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Crystal structures of HIV-1 reverse transcriptase complexes with thiocarbamate non-nucleoside inhibitors

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Abstract

O-Phthalimidoethyl-*N*-arylthiocarbamates (TCs) have been recently identified as a new class of potent HIV-1 reverse transcriptase (RT) non-nucleoside inhibitors (NNRTIs), by means of computer-aided drug design techniques [Ranise A. Spallarossa, S. Cesarini, F. Bondavalli, S. Schenone, O. Bruno, G. Menozzi, P. Fossa, L. Mosti, M. La Colla, et al., Structure-based design, parallel synthesis, structure–activity relationship, and molecular modeling studies of thiocarbamates, new potent non-nucleoside HIV-1 reverse transcriptase inhibitor isosteres of phenethylthiazolylthiourea derivatives, J. Med. Chem. 48 (2005) 3858–3873]. To elucidate the atomic details of RT/TC interaction and validate an earlier TC docking model, the structures of three RT/TC complexes were determined at 2.8–3.0 Å resolution by X-ray crystallography. The conformations adopted by the enzyme-bound TCs were analyzed and compared with those of bioisosterically related NNRTIs.

Keywords: HIV-1; Reverse transcriptase; X-ray crystallography; Non-nucleoside reverse transcriptase inhibitors; Thiocarbamates

HIV-1 reverse transcriptase (RT) catalyzes the conversion of viral RNA into double stranded DNA that is then integrated in the host genome [1]. Due to its crucial role in HIV-1 life cycle RT was identified as a privileged target for anti-AIDS highly active anti-retroviral therapy (HAART) [2]. Non-nucleoside inhibitors (NNRTIs) are selective, non-competitive, low toxicity RT-targeting agents that bind to an allosteric hydrophobic pocket (non-nucleoside inhibitor binding site, NNBS) located at about 10 Å from the RT polymerase active site [3]. The NNBS is created upon inhibitor interaction by rotations of the side chains of Tyr181 and Tyr188 and by repositioning of the β12–β13–β14 sheet [4]. The binding cavity is less conserved than the polymerase active site [4]; its conformation depends on the size, specific chemical structure and binding mode of the NNRTI [5]. As assessed by X-ray crystallography, NNRTIs lock RT into an inactive form by affecting the geometry of the catalytic Asp triad [6,7].

The emergence of HIV-1 NNRTI resistant mutants selected by first-generation inhibitors calls for the design and synthesis of second- and third-generation molecules with an improved resistance profile. However, rational drug design of new NNRTIs is challenging due to the plasticity and mutability of the NNBS [5].

O-Phthalimidoethyl-*N*-4-substituted-phenylthiocarbamates (TCs) have been recently identified as a new class of potent NNRTIs [8], bioisosterically related to phenylethylthiazolylthiourea (PETT) [9,10] and imidoylthiourea (ITU) [11] RT inhibitors (Fig. 1A). In particular, TC-1-3 fall among the most potent analogues, being active in the nanomolar concentration range ($EC_{50} = 40$, 30 and

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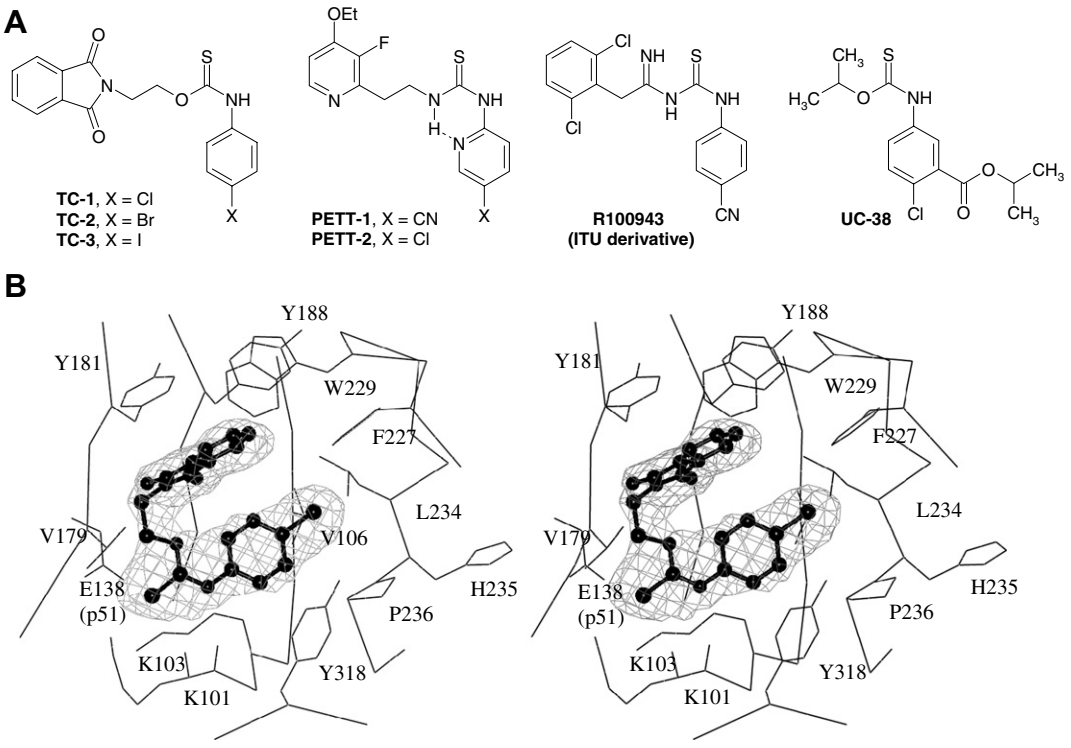

Fig. 1. Thiocarbamate/thiourea NNRTIs. (A) Structures of TC-1-3 and their bioisosters. (B) Stereo view of the electron density map showing the bound TC-1 inhibitor at the NNBS. The map is contoured at 1.2σ.

20 nM, respectively) [8]. TC-1 shares the thiocarbamate functionality with UC-38 [12], and the 4-chloroaniline substructure with other potent NNRTIs, such as TIBO (R-82913, R-86183), PETT MSC-127, dihydroquinazolinethiones, quinoxalines (S-2720) and oxathiin carboxanilide NSC-615985 [13].

In order to clarify the molecular bases of RT/TC interaction and collect relevant information for the design of new TCs with improved potency against HIV-1 mutants, the crystal structures of TC-1-3 in complexes with RT have been determined at 2.8 Å, 3.0 Å, and 2.8 Å resolution, respectively (Table 1). These derivatives were selected not only for their potency, but also to investigate the potential formation of short oxygen–halogen bonds [14] involving the different sterically demanding Cl, Br, and I atoms at the *para* position of TC *N*-phenyl ring.

Materials and methods

Protein expression, purification, and crystallization. HIV-1 RT was expressed and purified as previously reported [15]. For crystallization, RT was concentrated to 20 mg mL^{-1} and mixed with two-fold molar excess of inhibitor (30 μM in DMSO). Crystals were grown by the hanging-drop vapour-diffusion method by mixing an equal amount (5 μL) of the RT-inhibitor solution and the crystallization buffer [1.4 M $(NH_4)_2SO_4$, 50 mM HEPES, pH 7.2, 5 mM $MgCl_2$, 300 mM KCl]. After two weeks equilibration at room temperature crystals appeared and grew to a size of $0.3 \times 0.2 \times 0.2$ mm^3 within two months.

Data collection and processing, structure solution, and refinement. X-ray diffraction data were collected at 100 K at the Lund Max-Laboratory beam lines 7–11. The data were indexed and processed using DENZO and SCALEPACK, respectively [16,17]. Further data manipulations were carried out with the CCP4 program suite [18]. RT/TC crystals belong to the orthorhombic space group $C222_1$; data collection statistics are reported in Table 1. The protein model of HIV-1 RT (PDB entry 1EET) [19] was used for structure solution through molecular replacement searches (AMoRe program). The initial phases were refined using REFMAC 5.2 [20], monitoring progress of the refinement through the R_{work}/R_{free} values [21] (Table 1). Model building was carried out using the program O [22]. All figures were produced using CCP4MG [23], Molscript [24], and Raster3D [25].

Results and discussion

RT/TC-1, RT/TC-2, and RT/TC-3 complexes

The quality of the electron density map allowed an unambiguous assignment of the positions, orientations, and conformations of TC-1-3 within the NNBS (Fig. 1B). The TC binding mode approximates the bioactive conformations observed for PETT and ITU derivatives [5] (*vide infra*), with the phthalimide scaffold and the *N*-phenyl ring positioned at approximately right angle. The binding site volume in the three structures analyzed is about 500 $Å^3$, smaller than those reported for HEPT (700 $Å^3$), MKC-442 (640 $Å^3$) and TNK-651 (660 $Å^3$) [26]. All three RT/TC complexes show a conserved hydrogen bond involving the thiocarbamic NH group and the Lys101 main chain carbonyl, while π–π stacking interactions are observed between the phthalimide fragment and Tyr181. This evidence structurally rationalized the weak potency expressed by TC against the strain bearing the Tyr181Cys