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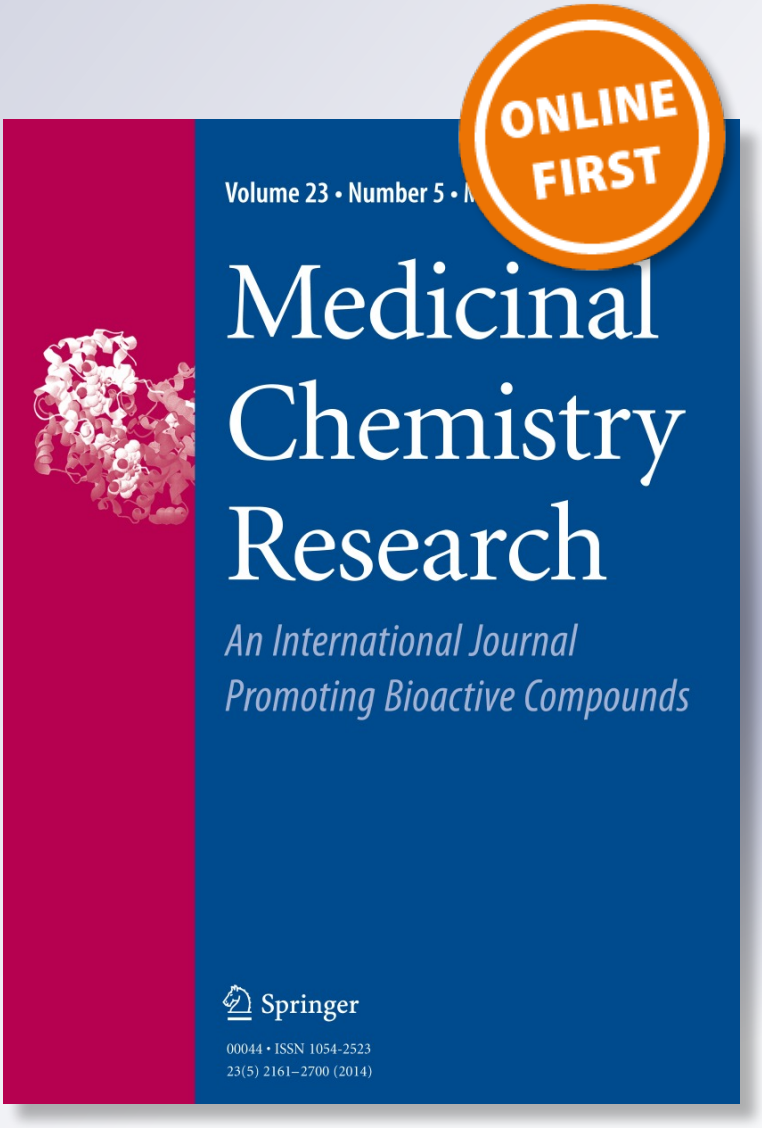
Medicinal Chemistry Research

ISSN 1054-2523

Med Chem Res
DOI 10.1007/s00044-014-0964-6



Springer

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MEDICINAL CHEMISTRY RESEARCH

REVIEW ARTICLE

Recent progress and challenges in the computer-aided design of inhibitors for influenza A M2 channel proteins

Linh Tran · Ly Le

Received: 11 September 2013 / Accepted: 17 February 2014

Abstract The M2 channel protein has become an attractive target for the design of new drugs against influenza because it plays a crucial role in the replication cycle of influenza A virus. Several adamantane-based drugs have recently been developed to inhibit the activity of the M2 channel and overcome the drug resistance issues observed in amantadine and rimantadine. Computer-aided drug design continues to play a critical role in the drug discovery process in terms of its contribution to the identification and development of new therapeutic agents. Scientists working in this field are currently facing significant challenges with regard to creating novel platforms capable of enhancing our understanding of these proteins, with computational techniques being used to search for new potential drugs against influenza. This review provides a summary of recent progress in drug discovery toward the development therapeutic agents targeting M2 channel proteins. It is hoped that this review will stimulate the development of new strategies for overcoming drug resistance problems and encourage the design of new and improved drugs against influenza A virus.

Keywords M2 channel protein · Drug development · Influenza A virus · Inhibition mechanism · Structural characteristics · M2 inhibitor

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Introduction

Among the three main components on the surface membrane proteins of influenza A viruses, including hemagglutinin (HA), neuraminidase (NA), and M2 channel protein, the M2 channel protein plays a central role in the replication of the virus (Cady *et al*., 2009). For this reason, the M2 channel protein has become a key target for rational drug design against influenza A virus. Several studies and reviews have recently been reported pertaining to M2 channel proteins that have provided an in-depth understanding of its characteristic features, including its inhibition mechanisms and general structure (Schnell and Chou, 2008; Stouffer *et al*., 2008). The structure and function of the M2 channel protein have been investigated extensively, with the results of these studies showing that the M2 protein is a 97-residue membrane protein with its amino and carboxyl termini directed toward the outside and inside of the virion, respectively (Du and Huang, 2012; Du *et al*., 2009). Furthermore, the M2 channel protein is activated by low pH and consists of three distinct segments, including (1) an extracellular *N*-terminal segment (residues 1–23); (2) a transmembrane (TM) segment (residues 24–46); and (3) an intracellular C-terminal segment (residues 47–97) (Pielak and Chou, 2011; Sharma *et al*., 2010). The four TM helices create a channel where the His37 residue acts as a pH sensor, the Trp41 residue acts as proton gate, and the Asp44 residue acts as a channel lock (Pinto *et al*., 1992).

Considerable research efforts have been focused on determining the three-dimensional (3D) structure of the M2 proton channel protein (Du and Huang, 2012). The structure of the M2 channel protein has been determined by two different groups using two different techniques. In one case, the structure of the protein was determined by NMR analysis, which revealed that the structure was composed

Published online: 02 March 2014

of four identical polypeptide chains, with each individual chain containing 43 amino acids from Arg18 to Lys60 (Schnell and Chou, 2008). In the second case, X-ray crystallography was used to determine the structure of only 25 amino acids of the M2 protein from Val22 to Lys46 with an unnatural mutation at G34A (Stouffer *et al*., 2008). A full-length 3D model of the M2 proton channel protein is shown in Fig. 1, together with the structures of amantadine and rimantadine. This model of the M2 protein has allowed for the M2 channel mechanism to be evaluated in greater detail, and has also provided a platform for the development of novel therapeutic agents using computational approaches, as well as enabling the rationally design of more effective drugs against influenza A viruses that can overcome the issues associated with drug resistance (Stouffer *et al*., 2008). There is an urgent need for the development of a universal avian influenza vaccine. For this reason, a live bacterial vaccine against avian influenza has been constructed by expressing a conserved peptide from the ectodomain of M2 antigen (M2e) on the surface of *Lactococcus lactis* (LL) (Reese *et al*., 2013). Furthermore, influenza A viruses belonging to a different subtypes, and human-infected H5N1 strains contain highly conserved M2 sequences at the HuScFv binding sites. The human monoclonal single-chain antibody variable fragments (HuScFv) have been reported to bind specifically to the recombinant and native M2 proteins of the A/H5N1 strains of the influenza virus, which were produced using phage display technology (Pissawong *et al*., 2013).

In a high-pH environment, the M2 channel is closed by the TM helices and the constrictive gates, which are controlled by the Val27 and Trp41 residues at the N-terminal ion entrance and C-terminal ion exit, respectively (Sharma *et al*., 2010). Under low-pH conditions, the highly proton-selective His37 residue senses the acidification at the N-terminal and allows for the inward flow of protons through the channel, whereas the gate formed by the linking together of the Trp41, Asp44, and Arg45 residues remains open, allowing the outward flow of protons toward the C-terminal (Acharya *et al*., 2010). Amantadine derivatives have been identified as a new class of potential M2 channel inhibitors (Kolocouris *et al*., 2007; Stamatiou *et al*., 2001). Amantadine itself obstructs the ion channel of the M2 protein by binding to Ser31 and the surrounding Val27, Ala30, and Gly34 residues (Stouffer *et al*., 2008). In contrast, rimantadine binds to a lipid-facing pocket of the channel, which is close to the Trp41, Ile42, and Arg45 residues of one TM helix, as well as the Leu40, Leu43, and Asp44 residues of another helix (Schnell and Chou, 2008). In light of the high levels of resistance shown toward these two drugs, however, the threat to human health from influenza A viruses remains a significant problem (Laohpongspaisan *et al*., 2009; Rungrotmongkol *et al*., 2009; Cox and Subbarao, 2000). Several potential compounds have been reported as inhibitors of the V27A and L26F mutants, and their inhibitory activities evaluated in vitro (Balannik *et al*., 2009). Extensive medicinal chemistry efforts have been applied to the identification and development of new inhibitors of the M2 channel protein, with high-throughput screening being used as a tool to establish suitable starting points for drug discovery. Using this approach, Wang *et al*., (2011b) identified a series of potent spirane amine inhibitors targeting not only the wild-type (WT) M2 channel protein, but also the A/M2-27A and L26F mutants. Furthermore, these compounds exhibited IC_{50} values that were similar to that of amantadine toward the WT protein. One of these compounds, known as M2WJ332, was recently found to be an effective inhibitor

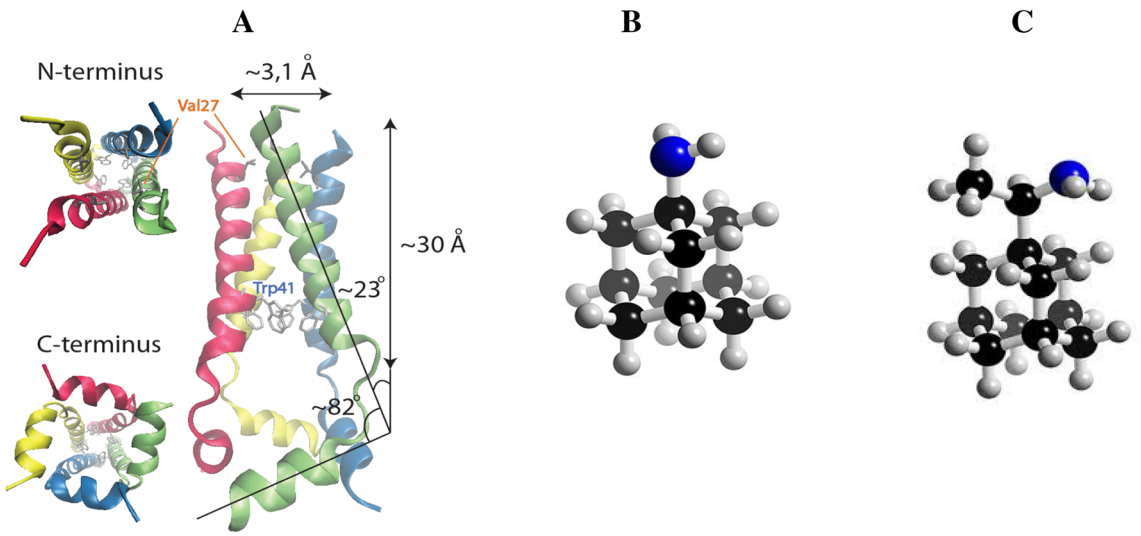

Fig. 1 Model structure of M2 channel protein (**a**), amantadine (**b**), and rimantadine(**c**)

of the S31N mutant, and gave an electrophysiological IC_{50} of 16 uM (Wang *et al*., 2013b). Given its ability to control the flow of protons into the virion, the M2 channel protein plays a crucial role in the replication of the influenza A virus. For this reason, significant research efforts are currently being focused on the development of more effective drugs against influenza A that work by inhibiting M2 channel proteins. Scientists working in this field are, therefore, facing considerable challenges with regard to developing an thorough understanding of the mechanisms of M2 protein inhibitions, with computational techniques being used to search for new potential drugs against a potential influenza pandemic (Du and Huang, 2012).

There is an urgent need for the development of novel inhibitors that are active against amantadine-resistant channels, as well as an assay capable of evaluating the effects of these inhibitors on the M2 membrane protein under natural conditions. In 2011, 3,7-dimethyl-1-bisnoradamantyl amine was reported as an effective inhibitor of the S31N mutant M2 channel, with a potency similar to that of amantadine (IC_{50} values of 252 and 200 μM, respectively) (Duque *et al*., 2011). In 2013, as part of a study aimed at discovering inhibitors of the WT and S31N mutant M2 channel proteins, Li *et al*., (2013) used a stable cell line that co-expressed the M2 ion channel with enhanced green fluorescent protein (EGFP) to develop an EGFP-based high-throughput assay, which was dependent on and sensitive to pH, for screening M2 ion channel inhibitors. Using this assay, Li et al. identified (1S,2S,3S,5R)-(+)-3-isopinocampheyl amine and (1R,2R,3R,5S)-(-)-3-isopinocampheyl amine as two novel inhibitors of the M2 channel protein. These compounds gave IC_{50} values of 6.7 and 4.3 μM in an electrophysiological assay, and 6.0 and 1.4 μM in A/Hong Kong/8/68 viral inhibition assays, respectively. These two compounds, however, only showed inhibitory activity toward the WT M2 ion channel, and not toward the drug-resistant mutants. In light of the fact that there are currently no drugs in clinical trials targeting mutated M2 channel proteins, Balgi et. al. (2013) developed a yeast growth restoration assay that was amenable to high-throughput screening and screened over 250,000 pure chemicals and semi-purified fractions from natural extracts. This screening process resulted in the identification of 21 active compounds, including amantadine and rimantadine, as well as 13 adamantane-type compounds and 6 non-adamantanes. Hexamethylene amiloride and a triazine derivative were identified as two of the non-adamantane compounds and represent two new M2 inhibitory chemotypes. These compounds also showed antiviral activity in a plaque reduction assay, and could be used as good starting points for a drug discovery program toward the development of new M2 channel inhibitors.

The importance of computer-aided drug design has grown significantly in terms of its contribution to the drug development process (Sukumar and Das, 2011; Andricopulo *et al*., 2009; Laurie and Jackson, 2006). Furthermore, numerous reports have demonstrated that theoretical computational studies, including molecular modeling, molecular docking, molecular dynamics simulations, phylogenetic analysis, quantum mechanical calculations, pharmacophore modeling, QSAR, and bioinformatics techniques can provide useful information for research in drug development (Wang and Chou, 2012; Du *et al*., 2010; Nguyen *et al*., 2009; Le and Leluk, 2011). Several computational studies have been conducted on the mechanisms associated with M2 channel proteins, and the results of these studies have provided valuable insights into the activity of the M2 channel proteins and enhanced efforts toward the targeting of M2 channel proteins through rational drug design. This review summarizes the most up-to-date and significant findings from the field of computational chemistry with regard to the targeting of M2 channel proteins using rational drug design.

Drug inhibition mechanisms of M2 channel proteins

Developing an understanding of the mechanisms involved in the inhibition of M2 channel proteins is essential for defining a basic research strategy and conducting a drug development program against this target. During the past 10 years, however, there have been several conflicting and incomplete reports in the literature pertaining to the mechanism of M2 protein inhibition, and the exact mechanism of inhibition remains unclear. The exact location of the functional adamantane binding site had also been a source of controversy (Pielak *et al*., 2009), and two mechanisms have been proposed based on experimental structures, including (1) the pore-block mechanism, which was revealed by an X-ray structure (Stouffer *et al*., 2008); and (2) the allosteric mechanism, which was revealed by an NMR structure (Schnell and Chou, 2008). In the pore-block mechanism, the amantadine drug physically blocks the pore (Stouffer *et al*., 2008) and interacts with the three pore-lining residues L26, A30, and S31. V27, S31, G34, and H37 are known to have a high affinity for adamantane and are located in close proximity to the adamantane binding site. Furthermore, the S31N mutant M2 protein accounts for a large proportion of adamantane drug resistance in all influenza subtypes (Rungrotmongkol *et al*., 2009; Simonsen *et al*., 2007). It has been suggested that this mutation leads to an indirect increase in the mobility of the M2 protein (Bright *et al*., 2006), and that this change prevents the inhibitors from being able to bind to the M2 channel and block proton transport in influenza A (H1N1).

In contrast, according to the allosteric mechanism, inhibitors of the M2 protein effectively stabilize the closed conformation of the C-terminal helices (Schnell and Chou, 2008) and facilitate the orientation of the H37 residue. Possible binding sites of the inhibitors in the M2 channel are T43, D44, and R45. According to the mechanism proposed by Schnell and Chou (2008), rimantadine binds to four equivalent sites near the "tryptophan gate" on the lipid-facing side of the channel, and stabilizes the closed conformation of the pore. Pielak et al. (2009) concluded that the lipid-facing pocket near the W41 gate was more relevant to the mechanism of adamantane inhibition. In a separate study, Du et al. (2009) conducted a structural analysis of the M2 protein by NMR and reported results that were in agreement with the allosteric mechanism.

Based on all of these reports, we believe that the functional binding site of M2 channel proteins has finally been identified. Following their study aimed at understanding the mechanism of adamantane inhibition in M2 channel proteins, Intharathep et al. (2008) stated that the drug molecules were likely to bind within either the inner cavity (i.e., near the H37 gate) or the external mouth of the channel (i.e., the opening pore residues L26, A30, and S31). More specifically, the drug molecules were oriented in such a way as to lie along the inner surface of the M2 channel. The results of several other studies have also supported the suggestion that the preferential binding position is inside the M2 channel protein (Nguyen *et al.*, 2013; Tran *et al.*, 2013; Tran *et al.*, 2011; Du *et al.*, 2012). Following a period of uncertainty and controversy concerning the location of the drug-binding site (Schnell and Chou, 2008; Stouffer *et al.*, 2008), it is now generally accepted that the inhibitors of the M2 protein exert their inhibitory activity by blocking the channel through a series of binding interactions inside the pore of the channel (Sharma *et al.*, 2010; Du *et al.*, 2012; Stouffer *et al.*, 2008; Hu *et al.*, 2007; Yi *et al.*, 2008; Cady *et al.*, 2010; Cross *et al.*, 2012). To date, progress toward the development of M2 channel inhibitors with improved properties and activities has been limited by a poor understanding of the mechanism of drug inhibition and the drug-binding site. In conclusion, the amino acid residues inside the M2 channel proteins (i.e., the residues from V27 to G34) have been identified as being particularly important to favorable adamantane inhibition. These results also providing an understanding of why most of the residues inside the M2 proton channel are highly conserved.

M2 sequence characteristics toward evolutionary diversity trend

The 3D structures of the M2 channel proteins have made it easier to understand why the influenza virus has become increasingly resistant to treatment with amantadine and rimantadine, which are two of the four antiflu drugs approved by the US Food and Drug Administration (FDA) (Kolocouris *et al.*, 2007). These results of all structural studies directed toward the M2 proteins have also provided detailed structural information, which has led to a deeper mechanistic understanding of the inhibition process that could prove invaluable in the rational design of new and more effective M2 drug candidates. Significant research efforts have been applied to determining the 3D structures of the M2 channel proteins. In 2008, two independent research groups simultaneously reported the high-resolution structures of the M2 proton channels from influenza A virus by solution NMR spectroscopy (Schnell and Chou, 2008) and X-ray crystallography (Stouffer *et al.*, 2008). Several studies have also been reported aimed at definitively identifying the residues involved in the M2 mechanism, as well as identifying the residues that interact with the inhibitors and prevent proton transport through the membrane. Several studies have also been conducted with the aim of developing a greater understanding of implications of mutational variability in the viral M2 proteins.

The identification and characterization of correlated mutations within the M2 protein could provide an additional source of information with regard to the functional significance of specific residues and regions of the viral proton channel (Jimenez *et al.*, 2011). Interestingly, the viral M2 channel proteins show extremely high sequence similarity, with approximately three-quarters of the positions being occupied by only one residue in over 95 % of the cases. Nine M2 proteins from the H3N2 strain, which have the following UniProt accession numbers: Q288Z5 (A/Swine/Colorado/1/1977 H3N2), Q6XT43 (A/England/878/1969 H3N2), P0C2M3 (A/Memphis/101/1972 H3N2), Q6XTV0 (A/Tokyo/3/1967 H2N2), Q2PIM1 (A/Memphis/18/1978 H3N2), P63231 (A/Udorn/307/1972 H3N2), Q2PIK5 (A/Memphis/110/1976 H3N2), Q30NQ0 (A/Beijing/39/1975 H3N2), and Q3YPZ4 (A/Memphis/1/1971 H3N2), and possibly two proteins from the H2N2 strain, make a phylogenic cluster that is closely related to 2RLF (Fig. 2). Importantly, the results of this study revealed that there are significant mutational correlations between some of the positions in the proteins that have not been studied previously in the context of being functionally important. Furthermore, several other residues believed to be important to the M2 activity were not highly conserved (Le and Leluk, 2011). The correlated mutations within the M2 channel protein family should be discussed in greater detail in terms of their functional significance.

Current drug-resistant mutations

The anti-influenza drugs amantadine and rimantadine, which target the M2 channel protein of the influenza A

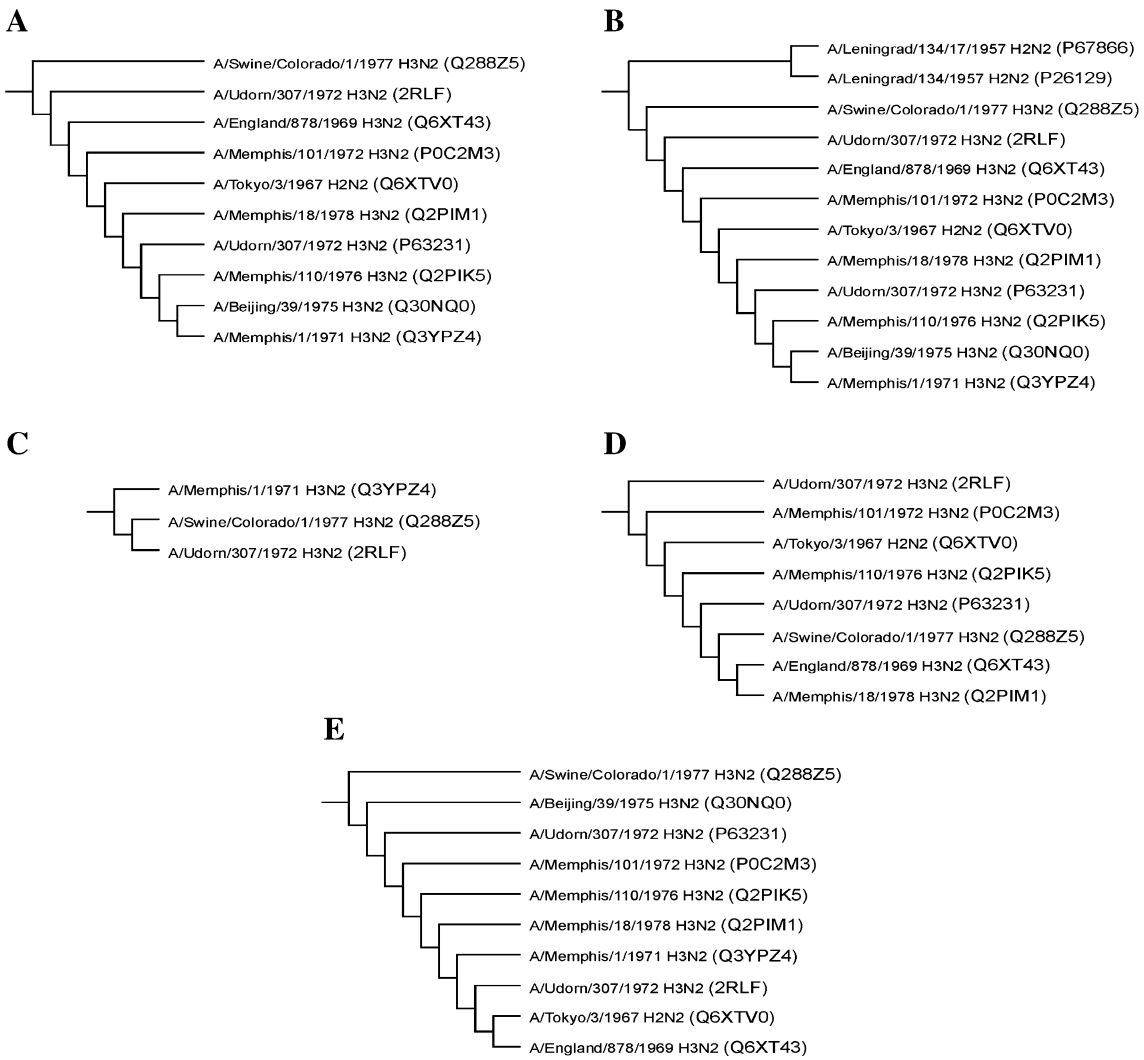

Fig. 2 Phylogenetic trees for the 2RLF cluster that were created via five different approaches. **a** ClustalX, **b** SSSg, **c** Phylip (maximum parsimony), **d** Phylip (maximum likelihood), and **e** Consurf. Reproduced from (Le and Leluk, 2011) with permission

virus, are no longer effective against this virus because of the spread of drug resistance (Bright *et al*., 2006; Deyde *et al*., 2007). Several studies of these FDA-approved drugs have shown that they have become ineffective as a consequence of five key mutation points including S31N, L26F, V27A, A30T, G34E, and L38F (Jimenez *et al*., 2011). The S31N is known to be the most dominant of these mutations, and is present in almost all of the circulating influenza A strains (Wang *et al*., 2013a). The results of recent research (Schnell and Chou, 2008) have demonstrated that S31 is a significant helix–helix packing interface factor, and that V27 is a pore-lining residue involved in the interaction of the protein with the proton channel inhibitors such as amantadine and rimantadine (Cady *et al*., 2010). Interestingly, mutation of the S31 residue results in drug resistance (Yi *et al*., 2008) even though this residue does not directly interact with the drug (Pielak *et al*., 2009). Williams et al. (2013b) successfully determined the side-chain conformation and dynamics of W41 in the M2 transmembrane peptide using high-resolution solid-state NMR spectroscopy to measure the chemical shifts of the W41 residue, the H37-W41 distances, and indole dynamics at high and low pH. The same group also used solid-state NMR spectroscopy to investigate the effects of amantadine- and aryl-substituted isoxazole compounds on the conformation of the S31N transmembrane segment and the dynamics of the proton-selective H37 residue (Williams *et al*., 2013a). Phylogenetic study also revealed that several specific positions, including 27, 28, 31, 36, 43, 50, 54, and 57, were involved in mutational correlations clusters (Le and Leluk, 2011). Only a few of these mutational points have previously been described as being significant to the proton transfer mechanism. The roles played by the residues at positions 28, 36, 50, 54, and 57, however, still

Table 1 Overview of M2 channel protein characteristics

Name	M2 channel protein
Functions	Virus assembly and budding
Drug-binding sites	Inside channel around S31 residue
Resistance mutations	S31N, L26F, V27A, A30T, G34E, L38F
Inhibitors	Amantadine, rimantadine, adamantane-based scaffold

remain unknown. Further studies are, therefore, required to investigate the contributions of these mutational points, as well as several other related residues, to develop a deeper understanding of the drug resistance mechanisms, which currently remain unclear in some cases (Betakova *et al*., 2005; Witter *et al*., 2008).

Recent strategies in drug design for new M2 channel inhibitors

There is an urgent need for the identification and development of new drug candidates for the treatment of influenza, and recent increases in the occurrence of oseltamivir resistant strains of the avian H5N1 and H1N1 viruses have prompted calls for new M2 channel inhibitors. For this reason, significant research efforts have been directed toward the design of new antiviral drugs that have a broader effect on different strains of the influenza virus, as well as proven efficacy against the mutant strains (Wang *et al*., 2011b). The M2 channel protein has traditionally been targeted with amantadine- and rimantadine-based drugs. Recent increases in the incidence of drug-resistant mutations (Bright *et al*., 2006; Deyde *et al*., 2007) have led to amantadine and rimantadine no longer being recommended for use by the Centers for Disease Control and Prevention. The most common drug-resistant mutation is S31N, but V27A and L26F are also found quite regularly (Du *et al*., 2012) (Table 1).

Three new inhibitors of the M2 protein channel were recently proposed by Du et al. (2010). The results of this study showed that the binding energies of these compounds were higher than those of amantadine and rimantadine. The results of two separate studies revealed that adamantane-based inhibitors possessed more than one pharmacophore (Stamatiou *et al*., 2001; Tataridis *et al*., 2007). Several other M2 inhibitors, including compound BL1743 (Fig. 3), have also been reported in the literature (Kurtz *et al*., 1995). Several studies involving the use of amantadine (Hu *et al*., 2010; Zhao *et al*., 2011; Wang *et al*.,. 2011a; Duque *et al*., 2011) or compound BL1743 (Wang *et al*., 2009; Balannik *et al*., 2009; Wang *et al*., 2011b, c) as a template for the design and synthesis of new inhibitors targeting M2 channel proteins have also been described in the literature. Molecular dynamics simulations have suggested that the channel pore site in the mutants is expanded, with the bulky hydrophobic group of compound A appearing to be well accommodated within the cavity (Fig. 3). In 2013, Jun Wang et al. (2013b) reported the discovery of a series of small-molecule drugs capable of inhibiting the S31N mutant of the M2 channel protein with potencies greater than that of amantadine. The binding of these drug molecules to the M2 protein effectively locked the protein into a well-defined conformation, with NMR structural analysis experiments revealing that the drug molecules were bound within the homotetrameric channel next to the A31 residue of the side chain.

In 2011, we presented a new design principle for the construction of potential inhibitors of drug-resistant strains of influenza A, which involved the introduction of an additional functional group to the amantadine scaffold, including amine, hydroxyl, cyclohexane, and aromatic groups, to increase the binding affinity of these compounds as M2 inhibitors (Tran *et al*., 2011). Most of the recently reported rational drug design studies have targeted M2 channel proteins bearing significant mutations inside the pore of the M2 channel, with amantadine being used as a template to create new inhibitors (Hu *et al*., 2010; Zhao *et al*., 2011; Wang *et al*., 2011a; Duque *et al*., 2011). For example, in our most recent study, we tested the binding affinity of 200 new adamantane compounds against the M2 channel proteins of H3N2 and 2009H1N1 using a virtual screening approach to search for the most potent M2 channel inhibitors based on an amantadine scaffold (Tran *et al*., 2011). Following on from this work, several other research groups also had a similar structure-based drug design strategy to identify new M2 channel inhibitors and reduce the false positives and negatives with the binding modes of 200 adamantane-based drugs in four different types of M2 channel protein structures, including structures from high-resolution X-ray crystallography, solution NMR, and solid-state NMR experiments (Tran *et al*., 2013). The results of these studies revealed that the favorable drug-binding sites, including those of the interaction residues and the resistance mutation residues, were Leu26, Val27, Ala30, Ser31, Gly34, and His37, and that these sites always play important roles and could, therefore, become promising targets for the design of new M2 channel inhibitors at the atomic level.

In our most recent study, we evaluated the binding modes of 200 adamantane-based drugs in four different types of M2 channel protein structures, including 2KQT, 2L0J, 2RLF, and 3C9J, to determine the critical binding interactions based on ligand-binding affinity properties. Using these data, we proposed three new candidates (i.e.,

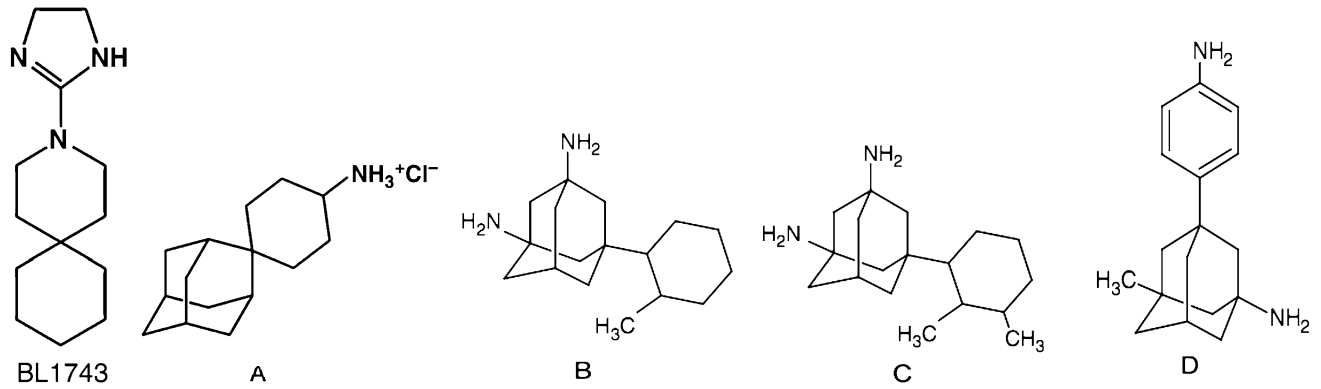

Fig. 3 Chemical structure of new potential M2 channel inhibitors

compounds B, C, and D), which had the highest binding affinities (in Fig. 3). The results of this study successfully revealed that there are two binding interaction mechanisms to the critical residues, including S31 and A30 in the holo and apo structures, respectively. Computational research is now playing a significant role in structure-based drug design (Du *et al*., 2012; Winkler *et al*., 2001; Wilson and Lill, 2011). Ligand docking and virtual screening approaches can be used to handle datasets and libraries containing large numbers of compounds (Heikamp and Bajorath, 2013; Waszkowycz, 2002; Lyne, 2002; Cavasotto and Orry, 2007). With regard to searching a database for new inhibitors of M2 channel proteins, we believe that further studies should be conducted to allow for broader searches to be performed using large-scale virtual screening techniques with other databases, such as ZINC, the CN chemical library, NCI diversity set, and DrugBank compounds to identify brand new scaffolds for M2 channel inhibitors that are not based on adamantane.

In 2009, Wei et al. (2009) constructed a fragment-based QSAR model for adamantane-based M2 channel inhibitors, with particular emphasis on the modeling of 34 M2 channel inhibitors against the H3N2 strain of the influenza A virus. Based on their fragment-based QSAR analyses, the authors of this report concluded that more research should be conducted toward exemplifying the F2 fragment of these adamantane-based drugs to create novel drugs for the treatment of influenza. In 2010, Zarubaev et al. (2010) synthesized a series of azolo-adamantane compounds with activity against the influenza A virus. They used both chemical and biological experiments to confirm the previously reported computational results, and consequently developed a new QSAR model.

In 2011, Lai et al. (2011) performed molecular docking experiments using the traditional Chinese medicine database (http://tcm.cmu.edu.tw/index.php) in an attempt to identify novel inhibitors of the M2 proteins. Following the docking and molecular dynamics simulation processes, the top three derivatives were shown to form stable interactions with three of the M2 residues, including S31, A30, and H37. The results of a separate study from Lin et al. (2011) suggested that the derivatives of genipin and methyl isoferulate (from traditional Chinese medicine) could have enhanced binding affinities toward the M2 channel. Selected molecular dynamics simulations of M2-derivative complexes revealed the presence of stable hydrogen-bonding interactions between these derivatives and the S10 and A9 residues of the M2 protein. The virtual screening of the traditional Chinese medicine database to identify new compounds that target M2 channel proteins, therefore, represents a viable option for overcoming the current difficulties associated with virus mutations (Chang *et al*., 2011), and these derivatives should be used as the basis for further biomolecular experiments and clinical studies.

Conclusions and outlook

This review provides a solid overview of the current level of understanding surrounding M2 channel protein structures, the mechanisms associated with their inhibition, and the origins of drug resistance in terms of structural characteristics and phylogenetic analysis. We have also reviewed several new strategies in computational drug design that have used state-of-the-art techniques, as well as a review of recent progress in both computational and experimental studies aimed at identifying new lead compounds for the development of M2 channel inhibitors. The gap in our current understanding of the 3D structures of M2 channel proteins and their inhibition mechanisms has recently been established, which has led to an increase in the amount of research being conducted toward drug resistance and novel rational drug design. Most notably, these newly designed M2 channel inhibitors possess more hydrophobic features.

We have also provided a review of the mutations inducing M2 inhibitor drug resistance, including the S31N, L26F, V27A, A30T, G34E, and L38F mutations. Based on the topics covered in this review, we believe that future studies should be focused on other mutational points, such as those at the 28, 36, 50, 54, and 57 positions. With regard to the development of new strategies for the design of novel M2 inhibitors, the virtual screening of larger databases composed of non-adamantane-based scaffolds represents an interesting approach that should be explored in greater detail. Furthermore, the flexible combination of M2 channel and neuraminidase inhibitors should be investigated in greater details to provide more effective inhibitors in the global battle against influenza. We hope that this review will encourage new opportunities and strategies for the development of more effective drugs against the influenza A virus.

Acknowledgments This work was supported by Vietnam's National Foundation for Science and Technology Development (NAFOSTED) through grant numbers 106.01–2012.66. The computing resources and support provided by the Institute for Computational Science and Technology, Ho Chi Minh City is gratefully acknowledged.

References

Acharya R, Carnevale V, Fiorin G, Levine BG, Polishchuk AL, Balannik V, Samish I, Lamb RA, Pinto LH, DeGrado WF, Klein ML (2010) Structure and mechanism of proton transport through the transmembrane tetrameric M2 protein bundle of the influenza A virus. Proc Natl Acad Sci USA 107(34):15075–15080. doi:10.1073/pnas.1007071107

Andricopulo AD, Salum LB, Abraham DJ (2009) Structure-based drug design strategies in medicinal chemistry. Curr Top Med Chem 9(9):771–790

Balannik V, Wang J, Ohigashi Y, Jing X, Magavern E, Lamb RA, Degrado WF, Pinto LH (2009) Design and pharmacological characterization of inhibitors of amantadine-resistant mutants of the M2 ion channel of influenza A virus. Biochemistry 48(50):11872–11882. doi:10.1021/bi9014488

Balgi AD, Wang J, Cheng DY, Ma C, Pfeifer TA, Shimizu Y, Anderson HJ, Pinto LH, Lamb RA, DeGrado WF, Roberge M (2013) Inhibitors of the influenza A virus M2 proton channel discovered using a high-throughput yeast growth restoration assay. PLoS One 8(2):e55271. doi:10.1371/journal.pone.0055271

Betakova T, Ciampor F, Hay AJ (2005) Influence of residue 44 on the activity of the M2 proton channel of influenza A virus. J Gen Virol 86(Pt 1):181–184. doi:10.1099/vir.0.80358-0

Bright RA, Shay DK, Shu B, Cox NJ, Klimov AI (2006) Adamantane resistance among influenza A viruses isolated early during the 2005–2006 influenza season in the United States. JAMA 295(8):891–894. doi:10.1001/jama.295.8.joc60020

Cady SD, Luo W, Hu F, Hong M (2009) Structure and function of the influenza A M2 proton channel. Biochemistry 48(31):7356–7364. doi:10.1021/bi9008837

Cady SD, Schmidt-Rohr K, Wang J, Soto CS, Degrado WF, Hong M (2010) Structure of the amantadine binding site of influenza M2 proton channels in lipid bilayers. Nature 463(7281):689–692. doi:10.1038/nature08722

Cavasotto CN, Orry AJ (2007) Ligand docking and structure-based virtual screening in drug discovery. Curr Top Med Chem 7(10):1006–1014

Chang SS, Huang HJ, Chen CY (2011) Two birds with one stone? Possible dual-targeting H1N1 inhibitors from traditional Chinese medicine. PLoS Comput Biol 7(12):e1002315. doi:10.1371/journal.pcbi.1002315

Cox NJ, Subbarao K (2000) Global epidemiology of influenza: past and present. Annu Rev Med 51:407–421. doi:10.1146/annurev.med.51.1.407

Cross TA, Dong H, Sharma M, Busath DD, Zhou HX (2012) M2 protein from influenza A: from multiple structures to biophysical and functional insights. Curr Opin Virol 2(2):128–133. doi:10.1016/j.coviro.2012.01.005

Deyde VM, Xu X, Bright RA, Shaw M, Smith CB, Zhang Y, Shu Y, Gubareva LV, Cox NJ, Klimov AI (2007) Surveillance of resistance to adamantanes among influenza A(H3N2) and A(H1N1) viruses isolated worldwide. J Infect Dis 196(2):249–257. doi:10.1086/518936

Du QS, Huang RB (2012) Recent progress in computational approaches to studying the M2 proton channel and its implication to drug design against influenza viruses. Curr Protein Pept Sci 13(3):205–210

Du QS, Huang RB, Wang CH, Li XM, Chou KC (2009) Energetic analysis of the two controversial drug binding sites of the M2 proton channel in influenza A virus. J Theor Biol 259(1):159–164. doi:10.1016/j.jtbi.2009.03.003

Du QS, Huang RB, Wang SQ, Chou KC (2010) Designing inhibitors of M2 proton channel against H1N1 swine influenza virus. PLoS One 5(2):e9388. doi:10.1371/journal.pone.0009388

Du J, Cross TA, Zhou HX (2012) Recent progress in structure-based anti-influenza drug design. Drug Discov Today 17(19–20):1111–1120. doi:10.1016/j.drudis.2012.06.002

Duque MD, Ma C, Torres E, Wang J, Naesens L, Juarez-Jimenez J, Camps P, Luque FJ, DeGrado WF, Lamb RA, Pinto LH, Vazquez S (2011) Exploring the size limit of templates for inhibitors of the M2 ion channel of influenza A virus. J Med Chem 54(8):2646–2657. doi:10.1021/jm101334y

Heikamp K, Bajorath J (2013) The future of virtual compound screening. Chem Biol Drug Des 81(1):33–40. doi:10.1111/cbdd.12054

Hu J, Asbury T, Achuthan S, Li C, Bertram R, Quine JR, Fu R, Cross TA (2007) Backbone structure of the amantadine-blocked transmembrane domain M2 proton channel from Influenza A virus. Biophys J 92(12):4335–4343. doi:10.1529/biophysj.106.090183

Hu W, Zeng S, Li C, Jie Y, Li Z, Chen L (2010) Identification of hits as matrix-2 protein inhibitors through the focused screening of a small primary amine library. J Med Chem 53(9):3831–3834. doi:10.1021/jm901664a

Intharathep P, Laohpongspaisan C, Rungrotmongkol T, Loisruangsin A, Malaisree M, Decha P, Aruksakunwong O, Chuenpennit K, Kaiyawet N, Sompornpisut P, Pianwanit S, Hannongbua S (2008) How amantadine and rimantadine inhibit proton transport in the M2 protein channel. J Mol Graph Model 27(3):342–348. doi:10.1016/j.jmgm.2008.06.002

Jimenez S, Torres M, Vizuete M, Sanchez-Varo R, Sanchez-Mejias E, Trujillo-Estrada L, Carmona-Cuenca I, Caballero C, Ruano D, Gutierrez A, Vitorica J (2011) Age-dependent accumulation of soluble amyloid beta (Abeta) oligomers reverses the neuroprotective effect of soluble amyloid precursor protein-alpha (sAPP(alpha)) by modulating phosphatidylinositol 3-kinase (PI3 K)/Akt-GSK-3beta pathway in Alzheimer mouse model. J Biol Chem 286(21):18414–18425. doi:10.1074/jbc.M110.209718

Kolocouris N, Zoidis G, Foscolos GB, Fytas G, Prathalingham SR, Kelly JM, Naesens L, De Clercq E (2007) Design and synthesis

of bioactive adamantane spiro heterocycles. Bioorg Med Chem Lett 17(15):4358–4362. doi:10.1016/j.bmcl.2007.04.108

Kurtz S, Luo G, Hahnenberger KM, Brooks C, Gecha O, Ingalls K, Numata K, Krystal M (1995) Growth impairment resulting from expression of influenza virus M2 protein in Saccharomyces cerevisiae: identification of a novel inhibitor of influenza virus. Antimicrob Agents Chemother 39(10):2204–2209

Lai C-Y, Chang T–T, Sun M-F, Chen H-Y, Tsai F-J, Lin J-G, Chen CY-C (2011) Molecular dynamics analysis of potent inhibitors of M2 proton channel against H1N1 swine influenza virus. Mol Simul 37(3):250–256. doi:10.1080/08927022.2010.543972

Laohpongspaisan C, Rungrotmongkol T, Intharathep P, Malaisree M, Decha P, Aruksakunwong O, Sompornpisut P, Hannongbua S (2009) Why amantadine loses its function in influenza m2 mutants: MD simulations. J Chem Inf Model 49(4):847–852. doi:10.1021/ci800267a

Laurie AT, Jackson RM (2006) Methods for the prediction of protein-ligand binding sites for structure-based drug design and virtual ligand screening. Curr Protein Pept Sci 7(5):395–406

Le L, Leluk J (2011) Study on phylogenetic relationships, variability, and correlated mutations in M2 proteins of influenza virus A. PLoS ONE 6(8):e22970. doi:10.1371/journal.pone.0022970

Li C, Long Y, Lin Z, Jie Y, Xiao Y, Yang L, Sun J, Ren Y, Chen L, Li Z (2013) New strategy for high throughput screening of anti-influenza virus m2 ion channel inhibitors. Curr Pharm Des 19(28):5146–5155

Lin CH, Chang TT, Sun MF, Chen HY, Tsai FJ, Chang KL, Fisher M, Chen CY (2011) Potent inhibitor design against H1N1 swine influenza: structure-based and molecular dynamics analysis for M2 inhibitors from traditional Chinese medicine database. J Biomol Struct Dyn 28(4):471–482. doi:10.1080/07391102.2011.10508589

Lyne PD (2002) Structure-based virtual screening: an overview. Drug Discov Today 7(20):1047–1055

Nguyen H, Le L, Truong TN (2009) Top-hits for H1N1pdm Identified by Virtual Screening Using Ensemble-based Docking. PLoS Curr 3:RRN1030. doi:10.1371/currents.RRN1030

Nguyen TH, Hoang D, Le L (2013) Binding Drugs on Two Position of M2 Proton Channel and Its Mutants. In: Toi VV, Toan NB, Dang Khoa TQ, Lien Phuong TH (eds) 4th International Conference on Biomedical Engineering in Vietnam, vol 40. IFMBE Proceedings. Springer Berlin Heidelberg, pp 384-390. doi:10.1007/978-3-642-32183-2_93

Pielak RM, Chou JJ (2011) Influenza M2 proton channels. Biochim Biophys Acta 1808 2:522–529. doi:10.1016/j.bbamem.2010.04.015

Pielak RM, Schnell JR, Chou JJ (2009) Mechanism of drug inhibition and drug resistance of influenza A M2 channel. Proc Natl Acad Sci USA 106(18):7379–7384. doi:10.1073/pnas.0902548106

Pinto LH, Holsinger LJ, Lamb RA (1992) Influenza virus M2 protein has ion channel activity. Cell 69(3):517–528 0092-8674(92)90452-I

Pissawong T, Maneewatch S, Thueng-In K, Srimanote P, Dong-din-on F, Thanongsaksrikul J, Songserm T, Tongtawe P, Bangphoomi K, Chaicumpa W (2013) Human monoclonal ScFv that bind to different functional domains of M2 and inhibit H5N1 influenza virus replication. Virol J 10:148. doi:10.1186/1743-422X-10-148

Reese KA, Lupfer C, Johnson RC, Mitev GM, Mullen VM, Geller BL, Pastey M (2013) A Novel Lactococcal Vaccine Expressing a Peptide from the M2 Antigen of H5N2 Highly Pathogenic Avian Influenza A Virus Prolongs Survival of Vaccinated Chickens. Vet Med Int 2013:316926. doi:10.1155/2013/316926

Rungrotmongkol T, Intharathep P, Malaisree M, Nunthaboot N, Kaiyawet N, Sompornpisut P, Payungporn S, Poovorawan Y, Hannongbua S (2009) Susceptibility of antiviral drugs against 2009 influenza A (H1N1) virus. Biochem Biophys Res Commun 385(3):390–394. doi:10.1016/j.bbrc.2009.05.066

Schnell JR, Chou JJ (2008) Structure and mechanism of the M2 proton channel of influenza A virus. Nature 451(7178):591–595. doi:10.1038/nature06531

Sharma M, Yi M, Dong H, Qin H, Peterson E, Busath DD, Zhou HX, Cross TA (2010) Insight into the mechanism of the influenza A proton channel from a structure in a lipid bilayer. Science 330(6003):509–512. doi:10.1126/science.1191750

Simonsen L, Viboud C, Grenfell BT, Dushoff J, Jennings L, Smit M, Macken C, Hata M, Gog J, Miller MA, Holmes EC (2007) The genesis and spread of reassortment human influenza A/H3N2 viruses conferring adamantane resistance. Mol Biol Evol 24(8):1811–1820. doi:10.1093/molbev/msm103

Stamatiou G, Kolocouris A, Kolocouris N, Fytas G, Foscolos GB, Neyts J, De Clercq E (2001) Novel 3-(2-adamantyl)pyrrolidines with potent activity against influenza A virus-identification of aminoadamantane derivatives bearing two pharmacophoric amine groups. Bioorg Med Chem Lett 11(16):2137–2142

Stouffer AL, Acharya R, Salom D, Levine AS, Di Costanzo L, Soto CS, Tereshko V, Nanda V, Stayrook S, DeGrado WF (2008) Structural basis for the function and inhibition of an influenza virus proton channel. Nature 451(7178):596–599. doi:10.1038/nature06528

Sukumar N, Das S (2011) Current trends in virtual high throughput screening using ligand-based and structure-based methods. Comb Chem High Throughput Screen 14(10):872–888

Tataridis D, Fytas G, Kolocouris A, Fytas C, Kolocouris N, Foscolos GB, Padalko E, Neyts J, De Clercq E (2007) Influence of an additional 2-amino substituent of the 1-aminoethyl pharmacophore group on the potency of rimantadine against influenza virus A. Bioorg Med Chem Lett 17(3):692–696

Tran L, Choi SB, Al-Najjar BO, Yusuf M, Wahab HA, Le L (2011) Discovery of potential M2 channel inhibitors based on the amantadine scaffold via virtual screening and pharmacophore modeling. Molecules 16(12):10227–10255. doi:10.3390/molecules161210227

Tran N, Tran L, Le L (2013) Strategy in structure-based drug design for influenza A virus targeting M2 channel proteins. Med Chem Res 22(12):6078–6088. doi:10.1007/s00044-013-0599-z

Wang JF, Chou KC (2012) Recent advances in computational studies on influenza a virus M2 proton channel. Mini Rev Med Chem 12(10):971–978

Wang J, Cady SD, Balannik V, Pinto LH, DeGrado WF, Hong M (2009) 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(23):8066–8076. doi:10.1021/ja900063s

Wang J, Ma C, Balannik V, Pinto LH, Lamb RA, Degrado WF (2011a) Exploring the requirements for the hydrophobic scaffold and polar amine in inhibitors of M2 from influenza A Virus. ACS Med Chem Lett 2(4):307–312. doi:10.1021/ml100297w

Wang J, Ma C, Fiorin G, Carnevale V, Wang T, Hu F, Lamb RA, Pinto LH, Hong M, Klein ML, DeGrado WF (2011b) Molecular dynamics simulation directed rational design of inhibitors targeting drug-resistant mutants of influenza A virus M2. J Am Chem Soc 133(32):12834–12841. doi:10.1021/ja204969m

Wang J, Ma C, Wu Y, Lamb RA, Pinto LH, DeGrado WF (2011c) Exploring organosilane amines as potent inhibitors and structural probes of influenza a virus M2 proton channel. J Am Chem Soc 133(35):13844–13847. doi:10.1021/ja2050666

Wang J, Ma C, Jo H, Canturk B, Fiorin G, Pinto LH, Lamb RA, Klein ML, DeGrado WF (2013a) 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(7):2804–2812. doi:10.1021/jm301538e

Wang J, Wu Y, Ma C, Fiorin G, Pinto LH, Lamb RA, Klein ML, Degrado WF (2013b) Structure and inhibition of the drug-resistant S31N mutant of the M2 ion channel of influenza A virus. Proc Natl Acad Sci USA 110(4):1315–1320. doi:10.1073/pnas.1216526110

Waszkowycz B (2002) Structure-based approaches to drug design and virtual screening. Curr Opin Drug Discov Devel 5(3):407–413

Wei H, Wang CH, Du QS, Meng J, Chou KC (2009) Investigation into adamantane-based M2 inhibitors with FB-QSAR. Med Chem 5(4):305–317

Williams JK, Tietze D, Wang J, Wu Y, DeGrado WF, Hong M (2013a) Drug-induced conformational and dynamical changes of the S31N mutant of the influenza M2 proton channel investigated by solid-state NMR. J Am Chem Soc 135(26):9885–9897. doi:10.1021/ja4041412

Williams JK, Zhang Y, Schmidt-Rohr K, Hong M (2013b) pH-dependent conformation, dynamics, and aromatic interaction of the gating tryptophan residue of the influenza M2 proton channel from solid-state NMR. Biophys J 104(8):1698–1708. doi:10.1016/j.bpj.2013.02.054

Wilson GL, Lill MA (2011) Integrating structure-based and ligand-based approaches for computational drug design. Future Med Chem 3(6):735–750. doi:10.4155/fmc.11.18

Winkler FK, Banner DW, Bohm HJ (2001) Structure-based approaches in modern drug discovery research. Ernst Schering Res Found Workshop 34:123–142

Witter R, Nozirov F, Sternberg U, Cross TA, Ulrich AS, Fu R (2008) Solid-state 19F NMR spectroscopy reveals that Trp41 participates in the gating mechanism of the M2 proton channel of influenza A virus. J Am Chem Soc 130(3):918–924. doi:10.1021/ja0754305

Yi M, Cross TA, Zhou HX (2008) A secondary gate as a mechanism for inhibition of the M2 proton channel by amantadine. J Phys Chem B 112(27):7977–7979. doi:10.1021/jp800171m

Zarubaev VV, Golod EL, Anfimov PM, Shtro AA, Saraev VV, Gavrilov AS, Logvinov AV, Kiselev OI (2010) Synthesis and anti-viral activity of azolo-adamantanes against influenza A virus. Bioorg Med Chem 18(2):839–848. doi:10.1016/j.bmc.2009.11.047

Zhao X, Li C, Zeng S, Hu W (2011) Discovery of highly potent agents against influenza A virus. Eur J Med Chem 46(1):52–57. doi:10.1016/j.ejmech.2010.10.010