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Bioorganic & Medicinal Chemistry Letters
Bioorganic & Medicinal Chemistry Letters 17 (2007) 4358–4362

Design and synthesis of bioactive adamantane spiro heterocycles

Nicolas Kolocouris,[a,*] Grigoris Zoidis,[a] George B. Foscolos,[a] George Fytas,[a] S. Radhika Prathalingham,[b] John M. Kelly,[b] Lieve Naesens[c] and Erik De Clercq[c]

[a]*Faculty of Pharmacy, Department of Pharmaceutical Chemistry, University of Athens, Panepistimioupoli-Zografou, GR-15771 Athens, Greece*

[b]*London School of Hygiene and Tropical Medicine, Department of Infectious and Tropical Diseases, Keppel Street, London WC1E 7HT, UK*

[c]*Rega Institute, Department of Microbiology and Immunology, Katholieke Universiteit Leuven, Minderbroedersstraat 10, 3000 Leuven, Belgium*

Received 18 March 2007; revised 27 April 2007; accepted 30 April 2007
Available online 6 May 2007

Abstract—Spiro[aziridine-2,2′-adamantanes] **1** and **2**, spiro[azetidine-2,2′-adamantanes] **3** and **5**, spiro[azetidine-3,2′-adamantane] **13**, spiro[piperidine-4,2′-adamantanes] **25** and **27**, and spiro barbituric analog **18** were synthesized and tested for their anti-influenza A virus properties and for trypanocidal activity. The effect of ring size on potency was investigated. Piperidine **25** showed significant anti-influenza A virus activity, being 12-fold more active than amantadine, about 2-fold more active than rimantadine, and 54-fold more potent than ribavirin. It also proved to be the most active of the compounds tested against bloodstream forms of the African trypanosome, *Trypanosoma brucei*, being 1.5 times more potent than rimantadine and at least 25 times more active than amantadine.

During the 20th century influenza A caused more deaths than any other infectious disease. The Spanish influenza pandemic of 1918 was known as the most devastating epidemic in recorded history. Recent epidemics (Asian influenza in 1957, Hong Kong influenza in 1968, and Russian influenza in 1977), while not as lethal as the 1918 pandemic, infected significant portions of the population (up to at least 10%), resulting in considerable morbidity, which is unsurpassed by any other human disease. Since 2003, an avian influenza virus strain that first appeared in China in 1997 has infected more than 272 persons in countries including Vietnam, Thailand, and Cambodia, and has killed more than half of them.[1] In the face of the persistent threat of human influenza A (H3N2, H1N1) infections, the outbreaks of avian influenza (H5N1) in Southeast Asia, and the potential for a new human or avian influenza A subtype to unleash a pandemic, there is much concern about the shortages in both the number and supply of effective anti-influenza virus agents.[2–5]

Amantadine and rimantadine (α-methyl adamantanemethanamine) are anti-influenza A drugs, which in vitro inhibit virus replication at micromolar concentrations.[6] During the past 12 years our group has synthesized many potent aminoadamantane derivatives, mainly heterocycles and carbocycles, the most potent of which are shown in Figure 1.[7–9] These compounds, in their protonated form, are considered to occlude the M2 protein ion channel pore[10] and block its proton pump function[11] in early and late endosomes,[6b] which is essential for virus uncoating during viral replication.[6b,c,12,13]

Another major public health problem in many areas of sub-Saharan Africa is sleeping sickness, with recent estimates of 300,000 people affected.[14] In humans, the disease is caused by infection with the tsetse fly-transmitted protozoan parasites *Trypanosoma brucei gambiense* (western and central Africa) and *Trypanosoma brucei rhodesiense* (eastern and southern Africa). Untreated, sleeping sickness is invariably fatal. The drugs which are currently available are unsatisfactory[15,16] because their administration requires hospitalization; they are expensive; can fail to eradicate parasitemia, and often produce toxic side effects. Melarsoprol the most widely used drug for the treatment of

Keywords: Adamantane spiro heterocycles; Anti-influenza A virus agent; H3N2; Rimantadine; Trypanocidal activity; NMR.
* Corresponding author. Tel.: +30 210 7274809; fax: +30 210 7274747; e-mail: Zoidis@pharm.uoa.gr

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doi:10.1016/j.bmcl.2007.04.108

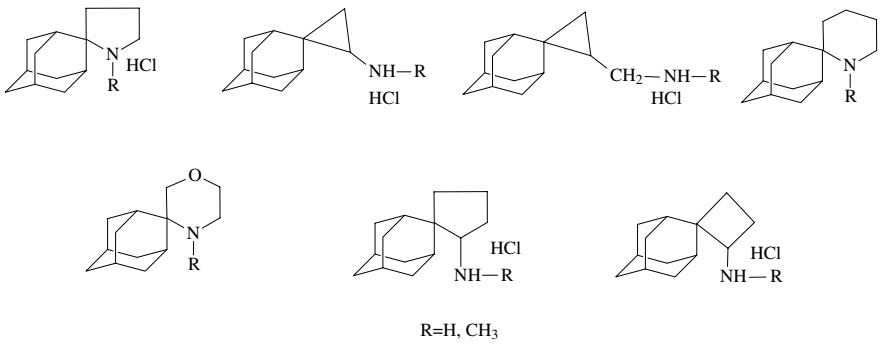


Figure 1. The most potent adamantane spiro heterocycles and carbocycles synthesized by our group.

advanced stage of sleeping sickness, which occurs once parasites have invaded the central nervous system, causes arsenic encephalopathy, with 5–10% patient mortality. Consequently, the development of new trypanocidal drugs is a World Health Organization (WHO) priority.

During the last decade, there have been reports that bloodstream forms of the African trypanosome, *T. brucei*, are sensitive to the anti-influenza virus drug rimantadine (IC_{50}: 7 μM) and to a lesser extent amantadine and that trypanocidal activity is enhanced with increasing pH (the trypanocidal effect was pH dependent and was enhanced in an alkaline environment). Rimantadine is also toxic to the trypanosomatid parasites *Trypanosoma cruzi* and *Leishmania major*.[17] More recently, a number of other aminoadamantane derivatives were evaluated for their trypanocidal properties using various assays, which revealed a correlation between lipophilicity and potency against *T. brucei*. Specifically, increased hydrophobicity was associated with enhanced activity.[18] We therefore reasoned that by investigating the trypanocidal properties of other lipophilic aminoadamantane derivatives, we could gain more insight into the chemical features responsible for activity. Here, we report the identification of a spiro-piperidine-4,2′-adamantane derivative, which displays considerable activity in vitro against bloodstream form *T. brucei*.

As a continuation of our efforts to explore the stereoelectronic requirements for optimal antiviral activity of adamantanes, we present herein the synthesis and biological evaluation of some spiro heterocyclic adamantane derivatives and specifically spiro[aziridine-2,2′-adamantanes] **1** and **2**, spiro[azetidine-2,2′-adamantanes] **3** and **5**, spiro[azetidine-3,2′-adamantane] **13**, spiro[piperidine-4,2′-adamantanes] **25** and **27**, and spiro barbituric analog **18** (Fig. 2).

Both spiro[aziridine-2,2′-adamantane] **1** and spiro[azetidine-2,2′-adamantane] **3** were synthesized using methyleneadamantane as starting material, which on treatment with bromine azide, generated in situ, and reduction of the azidobromide formed with $LiAlH_4$ afforded aziridine **1**.[19] The synthesis of azetidine **3** was achieved by treating methyleneadamantane with chlorosulfonyl isocyanate. The *N*-chlorosulfonyl β-lactam formed by reductive hydrolysis with sodium sulfite gave the respective azetidinone, which was reduced with $LiAlH_4$ to give the desired azetidine **3**.[19]

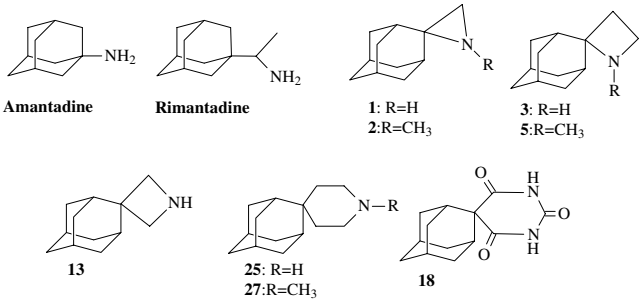
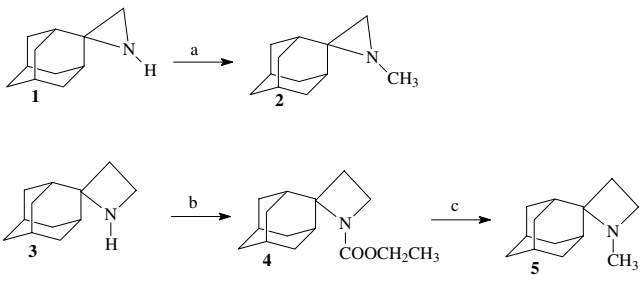


Figure 2. The bioactive adamantane spiro heterocycles presented herein.

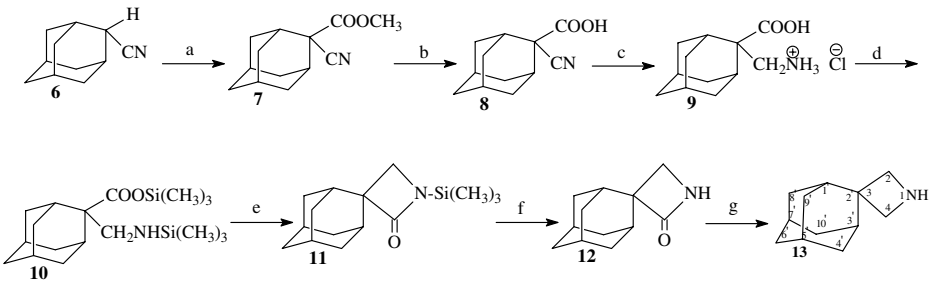
N-Methylation of the aziridine **1** according to Borch and Hassid's reductive methylation ($NaCNBH_3$, CH_2O, and CH_3CN)[20] afforded the desired *N*-methyl aziridine **2**. N-Acylation of the azetidine **3** followed by reduction of the intermediate carbamate **4** with $LiAlH_4$ gave the *N*-methyl derivative **5** (Scheme 1).

The synthetic route followed for the synthesis of azetidine **13** is presented in Scheme 2.

In order to synthesize the spiro-azetidine **13**, 2-adamantanecarbonitrile **6** was used as a starting material.[8c] Thus, lithiation at C-2 using LDA and reaction of the resulting carbanion with methyl chloroformate gave cyanoester **7** in good yield. Saponification of the latter



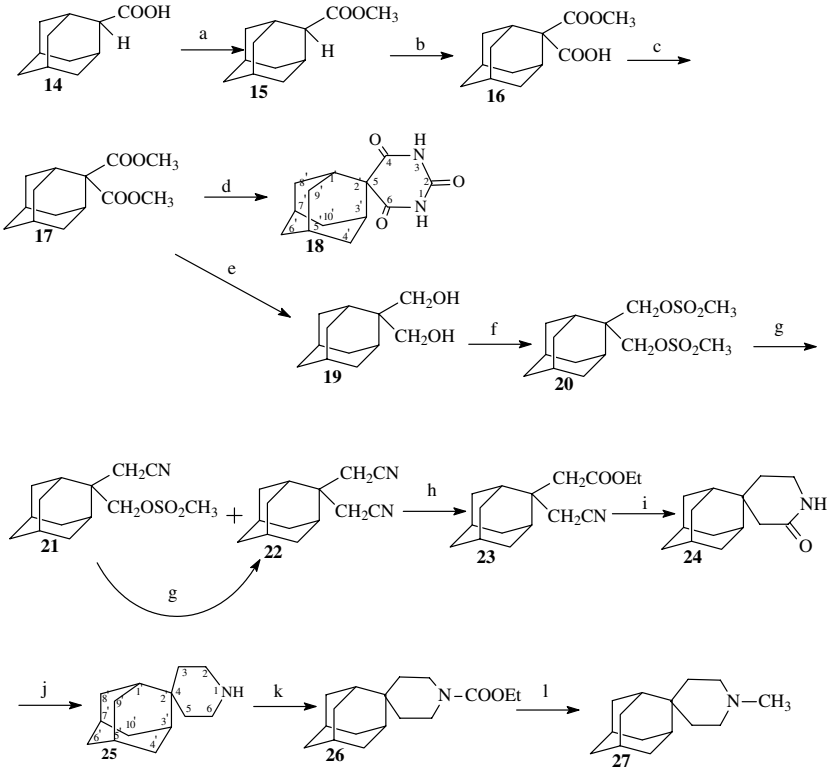
Scheme 1. Reagents and conditions: (a) CH_3CN, 37% CH_2O (aq), $NaCNBH_3$ and then CH_3COOH, 2 h, 25 °C (90%); (b) Et_3N, $ClCOOC_2H_5$, ether, 24 h, 25 °C (98%); (c) $LiAlH_4$, THF, 24 h, 25 °C (95%).



Scheme 2. Reagents and conditions: (a) LDA, −70 °C, THF, $ClCOOCH_3$, 24 h, 20 °C, (88%); (b) EtOH, NaOH, H_2O, reflux and then HCl (85%); (c) EtOH, HCl, H_2/PtO_2, 50 psi, 20 °C, 5 h (32%); (d) $(CH_3)_3SiCl$, C_6H_6, Et_3N, 3 h, 80 °C (quant); (e) CH_3MgI, ether 24 h, 20 °C and then NH_4Cl (69%); (f) CH_3OH, 10 min, 90 °C (96%); (g) $LiAlH_4$, THF, 5 h, reflux (90%).

afforded the desired cyanoacid **8**, however, subsequent catalytic hydrogenation over Raney nickel catalyst at 180 °C did not give the desired product, because of extensive decarboxylation. Hydrogenation under mild conditions (H_2/PtO_2, HCl) did give aminoacid **9** albeit in low yield (32% based on recovered cyanoacid **8**). *N*,*O*-Bistrimethylsilylation of aminoacid **9** was accomplished using $(CH_3)_3SiCl$ in Et_3N. Reaction of the silylated product **10** with an ethereal solution of CH_3MgI resulted in the formation of the trimethylsilyl analog of β-lactam **11**, which was hydrolyzed upon heating with CH_3OH/H_2O to the azetidinone **12**. This was then reacted with $LiAlH_4$ to give the desired azetidine **13**. The synthetic route mentioned above[21] was used for the first time in the synthesis of spiro-β-lactams and offers a new pathway for the preparation of complex spiro-β-lactams starting from a nitrile of a structure corresponding to complex spiromotif, and then building the β-lactam ring (Scheme 3).

For the synthesis of piperidine **25**, adamantane-2-carboxylic acid **14**[22] was used as starting material. This was successively carboxylated to the ester **16** using LDA and CO_2. The acid was then esterified to the diester **17**, which upon heating with urea in the presence of sodium ethoxide afforded the barbituric derivative **18** in low yield. Moreover, the preparation of diol **19** through reduction of ester **16** with $LiAlH_4$ was not successful,



Scheme 3. Reagents and conditions: (a) $SOCl_2$, 45 min, 45 °C and then CH_3OH, 24 h, 20 °C, (quant); (b) LDA, −70 °C, THF, CO_2, 24 h, 20 °C, (quant); (c) $SOCl_2$, 20 min, 35 °C and then CH_3OH, 1 h, reflux (quant); (d) Na, abs EtOH, NH_2CONH_2, 20 min 190 °C (20%); (e) $LiAlH_4$, ether, 8 h, reflux (93%); (f) MsCl, pyridine, CH_2Cl_2, 24 h, 25 °C (quant.); (g) NaCN, DMSO, 24 h, 105 °C (70%); (h) EtOH–HCl, 20 days, 20 °C and then H_2O, HCl, ether, 24 h, 20 °C; (i) EtOH, H_2, Ni–Raney, 60 psi, 120 °C, 6 h (96%); (j) $LiAlH_4$, THF, reflux, 25 h (93%); (k) Et_3N, $ClCOOC_2H_5$, ether, 70 h, 25 °C (75%); (l) $LiAlH_4$, THF, 24 h, 20 °C (92%).

due to decarboxylation. Conversely, reduction of **17** with $LiAlH_4$ gave diol **19** in very good yield (93%). Ester **16**, diester **17**, and diol **19** have been synthesized in the past by a less facile synthetic route in very low overall yields.[22]

The reaction of diol **19** with CH_3SO_2Cl in pyridine gave dimethanesulfonate **20**. The transformation of the latter to dinitrile **22** was found to be troublesome. Heating dimethanesulfonate **20** for 24 h with NaCN/DMSO at 72 °C and 3 h at 105 °C resulted in the formation of a separable mixture consisting of cyanoester **21**, dinitrile **22**, and unreacted diester **20** in a 52/32/14 ratio. It is noteworthy though that cyanoester **21** can be converted to **22** by the above-mentioned reaction. Cyanoester **23** was obtained from the reaction of dinitrile **22** with a saturated ethanolic solution of gaseous HCl for 18 days at 25 °C.[23] Hydrogenation of **23** over Raney nickel catalyst afforded the spiro[piperidine-4,2′-adamantan]-2-one **24**, which was reduced with $LiAlH_4$ in THF to give the parent piperidine **25**. N-Acylation of the latter followed by reduction of the intermediate carbamate **26** with $LiAlH_4$ gave the *N*-methyl derivative **27**.[24]

The antiviral efficacy of each of new aminoadamantane heterocycles **1**, **2**, **3**, **5**, **13**, **25**, and **27** was examined in vitro against influenza A (H3N2) and was compared to the activity of amantadine, rimantadine, and ribavirin (Table 1). The antiviral assay used was identical to that previously reported.[25]

The data presented in Table 1 indicate that compounds **3**, **13**, and **25** elicit potent anti-influenza virus A activity, with a selectivity index (SI) of 694, 342, and 106, respectively. Piperidine **25** was endowed with the most potent anti-influenza A virus activity; it proved to be at least 12-fold more potent than amantadine, about 2 times more potent than rimantadine, and 54-fold more active than ribavirin. Likewise, azetidine **13** exhibited a 6-fold higher potency than amantadine and had slightly higher potency and selectivity than rimantadine. It is noteworthy that azetidine **3** was equipotent with rimantadine, but displayed the highest selectivity index (more than 13-fold higher than that of amantadine) of any compound tested. Finally, methyl substitution at the nitrogen atom of all heterocycles caused reduction in anti-influenza virus A potency.

Table 1. Anti-influenza virus A (H3N2) activity and cytotoxicity of heterocyclic adamantane analogues-aziridines **1** and **2**, azetidines **3**, **5**, and **13**, and piperidines **25** and **27**[a]—in MDCK cells[b]

Compound	EC_{50}[c](μM)	MCC[d](μM)	SI (ratio MCC/EC_{50})
1	>5	25	<5
2	>4.5	23	<5
3	0.36	250	694
5	0.59	50	85
13	0.33 ± 0.05 (4)	113	342
25	0.16 ± 0.08 (4)	17	106
27	4.30 ± 7.31(4)	4	1
Amantadine	1.98	>100	>51
Rimantadine	0.362	>100	>276
Ribavirin	8.68	20	2

[a] Aziridines **1**, **2** and azetidines **3**, **5**, and **13** were tested as free bases. Piperidines **25** and **27** were tested as hydrochlorides.

[b] MDCK, Madin–Darby canine kidney cells; virus strain: influenza A/Hong Kong/7/87 (H3N2).

[c] Concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE or by measuring the cell viability with the colorimetric formazan-based MTS assay. For the compounds showing reproducible activity, data are shown as means ± SD (in brackets: number of independent determinations).

[d] Minimal cytotoxic concentration, or concentration that causes microscopically detectable changes in cell morphology. For the compounds showing reproducible activity, data are shown as means ± SD (in brackets: number of independent determinations).

Numbered compounds were first tested at 5 μg ml^{-1} (20–30 μM, depending on the compound) against bloodstream form *T. brucei* (strain 221) cultured at pH 7.4. At this concentration, compound **25** killed 100% of the parasites. The IC_{50} and IC_{90} were then calculated by investigating growth inhibition over a range of concentrations, from 1 to 20 μM. The values shown are means ± standard deviation from three experiments.

In a preliminary screen to identify the most active compounds, bloodstream form *T. brucei*, seeded at an initial density of 0.5×10^5 ml^{-1}, were cultured for 3 days in the presence of spiro aminoadamantane derivatives at 5 μg ml^{-1}. At this concentration, compounds **1**, **5**, and **27** displayed only minimal trypanocidal activity, while the spiro barbituric analog (compound **18**) inhibited parasite growth by 61%. This analog is at least 7-fold more potent than amantadine, although slightly less active than rimantadine.[17] Piperidine **25** was the most active of the compounds tested, resulting in lysis of all trypanosomes in the culture at 5 μg ml^{-1}. We therefore established its IC_{50} and IC_{90} values (Table 2); piperidine **25** was found to be 1.5 times more active than rimantadine and at least 25 times more active than amantadine. This contrasted with lack of activity displayed by compound **27**, the *N*-methyl derivative of piperidine **25**. Interestingly, methyl substitution at this nitrogen also reduced potency against influenza A virus (Table 1).

Synopsis. The aim of this study was to examine the anti-influenza A virus and trypanocidal activity of spiro adamantane analogs **1**, **2**, **3**, **5**, **13**, **25**, **27**, and **18**, and to correlate their potency to the size of the heterocyclic ring. Two interesting points arise from this analysis: (i) moving from six-, to four- to a three-membered ring reduces the activity both against influenza A virus and *T. brucei*; (ii) nonsubstituted heterocyclic compounds are more active, whereas N-methylation causes a dra-

Table 2. Susceptibility of cultured bloodstream form *T. brucei* to aminoadamantane derivatives

Compound	IC_{50}(μM)	IC_{90}(μM)
1	>30	—
5	>25	—
18	~20	—
25	5.28 ± 0.57	9.99 ± 0.94
27	>20	—
Amantadine	>130	—
Rimantadine	7.04 ± 0.12	13.97 ± 1.68

matic reduction in their potency. The N-substitution of the parent amines with other alkyl substituents was not attempted, as it has already been reported that the small size of the *N*-alkyl group enhances the activity of the respective compounds against influenza A H3N2 strains.[8a]

Acknowledgments

Dr. Zoidis would like to thank the State Scholarship Foundation of Greece and the University of Athens (ELKE Account KA: 70/4/5858) for financial support. We wish to express our appreciation to Leentje Persoons for excellent technical assistance.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.04.108.

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24. Spectra and elemental analysis of all the synthesized compounds were in accordance with their structure. The ^{1}H and ^{13}C NMR spectra were assigned using COSY and CHCORR spectroscopy. The NMR spectra assignment of the spiro[azetidine-3,2′-adamantane] **13**, spiro[piperidine-4,2′-adamantane] **25**, and spiro barbituric analog **18** is given as Supplementary information.
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