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2-(2,6-Dihalophenyl)-3-(pyrimidin-2-yl)-1,3-thiazolidin-4-ones as non-nucleoside HIV-1 reverse transcriptase inhibitors

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Abstract

Several 1,3-thiazolidin-4-ones bearing a 2,6-dihalophenyl group at C-2 and a substituted pyrimidin-2-yl ring at the N-3 were synthesised and evaluated as anti-HIV agents. The results of the in vitro tests showed that some of them were highly effective inhibitors of human immunodeficiency virus type-1 (HIV-1) replication at 10–40 nM concentrations with minimal cytotoxicity. Structure–activity relationship studies revealed that the nature of the substituents at the 2 and 3 positions of the thiazolidinone nucleus had a significant impact on the in vitro anti-HIV activity of this class of potent antiretroviral agents. The compounds had significantly reduced activity against the characteristic NNRTI-resistant virus mutants (bearing the K103N and Y181C RT mutations), thereby acting as non-nucleoside HIV-1 reverse transcriptase (RT) inhibitors (NNRTIs).

Keywords: 1,3-Thiazolidin-4-ones; NNRTIs; Anti-HIV activity

1. Introduction

Human immunodeficiency virus type-1 (HIV-1) is the causative agent for the transmission and development of the acquired immune deficiency syndrome (AIDS). Current treatment strategies for HIV infection are mainly based on drugs that interfere with virus replication by inhibiting either HIV protease or HIV reverse transcriptase (RT) (Jonckheere et al., 2000).

The RT inhibitors known to date are of two types: nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs, NtRTIs) and non-nucleoside reverse transcriptase inhibitors (NNRTIs). The NRTIs and NtRTIs are competitive inhibitors of the 2′-deoxy-nucleoside triphosphate (dNTP) binding site on RT and act as substrate decoys and chain terminators (Tantillo et al., 1994), whereas the NNRTIs bind at an allosteric site and are non-competitive with respect to the dNTP binding site (Smith et al., 1995).

NNRTIs are more vulnerable to HIV's high mutation rate which leads to rapid selection of virus strains that are resistant to drug inhibition (De Clercq, 1998). Recently, the development of a second generation of NNRTIs, able to inhibit some of the mutant virus strains resistant to its predecessors, has revived interest in the search for novel and potent NNRTIs (De Clercq, 2002).

The present work is an extension of our ongoing efforts towards the development and identification of new molecules with anti-HIV activity which have previously led to the discovery of 1*H*,3*H*-thiazolo[3,4-*a*]benzimidazoles (TBZs, Fig. 1) as highly active NNRTIs (Barreca et al., 1999; Chimirri et al., 1991, 1997, 1998; Monforte et al., 1993). More recent publications from our laboratory have documented a new class of NNRTIs, 2,3-diaryl-1,3-thiazolidin-4-one derivatives (Fig. 1), that proved to be more effective than TBZs in inhibiting HIV-1 replication (Barreca et al., 2001, 2002; Rao et al., 2002, 2003), but were still inferior to the clinically-used efavirenz.

These compounds may be envisaged as "open models" of TBZs since they maintain the plausible pharmacophoric elements of the TBZ derivatives necessary for an efficient RT inhibition (Barreca et al., 2002).

Structure–activity relationship (SAR) studies showed that the anti-HIV activity is strongly dependent on the nature of the substituents at C-2 and N-3 of the thiazolidinone

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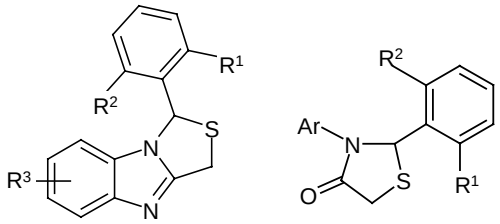


Fig. 1. Structure of TBZs and 2,3-diaryl-1,3-thiazolidin-4-one derivatives.

ring. In particular, a high activity level was found for compounds possessing a 2′,6′-dihalophenyl group at C-2 and a pyridin-2-yl ring at N-3 (Barreca et al., 2001, 2002).

Starting from these findings, to acquire more insight into the SAR of this class of compounds and also to obtain compounds that are more potent and more active against mutant virus strains, we extended our studies to the synthesis and the anti-HIV activity evaluation of a new series of 1,3-thiazolidin-4-ones in which the 2,6-dihalophenyl ring at C-2 was retained as an essential moiety, whereas the pyridine ring was replaced by a suitably substituted pyrimidine nucleus.

2. Materials and methods

2.1. Chemicals

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Elemental analyses (C, H, N) were carried out on a Carlo Erba Model 1106 Elemental Analyzer and the results are within ±0.4% of the theoretical values. Merck silica gel 60 F_{254} plates were used for analytical TLC; column chromatography was performed on Merck silica gel 60 (230–400 mesh). ^{1}H NMR spectra were recorded in $CDCl_3$ on a Varian Gemini-300 spectrometer. Chemical shifts were expressed in δ (ppm) relative to tetramethylsilane (TMS) as internal standard and coupling constants (J) in Hz.

2.2. General procedure for the synthesis of 2-(2,6-dihalophenyl)-3-(pyrimidin-2-yl)-1,3-thiazolidin-4-ones (1–18)

The synthesis of compounds **1**–**18** was performed according to a previously reported procedure (Barreca et al., 2002). 2-Mercaptoacetic acid (16 mmol) and the appropriate aromatic aldehyde (8 mmol) were added to a stirred solution of 2-aminopyrimidine (8 mmol) in dry toluene (50 ml). The reaction mixture was refluxed for 48 h and then neutralised by a solution of sodium hydrogen carbonate. After removal of the solvent under reduced pressure, the oily residue was powdered by treatment with a mixture of ethanol and diethyl ether to afford compounds **1**–**3**, **5**, **7**–**8** and **10**–**18**. Compounds **4**, **6** and **9** were isolated by silica gel column chromatography eluting with chloroform/methanol 98:2. All compounds were recrystallised from ethanol. Compounds **1**–**18** were characterised by means of ^{1}H NMR spectroscopy; physico-chemical data for the synthesised compounds are summarised in Table 1.

2.3. Antiviral activity assays

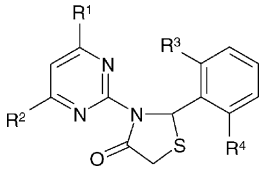
The methodology of the anti-HIV assays has been previously described (Balzarini et al., 1993; Pauwels et al., 1988). Briefly, MT-4 cells were infected with HIV-1 (III_B) and HIV-2 (ROD) at 100-times the $CCID_{50}$ (50% cell culture infective dose) per millilitre of cell suspension. One-hundred microlitres of the infected cell suspension were then transferred to microtiter plate wells, mixed with 100 μl of the appropriate dilutions of test compounds, and further incubated at 37 °C. After 5 days of incubation, the number of viable cells was determined. The 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}) were defined as the concentrations of compound required to reduce the number of viable cells in the virus-infected and mock-infected cell cultures, respectively.

To determine cross-resistance to different NNRTIs, CEM cells were infected with 100 $CCID_{50}$ of HIV-1 (III_B) or mutant HIV-1 strains selected for resistance to TIBO R82150 (L100I), TIBO R82913 (K103N), TSAO-m^{3}T (E138K), pyridinone L697,661 (Y181C) or HEPT (Y188H). One-hundred microlitres of the infected cell suspension were then added to microtiter plate wells containing 100 μl of an appropriate dilution of the test compounds. After 4 days of incubation, HIV-1-induced syncytium formation was recorded. The EC_{50} was defined as the concentration of compound required to reduce giant cell formation by 50%.

2.4. HIV-1 RT assay

The procedure for assessing the reverse transcriptase inhibition has been described previously (Balzarini et al., 1992). The reaction mixture (50 μl) contained 50 mM Tris–HCl (pH 7.8), 5 mM dithiothreitol, 30 mM glutathione, 50 μM EDTA, 150 mM KCl, 5 mM $MgCl_2$, 1.25 μg of bovine serum albumin, an appropriate concentration of the radiolabelled substrate [^{3}H]dGTP, 0.1 mM poly(C)·oligo(dG) as the template/primer, 0.06% Triton X-100, 10 μl of inhibitor solution (containing various concentrations of compounds), and 1 μl of RT preparation. The reaction mixtures were incubated at 37 °C for 15 min, at which time 100 μl of calf thymus DNA (150 μg/ml), 2 ml of $Na_4P_2O_7$ (0.1 M in 1 M HCl), and 2 ml of trichloroacetic acid (10% v/v) were added. The solutions were kept on ice for 30 min, after which the acid-insoluble material was washed and analysed for radioactivity. In the experiments in which 50% inhibitory concentration (IC_{50}, that is the concentration required to inhibit RT activity by 50%) of the test compounds was determined, a fixed concentration of 2.5 μM [^{3}H]dGTP was used.

Table 1
Physico-chemical data for newly synthesised compounds **1**–**18**



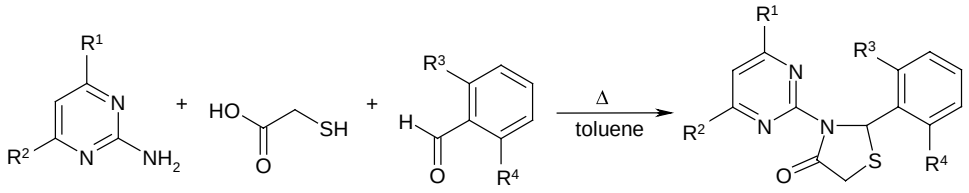
Compounds	R^1	R^2	R^3	R^4	Yield (%)	m.p. (°C)	^{1}H NMR
1	H	H	Cl	Cl	76	173–175	3.95 (d, 1H, $J = 15.9$ Hz, 5-H_A), 4.19 (dd, 1H, $J = 1.9$ and 15.9 Hz, 5-H_B), 7.01–8.62 (m, 7H, ArH and H-2)
2	H	H	Cl	F	60	146–147	3.87 (d, 1H, $J = 15.9$ Hz, 5-H_A), 4.19 (dd, 1H, $J = 1.4$ and 15.9 Hz, 5-H_B), 6.89–8.62 (m, 7H, ArH and H-2)
3	H	H	F	F	70	145–148	3.86 (d, 1H, $J = 15.9$ Hz, 5-H_A), 4.19 (dd, 1H, $J = 0.8$ and 15.9 Hz, 5-H_B), 6.89–8.64 (m, 7H, ArH and H-2)
4	Me	H	Cl	Cl	24	125–128	2.36 (s, 3H, Me), 3.96 (d, 1H, $J = 15.7$ Hz, 5-H_A), 4.19 (dd, 1H, $J = 1.9$ and 15.7 Hz, 5-H_B), 6.85–8.52 (m, 6H, ArH and H-2)
5	Me	H	Cl	F	62	136–139	2.39 (s, 3H, Me), 3.88 (d, 1H, $J = 15.9$ Hz, 5-H_A), 4.21 (dd, 1H, $J = 1.6$ and 15.7 Hz, 5-H_B), 6.86–8.52 (m, 6H, ArH and H-2)
6	Me	H	F	F	46	129–130	2.39 (s, 3H, Me), 3.86 (d, 1H, $J = 15.7$ Hz, 5-H_A), 4.24 (dd, 1H, $J = 1.6$ and 15.7 Hz, 5-H_B), 6.81–8.51 (m, 6H, ArH and H-2)
7	Me	Cl	Cl	Cl	20	244–246	1.98 (s, 3H, Me), 3.98 (d, 1H, $J = 16.8$ Hz, 5-H_A), 4.21 (dd, 1H, $J = 1.9$ and 16.8 Hz, 5-H_B), 5.95–7.37 (m, 5H, ArH and H-2)
8	Me	Cl	Cl	F	22	158–159	2.04 (s, 3H, Me), 3.89 (d, 1H, $J = 16.8$ Hz, 5-H_A), 4.24 (dd, 1H, $J = 1.6$ and 16.8 Hz, 5-H_B), 5.96–7.24 (m, 5H, ArH and H-2)
9	Me	Cl	F	F	32	144–148	2.06 (s, 3H, Me), 3.88 (d, 1H, $J = 16.8$ Hz, 5-H_A), 4.28 (dd, 1H, $J = 2.2$ and 16.8 Hz, 5-H_B), 5.97–7.32 (m, 5H, ArH and H-2)
10	Me	Me	Cl	Cl	38	198–201	2.37 (s, 6H, Me), 3.95 (d, 1H, $J = 15.7$ Hz, 5-H_A), 4.18 (dd, 1H, $J = 1.9$ and 15.7 Hz, 5-H_B), 6.70–7.30 (m, 4H, ArH), 7.49 (d, 1H, $J = 1.9$ Hz, H-2)
11	Me	Me	Cl	F	22	165–166	2.37 (s, 6H, Me), 3.86 (d, 1H, $J = 15.4$ Hz, 5-H_A), 4.20 (dd, 1H, $J = 1.4$ and 15.4 Hz, 5-H_B), 6.74 (d, 1H, $J = 1.4$ Hz, H-2), 6.89–7.18 (m, 4H, ArH)
12	Me	Me	F	F	34	141–143	2.38 (s, 6H, Me), 3.85 (d, 1H, $J = 15.7$ Hz, 5-H_A), 4.23 (dd, 1H, $J = 1.9$ and 15.7 Hz, 5-H_B), 6.74–7.24 (m, 5H, ArH and H-2)
13	Me	MeO	Cl	Cl	24	137–140	2.39 (s, 3H, Me), 3.77 (s, 3H, OMe), 3.94 (d, 1H, $J = 15.9$ Hz, 5-H_A), 4.19 (dd, 1H, $J = 1.6$ and 15.9 Hz, 5-H_B), 6.31–7.35 (m, 4H, ArH), 7.39 (d, 1H, $J = 1.6$ Hz, H-2)
14	Me	MeO	Cl	F	58	156–159	2.37 (s, 3H, Me), 3.74 (s, 3H, OMe), 3.83 (d, 1H, $J = 15.7$ Hz, 5-H_A), 4.15 (dd, 1H, $J = 1.6$ and 15.7 Hz, 5-H_B), 6.28–7.20 (m, 5H, ArH and H-2)
15	Me	MeO	F	F	68	173–175	2.40 (s, 3H, Me), 3.82 (s, 3H, OMe), 3.87 (d, 1H, $J = 15.9$ Hz, 5-H_A), 4.24 (dd, 1H, $J = 1.9$ and 15.9 Hz, 5-H_B), 6.32–7.31 (m, 5H, ArH and H-2)
16	Me	OH	Cl	Cl	50	190(dec)	1.98 (s, 3H, Me), 3.99 (d, 1H, $J = 16.8$ Hz, 5-H_A), 4.22 (dd, 1H, $J = 1.9$ and 16.8 Hz, 5-H_B), 5.96–7.37 (m, 5H, ArH and H-2), 11.84 (br.s, 1H, OH)
17	Me	OH	Cl	F	32	156–159	2.05 (s, 3H, Me), 3.89 (d, 1H, $J = 16.8$ Hz, 5-H_A), 4.26 (dd, 1H, $J = 2.1$ and 16.8 Hz, 5-H_B), 5.97–7.25 (m, 5H, ArH and H-2), 11.87 (br.s, 1H, OH)
18	Me	OH	F	F	26	145–148	2.05 (s, 3H, Me), 3.88 (d, 1H, $J = 16.8$ Hz, 5-H_A), 4.29 (dd, 1H, $J = 2.2$ and 16.8 Hz, 5-H_B), 5.97–7.30 (m, 5H, ArH and H-2), 11.87 (br.s, 1H, OH)

3. Results

The synthesis of the new 2-(2,6-dihalophenyl)-3-(pyrimidin-2-yl)-1,3-thiazolidin-4-ones (**1**–**18**) was carried out by reacting a 2,6-dihalobenzaldehyde with an equimolar amount of a suitably substituted 2-aminopyrimidine in the presence of an excess of mercaptoacetic acid in refluxing toluene (Fig. 2). The products obtained were isolated by conventional work-up in satisfactory yields. Both analytical and spectral data (^{1}H NMR) of all the synthesised compounds are in full agreement with the proposed structures (Table 1).

Compounds **1**–**18** were evaluated for prevention of the cytopathic effects of HIV-1 (III_B) and HIV-2 (ROD) in MT-4 cells. Compound-induced cytotoxicity was also measured in MT-4 cells in parallel with the antiviral activity. A select group of inhibitors of HIV replication in the MT-4 cell culture were also tested for inhibition of HIV-1 RT. The results from the cell-based and RT assays are summarised in Table 2 in which the lead compound of the TBZ series, i.e. 1-(2,6-difluorophenyl)-1*H*,3*H*-thiazolo[3,4-*a*]benzimidazole (**TBZ-1**), was also included for comparison.

Several of the new compounds prevented the cytopathic effect of HIV-1 III_B at nanomolar concentrations and were minimally toxic to MT-4 cells resulting in remarkably high selectivity indices. It is worth noting that compound **10**, one of the most promising of the series, was approximately 20-fold more active than **TBZ-1** and possessed a selectivity index of about 10,000.



1-18

Fig. 2. Synthesis of 2,3-diaryl-1,3-thiazolidin-4-ones (**1**–**18**).

Table 2
Anti-HIV-1 activity, cytotoxicity, selectivity index, in MT-4 cells, and HIV-1 RT inhibition by compounds **1**–**18**

Compounds	EC_{50} (μM)[a]	CC_{50} (μM)[b]	SI[c]	IC_{50} (μM)[d]
1	0.227 ± 0.006	173 ± 4.4	758	
2	0.581 ± 0.258	136 ± 15.2	234	
3	3.65 ± 0.44	>426.2	>118	
4	0.044 ± 0.021	180 ± 7.5	2454	0.91 ± 0.03
5	0.074 ± 0.028	134 ± 27.1	1809	1.79 ± 0.60
6	0.39 ± 0.13	>406.7	>1100	
7	51.8 ± 20.0	81.7 ± 72.8	2	
8	NA	0.893 ± 0.167		
9	NA	>73.1		
10	0.017 ± 0.004	161 ± 15.3	9521	0.87 ± 0.34
11	0.038 ± 0.009	174 ± 11.8	4533	2.50 ± 0.44
12	0.171 ± 0.062	152 ± 23.8	886	
13	0.024 ± 0.005	52.5 ± 14.2	2187	0.97 ± 0.14
14	0.056 ± 0.011	215 ± 49.5	3840	0.85 ± 0.00
15	0.264 ± 0.098	>370.5	>1400	
16	32.84 ± 3.56	179 ± 11.8	5.5	
17	NA	1.32		
18	NA	6.55		
TBZ-1	0.352 ± 0.14	19.2 ± 2.8	54.5	14.6 ± 2.6

NA: not active.

[a] Concentration required to reduce HIV-1-(III_B) induced cytopathic effect by 50% in MT-4 cells.

[b] Concentration required to reduce MT-4 cell viability by 50%.

[c] Selectivity index: ratio CC_{50}/EC_{50}.

[d] Concentration required to inhibit HIV-1 RT activity by 50%. Poly(C)·oligo(dG) was used as the template/primer and [^{3}H]dGTP as the radiolabelled substrate.

As observed for other classes of NNRTIs, none of the tested compounds inhibited the replication of HIV-2 (ROD) in MT-4 cells at subtoxic concentrations (data not shown).

The in vitro IC_{50} values for HIV-1 RT with poly(rC)·oligo(dG) as the template/primer were significantly higher than the corresponding EC_{50} values for inhibition of the cytopathic effect of HIV-1 in MT-4 cell culture. This discrepancy is not unusual for NNRTIs as it may reflect the differences between the in vitro HIV-1 RT assay, in which a synthetic template/primer is used, and the cellular systems (Casimiro-Garcia et al., 1999).

Table 3
Anti-HIV-1 activity of compounds **4**, **10** and **13** against mutant HIV-1 strains in CEM cells

Compounds	EC_{50} (μM)[a]					
	$HIV\text{-}1_{IIIB}$	L100I	K103N	E138K	Y181C	Y188H
4	0.032 ± 0.003	0.44 ± 0.09	17.9 ± 2.8	0.050 ±0.006	4.41 ± 3.23	7.35 ± 0.00
10	0.025 ± 0.003	0.23 ± 0.05	16.9 ± 2.1	0.039 ± 0.017	2.54 ± 0.40	7.90 ± 5.08
13	0.041 ± 0.035	0.38 ± 0.06	21.6 ± 3.8	0.070 ± 0.022	6.75 ± 0.00	6.21 ± 3.24
TBZ-1	1.39 ± 0.56	3.12 ± 0.94	15.6 ± 3.2	1.73 ± 1.02	13.8 ± 4.2	4.16 ± 1.12
Nevirapine	0.044 ± 0.010	0.24 ± 0.05	3.04 ± 0.47	0.044 ± 0.010	3.04 ± 1.42	>15
AZT	0.007 ± 0.003	0.008 ± 0.002	0.011 ± 6.006	0.016 ± 0.014	0.019 ± 0.014	0.004 ± 0.001

[a] 50% Effective concentration, or concentration required to protect CEM cells against the HIV-induced cytopathogenicity by 50%.

Compounds **4**, **10** and **13** were also evaluated in CEM cell cultures against HIV-1 wild-type and HIV-1 strains that contain mutations in the NNRTI pocket of the RT (Table 3). Both the antiviral activity and the cytotoxicity of the compounds against MT-4 and CEM cell cultures was fairly comparable (CC_{50} in CEM cells: 107±3.8 μM for **4**, 97±7.0 μM for **10** and 63±13 μM for **13**). The compounds generally kept activity against E138K RT HIV-1 but lost 10-fold antiviral activity against L100I RT HIV-1, 100-fold activity against Y181C and Y188H RT HIV-1 strains, but at least 3 orders of magnitude against K103N RT HIV-1. The trend of nevirapine activity against the above-mentioned HIV-1 mutant strains was analogous to that of our compounds, except for Y188H RT HIV-1 strain against which nevirapine was totally inactive.

4. Discussion

In our previous papers (Barreca et al., 2001, 2002; Rao et al., 2002, 2003) we demonstrated that in a series of 2,3-diaryl-1,3-thiazolidin-4-ones, highly effective as non-nucleoside RT inhibitors, a high activity level was associated with both the presence of a pyridin-2-yl nucleus at N-3 position and the 2,6-dihalo-substituted phenyl ring at C-2. Indeed, the presence of the two halogen atoms at the 2 and 6 positions restricted the rotation of the phenyl ring and allowed the molecules to assume the characteristic butterfly-like conformation present in many other known NNRTIs (Ding et al., 1995).

In view of these results and in order to better determine the structural characteristics able to improve the anti HIV-1 activity of this class of compounds, the SAR of a new series of 1,3-thiazolidin-4-ones was examined by varying the nature of the halogen atoms at the 2 and 6 positions of the phenyl ring at C-2 and the substitution pattern on the pyrimidin-2-yl nucleus at N-3.

The influence of these structural changes on the anti-HIV activity is shown in Table 2 and discussed below.

Considering first of all the effect of the halogen substituents on the phenyl ring at C-2, the 2,6-dichorophenyl derivatives were always more active than the corresponding 2,6-difluoro-substituted ones. The favourable effect of the chlorine is confirmed by the finding that 2-chloro,6-fluoro-derivatives possessed an intermediate activity between dichloro and difluoro analogues.

Turning to the biological effects of substituent variations on the pyrimidin-2-yl ring at N-3, the moderate anti-HIV-1 activity of unsubstituted compounds **1**–**3** was enhanced by the introduction of a methyl group at C-4′. Starting from this point, we explored the effect of the addition of a second substituent at C-6′ position of the pyrimidine ring. The presence of a second methyl group led to compounds **10**–**12** that were among the most active in the series, thus underlining the importance of lipophilicity.

However, the electronic properties too of the substituents seem to have a significant impact on the resultant antiviral activity. In fact, the 6′-methoxy analogues **13**–**15** showed antiviral activity comparable to that of the 6′-methyl compounds **10**–**12** and much higher than that of the derivatives **7**–**9** bearing a lipophilic but electron-withdrawing chlorine atom at position 6′. The markedly-reduced activity of the 6′-hydroxy-substituted compounds **16**–**18** pointed out that a polar group in this position had a detrimental effect on the affinity of the compounds for the hydrophobic NNRTI-binding site.

Compounds **4**, **10** and **13**, which ranked among the most potent inhibitors of HIV-1 in MT-4 cell cultures, were found to have very comparable anti-HIV-1 activity in CEM cell cultures. Therefore they were also evaluated in CEM cell cultures against an extensive panel of mutant virus strains containing in their RT a single mutation that is characteristic for the HIV-NNRTI resistance profile (Table 3). The compounds retained activity against some mutant HIV-1 strains, whereas against other mutant viruses their activity was reduced by 100 to 1000-fold relative to that recorded for the wild-type strain. Such a reduction was comparable to that of nevirapine. Therefore, the 1,3-thiazolidin-4-one derivatives could be considered to behave as first-generation NNRTIs, which may become very insensitive to virus strains that contain single mutation in their RT.

In conclusion, novel 2,3-diaryl-1,3-thiazolidin-4-ones were synthesised and characterised. Some of the compounds proved to be potent anti-HIV-1 agents, inhibiting virus replication at 10–40 nM concentrations. They behaved as typical NNRTIs in that their activity was restricted to HIV-1, with significantly reduced potency against the characteristic NNRTI-resistant mutants K103N and Y181C.

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