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Spiro[pyrrolidine-2,2′-adamantanes]: Synthesis, Anti-Influenza Virus Activity and Conformational Properties

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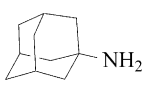
Abstract—Synthetic spiro[pyrrolidine-2,2′-adamantanes] **2**, **3**, **11**, **15**, **12**, **16**, **18**, **20** were evaluated in vitro and found to be active anti-influenza virus A compounds; the effect of the position of *C*-Me pyrrolidine ring substituent on antiviral activity was examined. Pyrrolidine 5-Me substitution appears to be optimal for H_2N_2 strain activity. From the four different possible protonated conformers, experimental observation using NMR spectroscopy and molecular mechanics calculations demonstrated only a pair of conformers $\mathbf{A}^+\mathbf{H}$ (*N*-Me (*ps*-ax), *C*-Me (*ps*-eq)) and $\mathbf{B}^+\mathbf{H}$ ((*N*-Me *ps*-ax, *C*-Me *ps*-ax)) which can contribute to the biological activity of *C*-Me, *N*-Me protonated derivatives $\mathbf{15}^+\mathbf{H}$, $\mathbf{16}^+\mathbf{H}$ and $\mathbf{20}^+\mathbf{H}$. The relative populations were calculated from NMR spectra. For compounds $\mathbf{15}^+\mathbf{H}$ and $\mathbf{20}^+\mathbf{H}$ conformer $\mathbf{A}^+\mathbf{H}$ (*cis* dimethyl orientation) is the major one whereas a similar population of conformers $\mathbf{A}^+\mathbf{H}$ and $\mathbf{B}^+\mathbf{H}$ (*trans* dimethyl orientation) was observed for compound $\mathbf{16}^+\mathbf{H}$. Since this new series is characterized by a lipophilic part, that is the pyrrolidine ring, in addition to adamantane, that can interact with influenza A M2 protein, an ultimate future goal would be the in vitro mapping of M2 lipophilic pocket.

Introduction

Influenza A as an infectious disease inflicted more casualties than any other infectious disease in Europe in the previous century. In fact, the Spanish flu pandemic of 1918 was the most lethal of all infectious epidemics in the history of mankind: Twenty millions of people died worldwide. The 1957 Asian influenza A (H_2N_2) and the 1968 Hong Kong influenza A (H_3N_2) have killed together up to two millions of people and resulted in an estimated 32 billion Euros in economic damages due to productivity loss and treatment costs.[1,2]

Given that influenza pandemics occur every 20–30 years and that a new lethal variant of influenza A (H_5N_1) appeared in Hong Kong in 1997,[2] the danger of future influenza A pandemics is real and new more effective drugs are needed.

Amantadine **1** is *anti*-influenza A drug that inhibit virus replication at micromolar concentrations;[3] its protonated form blocks the influenza A M2 ion channel protein.[4] Several active carbocyclic and heterocyclic aminoadamantanes have been synthesized in our lab during the last eight years.[5,6] From them spiro[pyrrolidine-2,2′-adamantanes] **2**, **3** were found to exhibit a strong activity against influenza A.[5,6a,b]



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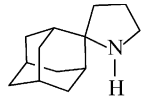
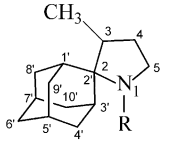


2: R=H
3: R=CH_3



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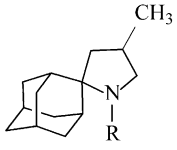
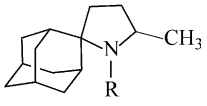
15: R=CH_3



12: R=H
16: R=CH_3



18: R=H
20: R=CH_3

Having in mind that adamantane ring substitution chemistry is difficult enough, the pyrrolidine ring of the above compounds can be used as a scaffold upon new

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groups can be mounted; the new *C*-substituted compounds will contribute in developing interesting SAR in aminoadamantane series. We now present some results of *C*-methylation of the pyrrolidine ring, resulting in novel compounds **11**, **15**, **12**, **16**, **18**, **20**.

Results and Discussion

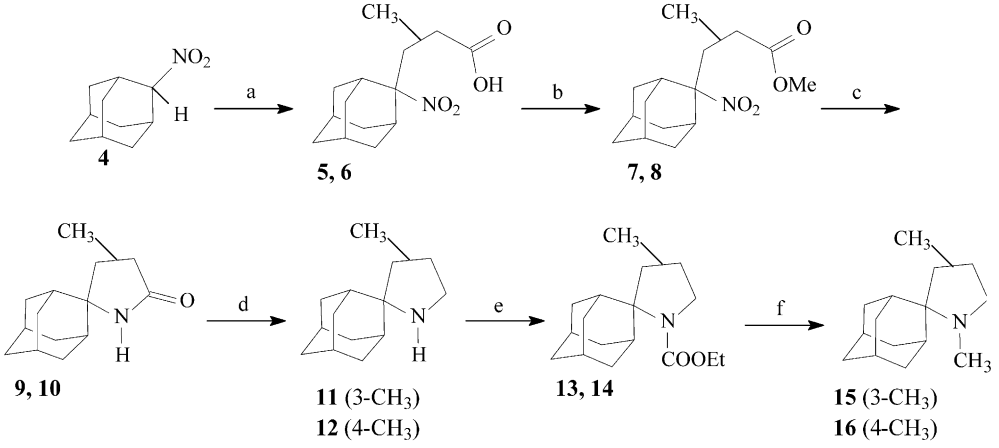
Synthetic chemistry

The Michael addition between 2-nitroadamantane **4** and ethyl crotonate or methacrylate afforded the corresponding ester adducts (Scheme 1). Product purification from the toxic conjugated esters was accomplished through saponification of the reaction mixture; the nitroacids **5** or **6** were obtained, which were further converted to the methyl esters **7** or **8**. Catalytic hydrogenation over Ni-Raney gave 4-methyl or 5-methylspiro-[pyrrolidine-2,2′-adamantan]-5-ones **9**, **10**. Lactams **9** or **10** were then reacted with $LiAlH_4$ to afford the parent pyrrolidines **11** or **12**. The *N*-methylated derivatives **15**, **16** were obtained through reduction of the carbamates **13**, **14**.

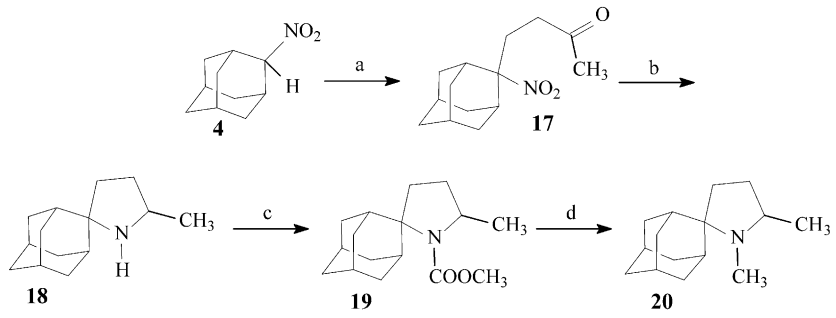
The synthesis of 5-methylspiro[pyrrolidine-2,2′-adamantanes] **18**, **20** is illustrated in Scheme 2. The Michael addition between 2-nitroadamantane **4** and methyl vinyl ketone using Triton-B as basic catalyst led to a gummy product, possibly formed from methyl vinyl ketone polymerization. The difficulty was overriden using the $-NR_3^+OH^-$ form of resin as basic catalyst.[7] This resin was prepared by treating the commercial $-NR_3^+Cl^-$ form of Amberlyst A-27 resin with aqueous NaOH 1 M.[8] Using this methodology, the nitroketone **17** was obtained. This compound was hydrogenated under Ni-Raney to afford pyrrolidine **18**. *N*-methylpyrrolidine **20** was obtained using standard methodology.[9]

Conformational analysis

Four conformers are possible for protonated spiropyrrolidines $\mathbf{15}^+\mathbf{H}$, $\mathbf{16}^+\mathbf{H}$ and $\mathbf{20}^+\mathbf{H}$, where *N*-Me and *C*-Me group can be *pseudo*-axial or *pseudo*-equatorial: $\mathbf{A}^+\mathbf{H}$ (*N*-Me ps-ax, *C*-Me ps-eq), $\mathbf{B}^+\mathbf{H}$ (*N*-Me ps-ax, *C*-Me ps-ax), $\mathbf{C}^+\mathbf{H}$ (*N*-Me ps-eq, *C*-Me ps-ax), $\mathbf{D}^+\mathbf{H}$ (*N*-Me ps-eq, *C*-Me ps-eq (Scheme 3). Molecular mechanics calculations, using the MM+ force field,[10] predict that conformers $\mathbf{C}^+\mathbf{H}$ and $\mathbf{D}^+\mathbf{H}$ with *N*-Me (eq) are of high energy, because of the severe steric crowding between adamantane and *N*-Me, and thus unpopulated. Interconversion of conformers $\mathbf{A}^+\mathbf{H}$ and $\mathbf{B}^+\mathbf{H}$, which requires nitrogen and ring inversion and dissociation/reassociation of a proton, is slow even at room temperature; when compounds **15**, **16** and **20** were protonated at room temperature by addition of a drop of



Scheme 1. Reagents and conditions: (a) (i) $CH_3CH{=}CHCO_2Et$ or $CH_2{=}CH(CH_3)CO_2Et$, Triton-B, *t*-BuOH, 80 °C, 12 h (ii) NaOH, EtOH–H_2O 3:1, reflux (**4**→**5**, 56% or **4**→**6**, 67%); (b) MeOH/HCl(g), 60 °C (94% for **7** or 70% for **8**); (c) H_2/Ni-Raney, EtOH, 50 psi, 60 °C, 10 h (83% for **9** or 95% for **10**); (d) $LiAlH_4$, THF, reflux, (65 h, 47% for **11** or 46 h, 87% for **12**); (e) $ClCO_2Et$, Et_3N, ether, rt, 25 h (65% for **13** or 97% for **14**); (f) $LiAlH_4$, THF, reflux, 21 h (76% for **15** or 88% for **16**).



Scheme 2. Reagents and conditions: (a) $CH_2{=}CHCOCH_3$, Amberlyst A-27 (-$NR_3^+OH^-$), ether, rt, 10 h (36%); (b) H_2/Ni-Raney, EtOH, 50 psi, 50 °C, 10 h (92%); (c) $ClCO_2Et$, Et_3N, ether, rt, 25 h (50%); (d) $LiAlH_4$, DME, reflux, 24 h (53%).

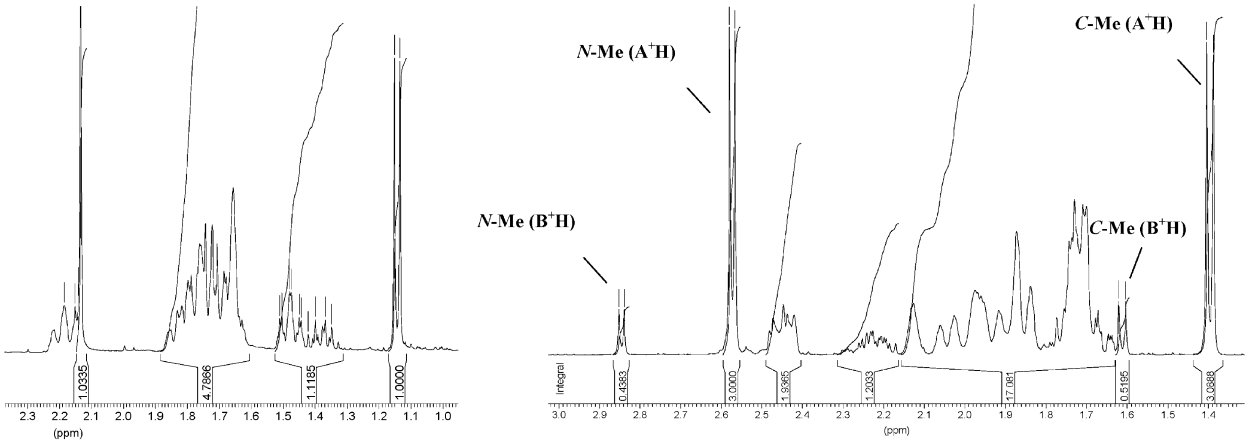


Figure 1. Region of the ^{1}H NMR spectrum of 1,5-dimethylspiro[pyrrolidine-2,2′-adamantane] **20** in a $CDCl_3$ solution (400 MHz) at 298 K (left-hand part); region of the ^{1}H NMR (400 MHz) in a $CDCl_3$ solution of compound **20** after adding a drop of trifluoroacetic acid at 298 K (right-hand part). Some peaks corresponding to major $\mathbf{A}^+\mathbf{H}$ and minor conformer $\mathbf{B}^+\mathbf{H}$ are annotated.

trifluoroacetic acid to the NMR tube, two sets of signals for $\mathbf{15}^+\mathbf{H}$, $\mathbf{16}^+\mathbf{H}$ and $\mathbf{20}^+\mathbf{H}$ were seen, corresponding to conformations $\mathbf{A}^+\mathbf{H}$ and $\mathbf{B}^+\mathbf{H}$. The *N*-Me or *C*-Me pair of signals in ^{1}H and several pairs in ^{13}C NMR spectrum were offered to measure the relative population of conformers. Representatively the ^{1}H NMR spectra for compound **20** are shown in Figure 1. By measuring the relative signal intensities the ratio $\mathbf{A}^+\mathbf{H}$:$\mathbf{B}^+\mathbf{H}$ for $\mathbf{15}^+\mathbf{H}$, $\mathbf{16}^+\mathbf{H}$ and $\mathbf{20}^+\mathbf{H}$ was found to be 87:13, 46:54 and 86:14, respectively (Scheme 3).[11]

Antiviral results

The activity of the new aminoadamantane heterocycles **11**, **12**, **15**, **16**, **18**, **20** were examined against influenza virus A H_2N_2 strain, which is sensitive against amantadine **1**, and influenza B according to previously reported methods (Table 1).[12] Amantadine **1** and pyrrolidines **2**, **3**, the last synthesized as described in the past,[6a] were included as controls. All protonated compounds[13] were found to be active against H_2N_2 strain; pyrrolidines **2**, **3**, **11**, **15**, **16**, **18**, **20** exhibited a slightly higher EC_{50} value than amantadine **1**.

For either *C*-Me, *N*-H or *C*-Me, *N*-Me pyrrolidines the activity was increased by moving the *C*-Me substituent from the 3- to the 5-position of the pyrrolidine ring (**18** > **12** > **11** and **20** > **16** > **15**). *N*-Methylated derivatives showed a somewhat lower activity than the corresponding *N*-H pyrrolidines, except for the pair **11**, **15**, with a 4-fold reduction in potency for the *N*-methyl derivative **15**.

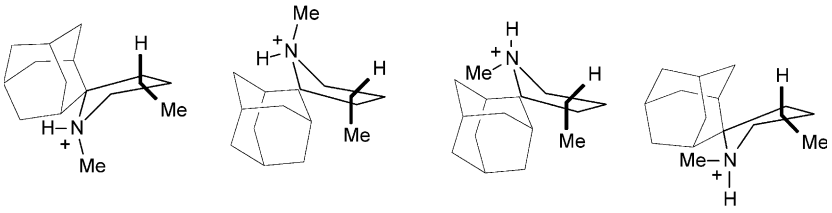
C-methyl substitution of *N*-H pyrrolidine **2**, resulting in compounds **11**, **12**, **18**, slightly lowered the biological activity against this strain. *C*-Methylation of *N*-CH_3 pyrrolidine **3** resulted in 1,4- and 1,5-dimethylpyrrolidines **16**, **20** with a similar or a little better activity than the parent *N*-methylpyrrolidine **3**, whereas 1,3-dimethylpyrrolidine **15** exhibited a 4-fold lower activity.

Among the *C*-methyl substituted compounds the best selectivity index was exhibited by 5-methylpyrrolidine **18** (Table 1). However, among all spiropyrrolidines, the unsubstituted spiropyrrolidine **2** had the best selectivity. In addition, all pyrrolidines had lower selectivity than amantadine **1**.

Table 1. Anti-influenza virus A and B activity and cytotoxicity of aminoadamantanes **1**, **2**, **3**, **11**, **12**, **15**, **16**, **18**, **20**[a] in MDCK cells[b]

Compd	EC50[c] (μM)		MCC_{50}[d] (μM)	SI[e]
	Influenza A H_2N_2[b]	Influenza B[b]		Influenza A H_2N_2
11 (3-Me, *N*-H)	3.7	>1035.2	>280	>280
15 (3-Me, *N*-Me)	12.5	>746.3	>60	>60
12 (4-Me, *N*-H)	3.3	1035.2	313.6	313.6
16 (4-Me, *N*-Me)	3.3	>746.3	>226	>226
18 (5-Me, *N*-H)	2.5	>1035.2	>414	>414
20 (5-Me, *N*-Me)	2.7	149.3	55.3	55.3
2 (*N*-H)	1.8	>1098.9	>610.5	>610.5
3 (*N*-Me)	2.9	>1035.2	357.0	357.0
Amantadine, **1**	1.1	>1333	>1211.8	>1211.8
Ribavirin	3.7	≥1024.6	≥276.9	≥276.9

[a]Aminoadamantane*s* **1**, **2**, **3**, **11**, **12**, **18** were tested as hydrochlorides and **15**, **16**, **20** as fumarates.[13]
[b]Abbreviations and strains used: MDCK, Madin-Darby canine kidney cells, Human epithelial cells; influenza A H2N2 (A2 Japan/305/57); influenza B (Hong Kong/5/72).
[c]Effective concentration, or concentration required to reduce virus-induced cytopathogenicity by 50%.
[d]Minimum cytotoxic concentration, or concentration required to cause a microscopically detectable alteration of normal cell morphology.
[c,d]All data represent mean values for at least two separate experiments.
[e]SI represents the ratio of MCC_{50} to EC_{50}.



A^+H (ax, eq)	B^+H (ax, ax)	C^+H (eq, ax)	D^+H (eq, eq)
15: 87 %	**15:** 13 %		
16: 46 %	**16:** 54 %		
20: 86 %	**20:** 14 %		

Scheme 3.

All compounds tested were inactive against influenza virus B which lacks the influenza A typical M2 protein, the target of aminoadamantanes biological action.

Conclusion

The pyrrolidine scaffold of the potent anti-influenza virus A spiro[pyrrolidine-2,2′-adamantane] **2**[6a] was mounted with methyl groups resulting in the novel biologically active 3-, 4- and 5-methylsubstituted compounds **11**, **15**, **12**, **16**, **18**, **20**. 5-Me Substitution was optimal for biological activity against H_2N_2 strain.

All these new agents are considered to act biologically through their protonated form; they represent cationic compounds possessing a lipophilic moiety. Studies of the interaction of amantadine **1** with the membrane spanning domain of M2 protein based on neutron diffraction, solid state NMR and dynamics simulations have revealed that the drug interacts with the pore of the channel. It has been proposed that possibly the ammonium group of amantadine forms a hydrogen bond with His-37 imidazole while adamantyl group of the drug fits in a lipophilic pocket formed in the vicinity of Gly-34.[4] Our new series of compounds possess a lipophilic part, that is the pyrrolidine ring, in addition to adamantane, that can possibly interact with influenza A M2 protein lipophilic pocket around Gly-34, contributing to antiviral activity.

From the four different protonated conformations which are possible for compounds $\mathbf{15}^+\mathbf{H}$, $\mathbf{16}^+\mathbf{H}$ and $\mathbf{20}^+\mathbf{H}$, experimental observation using NMR spectroscopy and molecular mechanics calculations demonstrate only a pair of conformers $\mathbf{A}^+\mathbf{H}$ (*N*-Me *ps*-ax, *cis*-dimethyl orientation) and $\mathbf{B}^+\mathbf{H}$ (*N*-Me *ps*-ax, *trans*-dimethyl orientation), which can contribute to the biological activity. For compounds $\mathbf{15}^+\mathbf{H}$ and $\mathbf{20}^+\mathbf{H}$, conformer $\mathbf{A}^+\mathbf{H}$ is the major one whereas an equal population was observed for compound $\mathbf{16}^+\mathbf{H}$. The different interaction of these conformers with M2 protein will be investigated using molecular modeling.

Inspired from the above results and since the pyrrolidine ring can be used as the scaffold upon which new groups can be mounted, future attempts should be directed towards the synthesis of more compounds aiming at mapping in vitro the lipophilic pocket of the M2 protein channel.

Acknowledgements

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9. The full ^{1}H and ^{13}C spectra assignment of the new compounds **11**, **12**, **15**, **16**, **18**, **20** was accomplished using DEPT, COSY and HMQC NMR experiments; all compounds gave satisfactory elemental analyses data.
10. Molecular mechanics were performed using the MM+ force field provided by the software Hyperchem; MM+ is an extension of MM2 force field. Molecular mechanics MM2 calculations give the most consistent results with experimental data for pyrrolidine conformers. An initial structure was constructed and minimized using conjugate gradient and Newton–Raphson algorithms and an energy gradient tolerance of $0.01\,kcal{\cdot}mol^{-1}{\cdot}Å^{-1}$. This structure was then manipulated using Hyperchem software modules to produce the target structures.
11. A detailed description of the conformational analysis results will be published elsewhere.
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13. Aminoadamantanes tested existed vastly in their protonated form in the physiological pH and testing medium. The starting pH value of the MDCK cells medium that was used for the anti-influenza viruses testing was neutral, and as the cultures progress over time the medium became more acidic.