Supporting Information

Allosteric site on SHIP2 identified through fluorescent ligand screening and crystallography: a potential new target for intervention

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Docking files: pdb files for receptor (SHIP2), SHIP2-monomer.pdb; 2-FAM-InsP5, pose-P3.pdb; pose-P4.pdb.
Table S1: Binding of FAM/FITC-InsPs to SHIP2cd

|                      | EC₅₀¹ (nM) |
|----------------------|------------|
| 2-FAM-InsP₅          | 57±1       |
| 5-FAM-InsP₅          | 27±2       |
| 2-FAM-Ins(1,4,5)P₃   | 781±1      |
| 1-FITC-InsP₃         | 410±1      |
| 2-FITC-Ins(1,4,5)P₃  | 611±1      |
| scyllo-FITC-InsP₃    | 1018±1     |

¹ EC50 determined in 20mM HEPES, pH 7.3, 1mM MgCl₂
Table S2: X-ray data collection and refinement statistics¹

|                  | 6SRR | 6SQU |
|------------------|------|------|
| **PDB ID**       | 6SRR | 6SQU |
| **Ligands**      | Apo  | 1,2,4-Dimer |
| **Data collection** |     |       |
| Wavelength (Å)   | 0.9762 | 0.9763 |
| Space group      | P 1 21 1 | P 1 21 1 |
| Unit cell        | 45.1, 62.1, 115.6, 90.0, 92.4, 90.0 | 44.4, 60.2, 114.0, 90.0, 92.8, 90.0 |
| Resolution (Å)   | 42.30 - 2.45 (2.51 - 2.45) | 41.35 - 2.27 (2.33 - 2.27) |
| Number of unique reflections | 23610 (1733) | 27911 (2067) |
| Completeness (%) | 99 (100) | 100 (100) |
| Multiplicity     | 4.4 (4.4) | 3.4 (3.5) |
| Rmerge           | 0.096 (1.00) | 0.079 (1.061) |
| Rpim             | 0.052 (0.541) | 0.050 (0.661) |
| Rmeas            | 0.109 (1.141) | 0.094 (1.253) |
| <l/sigma(I)>     | 9.5 (1.7) | 9.6 (1.1) |
| CC1/2            | 1.00 (0.51) | 1.00 (0.58) |
| Wilson B factor (Å²) | 59.79 | 49.57 |
| **Refinement**   |     |       |
| Resolution range | 36.28 – 2.45 (2.54 – 2.45) | 40.68 – 2.27 (2.35 – 2.27) |
| Reflections used in refinement | 23586 (2329) | 27890 (2770) |
| Reflections used for R-free | 1112 (137) | 1344 (134) |
| R-work (%)       | 18.7 (28.5) | 20.1 (29.7) |
| R-free (%)       | 23.7 (34.5) | 25.4 (35.1) |
| Number of non-hydrogen atoms | 4831 | 4781 |
| Protein residues | 600 | 580 |
| RMS(bonds)       | 0.009 | 0.002 |
| RMS(angles)      | 1.00 | 0.52 |
| Ramachandran favoured (%) | 93 | 96 |
| Ramachandran outliers (%) | 0.34 | 0.18 |
| Rotamer outliers (%) | 2.3 | 1.3 |
| Average B-factor (Å²) | 80.38 | 66.09 |
| **Ligand²**      |     |       |
| Occupancy        | -    | 0.91 |
| RSCC²            | -    | 0.89 |
| RSR³             | -    | 0.17 |

¹Data in brackets refers to high resolution bin. ²RSCC = Real space correlation coefficient, ³RSR = Real space R-factor
### Table S3: Top 10 ranked hits from Phenix ligand identification

| Rank | PDB Chemical ID | Ligand                                | No. of atoms | PubChem   | CC\(^1\) | Z-score |
|------|-----------------|---------------------------------------|--------------|-----------|----------|---------|
| 1    | U10             | Ubiquinone-10                         | 63           | 5281915   | 0.42     | 1.77    |
| 2    | FPP             | Farnesyl pyrophosphate                | 24           | 445713    | 0.61     | 1.76    |
| 3    | ARG             | Arginine                              | 12           | 52941769  | 0.64     | 1.66    |
| 4    | 1PG             | Pentaethylene glycol monomethyl ether | 17           | 90255     | 0.63     | 1.52    |
| 5    | TDP             | Thiamin diphosphate                   | 26           | 5431      | 0.57     | 1.45    |
| 6    | 1PE             | Pentaethylene glycol                  | 16           | 62551     | 0.63     | 1.36    |
| 7    | NBN             | N-butyl isocyanide                    | 6            | 76008     | 0.77     | 1.34    |
| 8    | NIO             | Nicotinic acid                        | 9            | 938       | 0.69     | 1.22    |
| 9    | BEZ             | Benzoic acid                          | 9            | 243       | 0.67     | 1.17    |
| 10   | POP             | Pyrophosphate                         | 9            | 4995      | 0.67     | 1.16    |

\(^1\)CC - Local correlation coefficient
Figure S1: Structures of drug-like compounds tested in this study
Figure S2. HPLC assay of Ins(1,3,4,5)P₄-directed SHIP2 5-phosphatase activity. A) reaction catalyzed by SHIP2, and HPLC of products of reaction of SHIP2cd (black trace, duplicated in B and C. A), B) and C) additionally show reaction products that were subsequently spiked with Ins(1,3,4)P₃, (A), Ins(1,4,5)P₃ (B) or Ins(3,4,5)P₃ (C) (gray traces). The product of dephosphorylation co-elutes with Ins(1,3,4)P₃.
Figure S3. Dose-response of inhibition of SHIP2 5-phosphatase activity by biphenyl phosphates and related compounds. BiPh(2,3′,4,5′,6)P₅ (1), BiPh(2′,4,4′,5,5′)P₆ (2), BiPh(3,3′,4,4′,5,5′)P₆ (3), 6,6′-F₂-BiPh(3,3′,4,4′)P₄ (4), 1,2,4-dimer (5). Inositol phosphate reaction products were analysed by HPLC. Conversion of Ins(1,3,4,5)P₄ to Ins(1,3,4)P₃ was determined from integrated peak areas and data fitted to a 4-parameter logistic.
Figure S4. Dose-response of inhibition of SHIP2 5-phosphatase activity. Estramustine sodium phosphate (12), galloflavin (11), purpurogallin (10), 5,6,7,8,4’-pentahydroxyflavone (13), AS1949490 (7) and valrubicin (14). Inositol phosphate reaction products were analysed by HPLC. Conversion of Ins(1,3,4,5)P₄ into Ins(1,3,4)P₃ was determined from integrated peak areas and data fitted to a 4-parameter logistic.
**Figure S5.** Electron density maps for 1,2,4-dimer (5) in its complex with SHIP2cd (PDB 6SQU). A) $2|m|F_o| - |D|F_c|$ difference Fourier electron density map contoured at 1.0σ (grey hatching) for the inhibitor (carbons in white). The view is approximately along the pseudo 2-fold axis relating protein monomers in the asymmetric unit. Individual, protein monomers are shown in wheat (monomer B) and green (monomer A). B) Omit map, $m|F_o| - |D|F_c|$ difference Fourier electron density omit map contoured at 2.5σ (grey hatching). The refined coordinate of 1,2,4-dimer (carbons shown in green) are shown overlayed.
Figure S6. Comparison of binding sites for SHIP1/2 allosteric ligands. Orthogonal views of a molecular surface representation of SHIP1 (PDB entry 6DLG) colored by domain (phosphatase domain in cyan and C2 domain in yellow) with molecular surface contribution of residue K681 colored magenta. K681 forms part of the binding pocket for the allosteric inhibitor ZPR-MN100 \(^{26}\). Overlaid is the equivalent position of the allosteric SHIP2 inhibitor 1,2,4-dimer (5) (PDB 6SQU, carbons in green). The position of the competitive inhibitor BiPh(2,3′,4,5′,6)P\(_5\) (1) (PDB 4A9C, grey carbons) is shown for reference.
Figure S7. Substrate activity of SHIP2 towards inositol phosphate, biphenyl phosphate and related compounds. Phosphate release from Ins(1,3,4,5)P$_4$ and InsP$_6$ assayed with 1 μM SHIP2cd; biphenyl and related compounds with 10 μM SHIP2cd (mean and s.d.).
Figure S8. Fit of geranyl pyrophosphate (19) to active site residual electron density in the crystal structure of the SHIP2cd complex with 1,2,4-dimer (PDB 6SQU). The SHIP2cd dimer is shown in cartoon format (green) with a semi-transparent molecular surface. Selected active site resides are shown in stick format and labeled, as is 1,2,4-dimer shown binding at an allosteric site. Residual difference electron density (pale blue hatching; 2m|Fo| − D|Fc|) contoured at 2.5σ is seen in the active site overlaying the docked position of farnesyl diphosphate (stick format) as determined by the ligand_fit method of Phenix.