

**Making Protein-Protein Interactions Drugable:
Discovery of Low-Molecular-Weight Ligands
for PDZ Domains**

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„Nothing in the world is as pure and holy as knowledge“
- Swami Vivekanand

To my family

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Abstract

Despite their central role in most regulatory processes and disease mechanisms, protein-protein interactions (PPIs) remain largely unconquered ground for drug discovery and chemical tool generation. In many cases, these interactions are mediated by protein interaction domains like Src homology 2 (SH2), Src homology 3 (SH3), WW, and postsynaptic density/Discs large/zona occludens-1 (PDZ). PDZ domains may be considered “drugable” because of a shallow ridge on their surface which is, however, not a proper cavity. They are hence good test cases for the development of PPI inhibitors.

Prior to this study no small molecule reversible inhibitors for the PDZ domains were known. In this thesis we describe the discovery of competitive small molecule modulators for the AF6 PDZ domain. Also the 3D solution NMR structure of the AF6 PDZ domain and AF6 PDZ domain in complex with most active ligand is described.

In our quest to find small molecule inhibitors for the PDZ domain, a small molecule library consisting of ~5000 compounds was screened against the AF6 PDZ domain. 2-thioxo-4-thiazolidinone scaffold binding to the protein was identified and chosen for further optimization by simple chemical procedures. These modifications lead to the design of a compound with 100 μ M binding affinity and a molecular weight of 291 Da.

To understand the mode of binding of this compound to the AF6 PDZ domain and to guide further structure based ligand design 3D structure of the complex was determined by NMR spectroscopy. The 3D structure reveals a new hydrophobic subpocket formed through induced-fit binding of the small molecule ligand. This finding redefines the drugability of PDZ domains and discloses 5-aryl-2-thioxo-4-thiazolidinones and related frameworks as promising candidates for the development of potent and selective small-molecule modulators of individual domains from the large PDZ family.

Table of contents

Abbreviations.....	V
1. Introduction	
1.1 Protein-Protein Interactions	1
1.2 Protein interaction domains	1
1.2.1 Inhibition of PPIs – challenges	2
1.3 PDZ domains	3
1.3.1 Structure of the PDZ domains and their mechanism of peptide recognition.....	4
1.3.2 Specificity of peptide recognition.....	6
1.3.3 Functions of PDZ domains.....	7
1.3.4 Regulation of interactions mediated by PDZ domains	7
1.3.5 PDZ domains as a model for design of PPI inhibitors	8
1.4 The AF6 PDZ domain	8
1.5 Ligand screening.....	10
1.6 NMR spectroscopy as a tool for ligand screening and high resolution structure determination	12
1.6.1 NMR-based screening of ligands.....	12
1.6.2 Ligand-based NMR screening methods	14
1.6.2.1 Transverse relaxation	14
1.6.2.2 Nuclear Overhauser Effect (NOE) based methods.....	14
1.6.2.3 Diffusion.....	18
1.6.3 Protein-based NMR screening methods	18
1.6.3.1 Chemical shift mapping.....	19
1.6.3.2 Selective active site isotope labeling.....	19
1.6.4 Screening of ligands for protein mixtures	21
1.6.5 Comparison of protein-based and ligand-based methods	21
1.7 High resolution structure determination by NMR spectroscopy.....	23

1.8 Objectives of this research	24
1.9 References	25
2. Material and Methods	
2.1 Expression and Purification of the AF6 PDZ domain	31
2.2 NMR instrumentation	32
2.3 NMR sample preparation	32
2.4 General experimental details	33
2.5 NMR based screening of ligands for the AF6 PDZ domain ..	33
2.6 Determination of binding constants	35
2.7 Relaxation and Dynamics	37
2.7.1 ¹⁵ N T1 measurements	37
2.7.2 ¹⁵ N T2 measurements	37
2.7.3 ¹ H - ¹⁵ N Heteronuclear NOE measurements	38
2.8 Resonance assignment of the AF6 PDZ domain	39
2.9 NMR experiments for the Structure determination	41
2.9.1 NMR experiments for intra-molecular NOE restraints	41
2.9.2 NMR experiments for inter-molecular NOE restraints	41
2.9.2.1 The filter element.....	42
2.9.2.2 2D-F2 ¹³ C-filtered NOESY.....	43
2.9.2.3 2D-F2 ¹³ C-filtered HMQC-NOESY.....	43
2.9.2.4 3D-F1 ¹³ C-suppressed NOESY-HMQC.....	44
2.10 Automated NOE cross peak assignment using CANDID....	47
2.11 Backbone φ and ψ angle determination	47
2.12 Structure Calculation	48
2.12.1 Structure calculation using CYANA	48

2.13 Docking of 5-(4-trifluoromethylbenzyl)-2-thioxo-4-thiazolidinone (7i) on to the AF6 PDZ domain.....	49
2.13.1 Ligand parameterization using Antechamber	49
2.13.2 Docking of 5-(4-trifluoromethylbenzyl)-2-thioxo-4-thiazolidinone (7i) onto the AF6 PDZ domain.....	49
2.14 References.....	51
3. Results and Discussion	
3.1 Domain architecture of the human AF6 Protein	53
3.2 Expression and purification of recombinant AF6 PDZ domain	53
3.3 NMR based screening of ligands for the AF6 PDZ domain ..	55
3.3.1 Screening results	55
3.3.2 Binding constant and CSP map of 5-(4-trifluoromethylbenzyl)-2-thioxo-4-thiazolidinone (7i).	63
3.3.3 5-(4-trifluoromethylbenzyl)-2-thioxo-4-thiazolidinone (7i) interacts competitively with the AF6 PDZ domain.....	63
3.4 Structure determination of the AF6 PDZ domain and AF6 PDZ 5-(4-trifluoromethylbenzyl)-2-thioxo-4-thiazolidinone (7i) complex.....	66
3.4.1 Resonance assignments	66
3.4.1.1 Backbone chemical shift chemical shift assignment....	66
3.4.1.2 Side-chain ¹ H and ¹³ C Resonance Assignments.....	69
3.4.2 ¹⁵ N relaxation measurements.....	70
3.4.3 Dihedral angle restraints	71
3.4.4 Secondary structure prediction form the chemical shifts ..	71
3.4.5 NOE assignments and structure calculation for the AF6 PDZ domain	73
3.4.5.1 Application of CANDID for structure determination of the AF6 PDZ domain.....	73
3.4.6 Structural Analysis.....	79
3.4.7 Structure determination of the AF6 PDZ - 5-(4-trifluoromethylbenzyl)-2-thioxo-4-thiazolidinone (7i) complex.....	82

3.4.8 Structural analysis of the AF6 PDZ - 5-(4-trifluoromethylbenzyl)-2-thioxo-4- thiazolidinone (7i) complex.....	91
3.5 Comparison of the ligand-free and ligand-bound AF6 PDZ domain	94
3.6 Comparison of the ligand-bound AF6 PDZ domain to the syntrophin and erbin PDZ domains	96
3.7 References.....	99
4. Summary and outlook	
4.1 Summary.....	101
4.2 Outlook.....	102
5. Zusammenfassung.....	104

Abbreviations

1D, 2D, 3D	one dimensional, two dimensional, three dimensional
1J	one bond scalar couplings
Å	Angstrom
COSY	correlation spectroscopy
Da	Dalton
DMSO	dimethylsulfoxide
<i>E. coli</i>	<i>Escherichia coli</i>
HMQC	heteronuclear multiple quantum correlation
HSQC	heteronuclear single quantum correlation
kDa	kilo Dalton
MHz	megahertz
NMR	nuclear magnetic resonance
NOESY	nuclear Overhauser enhancement spectroscopy
PDB	brookhaven protein data bank
ppm	parts per million
RMSD	root mean squared deviation
SAR	structure activity relationship
SDS-PAGE	sodium dodecyl sulphate- polyacrylamide gel electrophoresis
STD	saturation transfer difference
T_1	longitudinal relaxation time
T_2	Transverse relaxation time
TOCSY	total correlation spectroscopy
TROSY	transverse relaxation optimized spectroscopy
WATERGATE	water suppression by gradient- tailored excitation