Supplementary data

Mutual Influence of Backbone Proline Substitution and Lipophilic Tail Character on the Biological Activity of Simplified Analogues of Caspofungin

Monique P.C. Mulder[a], Peter Fodran[b], Johan Kemmink[a], Eefjan Breukink[c], John A.W. Kruijtzer[a], Adriaan J. Minnaard[b] and Rob M.J. Liskamp[a]

[a] Medicinal Chemistry and Chemical Biology, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, PO Box 80.082, 3508 TB Utrecht, The Netherlands

[b] Department of Bio-organic Chemistry, Stratingh Institute for Chemistry, University of Groningen, Nijenborgh 7, 9747 AG, Groningen, The Netherlands

[c] Biochemistry of Membranes, Bijvoet Center for Biomolecular Research and Institute of Biomembranes, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

Additional CD spectra:

Figure S.1. 22-membered ring size analogues 22 and 23 with a terphenyl and acetyl tail, respectively.

![Figure S1](image1.png)

Figure S 2. CD spectra of analogues (22 and 23). A) Measured in MeCN/H₂O (1/1, v/v); B) Measured in TFE/H₂O (1/1, v/v). All peptides were measured at 0.1 mM concentration.

A distinct different pattern in CD of analogue 22 with a terphenyl tail was observed, at wavelengths below 210 nm, compared to analogue 23 (Figure S 2). This corroborates the observation regarding the influence of the terphenyl side chain on CD spectra. And may be explained by a large contribution of the aromatic residues present in the terphenyl side chain.
Modelling:

Modeling of the structures 3 and 6 was accomplished using the YASARA Structure 10.5.2.1 software package. Structures were energy minimized using the simulated annealing protocol employing the AMBER99 force field. A 2500 ps MD in water was run of each structure. After 250 ps equilibration, the structure with the lowest energy trajectory (between 250-2500 ps) was saved as a yob file. Molecules were superimposed, with the reported crystal structure of ECBN, by minimizing the rmsd between the backbone atoms in the ring.

Figure S 3. Superimposition of ECBN (carbons colored green) with analogues 3 (right) and 6 (left) (carbons colored orange). The backbone atoms have been used as fixed coordinates for superimposition.

NMR

Cyclo[(DMT-Myr)-Orn-Thr-Hyp-hTyr-Orn-3-Hyp] (4)

Cyclo[(Me-Myr)-Orn-Thr-Hyp-hTyr-Orn-3-Hyp] (5)
(3S,5S)-N-ethyl 3,5-dimethylheptanethioate (13)

![NMR spectrum of (3S,5S)-N-ethyl 3,5-dimethylheptanethioate (13)]

(3S,5S)-N-ethyl 3,5-dimethylheptanethioate (13)

![NMR spectrum of (3S,5S)-N-ethyl 3,5-dimethylheptanethioate (13)]
(S)-12-methyltetradec-9-enoic acid (15)

(S)-12-methyltetradec-9-enoic acid (15)
(10R,12S)-10,12-dimethyltetradecanoic acid (16)

(10R,12S)-10,12-dimethyltetradecanoic acid (16)
Cycle-[Myc-Orn-Thr-Hyp-Tyr-Orn-Hyp] (21)