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-thazolidinone 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 μM 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.

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Abbreviations

1D, 2D, 3D	one dimensional, two dimensional, three dimensional
^1J	one bond scalar couplings
Å	Angstrom
COSY	correlation spectroscopy
Da	Dalton
DMSO	dimethylsulfoxide
E. coli	*Escherichia coli*
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