Mini-Reviews in Medicinal Chemistry, 2025, *25*, 1463-1473

MINI-REVIEW ARTICLE

Small Molecule Influenza Virus Fusion Inhibitors Targeting Viral Hemagglutinin: Chemical Insights and Antiviral Evaluation

Gözde Çınar [1,2,*], Mahmut Can Tekin[3] and Gökçe Cihan Üstündağ[3]

[1]*Institute of Health Sciences, Istanbul University, Istanbul 34126, Türkiye;* [2]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Istanbul Health and Technology University, Istanbul 34275, Türkiye;* [3]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Istanbul University, Istanbul 34116, Türkiye*

ARTICLE HISTORY

Received: April 30, 2025
Revised: August 05, 2025
Accepted: August 07, 2025

DOI:
10.2174/0113895575401074250923075239

Abstract: Influenza viruses are major human pathogens that cause widespread respiratory infections, affecting millions of people globally and contributing to significant morbidity and mortality. Several currently available anti-influenza drugs are facing increasing levels of viral resistance. Therefore, the discovery of therapeutics targeting novel mechanisms of action is becoming increasingly important. A key viral protein involved in the infection process is the envelope glycoprotein Hemagglutinin (HA), which facilitates both host cell receptor binding and membrane fusion, two essential steps required for viral entry and replication. Due to its central role in the early stages of infection, HA has emerged as a highly promising target for antiviral drug development. Many small-molecule HA inhibitors have been identified with potential anti-influenza activity by stabilizing the HA structure and preventing its conformational change during the membrane fusion process. This review presents a detailed chemical evaluation of these HA-targeting compounds based on studies reported in the literature, highlighting their core chemical scaffolds and structural features. The antiviral efficacy of these compounds is discussed based on *in vitro* and *in vivo* data, along with insights into their mechanisms of action. A comprehensive literature search was conducted, and studies meeting the predefined inclusion criteria were thoroughly reviewed. By focusing on the chemical structure of these inhibitors, this review provides information for the rational design of new therapeutic agents aimed at preventing or limiting influenza virus infections.

Keywords: Influenza virus, antiviral evaluation, anti-influenza, Hemagglutinin, polyphenols, zanamivir.

1. INTRODUCTION

Influenza viruses are single-stranded RNA viruses, enclosed in an envelope, and belong to the *Orthomyxoviridae* family, which is classified into four genera: Influenza A, B, C, and D. Seasonal influenza is a major health problem caused by influenza viruses that circulate widely around the world, leading to 3 to 5 million cases of severe illness and between 290,000 and 650,000 respiratory deaths annually [1]. Influenza viruses cause seasonal epidemics every year and occasional global pandemics with significant levels of morbidity and mortality. While influenza A and B viruses are responsible for seasonal epidemics, only the Influenza A Virus (IAV) is known to cause pandemics. In the 20th and 21st centuries, IAVs have led to four pandemics (1918, 1957, 1968, 2009) [2]. These pandemics emerge as a result of new viruses forming after antigenic shift [3, 4]. Vaccination remains the best defense against seasonal influenza virus outbreaks and is the primary strategy to prevent the virus's spread, while antivirals provide a complementary approach to combat infections, especially for individuals who are unable to be vaccinated. However, currently available seasonal influenza vaccines fail to provide long-lasting and broadly protective immunity and lack efficacy against drifted or pandemic influenza strains [5]. Therefore, discovering new antiviral strategies has become increasingly important. In this regard, plants could be a supportive therapeutic option in the fight against influenza pandemics, offering complementary benefits through natural compounds such as polyphenols, flavonoids, and essential oils, which have demonstrated significant antiviral activity by targeting both the structure of viruses and the processes of viral biosynthesis, in addition to the host response [6, 7]. Additionally, plant-derived immune adjuvants, recognized for their low toxicity, high stability, and cost-effectiveness, also demonstrate significant potential to enhance vaccine efficacy and provide new avenues for future vaccine development [8].

Hemagglutinin (HA) and Neuraminidase (NA) are two key glycoproteins found on the surface of both Influenza A

*Address correspondence to this author at the Institute of Health Sciences, Istanbul University, Istanbul 34126, Türkiye;
E-mail: gozde.cinar@istun.edu.tr

1875-5607/25 $65.00+.00

and Influenza B viruses, both of which play crucial roles in the processes of viral entry and release. HA is responsible for viral entry by attaching to receptors on the host cell and triggering fusion between the viral and cellular membranes, while NA plays a role in the release of newly formed virions. In addition to these glycoproteins, the viral envelope contains the matrix 2 (M2) protein, which plays a crucial role in regulating pH across the viral membrane during entry into the host cell and across the trans-Golgi network during viral maturation [9].

Currently, three classes of anti-influenza drugs have been approved by the Food and Drug Administration (FDA), targeting the viral proteins NA (oseltamivir, zanamivir, peramivir, and laninamivir), the M2 ion channel (amantadine and rimantadine), and the viral polymerase complex (baloxavir marbil and favipiravir) [10]. Oseltamivir, zanamivir, peramivir, laninamivir, and baloxavir marboxil are effective against influenza A and B viruses; amantadine and rimantadine target only influenza A, while favipiravir is active against influenza A, B, C, and other RNA viruses [11, 12]. Due to the high prevalence of resistance among present Influenza A strains, the use of M2 ion channel blockers is no longer considered effective or recommended [13]. Although NA inhibitors remain the standard of care in most countries, their effectiveness is limited by a short therapeutic window and the potential emergence of resistant viral strains [14]. Moreover, following its approval in Japan in 2018, baloxavir rapidly led to the emergence of resistant IAV strains during the first influenza season [15]. These facts underscore the importance of developing new anti-influenza treatments that target distinct viral pathways.

An attractive antiviral strategy is to prevent initial viral entry into the host cell, a process in which HA plays a key role. Many small-molecule HA inhibitors have been identified as having potential anti-influenza activity by stabilizing the HA structure and preventing its conformational change during the membrane fusion process. As highlighted by the recent COVID-19 pandemic, the urgent need for broad-spectrum antivirals that can be rapidly deployed against emerging respiratory viruses has become even more apparent. In this regard, small molecule HA inhibitors may serve as a valuable addition to pandemic preparedness strategies, offering a complementary approach alongside vaccines and other antiviral agents. In this review paper, we focus on the preclinical studies and Structure-Activity Relationships (SAR) of small molecule fusion inhibitors targeting the influenza virus surface protein HA.

2. THE ROLE OF HEMAGGLUTININ IN VIRAL ENTRY: STRUCTURE AND FUNCTION

The influenza virus Hemagglutinin (HA) is a homotrimeric membrane glycoprotein that mediates viral entry and belongs to the class I fusion proteins. Proteolytic cleavage of HA by host proteases generates the HA1 and HA2 subunits, which are covalently linked by disulfide bonds [16]. The mature HA1-HA2 complex involves two domains: the globular head domain and the stem domain [17]. HA1 forms the head domain, which binds to host cell receptors, whereas HA2 makes up the stem region, playing a key role in membrane fusion during viral infection [18]. The low pH inside the maturing endosome leads to a series of conformational changes of HA, including the exposure and release of the fusion peptide subdomain of HA2 from the inner pocket. As a result, low pH causes the fusion of the viral membrane and the endosomal membrane [19]. The fusion pore formed allows the release of viral ribonucleoproteins into the cytosol, leading to the completion of the viral entry step [20]. The HA head region exhibits high genetic variability driven by immune selection, in contrast to the stem domain, which is relatively stable and therefore considered a valuable target in vaccine and antiviral research [21].

The HA surface antigens are classified into 18 subtypes, each showing significant differences in both sequence and antigenic profiles. These 18 antigenic subtypes of HA are divided into two groups: Group 1 and Group 2. HA subtypes are classified into two groups: Group 1 includes HA subtypes H1, H2, H5, H8, H9, H11, H12, H13, H16, H17, and H18, while Group 2 includes subtypes H3, H4, H7, H10, H14, and H15 [22]. There are significant structural differences in the HA proteins of influenza virus subtypes responsible for past pandemics, such as the 1918 H1N1 Spanish influenza [23], 1957 H2N2 Asian influenza [24], 2009 H1N1 swine-origin pandemic [25], as well as in emerging subtypes of concern to human health, including H5N1 [26], H6N1 [27], H7N9 [28] and H10N8 [29]. Infection with the H5N1 and H7N9 subtypes can result in severe respiratory disease in humans, characterized by high mortality rates; fortunately, to date, these viruses have not caused sustained human-to-human transmission [30, 31].

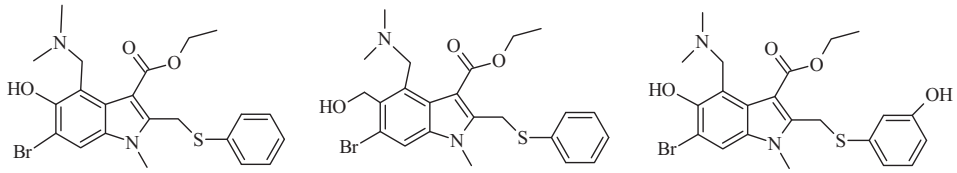
Although HA is more abundant than Neuraminidase (NA) on the virus surface and is the primary target for the immune response, NA remains the key target for small-molecule anti-influenza drugs such as oseltamivir and zanamivir. This is because the NA active site is more accessible for drug targeting compared to the shallow receptor-binding site of HA [32]. Additionally, the development of HA inhibitors is complicated by the high rate of HA evolution and its high pleomorphism [33].

3. SMALL MOLECULE INHIBITORS OF HA-MEDIATED FUSION

The development of orally bioavailable small molecule fusion inhibitors, especially those targeting the conserved stem region of HA, has great value for the treatment of drug-resistant influenza infections. Several small-molecule inhibitors targeting the fusion machinery of the influenza virus have been developed. However, for many of these, development has been hindered by their group-specific or even subtype-specific activities. Aside from Arbidol, no small-molecule inhibitors targeting HA fusion have progressed to clinical trial stages [34]. In the ensuing subsections, we will briefly review the molecular structures, classify them based on chemical features, highlight common structural motifs, and summarize the *in vitro/in vivo* antiviral activity and mechanism of action studies of these influenza fusion inhibitors.

3.1. Arbidol and Its Derivatives

Arbidol (Umifenovir) is a broad-spectrum antiviral agent that has been approved for the prevention and treatment of



Arbidol **15** **11**

Fig. (1). Arbidol and its derivatives, compound **15** bearing 5-hydroxymethyl substitution on the indole ring and compound **11** featuring a *m*-hydroxy substitution on the phenyl ring.

***tert*-butylhydroquinone (TBHQ)** **3-*tert*-butyl-4-methoxyphenol**

Fig. (2). TBHQ and its 4-O-methylated derivative.

influenza in Russia and China, showing a favorable safety profile with no major adverse effects (Fig. **1**). Clinically proven to be an effective antiviral drug, it features a low side effect profile, high safety, and minimal potential for resistance, making it a promising candidate for clinical use [35]. Arbidol is the only influenza entry inhibitor currently available on the market that inhibits HA conformational changes triggered by acidic pH, which are essential for the fusion activity mediated by HA [36, 37]. Arbidol interacts with a hydrophobic pocket located at the interface of two protomers within the stem region of the HA trimer, thereby blocking the conformational changes required for membrane fusion under the acidic conditions of the endosome. This unique mode of action sheds light on the design of new agents targeting HA [38]. A recent computational study demonstrated that Arbidol exhibits the highest affinity to H7 among 16 HA subtypes [39]. Arbidol not only suppresses HA-mediated fusion of influenza viruses but also exhibits antiviral activity against a broad range of viruses, acting at various stages, including viral attachment, internalization, and replication [40, 41]. *In vitro* studies revealed that arbidol effectively inhibited various influenza A strains (H1N1, H3N2, H9N2) with IC_{50} values ranging from 4.4 to 12.1 μM, demonstrating its ability by blocking HA-mediated hemolysis at concentrations of 3.91–15.63 μg/mL [42]. Arbidol was also found to be effective *in vivo* against two influenza A H1N1 strains, responsible for seasonal and pandemic flu, by significantly reducing mortality, alleviating virus-induced lung lesions, and decreasing viral titers in infected mice treated with doses of 90 and 180 $mg \cdot kg^{-1}d^{-1}$ [43].

Brancato *et al.* designed and synthesized two series of indole derivatives structurally related to Arbidol, and compound **15** indicated a greater therapeutic index than Arbidol for most of the influenza viruses tested. However, unlike arbidol, which targets both groups of HAs, compound **15** selectively targeted group 2 HAs (Fig. **1**) [44]. Wright *et al.* synthesized a series of Arbidol analogues in another study, identifying compound **11,** containing a *meta*-hydroxy group on its aromatic ring, as having increased affinity for both H3 and H1 HA subtypes (Fig. **1**) [45].

3.2. *tert*-Butylhydroquinone (TBHQ)

A well-known molecule, *tert*-butylhydroquinone, is commonly used as a synthetic food antioxidant because of its anti-lipid peroxidation activity (Fig. **2**) [46]. This small molecule has also been shown to inhibit both the acid-induced conformational change of H3 subtype HA and viral infectivity in cell culture at low micromolar concentrations (5-10 μM) [47-49]. Cocrystallization of H3 HA with TBHQ demonstrated that the compound bound in a hydrophobic interface between HA monomers, and only group 2 HAs were sensitive to TBHQ [50]. Antanasijevic *et al.* showed that TBHQ's antioxidant properties were not responsible for the inhibition of HA-mediated entry. In addition, structure-activity relationship studies demonstrated that the 3-*tert*-butyl-4-methoxyphenol derivative exhibited enhanced potency against H7 HA (IC_{50} = 6 μM), decreased toxicity, and increased stability (Fig. **2**) [51].

3.3. BMY-27709 and Salicylamide Derivatives

BMY-27709 is a salicylamide derivative, characterized by an amide bond linking an aromatic ring system to an aliphatic cyclic structure (Fig. **3**). The compound was recognized as an effective inhibitor of H1 and H2 subtypes of IAVs [52], and further studies confirmed that its antiviral effect results from targeted binding to the HA protein [53]. Yu *et al.* synthesized new quinolizidine salicylamides, and among the new series, compound **16** emerged as the most potent fusion inhibitor of the influenza A/WSN/33 strain (H1N1 subtype) with an EC_{50} of 0.25 μg/mL, which was fivefold more potent than BMY-27709 (Fig. **3**) [54]. SAR analysis of BMY-27709 and its derivatives highlighted the importance of the quinolizidine stereochemistry and aromatic ring substitution patterns for antiviral efficacy.

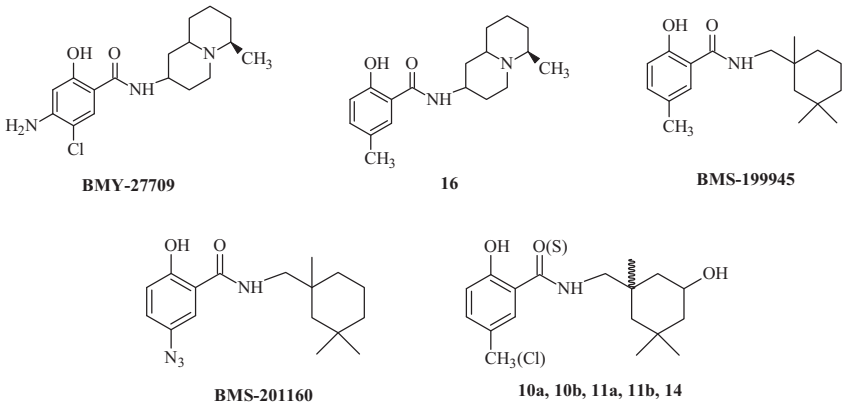


Fig. (3). Salicylamide derivatives exhibit structural similarity based on a salicylamide core; variations mainly occur in the side chains attached to the amide nitrogen or phenyl ring, but the salicylamide scaffold is conserved across the series.

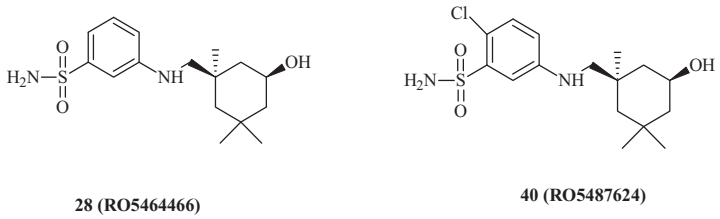


Fig. (4). Benzensulfonamide Derivatives **RO5464466** and its phenyl-chlorinated analogue **RO5487624**.

As a continuation of previous studies, the structurally related BMS-199945 and BMS-201160 were designed and synthesized by replacing the cyclic quinolizidine structure with a cyclohexane ring [55]. BMS-199945 and BMS-201160 were found to be more potent fusion inhibitors than BMY-27709 (Fig. **3**). Compounds were able to inhibit the low-pH-induced conformational change of isolated HA trimers, as detected by resistance to digestion with trypsin. The IC_{50} values for influenza A/WSN/33 virus-induced red blood cell hemolysis were 7, 0.57, and 1.1 μM for BMY-27709, BMS-199945, and BMS-201160, respectively [55]. Furthermore, SAR on BMY-27709 and its derivatives led to the design and synthesis of a series of salicylamide and thioamides, compounds **10a**, **10b**, **11a**, **11b,** and **14** (Fig. **3**). The most effective inhibitors exhibited EC_{50} values ranging from 0.02 to 0.14 μg/mL in cell culture against the H1 subtype of influenza A viruses. Hemolysis assay data (IC_{50s} = 0.025–0.44 μg/mL) confirmed the fusion inhibitory activity of these compounds [56].

3.4. Benzensulfonamide Derivatives

Structural optimization of salicylamide-based HA inhibitors resulted in the identification of benzenesulfonamide **28** (RO5464466) and its 2-chloro analogue **40** (RO5487624) as potent anti-influenza agents (Fig. **4**). RO5464466 and RO5487624 prevented the cytopathic effects caused by the infection of influenza A/Weiss/43 strain (H1N1) with EC_{50} values of 210 and 86 nM, respectively. F- or Cl- substitution on the aromatic ring showed a 3-5-fold increase in inhibitory potency, while extra substitution groups such as methyl, methoxy, and CF_3 led to reduced anti-influenza activities. Mechanism of action studies indicated that benzensulfonamides inhibited the virus fusion by binding to HA and stabilizing the prefusion HA structure [57]. RO5487624, which exhibited pharmacokinetics compatible with *in vivo* testing, conferred protection to mice exposed to an H1N1 virus [57, 58].

3.5. JNJ4796 and Benzylpiperazine Derivatives

JNJ4796, a small molecule fusion inhibitor that mimics the binding and functionality of a broadly neutralizing antibody, was discovered by researchers from Janssen Pharma and the Scripps Institute (Fig. **5**). Benzylpiperazines have emerged as a promising class of fusion inhibitors, with JNJ7918 (Fig. **5**) being the lead candidate, exhibiting IC_{50} values of 1.39 and 13.06 μM against H1N1 A/California/07/2009 and H5N1 A/Vietnam/1203/2004 HAs, respectively. Key chemical modifications to enhance its drug-like properties and increase molecular interactions with the HA stem led to the development of JNJ4796, which exhibited a favorable *in vivo* pharmacokinetics profile. JNJ4796 was found to effectively neutralize a wide range of influenza A group 1 viruses *in vitro,* inhibit the pH-sensitive conformational change of HA, and shield mice from both lethal and non-lethal influenza after oral dosing. The crystal structure of JNJ4796, in complex with H1N1 A/Solomon Islands/3/2006 and H5N1 A/Vietnam/1203/2004 hemagglutinins, revealed its interaction with a conserved hydrophobic groove at the HA1/HA2 interface in the HA stem [59].

Wang *et al.* designed new molecules by modifying the piperazine and phenyl rings of JNJ4796, and the

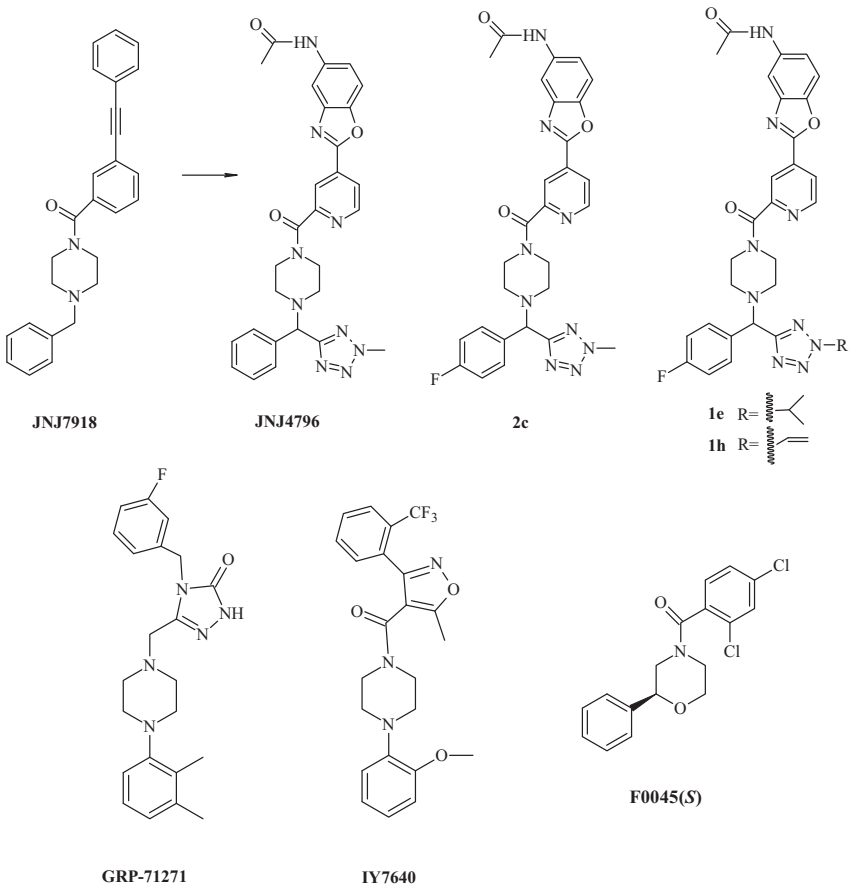


Fig. (5). Common structural feature of **JNJ4796** and its derivatives, which contain a piperazine ring (except for **F0045** with a morpholine ring) as the core scaffold, with side chains connected to the aromatic ring *via* a carbonyl or methyl group.

4-fluorophenyl analogue 2c showed *in vitro* activity against IAV H1N1 and oseltamivir-resistant IAV H1N1 strains (**with** IC_{50} values of 0.03–0.06 μM), comparable to JNJ4796 (Fig. **5**). Compound **2c** displayed lower cytotoxicity and better oral pharmacokinetic profiles than JNJ4796 [60]. In another study conducted by the same research group, the SAR of the benzoxazole and tetrazole rings of JNJ4796 was discussed. The *in vitro* profiles of (*R*)-1e and (*R*)-1h were highly favorable, with strong activity and low hERG inhibition, yet their oral performance was inferior to that of the lead molecule (Fig. **5**) [61].

Two chemically related compounds with phenylpiperazine structure, GRP-71271 and IY7640, were identified as fusion inhibitors by two different research groups (Fig. **5**). GRP-71271 was active against the H1N1 and H2N2 test strain [62]. IY7640, at concentrations below 1 μM, effectively inhibited multiple H1N1 strains, including pH1N1 (2009 pandemic) and oseltamivir-resistant variants, and conferred protection to mice against lethal infection [63]. Both of the compounds were predicted to target the stalk region of the HA protein. In an escape mutant analysis of IY7640 in cells, amino acid mutations were identified at the HA stalk region of the pH1N1 virus [63].

F0045(*S*) was identified through a competitive fluorescence polarization assay, which tested the binding interaction between a cyclic HR2-mimicking peptide (P7-TAMRA) and the stem region of H1/PR8 HA (Fig. **5**). It demonstrated strong *in vitro* inhibition against various H1N1 strains and an H5N1 pseudovirus, with IC_{50} values ranging from 1.6 to 22.8 μM. Co-crystal structures and biochemical evidence demonstrated that F0045(*S*) mimics the action of JNJ4796 by binding to the hydrophobic groove between HA1 and HA2 subunits, stabilizing the HA prefusion state, and preventing the conformational changes necessary for fusion with the host cell membrane [64].

3.6. MBX2329 and MBX2546

Basu *et al.* performed High-Throughput Screening (HTS) of a library containing more than 100,000 small molecules to identify potential inhibitors of influenza virus entry. From this screen, two potent compounds, MBX2329 and MBX2546, were selected for further study (Fig. **6**). MBX2329 and MBX2546 inhibited different influenza A H1N1 strains, including the oseltamivir-resistant strain and 2009 pandemic strain, with IC_{50}s ranging from 0.29 to 5.8 μM. Both MBX2546 and MBX2329 inhibited the A/Hong Kong/H5N1 virus strain with IC_{50}s of 3.6 and 5.9 μM, respectively. The compounds exhibited high Selectivity İndex (SI) values and large volumes of synergy with oseltamivir. According to mechanism of action studies, both compounds attach to the stem region of the HA trimer and inhibit the fusion process mediated by HA [65].

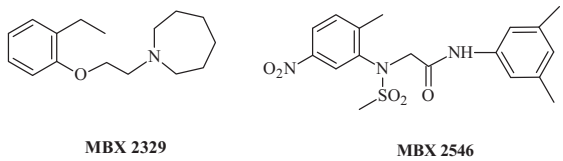


Fig. (6). MBX2329 and MBX2546.

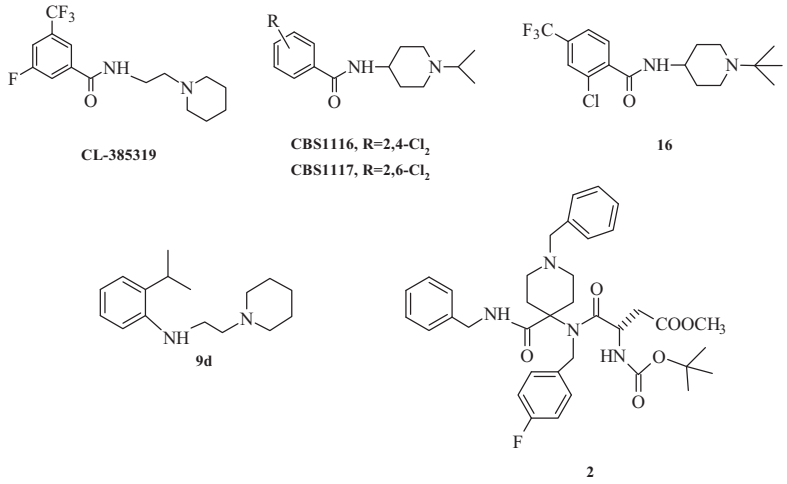


Fig. (7). Piperidine derivatives.

3.7. Piperidine Derivatives

CL-385319, bearing an *N*-[2-(piperidin-1-yl)ethyl]benzamide core structure, inhibited the infectivity of several H1 and H2 influenza A viruses (Fig. **7**). Inhibition appeared to result from the compound's ability to block the fusogenic function of the HA [66]. Liu *et al.* found that CL-385319 was potent against H5N1 influenza A virus infection in Madin-Darby Canine Kidney (MDCK) cells, with an IC_{50} of 27.03±2.54 μM and low cytotoxicity (CC_{50} = 1.48±0.01 mM) [67]. Subsequent studies identified the critical binding residues for the compound, which clustered in the stem region of the HA trimer. It was found that the recognition and binding of the compound to HA occurred through an 'induced fit' pathway in which the binding pocket was formed during their interaction [68].

Hussein *et al.* reported the identification of novel piperidine derivatives, among which CBS1116 (2,4-dichloro-N-(1-isopropyl-4-piperidinyl)benzamide) specifically inhibited the infection of H1N1 and H5N1 IAVs of group 1 in A549 and MDCK cells by disrupting the HA-mediated membrane fusion mechanism (Fig. **7**). Advanced Structure–Activity Relationship (SAR) investigations led to the identification of CBS1117 (2,6-dichloro-*N*-(1-isopropyl-4-piperidinyl) benzamide) as a highly potent inhibitor (Fig. **7**). This compound demonstrated a low IC_{50} of 0.07 μM, a high CC_{50} of 274.3 μM, and an improved Selectivity Index (SI) of approximately 4000, indicating reduced cytotoxicity and strong antiviral potential [69]. Furthermore, structural and biochemical analyses, including X-ray crystallography, NMR spectroscopy, and mutagenesis, revealed its interaction with avian H5 Hemagglutinin (HA), providing novel insights into its mechanism of action and subtype-specific activity [70].

Gaisina *et al.* described the synthesis of similar acylated 4-aminopiperidines, and the compounds were shown to be influenza A inhibitors *in vitro* with high potency (EC_{50} < 1 μM) and low cytotoxicity (SI > 100). Molecular modeling indicated that these ligands are capable of establishing multiple interactions within a distinct hydrophobic pocket on HA, a region crucial for membrane fusion during viral entry. The combination of the most effective compound of the series, compound **16,** with the NA inhibitor oseltamivir resulted in synergistic antiviral activity (Fig. **7**) [71].

Two novel aniline series were synthesized and evaluated as antivirals, with several showing low micromolar EC_{50} against influenza A/H1N1. Lead compound **9d,** a piperidine derivative, disrupted HA-mediated fusion by binding to the HA stem and blocking its low pH refolding (Fig. **7**). Molecular dynamics and virus resistance studies indicated that the binding site of **9d** overlapped with the pocket targeted by TBHQ, arbidol, and other H3 HA-specific inhibitors, underlining the relevance of this domain in drug design [72]. A few years later, De Castro *et al.* synthesized and evaluated novel N-benzyl-4,4-disubstituted piperidines, and several showed low micromolar potency against A/H1N1 (A/PR/8/34). Mechanistic studies identified compound **2** as a novel H1 HA-specific membrane fusion inhibitor **(**Fig. **7**) [73].

Compound **2a,** a camphene-like compound containing a pyrrolidine ring, showed an EC_{50} of 45.3 μM against H1N1 *in vitro*, and a molecular modeling study suggested that the compound could bind to both the TBHQ/arbidol region and a potential alternative binding site on the HA stem (Fig. **8**) [74]. Zhao *et al.* explored the SAR of pinanamine-based antivirals and identified **M090** as a potent inhibitor of aman-

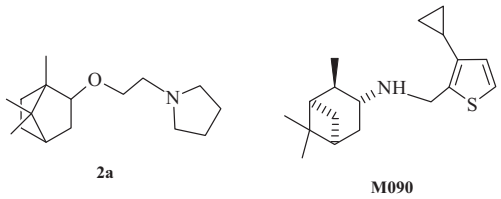


Fig. (8). Camphene-like derivative **2a** and pinanamine-based inhibitor **M090.**

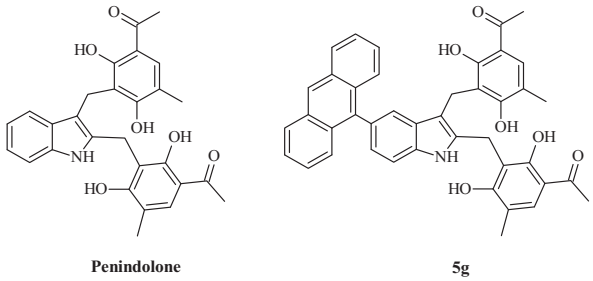


Fig. (9). Penindolone and its derivative bearing an anthracene group at the 5-position of the indole ring (**5g**).

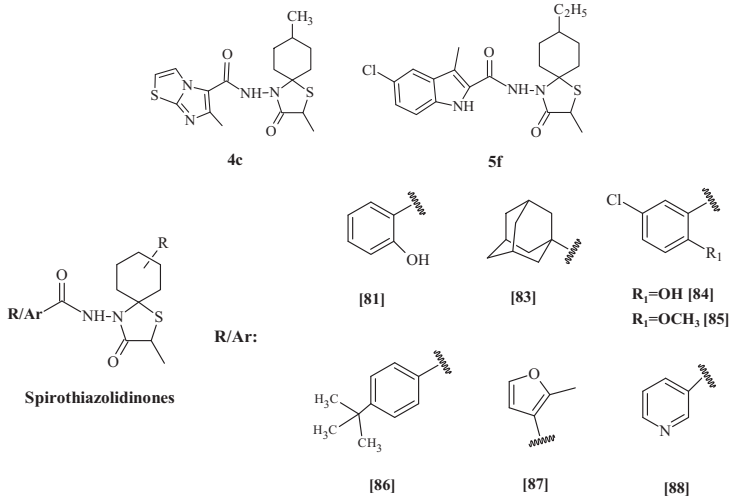


Fig. (10). Common structural feature of spirothiazolidinone derivatives, consisting of an aromatic or alicyclic ring connected to the spirothiazolidinone scaffold *via* an amide linker.

tadine- and oseltamivir-resistant H1N1 strains together with the H3N2 viral strain (Fig. **8**). Mechanistic studies demonstrated that M090 interacts with a highly conserved pocket in the HA2 domain, preventing virus-induced membrane fusion by "locking" HA2 in its bent conformation during rearrangement. It was also found that this domain is not fully group-specific, which may allow for the design of broad-spectrum anti-influenza drugs that target both phylogenetic groups of HAs [75]. A recent computational study examining the compound's effect on HA revealed the hydrophobic shielding effect of the 3-cyclopropylthiophene group in **M090** [76].

3.8. Penindolone and Derivatives

Penindolone (PND), representing a novel class of diclavatol-indole adducts, demonstrated strong anti-influenza A virus activity across various strains, coupled with a low resistance profile (Fig. **9**). Unlike existing anti-IAV drugs, PND features a unique scaffold and is the first inhibitor to target both the HA1 and HA2 subunits of viral hemagglutinin, effectively blocking both viral adsorption and membrane fusion [77]. To enhance bioactivity and better understand structure–activity relationships, a series of 65 PND derivatives was designed and synthesized. Their anti-IAV properties and ability to target Hemagglutinin (HA) were then evaluated. Among these, compound **5g** (Fig. **9**) demonstrated strong HA-binding affinity and surpassed the original PND in inhibiting HA-mediated membrane fusion [78].

3.9. Spirothiazolidinones

Spirocyclic scaffolds are frequently found in natural products and have garnered growing attention in drug discovery due to their unique three-dimensional structure and novel composition [79, 80]. Over the last twenty years, ef-

forts to discover new fusion inhibitors for Influenza A Virus (IAV) have led to the identification of several compounds featuring a spirothiazolidinone core (1-thia-4-azaspiro[4.5] decane) with potent activity against the A/H3N2 strain in cell-based assays. Mechanistic investigations of two lead candidates, **4c** and **5f**, demonstrated that they block the low pH-triggered conformational change of the H3 hemagglutinin (HA) protein (Fig. **10**) [81, 82]. Resistance selection experiments, combined with *in silico* predictions, suggest that the HA binding pocket of 4c and 5f overlaps with that of arbidol and TBHQ [81, 82].

Compounds 4c and 5f contain imidazo[2,1-b]thiazole and 5-chloro-3-methyl-1H-indole as their aromatic groups. Studies involving the synthesis of various analogues revealed that the anti-A/H3N2 activity persisted when the aromatic moiety was replaced with 1-adamantyl, substituted phenyl groups such as o-hydroxyphenyl, 5-chloro-2-hydroxyphenyl, 5-chloro-2-methoxyphenyl, 4-*tert*-butylphenyl, 2-methylfuran-3-yl, or pyridine-3-yl (Fig. **10**) [81-88].

CONCLUSION

Hemagglutinin, the envelope protein of influenza viruses, is essential for mediating viral adsorption and membrane fusion, thereby facilitating the virus's entry into host cells. While several anti-influenza drugs targeting the NA and M2 ion channel are available for clinical use, the frequent emergence of drug-resistant strains has heightened concerns about the potential appearance of novel multidrug-resistant influenza variants. Therefore, the development of anti-influenza drugs targeting new mechanisms has become a significant priority. The development of small-molecule inhibitors targeting the refolding of the HA membrane protein in influenza viruses has been a focus of research for over 20 years. Recent breakthroughs, particularly the discovery of broadly neutralizing anti-HA antibodies (bnAbs), have reignited interest in these inhibitors, with the goal of blocking HA-mediated fusion. The small molecules summarized in this review have shown promising activity against specific HA subtypes through various mechanisms of inhibiting hemagglutinin fusion (preventing the conformational changes of HA at low pH and binding to different regions of the HA stem region). These promising influenza virus inhibitors could complement NA inhibitors and M2 ion channel blockers by providing additional options for the treatment and prevention of severe respiratory infections caused by drug-resistant viruses and potential pandemic outbreaks.

AUTHORS' CONTRIBUTIONS

The authors confirm their contributions to the paper as follows: G.Ç. contributed to writing the original draft and collecting data, M.C.T. contributed to data collection, and G.C.Ü. contributed to writing, reviewing, and editing the manuscript. All authors reviewed the results and accept the final version of the manuscript.

LIST OF ABBREVIATIONS

CC_{50}	=	50% Cytotoxic Concentration
EC_{50}	=	Half Maximal Effective Concentration
FDA	=	Food and Drug Administration
HA	=	Hemagglutinin
IAV	=	Influenza A Virus
IC_{50}	=	Half Maximal Inhibitory Concentration
M2	=	Matrix 2
MDCK	=	Madin-Darby Canine Kidney
NA	=	Neuraminidase
PND	=	Penindolone
SI	=	Selectivity Index
TBHQ	=	*tert*-Butylhydroquinone

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

This work was supported by the Scientific Research Projects Coordination Unit of Istanbul University (Grant Number: THZ-2024-41094).

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

REFERENCES

[1] Influenza (seasonal) Fact Sheets. **2025**. Available from: https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal).

[2] Monto, A.S.; Fukuda, K. Lessons from influenza pandemics of the last 100 years. *Clin. Infect. Dis.,* **2020**, *70*(5), 951-957. http://dx.doi.org/10.1093/cid/ciz803 PMID: 31420670

[3] Smith, G.J.D.; Bahl, J.; Vijaykrishna, D.; Zhang, J.; Poon, L.L.M.; Chen, H.; Webster, R.G.; Peiris, J.S.M.; Guan, Y. Dating the emergence of pandemic influenza viruses. *Proc. Natl. Acad. Sci. USA,* **2009**, *106*(28), 11709-11712. http://dx.doi.org/10.1073/pnas.0904991106 PMID: 19597152

[4] Cheng, V.C.C.; To, K.K.W.; Tse, H.; Hung, I.F.N.; Yuen, K.Y. Two years after pandemic influenza A/2009/H1N1: What have we learned? *Clin. Microbiol. Rev.,* **2012**, *25*(2), 223-263. http://dx.doi.org/10.1128/CMR.05012-11 PMID: 22491771

[5] Becker, T.; Elbahesh, H.; Reperant, L.A.; Rimmelzwaan, G.F.; Osterhaus, A.D.M.E. Influenza vaccines: Successes and continuing challenges. *J. Infect. Dis.,* **2021**, *224*(12), S405-S419. http://dx.doi.org/10.1093/infdis/jiab269 PMID: 34590139

[6] Chon, H. Medicinal herbs and plant extracts for influenza: Bioactivity, mechanism of anti-influenza effects, and modulation of immune responses. In: *Studies in Natural Products Chemistry*; Elsevier: Amsterdam, **2012**; pp. 305-323. http://dx.doi.org/10.1016/B978-0-444-59530-0.00011-3

[7] Zhang, Z.J.; Morris-Natschke, S.L.; Cheng, Y.Y.; Lee, K.H.; Li, R.T. Development of anti-influenza agents from natural products. *Med. Res. Rev.,* **2020**, *40*(6), 2290-2338. http://dx.doi.org/10.1002/med.21707 PMID: 32677056

[8] Zou, M.; Lei, C.; Huang, D.; Liu, L.; Han, Y. Application of plant-derived products as adjuvants for immune activation and vaccine development. *Vaccine,* **2024**, *42*(25), 126115. http://dx.doi.org/10.1016/j.vaccine.2024.07.016 PMID: 38987109

[9] Pielak, R.M.; Chou, J.J. Influenza M2 proton channels. *Biochim. Biophys. Acta. Biomembr.,* **2011**, *1808*(2), 522-529. http://dx.doi.org/10.1016/j.bbamem.2010.04.015 PMID: 20451491

[10] O'Hanlon, R.; Shaw, M.L. Baloxavir marboxil: The new influenza drug on the market. *Curr. Opin. Virol.,* **2019**, *35*, 14-18. http://dx.doi.org/10.1016/j.coviro.2019.01.006 PMID: 30852344

[11] Meseko, C.; Sanicas, M.; Asha, K.; Sulaiman, L.; Kumar, B. Antiviral options and therapeutics against influenza: History, latest developments and future prospects. *Front Cell. Infect. Microbiol.,* **2023**, *13*, 1269344. http://dx.doi.org/10.3389/fcimb.2023.1269344 PMID: 38094741

[12] Bonomini, A.; Mercorelli, B.; Loregian, A. Antiviral strategies against influenza virus: An update on approved and innovative therapeutic approaches. *Cell. Mol. Life. Sci.,* **2025**, *82*(1), 75. http://dx.doi.org/10.1007/s00018-025-05611-1 PMID: 39945883

[13] Batool, S.; Chokkakula, S.; Song, M.S. Influenza treatment: Limitations of antiviral therapy and advantages of drug combination therapy. *Microorganisms,* **2023**, *11*(1), 183. http://dx.doi.org/10.3390/microorganisms11010183 PMID: 36677475

[14] Takashita, E.; Meijer, A.; Lackenby, A.; Gubareva, L.; Rebelo-de-Andrade, H.; Besselaar, T.; Fry, A.; Gregory, V.; Leang, S.K.; Huang, W.; Lo, J.; Pereyaslov, D.; Siqueira, M.M.; Wang, D.; Mak, G.C.; Zhang, W.; Daniels, R.S.; Hurt, A.C.; Tashiro, M. Global update on the susceptibility of human influenza viruses to neuraminidase inhibitors, 2013–2014. *Antiviral Res.,* **2015**, *117*, 27-38. http://dx.doi.org/10.1016/j.antiviral.2015.02.003 PMID: 25721488

[15] Takashita, E.; Kawakami, C.; Morita, H.; Ogawa, R.; Fujisaki, S.; Shirakura, M.; Miura, H.; Nakamura, K.; Kishida, N.; Kuwahara, T.; Mitamura, K.; Abe, T.; Ichikawa, M.; Yamazaki, M.; Watanabe, S.; Odagiri, T. Detection of influenza A(H3N2) viruses exhibiting reduced susceptibility to the novel cap-dependent endonuclease inhibitor baloxavir in Japan, December 2018. *Euro. Surveill,* **2019**, *24*(3), 1800698. http://dx.doi.org/10.2807/1560-7917.ES.2019.24.3.1800698 PMID: 30670142

[16] Liu, H.Y.; Yang, P.L. Small-molecule inhibition of viral fusion glycoproteins. *Annu. Rev. Virol.,* **2021**, *8*(1), 459-489. http://dx.doi.org/10.1146/annurev-virology-022221-063725 PMID: 34197186

[17] Skehel, J.J.; Wiley, D.C. Receptor binding and membrane fusion in virus entry: The influenza hemagglutinin. *Annu. Rev. Biochem.,* **2000**, *69*(1), 531-569. http://dx.doi.org/10.1146/annurev.biochem.69.1.531 PMID: 10966468

[18] White, K.; Esparza, M.; Liang, J.; Bhat, P.; Naidoo, J.; McGovern, B.L.; Williams, M.A.P.; Alabi, B.R.; Shay, J.; Niederstrasser, H.; Posner, B.; García-Sastre, A.; Ready, J.; Fontoura, B.M.A. Aryl sulfonamide inhibits entry and replication of diverse influenza viruses *via* the hemagglutinin protein. *J. Med. Chem.,* **2021**, *64*(15), 10951-10966. http://dx.doi.org/10.1021/acs.jmedchem.1c00304 PMID: 34260245

[19] Hamilton, B.S.; Whittaker, G.R.; Daniel, S. Influenza virus-mediated membrane fusion: Determinants of hemagglutinin fusogenic activity and experimental approaches for assessing virus fusion. *Viruses,* **2012**, *4*(7), 1144-1168. http://dx.doi.org/10.3390/v4071144 PMID: 22852045

[20] Sempere Borau, M.; Stertz, S. Entry of influenza A virus into host cells — Recent progress and remaining challenges. *Curr. Opin. Virol.,* **2021**, *48*, 23-29. http://dx.doi.org/10.1016/j.coviro.2021.03.001 PMID: 33838498

[21] Chen, G.L.; Subbarao, K. Attacking the flu: Neutralizing antibodies may lead to 'universal' vaccine. *Nat. Med.,* **2009**, *15*(11), 1251-1252. http://dx.doi.org/10.1038/nm1109-1251 PMID: 19893556

[22] Kirkpatrick, E.; Qiu, X.; Wilson, P.C.; Bahl, J.; Krammer, F. The influenza virus hemagglutinin head evolves faster than the stalk domain. *Sci. Rep,* **2018**, *8*(1), 10432. http://dx.doi.org/10.1038/s41598-018-28706-1 PMID: 29992986

[23] Gamblin, S.J.; Haire, L.F.; Russell, R.J.; Stevens, D.J.; Xiao, B.; Ha, Y.; Vasisht, N.; Steinhauer, D.A.; Daniels, R.S.; Elliot, A.; Wiley, D.C.; Skehel, J.J. The structure and receptor binding properties of the 1918 influenza hemagglutinin. *Science,* **2004**, *303*(5665), 1838-1842. http://dx.doi.org/10.1126/science.1093155 PMID: 14764886

[24] Liu, J.; Stevens, D.J.; Haire, L.F.; Walker, P.A.; Coombs, P.J.; Russell, R.J.; Gamblin, S.J.; Skehel, J.J. Structures of receptor complexes formed by hemagglutinins from the Asian Influenza pandemic of 1957. *Proc. Natl. Acad. Sci. USA,* **2009**, *106*(40), 17175-17180. http://dx.doi.org/10.1073/pnas.0906849106 PMID: 19805083

[25] Zhang, W.; Qi, J.; Shi, Y.; Li, Q.; Gao, F.; Sun, Y.; Lu, X.; Lu, Q.; Vavricka, C.J.; Liu, D.; Yan, J.; Gao, G.F. Crystal structure of the swine-origin A (H1N1)-2009 influenza A virus hemagglutinin (HA) reveals similar antigenicity to that of the 1918 pandemic virus. *Protein Cell.,* **2010**, *1*(5), 459-467. http://dx.doi.org/10.1007/s13238-010-0059-1 PMID: 21203961

[26] Stevens, J.; Blixt, O.; Tumpey, T.M.; Taubenberger, J.K.; Paulson, J.C.; Wilson, I.A. Structure and receptor specificity of the hemagglutinin from an H5N1 influenza virus. *Science,* **2006**, *312*(5772), 404-410. http://dx.doi.org/10.1126/science.1124513 PMID: 16543414

[27] Tzarum, N.; de Vries, R.P.; Zhu, X.; Yu, W.; McBride, R.; Paulson, J.C.; Wilson, I.A. Structure and receptor binding of the hemagglutinin from a human H6N1 influenza virus. *Cell. Host Microbe.,* **2015**, *17*(3), 369-376. http://dx.doi.org/10.1016/j.chom.2015.02.005 PMID: 25766295

[28] Shi, Y.; Zhang, W.; Wang, F.; Qi, J.; Wu, Y.; Song, H.; Gao, F.; Bi, Y.; Zhang, Y.; Fan, Z.; Qin, C.; Sun, H.; Liu, J.; Haywood, J.; Liu, W.; Gong, W.; Wang, D.; Shu, Y.; Wang, Y.; Yan, J.; Gao, G.F. Structures and receptor binding of hemagglutinins from human-infecting H7N9 influenza viruses. *Science,* **2013**, *342*(6155), 243-247. http://dx.doi.org/10.1126/science.1242917 PMID: 24009358

[29] Wang, M.; Zhang, W.; Qi, J.; Wang, F.; Zhou, J.; Bi, Y.; Wu, Y.; Sun, H.; Liu, J.; Huang, C.; Li, X.; Yan, J.; Shu, Y.; Shi, Y.; Gao, G.F. Structural basis for preferential avian receptor binding by the human-infecting H10N8 avian influenza virus. *Nat. Commun,* **2015**, *6*(1), 5600. http://dx.doi.org/10.1038/ncomms6600 PMID: 25574798

[30] Sutton, T.C. The pandemic threat of emerging H5 and H7 avian influenza viruses. *Viruses,* **2018**, *10*(9), 461. http://dx.doi.org/10.3390/v10090461 PMID: 30154345

[31] Harrington, W.N.; Kackos, C.M.; Webby, R.J. The evolution and future of influenza pandemic preparedness. *Exp. Mol. Med.,* **2021**, *53*(5), 737-749. http://dx.doi.org/10.1038/s12276-021-00603-0 PMID: 33953324

[32] Wu, N.C.; Wilson, I.A. Structural insights into the design of novel anti-influenza therapies. *Nat. Struct. Mol. Biol.,* **2018**, *25*(2), 115-121. http://dx.doi.org/10.1038/s41594-018-0025-9 PMID: 29396418

[33] Kilbourne, E.D.; Johansson, B.E.; Grajower, B. Independent and disparate evolution in nature of influenza A virus hemagglutinin and neuraminidase glycoproteins. *Proc. Natl. Acad. Sci. USA,* **1990**, *87*(2), 786-790. http://dx.doi.org/10.1073/pnas.87.2.786 PMID: 2300562

[34] Chen, Z.; Cui, Q.; Caffrey, M.; Rong, L.; Du, R. Small Molecule Inhibitors of Influenza Virus Entry. *Pharmaceuticals,* **2021**, *14*(6), 587. http://dx.doi.org/10.3390/ph14060587 PMID: 34207368

[35] Kang, Y.; Shi, Y.; Xu, S. Arbidol: The current demand, strategies, and antiviral mechanisms. *Immun. Inflamm. Dis.,* **2023**, *11*(8), 984. http://dx.doi.org/10.1002/iid3.984 PMID: 37647451

[36] Leneva, I.A.; Russell, R.J.; Boriskin, Y.S.; Hay, A.J. Characteristics of arbidol-resistant mutants of influenza virus: Implications for the mechanism of anti-influenza action of arbidol. *Antiviral Res.,* **2009**, *81*(2), 132-140. http://dx.doi.org/10.1016/j.antiviral.2008.10.009 PMID: 19028526

[37] Boriskin, Y.; Leneva, I.; Pécheur, E.I.; Polyak, S. Arbidol: A broad-spectrum antiviral compound that blocks viral fusion. *Curr. Med. Chem.,* **2008**, *15*(10), 997-1005. http://dx.doi.org/10.2174/092986708784049658 PMID: 18393857

[38] Kadam, R.U.; Wilson, I.A. Structural basis of influenza virus fusion inhibition by the antiviral drug Arbidol. *Proc. Natl. Acad. Sci. USA,* **2017**, *114*(2), 206-214. http://dx.doi.org/10.1073/pnas.1617020114 PMID: 28003465

[39] Ahmed, A.A.; Abouzid, M. Arbidol targeting influenza virus A Hemagglutinin; A comparative study. *Biophys. Chem.,* **2021**, *277*, 106663.

http://dx.doi.org/10.1016/j.bpc.2021.106663 PMID: 34388678

[40] Blaising, J.; Polyak, S.J.; Pécheur, E.I. Arbidol as a broad-spectrum antiviral: An update. *Antiviral Res.,* **2014**, *107*, 84-94. http://dx.doi.org/10.1016/j.antiviral.2014.04.006 PMID: 24769245

[41] Fink, S.L.; Vojtech, L.; Wagoner, J.; Slivinski, N.S.J.; Jackson, K.J.; Wang, R.; Khadka, S.; Luthra, P.; Basler, C.F.; Polyak, S.J. The antiviral drug arbidol inhibits zika virus. *Sci. Rep.,* **2018**, *8*(1), 8989. http://dx.doi.org/10.1038/s41598-018-27224-4 PMID: 29895962

[42] Wang, Y.; Ding, Y.; Yang, C.; Li, R.; Du, Q.; Hao, Y.; Li, Z.; Jiang, H.; Zhao, J.; Chen, Q.; Yang, Z.; He, Z. Inhibition of the infectivity and inflammatory response of influenza virus by Arbidol hydrochloride *in vitro* and *in vivo* (mice and ferret). *Biomed. Pharmacother.,* **2017**, *91*, 393-401. http://dx.doi.org/10.1016/j.biopha.2017.04.091 PMID: 28475918

[43] Liu, Q.; Xiong, H.; Lu, L.; Liu, Y.; Luo, F.; Hou, W.; Yang, Z. Antiviral and anti-inflammatory activity of arbidol hydrochloride in influenza A (H1N1) virus infection. *Acta. Pharmacol. Sin.,* **2013**, *34*(8), 1075-1083. http://dx.doi.org/10.1038/aps.2013.54 PMID: 23770981

[44] Brancato, V.; Peduto, A.; Wharton, S.; Martin, S.; More, V.; Di Mola, A.; Massa, A.; Perfetto, B.; Donnarumma, G.; Schiraldi, C.; Tufano, M.A.; de Rosa, M.; Filosa, R.; Hay, A. Design of inhibitors of influenza virus membrane fusion: Synthesis, structure–activity relationship and *in vitro* antiviral activity of a novel indole series. *Antiviral Res.,* **2013**, *99*(2), 125-135. http://dx.doi.org/10.1016/j.antiviral.2013.05.005 PMID: 23707194

[45] Wright, Z.V.F.; Wu, N.C.; Kadam, R.U.; Wilson, I.A.; Wolan, D.W. Structure-based optimization and synthesis of antiviral drug Arbidol analogues with significantly improved affinity to influenza hemagglutinin. *Bioorg. Med. Chem. Lett.,* **2017**, *27*(16), 3744-3748. http://dx.doi.org/10.1016/j.bmcl.2017.06.074 PMID: 28689973

[46] Gharavi, N.; Haggarty, S.; El-Kadi, A.O.S. Chemoprotective and carcinogenic effects of tert-butylhydroquinone and its metabolites. *Curr. Drug Metab.,* **2007**, *8*(1), 1-7. http://dx.doi.org/10.2174/138920007779315035 PMID: 17266519

[47] Bodian, D.L.; Yamasaki, R.B.; Buswell, R.L.; Stearns, J.F.; White, J.M.; Kuntz, I.D. Inhibition of the fusion-inducing conformational change of influenza hemagglutinin by benzoquinones and hydroquinones. *Biochemistry,* **1993**, *32*(12), 2967-2978. http://dx.doi.org/10.1021/bi00063a007 PMID: 8457561

[48] Hoffman, L.R.; Kuntz, I.D.; White, J.M. Structure-based identification of an inducer of the low-pH conformational change in the influenza virus hemagglutinin: Irreversible inhibition of infectivity. *J. Virol.,* **1997**, *71*(11), 8808-8820. http://dx.doi.org/10.1128/jvi.71.11.8808-8820.1997 PMID: 9343241

[49] Antanasijevic, A.; Cheng, H.; Wardrop, D.J.; Rong, L.; Caffrey, M. Inhibition of influenza H7 hemagglutinin-mediated entry. *PLoS One,* **2013**, *8*(10), 76363. http://dx.doi.org/10.1371/journal.pone.0076363 PMID: 24194835

[50] Russell, R.J.; Kerry, P.S.; Stevens, D.J.; Steinhauer, D.A.; Martin, S.R.; Gamblin, S.J.; Skehel, J.J. Structure of influenza hemagglutinin in complex with an inhibitor of membrane fusion. *Proc. Natl. Acad. Sci. USA,* **2008**, *105*(46), 17736-17741. http://dx.doi.org/10.1073/pnas.0807142105 PMID: 19004788

[51] Antanasijevic, A.; Hafeman, N.J.; Tundup, S.; Kingsley, C.; Mishra, R.K.; Rong, L.; Manicassamy, B.; Wardrop, D.; Caffrey, M. Stabilization and improvement of a promising influenza antiviral: Making a PAIN PAINless. *ACS Infect. Dis.,* **2016**, *2*(9), 608-615. http://dx.doi.org/10.1021/acsinfecdis.6b00046 PMID: 27759373

[52] Luo, G.; Colonno, R.; Krystal, M. Characterization of a hemagglutinin-specific inhibitor of influenza A virus. *Virology,* **1996**, *226*(1), 66-76. http://dx.doi.org/10.1006/viro.1996.0628 PMID: 8941323

[53] Luo, G.; Torri, A.; Harte, W.E.; Danetz, S.; Cianci, C.; Tiley, L.; Day, S.; Mullaney, D.; Yu, K.L.; Ouellet, C.; Dextraze, P.; Meanwell, N.; Colonno, R.; Krystal, M. Molecular mechanism underlying the action of a novel fusion inhibitor of influenza A virus. *J. Virol.,* **1997**, *71*(5), 4062-4070. http://dx.doi.org/10.1128/jvi.71.5.4062-4070.1997 PMID: 9094684

[54] Yu, K.L.; Ruediger, E.; Luo, G.; Cianci, C.; Danetz, S.; Tiley, L.; Trehan, A.K.; Monkovic, I.; Pearce, B.; Martel, A.; Krystal, M.; Meanwell, N.A. Novel quinolizidine salicylamide influenza fusion inhibitors. *Bioorg. Med. Chem. Lett.,* **1999**, *9*(15), 2177-2180. http://dx.doi.org/10.1016/S0960-894X(99)00361-3 PMID: 10465540

[55] Cianci, C.; Yu, K.L.; Dischino, D.D.; Harte, W.; Deshpande, M.; Luo, G.; Colonno, R.J.; Meanwell, N.A.; Krystal, M. pH-dependent changes in photoaffinity labeling patterns of the H1 influenza virus hemagglutinin by using an inhibitor of viral fusion. *J. Virol.,* **1999**, *73*(3), 1785-1794. http://dx.doi.org/10.1128/JVI.73.3.1785-1794.1999 PMID: 9971755

[56] Deshpande, M.S.; Wei, J.; Luo, G.; Cianci, C.; Danetz, S.; Torri, A.; Tiley, L.; Krystal, M.; Yu, K.L.; Huang, S.; Gao, Q.; Meanwell, N.A. An approach to the identification of potent inhibitors of influenza virus fusion using parallel synthesis methodology. *Bioorg. Med. Chem. Lett.,* **2001**, *11*(17), 2393-2396. http://dx.doi.org/10.1016/S0960-894X(01)00459-0 PMID: 11527739

[57] Tang, G.; Lin, X.; Qiu, Z.; Li, W.; Zhu, L.; Wang, L.; Li, S.; Li, H.; Lin, W.; Yang, M.; Guo, T.; Chen, L.; Lee, D.; Wu, J.Z.; Yang, W. Design and synthesis of benzenesulfonamide derivatives as potent anti-influenza hemagglutinin inhibitors. *ACS Med. Chem. Lett.,* **2011**, *2*(8), 603-607. http://dx.doi.org/10.1021/ml2000627 PMID: 24900355

[58] Zhu, L.; Li, Y.; Li, S.; Li, H.; Qiu, Z.; Lee, C.; Lu, H.; Lin, X.; Zhao, R.; Chen, L.; Wu, J.Z.; Tang, G.; Yang, W. Inhibition of influenza A virus (H1N1) fusion by benzenesulfonamide derivatives targeting viral hemagglutinin. *PLoS One,* **2011**, *6*(12), 29120. http://dx.doi.org/10.1371/journal.pone.0029120 PMID: 22195002

[59] van Dongen, M.J.P.; Kadam, R.U.; Juraszek, J.; Lawson, E.; Brandenburg, B.; Schmitz, F.; Schepens, W.B.G.; Stoops, B.; van Diepen, H.A.; Jongeneelen, M.; Tang, C.; Vermond, J.; van Eijgen-Obregoso Real, A.; Blokland, S.; Garg, D.; Yu, W.; Goutier, W.; Lanckacker, E.; Klap, J.M.; Peeters, D.C.G.; Wu, J.; Buyck, C.; Jonckers, T.H.M.; Roymans, D.; Roevens, P.; Vogels, R.; Koudstaal, W.; Friesen, R.H.E.; Raboisson, P.; Dhanak, D.; Goudsmit, J.; Wilson, I.A. A small-molecule fusion inhibitor of influenza virus is orally active in mice. *Science,* **2019**, *363*(6431), eaar6221. http://dx.doi.org/10.1126/science.aar6221 PMID: 30846569

[60] Wang, A.; Li, Y.; Lv, K.; Gao, R.; Wang, A.; Yan, H.; Qin, X.; Xu, S.; Ma, C.; Jiang, J.; Wei, Z.; Zhang, K.; Liu, M. Optimization and SAR research at the piperazine and phenyl rings of JNJ4796 as new anti-influenza A virus agents, part 1. *Eur. J. Med. Chem.,* **2021**, *222*, 113591. http://dx.doi.org/10.1016/j.ejmech.2021.113591 PMID: 34126455

[61] Wu, W.; Yan, H.; Jiang, B.; Wang, A.; Li, X.; Zhang, Y.; Wu, J.; Zhong, X.; Gao, R.; Wang, A.; Lv, K.; Li, Y.; Liu, M. Optimization and SAR research at the benzoxazole and tetrazole rings of JNJ4796 as new anti-influenza A virus agents, part 2. *Eur. J. Med. Chem.,* **2023**, *245*(Pt 1), 114906. http://dx.doi.org/10.1016/j.ejmech.2022.114906 PMID: 36395647

[62] Weisshaar, M.; Cox, R.; Morehouse, Z.; Kumar Kyasa, S.; Yan, D.; Oberacker, P.; Mao, S.; Golden, J.E.; Lowen, A.C.; Natchus, M.G.; Plemper, R.K. Identification and characterization of influenza virus entry inhibitors through dual Myxovirus high-throughput screening. *J. Virol.,* **2016**, *90*(16), 7368-7387. http://dx.doi.org/10.1128/JVI.00898-16 PMID: 27252534

[63] Kim, J.I.; Lee, S.; Lee, G.Y.; Park, S.; Bae, J.Y.; Heo, J.; Kim, H.Y.; Woo, S.H.; Lee, H.U.; Ahn, C.A.; Bang, H.J.; Ju, H.S.; Ok, K.; Byun, Y.; Cho, D.J.; Shin, J.S.; Kim, D.Y.; Park, M.S.; Park, M.S. Novel small molecule targeting the hemagglutinin stalk of influenza viruses. *J. Virol.,* **2019**, *93*(17), e00878-19. http://dx.doi.org/10.1128/JVI.00878-19 PMID: 31167918

[64] Yao, Y.; Kadam, R.U.; Lee, C.C.D.; Woehl, J.L.; Wu, N.C.; Zhu, X.; Kitamura, S.; Wilson, I.A.; Wolan, D.W. An influenza A hemagglutinin small-molecule fusion inhibitor identified by a new high-throughput fluorescence polarization screen. *Proc. Natl. Acad. Sci. USA,* **2020**, *117*(31), 18431-18438. http://dx.doi.org/10.1073/pnas.2006893117 PMID: 32690700

[65] Basu, A.; Antanasijevic, A.; Wang, M.; Li, B.; Mills, D.M.; Ames, J.A.; Nash, P.J.; Williams, J.D.; Peet, N.P.; Moir, D.T.; Prichard, M.N.; Keith, K.A.; Barnard, D.L.; Caffrey, M.; Rong, L.; Bowlin, T.L. New small molecule entry inhibitors targeting hemagglutinin-

mediated influenza a virus fusion. *J. Virol.,* **2014**, *88*(3), 1447-1460.
http://dx.doi.org/10.1128/JVI.01225-13 PMID: 24198411

[66] Plotch, S.J.; O'Hara, B.; Morin, J.; Palant, O.; LaRocque, J.; Bloom, J.D.; Lang, S.A.; DiGrandi, M.J.; Bradley, M.; Nilakantan, R.; Gluzman, Y. Inhibition of influenza A virus replication by compounds interfering with the fusogenic function of the viral hemagglutinin. *J. Virol.,* **1999**, *73*(1), 140-151.
http://dx.doi.org/10.1128/JVI.73.1.140-151.1999 PMID: 9847316

[67] Liu, S.; Li, R.; Zhang, R.; Chan, C.C.S.; Xi, B.; Zhu, Z.; Yang, J.; Poon, V.K.M.; Zhou, J.; Chen, M.; Münch, J.; Kirchhoff, F.; Pleschka, S.; Haarmann, T.; Dietrich, U.; Pan, C.; Du, L.; Jiang, S.; Zheng, B. CL-385319 inhibits H5N1 avian influenza A virus infection by blocking viral entry. *Eur. J. Pharmacol.,* **2011**, *660*(2-3), 460-467.
http://dx.doi.org/10.1016/j.ejphar.2011.04.013 PMID: 21536025

[68] Li, R.; Song, D.; Zhu, Z.; Xu, H.; Liu, S. An induced pocket for the binding of potent fusion inhibitor CL-385319 with H5N1 influenza virus hemagglutinin. *PLoS One,* **2012**, *7*(8), 41956.
http://dx.doi.org/10.1371/journal.pone.0041956 PMID: 22876294

[69] Hussein, A.F.A.; Cheng, H.; Tundup, S.; Antanasijevic, A.; Varhegyi, E.; Perez, J.; AbdulRahman, E.M.; Elenany, M.G.; Helal, S.; Caffrey, M.; Peet, N.; Manicassamy, B.; Rong, L. Identification of entry inhibitors with 4-aminopiperidine scaffold targeting group 1 influenza A virus. *Antiviral Res.,* **2020**, *177*, 104782.
http://dx.doi.org/10.1016/j.antiviral.2020.104782 PMID: 32222293

[70] Antanasijevic, A.; Durst, M.A.; Cheng, H.; Gaisina, I.N.; Perez, J.T.; Manicassamy, B.; Rong, L.; Lavie, A.; Caffrey, M. Structure of avian influenza hemagglutinin in complex with a small molecule entry inhibitor. *Life. Sci. Alliance,* **2020**, *3*(8), 202000724.
http://dx.doi.org/10.26508/lsa.202000724 PMID: 32611549

[71] Gaisina, I.N.; Peet, N.P.; Cheng, H.; Li, P.; Du, R.; Cui, Q.; Furlong, K.; Manicassamy, B.; Caffrey, M.; Thatcher, G.R.J.; Rong, L. Optimization of 4-aminopiperidines as inhibitors of influenza a viral entry that are synergistic with oseltamivir. *J. Med. Chem.,* **2020**, *63*(6), 3120-3130.
http://dx.doi.org/10.1021/acs.jmedchem.9b01900 PMID: 32069052

[72] Leiva, R.; Barniol-Xicota, M.; Codony, S.; Ginex, T.; Vanderlinden, E.; Montes, M.; Caffrey, M.; Luque, F.J.; Naesens, L.; Vázquez, S. Aniline-based inhibitors of influenza H1N1 virus acting on hemagglutinin-mediated fusion. *J. Med. Chem.,* **2018**, *61*(1), 98-118.
http://dx.doi.org/10.1021/acs.jmedchem.7b00908 PMID: 29220568

[73] de Castro, S.; Ginex, T.; Vanderlinden, E.; Laporte, M.; Stevaert, A.; Cumella, J.; Gago, F.; Camarasa, M.J.; Luque, F.J.; Naesens, L.; Velazquez, S. N-benzyl 4,4-disubstituted piperidines as a potent class of influenza H1N1 virus inhibitors showing a novel mechanism of hemagglutinin fusion peptide interaction. *Eur J. Med. Chem.,* **2020**, *194*, 112223.
http://dx.doi.org/10.1016/j.ejmech.2020.112223 PMID: 32220685

[74] Sokolova, A.S.; Putilova, V.P.; Yarovaya, O.I.; Zybkina, A.V.; Mordvinova, E.D.; Zaykovskaya, A.V.; Shcherbakov, D.N.; Orshanskaya, I.R.; Sinegubova, E.O.; Esaulkova, I.L.; Borisevich, S.S.; Bormotov, N.I.; Shishkina, L.N.; Zarubaev, V.V.; Pyankov, O.V.; Maksyutov, R.A.; Salakhutdinov, N.F. Synthesis and antiviral activity of camphene derivatives against different types of viruses. *Molecules,* **2021**, *26*(8), 2235.
http://dx.doi.org/10.3390/molecules26082235 PMID: 33924393

[75] Zhao, X.; Li, R.; Zhou, Y.; Xiao, M.; Ma, C.; Yang, Z.; Zeng, S.; Du, Q.; Yang, C.; Jiang, H.; Hu, Y.; Wang, K.; Mok, C.K.P.; Sun, P.; Dong, J.; Cui, W.; Wang, J.; Tu, Y.; Yang, Z.; Hu, W. Discovery of highly potent pinanamine-based inhibitors against Amantadine- and Oseltamivir-resistant influenza A viruses. *J. Med. Chem.,* **2018**, *61*(12), 5187-5198.
http://dx.doi.org/10.1021/acs.jmedchem.8b00042 PMID: 29799746

[76] Valdivia, A.; Rocha, M.; Luque, F.J. Mining druggable sites in influenza A hemagglutinin: Binding of the pinanamine-based inhibitor M090. *ACS Med. Chem. Lett.,* **2025**, *16*(1), 126-135.
http://dx.doi.org/10.1021/acsmedchemlett.4c00502 PMID: 39811135

[77] Wu, G.; Yu, G.; Yu, Y.; Yang, S.; Duan, Z.; Wang, W.; Liu, Y.; Yu, R.; Li, J.; Zhu, T.; Gu, Q.; Li, D. Chemoreactive-inspired discovery of influenza a virus dual inhibitor to block hemagglutinin-mediated adsorption and membrane fusion. *J. Med. Chem.,* **2020**, *63*(13), 6924-6940.
http://dx.doi.org/10.1021/acs.jmedchem.0c00312 PMID: 32520560

[78] Li, B.; Huang, L.; Lin, J.; Ma, X.; Luo, Y.; Gai, W.; Xie, Y.; Zhu, T.; Wang, W.; Li, D. Design, synthesis, and biological evaluation of novel penindolone derivatives as potential inhibitors of hemagglutinin-mediated membrane fusion. *Eur. J. Med. Chem.,* **2023**, *258*, 115615.
http://dx.doi.org/10.1016/j.ejmech.2023.115615 PMID: 37413878

[79] Zheng, Y.; Tice, C.M.; Singh, S.B. The use of spirocyclic scaffolds in drug discovery. *Bioorg. Med. Chem. Lett.,* **2014**, *24*(16), 3673-3682.
http://dx.doi.org/10.1016/j.bmcl.2014.06.081 PMID: 25052427

[80] Müller, G.; Berkenbosch, T.; Benningshof, J.C.J.; Stumpfe, D.; Bajorath, J. Charting biologically relevant spirocyclic compound space. *Chemistry,* **2017**, *23*(3), 703-710.
http://dx.doi.org/10.1002/chem.201604714 PMID: 27859909

[81] Vanderlinden, E.; Göktaş, F.; Cesur, Z.; Froeyen, M.; Reed, M.L.; Russell, C.J.; Cesur, N.; Naesens, L. Novel inhibitors of influenza virus fusion: Structure-activity relationship and interaction with the viral hemagglutinin. *J. Virol.,* **2010**, *84*(9), 4277-4288.
http://dx.doi.org/10.1128/JVI.02325-09 PMID: 20181685

[82] Cihan-Üstündağ, G.; Zopun, M.; Vanderlinden, E.; Ozkirimli, E.; Persoons, L.; Çapan, G.; Naesens, L. Superior inhibition of influenza virus hemagglutinin-mediated fusion by indole-substituted spirothiazolidinones. *Bioorg. Med. Chem.,* **2020**, *28*(1), 115130.
http://dx.doi.org/10.1016/j.bmc.2019.115130 PMID: 31753804

[83] Göktaş, F.; Vanderlinden, E.; Naesens, L.; Cesur, N.; Cesur, Z. Microwave assisted synthesis and anti-influenza virus activity of 1-adamantyl substituted N-(1-thia-4-azaspiro[4.5]decan-4-yl)carboxamide derivatives. *Bioorg. Med. Chem.,* **2012**, *20*(24), 7155-7159.
http://dx.doi.org/10.1016/j.bmc.2012.09.064 PMID: 23117173

[84] Göktaş, F.; Vanderlinden, E.; Naesens, L.; Cesur, Z.; Cesur, N.; Taş, P. Synthesis and structure-activity relationship of n-(3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)carboxamide inhibitors of influenza virus hemagglutinin mediated fusion. *Phosphorus Sulfur Silicon Relat. Elem.,* **2015**, *190*(7), 1075-1087.
http://dx.doi.org/10.1080/10426507.2014.965819

[85] Göktaş, F.; Özbil, M.; Cesur, N.; Vanderlinden, E.; Naesens, L.; Cesur, Z. Novel *N* -(1-thia-4-azaspiro[4.5]decan-4-yl)carboxamide derivatives as potent and selective influenza virus fusion inhibitors. *Arch. Pharm.,* **2019**, *352*(11), 1900028.
http://dx.doi.org/10.1002/ardp.201900028 PMID: 31531897

[86] Çınar, G.; Alikadıoğlu, Z.; Soylu-Eter, Ö.; Naesens, L.; Cihan-Üstündağ, G. Design, synthesis and anti-influenza virus activity of 4-*Tert*-Butyl-*N*-(3-Oxo-1-Thia-4-Azaspiro[4.5]Dec-4-yl)benzamide derivatives that target hemagglutinin-mediated fusion. *Drug Dev. Res.,* **2025**, *86*(2), 70080.
http://dx.doi.org/10.1002/ddr.70080 PMID: 40125625

[87] Apaydın, Ç.B.; Tansuyu, M.; Cesur, Z.; Naesens, L.; Göktaş, F. Design, synthesis and anti-influenza virus activity of furan-substituted spirothiazolidinones. *Bioorg. Chem.,* **2021**, *112*, 104958.
http://dx.doi.org/10.1016/j.bioorg.2021.104958 PMID: 33979734

[88] Cihan-Üstündağ, G.; Acar, Ç.; Naesens, L.; Erköse-Genç, G.; Şatana, D. Synthesis of new *N*-(3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)pyridine-3-carboxamide derivatives and evaluation of their anti-influenza virus and antitubercular activities. *Arch. Pharm.,* **2022**, *355*(10), 2200224.
http://dx.doi.org/10.1002/ardp.202200224 PMID: 35849096