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(3Z)-3-(2-[4-(aryl)-1,3-thiazol-2-yl]hydrazin-1-ylidene)-2,3-dihydro-*1H*-indol-2-one derivatives as dual inhibitors of HIV-1 reverse transcriptase

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ABSTRACT

The HIV-1 Reverse Transcriptase (RT) is a validated and deeply explored biological target for the treatment of AIDS. However, only drugs targeting the RT-associated DNA polymerase (DP) function have been approved for clinical use. We designed and synthesised a new generation of HIV-1 RT inhibitors, based on the (3Z)-3-(2-[4-(aryl)-1,3-thiazol-2-yl]hydrazin-1-ylidene)-2,3-dihydro-*1H*-indol-2-one scaffold. These compounds are active towards both RT-associated functions, DNA polymerase and ribonuclease H. The structure, biological activity and mode of action of the new derivatives have been investigated. In particular, the nature of the aromatic group in the position 4 of the thiazole ring plays a key role in the modulation of the activity towards the two RT-associated functions.

1. Introduction

The current approved treatment for Human Immunodeficiency Virus type 1 (HIV-1) infection is based on the highly active antiretroviral therapy (HAART), that associates a combination of antiviral agents, targeting different steps of the virus replication cycle [1,2]. This multidrug therapeutic regimen leads to the reduction of the amount of circulating virus, in some cases below the current blood testing techniques detectable level, and allows high control of the infection. Moreover, it leads to the reduction of drug resistance occurrence, decrease of mortality and morbidity rates, and an overall improvement of patients quality of life [3]. However, due to the chronic nature of HIV infection, a lifelong therapy is required and both adherence to treatment and the management of drug-related toxicities are issues to deal with.

Although numerous strategies of simplified treatment have been explored in order to further improve patient quality of life, while maintaining treatment success, RT inhibitors (RTIs), both Nucleoside/Nucleotide RTIs (NRTIs/NtRTIs) and Non-Nucleoside RTIs (NNRTIs) are always included in the HAART regimen [1,2], due to the key role of RT in the early phase of the HIV-1 life cycle.

Abbreviations: HIV-1, human immunodeficiency virus type 1; HAART, highly active antiretroviral therapy; RT, reverse transcriptase; RTIs, RT inhibitors; NRTIs/NtRTIs, nucleoside/nucleotide RTIs; NNRTI, non-nucleoside RTIs; DP, DNA polymerase; RNase H, ribonuclease H; RHIs, RNase H inhibitors; NNRTIBP, NNRTIs binding pocket; PLK4, polo-like kinase 4; BACE1, beta-secretase 1; CRTH2 (DP2), chemoattractant receptor-homologous molecule expressed on Th2 cells (D2 prostanoid receptor); NOE, nuclear overhauser effect; NMR, nuclear magnetic resonance; R.T., room temperature; RNase H, ribonuclease H; MM-GBSA, the molecular mechanics generalized born/surface area; MMFFs, Merck molecular force field; GB/SA, generalized born/surface area; PRCG, Polak-Ribier coniugate gradient; QPLD, quantum mechanic-polarized ligand docking.

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HIV-1 RT catalyses the reverse transcription process which consists of the conversion of a single strand viral RNA into a double strand viral DNA via the formation of a RNA-DNA hybrid. To perform this activity two main catalytic functions are associated in one enzyme, DNA polymerase (DP) and ribonuclease H (RNase H).

However, despite the fact that both functions are validated drug targets, since also RNase H function is essential for the reverse transcription process [4,5], no inhibitor that target this enzymatic activity has been introduced in therapy until now [6,7].

Most RNase H inhibitors (RHIs), designed and studied so far, act by chelating the Mg^{2+} ions within the active site [8–15]. However other mechanisms of action have been reported for hydrazone [16], naphthyridinone [17], anthraquinone [18] and propenone [19] derivatives.

The latter compounds are capable of binding an allosteric pocket close to the NNRTIs binding pocket (NNRTIBP) and of inhibiting the RNase H function by a long range allosteric effect.

We have recently reported the identification of a new scaffold for dual inhibition of both HIV-1 RT-associated RNase H and DP functions [20]. Among all the identified molecules, compound **1** (3Z)-3-(2-[4-(3,4-dihydroxyphenyl)-1,3-thiazol-2-yl]hydrazin-1-ylidene)-2,3-dihydro-*1H*-indol-2-one (Fig. 1) resulted as the best performing derivative with IC_{50} value in the low micromolar range [20].

Firstly, to gain insights into the drug novelty potential of compound **1**, we wanted to investigate its mode of action by measuring its capability to chelate Mg^{2+} ions, since RNase H active site inhibitors have been reported to chelate the Mg^{2+} [21]. Secondly, we examined if cross resistance with NNRTI resistant RTs could be observed for compound **1**, since this behaviour has been reported for those compounds that interact with the allosteric RNase H pocket, close to the NNRTIBP [16,18,22]. Results showed that compound **1** did not chelate Mg^{2+} (Fig. 2), but its activity was slightly affected when tested on the Lys103Asn and Tyr181Cys RTs, two NNRTI resistant mutant enzymes [20]. These results suggested that compound **1** could have a mode of action similar to previously reported hydrazone derivatives [16,17]. Hence, we performed biochemical and docking experiments that, in agreement with the biochemical data, indicated compound **1** able to bind RT in the region indicated by Himmel et al. in previous works [16,17]. Therefore, in order to investigate the influence of structural modifications on the biological activity of compound **1**, we synthesised a small library of (3Z)-3-(2-[4-(aryl)-1,3-thiazol-2-yl]hydrazin-1-ylidene)-2,3-dihydro-*1H*-indol-2-ones.

As in the original compound **1**, we have conserved the indolinone and thiazole moieties that have been reported as important fragments in a number of bioactive molecules, such as selective chymase inhibitors [23], polo-like kinase 4 (PLK4) inhibitors [24], Beta-secretase 1 (BACE1) inhibitors [25], chemoattractant receptor-homologous molecule expressed on Th2 cells (D2 prostanoid receptor) (CRTH2 (DP2)) antagonists [26], MAO inhibitors [27] antimycobacterial [28,29], anti-Candida [30], and antimicrobial [31]. In the present work, we introduced differently substituted phenyl moieties at the position 4 of the thiazole ring in place of the 3,4-dihydroxyphenyl group, and evaluated their ability to behave as dual RT-associated functions inhibitors (Fig. 1).

2. Chemistry

(3Z)-3-(2-[4-(aryl)-1,3-thiazol-2-yl]hydrazin-1-ylidene)-2,3-dihydro-*1H*-indol-2-ones (**EMAC2072-2082**) were synthesized starting from 2,3-dihydro-*1H*-indole-2,3-dione. This was reacted with thiosemicarbazide in 2-propanol to give 2-oxo-2,3-dihydro-*1H*-indol-3-thiosemicarbazone. Equimolar amounts of synthesized semicarbazone and of the appropriate α-halogeno-arylketone were then stirred in 2-propanol to give the desired final compounds, as depicted in Scheme 1.

Compounds **EMAC2072-2082** were characterised by means of both analytical and spectroscopic methods. Their analytical and spectroscopic properties are reported in Tables S1 and S2.

The possible formation of both *E* and *Z* diastereoisomers along the C=N double bond was investigated by Nuclear Overhauser Effect (NOE) experiments.

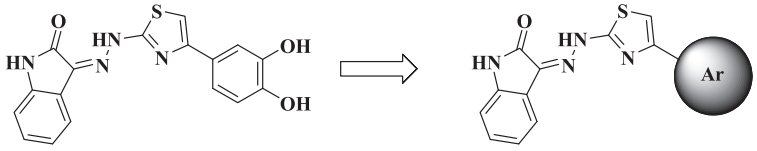
In each compound the irradiation of the indole CH-4 only changed the intensity of the neighbouring CH protons, whereas upon irradiation of the hydrazone NH signal, no intensity change was observed. This indicated that the two protons have no measurable NOEs since they are spatially too far from each other. This distance is in agreement with the "Z" configuration. Therefore, *Z*-configuration of each compound could be confirmed (Fig. S1).

3. Results and discussion

Compounds **EMAC2072-2082**, were evaluated for their ability to inhibit both HIV-1 RT associated RNase H and DP functions (Table 1) using as reference controls compound **1**, **Efavirenz (EFV)**, and **RDS 1643** [13], a diketoacid inhibitor of the RNase H function that binds the catalytic site.

All tested compounds, with the exception of compound **EMAC2081**, exhibited dual inhibitory activity towards both HIV-1 RT functions, confirming that the (3Z)-3-(2-[4-(aryl)-1,3-thiazol-2-yl]hydrazin-1-ylidene)-2,3-dihydro-*1H*-indol-2-one scaffold could be considered a promising candidate for the design of HIV-1 RT dual functions inhibitor [20]. Interestingly, while compound **1** showed a specificity index (SpI), expressed as the ratio of the RNase H IC_{50} value *vs* the DP IC_{50} value, favourable to the DP function (SpI = 3,5), all new derivatives exhibited a SpI favourable to the RNase H function. These results suggest that the compounds activity and specificity on the two functions is strongly influenced by the presence and the nature of the substituents in the 4-phenylthiazole system.

In fact, in the case of compound **EMAC2081**, bearing the unsubstituted 4-phenylthiazole moiety, almost no activity towards both functions was observed. Conversely, the introduction of a 4-(4-biphenyl)thiazole moiety (**EMAC2076**) led to one of the most potent compounds, with a SpI of 0.11. However, the introduction of a 4-(3-nitrophenyl)thiazole (**EMAC2078**) appears as the most performing substitution with respect to DP and RNase H inhibition.



HIT compound 1 EMAC 2072-2082

Fig. 1. Schematic representation of **compound 1** and new derivatives.