STRUCTURAL OPTIMIZATION OF TUBULIN INHIBITORS

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

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

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

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**

- **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)

### Cell line Table

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Description</th>
<th>IC&lt;sub&gt;50&lt;/sub&gt; [µM]</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549</td>
<td>Human lung adenocarcinoma</td>
<td>0.033</td>
</tr>
<tr>
<td>CCRF-CEM</td>
<td>T-lymphoblastic leukaemia</td>
<td>0.058</td>
</tr>
<tr>
<td>CEM-DNR</td>
<td>T-lymphoblastic leukaemia, daunorubicin resistant</td>
<td>0.097</td>
</tr>
<tr>
<td>HCT116</td>
<td>Human colorectal cancer</td>
<td>0.029</td>
</tr>
<tr>
<td>HCT116p53-/</td>
<td>Human colorectal cancer, p53 deficient</td>
<td>0.029</td>
</tr>
<tr>
<td>K562</td>
<td>acute myeloid leukaemia</td>
<td>0.029</td>
</tr>
<tr>
<td>K562-TAX</td>
<td>acute myeloid leukaemia, paclitaxel resistant</td>
<td>0.087</td>
</tr>
<tr>
<td>U2OS</td>
<td>human osteosarcoma</td>
<td>0.038</td>
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<tr>
<td>BJ</td>
<td>human fibroblast</td>
<td>&gt;50</td>
</tr>
</tbody>
</table>

### Physicochemical Properties

<table>
<thead>
<tr>
<th>RTB</th>
<th>logP</th>
<th>MW</th>
<th>QED</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>5.09</td>
<td>336.82</td>
<td>0.485</td>
</tr>
</tbody>
</table>

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.
MD study allowed to establish that the majority of protein-ligand contacts have \textit{hydrophobic} nature, but it was found that a \textit{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. The binding pose was additionally confirmed by \textit{100 ns molecular dynamic (MD) simulations.}
To design new compounds we preserved important scaffold features and enumerated possible analogs by CReM tool*

**Suggested modifications**

*Totally 2,373,726 new compounds were generated*

**Docking score:**

-10.2

-10.7

-10.4

-10.0

*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.*

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 exhibit 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

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Thank you for your attention!