Respiratory syncytial virus (RSV) is the major cause for respiratory tract disease in infants and young children. Currently, no licensed vaccine or a selective antiviral drug against RSV infections are available. Here, we describe a structure-based drug design approach that led to the synthesis of a novel series of zinc-ejecting compounds active against RSV replication. 30 compounds, sharing a common dithiocarbamate moiety, were designed and prepared to target the zinc finger motif of the M2-1 protein. A library of ~12,000 small fragments was docked to explore the area surrounding the zinc ion. Among these, seven ligands were selected and used for the preparation of the new derivatives. The results reported here may help the development of a lead compound for the treatment of RSV infections.

A B S T R A C T

1. Introduction

RSV is an enveloped, single-stranded, negative RNA virus belonging to the Paramyxoviridae family (Easton and Pringle, 2011). Its genome encodes 11 proteins, and among these the M2-1 protein is an essential transcription antitermination cofactor of the viral RNA-dependent RNA polymerase (RdRP) complex of which the crystal structure has been recently released (PDB code: 43CB) Tanner et al., 2014. This protein increases the polymerase function and prevents the termination of the transcription event, which is an essential event for the synthesis of full-length mRNA (Collins et al., 1996) and for the synthesis of polycistronic readthrough mRNAs (Sutherland et al., 2001; Fears and Collins, 1999; Hardy and Wertz, 1998). M2-1 contains a zinc binding domain (ZBD) that is situated at the N-terminus of the protein. It has been demonstrated that its integrity is a key element for maintaining the functional integrity of the M2-1 (Hardy and Wertz, 2000). Mutants of some of the residues that are coordinated with the zinc ion alter the activity of the protein by reducing the transcription event, changing the phosphorylation state of the protein and preventing interactions between M2-1 and the nucleocapsid protein (Collins et al., 1996; Sutherland et al., 2001; Fears and Collins, 1999; Hardy and Wertz, 1998, 2000; Tang et al., 2001). The sequence of the zinc finger motif (C-X$_3$-C-X$_2$-C-X$_3$-H) slightly differs from other ZBDs, e.g. the one in the nucleocapsid (NC) protein of the human immunodeficiency virus 1 (HIV-1). Nevertheless, Zn$^{2+}$ ion is linked to the Cys$_3$-His$_3$ motif in a similar way as its binds the zinc domain of HIV-1 by coordinating three cysteine and one histidine residues (Fig. 1) Berg, 1986; Henderson et al., 1981.

Zinc ejection has already been described in the literature as a method of enzyme inhibition (Rice et al., 1993). A successful example of metal chelation by zinc-ejecting compounds is represented by the effect of azodicarbonamides on the HIV nucleocapsid (NCp7) that selectively eject zinc ions (Loo et al., 1996; Rice et al., 2000). Recently, the crystal structure of an M2-1 dimer bound to a zinc-ejecting compound has been determined (Sutherland et al., 2001). Zinc ejection has been already described in the literature as a method of enzyme inhibition (Rice et al., 1993). A successful example of metal chelation by zinc-ejecting compounds is represented by the effect of azodicarbonamides on the HIV nucleocapsid (NCp7) that selectively eject zinc ions (Loo et al., 1996; Rice et al., 2000). Recently, the crystal structure of an M2-1 dimer bound to a zinc-ejecting compound has been determined (Sutherland et al., 2001).

In silico structure-based design and synthesis of novel anti-RSV compounds

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et al., 1995, 1997; Yu et al., 1995; Tummino et al., 1996). One of these NCP7 zinc-ejecting compounds is currently in phase I/II clinical trials to evaluate its potential to treat advanced acquired immunodeficiency disease syndrome (AIDS) Vandevelde et al., 1996. Based on the common function and geometry of the zinc finger motif between Retroviruses and RSV, a recent study evaluated the effect of 2,2'-dithiopyridine (aldrithiol, AT-2), known to inactivate HIV-1 by modifying the NC zinc finger, on the RSV infectivity. The results showed the ability of this molecule to inactivate RSV at a concentration of 10 mM, presumably by modification of the M2-1 protein (Boukhvalova et al., 2010). Considering these data, we used a structure-based drug design approach to generate a series of compounds containing a zinc-chelating moiety. In this study, we initially docked a number of fragments into the targeted area to evaluate their potential interactions around the zinc atom. Selected fragments were then linked to the dithiocarbamate group, which is known to have zinc ejecting properties (Thorn and Ludwig, 1962). Furthermore, this group has also been reported as an essential feature for the antiviral activity of pyrrolidine dithiocarbamate (PDTC) in several RNA viruses (Lanke et al., 2007), therefore it was chosen as the core feature for our compounds. Based on the initial results, different series of new potential zinc-ejecting compounds were synthesised and tested for their antiviral activity in cell-based RSV infection assays.

2. Materials and methods

Detailed molecular modeling and chemical procedures are reported in the Supplemental information file.

3. Results and discussion

3.1. Computer-aided design

The ZBD of M2-1 protein is a very tight binding pocket, defined by three cysteine residues and a histidine that have a simple geometry and form a planar ring which surrounds the Zn ion. Due to the small size of the region, our approach was to use a library of small fragments instead of traditional screening libraries that tend to be constituted by a variety of large and lipophilic molecules that could not fit the binding area. On the other hand, generating hits using libraries of smaller dimensions increase the chance of observing favorable interactions. The ZINC database is a collection of commercially available chemical compounds organized into different subsets of molecules filtered according to various parameters (Irwin et al., 2012). The ‘clean fragments’ subset, which is composed of 1,611,889 small molecules, was downloaded and used in this study. In this database, aldehydes and thiols are not present, the compounds have a logP less or equal to 3.5, a molecular weight less than 250 and rotatable bonds equal or less than 5. We further filtered the database by molecular weight: only those fragments with a molecular weight less than 150 were taken into account for docking studies. A total of 12,420 ligands were then docked in the proximity of the zinc binding domain. A series of docking simulations using MOE (Molecular Operating Environment, version 2009.10, Chemical Computing Group Inc.) was carried out to assess which fragments could best fit two different pocket subites, in particular the areas that are in close proximity to the Cys3-His1 motif: a first region defined by Gly18, Lys19, His22, Ser24 and His25 (Fig. 2) and a second area defined by Glu10, His14 and Cys15. After evaluating their binding scores and interactions by a visual inspection of the generated poses, the original input database was reduced to 100 fragments, of which seven were selected according to the synthetic feasibility of the corresponding compounds. A general unsymmetrical three-membered scaffold was designed to contain a common central dithiocarbamate linker, functionalized on its two sides with different moieties that were further validated by another set of docking studies (Fig. 2).
The designed molecules were characterized by an aromatic portion to target residues Glu10, His14, a central part formed by the dithiocarbamate moiety that occupies the area around the zinc atom and an alicyclic amine that resides in the region defined by residues Gly18, Lys19, Cys21, His22, Ser24 and His25. Six aromatic fragments were chosen, corresponding to 2-chloromethyl benzothiazole, 2-chloromethyl benzoxazole, 4-(trifluoromethyl)benzyl bromide, 3-nitrobenzyl bromide and 4-bromomethyl-3-nitrobenzoic acid. For the alicyclic amines, piperidine, morpholine, N-methyl piperazine, piperazine and benzyl piperazine were chosen. All the designed compounds were docked in the targeted area using PLANTS (version 1.1) software package (Korb et al., 2009) and they all nicely fit into the selected binding region. The predicted binding mode for compound 10e is shown in Fig. 3 as a representative example. From these results, it is possible to see how 10e establishes a stacking interaction with the imidazole ring of His14 through the benzoxazole ring, while the N(C=S)–S group is in close proximity to the Zn ion. Furthermore, the benzyl piperazine moiety establishes hydrophobic interactions with Lys19.

3.2. Chemistry

A common synthetic pathway was followed for the synthesis of new small families of compounds. These structures share the five types of secondary alicyclic amines, but are differentiated from each other by the aromatic moiety defined by the six selected fragments. Final compounds 8–13a–c, e were prepared in a one-pot reaction using an equimolar ratio of the appropriate alicyclic secondary amine (1a–c, e) and carbon disulfide, which were stirred at 0 °C for 30 min in the presence of sodium methoxide, in order to obtain the formation of the dithiocarbamate salt. The different aryl chloride or bromide 2–7 was successively added in order to get the final compounds through displacement of the chloride or bromide leaving group by the dithiocarbamate salt (Scheme 1). Compounds 8, 11–12d were obtained by partially changing the reaction conditions already described, through the addition of 0.5 equivalents of carbon disulfide to piperazine in order to prevent a double nucleophilic attachment on the two free amino groups.

Starting materials 3, 4 and 7 were not commercially available and were synthesized according to reported procedures (Vlahakis et al., 2013; Soares et al., 2010; Lopes et al., 2011). Compounds 15a–c, e were prepared by a base-catalyzed hydrolysis with lithium hydroxide of the correspondent methyl ester derivatives 14a–c, e.

3.3. Biology

The newly synthesized compounds were then evaluated for their antiviral activity in an in vitro cell-based assay, in which HEp-2 cells were infected with luciferase-expressing RSV (Hotard et al., 2012) in the presence of the compounds. The luciferase activity driven by RSV is a measure for virus replication. Cells were treated with different dilutions (30–0.5 μM) of RSV inhibitors 30 min prior to and during infection with RSV. As a control in our assay, cells were treated with DMSO, which was used to prepare the inhibitor stock solutions. Twenty-four hours post infection, the luciferase activity was measured and the number of viable cells were determined. The compound concentrations that gave 50% reduction of RSV-driven luciferase activity (IC50) or cell viability (CC50) compared to the DMSO control were determined by extrapolation. The reference compound AT-2 was tested in our RSV assay at several dilutions (10,000–0.5 μM). Previously, Boukhvalova et al.
demonstrated the inactivation of RSV particles by incubation in the presence of 10 mM AT-2. In their study, the compound was removed prior to analyzing the remaining RSV infectivity. In a recent paper that demonstrated inactivation of RSV by 10 mM AT-2, a zinc-ejecting compound, presumably by modification of the M2-1 protein (Boukhvalova et al., 2010). Using seven selected small fragments as building blocks for the design of new scaffolds, 30 derivatives were prepared. Their chemical structure is defined by a common dithiocarbamate central linker, while an alicyclic secondary amine and an aromatic substituent are on the two lateral parts. Among them, six compounds inhibited the viral replication at low or moderate micromolar concentrations in a cell-based assay. The most active compound, 10e, exhibited an IC50 of 6 μM, which represents a considerable improvement compared to AT-2 (IC50 of 841 μM in our RSV inhibitory assay), a previously reported inhibitor (Fig. 4). Common features among the active compounds suggest the importance of the benzyl piperazine moiety on one side of the scaffold. Although further studies are required to prove the mechanism of action of these compounds, these results represent a very promising starting point for the development of a novel class of RSV inhibitors.

Table 1
IC50 and CC50 values of the new compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (μM)a</th>
<th>CC50 (μM)b</th>
<th>Si c</th>
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<tr>
<td>AT-2</td>
<td>841</td>
<td>733</td>
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</tr>
<tr>
<td>9a</td>
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<tr>
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<tr>
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<td>NI</td>
<td>–</td>
</tr>
<tr>
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</tr>
<tr>
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<td>NI</td>
<td>–</td>
</tr>
<tr>
<td>10b</td>
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</tr>
<tr>
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<tr>
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<td>NI</td>
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</tr>
<tr>
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a NI indicates no significant inhibition at concentration >30 μM.
b Different dilutions of AT-2 (10,000–0.5 μM) were tested.
c Si = CC50/IC50.
Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.antiviral.2015.08.003.

References


