

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

Dissertation zur Erlangung des akademischen Grades des
Doktors der Naturwissenschaften (Dr. rer. nat)

Eingereicht im Fachbereich Biologie, Chemie, Pharmazie
der Freien Universität Berlin

vorgelegt von

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Januar 2006

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Thesis defence: 7th Aug. 2006

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

To my family

Acknowledgements

This work was carried out at Department of NMR Supported Structural Biology, Research Institute for Molecular Pharmacology (FMP), Berlin, Germany, from 1st June 2001 to 31st January 2006 under the supervision of **Prof. Dr. Hartmut Oschkinat**. This thesis would not have been possible without the help of many people. I sincerely acknowledge them.

First, I would like to express my deep gratitude to my guide **Prof. Dr. Hartmut Oschkinat** who gave me this opportunity to work at the FMP. His encouragement and guidance were the driving forces of my success. Under his supervision, I enjoyed my learning at FMP. I am indebted to **Dr. Markus Schade**, Combinature Biopharm AG, Berlin, who was a constant source for motivation throughout this work and introduced me to the field of NMR based screening of ligands. I am also thankful to **Ms. Carolyn Vargas** who apart from being a very nice colleague is also a dear friend. She was responsible for the synthesis of all the nice compounds without which this work would had been incomplete.

I am grateful to **Dr. Peter Schmieder** and **Dr. Dietmar Leitner** for providing ample measurement time, sometimes running into weeks, for my work. I am extremely thankful to them for teaching me most of the things that I know about multi-dimensional NMR and their patience to solve all my problems.

Many thanks to **Dr. Gerd Krause**, **Jens Laettig** and **Dr. Arvid Söderhäll** for their help during the MD simulations. I would also like to thank **Dr. Ludwig Krabben** for his support and help in getting me settled in the lab during the early days. My sincere thanks to **Dr. Prisca Boisguerin** for providing the plasmid for the AF6 PDZ domain. I have to thank **Dr. Annette Diehl** and all the technicians especially **Lilo** of the “wet” lab for the expression and purification of the protein. I thank **Dr. Frank Eisenmenger**, our system administrator, who helped me out with numerous problems related to the FMP servers and also taught me a lot of things about linux and unix. Thanks to **Urs** for help with the windows PC's even though he had to do so at the cost of his time during his PhD work. Discussions

with him about the PDZ domains and their interactions were always helpful. I also thank **Andrea Steuer** for helping me out of all the bureaucracy.

Michael “Micha” Soukenik deserves special thanks for helping me out with the day-to-day stuff in the lab as well as getting me settled in Berlin. He helped me out with everything in those early days, from registration in the Rathaus to finding a apartment. At FMP, I acquired many friends. **Sarav**, who is now in USA, is a very special person with whom I could discuss any topics for hours on end. **Kirill** and me discuss a lot of scientific and non-scientific stuff and matters that are totally out of this world, he is also my 1st target when it comes to translating German stuff to English. I thank **Mark** of the Christian Freund group for doing the translation of the Summary of my thesis.

I take this opportunity to thank **Prof. Dr. Bernd Reif** and his entire group for their friendship and support during the last months of my PhD. I would also like to thank the entire Oschkinat group for a fantastic working atmosphere and lively discussions during the seminars.

Thank you very much to my friends in Berlin, **Deepti, Sanjeev** and especially, **Rohit, Anvita** for their help during this work. We have had some of the most memorable days of our stay in Berlin with them. Also, thanks a lot to all other friends who were very nice to tolerate me and share with me lots and lots of movies.

This list would by no means be complete without thanking my family members. My warmest thanks to my brother and sister-in-law and to my in-laws for their love and support. Last but not least I have to thank three people without them this task would had been impossible: my wife, **Pradnya** and my **parents**. They have sacrificed greatly over the course of these five years. I would be eternally grateful to them for their love and support.

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.

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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
<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