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Supporting Information

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Discovery of Low-Molecular-Weight Ligands for the AF6 PDZ Domain

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Materials All reagents and starting materials were purchased from commercial sources and used without further purification. TLC was performed on plastic-backed plates pre-coated with silica (0.2 mm, 60 F_{254}) and visualized using an UV lamp (254 and 366 nm). Silica gel 60 (30-63 μm) was used for flash chromatography. Mass spectra were recorded on a LC-MS Agilent 1100 series spectrometer. The compounds were further checked by recording ^{1}H and ^{13}C NMR spectra on Bruker DRX600 and Bruker AV300 NMR spectrometers using DMSO-d_6 solutions. The chemical shifts are given in ppm and *J* values in Hz.

Synthesis of (*Z*)-5-Arylidene-2-thioxo-4-thiazolidinones and (*Z*)-5-isopropylidene-2-thioxo-4-thiazolidinone. Compounds **4a-f** and **4h-i** were synthesized according to the procedure described in [1] while compounds **4g** and **4j** were prepared according to the procedure in [2]. Twenty millimoles of 2-thioxo-4-thiazolidinone and 20 mmol of aldehyde were used as starting materials. The products were recrystallized from MeOH or mixtures of H_2O/MeOH or H_2O/EtOH when appropriate.

***Reduction of* 4.** Compounds **5** were prepared according to the procedure described for 5-benzyl-2,4-thiazolidinediones.[3] The final products were obtained after flash chromatography using mixtures of hexane/ethyl acetate as eluant.

Table 1. ^{1}H NMR data of 2-thioxo-4-thiazolidinone derivatives **4.**

Entry	R^1	=C*H*-	R^1 moiety	N*H*	Others
4a	3-thienyl	7.92	7.30, 7.71, 8.08	13.78	
4b	iPr	6.67	-	13.59	1.07 (CH_3) 2.38 (C*H*)
4c	C_6H_5	7.64	7.51, 7.55, 7.60	13.85	
4d	4-MeC_6H_4	7.60	7.36, 7.48	13.79	2.36 (CH_3)
4e	3-MeC_6H_4	7.59	7.32, 7.39, 7.41, 7.44	13.82	2.37 (CH_3)
4f	4-$CF_3C_6H_4$	7.70	7.79, 7.88	13.93	
4g	3-$CF_3C_6H_4$	7.98	7.77, 7.79, 7.85, 7.86,	13.93	
4h	4-BrC_6H_4	7.61	7.53, 7.74	13.86	
4i	3-indolyl	7.93	7.21, 7.26, 7.51, 7.83, 7.93	13.56, 12.30	
4j	4-PhC_6H_4	7.72	7.41, 7.49, 7.68, 7.73, 7.80	13.84	

Table 2. ^{1}H NMR data of derivatives **5.**

Entry	R^1	C*H*	CH_2	R^1 moiety	N*H*
5a	3-thienyl	5.00	3.51, 3.52	6.93, 7.40	13.16
5c	C_6H_5	5.03	3.17, 3.37	7.24, 7.26, 7.31	13.85
5f	4-$CF_3C_6H_4$	5.09	3.46, overlapped with H_2O signal	7.48, 7.69	13.20
5g	3-$CF_3C_6H_4$	5.09	3.46, overlapped with H_2O signal	7.56, 7.57, 7.63	13.19
5h	4-BrC_6H_4	5.02	3.19, 3.33	7.21, 7.51	13.87

Table 3. ^{13}C NMR data of 2-thioxo-4-thiazolidinone derivatives **4.**

Entry	R^1	C=S	C=O	C-5	*C*H	R^1 moiety	Others
4a	3-thienyl	194.6	169.1	123.0	124.8	129.3, 135.4, 134.3, 137.4	
4b	iPr	196.1	168.0	127.0	143.1	-	20.8, 31.7
4c	C_6H_5	195.7	169.4	125.5	131.6	129.4, 130.5, 130.8, 133.0	
4d	4-MeC_6H_4	195.7	169.5	124.3	131.8	130.1, 130.6, 141.2	21.1 (*C*H_3)
4e	3-MeC_6H_4	195.8	169.4	125.4	131.6	127.7, 129.4, 130.9, 131.8, 133, 138.9	20.9 (*C*H_3)
4f	4-$CF_3C_6H_4$	195.4	169.3	128.6	129.4	126.1, 126.2, 129.8 (q, J_{CF}=32.5), 130.9, 136.9	123.8 (q, J_{CF} =273.4, *C*F_3)
4g	3-$CF_3C_6H_4$	195.3	169.3	129.7	127.8	126.8 (q, J_{CF}=3.3), 127.3 (q, J_{CF}=3.3), 129.7, 130.1 (q, J_{CF}=32), 133.2, 134.2	123.8 (q, J_{CF} =273.4, *C*F_3)
4h	4-BrC_6H_4	195.4	169.4	124.3	130.2	126.4, 130.3, 132.2, 132.4	
4i	3-indolyl	194.7	169.2	123.5	124.8	112.6, 118.0, 118.5, 121.5, 123.4, 126.8, 130.1, 136.4	

Table 4. ^{13}C NMR data of derivatives **5.**

Entry	R^1	C=S	C=O	C-5	*C*H_2	R^1 moiety	Others
5a	3-thienyl	203.5	177.7	55.6	30.7	125.5, 127.1, 127.0, 137.9	
5c	C_6H_5	203.3	177.9	55.6	36.5	127.1, 128.5, 129.2, 136.6	
5f	4-$CF_3C_6H_4$	203.2	177.9	55.0	36.1	125.3, 127.8 (q, J_{CF}=32), 130.2, 141.6	124.3 (q, J_{CF}=272.6,*C*F_3)
5g	3-$CF_3C_6H_4$	203.3	178.1	55.1	36.0	123.9, 124.1, 125.9, 129.1 (q, J_{CF}=32), 129.4, 133.5, 138.1	
5h	4-BrC_6H_4	203.2	177.8	55.2	35.7	120.4, 131.3, 131.5, 136.0	

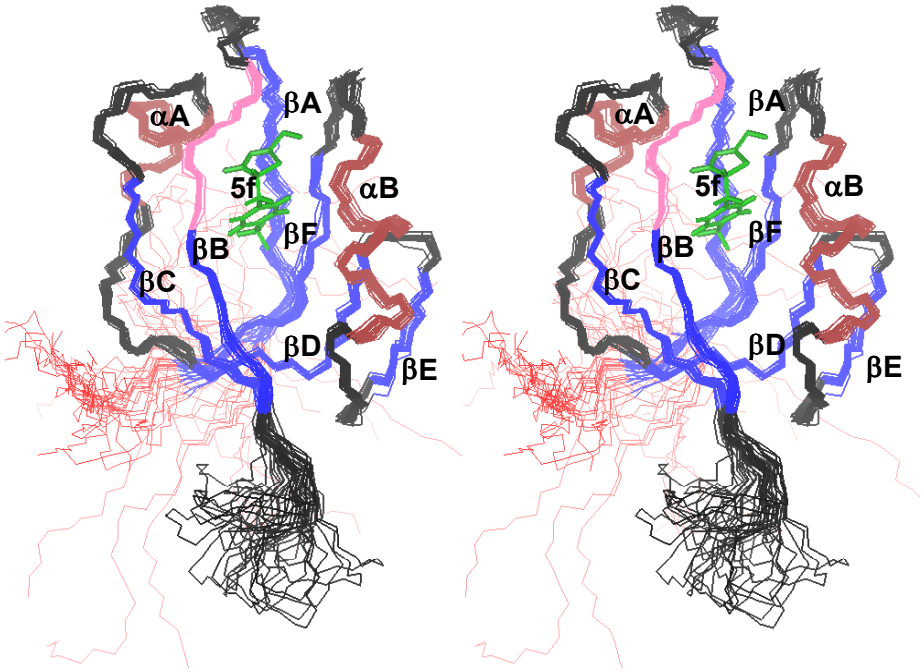

Figure 1. Solution NMR structure of AF6 PDZ-**5f** complex: Superposition of the backbone (N, CA, and C´) atoms for the 20 lowest-energy structures (stereoview). Color coding: α-helix: brown (αA: 51-55, αB: 77-86), β-strands: blue (βA: 11-17, βB: 25-29, βC: 40-45, βD: 62-66, βE: 69-71, βF: 90-95), loops: black and the conserved GMGL loop: pink (22-25). **5f** is colored green.

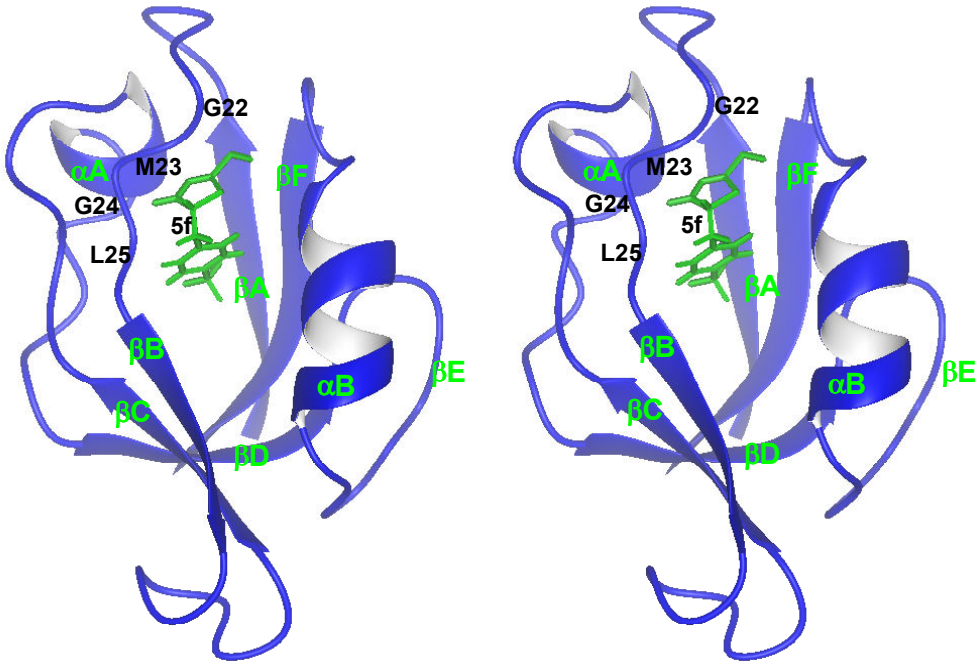

Figure 2. Ribbon representation of the AF6 PDZ-**5f** complex. Only residue 11-95 are shown. **5f** is colored green.

Table 5. Structural statistics of the AF6 PDZ domain.

Restraints	
total no. of experimental restraints	1506
total no. of NOE restraints	1426
intraresidue (i = j)	449
sequential (| i – j | = 1)	364
medium-range (2 ≤ | i – j | ≤ 5)	209
long-range (| i – j | > 5)	404
no. of H-bond restraints	20
no. of dihedral angle restraints (TALOS)	60
average inter-residue NOE's per residue	11.4
no. of NOE violations > 0.3 Å	0
no. of dihedral angle violations > 5°	0
φ – ψ Space (residues)[a,b]	
most favored regions (%)	68.1
additionally allowed regions (%)	21.3
generously allowed regions (%)	10
disallowed regions (%)	0.7
rmsd values[c,d]	
Cα	0.30 ± 0.09
heavy atoms	1.38 ± 0.16

[a] Residues considered: 11-95. [b] From Procheck-NMR.[4] [c] Residues considered: 11-17, 25-29, 40-46, 60-66, 69-72, 77-95. [d] Calculated using MOLMOL.[5]

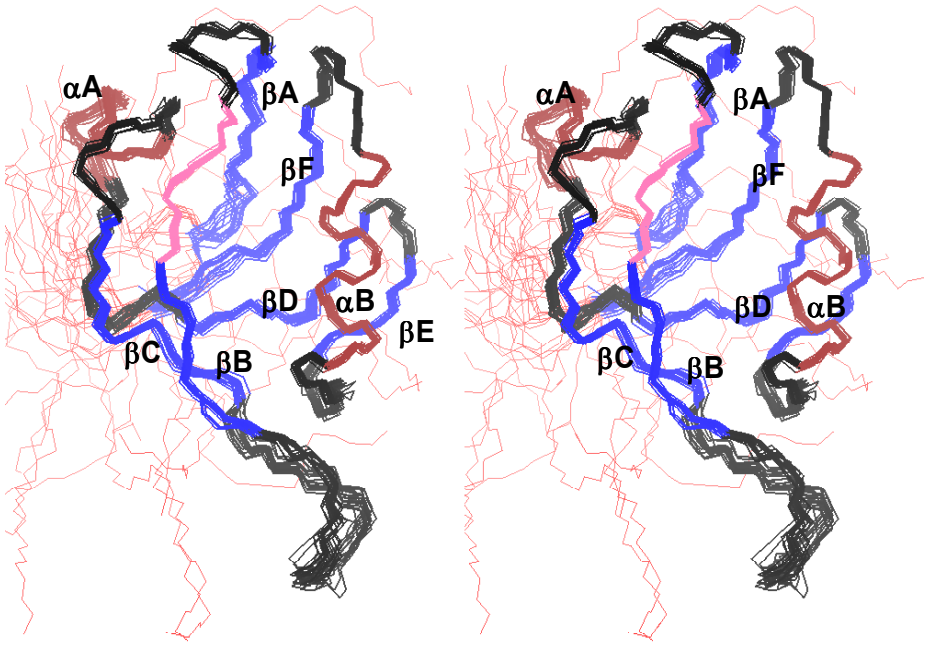

Figure 3. Solution NMR structure of AF6 PDZ domain (1XZ9): Superposition of the backbone (N, CA, and C′) atoms for the 20 lowest-energy structures (stereoview). Color coding: α-helix: brown (αA: 51-55, αB: 77-86), β-strands: blue (βA: 11-17, βB: 25-29, βC: 40-45, βD: 62-66, βE: 69-71, βF: 90-95), loops: black and the conserved GMGL loop: pink (22-25).

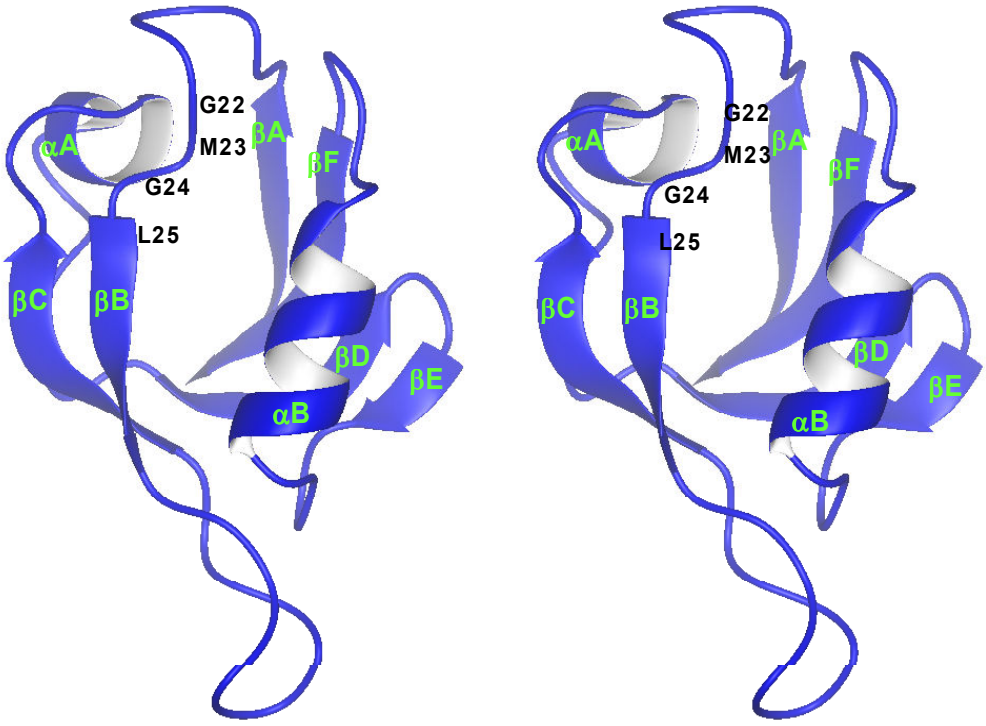

Figure 4. Ribbon representation of the AF6 PDZ domain. Only residues 11-95 are shown.

References

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