**Electronic Supplementary Information related to the article**

# High-affinity ligands of the colchicine domain in tubulin based on a structure-guided design

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Includes:

**Table S1.** Data collection and refinement statistics.

| | $T_2$R-TTL-TUB075 | $T_2$R-TTL-TUB015 |
|---|---|---|
| **Data collection**[a] | | |
| Space group | $P2_12_12_1$ | $P2_12_12_1$ |
| Cell dimensions | | |
| *a, b, c* (Å) | 104.7, 157.5, 180.0 | 104.8, 157.8, 180.0 |
| Resolution (Å) | 49.45 – 2.15 (2.21 – 2.15) | 49.44 – 2.10 (2.15 – 2.10) |
| $R_{meas}$ (%) | 11.8 (257.7) | 11.4 (228.1) |
| $R_{pim}$ (%) | 3.4 (88.5) | 3.3 (80.3) |
| $CC_{1/2}$[b] | 99.9 (30.0) | 99.9 (34.9) |
| I/σI | 16.5 (0.8) | 16.7 (0.8) |
| Completeness (%) | 99.5 (96.3) | 98.5 (91.8) |
| Redundancy | 12.5 (8.9) | 12.3 (8.5) |
| **Refinement** | | |
| Resolution (Å) | 49.45 – 2.15 | 49.44 – 2.10 |
| No. unique reflections | 161351 | 171487 |
| $R_{work}/R_{free}$ (%) | 17.5 / 20.6 | 17.8 / 21.7 |
| Average B-factors (Å$^2$) | | |
| Complex | 60.7 | 57.0 |
| Solvent | 53.8 | 49.7 |
| Ligand (chain B/D) | 39.6 / 50.2 | 37.9/ 52.6 |
| Wilson B-factor | 42.5 | 39.7 |
| Root mean square deviation from ideality | | |
| Bond length (Å) | 0.004 | 0.003 |
| Bond angles (°) | 0.719 | 0.613 |
| Ramachandran statistics[c] | | |
| Favored regions (%) | 98.20 | 97.9 |
| Allowed regions (%) | 1.8 | 2.1 |
| Outliers (%) | 0 | 0 |

[a]Highest shell statistics are in parentheses. [b]CC1/2= percentage of correlation between intensities from random half-datasets[1] [c]As defined by MolProbity[2].

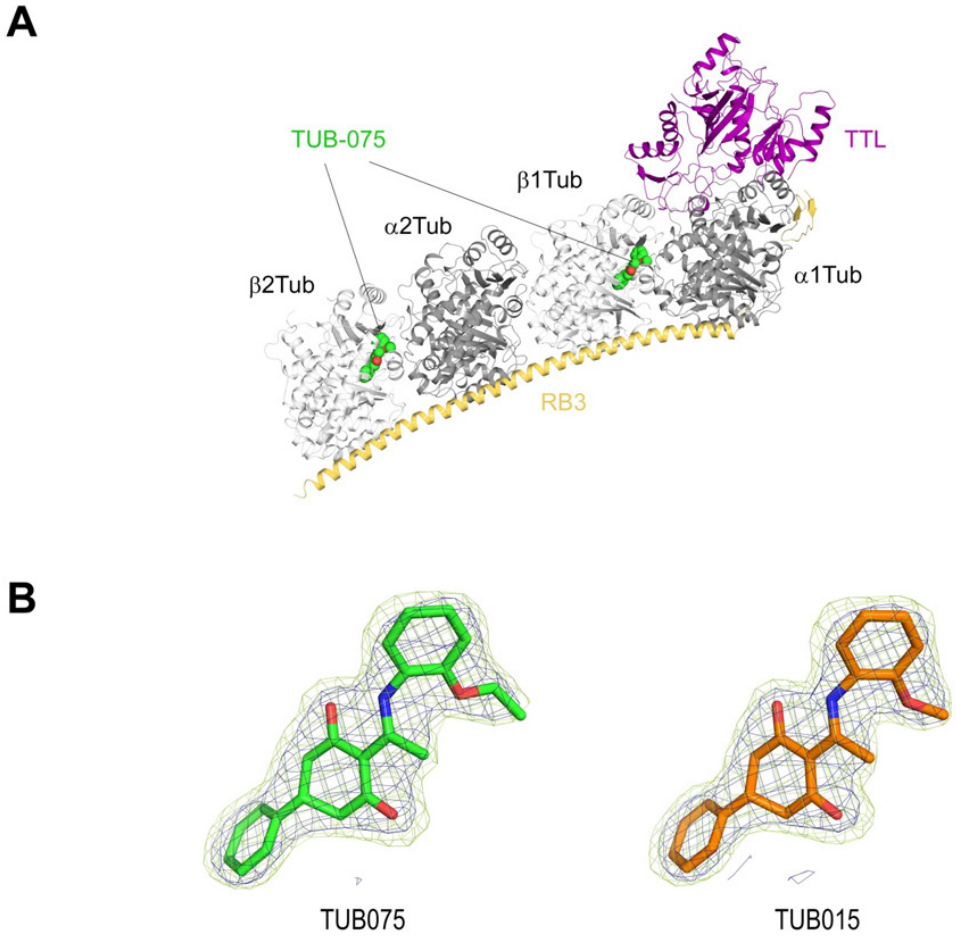


**Figure S1. The T2R-TTL-TUB075 complex and ligand details.** (**A**) Overall view of the $T_2$R-TTL-TUB075 complex structure. The α-tubulin and β-tubulin chains are in dark and light grey, TTL is in purple and RB3 is in yellow orange ribbon representation, respectively. The tubulin-bound TUB075 molecules are represented as green spheres, respectively. (**B**) Electron-density maps of both the TUB075 and TUB015 ligands bound to tubulin in their corresponding $T_2$R-TTL complexes. The SigmaA-weighted 2mFo - DFc (dark blue mesh) and mFo - DFc (light green mesh) omit maps are contoured at +1.0s and +3.0s, respectively. The map calculations excluded the atoms of the TUB ligands.

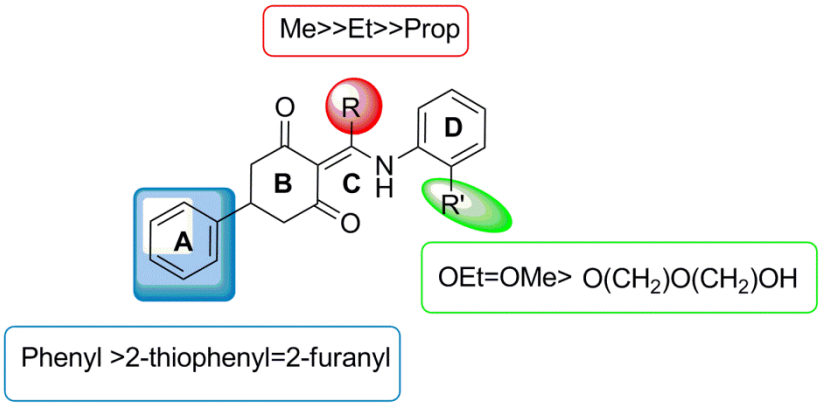


**Figure S2**. **Established SAR for cyclohexanediones**. Fragments of the cyclohexanedione derivatives and schematic representation of the most relevant structure-activity relationships established.[3,4]

**References**

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