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Discovery of 2,3-Diaryl-1,3-thiazolidin-4-ones as Potent Anti-HIV-1 Agents

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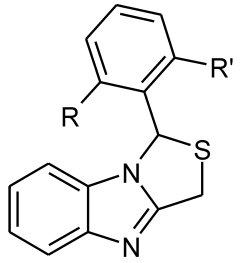
Abstract—Design, synthesis and anti-HIV activity of a series of 2,3-diaryl-1,3-thiazolidin-4-ones are reported. Some derivatives proved to be highly effective in inhibiting HIV-1 replication at nanomolar concentrations thereby acting as non-nucleoside HIV-1 RT inhibitors (NNRTIs). SAR studies evidenced that the nature of the substituents at the 2 and 3 positions of the thiazolidinone nucleus largely influenced the in vitro anti-HIV activity of this new class of potent antiviral agents.

Human immunodeficiency virus type 1 (HIV-1) was identified as the causative agent in the transmission and the development of acquired immune deficiency syndrome (AIDS).[1] The unique nature of the replicative cycle of HIV-1 provides many potential targets for therapeutic intervention. One of these, reverse transcriptase (RT) is a key enzyme, packaged within the HIV virion capsid, which plays an essential and multifunctional role in the replication of the virus.[2] Combinations of RT nucleoside inhibitors (NRTIs), RT non-nucleoside inhibitors (NNRTIs) and protease inhibitors (PIs), clinically used for the treatment of HIV infections, dramatically decrease viral load in most infected persons, but resistance to the currently available chemotherapeutics invariably emerges.[3] Consequently, there is a high medical need to develop novel, selective, potent, safe, inexpensive antiviral agents, also effective against mutant strains of HIV.

In previous papers,[4–7] we reported a series of 1-aryl-substituted 1*H*,3*H*-thiazolo[3,4-*a*]benzimidazole derivatives (TBZs) highly active as NNRTIs.

Extensive structure–activity relationship (SAR) studies have been performed within this family of compounds and it was observed that stringent requirements exist with regard to the structural determinants for optimum anti-HIV activity. The C-1 substituent plays a decisive and crucial role in the interaction of TBZ compounds with the target HIV-1 RT enzyme: in particular a 2,6-dihalo substituted phenyl ring at C-1 furnished rewarding results and gave a large improvement in potency.



TBZ1, R=R'=F
TBZ2, R=R'=Cl

We have also previously demonstrated[8] that the biological activity of TBZs is associated with their ability to assume a 'butterfly-like' conformation, which allows a binding mode similar to other NNRTIs by means of a suitable spatial location of lipophilic and electron-rich groups. Superimposition studies on a set of NNRTIs were also performed and, in the best alignment, two unsaturated zones corresponding to the wings of the

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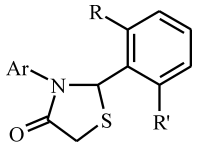
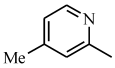
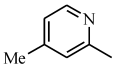
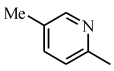
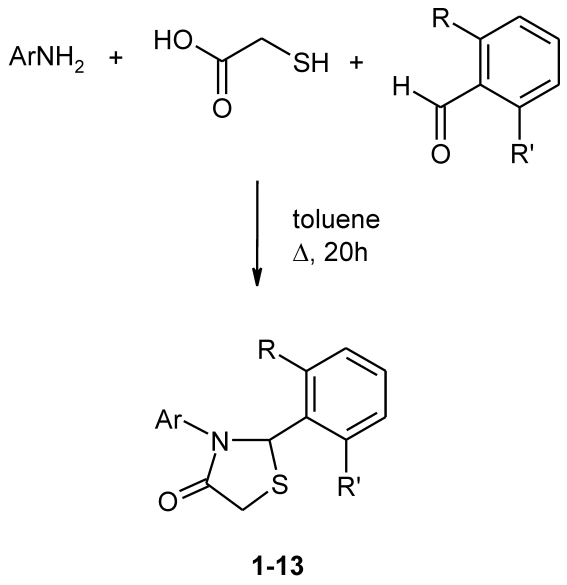
Table 1. Anti-HIV-1 activity, cytotoxicity and selectivity index for compounds **1**–**13**



Compd	Ar	R	R′	EC_{50} (μM)[a]		CC_{50} (μM)[b]	SI[c]	IC_{50} (μM)[d]
				HIV-1 III_B	HIV-2 ROD			
1		F	F	2.30±0.75	>429	>429	>186	
2		Cl	Cl	0.401±0.093	>39	38.1±4.5	95	
3		F	F	0.855±0.068	>427	>427	>500	
4		Cl	Cl	0.178±0.009	>38.5	38.5±4.9	216.3	
5		F	F	95.1±65.3	>427	>427	>4.5	
6		Cl	Cl	NA	>23	23.4±6.9	—	
7		F	F	0.082±0.029	>126	126.0±34.9	1536	2.90±0.49
8		Cl	Cl	0.044±0.003	>284.7	284.7±33.6	6470	2.24±0.38
9		F	F	0.248±0.026	>242.2	242.2±0.3	976	2.84±0.39
10		Cl	Cl	0.147±0.050	>368.5	>368.5	>2500	
11		F	F	53.5±39.1	>270	>319.3	>6	
12		Cl	Cl	NA	>31.5	42.6±4.8	—	
13		F	F	5.13±0.0	>170.7	201.6±36.7	39.3	
TBZ1				0.352±0.14		19.2±2.8	54.5	14.6±13.2
TBZ2				0.589±0.087		9.43±6.55	16	

NA, not active.
[a]Concentration required to reduce HIV-1-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]Poly(C)/oligo(dG) was used as the template/primer and [^{3}H]dGTP as the radiolabelled substrate.



1-13

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

butterfly and a hydrophobic region between the two wings were suggested as very important for the interaction with the RT binding site. In particular the following binding mode for TBZ was observed: the benzene ring of the benzimidazole moiety interacted hydrophobically with the side chain of Phe227 and Tyr188 and the dihalophenyl group bound close to the hydrophobic pocket. In conclusion, from the best geometry fit between different classes of NNRTIs, it resulted that the plausible pharmacophoric elements for TBZ were: the benzene fused ring, the aryl group at C-1 and the nitrogen atom of the thiazole nucleus.

On this basis, using the comparative molecular field analysis (CoMFA) model and the thiazolobenzimidazole system as a scaffold, we designed 2,3-diaryl-1,3-thiazolidin-4-one derivatives as new NNRTIs. The opening of the imidazole nucleus allowed keeping all key structural requirements, that is two π-systems and the nitrogen atom, for potent enzyme inhibition.

The synthesis of the new 2,3-diaryl-1,3-thiazolidin-4-ones (**1**–**13**) was carried out, according to reported procedures,[9] by reacting a suitable 2,6-dihalo benzaldehyde with an equimolar amount of a (hetero)aromatic amine in the presence of an excess of mercaptoacetic acid in refluxing toluene (Scheme 1). The obtained products were isolated by conventional workup in satisfactory yields. Both analytical and spectral data (^{1}H NMR) of all the synthesized compounds are in full agreement with the proposed structures.[10]

Compounds **1**–**13** were evaluated for anti-HIV activity by determining their ability to inhibit the replication of HIV-1 (III_B) or HIV-2 (ROD) in MT-4 cells and compared with TBZ1 and TBZ2.[11,12] The results are presented in Table 1.

Our results show that our approach has led to the development of highly potent anti-HIV agents, up to 10-fold more active than the corresponding TBZ lead compounds, probably because conformational changes may allow the correct positioning of the new molecules for a facile attack at the active site residues.

Their mechanism of action was elucidated and found to be based upon inhibition of HIV-1 RT (Table 1).

As observed for other classes of NNRTIs, none of the compounds showed activity against HIV-2 (ROD).

In addition, these compounds were minimally toxic to MT-4 cells and their selectivity indices were remarkably high. In particular, compound **8**, the most promising of the series, possessed a selectivity index of 6470.

In terms of SARs, we can say that the anti-HIV activity was strongly enhanced by introducing a 2-pyridinyl substituent at the N-3 atom of the thiazolidinone ring and in particular by two chlorine atoms at 2′ and 6′ positions of the phenyl ring at C-2: in fact derivative **3** was more active than its analogues **1** and **5**, while compound **4** was more active than **2** and **6** as well as **3**. Moreover we were very happy to learn that, as we had expected by using molecular modelling, the introduction of a methyl group at the 6 position of the pyridin-2-yl group led to a substantial increase in potency, thus confirming that increasing the steric bulk of this aromatic part led to improved antiviral activity. In fact, 6-methylpyridin-2-yl derivatives **7** and **8** possessed the most potent activity with EC_{50} values of 82 and 44 nM, respectively.

In conclusion, the data presented here suggest that the 2,3-diaryl-1,3-thiazolidin-4-ones should be considered a new family of antiviral agents acting as NNRTIs with minimal cytotoxicity. However, further studies are needed and are in progress to better define the precise antiviral mechanism and to correlate molecular structure with bioactivity.

Acknowledgements

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10. Selected data for compound **8**: mp 142–144 °C (EtOH). ^{1}H NMR (300 MHz, $CDCl_3$) 2.29 (s, 3H, Me), 3.94 (d, 1H, J = 15.9 Hz, 5-H_A), 4.16 (dd, 1H, J = 2.2 and 15.9 Hz, 5-H_B), 6.82–7.96 (m, 7H, ArH and H-2).
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