Fragment growing exploiting dynamic combinatorial chemistry of inhibitors of the aspartic protease endothiapepsin
Mondal, Milon; Groothuis, Daphne E.; Hirsch, Anna K. H.

Published in:
MedChemCommun

DOI:
10.1039/c5md00157a

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2015

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Fragment growing exploiting dynamic combinatorial chemistry of inhibitors of the aspartic protease endothiapepsin†

Milon Mondal, Daphne E. Groothuis and Anna K. H. Hirsch*

Fragment-based drug design (FBDD) has emerged as an efficient hit-identification and/or -optimization strategy with a higher hit rate than high-throughput screening (HTS). Whereas fragment linking is more challenging, fragment growing has become the preferred fragment-optimization strategy, requiring synthesis of derivatives and validation of their binding mode at each step of the optimization cycle. Dynamic combinatorial chemistry (DCC) is a powerful and efficient strategy to identify ligands for biological targets. Here, we have demonstrated that the novel combination of fragment-growing and DCC is a highly powerful strategy to grow a fragment into a more potent, non-covalent inhibitor of the aspartic protease endothiapepsin. We have designed a library of acylhydrazones using fragment growing starting from a known fragment in complex with endothiapepsin. We have used DCC and a fluorescence-based enzymatic assay to identify the best hit(s) from the dynamic combinatorial libraries, displaying double-digit micromolar inhibition of endothiapepsin. In addition, each DCC experiment requires only very small amounts of protein compared with established methods of analysis and the protein needs to be in the assay mixture only for a short period of time, making this protocol ideal for precious and unstable proteins. These results constitute a proof of concept that the combination of fragment growing and DCC is a powerful and efficient strategy to convert a fragment into a hit.

Introduction

Over the past decades, fragment-based drug design (FBDD) has become a well-established strategy for the identification of inhibitors of numerous biological targets.1–3 FBDD has higher hit rates than high-throughput screening (HTS), with better coverage of the chemical space.1 Once a fragment has been identified, it has to be optimized to a lead compound by fragment growing or linking. On the one hand, fragment growing has become the preferred fragment-optimization strategy.2,4–8 Preserving the binding modes of two fragments bound to adjacent pockets, as required in fragment linking, is considered attractive given the potential for super-additivity but at the same time very challenging.7 On the other hand, fragment growing is time-consuming as it requires synthesis of derivatives and validation of their binding mode at each step of the fragment-optimization cycle. To overcome this hurdle, we developed a strategy in which we combine fragment growing with dynamic combinatorial chemistry (DCC) for the optimization of a fragment to a hit. Over the past decade, DCC has emerged as an efficient and innovative approach in drug discovery as it allows for the formation of a dynamic combinatorial library (DCL), in which the chemical bonds that connect multiple building blocks are continuously being made and broken, allowing for the in situ formation of a great variety of compounds.10,11 There are numerous reports of DCC to facilitate hit/lead identification or optimization.11 A proof of concept for fragment linking facilitated by DCC has been provided retrospectively when letting disconnected known hydrazine inhibitors reassemble in the presence of crystals of the protein target.12 Linking of an intermediate derived from biosynthesis to fragments designed to occupy adjacent pockets by disulfide-based DCC13 as well as covalent tethering have been reported over the past decade.14–16 FBDD in combination with DCC has, however, never been explicitly reported for conventional fragment growing in a strict FBDD context. We introduce here a fluorescence-based enzymatic assay to conveniently screen the DCL for active inhibitors, which requires small amounts of the protein target for a short period of time, making it ideally suited for precious and unstable proteins.17

In the present study, we have applied this strategy to optimize a fragment into a hit for endothiapepsin. Endothiapepsin
is a pepsin-like aspartic protease. Members of this class of enzymes play a causative role in numerous diseases such as malaria (plasmepsins), Alzheimer’s disease (β-secretase), fungal infections (secreted aspartic proteases), and hypertension (renin). Endothiapepsin has been used as a model enzyme for the development of fragments of renin\(^{19}\) and β-secretase\(^{20}\) as well as to better understand the mechanism of action of aspartic proteases.\(^{21–23}\) Aspartic proteases consist of two similar subunits, each of which contributes an aspartic acid residue to the catalytic dyad (D35 and D219) for endothiapepsin) that hydrolyzes the peptide bond of the substrate using a catalytic water molecule.

Imine-based chemistry has been extensively used for protein-templated DCC in drug design projects.\(^{24}\) Due to the inherent instability of imines in aqueous media, acylhydrazones have become the workhorse for imine-type biomed-ical DCC projects.\(^{25–27}\) Acylhydrazone-based DCC is particularly attractive given that the resulting products provide both H-bond donor and acceptor sites and are stable enough under acidic and physiological conditions to enable direct analysis. In our previous work, we showed that the acylhydrazone moiety is a suitable central scaffold to address the catalytic dyad of endothiapepsin,\(^{27}\) we decided to use an acylhydrazone moiety to grow 1 towards the S1 and S3 pockets. We introduced an acylhydrazone linker pointing towards the S1 & S3 pockets. According to our modeling, the NH group of the newly designed acylhydrazone moiety is engaged in an H-bonding interaction with D35, while the carbonyl group of the acylhydrazone linker accepts an H bond from the backbone of G80. Careful investigation of known co-crystal structures of endothiapepsin\(^{28}\) and hotspot analysis\(^{22}\) of the active site of endothiapepsin suggested that both aliphatic and aromatic substituents can be introduced through the aldehyde of the acylhydrazone linker, which can be involved in hydrophobic interactions with L125, I122, F116 and Y79 in the S1 & S3 pockets (Fig. 2). Based on molecular modeling and docking studies, we selected a series of nine acylhydrazone-based inhibitors (Scheme 1a). All the derivatives can be conveniently synthesized starting from hydrazide 2 by reacting it with nine aldehydes (3a–11a) (Scheme 1b). While all aldehydes are commercially available, we synthesized 2 via an asymmetric Strecker reaction starting from commercially

![Fig. 1 X-ray crystal structure of endothiapepsin in complex with fragment 1 (Protein Data Bank (PDB) code: 3PCW).\(^{28}\) Color code: (protein surface) C: gray, O: red and N: blue; (fragment skeleton) C: green, N: blue and F: cyan. Hydrogen bonds below 3.0 Å are shown as black, dashed lines.](image1)

![Fig. 2 Superimposition of MOLOC-generated molecular models of potential acylhydrazone inhibitors featuring direct H-bonding interactions with the catalytic dyad (D35 and D219) in the active site of endothiapepsin (PDB code: 3PCW).\(^{28}\) Color code: (protein skeleton) C: gray; (inhibitor skeletons) C: green, violet, purple, orange, and yellow, N: blue, F: cyan, and O: red. Hydrogen bonds below 3.4 Å are shown as black, dashed lines.](image2)
We set up nine DCLs, each containing hydrazide 2 and one of the nine aldehydes 3a–11a to form the corresponding acylhydrazones 3h–11h (Scheme 1b). We used a fluorescence-based enzymatic activity assay to conveniently screen the DCLs for active inhibitors as this method requires very small amounts of protein.17 In the assay, we used the fluorogenic substrate of HIV protease (2-aminobenzoyl-Thr-Ile-Nle-Phe(p-NO2)-Gln-Arg-NH2, 12), which is hydrolyzed by endothiapepsin and generates two fluorescent fragments, namely 13 and 14, enabling convenient spectrophotometric monitoring (Scheme S2 in the ESI†). Having investigated the DCLs using the fluorescence-based assay, we identified a total of two DCLs out of nine displaying considerably higher inhibition of endothiapepsin than 1, showing 75% (IC50 = 407 μM) and 79% (IC50 = 252 μM) inhibition at an inhibitor concentration of 1 mM (based on the acylhydrazones formed in situ).
Conclusions

In conclusion, we have demonstrated for the first time that the combination of fragment growing and DCC is a powerful technique for the rapid and efficient identification of novel hits for the aspartic protease endothiapepsin. Moreover, by using a fluorescence-based assay, we could directly screen the DCLs for active inhibitors. The advantages of this approach are that only very small amounts of protein are required compared with established methods of analysis and that the protein needs to be in the assay mixture only for a short period of time, making this protocol ideal for precious and unstable proteins. Among the acylhydrazones identified, the most potent inhibitor 9h displays an IC_{50} value of 85 μM. This synergistic combination of computational and analytical methodologies proved to be successful for the development of inhibitors of the aspartic protease endothiapepsin and could be applied to a wide range of targets.

Acknowledgements

Funding was granted by the Netherlands Organisation for Scientific Research (NWO-CW, ChemTherm grant to A. K. H. H.) and the Dutch Ministry of Education, Culture, and Science (Gravitation program 024.001.035).

References