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Original article

Synthesis and anti-HIV studies of 2-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, and versatile substituents on the nitrogen atom of the thiazolidine ring, were synthesized whereas several compounds exhibited a modest anti-HIV-1 activity, (±)-2-adamantan-1-yl-3-(4,6-dimethyl-pyridin-2-yl)-thiazolidin-4-one **22** was endowed with a remarkable antiviral potency ($EC_{50} = 0.35$ μM). 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 against RT with respect to the substrate ($K_i = 12$ μM). Separation of the enantiomers via diastereoisomeric salts was performed for **22**. X-ray studies enabled us to ascribe an *S* configuration to (−)-2-adamantan-1-yl-3-(4,6-dimethyl-pyridin-2-yl)-thiazolidin-4-one (−)-**22**. Furthermore, it was found that the (+)-**22** isomer 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.

Keywords: Thiazolidin-4-ones; Adamantane derivatives; Anti-HIV studies

1. Introduction

Thiazolidin-4-ones have been reported to posses a wide range of biological activities including antibacterial (inhibitors of the bacterial enzyme MurB) [1], antituberculosis [2], antitumor [3], antihistaminic (H_1 antagonist) [4], and anti-inflammatory (COX-1 inhibitors) [5] or anticonvulsant activity [6].

Several 2,3-diaryl-1,3-thiazolidin-4-ones have proved to be particularly effective non-nucleoside HIV reverse transcriptase inhibitors (NNRTIs) [7]. Barrecca et al. [8] have stated that these compounds may be considered as an ‘‘open model’’ of previously described 1*H*,3*H*-thiazolo[3,4-*a*]benzimidazoles (TBZs) [9] because they contain necessary pharmacophoric elements of those HIV-1 NNRTIs, namely a benzene-fused ring, an aryl group at C-1 and the nitrogen atom of the thiazole nucleus. Structure–activity relationship (SAR) studies have shown that the anti-HIV activity strongly depends on the nature of substituents at C-2 and N-3 of the thiazolidinone ring. It has been demonstrated that a high antiviral activity was associated with the presence of a 2,6-dihalo-substituted phenyl ring at C-2 and pyridin-2-yl or pyrimidin-2-yl rings at N-3 [8,10]. Moreover in the case of most potent TBZ i.e. 1-(2,6-difluorophenyl)-1*H*,3*H*-thiazolo[3,4-*a*]benzimidazole shown in Fig. 1, the *R*-(+)-enantiomer was more active than the *S*-(−)-isomer [11].

In 2002, Rao et al. obtained a mixture of diastereoisomers of *trans*- and *cis*-5-methyl-2,3-diaryl-1,3-thizolidin-4-ones from 2,6-dihalobenzaldehyde, heteroaromatic amine and

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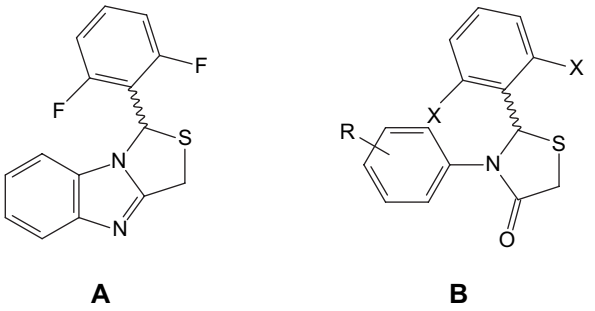


Fig. 1. Structures of TBZ (A) and 2,3-diaryl-1,3-thiazolidin-4-ones (B).

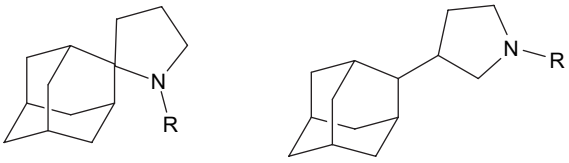


Fig. 2. Structures of antiviral aminoadamantane derivatives.

racemic 2-mercaptopropionic acid [12], but stereochemistry has not influenced the anti-HIV activity of the compounds. Surprisingly, the stereoselectivity of thiazolidin-4-ones has not yet been profoundly studied.

On the other hand, adamantane derivatives have many interesting activity profiles. The most prominent adamantadine (1-aminoadamantane), an effective therapeutic agent against influenza A [13], is also used in Parkinson's disease treatment [14]. Other aminoadamantane derivatives presented in Fig. 2 have been found to exhibit borderline activity against HIV-1 and HIV-2 [15].

In previous papers, TNF-α production-enhancing properties of certain adamantylated heterocycles [16] and significant antimicrobial activity of some adamantyl-substituted imides were reported [17]. The presence of an adamantyl moiety improves the lipophilicity of the molecule due to the aliphatic cage-like structure. Such compounds might be much better taken up by cells and have enhanced blood–brain barrier penetration because of their increased accumulation in lipids [18].

In 1979, Fenech et al. [19] screened three 3-substituted-2-adamantyl-4-thiazolidinones (see Fig. 3) against 12 bacterial strains. The minimal inhibitory concentrations (MIC $\geq$ 125 μg/mL) indicated that these compounds were poor antibacterial agents.

Prompted by the above observations, we designed and synthesized a series of novel thiazolidin-4-one derivatives **2–25** bearing the lipophilic adamantyl substituent at position 2, and several substituents on the nitrogen atom in the thiazolidine ring.

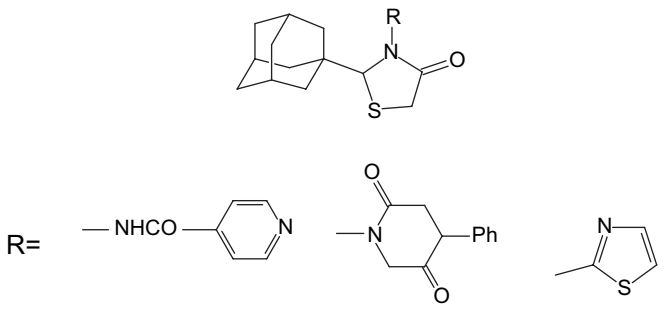


Fig. 3. Structures of 2-adamantan-1-yl-thiazolidin-4-ones.

2. Results and discussion

2.1. Chemistry

The starting reagent for the synthesis of title thiazolidinones was adamantane-1-carbaldehyde **1**. Although, there are many ways to obtain this compound [20,21] we chose the Swern method as the most useful and productive procedure [22]. An oxidation of adamantanemethanol in the presence of oxalyl chloride, DMSO and triethylamine led to the aldehyde **1** in 90% yield. The purity of the product controlled by GC was 99%. The melting point of **1** was in agreement with the literature data (141 °C) [23].

2-Adamantyl-substituted thiazolidin-4-ones **2–22** were prepared according to the scheme (Fig. 4) by an one-pot two-step methodology [24] involving formation of imines of adamantane-1-carbaldehyde **1** and different aliphatic, aromatic and heteroaromatic amines, followed by a condensation of resulting Schiff bases, with mercaptoacetic acid. To estimate the antiviral contribution of the adamantyl moiety, a reference compound, the thiazolidinone derivative **25**, has been also synthesized. This compound has a methyl group instead of the adamantyl moiety. Reactions were conducted in boiling benzene or toluene for 3–30 h using Dean–Stark trap to remove water. The reaction time for each case was established by means of GC method. The structures of the compounds obtained are given in Table 1.

As can be seen, all novel thiazolidinones have chiral C-2 atoms in the heterocyclic ring and were obviously obtained as racemates. For (±)-2-adamantan-1-yl-3-(4,6-dimethylpyridin-2-yl)-thiazolidin-4-one **22**, which showed highest activity in preliminary anti-HIV studies (see Table 2), a separation of both enantiomers via diastereoisomeric salts was performed neutralizing the methanol solution of **22** with

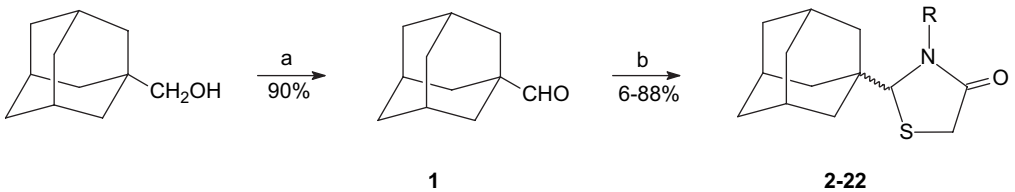
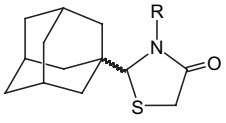
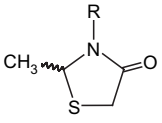


Fig. 4. Synthesis of 2-adamantyl-substituted thiazolidin-4-ones **2–22**. (a) DMSO, $(COCl)_2$, CH_2Cl_2, −65 °C, then Et_3N, rt.; (b) R-NH_2, $HSCH_2COOH$, benzene or toluene, reflux.

Table 1
Chemical structures of thiazolidinones

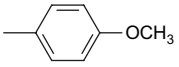
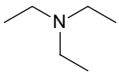
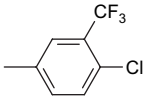
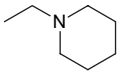
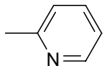
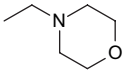
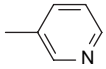
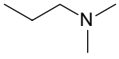
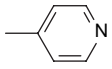
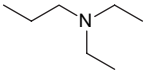
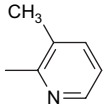
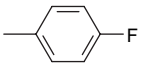
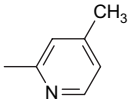
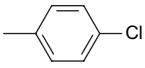
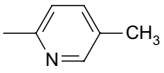
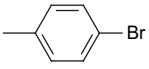
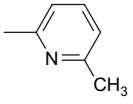
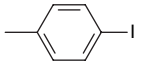
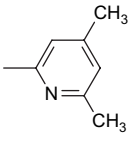
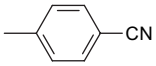
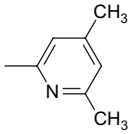
Compound	R	Compound	R
2		**13**	
3		**14**	
4		**15**	
5		**16**	
6		**17**	
7		**18**	
8		**19**	
9		**20**	
10		**21**	
11		**22**	

Table 1 (*continued*)

Compound	R	Compound	R
12		**25**	

(1*R*)-(−)-10-camphorsulfonic acid or (1*S*)-(+)-10-camphorsulfonic acid. In such conditions, only one of the diastereoisomeric salts crystallized while the other one remained in solution. After recrystallization from methanol, in order to obtain free pyridine derivatives, salts were treated with 2 M NaOH. Then specific rotations of the isolated enantiomers were measured. It appeared that (1*S*)-(+)-10-camphorsulfonic acid gave crystals of **23** with the sinistral enantiomer while the salt of the (+)-isomer remained in methanolic solution. On the contrary, (1*R*)-(−)-10-camphorsulfonic acid formed crystals of **24** with the (+)-enantiomer. The compounds (+)-**22** and (−)-**22** were tested by HPLC using the chiral column. It was found that enantiomeric excess was very high for both compounds; 96.8% for (−)-**22** and over 99% for (+)-**22**.

The (1*S*)-(+)-10-camphorsulfonic acid salt of (−)-2-adamantan-1-yl-3-(4,6-dimethyl-pyridin-2-yl)-thiazolidin-4-one was obtained as single crystals suitable for X-ray study. The orthorhombic crystals belong to the $P2_12_12$ space group and are formed of the salt components of the geometry shown in Fig. 5. The crystal is assembled of alternate, perpendicular to the *c*-direction, cationic and anionic layers. The voids in the structures found around ½ ½ ½ and 0 0 ½ points are occupied by pairs of rotationally disordered solvent (methanol) molecules which supplement the less voluminous camphorsulfonic acid anions. These pairs are related by the 2-fold symmetry. The cationic and anionic layers are bonded together by a series of strong N−H···O hydrogen bonds between the protonated pyridinium nitrogen and deprotonated sulphonate oxygen atoms. The thiazolidinone fragment is almost flat (the RMS deviation from the best plane for six atoms is of 0.1 Å). The flat pyridine ring is twisted from this plane around the N(3)−C(6) bond by 53.6(2)°. Such orientation of the dimethyl pyridine fragment is a result of repulsion of methyl groups at C(8) and C(10) atoms and the adamantyl moiety bound to the C(2) atom of the thiazolidine ring as well as of the repulsive interaction between the carbonyl oxygen and the aromatic ring. In literature the biological and pharmacological activity of molecules containing the thiazolidine fragment and many crystallographic studies for such systems are reported. From 20 crystal structures deposited in the Cambridge Structural Data base of molecules containing the *N*-substituted thiazolidinone-4 fragment, nine of them have an aromatic ring at the nitrogen atoms and at least a second substituent at the C(2) atom. For these molecules the dihedral angle between two rings lies between 45° and 60° as in the presented structure.

Table 2
Anti-HIV-1 and -HIV-2 activity of racemic thiazolidinones **2**–**22** and **25**

Compound	EC_{50} (μM)[a]		CC_{50} (μM)[b] (CEM)	SI[c]	IC_{50} (μM)[d]	
	HIV-1	HIV-2			HIV-1 RT	
					Poly rC.dG	Poly rA.dT
2	65 ± 0.00	>65	125 ± 4.2	1.9	>648	>648
3	7.7 ± 5.0	>59	56 ± 14	7.2	475 ± 54	481 ± 116
4	>11	>11	44 ± 19	<4	–	–
5	>57	>57.1	140 ± 7.7	<3	–	–
6	31 ± 0.0	>62.0	122 ± 6.2	4.0	≥620	>620
7	28 ± 7.9	>57.0	89 ± 3.9	3.1	>571	>571
8	>302	>301.7	≥302	≤1	–	–
9	>57	>57.5	≥287	≤5	–	–
10	>255	>255	≥255	<1	–	–
11	>228	>45	≥228	≤1	–	–
12	>295	>295	≥295	≤1	–	–
13	>12	>12	74 ± 17	<6	–	–
14	>48	>48	>240	<1	–	–
15	>318	>318	>318	<1	–	–
16	≥32	>63	105 ± 5.7	≤3	≥636	≥636
17	>63	>63	159 ± 32	<2.5	–	–
18	>12	>12	29 ± 2.0	<2.5	–	–
19	5.6 ± 2.8	>304	>304	>54	40 ± 3.0	88 ± 43
20	>12	>12.4	59 ± 40	<4.8		
21	21 ± 4.2	>304	>304	14	40 ± 0	43 ± 3.0
22	0.350 ± 0.175	>11.7	42 ± 8.1	120	29 ± 15	38 ± 2.9
25	>450	>450	>450	>450	≥900	>900
nevirapine	0.12 ± 0.11	>50	>50	417	2.5 ± 0.01	23 ± 11
ddI	4.6 ± 2.6	–	>250	>54	–	–

[a] Effective concentration required to protect CEM cells against the cytopathicity (giant cell formation) of HIV by 50%.
[b] Cytostatic concentration required to reduce CEM cell proliferation by 50%.
[c] Selectivity index; ratio CC_{50}/EC_{50}.
[d] Inhibitory concentration required to inhibit the HIV-1 reverse transcriptase-catalyzed reaction by 50%.

The data presented below led to establish absolute configurations of the enantiomers. As can be seen, for (−)-2-adamantan-1-yl-3-(4,6-dimethyl-pyridin-2-yl)-thiazolidin-4-one an *S* configuration should be attributed.

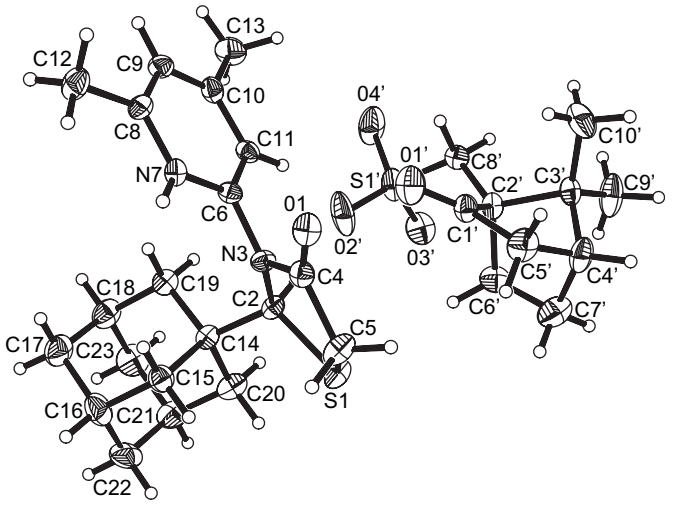


Fig. 5. ORTP plot of (1*S*)-(+)-10-camphorsulfonic acid salt of (*S*)-(−)-2-adamantan-1-yl-3-(4,6-dimethyl-pyridin-2-yl)-thiazolidin-4-one (**23**) (independent part of the unit cell). The non-H atoms are shown as 30% probability ellipsoids.

2.2. Biological testing

Compounds **2**–**22** and **25** were evaluated for activity against HIV-1(III_B) and HIV-2(ROD) in CEM cell cultures. The results are given in Table 2. Nevirapine and ddI were included as reference compounds. None of the compounds were active against HIV-2. Compounds **2**, **3**, **6**, **7**, **16**, **19** and **21** showed modest anti-HIV-1 activity ($EC_{50} = 5.6$–64 μM). In contrast, the thiazolidinone **22** was active in the submicromolar range ($EC_{50} = 0.350$ μM). It should be noted that compound **25** bearing the same *N*-substituent (i.e. 4, 6-dimethyl-2-pyridyl), but lacking the adamantyl moiety, is devoid of antiviral activity.

The compounds that showed anti-HIV-1 activity have also been evaluated on their inhibitory activity of recombinant HIV-1 RT using two different homopolymeric templates. The most antivirally active compound **22** was also most inhibitory to RT. The least active compounds (i.e. **2**, **6**, **7**, **16**) were also hardly inhibitory to the reverse transcriptase. However, the activity range was much smaller for HIV-1 RT than for the virus replication in cell culture (i.e. 10- to 100-fold activity differences against HIV-1 replication between **22** and **21** or **19** corresponded to an activity difference against RT by less than 2- to 3-fold) (IC_{50}: 29 → 40 μM or 38 → 88 μM) (Table 2). This may suggest that, depending on the nature of the compounds, differences in uptake or intracellular metabolism may exist.

Table 3
Anti-HIV-1 and -HIV-2 activities of enantiomers of thiazolidinone **22** in CEM cell cultures

Compound	EC_{50} (μM)[a]		CC_{50} (μM)[b]	SI[c]	IC_{50} (μM)[d]	
	HIV-1	HIV-2			HIV-1 RT	
					Poly rC.dG	Poly rA.dT
(*R*,*S*)-2-Adamantan-1-yl-3-(4,6-dimethyl-pyridin-2-yl)-thiazolidin-4-one **22**	0.350 ± 0.175	>12	42 ± 8.1	120	29 ± 15	38 ± 2.9
(*R*)-(+)-2-Adamantan-1-yl-3-(4,6-dimethyl-pyridin-2-yl)-thiazolidin-4-one (+)-**22**	0.178 ± 0.12	>12	35 ± 12	198	19 ± 11	20 ± 15
(*S*)-(−)-2-Adamantan-1-yl-3-(4,6-dimethyl-pyridin-2-yl)-thiazolidin-4-one (−)-**22**	12	>58	44 ± 11	3.8	>584	>584

[a] Effective concentration required to protect CEM cells against the cytopathicity (giant cell formation) of HIV by 50%.
[b] Cytostatic concentration required to reduce CEM cell proliferation by 50%.
[c] Selectivity index; ratio CC_{50}/EC_{50}.
[d] Inhibitory concentration required to inhibit the HIV-1 reverse transcriptase-catalyzed reaction by 50%.

Interestingly, **25** which represents a derivative of the most active compound **22** in which the adamantyl group has been removed and replaced by a methyl group, completely lost its anti-RT activity and also proved inactive against the virus replication in cell culture ($EC_{50} > 450$ μM). These data strongly illustrate the crucial role the adamantane plays in the eventual antiviral activity of the tested compounds.

The anti-HIV activity evaluation of separated enantiomers has revealed that the (*R*)-(+)-isomer was more active against HIV-1 than the (*S*)-(−)-isomer and 2-fold more active than the racemic mixture (see Table 3). The calculated eudismic ratio (ratio of the activity of eutomer versus distomer) is about 123 ± 83. However, according to the HPLC analysis, the distomer of an enantiomer pair has been contaminated with 1.6% of the eutomer, so the true eudismic ratio (which is critically dependent on the enantiomeric purity of the compounds used) is even higher [25]. A similar biological enantioselectivity has been described previously for *R*-(+)-isomer of TBZ's [11].

The most active adamantyl thiazolidinone (+)-**22** has been further investigated for its kinetic interaction with HIV-1 RT. As also characteristic for NNRTIs in general, compound (+)-**22** showed non-competitive interaction with the enzyme when using poly rC.dG as the template/primer and dGTP as the competing substrate. The K_i was calculated to be 12 μM (Fig. 6). Given its inactivity against HIV-2 and its stereoselectivity (active (+) versus inactive (−) derivative), the adamantyl thiazolidinone(s) belongs to a novel subclass of NNRTIs. The presence of a bulky lipophilic adamantyl entity is unique among the known NNRTIs, and is obligatorily required for antiviral (and anti-RT) activity since replacement of this moiety by a methyl (i.e. **25**) results in a completely inactive molecule. Also, the adamantyl derivatives were found inactive against influenza virus (H_3N_2) in MDCK cell cultures. These observations further add to the HIV-1 selectivity and uniqueness of these derivatives. When mutant HIV-1(III_B) strains, containing different NNRTI-characteristic mutations in the RT, were exposed to the (+)-**22** derivative, its antiviral potential was markedly diminished. The EC_{50} values were 2.3, 1.5, 8.2, >12 and >12 μM for Leu100Ile, Lys103Asn, Tyr181Cys, Tyr181Ile and Tyr188His RT-mutated virus strains. This observation may be due to a decreased flexibility of the adamantyl part of the molecule to adapt to the appearance of the mutated amino acids in the HIV-1 RT.

Structures of the most active compounds against HIV-1 are presented in Fig. 7. As can be seen all of these thiazolidinones have similar pharmacophores. Amide nitrogen atoms are connected by one, or two, or three carbon chains to nitrogen atoms in the substituted amines or pyridines. Our SAR studies should allow further optimization of the lead structures.

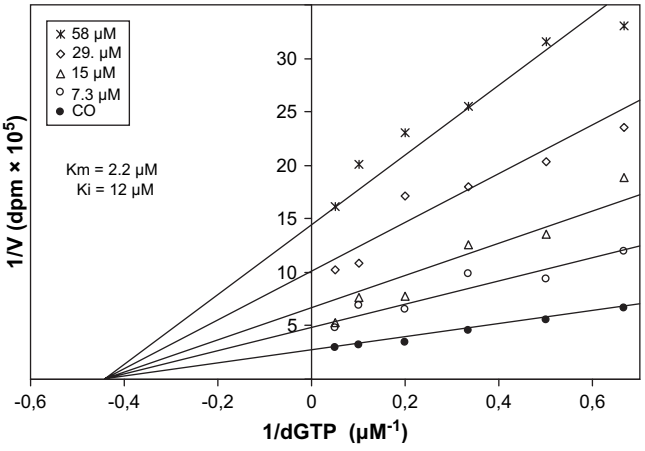


Fig. 6. Lineweaver–Burk diagram for the kinetic interaction of compound (+)-**22** against recombinant HIV-1 RT using poly rC.dG as the template/primer and [^{3}H]dGTP as the radiolabeled substrate.

3. Experimental section

3.1. Chemistry

All commercial reagents and solvents were used without further purification. Reactions were monitored by GC and TLC. Analytical thin layer chromatography was performed on Kieselgel 60 F_{254} 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. Melting points (uncorrected) were measured in open capillary tubes on a Gallenkamp-5 apparatus. ^{1}H NMR spectra were recorded on a Varian Mercury (400 MHz) and ^{13}C NMR on Varian Gemini (200 MHz) spectrometers in $CDCl_3$ and DMSO-d_6, chemical shifts (δ) were expressed in parts per million 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 (Table 4). Optical rotations $[\alpha]_D$ were measured on a JASCO

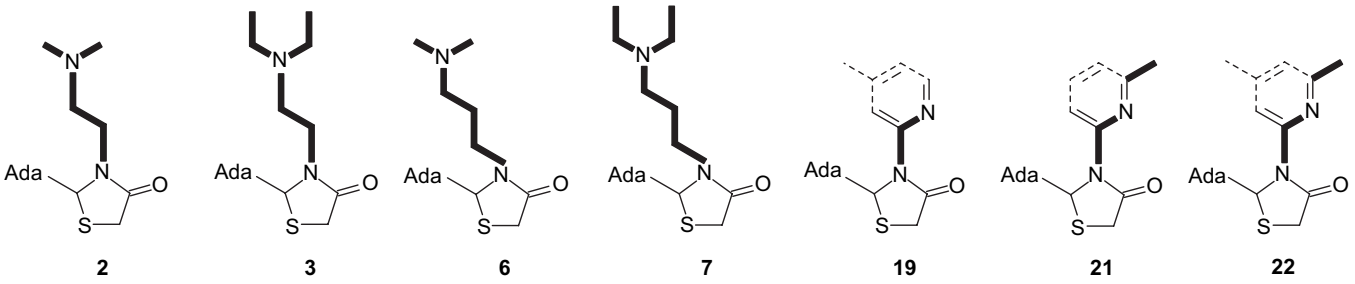


Fig. 7. Structures of anti-HIV-1 thiazolidin-4-ones.

P-1020 polarimeter. Specific rotations are given as deg/dm; the concentration values are reported as g/mL of chloroform and were recorded at 25 °C. Gas chromatography analysis was carried out using an Agilent 6850 Series − GC System, equipped with an HP-50+ (30 m) column. HPLC was performed using a Spectra series P100 apparatus equipped with a Spectra series UV100 detector (peak detection at 282 nm) and a chiral column Chiralcel OD-H with hexane/isopropanol (95/5) as an eluent. Elemental analysis measured on a CHN/S Perkin–Elmer 2400 element analyzer, was within ±0.4% of the theoretical values. X-ray measurements were performed on a Kuma KM-4 κ-axis single crystal X-ray diffractometer.

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

Oxalyl chloride (4.12 mL, 48 mmol) was dissolved in dichloromethane (60 mL) and cooled to −70 °C, using dry ice–acetone bath. Then DMSO (7.40 mL, 104 mmol) in dichloromethane (12 mL) was added dropwise, and stirred for 30 min keeping the temperature below −65 °C. After that, the solution of adamant-1-yl-methanol (6.65 g, 40 mmol) in dichloromethane (60 mL) was added slowly. The resulting mixture was stirred for additional 60 min at −65 °C. Next, triethylamine (27.8 mL, 200 mmol) was added and stirring was continued until reaction reached room temperature. Finally, the mixture was diluted with water (40 mL), stirred for 15 min, the phases were separated and the water layer was extracted with dichloromethane (2 × 40 mL). Combined organic layers were washed with water (5 × 100 mL) and dried over magnesium sulphate. The solvent was evaporated to give a pale yellow solid. All operations should be carried out in a well-ventilated hood, because of production of a malodorous side product dimethyl sulphide. The crude product was crystallized from petroleum ether to provide 5.91 g (90%) of compound **1** as white crystals: mp 142 °C (lit. mp 141 °C) [23].

3.3. General procedure for the preparation of 2-adamantyl substituted thiazolidin-4-ones (*2*–*22*)

A mixture of adamantane-1-carbaldehyde (329 mg, 2 mmol) and the appropriate amine (2 mmol) was refluxed for 0.5–2.5 h in benzene or toluene (10 mL) with simultaneous azeotropic removal of water. Then mercaptoacetic acid (0.28 mL, 4 mmol) was added and the mixture was refluxed for further 2–28 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 solvent was evaporated. The residue (oil or solid) was purified by flash column chromatography using $CHCl_3$/MeOH: 10/1 for (**2**–**7**), $CHCl_3$/MeOH: 50/1 for (**8**–**14**), or $CHCl_3$ for (**15**–**22**) as an eluent.

3.3.1. 2-Adamantan-1-yl-3-(2-dimethylamino-ethyl)-thiazolidin-4-one (*2*)

The reaction was performed in benzene for over 3 h. White crystals (413 mg, 67%); mp 92–93 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{17}H_{28}N_2OS$) C, H, N.

3.3.2. 2-Adamantan-1-yl-3-(2-diethylamino-ethyl)-thiazolidin-4-one (*3*)

The reaction was performed in benzene for over 6 h. White crystals (310 mg, 46%); mp 46–47 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{19}H_{32}N_2OS$) C, H, N.

3.3.3. 2-Adamantan-1-yl-3-(2-piperidin-1-yl-ethyl)-thiazolidin-4-one (*4*)

The reaction was performed in benzene for over 21 h. White crystals (397 mg, 57%); mp 123–125 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{20}H_{32}N_2OS$) C, H, N.

3.3.4. 2-Adamantan-1-yl-3-(2-morpholin-1-yl-ethyl)-thiazolidin-4-one (*5*)

The reaction was performed in benzene for over 21 h. White crystals (568 mg, 81%); mp 139–140 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{19}H_{30}N_2O_2S$) C, H, N.

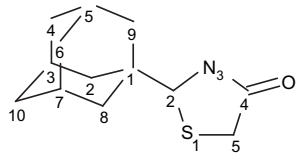
3.3.5. 2-Adamantan-1-yl-3-(3-dimethylamino-propyl)-thiazolidin-4-one (*6*)

The reaction was performed in benzene for over 4.5 h. White crystals (510 mg, 79%); mp 87–88 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{18}H_{30}N_2OS$) C, H, N.

3.3.6. 2-Adamantan-1-yl-3-(3-diethylamino-propyl)-thiazolidin-4-one (*7*)

The reaction was performed in benzene for over 5 h. White crystals (386 mg, 55%); mp 47–48 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{20}H_{34}N_2OS$) C, H, N.

Table 4
^{1}H NMR ($CDCl_3$) and ^{13}C NMR ($CDCl_3$) data of new compounds prepared (**2**−**25**)



Compound	^{1}H NMR δ (ppm)	^{13}C NMR δ (ppm)
2	4.25 (s, 1H), 3.96 (m, 1H), 3.51 (d, $J = 15.6$ Hz, 1H), 3.35 (m, 1H), 3.24 (d, $J = 15.6$ Hz, 1H), 2.73 (m, 1H), 2.59 (m, 1H), 2.38 (s, 6H), 2.00−1.61 (m, 15H)	173.7 (C-4), 72.6 (C-2), 55.1 (NCH_2), 44.8 ($2 \times CH_3$), 44.2 (CH_2N), 41.7 (C-1), 38.3 (C^{Ad}-2,8,9), 36.6 (C^{Ad}-4,6,10), 33.2 (C-5), 27.9 (C^{Ad}-3,5,7)
3	4.32 (d, $J = 1.3$ Hz, 1H), 3.89 (m, 1H), 3.52 (dd, $J = 15.6$, 1.3 Hz, 1H), 3.26 (d, $J = 15.6$ Hz, 1H), 3.21 (m, 1H), 2.71−2.45 (m, 6H), 2.01−1.60 (m, 15H), 1.04 (t, $J = 7.2$ Hz, 6H)	173.6 (C-4), 73.3 (C-2), 50.1 (CH_2N), 47.4 ($2 \times NCH_2$), 45.5 (NCH_2), 41.7 (C-1), 38.5 (C^{Ad}-2,8,9), 36.7 (C^{Ad}-4,6,10), 33.3 (C-5), 28.0 (C^{Ad}-3,5,7), 12.0 ($2 \times CH_3$)
4	4.32 (s, 1H), 3.97 (m, 1H), 3.52 (d, $J = 15.6$ Hz, 1H), 3.26 (d, $J = 15.6$ Hz, 1 + 1H), 2.58−2.29 (m, 6H), 2.01−1.40 (m, 15H + 6H)	173.7 (C-4), 72.8 (C-2), 55.8 (CH_2N), 54.7 ($2 \times NCH_2$), 44.5 (C-1), 41.7 (NCH_2), 38.4 (C^{Ad}-2,8,9), 36.6 (C^{Ad}-4,6,10), 33.4 (C-5), 28.0 (C^{Ad}-3,5,7), 25.9 ($2 \times CH_2$), 24.14 (CH_2)
5	4.27 (s, 1H), 4.00 (m, 1H), 3.68 (m, 6H), 3.53 (dd, $J = 15.6$, 1.2 Hz, 1H), 3.27 (d, $J = 15.6$ Hz, 1H), 2.64−2.49 (m, 5H), 2.02−1.60 (m, 15H)	173.9 (C-4), 72.8 (C-2), 67.0 ($2 \times CH_2O$) 55.4 (CH_2N), 53.7 ($2 \times NCH_2$), 44.1 (C-1), 41.7 (NCH_2), 38.4 (C^{Ad}-2,8,9), 36.6 (C^{Ad}-4,6,10), 33.3 (C-5), 28.00 (C^{Ad}-3,5,7)
6	4.14 (s, 1H), 3.81 (m, 1H), 3.68 (m, 1H), 3.56 (d, $J = 15.7$ Hz, 1H), 3.25 (d, H-11, $J = 15.7$ Hz, 1H), 2.94 (t, $J = 7.3$ Hz, 2H), 2.74 (s, 6H), 2.00−1.60 (m, 15H + 1H) 1.20 (t, 1H, $J = 7.3$ Hz)	174.0 (C-4), 72.5 (C-2), 55.0 (CH_2N), 44.3 ($2 \times CH_3$), 42.3 (NCH_2), 41.8 (C-1), 38.2 (C^{Ad}-2,8,9), 36.5 (C^{Ad}-4,6,10), 33.2 (C-5), 27.9 (C^{Ad}-3,5,7), 22.1 (CH_2)
7	4.21 (d, $J = 1.2$ Hz, 1H), 3.85 (m, 1H), 3.53 (dd, $J = 15.6$, 1.2 Hz, 1H), 3.31 (d, $J = 15.6$ Hz, 1H), 3.17 (m, 1H), 2.56 (q, $J = 7.2$ Hz, 4H), 2.45 (m, 2H), 2.01−1.59 (m, 15H + 2H), 1.03 (t, $J =$ 7.2 Hz, 6H)	173.5 (C-4), 72.5 (C-2), 49.6 (CH_2N), 46.5 ($2 \times NCH_2$), 45.2 (NCH_2), 41.7 (C-1), 38.3 (C^{Ad}-2,8,9), 36.6 (C^{Ad}-4,6,10), 33.4 (C-5), 28.0 (C^{Ad}-3,5,7), 24.1 (CH_2), 11.2 ($2 \times CH_3$)
8	7.33 (d, $J = 8.8$ Hz, 2H), 7.09 (d, $J = 8.8$ Hz, 2H), 4.83 (d, $J = 1.2$ Hz, 1H), 3.76 (d, $J = 15.6$ Hz, 1H), 3.51 (d, $J = 15.6$ Hz, 1H), 1.93−1.44 (m, 15H)	171.8 (C-4), 160.8 (d, $J = 246$ Hz, C−F), 136.3 (d, $J = 3.1$Hz, C^{Ar}−N), 127.3 (d, $J = 8.4$ Hz, $2 \times$ C), 116.0 (d, $J = 22$ Hz, $2 \times$ C), 74.3 (C-2), 41.6 (C-1), 38.6 (C^{Ad}-2,8,9), 36.6 (C^{Ad}-4,6,10), 33.3 (C-5), 28.0 (C^{Ad}-3,5,7)
9	7.37 (d, $J = 9.2$ Hz, 2H), 7.31 (d, $J = 9.1$ Hz, 2H), 4.85 (d, $J = 1.3$ Hz, 1H), 3.76 (dd, $J = 16.1$, 1.3 Hz, 1H), 3.50 (d, $J = 16.1$ Hz, 1H), 1.93−1.43 (m, 15H)	171.6 (C-4), 138.8 (C^{Ar}−N), 132.2 (C−Cl), 129.2 ($2 \times$ C), 126.8 ($2 \times$ C), 74.0 (C-2), 41.7 (C-1), 38.6 (C^{Ad}-2,8,9), 36.6 (C^{Ad}-4,6,10), 33.4 (C-5), 28.0 (C^{Ad}-3,5,7)
10	7.52 (d, $J = 8.5$ Hz, 2H), 7.25 (d, $J = 8.5$ Hz, 2H), 4.85 (d, $J = 1.2$ Hz, 1H), 3.75 (d, $J = 15.6$ Hz, 1H), 3.50 (d, $J = 15.6$ Hz, 1H), 1.93−1.43 (m, 15H)	171.6 (C-4), 139.4 (C^{Ar}−N), 132.2 ($2 \times$ C), 127.1 ($2 \times$ C), 120.1 (C−Br), 74.0 (C-2), 41.8 (C-1), 38.6 (C^{Ad}-2,8,9), 36.5 (C^{Ad}-4,6,10), 33.4 (C-5), 28.0 (C^{Ad}-3,5,7)
11	7.71 (d, $J = 8.8$ Hz, 2H), 7.12 (d, $J = 8.8$ Hz, 2H), 4.85 (d, $J = 1.2$ Hz, 1H), 3.74 (dd, $J = 15.6$, 1.2 Hz, 1H), 3.49 (d, $J = 15.6$ Hz, 1H), 1.93−1.43 (m, 15H)	171.5 (C-4), 140.1 (C^{Ar}−N), 138.2 ($2 \times$ C), 127.3 ($2 \times$ C), 91.36 (C−I), 73.9 (C-2), 41.8 (C-1), 38.6 (C^{Ad}-2,8,9), 36.6 (C^{Ad}-4,6,10), 33.5 (C-5), 28.0 (C^{Ad}-3,5,7)
12	7.70 (d, $J = 9.0$ Hz, 2H), 7.53 (d, $J = 9.0$ Hz, 2H), 4.93 (s, 1H), 3.78 (d, $J = 16.3$ Hz, 1H), 3.52 (d, $J = 16.3$ Hz, 1H), 1.94−1.40 (m, 15H)	171.5 (C-4), 144.2 (C^{Ar}−N), 133.0 ($2 \times$ C), 125.6 ($2 \times$ C), 118.3 (C≡N), 110.0 (C−CN), 73.4 (C-2), 42.1 (C-1), 38.7 (C^{Ad}-2,8,9), 36.5 (C^{Ad}-4,6,10), 33.5 (C-5), 27.9 (C^{Ad}-3,5,7)
13	7.25 (d, $J = 9.1$ Hz, 2H), 6.91 (d, $J = 9.1$ Hz, 2H), 4.82 (d, $J = 1.5$ Hz, 1H), 3.81 (s, 3H), 3.75 (dd $J = 15.7$, 1.5 Hz, 1H), 3.50 (d, $J = 15.7$ Hz, 1H), 1.92−1.46 (m, 15H)	171.8 (C-4), 158.0 (C−OMe), 132.2 (C^{Ar}−N), 126.8 ($2 \times$ C), 114.2 ($2 \times$ C), 74.4 (C-2), 55.2 (OCH_3), 41.4 (C-1), 38.5 (C^{Ad}-2,8,9), 36.6 (C^{Ad}-4,6,10), 33.3 (C-5), 28.0 (C^{Ad}-3,5,7)
14	7.68 (d, $J = 1.6$ Hz, 2H), 7.54 (m, 2H), 4.87 (d, $J = 1.2$ Hz, 1H), 3.77 (dd, $J = 16.0$, 1.2 Hz, 1H), 3.52 (d, $J = 16$ Hz, 1H), 1.95−1.42 (m, 15H)	171.7 (C-4), 132.1 (C−Cl, C^{Ar}), 130.0 (C^{Ar}), 128.6 (q, $J = 32$ Hz, C−CF_3), 124.1 (q, $J = 5$ Hz, C^{Ar}), 122.3 (q, $J = 272$ Hz, CF_3), 73.7 (C-2), 41.9 (C-1), 38.7 (C^{Ad}-2,8,9), 36.5 (C^{Ad}-4,6,10), 33.3 (C-5), 27.9 (C^{Ad}-3,5,7)
15	8.39 (ddd, $J = 4.9$, 2.0, 0.8 Hz, 1H), 7.84 (ddd, $J = 8.1$, 1.2, 0.8 Hz, 1H), 7.75 (ddd, $J = 8.1$, 7.3, 2 Hz, 1H), 7.11 (ddd, $J = 7.3$, 4.9, 1.2 Hz, 1H), 5.88 (d, $J = 1.0$ Hz, 1H), 3.83 (dd, $J = 16.2$, 1.0 Hz, 1H), 3.52 (d, $J = 16.2$ Hz, 1H), 1.91−1.39 (m, 15H)	171.2 (C-4), 152.4, 147.4, 138.0, 121.0, 118.5 (C^{Ar}), 70.4 (C-2), 41.7 (C-1), 38.3 (C^{Ad}-2,8,9), 36.6 (C^{Ad}-4,6,10), 35.0 (C-5), 28.0 (C^{Ad}-3,5,7)
16	8.66 (d, $J = 2.5$ Hz, 1H), 8.48 (dd, $J = 4.8$, 1.3 Hz, 1H), 7.74 (ddd, $J = 8.4$, 2.5, 1.3 Hz, 1H), 7.36 (dd, $J = 8.4$, 4.8 Hz, 1H), 4.92 (d, $J = 1.4$ Hz, 1H), 3.77 (dd, $J = 15.8$, 1.4 Hz, 1H), 3.52 (d, $J =$ 15.8 Hz, 1H), 1.92−1.42 (m, 15H)	171.8 (C-4), 147.2, 146.4, 137.1, 132.8, 123.6 (C^{Ar}), 73.6 (C-2), 41.8 (C-1), 38.7 (C^{Ad}-2,8,9), 36.5 (C^{Ad}-4,6,10), 33.2 (C-5), 27.9 (C^{Ad}-3,5,7)
17	8.64 (d, $J = 6.0$ Hz, 2H), 7.50 (dd, $J = 6.0$ Hz, 2H), 4.99 (s, 1H), 3.79 (dd, $J = 16.0$, 1.0 Hz, 1H), 3.53 (d, $J = 16.0$ Hz, 1H), 1.95−1.42 (m, 15H)	171.8 (C-4), 149.8, 118.6 (C^{Ar}), 72.4 (C-2), 42.5 (C-1), 38.7 (C^{Ad}-2,8,9), 36.7 (C^{Ad}-4,6,10), 33.8 (C-5), 27.9 (C^{Ad}-3,5,7)
18	8.30 (d, $J = 4.3$ Hz, 1H), 7.60 (ddd, $J = 7.7$, 0.8 Hz, 1H), 7.16 (dd, $J = 7.7$, 4.3 Hz, 1H), 5.51 (s, 1H), 3.79 (ddd, $J = 15.2$, 0.8 Hz, 1H), 3.46 (d, $J = 15.2$ Hz, 1H), 2.36 (s, 3H), 1.92−1.44 (m, 15H)	172.0 (C-4), 152.1, 145.8, 140.4, 130.2, 122.7 (C^{Ar}), 72.6 (C-2), 41.0 (C-1), 38.1 (C^{Ad}-2,8,9), 36.7 (C^{Ad}-4,6,10), 33.8 (C-5), 28.0 (C^{Ad}-3,5,7), 18.5 (CH_3)

Table 4 (*continued*)

Compound	^{1}H NMR δ (ppm)	^{13}C NMR δ (ppm)
19	8.23 (d, J = 5.0 Hz, 1H), 7.65 (s, 1H), 6.94 (d, J = 5.0 Hz, 1H), 5.85 (s, 1H), 3.83 (d, J = 16.0 Hz, 1H), 3.51 (d, J = 16.0 Hz, 1H), 2.40 (s, 3H), 1.91–1.40 (m, 15H)	172.2 (C-4), 152.5, 149.3, 147.1, 122.3, 119.1 (C^{Ar}), 70.5 (C-2), 41.7 (C-1), 38.3 (C^{Ad}-2,8,9), 36.6 (C^{Ad}-4,6,10), 35.0 (C-5), 28.0 (C^{Ad}-3,5,7), 21.3 (CH_3)
20	8.19 (d, J = 2.2 Hz, 1H), 7.68 (d, J = 8.4 Hz, 1H), 7.56 (dd, J = 8.4, 2.2 Hz, 1H), 5.80 (s, 1H), 3.81 (dd, J = 16.0, 0.8 Hz, 1H), 3.50 (d, J = 16.0 Hz, 1H), 2.32 (s, 3H), 1.91–1.39 (m, 15H)	172.1 (C-4), 150.2, 147.4, 138.6, 130.6, 118.2 (C^{Ar}), 70.6 (C-2), 41.6 (C-1), 38.3 (C^{Ad}-2,8,9), 36.6 (C^{Ad}-4,6,10), 34.9 (C-5), 28.0 (C^{Ad}-3,5,7), 17.9 (CH_3)
21	7.63 (m, 1H), 7.61 (m, 1H), 6.95 (dd, J = 6.0, 2.0 Hz, 1H), 5.90 (d, J = 0.9 Hz, 1H), 3.82 (dd, J = 16.0, 0.9 Hz, 1H), 3.50 (d, J = 16.0 Hz, 1H), 2.48 (s, 3H), 1.91–1.39 (m, 15H)	172.1 (C-4), 156.6, 151.6, 138.0, 120.3, 115.3 (C^{Ar}), 70.4 (C-2), 41.7 (C-1), 38.4 (C^{Ad}-2,8,9), 36.6 (C^{Ad}-4,6,10), 35.0 (C-5), 28.0 (C^{Ad}-3,5,7), 24.2 (CH_3)
22	7.42 (s, 1H), 6.79 (s, 1H), 5.88 (s, 1H), 3.81 (d, J = 15.8 Hz, 1H), 3.50 (d, J = 15.8 Hz, 1H), 2.43 (s, 3H), 2.34 (s, 3H), 1.91–1.40 (m, 15H)	172.1 (C-4), 156.2, 151.8, 149.3, 121.6, 116.0 (C^{Ar}), 70.6 (C-2), 41.7 (C-1), 38.4 (C^{Ad}-2,8,9), 36.7 (C^{Ad}-4,6,10), 35.1 (C-5), 28.1 (C^{Ad}-3,5,7), 24.0, 21.2 (2 × CH_3)
23	7.41 (s, 1H), 7.01 (s, 1H), 5.68 (s, 1H), 5.64 (bs, 1H), 3.84 (d, J = 16.2 Hz, 1H), 3.58 (d, J = 16.2 Hz, 1H), 2.92 (dd, J = 14.9, 1 Hz, 1H), 2.45 (dd, J = 14.9, 1 Hz, 1H), 2.40 (s, 3H), 2.32 (s, 3H), 1.94 (t, J = 4.6 Hz, 1H), 1.85–1.27 (m, 21H), 1.03 (s, 3H), 0.73 (s, 3H)	216.0 (C=O), 171.5 (C-4), 155.5, 150.7, 148.6, 122.1, 116.4 (C^{Ar}), 69.8 (C^*-2), 58.1 [1*S*-(+)], 47.2 (C-acid), 46.9 ($CH_2SO_3^-$), 42.2 (CH-acid), 42.1 (CH_2-acid), 41.1 (C-1), 37.8 (C^{Ad}-2,8,9), 36.2 (C^{Ad}-4,6,10), 33.8 (C-5), 27.5 (C^{Ad}-3,5,7), 26.4, 24.2 (CH_2-acid), 23.2, 20.8 (2 × CH_3), 20.0, 19.5 (2 × CH_3-acid)
24	7.41 (s, 1H), 7.01 (s, 1H), 5.68 (s, 1H), 5.64 (bs, 1H), 3.84 (d, J = 16.2 Hz, 1H), 3.58 (d, J = 16.2 Hz, 1H), 2.92 (dd, J = 14.9, 1 Hz, 1H), 2.45 (dd, J = 14.9, 1 Hz, 1H), 2.40 (s, 3H), 2.32 (s, 3H), 1.94 (t, J = 4.6 Hz, 1H), 1.85–1.27 (m, 21H), 1.03 (s, 3H), 0.73 (s, 3H)	216.0 (C=O), 171.5 (C-4), 155.5, 150.7, 148.6, 122.1, 116.4 (C^{Ar}), 69.8 (C^*-2), 58.1 [1*S*-(+)], 47.2 (C-acid), 46.9 ($CH_2SO_3^-$), 42.2 (CH-acid), 42.1 (CH_2-acid), 41.1 (C-1), 37.8 (C^{Ad}-2,8,9), 36.2 (C^{Ad}-4,6,10), 33.8 (C-5), 27.5 (C^{Ad}-3,5,7), 26.4, 24.2 (CH_2-acid), 23.2, 20.8 (2 × CH_3), 20.0, 19.5 (2 × CH_3-acid)
25	7.63 (s, 1H), 6.79 (s, 1H), 5.88 (qd, J = 6.0, 1.2 Hz, 1H), 3.91 (dd, J = 16.0, 1.2 Hz, 1H), 3.67 (d, J = 16.0 Hz, 1H), 2.43 (s, 3H), 2.34 (s, 3H), 1.60 (d, J = 6.0 Hz, 3H)	170.6 (C-4), 156.5, 150.1, 149.3, 121.3, 114.9 (C^{Ar}), 57.6 (C-2), 33.9 (C-5), 24.0 (CH_3-pyr), 23.9 (CH_3), 21.1 (CH_3-pyr)

*3.3.7. 2-Adamantan-1-yl-3-(4-fluoro-phenyl)-thiazolidin-4-one (**8**)*

The reaction was performed in benzene for over 11 h. White crystals (563 mg, 85%); mp 228–229 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{19}H_{22}FNOS$) C, H, N.

*3.3.8. 2-Adamantan-1-yl-3-(4-chloro-phenyl)-thiazolidin-4-one (**9**)*

The reaction was performed in benzene for over 13 h. White crystals (501 mg, 72%); mp 216–217 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{19}H_{22}ClNOS$) C, H, N.

*3.3.9. 2-Adamantan-1-yl-3-(4-bromo-phenyl)-thiazolidin-4-one (**10**)*

The reaction was performed in benzene for over 20 h. White crystals (675 mg, 86%); mp 205–206 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{19}H_{22}BrNOS$) C, H, N.

*3.3.10. 2-Adamantan-1-yl-3-(4-iodo-phenyl)-thiazolidin-4-one (**11**)*

The reaction was performed in benzene for over 24 h. White crystals (773 mg, 88%); mp 208–209 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{19}H_{22}INOS$) C, H, N.

*3.3.11. 4-(2-Adamantan-1-yl-4-oxo-thiazolidin-3-yl)-benzonitrile (**12**)*

The reaction was performed in benzene for over 19 h. White crystals (284 mg, 42%); mp 224–225 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{20}H_{22}N_2OS$) C, H, N.

*3.3.12. 2-Adamantan-1-yl-3-(4-methoxy-phenyl)-thiazolidin-4-one (**13**)*

The reaction was performed in benzene for over 24 h. White crystals (570 mg, 83%); mp 160–161 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{20}H_{25}NO_2S$) C, H, N.

*3.3.13. 2-Adamantan-1-yl-3-(4-chloro-3-trifluoromethyl-phenyl)-thiazolidin-4-one (**14**)*

The reaction was performed in benzene for over 26 h. White crystals (408 mg, 49%); mp 205–206 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{20}H_{21}ClF_3NOS$) C, H, N.

*3.3.14. 2-Adamantan-1-yl-3-pyridin-2-yl-thiazolidin-4-one (**15**)*

The reaction was performed in toluene for over 30 h. White crystals (258 mg, 41%); mp 150–151 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{18}H_{22}N_2OS$) C, H, N.

*3.3.15. 2-Adamantan-1-yl-3-pyridin-3-yl-thiazolidin-4-one (**16**)*

The reaction was performed in toluene for over 29 h. White crystals (447 mg, 71%); mp 160–161 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{18}H_{22}N_2OS$) C, H, N.

*3.3.16. 2-Adamantan-1-yl-3-pyridin-4-yl-thiazolidin-4-one (**17**)*

The reaction was performed in toluene for over 29 h. White crystals (44 mg, 7%); mp 176–178 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{18}H_{22}N_2OS$) C, H, N.

*3.3.17. 2-Adamantan-1-yl-3-(3-methyl-pyridin-2-yl)-thiazolidin-4-one (**18**)*

The reaction was performed in toluene for over 28 h. White crystals (218 mg, 33%); mp 130–131 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{19}H_{24}N_2OS$) C, H, N.

*3.3.18. 2-Adamantan-1-yl-3-(4-methyl-pyridin-2-yl)-thiazolidin-4-one (**19**)*

The reaction was performed in toluene for over 24 h. White crystals (309 mg, 47%); mp 131–132 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{19}H_{24}N_2OS$) C, H, N.

*3.3.19. 2-Adamantan-1-yl-3-(5-methyl-pyridin-2-yl)-thiazolidin-4-one (**20**)*

The reaction was performed in toluene for over 25 h. White crystals (250 mg, 38%); mp 148–149 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{19}H_{24}N_2OS$) C, H, N.

*3.3.20. 2-Adamantan-1-yl-3-(6-methyl-pyridin-2-yl)-thiazolidin-4-one (**21**)*

The reaction was performed in toluene for over 20 h. White crystals (427 mg, 65%); mp 165–166 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{19}H_{24}N_2OS$) C, H, N.

*3.3.21. 2-Adamantan-1-yl-3-(4,6-dimethyl-pyridin-2-yl)-thiazolidin-4-one (**22**)*

The reaction was performed in toluene for over 22 h. White crystals (349 mg, 51%); mp 130–131 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{20}H_{26}N_2OS$) C, H, N.

*3.3.22. 3-(4,6-Dimethyl-pyridin-2-yl)-2-methylthiazolidin-4-one (**25**)*

A mixture of acetaldehyde (0.56 mL, 10 mmol) and 4,6-dimethyl-pyridin-2-ylamine (611 mg, 5 mmol) was stirred for 4 h in benzene (20 mL) at 5–10 °C. Then mercaptoacetic acid (1.05 mL, 15 mmol) was added and the mixture was refluxed for further 2–28 h with simultaneous azeotropic removal of water. After cooling, the solution was washed two times with the saturated solution of $NaHCO_3$ and brine. The mixture was dried over magnesium sulphate and solvent was evaporated. The residue (yellow oil) was purified by flash column chromatography using $CHCl_3$/MeOH: 50/1 as an eluent. White crystals (289 mg, 26%); mp 78–79 °C; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{11}H_{14}N_2OS$) C, H, N.

*3.4. Separation of enantiomers of **22***

*3.4.1. (*S*)-(−)-2-Adamantan-1-yl-3-(4,6-dimethyl-pyridin-2-yl)-thiazolidin-4-one (1*S*)-(+)-10-camphorsulfonic acid salt (**23**)*

To the solution of **22** (342 mg, 1 mmol) in methanol (3 mL) 260 mg (1.1 mmol) of (1*S*)-(+)-10-camphorsulfonic acid monohydrate dissolved in 3 mL of hot methanol was added and the mixture was placed in a refrigerator overnight. Then the crystals were filtered off and recrystallized from methanol. White crystals (250 mg, 87.5%); mp 158 °C; $[\alpha]_D^{25} = -64.6°$, $c = 0.01$; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{19}H_{24}N_2OS$) C, H, N.

*3.4.2. (R)-(+)-2-Adamantan-1-yl-3-(4,6-dimethyl-pyridin-2-yl)-thiazolidin-4-one (1R)-(−)-10-camphorsulfonic acid salt (**24**)*

The salt **24** was obtained according to the same procedure as for **23** using (1*R*)-(−)-10-camphorsulfonic acid monohydrate. White crystals (250 mg, 87.5%); mp 158–159 °C; $[\alpha]_D^{25} = +64.6°$, $c = 0.01$; ^{1}H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); Anal. ($C_{19}H_{24}N_2OS$) C, H, N.

*3.4.3. (*S*)-(−)-2-Adamantan-1-yl-3-(4,6-dimethyl-pyridin-2-yl)-thiazolidin-4-one [(−)-**22**]*

The camphorsulfonic acid salt (**23**) was dissolved in methanol/water (1/1) mixture and neutralized with 2 M NaOH. The white crystals of (*S*)-(−)-2-adamantan-1-yl-3-(4,6-dimethyl-pyridin-2-yl)-thiazolidin-4-one slowly precipitated (140 mg, 82.0%); mp 138–139 °C; $[\alpha]_D^{25} = -187°$, $c = 0.01$. Enantiomeric excess detected by HPLC analysis was 96.8%.

*3.4.4. (R)-(+)-2-Adamantan-1-yl-3-(4,6-dimethyl-pyridin-2-yl)-thiazolidin-4-one [(+)-**22**]*

The analogues procedure as for (−)-**22** was applied. White crystals (145 mg, 85%); mp 138–139 °C; $[\alpha]_D^{25} = +188°$, $c = 0.01$. Enantiomeric excess detected by HPLC analysis was >99%.

3.5. X-ray measurements

The data were collected using MoKα radiation. After mounting and centering the single crystal of dimensions $0.25 \times 0.6 \times 0.6$ mm on the diffractometer the unit cell parameters were obtained by a least-squares treatment for 42 strong reflections with $11.6 < 2\theta < 23°$. Up to $2\theta < 50°$ 3762 reflections were collected. Structure was solved by direct methods from SHELXS97 [26] and then refined on F^2 using SHELXL97 software [27]. The final R and wR for 2335 reflections with $I > 2\sigma(I)$ were 0.0574 and 0.1740, respectively. The detailed structural data have been deposited with CCDC under the number CCDC 613396.

3.6. Biological methods

3.6.1. Cells and viruses

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

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

A total of 4×10^4 CEM cells/mL were infected with HIV-1(III_B) or HIV-2(ROD) at ~100 $CCID_{50}$ (50% cell culture infective dose) per milliliter of cell suspension. Testing of compound (+)-**22** was also performed for virus strains that contained NNRTI-characteristic mutations in the reverse transcriptase (i.e. Leu100Ile, Lys103Asn, Tyr181Cys, Tyr181Ile and Tyr188His). Then, 100 μL of the infected cell suspension was transferred to microtiter plate wells and mixed with 100 μL of the appropriate dilutions of the tested compounds. Giant cell formation was recorded microscopically in the HIV-infected cell cultures after four days. The 50% effective concentration (EC_{50}) of the tested compounds was defined as the compound concentration required for inhibiting virus-induced cytopathicity (CEM). The 50% cytostatic or cytotoxic concentration (CC_{50}) was defined as the compound concentration required for inhibiting CEM cell proliferation by 50%.

3.6.3. Anti-reverse transcriptase assays

The source of the reverse transcriptases used was recombinant HIV-1 RT [derived from HIV-1(III_B)], constructed and prepared as described before. The RT assays contained a total reaction mixture volume (50 μL) 50 mM Tris–HCl (pH 7.8), 5 mM dithiothreitol, 300 mM glutathione, 500 μM EDTA, 150 mM KCl, 5 mM $MgCl_2$, 1.25 μg of bovine serum albumin, labeled substrate [8-^{3}H]dGTP (1 μCi) (specific radioactivity 11 Ci/mmol) (2.5 μM) or [methyl-^{3}H]dTTP (1 μCi) (specific radioactivity 48 Ci/mmol) (0.6 μM) (Moravek Biochemicals, Brea, CA), a fixed concentration of the template/primer poly(C).oligo$(dG)_{12-18}$ or poly(A).oligo$(dT)_{12-18}$ (0.1 mM), 0.06% Triton X-100, 10 μL of inhibitor at various concentrations, and 1 μL of the RT preparation. The reaction mixtures were incubated at 37 °C for 30 min, at which time 200 μL of yeast RNA (150 μg/mL) and 1 mL of trichloroacetic acid (5% v/v) in saturated $Na_4P_2O_7$ (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 IC_{50} for each test compound was determined as the compound concentration that inhibited HIV RT activity by 50%. For the kinetic experiments with compound (+)-**22**, a variety of dGTP concentrations were tested in the presence of a fixed concentration (0.1 mM) of poly rC.dG template/primer and different concentrations (58.29, 29 and 7.3 μM) of (+)-**22**. The K_i value was calculated from the Lineweaver–Burk plots using the formula:

$$K_i = \frac{[\text{inhibitor}]}{\frac{V}{V_{max}} - 1}$$

3.6.4. Cytostatic assays

Murine leukeia L1210, murine mammary carcinoma FM3A, human T-lymphocyte Molt 4/C8, and CEM cells were suspended at 300,000–500,000 cells/mL of culture medium, and an amount of 100 μL of these cell suspensions was added to 200 μL microtiter plate wells containing 100 μL of an appropriate dilution of the tested compounds. After two days (L1210 and FM3A) or three days (Molt 4/C8 and CEM) of incubation at 37 °C, the cell number was determined using a Coulter counter. The 50% cytostatic concentration (CC_{50}) was defined as the compound concentration required inhibiting cell proliferation by 50%.

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejmech.2007.01.003.

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