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Influence of an additional 2-amino substituent of the 1-aminoethyl pharmacophore group on the potency of rimantadine against influenza virus A

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Abstract—We examined whether the incorporation of a second amino group into the 1-aminoethyl pharmacophore of rimantadine **2** and into the piperidine pharmacophore of the heterocyclic rimantadine **4** was compatible with anti-influenza virus A activity. The new synthetic molecules are capable of forming two hydrogen bonds within the receptor. We identified molecules **8** and **16**, bearing the adamantyl and 1,2-diaminoethyl groups, which are equipotent to rimantadine **2** bearing the adamantyl and 1-aminoethyl pharmacophore groups. Interestingly, diamino compound **16** is a 4-fold more potent inhibitor than its parent monoamino heterocyclic rimantadine **4** propably because of additional hydrogen bonding interactions with the M2 protein receptor.

Influenza A viruses have the ability to undergo changes by the mechanisms of antigenic drift and shift and new evolving strains can be a serious threat to the human population.[1,2] The most lethal pandemic, the so-called Spanish influenza A of 1918–1919, caused the death of 20 millions of people worldwide.[3] The Asian influenza in 1957, Hong Kong influenza in 1968 and Russian influenza in 1977 also caused serious pandemics. Since 2003, an avian strain that first appeared in China has infected more than 130 persons in Vietnam, Thailand and Cambodia and has killed more than half of them.[4] Given that influenza shifts may occur every 20–30 years the danger of future influenza A pandemics highlights the need to develop more effective drugs.

Amantadine **1** and rimantadine **2** (*a*-methyl adamantanemethanamine) are anti-influenza virus A drugs which inhibit virus replication at micromolar concentrations.[5] During the past 12 years we have synthesized many potent aminoadamantane derivatives.[6,7] These compounds, in their protonated form, are considered to occlude the M2 protein ion channel pore[8] and block its proton pump function[9] in early and late endosomes,[5b] which is critical for the virus replication.[5b,c,10]

Recently, we observed that the inclusion of the 1-aminoethyl pharmacophore group of rimantadine **2** into the pyrrolidine or the piperidine ring resulted in **3**, **4**, respectively, which are active compounds against influenza A virus.[7g] We have now examined whether the incorporation of a second amino group into the 1-aminoethyl pharmacophore group of rimantadine **2**, resulting in a 1,2-diaminoethyl group, is compatible with biological activity. It has been proposed that the amantadine **1**-receptor complex is stabilized through formation of hydrogen bonds between the drug's ammonium group and the cluster formed by four acceptor groups of the tetrameric M2 receptor.[11] Thus, the new amino analogues of rimantadine **2** can possibly act through the formation of two hydrogen bonds with the acceptor group cluster. In order to test our hypothesis, the *a*-aminomethyl adamantanemethanamines **8**, **11** and their cyclic analogues **9**–**13**, **15**–**19** were synthesized and their activity was evaluated against influenza virus A H3N2, this

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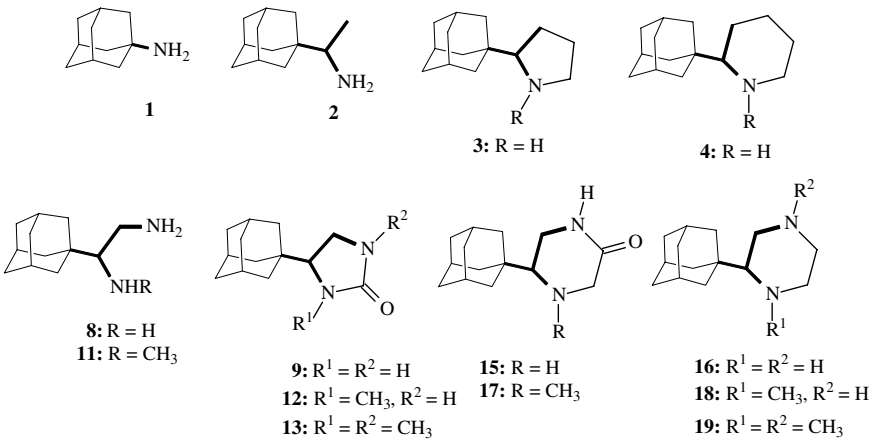
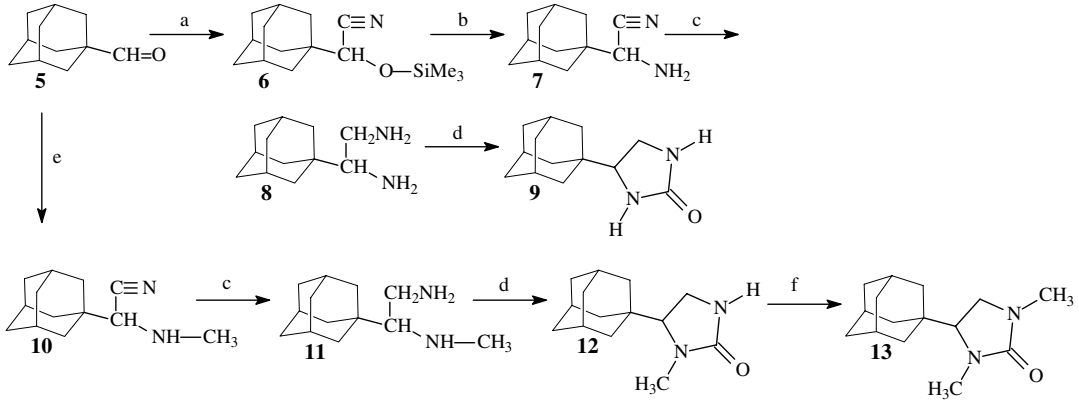


strain being responsible for the current every year epidemics.

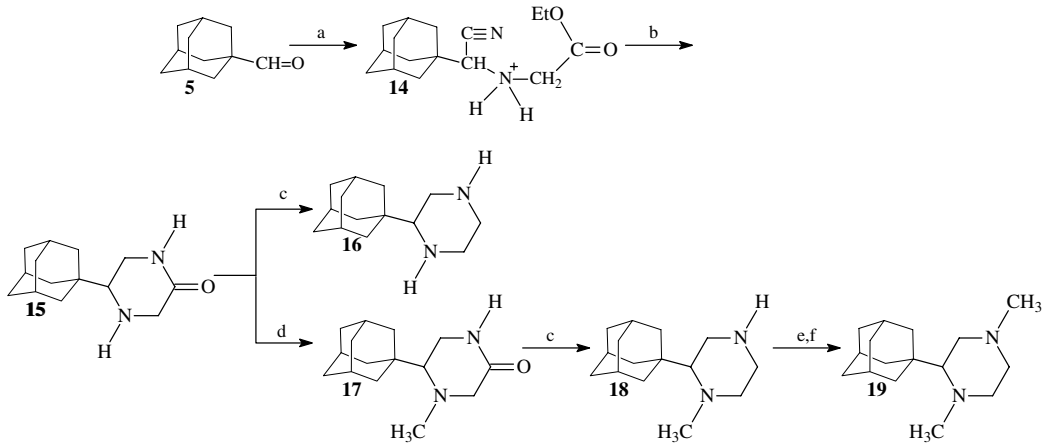
To synthesize the novel compounds, convenient methods for the preparation of the α-aminonitriles **7**, **10** and **14** were needed (Schemes 1 and 2). The preferred synthetic routes leading to α-aminonitriles are still based on the Strecker reaction.[12] The Strecker reaction conditions for preparing compounds **7**, **10** or **14** are consistent with mixing the bulky 1-adamantanecarboxaldehyde **5** with NaCN and NH_3, CH_3NH_2 or NH_2CH_2COOEt, respectively, in a mixture of DMSO/water, and leaving the mixture to react at ambient temperature. Using standard Strecker reaction conditions, the α-substituted aminonitriles **7**, **10** and **14** were synthesized in yields ranging from 23% to 66% (Schemes 1 and 2). In order to improve the yield of the compound **7** (isolated in 23% yield), we looked at several modifications which have been reported to increase the efficiency of the Strecker reaction. We choose to proceed by treating the bulky aldehyde **5** with TMSCN as the reactive cyanide in the presence of ZnI_2 catalyst.[13] But under mild conditions the yield was not improved. However, when a mixture of the α-trimethylsilyloxylnitrile **6** in a saturated $MeOH/NH_3$(g) solution was heated in an autoclave, the α-aminonitrile **7** was obtained in 62% yield.

Catalytic hydrogenation of the *a*-aminonitriles **7**, **10** over PtO_2 provided the α-aminomethyl adamantanemethanamines **8** and **11**. Reaction of the diamines **8** and **11** with 1,1′-carbonyldiimidazole[14] yielded the corresponding urea derivatives **9** and **12**. The *N*,*N*-dimethyl urea derivative **13** was prepared by methylation of **12** using NaH/CH_3I. The unstable α-aminonitrile **14** was reduced promptly after its preparation with H_2/PtO_2 to the corresponding aminoester which spontaneously cyclized to the piperazinone **15**. The reductive methylation of **15** with $NaBH_4/CH_2{=}O$ afforded the 1-Me derivative **17**. The piperazines **16** and **18** were obtained by means of a $LiAlH_4$ reduction under mild conditions of **15** and**17**, respectively. The *N*,*N*-dimethylpiperazine **19** was prepared by a $LiAlH_4$ reduction of the carbamate derivative of **18**.[15]

The potency of the new compounds **8**, **9**, **11**–**13**, and **15**–**19** was examined in vitro against influenza A (H_3N_2) and B viruses, and was compared to the activity of amantadine **1** and rimantadine **2** (Table 1).[16] The heterocyclic rimantadine analogues **3**, **4** were also included as controls.[7c,g] Several compounds, that is, compounds **8**, **11**, **9**, **15** and **16** were found to be inhibitors of influenza A H3N2 virus replication. All compounds were inactive against influenza B virus which is in accordance to their mode of action, that is, their interaction with



Scheme 1. Reagents and conditions: (a) Me_3SiCN, ZnI_2, $CHCl_3$, 1 h; (b) NH_3, MeOH, 70–75°C, in autoclave for 15 h, and then HCl(g)/Et_2O (62% from **5**); (c) H_2, PtO_2, HCl(g)/MeOH, 45lb/in^2, rt, 6 h, and then NaOH 20% (85% for **8**, 90% for **11**); (d) 1,1′-carbonyldiimdazole, pyridine, CH_2Cl_2, reflux, 15 h (87% for **9**, 89% for **12**); (e) NaCN, $CH_3NH_3^+Cl^-$, DMSO/H_2O 29:1, rt, 48 h, and then HCl(g)/Et_2O (66%); (f) NaH, CH_3I, DMF, rt, 18 h (94%).



Scheme 2. Reagents and conditions: (a) NaCN, HCl·$H_2NCH_2CO_2Et$, DMSO/H_2O 29:1, rt, 48 h, and then HCl(g)/Et_2O (52%); (b) H_2, PtO_2, EtOH/HCl(g), 45 lb/in^2, 15–20 °C, 6 h, and then Na_2CO_3 (92%); (c) $LiAlH_4$, THF, rt, Ar, 5 h (98% for **16**, 90% for **18**); (d) CH_2=O (aq) 37%, $NaBH_4$, MeOH, rt for 24 h, and 50 °C for 30 min (75%); (e) $ClCO_2Et$, Et_3N, rt, 24 h (74%); (f) $LiAlH_4$, THF, rt, 30 h, and then reflux for 1 h (67%).

influenza A M2 protein which is absent from influenza B virions.

We recall that in order to inhibit virus replication, the prototype amantadine drug **1**, existing mostly in its protonated form even at neutral pH,[17] must first be solvated in the lipid bilayer[18] prior to the blockage of the M2 proton pump inside the acidic endosomes. Molecular modelling studies predicted that amantadine **1** anchors inside M2 protein pore either between Leu26 and Ser31[11a] or between Gly34 and His37;[11b] in the binding site the adamantyl group fits the lipophilic pocket around Leu26 or Gly 34 with the ammonium group interacting favourably with the ring formed by the four Ser31 hydroxyl groups or His37 imidazole groups. Thus, the in vitro activity of an aminoadamantane analogue results from its favourable hydrogen bonding and van der Waals interaction with the M2 receptor; compounds **8**, **11**, and **16** act through their diprotonated form at the low pH environment of endosomes.

The most active agents were compounds **8** and **16** with EC_{50} values of 18.3 and 24.1 μM, respectively. Compounds **8** and**16** exhibited about 3- and 2-fold, respectively, higher potency than amantadine **1** and were equipotent to rimantadine **2**. In **8**, the *a*-methyl group of rimantadine is replaced with an *a*-(aminomethyl) group. Similarly, **16** resulted by replacing the 4-methylene unit of **4** with an NH group, the latter being a piperidine analogue of rimantadine **2**. Compound **16** contains the 1,2-diaminoethyl pharmacophore group of the parent molecule **8** in a piperazine ring.

Compounds **8** and **16** have two primary and two secondary amino groups, respectively, that upon protonation are capable of forming two hydrogen bonds within the pore of the M2 protein receptor; the second amino group was added into rimantadines' **2**, **4** framework to boost biological activity. Nevertheless, the result of adding the second amino group in the 1-aminoethyl pharmacophore group of rimantadine **2** was the in vitro activity retention. This may be due to a compromise between the membrane penetration ability of **8** and its binding affinity properties or simply to an uncorrect orientation of the molecule inside the M2 receptor. But interestingly, adding a second amino group in the piperidine pharmacophore ring of rimantadine **4** improves 4-fold the activity, as becomes clear by the comparison of the EC_{50} values of the monoamino compound **4** and its diamino analogue **16**. Judging that similar changes occurred in the membrane penetration ability when the structure changes from **2** to **8** and **4** to **16**, the enhanced activity of **16** compared to that of **4** could be interpreted in terms of additional hydrogen bonding interactions.

Compounds **9** and**15** with EC_{50} values of 72.6 and 91.8 μM were 4- and 5-fold less active than rimantadine **2**; compound **16** exhibited a 4-fold enhanced activity than its lactame precursor **15**. Since **9**, **15** could also form two hydrogen bonds with the receptor, their diminished potency must be explained in terms of an uncorrect binding orientation of these molecules.

N-Methylation of the parent N–H compound **8** caused a 3-fold reduction in anti-influenza virus A potency (see compound **11**). However, the result of potency reduction upon N-methylation was striking in the case of **16** or **15**, with compounds**18**, **19** or **17**, respectively, being almost inactive. The same dramatic reduction on anti-influenza A activity was also observed when the piperidine **4** was N-methylated.[7g] Conformational studies have revealed that the most populated conformer (~95% at ambient temperature) of the protonated *N*-methyl derivative of piperidine **4** has adamantyl group in equatorial position and N–Me group in axial position.[19] This preference leaves the N^+–H pharmacophore group in equatorial orientation and any hydrogen bonding interaction is distorted by the voluminous adamantyl group.

Synopsis. The active agents **8**, **16** have a second amino group only two carbons next to the amino group of

Table 1. Anti-influenza virus A (H3N2), B activity and cytotoxicity of amantadine **1**, rimantadines **2**–**4**, and amino analogues of rimantadine **8**, **9**, **11**–**13**, **15**–**19** in MDCK[b]

Compound[a]	Influenza A H3N2[b]	
	EC_{50}[c] (μM)	MTC[d] (μM)
8	18.3	>586
11	56.8	>568
9	72.6	>1135
12	269	>1067
13	107	1007
15	91.8	>1067
17	604	>1007
16	24.1	>553
18	197	>535
19	351	>520
Amantadine, **1**	49.1	>1333
Rimantadine, **2**	19.1	>1160
3	51.3	1035
4	70.5	978

[a] Aminoadamantanes **1**–**4** were tested as hydrochloride salts and **8**, **11** and **16**, **18** and **19** as dimaleate salts.
[b] Influenza A H_2N_2(X-31).
[c] Concentration required to reduce virus-induced CPE in MDCK (Madin-Darby canine kidney) cells by 50% as determined by the MTS method.
[d] Minimal toxic concentration, or concentration that causes microscopically detectable toxicity in uninfected cell cultures.

rimantadines **2**, **4**. The biological activity of **8** or **16** is exerted by the fraction of their in vitro effective drug concentration (Table 1) corresponding to the concentration of the molecules that bind acceptor groups inside the M2 pore after passing the membrane barriers; the latter concentration could be determined from direct binding experiment against M2 tetramer. Whatever is this concentration, the whole cell antiviral assay showed that the additional amino group is compatible with biological activity and thus the corresponding charged protonated amino group should have a complementary acceptor group inside the M2 lumen; otherwise a reduction in potency should be the reasonable result. Future binding studies against M2 protein and molecular modelling simulations will help to understand the molecular interactions through which the new aminoadamantane derivatives exerted their biological activity.

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15. Spectra and elemental analysis of all the synthesized compounds were in accordance with their structure. The ^1H and ^{13}C NMR spectra were assigned using COSY and CHCORR spectroscopy. The NMR spectra assignment of the α-aminomethyl adamantanemethanamine **8** and 5-(1-adamantyl)-2-piperazinone **15** is given below. ^1H NMR ($CDCl_3$, 400 MHz): δ 1.12–1.71 (complex m, 16H, 2,4,6,8,9,10-adamantane H, 2×NH_2), 1.93 (br s, 3H, 3,5,7-adamantane H), 2.06 (∼q, 1H, AMX, $J_{AX} \sim 3$ Hz, $J_{MX} \sim 12$ Hz, H_2N–C*H*–CH_2–NH_2), 2.25 (∼t, 1H, AMX, $J_{AM} = J_{MX} \sim 12$ Hz, H_2N–CH–CH_2–NH_2), 2.87 (∼q, 1H, AMX, $J_{AX} \sim 3$ Hz, $J_{AM} \sim 12$ Hz, H_2N–CH–CH_2–NH_2); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 28.35, (3,5,7-adamantane C), 35.48 (1-adamantane C), 37.15 (4,6,10-adamantane C), 38.51 (2,8,9-adamantane C), 42.30 (H_2N–CH–*C*H_2–NH_2), 63.51 (H_2N–*C*H–CH_2–NH_2). **15**; ^1H NMR ($CDCl_3$, 400 MHz): δ 1.40–1.83 (complex m, 13H, 2,4,6,8,9,10-adamantane H, N-H), 1.95 (s, 3H, 3,5,7-adamantane

H), 2.37 (dd, 1H, $J = 5.5$, 9.5 Hz, 5-piperazinone H), 3.20–3.35 (m, 2H, 6-piperazinone H), 3.50 (q, 2H, $J = 17.5$ Hz, 3-piperazinone H), 7.11 (bs, 1H, NH–CO); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 28.15, (3,5,7-adamantane C), 34.38 (1-adamantane C), 36.96 (4,6,10-adamantane C), 38.56 (2,8,9-adamantane C), 42.48 (6-piperazinone C), 50.0 (3-piperazinone C), 61.23 (5-piperazinone C), 170.9 (C=O).

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