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Off targets toxicological investigation of anti-cancer tubulin inhibitors

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ABSTRACT

A class of novel tubulin inhibitors was synthesized based on NSC751382 (Figure 1), Benzof[1,3]dioxole-5-carboxylic acid [3-(2,5-dimethyl-benzyl(oxyl)-4-(methanesulfonyl-ethyl-amino)-phenyl] -amide, as the lead compound. This compound showed potent tubulin polymerization inhibitory activity by binding at the colchicine’s binding domain, and suppressed cancer cell growth with an IC50 of 200 nM. It has a molecular weight of 482 g/mol, logP of 4.1, only one hydrogen bond donor, and eight hydrogen bond acceptors. The compound meets the Lipinski’s Rule of Five and is a highly drug-like molecule. In addition, NSC751382 significantly inhibited the growth of Taxol resistant cancer cells, suggesting it is not a substrate for P-glycoprotein. Furthermore, it exhibited potent in vivo anti-cancer activity and excellent pharmacokinetic parameters. We further optimize the structure of the compound and generated a new analog with much improved potency (IC50 of 1 nM) to inhibit cancer cell growth (Figure 1). The new compound also showed much better potency to inhibit tubulin polymerization, cause cell cycle arrest, and inhibit in vivo tumor growth as well. However, we did notice mouse weight lost during the treatment, suggesting toxicity to the animals. We speculate that the lead optimization may result in off target effect, i.e., the new compound is binding to other proteins besides tubulin and cause toxicity. It seems that the structural optimization might cause target changing of the lead compound, and the new off target proteins may cause the toxicity. To investigate the toxicity, we synthesized 4 structures which are very similar analogs to compound A, and all the compounds showed similar in vitro activity to inhibit cancer cell growth. These compounds will be tested in the animals to correlate the toxicity to the structures, and elucidate the toxic moiety of the compounds. We also synthesized a biotinylated compound A to investigate the potential off target proteins that bind to the compounds, and explore the toxic inducing factors. This analysis can help us understand what structural characteristics lead to the target switching phenomenon. Understanding the structural difference correlated to the molecular targets will help us to design new analogs with reduced toxicity.

METHODS

Cell Culture and MTT Assay

- A172 and U373 brain cancer cell lines were maintained in DMEM media containing cipro, FBS and penicillin streptomycin.
- Cells grown in 96-well plates for 24 h.
- Cells then treated with various agents’ concentrations for 48 h.
- MTT Assay assessed for the cell lines.
- The final absorbance reading recorded at 570 nm using plate reader.
- Determination of IC50 values using Graph Pad and normalize the data by nonlinear regression analysis.

Design and synthesis of compound A biotinylated probe

RESULTS

<table>
<thead>
<tr>
<th>Compounds No.</th>
<th>Structure</th>
<th>IC50 (μM) - A172</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>[Diagram]</td>
<td>16.28±0.71</td>
</tr>
<tr>
<td>2</td>
<td>[Diagram]</td>
<td>8.33±0.416</td>
</tr>
<tr>
<td>3</td>
<td>[Diagram]</td>
<td>9.52±0.75</td>
</tr>
<tr>
<td>4</td>
<td>[Diagram]</td>
<td>19.77±1.68</td>
</tr>
</tbody>
</table>

CONCLUSIONS AND FUTURE DIRECTIONS

Results of MTT assay suggest that the four synthesized compounds showed different activity to U373 and A172 cells, suggesting they may target different pathways within these cells. The activity of four compounds in A172 cells are in a similar range, indicating that their tubulin targeting potency is similar. The in vivo toxicity of these compounds is hypothesized to have an off target effect, which is the future work.

Future experiments will be conducted to perform the in vivo toxicity study and protein pull down assay to identify the off target proteins.

ACKNOWLEDGEMENTS

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