Supporting Information

Small-molecule inhibitors of the PDZ domain of Dishevelled proteins interrupt Wnt signalling

Nestor Kamdem [1,2], Yvette Roske [3], Dmytro Kovalskyy [4,6] Maxim O. Platonov [4,6], Oleksii Balinskyi [4,6], Annika Kreuchwig [1,2], Jörn Saupe [1,2], Liang Fang [2,3], Anne Diehl [1], Peter Schmieder [1], Gerd Krause [1], Jörg Rademann [1,5], Udo Heinemann [2,3], Walter Birchmeier [3] and Hartmut Oschkinat [1,2].

[1] Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Robert-Rössle-Straße 10, 13125 Berlin, Germany
[2] Institut für Chemie und Biochemie, Freie Universität Berlin, Takustraße 3, 14195 Berlin, Germany
[3] Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Straße 10, 13125 Berlin, Germany
[4] Enamine Ltd., Chervonotkatska Street 78, Kyiv 02094, Ukraine
[5] Institut für Pharmazie, Freie Universität Berlin, Königin-Luise-Straße 2 + 4, 14195 Berlin, Germany
[6] Taras Shevchenko National University, 62 Volodymyrska, Kyiv 01033, Ukraine

Corresponding Author *Mail: oschkinat@fmp-berlin.de; phone: +49 030 94793160

Leibniz-Forschungsinstitut für Molekulare Pharmakologie, Robert-Rössle-Straße 10, 13125 Berlin, Germany

Tables of contents

1. Structure-based alignment of the amino acid sequences of Dvl-1,2,3 PDZ ; PSD95-PDZ-1,2,3 ; Af-6 and Syn PDZ domains.

```
hDVL1          TVTLNMERHH FLGISIVGQS N--DRGDG-- ------GIYI GSIMKGGAVA ADGRIEPGDM
hDVL2          TVTLNMEKYN FLGISIVGQS N--ERGDG-- ------GIYI GSIMKGGAVA ADGRIEPGDM
hDVL3          TVTLNMEKYN FLGISIVGQS N--ERGDG-- ------GIYI GSIMKGGAVA ADGRIEPGDM
PSD-95 PDZ1      EITLERGN-S GLGFSIAGGT DNPHIGDDP- ------SIFI TKIIPGGAAA QDGRLRVNDS
PSD-95 PDZ2      EIKLIKGP-K GLGFSIAGGV GNQHIPGDN- ------SIYV TKIIEGGAAH KDGRLQIGDK
PSD-95 PDZ3      RIVIHRGS-T GLGFNIVGG- -----EDGE- ------GIFI SFILAGGPAD LSGELRKGDQ
hAF6          -ITVTLKKQN GMGLSIVAAK G--AGQDKL- ------GIYV KSVVKGGAAD VDGRLAAGDQ
h_alpha_Syn PDZ  RVTVRKADAG GLGISIKG-- ---GRENKM- ------PILI SKIFKGLAAD QTEALFVGDA
mShank3 PDZ      VAILQKRDHE GFGFVLRGAK AETPIEEFTP TPAFPALQYL ESVDVEGVAW RAG-LRTGDF

hDVL1          LLQVNDVNFE NMSNDDAVRV LREIVSQTGP ISLTVAKCWD PT
hDVL2          LLQVNDMNFE NMSNDDAVRV LRDIVHKPGP IVLTVAKCWD PS
hDVL3          LLQVNEINFE NMSNDDAVRV LREIVHKPGP ITLTVAKCWD PS
PSD-95 PDZ1      ILFVNEVDVR EVTHSAAVEA LKEAGS---I VRLYVMRR-- --
PSD-95 PDZ2      ILAVNSVGLE DVMHEDAVAA LKNTYD---V VYLKVAKP-- --
PSD-95 PDZ3      ILSVNGVDLR NASHEQAAIA LKNAGQ---T VTIIAQYK-- --
hAF6          LLSVDGRSLV GLSQERAAEL MTRTSS---V VTLEVAKQG- --
h_alpha_Syn PDZ  ILSVNGEDLS SATHDEAVQV LKKTGK---E VVLEVKYMK- --
mShank3 PDZ      LIEVNGVNVV KVGHKQVVGL IRQGGN---R LVMKVVSVT- --
```

Figure S1: Structure-based alignment of the amino acid sequences of Dvl1,2 and 3 PDZ, Psd-1,2,3 PDZ, Af-6 and Syn PDZ domains. For Dvl PDZ, differences are highlighted in blue and similarities are highlighted in purple. UNIPROT codes: O14640 (Dvl-1 PDZ); O14641 (Dvl-2 PDZ); Q92997 (Dvl-3 PDZ), P78352 (Psd-1, Psd-2, Psd-3 PDZ); Q13424 (Alpha-1 Sytr PDZ); P55196 (Af6 PDZ); Q4ACU6 (mShank-3 PDZ)

2. 1H-15N HSQC spectra of Dvl-3 PDZ domain alone and in the presence of varying concentrations of compound 3

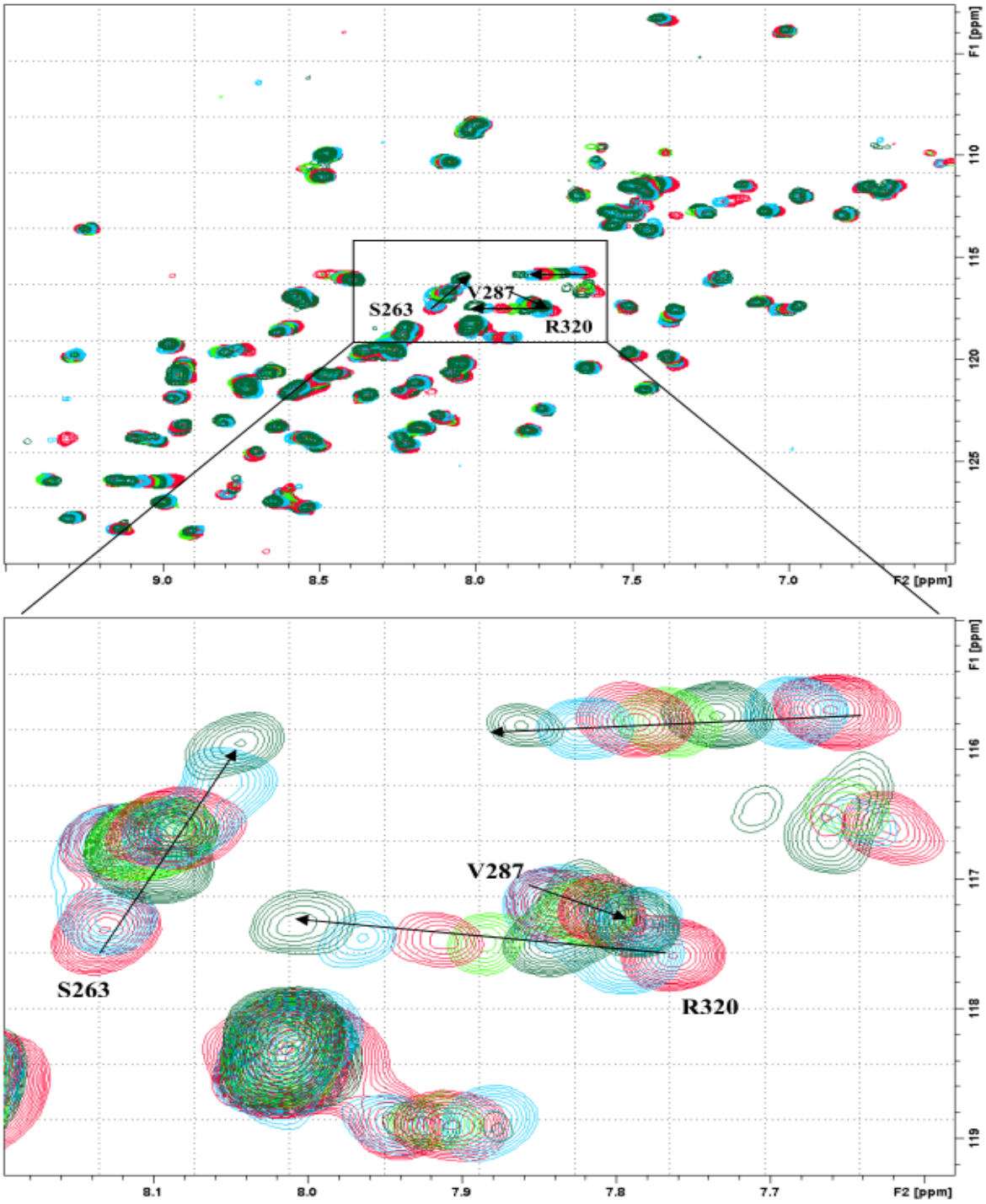


Figure S2: ^{1}H-^{15}N HSQC spectra of Dvl-3 PDZ domain alone and in the presence of varying concentrations of compound **1**. The zoom shows the gradual increase of shifts with residues surrounding the binding pocket of Dvl-3 PDZ.

3. Detailed views of diverse compounds bound to the Dvl-3 PDZ domain

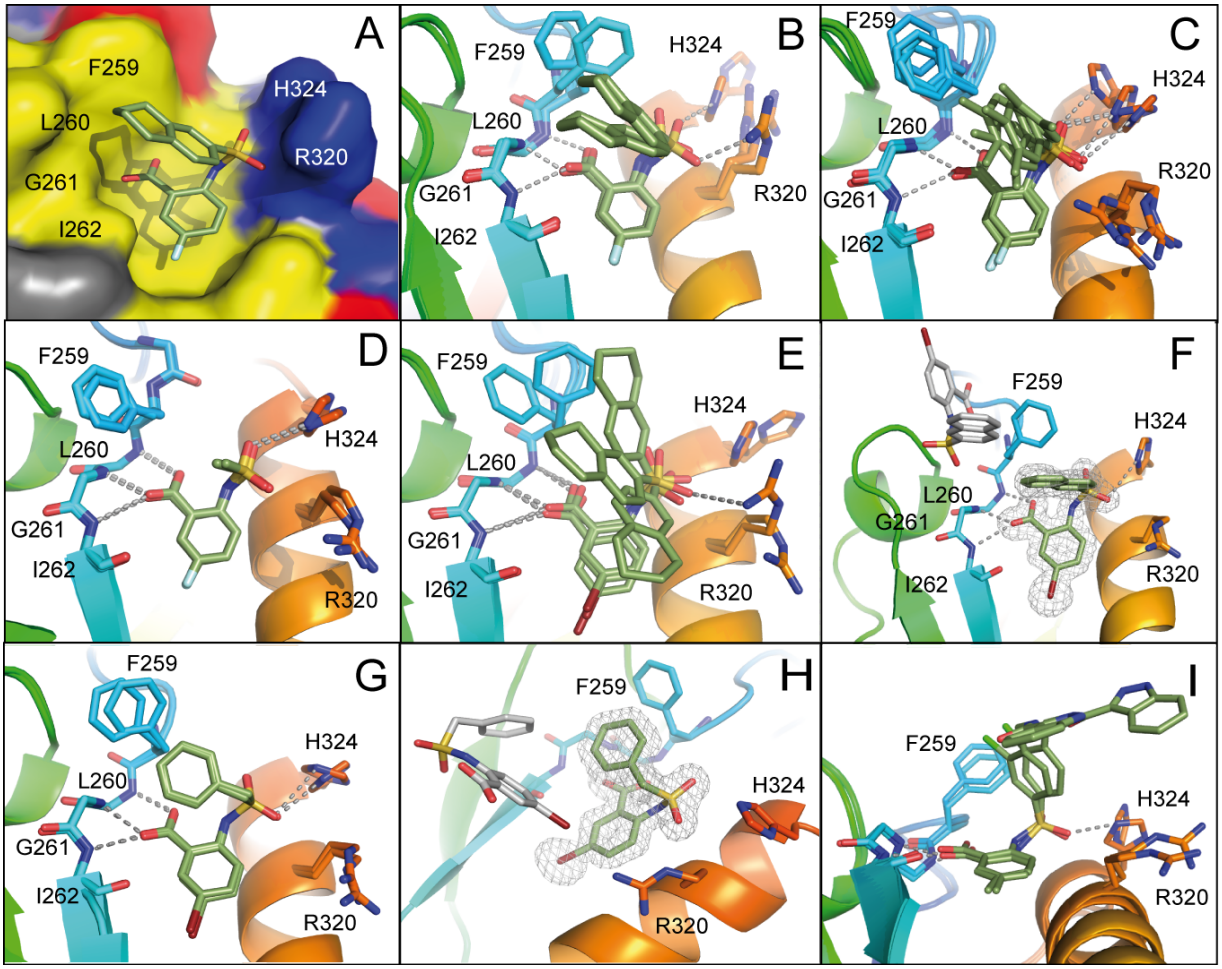


Figure S3: Detailed views of diverse compounds bound to the Dvl-3 PDZ domain. **A)** Surface representation of the Dvl-3 PDZ binding pocket with bound compound **3**. Positively charged amino acids are highlighted in blue and negatively charged amino acids in red. The hydrophobic Dvl-3 residues, contributing to compound binding, are colored yellow. **B-E), G) and I)** show detailed views of the binding pocket with bound compounds **3 (B)**, **5 (C), 6 (D), 7 (E), and 12 (G)**. Here, all Dvl-3 PDZ molecules per AU with their bound compounds are superimposed per species to demonstrate the binding variations per compound. Panels **F** and **H** present the additional unspecific compound binding to the Dvl-3 PDZ complex structures observed with compound **11 (F)** and compound **12 (H)**. Compound 18 (**I**) The non-specifically bound compounds are presented with grey sticks for covalent bonds to carbon atoms, and compounds bound to the canonical binding pocket of Dvl-3 PDZ domain are shown as green stick models enclosed in 2Fo-Fc electron density contoured at 1 sigma. >>>

4. Cell viability assays of compounds 3, 7,8, 9, 10, (A) and 18, 20, 21 (B)

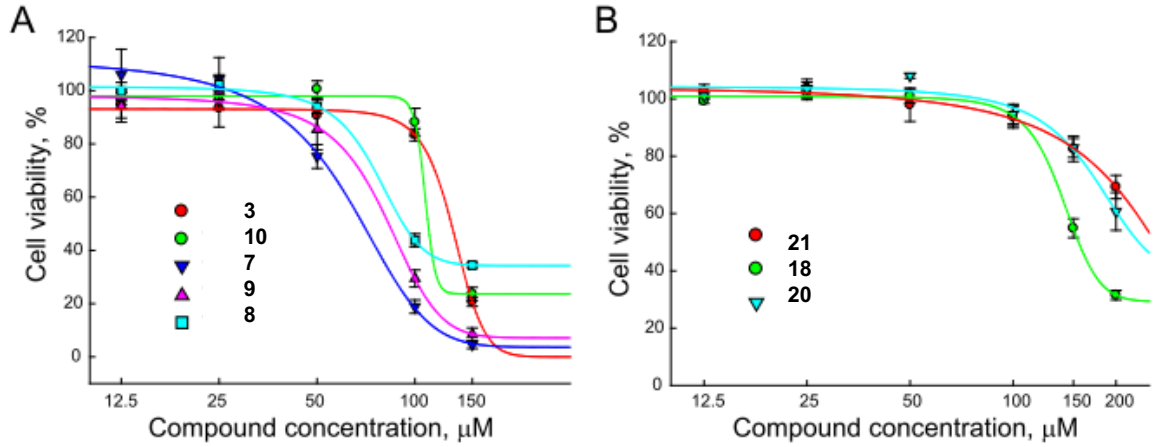


Figure S4: Cell viability assays of compounds 3, 7,8, 9, 10, (**A**) and 18, 20, 21 (**B**).

5. ITC binding assays of compound 18 with Dvl-3 PDZ (A) and with Dvl-1 PDZ (B)

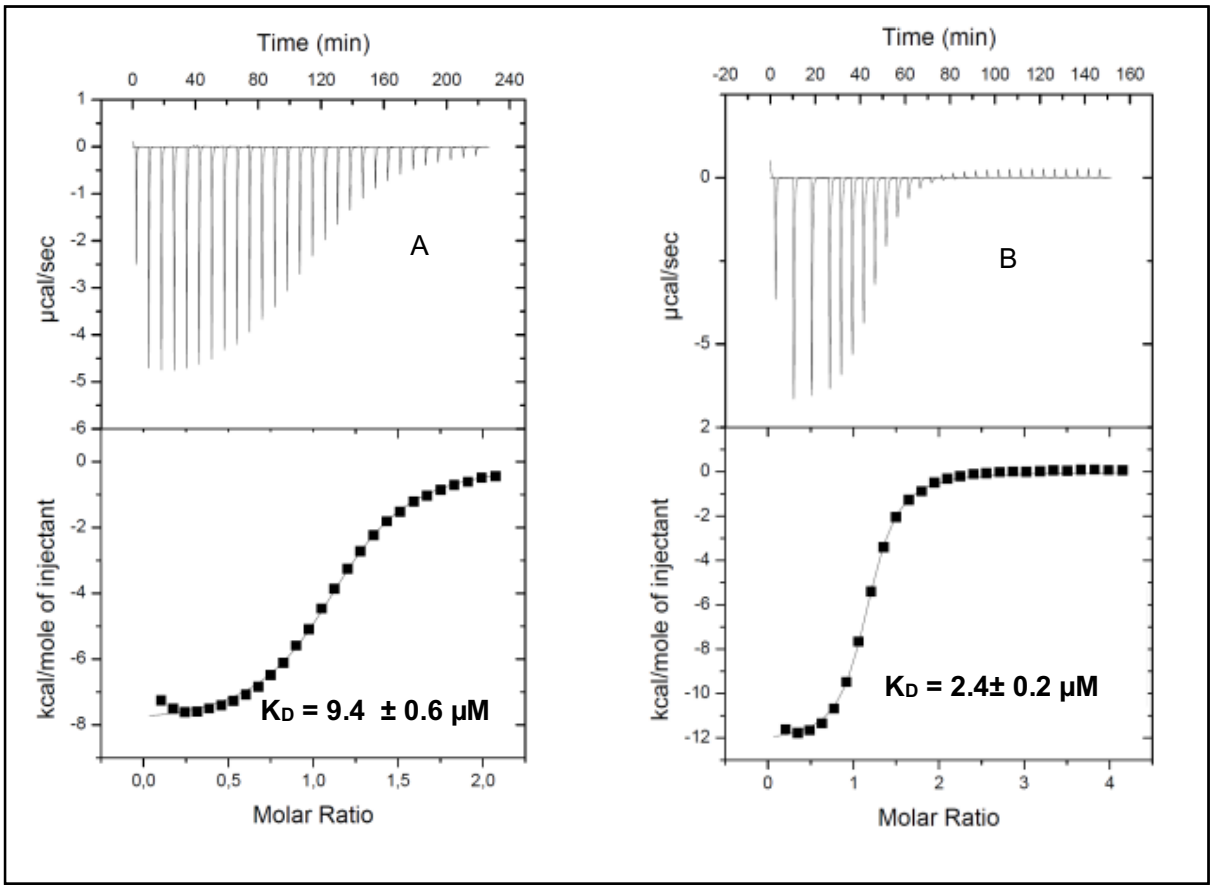


Figure S5: ITC binding assays of compound 18 with Dvl-3 PDZ (**A**) and with Dvl-1 PDZ (**B**). A 200 µM ligand solution containing 2% DMSO was injected 30 times in 10 µL aliquots at 120 s intervals with a stirring speed of 1000 rpm into a 1.4 mL sample cell containing the Dvl PDZ domain at a concentration of 20 µM and 2% DMSO.

6. Structures of selected compounds used for comparison to our compounds

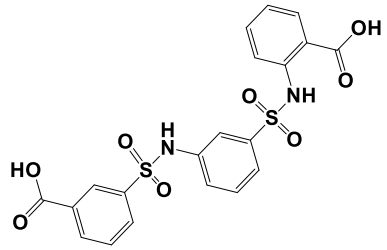
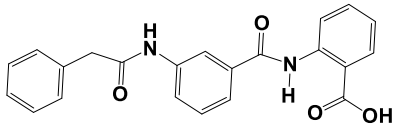
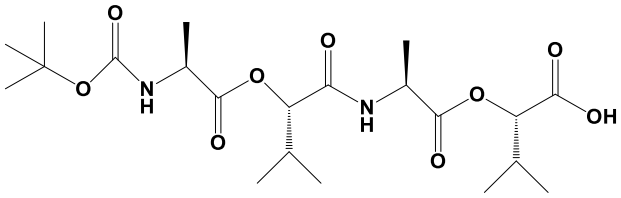
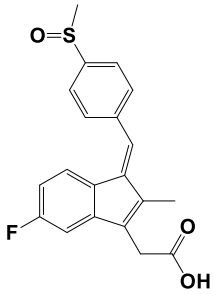
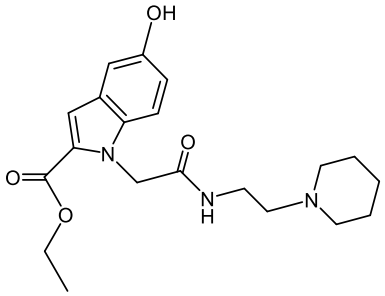
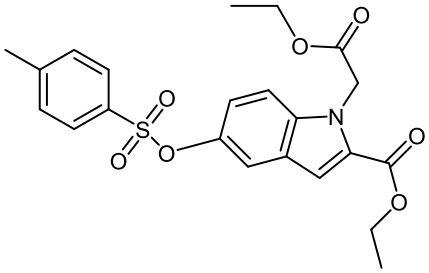
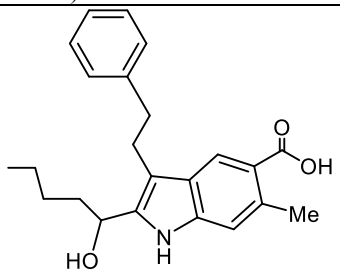
 3-({3-[(2-carboxyphenyl)sulfamoyl]phenyl}sulfamoyl)benzoic acid (NPL-1011)[16i]	 2-((3-(2-Phenylacetyl)amino)benzoyl)amino)benzoic acid, (CBC-322338/3289-8625)[16e]
 NSC668036[16a]	 Sulindac [16d]
 Ethyl 5-hydroxy-1-(2-oxo-2-((2-(piperidin-1-yl)ethyl)amino)ethyl)-1H-indole-2-carboxylate (KY-02327)[16g]	 Ethyl 1-(2-ethoxy-2-oxoethyl)-5-(tosyloxy)-1H-indole-2-carboxylate (KY-02061)[16g]
 2-(1-Hydroxypentyl)-6-methyl-3-phenethyl-1H-indole-5-carboxylic acid (FJ9)[16c]	

Figure S6: Structures of selected compounds used for comparison to our compounds.

7. ITC data of selected compounds used for comparison to our compounds

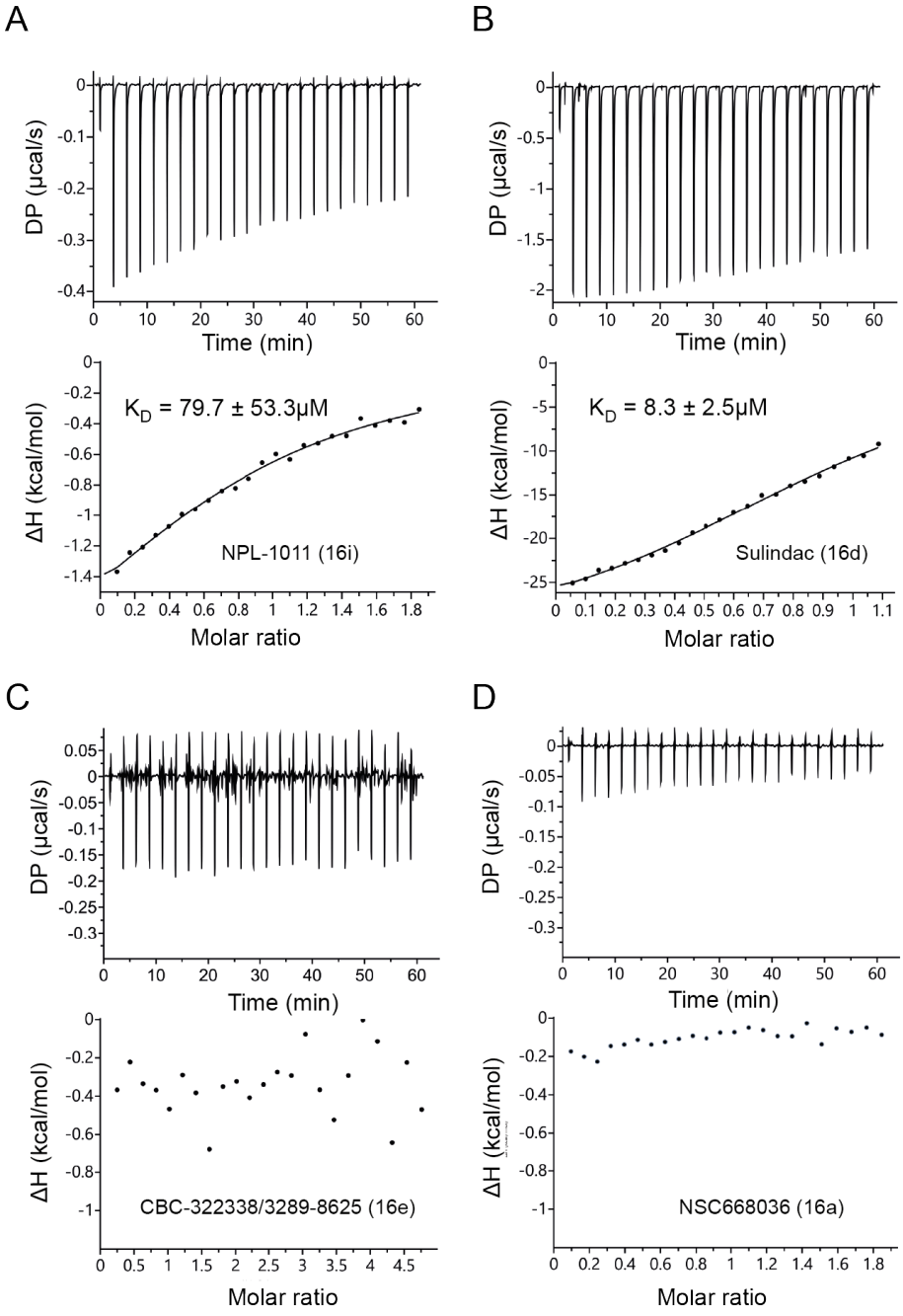
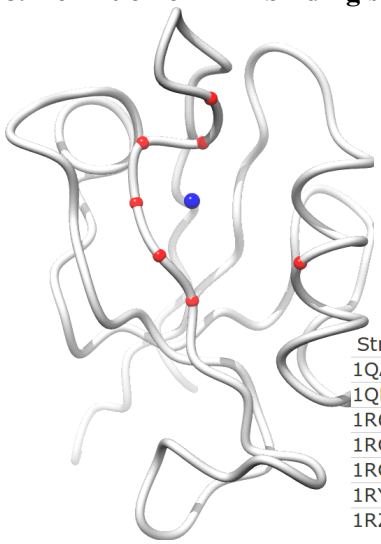


Figure S7: ITC data of A) NPL-1011[16i]; B) Sulindac[16d]; C) CBC-322338/3289-8625[16e] and D) NSC668036[16a] A) NPL-1011[16i] revealed a binding of 79.7 ± 53.3 µM to DVL3-PDZ with N= 0.90 ± 0.08, ΔH = -2.7 ± 1.2 kcal/mol, ΔG -5.5 kcal/mol, -tΔS = -2.8 kcal/mol, whereas Sulindac[16d] shown in B) displayed an KD = 8.3 ± 2.5 µM with N=0.97 ±0.14, ΔH1 = -31.9 ± 5.3 kcal/mol, -tΔS1 = 24.9 kcal/mol. C) Compound CBC-322338/3289-8625[16e] and D) NSC668036[16a] did not show any binding to the DVL3-PDZ domain.

8. Definition of PDZ binding site



Structure ID	βB	αB
1QAV_B	GLGFLV	ALEVLR
1QLC_A	GLGFSI	AVAALK
1R6J_A	HVGFIF	IADILS
1RGR_A	GLGFSI	AVEALK
1RGW_A	PWGFRL	AQNKIK
1RY4_A	PLGFYI	VTDMMV
1RZX_A	PLGFYI	VTDMMV

Figure S8: Definition of PDZ binding site. The center of the binding site (blue sphere) is defined as the geometric center of Cα atoms (red spheres) of 7 residues (typed in red) defined by multiple sequence alignment.

9. Purity check of compounds

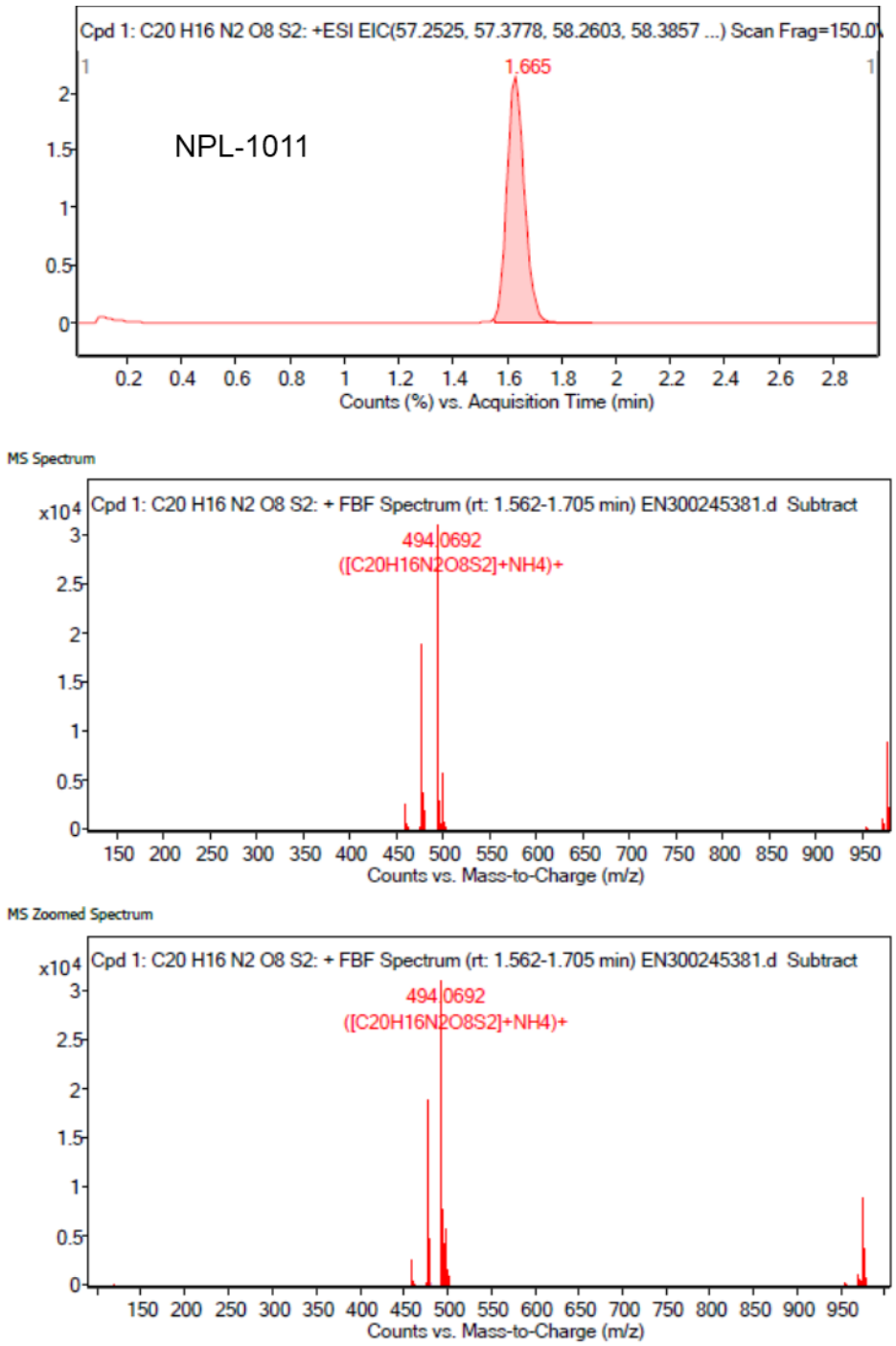


Figure S9a : Purity check of NPL-1011 compound

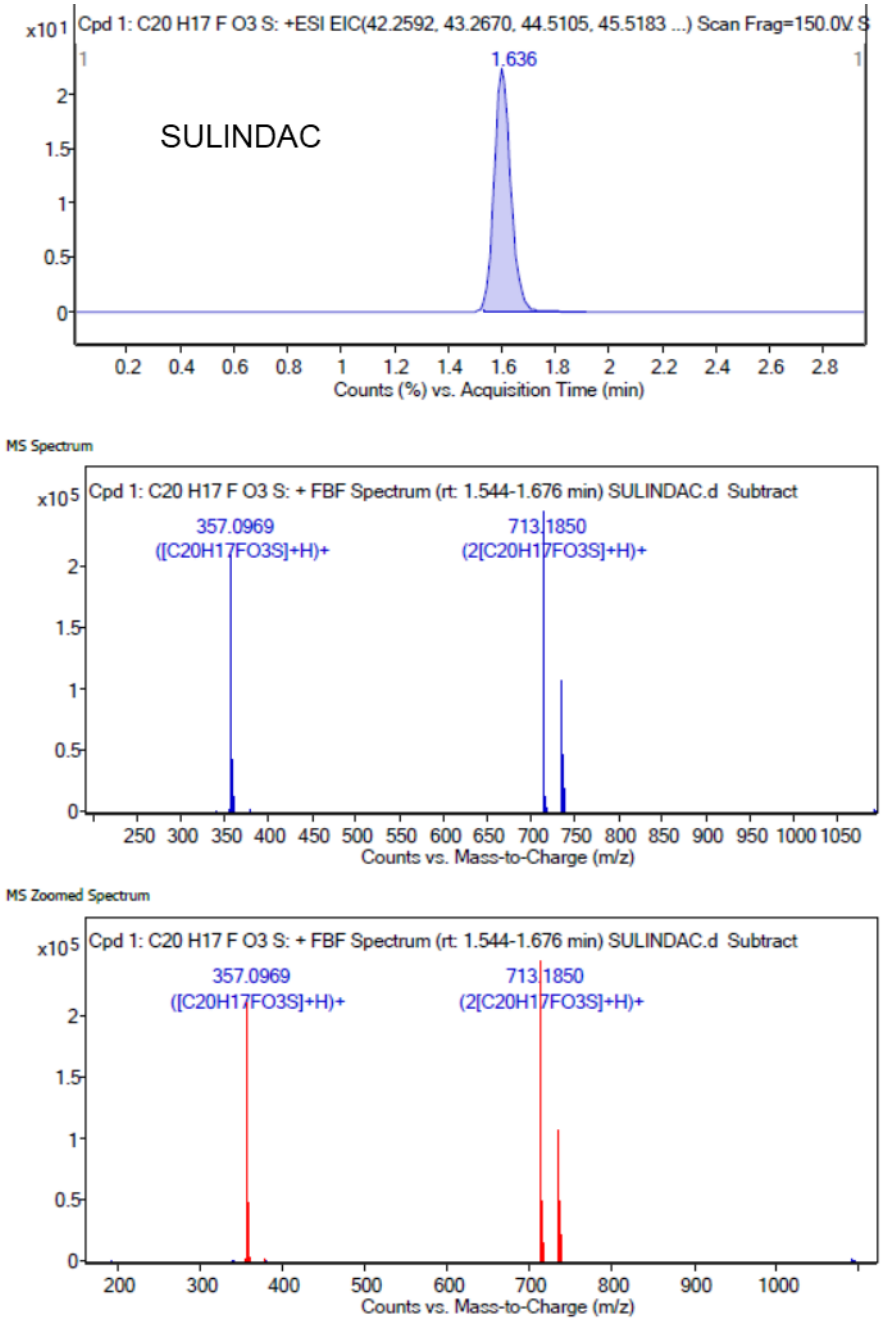


Figure S9b : Purity check of Sulindac compound

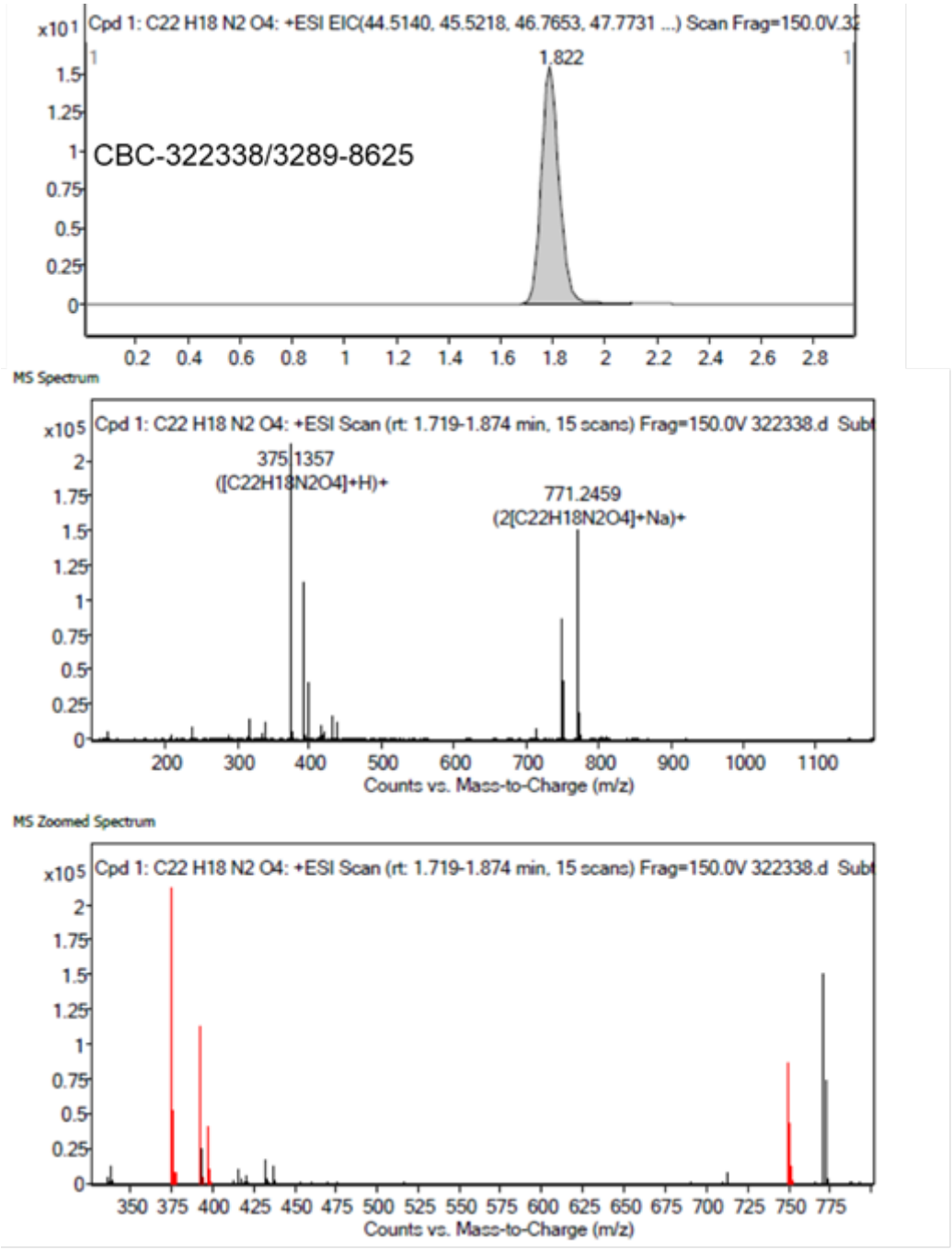


Figure S9c : Purity check of CBC-322338/3289-8625 compound

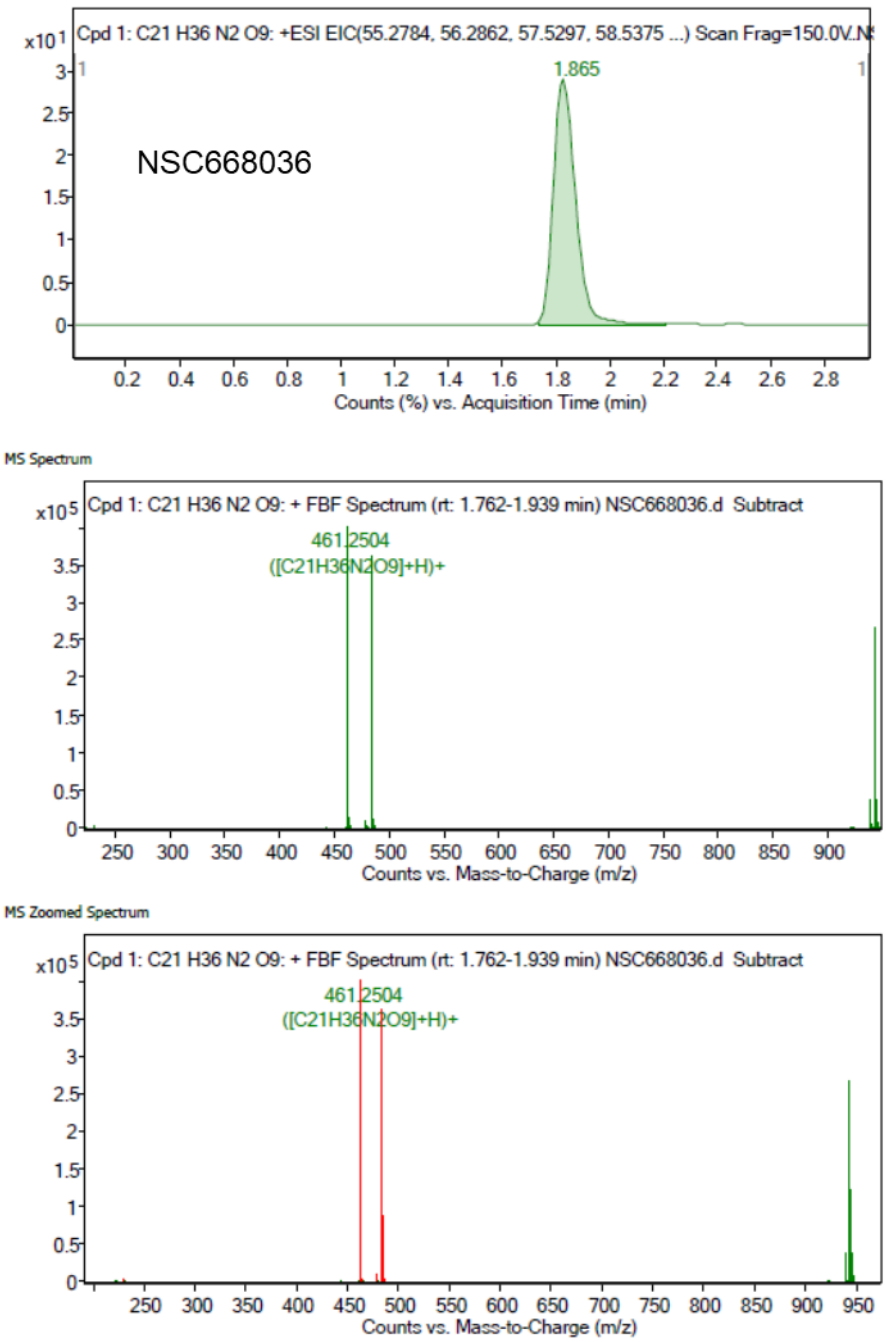


Figure S9d : Purity check of NSC668036 compound

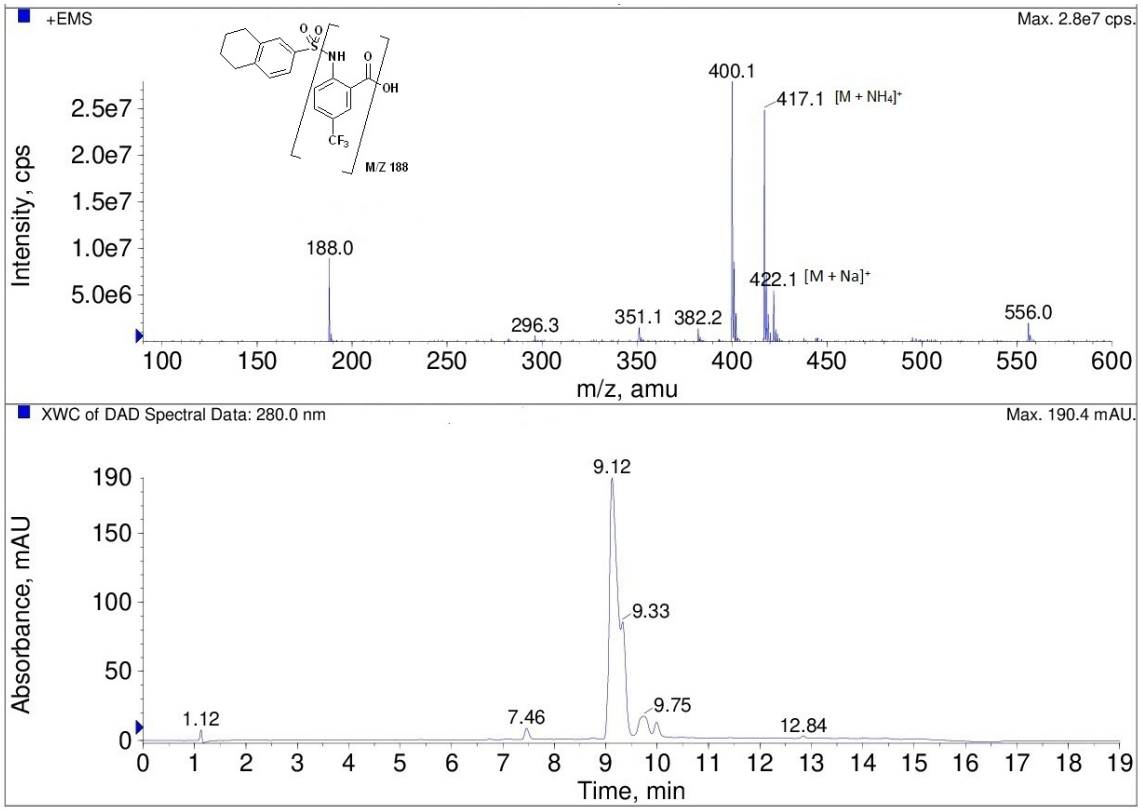


Figure S9e: LCMS of intermediate compound 8 : Peak at 1.1 refer to the instrumental signal prior to sample injection

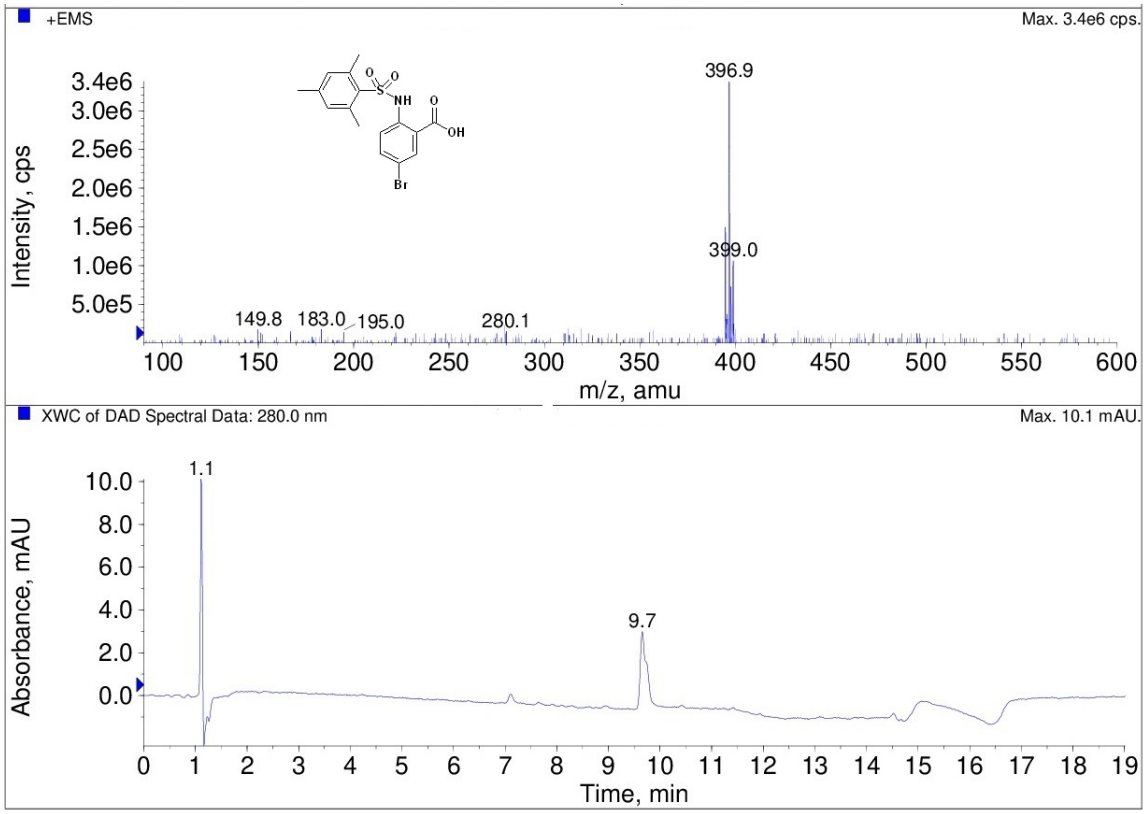


Figure S9f: LCMS of intermediate compound 14 : Peak at 1.1 refer to the instrumental signal prior to sample injection

10. Chemical shift perturbation values of Dvl-3 PDZ and Dvl-1 PDZ for compounds (3-21)

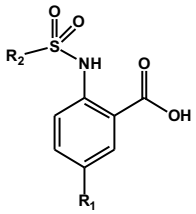
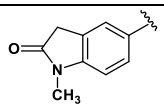
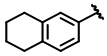
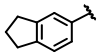
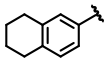
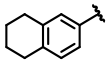
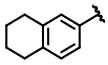
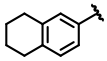
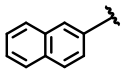
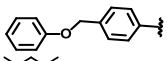
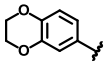
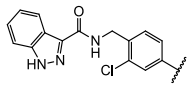
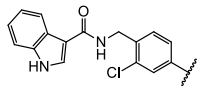
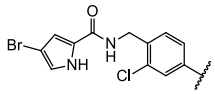
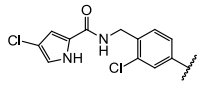
	ID	*R_1*	*R_2*	*ΔCSP(ppm) Dvl-3PDZ*	**ΔCSP(ppm) Dvl-1 PDZ**
	2	F		0.18	0.2
	3	F		0.27	0.086
	4	F		0.26	0.3
	5	F		0.23	0.15
	6	F	CH_3	0.11	
	7	Br		0.23	0.3
	8	CF_3		0.38	0.26
	9	Cl		0.28	0.34
	10	CH_3		0.26	0.31
	11	Br		0.31	0.18
	12	Br		0.21	0.29
	13	Br		0.2	0.22
	14	Br		0.31	0.26
	15	CF_3		0.28	0.24
	16	CF_3		0.36	0.08
	17	CF_3		0.21	0.23
	18	CH_3		0.30	0.36
	19	CH_3		0.36	0.32
	20	CH_3		0.35	0.36
	21	CH_3		0.34	0.34

Table S1: Chemical shift perturbation values of Dvl-3 PDZ and Dvl-1 PDZ for compounds (3 – 21). ΔCSP is the mean value of 3 amino acid residues showing strong chemical shift perturbations.

11. Data collection and refinement statistics of compounds 3, 5, 6, 7

Dvl3 with compound	**3**	**5**	**6**	**7**
Data collection				
Space group	I4	$P2_12_12_1$	$P6_1$	I4
a, *b*, *c* (Å)	76.3, 76.3, 72.4	56.8, 70.0, 87.2	87.3, 87.3, 57.8	76.3, 76.3, 72.6
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 120.0	90.0, 90.0, 90.0
Resolution (Å)*	30.0-1.43 (1.47-1.43)	34.6-1.60 (1.64-1.60)	34.8-1.67 (1.71-1.67)	30.9-1.85 (1.90-1.85)
R_{meas}*	4.4 (57.9)	3.8 (80.0)	5.5 (77.4)	5.8 (105.0)
$< I / \sigma(I) >$*	22.1 (3.2)	23.6 (2.3)	19.1 (2.5)	20.5 (2.1)
Completeness (%)*	100 (100)	99.7(99.8)	99.9 (100)	99.8 (99.6)
Redundancy*	5.4 (5.3)	4.8 (4.8)	5.7 (5.7)	7.4 (7.3)
Refinement				
No. total reflections	207003 (15053)	223464 (16344)	165069 (12220)	133118 (9391)
No. unique reflections	38358 (2826)	46555 (3405)	29202 (2161)	17796 (1282)
R_{work} / R_{free}	0.160 / 0.204	0.199/0.249	0.179/0.218	0.197/0.246
Mean B factor ($Å^2$)	16.1	24.3	21.4	20.6
Bond lengths (Å)	0.016	0.017	0.018	0.018
Bond angles (º)	1.867	1.753	1.762	1.805
Molecules in AU	2	4	2	2
Ramachandran				
Favoured region (%)	97.0	98.0	96.6	96.4
Outlier region (%)	0	0.3	0	0

* Data in highest resolution shell are indicated in parenthesis.

Table S2: Data collection and refinement statistics.

241 **12. Data collection and refinement statistics of compounds 11, 12, 18**
242

Dvl3 with compound	**11**	**12**	**18**
Data collection			
Space group	I422	$P6_1$	$P6_422$
a, *b*, *c* (Å)	78.6, 78.6, 77.8	85.3, 85.3, 58.9	89.3, 89.3, 131.6
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 120.0	90.0, 90.0, 120.0
Resolution (Å)*	32.0-1.58 (1.62-1.58)	34.6-1.48 (1.52-1.48)	34.8-2.76 (2.83-2.76)
R_{meas}*	6.4 (69.0)	6.7 (80.5)	14.2 (82.6)
$< I / \sigma(I) >$*	18.1 (2.9)	18.4 (3.2)	21.4 (4.1)
Completeness (%)*	99.5 (100)	100 (100)	99.9 (100)
Redundancy*	7.1 (7.2)	8.0 (8.0)	12.6 (13.3)
Refinement			
No. total reflections	120373.4 (8848.8)	326040 (24096)	107037 (8073)
No. unique reflections	16954 (1229)	40755 (3012)	8495 (607)
R_{work} / R_{free}	0.182 / 0.221	0.148/0.178	0.242/0.299
Mean B factor (Å^2)	23.0	22.7	36.6
Bond lengths (Å)	0.021	0.019	0.013
Bond angles (º)	2.028	1.933	1.442
Molecules in AU	1	2	2
Favoured region (%)	98.0	97.8	98.0
Outlier region (%)	0	0.0	0

243 * Data in highest resolution shell are indicated in parenthesis.

244 **Table S3**: Data collection and refinement statistics.
245

13. Selectivity of ligands derived from chemical shift perturbation of compounds tested at other PDZ domains

CP Id	PDZ							
	Dvl-1	Dvl-3	PSD95-1	PSD95-2	PSD95-3	Shank-3	α-1-Syn	AF-6
18	0.32	0.30	0.05	0.1	0.05	0.01	0.08	0.01
20	0.3	0.36	0.06	0.09	0.06	0.05	0.07	0.01
21	0.3	0.36	0.07	0.09	0.1	0.05	0.08	0.01

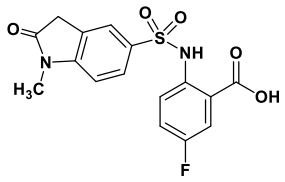
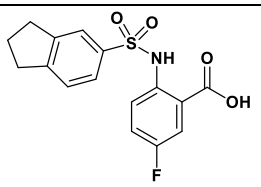
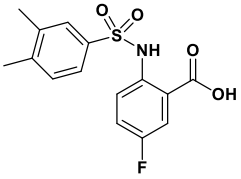
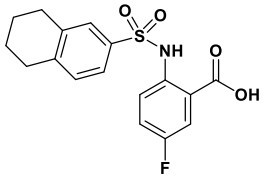
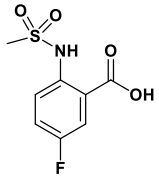
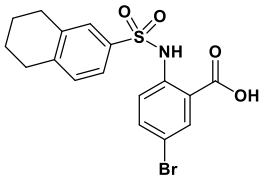
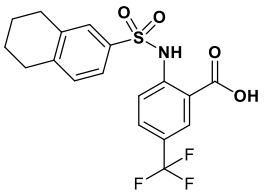
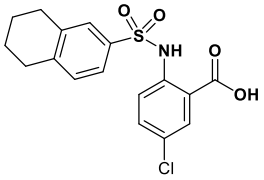
Table S4: Selectivity of ligands derived from chemical shift perturbation of compounds tested at other PDZ domains. The PDZ domain set includes PSD95-1, PSD95-2, PSD95-3, Shank-3, α-1 Syn and AF-6.ΔCSP is the mean value of 3 amino-acid residues showing chemical shift perturbation

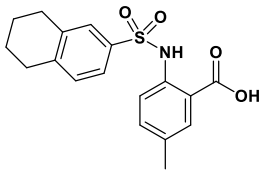
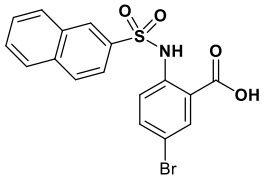
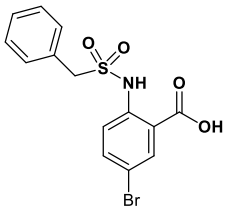
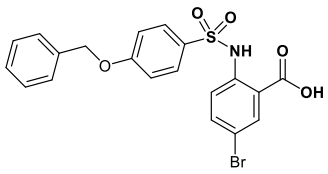
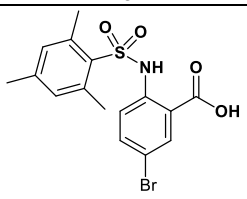
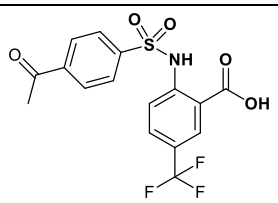
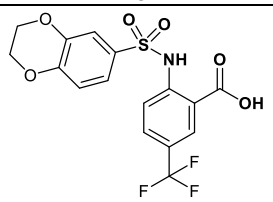
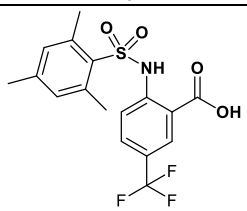
14. Details of Multifilter routines

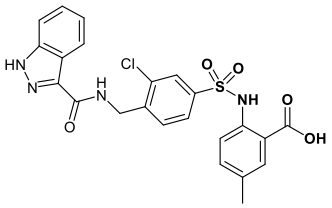
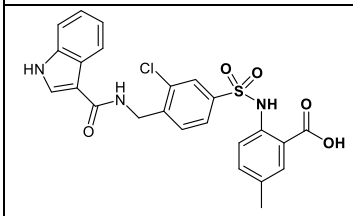
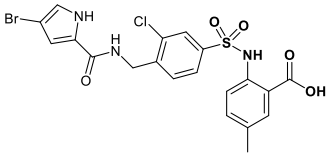
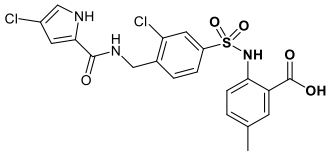
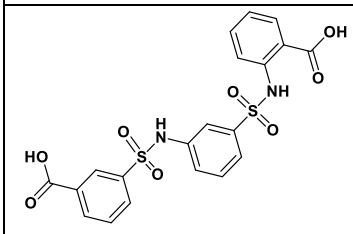
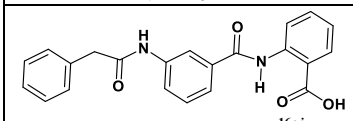
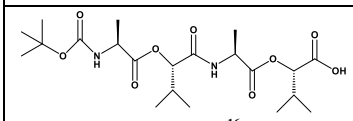
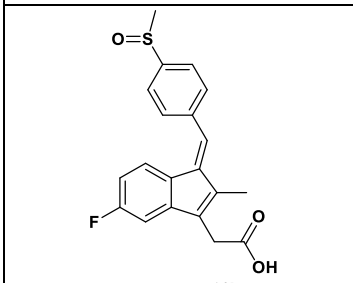
PDB structure ID	Distance from a ligand atom to PDZ atom			H-bond threshold	Resulting number of compounds
	2.5 Å		4 Å		
2os6, model 8	Gly21 HN	Leu22 HN	Leu22 CD1	3	228
2dlu, model 1	Gly29 HN	Phe30 HN	Phe30 CE1	4	204
2o2t, chain B	Gly149 HN	Phe150 HN	Phe150 CE1	4	332
1va8, model 3	Gly40 HN	Ala41 HN	Leu93 CG	4	284
1uhp, model 8	Gly22 HN	Phe23 HN	Phe86 CD2	3	329
3lnx, chain A	Leu18 HN	Gly19 HN	Ile20 CG1	4	220

Table S5: Details of Multifilter routines.

15. Smiles codes and Compounds ID

STRUCTURE / ID in paper	MOLECULE	MW	COMPANY ID	SMILES CODE
 **2**	$C_{16}H_{13}FN_2O_5S$	364,3	ENAMINE T58 630 40	CN1C(=O)CC2=C1C=CC(=C2)S(=O)(=O)NC3=C(C=C(C=C3)F)C(=O)O
 **3**	$C_{17}H_{16}FNO_4S$	349.4	ENAMINE T6324911	O=C(O)c1cc(F)ccc1NS(=O)(=O)c3ccc2CCCCc2c3
 **4**	$C_{16}H_{14}FNO_4S$	335.4	ENAMINE T6324915	O=C(O)c1cc(F)ccc1NS(=O)(=O)c3ccc2CCCc2c3
 **5**	$C_{17}H_{16}FNO_4S$	349.4	ENAMINE T6305470	C1CCC2=C(C1)C=CC(=C2)S(=O)(=O)NC3=C(C=C(C=C3)F)C(=O)O
 **6**	$C_8H_8FNO_4S$	233.22	FMP	CS(=O)(=O)Nc1ccc(F)cc1C(=O)O
 **7**	$C_{17}H_{16}BrNO_4S$	410.3	ENMINE 28744264	O=C(O)c1cc(Br)ccc1NS(=O)(=O)c3ccc2CCCCc2c3
 **8**	$C_{18}H_{16}F_3NO_4S$	399,383	FMP	O=C(O)c1cc(C(F)(F)F)ccc1NS(=O)(=O)c3ccc2CCCCc2c3
	$C_{17}H_{16}ClNO_4S$	365.8	ENAMINE 28775339	O=C(O)c1cc(Cl)ccc1NS(=O)(=O)c3ccc2CCCCc2c3

9				
 10	$C_{18}H_{19}NO_4S$	345.4	ENAMINE 233895416	Cc3ccc(NS(=O)(=O)c2ccc1CCCCc1c2)c(C(=O)O)c3
 11	$C_{17}H_{12}BrNO_4S$	406.3	FMP	O=C(O)c1cc(Br)ccc1NS(=O)(=O)c3ccc2ccccc2c3
 12	$C_{14}H_{12}BrNO_4S$	370.22	FMP	O=C(O)c1cc(Br)ccc1NS(=O)(=O)Cc2ccccc2
 13	$C_{20}H_{16}BrNO_5S$	462.3141	FMP	O=C(O)c1cc(Br)ccc1NS(=O)(=O)c3ccc(COc2ccccc2)cc3
 14	$C_{16}H_{16}BrNO_4S$	398.3	FMP	Cc2cc(C)c(S(=O)(=O)Nc1ccc(Br)cc1C(=O)O)c(C)c2
 15	$C_{16}H_{12}F_3NO_5S$	387.3297	FMP	CC(=O)c2ccc(S(=O)(=O)Nc1ccc(C(F)(F)F)cc1C(=O)O)cc2
 16	$C_{16}H_{12}F_3NO_6S$	403,329	FMP	O=C(O)c1cc(C(F)(F)F)ccc1NS(=O)(=O)c3ccc2OCCOc2c3
 17	$C_{17}H_{16}F_3NO_4S$	387,372	FMP	Cc2cc(C)c(S(=O)(=O)Nc1ccc(C(F)(F)F)cc1C(=O)O)c(C)c2

 **18**	$C_{23}H_{19}ClN_4O_5S$	498,939	ENAMINE Z1098340488	Cc4ccc(NS(=O)(=O)c3ccc(CNC(=O)c1n[nH]c2ccccc12)c(Cl)c3)c(C(=O)O)c4
 **19**	$C_{24}H_{20}ClN_3O_5S$	497,952	ENAMINE Z1098340555	Cc4ccc(NS(=O)(=O)c3ccc(CNC(=O)c1c[nH]c2ccccc12)c(Cl)c3)c(C(=O)O)c4
 **20**	$C_{20}H_{17}BrClN_3O_5S$	526,788	ENAMINE Z1098340559	Cc3ccc(NS(=O)(=O)c2ccc(CNC(=O)c1cc(Br)c[nH]1)c(Cl)c2)c(C(=O)O)c3
 **21**	$C_{20}H_{17}Cl_2N_3O_5S$	482,337	ENAMINE Z1098340560	Cc3ccc(NS(=O)(=O)c2ccc(CNC(=O)c1cc(Cl)c[nH]1)c(Cl)c2)c(C(=O)O)c3
 **NPL-1011**	$C_{20}H_{16}N_2O_8S_2$	476.5	ENAMINE EN300 -245381	C1=CC=C(C(=C1)C(=O)O)NS(=O)(=O)C2=CC=CC(=C2)NS(=O)(=O)C3=CC=CC(=C3)C(=O)O
 **CBC-322338/3289-8625**[16e,i]	$C_{22}H_{18}N_2O_4$	374.4	MERCK 322338-10MG	C1=CC=C(C=C1)CC(=O)NC2=CC=CC(=C2)C(=O)NC3=CC=CC=C3C(=O)O
 **NSC668036**[16a]	$C_{21}H_{36}N_2O_9$	460.5	SIGMA SML0046	CC(C)C(C(=O)NC(C)C(=O)OC(C(C)C)C(=O)O)OC(=O)C(C)NC(=O)OC(C)(C)C
 **Sulindac**[16d]	$C_{20}H_{17}FO_3S$	356.4	SIGMA S8139-5G	CC1=C(C2=C(C1=CC3=CC=C(C=C3)S(=O)C)C=CC(=C2)F)CC(=O)O

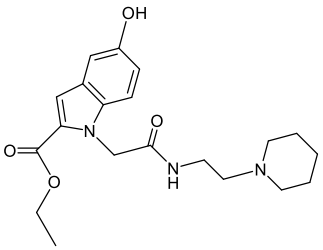
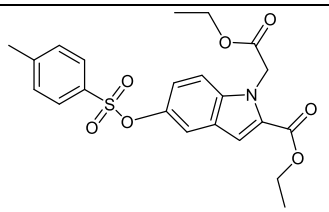
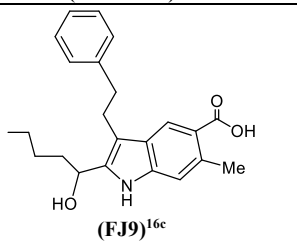
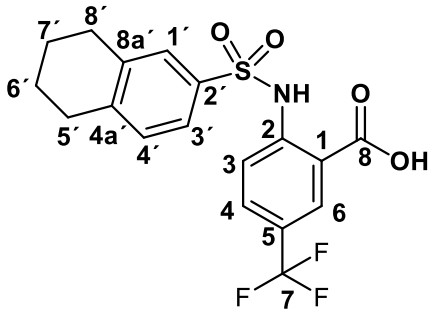
 **(KY-02327)**[16g]	$C_{20}H_{27}N_3O_4$	373.4	D&C Chemicals DC21213	CCOC(=O)C1=CC2=C(N1CC(=O)NCCN3CCCCC3)C=CC(=C2)O
 **(KY-02061)**[16g]	$C_{22}H_{23}NO_7S$	445.5	D&C Chemicals **DC21213**	CCOC(=O)CN1C2=C(C=C(C=C2)OS(=O)(=O)C3=CC=C(C=C3)C)C=C1C(=O)OCC
 **(FJ9)**[16c]	$C_{23}H_{27}NO_3$	365.5	Toronto Research Chemicals **H939980**	CCCCC(C1=C(C2=C(N1)C=C(C(=C2)C(=O)O)C)CCC3=CC=CC=C3)O

Table S6: Smiles codes and Compounds ID. Compounds containing literature indication are those used for comparison to our compounds.

16: NMR characterization of synthesized compounds (8 , 11 , 13 , 14 , 15 , 16 , 17)

2-(5,6,7,8-tetrahydronaphthalene-2-sulfonamido)- 5- (trifluoromethyl) benzoic acid (8)



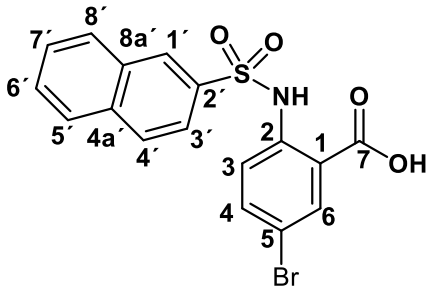
$C_{18}H_{16}F_3NO_4S$

M=399,4 g/mol

(0.52 g, 74% yield) **^{1}H-NMR** (300 MHz, DMSO-d6): δ = 11.77 [s, 1H, CO*OH*], 8.13 [s, 1H, *NH*], 7.85 [d, $^3J_{6,4}$ = 2.1 Hz, 1H, 6-H_{Ar}] 7.62 [d, $^4J_{1',3'}$ = 2.1Hz , 1H , 1′-H_{Ar}] 7.53 [dd, $^3J_{4,3}$ = 7.1 Hz, $^4J_{4,6}$ = 2.1 Hz, 4-H_{Ar}] 7.36 [dd, $^3J_{3'4'}$ = 7.5 Hz, $^4J_{3',1'}$ = 2.4 Hz, 1H, 3′-H_{Ar}] 7.15 [d, $^3J_{4',3'}$ = 7.5Hz, 1H,4′-H_{Ar}] , 6.90 [d, $^3J_{3,4}$ = 7.1Hz, 1H , 3-H_{Ar}] 2.73 (m, 4H, CH_2); 1.6 (m, 4H, CH_2);-**^{13}C-NMR** (75 MHz, DMSO-

d6): δ = 169.1(C, C_{Ar}-8], 152.7(C, C_{Ar}-2), 143.8 (C, C_{Ar}-4a´), 138.7(C, C_{Ar}-2´), 135.9 (C, C_{Ar}-8a´), 130.4(CH, C_{Ar}-4), 128.7 (CH, C_{Ar}-6), 127.5 (CH, C_{Ar}-1´), 124.0 (CH, C_{Ar}-4´), 121.6 (C, C-6), 118.2 (C, C_{Ar}-5), 116.9 (C, C_{Ar}-3), 29.0 (CH_2, C-8´), 28.8 (CH_2, C-5´), 22.3 (CH_2, C-6´), 22.2 (CH_2, C-7´); mp: 177°C; MS (ESI) *m*/z:calcd. for $C_{18}H_{16}F_3NO_4S$, 399; found 400 $[M+H]^+$.

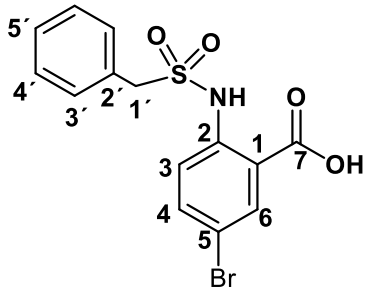
5-bromo-2-(naphthalene-2-sulfonamido) benzoic acid (11)



$C_{17}H_{12}BrNO_4S$
M = 406.3g/mol

(0.13 g, 67% yield) **^{1}H-NMR** (300 MHz, DMSO-d6): δ = 10.2 [s, 1H, *COOH*], 9.8 [s, 1H, *NH*] 8.59 [d, $^4J_{1´,3´}$ = 1.4 Hz, 1 H, 1´-H_{Ar}], 8.17 [d, $^3J_{8´,7´}$ = 7.8 Hz, 1 H, 8´-H_{Ar}], 8.10 [d, $^3J_{4´3´}$ = 8.8 Hz, 1 H, 4´-H_{Ar}], 8.02 [d, $^3J_{5´,6´}$ = 7.8 Hz, 1 H, 5´-H_{Ar}], 7.93 [d, $^4J_{6,4}$ = 2.4 Hz, 1 H, 6-H_{Ar}], 7.77 [dd, $^3J_{3´,4´}$ = 8.8 Hz, $^4J_{3´,1´}$ = 1.4Hz ,1 H, 3´-H_{Ar}], 7.72 – 7.65 [m, 3 H, 4-H_{Ar}, 6´-H_{Ar}, 7´-H_{Ar}] , 7.51 [d, $^3J_{3,4}$ = 8.9 Hz, 1 H, 3-H_{Ar}]. – **^{13}C-NMR** (75 MHz, DMSO-d6): δ = 168.2 (C, C-7) ,138.8 (C, C_{Ar}-2),136.8 (CH, C_{Ar}-4),135.3 (C, C_{Ar}-4a´),134.4 (C, C_{Ar}-8a´) ,133.4 (CH, C_{Ar}-6) ,131.4 (CH, C_{Ar}-6´), 129.3 (CH, C_{Ar}-4´) ,128.5 (CH, C_{Ar}-8´),127.8 (2xCH, C_{Ar}-5´, C_{Ar}-7´) 121.6 (CH, C_{Ar}-3´),120.6 (CH, C_{Ar}-3),119.0(C, C_{Ar}-1) ,114.9 (C, C_{Ar}-5). Mp: 199°C; (ESI) *m*/z: calcd.for $C_{17}H_{11}BrNO_4S^-$; 403.9560: found 403.9613 $[M-H]^-$.

5-bromo-2-(phenylmethylsulfonamido)benzoic acid (12)

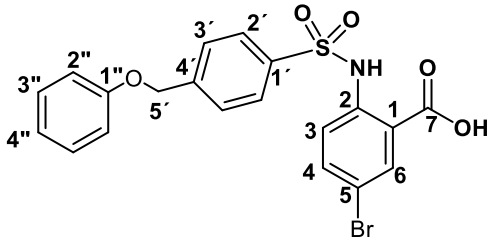


$C_{14}H_{12}BrNO_4S$

M=370.2 g/mol

(0.07g, 42% yield) **^{1}H-NMR** (300 MHz, DMSO-d6): δ = 10.57 [s, 1 H, COO*H*], 8.05 [d, $^4J_{6,4}$ = 2.4 Hz, 1 H, 6-H_{Ar}], 7.75 [dd, $^3J_{4,3}$ = 8.9 Hz, $^4J_{4,6}$ = 2.4Hz ,1 H, H-4$_{Ar}$], 7.49 [d,$^3J_{3,4}$ = 8.9 Hz,1 H, 3-H_{Ar}], 7.33 – 7.28 [m, 3 H, 3′-H_{Ar} , 5′-H_{Ar}], 7.23 – 7.20 [m, 2 H, 4′-H_{Ar}], 5.75 [s, 1 H, N*H*], 4.72 [s, 2 H, 1′-H] **^{13}C-NMR** (75 MHz, DMSO-d6): δ = 168.3 (C, C-7) , 139.9 (C, C_{Ar}-2), 137(CH, C_{Ar}-4), 133.4 (CH, C_{Ar}-6), 130.7 (CH, C_{Ar}-3′) , 128.6 (C, C_{Ar}-2′) , 128.4 (CH, C_{Ar}-5′) , 128.3 (CH, C_{Ar}-4′) , 119.5 (CH, C_{Ar}-3) , 117.5 (C, C_{Ar}-1) , 113.9 (C, C_{Ar}-5) , 57.4 (CH_2, C-1′). Mp: 216°C; (ESI) *m*/z: calcd.for $C_{14}H_{11}BrNO_4S^-$ 367.9860; found 367.9878 $[M-H]^-$.

5-bromo-2-(4-(phenoxymethyl)phenylsulfonamido)benzoic acid (13)



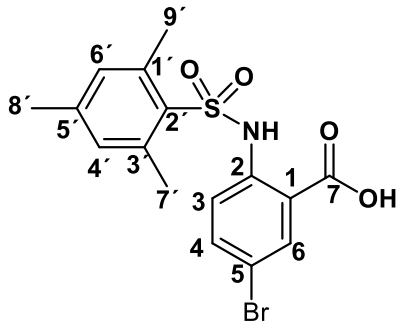
$C_{20}H_{16}BrNO_5S$

M=462.3 g/mol

(0.6 g, 29% yield) 1H-NMR (300 MHz, DMSO-d6): δ = 7.97 [d, $^4J_{6,4}$ = 2.4 Hz, 1 H, 6-H_{Ar}), 7.85 (d, $^3J_{2',3'}$ = 8.3 Hz, 2 H, 3′-H_{Ar}), 7.73 [dd, $^3J_{4,3}$ = 8.9 Hz, $^4J_{4,6}$ = 2.4Hz , 1 H,4-H_{Ar}], 7.63 [d, $^3J_{2',3'}$ = 8.3 Hz, 2 H, 2′-H_{Ar}], 7.47 [d, $^3J_{3,4}$ = 8.9 Hz, 1 H, 3-H_{Ar}], 7.29 [dd, $^3J_{3",2"}$ = $^3J_{3",4"}$ =7.3 Hz, 2 H, 3"-H_{Ar}], 7.00 – 6.92 [m, 3 H, 4"-H_{Ar} , 2"-H_{Ar}], 5.17 [s, H, 5′-H]. − **13C-NMR** (75 MHz, DMSO-d6): δ = 168.2 (C, C-7) , 157.9 (C, C_{Ar}-1") , 143.2 (C, C_{Ar}-4′), 138.8 (C, C_{Ar}-2), 137.5 (C, C_{Ar}-1′) , 136.9 (CH, C_{Ar}-4) 133.5 (CH, C_{Ar}-6), 129.4(CH, C_{Ar}-3"), 128.1(CH, C_{Ar}-2′),127.0 (CH, C_{Ar}-3′) , 120.9 (CH, C_{Ar}-4") , 120.5 (CH, C_{Ar}-

3) , 119.0 (C, C_{Ar}-1) , 114.9(CH, C_{Ar}-5), 114.7 (CH, C_{Ar}-2"), 68.0 (CH_2, C-5′) Mp: 175°C; (ESI) m/z: calcd for C20H15BrNO5S- 459.9860 found 459.9878 [M-H]-.

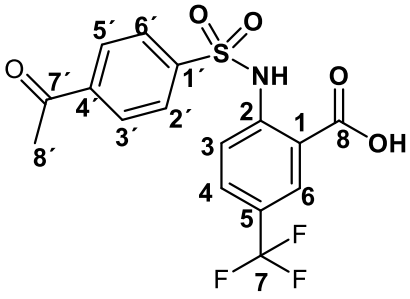
5-bromo-2-(2,4,6-trimethylphenylsulfoamido)benzoic acid (14)



$C_{16}H_{16}BrNO_4S$
M=398.3g/mol

(0.6 g, 78% yield) **^{1}H-NMR** (300 MHz, DMSO-d_6): δ = 11.77 [s,1H, *COOH*], 9.98 [s, 1H, *NH*], 7.68 [d, $^3J_{6,4}$ = 7.4 Hz, 1H, 6-H_{Ar}], 7.51[dd, $^3J_{4,3}$ =7.1Hz, $^4J_{4,6}$ =7.4 Hz, 1H 4-H_{Ar}], 7.17 [d, 2H, 4′-H_{Ar} , 6′-H_{Ar}], 7.14 [d, $^3J_{3,4}$= 1H,3-H_{Ar}] , 2.56 [s, 6H, CH_3, 9′-H, 7′-H], 2.21 [s, 3H, CH_3, 8′-H];-**^{13}C-NMR** (300 MHz, DMSO-d_6): δ = 168.8 (C, C-7), 143.3 (C, C_{Ar}-2), 139.5 (C, C_{Ar}-2′), 139.0 and 139.0 (2xC, C_{Ar}-3′, C_{Ar}-1′) 137.3 (CH, C_{Ar}-4), 134.0 (CH, C_{Ar}-6′), 133.0 (CH, C_{Ar}-6), 132.5 and 132.5 (2XCH, C_{Ar}-4′, C_{Ar}-6′) 119.1(CH, C_{Ar}-3), 117.9(C, C_{Ar}-5), 114.3 (C, C_{Ar}-1), 22.5 and 22.5 (2 x CH_3, C-7′, C-9′) 20.7 (CH_3, C-8′) ; mp: 185; MS (ESI): m/z 399 [M+H]+.

2-(4-acetylphenylsulfoamido)-5-(trifluoromethyl)benzoic acid (15)

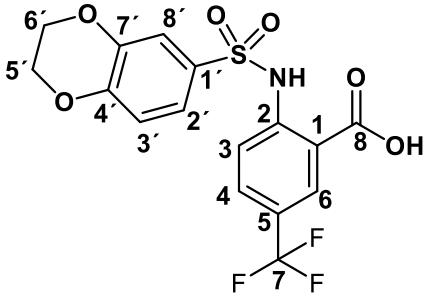


$C_{16}H_{12}F_3NO_5S$
M=387.3g/Mol

(0.4 g, 63% yield) **^{1}H-NMR** (300 MHz, DMSO-d6): δ = 12.28 [s, 1H, *COOH*]; 12.10 [s, 1H, *NH*], 8.11 [d, $^4J_{6,4}$= 2.5 Hz, 1H, 6-H_{Ar}], 8.08 [d, $^3J_{3'2'}$ = 7.5 Hz, 2H, 3′-H_{Ar}], 7.86 [dd, $^4J_{4,6}$ = 2.5 Hz, $^3J_{4,3}$ = 7.3Hz, 1H, 4-H_{Ar}], 7.64 [d, $^3J_{4,3}$ = 7.3 Hz, 1H, 3-H_{Ar}] , 7.56 [dd $^3J_{2',3'}$ = 7.5Hz, $^4J_{2',6'}$ =2.3Hz, 2H, 2′-H_{Ar}, 6′-H_{Ar}] 7.22 [dd , $^3J_{3',2'}$ = 7.5Hz , $^4J_{3',5'}$ = 2.1Hz , 2H, 3′-H_{Ar}, 5′-H_{Ar}] 2.50 [s, 3H, CH_3, 8′-H]; - **^{13}C-NMR**

(75 MHz, DMSO-d6): δ = 197.9 (C, C-7′), 169.1(C, C-8), 151.8 (C, C_{Ar}-2) 143.5 (C, C_{Ar}-1′), 142.5 (C, C_{Ar}-4′), 140.6 (CH, C_{Ar}-4), 131.4 (CH, C_{Ar}-7), 129.6 (2XCH, C_{Ar}-3′, C_{Ar}-5′), 128.6 (2xCH, C_{Ar}-2′, C_{Ar}-6′), 127.6 (C, C_{Ar}-6), 123.0 (C, C-$_{Ar}$-5), 118.7 (CH, C_{Ar}-3), 27.3 (CH_3, C-8′); mp: 170°C; MS (ESI) *m*/z : calcd. for $C_{16}H_{12}F_3NO_5S$. 387; found 388 $[M+H]^+$.

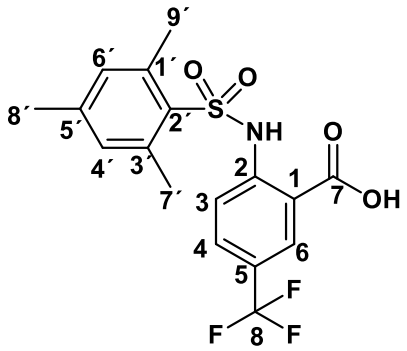
2-(2,3-dihydrobenzo[*b*][1,4]dioxine-6-sulfonamido)-5-(trifluoromethyl)benzoic acid (16)



$C_{16}H_{12}F_3NO_6S$
M=403.3g/mol

(0.4 g, 65% yield) **^{1}H-NMR** (300 MHz, DMSO-d6): δ = 11.48 [s, 1H, *COOH*], 8.13[s, 1H, *NH*] , 7.89 [d, $^4J_{6,4}$ = 3,9 Hz , 1H , 6-H_{Ar}] 7.66 [dd, $^3J_{4,3}$ = 7.2 Hz, $^4J_{4,6}$ = 4.3 Hz, 1H, 4-H_{Ar}], 7.23 [d, $^3J_{4,3}$ = 7,2 Hz 1H, 3-H_{Ar}], 7.11 [dd, $^3J_{2',3'}$ = 7.3Hz , $^4J_{2',8'}$= 3.2Hz , 1H, 2′-HAr] 6.95 [d, $^4J_{2',8'}$= 3.2 Hz, 1H , 8′-H_{Ar}] 4.23 – 4.31 [m, 4H, 5′-H, 6′-H]; - **^{13}C-NMR** (75-MHz, DMSO-d6): δ = 168.9(C, C-8), 148.3(C, C-4′), 143.8 (C, C-2), 143.5 (C, C-7′) , 131.3 (C, C-1′), 130.8 (CH, C-4), 128.6(CH, C-6), 125.7 (C, C-7), 122.1(C, C-5), 120.9(CH, C-2′), 118.3 (CH, C-3), 118.1(CH, C-3′), 116.8 (CH, C-8′) , 64.7(CH_2, C-5′) 64.3 (CH_2, C-6′); mp: 178°C; MS (ESI) *m*/z: calcd. for $C_{16}H_{12}F_3NO_6S$. 403; found 404 $[M+H]^+$.

5-(trifluoromethyl)-2-(2,4,6-trimethylphenylsulfoamido)benzoic acid (17)



$C_{17}H_{16}F_3NO_4S$
M=387.4g/mol

(0.38 g, 62% yield) **^{1}H-NMR** (300 MHz, DMSO-d6): δ = 12.28 [s, 1H, *COOH*], 11.60 [s, 1H, *NH*], 8.15 [d, $^4J_{6,4}$ = 4.3 Hz, 1H, 6-H_{Ar}] 7.92 [dd, $^3J_{4,3}$ =7.9 Hz, $^4J_{4,6}$= 2.1 Hz, 1H,4-H_{Ar}] 7.87 [d, $^4J_{6',4'}$= 1.9 Hz, 2H, 4´-H_{Ar} , 6´-H_{Ar}], 7.48 [d, $^3J_{3,4}$ = 7.9 Hz, 1H, 3-H_{Ar}] , 2.60 [s, 6H, CH_3 , 9´-H, 7´-H], 2.23 [s, 3H, CH_3, 8´-H]; - **^{13}C-NMR** (75 MHz, DMSO-d6): δ = 169.3 (C, C-7), 154.2 (C, C-2), 143.6 (C, C-2´) , 139.1 and 139.1 (2xC, C-1´, C-3´) 132.9 (C, C-5´), 132.5 (CH, C-4), 131.5 and 131.5 (2xCH, C-4´, C-6´) , 130.1(CH, C-6), 128.7 (C, C-8), 122.5 (C, C-5), 117.0 (CH, C-3), 109.0 (C, C-1) ,22.4 and 22.4 (2xCH_3,C-7´, C-9´), 20.8 (CH_3, C-8´) ; mp:184°C; MS (ESI) *m*/z: calcd. for $C_{17}H_{16}F_3NO_4S$; 387: found 388 $[M+H]^+$.