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Original article

Identification of HIV-1 reverse transcriptase dual inhibitors by a combined shape-, 2D-fingerprint- and pharmacophore-based virtual screening approach

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ABSTRACT

We report the first application of ligand-based virtual screening (VS) methods for discovering new compounds able to inhibit both human immunodeficiency virus type 1 (HIV-1) reverse transcriptase (RT)-associated functions, DNA polymerase and ribonuclease H (RNase H) activities. The overall VS campaign consisted of two consecutive screening processes. In the first, the VS platform Rapid Overlay of Chemical Structures (ROCS) was used to perform *in silico* shape-based similarity screening on the NCI compounds database in which a hydrazone derivative, previously shown to inhibit the HIV-1 RT, was chosen. As a result, 34 hit molecules were selected and assayed on both RT-associated functions. In the second, the 4 most potent RT inhibitors identified were selected as queries for parallel VS performed by combining shape-based, 2D-fingerprint and 3D-pharmacophore VS methods. Overall, a set of molecules characterized by new different scaffolds were identified as novel inhibitors of both HIV-1 RT-associated activities in the low micromolar range.

1. Introduction

Reverse transcription occurs in the early steps of the human immunodeficiency virus type 1 (HIV-1) life cycle and necessitates the conversion of the viral single-stranded RNA genome into a double-stranded DNA, subsequently translocated to the cell nucleus and integrated into the host DNA [1,2]. The process is accomplished by the virally encoded reverse transcriptase (RT), a multifunctional enzyme with distinct associated activities, including RNA- and DNA-dependent DNA polymerase (RDDP and DDDP, respectively), ribonuclease H (RNase H), strand transfer, strand displacement synthesis and nucleotide excision [1–3]. The two RT catalytic sites are inter-dependent and mutations in the polymerase domain affect the RNase H activity, and *vice versa* [4]. The pivotal role of RT in the HIV-1 life cycle made it a druggable target for the AIDS chemotherapy. In fact, several classes of RT inhibitors (RTIs) have been approved for the treatment of HIV-1 infected patients [5,6]. However, all RTIs in the market actually target only the RT-associated polymerase function [7]. Hence, the identification of new RTIs, targeting other RT-associated functions, or more than one function, could represent an attractive challenge [8,9]. Until now, only very few compounds are known to inhibit the RT-associated RNase H function [8,10], and even fewer both RT functions [11–13]. In particular, the identification of dual inhibitors is mostly interesting since, while the approved NNRTIs inhibit the RT-associated RDDP activity and increase the RT-associated RNase H activity [14], dual RT inhibitors may bind a pocket different from the NNRTI-binding site and show a different drug resistance profile. The state-of-the-art of HIV-1 RNase H inhibitors (RNase HIs) has

Abbreviations: BNMB, (Z)-4-(tert-butyl)-N′–((2-methoxynaphthalen-1-yl) methylene)benzohydrazide; LBVS, ligand based virtual screening; DDDP, DNA-dependent DNA polymerase; DHBNH, dihydroxy benzoyl naphthyl hydrazone; DKA, diketo acid; HIV-1, human immunodeficiency virus type; NNRTI, non-nucleoside RT inhibitors; RDDP, RNA-dependent DNA polymerase; RNase H, ribonuclease H; RNase HI, RNase H inhibitor; ROCS, rapid overlay of chemical structures; RT, reverse transcriptase; SAR, structure–activity relationship; VS, virtual screening; MMFFs, Merck molecular force field; GB/SA, generalized born/surface area; PRCG, Polak-Ribier coniugate gradient; RMSD, root-mean-square deviation; Glide, grid based ligand docking with energetics; NDDO, neglect of diatomic differential overlap; MOPAC, molecular orbital package; GBPM, grid based pharmacophore model.

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been recently reviewed [15]. RNase HIs are characterized by heterogeneous scaffolds and different mechanism of action. Representative structures of RNase HIs are reported in Fig. 1: RDS1643 is a diketo acid derivative (DKA) that selectively inhibits RNase H function [8]. This compound is able to block the replication of wild type HIV-1, showing an EC_{50}/CC_{50} of 13 μM/63 μM and also the replication of three HIV-1 non-nucleoside RT inhibitor (NNRTI) resistant viral mutants. DKAs were reported to bind to the RNase H catalytic site chelating the Mg^{2+} ion [6,8,10]. Tropolone derivatives are natural compounds with many biological activities. The hydroxylated tropolone derivative β-thujaplicinol is a potent and selective inhibitor of the HIV-1 RNase H activity. However, it is known to exhibit high cytotoxicity [6,8,10]. The co-crystallized structure of the RNase H domain with this compound has been recently resolved and confirmed the importance of metal chelation in protein-ligand binding [16,17]. An analogous binding mode was identified for some naphthyridinone-containing inhibitors and dihydroxypyrimidine-4-carboxylic acid derivatives [18,19]. Vinylogous urea derivatives, such as NSC727447, were proposed to bind to an allosteric pocket outside the RNase H catalytic site. NSC727447 does not inhibit the RT-associated RDDP activity and does not show metal-chelating properties [20]. Another interesting class of inhibitors are the hydrazones, whose mechanism of action is not perfectly clear although there are evidences that they may bind to a site proximate to the catalytic portion [21]. Recently, the structure of the complex RT-DHBNH (dihydroxy benzoyl naphthyl hydrazone) was published [11] and it was found that this compound could also bind to a site which is 50 *Å* away from the RNase H catalytic domain, located between the non-nucleoside (NNRTI)-binding pocket and the polymerase active site [11]. The same pocket is also occupied by MK3, a naphthyridinone derivative recently co-crystallized with RT [18]. Interestingly, DHBNH inhibits selectively the HIV-1 RNase H function, being inactive on the RDDP activity. Conversely, some DHBNH derivatives with bulky substituent in para position of benzoyl ring were found to exhibit activity on both RT-associated activities [11].

In the last decades, virtual screening (VS) approaches proved to be important tools in medicinal chemistry to speed up drug development [22,23]. Success stories have been reviewed [24], recently also with particular focus on antiviral targets [25]. However, it has to be pointed out that VS methods are not a "panacea": comparisons of VS methods performance highlight their advantage and pitfall, strength and weakness [23,26,27]. Synergism arising from multi-disciplinary approaches including VS and medicinal chemistry are particularly promising, as well as applying several VS approaches simultaneously [28–31]. The combination of VS methods can be done in a hierarchical, parallel or consensus way. The first approach is useful for reducing large collections of small organic molecules down to the most promising scaffolds. The second approach allows balancing differences and shortcomings in the ability of VS methods to search the chemical space. Another possibility is to exploit data fusion for prioritizing compounds to submit to biological test.

Therefore, with the aim to find new lead structures for HIV-1 RNase HIs, possibly inhibiting both RT functions, we have applied the shape-based screening method Rapid Overlay of Chemical Structures (ROCS) [32,33] on the National Cancer Institute (NCI) compounds database [34,35] in an initial VS run using the hydrazone analog, DHBNH, as query compound, and we have found a number of compounds which were able to inhibit both HIV-1 RT functions. Subsequently, the most-active compounds identified during this process were employed for a parallel Ligand Based Virtual Screening (LBVS). In particular, the methods applied were: ROCS, 2D-fingerprints and LB-pharmacophore search. When tested on the RT functions, several of the selected compounds characterized by new scaffolds showed to inhibit both RT-associated RNase H and RDDP activities, which may open the way to a new approach for drug development. In fact, the double activity of some anti-HIV agents has already been described in literature, i.e., in the case RNase H- integrase inhibitors [36,37], and also RNase H-RDDP activity [11–13].

2. Experimental section

2.1. Hardware specifications

An Intel Pentium 6400 Core 2 Duo equipped with 4 GB RAM running Linux Centos 5.4 was utilized for the molecular modeling studies. ROCS virtual screening was performed on four Intel 6600 Core 2 Duo processor machines with 2 GB RAM.

The workflows we followed for the two selections are given in Figs. 2 and 3. All data manipulations were made with specific protocols using Pipeline Pilot Student Edition [38].

2.2. Shape based method: first and second selection

DHBNH was selected as query for the initial ROCS screening. Conformational sampling was performed with OMEGA [39,40]. The predicted DHBNH lowest-energy conformation was employed to virtually screen the multi-conformer NCI database obtained with OMEGA, using default settings.

The best 200 hits were selected, considering ComboScore values. This score takes into account two distinct aspects: the shape similarity (ShapeTanimoto) and chemical pattern (ColorScore)

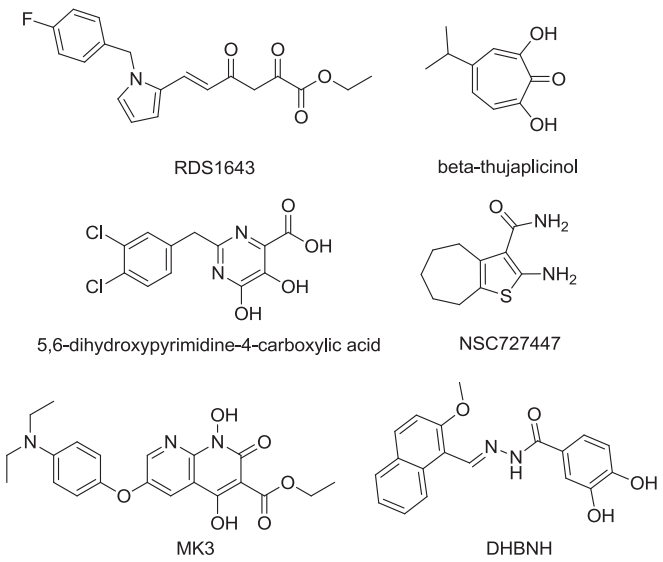

Fig. 1. Examples of HIV-1 RNase H inhibitors.

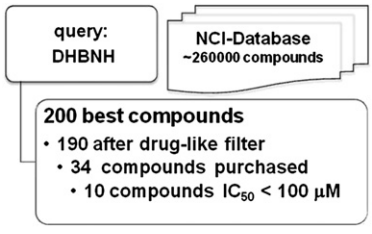

Fig. 2. Workflow followed for the first selection after ROCS screening.