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Original article

Synthesis and anti-HIV studies of 2- and 3-adamantyl-substituted thiazolidin-4-ones

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Abstract

A series of novel thiazolidin-4-ones bearing a lipophilic adamantyl substituent at position 2 or 3 were synthesized. A majority of them showed a modest anti-HIV-1 activity, whereas 2-adamantan-1-yl-3-(4,6-dimethylpyrimidin-2-yl)-thiazolidin-4-one (**8**) was endowed with a remarkable antiviral potency ($EC_{50} = 0.67 \mu\text{M}$). The new series of compounds (**22–29**) with an adamantyl moiety at the 3-position of the thiazolidinone ring showed good to modest anti-HIV-1 activity ($EC_{50} = 1.0–11 \mu\text{M}$) but also pronounced cytostatic activity. For example **24**, **26** and **29** showed an EC_{50} of 1.0–2.0 μM , while the 50% effective concentrations for **23** and **28** were 7.8 and 11.0 μM , respectively. X-ray studies and quantum chemical calculations revealed that the anti-HIV activity of the compounds strongly depends on their dipole moments and conformation of the thiazolidinones.

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1. Introduction

We previously reported on several 2-adamantyl-substituted thiazolidin-4-ones showing modest anti-HIV-1 activity [1]. In the case of racemic (\pm)-2-adamantan-1-yl-3-(4,6-dimethylpyridin-2-yl)-thiazolidin-4-one, a remarkable antiviral potency ($EC_{50} = 0.35 \mu\text{M}$) was observed. The presence of the adamantane moiety played an important role in the eventual antiviral activity of the compound. This compound behaved as a typical non-nucleoside reverse transcriptase (RT) inhibitor (NNRTI) with non-competitive inhibition of RT with respect to the nucleotide substrate ($K_i = 12 \mu\text{M}$; $K_i/K_m = 5.5$). Separation of the enantiomers via diastereoisomeric salts was performed for

this thiazolidinone. X-ray studies enabled us to ascribe the *R* configuration to (+)-2-adamantan-1-yl-3-(4,6-dimethylpyridin-2-yl)-thiazolidin-4-one. Furthermore, it was found that the (+)-isomer shown in Fig. 1, was predominantly responsible for the potent anti-HIV-1 activity (EC_{50} value of 0.178 μM), while the levo isomer was more than 60-fold less active. These findings confirm the general rule, that only one defined NNRTI enantiomer inhibits RT while the other one is usually markedly less potent or even inactive [2,3].

Moreover we found that all thiazolidinones investigated showing antiviral activity have similar pharmacophores i.e. an amide nitrogen atoms connected via one, two or three carbon chains to nitrogen atoms in the substituted amines or pyridines.

Prompted by the above observations, we decided to extend this group of novel non-nucleoside reverse transcriptase inhibitors bearing a bulky lipophilic adamantyl entity. We designed and synthesized three series of thiazolidinone derivatives.

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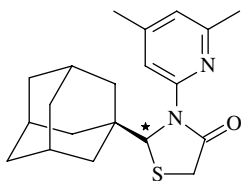


Fig. 1. 2-Adamantan-1-yl-3-(4,6-dimethylpyridin-2-yl)-thiazolidin-4-one (7).

Compounds **8–11** represent additional 2-adamant-1-yl-substituted thiazolidin-4-ones not presented previously. Compounds **12–21** are 2-adamant-1-yl-methylthiazolidin-4-ones. The thiazolidinones **22–29** have fluorine or chlorine substituted phenyl ring at the 2-position instead of the adamantane moiety and consist of the adamantyl group connected via methylene or ethylene bridges at the nitrogen atom of the thiazolidinone ring. It should be mentioned that such haloaromatic substituents were present in the most active thiazolidinones described by Monforte et al. [4–8]. Many of these thiazolidinones had the pyridyl or pyrimidyl groups as *N*-substituents [7,8]. Results obtained by these authors showed that compounds with a pyrimidine nucleus were mostly 2–3 times less active against HIV-1. The most active thiazolidinones contained MeO or dihalo substituents attached to the aromatic ring and a methyl group at the pyridyl nucleus.

2. Results and discussion

2.1. Chemistry

The compounds 2-adamantyl-substituted thiazolidin-4-ones **8–11** and 2-adamantylmethyl thiazolidin-4-ones **12–21** were prepared according to the scheme shown in Fig. 2 by a one-pot two-step methodology described previously [1]. The starting reagents for the synthesis of the title thiazolidinones were adamantane-1-carbaldehyde (**1**) and adamantan-1-yl-acetaldehyde (**2**), respectively. These aldehydes were prepared by Swern oxidation from adamantanemethanol and 2-adamantaneethanol [9]. The general pathway of the 2-adamantylthiazolidin-4-one syntheses is presented in the scheme given in Fig. 2.

Compounds **22–29** were obtained from commercially available 2,6-dihalobenzaldehydes as well as 3,4,5-trimethoxybenzaldehyde. As amine components 1-aminomethyladamantane (**5**) and 2-aminoethyladamantane (**6**) were used. These reagents were synthesized from the respective adamantane alcohols by the Mitsunobu condensation with phthalimide [10] and consecutive hydrolysis of imides by the Gabriel

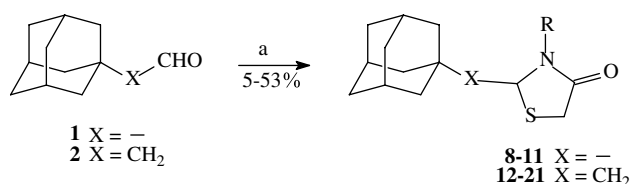


Fig. 2. Synthesis of adamantylthiazolidin-4-ones **8–21**: (a) $R-NH_2$, HSC_2COOH , toluene, reflux.

method. The general pathway of obtaining 3-adamantylmethyl and 3-adamantylethylthiazolidin-4-ones is presented in Fig. 3.

The structural formulas of the obtained compounds are given in Table 1.

Structures and purity of novel compounds were confirmed by elemental analysis, NMR and FTIR spectroscopy, as well as by chromatographic methods. In some cases we performed X-ray studies for structure and conformation determination of the obtained thiazolidinones.

2.2. Biological testing

Compounds **8–29** were evaluated for their inhibitory activity against HIV-1(III_B) and HIV-2(ROD)-induced cytopathicity in CEM cell cultures. The results are displayed in Table 2. The parent pyridine leading derivative **7**, the non-nucleoside RT inhibitor nevirapine and the nucleoside RT inhibitor ddI (didanosine) were included as reference compounds (Table 2).

Compounds **8–11** belong to the same group as the most active agent described in our previous publication, i.e. 2-adamantan-1-yl-3-(4,6-dimethylpyridin-2-yl)-thiazolidin-4-one ($EC_{50} = 0.35 \mu M$) (**7**) [1]. The structural difference between compounds **8–11** and compound **7** consists only in the replacement of a pyridin-2-yl by a pyrimidin-2-yl or pyrimidin-4-yl nucleus in **11**, as the *N*-substituent. The thiazolidinone **8** ($EC_{50} = 0.67 \mu M$), bearing a 4,6-dimethylpyrimidinyl group, is only ~two times less potent than the parent reference compound **7**. Its antiviral potency and selectivity was close to that of the clinically-used compound nevirapine. The replacement of the 4,6-dimethylpyrimidinyl by the 4-methylpyrimidinyl in **10** ($EC_{50} = 4.50 \mu M$) results in a 6-fold reduction in antiviral potency. A similar tendency was observed in our previous work on thiazolidinones containing 4,6-dimethylpyridinyl- and 4-methylpyridinyl-rings. Thus, 4,6-dimethyl-substituents connected to a heterocycle seem to be crucial for the activity. It was also striking to notice that minor modifications such as the inclusion of a methyl and/or chloro group at the pyrimidine ring may have a dramatic effect on the eventual antiviral activity of compounds (Table 2).

The second group of compounds studied belonged to the thiazolidinones **12–21**. In contrast to **8–11**, these compounds contain an adamantylmethylene instead of an adamantyl substituent connected to the heterocyclic ring at the 2-position. This little modification of structure invariably causes reduction of antiviral activity ($EC_{50} = 8.6–53 \mu M$). For example, when a methylene bridge was inserted between the 1-adamantyl and the heterocyclic ring in **8** giving compound **12** ($EC_{50} = 8.60 \mu M$), a 13-fold reduction of potency was observed. This tendency is also evident when comparing, for instance, **10** ($EC_{50} = 4.50 \mu M$) and **17** or **18** (completely inactive), with their respective anti-HIV-1 agents reported previously [1]. These data point to crucial and rather fixed interaction points of adamantane and other parts of the inhibitory molecule with the HIV-1 RT enzyme, a property which is often observed for many other non-nucleoside reverse transcriptase inhibitors. It is interesting that compounds **8** and **12** having a pyrimidin-2-yl ring at the N-3 position showed activity

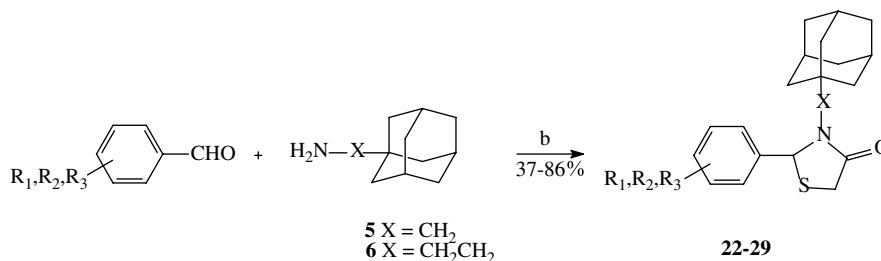


Fig. 3. Synthesis of adamantyl-substituted thiazolidin-4-ones **22–29**: (b) HSCH₂COOH, toluene, reflux.

against HIV-1 whereas **11** and **21** with a pyrimidin-4-yl substituent lost their antiviral properties.

The third series of compounds (**22–29**) is structurally entirely new. These compounds showed good to modest anti-HIV-1 activity ($\text{EC}_{50} = 1.0\text{--}11 \mu\text{M}$) but also pronounced cytostatic activity. For example **24**, **26** and **29** showed EC_{50} 's of $1.0\text{--}2.0 \mu\text{M}$, while the 50%-effective concentration for **23** and **28** were 7.8 and $11.0 \mu\text{M}$, respectively. In this series the effect of the alkylene bridge length is not unambiguous, since the ethyl results in a more pronounced anti-HIV-1 activity than methyl in compounds **23** and **29** (compare with **22** and **28**), whereas the presence of a methyl resulted in more active agents than ethyl in compounds **25** and **27** (compare with **24** and **26**). It should also be noticed that these thiazolidinones exhibit a certain antiviral activity against HIV-2 ($\text{EC}_{50} = 6.5\text{--}46 \mu\text{M}$), but it is currently unclear whether this activity reflects a specific antiviral effect rather than (and most likely) a toxic effect of the compounds. It is indeed evident from the data in Table 2 that the antiviral activities against HIV-2 were at compound concentrations that are close to the cytostatic concentrations (CC_{50}) obtained in mock-infected cell cultures.

Four 3-adamantyl-substituted thiazolidin-4-ones from this series, as well as *R* enantiomer of **7**, were also evaluated for their inhibitory activity against recombinant HIV-1 and HIV-2 RT. They showed pronounced inhibition of the HIV-1 RT-catalysed reaction but no appreciable activity against HIV-2 RT, pointing to the selectivity of the compounds that seem to behave as NNRTIs. The IC_{50} for each test compound are presented in Table 3. Moreover, there was fair correlation between the $\log \text{IC}_{50}$ HIV-1 RT and the $\log \text{EC}_{50}$ HIV-1 ($r = 0.89$).

It would have been obvious to synthesize also 1-adamantyl derivatives without a methylene group between the nitrogen atom and the thiazolidine ring (see Fig. 3). Unfortunately, 1-aminoadamantane does not react at all with aromatic aldehydes. An amino-group attached via a tertiary carbon atom to an adamantyl skeleton is completely inactive in condensation with aromatic aldehydes, so obtaining of expected compounds was impossible.

All compounds were also evaluated against a broad variety of DNA and RNA viruses (i.e. herpes simplex virus type 1 and type 2, vaccinia virus and vesicular stomatitis virus (VSV) in HEL cell cultures; parainfluenza-3 virus, reovirus-1, Sindbis virus, Cocksackie virus B4 and Punta Toro virus in Vero cell cultures; VSV, respiratory syncytial virus and Cocksackie virus B4 in HeLa cell cultures; feline corona virus (FIPV) in Crandel feline

kidney cells, and influenza virus A (H1N1, H3N2) and B in MDCK cell cultures). None of the compounds showed appreciable activity at subtoxic concentrations. Since adamantane and heterocyclic rimantadine analogues recently obtained display the anti-influenza virus activity, the inactivity of the adamantyl derivatives against this virus is most likely due to the bulky moieties that are linked to the adamantane [11,12].

2.3. X-ray studies

X-ray measurements are only possible for high-quality monocrystals with proper dimensions. Only few thiazolidinones from the three compound series could form suitable crystals. The majority of the compounds obtained are fine-crystalline, amorphous or liquid. So, for a better understanding of the differences between the antiviral activities of the studied compounds, we performed both X-ray structural studies and quantum chemical calculations only for a number of selected compounds (i.e. **8**, **12**, **18**, **21** and **26** (for **8** only calculations)). The molecular geometry of **26** is shown in Fig. 4. The plots revealed the “butterfly-like” conformation typical for most NNRTIs [13,14]. The geometries of the remaining three structures (**12**, **18**, **21**) are visualized in Fig. 5 together with the results of potential density calculations.

2.4. Quantum chemical calculations

Quantum chemical calculations were performed using the Spartan'04 program. Single point RHF calculations using 6-31G** basis set were performed for molecular structures taken from crystallographic studies. The results served as a basis for potential density and dipole moment calculations for the given structures. In Table 4 volumes, surfaces as well as dipole moments calculated for thiazolidinones **8**, **12**, **18**, **21**, **26** are presented. For comparison, calculations for 2-adamantan-1-yl-3-(4,6-dimethylpyridin-2-yl)-thiazolidin-4-one (**7**) were also performed.

The results are shown graphically in Fig. 5. The molecules **12**, **18** and **21** are topologically non-distinguishable, what can be seen from values of both volumes and surfaces of the molecules. Slightly smaller is molecule **7** (see Table 4). In our opinion the dipole moment might be treated as a global measure of electrical properties of molecules. From Fig. 5 one can see that the four molecules have distinct electro-negative regions (shown in red to yellow) and electro-positive ones (shown in blue to green). The molecules in the upper row of

Table 1
Chemical structures of thiazolidinones

General Structure of 8-11			
Compound	R	Compound	R
8		10	
9		11	
General Structure of 12-21			
Compound	R	Compound	R
12		17	
13		18	
14		19	
15		20	

Table 1 (continued)

Compound	R	Compound	R	
16		21		
General Structure of 22-29				
Compound	X	R ₁	R ₂	R ₃
22	CH ₂	3-OCH ₃	4-OCH ₃	5-OCH ₃
23	CH ₂ CH ₂	3-OCH ₃	4-OCH ₃	5-OCH ₃
24	CH ₂	2-Cl	6-Cl	
25	CH ₂ CH ₂	2-Cl	6-Cl	
26	CH ₂	2-Cl	6-F	
27	CH ₂ CH ₂	2-Cl	6-F	
28	CH ₂	2-F	6-F	
29	CH ₂ CH ₂	2-F	6-F	

Fig. 5 are endowed with higher dipole moments with the higher electrostatic potential localized in the groove region between the carbonyl oxygen and the aromatic nitrogen atoms. The major difference between molecules in the upper and those in the bottom row of Fig. 5 is that the latter have hydrogen atoms of the pyridine/pyrimidine ring in the close vicinity of the carbonyl oxygen of the thiazolidinone whereas 7 and 12 have on these sites nitrogen atoms and hence a more negative potential. One can also see that the thiazolidinone fragment of both molecules is more co-planar with the aromatic ring than it is for the upper-row molecules. These facts might correlate with the anti-HIV activity of compounds listed in Table 4. Compound 26 belongs to a completely different class of molecules but from the topological point of view it is very similar to the others and it has a very similar volume and surface. This molecule has the dipole moment similar to 7 and 12. The crystal structure of 8 hasn't been studied. However, because of the similar activity to the previously studied molecule 7 [1], it was essential to compare values such as the dipole moment and the electrostatic potential distribution for this molecule. We therefore performed simple quantum-mechanical calculations to evaluate these values. As can be derived from Table 4 and Fig. 4 compounds having higher dipole moments and twisted aromatic rings in relation to the heterocyclic one showed anti-HIV activity whereas the remaining thiazolidinones are completely inactive.

3. Experimental section

3.1. Chemistry

All commercial reagents and solvents were used without further purification. Reactions were monitored by GC and

Table 2
Anti-HIV-1 and anti-HIV-2 activity and cytostatic properties of the compounds in human T-lymphocyte (CEM) cells

Compound	EC ₅₀ (μM) ^a		CC ₅₀ (μM) ^b	SI ^c
	HIV-1(III _B)	HIV-2(ROD)		
8	0.67 ± 0.49	≥58	122 ± 5.2	182
9	≥54.9	120 ± 21.0	>275	<5
10	4.5 ± 0.6	>60.7	136 ± 4.2	30
11	≥58.2	≥58.2	94 ± 10.1	<1.6
12	8.6 ± 3.6	30 ± 11	27 ± 2.8	3
13	>53	≥53	102 ± 4.0	<1.9
14	≥11.6	23.5 ± 4.0	27.3 ± 1.8	<2.3
15	48.7 ± 14	≥57	77 ± 4.0	1.6
16	18.1 ± 0.2	21.9 ± 2.8	29.3 ± 3.8	1.6
17	17.8 ± 0	21.9 ± 5.8	35 ± 2.0	1.9
18	>54	≥54	115 ± 1.9	<2
19	53.0 ± 15.2	≥62	77 ± 2.2	<1.4
20	30.8 ± 5.7	46 ± 20.3	43 ± 20.1	<1.4
21	≥11.2	≥11.2	22.1 ± 3.1	<1.9
22	≥10.9	≥10.9	18 ± 1.0	<1.6
23	7.8 ± 2.1	6.5 ± 3.9	11.3 ± 0.97	1.4
24	1.0 ± 0.6	≥10.1	21.1 ± 2.3	21
25	3.4 ± 1.2	≥9.7	19.0 ± 1.9	5.5
26	2.0 ± 1.6	≥10.5	20.2 ± 1.3	10
27	3.8 ± 0.8	≥10.1	18.5 ± 3.6	4.7
28	11.0 ± 2.7	35.7 ± 4.4	>275	>25
29	2.0 ± 0.36	≥4	7.9 ± 0.28	4
7^d	0.35 ± 0.175	>12	42 ± 8.1	120
Nevirapine	0.12 ± 0.11	>50	>50	417
ddl ^e	4.6 ± 2.6	–	>250	>54

^a EC₅₀: effective concentration or concentration required to protect CEM cells against the cytopathogenicity of HIV by 50%.

^b CC₅₀: cytotoxic concentration or concentration required to reduce mock-infected CEM cell viability by 50%.

^c SI: selectivity index; ratio CC₅₀/EC₅₀.

^d Data taken from Ref. [1]. Results of **7** represent the biological activity noticed for the racemic mixture.

^e Didanosine.

TLC. Analytical thin-layer chromatography was performed on Kieselgel 60 F₂₅₄ plates (Merck); the spots were located by UV (254 nm) or iodine. Preparative flash-column chromatography was performed using silica gel (Merck) 230–400 mesh. FTIR spectra were recorded on a Perkin–Elmer 2000 spectrometer in dichloromethane. Melting points (uncorrected) were measured in open capillary tubes on a Gallenkamp-5 apparatus. ¹H NMR spectra were recorded on a Varian Mercury (400 MHz) spectrometer in CDCl₃ and

Table 3
Anti-HIV-1 and anti-HIV-2 RT activity using polyrC.dG as the template/primer and [³H]dGTP as the radiolabeled substrate

Compound	IC ₅₀ ^a (μM)	
	HIV-1 RT	HIV-2 RT
7 (<i>R</i> enantiomer)	13.4 ± 13.6	>600
24	42.9 ± 3.2	≥600
25	226 ± 58	>600
28	495 ± 77	>600
29	31.7 ± 17.7	≥600

^a Fifty percent inhibitory concentration, or compound concentration required to inhibit the HIV RT reaction by 50%.

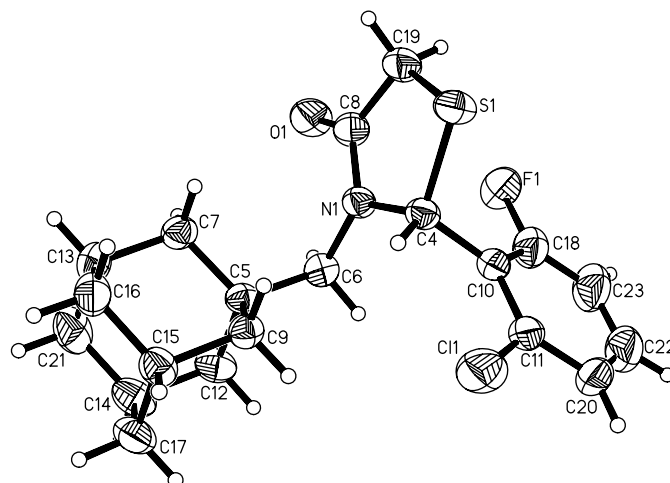


Fig. 4. “Butterfly-like” conformation of the **26** molecule and the numbering scheme. The non-H atoms are shown as 30% probability ellipsoids.

DMSO-*d*₆, chemical shifts (δ) were expressed in ppm relative to tetramethylsilane used as an internal standard, *J* values are in hertz, and the splitting patterns were designed as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet.

Elemental analysis measured on a CHN/S Perkin–Elmer 2400 element analyzer, were within $\pm 0.4\%$ of the theoretical values.

X-ray measurements were performed on an Oxford Diffraction Xcalibur κ -axis diffractometer with a Ruby ccd-detector. The Cu K α characteristic radiation was used to collect the data.

3.2. Adamantane-1-carbaldehyde (**1**)

Adamantane-1-carbaldehyde (**1**) was obtained in the same way as it was described in our previous publication [1].

3.3. Adamantan-1-yl-acetaldehyde (**2**)

This compound was synthesized according to the method published for aldehyde **1**. The crude product of the Swern reaction was purified by flash-column chromatography using CHCl₃ as an eluent. Colourless oil (80%) [15]. FTIR (CH₂Cl₂) (cm⁻¹): 1716, 2851, 2908.

3.4. 2-Adamantan-1-yl-methylphthalimide (**3**) and 2-(2-adamantan-1-yl-ethyl)phthalimide (**4**)

Adamantanemethanol (3.33 g, 20 mmol) or adamantaneethanol (3.60 g, 20 mmol) were dissolved in 100 mL of dry THF. Next, phthalimide (2.95 g, 20 mmol), triphenylphosphine (5.25 g, 20 mmol) and diethyl azodicarboxylate (3.48 g, 20 mmol) were added to the solution. The mixtures were stirred for 18 h at room temperature. Then THF was carefully evaporated and residues were crystallized from methanol. White crystals of imide **3** (4.5 g, 76%); mp 137 °C; ¹H NMR (CDCl₃) δ (ppm): 7.84 (m, 4H), 3.23 (s, 2H), 1.86 (s, 3H), 1.51–1.59 (m, 12H); FTIR (CH₂Cl₂) (cm⁻¹): 1353, 1395, 1710, 1771, 2850, 2908. White crystals

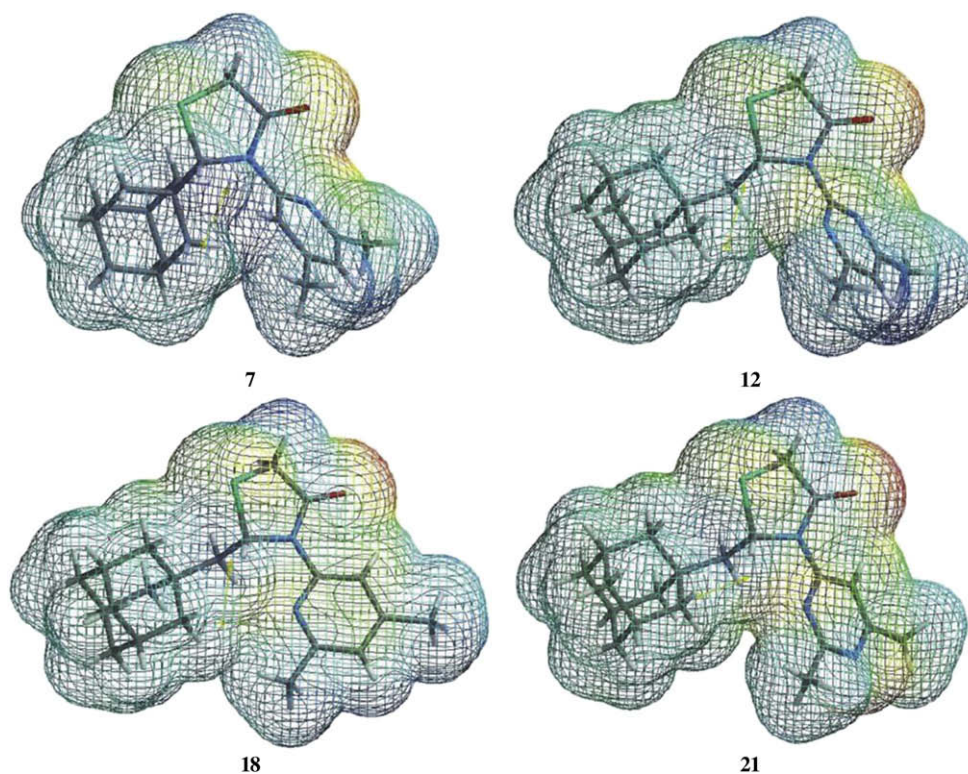


Fig. 5. The results of potential density calculations. The values of the electrostatic potential coded in colours are displayed on the “water accessible” surfaces of the molecules. The geometry of the molecules shown in the drawing was taken from the X-ray structural studies. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of imide **4** (4.4 g, 70%); mp 155 °C; ^1H NMR (CDCl_3) δ (ppm): 7.80 (m, 4H), 3.23 (t, $J = 7.6$ Hz, 2H), 2.40 (t, $J = 7.6$ Hz, 2H), 1.85 (s, 3H), 1.48–1.59 (m, 12H); FTIR (CH_2Cl_2) (cm^{-1}): 1350, 1395, 1712, 1770, 2852, 2908.

3.5. Adamantan-1-ylmethylamine (**5**) and 2-adamantan-1-yl-ethylamine (**6**) hydrochlorides

2-Adamantan-1-yl-methylphthalimide (4.1 g, 14 mmol) or 2-(2-adamantan-1-yl-ethyl)phthalimide (4.3 g, 14 mmol) were heated for 1 h in 40 mL of dry ethanol with hydrazine hydrate (0.7 g, 14 mmol). After that 20 mL of 10% HCl was added and the mixture was refluxed for 30 min. Then white deposits were filtered off and filtrates were concentrated and filtered again. These solutions were extracted with three portions of diethyl ether (3×50 mL). The organic layers were dried over MgSO_4 and ether was evaporated. Next, to colourless

oils HCl in methanol (15 mL) was added and the solvents were evaporated. Crude products were crystallized from dry methanol. White crystals of adamantan-1-yl-methylamine hydrochloride (1.5 g, 50%); mp 325 °C [16] and white crystals of 2-adamantan-1-yl-ethylamine hydrochloride (1.9 g, 65%); mp >330 °C [16] were obtained, respectively.

3.6. General procedure of preparation of adamantyl-substituted thiazolidin-4-ones (**8–29**)

All thiazolidin-4-ones were synthesized using the same method described below. A mixture of appropriate aldehyde (2 mmol) and respective amine (2 mmol) was refluxed for 0.5–2.5 h in toluene (10 mL) with simultaneous azeotropic removal of water. Then mercaptoacetic acid (0.28 mL, 4 mmol) was added and the mixture was refluxed further for 6–28 h. After cooling, the solution was washed two times with a saturated solution of NaHCO_3 and brine. The mixture was dried over magnesium sulphate and the solvent was evaporated. The residue (oil or solid) was purified by flash-column chromatography using chloroform or chloroform:methanol mixture as eluent. For X-ray studies single crystals were obtained by recrystallization of respective thiazolidinones from heptane.

3.6.1. 2-Adamantan-1-yl-3-(4,6-dimethylpyrimidin-2-yl)-thiazolidin-4-one (**8**)

The reaction was performed for over 22 h. White crystals were obtained (350 mg, 51%); mp 205 °C; ^1H NMR

Table 4
Geometrical and electrical properties of chosen compounds

Molecule	Volume (\AA^3)	Surface (\AA^2)	Dipole moment (D)	EC_{50} (μM) ^a
7	335.59	334.22	4.38	0.35
8	—	—	3.79	0.67
12	347.86	353.01	4.87	8.6
18	354.22	354.99	2.87	na ^b
21	348.65	351.59	2.65	na
25	343.48	339.00	4.31	2.0

^a EC_{50} : effective concentration or concentration required to protect CEM cells against the cytopathogenicity of HIV by 50%.

^b na: no activity.

(CDCl₃): 6.88 (s, 1H), 5.97 (s, 1H), 3.80 (d, *J* = 15.8 Hz, 1H), 3.48 (d, *J* = 15.8 Hz, 1H), 2.43 (s, 3H), 2.32 (s, 3H), 1.92–1.44 (m, 15H); Anal. (C₁₉H₂₅N₃OS) C, H, N.

3.6.2. 2-Adamantan-1-yl-3-(4-chloro-6-methylpyrimidin-2-yl)-thiazolidin-4-one (**9**)

The reaction was performed for over 28 h. White crystals were obtained (50 mg, 5.5%); mp 192 °C; ¹H NMR (CDCl₃): 6.98 (s, 1H), 5.99 (s, 1H), 3.83 (d, *J* = 15.4 Hz, 1H), 3.48 (d, *J* = 15.4 Hz, 1H), 2.32 (s, 3H), 1.94–1.45 (m, 15H); Anal. (C₁₈H₂₂ClN₃OS) C, H, N.

3.6.3. 2-Adamantan-1-yl-3-(4-methylpyrimidin-2-yl)-thiazolidin-4-one (**10**)

The reaction was performed for over 20 h. White crystals were obtained (140 mg, 15%); mp 163 °C; ¹H NMR (CDCl₃): 8.61 (m, 1H), 7.37 (dd, *J* = 6.0 Hz, *J* = 2.0 Hz, 1H), 5.45 (d, *J* = 0.9 Hz, 1H), 3.82 (dd, *J* = 16.0 Hz, *J* = 0.9 Hz, 1H), 3.50 (d, *J* = 16.0 Hz, 1H), 2.48 (s, 3H), 1.88–1.39 (m, 15H); Anal. (C₁₈H₂₃N₃OS) C, H, N.

3.6.4. 2-Adamantan-1-yl-3-(2,6-dimethylpyrimidin-4-yl)-thiazolidin-4-one (**11**)

The reaction was performed for over 22 h. White crystals were obtained (200 mg, 34%); mp 176 °C; ¹H NMR (CDCl₃): 6.99 (s, 1H), 5.97 (s, 1H), 3.80 (d, *J* = 15.8 Hz, 1H), 3.48 (d, *J* = 15.8 Hz, 1H), 2.45 (s, 3H), 2.37 (s, 3H), 1.90–1.40 (m, 15H); Anal. (C₁₉H₂₅N₃OS) C, H, N.

3.6.5. 2-Adamantan-1-ylmethyl-3-(2,6-methylpyrimidin-2-yl)-thiazolidin-4-one (**12**)

The reaction was performed for over 22 h. White crystals were obtained (220 mg, 31%); mp 160 °C; ¹H NMR (CDCl₃): 7.33 (s, 1H), 5.90 (s, 1H), 3.93 (d, *J* = 15.8 Hz, 1H), 3.66 (d, *J* = 15.8 Hz, 1H), 2.51 (s, 3H), 2.37 (s, 3H), 1.93–1.41 (m, 17H); Anal. (C₂₀H₂₇N₃OS) C, H, N.

3.6.6. 2-Adamantan-1-ylmethyl-3-(4-chloro-6-methylpyrimidin-2-yl)-thiazolidin-4-one (**13**)

The reaction was performed for over 28 h. White crystals were obtained (80 mg, 10.5%); mp 168 °C; ¹H NMR (CDCl₃): 8.03 (s, 1H), 5.90 (s, 1H), 3.93 (m, 1H), 3.62 (m, 1H), 2.44 (s, 3H), 1.93–1.41 (m, 17H); Anal. (C₁₉H₂₄ClN₃OS) C, H, N.

3.6.7. 2-Adamantan-1-ylmethyl-3-(4-methylpyrimidin-2-yl)-thiazolidin-4-one (**14**)

The reaction was performed for over 28 h. White crystals were obtained (70 mg, 10%); mp 103 °C; ¹H NMR (CDCl₃): 8.60 (s, 1H), 7.31 (s, 1H), 5.92 (s, 1H), 3.90 (m, Hz, 1H), 3.60 (m, 1H), 2.44 (s, 3H), 1.93–1.41 (m, 17H); Anal. (C₁₉H₂₅N₃OS) C, H, N.

3.6.8. 2-Adamantan-1-ylmethyl-3-(2-pyrrolidin-1-ylethyl)-thiazolidin-4-one (**15**)

The reaction was performed for over 12 h. Colourless oil was obtained (180 mg, 26%); ¹H NMR (CDCl₃): 4.41 (s,

1H), 3.82 (m, 1H), 3.46 (m, 1H), 3.25 (s, 1H), 3.15 (m, 1H), 2.65 (m, 1H), 2.48 (m, 4H), 1.95–1.55 (m, 22H); Anal. (C₂₀H₃₂N₂OS) C, H, N.

3.6.9. 2-Adamantan-1-ylmethyl-3-(4-methylpyridin-2-yl)-thiazolidin-4-one (**16**)

The reaction was performed for over 24 h. White crystals were obtained (344 mg, 50%); mp 95 °C; ¹H NMR (CDCl₃): 8.28 (s, 1H), 7.77 (s, 1H), 7.02 (s, 1H), 5.92 (m, 1H), 3.94 (m, Hz, 1H), 3.60 (m, 1H), 2.30 (s, 3H), 1.93–1.40 (m, 17H); Anal. (C₂₀H₂₆N₂OS) C, H, N.

3.6.10. 2-Adamantan-1-ylmethyl-3-(6-methylpyridin-2-yl)-thiazolidin-4-one (**17**)

The reaction was performed for over 24 h. White crystals were obtained (300 mg, 44%); mp 116 °C; ¹H NMR (CDCl₃): 8.20 (s, 1H), 7.37 (s, 1H), 7.00 (s, 1H), 5.91 (m, 1H), 3.94 (m, Hz, 1H), 3.60 (m, 1H), 2.30 (s, 3H), 1.93–1.33 (m, 17H); Anal. (C₂₀H₂₆N₂OS) C, H, N.

3.6.11. 2-Adamantan-1-ylmethyl-3-(4,6-dimethylpyridin-2-yl)-thiazolidin-4-one (**18**)

The reaction was performed for over 24 h. White crystals were obtained (378 mg, 53%); mp 159 °C; ¹H NMR (CDCl₃): 7.64 (s, 1H), 6.92 (s, 1H), 5.84 (m, 1H), 4.04 (m, 1H), 3.66 (m, Hz, 1H), 2.50 (s, 3H), 2.35 (s, 3H), 1.93–1.33 (m, 17H); Anal. (C₂₁H₂₈N₂OS) C, H, N.

3.6.12. 2-Adamantan-1-ylmethyl-3-(2-dimethylaminoethyl)-thiazolidin-4-one (**19**)

The reaction was performed for over 12 h. Colourless oil was obtained (320 mg, 50%); ¹H NMR (CDCl₃): 4.40 (s, 1H), 3.82 (m, 1H), 3.46 (m, 2H), 3.25 (s, 1H), 3.15 (m, 1H), 2.65 (m, 1H), 2.29 (s, 6H), 1.95–1.55 (m 1.93–1.41 17H); Anal. (C₁₈H₃₀N₂OS) C, H, N.

3.6.13. 2-Adamantan-1-ylmethyl-3-(2-dimethylaminoethyl)-thiazolidin-4-one (**20**)

The reaction was performed for over 12 h. Colourless oil was obtained (300 mg, 47%); ¹H NMR (CDCl₃): 4.22 (m, 1H), 3.82 (m, 1H), 3.46 (m, 1H), 3.25 (s, 1H), 3.15 (m, 1H), 2.65 (m, 1H), 1.95–1.55 (m 1.93–1.41 22H), 1.00 (t, *J* = 7.2 Hz, 6H); Anal. (C₂₀H₃₄N₂OS) C, H, N.

3.6.14. 2-Adamantan-1-ylmethyl-3-(2,6-dimethylpyrimidin-4-yl)-thiazolidin-4-one (**21**)

The reaction was performed for over 22 h. White crystals were obtained (36 mg, 5%); mp 118 °C; ¹H NMR (CDCl₃): 6.99 (s, 1H), 5.97 (s, 1H), 3.80 (d, *J* = 15.8 Hz, 1H), 3.48 (d, *J* = 15.8 Hz, 1H), 2.45 (s, 3H), 2.37 (s, 3H), 1.90–1.40 (m, 17H); Anal. (C₂₀H₂₇N₃OS) C, H, N.

3.6.15. 3-Adamantan-1-ylmethyl-2-(3,4,5-trimethoxyphenyl)-thiazolidin-4-one (**22**)

The reaction was performed for over 6 h. White crystals were obtained (477 mg, 58%); mp 67 °C; ¹H NMR (CDCl₃): 7.11 (s, 2H), 6.30 (m, 1H), 3.74 (s, 2H), 3.71 (s, 9H), 3.33

(m, 1H), 3.21 (m, 1H), 1.92–1.34 (m, 15H); Anal. (C₂₃H₃₁NO₄S) C, H, N.

3.6.16. 3-(2-Adamantan-1-ylethyl)-2-(3,4,5-trimethoxyphenyl)-thiazolidin-4-one (23)

The reaction was performed for over 6 h. White crystals were obtained (677 mg, 78%); mp 63 °C; ¹H NMR (CDCl₃): 7.10 (s, 2H), 6.30 (m, 1H), 3.71 (s, 9H), 3.33 (m, 1H), 3.21 (m, 1H), 3.14 (s, 2H), 1.92–1.34 (m, 17H); Anal. (C₂₄H₃₃NO₄S) C, H, N.

3.6.17. 3-Adamantan-1-ylmethyl-2-(2,6-dichlorophenyl)-thiazolidin-4-one (24)

The reaction was performed for over 6 h. White crystals were obtained (688 mg, 86%); mp 149 °C; ¹H NMR (CDCl₃): 7.51 (m, 3H), 6.68 (m, 1H), 3.33 (m, 1H), 3.81 (m, 1H), 3.30 (m, 2H), 1.92–1.34 (m, 15H); Anal. (C₂₀H₂₃Cl₂NOS) C, H, N.

3.6.18. 3-(2-Adamantan-1-ylethyl)-2-(2,6-dichlorophenyl)-thiazolidin-4-one (25)

The reaction was performed for over 6 h. Amorphous glass was obtained (650 mg, 79%); ¹H NMR (CDCl₃): 7.61 (m, 3H), 6.62 (m, 1H), 3.78 (s, 2H) 3.57 (m, 1H), 2.41 (m, 1H), 1.92–1.05 (m, 17H); Anal. (C₂₁H₂₅Cl₂NOS) C, H, N.

3.6.19. 3-Adamantan-1-ylmethyl-2-(2-chloro-6-fluorophenyl)-thiazolidin-4-one (26)

The reaction was performed for over 6 h. White crystals were obtained (688 mg, 86%); mp 136 °C; ¹H NMR (CDCl₃): 7.80–7.70 (m, 3H), 6.69 (m, 1H), 3.81 (m, 1H), 3.54 (m, 1H), 3.30 (m, 2H), 1.99–1.30 (m, 15H); Anal. (C₂₀H₂₃ClF₂NOS) C, H, N.

3.6.20. 3-(2-Adamantan-1-ylethyl)-2-(2-chloro-6-fluorophenyl)-thiazolidin-4-one (27)

The reaction was performed for over 6 h. Amorphous glass was obtained (550 mg, 69%); ¹H NMR (CDCl₃): 7.79–7.65 (m, 3H), 6.62 (m, 1H), 3.77 (s, 2H) 3.57 (m, 1H), 2.41 (m, 1H), 1.93–1.10 (m, 17H); Anal. (C₂₁H₂₅ClF₂NOS) C, H, N.

3.6.21. 3-Adamantan-1-ylmethyl-2-(2,6-difluorophenyl)-thiazolidin-4-one (28)

The reaction was performed for over 6 h. White crystals were obtained (270 mg, 37%); mp 145 °C; ¹H NMR (CDCl₃): 7.77–7.67 (m, 3H), 6.69 (m, 1H), 3.81 (m, 1H), 3.53 (m, 1H), 3.30 (m, 2H), 1.99–1.32 (m, 15H); Anal. (C₂₀H₂₃F₂NOS) C, H, N.

3.6.22. 3-(2-Adamantan-1-ylethyl)-2-(2,6-difluorophenyl)-thiazolidin-4-one (29)

The reaction was performed for over 6 h. Amorphous glass was obtained (350 mg, 46%); ¹H NMR (CDCl₃): 7.74–7.65 (m, 3H), 6.60 (m, 1H), 3.75 (s, 2H) 3.58 (m, 1H), 2.40 (m, 1H), 1.89–1.10 (m, 17H); Anal. (C₂₁H₂₅F₂NOS) C, H, N.

3.7. X-ray measurements

The data were collected using Cu K α radiation. After mounting and centering the single crystal on the diffractometer reflections were collected up to $2\theta < 140^\circ$. Structures were solved by direct methods from SHELXS-97 program [17] and then refined on F^2 using SHELXL-97 software [18]. The detailed structural data have been deposited with CCDC under the numbers 669067, 669068, 669069 and 669070, respectively.

3.8. Biological methods

3.8.1. Cells and viruses

Human immunodeficiency virus type 1 [HIV-1(III_B)] was obtained from Dr. R.C. Gallo (when at the National Cancer Institute, Bethesda, MD). HIV-2(ROD) was provided by Dr. L. Montagnier (when at the Pasteur Institute, Paris, France).

3.8.2. Activity assay of test compounds against HIV-1 and HIV-2 in cell culture

CEM cells (4×10^4) per millilitre were infected with HIV-1(III_B) or HIV-2(ROD) at ~ 100 CCID₅₀ (50% cell culture infective dose) per millilitre of cell suspension. Then 100 μ l of the infected cell suspension was transferred to microlitre plate wells and mixed with 100 μ l of the appropriate dilutions of the tested compounds. Giant cell formation was recorded microscopically in the HIV-infected cell cultures after 4 days. The 50% effective concentration (EC₅₀) of the tested compounds was defined as the compound concentration required to inhibit virus-induced cytopathicity (CEM) by 50%. The 50% cyto-static concentration (CC₅₀) was defined as the compound concentration required inhibiting mock-infected CEM cell proliferation by 50%.

3.8.3. Anti-reverse transcriptase assays

The source of the reverse transcriptases used was recombinant HIV RT [derived from HIV-1(III_B) or HIV-2(ROD)], constructed and prepared as described before [1]. The RT assays contained a total reaction mixture volume (50 μ l) of 50 mM Tris-HCl (pH 7.8), 5 mM dithiothreitol, 300 mM glutathione, 500 μ M EDTA, 150 mM KCl, 5 mM MgCl₂, 1.25 μ g of bovine serum albumin, labelled substrate [8-³H]dGTP (1 μ Ci) (specific radioactivity 11 Ci/mmol) (2.5 μ M) (Moravek Biochemicals, Brea, CA), a fixed concentration of the template/primer poly(C).oligo(dG)_{12–18} (0.1 mM), 0.06% Triton X-100, 10 μ l of inhibitor at various concentrations, and 1 μ l of the RT preparation. The reaction mixtures were incubated at 37 °C for 30 min, at which time 200 μ l of yeast RNA (150 μ g/ml) and 1 ml of trichloroacetic acid (5% v/v) in saturated Na₄P₂O₇ (0.1 M in 1 M HCl) were added. The solutions were kept on ice for 30 min, after which the acid-insoluble material was washed and analyzed for radioactivity. The IC₅₀ for each test compound was determined as the compound concentration that inhibited HIV RT activity by 50%.

4. Summary

In the presented publication, as well as in our previous paper, we performed the synthesis of two new subclasses of NNRTIs. The first one includes the thiazolidine-4-ones with the adamantyl moiety attached directly, or via methylene or ethylene bridges, to the thiazolidinone ring. Compounds with the adamantyl group at the 3-position of the heterocyclic ring belong to the second subclass of NNRTIs. Compounds **7** and **8** bearing a 4,6-dimethylpyridinyl and 4,6-dimethylpyrimidinyl nucleus showed the highest anti-HIV activities ($EC_{50} = 0.35$ and $0.67 \mu\text{M}$, respectively). The X-ray measurements revealed that these agents exist in a “butterfly conformation” common for many NNRTIs. Quantum chemical calculations revealed also that the anti-HIV activity of the compounds strongly depends on their dipole moments.

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Appendix. Supplementary data

Supplementary data associated with this article can be found in the online version, at [doi:10.1016/j.ejmech.2008.02.039](https://doi.org/10.1016/j.ejmech.2008.02.039).

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