Amantadine Analogs That Inhibit MDCK Cell Infection By Influenza A With M2(S31N)

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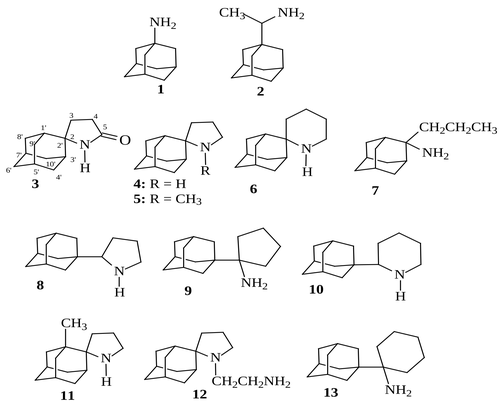
Abstract

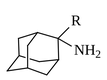
A mutation of Ser31 to Asn has become dominant globally in human infections by Influenza A since 2005, rendering the virus resistant to the FDA approved prophylactics amantadine and rimantadine. Attempts to identify alternative M2 blockers have reportedly been futile so far. Here we report that infection of cultured Madin-Darby canine kidney cells by Influenza A/California/04/2009 (H1N1 swine flu), which bears the S31N mutation, is not blocked effectively by amantadine (242 μM EC_{50}) nor rimantadine (106 μM EC_{50}), but is blocked by 16 amantadine variants, 9 of which were previously reported to also block wild type Influenza A. EC_{50}s range from 0.79-36 μM. As another control, an H3N2 strain of influenza A with wild type M2 (Strain Victoria) was found to be blocked by amantadine with an EC_{50} of 3 μM, as well as by rimantadine. We suggest that the amantadine variants block viral reproduction by blocking the S31N strain of the M2 proton channel.

Methods

We measured drug EC_{50}s by infecting MDCK cells with Influenza A(S31N) in the presence, or absence, of amantadine analogs, and then counting the number of miniplaques formed. Scheme 1 shows the compounds tested. Scheme 2 shows variants of compound **7** that were also tested and found to be effective. Proton uptake was determined using liposome assays (1). Solid state NMR was used to show binding of compound **4** to residues in the amantadine binding site.



Scheme 1. Amantadine **1**, rimantadine **2**, and aminoadamantane derivatives **3-13** found to be effective against H1N1 2009 Influenza A infection. Syntheses and testing in wild-type influenza A were previously reported(2-6) for all but compounds **11, 12**, and **13**.



14: R = H
15: R = CH_3
16: R = CH_2CH_3
17: R = $CH_2CH_2CH_2CH_3$
18: R = $CHCH(CH_3)_2$
19: R = Ph
20: R = CH_2Ph

Scheme 2. Variants of compound **7** tested and found to be effective against H1N1 2009 Influenza A infection.

Results

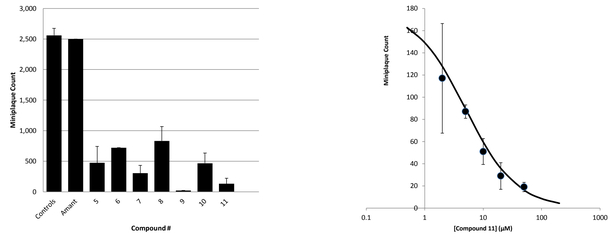


Figure 1. H1N1 infections of MDCK cells with compounds from Scheme 1. A) Screening using 100 μM drug in the culture medium. Compared to drug-free controls (left), amantadine was ineffective as expected, while test compounds blocked cell infection. Error bars, where present represent the standard deviation for the two replicas; otherwise N=1. B) Dose-response study for compound **7**. Error bars are ±1 S.D.

Compound#	EC_{50} ±SE (μM) (N)	H^+ Uptake Rate ±SD (%) (N)
Amantadine	242 ± 9 1 (13)	77 ± 29 (8)
Rimantadine	106 ± 41 (13)	ND
3	15.6 ± 3.3 (13)	ND
4	7.6 ± 1.8 (13)	6.4 ± 18 (6)
5	7.9 ± 1.5 (16)	ND
6	19.8 ± 2.5 (15)	1.3 ± 5.7 (5)
7	4.71 ± 0.92 (20)	24 ±17 (6)
8	15.4 ± 2.4 (16)	12 ± 13 (5)
9	0.79 ± 0.14 (18)	11 ± 25 (3)
10	7.0 ± 1.2 (14)	ND
11	36.0 ± 17.1 (17)	17 ± 8.7 (6)
12	2.66 ± 0.33 (17)	11 ± 18 (4)
13	3.62 ± 0.49 (20)	ND

Table 1. EC_{50} 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. H+ uptake rate by liposomes, comprised of 0.1 mg M2 22-62 (S31N) and 20 mg lipid per ml electrolyte, is given as %control ± S.D.(%control) (N) (no drug, 9.7±2.0 (40) H^+/tetramer/s) at a dosage of 100 μM in the internal and external electrolytes. The S.D.(% control) was calculated using propogation of errors.

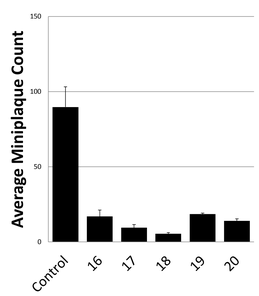


Figure 2. Anti-H1N1 screen results for compounds in Scheme 2. Drug concentrations: 50 μM. N=4 (drug-free control) or N=2 (with drug).

Compound #	16	17	18	19
EC_{50} (μM)	25	9	8	21
SE(EC_{50}) (μM)	3	0.6	0.3	1.7
N	21	20	21	21

Table 2. EC50s, their uncertainties and assay counts for the compounds in Scheme 2.

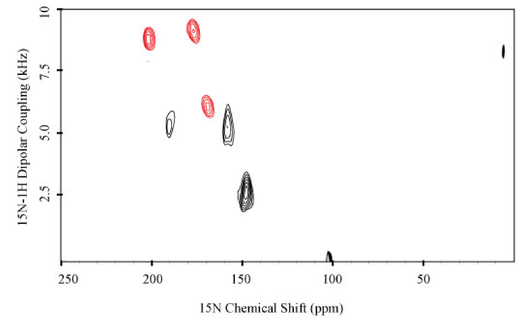


Figure 3. Superimposed PISEMA spectra of 15N-Val28, Ala30, Ile42 S31N M2 transmembrane domain (residues 22-46) in dimyristoylphosphatidylcholine bilayers aligned on glass slides with (red) and without (black) compound **4**. The sample composition is 1 mg drug:60 mg lipid:8 mg peptide with 40-50% hydration.

Summary

- Nine drugs (compounds **3-10,** Scheme 1 and **16**, Scheme 2), which were previously tested against WT influenza A, are effective against the H1N1 (M2 S31N) amantadine-resistance virus.
- Seven novel variants are also effective (compounds **11-13** of Scheme 1 and compounds **17-20** in Scheme 2) against H1N1.
- These novel drugs, perhaps in combination therapy, could be useful as resistance-proof therapeutics.

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