HHS Public Access

Author manuscript

Bioorg Med Chem Lett. Author manuscript; available in PMC 2020 December 11.

Published in final edited form as:

Bioorg Med Chem Lett. 2019 October 15; 29(20): 126639. doi:10.1016/j.bmcl.2019.126639.

Novel influenza polymerase PB2 inhibitors for the treatment of influenza A infection

Hongwang Zhang, **Longhu Zhou**, **Sarah Amichai**, **Keivan Zandi**, **Bryan Cox**, **Raymond Schinazi**, **Franck Amblard**

Center for AIDS Research, Global Virus Network, Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, GA 30322, USA

Abstract

Exploration of the chemical space of known influenza polymerase PB2 inhibitor Pimodivir, was performed by our group. We synthesized and identified compounds **16a** and **16b**, two novel thienopyrimidine derivatives displaying anti-influenza A activity in the single digit nanomolar range in cell culture. Binding of these unique compounds in the influenza polymerase PB2 pocket was also determined using molecular modeling.

Keywords

Flu; Influenza A; Antiviral; Virus

Introduction:

Seasonal influenza outbreaks are estimated to impact more than 3.5 million people every year and be responsible for over 290,000 to 650,000 death worldwide. Although influenza vaccines remain the most effective means to prevent seasonal influenza, they typically provide suboptimal protection. Moreover, they may be completely ineffective in the event of an antigenic mismatch between viruses in the seasonal vaccine and those circulating in the community, and their utility in response to rapid and pandemic spreads remain uncertain.[1] Only three classes of antiviral agents are currently approved in the US for the treatment of influenza: Adamantanes (amantidine, rimantadine), neuramidinase (NA) inhibitors (Oseltamivir, Peramivir, Zanamivir) and baloxavir marboxil (BXM), which is the prodrug of baloxavir acid (BXA), an endonuclease (PA) inhibitor. However, adamantanes, which target the viral M2 protein involved in the uncoating of the virus during replication, are not part of the standard of care for seasonal influenza A viruses due to widespread viral resistance.[2] The use of NA inhibitors, which impair the release of virus from infected cells, is also limited since their antiviral potency is relatively modest. Importantly, oseltamivir, the most prescribed NA drug, was completely ineffective during the 2008–2009 H1N1 flu outbreak.[3, 4] BXM is the only endonuclease inhibitor approved for the treatment of both influenza A

and B. Even though a single dose of BXM proved to positively improve outcomes, treatment-emergent monitoring in the phase 2 study identified PA I38T/F substitutions in A/H3N2 viruses conferring more than 10-fold reduction in BXM susceptibility.[5] Furthermore, this loss of susceptibility occurred in 10% of the persons treated with BXM in phase III trials.[6] Drugs targeting viral proteins are currently in the influenza drug pipeline, including Pimodivir, an influenza polymerase PB2 inhibitor which is now in phase III clinic trials.[7,8] The PB2 subunit specifically plays a role in generating 5'-capped RNA fragments from cellular pre-mRNA molecules that are used as primers for viral transcription. Known inhibitors bind to active site of the PB2 cap-binding domain preventing the binding of the natural ligand, 7-methyl GTP, thereby preventing viral RNA synthesis.[9] It is worth noting that because of major sequence difference between influenza A and B PB2 domains, PB2 inhibitor remains only active against influenza A.[10] As part of our influenza research program, we divided Pimodivir into 3 key components (Parts A, B and C, Figure 1) and evaluated the influence of novel structural modifications for each part. Herein, we wish to report the synthesis of a small library of influenza polymerase PB2 inhibitor along with their antiviral evaluation.

Part A:

Key amine **9d** was purchased while amines **9a** and **9c** were prepared by following reported procedures[11]. **9b** was synthesized according to the chemistry described in scheme 1[12].

Dichloropyrimidine **10** was treated with amines **9a-d** and DIPEA to give intermediates **11a-d** which were then reacted with fluoro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-tosyl-1H-pyrrolo[2,3-b]pyridine **12**[13] in presence of $Pd_2(dba)_3$, X-Phos and K_3PO_4. Final saponification and deprotection with LiOH afforded the desired derivatives **13a-d** (Scheme 2).

Part B:

Compounds **16a-f** were synthesized from dichloro heteromatic moieties **14a-f**[14] following the same coupling, saponification, deprotection sequence (Scheme 3)

Compound **21** was prepared by following the chemistry described in Scheme 4. Dichloro pyrimidine **17** was treated with KSCN under acidic condition to form **18**. Compound **18** was further brominated and then coupled with amine **9a** in presence of DIPEA. Finally, Suzuki type coupling with 5-fluoro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-tosyl-1H-pyrrolo[2,3-b]pyridine **12** in presence of X-Phos and K_3PO_4, followed by treatment with LiOH gave compound **21**.

Triazolopyrimidine derivative **27** was synthesized by first reacting dichlorofluoro pyrimidine **22** with hydrazine followed by coupling of intermediate **23** with carboxylic acid derivative **24**[13] in presence of EDCI and DMAP. Subsequent reaction with $POCl_3$ gave chlorinated intermediate **26** which was reacted with 5-fluoro-3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1-tosyl-1H-pyrrolo[2,3-b]pyridine **12** in presence of X-Phos and K_3PO_4. Final treatment with LiOH afforded the desired compound **27** (Scheme 5).

Compound **32** was prepared by first reacting trichloropyrimidine **28** with formic hydrazide and cyclization of intermediate **29** in presence of $POCl_3$. Subsequent coupling of **30** with **9a** in presence of DIPEA, followed by palladium mediated coupling with 5-fluoro-3-(4,4,5,5tetramethyl-1,3,2-dioxaborolan-2-yl)-1-tosyl-1H-pyrrolo[2,3-b]pyridine **12** and treatment with LiOH gave targeted compound **32** (Scheme 6).

Part C:

Compound **33**[15] was first protected with a THP group then reacted with bis(pinacolato) diboron in presence of potassium acetic and $Pd(dppf)_2Cl_2$ to form boronic ester derivative **35**. Compound **35** was then coupled with chlorinated scaffolds **11a** or **15a-b** to give, after deprotection under acid condition and saponification of the ester group with LiOH, the desired compounds **36a-b** and **37** (Scheme 7).

The synthesized compounds were evaluated against influenza A virus PR8-GLuc (H1N1) strain. Briefly, A549 cells were seeded at a density of 40,000 cells/well and incubated at 37 °C and 5% CO_2 overnight followed by inoculating with 0.1 MOI of the virus for 1 h and then were washed with 2 acidic washes (PBS, pH 2.2) and 3 neutral washes (PBS, pH 7.4) to remove unbound virus. Infected cells then were treated with test compound in a single concentration lower than maximum non-toxic concentration. (MNTC is defined as the maximum concentration at which at least 90% of the treated cells are viable and is determined by treating cells with increasing concentrations of tested compound). Those which showed 50% inhibitory activity against virus replication were then retested in a dose response manner to determine EC_{50} and EC_{90} values. In addition, cytotoxicity was assessed against a panel of cell lines including A459 cells, primary human peripheral blood mononuclear (PBM) cells, human lymphoblastoid cells (CEM), and African Green monkey Vero cells to determine therapeutic indexes. The results are summarized in Table 1.

Increasing the size of Pimodivir's bicyclic part A (Figure 1) by adding another ring led to a 12–230 times decrease of potency (compounds **13b** and **13d**). A similar pattern was observed when the bicyclooctane ring of Pimodivir was changed to a smaller bicycloheptane ring (compound **13c**, EC_{50} = 0.05 μM). On the other hand, introduction of an unsaturated bicylooctane ring led to the discovery of compound **13a** which displayed an activity profile similar to Pimodivir (EC_{90} = 0.027 μM for both compounds). However, unlike Pimodivir, which displays a CC_{50} of 20 μM in A549 cells, **13a** did not display toxicity in A549 cells to 100 μM. Further optimization of compound **13a** was performed by evaluating the replacement of the central pyrimidine moiety (Figure 1 - Part B) by various bicylic heterocyclic rings. Introduction of a triazolopyrimidine ring in compounds **27** and **32**, an imidazolopyrimidine in compound **16e** and **16f** or a thiazolopyrimidine ring in compound **21** was counterproductive and led to a significant loss of activity. However introduction of a thienopyrimidine (compounds **16a**[16] and **16b**[17]) or a methylated thienopyrimidine (compounds **16c** and **16d**) was well tolerated and compounds **16a** and **16b** displayed activities similar to that of Pimodivir (EC_{50} = 0.006 and 0.017 μM respectively). It is worth noting that despite their high potencies, compounds **16a** and **16b** exhibited toxicity in the micromolar range in our panel of cell lines. Because oxidation of the 2-position of the 7-azaindole ring *via* aldehyde oxidase has been previously established in that series of

compounds,[18] introduction of a nitrogen at this position, to eventually block the formation of metabolites, was also evaluated (Figure 1 - Part C). Unfortunately, this modification led to significant loss of potency as seen in compounds **36a-b** and **37**.

Based on the high potency and unusual structures of the thienopyrimidine derivatives **16a** and **16b**, we used molecular modeling to establish how these compounds bind to the influenza A PB2 pocket. The crystal structure of the PB2 subunit complexed with Pimodivir served as a locus for induced fit docking (PDBID 6EUV).[19] The results of induced fit docking yielded poses that maintain key interactions observed for Pimodivir (Figure 2). The azaindole ring of **16a/16b** acted as a hydrogen bond donor to Glu361 and a hydrogen bond acceptor from Lys376 while the bicyclic carboxylate formed an electrostatic interaction with Arg355. Interestingly, to accommodate the thienopyrimidine ring in both compounds, Leu512 rotated away from the agent thereby enlarging the pocket. The model also suggested that in the case of **16b**, Arg508 and Arg332 position closer to the inhibitor than observed in the crystal structure.

A small library of influenza polymerase PB2 inhibitors were designed by evaluating novel structural modifications of the 3 key portions of reference compound Pimodivir. Antiviral activity along with cytotoxicity of each compounds was assessed. Among them, thienopyrimidine derivatives **16a** and **16b** showed antiviral activities in the low nanomolar range, similar to that of Pimodovir. Further modifications of compounds **16a** and **16b** are currently being investigated in order to further improve potency and decrease observed toxicities.

Acknowledgments

This work was supported in part by NIH Grant 1-R01-AI-132833, and 5P30-AI-50409 (CFAR).

References

1. Harding AT; Heaton NS Efforts to Improve the Seasonal Influenza Vaccine. Vaccines (Basel). 2018, 6(2),19.
2. Bright RA; Medina MJ; Xu X; Perez-Oronoz G; Wallis TR; Davis XM; Povinelli L; Cox NJ; Klimov AI Incidence of adamantane resistance among influenza A (H3N2) viruses isolated worldwide from 1994 to 2005: a cause for concern. Lancet. 2005, 366 (9492), 1175–81. [PubMed: 16198766]
3. Hurt AC; Ernest J; Deng YM; Iannello P; Besselaar TG; Birch C; Buchy P; Chittaganpitch M; Chiu SC; Dwyer D; Guigon A; Harrower B; Kei IP; Kok T; Lin C; McPhie K; Mohd A; Olveda R; Panayotou T; Rawlinson W; Scott L; Smith D; D'Souza H; Komadina N; Shaw R; Kelso A; Barr IG Emergence and spread of oseltamivir-resistant A(H1N1) influenza viruses in Oceania, South East Asia and South Africa. Antiviral Res. 2009, 83(1), 90–3. [PubMed: 19501261]
4. Hurt AC; Hardie K; Wilson NJ; Deng YM; Osbourn M; Gehrig N; Kelso A. Community transmission of oseltamivir-resistant A(H1N1)pdm09 influenza. N. Engl. J. Med 2011, 365(26), 2541–2. [PubMed: 22204735]
5. Omoto S; Speranzini V; Hashimoto T; Noshi T; Yamaguchi H; Kawai M; Kawaguchi K; Uehara T; Shishido T; Naito A; and Cusack S. Characterization of influenza virus variants induced by treatment with the endonuclease inhibitor baloxavir marboxil. Sci. Rep 2018, 8(1), 9633–48. [PubMed: 29941893]
6. Hayden FG; Sugaya N; Hirotsu N; Lee N; de Jong MD; Hurt AC; Ishida T; Sekino H; Yamada K; Portsmouth S,; Kawaguchi K; Shishido T; Arai M; Tsuchiya K; Uehara T; Watanabe A; Baloxavir

Marboxil Investigators Group. Baloxavir Marboxil for Uncomplicated Influenza in Adults and Adolescents. N. Engl. J. Med 2018, 379(10), 913–23. [PubMed: 30184455]

7. a)Shaw ML The Next Wave of Influenza Drugs. ACS Infect. Dis 2017, 3 (10), 691–4. [PubMed: 28892353] b)Trevejo JM; Asmal M; Vingerhoets J; Polo R; Robertson S; Jiang Y; Kieffer TL; Leopold L. Pimodivir treatment in adult volunteers experimentally inoculated with live influenza virus: a Phase IIa, randomized, double-blind, placebo-controlled study. Antivir. Ther 2018, 23(4), 335–44. [PubMed: 29244026] c)Finberg RW; Lanno R; Anderson D; Fleischhackl R; van Duijnhoven W; Kauffman RS; Kosoglou T; Vingerhoets J; Leopold L. Phase 2b study of pimodivir (JNJ-63623872) as monotherapy or in combination with oseltamivir for treatment of acute uncomplicated seasonal influenza A: TOPAZ trial. J. Infect. Dis 2019, 219(7),1026–34. [PubMed: 30428049]
8. a)Clark MP; Ledeboer MW; Davies I; Byrn RA; Jones SM; Perola E; Tsai A; Jacobs M; Nti-Addae K; Bandarage UK; Boyd MJ; Bethiel RS; Court JJ; Deng H-B; Duffy JP; Dorsch WA; Farmer LJ; Gao H; Gu W-X; Jackson K; Jacobs DH; Kennedy JM; Ledford B; Liang J-L; Maltais F; Murcko M; Wang T-S; Wannamaker MW; Bennett HB; Leeman JR; McNeil C; Taylor WP; Memmott C; Jiang M; Rijnbrand R; Bral C; Germann U; Nezami A; Zhang Y-G; Salituro FG; Bennani YL; Charifson PS Discovery of a Novel, First-in-Class, Orally Bioavailable Azaindole Inhibitor (VX-787) of Influenza PB2. J. Med. Chem 2014, 57 (15), 6668–78. [PubMed: 25019388] b)Farmer LJ; Clark MP; Boyd MJ; Perola E; Jones SM; Tsai A; Jacobs MD;Bandarage UK; Ledeboer MW; Wang T-S; Deng H-B; Ledford B; Gu W-X; Duffy JP; Bethiel RS; Shannon D; Byrn RA; Leeman JR; Rijnbrand R; Bennett HB; O'Brien C; Memmott C; Nti-Addae K; Bennani YL; Charifson PS Discovery of Novel, Orally Bioavailable β−Amino Acid Azaindole Inhibitors of Influenza PB2. ACS Med. Chem. Lett 2017, 8, 256–60. [PubMed: 28197322]
9. Palese P; Shaw ML Orthomyxoviridae: The Viruses and Their Replication In Fields Virology: Knipe DM, Howley PM, Eds.; Lippincott Williams & Wilkins: Philadelphia, PA, 2007; pp 1647–89.
10. Yuan S-F; Wen L; Zhou J. Inhibitors of influenza A virus polymerase. ACS infect. Dis 2018, 4, 218–23. [PubMed: 29355011]
11. Tanoury GJ; Nugent WA; Dvornikovs V; Rose PJ Preparation of pyrimidinylpyrrolopyridine derivatives for use as influenza viruses replication inhibitors. WO2015073481, 2015, 165pp.
12. Jordan R; Bailey TR; Rippin SR; Dai D-C Preparation of isoindole derivatives for treatment and prevention of orthopoxvirus infections. WO2008130348, 2008, 82 pp.
13. Nti-Addae KW; Waldo M; O'Neil SA; Van Alsten JG; Macikenas D; Mudunuri P; Shi Y; Ledeboer MW; Jurkauskas V; Medek A; Jones S; Byrn R; Asmal M; Robertson SM; Tsai W-J Preparation of pyrimidinylpyrrolopyridine derivatives for use as influenza viruses replication inhibitors. WO2015073476, 2015, 126pp.
14. 14a-d are commercially available; 14e-f are synthesized by following the chemistry described in : Janssen PAJ; Lewi PJ; De Jonge MR; Koymans LMH; Daeyaert FFD; Heeres J; Vinkers HM; Leenders RGG; Vandenput DAL Preparation of purine derivatives for use in pharmaceutical compositions as HIV replication inhibitors. WO2005028479, 2005, 108pp.
15. Charifson PS; Clark MP; Bandarage UK; Bethiel RS; Boyd MJ; Davies I; Deng H-B; Duffy JP; Farmer LJ; Gao H; Gu W-X; Kennedy JM; Ledford B; Ledeboer MW; Maltais F; Perola E; Wang T-S Preparation of pyrrolopyridinylpyrimidinylamine derives and analogs for use as influenza viruses replication inhibitors. WO2013019828, 2013, 174pp.
16. Compound **16a**: 1H NMR (400MHz, DMSO-d_6): δ 12.24 (s, 1H), 8.71 (dd, J = 2.8 Hz, J = 10.0 Hz, 1H), 8.35 (s, 1H), 8.29 (s, 1H), 8.06 (d, J = 5.2 Hz, 1H), 7.39 (d, J = 5.6 Hz, 1H), 6.55 (t, J = 7.2 Hz, 1H), 6.25 (t, J = 7.2 Hz, 1H), 4.84 (m, 1H), 3.10 (brs, 1H), 2.93 (brs, 1H), 2.65 (brs, 1H), 1.81 (m, 1H), 1.61 (m, 1H), 1.34 (m, 1H), 1.17 (m, 1H); 13C NMR (100 MHz, DMSO-d_6): δ 175.2, 159.0, 157.2, 156.5, 154.8, 146.4, 135.2, 132.6, 131.8, 131.1, 128.5, 125.9, 124.6, 119.0,115.9, 114.8, 72.7, 60.7, 52.8, 50.5, 23.42, 20.26; 19F NMR (376 MHz,DMSO-d6): δ −138.42; MS: m/z = 436.4 [M+ H]$^+$**16a**$^+$
17. Compound **16b**: 1H NMR (400MHz, DMSO-d_6): δ 12.29 (s, 1H), 8.67 (dd, J = 2.8 Hz, J = 10.0 Hz, 1H), 8.35 (s, 1H), 8.29 (m, 1H), 7.65 (d, J = 6.0 Hz, 1H), 7.40 (d, J = 6.0 Hz, 1H), 6.56 (t, J = 7.6 Hz, 1H), 6.25 (t, J = 7.2 Hz, 1H), 4.83 (s, 1H), 3.11 (m, 1H), 2.94 (m, 1H), 2.56 (m, 1H), 1.83 (m, 1H), 1.65 (m, 1H), 1.36 (m, 1H), 1.20 (m, 1H); 13C NMR (100 MHz, DMSO-d_6): δ 167.1, 158.3, 157.2, 156.5, 154.8, 146.4, 135.4, 132.5, 131.8, 131.4, 121.0, 120.3, 119.0, 115.8, 114.7,

113.9, 52.8, 51.0, 34.0, 33.2, 23.4, 20.2; 19F NMR (376 MHz, DMSO-d_6): δ −138.32; MS: m/z = 436.5 $[M+H]^+$ **16b**$^+$

18. Bandarage UK; Clark MP; Perola E; Gao H; Jacobs MD; Tsai A; Gillespie J; Kennedy JM; Maltais F; Ledeboer MW; Davies I; Gu W; Byrn RA; Nti Addae K; Bennett H; Leeman JR; Jones SM; O'Brien C; Memmott C; Bennani Y; Charifson PS Novel 2-Substituted 7-Azaindole and 7-Azaindazole Analogues as Potential Antiviral Agents for the Treatment of Influenza. ACS Med. Chem. Lett 2017, 8, 261–265.
19. a)Pflug A, Gaudon S, Resa-Infante P, Lethier M, Reich S, Schulze WM, & Cusack S. (2017). Capped RNA primer binding to influenza polymerase and implications for the mechanism of cap-binding inhibitors. Nucleic acids res, 46(2), 956–971.b)Sherman W, Beard HS, Farid R. Use of an induced fit receptor structure in virtual screening. Chemical biology & drug design, 2006, 67(1), 83–84. [PubMed: 16492153] c)Sherman W; Day T; Jacobson MP; Friesner RA; Farid R, "Novel Procedure for Modeling Ligand/Receptor Induced Fit Effects," J. Med. Chem, 2006, 49, 534–553. [PubMed: 16420040]

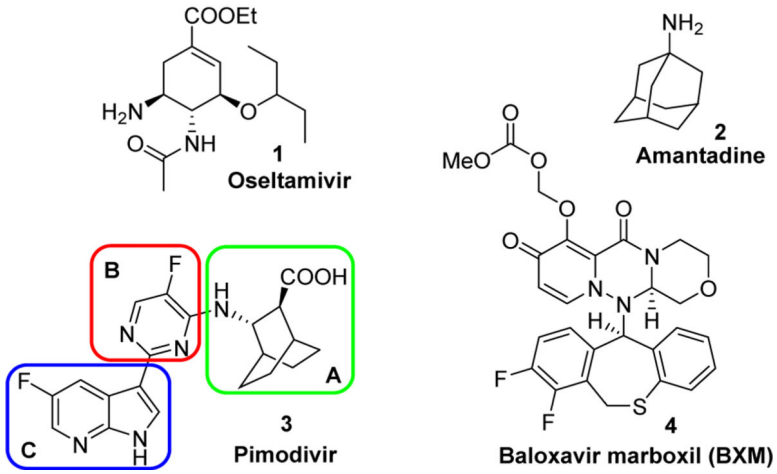


Figure 1.

Structure of approved drugs oseltamivir, amantadine, baloxavir marboxil and experimental drug Pimodivir.

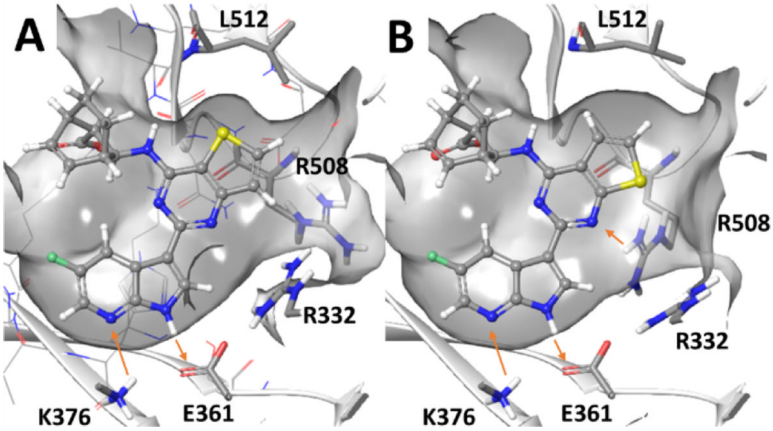
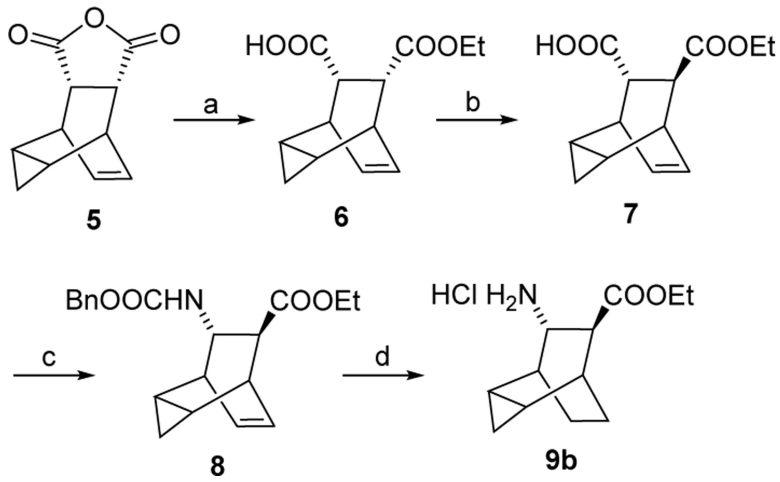


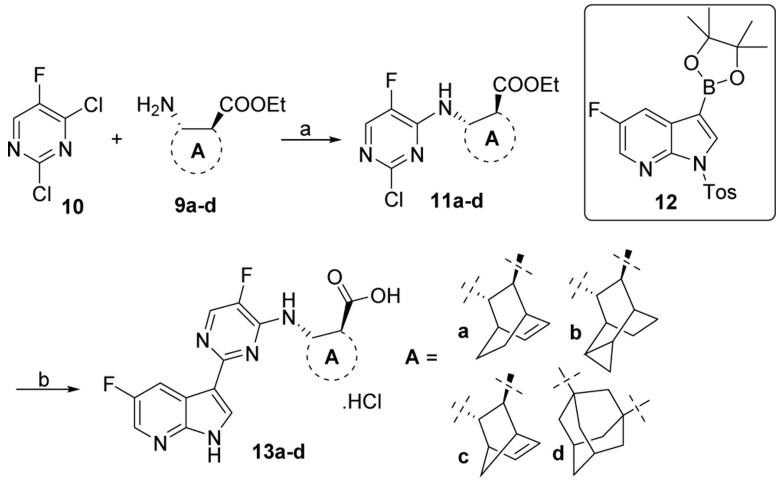
Figure 2.

Molecular model of **(A) 16a** or (B) **16b** bound to the influenza PB2 pocket obtained from induced fit into Pimodivir complexed crystal structure (PDBID 6EUV).



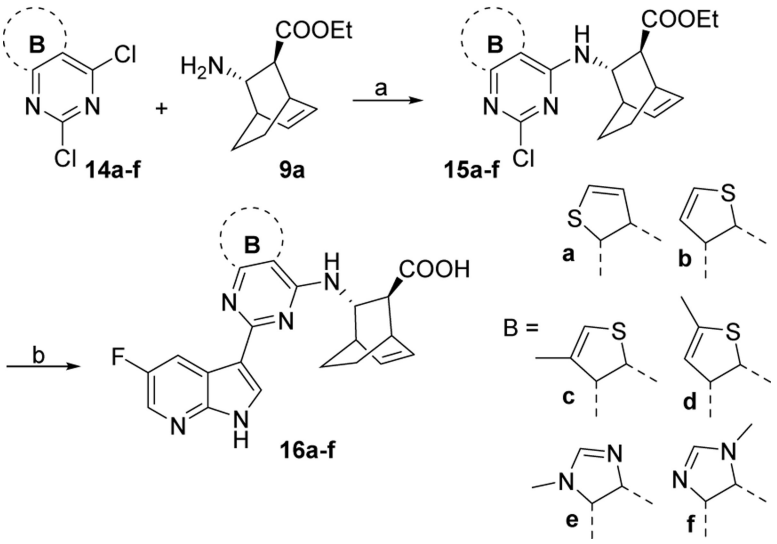
Scheme 1. Reagents and conditions:

a) quinine, toluene, EtOH, −15 °C, two days; b) i) 6N HCl, toluene, rt, 1 h; ii) KOtAmyl, toluene, −20 °C to −10 °C, 2.5 h; iii) HOAc, HCl, 45 min, 41 % over 2 steps; c) Et_3N, DPPA, BnOH, toluene, 96 °C, overnight, 71 %; d) $Pd(OH)_2$ /C, H_2, HCl/EtOH, overnight, 80 %.



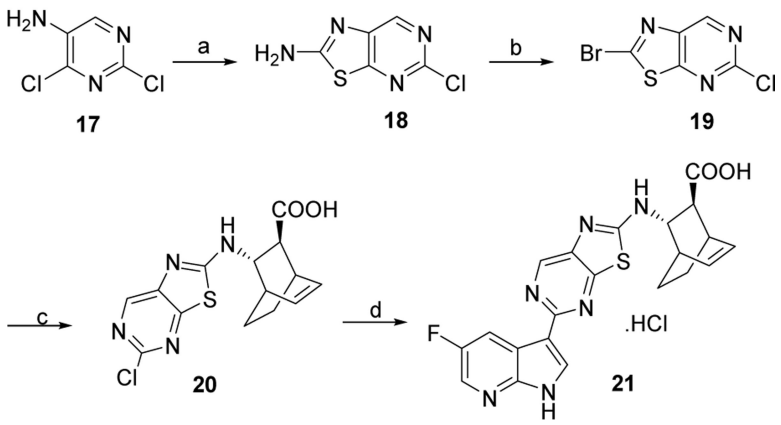
Scheme 2. Reagents and conditions:

a) DIPEA, dichloroethane, 70 °C, overnight, for **11a**, 67%; for **11b**, 74%; for **11c**, 56%; for **11d**, 45%; b) i) **12**, $Pd_2(dba)_3$, X-Phos, K_3PO_4, 2-methylTHF/H_2O, 110 °C, overnight, 45–70%; ii) LiOH, THF, 70 °C, 48 h, for **13a**, 50%; for **13b**, 65%; for **13c**, 35%; for **13d**, 67%.



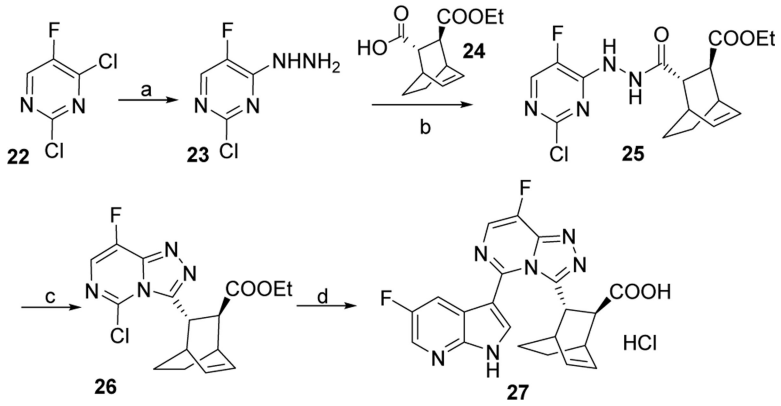
Scheme 3. Reagents and conditions:

a) DIPEA, dichloroethane, 70 °C, overnight, for **15a**, 55%; for **15b**, 62%; for **15c**, 54%; for **15d**, 50%; for **15e**, 36%; for **15f**, 40%; b) i) **12**, $Pd_2(dba)_3$, X-Phos, K_3PO_4, 2-methylTHF/H_2O, 110 °C, overnight, 25–67%; ii) LiOH, THF, 70 °C, 2 days, for **16a**, 65%; for **16b**, 64%; for **16c**, 58%; for **16d**, 60%; for **16e**, 21%; for **16f**, 18%.



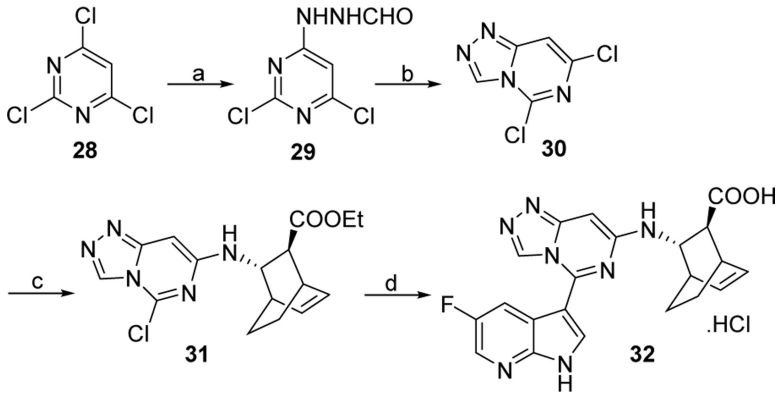
Scheme 4. Reagents and conditions:

a) KSCN, acetic acid, reflux, 3 h, 89%;b) *t*-Butyl nitrite, $CHBr_3$, 60 °C for 1 h then 90 °C for 1 h, 50%; c) **9a**, DIPEA, dichloroethane, 70 °C, overnight, 65%; d) i) **12**, $Pd_2(dba)_3$, X-Phos, K_3PO_4, 2-methylTHF/H_2O, 110 °C, overnight, 43%; ii) LiOH, THF, 70 °C, 2 days, 42%.



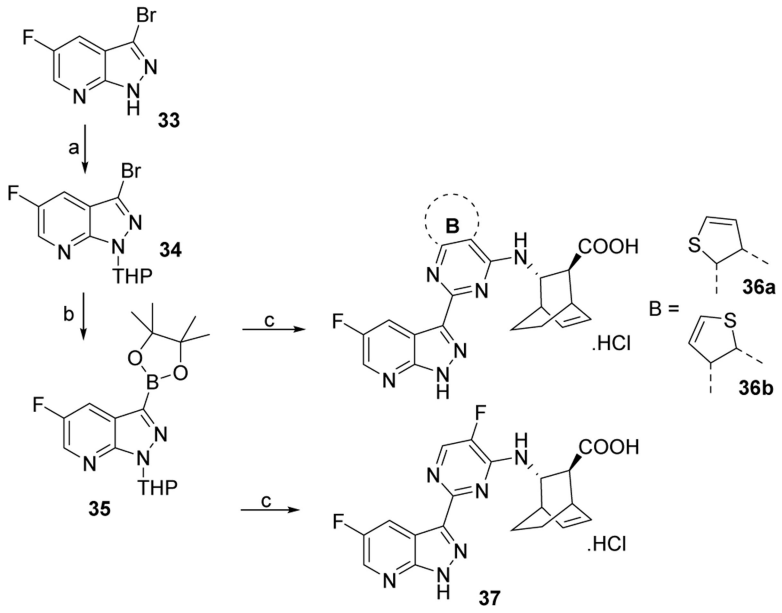
Scheme 5. Reagents and conditions:

a) NH2NH2, ethanol, 0°C to rt, overnight, 68%; b) EDCI, DMAP, dichloromethane, overnight, 51%; c) POCl3, 80 °C, overnight, 80%; d) i) **12**, Pd2(dba)3, X-Phos, K3PO4, 2-methylTHF/H2O, 110 °C, overnight, 25%; ii) LiOH, THF, 70°C, 2 days, 40%.



Scheme 6. Reagents and conditions:

a) NH_2NHCHO, ethanol, rt, overnight, 54 %; b) $POCl_3$, 80 °C, 10 h, 78%; c) **9a**, DIPEA, dichloroethane, 70 °C, overnight, 50 %. d) i) **12**, $Pd_2(dba)_3$, X-Phos, K_3PO_4, 2-methylTHF/H_2O, 110 °C, overnight, 56%; ii) LiOH, THF, 70°C, 2 days, 42%.



Scheme 7. Reagents and conditions:

a) dihydropyran (DHP), TsOH, dichloromethane, rt, overnight, 65%; b) bis(pinacolato)diboron, potassium acetic, $Pd(dppf)_2Cl_2$, 80 °C, overnight, 70%; c) i) **11a** or **15a-b**, $Pd_2(dba)_3$, X-Phos, K_3PO_4, 2-methylTHF/H_2O, 110°C, overnight, 30–40%; ii) trifluoroacetic acid, dichloromethane, rt, overnight, 63–72%; iii) LiOH, THF, 70°C, 2 days, for **36a**, 35%; for **36b**, 45%; for **37**, 40%.

Table 1.

Anti-influenza A activity in A549 cells and cytotoxicity of compounds **13a-d**, **16a-f**, **21**, **27**, **32**, **36a-b** and **37**.

cpd	Anti-Influenza A activity in A549 cells (μM ± SD)		Toxicity (μM) in				
			PBM	CEM	Vero	Huh7	A549
	EC_{50}	EC_{90}	CC_{50}	CC_{50}	CC_{50}	CC_{50}	CC_{50}
pimodivir	0.004 ± 0.002	0.027 ± 0.01	> 100	48.9	> 100	95.8	20.0
13a	0.007 ± 0.005	0.027 ± 0.01	> 100	91.1	> 100	> 100	≥ 100
13b	0.05 ± 0.04	0.21 ± 0.07	> 100	62.9	> 100	ND	94.6
13c	0.05 ± 0.03	0.28 ± 0.05	> 100	> 100	> 100	> 100	> 100
13d	0.95 ± 0.14	5.1 ± 2.1	23.1	10.1	30.3	14.3	16.2
16a	0.0065 ± 0.0011	0.047 ± 0.008	9.3	4.2	65.9	24.0	14.5
16b	0.017 ± 0.01	0.10 ± 0.06	64.6	31.6	74.9	68.7	14.7
16c	0.029 ± 0.006	0.082 ± 0.01	> 100	> 100	> 100	> 100	> 100
16d	0.027 ± 0.014	0.14 ± 0.11	78.4	12.9	90.3	31.2	41.7
16e	0.4 ± 0.23	2 ± 0.8	>100	29.1	> 100	> 100	≥ 100
16f	>10	ND	>100	> 100	> 100	> 100	> 100
21	>100	ND	84.8	17.3	86.1	70.2	31.3
27	>40	ND	≥ 100	41.7	≥ 100	≥ 100	65.4
32	0.68 ± 0.17	2.5 ± 0.79	> 100	30.8	> 100	71.0	51.9
36a	0.012 ± 0.001	0.036 ± 0.007	> 100	13.7	> 100	62.7	> 100
36b	0.042 ± 0.007	0.14 ± 0.04	> 100	67.0	52.5	70.7	32.5
37	0.016 ± 0.003	0.044 ± 0.011	> 100	48.9	> 100	83.0	30.6

ND: Not determined