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Research paper

Computational and synthetic approaches for developing Lavendustin B derivatives as allosteric inhibitors of HIV-1 integrase

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ABSTRACT

Through structure-based virtual screening and subsequent activity assays of selected natural products, Lavendustin B was previously identified as an inhibitor of HIV-1 integrase (IN) interaction with its cognate cellular cofactor, lens epithelium-derived growth factor (LEDGF/p75). In order to improve the inhibitory potency we have employed *in silico*-based approaches. Particularly, a series of new analogues was designed and docked into the LEDGF/p75 binding pocket of HIV-1 IN. To identify promising leads we used the Molecular Mechanics energies combined with the Generalized Born and Surface Area continuum solvation (MM-GBSA) method, molecular dynamics simulations and analysis of hydrogen bond occupancies. On the basis of these studies, six analogues of Lavendustine B, containing the benzylamino-hydroxybenzoic scaffold, were selected for synthesis and structure activity-relationship (SAR) studies. Our results demonstrated a good correlation between computational and experimental data, and all six analogues displayed an improved potency for inhibiting IN binding to LEDGF/p75 *in vitro* to respect to the parent compound Lavendustin B. Additionally, these analogs show to inhibit weakly LEDGF/p75-independent IN catalytic activity suggesting a multimodal allosteric mechanism of action. Nevertheless, for the synthesized compounds similar profiles for HIV-1 inhibition and cytoxicity were highlighted. Taken together, our studies elucidated the mode of action of Lavendustin B analogs and provided a path for their further development as a new promising class of HIV-1 integrase inhibitors.

1. Introduction

An essential step of the retroviral lifecycle is the insertion of the reverse-transcribed viral genome into the host chromosome. This process is catalyzed by HIV-1 integrase (IN), that has gained popularity as a promising target for the discovery of novel anti-HIV drugs.

IN is comprised of three domains: the N-terminal domain (NTD), the catalytic core domain (CCD) that coordinates two catalytic Mg^{2+} ions and the C-terminal domain (CTD) [1,2]. Initial drug discovery efforts for IN inhibitors have focused on small molecules able to inhibit the catalytic activity of IN and have resulted in three FDA approved IN inhibitors currently in clinical use, raltegravir, elvitegravir and dolutegravir [3–8]. These compounds all share a similar mechanism of action in that all bind at the IN active site in the presence of the viral DNA and inhibit the strand transfer (ST) activity. While these inhibitors have been highly effective against HIV, resistance mutations have emerged in patients [9–11]. Therefore, there is a continual need for the development of new IN inhibitors with innovative scaffolds that target alternative sites of

Abbreviations: ALLINI, allosteric integrase inhibitor; CCD, catalytic core domain; CTD, C-terminal domain; IBD, IN-binding domain; IN, Integrase; INLAIs, Integrase lens epithelium-derived growth factor Allosteric Inhibitors; vDNA, viral DNA; GOLD, Genetic Optimization for Ligand Docking; LEDGF/p75, lens epithelium-derived growth factor; LEDGINs, lens epithelium-derived growth factor Integrase Inhibitor; MD, molecular dynamic; MM-GBSA, Molecular Mechanics energies combined with the Generalized Born and Surface Area continuum solvation; MINIs, Multimerization Integrase Inhibitors; NPs, Natural Products; NTD, N-terminal domain; NCINIs, Non Catalytic Integrase Inhibitors; PPI, protein-protein interaction; PPIIs, protein-protein interaction inhibitors; 3′P, 3′processing; ST, strand-transfer; VSV-g, vesicular stomatitis virus g.

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the enzyme.

The integration process comprises two catalytic steps: the first is a hydrolytic reaction termed 3'processing (3′ P), followed by a transesterification reaction (also referred as to ST) [12–14]. The cellular chromatin associated protein lens epithelium-derived growth factor (LEDGF/p75) associates with IN and significantly enhances integration efficacy by tethering preintegration complexes to active genes during integration [15–19]. LEDGF/p75 is a transcriptional co-activator strongly associated with chromatin throughout the cell cycle [20–22]. Its C-terminal domain contains the IN-binding domain (IBD), allowing it to not only interact with natural cellular binding partners, but also HIV-1 IN [18,19,23,24].

Recent efforts have led to the discovery of a new class of allosteric IN inhibitors (ALLINIs, also known as LEDGINs, NCINIs, INLAIs, or MINIs) [25–30] targeting the IN dimer interface at the principal LEDGF/p75 binding pocket. Interestingly, two alternative approaches have identified a similar class of quinoline-based ALLINIs: a high throughput screen was used to discover compounds inhibiting 3'-processing activity of IN Ref. [26] and the rational drug design was exploited to develop small molecules to block the IN-LEDGF/p75 interaction [25]. Of note, the rational design approach was made possible by the crystal structure of a CCD-CCD dimer bound to the IBD [31]. Furthermore, the ability to solve the structures of ALLINIs bound at the CCD-CCD dimer [25–28] has facilitated the rapid expansion of this class of inhibitors.

ALLINIs exhibit a multimodal mechanism of action in that they not only inhibit IN-LEDGF/p75 interaction but they also promote higher order aberrant IN multimerization, resulting in inactive protein [2,32,33]. Surprisingly, ALLINIs exhibit higher potency when present during virion morphogenesis compared with the early stage of viral replication [29,34–37]. In the virions, where due to the lack of competing LEDGF/p75 binding to the IN dimer, ALLINIs potently induce aberrant IN multimerization and result in eccentric, non-infectious virions; whereas during the early stage of HIV-1 replication LEDGF/p75 effectively competes with ALLINI binding to IN and reduces the inhibitor potency [38]. Selection of HIV-1 phenotypes under the genetic pressure of various ALLINIs have identified substitutions at the IN dimer interface at the inhibitor binding sites that confer resistance to these compounds [25,28,39,40]. Collectively, the studies with ALLINIs have shown that the potent inhibitors that target IN sites distinct from the active site can be developed. At the same time, there is a need to further improve these compounds to overcome the resistance seen in cell culture assays.

Natural Products (NPs) have historically been an extraordinary source for new medicines and are continuing to be the origin of lead compounds for drug discovery [41,42]. Previously, we have reported the application of a structure-based virtual screening strategy for the identification of NPs as potential protein-protein interaction inhibitors (PPIIs) targeting the IN-LEDGF/p75 protein complex [43]. Among them, we focused our interest on the Lavendustin B (Fig. 1A), which inhibited IN binding to LEDGF/p75 in Alphascreen assay [43]. This novel scaffold is unique from all reported ALLINIs and could represent an encouraging new hit compound warranting further improvement and investigation. Therefore, to exploit this novel scaffold and improve its potency as an IN inhibitor, we have employed *in silico* approaches to identify promising Lavendustin B derivatives and examine inhibitory activities using *in vitro* and *cell based* assays.

2. Results and discussion

2.1. Rational design

By using a combination of docking and ultrashort molecular dynamics (MD), we have generated a weighted ensemble of protein-ligand configurations for IN-LEDGF/p75 protein-protein interaction inhibitors. Therefore we estimated their binding affinities by averaging snapshots taken from the MD trajectories, together with the presence of fundamental hydrogen bonds [44]. These *in silico* studies, followed by experimental analysis of selected compounds, have led to the identification of Lavendustin B with an IC_{50} of 94.07 μM for inhibiting IN binding to LEDGF/p75 *in vitro* [43]. *In silico* docking studies (Fig. 1 B) have highlighted the following interactions: the carboxylic group of Lavendustin B establishes H-bond interactions with the backbone nitrogen atoms of Glu170 and His171 residues, similar to the interactions seen with LEDGF/p75 hotspot residue of Asp366 [24]. Additionally, there is the formation of a potential hydrogen bond with the hydroxyl group side chain of the Thr174 residue. The remaining portion of Lavendustin B is housed within the dimer interface cleft comprised of IN subunit A residues of Thr174, Gln168, Ala169 and Met178 and IN subunit B residues of Ala128, Ala129, Trp131 and Trp132 allowing the molecule to establish hydrophobic contacts with both subunits. We have used these *in silico* predicated interactions as the basis for our current study.

In order to design new analogs, we utilized a published X-ray crystal structure of the active compound KF115 (PDB-400J) which shares a similar binding mode with Lavendustin B (Fig. 1A and C) [28]. KF115 is a pyridine-based inhibitor in the class of ALLINIs that preferentially promoted aberrant IN multimerization over inhibiting the IN-LEDGF/p75 interaction. The superimposition of the crystal structure of KF115 bound to the CCD-CCD dimer with the docked model of Lavendustin B (Fig. 1B) reveals a high degree of similarity with the carboxylic groups of the both compounds interacting with Glu170, His171 and Thr174. In addition, the 4-chlorophenyl and the 3,4-dimethylphenyl groups of KF115 occupy the hydrophobic pockets in a similar manner of the two 2-hydroxyphenyl portions of Lavendustin B. Considering these results, structural modifications on Lavendustin B were introduced *in silico*: the 2-hydroxy group was removed, and halogen atoms (chlorine and fluorine) and methyl substituent were added to explore the hydrophobic areas. The planned modifications on Lavendustin B are depicted in Fig. 1 D for compounds (**1**–**10**).

2.2. Docking and molecular dynamics (MD)

Before carrying out the synthesis of the designed compounds (**1**–**10**) we wanted to predict the potential binding mode of the analogs by means of the reported computational procedure [43,45]. First a docking simulation into the principal LEDGF/p75 binding pocket on IN [24] was performed using GOLD (Genetic Optimization for Ligand Docking) [46]. In order to take into account the flexible side chain of residue Gln95 two different conformations of IN CCD were used (PDB ID: 3LPU [25] and 2B4J [32]). More than two clusters were taken for additional analysis. To eliminate potentially unfavorable contacts, the geometry of the system was minimized using the steepest descent algorithm followed by a conjugate gradient. The solvent effects were considered through the generalized Born implicit solvent model. The output complex was employed to estimate ligand binding free energy using the MM-GBSA method. The obtained results for both CCD conformations (complex 1 and 2) are shown in Table 1.

Since the calculated binding energy of the complex 1 and 2 were similar we decided to consider only the conformation of the protein retrieved by 2B4J (complex 1) for further or more complete analysis. Fig. 2 shows the binding orientations of the designed analogs. We observed that six compounds, namely (**1**–**3**, **5**, **6** and **8**), share the binding mode with parent Lavendustin B. The other derivatives (**4**, **7**, **9** and **10**) assume a binding pose for which they mimic the