Letter

Discovery of Novel, Orally Bioavailable #-Aminoacid Azaindole Inhibitors of Influenza PB2

Luc J. Farmer, Michael P. Clark, Michael J. Boyd, Emanuele Perola, Steven M Jones, Alice Tsai, Marc D. Jacobs, Upul K. Bandarage, Mark Willem Ledeboer, Tiansheng Wang, Hongbo Deng, Brian Ledford, Wenxin Gu, John P Duffy, Randy S Bethiel, Dean Shannon, Randal A Byrn, Joshua R. Leeman, Rene Rijnbrand, Hamilton B Bennett, Colleen O'Brien, Christine Memmott, Kwame Nti-Addae, Youssef L Bennani, and Paul S. Charifson

ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.6b00486 • Publication Date (Web): 20 Jan 2017

Discovery of Novel, Orally Bioavailable β-Aminoacid Azaindole Inhibitors of Influenza PB2

Luc J. Farmer,[a*] Michael P. Clark[*], Michael J. Boyd, Emanuele Perola, Steven M. Jones,[b] Alice Tsai, Marc D. Jacobs, Upul K. Bandarage, Mark W. Ledeboer,[c] Tiansheng Wang, Hongbo Deng, Brian Ledford, Wenxin Gu, John P. Duffy, Randy S. Bethiel, Dean Shannon,[d] Randal A. Byrn, Joshua R. Leeman, Rene Rijnbrand,[e] Hamilton B. Bennett,[f] Colleen O'Brien, Christine Memmott, Kwame Nti-Addae, Youssef L. Bennani,[g] Paul S. Charifson.

Vertex Pharmaceuticals Inc., 50 Northern Ave, Boston, Massachusetts 02210, United States

Present Addresses

[a]Paraza Pharma. Inc., 7171 Frederick-Banting, Montreal, Quebec H4S 1Z9, Canada

[b]Contrafect Corporation, 28 Wells Ave, 3rd floor, Yonkers, New York 10701, United States

[c]Goldfinch Bio., 215 First St, 4th floor, Cambridge, MA 02142, United States

[d]DE Synthetics Inc., 30 Dineen Drive, Fredericton, New Brunswick E3B 5A3, Canada

[e]Arbutus Biopharma Inc., 3805 Old Easton Road, Doylestown, Pennsylvania 18902, United States

[f]Moderna Therapeutics, Inc. 200 Technology Square, Cambridge, Massachusetts, 02139, United States

[g]Vertex Pharmaceuticals (Canada) Inc., 275 Armand-Frappier, Laval, Quebec H7V 4A7, Canada

ABSTRACT: In our efforts to develop novel small-molecule inhibitors for the treatment of influenza, we utilized molecular modeling and the X-ray crystal structure of the PB2 subunit of the influenza polymerase to optimize a series of acyclic β-aminoacid inhibitors, highlighted by compound **4**. Compound **4** showed good oral exposure in both rat and mouse. More importantly, it showed strong potency *versus* multiple influenza-A strains, including pandemic 2009 H1N1 and avian H5N1 strains and showed a strong efficacy profile in a mouse influenza model even when treatment was initiated 48h after infection. Compound **4** offers good oral bioavailability with great potential for the treatment of both pandemic and seasonal influenza.

KEYWORDS: PB2 inhibitor, azaindole, influenza

Seasonal and pandemic influenza outbreaks remain a significant challenge worldwide. Influenza is a common and potentially deadly infectious disease that has afflicted large human populations in terms of morbidity and mortality at a range of 3000 to 49000 deaths per year, in the US over the last 31 years. [1] Recent statistics also suggest that each year in the United States, 5-20% of the population becomes infected with influenza resulting in more than 200,000 hospitalizations.[2] Swine and avian hosts can also serve as a reservoir of influenza A that can occasionally transmit influenza variants from others species to humans, which can lead to a global epidemic such as the 2009 H1N1 swine flu pandemic. [3,4] Finally, the emergence of H5N1 ('bird flu'), an extremely virulent strain to humans, could potentially become a global threat if it were to acquire the ability for human-to-human transmission. Since there are no efficient ways to accurately predict the exact viral strains prior to each influenza season or pandemic, targeting pathways to stop viral replication is an area of high interest for drug discovery. The mostly known and currently used standard of care (SOC) to treat influenza are the neuraminidase inhibitors (NA), oseltamivir **1** (Figure 1), zanamivir and peramivir. While this small-molecule drug can effectively treat a variety of type A and B influenza viruses, it suffers limitations from resistance and has to be administered within 24-48h post infection to be efficacious.[5] Moreover, recent reports have shown that some H5N1 influenza strains are resistant to oseltamivir.[6]

To circumvent some of these limitations and address unmet medical needs in this arena, we recently reported on the discovery of a novel class of compounds from a phenotypic cell protection (CPE) assay screen that have shown survival benefits in the mouse model when administered 48 h post infection.[7] Compound **2** was our initial hit from the CPE assay. Our early lead optimization efforts culminated in the discovery of a novel 7-azaindole based first-in-class inhibitor of influenza polymerase-B2 (PB2), **JNJ-63623872** (**3**) (formerly known as VX-787) (Figure 1).[7]

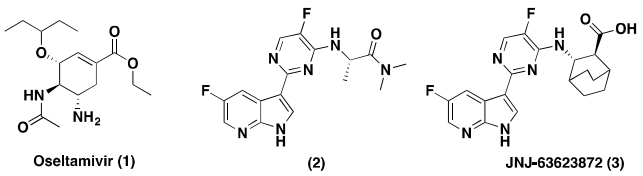


Figure 1. Inhibitors of influenza virus: NA inhibitor oseltamivir (**1**) azaindole (2) and JNJ-63623872 (**3**).

PB2 is part of a heterotrimeric viral complex that is essential for viral RNA replication. This viral polymerase complex assembly, comprised of PA, PB1 and PB2 subunits, is responsible for replication and transcription of the eight separate segments of the viral RNA genome in the nuclei of infected cells. [8,9,10] The polymerase complex synthesizes viral mRNAs using short, capped primers derived from cellular transcripts by a unique 'cap-snatching' mechanism, where the virus utilizes host pre-mRNA as a primer for transcription.[11] The PB2 subunit binds the 5' cap domain for 7-methyl GTP (m^7GTP) of host pre-mRNAs and positions it for cleavage by the PA subunit, leaving a 10-13 nucleotide primer. Then, elongation of the chimeric viral mRNA in PB1 by poly-adenylation of viral mRNA polymerase completes the replication cycle. Since compound **3** targets the viral transcription complex, it offers an alternative mechanism of action relative to oseltamivir, and for combination therapy with other antivirals.

Identity of PB2 as a target was further confirmed with the X-ray crystal structure of screening hit **2** bound to the PB2 cap-binding domain [7] (Figure 2).

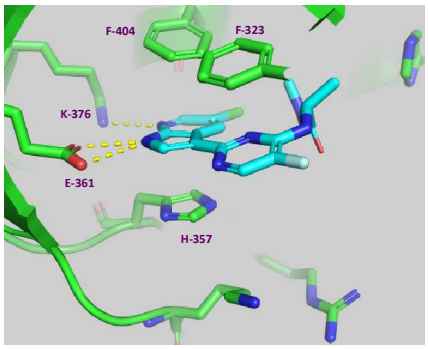


Figure 2. X-ray structure of cap-binding domain of PB2 containing PB2 inhibitor **2**. PDB accession code is 4NCM; reprint[7].

As shown in Figure 2, compound **2** forms hydrogen bonds to both Glu361 and Lys376 side chains, with the 7-azaindole ring sandwiched between His357 and Phe404. The pyrimidine ring pi-stacks with Phe323.

In our effort to generate novel inhibitors of PB2 we focused our attention on the alanine dimethylamide side chain. Our goal was to design simple acylic beta-amino acid analogs. We first envisioned that a (*R*)-*t*-butyl group could fill the hydrophobic space in the PB2 binding pocket, while retaining the key interactions of compound **2**. To support this hypothesis, we docked our first proposed analog, compound **4** (**Table 1**), into the active site of PB2 and then superimposed the docking model with the X-ray structure of PB2 in complex with **2** (Figure 3).

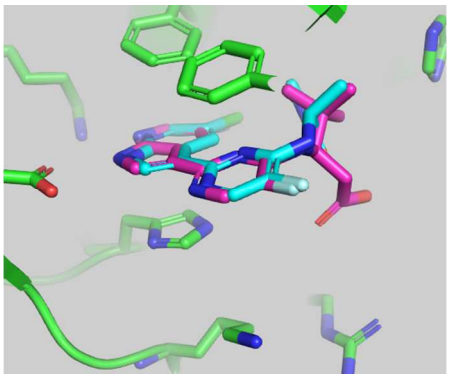
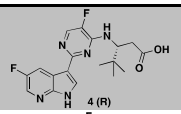
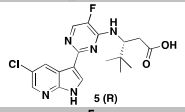
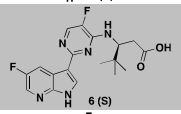
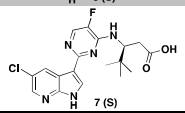


Figure 3. Superposition of the crystallographic pose of **2** and the docking model of compound **4** in the active site of PB2. Compound **4** was docked into the crystallographic conformation of PB2 from the complex with 2 using the Glide program (Schrodinger, Inc.).

The overlay of the two compounds in the active site of PB2 showed good superposition of the key pharmacophoric elements, within the azaindole moiety and carboxylic acid group. Additionally, the *tert*-butyl group of compound **4** occupied the same region occupied by the alpha-methyl dimethylamide functionality of compound **2**. To test the validity of the model, two enantiomeric pairs (**4** and **6**, **5** and **7**) were synthesized and evaluated for their binding affinity for PB2 (K_d) and their ability to inhibit viral RNA replication (EC_{90}) in a branched DNA (bDNA) cell assay.[11] Interestingly, and in agreement with the model (Figure 3), the expected (R)-enantiomers of both 5-fluoro (**4**) and 5-chloro (**5**) azaindole exhibited excellent binding activity and potency whereas the (S)-enantiomers (**6** and **7**) showed reduced activity (Table 1).

Table 1. In Vitro Potency of (R)-3-Amino-4,4-dimethylpentanoic acids and Their Enantiomers

Compounds	bDNA[a] EC_{90} (uM)	PB2[b] Kd (uM)
 4 (R)	0.03	0.003
 5 (R)	0.023	<0.003
 6 (S)	>6.65	0.15
 7 (S)	>3.3	0.2

[a] The concentration of test compounds resulting in viral RNA levels equal to that of 10% of the control wells was reported as EC_{90}. [b] Affinity for cap-binding domain of the PB2 subunit as measured in a fluorescence polarization competition binding assay.

The docked binding mode was subsequently confirmed by the X-ray structure of compound **4** bound to PB2 (Figure 4). Consistent with the model depicted in Figure 3, the compound **4** forms hydrogen bonds to both the Glu361 and Lys376 side chains, the azaindole ring is sandwiched between His357 and Phe404, while the pyrimidine ring forms a pi-stacking interaction with Phe323.

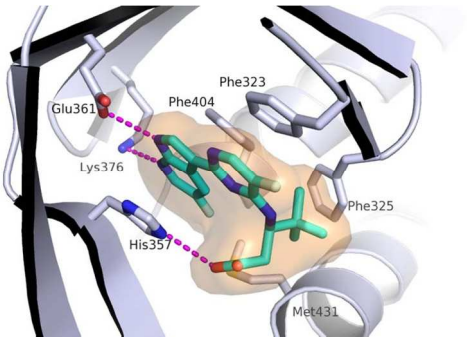
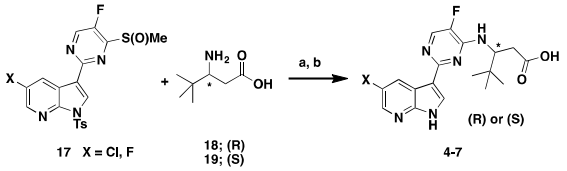


Figure 4. X-ray structure of cap-binding domain of PB2 containing PB2 inhibitor **4**. PDB accession code is 5JUR.

Synthesis of the two enantiomeric pairs compounds **4-7** from (Table 1) were accomplished in a two-step protocol from the coupling of commercially available (R)- or (S)-3-amino-4,4-dimethyl pentanoic acid **18** or **19** *via* a facile displacement with the previously prepared sulfoxide **17**[7] according to Scheme 1.

Scheme 1. Synthesis of Enantiomeric Pairs Compounds 4-7 [a]

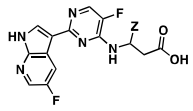


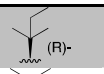
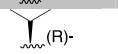
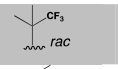
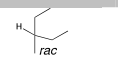
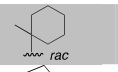
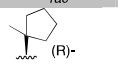
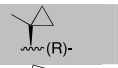
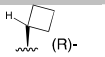
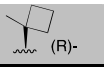
17 X = Cl, F 18; (R) 19; (S) 4-7

[a]Reagents and conditions: (a) Na_2CO_3, THF, MeCN, MW, 135°C, 30 min.; (b) i) MeONa, MeOH, 30 min., ii) NH_4Cl 45-55% (2-steps).

This structure suggests that the hydrophobic pocket defined by the three phenylalanine residues (F323, F325 and F404) could potentially accommodate larger side chains. In order to test such a hypothesis, additional compounds with side chain variations to replace the *t*-butyl group were prepared to further explore this hydrophobic pocket. We focused our optimization on the preparation of analogues bearing a fluoro substitution at the 5-position of the azaindole ring system (Table 2)[7]. A slight increase in size with the addition of a methyl group as exemplified with the (R)-3-amino-4-4, dimethylhexanoic acid side chain based compound, **8**, led to comparable potency with compound **4**. The removal of a methyl group as shown with the (R)-3-amino-4-methylpentanoic acid based compound **9** led to a 20-fold loss in cellular activity (EC_{90} = 0.59 μM) with respect to **4**. Interestingly, the replacement of one methyl group on the side chain of **4** for the larger and more lipophilic trifluoromethyl in compound **10** was also less active. The *iso*-pentyl extended side chain version of analog **9**, as in compound **11**, was slightly better tolerated (EC_{90} = 0.34 μM). Cyclizing both ethyl side chains of **11** as a cyclohexane ring **12** restored activity within the 100 nM range. With this result, more cycloalkyls were prepared to explore optimization. Cycloalkyl based side chains with the (R)-absolute configuration (compounds **13-16**) all showed <1 μM potency (EC_{90} = 0.007 to 0.780 μM) in the bDNA assay. Spirocyclobutane analog **16** was shown to have the highest anti-influenza activity (EC_{90} = 0.007 μM). It is worth noting that a single point substitution from a methyl to hydrogen, from compounds **16** to **15** led to over a 100-fold decrease in activity. The above results indicate that a balance of both size and shape of the side chains bearing a tertiary carbon appear to be important to fill this hydrophobic pocket. All the corresponding enantiomers showed less affinity for PB2 or weaker potency (Table 4; Supporting Information), which correlated well with results initially observed with the 3-amino-4,4-dimethylpentanoic acids, based enantiomeric pairs (compound **4**:**6** and **5**:**7**). Scheme 2 depicts the synthesis of 1-methyl-1-cyclobutane **16**. Coupling of *rac* aminoester **21** (commercially available or prepared in 7 steps; see Supporting Information) with 2,4-dichloro-5-fluoropyrimidine **20** provided the desired intermediate **22**.

Table 2. Optimization of In Vitro Potency of Various Substituted Butanoic Acid Side Chains



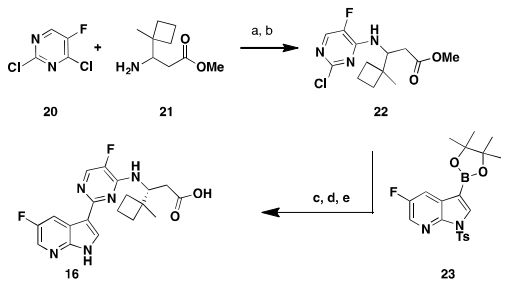
Compounds	Z	bDNA[a] EC_{90} (uM)	$PB2^{b}$ Kd (uM)
8		0.06	<0.003
9		0.59	ND
10		0.52	ND
11		0.34	ND
12		0.12	<0.003
13		0.02	<0.003
14		0.67	ND
15		0.78	ND
16		0.01	<0.003

[a]The concentration of test compounds resulting in viral RNA levels equal to that of 10% of the control wells was reported as EC_{90}. [b]Affinity for cap-binding domain of the PB2 subunit as measured in a fluorescence polarization competition binding assay. ND = not determined.

Suzuki cross coupling reaction with 5-fluoroazaindole boronate ester fragment **23**[12] gave the fully assembled scaffold, which upon removal of the tosylate protecting group and hydrolysis of the ester followed by chromatographic chiral resolution delivered the desired product **16**. The above general route was also used to prepare all other spirocyclic analogs (see Supporting Information).

The overall cellular potency and target-affinity of analogs **4** and **16** prompted us to further investigate their pharmacokinetic profiles and *in vivo* potency. The pharmacokinetic profile of compound **4** showed desirable iv and oral exposure in both rat (1 mg/kg iv: Cl = 8 mL min^{-1} kg^{-1} and $t_{1/2}$ = 9.9h; po (3 mg/kg) AUC = 3.3 □g-h/mL) and mouse (30 mg/kg dose, po AUC = 7.2 □g-h/mL and Cmax = 11.8 □g/mL). Compound **16** also showed good oral exposure in mouse (30 mg/kg dose, po AUC = 6.7 □g-h/mL and Cmax = 7.4 □g/mL). Additionally, evaluation in S9 fractions shows similar stability in rat (100% remaining at 1 μM) vs human (94% remaining).

Scheme 2. Synthesis of varied β-amino acid analogs



[a]Reagents and conditions: (a) Et_3N, THF, *t*-BuOH, rt, 5 min.; (b) 55°C, **62%** (2-steps); (c) **23**, K_3PO_4, X-Phos, 2-Me-THF, H_2O, Pd2(dba)3, 75°C, 3h, **82%**; (d) THF, MeOH, NaOH (2M), 50°C, **94%**; (e) SFC chiral chromatography.

Compound **4** demonstrates potent, antiviral activity *in vitro* (cell protection assay; CPE) against a broad range of influenza type A strains,[14] including oseltamivir carboxylate resistant isolates and current pandemic H1N1 and H5N1 strains (Table 3; see also Table 4 in supporting information for comparative zanamivir and oseltamivir carboxylate activities in neuraminidase enzyme assay with these influenza strains/types). Compound 4 shows limited activity against influenza B (data not shown).

Compound 4 was advanced into a mouse influenza model (Figure 5). It was dosed orally at 10, 30 and 60 mg/kg twice a day for 10 days starting 48 hours after infection and it showed complete survival benefit at all three doses. Additionally, compound 4 provided a dose-dependent decrease in body weight loss as compared to untreated controls.[14] It is worth noting that oseltamivir (dosed 10 mg/kg BID) is known to be devoid of efficacy in this +48h delay to treatment model.[7, 8, 15]

Table 3. Compound 4 Activity Against Oseltamivir Sensitive and Resistant Influenza A Viruses.

Virus Name	Type	Oseltamivir R/S	4 CP EC_{50}(μM)[a]
A/Georgia/17/2006	A(H1N1)[b]	S	0.009
A/Georgia/20/2006	A(H1N1)[b]	R	0.002
A/Puerto Rico/8/34	A(H1N1)[b]	S	0.014
A/Henan/Jinshui/147/2007	A(H3N2)[b]	S	0.019
A/Texas/12/2007	A(H3N2)[b]	R	0.030
A/California/07/2009	A(H1N1)[c]	S	0.004
A/Texas/48/2009	A(H1N1)[c]	R	0.002
A/Vietnam/1203/2004	A(H5N1)[d]	S	0.004

[a]Mean MDCK cell 3-day CP (cell protection) assay (MDCK cells incubated with test compounds and influenza A virus for 72h and the concentration of test compound resulting in 50% cell protection was reported as EC50) N=3; [b] Seasonal subtype; [c] Pandemic subtype;
[d]Highly pathogenic avian influenza (H5N1) strain Oseltamivir R, resistant (determined via externally validated phenotypic or genetic analysis); S, sensitive.

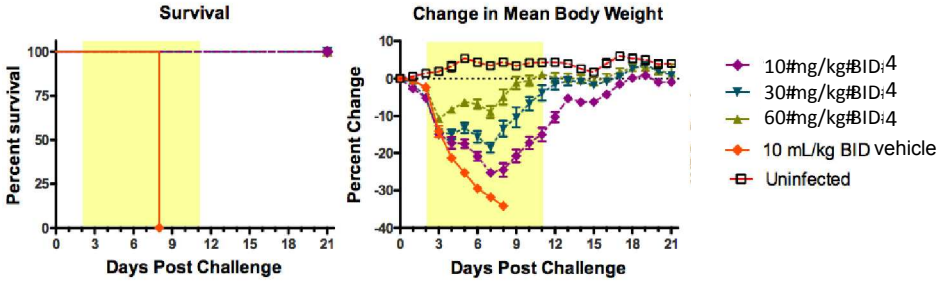


Figure 5. *In-vivo* activity of **4** and oseltamivir in mouse influenza A model when administered 48h post infection. Survival and body weight curves of male BALB/C mice (8 mice/group) inoculated with mouse-adapted influenza viruses A/PR/8/34 (5e3 $TCID_{50}$/mouse) by intranasal instillation.

Similarly compound **16** showed complete survival benefit in the mouse influenza model when administered at 3, 10 and 30 mpk BID 48h post infection (see Figure 6; Supporting Information).

In Summary, structure-guided optimization led to a set of novel tertiary beta-amino acid analogs. The most potent compounds **4** and **16** were found to be orally bioavailable and efficacious inhibitors *in vivo*. Further testing showed that compound **4** is very potent against all influenza A strains tested, including pandemic H1N1 and avian H5N1 flu strains. These data clearly indicate that both **4** and **16** are promising inhibitors of PB2 mediated viral influenza replication with respect to overall potency, efficacy, and extended treatment window. Both compounds possess therapeutic potential.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: xxxxxxx

Protocols for PB2 binding assay; bDNA cellular assay; experimentals for key compounds and Figure 6.

AUTHOR INFORMATION

Corresponding Authors

*(M.P.C.) Tel: 1-617-961-7727. Fax: 1-617-366-3908. E-mail: michael_clark@vrtx.com.

ORCID

Michael P. Clark: 0000-0003-1628-1671
Luc J Farmer: 0000-0001-8174-173X

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGEMENTS

The authors thank Barry Davis for analytical chemistry support and Greg May for SFC chiral separation.

ABBREVIATIONS

Dimethylformamide (DMF), tetrahydrofuran (THF), trifluoroacetic acid (TFA), triethylamine (TEA), lithium diisopropylamide (LDA), 2-dicyclohexylphosphino-2'4'6'-triisopropylbiphenyl (X-Phos), dimethoxyethane (DME).

REFERENCES

(1) Centers for Disease Control and Prevention. Estimates of deaths associated with seasonal influenza --- United States, 1976--2007. August 27, **2010** / 59(33); 1057-1062.

(2) Thompson, W. W.; Shay, D. K.; Weintraub, E.; Brammer, L.; Bridges, C. B.; Cox, N. J.; Fukuda, K. Influenza-associated hospitalizations in the United States. *JAMA* **2004**, *292*, 1333-1340.

(3) Cohen, J. Surprising Twist in Debate Over Lab-Made H5N1. *Science* **2012**, *335*, 1155-1156.

(4) Herfst, S.; Schrauwen, J. A.; Linster, M.; Chutinimitkul, S.; deWit, E.; Munster, V. J.; Sorrell, E. M.; Bestebroer, T. M.; Burke, D. F.; Smith, D. J.; Rimmelzwaan G. F.; Osterhaus, A. D. M. E.; Fouchier, R. A. M. Airborne transmission of influenza A/H5N1 virus between ferrets. *Science* **2012**, *336*, 1524-1541.

(5) Moscona, A. Neuraminidase inhibitors for influenza. *N. Engl. J. Med*. 2005, *353*, 1363-1373.

(6) Nitsch-Osuch, A., Byrdak, L.B. Influenza viruses resistant to neuraminidase inhibitors. *Acta Biochim Pol*, **2014** (61) 505-508.

(7) Clark, M. P.; Ledeboer, M. W.; Davies, I.; Byrn, R. A.; Jones, S. M.; Perola, E.; Tsai, A.; Jacobs, M.; Nti-Addae, K.; Bandarage,

U. K.; Boyd, M. J.; Bethiel, R. S.; Court, J. J.; Deng, H.; Duffy, J. P.; Dorsch, W. A.; Farmer, L. J.; Gao, H.; Gu, W.; Jackson, K.; Jacobs, D. H.; Kennedy, J. M.; Ledford, B.; Liang, J.; Maltais, F.; Murcko, M.; Wang, T.; Wannamaker, M. W.; Leeman, J. R.; McNeil, C.; Taylor, W. P.; Memmott, C.; Rijnbrand, R.; Bral, C.; Germann, U.; Nezami, A.; Zhang, Y.; Salituro, F. G.; Bennani, Y. L.; Charifson; P. S. Discovery of a novel, first-in-class, orally bioavailable Azaindole Inhibitor (VX-787) of Influenza PB2. *J. Med. Chem.* **2014**, 57(15), 6668-6678.

(8) Guilligay, D.; Tarendeau, F.; Resa-Infante, P.; Coloma, R.; Crepin, T.; Sehr, P.; Lewis, J.; Ruigrok, R. W.; Ortin, J.; Hart, D. J.; Cusack, S. The structural basis for cap binding by influenza virus polymerase subunit PB2. Nat. Struct. Mol. Biol. **2008**, 15, 500–506.

(9) Pautus, S.; Sehr, P.; Lewis, J.; Fortune, A.; Wolkerstorfer, A.; Szolar, O.; Guilligay, D.; Lundardi, T.; Decout, J.-L.; Cusack, S. New 7- methylguanine derivatives targeting the influenza polymerase PB2 cap- binding domain. *J. Med. Chem*. **2013**, 56, 8915–8930.

(10) Fechter, P.; Mingay, L.; Sharps, J.; Chambers, A.; Fodor, E.; Brownlee, G. G. Two aromatic residues in the PB2 subunit of influenza A RNA polymerase are crucial for cap binding. *J. Biol. Chem*. **2003**, 278, 20381–20388.

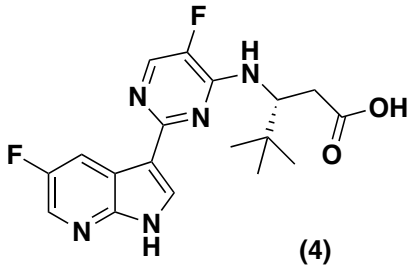
(11) Palese, P.; Shaw, M. L. Orthomyxoviridae: The viruses and their Replication. In Fields Virology; Knipe, D. M., Howley, P. M., Eds.; Lippincott Williams & Wilkins: Philadelphia, PA, **2007**; pp. 1647–1689.

(12) Wagaman, P. C.; Leong, M. A.; Simmen, K. A. Development of a novel influenza A antiviral assay. *J. Virol. Methods* **2002**, 105, 105-114.

(13) Charifson, P. S.; Clark, M. P.; Bandarage, U. K.; Bethiel, R. S.; Boyd, M. J.; Davies, I.; Deng, H.; Duffy, J. P.; Farmer, L. J.; Gao, H.; Gu, W.; Kennedy, J. M.; Ledford, B.; Ledeboer, M. W.; Maltais, F.; Perola, E.; Wang, T.; Inhibitors of influenza viruses replication. WO 2013/019828, February 2, **2013**.

(14) Byrn, R. A.; Jones, S.; Bennett, H. B.; Bral, C.; Clark, M. P.; Jacobs, M. D.; Kwong, A. D.; Ledeboer, M. W.; Leeman, J. R.; McNeil, C. F.; Murcko, M. A.; Nezami, A.; Perola, E.; Rijnbrand, R.; Saxena, K.; Tsai, A. W.; Zhou, Y.; Charifson, P. S.; Preclinical activity of VX-787, a first-in-class, orally bioavailable inhibitor of the influenza viral polymerase PB2 subunit. *Antimicrob Agents Ch.* **2015** 59(3), 1569-1582

(15) Tsai, A. W.; McNeil, C. F.; Leeman, J. R.; Bennett, H. B.; Nti-Addae, K.; Huang, C.; Germann, U. A.; Byrn, R. A.; Berlioz-Seux, F.; Rijnbrand, R.; Clark, M. P; Charifson, P. S; Jones, S. M. Novel ranking system for identifying efficacious anti-influenza virus PB2 inhibitors. *Antimicrob Agents Ch.* **2015**, 59(10), 6007-6016.



(4)

bDNA EC_{90} = 0.030 μM
PB2 Kd = 0.003 μM