De Novo Design of Novel Aspartic Protease Inhibitors Exploiting Dynamic Combinatorial Chemistry

Milon Mondal,[a] *Helene Köster,*[b] *Nedyalka Radeva,*[b] *Costas Potamitis,*[c] *Maria Zervou,*[c] *Gerhard Klebe*[b] *and Anna K. H. Hirsch*[a]

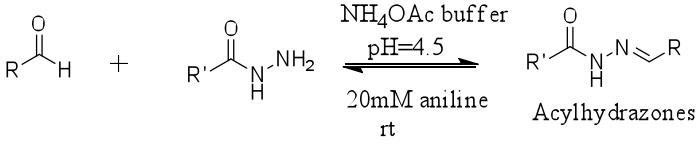
[a] *Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 7, Groningen, NL-9747 AG, THE NETHERLANDS*

[b] *Institut für Pharmazeutische Chemie, University of Marburg, Marbacher Weg 6, Marburg, D-35032, GERMANY*

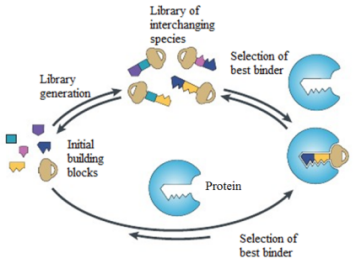
[c] *Institute of Biology, Medicinal Chemistry and Biotechnology National Hellenic Research Foundation, 48, Vas. Constantinou Ave, 11635 Athens, GREECE*

a.k.h.hirsch@rug.nl

Dynamic combinatorial chemistry (DCC) offers an efficient and innovative approach to accelerate identification of novel ligands for biological targets. In a dynamic combinatorial library (DCL) of acylhydrazones, the connection bonds between the building blocks (aldehydes and hydrazides) are reversible and continuously being made and broken. The composition of a DCL will respond to the addition of a target protein that selectively binds one or more library members and will extract such member(s) from the DCL.[1]



R = aromatic ring
R' = aromatic ring or aliphatic substituent



Using a protein–ligand co-crystal structure of endothiapepsin,[2] a model system for the pepsin-like aspartic proteases that are involved in numerous diseases such as hypertension, Alzheimer's disease and malaria, we designed a library of potential inhibitors (acylhydrazones) by structure-based design. We have used DCC approach to identify the best binder(s) using a DCL generated from five aldehydes and five hydrazides in ammonium acetate buffer at pH 4.5. Upon addition of endothiapepsin, the protein-bound library member(s) were characterised by STD-NMR.[3] The ligands identified were synthesised separately and tested for their biological activity using an enzyme-based fluorescence assay and shown to have IC_{50} values in the double-digit micromolar range. Co-crystallisation experiments validated the predicted binding mode and constitute a proof-of-concept that combination of *de novo* structure-based design and DCC constitute an efficient starting point for lead-compound identification and optimisation.

1. J.-M. Lehn, O. Ramström, *Nat. Rev. Drug Discov.* **2002**, *1*, 26–36.
2. J. B. Cooper, *Curr. Drug Targets* **2002**, *3*, 155–174.
3. J. Angulo, P.-M. Nieto, *Eur. Biophys. J.* **2011**, *40*, 1357–1369.