DOI: 10.1002/cmdc.201300553

# Small-Molecule Inhibitors of AF6 PDZ-Mediated Protein–Protein Interactions

Carolyn Vargas,[a, b] Gerald Radziwill,*[c] Gerd Krause,[a] Anne Diehl,[a] Sandro Keller,[a, b] Nestor Kamdem,[a] Constantin Czekelius,[d] Annika Kreuchwig,[a] Peter Schmieder,[a] Declan Doyle,[f] Karin Moelling,[e] Volker Hagen,[a] Markus Schade,*[g] and Hartmut Oschkinat*[a]

PDZ (PSD-95, Dlg, ZO-1) domains are ubiquitous interaction modules that are involved in many cellular signal transduction pathways. Interference with PDZ-mediated protein–protein interactions has important implications in disease-related signaling processes. For this reason, PDZ domains have gained attention as potential targets for inhibitor design and, in the long run, drug development. Herein we report the development of small molecules to probe the function of the PDZ domain from human AF6 (ALL1-fused gene from chromosome 6), which is an essential component of cell–cell junctions. These compounds bind to AF6 PDZ with substantially higher affinity than the peptide (Ile-Gln-Ser-Val-Glu-Val) derived from its natural ligand, EphB2. In intact cells, the compounds inhibit the AF6–Bcr interaction and interfere with epidermal growth factor (EGF)-dependent signaling.

## Introduction

Most biological processes are governed by the transient formation of protein complexes that are established through weak yet specific protein–protein interactions (PPIs).[1,2] Malfunctions within these networks can bring about pathological conditions and thus have important implications for the treatment of human diseases. Such PPIs represent a particularly challenging class of targets for the development of bioactive small molecules.[3,4] One approach to their modulation is to target the respective interaction-mediating protein domains.[5] Although the task of finding and developing such modulators is formidable,[4,6] there have been several examples of successful inhibition of protein–protein interactions by small molecules.[4,7] We have undertaken a small-molecule approach toward understanding PPIs involving PDZ (postsynaptic density/discs large/zona occludens-1) domains.[8] They consist of six β strands (βA–βF) flanked by two α helices (αA and αB)[9] and expose a characteristic, well-defined binding pocket that is an attractive target for inhibitor design. For DVL and Pick1 PDZ domains, small-molecule inhibitors have been identified, the binding affinities of which are similar to those of the endogenous peptide ligands.[10,11] Herein we describe the development of interaction modulators for the disease-related cell-junction protein AF6 (ALL-1 fused gene from chromosome 6, also known as l-afadin). AF6 is also a scaffolding protein that connects membrane-associated proteins to the actin cytoskeleton.[8,12] It binds the protein kinase break-point-cluster region (Bcr) via its PDZ domain and the GTPase Ras via its Ras binding domain, which leads to down-regulation of Ras signaling at sites of

[a] Dr. C. Vargas, Dr. G. Krause, Dr. A. Diehl, Prof. Dr. S. Keller, Dr. N. Kamdem, A. Kreuchwig, Dr. P. Schmieder, Dr. V. Hagen, Prof. Dr. H. Oschkinat
Leibniz Institute of Molecular Pharmacology (FMP)
Robert-Rössle-Str. 10, 13125 Berlin (Germany)
E-mail: oschkinat@fmp-berlin.de

[b] Dr. C. Vargas, Prof. Dr. S. Keller
Current address: Molecular Biophysics, University of Kaiserslautern
Erwin-Schrödinger-Str. 13, 67663 Kaiserslautern (Germany)

[c] Prof. Dr. G. Radziwill
Institute of Medical Virology, University of Zurich (Switzerland)
and
Current address: Faculty of Biology
BIOSS Centre for Biological Signalling Studies, University of Freiburg
Schaenzlestr. 1, 79104 Freiburg (Germany)
E-mail: gerald.radziwill@biologie.uni-freiburg.de

[d] Prof. Dr. C. Czekelius
Institut für Chemie und Biochemie, Freie Universität Berlin (Germany)
and
Current address: Instiut für Organische Chemie und Makromolekulare Chemie
Heinrich-Heine-Universität Düsseldorf
Universitätsstr. 1, 40225 Düsseldorf (Germany)

[e] Prof. Dr. K. Moelling
Institute of Medical Virology
University of Zurich, Gloriastr. 30, 8006 Zurich (Switzerland)

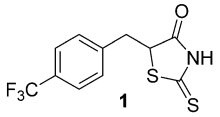
[f] Dr. D. Doyle
Structural Genomics Consortium
Oxford University, Botnar Research Center, Oxford, OX3 7LD (UK)
and
Current address: Trinity College Dublin
School of Biochemistry and Immunology, College Green, Dublin 2 (Ireland)

[g] Dr. M. Schade
Evotec AG, Berlin (Germany)
and
Current address: Grünenthal GmbH, 52099 Aachen (Germany)
E-mail: Markus.Schade@grunenthal.com

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201300553.

cell–cell contact to maintain cells in a non-proliferative state.[13] The PDZ domain (AF6 PDZ) mediates clustering of Eph receptor tyrosine kinases[14] and binds c-Src, a non-receptor tyrosine kinase.[15] Furthermore, AF6 PDZ interacts with Jagged-1[16] and connexin 36.[17] Although a number of these proteins that interact with the AF6 PDZ have been identified, the physiological roles of these interactions have yet to be established.[18] Low-molecular-weight regulators may help address this issue. We previously reported the three-dimensional NMR structure of compound **1** (5-(4-trifluoromethylbenzyl)-2-thioxo-4-thiazolidinone) in complex with AF6 PDZ.[19] The binding affinity of **1** for AF6 PDZ ($K_D = 100$ μM) is similar to that of the peptide (Ile-Gln-Ser-Val-Glu-Val, $K_D = 137$ μM)[20] derived from its natural ligand, EphB2.[14] In our previous study, compound **1** was found to bind α1-syntrophin PDZ in a similar manner as to AF6 PDZ, with approximately threefold lower affinity.[19] Therefore, in this work, we aimed at developing compounds that bind more avidly to AF6 PDZ but not to α1-syntrophin PDZ. For selectivity testing, we initially performed a screen of **1** against a limited set of PDZ domains (Dlg2 PDZ-2, Dlg3 PDZ-2, MPDZ-3, MPDZ-5, MPDZ-11, MPDZ-12, and Shank3). Then, guided by molecular modeling, we designed and synthesized derivatives of our lead scaffold **1** and estimated binding affinity by NMR to ascertain ligand selectivity and established structure–activity relationships on the basis of the solved complex structure. We investigated the selectivity of the tightest-binding compounds further by testing them against a second set of PDZ domains, namely DVL-1, PSD95 PDZ-1, -2 and -3, and Shank3 PDZ. We then tested the effects of the compounds in cellular assays.



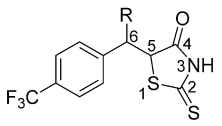
**1**

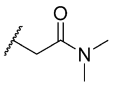
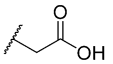
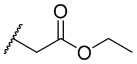
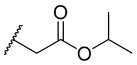
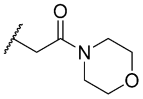
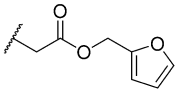
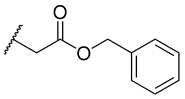
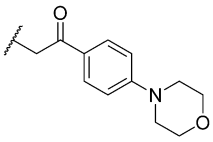
5-(4-trifluoromethylbenzyl)-2-thioxo-4-thiazolidinone

## Results and Discussion

As a prerequisite for deriving extended derivatives by molecular modeling, we screened lead scaffold **1** for potential binding to a variety of selected PDZ domains. Because the ligands are positioned largely in the space occupied by the last two residues of peptide ligands, PDZ domain classification is not relevant for specificity testing (class I: S/T-X-Φ; class II: Φ-X-Φ). In the absence of $^{15}$N-labelled protein, two-dimensional total correlated spectroscopy (TOCSY) NMR experiments were used for this purpose, and the AF6 PDZ chemical shift perturbations (CSP, $\Delta\delta$) were taken as reference ($\Delta\delta = 0.06$ ppm). Dlg2 PDZ2, Dlg3 PDZ2, MPDZ-3, MPDZ-5, MPDZ-11, and MPDZ-12 showed intermediate binding affinities ($\Delta\delta = 0.03$, 0.02, 0.04, 0.06, 0.03, and 0.03 ppm, respectively), whereas no binding was detected for both Dlg2 PDZ1 and Shank3 PDZ ($\Delta\delta = < 0.02$ ppm). Evidently, **1** binds to various PDZ domains. Nonetheless, the differential binding exhibited by **1** is an indication that selectivity may be achieved by rational derivatization. To this end, we synthesized derivatives of **1** with the aid of molecular modeling based on the AF6 PDZ–**1** complex structure (PDB code 2EXG) and NMR chemical shift data. Selectivity was introduced through substituents that recognize a unique "Ala80 pocket" in the binding groove of AF6 PDZ, absent in other PDZ domains (Supporting Information, Figure S1). This was achieved by extending the lead scaffold at the 6-position by substituents of various bulk (Table 1). As ex-

**Table 1.** Lead scaffold **1** and its derivatives **2**–**10** with dissociation constants and selectivity indexes (SI) for AF6 and α1-syntrophin PDZ domains.



| R | Compd | $K_D$ [μM][a] AF6 | α1-Syntrophin | SI |
|---|---|---|---|---|
| H | **1** | 100 ± 12 | 270 ± 21 | 3 |
|  | **2** | 256 ± 90 | 481 ± 128 | 2 |
|  | **3** | 274 ± 47 | > 1000 | > 4 |
|  | **4** | 91 ± 16 | 307 ± 60 | 3 |
|  | **5** | 36 ± 7 | 504 ± 95 | 14 |
|  | **6** | 32 ± 3 | > 1000 | > 31 |
|  | **7** | 30 ± 6 | 773 ± 137 | 26 |
|  | **8** | 27 ± 5 | 400 ± 152 | 15 |
|  | **9** | 25 ± 5 | > 1000 | > 40 |
|  | **10** | 10 ± 2 | 71 ± 16 | 7 |
| | **10a** | no binding* | – | |
| | **10b** | 4.9 ± 2* | – | |

[a] $K_D$ values were measured by $^1$H–$^{15}$N HSQC NMR titration (Figure S2) using the rapidly interconverting racemate **1** and racemic mixtures of diastereomers **2**–**10**; values are reported as means ± SD of at least six residues influenced by ligand binding. *: Denotes the value obtained after enantiomeric separation of compound **10** into the pairs **10a** (5*S*,6*S* and 5*R*,6*S*) and **10b** (5*R*,6*R* and 5*S*,6*R*). Mixtures **10a** and **10b** were assigned on the basis of molecular modeling using the solution structure of **1** complexed with AF6 PDZ (PDB code 2EXG) as template.

pected, the modification of **1** at position 6 resulted in a number of racemic mixtures of diastereomers, **5**–**10**. Separation of the four stereoisomers of each diastereomeric mixture proved difficult, because of rapid racemization. However, this was performed for one stereocenter of **10** at the state of precursors resulting in the mixture (5*R*,6*R*) and (5*S*,6*R*) or (5*S*,6*S*) and (5*R*,6*S*), as discussed below. In agreement with our initial hypothesis, bulky substituents at position 6 of compound **1** had the most pronounced influence on the binding affinity to AF6 PDZ. Short extensions such as a methyl (**2**), *N*,*N*-dimethylcarboxymethyl (**3**), or carboxymethyl (**4**) groups showed no improvement, whereas substituents such as ethoxycarbonylmethyl (**5**), isopropyloxycarbonylmethyl (**6**), morpholinocarbonylmethyl (**7**), 2-furanylmethoxycarbonylmethyl (**8**), and benzyloxycarbonylmethyl (**9**) improved binding. The binding affinities of **4**–**9** clustered in the $K_D$ range of 25–36 μM, suggesting that these molecules occupy a hydrophobic interaction area of similar size. Additionally, the bulkier and longer morpholinophenylcarbonylmethyl moiety of **10** shows the highest binding affinity ($K_D = 10$ μM).

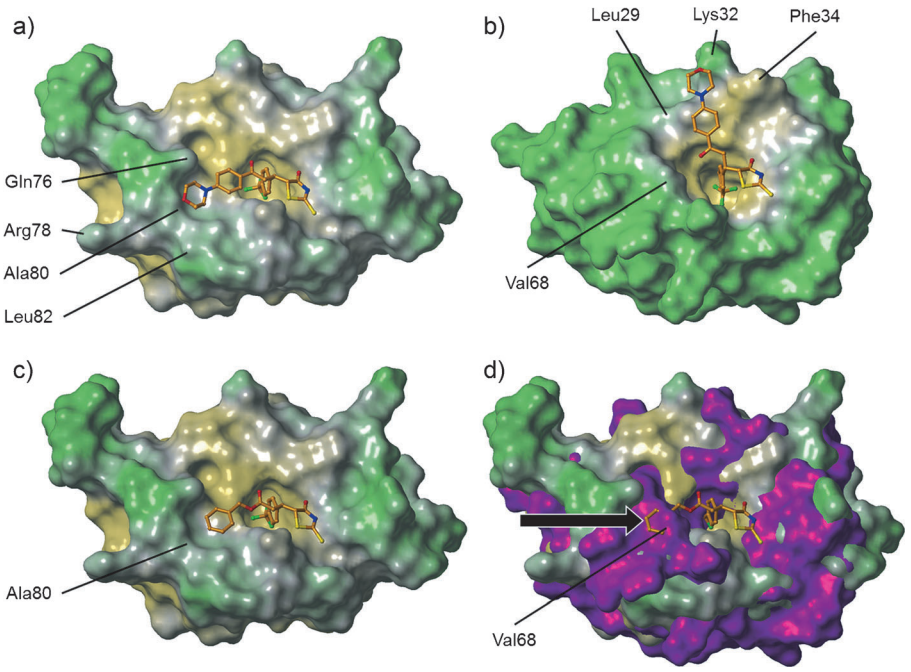



**Figure 1.** Structural models of PDZ–ligand complexes: a) AF6 PDZ–(5*R*,6*R*)-**10**; b) α1-syntrophin PDZ–(5*R*,6*S*)-**10**; and c) AF6 PDZ–(5*R*,6*R*)-**9**. d) Superposition of the surfaces of the AF6 PDZ–(5*R*,6*R*)-**9** complex and the α1-syntrophin PDZ domain (violet surface, presence of bulkier Val68 instead of Ala80, black arrow). Surface representations of AF6 PDZ are shown as hydrophobic color potential (yellow = hydrophobic, green = hydrophilic); ligands are depicted in orange. The indicated Val68 is of α1-syntrophin PDZ.

In contrast to AF6 PDZ, $K_D$ values for α1-syntrophin PDZ ranged from 70 μM to >1 mM (Table 1). The preferential binding of the compounds to AF6 PDZ relative to α1-syntrophin PDZ is given in the right column of Table 1 by a selectivity index (ratio of $K_D$(α1-syntrophin PDZ) over $K_D$(AF6 PDZ)). Compounds **6** and **9** bound 30- and 40-fold more tightly to AF6 PDZ than to α1-syntrophin PDZ, respectively. Although **10** discriminates only by a factor of 7, it has the highest affinity for AF6 PDZ, showing a 10-fold improvement over compound **1**. Considering that **10** is a racemic mixture of diastereomers, we attempted to separate the stereoisomers. Enantiomeric separation of **10** was performed by chromatography on the level of a protected precursor and resulted in **10a** and **10b**, each a diastereomeric mixture of enantiomerically pure material with respect to the benzylic position (Figure S3). No binding was detected for **10a** (Table 1, Figure S4a), whereas **10b** induced strong CSPs with $K_D = 4.9$ μM (Table 1, Figure S4b). Because **1** and its derivatives **5**–**10** induced the same strong CSPs to residues located along the binding pocket (Met23, Leu25, Ile27, Met83, Val90, and Leu92), they are all likely to bind in a very similar way.[19] This allowed us to use the AF6 PDZ–**1** complex structure (PDB code 2EXG) and the ligand-bound NMR structure of α1-syntrophin PDZ (PDB code 2PDZ) as templates for modeling the PDZ–ligand complexes of all four stereoisomers of **9** (which selectively binds AF6 PDZ over α1-syntrophin PDZ) and **10** (which has the highest affinity for AF6 PDZ). This yielded the (5*R*,6*R*) isomers of **9** and **10** as the best binding partners (Figure 1a,c,d). Docking of isomers (5*R*,6*R*) and (5*S*,6*R*) resulted in a larger number of productive interactions than complexes involving isomers (5*S*,6*S*) and (5*R*,6*S*). For the latter, no energetically relevant interactions could be found in which the five-membered ring interacts appropriately with the GLGF loop, the conserved sequence motif critical for PDZ interactions. Therefore, we assigned **10b** to consist of the (5*R*,6*R*) and (5*S*,6*R*) isomers, whereas **10a** consisted of (5*S*,6*S*) and (5*R*,6*S*).

In the models of the complex AF6 PDZ–**10** (Figure 1a), the R group of the 5*R*,6*R* isomer extends across the peptide binding groove toward the region where αB starts and where the morpholino ring makes contact with the Gln76 side chain. The morpholino ring occupies a surface indentation primarily formed by Ala80, which we designate as the Ala80 pocket. The experimental CSPs of Gln76, Arg78, Ala80, and Leu82 confirmed binding of **10b** to the Ala80 pocket. Similar arguments explain the binding of **9** to AF6 PDZ (Figure 1c).

In contrast, modeling complexes involving ligands **10** and α1-syntrophin PDZ (PDB code 2PDZ) yielded a satisfying result only for the (5*R*,6*S*) isomer of **10**. The α1-syntrophin PDZ–**10** models (Figure 1b) show the R group of **10** oriented toward βB, where the oxygen atom of the morpholino ring could form hydrophilic contacts with the NH groups of the Lys32 side chain. In this region, confirmatory CSPs were observed for

Leu29, Lys32, and Phe34. This alternative Lys32 binding geometry is very likely caused by the occlusion of the Ala80 pocket in α1-syntrophin PDZ with the side chain of residue Val68. Figure 1d shows a superposition of the AF6 PDZ and α1-syntrophin surfaces including the (5*R*,6*R*) isomer of **9**, suggesting that the low affinity between α1-syntrophin PDZ and **9** may be due to both the absence of the Ala80 pocket and the lack of other attractive protein contacts. Intriguingly, the −2 position of the natural peptide ligand partially targets the Ala80 pocket and is considered a critical determinant of PDZ subclass selectivity.[21]

To obtain further selectivity profiles for **9** and **10**, we screened them against $^{15}$N-labeled PDZ domains of PSD95 (PDZ-1, -2, and -3), DVL1 and Shank3, applying $^{1}$H–$^{15}$N heteronuclear single quantum correlation (HSQC) spectroscopy and comparing the observed CSPs to the changes observed on AF6 PDZ ($\Delta\delta = 0.28$ and 0.31 ppm for **9** and **10**, respectively). Compound **9** did not bind PSD95 PDZ-1, -2, and -3, and Shank3 PDZ ($\Delta\delta < 0.02$ ppm), whereas very weak binding to both DVL1 and α1-syntrophin PDZ ($\Delta\delta = 0.04$ ppm) was observed. Compound **10** did not bind Shank3 and PSD95 PDZ-1 and -3 ($\Delta\delta < 0.02$ ppm), but showed weak binding to DVL1 ($\Delta\delta = 0.07$ ppm), PSD95 PDZ-2 ($\Delta\delta = 0.12$ ppm), and α1-syntrophin PDZ ($\Delta\delta = 0.10$ ppm). Taken together, we have shown examples of the preferential binding of **9** to AF6 PDZ within a panel of PDZ domains investigated, and binding of **10b** to the AF6 PDZ domain with considerable affinity ($K_D = 4.9$ μM), suggesting that thiazolidinone derivatives may be developed as specific binders for PDZ domains.

Compounds **1** (the lead scaffold), **5**–**7** ($K_D \sim 30$ μM), and **10** (highest-affinity AF6 PDZ binder in the series) were tested in biological assays. Compounds **5**, **6**, and **7** were included in individual assays for comparison, to obtain data points with weaker binding ligands. In pull-down assays, we observed that **1**, **5**–**7**, and **10** interfere with the binding of Bcr (one of the AF6 PDZ interaction partners) to the isolated AF6 PDZ (Figure 2a). Follow-up co-immunoprecipitation assays conducted on **1**, **5**, and **10** demonstrated that **1** and **5** indeed interfere with the interaction between full-length proteins AF6 and Bcr in HEK293 cells (Figure 2b), thus supporting the results of pull-down assays. Surprisingly, **10** induced degradation of AF6 and Bcr in cells (Figure 2b, right panel; Figure S5a), a case that requires further elaborative studies.

Furthermore, we used epidermal growth factor (EGF)-stimulated cells to analyze the effect of compounds on signaling, i.e., EGFR phosphorylation and ERK (extracellular signal-regulated kinase) phosphorylation. Compound **1** was most effective and decreased EGF-induced ERK1/2 phosphorylation at 100 μM, whereas **1** and **6** inhibited ERK1/2 phosphorylation at 300 μM and 1 mM, respectively (Figure S5a). Furthermore, **6** and **10** were shown to abrogate EGF-induced receptor tyrosine phosphorylation (Figure S5b). Therefore, we postulate that under these conditions the compounds act on the level of EGFR phosphorylation, interfering with downstream phosphorylation of ERK. Generally, phosphorylation of both EGFR and ERK correlates with increased cell proliferation. Accordingly, both **1** and **6** decreased cell proliferation (Figure S6). It is possible that the negative influence of **1** and **6** on EGF-induced signaling involves receptor-type protein tyrosine phosphatases such as RPTPβ/ζ and RPTPγ.[22] Both carry a C-terminal sequence, Glu-Ser-Leu-Val, that binds to AF6 PDZ,[20] which in effect may restrict their phosphatase activity. We hypothesize that compounds competing for binding of RPTPs to AF6 PDZ could release RPTPs, thereby inhibiting receptor tyrosine phosphorylation and ERK activation, as depicted in a model (Figure 3).

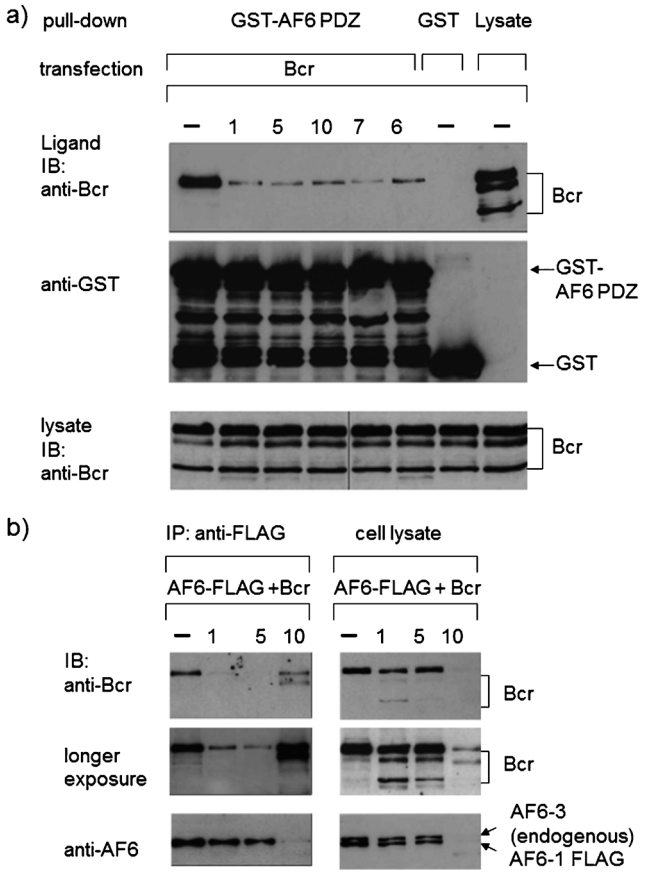



**Figure 2.** Effect of compounds **1**, **5**–**7**, and **10** on the interaction of AF6 PDZ with Bcr. a) Pull-down assay: Incubated GST–AF6 PDZ and GST bound to glutathione sepharose with HEK293 lysate overexpressing Bcr pretreated with **1**, **5**–**7**, and **10** or DMSO (1 mM, 1 h) as indicated. Bound Bcr was detected by immunoblotting with anti-Bcr antibody (top panel). Levels of GST proteins and Bcr were controlled by immunoblotting (middle and bottom panels). b) Co-immunoprecipitation: HEK293 cells transiently transfected with full-length AF6–FLAG and Bcr were treated before cell lysis with **1**, **5**, and **10** (1 mM, 1 h). Interactions were monitored by immunoprecipitation with anti-FLAG antibody and immunoblotting with anti-Bcr antibody (left panels); right panels show control of protein expression. All compounds were soluble under the assay conditions.

Compound **10** was also shown to inhibit ERK1/2 and EGF receptor phosphorylation (Figures S5a and S5b). Additionally, **10** induces degradation of both AF6 and Bcr (Figure 2b and Figure S5a). Compound-induced degradation was observed in experiments with intact cells, and it was not detectable in pull-down assays (Figure 2a). Additionally, in contrast to **1** and **6**, **10** slightly elevated the HEK293 cell count, presumably through disruption of cell–cell contacts and overgrowth of cells, as previously observed after down-regulation of AF6.[23]

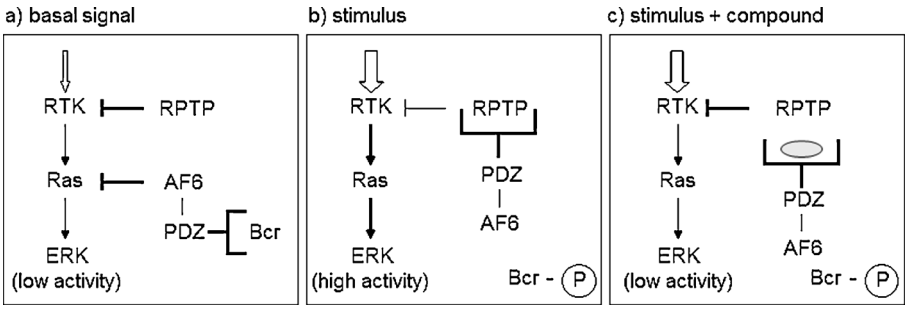



**Figure 3.** Model depicting how AF6 PDZ and chemical interference may act on signaling. a) In non-stimulated cells, binding of Bcr to AF6 PDZ down-regulates Ras signaling.[13] In addition, RPTP inhibits activation of RTK-dependent ERK activation. b) Upon stimulation of cells, phosphorylated Bcr is released from the AF6 PDZ domain that now sequesters RPTP, resulting in activation of ERK. c) Inhibitory compounds (grey) interfere with the RPTP–AF6 PDZ interaction and allow RPTP-dependent inhibition of RTK/ERK signaling.

Therefore, AF6 degradation may overcompensate for ERK pathway inhibition, resulting in enhanced proliferation in the presence of **10** (Figure S6).

## Conclusions

In summary, we have demonstrated inhibition of the Bcr–AF6 PDZ interaction, EGF-dependent signaling, and cell proliferation by our compounds. These findings show that small-molecule binders can be developed to differentially modulate the activity of individual PDZ domains. In spite of the rather modest affinities of the improved compounds, they have provided us with a better assessment of AF6 PDZ-mediated PPIs, thereby opening up new avenues for the development of tailored chemical probes and novel chemotherapeutics.

## Experimental Section

Chemical synthesis, NMR protein sample preparation, quantification of ligand binding, molecular modeling, and cell-based assays are described in the Supporting Information.

## *Acknowledgements*

*This research was supported by the Deutsche Forschungsgemeinschaft (DFG) Research Group 806 and EXC-294 BIOSS (G.R.). We thank M. Leidert and S. Radetzki for cloning and protein preparation, Dr. V. Martos for help in some HPLC runs, and B. Schlegel and J. Fritz for technical assistance. We thank Prof. M. Stubbs (Martin Luther University Halle-Wittenberg) and Dr. D. Geissler (FMP Berlin) for constructive discussions.*

**Keywords:** chemical shift perturbation · chemical synthesis · molecular modeling · NMR spectroscopy · signal transduction

[1] A. C. Gavin, M. Bosche, R. Krause, P. Grandi, M. Marzioch, A. Bauer, J. Schultz, J. M. Rick, A. M. Michon, C. M. Cruciat, M. Remor, C. Hofert, M. Schelder, M. Brajenovic, H. Ruffner, A. Merino, K. Klein, M. Hudak, D. Dickson, T. Rudi, V. Gnau, A. Bauch, S. Bastuck, B. Huhse, C. Leutwein, M. A. Heurtier, R. R. Copley, A. Edelmann, E. Querfurth, V. Rybin, G. Drewes, M. Raida, T. Bouwmeester, P. Bork, B. Seraphin, B. Kuster, G. Neubauer, G. Superti-Furga, *Nature* **2002**, *415*, 141 – 147.
[2] P. Bork, L. J. Jensen, C. Mering, A. K. Ramani, I. Lee, E. M. Marcotte, *Curr. Opin. Struct. Biol.* **2004**, *14*, 292 – 299.
[3] A. J. Wells, C. L. McClendon, *Nature* **2007**, *450*, 1001 – 1009.
[4] M. R. Arkin, M. Randal, W. L. Delano, J. Hyde, T. N. Luong, J. D. Oslob, D. R. Raphael, L. Taylor, J. Wang, R. S. McDowell, J. A. Walls, A. C. Braisted, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 1603 – 1608.
[5] K. K. Dev, *Nat. Rev. Drug Discovery* **2004**, *3*, 1047 – 1056.
[6] P. M. Fischer, D. P. Lane, *Trends Pharmacol. Sci.* **2004**, *25*, 343 – 346.
[7] a) L. Pagliaro, J. Felding, K. Audouze, S. J. Nielsen, R. B. Terry, C. Krog-Jensen, S. Butcher, *Curr. Opin. Chem. Biol.* **2004**, *8*, 442 – 449; b) A. G. Cochran, *Curr. Opin. Chem. Biol.* **2001**, *5*, 654 – 659; c) H. Yin, A. D. Hamilton, *Angew. Chem. Int. Ed.* **2005**, *44*, 4130 – 4163; *Angew. Chem.* **2005**, *117*, 4200 – 4235.
[8] a) R. Prasad, Y. Gu, H. Alder, T. Nakamura, O. Canaani, H. Saito, K. Huebner, R. P. Gale, P. C. Nowell, K. Kuriyama, Y. Miyazaki, C. M. Croce, E. Canaani, *Cancer Res.* **1993**, *53*, 5624 – 5628; b) A. B. Zhadanov, D. W. J. Provance, C. A. Speer, J. D. Coffin, D. Goss, J. A. Blixt, C. M. Reichert, J. A. Mercer, *Curr. Biol.* **1999**, *9*, 880 – 888; c) T. Yamamoto, N. Harada, K. Kano, S. Taya, E. Canaani, Y. Matsuura, A. Mizoguchi, C. Ide, K. Kaibuchi, Y. Miyazaki, C. M. Croce, E. Canaani, *J. Cell Biol.* **1997**, *144*, 361 – 371.
[9] a) B. Z. Harris, W. A. Lim, *J. Cell Sci.* **2001**, *114*, 3219 – 3231; b) M. Sheng, C. Sala, *Annu. Rev. Neurosci.* **2001**, *24*, 1 – 29.
[10] H. Lee, N. X. Wang, D. Shi, J. J. Zheng, *Angew. Chem. Int. Ed.* **2009**, *48*, 6448 – 6452; *Angew. Chem.* **2009**, *121*, 6570 – 6574.
[11] T. S. Thorsen, K. L. Madsen, N. Rebola, M. Rathje, V. Anggono, A. Bach, I. S. Moreira, N. Stuhr-Hansen, T. Dyhring, D. Peters, T. Beuming, R. Huganir, H. Weinstein, C. Mulle, K. Stromgaard, L. C. B. Ronn, U. Gether, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 413 – 418.
[12] A. B. Zhadanov, D. W. J. Provance, C. A. Speer, J. D. Coffin, D. Goss, J. A. Blixt, C. M. Reichert, J. A. Mercer, *Curr. Biol.* **1999**, *9*, 880 – 888.
[13] G. Radziwill, R. A. Erdmann, U. Margelisch, K. Moelling, *Mol. Cell. Biol.* **2003**, *23*, 4663 – 4672.
[14] B. Hock, B. Bohme, T. Karn, T. Yamamoto, K. Kaibuchi, U. Holtrich, S. Holland, T. Pawson, H. Rubsamen-Waigmann, K. Strebhardt, *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 9779 – 9784.
[15] G. Radziwill, A. Weiss, J. Heinrich, M. Baumgartner, P. Boisguerin, K. Owada, *EMBO J.* **2007**, *26*, 2633 – 2644.
[16] M. Popovic, J. Bella, V. Zlatev, V. Hodnik, G. Anderluh, P. N. Barlow, A. Pintar, S. Pongor, *J. Mol. Recognit.* **2011**, *24*, 245 – 253.
[17] X. Li, B. D. Lynn, J. I. Nagy, *Eur. J. Neurosci.* **2012**, *35*, 166 – 181.
[18] a) G. Udugamasooriya, D. Saro, M. R. Spaller, *Org. Lett.* **2005**, *7*, 1203 – 1206; b) M. Paduch, M. Biernat, P. Stefanowicz, Z. S. Derewenda, Z. Szewczuk, J. Otlewski, *ChemBioChem* **2007**, *8*, 443 – 452.
[19] M. Joshi, C. Vargas, P. Boisguerin, A. Diehl, G. Krause, P. Schmieder, K. Moelling, V. Hagen, M. Schade, H. Oschkinat, *Angew. Chem. Int. Ed.* **2006**, *45*, 3790 – 3795; *Angew. Chem.* **2006**, *118*, 3874 – 3879.
[20] U. Wiedemann, P. Boisguerin, R. Leben, D. Leitner, G. Krause, K. Moelling, R. Volkmer-Engert, H. Oschkinat, *J. Mol. Biol.* **2004**, *343*, 703 – 718.
[21] Q. Chen, X. Niu, Y. Xu, J. Wu, Y. Shi, *Protein Sci.* **2007**, *16*, 1053 – 1062.
[22] M. Marzec, M. Kasprzycka, X. Liu, P. N. Raghunath, P. Wlodarski, M. A. Wasik, *Oncogene* **2007**, *26*, 813 – 821.
[23] M. Lorger, K. Moelling, *J. Cell Sci.* **2006**, *119*, 3385 – 3398.

Received: December 23, 2013
Revised: February 13, 2014
Published online on March 25, 2014