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# An allosteric pan-TEAD inhibitor blocks oncogenic YAP/TAZ signaling and overcomes KRAS G12C inhibitor resistance

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**Supplementary Table S1.** Small molecule screening data

| Category | Parameter | Description |
|---|---|---|
| Assay | Type of assay: | In vitro biochemical binding assay using recombinant proteins |
| | Target: | $YAP_{50-100}$ (P46937) - TEAD3 (Q99594) PPI. |
| | Primary measurement: | Detection of TR-FRET signal from the disruption of the YAP-TEAD complex |
| | Key reagents | His6-TEAD3 (217-447), Genentech<br>Biotin-YAP (50-100), Genentech<br>Streptavidin-XL665 (CIS Bio)<br>Anti-His-europium (PerkinElmer) |
| | Assay protocol | See Methods "TEAD YAP/TAZ TR-FRET Assay" |
| | Additional comments | None |
| Library | Library size | 2.2 million compounds |
| | Library composition | Roche and Genentech internal compound collection. Compounds include internally made molecules for all internal programs and externally sourced compounds. |
| | Source | Internal and External |
| | Additional comments | None |
| Screen | Format | Competitive binding assay with a proximity-based readout of time-resolved fluorescencence resonance energy transfer |
| | Concentration(s) tested | 5 uM (2,130,060 compounds), 20 uM (334 compounds), 25 uM (59,156 compounds), 100 uM (5991 compounds). DMSO: 0.5%. |
| | Plate controls | Neutral control: all reaction components with DMSO as sample<br>Blank: all reaction components except TEAD3 with DMSO as sample<br>Reference inhibitor: peptide 17 |
| | Reagent/ compound dispensing system | Reagent: BioRapTR (Beckman); Compound: Echo 555 acoustic dispenser (Beckman) |
| | Detection instrument and software | ViewLux (PerkinElmer). Analysis software: Genedata Screener |
| | Assay validation/QC | RZ' range: 0.44-0.94, RZ' average = 0.68.<br>Signal/Background range: 4.0-11.2;<br>Signal/Background average = 6.8 |
| | Correction factors | None |
| | Normalization | Neutral control – Blank. Analyzed using Genedata Screener software |
| | Additional comments | |
| Post-HTS analysis | Hit criteria | Inhibition >/= 20% |
| | Hit rate | 0.085% (1870 of 2,200,000) |
| | Additional assay(s) | Confirmation in dose-response in YAP/TEAD3 PPI TR-FRET assay; counterscreen in dose-response in TR-FRET assay with 6x-His-Biotin in place of YAP/TEAD3 complex |
| | Confirmation of hit purity and structure | All hits where resynthesized and compounds tested for purity via NMR and LC-MS. |
| | Additional comments | Please see chart for HTS workflow. |

**Supplementary Table S2. X-ray crystallography data collection and refinement statistics**

| | TEAD2/Compound 1<br>PDB: 7TYQ | TEAD2/Compound 2<br>PDB: 7TYU | TEAD2/GNE-7883<br>PDB: 7TYP |
|---|---|---|---|
| **Data collection** | | | |
| Space group | C121 | C121 | C121 |
| Cell dimensions | | | |
| *a*, *b*, *c* (Å) | 114.85, 61.18, 79.95 | 115.27, 61.53, 79.41 | 122.97, 61.43, 80.05 |
| α, β, γ (°) | 90, 111.61, 90 | 90, 111.759, 90 | 90, 117.18, 90 |
| Wavelength (Å) | 1.00000 | 0.97946 | 0.99999 |
| Resolution (Å) | 53.74-1.88 (ellipsoidal)<br>53.74-2.30 (isotropic)<br>(1.91-1.88)* | 53.84-1.78 (ellipsoidal)<br>53.84-2.16 (isotropic)<br>(1.82-1.78) | 71.21-1.60 (ellipsoidal)<br>71.21-1.90 (isotropic)<br>(1.76-1.60) |
| $R_{merge}$ | 0.059 (0.991) | 0.025 (0.576) | 0.032 (0.741) |
| $I / \sigma(I)$ | 16.2 (1.7) | 14.0 (1.5) | 17.2 (1.5) |
| CC(1/2) | 0.999 (0.711) | 1.00 (0.783) | 0.999 (0.652) |
| Completeness | 94.4 (73.1) | 92.2 (61.3) | 89.2 (68.4) |
| Redundancy | 6.8 (7.2) | 3.4 (3.6) | 3.3 (3.5) |
| | | | |
| **Refinement** | | | |
| Resolution (Å) | 74.33-1.88 (anisotropic) | 53.84-1.78 (anisotropic) | 71.21-1.60 (anisotropic) |
| No. reflections | 28082 (1405) | 31238 (1563) | 42783 (2140) |
| $R_{work}$ / $R_{free}$ | 0.2061/0.2472 | 0.1972/0.2298 | 0.1840/0.2156 |
| No. atoms | | | |
| Protein | 3251 | 3268 | 3361 |
| Ligand/ion | 50 | 70 | 90 |
| Water | 66 | 81 | 190 |
| *B*-factors | | | |
| Protein | 49.2 | 51.9 | 37.5 |
| Ligand/ion | 41.3 | 45.2 | 26.7 |
| Water | 45.1 | 48.1 | 44.0 |
| R.m.s. deviations | | | |
| Bond lengths (Å) | 0.004 | 0.004 | 0.01 |
| Bond angles (°) | 0.67 | 0.71 | 1.38 |

*Values in parentheses are for highest-resolution shell.