Supplement of

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

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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.

**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: 1H-15N HSQC spectra of Dvl-3 PDZ domain alone (concentration of 50 µM) and in the presence of varying concentrations of compound 1 (25, 75, 100, 150, 200, 300, 400 µM). The arrows indicate the gradual change of chemical shifts with increasing ligand concentration for 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). Three independent biological replicas were performed in each case and error bars represent standard deviations.

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. The data in A and B fitted to a one-site binding model with \( K_D \) determined by \( 1/K_A \) and \( \Delta K_D = \Delta K_A/K_c^2 \) and with \( n=1.14 \) and 1.12, respectively.
6. Structures of selected compounds used for comparison to our compounds

| Compound                                                                                           | Reference(s)                                                                 |
|----------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| 3-((3-[(2-carboxyphenyl)sulfamoyl][phenyl]sulfamoyl)benzoic acid (NPL-1011); (Hori et al., 2018) |                                                                                           |
| NSC668036; (Shan et al., 2005)                                                                      |                                                                                           |
| Ethyl 5-hydroxy-1-(2-oxo-2-((2-(piperidin-1-yl)ethyl)amino)ethyl)-1H-indole-2-carboxylate (KY-02327); (Kim et al., 2016) |                                                                                           |
| Ethyl 1-(2-ethoxy-2-oxoethyl)-5-(tosyloxy)-1H-indole-2-carboxylate (KY-02061); (Kim et al., 2016) |                                                                                           |
| 2-((3-(2-Phenylacetyl)amino)benzoyl)amino)benzoic acid, (CBC-322338/3289-8625); (Grandy et al., 2009, Hori et al., 2018) |                                                                                           |
| NSC668036; (Shan et al., 2005)                                                                      |                                                                                           |
| Ethyl 1-(2-ethoxy-2-oxoethyl)-5-(tosyloxy)-1H-indole-2-carboxylate (KY-02061); (Kim et al., 2016) |                                                                                           |
| Ethyl 5-hydroxy-1-(2-oxo-2-((2-(piperidin-1-yl)ethyl)amino)ethyl)-1H-indole-2-carboxylate (KY-02327); (Kim et al., 2016) |                                                                                           |
| Ethyl 1-(2-ethoxy-2-oxoethyl)-5-(tosyloxy)-1H-indole-2-carboxylate (KY-02061); (Kim et al., 2016) |                                                                                           |

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 (Hori et al., 2018), B) Sulindac (Lee et al., 2009a); C) CBC-322338/3289-8625 (Grandy et al., 2009, Hori et al., 2018) and D) NSC668036 (Shan et al., 2005), A) NPL-1011 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, ΔS = -2.8 kcal/mol, whereas Sulindac shown in B) displayed an KD = 8.3 ± 2.5 µM with N=0.97 ±0.14, ΔH1 = -31.9 ± 5.3 kcal/mol, ΔS1 = 24.9 kcal/mol. C) Compound CBC-322338/3289-8625 and D) NSC668036 did not show any binding to the Dvl-3-PDZ domain under the assay conditions applied.
8. NMR binding assay with 8-fold excess of reference compound 3289-8625

Figure S8: $^1$H$^{15}$N HSQC spectra of Dvl-3 PDZ domain alone (black, concentration of 50 µM) and in the presence of eight-fold excess of compound 3289-8625. For a comparison of effect strength see Figure S2 (8-fold excess is the maximum ligand concentration used there), Table S1 and Table 1.
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 S9c: 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 (2-21)

| ID | R₁ | R₂     | 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  | CH₃    | 0.11              |                   |
| 7  | Br |        | 0.23              | 0.3               |
| 8  | CF₃|        | 0.38              | 0.26              |
| 9  | Cl |        | 0.28              | 0.34              |
| 10 | CH₃|        | 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 | CF₃|        | 0.28              | 0.24              |
| 16 | CF₃|        | 0.36              | 0.08              |
| 17 | CF₃|        | 0.21              | 0.23              |
| 18 | CH₃|        | 0.30              | 0.36              |
| 19 | CH₃|        | 0.36              | 0.32              |
| 20 | CH₃|        | 0.35              | 0.36              |
| 21 | CH₃|        | 0.34              | 0.34              |

**Table S1**: Chemical shift perturbation values of Dvl-3 PDZ and Dvl-1 PDZ for compounds (2 – 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       | P2₁₂₂₁  | P6₁     | 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<sub>meas</sub>*  | 4.4 (57.9) | 3.8 (80.0) | 5.5 (77.4) | 5.8 (105.0) |
| < I / σ(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<sub>work</sub> / R<sub>free</sub> | 0.160 / 0.204 | 0.199/0.249 | 0.179/0.218 | 0.197/0.246 |
| Mean B factor (Å²)  | 16.1     | 24.3     | 21.4     | 20.6     |
| Bond lengths (Å)    | 0.016    | 0.017    | 0.018    | 0.018    |
| Bond angles (°)     | 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.
### 12. Data collection and refinement statistics of compounds 11, 12, 18

| Dvl3 with compound | 11 | 12 | 18 |
|-------------------|----|----|----|
| **Data collection** |    |    |    |
| Space group       | I422 | P6$_1$ | P6$_3$22 |
| $a, b, c$ (Å)     | 78.6, 78.6, 77.8 | 85.3, 85.3, 58.9 | 89.3, 89.3, 131.6 |
| $\alpha, \beta, \gamma$ (°) | 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) |
| $R_{\text{meas}}$* | 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) |
| $R_{\text{work}} / R_{\text{free}}$ | 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 (°)    | 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 |

* Data in highest resolution shell are indicated in parenthesis.

**Table S3**: Data collection and refinement statistics.
13. Selectivity of ligands derived from chemical shift perturbation of compounds tested at other PDZ domains

| CP Id | Dvl-1 | Dvl-3 | PSD95-1 | PSD95-2 | PSD95-3- | Shank-3 | a-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.
| MOLECULE | MW  | COMPANY ID | SMILES CODE |
|----------|-----|------------|-------------|
| $C_6H_7FNO_2S$ | 364.3 | ENAMINE T58 630 40 | $CN1C(=O)CC2=C1C=CC(=C2)S(=O)(=O)NC3=C(C=C(C3)F)C(=O)O$ |
| $C_7H_8FNO_2S$ | 349.4 | ENAMINE T6324911 | $O=C(O)c1ccc(F)cc1NS(=O)(=O)c3eee2CCCCc2c3$ |
| $C_{13}H_{14}FNO_2S$ | 335.4 | ENAMINE T6324915 | $O=C(O)c1ccc(F)cc1NS(=O)(=O)c3eee2CCCCc2c3$ |
| $C_{17}H_{16}FNO_2S$ | 349.4 | ENAMINE T6305470 | $C1CCC2=C(C1)C=CC(=C2)S(=O)(=O)NC3=C(C=C(C3)F)C(=O)O$ |
| $C_6H_7FNO_2S$ | 233.22 | FMP | $CS(=O)(=O)c1ccc(F)cc1C(=O)O$ |
| $C_{17}H_{16}BrNO_2S$ | 410.3 | ENMINE 28744264 | $O=C(O)c1ccc(Br)cc1NS(=O)(=O)c3eee2CCCCc2c3$ |
| $C_{13}H_{14}FNO_2S$ | 399.383 | FMP | $O=C(O)c1ccc(F)cc1NS(=O)(=O)c3eee2CCCCc2c3$ |
| $C_{17}H_{18}ClNO_2S$ | 365.8 | ENAMINE 28775339 | $O=C(O)c1ccc(Cl)cc1NS(=O)(=O)c3eee2CCCCc2c3$ |
| Compound | Formula | MW | Name | Structure |
|----------|---------|-----|------|-----------|
| C₁₀H₁₀NO₅S | 345.4 | ENAMINE | Cc3ee(N(S(=O)=O)c2ee1CCCe1c2)c(C(=O)O)c3 |
| C₁₀H₆BrNO₅S | 406.3 | FMP | O=C(O)c1ee(Br)ce1NS(=O)(=O)ce3cc2eeccc2c3 |
| C₁₀H₆BrNO₅S | 370.22 | FMP | O=C(O)c1ee(Br)ce1NS(=O)(=O)ce3cc2eeccc2c3 |
| C₁₀H₆BrNO₅S | 462.314 | FMP | O=C(O)c1ee(Br)ce1NS(=O)(=O)ce3cc2eeccc2c3 |
| C₁₀H₆BrNO₅S | 398.3 | FMP | O=C(O)c1ee(Br)ce1NS(=O)(=O)ce3cc2eeccc2c3 |
| C₁₀H₆BrNO₅S | 398.3 | FMP | O=C(O)c1ee(Br)ce1NS(=O)(=O)ce3cc2eeccc2c3 |
| C₁₀H₆BrNO₅S | 387.329 | FMP | O=C(O)c1ee(Br)ce1NS(=O)(=O)ce3cc2eeccc2c3 |
| C₁₀H₆BrNO₅S | 403.329 | FMP | O=C(O)c1ee(Br)ce1NS(=O)(=O)ce3cc2eeccc2c3 |
| C₁₀H₆BrNO₅S | 387.372 | FMP | O=C(O)c1ee(Br)ce1NS(=O)(=O)ce3cc2eeccc2c3 |
| Chemical Structure | Formula | Molecular Weight | Database | Code | Structure |
|--------------------|---------|------------------|----------|------|-----------|
| ![Chemical Structure 1](image1.png) | C₃₂H₆₄Cl₅N₁₀O₁₉S₁₀ | 498,939 | ENAMINE | Z1098340488 | Cc4ee(NS(=O)(=O)c3ee(CNC(=O)c1n[nH]e2eeec12)e(Cl)c3)hc(C(=O)O)hc4 |
| ![Chemical Structure 2](image2.png) | C₃₂H₆₄Cl₅N₁₀O₁₉S₁₀ | 497,952 | ENAMINE | Z1098340555 | Cc4ee(NS(=O)(=O)c3ee(CNC(=O)c1n[nH]e2eeec12)e(Cl)c3)hc(C(=O)O)hc4 |
| ![Chemical Structure 3](image3.png) | C₃₂H₆₄Cl₅N₁₀O₁₉S₁₀ | 526,788 | ENAMINE | Z1098340559 | Cc3ee(NS(=O)(=O)c2ee(cNC(=O)c1c[nH]c2)c(Cl)c2)c(C(=O)O)c3 |
| ![Chemical Structure 4](image4.png) | C₃₂H₆₄Cl₅N₁₀O₁₉S₁₀ | 482,337 | ENAMINE | Z1098340560 | Cc3ee(NS(=O)(=O)c2ee(cNC(=O)c1c[nH]c2)c(Cl)c2)c(C(=O)O)c3 |
| ![Chemical Structure 5](image5.png) | C₃₂H₆₄Cl₅N₁₀O₁₉S₁₀ | 476.5 | ENAMINE | EN300-245381 | Cl=CC(c(C(=C1)C(=O)O)NS(=O)(=O)c2=CC=CC(=C2)NS(=O)(=O)c3=C=C(=C3)C(=O)O) |
| ![Chemical Structure 6](image6.png) | C₃₂H₆₄Cl₅N₁₀O₁₉S₁₀ | 374.4 | MERCK | 322338-10MG | Cl=CC(c(C(=C1)C(=O)N)c2=CC=CC(=C2)C(=O)N)c3=CC=c(C(=O)N) |
| ![Chemical Structure 7](image7.png) | C₃₂H₆₄Cl₅N₁₀O₁₉S₁₀ | 460.5 | SIGMA | SML0046 | C(c(C(=O)N)c(C(=O)N)c(O)c(C(=O)N)c(O)c(C(=O)N)c(O)c(C(=O)N)c(C(=O)N)c(O)c(C(=O)N)c(O)c(C(=O)N)c(O) |
| ![Chemical Structure 8](image8.png) | C₃₂H₆₄Cl₅N₁₀O₁₉S₁₀ | 356.4 | SIGMA | S8139-5G | CC1=C(C(=C1)C(=O)O)NC(=O)c(=O)c(C(=O)N)c(O)c(C(=O)N)c(O)c(C(=O)N)c(O)c(C(=O)N)c(O)c(C(=O)N)c(O)c(C(=O)N)c(O) |

*CBC*-322338/3289-8625 (Grandy et al., 2009, Hori et al., 2018)

NSC568036 (Shan et al., 2005)

Sulindac (Lee et al., 2009a)
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)

C₁₈H₁₆F₃NO₄S
M=399.4 g/mol

(0.52 g, 74% yield) ¹H-NMR (300 MHz, DMSO-d6): δ = 11.77 [s, 1H, COOH], 8.13 [s, 1H, NH], 7.85 [d, ⁴J₁₋₃= 2.1 Hz , 1H , 1'⁻-H₉], 7.62 [d, ⁴J₁₋₃= 2.1 Hz , 1H , 1'⁻-H₉], 7.53 [dd, ³J₄₋₃= 7.1 Hz, ⁴J₄₋₆= 2.1 Hz, 4-H₉], 7.36 [dd, ³J₃₋₂= 7.5 Hz, ⁴J₃₋₂= 2.4 Hz, 1H, 3'⁻-H₉], 7.15 [d, ³J₃₋₂= 7.5Hz, 1H,4'-H₉],
6.90 [d, J = 7.1 Hz, H, 3-HAr] 2.73 (m, 4H, CH$_2$); 1.6 (m, 4H, CH$_2$). ¹³C-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$_3$NO$_4$S, 399; found, 400 [M+H].

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

(0.13 g, 67% yield) ¹H-NMR (300 MHz, DMSO-d6): δ = 10.2 [s, 1H, COOH], 9.8 [s, 1H, NH] 8.59 [d, J = 8.9 Hz, H, 3-HAr], 8.26 [d, J = 8.9 Hz, H, 4-HAr], 8.13 [d, J = 8.9 Hz, H, 5-HAr], 7.99 [d, J = 2.4 Hz, H, 6-HAr], 7.77 [dd, J = 8.8 Hz, J = 1.6 Hz, H, 1-H], 7.72 – 7.65 [m, 3H, 4-HAr, 6’-HAr, 7’-HAr], 7.51 [d, J = 8.9 Hz, H, 3-HAr]. ¹³C-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$_{12}$BrNO$_4$S, 403.9560; found, 403.9613 [M-H].
5-bromo-2-(phenylmethylsulfonamido)benzoic acid (12)

\[
\text{C}_{14}H_{12}BrNO_4S
\]

\[M=370.2 \text{ g/mol}\]

(0.07 g, 42% yield) \(^1\text{H-NMR}\) (300 MHz, DMSO-d6): \(\delta = 10.57 \text{ [s, 1H, COOH], 8.05 [d, } ^4\text{J}_{6,4} = 2.4 \text{ Hz, 1H, } \text{H-6}], 7.75 \text{ [dd, } ^3\text{J}_{4,3} = 8.9 \text{ Hz, } ^4\text{J}_{6,4} = 2.4 \text{ Hz ,1H, } \text{H-4}], 7.49 \text{ [d, } ^3\text{J}_{3,4} = 8.9 \text{ Hz,1H, } \text{H-3}], 7.33 – 7.28 \text{ [m, 3H, 3´-H}_{\text{Ar}], 7.23 – 7.20 \text{ [m, 2H, 4´-H}_{\text{Ar}], 5.75 [s, 1H, NH], 4.72 [s, 2H, 1´-H].}\]

\(^{13}\text{C-NMR}\) (75 MHz, DMSO-d6): \(\delta = 168.3 \text{ (C, C-7) , 139.9 (C, C}_{\text{Ar}-2), 137\text{(CH, C}_{\text{Ar}-4), 133.4 (CH, C}_{\text{Ar}-6), 130.7 (CH, C}_{\text{Ar}-3´), 128.6 (C, C}_{\text{Ar}-2´), 128.4 (CH, C}_{\text{Ar}-5´), 128.3 (CH, C}_{\text{Ar}-4´), 119.5 (CH, C}_{\text{Ar}-3), 117.5 (C, C}_{\text{Ar}-1) , 113.9 (C, C}_{\text{Ar}-5) , 57.4 (CH}_{2}, C-1´). \text{Mp: 216°C; (ESI) m/z: calcd.for C}_{14}H_{12}BrNO_4S^-, 367.9860; found , 367.9878 [M-H].}\]

5-bromo-2-(4-(phenoxy)methyl)phenylsulfonamido)benzoic acid (13)

\[
\text{C}_{20}H_{16}BrNO_5S
\]

\[M=462.3 \text{ g/mol}\]

(0.6 g, 29% yield) \(^1\text{H-NMR}\) (300 MHz, DMSO-d6): \(\delta = 7.97 \text{ [d, } ^4\text{J}_{6,4} = 2.4 \text{ Hz, 1H, 6-H]}, 7.85 (d, ^3\text{J}_{2,3} = 8.3 \text{ Hz, 2H, 3´-H}_{\text{Ar}], 7.73 [dd, } ^3\text{J}_{4,3} = 8.9 \text{ Hz, } ^4\text{J}_{6,4} = 2.4 \text{ Hz ,1H, } \text{H-4}], 7.63 [d, } ^3\text{J}_{2,3} = 8.3 \text{ Hz, 2H, 2´-H}_{\text{Ar}], 7.47 [d, } ^3\text{J}_{4,3} = 8.9 \text{ Hz, 1H, 3-H]}, 7.29 [dd, ^3\text{J}_{3,4} = 7.3 \text{ Hz, } ^3\text{J}_{3,4} = 7.3 \text{ Hz, 2H, 3´-H}_{\text{Ar}], 7.00 – 6.92 [m, 3H, 4´-H}_{\text{Ar}], 5.17 [s, 2H, 5´-H]. \text{13C-NMR}\) (75 MHz, DMSO-d6): \(\delta = 168.2 \text{ (C, C-7) , 157.9 (C, C}_{\text{Ar}-1), 143.2 (C, C}_{\text{Ar}-4´), 138.8 (C, C}_{\text{Ar}-2), 137.5 (C, C}_{\text{Ar}-1´) , 136.9 (CH, C}_{\text{Ar}-4), 133.5 (CH, C}_{\text{Ar}-6), 129.4(CH, C}_{\text{Ar}-3´), 128.1(CH, C}_{\text{Ar}-2´),127.0 (CH, C}_{\text{Ar}-3´), 120.9 (CH, C}_{\text{Ar}-4´), 120.5 (CH, C}_{\text{Ar}-3), S. 23}
5-bromo-2-(2,4,6-trimethylphenylsulfoamido)benzoic acid (14)

\[
\begin{align*}
\text{C}_{16}\text{H}_{16}\text{BrNO}_4\text{S} \\
M=398.3 \text{ g/mol}
\end{align*}
\]

(0.6 g, 78% yield) \textsuperscript{1}H-NMR (300 MHz, DMSO-d\text{6}): $\delta = 11.77$ [s, 1H, COOH], 9.98 [s, 1H, NH], 7.68 [d, $^4J_{6,4} = 2.4$ Hz, 1H, 6-H\text{Ar}], 7.51 [d, $^3J_{J,3} = 7.1$ Hz, 1H 4-H\text{Ar}], 7.17 [d, 2H, 4'-H\text{Ar}, 6'-H\text{Ar}], 7.14 [d, $^3J_{J,3} = 1\text{H}, 3$-H\text{Ar}], 2.56 [s, 6H, CH\text{3}, 9'-H, 7'-H], 2.21 [s, 3H, CH\text{3}, 8'-H]. \textsuperscript{13}C-NMR (300 MHz, DMSO-d\text{6}): $\delta = 168.8$ (C, C-7), 143.3 (C, C\text{Ar}-2), 139.5 (C, C\text{Ar}-2'), 139.0 and 139.0 (2xC, C\text{Ar}-3', C\text{Ar}-1') 137.3 (CH, C\text{Ar}-4), 134.0 (CH, C\text{Ar}-6'), 133.0 (CH, C\text{Ar}-6), 132.5 and 132.5 (2xC, C\text{Ar}-4', C\text{Ar}-6') 119.1(CH, C\text{Ar}-3), 117.9(C, C\text{Ar}-5), 114.3 (C, C\text{Ar}-1), 22.5 and 22.5 (2 x CH\text{3}, C-7', C-9') 20.7 (CH\text{3}, C-8'); mp: 185; MS (ESI): $m/z$ calcd for C\text{16}H\text{16}BrNO\text{4}S, 397; found, 398 [M+H]\text{+}.

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

\[
\begin{align*}
\text{C}_{16}\text{H}_{12}\text{F}_3\text{NO}_5\text{S} \\
M=387.3 \text{ g/mol}
\end{align*}
\]

(0.4 g, 63% yield) \textsuperscript{1}H-NMR (300 MHz, DMSO-d\text{6}): $\delta = 12.28$ [s, 1H, COOH], 12.10 [s, 1H, NH], 8.11 [d, $^4J_{6,4} = 2.5$ Hz, 1H, 6-H\text{Ar}], 8.08 [d, $^3J_{J,3} = 7.5$ Hz, 2H, 3'-H\text{Ar}], 7.86 [dd, $^4J_{4,6} = 2.5$ Hz, $^3J_{J,3} = 7.3$Hz, 1H, 4-H\text{Ar}], 7.64 [d, $^3J_{J,3} = 7.3$ Hz, 1H, 3-H\text{Ar}], 7.56 [dd, $^4J_{J,3} = 7.5$Hz, $^4J_{2,6} = 2.3$Hz, 2H, 2'-H\text{Ar}, 6'-H\text{Ar}] 7.22 [dd, $^3J_{J,3} = 7.5$Hz, $^4J_{J,3} = 2.1$Hz, 2H, 3'-H\text{Ar}, 5'-H\text{Ar}] 2.50 [s, 3H, CH\text{3}, 8'-H]. \textsuperscript{13}C-NMR
(75 MHz, DMSO-d6): $\delta = 197.9$ (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-3), 140.6 (2xCH, C_Ar-4), 131.4 (CH, C_Ar-5), 129.6 (2xCH, C_Ar-6, C_Ar-5'), 128.6 (2xCH, C_Ar-2', C_Ar-6'), 128.4 (2xCH, C_Ar-3, C_Ar-4'), 127.6 (C, C_Ar-6), 123.0 (C, C_Ar-5), 118.7 (CH, C_Ar-3), 7.89 (d, $J_{6,4}=2.5$ Hz, 1H, 6-HAr) ; mp: 178°C; MS (ESI) $m/z$ calcld. for C_{16}H_{12}F_3NO_6S, 403; found, 404 [M+H]^+.

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

$^{1}H$-NMR (300 MHz, DMSO-d6): $\delta = 11.48$ [s, 1H, COOH], 8.13 [s, 1H, NH], 7.89 [d, $J_{6,4}=2.5$ Hz, 1H, 6-HAr], 7.66 [d, $J_{4,3}=7.2$ Hz, $J_{4,6}=2.5$ Hz, 1H, 4-HAr], 7.23 [d, $J_{4,3}=8.1$ Hz 1H, 3-HAr], 7.11 [dd, $J_{2,3}=7.3$Hz, $J_{2,8}=3.2$Hz, 1H, 2'-HAr] 6.95 [d, $J_{2,8}=3.2$ Hz, 1H, 8'-HAr]. $^{13}C$-NMR (75-MHz, DMSO-d6): $\delta = 168.9$(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) $m/z$: calcld. for C_{16}H_{12}F_3NO_6S, 403; found, 404 [M+H]^+. 

S. 25
5-(trifluoromethyl)-2-(2,4,6-trimethylphenylsulfoamido)benzoic acid (17)

\[
\begin{align*}
\text{C}_{17}\text{H}_{16}\text{F}_{3}\text{NO}_{4}\text{S} \\
M=387.4\text{g/mol}
\end{align*}
\]

(0.38 g, 62% yield) \(^1\text{H-NMR}\) (300 MHz, DMSO-d6): \(\delta = 12.28 \text{[s, 1H, COOH]}, 11.60 \text{[s, 1H, NH]}, 8.15 \text{[d, } \^4J_{6,4}=2.1 \text{Hz, 1H, } 6\text{-H}\text{Ar}]) 7.92 \text{[dd, } \^3J_{4,3}=7.9 \text{Hz, } \^4J_{4,6}=2.1 \text{Hz, 1H, } 4\text{-H}\text{Ar}]) 7.87 \text{[d, } \^4J_{6',4}=1.9 \text{Hz, 2H, } 4'\text{-H}\text{Ar, 6'}\text{-H}\text{Ar}]), 7.48 \text{[d, } \^3J_{3,4}=7.9 \text{Hz, 1H, } 3\text{-H}\text{Ar}], 2.60 \text{[s, 6H, } \text{CH}_3\text{, 9'-H, 7'-H}], 2.23 \text{[s, 3H, } \text{CH}_3\text{, 8'-H}]. \(^{13}\text{C-NMR}\) (75 MHz, DMSO-d6): \(\delta = 169.3 \text{(C, C-7), 154.2 (C, C-2), 143.6 (C, C-2'), 139.1 and 139.1 (2xC, C-1', C-3')}\), 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\text{, C-7', C-9'}), 20.8 (CH\text{, C-8'}) \text{; mp:184°C; MS (ESI) } m/z: \text{calcd. for } \text{C}_{17}\text{H}_{16}\text{F}_{3}\text{NO}_{4}\text{S, 387; found, 388 [M+H]^+}.\]