

Novel chemical scaffolds for influenza A M2 ion channel blockers beyond adamantanes

Vanessa A. Shu

Martin Luther University Halle-Wittenberg

Ian Tietjen

Delaware Valley College

Wolfgang Sippl

Martin Luther University Halle-Wittenberg

Fidele Ntie-Kang

fidele.ntie-kang@ubuea.cm

University of Buea

Systematic Review

Keywords:

Posted Date: October 19th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11157727/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Novel chemical scaffolds for influenza A M2 ion channel blockers beyond adamantanes

Vanessa A. Shu,¹ Ian Tietjen,² Wolfgang Sippl^{1*} and Fidele Ntie-Kang^{1,3,4*}

¹Institute of Pharmacy, Martin-Luther University Halle-Wittenberg, Wolfgang-Langenbeck Strasse 4, 06120, Halle (Saale), Germany

²School of Business, Arts, and Sciences, Delaware Valley University, Doylestown, PA, 18901, USA

³Center for Drug Discovery, Faculty of Science, University of Buea, P. O. Box 63, Buea. Cameroon

⁴Department of Chemistry, Faculty of Science, University of Buea, P. O. Box 63, Buea. Cameroon

Corresponding author: wolfgang.sippl@pharmazie.uni-halle.de (WS); fidele.ntie-kang@ubuea.cm (FNK)

Abstract

The influenza A M2 proton-gated proton channel is an established antiviral target whose inhibition disrupts essential steps of viral replication including uncoating and egress. Although several antiviral agents targeting other viral proteins are currently available for the treatment of influenza, the emergence of antiviral resistance and the limited number of mechanistically distinct therapeutic targets underscore the need for new influenza antivirals. Adamantanes including amantadine and rimantadine provided the original foundation for M2-directed therapy. However, development of widespread adamantane resistance due to mutations in M2 has substantially limited their clinical utility and fueled the search for structurally diverse M2 ion channel blockers capable of retaining activity against both wild-type and drug-resistant variants. Novel chemical scaffolds beyond adamantanes offer opportunities to overcome the limitations associated with adamantane-based inhibitors by establishing alternative interactions within the M2 transmembrane channel to inhibit proton conduction. This review examines emerging non-adamantane scaffolds reported as influenza A M2 inhibitors, with particular emphasis on their structural features, binding modes, antiviral activity, mechanisms of inhibition, and how scaffold diversification can provide new opportunities for targeting conserved and mutation-tolerant regions of the channel. Collectively, these studies highlight the potential

of non-adamantane chemotypes to expand the chemical space of M2 inhibitors and provide a foundation for the development of next-generation influenza antivirals with improved profiles against adamantane-resistant influenza A viruses.

1. Introduction

Influenza viruses remain an ongoing threat to human health and wellness, being responsible for annual respiratory diseases in humans with varying degrees of widespread symptoms spanning fever, severe muscle and body aches, fatigue, headache, and in extreme cases respiratory failure and death. The World Health Organization (WHO) estimates over 1 billion seasonal influenza cases occur each year globally, resulting in about 5 million severe cases and 650,000 respiratory deaths. These numbers can escalate dramatically during recurring pandemics [1-3].

Novel influenza viruses continue to emerge from animal reservoirs and spread among human populations, driven by increasing global travel, intensified human–animal interfaces, and the high genetic plasticity of influenza viruses [4-5]. Although annual influenza vaccines provide effective protection against many seasonal strains, continuous viral evolution through antigenic drift results in strain mismatch, thereby impacting vaccine effectiveness [6]. Seasonal influenza vaccines are formulated to target currently circulating strains but may provide limited protection against novel viruses with distinct antigenic properties. Consequently, the emergence of novel pandemic strains necessitates the development of strain-specific vaccines. However, the development of licensed influenza vaccines involve lengthy manufacturing approaches that require a significant time lag from the advent of a new pandemic virus strain to the widespread availability of an effective vaccine [7]. These constraints drive the need for the discovery of antivirals effective against both seasonal and pandemic influenza viruses.

Influenza viruses are a group of single stranded negative sense RNA genome viruses, belonging to the family Orthomyxoviridae. Based on the variation of their nucleoprotein (NP), influenza viruses are classified into four subtypes: Influenza A, B C and D, henceforth referred to as IAV, IBV, ICV and IDV, respectively [8]. IAV and IBV are responsible for seasonal influenza infection epidemics, while ICV causes only mild forms of infection. IDV, on the other hand, does not infect humans, but has been reported to infect cattle [9-10]. IAV can also infect other species such as birds, pigs, ferrets, horses, seals, whales, mink, giant anteaters, cats and dogs. Due to its wide host range and the ability to spread from its reservoir to different species, IAV is the most common and virulent species, with wild aquatic birds being its primary reservoir, and is the driver of influenza pandemics in humans, birds, and other species [11].

IAVs are the most diverse and epidemiological impactful species of influenza viruses. They are also connected with the most severe manifestations of the disease in humans. Several pandemics due to IAV have been recorded in the 20th and 21st centuries: the 1918 Spanish Flu (A/H1N1), 1957 Asian Influenza (A/H2N2), 1968 Hong Kong Influenza (A/1968), and the most recent being the 2009 pandemic (A/H1N1pdm09) [12]. Influenza pandemics arise when zoonotic viruses acquire sustained human-to-human transmission. The extensive circulation of zoonotic avian influenza viruses, particularly highly pathogenic H5N1, represents a pandemic concern, with sporadic detections in other mammalian species including marine mammals and dairy cattle, likely as a result of spillover infections from wild birds to mammals [13–14]. Other avian influenza viruses, including H7N9, H9N2 and H5N6, have also caused occasional human infections [15–17]. Although sustained human-to-human transmission has not been observed, the continued evolution and cross-species transmission of these viruses remain major concerns. Notably, the majority of the reported human H5N1 infections since 2003 have been fatal, emphasizing the potential severity of zoonotic influenza [18].

The emergence of these pandemics rely on the ability of IAV to develop adaptive mutations *via* antigenic changes, thus permitting it to evade human immune response. A few factors have been reported to favor these antigenic changes; for instance, owing to the lack of a proofreading function on the viral RNA polymerase, the virus is liable to coding errors resulting in high rates of wrong nucleotide integration during viral replication. This results in accumulating point mutations in the viral genome which is known as antigenic drift. Furthermore, due to the unique arrangement of viral genetic information on multiple RNA segments, the virus is able to mix genetic information of different IAV strains via co-infection leading to the generation of novel, gene-reassorted virus strains. This process is known as antigenic shift and is responsible for the emergence of viral strains with largely modified characteristics and the potential to infect humans with increased pathogenicity during pandemic outbreaks [19–21]. Antigenic shift often results in novel strains with different combinations of hemagglutinin (HA) and neuraminidase (NA), with 18 novel hemagglutinins (H1–H18) and 11 neuraminidases (N1–N11), as reported by the Center for Diseases Control and Prevention [22].

IAV consists of 8 negative-sense single stranded viral RNA (vRNA) gene segments, which encode ten core proteins along with non-essential accessory proteins that contribute to virulence and host-interaction. These 8 genome segments carry the genetic information to make the following viral proteins: matrix1 (M1), matrix2 (M2), hemagglutinin (HA), neuraminidase (NA), nucleoprotein (NP), non-structural protein 1 (NS1), non-structural protein 2 (NS2: also described as nuclear export protein, NEP), polymerase acidic protein (PA), polymerase basic protein 1 (PB1), and polymerase basic protein 2 (PB2), which are

essential for the survival, replication and eventual infection of new cells by the virus (Fig. 1) [23-25]. The matrix2 protein, or M2 protein, is regarded as a key component for both the early and late stages of the viral replication cycle. M2 is a tetrameric, single pass, type III integral transmembrane (TM) protein [26-27]. The M2 protein also exhibits other key ion channel-independent roles such as modulating host innate immunity by interfering with the cell death and autophagy pathways of the host. During the viral budding process, the M2 protein also alters membrane curvature by inserting its amphipathic helix into the membrane, thereby facilitating membrane scission and release of budding virions [28-29].

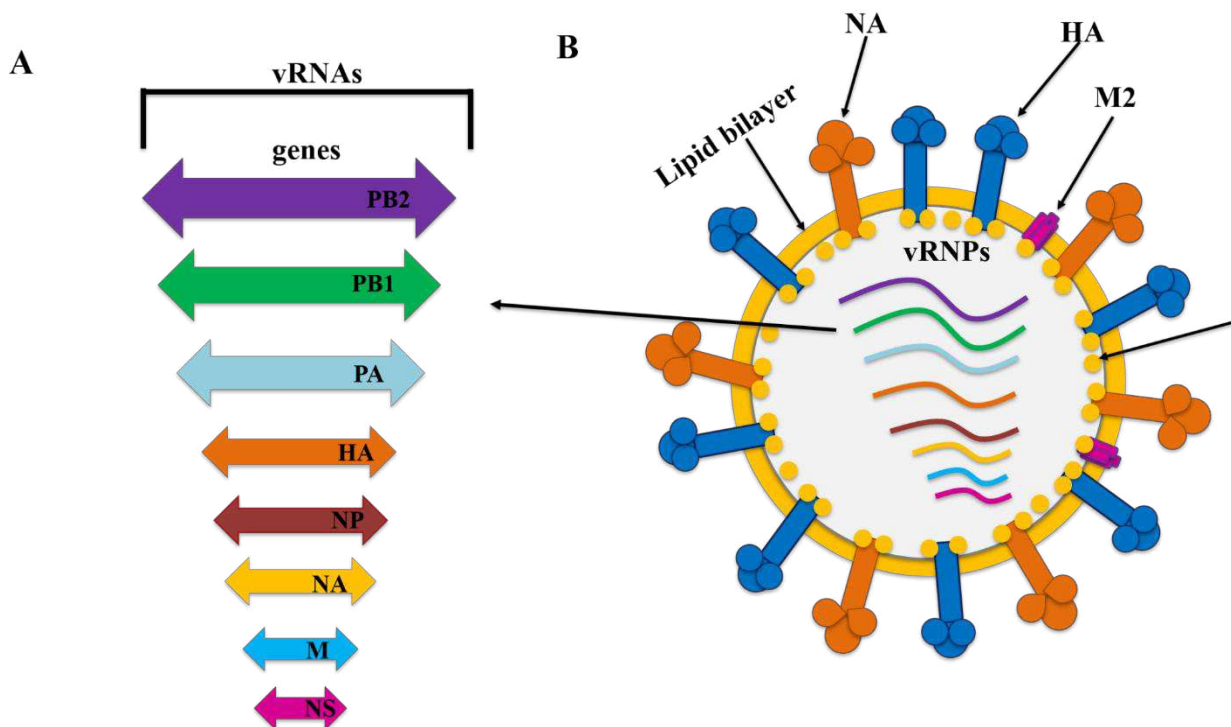


Fig. 1: (A) A schematic representation of the 8 viral RNA genomes that make up the IAV M2 protein. (B) A schematic representation of the morphological structure of IAV showing viral membrane proteins [30].

The influenza A M2 protein functions as an ion channel and plays a pivotal role during viral replication. Upon entry of the virus into the host cell, the interior of the virus requires an acidic environment to facilitate the release of genetic material (uncoating) into the host cytoplasm. The M2 proton ion channel plays this role by opening up in response to low pH permitting the influx of protons into the virus, which then causes the viral RNA to be dissociated from the matrix protein and eventual fusion of the viral and endosomal membranes. The viral RNA is then released into the cytoplasm of the host cell for replication [31-32]. The M2 protein also functions in equilibrating the pH of the trans-Golgi network, preventing premature conformational changes of Hemagglutinin in viruses, thus

requiring a higher pH to trigger hemagglutinin fusion. Collectively, these properties make M2 an attractive biological target for antiviral drug development [33-34].

Structure of the Influenza A M2 protein and its drug binding site

The M2 protein is a homotetrameric 97-residue single pass membrane protein with its 4 transmembrane helices forming an ion channel in which His37 and Trp41 function as the pH sensor and gate respectively. It is composed of 3 main domains, a short N-terminal ecto domain (residues 1-24) which is highly conserved and serves as an attractive target for universal influenza vaccine development, a transmembrane domain (TM, residues 25-43) which functions in proton regulation and a C-terminal domain (residues 44-97) which is essential for virus budding. The C-terminal domain is subdivided into the amphipathic helix (residues 45 to 62) and the cytoplasmic tail (residues 63 to 97) (Fig. 2) [35]. Studies have shown that though the ecto and C-terminal domains of the M2 protein play essential roles in the functioning of the channel, elimination of these domains result in no impact on the proton transport activity of the channel [36]. Despite its high degree of conservation, certain regions (residues 10 to 28, 54 to 57 and 77 to 93) of the M2 protein have been reported to undergo relatively high sequence variations, and only His37 and Trp41 are absolutely conserved across all IAVs. Naturally occurring mutations of the M2 protein, found in different influenza A viruses include, S31N, V27A, L26F, A30T, G34E, and L38F [37-39]. Extensive structural investigations using X-ray crystallography, solution NMR, ssNMR and electron paramagnetic resonance have generated multiple models of the M2 channel. The TM helix and membrane surface-bound amphipathic helix of the M2 channel constitute the proton conductance domain of the channel and have been reported to adequately retain the principal ion channel functional properties of the full-length protein. Hence, the majority of the structure investigation studies conducted on the M2 protein were carried out on the isolated TM construct [40-41].

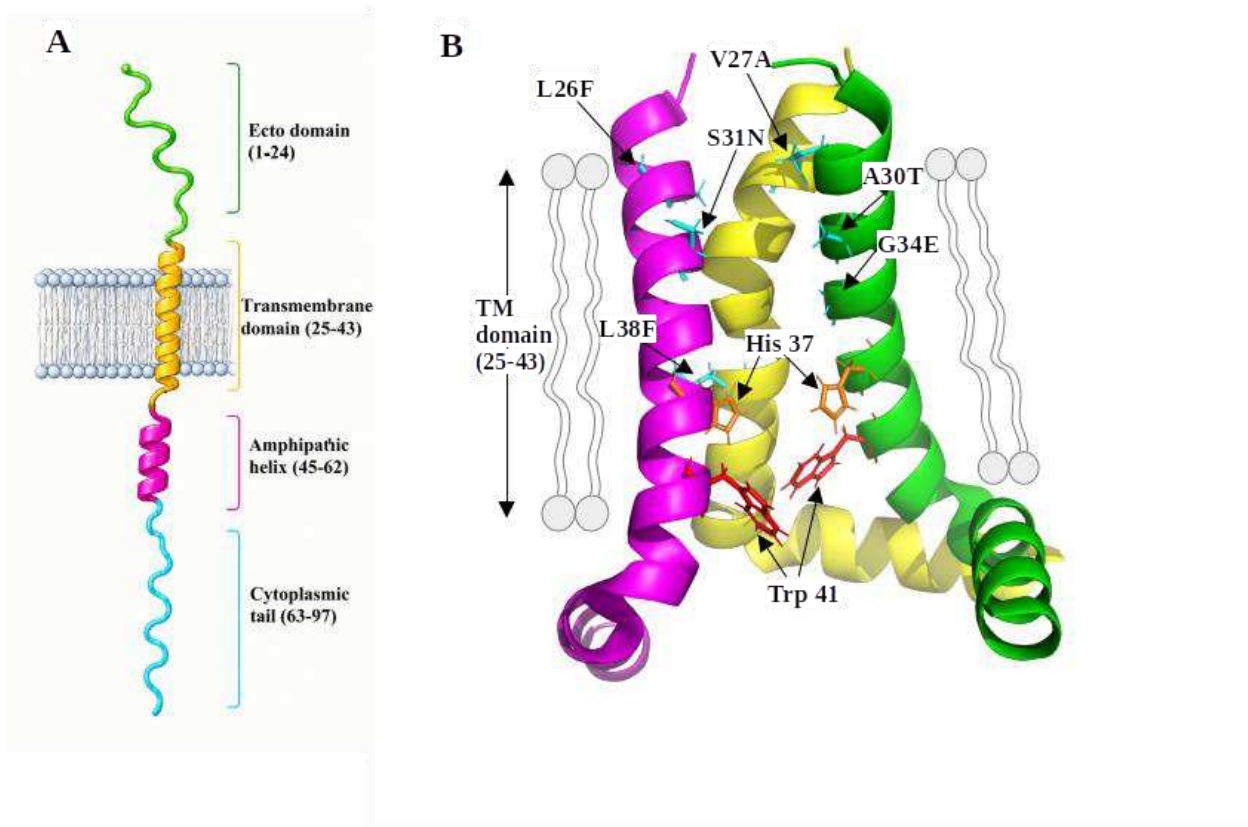


Fig. 2: (A) Schematic representation of the full-length M2 protein showing the different domains (B) Representation of the tetrameric M2 channel (one chain omitted for clarity), showing the functionally important residues His37 and Trp41 and mutation sites within the transmembrane region (PDB ID: 2L0J) [42-43].

Early solid-state NMR studies on the TM domain of the M2 protein revealed that the 4 alpha helices of the TM assemble into a left-handed tetrameric bundle in which the helices are tilted by more than 30° relative to the membrane bilayer normal [41]. Computational modeling and crosslinking studies further supported this proposed tetrameric arrangement of the channel and further demonstrated that the polar residues Ser31, His37, and Trp41 are oriented towards the aqueous pore of the channel and facilitate proton conduction [44]. Subsequent structural studies provided more insights into the conformational variability of the M2 channel, for instance, the crystal structure of the G34A mutant (PDB ID: 3LBW) which was identified in 2010 lacked distortions present in previously identified crystal structures but displayed a helix tilt angle similar to that of the ssNMR structure (PDB ID: 2L0J) [45-46].

Early structural studies identified the pore and lipid-facing site as the two possible adamantane binding sites. The first X-ray crystal structure of the M2 transmembrane

domain in complex with amantadine (PDB ID: 3C9J) suggested a pore binding mechanism based on electron density mapping. However, this low resolution of the derived structure limited the assignment of the ligand and surrounding water network [47]. Schnell and co on the other hand later reported the solution NMR structure of the M2 (18-60), suggesting 4 equivalent rimantadine sites on the lipid-facing side near the channel [48]. Using solid state NMR spectroscopy, Cady et al. identified the existence of 2 amantadine-binding sites in the M2 protein in phospholipid bilayers. A high affinity site located at the N-terminal end of the channel in the region occupied by residues and a low affinity site at the C-terminal protein surface which was only observed at high drug concentrations in the bilayer [49]. High resolution X-ray structures of M2TM in complex with amantadine, rimantadine and other adamantane compounds confirmed that drugs occupy the channel pore and interact with the structured water network extending towards His 37 [50]. Subsequently, these studies established that the pore binding site represents the high-affinity, physiologically relevant binding site for adamantane inhibitors, whereas binding at the lipid-facing surface occurs predominantly at higher drug concentrations and is considerably weaker and largely nonspecific [51].

Mechanism of Drug binding and resistance

The M2 channel plays a key during viral entry and maturation by equilibrating the pH across the viral and trans-Golgi membranes of infected cells. It is the main target for the first licensed drugs which were approved for influenza treatment (amantadine and rimantadine). However, due to the emergence of drug-resistant M2 mutants, the use of these drugs have been discontinued. Different studies have been conducted to better understand the mechanism by which these drugs inhibit the M2 channel. Classical M2 inhibitors such as Amantadine and rimantadine bind by fitting into the central lumen of the M2 channel and inhibiting the channel activity by physical occlusion. The adamantane scaffold occupies the hydrophobic region formed by residues Val27, Ala30, Ser31, and Gly34, while the polar amine group is positioned towards His37. Binding of the inhibitor within the M2 transmembrane pore induces structural and dynamic changes in the channel thereby dehydrating the channel by disrupting the continuous hydrogen-bonded water-wire network required for proton transport. Consequently, the protonation equilibrium of the His37 tetrad is perturbed, impairing the smooth flow of protons through the channel. This inhibition prevents acidification of the viral interior, thereby disrupting viral uncoating and interrupting the IAV life cycle [52-53].

The widespread emergence of drug-resistant influenza A strains has largely eliminated the clinical utility of adamantane-based antivirals. Resistance most commonly arises due to point mutations within the transmembrane drug-binding pocket, with the S31N mutation

being the most common and accounting for over 98% of circulating adamantane-resistant IAVs. The naturally occurring unmodified M2 protein sequence is referred to as the wild-type (WT). Unlike resistance mechanisms that simply prevent ligand access through steric hindrance, M2 resistance primarily results from a collection of structural and dynamic changes within the channel. For instance, the replacement of serine with asparagine at position 31 introduces a larger, polar side chain that reorganizes the local hydrogen-bonding network and alters the hydration pattern of the pore [54]. These changes lead to weaker hydrophobic interactions with adamantane derivatives, hence a reduction in ligand residence time, and an increase in competition between inhibitors and water molecules occupying the binding site [55-57]. Other mutations such as L26F, V27A, A30T, G34E, and L38F also confer drug resistance IAVs (Fig. 2), similarly modifying the size, polarity, and conformational flexibility of the pore, leading to diminished inhibitor affinity while preserving proton conduction and viral fitness. For example, the V27A mutation enlarges the binding cavity, reducing van der Waals contacts with hydrophobic inhibitors, whereas A30T introduces a hydroxyl-containing side chain which increases the overall polarity of the pore and perturbs local water organization. Consequently, resistance is concluded to arise from a combination of altered pore geometry, changes in channel dynamics, and reorganization of the internal water network rather than from a simple loss of steric complementarity [58].

2. Antivirals targeting human influenza viruses

There are 2 main approaches currently implemented as countermeasures against IAV infections: the use of prophylactic vaccines and antivirals for prophylactic and therapeutic remedy. Three classes of anti-influenza drugs have been approved by the US Food and Drug Administration (FDA) for influenza A therapy, based on the viral protein on which they interact. These include NA inhibitors, viral polymerase inhibitors, and M2 ion channel blockers. That notwithstanding, other viral proteins are also being investigated for their potential as biological targets in the fight against influenza virus infection. The two M2 ion channel inhibitors (Amantadine and Rimantadine), which were previously used in therapy are no longer recommended for use due to the development of drug resistance. Table 1 highlights some antiviral drugs that target influenza virus infections [59].

Table 1: Some Anti-influenza virus drugs and their mode of action

Drug	Viral target	Virus type	Mode of action	Status	Reference
Amantadine	M2 ion channel	IAV	Inhibits viral uncoating replication	Discontinued	[60]
Rimantadine	M2 ion channel	IAV	Inhibits viral uncoating replication	Discontinued	[60]
TCN-032	Antibody against conserved M2 extracellular epitope	IAV	Inhibits viral budding	Phase II clinical trial	[61]
Radavirsen	Antisense oligonucleotide to M1 and 2 segment	IAV	Blocks translation and processing of pre-mRNA	Phase I clinical trial	[62]
Zanamivir	NA inhibitor	IAV and IBV	Disrupts viral release	FDA approved	[60]
Oseltamivir	NA inhibitor	IAV and IBV	Disrupts viral release	FDA approved	[60]
Peramivir	NA inhibitor	IAV and IBV	Disrupts viral	FDA approved	[60]

Laninamivir	NA inhibitor	IAV and IBV	Disrupts NA activity	FDA approved (specific countries)	[63]
Nitazoxamide	HA inhibitor	IAV and IBV	Blocks maturation of viral HA at post-translational phase.	Phase III clinical trial [C]	[64]
Arbidol	HA inhibitor	IAV and IBV	Blocks viral entry	FDA approved (specific countries)	[65]
Flufirvitide-3	HA inhibitor	IAV and IBV	Inhibits viral entry	Phase II clinical trial	[66]
MHAA4549A	HA	IAV	Inhibits HA protein maturation	Phase II clinical trial	[66]
MEDI8852	HA inhibitor	IAV	Inhibits membrane fusion	Phase II clinical trial	[67]
VIS410	HA inhibitor	IAV		Phase II clinical trial	[68]
CR6261	HA	IAV	Inhibits membrane fusion	Phase II clinical trial	[69]
CR8020	HA	IAV	Inhibits membrane fusion	Phase II clinical trial	[70]

CT-P27	HA	IAV	Inhibits HA-mediated viral fusion	Phase II clinical trial	[71]
Favipiravir	RdRp	IAV, IBV and ICV	Inhibits transcription and replication	FDA approved (specific countries)	[72]
Enisamium iodide	RdRp inhibitor	IAV and IBV	Inhibits viral RdRp	Phase II clinical trial	[73]
Ribavirin	RdRp inhibitor	IAV and IBV		Phase II clinical trial (in combination with other drugs)	[74]
ZX-7101	PA	IAV	Inhibits Cap-dependent endonuclease	Phase I clinical trial	[75]
TG-1000	PA		Inhibits Cap-dependent endonuclease	Phase II clinical trial	[76]
AL-794	PA	IAV and IBV	Inhibits Cap-dependent endonuclease	Phase I clinical trial	[77]
Ingavirin	NP inhibitor	IAV and IBV	Blocks the transport of NP to the nucleus	FDA approved (specific countries)	[78]

Molnupiravir	PB1	IAV	Acts as a nucleoside analogue	Phase II clinical trial	[76]
Pimodivir	PB2 inhibitor	IAV	Disrupts the process of viral protein synthesis	Phase III clinical trial	[79]
ZSP1273	PB2	IAV	Inhibits PB2 cap-binding domain	Phase III clinical trial	[80]
CC-42344	PB2	IAV	Inhibits PB2 cap-binding domain	Phase II clinical trial	[81]
Atorvastatin	HMG-CoA inhibitor	IAV	Inhibits the formation of lipid-droplets in viral envelope	Phase II clinical trial	[82]
Danirixin	Blocks CXCR2	IAV and IBV	Inhibits neutrophil chemotaxis during inflammation	Phase II clinical trial	[83]
Baloxavir marboxil	Polymerase acidic protein	IAV and IBV	Blocks viral transcription	FDA approved	[84]
DAS181	Host	IAV and IBV	Inhibits viral entry	Phase III clinical trial	[85]

3. The adamantane era: successes, mode of action and limitations

Adamantane (tricyclo[3.3.1.1^{3,7}]decane **1**, Fig. 3), is the smallest member of the diamonoid family of compounds; *i.e.*, hydrocarbons with a spatial arrangement carbon atoms mimicking a diamond crystal lattice. Adamantane has a cage-like hydrogen skeleton containing 10 carbon atoms. The addition of isobutyl fragments onto the adamantane ring in a cyclohexane ring-forming leads to higher diamonoid analogues [86]. Just like other diamonoids, adamantane possesses numerous properties which stem directly from their 3 dimensional (3D) diamond-like structure. These include high lipophilicity, high melting points, high symmetry, thermodynamic stability, facile crystallization and structural rigidity [87]. After the identification of the antiviral activity of amantadine, adamantane-containing compounds have gained huge attention and this has led to their exploration towards applications in medicinal chemistry and drug discovery research [88]. Several adamantyl-based drugs (Fig. 3) are currently being used in the treatment of different diseases such as type 2 diabetes mellitus, acne vulgaris, viral infections and neurodegenerative disorders. The rigid, highly symmetrical, 3 dimensional structure of the adamantane scaffolds equips it to control the orientation of functional groups, thereby improving both activity and selectivity at specific targets. These properties have led to the inclusion of adamantane and its derivatives in different drug discovery programs, especially those targeting ion channels [89].

The first report of the anti-influenza activity of 1-adamantamine (amantadine, **2**) dates back to over six decades ago, where the compound was found to cause dose-related inhibition of influenza infections in tissue culture, chick embryos and mice [90]. Shortly after that, the antiviral activity of this compound class against several viral targets was identified resulting in the introduction of numerous adamantane based drugs into the market [88]. The adamantane drugs (Amantadine and Rimantadine **3**) act as inhibitors by binding within the IAV M2 channel pore, thereby preventing proton transport and M2-mediated pH changes [91]. As a result, these drugs also inhibit the release of viral ribonucleoprotein complex (NP-RNA) complex into the host cytoplasm during viral replication by disrupting acid mediated dissociation, thereby interrupting the viral life cycle. Also, amantadine has been reported to interfere with viral assembly by altering the pH of secretory vesicles responsible for the transport of viral glycoproteins. However, these drugs are reported to be active only against the wild type form of the M2 ion channel [92]. However, they have acquired resistance to influenza viruses resulting in the discontinuation of their use in influenza therapy [93]. Bearing in mind that the M2 protein is an already validated clinical target and plays a key role in the viral lifecycle, numerous research work has been carried out over the years on this target in an attempt to generate novel bioactive compounds capable of inhibiting the activity of this protein. The majority of the M2 inhibitors which have been

reported are compounds containing the adamantane ring. Herein, we will discuss non adamantane based M2 ion channel inhibitors acting against influenza A which have been recently reported.

The presence of a polar head group (a primary or secondary amine), attached to a hydrophobic tail appears to be a unique feature found in all M2 channel inhibitors. In an attempt to develop novel bioactive molecules capable of overcoming the challenge of drug resistance currently being faced by existing M2 inhibitors, Wang et al, decided to investigate the outcome of substituting the polar head group of amantadine with other polar molecules. Different molecules were designed (the amine group of amantadine was substituted with other polar groups), synthesized and screened on the IAV (A/Udorn/72) M2 protein using two-electrode voltage patch clamp assays (TEVC) on *Xenopus laevis* oocytes which were micro-injected with RNA expressing the A/M2 protein. The adamantane based compounds **4-6** in which the amine group was replaced by amidine, guanidine, and hydroxyl head groups respectively revealed improved activities, with the activity of compound **4** (4 μ M) increased by nearly 4 fold when compared to that of Amantadine (IC_{50} = 16 μ M). Based on these findings, it was concluded that besides the amine group, other polar groups can be accommodated at the M2 binding site. However, it is worth noting that these compounds were only active against the influenza A M2 WT with little to no activity against the S31N and V27A mutants [94].

Stankova and coworkers synthesized a series of amantadine and rimantadine analogues bearing modified peptides and thiazole rings [95]. The compounds were tested for antiviral properties on the IAV A/Hong Kong/68 (H1N1) strain by cytopathic assay (CPE). The rimantadine analogue **7** (with thiazole ring Gly-Thz-rimantadine) portrayed moderate activity with IC_{50} value of 0.11 μ g/mL against the A/Hong Kong/68 (H1N1) and a CC_{50} value of 50 μ g/mL in MDCK (Madin Darby canine kidney cell monolayers) cells. All other analogues were inactive suggesting that the presence of a bulky group does not improve antiviral activity.

Steered by molecular dynamics simulation studies of the impact of drug binding on AM2 protein, a series of spirane amine compounds were designed, synthesized and tested for their inhibitory activity against influenza A M2 WT, V27A and L26F. The compounds were tested for their antiviral activity through TEVC and plaque reduction assay. The spiroadamantane amine compound **8** was identified as the most active, inhibiting the M2-WT, V27A and L26F channels with IC_{50} values of 18.7 ± 1.4 μ M, 0.3 ± 0.0 μ M and 5.6 ± 0.5 μ M respectively [96]. Further *in vitro* and *in vivo* studies were conducted on this compound and interestingly compound **8** showed similar antiviral activity to amantadine against the influenza M2-WT A/Udorn/72 (H3N2) strain with an EC_{50} value of 0.3 ± 0.1 μ M, and was able

to rescue mice from lethal M2-WT and V27A-containing influenza A virus infections [97], suggesting that some adamantane derivatives still hold a promise in lead compound discovery against influenza A M2.

Structure activity relationship studies revealed that strong influenza A M2 S31N inhibitors generally possess the following properties; a hydrophobic such as the adamantane scaffold, an ammonium linker and an aryl headgroup bearing a hydrophobic substituent. Taking into account these requirements, Li et al. synthesized a series of compounds **9-11** bearing the adamantane ring connected via an ammonium linker to isoxazole, while making variations at the 5-position of isoxazole. The synthesized compounds were tested for their ability to block the M2 S31N channel as well as their antiviral activity against IAV A/California/07/2009 (H1N1) via TEVC and plaque assays respectively. Compounds **9** and **10** with benzene and thiophene substituents at the C-5 position were identified as the preferred aryl substitutions. Further modifications were made on the benzene and thiophene rings by introducing substituents at different positions. It is worth noting that compound **11**, with a naphthalene substituent at the C-5 position of the isoxazole ring revealed poor channel blockage activity which is likely associated with steric hindrance. Lead compounds identified from the channel inhibition assay were further screened in a plaque reduction assay against human clinical isolates of IAV. compound **10** was found to be the most active compound with submicromolar activity against 4 influenza A S31N viruses including A/California/07/2009 (H1N1), A/Washington/29/ 2009 (H1N1), A/Denmark/528/2009 (H1N1), and A/ Switzerland/9715293/2013 (H3N2) and also nontoxic with CC_{50} values of $190.5 \pm 14.2 \mu\text{M}$ and $208.6 \pm 20.9 \mu\text{M}$ in MDCK cells and A549 cells respectively [98].

A study geared towards the design and development of novel conjugates of drugs with metal ions, led to the adamantane-copper chloride complex **12** $\{[\text{AdNH}_3^+].[\text{CuCl}_3]^{-}\}$ [99]. The compound was evaluated *in vitro* for its anti-influenza activity against the A M2 WT and some mutated strains (S31N, A30T, and L26F). Interestingly, it demonstrated improved antiviral activity against S31N and M2 L26F strains, with over 1.5 and 3.2 fold activity, respectively, when compared to amantadine. Furthermore, compound **12** inhibited the A/WSN/33 M2 N31 and A30T strains with EC_{50} values 21.95 and 11.58 μM respectively, whereas no adamantane derivative has been reported to inhibit these variants. Additional analysis of the toxicity of the compound *in vivo* by using brine shrimp *Artemia salina* assay was carried out. Unlike free Cu (II) ions which are toxic to cells, similar survival rates of the brine shrimp larvae were observed in cells treated with over 150 μM of compound **12** as well as untreated cells indicating more limited toxicity [99].

A series of diaza-adamantane-monoterpenoid compounds were further synthesized and tested for their antiviral activity against rimantadine-resistant strains of the IAV A/Puerto Rico/8/34 (H1N1) [100]. Interestingly, compound **13**, which contains a diaza-adamantane ring combined with a citronellal monoterpene, was the most active with high antiviral activity ($IC_{50} = 8 \mu M$), moderate cytotoxicity in MDCK cell lines ($CC_{50} = 239 \mu M$), and good drug-like characteristics with a high binding affinity towards the influenza A M2 protein (PDB ID: 3C9J). Molecular modeling studies conducted on the M2 protein revealed that the diaza-adamantane moiety of **13** binds in the inner region of the channel where the four monomers of the protein converge, while the bulky substituent binds to the outer region of the channel where the monomers diverge. Besides, 2 non-classical hydrogen bonds were observed between the side chain carbonyl group of Asparagine and the C-H bond of diaza-adamantane as well as the backbone carbonyl oxygen of Alanine and the amine hydrogen on **13**.

In the quest for novel compounds with potential of not only inhibiting the WT but acting as dual inhibitors of IAVs, Barniol-Xicota et al. synthesized a series of adamantane-piperidine derivatives **14-16** and evaluated their channel blockage ability by TEVC and antiviral activity. Compound **14** (4-(1-adamantyl)piperidine and its guanidine derivative **15** portrayed low micromolar blocking activity against the WT and V27A mutants of the influenza A M2 protein. Electrophysiological studies conducted on these compounds revealed that compound **14** binds strongly to the WT M2 channel and binds fast but weak to the V27A M2 channel. Compound **15** on the other hand is a fast and weak binder on both channels. Structure activity relationship studies identified that positioning the adamantane ring on the C-4 position of the piperidine ring improves the association rate of the compound to the M2 channel, and placing the piperidine ring on the C-1 position of the adamantane ring favors inhibitory activity. Anti-influenza virus activity screening of the compounds against the M2-WT A/HK/7/87 virus (H3N2 subtype) in a plaque reduction assay was carried out and compound **14** emerged as the most active compound with an EC_{50} value of $0.14 \mu M$ while compound **15** was inactive [101].

Zarubaev et al. in 2015 synthesized different compounds composed of the adamantane ring and heterocyclic azole (di-, tri- and tetra-) moieties, evaluated their cytotoxicity and inhibitory activity against the adamantane-resistant strain A/Puerto Rico/8/34.

Interestingly, of all the different compound classes synthesized, only the tetrazole compound **17** possessed antiviral properties. Structure activity relationship studies revealed that the position of the adamantyl group on the azole ring is critical for antiviral activity with the N-1 position being the preferred position with over 5-fold improved activity. Based on this study, it was observed that the presence of a free amino group is not a

prerequisite for anti-influenza activity and that combining a 5 or 6 membered amino heterocycle with adamantane also leads to compounds with more inhibitory activity [102].

A group of aminoadamantane heterocycles **18-20** were synthesized and evaluated for their antiviral activity against IAV (H3N2 subtype) by Zoidis and coworkers in 2009. Compound **18** was found to be slightly less active than amantadine but with over 4 fold increased activity when compared to ribavirin. Compound **19** on the other hand was the most active of all the synthesized compounds with better activity than amantadine, and similar activity to rimantadine in both antiviral and cytotoxicity evaluation [103].

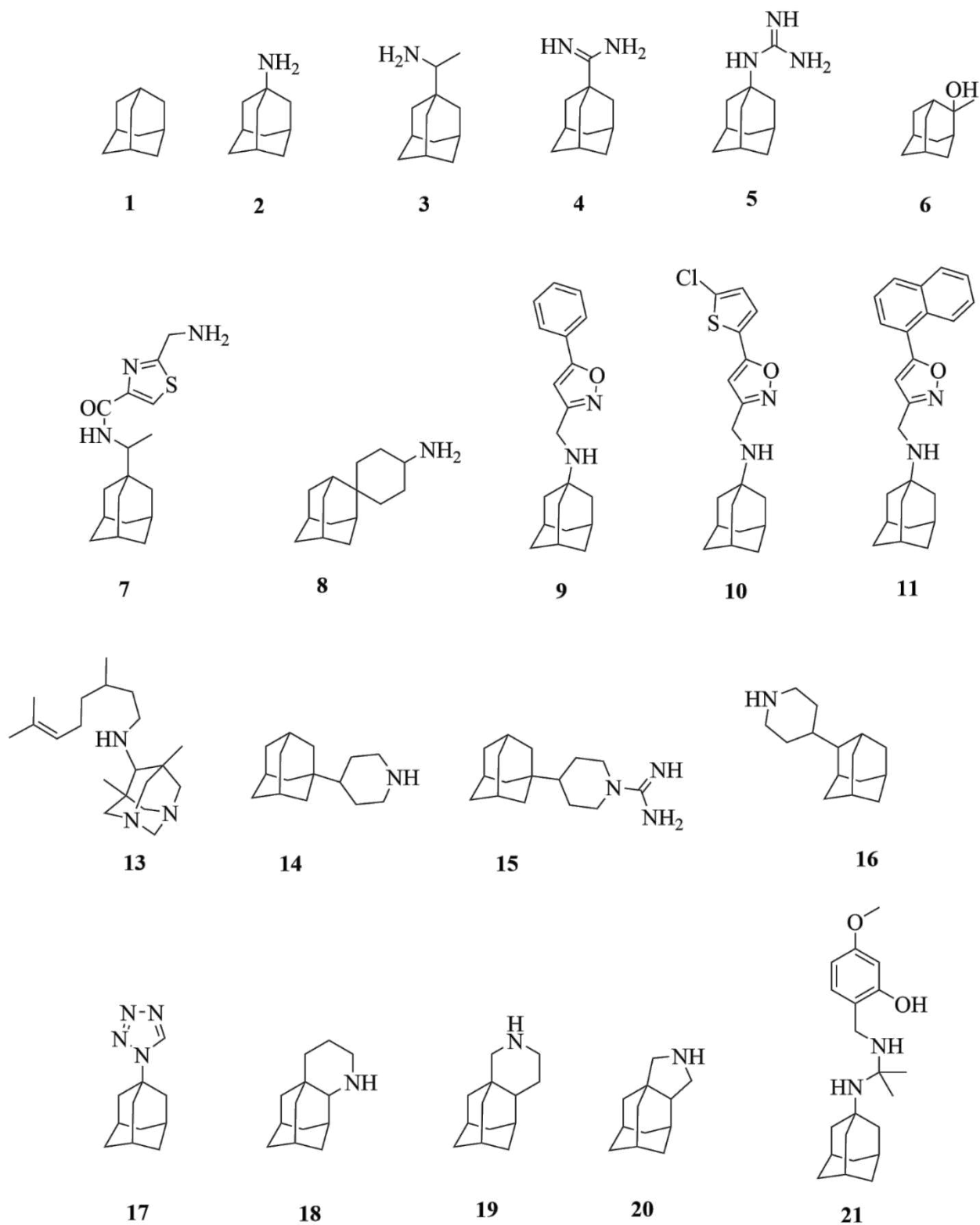


Fig. 3: Chemical structures of adamantane-containing compounds active against IAV M2.

Aiming to identify novel compounds capable of inhibiting multiple amantadine-resistant M2 mutants, several amantadine-aryl conjugates (**21-23**) were designed, synthesized and evaluated *in vitro* against influenza M2 WT and the five amantadine resistant viruses (S31N, L26F, V27A, A30T, and G34E). The CPE assay in MDCK cells was used to measure the inhibitory activity of the compounds, while TEVC was used to determine the direct blocking ability against the M2 protein. Compound **21** efficiently blocked the M2 WT (80.0%) and L26F (89.9%) channels, compound **22** (Fig. 4) blocked the M2 WT (81%) channel, while compound **23** blocked the M2 WT (81%), L26F (37%) and V27A (48.8%) channels. Compounds **21-23** also demonstrated improved anti-influenza properties in the *in vitro* antiviral assays bearing different M2 mutants with submicromolar EC_{50} (0.13 to 0.66 μ M) values against the M2 WT. At micromolar concentrations ($EC_{50} < 20 \mu$ M), compound **21** inhibited M2 L26F, S31N, G34E viruses, **22** inhibited the M2 L26F, S31N viruses and **23** the M2 L26F, SN31, A30T viruses. Intriguingly, some of these compounds portrayed *in vitro* inhibition but no M2 channel blocking property suggesting a probable alternative mechanism of antiviral activity [104].

In the quest to develop influenza AM2-inhibitors as oral influenza antivirals, Wang and coworkers optimized the *in vitro* pharmacokinetic properties of AM2-S31N inhibitors and identified many AM2-S31N inhibitors with remarkable channel blocking and antiviral activity. Guided by the NMR structure of the AM2-S31N protein (PDB ID: 2LY0) in complex with its native ligand, the mouse microsomal stability of the ligand was profiled and used as a guide to optimize the *in vitro* pharmacokinetic properties of the AM2-S31N inhibitors, which were designed and synthesized, with compound **24** being the most interesting with submicromolar antiviral activity ($EC_{50} < 1 \mu$ M) against 6 IAV strains containing the AM2-S31N mutant [A/Denmark/528/2009 (H1N1), A/Washington/29/2009 (H1N1), A/Texas/04/2009 (H1N1), A/North Carolina/29/2009 (H1N1), A/Switzerland/9715293/2013 (H3N2), and A/California/07/2009 (H1N1)], strong channel blocking ability, and good *in vitro* pharmacokinetic properties. However, compound **24** revealed a weak S31N M2 channel blockage activity (47.9 %) after 100 μ M of the compound was applied at the 2 minutes time point in the TEVC assay. This notable difference in channel blockage and antiviral activity was suggested to be most likely as a result of a slow-binding inhibitor which was further confirmed by kinetic studies on the M2 S31N channel [105].

Driven by an understanding of the structure and mechanism of drug inhibition of the AM2 protein, a set of organosilane-based AM2-S31N inhibitors were designed, synthesized and evaluated for their channel blockage (TEVC assay), antiviral activity (plaque assay) and cytotoxicity (neutral red assay). Compound **25** was found to be the most active compound blocking the AM2-S31N channel by $86.7 \pm 0.7\%$ after 2 minutes incubation with 100 μ M of the compound and portraying very good antiviral activity (single to submicromolar) against

all investigated multi-drug resistant strains (A/Denmark/528/2009 (H1N1), A/Washington/29/2009 (H1N1), and A/Texas/04/2009 (H1N1), with a high selectivity index of (SI) of 101.8 [106].

Structure-activity-relationship and molecular dynamics simulation studies on the influenza A M2 protein revealed that, irrespective of the mutant they target, strong M2 inhibitors generally possess a positively charged ammonium group which acts as a mimic of hydronium ions which form water-mediated hydrogen bond interactions with backbone carbonyls of the M2 protein. Also, M2 inhibitors bind via hydrophobic interactions to the M2 protein and 3-dimensional-shaped molecules are often preferred. Furthermore, studies of the NMR structure of M2 inhibitors indicated that M2 S31N inhibitors bind in a different orientation from M2 WT inhibitors. Taking into consideration these different points, dual inhibitors which target both the WT and S31N mutant of the influenza A M2 protein were designed, synthesized and tested in TEVC and plaque reduction assays. Compound **26** was the most active against influenza A M2 S31N and WT with 76% and 77% inhibition respectively, which is comparable to the inhibition of M2 WT by amantadine (91%) [107].

With the aid of structural information and medicinal chemistry approaches, a series of benzyl substituted amantadine derivatives were designed and synthesized by Wang et al. The compounds were evaluated for their inhibitory activity in TEVC and plaque reduction assays. The benzene-1,3-diol derivative **27** was the most active compound acting as a dual inhibitor of the M2 WT and S31N mutant. Compound **27** inhibited the S31N mutant in the plaque reduction assay with an EC_{50} value of 3.2 μ M which is about 7 fold more than amantadine (EC_{50} = 22.5 μ M). **27** also portrayed promising activity against the M2 WT (IC_{50} = 59 μ M) which is 3.7 fold less active than amantadine (IC_{50} = 16.1 μ M) [108].

In view of the fact that the adamantane scaffold and ammonium linker are key pharmacophores for M2 inhibition and replacement of the adamantane ring with other scaffolds leads to a decrease in channel inhibition activity, Li et al. while seeking to generate structurally diverse M2 inhibitors studied the structure activity relationships of M2-S31N inhibitors and designed a series of novel compounds by diversifying the aromatic head group and hydrophobic substitutions on M2 inhibitor. The compounds were synthesized and evaluated for their channel blocking and antiviral activity against influenza A M2 S31N virus using TEVC and antiviral assays. Compounds **28** and **29** were the most active compounds against all tested M2-S31N containing viruses, A/WSN/33 (H1N1), A/Switzerland/9715293/2013 (H3N2), and A/California/07/2009 (H1N1) [109].

Based on the privileged scaffold structure design strategy, a series of matrinic derivatives **30-36** bearing the 11-adamantyl group were designed, synthesized and tested for the ability to inhibit the wild-type IAV A/hanfang/359/95 (H3N2). Compound **31** portrayed promising

inhibitory activity with an IC_{50} value of 5.14 μ M and an SI value of 9.31, which was better than the control drug adamantine. Structure activity relationship studies revealed that connecting the adamantyl group at the C-11 position by an ester linker might be accountable for better activity [110].

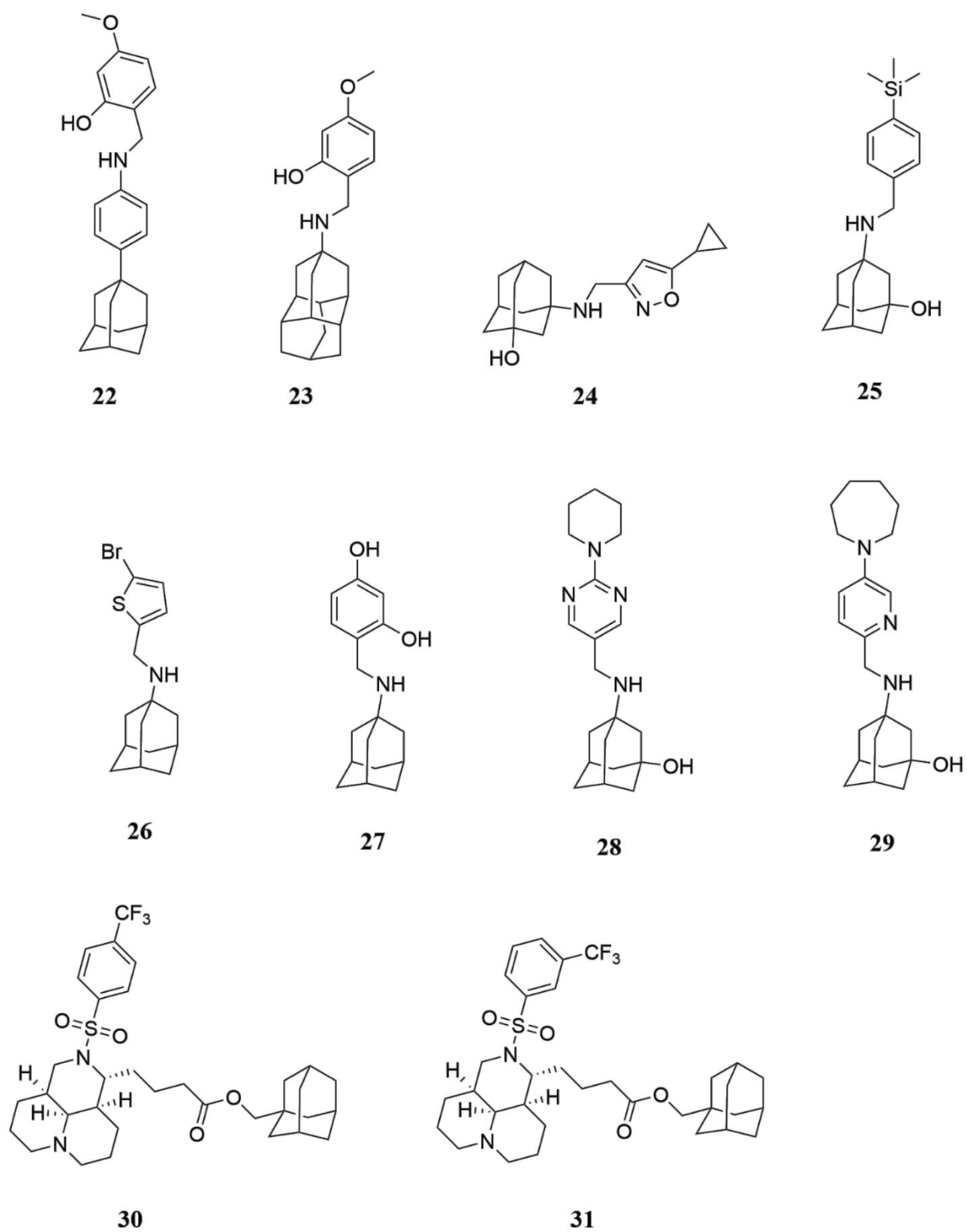
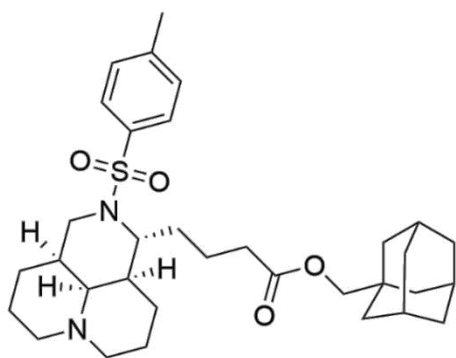
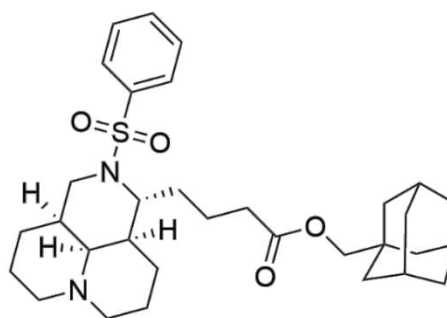


Fig. 4: Chemical structures of adamantane-containing compounds active against IAV M2.

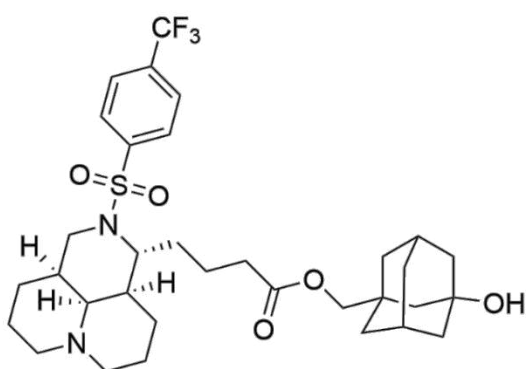
While the influenza A M2 ion channel remains the principal target of adamantane-based antivirals, evidence involving the application of the adamantane scaffold to other viral proteins involved in the influenza virus life cycle exist, opening new opportunities for the development of antiviral agents with alternative mechanisms of action. A number of novel adamantane-containing *N*-(1-thia-4-azaspiro[4.5]decan-4-yl)carboxamide compounds were synthesized and tested against influenza viruses A/PR/8/34(A/H1N1); A/X-31 (A/H3N2) and B/HK/5/72. The antiviral activity was evaluated by a cytopathic effect reduction assay using MDCK cells infected with the different viral strains and a hemolysis inhibition assay. Compound **37** (Fig. 5) was the most active compound with an EC₅₀ value of 1.4 μM against influenza A/H3N2 virus, and a strong inhibitory effect in the hemolysis assay suggesting **37** inhibits influenza virus fusion by preventing the low-pH-induced conformational change of hemagglutinin [111]. The results described in the preceding paragraphs have been summarized in Table 2.



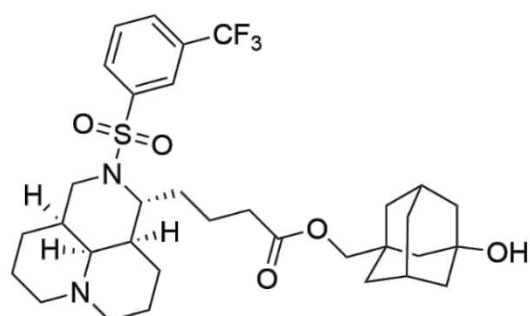
32



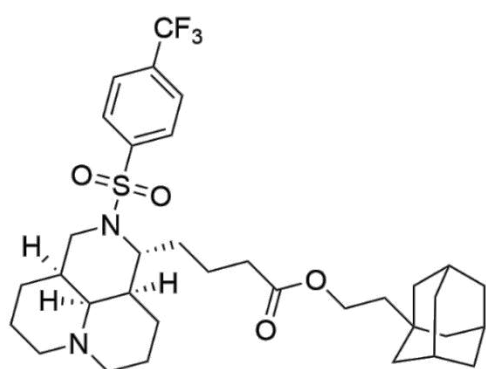
33



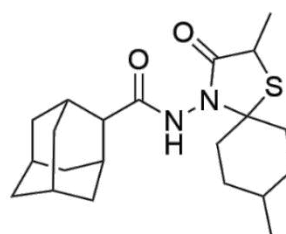
34



35



36



37

Fig. 5: Chemical structures of adamantane-containing compounds active against IAV M2.

Table 2: Summary of all the TEVC and antiviral results, including which subtypes of M2 or virus were assessed.

Compound	% Inhibition in TEVC	Antiviral activity, EC ₅₀ (μM)	IAV subtype
2	91.0 ± 2.1	0.337 ± 0.06	A/Albany/1/98 (H3N2)
3	92.7 ± 0.7	0.016	A/HK/7/87 (H3N2)
4	95 ± 1	IC ₅₀ : 4.0	A/Udorn/72 (H3N2)
5	97 ± 1	IC ₅₀ : 15.0	A/Udorn/72 (H3N2)
6	86 ± 2	IC ₅₀ : 14.0	A/Udorn/72 (H3N2)
7		IC ₅₀ : 0.11	A/Hong Kong/68 (H1N1)
8	89.1 ± 0.4 (WT), 95.2 ± 0.3 (V27A)	0.3 ± 0.1 (WT), 1.8 ± 0.2 (V27A)	A/Udorn/72 (H3N2)
9	91.2 ± 1.5	0.3 ± 0.1	A/California/07/2009 (H1N1)
10	78.8 ± 3.0	0.1 ± 0.03	A/California/07/2009 (H1N1)
11	56.8 ± 2.4		A/California/07/2009 (H1N1)
12	73.0 ± 1.4	21.95 (S31N), 11.58 (A30T)	A/WSN/33 (H1N1)
13		8 ± 2	A/Puerto Rico/8/34 (H1N1)
14	92.0 ± 1.4	0.14	A/HK/7/87 (H3N2)
15	91.8 ± 1.0		A/HK/7/87 (H3N2)

16	70.7 ± 2.4	>50	A/HK/7/87 (H3N2)
17		53.84	A/Puerto Rico/8/34 (H1N1)
18		4.1 ± 3.6	A/Hong Kong/ 7/87 (H3N2)
19		0.6 ± 0.4	A/Hong Kong/ 7/87 (H3N2)
20		0.5 ± 0.3	A/Hong Kong/ 7/87 (H3N2)
21	80.0 (WT), 89.9 (L26F)	0.13 ± 0.02 (WT), 1.80 ± 0.29 (L26F)	A/Udorn/72 (H3N2)
22	81 (WT)	0.66 ± 0.21 (WT)	A/Udorn/72 (H3N2)
23	81 (WT), 37 (L26F), 48.8 (V27A)	0.29 ± 0.11 (WT)	A/Udorn/72 (H3N2)
24	47.9 ± 1.0 (S31N)	0.8 ± 0.05	A/North Carolina/29/2009 (H1N1)
25	86.7 ± 0.7 (S31N)	0.4 ± 0.2	A/WSN/33 (H1N1)
26	76 (S31N), 77 (WT)	1.8 (S31N)	A/WSN/33 (H1N1)
27	67 (S31N)	3.2 (S31N)	A/WSN/33 (H1N1)
28	67.2 ± 1.5	4.3 ± 0.3	A/WSN/33 (H1N1)
29	85.8 ± 0.9	2.7 ± 0.1	A/WSN/33 (H1N1)

30		21.1 ± 3.91	A/hanfang/359/95 (H3N2)
31		5.14 ± 0.4	A/hanfang/359/95 (H3N2)
32		16.2	A/hanfang/359/95 (H3N2)
33		16.3 ± 1.83	A/hanfang/359/95 (H3N2)
34		9.53 ± 0.47	A/hanfang/359/95 (H3N2)
35		11.1 ± 1.84	A/hanfang/359/95 (H3N2)
36		16.9 ± 1.55	A/hanfang/359/95 (H3N2)
37		1.4	A/X-31 (A/H3N2)

Non-Adamantane M2 channel blockers

Extensive medicinal chemistry efforts have been devoted to identifying A/M2 channel inhibitors with improved activity and reduced side effects. However, very few molecular cores other than the adamantane scaffold have been studied for A/M2 channel inhibition. This section of this work reports some of the non-adamantane compounds which have been reported to portray interesting inhibitory activities against the influenza M2 protein.

Motivated by the lack of a robust assay for the screening of novel compounds, Balgi et al adapted a yeast growth inhibition assay, in which yeast growth was inhibited by expression of the M2 channel and restored by exposure to an M2 inhibitor, into an efficient high-throughput screen for M2 inhibitors. Thousands of compounds were screened and 21 active compounds were identified, some of which are non-adamantane containing compounds (**38-42**) (Fig. 6). The non-adamantane compounds **38** and **43** (hexamethylene amiloride and a triazine derivative respectively), both also revealed antiviral activity against influenza A/Udorn/72 in MDCK plaque reduction assay [112].

The screening of thousands of compounds using a yeast-based assay by Arns and coworkers led to the identification of the spirothiazamenthane compound **43** with similar M2 inhibitory activity to amantadine against the influenza A WT. Several derivatives of compound **43** were then synthesized and tested against M2 WT, V27A and S31N strains in yeast assay and further in an antiviral assay composed of engineered WT, V27A and S31N

M2 influenza A/Puerto Rico/8/34 (PR8) strains. Compound **44** revealed micromolar inhibitory activity against all three strains in the antiviral assay [113].

A series of pyrrolidine derivatives were designed as analogues of amantadine, synthesized and evaluated for their inhibitory activity against the influenza A M2/WT and other mutants (S31N, V27A and L26F). The compounds were tested using a TEVC technique on influenza A M2 channels expressed in *Xenopus oocytes* and their IC₅₀ values measured in an isochronic inhibition assay. Most of the compounds inhibited the M2 WT channel in low micromolar range with compounds **45** and **46** also inhibiting the amantadine-resistant mutants A/M2-V27A and A/M2-L26F with submicromolar and micromolar IC₅₀ values, respectively [114].

A number of compounds containing the azatetracyclo[5.2.1.15,8.01,5]undecane scaffold were designed and synthesized as analogues of amantadine. They were tested for their ability to inhibit the influenza A M2 WT channel as well as other amantadine-resistant mutants (A/M2-S31N and A/M2-V27A) in *Xenopus oocytes* using two-electrode voltage clamp (TEV) assays. None of the synthesized compounds exhibited inhibitory activity against the A/M2 S31N mutant channel. However, most compounds demonstrated low micromolar inhibitory activity against the wild-type A/M2 channel, and several also inhibited the A/M2 V27A mutant, with compound **47** displaying submicromolar activity against the V27A channel [115].

In search for new classes of M2 inhibitors, structure activity relationship studies were conducted on the 2-[3-azaspiro(5,5)undecanol]-2-imidazoline compound class and a series of compounds were synthesized and tested for their ability to inhibit the full length Udorn AM2 protein as measured by TEVC. Interestingly, the spiro-piperidine compound **48** emerged as the most active compound with an IC₅₀ value of $0.92 \pm 0.11 \mu\text{M}$. SSNMR studies revealed that compound **48** interacts with the AM2 TM protein by inducing a homogeneous conformation on the peptide and immobilizing the G34-I35 region which is a hot spot for conformational changes [116].

Further structural modifications conducted by Balannik et al. on the 2-[3-azaspiro(5,5)undecanol]-2-imidazoline compound class led to the identification of a triple inhibitor **49** (Fig. 6) of the influenza A M2 WT, L26F and V27A variants. The compounds were tested in vitro on AM2 channels expressed in *Xenopus oocytes* using TEVC and their inhibitory effects confirmed through an in vivo plaque reduction of IAV (A/Udorn/72). Compound **49** revealed promising inhibitory properties against the AM2 WT, L26F and V27A mutants as well as the replication of mutant viruses carrying these mutants in the plaque reduction assay [117].

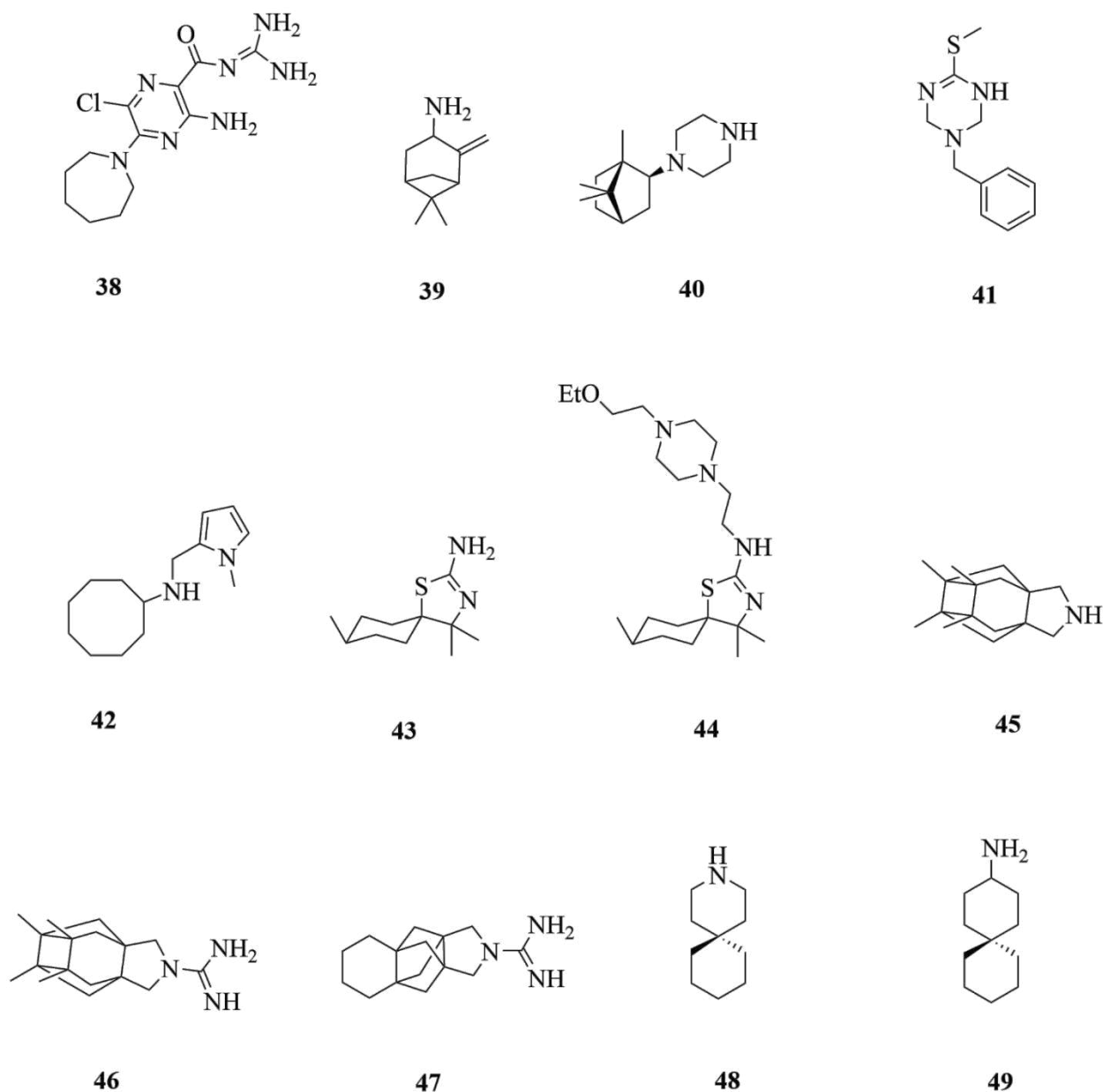


Fig. 6: Chemical structures of non adamantane-containing compounds active against IAV M2.

A small primary amine library (consisting of 70 compounds) based on the amantadine scaffold was designed using scaffold-hopping technique by Hu et al and the compounds were screened via cell viability and viral inhibition assays on the influenza A/WS/33 (H1N1, amantadine resistant) and A/Hong Kong/8/68 (H3N2, amantadine sensitive) viruses. Compound **50** (Fig. 7) was found to be the most active compound with comparable inhibitory activity to amantadine against the influenza A M2 WT in the cell based assay

while compounds **51-53** inhibited the amantadine sensitive strain of in the viral inhibition assay with IC₅₀ values of 6.02, 1.36 and 2.30 respectively. None of the identified compounds were active against the amantadine resistant virus A/WS/33 (H1N1) [118].

While seeking to identify alternative chemotypes for M2 inhibitors other than the adamantane scaffold, camphor derivatives were synthesized and evaluated against WT IAV/Hong Kong/68 using a plaque reduction assay. The compounds **54-55** revealed inhibitory activities against the M2 WT with IC₅₀ values ranging 0.10 ± 0.05 to 0.49 ± 0.16 µM. Compound **54** was found to be 5 folds more active than amantadine which inhibited the same assay with an IC₅₀ value of 0.50 ± 0.16 µM. Molecular docking studies conducted on the compounds indicated that compound **54** occupies a greater space with better interactions in the M2 channel when compared to amantadine. The compounds were further tested for their cytotoxicity in MDCK cells and all were found to be nontoxic with the least CC₅₀ >300 µM [119].

Guided by molecular dynamics simulation studies on the mechanism and influence of drug binding on the influenza A M2 protein, a series of spiroamine compounds **57-61** were designed and synthesized as potential M2 inhibitors. Ion channel inhibition was measured in *Xenopus laevis* oocytes expressing WT A/M2 or A/M2-V27A and antiviral activity confirmed via a plaque reduction assay on the influenza A virus (A/Udorn/72). The spiroamine compounds revealed quite promising inhibitory activity against both the M2 WT and V27A mutants with compound **59** showing over 2-fold increased activity against the M2-WT when compared to amantadine and also good activity against the AM2-V27A mutant [96].

Silicon-containing compounds generally lack the toxicities commonly associated with heavy metals and exhibit metabolic profiles comparable to those of their carbon-based analogues. They also possess properties such as increased size and hydrophobicity which are vital in designing A/M2 inhibitors. Considering these points, organosilane silane amine compounds were designed, synthesized and tested against the influenza A M2 WT and the drug-resistant A/M2-V27A mutant by Wang et al. Structure activity relationship studies revealed that replacing the quaternary carbon of spirane amine with silicon resulted in an improvement in M2 activity. For instance, compounds **62** and **63** were found to be over 2.7 and 3.3 fold more active against the A M2 V27A mutant respectively when compared to their corresponding carbon analogues **64** and **65** [120].

A series of imidazole-linked pinanamine compounds were designed, synthesized and tested for their activity as M2 blockers. The cytopathic effect (CPE) assay and an MTT cell viability test were used to investigate the antiviral properties of the compounds on the influenza A/HK/68 strain containing an A/M2-WT protein, and the A/WSN/33 strain

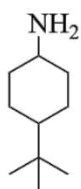
containing A/M2-S31N mutant. Compound **66** demonstrated dual inhibitory activity with low micromolar concentrations (IC_{50} values of 2.5 ± 0.2 and $3.4 \pm 0.5 \mu M$) against WT IAV (A/HK/68) and S31N (A/WSN/33) respectively. Compound **66** also blocked the A M2-WT ion channel at $100 \mu M$ by $97.9 \pm 0.2 \%$ and the A M2 S31N by $3.47 \pm 0.31 \%$. These results suggest that blocking of the M2 channel is not the primary mechanism of action of compound **66** against the amantadine-resistant strain [121].

Aiming to develop non-adamantane compounds capable of inhibiting the A-M2 S31N mutant, a series of pinane oxime derivatives were designed and synthesized by Dong et al. The compounds were tested for their in vitro antiviral activity against IAV (A/Hong Kong/8/68) strain (H3N2, M2-WT) and A/WS/33 strain (H1N1, M2-S31N) using a patch clamp assay and their cytotoxicity ascertained via MTT assay using Madin Darby canine kidney (MDCK) cells. Compound **67** emerged as the most active inhibitor with an EC_{50} value of $3.29 \pm 0.10 \mu M$ and $2.45 \pm 0.06 \mu M$ against H3N2 and H1N1 respectively. Molecular docking studies conducted on compound **67** revealed that it binds tightly to the drug binding via the formation of hydrophobic interactions, halogen and hydrogen bonds with the M2 protein [122].

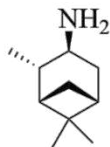
A high-throughput screening campaign using a chemical library of over 2000 synthetic compounds led to discovery of salinomycin **68** as a notable inhibitor of influenza viruses A and B. The compounds were screened in a plaque reduction assay against influenza viruses A/Puerto Rico/8/1934 (PR8; H1N1), A/Hong Kong/8/1968 (HK; H3N2), and B/Lee/1940 (Lee) to investigate antiviral activity and were further tested for their ion channel blocking activity in purified retroviral Gag-based virus-like particles (VLPs). **68** revealed antiviral activity against influenza viruses A and B with EC_{50} values ranging from 0.4 to $4.3 \mu M$, and acts by preventing M2-mediated proton transfer from virus-containing endosomes into the virion. Furthermore, an improved antiviral effect was observed upon administration of salinomycin together with oseltamivir in a mouse model, than when administered alone [123].

Duncan et al. conducted virtual screening of a library of natural product compounds derived from marine and terrestrial sources, and this resulted in the identification of compound **69** (chebulagic acid) as an active M2-S31N inhibitor. The compounds were tested for their M2 inhibitory activity using a yeast growth restoration assay on *Saccharomyces cerevisiae* strains bearing plasmid of M2(S31N) or M2(S31) from the Udorn strain of influenza A. Compound **69** restored yeast growth of M2(S31N)-expressing yeast at $25 \mu g/mL$ by $29.5 \pm 4.4\%$ suggesting that it acts by interfering with the adverse effects of M2 on yeast growth. **69** was further tested for its antiviral activity in a plaque reduction assay against A/Puerto Rico/8/34 (PR8) strains. Interestingly, **69** was found to inhibit both the M2

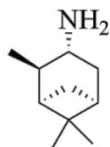
S31N and M2 S31 strains of the virus with EC_{50} values of $60.9 \pm 22.0 \mu\text{M}$ and $50.3 \pm 26.4 \mu\text{M}$ respectively [124]. The findings discussed in the preceding paragraphs are summarized in Table 3.



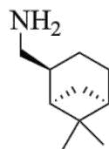
50



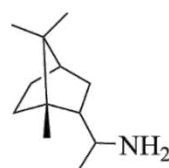
51



52



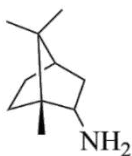
53



54



55



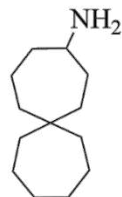
56



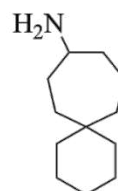
57



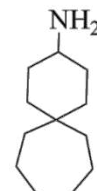
58



59



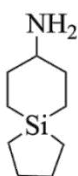
60



61



62



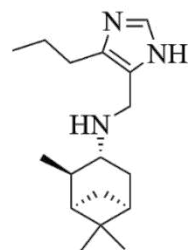
63



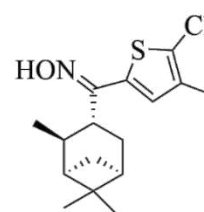
64



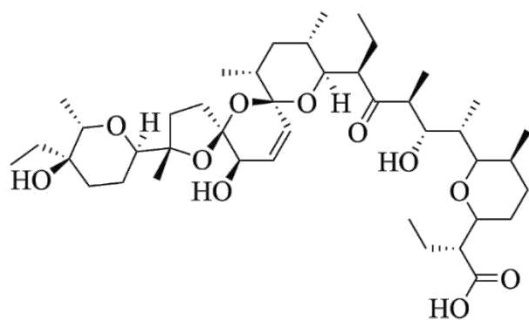
65



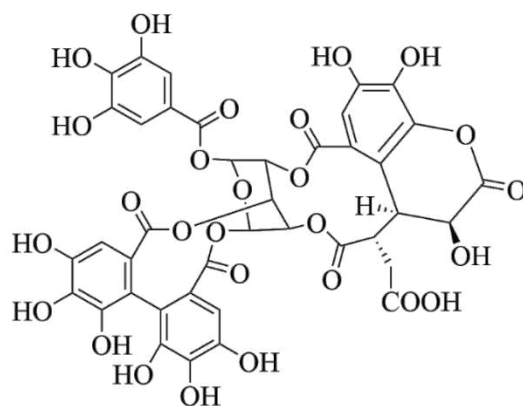
66



67



68



69

Fig. 7: Chemical structures of non adamantane-containing compounds active against IAV M2.

Table 3: Summary of all the TEVC and antiviral results, including which subtypes of M2 or virus were assessed.

Compound	% Inhibition in TEVC	Antiviral activity, EC_{50} / $IC_{50}(\mu M)$	IAV subtype
38	89.9 $\pm$ 0.7	1.1	A/Udorn/72 (H3N2)
39		0.5	A/Udorn/72 (H3N2)
40		5.0	A/Udorn/72 (H3N2)
41		12	A/Udorn/72 (H3N2)
42		30	A/Udorn/72 (H3N2)
43		0.03	A/Puerto Rico/8/34 (H1N1)
44		2.1	A/Puerto Rico/8/34 (H1N1)
45	90.2 $\pm$ 0.9 (WT), 96.4 $\pm$ 0.5 (V27A), 90.6 $\pm$ 1.1 (L26F)	1.8	A/PR/8/34 (H1N1)
46	91.7 $\pm$ 1.2 (WT), 96.7 $\pm$ 0.5 (V27A), 88.4 $\pm$ 0.6 (L26F)	>100	A/PR/8/34 (H1N1)
47	93.1 $\pm$ 2.5 (WT), 93.8 $\pm$ 0.9 (V27A)		A/Udorn/72 (H3N2)
48		0.92 $\pm$ 0.11	A/Udorn/72 (H3N2)

49		12.6 (WT), 84.9 (V27A)	A/Udorn/72 (H3N2)
50		3.37	A/Hong Kong/8/68 (H3N2)
51		6.02	A/Hong Kong/8/68 (H3N2)
52		1.36	A/Hong Kong/8/68 (H3N2)
53		2.30	A/Hong Kong/8/68 (H3N2)
54		0.10 ± 0.05	A/Hong Kong/8/68 (H3N2)
55		0.29 ± 0.10	A/Hong Kong/8/68 (H3N2)
56		0.49 ± 0.16	A/Hong Kong/8/68 (H3N2)
57		3.3 ± 0.2 (WT)	A/Udorn/72 (H3N2)
58		12.6 ± 1.2 (WT)	A/Udorn/72 (H3N2)
59		8.7 ± 0.6 (WT), 11.3 ± 0.7 (V27A)	A/Udorn/72 (H3N2)
60		13.2 ± 1.0 (WT)	A/Udorn/72 (H3N2)
61		13.0 ± 1.1 (WT)	A/Udorn/72 (H3N2)
62	94.5 ± 0.6(WT), 67.4 ± 1.1 (V27A)	13.7 ± 1.7 (WT)	A/Udorn/72 (H3N2)
63	93.9 ± 1.8 (WT), 47.8 ± 0.5 (V27A)	7.8 ± 0.6 (WT)	(H3N2)

64	89.0 ± 1.5 (WT), 53.2 ± 2.3 (V27A)	12.6 ± 1.1 (WT)	(H3N2)
65	95.9 ± 0.9 (WT), 25.2 ± 0.9 (V27A)	3.3 ± 0.2 (WT)	(H3N2)
66	97.9 ± 0.2 (WT)	2.5 ± 0.2 (WT), 3.4 ± 0.5 (S31N)	A/Hong Kong/8/68 (H3N2), A/WSN/33 (H1N1)
67		3.29 ± 0.10 (WT), 2.45 ± 0.06 (S31N)	A/Hong Kong/8/68 (H3N2), A/WS/33 (H1N1)
68		0.7 ± 0.1, 0.7 ± 0.1 (0.4 ± 0.1)	A/Puerto Rico/8/34 (H1N1), A/Hong Kong/8/1968 (H3N2)
69		60.9 ± 22.0 (S31N), 50.3 ± 26.4 (S31)	A/Puerto Rico/8/34 (H1N1)

Computational and Structure-Based Design Approaches as Techniques for the Identification of Chemically Diverse Leads

Adamantanes and their derivatives have gained huge attention by medicinal chemists over the years owing to their unique physicochemical chemical properties and structural backbone. Replacing phenyl rings with adamantane or its derivatives offers a promising approach to enhance lipophilicity while evading the predominantly planar chemical space of modern drug discovery. Furthermore, several key properties associated with adamantanes include their 3-dimensional cage, lipophilicity, rigidity and high symmetry [125, 126]. The widespread emergence of amantadine- and rimantadine-resistant IAV strains has significantly limited the utility of adamantane-based antivirals, underscoring the need to identify structurally diverse chemotypes beyond the adamantane scaffold. Accordingly, the development and application of complementary drug-discovery strategies capable of identifying novel IAV inhibitors with distinct chemical scaffolds is essential for expanding the antiviral chemical space and overcoming existing resistance. The following section highlights representative approaches and case studies that illustrate how some of these strategies have contributed to the discovery and optimization of non-adamantane M2 inhibitors [127].

The application of computer-aided drug design techniques in the discovery of bioactive molecules cannot be emphasized. Computational techniques facilitate the exploration of a chemical space and save time by providing insights on structural and pharmacophoric requirements for ligands and biological targets. We will be looking at some computational techniques which have been implemented and proven to be quite useful in the identification of influenza A M2 inhibitors.

Tran and collaborators employed docking-based virtual screening and pharmacophore modeling to identify novel influenza A M2 inhibitors. 200 new compounds were designed and virtual screening conducted to evaluate the free energy of binding of the designed compounds and the M2 channel protein. Next, pharmacophore modeling was carried out on the top binding compounds to identify common features and key interactions between the ligands and the target protein. These analyses led to the identification of 2 novel compounds (Fig. 8) with promising influenza A M2 H3N2 and 2009-H1N1 inhibitory activity requiring further investigation [128].

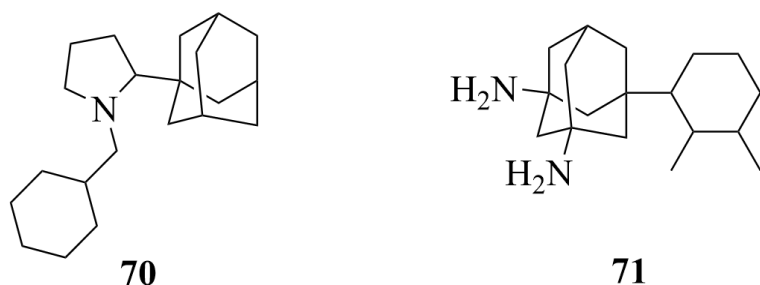


Fig. 8: Chemical structures of predicted M2 inhibitors identified by virtual screening and pharmacophore modeling.

Virtual screening of natural product derived libraries led to the identification of an influenza A M2 S31N inhibitor by Duncan et al. First, a set of reported adamantane and non-adamantane M2 S31N inhibitors was compiled and subjected to pharmacophore analysis to identify shared molecular features potentially responsible for M2 S31N inhibition. The common pharmacophore features were used in the virtual screening of the natural product compound libraries resulting in the identification of a number of compounds which were evaluated for their *in vitro* M2 S31N inhibitory activity using a yeast growth restoration assay. These studies led to the identification of chebulagic acid **69** (Fig. 7), which selectively restored the growth of yeast expressing M2 S31N. Molecular docking studies were conducted on chebulagic acid to better understand how it interacts with the M2 protein (PDB ID: 2LY0). Given the large size of the compound, molecular docking studies on 2 predicted hydrolysis reaction products of the compound (the galloyl and chebuloyl units)

(Fig. 9). These analyses revealed that **69** interacts with the highly conserved His 37 amino acid residue within the pore of the M2 S31N channel [124].

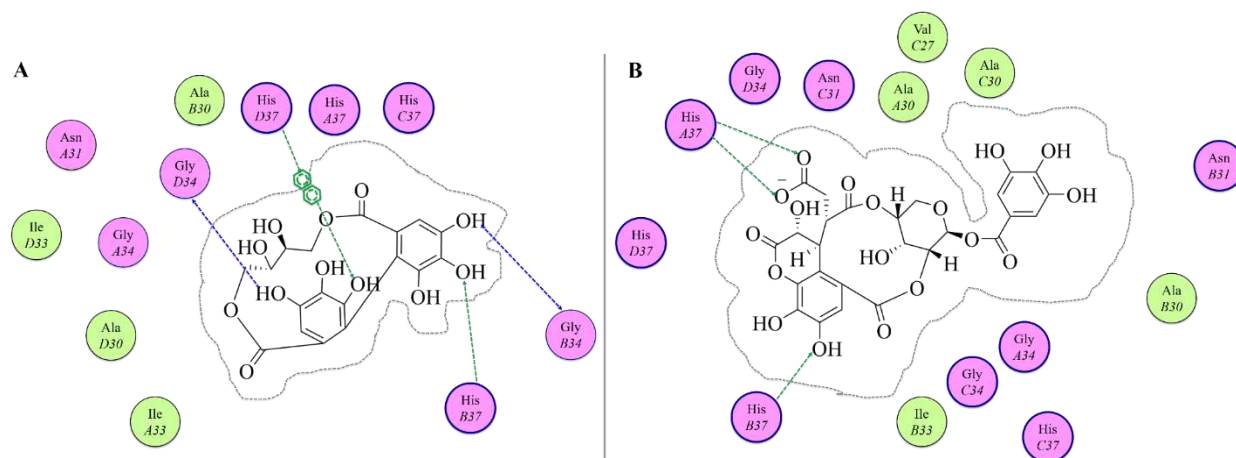


Fig 9: Two-dimensional protein–ligand interaction diagrams illustrating the interactions of the chebulagic acid units with the M2 S31N protein: (A) galloyl unit and (B) chebuloyl unit [124].

Wang and coworkers combined molecular dynamics and classical medicinal chemistry approaches to design some M2 V27A and L26F inhibitors. Molecular dynamics simulations were conducted on the influenza A M2 protein (PDB ID: 3LBW) in complex with amantadine, to better understand the mode of binding and mechanism of proton conduction. Based on the results obtained from the MD simulation studies, they designed a series of novel compounds which were synthesized and found to be active inhibitors of the influenza A M2, WT, V27A and L26F mutants **57-61** (Fig. 7) [96].

An integrated virtual screening and structure-guided molecular growth strategy employed by Deng et al. led to the identification of a potential inhibitor of the influenza A M2-V27A/S31N double mutant. The authors initially conducted pharmacophore-based virtual screening of the ZINC15 database using a pharmacophore model incorporating the key features of amantadine. The resulting top-ranked hits were subsequently subjected to molecular descriptor-based filtering to identify compounds with physicochemical properties comparable to those of amantadine, leading to the selection of a promising starting scaffold **72** (Fig. 10). Its binding mode within the M2-V27A/S31N channel was then evaluated through molecular docking followed by 10 ns molecular dynamics (MD) simulations. The simulations indicated that the ligand was accommodated within the binding pocket but did not fully exploit the available interaction space, suggesting opportunities for structural optimization. Structure-guided molecular growth was therefore employed to extend the scaffold and enhance interactions with the binding site, ultimately leading to the identification of a potential M2-V27A/S31N inhibitor **73-76** [129].

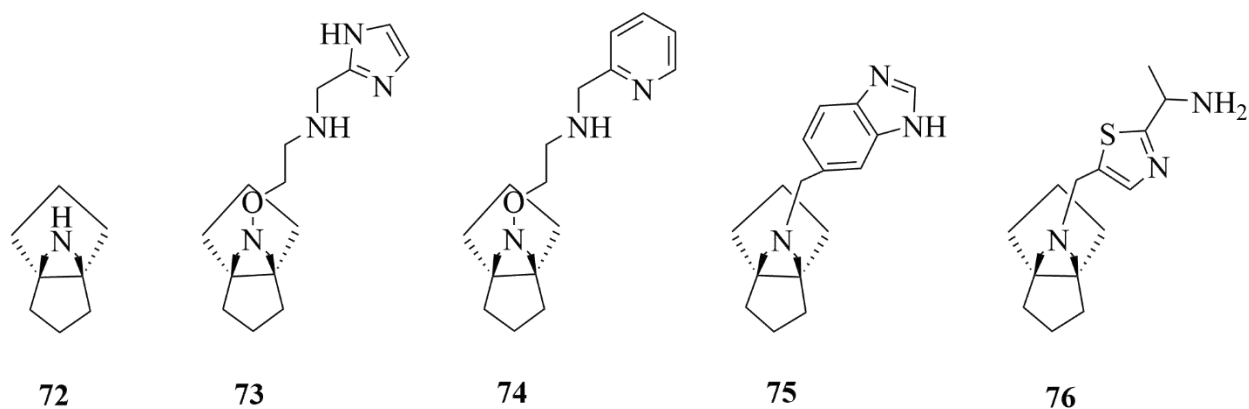


Fig. 10: Chemical structures of compounds obtained after pharmacophore modeling and molecular growth.

High-throughput screening of a library of over 250,000 pure and semi-purified fractions from natural product extracts was conducted on a developed yeast growth restoration assay by Balgi et al. They adapted a yeast growth assay, such that expression of M2 channel inhibits yeast growth meanwhile growth restoration occurs in the presence of an M2 inhibitor. This study led to the identification of 6-non adamantane compounds **38-42** (Fig. 6) with good M2 inhibitory activity [112].

Sulli and collaborators developed a free-cell ion channel assay by incorporating M2 ion channels into virus-like particles (VLPs) while measuring the proton conductance across the viral lipid bilayer, membrane potential and ion channel function. High-throughput screening of 100,000 compounds was then carried out on this assay resulting in the identification of 2 M2 inhibitors with novel chemotypes **77-78** (Fig. 11). The compounds portrayed good M2 inhibitory properties against the adamantane-sensitive strain HK/156/1997 as well as inhibition of live influenza virus (Udorn/307/1972) in plaque reduction assay [130].

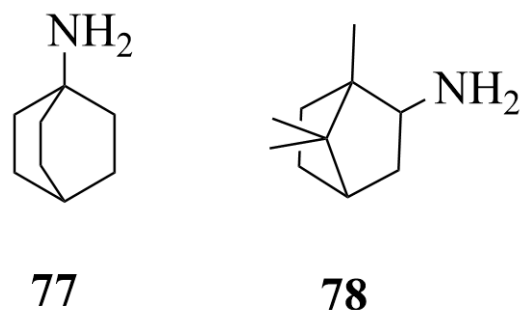


Fig. 11: Chemical structures of compounds obtained by M2-VLP High-Throughput Screening

Radošević et al. employed virtual screening 2,627 approved drugs from the DrugBank database as a drug-repurposing strategy, leading to the identification of guanethidine as a dose-dependent inhibitor of the influenza A/CA/07/2009 (H1N1) virus. Prior to *in silico* screening, a learning set comprising compounds previously reported as active against both wild-type (WT) and S31N mutant influenza A M2 proteins was assembled to establish predictive criteria for compound selection. The compounds in the learning set and DrugBank were first subjected to EIIP (Electron-Ion Interaction Potential)/AQVN (Average Quasivalence Number)-based filtering, followed by ligand-based virtual screening. The highest-ranked candidates were subsequently evaluated by molecular docking against both influenza A M2 WT and S31N proteins, with docking scores used to prioritize potential inhibitors. Guanethidine **79** (Fig. 12) emerged as the top-ranked candidate, and subsequent *in vitro* studies confirmed its inhibitory activity against the influenza A/CA/07/2009 (H1N1) strain [131].

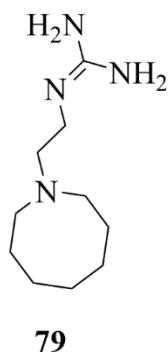


Fig. 12: Chemical structure of the M2 inhibitor obtained after virtual screening of approved drugs.

Challenges and Future Perspectives

Despite substantial progress in the discovery of chemically diverse M2 ion channel blockers beyond the classical adamantane framework, the development of clinically viable M2-targeted antivirals remains challenging. The identification of novel chemical scaffolds capable of inhibiting drug-resistant M2 variants represents an important advancement in this research area. However, strong M2 channel inhibition alone is insufficient to ensure successful antiviral development as there exist several other interconnected challenges hindering the translation of activity observed in biochemical or cellular models into meaningful *in vivo* efficacy. Addressing these limitations will require an integrated approach that combines structural and mechanistic studies with medicinal chemistry, resistance profiling, advanced computational methods, and rigorous pharmacological

evaluation. In this context, future development should focus not only on expanding the chemical diversity of M2 inhibitors but also on establishing design principles that can deliver broad-spectrum, resistance-resilient, drug-like, and clinically effective M2 channel blockers. In this section of the work, we will be looking at some of these challenges and possible ideas on how to navigate through them.

Resistance to existing M2 inhibitors represents one of the major challenges in the development of novel M2-targeted antiviral agents. This resistance is primarily associated with mutations within the transmembrane domain of the M2 channel. Given the tetrameric architecture of the protein, a single mutation in a pore-lining residue is propagated across the four equivalent subunits, thereby substantially altering the physicochemical environment of the inhibitor-binding site. Among the numerous resistance-associated mutations identified in circulation, the S31N mutant is particularly prominent and present in the majority of currently circulating transmissible amantadine-resistant IAV strains. Replacement of serine with the bulkier asparagine residue at position 31 alters the steric and physicochemical characteristics of the channel pore and partially occupies the adamantane-binding region, thereby substantially reducing inhibitor binding region. Consequently, the S31N variant represents a major obstacle in M2 inhibitor discovery and is considered a highly challenging target for structure-based antiviral development [127].

The M2 protein is a dynamic membrane protein which changes conformations depending on pH, bilayer thickness and drug binding. This poses a challenge in drug design because a ligand may appear to fit a static structure in computational studies but fail to block the channel experimentally. Furthermore, because the M2 protein is an ion channel, effective inhibition doesn't rely solely on if the ligand binds to the pore, but also on if proton conductance is disrupted upon ligand binding [130]. An understanding of what happens to the ligand once it binds to the pore is quite essential in the development of novel M2 inhibitors, and ensemble docking and molecular dynamics simulation studies are useful approaches which could enhance this process.

Another major challenge in the development of M2 ion channel blockers is the translation of channel inhibition into meaningful antiviral efficacy. Although electrophysiological and biochemical assays provide valuable information regarding the ability of a compound to bind to and block M2-mediated proton conductance, activity in these simplified systems does not necessarily predict antiviral activity in infected cells or therapeutic efficacy *in vivo*. A number of factors such as membrane composition, M2 conformational state, cellular uptake, physicochemical properties, compound stability, and viral genotype can all influence the relationship between channel activity and antiviral activity. Hence, a stepwise

strategy involving channel assays, cellular antiviral studies and assessment against clinically relevant M2 variants is important [132].

Most influenza A M2 ion channel blockers are designed to target the predominant S31N mutant, although activity against this mutant does not guarantee broad spectrum antiviral activity. There exist other naturally occurring M2 transmembrane mutations, including L26F, V27A, A30T, and G34E, which also pose a health threat requiring attention. Therefore, in developing novel M2 inhibitors, resistance profiling should be introduced at an early stage in the discovery process and candidate compounds should be screened against multiple resistance-associated mutations as well as recombinant influenza viruses bearing these mutations [133].

Studies of the M2 transmembrane protein revealed multiple regions of drug binding including the central pore and the lipid-facing sites [36]. Expanding beyond the classical adamantane core and exploring alternative binding regions could be a potential technique in tackling this highly challenging M2 protein. Hence, future M2 inhibitor discovery could seek to design novel scaffolds with unique chemotypes other than the adamantane scaffold which binds to other unexplored binding sites of the M2 protein.

Conclusion

The influenza A M2 protein is a highly promising biological target in the quest for anti influenza compounds. The emergence of widespread adamantane-resistance has fueled the quest for the discovery of chemically diverse M2 ion channel blockers beyond the adamantane scaffold. Based on the studies conducted and reported in literature, the M2 protein can be targeted via alternative chemotypes, binding interactions and design techniques aimed at targeting numerous resistance associated mutations. Nevertheless, owing to the dynamic nature of the protein, membrane environment, and drug resistance, translating M2 channel inhibition to antiviral activity still remains a major challenge. This review summarizes recent advances in influenza A M2 ion channel inhibitors, emphasizing non-adamantane chemotypes as promising strategies to address antiviral resistance. It further discusses emerging design strategies for expanding chemical diversity and overcoming key limitations in M2 inhibitor discovery and development. Hence, future efforts focused on identifying chemically distinct scaffolds with broad spectrum activity and improved pharmacological properties, involving an integration of efforts from structural biology, computational design, screening, and medicinal chemistry could go a long way in the development of next generation M2 blockers with better therapeutic applications.

Funding:

We acknowledge the German Academic Exchange Services (DAAD) for the doctoral scholarship awarded to VAS (grant number: 57730835). FNK acknowledges the Calestous Juma Science Leadership Fellowship from the Gates Foundation (award number: INV-036848)

Conflicts of interest:

The authors declare no competitive or financial conflict of interest.

Author contributions:

V.A.S. wrote the main manuscript text with support from I.T., W.S., and F.N.K. All authors reviewed the manuscript.

References

1. Dawood, F. S. et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. *N Engl J Med* 360, 2605–2615 (2009).
2. Li, K. S. et al. Genesis of a highly pathogenic and potentially pandemic H5N1 influenza virus in eastern Asia. *Nature* 430, 209–213 (2004).
3. World Health Organization. Influenza (seasonal). (2025).
4. Zhang, W. et al. Research priorities for zoonotic and pandemic influenza vaccines: evidence and recommendations from the WHO Public Health Research Agenda for Influenza (2024 update). *Vaccines* 13, 1206 (2025). doi.org/10.3390/vaccines13121206.
5. Bright, R. A. mRNA and next-generation vaccine platforms for pandemic influenza preparedness. *Vaccines* 14, 247 (2026). doi.org/10.3390/vaccines14030247.
6. Russell, C. A. et al. Seasonal influenza vaccine performance and the potential benefits of mRNA vaccines. *Hum. Vaccin. Immunother.* 20, 2336357 (2024). doi.org/10.1080/21645515.2024.2336357.
7. Hamamoto, I. Developments and current challenges in the process of cell culture-based seasonal influenza vaccine manufacture in Japan. *Glob. Health Med.* 6, 93–100 (2024). doi.org/10.35772/ghm.2023.01070.
8. Hause, B. M. et al. Characterization of a novel influenza virus in cattle and swine: proposal for a new genus in the Orthomyxoviridae family. *mBio* 5, e00031-14 (2014).
9. Chiapponi, C. et al. Detection of influenza D virus among swine and cattle, Italy. *Emerg Infect Dis* 22, 352–354 (2016).

10. Mostafa, A. et al. Zoonotic potential of influenza A viruses: a comprehensive overview. *Viruses* 10, 497 (2018).
11. Belshan, M. A., Knoop, F. C. & Huggett, K. N. Influenza A. Reference Module in Biomedical Sciences (2014). doi:10.1016/B978-0-12-801238-3.05048-0.
12. Kilbourne, E. D. Influenza pandemics of the 20th century. *Emerg Infect Dis* 12, 9–14 (2006).
13. Peacock, T. P. et al. The global H5N1 influenza panzootic in mammals. *Nature* 637, 304–313 (2025). doi.org/10.1038/s41586-024-08054-z.
14. Krammer, F., Hermann, E. & Rasmussen, A. L. Highly pathogenic avian influenza H5N1: history, current situation, and outlook. *J. Virol.* 99, e02209-24 (2025). doi.org/10.1128/jvi.02209-24.
15. Song, W. & Qin, K. Human-infecting influenza A (H9N2) virus: a forgotten potential pandemic strain? *Zoonoses Public Health* 67, 203–212 (2020). doi.org/10.1111/zph.12685.
16. Dabrera, G. H5 and H9 avian influenza—potential re-emergent zoonotic threats to humans. *Curr. Opin. Infect. Dis.* 37, 431–435 (2024). doi.org/10.1097/QCO.0000000000001019.
17. Li, F. et al. Epidemiological characterization of human infection with H5N6 avian influenza. *Front. Public Health* 12, 1398365 (2024). doi.org/10.3389/fpubh.2024.1398365.
18. Lai, S. et al. Global epidemiology of avian influenza A H5N1 virus infection in humans, 1997–2015: a systematic review of individual case data. *Lancet Infect. Dis.* 16, e108–e118 (2016). doi.org/10.1016/S1473-3099(16)00153-5.
19. Peteranderl, C., Herold, S. & Schmoldt, C. Human influenza virus infections. *Semin Respir Crit Care Med* 37, 487–500 (2016).
20. Vasin, A. V. et al. Molecular mechanisms enhancing the proteome of influenza A viruses: an overview of recently discovered proteins. *Virus Res* 185, 53–63 (2014).
21. Low, Z. Y. et al. The convergent evolution of influenza A virus: implications, therapeutic strategies and what we need to know. *Curr Res Microb Sci* 5, 100202 (2023).
22. Fouchier, R. A. M. et al. Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls. *J Virol* 79, 2814–2822 (2005).
23. Han, C. W., Jeong, M. S. & Jang, S. B. Structure and function of the influenza A virus non-structural protein 1. *J Microbiol Biotechnol* 29, 1184–1192 (2019).

24. Nieva JL, Madan V, Carrasco L. Viroporins: Structure and biological functions. *Nat Rev Microbiol* 10, 563–574 (2012). doi: 10.1038/nrmicro2820.
25. McCown MF, Pekosz A. Distinct domains of the influenza A virus M2 protein cytoplasmic tail mediate binding to the M1 protein and facilitate infectious virus production. *J Virol* 80, 8178–8189 (2006).
26. Ma, C. et al. Identification of the functional core of the influenza A virus A/M2 proton-selective ion channel. *Proc Natl Acad Sci U.S.A.* 106, 12283–12288 (2009).
27. Manzoor, R., Igarashi, M. & Takada, A. Influenza A virus M2 protein: roles from ingress to egress. *Int J Mol Sci* 18, 2649 (2017).
28. Ren, W., Wang, Q., Yi, Y. & Li, J. Roles of the M2 protein of influenza A virus. *Virus Genes* (2026). doi:10.1007/s11262-026-02243-w.
29. Rossman, J. S. & Lamb, R. A. Influenza virus assembly and budding. *Virology* 411, 229–236 (2011).
30. Dou, D. et al. Influenza A virus cell entry, replication, virion assembly and movement. *Front. Immunol.* 9, 1581 (2018). <https://doi.org/10.3389/fimmu.2018.01581>.
31. Cady SD, Luo W, Hu F, Hong M. Structure and function of the influenza A M2 proton channel. *Biochemistry* 48, 7356–7364 (2009). doi: 10.1021/bi9008837.
32. Mould JA, Shuck K, Drury JE, Frings SM, Kaupp UB, Pekosz A, Lamb RA, Pinto LH. Structure–function relationship of the M2 ion channel of influenza A virus. *Int Congr Ser* 1219, 389–396 (2001). doi: 10.1016/S0531-5131(01)00641-0.
33. Sugrue RJ, Bahadur G, Zambon MC, et al. Specific structural alteration of the influenza haemagglutinin by amantadine. *EMBO J* 9, 3469–3476 (1990). doi: 10.1002/j.1460-2075.1990.tb07555.x.
34. Lamb RA. The structure, function, and pathobiology of the influenza A and B virus ion channels. *Cold Spring Harb Perspect Med* 10, a038505 (2020). doi: 10.1101/cshperspect.a038505.
35. Sugrue, R. J. & Hay, A. J. Structural characteristics of the M2 protein of influenza A viruses: evidence that it forms a tetrameric channel. *Virology* 180, 617–624 (1991).
36. Schnell, J. R. & Chou, J. J. Structure and mechanism of the M2 proton channel of influenza A virus. *Nature* 451, 591–595 (2008).
37. Manzoor, R., Igarashi, M. & Takada, A. Influenza A virus M2 protein: roles from ingress to egress. *Int. J. Mol. Sci.* 18, 2649 (2017).

38. Deng L, Cho KJ, Fiers W, Saelens X. M2e-based universal influenza A vaccines. *Vaccines* 3, 105–136 (2015). doi: 10.3390/vaccines3010105.
39. Ito T, Gorman OT, Kawaoka Y, Bean WJ, Webster RG. Evolutionary analysis of the influenza A virus M gene with comparison of the M1 and M2 proteins. *J Virol* 65, 5491–5498 (1991).
40. Peterson, E., Ryser, T., Funk, S., Inouye, D., Sharma, M., Qin, H., Cross, T. A. & Busath, D. D. Functional reconstitution of influenza A M2(22–62). *Biochim. Biophys. Acta Biomembr.* 1808, 516–521 (2011).
41. Cross, T. A., Sharma, M., Yi, M. & Zhou, H. X. Influence of solubilizing environments on membrane protein structures. *Trends Biochem. Sci.* 36, 117–125 (2011).
42. Sharma, M., Yi, M., Dong, H., Qin, H., Peterson, E., Busath, D. D., Zhou, H.-X. & Cross, T. A. Insight into the mechanism of the influenza A proton channel from a structure in a lipid bilayer. *Science* **330**, 509–512 (2010). <https://doi.org/10.1126/science.1191750>
43. Kostolanský, F. *et al.* Universal anti-influenza vaccines based on viral HA2 and M2e antigens. *Acta Virol.* **64**, 417–426 (2020). https://doi.org/10.4149/av_2020_408
44. Nishimura, K., Kim, S., Zhang, L. & Cross, T. A. The closed state of a H⁺ channel helical bundle combining precise orientational and distance restraints from solid-state NMR. *Biochemistry* 41, 13170–13177 (2002).
45. Acharya, R., Carnevale, V., Fiorin, G., Levine, B. G., Polishchuk, A. L., Balannik, V., Samish, I., Lamb, R. A., Pinto, L. H., DeGrado, W. F. & Klein, M. L. Structure and mechanism of proton transport through the transmembrane tetrameric M2 protein bundle of the influenza A virus. *Proc. Natl. Acad. Sci. U.S.A.* 107, 15075–15080 (2010).
46. Stouffer, A. L., Ma, C., Cristian, L., Ohigashi, Y., Lamb, R. A., Lear, J. D., Pinto, L. H. & DeGrado, W. F. The interplay of functional tuning, drug resistance, and thermodynamic stability in the evolution of the M2 proton channel from the influenza A virus. *Structure.* 16, 1067–1076 (2008).
47. Stouffer, A. L., Acharya, R., Salom, D., Levine, A. S., Di Costanzo, L., Soto, C. S., Tereshko, V., Nanda, V., Stayrook, S. & DeGrado, W. F. Structural basis for the function and inhibition of an influenza virus proton channel. *Nature* **451**, 596–599 (2008). doi.org/10.1038/nature06528.
48. Schnell, J. R. & Chou, J. J. Structure and mechanism of the M2 proton channel of influenza A virus. *Nature* **451**, 591–595 (2008). doi.org/10.1038/nature06531.

49. Cady, S. D., Schmidt-Rohr, K., Wang, J., Soto, C. S., DeGrado, W. F. & Hong, M. Structure of the amantadine binding site of influenza M2 proton channels in lipid bilayers. *Nature* **463**, 689–692 (2010). doi.org/10.1038/nature08722.
50. Thomaston, J. L., Polizzi, N. F., Konstantinidi, A., Wang, J., Kolocouris, A. & DeGrado, W. F. Inhibitors of the M2 proton channel engage and disrupt transmembrane networks of hydrogen-bonded waters. *J. Am. Chem. Soc.* **140**, 15219–15226 (2018). doi.org/10.1021/jacs.8b06741.
51. Rosenberg, M. R. & Casarotto, M. G. Coexistence of two adamantane binding sites in the influenza A M2 ion channel. *Proc. Natl Acad. Sci. USA* **107**, 13866–13871 (2010). doi.org/10.1073/pnas.1002051107.
52. Cady, S. D., Schmidt-Rohr, K., Wang, J., Soto, C. S., DeGrado, W. F. & Hong, M. Structure of the amantadine binding site of influenza M2 proton channels in lipid bilayers. *Nature*. **463**, 689–692 (2010).
53. Hong, M. & DeGrado, W. F. Structural basis for proton conduction and inhibition by the influenza M2 protein. *Protein Sci.* **21**, 1620–1633 (2012).
54. Thomaston, J. L., Wu, Y., Polizzi, N., Liu, L., Wang, J. & DeGrado, W. F. X-ray crystal structure of the influenza A M2 proton channel S31N mutant in two conformational states: an open and shut case. *J. Am. Chem. Soc.* **141**, 11481–11488 (2019).
55. Kozakov, D., Chuang, G. Y., Beglov, D. & Vajda, S. Where does amantadine bind to the influenza virus M2 proton channel? *Trends Biochem. Sci.* **35**, 471–475 (2010).
56. Gleed, M. L. & Busath, D. D. Why bound amantadine fails to inhibit proton conductance according to simulations of the drug-resistant influenza A M2 (S31N). *J. Phys. Chem. B* **119**, 1225–1231 (2015).
57. Tzitzoglaki, C. *et al.* Binding and proton blockage by amantadine variants of the influenza M2WT and M2S31N explained. *J. Med. Chem.* **60**, 1716–1733 (2017). doi.org/10.1021/acs.jmedchem.6b01115.
58. Gianti, E., Carnevale, V., DeGrado, W. F., Klein, M. L. & Fiorin, G. Hydrogen-bonded water molecules in the M2 channel of the influenza A virus guide the binding preferences of ammonium-based inhibitors. *J. Phys. Chem. B* **119**, 1173–1183 (2015).
59. Kumari R, Sharma SD, Kumar A, Ende Z, Mishina M, Wang Y, et al.. Antiviral approaches against influenza virus. *Clin Microbiol Rev* **36**, e0004022 (2023). doi: 10.1128/cmr.00040-22.

60. Xu J, Luo Q, Huang Y, Li J, Ye W, Yan R, et al. Influenza neuraminidase mutations and resistance to neuraminidase inhibitors. *Emerg Microbes Infect* 13, 2429627 (2024). doi: 10.1080/22221751.2024.2429627.
61. Granda AG, Olsen OA, Cox TC, et al. Human antibodies reveal a protective epitope that is highly conserved among human and nonhuman influenza A viruses. *Proc Natl Acad Sci U S A* 107, 12658–12663 (2010). doi: 10.1073/pnas.0911806107.
62. Beigel JH, Voell J, Muñoz P, et al. Safety, tolerability, and pharmacokinetics of radavirsen (AVI-7100), an antisense oligonucleotide targeting influenza A M1/M2 translation. *Br J Clin Pharmacol* 84, 25–34 (2018). doi: 10.1111/bcp.13405.
63. McKimm-Breschkin JL, Barrett S. Neuraminidase mutations conferring resistance to laninamivir lead to faster drug binding and dissociation. *Antiviral Res* 114, 62–66 (2015). doi: 10.1016/j.antiviral.2014.12.004.
64. Rossignol JF. Nitazoxanide: a first-in-class broad-spectrum antiviral agent. *Antiviral Res* 110, 94–103 (2014). doi: 10.1016/j.antiviral.2014.07.014.
65. Kadam RU, Wilson IA. Structural basis of influenza virus fusion inhibition by the antiviral drug Arbidol. *Proc Natl Acad Sci U S A* 114, 206–214 (2017). doi: 10.1073/pnas.1617020114.
66. Ali, S., Takas, T., Nyborg, A., Shoemaker, K., Kallewaard, N., Chiong, R., Dubovsky, F. & Mallory, R. Evaluation of MEDI8852, an anti-influenza A monoclonal antibody, in treating acute uncomplicated influenza. *Antimicrob Agents Chemother* 62 (2018).
67. Paules, C. I., Lakdawala, S., McAuliffe, J. M., Paskel, M., Vogel, L., Kallewaard, N. L., Zhu, Q. & Subbarao, K. The hemagglutinin A stem antibody MEDI8852 prevents and controls disease and limits transmission of pandemic influenza viruses. *J Infect Dis* 216, 356–365 (2017).
68. Hershberger, E., Sloan, S., Narayan, K. et al. Safety and efficacy of monoclonal antibody VIS410 in adults with uncomplicated influenza A infection: results from a randomized, double-blind, phase-2, placebo-controlled study. *eBioMedicine* 40, 574–582 (2019).
69. Throsby, M. et al. Heterosubtypic neutralizing monoclonal antibodies cross-protective against H5N1 and H1N1 recovered from human IgM+ memory B cells. *PLoS ONE* 3, e3942 (2008). doi.org/10.1371/journal.pone.0003942
70. Ekiert, D. C. et al. A highly conserved neutralizing epitope on group 2 influenza A viruses. *Science* 333, 843–850 (2011).

71. Yi, K. S., Choi, J. A., Kim, P., Ryu, D. K., Yang, E., Son, D., Shin, J., Park, H., Lee, S., Lee, H., Im, B. H., Chae, J. S., Lee, E. B., Lee, S. Y. & Song, M. Broader neutralization of CT-P27 against influenza A subtypes by combining two human monoclonal antibodies. *PLoS One* 15, e0236172 (2020).
72. Shiraki, K. & Daikoku, T. Favipiravir, an anti-influenza drug against life-threatening RNA virus infections. *Pharmacol Ther* 209, 107512 (2020).
73. Te Velthuis, A. J. W., Zubkova, T. G., Shaw, M., Mehle, A., Boltz, D., Gmeinwieser, N. et al. Enisamium reduces influenza virus shedding and improves patient recovery by inhibiting viral RNA polymerase activity. *Antimicrob Agents Chemother* 65, e02605-20 (2021).
74. Beigel, J., Bao, Y., Beeler, J. et al. Oseltamivir, amantadine, and ribavirin combination antiviral therapy versus oseltamivir monotherapy for the treatment of influenza: a multicentre, double-blind, randomised phase 2 trial. *Lancet Infect Dis* 17, 1255–1265 (2017).
75. Luo, D., Ye, Q., Li, R. T., Zhou, H. Y., Guo, J. J., Zhao, S. Q., Zhang, S., Jiang, T., Deng, Y. Q. & Qin, C. F. PA-E18G substitution in influenza A virus confers resistance to ZX-7101, a cap-dependent endonuclease inhibitor. *Virol Sin* 38, 559–567 (2023).
76. Drapkina, O. M., Gorshkov, A. Yu. & Chudinovskikh, T. I. Molnupiravir in the treatment of patients with influenza or acute respiratory viral infections: a multicenter comparative randomized double-blind placebo-controlled trial. *Pharmacy & Pharmacology* 13, 297–315 (2025).
77. Kakuda, T. N., Yogaratnam, J., Rito, J., Boyce, M., Mitchell, T., Gupta, K., Symons, J. A., Chanda, S., Van Remoortere, P. & Fry, J. Phase I study on safety and pharmacokinetics of a novel influenza endonuclease inhibitor, AL-794 (JNJ-64155806), following single- and multiple-ascending doses in healthy adults. *Antiviral Therapy* (2018).
78. Shishkina, L. N. et al. In vitro and in vivo efficacy of Ingavirin against strains of pandemic influenza virus A(H1N1/09)v. *Zh. Mikrobiol. Epidemiol. Immunobiol.* 2, 93–96 (2011).
79. Finberg, R. W., Lanno, R., Anderson, D., Fleischhackl, R., van Duijnhoven, W., Kauffman, R. S., Kosoglou, T., Vingerhoets, J. & Leopold, L. Phase 2b study of pimodivir (JNJ-63623872) as monotherapy or in combination with oseltamivir for treatment of acute uncomplicated seasonal influenza A: TOPAZ trial. *J Infect Dis* 219, 1026–1034 (2019).
80. Chen, X., Ma, Q., Zhao, M., Yao, Y., Zhang, Q., Liu, M., Yang, Z. & Deng, W. Preclinical study of ZSP1273, a potent antiviral inhibitor of cap binding to the PB2 subunit of influenza A polymerase. *Pharmaceuticals* 16, 365 (2023).

81. Beigel, J. H., Nam, H. H., Adams, P. L., Krafft, A., Ince, W. L., El-Kamary, S. S. & Sims, A. C. Advances in respiratory virus therapeutics – a meeting report from the 6th ISIRV Antiviral Group conference. *Antiviral Res* 167, 45–67 (2019).
82. Episcopio, D., Aminov, S., Benjamin, S., Germain, G., Datan, E., Landazuri, J. et al. Atorvastatin restricts the ability of influenza virus to generate lipid droplets and severely suppresses the replication of the virus. *FASEB J* 33, 9516–9525 (2019).
83. Miller, B. E., Mistry, S., Smart, K., Connolly, P., Carpenter, D. C., Cooray, H. et al. The pharmacokinetics and pharmacodynamics of danirixin (GSK1325756)—a selective CXCR2 antagonist—in healthy adult subjects. *BMC Pharmacol Toxicol* 16, 18 (2015).
84. Omoto, S. et al. Characterization of influenza virus variants induced by treatment with the endonuclease inhibitor baloxavir marboxil. *Sci. Rep.* 8, 9633 (2018).
85. Moss, R. B., Hansen, C., Sanders, R. L., Hawley, S., Li, T. & Steigbigel, R. T. A Phase II study of DAS181, a novel host-directed antiviral for the treatment of influenza infection. *J. Infect. Dis.* 206, 1844–1851 (2012).
86. Sekutor, M., Fokin, A. A. & Schreiner, P. R. *The Chemistry of Diamondoids: Building Blocks for Ligands, Catalysts, Pharmaceuticals, and Materials*. Wiley-VCH GmbH, Germany (2024).
87. Fort, R. C. & Schleyer, P. von R. Adamantane: Consequences of the Diamondoid Structure. *Chem. Rev.* 64, 277–300 (1964).
88. Jackson, G. G., Muldoon, R. L. & Akers, L. W. Serological evidence for prevention of influenzal infection in volunteers by an anti-influenzal drug adamantanamine hydrochloride. *Antimicrob. Agents Chemother.* 161, 703–707 (1964).
89. Liu, J., Obando, D., Liao, V., Lifa, T. & Codd, R. The many faces of the adamantyl group in drug design. *Eur. J. Med. Chem.* 46, 1949–1963 (2011).
90. Davies, W. L. et al. Antiviral activity of 1-adamantanamine (amantadine). *Science* 144, 862–863 (1964).
91. Akole, A. & Warner, J. M. Model of influenza virus acidification. *PLoS One* 14, e0214448 (2019).
92. Amarelle, L., Lecuona, E. & Sznajder, J. I. Anti-influenza treatment: drugs currently used and under development. *Arch. Bronconeumol.* 53, 19–26 (2017).
93. Pielak, R. M. & Chou, J. J. Flu channel drug resistance: a tale of two sites. *Protein Cell* 1, 246–258 (2010).

94. Wang, J. et al. Exploring the requirements for the hydrophobic scaffold and polar amine in inhibitors of M2 from influenza A virus. *ACS Med. Chem. Lett.* 2, 307–312 (2011).
95. Stankova, I. et al. Adamantane derivatives containing thiazole moiety: synthesis, antiviral and antibacterial activity. *Int. J. Pept. Res. Ther.* 26, 1781–1787 (2020).
96. Wang, J. et al. *J. Am. Chem. Soc.* 133, 12834–12841 (2011).
97. Hu, Y. et al. An M2-V27A channel blocker demonstrates potent *in vitro* and *in vivo* antiviral activities against amantadine-sensitive and -resistant influenza A viruses. *Antiviral Res.* 140, 45–54 (2017). doi.org/10.1016/j.antiviral.2017.01.006
98. Li, F. et al. Expedient lead optimization of isoxazole-containing influenza A virus M2-S31N inhibitors using the Suzuki–Miyaura cross-coupling reaction. *J. Med. Chem.* 60, 1580–1590 (2017).
99. Banti, C. N. et al. Amantadine copper(II) chloride conjugate with possible implementation in influenza virus inhibition. *Polyhedron*. 185, 114590 (2020).
100. Suslov, E. et al. Anti-influenza activity of diazaadamantanes combined with monoterpene moieties. *Bioorg. Med. Chem. Lett.* 27, 4985–4989 (2017).
101. Barniol-Xicota, M. et al. Slow but steady wins the race: dissimilarities among new dual inhibitors of the wild-type and the V27A mutant M2 channels of influenza A virus. *J. Med. Chem.* 60, 3727–3738 (2017). doi.org/10.1021/acs.jmedchem.6b01758.
102. Zarubaev, V. V. et al. Synthesis and anti-viral activity of azolo-adamantanes against influenza A virus. *Bioorg. Med. Chem.* 18, 839–848 (2010). doi.org/10.1016/j.bmc.2009.11.047.
103. Zoidis, G., Kolocouris, N., Naesens, L. & De Clercq, E. Design and synthesis of 1,2-annulated adamantane piperidines with anti-influenza virus activity. *Bioorg. Med. Chem.* 17, 1534–1541 (2009). doi.org/10.1016/j.bmc.2009.01.009.
104. Tzitzoglaki, C. et al. Amantadine variant–aryl conjugates that inhibit multiple M2 mutant–amantadine-resistant influenza A viruses. *Eur. J. Med. Chem. Rep.* 6, 100083 (2022). doi.org/10.1016/j.ejmcr.2022.100083.
105. Wang, Y. et al. In vitro pharmacokinetic optimizations of AM2-S31N channel blockers led to the discovery of slow-binding inhibitors with potent antiviral activity against drug-resistant influenza A viruses. *J. Med. Chem.* 61, 1074–1085 (2018). doi.org/10.1021/acs.jmedchem.7b01536.

106. Hu, Y., Wang, Y., Li, F., Ma, C. & Wang, J. Design and expeditious synthesis of organosilanes as potent antivirals targeting multidrug-resistant influenza A viruses. *Eur. J. Med. Chem.* 135, 70–76 (2017). doi.org/10.1016/j.ejmech.2017.04.038.
107. Wu, Y. *et al.* Flipping in the pore: discovery of dual inhibitors that bind in different orientations to the wild-type versus the amantadine-resistant S31N mutant of the influenza A virus M2 proton channel. *J. Am. Chem. Soc.* 136, 17987–17995 (2014).
https://doi.org/10.1021/ja508461m.
108. Wang, J. *et al.* Discovery of novel dual inhibitors of the wild-type and the most prevalent drug-resistant mutant, S31N, of the M2 proton channel from influenza A virus. *J. Med. Chem.* 56, 2804–2812 (2013). doi.org/10.1021/jm301538e
109. Li, F., Ma, C., Hu, Y., Wang, Y. & Wang, J. Discovery of potent antivirals against amantadine-resistant influenza A viruses by targeting the M2-S31N proton channel. *ACS Infect. Dis.* 2, 726–733 (2016). doi.org/10.1021/acsinfecdis.6b00130.
110. Jiang, J. *et al.* Construction and synthesis of tricyclic matrinic derivatives against influenza A virus by privileged structure strategy. *Pharmazie* 74, 265–269 (2019).
doi.org/10.1691/ph.2019.8968.
111. Gökteş, 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.* 20, 7155–7159 (2012). doi.org/10.1016/j.bmc.2012.09.064.
112. Balgi, A. D. *et al.* Inhibitors of the influenza A virus M2 proton channel discovered using a high-throughput yeast growth restoration assay. *PLoS ONE*. 8, e55271 (2013).
doi.org/10.1371/journal.pone.0055271
113. Arns, S. *et al.* Novel spirothiazamenthane inhibitors of the influenza A M2 proton channel. *Eur. J. Med. Chem.* 120, 64–73 (2016). doi.org/10.1016/j.ejmech.2016.05.008
114. Rey-Carrizo, M. *et al.* Easily accessible polycyclic amines that inhibit the wild-type and amantadine-resistant mutants of the M2 channel of influenza A virus. *J. Med. Chem.* 57, 5738–5747 (2014). doi.org/10.1021/jm5005804
115. Rey-Carrizo, M. *et al.* 3-Azatetracyclo[5.2.1.1^{^5},8.0^{^1},5]undecane derivatives: from wild-type inhibitors of the M2 ion channel of influenza A virus to derivatives with potent activity against the V27A mutant. *J. Med. Chem.* 56, 9265–9274 (2013).
doi.org/10.1021/jm401340p

116. Wang, J. *et al.* Discovery of spiro-piperidine inhibitors and their modulation of the dynamics of the M2 proton channel from influenza A virus. *J. Am. Chem. Soc.* 131, 8066–8076 (2009). doi.org/10.1021/ja900063s
117. Balannik, V. *et al.* Design and pharmacological characterization of inhibitors of amantadine-resistant mutants of the M2 ion channel of influenza A virus. *Biochemistry.* 48, 11872–11882 (2009). doi.org/10.1021/bi9014488
118. Hu, W. *et al.* Identification of hits as matrix-2 protein inhibitors through the focused screening of a small primary amine library. *J. Med. Chem.* 53, 3831–3834 (2010). doi.org/10.1021/jm901664a
119. Zhao, X. *et al.* Identification of camphor derivatives as novel M2 ion channel inhibitors of influenza A virus. *Med. Chem. Comm.* 6, 727–731 (2015). doi.org/10.1039/C4MD00515E
120. Wang, J. *et al.* Exploring organosilane amines as potent inhibitors and structural probes of influenza A virus M2 proton channel. *J. Am. Chem. Soc.* 133, 13844–13847 (2011). doi.org/10.1021/ja2050666
121. Dong, J. *et al.* Imidazole-based pinanamine derivatives: discovery of dual inhibitors of the wild-type and drug-resistant mutant of the influenza A virus. *Eur. J. Med. Chem.* 108, 605–615 (2016). doi.org/10.1016/j.ejmech.2015.12.013
122. Dong, J. *et al.* Design and synthesis of pinane oxime derivatives as novel anti-influenza agents. *Bioorg. Chem.* 102, 104106 (2020). doi.org/10.1016/j.bioorg.2020.104106
123. Jang, Y. *et al.* Salinomycin inhibits influenza virus infection by disrupting endosomal acidification and viral matrix protein 2 function. *J. Virol.* 92, e01441-18 (2018). doi.org/10.1128/JVI.01441-18
124. Duncan MC, *et al.* Virtual screening identifies chebulagic acid as an inhibitor of the M2(S31N) viral ion channel and influenza A virus. *Molecules.* 2020;25:2903.
125. Dane, C. *et al.* Unlocking therapeutic potential: the role of adamantane in drug discovery. *Aust. J. Chem.* 77, CH24075 (2024). doi.org/10.1071/CH24075
126. Dane, C. *et al.* The adamantane scaffold: beyond a lipophilic moiety. *Eur. J. Med. Chem.* 291, 117592 (2025). doi.org/10.1016/j.ejmech.2025.117592
127. Wang, J., Li, F. & Ma, C. Recent progress in designing inhibitors that target the drug-resistant M2 proton channels from the influenza A viruses. *Biopolymers.* 104, 291–309 (2015). doi.org/10.1002/bip.22623

128. Tran, L. et al. Discovery of potential M2 channel inhibitors based on the amantadine scaffold via virtual screening and pharmacophore modeling. *Molecules*. 16, 10227–10255 (2011). doi.org/10.3390/molecules161210227
129. Deng, C. et al. Virtual screening and molecular growth guide the design of inhibitors for the influenza virus drug-resistant mutant M2-V27A/S31N. *J. Biomol. Struct. Dyn.* 42, 5253–5267 (2024). doi.org/10.1080/07391102.2023.2233026
130. Sulli, C. et al. Detection of proton movement directly across viral membranes to identify novel influenza virus M2 inhibitors. *J. Virol.* 87, 10679–10686 (2013). doi.org/10.1128/JVI.01190-13
131. Radosevic, D. et al. Virtual screen for repurposing of drugs for candidate influenza A M2 ion-channel inhibitors. *Front. Cell. Infect. Microbiol.* 9, 67 (2019). doi.org/10.3389/fcimb.2019.00067.
132. Hu F, et al. Conformational plasticity of the influenza A M2 transmembrane helix in lipid bilayers under varying pH, drug binding, and membrane thickness. *Biochim Biophys Acta*. 2011;1808:415–423
133. Musharrafieh, R., Ma, C. & Wang, J. Discovery of M2 channel blockers targeting the drug-resistant double mutants M2-S31N/L26I and M2-S31N/V27A from the influenza A viruses. *Eur. J. Pharm. Sci.* 141, 105124 (2020). https://doi.org/10.1016/j.ejps.2019.105124