Implication of cysteine residues in the selection of oxorhenium inhibitors of cyclophilin hCyp18

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Supplementary Information

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ABBREVIATIONS:

Aac-pNA: amino acyl-4-nitroanilide; Acm: carbamidomethylated; CEE: cyclophilin enhancing effect; CsA: cyclopsporine A; DMSO: dimethylsulfoxide; DTT: dithiothreitol; GSH: glutathione; IIC: integrated ionic current; Kd: dissociation constant; LC-ES/MS: liquid chromatography coupled to on-line electrospray mass spectrometry; NS₂: N-bis(2-thioethyl); pNA: 4-nitroanilide; PPIase: peptidyl-prolyl isomerase; RP-HPLC: reverse phase high performance liquid chromatography.



10 and 6 (--O--) (designated as 'Ligand').

Fig. S2: Apparent Kds of module 11 (----------) and of an equimolar mixture of modules 10 and 7 (-- --) and modules 10 and 8 (--- --) (designated as 'Ligand').



y = m4+((m1-m4)*(1/(1+(m0/m2.. Error 1,2645 1.1569e-5 0,067195 1,591 NA NA y = m4 + ((m1 - m4)*(1/(1 + (m0/m2)))

	value	Error		
m1	98,542	1,5184		
m2	0,00013834	1,2771e-5		
m3	0,89039	0,070397		
m4	-2,2388	1,8892		
Chisq	73,615	NA		
R	0,99837	NA		
y = m4+((m1-m4)*(1/(1+(m0/m2				
y = m4	+((m1-m4)*(1/(1+(m0/m2		
y = m4	+((m1-m4)*(1/(Value	1+(m0/m2 Error		
y = m4 m1	+((m1-m4)*(1/(Value 99,612	1+(m0/m2 Error 1,4573		
y = m4 m1 m2	+((m1-m4)*(1/(Value 99,612 0,00011198	1+(m0/m2 Error 1,4573 8,9212e-6		
y = m4 m1 m2 m3	+((m1-m4)*(1/(Value 99,612 0,00011198 0,97472	1+(m0/m2 Error 1,4573 8,9212e-6 0,074934		
y = m4 m1 m2 m3 m4	+((m1-m4)*(1/(Value 99,612 0,00011198 0,97472 0,20605	1+(m0/m2 Error 1,4573 8,9212e-6 0,074934 1,5889		
y = m4 m1 m2 m3 m4 Chisq	+((m1-m4)*(1/(Value 99,612 0,00011198 0,97472 0,20605 57,142	1+(m0/m2 Error 1,4573 8,9212e-6 0,074934 1,5889 NA		



Fig. S3: Apparent Kds of module 12 (---) and of an equimolar mixture of modules 12 and 9 (----) (designated as 'Ligand').

Fig. S4: Circular dichroism spectra of 10 μ M native hCyp18 (----) and 10 μ M Acm₂-hCyp18 (----) in water at 20 °C.



Fig. S5: kobs of hCyp18 (A) and Acm₂-hCyp18 (B) calculated from the trypsin-coupled PPIase assay using Suc-Ala-Ala-Pro-Arg-pNA as a substrate.



Fig. S6: Affinity constants Kd of Suc-Ala-Ala-Pro-Arg-*p*NA determined using the fluorescence quenching assay for hCyp18 (----) and Acm₂-hCyp18 (----).







y = m4+((m1-m4)*(1/(1+(m0/m2			
	Value	Error	
m1	99,698	1,4144	
m2	3,4857e-7	1,6424e-8	
m3	1,9602	0,14037	
m4	1,3584	1,0089	
Chisq	49,424	NA	
R	0,99896	NA	

y = m4+((m1-m4)*(1/(1+(m0/m2				
	Value	Error		
m1	101,77	1,5195		
m2	4,5047e-6	2,7416e-7		
m3	1,4715	0,123		
m4	0,75816	1,3781		
Chisq	56,533	NA		
R	0,99876	NA		