Identification and activity of inhibitors of the essential nematode-specific metalloprotease DPY-31

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Abstract

Infection by parasitic nematodes is widespread in the developing world causing extensive morbidity and mortality. Furthermore, infection of animals is a global problem, with a substantial impact on food production. Here we identify small molecule inhibitors of a nematode-specific metalloprotease, DPY-31, using both known metalloprotease inhibitors and virtual screening. This strategy successfully identified several μM inhibitors of DPY-31 from both the human filarial nematode Brugia malayi, and the parasitic gastrointestinal nematode of sheep Teladorsagia circumcincta. Further studies using both free living and parasitic nematodes show that these inhibitors elicit the severe body morphology defect 'Dumpy' (Dpy; shorter and fatter), a predominantly non-viable phenotype consistent with mutants lacking the DPY-31 gene. Taken together, these results represent a start point in developing DPY-31 inhibition as a totally novel mechanism for treating infection by parasitic nematodes in humans and animals.

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More than 1 billion people, predominantly in the developing world, are infected by parasitic nematodes (helminths). The primary strategy for eliminating these infections is preventive chemotherapy by mass anthelmintic drug administration, an approach that will select for drug resistance.1 Furthermore, helminth infection also represents a significant global burden to livestock.2 Resistance to anthelmintic drugs is increasing in gastrointestinal (GI) parasites of livestock, causing concern that this will also occur in human parasites. This increased resistance coupled with the limited availability of new drugs and absence of vaccines means that the identification of new potential targets for drug intervention is critical.3

The life cycle of all nematodes requires cyclical repetitive shedding of the organism’s protective cuticle and concomitant generation of a new cuticle at several points during maturation. This molting process involves a specific class of well-characterized astacin metalloproteases.4 The zinc endopeptidase DPY-31 is a nematode-specific member of this class that is essential for cuticle formation.4d A mutant suppressor screen in Caenorhabditis elegans identified the target of DPY-31 to be the cuticle collagen SQT-3, and specified the C-terminal cleavage domain of this crucial structural protein where DPY-31 acts.5 Critically, without the ability to molt, a nematode will fail to develop and ultimately die prematurely.

Here we describe the identification of small molecule inhibitors of DPY-31 employing two different approaches: use of known metalloprotease inhibitors and virtual screening. These compounds were screened for activity against recombinantly expressed DPY-31 from both the human filarial nematode Brugia malayi, and the parasitic GI nematode of sheep Teladorsagia circumcincta. Active compounds were then tested against both free living and parasitic nematodes themselves.

Seven known zinc protease inhibitors were included for testing (Fig. 1). The phosphinic pseudopeptide 1 used in the virtual screening described below is a mid-μM inhibitor of crayfish astacin and is studied here in the context of the nematode astacin DPY-31.6 Furthermore, the antibiotic and CD13/aminopeptidase N inhibitor actinonin (2),7 and the broad-spectrum matrix metalloprotease inhibitor marimastat (3),8 were examined. Four non-peptidic inhibitors of human procollagen C-proteinase (4–7) developed by Pfizer were also screened for in vitro activity against DPY-31.9

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Amidic acids were installed by CDI coupling of 1,2-aminophenol with corresponding tripeptides, followed by hydrolysis.10 Two different carbamates on the N-terminus (Fig. 2). The hydroxamate transition state analogs are complementary to the transition state analog I (Fig. 1, PDB 1QJI).6 This reinforces the conserved nature of this metalloprotease.

As DPY-31 has not been crystallized, structure-based virtual screening was carried out using a 3D homology model of C. elegans DPY-31 constructed using Modeller13 (Fig. 3A–C), and the structure of crayfish astacin in complex with phosphinic pseudopeptide transition state analog 1 (Fig. 1, PDB 1QJI).7 This resulted in a model with Modeller objective function of 1342.4 (Fig. 3D).

The rigid-body docking program LiDAEUS was used to dock the conformer virtual library into the substrate binding groove of the DPY-31 model. The results were ranked and merged with the results from the ligand-based methods described above. These unique molecules were then docked into DPY-31 using Vina. The top compounds were then docked using Autodock and compounds whose predicted binding modes differed between the programs were discarded.8 Predicted binding poses were also scored using DrugScore 1.2.15 A final ranked list was prepared via a rank-by-rank consensus scheme,16 taking the Vina, Autodock, X-Score and DrugScore scores into account. The top 200 virtual hits were clustered according to similarity (Tanimoto < 0.7) and one compound from each cluster was selected for purchase (46 compounds). A further 28 compounds were selected for purchase as structural analogs of the molecules that were identified using the virtual screening techniques described above.

In total, 104 compounds were screened against recombinant DPY-31 from both the human parasite B. malayi as well as the sheep GI parasite T. circumcincta using an absorbance assay.3,10 Data for four of the most active compounds are given in Table 1. In keeping with the high level of sequence homology of DPY-31 across species,4–6 these inhibitors displayed broadly similar efficacy between the two species. Surprisingly, the phosphinic pseudopeptide 1 was inactive in this in vitro assay (IC50 > 500 μM). This may be due to the extremely slow binding kinetics of these inhibitors.17 Furthermore, shorter dipeptide hydroxamic acids (cf. 8 and 9) were inactive in this assay.

Having successfully demonstrated small molecule inhibition of isolated DPY-31, we selected tripeptide hydroxamic acids 8 and 9 for phenotypic screening. These compounds were tested against three strains: free-living wild-type C. elegans N2, the T. circumcincta dpy-31 transgenically-rescued C. elegans dpy-31 mutant TP224, and parasitic T. circumcincta (Fig. 4). Phenotypes were evaluated in 96 well plate format over the course of 3 days using concentrations ranging from 50 μM to 2 mM. Both compounds were able to induce the Dpy phenotype that is consistent with loss of function of DPY-31.6,4 The similarity of these effects between wild-type C. elegans, the mutant strain, and T. circumcincta reinforces the conserved nature of this metalloprotease.

In conclusion, using a combination of in silico and experimental methods, we have identified small molecule inhibitors of the nematode-specific astacin metalloprotease DPY-31, which is essential for cuticle collagen biogenesis. These compounds are active against recombinant DPY-31 from both human and livestock nematode parasites. Furthermore, we have shown that these compounds can elicit the specific body morphology defect associated with deficiency of this essential protein in both free-living and parasitic nematodes. In C. elegans, these compounds replicate the phenotype
associated with mutation of the dpy-31 metalloprotease encoding gene.4d,g These results represent a first step toward validation of DPY-31 as a totally novel target for drug intervention in the treatment and control of parasitic nematodes of medical and veterinary significance. Future SAR work is expected to enhance potency while ensuring selectivity for DPY-31.

Acknowledgments

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Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>pIC50 rDPY-31 B. malayi ± standard error</th>
<th>pIC50 rDPY-31 T. circumcincta ± standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3.7 ± 0.2</td>
<td>4.1 ± 0.5</td>
</tr>
<tr>
<td>6</td>
<td>4.7 ± 0.3</td>
<td>4.4 ± 0.3</td>
</tr>
<tr>
<td>8</td>
<td>4.1 ± 0.4</td>
<td>4.6 ± 0.3</td>
</tr>
<tr>
<td>9</td>
<td>4.6 ± 0.4</td>
<td>4.5 ± 0.2</td>
</tr>
</tbody>
</table>

Figure 3. 3D homology models of C. elegans DPY-31 alone, and crayfish astacin in complex with a phosphinic pseudopeptide transition state analog. (A) 3D homology model of C. elegans DPY-31, with (B) and (C) showing a closer view of the catalytic zinc-binding site, (D) 3D homology model of crayfish astacin in complex with phosphinic pseudopeptide transition state analog 1.

Figure 4. (a) WT L1 C. elegans (N2). (b) Dpy phenotype in L1 C. elegans (N2) with 50 μM 8. (c) WT L1 transgenic rescue strain TP224. (d) Dpy L1 phenotype in TP224 with 100 μM 8. (e) WT T. circumcincta L3. (f) Dpy phenotype in T. circumcincta L3 with 500 μM 9.

Supplementary data

Supplementary data (the in silico identification of potential astacin metalloprotease inhibitors, protocols for the recombinant expression of DPY-31, synthetic procedures and characterization for compounds 8 and 9, and descriptions of the techniques used for absorbance assays and phenotypic screening. $^1$H and $^{13}$C NMR spectra for compounds 8 and 9) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.10.077.

References and notes


10. See Supporting information for details.


