ACS Medicinal Chemistry Letters

Letter

Drug Design and Synthesis of First in Class PDZ1 targeting NHERF1 Inhibitors as Anticancer Agents

Antonio Coluccia, Giuseppe La Regina, Valentina Naccarato, Marianna Nalli, viviana orlando, Stefano Biagioni, Maria Laura De Angelis, Marta Baiocchi, Candice Gautier, Stefano Gianni, Fiorella Di Pastena, Laura Di Magno, Gianluca Canettieri, Addolorata Maria Luce Coluccia, and Romano Silvestri

Just Accepted

ACS Publications

Drug Design and Synthesis of First in Class PDZ1 targeting NHERF1 Inhibitors as Anticancer Agents

Antonio Coluccia,[†] Giuseppe La Regina,[†] Valentina Naccarato,[†] Marianna Nalli,[†] Viviana Orlando,[fi] Stefano Biagioni,[fi] Maria Laura De Angelis,[ß] Marta Baiocchi,[ß] Candice Gautier,[§] Stefano Gianni,[§] Fiorella Di Pastena,[∞] Laura Di Magno,[¢] Gianluca Canettieri,[∞] Addolorata Maria Luce Coluccia,[‡] Romano Silvestri[†,*]

[†]Department of Drug Chemistry and Technologies, Sapienza University of Rome, Laboratory affiliated to Istituto Pasteur Italia – Fondazione Cenci Bolognetti, Piazzale Aldo Moro 5, I-00185 Roma, Italy

[∞]Department of Molecular Medicine, Sapienza University of Rome, Viale Regina Elena, 291, I-00161 Roma, Italy, Laboratory affiliated to Istituto Pasteur Italia – Fondazione Cenci Bolognetti, Piazzale Aldo Moro 5, I-00185 Roma, Italy

[‡]Department of Biological and Environmental Sciences and Technologies, University of Salento, I-73100 Lecce, Italy

[§]Department of Biochemistry, Sapienza University of Rome, Laboratory affiliated to Istituto Pasteur Italia – Fondazione Cenci Bolognetti, Piazzale Aldo Moro 5, I-00185 Roma, Italy

[ß]Department of Oncology and Molecular Medicine, Istituto Superiore di Sanità, Viale Regina Elena, 299, 00161 Rome, Italy

[fi]Department of Biology and Biotechnologies "Charles Darwin", Piazzale Aldo Moro 5, I-00185 Roma, Italy

[¢]Center for Life Nano Science@Sapienza, Istituto Italiano di Tecnologia, 00161 Rome, Italy

KEYWORDS: Cancer, NHERF1, PDZ domain, β-Catenin, drug design, synthesis

ABSTRACT: Targeted approaches aiming at modulating NHERF1 activity, rather than its overall expression, would be preferred to preserve the normal functions of this versatile protein. We focused our attention on the NHERF1/PDZ1 domain that governs its membrane recruitment/displacement through a transient phosphorylation switch. We herein report the design and synthesis of novel NHERF1 PDZ1 domain inhibitors. These compounds deserve potential therapeutic value when used in combination with antagonists of β-Catenin to augment apoptotic death of colorectal cancer cells refractory to currently available Wnt/β-Catenin-targeted agents.

NHERF1 (Na$^+$/H$^+$ exchanger 3 regulating factor 1) is an integral membrane adaptor protein of 358 amino acids carrying two NH$_2$-terminal PDZ (post-synaptic density 95/discs large/zona occludens 1) tandem domains.[1] PDZ1 (11-97 amino acids) and PDZ2 (150-237 amino acids) show 74% identity to each other and bind to specific carboxyl-terminal motifs on target proteins, such as β-Catenin and PTEN, that may have a pivotal role in tumorigenesis. Oncogenic activity of NHERF1 is strictly dictated by changes on its subcellular localization.[1,2] Whilst a loss of its physiologic membranous expression usually occurs in early dysplastic human adenomas, especially those of colon and breast, NHERF1 is progressively re-expressed throughout the cytoplasm and nuclei in highly invasive/metastatic carcinomas. Recent evidence also earmarked NHERF1 as a major driver of a cytoprotective autophagic responses elicited by therapeutic inhibition of the Wnt/β-Catenin signaling cascade which triggers colorectal cancers (CRC).[3] Notably, these studies have started to explore a combined β-Catenin/NHERF1-inhibitory strategy as a fruitful approach to augment apoptotic death of CRC cells refractory to currently

available Wnt-targeted agents, in particular at early stages of the disease

Previous studies focused on small molecule ligands of the PDZ1-domain of NHERF1 to prevent its ectopic cytoplasmic/nuclear *mis*localization and oncogenic function.[4-6] These studies predicted the canonical inactivating binding motif for the NHERF1 PDZ1-domain on four D/E-(S/T)-X-(L/V/I/M) residues that are commonly contained at the C-terminal of its specific ligands, such as DSLL for the β2-adrenergic receptor (β2-AR) or ETWM for the parathyroid hormone receptor (PTHR).[7]

The crystal structure of the PDZ1-β2-AR C-terminal peptide is available at the protein data bank (PDB code 1GQ4).[8] The complex was fixed cutting the chimeric covalent bond between PDZ1 and the substrate (-NDSLL) and submitted to molecular dynamics (MD) simulations to obtain the reasonable binding conformations. MD of the complex PDZ1-PTH1R C-terminal peptide (WETVM) was also performed (Figures 1S and 2S, Supporting Information). Trajectory analyses showed that the systems were stable during simulation time of 50 ns, with an

average PDZ1 Ca RMSD of 1.66 and 1.73 Å for PDZ1-NDSLL-COOH and PDZ1-WETVM-COOH respectively. The main interactions between the PDZ1 and the -β2-AR substrate observed at the crystal structures were retained during the MD simulations (Supporting Information). The trajectories were clustered by the RMSDs of the binding site residues giving 5 clusters for the two studied complexes, each containing representative structures. The obtained ten structures guided the design of a pharmacophore model (Figure 3S, Supporting Information) that was used to filter out an in-house training set of about 6000 compounds. One out of six selected compounds showed significant NHERF1 inhibition.[3] We herein report the design and synthesis of new NHERF1 inhibitors **1-14**. In preliminary docking studies, these compounds matched the requirements of the pharmacophore model, in particular the interactions at the t-COOH binding pocket.[9]
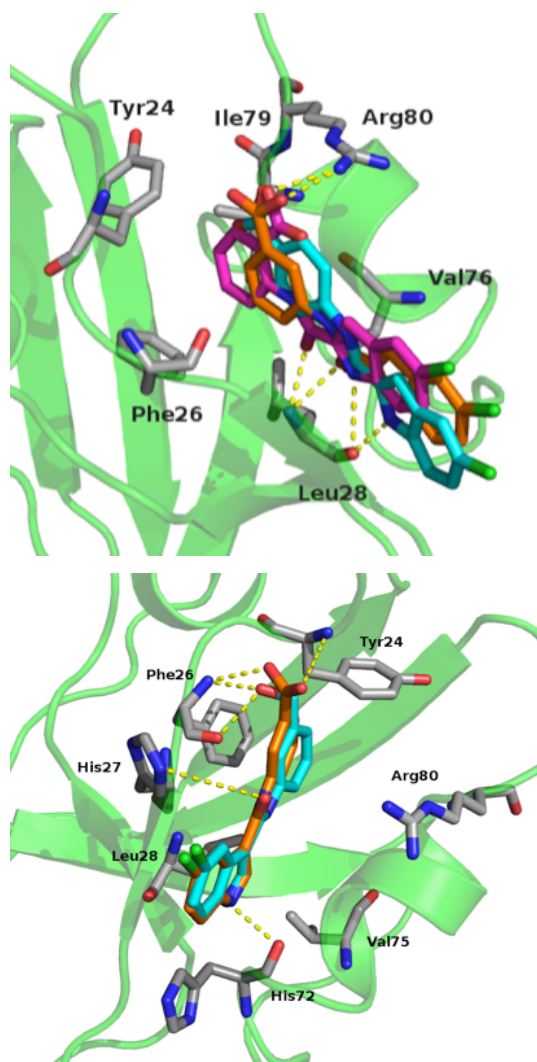

Figure 1. Proposed binding modes. Top panel: derivatives **5** (cyan), **6** (orange) and **7** (magenta); bottom panel: derivatives **9** (cyan) and **12** (orange). Residues involved in interactions are reported as stick. PDZ1 is reported as cartoon (green). The H-bonds are depicted as yellow dot lines.

Table 1. Structure and Activity of Compounds 1-14 in Ls174Tshβ Cat Cells Expressing Low (-DOX) or High (+DOX) Levels of the Protein Target NHERF1.[a]

Compd	R	Ls174Tsh IC$_{50}$ (µM)		
		-Dox	+Dox	Δ
1		62 ± 4	24 ± 4	38
2		66 ± 2	25 ± 2	41
3		65 ± 2	24 ± 5	41
4		62 ± 2	22 ± 3	40
5		74 ± 6	12 ± 4	62
6		66 ± 4	25 ± 2	41
7		64 ± 8	22 ± 6	42
8		86 ± 24	55 ± 21	31
9		82 ± 4	8 ± 4	74
10		84 ± 4	5 ± 2	79
11		65 ± 6	22 ± 2	43
12		66 ± 2	25 ± 2	41
13		85 ± 2	10 ± 2	75
14		68 ± 4	25 ± 2	43

[a]Ls174T cells stably transfected with Dox-inducible shRNA for β-Catenin (Ls174Tshβ-Cat) were cultured without or with 2 µg/mL of Dox (-Dox/+Dox) for 1, 3 or 5 days.

Compounds **1-14** were synthesized starting from 5-chloro-1*H*-indole-2-carboxylic acid (**18**) or its 3-isomer **19** (Scheme 1, Supporting Information). The acids were transformed into the corresponding acyl chlorides with thionyl chloride and coupled with an appropriate aminobenzoate in dichloromethane in the presence of pyridine to afford carboxamides **20-24**. Hydrolysis of the ester functionality of **20-24** was performed with lithium hydroxide monohydrate in tetrahydrofuran (**5** and **6**) or with 3N sodium hydroxide in ethanol at 25 °C (**7**, **11** and **12**). Alcohol derivatives **1**, **3**, **4** and **8-10** were obtained by tetrabutylammonium fluoride cleavage in tetrahydrofuran at 25 °C of the corresponding *tert*-butyldimethylsilyl derivatives **25-30**. The latter compound were prepared by reaction of **18** or **19** with *tert*-butyl

dimethylsilyloxymethylanilines **31-33**. Carboxamides **2**, **13**, **25-30** and **34** were synthesized from the corresponding anilines in the presence of tripyrrolidinophosphonium hexafluorophosphate (PyBOP) and trietylamine in DMF at 25 °C. Compound **14** was obtained after lithium hydroxide of the intermediate methyl tyrosinate **34**.

The ability of **1-14** to inhibit NHERF1 was assessed in Ls174T CRC cells harboring a Doxycycline hyclate (Dox)-inducible small hairpin RNA (shRNA) for β-Catenin (Ls174Tshβ-Cat) (Table 1). Our published data indicated that such CRC cells exhibited low/undetectable NHERF1 levels at baseline (*i.e.* in the absence of Dox), whilst they markedly increase the expression of the protein as well as a NHERF1-mediated survival response under Dox treatment.[3] The relative activity of **1-14** can be esplicited by Δ values obtained by subtracting the IC_{50} values of the Ls174Tshβ-Cat cells cultured in the presence or in the absence of Dox. Indeed, NHERF1/PDZ-1 ligand inhibitors are expected to exert their growth-inhibitory effects only on *β*-Catenin-depleted CRC cells which highly re-expressed the protein target NHERF1, thus showing the highest Δ values with regard to Dox-untreated cells. Introduction of a methylene spacer between the 2-carboxamide function and the phenyl group of **1** (-Dox IC_{50} = 62 µM; +Dox IC_{50} = 24 µM) to afford **4** gave a slight improvement of activity. Moving the hydroxymethyl group of **1** from *para* to *meta* (**3**) or *orto* (**4**) only minimally affected the difference (Δ) of activity between the two cell lines. Replacement of the hydroxymethyl of **1** with a carboxylic acid function (**5**) (-Dox IC_{50} = 74 µM; +Dox IC_{50} = 12 µM) improved the Δ value from 38 to 62, while the corresponding *meta* (**6**) or *orto* (**7**) carboxylic acids gave similar results to **2-4**. We synthesized the corresponding analogs **8-14** at position 3 of the indole. Derivatives **9** (-Dox IC_{50} = 82 µM; +Dox IC_{50} = 8 µM) and **10** (-Dox IC_{50} = 84 µM; +Dox IC_{50} = 5 µM) bearing the hydroxymethyl at position *meta* and *orto* (Δ of 74 and 79 respectively) were superior to the *para* analogue **7** as well as to the carboxylic acids **11** and **12**. The 3-indolecarboxamide **13** (-Dox IC_{50} = 85 µM; +Dox IC_{50} = 10 µM) yielded a pleasant Δ value of 75, that was 1.8 fold superior to the isomeric derivative **1**. In sum, compounds **5**, **9**, **10** and **13** exhibited a remarkable cytotoxicity and the highest Δ values on Ls174Tshβ-Cat which highly re-express the specific protein target NHERF1.

The indole NH and the carboxamide oxygen formed H-bonds with the Leu27 backbone. Hydrophobic contacts were observed between the phenyl ring and Tyr24, Phe26 and Ile78, as well as between the indole ring and Val75 and Val76. Noteworthy, the carboxylic group of derivatives **5-7** formed polar contacts with the Arg80 (Figure 1, top panel). Derivatives **8-14** showed binding modes with different patterns in H-bonding. In comparing the alcohol **9** and its corresponding carboxylic acid **12**, the indole NH formed an H-bond with His72 backbone, the amide oxygen atom formed an H-bond with His27 side chain, the indole ring established hydrophobic contacts with Leu28 and Val75, the phenyl ring arranged hydrophobic contacts with Tyr24 and Phe26 and a pi-cation interaction with the guanidine moiety of Arg80. The alcohol and the carboxyl moieties

were involved in H-bond/polar contact with the terminal COOH binding pocket (Figure 1, bottom panel).

The binding of **9**, **10** and **13** to NHERF1 PDZ1 was assessed by means of a dansylated peptide corresponding to the C-terminal sequence of β2-AR (DNDSLL) using a a fluorescent pseudo-wild type produced by replacing Tyr38 of PDZ1 with a Trp.[9] The presence of a constant concentration of **9**, **10** or **13** abolished the binding C-terminal sequence of β2-AR with an affinity of about 10 µM, thus validating these compounds as specific inhibitors of NHERF1/PDZ1 domain (Figure 2). To further test the ability of **9**, **10** and **13** to bind NHERF1 PDZ1, we performed urea-induced denaturation in the presence of the inhibitors. In fact, the ability to bind the native, rather than the denatured, state of the protein induces a stabilization of the domain. Such a stabilization is correlated to the affinity of the compounds to PDZ1 NHERF1 (Figure 4S, Supporting Information). The results provide compelling evidence of the inhibition of NHERF1 PDZ1 by derivatives **9**, **10** and **13.**
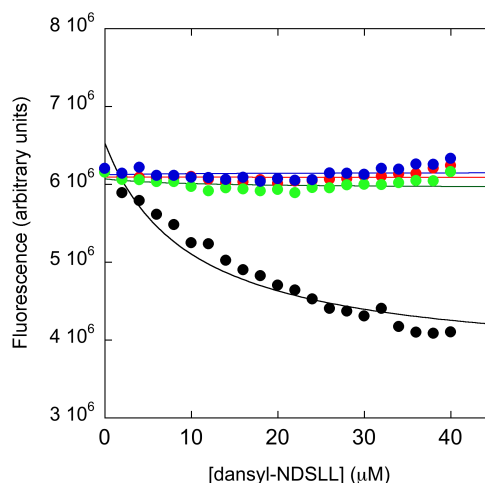

Figure 2. Binding of PDZ1 NHERF1 Y38W to the ligand Dansyl-NDSLL in the presence (black circles) and in the absence of 5 µM of **9** (green), **10** (red) or **13** (blue). Fluorescence data were recorded in the presence of 50 mM Na phosphate pH 7.2, 300 mM NaCl, 5 mM DTT, 20% DMSO, at 25 °C. Lines are the best fir to a hyperbolic binding transition. The binding between PDZ1 NHERF1 Y38W to the ligand Dansyl-NDSLL was abolished in the presence of **9**, **10** or **13**.

Table 2. Cell Growth Inhibition of DLD-1, SW480 and SW620 Cell Lines by Compounds 5, 9, 10 and 13.[a]

Compd	IC_{50} (µM)		
	DLD-1	SW480	SW620
5	68 ± 1	17 ± 1	22 ± 1
9	82 ± 2	13 ± 1	27 ± 1
10	85 ± 2	15 ± 1	30 ± 1
13	84 ± 2	16 ± 1	31 ± 1

[a]Cytotoxic concentrations for the indicated cell lines. Experiments were performed in triplicate and repeated at least twice.

Derivatives **5**, **9**, **10** and **13** with the highest Δ values as inhibitors of Ls174Tshβ-Cat cells were also tested in three

CRC cell lines, such as DLD-1, SW480 and SW620. It is known that NHERF1 is expressed at detectable baseline levels in SW480 and SW620 but not in DLD-1 cells.[3] This relatively explains why DLD-1 cells showed the highest IC_{50} values compared to SW480 and SW620 cells (Table 2), at micromolar concentrations quite similar to those previously obtained in Dox-untreated Ls174Tshβ-Cat cells (Table 1) (Additional cancer cell lines are reported in Table 1S, Supporting Information).

We previously reported that 3-benzyl-5-chloro-*N*-(4-(hydroxymethyl)-phenyl)-1Hindole-2-carboxamide (**15**), a NHERF1 PDZ1 inhibitor with -Dox IC_{50} = 52 μM; +Dox IC_{50} = 8 μM, synergized *N*-(2-methyl-4-nitrophenyl)-2,5-dichlorobenzen-sulfonamide (FH535, **16**) and pyrvinium pamoate inhibitor of β-Catenin-dependent transcription in triggering massive apoptosis of Ls174T and DLD1 CRC cells, thereby preventing persistent ERK1/2 phospho-activation mediated by single β-Catenin targeting.[3,10,11] We therefore speculated the potential benefit of a synergic activity of NHERF1 PDZ1 and β-Catenin inhibitors in CRC therapy.

Table 3. Synergistic Cell Growth Inhibition of SW480 and SW620 Cell Lines by Compounds 10, 15, 16 and 17.[a,b]

Compd	Inhibitor	IC_{50} (μM)		
		DLD-1	SW480	SW620
10	NHERF1	85 ± 2	15 ± 1	30 ± 1
15	NHERF1	65 ±11	8 ± 1	13 ± 2
16	β-Cat	28 ± 5	10 ± 2	19 ± 1
17	β-Cat	21 ± 2	8 ± 2	17 ± 1
10 + 16	NHERF1 + β-Cat	0.8 ± 2	3.0 ± 2	22 ± 2
10 + 17	NHERF1 + β-Cat	0.1 ± 2	0.3 ± 2	12 ± 2
15 + 16	NHERF1 + β-Cat	0.5 ± 3	0.9 ± 2	18 ± 1
15 + 17	NHERF1 + β-Cat	0.1 ± 2	0.1 ± 2	0.5 ± 2

[a]Cytotoxic concentrations for the indicated cell lines. Experiments were performed in triplicate repeated at least twice. [b]Compound combinations were in 1:1 ratio.

We compared **10**, the compound with the highest Δ value within this series, and **15**,[3] with two *β*-Catenin inhibitors, FH535 (**16**),[12] and **17** (Chart 1S, Supporting Information). We tested the effect of increasing concentrations of **17** on the Wnt signaling pathway, by performing Wnt reporter assays in 293T cells. We transfected TOPFlash (TOP) reporter plasmid, containing 8 repeats of TCF/LEF binding site or FOPFlash (FOP) plasmid, where the TFC/LEF binding sites were mutated as negative control.[13] Wnt pathway was activated using the GSK3beta inhibitor LiCl. **17** inhibited the activity of TOP reporter, with an IC_{50} of 14.1 μM (Figure 5S, Supporting Information). Conversely, this compound failed to prevent the activity of FOP reporter, indicating the specificity of the inhibition of the Wnt pathway.

Indeed, compound 1:1 combination of a NHERF1 inhibitor with a β-Catenin inhibitor significantly improved the growth inhibition of all CRC cells. All combinations were superior to each single tested compound in DLD-1 and SW480 cells. Compound combinations **10+17**, **15+16** and **15+17** inhibited the SW480 cells at submicromolar

concentration, the most potent combination was **15+17** (IC_{50} = 0.1 μM). Combinations **10+17** and **15+17** showed the higher effectiveness, **15+17** was the most potent with IC_{50} values of 0.1 and 0.5 μM (Table 3).

Cancer stem cells (CSCs) are receiving growing interest during the last years, because they have proved to be selectively resistant to chemotherapics, thus giving an explanation to the relapse that in most cases follows fast tumor shrinkage induced by standard therapies. In this frame, the identification of targeted drugs active on CSCs may give hope of a full tumor eradication. Preliminary screening of two APC-mutated CSCs indicated that their sensitivity to compounds **10** and **17** was broadly comparable to that of standard cell lines (Table 4). Analysis on a wider panel of CSCs is ongoing, in order to verify a possible role of different mutational profiles of individual CSCs on drug sensitivity.

Table 4. Cell Growth Inhibition of CSC13 and CSC8 Cancer Stem Cell Lines by Compounds 10 and 17.[a]

Compd	Inhibitor	IC_{50} (μM)	
		CSC13	CSC8
10	NHERF1	12 ± 1	8 ± 1
17	β-Cat	22 ± 2	13 ± 2

[a]Cytotoxic concentrations for the indicated CSCs. Experiments were performed in triplicate repeated at least twice.

Since physiological or pathological roles of NHERF1 are related to its subcellular localization, targeted approaches aiming at modulating NHERF1 activity, rather than its overall expression, would be preferred to preserve the normal functions of this versatile protein. By far, particular attention has been paid to the NHERF1/PDZ1-domain that governs its membrane recruitment/displacement through a transient phosphorylation switch. We herein characterized novel NHERF1 PDZ1 domain inhibitors with therapeutic value when used in combination with antagonists of β-Catenin. This double targeted strategy deserves potential to augment apoptotic death of CRC cells refractory to currently available Wnt-targeted agents, particularly at early stages of the disease.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.

Additional information for MD simulations and binding modes, experimental procedures of synthesis, computational studies, NHERF1 PDZ1 binding studies, Wnt reporter assay, cell cultures and biological assays, additional cancer cell growth inhibition".

AUTHOR INFORMATION

Corresponding Author

*Phone: +39 06 4991 3800. Fax: +39 06 4991 3133. E-mail: romano.silvestri@uniroma1.it.

Author Contributions

Chemistry: A.C., computational studies; G.L.R., V.N. and M.N., chemical synthesis. Biology: V.O., cancer cells; M.B., cancer stem cells; C.G. and S.G., PDZ domain; F.D.P., L.D.M. and G.C., β-Catenin; A.M.L.C., NHERF1.

Funding Sources

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

ABBREVIATIONS

NHERF1, Na^+/H^+ exchanger 3 regulating factor 1; PDZ, post-synaptic density 95/discs large/zona occludens 1; CRC, colorectal cancer; Dox, doxycycline.

REFERENCES

(1) Vaquero, J.; Nguyen Ho-Bouldoires T. H.; Claperon, A.; Fouassier, L. Role of the PDZ scaffold protein NHERF1/EBP50 in cancer biology: from signaling regulation to clinical relevance. *Oncogene* **2017**, *36*, 3067-3079.

(2) Georgescu, M. M.; Morales, F. C.; Molina, J. R.; Hayashi, Y. Roles of NHERF1/EBP50 in cancer. *Curr. Mol. Med.* **2008**, *8*, 459-468.

(3) Saponaro, C.; Sergio, S.; Coluccia, A.; De Luca, M.; La Regina, G.; Mologni, L.; Famiglini, V.; Naccarato, V.; Bonetti, D.; Gautier, C.; Gianni, S.; Vergara, D.; Salzet, M.; Fournier, I.; Bucci, C.; Silvestri, R.; Gambacorti Passerini, C.; Maffia, M.; Coluccia, A. M. L. AML. β-Catenin knockdown promotes NHERF1-mediated survival of colorectal cancer cells: implications for a double-targeted therapy. *Oncogene* **2018**, *37*, 3301-3316.

(4) Mayasundari, A.; Ferreira, A. M.; He, L.; Mahindroo, M.; Bashford, D.; Fujii, N. Rational design of the first small-molecule antagonists of NHERF1/EBP50 PDZ domains. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 942-945.

(5) Du, G.; Gu, Y.; Hao, C.; Yuang, Z.; He, J.; Jiang, W. G.; *et al.* The cellular distribution of Na^+/H^+ exchanger regulatory factor 1 is determined by the PDZ-I domain and regulates the malignant progression of breast cancer. *Oncotarget* **2016**, *7*, 29440-29453.

(6) Fitzpatrick, J. M.; Pellegrini, M.; Cushing, P. R.; Mierke, D. F. Small molecule inhibition of $Na(^+)/H(^+)$ exchange regulatory factor 1 and parathyroid hormone 1 receptor interaction. *Biochemistry* **2014**, *53*, 5916-5922.

(7) Mamonova, T.; Kunikova, M.; Friedman, P. A. Structural basis for NHERF1 PDZ domain binding. *Biochemistry* **2012**, *51*, 3110-3120.

(8) Karthikeyan, S.; Leung, T.; Ladias, J. A. A. Structural determinants of the Na^+/H^+ exchanger regulatory factor interaction with the beta 2 adrenergic and platelet-derived growth factor receptors. *J. Biol. Chem.* **2002**, *277*, 18973-18987.

(9) Gianni, S.; Engström, A.; Larsson, M.; Calosci, N.; Malatesta, F.; Eklund, L.; Ngang, C. C.; Travaglini-Allocatelli, C.; Jemth, P. The kinetics of PDZ domain-ligand interactions and implications for the binding mechanism. *J. Biol. Chem.* **2005**, *280*, 34805-34812.

(10) Handeli, S.; Simon, J. A. A small-molecule inhibitor of Tcf/beta-catenin signaling down-regualtes PPARγ and PPARδ activities. *Mol. Cancer Ther.* **2008**, *7*, 521-529.

(11) Thorne, C. A.; Hanson, A. J.; Schneider, J.; Tahinci, E.; Orton, D.; Cselenyi, C. S.; Jernigan, K. K.; Meyers, K. C.; Hang, B. I.; Waterson, A. G.; Kim, K.; Melancon, B.; Ghidu, V. P.; Sulikowski, G. A.; La Fleur, B.; Salic, A.; Lee, L, A.; Miller, D. M. 3rd; Lee, E. Small-molecule inhibition of Wnt signaling through activation of casein kinase 1α. *Nat. Chem. Biol.* **2010**, *6*, 829-836.

(12) Chen, Y.; Rao, X.; Huang, K.; Jiang, X.; Wang, H.; Teng, L. FH535 inhibits proliferation and motility of colon cancer cells by targeting Wnt/β-catenin signaling pathway. *J. Cancer* **2017**, *8*, 3142-3153.

(13) Veeman, M. T.; Slusarski, D. C.; Kaykas, A.; Louie, S. H.; Moon, R. T. Zebrafish prickle, a modulator of noncanonical Wnt/Fz signaling, regulates gastrulation movements. *Curr. Biol.* **2003**, *13*, 680-685.

Table of Contents Graphic