Available online at www.sciencedirect.com
SCIENCE DIRECT®
Il Farmaco 58 (2003) 259–263
IL FARMACO
www.elsevier.com/locate/farmac

Short Communication

Anti-HIV agents: design and discovery of new potent RT inhibitors

Maria Letizia Barreca [a], Alba Chimirri [a], Erik De Clercq [b], Laura De Luca [a], Anna-Maria Monforte [a,*], Pietro Monforte [a], Angela Rao [a], Maria Zappalà [a]

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

Accepted 4 July 2002

Abstract

This paper reports our work in the field of nonnucleoside RT inhibitors (NNRTIs). On the basis of extensive studies on 1*H*,3*H*-thiazolo[3,4-*a*]benzimidazole derivatives (TBZs) followed by structure–activity relationship (SAR) considerations and molecular modeling, the design and synthesis of a series of 2,3-diaryl-1,3-thiazolidin-4-ones have been performed. Some derivatives proved to be highly effective in inhibiting human immunodeficiency virus type-1 (HIV-1) replication at nanomolar concentrations with minimal toxicity, acting as reverse transcriptase (RT) inhibitors. Computational studies were used in order to probe the binding of our ligands to HIV-1-RT.

Keywords: NNRTIs; anti-HIV agents; 2,3-Diaryl-1,3-thiazolidin-4-ones

Reverse transcriptase (RT) is a key enzyme which plays an essential and multifunctional role in the replication of the human immunodeficiency virus type-1 (HIV-1) and thus constitutes an attractive target for the development of new drugs useful in AIDS therapy [1].

The first antiretroviral drugs approved in the USA and Europe were nucleoside RT inhibitors (NRTIs) which compete with normal nucleoside substrates for incorporation into the viral genome, thus behaving as chain terminators [2].

Unlike nucleoside analogues, nonnucleoside RT inhibitors (NNRTIs) bind in a noncompetitive manner to a specific 'pocket' of the HIV-1 RT altering its ability to function [3,4].

X-ray crystallographic studies of NNRTI–RT complexes have shown that the NNRTIs present a very similar conformational 'butterfly-like' shape and appear to function as π-electron donors to aromatic side-chain residues surrounding the binding pocket [5].

Presently, three NNRTIs namely nevirapine, delavirdine and efavirenz are available in clinical practice. Combination of these drugs with NRTIs and protease inhibitors (PIs) leads to a dramatic decrease of the viral load in most of the HIV-infected patients. However, in view of the increasing incidence of resistance to current drug regimens and the frequency of adverse events, the development of novel antiviral agents, also effective against mutant HIV strains, remains a high priority for medical research.

In previous papers [6,7] we reported the synthesis and biological activity of a series of 1-aryl-1*H*,3*H*-thiazolo[3,4-*a*]benzimidazoles (TBZs) highly active as HIV-1 NNRTIs.

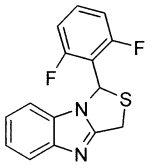
We demonstrated that, analogously to other NNRTIs, their biological activity was associated with the possibility of assuming a 'butterfly-like' shape that allows the interactions between the aromatic groups of TBZs and aminoacid residues in the non-nucleoside inhibitor binding pocket (NNIBP) of RT [8].

The lead compound of this class, 1-(2,6-difluorophenyl)-1*H*,3*H*-thiazolo[3,4-*a*]benzimidazole (TBZ, NSC 625487), proved to be a highly potent inhibitor of HIV-1 induced cytopathic effect; it inhibited the replication of various strains of HIV-1, including a zidovudine-resistant strain (G910-6), in a variety of human cell lines [8]. In addition, combination of TBZ with either

* Correspondence and reprints.
E-mail address: monforte@pharma.unime.it (A.-M. Monforte).

0014-827X/03/$ - see front matter
doi:10.1016/S0014-827X(03)00024-7

zidivudine or didanosine synergistically inhibited HIV-1 replication and HIV-1 induced cythopathicity. Furthermore, a stereoselectivity of action was evidenced: in fact, the *R*-(+) isomer was more active than the *S*-(−) enantiomer.



TBZ, NSC 625487

Starting from these promising results, we designed and synthesized several new potential RT-inhibitors. Extensive structure–activity relationship (SAR) studies evidenced that specific requirements exist with regard to the determinants for an optimal anti-HIV activity (Fig. 1). In particular, the C-1 substituent plays a crucial role in the interaction of TBZs with the target HIV-1 RT. In fact, it was demonstrated that a 2,6-dihalo substituted phenyl ring at C-1 largely improved potency. On the contrary, the replacement of the benzene nucleus with a naphthyl moiety [9] or the introduction of bulky substituents at different positions of the benzene-fused ring [10] negatively influenced the activity. Only a methyl group at C-5 resulted in a remarkable increase in anti-HIV potency. Moreover, the presence of a methyl function at 3 position led to active and less toxic derivatives [11]. The biological results suggested that the C-3 stereochemistry influences the HIV-1 inhibitory activity: in fact, the *trans* derivatives were more active than the corresponding *cis* isomers. This behavior may be related to the influence of the substituent at C-3 that in the *trans* arrangement allows the formation of two intramolecular hydrogen-bonds between the two 2′,6′-halogen atoms and the hydrogens at C-1 and C-3, stabilizing the butterfly-like conformation of the molecule. Furthermore, in order to characterize a possible biological stereoselectivity, the two enantiomers of the most active compound were evaluated: the 1*R*,3*R*-enantiomer possessed the same significant anti-HIV-1 activity as the racemic mixture whereas the 1*S*,3*S*-enantiomer was completely inactive, thus confirming that the stereochemistry both at C-1 and C-3 may play a role in the anti-HIV-1 activity.

When the thiazole nucleus was replaced by sulfur-containing six or seven-membered rings fused to the '*a*' edge of the benzimidazole system, less active derivatives were obtained [12], whereas the removal of the thiazole ring afforded benzimidazole derivatives in some cases more active that TBZs [13,14].

Finally, since the potential therapeutic utility of TBZ derivatives was hampered by the metabolic oxidation of the sulfur atom leading to the formation of less potent sulfoxide and sulfone metabolites, a series of 1*H*,3*H*-oxazolo[3,4-*a*]benzimidazoles (OBZs) was synthesized [15]. The substitution of the sulfur of the thiazolidine nucleus by the oxygen atom provided compounds with inhibitory activity against the replication of various HIV-1 strains, including NNRTIs-resistant strains.

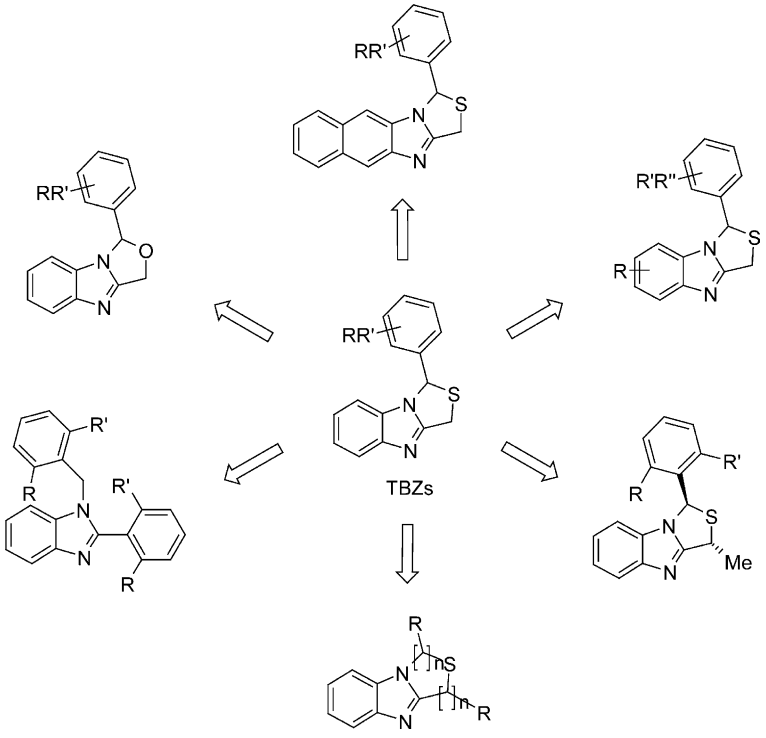
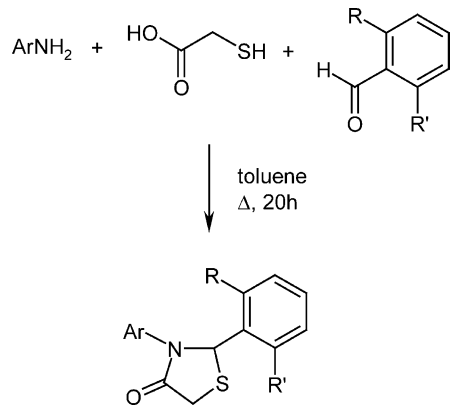


Fig. 1. Structural modifications on TBZ derivatives.



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

Testing of these compounds in an HPLC assay on biological fluids indicated that this kind of structural modification appreciably improved the metabolic stability.

In order to obtain useful information for the design of new and more potent RT inhibitors, a molecular modeling study was carried out [16]. A very informative and statistically significant CoMFA model was developed for a large series of TIBO derivatives. The main molecular determinants responsible for high inhibitory activity were identified and proved valid also for a series of TBZ derivatives. The butterfly-like conformation and a suitable spatial location of lipophilic and electron-rich groups are the key structural requirements for a potent enzyme inhibition by the two classes of NNIs, TIBO and TBZ. Flexible docking experiments showed that TIBO, TBZ and other NNRTIs have very similar structural and binding properties. In particular, in the best alignment, two unsaturated zones corresponding to the wings of the butterfly and a hydrophobic region between the two wings were suggested as very important for the interaction with the RT binding site.

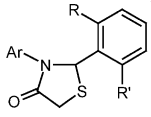
Therefore, the plausible pharmacophoric elements for TBZ were found to be the benzene-fused ring, the aryl group at C-1 and a part of the thiazole nucleus.

On this basis, using the thiazolobenzimidazole system as a scaffold, we designed 2,3-diaryl-1,3-thiazolidin-4-one derivatives as new NNRTIs [17]. The opening of the imidazole nucleus allowed the keeping of all key structural requirements, that is two π-systems and a nitrogen atom, for potent enzyme inhibition.

The synthesis of the designed 2,3-diaryl-1,3-thiazolidin-4-ones was carried out by reacting a suitable 2,6-dihalosubstituted benzaldehyde with an equimolar amount of a (hetero)aromatic amine in the presence of an excess of mercaptoacetic acid (Scheme 1).

This approach led to the development of highly potent anti-HIV agents, active at nanomolar concentrations, up to ten-fold lower than those of the corresponding TBZ lead compounds (Table 1), probably because the new molecules, being more flexible, are able to better accommodate into the RT binding pocket.

Table 1
Anti-HIV-1 activity, cytotoxicity and selectivity index in MT-4 cells of selected 2,3-diaryl-1,3-thiazolidin-4-ones



Comp.	Ar	R	R'	EC_{50} (μM)[a]	CC_{50} (μM)[b]	SI[c]	Comp.	Ar	R	R'	EC_{50} (μM)[a]	CC_{50} (μM)[b]	SI[c]
1		Cl	Cl	0.401 ± 0.093	38.1 ± 4.5	95	**8**		Cl	Cl	0.147 ± 0.050	>368.5	>2500
2		F	F	2.30 ± 0.75	>429	>186	**9**		Cl	F	0.099 ± 0.037	33.9 ± 0.9	343
3		Cl	Cl	0.178 ± 0.009	38.5 ± 4.9	216.3	**10**		F	F	0.248 ± 0.026	242.2 ± 0.3	976
4		F	F	0.855 ± 0.068	>427	>500	**11**		Cl	Cl	0.044 ± 0.003	284.7 ± 33.6	6470
5		Cl	Cl	0.272±0.025	67.4 ± 37.7	248	**12**		Cl	F	0.053 ± 0.009	31.9 ± 2.0	601
6		Cl	F	0.064 ± 0.0	30.7 ± 1.5	484	**13**		F	F	0.082 ± 0.029	126.0 ± 34.9	1536
7		F	F	0.030 ± 0.013	32.0 ± 0.54	1066	**TBZ**				0.352 ± 0.14	19.2 ± 2.8	54.5

[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}.

Table 2
Anti-HIV-1 activity of compounds **7** and **11**–**13** against mutant HIV-1 strains in CEM cells

Comp.	EC_{50} [a] (μM)					
	HIV-1_{IIIB}	100 Leu?Ile	103 Lys?Asn	138 Glu?Lys	181 Tyr?Cys	188 Tyr?His
7	0.026±0.018	0.94±0.18	≥11	0.073±0.089	1.88±1.07	1.02±0.83
11	0.073±0.029	0.150±0.079	≥60	0.117±0.117		16.2±6.2
12	0.031±0.015	0.102±0.074	10.5±2.8	0.124±0.124	7.28±0.65	4.64±0.00
13	0.117±0.019	0.75±0.32	35.9±2.28	0.39±0.19	≥13	≥13

[a] Concentration required to protect CEM cells against the HIV-induced cytopathogenicity by 50%.

These compounds are minimally toxic to MT-4 cells and their selectivity indices are remarkably high; in particular the most promising compound of the series (**7**) possessed a selectivity index of 6470. Worth noting, some of the compounds kept antiviral activity against mutant HIV-1 strains (Table 2), whereas, as observed for other classes of NNRTIs, none of the compounds inhibited the replication of HIV-2 in MT-4 cells at subtoxic concentrations.

From the SAR point of view, the anti-HIV activity is strongly influenced by the nature of the substituent at 2 and 3 positions of the thiazolidinone nucleus. The presence of a 2-pyridinyl substituent at *N*-3 atom of the thiazolidinone ring enhances the anti-HIV activity. The best results were obtained by the introduction of a methyl function or a bromine atom at the 6 position of the pyridin-2-yl group, as suggested by molecular modeling studies. In fact, the compounds with the best combination of high potency and low toxicity were 6-methyl or 6-bromo substituted-pyridin-2-yl derivatives, such as **6**–**7** and **11**–**12**.

In order to have more specific information about the mechanism of inhibition of NNRTIs and to identify the correct binding mode of this new class of antiviral agents, computational methods have been used to map the drug–enzyme interaction zones.

The main intermolecular interactions involved in the RT inhibition were investigated using the GRID program [18]. The X-ray structure of the enzyme from nevirapine–RT complex was used as target for our modeling studies, because the Nevirapine is one of the three NNRTIs approved by FDA and also because of the high resolution of the crystal. Several probes with different chemical characteristics were chosen and their atom affinity potentials calculated on a grid that surrounded the allosteric pocket. The GRID approach allowed the detection of three lipophilic regions within the NNIBP as determinants for high inhibitory activity [19].

Moreover, an automated docking approach was performed in order to understand how the 2,3-diaryl-1,3-thiazolidin-4-ones bind within the NNRTIs pocket and to obtain information for the design of new potential NNRTIs.

The most active compounds of the series (i.e. **7** and **11**) were chosen as ligand test for an automated molecular docking procedure and the results were superimposed with the Nevirapine bound to the RT enzyme and compared with the GRID molecular interaction fields. Docking experiments suggested that RT inhibition by 2,3-diaryl-1,3-thiazolidin-4-ones might occur through a binding mode very similar to that observed for other known HIV-1 NNRTIs such as Nevirapine [20].

The results obtained could be utilized for an improvement of the anti-HIV activity by changing some structural features of the thiazolidinone derivatives and particularly could be useful for the design of new and more potent RT inhibitors.

References

[1] H. Jonckheere, J. Anné, E. De Clercq, The HIV-1 reverse transciption (RT) process as target for RT inhibitors, Med. Res. Rev. 20 (2000) 129–154.
[2] E. De Clercq, Novel compounds in preclinical/early clinical development for the treatment of HIV infections, Rev. Med. Virol. 10 (2000) 255–277.
[3] G. Hajos, S. Riedi, J. Molnar, D. Szabo, Nonnucleoside reverse transcriptase inhibitors, Drugs Future 25 (2000) 47–62.
[4] J. Ren, J. Milton, K.L. Weaver, S.A. Short, D.I. Stuart, D.K. Stammers, Structural basis for the resilience of Efavirenz (DMP-266) to drug resistance mutations in HIV-1 reverse transcriptase, Strucure 8 (2000) 1089–1094.
[5] O.S. Pedersen, O.B. Pedersen, Non-nucleoside reverse transcriptase inhibitors: the NNRTI boom, Antiv. Chem. Chemother. 10 (1999) 285–314.
[6] A. Chimirri, S. Grasso, A.M. Monforte, P. Monforte, M. Zappalà, Anti-HIV agents II. Synthesis and in vitro anti-HIV activity of novel 1*H*,3*H*-thiazolo[3,4-*a*]benzimidazoles, Farmaco 46 (1991) 925–933.
[7] P. Monforte, A.M Monforte, M. Zappalà, G. Romeo, S. Grasso, A. Chimirri, 1-Phenyl substituted 1*H*,3*H*-thiazolo[3,4-*a*]benzimidazoles and pharmaceutical compositions containing them, US Patent, 5 (1993) 217,984.
[8] A. Chimirri, S. Grasso, C. Molica, A.M. Monforte, P. Monforte, M. Zappalà, G. Bruno, F. Nicolò, M. Witvrouw, H. Jonckeere, J. Balzarini, E. De Clercq, Structural features and anti-human immunodeficiency virus (HIV) activity of the isomers of 1-(2′,6′-

difluorophenyl)-1*H*,3*H*-thiazolo[3,4-*a*]benzimidazole, a potent non-nucleoside HIV-1 reverse transcriptase inhibitor, Antivir. Chem. Chemother. 8 (1997) 363–370.

[9] A. Chimirri, S. Grasso, A.M. Monforte, P. Monforte, A. Rao, M. Zappalà, G. Bruno, F. Nicolò, R. Scopelliti, Anti-HIV agents. V. Synthesis, structure and in vitro anti-HIV activity of novel 1*H*,3*H*-naphtho[2′,3′:4,5]imidazo[1,2-*c*]thiazoles, Farmaco 52 (1997) 673–677.

[10] A. Chimirri, S. Grasso, P. Monforte, A. Rao, M. Zappalà, A.M. Monforte, C. Pannecouque, M. Witvrouw, J. Balzarini, E. De Clercq, Synthesis and biological activity of novel 1*h*,3*h*-thiazolo[3,4-*a*]benzimidazoles: non-nucleoside human immunodeficiency virus type 1 reverse transcriptase inhibitors, Antivir. Chem. Chemother. 10 (1999) 211–217.

[11] A. Chimirri, S. Grasso, A.M. Monforte, P. Monforte, A. Rao, M. Zappalà, G. Bruno, F. Nicolò, C. Pannecouque, M. Witvrouw, E. De Clercq, Synthesis, structure and in vitro anti-human immunodeficiency virus activity of novel 3-methyl-1*H*,3*H*-thiazolo[3,4-*a*]benzimidazoles, Antivir. Chem. Chemother. 9 (1998) 431–438.

[12] A. Chimirri, A.M. Monforte, P. Monforte, F. Nicolò, A. Rao, M. Zappalà, Synthesis and structural features of new cyclofunctionalized benzimidazoles, Heterocycles 3 (2000) 613–620.

[13] T. Roth, M.L. Morningstar, P.L. Boyer, S.H. Hughes, R.W. Buckheit, C.J. Michejda, Synthesis and biological activity of novel nonnucleoside inhibitors of HIV-1 reverse transcriptase. 2-Aryl-substituted benzimidazoles, J. Med. Chem. 40 (1997) 4199–4207.

[14] A. Rao, A. Chimirri, E. De Clercq, A.M. Monforte, P. Monforte, C. Pannecouque, M. Zappalà, Synthesis and anti-HIV activity of TBZ structurally-related 1,2-substituted benzimidazoles, Il Farmaco 57 (2002) 819–823.

[15] A. Chimirri, P. Monforte, A. Rao, M. Zappalà, A.M. Monforte, G. De Sarro, C. Pannecouque, M. Witvrouw, J. Balzarini, E. De Clercq, Synthesis, biological activity, pharmacokinetic properties and molecular modelling studies of novel 1*H*,3*H*-oxazolo[3,4-*a*]benzimidazoles: non-nucleoside HIV-1 reverse transcriptase inhibitors, Antivir. Chem. Chemother. 12 (2001) 169–174.

[16] M.L. Barreca, A. Chimirri, A. Carotti, A. Carrieri, A.M. Monforte, M. Pellegrini Calace, A. Rao, Comparative molecular field analysis (CoMFA) and docking studies of non-nucleoside HIV-1 RT inhibitors (NNRTIs), Bioorg. Med. Chem. 7 (1999) 2283–2292.

[17] M.L. Barreca, A. Chimirri, L. De Luca, A.M. Monforte, P. Monforte, A. Rao, M. Zappalà, J. Balzarini, E. De Clercq, C. Pannecouque, M. Witvrouw, Discovery of 2,3-diaryl-1,3-thiazolidin-4-ones as potent anti-HIV-1 agents, Bioorg. Med. Chem. Lett. 11 (2001) 1793–1796.

[18] P.J. Goodford, A computational procedure for determining energetically favorable binding sites on biologically important macromolecules, J. Med. Chem. 28 (1985) 849–857.

[19] M.L. Barreca, A. Chimirri, E. De Clercq, L. De Luca, H.D. Höltje, M. Höltje, A.M. Monforte, P. Monforte, A. Rao, M. Zappalà, Discovery of 2,3-diaryl-1,3-thiazolidin-4-ones as potent HIV-1 NNRTIs by molecular modeling studies, First Magna Graecia Medicinal Chemistry Workshop on New Perspectives in Medicinal Chemistry, Copanello (CZ), 2001, p. 87.

[20] M.L. Barreca, L. De Luca, A.M. Monforte, P. Monforte, A. Rao, M. Zappalà, A. Chimirri, Computational approaches in discovering novel anti-AIDS agents, Biomolecular Interactions MGMS Annual international Meeting, Bristol, UK, P3, 2002, p. 47.