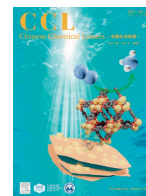




Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

New techniques and strategies in drug discovery (2020–2024 update)



Qijie Gong^{a,1}, Jian Song^{b,1}, Yihui Song^{c,1}, Kai Tang^{d,1}, Panpan Yang^{e,1}, Xiao Wang^b,
Min Zhao^e, Liang Ouyang^{e,*}, Li Rao^{f,*}, Bin Yu^{g,*}, Peng Zhan^{d,*}, Saiyang Zhang^{b,*},
Xiaojin Zhang^{a,*}

^aJiangsu Key Laboratory of Drug Design and Optimization, and Department of Chemistry, China Pharmaceutical University, Nanjing 211198, China

^bSchool of Basic Medical Sciences, Zhengzhou University, Zhengzhou 450001, China

^cSchool of Pharmaceutical Sciences & Key Laboratory of Advanced Drug Preparation Technologies, Zhengzhou University, Zhengzhou 450001, China

^dDepartment of Medicinal Chemistry, Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Shandong University, Ji'nan 250012, China

^eState Key Laboratory of Biotherapy and Cancer Center, National Clinical Research Center for Geriatrics, West China Hospital, and Collaborative Innovation Center of Biotherapy, Sichuan University, Chengdu 610041, China

^fNational Key Laboratory of Green Pesticide, Key Laboratory of Pesticide & Chemical Biology of Ministry of Education, Hubei International Scientific and Technological Cooperation Base of Pesticide and Green Synthesis, College of Chemistry, Central China Normal University, Wuhan 430079, China

^gCollege of Chemistry, Pingyuan Laboratory, Zhengzhou University, Zhengzhou 450001, China

ARTICLE INFO

Article history:

Received 12 July 2024

Revised 11 September 2024

Accepted 12 September 2024

Available online 13 September 2024

Keywords:

Drug discovery

Covalent inhibitors

Computational drug design

Protein scaffolding

Pro-PROTACs

Programmed cell death

C-C cleavage

ABSTRACT

In the realm of drug discovery, recent advancements have paved the way for innovative approaches and methodologies. This comprehensive review encapsulates six distinct yet interrelated mini-reviews, each shedding light on novel strategies in drug development. (a) The resurgence of covalent drugs is highlighted, focusing on the targeted covalent inhibitors (TCIs) and their role in enhancing selectivity and affinity. (b) The potential of the quantum mechanics-based computational aid drug design (CADD) tool, Cov_DOX, is introduced for predicting protein-covalent ligand binding structures and affinities. (c) The scaffolding function of proteins is proposed as a new avenue for drug design, with a focus on modulating protein-protein interactions through small molecules and proteolysis targeting chimeras (PROTACs). (d) The concept of pro-PROTACs is explored as a promising strategy for cancer therapy, combining the principles of prodrugs and PROTACs to enhance specificity and reduce toxicity. (e) The design of prodrugs through carbon-carbon bond cleavage is discussed, offering a new perspective for the activation of drugs with limited modifiable functional groups. (f) The targeting of programmed cell death pathways in cancer therapies with small molecules is reviewed, emphasizing the induction of autophagy-dependent cell death, ferroptosis, and cuproptosis. These insights collectively contribute to a deeper understanding of the dynamic landscape of drug discovery.

© 2025 Published by Elsevier B.V. on behalf of Chinese Chemical Society and Institute of Materia Medica, Chinese Academy of Medical Sciences.

1. Targeting noncatalytic cysteine residues for antiviral drug design

Kai Tang, Peng Zhan*

Over the past few decades, significant achievements have been witnessed in the field of drug discovery, with the widespread utilization of novel techniques and strategies [1]. The role of covalent

targeting in drug discovery has historically been subordinate. Even though covalent modification of proteins and nucleobases is crucial for regulating biological systems, the systematic development of reactive drugs has been considered highly adventurous due to potential toxicity arising from haptenization, indiscriminate labeling, and idiosyncratic drug reactions [2,3]. However, >40 targeted covalent inhibitors (TCIs), such as omeprazole, clopidogrel, osimertinib, voxelotor, sotorasib and nirmatrelvir have been granted approval by the U.S. Food and Drug Administration (FDA) for treating various conditions including cancers, gastrointestinal disorders, hepatitis C virus (HCV) infection, cardiovascular indications, the novel coronavirus disease 2019 (COVID-19) and other diseases [4–7].

As shown in Fig. 1A, the covalent mechanism of TCIs could generally be described in two steps. Firstly, the small molecule ligand

* Corresponding authors.

E-mail addresses: ouyangliang@scu.edu.cn (L. Ouyang), raoli@ccnu.edu.cn (L. Rao), yubin@zsu.edu.cn (B. Yu), zhanpeng1982@sdu.edu.cn (P. Zhan), saiyangz@zsu.edu.cn (S. Zhang), zxj@cpu.edu.cn (X. Zhang).

¹ These authors contributed equally to this work.

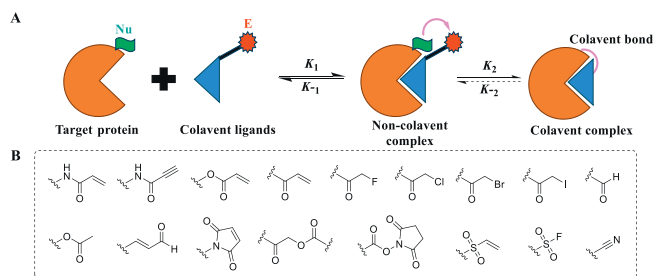


Fig. 1. (A) The two-step process of covalent complex formation. (B) Representative covalent warheads targeting specific amino acids.

can reversibly interact with the target protein through the specific binding pocket of the seeker to form a non-covalent complex. Subsequently, the electrophilic warhead of the intermediate complex will undergo covalent interaction with nucleophilic amino acid residues of the target protein through addition, substitution, oxidation, and other reactions to generate a covalent complex [8–10]. The warhead, also known as a covalent reactive group (CRG), serves as the central component of a covalent ligand, distinguishing it from non-covalent binders [11]. Of the specific nucleophilic amino acids, with cysteine being the most nucleophilic and also most frequently addressed proteinogenic amino acid, developing reactive warheads with cysteine is the most common approach for designing covalent drugs, and the specific types of covalent warheads are shown in Fig. 1B [12].

In the past few years, COVID-19 caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has spread worldwide, the application of covalent strategy in the field of antiviral mainly focuses on the study of 3CL protease inhibitors of SARS-CoV-2 [13]. The 3CL protease (3CL^{pro}, also known as main protease M^{pro}) is a key enzyme that hydrolyzes the precursors of coronavirus polymerase to produce functional non-structural proteins. It is composed of three domains of about 300 amino acids with a nonclassical Cys-His catalytic binary (Cys145 and His41) in the gap between domains I and II, the active site of M^{pro} is composed of four sites (S1, S1', S2, and S4). Due to the highly conserved substrate and central role in viral replication, it has been identified as an attractive broad-spectrum antiviral drug target [14,15].

The antiviral covalent inhibitors reported in the past primarily focus on covalent proteases, which can be categorized into peptidomimetics and nonpeptidic inhibitors based on their structural characteristics. The mechanism of action for peptide-like covalent inhibitors typically involves two steps: firstly, peptide-like molecules that mimic natural peptide substrates bind to M^{pro} forming non-covalently, the covalent warhead is positioned close to the catalytic residue of M^{pro}'s active site, allowing for nucleophilic attack by Cys145's sulfhydryl group to form a covalent bond with the warhead. In other words, these inhibitors initially interact with the protein surface through non-binding interactions before undergoing chemical reactions to establish covalent bonds [16].

As depicted in Fig. S1 (Supporting information), common types of warheads include Michael acceptors, aldehydes, cyanides, and α -ketoamides. In 2020, Ho *et al.* applied the established enzymatic analysis method based on fluorescence resonance energy transfer (FRET) to the screening of SARS-CoV-2 M^{pro} inhibitors and found that GC376 showed excellent anti-SARS-CoV-2 M^{pro} activity (half maximal inhibitory concentration (IC₅₀) = 0.160 ± 0.034 μ mol/L) and could block viral infection in Vero-E6 cells in a cytopathic effect reduction assay (median effective concentration (EC₅₀) = 2.189 ± 0.092 μ mol/L) [17]. Regarding GC376, the sulfite complex can undergo conversion into the aldehyde intermediate and subsequently react with Cys145 via nucleophilic addition. As early as the SARS outbreak in 2002, Pfizer

identified the inhibitory activity of PF-00835231 against SARS-CoV-1 M^{pro}. Given the 100% sequence homology between SARS-CoV-1 and SARS-CoV-2 M^{pro} at the substrate binding site, it can effectively inhibit SARS-CoV-2 M^{pro} as well (K_i = 0.271 nmol/L). Moreover, it exhibited significant anti-SARS-CoV-2 activity in Vero-E6 cells (EC₅₀ = 231 nmol/L). To address the issues of poor membrane permeability and oral bioavailability, the researchers attempted to introduce a cyanogroup covalent warhead and conducted several rounds of optimization, finally obtaining Nirmatrelvir (Fig. S1A, Paxlovid) [18]. The covalent modification not only maintained its *in vitro* inhibitory activity against M^{pro} (K_i = 3.11 nmol/L) but also improved its anti-SARS-2 activity (EC₅₀ = 74.5 nmol/L), metabolic stability, and oral bioavailability. The co-crystal structure analysis of the complex formed by Nirmatrelvir with SARS-CoV-2 revealed that it fully occupies the S1, S2, and S4 sites while covalently binding to the Cys145. Subsequently, Jin *et al.* reported the compound **1** with the Michael acceptor based on the *de novo* design strategy (Fig. S1A). The results showed that Michael addition occurred between the vinyl group and the sulfur atom of Cys145 and a C-S covalent bond was formed, which confirmed the covalent binding mode of compound **1** [19]. Drug repositioning is also an effective approach for drug discovery. Ma *et al.* discovered that Boceprevir, an anti-HCV drug, exhibited moderate inhibitory activity against the M^{pro} (Fig. S1A, IC₅₀ = 4.13 μ mol/L) [20].

The majority of cervical cancer cases, the fourth most prevalent cancer in women globally, are attributed to human papillomavirus (HPV) infections. Recently, Bradley *et al.* developed the first covalent peptide inhibitor **2** (also termed reactide, K_i = 17 ± 3.9 nmol/L) by selectively targeting cysteine 58 in HPV16 early protein 6 (E6), achieving quantitative conversion and providing a starting point for the development of covalent peptidomimetic inhibitors to combat HPV-driven cancers [21].

Our group has been engaged in the discovery and optimization of antiviral TCIs. In 2022, we reported a series of nonpeptidic covalent M^{pro} inhibitors with a piperazine scaffold containing different warheads [22]. Among them, GD-9 exhibited a good inhibition effect of M^{pro} (Fig. S1B, IC₅₀ = 0.18 ± 0.11 μ mol/L) and significant antiviral potency against SARS-CoV-2 (EC₅₀ = 2.64 ± 0.62 μ mol/L), which is comparable to that of remdesivir. The co-crystal structure revealed that GD-9 formed a covalent bond between the methylene carbon of the N4-acyl group with Cys145 (PDB code: 8B56). In 2020, Rao *et al.* discovered that Eblesen effectively inhibited the M^{pro} and exhibited potent anti-SARS-CoV-2 activity in Vero-E6 cells. Structural biology studies revealed that the selenium atom of Eblesen could form a covalent bond with Cys145. Encouragingly, Ampornanai *et al.* optimized the compound MR6-31-2 with better antiviral potency (EC₅₀ = 2.64 ± 0.62 μ mol/L) [23]. In addition to organic covalent binders, the use of inorganic, coordinate covalent binders is also proposed that could attenuate the activity of the protease. In 2021, Cohen *et al.* reported the first metal complex **3** as an M^{pro} cysteine protease inhibitor, Mass spectrometry experiments verified the coordinate covalent binding of a single Re^I tricarbonyl complex to 3CL^{pro}, which provided a novel idea for the development of anti-SARS-CoV-2 drugs [24].

In addition to the aforementioned potential candidates for anti-SARS-CoV-2 drugs, there have also been reports on covalent small molecules targeting other viruses. The capsid core (CA), among the targets for human immunodeficiency virus type 1 (HIV-1) therapeutics, remains relatively unexplored yet highly enticing as a drug target due to its indispensable role throughout virus replication. Recently, Stefan *et al.* discovered the compound BBS-103 that covalently bound CA via a sulfur(VI) fluoride exchange (SuFEx) reaction to Tys145. It exerted antiviral activity by perturbing virus production, not uncoating (EC₅₀ = 7.3 μ mol/L) [25]. The aforementioned compound eblesen is also a covalent binder attached to Cys198 and Cys218 of CA via a selenylsulfide linkage. Unlike BBS-

103, which was identified to impaired *in vitro* assembly, ebselen impaired capsid disassembly [26].

On the other hand, several antiviral covalent compounds targeting human host targets have also been reported. The tumor susceptibility gene 101 (Tsg101) is a crucial component of the endosomal sorting complexes required for transport-I (ESCRT-I) complex, serving as both an adaptor to facilitate interactions with other ESCRT members and a sensor for ubiquitin (Ub). By binding to HIV-1 Gag proteins, TSG101 actively facilitates the release of HIV-1 virions from infected cells, thereby highlighting its potential as a novel therapeutic target against HIV-1 [27]. Lately, Nico *et al.* conducted an *in vitro* characterization of a family of prazole derivatives, such as lansoprazole, that covalently bind to the Cys73 site on Tsg101. And they also assessed their ability to inhibit viral particle production by SARS-CoV-2 and HIV-1, thereby opening up new avenues for designing prazole derivatives with antiviral applications [28].

Compared to traditional non-covalent inhibitors, covalent inhibitors offer significant advantages in terms of prolonged efficiency, enhanced activity, and reduced dosage. Therefore, it is predicted that the study of covalent binding will be crucial for the development of broad-spectrum anti-drug resistance antiviral drugs represented by SARS-CoV-2 M^{pro} inhibitors. At present, the successful strategy in drug design involves incorporating warheads into non-covalent M^{pro} inhibitors, with most covalent M^{pro} inhibitors targeting catalytic site residues like cysteine. As a result, the “warhead” priority structure evolution strategy based on covalent fragments, covalent modification targeting non-catalytic site residues, and the development of a novel “warhead” may become novel hotspots in the design of antiviral covalent drug design.

Acknowledgments: The work of this section was supported by the Postdoctoral Fellowship Program of CPSF (No. GZC20231489), China Postdoctoral Science Foundation (No. 2023M742101), the Ministry of Science and Technology of the People's Republic of China (No. 2023YFC2606500), and the Shandong Laboratory Program (No. SYS202205).

2. Cov_DOX: an example of employing first principle level theory in covalent drug CADD

Li Rao*

In recent years, the resurgence of covalent drugs has been a major topic of drug design. TCI is a newly developed covalent drug concept with a significant advantage in selectivity and affinity [9], while structure-based drug design (SBDD) is the key to the critical selectivity of targeted covalent inhibitor. Understandably, a successful SBDD relies on accurate binding structure information. At present, TCI design heavily relies on demanding X-ray structure for accurate binding structure information and a large amount of activity assay for affinity screen and improve. Molecular docking tools are much cheaper alternatives to the experiments. However, traditional docking methods were limited by the rough theories and lack of electronic structure description. Therefore, the application of those traditional docking methods on covalent ligands was considerably not so reliable [29,30].

Aiming at filling the gap between docking and demanding experiments, we developed a quantum mechanics-based CADD tool, dubbed Cov_DOX, for accurate protein-covalent ligand binding structure and binding affinity prediction [31]. Based on a funnel-like conformation search across multiple potential energy surface strategies [32], Cov_DOX employs three levels of calculations (molecular mechanics, semi-empirical quantum mechanics, and density functional theory) to produce both reliable and affordable predictions of covalent binding structures, taking advantage of first principle level of theory while limiting the computational cost.

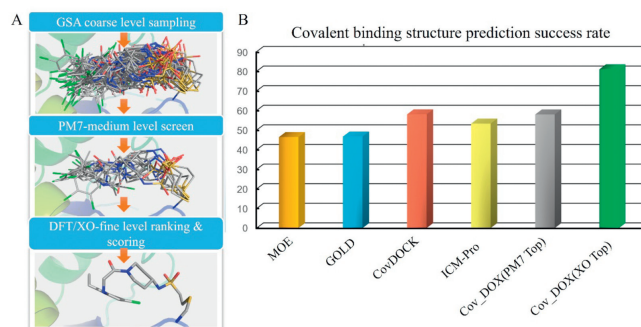


Fig. 2. (A) A schematic illustration of Cov_DOX protocol. (B) The success rates for the Top 1 pose prediction with some covalent docking front runners and Cov_DOX, see Ref. 31 for more details. Reproduced with permission [31]. Copyright 2022, American Chemical Society.

As shown in Fig. 2A, the coarse-level theory generalized simulated annealing (GSA), based on molecular mechanics potential energy surface, is used to search the possible binding pose in the first step of Cov_DOX calculation. The coarse level theory only needs to make sure that the conformation space has encompassed at least one “Good Pose”. Next, geometry optimizations (OPT) and energy evaluations are applied to a cluster including the ligand and its surrounding (<3 Å) protein residues for all of the generated binding poses at the medium-level PM7/COSMO theory, while only the ligand atoms were relaxed during the optimization. Here, the purpose of the medium-level calculation is to narrow down the GSA-generated conformation library, for which the accuracy requirement is to make sure that at least one “Good Pose” is included in a total of 10 candidates. Finally, the Top 10 candidates picked out by the medium-level calculations are re-ranked by the fine-level calculations to identify the optimal binding pose. In Cov_DOX, the fine level theory is the first principle density functional theory (DFT) which allows the proper description of both the critical covalent bond and the non-covalent interactions between protein and the ligand [33]. In addition, the hybrid chemistry method extended ONIOM(XO) [34] is used in companies with DFT to reduce the computational cost, which significantly increases the practical value of Cov_DOX.

To validate its performance on covalent ligands, the Cov_DOX protocol was tested against a total of 405 protein-covalent ligand complexes, covering various types of covalent warhead chemistry, target protein, and ligand chemistry. As shown in Fig. 2B, Cov_DOX achieves an overall success rate of 81% for the Top 1 pose prediction. Such an accuracy is not far from the much more demanding crystallographic experiments, in sharp contrast to the performance of some frequently used covalent docking front runners (success rate 40%–60%). To be more specific, we found that the Cov_DOX prediction ability is independent of the receptor classes, the warhead types, and the reaction mechanisms, indicating a strong universality alongside the relatively high accuracy.

The Cov_DOX protocol can be assessed at a web server, whose URL is <http://doxwebserver.ccn.edu.cn/>. Our previously developed DOX2.0 protocol [32], designed for non-covalent ligands only, was also implemented in the current Cov_DOX. Recently, we focused on adding protein-covalent ligand binding strength prediction ability to Cov_DOX. Based on an inhomogeneous solvation model, a non-fitting method at the first-principles level for binding affinity prediction was developed by taking the critical protein cavity water molecules into account explicitly. The newly developed method, DOX_BDW [35], was validated against 622 non-covalent protein-ligand binding pairs and 143 covalent protein-ligand binding pairs. The results found that the DOX_BDW method was able to produce binding affinity predictions that were strongly correlated with the

corresponding experimental data ($R=0.66\text{--}0.85$), better than the current empirical scoring functions that are heavily parametrized, especially for covalent ligands. See our recent publication for more details [35].

However, we would like to emphasize that we do not expect to replace the covalent docking tools with Cov_DOX in virtual screening, considering its relatively high computational cost. For the moment, a typical Cov_DOX binding affinity prediction job consumes ~ 500 core-hour, while a binding structure prediction job consumes ~ 2000 core-hour. Although the accuracy of Cov_DOX is worthy of the payment, the expense is not comparable with the docking tools focused on large-scale virtual screening. On the other hand, it is worth mentioning that many of the SBDD scenarios still heavily rely on the accuracy of the X-ray structures, such as optimization of the lead structures. Compared with the difficulty of crystalizing the protein-ligand complexes and acquiring user time on synchrotron radiation facilities, the expense of Cov_DOX is just a drop in the bucket. We conclude that Cov_DOX is very useful for covalent protein-ligand complex investigations, where the accuracy of the X-ray structure is badly needed but is unavailable and/or unaffordable.

Acknowledgment: The work of this section is supported by the National Natural Science Foundation of China (Nos. 22373039 and 21603081).

3. Targeting the scaffolding function of proteins for new drug design

Yihui Song, Bin Yu*

The scaffold function of proteins plays pivotal roles in assembling and organizing protein-protein interactions within cellular signaling pathways [36]. Beyond the classic catalytic function, the scaffold role of proteins involved in various cellular processes (e.g., RNA metabolism, DNA repair, cell division, differentiation and genome stability) is also closely linked to the pathogenesis of diseases. These findings offer fresh insights into the comprehensive investigation of the scaffolding functions and small molecule regulation of traditional targets. Disrupting or modulating the scaffolding function of these proteins holds therapeutic promise for a variety of diseases [37]. Historically, drug discovery efforts targeting primarily focused on developing small-molecule inhibitors that target the catalytic functions of proteins. However, the clinical progress of these inhibitors has often been impeded by selectivity issues stemming from the highly conserved catalytic mechanism within homologous protein families [36]. Targeting the scaffolding function of proteins for drug discovery represents an innovative alternative approach in the field of pharmacology and drug development. This approach involves the identification and development of small molecular compounds or proteolysis-targeting chimeras (PROTACs)-based degraders that specifically interfere with signaling networks organized by scaffolding function of proteins or protein stability (Fig. 3). These compounds with selective advantage

may offer promising prospects for therapeutic application [37,38]. In this section, we use representative targets as examples to provide a brief overview of recent advancements in drug discovery by modulating the scaffolding function of proteins.

Focal adhesion kinase (FAK), also known as protein tyrosine kinase 2 (PTK2), is a non-receptor tyrosine kinase encoded by the *PTK2* gene. It functions as a focal adhesion-associated protein kinase, playing vital roles in integrin-mediated signal transduction [39]. FAK is a protein of 125 kD, consisting of the N-terminal FERM (F for the 4.1 protein, ezrin, radixin and moesin) domain, the central kinase domain, two C-terminal proline-rich motifs (PR1 and PR2) and the focal adhesion targeting (FAT) domain [39]. The central kinase domain is responsible for its enzymatic catalytic functions. Serving as a scaffold protein, the N-terminal FERM domain directly interacts with partner proteins (e.g., integrins and growth factor receptors). It also binds to the central kinase domain to block substrate access, thus protecting FAK from activation of sarcoma protein kinase (sarcoma, Src) phosphorylation. The C-terminal FAT domain, containing multiple binding sites for protein-protein interactions, recruits FAK to focal adhesion complexes [39]. Under resting conditions, the FERM domain and the central kinase domain form an auto-inhibitory intramolecular interaction, keeping FAK in an inactive state. Once this intramolecular interaction is disrupted, FAK becomes activated and enhances the auto-phosphorylation at Tyr397. Subsequently, FAK-dependent signaling pathways involved in cell growth, proliferation, survival and migration cellular processes are activated [39]. Overexpression of FAK has been observed in various metastatic tumors and is associated with oncogenic signals, making FAK a significant target in anti-cancer therapy [39]. Reported FAK inhibitors can be categorized into two types: ATP-binding site inhibitors and kinase-independent inhibitors. ATP binding site inhibitors (e.g., TAE-226, PF-573228, PF-562271, PF-4554878 and GSK-2256098) competitively bind to the ATP binding site of FAK' kinase domain. These inhibitors exert their inhibitory activity against FAK by suppressing the phosphorylation of Tyr397 or Tyr861 sites. However, ATP-binding site inhibitors have certain limitations in inhibiting cell growth and inducing apoptosis [39]. Other kinase-independent inhibitors and PROTAC-based degraders have been developed by targeting the scaffold function of FAK (Fig. S2A in Supporting information) [40]. Chloropyramine hydrochloride is a protein-protein interaction inhibitor targeting the interaction between FAK and vascular endothelial growth factor receptor 3 (VEGFR-3). It binds to the VEGFR-3 binding site within the FAT domain of FAK. Preclinical studies have demonstrated that chloropyramine hydrochloride exhibits therapeutic efficacy in multiple cancer types, including pancreatic cancer, breast cancer, neuroblastoma, and advanced melanoma, by disrupting the interaction between FAK and VEGFR-3. It has been received orphan drug designation from FDA [40]. Moreover, chloropyramine hydrochloride has shown significant synergetic anti-cancer effects against breast and pancreatic cancer in combination with chemotherapy drugs [40,41]. Furthermore, a variety of FAK PROTAC degraders (e.g., PROTAC-3, A13, FC-11, B5, BI-3633 and GSK215) have been synthesized, which can directly degrade FAK protein. These degraders are designed by connecting a FAK inhibitor (such as PF562271, TAK-226 or VS-4718) with an E3 ligase ligand (CRBN or VHL) using different linkers. The linker type and length are meticulously optimized to enhance the degradation efficacy of FAK degraders [42,43]. These innovative degraders hold the promise of expanding the druggable landscape and exerting control over protein functions that are conventionally challenging to address with traditional small-molecule therapeutics [44]. Collectively, efforts toward modulating the scaffolding function of FAK, whether through small molecular compounds or PROTAC-based degraders, represents a compelling and promising strategy for the development of highly selective drugs for cancer therapy.

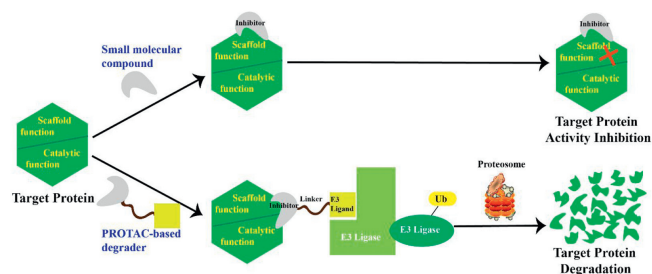


Fig. 3. Drug design strategy by modulating the scaffolding role of proteins.

The histone lysine-specific demethylase 4 (KDM4) can catalyze the demethylation of histone H3 lysine 9 (H3K9) and histone H3 lysine 36 (H3K36). As a member of the Jumonji C domain-containing histone demethylase (JHDM) family, KDM4 is classified into five subtypes (A, B, C, D and E) [45]. The N-terminus of KDM4 enzymes shares conserved Jumonji N and Jumonji C catalytic domains, while the C-termini exhibit notable differences. Only three subtypes, 4A, 4B and 4C, feature the distinctive tandem plant homeodomain (PHD) and TUDOR domains at the C-terminal [45]. Studies have demonstrated that overexpression of KDM4A, 4B and 4C is closely correlated to the pathogenesis of various cancers, underscoring their therapeutic potential in anti-cancer drug development [46]. The reported KDM4 inhibitors primarily target their conserved catalytic domain and can be categorized into three types based on their mechanism of action: (1) Metal chelating inhibitors (e.g., *N*-oxalylglycine) serve as α -ketoglutarate (α -KG) or 2-oxoglutarate (2-OG) cofactor mimetic, competitively binds to the Fe(II). Their hydrophilic property limits the clinical application due to the poor cell penetration; (2) Metal cofactor disruptors (e.g., disulfiram and ebselen) inhibit KDM4's enzymatic activity by blocking the binding of cofactors such as iron and zinc; (3) Competitive inhibitors of histone substrates (e.g., MS275) achieve their inhibitory effects by competitively binding to substrate histones [47]. Nonetheless, these inhibitors face challenges related to poor selectivity due to the conserved catalytic mechanism of the KDM4 family. Evidence has shown that the unique C-termini of the KDM4 family also plays crucial roles in RNA metabolism, DNA damage response, chromatin localization, substrate specificity, etc. Hence, focusing on the distinct tandem TUDOR domain may favor the development of highly selective KDM4 inhibitors [46,48]. Lysine-specific demethylase 4A (KDM4A), also known as Jumonji domain-containing 2A (JMJD2A), plays vital roles in cell proliferation, differentiation and tumorigenesis [45]. Amiodarone, a well-known antiarrhythmic drug, has been recognized as a KDM4A scaffold inhibitor. A series of amiodarone analogs have been developed via structural modification. Among them, WAG-003 ($IC_{50} = 34 \mu\text{mol/L}$) and *N,N*-desethylami ($IC_{50} = 72 \mu\text{mol/Lol/L}$) exhibit a moderate inhibitory effect on the enzymatic activity of KDM4A by targeting the TUDOR domain of KDM4A (Fig. S2B in Supporting information) [46]. Additionally, fragment **54** ($K_i = 170 \mu\text{mol/Lol/L}$) was identified as a KDM4A scaffold inhibitor with a 2D-NMOL/LR based fragment screening approach (Fig. S2B). It exerts inhibitory effect through competitively binding to the H3-K4me3 binding pocket in KDM4A's tandem TUDOR domain. The co-crystal structure of KDM4A TUDOR domain/fragment **54** (PDB code: 5VAR) reveals that the fragment forms a robust network of hydrogen bonds and hydrophobic interactions with KDM4A, providing novel structure skeleton for the development of KDM4A scaffold inhibitors [48].

Protein disulfide isomerase A1 (PDIA1) is a thiol disulfide oxidoreductase located in the endoplasmic reticulum. Functioning as a crucial folding catalyst, PDIA1 catalyzes the oxidation, reduction and isomerization of disulfide bonds between cysteine residues on its substrate protein [49]. PDIA1 possesses two distinct types of sulfur-like redox domains, namely the catalytic domains (a and a') and the non-catalytic domains (b and b'). These sulfur-like redox domains share 37% sequence homology and all of them feature the active site motif Cys-Gly-His-Cys (CGHC). However, they independently carry out functions related to disulfide bond oxidation, reduction and isomerization. Specifically, the catalytic domains a and a' are responsible for catalytic functions, while the non-catalytic domains b and b' are involved in substrate recruitment through their scaffold function [49]. PDIA1 is implicated in various biological processes such as platelet activation, thrombosis, and viral infection. Dysregulation of PDIA1 is closely linked to the pathogenesis of numerous diseases, such as cancer, cardiovascular diseases and neurodegenerative disorders [49]. To date, two different

types of PDIA1 inhibitors, including catalytic inhibitors targeting the a-type domains (e.g., RB-11-ca and KSC-34) and non-catalytic inhibitors targeting the b-type domains (e.g., BAP2 and its derivatives), have been developed [49,50]. Serving as allosteric PDIA1 inhibitors, chalcone-containing BAP2 and its analogs bind to the b' domain and induce a conformational change in PDIA1, subsequently blocking the a' active sites (Fig. S2C in Supporting information). BAP2 exhibits anti-tumor effects against malignant glioblastoma by significantly downregulating gene transcription associated with DNA repair, estrogen receptor (ER) stress response, and apoptosis. Moreover, BAP2 can synergize with DNA alkylating agents to overcome resistance by upregulating DNA repair mechanisms [50].

While inhibitors aimed at the enzymatic catalytic functions of disease-related protein targets constitute a crucial element of clinical drugs, the exploration of scaffolding functions targeting traditional targets has emerged as a novel avenue in innovative drug discovery. However, this field is still in its nascent stage. The pharmacokinetics and toxicity profiles and clinical therapeutic potential of scaffold compounds remain to be discussed. There is a pressing need for a concerted effort to comprehensively understand pathological mechanisms involving proteins' scaffolding function to accelerate new drug discovery by modulating the scaffolding function of traditional targets.

Acknowledgments: This work is supported by the National Natural Science Foundation of China (Nos. 32371317, 22277110 and 82473761), "Chunhui Plan" Cooperative Scientific Research Project of the Ministry of Education (No. HZKY20220280) and Core teacher of Zhengzhou University.

4. Pro-PROTACs: a promising strategy for cancer therapy derived from prodrug and PROTACs

Jian Song, Xiao Wang, Sai-Yang Zhang*

The proteolysis targeting chimera (PROTAC) is a heterobifunctional ternary complex composed of protein of interest ligand (POI), linker, and E3 ligase [51]. By recruiting E3 ubiquitin ligase, it facilitates polyubiquitination of the target protein and subsequently promotes its degradation through the ubiquitin proteasome system (UPS) [52]. Since the proposal of PROTAC by Professor Crew's research group, numerous research teams have dedicated substantial efforts to expanding the application scope of this technology owing to its precise targeting and efficient degradation. With the advancement of research, it has been discovered that this technology exhibits non-selective protein degradation effects on cells or tissues, thereby potentially inducing off-target toxicity and resulting in the degradation of POI in normal tissues or cells. Furthermore, due to its non-compliance with Lipinski's Five Laws of Pharmacology (Ro5), PROTACs demonstrate suboptimal drug permeability, solubility, and oral bioavailability, which may adversely impact drug efficacy [53,54].

To mitigate the non-specific toxic side effects resulting from protein degradation induced by PROTACs, researchers have proposed a prodrug-based strategy for PROTAC delivery (Pro-PROTACs) to achieve precise targeting of active PROTACs in tumor tissues while minimizing off-target toxicity [53]. Pro-PROTAC based on prodrugs could conditionally regulate the targeted degradation activity of PROTAC molecules through different response modes, thereby reducing the potential toxicity of PROTACs to normal cells. Therefore, the development of PROTACs based on prodrugs is considered one of the effective solutions. Currently, multiple types of Pro-PROTACs have been reported (Fig. 4), including photo-responsive Pro-PROTACs [55,56], degrader-antibody conjugates (DAC) [57,58], folic acid cage Pro-PROTACs [59,60], aptamer couplings Pro-PROTACs [61,62], nitroreductase (NTR)-responsive Pro-PROTACs [63,64], radiation triggered-responsive Pro-PROTACs

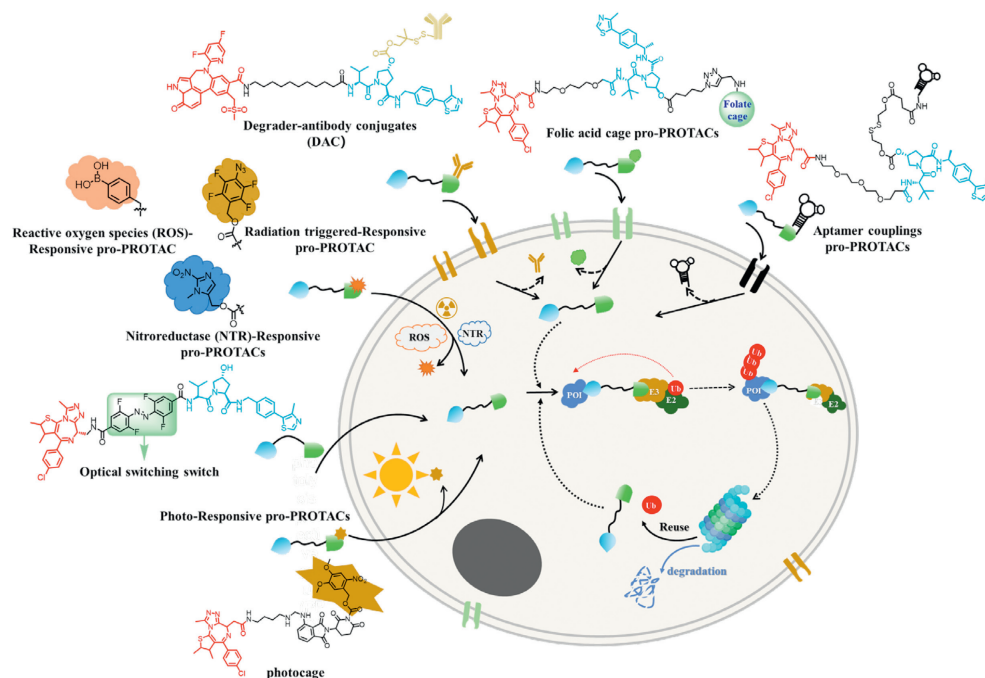


Fig. 4. The mechanisms by which various response types of Pro-PROTACs penetrate tumor cells and exert their effects.

[65] and reactive oxygen species (ROS)-responsive Pro-PROTACs [66,67]. These strategies have been successfully applied in the development of pro-PROTACs, achieving efficient degradation of a range of targets such as epidermal growth factor receptor (EGFR), Bruton's tyrosine kinase (BTK), and anaplastic lymphoma kinase (ALK). Moreover, these strategies could also partially mitigate off-target toxicity in tumor targeting, and enhance the drug metabolism and pharmacokinetic (DMPK) properties.

In conclusion, Pro-PROTACs synthesized with diverse regulatory elements could mitigate the potential toxicity of conventional PROTAC drugs towards normal cells and achieve precise drug release. Despite being full of potential, it is still filled with challenges, such as the complexity of Pro-PROTACs structures which poses significant challenges for optimizing and manufacturing, as well as the possibility that larger molecular weight may impair cell membrane permeability. Although Pro-PROTACs have partially enhanced tissue specificity and achieved selective degradation, current research primarily remains at the cellular level, necessitating further validation in tissues or organisms. Overall, the investigation of novel Pro-PROTACs could address the limitations of traditional degraders and represent an effective approach to overcoming current challenges in the clinical application of PROTACs.

Acknowledgments: This work was supported by the National Natural Science Foundation of China (Nos. U2004123 and 82273782), the National Natural Science Foundation of Henan Province (No. 242300421472) and the Training Program for Young Key Teachers of Colleges and Universities in Henan Province (No. 2023GGJS008).

5. Carbon-carbon bond cleavage-based uncaging strategies for prodrug design

Qijie Gong, Xiaojin Zhang*

The application of prodrug strategies is extensively used in the development of targeted therapeutic drugs to overcome deficiencies in the physicochemical properties of a molecule, especially in the field of anticancer drugs [68]. Prodrugs typically comprise a trigger group and a parent drug (Fig. S3 in Supporting informa-

tion). In some cases, they may also incorporate a self-immolative linker. It is well-reported that, in modern medicinal chemistry, self-immolative linkers have been widely used in the design of prodrugs and probes for connecting targeted responsive trigger groups and released groups, such as parent drugs and fluorophores [69]. For example, upon enzymatic- or chemical-triggered transformation, *para*-aminobenzyl carbamate linkers are cleaved, undergoing a rapid 1,6-elimination to release amines [69]. In the previous strategies, the parent drug and the trigger group or linker are commonly bridged by a C–N or C–O bond (Fig. S3). Activation of such prodrugs can be achieved by the cleavage of the C–N or C–O bond mediated by enzymatic- or chemical-triggered transformation [68]. However, the application of the traditional prodrug strategy is limited due to the necessity of special modifiable groups such as amino, carboxyl, and hydroxyl groups on the structure of the parent drug.

Natural products are important sources for drug discovery and rational design due to the wide range of pharmacological activities and diverse structural skeletons [70]. Numerous studies have revealed that *ortho*-quinones are promising cytotoxic natural products [71–73]. *Ortho*-quinones tend to participate in NAD(P)H: quinone oxidoreductase 1 (NQO1)-dependent redox-cycling, which results in large and rapid production of ROS [72]. The production of ROS in cancer cells is of great use for antiproliferative activity, while the dissemination of ROS systemically is deleterious, which may lead to methemoglobinemia and anemia. However, the majority of *ortho*-quinones solely possess the *ortho*-quinone pharmacophore, lacking the presence of conventional modifiable groups. Consequently, this deficiency impedes the successful implementation of traditional prodrug strategies. For example, β -lapachone (β -lap, Fig. 5) has entered several clinical phase I/II trials due to its potent antiproliferative activities against various human cancers such as lung, pancreatic, liver, breast, and prostate [72]. However, β -lap exhibited significant toxic side effects during clinical trials and, as a result, was not successfully brought to market.

The traditional prodrug strategy is generally not applicable to the majority of *ortho*-quinones, including β -lap, primarily due to the absence of conventional modifiable functional groups in these compounds. The pharmacophore of *ortho*-quinone is a classical

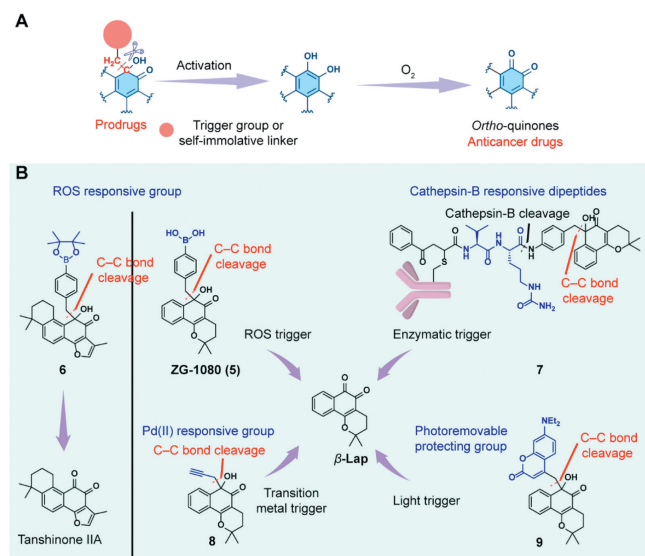


Fig. 5. (A) Carbon-carbon bond cleavage-based uncaging strategies for prodrug design of ortho-quinones. (B) Examples of C-C bond cleavage-based prodrugs triggered by enzymes, chemical molecules, or light irradiation.

toxophore that leads to systemic side effects. However, the toxophore present in ortho-quinones could not be fully bypassed using the previously reported prodrug strategies. In this section, we briefly review a carbon-carbon bond cleavage-based uncaging strategy for prodrug design and its prospective application on ortho-quinones (Fig. 5A), such as β -lap.

To date, the characterization of the tumor-specific microenvironment has been widely used in the design of antitumor prodrugs [74]. For example, the level of ROS is excessive in cancer cells, while the concentration in normal cells is extremely low [75]. The boronic acid (ester) group is a classical ROS-specific trigger group and has been widely used in the design of prodrug targeting cancer cells. In 2022, Zhang *et al.* reported a small-molecule β -lap prodrug ZG-1080 (**5**, Fig. 5B) based on a C-C bond cleavage-based uncaging strategy by attaching a boronic acid (ester) trigger group to β -lap via a self-immolative linker [76]. In the presence of ROS, the small-molecule prodrug can rapidly release β -lap under physiological conditions with very low activation energy for C-C bond cleavage (Fig. 5B and Fig. S4A in Supporting information). In the *in vitro* models, the prodrug selectively exhibited potent antitumor effects in lung and pancreatic cells. In the *in vivo* study, the prodrug significantly inhibited the growth of Mia PaCa-2 pancreatic cells in nude mice. Moreover, the prodrug showed dramatically enhanced safety *in vivo*, which could solve the problem of the narrow therapeutic window of β -lap in clinical trials. This rational carbon-carbon bond cleavage-based uncaging strategy can also be applied to other ortho-quinone natural products, providing a novel insight into the prodrug design of ortho-quinones. In addition, Zhang *et al.* employed the C-C bond cleavage-based uncaging strategy to design a ROS prodrug (referred to as prodrug **6**) derived from another ortho-quinone, tanshinone IIA [76]. This strategy enables the release of tanshinone IIA upon activation by ROS, thereby exerting antitumor activity.

Similarly, Bernardes *et al.* report a C-C bond cleavage-based uncaging strategy using a self-immolative para-aminobenzyl linker to protect and control the release of β -lap [77]. Inspired by the concept of antibody-drug conjugates (ADCs), they designed a new class of antibody-drug conjugates (such as **7**, Fig. 5B) of β -lap for the treatment of acute myeloid leukaemia (AML). Compound **7** was prepared by condensation of a cathepsin B-responsive dipeptide group with a self-immolative linker [77]. Cathepsin B is a

cysteine protease that is overexpressed in many cancer cells. The antibody conjugation of these ADCs can enable targeting and assisted delivery of payloads inside target cancer cells, then triggering the release of β -lap. Both *in vitro* and *in vivo* models, these antibody conjugates exhibit potent antiproliferative activity against AML cells with little side effects on normal tissues. This study proposed a strategy based on C-C bond cleavage to achieve prodrug activation to generate the ADCs, which applies to any peptide promoiety.

Transition metals including palladium, ruthenium, gold, copper, and platinum can trigger bioorthogonal cleavage, thereby activating drug release [78]. For example, Unciti-Broceta *et al.* reported the application of palladium to generate 5-Fu *in vitro* from a biologically inert precursor through a bioorthogonal reaction [79]. Based on this, Bernardes *et al.* reported a palladium trigger-based bioorthogonal cleavage from the prodrug **8** (Fig. 5B), leading to C-C bond cleavage and ultimately releasing β -lap [80]. In this study, non-toxic doses of nano-palladium were applied to decage the C-C bond between propargyl and ortho-quinones in aqueous buffers and living cells. Meanwhile, this strategy can be successfully applied in a zebrafish tumor model to release β -lap *in vivo*, exhibiting a significant antiproliferative potency.

Recently, the application of photoremovable protecting groups has played an important role in modern medicinal chemistry, which controls the activation and release of drugs and could be used as a rational strategy to address the problem of off-target effects of drugs [81]. As an external stimulus, light is characterized by highly spatiotemporal control of drug activation. Given this, Beharry *et al.* combined the photoactivation strategy with the C-C bond cleavage-based uncaging strategy to design a photoactivatable prodrug **9** of β -lap (Fig. 5B), which can be activated with high spatiotemporal control, thereby avoiding its systemic side effects [82]. The coumarin photocage was selected as the light-trigger group, which was linked to the hydroxyketone through a C-C bond, which was activated to release β -lap under photostimulation.

The cleavage of the chemical bond between the ortho-quinone parent drug and the trigger group or the self-immolative linker is primarily driven by the formation of an aromatic intermediate (Fig. S4B in Supporting information). The ortho-diphenol intermediates will spontaneously oxidize into ortho-quinones in the presence of oxygen. Therefore, the development of carbon-bonded ortho-quinone prodrugs could be a promising design strategy for avoiding the toxophore of ortho-quinones.

In general, the carbon-carbon bond cleavage-based uncaging strategy can be applied to other quinones including para- and ortho-quinones, enabling the application of compounds previously considered unsuitable for therapeutic use. Furthermore, this strategy can be applied to other structural skeletons capable of forming aromatic rings upon cleavage of the C-C bond. This rational prodrug strategy via activation through C-C bond cleavage provides novel insights into the prodrug design and also provides a valuable addition to the existing prodrug strategies.

Acknowledgments: This work was supported by grants from the National Natural Science Foundation of China (Nos. 82322062 and 82273769) and Jiangsu Provincial Funds for Distinguished Young Scientists (No. BK20211527).

6. Small molecules targeting programmed cell death in cancer therapies

Panpan Yang, Min Zhao, Liang Ouyang*

Programmed cell death (PCD) was a precise and orderly self-destruct process that played a key role in physiological and pathological processes such as maintaining homeostasis and inhibiting excessive proliferation of tumor cells [83]. Regulation of PCD was

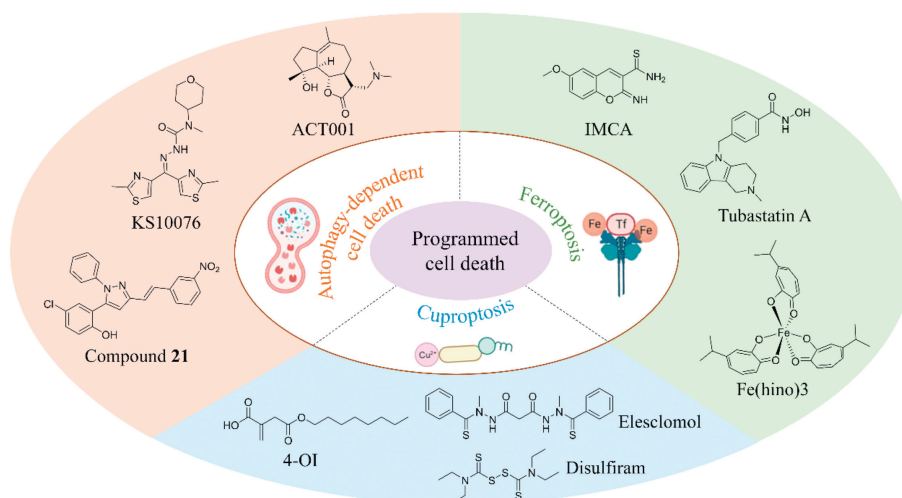


Fig. 6. Small molecule inhibitors developed to target novel programmed cell death pathways in cancer therapy.

a tumor treatment strategy that has been proposed in the early stage of radiotherapy and chemotherapy. The discovery of new cell death pathways, such as autophagy-dependent cell death (ADCD), ferroptosis, cuproptosis, indicated that tumor cells were not only triggered by a single death pathway [84]. Therefore, the combination of drugs to target multiple death pathways has gradually become an important research direction in anticancer therapy. The development of small molecule compounds based on novel death pathways benefited to the design of drug combination strategies. Herein, we summarize the anti-tumor small-molecule compounds targeting novel PCDs developed in the last five years.

Autophagy was an evolutionarily conserved and lysosomal-dependent degradation process, which is divided into cytoprotective autophagy and ADCD. As defined by Nomenclature Committee on Cell Death, ADCD was a type of cell death that is strictly dependent on the mechanism of autophagy or its key components [85]. ADCD was characterized by excessive activation of autophagy, resulting in self-digestion of cellular materials in autophagolysosomes beyond the scope of cell survival. Therefore, pharmacological regulation of core autophagy factors in cancer cells can induce ADCD and thus exert anticancer effects [86]. Leong *et al.* has reported compound **10**, a novel dual functional phenylpyrazole-styryl hybrid, showed potent inhibitory activity against EJ28 cell ($IC_{50} = 2.8 \mu\text{mol/L}$). Compound **10** simultaneously induced ROS-independent apoptosis and ADCD through EGFR/AKT/mTOR pathway in EJ28 cells [87]. Kim *et al.* identified a metal chelator, KS10076, with tumor inhibitory effect in HCT116 and SW480 murine xenograft models. KS10076 destabilized STAT3 and thereby triggering ADCD via the EIF2A/LC3B signaling pathway [88]. ACT001 was capable of inducing ADCD by binding to MEK4 to activate JNK and p38 phosphorylation, and inhibited the growth of pituitary tumor cells GH3 cells ($IC_{50} = 9.56 \mu\text{mol/L}$) and MMQ cells ($IC_{50} = 22.65 \mu\text{mol/L}$) (Fig. 6) [89].

Ferroptosis was a new form of regulatory non-apoptotic cell death characterized by iron-dependent lipid peroxidation induced cell death. The occurrence of ferroptosis was affected by iron metabolism, lipid metabolism and amino acid metabolism, which was mainly regulated by the system Xc^- -glutathione-glutathione peroxidase 4 (Xc^- -GSH-GPX4) axis and the NAD(P)H-ferroptosis suppressor protein 1-ubiquinone (NAD(P)H-FSP1-CoQ10) pathway. Thus, small molecules that regulated relevant metabolic pathways harbored the potential to induce ferroptosis in cancer cells, including inhibitors targeting the Xc^- -system and GPX4 and other stimulators that caused lipid peroxidation [90]. Pharmacological study identified that Tubastatin A was able to overcome ferroptosis

resistance and radioresistance of cancer cells by inhibiting GPX4 enzyme activity, promoting radiation-induced lipid peroxidation and tumor suppression in mouse breast cancer xenograft [91]. Fe(hino)_3 , formed by the complexation of iron with the iron chelator Hinokitiol (hino) *in vitro*, was commonly used as ferroptotic inducer. Fe(hino)_3 can promote free radical production and lipid peroxidation to induce ferroptosis and significantly inhibit the growth of TNBC cell-derived tumors [92]. Zhang *et al.* found that the benzopyran derivative 2-imino-6-methoxy-2H-chromene-3-carbothioamide (IMCA) can downregulate the expression of SLC7A11 (the subunit of system Xc^-) and decrease levels of cysteine and glutathione in colon cancer cells, resulting in ROS accumulation and ferroptosis occurrence. The IC_{50} values of IMCA for DLD-1 and HCT116 cells were 50.2 and 44.5 $\mu\text{mol/L}$, respectively (Fig. 6) [93].

Cuproptosis, proposed by Tsvetkov *et al.* in 2022, was a novel PCD that differs from the known cell death pathway [94]. Excess intracellular Cu(II) was transported to the mitochondria by ionophore and subsequently reduced to Cu(I) by mitochondrial ferredoxin 1 (FDX1). Cu(I) can bind directly to the fatty acylated components of the tricarboxylic acid cycle (TCA) cycle, such as dihydrolipoamide S-acetyltransferase (DLAT), and induce the aggregation of fatty acylated proteins and the instability of Fe-S cluster proteins, leading to protein toxic stress and cell death [95]. Therefore, the occurrence and development of cuproptosis was closely related to the TCA cycle. Itaconate was a metabolite of the TCA cycle. 4-Octyl itaconate (4-OI) was a cell-permeable itaconate derivative that promoted cuproptosis in colon cancer cells by inhibiting aerobic glycolysis by targeting glyceraldehyde-3-phosphate dehydrogenase (GAPDH) [96]. Copper ionophore, like elesclomol and disulfiram (DSF), can reversibly bind to copper ion, which was thought to be able to induce cuproptosis. Recent study developed a ROS responsive nanomide loaded with elesclomol and Cu (NP@ESCu), it exhibited excellent anti-tumor activity by inducing cuproptosis in a mouse subcutaneous bladder cancer model (Fig. 6) [97]. However, as for the anti-cancer effect of copper ionophores by inducing cuproptosis, further studies are needed to clarify its specific mechanism, such as exploring the biomarkers of cuproptosis and elucidating the copper dependent signaling pathways [97].

In recent decades, small-molecule drugs targeting programmed cell death, especially apoptosis, have developed rapidly, but the resistance of cancer cells to apoptosis has severely limited the development and application of related drugs. The emergence of new cell death modes, such as ADCD, ferroptosis and cuproptosis, has provided new options for cancer treatment, and some small-molecule

compounds regulating new PCD have shown good anti-cancer effects *in vitro* and *in vivo*. The studies have found that different subprograms that regulate cell death often share some key genes or regulatory factors. The hope for future cancer therapy may lie in the discovery of more small-molecule compounds that target different signaling pathways in the PCD subprogram, which could also help prevent cancer cells from being resistant to specific types of PCD, thus achieving better efficacy in clinical therapy. Therefore, in-depth study of the complexity of PCD *in vivo* and focusing on the crosstalk between different PCD pathways may be a new direction for future research.

Acknowledgments: This work was supported by grants from the National Natural Science Foundation of China (No. 82273770) and the Foundation for Innovative Research Groups of the National Natural Science Foundation of Sichuan Province (No. 24NSFTD0051).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Qijie Gong: Writing – original draft. **Jian Song:** Writing – original draft. **Yihui Song:** Writing – original draft. **Kai Tang:** Writing – review & editing, Writing – original draft. **Panpan Yang:** Writing – original draft. **Xiao Wang:** Writing – original draft. **Min Zhao:** Writing – original draft. **Liang Ouyang:** Writing – review & editing. **Li Rao:** Writing – review & editing, Writing – original draft. **Bin Yu:** Writing – review & editing. **Peng Zhan:** Writing – review & editing. **Saiyang Zhang:** Writing – review & editing. **Xiaojin Zhang:** Writing – review & editing.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ccl.2024.110456.

References

- [1] J. Du, J. Guo, D. Kang, et al., *Chin. Chem. Lett.* 31 (2020) 1695–1708.
- [2] J. Singh, R.C. Petter, T.A. Baillie, A. Whitty, *Nat. Rev. Drug Disc.* 10 (2011) 1474–1784.
- [3] J. Uetrecht, *Annu. Rev. Pharmacol. Toxicol.* 47 (2007) 513–539.
- [4] L. Boike, N.L. Henning, D.K. Nomura, *Nat. Rev. Drug Disc.* 21 (2022) 881–898.
- [5] H. Kim, Y.S. Hwang, M. Kim, S.B. Park, *RSC Med. Chem.* 12 (2021) 1037–1045.
- [6] X. Shao, S. Xu, X. Wan, et al., *Chin. Chem. Lett.* 34 (2023) 108349.
- [7] S. Gao, T. Huang, L. Song, et al., *Acta Pharm. Sin.* B 12 (2023) 581–599.
- [8] T.A. Baillie, *Angew. Chem. Int. Ed.* 55 (2016) 13408–13421.
- [9] E. Mons, S. Roet, R.Q. Kim, M.P.C. Mulder, *Curr. Protoc.* 2 (2022) e419.
- [10] J.M. Strelow, *SLAS Discov.* 22 (2017) 3–20.
- [11] M.E. Flanagan, J.A. Abramite, D.P. Anderson, et al., *J. Med. Chem.* 57 (2014) 10072–10079.
- [12] M. Gehring, S.A. Laufer, *J. Med. Chem.* 62 (2019) 5673–5724.
- [13] X. Pang, W. Xu, Y. Liu, H. Li, L. Chen, *Eur. J. Med. Chem.* 257 (2023) 115491.
- [14] M. Berry, B.C. Fielding, J. Gamielien, *Viruses* 7 (2015) 6642–6660.
- [15] D.W. Kneller, G. Phillips, H.M. O'Neill, et al., *Nat. Commun.* 11 (2020) 3202.
- [16] A. Citarella, A. Dimasi, D. Moi, et al., *Biomolecules* 13 (2023) 1339.
- [17] S. Iketani, F. Forouhar, H. Liu, et al., *Nat. Commun.* 12 (2021) 2708.
- [18] D.R. Owen, C.M.N. Allerton, A.S. Anderson, et al., *Science* 374 (2021) 1586–1593.
- [19] Z. Jin, X. Du, Y. Xu, et al., *Nature* 582 (2020) 289–293.
- [20] C. Ma, M.D. Sacco, B. Hurst, et al., *Cell Res.* 30 (2020) 678–692.
- [21] X. Ye, P. Zhang, J. Tao, et al., *Pentelute, Chem. Sci.* 14 (2023) 12484–12497.
- [22] S. Gao, L. Song, T. Claff, et al., *J. Med. Chem.* 65 (2022) 16902–16917.
- [23] K. Ampornadani, X. Meng, W. Shang, et al., *Nat. Commun.* 12 (2021) 3061.
- [24] J. Karges, M. Kalaj, M. Gembicky, S.M. Cohen, *Angew. Chem. Int. Ed.* 60 (2021) 10716–10723.
- [25] W.M. McFadden, M.C. Casey-Moore, G.A.L. Bare, et al., *Cell Chem. Biol.* 31 (2024) 477–486.
- [26] S. Thenin-Houssier, I.M. de Vera, L. Pedro-Rosa, et al., *Antimicrob. Agents Chemother.* 60 (2016) 2195–2208.
- [27] D.A. Nyenhuis, R. Rajasekaran, S. Watanabe, et al., *J. Biol. Chem.* 299 (2023) 102901.
- [28] D.A. Nyenhuis, S. Watanabe, R. Bernstein, et al., *Adv. Sci.* 11 (2024) e2308312.
- [29] A. Scarpino, G.G. Ferenczy, G.M. Keseru, *J. Chem. Inf. Model.* 58 (2018) 1441–1458.
- [30] C. Wen, X. Yan, Q. Gu, et al., *Molecules* 24 (2019) 2183.
- [31] L. Wei, Y. Chen, J. Liu, et al., *J. Med. Chem.* 65 (2022) 5528–5538.
- [32] L. Wei, B. Chi, Y. Ren, et al., *J. Chem. Theory Comput.* 7 (2019) 4264–4279.
- [33] J. Chai, M. Head-Gordon, *Phy. Chem. Chem. Phys.* 10 (2008) 6615.
- [34] W. Guo, A. Wu, I.Y. Zhang, X. Xu, *J. Comput. Chem.* 33 (2012) 2142–2160.
- [35] J. Liu, J. Wan, Y. Ren, et al., *J. Chem. Inf. Model.* 63 (2023) 4850–4863.
- [36] P.D. Mace, J.M. Murphy, *J. Biol. Chem.* (2021) 100705.
- [37] Z. Wang, W. Huang, K. Zhou, X. Ren, K. Ding, *J. Med. Chem.* 65 (2022) 1735–1748.
- [38] C. Kim, X.D. Wang, Z. Liu, S. Zha, Y. Yu, *Biochemistry* 62 (2023) 561–563.
- [39] E. Shanthi, M.H. Krishna, G.M. Arunesh, et al., *Expert Opin. Ther. Pat.* 24 (2014) 1077–1100.
- [40] E.V. Kurenova, D.L. Hunt, D. He, et al., *J. Med. Chem.* 52 (2009) 4716–4724.
- [41] E. Kurenova, J. Liao, D.H. He, et al., *Oncotarget* 4 (2013) 1632–1646.
- [42] Y. Sun, R. Wang, Y. Sun, et al., *Eur. J. Med. Chem.* 237 (2022) 114373.
- [43] Q. Qin, R. Wang, Q. Fu, et al., *J. Enzyme Inh. Med. Chem.* 37 (2022) 2241–2255.
- [44] P.M. Cromm, K.T.G. Samarasinghe, J. Hines, C.M. Crews, *J. Am. Chem. Soc.* 140 (2018) 17019–17026.
- [45] G. Yang, C. Li, F. Tao, et al., *Genes Dis.* 11 (2024) 645–663.
- [46] R.M. Labbé, A. Holowatyj, Z.Q. Yang, *Am. J. Transl. Res.* 6 (2014) 1–15.
- [47] Q. Wu, B. Young, Y. Wang, et al., *J. Med. Chem.* 65 (2022) 9564–9579.
- [48] A.K. Upadhyay, R.A. Judge, L. Li, et al., *Bioorg. Med. Chem. Lett.* 28 (2018) 1708–1713.
- [49] L.E. Powell, P.A. Foster, *Cancer Med.* 10 (2021) 2812–2825.
- [50] S. Xu, Y. Liu, K. Yang, et al., *Theranostics* 9 (2019) 2282–2298.
- [51] K. Li, C.M. Crews, *Chem. Soc. Rev.* 51 (2022) 5214–5236.
- [52] M. Békés, D.R. Langley, C.M. Crews, *Nat. Rev. Drug Disc.* 21 (2022) 181–200.
- [53] C. Chen, Y. Yang, Z. Wang, et al., *J. Med. Chem.* 66 (2023) 8428–8440.
- [54] C. Pu, S. Wang, L. Liu, et al., *Chin. Chem. Lett.* 34 (2023) 107927.
- [55] G. Xue, K. Wang, D. Zhou, H. Zhong, Z. Pan, *J. Am. Chem. Soc.* 141 (2019) 18370–18374.
- [56] H. Xu, N. Ohoka, T. Inoue, H. Yokoo, Y. Demizu, *Bioorg. Med. Chem. Lett.* 107 (2024) 129778.
- [57] P.S. Dragovich, *Chem. Soc. Rev.* 51 (2022) 3886–3897.
- [58] K.B. Hong, H. An, *J. Med. Chem.* 66 (2023) 140–148.
- [59] J. Liu, H. Chen, Y. Liu, et al., *J. Am. Chem. Soc.* 143 (2021) 7380–7387.
- [60] H. Chen, J. Liu, H. Kaniskan, W. Wei, J. Jin, *J. Med. Chem.* 64 (2021) 12273–12285.
- [61] S. He, F. Gao, J. Ma, et al., *Angew. Chem. Int. Ed.* 60 (2021) 23299–23305.
- [62] S. He, Y. Fang, Y. Zhu, et al., *Adv. Sci.* 11 (2024) e2401623.
- [63] W. Cheng, S. Li, X. Wen, et al., *Chem. Commun.* 57 (2021) 12852–12855.
- [64] S. Shi, Y. Du, Y. Zou, et al., *J. Med. Chem.* 65 (2022) 5057–5071.
- [65] C. Yang, Y. Yang, Y. Li, et al., *J. Am. Chem. Soc.* 145 (2023) 385–391.
- [66] C. Liang, Q. Zheng, T. Luo, et al., *CCS Chem.* 4 (2022) 3809–3819.
- [67] H. Liu, C. Ren, R. Sun, et al., *Chem. Commun.* 58 (2022) 10072–10075.
- [68] J. Rautio, N.A. Meanwell, L. Di, M.J. Hageman, *Nat. Rev. Drug Disc.* 17 (2018) 559–587.
- [69] S. Gnaim, D. Shabat, *Acc. Chem. Res.* 52 (2019) 2806–2817.
- [70] A.G. Atanasov, S.B. Zotchev, V.M.C. Dirsch, C.T. Supuran, *Nat. Rev. Drug Disc.* 20 (2021) 200–216.
- [71] D. Dey, M.M. Hasan, P. Biswas, et al., *Front. Oncol.* 12 (2022) 899009.
- [72] Q. Gong, J. Hu, P. Wang, X. Li, X. Zhang, *Eur. J. Med. Chem.* 210 (2020) 112962.
- [73] M.A. Ansari, F.B. Khan, H.A. Safdari, et al., *Pharmacol. Res.* 164 (2021) 105364.
- [74] X. Zhang, X. Li, Q. You, X. Zhang, *Eur. J. Med. Chem.* 139 (2017) 542–563.
- [75] P. Wang, Q. Gong, J. Hu, X. Li, X. Zhang, *J. Med. Chem.* 64 (2021) 298–325.
- [76] Q. Gong, X. Li, T. Li, et al., *Angew. Chem. Int. Ed.* 61 (2022) e202210001.
- [77] L. Dunsmore, C.D. Navo, J. Becher, et al., *Nat. Chem.* 14 (2022) 754–765.
- [78] J. Wang, X. Wang, X. Fan, P. Chen, *ACS Cent. Sci.* 7 (2021) 929–943.
- [79] J.T. Weiss, J.C. Dawson, K.G. Macleod, et al., *Nat. Commun.* 5 (2014) 3277.
- [80] G.M. Dal Forno, E. Latocheski, A. Beatriz Machado, et al., *J. Am. Chem. Soc.* 145 (2023) 10790–10799.
- [81] H.H. Han, H.M. Wang, P. Jangili, et al., *Chem. Soc. Rev.* 52 (2023) 879–920.
- [82] E.G. Kaye, B. Mirabi, I.R. Lopez-Miranda, et al., *J. Am. Chem. Soc.* 145 (2023) 12518–12531.
- [83] S. Bedoui, M.J. Herold, A. Strasser, *Nat. Rev. Mol. Cell Biol.* 21 (2020) 678–695.
- [84] K. Hadian, B.R. Stockwell, *Nat. Rev. Drug Disc.* 22 (2023) 723–742.
- [85] L. Galluzzi, I. Vitale, S.A. Aaronson, et al., *Cell Death Diff.* 25 (2018) 486–541.
- [86] D. Tang, R. Kang, T. Bergh, P. Vandenabeele, G. Kroemer, *Cell Res.* 29 (2019) 347–364.
- [87] S.W. Leong, J. Wang, K.S. Okuda, et al., *Eur. J. Med. Chem.* 254 (2023) 115335.
- [88] J. Kim, A. Park, J. Hwang, et al., *Cell Rep.* 40 (2022) 111077.
- [89] L. Cai, Z.R. Wu, L. Cao, et al., *Acta Pharmacol. Sin.* 43 (2022) 2386–2396.
- [90] X. Tong, R. Tang, M. Xiao, et al., *J. Hematol. Oncol.* 15 (2022) 174.
- [91] S. Liu, H.L. Zhang, J. Li, et al., *Redox Biol.* 62 (2023) 102677.
- [92] H. Zhao, M. Zhang, J. Zhang, et al., *Cell Biosci.* 13 (2023) 87.
- [93] L. Zhang, W. Liu, F. Liu, et al., *Oxid. Med. Cell. Longev.* 2020 (2020) 1675613.
- [94] P. Tsvetkov, S. Coy, B. Petrova, et al., *Science* 375 (2022) 1254–1261.
- [95] Y. Wang, L. Zhang, F. Zhou, *Cell. Mol. Immunol.* 19 (2022) 867–868.
- [96] W. Yang, Y. Wang, Y. Huang, et al., *Biomed. Pharmacother.* 159 (2023) 114301.
- [97] Y. Wang, Y. Chen, J. Zhang, et al., *Drug Resist. Updat.* 72 (2024) 101018.