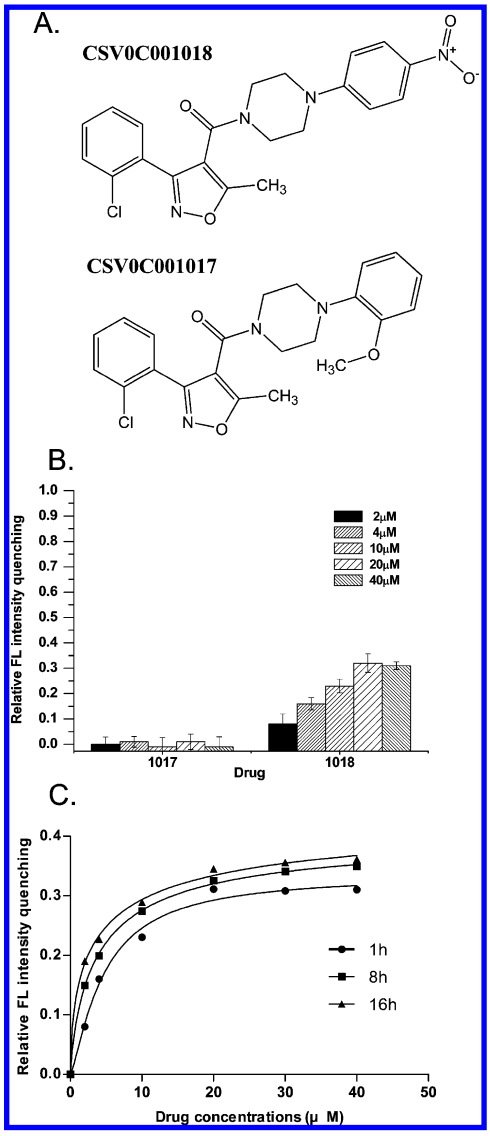

Figure 6. (A) Chemical structures of CSV0C001018 and CSV0C001017. (B) Relative FL intensity quenching of the NP upon CSV0C001018 and CSV0C001017 binding at various concentrations. (C) Nonlinear regression analysis of the relative fluorescence intensity quenching of the H1N1 NP in the presence of various concentrations of CSV0C001018 at various time points.

concentration (Figure 6B). Similar effects were observed in the cell-based CPE assay. These results further demonstrate that CSV0C001018 inhibited influenza replication with an IC_{50} of 1.4 ± 0.7 μM. According to the cytoxicity assay, the CC_{50} of CSV0C001018 was greater than 100 μM. Therefore, the selective index ($SI = IC_{50}/CC_{50}$) for CSV0C001018 was greater than 71. In contrast, no significant reduction in CPE was observed in the presence of another nucleozin analog CSV0C001017 at concentrations of up to 50 μM. Thus, these results indicate that CSV0C001017 did not affect the influenza virus-induced cytopathic effect in MDCK cells. In addition to CSV0C001018, several potential non-nucleozin analogue compounds with anti-influenza activity were identified by the tryptophan fluorescence-quenching assay. Two of the potential natural compounds, curcumin and amodiaquine dihydrochloride (Figure 1S of the Supporting Information), also caused significant quenching of NP (more than 10%). Although curcumin and amodiaquine dihydrochloride possessed anti-influenza activity in the cell-based CPE reduction assay, their SI values are far less than the SI value for CSV0C001018 (Table 1).

DISCUSSION

Drugs that have been approved to treat influenza virus infections primarily target M2 channels and neuraminidase. The use of M2 inhibitors, such as amantadine and rimantadine, has been limited by the propensity of these drugs to cause CNS side effects and the rapidly increased number of drug-resistant viral strains.[30,31] Neuraminidase, or sialidase, has an important role during the influenza virus replication cycle in infected cells.[32] The neuraminidase inhibitors (zanamivir and oseltamivir) are commonly employed to treat influenza virus infections with minimal adverse effects.[33] However, recent studies have reported that the influenza virus has developed resistance to oseltamivir and zananimivir.[34] Therefore, the development of novel antiviral strategies is required to combat the drug-resistant influenza viruses.

The influenza virus nucleoprotein (NP) is a multifunctional RNA-binding protein that is associated with genome and antigenome RNA, and it is necessary for viral RNA transcription and replication.[35] Recent studies have reported that NPs represent a potential anti-influenza drug target because of their many crucial functions in the viral cycle. Kao et al. identified a small-molecule compound called nucleozin that can trigger oligomeric NP aggregates and inhibit their nuclear entry.[23] In addition, Su et al. identified several nucleozin analogues that can inhibit the replication of the influenza virus in MDCK cells by inducing the severe aggregation of NPs and by preventing RNP formation during the production of viral particles.[29]

Fluorescence-based assays are efficient and can be easily used to identify intermolecular interactions and to screen for drugs. Parikh et al. reported that the intrinsic fluorescence property of serum can be used to analyze drug/serum protein binding and is suitable for high-throughput screening.[36] The influenza virus A NP contains six tryptophan residues that can be utilized by intrinsic fluorescence technology for drug screening. As an example, the nucleozin/NP interactions and the subsequent nucleozin-induced conformational changes in NP can be monitored with tryptophan quenching. The resistance of SOIV to nucleozin is indicated by the minimal fluorescence-quenching effect and weaker binding affinity between nucleozin and the SOIV NP harboring the Y289H mutation.[23] According to the protein sequence alignment, the 498-amino acid NP is highly conserved among all strains of influenza viruses. When the location of the ligand-binding site of NP is highly conserved among influenza viruses, the inhibitors obtained from the current method are likely to display a broad-spectrum antiviral activity and to be useful against all strains of influenza virus including the H3N2, H5N1, and influenza B.

These findings can be easily applied to high-throughput, drug-specific assays for NP binding in a multiwell format. The advantages of our fluorescence-quenching screening system are that it can identify promising compounds that affect NP function; it does not require that the compounds are permeable to cells. On the basis of the fluorescence-quenching assay of the NPs in 96-well plates, we identified several potential compounds that target influenza, including CSV0C001018, curcumin, and amodiaquine dihydrochloride, which caused significant NP quenching. The docking model suggested that the nitro functional group of CSV0C001018 could form

dx.doi.org/10.1021/ac2022426 | Anal. Chem. 2012, 84, 6391−6399

hydrogen bonds with S309 and S310 at the C-terminal end of the NP α helix 13. The model also suggested that its benzene ring, which is located 45 degrees below the aromatic ring of Y289, also formed a weak $\pi-\pi$ stacking interaction (Figure 2S of the Supporting Information). However, the binding sites of curcumin and amodiaquine dihydrochloride in NPs are still unknown and should be explored in future experiments.

CONCLUSION

In this study, we developed a convenient method that can be used for screening anti-influenza drugs. After screening for approximate 200 compounds, a new anti-influenza drug, CSV0C001018, was identified. The importance for considering drug resistance and the persistence of latent infection has been highlighted in the field of HIV therapy.[37,38] One approach to overcome the resistance problem is to develop novel NP-targeting agents that can be added to combination regimens. Spectrofluorometry may prove to be a useful method for rapidly determining the NP binding of drug libraries in these pathogenic viruses.

ASSOCIATED CONTENT

Supporting Information

Supplementary data that include the structures of curcumin and amodiaquine dihydrochloride is shown in Figures 1S, respectively. In addition, the predicted 3D model of CSV0C001018 bound to H1N1 NP is shown in Figures 2S. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

◆These authors equally contributed to this work.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by NSC Grants 97-2311-B-005-003-MY3, 99-2323-B-400-002, and ID-100-SP-10 to M.-H.H. and J.T.-A.H., respectively.

ABBREVIATIONS:

Nucleoprotein, NP; swine-origin influenza virus H1N1pdm09, SOIV; neuraminidase, NA

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