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Chemical and Biological Insights into Belactosin-Based Proteasome Inhibitors

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Abstract

Belactosin is a naturally occurring peptide-derived secondary metabolite isolated from *Streptomyces* species and is recognized for its potent proteasome inhibitory activity. The proteasome plays a crucial role in intracellular protein degradation and regulation of cell cycle, apoptosis, and immune responses. Inhibition of the proteasome has emerged as a validated therapeutic strategy, particularly in cancer treatment. Belactosin A and its analogues possess a unique β-lactone moiety responsible for irreversible binding to the catalytic threonine residue of the proteasome. This paper presents a comprehensive review of belactosin, covering its discovery, chemical structure, biosynthesis, mechanism of action, biological activities, structure–activity relationships, pharmaceutical relevance, and future prospects. The study highlights belactosin as an important lead compound in natural product–based drug discovery.

Keywords: Belactosin, proteasome inhibitor, β-lactone, *Streptomyces*, natural products, anticancer agents

1. Introduction

Natural products have historically played a central role in the development of therapeutic agents. A significant proportion of modern drugs, especially anticancer and antibiotic agents, are either natural products or their derivatives. Microorganisms, particularly actinomycetes of the genus *Streptomyces*, are prolific producers of structurally diverse bioactive metabolites. These organisms synthesize secondary metabolites that provide competitive advantages in their ecological niches and often display potent biological activities relevant to human health.

Belactosin belongs to a class of peptide-derived natural products characterized by the presence of a reactive β-lactone ring. Since its discovery, belactosin has attracted attention due to its ability to inhibit the proteasome, a multicatalytic protease complex responsible for the degradation of ubiquitinated

proteins. Proteasome dysfunction is associated with several diseases, including cancer, neurodegenerative disorders, and inflammatory conditions. The identification of belactosin has therefore contributed to the expanding field of proteasome inhibitor research.

2. Discovery and Source

Belactosin A was first isolated during microbial screening programs aimed at identifying novel proteasome inhibitors from fermentation broths. The producing organism was identified as a *Streptomyces* species. Subsequent investigations revealed additional congeners, including belactosins B, C, and D, which differ slightly in their amino acid composition and side-chain modifications.

The production of belactosin occurs during the stationary phase of microbial growth, consistent with its classification as a secondary metabolite. Optimization of fermentation conditions such as carbon source, nitrogen source, pH, and temperature has been shown to influence belactosin yield. Advances in microbial genetics have enabled partial elucidation of the gene clusters responsible for belactosin biosynthesis.

3. Chemical Structure and Properties

Belactosins are small peptide-based molecules containing a characteristic β-lactone ring fused with amino acid residues. The β-lactone moiety is chemically strained and highly reactive, enabling covalent interaction with nucleophilic residues in target enzymes. Spectroscopic techniques such as nuclear magnetic resonance (NMR), mass spectrometry (MS), and infrared spectroscopy (IR) have been employed to elucidate the structure of belactosin[2].

Structural variations among belactosin analogues influence their biological activity. Minor changes in side chains can alter binding affinity, selectivity, and stability. The presence of hydrophobic amino acid residues contributes to effective interaction with the proteasome active site, while the β-lactone ring is essential for irreversible inhibition.

Belactosin A incorporates a unique 3-(trans-2'-aminocyclopropyl)alanine moiety (also known as trans-3,4-methano-L-ornithine) in its backbone. Its molecular formula is $C_{17}H_{27}N_3O_6$

Belactosin B is a natural product dipeptide with a reactive β-lactone group, isolated from *Streptomyces.* Its molecular formula is $C_{18}H_{31}N_3O_7$. A dipeptide containing L-alanine, a cyclopropane-containing amino acid (trans-3,4-methano-L-ornithine), and a chiral β-lactone carboxylic acid.

Belactosin C has a simpler L-ornithine residue in the corresponding position, without the cyclopropane ring. Its molecular formula is $C_{16}H_{27}N_3O_6$

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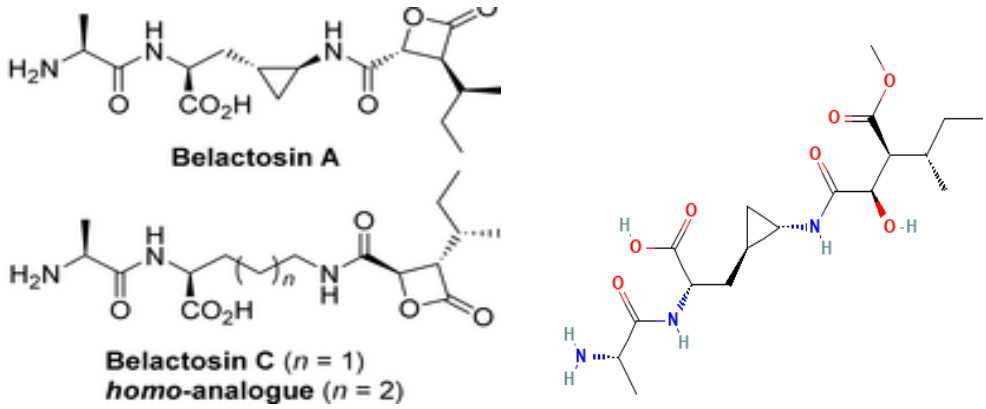

Belactosin B

4. Biosynthesis and Synthetic Approaches

The biosynthesis of belactosin is proposed to involve a non-ribosomal peptide synthetase (NRPS) pathway[1]. NRPS enzymes are large, multi-domain complexes capable of assembling peptide chains independently of ribosomal machinery. In belactosin biosynthesis, specific amino acid residues are activated, tethered to thiolation domains, and condensed in a stepwise manner.

Following peptide assembly, tailoring enzymes catalyze intramolecular cyclization reactions leading to the formation of the characteristic β-lactone ring[2]. Additional post-synthetic modifications, such as oxidation or methylation, contribute to the structural diversity observed among belactosin analogues. Genetic studies suggest that manipulation of the biosynthetic gene cluster may enable the generation of structurally novel belactosin derivatives.

Steps involved in biosynthesis of belactosin:

i. Amino Acid Activation (Adenylation Domain – A)

$$\text{Amino Acid (AA)} + \text{ATP} \xrightarrow{\text{A domain}} \text{AA–AMP} + PP_i$$

The adenylation (A) domain selectively recognizes the substrate amino acid (e.g., Leu, Val, or a non-proteinogenic amino acid involved in belactosin) and converts it into an aminoacyl-adenylate (AA–AMP).

ii**. Thiolation (Peptidyl Carrier Protein – PCP / T Domain)**

Thioester formation

$$\text{AA–AMP} + \text{PCP–SH} \xrightarrow{\text{T domain}} \text{AA–S–PCP} + \text{AMP}$$

The activated amino acid is transferred to the 4′-phosphopantetheine arm of the thiolation (T) domain, forming a thioester-linked intermediate.

iii. Peptide Bond Formation (Condensation Domain – C)

$$AA_1\text{–S–}PCP_1 + AA_2\text{–S–}PCP_2 \xrightarrow{\text{C domain}} AA_1\text{–}AA_2\text{–S–}PCP_2 + PCP_1\text{–SH}$$

The condensation (C) domain catalyzes amide bond formation, extending the peptide chain in a stepwise manner.

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iv. Chain Release and Cyclization (Thioesterase or Cyclase Domain)

iva. Intramolecular Cyclization Leading to β-Lactone Formation

β-Lactone ring closure

$$\beta\text{-Hydroxyacyl–S–PCP} \xrightarrow{\text{TE/Cyclase}} \text{Belactosin β-lactone} + \text{PCP–SH}$$

Mechanism (key step):

- Nucleophilic attack of a β-hydroxyl group on the thioester carbonyl
- Intramolecular esterification → four-membered β-lactone ring

v. Post-NRPS Tailoring Reactions

va. Oxidation (Cytochrome P450 or Dehydrogenase)

$$\text{Belactosin intermediate} \xrightarrow{[O],\ \text{P450}} \text{Oxidized belactosin analogue}$$

Examples:

- Hydroxylation of alkyl side chains
- Oxidation of secondary alcohols

vb. Methylation (SAM-Dependent Methyltransferase)

$$\text{Belactosin–OH / –NH} + \text{SAM} \xrightarrow{\text{MT}} \text{Belactosin–OCH}_3\text{/–NCH}_3 + \text{SAH}$$

Methylation contributes to structural diversity and biological activity among belactosin analogues[8].

vi. Genetic Manipulation for Novel Derivatives

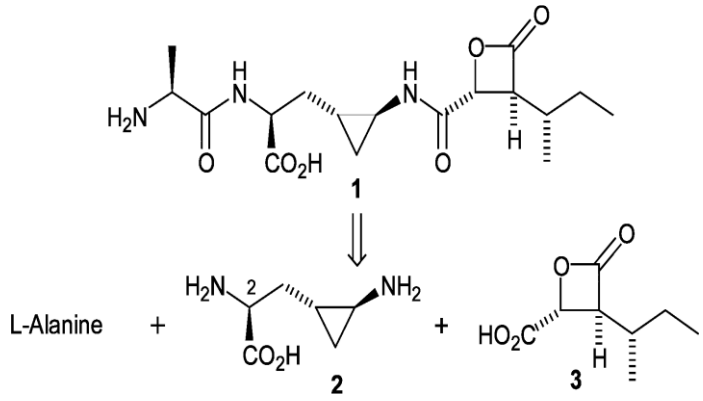
Reaction concept (combinatorial biosynthesis):

Modified NRPS A-domain specificity ⇒ New AA incorporation ⇒ Novel belactosin derivatives

In addition to biosynthetic studies, several total and semi-synthetic approaches have been explored to access belactosin and its analogues for structure–activity relationship (SAR) investigations.

Total synthesis of (+)-belactosin A

A concise first total synthesis of the antitumour antibiotic belactosin A is reported[3], involving coupling of β-lactone carboxylic acid 3 with *N*-Ala-aminocyclopropyl alanine 11.



Retrosynthetic analysis of belactosin A

Coupling of L-alanine and the β-lactone carboxylic acid 3 to the intriguing and unique central (2*S*,1'*R*,2'*S*)-3-(*trans*-2-aminocyclopropyl) alanine core 2 constituted an effective and flexible synthetic

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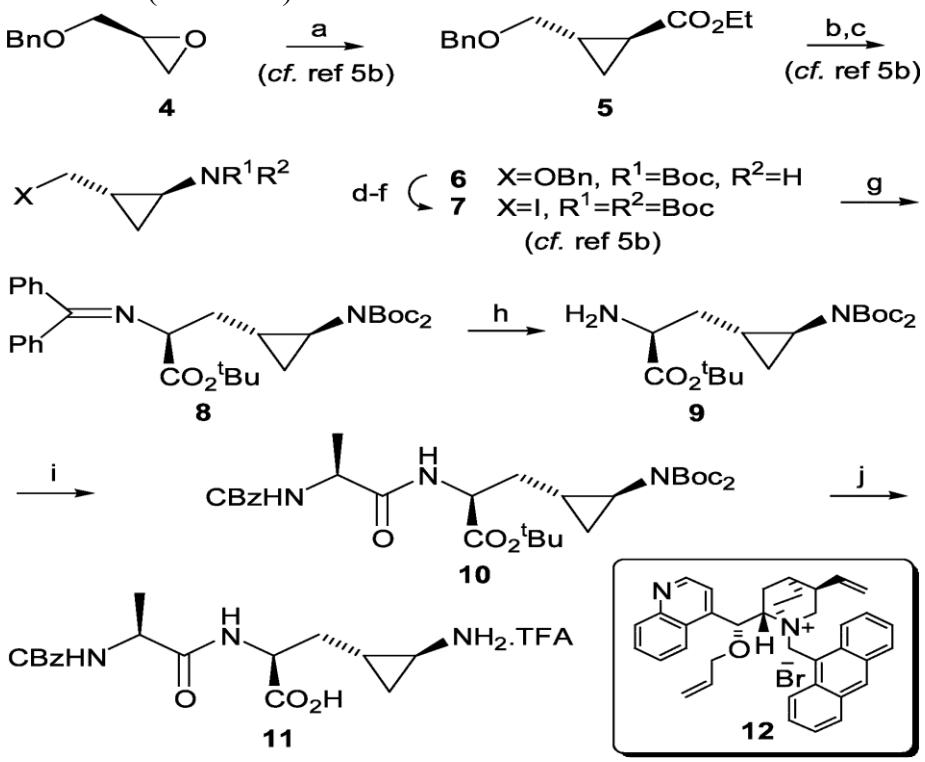
strategy[10]. Stereo controlled synthesis of the β-lactone 3, and coupling of these fragments to complete the first total synthesis of 1 (Scheme 1)



Scheme 1 (a) Triethyl phosphonoacetate, NaH, toluene, 110 °C, 14 h, 63%;(b)NaOH(aq), EtOH, 96%; (c) DPPA, tBuOH, NEt_3, reflux, 53%; (d)Boc_2O, MeCN, DMAP, 95%; (e) Pd/C, H2, cat. AcOH, THF, 98%; (f)Bu_4NI, DDQ, PPh_3, $CHCl_3$, rt; (g) 2 eq *N*-(diphenyl methylene) glycine *tert*-butylester, 20 mol% 12, 10 eq CsOH·H2O (s), toluene/CH_2Cl_2 (1 : 1), -40°C, 40 h; 67%; (h) 15% citric acid/THF, 84%; (i) 2 eq *N*-CBz-Ala, DCC/HOBt/DMF, 100%; (j) TFA/CH_2Cl_2, 15 °C, 20 h, 90%.

Synthesis of β-lactone **3** is possible *via* stereoselective chlorination of the monosubstituted succinate **18** followed by cyclisation. ^{1}H NMR and crystallographic analysis of *tert*-butyl ester **19** confirmed the relative stereochemistry to be in agreement with that found for the natural product. Lactone **19** was then converted to its acid **3** with anhydrous TFA/CH_2Cl_2 in high yield, maintaining the integrity of the β-lactone ring (**Scheme 2).**

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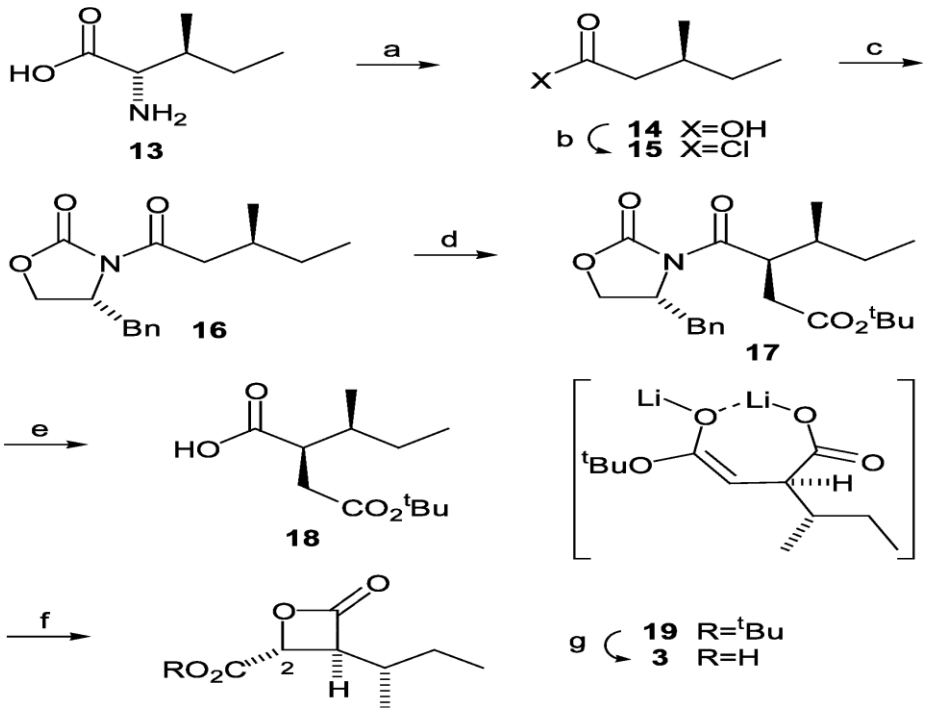
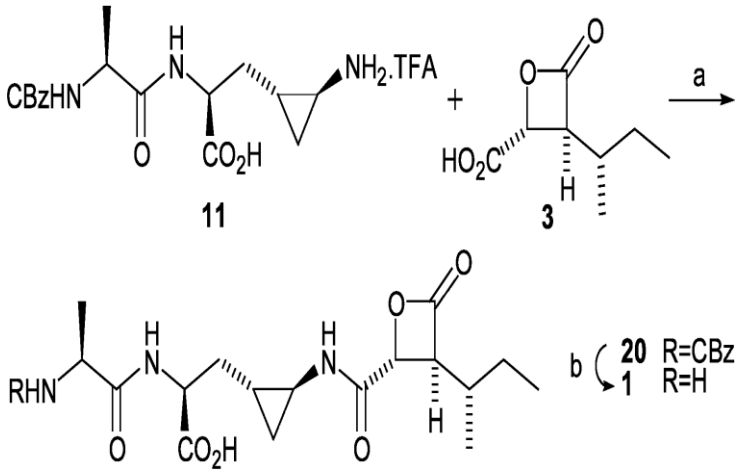


Scheme 2 (a) H_2NOSO_3H/KOH (aq), 0 °C to rt, 74%; (b) $(COCl)_2$, CH_2Cl_2,0 °C to rt, 80%; (c) (4*R*)-benzyl-2-oxazolidinone, nBuLi 278 °C, 79%; (d)*tert*-butyl bromoacetate, NaHMDS, -78 °C, 82%; (e) LiOH (aq), H_2O_2, 0°C to rt, 92%; (f) 2 eq LiHMDS, CCl_4, -78 °C to rt; then ether/$NaHCO_3$,55%; (g) TFA/CH_2Cl_2, 0 °C, 20 h, 90%.



Scheme 3

Reaction of 11 & 3 gave Amide **20** obtained in 50% yield as a single diastereoisomer after purification. final deprotection of **20** was achieved by hydrogenation in the presence of Pd/C in THF under TFA activation, yielding the TFA salt of belactosin A (**Scheme 3).**

5. Mechanism of Action

Belactosin A exerts its biological activity primarily through inhibition of the 20S proteasome core particle. The proteasome contains multiple proteolytic active sites, with the catalytic threonine residue playing a key role in peptide bond hydrolysis[9]. The β-lactone ring of belactosin reacts covalently with this threonine residue, resulting in irreversible inhibition of proteasomal activity.

Proteasome inhibition leads to the accumulation of ubiquitinated and misfolded proteins within the cell. This accumulation disrupts cellular homeostasis, interferes with cell cycle progression, and triggers apoptotic pathways. Cancer cells, which rely heavily on proteasome function for rapid proliferation, are particularly sensitive to proteasome inhibitors.

6. Biological Activities

6.1 Anticancer Activity

Belactosin A has demonstrated cytotoxic effects against a variety of cancer cell lines, including leukaemia and solid tumour models. Its mechanism of action is comparable to that of clinically used proteasome inhibitors such as bortezomib, although belactosin possesses a distinct chemical scaffold[9]. Studies suggest that belactosin induces apoptosis through activation of caspase pathways and inhibition of nuclear factor kappa B (NF-κB) signalling.

6.2 Antimicrobial and Other Activities

In addition to anticancer properties, belactosins have shown moderate antimicrobial activity against certain bacterial strains. Preliminary studies also suggest potential immunomodulatory and anti-inflammatory effects, although these activities require further investigation.

7. Structure–Activity Relationship (SAR)

SAR studies confirm that the intact β-lactone ring is indispensable for proteasome inhibition. Replacement of the lactone with lactam or open-chain analogues leads to drastic loss of activity. Hydrophobic substitutions enhance cellular permeability, while polar substitutions reduce cytotoxicity.

Based on the structure–activity relationship studies of belactosin A, highly potent derivative 1(Figure 1) was developed. Further, by the topology-based scaffold hopping of 1 based on (SAR) and binding-mode analyses results a significantly simplified non-peptide inhibitor 2 (Figure 1) was developed. But it showed moderate cell growth inhibitory effects. This was due to its unstable β-lactone warhead and therefore it was replaced by a more stable and irreversible warhead to develop potent cell growth inhibitors[11].

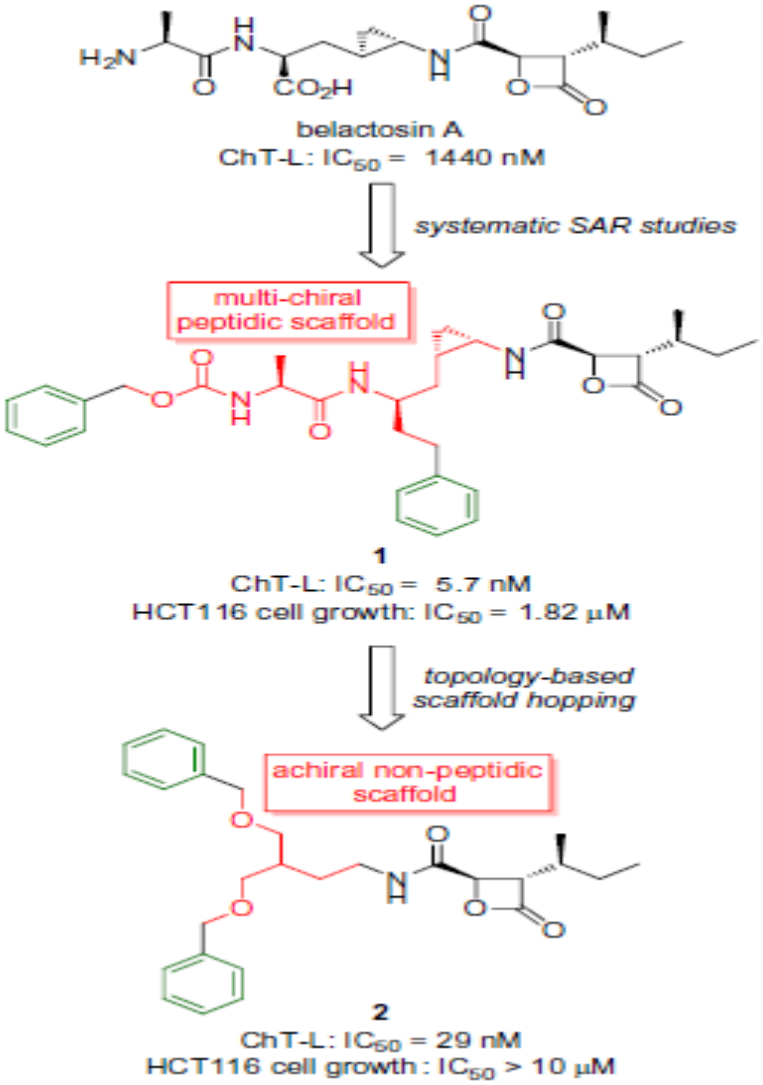


Figure 1 Belactosin A derivatives

Figure 2, shows the simulated binding mode, the moiety derived from the non-peptidic belactosin A derivative ligated with the P2 side-chain is effectively accommodated in the primed binding site[11], as expected. Therefore, these molecules were synthesised and their biological effects was evaluated.

8. Pharmaceutical Importance

Belactosin represents an important lead compound in the development of proteasome inhibitors. Although it has not yet progressed to clinical use, belactosin has contributed to the understanding of proteasome inhibition mechanisms and inspired the synthesis of novel β-lactone–containing inhibitors[10]. Its study underscores the importance of microbial natural products in modern drug discovery.

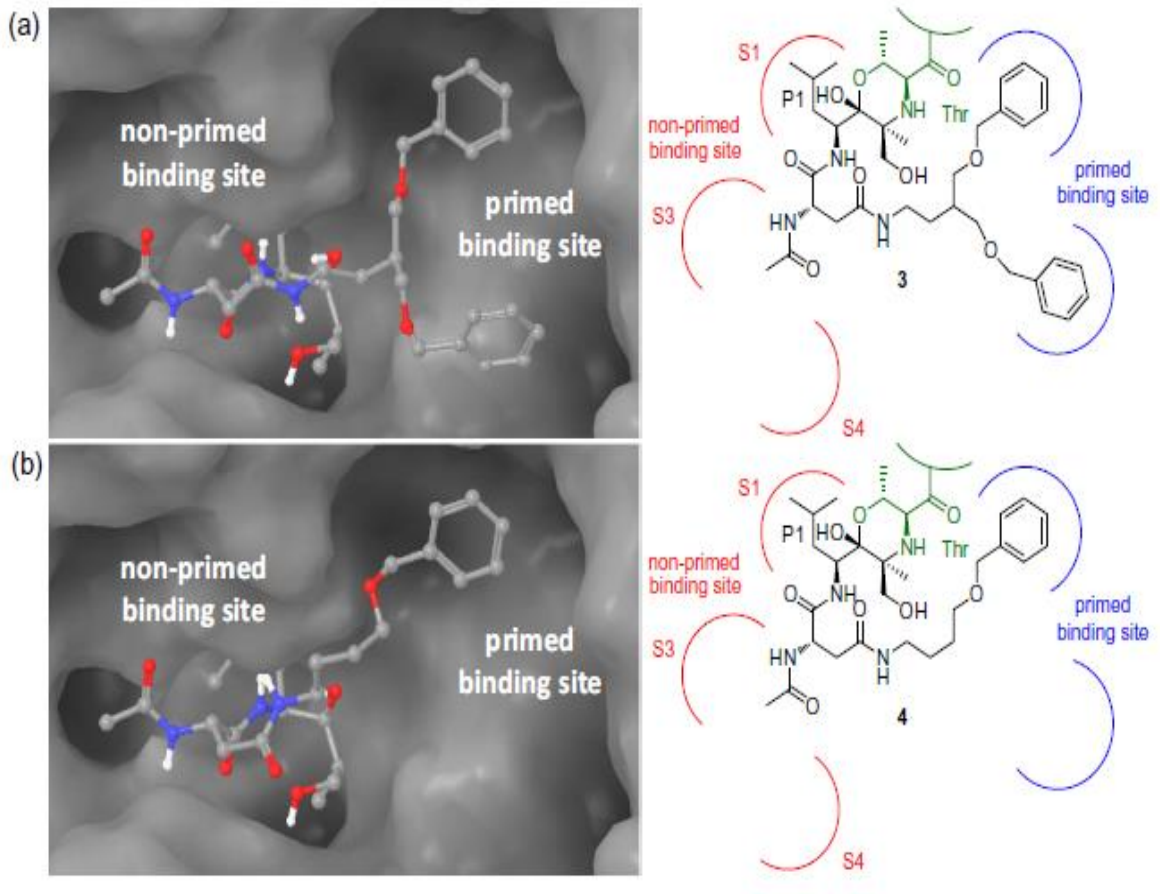


Figure 2 Computationally predicted binding mode of belactosin A derivatives

9. Challenges and Future Perspectives

Despite its promising biological activity, belactosin faces several challenges, including chemical instability, limited bioavailability, and potential off-target effects. Future research should focus on semi-synthetic modification, biosynthetic engineering, and formulation strategies to enhance stability and selectivity. Advances in genomics and synthetic biology may enable the production of optimized belactosin analogues with clinical potential.

10. Conclusion

Belactosin is a structurally unique and biologically significant natural product with potent proteasome inhibitory activity. Its discovery has enriched the field of proteasome research and provided valuable insights into natural product–based anticancer drug development. Continued interdisciplinary research is essential to fully exploit belactosin's therapeutic potential.

References

1. Wolf, F., Bauer, J. S., Bendel, T. M., Kulik, A., Kalinowski, J., Gross, H., & Kaysser, L. (2017). **Biosynthesis of the β-lactone proteasome inhibitors belactosin and cystargolide**. *Angewandte Chemie International Edition, 56*(23), 6665–6668. https://doi.org/10.1002/anie.201612076
2. Kudo, F., Miyanaga, A., & Eguchi, T. (2024). **Discovery of a cryptic nitro intermediate in the biosynthesis of the 3-(trans-2′-aminocyclopropyl)alanine moiety of belactosin A**. *Nature Chemical Biology, 20*(10), 1371–1379. https://doi.org/10.1038/s41589-024-01657-7
3. Kuttenlochner, W., Beller, P., Kaysser, L., & Groll, M. (2024). **Deciphering the SAM- and metal-dependent mechanism of O-methyltransferases in cystargolide and belactosin biosynthesis**. *Journal of Biological Chemistry, 300*, 107646. https://doi.org/10.1016/j.jbc.2024.107646
4. Gaudelli, N. M., Long, D. H., & Townsend, C. A. (2015). **β-Lactam formation by a nonribosomal peptide synthetase during antibiotic biosynthesis**. *Nature, 520*(7547), 383–387. https://doi.org/10.1038/nature14100
5. Sunazuka, T., Hirose, T., Shirahata, T., Harigaya, Y., Kuwajima, I., & Ōmura, S. (2004). **Total synthesis of (+)-belactosin A**. *Chemical Communications*, (18), 2064–2065. https://doi.org/10.1039/B316142K
6. Hirata, Y., Ishikawa, T., Sunazuka, T., Hirose, T., Shirahata, T., Harigaya, Y., & Ōmura, S. (2013). **Design and stabilization of belactosin cis-cyclopropane analogues**. *Organic & Biomolecular Chemistry, 11*(39), 6615–6622. https://doi.org/10.1039/C3OB41338A
7. Hirata, Y., Ishikawa, T., Sunazuka, T., Hirose, T., Shirahata, T., Harigaya, Y., & Ōmura, S. (2014). **Hybrid proteasome inhibitors linking belactosin derivatives and peptide boronates**. *Journal of Medicinal Chemistry, 57*(6), 2726–2735. https://doi.org/10.1021/jm500045x
8. Kuttenlochner, W., Beller, P., Kaysser, L., & Groll, M. (2024). **Mechanistic study of O-methyltransferases in β-lactone natural product biosynthesis**. *Journal of Biological Chemistry, 300*(9), 107646. https://doi.org/10.1016/j.jbc.2024.107646
9. Kawamura, S., Hirata, Y., Ishikawa, T., Sunazuka, T., Hirose, T., Shirahata, T., Harigaya, Y., & Ōmura, S. (2014). **Rational scaffold hopping from belactosin to potent non-peptidic inhibitors**. *Chemical Communications, 50*(18), 2445–2447. https://doi.org/10.1039/C3CC48818G
10. Armstrong, A., & Scutt, J. N. (2004). **Total synthesis of (+)-belactosin A**. *Chemical Communications*, (18), 2064–2065. https://doi.org/10.1039/B316142K
11. Kawamura, S., Unno, Y., Asai, A., Arisawa, M., & Shuto, S. (2013). *Title of the article*. **Organic & Biomolecular Chemistry, 11**, 6615–6623