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COMMUNICATION

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] The introduction and/or modification of substituents of a lead are commonly investigated to optimize these properties, but, a more drastic structural change, i.e., scaffold hopping, is occasionally needed to identify drug candidates with the desired properties.[3-7] Although natural products are useful sources of drug candidates, their complex structures sometimes limit their use as clinical drugs.[8] Peptides are also potentially useful for the treatment of diseases, but their low membrane permeability and biological stability often limit their application as clinical drugs.[9] To create promising drug candidates from these leads, including natural products and peptides, development of a rational methodology for scaffold hopping to address these limitations is required. Here we report development of potent non-peptidic proteasome inhibitors derived from a peptidic natural product, belactosin A, based on a rational scaffold hopping.[10]

The term "scaffold hopping" was defined by Schneider *et al.* in 1999 as the "*identification of isofunctional molecular structures with significantly different molecular backbones*".[11] Recently, Sun and co-workers classified scaffold hopping approaches into four major categories based on the degree of structural change: a) 1° hop: heterocycle replacement, b) 2° hop: ring opening or closure, c) 3° hop: peptidomimetics, and d) 4° hop: topology-based hopping, in which a scaffold is converted into a completely new chemical backbone retaining the required topology of the key functional groups.[7] In general, the higher the hop level becomes, the more drastic the change in molecular properties, while the success rate inversely correlates with the magnitude of the structural change. Thus, success examples of topology-based hopping are significantly

rare,[7] despite its potential to improve the molecular properties drastically.

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,[12] and inhibits the proteasome ChT-L activity[13] by acylating the active-site Thr residue *via* its strained β-lactone opening, as confirmed by X-ray crystallographic analysis.[14-16] 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.[12,13]

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.
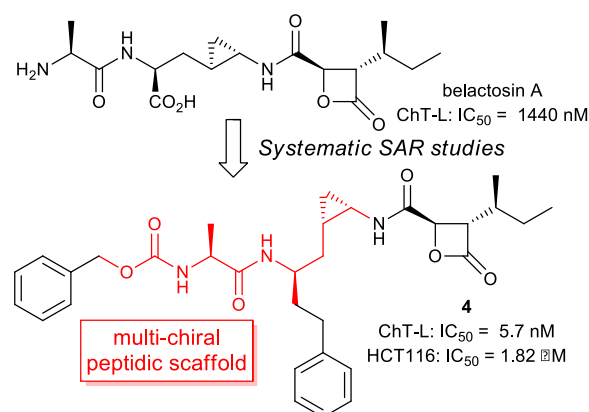

Fig. 1 Potent proteasome inhibitor **4** developed by us

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.
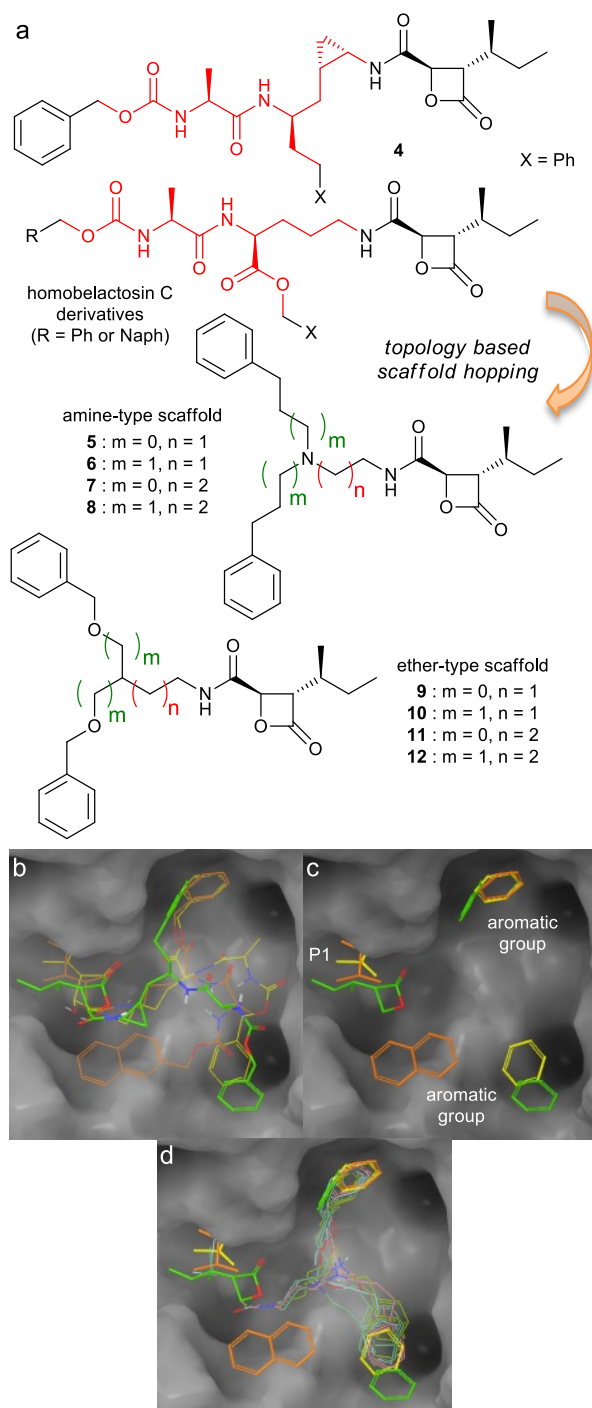

Fig. 2 Rational design of the non-peptidic belactosin A derivatives by topology-based scaffold hopping (a) Structure of the compound **4**, homobelactosin C derivatives[14, 15] and newly designed non-peptide

derivatives **5-12** (b) Previously analyzed non-covalent binding mode of **4** (green tube) around the transition state[20] and X-ray analyzed binding mode of the homobelactosin C derivatives (yellow and orange wires)[14, 15] (c) Plausible pharmacophore model constructed (d) Results of flexible alignment of the designed compounds (**5** and **9**, aquamarine; **6** and **10**, turquoise; **7** and **11**, yellow green; **8** and **12**, pink) onto the binding conformation of the belactosin derivatives shown in Fig. 2b.

These structural information and the previous SAR studies[15-18] suggested that the only β-lactone moiety and two aromatic groups are essential for the strong binding, so that we constructed a pharmacophore model composed of these three moieties, as shown in Fig. 2c. Based on the pharmacophore model, we designed the non-peptide derivatives **5-12**, in which two aromatic groups were placed symmetrically to eliminate chiral centers. The designed compounds were flexibly superimposed onto the bioactive conformation of **4** and homobelactosin C derivatives in Fig 2b. As shown in Fig. 2d, the β-lactone moiety and two hydrophobic groups were well superimposed in all these compounds without steric repulsion with proteasome, suggesting that these newly designed compounds can retain strong inhibitory activity of **4**.

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 (**5-8**) showed potent proteasome inhibitory activity (IC_{50} = 175-393 nM). Compounds **9-12** with ether-type scaffolds showed further strong 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**

cpd	ChT-L activity			properties compared with **4**		cell growth IC_{50} [μM][a]
	IC_{50} [nM][a]	BEI[b]	SEI[c]	Mw.	tPSA[d]	
5	175 ± 32	37	26	- 141	- 64	8.90
6	393 ± 24	33	25	- 113	- 64	> 10
7	215 ± 40	35	26	- 127	- 64	2.20
8	352 ± 25	32	25	- 99	- 64	3.80
9	28 ± 2.2	41	23	- 138	- 49	4.10
10	29 ± 13	38	23	- 110	- 49	> 10
11	26 ± 6.1	40	24	- 124	- 49	1.80
12	56 ± 16	36	22	- 96	- 49	4.20
4	5.7 ± 1.2	34	15	-	-	1.82

[a] Based on three experiments. [b] pIC_{50} per molecular weight (kDa). [c] pIC_{50} per polar surface area (PSA) normalized to 100 Å^2. [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**.[21]

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.[22] Because molecular weight and PSA are well known physicochemical properties well-related to membrane permeability and bioavailability,[23-27] 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.[22]

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**.[16] The BEI of the peptidic inhibitor **4** and its congeners varies considerably (BEI = 25-43), but their SEI are analogous (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.
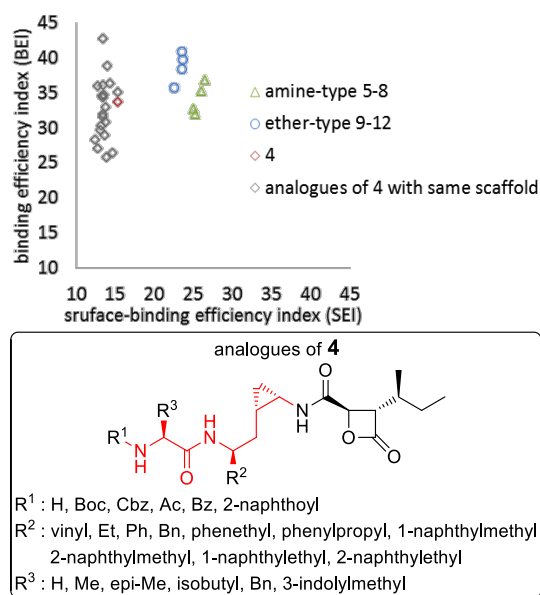

Fig. 3 BEI and SEI values of belactosin derivatives synthesized by our group

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).

Notes and references

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