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Rational design of the first small-molecule antagonists of NHERF1/EBP50 PDZ domains

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Abstract—This report describes the first small-molecule antagonists that specifically target the ligand-binding pocket of PDZ domains of NHERF1 multi-functional adaptor protein. Comparison of the peptide sequence homology between the native ligand of NHERF1 PDZ domains and an indole-based non-peptide chemical scaffold allowed the design of a small-molecule antagonist of NHERF1 PDZ domains.

Na^+/H^+ exchanger regulatory factor 1 (NHERF1), also known as ERM-binding phosphoprotein 50 (EBP50), is a protein that possesses two postsynaptic density 95/disc-large/zona occludens-1 (PDZ) domains that recruit membrane receptors, transporters, and cytoplasmic signaling proteins to coordinate diverse functions.[1] NHERF1 is thought to be a crucial component for recycling and sorting of several receptors, ion channels, and transporters. NHERF1 expression is altered in several cancers.[2] Its role in mammary carcinogenesis, which is dependent on its PDZ domains, is controversial, as it appears to be either tumor-promoting[3,4] or tumor-suppressing,[5–7] depending on the cellular context. One way to define this role would be to observe the effect of inhibition of NHERF1 function by a chemical antagonist.

The NHERF1 PDZ1 domain binds to the PDZ binding motifs on the extra carboxyl termini of β2-adrenergic receptor (β2AR),[8] cystic fibrosis transmembrane conductance regulator (CFTR),[9,10] platelet-derived growth factor receptor (PDGFR),[11] and epidermal growth factor receptor (EGFR).[12] The peptide sequences of these NHERF1 PDZ1-binding motifs share the D(−3)-T/S(−2)-X(−1)-L(0) motif. Those amino acid side chains are known to be important in the interaction of NHERF1 PDZ1.[13–15] The L(0) side chain inserts into a deep hydrophobic cavity formed by Tyr24 and Phe26, and the hydroxyl group of the T/S(−2) interacts to His72. In addition to those two interactions, which are common in class I PDZ domains, the NHERF1 PDZ1 domain forms a strong salt bridge with the D(−3) by surrounding it with His27 and Arg40. These three interactions should be considered in designing a specific antagonist for the NHERF1 PDZ1 domain.

We have reported indole-based chemical scaffolds that target PDZ domains, including MAGI3 PDZ2 and Dishevelled PDZ.[16–19] Those scaffolds have been designed to mimic the tetrapeptide sequence of the beta-strand of PDZ domain ligands. An important feature of the chemistry is the feasibility of generating diverse libraries that are highly variable on the indole core in order to discover class- and domain-selective inhibitors. This scaffold offers several opportunities for optimization toward targeting specific PDZ domains. We postulated that introduction of a carboxylic acid moiety on the side chain substituted on the indole-2-position would mimic the D(−3) in the PDZ1 ligands and make the molecule fit between His27 and Arg40 of the NHERF1 PDZ1, and designed analogs of indole-2-carbinol (**1**–**2**),

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-2-amide (**3**–**4**), and -3-carbinol (**5**–**6**), to verify this hypothesis (Fig. 1).

To evaluate our hypothesis regarding chemical modification of the substituent at the indole-2-position, we initiated a docking study with compound **2** based on a crystal structure of the PDZ1 domain bound to the NDSLL pentapeptide.[15] Our docking experiments with **2** indicate that critical interactions with the β-strand conformation of the bound peptide ligand in the crystal structure are retained (Fig. 2A). The phenethyl group at the indole-3-position occupies the large hydrophobic pocket directed toward Val59, Val62, and Leu88. Specifically, the carboxylate at the indole-2-position interacts with His27 and Arg40 of the PDZ1 domain via the formation of salt bridges similar to that seen with D(−3) of the peptide ligand. Furthermore, the hydrogen bonding seen between S(−2) of the peptide ligand and His72 of the PDZ1 domain is mimicked through the alcoholic moiety of the same side chain (Fig. 2A and B).

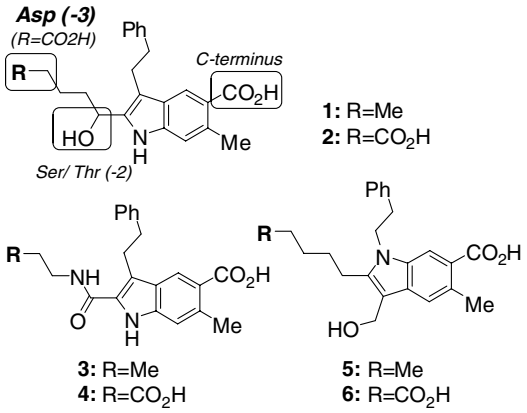

Figure 1. Design of novel scaffolds mimicking the side-chain presentation of the tetrapeptide sequence of the PDZ domain ligand.

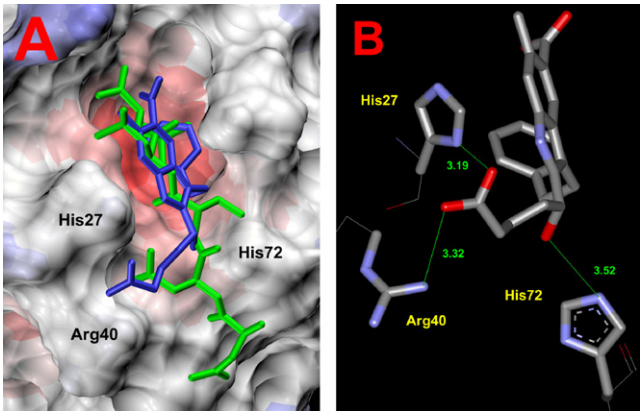

Figure 2. DOCK fitting of **2** to the NHERF1 PDZ1 domain (PDB: 1GQ4[15]). (A) Designed compound **2** (blue) fits to the PDZ1 domain by hydrophobic interaction and salt bridge formation (see text), similar to the NDSLL peptide ligand (green). Hydrogens are omitted for clarifying. (B) Superimposition of the interaction network on the indole-2-substituent. Numbers (green) represent the distance (angstrom) between nitrogen and oxygen.

Preparation of these compounds proceeded smoothly (Fig. 3). Iodoaniline **7** underwent palladium-catalyzed coupling with a silylalkyne to generate 2-silylindole **8**, which was acylated under mild conditions to produce 2-ketoindole **9**. Reduction of the 2-keto group and ester hydrolysis yielded **2**. Alternatively, indole-2-carboxylic acid **10** was generated by coupling **7** with a pyruvic acid derivative **13**. Acid **10** was coupled with amines to derive **11**–**12**, which were converted to **3**–**4**. Preparation of compounds **1**, **5**, and **6** has been reported previously.[17–19] All compounds for assays were prepared as sodium salts and were dissolved in water without DMSO.

We conducted AlphaScreen energy transfer assays[19,20] to evaluate the potency of these compounds as biochemical antagonists of the PDZ1 domain interactions of NHERF1. The signal generated by the interaction of a biotinylated peptide derived from the carboxy-terminal sequence of β2AR and GST-fused human NHERF1 PDZ1 domain was used to measure the antagonism activity of the compounds. Reduction of the signal level with increasing concentration of compound signified antagonism. Comparison of pairs of the same scaffold with and without the carboxylic acid on the indole-2-position (i.e., R=Me vs CO_2H in Fig. 1) showed that the carboxylic acid moiety improves the potency of antagonism in each scaffold (Table 1). The weak potency in the

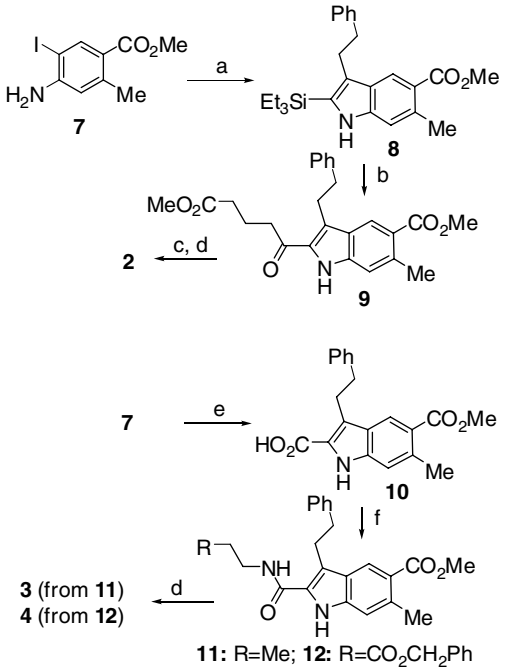

Figure 3. Preparation of NHERF1 PDZ-domain antagonists. (**a**) $PhCH_2CH_2C \equiv CSiEt_3$ (1.5 equiv), $Pd(OAc)_2$ (15 mol%), Na_2CO_3 (5 equiv), DMF, 100 °C, 5 h. Yield: 76%; (**b**) $MeO_2C(CH_2)_3COCl$ (3 equiv), $ZnCl_2$ in Et_2O (1.5 equiv), CH_2Cl_2, 0 °C, 5 h. Yield: 74%; (**c**) $NaBH_4$ (excess), MeOH, 5 °C; (**d**) NaOH, H_2O, MeOH, 65 °C, 12 h. Yield: 92% (**2**) for two steps, 20% (**3**), 35% (**4**); (**e**) $Ph(CH_2)_3COCO_2H$ (**13**, 5 equiv), $Pd(OAc)_2$ (15 mol%), DABCO (5 equiv), DMF, 100 °C, 18 h. Yield: 64%; (**f**) *n*-$PrNH_2$ (for **11**) or $PhCH_2O_2C(CH_2)_2NH_2$.p-tosylate (for **12**) (3 equiv), HBTU (2.5 eq.), DIPEA (3 equiv), DMF, room temperature, 18 h. Yield: 89% (**3**), 45% (**4**).

Table 1. IC_{50} (μM) of the binding of NHERF1 PDZ domains to their cognate ligands measured by AlphaScreen assay

Compound	GST-PDZ1: β2AR[a]	GST-PDZ2: CFTR[b]
1	1540	160
2	777	330
3	NA[c]	NA[c]
4	820	490
5	280	380
6	15	170

[a] Biotin-SQGRNCSTNDSLL.
[b] Biotin-KEETEEEVQDTRL.
[c] NA, No competition activity was observed up to 300 μM.

indole-2-carbinol and -2-amide scaffolds may be due to the position of the hydroxyl group that mimics the S(−2) not being enough appropriately positioned (**1**–**2**), or replacement of the hydroxyl group with an amide (**3**–**4**). Actually, distance of hydrogen bonds observed in between His27/ Arg40/ His72 of the PDZ1 and carboxylate/ hydroxyl groups of **2** (3.19, 3.32, 3.52 Å, respectively; Fig. 2B) is slightly longer than that of the NDSLL peptide (2.71, 2.86, 2.52 Å, respectively[13]). On the other hand, indole-3-carbinol (**5**–**6**) showed not only the greatest potency but also the greatest comparative improvement (Fig. 4). This may be due to the proper orientation of the 3-carbinol group to make better replacement of the hydroxyl group of S(−2), that forms essential interaction of the NDSLL peptide to the PDZ1 domain, in irreversible manner.[17]

The NHERF1 PDZ2 binds to CFTR[10] and β-catenin,[3] which possesses the D-T-X-L motif, but also binds to ligands that have amino acid residues other than D(-3), such as parathyroid hormone receptor (PTH1R, -E-T-V-M)[21] and Yes-associated protein (YAP)-65 (-L-T-W-L).[22] Therefore, we anticipated that the binding preference of the PDZ2 domain would be less specific to ligand possessing the carboxylic acid corresponding to D(-3). We used a similar AlphaScreen protocol to assay competition against the interaction of a biotinylated peptide derived from the carboxy-terminal sequence of CFTR and GST-fused human NHERF1 PDZ2 domain (Table 1). As expected, the PDZ2 domain showed less significant preference for compounds with the carboxylic acid at the indole-2-position (**2**, **4**, **6**).

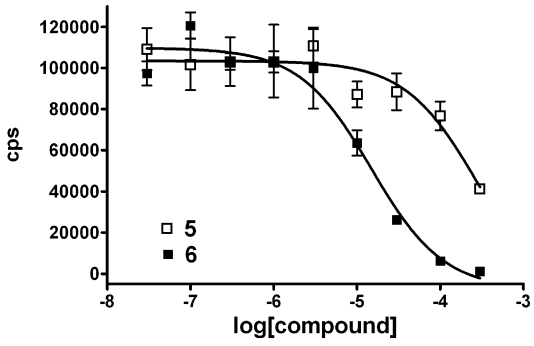

Figure 4. The effects of the compounds' carboxylic acid moiety of the indole-3-carbinol compound on the potency of their biochemical antagonism. The GST-fused NHERF1 PDZ1 domain and biotin-SQGRNCSTNDSLL peptide (β2AR carboxy-terminal sequence) were allowed to equilibrate with the test compounds and were then incubated for 30 and 45 min with anti-GST and streptavidin beads, respectively, to titrate inhibition of PDZ1 domain ligand binding as measured by AlphaScreen.

In summary, we have created the first non-peptide small-molecule antagonist of NHERF1 PDZ domain interactions by incorporating an extra carboxylic acid on the 2-position of the indole carboxylate scaffolds that we had utilized to design antagonists for MAGI3 PDZ2[16,17] and Dishevelled PDZ[19] domains. Among the three indoles, the indole-3-carbinol scaffold offered the most potent antagonism. These results suggest that incorporation of functional groups to mimic native ligands for each PDZ domain is a promising strategy to discover new antagonists of these domains with increased potency and selectivity. This method also offers new small-molecule tools to investigate the pharmacological function of these PDZ domains. Pharmacological studies using these compounds are under way and will be reported in due course.

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Supplementary data

DOCK modeling method, chemical syntheses procedure, analysis data, protein preparation, and protocols for AlphaScreen biochemical assays associated with this article can be found in the online version. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.12.038.

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