Small Molecule Inhibitor Screen for Fibroblast Growth Factor Receptor 4 Activity

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Results

Within the same library, another approach was made where compounds synthesized by Vichem Chemie in the focus library of known kinase inhibitors were screened for FGFR4 inhibition in both the cell-based and in vitro kinase assays. To date, more than 600 compounds have completed the process.

In this project, compounds synthesized by Vichem Chemie in the focus library of known kinase inhibitors were indiscriminately screened for FGFR4 inhibition in the cell-based and in vitro kinase assays. Similar to the compounds in this focus library, the compounds were screened for FGFR4 inhibition in both the cell-based and in vitro kinase assays.

Potential candidates from the screens were further tested in the cell viability assay to validate their toxicity effect in breast cancer cells (MDA-MB43) versus that of normal human breast cells, HMEC (Human Mammary Epithelial Cell).

Table 1. In vitro kinase and cell-based FGFR4 inhibition by potential in silico FGF hits, with their respective IC50 in MDA-MB43 and HMEC (Human Mammary Epithelial Cell).

<table>
<thead>
<tr>
<th>Compound name</th>
<th>In vitro FGFR4 inhibition @ 10µM (%</th>
<th>Cell-based FGFR4 inhibition @ 10µM (%</th>
<th>IC50 (µM)</th>
</tr>
</thead>
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<tr>
<td>V2-153</td>
<td>135.0</td>
<td>115.0</td>
<td>&gt;10</td>
</tr>
<tr>
<td>V4-007</td>
<td>100.0</td>
<td>100.0</td>
<td>&gt;10</td>
</tr>
<tr>
<td>V4-013</td>
<td>98.0</td>
<td>98.0</td>
<td>&gt;10</td>
</tr>
<tr>
<td>V4-015</td>
<td>98.0</td>
<td>98.0</td>
<td>&gt;10</td>
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Table 2. In vitro and cell-based FGFR4 inhibition of potential FGFR4 hits, with their respective IC50 in MDA-MB43 and HMEC (Human Mammary Epithelial Cell).

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Figure 3. FGFR4’s phosphorylation is inhibited by potential compounds from the Nested Chemical Library. MDA-MB43 was treated with increasing dose of V4-015 for 72 hours. The ratio of pFGFR4 to total FGFR4 is shown as a percentage of DMSO treated cells. (A) V4-015 reduces cell viability of MDA-MB43 more efficiently than HMEC. Cells were seeded and a cell viability assay was performed using CellTiter-Glo® Assay (Promega), after treatment with increasing dose of V4-015 over 72 hours.

Figure 4. Compounds V4-015 reduces cell viability of MDA-MB43 more efficiently than HMEC. Cells were seeded and a cell viability assay was performed using CellTiter-Glo® Assay (Promega), after treatment with increasing dose of V4-015 over 72 hours.

Figure 5. FGFR4’s phosphorylation is inhibited by potential compound from the Nested Chemical Library. MDA-MB43 was treated with increasing dose of V4-015 for 72 hours. The ratio of pFGFR4 to total FGFR4 is shown as a percentage of DMSO treated cells. (A) V4-015 reduces cell viability of MDA-MB43 more efficiently than HMEC. Cells were seeded and a cell viability assay was performed using CellTiter-Glo® Assay (Promega), after treatment with increasing dose of V4-015 over 72 hours.

Figure 6. Compound V4-036 reduces cell viability. Cell viability of HMEC and MDA-MB43, performed using CellTiter-Glo® Assay (Promega), after treatment with increasing dose of V4-036 for 72 hours.

Conclusions

• FGFR4 is an attractive target for cancer therapy.
• The screening of selected in silico FGFR3 compounds and compounds from the focus library have resulted in two potential FGFR4 hits, V4-015 and V4-036, respectively.
• V4-015 and V4-036 appear to be promising for further characterization, based on their high FGFR4 inhibition in the cell-based and in vitro kinase assay. This is in addition to their greater cytotoxic effect on breast carcinoma cell line in contrast to normal breast epithelial cell line.

Acknowledgement

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References