Supporting Information

Prota et al. 10.1073/pnas.1408124111

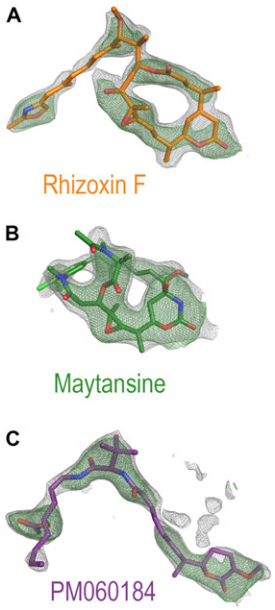

Fig. S1. Simulated annealing omit maps of the three microtubule-destabilizing agents investigated in this study. Simulated annealing omit maps of rhizoxin F (*A*), maytansine (*B*), and PM060184 (*C*) in the corresponding tubulin tyrosine ligase complex structures. The SigmaA-weighted 2mFo–DFc (gray mesh) and mFo–DFc (green mesh) electron density maps are contoured at 0.7σ and +3.0σ, respectively. The rhizoxin F, maytansine, and PM060184 molecules are shown in stick representation.

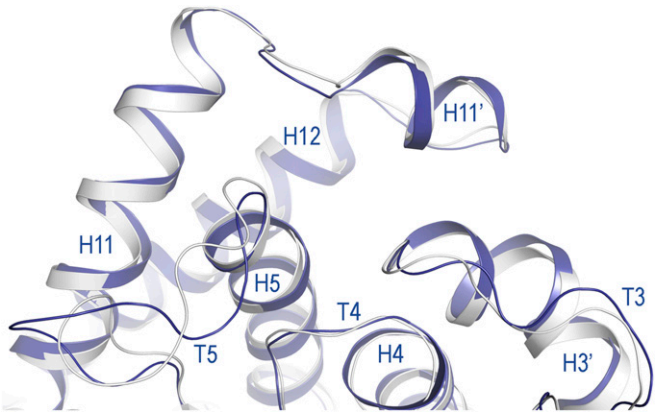

Fig. S2. Maytansine site in the straight and curved conformational states of tubulin. Superimposition of the maytansine site in the curved (gray) and straight (blue; PDB ID no. 1JFF) conformational states of tubulin are shown in ribbon representation. Only the backbone of the rhizoxin complex is displayed. Selected secondary structure elements are labeled. The binding sites of rhizoxin F, maytansine, and PM060184 superimpose onto straight tubulin with an rmsd of 0.65 Å (70 C_α atoms), 0.63 Å (72 C_α atoms), and 0.68 Å (74 C_α atoms), respectively.

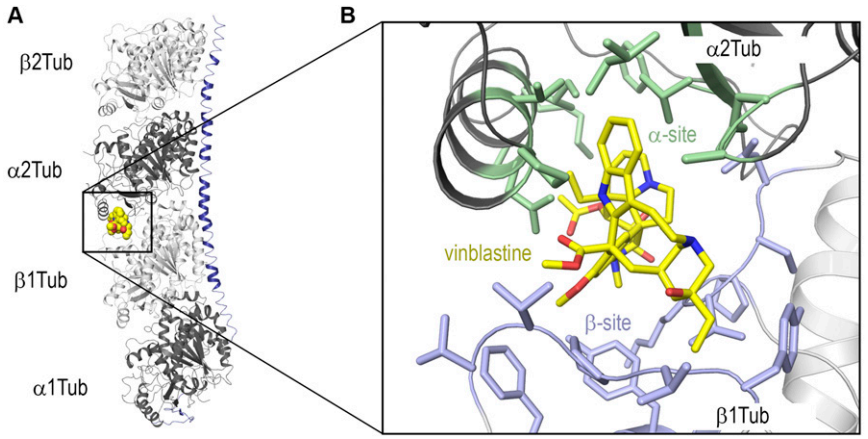

Fig. S3. Structure of the tubulin–vinblastine complex highlighting the composite binding site of the drug. (*A*) Overall view of the T_2R–vinblastine complex (PDB ID no. 1Z2B). Tubulin (gray) and RB3 (blue) are in ribbon and the ligand vinblastine (yellow) is in sphere representation, respectively. The box marks the area shown in detail in *B*. (*B*) Zoom into the composite vinblastine binding site. Vinblastine (yellow) and the binding site residues contributed by β-tubulin (β-site, slate) and α-tubulin (α-site, green) are shown in stick representation.

Table S1. Data collection and refinement statistics

	Tubulin–Rhizoxin F (PDB ID no. 4TUY)	Tubulin–Maytansine (PDB ID no. 4TV8)	Tubulin–PM060184 (PDB ID no. 4TV9)[§]
Data collection*			
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions			
a, *b*, *c*, Å	104.3, 156.9, 181.1	103.9, 155.6, 180.6	104.2, 157.3, 180.2
Resolution, Å[†]	68.4–2.1 (2.15–2.10)	62.4–2.1 (2.16–2.10)	52.0–2.0 (2.05–2.00)
R_{meas}, %	17.1 (252.0)	14.4 (479.7)	10.5 (278.4)
R_{pim}, %	4.0 (95.7)	4.2 (148.0)	1.7 (66.4)
$CC_{1/2}$[‡]	99.9 (58.2)	99.9 (20.6)	100.0 (18.4)
I/σI	16.9 (1.3)	14.2 (0.6)	23.7 (1.1)
Completeness, %	100 (100)	99.6 (94.5)	99.9 (99.1)
Redundancy	13.6 (14.3)	13.4 (12.1)	37.6 (20.1)
Refinement			
Resolution, Å	62.7–2.1	62.4–2.1	52.0–2.2
No. unique reflections	173178	169427	199354
R_{work}/R_{free}, %	18.6/21.7	19.9/21.7	18.0/20.7
Average B-factors, Å^2			
Complex	51.3	66.8	60.9
Solvent	52.3	63.2	57.7
Ligand	63.3	93.8	70.9
Wilson B-factor	36.8	50.0	44.9
rmsd from ideality			
Bond length, Å	0.002	0.005	0.003
Bond angles, °	0.658	0.979	0.710
Ramachandran statistics[¶]			
Favored regions, %	98.0	97.6	97.7
Allowed regions, %	2.0	2.4	2.2
Outliers, %	0	0	0.1

*Highest shell statistics are in parentheses.
[†]The resolution cutoff was selected based on I/σI and $CC_{1/2}$ according to Karplus and Diederichs (1).
[‡]$CC_{1/2}$ = percentage of correlation between intensities from random half-data sets (1).
[¶]As defined by MolProbity (2).
[§]Diffraction data sets were collected and merged from three crystals.

1. Karplus PA, Diederichs K (2012) Linking crystallographic model and data quality. *Science* 336(6084):1030–1033.
2. Davis IW, Murray LW, Richardson JS, Richardson DC (2004) MOLPROBITY: Structure validation and all-atom contact analysis for nucleic acids and their complexes. *Nucleic Acids Res* 32 (Web Server issue):W615-9.