Supporting Information

Supplemental Experimental Section

Biochemical characterization

The constants defining the binding of the compounds to stabilized microtubules were measured as described[1]. The critical concentration (Cr) for tubulin assembly in the presence of the compounds in GAB buffer (3.4 M glycerol, 10 mM sodium phosphate (NaPi), 1 mM EGTA, 6 mM MgCl2, 1 mM GTP, pH 6.7) was measured as reported earlier[2].

Crystallization, data collection and structure determination

Crystals of T_2R-TTL were grown as described by Prota et al.[3]. Briefly, crystals were grown in the presence of 0.5 mM compound (0-3% PEG 4K, 1-6% glycerol, 30 mM $CaCl_2$, 30 mM $MgCl_2$, 0.1 M MES/imidazole pH 6.7, 1 mM AMPPCP, 10 mM DTT, 0.1 mM GDP). Before cryo-cooling, the crystal containing drop was supplemented stepwise with cryo-solution (crystallization buffer containing 10% PEG 4K and 20% glycerol and 1 mM DDM or KS-1-199-32). After five min, crystals were transferred to a fresh drop of cryo-solution (see above) for a short incubation and were flash cooled. Standard data collection at beamline X06DA at the Swiss Light Source (Paul Scherrer Institut, Villigen PSI, Switzerland), data processing and structure solution using the difference Fourier method were performed as described previously[3]. Data collection and refinement statistics are given in Table S1.

Chains in the T_2R-TTL complex were defined as follows: chain A, α1-tubulin; chain B, β1-tubulin; chain C, α2-tubulin; chain D, β2-tubulin; chain E, RB3; chain F, TTL (Figure S1A). Chain D was used throughout for the structural analysis and figure preparation. Structure visualization, molecular editing and figure preparation were performed with The PyMOL molecular graphics system (The PyMOL Molecular Graphics System, Version 1.5.0.5. Schrödinger, LLC).

Supplemental Table S1. Data collection and refinement statistics.

	T_2R-TTL-DDM	T_2R-TTL-KS-1-199-32
Data collection[a]		
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a, b, c (Å)	104.5, 157.8, 180.2	104.6, 157.8, 179.4
Resolution (Å)	52.25 - 1.9 (1.95 – 1.90)	72.2 – 2.2 (2.26 – 2.20)
R_{meas} (%)	10.6 (320.9)	22.9 (411.6)
R_{pim} (%)	3.3 (95.1)	7.0 (119.7)
$CC_{1/2}$[b]	100.0 (33.3)	99.8 (30.9)
I/σI	18.7 (0.9)	12.5 (0.75)
Completeness (%)	100.0 (99.8)	100.0 (100.0)
Redundancy	13.3 (12.5)	13.3 (12.9)
Refinement		
Resolution (Å)	52.25 - 1.9	72.2 – 2.2
No. unique reflections	233651	150705
R_{work}/R_{free} (%)	17.3 / 20.8	18.9 / 22.9
Average B-factors (Å^2)		
Complex	48.9	52.9
Solvent	51.9	50.7
Ligands (chain B/D)	65.7 / 58.9	65.2 / 54.7
Wilson B-factor	32.8	36.7
Root mean square deviation from ideality		
Bond length (Å)	0.005	0.002
Bond angles (°)	0.912	0.627
Ramachandran statistics[c]		
Favored regions (%)	97.9	97.4
Allowed regions (%)	2.1	2.5
Outliers (%)	0	0.1

[a]Highest shell statistics are in parentheses. [b]$CC_{1/2}$= percentage of correlation between intensities from random half-datasets[4]. [c]As defined by MolProbity[5].

Supplemental Figure Legends

Supplemental Figure 1. Chemical structures of (**A**) (+)-Discodermolide, (**B**) KS-1-199-32[6] and (**C**) paclitaxel. The section of the C13-sidechain moiety of paclitaxel that was modelled into DDM to generate the hybrid KS-1-199-32 envisaging to occupy the aromatic pocket on β-tubulin is highlighted in red in (**B**), (**C**) and (**D**).

Supplemental Figure 2. (**A**) Overall view of the T_2R-TTL-DDM complex structure. The α-tubulin and β-tubulin chains are in dark and light grey, TTL is in light blue and RB3 is in yellow ribbon representation, respectively. The tubulin-bound DDM molecules are represented as purple spheres. The nucleotides in each of the four tubulin subunits are in stick representation and are labelled. (**B**) Superposition of the NMR (grey), the docked (light blue) and the X-ray (purple) DDM structures. (**C**) Superposition of the taxane sites of both the T_2R-TTL-DDM (grey/purple) and the tubulin-DDM complex suggested from docking studies (light blue) highlighting the agreement in binding mode.

Supplemental Figure 3.

Close-up view of the superimposed paclitaxel-stabilized microtubule (green ribbon and sticks, white surface, PDB ID 3J6G) and tubulin–DDM (purple ribbon and sticks) complexes. The structures are superimposed on their taxane pockets (Gly225-Ser236, Leu208-Leu219, Pro360-Ser374, Phe272-Thr276, and Leu286-Met295) rmsd 0.870 (46 Cα atoms). Key interacting residues are in stick representation and are labelled.

Supplemental References

[1] R. Matesanz, I. Barasoain, C. G. Yang, L. Wang, X. Li, C. de Ines, C. Coderch, F. Gago, J. J. Barbero, J. M. Andreu, W. S. Fang, J. F. Diaz, *Chem Biol* **2008**, *15*, 573-585.

[2] R. M. Buey, I. Barasoain, E. Jackson, A. Meyer, P. Giannakakou, I. Paterson, S. Mooberry, J. M. Andreu, J. F. Diaz, *Chem Biol* **2005**, *12*, 1269-1279.

[3] a) A. E. Prota, K. Bargsten, D. Zurwerra, J. J. Field, J. F. Diaz, K. H. Altmann, M. O. Steinmetz, *Science* **2013**, *339*, 587-590; b) A. E. Prota, M. M. Magiera, M. Kuijpers, K. Bargsten, D. Frey, M. Wieser, R. Jaussi, C. C. Hoogenraad, R. A. Kammerer, C. Janke, M. O. Steinmetz, *J Cell Biol* **2013**, *200*, 259-270.

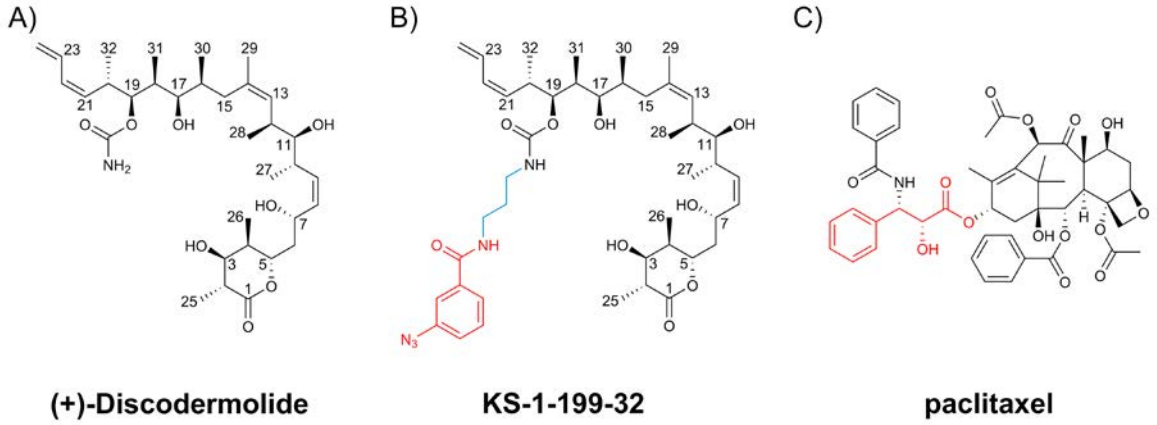
[4] P. A. Karplus, K. Diederichs, *Science* **2012**, *336*, 1030-1033.

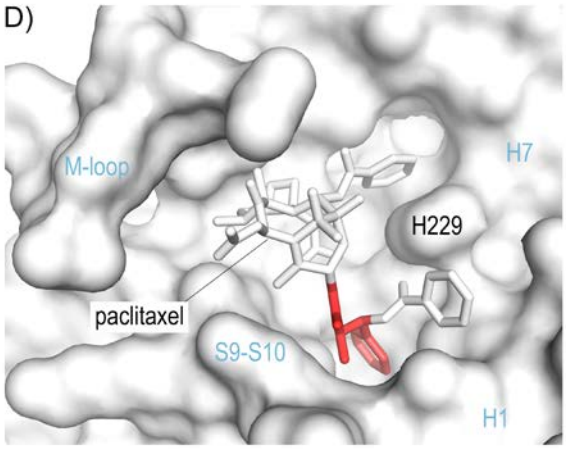
[5] I. W. Davis, L. W. Murray, J. S. Richardson, D. C. Richardson, *Nucleic Acids Res* **2004**, *32*, W615-619.

[6] A. B. Smith, K. Sugasawa, O. Atasoylu, C. P. Yang, S. B. Horwitz, *J Med Chem* **2011**, *54*, 6319-6327.

Supplemental Figures

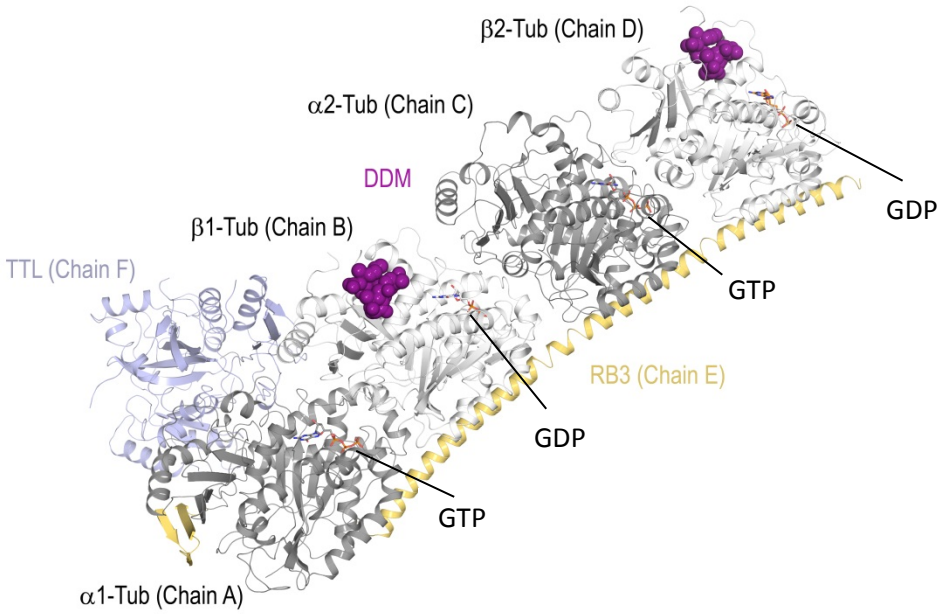
Suppl. Fig.1





Suppl. Fig.2

A)



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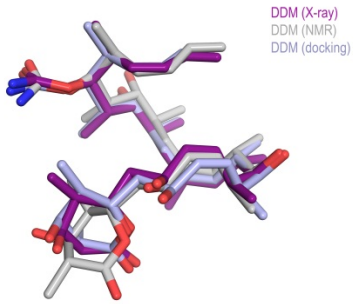
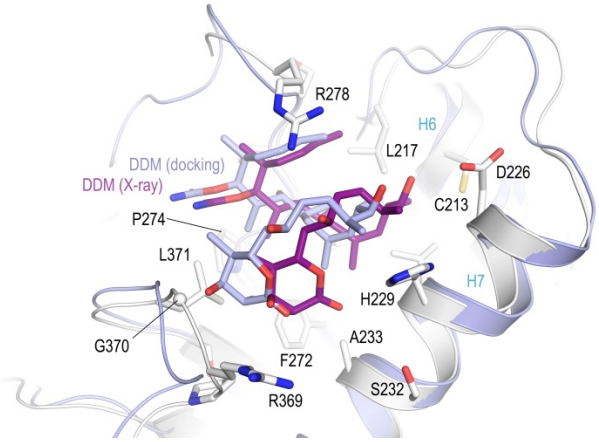
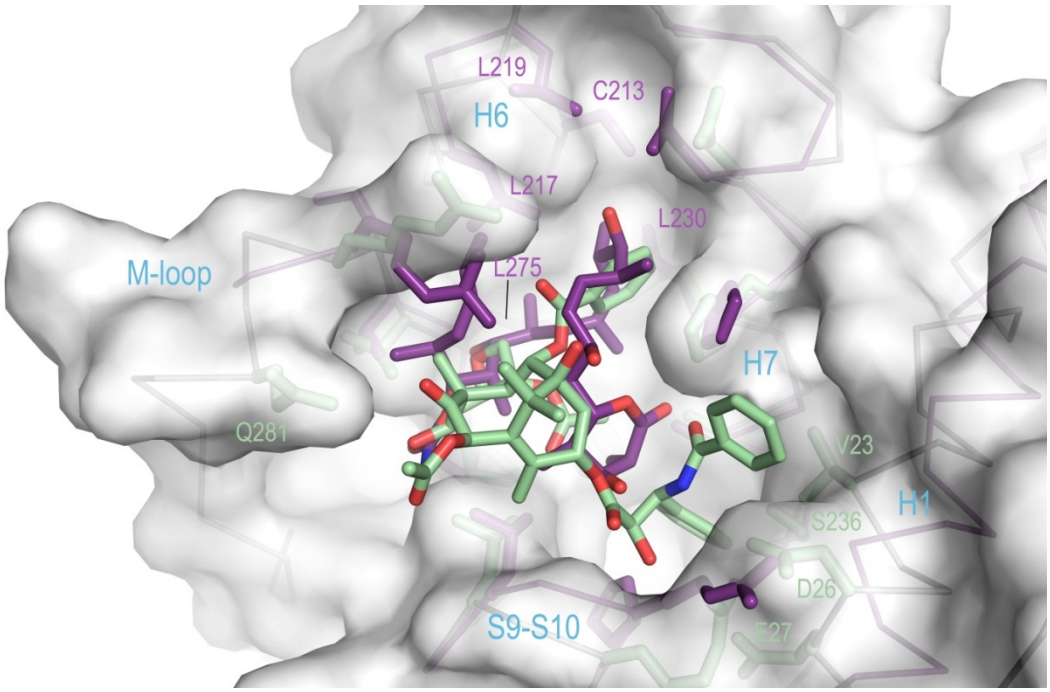


C)



Suppl. Fig.3


L219
C213
H6
L217
L230
M-loop
L275
H7
Q281
V23
H1
S236
D26
S9-S10
E27