Table I. Antifungal peptides activity[1,2] against fungal strains determined by the broth microdilution method.

Antifungal agent	Yeasts			
	C.albicans ATCC 10231		*C.albicans* SA40	
	IC_{50}[1] µg/ml	MFC[2] µg/ml	IC_{50} µg/ml	MFC µg/ml
Histatin 5	6.25[3]	12.5	6.25	12.5
ATCUN-C16	6.25	12.5	6.5	12.5

[1] Growth inhibition obtained with fungal pathogens yeasts in the presence of synthetic proteins. Fungal cells (1×10^4CFU/ml) were incubated with a dilution series of proteins in SDB (10%) supplemented with dialysed yeast extract, and growth inhibition was assessed spectrophotometrically at 620nm after 48 of incubation at 30 C (Helmerhorst E.J.,Venuleo C., Beri A., Oppenheim F.G.,Yeast 2005;22:705-14). The IC_{50} values corresponded to the concentration of proteins that inhibited the maximum growth of that particular strain by 50%.

[2] Cells were exposed to a dilution series of synthetic proteins in 10% SDB, pH7.0, after which cells were plated on SDA and incubated at 37 C for 48h. Cell viability was determined by colony counting. The minimum fungicidal concentration (MFC) was the lowest drug concentration resulting in the death of 99.9% or more of the initial inoculum.

[3] Average of three replicate experiments, each performed in duplicate.

Table II. $^{1}J_{C\alpha H\alpha}$ values in Hz for the ACTUN-C16 peptide measured at 25°C in a 1:1 TFE:H_2O solution, pH 6.0.[a]

Residue	$^{1}J_{C\alpha H\alpha}$ (Hz)
D1	142.50
S2	142.10
H3	144.20
A4	144.20
G5	nd [b]
Y6	147.00
K7	144.60
R8	146.00
K9	145.50
F10	146.80
H11	147.10
E12	144.10
K13	145.60
H14	145.60
H15	145.00
S16	143.60
H17	143.70
R18	141.80
G19	nd [b]
Y20	142.90

[a]: Coupling constants are reported with an accuracy of ±0.5 Hz.
[b]: nd: not determined.

Table III. ^{1}H and ^{13}C Chemical Shifts Assignments of the ACTUN-C16 peptide in a 1:1 TFE:H_2O solution at 25°C and pH 6.0.[a]

Residue	^{15}N	NH	$^{13}C_{\alpha}$	$^{1}H_{\alpha}$	$^{13}C_{\beta}$	$^{1}H_{\beta}$	Others[b]
D1	-	-	51.9	3.97	49.5	2.69/2.69	
S2	116.7	7.92	57.1	4.37	61.42	3.82/3.82	
H3	118.0	7.98	55.2	4.60	28.0	3.11/3.24	
A4	122.6	7.98	52.4	4.09	16.0	1.43	
G5	105.5	8.36	44.3	3.83/3.83	-	-	
Y6	120.5	7.78	58.2	4.28	35.9	3.07/3.07	
K7	119.0	7.98	57.1	4.00	30.1	1.86/1.86	γ 23.2 (1.38/1.55), δ 27.2 (1.66/1.66), ε 39.7 (2.91/2.91)
R8	119.0	7.89	56.5	4.12	25.0	1.72/1.64	γ 27.7 (1.90/1.90), δ 41.0 (3.15/3.15)
K9	118.8	7.89	56.0	4.10	29.6	1.82/1.82	γ 22.5 (1.40/1.44), δ 26.4 (1.63/1.63), ε 39.7 (2.89/2.89)
F10	119.4	8.28	58.5	4.20	36.7	3.12/2.96	
H11	118.5	8.21	57.1	4.34	28.1	3.23/3.23	
E12	119.2	8.31	56.4	4.03	27.7	2.11 /2.24	γ 34.1 (2.46/2.24)
K13	118.6	8.01	55.5	4.08	30.2	1.68/1.74	γ 22.2 (1.26/1.41), δ 26.6 (1.55/1.55), ε 39.7 (2.88/2.88)
H14	118.8	7.89	55.7	4.36	28.0	2.94/2.73	
H15	119.0	7.98	54.9	4.49	28.0	2.94/2.94	
S16	114.6	7.95	57.1	4.35	61.7	3.86/3.83	
H17	118.3	7.97	54.4	4.55	27.8	3.13/3.03	
R18	120.2	7.95	54.1	4.24	28.5	1.73/1.64	γ 24.4 (1.42/1.42), δ 41.0 (3.01/3.01)
G19	108.0	8.14	42.9	3.96/3.74	-	-	
Y20	122.8	7.29	56.6	4.39	36.7	2.91/3.02	

[a]: ^{1}H Chemical shifts are reported in ppm with an accuracy of ±0.02 ppm. ^{13}C Chemical shifts are reported in ppm with an accuracy of ±0.1 ppm.
[b]: Carbon chemical shifts first, in brackets the proton chemical shift.

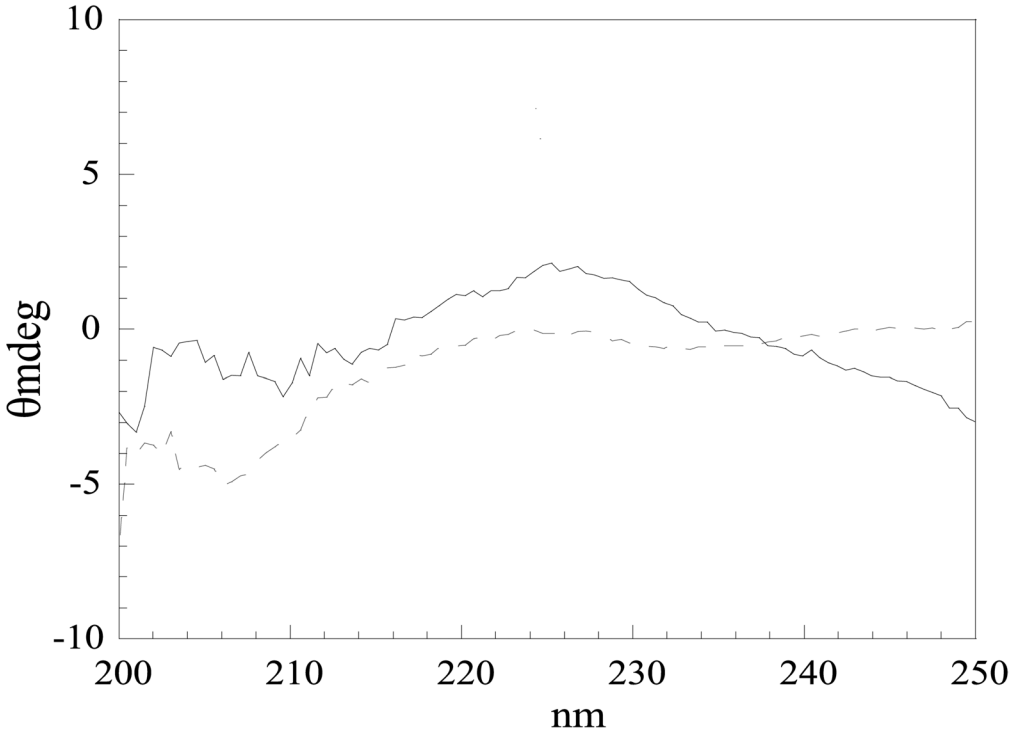


Fig. 1 Circular dichroism spectra of the peptide with or without the plasmid DNA. CD spectra of ATCUN-C16 (50 μM) in presence (—) and in absence (- - -) of pDNA (Peptide/DNA 1:1 w/w) in 17mM Tris-HCl, pH 7.4, buffer. The ATCUN-C16-pDNA spectrum was subtracted of the contribution of the free pDNA.

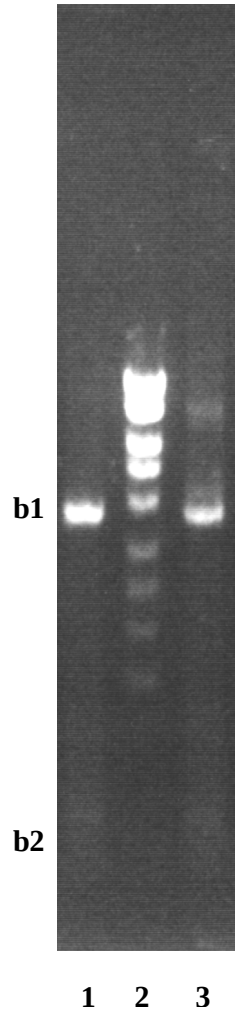
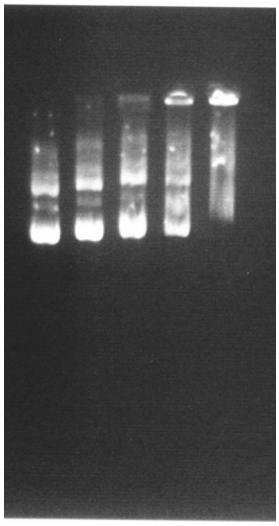


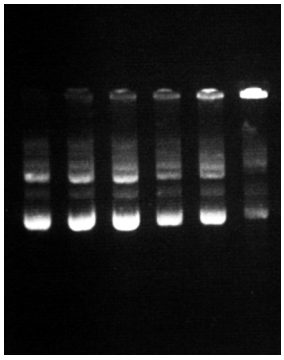
Fig. 2 Agarose gel electrophoresis (1% agarose) of the 0.25 μg pQE30-rhdA after cleavage with SmaI (1U/μg) for 1h at 30°C, in the absence (lane 1) and in the presence (lane 3) of RTN32 peptide, in a ratio pDNA/peptide 1:10 w/w. **b_1** and **b_2** bands correspond to the SmaI digest fragments of about 3900 bp and 600 bp respectively. Molecular weight markers are shown in the lane 2.



1 2 3 4 5

Fig. 3 Gel retardation shift assay at different Hst5/pDNA w/w ratios, using 0.9 μg pQE30-rhda and 1% agarose gel. Lane 1 (0), lane 2 (0.5), lane 3 (1), lane 4 (2) and lane 5 (5).

0’ 15’ 90’ 3h 5h 20h



1 2 3 4 5 6

Fig. 4 Agarose gel electrophoresis of pDNA treated with ATCUN-C16 peptide in 50 mM Tris-HCl buffer, pH 7.5, lanes 0-6 pDNA/ATCUN-C16 peptide mixture (ratio 1:1 w/w) in the presence of $ZnCl_2$ (ATCUN-C16:Zn^{2+} 1:1 c/c) at different times (0, 15’, 90’, 3h, 5h and 20h).

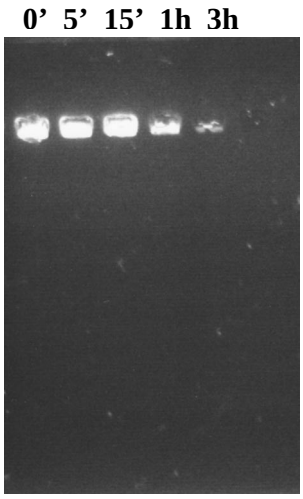


Fig. 5 Agarose gel electrophoresis of pDNA treated with ATCUN-C16 peptide in 50 mM Tris-HCl buffer, pH 7.5, A) pDNA/ATCUN-C16 peptide mixture (ratio 1:1 w/w) in the presence of $NiCl_2$ and magnesium-monoperoxyphthalate (MMPP), at a molar ratio peptide/Ni^{2+}/ MMPP 1:1:10 c/c/c, incubated for 0 (lane 1), 5 (lane 2), 15 (lane 3), 60 (lane 4), 180 (lane 5) min at 37° C.

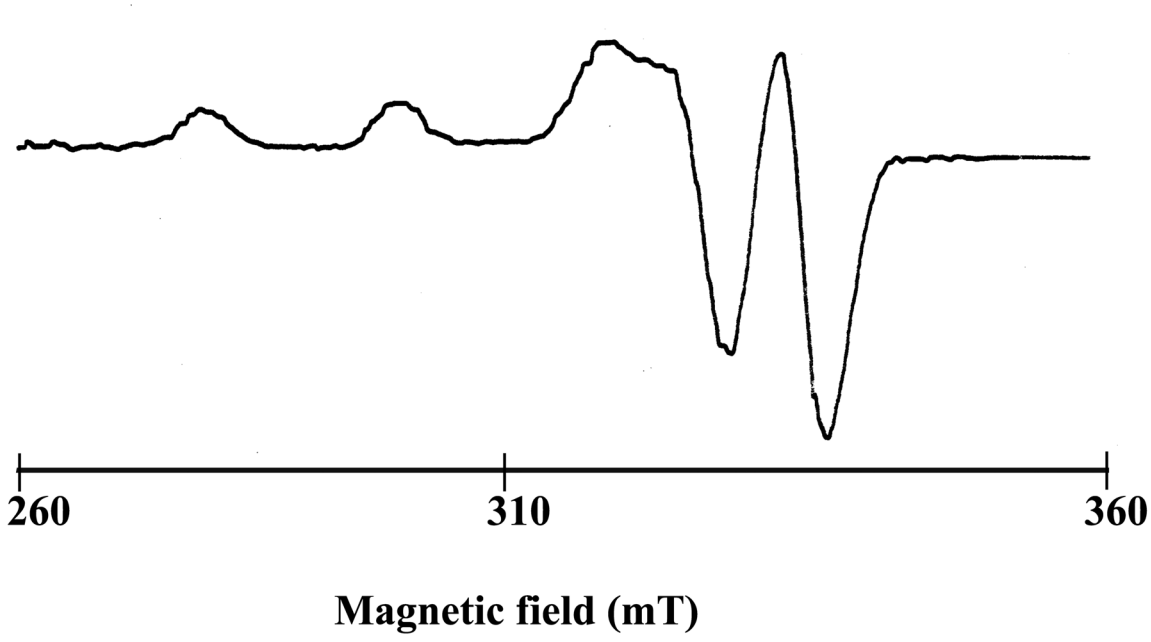


Fig.6 Low-temperature EPR spectrum of Cu-ATCUN-C16 complex, 1:1 Cu/peptide molar ratio, in 50mM Hepes pH 7.2 buffer.