SUPPLEMENTARY MATERIALS

Structure-Activity Relationship investigations of novel constrained chimeric peptidomimetics of SOCS3 protein targeting JAK2

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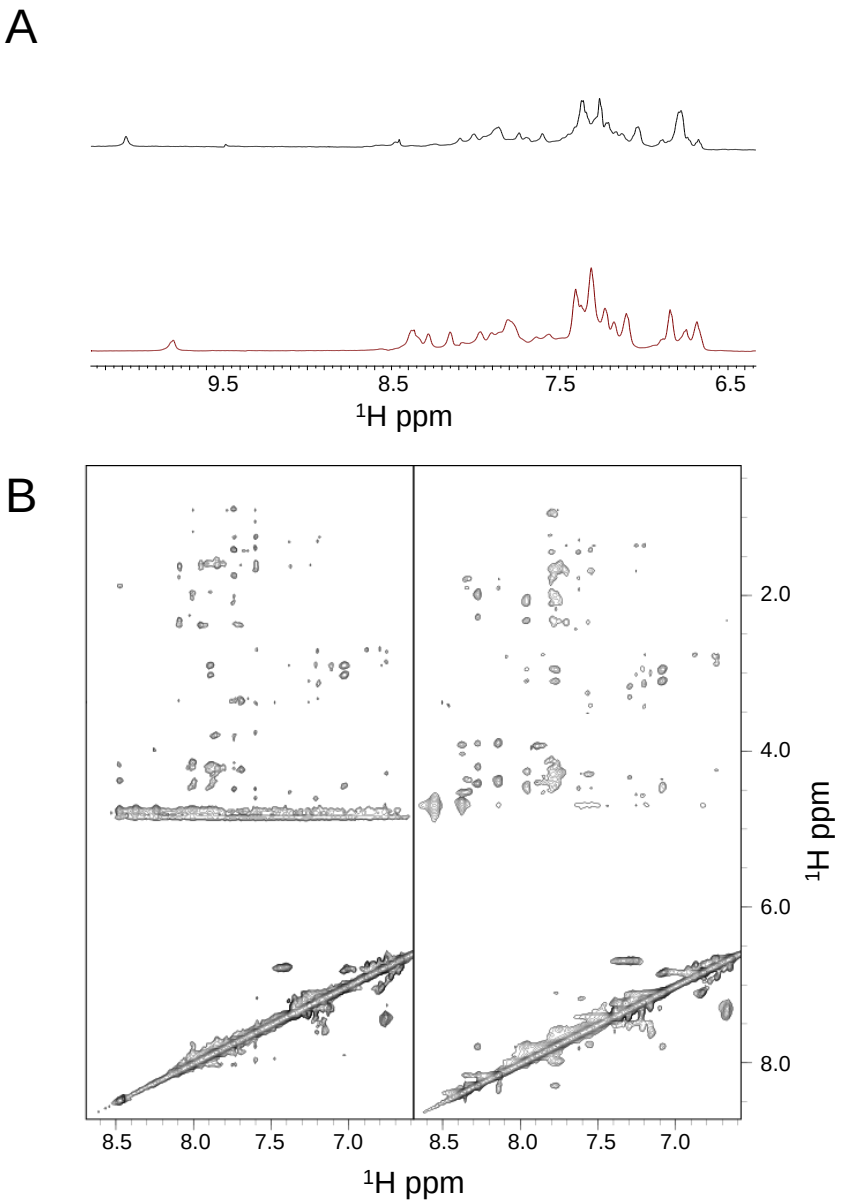

Figure S1. (**A**) Comparison of 1D [^{1}H] spectra of KIRCONG *i*/*i*+*5* in H_2O/TFE 85/15 v/v (black) and KIRCONG *i*/*i*+*5* in H_2O/TFE (60/40 v/v) (red). Spectral regions containing peaks from H_N and aromatic protons are shown. (**B**) 2D [^{1}H-^{1}H] NOESY 300 spectra of KIRCONG *i*/*i*+*5* in H_2O/TFE 85/15 v/v (left panel) and KIRCONG *i*/*i*+*5* in H_2O/TFE 60/40 v/v (right). The figure shows spectral regions containing correlations involving H_N, aromatic and aliphatic protons.

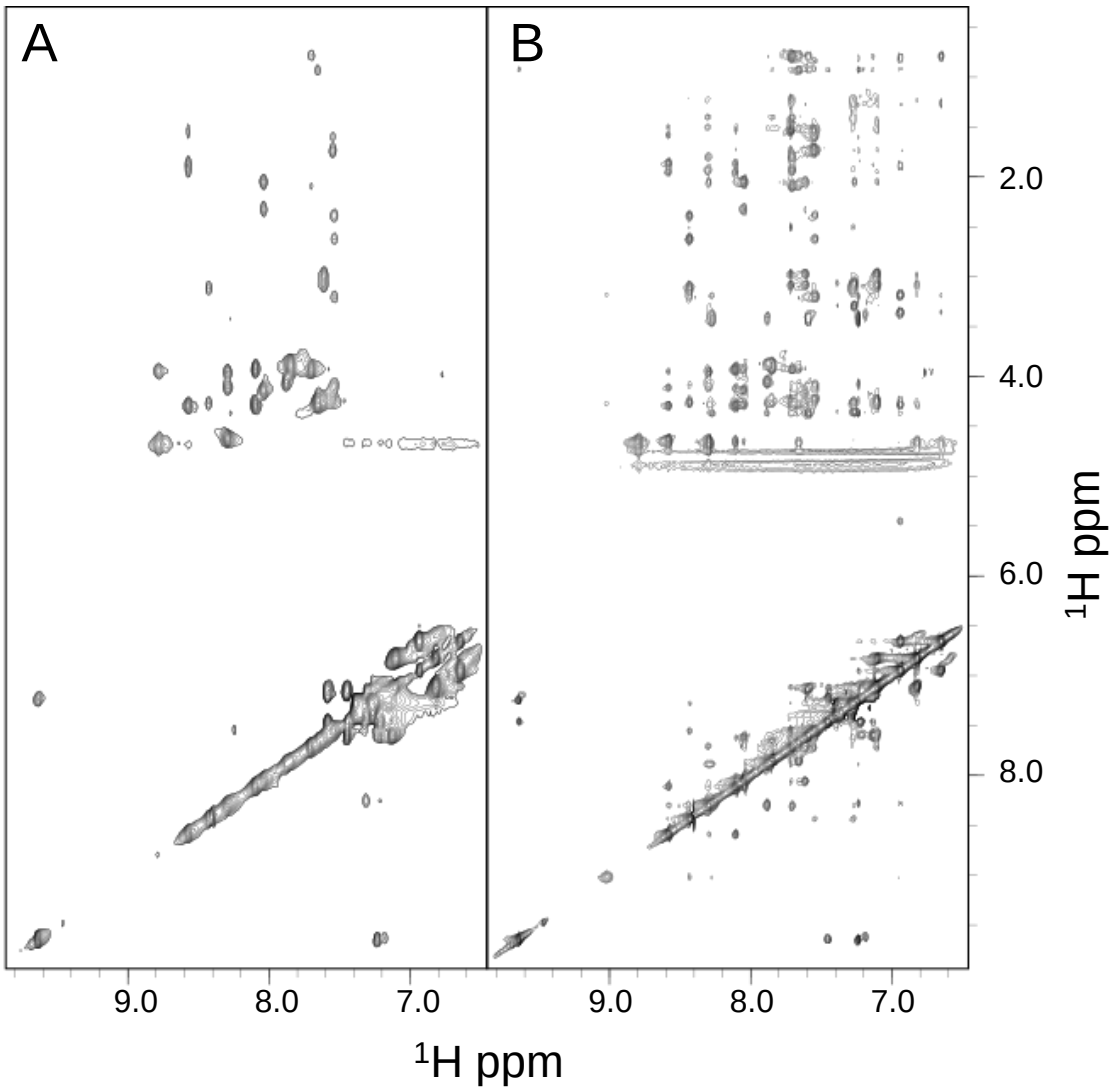

Figure S2. 2D [^{1}H-^{1}H] TOCSY (**A**) and NOESY 300 (**B**) spectra of KIRCONG *i*/*i+7* in H_2O/TFE 60/40 v/v. The figure shows spectral regions containing correlations involving H_N, aromatic and aliphatic protons.

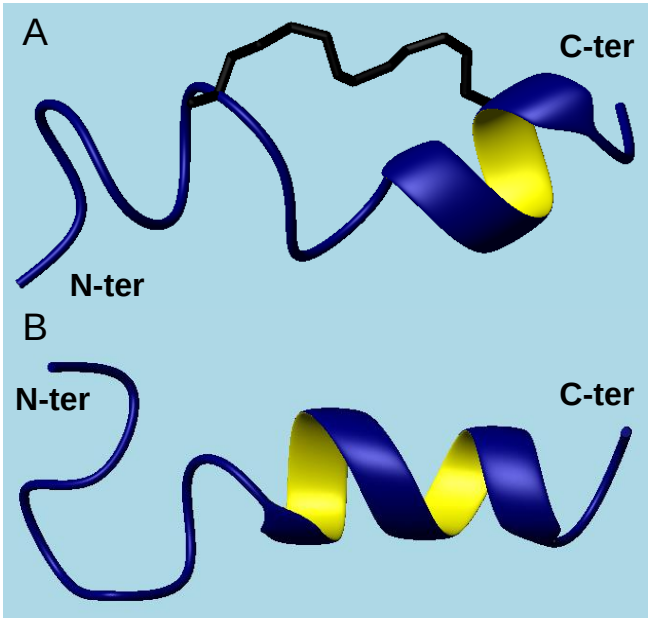

Figure S3. NMR structures (conformers n. 1) of KIRCONG *i*/*i*+*7* in H_2O/TFE (60/40, v/v) (**A**), and KIRCONG chim in H_2O/TFE (60/40, v/v) (**B**).

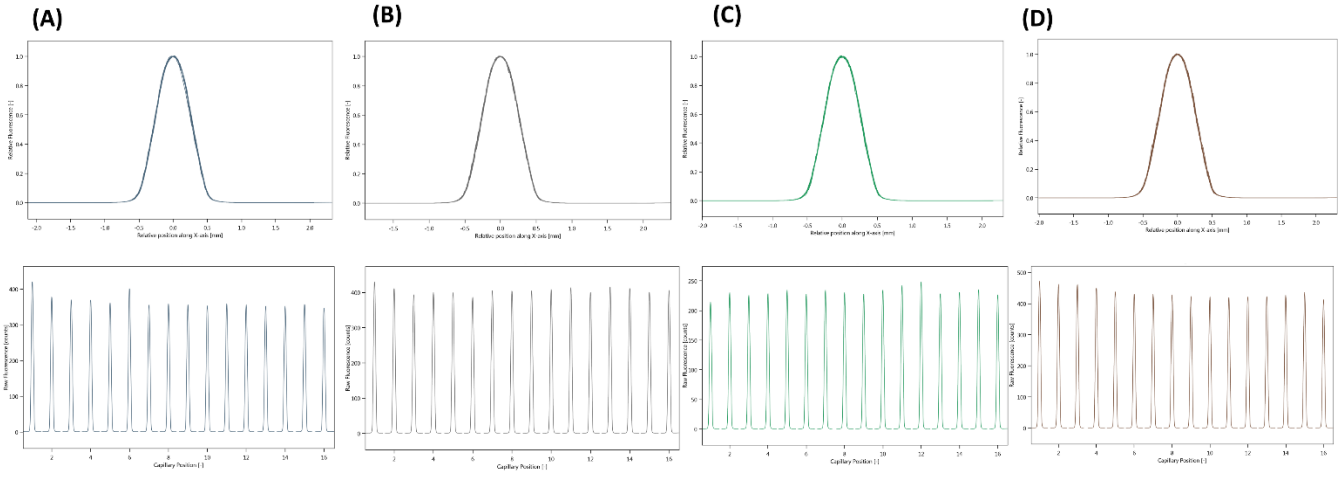

Figure S4. Upper: capillary shape and lower: capillary scan of: (**A**) KIRCONG *amide*, (**B**) KIRCONG *disulfide*, (**C**) KIRCONG *i*/*i*+*5* and (**D**) KIRCONG *i*/*i*+*7*.

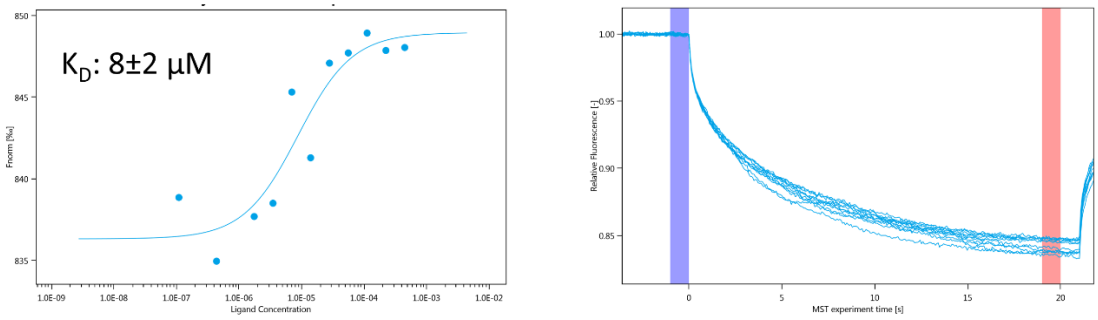

Figure S5. Binding isotherms for MST signals versus linear KIRCONG *i*/*i+5* concentrations.

Table S1. Deconvolution of CD spectra.

	%TFE	HELIX	BETA	TURN	OTHERS
KIRCONG *amide*	15	0	42.0	12.7	45.4
	25	0	41.5	13.6	44.9
	45	1.3	39.7	14.9	44.1
	65	8.3	31.8	14.5	45.4
KIRCONG *disulfide*	15	0.6	43.8	12.9	42.7
	25	0	42.6	13.6	43.8
	45	9.8	35.0	13.4	41.8
	65	8.3	34.7	13.4	43.6
KIRCONG *i/i+5*	15	8.2	37.4	13.6	40.8
	25	6.1	37.5	13.9	42.4
	45	22.6	21.0	13.3	43.1
	65	41.5	16.8	9.9	31.7
KIRCONG i/*i+7*	15	3.8	43.7	13.6	39
	25	12.1	31.8	13.5	42.7
	45	24.4	23.5	13.4	38.7

Table S2. ^{1}H chemical shifts of KIRCONG *i*/*i*+5 in H_2O/TFE (60/40, v/v), pH 4.52, and T=25°. In red tentative assignments are reported. The β-Alanine and (S)-N- 2-(4'-pentenyl) alanine are indicated as "BAL" and "X", respectively. N.D. stands for not determined.

Residue	HN	Hα	Hβ	Hγ	Others
1F	N.D.	4,34	3,17-3,30		δ 7,30 ε 7,40
2S	8,55	4,64	3,90-3,94		
3S	8,38	4,52	3,92-4,02		
4K	8,34	4,36	1,79-1,91	1,50	δ 1,72 ε 3,01
5S	8,15	4,42	3,89-3,91		
6E	8,28	4,21	1,96-2,01	2,29	
7Y	7,78	4,50	2,95-3,10		δ 7,09 ε 6,83
8Q	7,97	4,26	2,06-2,11	2,34	ε 6,68-7,31
9L	7,75	4,31	1,59-1,66	1,65	0,87-0,92
10BAL	7,57	2,35	3,28-3,51		
11X	7,70		βCH3 1,36 1,70-1,77	1,36	δ 1,69–1,78 Hε 5,26
12F	7,86	4,56	2,76-2,82		δ 7,22 ε 7,26
13Y	8,10	4,23	2,80-2,88		δ 6,74 ε 6,90
14W	7,53	4,55	2,95-3,03		Hε1 9,80 Hδ1 7,21 Hη2 7,29 Hε3 7,61 Hζ3 7,17 Hζ2 7,31
15S	7,85	4,47	3,94		
16X	7,63		βCH3 1,46 1,78-1,94	1,33	δ 1,77–1,92 Hε 5,40
17V	7,80	4,10	2,09	0,97-1,01	
18T	7,83	4,36	4,38	1,24	
19G	7,90	3,93			

Table S3. ^{1}H chemical shifts of KIRCONG *i/i+5* in H_2O/TFE (85/15, v/v), pH 6.84, and T=25°. In red tentative assignments are reported. "BAL" and "X" are used for β-Alanine and (S)-N- 2-(4'-pentenyl) alanine, respectively. N.D. stands for not determined.

Residue	HN	Hα	Hβ	Hγ	Others
1F	N.D.	4,34	3,17-3,30		δ 7,13 ε 7,30
2S	N.D.	4,74	3,97		
3S	N.D.	4,56	3,84		
4K	8,37	4,32	1,75-1,86	1,41	δ 1,64 ε 2,96
5S	8,24	4,37	3,83-3,87		
6E	8,47	4,16	1,88-1,93	2,14	
7Y	7,89	4,44	2,89-3,02		δ 7,03 ε 6,79
8Q	8,00	4,21	1,96-2,02	2,27	ε 6,77-7,42
9L	7,85	4,24	1,54-1,61	1,58	0,83-0,89
10BAL	7,69	2,38	3,35		
11X	7,60		βCH3 1,36 1,73	1,66	δ 1,651,78 Hε 5,27
12F	7,86	4,60	2,69-2,72		δ 7,22 ε 7,34
13Y	8,10	4,26	2,72-2,85		δ 6,76 ε 6,89
14W	7,59	4,59	2,70-2,91		Hε1 10,08 Hδ1 7,21 Hη2 7,44 Hε3 7,59 Hζ3 7,17 Hζ2 7,41
15S	7,85	4,47	3,94		
16X	7,74		βCH3 1,40 1,73-1,76	1,07	δ 1,68–1,78 Hε 5,34
17V	7,75	4,13	2,11	0,89-0,91	
18T	8,00	4,37	4,31	1,20	
19G	7,86	3,79			

Table S4. ^{1}H chemical shifts of KIRCONG *i/i+7* in H_2O/TFE (60/40, v/v), pH 4.65, and T=25°C. "BAL", "X" and "Z" indicate β-Alanine, (S)-N-2-(4'-pentenyl) alanine, and (R)-N- 2-(7'-octenyl) alanine, respectively. N.D. stands for not determined.

Residue	HN	Hα	Hβ	Hγ	Others
1F	N.D.	4,35	3,07-3,31		δ 7,27 ε 7,39
2S	8,78	4,68	3,95-3,97		
3S	8,29	4,63	3,96-4,11		
4K	8,57	4,28	1,88-1,96	1,51-1,58	δ 1,72 ε 3,00
5S	8,10	4,29	3,91-3,95		
6E	8,04	4,13	2,03-2,06	2,32-2,35	
7Y	7,72	4,23	2,98-3,08		δ 7,40 ε 6,83
8Z	8,00		βCH3 1,54 1,74	1,22-1,45	δ 1.47–2.51 ε 1,93 η 1.28 ζ 1.89 Hζ1 5.45
9L	7,56	4,24	1,59-1,75	1,71	0,86-0,94
10BAL	7,55	2,39-2,62	3,19-4,10		
11F	8,44	4,27	3,12		δ 7,27 ε 7,38
12Y	N.D.	4,28	3,18-3,36		δ 6,94 ε 6,66
13W	8,27	4,36	3,41-3,46		Hε1 9,63 Hδ1 7,25 Hη2 7,21 Hε3 7,58 Hζ3 7,13 Hζ2 7,45
14S	7,88	4,07	3,87		
15X	8,31		βCH3 1,41 1,80-1,93	1,24-1,51	δ 1,87–2,10 Hε 5,37
16V	7,70	3,93	2,09	0,79-0,82	
17T	7,66	4,27	4,19	0,94	
18G	7,85	3,89			

Table S5. Structure statistics of KIRCONG *i*/*i*+*7* NMR conformers in H_2O/TFE (60/40, v/v).

Residual target function, Å²	0.27±0.05
Residual NOE violations	1
Number ≥ 0.1 Å*	0
Residual angle violations	0
Atomic pairwise RMSD, Å	
Backbone atoms (residues 3-17)	0.53±0.15
Heavy atoms (residues 3-17)	1.03±0.16
Procheck analysis (all residues)#	
Residues in core regions	49.7%
Residues in allowed regions	35.3%
Residues in generous regions	14.1%
Residues in disallowed regions	0.9%

*CYANA [1] average violations
#PROCHECK_NMR [2] statistics

References

1. Herrmann, T.; Guntert, P.; Wuthrich, K. Protein NMR structure determination with automated NOE assignment using the new software CANDID and the torsion angle dynamics algorithm DYANA. *J Mol Biol* **2002**, *319*, 209-227.
2. Laskowski, R.A.; Rullmannn, J.A.; MacArthur, M.W.; Kaptein, R.; Thornton, J.M. AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR. *J Biomol NMR* **1996**, *8*, 477-486.