Rational Hopping of a Peptidic Scaffold into Non-Peptidic Scaffolds: Structurally Novel Potent Proteasome Inhibitors Derived from a Natural Product, Belactosin A

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Rational scaffold hopping of the natural product belactosin A derivative was successfully achieved based on the pharmacophore model constructed. The peptidic scaffold was replaced by significantly simplified non-peptidic scaffolds, by which weak belactosin A (IC_{50} = 1440 nM) was converted into highly potent non-peptidic inhibitors (IC_{50} = 26–393 nM).

In the drug discovery process, not only desired biological effect on target biomolecule, but also other various properties such as metabolic stability, membrane permeability, solubility, toxicity profile, and synthetic accessibility of leads, have to be optimized. 1, 2

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Belactosin A is a tripeptide natural product produced by Streptomyces sp., that comprises L-alanine, 3-(trans-2-aminocyclopropyl)-L-alanine, and a chiral carboxy-β-lactone moiety, 2, 3 and inhibits the proteasome ChT-L activity 4 by acylating the active-site Thr residue via its strained β-lactone opening, as confirmed by X-ray crystallographic analysis. 5-10 Due to its proteasome inhibitory activity, belactosin A prevents cell cycle progression at the G2/M stage in tumor cells and is therefore a novel lead for developing potent anticancer agents. 11, 12

In recent years, we have performed systematic structure-activity relationship (SAR) studies of belactosin A 16-19 and identified a highly potent proteasome inhibitor 4 (IC_{50} = 5.7 nM). However, synthetic accessibility of 4 is too low (total synthetic steps 26) due to its multi-chiral peptidic scaffold. Furthermore, peptidic scaffolds generally impair membrane permeability and bioavailability. 9 Therefore, to identify novel leads with superior drug-like properties, we planned to convert the multi-chiral peptidic scaffold of 4 into an achiral non-peptidic scaffold by topology-based hopping.

Fig. 1 Potent proteasome inhibitor 4 developed by us

Previously, we analyzed binding mode of 4 around the transition state (Fig. 2b, green tube). 20 In addition, two crystal structures of homobelactosin C derivatives in complex with proteasome (Fig. 2b, yellow and orange wires) have been analyzed by Meijere et al. 14, 15 These analyses importantly demonstrates that no hydrogen bond is
formed between peptidic scaffolds of these belactosin derivatives and proteasome, suggesting that these peptidic scaffolds can be replaced with non-peptidic scaffolds. Furthermore, in these binding structures, the peptidic moieties are bended to place aromatic rings onto hydrophobic proteasome surfaces, which implicates these peptidic scaffolds can be shortened.

The inhibitory effects of the compounds on the ChT-L activity of human 20S proteasomes were measured using a chromophoric substrate Suc-LLVY-AMC, and the results are summarized in Table 1. All of the compounds with amine-type scaffolds (5-12) showed potent proteasome inhibitory activity (IC_{50} = 26-56 nM), in spite of their achiral non-peptidic scaffolds, which were markedly simplified in comparison with the multi-chiral peptidic scaffold of 4. In these compounds, the linker length only weakly impacts on their proteasome inhibitory activities. The flexible nature of these scaffolds would allow the two aromatic rings to be placed into the proteasome binding sites regardless of their linker length as expected (Fig. 2d).

Table 1. Proteasome and cell growth inhibitory effects and molecular properties of novel non-peptide proteasome inhibitors 5-12

<table>
<thead>
<tr>
<th>cpd</th>
<th>ChT-L activity</th>
<th>properties compared with 4</th>
<th>cell growth</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IC_{50} [nM]</td>
<td>BEI [ES]</td>
<td>SEI [ES]</td>
</tr>
<tr>
<td>5</td>
<td>175 ± 32</td>
<td>37</td>
<td>26</td>
</tr>
<tr>
<td>6</td>
<td>393 ± 24</td>
<td>33</td>
<td>25</td>
</tr>
<tr>
<td>7</td>
<td>215 ± 40</td>
<td>35</td>
<td>26</td>
</tr>
<tr>
<td>8</td>
<td>352 ± 25</td>
<td>32</td>
<td>25</td>
</tr>
<tr>
<td>9</td>
<td>28 ± 2.2</td>
<td>41</td>
<td>23</td>
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<tr>
<td>10</td>
<td>29 ± 13</td>
<td>38</td>
<td>23</td>
</tr>
<tr>
<td>11</td>
<td>26 ± 6.1</td>
<td>40</td>
<td>24</td>
</tr>
<tr>
<td>12</td>
<td>56 ± 16</td>
<td>36</td>
<td>22</td>
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<tr>
<td>4</td>
<td>5.7 ± 1.2</td>
<td>34</td>
<td>15</td>
</tr>
</tbody>
</table>

[a] Based on three experiments. [b] pIC_{50} per molecular weight (kDa). [c] pIC_{50} per polar surface area (PSA) normalized to 100 Å².
[d] Calculated by ChemBioDraw Ultra 12

We also evaluated the cell growth inhibitory effect of these compounds on HCT116 (Table 1). Most of these compounds retained the potent cell growth inhibitory effects of their parent compound 4, and, in particular, the cell growth inhibitory effect of 11 was as potent as 4.

To clarify the impact of the scaffold hopping on the molecular properties as leads, we calculated binding efficiency index (BEI) and surface-binding efficiency index (SEI) of these compounds (Table 1): BEI and SEI indicate binding efficiency per molecular weight and polar surface area (PSA), respectively. Because molecular weight and PSA are well known physicochemical properties well-related to membrane permeability and bioavailability, BEI and
SEI can be effective indicators of drug-likeness. The desirable leads in the drug discovery process generally have high BEI and SEI values.\(^2\)

Fig. 3 shows the plot of BEI vs. SEI of the newly identified non-peptide inhibitors 5-12 and the previously developed inhibitors with the peptide scaffold identical to that of 4.\(^6\) The BEI of the peptidic inhibitor 4 and its congeners varies considerably (BEI = 25-43), but their SEI are comparable (SEI = 13-16), indicating that the substituent modifications effectively improved the affinity for the proteasome but not the molecular properties arising from the scaffold. On the other hand, the newly identified non-peptidic inhibitors 5-12 show remarkably higher SEI compared with their parent 4. Furthermore, their BEI are comparable with or superior to 4 due to their significantly lower molecular weight (Table 1). These improved BEI and SEI suggest that these novel non-peptide inhibitors are clearly superior to 4 as lead compounds for optimization.

![Fig. 3](image_url)

**Fig. 3** BEI and SEI values of belactosin derivatives synthesized by our group

Furthermore, synthetic accessibility of these novel non-peptide derivatives is significantly superior to that of 4 due to their significantly simplified scaffolds with no chiral center.

We successfully achieved the topology-based scaffold hopping of 4. The multi-chiral peptidic scaffold of 4 was rationally replaced with achiral non-peptidic scaffolds to identify the superior leads suitable for further optimization. The present study demonstrates that topology-based hopping is a highly effective strategy to change the undesired molecular properties of leads, including natural products and peptides. This study is also of vital importance in terms of the use of a natural product as a lead. Thus, a weak peptidic natural product inhibitor belactosin A (IC\(_{50} = 1440\) nM) was converted into a highly potent non-peptide inhibitor 11 (IC\(_{50} = 26\) nM).

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**Notes and references**


10. During our study, simplified belactosin C analogues were reported by Meijere et al.: *Org. Biomol. Chem.*, 2012, 10, 6363.


21. The cell growth inhibitory activity of the belactosin derivatives may be significantly simplified scaffolds with no chiral center.


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