automatic preparation with manual addition of nutrients, with no supplementation with drugs. An automatic Hyperformer nutrient mixer was used (Mc Gaw). The mixture was passed through a prefilter and a final 0.22 μm filter with nitrogen pressure.

### *2. L-Tryptophan analysis*

The analysis was performed on a LiChroCART$^{R}$ 250 · 4 mm HPLC Cartridge LiChrospher® 100 RP-18 (5 μm) Merck, coupled with a column guard of LiChroCART® 4 · 4 mm LiChrosorb® RP-18 (5 μm) Merck (Darmstadt, Germany). The mobile phase consisted of 96% solution A and 4% solution B. Solution A was 0.01 M sodium acetate buffer and solution B was acetonitrile, pH 5.45. Chromatography was performed at room temperature using a 1.8 ml/min-flow rate and a 13 min run time. Detector sensitivity was set at 0.01 a.u.f.s and eluates monitored at 254 nm. The volume of each injection was 100 μl. Under these conditions L-Tryptophan retention time was roughly 8 min. All samples containing TPN suspension were prepared as below. An accurately measured 1 ml of the TPN suspension was placed in an Altech Maxi-Clean C C18 cartridge and eluted with mobile phase to bring it to volume in a 100 ml measuring flask. This procedure was previously validated by skiping cartridges with 1 ml of standard containing an L-Tryptophan concentration similar to that of the TPN and eluting with 100 ml of mobile phase, to obtain recovery values of 97.8; 100.3; 99.6; 100,4% (mean recovery, 99.5%; SD, 1.2%; CV, 1.2%).

### *3. Emulsion analysis*

Samples were inspected against a black background for creaming, coalescence and color change. Samples were filtered with nylon filters (1.2 μm-pore size) Micropore Separations Inc. Cat N° DDR12025SO to determine the presence of crystals.

The pH of the TPN was measured in triplicate by using an Altronix device (Model TPX). Particle size and particle size distribution of the oil droplets in the emulsion were determined by Low Angle Laser Light Scattering (Mastersizer μ, Malvern Instruments Ltd., Spring Lane South, Worcestershire WR14 1AT, UK). Electrophoretic mobilities of particles in suspension were determined in samples of parenteral solution (5 μl) diluted in 5 ml of 1 mM KCl, in a capillary H-cell with Ag/AgCl electrodes. The electrodes were connected to a direct current source fixed at 40 V. Measurements were obtained with alternating changes of the polarity of electrodes to avoid polarization. The conditions were standardized by determining the zeta potential (ξ) of a phosphatidylserine liposome whose value was roughly ±120 mV in 0.01 mM NaCl at pH 7.4. The rate of migration was determined by light microscope observation of individual particle displacement showing rectilinear and uniform movement along a reticular lattice (length 1 mm). The temperature was maintained at 25 °C in all cases. Zeta potential was calculated by the Smoluchowski equation [15].

**References**


1 Driscoll, D.; Bhargava, H.; Li, L.; Zaim, R.; Babayan, V.; Bistrian, B.: Am. J. Health-Syst. Pharm. **52**, 623 (1995)
2 Martínez Tutor, M.: J, Farm. Hosp. Spain, **6**, 26 (1995)
3 Kumpf, V. J.: Am. J. Health-Syst. Pharm. **56**, 815 (1999)
4 Ambados, F.; Brealey, J.: Aust. J. Hosp. Pharm. **28**,112 (1998)
5 Rollins, C. J.: J. Intraven. Nurs. **20**, 299 (1997)
6 Driscoll, D. F.; Bacon, M. N.; Bistrian, B. R.: J. Parenter. Enteral Nutr. **20**, 296 (1996)
7 Chadwick, C. L.; Forbes, A.: Hosp. Pharm. **3**, 139 (1996)
8 Klang, M. G.: NY Health-Syst Pharm. **15**, 5 (1996)
9 Ricard, C.; Fortune, R.; Florent, M.; Bardet, L.: J. Pharm. Clin. **15**, 72 (1996)
10 Driscoll, D. F.: Nutr. Clin. Pract. **10**, 114 (1995)
11 Martinez, M. J.; Alfaro, A.; Obaldia, M. C.; Monzon, J. L.; Gonzalez, L.: Farm. Hosp. Spain. **6**, 50 (1995)
12 Koycha, M.; Ozil, P.; Rochat, M. H.; Harraki, B.: STP Pharma Sci. **4**, 349 (1994)
13 Li, L. C.; Sampogna, T. P.: J. Pharm. Pharmacol. **45**, 985 (1993)
14 Koycha, M.; Rochat, M. H.; Ozil, P.: STP Pharma Sci. **4**, 169 (1994)
15 Mc Laughlin, S.: Curr. Top. Membr. Transp. **9**, 71, (1977)


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## Mechanism of a anti-fungal action of selected cyclic dipeptides

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Cyclic dipeptides are amongst the most common derivatives found in nature and yet only few have been tested for their biological activity [1]. Previously we reported on the anti-fungal activity of cyclic dipeptides but were only able to speculate on the mechanism of action of these compounds [2]. We are now able to assess the mechanism of action of cyclo(Pro-Trp), cyclo(Trp-Pro), cyclo(Tyr-Pro), cyclo(Trp-Trp), cyclo(Phe-Phe), cyclo(Trp-Tyr), cyclo(Phe-Trp) and cyclo(Pro-Pro) on *Candida albicans*. The yeast *C.albicans* was selected as the representative of fungal pathogens as it has gained prominence as an opportunistic pathogen in AIDS patients.

Four response curves were observed that indicate that two mechanisms of action of anti-fungal activity are exhibited by cyclic dipeptides. The first mechanism is that of receptor mediation and activation of intracellular signalling and the second is that of membrane disruption. The first mechanism is effected by molecules with a negative partition coefficient (log P) (−0.31 to −2.11) [3] and those with a highly lipophilic nature (log P = 3.57) whilst the second is effected by molecules exhibiting low to increasing lipophilic nature (log P = 0.12 to 2.53). Using a transformed regression model and developing the structure – activity relationships by applying fifth order polynomials it was shown that the molecular weight of the molecules has the most significant effect on their anti-fungal activity ($r^2 = 0.998$) whilst the partition coefficient has a slightly less significant effect ($r^2 = 0.815$) [4].

The first response curve (Fig. A) is a result of those cyclic dipeptides containing an imino acid. Proline imposes conformational restraints on the peptides in which it is found [5]. Furthermore, the *cis-trans* isomerism of the *N*-alkylamide bond in the Proline containing DKPs has been implicated in the receptor mediated biological activity of proline containing compounds. This is supported by the fact that the cyclic dipeptides have a significant effect on the consumption of ATP ($r^2 = 0.869$; results not shown and [6]) and the phosphorylation of the CREB proteins ($r^2 = 0.938$; results not shown). The second response curve caused by cyclo(Trp-Tyr) (Fig. B) is found when the molecule is monofunctional. This causes the molecule to orientate itself vertically and compete with the solvent for adsorption sites. Thus cyclo(Trp-Tyr) could bind to surface or internal receptors causing the effects recorded [7].

The third response curve (Fig. C) describes concentration dependence of the activity of the cyclic dipeptides. The dipeptides causing such a response all contain only aromatic amino acids, which can participate in hydrophobic interactions [7]. The delocalized π-electron clouds that enable phenylalanine and tryptophan to interact with other π-systems and allow them to transfer electrons may allow the disruption of the negative charge maintained on biological membranes. The final response (Fig. D) is caused by the hydrophobic cyclo(Phe-Phe) due to the increasing difficulty of the dipeptide to find a vacant binding site on the *C.albicans* surface. Furthermore, the hydrophobic nature of these dipeptides would suggest that cyclo(Phe-Phe) would interfere with plasmamembrane integrity [8].

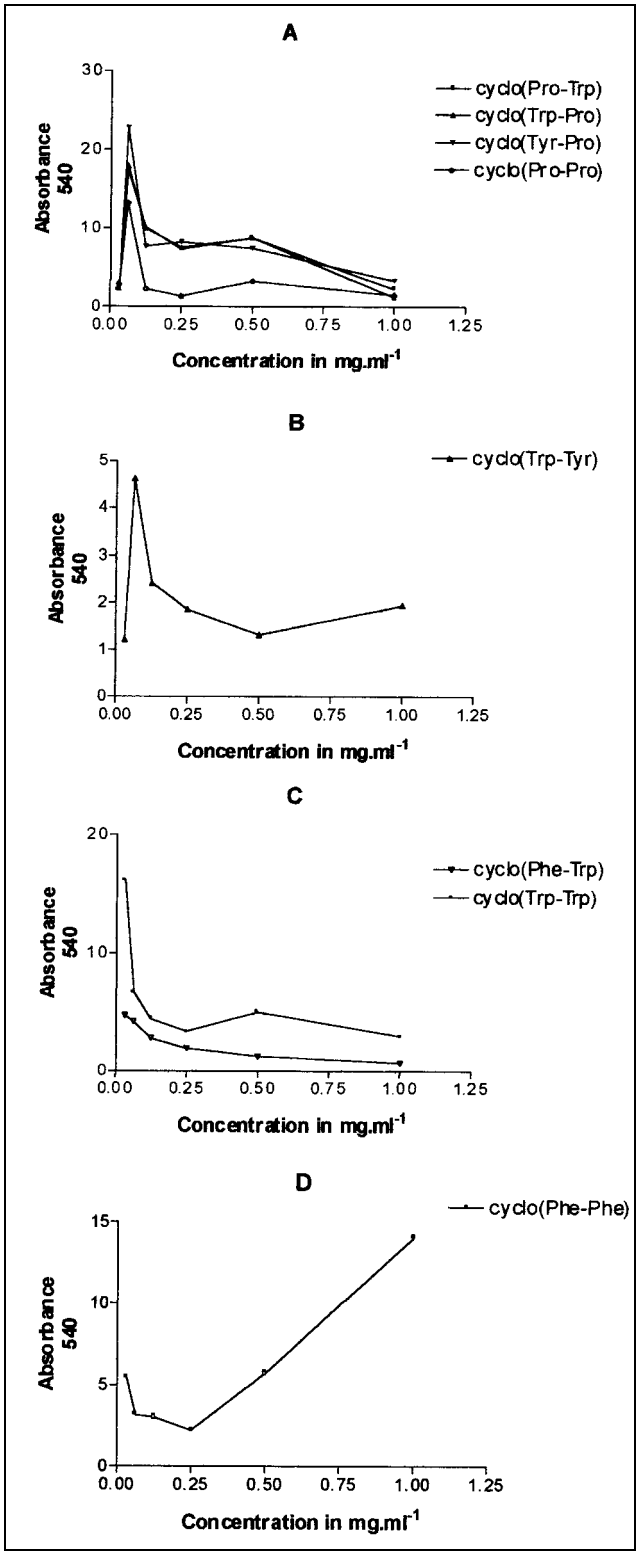


Fig.: The four response curves exhibited by *C. albicans*

The practical value of data describing the effect of cyclic dipeptides on the growth of *C.albicans* in batch culture is leading to a better understanding of the dipeptides' mechanisms of action for facilitation of improvement or potentiation of the activity of the dipeptides.

## Experimental

The activity of the cyclic dipeptides on *C.albicans* was determined using the method of Severina et al. [9] with the following modifications. *C.albicans* was picked from an overnight culture on Sabouraud Agar (Oxoid) and placed into 50 ml of Sabouraud broth (Oxoid). As the external environment has a major effect on the efficacy of antimicrobial agents, the growth conditions of the *C.albicans* were standardized to minimize the stress on the organisms [7]. The cyclic dipeptides used were synthesized according to the method of Grant et al. [5]. All other reagents used in this study were of analytical grade and were not purified further prior to use. Partition coefficients were calculated using the Hyperchem™ suite of software and confirmed according to Tute [4].

### References

1 Prasad, C.: Peptides **16**, 151 (1994)
2 Graz, M.; Hunt, A.; Jamie, H.; Grant, G.; Milne, P.: Pharmazie **54**, 772 (1999)
3 Leo, A.; Hansch, C.; Elkins, D.: Chem. Rev. **71**, 525 (1971)
4 Tute, M. S.: Adv. Drug. Res. **6**, 1 (1971)
5 Grant, G.; Hunt, A.; Milne, P.; Roos, H. M.; Joubert, J. A.: J. Chem. Crystall. **29**, 435 (1999)
6 Graz, M.; Grant, G.; Brauns, C.; Hunt, A.; Jamie, H.; Milne, P.: J. Pharm. Pharmacol. **52**, 75 (2000)
7 Hancock, R. E. W.; Lehrer, R.: Tibtech. **16**, 82 (1998)
8 Bodansky, M.; Singler, G. F.; Bodansky, A.: J. Am. Chem. Soc. **95**, 2352 (1973)
9 Severina, E.; Severin, A.; Tomasz, A.: J. Antimicrob. Chemother. **41**, 341 (1998)

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