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Synthesis of new 2,3-diaryl-1,3-thiazolidin-4-ones as anti-HIV agents

Angela Rao [a], Jan Balzarini [b], Anna Carbone [a], Alba Chimirri [a], Erik De Clercq [b], Anna Maria Monforte [a], Pietro Monforte [a,*], Christophe Pannecouque [b], Maria Zappalà [a]

[a] *Dipartimento Farmaco-Chimico, Università di Messina, viale Annunziata, Messina 98168, Italy*
[b] *Rega Institute for Medical Research, Katholieke Universiteit Leuven, 10 Minderbroedersstraat, Leuven B-3000, Belgium*

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Abstract

Several 2,3-diaryl-1,3-thiazolidin-4-ones were synthesized and evaluated as anti-HIV agents. The results of the in vitro tests showed that some of them were highly effective inhibitors of HIV-1 replication at 30–50 nM concentrations with minimal cytotoxicity, thereby acting as non-nucleoside HIV-1 reverse transcriptase inhibitors (NNRTIs).

Keywords: 2,3-diaryl-1,3-thiazolidin-4-ones; Anti-HIV activity; NNRTIs

1. Introduction

The therapeutic agents currently approved for the treatment of HIV-1 infections include three non-nucleoside reverse transcriptase inhibitors (NNRTIs): nevirapine, delavirdine and efavirenz [1]. Unlike the nucleosides/nucleotides that act at the catalytic site of HIV reverse transcriptase (RT) by terminating DNA synthesis, NNRTIs bind in a region of the enzyme, which is approximately 10 Å away from the catalytic site. Their binding appears to result in a distortion of the catalytic site, which affects the ability of the enzyme to carry out its catalytic functions [2].

Since they are not analogues of natural compounds and do not utilize the biochemical machinery of the host cells, NNRTIs usually manifest side effects milder than those resulting from treatment with nucleosides. Although the therapeutic potential of NNRTIs has been compromised by the rapid development of resistance, they have proven to be useful in combination therapy with nucleoside RT and protease inhibitors [3].

A multiple-drug treatment approach avoids or delays emergence of resistant viral strains and has indeed contributed to the declining morbidity and mortality among HIV-infected patients [4]. However, there are certain factors that restrict the selection of the agents for combination therapy, including drug compatibilities, adverse effects and cross resistance. Therefore, the synthesis of new effective NNRTIs remains a worthwhile goal.

In the course of our studies aimed at the discovery of new NNRTIs [5–7], we have found that members of a series of 2,3-diaryl-1,3-thiazolidin-4-ones [8–12] were highly effective in inhibiting the cytopathic effect of HIV-1 in human T-lymphocyte cells. The RT activity inhibition assays demonstrated that these compounds represent a new class of NNRTIs [8]. Preliminary molecular modelling studies suggested that the binding site of these compounds, similarly to other NNRTIs, is the non-nucleoside-binding pocket [10].

We have demonstrated that a high activity level was associated with the presence of a 2,6-dihalosubstituted phenyl ring at C-2. Moreover, we found that an increase in antiviral potency was dependent on the presence of a (hetero)aromatic nucleus at N-3 bearing a small lipophilic substituent at the *meta* position.

Following these results and in order to better determine the structural characteristics able to improve the anti-HIV-1 activity of this class of compounds, we extended our studies to the synthesis of a new series of 1,3-thiazolidin-4-ones in which the optimal (hetero)aromatic ring at N-3 was maintained, whereas the 2,6-dihalophenyl ring at C-2 was modified by varying the substitution pattern in terms of nature and number of the substituents.

* Corresponding author.
E-mail address: monforte@pharma.unime.it (P. Monforte).

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Compounds synthesized were evaluated for prevention of the cytopathic effect of HIV in cell-based assays. In addition, to confirm their mechanism of action, the inhibitory effects on HIV-1 RT were examined.

2. Results and discussion

The synthesis of the new 2,3-diaryl-1,3-thiazolidin-4-ones was carried out by reacting a properly-substituted benzaldehyde with an equimolar amount of a suitable (hetero)aromatic amine in the presence of an excess of mercaptoacetic acid in refluxing toluene (Scheme 1). The products obtained were isolated by conventional workup in satisfactory yields. Both analytical and ^{1}H NMR spectral data of all the synthesized compounds are in full agreement with the proposed structures.

All compounds obtained were tested for anti-HIV activity by determining their ability to inhibit the replication of HIV-1 (III_B) or HIV-2 (ROD) in human T-lymphocyte (MT-4) cells and the results are reported in Table 1, in which the data of some 2-(2,6-difluorophenyl)-derivatives (i.e. **1, 7, 13, 19, 25** and **31**) have been included for comparison purposes. The anti-HIV activity of several compounds was also measured in HIV-1 (III_B) infected CEM cells (Table 2). Compound-induced cytotoxicity was also measured in MT-4 and CEM cells parallel with the antiviral activity. A select group of compounds was moreover evaluated for the inhibitory effects on HIV-1 RT enzymatic activity and the results are also reported in Table 2.

Several of the new compounds prevented the cytopathic effect of HIV-1 III_B at nanomolar concentrations and were minimally toxic to MT-4 or CEM cells resulting in remarkably high selectivity indices. It is worth noting that compound **24**, one of the most promising of the series, possessed a selectivity index >7000.

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 [13].

From the structure–activity relationship point of view, the antiviral activity varies considerably with the nature of both (hetero)aromatic nucleus at N-3 position and the substituents on the phenyl ring at C-2. In fact, pyridin-2-yl derivatives are more active than the corresponding pyrimidin-2-yl and phenyl ones. It is also worth noting that in the pyridin-2-yl series a bromine atom or a methyl group at 6-position is an important feature for potent anti-HIV agents and that the shift of the methyl group from 6- to 4-position negatively influences the activity. Considering the substitution pattern on the C-2 phenyl ring, the results obtained confirm that the presence of two halogen atoms at 2- and 6-positions is of paramount importance to increase the activity. Indeed, as previously reported [12], these structural features restrict the rotation of the phenyl ring and allow the molecules to assume the characteristic butterfly-like conformation present in many other known NNRTIs [14]. The replacement of one fluorine atom with a methoxy group maintains the activity (**21** vs. **19, 33** vs. **31**), whereas the presence of two methoxy groups induces a decrease in activity with the sole exception of **24** which is one of the most promising derivatives of the series.

Furthermore, the substitution of both fluorine atoms with methyl groups is detrimental for anti-HIV activity leading to loss of potency of one order of magnitude.

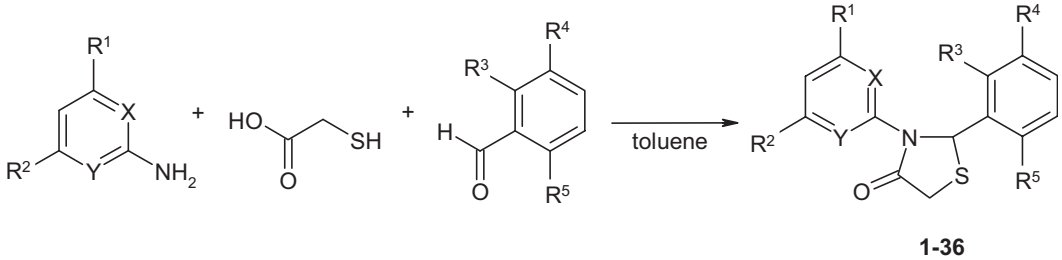
Of the 2,3,5-trisubstituted congeners, the best results have been recorded among the 2-chloro,3-methyl,6-fluoro derivatives, the most active of which is compound **17** that possesses an EC_{50} = 0.050 μM.

In conclusion, a new series of 2,3-diaryl-1,3-thiazolidin-4-ones was synthesized and characterized and some of them proved to be potent anti-HIV agents. Once again, the results reported in this study confirm that the anti-HIV activity in this class of NNRTIs is strongly dependent on the nature of the substituents at C-2 and N-3 of the thiazolidinone ring.

3. Experimental

3.1. Chemistry

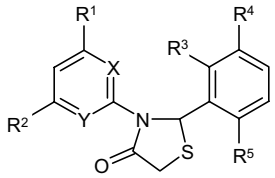
Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. Elemental analyses (C, H and N) were carried out on a Carlo Erba Model 1106 Elemental Analyzer and the results are within ±0.4% of the



1-36

Scheme 1. Synthesis of 2,3-diaryl-1,3-thiazolidin-4-ones.

Table1
Anti-HIV-1 activity, cytotoxicity and selectivity index in MT-4 cells for compounds **1–36**



Compound	X	Y	R^1	R^2	R^3	R^4	R^5	EC_{50} (μM) [a]	CC_{50} (μM) [b]	SI [c]
1 [d]	CH	CH	Me	H	F	H	F	0.688±0.164	207.8±54.2	302
2	CH	CH	Me	H	Me	H	Me	NA	36.5±10.1	
3	CH	CH	Me	H	F	H	MeO	1.16±0.09	144.4±55.2	124
4	CH	CH	Me	H	F	Cl	CF_3	**NA**	32.3±4.7	
5	CH	CH	Me	H	Cl	Me	F	1.49±0.36	40.9±1.5	27
6	CH	CH	Me	H	MeO	H	MeO	1.91±1.15	208.7±34.1	109
7 [e]	N	CH	H	Me	F	H	F	0.248±0.026	242.2±0.3	976
8	N	CH	H	Me	Me	H	Me	5.73±0.13	41.5±1.67	7
9	N	CH	H	Me	F	H	MeO	0.879±0.439	≥220	≥250
10	N	CH	H	Me	F	Cl	CF_3	2.12±0.05	92.6±96.0	44
11	N	CH	H	Me	Cl	Me	F	0.322±0.128	295.3±102.6	917
12	N	CH	H	Me	MeO	H	MeO	3.33±0.60	249.0±15.4	75
13 [e]	N	CH	Me	H	F	H	F	0.082±0.029	126.0±34.9	1536
14	N	CH	Me	H	Me	H	Me	1.54±0.47	284.0±76.3	184
15	N	CH	Me	H	F	H	MeO	0.288±0.216	165.9±84.3	576
16	N	CH	Me	H	F	Cl	CF_3	3.02±1.74	121.5±104.3	40
17	N	CH	Me	H	Cl	Me	F	0.050±0.002	38.0±3.3	760
18	N	CH	Me	H	MeO	H	MeO	1.36±0.06	241.5±28.7	178
19 [e]	N	CH	Br	H	F	H	F	0.030±0.013	32.0±0.54	1066
20	N	CH	Br	H	Me	H	Me	6.33±1.57	268.3±49.4	42
21	N	CH	Br	H	F	H	MeO	0.034±0.021	100.8±98.3	2964
22	N	CH	Br	H	F	Cl	CF_3	0.658±0.395	86.4±56.5	131
23	N	CH	Br	H	Cl	Me	F	0.349±0.423	21.1±13.8	60
24	N	CH	Br	H	MeO	H	MeO	0.045±0.002	>316.2	>7026
25 [e]	N	CH	Me	Me	F	H	F	0.090±0.037	64.1±71.1	712
26	N	CH	Me	Me	Me	H	Me	4.35±2.78	>212.5	>49
27	N	CH	Me	Me	**F**	**H**	MeO	0.202±0.123	≥209.4	≥1037
28	N	CH	Me	Me	F	Cl	CF_3	5.88±4.35	163.0±65.6	28
29	N	CH	Me	Me	Cl	Me	F	0.234±0.111	35.2±1.8	150
30	N	CH	Me	Me	MeO	H	MeO	1.13±0.32	310.0±50.5	274
31 [f]	N	N	Me	H	F	H	F	0.39±0.13	>406.7	>1100
32	N	N	Me	H	Me	H	Me	3.41±2.24	193.3±21.7	57
33	N	N	Me	H	F	H	MeO	0.532±0.185	317.4±37.2	597
34	N	N	Me	H	F	Cl	CF_3	0.918±0.153	192.3±28.0	209
35	N	N	Me	H	Cl	Me	F	0.769±0.615	155.9±41.0	203
36	N	N	Me	H	MeO	H	MeO	0.905±0.199	231.2±11.7	255

[a] 50% Effective concentration or concentration required to reduce HIV-1-(III_B)-induced cytopathic effect by 50% in MT-4 cells.
[b] Cytotoxic concentration or concentration required to reduce MT-4 cell viability by 50%.
[c] Selectivity index: ratio of CC_{50} to EC_{50}.
[d] Data from Ref. [11].
[e] Data from Ref. [10].
[f] Data from Ref. [12]. NA, not active.

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 TMS as internal standard and coupling constants (J) in Hz.

3.1.1. General procedure for the synthesis of 2,3-diaryl-1,3-thiazolidin-4-ones

To a stirred solution of the aromatic amine (8 mmol) in dry toluene (50 ml), 2-mercaptoacetic acid (16 mmol) and the appropriate aldehyde (8 mmol) were added. The reaction mixture was refluxed for 48 h and then neutralized by a

Table2
Anti-HIV-1 activity, cytotoxicity and selectivity index in CEM cells and RT inhibitory activity for some 2,3-diaryl-1,3-thiazolidin-4-ones

Compound	EC_{50}(μM)[a]	CC_{50}(μM)[b]	SI[c]	IC_{50}(μM)[d]
2	NA	30.1 ± 2.48		
3	3.25 ± 2.65	39.38 ± 7.25	12	28.4 ± 24.4
4	NA	23.5 ± 2.2		
5	0.99 ± 0.35	30.9 ± 10.7	31	40.2 ± 8.6
9	0.391 ± 0.33	276 ± 52	340	
15	0.219 ± 0.043	162 ± 8.8	741	
16	1.15 ± 0.82	79.1 ± 72.2	69	
17	0.068 ± 0.044	30.9 ± 3.2	454	0.83 ± 0.00
20	NA	> 275		
21	0.065 ± 0.031	192 ± 18.5	2950	
22	0.329 ± 0.175	24.5 ± 10.5	75	
23	0.273 ± 0.174	27.6 ± 8.5	101	
26	1.12 ± 0.22	> 320	> 286	
27	0.391 ± 0.33	209 ± 42	535	
28	5.92 ± 3.69	130 ± 35.8	22	
29	0.128 ± 0.019	27.9 ±3.2	220	
32	0.837 ± 0.23	144.8 ± 12.1	173	
33	0.469 ± 0.219	> 313	>667	9.70 ± 0.94
35	0.338 ± 0.246	158.5 ± 51.4	489	

[a] 50% Effective concentration or concentration required to protect CEM cells against the cytopathicity of HIV-1–(III_B) by 50%.
[b] Cytotoxic concentration or concentration required to reduce CEM cell viability by 50%.
[c] Selectivity index: Ratio CC_{50}/EC_{50}.
[d] Poly(C)/oligo(dG) was used as the template/primer and [^{3}H]dGTP as the radiolabelled substrate. NA = not active

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 **4–5, 8–9, 11–12, 14–17, 20–23, 26–30, 33** and **35**. The remaining compounds were isolated by silica gel column chromatography eluting with $CHCl_3$ (**2–3, 10** and **34**), $CHCl_3$/MeOH 99:1 (**6, 18** and **32**), $CHCl_3$/MeOH 98:2 (**35**) or cyclohexane/EtOAc 70:30 (**24**). All compounds were recrystallized from EtOH. Data of compounds **1, 7, 13, 19, 25** and **31** have been previously reported [10–12].

*3.1.1.1. 2-(2,6-Dimethylphenyl)-3-(3-methylphenyl)-1,3-thiazolidin-4-one (**2**).* Yield: 35%, m.p.: 103–105 °C; ^{1}H NMR (δ) 2.26, 2.38 and 2.53 (3s, 9H, Me), 3.95 (s, 2H, CH_2-5), 6.80–7.15 (m, 8H, ArH and H-2). Anal. ($C_{18}H_{19}NOS$) C, H, N.

*3.1.1.2. 2-(2-Fluoro,6-methoxyphenyl)-3-(3-methylphenyl)-1,3-thiazolidin-4-one (**3**).* Yield: 32%, m.p.: 102–104°C; ^{1}H NMR (δ) 2.27 (s, 3H, Me), 3.80–4.10 (m, 5H, CH_2-5 and OMe), 6.60–7.20 (m, 8H, ArH and H-2). Anal. ($C_{17}H_{16}FNO_2S$) C, H, N.

*3.1.1.3. 2-(3-Chloro,2-fluoro,6-trifluoromethylphenyl)-3-(3-methylphenyl)-1,3-thiazolidin-4-one (**4**).* Yield: 83%, m.p.: 127–129 °C; ^{1}H NMR (δ) 2.28 (s, 3H, Me), 3.88 (d, 1H, J = 15.4 Hz, 5-H_A), 4.13 (dd, 1H, J = 1.9 and 15.4 Hz, 5-H_B), 6.52 (d, 1H, J = 1.9 Hz, H-2), 6.98–7.46 (m, 6H, ArH). Anal. ($C_{17}H_{12}ClF_4NOS$) C, H, N.

*3.1.1.4. 2-(2-Chloro,6-fluoro,3-methylphenyl)-3-(3-methylphenyl)-1,3-thiazolidin-4-one (**5**).* Yield: 85%, m.p.: 130–133 °C; ^{1}H NMR (δ) 2.22 and 2.28 (2s, 6H, Me), 3.86 (d, 1H, J = 15.4 Hz, 5-H_A), 4.12 (dd, 1H, J = 1.9 and 15.4 Hz, 5-H_B), 6.78 (d, 1H, J = 1.9 Hz, H-2), 6.87–7.20 (m, 6H, ArH). Anal. ($C_{17}H_{15}ClFNOS$) C, H, N.

*3.1.1.5. 2-(2,6-Dimethoxyphenyl)-3-(3-methylphenyl)-1,3-thiazolidin-4-one (**6**).* Yield: 24%, m.p.: 130–135 °C; ^{1}H NMR (δ) 2.25 (s, 3H, Me), 3.73 and 3.91 (2s, 6H, OMe), 3.80 (d, 1H, J = 15.1 Hz, 5-H_A), 4.05 (dd, 1H, J = 1.9 and 15.1 Hz, 5-H_B), 6.39–7.16 (m, 8H, ArH and H-2). Anal. ($C_{18}H_{19}NO_3S$) C, H, N.

*3.1.1.6. 2-(2,6-Dimethylphenyl)-3-(4-methylpyridin-2-yl)-1,3-thiazolidin-4-one (**8**).* Yield: 33%, m.p.: 110–113 °C; ^{1}H NMR (δ) 2.33, 2.41 and 2.59 (3s, 9H, Me), 3.98 (s, 2H, CH_2-5), 6.79–8.05 (m, 7H, ArH and H-2). Anal. ($C_{18}H_{18}N_2OS$) C, H, N.

*3.1.1.7. 2-(2-Fluoro,6-methoxyphenyl)-3-(4-methylpyridin-2-yl)-1,3-thiazolidin-4-one (**9**).* Yield: 28%, m.p.: 136–139 °C; ^{1}H NMR (δ) 2.32 (s, 3H, Me), 3.81 (d, 1H, J = 15.7 Hz, 5-H_A), 3.88 (s, 3H, OMe), 4.15 (dd, 1H, J = 1.4 and 15.7 Hz, 5-H_B), 6.56–8.11 (m, 7H, ArH and H-2). Anal. ($C_{17}H_{15}FN_2O_2S$) C, H, N.

*3.1.1.8. 2-(3-Chloro,2-fluoro,6-trifluoromethylphenyl)-3-(4-methylpyridin-2-yl)-1,3-thiazolidin-4-one (**10**).* Yield: 81%, m.p.: 125–127 °C; ^{1}H NMR (δ) 2.35 (s, 3H, Me), 3.85 (d, 1H, J = 15.9 Hz, 5-H_A), 4.21 (dd, 1H, J = 1.6 and 15.9 Hz, 5-H_B),

6.82–8.01 (m, 6H, ArH and H-2). Anal. ($C_{17}H_{11}ClF_4N_2OS$) C, H, N.

*3.1.1.9. 2-(2-Chloro,6-fluoro,3-methylphenyl)-3-(4-methylpyridin-2-yl)-1,3-thiazolidin-4-one (**11**)*. Yield: 83%, m.p.: 145–148 °C; ^{1}H NMR (δ) 2.14 and 2.34 (2s, 6H, Me), 3.85 (d, 1H, J = 15.7 Hz, 5-H_A), 4.19 (dd, 1H, J = 1.6 and 15.7 Hz, 5-H_B), 6.82–8.10 (m, 6H, ArH and H-2). Anal. ($C_{17}H_{14}ClFN_2OS$) C, H, N.

*3.1.1.10. 2-(2,6-Dimethoxyphenyl)-3-(4-methylpyridin-2-yl)-1,3-thiazolidin-4-one (**12**)*. Yield: 32%, m.p.: 144–146 °C; ^{1}H NMR (δ) 2.30 (s, 3H, Me), 3.76–3.90 (m, 7H, OMe and 5-H_A), 4.13 (dd, 1H, J = 1.6 and 15.4 Hz, 5-H_B), 6.45–8.10 (m, 7H, ArH and H-2). Anal. ($C_{18}H_{18}N_2O_3S$) C, H, N.

*3.1.1.11. 2-(2,6-Dimethylphenyl)-3-(6-methylpyridin-2-yl)-1,3-thiazolidin-4-one (**14**)*. Yield: 43%, m.p.: 143–146 °C; ^{1}H NMR (δ) 2.27, 2.39 and 2.63 (3s, 9H, Me), 3.97 (s, 2H, CH_2-5), 6.80–7.69 (m, 7H, ArH and H-2). Anal. ($C_{18}H_{18}N_2OS$) C, H, N.

*3.1.1.12. 2-(2-Fluoro,6-methoxyphenyl)-3-(6-methylpyridin-2-yl)-1,3-thiazolidin-4-one (**15**)*. Yield: 34%, m.p.: 113–115 °C; ^{1}H NMR (δ) 2.32 (s, 3H, Me), 3.82 (d, 1H, J = 15.7 Hz, 5-H_A), 3.86 (s, 3H, OMe), 4.17 (dd, 1H, J = 1.6 and 15.7 Hz, 5-H_B), 6.58–7.97 (m, 7H, ArH and H-2). Anal. ($C_{17}H_{15}FN_2O_2S$) C, H, N.

*3.1.1.13. 2-(3-Chloro,2-fluoro,6-trifluoromethylphenyl)-3-(6-methylpyridin-2-yl)-1,3-thiazolidin-4-one (**16**)*. Yield: 86%, m.p.: 125–127 °C; ^{1}H NMR (δ) 2.22 (s, 3H, Me), 3.85 (d, 1H, J = 15.9 Hz, 5-H_A), 4.20 (dd, 1H, J = 1.6 and 15.9 Hz, 5-H_B), 6.84–8.02 (m, 6H, ArH and H-2). Anal. ($C_{17}H_{11}ClF_4N_2OS$) C, H, N.

*3.1.1.14. 2-(2-Chloro,6-fluoro,3-methylphenyl)-3-(6-methylpyridin-2-yl)-1,3-thiazolidin-4-one (**17**)*. Yield: 76%, m.p.: 127–128°C; ^{1}H NMR (δ) 2.15 and 2.27 (2s, 6H, Me), 3.85 (d, 1H, J = 15.7 Hz, 5-H_A), 4.21 (dd, 1H, J = 1.1 and 15.7 Hz, 5-H_B), 6.97–8.29 (m, 6H, ArH and H-2). Anal. ($C_{17}H_{14}ClFN_2OS$) C, H, N.

*3.1.1.15. 2-(2,6-Dimethoxyphenyl)-3-(6-methylpyridin-2-yl)-1,3-thiazolidin-4-one (**18**)*. Yield: 25%, m.p.: 143–145 °C; ^{1}H NMR (δ) 2.30 (s, 3H, Me), 3.78 and 3.83 (2s, 6H, OMe), 3.84 (d, 1H, J = 15.4 Hz, 5-H_A), 4.15 (dd, 1H, J = 1.6 and 15.4 Hz, 5-H_B), 6.45–7.86 (m, 7H, ArH and H-2). Anal. ($C_{18}H_{18}N_2O_3S$) C, H, N.

*3.1.1.16. 3-(6-Bromopyridin-2-yl)-2-(2,6-dimethylphenyl)-1,3-thiazolidin-4-one (**20**)*. Yield: 32%, m.p.: 159–162 °C; ^{1}H NMR (δ) 2.36 and 2.66 (2s, 6H, Me), 3.98 (s, 2H, CH_2), 6.82–7.92 (m, 7H, ArH and H-2). Anal. ($C_{17}H_{15}BrN_2OS$) C, H, N.

*3.1.1.17. 3-(6-Bromopyridin-2-yl)-2-(2-fluoro,6-methoxyphenyl)-1,3-thiazolidin-4-one (**21**)*. Yield: 52%, m.p.: 110–112 °C; ^{1}H NMR (δ) 3.81 (d, 1H, J = 15.9 Hz, 5-H_A), 3.90 (s, 3H, OMe), 4.18 (dd, 1H, J = 1.6 and 15.9 Hz, 5-H_B), 6.63–8.25 (m, 7H, ArH and H-2). Anal. ($C_{16}H_{12}BrFN_2O_2S$) C, H, N.

*3.1.1.18. 3-(6-Bromopyridin-2-yl)-2-(3-chloro,2-fluoro,6-trifluoromethylphenyl)-1,3-thiazolidin-4-one (**22**)*. Yield: 75%, m.p.: 149–151 °C; ^{1}H NMR (δ) 3.84 (d, 1H, J = 15.9, 5-H_A), 4.20 (dd, 1H, J = 1.6 and 15.9 Hz, 5-H_B), 6.98–8.28 (m, 6H, ArH and H-2). Anal. ($C_{16}H_8BrClF_4N_2OS$) C, H, N.

*3.1.1.19. 3-(6-Bromopyridin-2-yl)-2-(2-chloro,6-fluoro,3-methylphenyl)-1,3-thiazolidin-4-one (**23**)*. Yield: 64%, m.p.: 128–129 °C; ^{1}H NMR (δ) 2.33 (s, 3H, Me), 3.84 (d, 1H, J = 15.9 Hz, 5-H_A), 4.20 (dd, 1H, J = 1.6 and 15.9 Hz, 5-H_B), 6.82–8.05 (m, 6H, ArH and H-2). Anal. ($C_{16}H_{11}BrClFN_2OS$) C, H, N.

*3.1.1.20. 3-(6-Bromopyridin-2-yl)-2-(2,6-dimethoxyphenyl)-1,3-thiazolidin-4-one (**24**)*. Yield: 23%, m.p.: 126–129 °C; ^{1}H NMR (δ) 3.46–3.95 (m, 7H, OMe and 5-H_A), 4.15 (dd, 1H, J = 1.6 and 15.7 Hz, 5-H_B), 6.51–8.20 (m, 7H, ArH and H-2). Anal. ($C_{17}H_{15}BrN_2O_3S$) C, H, N.

*3.1.1.21. 2-(2,6-Dimethylphenyl)-3-(4,6-dimethylpyridin-2-yl)-1,3-thiazolidin-4-one (**26**)*. Yield: 42%, m.p.: 155 °C dec.; ^{1}H NMR (δ) 2.23, 2.27, 2.40 and 2.62 (4s, 12H, Me), 3.96 (s, 2H, CH_2), 6.65–7.46 (m, 6H, ArH and H-2). Anal. ($C_{19}H_{20}N_2OS$) C, H, N.

*3.1.1.22. 3-(4,6-Dimethylpyridin-2-yl)-2-(2-fluoro,6-methoxyphenyl)- 1,3-thiazolidin-4-one (**27**)*. Yield: 33%, m.p.: 139–141 °C; ^{1}H NMR (δ) 2.26 (s, 6H, Me), 3.81 (d, 1H, J = 15.4 Hz, 5-H_A), 3.87 (s, 3H, OMe), 4.16 (dd, 1H, J = 1.6 and 15.4 Hz, 5-H_B), 6.57–7.76 (m, 6H, ArH and H-2). Anal. ($C_{18}H_{17}FN_2O_2S$) C, H, N.

*3.1.1.23. 2-(3-Chloro,2-fluoro,6-trifluoromethylphenyl)-3-(4,6-dimethylpyridin-2-yl)-1,3-thiazolidin-4-one (**28**)*. Yield: 58%, m.p.: 144–146 °C; ^{1}H NMR (δ) 2.18 and 2.30 (2s, 6H, Me), 3.83 (d, 1H, J = 15.9 Hz, 5-H_A), 4.19 (dd, 1H, J = 1.6 and 15.9 Hz, 5-H_B), 6.69–7.82 (m, 5H, ArH and H-2). Anal. ($C_{18}H_{13}ClF_4N_2OS$) C, H, N.

*3.1.1.24. 2-(2-Chloro,6-fluoro,3-methylphenyl)-3-(4,6-dimethylpyridin-2-yl)-1,3-thiazolidin-4-one (**29**)*. Yield: 75%, m.p.: 110–113 °C; ^{1}H NMR (δ) 2.15 and 2.28 (2s, 9H, Me), 3.84 (d, 1H, J = 15.9 Hz, 5-H_A), 4.21 (dd, 1H, J = 1.6 and 15.9 Hz, 5-H_B), 6.67–7.86 (m, 5H, ArH and H-2). Anal. ($C_{18}H_{16}ClFN_2OS$) C, H, N.

*3.1.1.25. 2-(2,6-Dimethoxyphenyl)-3-(4,6-dimethylpyridin-2-yl)-1,3-thiazolidin-4-one (**30**)*. Yield: 28%, m.p.: 158–160 °C; ^{1}H NMR (δ) 2.24 and 2.26 (2s, 6H, Me), 3.77–3.90

(m, 7H, OMe and 5-H_A), 4.14 (dd, 1H, J = 1.6 and 15.4 Hz, 5-H_B), 6.45–7.64 (m, 6H, ArH and H-2). Anal. ($C_{19}H_{20}N_2O_3S$) C, H, N.

*3.1.1.26. 2-(2,6-Dimethylphenyl)-3-(4-methylpyrimidin-2-yl)-1,3-thiazolidin-4-one (**32**).* Yield: 28%, m.p.: 137–140 °C; ^{1}H NMR (δ) 2.36, 2.51 and 2.59 (3s, 9H, Me), 3.99 (s, 2H, CH_2), 6.82–8.48 (m, 6H, ArH and H-2). Anal. ($C_{17}H_{17}N_3OS$) C, H, N.

*3.1.1.27. 2-(2-Fluoro,6-methoxyphenyl)-3-(4-methylpyrimidin-2-yl)-1,3-thiazolidin-4-one (**33**).* Yield: 32%, m.p.: 148–150 °C; ^{1}H NMR (δ) 2.38 (s, 3H, Me), 3.84 (d, 1H, J = 15.7 Hz, 5-H_A), 3.87 (s, 3H, OMe), 4.17 (dd, 1H, J = 1.1 and 15.7 Hz, 5-H_B), 6.61–8.49 (m, 6H, ArH and H-2). Anal. ($C_{16}H_{14}FN_3O_2S$) C, H, N.

*3.1.1.28. 2-(3-Chloro,2-fluoro,6-trifluoromethylphenyl)-3-(4-methylpyrimidin-2-yl)-1,3-thiazolidin-4-one (**34**).* Yield: 23%, m.p.: 148–151 °C; ^{1}H NMR (δ) 2.32 (s, 3H, Me), 3.87 (d, 1H, J = 15.9 Hz, 5-H_A), 4.21 (dd, 1H, J = 1.6 and 15.9 Hz, 5-H_B), 6.88–8.51 (m, 5H, ArH and H-2). Anal. ($C_{16}H_{10}ClF_4N_3OS$) C, H, N.

*3.1.1.29. 2-(2-Chloro,6-fluoro,3-methylphenyl)-3-(4-methylpyrimidin-2-yl)-1,3-thiazolidin-4-one (**35**).* Yield: 38%, m.p.: 126 °C dec.; ^{1}H NMR (δ) 2.17 and 2.39 (2s, 6H, Me), 3.89 (d, 1H, J = 15.7 Hz, 5-H_A), 4.23 (dd, 1H, J = 1.9 and 15.7 Hz, 5-H_B), 6.86–8.51 (m, 5H, ArH and H-2). Anal. ($C_{16}H_{13}ClFN_3OS$) C, H, N.

*3.1.1.30. 2-(2,6-Dimethoxyphenyl)-3-(4-methylpyrimidin-2-yl)-1,3-thiazolidin-4-one (**36**).* Yield: 23%, m.p.: 147–149 °C; ^{1}H NMR (δ) 2.34 (s, 3H, Me), 3.47–3.91 (m, 7H, OMe and 5-H_A), 4.16 (dd, 1H, J = 1.4 and 15.4 Hz, 5-H_B), 6.48–8.47 (m, 6H, ArH and H-2). Anal. ($C_{17}H_{17}N_3O_3S$) C, H, N.

3.2. Pharmacology

3.2.1. In vitro anti-HIV assay

The methodology of the anti-HIV assays has been previously described [15,16]. Briefly, MT-4 or CEM cells were infected with HIV-1 (III_B) and HIV-2 (ROD) at 100 $CCID_{50}$ (50% cell culture infective dose) per ml of cell suspension. Then, 100 µl of the infected cell suspension was added to microtitre plate containing 100 µl of an appropriate dilution of the test compounds. In MT-4 cells, after 5 d of incubation, the number of viable cells was determined. In CEM cells, after 4 d of incubation, HIV-1-induced syncytium formation was recorded. The 50% effective concentration (EC_{50}) and 50% cytotoxic concentration (CC_{50}) were defined as the compound concentrations required to reduce cell viability (MT-4) or to inhibit virus-induced cytopathicity (CEM) by 50%, or to reduce by 50% the number of viable cells in mock-infected MT-4 and CEM cell cultures, respectively.

3.2.2. HIV-1 RT assay

The procedure for assessing the reverse transcriptase inhibition has been described previously [17]. 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 albumine, 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. For the experiments in which 50% inhibitory concentration (IC_{50}) of the test compounds was determined, fixed concentration of 2.5 µM [^{3}H]dGTP was used.

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