STRUCTURAL OPTIMIZATION OF TUBULIN INHIBITORS

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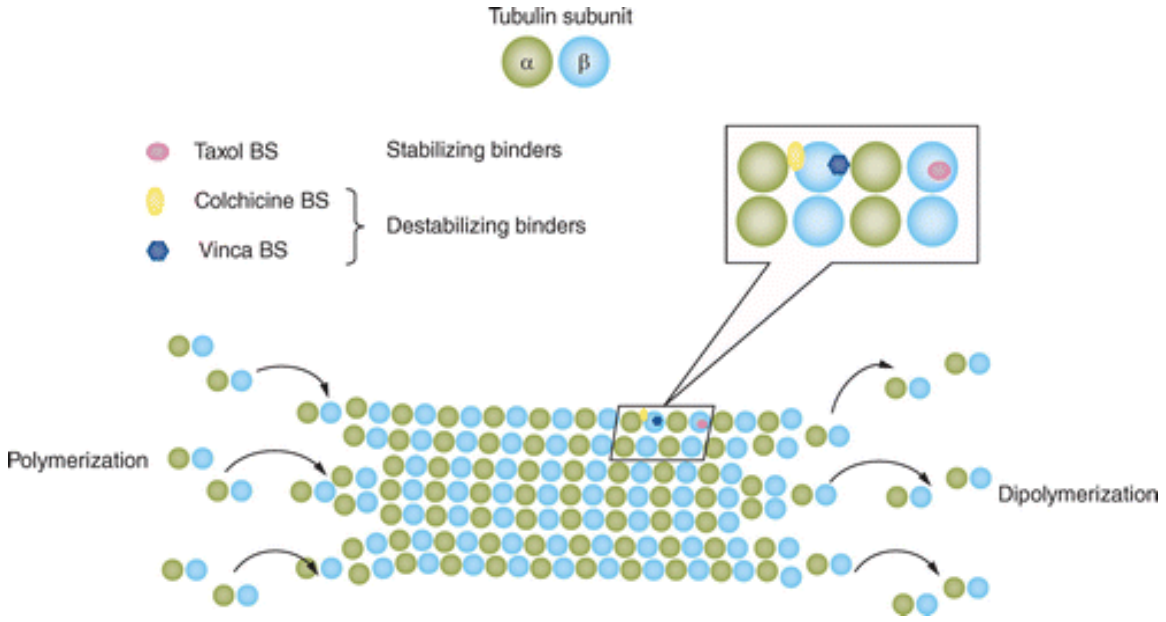
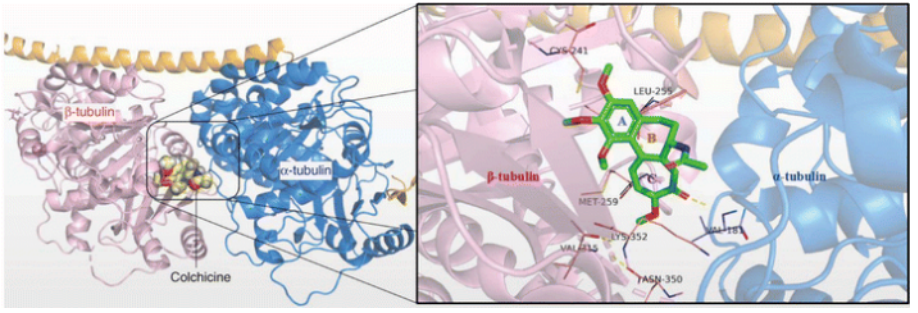
25.05.2022

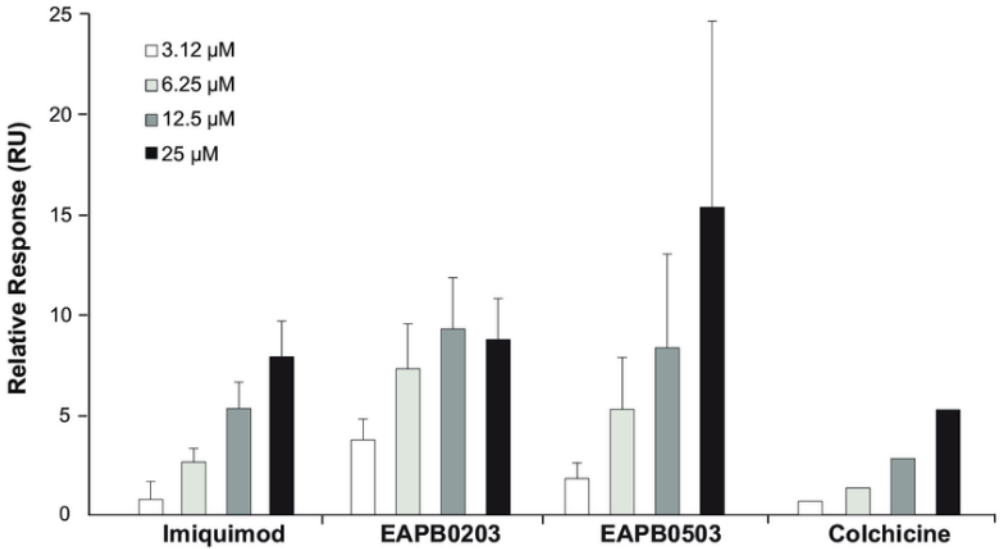




Tubulin inhibitors prevent microtubule formation and mitosis progression making them useful for anticancer therapy.

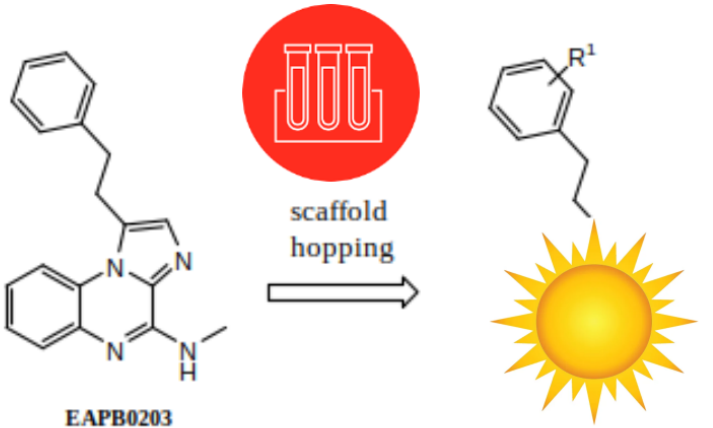
Recent advances in research of colchicine binding site inhibitors and their interaction modes with tubulin. Kewei Sun, Zhonghao Sun, Fenglan Zhao, Guangzhi Shan, and Qingguo Meng. Future Medicinal Chemistry 2021 13:9, 839-858

**Binding levels of EAPB0203, EAPB0503 and imiquimod were determined by surface plasmon resonance on immobilized tubulin at different concentrations*



Compounds bearing imidazo[1,2-a]quinoxalines scaffold were proven to inhibit microtubule polymerization by the interaction with colchicine-binding site.

isosteric analogues



*Our chemists applied **the scaffold hopping** approach to the previously reported inhibitor EAPB020330 and suggested its isosteric analogues*

*Courbet A, Bec N, Constant C, et al. Imidazoquinoxaline anticancer derivatives and imiquimod interact with tubulin: Characterization of molecular microtubule inhibiting mechanisms in correlation with cytotoxicity. PLoS One. 2017;12(8):e0182022. Published 2017 Aug 10. doi:10.1371/journal.pone.0182022

Our goals:

i. Study the **structure-activity relationship** of highly active and selective tubulin inhibitors previously synthesized in our institute;

ii. Establish their **binding mode** and suggest possible **directions of modifications**;

iii. Design **new analogs** with improved physicochemical properties.

The lead compound

Cell line	Description	IC_{50} [μM]
A549	Human lung adenocarcinoma	0.033
CCRF-CEM	T-lymphoblastic leukaemia	0.058
CEM-DNR	T-lymphoblastic leukaemia, daunorubicin resistant	0.097
HCT116	Human colorectal cancer	0.029
HCT116p53-/-	Human colorectal cancer, p53 deficient	0.029
K562	acute myeloid leukaemia	0.029
K562-TAX	acute myeloid leukaemia, paclitaxel resistant	0.087
U2OS	human osteosarcoma	0.038
BJ	human fibroblast	>50

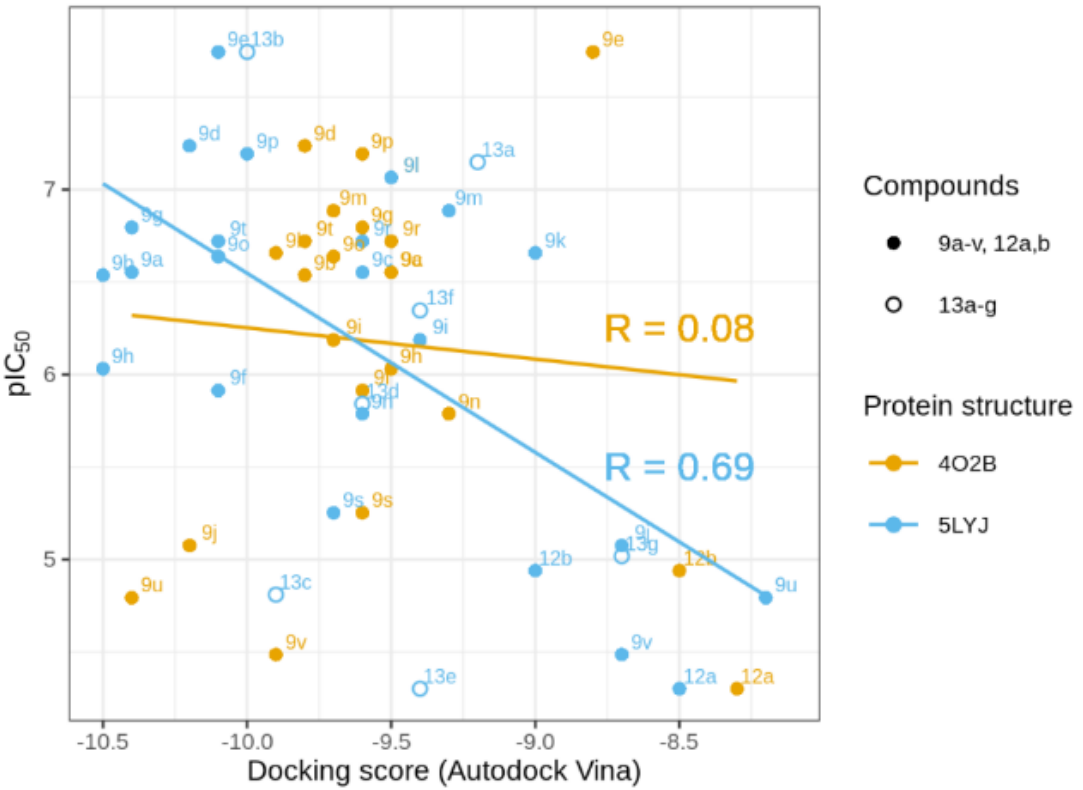
✓ **Low nanomolar cytotoxicity** against multiple cancer cells including clones resistant to clinically used drugs

✓ **Low toxicity toward human fibroblasts** was observed with the high selectivity index exceeding three orders of magnitude

✘ **Unfavorable physicochemical properties** (in particular high lipophilicity)

RTB	logP	MW	QED
4	5.09	336.82	0.485

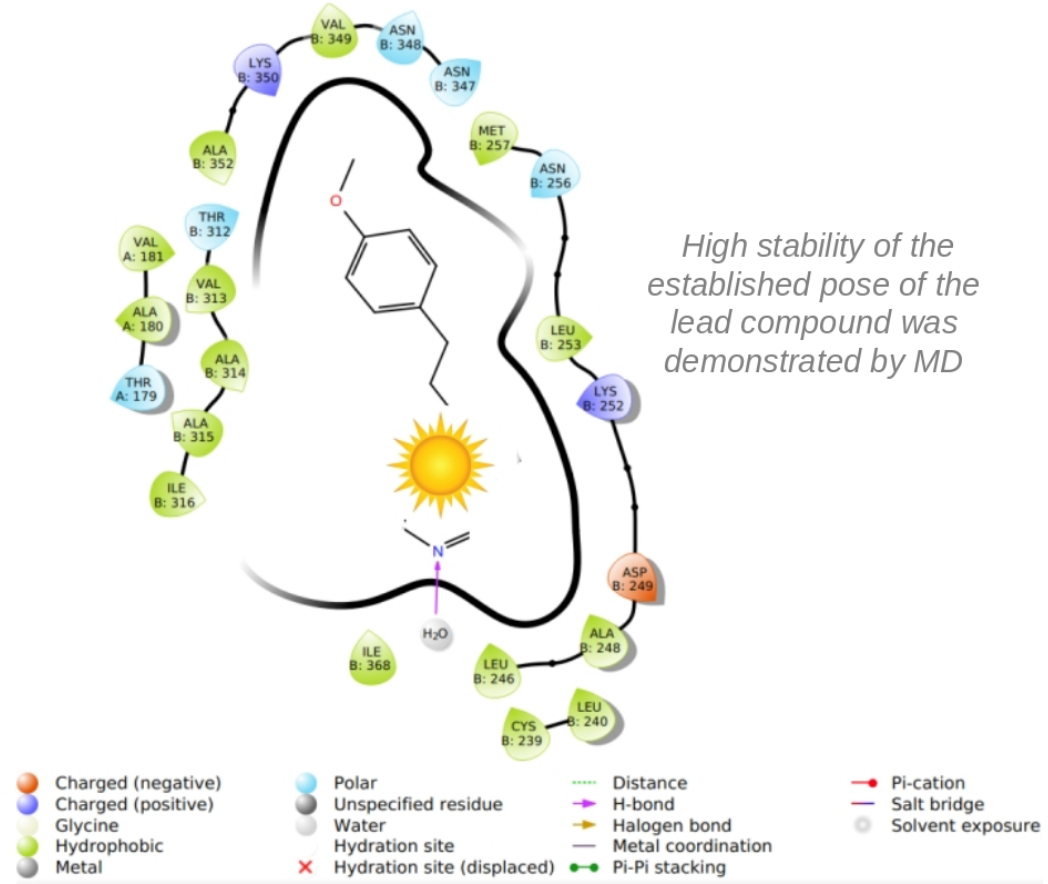
Cross-linking study confirmed interaction of the synthesized derivatives in the **colchicine-binding site**



Molecular docking study

- Three complexes with colchicine (**4O2B**), nocodazole (**5CA1**) and combretastatin A4 (**5LYJ**) were used

The protein structure from the complex with combrestatine-A4 (PDB: 5LYJ) is more suitable and results in higher correlation of activity with calculated docking scores than docking to other tubulin structures.



High stability of the established pose of the lead compound was demonstrated by MD

*The binding pose was additionally confirmed by **100 ns molecular dynamic (MD)** simulations.*

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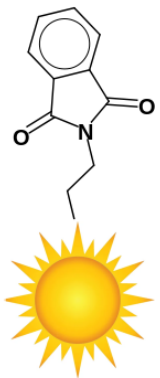
MD study allowed to establish that the majority of protein-ligand contacts have **hydrophobic** nature, but it was found that a **nitrogen in the core part** of the lead molecule can form a hydrogen bond through a water bridge and this contact persists in course of the simulation

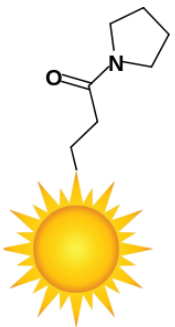
Lead

docking score:
-10.2

Suggested modifications

Totally 2 373 726 new compounds were generated

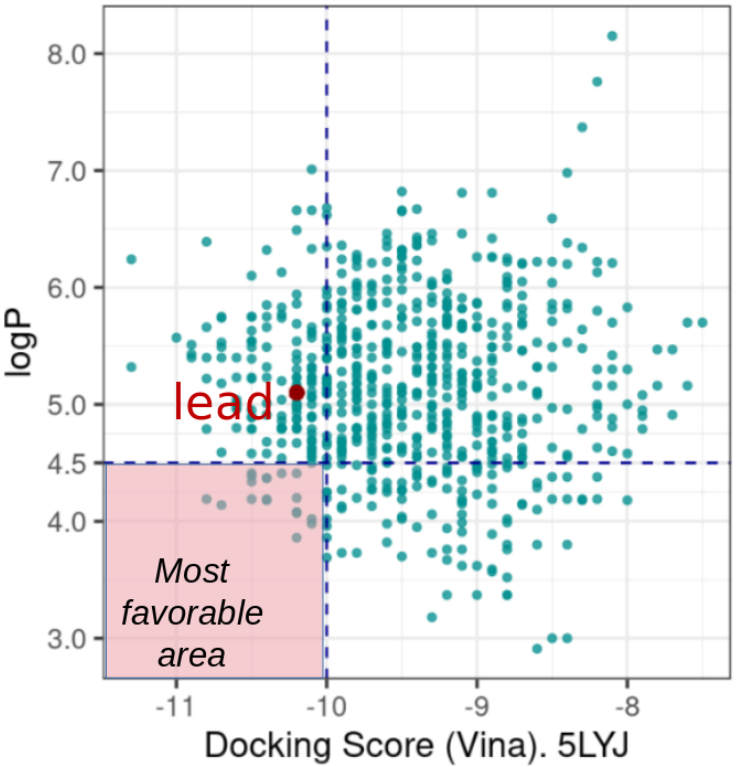




-10.7 -10.4 -10.0

docking score:

To design new compounds we preserved important scaffold features and enumerated possible analogs by CReM tool*



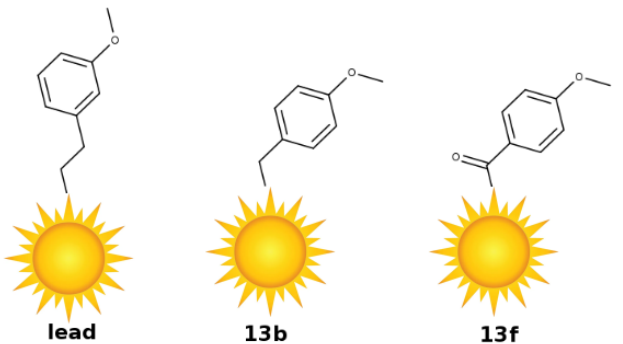
Finally compounds with desired physicochemical properties, **were selected** and **evaluated by docking procedure** and the most promising ones **were suggested** for synthesis and biological experiments.

**Polishchuk, P. CReM: chemically reasonable mutations framework for structure generation. J Cheminform 12, 28 (2020).*

Tested suggested modifications

Cmpd.	logP	QED
lead	5.09	0.485
13b	4.9	0.502
13f	4.54	0.505

	IC_{50} [µM]							
Cmpd.	**CEM**	**CEM-DNR**	**K562**	**K562 Tax**	**A549**	**HCT116**	**HCT116 p53-/-**	**BJ**
lead	**0.018**	0.097	0.029	0.087	**0.033**	0.029	0.029	>50
13b	**0.018**	**0.029**	**0.013**	**0.03**	**0.034**	**0.017**	**0.021**	≥ 50
13f	0.45	0.57	9.57	0.40	3.33	0.45	0.64	≥ 50



__13b__ was identified as the most active inhibitor with low nanomolar cytotoxicity against various cancer cell lines including drug-resistant mutants. Importantly, this compound did not exhibited any in vitro toxicity.

Although there is still a significant part of molecules in the queue for synthesis and experimental validation.

Conclusions:

1) Systematic **SAR** revealed the optimal substitution pattern
2) **Binding mode** was established by molecular docking and molecular dynamics.
3) Promising *in silico* **modifications** were suggested and some of them have already tested
4) From the whole set of tested compounds, **13b was identified as the most active inhibitor** with low nanomolar cytotoxicity against various cancer cell lines including drug-resistant mutants and compound did not exhibited any in vitro toxicity.
5) **A significant part of the suggested modifications is in the queue** for synthesis and experimental validation.

Acknowledgments

Institute of Molecular and Translational Medicine, Olomouc:

- Soural Miroslav Ph.D.
- Řehulka Jiří Ph.D.
- Gurská Soňa Ph.D.
- Džubák Petr M.D., Ph.D.
- Hajdúch Marián M.D., Ph.D.

colleagues:

- Olena Mokshyna, Ph.D.
- Guzel Minibaeva

Supervisor:

- **Polishchuk Pavel Ph.D., M.Sc.**

This work was funded by the:

- INTER-EXCELLENCE LTARF18013 project (MEYS)
- the European Regional Development Fund - Project ENOCH (No. CZ.02.1.01/0.0/0.0/16_019/0000868)
- ELIXIR CZ research infrastraucture project (MEYS Grant No: LM2018131)

This work was supported by the Ministry of Education, Youth and Sports of the Czech Republic through the e-INFRA CZ (ID:90140).

Thank you for your attention!