Supporting information

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

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Synthetic Chemistry

All chemicals were purchased from commercial vendors and used without further purification unless otherwise noted. $^1$H and $^{13}$C NMR spectra were recorded on a Bruker-300 NMR spectrometer. Chemical shifts are reported in parts per million referenced with respect to residual solvent (CD$_3$OD) 3.31 ppm and (DMSO-d$_6$) 2.50 ppm or from internal standard tetramethylsilane (TMS) 0.00 ppm. The following abbreviations were used in reporting spectra: s) singlet, d) doublet, t) triplet, q) quartet, m) multiplet, dd) doublet of doublets, ddd) doublet of triplets,
doublet of doublets. All reactions were carried out under N\textsubscript{2} atmosphere, unless otherwise stated. HPLC grade solvents were used for all the reactions. Flash column chromatography was performed using silica gel (230-400 mesh, Merck). Low-resolution mass spectra were obtained using an ESI technique on 3200 Q Trap LC/MS/MS system (applied biosystem). The purity was assessed by using Shimadzu LC-MS with Waters X Terra MS C-18 column (part # 186000538), 50 x 2.1 mm, at a flow rate of 0.3 ml/min; \( \lambda = 250 \) and 220 nm; mobile phase A, 0.1\% formic acid in H\textsubscript{2}O, and mobile phase B, 0.1\% formic in 60\% isopropanol, 30\% CH\textsubscript{3}CN and 9.9\% H\textsubscript{2}O. The purified fractions were lyophilized. All compounds submitted for testing in TEVC assay and plaque reduction assay were confirmed to be > 95.0\% purity by LC-MS traces. All compounds were characterized by proton NMR, and selected compounds were also characterized by carbon NMR.

**Method I: General procedure for the reductive amination with amantadine.**

Adamantane (1 eq) and aldehyde (1 eq) were mixed with 2 mL of titanium (IV) isopropoxide. The resulting slurry was heated to 100 °C and stirred overnight. Then the solution was cooled to 0 °C using an ice-water bath, CH\textsubscript{3}OH was added and NaBH\textsubscript{4} (4 eq) was added portionwise over 10 mins. The solution was warmed to room temperature and stirred for 4 hours. The reaction was quenched with 1M NaOH and filtered through Celite. The filtrate was concentrated under
reduced pressure and purified by silica gel flash column chromatography (5-10% \( \text{CH}_3\text{OH/CH}_2\text{Cl}_2 \)) to give the final product.

Method II: General procedure for the alkylation of aryl chloride/bromide with amantadine.

\[
\begin{align*}
\text{NH}_2 + \text{X} & \xrightarrow{\text{CsI, DIEA, iPrOH, reflux overnight}} \text{R} \\
\text{X} = \text{Cl, Br} 
\end{align*}
\]

The chloride/bromide (1 eq), and amantadine (1.5 eq) were dissolved in isopropanol, CsI (0.1 eq) and triethyl amine (2 eq) were then added. The reaction mixture was heated to reflux overnight. The solvent was removed under reduced pressure, and the resulting residue was extracted with ethyl acetate and water. The organic layer was separated, dried over anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The mixture was then purified by silica gel flash column chromatography (5-10% \( \text{CH}_3\text{OH/CH}_2\text{Cl}_2 \)) to give the final product.

Cross-coupling for the synthesis of 31.
Compound 31 was synthesized using Suzuki cross-coupling reaction.\(^1\) Nitrogen was bubbled through a mixture of toluene (4 ml) and H\(_2\)O (200 µL) for 30 mins. Then N-[(5-bromothiophen-2-yl)methyl]adamantan-1-amine (11) (0.33 g, 1 mmol), cyclopropylboronic acid (0.11 g, 1.3 mmol), potassium phosphate (0.79g, 3.7 mmol), tricyclohexylphosphine (0.028 g, 0.1 mmol), and palladium acetate (0.022 g, 0.05 mmol) was added sequentially. The resulting mixture was heated to 100 °C for 3 h then cooled to room temperature. Solvent was removed under reduced pressure. Water was added to the mixture and extracted with ethyl acetate (X3). The combined ethyl acetate was dried with MgSO\(_4\), filtered and concentrated under reduced pressure. The mixture was then purified by silica gel flash column chromatography (5-10% CH\(_3\)OH/CH\(_2\)Cl\(_2\)) to give the final product 31 as a yellow solid (0.21 g, yield: 72%).\(^1\)\(^\text{HNMR (300 MHz, CD\(_3\)OD):}\) \(\delta\) 7.03 (d, \(J = 3.45\) Hz, 1H), 6.76 (d, \(J = 3.45\) Hz, 1H), 4.31 (s, 2H), 2.24-2.20 (m, 3H), 2.14-2.09 (m, 1H), 2.00-1.95 (m, 6H), 1.84-1.72 (m, 6H), 1.05-1.02 (m, 2H), 0.71-0.69 (m, 2H). \(\text{EI-MS: } m/z (M+H^+): 288.5 \text{ (calculated), 288.4 (found).}\)

The following compounds were synthesized using method I: 6, 7, 9, 11, 13, 14, 16, 18, 19, 20, 24, 25, 26, 27, 28, 29, 30, 32, 33, and 34.

The following compounds were synthesized using method II: 8, 10, 12, 15, 17, 21, 22, and 23.

**Synthesis of compound 27.**

\[\text{Br} \quad \text{S} \quad \text{HN} \quad + \quad \text{I}_2 \quad \text{n-BuLi, -78°C} \quad \rightarrow \quad \text{HN} \quad \text{S} \quad \text{Br} \quad \text{I} \]
N-[(5-Iodothiophen-2-yl)methyl]adamantan-1-amine. To a solution of N-((5-bromothiophen-2-yl)methyl)adamantan-1-amine (11) (1 mmol) in THF (12 mL) was added n-BuLi in hexane (2.5M 1.8 mL) at -78 °C under N₂. The reaction mixture was stirred for 30 min and then I₂ was added and stirred for 30 min at -78 °C. The mixture was quenched with sodium thiosulfate, and the crude mixture was extracted with diethyl ether (x 3). The combined organic layers were dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude reaction mixture was purified by prep HPLC to give a light yellow solid. ¹H NMR (500 MHz, CDCl₃) δ 7.05 (d, J = 3.5 Hz, 1H), 6.63 (d, J = 3.7 Hz, 1H), 3.96 (s, 2H), 2.20-1.96 (m, 3H), 1.86-1.47 (m, 12H). ¹³C NMR (126 MHz, CDCl₃) δ 152.95, 136.46, 125.40, 71.50, 51.04, 42.89, 40.37, 36.78, 29.67. Data: LC/MS (ESi) m/z 374.01 [M+1]. HRMS (ESI) m/z calcd. for C15H21NSI (M+H⁺) 374.0439, found 374.0436.

N-[(2-Chloro-1,3-thiazol-5-yl)methyl]adamantan-1-amine. (Yield: 71 %). ¹H NMR (300 MHz, CD₂OD): δ 7.44 (s, 1H), 3.93 (s, 2H), 2.09-2.07 (m, 3H), 1.72-1.69 (m, 12H). EI-MS: m/z (M+H⁺): 283.8 (calculated), 283.4 (found).

N-[(5-Chloro-1,2,4-thiadiazol-3-yl)methyl]adamantan-1-amine. (Yield: 68 %). ¹H NMR (300 MHz, CD₂OD): δ 4.05 (s, 2H), 2.09-2.07 (m, 3H), 1.74-1.66 (m, 12H). EI-MS: m/z (M+H⁺): 284.8 (calculated), 284.8 (found).

N-[(2-Bromo-1,3-thiazol-5-yl)methyl]adamantan-1-amine. (Yield: 67 %). ¹H NMR (300 MHz, DMSO-d6): δ 7.51 (s, 1H), 3.91 (s, 2H), 2.32 (br s, 1H), 2.05-2.02 (m, 3H), 1.62-1.58 (m, 12H). EI-MS: m/z (M+H⁺): 327.1, 329.0 (calculated), 327.6, 329.6 (found).
| **Figure** | **Chemical Structure** | **Chemical Name** | **Yield** | **¹HNMR** | **EI-MS** |
|------------|------------------------|-------------------|-----------|-----------------|----------|
| ![Figure 8](image1.png) | N-[(2-Bromo-1,3-thiazol-4-yl)methyl]adamantan-1-amine. (Yield: 65 %). | ¹HNMR (300 MHz, DMSO-d6): δ 7.44 (s, 1H), 3.76 (s, 2H), 2.01 (s, 3H), 1.60-1.50 (m, 12H). El-MS: m/z (M+H⁺): 327.1, 329.0 (calculated), 327.1, 329.1 (found). |
| ![Figure 10](image2.png) | N-[(3-Bromo-1,2-oxazol-5-yl)methyl]adamantan-1-amine. (Yield: 65 %). | ¹HNMR (300 MHz, CD₃OD): δ 6.50 (s, 1H), 3.91 (s, 2H), 2.09-2.07 (m, 3H), 1.74-1.68 (m, 12H). El-MS: m/z (M+H⁺): 311.0, 313.0 (calculated), 311.5, 313.3 (found). |
| ![Figure 11](image3.png) | N-[(5-Bromothiophen-2-yl)methyl]adamantan-1-amine. (Yield: 74 %) ¹H NMR (400 MHz, CDCl₃): δ 6.88 (d, J = 3.6 Hz, 1H), 6.68 (d, J = 3.6 Hz, 1H), 3.93 (s, 2H), 2.12-2.09 (m, 3H), 1.70-1.65 (m, 12H). LC/MS (ESCI) m/z 325.86/328.00 [M+1]⁺. HRMS (ESI) m/z calcd. for C₁₅H₂₁NSBr (M+H⁺) 326.0578, found 326.0590. |
| ![Figure 12](image4.png) | N-(1,2,4-Oxadiazol-3-ylmethyl)adamantan-1-amine. (Yield: 78 %) ¹H NMR (400 MHz, CDCl₃): δ 8.65 (s, 1H), 4.00 (s, 2H), 2.11-2.08 (m, 3H), 1.71-1.60 (m, 12H). El-MS: m/z (M+H⁺): 234.3 (calculated), 234.3 (found). |
| ![Figure 13](image5.png) | N-(Furan-2-ylmethyl)adamantan-1-amine. (Yield: 61 %). ¹H NMR (300 MHz, DMSO-d₆): δ 7.50 (s, 1H), 6.35-6.34 (dd, J=1.84 Hz, 1.84 Hz, 1H), 6.19-6.18 (d, J=2.96Hz, 1H), 3.66 (s, 2H), 2.00 (s, 3H), 1.63-1.54 (m, 12H). El-MS: m/z (M+H⁺): 232.3 (calculated), 232.3 (found). |
| ![Figure 14](image6.png) | N-[(5-Methylfuran-2-yl)methyl]adamantan-1-amine. (Yield: 47 %). ¹H NMR (300 MHz, DMSO-d₆): δ 6.02 (s, 1H), 5.93 (s, 1H), 3.59 (s, 2H), 2.20 (s, 3H), 2.00 (s, 3H), 1.63-1.54 (m, 12H). El-MS: m/z (M+H⁺): 246.3 (calculated), 246.1 (found). |
| Structure | Description | Yield | NMR (300 MHz, CD$_3$OD) | EI-MS: m/z (M+H$^+$) | Found   |
|-----------|-------------|-------|--------------------------|----------------------|---------|
| ![Structure](image1) | $N$-[(2-Methyl-1,3-thiazol-5-yl)methyl]adamantan-1-amine. (Yield: 82 %). $^1$HNMR | $82\%$ | $\delta$ 7.48 (s, 1H), 3.93 (s, 2H), 2.66 (s, 3H), 2.09-2.07 (m, 3H), 1.75-1.70 (m, 12H). | 263.4 (calculated), 263.4 (found). |
| ![Structure](image2) | 5-[(Adamantan-1-ylamino)methyl]-1,3-thiazol-2-amine. (Yield: 66 %). $^1$HNMR | $66\%$ | $\delta$ 6.82 (s, 1H), 3.80 (s, 2H), 2.10-2.07 (m, 3H), 1.76-1.66 (m, 12H). | 264.4 (calculated), 264.4 (found). |
| ![Structure](image3) | $N$-[(2-Methyl-1,3-thiazol-4-yl)methyl]adamantan-1-amine (Yield: 77 %). $^1$HNMR | $77\%$ | $\delta$ 7.23 (s, 1H), 3.87 (s, 2H), 2.70 (s, 3H), 2.10-2.08 (m, 3H), 1.79-1.71 (m, 12H). | 263.4 (calculated), 263.0 (found). |
| ![Structure](image4) | $N$-[(2-Methyl-1H-imidazol-4-yl)methyl]adamantan-1-amine. (Yield: 51 %). $^1$H NMR | $51\%$ | $\delta$ 6.62 (s, 1H), 3.53 (s, 2H), 2.19 (s, 3H), 2.01 (s, 3H), 1.63-1.54 (m, 12H). | 245.2 (calculated), 246.3 (found) |
| ![Structure](image5) | $N$-[(5-Methyl-1,2-oxazol-3-yl)methyl]adamantan-1-amine. (Yield: 88 %). $^1$HNMR | $88\%$ | $\delta$ 6.19 (s, 1H), 3.77 (s, 2H), 3.40 (s, 3H), 2.09-2.06 (m, 3H), 1.76-1.70 (m, 12H). | 247.4 (calculated), 247.2 (found). |
| ![Structure](image6) | $N$-[(1-Methyl-1,2,3-triazol-4-yl)methyl]adamantan-1-amine. (Yield: 81 %). $^1$HNMR | $81\%$ | $\delta$ 7.82 (s, 1H), 4.09 (s, 3H), 3.85 (s, 2H), 2.10-2.08 (m, 3H), 1.77-1.71 (m, 12H). | 247.4 (calculated), 247.4 (found). |
| ![Structure](image7) | $N$-[(5-Methyl-1,2,4-oxadiazol-3-yl)methyl]adamantan-1-amine. (Yield: 89 %). $^1$HNMR | $89\%$ | $\delta$ 3.85 (s, 2H), 2.58 (s, 3H), 2.10-2.07 (m, 3H), 1.76-1.66 (m, 12H). | 248.3 (calculated), 248.4 (found). |
| Compound                                                                 | Yield (%) | NMR (300 MHz, CD3OD) | EI-MS: m/z (M+H+) |
|-------------------------------------------------------------------------|-----------|----------------------|------------------|
| N-[(5-Methyl-1,3,4-oxadiazol-2-yl)methyl]adamantan-1-amine              | 89        | δ 3.97 (s, 2H), 2.53 (s, 3H), 2.10-2.07 (m, 3H), 1.77-1.71 (m, 12H) | 248.3 (calculated), 248.4 (found). |
| N-[(Trifluoromethyl)-1,3,4-oxadiazol-2-yl)methyl adamantan-1-amine      | 77        | δ 4.04 (s, 2H), 2.11-2.07 (m, 3H), 1.63-1.53 (m, 12H) | 302.3 (calculated), 302.3 (found). |
| N-[(2-Tert-butyl-1,3-thiazol-5-yl)methyl]adamantan-1-amine              | 78        | δ 7.51 (s, 1H), 3.94 (s, 2H), 2.09-2.07 (m, 3H), 1.76-1.72 (m, 12H), 1.42 (s, 9H) | 305.5 (calculated), 305.5 (found). |
| N-(Thiophen-2-ylmethyl)adamantan-1-amine                                | 86        | δ 7.19-7.16 (m, 1H), 6.94-6.92 (m, 2H), 3.98 (s, 2H), 2.10-2.07 (m, 3H), 1.71-1.61 (m, 12H) | 248.4 (calculated), 248.4 (found). |
| N-[(5-Chlorothiophen-2-yl)methyl]adamantan-1-amine                       | 54        | δ 6.86 (s, 1H), 6.75 (s, 1H), 3.79 (s, 2H), 1.98 (s, 3H), 1.63-1.61 (m, 12H) | 282.1 (calculated), 282.2 (found). |
| N-[(5-Methylthiophen-2-yl)methyl]adamantan-1-amine                       | 68        | δ 6.67 (s, 1H), 6.56 (s, 1H), 3.78 (s, 2H), 2.36 (s, 3H), 1.99 (s, 3H), 1.63-1.61 (m, 12H) | 262.1 (calculated), 262.1 (found). |
| N-[(5-Methoxythiophen-2-yl)methyl]adamantan-1-amine                      | 58        | δ 6.52-6.50 (d, J=3.16 Hz, 1H), 5.99-5.98 (d, J=3.4 Hz, 1H), 3.83 (s, 3H), 3.81 (s, 2H), 2.06 (s, 3H), 1.67-1.53 (m, 12H) | 278.1 (calculated), 278.2 (found). |
$N$-[5-Ethylthiophen-2-yl)methyl]adamantan-1-amine. (Yield: 68 %). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 6.73 (s, 1H), 6.64 (s, 1H), 3.84 (s, 2H), 2.77-2.73 (m, 2H), 2.04 (s, 3H), 1.64-1.58 (m, 12H), 1.26-1.21 (m, 3H). EI-MS: $m/z$ (M+H$^+$): 276.1 (calculated), 276.4 (found).

$N$-[4-Bromothiophen-2-yl)methyl]adamantan-1-amine (Yield: 78 %). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 7.45 (s, 1H), 6.95 (s, 1H), 3.89 (s, 2H), 2.11 (br s, 1H), 2.06-2.02 (m, 3H), 1.64-1.60 (m, 12H). EI-MS: $m/z$ (M+H$^+$): 326.1, 328.1 (calculated), 326.2, 328.5 (found).

$N$-[3-Methylthiophen-2-yl)methyl]adamantan-1-amine. (Yield: 68 %). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 7.20-7.18 (d, $J=4.92$ Hz, 1H), 6.80-6.77 (d, $J=4.92$Hz, 1H), 3.76 (s, 2H), 2.13 (s, 3H), 2.03 (s, 3H), 1.65-1.57 (m, 12H). EI-MS: $m/z$ (M+H$^+$): 262.2 (calculated), 262.2 (found).

$N$-[5-Bromo-4-methylthiophen-2-yl)methyl]adamantan-1-amine (Yield: 71 %). $^1$H NMR (300 MHz, DMSO-$d_6$): $\delta$ 6.74 (s, 1H), 3.82 (s, 2H), 2.09 (s, 3H), 2.06-2.03 (m, 3H), 1.73-1.62 (m, 12H). EI-MS: $m/z$ (M+H$^+$): 340.1, 342.1 (calculated), 340.2, 342.3 (found).

Optimization of CHARMM force field parameters for the halogenated compound 11, and molecular dynamics simulations of A/M2 WT and S31N proton channels bound to 11.

SI-1. Charge optimization based on QM data

Due to the presence of halogens in several potential inhibitors of the M2 proton channel, electronic structure calculations were executed to model possible occurrences of chemically
specific interactions. In particular, given the pressing consideration that recently emerged in medicinal chemistry on the role played by halogen bonding in molecular recognition,\textsuperscript{2,3} the need for biomolecular force fields that can account for this particular behavior becomes crucial for accurate predictions of drug protein interactions.

In the current context, it was required to model halogen bonding regardless of whether it is a main component of the structure-activity relationship (SAR) for the chemical class of interest. Indeed, the call for reliable parameters for halogenated compounds still remains open, and this poses a significant challenge in performing molecular simulations to evaluate the effects of halogen substituted small molecules binding to drug targets.

Here, quantum mechanical (QM) calculations for the brominated compound 11 were used to optimize the partial atomic charges on the halogen provided with the existing CHARMM general force field (CGenFF/Paramchem) parameters.\textsuperscript{4-8}

Calculations were carried out using the NWChem program\textsuperscript{9} at both the density-functional theory (DFT) and the second-order Møller-Plesset perturbation theory (MP2) levels. Multiple basis sets were used, including split-valence Pople\textsuperscript{10} (i.e. 6-311G**) and correlation consistent\textsuperscript{11} (such as cc-pVDZ and cc-pVTZ). Reproducing the interaction energies of 11 and a similar halogenated compound in complex with model systems, based on QM data, reassigned partial atomic charges.

\textit{(a) Interactions with Water}

\textit{System 1. Di-hydrated 11 - Water}

First, charges were calculated of a di-hydrated compound 11 in complex with a water molecule (Fig. S1A). Indeed, while a water molecule was introduced to evaluate the intermolecular
interactions provided by the bromine on the substituted thiophene ring, two additional waters were added to hydrate the quaternary ammonium moiety bearing the adamantly group of 11.

Interaction energies were determined upon geometry optimization of system 1 at both DFT and MP2 levels of theory using different basis sets (Table S1), and were calculated in the order of −5.419 kcal/mol (6-311G** basis set; Fig. S1A).

QM optimized partial atomic charges were then derived for the bromine atom.

System 2. Di-hydrated MBT – Water

To test the resultant charges for reproducibility (and ultimately transferability) across similar halogenated-compounds, a second system was submitted to the same calculations. The latter consisted in a di-hydrated derivative of 11 where the adamantane ring is substituted for a methyl-group (hereafter called MBT) in complex with a water molecule. Interaction energies were calculated using different basis sets (Table S2), and were estimated at −6.145 kcal/mol (MP2/6-311G** basis set; Fig. S1B).

(b) Interactions with Protein Groups

Traditionally perceived as Lewis-base moieties with strong preferences for establishing hydrophobic contacts in the context of protein-ligand interactions, covalently bound halogens have recently gained increasing attention in drug design for their ability to establish close contacts with biomolecular counterparts through an electrostatically-driven behavior known as halogen bonding.

In that case, halogens act as Lewis acids, and therefore can interact with certain electron donors in protein systems located at suitable distance along the electrostatic potential defined by covalently bound halogens. The resulting charges were tested on model
systems involving potential halogen bond with protein groups using a di-hydrated MBT in complex with either N-methyl-acetamide (NMA) or imidazole.

**System 3. Di-hydrated MBT – Carbonyl oxygen moiety (backbone carbonyl system)** Among the most frequently occurring interaction types in biomolecular systems, carbonyl oxygen moieties certainly stand up, whether belonging to the backbone of proteins (in many instances) or to the side chains of certain amino acids. Here, calculations involving a di-hydrated MBT compound interacting with the carbonyl oxygen moiety of an NMA molecule, targeting the backbone carbonyl structural motif, were conducted. Interaction energies were calculated using different basis sets (**Table S2**), and were estimated at −8.528 kcal/mol (MP2/6-311G** basis set; **Fig. S1C**).

**System 4. Di-hydrated MBT – Imidazole (Histidine nitrogen system)**

Given the importance exerted by H37 on the functional mechanism of the M2 proton channel, a system comprising a di-hydrated MBT compound targeting the unprotonated nitrogen of an imidazole ring (featuring the Nδ or Nε atoms of an histidine) was also evaluated by quantum force fields. Interaction energies were calculated using different basis sets (**Table S2**), and were estimated at −9.753 kcal/mol (MP2/6-311G** basis set; **Fig. S1D**).

Optimized partial charges, together with parameters from CGenFF, were then used to perform molecular dynamics.
Figure S1. QM optimized complex geometries (at the MP2/6311G** level) of a dy-hydrated 11 with water (A); a dy-hydrated MBT with water (B); a dy-hydrated MBT with NMA (C); a dy-hydrated MBT with imidazole (D). For each complex, angles are in degrees (deg) and distances in angstroms (Å).
Table S1. Interaction energies of a di-hydrated 11 in complex with one water molecule obtained using different basis sets. \[^{[a]}\]

| System | Interaction Partners | Theory | Basis Set     | Interaction Energy (kcal/mol) |
|--------|----------------------|--------|---------------|-------------------------------|
| 1      | 2W- 11 \cdots OH2   | MP2    | cc-pVDZ       | −6.249                        |
| 1      | 2W- 11 \cdots OH2   | MP2    | cc-pVTZ       | −5.679                        |
| 1      | 2W-11 \cdots OH2    | MP2    | 6-311G**      | −5.419                        |
| 1      | 2W-11 \cdots OH2    | MP2    | aug-cc-pVDZ   | −6.424                        |

\[^{[a]}\] 11 = N-[(5-bromothiophen-2-yl)methyl]adamantan-1-amine; interaction energy with aug-cc-pVDZ was calculated on the geometry optimized with cc-PVDZ
Table S2. Interaction energies of a di-hydrated 11 derivative (MBT) in complex with one water molecule, an NMA and an imidazole obtained using different basis sets. [a]

| System | Interaction Partners | Theory | Basis Set | Interaction Energy (kcal/mol) |
|--------|-----------------------|--------|-----------|-----------------------------|
| 2      | 2W-MBT … OH2          | CGenFF | na        | −5.023                      |
| 2      | 2W-MBT … OH2          | MP2    | cc-pVTZ   | −5.800                      |
| 2      | 2W-MBT … OH2          | MP2    | 6-311G**  | −6.145                      |
| 3      | 2W-MBT … O(BCB)       | CGenFF | na        | −7.281                      |
| 3      | 2W-MBT … O(BCB)       | MP2    | cc-pVTZ   | −8.463                      |
| 3      | 2W-MBT … O(BCB)       | MP2    | 6-311G**  | −8.528                      |
| 4      | 2W-MBT … N(HIS)       | CGenFF | na        | −7.386                      |
| 4      | 2W-MBT … N(HIS)       | MP2    | cc-pVTZ   | −10.087                     |
| 4      | 2W-MBT … N(HIS)       | MP2    | 6-311G**  | −9.753                      |

[a] MBT = derivative of 11 where the adamantane ring is substituted for a methyl-group; 2W-MBT = di-hydrated MBT; NMA = N-methyl-acetamide; O(BCB) = carbonyl oxygen in NMA; N(HIS) = un-protonated nitrogen on the imidazole ring of a histidine. The charge on the bromine atom of 11 was tuned to reproduce interactions with one water molecule; na = not applicable
SI-2. Molecular Dynamics Simulations.

Simulations involving the M2 channel were conducted on a 22-46 segment of the tetrameric M2 protein bundle of the Influenza A Virus (Udorn sequence), spanning residues SSDPLVVAASIIGILILWILDRL and SSDPLVVAANIIGILILWILDRL for the WT protein and the S31N mutant, respectively.

The segment was constructed upon adding the amino acid range 22 to 25 (SSD) to the transmembrane region of the A/M2 bundle (residues 25 to 46). Upon A34G reversion, the protein bundle was modeled at intermediate pH, with two of the four H37 residues doubly protonated and the others mono-protonated at the N_ε position, as in the study of Acharya et al.\textsuperscript{15} To resolve potential steric clashes and bad contacts in the starting configuration, the system was energy minimized by adding harmonic restraints on residues 25 to 46. While 11, in complex with the WT channel, was inserted with the positively charged ammonium facing downwards the C-terminal H37, its initial configuration was flipped to face the positively charged ammonium upwards the N-terminal V27 in the S31N mutant complex.

In each complex, all water molecules overlapping the ligand were removed. Each system was embedded in a 1-palmitoyl-2-oleoylphosphatidylcholine (POPC) bilayer (8\times8 \text{ nm}^2), and then hydrated by a 150 mM KCl water solution. Simulations were performed using the CHARMM36,\textsuperscript{4,5} CGenFF,\textsuperscript{6-8} and TIP3P\textsuperscript{16} force fields for the treatment of protein, lipids, 11 and water molecules, respectively. Optimized partial atomic charge of the bromine atom of 11 was obtained by MP2 calculations, as detailed in the SI-1.

Systems, comprising protein segments, ligands and water molecules, were energy minimized before equilibration. Position restraints were initially applied upon setting harmonic potentials,
which were gradually released during the first 50-ns simulation time, on protein residues, as well as on the ligand 11. In particular, an initial force constant $K_1 = 20$ kcal/mol/Å$^2$ was applied to protein backbone and 11 atoms, while $K_1 = 5$ kcal/mol/Å$^2$ was set to protein side chains. Equilibrated systems were then simulated for additional 150 ns of unrestrained dynamics.

The velocity Verlet integration method was used to solve the equations of motion, implementing a time step of 2 fs, for a total of 200 ns simulation. The Particle mesh Ewald (PME) method was used to solve the electrostatic potential. The Langevin temperature and Langevin piston coupling schemes were applied. The systems were run at 310 K temperature and 1 atm pressure.

For the binding mode of compound 11 in complex with the S31N channel, the pair correlation function of the bromine atom with surrounding water oxygens was generated over all the trajectory frames of the simulation (Fig. S2). The distribution shows a first hydration shell centered at 3.5 Å of distance from the bromine, with a second peak centered at 6 Å (integrals at 5 Å and 8 Å, respectively).

The programs NAMD$^{17}$ (version 2.9) and VMD$^{18}$ (version 1.9) were used for simulation and analysis.
Figure S2. Pair correlation function between the bromine atom of compound 11 and waters oxygens.
Figure S3. Assignments for I33 (blue) and V27 (red) in 2D C(C)H-TOCSY. For clarity, only assignments for residues I33 and V27 are shown, which are the most important ones to determine drug orientations. *: the I33 Hγ12-Cγ2 peak overlaps with the A30 Hβ-Cβ.
Figure S4. The direct comparison of 2D $^{13}$C-$^1$H NOESY (A, part of Figure 2 in main text) and 2D C(C)H-TOCSY (B) shows the band of intensities between I33 $\delta$1 and amantadine H6,H7 and H8 are most likely protein-protein NOEs, mainly between I33 $\delta$1 and I33 H$\beta$, and between I33 $\delta$1 and I33 H$\gamma$12. Peak labels in blue and red are for I33 and V27, respectively. Peaks #1 and #2 could be assigned to I33 $\delta$1-H7, and I33 $\delta$1-H6, respectively. However, the peaks #1 and #2 don’t align well with the drug peaks H7 and H6. Moreover, the peaks are too close to the strong intra-residue peaks from I33, in particular, Peak #3, which could be for I33 $\delta$1 and H8, completely overlaps with the H$\gamma$12-$\delta$1 peak. As a comparison, the assignments in the dashed magenta frame between V27 methyl groups and the amantadine protons can be obtained unambiguously.
Table S3. Peptide-drug NOEs for the WT and S31N M2TM in presence of 11*

| Peptide proton | Drug proton | Distance( Å) |
|----------------|-------------|--------------|
| Val27 Hγ1     | H8          | 3.5          |
| Val27 Hγ1     | H7          | 4.0          |
| Val27 Hγ1     | H6          | 5.0          |
| Val27 Hγ2     | H8          | 3.5          |
| Val27 Hγ2     | H7          | 4.0          |
| Val27 Hγ2     | H6          | 5.0          |
| Val27 Hα      | H8          | 5.0          |
| Val27 Hα      | H7          | 5.0          |
| Val27 Hα      | H6          | 5.0          |
| Ala30 Hα      | H6          | 6.0          |
| Ala30 Hβ      | H5          | 4.0          |
| Ala30 Hβ      | H2          | 5.0          |
| Asn31 Hα      | H6          | 6.0          |
| Ile33 Hγ2     | H1          | 5.0          |
| Ile33 Hδ1     | H1          | 6.0          |

| Peptide proton | Drug proton | Distance( Å) |
|----------------|-------------|--------------|
| Val27 Hγ1     | H1          | 4.0          |
| Val27 Hγ1     | H2          | 5.0          |
| Val27 Hγ2     | H1          | 5.5          |
| Ala30 Hβ      | H5          | 4.0          |
| Ala30 Hβ      | H6          | 4.0          |
| Ala30 Hβ      | H8          | 5.0          |
| ASN31 Hα      | H6          | 4.0          |
| Ile33 Hγ2     | H8          | 5.5          |
| Ile33 Hδ1     | H8          | 6.0          |
| Gly34 Hα1/ Hα2| H7          | 5.0          |
| Gly34 Hα1/ Hα2| H8          | 4.0          |

*: Distances were derived from NOEs in the 150ms 2D 13C-(1H)-1H NOESY shown in Figure 2 A and B. NOE cross peaks were classified as strong (1.8 to 3.5 Å), medium (1.8 to 4.0 Å), weak (1.8 to 5.0 Å) and very weak (1.8 to 6.0 Å) by referencing to the NOE intensities observed for drug protons at fixed distances.
NMR spectra for the synthesized compounds:
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