Acta Pharmaceutica Sinica B 2026;16(4):2587−2592

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com

LETTER TO THE EDITOR

Identification of HMGB1 as a target of 3-*O*-benzoyl-20-deoxyingenol in NSCLC therapy using integrated ABPP and SIP

KEY WORDS

NSCLC;
3-*O*-Benzoyl-20-deoxyingenol;
HMGB1;
Autophagy-mediated apoptosis;
Immunogenic cell death

To the Editor:

Current therapies for non-small cell lung cancer (NSCLC, ∼85% of lung malignancies) are limited by high recurrence, metastasis, and drug resistance, leading to poor long-term efficacy. These challenges underscore the urgent need for novel small-molecule agents[1]. Autophagy-induced tumor cell death is a promising therapeutic strategy[2]. Enhanced interaction between HMGB1 and Beclin1 promotes autophagy activation[3,4]. Nevertheless, the therapeutic potential of this mechanism in NSCLC remains to be fully elucidated and warrants further investigation. Notably, HMGB1 is not only a pivotal mediator of autophagy but also a key marker of immunogenic cell death (ICD), specifically promoting dendritic cell (DC) maturation[5]. However, there is an absence of clinically available drugs that directly target HMGB1 to synergistically regulate these processes[6]; this very gap renders HMGB1 a compelling therapeutic target at the nodes of autophagy, ICD, and NSCLC.

Euphorbia kansui, a medicinal plant used for lung cancer-related fluid accumulation, contains antitumor ingenol diterpenoids, some of which induce autophagic cell death. Herein, we identified a naturally active small molecule, 3-*O*-benzoyl-20-deoxyingenol (**3-Bz-20-DOI**), from *E. kansui*, which effectively inhibited the growth of NSCLC cells with reduced toxicity. Integrative ABPP and SIP profiling revealed that **3-Bz-20-DOI** specifically targets HMGB1. Notably, licorice-derived glycyrrhizic acid (GA) antagonizes **3-Bz-20-DOI** and *E. kansui via* HMGB1, which explains the molecular mechanism underlying the neutralization of efficacy in the "Eighteen Incompatible Medicaments" between *E. kansui* and licorice. Our work confirms that targeting HMGB1 is a therapeutic strategy for NSCLC.

1. HMGB1 was identified as a therapeutic target of 3-Bz-20-DOI in NSCLC using ABPP combined with SIP technology

To investigate bioactive ingenane diterpenoids from *E. kansui*, we isolated and identified three compounds: **3-Bz-20-DOI**, 5-*O*-benzoyl-20-deoxyingenol, and 3-*O*-(2′*E*,4′*Z*-decadienoyl)-20-deoxyingenol. Bioactivity screening revealed that **3-Bz-20-DOI** had the strongest antiproliferative activity against NSCLC cells (Fig. 1A and Supporting Information Fig. S1). To evaluate the *in vivo* pharmacological efficacy and elucidate the mechanism of action of **3-Bz-20-DOI**, we obtained a substantial quantity of the compound *via* semi-synthesis from 20-deoxyingenol (Supporting Information Scheme S1). In nude mice with axillary tumors (Fig. 1B), **3-Bz-20-DOI** significantly suppressed tumor growth with lower toxicity compared to CDDP (Fig. 1C−E, Supporting Information Fig. S2). To identify the key anti-NSCLC target of **3-Bz-20-DOI**, we synthesized a photoaffinity probe **DOI-P** (Fig. 1F). Equivalence and gel labeling experiments confirmed that the antitumor activity of **DOI-P** matched that of **3-Bz-20-DOI** and that **DOI-P** has high labeling efficiency (Fig. 1G and H). Using ABPP combined with SIP (Fig. 1I and Supporting Information Fig. S3), 6 targets were finalized by intersecting enriched proteins to exclude false positives (Fig. 1J). HMGB1 was validated as the key target *via* SIP, CETSA, DARTS, and Pull-down assays (Fig. 1K−N, Supporting Information Fig. S4A and S4C). Pull-down experiments revealed that **DOI-P** labeled HMGB1 in A549 cells/tumor lysates, and this labeling was competitively

Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2026.01.043

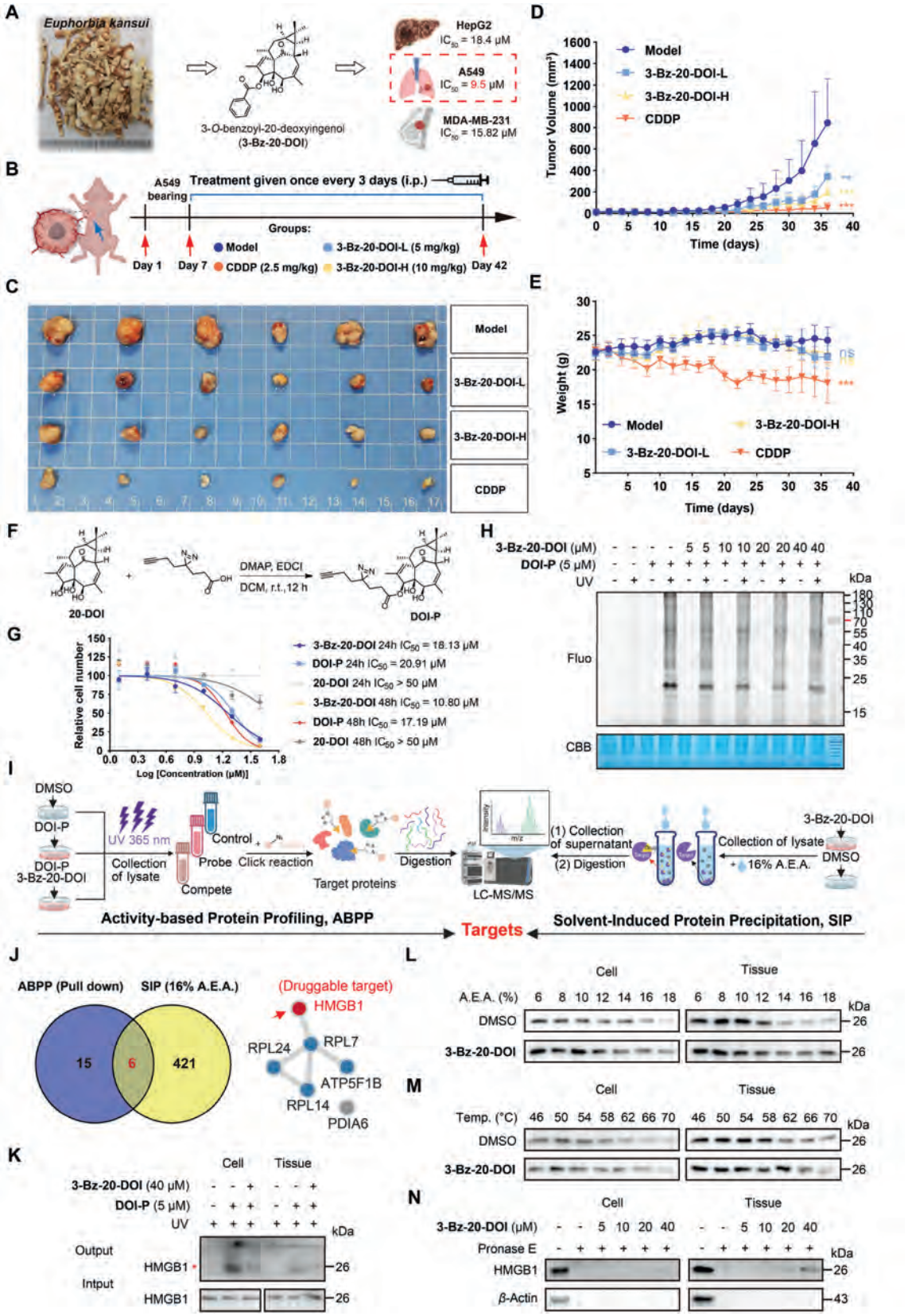

Figure 1 Evaluation of the efficacy of **3-Bz-20-DOI** against NSCLC and identification of its target. (A) **3-Bz-20-DOI** significantly inhibited A549 cell growth. (B) Experimental protocol for assessing antitumor activity *in vivo*. (C) Tumors were harvested and photographed. (D) Effect of **3-Bz-20-DOI** on mice A549 tumor volume (mean ± SD, $n = 6$). $^{**}P < 0.01$, $^{***}P < 0.001$. (E) Effect of **3-Bz-20-DOI** on mice body weight (mean ± SD, $n = 6$). $^{***}P < 0.001$. (F) Schematic representation of **DOI-P** synthesis. (G) Evaluation of the cytotoxicity of **3-Bz-20-DOI**, **DOI-**

inhibited by **3-Bz-20-DOI** (Fig. 1K). Colocalization assays further corroborated this interaction (Fig. S4B). Non-labeling experiments showed that **3-Bz-20-DOI** enhanced the solvent/thermal stability of HMGB1 and increased its resistance to Pronase E hydrolysis (Fig. 1L−N). Overexpression of HMGB1 in A549 cells (*via* the pcDNA3.1(−) plasmid) resulted in an increased **3-Bz-20-DOI** recognition and binding to the target protein HMGB1, thereby amplifying its cytotoxic effect in A549 cells (Fig. S4D). This enhanced cytotoxicity further demonstrated that HMGB1 is the primary target of **3-Bz-20-DOI**.

2. 3-Bz-20-DOI inhibits HMGB1 nuclear translocation by binding to Lys-44 in NLS1, promotes HMGB1 binding to Beclin1, and ultimately induces autophagy-mediated apoptosis and immunogenic cell death in NSCLC cells

To further elucidate the pharmacological mechanism of **3-Bz-20-DOI**, we performed gel-labeling of the A- and B-box domains of purified HMGB1 with **DOI-P**, which revealed preferential binding to the A-box domain (Supporting Information Figs. S5 and S6A). Molecular docking and dynamics simulations showed that **3-Bz-20-DOI** interacts intermolecularly with the A-box residues Cys-23, His-27, Lys-30, and Lys-44 (Fig. 2A−E). Comparative analysis of the binding modes at 0 ns and 100 ns revealed significant conformational changes in HMGB1 (Fig. 2B−D and Fig. S6C). Circular dichroism (CD) experiments further confirmed that **3-Bz-20-DOI** dose-dependently induced α-helix changes in HMGB1 (Fig. 2E). Single-point mutations of HMGB1-expressing plasmids (Cys-23, His-27, Lys-30, Glu-40, Phe-41, and Lys-44 to Ala) were stably transfected and probed *in situ* (Supporting Information Fig. S7), with the purified mutants validated *via in vitro* labeling (Fig. 2F and Fig. S7D). BLI/ITC analyses confirmed significantly reduced affinity between **3-Bz-20-DOI** and the His-27 and Lys-44 mutants of HMGB1 (Fig. 2G and Supporting Information Fig. S8).

In addition, referring to previous work[7], nickel column pull-down assays revealed that **3-Bz-20-DOI** disrupted A box/C-tail interactions (Fig. S6B−S6F). Finally, we found that **3-Bz-20-DOI** induces protein conformational changes primarily through binding to His-27 and Lys-44. Notably, Lys-44 of HMGB1 is also a key amino acid residue in nuclear localization signal 1 (NLS1) of the HMGB1 protein. Mutation of Lys-44 enhances cytoplasmic transport of HMGB1, and the effect of **3-Bz-20-DOI** on $HMGB1^{K44A}$ is weakened (Fig. 2H), indicating that **3-Bz-20-DOI** disrupts HMGB1 nuclear localization by binding Lys-44 and promoting cytoplasmic accumulation.

To investigate the mechanism of action of **3-Bz-20-DOI** in A549 cells, we performed whole proteomic analysis (Supporting Information Fig. S9). The analysis revealed 252 upregulated proteins and 345 downregulated proteins (Fig. S9B). KEGG and GO enrichment analyses of these differentially expressed proteins (DEPs) indicated that **3-Bz-20-DOI** exerts its effects primarily by inducing autophagy and apoptosis (Fig. S9C and S9D). Moreover, GSEA revealed that **3-Bz-20-DOI** significantly downregulated NSCLC-related markers and pathways such as cell adhesion molecules (Fig. S9E and S9F). We subsequently conducted a preliminary validation of the aforementioned analytical results. **3-Bz-20-DOI** enhanced autophagic flux (Supporting Information Fig. S10A), induced apoptosis in NSCLC cells (Supporting Information Fig. S11A and S11B), and inhibited cell adhesion (Supporting Information Figs. S12 and S13). **3-Bz-20-DOI** dose-dependently increased LC3 expression, with chloroquine (CQ) further promoting LC3 accumulation (Supporting Information Fig. S14E), confirming that it is an autophagy inducer. Following treatment with three well-established autophagy inhibitors (3-MA, CQ, and BafA1), only 3-MA significantly reversed the inhibitory activity of **3-Bz-20-DOI** on NSCLC (Fig. S10D and S10E). These findings indicate that **3-Bz-20-DOI** enhances autophagic flux at the early stage of autophagy (Fig. S11C and S11D). Notably, 3-MA significantly reversed the autophagy-mediated apoptotic effects of **3-Bz-20-DOI**.

Previous research has shown that corynoxine B, an active monomer from the traditional Chinese medicine *Uncaria rhynchophylla* (Miq.) Jacks., promotes autophagy by enhancing the interaction between HMGB1/2 and Beclin1, thereby improving Parkinson's disease[8]. In our study, we found that **3-Bz-20-DOI** also promoted the interaction between HMGB1 and Beclin1 to activate autophagy; however, this autophagy induced autophagic cell death in NSCLC cells. Moreover, **3-Bz-20-DOI** protected HMGB1 from degradation by cycloheximide (CHX), indicating that it enhances protein stability (Fig. S14F). Transmission electron microscopy revealed that it promotes autophagic lysosome formation (Supporting Information Fig. S15). Quantitative comparison with known autophagy activators by flow cytometry and western blotting showed that **3-Bz-20-DOI** induced stronger effects (Fig. 2I and J). Co-immunoprecipitation showed that **3-Bz-20-DOI** promotes HMGB1−Beclin1 binding (Fig. 2K). Cell colocalization assays indicated that **3-Bz-20-DOI** inhibited HMGB1 nuclear translocation, prompting more cytoplasmic HMGB1 to bind Beclin1 and induce autophagy (Fig. 2L and Fig. S10B). **3-Bz-20-DOI** also induced the extracellular release of HMGB1, a key marker of ICD (Fig. 2L). Compared with classical positive controls (doxorubicin and oxaliplatin), **3-Bz-20-DOI** triggered ICD characterized by significantly elevated cell membrane CRT expression, intracellular ATP levels, HMGB1 secretion, and increased DC maturation rate (Fig. 2M and N, Supporting Information Figs. S16 and S17). Furthermore, compared with cells overexpressing $HMGB1^{WT}$, those overexpressing $HMGB1^{K44A}$ did not exhibit the ICD effects of **3-Bz-20-DOI** (Supporting Information Fig. S18). In summary, **3-Bz-20-DOI** inhibited HMGB1 nuclear translocation by binding NLS1 Lys-44, promoted HMGB1-Beclin1 binding, induced autophagy-mediated apoptosis, and activated ICD in NSCLC cells.

As a classic pair in traditional Chinese medicine, "Eighteen Incompatible Medicaments", licorice and *E. kansui* are efficacious and may cause toxicity when coadministered. Previous studies have focused on their ability to induce cardiac, hepatic, and renal injury, but how licorice weakens *E. kansui* efficacy has rarely been investigated. Our research revealed that the major component of

P, and **20-DOI** ($n = 3$). (H) Fluorescent labeling of proteins by excess **3-Bz-20-DOI** competition with **DOI-P** in A549 cells. (I) Identification of the targets underlying the anti-NSCLC effects of **3-Bz-20-DOI** by ABPP and SIP analysis. (J) A total of 6 targets were identified by cross-analyzing proteins from ABPP and SIP analysis. (K−N) Pull-down, SIP, CETSA, and DARTS assays were performed to investigate the effect of **3-Bz-20-DOI** on HMGB1 binding or protein stability. (validated in cell lysate and tumor tissue lysate, respectively). Fig. 1L and M Statistical analyses of the quantitative plots in the Supporting Information (Fig. S4). M, mol/L.

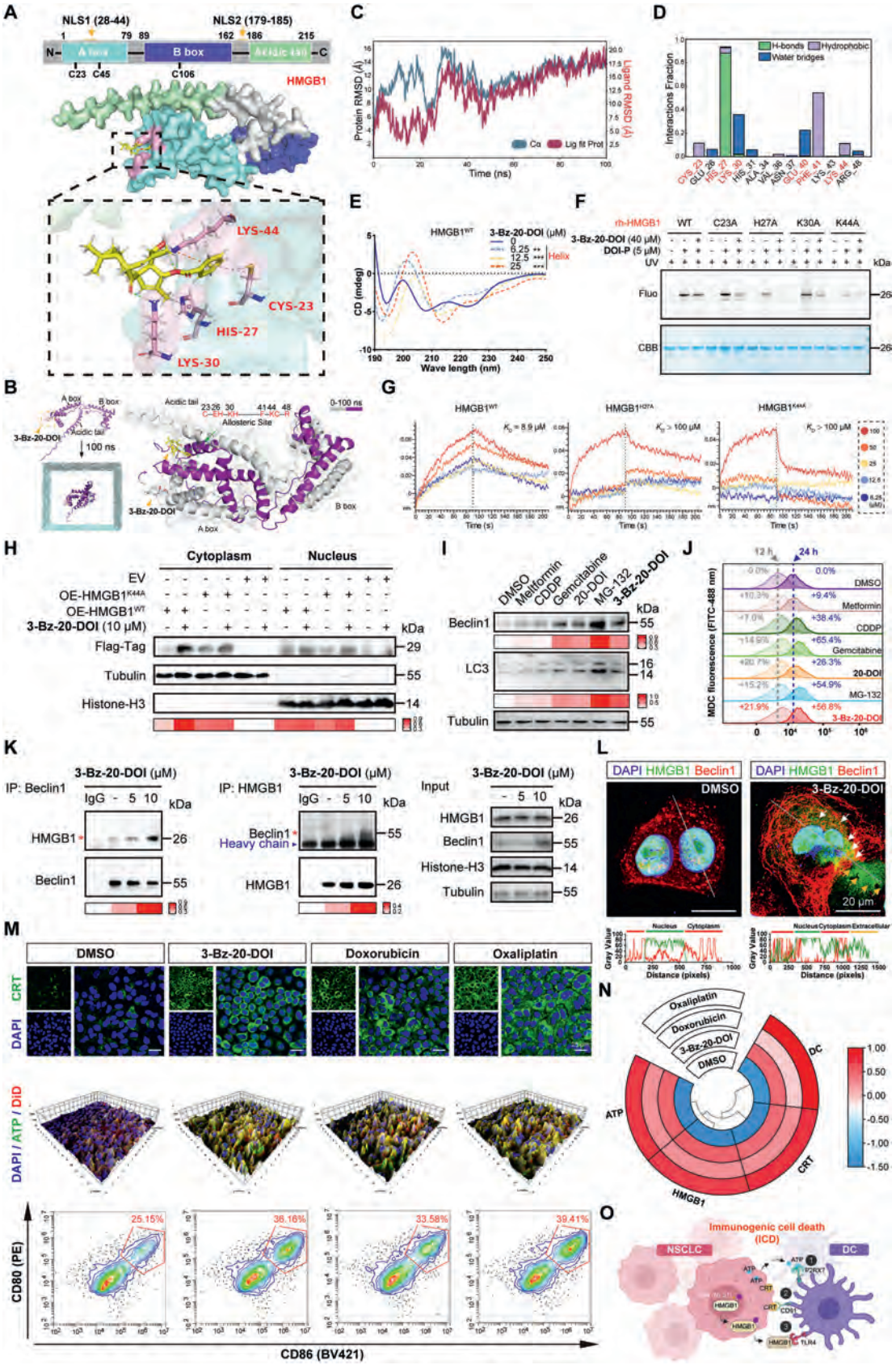

Figure 2 Pharmacological mechanism study of **3-Bz-20-DOI** against NSCLC. (A) Interactions of **3-Bz-20-DOI** with HMGB1 (AF-P09429-F1) investigated by molecular docking. (B) Molecular dynamics simulations of **3-Bz-20-DOI** and HMGB1 proteins (100 ns), and accurately predicted protein allosteric site by the AutoML learning model in PASSer. (C, D) Visualization of molecular dynamics simulations (RMSD and

liquorice, GA, significantly reversed the antitumor activity of **3-Bz-20-DOI**. Notably, **3-Bz-20-DOI** treatment promoted cytoplasmic HMGB1 accumulation, which was reversed by the HMGB1 inhibitor GA (Fig. S14D). Both **3-Bz-20-DOI** and GA target HMGB1, antagonizing each other in regulating its nuclear translocation, as validated with *E. kansui* extract: 10 μmol/L GA reversed its HMGB1 regulation, while 10 μmol/L **3-Bz-20-DOI** acted synergistically with it (Supporting Information Fig. S19). Whether these differences in HMGB1 regulation are related to gastrointestinal, liver, or kidney injury requires urgent investigation. In summary, HMGB1 is key to this mutual antagonism, but its role in the characteristic hepatorenal toxicity requires urgent investigation.

We found that **3-Bz-20-DOI** from *E. kansui* inhibited NSCLC growth by targeting HMGB1. *In vivo* experiments demonstrated that, compared with CDDP, **3-Bz-20-DOI** effectively inhibited A549 cell growth with lower toxicity. Based on the ABPP combined with the SIP technique, we identified its anti-NSCLC targets, mainly ribosomal proteins and HMGB1, with HMGB1 emerging as the most attractive druggable target. Western blotting experiments of cells and tumor tissues indicated that **3-Bz-20-DOI** did not affect HMGB1 protein expression but rather exerted functional regulation (Fig. 2O and Fig. S14B−S14E). Autophagy has a dual function at different stages of tumorigenesis: its activation promotes both the survival of cancer cells during tumor progression (protective autophagy) and the death of cancer cells in the initial cancer lesion (cytotoxic/nonprotective autophagy)[9]. Overall, excessive autophagy activation can be an effective strategy to prevent cancer progression[10-12]. The results of the present study demonstrate that **3-Bz-20-DOI** is an autophagy activator that is dependent on the HMGB1−Beclin1 pathway and promotes the assembly of Beclin1 into autophagosomes during the early stage of autophagy. Furthermore, studies examining autophagy stages revealed that a specific dose of 3-MA reversed the killing effect of **3-Bz-20-DOI** on NSCLC cells (Fig. S10C−S10E). Mechanistically, 3-MA significantly reduced intracellular HMGB1 protein expression. The key finding elucidates that the reversal effect of 3-MA stems from its discruption of the **3-Bz-20-DOI**-induced interaction between HMGB1 and Beclin1 during early stage of autophagy, thereby inhibiting the autophagic flux and ultimately reversing apoptosis (Fig. S11C and S11D). Whole proteomics analysis further revealed that **3-Bz-20-DOI** could regulate multiple signaling pathways, including activation of the P53 and PPAR signaling pathways, to inhibit NSCLC cell proliferation (Fig. S9). Overall, we demonstrated that **3-Bz-20-DOI** regulated nuclear transport by binding to the Lys-44 site of HMGB1, thereby modulating nuclear translocation, promoting autophagy, and inducing apoptosis and ICD in NSCLC cells.

Acknowledgments

This research work was supported by Scientific Research Fund Project of Liaoning Provincial Department of Education (LJ212410163058, China), Natural Science Foundation of Liaoning Province (2025-MS-266, China), Shenyang Pharmaceutical University Excellent Youth Lift Plan (YQ202202, China), National Key R&D Program of China (Grant No. SQ2024YFC3500039, China), Song Shaojiang Expert Workstation of Yunnan Province (No. 202305AF150030, China), Scientific and technological innovation project of China Academy of Chinese Medical Sciences (CI2023E002 and CI2023E005TS07, China), Beijing Municipal Natural Science Foundation (7252252, China).

Author contributions

Yaxu Wang: Writing — original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation. Yan Liu: Methodology, Investigation. Yuan Zhuang: Methodology, Formal analysis. Wennan Luo: Methodology, Investigation. Lu Pan: Methodology, Data curation. Liwei Gu: Writing — review & editing, Funding acquisition. Jigang Wang: Writing — review & editing, Funding acquisition. Qingbo Liu: Writing — review & editing, Project administration, Investigation, Funding acquisition. Shao-Jiang Song: Writing — review & editing, Project administration, Investigation, Funding acquisition.

Conflicts of interest

The authors declare no conflicts of interest.

Appendix A. Supporting information

Supporting information to this article can be found online at https://doi.org/10.1016/j.apsb.2026.01.043.

References

1. Han B, Zheng R, Zeng H, Wang S, Sun K, Chen R, et al. Cancer incidence and mortality in China, 2022. *J Natl Cancer Cent* 2024;**4**:47−53.
2. Liu S, Yao S, Yang H, Liu S, Wang Y. Autophagy: regulator of cell death. *Cell Death Dis* 2023;**14**:648.
3. Ye J, Zhang J, Zhu Y, Wang L, Jiang X, Liu B, et al. Targeting autophagy and beyond: deconvoluting the complexity of Beclin-1 from biological function to cancer therapy. *Acta Pharm Sin B* 2023;**13**:4688−714.
4. Kang R, Livesey KM, Zeh HJ, Loze MT, Tang D. HMGB1: a novel Beclin 1-binding protein active in autophagy. *Autophagy* 2010;**6**:1209−11.

protein−ligand interactions). (E) CD spectra analysis for **3-Bz-20-DOI**-mediated recombinant HMGB1 conformational change (Analysis of protein secondary structure using BeStSel) (mean ± SD, $n = 3$). $^{**}P < 0.01$, $^{***}P < 0.001$. (F) *In vitro* fluorescent labeling of several HMGB1 mutants. (G) Binding of **3-Bz-20-DOI** to $HMGB1^{WT}$, $HMGB1^{H27A}$ and $HMGB1^{K44A}$ determined by BLI assay. (H) Expression level of HMGB1 protein in HEK-293T cells nuclear and cytoplasmic extracts after treatment with **Bz-20-DOI** (HEK-293T cells were transfected to express $HMGB1^{WT}$ and $HMGB1^{K44A}$) ($n = 3$). (I) Western blot analysis of the expression of Beclin1 and LC3 protein in the A549 cells after treatment with several autophagy agonists. (J) After treatment with several autophagy agonists, intracellular autophagy flux was observed by flow cytometry (expressed as mean, $n = 3$). (K) Effects on the interaction between HMGB1 and Beclin1 after **3-Bz-20-DOI** intervention. (L) Immunofluorescence analysis of the interaction between HMGB1 and Beclin1 (scale bar = 20 μm). (M) CRT protein expression, ATP content detection and proportion of mature DCs (scale bar = 25 μm). (Doxorubicin and oxaliplatin as positive controls). (N) Quantitative results for ATP, HMGB1, CRT and DC maturation ratio are presented as a heatmap. (O) Schematic diagram of the mechanism by which **3-Bz-20-DOI** induces ICD in NSCLC cells. M, mol/L.

5. Chen R, Kang R, Tang D. The mechanism of HMGB1 secretion and release. *Exp Mol Med* 2022;**54**:91−102.
6. Chen R, Zou J, Zhong X, Li J, Kang R, Tang D. HMGB1 in the interplay between autophagy and apoptosis in cancer. *Cancer Lett* 2024;**581**:216494.
7. Li Y, Fu Y, Zhang Y, Duan B, Zhao Y, Shang M, et al. Nuclear fructose-1,6-bisphosphate inhibits tumor growth and sensitizes chemotherapy by targeting HMGB1. *Adv Sci (Weinh)* 2023;**10**:e2203528.
8. Zhu Q, Song J, Chen JY, Yuan Z, Liu L, Xie LM, et al. Corynoxine B targets at HMGB1/2 to enhance autophagy for alpha-synuclein clearance in fly and rodent models of Parkinson's disease. *Acta Pharm Sin B* 2023;**13**:2701−14.
9. Huang Y, You X, Wang L, Zhang G, Gui S, Jin Y, et al. Pyridinium-substituted tetraphenylethylenes functionalized with alkyl chains as autophagy modulators for cancer therapy. *Angew Chem Int Ed Engl* 2020;**59**:10042−51.
10. Yang H, Zhang X, Wang C, Zhang H, Yi J, Wang K, et al. Microcolin H, a novel autophagy inducer, exerts potent antitumour activity by targeting PITPalpha/beta. *Signal Transduct Target Ther* 2023;**8**:428.
11. Wang M, Liao J, Wang J, Xu M, Cheng Y, Wei L, et al. HDAC2 promotes autophagy-associated HCC malignant progression by transcriptionally activating LAPTM4B. *Cell Death Dis* 2024;**15**:593.
12. Biswas U, Roy R, Ghosh S, Chakrabarti G. The interplay between autophagy and apoptosis: its implication in lung cancer and therapeutics. *Cancer Lett* 2024;**585**:216662.

Yaxu Wang[a,b], Yan Liu[a], Yuan Zhuang[a,b], Wennan Luo[b], Lu Pan[a,b], Liwei Gu[b,*], Jigang Wang[a,b,*], Qingbo Liu[a,*], Shao-Jiang Song[a,*]

[a]*Key Laboratory of Computational Chemistry Based Natural Antitumor Drug Research & Development, Liaoning Province, School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China*

[b]*State Key Laboratory for Quality Ensurance and Sustainable Use of Dao-di Herbs, Artemisinin Research Center, and Institute of Chinese Materia Medica, China Academy of Chinese Medical Sciences, Beijing 100700, China*

*Corresponding authors.
E-mail addresses: lwgu@icmm.ac.cn (Liwei Gu), jgwang@icmm.ac.cn (Jigang Wang), liuqingbolily@163.com (Qingbo Liu), songsj99@163.com (Shao-Jiang Song)

Received 11 August 2025
Received in revised form 16 November 2025
Accepted 24 December 2025