PACKING OF AMANTADINE-LIKE COMPOUNDS IN THE CENTRAL CAVITY OF INFLUENZA A M2

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ABSTRACT

Over 291 variants of amantadine, mw 113.2 to 437.5, have been proposed as therapeutic agents for influenza A. These amantadine-like compounds (ALCs) have been thought to function as intrachannel blockers in the M2 channel of the influenza A virus. We explored whether these drugs fit in the central cavity of the channel using simple simulation methods with the CHARMM force field, the atomic coordinates of the solid state NMR structure for the M2 channel (PDB ID 2L0J, model 1), and a full set of ALCs, with variations and titration states, gleaned from the literature. First, we examined the optimal water packing in the central cavity of the rigid channel using randomized, annealed water insertion, which indicates that optimally it holds 882 to 1116 Daltons of water. The ALCs from the literature nearly all fit sterically within the central cavity, judging from ALCs potential energies, water volume displacement capacity, and ALC compression analysis. Future work will focus on the relative energetics of drug binding and configuration and the solvation of ALCs in explicit water within the central cavity.

INTRODUCTION

30,000 a year die from seasonal influenza infections in the United States. In 1968, amantadine was approved by the FDA to help combat the flu. Influenza A M2 is a target for anti-flu drugs. There are 291 amantadine and spiroamine compounds which have been synthesized to target the M2 channel in Influenza A. 116 of these ALCs show an improved efficacy over amantadine. As new drugs are developed the binding site location is of interest and the controversy over this location has been resolved in favor of the central cavity. We report initial efforts for screening all 291 previously tested compounds in central cavity of the solid state NMR apo structure (Sharma et al 2010) of the Influenza M2 channel.

Central Cavity Water Packing

Materials: Model 1 of PDB ID 2L0J for M2 channel, TIP3 water, CHARMM 35b

Methods:

The Fulton Super Computer at BYU was used to run CHARMM. TIP3 water molecules were systematically added to the central cavity of a rigid 2L0J M2 channel using a NOE constraint to disperse the newly added water molecule approximately one diameter from the insertion point in a random direction, and minimizations to allow the previously added waters to accommodate to the addition. This produced a well randomized and packed central cavity. 80 such systems, with water contents of 1 to 80 molecules, were further equilibrated with 12.4 ps of simulated annealing and 10.0 ps of dynamics simulation at a constant temperature of 310 K. During all phases of dynamics, TIP3 were under geometric planar constraints, which allowed waters to fill the cavity between valine 27 and tryptophan 41 (see Figure 1). The dynamic average potential energy of water including its interaction with the channel is plotted in Figure 2.

Results:

A range of 49 to 61 TIP3 molecules positioned within the central cavity produced equipotential van der Waals and electrostatic interactions. This wide base of favorable water conformations in the rigid channel represents a flexible background for future simulations, where ALCs would displace some of these water molecules. The high end of the water content range is 1116 Daltons, which represents a conservative estimate of the upper limit on the size of drugs that could fit in the central cavity. This maximum water volume displacement capacity would easily accommodate any one of the ALCs whose weights range from 113.2 to 437.5 Daltons.

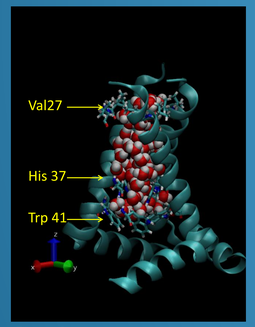

Figure 1
Water cluster in central cavity of M2 . One subunit has been removed to allow view of the cluster. The landmark side chains of residues 27, 37, and 41 are included.

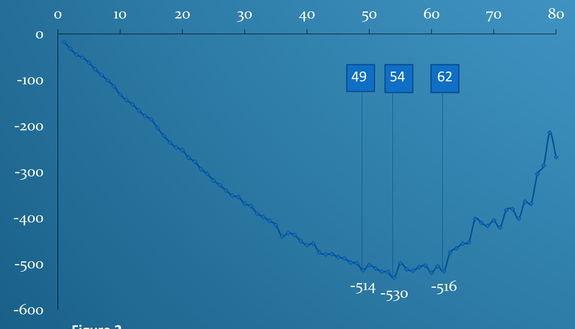

Figure 2
Total potential energy for increasing H_2O in central cavity (between valine 27 and tryptophan 41)

ALC GIRTH

Materials: 291 drugs comprising 765 titratable and chiral ALCs with a total of 15,300 conformations, 2L0J, Surflex, CHARMM

Methods:

Each of the 765 ALCs of the 291 previously tested drugs were docked to 2L0J using Surflex (Steve Kearns) and the best 20 conformations for each ALC were recorded as mol2 files. These 15,300 conformations were converted to CHARMM topology, parameter, and pdb coordinate files using SwissParam (Vincent Zoete). Once in CHARMM these conformations were oriented so that the longest elliptical axis was along the X axis and the second longest elliptical axis along Y. Assuming drugs enter the channel with their longest dimension aligned with the channel axis, the next largest dimension would be the second longest elliptical axis. Therefore the ALC girth was calculated by subtracting the minimum Y atomic coordinate value from the maximum Y atomic coordinate value. This drug dimension has the potential to limit the drug access into the cylindrical channel.

The ALCs were then allowed to minimize for 500 steps of steepest decent and a new relaxed ALC girth was calculated. The change in girth due to relaxation was plotted against relative efficacy compared to amantadine to illustrate the extent to which the ALCs had been compressed within M2 during Surflex construction (see Figure 3).

Results:

Figure 2.1 shows the largest change in girth among the 20 conformations for each of the 765 ALCs plotted against the experimental binding score. The reference is amantadine. The binding energy (relative to amantadine in kcal/mol) is equal to RT (0.6) times this binding score. The ALC girths range from 3.8 to 8.6 Å while the average girth is 5.0 Å. The maximum change in girth was 0.3 Å and minimum was -0.7 Å with the average being -0.2 Å. The lack of relation between compression and relative efficacy suggests that even the worst binders are not too wide for the central cavity.

Internal Cavity Size

Materials: 2L0J, Hole2 (Smart)

Methods:

Hole2 was used to calculate the radius of a set of spheres placed 0.25 Å apart along the channel axis. Every sphere was allowed to move perpendicular to the channel axis while the sphere radius expanded until it could not increase without contacting two channel atoms.

Each of the 8 2L0J models were analyzed using hole2 and the profiles were all overlaid to display the internal radius of the M2 channel and the variations consistent with the solid state NMR measurements for the M2 channel (Figure 4).

Results:

The diameter of the internal cavity ranges from 5-7 Å over a length of 10 Å indicated by the black bar. The blue arrows indicate the positions of the side chains that form the N-terminal (Val27) and C-terminal (His37/Trp41) boundaries of the cavity.

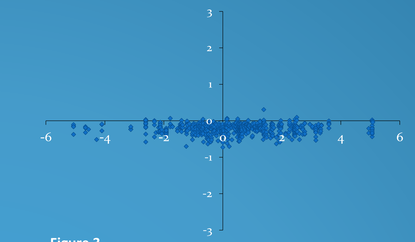

Figure 3
Y axis: the change in girth required to fit in the central cavity. Compression is shown as a negative value.

X axis: $\ln(EC50_{ALC}/EC50_{amantadine})$
EC50 represents IC50 or AVI50 depending on the experiment represented

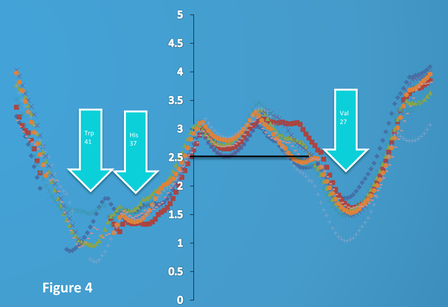

Figure 4

Note:
The Y girth does not take into account the VDW radius of surface hydrogen atoms and thus girths should be increased by ~2 Å to have a VDW girth for the ALC

Although the central cavity has a diameter of 5.0 Å for 10 Å along the channel axis, some efficacious ALCs' girths may exceed the Hole2 radius. Hole2 fails to capture the corners created by a tetramer of alpha helices.

Figure 6 shows two interesting examples of ALCs positioned in the central cavity and their relevant parameters.

References:
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**:509-512.
Smart, O.S., J.M. Goodfellow and B.A. Wallace. 1993. The Pore Dimensions of Gramicidin A. *Biophysical Journal* **65**:2455-2460.

Acknowledgments:
Steven Kearns compiled the ALCs structure an efficacy data set and constructed mol2 files for each ALC including enantiomeric and titration variants. Vincent Zoete and SwissParam converted mol2 structures to CHARMM topology an d parameter files. Support is from NIH AI23007 and a Mentoring Grant from BYU.

Figure 6

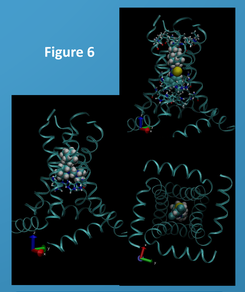

Figure 6	Left	Right
Drug	ALC-733	ALC-708
Girth (Å)	9.4	5.7
M. Weight (D)	306.5	437.5
Binding Score	-2.1	-4.3

STRAIN AND BINDING ENERGY

Materials: 2L0J, 765 ALCs comprising 15300 conformations

Methods:

At three stages, the potential energy of the drug and its interaction with a water-free, rigid channel was computed after optimization. The stages include; energy minimization in site (**bound**), **translated** far from the channel, and minimized ***in vacuo***.

Once the ALC was placed in the Surflex docked position, its configuration was optimized to achieve a **bound** state. While maintaining its bound internal coordinates the ALC was **translated** beyond the nonbond distances and again minimized to reach an ***in vacuo*** (reference) configuration. Each phase of minimization consisted of 5,000 steps of steepest descents, followed by 5,000 steps of Adopted Basis Newton Raphson.

The strain energy is the amount of internal energy incurred by the ALC while in a bound state within the channel. This was calculated by subtracting the ***in vacuo*** (reference) energy from the **translated** energy.

The potential energy of binding was calculated by subtracting the ***in vacuo*** *(*reference) energy from the **bound** energy.

ALCs were grouped into four sets based on their binding scores (Figure 3), better (<-0.7), comparable (-0.7 to +0.7), weak (+0.7 to +2.3) and worst (>2.3). Strain energy and potential energy of binding were plotted, for the best of 20 binding configurations, in a separate chart for each group (Figure 5).

Results:

ALCs with net negative potential energy of binding demonstrate that despite their strain energies (X axis) they lower the total potential energy of the system when they are in a bound state (Y axis). Of the 765 ALCs tested, all but 38 yielded negative binding energies, none of 167 in the group of "Better" antivirals, 7 of 276 "Comparable" blockers (to amantadine), 17 of 129 "Weak" blockers, and 4 of 193 "Worst" blockers. The slopes of fitted lines correlate with blocker class, with the Best blockers having the most shallow relationship between net binding energy and drug energy, suggesting the best blockers may be most able to adapt to the central cavity by improving interactions (through high stress) at the expense of strain, as if they have a small modulus of expansion.

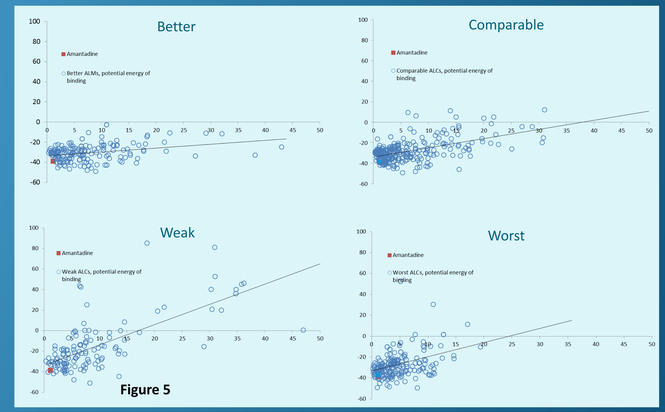

Figure 5

CONCLUSION

- 1116 Daltons of water is a conservative estimate the upper limit of which the central cavity can hold and all drugs designed to target the M2 channel should fall below this weight.
- Based on three criteria to evaluate whether ALCs fit in the channel, 1) MW<1116 Daltons, minimal (<.7 Å) compression when conformed to the central cavity, and negative net binding energy. All ALCS meet the first two criteria and 96.3% meet the third.

Here we show that the central cavity is sufficiently large for almost all antiviral candidates synthesized to date, but not how ineffective blockers fail. Failure to block may be related to difficulty entering the channel, which we didn't explore. Further research includes conducting dynamic simulations with a hydrated channel and ALCs, using coordinates from a solid state NMR model of M2 which has amantadine bound, and umbrella sampling of drugs passing through the Val27 sphincter.