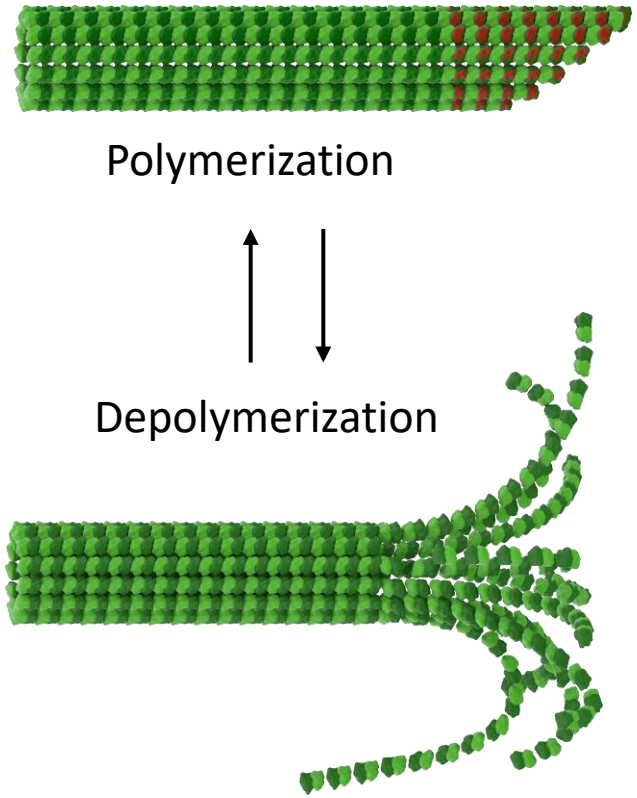
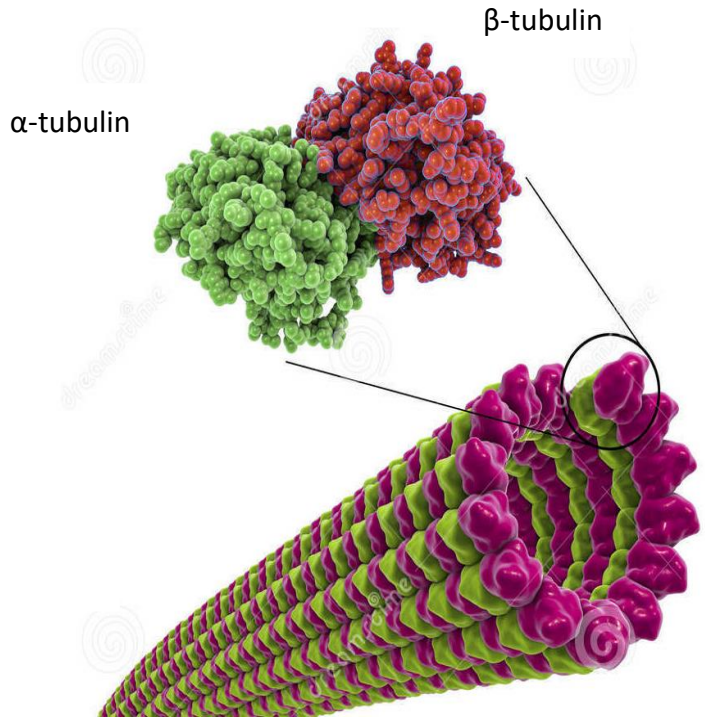
AN INSIGHT INTO THE ORIGIN OF MICROTUBULE-CURLING EFFECT OF PODOPHYLLOTOXIN ESTERS: MOLECULAR DYNAMICS STUDY

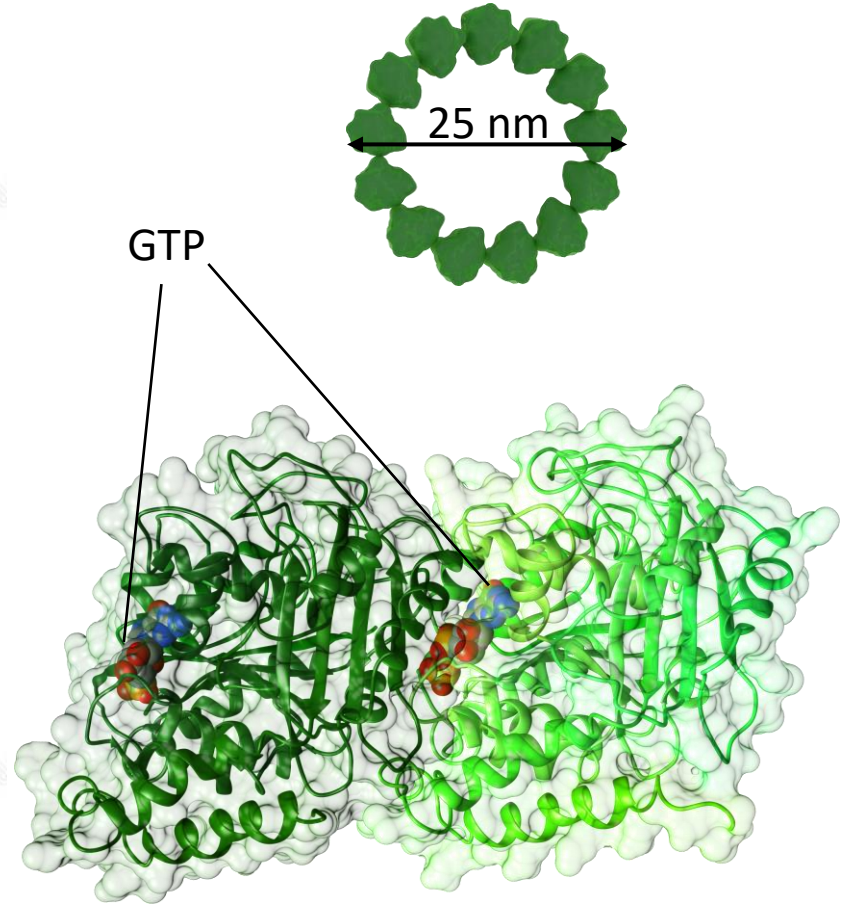
Anastasia Nikolaevna Borovik

Co-authors: Nikolay A. Zefirov, Apollonia Glaßl, Evgeniy V. Radchenko, Vladislav V. Stashevsky, Elena R. Milaeva, Sergei A. Kuznetsov and Olga N. Zefirova

Tubulin. Microtubule dynamics instability

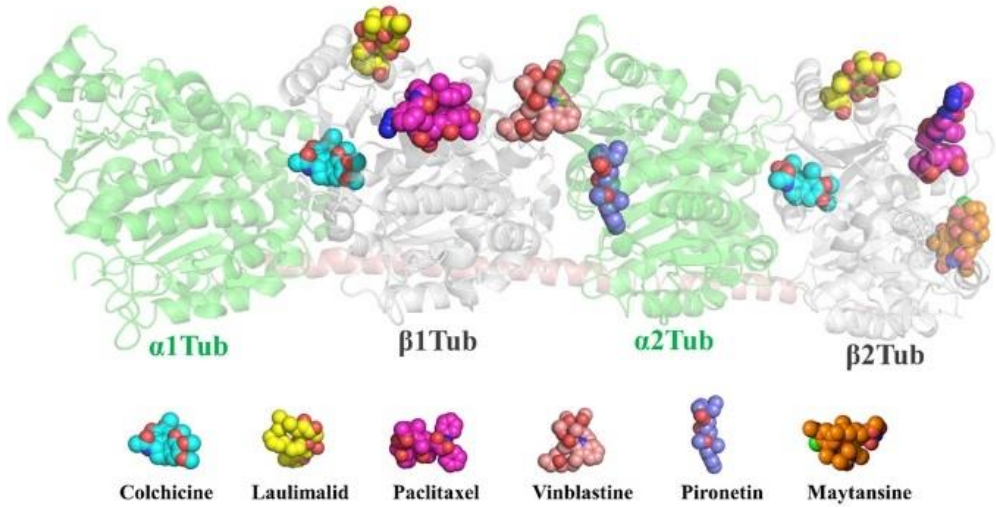




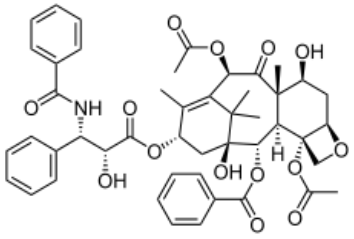


Tubulin heterodimers composed of GTP-bound α- and β-tubulin molecules polymerize to form microtubule protofilaments, which associate laterally to form a hollow microtubule. Periods of rapid microtubule polymerization alternate with periods of shrinkage or depolymerization in a process known as dynamic instability.

Binding sites of anticancer agents in tubulin

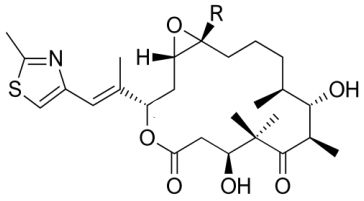


Taxol binding site



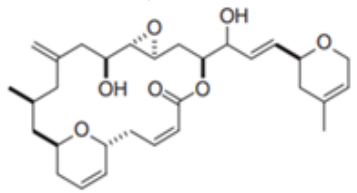
Paclitaxel

Epothilone binding site



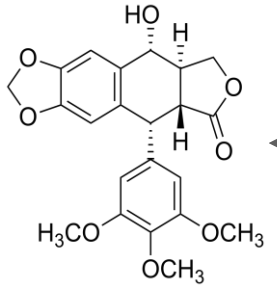
Epothilone B

Laulimalide binding site

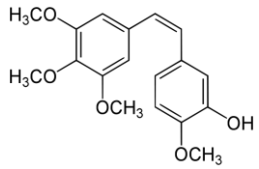


Laulimalide

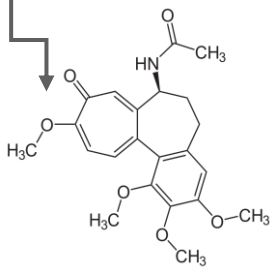
Colchicine binding site



Podophyllotoxin

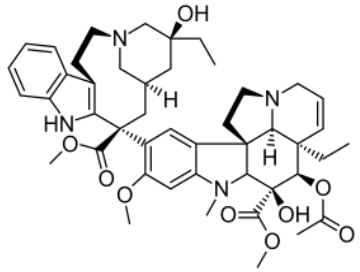


Combretastatin A-4



Colchicine

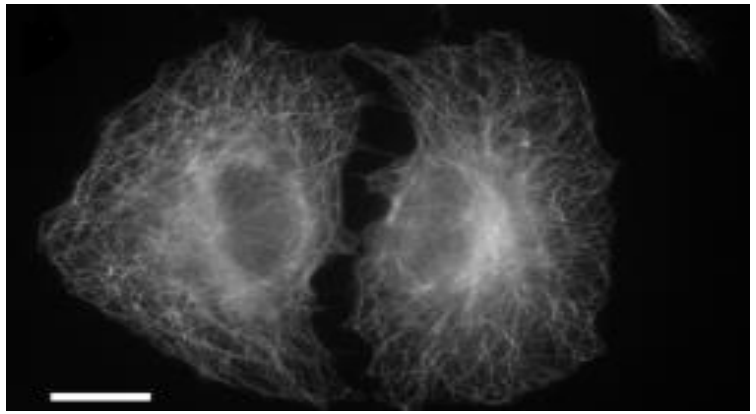
Vinblastine binding site



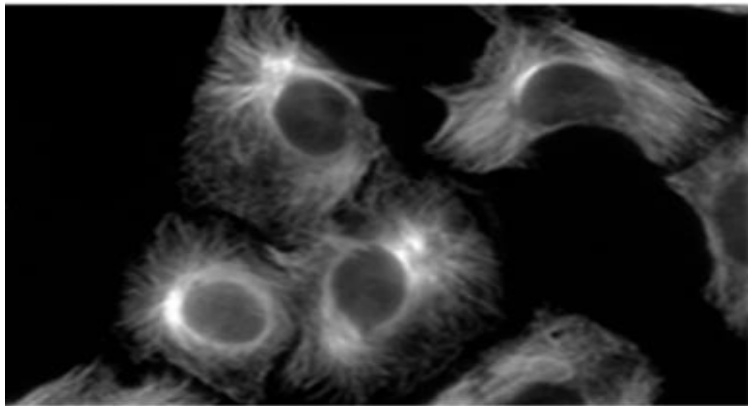
Vinblastine

Different types of standard anticancer agents action on microtubule net

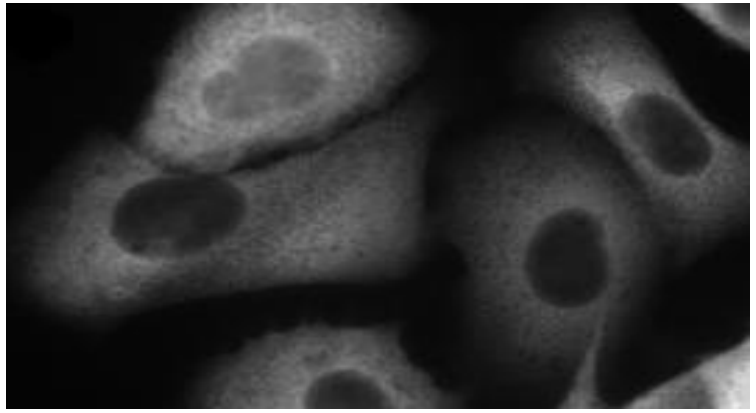
Normal MT (A549)



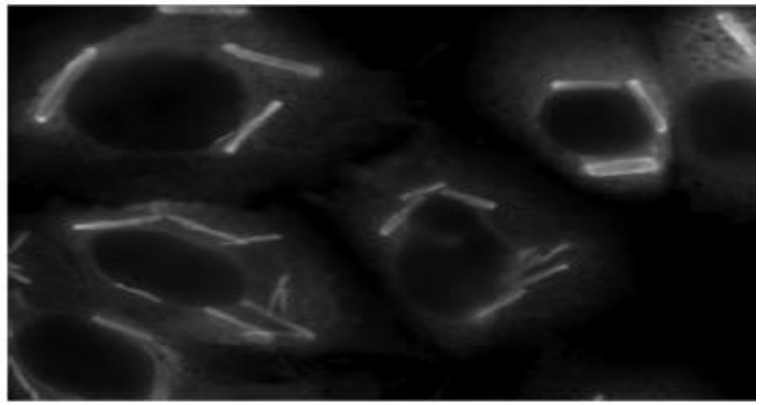
MT collected into bundles (taxol action)



Depolymerized MT (colchicine action)

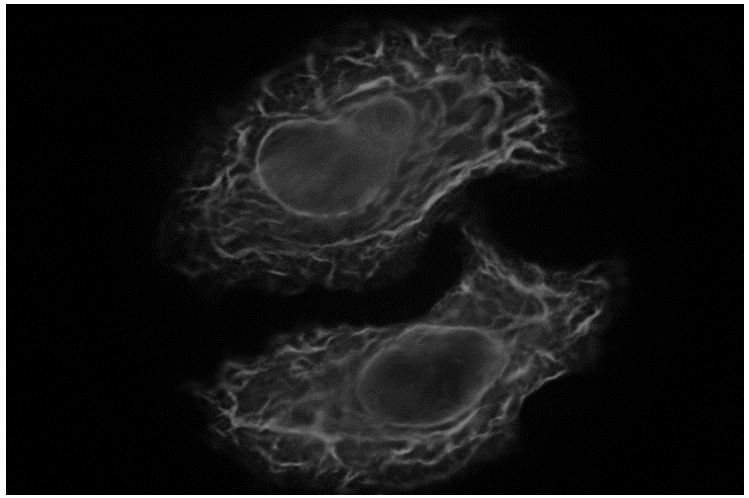


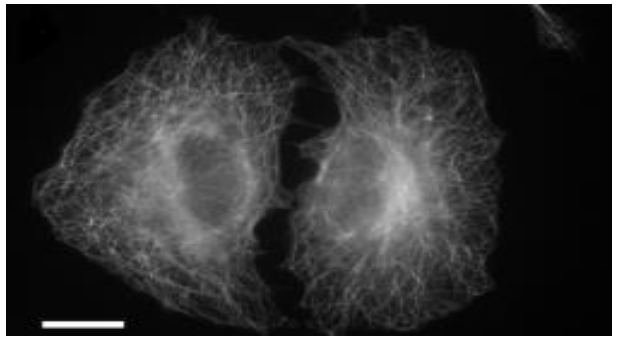
Depolymerized MT and paracrystals (vinblastine action)



Non-standard action of colchicine binding site ligands

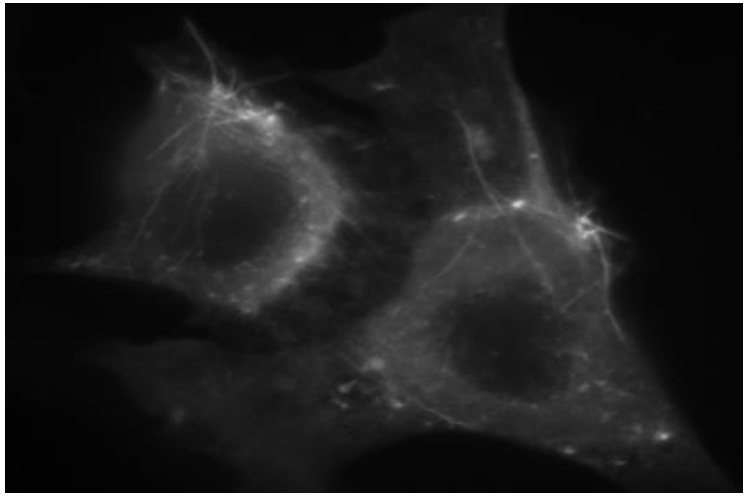
Tubulin-Clustering effect





Normal MT (A549)

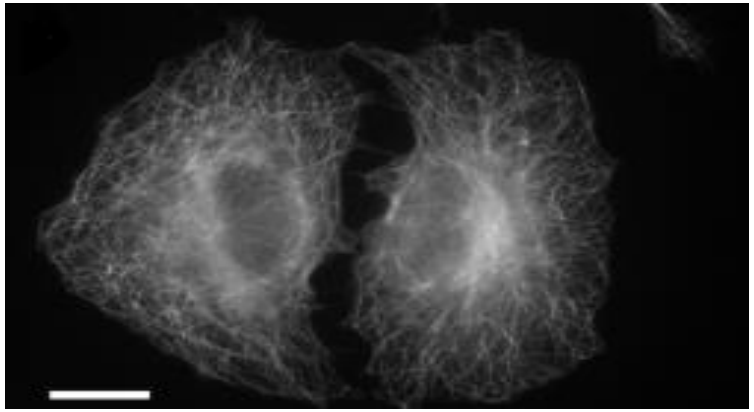
Tubulin filaments



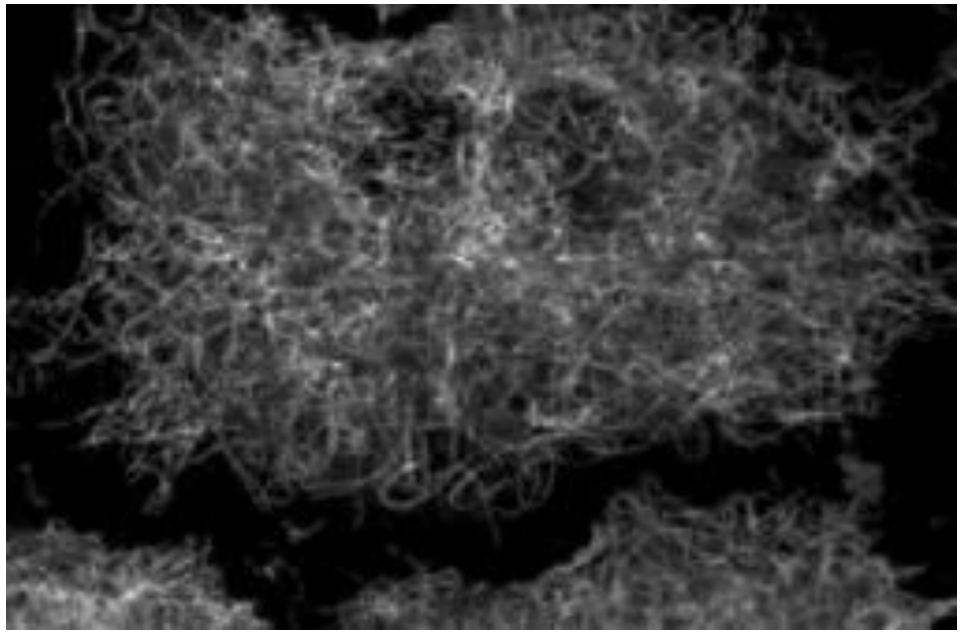
- *Thomopoulou P et al. (2015) New colchicine-derived triazoles and their influence on cytotoxicity and microtubule morphology. ACS Med Chem Lett 7:188–191*
- *Olga N. Zefirova et al. Unusual Tubulin-Clustering Ability of Specifically C7- Modified Colchicine Analogues ChemBioChem 2013, 14, 1444 – 1449*
- *Sung-Kuk Kim et al. The colchicine derivative CT20126 shows a novel microtubule-modulating activity with apoptosis Experimental & Molecular Medicine (2013) 45, e19*
- *Cristina C. Rohena et al. Janus Compounds, 5-Chloro-N4 -methyl-N4 -aryl-9Hpyrimido[4,5-b]indole-2,4-diamines, Cause Both Microtubule Depolymerizing and Stabilizing Effects Molecules 2016, 21, 1661*
- *Olga N. Zefirova et al. Novel colchicine conjugate with unusual effect on the microtubules of cancer cells Pure Appl. Chem. 2020; 92(8): 1217–1226*

Non-standard action of colchicine binding site ligands

Normal MT (A549)

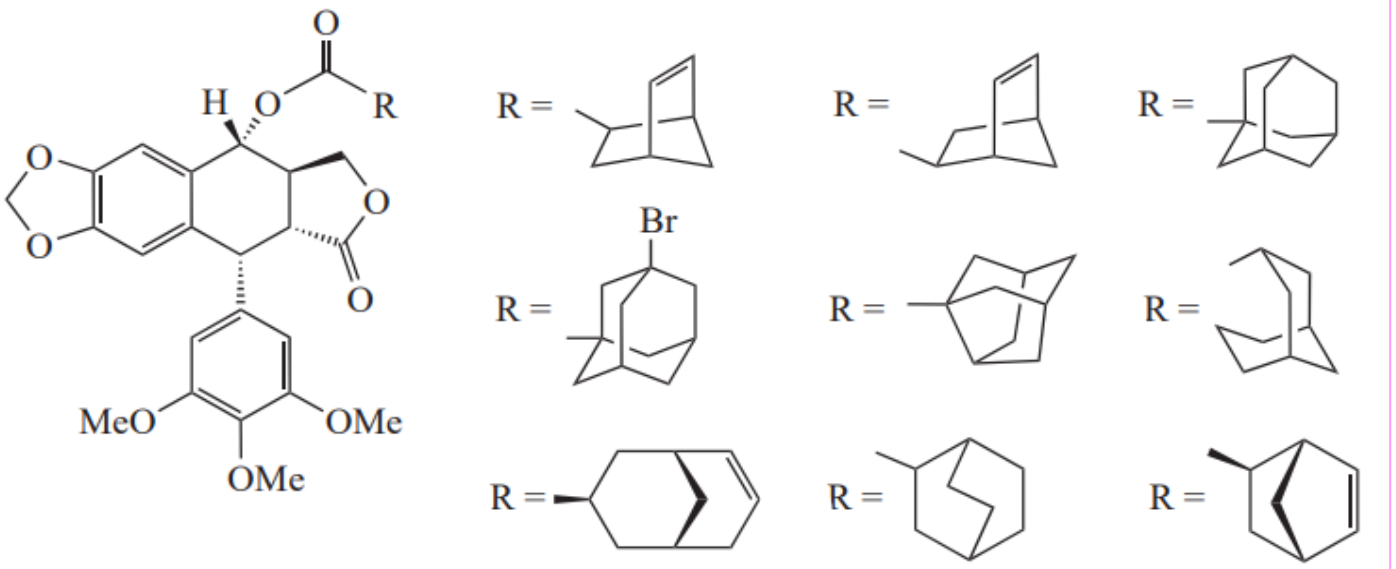


“Curling” effect

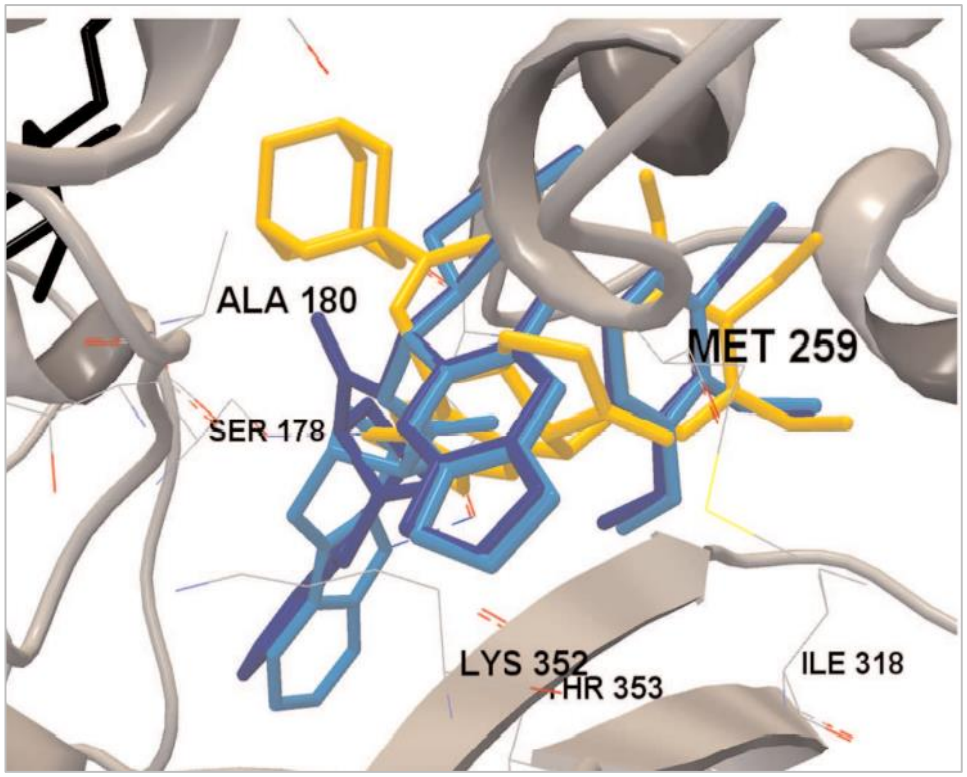


- ***Nikolai A. Zefirov et al. Adamantyl-substituted ligands of colchicine binding site in tubulin: different effects on microtubule network in cancer cells. Structural Chemistry (2019) 30:465–471***
- ***Nikolay A. Zefirov et al. Novel bridged and caged C4-podophyllotoxin derivatives as microtubule disruptors: synthesis, cytotoxic evaluation and structure–activity relationship Mendeleev Commun., 2018, 28, 475–478***
- ***Nikolay A. Zefirov et al. Podophyllotoxin analogues with bicyclo[3.2.1] octane moiety, annelated with indole: synthesis, molecular modeling and biotesting. Biomedicinal chemistry (in Russ.), 2019 vol. 65, 2.***

C4 podophyllotoxin esters providing MT curling effect



MT curling effect was observed for podophyllotoxin esters with adamantane and bicyclo[3.3.1]nonane moieties



Preliminary molecular docking: Hypothesis 1:

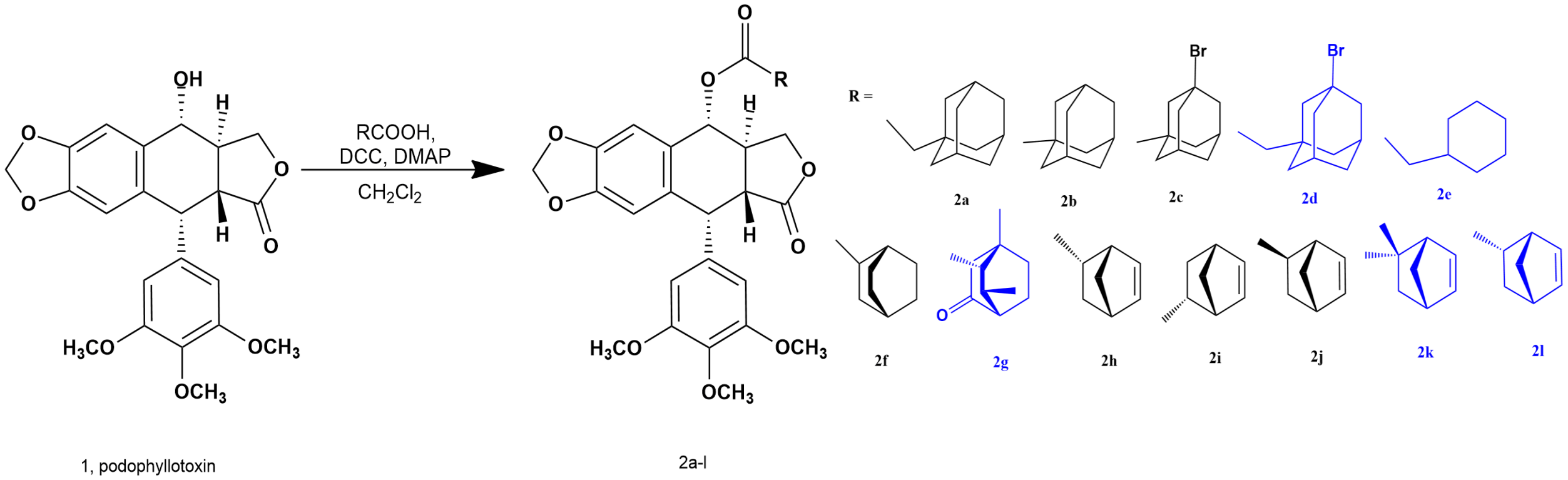
MT curling effect depends on the location of bridged substituents near GTP binding site of α-subunit

Subject of present investigation:

Increasing the number of synthesized alicyclic podophyllotoxin esters and their testing at different concentrations to check the hypothesis 1.

An attempt to explain “curling” action of some podophyllotoxin esters on microtubular net of cancer cells by carrying out molecular dynamics simulation.

Synthetic procedure



1, podophyllotoxin

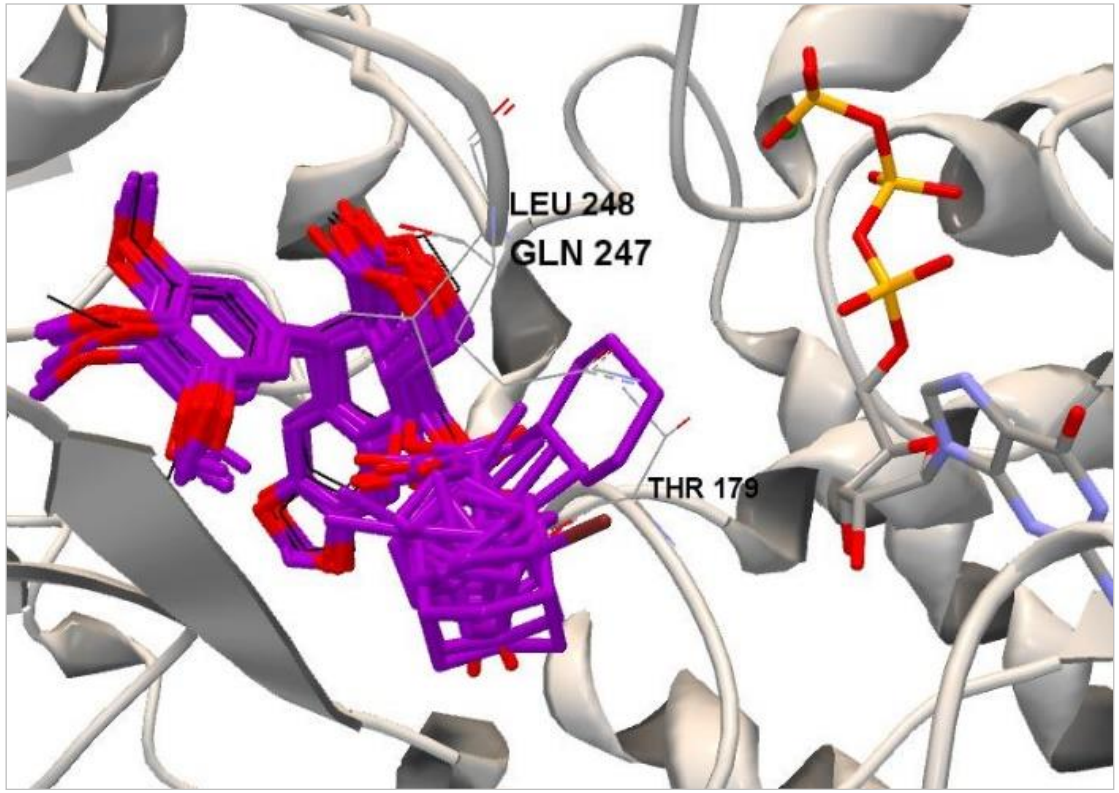
2a-l

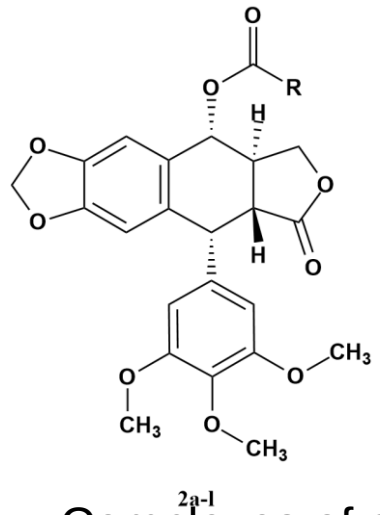
Podophyllotoxin esters with alicyclic residues. Compounds marked in blue (2d, 2e, 2g, 2k, 2l) were synthesized in our work, the rest of compounds were described earlier.

For esters 2f, 2g, 2j–k relative configuration is indicated, since they were obtained as diastereomeric mixtures.

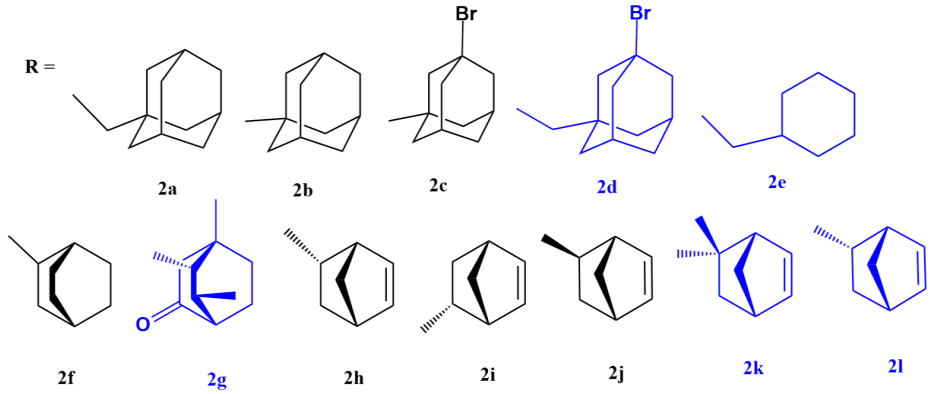
Molecular modeling

In order to explain differences in action on microtubular net molecular docking of all synthesized analogues was performed



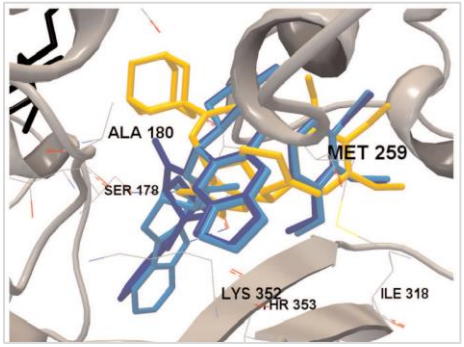


2a-l



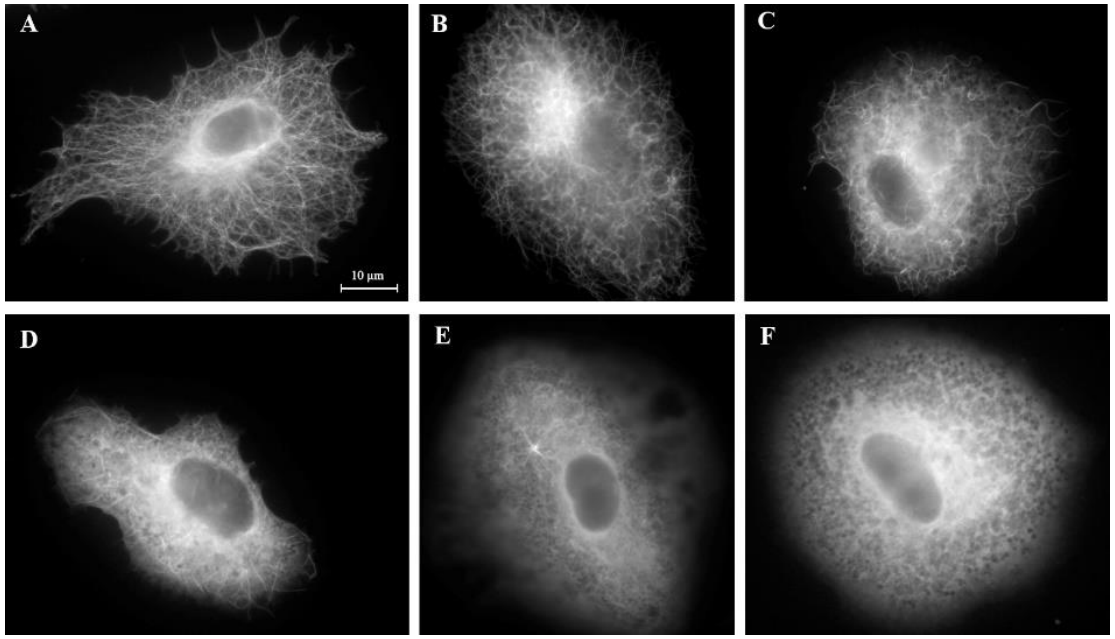
Complexes of esters **2a-2l** ((including diastereomers of diastereomeric pairs) with colchicine binding site in α,β-tubulin (PDB ID 1SA1). Docking procedure was performed using AutoDock Vina 1.1.2 software.

Result: docking data is not enough to explain the curling effect: all the alicyclic substituents (except cyclohexane) are located in the same area



~~**Hypothesis 1: MT curling effect depends on the location of bridged substituents near GTP binding site of α-subunit**~~

Biological tests results



Immunofluorescence microscopy image of the microtubules in carcinoma A549 cells treated with 2 µM of tested compounds and DMSO as negative control. A) 0.5% DMSO (intact MTs); B) **2d** (slightly curled MTs), C) **2d** (curled MTs), D) **2l** (shorted Mts); E) **2e** (full depolymerization of MTs, the rests of MTs near the centrosomes are seen as star-like structures); F) **1** (full depolymerization of MTs).

Ester	Microtubules			Cell growth inhibition IC_{50} $(\mu M)^2$	Cytotoxicity, EC_{50} $(\mu M)^2$	Induction of apoptosis, % (2 µM, 48 h)
	2 µM	10 µM	100 µM			
2d	+++	++	+	1.6 ± 0.01	2.2 ± 0.4	3
2e	-	-	-	0.109 ± 0.007	0.29 ± 0.08	51
2g	++++	++	-	1.8 ± 0.35	2.5 ± 0.1	3
2k	++/+	+	-	0.7 ± 0.1	0.7 ± 0.2	53
2l	++/+	-	-	0.55 ± 0.2	0.8 ± 0.1	58
1	-	n.d.	n.d.	0.02	0.02 ± 0.005	48
DMSO	++++	++++	++++			

Effect on MTs of lung carcinoma A549 cells at the marked concentration after cell treatment for 24 h; the symbols indicate: «–» – no MTs; «+» – shorted MTs, «++» – curled MTs, «+++» – slightly curled MTs, «++++» – intact MTs; n.d. – not determined;

Result: the "curling" precedes full MTs depolymerization and passes before or in parallel with MTs shortening

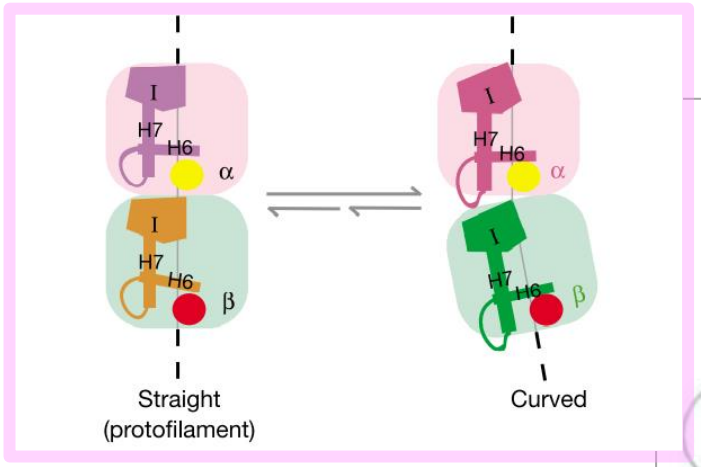
Reflections on the biological tests results

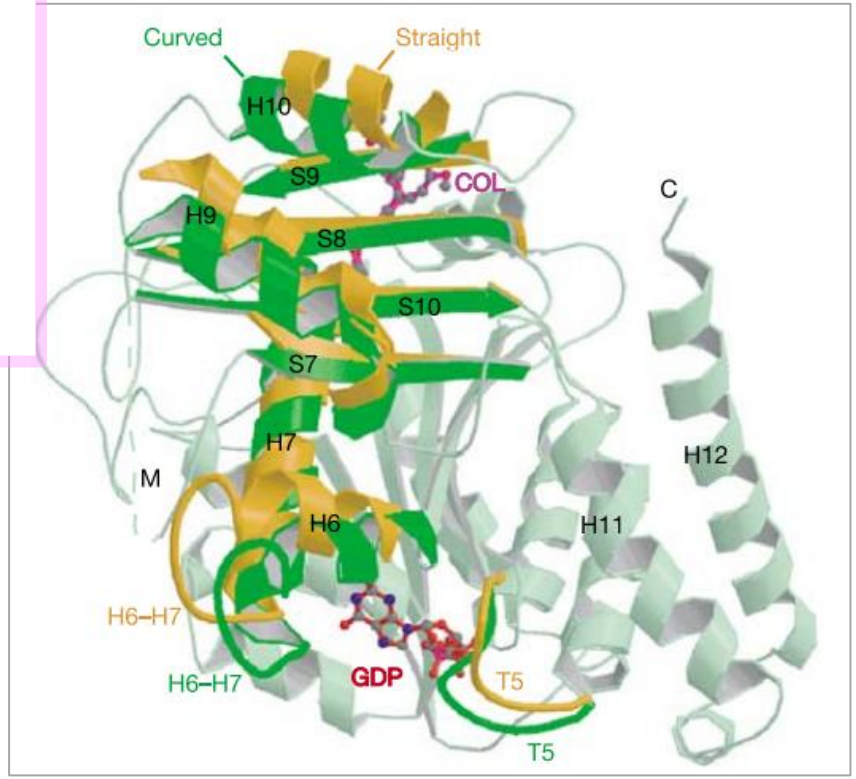
If “curling” precedes full MTs depolymerization and passes before or in parallel with MTs shortening, then the division of bridged podophyllotoxin esters into “only depolymerizing” and “only curling” is invalid, because the effect is concentration dependent.

Kinetic studies carried out in the early works revealed the ability of substoichiometric concentrations of podophyllotoxin to suppress the dynamic instability of MTs due to the weak association of free tubulin–PF complex with shortened MTs.

Another hypothesis

Free tubulin complexes with bridged podophyllotoxin esters may have noticeable structural differences that enable them to copolymerize with shortened MTs leading to "MTs" with different dynamics and "curled" morphology.



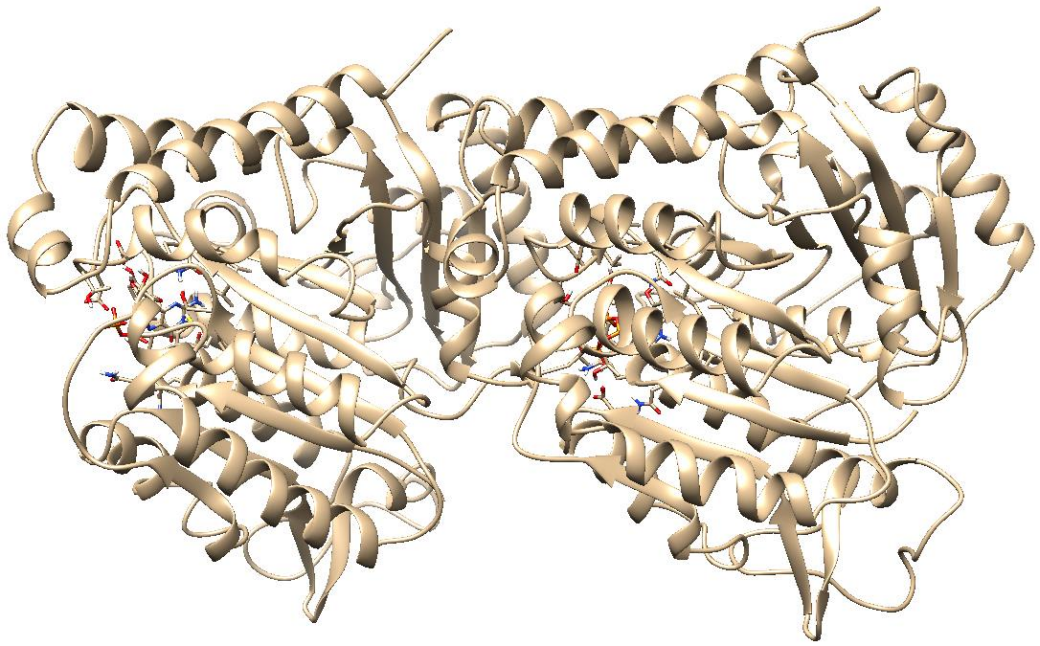


Hypothesis 2: "curling" effect depends on the angle of curvature between α and β subunits

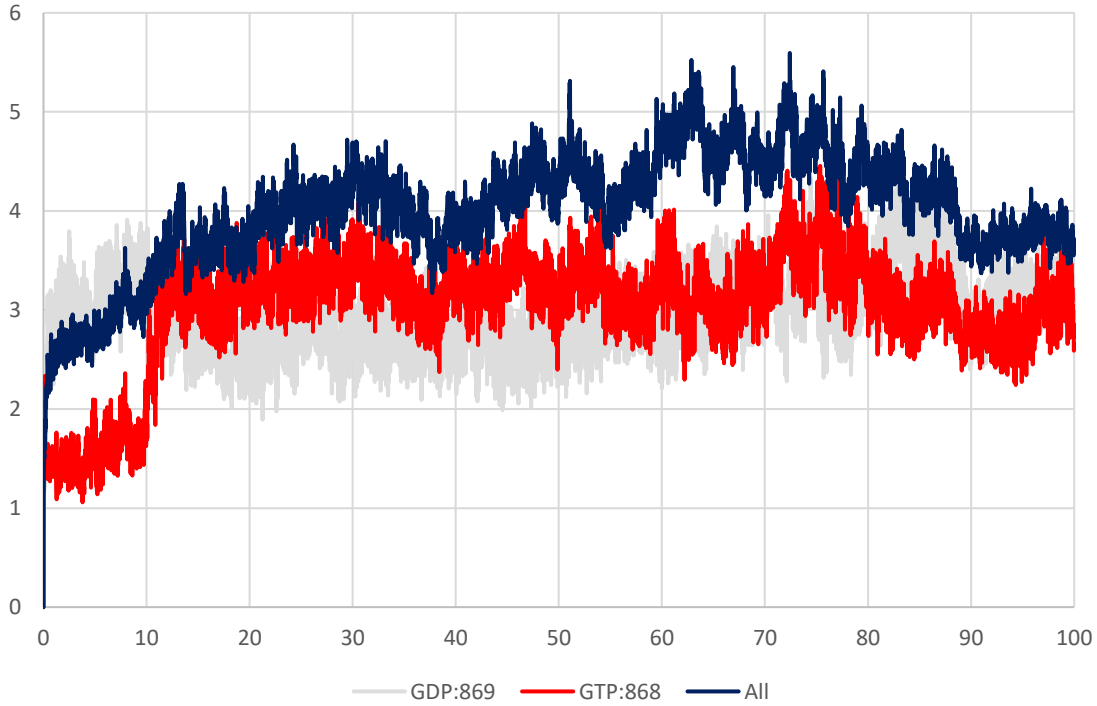
To check if this is possible in principle, we carried out molecular modeling study.

Molecular modeling

Molecular dynamics simulation was performed using GROMACS simulation package*



A reference model of α,β-tubulin dimer with GTP and GDP was obtained using a molecular dynamic procedure



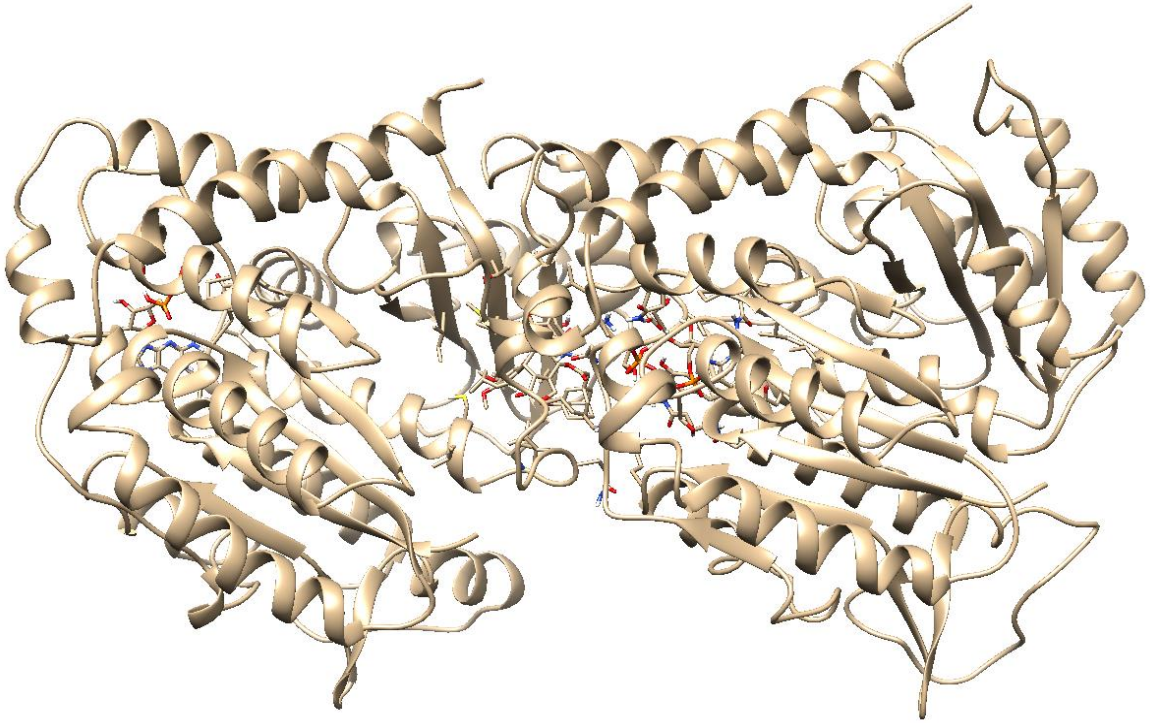
RMSD of the model of “free” tubulin and graphs for each ligand of the protein

*** Huang J., MacKerell A.D.J. (2013) J. Comput. Chem., 34, 2135-2145; Vanommeslaeghe K., Hatcher E., Acharya C., Kundu S., Zhong S., Shim J., Darian E., Guvench O., Lopes P., Vorobyov I., Mackerell A.D. Jr. (2010) J. Comput. Chem., 31, 671–690.**

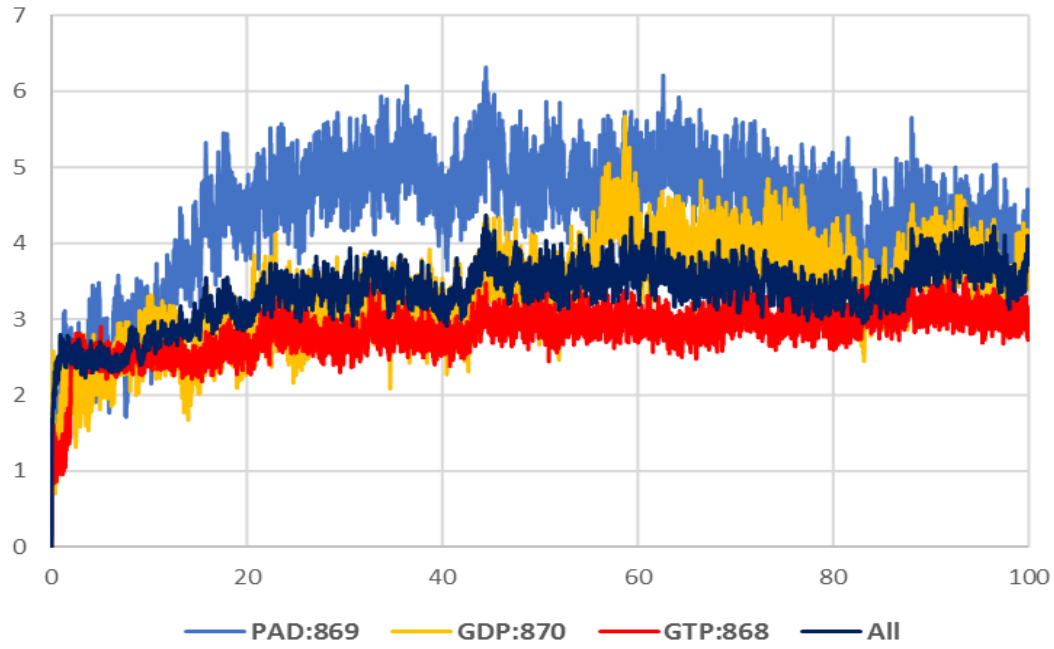
*** Lee J., Cheng X., Swails J.M., Yeom M.S., Eastman P.K., Lemkul J.A., Wei Sh., Buckner J., Jeong J.C., Qi Y., Jo S., Pande V.S., Case D.A., Brooks C.L. 3rd, MacKerell**

Molecular modeling

Molecular dynamics simulation was first performed for two substances: ester with adamantaneacetic moiety (demonstrating strongest curling) and podophyllotoxin as a reference compound



Initial models were built by moving the best conformation of each ligand (obtained from docking data) into reference tubulin model (obtained from molecular dynamics study)

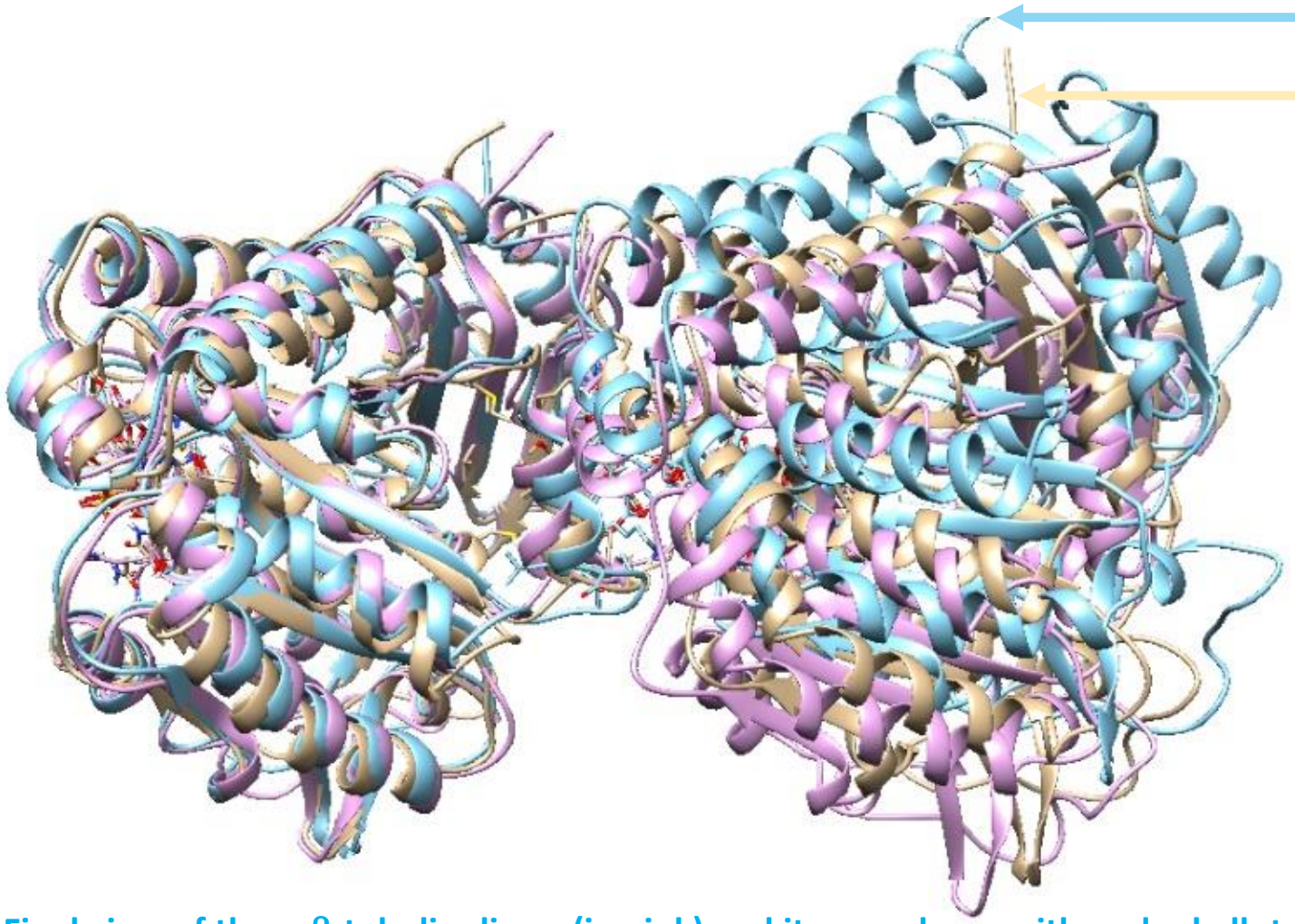


RMSD of the model of tubulin complex with adamantaneacetic ester and graphs for each ligand of the protein

The location of ligands which provide curling or not is not outwardly different

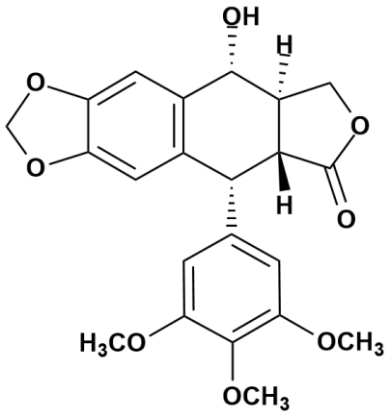
Molecular modeling

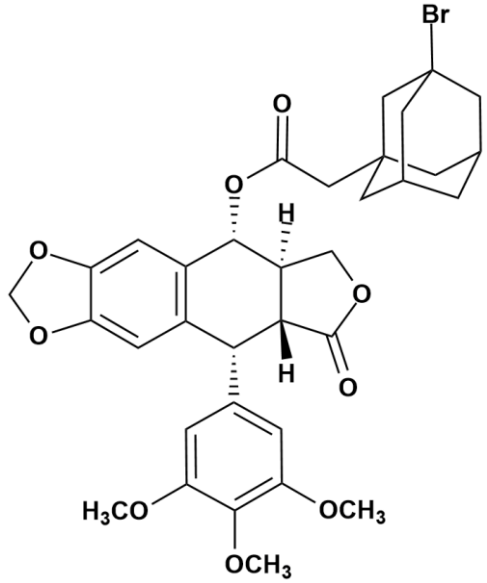
Superimposing models obtained from MD study by the best match of β-subunits proves the hypothesis that curling effect might depend on the angle of curvature between α and β subunits in complex with each ligand



The biggest angle of deviation is observed for 2a-podophyllotoxin complex which demonstrated strong curling effect

Angle of the podophyllotoxin-tubulin complex is less then for 2a





Final view of the α,β-tubulin dimer (in pink) and its complexes with podophyllotoxin (in beige) or adamantaneacetic ester (in blue) predicted by molecular dynamics in the CHARMM36 / CGenFF 4.4 force field

Conclusion

The “curling” effect caused by bridged podophyllotoxin esters takes place at one of the first steps of their depolymerization and may occur due to a change in the curvature of curved conformation of tubulin dimer. Analogously to podophyllotoxin such “strongly curved” tubulin complexes at substoichiometric concentrations may copolymerize with shortened microtubules and their copolymerization leads to “curled” morphology of tubulin associates.

Thank you for your attention!