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Chapter X:

Anti-influenza drug discovery and development: *targeting the virus and its host by all possible means*

Olivier Terrier¹, Anny Slama-Schwok²

Abstract

Influenza infections remain a major and recurrent source of public health concern. Together with vaccines, antiviral drugs play a key role in the prevention and treatment of influenza virus infection and disease. Today, the number of antiviral molecules approved for the treatment of influenza is relatively limited and their use is threatened by the emergence of viral strains with resistance mutations. There is therefore a real need to expand the prophylactic and therapeutic arsenal. This chapter summarizes the state of the art in drug discovery and development for the treatment of influenza virus infections, with a focus on both virus-targeting and host-cell-targeting strategies. Novel antiviral strategies targeting other viral proteins or targeting the host cell, some of which are based on drug repurposing, may be used in combination to strengthen our therapeutic arsenal against this major pathogen.

Keywords: antiviral, drug repurposing, replication, entry, immune modulator

Abbreviations:

CoV: Coronavirus

COX: cyclo-oxygenase

HA: hemagglutinin,

IAV: Influenza A virus,

IFN: Interferon

M2: Matrix 2

NA: neuraminidase,

NOX: NADPH oxydase

NP: nucleoprotein,

p09: H1N1 2009-pandemic strain

PA: polymerase acidic subunit,

PB1: polymerase basic subunit 1,

PB2: polymerase basic subunit 2,

PPI: protein–protein interaction

RdRP: RNA-dependent ribonucleoprotein complex

RIG-I: retinoic acid inducible gene–I

TNF- α : Tumor necrosis factor- α

vRNP: viral ribonucleoproteins

-
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1 **1. Introduction**

2 Influenza infections remain a major and recurrent source of public health concern. Influenza viruses are
3 the causative agents of annual flu epidemics, marked by up to 1 billion infections and 300,000-650,000
4 deaths worldwide, with a huge economic burden in terms of hospitalization costs and work/school
5 absenteeism (WHO, 2018; [56]). In addition, Influenza A viruses (IAV) have been the cause of several
6 pandemics in recent human history, from the Spanish Flu H1N1 in 1918 to the more recent H1N1 2009
7 pandemic [71].

8 Together with vaccines, antiviral drugs play a vital part in the prevention and treatment of influenza
9 virus infection and disease. During a normal influenza season, antiviral drugs are mainly used to treat
10 critically ill patients, such as those hospitalized in intensive care. In a pandemic context, pending the
11 availability of a vaccine, antiviral drugs are essential both to treat patients who have been infected and
12 to prevent infection in those exposed, including healthcare workers. Today, the number of antiviral
13 molecules approved for the treatment of influenza, based on the targeting of viral proteins, is relatively
14 reduced and threatened by the emergence of strains with resistance mutations. There is therefore a real
15 need to expand the prophylactic and reinforce the current therapeutic arsenal. This chapter summarizes
16 the state of the art in drug discovery and development for the treatment of influenza virus infections,
17 with a focus on both virus-targeting and host-cell-targeting strategies (**Figure 1**). Novel antiviral
18 strategies targeting other viral proteins or targeting the host cell, some of which are based on drug
19 repurposing, may be used in combination to strengthen our therapeutic arsenal against this major
20 pathogen.

21

22 **2- From existing classic antiviral drugs to new pre-clinical candidates**

23

24 **2.1 M2 ion channel blockers (amantadine/rimantadine)**

25 Influenza A M2 is a multifunctional viral homo-tetramer protein [57]. Its transmembrane (TM) domain
26 forms a proton channel. This channel is required for acidification of the viral endosome formed after
27 fusion and endocytosis of the virus within the host cell. This process allows viral ribonucleoproteins
28 (vRNPs) to dissociate from the matrix 1 (M1) protein. The proton conductance mechanism relies on the
29 conserved H37XXXW41 sequence which is responsible for selectively gating H⁺ ions [89, 132], [52,

30 121]. Channel blockers interfere with the proton conductance mechanism by binding to the
31 transmembrane pore [137] (**Figure 2**). When proton conductance through M2 is blocked by the
32 adamantane drug, this dissociation is prevented and the virus is no longer able to replicate. In recent
33 years, adamantane drug-resistant mutants have become prevalent in circulating viruses. The two most
34 prevalent drug-resistant mutants are S31N, L26F and V27A, all of which are located in the
35 transmembrane region of M2 [138]. **Figure 2A** shows the strong interaction of amantadine with V27 in
36 the upper part of the pore. Upon drug-resistance V27A mutation, this interaction is lost. Recently-
37 developed spiro-amantadyl amine effectively binds to A27 of the pore (**Figure 2B**) [136]. Recently,
38 new amantadine derivatives effective against double mutants M2-S31N/L26I and M2-S31N/V27A viral
39 strains have been developed by Musharrafieh et al [91]. The antiviral efficacy of such compounds is
40 summarized In **Table 1**. M2 resistance mutations in H1N1/H3N2 circulating strains prompted the WHO
41 to remove both amantadine and rimantadine from the list of recommended anti-influenza agents for
42 clinical use in 2009 [34].

43

44 **2.2 Neuraminidase (NA) and hemagglutinin (HA) inhibitors**

45

46 **2.2.1 NA inhibitors**

47 **NA inhibitors** competitively inhibit and prevent cleavage of the terminal sialic acid residues from
48 glycoproteins and carbohydrates displayed on the surface of mammalian cells and influenza virus
49 particles. Binding of virions to uncleaved sialic acid then impairs virion release and dissemination.
50 Among these NA inhibitors, peramivir, zanamivir, oseltamivir carboxylate are the most frequently
51 prescribed drugs and considered standard-of-care for influenza management (**Table 1 and Figure 3**).
52 Resistance to oseltamivir can develop rapidly in both experimental settings and the clinic, and typically
53 originates from substitutions at signature resistance sites in the viral NA protein such as H274Y and
54 I223R (predominant in H1N1 and H5N1 viruses), and E119V, R292K, or N294S (predominant in H3N2
55 viruses). These three NA inhibitors are currently licensed worldwide for the treatment of influenza A
56 and B infections, oseltamivir being the most widely used. There is still a lot of debate about the
57 effectiveness and real impact of inhibitors on the prevention and treatment of influenza. New oseltamivir

58 derivatives, targeting either multiple sites or different NA cavities (as the “430” or the “150” cavity)
59 have been recently developed. Some of these derivatives are very potent against multiple IAV and IBV
60 strains, including oseltamivir-resistant ones (**Table 1**).

61

62 **2.2.2 Hemagglutinin inhibitors**

63 The surface glycoprotein HA enables viral entry into host cells by binding to cell-surface, sialic-acid-
64 containing glycans and mediating fusion between the viral and host membranes in endosomal
65 compartments. HA is composed of head (HA1) and stem (HA2/HA1) domains. As the regions on HA
66 involved in binding and fusion are highly conserved, they are attractive sites for the design of new
67 antivirals (Table 2). The broad-spectrum antiviral drug arbidol shows efficacy against influenza viruses
68 by targeting the hemagglutinin (HA) stem region [63]. This molecule is currently licensed in Russia and
69 China for the treatment of influenza and other infections [8]. A challenging strategy aiming at
70 mimicking antibodies binding sites was successfully developed by Wilson et al, targeting the conserved
71 stem region and more recently at the interface of the trimeric head region [4, 141, 155] (**Figure 4A**).
72 The binding sites of the binding sites for CBS1117 and JNJ4796 were both found in the stem region
73 close to the fusion peptide, highlighting the possibility of further structure-based designed compounds
74 [2]. De novo design of high-affinity trimeric proteins called “HA mini-binders” that bind influenza A
75 hemagglutinin trimer at a conserved region binding site (**Figure 4B**) [129]. These molecules were
76 developed as alternative to antibodies. These and other compounds are summarized in **Table 2**.

77

78 **2.3 Polymerase – nucleoprotein- RNA inhibitors**

79

80 **2.3.1 Polymerase/endonuclease inhibitor (favipiravir, Baloxavir marboxil)**

81

82 Influenza virus transcribe and replicate their genome in the nucleus of infected cells by the
83 means of a hetero-trimeric polymerase, PA, PB1 and PB2. The polymerase complex function requires
84 the nucleoprotein NP, a protein associated with- and protecting the segmented genomic RNA. Therefore,
85 all four proteins are essential for replication. Whereas replication requires the generation of
86 complementary positive polarity RNA intermediates (cRNA) that are then copied into progeny negative

87 polarity segments (vRNPs), viral message is directly synthesized from vRNPs. Since the influenza virus
88 RdRP lacks enzymatic activity to form 5' mRNA cap structures, endonuclease activity of the PA subunit
89 is necessary for the generation of viral mRNAs through transfer of 5'-capped RNA primers derived from
90 host mRNAs in a cap-snatching mechanism. The endonuclease active site of PA-N terminal comprises
91 a histidine and a cluster of three strictly conserved acidic residues (Glu80, Asp108, Glu119), which
92 coordinate (together with Ile120) one or two manganese or magnesium ions [32] (**Figure 5A**). PB2 is
93 involved in binding of the capped primers, whereas the PB1 subunit harbors enzymatic activity for
94 phosphodiester bond formation.

95 Several classes of inhibitors are in the clinics (**Figure 6**): baloxavir (PA), favipiravir (PB1)
96 and pimodivir (PB2, **Figure 5B**).

97
98 **2.3.2 Pre-clinical compounds targeting the polymerase PA-PB1 and PA subunits; escape mutations**
99 **and resistance.**

100
101 Pre-clinical candidates, some of them being listed in Tables 3 and 4, are in development, benefiting from
102 the recent insight provided by the structures of PA-PB1, PB1-PB2 and
103 whole polymerase complex with or without RNA by X-ray crystallography [26, 48, 95, 102, 110, 130,
104 146] and cryo-electron microscopy [23, 42, 44, 111, 144]. The error-prone nature of influenza viral
105 replication can rapidly generate point mutants for the selection of resistance that have seriously
106 compromised the efficacy of influenza therapeutics. Escape mutations were identified under the pressure
107 selection of PA inhibitors: the signature hotspot for escape from baloxavir marboxil is PA residue 38,
108 for which several substitutions (PA I38T/M/F) have been described [96]. Similarly, escape mutations
109 from L-742.001[127] and RO-7 [70] treatments were also characterized although in laboratory
110 resistance-assays, escape mutants were not detected after multiple passages for L-742.001. While very
111 tight affinities have been achieved by designing metal binding inhibitors to block the active site of the
112 endonuclease activity in PA N-terminal (**Table 2**), the appearance of escape mutants often rapidly
113 decrease their efficacy. Several recent reviews focus on the development of PA and polymerase
114 inhibitors [61, 62, 87, 170].

115

116 Different strategies have been undertaken to attempt overcoming induced resistance. Interfering with its
117 proper assembly of the RdRP polymerase to inhibit function is pursued using protein–protein interaction
118 (PPI) inhibitors. The advantage of such an approach is the relatively large interacting surface between
119 the two proteins as compared to the binding site of an active-site ligand. Indeed, inducing simultaneous
120 mutation of at least one residue on both proteins while maintaining their interaction is less likely to
121 develop resistance and suggests that PPI inhibitors could be less prone to drug resistance than inhibitors
122 of enzyme active sites. The recent identification of a single- domain antibody (nanobody) allowing to
123 disrupt dimerization of FluA polymerase is among these lines [42]. PPI inhibitors have been developed
124 based on the structural insight given by PA–PB1 crystal structures in 2012 [81]. The inhibition of the
125 polymerase PA–PB1 subunit interface has become an active field of research with the goal of remaining
126 active against resistant strains to amantadine and to oseltamivir (**Table 3**). Recently, compound 12 was
127 identified by structure-based screening of compounds targeting the PA-PB1 structure. No resistant virus
128 was selected in vitro under drug selection pressure of compound 12a [164]. Moreover, derivatives of
129 cyclothiophene and R151785 were found active against multiple strains of Influenza A and B [31, 94,
130 165].

131 Based on the ability of PA-PB1 to bind viral RNA, it is likely that novel types of inhibitors could be
132 developed by structure-based design [131]. Additionally, inhibitors targeting PA C-terminal [78] and its
133 interactions with vRNA or with PolIII could be effective targets, based on the accumulating wealth of
134 structural data [42, 102, 110, 144] and deeper insight in the multi-protein assembly required for during
135 replication / transcription.

136

137 ***2.3.4 Broad-spectrum inhibitors***

138 Favipavir is a drug with broad-spectrum antiviral activity in cell culture, inhibiting RNA viruses of the
139 arenavirus, bunyavirus, flavivirus, alphavirus, norovirus, picornavirus, paramyxovirus, and rhabdovirus
140 families, in addition to influenza viruses [160]. This drug is incorporated into newly synthesized RNA
141 by the viral polymerase in place of purines but not pyrimidines, resulting in increased frequencies of C-
142 to-U and G-to-A transition mutations. Although the barrier for resistance is relatively high, this drug
143 seems to present toxicity issues. N 4-hydroxycytidine (NHC) is also a broad-spectrum antiviral

144 candidate, which showed oral efficacy against RSV and both highly-pathogenic avian and seasonal
145 influenza viruses as well as SARS-CoV-2 virus [140].

146 *2.3.5 Pre-clinical compounds targeting the polymerase PB2 subunit*

147 Crystal structure of the PB2 cap-binding domain have been exploited to develop different 7-
148 methylguanine derivatives [100]. Pimodivir (VX-787) is an inhibitor targeting the polymerase PB2
149 subunit at the m⁷ GTP-binding site, forming extensive stacking interactions with several aromatic
150 residues His (**Figures 5B and 6**). It inhibits influenza virus replication and reduced viral load in animal
151 infection models of H3N2 and H1N1 viruses, although potency was highest against H1N1 strains [9,
152 20]. Phase-2 clinical studies indicated that this drug is well-tolerated, reduced viral load, and resulted in
153 slightly faster resolve of clinical signs. Further derivatives of pimodivir have been designed [84].
154 Targeting the PB1-PB2 interface by PPI inhibitors has been challenging: although PP7 exhibited
155 antiviral activities against influenza virus subtypes A pandemic H1N1, H7N9 and H9N2, resistances
156 have been unexpectedly detected in laboratory assays [162].

157 *2.3.6 Pre-clinical compounds targeting the nucleoprotein or the nucleoprotein-RNA interactions*

158 The nucleoprotein associated with viral RNA and the polymerase complex is essential for transcription
159 and replication [22, 145, 146]. The assembly of NP-RNA oligomers into RNP has been determined by
160 cryo-electron microscopy studies [3, 22, 23, 146]. In the X-rays structures of the NP [156], the protein
161 adopts a trimeric structure. NP self-association to achieve trimer formation is mediated by a flexible
162 tail-loop that protrudes into a pocket of the adjacent subunit, via the formation of a critical interaction
163 between R416 of one subunit and E339 of the adjacent subunit. The R416A mutant lacking this
164 interaction adopts a monomeric structure [16]. The native protein can also be purified in a monomeric
165 form at low salt and concentration conditions [15, 16, 133]. The ability to modify the oligomeric state
166 of NP is the structural basis of most NP inhibitors presently developed. Nucleozin was the first NP
167 inhibitor developed as a molecule impeding nuclear accumulation. Nucleozin enhanced higher order
168 structures [64][46]. **Figure 5C** shows the interactions of one of the nucleozin ligands found in the X-

169 ray structure (PDB ID 5B7B) stabilizing the interface between two NP subunits [98]. Escape mutants to
170 nucleozin have been identified in laboratory assays. The opposite approach to impede nucleoprotein
171 self-association has also been pursued by disrupting the important salt bridge R416-E339 mediating NP
172 oligomerization [123]. Recently, new compounds with high affinity for NP were designed stabilizing
173 monomeric NP [150]. Impeding NP binding to viral RNA has been achieved by naproxen drug
174 repurposing, naproxen being a known inhibitor of cyclo-oxygenase (COX)[73]. As NP oligomerization
175 is enhanced by the presence of RNA, naproxen binding to NP reduced NP oligomers and favored
176 monomeric NP. Docking and single mutations studies identified Tyr148, the only aromatic residue
177 within the RNA binding groove and residues of the C-terminal part of NP R355, R361, Phe489 being
178 involved in the interaction of naproxen with NP. Laboratory assays showed no resistance after 8 cell
179 passages infected with Influenza A. Naproxen exhibited antiviral effects in mice models of Influenza A
180 infection [33, 73] as well as Influenza B virus [168]. Further structure-based design yielded new
181 naproxen derivatives with improved antiviral effects and selectivity for NP without COX inhibition
182 (**Figures 5D and 6**) [33, 134] (**Table 4**). Some of these derivatives were found inhibiting NP-PA
183 interactions [33, 143]. Naproxen derivatives also present antiviral properties against oseltamivir-
184 resistant strains [33]. Additional compounds with some similarity of their hydroxyquinoline scaffold to
185 the methoxy naphthalene scaffold of naproxen called NUD were designed and were also found to be
186 resistant in escape mutation laboratory assays [79].

187 ***2.4 Drugs targeting the non-structural protein-1 (NS1)***

188 NS1 has a plethora of strategies to inhibit the host immune response due to its ability to establish
189 multiple protein–protein and protein–RNA interactions. NS1 hampers different pathways both in the
190 cytoplasm and in the nucleus of infected cells. NS1 antagonizes interferon-mediated antiviral host
191 response by binding to double-stranded (ds) viral RNA, thus protecting it from cellular factors, by
192 blocking retinoic acid inducible gene-I (RIG-I) and NF- κ B activation. One pathway by which NS1
193 increases virulence is through the activation of phosphoinositide 3-kinase (PI3K) by binding to its p85 β
194 subunit [17]. NS1 has two structural domains—RNA-binding domain (RBD) and the effector domain

195 (ED)—connected by a short linker (LR), and a disordered C-terminal tail. New drugs binding to NS1
196 effector domain have been designed with low micromolar antiviral efficacy [68] (**Table 4**).

197 **3. Host-targeting & drug repurposing approaches for the treatment of influenza**

198 Considerable progress has been made in understanding the interactions between influenza viruses and
199 the host cell in recent years. In this context, and in light of the emerging problem of resistance to
200 available classical antivirals, many studies have focused on targeting host factors to limit virus
201 replication, but also to modulate host immune response. The targeting of host-factors and/or signaling
202 pathways makes sense in the context of virally-induced hypercytokinemia (also known as “cytokine
203 storm”), which is directly correlated with tissue injury and an unfavorable prognosis of severe influenza
204 [76]. Indeed, approaches to control or attenuate this disproportionate immune response are of particular
205 interest and are the subject of numerous pre-clinical and clinical studies. As with all viruses, influenza
206 viruses depend on cellular machinery for their replication and propagation. Many cellular factors
207 essential for the replication of influenza viruses have been uncovered through genome-wide RNA
208 interference approaches [65, 69, 86, 128] but also more broadly through different integrated cell biology
209 approaches using interactome and transcriptome data, for example [109, 148]. In order to list the
210 different host-targeting strategies developed, a distinction can be made between molecules with a mode
211 of action associated with a relatively well-defined stage of the viral cycle, and molecules associated with
212 the modulation of signaling pathways. It is these two main classes that will be described in the following
213 sections.

214

215 ***3.1 Drugs targeting host cell component at different stages of influenza replication cycle***

216 The replication cycle of influenza viruses can be divided into several distinct phases, 1) entry 2) nuclear
217 import of viral genome (viral ribonucleoprotein; vRNPs) 2) genome replication and protein synthesis,
218 3) Nucleo-cytoplasmic export of vRNPs, and 4) plasma membrane transport and budding of neo-virions
219 (**Figure 1**). A number of molecules targeting host factors in these different steps, at different
220 preclinical/clinical development stages, are known today.

221 **Viral entry** is a target of great interest, as it is likely to allow prophylactic approaches, by blocking the
222 infection in its early stages. One of the most advanced strategy consists to target the viral receptor.
223 DAS181 (**Table 5**) (Fludase, Ansun BioPharma) is a sialidase fusion protein that cleaves both the
224 Neu5Ac $\alpha(2,3)$ - and Neu5Ac $\alpha(2,6)$ -Gal linkages of sialic acid on host cells. DAS181 is administered
225 as an inhalable dry powder to deliver sialidase to the pulmonary epithelium for cleavage of sialic acids,
226 which renders the cells inaccessible to infection by virus [80]. DAS181 was demonstrated to have broad-
227 spectrum activity, given the conserved nature of influenza and parainfluenza viruses binding to
228 respiratory epithelium. Preclinical in vitro and in vivo studies demonstrated that DAS181 has activity
229 against a number of seasonal influenza strains including those containing the H274Y mutation
230 (conferring resistance to oseltamivir), highly pathogenic avian influenza strains (H5N1), and pandemic
231 2009 influenza A (H1N1). This compound was assessed in different Phase I and Phase II clinical trials
232 (NCT 00527865, NCT 01651494, NCT01037205) with results indicating a significant reduction of viral
233 load in treated influenza patients [88] but with identification of respiratory adverse events and rapid
234 clearance of the drug being consistent with the induction of antibodies against DAS-181 - this could be
235 a limitation in the duration and dosages of such treatment [163]. Other approaches targeting viral entry
236 have also been described (**Table 5**), e.g. targeting the endosome acidification step by inhibition of V-
237 ATPase (ex: bafilomycin A1, concanamycin), or inhibition of the internalization (ex: Dynasore) or
238 cleavage steps of haemagglutinin (ex: camostat). Most of these strategies were primarily evaluated at
239 the preclinical stage and have not been further evaluated as their efficacy was either limited or
240 accompanied by cytotoxicity. One exception is the protease inhibitor aprotinin, which was approved as
241 anti-influenza drug in Russia [169].

242 The step of **nuclear import of vRNPs** is a crucial one, for which there are today very few molecules
243 with antiviral potential described in literature. Interestingly, it has been shown in vitro that ivermectin
244 (**Table 5**), a well-known anti-parasite drug, was able to inhibit viral replication via inhibition of
245 importins (IMP α/β), and therefore the nuclear import of vRNPs [47].

246

247 Targeting the **replication** stage of the virus is one of the earliest host-targeting strategies, with pioneer
248 works on the antiviral efficacy of ribavirin in the 1970s [38]. However, this nucleoside analogue or its

249 prodrug, less toxic, do not appear to be options being considered for the treatment of influenza virus
250 infections. of influenza viruses, despite interesting preliminary in vitro and in vivo results [125]. (**Table**
251 **5**). Other, more recent strategies propose to target **mRNA splicing**. Influenza viruses are known to
252 hijack cellular splicing machinery to their benefit, making them extremely dependent on it [36, 37].
253 Several studies show that the inhibition of Cdc2-like kinase 1 (CLK1), involved in the alternative
254 splicing of M2 gene of influenza, appears to be an interesting antiviral option, with several molecules
255 available (TG003, Clypearin, Corilagin, **Table 5**). Of all its molecules, Clypearin has relatively low
256 EC50s and very low toxicity, making it an attractive potential antiviral candidate. [65, 171].

257 While strategies to prevent the nuclear import of vRNPs are relatively uncommon, paradoxically there
258 are many more therapeutic approaches to block the **nuclear-cytoplasmic transport of vRNPs**. Indeed,
259 in contrast to the inhibition of importins, the inhibition of exportin 1 (XPO1) by Verdinexor (XPO1
260 antagonist KPT-335) allows to significantly reduce viral production in vitro and in vivo [101]. Another
261 compound, DP2392-E10, inhibits nuclear export of both viral NP and nuclear export protein (NEP).
262 More specifically, in vitro pull-down assays revealed that DP2392-E10 directly binds cellular CRM1,
263 which mediates nuclear export of NP and NEP – highlighting CRM1 as a target of interest [19]. With
264 the same objective, other strategies consist to target the Raf/MEK/ERK signaling pathway, known to be
265 involved in the export of vRNPs [120]. Several MEK inhibitor molecules have been studied for their
266 ability to inhibit the replication of influenza viruses, such as CI-1040 or U0126 [24, 108]. Interestingly,
267 Schröder and colleagues have demonstrated that Trametinib (GSK-1120212), a licensed MEK inhibitor
268 used for the treatment of malignant melanoma, efficiently blocks influenza viral replication of different
269 subtypes in vitro and in vivo [119] (**Table 5**).

270 **Apical transport and budding**, the last part of the last major step of the replication cycle is also the
271 object of several antiviral strategies, notably by blocking the transport of viral proteins to the plasma
272 membrane (ex: Clonidine; [82]), or the cholesterol pathway, which would reduce virion egress.
273 (U18666A; [92]). One of the most advanced strategies is Nitazoxanide, initially licensed for the
274 treatment of parasitic infections, for which anti- influenza properties were first documented by
275 Rossignol et al. [113]. Interestingly, the proposed mode of action of nitazoxanide toward influenza is
276 clearly distinct to that for which it was designed in its initial indication, acting at the post-translational

277 level by selectively blocking the maturation of the viral glycoprotein HA, with a consecutive impact on
278 its intracellular trafficking and insertion into the host plasma membrane [112]. This drug presents potent
279 antiviral activity against a large panel of circulating strains [139]. The effectiveness of nitazoxanide in
280 treating patients with non-complicated influenza was successful in a Phase IIb/III trial [50] and is
281 currently being assessed in a Phase III clinical trial (NCT01610245).

282

283 *3.2 Drugs targeting host cell signaling pathways and host-response that are crucial for influenza* 284 *replication cycle.*

285 Our increased knowledge of signaling pathways that are crucial in the response to infection and/or those
286 hijacked by the virus has allowed many research teams to explore complementary antiviral strategies
287 that can be described here (**Table 6**). The targeting of the raf/MEK/ERK channel, mentioned above,
288 could of course also have been listed here. At the crossroads of the regulatory pathways of the immune
289 response and the stress response, the **NF-kB pathway** was one of the first to be studied (**Table 6**). In
290 the context of cell biology approaches, it was initially shown that the anti-inflammatory drug
291 acetylsalicylic acid (ASA) had interesting antiviral effect against influenza viruses in vitro and in vivo,
292 via inhibition of the NF-kB activating IkkB kinase [83, 153, 159]. Several drugs targeting the NF-kB
293 pathway have been evaluated since then, such as pyrrolidine dithiocarbamate or SC7574; with
294 encouraging in vivo results [39, 49, 149]. BAY81-8781/LASAG (D, L-Lysine acetylsalicylate-glycine)
295 (**Table 6**), a modified version of ASA demonstrates antiviral activity against several human and avian
296 influenza viruses in vitro. In a mouse infection model, inhalation of LASAG resulted in reduced lung
297 viral titers and protection of mice from lethal infection [35]. More recently, a Phase II proof-of-concept
298 study comparing LASAG versus placebo in patients with severe influenza demonstrated that aerosolized
299 LASAG improved the time to symptom alleviation compared to placebo, despite the absence of a
300 statistically significant reduction of viral load in LASAG-treated group [116].

301 Based on clinical observations, hydroxyl methylglutaryl-coenzyme A (**HMG-CoA**) reductase inhibitors
302 such as statins (**Table 6**), approved for their use as cholesterol metabolism regulators, have demonstrated
303 pleiotropic anti-inflammatory and immunomodulatory properties, which could be of benefit to improve
304 survival of patients with severe influenza [43, 85]. However, most in vivo studies reported so far failed

305 to clearly demonstrate such a beneficial effect in the specific context of influenza infection [6, 72, 115].
306 On the other hand, a few observational studies highlighted an association between statin treatment with
307 a reduction of mortality in patients hospitalized with laboratory-confirmed seasonal influenza [40, 142].
308 A randomized placebo-controlled Phase II clinical trial (NCT02056340) aimed at evaluating the
309 potential effect of atorvastatin to reduce the severity of illness in influenza-infected patients is currently
310 undergoing. The combination of naproxen with clarithromycin and oseltamivir twice daily reduced the
311 both 30- and 90-day mortality and length of hospital stay of patients hospitalized for A(H3N2) influenza
312 [54]. Other approaches, at the preclinical validation stages, propose to target the **TNF-alpha**
313 (Etanercept);) or **NOX2** (Apocynin/Ebselen) or lipoxygenase/COX pathway (Celecoxib/Mesalazine)
314 pathways [12, 28, 74, 97, 124, 157, 167]. A phase III clinical trial is currently investigating the benefit
315 of celecoxib for the treatment of severe influenza (NCT02108366). These molecules could be of interest
316 to better control the inflammatory response, which is a very important aspect of the pathology.

317 Modulation of immune and inflammatory responses is a therapeutic avenue that has been much
318 explored, but which may present risks given the ambivalent aspect of these pathways in relation to viral
319 replication and the evolution of the pathology. Indeed, such treatment should stimulate induction of
320 antiviral genes to control IAV spread, without driving immunopathology. In this context, **IFN-lambda**
321 (**Table 6**) appears as a potent anti-influenza therapeutic, without the inflammatory side effects of IFN-
322 alpha treatment [29]. Intranasal administration of IFN- λ 2/3 was shown to significantly suppress
323 infection of various influenza strains, including WS/33 (H1N1), PR (H1N1), and H5N1 in the mouse
324 lung, and was accompanied by greater up-regulation of ISGs [67]. More recently, using a transcriptome-
325 based screening approaches, we identified and validated diltiazem, a calcium channel blocker used as
326 an anti-hypertensive drug, as a very promising host-targeted inhibitor of influenza infection.
327 Interestingly, the study of the mode of action revealed that diltiazem was a strong induced or type III
328 IFN [107]. An ongoing French multicenter randomized clinical trial is investigating the effect of
329 diltiazem- oseltamivir bi-therapy compared with standard oseltamivir monotherapy for the treatment of
330 severe influenza infections in intensive care units (FLUNEXT trial NCT03212716).

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332

333 **4. Perspectives and concluding remarks**

334 Among all the molecules listed in this chapter, some are already available on the market for other
335 therapeutic indications and fall within the scope of drug repurposing. This is the case for naproxen,
336 diltiazem, LASAG or Nitazoxanide, for example. The basis of drug repurposing relies on bypassing the
337 long, risky and expensive preclinical and early clinical evaluation stage conventionally used for de novo
338 drug development and exploiting available extensive human clinical, pharmacokinetics and safety data
339 as the starting point for the development [106]. All these aspects make the repositioning of drugs a very
340 interesting approach, in particular to enable a rapid response to the need for new antiviral strategies in
341 the context of the emergence of a virus with pandemic potential.

342 Another very interesting perspective is the interest in combining different antiviral approaches with each
343 other, including classical approaches targeting the virus with those targeting the host cell. The concept
344 of combining therapies has already been used successfully, notably in the design of antiretroviral
345 treatments [13]. Combination therapy can have several objectives, such as reducing the risk of the
346 emergence of resistance by simultaneously targeting several viral proteins and/or key host factors, but
347 also increasing the effectiveness of the treatments by obtaining additive or synergistic effects.

348 While there is relatively little convincing evidence to support the use of conventional virus-targeting
349 antivirals in combination [41, 103], there are interestingly a growing number of examples of
350 combinations of combination host-targeted approaches with oseltamivir. For example, we have shown
351 that the combination of diltiazem and oseltamivir provides a much greater reduction in viral titers in a
352 reconstructed human epithelium model compared to single treatments [107]. More recently Schloer and
353 colleagues have shown that a combination treatment of an antifungal molecule, itraconazole, with
354 oseltamivir, achieves much greater antiviral activity compared to monotherapy, making it possible to
355 consider reducing the concentrations of drugs used, and thus possibly reducing the problems of adverse
356 effects and emergence of resistance mutations [117]. These results open up interesting prospects for the
357 development of future therapeutic strategies, particularly for the treatment of severe forms of influenza.
358 The potential arsenal for fighting influenza virus infections is potentially very extensive, in particular
359 thanks to the combination of new molecules targeting the virus, resulting from docking and structure-
360 based design strategies, with approaches targeting cellular factors and signaling pathways. In this

361 context, the quality and relevance of the preclinical models, as well as the quality of the tools for
362 evaluating combinations of molecules, are important critical elements.

363 Beyond influenza viruses, many of the antiviral molecules described in this chapter have the potential
364 for broader-spectrum use. Indeed, some virus-targeted strategies can target viral determinants with very
365 strong similarities between different viruses. This is particularly the case with Naproxen for which we
366 have previously demonstrated antiviral activity against both influenza viruses and SARS-CoV-2 [73,
367 135]. This property is explained by the fact that the nucleoproteins N of enveloped, positive-sense,
368 single-stranded viruses Coronavirus (CoV) share with negative-sense single-stranded viruses such as
369 Influenza A virus the ability to bind to- and protect genomic viral RNA without sequence specificity
370 and to form self-associated oligomers. Despite their differences, viruses induce and divert many
371 common cellular pathways. As a result, host-targeted approaches can identify molecules with a broad
372 spectrum of antiviral activity. An example is diltiazem, for which we have shown antiviral activity
373 against influenza viruses [107], but which has been shown to be effective against other respiratory
374 viruses, such as SARS-CoV-2 [104, 105], due to its mode of action involving the type III interferon
375 response. Efforts to identify anti-influenza molecules therefore open up very interesting prospects for
376 the broader development of antivirals. In many ways, antiviral research on influenza viruses is
377 pioneering in this area and provides a starting point for the study of other emerging viruses.

378

379 **5. References**

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- 826

827 **Figure legends**

828 **Figure 1. Influenza viral particle and viral cycle; current state of anti-influenza drug discovery**
829 **and development.** (A) Influenza A virus (IAV) particle. The IAV genome is composed of eight
830 ribonucleoprotein complexes (vRNPs). Each one consists of single-stranded negative-sense viral RNA
831 (vRNA) encapsidated by viral nucleoprotein (NP) and a viral polymerase complex (PA, PB1, and PB2)
832 positioned at the extremity of the vRNA segment. Three viral proteins are embedded within the viral
833 membrane, hemagglutinin (HA), neuraminidase (NA), and ion channel protein (M2). Matrix protein 1
834 (M1) underlies the viral envelope and holds the vRNPs inside the virion. (B) The viral particle binds to
835 sialic acid receptors and enters the cell via receptor-mediated endocytosis. Acidification of the endocytic
836 vesicles leads to virus uncoating mediated by the M2 ion channel. vRNPs are then released into the
837 cytoplasm and transported into the nucleus. There, the viral RNA-dependent RNA polymerase complex
838 snatches the host mRNA caps to initiate the negative vRNA transcription. Transcribed vRNAs then need
839 to undergo an mRNA maturation phase, including the pre-mRNA splicing, before export to the
840 cytoplasm to be translated. vRNAs are also replicated in the nucleus to generate new vRNPs in
841 association with neosynthesized viral proteins. Progeny vRNPs are transported toward the cytoplasmic
842 membrane with viral components to be packaged into new infectious particles which are formed by
843 cellular envelope budding. Classic virus-targeting strategies are highlighted in red, and virus-host-
844 targeted strategies in blue. Figure created by BioRender.com

845
846 **Figure 2:** Looking down the M2 channel in the presence of inhibitors: Structure of M2 WT and VA27
847 mutant in complex with amantadine and spiroamantadine. View down the pore channel in A- WT-
848 amantadine (V27 is colored in yellow, PDB ID 6BKK[137]) and B- V27A-spiroamantadine complexes
849 (A27 is colored in yellow, PDB ID 6NV1[136])

850
851 **Figure 3: Structures of the approved NA inhibitors**

852
853 **Figure 4: Structure of some of the pre-clinical candidates targeting HA:** A: Structure of HA in
854 complex with JNJ4796 shown in orange (PDB ID 6CF7)[141] B: Structure of trimeric HA in complex
855 with mini-binder highlighted in yellow (PDB ID 6KUY)[129].

856 **Figure 5: Structure of some of the pre-clinical candidates targeting the polymerase** A: Active-site
857 PA N-terminal inhibitor compound 22[25]; B: PB2 inhibitor Pimodivir [20] (the numbering are
858 associated with this structure corresponding to the full-length PB2, C: nucleozin-NP oligomeric
859 complex PDB ID 5B7B, monomers A and B are in cyan and yellow, respectively; D: Naproxen F1-NP
860 monomeric complex from docking studies[33].

861 **Figure 6: Structures of the approved polymerase inhibitors and some pre-clinical candidates**

Tables

Table 1. Summary of the activity and structures of the main antiviral compounds bound to their target, the proton channel M2 of Influenza A or the neuraminidase NA of Influenza A and B.

Target	Compound	IC ₅₀	PDB ID	Stage (year approval)	references
M2	Amantadine	100 μM (H1N1 WT) > 500μM (S31N) 15.7 μM (WT channel ^a) [155]	6BKK	Approved (1976)	[137], [10]
	Rimantadine	0.1 μM (H1N1 WT) > 200μM (S31N)	2RLF	Approved (1994)	[118]
	Spiro-adamantyl amine	18.7 μM (WT channel ^a) 0.2 μM (V27A ^a)	6BMZ 6NV1 6OUG	Pre-clinical	[136, 137]
NA	Oseltamivir (Tamiflu)	0.8 nM (N5 NA)	2HT7	Approved (1999)	[114]
	Peramivir	3.4 nM	2HTU	Approved (2014)	[114]
	Zanamivir	0.6 nM (N5 NA)	3CKZ	Approved (1999)	[21]
	Chebulinic acid Chebulagic Acid	1.36 ± 0.36 μM (H1N1 PR8) (Oseltamivir resistant and H1N1 pdm09 viruses) CC ₅₀ > 100μM		Pre-clinical	[75]
	Oseltamivir derivatives	0.66 μM (H5N1)	Docking 150/430 cavity	Pre-clinical	[1, 58, 166]
	Triazol oseltamivir derivatives C1-modified oseltamivir derivatives	0.05-0.15 μM (H5N1, H5N2 and H5N6) 0.1 μM (H5N1, H5N6) 0.7 μM (Oseltamivir resistant virus)	Docking 430 cavity	Pre-clinical	[60]

a: Patch clamp assays [136]

Table 2: Recent antiviral candidates targeting HA, their activity and structures of their complexes with HA

Target	Compound / binding site	IC ₅₀ / CC ₅₀	PDB ID	Stage	references
HA	Arbidol / Stem region	4-12 μ M CC ₅₀ = 59 μ M	5T6S, 5T6N	Pre-clinical and clinical NCT03787459	[63, 147, 151]
	F0045(S) / Stem region	0.5-2 μ M (H1 HA)	6WCR	Pre-clinical	[155]
	JNJ4795 / Stem region	0.01-0.07 μ M (H1 HA)	6CF7	Pre-clinical	[141]
	IY7640 / Stem region	0.5-7 μ M (H1 HA) CC ₅₀ > 800 μ M	Docking studies	Pre-clinical	[66]
	CBS1117 / Stem region	3 μ M For H5 HA	6VMZ	Pre-clinical	[2, 45, 55]
	MB2746 / Stem region	0.3 μ M (H1 HA) CC ₅₀ > 100 μ M	Docking studies	Pre-clinical	[5]
	De novo design of “Mini-binder” proteins	0.15-0.19 nM (H3 and H1 HA)	6KUY		[129]
	Peninddone		HA1 and HA2	Pre-clinical	[152]

Table 3: Inhibitors of PA, PA-PB1 interactions and PB1

Target	Compound	IC ₅₀ / CC ₅₀	PDB ID	Stage	References
PA	Baloxavir marboxil	0.3-1 μ M (H1N1/H3N2)	6FS6 6FS9	Approved (2019) NCT02954354 NCT0294901	[96]
	L-742,001	3 μ M (WT H1N1) 24 μ M (WT H1N1 pdm09) 236 μ M (H1N1 pdm09 PA F105S)	5CGV 5D9J	Clinical trial NCT01526785	[127]
	RO7	16 nM (WT H1N1) 3 nM (H1N1 pdm09)	5VPX	Pre-Clinical	[59, 70]
	Ana-0	0.8 μ M	Docking	Pre-clinical	[161]
	Compound 22	110 pM	6E6W	Pre-clinical	[25]
	N-acylhydrazone derivatives	11 μ M	5EGA	Pre-clinical	[11]
	”312”	37 μ M (H1N1, H2N2 and H3N2)	PA –C-terminal	Pre-Clinical	[78]
PA-PB1	Compound 12a	0.9-2.7 μ M (FluA amantadine-& oseltamivir resistant, FluB)	Docking	Pre-clinical	[164]
	Amino-acids adducts of diphenyl-pyridine derivatives	39 \pm 2 μ M (H1N1)	Docking	Pre-clinical	[27]
	Cycloheptathiophene-3-carboxamide	0.2 μ M-0.7 μ M H1N1 pdm09, H1N1 oseltamivir-resistant, H3N2, Influenza B	Docking	Pre-clinical	[31],[94]
	R151785	2.5, 5.0 μ M p09, H1N1 oseltamivir- & amantadine resistant Influenza B	Docking	Pre-clinical	[165]
PB1	Favipiravir	Broad-spectrum		Approved (2014)	[160]
	β -d-N4-Hydroxycytidine/ EIDD-2801	Broad-spectrum Influenza, SARS-CoV2		Clinical trial NCT04405739	[140], [122]

Table 4. Inhibitors of PB2 cap-binding, PB1-PB2, NP and NS1

Target	Compound / binding site	IC ₅₀ / CC ₅₀	PDB ID	Stage	References
PB2	Pimodividir (VX787)	2.6 nM	4P1U	Approved (2017)	[9, 20]
	5,7-difluoroindole derivative of pimodivir	11 nM	6S5V	Pre-clinical	[84]
	D 715-2441	3.6-4.4 μM (H1N1, H3N2, H5N1, H7N9)	Docking	Pre-clinical	[77]
	Cap analogs	7.5 μM H3N2	4CB5	Pre-clinical	[100]
PB1-PB2	PP7	1.4-9.5 μM (Strain-specific)	Docking	Pre-clinical	[162]
NP	Nucleozin	0.07 μM (H1N1) 0.16 μM (H3N2) 0.33μM (H5N1Y287H)	5B7B	Pre-clinical	[64, 98]
	Compound 3	0.1 μM (H1N1 and H5N1)	3RO5	Pre-clinical	[46]
	2-(4-chloro-3,5-difluorophenylamino)thiazole-4-carboxamide derivatives	0.11μM	Docking	Pre-clinical	[123], [150]
	Naproxen Naproxen C0 (Naproxen derivative 2) Naproxen F1 (Naproxen derivative 4)	Broad-spectrum FluA & Sars-CoV2 16±5 μM (H1N) 2.9±0.3μM (H1N1) 1.8μM (H1N1 pdm09) 1.3±0.2μM (H1N1) 0.7μM (H1N1 pdm09, H3N2, resistant to oseltamivir)	Docking	Pre-clinical	[33, 73, 134]
	Hydroquinolinone derivatives (NUD)	1.8-7.0 μM (H1N1)	Docking	Pre-clinical	[79]
	NS1	A22	≈ 1 μM (H1N1 PR8)	Docking	Pre-clinical
	ML303	0.7-17 μM (H1N1 pdm09, H3N2)	HTS	Pre-clinical	[99]

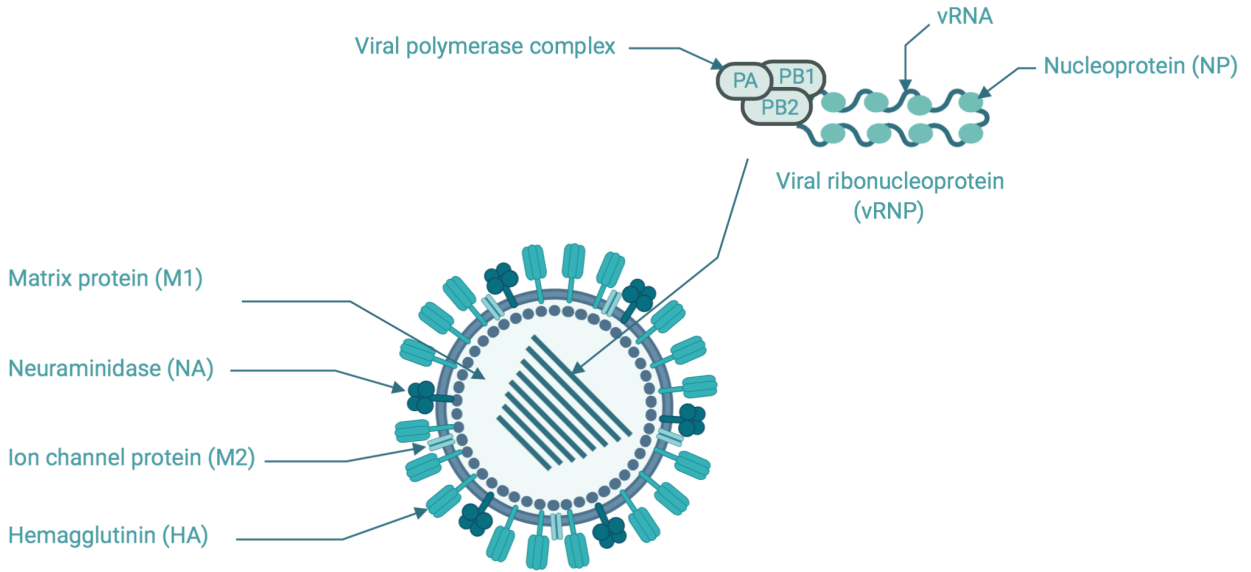
Table 5. Drugs targeting host-cell component at different level of viral cycle stages.

Viral cycle stage	Drug name	Mode of action	Research phase	References	
Viral entry	DAS181	Sialidase – removes sialic acid receptors	Phase I/II	Moss <i>et al.</i> 2012[88] Zenilman <i>et al.</i> 2015[163]	
	Bafilomycin A1	V-ATPase inhibitors – inhibits endosomal acidification	preclinical	Yeganeh <i>et al.</i> 2015[158]	
	Concanamycin			Müller <i>et al.</i> 2011[90]	
	Diphyllin			Chen <i>et al.</i> 2013[14]	
	Saliphenylthalamide			Bimbo <i>et al.</i> 2013[7]	
	Aprotinin	Protease inhibitors – inhibit HA0 cleavage	Approved (2011)	Zhirnov <i>et al.</i> 2011[169]	
	Camostat			Yamaya <i>et al.</i> 2015[154]	
	Dynasore	Inhibition of internalization	Preclinical	de Vries <i>et al.</i> 2011[30]	
	EIPA			Harada <i>et al.</i> 2007[51]	
	Fattiviracin			Gotz <i>et al.</i> 2016[47]	
Nuclear import of vRNP	Ivermectin	Inhibits importin- α/β		Gotz <i>et al.</i> 2016[47]	
Genomic replication & protein synthesis	TG003	CLK1 inhibitors -Regulation of splicing – decrease in M2 mRNA expression		Karlas <i>et al.</i> 2010[65]	
	Clypearin			Zu <i>et al.</i> 2015[171]	
	Corilagin			Slaine <i>et al.</i> 2017[126]	
	Sylvestrol	eIF4A inhibitors – inhibit viral protein synthesis	Approved (1986)	Durr <i>et al.</i> 1975[38]	
	Pateamine			Sidwell <i>et al.</i> 2005[125]	
	Ribavirin	Nucleoside analogue	Phase III (HCV)		
	Viramidine (ribavirin prodrug)				
vRNP nuclear export	Cyclosporin A	Inhibits host RNA polymerase II	preclinical	Liu <i>et al.</i> 2012[77]	
		Inhibits nuclear export of vRNPs			
	Verdinexor	Exportin 1 inhibitors		Perwitasari <i>et al.</i> [101] 2014	
	DP2392-E10			Chutiwitoonchai <i>et al.</i> 2017[19]	
	CI-1040			Haasbach <i>et al.</i> 2017[49]	
	UO126	MEK inhibitor – nuclear retention of vRNP complex		Pleschka <i>et al.</i> 2001[108]	
	PBP10/BOC2	Formyl peptide receptor 2 antagonists – Raf/MEK/ERK inhibition		Courtin <i>et al.</i> 2017[24]	
	Trametinib	MEK1/2 inhibitor – inhibition of vRNP export		Approved (cancer)	Schröder <i>et al.</i> 2018[119]
	Dapivirine	Reverse transcriptase inhibitor - inhibition of vRNP export		Phase III (HIV)	Hu <i>et al.</i> 2017[53]
	Apical transport and budding	Nitazoxanide		Anti-parasitic – Inhibition of HA maturation & transport	Phase III
Ruxolitinib		Virion formation & vRNA incorporation inhibition	Approved (myelofibrosis)	Watanabe <i>et al.</i> 2015 [148]	
U18666A		Hydrophobic polyamine - Reduces plasma membrane cholesterol level and decreases virion egress	preclinical	Musiol <i>et al.</i> 2013[92]	
Clonidine		alpha2-adrenergic receptors inhibitor - Inhibits transport of HA transport to plasma membrane		Matsui <i>et al.</i> 2018[82]	

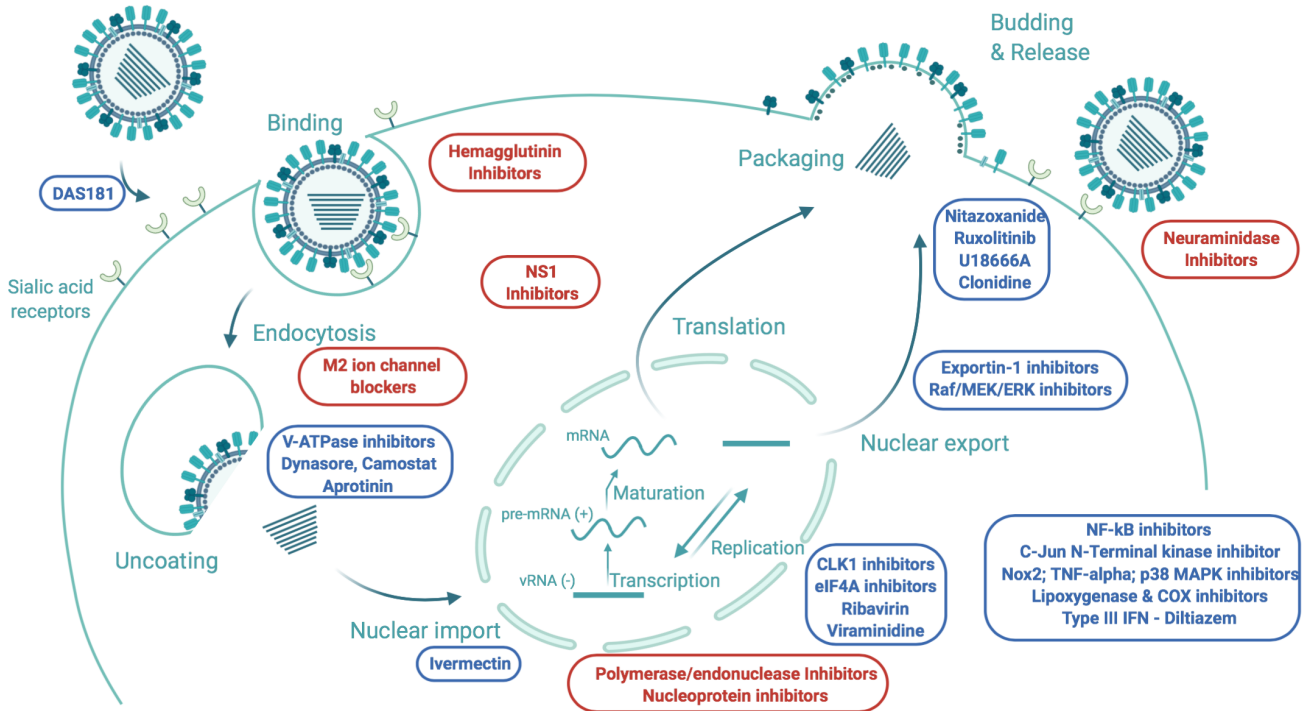
Table 6. Drugs targeting host-cell signaling pathway and host-responses that are crucial for influenza replication cycle.

Host signalling pathway/response	Drug name	Mode of action	Research phase	References
NF-kB pathway	Acetylsalicylic acid	Immune dysregulation Inhibition of caspase/vRNP export inhibition	Approved	Mazur <i>et al.</i> 2007[83]
	pyrrolidine dithiocarbamate		preclinical	Wiesener <i>et al.</i> 2011[149]
	SC75741		preclinical	Ehrhardt <i>et al.</i> 2013[39] Haasbach <i>et al.</i> 2013[49]
	LASAG		Phase II	Droebner <i>et al.</i> 2017[35] Scheuch <i>et al.</i> 2018[116]
C-Jun-N-terminal-kinase	SP600125	C-Jun N-Terminal kinase inhibitor – Immune dysregulation	preclinical	Nacken <i>et al.</i> 2012[93]
p38 MAPK	NJK14047	Immune dysregulation	preclinical	Choi <i>et al.</i> 2016[18]
HMG-CoA	Statins	Immunomodulation	Phase II	Fedson <i>et al.</i> 2013[43] Mehrbod <i>et al.</i> 2014[85] Fedson <i>et al.</i> 2018[43]
TNF-alpha	Etanercept	Anti-inflammatory drug - Prevents TNF-mediated lung injury and edema	preclinical	Shi <i>et al.</i> 2013[124]
Nox2	Apocynin	ROS scavenger, inhibits Nox2 activity	preclinical	Ye <i>et al.</i> 2015[157] Oostwoud <i>et al.</i> 2016
	Ebselen	ROS scavenger and glutathione peroxidase mimetic, inhibits Nox2	preclinical	
Lipoxygenase & COX pathways	Celecoxib	Immune dysregulation	Phase III	Davidson <i>et al.</i> 2018[28] Carey <i>et al.</i> 2010[12] Zheng <i>et al.</i> 2008[167]
	Mesalazine	Immune dysregulation	preclinical	
Type III IFN response	Type III IFN	Induction of type III IFN response	preclinical	Davidson <i>et al.</i> 2016[29] Kim <i>et al.</i> 2017[67]
	Diltiazem		Phase II	Pizzorno <i>et al.</i> 2019[106, 107]

A



B



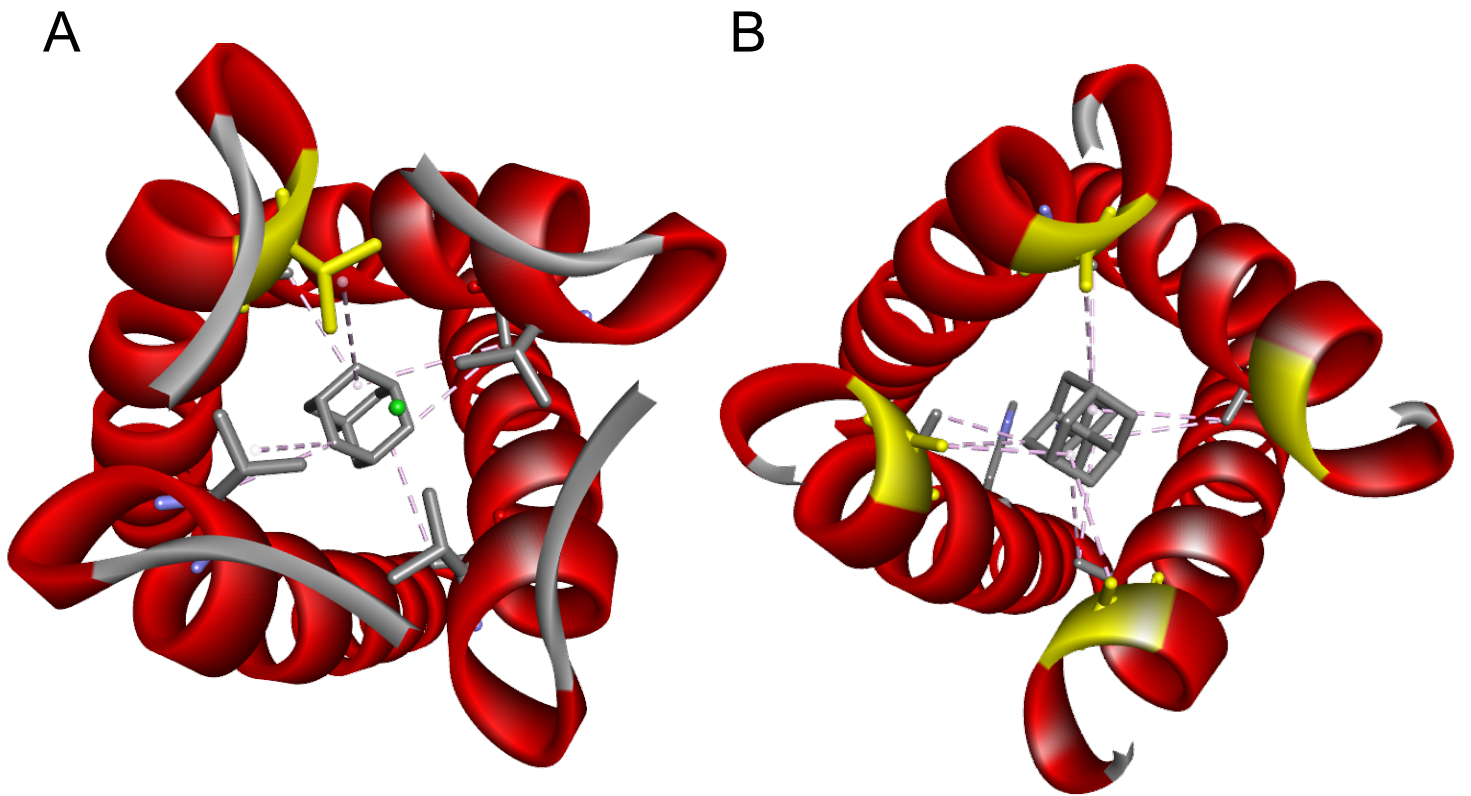
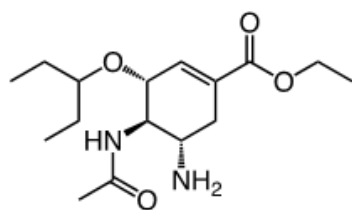
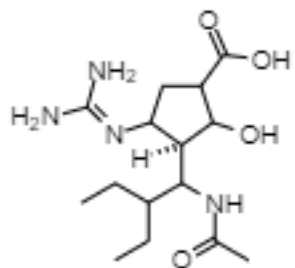


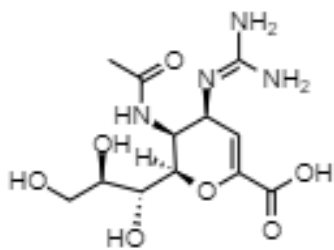
Figure 2



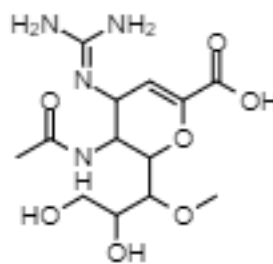
Oseltamivir



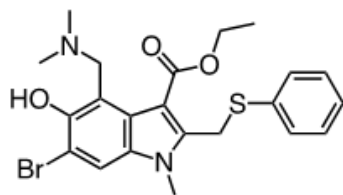
Peramivir



Zanamivir

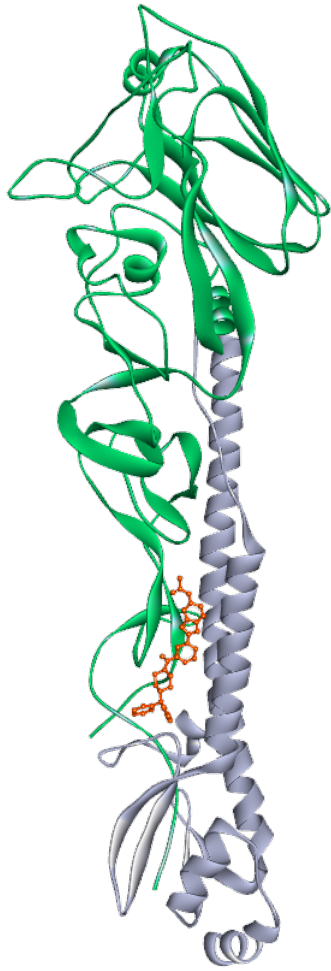


Laninamivir



Ardibol

A



B

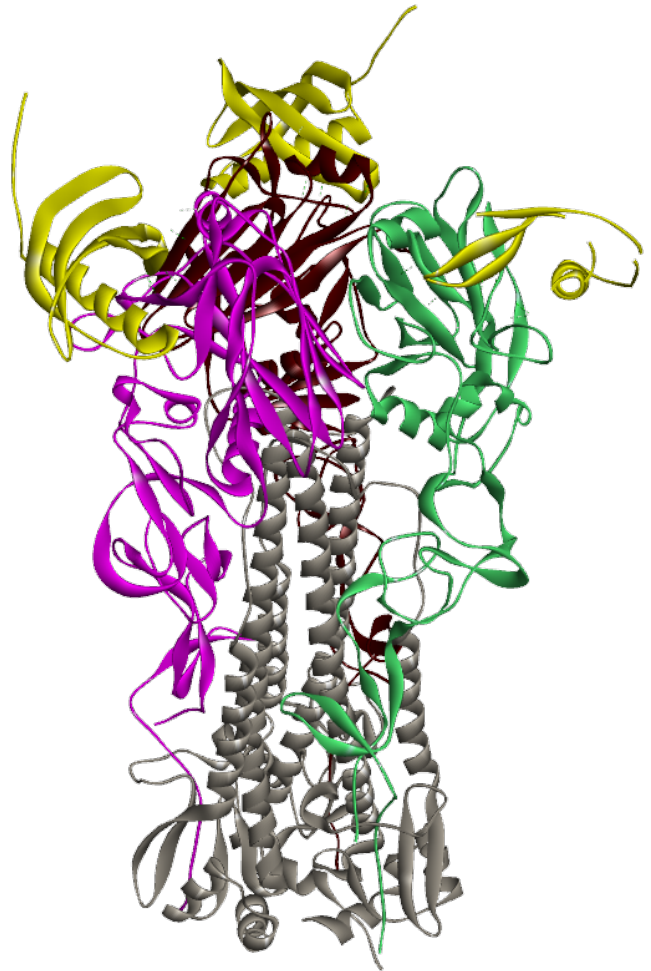
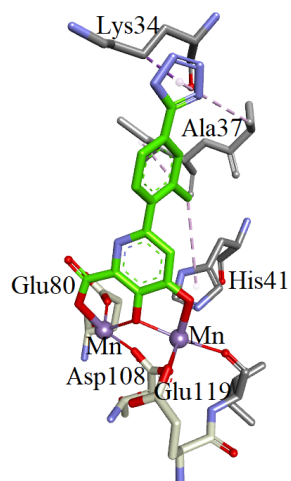
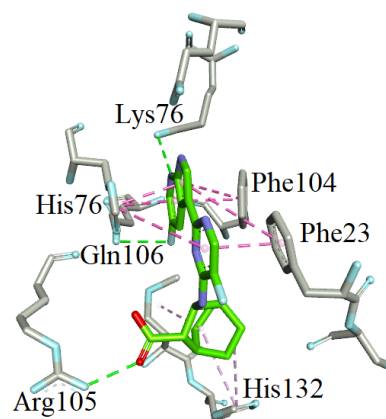
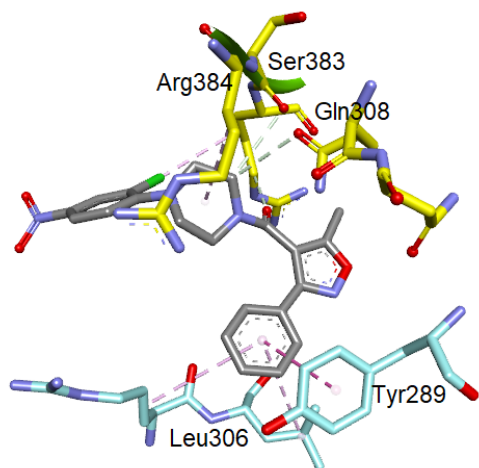
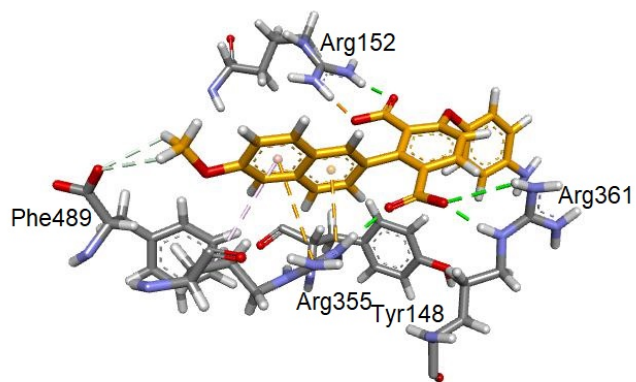
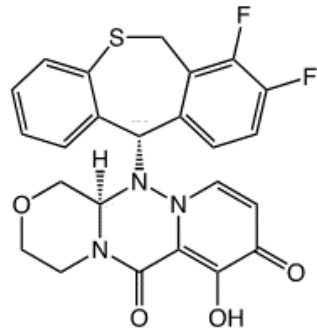
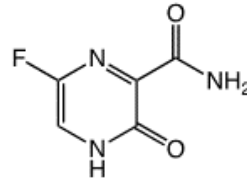


Figure 4

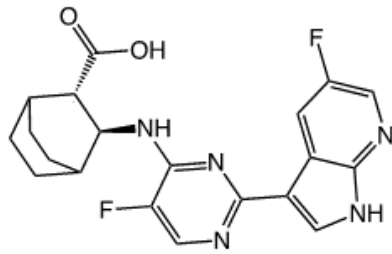
A**B****C****D****Figure 5**



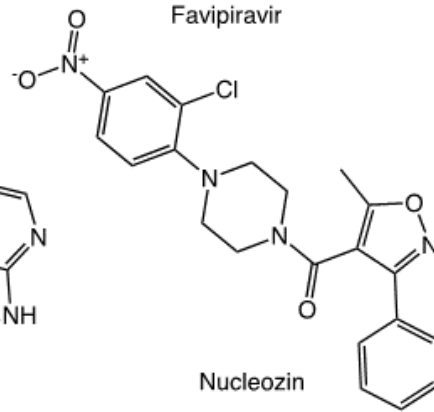
Baloxavir



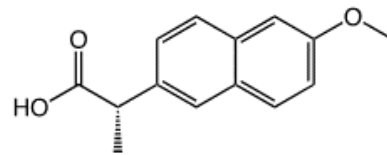
Favipiravir



Pimodivir



Nucleozin



Naproxen