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Inhibition of HIV-1 RT activity by a new series of 3-(1,3,4-thiadiazol-2-yl) thiazolidin-4-one derivatives

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ABSTRACT

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) represent potent anti-HIV agents targeting HIV-1 reverse transcriptase (RT), a crucial enzyme for the viral life cycle. We have previously identified a series of NNRTIs bearing a 2,3-diaryl-1,3-thiazolidin-4-one core and some compounds proved to be effective in inhibiting HIV-1 replication at micromolar concentration. As a continuation in this research work we report the design, the synthesis and the structure–activity relationship studies of a further series of 3-(1,3,4-thiadiazol-2-yl)thiazolidin-4-one derivatives containing an arylthioacetamide group as pharmacophoric structural requirement for binding to the RT catalytic area. The new compounds proved to be effective to inhibit RT activity at micromolar concentrations. Finally, docking studies were carried out in order to rationalize the biological results of the new synthesized inhibitors.

1. Introduction

The global pandemic of human immunodeficiency virus (HIV) remains an important issue to human health worldwide and type 1 is the main causative agent of acquired immunodeficiency syndrome (AIDS). Nowadays, the treatment for HIV/AIDS consists of a combination antiretroviral therapy (ART) including two or more antiretroviral drugs belonging to different drug classes. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) disrupt the normal functions of HIV-1 reverse transcriptase (RT) enzyme via binding to NNRTI binding pocket (NNIBP) close to the polymerase active site and are considered essential components of ART due to their good tolerability, low toxicity and the high selectivity.[1–3] US Food and Drug Administration approved the first generation six NNRTIs for clinical use: nevirapine (NVP), delavirdine (DLV), efavirenz (EFV) as first generation NNRTIs and etravirine (TMC125), rilpivirine (TMC278)[4,5] and doravirine (DOR) as second generation NNRTIs (Fig. 1). Unfortunately, extensive adverse effects combined with a high mutation rate of HIV-1 RT limit the clinical use of NNRTIs.[6–10] Wherefore, there is still needed a development of a next-generation of NNRTIs with increased safety and tolerability profile.

Thiazolidinediones (TZDs) exhibit a wide range of pharmacological activities including antihyperglycemic,[11] antimicrobial,[12] antiviral,[13] antioxidant,[14] anticancer,[15] anti-inflammatory,[16] neuroprotective,[17,18] as well as tyrosinase inhibitory effect[19,20] Among the large library of TZDs, we have discovered several 2,3-diaryl-1,3-thiazolidin-4-one derivatives (Fig. 2) containing different aryl/heteroaryl substituents at C-2 and N-3 of the nucleus demonstrating anti-HIV activity as NNRTIs.[21–25]

In order to expand this series of promising anti-HIV-agents, we decided to introduce new chemical moieties thus decorating the 1,3,4-thiadiazole ring of previously reported 1,3-thiazolidin-4-one derivatives.[25–27] Our choice fell on the N-(hetero)arylacetamide “tail” that has been identified as a structural chemical feature of compounds I-III as promising NNRTIs (Fig. 3).[28–30]

In this context, herein we examined the structure-activity relationships (SARs) for 1,3-thiazolidin-4-ones that demonstrated that their NNRT inhibitory effects were strongly dependent on the nature of the substituents at C-2 and N-3 of the thiazolidinone ring (see Fig. 2).[25,31,32]. Specifically, we speculated that the introduction of a halogen atom on the C-2 phenyl ring was able to positively influence the inhibitory activity. This behavior might be explained by considering the relevant role of hydrophobic interactions with residues Phe227 and Trp229 on the opening hydrophobic channel of the NNIBP in HIV-1 RT.[1,33,34]. Furthermore considering the N-(hetero)arylacetamide “tail” as a structural chemical feature for promising NNRTIs, we combined the versatile scaffold ‘2-aryl-3-(1,3,4-thiadiazol-2-yl)-1,3-thiazolidine-4-one’ with S-linked *N*-arylacetamide.

The designed compounds were synthesized and screened as

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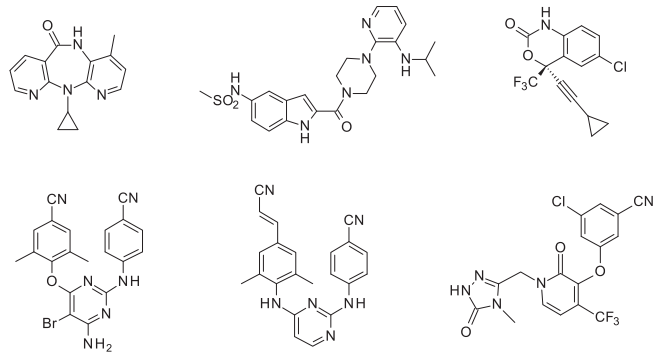


Fig. 1. Chemical structure of first and second generation approved NNRTIs.

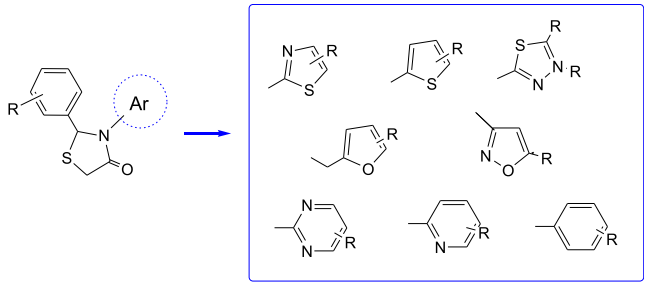


Fig. 2. 2,3-Diaryl-1,3-thiazolidin-4-one derivatives with anti-HIV activity (NNRTIs).

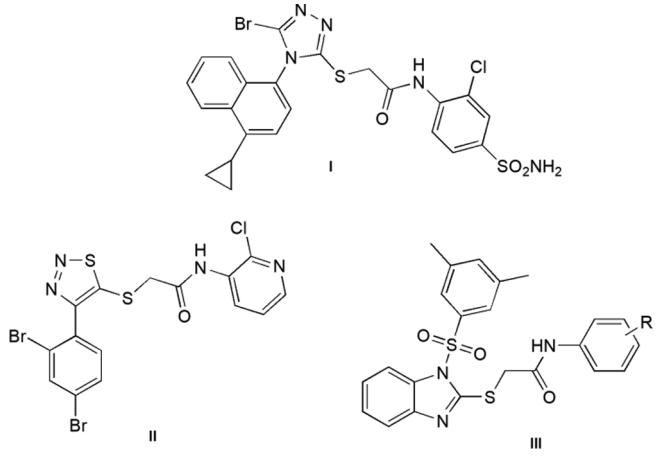


Fig. 3. Previously reported NNRTIs (I, II, and III) bearing N-(hetero)arylacetamide "tail".

inhibitors of polymerase activity of HIV-1 RT in an enzymatic assay and as inhibitors of HIV-1 replication in MT-4 cell culture. Finally, docking studies were carried to clarify the binding mode of the newly synthetized compounds.

2. Results and discussion

On the basis of above mentioned rational design, we report a series of compounds obtained for the combination of the 1,3-thiazolidin-4-one nucleus with the 1,3,4-thiadiazol-2-yl]sulfanyl]-*N*-arylacetamide portion. Inspired by previously optimized SARs for NNRTIs[27] we introduced a sulfonamide or methylsulfonic group in para position of phenyl ring of *N*-arylacetamide (Fig. 4).

To avoid false positive results, we preliminary explored possible assays interference; all designed compounds passed the PAINS (pan-assay interference compounds) filter investigated by means of the free web tool (http://www.swissadme.ch/). Therefore, the target

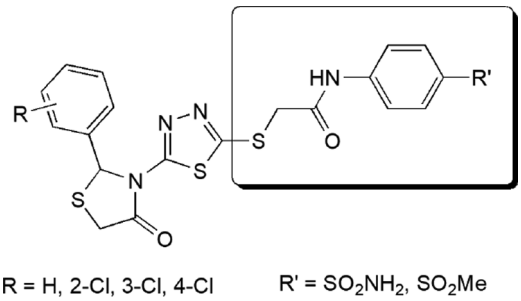
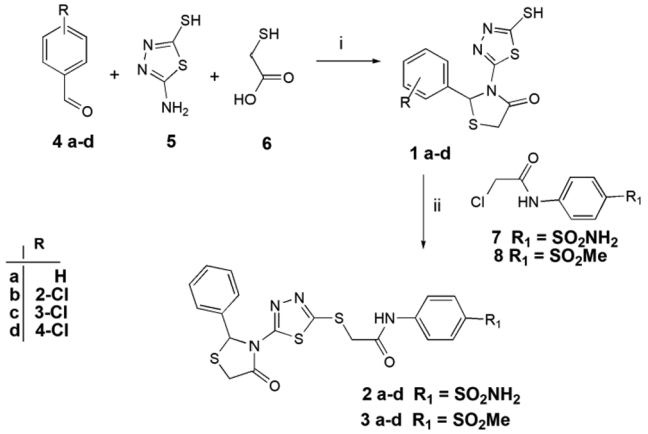


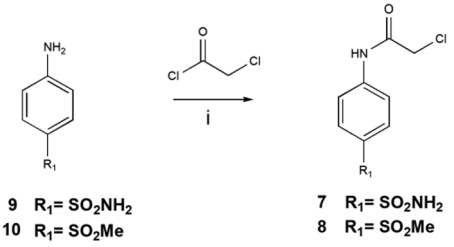
Fig. 4. Chemical structure of the new designed 3-(1,3,4-thiadiazol-2-yl)-1,3-thiazolidin-4-ones.



Scheme 1. Reagents and conditions: (i) CH_3CH_2OH, reflux, 72 h; (ii) K_2CO_3, DMF, r.t., 24 h.

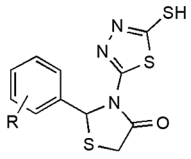
thiazolidin-4-one derivatives were synthesized straightforwardly following synthetic strategy depicted in Scheme 1. The required 2-aryl-3-(5-sulfanyl-1,3,4-thiadiazol-2-yl)thiazolidin-4-ones (**1 a-d**) were prepared by reaction of commercially available 5-amino-1,3,4-thiadiazole-2-thiol (**5**) with 2-mercaptoacetic acid (**6**) and the appropriate benzaldehyde **4a-d**.[35] In turn, key reagents 1a-d were coupled with *N*-arylacetamides 7 and 8, which were prepared by reaction of appropriate commercially available anilines **9–10** with chloroacetyl chloride (see Scheme 2). Finally, the coupling reaction between the 2-aryl-3-(5-sulfanyl-1,3,4-thiadiazol-2-yl)thiazolidin-4-ones (**1 a-d**) and obtained 2-chloro-N-(phenyl)acetamides (**7–8**) afforded in good yields target compounds 2-([5-(4-oxo-2-phenyl-1,3-thiazolidin-3-yl)-1,3,4-thiadiazol-2-yl]sulfanyl)-N-(4-sulfamoyphenyl)acetamide (**2 a-d**) and N-(4-methylsulfonylphenyl)-2-[[5-(4-oxo-2-phenyl-thiazolidin-3-yl)-1,3,4-thiadiazol-2-yl]sulfanyl]acetamide (**3 a-d**). Analytical and spectral data (1H NMR) of all synthesized compounds were in full agreement with the proposed structures.

The newly synthesized compounds were evaluated to assess their ability to inhibit the polymerase activity of HIV-1 RT as well as HIV-1 replication in MT-4 cell cultures. We collected in Table 1 summarizes biological data of target compounds **2 a-d** and **3 a-d**. We also examined

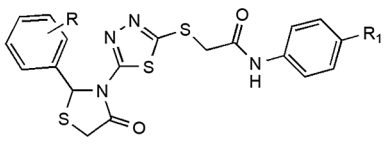


Scheme 2. Reagents and conditions: (i) DIPEA 0 °C, CH_2Cl_2, r.t., 24 h.

Table 1
Anti-RT and anti-HIV-1 activities, cytotoxicity and selectivity index in MT-4 cells.[a]



1 a-d



2 a-d
3 a-d

Comp.	R	R1	IC_{50} (μM)[b]	EC_{50} (μM)[c]	CC_{50} (μM)[d]	SI[e]
1a	H	–	> 100	31.99 ± 0.78	> 423.1	13
1b	2-Cl	–	> 100	>199.78	199.8 ± 18.06	< 1
1c	3-Cl	–	> 100	>238.22	238.2 ± 49.76	< 1
1d	4-Cl	–	> 363	> 194.63	194.6 ± 18.34	< 1
2a	H	SO_2NH_2	30.56 ± 6.12	> 99.24	99.24 ± 15.30	< 1
2b	2-Cl	SO_2NH_2	21.84 ± 2.89	> 18.81	≥18.81	–
2c	3-Cl	SO_2NH_2	19.17 ± 3.86	> 118.17	118.2 ± 11.05	< 1
2d	4-Cl	SO_2NH_2	71.95 ± 7.0	≥225.3	≥222.5	–
3a	H	SO_2CH_3	40.64 ± 6.72	> 130.46	130.5 ± 26.70	< 1
3b	2-Cl	SO_2CH_3	11.83 ± 0.3	≥ 172.9	≥ 172.9	–
3c	3-Cl	SO_2CH_3	15.75 ± 3.95	> 112.88	112.9 ± 34.00	< 1
3d	4-Cl	SO_2CH_3	39.43 ± 13.8	> 115.5	115.5 ± 49.19	< 1
NVP			1.55 ± 0.25	0.18 ± 0.03	> 15.02	> 81

[a] Data reported as mean ± standard deviations.
[b] Concentration required to inhibit the in vitro RNA-dependent DNA polymerase activity of recombinant RT by 50%.
[c] Effective concentration required to reduce HIV-1-induced cytopathic effect by 50% in MT-4 cells.
[d] Cytotoxic concentration required to reduce MT-4 cell viability by 50%.
[e] Selectivity index: ratio CC_{50}/EC_{50}.

the biological effects of synthetic precursors 2-phenyl-3-(5-sulfanyl-1,3,4-thiadiazol-2-yl)thiazolidin-4-ones **1 a-d** as negative control to establish the role of *N*-arylacetamide tail. The results were expressed as IC_{50} values (anti-RT activity), EC_{50} values (anti-HIV-1 activity), CC_{50} values (cytotoxicity) and SI values (selectivity index, given by the CC_{50}/EC_{50} ratio). The FDA-approved drugs nevirapine (NVP) was used as reference compound in the same tests.

The preliminary biological testing for all target compounds **2 a-d** and **3 a-d** revealed that they were generally lower active inhibitors when compared with reference compound NVP. Among the two series of tested compounds **2 a-d** and **3 a-d**, the most promising molecules were derivatives **2c**, **3b** and **3c** showing IC_{50} value of 19.17 μM, 11.83 μM, 15.75 μM, respectively. Interestingly, target compounds **2 a-d** and **3 a-d** were more active than their synthetic precursors 2-phenyl-3-(5-sulfanyl-1,3,4-thiadiazol-2-yl)thiazolidin-4-ones (**1 a-d**), thus suggesting that the introduction of the arylacetamide substituent positively influences the RT enzymatic inhibition. Unfortunately, all the designed derivatives showed low anti-HIV activity in MT-4 cell culture (Table 1).

In order to investigate the binding mode of studied derivatives we performed docking studies into the non-nucleoside binding site of HIV-1 RT (PDB code: 3DLG)[36] using AutoDock Vina[37] following the same procedure described in our previous papers.[30] Fig. 5 displays the docking results of compounds **1c**, **2c** and **3c** that were selected as protype for each class of tested inhibitors. By analysis of the docking results, it was possible to observe that active inhibitors **2c** and **3c** shared a similar binding mode by occupying the hydrophobic pocket made by the residues of Tyr181, Tyr188 and Trp229 (see panels 5B and 5C); moreover they established a favourable hydrogen bond contact with Lys103 that represents an important residues for RT activity.[38,39] On the contrary, the inactive intermediate **1c** was not able to form this crucial hydrogen bond interaction with Lys103. Additionally, compound **1c** does not occupy the region lined by Lys101 and Pro236 (panel 5A) as relevant residues for the interaction of well-known active NNRTIs.[30,36,38,40,41]

3. Conclusions

Herein we reported design, synthesis and biological evaluation of 3-(1,3,4-thiadiazol-2-yl)thiazolidin-4-one derivatives. Biological tests highlighted that the presence of arylacetamide moiety positively influences the RT enzymatic inhibition. This evidence was supported also by docking studies that showed the contacts between our ligands and crucial residues controlling the recognition process in to the enzymatic cavity RT.

4. Experimental section

4.1. Chemistry

All starting materials and reagents commercially available (Sigma-Aldrich Milan, Italy; Alfa Aesar Karlsruhe, Germany) were used without further purification. Melting points were determined on a Buchi B-545 apparatus (BUCHI Labortechnik AG Flawil, Switzerland) and are uncorrected. Elemental analyses (C, H, N) were carried out on a Carlo Erba Model 1106 Elemental Analyzer (Carlo Erba Milano, Italy); the results confirmed a ≥95% purity. Merck silica gel 60 F254 plates were used for analytical TLC (Merck KGaA, Darmstadt, Germany). Flash Chromatography (FC) was carried out on a Biotage SP1 EXP (Biotage AB Uppsala, Sweden). 1H NMR spectra were measured in CDCl3 with a Gemini 300 spectrometer (Varian Inc. Palo Alto, California USA); chemical shifts are expressed in δ (ppm) and coupling constants (J) in Hz.

4.1.1. General procedure to synthesize 2-phenyl-3-(5-sulfanyl-1,3,4-thiadiazol-2-yl)thiazolidin-4-ones (1 a-d)

To a solution of commercially available 5-amino-1,3,4-thiadiazol-2-thiol (1 mmol) in ethanol (16 mL) the appropriate benzaldehyde (**4 a-d**) (1 mmol) and 2-mercaptoacetic acid (1 mmol, 70 μl) were added. The mixture was stirred 72 h at 100 °C; the evaporation of the solvent furnished the crude product that was crystallized from a mixture of Et_2O and EtOH. This synthesis was carried out following a previously reported procedure with slight modifications and the 1H NMR and 13C NMR spectral assignments were in good agreement with previous data.[18,35,42] For intermediates **1a**, **1b**, and **1d** registered CAS numbers have been already assigned.

4.1.1.1. Phenyl-3-(5-sulfanyl-1,3,4-thiadiazol-2-yl)thiazolidin-4-one (1a)

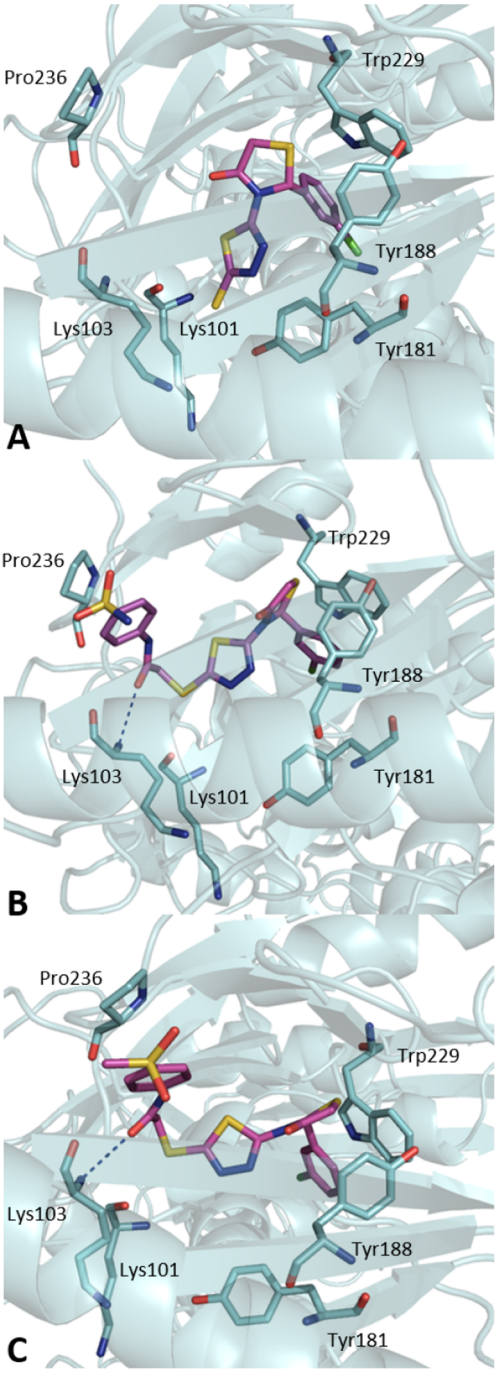


Fig. 5. Binding mode of derivatives **1c** (A), **2c** (B), **3c** (C) in complex with NNIBP (binding affinity values of −8,6 Kcal/mol, −8,8 Kcal/mol and −8,9 Kcal/mol, respectively). The hydrogen bonds are in dotted lines. The figure was created using PyMOL software.

CAS registry number 138225-69-1. Yield 65%; M.p. 262–264 °C. 1H NMR (DMSO-d_6): (δ) 3.95 (d, 1H, J = 17.8), 4.23 (d, 1H, J = 17.8), 6.51 (s, 1H, CH), 7.29–7.35 (m, 5H, ArH), 14.09 (bs, 1H, SH). Anal. Calcd for. ($C_{11}H_9N_3OS_3$): C 44.72, H 3.07, N 14.22; Found: C 44.54, H 3.20, N 14.00.

4.1.1.2. 2-(2-Chlorophenyl)-3-(5-sulfanyl-1,3,4-thiadiazol-2-yl) thiazolidin-4-one (1b) CAS registry number 106146–16-1. Yield 68%; M.p. 240–242 °C. 1H NMR (DMSO-d_6): (δ) 3.96 (d, 1H, J = 17.2), 4.15 (d, 1H, J = 17.2), 6.62 (s, 1H, CH), 7.26–7.36 (m, 3H, ArH), 7.52 (d, 1H, J = 9.3, ArH), 14.17 (bs, 1H, SH). Anal. Calcd for. ($C_{11}H_8ClN_3OS_3$): C 40.05, H 2.44, N 12.74; Found: C 40.16, H 2.81, N 12.33.

4.1.1.3. 2-(3-Chlorophenyl)-3-(5-sulfanyl-1,3,4-thiadiazol-2-yl) thiazolidin-4-one (1c). Yield 65%; M.p. 240–242 °C. 1H NMR (DMSO-d_6): (δ) 3.94 (d, 1H, J = 17.0), 4.27 (d, 1H, J = 17.0), 6.50 (s, 1H, CH), 7.33–7.36 (m, 3H, ArH), 7.53 (s, 1H, J = 9.3, ArH), 14.18 (bs, 1H, SH). Anal. Calcd for. ($C_{11}H_8ClN_3OS_3$): C 40.05, H 2.44, N 12.74; Found: C 40.00, H 2.66, N 12.45.

4.1.1.4. 2-(4-Chlorophenyl)-3-(5-sulfanyl-1,3,4-thiadiazol-2-yl) thiazolidin-4-one (1d) CAS registry number 2226509–73-3. Yield 67%; M.p. 248–250 °C. 1H NMR (DMSO-d_6): (δ) 3.93 (d, 1H, J = 17.0), 4.26 (d, 1H, J = 17.0), 6.49 (s, 1H, CH), 7.40 (d, 1H, J = 8.2, ArH), 7.53 (s, 1H, J = 8.2, ArH), 7.62–7.78 (m, 2H, ArH), 14.15 (bs, 1H, SH). Anal. Calcd for. ($C_{11}H_8ClN_3OS_3$): C 40.05, H 2.44, N 12.74; Found: C 40.25, H 2.31, N 12.50.

*4.1.2. General procedure to synthesize 2-chloro-N-arylacetamides (**7**–**8**)*

The 2-chloro-*N*-arylacetamide derivatives **7**–**8** were synthesized following a previously reported procedure[27] introducing slow modifications as reported below. To a suspension of appropriate aniline (**9**–**10**) (1 mmol) in DCM (2 mL) were added dropwise EDIPA (1 mmol, 174 µl) and chloroacetyl chloride (1 mmol, 80 µl). The reaction mixture was stirred at 0 °C for 5 min, and subsequently at room temperature for 24 h. The completion of the reaction was followed by TLC Cyclohexane/EtOAc 50:50. Afterwards, H_2O (5 mL) and a saturated solution of $NaHCO_3$ (5 mL) were added to quench the reaction. The obtained water layer was extracted with EtOAc (3x10 mL), dried over Na_2SO_4, filtered and evaporated *in vacuo*. Desired intermediates **7**–**8** were purified by crystallization from Et2O and MeOH. All spectral data of intermediates **7**–**8** were in good agreement with literature.[27]

*4.1.3. General procedure to synthesize compounds 2-([5-(4-oxo-2-phenyl-1,3-thiazolidin-3-yl)-1,3,4-thiadiazol-2-yl]sulfanyl)-N-(4-sulfamoylphenyl) acetamide (**2 a-d**) and N-(4-methylsulfonylphenyl)-2-[[5-(4-oxo-2-phenyl-thiazolidin-3-yl)-1,3,4-thiadiazol-2-yl]sulfanyl]acetamide (**3 a-d**)*

To a mixture of appropriate intermediate **1 a-d** (1 mmol) and K_2CO_3 (1 mmol, 138 mg) in DMF (2 mL) the suitable 2-chloro-N-(4-sulfamoylphenyl)acetamide (1 mmol, 248 mg, **7**) or 2-chloro-N-(4-methylsulfonylphenyl)acetamide (0.5 mmol, 123 mg, **8**) was added. The reaction mixture was stirred at room temperature for 24 h, then quenched with H_2O (5 mL) and solution of $NaHCO_3$ (5 mL). The water layer was successively extracted with EtOAc (3x10 mL) and the organic layer was washed with brine (3x5 mL), dried over Na_2SO_4, filtered and evaporated under reduced pressure. Final products **2 a-d** and **3 a-d** were obtained by chromatography on silica gel with DCM/MeOH 98:2 and subsequent crystallization from Et_2O at 0 °C.

*4.1.3.1. 2-[[5-(4-Oxo-2-phenyl-thiazolidin-3-yl)-1,3,4-thiadiazol-2-yl] sulfanyl]-N-(4 sulfamoylphenyl)acetamide (**2a**)*. Yield 30%, M.p.: 172 °C dec. 1H NMR (DMSO-d_6), (δ) 3.41 (d, 1H, J = 17.0); 4.21 (s, 2H, CH2); 4.28 (d, 1H, J = 17.0); 6.68 (s, 1H); 7.26 (bs, 2H, NH2); 7.29–7.33 (m, 5H, ArH); 7.68 (d, 2H, J = 8.8, ArH); 7.74 (d, 2H, J = 8.8, ArH); 10.63 (bs, 1H, NH). Anal. Calcd for.: ($C_{19}H_{17}N_5O_4S_4$): C 44.96; H 3.38; N 13.80; Found: C 44.80; H 3.15; N 13.60.

*4.1.3.2. 2-[[5-[2-(2-Chlorophenyl)-4-oxo-thiazolidin-3-yl]-1,3,4-thiadiazol-2-yl]sulfanyl]-N-(4-sulfamoylphenyl)acetamide (**2b**)*. Yield 52%, M.p.: 178 °C dec. 1H NMR (DMSO-d_6), (δ) 4.04 (d, 1H, J = 16.4); 4.17–4.23 (m, 3H); 6.79 (s, 1H); 7.26 (bs, 2H, NH2); 7.27–7.33 (m, 4H, ArH); 7.67 (d, 2H, J = 8.8, ArH); 7.75 (d, 2H, J = 8.8, ArH); 10.63 (bs, 1H, NH). Anal. Calcd for.: ($C_{19}H_{16}ClN_5O_4S_4$): C 42.10; H 2.98; N 12.92; Found: C 42.00; H 2.66; N 12.70.

*4.1.3.3. 2-[[5-[2-(3-Chlorophenyl)-4-oxo-thiazolidin-3-yl]-1,3,4-thiadiazol-2-yl]sulfanyl]-N-(4-sulfamoylphenyl)acetamide (**2c**)*. Yield

98%, M.p.: 215 °C dec. 1H NMR (DMSO-d_6), (δ) 4.01 (d, 1H, J = 17.0); 4.22 (s, 2H, CH2); 4.32 (d, 1H, J = 17.0); 6.67 (s, 1H); 7.25 (bs, 2H, NH2); 7.26–7.33 (m, 3H, ArH); 7.67 (s, 1H, ArH); 7.70–7.76 (m, 4H, ArH); 10.63 (bs, 1H, NH). Anal. Calcd for.: ($C_{19}H_{16}ClN_5O_4S_4$): C 42.10; H 2.98; N 12.92; Found: C 42.38; H 2.64; N 12.80.

4.1.3.4. 2-[[5-[2-(4-Chlorophenyl)-4-oxo-thiazolidin-3-yl]-1,3,4-thiadiazol-2-yl]sulfanyl]-N-(4-sulfamoylphenyl)acetamide ***(2d)***. Yield 88%, M.p.: 225 °C dec. 1H NMR (DMSO-d_6), (δ) 4.01 (d, 1H, J = 17.0); 4.22 (s, 2H, CH2); 4.26 (d, 1H, J = 17.0); 6.68 (s, 1H); 7.25 (bs, 2H, NH2); 7.33–7.40 (m, 4H, ArH); 7.71–7.77 (m, 4H, ArH); 10.63 (bs, 1H, NH). Anal. Calcd for.: ($C_{19}H_{16}ClN_5O_4S_4$): C 42.10; H 2.98; N 12.92; Found: C 42.42; H 2.77; N 12.63.

4.1.3.5. N-(4-Methylsulfonylphenyl)-2-[[5-(4-oxo-2-phenyl-thiazolidin-3-yl)-1,3,4-thiadiazol-2-yl]sulfanyl]acetamide ***(3a)***. Yield 98%, M.p.: 222 °C dec. 1H NMR (DMSO-d_6), (δ) 3.14 (s, 3H, CH3); 4.00 (d, 1H, J = 17.0); 4.22 (s, 2H, CH2); 4.26 (d, 1H, J = 17.0); 6.68 (s, 1H); 7.28–7.33 (m, 5H, ArH); 7.76 (d, 2H, J = 8.8, ArH); 7.84 (d, 2H, J = 8.8, ArH); 10.74 (bs, 1H, NH). Anal. Calcd for.: ($C_{20}H_{18}N_4O_4S_4$): C 47.41; H 3.58; N 11.06; Found: C 47.12; H 3.26; N 11.37.

4.1.3.6. 2-[[5-[2-(2-Chlorophenyl)-4-oxo-thiazolidin-3-yl]-1,3,4-thiadiazol-2-yl]sulfanyl]-N-(4-methylsulfonylphenyl)acetamide ***(3b)***. Yield 31%, M.p.: 195 °C dec. 1H NMR (DMSO-d_6), (δ) 3.12 (s, 3H, CH3); 4.00–4.22 (m, 4H), 6.77 (s, 1H); 7.13–7.31 (m, 3H, ArH); 7.48–7.82 (m, 5H); 10.74 (bs, 1H, NH). Anal. Calcd for.: ($C_{20}H_{17}ClN_4O_4S_4$): C 44.39; H 3.17; N 10.35; Found: C 44.18; H 3.00; N 10.49.

4.1.3.7. 2-[[5-[2-(3-Chlorophenyl)-4-oxo-thiazolidin-3-yl]-1,3,4-thiadiazol-2-yl]sulfanyl]-N-(4-methylsulfonylphenyl)acetamide ***(3c)***. Yield 98%, M.p.: 190–192 °C. 1H NMR (DMSO-d_6), (δ) 3.14 (s, 3H, CH3); 4.01 (d, 1H, J = 17.0); 4.24 (s, 2H, CH_2); 4.33 (d, 1H, J = 17.0); 6.77 (s, 1H); 7.25–7.33 (m, 3H, ArH); 7.51 (s, 1H, ArH); 7.76 (d, 2H, J = 8.8, ArH); 7.84 (d, 2H, J = 8.8, ArH); 10.74 (bs, 1H, NH). Anal. Calcd for.: ($C_{20}H_{17}ClN_4O_4S_4$): C 44.39; H 3.17; N 10.35; Found: C 44.30; H 3.00; N 10.17.

4.1.3.8. 2-[[5-[2-(4-Chlorophenyl)-4-oxo-thiazolidin-3-yl]-1,3,4-thiadiazol-2-yl]sulfanyl]-N-(4-methylsulfonylphenyl)acetamide ***(3d)***. Yield 34%, M.p.: 195 °C dec. 1H NMR (DMSO-d_6), (δ) 3.14 (s, 3H, CH3); 3.98–4.32 (m, 4H); 6.67 (s, 1H); 7.27–7.39 (m, 4H, ArH); 7.78–7.84 (m, 4H, ArH); 10.74 (bs, 1H, NH). Anal. Calcd for.: ($C_{20}H_{17}ClN_4O_4S_4$): C 44.39; H 3.17; N 10.35; Found: C 44.15; H 3.00; N 10.49.

4.2. In vitro anti-HIV assay

Evaluation of the antiviral activity of the compounds against HIV in MT-4 cells was performed using the MTT assay as previously described.[43] Stock solutions (10 × final concentration) of test compounds were added in 25 µl volumes to two series of triplicate wells so as to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial 5-fold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman instruments, Fullerton, CA). Untreated HIV- and mock-infected cell samples were included as controls. HIV stock (50 µl) at 100–300 $CCID_{50}$ (50% cell culture infectious doses) or culture medium was added to either the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effects of test compound on uninfected cells in order to assess the cytotoxicity of the test compounds. Exponentially growing MT-4 cells were centrifuged for 5 min at 220 g and the supernatant was discarded. The MT-4 cells were resuspended at 6 × 105 cells/ml and 50 µl volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock-and HIV-infected cells was examined spectrophotometrically using the MTT assay. The MTT assay is based on the reduction of yellow colored 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Acros Organics) by mitochondrial dehydrogenase activity in metabolically active cells to a blue-purple formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (Infinite M1000, Tecan), at two wavelengths (540 and 690 nm). All data were calculated using the median absorbance value of three wells. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the test compound that reduced the absorbance (OD540) of the mock-infected control sample by 50%. The concentration achieving 50% protection against the cytopathic effect of the virus in infected cells was defined as the 50% effective concentration (EC_{50}).

4.3. Reverse transcriptase assay

Recombinant wild type p66/p51 HIV-1 RT was expressed and purified as previously described.[43] The RT assay is performed with the EnzCheck Reverse Transcriptase Assay kit (Molecular Probes, Invitrogen), as described by the manufacturer. The assay is based on the dsDNA quantitation reagent PicoGreen. This reagent shows a pronounced increase in fluorescence signal upon binding to dsDNA or RNA-DNA heteroduplexes. Single-stranded nucleic acids generate only minor fluorescence signal enhancement when a sufficiently high dye: base pair ratio is applied.[44] This condition is met in the assay. A poly (rA) template of approximately 350 bases long, and an oligo(dT)16 primer, are annealed in a molar ratio of 1:1.2 (60 min. at room temperature). Fifty-two ng of the RNA/DNA is brought into each well of a 96-well plate in a volume of 20 µl polymerization buffer (60 mMTris-HCl, 60 mM KCl, 8 mM $MgCl_2$, 13 mM DTT, 100 µM dTTP, pH 8.1). Five µl of RT enzyme solution, diluted to a suitable concentration in enzyme dilution buffer (50 mM Tris-HCl, 20% glycerol, 2 mM DTT, pH 7.6), is added (final RT enzyme concentration in the reaction mixture is 25 nM). To test the activity of compounds against RT, 1 µl of compound in DMSO is added to each well before the addition of RT enzyme solution. Control wells without compound contain the same amount of DMSO (3.85% of the total reaction volume of 26 µl). The reactions are incubated at 25 °C for 40 min and then stopped by the addition of EDTA (15 mM fc). Heteroduplexes are then detected by addition of PicoGreen. Signals are read using an excitation wavelength of 490 nm and emission detection at 523 nm using a spectrofluorometer (Safire 2, Tecan). Results are expressed as relative fluorescence i.e. the fluorescence signal of the reaction mix with compound divided by the signal of the same reaction mix without compound.

4.4. Docking studies

The crystal structure of HIV-1 reverse transcriptase in complex with GW564511 inhibitor was retrieved from the RCSB Protein Data Bank (PDB code 3DLG).[35] The protein and ligands 1–3 a-d were prepared by Discovery Studio 2.5.5. The ligands were also minimized using CHARMm forcefield and used for docking by AutoDock Vina following the same protocol described in previous papers.[27,30]

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Kudalkar SN, Ullah I, Bertoletti N, et al. Structural and pharmacological evaluation of a novel non-nucleoside reverse transcriptase inhibitor as a promising long acting nanoformulation for treating HIV. *Antiviral Res.* 2019;167:110–116.
2. Kohlstaedt LA, Wang J, Friedman JM, Rice PA, Steitz TA. Crystal structure at 3.5 A resolution of HIV-1 reverse transcriptase complexed with an inhibitor. *Science.* 1992;256:1783–1790.
3. Kohlstaedt LA, Steitz TA. Reverse transcriptase of human immunodeficiency virus can use either human tRNA(3Lys) or Escherichia coli tRNA(2Gln) as a primer in an in vitro primer-utilization assay. *Proc Natl Acad Sci USA.* 1992;89:9652–9656.
4. Rawal RK, Murugesan V, Katti SB. Structure-activity relationship studies on clinically relevant HIV-1 NNRTIs. *Curr Med Chem.* 2012;19:5364–5380.
5. Prajapati DG, Ramajayam R, Yadav MR, Giridhar R. The search for potent, small molecule NNRTIs: a review. *Bioorg Med Chem.* 2009;17:5744–5762.
6. Mehellou Y, De Clercq E. Twenty-six years of anti-HIV drug discovery: where do we stand and where do we go? *J Med Chem.* 2010;53:521–538.
7. Das K, Arnold E. HIV-1 reverse transcriptase and antiviral drug resistance. Part 2. *Curr Opin Virol.* 2013;3:119–128.
8. Das K, Arnold E. HIV-1 reverse transcriptase and antiviral drug resistance. Part 1. *Curr Opin Virol.* 2013;3:111–118.
9. Croxtall JD. Etravirine: a review of its use in the management of treatment-experienced patients with HIV-1 infection. *Drugs.* 2012;72:847–869.
10. Asahchop EL, Wainberg MA, Sloan RD, Tremblay CL. Antiviral drug resistance and the need for development of new HIV-1 reverse transcriptase inhibitors. *Antimicrob Agents Chemother.* 2012;56:5000–5008.
11. Day C. Thiazolidinediones: a new class of antidiabetic drugs. *Diabet Med.* 1999;16:179–192.
12. Tuncbilek M, Altanlar N. Synthesis of new 3-(substituted phenacyl)-5-[3'-(4H-4-oxo-1-benzopyran-2-yl)-benzylidene]-2,4-thiazolidinediones and their antimicrobial activity. *Arch Pharm (Weinheim).* 2006;339:213–216.
13. Bahare RS, Ganguly S, Choowongkomon K, Seetaha S. Synthesis, HIV-1 RT inhibitory, antibacterial, antifungal and binding mode studies of some novel N-substituted 5-benzylidine-2,4-thiazolidinediones. *Daru.* 2015;23:6.
14. Reddy KA, Lohray BB, Bhushan V, et al. Novel euglycemic and hypolipidemic agents: Part-2. Antioxidant moiety as structural motif. *Bioorg Med Chem Lett.* 1998;8:999–1002.
15. Patil V, Tilekar K, Mehendale-Munj S, Mohan R, Ramaa CS. Synthesis and primary cytotoxicity evaluation of new 5-benzylidene-2,4-thiazolidinedione derivatives. *Eur J Med Chem.* 2010;45:4539–4544.
16. Prabhakar C, Madhusudhan G, Sahadev K, et al. Synthesis and biological activity of novel thiazolidinediones. *Bioorg Med Chem Lett.* 1998;8:2725–2730.
17. Heneka MT, Landreth GE. PPARs in the brain. *Biochim Biophys Acta.* 2007;1771:1031–1045.
18. Chimirri A, Grasso S, Monforte AM, Zappala M, De Sarro A, De Sarro GB. Synthesis and anticonvulsant properties of 3-(1,3,4-thiadiazol-2-yl) thiazolidin-4-ones. *Farmaco.* 1991;46:935–943.
19. Ha YM, Park YJ, Lee JY, et al. Design, synthesis and biological evaluation of 2-(substituted phenyl)thiazolidine-4-carboxylic acid derivatives as novel tyrosinase inhibitors. *Biochimie.* 2012;94:533–540.
20. Ha YM, Park YJ, Kim JA, et al. Design and synthesis of 5-(substituted benzylidene) thiazolidine-2,4-dione derivatives as novel tyrosinase inhibitors. *Eur J Med Chem.* 2012;49:245–252.
21. Zhan P, Liu X, Li Z, et al. Novel 1,2,3-thiadiazole derivatives as HIV-1 NNRTIs with improved potency: synthesis and preliminary SAR studies. *Bioorg Med Chem.* 2009;17:5920–5927.
22. Tian Y, Zhan P, Rai D, Zhang J, De Clercq E, Liu X. Recent advances in the research of 2,3-diaryl-1,3-thiazolidin-4-one derivatives as potent HIV-1 non-nucleoside reverse transcriptase inhibitors. *Curr Med Chem.* 2012;19:2026–2037.
23. Chimirri A, Grasso S, Monforte AM, Monforte P, Zappala M. Anti-HIV agents. I: Synthesis and in vitro anti-HIV evaluation of novel 1H,3H-thiazolo[3,4-a]benzimidazoles. *Farmaco.* 1991;46:817–823.
24. Barreca ML, Chimirri A, De Luca L, et al. Discovery of 2,3-diaryl-1,3-thiazolidin-4-ones as potent anti-HIV-1 agents. *Bioorg Med Chem Lett.* 2001;11:1793–1796.
25. Barreca ML, Balzarini J, Chimirri A, et al. Design, synthesis, structure-activity relationships, and molecular modeling studies of 2,3-diaryl-1,3-thiazolidin-4-ones as potent anti-HIV agents. *J Med Chem.* 2002;45:5410–5413.
26. Ravichandran V, Jain A, Kumar KS, Rajak H, Agrawal RK. Design, synthesis, and evaluation of thiazolidinone derivatives as antimicrobial and anti-viral agents. *Chem Biol Drug Des.* 2011;78:464–470.
27. Monforte AM, De Luca L, Buemi MR, Agharbaoui FE, Pannecouque C, Ferro S. Structural optimization of N1-aryl-benzimidazoles for the discovery of new non-nucleoside reverse transcriptase inhibitors active against wild-type and mutant HIV-1 strains. *Bioorg Med Chem.* 2018;26:661–674.
28. Zhan P, Chen X, Li D, Fang Z, De Clercq E, Liu X. HIV-1 NNRTIs: structural diversity, pharmacophore similarity, and implications for drug design. *Med Res Rev.* 2013;33(Suppl 1):E1–72.
29. Li W, Li X, De Clercq E, Zhan P, Liu X. Discovery of potent HIV-1 non-nucleoside reverse transcriptase inhibitors from arylthioacetanilide structural motif. *Eur J Med Chem.* 2015;102:167–179.
30. Ferro S, Buemi MR, De Luca L, Agharbaoui FE, Pannecouque C, Monforte AM. Searching for novel N1-substituted benzimidazol-2-ones as non-nucleoside HIV-1 RT inhibitors. *Bioorg Med Chem.* 2017;25:3861–3870.
31. Rao A, Balzarini J, Carbone A, et al. Synthesis of new 2,3-diaryl-1,3-thiazolidin-4-ones as anti-HIV agents. *Farmaco.* 2004;59:33–39.
32. Murugesan V, Tiwari VS, Saxena R, et al. Lead optimization at C-2 and N-3 positions of thiazolidin-4-ones as HIV-1 non-nucleoside reverse transcriptase inhibitors. *Bioorg Med Chem.* 2011;19:6919–6926.
33. Ren J, Stammers DK. Structural basis for drug resistance mechanisms for non-nucleoside inhibitors of HIV reverse transcriptase. *Virus Res.* 2008;134:157–170.
34. Das K, Bauman JD, Clark Jr AD, et al. High-resolution structures of HIV-1 reverse transcriptase/TMC278 complexes: strategic flexibility explains potency against resistance mutations. *Proc Natl Acad Sci USA.* 2008;105:1466–1471.
35. Dadlani VG, Somani RR, Tripathi PK. Design, synthesis and in-silico study of novel series of 2-phenyl-3-(5-sulfanyl-1,3,4-thiadiazol-2-Yl)-1,3-thiazolidin-4-one derivatives with potential anti-tubercular activity. *Int J Pharm Sci Res.* 2019;10:2565–2576.
36. Ren J, Chamberlain PP, Stamp A, et al. Structural basis for the improved drug resistance profile of new generation benzophenone non-nucleoside HIV-1 reverse transcriptase inhibitors. *J Med Chem.* 2008;51:5000–5008.
37. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *J Comput Chem.* 2010;31:455–461.
38. Wensing AM, Calvez V, Gunthard HF, et al. 2017 update of the drug resistance mutations in HIV-1. *Top Antivir Med.* 2017;24:132–133.
39. Hsiou Y, Ding J, Das K, et al. The Lys103Asn mutation of HIV-1 RT: a novel mechanism of drug resistance. *J Mol Biol.* 2001;309:437–445.
40. Ren J, Milton J, Weaver KL, Short SA, Stuart DI, Stammers DK. 'Structural basis for the resilience of efavirenz (DMP-266) to drug resistance mutations in HIV-1 reverse transcriptase'. *Structure (London, England: 1993).* 2000;8:1089–1094.
41. Das K, Ding J, Hsiou Y, et al. Crystal structures of 8-Cl and 9-Cl TIBO complexed with wild-type HIV-1 RT and 8-Cl TIBO complexed with the Tyr181Cys HIV-1 RT drug-resistant mutant. *J Mol Biol.* 1996;264:1085–1100.
42. Ragab FA, Heiba HI, El-Gazzar MG, Abou-Seri SM, El-Sabbagh WA, El-Hazek RM. Synthesis of novel thiadiazole derivatives as selective COX-2 inhibitors. *Medchemcomm.* 2016;7:2309–2327.
43. Pannecouque C, Daelemans D, De Clercq E. Tetrazolium-based colorimetric assay for the detection of HIV replication inhibitors: revisited 20 years later. *Nat Protoc.* 2008;3:427–434.
44. Singer VL, Jones LJ, Yue ST, Haugland RP. Characterization of PicoGreen reagent and development of a fluorescence-based solution assay for double-stranded DNA quantitation. *Anal Biochem.* 1997;249:228–238.