Supplementary Table

Supplementary Table 1. Related to Figure 6. X ray crystallography data collection and refinement statistics.	
Data Collection[a]	
Wavelength (Å)	1
Resolution range (Å) [b]	48.09 - 2.394 (2.479 - 2.394)
Space group	P 21 21 21
Unit cell a, b, c (Å) α, β, γ (°)	103.4 158.4 173.5 90 90 90
Total reflections	1501761 (135942)
Unique reflections	112238 (10823)
Multiplicity	13.4 (12.6)
Completeness (%)	99.7 (96.8)
Mean I/sigma(I)	18.6 (1.3)
Wilson B-factor	50.9
R-merge	0.142 (2.041)
R-meas	0.148
CC1/2[c]	0.999 (0.425)
CC*	1 (0.772)
Refinement	
R-work	0.2175 (0.3415)
R-free	0.2371 (0.3652)
Macromolecules	17264
Ligands	185
Water	361
Protein residues	2171
RMS(bonds) (Å)	0.009
RMS(angles) (°)	1.42
Ramachandran favored (%)[d]	98
Ramachandran outliers (%)[d]	0
B-factors	
Average B-factor	62.7
Macromolecules	62.9
Ligands	59.2
Solvent	53.4

[a] Highest resolution shell statistics are in parentheses.
[b] Resolution cutoffs were chosen based on CC1/2 and Mean I/sigma(I) (PMID: 22628654)
[c] As defined by Karplus and Diederichs (PMID: 22628654)
[d] As defined by MolProbity (PMID: 15215462)

Supplementary Figures.

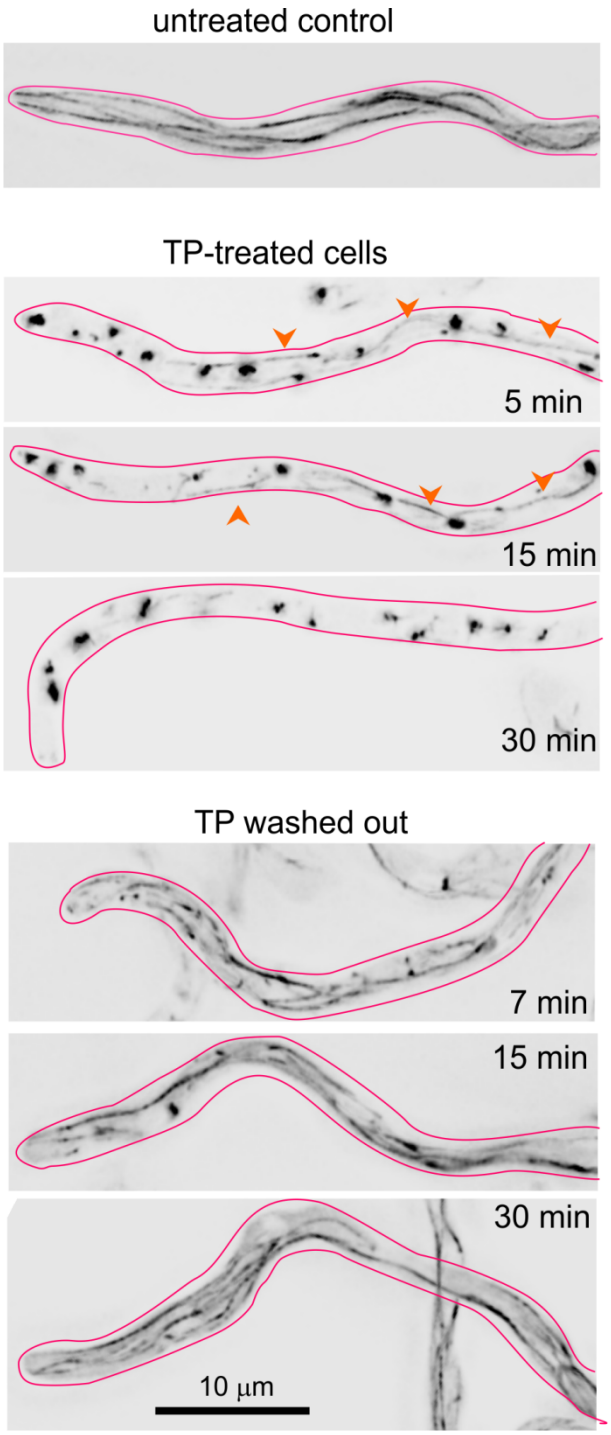

Figure S1. Related to Figure 2.- Effect of compound 1 on *Aspergillus nidulans* MTs visualized with alpha-tubulin-GFP. Cells were treated with 20 uM compound **1**, which resulted in disruption of the MT cytoskeleton and the formation of tubulin-GFP 'clumps' in 5 min, with some MTs persisting at the 5 min and 15 min time points (orange arrowheads). At 30 min MTs were hardly, if at all, visible and cells had acquired the characteristic 'hook morphology' resulting from inhibition of MT-dependent transport. This pronounced effect on MTs was quickly reversed after removing the drug. Recovery of the normal MT cytoskeleton was noticeable as early as 7 min after washing out the inhibitor with fresh medium, nearly complete after 15 min and complete after 30 min. Images were deconvolved with the 'blind' settings of Huygens deconvolution software and converted to inverted greyscale. Photographs are maximal intensity projections of z-stacks of images (z = 125 nm) covering the complete width of the tubular cells, which were contoured in magenta. All images as shown at the same magnification, indicated by the scale bar.

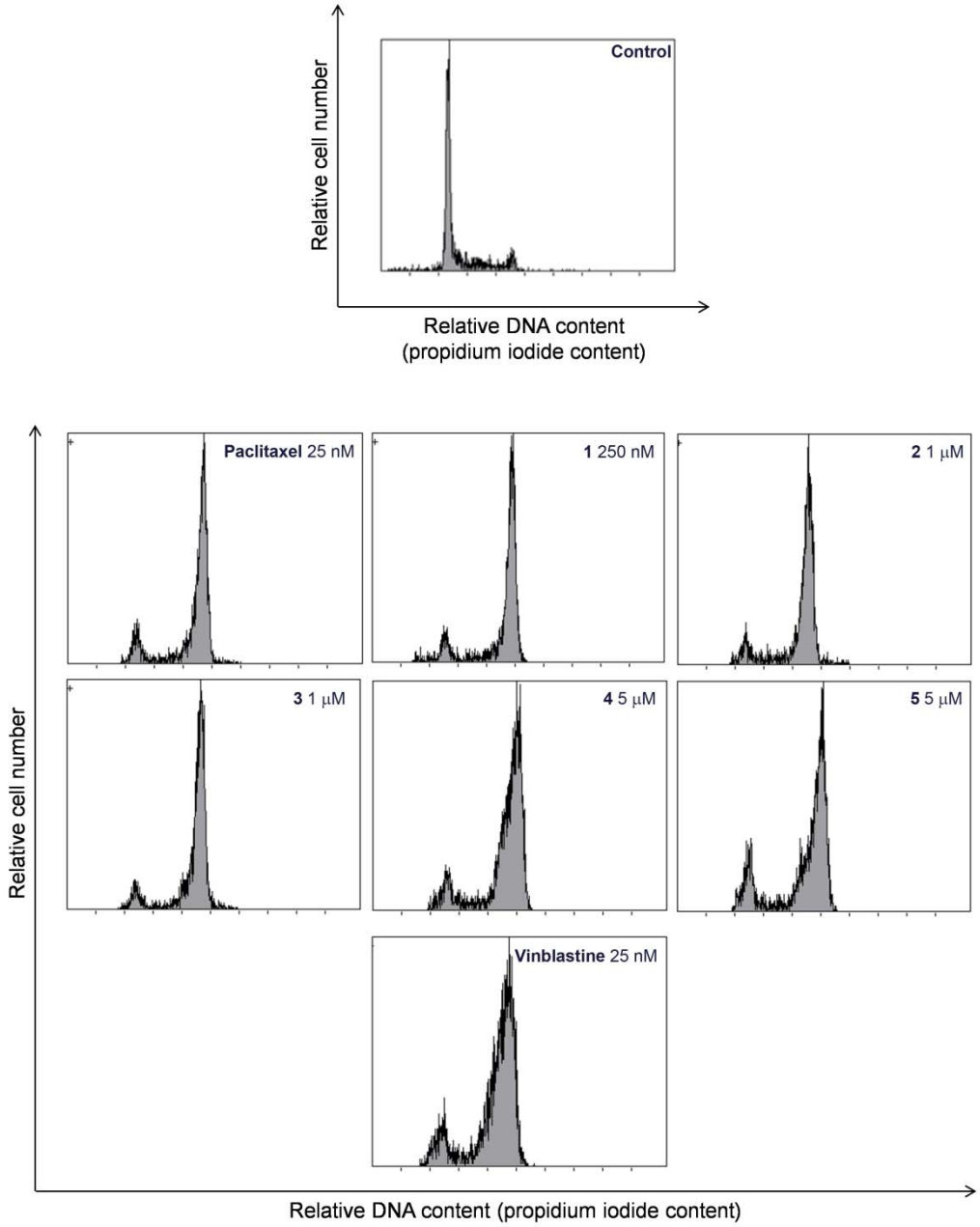

Figure S2. Related to Figure 2.- Effect of the compounds, vinblastine and paclitaxel in the cell cycle of A549 cells. The profiles shown are those in the presence of the minimal concentration of drug able to stop the cell cycle in the G_2/M phase.

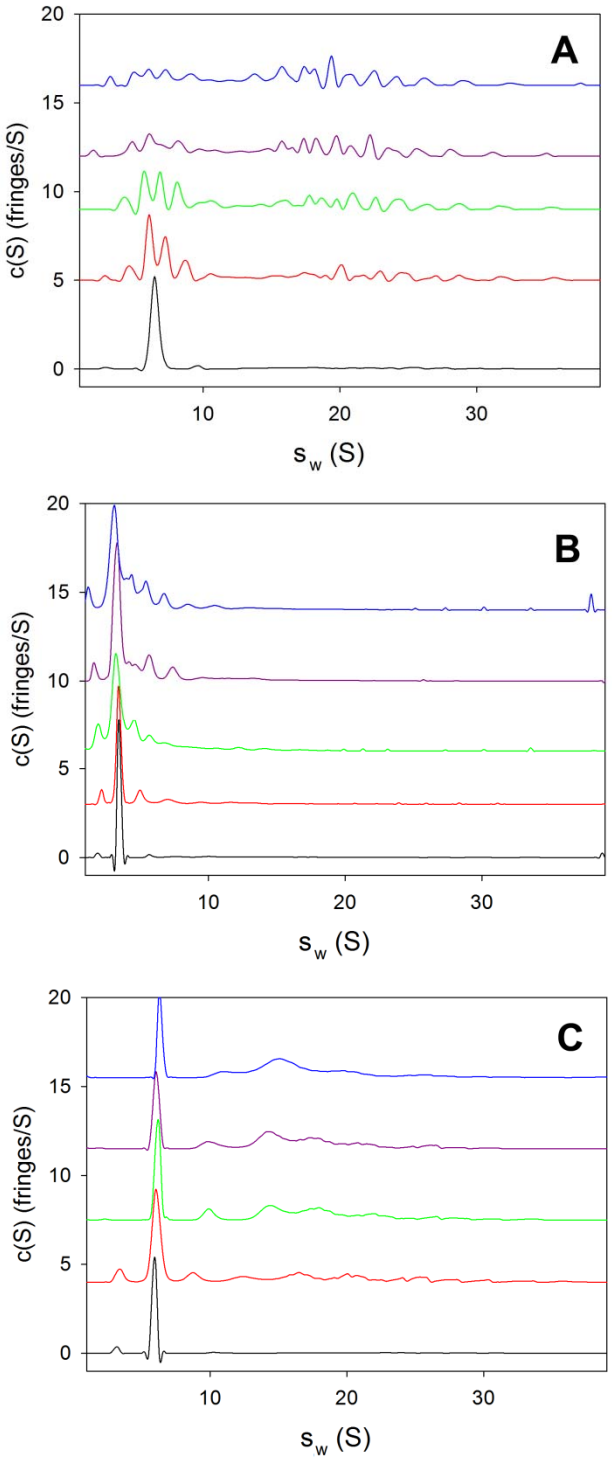

Figure S3. Related to Figure 4.- Effect of nucleotide, temperature and Mg^{+2} in TP-induced tubulin oligomerization. (A) Sedimentation coefficients distribution c(s) of 30 μM GDP-tubulin at 25ºC in PEDTA-GDP 1.5 in the presence of no ligand (black line), 15 μM of **1** (red line), 25 μM of **1** (green line), 35 μM of **1** (purple line) and 45 μM of **1** (blue line). (B) Sedimentation coefficients distribution c(s) of 30 μM GDP-tubulin at 4ºC in PEDTA-GTP 1.5 in the presence of no ligand (black line), 15 μM of **1** (red line), 25 μM of **1** (green line), 35 μM of **1** (purple line) and 45 μM of **1** (blue line). (C) Sedimentation coefficients distribution c(s) of 30 μM GTP-tubulin at 25ºC in PEDTA-GTP 1.5 after addition of 2 mM EDTA, in the presence of no ligand (black line), 15 μM of **1** (red line), 25 μM of **1** (green line), 35 μM of **1** (purple line) and 45 μM of **1** (blue line).

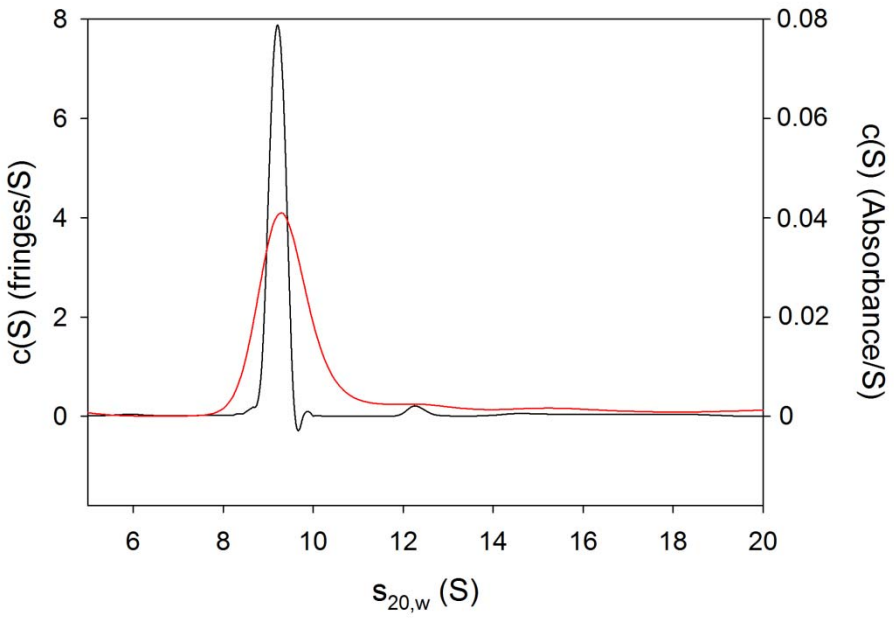

Figure S4. Related to Figure 4.- Binding of **1** to the T2R complex. Sedimentation coefficients distribution c(s) of 10 μM tubulin plus 7.5 μM RB3 and 12 μM of **1** in NAPI-DTT1.5 buffer. Black line.-profile calculated from the Rayleigh interference (protein). Red line.- corresponding sedimentation coefficients distributions of the ligand calculated from the absorbance at 320 nm (ligand). Related to Figure 4.

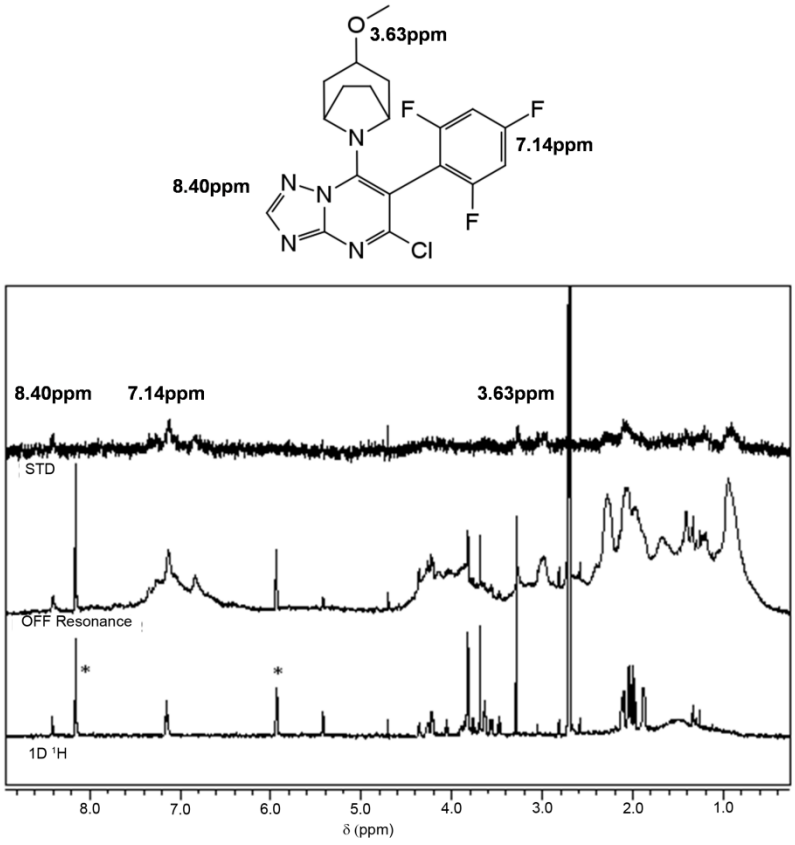

Figure S5. Related to Figure 6.- Upper panel. Structure of **1** showing the typical chemical displacements of the groups involved in the interaction with tubulin. Lower panel. Calculated STD spectra, off resonance and on resonance spectrum of 300 μM **1** in the presence of 10 μM tubulin in 10 mM Sodium Phosphate, 0.1 mM GTP in D_2O, pH* 7.0. The asterisks indicate the signals of the nucleotide bound.

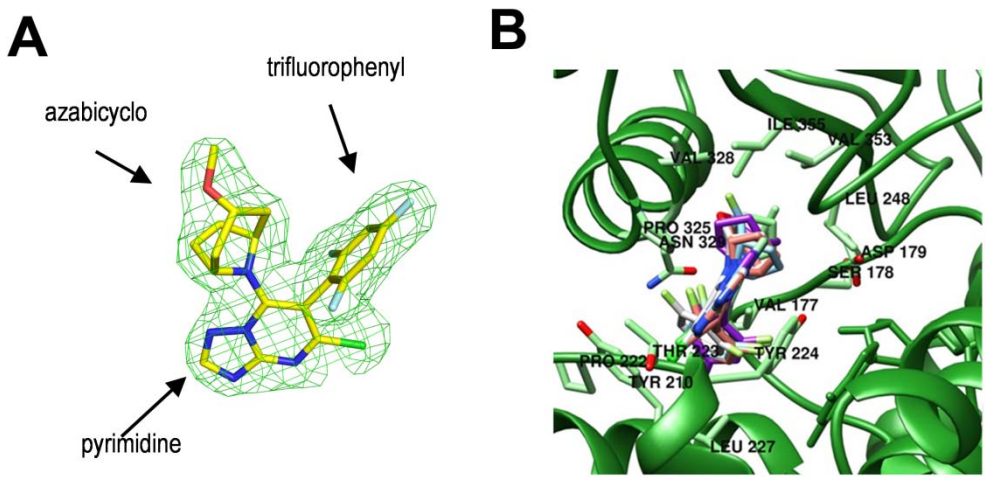

Figure S6. Related to Figure 7.- (A) SigmaA-weighted mFo-DFc (green mesh) electron density omit map of **1** in the TTL-T2R-**1** complex contoured at + 3.0σ. **(B)** Molecular models of the interaction of the ligands with the binding site. A Comparison of the calculated binding poses of **1** (purple), with those of **2**, **3**, **4** and **5**, residues labeled are those that interact with compound **1**.