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REVIEW

Target identification of natural products in cancer with chemical proteomics and artificial intelligence approaches

Guohua Li[1,2]*, Qian Shi[2]*, Qibiao Wu[1], Xinbing Sui[1,2]

[1]School of Pharmacy, Faculty of Medicine & Faculty of Chinese Medicine, Macau University of Science and Technology, Macau 999078, China; [2]School of Pharmacy, Hangzhou Normal University, Hangzhou 311121, China

ABSTRACT Natural products (NPs) have long been recognized for their therapeutic potential, especially in cancer treatment, due to an ability to interact with multiple cellular pathways. The identification of molecular targets for NPs is a critical step in understanding anticancer mechanisms, with chemical proteomics emerging as a powerful approach. Both label-based and -free proteomic techniques have been utilized to identify these targets, each with their own advantages and limitations. While label-based methods provide high specificity through chemical tagging, the requirement for labeling can be a limitation, potentially altering NP natural properties. Conversely, label-free techniques allow for the detection of NP-protein interactions without structural modification but may struggle with transient interactions or low-abundance targets. Recent advances in artificial intelligence (AI) have further enhanced the field by improving target prediction and streamlining data analysis. AI-driven models, especially machine learning algorithms, have proven effective in processing complex proteomic data and predicting potential NP-protein interactions. The integration of AI with chemical proteomics accelerates target identification and deepens our understanding of the molecular mechanisms underlying the anticancer effects of NPs. This review explores the application of chemical proteomics and AI in the identification of cancer-related targets for NPs, highlighting current challenges and future directions for clinical translation.

KEYWORDS Chemical proteomics; artificial intelligence; natural products; cancer; target identification

Introduction

Natural products (NPs) have long been an invaluable source of therapeutic agents, especially in the field of cancer treatment[1]. NPs serve as a cornerstone in drug discovery with > 60% of currently used anticancer agents being either directly sourced from natural origins or structurally emulated based on natural prototypes. The chemical diversity of NPs and their capacity to target multiple pathways within cancer cells make NPs ideal candidates for therapeutic development[2]. NPs, such as paclitaxel, vincristine, and camptothecin, have revolutionized cancer chemotherapy and new NP-derived agents continue to be explored in clinical trials[3]. This review focuses on several key classes of natural compounds (terpenoids, polyphenols, quinones, and alkaloids), each of which has demonstrated significant anticancer activity through various molecular pathways. The selected compounds have been extensively studied due to their strong anti-tumor effects, established clinical applications, or encouraging preclinical findings. However, despite their broad application, the precise molecular targets of many NPs remain largely unidentified, which limits our understanding of their mechanisms of action and impedes the development of more targeted therapies.

To address the complexity of NP-target interactions, chemical proteomics have emerged as a powerful tool for target identification. This strategy uses compounds that have been altered to serve as probes or affinity tags and bind to and capture target proteins, allowing for the isolation and subsequent identification of these proteins *via* mass spectrometry (MS). This technique can uncover both direct and indirect interactions between NPs and NP targets, providing a comprehensive profile of the protein networks involved[4]. Methods, such as compound-centric chemical proteomics (CCCP) and activity-based protein profiling (ABPP),

*These authors contributed equally.
Correspondence to: Qibiao Wu and Xinbing Sui
E-mail: qbwu@must.edu.mo and suilab@hznu.edu.cn
ORCID ID: https://orcid.org/0000-0002-1670-1050 and https://orcid.org/0000-0001-7330-0467
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are commonly used in target identification, which helps to delineate the precise molecular mechanisms by which NPs affect cancer cell functions, including survival, apoptosis, and metastasis[5,6]. In parallel, label-free target identification methods have gained significant traction due to an ability to analyze NP-protein interactions without the need for chemical modification or NP tagging. Techniques, like drug affinity responsive target stability (DARTS), thermal proteome profiling (TPP), and solvent-induced protein precipitation (SIP), have proven highly effective in capturing direct interactions in native cellular environments[7,8]. These label-free approaches allow for real-time monitoring of protein stability or accessibility changes upon NP treatment, providing valuable insight into the biological relevance of NP-target binding[4].

While chemical proteomics has significantly improved target identification, one of the ongoing challenges in drug discovery is the enormous and intricate biological datasets generated from these high-throughput techniques[9]. In response to this challenge, artificial intelligence (AI) has emerged as a transformative tool in the field of chemical proteomics and target identification. Machine learning (ML) and deep learning (DL) enable efficient analysis of extensive datasets by identifying potential protein targets and revealing complex patterns that are often difficult for human researchers to detect. These algorithms can integrate multiple types of omics data, such as proteomics, genomics, and transcriptomics, and provide deeper insight into the functional relationships between NPs and NP targets[10]. Researchers can generate predictive models that forecast the interactions between small molecules and proteins by using AI, which significantly speeds up the target discovery process.

The application of AI in drug discovery has gained traction in recent years. For example, *in silico* models have been developed to predict the binding affinities of natural compounds to specific proteins, which facilitates the identification of promising therapeutic candidates before experimental tesging[11]. Furthermore, AI can aid in the interpretation of chemical proteomics data by clustering protein targets based on biological functions or pathways, which provides a more holistic view of how an NP might influence cellular processes. This approach improves the efficiency of target identification and enhances the accuracy and reliability of predictions, which reduces the time and cost associated with traditional experimental methods[12]. The integration of chemical proteomics with AI-driven data analysis creates a powerful synergy that is capable of revolutionizing target identification and validation for NPs in cancer treatment. This combined approach enables a more systematic and comprehensive identification of molecular targets and enhances our understanding of the mechanisms by which NPs exert anticancer effects[13,14]. Additionally, AI can help identify novel targets that may not have been previously recognized, opening new possibilities for innovative cancer treatments and advancing next-generation therapies[15]. Recent studies have demonstrated the value of combining these two fields. For example, the use of AI to analyze data from chemical proteomics experiments has led to the identification of new targets for cancer drugs, such as proteins involved in immune modulation and pyroptosis therapy[16]. The fusion of chemical proteomics with AI tools has the potential to uncover previously unknown aspects of cancer biology, which have led to the discovery of novel therapeutic targets and more effective drug candidates.

In summary, the integration of chemical proteomics and AI holds great promise in expediting the target identification process for NPs in cancer treatment. Consequently, the integration of chemical proteomics and AI not only facilitates the development of more potent cancer therapies but also furnishes profound insights into the mechanisms underlying cancer biology. These cutting-edge technologies are certain to assume a pivotal position in the future of cancer drug discovery as the technologies continue to advance. Moreover, these technologies will offer innovative strategies to combat life-threatening cancers, potentially revolutionizing cancer treatment paradigms and bringing us closer to more effective and personalized cancer management.

NPs as effective cancer therapeutics

NPs represent a rich source of anti-cancer agents owing to their diverse chemical structures and biological activities. Herein we review compounds that are representative of several important classes of NPs (**Figure 1**) and systematically summarize the applications in various types of cancers by incorporating findings from the most recent reviews and key representative studies. **Table 1** provides an overview of the different types of cancer treated by NPs. This review offers a valuable resource for understanding how NPs can be harnessed in oncology and paves the way for future research in developing effective NP-based therapies based on the most recent literature.

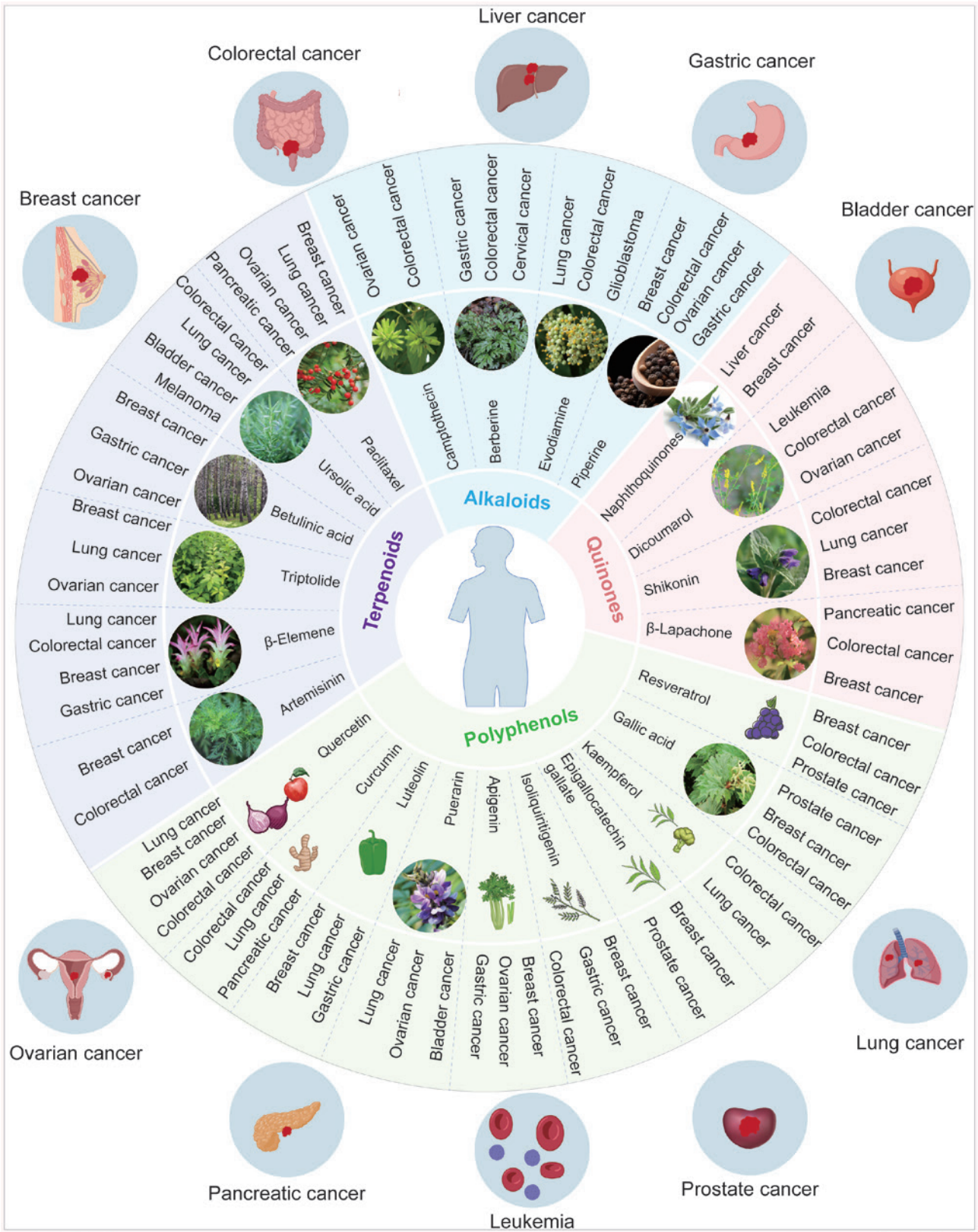


Figure 1 Continued

Figure 1 Schematic representation of the typical natural products (NPs) in different types of cancer. This figure provides an overview of representative NPs with anticancer properties and the applications in different types of cancer. The diagram illustrates four major classes of NPs (**terpenoids**, **polyphenols**, **quinones**, and **alkaloids**), highlighting key compounds from each class and the primary mechanisms of action in cancer treatment.

Terpenoids

Terpenoids, also known as isoprenoids, are a diverse class of NPs derived from five-carbon isoprene units. These compounds are widely distributed in nature (especially in plants) and have demonstrated significant pharmacologic potential, including anti-cancer properties. The structural diversity of terpenoids allows terpenoids to interact with various molecular targets, making terpenoids a promising class of compounds in cancer treatment. Several terpenoids have been successfully translated into clinical use or are under investigation in clinical trials[88].

Paclitaxel

Paclitaxel, commonly sold under the brand name Taxol, is a diterpenoid compound derived from the bark of the Pacific yew, *Taxus brevifolia*. Paclitaxel is one of the most well-known NPs used in cancer chemotherapy. Paclitaxel was first isolated from the bark of the yew in 1971 by Drs. Monroe Wall and Mansukh Wani[89]. The compound exerts anti-cancer effects by binding to β-tubulin, thus stabilizing microtubules. The resulting inability of cancer cells to depolymerize microtubules disrupts mitotic spindle formation, arrests cell cycle progression, and induces apoptosis[90].

Paclitaxel underwent a series of multi-phase clinical trials. Early phase I and II studies, which were conducted from the late 1970s to the early 1980s, primarily focused on evaluating the safety profile of paclitaxel and determining an effective dosing regimen. These preliminary studies helped in the identification of dose-limiting toxicities, such as neutropenia, and in the development of pre-medication protocols to mitigate severe hypersensitivity reactions. Subsequent phase III trials, which were conducted through 1992, further confirmed the promising anti-tumor activity of paclitaxel in a variety of solid tumors, including ovarian, breast, and lung cancers[91]. Paclitaxel was first approved by the FDA for the treatment of ovarian cancer in 1992, followed by approval for breast cancer in 1994 and pancreatic cancer in 2013[92]. However, development of paxlitaxel faced significant challenges, including scarcity of the compound, environmental concerns, and poor solubility. Despite these obstacles, sustainable production was later achieved through semi-synthetic and plant cell culture technologies, ensuring availability for widespread use[93]. Recent studies have explored new delivery systems and formulations to enhance the efficacy of paclitaxel and reduce side effects, such as the development of albumin-bound paclitaxel nanoparticles (Abraxane), which offer improved solubility and reduced hypersensitivity reactions[94,95].

Triptolide

Triptolide, a diterpenoid epoxide first isolated in 1972 from the Chinese herb, *Tripterygium wilfordii* Hook F, is another promising NP for cancer therapy. Triptolide was initially recognized for immunosuppressive and anti-inflammatory properties, and has gained attention for potent anticancer activities across a variety of malignancies[96]. The anti-cancer effects of triptolide are primarily mediated through inhibition of transcriptional machinery, induction of apoptosis, and suppression of metastasis. Triptolide targets multiple pathways that are critical for cancer cell survival and proliferation, including pathways involving NF-κB, p53, and heat shock proteins[97].

Triptolide has demonstrated particularly strong anti-cancer effects in breast cancer. Triptolide has been shown to inhibit breast cancer cell proliferation by inducing apoptosis and suppressing cell cycle progression[97]. Furthermore, triptolide inhibits angiogenesis by decreasing vascular endothelial growth factor A (VEGFA) expression in breast cancer cells[98]. Triptolide also exerts antimetastatic effects in breast cancer through a mechanism that is mediated by a microRNA. Specifically, triptolide induces miR-146a expression, which inhibits the production of RhoA and Rac1, Rho GTPase family members with key roles in cell migration and invasion[99]. The potent anti-cancer activity of triptolide in lung cancer has been attributed to multiple mechanisms and targeting multiple signaling pathways. For example, triptolide has been shown to inhibit the Wnt/β-catenin signaling pathway in lung cancer cells by increasing the expression of WIF1 and other Wnt inhibitors, resulting in reduced β-catenin activity and induction of apoptosis[100]. In addition, triptolide can enhance

Table 1 Types of cancers are treated by natural products

NPs	Classification	Source	Cancer types
Paclitaxel	Terpenoids	*Taxus brevifolia*	Ovarian cancer[17], breast cancer[18], lung cancer[19], pancreatic cancer[20]
Triptolide	Terpenoids	*Tripterygium wilfordii Hook F*	Breast cancer[21], lung cancer[22], ovarian cancer[23]
Artemisinin	Terpenoids	*Artemisia annua*	Breast cancer[24], colorectal cancer[25]
Betulinic acid	Terpenoids	*Betula species*	Breast cancer[26], gastric cancer[27], ovarian cancer[28]
Ursolic acid	Terpenoids	*Rosmarinus officinalis* and *Salvia officinalis*	Colorectal cancer[29], melanoma[30], lung cancer[31], bladder cancer[32]
β-elemene	Terpenoids	*Curcuma wenyujin, Kwangsiensis, and Curcuma phaeocaulis*	Breast cancer[33], lung cancer[34], colorectal cancer[35], gastric cancer[36]
Quercetin	Polyphenols (flavonoids)	Onions, apples, and broccoli	Lung cancer[37], breast cancer[38], ovarian cancer[39], colorectal cancer[40]
Curcumin	Polyphenols (flavonoids)	*Curcuma longa*	Colorectal cancer[41], lung cancer[42], pancreatic cancer[43]
Luteolin	Polyphenols (flavonoids)	*Chrysanthemum morifolium, Perilla frutescens, and Lonicera japonica*	Breast cancer[44], lung cancer[45], gastric cancer[46]
Puerarin	Polyphenols (flavonoids)	*Pueraria lobata*	Lung cancer[47], ovarian cancer[48], colorectal cancer[49]
Apigenin	Polyphenols (flavonoids)	*Centaurea caerulea, Apium graveolens L*	Breast cancer[50], ovarian cancer[51], gastric cancer[52]
Isoliquiritigenin	Polyphenols (flavonoids)	*Glycyrrhiza glabra and Glycyrrhiza uralensis*	Breast cancer[53], gastric cancer[54], colorectal cancer[55]
Epigallocatechin gallate	Polyphenols (flavonoids)	*Camellia sinensis*	Breast cancer[56], prostate cancer[57]
Kaempferol	Polyphenols (flavonoids)	*Gossypium herbaceum, Kaempferia galanga L*	Lung cancer[58], colorectal cancer[59]
Gallic acid	Polyphenols (Phenolic Acids)	*Rheum palmatum L, Eucalyptus robusta*	Breast cancer[60], prostate cancer[61], colorectal cancer[62]
Resveratrol	Polyphenols (phytoalexin)	*Veratrum album*	Colorectal cancer[63], breast cancer[64], prostate cancer[65]
β-Lapachone	Quinones	*Tabebuia species*	Breast cancer[66], colorectal cancer[67], pancreatic cancer[68]
Shikonin	Quinones	*Lithospermum erythrorhizon*	Breast cancer[69], lung cancer[70], colorectal cancer[71]
Dicoumarol	Quinones	*Melilotus officinalis*	Colorectal cancer[72], ovarian cancer[73], leukemia[74]
Naphthoquinones	Quinones	*Taxus, Cinnamomum*	Breast cancer, liver cancer[75]
Piperine	Alkaloids	*Piper nigrum, Piper longum*	Breast cancer[76], colorectal cancer[77], ovarian cancer[78], gastric cancer[79]
Evodiamine	Alkaloids	*Evodia rutaecarpa*	Lung cancer[80], gastric cancer[81], glioblastoma[82]
Berberine	Alkaloids	*Coptis chinensis, Berberis vulgaris*	Colorectal cancer[83], gastric cancer[84], cervical cancer[85]
Camptothecin	Alkaloids	*Camptotheca acuminata*	Colorectal cancer[86], ovarian cancer[87]

cisplatin-induced apoptosis in lung cancer cells by selectively inhibiting NER activity[101]. Similarly, triptolide modulates several critical pathways to exert therapeutic activity in ovarian cancer. For example, triptolide inhibits the growth of epithelial ovarian cancer (EOC) cells by targeting the Hedgehog signaling pathway[102]. Triptolide also inhibits the migration and invasion of ovarian cancer cells, suggesting its potential to reduce metastasis. This activity is partly due to an ability to suppress the activity of matrix metalloproteinases (MMPs), such as MMP-2 and MMP-9, which have key roles in cancer cell invasion and extracellular matrix degradation[103].

However, like paclitaxel, the clinical development of triptolide has faced significant challenges. Specifically, poor solubility, a narrow therapeutic range, and potential toxicity have limited the direct clinical application of triptolide. To address these issues, researchers have developed water-soluble prodrugs, including Minnelide, which exhibit enhanced bioavailability and reduced toxicity[104]. Preclinical and early-phase clinical trials have shown promising results, especially in pancreatic and liver cancers, with significant tumor growth inhibition and improved survival rates. Recent studies have focused on improving the delivery and efficacy of triptolide through nanoparticle formulations and combination therapies[105]. These efforts aim to overcome the limitations of triptolide and expand the utility of triptolide as an NP-based anticancer agent. The unique mechanisms of action and broad-spectrum anticancer activity continue to position triptolide as a valuable addition to the arsenal of natural compounds in oncology.

Artemisinin

Artemisinin is a sesquiterpene lactone derived from the plant, *Artemisia annua*[106]. The isolation of artemisinin in the 1970s by Tu and her team marked a groundbreaking discovery in the field of antimalarial therapeutics[107]. While use of artemisinin as a drug is hampered by limited bioavailability and rapid metabolism, preclinical and clinical studies have demonstrated the efficacy of artemisinin against various cancers, including leukemia, and lung, breast, and liver cancers[24,108-110]. Artemisinin and related compounds have been shown in preclinical studies to induce cancer cell death through a variety of mechanisms that involve oxidative stress, DNA damage, cell cycle arrest, and apoptosis[111]. Chen et al.[112] sensitizes cancer cells to ferroptosis by regulating iron homeostasis. In addition to modulating estrogen receptor activity, artemisinin has been shown to enhance the antitumor immune response in breast cancer. Artemisinin treatment induces apoptosis in 4T1 breast cancer cells and decreases levels of transforming growth factor-beta (TGF-β) *in vitro*. *In vivo* studies have further revealed that artemisinin impedes tumor growth in 4T1 tumor-bearing mice, which prolongs survival[24]. Furthermore, artemisinin inhibits breast cancer-induced osteolysis by suppressing osteoclast formation and breast cancer cell proliferation[113]. Artemisinin has been identified as a potent inhibitor of microsomal prostaglandin E synthase-2, an enzyme involved in the inflammatory response associated with colorectal cancer progression[114]. Furthermore, artemisinin has been investigated in combination with other natural compounds to enhance anticancer efficacy. Specifically, a study demonstrated that niosomal nanoparticles co-loaded with artemisinin and curcumin exhibit enhanced anticancer effects against human colon cancer cells[115].

Betulinic acid

Betulinic acid, a pentacyclic triterpenoid derived from the bark of birch trees (*Betula* spp.), was initially recognized for antiviral and anti-inflammatory properties but was later identified as a promising anticancer agent[116]. Betulinic acid has been shown to trigger cell cycle arrest and apoptosis in breast cancer cells *via* mitochondrial mechanisms and regulates the p53/p21 signaling cascade[117]. Additionally, betulinic acid has been shown to inhibit the expression of CD81, a protein associated with a poor prognosis in breast cancer[118].

Betulinic acid inhibits the NF-κB/VASP pathway in gastric cancer, which is involved in cell proliferation and migration[27]. Additionally, betulinic acid enhances the expression of ZBTB10, a transcription factor that represses transcription of several members of the PI3K/AKT signaling pathway an thereby reduces tumor progression[119]. Betulinic acid has been reported to significantly reduce the viability of SKOV3 ovarian cancer cells, induce apoptosis, and impair migration and invasion in tumor cells[120]. Additionally, a sequential regimen of betulinic acid followed by 5-fluorouracil has demonstrated enhanced cytotoxic effects in ovarian cancer cells, suggesting that betulinic acid could be used in combination with other chemotherapeutic drugs to enhance treatment efficacy[121].

Further research has also highlighted the potential of betulinic acid derivatives, especially an analogue known as 2c, in enhancing anticancer efficacy through targeted nanotherapy. Aptamer-conjugated nanoparticles (Apt-2cNPs) demonstrated enhanced bioavailability, tumor specificity, and anti-tumorigenic immune responses in colorectal cancer models, showing promise for reducing off-target toxicity and improving

therapeutic outcomes[122]. These findings underscore the significance of betulinic acid as an NP in cancer treatment by showing the versatility and potential of betulinic acid when engineered into advanced therapeutic systems.

Ursolic acid (UA)

UA, a pentacyclic triterpenoid compound extracted from plants, including *Rosmarinus officinalis* and *Salvia officinalis*, has gained attention as a promising NP for cancer therapy due to potent anti-inflammatory, antioxidant, and anticancer properties[123].

UA has been shown to inhibit cell proliferation and induce apoptosis through multiple mechanisms in colorectal cancer. For example, UA inhibits the growth of colorectal cancer cells by downregulating TGF-β levels through the action of miR-140-5p[124]. Additionally, UA and its natural derivative corosolic acid can upregulate the expression of adenomatous polyposis coli (APC) protein, promoting β-catenin degradation and inhibiting the Wnt/β-catenin pathway to suppress colon cancer cell growth and metastssis[125]. UA exhibits potent anticancer effects in lung cancer by suppressing vaccinia-related kinase 1 (VRK1) expression. The combination of UA and doxorubicin results in a more significant reduction in cell viability compared to either compound alone, indicating a synergistic effect[126]. UA induces apoptosis in bladder cancer by inhibiting the Akt/NF-κB pathway, reducing anti-apoptotic proteins, and enhancing caspase-3 expression, while decreasing cell proliferation in a dose-dependent manner[127].

However, UA is poorly soluble in water and use of UA is limited by other pharmacokinetic properties, including low oral bioavailability and weak membrane permeability[128]. To address these challenges, researchers have explored various strategies to improve the bioavailability and pharmacokinetic properties of UA. One approach involves the development of alternative UA-based formulations, such as nanoparticles, liposomes, or micelles, which can enhance solubility and increase the ability to reach tumor sites[129]. Additionally, prodrug strategies have been investigated to improve oral bioavailability and facilitate more effective systemic delivery[130]. Another promising method is the combination of UA with other compounds that may enhance absorption and therapeutic efficacy. For example, co-administration with agents (e.g., piperine) has been shown to improve the bioavailability of various compounds and could increase the therapeutic potential of UA[131].

β-*Elemene*

Elemene, a naturally occurring terpenoid compound, has been shown to exhibit broad-spectrum antitumor properties and low toxicity. Elemene is extracted primarily from traditional Chinese medicinal plants, such as *Curcuma wenyujin*, *C. kwangsiensis*, and *C. phaeocaulis*. Elemene (especially the active component, β-elemene) has been extensively studied for its potential in cancer therapy[132].

β-elemene has been reported to exhibit anticancer properties in lung cancer. For example, β-elemene activates transcription factor EB (TFEB), which enhances lysosomal degradation of GPX4 and promotes ferroptosis in NSCLC cells[34]. Additionally, β-elemene inhibits lung cancer cell growth by inactivating STAT-3, which leads to a reciprocal interaction between miRNA155-5p and FOXO3a, ultimately increasing IGFBP1 expression[133]. In addition to lung cancer, β-elemene has also been shown to enhance the sensitivity of KRAS-mutant colorectal cancer cells to cetuximab by promoting ferroptosis and suppressing epithelial-mesenchymal transition (EMT)[35]. In another study β-elemene was shown to induce apoptosis and autophagy by regulating the ROS/AMPK/mTOR pathway in colorectal cancer cells[134]. β-elemene has also shown promising effects in breast cancer. Specifically, β-elemene suppresses the migration and invasion of breast cancer cells by modulating the structure and function of PKM-2[33]. β-elemene promotes ferroptosis and reverses radio resistance in gastric cancer by inhibiting the interaction between OTUB1 and GPX4, thus reducing the GPX4 level, a key antioxidant enzyme, and enhancing the effectiveness of radiotherapy[36]. In practice, a β-elemene formulation (Elemene Emulsion) has been approved by the China State Food and Drug Administration. Elemene Emulsion is currently used as an adjuvant therapy to enhance anticancer efficacy and minimize side effects of chemotherapeutic agents[135].

Polyphenols

Polyphenols are widely present in plant-based foods and beverages such as fruits, vegetables, spices, soy, nuts, tea and wine. Polyphenols have gained attention as potential therapeutic agents for cancer prevention and treatment due to the antioxidant, anti-inflammatory, and anticancer properties[136]. Polyphenols are classified into several categories, including flavonoids, phenolic acids, stilbenes, and lignans. Among these categories, flavonoids [such as quercetin, epigallocatechin

gallate (EGCG), and curcumin] and phenolic acids (such as ellagic acid and chlorogenic acid) have been extensively studied for anticancer activities.

Quercetin

Quercetin, a naturally occurring flavonoid compound, has gained widespread recognition for multi-target anticancer properties and a favorable safety profile. As a prominent dietary polyphenol, it is widely distributed in fruits and vegetables like apples, onions, and broccoli, and has demonstrated significant efficacy in both preclinical and clinical cancer research[137,138].

Quercetin has been shown to directly bind to SIRT5, a mitochondrial NAD^+-dependent lysine deacylase, in NSCLC and inhibit PI3K kinase activity by blocking the interaction between SIRT5 and PI3K[37]. Quercetin inhibits cell proliferation and survival by targeting the AKT/mTOR/PTEN signaling pathway in breast cancer cells[139]. Additionally, wurtzite-type zinc oxide (ZnO) nanoparticles functionalized with quercetin (ZnO@Quercetin) have shown significant cytotoxic effects against ovarian cancer cells by generating oxidative stress and depolarizing mitochondrial membranes, suggesting that quercetin-loaded nanomaterials could be a promising strategy for cancer treatment[140]. Quercetin has also been used to enhance the activity of other anticancer agents in colorectal cancer. Quercetin achieved synergistic chemo-immunotherapy effects when co-delivered with ginsenoside Rg3 *via* a folate-targeted PEG-modified cyclodextrin nanoparticle, prolonging blood circulation and enhancing tumor targeting of Rg3 and improving survival in a mouse model when combined with anti-PD-L1 therapy[40].

Curcumin

Curcumin is a bioactive polyphenol naturally derived from the underground stems of *Curcuma longa* (turmeric) that exhibits diverse biological activities and has garnered strong attention in cancer treatment[141]. Curcumin exhibits potent anti-inflammatory, antioxidant, and anticancer properties, which positions curcumin as a promising candidate for preventive and therapeutic applications in oncology[142].

A prospective observational study involving colorectal cancer showed that combining curcumin with bevacizumab and folinic acid, fluorouracil, and irinotecan (FOLFIRI) resulted in similar long-term survival outcomes and manageable toxicity profiles in patients (NCT02439385)[143]. Additionally, a phase II clinical trial showed that a 4-g daily dose of curcumin significantly reduced aberrant crypt foci (ACF), which are precursors to colorectal cancer, by 40%. This finding suggested a potential chemopreventive effect[144]. Preclinical studies further demonstrated that curcumin inhibits colon cancer cell growth *via* inducing cell cycle arrest at the G2/M and G1 phases, activating apoptosis through intrinsic and extrinsic pathways, and promoting autophagy[41,145]. In addition to potential benefits in colorectal cancer, curcumin has demonstrated promising anticancer effects in lung cancer. Curcumin has been shown to induce apoptosis in NSCLC cells by upregulating BAX and BAD, leading to caspase activation and programmed cell death[146]. Curcumin has demonstrated potential anticancer effects in both preclinical and clinical studies involving pancreatic cancer. A phase II clinical trial that enrolled 25 patients with advanced pancreatic cancer evaluated the efficacy of oral curcumin and showed that curcumin was well-tolerated. Two patients exhibited clinical activity. Even though circulating curcumin was detectable, curcumin was present at low levels, which suggested limited oral bioavailability[147].

The poor solubility and bioavailability of curcumin pose significant challenges to a broader application as a therapeutic agent. In a phase II randomized, double-blind trial that evaluated the efficacy of adding curcumin to preoperative chemoradiation therapy for patients with locally advanced rectal cancer, there was no increase in the pathologic complete response rate, emphasizing the challenges associated with curcumin bioavailability[148]. To overcome these limitations, researchers have developed advanced drug delivery systems, such as liposomes and nanoparticles. A nanoparticle formulation of curcumin administered *via* several different routes reduced tumor volume *in vivo*, suggesting enhanced therapeutic efficacy[149]. Nanoliposomes co-loaded with curcumin and 5-FU and decorated with arginine-glycine-aspartic acid (RGD) peptide have been applied for enhanced synergistic chemotherapy in breast cancer[150]. A similar innovative approach utilizing RGD-modified nanoliposomes for the co-delivery of arsenic trioxide and curcumin in prostate cancer showed promising results because RGD-modified nanoliposomes significantly increase apoptosis and reduce EGFR expression in cancer cells[151].

Luteolin

Luteolin is a naturally occurring flavonoid found in vegetables, fruits, and medicinal herbs, including *Chrysanthemum morifolium*, *Perilla frutescens*, and *Lonicera japonica*, exhibits multiple biological activities, including antioxidant, anti-inflammatory, and anticancer effects, underscoring the potential of luteolin as an agent for cancer prevention and therapy[152].

Luteolin has been shown to suppress metastasis by reversing EMT through downregulation of β-catenin levels in breast cancer[153]. Additionally, luteolin has been reported to enhance the efficacy of chemotherapeutic agents. Luteolin-loaded phytosomes potentially sensitize human breast carcinoma cells to doxorubicin through inhibition of nuclear factor erythroid 2–related factor 2 (Nrf2)[154]. Luteolin significantly curbs cell growth and promotes programmed cell death in breast cancer models resistant to tamoxifen expressing estrogen receptors. Luteolin halts the cell cycle at the G2/M checkpoint, lowers mitochondrial potential, and diminishes the active components of the PI3K/AKT/mTOR cascade. These results imply that luteolin may have a key role in counteracting resistance to hormone-based treatments[155]. Similarly, luteolin has been shown to sensitize cancer cells to chemotherapeutic agents in lung cancer. Luteolin has been shown to enhance the anticancer effect of cisplatin in lung cancer cells by modulating the c-Jun NH2-terminal kinase (JNK) pathway, leading to increased apoptosis[156].

Puerarin

Puerarin is a prominent isoflavonoid derived from the root of *Pueraria lobata* (kudzu). Extensive research has been conducted to explore the efficacy of puerarin against various cancer types[157].

Notably, such research has demonstrated the efficacy of puerarin in inhibiting the growth of NSCLC cells. *In vitro* studies have shown that puerarin significantly suppresses NSCLC cell proliferation by inducing apoptosis and modulating key signaling pathways[158]. Additionally, puerarin suppresses tumor metastasis by inhibiting EMT, downregulating MMP-9 expression, and reducing phosphorylation of extracellular signal-regulated kinase (ERK)[159]. *In vivo* experiments using NSCLC xenograft models have confirmed that puerarin treatment leads to significant tumor growth inhibition, in part by modulating the tumor microenvironment and suppressing M2 macrophage polarization through inactivation of the MEK/ERK 1/2 pathway[47]. Puerarin has been investigated for its effects on ovarian cancer. Specifically, puerarin has been shown to inhibit cell growth in ovarian cancer by increasing apoptosis[48]. Moreover, puerarin regulates the expression of some microRNAs, such as miR-490, which has a crucial role in inhibiting lung cancer cell invasion and migration[160]. Puerarin has inhibitory effects on bladder cancer cells *via* apoptosis induction through various mechanisms, including modulation of signaling pathways[161]. While these findings highlight the potential of puerarin in cancer treatment, most studies are in the preclinical stage. Additional clinical investigations are essential to validate the safety profile and therapeutic effectiveness in human patients.

Other polyphenols

Apigenin (4′,5,7-trihydroxyflavone) is a lipophilic flavonoid abundant in various plant sources[162]. Apigenin has been shown to suppress the growth, movement, and spread of breast cancer cells by targeting molecular pathways, such as p38-MAPK, NF-κB, and JAK/STAT[163,164]. Sudhakaran et al.[165] reported that apigenin modifies alternative splicing in triple-negative breast cancer (TNBC) by interacting with splicing regulators, such as hnRNPA2. This interaction converts cancer-specific isoforms into forms typical of normal tissues, triggering cell death and curtailing tumor progression. Of note, a recent study developed and optimized apigenin-loaded polymer-lipid hybrid nanoparticles (AGN-PLHNPs) to enhance the therapeutic efficacy of apigenin, marking a significant step forward in the potential treatment of breast cancer[166]. In addition to the potential uses of apigenin in breast cancer, apigenin has been shown to reverse multi-drug resistance in Taxol-resistant ovarian cancer cells by targeting AXL and TYRO-3 receptor tyrosine kinases[51]. Apigenin induced endoplasmic reticulum stress and promote autophagic cell death in gastric cancer by suppressing HIF-1α and EZH-2, especially under low-oxygen conditions[167].

Isoliquiritigenin (ISL) is a natural flavonoid compound with diverse biological activities. ISL is primarily derived from the roots of *Glycyrrhiza* spp., such as *G. glabra* and *G. uralensis*. ISL has garnered significant attention due to its diverse biological activities, including antioxidant, anti-inflammatory, and anticancer properties[168]. ISL exerts several promising effects in breast cancer. For example, ISL downregulates miR-21 expression by inhibiting the STAT3 signaling pathway, leading to decreased tumor progression[53]. ISL has also been shown to exhibit significant antitumor effects in gastric cancer. ISL disrupts glucose metabolism by inhibiting GLUT4, leading to energy metabolic collapse through activation of PDHK1/PGC-1α[54]. ISL also reduces gastric cancer cell stemness by inhibiting GRP78-mediated stemness, regulating the tumor microenvironment, and enhancing chemosensitivity[169]. ISL promotes apoptosis and inhibits cell proliferation in colorectal cancer by upregulating p62/SQSTM1, leading to caspase-8 activation and enhanced apoptotic cell death[170]. ISL exerts antitumor effects in hepatocellular carcinoma *in vitro* and *in*

vivo by inducing apoptosis and autophagy through downregulation of the PI3K/AKT/mTOR pathway[171].

Epigallocatechin gallate (EGCG), a predominant catechin in green tea, is renowned for potent antioxidant and anti-inflammatory properties[172,173]. EGCG promotes autophagic cell death in breast cancer cells by mediating assembly of the ESCRT-III complex[174]. EGCG also modulates the Wnt/β-catenin pathway, which prevents the accumulation and nuclear translocation of β-catenin, thereby inhibiting cancer cell growth[175]. EGCG can disrupt the androgen receptor (AR) signaling pathway in prostate cancer cells. EGCG suppresses the growth of prostate cancer cells, which are often androgen-dependent, by binding to the AR and inhibiting nuclear translocation and transcriptional activity[176]. EGCG has been shown to reduce expression of the histamine receptor, H1R, to inhibit the growth and spread of oral cancer cells[177].

Kaempferol, a flavonoid found in various plants, inhibits TGF-β1-induced EMT in lung cancer cells by restoring E-cadherin expression and suppressing mesenchymal markers[58]. Additionally, kaempferol has the ability to reverse resistance to chemotherapy drugs. Kaempferol has the ability to induce apoptosis and cause cell cycle arrest in 5-FU-resistant colorectal cancer cells, effectively overturning 5-FU resistance[178]. Moreover, kaempferol exhibits significant effects in various cancer types, including hepatocellular carcinoma, where kaempferol promotes G2/M cell cycle arrest and enhances apoptosis by regulating the ATM/CHEK2/KNL1 pathway[179]. Kaempferol in combination with verapamil, enhances ROS production and disrupts lysosomal biogenesis in breast cancer, sensitizing cancer cells to chemotherapy[180].

Gallic acid, a trihydroxybenzoic acid, has been reported to exhibit anticancer effects in various cancer types[181]. Gallic acid induces G2/M phase arrest and enhances the therapeutic effects of chemotherapeutic agents, like camptothecin, in breast cancer[182,183]. Gallic acid has been shown to interact with G-quadruplexes in colon cancer, which are non-canonical DNA secondary structures involved in gene regulation[62]. Gallic acid exerts cytotoxic effects on DU145 prostate cancer cells by generating ROS and inducing mitochondria-mediated apoptosis[184].

Resveratrol, a natural phytoalexin, exerts anticancer effects through multiple mechanisms, including inhibition of cell proliferation, induction of apoptosis, modulation of angiogenesis, and suppression of metastasis[185,186]. Resveratrol promotes sensitization to doxorubicin by inhibiting EMT and modulating the SIRT1/β-catenin signaling pathway in breast cancer[187]. Resveratrol reduces cell viability and inhibits invasiveness in prostate cancer by downregulating oncogenic microRNA miR-21 and increasing tumor suppressors (PDCD4 and maspin)[188]. In addition, resveratrol-loaded nanoparticles have also demonstrated remarkable anti-cancer activity in hormone-sensitive and -insensitive prostate cancer models, suggesting a promising approach for improving therapeutic efficacy[189].

Quinones

Quinones represent a varied class of natural compounds found abundantly in various plants, fungi, and microorganisms. Quinones are characterized by a six-membered ring with two carbonyl groups. Quinones have crucial roles in biological processes, including electron transport and redox reactions[190]. The potent anticancer properties of quinones have been attributed to an ability to induce cytotoxicity, inhibit cell proliferation, and modulate various cellular pathways associated with cancer progression[88].

β-*Lapachone*

β-lapachone is a naturally occurring naphthoquinone compound derived from *Tabebuia* spp., which are commonly known as lapacho trees. Potent anticancer activity of β-lapachone has been demonstrated in various types of cancer. The anticancer effects of β-lapachone have primarily been attributed to an ability to induce DNA damage, generate ROS, and trigger apoptosis in cancer cells[66], with particularly strong effects in breast cancer. β-lapachone has been shown to selectively target cancer cells with high levels of NADPH-quinone oxidoreductase 1 (NQO1), an enzyme frequently overexpressed in breast cancer cells[191].

β-lapachone triggers ferroptosis, a regulated cell death process marked by iron-dependent lipid peroxidation, in colorectal cancer. Specifically, β-lapachone activates the JNK pathway, which in turn induces NCOA4-mediated ferritinophagy (the selective autophagic degradation of ferritin) and ferroptosis in colorectal cancer cells[192]. Additionally, the combination of β-lapachone with polyphenols from *A. annua L.* has been shown to enhance the anticancer effects of oxaliplatin in functionally oxaliplatin-resistant colorectal cancer cells. This combination therapy increases the sensitivity of cancer cells to treatment and reduces the potential for toxicity associated with higher doses of individual drugs[193]. A recent study revealed that knocking down the BER scaffold protein, XRCC1,

increased the sensitivity of pancreatic ductal adenocarcinoma (PDA) cells to β-lapachone[194]. Additionally, researchers have developed an albumin-binding β-lapachone prodrug that targets KRAS-mutant pancreatic ductal adenocarcinoma. This prodrug leverages the enhanced macropinocytosis of KRAS-mutant cells and the low expression of the neonatal Fc receptor to selectively deliver the drug to tumor cells[68].

Shikonin

Shikonin is a naturally occurring quinone extracted from the roots of *Lithospermum erythrorhizon*, a plant commonly used in traditional Chinese medicine, that exhibits a broad range of pharmacologic activities, including potent anticancer properties[195]. Shikonin has shown promising antitumor effects that occur through multiple pathways, such as induction of apoptosis, cell cycle arrest, inhibition of metastasis, and suppression of angiogenesis[196].

Shikonin has been shown to be a novel and selective IMPDH2 inhibitor, targeting TNBC and suppressing TNBC growth[197]. Shikonin induces apoptosis and autophagy in colorectal cancer by targeting galectin-1 and activating the JNK signaling pathway[198]. Shikonin suppresses small cell lung cancer (SCLC) by inducing ferroptosis *via* ATF3 upregulation. Shikonin increases ROS, reduces GPX4, and inhibits tumor growth by blocking c-myc-mediated HDAC1 recruitment[199]. These findings highlight the broad-spectrum anticancer effects of shikonin and the potential of shikonin as a therapeutic agent across various cancer types.

Dicoumarol

Dicoumarol, a naturally occurring anticoagulant derived from moldy sweet clover, has been investigated for potential anticancer properties in various cancer types. The mechanisms underlying dicoumarol action include inhibition of NQO1, modulation of mitochondrial function, and induction of apoptosis[200].

Dicoumarol suppresses the oncogenic capacities mediated by high mobility group protein AT-hook 2 (HMGA2) in colorectal cancer and inhibits cell proliferation by inducing apoptosis[72]. Dicoumarol inhibits pyruvate dehydrogenase kinase 1 (PDK1), a key enzyme involved in the Warburg effect, in ovarian cancer. The Warburg effect is a metabolic alteration commonly observed in cancer cells. This inhibition shifts glucose metabolism from aerobic glycolysis to oxidative phosphorylation, which generates higher levels of ROS, attenuates mitochondrial membrane potential, induces apoptosis, and reduces cell viability[73]. Dicoumarol inhibits mitochondrial oxidative phosphorylation complexes, stimulates superoxide anion release, and inhibits pyrimidine biosynthesis in human myeloid leukemia HL-60 cells, leading to cell cycle arrest in the S phase[74].

Naphthoquinones

Naphthoquinones, a class of natural compounds characterized by a naphthalene ring with two carbonyl groups, are mainly derived from plants of the *Taxus* (yew) and *Cinnamomum* genera. As a prominent dietary polyphenol, it is found in many fruits and vegetables, including apples, onions, and broccoli, and has shown efficacy in preclinical and clinical cancer research[201].

Recent studies have identified specific naphthoquinone derivatives that exhibit selective cytotoxicity against TNBC cells. For example, the 1,4-naphthoquinone derivative, ZSW-4B, has been shown to induce apoptosis in TNBC cells by specifically activating the AMPK signaling pathway[202]. Another study reported the development of 2-amino-1,4-naphthoquinone derivatives as potent aromatase inhibitors, which also had significant anticancer activity. These derivatives had nanomolar inhibitory activity against aromatase, a key enzyme in estrogen synthesis, indicating the potential use in estrogen-dependent cancers[203]. Naphthoquinones have also demonstrated significant anticancer activity in other cancers. For example, specific naphthoquinone derivatives have been shown to inhibit the proliferation of liver cancer cells by targeting the signaling pathway involving MAPK, AKT, and STAT3[75]. Naphthoquinones induce cell cycle arrest and apoptosis in melanoma cells by modulating the expression of key cell cycle regulators[204].

Alkaloids

Alkaloids are a diverse group of naturally occurring organic compounds that have significant therapeutic potential in cancer treatment. These nitrogen-based compounds, mainly sourced from plants, display diverse biological effects that contribute to cancer drug development[205].

The use of alkaloids in medicine dates back centuries with opium (containing morphine) being one of the earliest known examples. However, the specific role of alkaloids in cancer treatment was not fully recognized until more recent times. The 19th century saw advances in the scientific understanding of alkaloids with the isolation of quinine from cinchona bark being a notable example[206]. The identification of vincristine and vinblastine from *Catharanthus roseus* in the 1950s

represented a major breakthrough in cancer treatment. These alkaloids have shown remarkable efficacy in treating leukemias and lymphomas by inhibiting cellular proliferation[207]. This success spurred further exploration of other alkaloids with potential anticancer properties, revealing a broader potential for natural alkaloids in oncology.

Piperine

Piperine is a natural alkaloid that is mainly present in black (*Piper nigrum*) and long pepper (*P. longum*). Piperine is the compound responsible for the pungency of black pepper and has been traditionally used in medicine for anti-inflammatory, antioxidant, and thermogenic properties[208]. The potential anticancer activity of piperine has been linked to an ability to modulate various cellular pathways involved in tumorigenesis[209].

Specifically, piperine targets breast cancer by inhibiting TNBC growth, inducing cell cycle arrest, suppressing Akt activation, and promoting apoptosis. Piperine enhances γ radiation cytotoxicity, reduces metastasis by downregulating MMP-2/-9, and significantly suppresses TNBC tumor growth *in vivo*[210]. Piperine also influences redox homeostasis, inhibits cancer stem cell self-renewal, and modulates endoplasmic reticulum stress and autophagy[211,212]. In addition to direct anticancer effects, piperine enhances the efficacy of other cancer treatments. For example, peperine sensitizes breast cancer cells to chemotherapy drugs, like doxorubicin, thereby potentially reducing the required dosage and associated toxicities[213]. Piperine also acts as a bioavailability enhancer for various chemotherapeutic agents by inhibiting P-glycoprotein (P-gp) activity, which helps reverse multidrug resistance in cancer cells[214].

Piperine and its derivatives have shown promising anticancer effects in colorectal cancer through various mechanisms. For example, a novel piperine derivative (HJJ_3_5) has been shown to inhibit colorectal cancer cell proliferation, migration, and invasion by modulating EMT signaling pathways. HJJ_3_5 suppresses key EMT-related gene expression, reducing SNAI1 levels and thereby limiting the metastatic ability of colorectal cancer cells[215]. Another piperine derivative, HJ-23, has demonstrated significant anti-tumor effects by activating the p53 pathway and inhibiting the E2F transcription factor pathway[216]. In addition to colorectal cancer, piperine has shown potential in other cancer types. For example, piperine has been shown to inhibit cell proliferation and induce apoptosis in ovarian cancer by modulating JNK/p38 MAPK signaling pathways[217]. Piperine has demonstrated the ability to suppress tumor growth in gastric cancer through a mechanism involving mitochondrial ROS[218].

Evodiamine (EVO)

EVO is a naturally occurring indoloquinazolidine alkaloid derived from *Evodia rutaecarpa*, a key herb in traditional Chinese medicine. EVO has attracted interest for its broad pharmacologic activities, including anticancer, neuroprotective, immune-modulating, and anti-inflammatory effects[219].

EVO exerts potent anticancer activity in lung cancer by targeting multiple pathways and mechanisms. For example, EVO suppresses lung cancer cells and lung cancer stem cells by interfering with the heat shock protein (HSP) system, especially HSP70, through ubiquitin-dependent protein degradation[220]. Additionally, EVO inhibits the NOTCH3 signaling pathway in NSCLC by promoting DNA methyltransferase-induced NOTCH3 methylation, thereby exerting anti-lung cancer effects[221]. EVO also exhibits potential as a targeted agent that can complement immunotherapy for treating NSCLC. It has been reported that EVO inhibits NSCLC by boosting CD8+ T cell activity and suppressing the MUC1-C/PD-L1 axis[80].

In addition to lung cancer, EVO has shown anticancer effects in other types of cancer. For example, EVO has been shown to induce apoptosis and autophagy by inducing increased expression of LC3B-II and beclin-1[81]. Similarly, EVO inhibits the proliferation of glioblastoma cells by inducing apoptosis and autophagy but this phenomenon is associated with increased cytoplasmic accumulation of calcium[82].

Berberine

Berberine is a natural plant alkaloid in several medicinal herbs, including *Coptis chinensis* (Chinese goldthread) and *Berberis vulgaris* (barberry). Berberine has been traditionally used in various cultures for antimicrobial and anti-inflammatory properties; recent research has highlighted the potential of berberine in cancer therapy[222].

Berberine has been shown to inhibit the proliferation of colon cancer cells by inhibiting the WNT/β-catenin signaling pathway[223]. Additionally, berberine inhibits the proliferation and migration of colorectal cancer cells by downregulating the expression of glucose-regulated protein 78 (GRP78)[224]. Berberine inhibits high-fat diet-associated colorectal cancer by modulating the gut microbiota-regulated

lysophosphatidylcholine (LPC) level, thus decreasing cell proliferation and ameliorating cell junction impairment in colorectal cancer cells[225]. Berberine suppresses the growth of xenograft gastric tumors by downregulating HNF4α, WNT5a, and β-catenin expression in tumor and liver tissues. This downregulation leads to reduced tumor volume and weight, thereby retarding tumor growth[226]. Berberine also enhances the antitumor effects of epidermal growth factor receptor (EGFR) inhibitors, such as erlotinib and cetuximab, including STAT3 phosphorylation[227]. Similarly, berberine improves the sensitivity of gastric cancer cells to cisplatin by enhancing autophagic cell death. Berberine inhibits gastric cancer cell growth by triggering autophagy through suppression of MAPK/mTOR/p70S6K and Akt signaling pathways[228].

Berberine has shown promise in multiple cancer types other than colon and gastric cancers. Berberine facilitates the Warburg effect by enhancing TET3-related demethylation and increasing miR-145 expression in ovarian cancer, which in turn reduces tumor cell proliferation and invasion[229]. Berberine also reduces tumor-induced angiogenesis in cervical cancer by downregulating VEGF levels and enhances the anticancer effect of radiotherapy by regulating glucose metabolism through the PI3K/HIF-1 pathway[230]. Berberine inhibits cell proliferation by interacting with aldo-keto reductase 1C3 (AKR1C3) and decreasing androgen synthesis[231].

Camptothecin (CPT)

CPT is a naturally occurring pentacyclic alkaloid initially extracted from the Chinese tree, *Camptotheca acuminata*. CPT was shown to exhibit potent antitumor effects against various cancer cell lines, such as cervical cancer and leukemia cell lines, when first studied in the late 1960s [232]. A breakthrough came in 1985 when Hsiang et al.[233] discovered that CPT exerts anticancer effects by inhibiting DNA topoisomerase I, an enzyme crucial for DNA replication and transcription. Despite potent anticancer properties, the clinical application of CPT has been limited due to poor solubility and instability under physiologic conditions. Several CPT derivatives have been developed to address these challenges, including irinotecan and topotecan. These derivatives have been approved for clinical use and have been shown to be efficacious in treating various cancers, such as colorectal and ovarian cancers[234].

Irinotecan has been utilized as a second-line treatment in colorectal cancer, especially for patients with metastatic disease. Irinotecan has shown effectiveness in improving survival rates when combined with other chemotherapeutic agents[86]. Topotecan has been used as a second- or third-line treatment in ovarian cancer, especially for patients with recurrent disease. Use of topotecan is associated with improved progression-free survival in some patient populations[87].

Beyond these applications, CPT and its derivatives are being explored in combination therapies to enhance the anticancer effects. For example, studies have investigated the use of CPT in conjunction with oncolytic measles virus therapy to improve breast cancer treatment[235]. Additionally, research is ongoing to develop CPT-based prodrug nanomedicines. These nanomedicines aim to improve the pharmacokinetic and pharmacodynamic profiles of CPT, addressing issues related to solubility and stability, and thereby enhancing the therapeutic potential in cancer treatment[236].

Application of chemical proteomics to the identification of NP anticancer targets

Chemical proteomics has emerged as a transformative approach for the systematic identification of NP molecular targets[237]. Methods, such as CCCP, use NPs covalently attached to biocompatible resins, like agarose or magnetic beads, to isolate interacting proteins. These enriched proteins can then be analyzed using high-resolution mass spectrometry and bioinformatics to map NP-protein interactions and elucidate anticancer mechanisms. Advances in labeled and label-free chemical proteomics methods have significantly enhanced the precision and scope of target identification[4,8,238].

The principles, experimental workflows, and applications of chemical proteomics in target identification were reviewed herein, highlighting the advantages and limitations. Case studies on representative NPs illustrate the utility of these methods in uncovering novel anticancer targets and pathways. Moving forward, the integration of chemical proteomics with complementary technologies and bioinformatic tools holds great promise for accelerating NP-based drug discovery, enabling the development of targeted cancer therapies with optimized efficacy and minimized side effects. We have systematically compiled representative examples of anticancer target identification of NPs using label-based and label-free methods. **Table 2** summarizes the key target proteins, techniques employed, and cancer types investigated.

Table 2 Representative examples of natural product anticancer-labeled and label-free target identification

NPs	Target protein	Method	Cancer [ref.]
Hardwickiic acid	HSP27	CCCP	Melanoma[239]
Ainsliadimer A	IKKα/β	CCCP	Gastric cancer[240]
J13	MYH9, actin	CCCP	Liver cancer[241]
Quercetin	HSP70, HSP90	Biotin-labeled probes	Leukemia[242]
Arsenic trioxide	PKM2	Biotin-labeled probes	Leukemia[243]
Cucurbitacin B	IGF2BP1	Biotin-labeled probes	Liver cancer[244]
ART1	HSD17B4	Biotin-labeled probes	Lung cancer[245]
MJE3	PGAM1	Bioorthogonal strategies	Breast cancer[246]
Withangulatin A	PHGDH	Bioorthogonal strategies	Colorectal cancer[247]
Nimbolide	RNF114	Bioorthogonal strategies	Breast cancer[248]
Pladienolide	SF3b	Photoaffinity probes	Cervical cancer[249]
Arenobufagin	ATP1A1, MDR1, PARP1	Photoaffinity probes	Liver cancer[250]
Chlorogenic acid	ACAT1	Photoaffinity probes	Melanoma, lung cancer[251]
Bruceine D	ICAT	DARTS	Liver cancer [252]
Ginsenoside CK	KEAP1	DARTS	Leukemia[253]
Ertredin	NDUFA12	DARTS	Liver cancer [254]
Rotundifuran	CCN1/Cyr61	DARTS	Cervical cancer[255]
Vioprolide A	NOP14	TPP	Leukemia[256]
Triptolide	HnRNP A2/B1	TPP	Lung cancer[257]
Lycorine	IDH1	TPP	Colorectal cancer[258]
Alantolactone	AKR1C1	TPP	Lung cancer[259]
Ainsliadimer A	PRDX1, PRDX2	DARTS, TPP	Colorectal cancer[260]
Arenobufagin	HSP90β	TRAP	Colorectal cancer[261]
Silibinin	ACSL4	TRAP	Liver cancer[262]

Label target identification for NPs

Label-based methods have a critical role in unraveling the molecular targets of NPs, providing essential tools to map NP-protein interactions. These approaches use NP-derived molecular probes, which typically consist of three key elements: a reporter (e.g., biotin, bioorthogonal tags, or photolabels); a linker; and an active NP moiety[263]. The methods were classified into two main approaches: CCCP and ABPP. **Figures 2 and 3** illustrate the historical milestones of these label-based approaches from early affinity-based capture techniques and the development of sophisticated chemical probes to modern high-throughput screening methods that integrate click chemistry and quantitative proteomics. These breakthroughs have not only deepened our understanding of small molecule-protein interactions but have also significantly accelerated the discovery and validation of novel targets of NP-derived drugs.

CCCP

CCCP involves immobilizing NPs on biocompatible resins, such as agarose or magnetic beads, to enable the selective capture of binding proteins. These proteins are then enriched and identified using affinity purification and MS. This technique permits efficient and high-throughput target protein

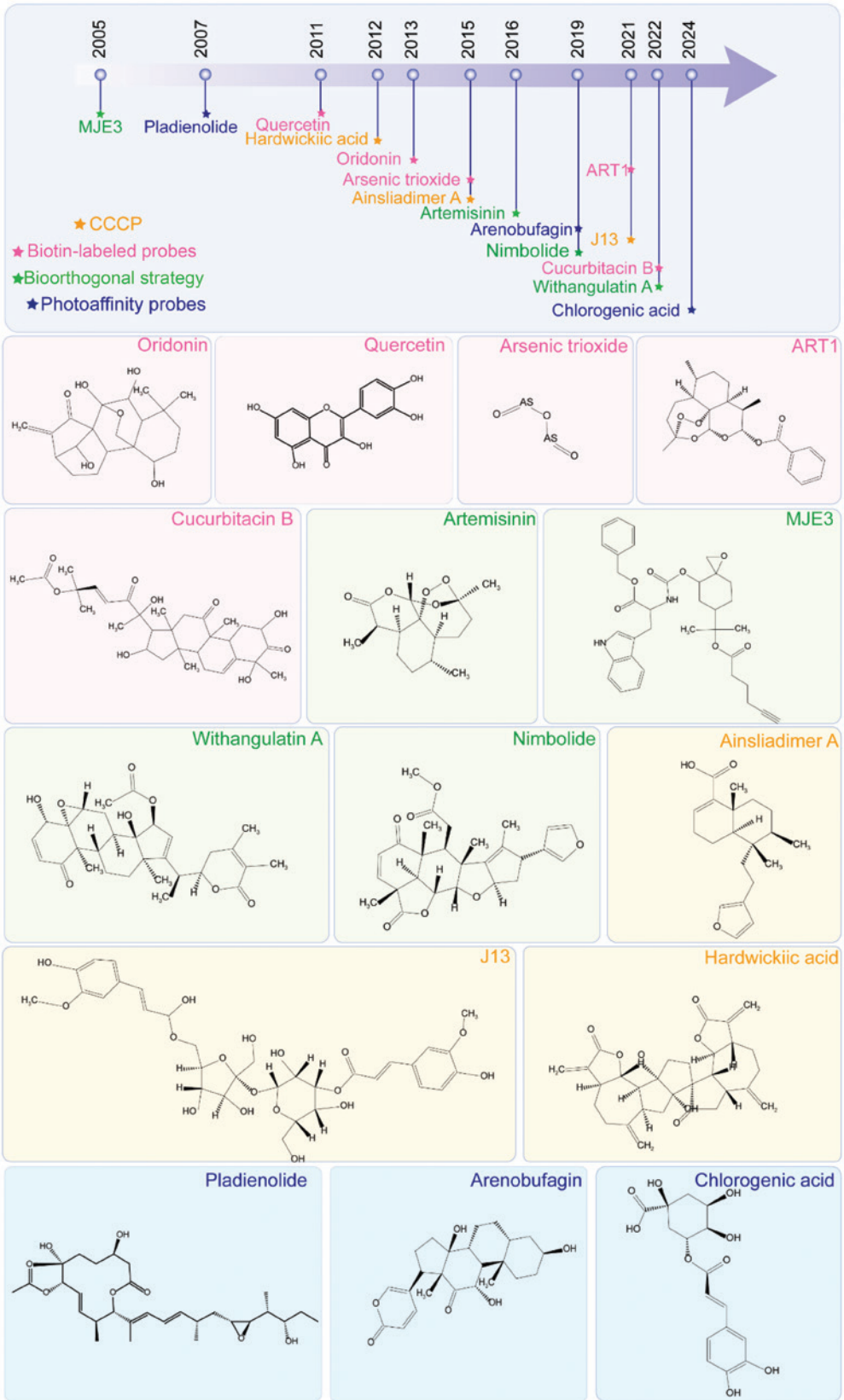


Figure 2 Continued

Figure 2 Exploring the development of natural product (NP) targets using the label target approach. This figure presents a timeline of the development of NP targets in cancer treatment from 2005 to 2024. Key NPs and technological advancements in target identification are highlighted, including the introduction of methods, such as **compound-centric chemical proteomics (CCCP)**, **biotin-labeled probes**, **bioorthogonal strategy**, and **photoaffinity probes**. The figure also shows the structural diversity of NPs, from simple molecules (e.g., quercetin) to more complex structures (e.g., artemisinin and withangulatin A). This timeline reflects the continuous progress in the exploration of NP targets for cancer therapy, serving as a historical record and a testament to the potential future directions in this field.

isolation[264]. As early as 1989, the Schreiber group utilized an affinity matrix containing the natural immunosuppressant, FK506, to conduct affinity chromatography, through which FK506-binding protein 12 (FKBP12) was identified as the relevant target[265]. Similarly, Evans et al.[246] used CCCP to identify the target of the small-molecule agent, MJE3. By immobilizing MJE3 onto a solid support and performing pull-down experiments, Evans et al.[246] identified phosphoglycerate mutase 1 (PGAM1) as the primary binding protein. Li et al.[266] employed a similar approach by immobilizing artemisinin, a derivative of the NP, on a solid support to explore its protein targets, identifying gephyrin as a key interacting protein in αTC1 cells. Faiella and colleagues immobilized hardwickiic acid (HAA) on a matrix carrier and incubated HAA with U937 cell lysates, which further illustrated the versatility of CCCP. HSP27 was shown to be the target of HAA, shedding light on the anti-cancer and stress-response modulating properties of the compound[239]. More recently, a natural-derived small-molecule (J13) has been investigated using CCCP to identify its protein targets. By immobilizing J13 onto epoxy-activated Sepharose 4B beads and performing pulldown assays, molecular motors consisting of myosin-9 and actin were identified as significant targets of the anti-cancer agent[241].

ABPP

CCCP is limited by reliance on immobilized compounds, which may have binding properties that are altered relative to the free compounds and the method is unable to directly assess protein activity. ABPP overcomes these limitations by targeting active sites and capturing functional protein states in native systems. To accomplish these goals, ABPP uses various labeling strategies. These strategies include biotin-labeled probes, which allow for efficient affinity purification, bioorthogonal tagging, which involves selective chemical reactions, and photoaffinity probes, which utilize light-activated crosslinking to capture protein targets[267,268]. Liu et al.[269] defined ABPP as a distinct approach and showcased its broad potential in 1999. Active-site-directed chemical probes are employed to label enzymes in an activity-dependent manner, coupled with visualization techniques for profiling.

Biotin-labeled probes exhibit a highly specific and strong binding affinity to streptavidin and neutravidin, making biotin one of the most robust protein-ligand interactions known. Related approaches involve allowing biotin-labeled NPs to interact comprehensively with cellular proteomes, including membrane-associated target proteins, prior to affinity purification[270]. The Crews group synthesized a biotinylated derivative of the NP parthenolide in 2001 and demonstrated the covalent interaction with cysteine 179 of IκB kinase β (IKKβ), elucidating the mechanism of anti-inflammatory activity[271]. Similarly, an O7-biotinylated quercetin derivative (BioQ) was developed as a photoaffinity proteomics probe. BioQ demonstrated quercetin-like activity, inhibiting HSP70 induction and photocrosslinking CK2 kinase, a key regulator of the heat shock response. Subsequent liquid chromatography-tandem mass spectrometry (LC-MS/MS) analyses revealed BioQ-binding proteins, such as HSP70, HSP90, ubiquitin-activating enzyme, a spliceosomal protein, RuvB-like 2 ATPases, eukaryotic translation initiation factor 3, and a mitochondrial ATPase. These findings provide insights into quercetin's anticancer mechanism by identifying proteins that have been implicated as potential anticancer targets[242]. A similar biotin-based methodology was applied in 2015 to identify arsenic-binding proteins in the context of acute promyelocytic leukemia (APL). Arsenic-binding proteins were isolated using streptavidin and identified *via* LC-MS/MS. Over 40 arsenic-binding proteins were detected with further analysis revealing that pyruvate kinase M2 (PKM2) had a strong affinity for arsenic, while glutathione S-transferase P1 (GSTP1) and heat shock 70 kDa protein 9 did not bind arsenic directly *in vitro*. These findings suggested a significant role of PKM2 in mediating the therapeutic effects of a natural-derived arsenic trioxide in APL[243]. Recently, researchers utilized biotin-labeled probes to explore the protein targets of the natural-derived small molecule, cucurbitacin B. Through this approach, IGF2BP1 was identified as a critical target, highlighting its potential role in modulating the tumor immune microenvironment[244].

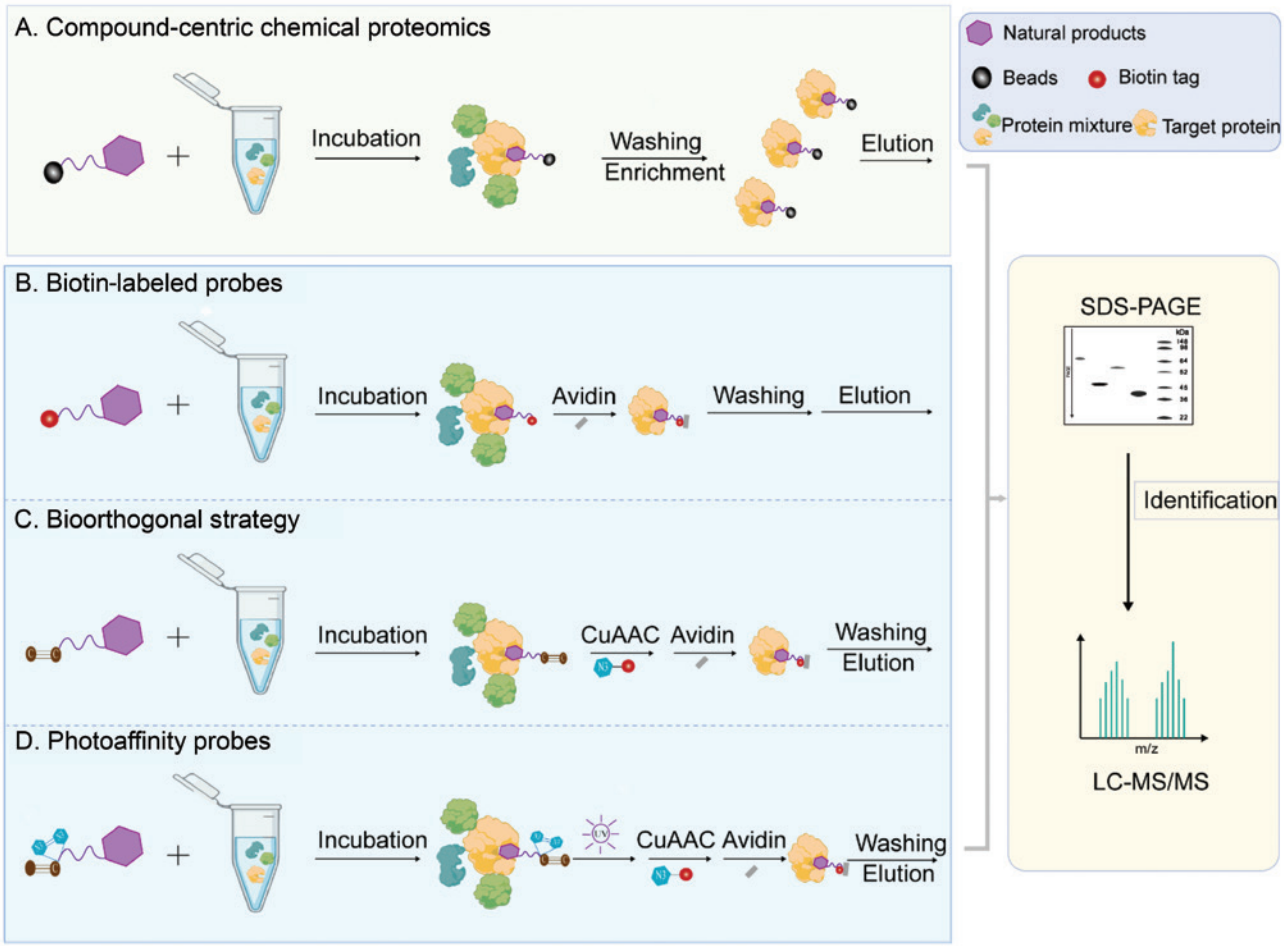


Figure 3 Labeled target identification strategies of natural products (NPs). (**A**) **Compound-centric chemical proteomics**: NPs are incubated with protein mixtures, allowing the NPs to bind to target proteins. The mixture is then washed to remove non-bound proteins and the bound proteins are eluted and identified using techniques, like mass spectrometry. (**B**) **Biotin-labeled probes**: NPs are modified with biotin tags and incubated with protein mixtures. The biotin-labeled target proteins are captured using avidin beads, washed to remove contaminants, then eluted for identification *via* sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). (**C**) **Bioorthogonal strategy**: Natural products are tagged with bioorthogonal groups that can react with specific reagents (e.g., CuAAC). After incubation with protein mixtures, the tagged target proteins are captured using avidin beads and identified through LC-MS/MS. (**D**) **Photoaffinity probes**: Natural products are equipped with photoaffinity labels. Upon incubation with protein mixtures and exposure to UV light, the probes form covalent bonds with target proteins. The protein complexes are then captured with avidin beads and identified using LC-MS/MS.

Biotin-labeled probes face several limitations, such as potential steric hindrance and the need for affinity purification, which may disrupt native protein complexes and introduce non-specific interactions. In contrast, bioorthogonal strategies leverage reactions and chemical groups that are biologically inert, ensuring compatibility with living systems and avoiding interference with endogenous biomolecules. This biocompatibility allows for safe and reliable *in vivo* applications[272]. Furthermore, bioorthogonal reactions exhibit exceptional selectivity, enabling precise labeling of target molecules in complex biological environments, while minimizing non-specific interactions. This reduces background noise and enhances the accuracy and sensitivity of experimental results, making bioorthogonal strategies a powerful tool for studying biological processes[273]. The bioorthogonal strategy was formally introduced by Bertozzi in 2003[274]. Specifically, Bertozzi and her team developed the copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction to label cell surface glycans. This technique was later extended to the identification of anticancer targets, in which the compounds were labelled with azide

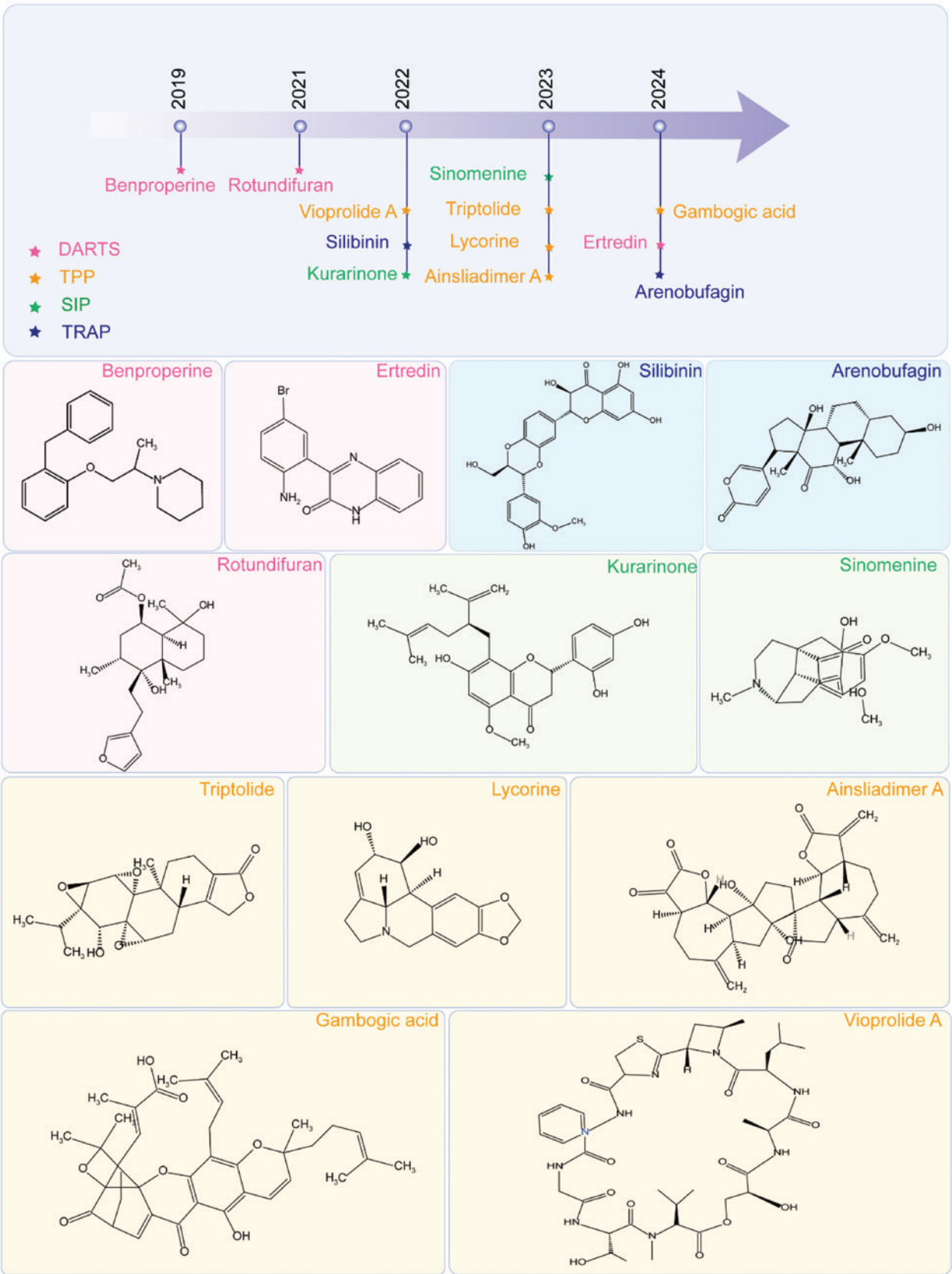


Figure 4 Continued

Figure 4 Exploring the development of natural product targets using the label-free target approach. This figure presents a timeline of the development of natural product (NP) targets in cancer treatment from 2019 to 2024 using label-free target identification strategies. These methods include **drug affinity responsive target stability** (DARTS), **thermal proteome profiling** (TPP), **solvent-induced protein precipitation** (SIP), and **translation activity profiling** (TRAP) and have allowed researchers to analyze NP-protein interactions without chemical modification or tagging. The structural diversity of these NPs is also showcased, illustrating the wide range of chemical structures that can be explored for anticancer properties.

and alkyne tags to investigate the targets within biological systems[275]. For example, Shi et al.[276] used cell-based proteomics with affinity probes in 2012 to analyze dasatinib targets. Shi et al.[276] developed DA-2, a cell-permeable kinase probe, for proteome-wide profiling. DA-2 identified Abl and Src kinases, as well as new targets (PCTK3 and STK25). Bioorthogonal methods have been applied to the exploration of NP targets. For example, Li et al.[277] aimed to identify the protein-binding partners of artemisinin and used a bioorthogonal-probe-based enrichment strategy to capture an artemisinin-binding peptide from the Plasmodium falciparum homolog of translationally controlled tumor protein. Subsequent MS analyses showed that artemisinin alkylates multiple amino acids within the N-terminal region of the target protein.

Bioorthogonal strategies can be limited due to the formation of relatively weak non-covalent interactions between the probe and the protein. Photoaffinity probes are particularly effective for studying the mechanisms underlying NP actions because photoaffinity probes can overcome this limitation. By generating photoactive intermediates under irradiation with light of a specific wavelength, photoaffinity groups form stable, irreversible covalent bonds with target proteins, which enables precise detection and capture[278]. For example, Zeng et al.[279] used affinity probes in 2021 to identify MAP2K7 as a direct target of berberine, ultimately revealing that MAP2K7 inhibition by berberine alters signaling through the JNK pathway and providing insight into the anti-inflammatory and antitumor effects of this NP. Similarly, photoaffinity probes have been used to study chlorogenic acid, a natural anticancer compound. Researchers identified acetyl-CoA acetyltransferase 1 as a primary target of chlorogenic acid and showed that the binding event impairs cancer cell proliferation by inhibiting protein activation[251].

Label-free target identification for NPs

Label-free methods represent a transformative and advantageous approach to identifying the molecular targets of NPs, offering several key benefits over traditional label-based strategies. Unlike label-based methods, which necessitate chemical modification of NPs and may thereby compromise the inherent bioactivity or interaction profiles, label-free techniques maintain the native structure and activity of NPs. This preservation is crucial for accurately studying compounds with complex or sensitive chemical properties, ensuring that the observed interactions are representative of the true biological context[280].

The most often used approaches can be broadly classified as DARTS and TPP[7,281]. Other label-free approaches, including SIP, target responsive accessibility profiling (TRAP), and tandem ion chromatography and co-elution (TICC), provide complementary insights into the interactions between NPs and NP targets[6]. **Figures 4 and 5** highlight milestones achieved in this application of these label-free strategies, from early studies that first measured ligand-induced changes in protein stability to studies that introduced the latest high-throughput, MS-based methods that capture dynamic, proteome-wide interactions under native conditions. These breakthroughs have advanced rapidly in recent years, preserving the native structure and bioactivity of NPs while offering unprecedented insights into molecular interactions and accelerating the discovery of novel therapeutic targets.

DARTS

DARTS is a label-free technique used to identify drug-protein interactions by exploiting the stabilization of target proteins upon ligand binding[281]. Proteins from biological samples, such as cell lysates or tissues, are treated with a small molecule of interest and then exposed to limited proteolysis in DARTS. Target proteins bound by the drug exhibit increased resistance to protease digestion compared to untreated controls, allowing for the discovery of potential targets in a relatively unperturbed biological context[282]. These protected fragments are subsequently analyzed using SDS-PAGE, Western blotting, or MS to identify the interacting proteins[282,283]. The proteolytic patterns that are generated in DARTS analyses enable a deeper understanding of the mechanisms underlying NP actions.

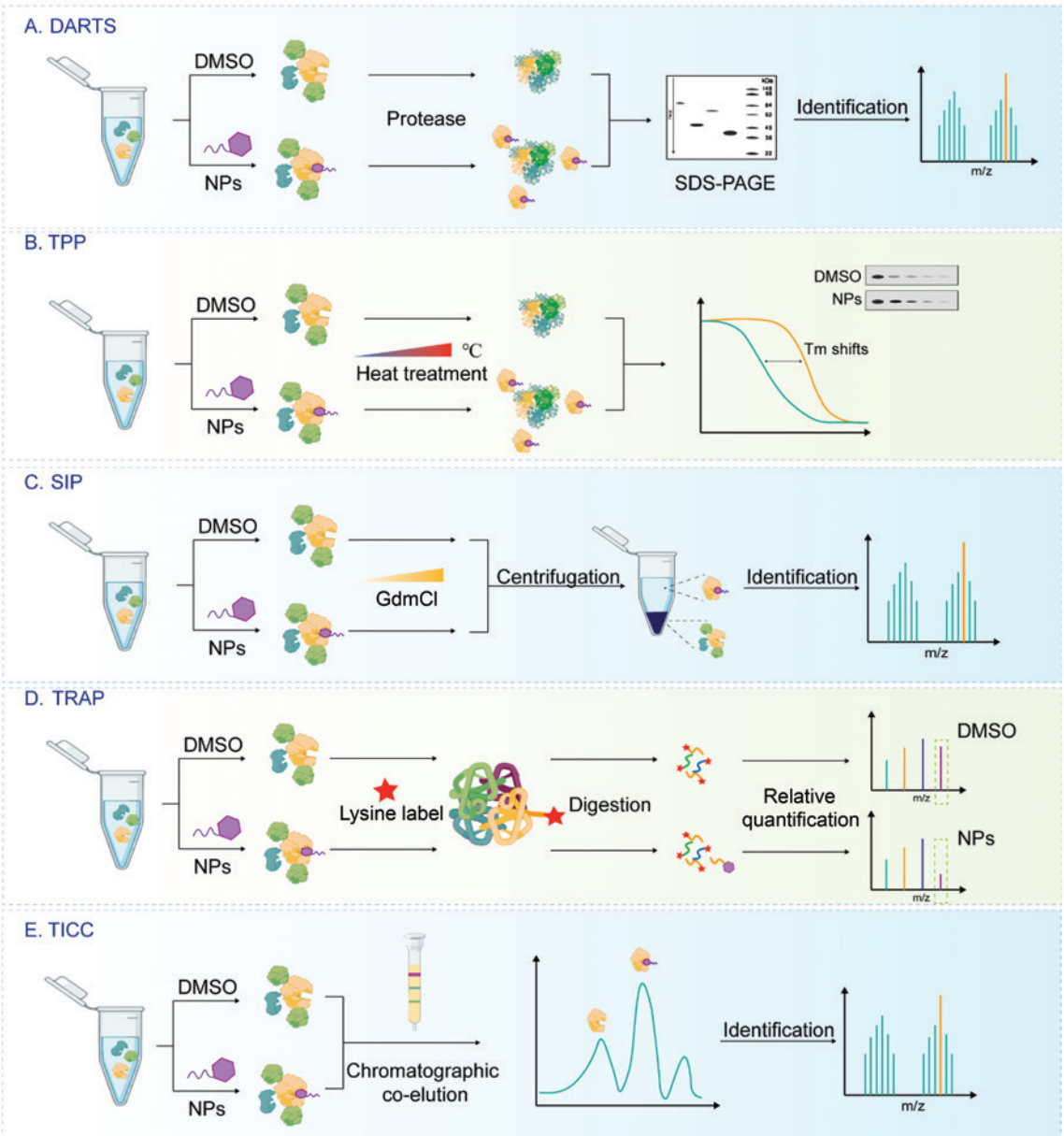


Figure 5 Label-free target identification strategies of NPs. (**A**) **Drug affinity responsive target stability** (DARTS): In this method, natural products are incubated with protein mixtures. The complexes are then treated with protease, which degrades unprotected proteins. The remaining protein fragments are analyzed *via* SDS-PAGE and LC-MS/MS to identify target proteins that have increased stability due to NP binding. (**B**) **Thermal proteome profiling** (TPP): Natural products are incubated with protein mixtures, followed by heat treatment. The thermal stability changes of proteins are monitored by SDS-PAGE and LC-MS/MS. Proteins showing altered melting temperatures are identified as potential targets of the NPs. (**C**) **Solvent-induced protein precipitation** (SIP): Natural products are incubated with protein mixtures, then a solvent (e.g., GdmCl) is added to precipitate proteins. The precipitated protein-NP complexes are separated by centrifugation and identified using LC-MS/MS. This method captures direct interactions in the native cellular environments. (**D**) **Target-responsive accessibility profiling** (TRAP): Natural products are incubated with protein mixtures and lysine residues are labeled. After digestion, the peptides are analyzed by LC-MS/MS for relative quantification. Changes in labeling patterns indicate NP-protein interactions, allowing identification of target proteins. (**E**) **Target identification by chromatographic co-elution** (TICC): Natural products are incubated with protein mixtures and the complexes are analyzed using chromatographic techniques. Co-elution patterns of proteins and NPs are monitored to identify target proteins that interact with the NPs. The identified proteins are further confirmed by LC-MS/MS.

Moreover, the simplicity of the method and adaptability to a variety of biological samples make DARTS a versatile tool for studying NP-induced modulation of cellular processes[6].

The Lomenick group first introduced DARTS in 2009 as a revolutionary technique to identify protein-ligand interactions in complex protein mixtures[282]. In 2014 researchers used DARTS to explore the molecular targets of grape seed extract, an NP with significant anticancer potential in human colorectal cancer cells. Endoplasmic reticulum stress response proteins were identified as critical targets and grape seed extract was shown to disrupt key signaling pathways, including PI3K-AKT-mTOR, through oxidative protein modifications[284]. In 2019, DARTS identified the antitussive drug benproperine as an ARPC2 inhibitor. Benproperine binds directly to ARPC2, a critical component of the ARP2/3 complex, suppressing actin polymerization and inhibiting cancer cell migration and metastasis[285]. Chen et al. utilized DARTS to identify purine-rich element binding protein A as a target of the NP 20(S)-protopanaxadiol (PPD) in 2023, showing that this agent enhances the transcription of genes involved in learning and memory[286]. More recently, Gu et al.[287] used DARTS to investigate the molecular glue, aurovertin B, identifying the ability of aurovertin B to promote neddylation and degradation of coronin-1A as mediated by tripartite motif-containing 4 in TNBC cells. This finding underscores the capability of DARTS in revealing novel mechanisms of NPs and the therapeutic promise against challenging diseases, like TNBC.

TPP

TPP is an innovative technique that combines the principles of the cellular thermal shift assay (CETSA) with quantitative MS to comprehensively analyze protein thermal stability across the proteome[288]. Like DARTS, TPP identifies interactions between proteins and small molecules, such as NPs, by assessing changes in protein stability upon ligand binding. While DARTS focuses on protease resistance, TPP measures shifts in protein thermal denaturation profiles caused by ligand binding. TPP identifies interactions between proteins and small molecules, such as NPs, by measuring changes to the extraction patterns as the temperature of a biological sample is increased. When an NP binds to a target protein, the NP often stabilizes the protein, leading to a shift in the thermal denaturation profile, which can be detected as changes to the temperature-dependent proteome. This high-throughput, label-free method enables the identification of NP targets without requiring chemical modification, providing insight into the mechanisms of action within a native cellular context. Furthermore, the high-throughput, label-free method allows for protein profiling across various biological contexts, providing a comprehensive understanding of how NPs modulate cellular proteomes[7,289].

In 2013 the Nordlund group introduced CETSA as a groundbreaking technique to detect protein-ligand interactions in cells and tissues. CETSA enables the detection of target engagement in a native biological environment by leveraging the principle that ligand binding increases the thermal stability of target proteins[290]. In 2015 Franken et al.[291] detailed the specific TPP methodology for unbiased identification of direct and indirect drug targets using multiplexed quantitative MS. Although this study did not specifically focus on NPs, the study provided a critical methodologic framework that would later be adapted for natural product research. In the years following these foundational works, both CETSA and TPP have been widely adopted for studying NP targets. For example, in 2017 Kitagawa et al.[292] demonstrated the power of these technologies in a novel pharmacologic strategy that involved the dual blockade of the lipid kinase, PIP4K, and mitotic pathways, leading to cancer-selective lethality. CETSA was used to identify PIP4K as a key target for a central compound in this strategy (a131). In 2023 CETSA and TPP approaches were further applied to study the anticancer effects of the NP, triptolide. Researchers used a streamlined thermal proteome profiling approach along with XGBoost to identify HnRNP A2/B1 as a potential antitumor target. Triptolide was shown to bind HnRNP A2/B1, leading to inhibition of the PI3K-AKT signaling pathway, which has a role in the anticancer activity[257]. More recently, Zhang et al.[293] used TPP to identify canopy FGF signaling regulator 3 (CNPY3) as a molecular target of gambogic acid in prostate cancer. The study demonstrated that gambogic acid binds to CNPY3, triggering pyroptosis through an interaction with SIRT1. This binding event leads to re-localization of CNPY3, altering its subcellular distribution and contributing to the pro-apoptotic effects of the NP.

TPP provides a global view of protein stability changes across the entire proteome, enabling the simultaneous identification of multiple potential targets. The high-throughput nature and compatibility of TPP with various biological samples make it a powerful tool for comprehensive target identification. However, TPP also has limitations. TPP requires sophisticated equipment and technical expertise for precise temperature control and quantitative MS analysis. Additionally, interpreting complex thermal shift data can be

challenging and the technique may not distinguish directly from indirect protein-ligand interactions as effectively as some other methods. TPP is particularly well-suited for applications in which a broad assessment of protein stability changes is beneficial, such as in the initial stages of drug discovery or when studying the effects of NPs with unknown mechanisms.

SIP

SIP builds on the principle of leveraging protein solubility changes to study protein-ligand interactions and identify drug targets on a proteome-wide scale[294]. Like TPP, SIP uses systematic perturbation (i.e., organic solvents rather than temperature to influence protein behavior). By introducing specific solvents, SIP alters the solubility of proteins based on structural properties and interactions with small molecules, such as drugs or NPs. Binding of a small molecule to a target protein often modulates protein stability against solvent-induced precipitation, resulting in a distinct solubility profile that can be quantitatively analyzed using MS[295]. This high-throughput, label-free method eliminates the need for chemical tagging or modification, making SIP particularly well-suited for studying protein interactions in native cellular environments. Furthermore, SIP provides complementary insight into how small molecules modulate protein function and stability, expanding our understanding of the mechanisms of action and potential therapeutic applications[295,296].

In 2019, Zhang et al. pioneered the use of the SIP technique to identify protein-ligand interactions in complex cell lysates and successfully identified dihydrofolate reductase (DHFR) as the target of methotrexate. In a subsequent study, Sun et al.[297] utilized SIP and TPP to investigate the potential targets of kurarinone in a Parkinson disease mouse model induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Subsequently, Li et al.[298] extended SIP application to the NP sinomenine from traditional Chinese medicine Sinomenii Caulis. Guanylate-binding protein 5 was identified as a direct sinomenine target in LPS-induced RAW264.7 cells, with validation via molecular docking and CETSA. Following these advances, Bizzarri et al.[299] developed the integral SIP method to assess target engagement of compounds in pathogenic microorganisms under near physiologic conditions.

Although SIP has been successfully used to uncover primary and off-target interactions for various small molecules, the application to NPs, particularly in cancer research, remains largely unexplored. Given the power of SIP in identifying protein-ligand interactions, SIP holds significant potential for revealing how natural compounds interact with cancer-related proteins in complex biological systems. This application could offer valuable insight into the mechanisms underlying the anticancer effects of NPs, paving the way for novel therapeutic strategies.

TRAP

TRAP is a cutting-edge technique that integrates the principles of protein accessibility with quantitative MS to comprehensively analyze the interactions between proteins and small molecules, such as NPs, across the proteome[8]. TRAP identifies these interactions by measuring changes in the accessibility of protein sites upon binding small molecules. When an NP binds to a target protein, the NP often alters the protein' conformation, leading to changes in the accessibility of specific amino acids, such as lysine. The patterns of exposed amino acids can be interrogated using amino acid-specific labelling reagents coupled with MS. This high-throughput method enables the identification of NP targets without requiring chemical modification of the NP, providing insight into the mechanisms of action within a native cellular context. Furthermore, TRAP allows for protein profiling across various biological contexts, offering a comprehensive understanding of how NPs modulate cellular proteomes[300]. TRAP shares some similarities with SIP, another technique used to study protein-ligand interactions. Both methods aim to identify interactions between proteins and small molecules in a native cellular context. However, TRAP offers distinct advantages. Unlike SIP, which relies on changes in protein solubility induced by ligand binding, TRAP focuses on changes in protein accessibility. This feature allows TRAP to provide detailed information on the specific regions of proteins affected by ligand binding, offering deeper insights into the mechanisms underlying NP actions. The ability of TRAP to detect changes in amino acid accessibility provides a more nuanced understanding of protein-ligand interactions, making TRAP particularly useful for studying conformational changes and binding specificities.

In 2022 the introduction of living cell (LC)-TRAP represented an important advance in the study of drug-protein interactions in live cells. This innovative technique was first applied to investigate the targetome of silibinin, a natural compound commonly used in liver disease treatment, in HepG2 cells. LC-TRAP successfully identified 65 potential silibinin targets with acyl-CoA synthetase long-chain family member 4, an enzyme associated with ferroptosis, emerging as a critical target[262]. Building on the success of LC-TRAP, the TRAP technique evolved in 2023 into a powerful tool

for mapping protein-ligand interactions by detecting solvent accessibility changes across the proteome. This development enabled researchers to apply TRAP to study glycolytic metabolites in cancer cells, uncovering 913 potential targets for 10 key metabolites. The study revealed the non-metabolic roles of these metabolites, highlighting the capacity to influence diverse cellular processes, such as enzyme activity, transcription factor regulation, and post-translational modifications[301].

A recent study explored the anticancer properties of arenobufagin by further expanding the potential of these methodologies. Arenobufagin was shown to enhance T-cell mediated antitumor immunity by altering the availability of the chaperone protein, HSP90β. Using a combination of advanced techniques, including single-cell multi-omics and TRAP, the study identified HSP90β as a direct target of arenobufagin[261]. This work demonstrated how the integration of modern techniques, such as TRAP, can deepen our understanding of NP mechanisms in cancer treatment, providing a pathway for more targeted and effective therapies in the future.

TICC

TICC represents a novel approach that holds promise for identifying protein targets. TICC is based on the principle that binding of a molecule to its target protein results in a shift in the molecule chromatographic retention time. This technique involves subjecting cell lysates containing the molecule of interest to non-denaturing high-performance liquid chromatography (HPLC). Subsequently, the fractions are analyzed using LC-MS/MS to identify the proteins that co-elute with the molecule, thereby potentially identifying the targets. TICC offers a label-free and potentially highly sensitive method for identifying targets in a native cellular environment[302,303].

The TICC method was first introduced in 2012 as a novel approach to monitor drug-protein interactions under near physiologic conditions without the need for immobilization or chemical derivatization. In this pioneering work the authors utilized liquid chromatography to separate cell-free lysates, followed by shotgun proteomics to analyze the fractions[302]. This approach enabled the identification of drug-bound protein fractions and established TICC as a straightforward and physiologically relevant technique for early-stage target identification in drug discovery. Further research in 2021 expanded the application of TICC in the field of antibiotic target identification. Specifically, the applicability of TICC in antibiotic research was evaluated with a focus on identifying potential target proteins in *Bacillus subtilis* by monitoring changes in the elution curves of target-bound and free drugs in ion exchange chromatography[303]. In addition to applications in drug discovery and antibiotic research, TICC has significant potential for identifying cancer-related targets of NPs. The ability of TICC to operate under near physiologic and non-denaturing conditions makes TICC particularly well-suited for investigating the interactions between natural compounds and the protein targets. TICC also enables the identification of both high- and low-affinity interactions, which is especially advantageous for NPs and often exhibits complex and subtle binding characteristics[304].

The ability of TICC' to detect both high- and low-affinity interactions is a significant advantage because TICC can capture the often subtle and complex binding characteristics of NPs. The high sensitivity and compatibility of the technique with non-denaturing conditions further enhance the utility of TICC in identifying targets within a physiologic context. However, TICC has some limitations. The method requires sophisticated instrumentation and technical expertise for accurate HPLC separation and subsequent LC-MS/MS analysis. Data interpretation can be complex because co-elution does not always confirm direct protein-ligand interactions and additional validation steps are often necessary to confirm target identification. Furthermore, the technique may have limitations in resolving proteins with very similar retention times, which could lead to potential false-positive results. TICC is particularly well-suited for applications in which the preservation of native protein-ligand interactions is crucial. TICC is a powerful tool for the initial stages of target identification in drug discovery, especially when studying NPs with unknown mechanisms of action. The ability of TICC to operate under near physiologic conditions makes TICC ideal for investigating the interactions of natural compounds with protein targets, offering a complementary approach to other target identification methods.

Integration of AI and chemical proteomics in target identification

AI has become a transformative tool in improving the identification of NP targets in cancer research. AI enables the prediction of potential protein targets based on the structural and physicochemical properties of NPs by leveraging ML algorithms. Techniques, such as DL and molecular docking simulations, facilitate the analysis of complex interaction networks, which help to uncover subtle binding relationships and prioritize candidates for experimental validation[305].

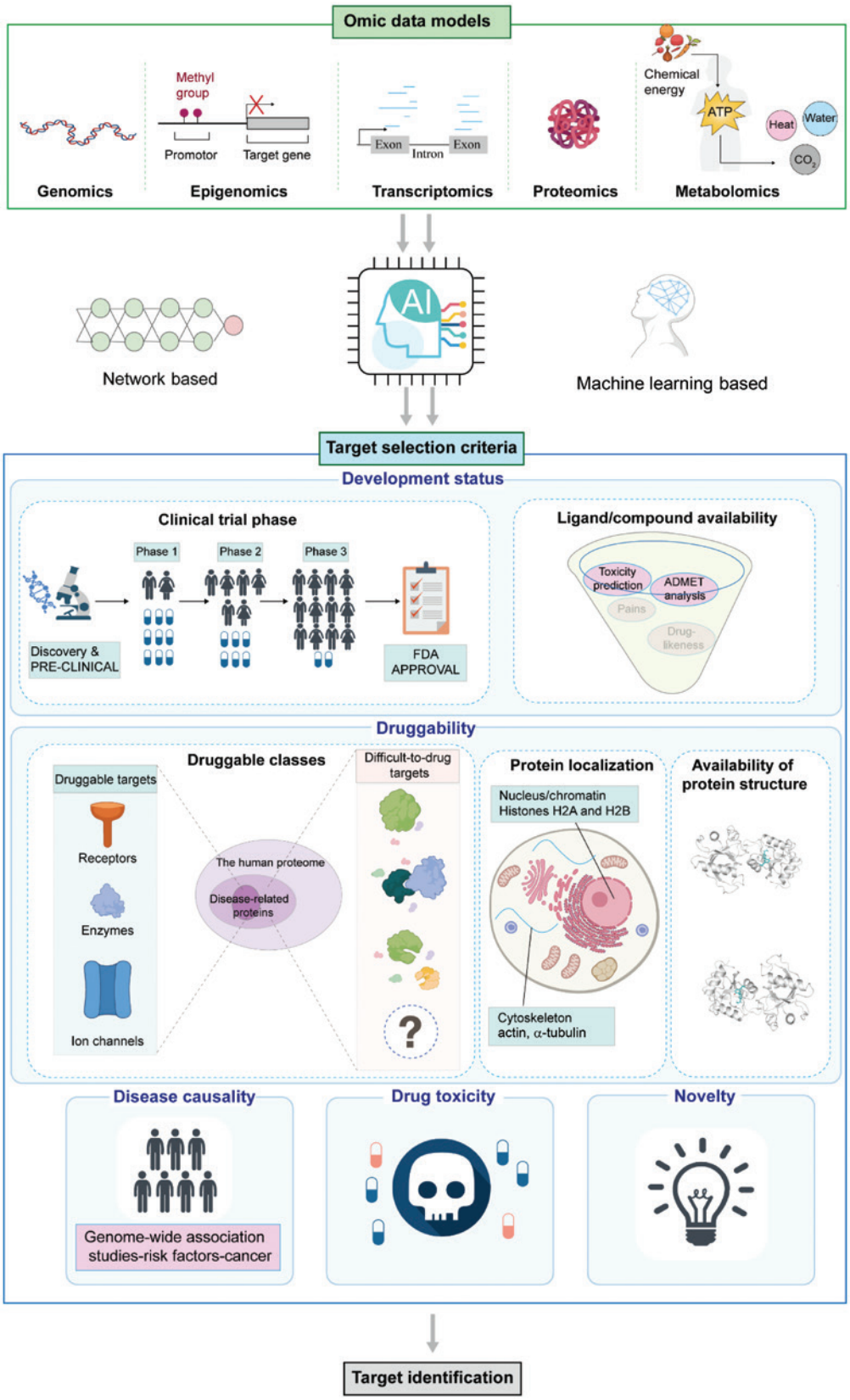


Figure 6 Continued

Figure 6 Workflow for AI-driven target identification using multi-omic data models. Artificial intelligence (AI)-driven target discovery follows a systematic workflow, integrating multi-omics data analysis, machine learning algorithms, and target selection criteria to efficiently identify and prioritize potential therapeutic targets. The process begins with the collection and integration of various **omics data**, including **genomics**, **epigenomics**, **transcriptomics**, **proteomics**, and **metabolomic**s. This comprehensive data integration provides a holistic view of the biological systems and disease mechanisms. The integrated data are then analyzed using advanced AI and machine learning algorithms, which help in understanding the complex interactions between different biological entities and enhance the predictive power by identifying patterns. Based on the analysis, potential targets are selected using predefined criteria that consider the development status, **druggability**, **disease causality**, **drug toxicity**, and **novelty**. This systematic approach ensures that the most promising targets are prioritized for further investigation and clinical development.

Figure 6 illustrates this workflow, highlighting the key steps involved in AI-driven target discovery for NPs. Building on this framework, the integration of advanced computational workflows with large-scale proteomic datasets-generated *via* chemical proteomics-not only refines target identification but also provides deeper insights into the underlying anticancer mechanisms (**Figure 7**). Representative case studies of NPs underscore the efficiency of AI in expediting target discovery and streamlining drug development pipelines.

Role of AI in target prediction

AI, particularly ML and DL, excels at processing large-scale datasets and identifying complex relationships that are often undetectable by traditional methods. In the context of target prediction, AI algorithms can analyze genomic, proteomic, and pharmacologic data to uncover potential therapeutic targets. For example, DL models, such as convolutional neural networks (CNNs) and recurrent neural networks (RNNs), have been used to predict drug-target interactions with high accuracy[306,307]. These models can process multi-omics data, integrating information from various biological layers to enhance the precision of target identification. In this review, a comprehensive inventory was compiled of AI/ML tools used for NP identification and drug lead discovery (**Table 3**). This summary serves as a quick reference for researchers, enabling selection of the most suitable tools for integrating AI into various stages of the drug discovery pipeline, from target prediction to validation of therapeutic candidates.

A key application of AI in target prediction is the creation of predictive models that can anticipate the interactions between drugs and potential targets. AI-driven models, such as those based on graph neural networks (GNNs), have shown remarkable performance in predicting drug-target binding affinities[320]. These models can incorporate molecular structures, protein sequences, and interaction networks to provide a comprehensive understanding of how drugs interact with biological targets. For example, Nguyen et al.[307] developed a GNN-based model (GraphDTA), which accurately predicts drug-target binding affinities by integrating molecular and interaction data. This model outperformed traditional methods by achieving higher prediction accuracy and requiring less manual feature engineering. In addition, Stokes et al.[321] utilized graph convolutional networks to establish a screening model and designed the novel broad-spectrum antibiotic, halicin. A comparison of different AI tools based on critical factors, such as prediction accuracy and computational efficiency, has been included. Studies comparing various AI models have shown that GNNs often achieve higher accuracy in predicting drug-target interactions compared to traditional ML methods like support vector machines (SVMs) and random forests (RFs)[322]. Furthermore, AI models (e.g., GraphDTA) have demonstrated superior performance in terms of both accuracy and efficiency, making AI models more suitable for large-scale target prediction tasks. For example, Huang et al.[323] proposed MolTrans, a molecular interaction transformer for drug-target interaction prediction, which also showed significant improvements in accuracy and efficiency. Additionally, the IGModel presents a novel framework for DL-based prediction of protein-ligand interactions. The IGModel uses the geometric information of protein-ligand complexes as input to predict the root mean square deviation of docking poses and binding strength (pKd) within the same framework, ensuring the output scores carry intuitive meaning[324].

AI has also been applied to predict the mechanisms underlying bioactive compound actions. Reker et al.[325] developed a cascaded self-organizing map algorithm to predict the targets of small molecules by comparing physicochemical properties and pharmacophoric features with those of known drugs. In another study Reher et al.[326] proposed a neural network model

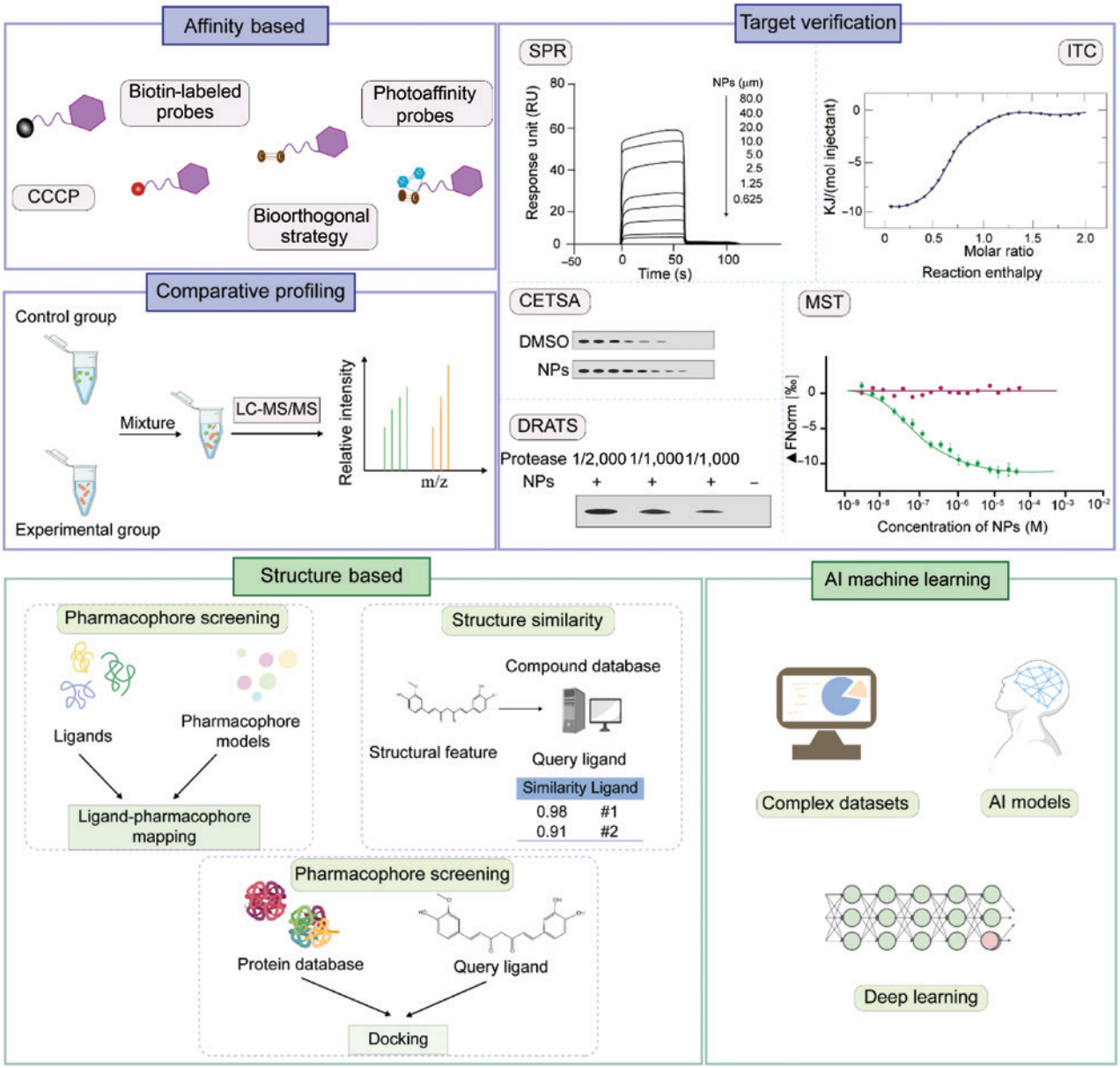


Figure 7 Schematic diagram of an AI-driven drug discovery framework based on chemical proteomics. This figure illustrates an integrated drug discovery framework combining chemical proteomics and artificial intelligence. The process encompasses affinity-based screening using techniques, such as **surface plasmon resonance** (SPR) and **isothermal titration calorimetry** (ITC) to assess binding affinity between compounds and target proteins. The process also includes target verification through methods, such as the **cell-based thermal shift assay** (CETSA) and **microscale thermophoresis** (MST), to confirm target engagement. Comparative profiling is done *via* liquid chromatography-mass spectrometry (LC-MS/MS) to compare protein profiles between control and experimental groups. Structure-based approaches, including pharmacophore screening and molecular docking, identify potential ligands based on structural features. Finally, AI-driven machine learning analyzes complex datasets with deep learning models to predict compound-target interactions and optimize drug candidates. This comprehensive strategy enables efficient identification and optimization of novel compounds for therapeutic development.

capable of directly annotating compound structures from LC-MS/MS data. This innovation has significantly accelerated the discovery of complex NPs, such as symplocolide A, a toxic carrot eneol compound. By applying the innovative NMR-based ML tool, small molecule accurate recognition technology (SMART 2.0), for mixture analysis, along with

Table 3 A comprehensive inventory of AI/ML tools used in NP identification and drug lead discovery

Tools	Features	Availability	Web links or references
SIRIUS 4	Software for analyzing mass spectrometry data and annotating molecular formulas and structures	Open source	https://bio.informatik.uni-jena.de/sirius[308]
CSI:FingerID	Predicts molecular fingerprints from MS/MS data using machine learning fingerprint methods	Open source	www.csi-fingerid.org[309]
GNPS	Builds and analyzes molecular networks from MS data; promotes data sharing and mining	Free online	http://gnps.ucsd.edu[310]
DeepChem	Open-source deep learning library for molecular property prediction and virtual screening	Open source	https://github.com/deepchem/deepchem[311]
RDKit	Chemical informatics tool for molecular data processing, feature extraction, and simulation.	Open source	https://github.com/rdkit[312]
Chemprop	Graph neural network-based tool for predicting molecular properties and activities	Open source	https://github.com/chemprop[313]
AutoDock Vina	Molecular docking tool that predicts binding modes between small molecules and target proteins	Open source	https://github.com/ccsb-scripps/AutoDock-Vina[314]
Schrödinger (Glide)	Integrated platform for virtual screening, molecular docking, and ADME prediction	Commercial	https://www.schrodinger.com/[315]
Exscientia	AI-driven platform for lead compound identification and optimization	Commercial	https://www.exscientia.com/
ChemTS	Uses generative models to design novel molecules and accelerate the discovery of drug candidates	Open source	https://github.com/tsudalab/ChemTS[316]
IBM RXN for Chemistry	AI-powered reaction prediction and synthesis planning platform to assess molecule synthesizability	Open source	https://rxn.app.accelerate.science/rxn
ASKCOS	Machine learning-based retrosynthesis tool that provides synthesis route design suggestions	Open source	https://askcos.mit.edu[317]
ADMETlab	Online tool for predicting compound absorption, distribution, metabolism, excretion, and toxicity (ADMET)	Open source	http://admet.scbdd.com/[318]
QSAR Toolbox	QSAR modeling tool for predicting biological activity and safety profile	Open access	https://qsartoolbox.org/[319]
DeepTox	Uses deep learning for toxicity prediction to support drug safety evaluations	Open source	http://shiny.bioinf.jku.at/Analoging/
Atomwise	Deep learning platform for large-scale virtual screening to accelerate lead identification	Commercial	https://www.atomwise.com/
BenevolentAI	Comprehensive AI platform integrating target discovery through lead optimization	Commercial	https://www.benevolent.com/
Insilico Medicine	Utilizes various AI algorithms for drug discovery and biomarker development	Commercial	https://insilico.com/

cancer cell cytotoxicity and MS2-based molecular networking, the researchers[326] were able to rapidly identify and characterize new NPs.

AI not only aids in the initial prediction of targets but also supports the validation process. By integrating AI with experimental data, researchers can identify potential off-target effects and validate the specificity of drug candidates[327]. This is especially crucial for complex diseases, like cancer, in which multiple pathways and targets are typically implicated. AI models can simulate the effects of drugs on cellular networks, predicting the impact on disease phenotypes and identifying potential biomarkers for therapeutic response[328]. For example, AI was used to simulate the effects of a potential drug on the complex network of neurons and glial cells. By incorporating genetic data from patients and data on key protein expression, the model predicted drug' impact on disease progression and identified specific biomarkers that could indicate a positive therapeutic response[329]. AI-driven analysis of large autoimmune disease datasets containing patient immune profiles and drug response data helped validate the specificity of a new immunomodulatory drug. The model predicted how the drug interacts with different immune cell subsets and molecular targets, enabling researchers to optimize the treatment strategy and improve the overall effectiveness of the therapy[330]. These examples illustrate the crucial role of AI in predicting potential targets and ensuring the safety and efficacy of drug candidates through comprehensive validation processes, thereby transforming the drug discovery and development process.

AI-driven workflow and criteria for anticancer target discovery

The integration of AI into anticancer target discovery has established a comprehensive framework that transforms how novel targets are identified and validated. AI-driven criteria for target discovery prioritizes the analysis of multi-omics data, including genomics, epigenetics, transcriptomics, proteomics, and metabolomics, to uncover complex biological interactions and patterns that traditional methods often miss[331,332]. These criteria emphasize the importance of data quality, biological relevance, and predictive power in selecting potential targets. For example, studies have shown that DL excels in cancer genomics by detecting subtle genetic variations and predicting treatment responses through genomic data analysis. Studies involving cancer histopathology have demonstrated the ability of DL to enhance diagnostic accuracy by identifying cancer types and stages from histologic images, offering precise prognostic predictions[333]. Studies have also highlighted the critical role of epigenetics, particularly age-dependent DNA methylation, in lipid metabolism dysfunction, which accelerates aging and may influence cancer development[334]. This advance has been further supported by recent studies in spatial transcriptomics, which illustrate how AI analyzes spatial transcriptomic data to detect prognostic regions in high-grade serous carcinoma and enhances our understanding of tumor biology and improving patient stratification[335]. In addition, recent research has shown that AI can effectively analyze spatial proteomics data with single-cell resolution in tissues. By integrating this data, AI can provide detailed insight into protein expression patterns and the spatial distribution within the tumor microenvironment, further enhancing the identification of potential anticancer targets[336]. Moreover, researchers have developed metabolomic ML predictors for the diagnosis and prognosis of gastric cancer. These tools utilize AI to analyze metabolomic data, identifying specific metabolic signatures that are associated with gastric cancer[337]. These tools utilize AI to analyze metabolomic data, identifying specific metabolic signatures that are associated with gastric cancer. The integration of such diverse data types provides a holistic view of potential targets within biological systems, enhancing the effectiveness of AI models (e.g., AlphaFold) in predicting protein structures with high accuracy[338]. Multiomics data offers a more comprehensive understanding of biological systems by integrating various omics layers, thereby enhancing the biological relevance and predictive power of AI models. The integration of AI with multi-omics data and experimental validation has become a powerful strategy for identifying and prioritizing anticancer targets, ultimately driving the development of more effective and personalized cancer therapies[339-341].

The successful integration of multi-omics data with AI has been demonstrated in various workflows designed for target discovery. AI-driven workflows typically begin with data collection and preprocessing from public databases and high-throughput experiments[342]. This is followed by feature extraction and selection, in which AI algorithms identify key features that distinguish potential targets. Predictive models are subsequently trained and validated using techniques, such as cross-validation and independent testing, to ensure robustness and generalizability. Finally, experimental validation is conducted to confirm the biological relevance and therapeutic potential of the predicted targets[343-345]. AI facilitates the application of network pharmacology and systems biology

principles in target discovery[346]. Through the construction and analysis of biological networks, researchers can identify key nodes and hubs that represent potential targets with high therapeutic value. These networks, which can include protein-protein interaction networks, gene regulatory networks, and metabolic networks, aid in pinpointing key nodes that may serve as potential therapeutic targets. For example, Guan et al.[347] used bioinformatics and ML methods to identify potential targets for preventing cancer progression through the PLA2G1B recombinant protein. Similarly, in a study of high-grade gliomas, researchers used AI-driven workflows to analyze multi-scale signaling and tumor evolution. By integrating proteomic, metabolomic, lipidomic, and post-translational modifications with genomic and transcriptomic measurements, AI models have been developed to predict molecular alterations, tumor grade, and prognosis in glioma patients[348]. In agreement with these approaches, deciphering gastric inflammation-induced tumorigenesis through multi-omics data and AI methods has also shown great potential[349]. The criteria for evaluating these networks involve assessing the topological properties, the connectivity of the nodes, and the biological significance of the identified targets within the context of network function in cancer biology.

Beyond the identification of targets through network analysis, the prioritization and validation of these targets represent another critical step in AI-driven target discovery. The development status of a target is assessed by examining progression through clinical trial phases, from discovery and preclinical studies to potential FDA approval[350]. This process involves evaluating the availability of ligands or compounds that can interact with the target, as well as conducting toxicity prediction and absorption, distribution, metabolism, excretion, and toxicity (ADMET) analysis to ensure the safety and efficacy of potential therapeutic agents[351]. Druggability is another critical criterion that focuses on target accessibility to therapeutic intervention. Druggability includes identifying druggable classes, such as receptors, enzymes, and ion channels, as well as assessing protein localization and the availability of structural information, which are essential for drug design and development[352,353]. Additionally, target expression and stability in different tissues and cell types are evaluated to determine feasibility as a drug target[354]. Computational tools and AI models are used to predict binding affinities and simulate interactions between small molecules or biologics and the target. For example, AI-driven high-throughput virtual screening techniques have been developed to identify potential hit compounds from large compound libraries more efficiently than traditional *in vitro* screening assays. These simulations accelerate the drug discovery process by enabling the rapid identification of promising compounds that can be further optimized into drug candidates[355]. Disease causality is evaluated through genome-wide association studies and risk factor analysis to establish target relevance to cancer development[356]. Drug toxicity is carefully assessed to minimize potential adverse effects, while novelty ensures the target offers a new avenue for therapeutic intervention, potentially leading to more effective and innovative cancer treatments[357]. AI-driven workflows enhance the efficiency and effectiveness of anticancer target discovery by systematically applying these criteria. This approach not only accelerates the identification of novel targets but also increases the likelihood of successful clinical outcomes, ultimately contributing to advances in personalized and targeted cancer therapies. The criteria for target prioritization include the strength of the predictive evidence from AI models, the availability of suitable validation tools, and target potential for clinical impact if successfully modulated.

AI has also been instrumental in constructing comprehensive databases that facilitate the discovery of anticancer targets. For example, the PandaOmics platform successfully utilized multi-omics data and biological network analysis to identify TRAF2 and NCK interaction kinases as potential targets for antifibrotic therapy, leading to the development of a specific inhibitor (INS018_055)[358]. This demonstrated how AI can integrate diverse data types to provide a holistic view of potential targets within biological systems. Another notable example is the study using AI methods to discover 370 new cancer drug targets across 27 different cancers. This research brought the field closer to completing the Cancer Dependency Map and opened new possibilities for targeted cancer therapies[359]. Another study has also leveraged the TCMSP database to predict effective components in Chinese herbal formulas[360]. We have summarized several key databases based on our comprehensive analysis, as shown in **Table 4**.

In addition to enhancing the general process of target discovery, AI also has a pivotal role in advancing personalized medicine and biomarker identification for patient stratification. The scope of AI in anticancer target discovery extends to personalized medicine and the identification of biomarkers for patient stratification. AI can identify targets that are relevant to individual patients or specific subgroups by analyzing patient-specific data, which enables the development of tailored therapeutic approaches. This involves the discovery

Table 4 Natural product-related database and analysis platform: AI-driven drug discovery and development

Database	Features	Web links or references
TCMSP	Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform provides information on drug metabolism pharmacokinetics of Chinese medicine components	https://www.tcmsp-e.com/
PharmMapper	A web server for potential drug target identification using pharmacophore mapping approach that is useful for natural product target prediction	http://lilab.ecust.edu.cn/pharmmapper/[361]
ChemMapper	Integrates multiple chemoinformatic algorithms for target prediction, which is applicable to NPs	http://lilab.ecust.edu.cn/chemmapper/[362]
BATMAN-TCM	Bioinformatics Analysis Tool for Molecular mechANism of Traditional Chinese Medicine focuses on TCM and NPs	http://bionet.ncpsb.org.cn/batman-tcm/[363]
SymMap	Maps genetic variants to potential target genes based on expression quantitative trait loci (eQTLs) can be used for NP research	http://www.symmap.org/[364]
SuperPred	Predicts protein-protein interactions and targets based on sequence similarity is useful for NP targets	https://prediction.charite.de/index.php[365]
TDR Targets	Integrates multiple algorithms and data sources for target prediction, including NPs	https://tdrtargets.org/[366]
Therapeutic Target Database	Provides information on therapeutic targets and associated drugs, including NPs	https://idrblab.org/ttd/[367]
DGLdb	Database of drug-gene interactions and literature that covers NPs	https://www.dgidb.org/[368]
chEMBL	Open-source database of bioactive molecules with a focus on medicinal chemistry	https://www.ebi.ac.uk/chembl[369]
DrugBank	Combines detailed drug data with comprehensive target information	https://go.drugbank.com[370]
TCMID	Traditional Chinese Medicine Integrated Database for network pharmacology with a focus on NPs	http://www.megabionet.org/tcmid/[371]
TargetHunter	Predicts potential targets for small molecules using machine learning, which is applicable to NPs	http://www.cbligand.org/TargetHunter[372]
Herb	Information on herbal medicines and their bioactive compounds with a focus on NPs	http://herb.ac.cn/[373]
Herbal Ingredients' Targets Platform (HIT)	An enhanced platform for Herbal Ingredients' Targets, which covers NPs	http://hit2.badd-cao.net[374]
CTD	Comparative Toxicogenomics Database linking chemicals, genes, and diseases, which includes NPs	http://ctdbase.org/[375]
ETCM	Encyclopaedia of Traditional Chinese Medicine with a focus on NPs	http://www.nrc.ac.cn:9090/ETCM/[376]
RF QSAR	Random Forest Quantitative Structure-Activity Relationship model for target prediction, which can be used for NPs	http://www.kaist.ac.kr

of predictive biomarkers that can indicate the probability a patient will respond to a particular treatment, as well as prognostic biomarkers that can provide information about disease outcomes. The criteria for biomarker discovery focus on biomarker association with treatment response or prognosis, measurability in clinical samples, and potential to guide treatment decisions and improve patient outcomes. Recent studies have shown that AI-based biomarkers derived from routine clinical data enhance the accessibility of personalized medicine by providing rapid and cost-effective alternatives to traditional molecular biomarkers[377]. For example, Shin et al.[378] developed an AI prediction model for hepatocellular carcinoma in patients with chronic hepatitis B. This model effectively classifies patients into different risk categories, offering a non-invasive and efficient method for early cancer detection and risk assessment. AI has been applied to predict remission or disease activity in ulcerative colitis. Lacucci et al.[379] developed a CNN-based AI model trained in histologic images. This model accurately distinguishes endoscopic remission/activity and predicts histologic remission and clinical outcomes from colonoscopy videos, achieving high sensitivity and specificity.

The clinical translation potential of AI is further underscored by the ability to process complex datasets and uncover intricate patterns with applications spanning from drug development to patient monitoring[380]. For example, a study developed and validated a clinical risk score to predict the occurrence of critical illness in hospitalized patients with COVID-19 and demonstrated the potential of AI in clinical risk assessment[381]. Additionally, Kate et al.[382] reported the development of a continual prediction model for inpatient acute kidney injury (AKI), which uses AI to analyze electronic health record data and predict AKI events in hospitalized patients. This model helps in early intervention and management of AKI, improving patient outcomes and reducing healthcare costs. A meta-analysis of AI algorithms for lung cancer diagnosis has shown a combined sensitivity and specificity of 87%, significantly reducing misdiagnosis rates compared to manual pathology section analysis[383].

Building on these clinical translation successes, the advantages of AI in drug discovery are also notable because AI optimizes resource allocation and enhances the efficiency of the drug development process[384]. For example, researchers developed a spatial architecture-embedding HLA signature to predict clinical response to immunotherapy in renal cell carcinoma by integrating spatial transcriptomics and ML to analyze tumor microenvironment interactions and HLA expression, which provided a more accurate prediction of treatment outcomes and improving patient stratification[385]. Furthermore, AI techniques have been particularly valuable in predicting drug-target interactions. An innovative end-to-end learning framework (EEG-DTI) for drug-target interaction predictions was introduced by Peng et al.[386] that demonstrated strong DTI prediction performance, even in cases in which the three-dimensional structures of drug targets were not used. Another model (DTI-HETA) utilizes a heterogeneous graph with an attention mechanism surpassed the performance of current state-of-the-art models[387]. These advances highlight the potential of AI to bridge research and clinical practice, accelerating the discovery and validation of novel anticancer targets and improving personalized therapeutic strategies. The ability of AI to analyze large and complex biological datasets and identify patterns that might be difficult for humans to discern has significantly enhanced drug development processes, making AI a transformative tool in cancer research[388].

AI-enhanced data analysis in chemical proteomics

AI has become a crucial tool in chemical proteomics by improving data analysis efficiency and accuracy, especially for NPs. By integrating AI with chemical proteomics, researchers can enhance data processing, pattern recognition, and predictive modeling, ultimately accelerating the discovery of novel therapeutic targets[389]. This integration is crucial for identifying novel therapeutic targets and understanding the complex interactions between natural compounds and biological systems. For example, AI algorithms can analyze complex MS data to identify protein-drug interactions with higher accuracy and efficiency. This analysis is particularly valuable for NPs, which often exhibit chemical complexity and diversity, making traditional analytical methods less effective[390].

AI-driven predictive models are increasingly being used to identify potential therapeutic targets of NPs. These models can integrate multi-omics data, including genomics, proteomics, and metabolomics, to forecast the biological activities and targets of natural compounds[391]. For example, ML algorithms can analyze spectroscopic data to identify and characterize bioactive molecules in NPs, speeding up target identification[392]. Additionally, AI-based generative models can predict drug candidates and optimize extraction processes, ensuring maximum yields of bioactive compounds[393]. In practice, these advances are already making a significant impact. The

TamGen model, for example, leverages a chemical language model to generate target-aware molecules, demonstrating the potential for AI to enhance the efficiency and specificity of drug design[394]. Similarly, the integration of multi-omics data through AI frameworks has transformed the way researchers approach combination therapies. By analyzing complex datasets, AI can identify synergistic interactions between compounds, optimizing therapeutic outcomes[395]. Moreover, AI-driven approaches have been instrumental in metabolomics, in which algorithms analyze metabolic pathways to uncover novel bioactive compounds. This capability is particularly valuable in the context of NPs, which often exhibit complex chemical structures and diverse biological activities[396]. By harnessing AI, researchers can now predict the biological activities of these compounds more accurately and identify previously overlooked therapeutic targets. For example, Liu et al.[397] used a transfer learning approach based on network target theory to efficiently predict drug-disease interactions. This model successfully identified a vast number of drug-disease associations, demonstrating the potential to enhance drug discovery and development. Other studies have showcased the power of AI in drug discovery. For example, DrugGPT uses the transformer architecture to generate SMILES strings of interacting small molecules based on the amino acid sequence of a given target protein. This approach accelerates the lead discovery phase by proposing new compounds with desired properties, learned from large datasets of known drugs and drug targets. The ability of AI to process and integrate diverse data types provides a significant advantage over traditional methods, which often struggle with the complexity and scale of modern biological datasets[398].

The PandaOmics and Insilico Medicine AI tools have been pivotal in integrating multiomics data to predict drug-target interactions and discover novel therapeutic targets[358]. For example, the STarFish tool enables the prediction of NP molecular targets by leveraging ML algorithms, significantly enhancing the efficiency of target identification. Natural microtubule inhibitors, such as paclitaxel and ixabepilone, are pivotal examples of NP success in drug discovery within the field of antitumor treatment[399]. DL models have recently identified additional β-microtubule inhibitors, such as eleutherobin, bruceine D, and phorbol 12-myristate 13-acetate, emphasizing the role of DL in uncovering potent NP-based drugs[400,401]. AI techniques have also facilitated broader bioactivity analyses by clustering chemical structures, thereby evaluating the therapeutic potential of NPs and integrating structural and bioactivity data to provide robust insight into drug discovery. For example, the identification of covalently bound berberine targeting PLK1, a protein central to cell proliferation, demonstrated the precision of AI in predicting molecular interactions[402].

AI has been instrumental in analyzing complex cancer datasets to identify potential therapeutic targets and predict drug responses. For example, in a study focused on breast cancer, an AI system was utilized to analyze the proteomic changes induced by a specific NP extract. By comparing the protein expression profiles of cancer cells before and after treatment with the extract, the AI tool was able to identify a set of proteins that were consistently affected[16]. Furthermore, AI models can analyze gene expression data to identify significant gene biomarkers associated with cancer progression. These biomarkers can then be used to develop classification and DL models for predicting and classifying various stages of cancer[403]. AI offers valuable assistance in patient stratification by leveraging the genetic profiles and responses to NP therapies. AI models possess the ability to precisely identify subgroups of patients who exhibit a higher probability of responding favorably to NPs based on an analysis of extensive patient data cohorts. This AI ability enables implementation of personalized medicine strategies in cancer treatment[404]. For example, a biologically informed deep neural network (P-NET) was developed in a recent study. This innovative model utilizes patient genomic profiles and treatment resistance states to stratify patients with prostate cancer. Not only does P-NET accurately predict the occurrence of metastatic disease, but P-NET also successfully identifies novel molecular drivers of treatment resistance, such as murine double minute 4 and fibroblast growth factor receptor 1. These identified molecular factors hold significant potential as therapeutic targets[405]. Furthermore, a breat cancer study utilized ML to predict localization of local recurrences and distant metastases after local recurrences[406]. Researchers analyzed data from 154 patients by applying 4 different mathematical models. The best-performing model predicted recurrence in the remaining breast tissue with 77% accuracy and recurrence in surgical scars with 69% accuracy[407]. Another study integrated clinical, digital pathology, genomic, and transcriptomic data from 168 breast cancer patients undergoing pre-surgical chemotherapy with or without HER2-targeted therapy. By correlating surgical pathology outcomes with pre-treatment tumor features, a multi-omic machine learning model was developed that predicted pathologic complete response in an external cohort with an AUC of

0.87, highlighting the potential of combining multi-omic data with AI for predictive modeling in breast cancer treatment[408]. AI models have been developed to predict outcomes in high-grade serious ovarian cancer by analyzing surgical images. These models distinguished between patients with favorable and unfavorable responses and helped to refine clinical treatment plans[406]. Additionally, a novel computational technique (SigMA) was introduced to accurately detect HR deficiency from targeted gene panels, expanding the pool of patients eligible for therapies targeting HR insufficiency[409]. These examples highlight the diverse and impactful applications of AI in cancer research, driving the development of new therapeutic strategies and improving patient outcomes. AI has also been integrated with chemical proteomics in cancer treatment, diagnosis, and prognosis. AI allows for a more comprehensive and in-depth understanding of the intricate molecular interactions that occur within cancer[410]. By examining these interactions, researchers can uncover valuable insights that aid in identifying new therapeutic targets. This integrated approach combines the power of AI data analysis capabilities with the detailed molecular information provided by chemical proteomics, opening new avenues for cancer research and treatment development.

Despite significant advances, several challenges remain in the application of AI in chemical proteomics and NP research. One of the primary challenges is the need for high-quality, diverse, and well-annotated datasets to train robust AI models. Additionally, the interpretability of AI models remains a critical concern, as understanding the reasoning behind predictions is essential for clinical adoption. Future research should prioritize creating more interpretable AI models, as well as integrating AI with other emerging technologies, such as single cell sequencing and synthetic biology.

Discussion

NPs have garnered significant attention in cancer research due to multi-target capabilities, which allow NPs to interact with various pathways involved in tumorigenesis[411]. The advent of chemical proteomics has greatly advanced our understanding of the molecular targets of these NPs with label-based and -free techniques proving to be valuable tools in this area[8]. Label-based approaches, while highly effective in identifying direct target interactions, can be limited due to the requirement of chemical tagging. Label-free techniques offer the advantage of detecting interactions without modifying the natural structure of the NP, although label-free techniques may face challenges in resolving transient interactions or low-abundance protein targets[412,413]. Despite these challenges, chemical proteins remain a cornerstone of NP target identification, shedding light on the complexity of the interactions between NPs and cellular proteins.

To validate the identified protein targets, a suite of complementary biophysical and biochemical methods has been used. For example, surface plasmon resonance (SPR) enables real-time monitoring of NP-protein interactions, which provides kinetic parameters and binding affinities. Isothermal titration calorimetry (ITC) further quantifies these interactions by measuring the heat changes during binding, thus offering detailed thermodynamic profiles. In addition, CETSA confirms target engagement within the native cellular environment by detecting ligan d-induced shifts in protein thermal stability[414]. DARTS leverages the protection of NP-bound proteins against proteolytic degradation to validate binding, while microscale thermophoresis (MST) monitors the movement of fluorescently labeled proteins under temperature gradients, offering another sensitive measure of interaction. Together, these methods form a robust target verification framework that bridges high-throughput chemical proteomics and functional validation, ensuring that identified interactions are specific and biologically relevant (**Figure 7**).

The integration of AI has significantly enhanced the ability to predict and validate potential molecular targets for cancer therapies in recent years. ML algorithms have been instrumental in processing large datasets from proteomic studies, identifying novel targets with greater precision than traditional methods. AI-driven approaches can also integrate diverse data types, such as gene expression profiles and chemical structures, enabling more accurate predictions of NP-target interactions[414]. This synergy between AI and chemical proteomics offers a promising pathway to overcome the inherent complexities of NP-target identification, especially in the context of cancer research where tumor heterogeneity often complicates target identification[300,415]. Furthermore, the application of AI in target prediction has shown great potential in streamlining the drug discovery process. By using algorithms trained on vast amounts of biological and chemical data, AI models can predict which proteins are most likely to interact with specific NPs. These predictions can then be validated experimentally, accelerating the development of new cancer therapies[416]. Additionally, AI can enhance the interpretation of proteomic data, especially when dealing with large, complex datasets

that would otherwise be difficult to analyze manually[417]. This AI-enhanced data analysis not only improves the speed and accuracy of NP-target identification but also allows for deeper insight into the molecular mechanisms through which NPs exert anticancer effects.

Looking ahead, the combination of single-cell proteomics and AI holds tremendous potential in advancing our understanding of NP-target interactions on a more granular level. Single-cell technologies, when combined with AI-based predictive models, could help unravel the complex dynamics of how NPs affect tumor microenvironments, offering more precise and individualized therapeutic strategies[300]. For example, AI can analyze single-cell proteomic data to identify specific cellular subpopulations within tumors that respond differently to NPs, enabling the development of targeted therapies that maximize efficacy while minimizing side effects. The study by Esteban-Medina et al.[418] exemplifies the importance of integrating diverse data sources and methodologies, using ML models to identify candidate drugs for melanoma treatment by contextualizing protein drug targets within the functional landscape of the disease. Additionally, the ability of AI to predict the potential repurposing of existing NPs for cancer treatment further emphasizes the role in accelerating drug discovery and expanding the therapeutic potential of natural compounds[417]. For example, Jiang and Li[419] developed a network-based DL model (DeepDR) that integrates drug-disease and drug-target networks to identify new therapeutic uses for existing drugs. Similarly, the network topology-based DL framework, NETTAG, uses an interpretable AI model to predict repurposable drugs by analyzing multi-omics data and integrating the data within the human protein-protein interaction network[420]. A comparative analysis of AI-assisted target identification methods with traditional approaches reveals significant advantages of AI. AI methods can process large datasets and identify patterns much faster than traditional methods. For example, the Atomwise AIMS Program[421] utilized AI for high-throughput screening of small molecules, demonstrating how AI can enhance the efficiency and accuracy of target identification by processing vast amounts of data quickly. This analysis highlights the benefits of AI in efficiency, accuracy, and applicability, helping researchers choose the most suitable methodologies for specific goals.

However, despite promising advances, there are still considerable challenges in translating these findings into clinical practice. The clinical translation of NP-based therapies faces challenges related to synthesis, formulation, and pharmacokinetic evaluation. Controllable and reproducible synthesis of NPs with optimal physicochemical parameters is crucial. Advances in synthetic biology and biotechnology offer new solutions, such as using microbial fermentation and enzymatic catalysis to achieve efficient synthesis of complex molecules. This approach optimizes preparation processes, improves yields, and enhances quality[422]. Many NPs also suffer from poor pharmacokinetics and low bioavailability. For example, curcumin, despite its broad therapeutic benefits, has limited clinical utility due to poor solubility and rapid degradation in the body. However, nanoparticle-based drug delivery strategies offer promising solutions to enhance curcumin effectiveness in clinical applications issues, such as the reproducibility of preclinical results, the variability in NP bioavailability, and the complexity of human tumor biology, must be addressed[423]. Similarly, the anticancer compound, zerumbone, which is derived from ginger, faces challenges in bioavailability and formulation stability. The molecular properties of zerumbone make it difficult to achieve appropriate tissue levels and the classification of zerumbone as a supplement rather than a pharmaceutical hinders stringent regulatory processes[424]. Liquid biopsy technologies offer real-time monitoring of treatment response and disease progression and provide insight into tumor biomarkers for more personalized treatment decisions[425]. AI analysis of clinical trial data can identify patient subgroups more likely to respond to NP-based therapies, improving trial design and success rates. Regulatory challenges also arise due to the complex nature of NPs, making it difficult to meet drug approval requirements. These challenges include the lack of clear regulatory guidelines specific for NPs and the limited understanding of the biological interactions in patients. Addressing these challenges requires continued research, innovation, and collaboration across disciplines. For example, combination therapy, structural optimization, and drug delivery systems are anticipated to enhance the efficacy of NP-based therapies and overcome clinical translation hurdles[426]. Collaborative efforts among computational scientists, biologists, and clinicians are crucial to overcoming these barriers and ensuring the effective integration of NPs into clinical oncology. The future of NP-based cancer therapies hinges on continued innovation in chemical proteomics and AI, as well as interdisciplinary collaboration to tackle the clinical challenges that remain.

In conclusion, the integration of chemical proteomics and AI holds great promise for revolutionizing the identification

of molecular targets for NPs in cancer treatment. As these technologies advance, the technologies will improve the precision and speed of target identification, while offering deeper understanding of the mechanistic pathways of natural compounds, thereby aiding the development of more personalized and effective cancer treatments.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

Conceived the study: Xinbing Sui.
Collected the data: Guohua Li, Qian Shi.
Wrote the paper: Guohua Li.
Revised the paper: Qibiao Wu, Xinbing Sui.

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