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COMMENTARY

Hypoxia-selective targeting of eEF1A1: A promising therapeutic strategy for triple-negative breast cancer

KEY WORDS

Triple-negative breast cancer;
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Hypoxia-selective cytotoxicity

Triple-negative breast cancer (TNBC) remains one of the most challenging malignancies in clinical oncology, characterized by aggressive behavior, high metastatic potential, and lack of effective targeted therapies[1-2]. The absence of estrogen receptor, progesterone receptor, and HER2 expression eliminates the utility of endocrine therapy and HER2-targeted agents, leaving conventional chemotherapy as the mainstay of treatment[3]. However, the modest efficacy and considerable toxicity of cytotoxic agents underscore the urgent need for novel therapeutic strategies with distinct mechanisms of action and improved safety profiles.

In this issue, Liu et al.[4] report a significant advance in TNBC drug discovery through the systematic optimization of boholamide A, a naturally occurring macrocyclic depsipeptide featuring the distinctive 4-amino-2,4-pentadienolate (APD) moiety. This study exemplifies how natural product-inspired medicinal chemistry to generate clinical candidates with unique pharmacological properties, specifically, hypoxia-selective cytotoxicity that exploits the metabolic vulnerabilities of solid tumors.

The boholamide A scaffold belongs to an extraordinary family of APD-containing cyclolipodepsipeptides. Those compounds share a common electrophilic APD moiety that undergoes Michael addition reactions with nucleophilic protein residues, and exhibit distinctive hypoxia selectivity[5-6]. Previous investigations by the authors' group and others established that BE-43547A$_2$ targets eukaryotic translation elongation factor 1 alpha 1 (eEF1A1) through covalent modification of Cys31, disrupting the eEF1A1/FoxO1 interaction and suppressing the JAK/STAT3 signaling pathway in pancreatic cancer[7-8]. The current study extends this mechanistic framework to TNBC and achieves substantial improvements in drug-like properties through strategic structural optimization.

The structure−activity relationship (SAR) investigation conducted by Liu et al.[4] demonstrates sophisticated medicinal chemistry design. Retaining the essential APD pharmacophore, the authors systematically modified the lipophilic chain and amino acid residues to optimize anti-TNBC potency. The most significant advancement was the identification of compound **1j**, which incorporates a *tert*-butyl substituent at the C11 position, replacing the isopropyl group in the parent natural product. This modification yielded remarkable improvements: the IC_{50} against MDA-MB-231 cells under hypoxia decreased to 0.15 μmol/L, representing an 8.3-fold enhancement over the initial lead **1c** and substantially surpassing the activity of natural boholamide A. Beyond potency, compound **1j** exhibited an improved hypoxia-selective index (HSI = 6.73), indicating enhanced preferential toxicity toward hypoxic cells compared to normoxic counterparts.

The mechanistic findings presented in this study provide crucial insights into the therapeutic potential of this compound class. Using a click chemistry-enabled activity-based protein profiling probe, the authors confirmed that **1j** maintains the parental mechanism of eEF1A1 covalent targeting at Cys31. The binding kinetics studies revealed dose-dependent and time-saturated binding consistent with irreversible inhibition, while cellular thermal shift assays validated the direct protein−ligand interaction. Notably, the authors demonstrated that **1j** disrupts the eEF1A1/FoxO1 interaction, promotes FoxO1 nuclear translocation, and consequently suppresses the pro-survival JAK/STAT3 pathway—mirroring the mechanism established for BE-43547A$_2$ in pancreatic cancer[8].

The functional consequences of eEF1A1 targeting in TNBC cells are particularly noteworthy. Beyond proliferation inhibition, **1j** effectively suppressed colony formation, induced apoptosis

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through the mitochondrial pathway (evidenced by Bax upregulation and Bcl-2/Mcl-1 downregulation), and inhibited cell migration by modulating epithelial−mesenchymal transition markers. The suppression of metastasis-related proteins including Vimentin and MMP9, coupled with E-cadherin upregulation, suggests potential anti-metastatic activity that could address one of the most lethal aspects of TNBC.

Translating from cellular activity to *in vivo* efficacy, the authors developed a water-soluble prodrug **16** to overcome the formulation challenges of the parent compound. The prodrug strategy employing *N*-methylpiperazine substitution maintained pharmacological activity while improving pharmaceutical properties. In the 4T1 syngeneic breast cancer model, **16** demonstrated dose-dependent tumor growth inhibition and reduced hepatosplenomegaly, an indicator of metastatic burden, without significant systemic toxicity at therapeutically relevant doses. The histopathological and serum biochemical analyses confirmed acceptable safety margins, supporting further preclinical development.

Several aspects of this study merit particular attention for the broader drug discovery community. First, the efficient 13-step synthetic route to **1j** with 7.92% overall yield represents a substantial improvement over the original 16-step synthesis of boholamide A, enhancing accessibility for further development. Second, the combinatorial SAR strategy—systematically varying three distinct structural regions while maintaining the APD pharmacophore—provides a template for optimizing other complex natural product scaffolds. Third, the identification of eEF1A1 as the molecular target unifies the mechanism of APD-containing cyclolipodepsipeptides and establishes this translation factor as a druggable node for hypoxia-selective cancer therapy.

The study also raises intriguing questions for future investigation. While the covalent binding mechanism ensures sustained target engagement, the potential for off-target reactivity with other cysteine-containing proteins warrants careful evaluation in subsequent optimization efforts. Additionally, the precise relationship between eEF1A1 inhibition and hypoxia-selective cytotoxicity—whether mediated by differential target expression, altered redox status, or other microenvironmental factors—deserves further elucidation. The observation that compound **1j** inhibits cancer cell migration and induces apoptosis through mitochondrial pathways suggests that multiple mechanisms may contribute to its antitumor efficacy.

From a broader perspective, this work exemplifies the power of integrating natural product chemistry, medicinal chemistry optimization, and chemical biology to discover innovative therapeutic candidates. The APD-containing depsipeptides, initially identified as microbial metabolites with intriguing biological properties, are now yielding optimized leads with genuine clinical potential through systematic analogue synthesis and target deconvolution. This approach may serve as a template for the development of other natural product-inspired therapeutics.

In conclusion, the study by Liu et al.[4] represents a significant contribution to TNBC drug discovery, demonstrating that natural product-inspired optimization can yield clinical candidates with differentiated mechanisms and improved pharmaceutical properties. The hypoxia-selective targeting of eEF1A1 through covalent modification offers a promising strategy to address the therapeutic challenges posed by the hypoxic tumor microenvironment. As compound **16** advances through preclinical development, this work reinforces the enduring value of natural products as inspiration for innovative cancer therapeutics and highlights the importance of mechanistically-informed drug design in addressing unmet medical needs.

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