THE DEVELOPMENT OF OLD AND NEW CLASS OF AMINOADAMANTANES DERIVATIVES AGAINST S31N H1N1 INFLUENZA A VIRUSES

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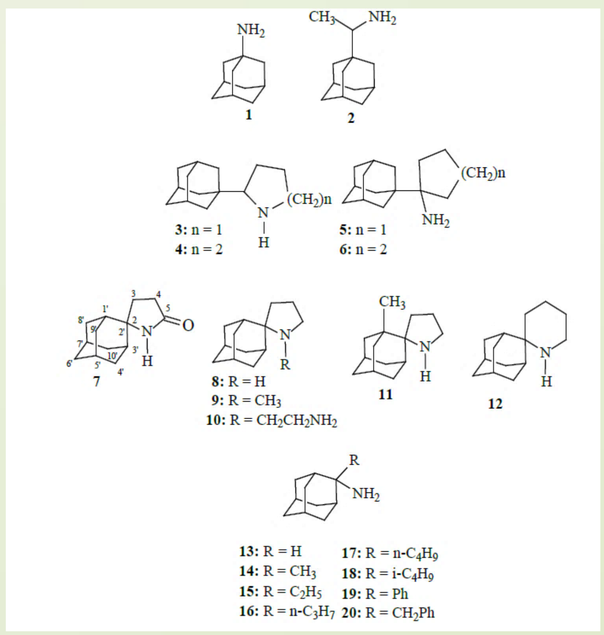
ABSTRACT

Simple analogues of amantadine (Amt) (Scheme 1) led to activity *in vitro* against M2 S31N viruses A/Calif/07/2009 (H1N1) and A/PR/8/34 (H1N1) (Table I), but not against M2 S31N A/WSN/33 (H1N1) (Table II); inhibition of this last strain is considered to be the target when drugs development is considered. The drugs were also active against the revertant S31 WSN/33 virus.



Scheme 1. Amantadine **1**, rimantadine**2**, and aminoadamantane derivatives **3**-**20**. Compounds bear a substitution at the adamantane-C1 carbon (1st and 2nd lines) or C2 carbon (3rd and 4th lines).

METHODS

We measured drug EC50s by infecting MDCK cells with Influenza A(S31N) in the presence, or absence, of amantadine analogs (Scheme **1**), and then counting the number of miniplaques formed. Resistance testing was done by 3-4 day passages at the EC50 followed by dose response testing. **6**,**10**-**11**, **17**-**20** were novel syntheses. Proton uptake was determined using electrophysiology. Solid state NMR was carried out with oriented bilayers using a PISEMA protocol. Molecular Dynamics (MD) simulations were performed on the complexes between M2TM (using 2kqt as the template) in DMPC bilayers and Amt derivatives shown in Scheme **1** .[1]

RESULTS

Antiviral assays showed that amantadine **1**, rimantadine **2**, and aminoadamantane derivatives **3-20** (Scheme 1) were all active against A/PR/8/34 (M2 S31N, V27A) and the analogues **3-11** and **15-20** were effective against A/California/04/2009(M2 S31N) (Table **I**). None of these were active against A/WSN/33 but against the revertant, S31N A/WSN/33 (Table **II**).

Table I: EC_{50} (for **A/Calif/07/2009** and **A/PR/8/34**) and its standard error from dose-response testing based on least-squares fitting of single-site binding curves; N is the number of assay counts fitted for each drug.

#	A/Calif/07/2009 (H1N1) EC_{50} ±SE (μM) (N)	A/PR/8/34 (H1N1) EC_{50}±SE (μM) (N)
M2	S31N	V27T/S31N
1	240 ± 90 (13)	24.1 ± 3.5 (21)
2	106 ± 41 (13)	3.3 ± 0.5 (2)
3	15.4 ± 2.4 (16)	N.D.
4	7.0 ± 1.2 (14)	N.D.
5	0.79 ± 0.14 (18)	N.D.
6	3.62±0.49 (20)	<0.3 ± 0.5 (2)
7	15.6 ±3.3 (13)	N.D.
8	7.6 ± 1.8 (13)	N.D.
9	7.9 ± 1.5 (13)	N.D.
10	2.66 ± 0.33 (17)	0.3 ± 0.5 (2)
11	36.0 ± 17.1 (17)	0.7 ± 0.5 (2)
13	150 ± 30 (20)	3.8 ± 1.0 (2)
14	54 ± 2 (20)	0.4 ± 0.4 (2)
15	25±3 (21)	1.8 ± 0.9 (2).
16	4.71 ± 0.92 (20)	0.5 ± 0.2 (2)
17	8.5±0.6 (20)	<0.3 ± 0.3 (2)
18	8.0± 0.3 (21)	0.3 ± 0.5 (2)
19	20.8±1.7 (21)	<0.3 ± 0.5 (2)
20	8.6±0.8 (21)	1.2 ± 1.1 (2)

Table II: EC_{50} (for **A/WSN/33**, S31N and its **revertant N31S**) and its standard error from dose-response testing based on least-squares fitting of single-site binding curves; N is the number of assay counts fitted for each drug.

#	A/ WSN/33 (H1N1) EC_{50} ±SE (μM) (N)	A/WSN/33 (S31) (revertant) IC_{50}
M2	S31N	N31S
1	24.3 ± 1.1 (21)	0.38
3	>100 (2)	0.30
4	>100 (2)	0.78
5	>100 (2)	3.92
6	>100 (2)	N.D
7	>100 (2)	50-100
8	>100 (2)	0.28
9	>100 (2)	0.91
10	>100 (2)	6.54
11	>100 (2)	6.20
12	>100 (2)	0.33
13	>100 (2)	0.38
16	390 ± 8 (2)	0.33
17	355 ± 4 (2)	3.80
18	210 ± 40 (2)	26.27
19	86 ± 19.6 (2)	>100
20	280 ± 150 (2)	19.64

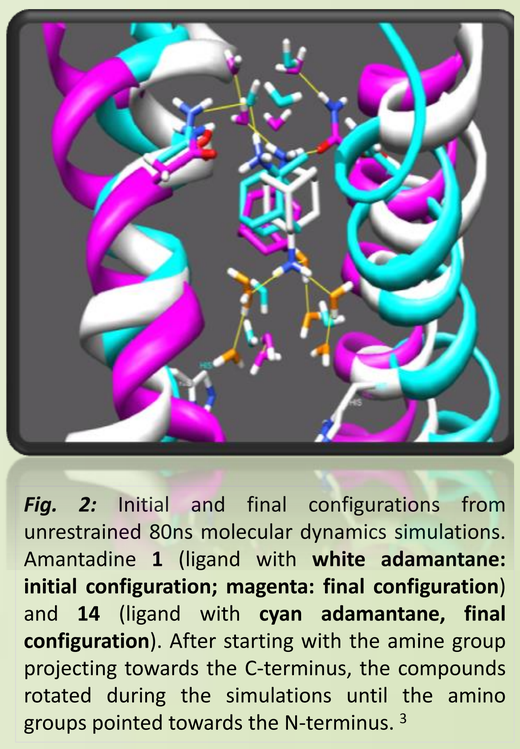


Fig. 2: Initial and final configurations from unrestrained 80ns molecular dynamics simulations. Amantadine **1** (ligand with **white adamantane: initial configuration; magenta: final configuration**) and **14** (ligand with **cyan adamantane, final configuration**). After starting with the amine group projecting towards the C-terminus, the compounds rotated during the simulations until the amino groups pointed towards the N-terminus. [3]

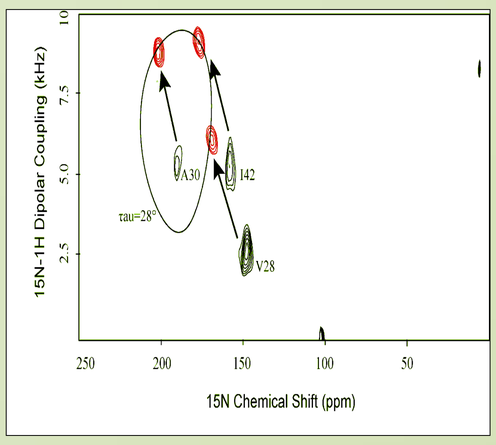


Fig. 1: Superimposed PISEMA spectra of ^{15}N-Val28, Ala30, Ile42 S31N M2 transmembrane domain (residues 22-46) in dimyristoylphosphatidylcholinebilayers aligned on glass slides with (**red**) and without (**black**) compound **8**. The sample composition is 1 mg drug:60 mg lipid:8 mg peptide with 40-50% hydration. Arrows indicate assignments and the curve is a PISA wheel indicating an 8º reduction in helix tilt relative to the membrane normal.

Solid state NMR of the transmembrane domain (TMD) with a site mutation corresponding to S31N and ITC measurements shows evidence of drug binding (***Fig. 1***). **MD simulations** of the complex of several aminoadamantane variants with S31N M2TM suggest a different orientation of aminoadamantane derivatives inside the pore compared to the wt sequence (***Fig. 2***). In particular for compounds **1,2, 14-16** in wt M2TM the ammonium group is oriented C-ward whereas in S31N M2TM ammonium group is oriented N-ward.[3]

Electrophysiology using the full length S31N M2 protein in HEK cells showed no blockade (Table **III**) .

A wild type strain, A/Victoria/3/75 (H3N2) developed resistance to representative drugs within one passage with mutations in M2 TMD, but A/Calif/07/2009 S31N was slow (>8 passages) to develop resistance *in vitro* revealing compounds with persistent *in vitro* efficacy against H1N1 (2009) Influenza A (**Table IV**). Interestingly potent cocktails of compounds showed enhanced resistance to mutation.

Strikingly the resistant virus had no mutations in M2 TMD and minor mutation in the other viral proteins.

Overall a bind no-block mechanism may responsible for these observations.

Table III: Proton Channel Block Measured in Transfected HEK Cells for compounds tested.
[α] % Block after 3 min exposures to 100 μM drug; similar values of block were observed after 30 min; v is the number of replicates.
[b]The M2 sequence for this strain is identical to that of **A/Calif/07/2009** M2.

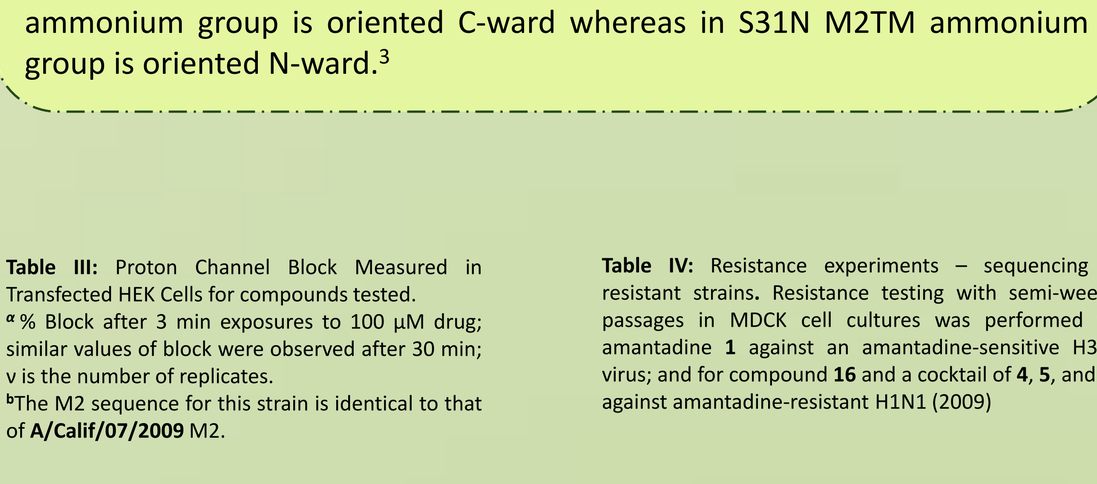
#	A/England/195/2009 (H1N1) M2: S31	A/England/195/2009 (H1N1)[b] M2: N31
	%Block [α] (v)	%Block 100μM
1	95±8% (10μM) (2)	14 ± 2% (2)
5	48±4% (10μM) (2)	4 ± 4% (2)
8	77.6% (100 μM) (2)	4 ± 2% (2)
13	63±5% (10μM) (2)	13 ± 3% (2)
14	75.8% (100 μM) (2)	0 ± 5% (2)

Table IV: Resistance experiments – sequencing of resistant strains**.** Resistance testing with semi-weekly passages in MDCK cell cultures was performed for amantadine **1** against an amantadine-sensitive H3N2 virus; and for compound **16** and a cocktail of **4**, **5**, and **12** against amantadine-resistant H1N1 (2009)

#	1 (5 μM) A/Victoria/3/75 EC_{50} ± S.E. (μM) (H3N2)	16 (5 μM) A/Calif/07/2009 EC_{50} ± S.E. (μM) (H1N1)	4, 5, 12 (5 μM) A/Calif/07/2009 EC_{50} ± S.E. (μM) (H1N1)
	WT	S31N	S31N
0	2.77 ± 0.29	4.71 ± 0.92	0.99x ± 0.15
1	Inactive	5.4 ± 1.4	-
2	Inactive	3.7 ± 0.5	-
5	N.D.	-	1.20x ± 0.07
6	N.D.	2.1 ± 1.6	-
8	N.D.	18.5 ± 1.0	-
10	N.D.	76 ± 9	7.9x ± 0.8
12	N.D.	149 ± 115	N.D.

CONCLUSIONS

The results indicate that 2-alkyl-2-aminoadamantane derivatives with sufficient adducts can persistently block p2009 influenza A *in vitro* through an alternative not blocking M2 mechanism. However when a polar head is added to aminoadamantane derivative the aminoadamantane - heterocycle conjugates were active against A/WSN/33 (H1N1) by blocking M2 (Sheme **2**).



Scheme 2: Aminoadamantanes derivativeswith polar group added to amantadine [2] rimantadine and 2-alkyl-2-aminoadamantane variants were found to be ctive against **A/WSN/33 (H1N1)**

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References

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