Bioorganic & Medicinal Chemistry 20 (2012) 7155–7159

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Microwave assisted synthesis and anti-influenza virus activity of 1-adamantyl substituted *N*-(1-thia-4-azaspiro[4.5]decan-4-yl)carboxamide derivatives

Füsun Göktaş [a,*], Evelien Vanderlinden [b], Lieve Naesens [b], Nesrin Cesur [a], Zafer Cesur [a]

[a] Istanbul University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 34116 Beyazıt, Istanbul, Turkey
[b] Rega Institute for Medical Research, University of Leuven, B-3000 Leuven, Belgium

ARTICLE INFO

Article history:
Received 11 June 2012
Revised 25 September 2012
Accepted 26 September 2012
Available online 12 October 2012

Keywords:
Adamantane
Spirothiazolidinone
Microwave
Antiviral
Influenza virus
Hemagglutinin

ABSTRACT

A microwave-assisted three-component one-pot cyclocondensation method was applied for the synthesis of novel *N*-(1-thia-4-azaspiro[4.5]decan-4-yl)carboxamide compounds carrying an adamantyl moiety. The structures of the compounds were confirmed by spectral and elemental analysis. All compounds were evaluated for antiviral activity against influenza A (H1N1 and H3N2) and influenza B virus in MDCK cell cultures. The compounds displayed a confined structure-activity relationship. The *N*-(2,8-dimethyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)adamantane-1-carboxamide **3b** was the most potent inhibitor [antiviral EC_{50}: 1.4 μM against influenza A/H3N2 virus]. Its strong inhibitory effect in a virus hemolysis assay supports that **3b** acts as an influenza virus fusion inhibitor by preventing the conformational change of the influenza virus hemagglutinin at low pH.

1. Introduction

Influenza viruses are important respiratory pathogens causing significant morbidity and mortality and considerable economic losses during the epidemic periods. Vaccination represents the primary protection but, due to the antigenic variability, the vaccine requires annual updating. Vaccine development is retarded when a new influenza virus, such as the 2009 pandemic variant, appears.[1] The influenza virions carry a lipid envelope in which three viral surface proteins are embedded: the hemagglutinin (HA), the neuraminidase (NA) and the M2 proton channel protein.[2] The latter two are validated antiviral targets: M2 channel activity is blocked by the adamantane compounds amantadine and rimantadine, whereas the NA is inhibited by zanamivir and oseltamivir.[3]

Besides its application in anti-influenza virus therapeutics, the adamantyl moiety represents a versatile pharmacophore, since adamantane derivatives have also been explored for the treatment of some neurological conditions, type 2 diabetes or acne vulgaris.[4] Furthermore, incorporation of an adamantyl moiety into a pharmacologically active molecule often improves the therapeutic profile of the parent agent.[5]

The anti-influenza virus action of the M2 blockers amantadine and rimantadine is situated during the viral entry process. After cellular uptake of the influenza virus by receptor-mediated endocytosis, the low pH inside the endosomes activates the M2 proton channel. Transport of protons into the virion interior leads to uncoating, that is, release of the viral ribonucleoprotein from the M1 capsid protein. In addition, the low endosomal pH provokes an irreversible change in the conformation of the HA protein, leading to fusion of the viral and endosomal membranes. Inhibition of M2 channel function is an explored strategy.[6,7] Although amantadine is effective in both prevention and prophylaxis of influenza A virus infections, its clinical use is limited by its neurological side effects and low barrier for resistance. Its analogue rimantadine has been considered a better antiviral drug because of its enhanced activity and fewer side effects.[8] Recently gained insight in the protein structure of M2 and the precise M2 binding mode of amantadine and rimantadine[9,10] may lead to more potent M2 inhibitors which retain activity against virus mutants carrying amantadine- or rimantadine-associated resistance mutations in the M2 protein.[11]

In comparison, the development of small molecule inhibitors of the HA-mediated fusion process has proven to be relatively

Abbreviations: HA, hemagglutinin; NA, neuraminidase; MDCK, madin darby canine kidney cells; EC_{50}, 50% effective concentration; RBC, red blood cells; DMSO, dimethyl sulfoxide; CPE, cytopathic effect; PFU, plaque forming unit; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium; MCC, minimal cytotoxic concentration; Mp, melting point; adm, adamantane; spd, spiro[4.5]decane.

* Corresponding author. Tel.: +90 212 4555700/13460; fax: +90 212 4400252.
E-mail address: fusung@istanbul.edu.tr (F. Göktaş).

0968-0896/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.09.064

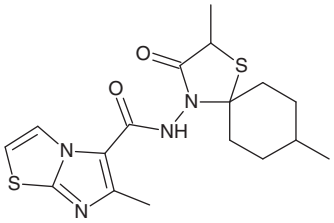


Figure 1. Structure of 6-methyl-*N*-(2,8-dimethyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl) imidazo[2,1-*b*][1,3]thiazole-5-carboxamide (**4c**).

difficult. Most reported fusion inhibitors suffer from having subtype-dependent antiviral activity and a low barrier for resistance selection.[12] We recently identified a new class of *N*-(1-thia-4-azaspiro[4.5]decan-4-yl)carboxamide derivatives with potent activity against influenza A/H3N2 viruses.[13] We demonstrated that the lead compound [6-methyl-*N*-(2,8-dimethyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)imidazo[2,1-*b*][1,3]thiazole-5-carboxamide; encoded **4c** in Ref. 13] (Fig. 1) inhibits the HA-mediated membrane fusion at low pH, due to its specific binding interaction with a hydrophobic pocket within the HA protein. We now report on related analogues carrying an 1-adamantyl substituent. This creates the possibility to combine two pharmacological activities (i.e., M2 and HA inhibition) into one single agent.

2. Results and discussion

2.1. Chemistry

Microwave (MW) irradiation has been recently introduced to perform diverse chemical reactions in a short time, and to produce compounds in high yields. In the green-chemistry area, the objective is to perform organic reactions solvent-free under dry media conditions, thereby reducing the use of toxic solvents, and preventing unnecessary pollution. Thus, MW reaction methods are convenient, clean and economical.[14]

A mixture of 1-adamantanecarbohydrazide, the appropriate cyclic ketone and sulfanylacetic acid or 2-sulfanylpropanoic acid in toluene, was heated under microwave irradiating conditions at 120 °C and 400 W for 10 min. After solvent evaporation, the residue was made alkaline and allowed to solidify.

The IR spectra of **2a–f** and **3a–f** showed common characteristic absorption bands at 3468–3265 cm^{-1} (NH), 1683–1658 cm^{-1} (NH–C=O) and 1720–1697 cm^{-1} (C=O), which provided evidence for the cycloaddition reaction between azomethine intermediates and sulfanylacetic or 2-sulfanylpropanoic acids. In the ^1H NMR spectra of **2a–f** and **3a–f**, CONH proton was observed at 9.57–9.25 ppm as a singlet. The resonance of the CH_2 protons **2a–f** at position 2 of the spiro system was observed at 3.57–3.49 ppm as a singlet. Additional support was obtained from the spectra of **3a–f**, which showed the same resonance with almost the same chemical shift values (3.84–3.78 ppm) as a quartet. CH_3 protons of **3a–f** resonated at 1.41–1.37 ppm as a doublet. Adamantil protons were observed as expected, together with the remaining protons of the spiro system in the range of C_{6-10}. Synthesis of **3a–f** was performed using racemic 2-sulfanylpropanoic acid. On the other hand, in spirocyclic compounds, two rings meet at a single atom. This means that the two rings are orthogonal about the tetrahedral atom. Even symmetrically looking compounds are unexpectedly chiral.[15] Thus, our target compounds structurally are stereoisomers. Enantiomeric separation was not performed. The detailed spectral data of the compounds (**2a–f** and **3a–f**) are given in the Section 4.

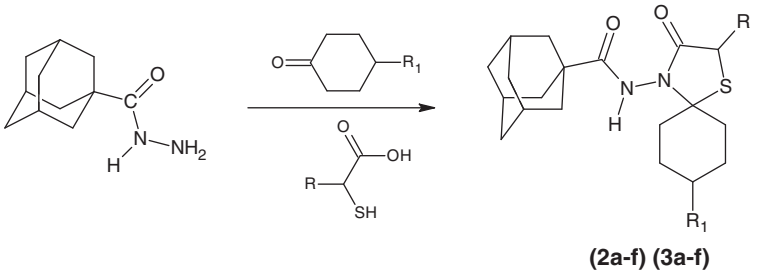
2.2. Biological activity

The test compounds were evaluated for anti-influenza virus activity by a cytopathic effect (CPE) reduction assay.[13] MDCK cells were infected with one of three influenza virus strains: A/PR/8/34 (A/H1N1); A/X-31 (A/H3N2) or B/HK/5/72. After 72 h incubation at 35 °C, microscopy followed by the MTS cell viability test were performed, to determine the following parameters for antiviral and cytotoxic activity: EC_{50}, or compound concentration producing 50% inhibition of virus-induced cytopathic effect; MCC, or minimum cytotoxic concentration (defined as the compound

Table 1
Anti-influenza virus activity and cytotoxicity in MDCK cell cultures

Compound	Cytotoxicity (μM)		EC_{50}[c] (μM) against influenza virus					
	CC_{50}[a]	MCC[b]	A/H1N1 (A/PR/8/34)		A/H3N2 (A/X-31)		B (B/HK/5/72)	
			CPE	MTS	CPE	MTS	CPE	MTS
2a	ND	ND	ND	ND	ND	ND	ND	ND
3a	>100	>100	>100	>100	>100	>100	>100	>100
2b	>100	>100	>100	>100	>100	>100	>100	>100
3b	>100	>100	>100	>100	1.6	1.4	>100	>100
2c	>100	>100	>100	>100	>100	21	>100	>100
3c	>100	>100	>100	>100	51	1.7	>100	>100
2d	>100	>100	>100	>100	>100	44	>100	>100
3d	>100	>100	>100	>100	29	23	>100	>100
2e	>100	>20	>100	53	>100	34	>100	>100
3e	>100	>100	>100	>100	>100	>100	>100	>100
2f	>100	>20	>100	>100	>100	>100	>100	>100
3f	42	20	>100	>100	>100	>100	>100	>100
4c [13]	>100	>100	>100	>100	6.8	9.0	>100	>100
Zanamivir	>100	>100	0.44	0.48	12	53	0.072	0.063
Ribavirin	>100	>20	8.9	8.4	8.9	8.5	0.094	0.14
Amantadine	>500	>500	58	75	224	208	>500	>500
Rimantadine	218	500	2.7	2.5	45	40	>500	>500

ND: Not done
MDCK cells: Madin Darby canine kidney cells.
[a] 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric MTS assay.
[b] Minimum cytotoxic concentration, causing a microscopically detectable alteration in cell morphology.
[c] 50% Effective concentration, or concentration producing 50% inhibition of virus replication,as determined by microscopical scoring of the cytopathic effect (CPE) or by the MTS cell viability assay.



Compound	R	R_1
2a	H	H
3a	CH_3	H
2b	H	CH_3
3b	CH_3	CH_3
2c	H	C_2H_5
3c	CH_3	C_2H_5
2d	H	C_3H_7
3d	CH_3	C_3H_7
2e	H	$C(CH_3)_3$
3e	CH_3	$C(CH_3)_3$
2f	H	C_6H_5
3f	CH_3	C_6H_5

Figure 2. Strategy for the synthesis of compounds **2a–f** and **3a–f**.

concentration causing minimal changes in cell morphology), and CC_{50} (the compound concentration causing 50% reduction in cell viability based on the MTS assay).

As shown in Table 1, compound **3b** was the most potent analogue among the series, with an antiviral EC_{50} value of 1.5 μM against the A/H3N2 virus. This compound produced no cytotoxic effects at 100 μM (the highest concentration tested), yielding a selectivity index (or ratio of cytotoxic to antiviral concentration) of >67. Since **3b** carries the *N*-(1-thia-4-azaspiro[4.5]decan-4-yl)carboxamide backbone that is characteristic for our series of influenza virus fusion inhibitors,[13] we further evaluated the activity of **3b** in a hemolysis inhibition assay. The A/H3N2 virus was preincubated with test compound prior to its addition to red blood cells. After lowering the pH, the degree of hemolysis was determined by spectrophotometry. In the absence of compound, low pH-induced refolding of HA resulted in perforation of the RBC membrane and hemolysis. Compound **3b** produced a dose-dependent inhibition of hemolysis (data not shown), with an IC_{50} of 3.7 μM. This value was in the same range as that obtained with our previously identified lead compound **4c**,[13] which was included as a reference (IC_{50} of 2.6 μM).

Structure–activity analysis on this new series of 1-adamantyl substituted *N*-(1-thia-4-azaspiro[4.5]decan-4-yl)carboxamides (Fig. 2) revealed which substituents are required for the antiviral activity. Overall, these data were in nice agreement with the structure–activity relationship that we previously obtained for **4c** and analogues.[13] An important new insight is that the anti-influenza virus activity is unchanged when the aromatic ring system in **4c** is replaced by a non-aromatic and bulky adamantyl moiety. With regard to the spiro moiety, substitution at position 2 and 8 is clearly essential. The methyl substituent at position 2 (*R*) is obviously required, since the analogues **2c** and **2d** lacking this group were less active than their 2-methylated counterparts **3c** and **3d**. Regarding position 8 of the spiro ring, a smaller alkyl group (i.e., methyl in **3b**, ethyl in **3c** or *n*-propyl in **3d**) is preferable over a larger alkyl (i.e., *t*-butyl; **3e**) or phenyl (**3f**) substituent. Compounds lacking a substituent at this position 8 (**3a**) were devoid of any anti-influenza virus activity. In analogy to our findings with **4c**, the novel *N*-(1-thia-4-azaspiro[4.5]decan-4-yl)carboxamide derivatives had activity against influenza A/H3N2 but not A/H1N1 or B viruses.

3. Conclusion

Among this novel series of 1-adamantyl substituted spirothiazolidinones, compound **3b** emerged as a potent and selective inhibitor of influenza A/H3N2 virus. Similar to its congener **4c** for which an inhibitory effect on the conformational change of HA was clearly demonstrated,[13] **3b** strongly inhibits influenza-virus induced hemolysis at low pH. Compound **3b** represents a hybrid molecule combining the adamantyl pharmacophore for inhibition of the influenza virus M2 protein, and the spirothiazolidinone backbone, which interferes with HA-mediated fusion. This creates the possibility to combine two antiviral modes of action into one single pharmacological agent.

4. Experimental protocols

4.1. Chemistry

Commercially available chemicals were obtained from Merck and Aldrich. Melting points (Mp) were determined on a Büchi 530 capillary melting point apparatus in open capillaries and were uncorrected. Compound purity was measured by thin-layer chromotography (TLC) on Silica gel 60 F_{254}. Infrared (IR) spectra were recorded on a Pelkin Elmer 1600 FT infrared spectrophotometer in potassium bromide pellets (υ in cm^{-1}). ^1H nuclear magnetic resonance (NMR) spectra were recorded on a Varian UNITY INOVA 500 MHz NMR spectrophotometer and Varian Mercury 400 MHz High Performance Digital FT-NMR Spectrometer using tetramethylsilane as internal standard. Microwave reactions were carried out using a Milestone Start S microwave apparatus. Elementary analyses were performed on a Thermo Finnigan Flash EA 1112 Series elemental analyzer.

4.2. General synthesis of 2a–f and 3a–f

A one-pot three component mixture of 1-adamantane carbohydrazide (0.005 mol), the appropriate cyclic ketone (0.005 mol) and sulfanylacetic acid or 2-sulfanylpropanoic acid in 12 mL of toluene was added to a glass tube with a magnetic stirring bar. The mixture was heated under microwave irradiating conditions at 120 °C and 400 W for 10 min. After cooling, completion of the reactions was checked by TLC, and the compounds were transferred to a glass flask. Excess toluene was evaporated in vacuo. The residue was triturated with saturated $NaHCO_3$ until CO_2 effervescence ceased and was allowed to stand overnight. The solid thus obtained was filtered, washed with H_2O, and crystallized from the C_2H_5OH–H_2O mixture.

4.2.1. *N*-(3-Oxo-1-thia-4-azaspiro[4.5]dec-4-yl)adamantane-1-carboxamide (2a)

Yield: 87.35%. Mp 245–7 °C. IR: 3280 (NH), 1705, 1683 (C=O). ^1H NMR (DMSO-d_6) δ (ppm): 0.96–1.98 (25H, m, adm-H and spd.

C_{6-10}-H), 3.50 (2H, s, spd. C_2-H), 9.45 (1H, s, CONH). Anal. calcd for $C_{19}H_{28}N_2O_2S{\cdot}H_2O$ (366.51): C, 62.26; H, 8.25; N, 7.64. Found: C, 63.03; H, 7.55; N, 8.13.

4.2.2. *N*-(8-Methyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)adamantane-1-carboxamide(2b)

Yield: 93.37%. Mp 229–31 °C. IR: 3265 (NH), 1705, 1681 (C=O). ^{1}H NMR (DMSO-d_6) δ (ppm): 0.84 (3H, d, J = 6.3 Hz, $-CH_3$), 1.09–1.97 (24H, m, adm-H and spd. C_{6-10}-H), 3.50 (2H, s, spd. C_2-H), 9.45 (1H, s, CONH). Anal. calcd for $C_{20}H_{30}N_2O_2S{\cdot}0.5H_2O$ (371.53): C, 64.65; H, 8.41; N, 7.54. Found: C, 64.49; H, 8.09; N, 8.23.

4.2.3. N-(8-Ethyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)adamantane-1-carboxamide (2c)

Yield: 88.82%. Mp 195–7 °C. IR: 3448 (NH), 1701, 1660 (C=O). ^{1}H NMR (DMSO-d_6) δ (ppm): 0.83 (3H, t, J = 7.3 Hz, $-CH_2CH_3$), 1.10–1.17 (2H, m, $-CH_2CH_3$), 1.66–1.97 (24H, m, adm-H and spd. C_{6-10}-H), 3.50 (2H, s, spd. C_2-H), 9.45 (1H, s, CONH). Anal. calcd for $C_{21}H_{32}N_2O_2S{\cdot}0.5C_2H_5OH$ (399.58): C, 63.11; H, 8.82; N, 7.01. Found: C, 62.85; H, 8.45; N, 7.65.

4.2.4. *N*-(3-Oxo-8-propyl-1-thia-4-azaspiro[4.5]dec-4-yl)adamantane-1-carboxamide(2d)

Yield: 93.33%. Mp 188–90 °C. IR: 3446 (NH), 1701, 1658 (C=O). ^{1}H NMR (DMSO-d_6) δ (ppm): 0.84 (3H, t, J = 7.3 Hz, $-CH_2CH_2CH_3$), 1.08–1.13 (4H, m, $-CH_2CH_2CH_3$ and adm-H), 1.26 (2H, q, J = 7.3 Hz, $-CH_2CH_2CH_3$), 1.64–1.97 (22H, m, adm-H and spd. C_{6-10}-H), 3.49 (2H, s, spd. C_2-H), 9.45 (1H, s, CONH). Anal. calcd for $C_{22}H_{34}N_2O_2S{\cdot}H_2O$ (408.59): C, 64.06; H, 8.88; N, 6.85. Found: C, 64.53; H, 8.75; N, 7.13.

4.2.5. *N*-(3-Oxo-8-tert-butyl-1-thia-4-azaspiro[4.5]dec-4-yl)adamantane-1-carboxamide (2e)

Yield: 83.66%. Mp 230–2 °C. IR: 3446 (NH), 1701, 1670 (C=O). ^{1}H NMR (DMSO-d_6) δ (ppm): 0.81 (9H, s, $-C(CH_3)_3$), 1.16–1.97 (24H, m, adm-H and spd. C_{6-10}-H), 3.49 (2H, s, spd. C_2-H), 9.45 (1H, s, CONH). Anal. calcd for $C_{23}H_{36}N_2O_2S{\cdot}H_2O$ (422.62): C, 65.36; H, 8.58; N, 6.92. Found: C, 64.99; H, 8.63; N: 7.05.

4.2.6. *N*-(3-Oxo-8-phenyl-1-thia-4-azaspiro[4.5]dec-4-yl)adamantane-1-carboxamide (2f)

Yield: 78.30%. Mp 252–4 °C. IR: 3292 (NH), 1697, 1681 (C=O). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.63–2.00 (24H, m, adm-H and spd. C_{6-10}-H), 3.57 (2H, s, spd. C_2-H), 7.18–7.31 (5H, m, phenyl-H), 9.57 (1H, s, CONH). Anal. calcd for $C_{25}H_{32}N_2O_2S{\cdot}H_2O$ (422.61): C, 67.83; H, 7.74; N, 6.33. Found: C, 68.28; H, 7.48; N, 6.77.

4.2.7. *N*-(2-Methyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)adamantane-1-carboxamide (3a)

Yield: 77.34%. Mp 241–3 °C. IR: 3304 (NH), 1708, 1670 (C=O). ^{1}H NMR (DMSO-d_6) δ (ppm): 0.98–1.98 (25H, m, adm-H and spd. C_{6-10}-H), 1.38 (3H, d, J = 6.8 Hz, spd. C_2-CH_3), 3.79 (1H, q, J = 6.8 Hz, spd. C_2-H), 9.50 (1H, s, CONH). Anal. calcd for $C_{20}H_{30}N_2O_2S$ (362.52): C, 66.26; H, 8.34; N, 7.73. Found: C, 66.02; H, 7.84; N, 7.91.

4.2.8. *N*-(2,8-Dimethyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)adamantane-1-carboxamide (3b)

Yield: 93.61%. Mp 255–6 °C. IR: 3273 (NH), 1708, 1668 (C=O). ^{1}H NMR (DMSO-d_6) δ (ppm): 0.85 (3H, d, J = 6.0 Hz, spd. C_8-CH_3), 1.08–1.98 (27H, m, adm-H, spd. C_2-CH_3 and spd. C_{6-10}-H), 3.81 (1H, q, J = 6.8 Hz, spd. C_2-H), 9.25 (1H, s, CONH). Anal. calcd for $C_{21}H_{32}N_2O_2S$ (376.55): C, 66.98; H, 8.57; N, 7.44. Found: C, 66.87; H, 8.82; N, 7.91.

4.2.9. *N*-(8-Ethyl-2-methyl-3-oxo-1-thia-4-azaspiro[4.5]dec-4-yl)adamantane-1-carboxamide (3c)

Yield: 89.23%. Mp 209–10 °C. IR: 3468 (NH), 1703, 1668 (C=O). ^{1}H NMR (DMSO-d_6) δ (ppm): 0.82 (3H, t, J = 7.5 Hz, $-CH_2CH_3$), 1.00–1.19 (5H, m, adm-H, spd. C_{6-10}-H and CH_2CH_3), 1.37 (3H, d, J = 6.8 Hz, C_2-CH_3), 1.64–1.97 (21H, m, adm-H and spd. C_{6-10}-H), 3.78 (1H, q, J = 6.8 Hz, spd. C_2-H), 9.50 (1H, s, CONH). Anal. calcd for $C_{22}H_{34}N_2O_2S$ (408.59): C, 66.14; H, 9.41; N, 7.04. Found: C, 66.12; H, 8.83; N, 7.01.

4.2.10. *N*-(2-Methyl-3-oxo-8-propyl-1-thia-4-azaspiro[4.5]dec-4-yl)adamantane-1-carboxamide (3d)

Yield: 88.11%. Mp 198–9 °C. IR: 3462 (NH), 1701, 1662 (C=O). ^{1}H NMR (DMSO-d_6) δ (ppm): 0.83 (3H, t, J = 7.3 Hz, $-CH_2CH_2CH_3$), 1.10–1.13 (4H, m, $-CH_2CH_2CH_3$ and adm-H), 1.26 (2H, q, J = 7.8 Hz, $-CH_2CH_2CH_3$), 1.37 (3H, d, J = 6.8 Hz, spd. C_2-CH_3), 1.66–1.97 (22H, m, adm-H and spd. C_{6-10}-H), 3.78 (1H, q, J = 6.8 Hz, spd. C_2-H), 9.50 (1H, s, CONH). Anal. calcd for $C_{23}H_{36}N_2O_2S{\cdot}H_2O$ (422.62): C, 65.36; H, 9.06; N, 6.63. Found: C, 65.58; H, 8.97; N, 6.93.

4.2.11. *N*-(2-Methyl-3-oxo-8-tert-butyl-1-thia-4-azaspiro[4.5]dec-4-yl)adamantane-1-carboxamide (3e)

Yield: 76.07%. Mp 256–7 °C. IR: 3273 (NH), 1720, 1670 (C=O). ^{1}H NMR (DMSO-d_6) δ (ppm): 0.81 (9H, s, $-C(CH_3)_3$), 1.12–1.22 (2H, m, adm-H and spd. C_{6-10}-H), 1.37 (3H, d, J = 6.8 Hz, spd. C_2-CH_3), 1.64–1.97 (22H, m, adm-H and spd. C_{6-10}-H), 3.78 (1H, q, J = 6.8 Hz, spd. C_2-H), 9.50 (1H, s, CONH). Anal. calcd for $C_{24}H_{38}N_2O_2S{\cdot}H_2O$ (436.64): C, 66.01; H, 9.23; N, 6.42. Found: C, 66.10; H, 8.91; N, 6.75.

4.2.12. *N*-(2-Methyl-3-oxo-8-phenyl-1-thia-4-azaspiro[4.5]dec-4-yl)adamantane-1-carboxamide (3f)

Yield: 84.01%. Mp 146–8 °C. IR: 3462 (NH), 1697, 1670 (C=O). ^{1}H NMR (DMSO-d_6) δ (ppm): 1.41 (3H, d, J = 6.8 Hz, spd. C_2-CH_3), 1.60–1.99 (24H, m, adm-H and spd. C_{6-10}-H), 3.84 (1H, q, J = 6.8 Hz, spd. C_2-H), 7.15–7.29 (5H, m, phenyl-H), 9.57 (1H, s, CONH). Anal. calcd for $C_{26}H_{34}N_2O_2S{\cdot}1.5\ H_2O$ (465.64): C, 67.05; H, 8.01; N, 6.01. Found: C, 66.98; H, 7.53; N, 6.27.

4.3. Antiviral assay

The multicycle CPE reduction assay for antiviral evaluation of the test compounds can be found elsewhere.[13] MDCK cells, seeded into 96-well plates, were exposed to serial dilutions of the compounds, together with the influenza virus (multiplicity of infection: 0.0004 PFU per cell). After three days incubation at 35 °C, microscopy was performed for visual scoring of the CPE and cytotoxic effects. The data were confirmed by the spectrophotometric MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium] cell viability assay.

4.4. Hemolysis inhibition assay

The detailed methodology has already been published.[13] An allantois stock of influenza virus A/H3N2 (strain A/X-31) was pre-incubated with serial dilutions of the test compounds, and incubated at 37 °C for 1 h. Chicken red blood cells (RBC) were added and, after 10 min incubation at 37 °C, the RBC (with adsorbed virus) were collected by centrifugation. The RBC pellets were resuspended in buffer containing the corresponding compound concentrations, and the pH was lowered to 5.0. After 25 min incubation at 37 °C, the samples were neutralized, and intact chicken RBC were removed by centrifugation. The supernatant was transferred to a 96-well plate, and the optical density was measured at 540 nm. Background values were derived from mock-infected

samples that underwent identical treatment. The inhibitory effect of the compounds was expressed as the IC_{50}, relative to the value observed in the no compound control.[13]

Acknowledgments

This work was supported by Istanbul University Scientific Research Project (Project Number: BYP-2906 and BYP-12603), the Fonds voor Wetenschappelijk Onderzoek Vlaanderen (FWO No. 9.0188.07) and the Geconcerteerde Onderzoeksacties (GOA/10/014). E.V. is a recipient of a PhD grant from the KULeuven. We appreciate the dedicated assistance from Leentje Persoons and Wim van Dam.

References and notes

1. Shih, S. R.; Chu, T. Y.; Reddy, G. R.; Tseng, S. N.; Chen, H. L.; Tang, W. F.; Wu, M. S.; Yeh, J. Y.; Chao, Y. S.; Hsu, J. T.; Hseieh, H. P.; Horng, J. T. *J. Biomed. Sci.* **2010**, *17*, 1.
2. Zarubaev, V. V.; Golod, E. L.; Anfimov, P. M.; Shtro, A. A.; Saraev, V. V.; Gavrilov, A. S.; Logvinov, A. V.; Kiselev, O. I. *Bioorg. Med. Chem.* **2010**, *18*, 839.
3. Russell, R. J.; Kerry, P. S.; Steinhauer, D. J.; Martin, S. R.; Gamblin, S. J.; Skehel, J. J. *Proc. Natl. Acad. Sci. U.S.A.* **2008**, *105*, 17736.
4. Liu, J.; Obando, D.; Liao, V.; Lifa, T.; Codd, R. *Eur. J. Med. Chem.* **2011**, *46*, 1949.
5. Hassan, G. S.; El. Emam, A. A.; Gad, L. M.; Barghash, A. E. M. *Saudi Pharm. J.* **2010**, *18*, 123.
6. Scholtissek, C.; Quack, G.; Klenk, H. D.; Webster, R. G. *Antiviral Res.* **1998**, *37*, 83.
7. Duque, M. D.; Ma, C.; Torres, E.; Wang, J.; Naesens, L.; Jiminez, J. J.; Camps, P.; Luque, F. J.; DeGrado, W. F.; Lamb, R. A.; Pinto, L. H.; Vázquez, S. *J. Med. Chem.* **2011**, *54*, 2646.
8. De Clercq, E. *Nat. Rev. Drug Disc.* **2006**, *5*, 1015.
9. Stouffer, A. L.; Acharya, R.; Salom, D.; Levine, A. S.; Di Costanzo, L.; Soto, C. S.; Tereshko, V.; Nanda, V.; Stayrook, S.; DeGrado, W. F. *Nature* **2008**, *451*, 596.
10. Cady, S. D.; Schmidt-Rohr, K.; Wang, J.; Soto, C. S.; DeGrado, W. F.; Hong, M. *Nature* **2010**, *463*, 689.
11. Wang, J.; Ma, C.; Fiorin, G.; Carnevale, V.; Wang, T.; Hu, F.; Lamb, R. A.; Pinto, L. H.; Hong, M.; Klein, M. L.; DeGrado, W. F. *J. Am. Chem. Soc.* **2011**, *133*, 12834.
12. Jiang, S.; Li, R.; Du, L.; Liu, S. *Protein Cell.* **2010**, *1*, 342.
13. Vanderlinden, E.; Göktaş, F.; Cesur, Z.; Froeyen, M.; Reed, M. L.; Russell, C. J.; Cesur, N.; Naesens, L. *J. Virol.* **2010**, *84*, 4277.
14. Dandia, A.; Singh, R.; Khaturia, S.; Mérienne, C.; Morgant, G.; Loupy, A. *Bioorg. Med. Chem.* **2006**, *14*, 2409.
15. Clayden, J.; Greeves, N.; Warren, S.; Wothers, P. *Org. Chem.*; Oxford University Press: New York, 2001. pp. 870.