Proteolysis Targeting Chimeras for the selective degradation of Mcl-1/Bcl-2 derived from nonselective target binding ligands

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Contents

<table>
<thead>
<tr>
<th>Contents</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Supplementary Figures</td>
<td>S2-S4</td>
</tr>
<tr>
<td>Figure S1</td>
<td>S2</td>
</tr>
<tr>
<td>Figure S2</td>
<td>S2</td>
</tr>
<tr>
<td>Figure S3</td>
<td>S2</td>
</tr>
<tr>
<td>Figure S4</td>
<td>S3</td>
</tr>
<tr>
<td>Figure S5</td>
<td>S3</td>
</tr>
<tr>
<td>Figure S6</td>
<td>S4</td>
</tr>
<tr>
<td>Figure S7</td>
<td>S4</td>
</tr>
<tr>
<td>Figure S8</td>
<td>S4</td>
</tr>
<tr>
<td>Figure S9</td>
<td>S5</td>
</tr>
<tr>
<td>The 1H/13C-NMR, HPLC and ESI-MS/MS spectrums of the key compounds.</td>
<td>S6-17</td>
</tr>
</tbody>
</table>
Figure S1. Inhibition curves of S1-6, Nap-1 and the PROTACs C1-C5 and H1-H5 to McI-1 and Bcl-2 measured by ELISA.

Figure S2. Ubiquitin adducts of McI-1 analyzed by Western blotting in Hela cells treated with H4 and H5 using FK2 antibody.

Figure S3. Protein levels of McI-1 and Bcl-2 after treatment with 10 μM C3, C5 or S1-5 over a 0-24 h time gradient in Hela cells. Repeated experiments (n=3) of Figure 3a.
Figure S4. Protein levels of Mcl-1 and Bcl-2 after treatment with C3, C5 or S1-5 in a 0-10 μM concentration gradient for 12 h in Hela cells. Repeated experiments (n=3) of Figure 3b.

Figure S5. mRNA levels of Mcl-1 (a) or Bcl-2 (b) after treatment with C3 (a) or C5 (b) at a 10 μM concentration gradient for 0-24 h in Hela cells. Data are expressed as the mean ± s.e.m. (n=3)
**Figure S6.** Protein levels after treatment with C3 or S1-5 in a 0-10 μM concentration gradient for 12 h in K562 cells. All western blot figures represent the results from n = 3 independent samples.

**Figure S7.** Protein levels after treatment with C3 or C5 at 10 μM concentration for 12 h in Hela cells. All western blot figures represent the results from n = 3 independent samples.

**Figure S8.** Stable interactions between Mcl-1/Bcl-2 and CRBN were induced by the addition of the PROTACs in Hela cells. Immobilized CRBN was used as a bait to trap Mcl-1 and Bcl-2 in the presence of a concentration gradient of H3. Protein levels of the trapped proteins were visualized by immunoblot in the pull-down reactions. All western blot figures represent the results from n = 3 independent samples.
Figure S9. The C5/C3-induced unfavourable complexes between Mcl-1/Bcl-2 and CRBN. (a) CRBN, C5 and Mcl-1 and (b) CRBN, C3 and Bcl-2 were docked and a 50-ns molecular dynamics simulation relaxed the structure (Mcl-1 PDB ID: 2PQK; Bcl-2 PDB ID: 2XA0; CRBN PDB ID: 4TZ4).
$^1$H-NMR spectra of compound H1.

$^1$H-NMR spectra of compound H2.
$^1$H-NMR spectra of compound H3.

$^1$H-NMR spectra of compound H4.
$^1$H-NMR spectra of compound H5.

$^1$H-NMR spectra of compound C1.
$^1$H-NMR spectra of compound C2.
$^1$H-NMR spectra of compound C3.
$^{13}$C-NMR spectra of compound C3
The purity of compound C3 determined by HPLC.

Mass spectrum of compound C3.
$^1$H-NMR spectra of compound C3-Me.

$^1$H-NMR spectra of compound C4.
$^1$H-NMR spectra of compound C5.
$^{13}$C-NMR spectra of compound C5.
The purity of compound **C5**.

Mass spectrum of compound **C5**.
$^1$H-NMR spectra of compound C5-Me.