

Summary and outlook

4.1 Summary

PDZ domains are highly abundant in the *H. sapiens* genome and are involved in forming complex signal transduction networks. The provisional role of PDZ domains in processes connected to disease states makes them prime targets for protein-protein interactions inhibitor design.

These ~90 residue domains mostly recognize the C-terminal four to seven residues of membrane receptors and ion channels. They are considered good targets for the development of protein-protein interaction (PPI) inhibitors because of the presence of a well defined but shallow binding cavity.

Prior to this study, no reversibly binding small molecule inhibitors for the PDZ domains were known. Hence discovering novel, non-covalent competitive small molecule inhibitors for these domains was the primary aim of this study.

As a model system, we used the AF6 PDZ domain as the target protein for ligand screening and development purposes. The human AF6 protein (*ALL-1* fusion partner on chromosome 6), also known as L-afadin, is involved in numerous signal transduction pathways including Ras signaling. This protein contains two N-terminal Ras-association domains, a fork head association domain (FHA), a class V myosin homology repeat, also known as dilute domain (DIL), a class II PDZ domain and a proline rich sequence.

In our quest to find small molecule inhibitors for the AF6 PDZ domain, a small molecule library consisting of ~5000 compounds was screened against the AF6 PDZ domain. NMR was applied for this purpose using ¹⁵N-labeled protein and 2D ¹H-¹⁵N-HSQC experiments.

From the screening experiments, 2-thioxo-4-thiazolidinone was identified as a scaffold that binds to the protein and chosen for further optimization by simple chemical procedures. Systematic modification of this scaffold lead to the design of (2R,5R)-2-sulfanyl-5-[4-(trifluoromethyl)benzyl]-1,3-thiazol-4-one (**7i**), a chiral 291 Da compound with 100 μ M binding affinity for the racemic mixture. The compound was shown to interact competitively using 15 N-filtered NMR experiments. Assuming that only one of the enantiomer binds preferentially, the affinity of the compound can be approximated to 50 μ M which is in the same range as that of the natural peptide ligands.

To understand the interaction between the AF6 PDZ domain and **7i**, and to aid future structure-based ligand design, solution structures of the PDZ domain with and without the ligand were determined. Analysis of the structure determined in presence of **7i** shows the formation of a hydrophobic subpocket in AF6 PDZ absent in published structures of both apo and peptide-bound PDZ domains. This unexpected ligand–subpocket formed through induced-fit binding redefines the protein’s drugability 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.

4.2 Outlook

The structure of the AF6 PDZ domain in complex with **7i** reveals several potential areas which can be targeted by functional groups of **7i** analogues. Figure 4.1 shows the structure of the AF6 PDZ – **7i** complex. Surface topology of the protein shows distinct patches which can be exploited for further ligand design. Most notable among them is the possible hydrophobic interaction area highlighted as EA4. Also, as described earlier, Gln76 of the AF6 PDZ domain points towards the ligand binding groove. The corresponding position in most of the other PDZ domains is occupied by a His residue. Ligands targeting specifically a glutamine residue are likely to be specific for the AF6 PDZ domain.

The second approach would be to use the structure of the PDZ domain in complex with a peptide as a template for developing non-peptidic ligands. Recently, Wiedemann and co-workers derived ‘specificity profiles’ for the AF6, α -1 syntrophin and erbin PDZ domains. These profiles, which describe the contribution of individual amino acids in the peptide ligands to affinity and specificity towards the PDZ domains also led to the development of best binding peptides, also known as “super-binders”, for the three PDZ domains. In the case of the AF6 PDZ domain, the peptide $\text{NH}_2\text{-LEGIFV-COOH}$ was found to have highest binding affinity ($\sim 10 \mu\text{M}$). Structure determination of the AF6 PDZ complex with this peptide will be helpful in further ligand development strategies. Efforts are currently underway to determine the structure of this complex.

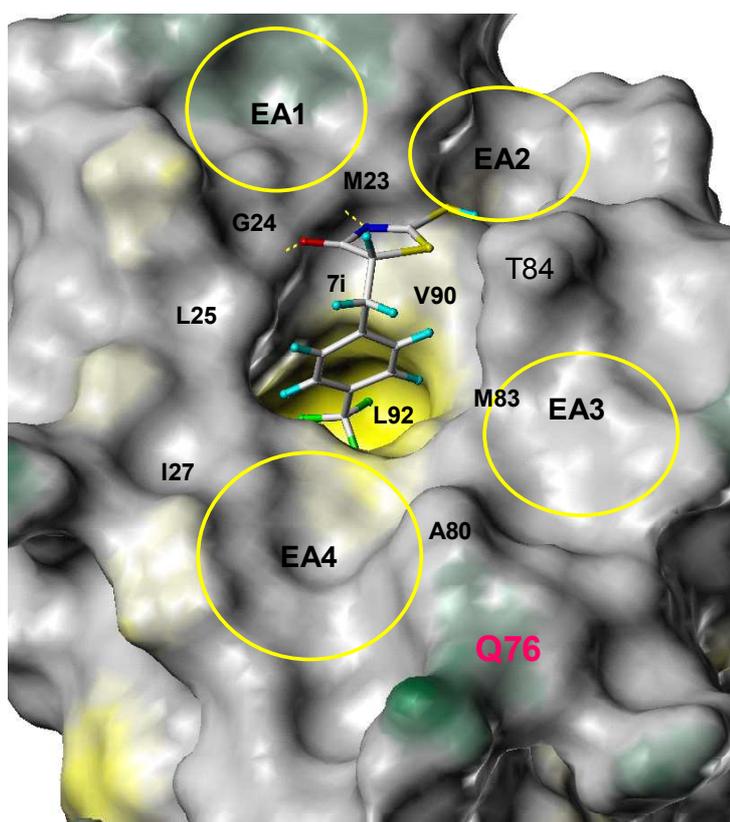


Figure 4.1: Surface topology of the AF6 PDZ – 7i complex. Protein surface coloring indicates yellow – hydrophobic areas and green – hydrophilic areas. Distinct patches which can be exploited for further ligand design are highlighted by yellow circles.