Nanoscale

ROYAL SOCIETY OF CHEMISTRY

REVIEW

View Article Online
View Journal | View Issue

Check for updates

Cite this: *Nanoscale*, 2025, **17**, 1812

Recent advances in biotin-based therapeutic agents for cancer therapy

Chao Wang,†[a] Yutao Xiu,†[a] Yujing Zhang,[b] Yanhong Wang,[a] Jiazhen Xu,[a] Wanpeng Yu*[c] and Dongming Xing *[a,b,d]

Biotin receptors, as biomarkers for cancer cells, are overexpressed in various tumor types. Compared to other vitamin receptors, such as folate receptors and vitamin B12 receptors, biotin receptor-based targeting strategies exhibit superior specificity and broader potential in treating aggressive cancers, including ovarian cancer, leukemia, colon cancer, breast cancer, kidney cancer, and lung cancer. These strategies promote biotin transport *via* receptor-mediated endocytosis, which is triggered upon ligand binding. Biotin, as the ligand of the biotin receptor, can be conjugated to anti-cancer drugs to form targeted therapies that bind to receptors overexpressed on tumor cells, thus increasing drug uptake. Despite these advantages, many candidate drugs have progressed slowly and remain in the preclinical stage, impeding clinical translation. This is mainly due to the effects of various conjugation methods and drug formulations on their functionality and efficacy. Therefore, developing novel biotin-based therapeutics is crucial. The innovation of this strategy lies in its multifunctionality—researchers can use different conjugation methods to design and synthesize these drugs, enabling precise targeting of various tumor types while minimizing toxicity to normal cells. These drugs include small-molecule-biotin conjugates (SMBCs) and nano-biotin conjugates (NBCs). This dual-platform approach represents a significant advancement in targeted therapy, offering unprecedented flexibility in drug design and delivery. Compared to chemotherapy drugs and traditional delivery systems, biotin-based drugs with tumor-specific targeting demonstrate enhanced targeting, improved efficacy, and reduced toxicity. This review examines strategies and applications for enhancing the delivery of chemotherapy drugs to cancer cells, highlighting the need for high-quality conjugates and strategies. It not only summarizes the latest progress but also provides key insights into how this emerging field could revolutionize personalized cancer treatment, especially in the context of precision medicine. Additionally, it offers perspectives on future research directions in this field.

Received 11th September 2024,
Accepted 5th December 2024

DOI: 10.1039/d4nr03729d

rsc.li/nanoscale

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

1. Introduction

In recent years, global cancer patients have been increasing. The International Agency for Research on Cancer (IARC) conducted a comprehensive assessment of cancer incidence and mortality across 185 countries, analyzing data from 36 types of cancer. The findings indicate a concerning trend of rising cancer incidence and mortality rates globally (Fig. 1).[1] Cancer treatment has garnered significant attention in the past, present, and future with the aim of reducing tumor mortality rates and improving treatment efficacy. However, the complex pathological nature of cancer presents challenges to effective treatment, leading to a persistent increase in mortality rates. Traditional cancer therapies are inadequate to address these challenges. Consequently, an increasing number of scientists are dedicating their efforts to ongoing research aimed at designing and developing innovative anti-tumor drugs and therapeutic modalities to address this pressing issue.

In consideration of cancer's primary etiological factors, which stem from genetic alterations resulting in specific mutations, unrestricted tumor proliferation, infiltration into normal cells, and ongoing genetic changes, achieving a cure is challenging. Additionally, treatment resistance often develops, rendering drugs ineffective.[2,3] The drug resistance of cancer cells during treatment and the recurrence and metastasis of residual cancer cells are also the leading causes of treatment failure. These factors together make tumors difficult to cure. At present, chemotherapy is a common form of cancer treatment.[4,5] It is a systemic treatment method to kill cancer

[a]*Cancer Institute, The Affiliated Hospital of Qingdao University, Qingdao University, Qingdao, 266071, China. E-mail: xdm_tsinghua@163.com*
[b]*The Affiliated Cardiovascular Hospital of Qingdao University, Qingdao University, Qingdao, 266071, China*
[c]*Qingdao Medical College, Qingdao University, Qingdao 266071, China. E-mail: yuwanpeng1987@163.com*
[d]*School of Life Sciences, Tsinghua University, Beijing, 100084, China*
†These authors contributed equally.

This journal is © The Royal Society of Chemistry 2025

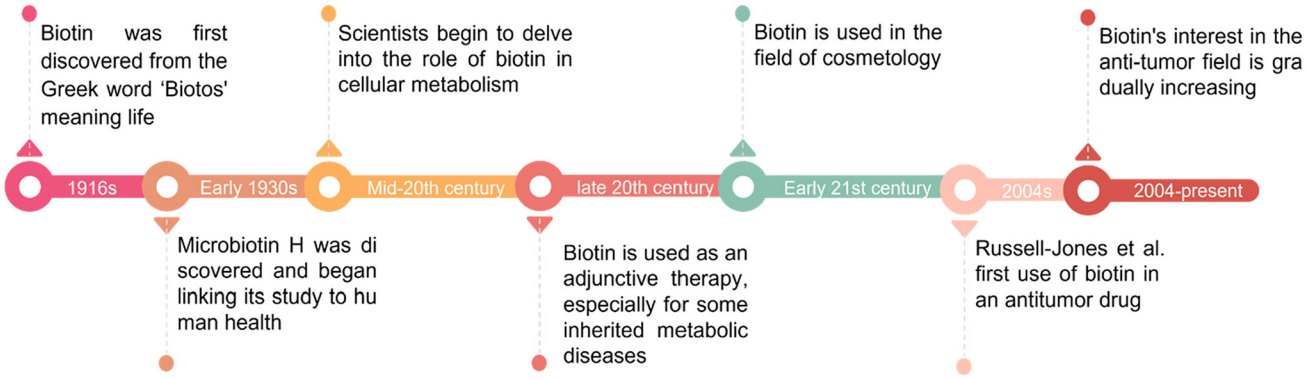


Fig. 1 Biotin is getting more and more attention. Biotin's journey since its development to the present day.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

cells using chemical drugs. After entering the human bodyn b, chemotherapy drugs will circulate throughout most body organs and tissues. Chemotherapy can have a lot of side effects. Simple chemotherapy treatments can significantly impact patients, and nowadays, the sole reliance on chemotherapeutic drugs for treatment has largely been abandoned. Scientists are initiating the design and modification of chemotherapy drugs to synergize with other substances. The integration of targeted agents has significantly enhanced drug efficacy while mitigating their toxic side effects. Recognition of the benefits of targeted drugs has spurred continuous development in this field. Both Chinese and Western pharmaceutical industries are actively engaged in the advancement of targeted drug therapies.[6]

Tumor-targeted drug delivery systems (TTDDSs) offer a promising cancer treatment strategy by selectively delivering cytotoxic agents to tumor cells, minimizing side effects.[7] For optimal efficacy, the chosen receptor should be highly expressed on tumor cells and efficiently localized to the cell surface. Biotin receptors, which are overexpressed in various tumor types such as ovarian, breast, and lung cancers, are ideal candidates due to their high affinity for biotin and their ability to facilitate tumor-specific uptake through endocytosis.[8] Biotin's small size, tumor specificity, and biocompatibility make it a valuable targeting agent for drug delivery (Fig. 3).[9,10]

The main challenge in current cancer treatment is how to maximize the destruction of cancer cells while minimizing damage to normal tissues. Traditional chemotherapy drugs often cause severe side effects due to their lack of selectivity. Although the emergence of targeted therapy strategies has provided a new direction for addressing this issue, there is still a need for the development of more effective targeting systems.[11] In recent years, research on biotin conjugates has received significant attention. Compared to other targeted ligands, biotin offers notable advantages. First, biotin receptors (Sodium-dependent multivitamin transporter, SMVT) are encoded by the SLC5A6 gene and are highly expressed in various cancer cells, with their uptake rate being much higher than that of normal cells.[12] Additionally, the biotin-targeting system demonstrates superior specificity compared to other vitamin receptors, such as folate receptors, and has shown significant efficacy in various aggressive cancers, including ovarian and breast cancers.[13] Third, biotin can conjugate to various therapeutic molecules (such as chemotherapy drugs, genes, and protein-based drugs), demonstrating great flexibility in application. Various biotin conjugates have been successful in cultured cells, animal models, and human clinical trials. These advantages have been confirmed in multiple studies. For example, in drug-resistant breast cancer cells

Chao Wang

Dr Wang received his PhD from Shenyang Pharmaceutical University. He joined the Affiliated Hospital of Qingdao University in 2020. Dr Wang's research focus is on developing novel macromolecules and active small molecules for cancer diagnostic and therapy. He has led and undertaken 5 research projects and published over 30 peer-reviewed papers.

Yutao Xiu

Yutao Xiu graduated from Shandong First Medical University with a bachelor's degree. He is currently pursuing a Master of Science degree at Qingdao University. He majored in bioinformatics. His research focuses on the development of novel macromolecules and active small molecules for cancer therapy.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

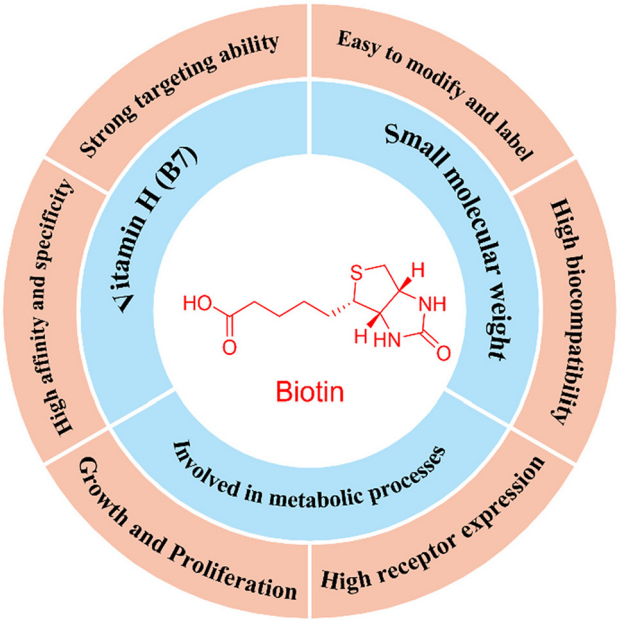


Fig. 2 Biotin has the advantages of small molecular weight, tumor specificity, simple biochemical structure, high affinity and specificity, easy modification and labeling, and biocompatibility.

(MCF-7/ADR), biotin-modified nanocarriers significantly increased drug uptake, reduced drug efflux, and showed a clear reversal of drug resistance.[14] In 2004, Russell-Jones compiled the information on different surface molecules and toxin-binding subunits from various organisms, proposing them as potential carriers for orally delivering other molecules. Specifically, the discussion highlighted the receptor-mediated absorption of molecules like folic acid, vitamins, and VB12, which can be conjugated with active drug substances.[15] Many ligands can act as such targeting agents, *e.g.*, micro-biotin B12, folic acid, and hyaluronic acid, but Gregory Russell-Jones *et al.* suggested that treatment of mice carrying Colo-26 with a doxorubicin–polymer coupling resulted in a greatly enhanced killing of the biotin-targeting drug-polymer complex, but no enhancement of killing was seen with either folic acid or vitamin B12.[16] In 2010, Shuyi Chen *et al.*[17] devised an efficient tumor-targeting drug delivery system relying on tumor-specific vitamin receptor-mediated endocytosis. Significantly, they pioneered the use of biotin conjugation with drugs to synthesize a biotin conjugate. Biotin-based therapeutic agents, a type of TTDDS, represent a method to mitigate the adverse side effects commonly associated with conventional chemotherapy. Biotin-based therapeutics not only reduce the systemic toxicity of chemotherapy drugs through specific targeting but also enhance therapeutic efficacy through multi-drug delivery strategies. Studies have shown that biotin-modified nanocarriers can deliver both chemotherapy drugs and chemosensitizers

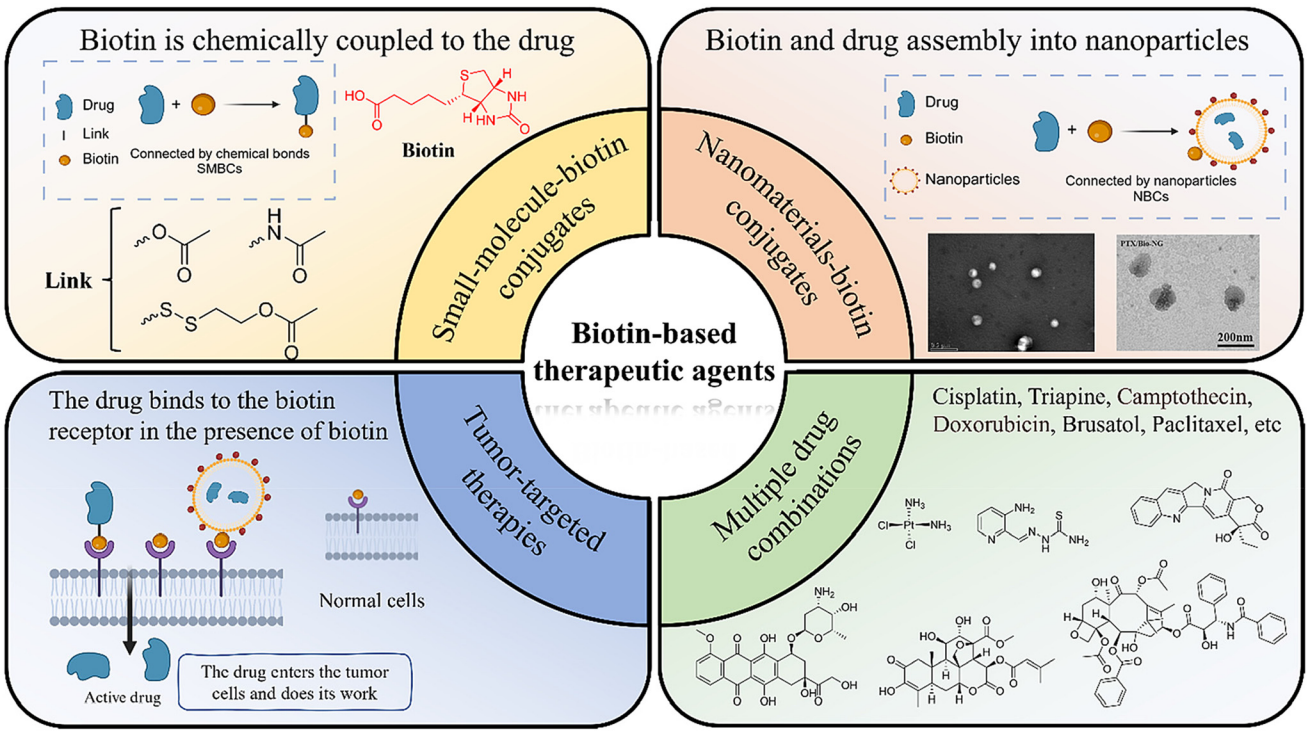


Fig. 3 Biotin has a variety of binding modes to drugs, which can be classified into two main groups: the SMBCs and the NBCs. Tumor cells have a greater abundance of biotin receptors guided by these receptors compared to normal cells. Biotin translocates and binds to them, facilitating the uptake of biotin by tumor cells.

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

simultaneously, significantly improving treatment outcomes for drug-resistant tumors. This multifunctionality gives biotin-based therapies a unique advantage in overcoming the limitations of current cancer treatments. This review will spotlight the recent strides made in leveraging biotin receptors, particularly the biotin receptor, as the primary target for tumor-targeted drug delivery systems. The understanding and applications of biotin have evolved since its discovery in 1916, and people are gradually discovering new functions for biotin. In 2004, Russell-Jones *et al.* discovered a new field of biotin by applying it for the first time in the field of anti-tumor. After that, biotin will be continuously used in anti-tumor applications, and people are more and more discovering how biotin goes hand in hand with organic molecules and conducting anti-tumor tests. However, the various binding modes between biotin and anti-tumor agents wield significant influence over the efficacy and toxicity of tumor treatment. Consequently, there is a continuous exploration of different binding modes to ascertain the optimal approach yielding the most favorable outcomes. However, this presents a significant challenge, encompassing the selection of targets, the variety of linking methods, drug selection, inefficient delivery, low release rates, and drug inactivation post-conjugation, all of which impede the development of patentable drugs (Fig. 2).

In conclusion, side effects greatly affect patient outcomes during conventional radiation therapy. Biotin-based therapeutic agents serve as a viable targeted drug that can significantly reduce the side effects of drugs on patients and enhance their effectiveness against tumors. However, the strength of this therapeutic strategy is highly dependent on the specific binding mode between biotin and antitumor drugs. Research efforts to explore the binding modes and coupling drugs of biotin couplers are ongoing, laying the groundwork for the introduction of biotin-based therapeutic agents.[18] This article provides an in-depth analysis of the recent discoveries of biotin couplers and the progress in the development of the new generation of biotin-based therapeutic agents (biosmall molecule couplers, biomacromolecule couplers). This review aims to increase our understanding of this emerging field and contribute substantially to the advancement of biotin coupler-based cancer therapy.

2. The advantages and limitations of biotin

Biotin-targeted drug delivery systems have shown significant potential in cancer treatment, but they also face multiple clinical challenges. In drug delivery, biotin can selectively deliver drugs to cancer cells through receptor-mediated endocytosis. This is because many cancer cells (such as leukemia L1210FR, ovarian cancer Ov2008, colon cancer Colo-26, lung cancer M109, and breast cancer 4T1, among others) overexpress biotin receptors compared to normal tissues.[17,19] However, studies have found that the expression patterns of biotin receptors exhibit significant cell specificity. For example, in breast cancer cell lines, the MCF-7 and MDA-MB-231 cell lines show higher levels of biotin receptor expression, while the MDA-MB-157 cell line demonstrates markedly lower expression. This expression difference is closely related to the molecular characteristics of the cells, especially in claudin-low subtype breast cancer. MDA-MB-157, as a typical claudin-low cell line, not only exhibits low biotin receptor expression but also shows enrichment of epithelial–mesenchymal transition (EMT) characteristics. This heterogeneity in expression profiles suggests that when developing biotin receptor-based targeted therapeutic strategies, the impact of tumor molecular subtypes must be carefully considered, providing crucial insights for the development of personalized treatment plans.[16] This finding also opens new directions for understanding the role of biotin receptors in tumor progression.[13]

Biotin, folate, vitamin B12, and riboflavin are essential for all cells, especially for tumor cell division, and have recently been tested experimentally as targeted drugs.[20] Biotin seems to be associated with sodium co-transport, as biotin uptake into PBMCs decreases when extracellular sodium is replaced by choline, lithium, or ammonium.[21] However, it is important to note that biotin-conjugated drugs have shown good targeting properties in cell culture and animal models, but their immunogenicity may affect their clinical application. Moreover, high concentrations of endogenous biotin can interfere with the effect of biotin-conjugated drugs, thus limiting their effectiveness as targeting agents. Attaching biotin to small molecules can also affect cell permeability and phenotypic outcomes, which could be a drawback when handling live cells.[22] Treating cells with biotinylated compounds can reduce IL-2 production, and in short-term cell culture assays, this reduction may not have immediate harmful effects, but it can limit immune cell activation and proliferation, potentially affecting their response to immune challenges.[23,24] These limitations are challenges that biotin-based drugs must overcome in their clinical application.

Biotin-targeted therapy has shown unique advantages in the field of anticancer treatment, but its effectiveness and applicability still need to be further explored compared to other targeted drugs. First, biotin receptors are commonly overexpressed in various cancer types (such as leukemia, ovarian cancer, and breast cancer), which allows biotin-based drugs to more effectively target tumor cells. For example, studies have shown that biotinylated antibodies or drugs can promote drug internalization by binding to biotin receptors on the surface of tumor cells, thereby enhancing therapeutic efficacy. Compared with targeted drugs such as folic acid, biotin has a wider applicability. Folate receptors are mainly expressed in certain types of tumors (such as ovarian cancer, breast cancer, lung cancer, endometrial cancer, colorectal cancer, brain tumors),[25,26] while biotin receptors are highly expressed in a wider range of cancer cells, and the expression level is higher than that of folate. This specificity allows biotin-based therapies to be effective across a broader patient population. Additionally, biotinylated drugs typically have a smaller molecular weight and better tissue permeability, which helps

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

enhance their accumulation in the tumor microenvironment.[27] In the case of antibody–drug conjugates (ADC), although ADCs achieve precise tumor targeting by linking cytotoxic drugs with specific antibodies, their efficacy is often influenced by the expression levels of the target antigen and the cellular internalization process. In contrast, biotinylated drugs can achieve more efficient cellular uptake through biotin receptor-mediated endocytosis, thereby enhancing the anticancer effect. For example, studies have shown that biotin-conjugated chemotherapy drugs exhibit significantly enhanced cytotoxicity in HER2-positive breast cancer cells, whereas traditional ADCs may lose their effectiveness due to insufficient antigen expression. However, despite the numerous advantages of biotin-targeted therapies, there are also some limitations. For instance, resistance mechanisms may affect their long-term efficacy, as certain tumor cells could evade treatment by down-regulating biotin receptors or altering metabolic pathways. Furthermore, the optimal dose and dosing regimen have yet to be determined, which may lead to uncertainty in clinical applications. Therefore, when further developing biotin-based therapeutic strategies, these factors must be carefully considered to ensure their efficacy and safety in clinical practice.

3. Small-molecule–biotin conjugates (SMBCs)

3.1. Biotin–drug conjugates

Traditional chemotherapy drugs, due to their unique molecular composition and structural characteristics, often exhibit low specificity, high cytotoxicity, and significant drug resistance. To address these limitations and improve the practicality of chemotherapeutic agents, biotin has been conjugated with chemotherapy drugs to synthesize biotin-based small molecule therapeutics, forming an implementable targeting strategy. This strategy involves conjugating biotin with chemotherapeutic drugs (such as antitumor drugs and photosensitizers[28]) through chemical bonds, such as disulfide bonds[29] and pH-sensitive bonds.[30] This strategy has clear advantages: using biotin allows for more flexible targeting of specific proteins while maximizing the reduction of off-target effects, enhancing selectivity towards the desired target proteins, and minimizing interactions with non-specific cellular components. Furthermore, this method allows for potential modifications or adjustments based on emerging scientific knowledge or new drug development strategies without the need to completely redesign the entire compound structure from scratch. This strategy offers a more practical approach to overcoming the challenges associated with traditional chemotherapy drugs.

3.1.1. Targeting DNA. Cisplatin, as the first metal-based anticancer drug to be approved, has been an effective treatment for various tumors since its approval by the FDA in 1978.[31] Cisplatin primarily exerts its anticancer effects by binding to DNA, thereby blocking cell division and proliferation, ultimately inducing necrosis or apoptosis. However, it also causes significant toxicity to normal tissues, leading to severe side effects during treatment such as nausea, vomiting, renal dysfunction, and neurotoxicity. Moreover, with continued treatment, cancer cells gradually develop resistance to cisplatin through mechanisms such as enhanced DNA repair, increased drug efflux, and alterations in the intracellular microenvironment, resulting in a marked reduction in drug efficacy. This issue is particularly pronounced in recurrent tumors. These challenges not only diminish patients' quality of life but also limit the long-term application of cisplatin. Consequently, reducing cisplatin's toxicity and overcoming drug resistance have become key driving forces in the development of next-generation platinum-based anticancer drugs. Researchers are actively exploring approaches such as chemical structure modifications or conjugation with other biomolecules to develop platinum compounds with better tumor selectivity, lower toxicity, and the ability to effectively overcome resistance.[32,33]

To alleviate the side effects of cisplatin, researchers have utilized the targeting properties of biotin by integrating it into platinum-based compounds, aiming to enhance their targeting capability and efficacy.[34] Leveraging this feature, in 2019, Suxing Jin *et al.* synthesized a novel Pt(IV) complex (DPB, Fig. 4, compound 1), which uniquely combined biotin, cisplatin, and dichloroacetic acid (DCA).[35] Pt(IV) complexes are typically synthesized by oxidizing Pt(II) precursors and conjugating axial ligands to incorporate biotin and DCA (Fig. 4A). Biotin played a critical role in this complex; experimental results demonstrated that DPB significantly increased tumor cell uptake due to biotin's tumor-targeting capability, which promoted drug accumulation in tumor cells, thereby enhancing efficacy. Additionally, the introduction of DCA allowed

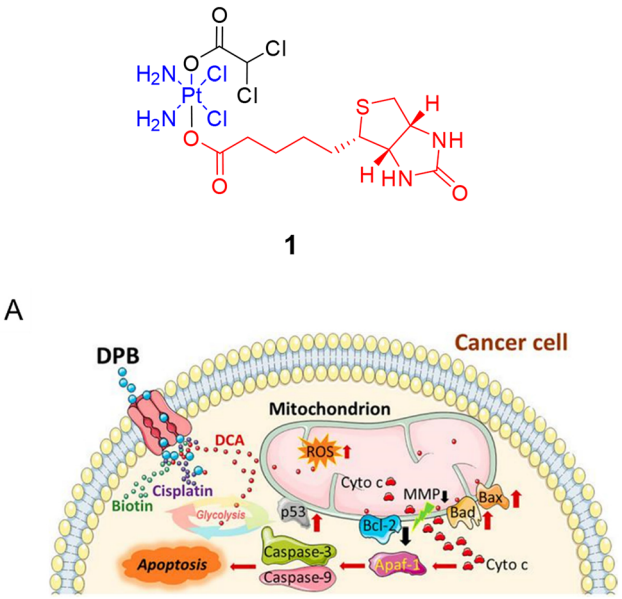


Fig. 4 Chemical structures of compound 1. (A) Mitochondrial pathway for the DPB-induced apoptosis in HeLa cells. Reproduced with permission.[35] Copyright 2019, ACS.

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

DPB to further disrupt cancer cell glycolysis, improving its therapeutic effects. DPB also exhibited enhanced lipophilicity and reactivity, interfering with cellular energy metabolism and deepening its tumor selectivity through biotin's targeting action, which reduced side effects on normal cells.

Subsequently, to further validate biotin's role in cisplatin-based therapies, in 2020, Xing Wang *et al.* developed a trifunctional Pt(II) complex incorporating biotin.[36] They synthesized compound **2** (Fig. 5) by introducing biotin into the Pt(II) complex DN604 and conjugating biotin with a group of naphthalimide compounds. Compound **3** (Fig. 5) was then synthesized by combining compound **2** with the known Pt(II) complex DN604. Compound **3** not only retained the targeting and fluorescent diagnostic capabilities of compound **2** but also introduced DN604's ability to induce apoptosis and inhibit DNA damage repair. Biotin again played a pivotal role in enhancing drug uptake in A549/cDDP and A2780/cDDP tumor cells, effectively overcoming these cells' resistance to cisplatin. In this complex, biotin binds to biotin receptors on the tumor cell surface, improving drug targeting, significantly reducing off-target toxicity, and mitigating the common off-target effects encountered during chemotherapy. In *in vitro* experiments, compound **3** exhibited a 21.41% higher apoptosis induction rate in A549/cDDP cells compared to cisplatin, and the accumulation of Pt(II) in DNA was 1.6 times that of cisplatin. This effect was directly attributed to biotin's efficient targeting action, which not only increased Pt drug accumulation in tumor cells but also elevated intracellular ROS levels, disrupting the redox balance in tumor cells. This eventually led to mitochondrial damage, accelerated mitochondrial membrane depolarization, and induced tumor cell death (Fig. 5A). These studies not only highlighted the immense potential of biotin in drug targeting delivery but also provided important directions for developing more effective platinum-based anticancer drugs in the future.

Despite the clinical success of platinum-based anticancer drugs, they face challenges of resistance and severe side effects.[37] To address these issues, ruthenium-based anticancer

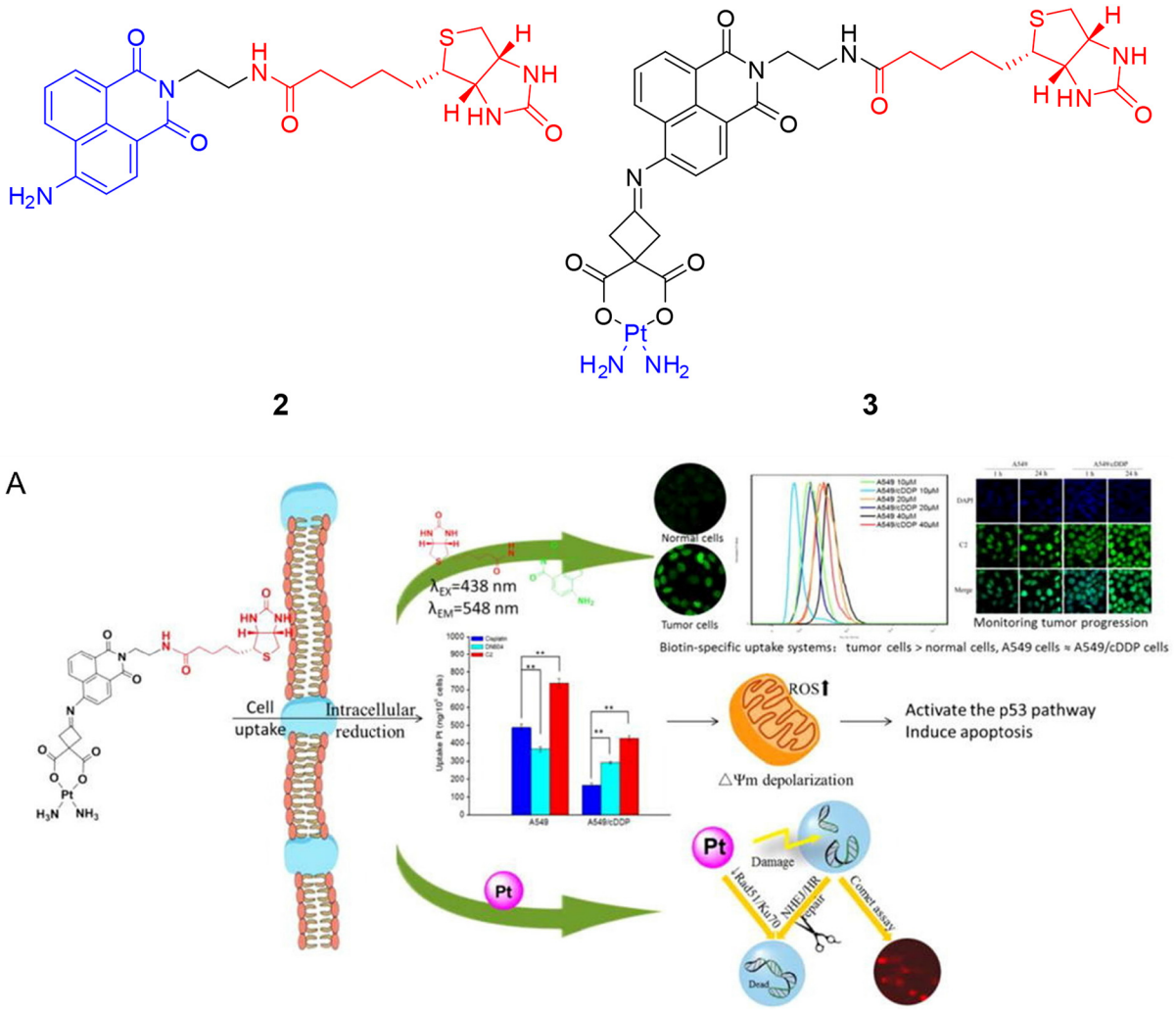


Fig. 5 (A) Chemical structures of compounds **2** and **3**. (A) Mechanisms of action and pharmacodynamic outcomes of drugs. Reproduced with permission.[36] Copyright 2020, Elsevier.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

complexes have recently emerged as alternative candidates, offering lower cytotoxicity and reduced cross-resistance to platinum drugs.[38] In 2019, Leonor Corte-Real *et al.* designed a series of biotin-conjugated ruthenium(II) complexes (Fig. 6).[39] To further investigate this family of compounds, the authors modified the structure of compound **4** and synthesized two novel compounds, compound **5** (Fig. 6) and compound **6** (Fig. 6), expanding the compound family. Biotin played a key role in these compounds as well, improving drug selectivity in invasive MDA-MB-231 cells by binding to biotin receptors on the cell membrane. Additionally, as a P-glycoprotein (P-GP) inhibitor, biotin exhibited pronounced selectivity and showed significant anti-metastatic potential. Studies demonstrated that when biotin was conjugated to ruthenium complexes, the drugs exhibited significant distribution on cancer cell membranes, improving drug accumulation and activity in tumor cells. In a zebrafish model, these compounds displayed lower IC_{50} values, induced apoptosis-mediated cell death, inhibited cancer cell colony formation, and exhibited higher tolerance than non-biotinylated compounds (Fig. 6A–D). Furthermore, the introduction of biotin enabled these ruthenium complexes to outperform non-biotinylated counterparts in inhibiting cancer cell colony formation, inducing apoptosis, and enhancing tolerance. This strategy offers valuable insights for the future development of metal-based anticancer drugs, especially as alternatives to platinum-based therapies.

In conclusion, biotin-conjugated platinum and ruthenium drugs have demonstrated excellent antitumor activity. Biotin's targeting capability not only enhanced the antitumor efficacy of these drugs but also significantly improved their utilization and selectivity. However, these biotin-based drugs have yet to reach the market, indicating that further optimization of synthesis techniques is needed. This discovery provides broad prospects for the application of biotin in targeted anticancer drug delivery and opens new avenues for future drug development.

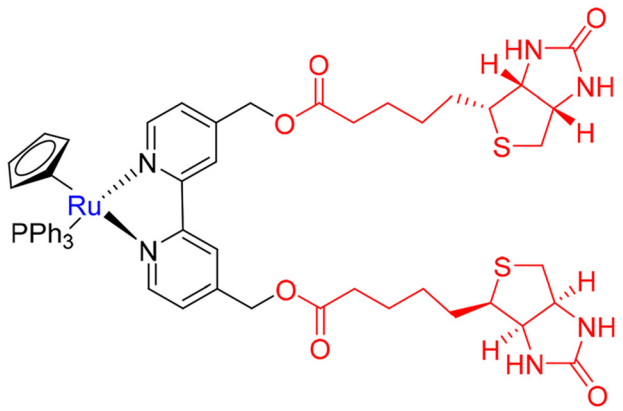
3.1.2. Targeting DNA topoisomerase I (TOP1). Topoisomerases resolve topological barriers during DNA replication, transcription, recombination, and chromatin remodeling by triggering transient single- or double-stranded DNA breaks, which are essential for maintaining the stability of DNA structure and therefore play an indispensable role in cellular growth, development, and reproduction.[40] Their main function is to prevent the accidental release of broken DNA strands by creating temporary covalent bonds between proteins and broken DNA strands and thus avoiding genomic damage.[41] This suggests that topoisomerase plays a crucial role within cells, and any abnormalities in it could potentially trigger apoptosis in both normal and tumor cells. Thus, inhibition of topoisomerases has become an effective method to suppress cancer. Its main function is to prevent the accidental release of broken DNA strands by creating temporary covalent bonds between proteins and broken DNA strands, thus avoiding genome damage. Inhibition of topoisomerases has thus become an effective method of suppressing cancer. In 1966, Wall and Wani discovered a pentacyclic monoterpene alkaloid, comedophylline (CPT), from the Chinese arborvitae tree, Tree of Hibiscus.[42] CPT was found to be effective in inhibiting topoisomerases, and has been shown to be effective in the treatment of tumors. However, CPT suffers from poor water solubility and low bioavailability, and the α-hydroxyl lactone ring in its chemical structure is prone to hydrolysis under neutral or alkaline conditions to form an inactive ring-opening carboxylic acid form, which results in reduced stability and efficacy *in vivo*.[43] In addition, CPT may also cause complications, such as hemorrhagic cystitis.[44] In an effort to overcome these limitations, in recent years researchers have attempted to conjugate biotin to CPT to improve its efficacy conjugated with biotin to improve its efficacy and stability.

In 2018, to address the side effects of CPT, Yuanwei Liang *et al.* integrated biotin, disulfide bonds, and CPT.[45] A compound named Biotin-SS-CPT was designed by fully utilizing the properties of disulfide bonds (35) and biotin (compound **7**, Fig. 7).[46] Biotin confers a targeting effect and S–S confers a controlled release capability. The S–S bond is reduced by thiols upon uptake, leading to intramolecular cyclization and subsequent cleavage of the adjacent organic carbonate bond, which releases CPT. The authors performed toxicity assessment, magnetic resonance imaging, and pathological analysis of compound **7** and free CPT. The experimental results showed that compound **7** exhibited excellent antitumor activity in MGC803 cells. Notably, compound **7** was able to significantly release CPT under specific pH (5.3, 6.8 and 7.4) conditions, suggesting that CPT may achieve efficient release in tumor cells. High pressure liquid chromatography (HPLC) analysis further confirmed the stability of compound **7** under physiological conditions. In the presence of biotin, the uptake of compound **7** in normal cells was significantly reduced. In addition, compound **7** exhibited inhibitory effects on both cell cycle progression and migration of tumor cells (Fig. 7). The pre-drug compound **7** synthesized by the authors was able to specifically and selectively identify cancer cells, effectively localize and accumulate at the tumor site, and precisely release CPT through biological response, showing good potential for clinical application. The present study not only validated the effectiveness of the drug, but also significantly demonstrated that the introduction of biotin reference enhanced its efficacy.

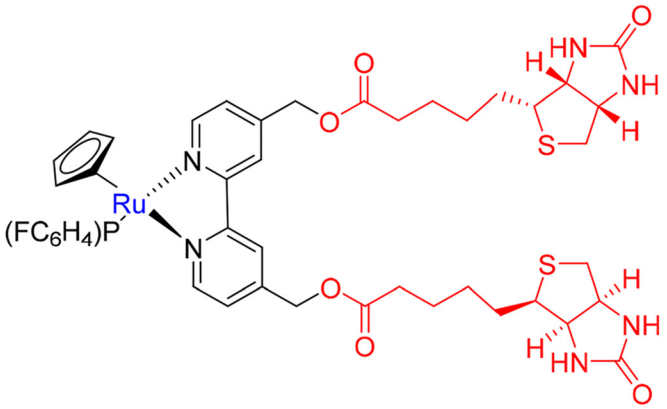
Unlike the approach of Yuanwei Liang *et al.*[45] in 2022, Lingyan Liu *et al.* developed a prodrug system activated by ROS in order to solve the problem of the low targeting efficiency of CPT on tumor cells, and four candidate products were obtained, which were named as Bio-(8)-MB-CPT (Fig. 8, compound **8**), Bio-(4)-MB-CPT, (8)-MB-CPT and (4)-MB-CPT.[47] Subsequent validation revealed that the fluorescence intensity of compound **8** was significantly enhanced in the presence of reactive oxygen species (ROS), which demonstrated that the release of CPT was triggered by ROS. Furthermore, stability analysis showed that the lactone ring of compound **8** was more stable than the parent CPT. The authors observed no significant pathologic damage to major organs by H&E staining, and the compound has a favorable safety profile and targeted efficacy. When biotin was present, the drug showed significant efficacy in inhibiting tumor cell viability (Fig. 8A–D). These

 This journal is © The Royal Society of Chemistry 2025

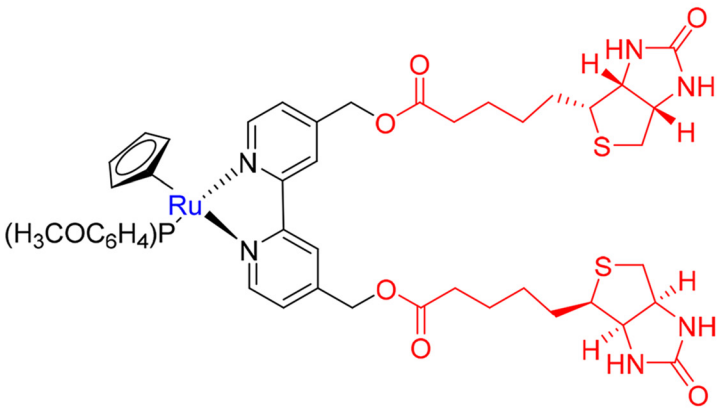
Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



4



5



6

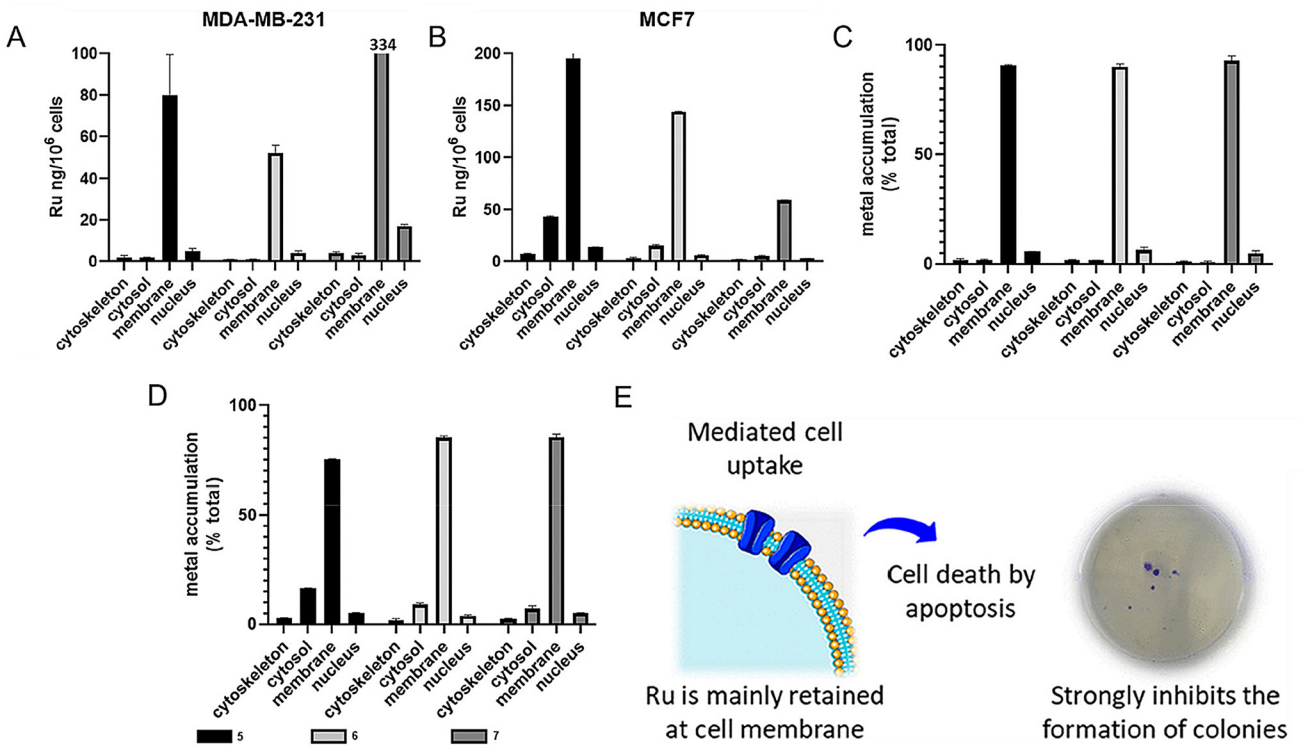
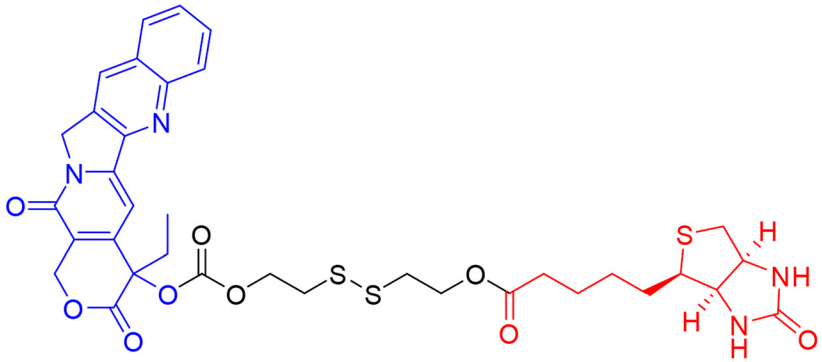


Fig. 6 Chemical structures of compounds **4**, **5** and **6**. (A–D) Distribution of Ru in cells after drug treatment. (E) Representative images of colony formation assay. Reproduced with permission.[39] Copyright 2019, ACS.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



7

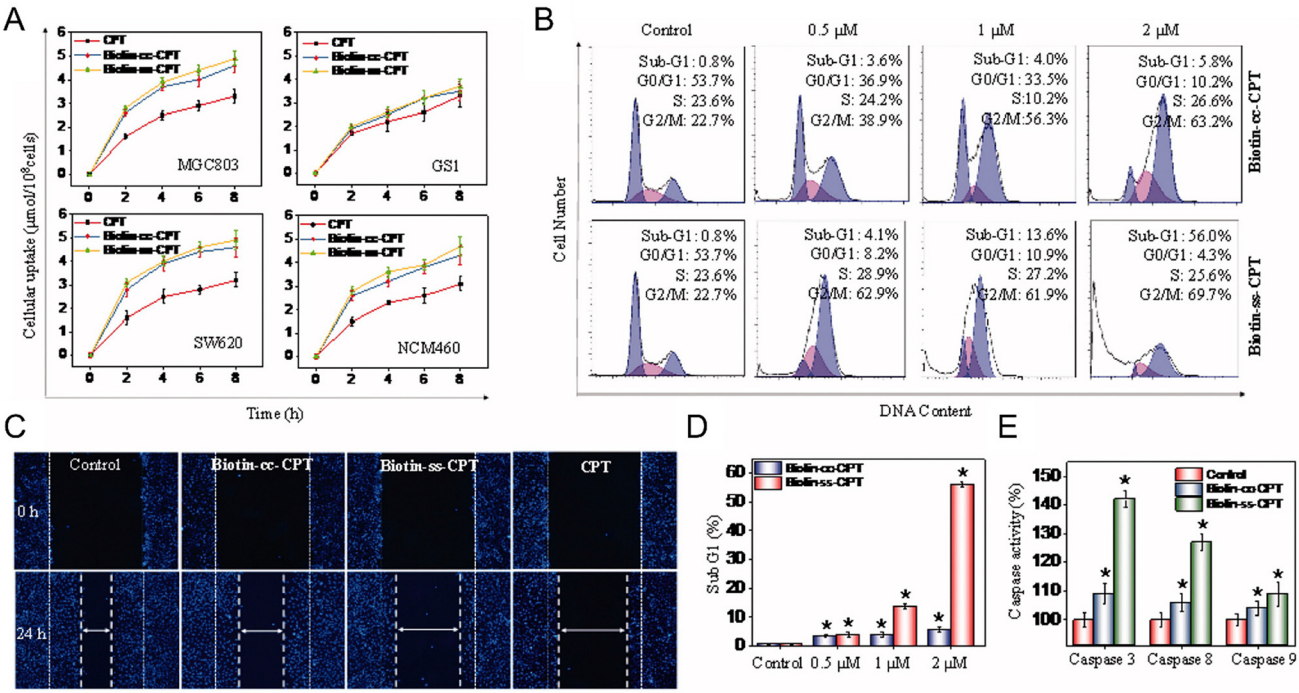


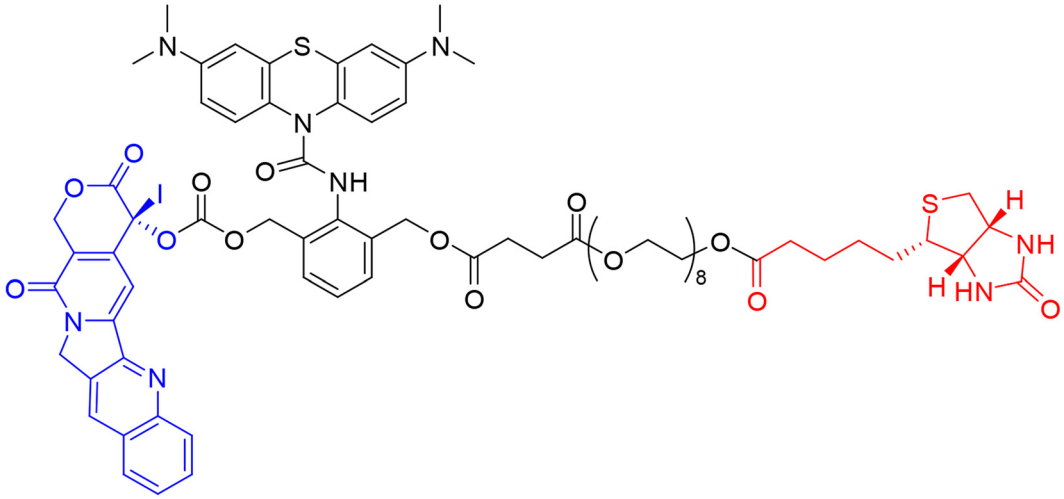
Fig. 7 Chemical structures of compound 7. (A) Cellular uptake of biotin couplers in normal and tumor cells. (B) Cell cycle analysis. (C) Drug-mediated inhibition of cell migration. (D) The proportional of Sub-G1 phase population. (E) Enzymatic activities of cysteine 8, 9, and 3. Reproduced with permission.[45] Copyright 2018, Taylor & Francis.

findings suggest that compound **8** can specifically target tumor cells, release CPT, and exert potent antitumor effects. The approach adopted by the authors provides a promising strategy for the development of effective treatments for regionally metastatic cancers.

3.1.3. Targeting DNA topoisomerase II (TOP II). DNA TOP II enzymes are ubiquitous in all branches of life and can modify the DNA superhelix and separating double-stranded DNA fragments during key cellular processes such as replication and transcription. Specifically, type II topoisomerases play a crucial role in unraveling newly replicated sister chromatids within the cell.[48] DOX,[49] which is a DNA topoisomerase II inhibitor, has been playing an important role in antitumor therapy for many years and has demonstrated broad efficacy in clinical applications. As an antitumor antibiotic, DOX disrupts RNA and DNA synthesis by interfering with TOP, leading to DNA insertion and disrupting TOP II-mediated DNA repair Due to its potent inhibition of RNA synthesis and broad antitumor spectrum, DOX efficiently hinders the growth of many tumor types, earning its status as a versatile anticancer agent. Due to its non-cell cycle specificity, it has cytotoxic effects at all stages of tumor cell growth. However, the clinical application of DOX has been hampered by its lack of tumor specificity, severe cardiotoxicity and hematotoxicity. However, the emergence of pre-targeting strategies offers a promising avenue for its highly targeted tumor therapy.[50]

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



8

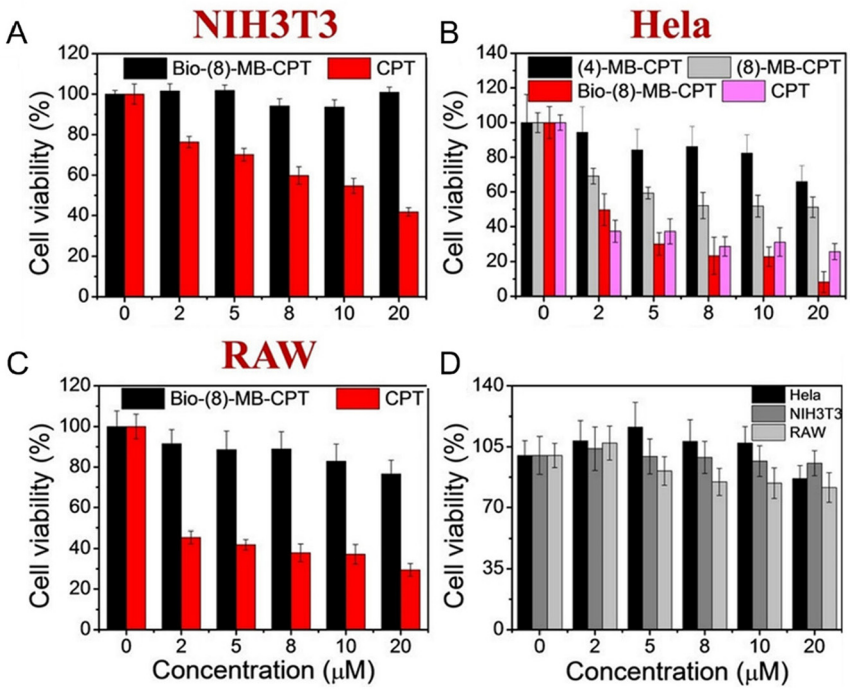
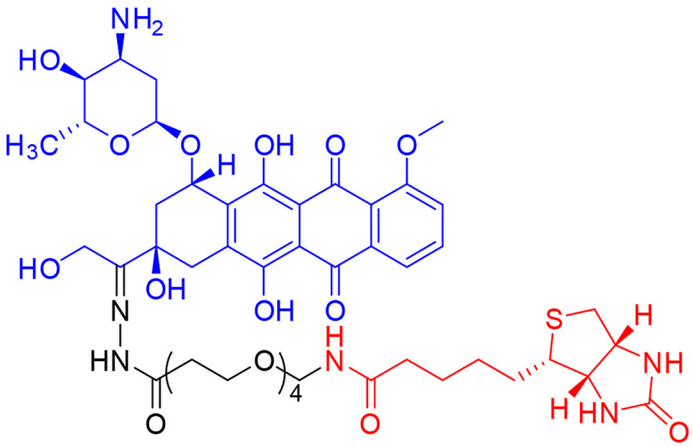


Fig. 8 Chemical structures of compound **8**. CCK-8 assay was used to determine the cell viability of free CPT and prodrug at different concentrations in (A) NIH3T3 cells, (B) HeLa cells, (C) RAW364.7 cells and (D) NAC pretreated cells. Reproduced with permission.[47] Copyright 2022, Wiley.

Therefore, in 2019, Meinan Yao *et al.*[51] designed a new pre-targeting system that couples DOX to mini-polyethylene glycolized biotin *via* pH-sensitive bonds to produce DOX prodrugs (bDOX) (Fig. 9, compound **9**). pH-Sensitive bond breaking varied with pH level; at pH 6.0, the release rate of DOX was approximately 64%, while at pH 5.0 at pH 5.0, the release rate of DOX increased to 97%. In pretargeted therapy, bDOX selectively enters tumor cells and is taken up in large quantities under the influence of affinities and biotin, followed by release of DOX in the uniquely acidic pH environment of the tumor cells (typically around pH 5.3). Pretargeted bDOX was uptaken by human colorectal tumor cells (LS180 and HT-29 cells) increased as compared to free DOX (Fig. 9A–C). *In vivo* studies using an LS180 xenograft animal model demonstrated that pre-targeted bDOX had better tumor inhibitory effects compared to free DOX. Notably, there was a significant reduction in cytotoxicity as evidenced by changes in body weight, cardiomyocyte apoptosis, routine blood parameters, and splenic pathologic changes. In summary, the pre-targeting strategy using bDOX provides a promising approach to enhance tumor-

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



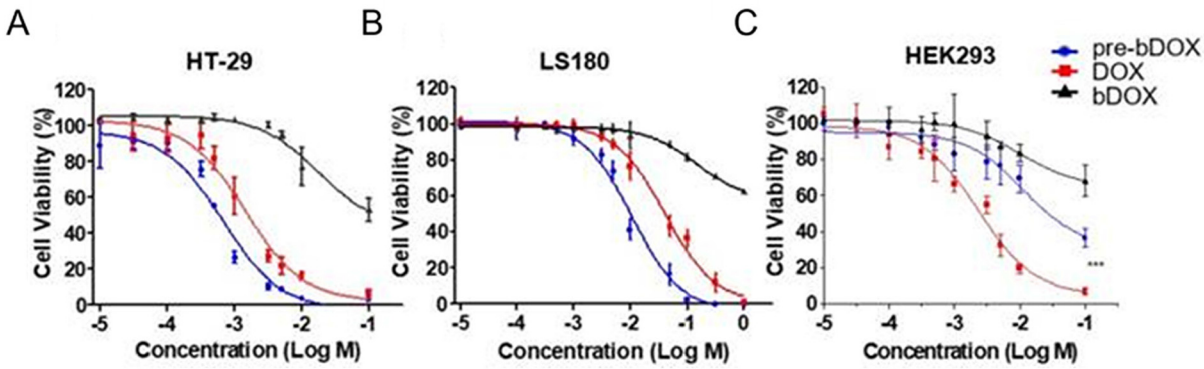


Fig. 9 Chemical structures of compound **9**. Chemotherapeutic effect of bDOX *in vitro*. Relative cell viability of (B) HT-29, (C) LS180 and (D) HEK293. Reproduced with permission.[51] Copyright 2019, Ivyspring International Publisher.

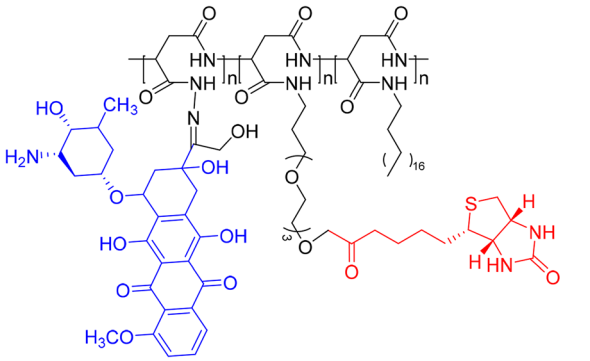
specific drug delivery and minimize systemic toxicity, thereby improving the therapeutic index of DOX in cancer therapy.

In 2020, Minh Phuong Nguyen *et al.*[52] in an effort to further advance this field, designed a multifunctional biopolymer (Fig. 10, compound **10**) to further mitigate the side effects associated with DOX on top of enhancing drug delivery efficiency. Compound **10** is a mixture of polyaspartic acid (PA) with biotin and DOX functional groups supplemented with superparamagnetic iron oxide nanoparticles (SPIONs) designed to improve targeting and drug delivery to cancer tumors. Validation of the *in vitro* uptake of the polymers consisted of examining cellular uptake under the influence of biotin *via* Prussian blue stained images. The results showed enhanced uptake of biotin by tumor cells, particularly 4T1 cells, indicating the efficacy of biotin in improving tumor cell uptake. *In vitro* drug release studies showed that less than 60% of DOX was released within 72 h at pH 6.0 and 7.4, whereas more than 70% of DOX was released at pH 5.0, suggesting that the polymer has the potential to promote significant drug release from the tumor microenvironment (TME). Cytotoxicity assessment using MTT assay in normal (3T3) and cancerous (4T1) cells showed that the cellular activity was higher than 85% in 3T3 cells and lower than 50% in 4T1 cells, suggesting that cancerous cells were selectively damaged (Fig. 10A–D). The results indicate that compound **10** has good selectivity and low toxicity to normal cells. Moreover, these results emphasize the important role of biotin in this regard. The authors further validated the efficacy of tumor treatment in BALB/c mice, in which tumor growth was significantly inhibited in mice injected with DOX polymer compared to controls. PA, as a multifunctional polymer, combined with biotin and DOX functional groups, enhanced the targeting of cancer cells and induced damage to cancer cells. *In vivo* tumor experiments demonstrated a three-fold reduction in tumor volume growth after injection of this polymer compared to controls, highlighting its potential for effective cancer treatment. The development of multifunctional biopolymers could improve DOX and increase its efficacy in cancer treatment.

3.1.4. Targeting nuclear factor erythroid 2-related factor 2 (Nrf2). Brusatol (Bru) is a potent inhibitor of the Nrf2 pathway, showing promise as an adjuvant chemotherapy compound for various tumor types. It induces rapid and transient depletion of Nrf2 protein in mouse Hepa-2c1c1 hepatoma cells through post-transcriptional mechanisms and also inhibits Nrf2 in

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



10

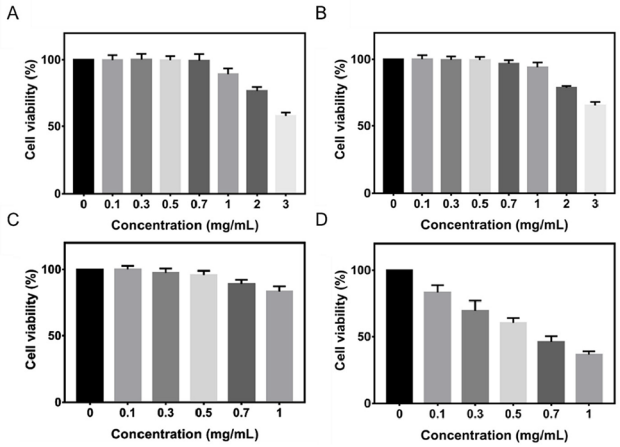


Fig. 10 Chemical structures of compound **10**. Cytoxicity of (A) SPIONs-PA without Dox on 3T3 cells; (B) SPIONs-PA without Dox on 4T1 cells; (C) SPIONs-PA with Dox on 3T3 cells; and (D) SPIONs-PA with Dox on 4T1 cells. Reproduced with permission.[52] Copyright 2020, Institute of Physics, IOP Publishing.

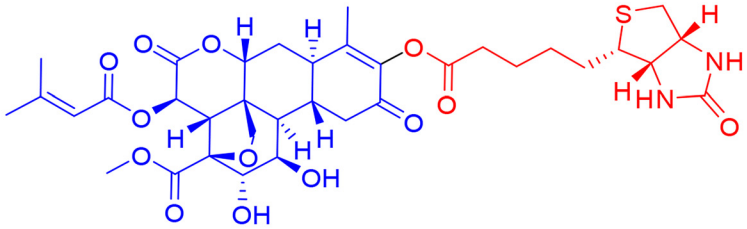
primary human hepatocytes.[53] While Bru has been demonstrated to disrupt redox homeostasis and act as a sensitizer in combination with other anti-cancer therapies, its efficacy as a monotherapy against certain cancers raises concerns about target specificity and systemic toxicity.[54]

In 2022, Shangping Xing *et al.*[55] synthesized a Bio-Bru complex by coupling biotin with Bru (Fig. 11, compound **11**). Notably, the IC_{50} values of Bru and Bio-Bru are 5 nM and 5000 nM, respectively, indicating a significant decrease in cytotoxicity for the complex compared to the parent compound. We investigate whether Bio-Bru exhibits comparable anticancer potential to Bru. The IC_{50} values of Bru at 48 h are 56.23 nM and 46.28 nM, while those of compound **11** are 980.12 nM and 762.65 nM for H1299 and A549 cells, respectively (Fig. 11A). Despite compound **11** demonstrating lower cytotoxicity compared to the parent compound, it still exhibits promising anticancer effects *in vitro*. Additionally, both Bru and compound **11** induce G0/G1 phase arrest in non-small cell lung cancer (NSCLC) cells, as depicted in Fig. 11B and C. Compound **11** exerts a potent effect on NSCLC cells, influencing their proliferation, migration, invasion, and Nrf2 protein levels. This suggests that the addition of biotin does not alter the anti-cancer mechanism of Bru and retains its anti-cancer activity. While the specific targeting effect of compound **11** requires further investigation, its potential merits attention.

3.1.5. Targeting ribonucleotide reductase (RR). Triapine is a synthetic heterocyclic carboxaldehyde thiosemicarbazone with potential antineoplastic activity (PubChem CID: 9571836). Triapine can inhibit RR, thereby blocking the conversion of diphosphoribonucleotides to deoxyribonucleotides necessary for DNA synthesis. Although Triapine has demonstrated tumor growth inhibition *in vitro*, its effectiveness in actual tumor treatment needs to be improved. In 2018, Sebastian Kallus *et al.*[56] synthesized three biotin-thiosemicarbazone conjugates: BioTriapine, BioFTSC1 and BioFTSC2 (Fig. 12, compounds **12**, **13** and **14**) and their metal complexes with biologically relevant metal ions, Cu-BioTriapine (Cu(II)) and Fe-BioTriapine (Fe(III)) (Fig. 12, compounds **15** and **16**). It is noteworthy that the anticancer activity of Triapine was slightly reduced after coupling with biotin (Fig. 12A). The anticancer activity of the ethylene-conjugated derivative (compound **13**) was lower than that of the butene-conjugated derivatives (compounds **14** and **12**). In addition, the coordination of the metal complexes Cu(II) and Fe(III) significantly weakened the activity of compound **12**. However, for the whole, the introduction of biotin improved its targeting properties. The authors evaluated the antitumor activity of compound **12** and triazepam using CT-26 colon cancer mice and showed that tumors treated with BioTriapine had a higher survival rate (Fig. 12B) and a higher safety profile compared to tumors treated with triazepam alone. To assess the role of SMVT in drug uptake by cancer cells, the authors performed inhibition experiments with the SMVT inhibitor indomethacin. Indomethacin significantly reduced the uptake of FITC-labeled biotin by MCF-7 cells, but not by HCT-116 cells, but did not prevent the accumulation of compound **15** (or Cu-Triapine) in the cells (Fig. 12C and D). Although biotin referencing reduced the antitumor activity of Triapine, it greatly improved its safety. Biotin–Triapine coupling strategies still need to be improved.

3.1.6. Targeting tubulin. Microtubules, composed of microtubulin dimers, form long, hollow cylinders and play crucial roles in cell structure and division.[57] These proteins, belonging to five distinct families, are primarily found in kinetoplastid protozoa. Tubulin family members α-tubulin and β-tubulin play essential roles in microtubule formation, distributed throughout the cytoplasm as vital components of the cytoskeleton. Alongside microfilaments and intermediate filaments, microtubules participate in various cellular processes, including cell structure maintenance and intracellular transport. They serve as tracks for the movement of organelles, secretory vesicles, and other cellular materials. Given their critical role in cell division, microtubules represent intriguing targets for anticancer drugs.[58] Colchicine is a natural compound known for its inhibitory properties. Derived from the toxic meadow saffron plant, colchicine was the first microtubule protein destabilizer discovered. It exhibits a strong affinity for microtubulin and can polymerize with it to form

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



11

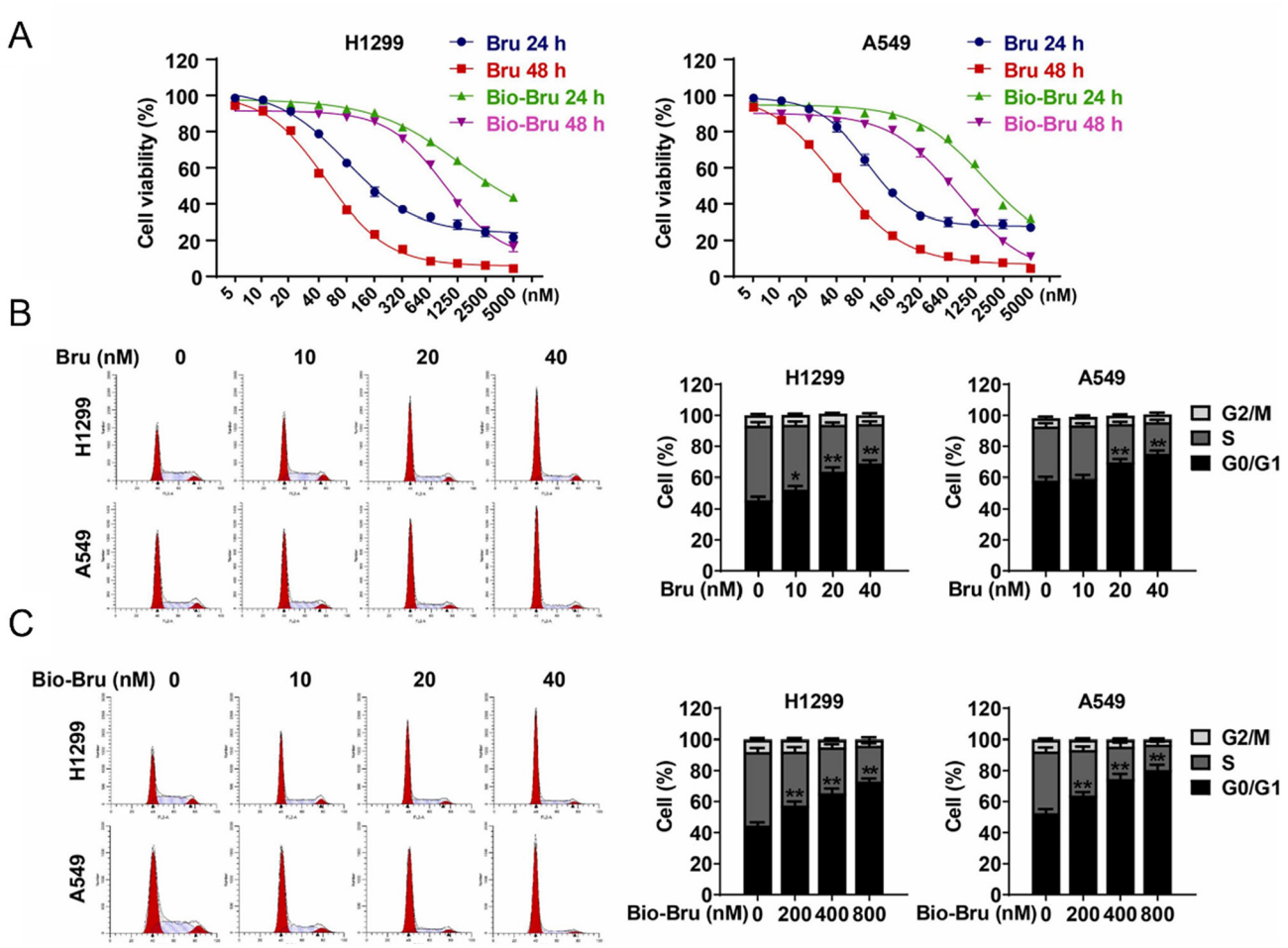


Fig. 11 Chemical structures of compound **11**. (A) The cytotoxicity of Bru or Bio-Bru towards H1299 and A549 cells was determined by CCK-8 assay. Flow cytometry analysis of cell cycle distribution after the treatment of Bru (B) or compound **11**; (C) in H1299 and A549 cells. Reproduced with permission.[55] Copyright 2022, Elsevier.

microtubules. When colchicine binds to β-tubulin, it induces a conformational change in the microtubule dimer, preventing it from assuming a linear structure due to spatial conflicts with α-tubulin, thus impeding microtubule assembly.[59] Despite its potential in tumor treatment, colchicine's broad cytotoxicity affects normal cells more severely, resulting in a lower therapeutic index that limits its precise application in cancer therapy.[60,61]

This side effect hinders the extensive clinical use of this drug. In 2022, Chao Wang *et al.*[62] addressed this issue by coupling biotin with colchicine using a reduction-sensitive disulfide bond as a linker to target colchicine enrichment in tumor cells. This strategy significantly reduces the side effects of colchicine on normal cells. The antiproliferative activity of Deac-SS-Biotin (depicted in Fig. 13, compound **17**) was evaluated on SGC-7901, A549, and HeLa cell lines (gastric adenocarcinoma SGC-7901 cells, lung adenocarcinoma A549 cells, and cervical cancer HeLa cells). Results from the CCK-8 assay demonstrated that compound **17** exhibited similar biological activity to colchicine alone, indicating its potent antitumor

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

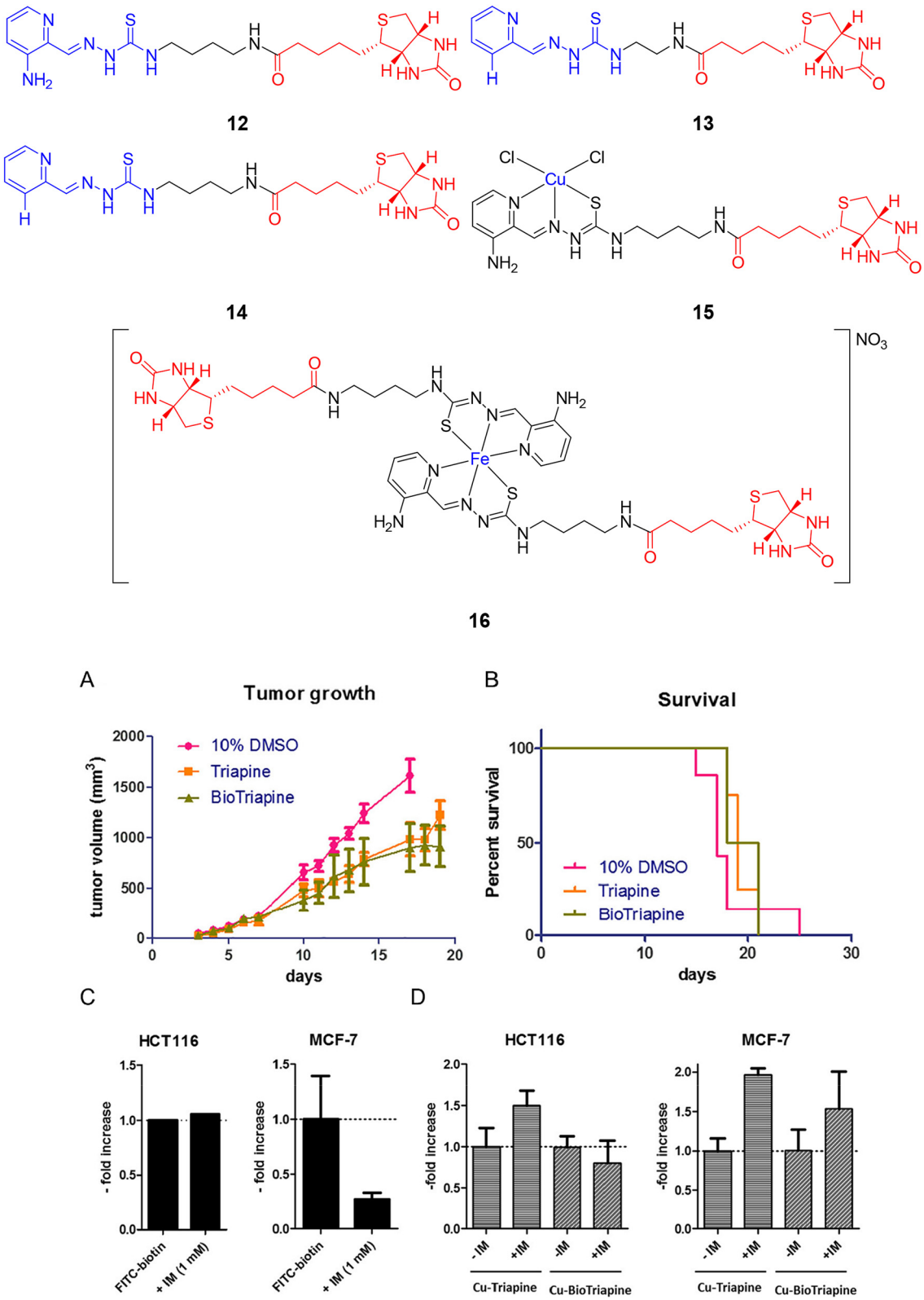


Fig. 12 Chemical structures of compounds **12**, **13**, **14**, **15** and **16**. Anticancer activity *in vivo*. (B) Tumor volumes. (C) Overall survival of the BioTriapine-treated animals was significantly prolonged. The indicated cell lines were treated with (D) FITC-biotin or (E) Cu-Triapine and Cu-BioTriapine with and indomethacin (IM). Reproduced with permission.[56] Copyright 2019, Elsevier.

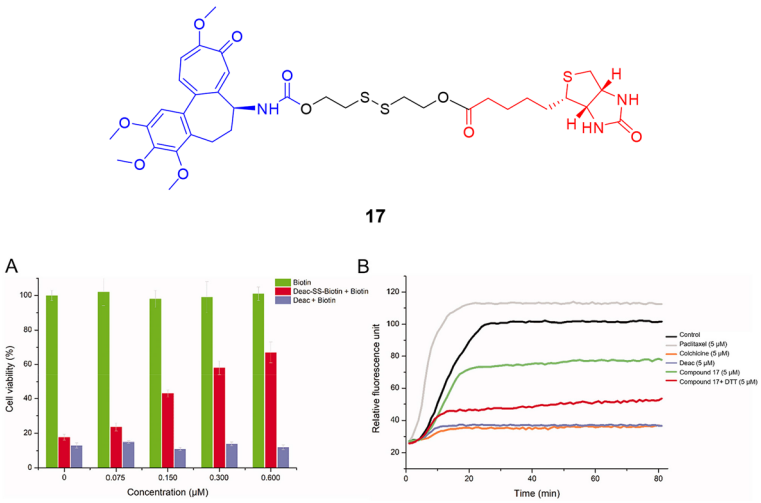


Fig. 13 Chemical structures of compound **17**. (A) *In vitro* cytotoxicity. (B) The effect of compound **17** on tubulin polymerisation. Reproduced with permission.[62] Copyright 2022, Taylor & Francis.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

efficacy. The study also investigates the release profile of compound **17**, revealing its slow release with significantly reduced reactivity. Mechanistically, compound **17** undergoes physiological cleavage by dithiothreitol (DTT), selectively releasing colchicine, which exhibits preferential targeting towards tumor cells. Moreover, the inclusion of biotin enhances the targeting ability of the compound. Tubulin polymerization experiments further confirmed that compound **17** (with DTT) effectively inhibits tubulin polymerization, highlighting its potential as a novel antitumor agent (Fig. 13A and B). These findings shed light on biotin receptor-targeted chemotherapy and offer promising avenues for the development of new antitumor drugs.

PTX is a naturally occurring anticancer agent, characterized by its tricyclic diterpene structure with a molecular formula of $C_{47}H_{51}NO_{14}$. Initially isolated from the stem bark of Taxus chinensis in 1971 by American chemists M.C. Wani and Monroe E. Wall, PTX has since been recognized for its therapeutic potential. PTX exerts its antitumor effects by disrupting the dynamic equilibrium of tubulin and tubulin dimers *in vivo*. It promotes tubulin polymerization and microtubule assembly while preventing depolymerization, thereby stabilizing microtubules. Within cells, microtubules play critical roles in maintaining cell shape, facilitating cellular movement, and transporting intracellular materials. By stabilizing tubulin, PTX inhibits cancer cell mitosis and induces apoptosis, effectively impeding cancer cell proliferation and exerting its anticancer activity. However, despite its therapeutic benefits, PTX suffers from limitations such as poor water solubility, limited tumor cell targeting, significant impact on normal cells, and notable side effects.[63]

DTX and PTX are both novel PTX analogs currently available in the clinic. They share major parts of their structure and mechanism of action, but there are differences between them and PTX. They differ in microtubule protein polymer generation, with DOX being twice as active in depolymerization inhibition. *In vitro*, DOX also tends to be more effective in various cell lines and research models.[64] Taxane are potent antitumor compounds isolated from plants, and a range of derivatives have been synthesized through structural modifications of these active ingredients. Major taxane drugs include PTX, DTX, and various derivatives with a taxane skeleton structure.

In 2014, Jacob G. Vineberg and colleagues developed new tumor-targeting dual-payload conjugates, compound **18** and compound **19** (Fig. 14), which used the targeting function of biotin for precise drug delivery to cancer cells.[65] These conjugates consist of paclitaxel and camptothecin as the two payloads, self-cleaving disulfide bonds for drug release, biotin for tumor targeting, and 1,3,5-triazine as a scaffolding module. Compounds **19** also included tetraethylene glycol diamine to improve water solubility. The functionalized 1,3,5-triazine TTDDS platform is versatile and suitable for various drug combinations and tumor-targeting modules. *In vitro* experiments showed that compound **19** had a significantly lower IC_{50} value for BR-positive cancer cells compared to normal cells, indicating that biotin's targeting ability effectively improved the drug's selectivity and anticancer activity. By combining the properties of nanomaterials, these conjugates achieved higher water solubility and drug loading efficiency through the tetraethylene glycol diamine and 1,3,5-triazine structures, enhancing the overall stability and effectiveness of the delivery system. The combination of biotin's targeting ability and the drug-carrying properties of nanomaterials opens promising possibilities for precise and efficient cancer treatment.

Jacob G. Vineberg and colleagues focused on using biotin as a targeting molecule to develop new therapeutic and diagnostic drugs by combining it with paclitaxel conjugates.[66] The authors designed and synthesized two tumor-targeting conjugates: compound **20** and compound **21** (Fig. 15). These conju-

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

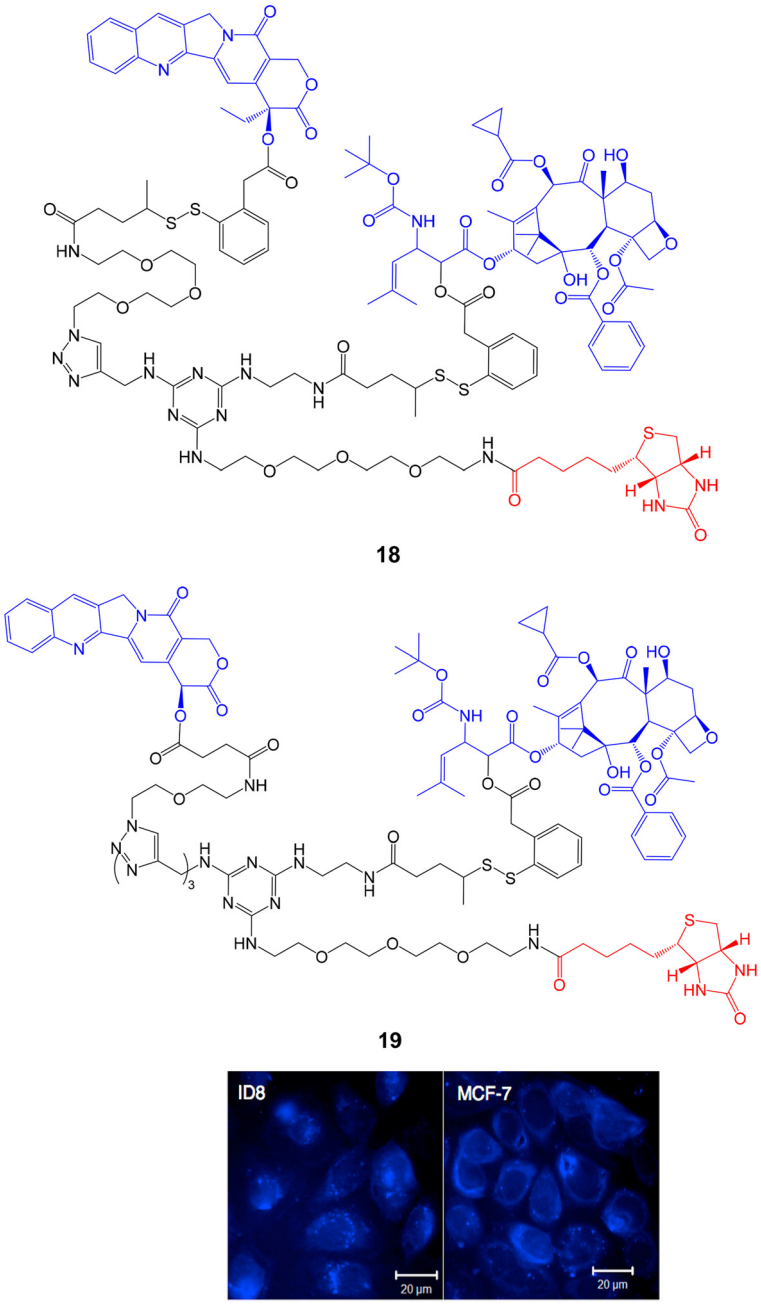
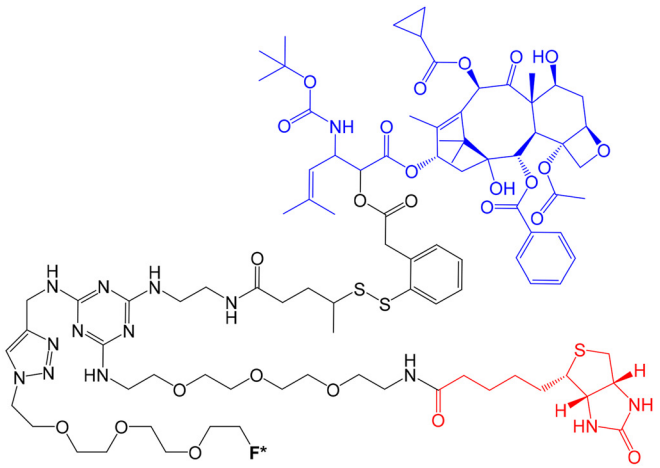


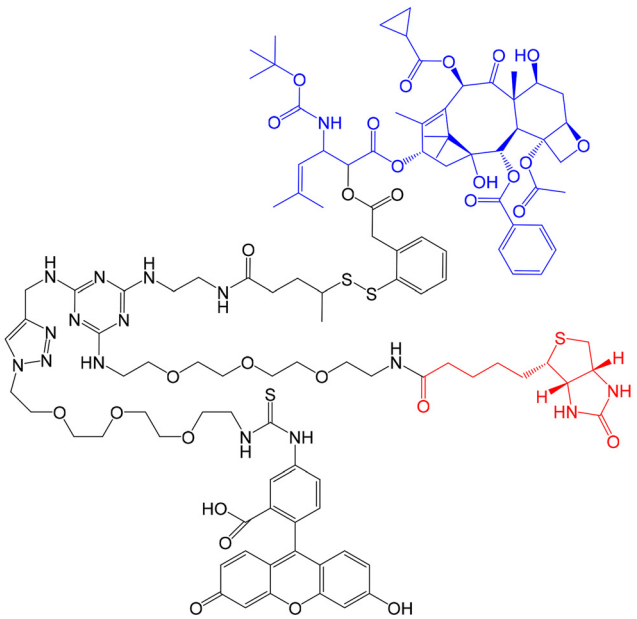
Fig. 14 Chemical structures of compound **18** and compound **19**. Confocal fluorescence microscopy images showing internalization of compound **18** in ID8 (left) and MCF-7 (right) after incubation at 37 °C for 10 h. Reproduced with permission.[65] Copyright 2014, ACS.

gates consist of a tumor-targeting module, a cytotoxic payload with a smart linker, and a triazine cleavable group with an acetylene tether. The fluorine-labeled therapeutic diagnostic compound **20** was designed for 18F-PET radioactive labeling and ^{19}F NMR analysis. Compound **21**, which carries fluorescein, was designed as a fluorescence imaging probe, creating a diagnostic–therapeutic system for precise targeted drug delivery. They also designed a smaller drug conjugate, compound **22**, which lacks the polyethylene glycol (PEG) oligomer and triazine separator module, for comparison based on cytotoxicity. In experiments, biotin as the targeting molecule ensured that the conjugates entered biotin receptor-positive (BR+) cancer cells specifically through receptor-mediated endocytosis (RME). Flow cytometry and confocal fluorescence microscopy (CFM) confirmed that compound **21** was efficiently internalized in BR+ cancer cells *via* RME, showing high specificity for these cells. The study also evaluated compound **1** on various cancer cell lines (MX-1, L1210FR, ID8 BR+) and normal human lung fibroblasts (L1210 BR−, WI38) to test its potency and selectivity. Notably, in the presence of glutathione ethyl ester

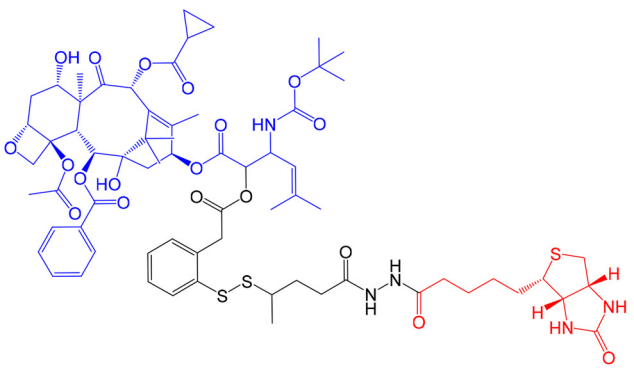
Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



20



21



22

Fig. 15 Chemical structures of compound **20**, compound **21** and compound **22**. Reproduced with permission.[66] Copyright 2015, ACS.

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

(GSH-OEt), only compound **20** entered the cells through RME in the first 24 hours and showed cytotoxic effects. Compared to normal cells, the conjugate showed significantly higher cytotoxicity against cancer cells. The intensity of cytotoxicity was closely related to the expression of biotin receptors, confirming the critical role of biotin in targeted drug delivery. This study further demonstrated the dual function of biotin in therapeutic diagnostic drugs: it enables both tumor imaging and efficient delivery of therapeutic agents, thus improving drug selectivity and efficacy.

In 2017, Tao Wang *et al.*[67] designed a novel type of tumor-targeted therapy drug conjugate using taxane and biotin as materials for SPECT (single photon emission computed tomography) and PET (positron emission tomography) imaging.[68] The former is a biotin-ligand-taxane conjugate ((Fig. 16) compound **23**) containing 99mTc for SPECT, while the latter is a biotin-ligand-DTX conjugate ((Fig. 16), compounds **24** and **25**) containing ^{64}Cu for PET imaging. Through chemical synthesis, the authors successfully obtained these two compounds. These tumor-targeted therapeutic drug conjugates hold promise for significantly enhancing the efficacy of next-generation chemotherapy.

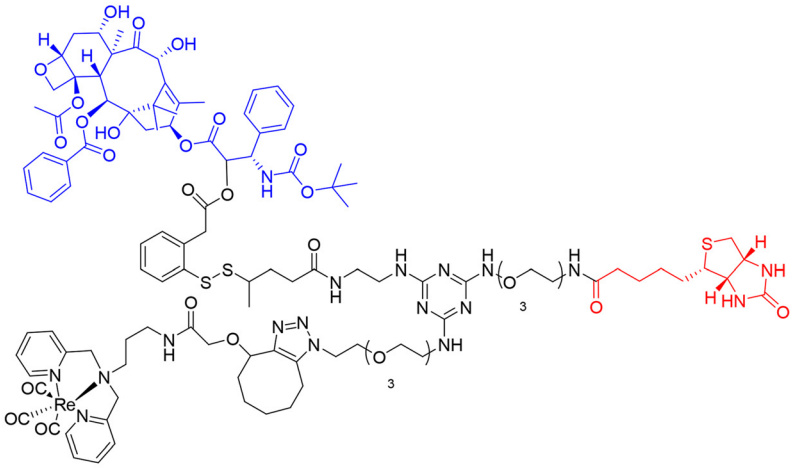
3.1.7. Targeting thioredoxin reductase (TrxR). Reactive oxygen species (ROS) are pivotal radiation effectors, typically elevated in tumor cells and their microenvironment, culminating in radiation-induced DNA damage and subsequent cancer cell demise.[69] The thioredoxin (Trx) system, comprising thioredoxin reductase (TrxR), NADPH, and Trx, stands as the primary endogenous antioxidant mechanism.[70] In various cancers, such as cervical cancer, Trx and thioredoxin reductase TrxR are commonly overexpressed to counteract oxidative stress, which correlates with the proliferation and progression of malignant tumor cells. TrxR plays a crucial role in scavenging ROS by catalyzing the NADPH-dependent reduction of Trx, providing electrons for antioxidant defense, especially in response to radiation and other forms of damage. Consequently, the redox TrxR/Trx system in cancer cells emerges as a potential target for radiobiological intervention. Gold (Au) compounds have demonstrated efficacy as TrxR inhibitors and antitumor agents. Notably, the Au(I) compound [Au (SCN)(PEt_3)] exhibits radiosensitizing properties by impeding TrxR activity in lung cancer cells. Furthermore, Au-containing compounds boast structural stability,[71] and their anticancer effects have been extensively investigated both *in vitro* and *in vivo*.[72] However, challenges related to targeting remain, warranting further exploration and refinement of these compounds.

In 2022, Zhibin Yang *et al.*[73] synthesized a series of novel biotin-targeted Au(I) complexes and conducted a comprehensive biological evaluation through a series of fundamental experiments. Following rigorous comparison, the most potent compound was identified: biotinylated Au(I) compound containing triphenylphosphine ligand (Fig. 17, compound **26**). By incorporating the biotin group into the compound, the absorption of the compound by tumor cells is enhanced, with notably stronger efficacy observed against HeLa cells in experiments. Furthermore, in normal H8 cells under identical conditions, the intracellular Au content after treatment with compound **26** is lower than that in HeLa cells, while no significant difference is observed with auranofin. This disparity suggests the guiding function of biotin, with lower biotin receptor content detected in normal cells compared to tumor cells. The introduction of the biotin structure in compound **26** significantly increased Au uptak(I). Their antiproliferative activity against cervical cancer cells (SiHa and HeLa) and normal cervical cells (H8) is evaluated using the MTT assay. Results indicate that all biotin-Au(I) complexes exhibit notable inhibitory effects on HeLa cell proliferation. Through *in vitro* studies on radiosensitization effects, the authors combine compound **26** with radiotherapy, leading to a significant inhibition of GPX4 expression and promotion of tumor cell ferroptosis (Fig. 17A). Treatment with compound **26** alone also markedly suppresses GPX4 expression and induces iron droop. Furthermore, the authors utilize zebrafish to validate the efficacy of compound **26** in enhancing radiotherapy. This research introduces a novel approach for developing tumor radiotherapy and chemotherapy drugs.

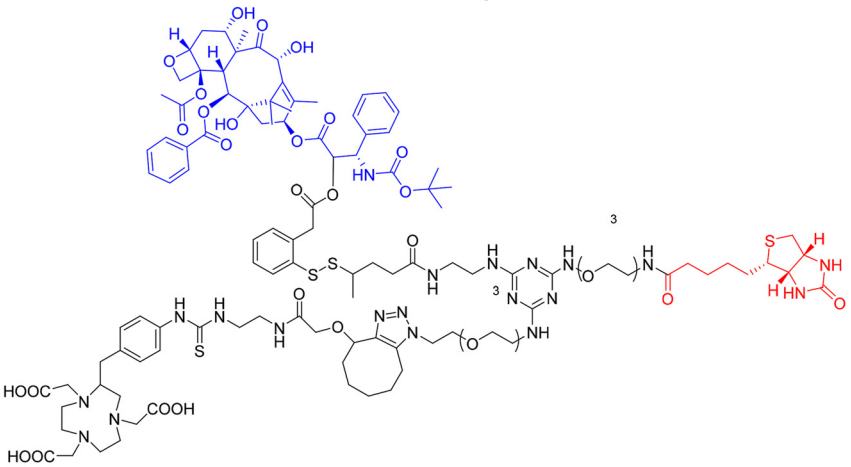
3.2. Biotin-photosensitizer conjugates

Photodynamic therapy (PDT) is a clinically approved, non-invasive treatment modality that has demonstrated significant efficacy across various medical disciplines, including oncology, ophthalmology, and dermatology. PDT relies heavily on the use of photosensitizers, which are integral to the treatment's mechanism of action. Photosensitizers, also referred to as initiators or light-activated agents, are compounds capable of absorbing energy from specific wavelengths in the ultraviolet (250–420 nm) or visible light (400–800 nm) spectra. Upon absorption, these compounds become excited and subsequently generate reactive oxygen species (ROS), such as free radicals and singlet oxygen. These reactive species are pivotal in initiating processes like monomer polymerization, cross-linking curing, and, crucially, inducing cytotoxic effects that lead to targeted cell death in malignant tissues. One particularly promising photosensitizer is Chlorin e6 (Ce6), typically synthesized from pheophorbide a. Ce6 has gained attention due to its high efficiency in producing singlet oxygen, making it an ideal candidate for tumor-targeted photodynamic therapy. The combination of PDT with chemotherapy has emerged as a potent strategy to overcome drug resistance in cancer treatment. Ce6, when used in conjunction with chemotherapy, enhances the anti-tumor response by exploiting its strong phototoxic properties to damage cancer cells more effectively.[74,75] Despite its potential, photosensitizers, including Ce6, face certain limitations, such as poor solubility, rapid clearance from the bloodstream, and non-specific distribution. To address these challenges, researchers have explored various strategies, including the conjugation of photosensitizers with biotin. Biotin-photosensitizer conjugates have shown promise in improving the targeted delivery and retention of photosensitizers within tumor tissues, thereby enhancing the therapeutic efficacy of PDT.[76,77] Recent studies have focused on optimizing

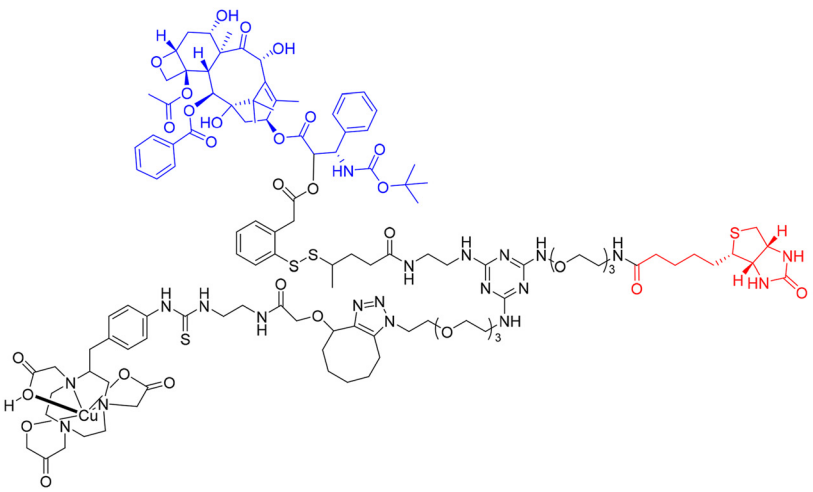
Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



23



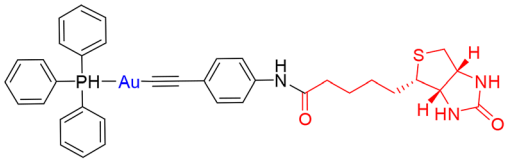
24



25

Fig. 16 Chemical structures of compounds **23**, **24** and **25**.

 This journal is © The Royal Society of Chemistry 2025



26

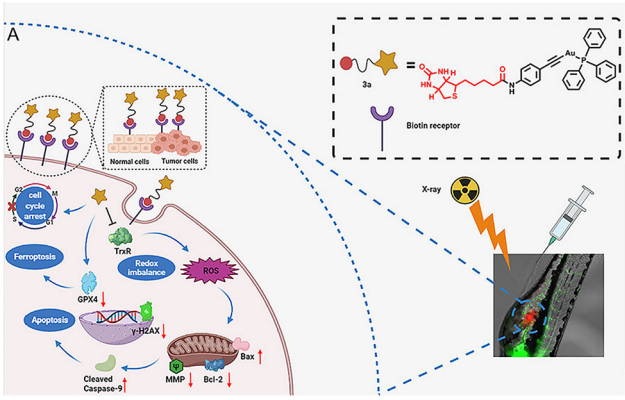


Fig. 17 Chemical structures of compound **26**. (A) Mechanism of compound **21**. Reproduced with permission.[73] Copyright 2022, ACS.

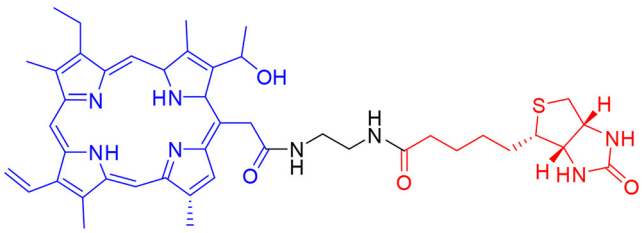
these conjugation strategies, exploring different linker chemistries, and evaluating the pharmacokinetics and biodistribution of these conjugates in preclinical models. These efforts are laying the groundwork for the development of next-generation anti-tumor photosensitizers, with the potential to advance to clinical trials. Such advancements could significantly enhance the effectiveness of PDT, offering a more precise and potent approach to cancer treatment. The incorporation of biotin into PDT has emerged as a pivotal advancement in the development of photosensitizers. Recognizing the limitations of conventional photosensitizers, such as poor selectivity and rapid clearance, researchers have turned to biotin, a naturally occurring vitamin with strong affinity for biotin receptors, which are often overexpressed in cancer cells. By conjugating biotin with photosensitizers, scientists aim to enhance the targeting and retention of these compounds within tumor tissues, thereby addressing some of the key shortcomings of traditional PDT. This biotin-enhanced approach not only improves the precision and efficacy of photodynamic therapy but also opens new avenues for the design of more effective anti-tumor treatments.

In 2021, Wei Liu *et al.*[78] developed a novel tumor-targeting photosensitizer, Ce6-biotin, by coupling biotin with Ce6 (Fig. 18, compound **27**). Upon coupling, compound **27** exhibits improved water solubility and reduced aggregation. The authors evaluated the photophysical properties of compound **27** and compared its fluorescence with that of Ce6 in PBS. Although the fluorescence of compound **27** is slightly weaker than that of Ce6, likely due to minimal alteration in Ce6's basic structure, it emits strong fluorescence in the infrared region, making it suitable as a fluorescent probe *in vivo*. Additionally, compound **27** demonstrates a slightly increased singlet oxygen generation rate compared to Ce6. Notably, at concentrations ranging from 1.0 μM to 2.0 μM, compound **27** exhibits significantly higher therapeutic efficacy than Ce6 at equivalent concentrations. Moreover, cytotoxicity assays conducted in HeLa (BR+) and B16 (BR−) cells show that under the same light dose (20 J cm^{-2}) and compound **27** concentration (2.0 μM), the cytotoxicity of compound **27** to HeLa cells is greater than that to B16 cells. The cellular uptake of Ce6 and compound **27** in HeLa cells is examined using flow cytometry, given the overexpression of biotin receptors in HeLa cells. Photodynamic sensitizers (PSs) at a concentration of 2 μM are incubated with HeLa cells for varying durations (0.5, 1, 2, 4, and 6 h). Following that, the internalized PSs were quantified *via* flow cytometry. As illustrated in Fig. 18B, the uptake of compound **27** in HeLa cells markedly surpasses that of Ce6 after 6 hours. Quantitative analysis (Fig. 18C) reveals that the cellular uptake of compound **27** following 6 h of incubation is approximately four times higher than that of Ce6. The pronounced disparity in cellular uptake between Ce6 and compound **27** supports the notion that conjugating Ce6 with biotin molecules enhances the affinity of Ce6 towards biotin receptor-positive HeLa cells. To evaluate the anticancer efficacy and dark toxicity of Ce6 and compound **27**, we utilize the 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to assess cell viability post-treatment with both photosensitizers (PS). In the absence of irradiation, Ce6 and compound **27** exhibit minimal inhibitory effects on HeLa cells at concentrations ranging from 0.25 to 4 μM (Fig. 18D and E). These findings demonstrate that compound **27** enhances therapeutic efficacy against BR-positive cancer cells without increasing dark cell toxicity. This biotin-conjugate effectively addresses the toxic and side effects of Ce6 while enhancing its therapeutic efficacy. Biotin plays a key role in this system, but the results show that the current coupling method needs to be further improved to continue to improve efficacy.

Curcumin-Pt(II) mixed ligand complexes and 1,10-phenanthroline bases have been developed as potent anticancer drugs and photosensitizers for targeted cancer cell phototoxicity.[79,80] In 2019, Aarti Upadhyay *et al.*[81] conjugated these compounds with biotin to form compound. The authors synthesized three compounds referred to as "complexes **2**, **3** and **4**" with complexes **3** (Fig. 19, compound **28**) demonstrating the most favorable efficacy, thus warranting detailed description herein. Upon interaction with tumor cells, these complexes exhibit robust cytotoxicity under visible light, as evidenced by their low IC_{50} values, leading to early apoptosis in HeLa, $HepG_2$, and A549 cancer cells with minimal dark toxicity. Notably, these complexes demonstrate efficacy against the multi-drug-resistant MDA-MB-231 cell line. The cancer-selective conjugates are successfully developed: compound **28** with biotin and sugar components, aiming to mitigate the adverse effects commonly associated with traditional Pt-based drugs. compound **28** incorporates biotin. compound **28** exhibits significant uptake in $HepG_2$ cells, while compound **28** demonstrates high cellular uptake in HeLa cells, albeit minimal uptake in

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



27

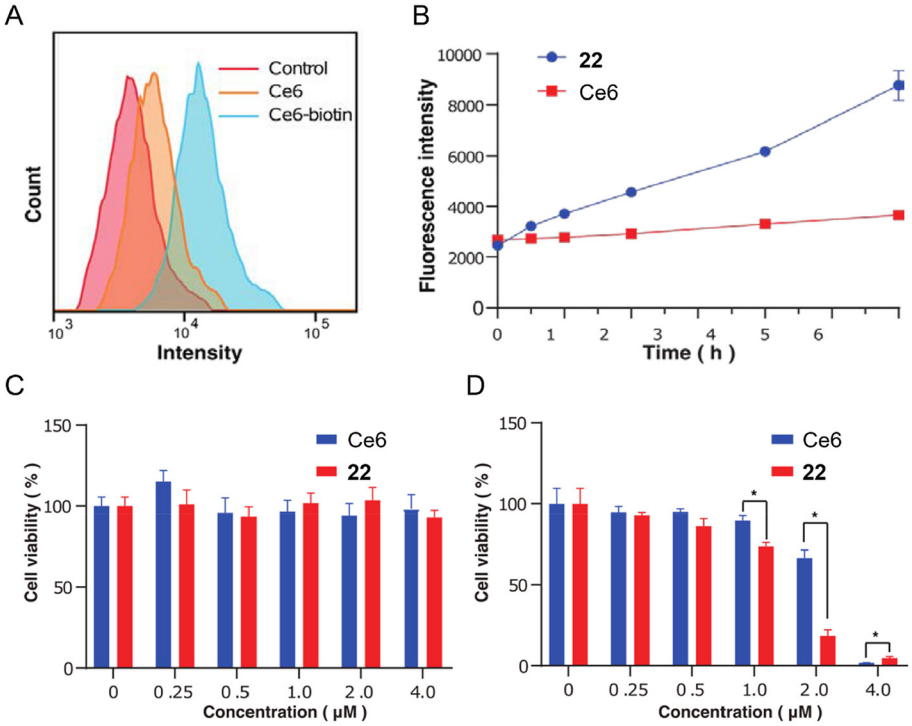


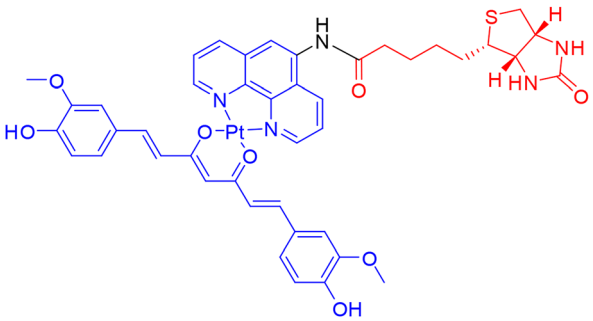
Fig. 18 Chemical structures of compound **27**. (A) Flow cytometry analyses of HeLa cells incubated with Ce6 and Ce6-biotin. (B) Uptake isotherms of Ce6 and Ce6-biotin toward HeLa cells. Cytotoxicity against HeLa cells of Ce6 and Ce6-biotin in the dark (C) or upon irradiation (D). Reproduced with permission.[78] Copyright 2021, MDPI.

HPL1D standard cell lines. It is imperative that drugs exhibit selectivity towards cancer cells rather than normal cells. To investigate this, the authors employ HPL1D cells to assess the uptake of complexes **2**, **3**, and **4** by normal cells. Additionally, HeLa and $HepG_2$ cell lines are chosen due to their documented overexpression of GLUT-1 (glucose transporter) and biotin receptors compared to complexes **2** (15 μM) and complexes **4** (15 μM), with compound **28** (15 μM) featuring a biotin component. Notably, complexes **2** exhibit enhanced uptake in HepG2 cells, indicating the targeting role of biotin (Fig. 19A–C). Furthermore, these complexes preferentially localize to the nucleus, as observed in Pt estimation studies using ICP-MS. Given DNA's susceptibility as a potential target, the complexes' DNA binding and photocleavage properties were thoroughly examined. Complexes containing 1,10-phenanthroline exhibit enhanced DNA binding tendencies. Mechanical DNA photolysis studies have demonstrated that light exposure is the primary inducer of reactive oxygen species (ROS). This mechanism involves light exposure inducing hydroxyl radicals, which in turn convert O_2 into the precursor of ROS, O_2^-. Overall, Pt (II) complexes demonstrate significant anticancer potential as curcumin-based phototoxic drugs. Their enhanced ability to selectively deliver the dye to cancer cells compared to normal cells underscores their potential for targeted cancer therapy.

In 2020, Summer Y. Y. Ha *et al.*[82] introduced an advanced multifunctional molecular therapeutic agent that integrates three key components: a tumor-targeting biotin moiety, a glutathione (GSH)-activated zinc(II) phthalocyanine (ZnPc)-based photosensitizer, and a reactive oxygen species (ROS)-responsive CA4 unit (Fig. 20, compound **29**).[83] This creative compound, referred to as compound **29**, is designed to combine dual chemical and photodynamic therapy, leveraging the unique properties of each component. The ZnPc unit in the compound features a DNBS substituent that enhances both fluo-

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



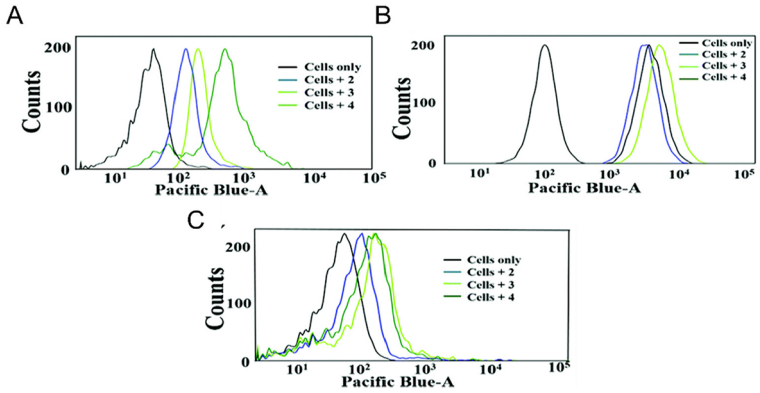


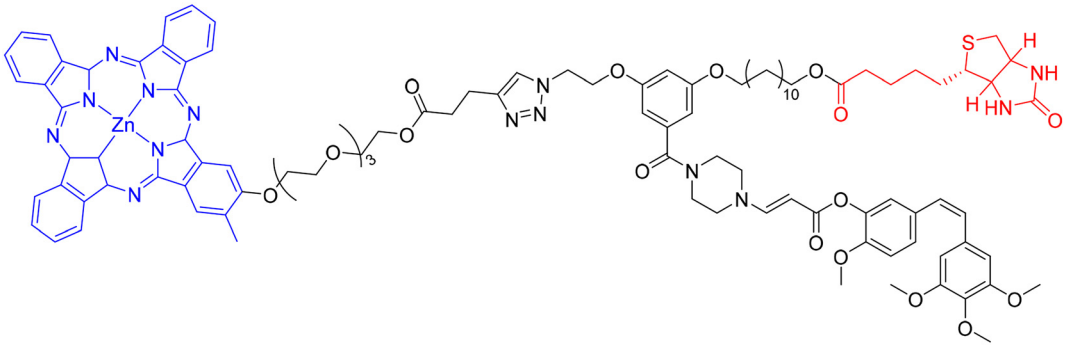
Fig. 19 Chemical structures of compound **28**. The data from the cellular uptake study: (A) uptake in HeLa cells, (B) uptake in HepG2 cells and (C) uptake in HPL1D cells. Reproduced with permission.[81] Copyright 2019, RSC.

rescence emission and singlet oxygen generation upon activation. The GSH activation further amplifies the intracellular fluorescence intensity and photocytotoxicity, illustrating the synergistic effects of biotin targeting and GSH reactivity. During the photodynamic process, the generated singlet oxygen not only induces cytotoxic effects but also cleaves the amino acrylate linker, triggering the release of the CA4 unit. This mechanism enables a synergistic dual therapeutic approach, combining chemical and photodynamic modalities. To evaluate the efficacy of this multifunctional agent, the authors conducted cellular uptake studies using $HepG_2$ human hepatoma cells, which exhibit high biotin receptor expression, and HCT-116 human colorectal cancer cells, characterized by low biotin receptor expression. The results demonstrated that the intracellular fluorescence intensity in $HepG_2$ cells was significantly higher than in HCT-116 cells, indicating effective biotin-mediated targeting. Importantly, the addition of GSH did not alter the photosensitizer's effect, confirming the robustness of the therapeutic agent. Moreover, the authors observed no cytotoxicity in the absence of light, while the IC_{50} value under light exposure was 48 nM, underscoring the potent cytotoxic effect of the photosensitizer on tumor cells (Fig. 20A). This study highlights the potential of integrating biotin targeting with GSH activation and ROS-responsive elements to develop highly effective multifunctional therapeutic agents for cancer treatment.

Johannes Karges *et al.*[84] devised a Ru(II)-based PDT anticancer drug delivery system. The metal compound were encapsulated within polymer nanoparticles featuring biotin groups at their termini (Fig. 21, compound **30**). This study unveiled that Ru nanoparticles (NPs) exhibited selective accumulation in two-dimensional monolayer cancer cells and three-dimensional multicellular tumorspheres. This unique design endows the particles with high selectivity towards cancer cells, surpassing non-cancerous cells in both 2D monolayer models and 3D multicellular tumor spheres. Employing an equivalent amount of Ru(II) polypyridine complex *in vivo*, the researchers note enhanced particle accumulation within tumors compared to the complex alone, confirming its effectiveness in targeting cancer. Under clinically relevant single-photon (500 nm) or two-photon (800 nm) excitation, the nanoparticles demonstrate notable phototoxic effects in both 2D monolayer cells and 3D multicellular tumor spheres, leading to tumor eradication in mouse models (Fig. 21A–D). The encapsulation method proposed for the Ru(II) polypyridine complex in this investigation holds significant promise for advancing cancer-targeted PDT.

3.3. Combination of biotin and immunotherapy

Immunotherapy has shown significant antitumor activity in cancer treatment. It can specifically target tumor cells, activate the body's immune system to enhance antitumor responses,



29

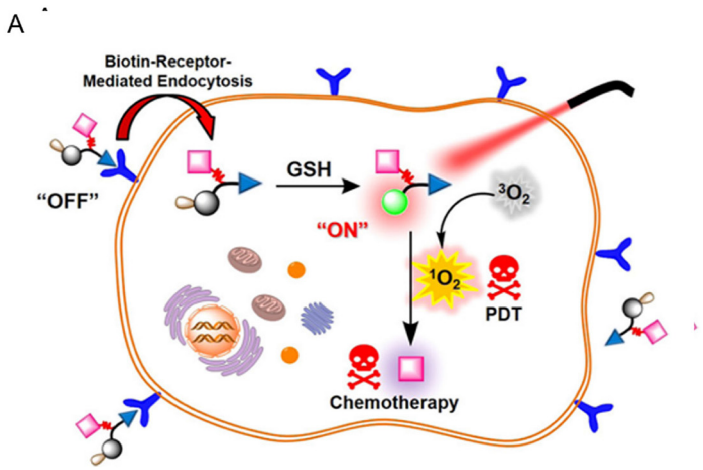


Fig. 20 Chemical structures of compound **29**. (A) Working principle of the biotinylated ZnPc–CA4 conjugate. Reproduced with permission.[82] Copyright 2020, ACS.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

and generally has fewer side effects and better long-term survival rates compared to traditional chemotherapy. Furthermore, immunotherapy can improve efficacy through personalized approaches, especially showing significant effects in certain cancer types. However, immunotherapy also faces several major drawbacks, including resistance caused by individual differences, potential immune-related adverse reactions (such as attacks on healthy cells), high treatment costs, and uncertainty in efficacy. Additionally, there is currently a lack of reliable biomarkers to predict patients' responses to immunotherapy, making the selection of the appropriate treatment plan more complex (high specificity of engineered T cells with third-generation CAR (CD28-4-1BB-CD3-ζ) based on biotin-bound monomeric streptavidin for potential tumor immunotherapy).

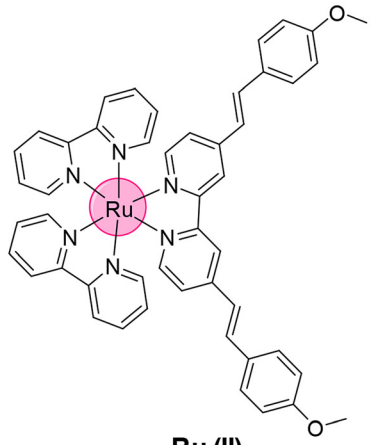
As an excellent targeting agent, biotin can effectively facilitate drug uptake in tumor cells through receptor-mediated endocytosis.[17] Thus, some have proposed using biotin to conjugate antibodies (such as biotinylated trastuzumab) to enhance targeting of HER2-positive tumor cells, thereby improving treatment efficacy. In immunotherapy, biotin can also act as a "molecular switch" to enhance immune responses by binding to specific antibodies or cytokines. For example, a biotin-based CAR T cell (UniCAR) has been developed, which can target various tumor antigens by recognizing antibodies bound to biotin. This method not only improves T cell recognition of tumor cells but also enhances its cytotoxicity. Notably, in three-dimensional tumor spheroid models, biotinylated CAR T cells can effectively penetrate the tumor microenvironment and induce cell death in the core region, overcoming the limitations of traditional CAR T cells in solid tumors. Currently, several preclinical studies are exploring biotin-based immunotherapy strategies.[85]

For example, in mouse models, researchers have shown significant antitumor activity using biotin-linked anti-PD-L1 inhibitors (such as SWS1). These inhibitors not only effectively block the PD-1/PD-L1 pathway but also promote an increase in tumor-infiltrating lymphocytes, thereby enhancing immune response.[86] Furthermore, these studies indicate that biotinylated drugs accumulate significantly more in tumor tissues than in normal tissues, further enhancing the therapeutic effect.

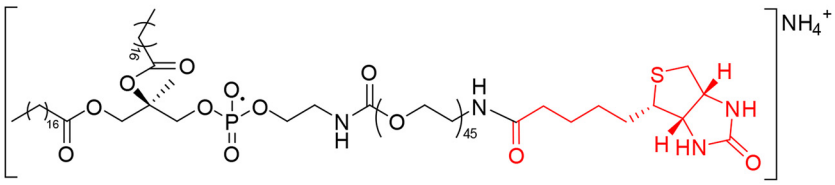
Although biotin has potential in enhancing immunotherapy, its clinical application still faces several challenges. First,

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



Ru (II)



30

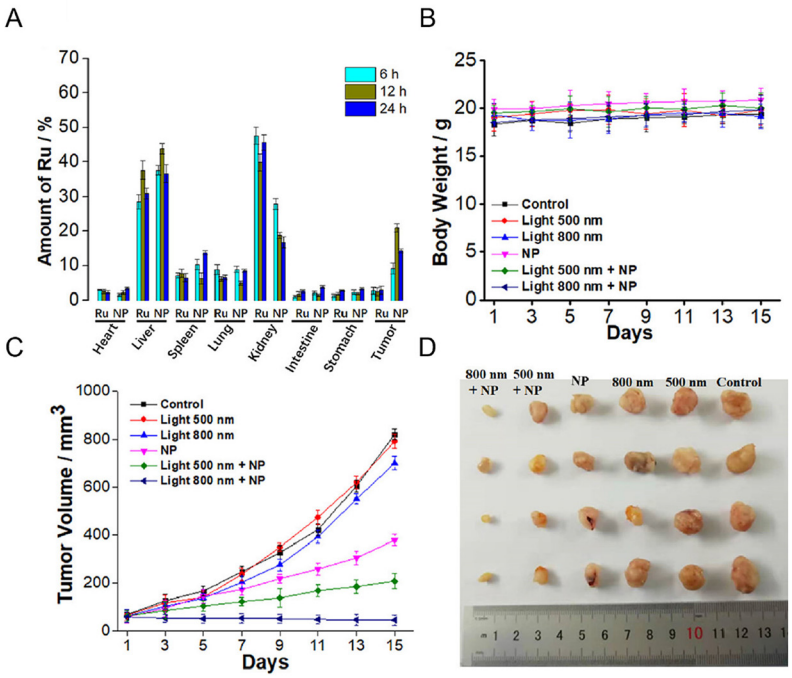
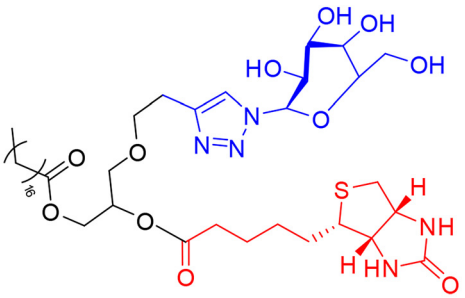


Fig. 21 Chemical structures of compound **30**. (A) The time-dependent biological distribution of NP and Ru was assessed using ICP-MS. (B) The average body weight of tumor-bearing mice. (C) The tumor growth inhibition curve. (D) Representative photographs of the tumors taken 15 days after treatment. Reproduced with permission.[84] Copyright 2020, ACS.

determining the optimal dose remains a key issue. Overuse of biotin may lead to off-target effects and interfere with the efficacy of other drugs. Additionally, resistance mechanisms represent a significant obstacle, as certain tumor cells may evade treatment by downregulating biotin receptors or altering metabolic pathways.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



31

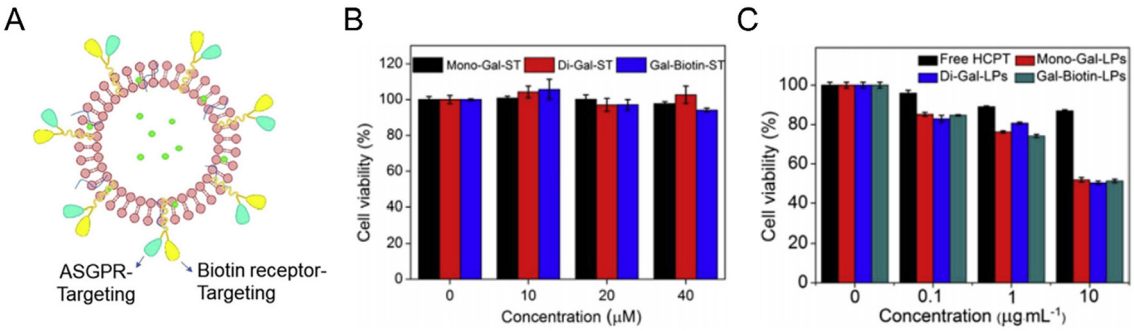


Fig. 22 The structure of compound **31**. (A) Schematic illustration for the liposomes modified with targeting lipid materials. (B and C) *In vitro* cells viability. Reproduced with permission.[90] Copyright 2019, Elsevier.

4. Nano-biotin conjugates (NBCs)

Nanomaterials consist of crystals or amorphous superparticles with a nanometer-scale (1–100 nm) as the basic structural unit. Nanodrug delivery systems (NDDS), utilizing nanomaterials to transport antitumor drugs, have emerged as promising cancer treatments with numerous advantages over traditional small-molecule drugs. NDDS has been extensively studied and utilized due to its ability to improve drug pharmacokinetic (PK) profiles. Recently, researchers have focused on the interaction between NDDS and anti-tumor drugs. By combining NDDS with antitumor drugs, several benefits can be achieved. Firstly, encapsulating antitumor drugs in nanocarriers significantly extends their blood circulation time compared to their free-form counterparts, allowing for continuous release at the tumor site. Secondly, NDDS can facilitate passive targeting by enhancing permeability and retention (EPR) effects, thereby improving tumor distribution. Lastly, NDDS strategies may promote cellular uptake of antitumor drugs, as nanoparticles possess unique properties that facilitate efficient internalization into cells through mechanisms such as endocytosis or receptor-mediated pathways. However, the combination of NDDS and antitumor drugs alone may not provide optimal targeting or therapeutic effects, posing a significant challenge in NDDS.[87] Therefore, the combination of biotin, NDDS, and antitumor substances has shown promise in targeted drug transport and enhancing tumor therapeutic performance. By enhancing tumor distribution efficiency, extending blood circulation duration, and facilitating cellular uptake, this combination may offer a novel therapeutic approach for cancer treatment, while mitigating adverse effects on healthy tissues. The advancements in nanomedicine and nanocarriers in recent years have provided valuable insights and directions for the future development of antitumor drugs using nanotechnology.[88]

4.1. Targeting asialoglycoprotein receptor (ASGPR)

Liposomes (Lip) are hollow vesicles prepared from lecithin and ceramide, possessing a bilayer structure identical to that of the cell membrane. Due to their unique structure, Lip serve several functions: targeting and lymphatic orientation, sustained release, reduction of drug toxicity, and improvement of stability. Lip consists of lipid bilayers with an aqueous phase inside, initially proposed as a model by British scholar Bangham in 1965 to study biofilms. This unique structure enables Lip to encapsulate various substances within their aqueous phase and membrane. Hemoglobin release from all Lip remained insignificant below 800 nM phospholipids, with a hemolysis rate of less than 10%, indicating their non-toxic and biocompatible nature. Consequently, liposomes began to be utilized as drug carriers to regulate drug release, aiming to minimize side effects and enhance efficacy. Ligand-modified liposomes are vital for targeted drug delivery, improving drug efficacy, and reducing chemotherapy-related side effects.

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

However, existing single-target ligand-modified liposomes have demonstrated inadequate precision in targeting tumor cells, coupled with suboptimal delivery and release efficiency. Consequently, it is imperative to develop and fabricate novel compounds with improved characteristics to overcome these shortcomings.[89]

In 2019, Ruihua Ding *et al.*[90] engineered several novel liver cancer cell-targeted lipid materials: Single-Gal-ST (monogalactose), Di-Gal-ST (bisgalactose), Gal-Biotin-ST (galactose-biotin, Fig. 22, compound **31**). They employ a creative synthesis method that preserves the properties of liposomes while utilizing the biotin-directed effect to enhance liposome accumulation in tumor cells (Fig. 22A). Like previous biotin-conjugated drugs, compound **31** exhibits reduced drug release rates, with HCPT-loaded liposomes releasing only about 5% of HCPT compared to 35% for the free 10-Hydroxy-CPT (HCPT) solution. Galactose, a monosaccharide composed of six carbons and one aldehyde, is introduced to improve the liver targeting effect by specifically recognizing the overexpressed ASGPR on hepatocyte surfaces. ASGPR autoantibodies are considered specific markers of autoimmune hepatitis (AIH).[91] Interestingly, uptake experiments on these liposomes reveal that the fluorescence intensity of compound **31** in $HepG_2$ (liver cancer cells) is 1.3 times higher than that of single-gal-LPs and 5.0 times higher than that of Lips. While its uptake ability is weaker than that without biotin, this demonstrates that the addition of biotin can more accurately target tumor cells, highlighting a synergistic effect between galactose and biotin. This synergy enhances liposomes' ability to distinguish between liver cancer cells and normal liver cells, showing a trend of Lips < single-gal-LPs < Di-Gal-Lips < compound **31** (Fig. 22B and C). Cytotoxicity experiments also demonstrate a survival rate of over 90% for normal cells. Galactose competition experiments further demonstrate that galactose-biotin-modified liposomes can penetrate hepatoma cells *via* ASGPR-mediated endocytosis. Additionally, *in vitro* cytotoxicity assays indicate high biosafety for these materials. Overall, this study develops a range of creative lipid materials targeting hepatoma cells for liposomal delivery, paving the way for more precise treatments for HCC.

4.2. Targeting ATF3

15,16-Dihydrotanshinone I (DI) occur extracted from the rhizome of Salvia Miltiorrhiza.[92] Salvia Miltiorrhiza itself has demonstrated efficacy in treating gastric cancer, liver cancer, cervical cancer, and other diseases. Dihydrotanshinone I (DHTS I, DI) has been shown to effectively improve the overall condition of mice with experimental ulcerative colitis, alleviating symptoms such as weight loss, diarrhea, bloody stool, and mental distress, while also exhibiting antitumor activity. Being a natural component derived from Salvia Miltiorrhiza, DI has been found to be safe and effective, with no noticeable side effects or toxic reactions observed during experiments. However, DI's poor solubility has limited its efficacy. DHTS has been observed to upregulate the expression of ATF3 (cyclic AMP-dependent transcription factor ATF-3), thereby facilitating DHTS-induced apoptosis in both non-malignant SW480 and malignant SW620 colorectal cancer cells.[93] In 2018, Jingjing Luo *et al.*[94] synthesizes copolymers PEG-PLGA (PPA) and Biotin-PEG-PLGA (BPA) by combining polylactic acid-glycolic acid (PLGA) with polyethylene glycol (PEG) and biotin (Fig. 23, compound **32**). Utilizing PPA and BPA to load DI improves its solubility and efficacy. DI-PPA-NPs and DI-BPA-NPs are obtained, respectively. Biophysical methods are employed to characterize the particle size, distribution, encapsulation efficiency, and *in vitro* release profile of DI-BPA-NPs (Fig. 23A). Altering the pH reveals that the cumulative release rate of DI is slightly higher at pH 5.3, consistent with the pH of the tumor microenvironment (TME). The antiproliferative activity of free DI, DI-PA-NPS, and DI-BPA-NPS on human cervical cancer HeLa cells is assessed using the MTT assay. Results show that DI-BPA-NPS exhibits the highest IC_{50} value for HeLa cells at 4.55 ± 0.631 μM, while the IC_{50} values of DI and DI-PPA-NPS at 72 h are 8.20 ± 0.849 and 6.14 ± 0.312 μM, respectively. The presence of biotin in DI-BPA-NPs facilitates robust targeting, preferentially taken up by tumor cells and accumulating within them to exert more potent antitumor activity. In conclusion, DI-BPA-NPS exhibits a significantly enhanced inhibitory effect on human cervical cancer cells compared to free DI. This underscores the targeting efficacy of biotin within DI-BPA-NPS, thereby enhancing the therapeutic potential of DI in cancer therapy and offering a novel delivery strategy for DI.

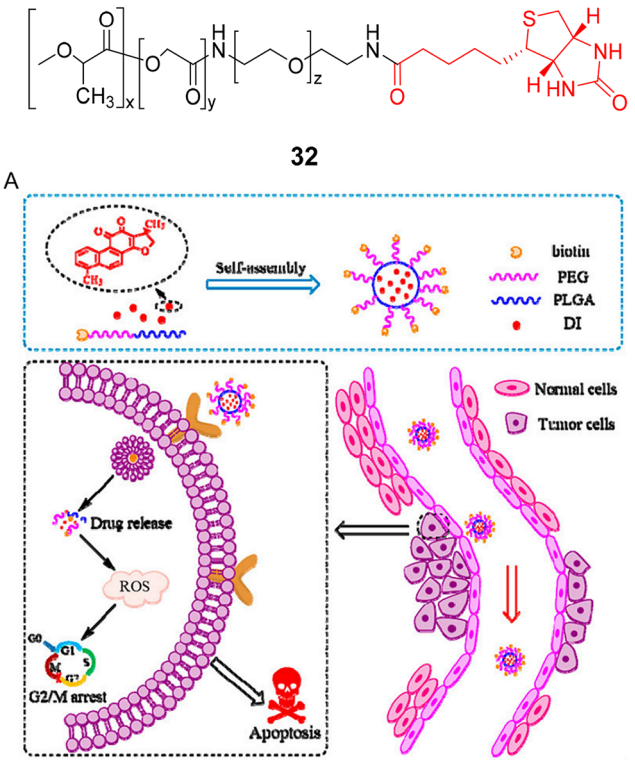


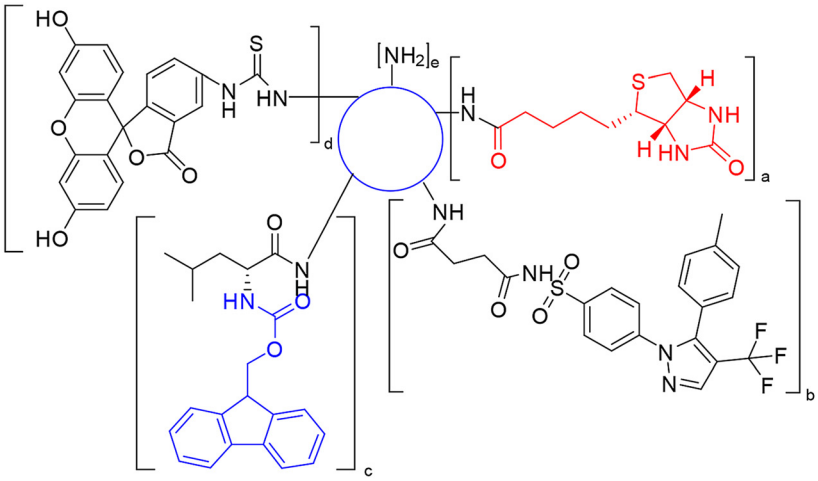
Fig. 23 The structure of compound **32**. (A) Assembly and mechanism of action of drugs. Reproduced with permission.[94] Copyright 2018, ACS.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

4.3. Targeting COX-2

COX-2 is released into the TME by cancer-associated fibroblasts, type 2 macrophages, and cancer cells. COX-2 induces cancer stem cell-like activity and promotes resistance to apoptosis, proliferation, angiogenesis, inflammation, invasion, and metastasis. In the TME, COX-2-mediated hypoxia interacts positively with YAP1 and anti-apoptotic mediators, diminishing cancer cell resistance to chemotherapy drugs.[95] The third-generation PAMAM dendrimer (G3 PAMAM), with 32 amino surface groups, is less toxic and easily modifiable. It exhibits intense penetration through the biofilm. Biotinylated dendrimers and their conjugates with anticancer drugs exhibit elevated cellular uptake and cytotoxicity when bound to biotin. COX-2 is an inducible enzyme that catalyzes the conversion of arachidonic acid to prostaglandins during the inflammatory response in cancer development. COX-2 plays a crucial role in cancer progression by promoting cell proliferation, migration, invasion, resistance to apoptosis, and angiogenesis.[96] Therefore, COX-2 inhibitors have potential in cancer prevention. Peroxisome proliferator-activated receptor (PPAR-γ) agonists are transcription factors that regulate cell functions by binding to various ligands. PPAR-γ is overexpressed in tumor cells and activation of PPAR-γ may inhibit tumor growth, induce apoptosis, cell cycle arrest, and redifferentiation, ultimately leading to tumor cell death. Both COX-2 inhibitors and PPAR-γ agonists exhibit strong anti-tumor activity.[97] However, there is substantial evidence suggesting that COX-2 inhibitors may increase the risk of cardiovascular events and elevate prothrombotic activity.[98]

In 2018, Lukasz Uram *et al.*[99] utilized the characteristics of biotinylated G3 PAMAM (Fig. 24, compound **33**) to synthesize, detect, and compare the effects of compound **33** dendrimers binding to selective COX-2 inhibitors (celecoxib)[100] or/and PPAR-γ antagonists (Fmoc-LLeucine)[101] on the cytotoxicity of four different types of human cell lines (Fig. 24A).



33

A

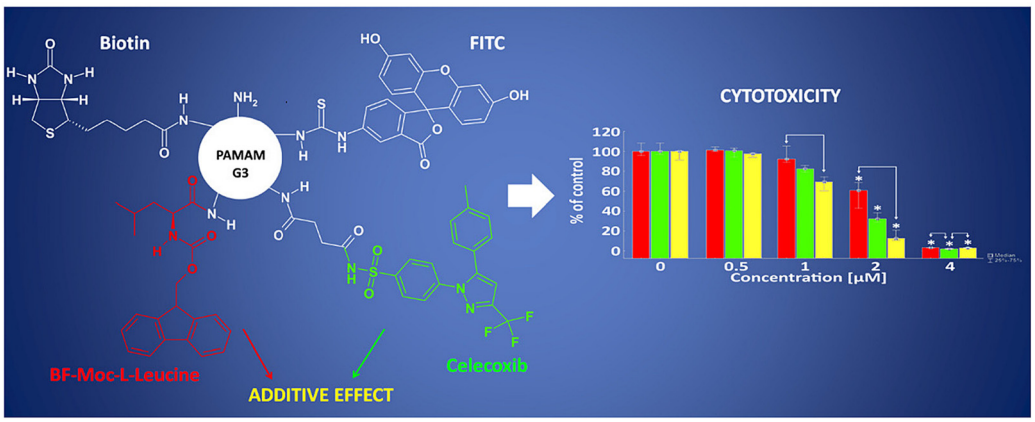


Fig. 24 The structure of compound **33**. Schematic formulae of studied conjugates: $G3^{1B31C}$ (a = 1, b = 31, c, d, e = 0); $G3^{1B31L}$ (a = 1, c = 31, b, d, e = 0); $G3^{1B16C15L}$ (a = 1, b = 16, c = 15, d, e = 0); $G3^{1B20C1F}$ (a = 1, b = 20, c = 0, d = 1, e = 10); $G3^{1B20L1F}$ (a = 1, b = 0, c = 20, d = 1, e = 10); $G3^{1B10C10LF}$ (a = 1, b = 10, c = 10, d = 1, e = 10). (A) Drugs and their effects on cell viability. Reproduced with permission.[99] Copyright 2018, Elsevier.

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

Additionally, the authors synthesize a series of drugs derived from compound **33**. Relatively high biotin uptake is observed in normal fibroblasts (BJ), immortalized keratinocytes (HaCaT), and glioblastoma (U-118 MG) cells, but significantly decreases in SCC15 cells. COX-2 protein is constitutively expressed in all studied cells, with substantially increased levels observed in cancer cells. The authors validate the cytotoxicity of the newly synthesized compound **28** dendrimer polymers by comparing them with the COX-2 inhibitor celecoxib and/or the PPAR-γ receptor antagonist Fmoc-L-Leucine. The dendrimer G3-1B16C15L combined with both drugs exhibits the highest cytotoxicity. The focus is on evaluating the cell's capacity to uptake biotin. Time-dependent uptake of biotin labeled with ATTO590 is carried out for 24 h. The cancer cell lines SCC-15 and U-118 MG did not show clear drug targeting effects in this study. In addition, when treating skin squamous cell carcinoma, the drug was highly toxic to L929 cells. This result was unexpected. This problem may be linked to the transport mechanism of biotin. The main transporter for biotin is SMVT. SMVT needs a free carboxyl group to work properly. This group is important for transporting biotin effectively. However, during the drug conjugation process, biotin is often modified, especially the carboxyl group. This modification may reduce the transport efficiency through SMVT. When the conjugated biotin cannot bind well to SMVT, it cannot enter the cancer cells efficiently. This results in poor drug targeting. Moreover, in skin squamous cell carcinoma (SCC), SMVT is not activated enough. This makes it harder for biotin to be taken up by the cells. Even if the drug is designed to target cancer cells, its effectiveness is reduced because of the limited transport function of SMVT.

These problems highlight a major challenge for biotin-based drugs in clinical use. It is difficult to balance drug modification and transport efficiency. In diseases like skin squamous cell carcinoma, where SMVT is not fully understood, biotin-based drugs face many challenges. More research is needed to improve this. Researchers may need to find ways to activate SMVT better or use alternative transport methods to improve the drug's targeting and delivery. In conclusion, although biotin-based drugs show potential, their different effects in various cancers show that improving drug design and transport mechanisms is important. More studies are needed to better understand these issues and find solutions for using biotin-based drugs in the clinic.

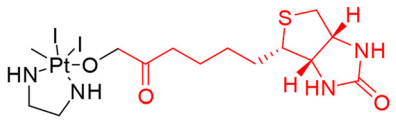
4.4. Targeting DNA

In the method of combining metal with chemotherapy, Pt-based chemotherapy therapeutics offer the ability to track biological distribution and pharmacokinetics while exerting therapeutic effects. In 2022, Jie Yu *et al.*[102] designed and synthesized reducing agent-sensitive Bio-Pt-I NPs (Fig. 25, compound **34**) with high Pt and iodine content as a therapeutic nano-drug aimed at improving the integration of tumor diagnosis and treatment (Fig. 25A). The incorporation of biotin enables specific targeting of tumor cells, thereby increasing the concentration of iodine-coupled Pt within tumor cells. The addition of iodine significantly enhances the CT imaging capability of Pt. CT imaging reveals observable tumor contours following injection of compound **34** NPs compared to compound **34**, demonstrating the effectiveness of iodine-enhanced CT imaging at the animal level (Fig. 25B). *In vitro* cytotoxicity experiments demonstrate that Bio-Pt-I exhibits a notable inhibitory effect on the growth of various tumor cells, including cisplatin-resistant tumor cells, underscoring its diagnostic and therapeutic potential. Compound **34** NPs (IC_{50} = 74.2 μM) and Pt(IV)-I (IC_{50} = 108.0 μM) significantly inhibit the proliferation of HepG2 cells, while compound **34** NPs (IC_{50} = 400.0 μM) exhibit lower cytotoxicity (Fig. 25C and D). Subsequently, the beneficial role of iodine in sensitizing A549/DDP cells to Pt-containing drugs is confirmed. The cytotoxicity of compound **34** against A549 and A549/DDP tumor cells is negligible, with the IC_{50} not detectable within the dose range. In compound **34** NPs, a synergistic effect is observed among Pt, iodine, and biotin, collectively enhancing the drug's toxicity, with IC_{50} values of 60.8 μM and 74.2 μM for A549 and A549/DDP, respectively (Fig. 25E and F). Moreover, both compound **34** NPs and Bio-Pt-Cl NPs exhibit enhanced drug uptake compared to their non-targeted counterparts at both 0.5 and 4 h (Fig. 25G). The authors further confirm that iodine can inhibit the expression of Bcl-2 in tumor tissues, thereby overcoming cisplatin resistance. This study represents a pioneering effort in combining diagnosis and treatment through CT imaging and tumor suppression. The development of this therapeutic system advances the potential clinical translation of compound **34** NPs and provides a solid foundation for future research in this area.

4.5. Targeting DNA TOP I

To address the challenges posed by CPT, scientists have explored nanomaterial-based solutions. In 2022, Diptendu Patra *et al.*[103] designed and synthesized a therapeutic multi-prodrug CP TP PG BN Fe (Fig. 26, compound **35**), co-targeted by biotin and mitochondria (Fig. 26A). The multi-prodrug spontaneously self-assembled into nanospheres in water, effectively targeting the biotin receptor and the mitochondria of cancer cells. The iron complex provides MRI tracking capability, aiding in dose estimation during treatment.[104] The diagnostic potential of compound **30** was assessed through relaxation and DOI imaging experiments.[105] Therapeutic multi-prodrugs can efficiently and precisely deliver CPT to mitochondria in cancer cells, a pivotal event in regulating tumor cell apoptosis. To validate the mitochondrial targeting ability of therapeutic multi-prodrug CP TP PG BN Fe, fluorescence cell imaging experiments were conducted on biotin receptor-positive human cervical cancer (HeLa) and breast cancer (MCF-7) cell lines. The results demonstrate that compound **35** exhibits continuous receptor and mitochondrial dual-targeting ability, delivering CPT effectively and accurately to cancer cells compared to CP PG BN. The fluorescence intensity is higher in compound **30**-treated cells than in CP PG BN-treated cells. The introduction of Fe plays a significant role in enhancing this effect. Cytotoxicity studies using MTT assay evaluate the effects

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



34

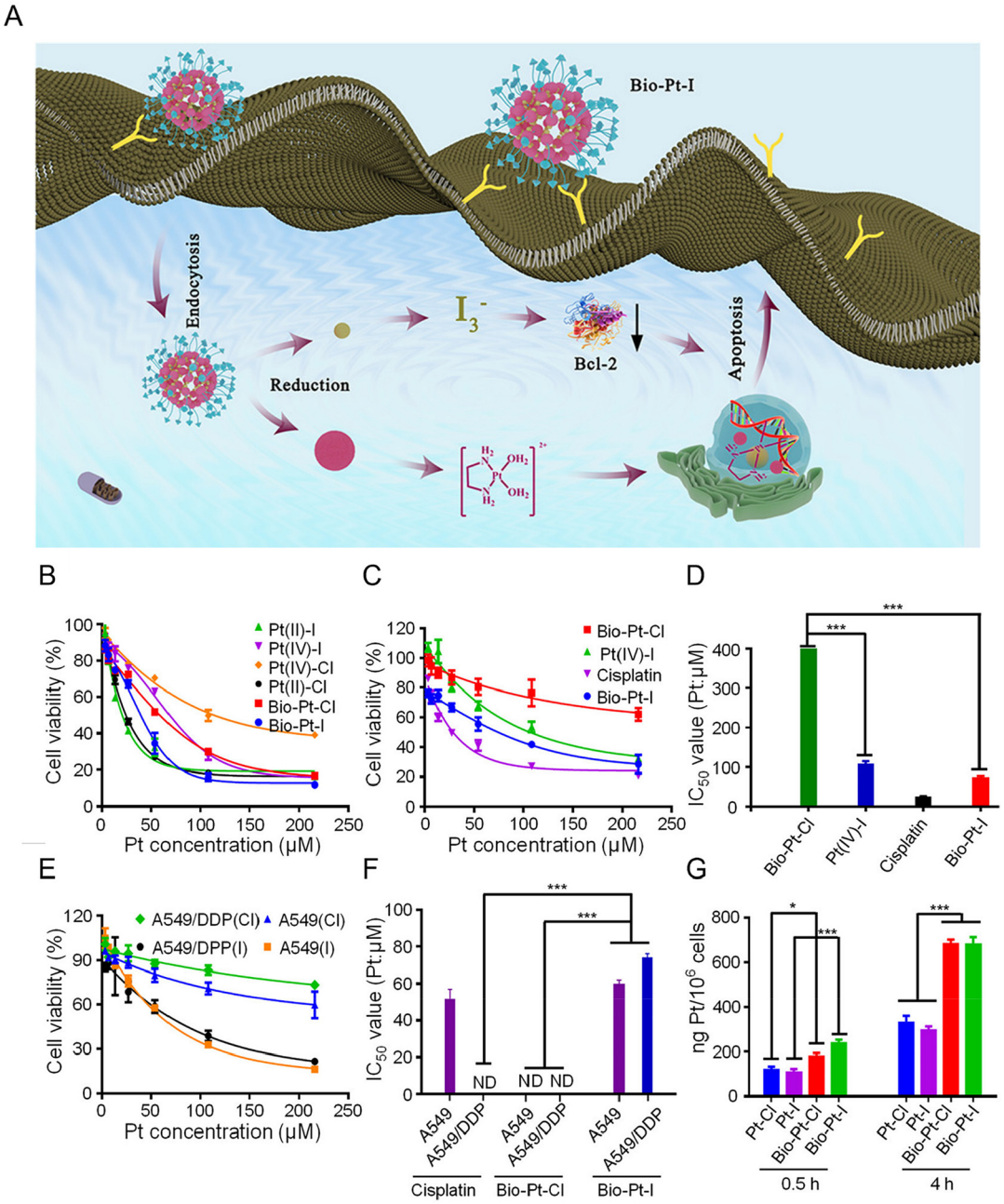
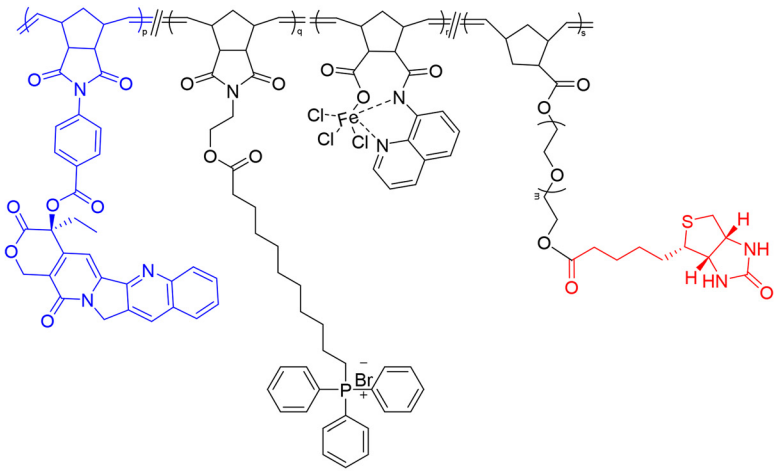


Fig. 25 The structure of compound **34**. (A) Assembly and mechanism of action of drugs. *In vitro* cytotoxicity profiles and action mechanism of Bio-Pt-I; (B) MCF-7 cells; (C) $HepG_2$ cells; (D) IC_{50} values of cisplatin, Pt(IV)-I, Bio-Pt-Cl, and compound **34** against $HepG_2$ cells. (E) A549 and A549/DDP tumor cells. (F) Comparison of IC_{50} values of cisplatin, Bio-Pt-Cl, and compound **34** against A549 and A549/DDP tumor cells. (G) The uptake of Pt in $HepG_2$ cells. Reproduced with permission.[102] Copyright 2022, ACS.

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



35

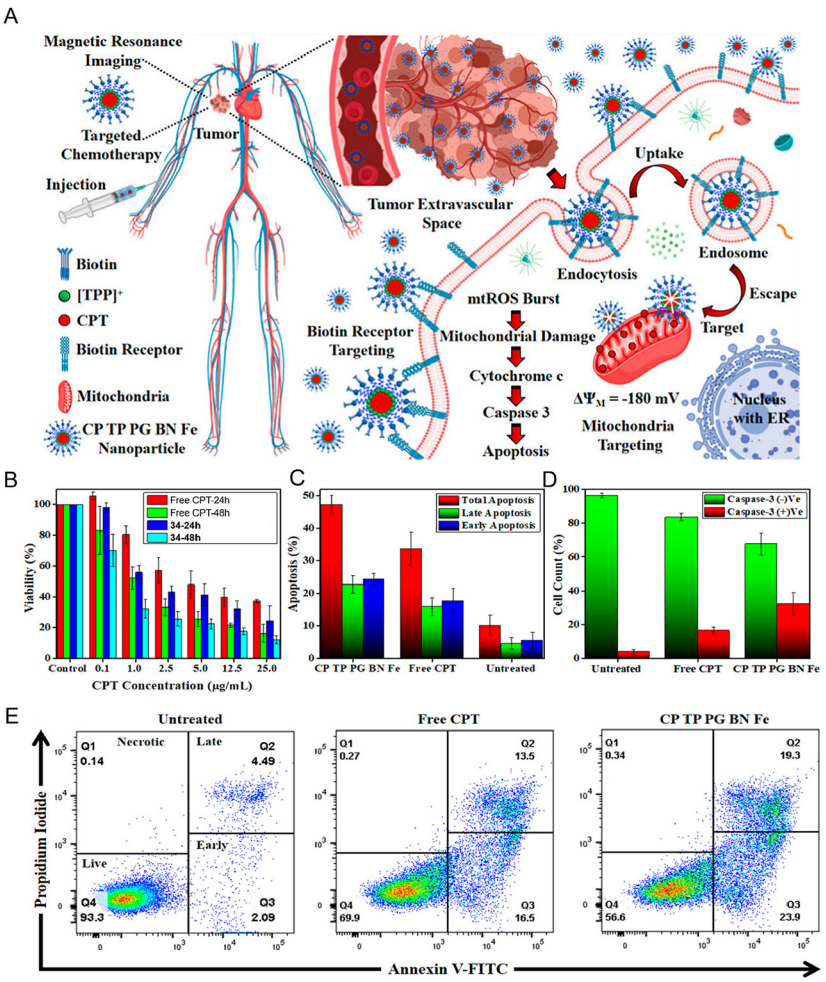


Fig. 26 The structure of compound **35**. (A) Pictorial illustration of MRI-based sequential receptor and mitochondria dual-targeting theranostic poly-prodrug, compound **35** for effective and precise chemotherapy. Cytotoxic effect evaluation using (B) MTT assay for cell viability, (C) apoptosis assay, and (D) analysis of caspase-3 expression. (E) The scattered plot further demonstrated the increased cell population in the apoptosis quadrant. Reproduced with permission.[103] Copyright 2022, ACS.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

of CPT (0.1–25 μg mL^{-1}) in compound **35** for two different incubation periods (24 h and 48 h). Additionally, apoptosis experiments show that the average percentage of active caspase-3 positive cells is 3% in untreated cells, 15% in free CPT-treated cells, and 35% in compound **35**-treated cells (Fig. 26B–E). The therapeutic multi-prodrug compound **35** demonstrates its ability to induce mitochondrial reactive oxygen species (mtROS) to a greater extent than free CPT. These experiments underscore the mitochondrial-targeted and potent characteristics of therapeutic multi-prodrug compound **35**, presenting a promising approach for tumor treatment.

In 2019, Cinzia Scialabba *et al.*[106] developed highly controlled, well-defined red-emitting carbon nanodots with surface-decorated biotin groups (Cyclodextrins (CDs)-PEG-BT) connected by discrete PEG2000 chains (Fig. 27, compound **36**).[107] This ligation method incorporates biotin, allowing the group to specifically bind to the biotin receptor (BR) and mediate endocytosis to target cancer cells. compound **36** were found to efficiently capture a large amount of irinotecan (>16%) released into the tumor through the NIR-triggered photothermal (PT) effect (Fig. 27A).[108] Moreover, facilitated by biotin action, it accumulates in numerous tumor cells, leading to cell death. The authors conducted the infrared photothermal conversion of compound **36** (compound **36** @ IT) loaded with imipridone and observed that the drug-loaded complex exhibited efficient near-infrared photothermal conversion (21.2%), enabling immediate payload release under low-power 810 nm laser irradiation (100% within 200 s). compound **36** demonstrates *in vitro* bioimaging capabilities in a transparent window, facilitating the identification and monitoring of cancer cells in complex tissues. Experimental findings confirm that the targeted PT effect of compound **36** @ IT can specifically act on tumor cells to induce local hyperthermia and a significant release of irinotecan, effectively eliminating cancer cells in the vicinity. The authors established an *in vivo* solid tumor model by co-culturing MCF7 or MDA-MB-231 cells with human dermal fibroblasts (HDFa) to generate spheroids. The cytotoxic effects of CDs-PEG-BT were evaluated after 48 hours of incubation using the MTS assay. This value was compared with the efficacy observed with free IT (Fig. 27B–I). Surprisingly, biotinylated CDs exhibited cytotoxic effects comparable to those of free drugs. Additionally, these nanothermal agents have demonstrated potent anti-cancer effects in human biopsy patient-like organs, offering insights into tumor metastasis potential and drug response in human subjects. This complex exhibit high potential as an effective therapeutic tool for image-guided PT treatment of BR-overexpressing cancers, including breast cancer.

4.6. Targeting DNA TOP II

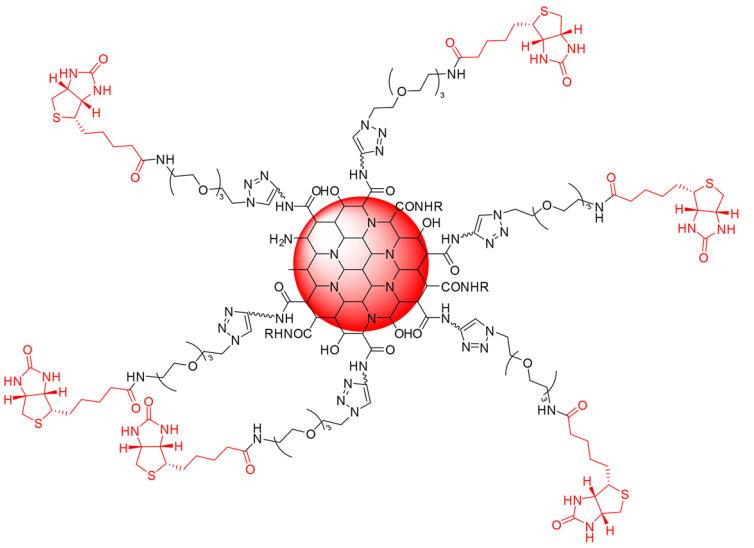
In 2017, Cenk Daglioglu *et al.*[109] developed Fe_3O_4@SiO_2(FITC)-BTN/FA/DOX multifunctional nanoparticles with pH sensing (Fig. 28, compound **37**) to address anti-cancer drug efflux activity and mitigate substantial side effects. These nanoparticles exhibit a higher release rate in environments with lower pH. At pH 5 and pH 7.4, the release rate in the first 4 h increased by about 6.5 times. The synthesized nanoparticles were equipped with two targeting groups, biotin and folic acid, enhancing their uptake by cancer cells and reducing the likelihood of drug resistance. DOX binds to the nanoparticle surface through an acid-sensitive Schiff base. Moreover, the presence of two active targeting ligands on the nanoparticle surface improves the accumulation of nanoparticles in cells and promotes their retention in the cytoplasm. The anti-cancer activity of the nanoparticles demonstrated that, compared with single-targeting agents, the dual-targeting agents have a solid potential to overcome drug resistance, resulting in increased toxicity and cancer cell apoptosis (Fig. 28A and B). This multifunctional nanoparticle platform with dual targeting and drug release characteristics holds promise as a promising anti-cancer nanomedicine.

In 2018, Zhen Li *et al.*[110] used methoxy polyethylene glycol-poly (L-histidine)-D-α-vitamin E succinate (MPEG-PLH-VES) copolymer as a material to load DOX to form MPEG-PLH-VES nanoparticles (NPs) containing DOX (Fig. 29A). Biotin was added to MPEG-PLH-VES nanoparticles to target tumor cells to form biotin-PEG-VES nanoparticles (MPEG-PLH-VES/B NPs) (Fig. 29, compound **38**). The loading rate of the two NPs to DOX was about 90%, and the average particle size was about 130 nm. They were sensitive to pH and could increase the drug release rate in an acidic environment. The results of confocal laser scanning microscopy (CLSM) showed that DOX-loaded NPs led to the effective delivery of DOX to MCF-/ADR cells and significantly promoted the escape of the carrier from endosomal encapsulation. *In vitro*, cytotoxicity evaluation showed that the toxicity of drug-loaded NPs was significantly higher than that of free DOX, and the nanoparticles compound **38** NPs containing biotin were significantly higher than MPEG-PLH-VES NPs. This result suggests that biotin does indeed play a targeting role in the system of action. It was also verified in the *in vivo* imaging study of MCF-7/ADR tumor transplanted mice. *In vitro*, results showed that although compound **38** NPs loaded with DOX had the most potent inhibitory effect on MCF-7/ADR xenograft tumors, its systemic toxicity could be ignored, which was proved by histological analysis and weight change. The authors also explained the principle that NPs can overcome multidrug resistance (MDR). DOX-loaded compound **38** NPs have efficient DOX cell uptake of MCF-7/ADR cells, drug-dependent release, PLH promotes endosomal escape, high cytotoxicity to MCF-7/ADR cells, selective tumor accumulation in MCF-7/ADR xenograft tumor model and significant tumor growth inhibition. These results indicate the great potential of compound **38** nanocarriers in overcoming multidrug resistance in cancer chemotherapy.

In the same year, Chandra Bhushan Tripathi *et al.*[111] engineered a molecularly targeted nanostructured lipid carrier (NLCs) designed to load Dox and deliver it to tumor cells, thereby inducing apoptosis in breast tumor cells. NLCs were crafted using perilla oil (ω3-FA) as a lipid, and the encapsulation of Dox was bolstered by molecular ion pairing.[112] *In vitro* and *in vivo* evaluation of biotinylated NLCs (Bio-Dox-NLCs) was conducted (Fig. 30, compound **39**). The nanoparticles

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



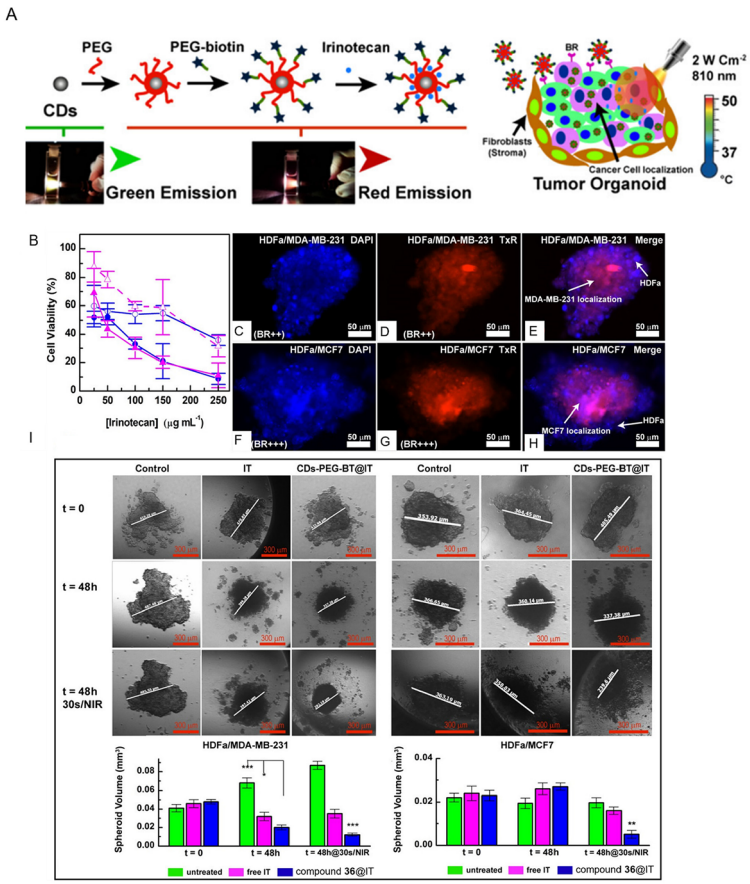


Fig. 27 The structure of compound **36**. (A) Assembly and mechanism of action of drugs. (B) Anticancer activity on 3D spheroids. (C–H) Cellular uptake. (I) NIR-triggered photothermal ablation of spheroids after 48 h of incubation followed by the photothermal treatment. Reproduced with permission.[106] Copyright 2019, ACS.

exhibited an entrapment efficiency of 99.15 ± 1.71%, a drug content of 19.67 ± 2.6 mg g^{-1}, and a biotin content of 5.85 ± 0.64 μg g^{-1}. Under acidic pH conditions (mimicking the internal tumor environment), the drug release rate was measured at 98.67 ± 2.43%. MTT assay and flow cytometry analysis revealed that compound **39** demonstrated enhanced antiproliferative activity compared to Dox alone in MCF-7 cell lines, inducing apoptosis. The slower rate of drug release can

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

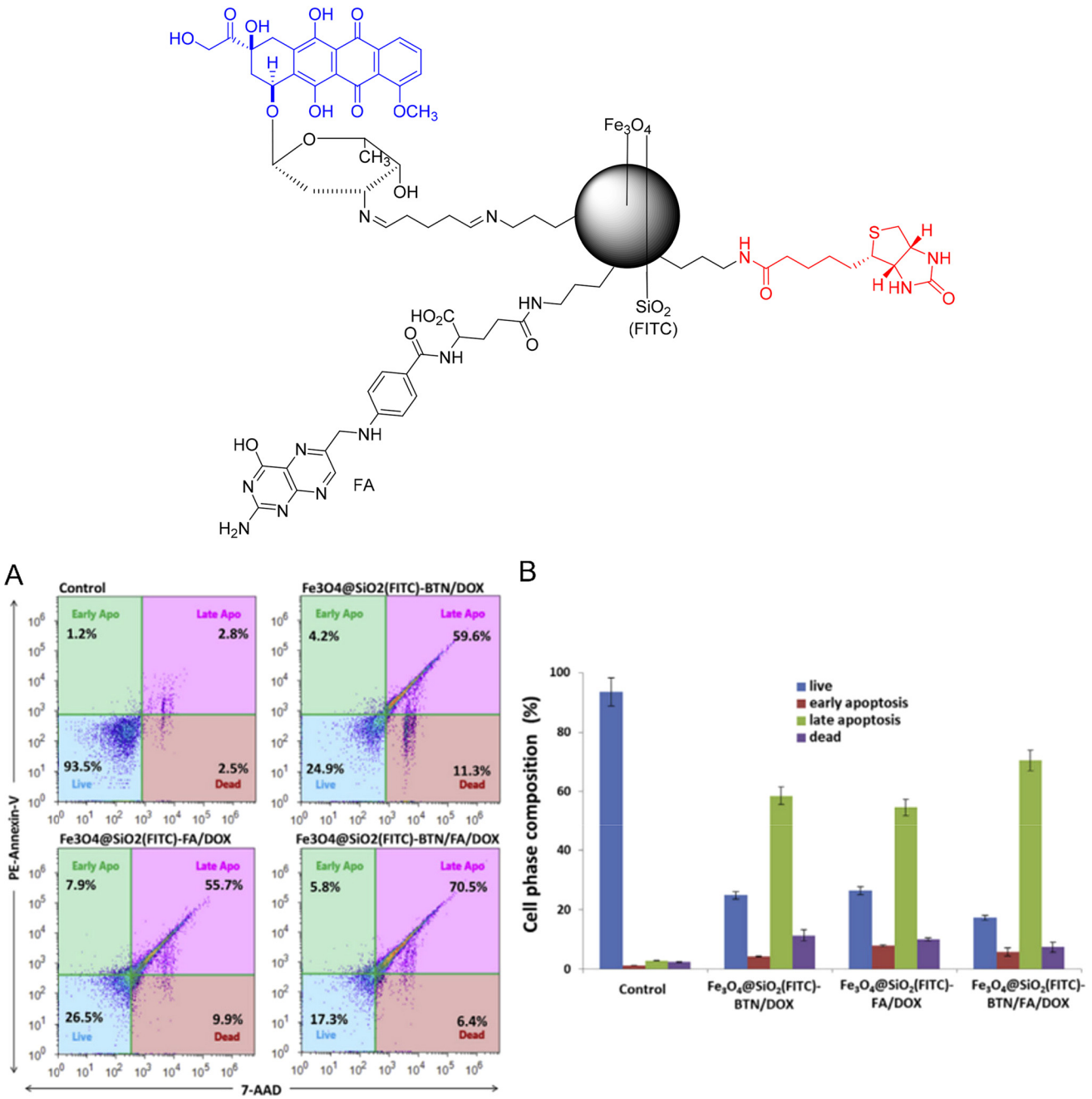


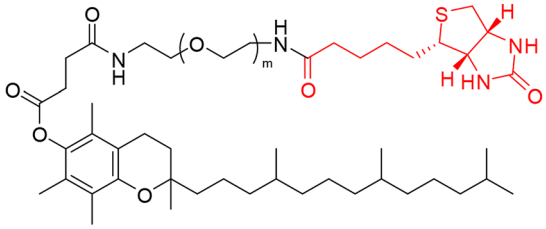
Fig. 28 The structure of compound **37**. (A) Representative images of nanoparticle induced apoptosis of HeLa cells were detected by flow cytometry. (B) Phase composition percentage of HeLa cells treated with nanoparticles. Reproduced with permission.[109] Copyright 2018, Elsevier.

maintain an effective drug concentration within tumor cells for an extended duration, leading to tumor cell death (Fig. 30A). Experimental findings highlighted that biotinylated ω-3 fatty acid-loaded Dox-enhanced NLCs could specifically induce programmed cell death in breast cancer cells. Simultaneously, this coupling form exhibited minimal impact on biotin and drugs, allowing biotin to effectively fulfill its targeting role, thereby increasing efficiency and decreasing toxicity. Hence, it can serve as a safe and effective delivery system with enhanced potential for breast cancer treatment.

In 2022, Wenzhi Yang *et al.*[113] synthesized a series of biotinylated β-cyclodextrin grafted pullulan (Bio-CDPu), characterized by Fourier transform infrared (FTIR) and hydrogen nuclear magnetic resonance (^{1}H NMR).[114,115] These were then self-assembled into spherical nanoparticles with a diameter ranging from 110 to 200 nm, intended for loading DOX for tumor therapy. Biotin within the Bio-CDPu served dual roles, functioning both hydrophobically and in targeting (Fig. 31, compound **40**). Consequently, it was speculated that compound **40** nanocarriers possessed amphiphilicity, safety, liver

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



38

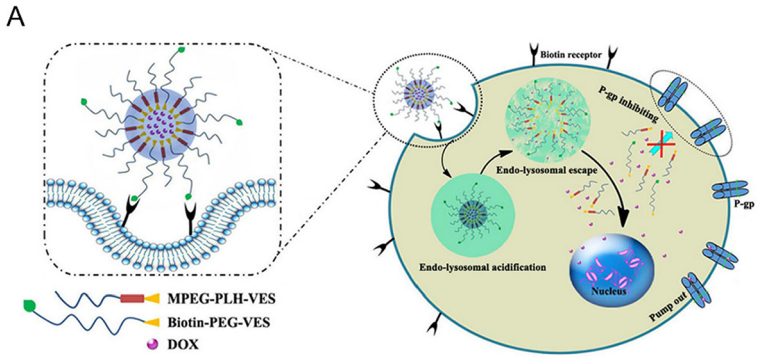
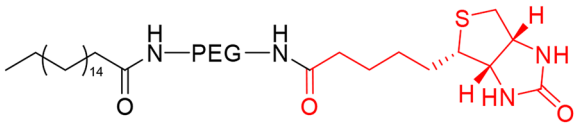


Fig. 29 The structure of compound **38**. (A) Pathways and modes of action of nanosystems. Reproduced with permission.[110] Copyright 2018, ACS.



39

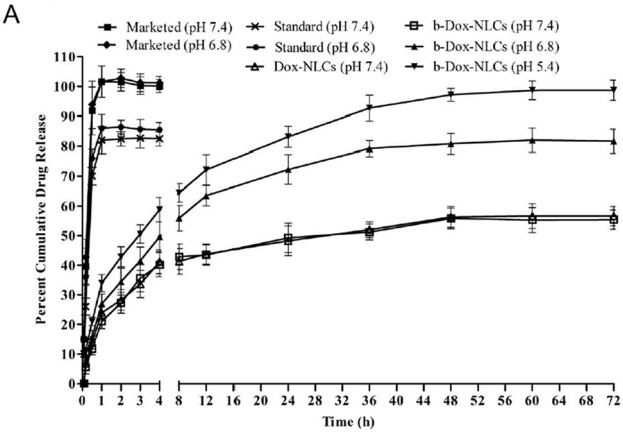


Fig. 30 The structure of compound **39**. (A) Percent cumulative drug release. Reproduced with permission.[111] Copyright 2020, Taylor & Francis.

targeting, and excellent anti-cancer drug loading performance, potentially effective for liver tumors and reducing drug side effects. Drug release experiments were conducted under different pH conditions. The results indicated that lower pH values correlated with increased solubility of DOX in nanoparticles, facilitating controlled drug release, particularly in tumor cells. Specifically, the release rate exceeded 60% at pH 7.4 and exceeded 80% at pH 3.5. The release duration of compound **40** NPs was approximately 12 h, with higher drug content correlating to lower release rates. Notably, the cytotoxicity of compound **40** NPs to normal cells was greatly reduced, even at concentrations in the range of 10–300 μg mL^{-1}. However, the cytotoxicity of compound **40** to tumor cells Bel-7404 did not decrease, and the efficacy of compound **40** was higher than that of DOX alone at 0.1–10 μg mL^{-1} concentration. To verify whether compound **40** NPs could target biotin receptors, FITC-labeled compound **40** was utilized for CLSM and FCM analysis. Results indicated enhanced uptake of compound **40** NPs by Bel-7404 cells compared to free drugs. Additionally, the authors demonstrated that the inhibitory effect on tumor cells was stronger with higher biotin content. Encapsulation of DOX by compound **40** NPs improved its action efficiency and reduced associated side effects, leading to improved bioavailability. Pharmacokinetic analysis of DOX/Bio-CDPu NPs indicated higher AUC (551.04 mg L^{-1} h^{-1}, 2.51 times), slower CL (0.007 kg L^{-1} h^{-1}, 5.57 times), longer $t_{1/2}$ (69.32 h, 5.96 times), and longer MRT0-t (21.28 h, 2.62 times). After two weeks of treatment, the tumor inhibition rate of the DOX/Bio-CDPu group was 64%, higher than that of the free DOX group (47%), underscoring the potent anti-tumor therapeutic effect of DOX/Bio-CDPu (Fig. 31A–D). This coupling not only ensures the proper functionality of DOX but also minimizes side effects, with biotin playing a pivotal role.

In 2019, Baskaran Purushothaman *et al.*[116] developed a nanoparticle-based drug delivery system (NDDS), a new self-

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

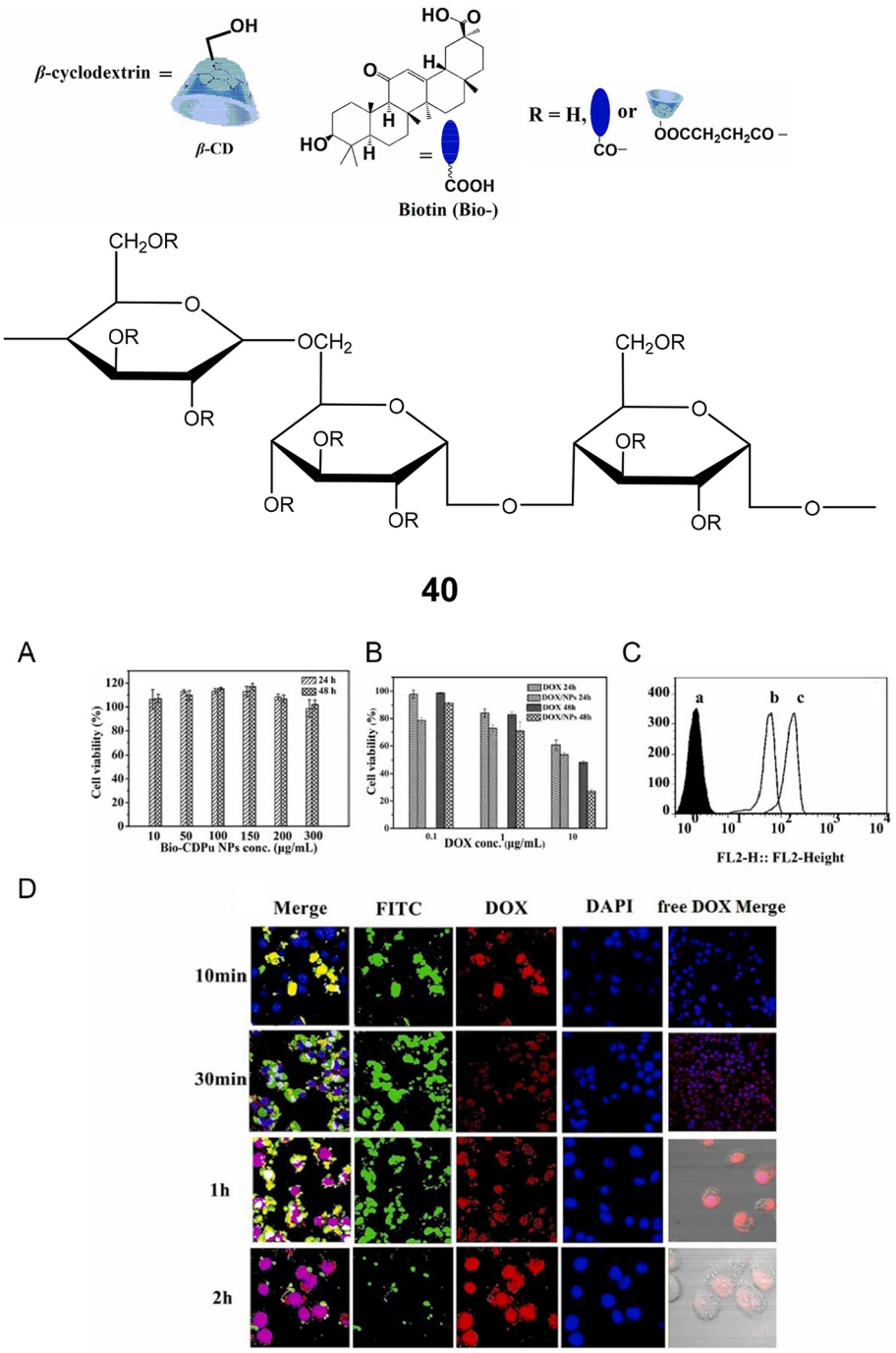
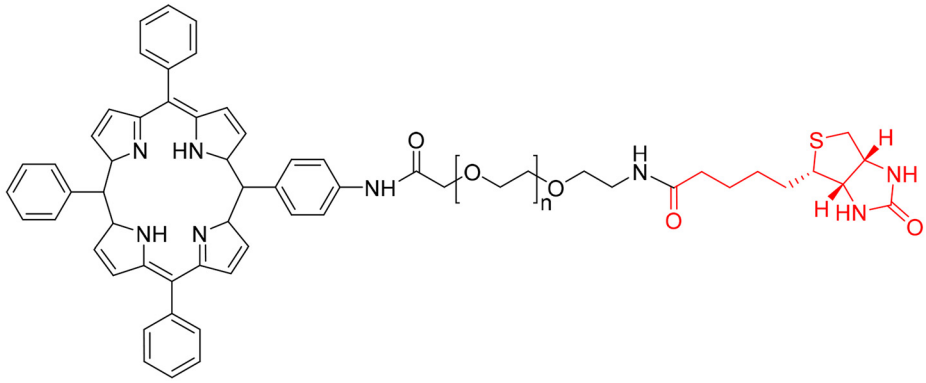


Fig. 31 The structure of compound **40**. (A) The cytotoxicity of Bio-CDPu NPs *in vitro*. (B) The cytotoxicity of free DOX and DOX/Bio-CDPu NPs. (C) FCM analysis results. (D) CLSM images. Reproduced with permission.[113] Copyright 2022, Elsevier.

assembled photosensitizer (PS) nanoparticle based on DDSs for chemical drugs.[117] The author chooses chlorophyll derivative tetraphenylporphyrin (TPP) as a PDT agent in the complex. PDT can selectively kill cancer cells without affecting adjacent normal cells, improving anticancer efficacy and reducing side effects. This nanoparticle is a PS-coupled amphiphilic biotinylated polymer (TPP-PEG-biotin) (Fig. 32, compound **41**) as a nanocarrier for DOX to develop new self-assembled NPs for combination therapy. After coupling biotin with TPP and PEG, the author synthesizes it into a nanocarrier compound **41** self-assembled nanoparticles (SANs). To determine the effectiveness of the complex, the nanocarrier-loaded DOX is a nanocarrier carrying drugs to form DOX@compound **41** SANs. The experimental findings demonstrate that DOX-loaded compound **41** SANs can effectively penetrate the tumor area and exert a stronger antitumor effect. Additionally, it is confirmed that compound **41** and its self-assembled nanoparticles target subcellular orga-

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



41

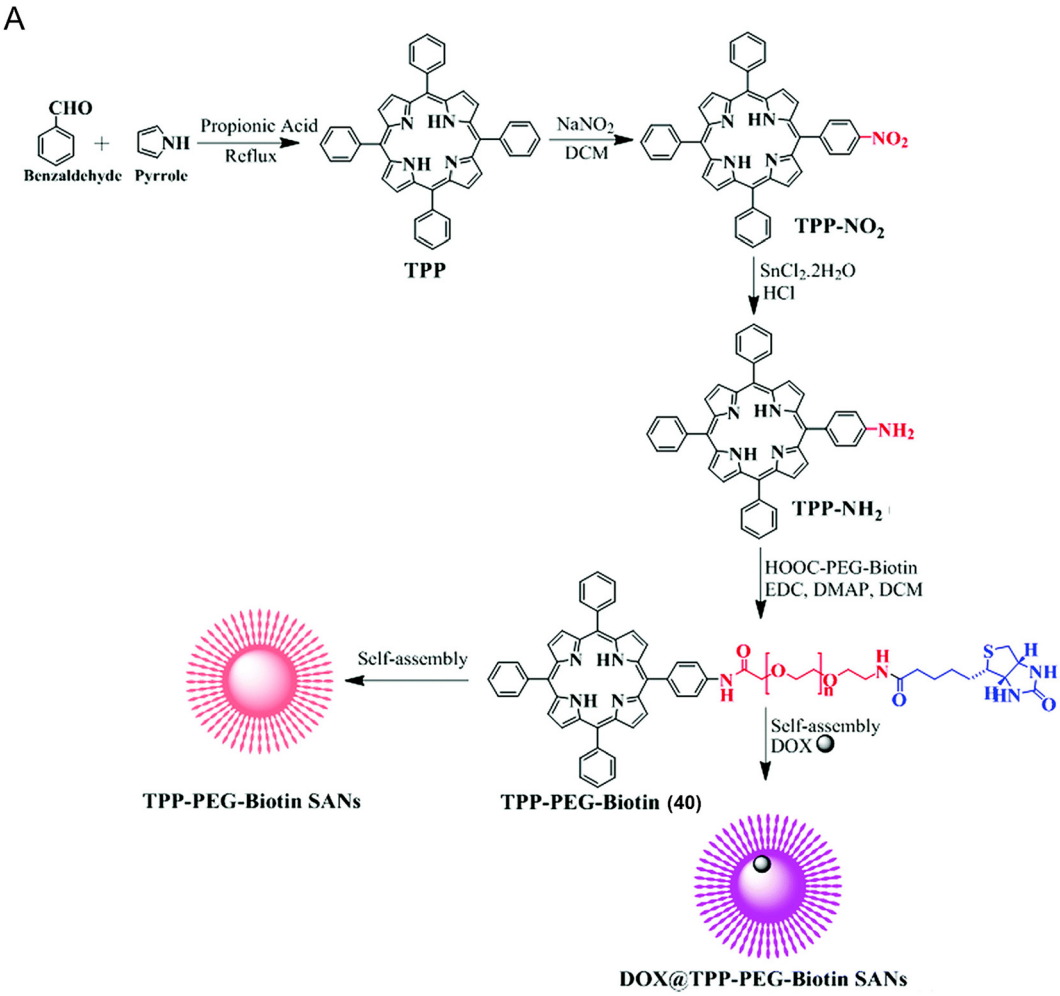


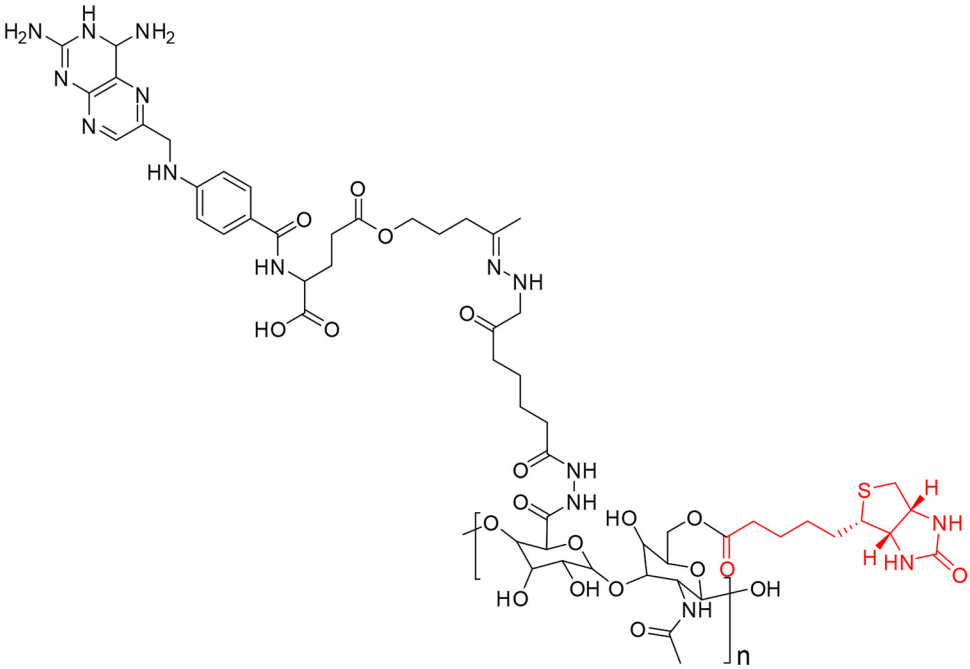
Fig. 32 The structure of compound **41**. Intracellular release of DOX. (A) Synthesis of compound **41** SANs and DOX@ compound **41** SANs. Reproduced with permission.[116] Copyright 2019, RSC.

nelles, including mitochondria and lysosomes (Fig. 32A and B). This paper proposes the synthesis of DOX@compound **41** SANs as a promising chemophotodynamic combination anticancer drug with the potential for synergistic anticancer effects and tumor targeting.

4.7. Targeting DYRK2 and CTL epitope peptide

The authors have proposed that two ligands bind to drugs or liposomes, which is more targeted than a single ligand. In 2019, Mengna Liu *et al.*[118] designed an anti-tumor drug with

icariin (ICA) and curcumin (Cur) based on low molecular weight hyaluronic acid-hydrazone bond-folate-biotin (Bio-oHA-Hyd-FA) (Fig. 33, compound **42**).[119] The compound synthesized by the author has low molecular weight hyaluronic acid and features a hydrazone bond that is sensitive to pH. Hence, the compound also exhibits pH sensitivity. The pH-sen-



42

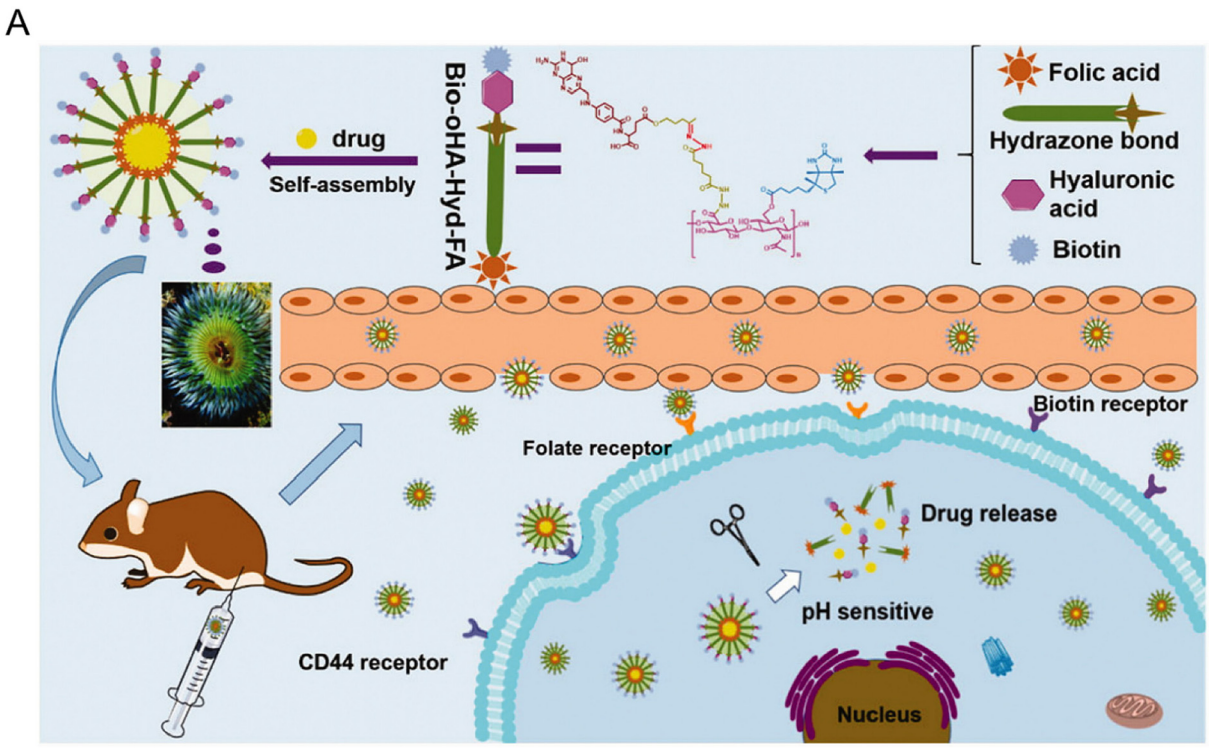


Fig. 33 The structure of compound **42**. (A) The sketch map of compound **42** carriers self-assembly into polymeric micelles ("nano-actiniaes") and the anti-cancer drugs released from nanomicelles in the weakly acidic environment. Reproduced with permission.[118] Copyright 2019, Taylor & Francis.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

sitive group in the nanocarrier is destroyed in an acidic environment, making it easier for anticancer drugs to be released in tumor cells. This method further enhances the targeting of tumor cells and reduces the toxic and side effects on normal cells. The addition of biotin and folic acid increases the targeting effect.[120] Folate receptor is overexpressed in tumor cells, which can preferentially uptake folic acid for tumor cells. In this study, the cytotoxicity of compound **42** material, free Cur, free ICA, free ICA & Cur, curs-loaded polymeric micelles, ICA-loaded polymeric micelles, ICA & curs-loaded polymeric micelles on MCF-7 cells and BCSCs is evaluated by MTT reagent. From the MTT experiment, when the concentration of compound **42** material reaches 500 lg mL^{-1}, the survival rate of BCSCs and MCF-7 cells is still higher than 75%, indicating that compound **42** has low toxicity and excellent safety. The cytotoxicity of ICA & curs-loaded polymeric micelles is significantly higher than that of the free Cur group, free ICA group, free ICA & Cur group, curs-loaded polymeric micelles group, and ICA-loaded polymeric micelles group, which may be due to the combination of icariin and curcumin. And the authors also demonstrate that the co-delivery of curcumin and epimedium glycosides has a significant effect on the invasion of MCF-7 cells and BCSCs (Fig. 33A). The above results indicate that the compound **42** drug carrier has pH sensitivity and multi-target characteristics and is reliable in targeting cancer cells and cancer stem cells. The dual-targeted drug proposed by the author utilizes the targeting of biotin and folic acid to carry anti-tumor drugs to the tumor cells. This strategy provides a new idea for the development of dual-targeted biotin antitumor drugs.

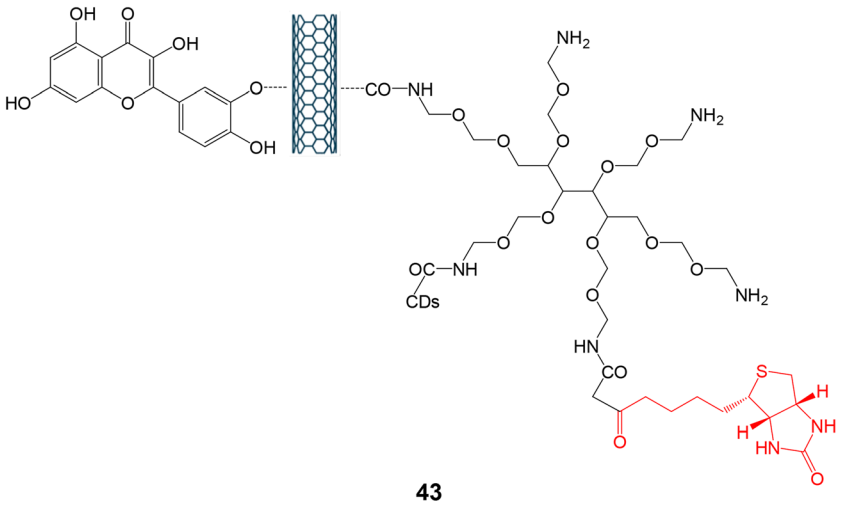
4.8. Targeting phosphodiesterase (PDE)

Nanotubular kaolin, also referred to as kaolin nanotubes (HNT) in certain materials science literature, represents the predominant natural form of kaolin.[121] HNTs have garnered significant interest in the biomedical realm. Possessing a negatively charged outer surface and a positively charged inner core under most natural pH conditions, kaolin allows for various modifications as drug delivery carriers. Furthermore, they exhibit commendable biocompatibility, cost-effectiveness, and environmental friendliness. Surface modification of nanoparticles with PEG serves the purpose of mitigating their immunogenicity, toxicity, protein adsorption, surface interaction, poor cell uptake, and cell adhesion. Quercetin (Que), a flavonoid alcohol compound found abundantly in the plant kingdom, is recognized for its diverse biological activities. However, despite its anticancer potential, Que was classified as a class 3 carcinogen by the International Agency for Research on Cancer of the World Health Organization in 2017 due to its carcinogenicity. In fact, Que exhibits anticancer properties. Yet, its efficacy is hindered by factors such as poor water solubility, low bioavailability, short half-life, and weak tumor accumulation, impeding its application and antitumor effectiveness *in vivo*.[122] The principal mechanism underlying quercetin's antitumor effects lies in its ability to inhibit PDE activity. Que has been shown to suppress the activity of phosphodiesterase, a group of enzymes responsible for regulating intracellular second messengers, including cAMP phosphodiesterase and cGMP phosphodiesterase. These enzymes play a pivotal role in modulating cell signaling pathways, and quercetin's inhibition may influence processes such as cancer cell proliferation and apoptosis, among other biological responses.

In 2018, Yamina Ait Mehdi *et al.*[123] synthesized HNTs-*g*-PEG-CDs-Biotin (CDs multi-carbon dots) (Fig. 34, compound **43**) using kaolin nanotubes as the base material, along with PEG and biotin for modification (Fig. 34A). The grafted halloysite nanotubes (HNTs-*g*-PEG) were decorated with carbon quantum dots for additive fluorescents properties. MTT analysis reveals that the cell viability remains above 80% even with increased concentrations of blank nanoparticles containing compound **43**, indicating the low toxicity of the prepared nanoparticles themselves (Fig. 34D and E). Que is released from both HNTs and compound **43** NPs in PBS solution at pH 7.4 (Fig. 34B and C). In addition, it is released from compound **43** in PBS solutions at pH 6.8 and pH 5.0 and compared to the release carried out at pH 7.4. Biotin-based therapeutic agents HNTs-*g*-PEG maintain the pharmacological effect of Que and enhance its anticancer efficacy. The compound **43** loaded with Que exhibits heightened cytotoxicity. This study underscores the potential of PEG-grafted CDs and biotin NPs as effective carriers for targeted drug delivery, both *in vitro* and *in vivo*.

In 2022, Kangkang Li *et al.*[124] devised Que-loaded mixed micelles (Que-MMICs) assembled from 1,2-distearoyl-*sn*-glycerol-3-phosphate ethanolamine-polyethylene glycol-biotin (DSPE-PEG-biotin) and poly(ethylene glycol) methyl ether methacrylate-poly[2-(dimethylamino)ethyl acrylate]-polycaprolactone (PEGMA-PDMAEA-PCL) (Fig. 35, compound **44**) for treating NSCLC (Fig. 35A). The blood compatibility of compound **44** is evaluated *via* a hemolysis test, revealing its good blood solubility and potential as a nanocarrier for drug loading. Comprising hydrophilic PEMMA, PDMAEA, and hydrophobic PCL, the copolymer facilitates enhanced encapsulation of Que in nanoparticles, thus improving its solubility and stability, as evidenced by replacing Que with an NLR fluorescent probe. The Que-loaded compound **44** mixed micelles (Que-MMICs) are prepared using the thin-film hydration method. Subsequent uptake experiments demonstrate a 1.2-fold increase in cellular uptake of biotin-conjugated nanoparticles compared to NLR-MICs. Que alone exhibits a concentration-dependent effect on A549 cells, albeit weakly. This suggests that Que's anti-tumor activity can be potentiated in the presence of nanoparticles. Treatment with 10 mg mL^{-1} Que-MMICs results in a significant increase in the percentage of A549 cells in the G2/M phase, indicating cell cycle arrest and apoptosis induction. Que-MMICs effectively inhibit A549 cells by inducing apoptosis and cell cycle arrest, demonstrating superior efficacy compared to Que-MICs and free Que. Additionally, migration ability determination, *in vivo* biodistribution, *in vivo* anti-tumor activity, ICH, and HE safety evaluations consistently affirm the tumor-targeting and anti-tumor effects of biotin-modified micelles. This enhancement is attributed to the introduction of biotin (DSPE-PEG-biotin), which facilitates drug accumulation in tumors *via* specific receptor-mediated endocytosis. Overall, Que-MMICs exhibited robust

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



43

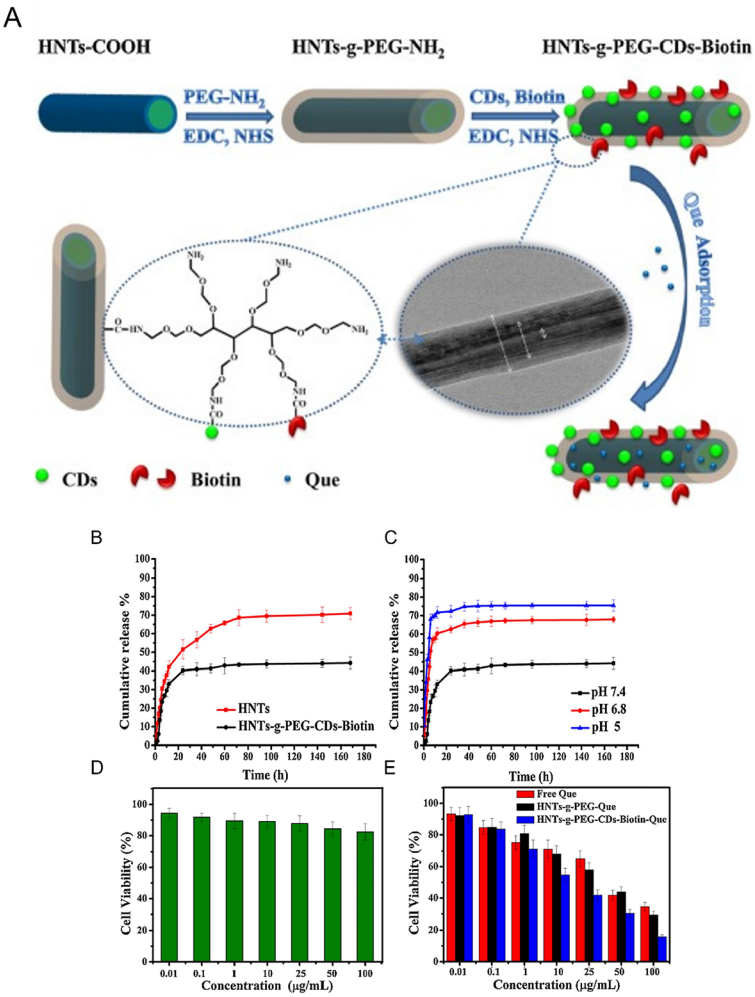
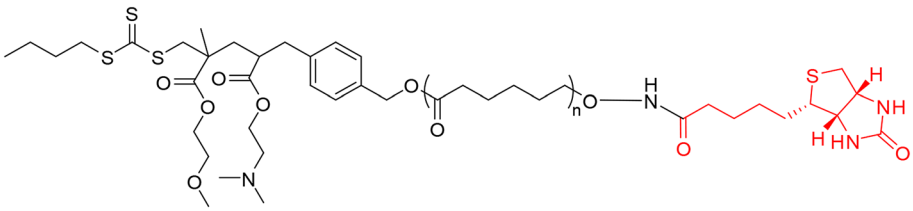


Fig. 34 The structure of compound **43**. (A) Schematic illustration of the chemical structure and preparation procedure of compound **43**, followed by Que adsorption. TEM images of HNTs-*g*-PEG with scale bare 100 nm with 20 nm insert in the right bottom (B), and 50 nm (C). *In vitro* cytotoxicity of HNTs-*g*-PEG-Biotin towards HeLa cells (D), free Que, Que-loaded HNTs-*g*-PEG and Que-loaded HNTs-*g*-PEG-CDs-Biotin (E) determined by MTT assay. Reproduced with permission.[123] Copyright 2018, Elsevier.

 This journal is © The Royal Society of Chemistry 2025



44

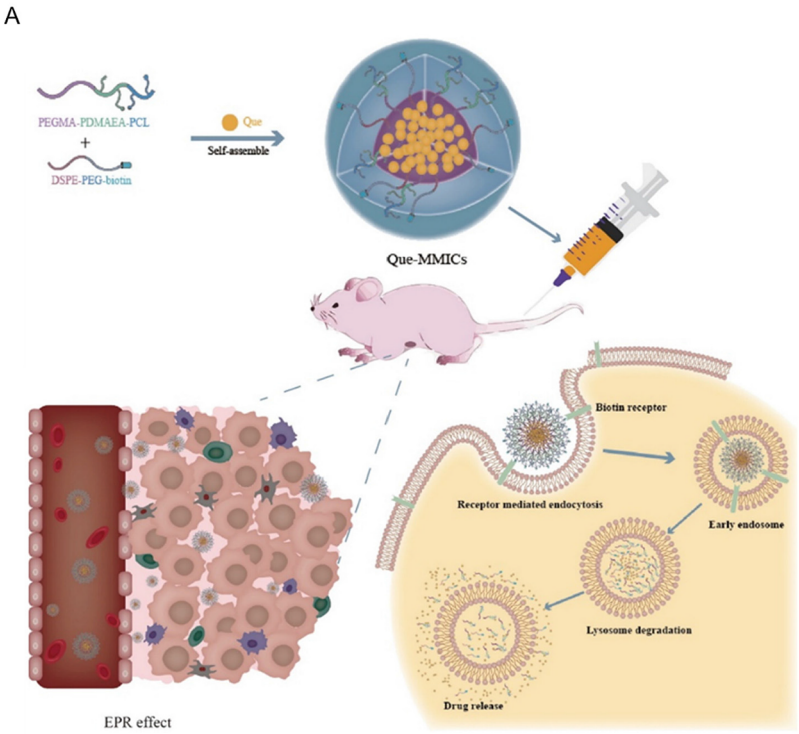


Fig. 35 The structure of compound **44**. (A) Assembly and mechanism of action of drugs. Reproduced with permission.[124] Copyright 2022, Taylor & Francis.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

anti-tumor efficacy against A549 cells *in vitro* and *in vivo*, promising further development as an effective drug delivery system.

4.9. Targeting T and B cells

CD20 × CD3, a T cell bispecific antibody, holds promise for treating B-cell non-Hodgkin's lymphoma by recruiting T cells around B cells, thereby inducing a lethal effect on the B cells. This dual-antibody approach targets tumor cell surface antigens (CD20) and effector T-cell surface antigens (CD3), bringing healthy T cells in close proximity to cancerous B cells to facilitate their elimination.[125,126] Chen Bai *et al.*[127] introduced a groundbreaking method in 2020 by coupling biotinylated CD20 and CD3 antibodies to the surface of streptavidin-modified ultrasmall Fe_3O_4 nanoparticles to create a bispecific nanoplatform (BSNP, CD20&CD3@Fe_3O_4) (Fig. 36A–D, compound **40**). *In vitro* experiments demonstrate that compound **45** exhibits a more potent cell-killing effect compared to monoclonal CD20 antibodies. During the 24 h, 48 h, and 72 h co-incubation period with Raji cells, there is a progressive decrease in the survival rate of RAJI cells, indicating significant cytotoxicity. When exposed to the same concentration of CD20 antibody, the survival rate of compound **45** against Raji cells is lower than that of the monoclonal CD20 antibody, thereby significantly enhancing the effect (Fig. 36E–G). Furthermore, the authors illustrate that although compound **45** exhibits greater cytotoxicity, it induces less toxicity in K562 cells (Fig. 36H–J). *In vivo* experiments demonstrate that the addition of biotin not only augments the effect of CD20 but also reduces toxicity to normal cells, thereby enhancing efficacy and minimizing adverse effects. Additionally, compound **45** not only directly eliminates cancer cells but also enhances antitumor immune function. Notably, *in vivo* experiments show that compound **45** treatment does not induce pathological damage to normal tissue in mice. This indicates the potential of compound **45** to selectively target tumor cells, thereby minimizing toxicity to normal cells. This is a promising finding that could significantly impact the development of

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

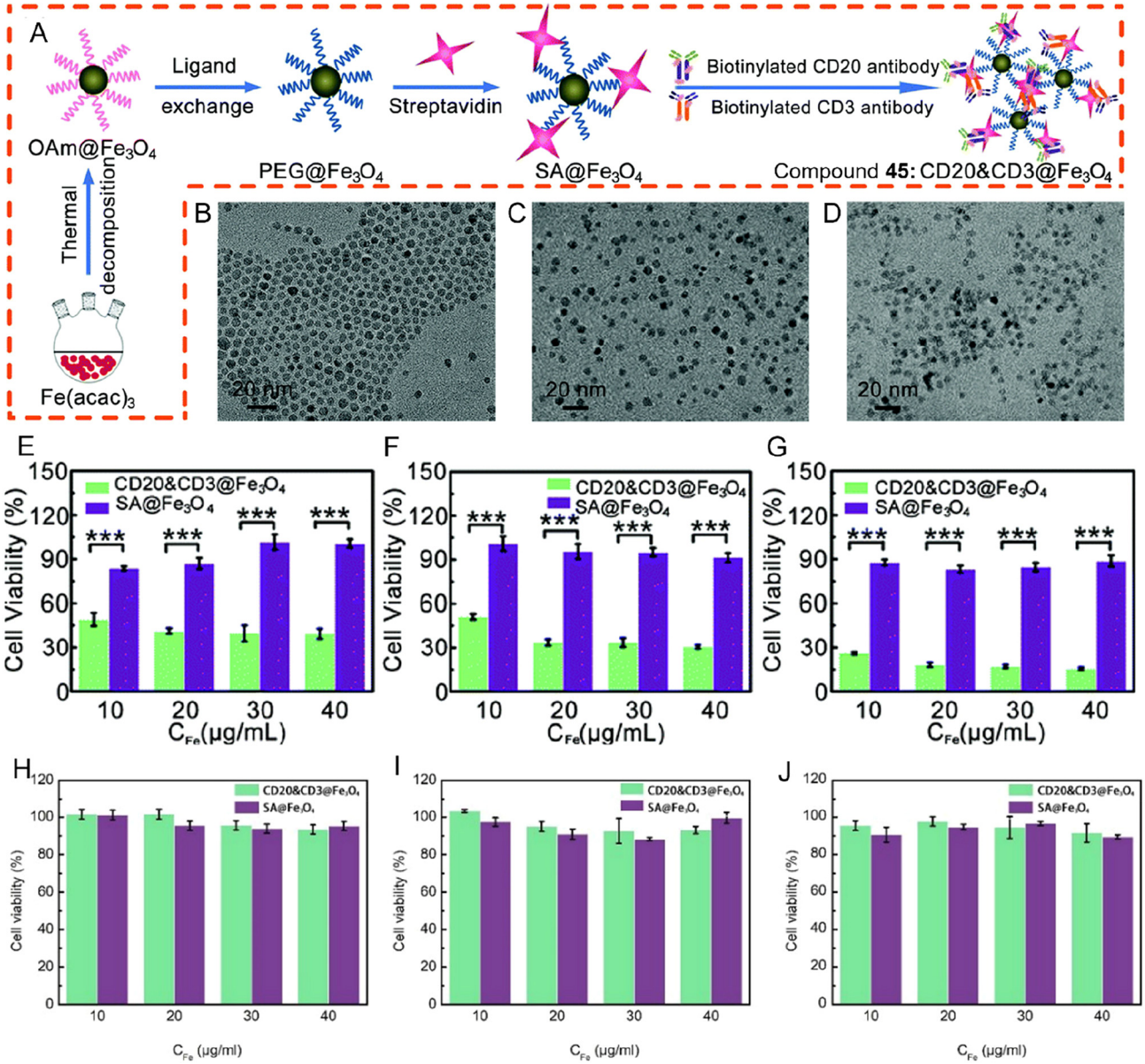


Fig. 36 (A) The structure of compound **45**. (B) PEG@Fe_3O_4, (C) SA@Fe_3O_4, and (D) CD20&CD3@Fe_3O_4. Cell viabilities of Raji cells incubated with different concentrations of SA@Fe_3O_4 and CD20&CD3@Fe_3O_4 for (E) 24 h, (F) 48 h, and (G) 72 h. Cell viabilities of K562 cells incubated with different concentration of SA@Fe_3O_4 and CD20@Fe_3O_4 for (H) 24 h, (I) 48 h, and (J) 72 h. Reproduced with permission.[127] Copyright 2020, RSC.

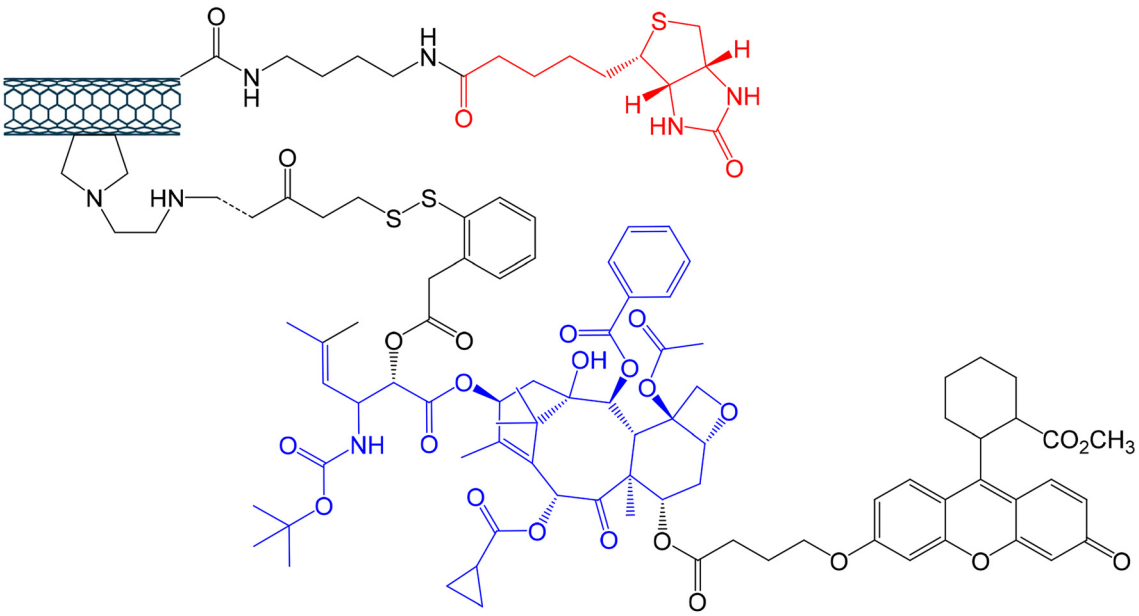
cancer drugs. Additionally, the authors employ physical methods to validate the stable physical and chemical properties of compound **45**, including an appropriate hydrodynamic size (approximately 30 nm), strong saturation magnetization (about 50 emus g^{-1} [Fe]), and enhanced $T1$ contrast ability for acquiring bimodal MRI-fluorescence images.[128]

4.10. Targeting tubulin

Microtubule inhibitors have significant effectiveness in cancer treatment, but traditional drug delivery methods lack tumor specificity, which can lead to systemic toxicity and side effects, limiting their therapeutic effect. To address this, researchers have turned to nanotechnology to improve the targeting and effectiveness of microtubule inhibitors. In 2008, Jingyi Chen and colleagues developed a biotin-functionalized single-walled carbon nanotube (SWNT) conjugate to enhance drug delivery efficiency and precision.[129] The nanometer size and high surface area of the SWNT increased the drug loading capacity, while biotin targeted the drug to cancer cells by binding to biotin receptors, which are highly expressed on the surface of cancer cells. This ensured that the drug was concentrated in the tumor cells, reducing its impact on normal tissues. In later studies, the authors combined paclitaxel with the SWNT to form a Biotin-SWNT-linker-taxoid-fluorescein conjugate

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



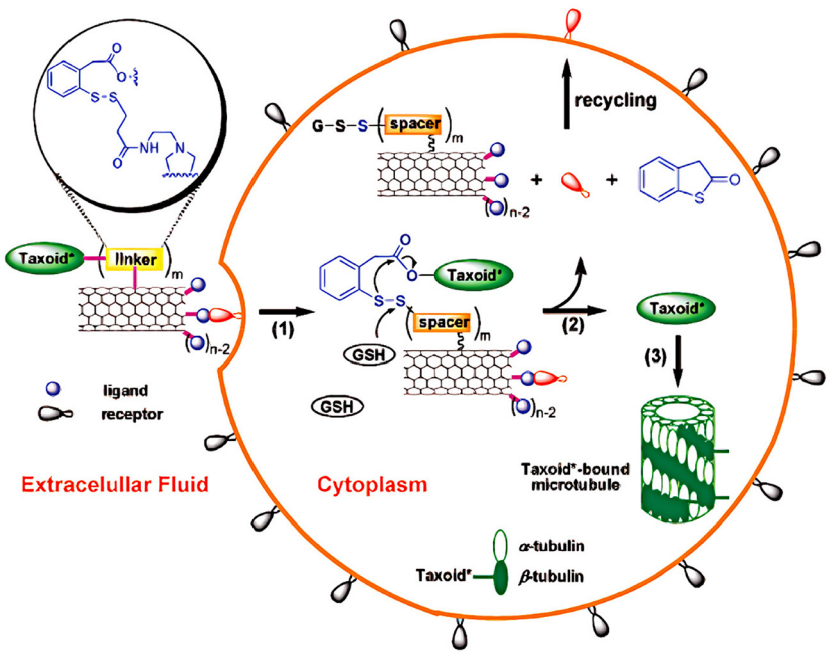


Fig. 37 The structure of compound **46**. Schematic illustration of three key steps involved in the tumor-targeting drug delivery of compound **46**. Reproduced with permission.[129] Copyright 2008, ACS.

(Fig. 37, compound **46**). Experimental results showed that this conjugate entered cancer cells through receptor-mediated endocytosis, and the released paclitaxel effectively bound to microtubules, triggering specific toxicity in tumor cells. Compared to direct drug exposure, this delivery system significantly increased the drug accumulation in cancer cells, demonstrating the great potential of biotin-functionalized SWNTs for tumor treatment.

In 2018, Tao Wang and colleagues studied the design and synthesis of asymmetric butterfly-shaped PAMAM dendrimers (ABTD).[130] The ABTD scaffold was constructed using two half-dendritic units (G3-HD and G1-HD), each functionalized to carry different drug payloads. The G3-HD unit was attached to 16 biotin molecules, while the G1-HD unit carried 4 paclitaxel molecules. The targeting ability of biotin allowed the ABTD conjugates to specifically deliver therapeutic drugs, such as

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

paclitaxel, to tumor cells, while avoiding damage to normal cells. They used this method to synthesize a variety of effective structures, of which compound **47**, compound **48**, and compound **49** (Fig. 38) were the most effective. This significantly improved the selectivity and effectiveness of the treatment. Experimental results showed that the compound **47** conjugate had remarkable selectivity, with an affinity for cancer cells 1400 to 7500 times higher than for normal cells, further proving the advantage of biotin in enhancing the targeting efficiency of drug delivery. Additionally, biotin's role was not limited to drug targeting; it also enhanced the internalization efficiency of the conjugate in tumor cells. When compared to paclitaxel labeled with a fluorescent probe, compound **49** showed stronger cellular uptake, highlighting the important role of the multiple biotin–biotin receptor binding effects in improving drug internalization and therapeutic efficacy (Fig. 38A–F). This demonstrates that biotin's multiple functions in dendrimer-based drug delivery systems make it a highly promising platform for targeted therapy.

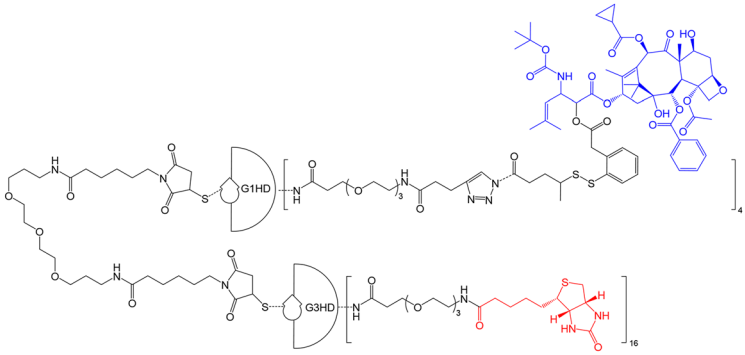
Microtubule inhibitors represent a highly effective strategy against tumors, yet conventional approaches to anti-tumor treatment possess certain limitations. Consequently, researchers have turned to nanotechnology to enhance the efficacy of microtubule inhibitors, aiming to unlock unforeseen therapeutic effects. In 2018, Sri Vishnu Kiran Rompicharla *et al.*[131] aimed to mitigate side effects and enhance drug efficacy by developing and investigating a biotin-coupled-PEG-modified multifunctional PAMAM dendrimer (G4-PTX-PEG-biotin) system (depicted in Fig. 39 compound **50**) for efficient delivery of the chemotherapeutic drug PTX, known for its poor physical and chemical properties. Fluorescence analysis revealed a significant increase in geometric mean fluorescence, with biotin-labeled dendritic molecular conjugates exhibiting 1.53 and 1.94 times higher absorption at 1 hour and 4 h, respectively (Fig. 39A).[132] These findings indicate that biotin coupling plays a crucial targeting role in this complex. The cytotoxicity of dendritic molecule-drug conjugates is evaluated in A549 cells using the MTT assay. Results demonstrate that compound **50** outperforms both G4-PTX-PEG and free PTX after 24 h and 48 h of exposure, indicating superior cytotoxicity (Fig. 39B). The heightened efficacy of compound **50** is attributed to its enhanced enrichment in A549 cells facilitated by biotin targeting, consequently inducing greater cell death. The study employs a 3D sphere model to emulate solid tumors and conducts a multicellular tumor sphere experiment to assess the efficacy of the complex (Fig. 39C and D). Results indicate that biotin receptor-mediated uptake of F-G4-PEG-biotin facilitates better diffusion of dendritic polymer conjugates within 3D tumorspheres, with fluorescence concentrated in the microsphere core. Additionally, surface biotin coupling significantly enhances dendritic conjugate absorption, leading to notable internalization into spheroids and increased drug availability at the target site. This active targeting approach results in superior PTX accumulation in cells, thus enhancing therapeutic effects. Variations in PTX treatment demonstrate significant differences in antitumor capacity, with free PTX exhibiting limited penetration into the sphere mass. Overall, the study suggests the potential of biotin-modified conjugates as a promising treatment strategy for solid tumors, warranting further investigation for clinical translation.

In 2018, Ishwor Poudel *et al.*[133] developed biotinylated chitosan-modified DTX loaded nano-acid salt (BI-CHI-DTX-NC) to achieve controlled drug release, improve bioavailability, targeted drug delivery, and enhance anti-cancer ability while reducing the systemic toxicity of DTX. Firstly, DTX is loaded onto nano-salts (DTX-NC) by calcium-catalyzed di-meat bean phosphatidylglycerol sodium (DMPG-Na) and cholesterol-loaded liposomes. Then DTX-NC is encapsulated with BI-CHI (Fig. 40, compound **51**) (BI-CHI-DTX-NC) and compared with DTX and DTX-NC. The release of DTX shows a strong pH dependence, and the release rate is highest in the acidic environment of tumor cells. The prepared compound **51** shows higher *in vitro* anticancer activity in biotin-overexpressing human breast cancer MCF-7 cells. *In vitro* experiments show that the drug concentration (GI_{50}) required for free DTX to inhibit 50% cell growth is 1.8 lg ml^{-1}, while DTX-NC decreases by 33.34% (1.2 lg ml^{-1}). The GI_{50} value of compound **51** is 0.2 lg ml^{-1}, 88.89% lower than that of the DTX solution. In addition, compound **51** shows a 10-fold increase in bioavailability, longer cycle time, slower plasma elimination, and lower tissue distribution than the free DTX solution. *In vitro* drug release studies demonstrate the favorable drug release kinetics and prolonged duration of action of the newly synthesized compounds. These results indicate that the presence of biotin effectively enhances both the drug release profile and sustained action properties (Fig. 40A). These all suggest that compound **51** has higher strength and accuracy. The presence of this compound can increase the anti-tumor efficiency of anticancer drugs and reduce side effects.

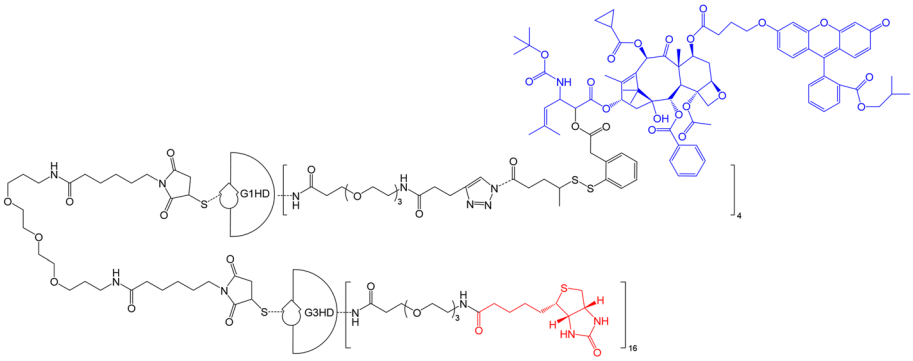
In 2020, Baolan Tang *et al.*[134] explored the impact of multiple biotin couplings to liposomes on their targeting efficacy. They synthesized four biotin-modified Lip ligands: biotin-cholesterols (Bio-Chol), Bio-Bio-Chol, Tri-Bio-Chol, and Tetra-Bio-Chol, containing one, two, three, and four biotins, respectively (Fig. 41, compounds **52**, **53**, **54** and **55**). Subsequently, Bio-Lip, Bio-Bio-Lip, Tri-Bio-Lip, and Tetra-Bio-Lip with varying densities of targeted molecules are prepared for breast cancer targeting (Fig. 41A). Their study of Lip-loaded PTX (PTX) reveals that PTX-compound **54** exhibits both enhanced anti-tumor efficacy and superior targeting ability. Notably, the enrichment capacity within tumor cells follows the order: compound **54** > compound **55** > compound **53** > compound **52** > Lip. This research underscores that the strength of targeting is not solely determined by the number of biotins; rather, optimal targeting is achieved with three biotin couplings. In the apoptosis assay, PTX-compound **54** modified with compound **54** exhibits a greater propensity to induce apoptosis in 4T1 cells compared to other groups, consistent with the findings of *in vitro* cytotoxicity assessments. Thus, this study not only enhances the targeting ability of biotin against breast cancer but also provides valuable insights for the advancement of targeted drug delivery systems.

 This journal is © The Royal Society of Chemistry 2025

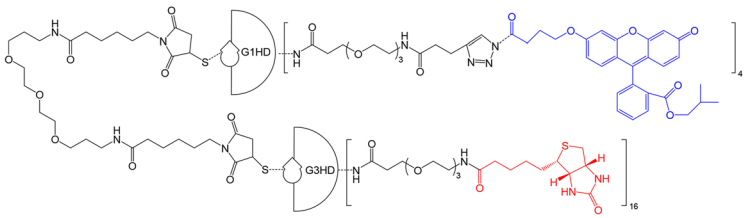
Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



47



48



49

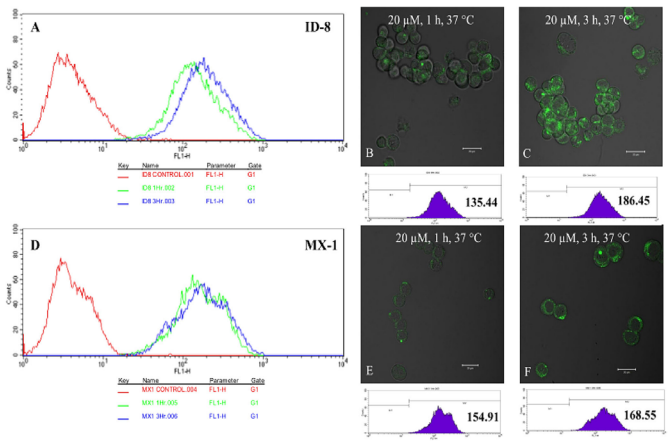


Fig. 38 The structure of compound **47**, compound **48** and compound **49**. CFM images and flow cytometry analysis of different types of cells after incubation with 3 at the final concentration of 20 μM at 37 °C for different periods. (A) Flow cytometry analysis of 3 in ID-8 at 0 h (red, control), 1 h (green), and 3 h (blue). (B, C) CFM images and flow cytometry analysis in ID8 at 1 and 3 h, respectively. (D) Flow cytometry analysis of 3 in MX-1 at 0 h (red, control), 1 h (green), and 3 h (blue). (E, F) CFM images and flow cytometry analysis in MX-1 at 1 and 3 h, respectively. Reproduced with permission.[130] Copyright 2018, ACS.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

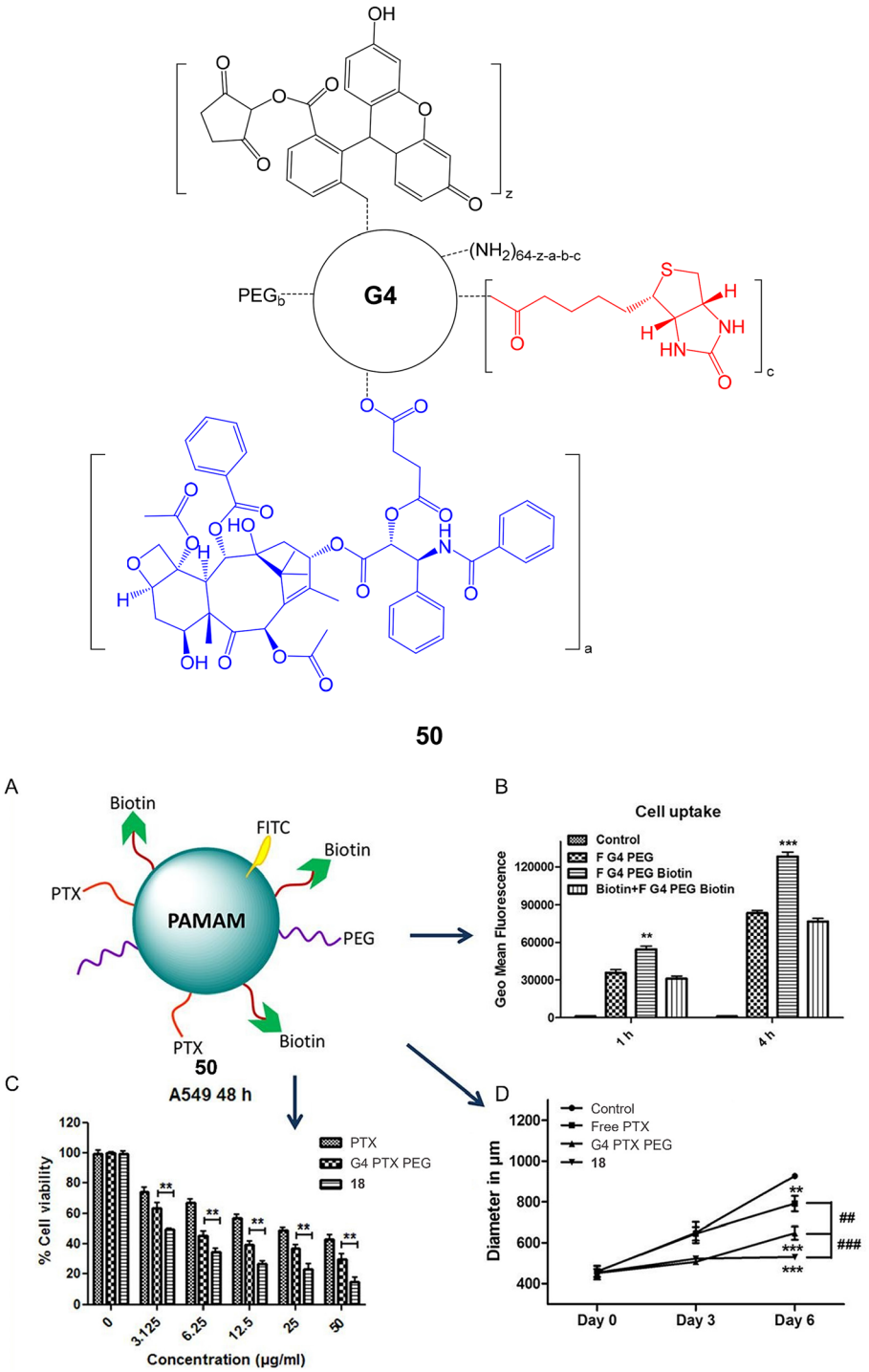
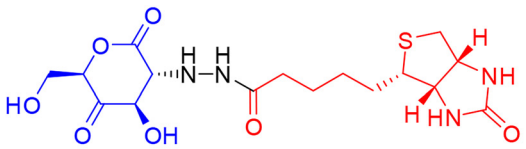


Fig. 39 The structure of compound **50**. (B) Cellular uptake. (C) Percentage cell viability. (D) Line graph of spheroid diameters depicting growth inhibition. Reproduced with permission.[131] Copyright 2019, Elsevier.

In the same year, Mengyi Huang *et al.*[135] introduced and designed biotin-glucose branched ligand-modified dual-targeted liposomes (Bio-Chol, compound **56**; Bio-Glu-Chol, compound **57**) (Fig. 42) and assessed their potential as targeted chemotherapy drug delivery systems both *in vitro* and *in vivo* (Fig. 42A). Cancer cells exhibit robust growth, development, and reproduction, necessitating increased glucose uptake to fulfill their energy demands. This heightened glucose uptake is attributed to the overexpression of glucose transporter 1 (GLUT1) in various cancer cells, a phenomenon known as the Warburg effect.[136] Compared to non-targeted Lip, Bio-Lip, and Glu-Lip, Bio-Glu-Lip demonstrates the highest uptake in 4T1

 This journal is © The Royal Society of Chemistry 2025



51

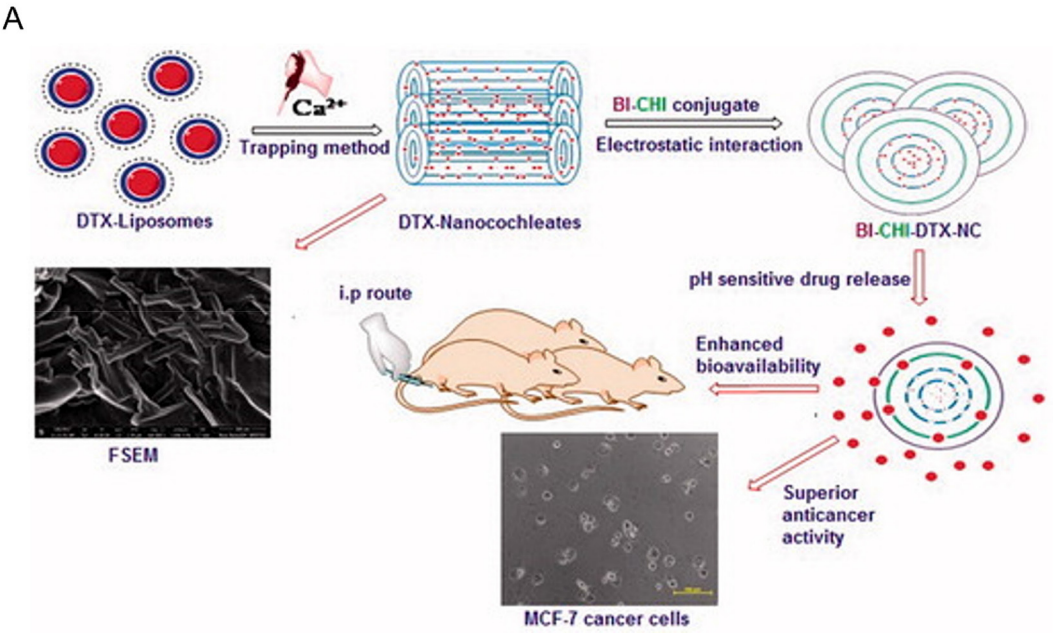


Fig. 40 The structure of compound **51**. (A) Compound **51** synthesis process and electron microscope images. Reproduced with permission.[133] Copyright 2018, Taylor & Francis.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

cells (3.00 times, 1.60 times, and 1.95 times higher, respectively) and MCF-7 cells (2.63 times, 1.63 times, and 1.85 times higher, respectively). The efficacy of Bio-Glu-Lip is further validated through stability and blood compatibility tests, crucial for ensuring the therapeutic efficacy of liposomes under biological conditions. Lip containing PTX demonstrates slow-release kinetics during the assessment of PTX release properties, with cumulative release rates remaining below 70% after 48 hours. Notably, PTX-Bio-Glu-Lip displays similar release properties to other PTX-loaded liposomes, indicating its safety profile *in vivo*. *In vivo* imaging results in 4T1 breast tumor-bearing BALB/c mice confirm the dual-targeted delivery capability of biotin and glucose-modified liposomes to breast tumors, thus validating the efficacy of PTX-Bio-Glu-Lip. Overall, these findings highlight the potential of Bio-Glu-Lip as a safe and effective drug delivery system for targeted chemotherapy, with promising implications for cancer treatment.

In 2020, Qijun Liu *et al.*[137] addressed the challenges of glioma chemotherapy, which often lacks specificity due to the blood–brain barrier restricting drug penetration into the central nervous system. To overcome this, they pioneered the development of multi-targeted liposomes utilizing glucose and biotin. Two ligands (Glu_3-Chol, Bio_2-Chol) were synthesized, and three modified liposomes (Glu_3-Chol; Bio-Chol, compound **58**; Bio_2-Chol, compound **59**) were prepared (Fig. 43). Their investigation focuses on the uptake mechanism of the PTX-loaded compound **59** (Fig. 43A). The findings reveal a significant enhancement in targeting efficiency on U87MG and C6 cells *in vitro*, showing 4.04 times and 3.49 times higher uptake compared to uncoated Lip, respectively. Qualitative research using confocal laser scanning microscopy (CLSM) and flow cytometry demonstrates effective transfer of the drug-loaded compound into the cytoplasm, with minimal entry into the nucleus, facilitating drug release.[138] Further analysis of the drug uptake mechanism reveals that pre-incubation with free glucose or free biotin competitively inhibits the uptake of compound **59** by both cell lines. The cellular uptake of the bitargeted compound **59** complex may be facilitated by GLUT1 and SMVT transporters,[139] which are involved in energy-dependent internalization through various endocytosis pathways (Fig. 43B and C). These results underscore the significant enhancement in tumor-targeting ability achieved through multi-targeting ligands. This creative design strategy holds promise for exploring multi-targeting systems as novel carriers for glioma treatment.

In 2021, Monalisa Chowdhury *et al.*[140] outlined the design and synthesis of covalently customized biotinylated Fe^{2+} doped carbon dots (FCD_b).[141] FCD_b (Fig. 44, compound **60**) were

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

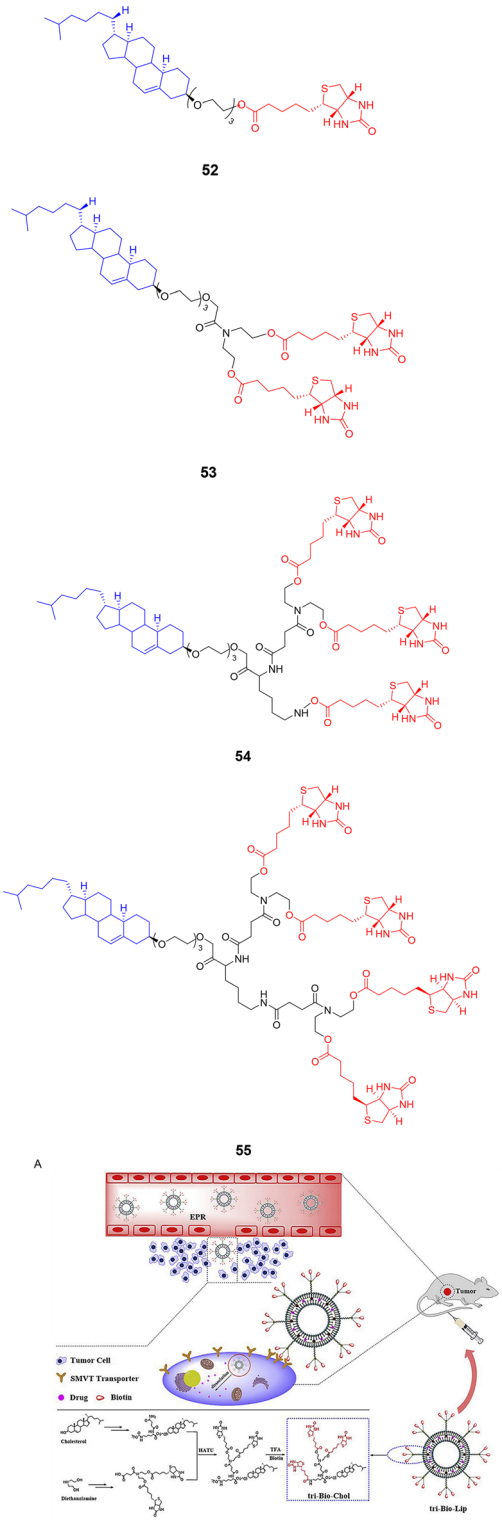


Fig. 41 The structure of compounds **52**, **53**, **54** and **55**. (A) Assembly and mechanism of action of drugs. Reproduced with permission.[134] Copyright 2020, Elsevier.

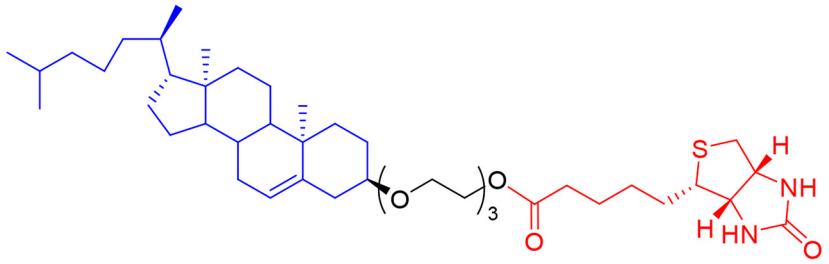
developed as a versatile platform for prodrug activation, diagnostic imaging, and targeted delivery of the anticancer drug PTX for prodrug-free combination therapy in cancer treatment. The authors engineer FCD_b as carriers for PTX, intended for use as an antitumor agent in drug-free combination therapy (Fig. 44A). Hydrogen peroxide (H_2O_2) is employed as a prodrug, with Fe^{2+} serving as a prodrug activator. FCD are synthesized using the hydrothermal method. The carrier-loaded PTX demonstrates precise delivery to cancer cells with robust stability. H_2O_2 acts as a prodrug within FCD_b, while the intrinsic fluorescence properties of FCD_b facilitate sensing and bioimaging. Sensing and imaging rely on Fe^{2+}-induced ROS (hydroxyl/superoxide radicals) generation from the precursor drug H_2O_2, inhibiting the intrinsic emission of FCD_b. Leveraging Fe-doped carbon dots-induced ROS production, biotin-functionalized FCDb effectively distinguishes malignant B16F10 cells from non-malignant NIH3T3 cells, as both biotin receptors and H_2O_2 are overexpressed in malignant cells. The presence of biotin and H_2O_2 in FCDb facilitates specific targeting of biotin receptors and H_2O_2, leading to increased uptake of FCD_b by malignant cells and eventual accumulation within them. FCD_b also exhibits excellent loading capacity for the anticancer drug PTX (FCD_b-PTX). Compared to current combination therapy in non-cancerous NIH3T3 cells, this newly developed drug-free prodrug formulation displays 2.7 to 3.5 times higher cytotoxicity against B16F10 cancer cells, primarily through early and late apoptotic pathways. Hence, biotin-modified Fe^{2+}-doped carbon dots hold promise as potential cancer-targeted therapeutic agents.

In 2021, Runxin Lu *et al.*[142] introduced a novel and creative TME-responsive nanocarrier termed Biotin/R8 peptide co-modified nanocarrier (Fig. 45, compound **61**) designed for loading PTX/Glucose oxidase (GOX) (Fig. 45A). This development represents a significant advancement in nanoparticle technology, allowing for efficient loading of both GOX and PTX. The nanoparticles are engineered to precisely target and penetrate solid tumors through their biotin coupling. Once internalized by cancer cells, GOX facilitates the decomposition of glucose within the TME, thereby disrupting tumor survival conditions. This process enhances the aggregation and internalization of nanocarriers within the tumor, accelerating the degradation of hydrazone bonds and amplifying the chemotherapy effect. Importantly, all components utilized in these nanosystems are biocompatible and biodegradable, ensuring minimal side effects. This creative multimodal synergistic treatment strategy holds immense potential to revolutionize cancer treatment paradigms and may pave the way for clinical applications in the future.

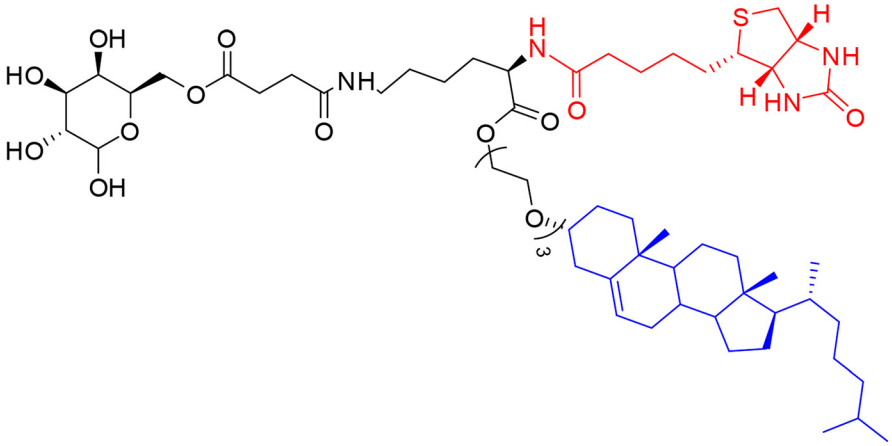
In the meantime, Caixia Yan *et al.*[143] developed a novel PTX nanoparticle system based on biotin and arginine-modified hydroxypropyl-β-cyclodextrin (Biotin-Arg (Pbf)-HP-β-CD) (Fig. 46, compound **62**). The synthesis method involves using arginine as a functional spacer to couple the hydroxyl group of the main surface HP-β-CD with the carboxyl group of biotins. PTX NPs are prepared by an improved emulsion solvent evaporation method using Biotin-Arg (Pbf)-HP-β-CD as a carrier (Fig. 46A). The average diameter of the optimized PTX-loaded compound **62** NPs is 121.9 nm, and the zeta potential is −57.7 mV. In the NPs system, biotin is introduced to target

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



56



57

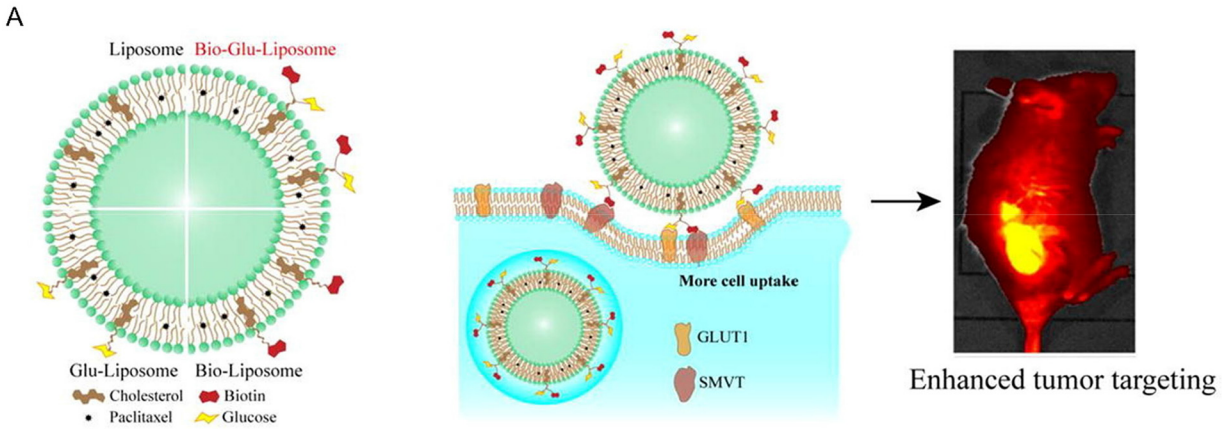
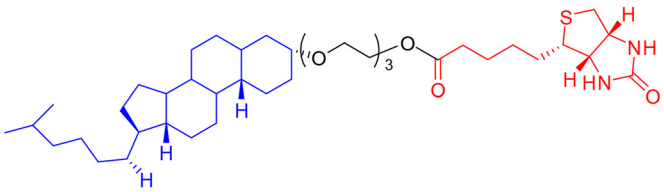


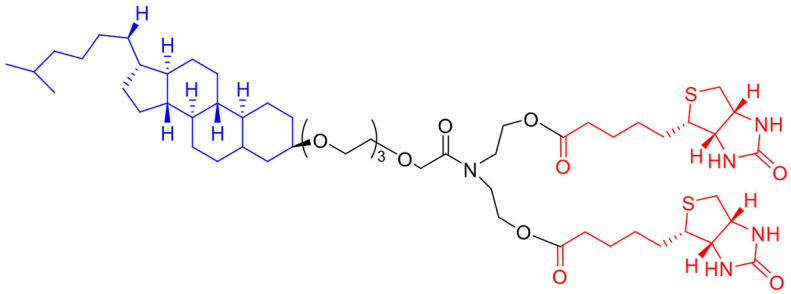
Fig. 42 The structure of compounds **56** and **57**. (A) Assembly and mechanism of action of drugs. Reproduced with permission.[135] Copyright 2020, Elsevier.

tumor cells specifically, while arginine is introduced to improve the cell membrane permeability of NPs, facilitating their cellular uptake. The synthesis process involves the preparation of Fmoc-Arg (Pbf)-HP-β-CD followed by the synthesis of biotin-NHS and the coupling reaction between biotin-NHS ester and Fmoc-Arg (Pbf)-HP-β-CD to form compound **62**.[144] The compound is then processed using an improved emulsion solvent evaporation method to prepare PTX-loaded compound **62** NPs. To verify its efficacy, the authors conduct *in vitro* hemolysis and cytotoxicity tests. The experimental results demonstrate that PTX-loaded NPs exhibit good blood compatibility, reducing the side effects associated with PTX use. Moreover, the cytotoxicity of the blank NPs is negligible, with a cell survival rate of no less than 98% in the studied concentration range, indicating their safety. These findings suggest that compound **62** NPs

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



58



59

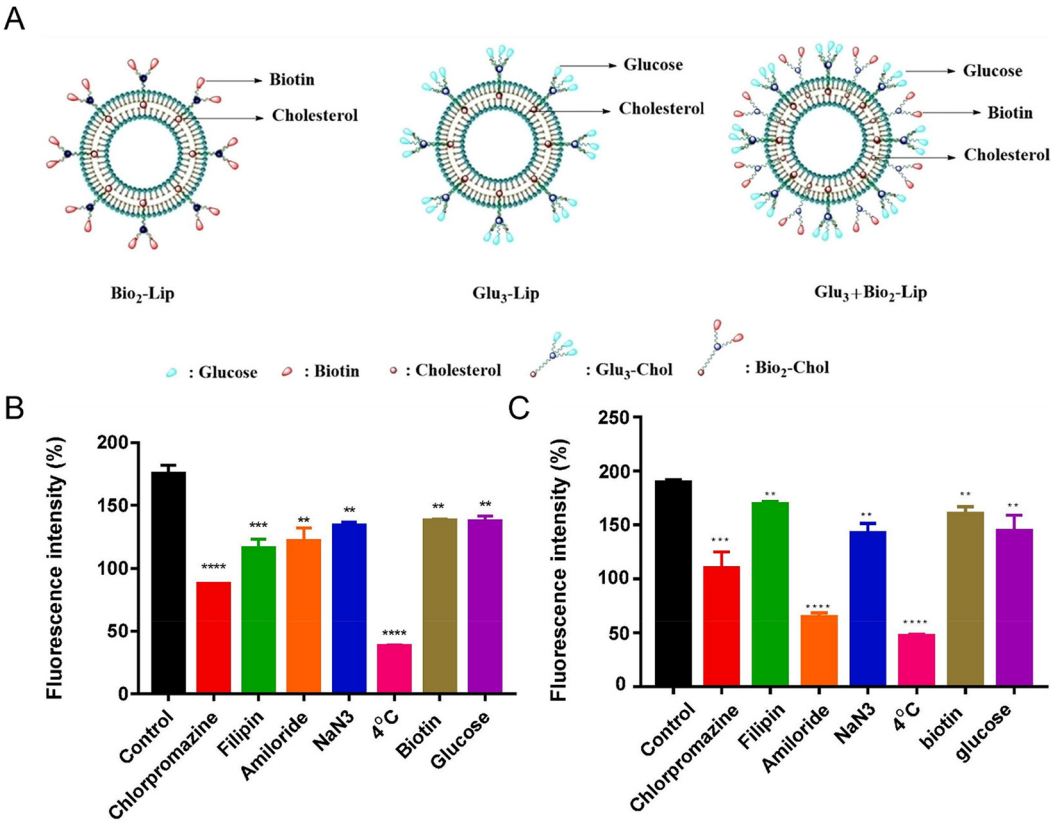


Fig. 43 The structure of compounds **58** and **59**. (A) The way drugs are assembled. (B and C) The relative uptake of CFPE-labeled. Reproduced with permission.[137] Copyright 2021, Elsevier.

could serve as a safe and effective PTX carrier system. *In vitro* cell uptake experiments suggest that compound **62** NPs can effectively facilitate PTX uptake by tumor cells, potentially enhancing their therapeutic efficacy. During the *in vitro* cellular uptake study, an increase in fluorescence intensity is observed for compound **62** NPs compared to Arg (Pbf)-HP-β-CD nanoparticles. This enhancement may be attributed to the presence of biotin. Furthermore, the inhibitory effect of PTX-loaded compound **62** NPs on tumor growth is evaluated in U14 tumor-bearing mice, revealing a significant inhibition of tumor growth. Overall, these experiments confirm that PTX-supported compound **62** NPs hold promise as a promising carrier system for tumor therapy, offering targeted delivery and potent antitumor effects.

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

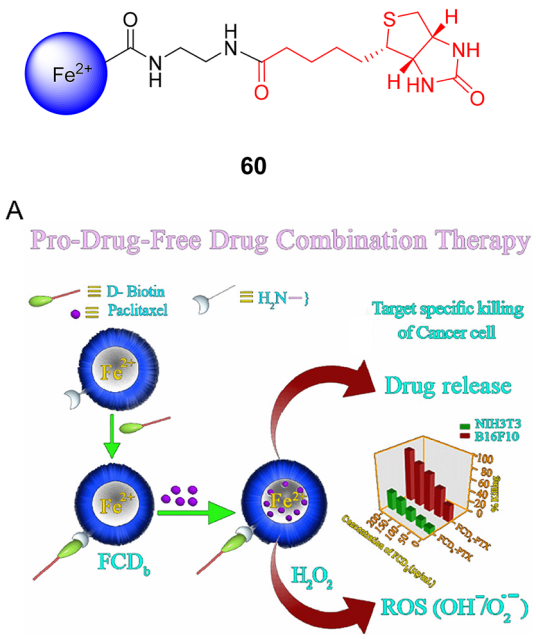


Fig. 44 The structure of compound **60**. (A) A picture showing PTX-loaded FCDb as a therapeutic agent. Reproduced with permission.[140] Copyright 2021, ACS.

Solid–liquid nanocarriers (SLNs) are at the forefront of rapidly developing medical applications and have a potential role in the delivery of bioactive drugs.[145] In 2020, Xianfu Sun *et al.*[146] reported a new natural deep eutectic solvent (NADES) and biotin-conjugated lysine-polyethylene glycol copolymer SLN. For the first time, the researchers develop biotin-targeted solid–lipid nanocarriers (SLNs) encapsulating PTX and 7-hydroxycoumarin (7-HC) using secondary metabolites to prepare natural deep eutectic solvents (NADES) (Fig. 47, compound **63**). As outlined in the introduction of the paper, it is observed that the inner core and outer shell of the SLNs exhibit hydrophobic and hydrophilic properties, respectively. This structural arrangement suggests the potential of SLNs to accommodate hydrophobic drugs in the core and hydrophilic drugs in the outer shell, thereby enhancing their antitumor efficacy. *In vitro* release experiments confirm the drug release profile at different pH values, revealing a higher release rate under acidic conditions. At pH 7.4, the release rate reaches 89%, while at pH 5.5, it reaches 92%, with a release duration of up to 320 minutes. These findings indicate the capacity of the drug to release effectively in the acidic TME, thereby exerting a sustained therapeutic effect. *In vitro* antitumor assays demonstrate that compound **63** exhibits significantly lower toxicity to normal cells (Fig. 47A) while displaying notable cytotoxicity against tumor cells (Fig. 47B). It can be seen from

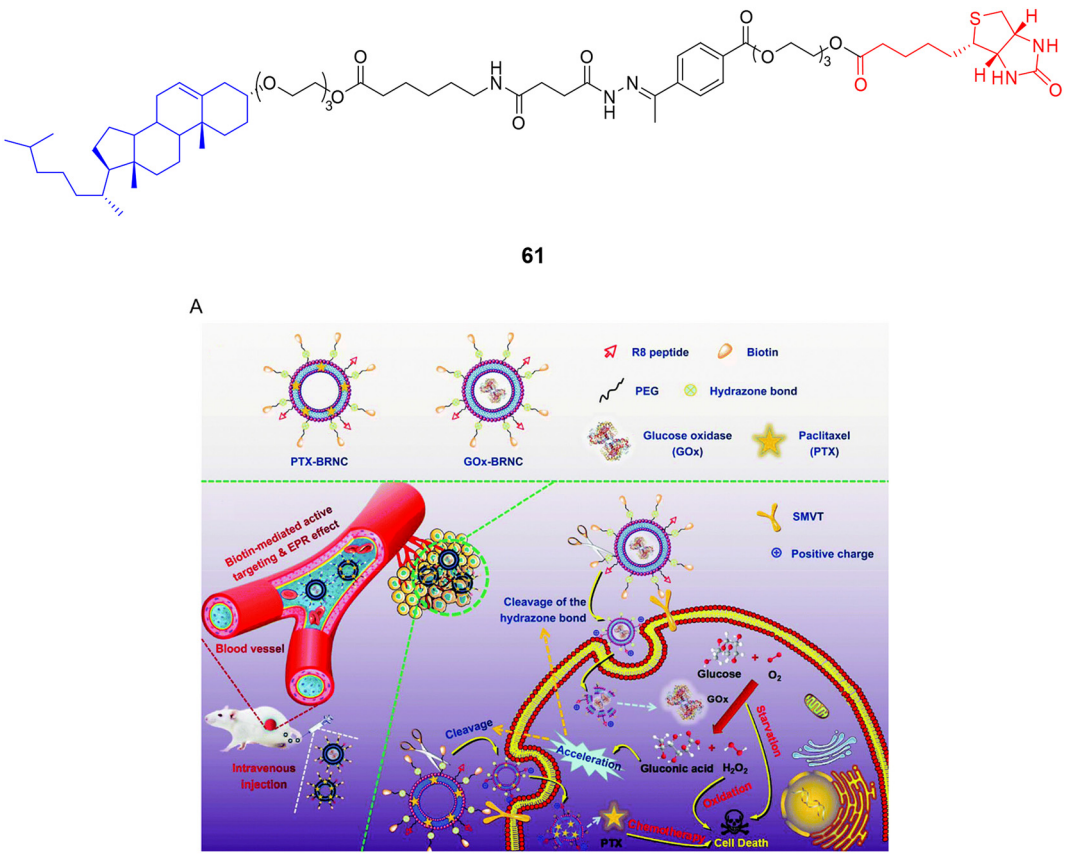
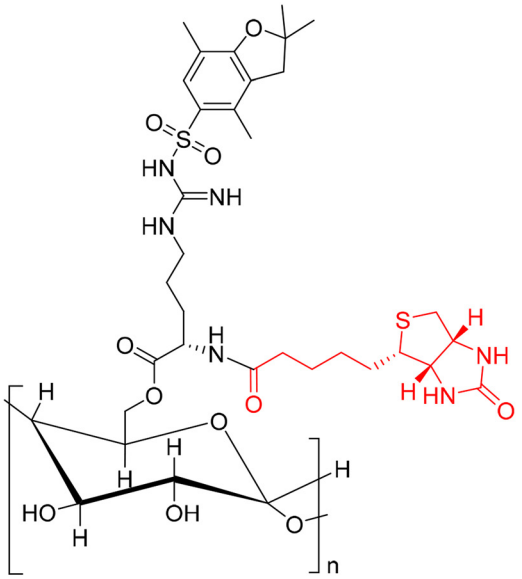


Fig. 45 The structure of compound **61**. (A) Assembly and mechanism of action of drugs. Reproduced with permission.[142] Copyright 2021, RSC.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



62

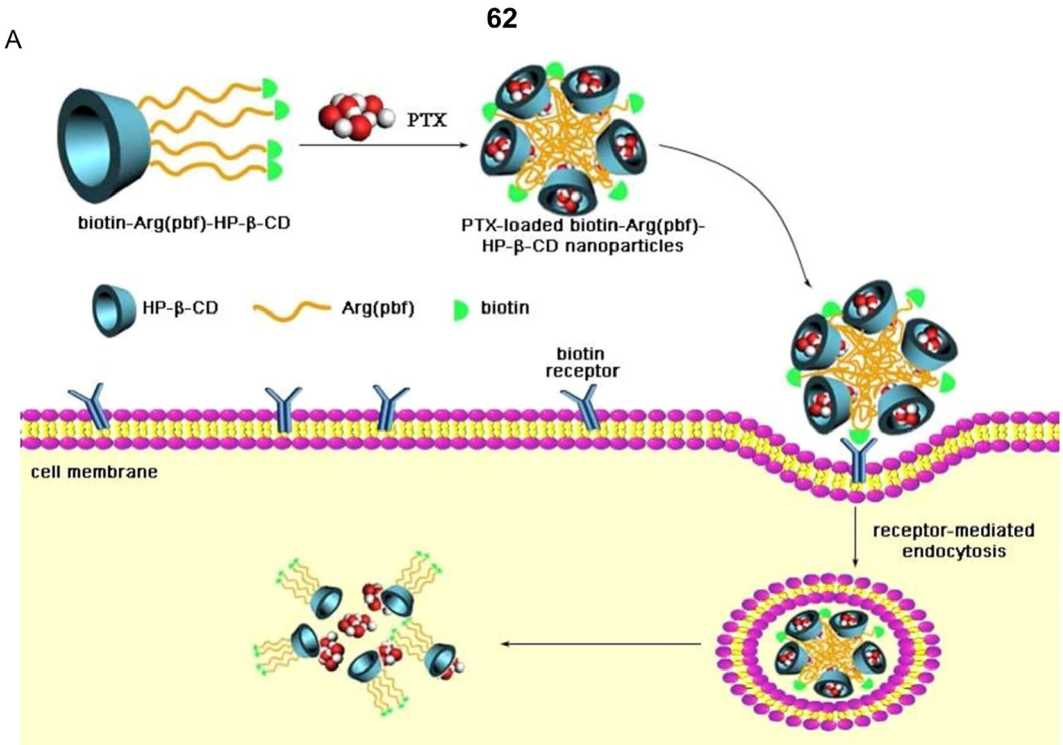


Fig. 46 The structure of compound **62**. (A) Assembly and mechanism of action of drugs. Reproduced with permission.[143] Copyright 2019, Elsevier.

these results that the synthesized PTX and 7-HC-loaded SLN system has a high value in preventing cancer cell proliferation.

In 2021, Haohui Shi *et al.*[147] designed and synthesized a novel amphiphilic polymer based on aggregation-induced emission (AIE) fluorine,[148] biotin, and disulfide-modified chitosan (TPE-bi(SS-CS-Bio)) (Fig. 48, compound **64**). The primary purpose of this polymer is to self-assemble into micelles, facilitating the loading of PTX and sealing it in the core area with high drug loading. Fluorescence studies demonstrate that the micelles exhibit good AIE characteristics and emit strong blue fluorescence. *In vitro* drug release studies reveal that the micelles can rapidly decompose in high glutathione levels. Biotin modification enhances the uptake of polymers by tumor cells, facilitating PTX release within tumor cells. Its main advantages include high efficiency and low toxicity to normal cells. Additionally, the toxicity to tumor cells is significantly enhanced (Fig. 48A). Additionally, the drug-loaded micelles exhibit significant cytotoxicity to MCF-7 cells, and

 This journal is © The Royal Society of Chemistry 2025

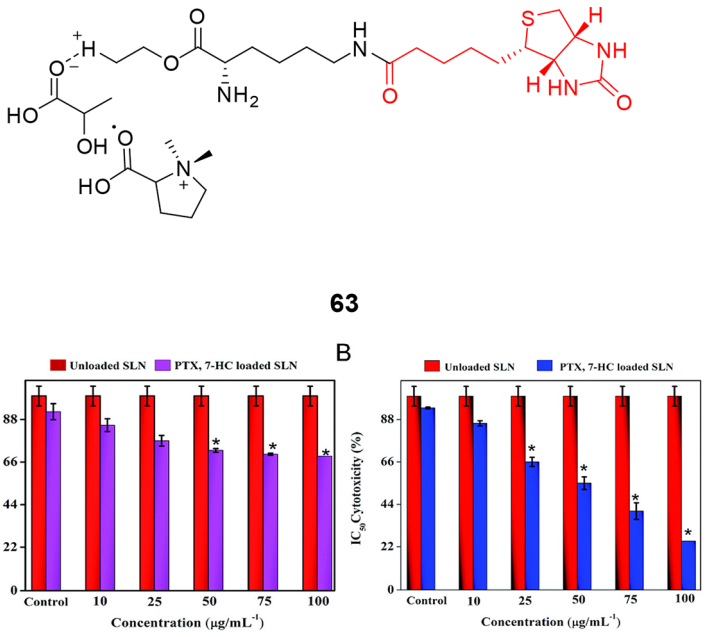


Fig. 47 The structure of compound **63**. (A) Cell viability of L929 cell lines. (B) Cytotoxicity of cancer cells. Reproduced with permission.[146] Copyright 2020, RSC.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

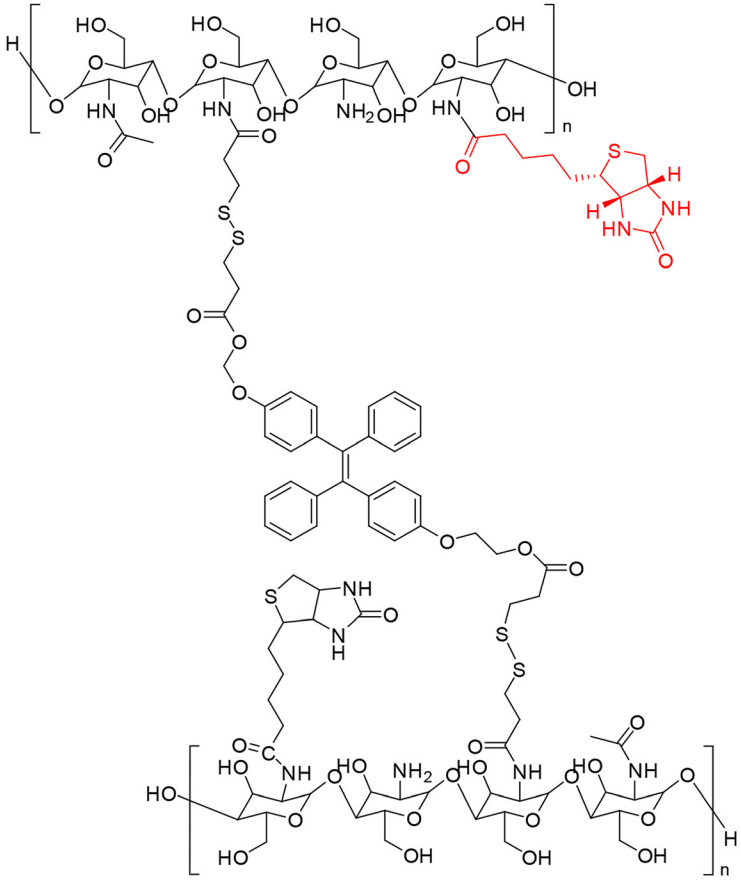
their distribution in cells can be tracked due to their excellent AIE characteristics. These results indicate that compound **64** possesses biological imaging ability and holds potential as a carrier for PTX. This result demonstrates that employing nanomaterials as drug carriers significantly enhances drug efficacy while mitigating side effects, indicating a promising research direction.

In 2022, Dan Gao *et al.*[149] formulated a dual-targeted PTX enzyme-sensitive hyaluronic acid nanogel (PTX/Biotin-NG), incorporating CD44 as the targeting moiety and utilizing biotin for tumor cell-specific guidance (Fig. 49A). The aim was to enhance the efficacy of CD44 against breast cancer while mitigating its adverse effects on normal cells (Fig. 49, compound **65**). Compound **65** is synthesized *via in situ* polymerization of di (ethylene glycol) diacrylate (DEGDA) and CMHA.[150] Amphiphilic cholesterol-grafted methacrylic acid hyaluronic acid is synthesized, and *in situ* free radical polymerization with DEGDA is utilized to form NG. Biotin is further conjugated to the surface of NG, serving as a targeting ligand. This step is essential to facilitate the coupling of biotin to the NG surface (Bio-NG, Fig. 49, compound **66**). The surface biological content of NG is measured to be 40.88 ± 1.2 nmol mL^{-1} using the UV-Vis method. It is observed that Bio-NG can specifically target tumor cells under the action of biotin. In the PTX loading process, the melting point of PTX is found to be 214.7 °C, and the decomposition temperature is 236.4 °C. PTX/Bio-NG and PTX/NG exhibit similar peaks to Bio-NG, indicating that PTX exists in NG in a molecular form. PTX/Bio-NG demonstrates potent cytotoxicity to 4T1 cells through endocytosis mediated by CD44 and biospecific receptors, primarily through clathrin and caveolin.[151] The nanomedical drugs synthesized by the authors exhibit excellent physical and chemical properties, characterized by large drug load, robust stability, and high encapsulation efficiency. These features are crucial for their effectiveness in the anti-tumor process. The targeting ability poses another challenge to the efficacy of nanogels. However, the experimental results show that the cell uptake rate of biotin-coupled compound **66** does not meet the ideal requirements. In competitive inhibition experiments, it is observed that the targeting ability of C6/Bio-NG is notably inadequate. Cells are treated with HA, biotin, and a combination of HA and biotin, respectively. The findings reveal that the uptake of C6/Bio-NG in HA-treated cells is only 54.9% compared to the control group, while in biotin-treated cells, it is 50.1% of the control group. These results suggest that while biotin contributes to cellular targeting, its effectiveness is still insufficient. This further demonstrates the effective targeting role of biotin in this context. HA and Bio contribute to the targeting effect, allowing Bio-NG to target tumor cells more effectively under these dual targeting mechanisms.

5. Clinical challenges

Biotin shows great potential in targeted drug delivery systems, but its clinical application still faces multiple challenges. The primary issue is dose-related uncertainty. Studies have shown that excessive use of biotin (5–100 mg daily) can cause serious interference with diagnostic tests. The FDA has set the biotin interference threshold at 3510 ng mL^{-1} (14 367 nM), which presents a significant challenge to immunoassay platforms based on biotin–streptavidin chemistry. The FDA has also

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .



64

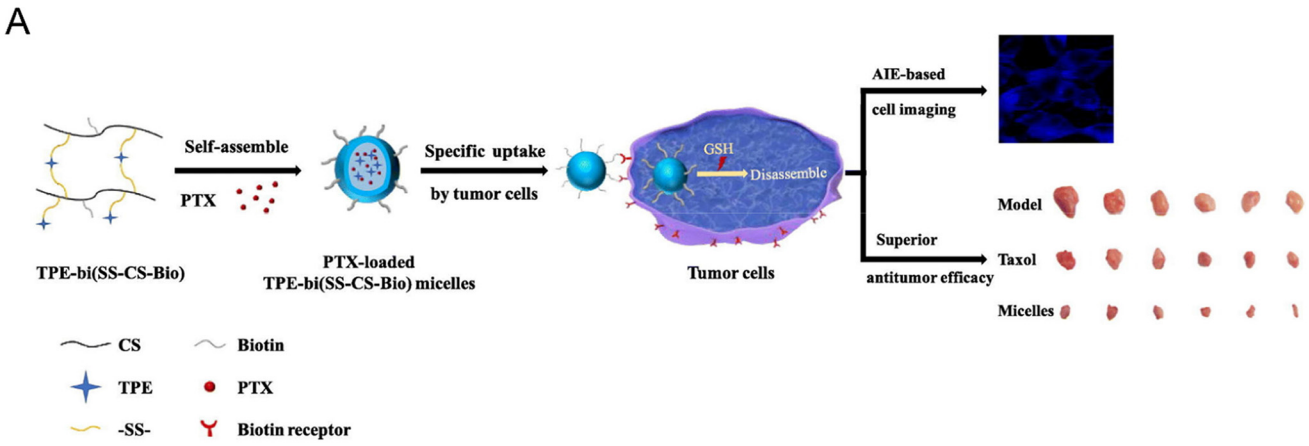
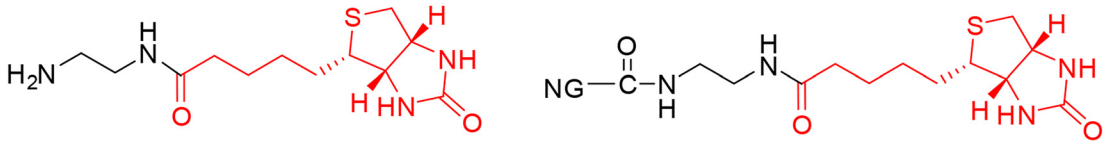


Fig. 48 The structure of compound 64. (A) Assembly and mechanism of action of drugs. Reproduced with permission.[147] Copyright 2021, Elsevier.

found that high concentrations of biotin can lead to numerous adverse reactions, including death in severe cases.[152] In addition, the use of biotin supplements can significantly affect the accuracy of clinical test results. In some cases, differences between results from various testing methods can reach up to 315-fold. Patients receiving high-dose biotin therapy may be misdiagnosed with Graves' disease, and thyroid function test results may show false abnormalities.[153,154]

More challenging is the uncertainty regarding the drug delivery mechanism of biotin-conjugated drugs. Research has shown that the main transporter of biotin, SMVT, requires a free carboxyl group to function properly. However, during drug conjugation, this carboxyl group often needs to be modified, which may significantly affect the transport efficiency. Additionally, the absorption and transport of biotin are influenced by various factors, and there are two primary uptake

 This journal is © The Royal Society of Chemistry 2025



65 66

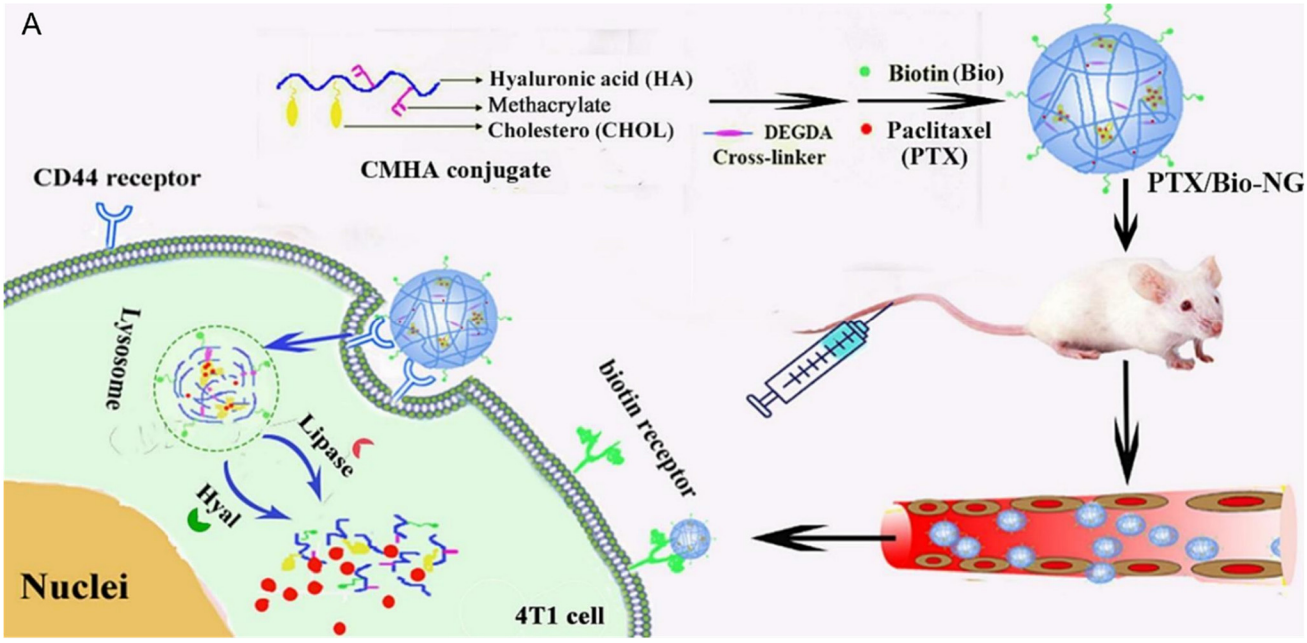


Fig. 49 The structure of compounds **65** and **66**. (A) Mechanism of action of compounds **65** and **66**. Reproduced with permission.[149] Copyright 2022, Elsevier.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

systems: the SMVT system and another specific transporter system. Some substances (*e.g.*, lipoic acid and panthothenic acid) can significantly inhibit sodium ion-dependent biotin uptake, which further complicates drug design and optimization of administration strategies (antenatal and postnatal radiologic diagnosis of holocarboxylase synthetase deficiency: a systematic review). Addressing these clinical challenges is critical for advancing the use of biotin in drug delivery systems. Future research needs to further clarify the mechanisms of biotin conjugates, optimize administration strategies, and establish more reliable clinical testing methods.

Moreover, the pharmacokinetic characteristics of biotin conjugates are highly complex. First, biotin conjugates must be transported through specific transporter systems, which can affect their bioavailability. Second, modifications to the biotin molecular structure during drug conjugation can impact its binding affinity to receptors. In drug design, the biotin-linker-drug conjugate is mainly used, but the choice and design of the linker directly impact the drug's release efficiency at the tumor site. Furthermore, dual-target strategies (such as folate/biotin dual-functionalized liposomes) can improve targeting to certain cancer cells, but they also increase formulation complexity and stability requirements. In terms of safety, it is important to consider the non-specific distribution of biotin conjugates in normal tissues, as well as potential immunogenicity issues. Addressing these challenges is crucial for improving the clinical efficacy of biotin-targeted drugs in cancer therapy.[155]

As of October 2023, biotin-based anticancer drugs have not yet entered clinical trials or been marketed, primarily due to the lack of sufficient preliminary research data and evidence of efficacy. The application of biotin in anticancer therapy has mostly been confined to *in vitro* experiments and animal models, and its mechanisms of action in tumor cells have not been fully understood or verified. Moreover, biotin's stability and bioavailability *in vivo* are limited, making it challenging to achieve effective drug delivery and tumor targeting, which presents technical hurdles in drug development. At the same time, safety and toxicity concerns are significant factors hindering its progress. High doses of biotin may cause side effects, affecting normal cell function and increasing the risks of clinical application. Additionally, market and economic factors also influence the research and development process. New drug development requires substantial financial investment, and the commercial potential of biotin-based drugs remains unclear, leading to a lack of sufficient interest and confidence from investors. Strict regulatory approval requirements and limited research resources further restrict the clinical development of biotin-based anticancer drugs.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

6. Conclusions

Many years ago, researchers studied the structures of chemotherapy drugs to improve their various side effects and explore how different structures affect drug efficacy. However, this approach faced multiple limitations. In recent years, targeted drug therapy has emerged as a highly promising treatment modality, with an increasing number of targeted drugs being developed. Biotin, an essential vitamin in the human body, has shown excellent potential in targeting cells. Consequently, biotin-based therapeutic drugs have demonstrated significant potential in cancer treatment by enhancing the efficacy of chemotherapy drugs and reducing side effects through specific targeting of biotin receptors overexpressed in tumor cells. These creative molecules offer fascinating potential in the field of drug development. This targeted delivery method holds great promise for overcoming the poor cell permeability typically encountered with traditional small-molecule drugs, increasing the uptake ratio between tumor cells and normal cells. Leveraging this advantage, researchers continuously strive to optimize the structure of biotin-based therapeutics through various conjugation methods and drug combinations. However, despite the bright prospects, biotin-based therapeutic drugs have yet to surpass the clinical stage to become mainstream medications. The ongoing exploration of biotin's potential in anti-tumor therapy opens new avenues for cancer treatment and drug discovery.

While the structure of biotin-based therapeutics continues to be refined, the complex conjugation mechanisms and diverse anti-tumor drugs present significant challenges to their development. Common strategies in this field include SMBCs and NBCs, each with unique advantages. SMBCs' simplicity and efficiency have garnered considerable attention, representing the traditional approach to synthesizing biotin-based therapeutics. Within this framework, strategies based on boron-sulfur, photo-triggered, enzyme-cleavable, and classical disulfide models are typically employed. However, traditional synthesis methods have proven insufficient to achieve optimal efficacy and safety. Compared to SMBCs, nanoparticles provide superior drug loading and release capabilities. Their inherent properties improve drug solubility and stability, which increases bioavailability and efficacy while reducing dosing frequency. NBCs provide a valuable research pathway, addressing challenges associated with payload and offering a versatile platform. Nanoparticle structures, such as nanoparticles and nanotubes, also exhibit cytotoxic activity and offer higher drug-loading capacity than SMBCs. Biotin-targeted therapy still faces several challenges, including resistance mechanisms, uncertainties about the optimal dosage and dosing regimen, and potential off-target effects. To overcome these challenges, future research should focus on developing new biotin–drug conjugates that increase drug concentration at tumor sites while reducing toxicity to normal tissues. For example, combining biotin with nanocarriers through nanotechnology can enable sustained targeting by adjusting the release rate. Additionally, exploring combinations with other targeting ligands, such as antibodies or peptides, may further enhance targeting and therapeutic efficacy. Furthermore, combining biotin-targeted drugs with other treatment modalities, such as chemotherapy, radiation, or immunotherapy, could improve overall efficacy. Studies have shown that biotinylated immune checkpoint inhibitors, for instance, can enhance immune responses and increase tumor cell clearance. This combination strategy holds great promise in overcoming the limitations of single treatments and improving patient prognosis.

Nevertheless, drugs developed using these methods have yet to reach the market, facing issues such as biotin interfering with drug action, suboptimal efficacy, lack of targeting, and significant side effects. Despite these challenges, the rapid development of biotin-based therapeutics remains hopeful with the ongoing advancement of nanomaterials. Scientists are tirelessly designing and synthesizing new biotin conjugates to improve drug delivery efficiency, efficacy, and safety, thereby opening new prospects for cancer treatment.

Author contributions

Chao Wang: conceptualization. Yutao Xiu: data curation. Jiazhen Xu: formal analysis. Yujing Zhang: investigation. Yanhong Wang: methodology. Wanpeng Yu: supervision. Yutao Xiu: writing – original draft. Dongming Xing: writing – review & editing. All authors contributed and approved the submitted version.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (82303590), the Natural Science Foundation of Shandong (ZR2021MC189), the Natural Science Foundation of Qingdao (23-2-1-141-zyyd-jch), the Youth Innovation Team Development Program of Shandong Province (2023KJ227), the China Postdoctoral Science Foundation (2023M741867), and the Qingdao Postdoctoral Application Project (QDBSH20230202076). The author thanks Dongming Xing and Wanpeng Yu for the support in preparing figures.

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

References

1 H. Sung, J. Ferlay, R. L. Siegel, M. Laversanne, I. Soerjomataram, A. Jemal and F. Bray, Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries, *CA-Cancer J. Clin.*, 2021, **71**(3), 209–249, DOI: 10.3322/caac.21660.

2 E. M. Packeiser, L. Engels, I. Nolte, S. Goericke-Pesch and H. Murua Escobar, MDR1 Inhibition Reverses Doxorubicin-Resistance in Six Doxorubicin-Resistant Canine Prostate and Bladder Cancer Cell Lines., *Int. J. Mol. Sci.*, 2023, **24**(9), DOI: 10.3390/ijms24098136.

3 V. Brabec and J. Kasparkova, Molecular aspects of resistance to antitumor platinum drugs, *Drug Resistance Updates*, 2002, **5**(3–4), 147–161, DOI: 10.1016/s1368-7646(02)00047-x.

4 W. Liu, Q. Lin, Y. Fu, S. Huang, C. Guo, L. Li, L. Wang, Z. Zhang and L. Zhang, Target delivering paclitaxel by ferritin heavy chain nanocages for glioma treatment, *J. Controlled Release*, 2020, **323**, 191–202, DOI: 10.1016/j.jconrel.2019.12.010.

5 X. Wei, Z. Dai, J. Yang, A. Khan, H. F. Yu, Y. L. Zhao, Y. F. Wang, Y. P. Liu, Z. F. Yang, W. Y. Huang, *et al.*, Unprecedented sugar bridged bisindoles selective inhibiting glioma stem cells, *Bioorg. Med. Chem.*, 2018, **26**(8), 1776–1783, DOI: 10.1016/j.bmc.2018.02.024.

6 W. Xu, B. Li, M. Xu, T. Yang and X. Hao, Traditional Chinese medicine for precancerous lesions of gastric cancer: A review, *Biomed. Pharmacother.*, 2022, **146**, 112542, DOI: 10.1016/j.biopha.2021.112542.

7 J. D. Seitz, J. G. Vineberg, E. Herlihy, B. Park, E. Melief and I. Ojima, Design, synthesis and biological evaluation of a highly-potent and cancer cell selective folate-taxoid conjugate, *Bioorg. Med. Chem.*, 2015, **23**(9), 2187–2194, DOI: 10.1016/j.bmc.2015.02.057.

8 N. Krall, F. Pretto, W. Decurtins, G. J. Bernardes, C. T. Supuran and D. Neri, A small-molecule drug conjugate for the treatment of carbonic anhydrase IX expressing tumors, *Angew. Chem., Int. Ed.*, 2014, **53**(16), 4231–4235, DOI: 10.1002/anie.201310709.

9 S. Maiti and P. Paira, Biotin conjugated organic molecules and proteins for cancer therapy: A review, *Eur. J. Med. Chem.*, 2018, **145**, 206–223, DOI: 10.1016/j.ejmech.2018.01.001.

10 W. Yang, Y. Cheng, T. Xu, X. Wang and L. P. Wen, Targeting cancer cells with biotin-dendrimer conjugates, *Eur. J. Med. Chem.*, 2009, **44**(2), 862–868, DOI: 10.1016/j.ejmech.2008.04.021.

11 Y. Singh, K. K. Durga Rao Viswanadham, A. Kumar Jajoriya, J. G. Meher, K. Raval, S. Jaiswal, J. Dewangan, H. K. Bora, S. K. Rath, J. Lal, *et al.*, Click Biotinylation of PLGA Template for Biotin Receptor Oriented Delivery of Doxorubicin Hydrochloride in 4T1 Cell-Induced Breast Cancer, *Mol. Pharm.*, 2017, **14**(8), 2749–2765, DOI: 10.1021/acs.molpharmaceut.7b00310.

12 S. Fathi-Karkan, S. Sargazi, S. Shojaei, B. Farasati Far, S. Mirinejad, M. Cordani, A. Khosravi, A. Zarrabi and S. Ghavami, Biotin-functionalized nanoparticles: an overview of recent trends in cancer detection, *Nanoscale*, 2024, **16**(27), 12750–12792, DOI: 10.1039/d4nr00634h.

13 P. Mikled, W. Chavasiri and M. Khongkow, Dual folate/biotin-decorated liposomes mediated delivery of methylnaphthazarin for anti-cancer activity, *Sci. Rep.*, 2024, **14**(1), 21796, DOI: 10.1038/s41598-024-72532-7.

14 L. Lv, C. Liu, C. Chen, X. Yu, G. Chen, Y. Shi, F. Qin, J. Ou, K. Qiu and G. Li, Quercetin and doxorubicin co-encapsulated biotin receptor-targeting nanoparticles for minimizing drug resistance in breast cancer, *Oncotarget*, 2016, **7**(22), 32184–32199, DOI: 10.18632/oncotarget.8607.

15 G. J. Russell-Jones, Use of targeting agents to increase uptake and localization of drugs to the intestinal epithelium, *J. Drug Targeting*, 2004, **12**(2), 113–123, DOI: 10.1080/10611860410001693760.

16 G. Russell-Jones, K. McTavish, J. McEwan, J. Rice and D. Nowotnik, Vitamin-mediated targeting as a potential mechanism to increase drug uptake by tumours, *J. Inorg. Biochem.*, 2004, **98**(10), 1625–1633, DOI: 10.1016/j.jinorgbio.2004.07.009.

17 S. Chen, X. Zhao, J. Chen, J. Chen, L. Kuznetsova, S. S. Wong and I. Ojima, Mechanism-based tumor-targeting drug delivery system. Validation of efficient vitamin receptor-mediated endocytosis and drug release, *Bioconjugate Chem.*, 2010, **21**(5), 979–987, DOI: 10.1021/bc9005656.

18 C. Muth, S. Harder, L. Uhlmann, J. Rochon, B. Fullerton, C. Güthlin, A. Erler, M. Beyer, M. van den Akker, R. Perera, *et al.*, Pilot study to test the feasibility of a trial design and complex intervention on PRIoritising MUltimedication in Multimorbidity in general practices (PRIMUMpilot), *BMJ Open*, 2016, **6**(7), e011613, DOI: 10.1136/bmjopen-2016-011613.

19 R. Tripathi, A. Guglani, R. Ghorpade and B. Wang, Biotin conjugates in targeted drug delivery: is it mediated by a biotin transporter, a yet to be identified receptor, or (an)other unknown mechanism(s)?, *J. Enzyme Inhib. Med. Chem.*, 2023, **38**(1), 2276663, DOI: 10.1080/14756366.2023.2276663.

20 R. Kolhatkar, A. Lote and H. Khambati, Active tumor targeting of nanomaterials using folic acid, transferrin and integrin receptors, *Curr. Drug Discovery Technol.*, 2011, **8**(3), 197–206, DOI: 10.2174/157016311796799044.

21 T. Giuseppe, M. Delia, C. Simona, R. Marta and R. Daniela, New Perspectives in Cancer Therapy: The Biotin-Antitumor Molecule Conjugates, *Med. Chem.*, 2014, **S1**(004), 1–8, DOI: 10.4172/2161-0444.S1-004.

22 Y. Tabana, D. Babu, R. Fahlman, A. G. Siraki and K. Barakat, Target identification of small molecules: an overview of the current applications in drug discovery, *BMC Biotechnol.*, 2023, **23**(1), 44, DOI: 10.1186/s12896-023-00815-4.

23 S. M. Wignall, N. S. Gray, Y. T. Chang, L. Juarez, R. Jacob, A. Burlingame, P. G. Schultz and R. Heald, Identification

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

of a novel protein regulating microtubule stability through a chemical approach, *Chem. Biol.*, 2004, **11**(1), 135–146.

24 J. Min, Y. Kyung Kim, P. G. Cipriani, M. Kang, S. M. Khersonsky, D. P. Walsh, J. Y. Lee, S. Niessen, J. R. Yates 3rd, K. Gunsalus, *et al.*, Forward chemical genetic approach identifies new role for GAPDH in insulin signaling, *Nat. Chem. Biol.*, 2007, **3**(1), 55–59, DOI: 10.1038/nchembio833.

25 H. J. Bax, J. Chauhan, C. Stavraka, A. Santaolalla, G. Osborn, A. Khiabany, M. Grandits, J. López-Abente, L. Palhares, C. Chan Wah Hak, *et al.*, Folate receptor alpha in ovarian cancer tissue and patient serum is associated with disease burden and treatment outcomes, *Br. J. Cancer*, 2023, **128**(2), 342–353, DOI: 10.1038/s41416-022-02031-x.

26 A. Cheung, H. J. Bax, D. H. Josephs, K. M. Ilieva, G. Pellizzari, J. Opzoomer, J. Bloomfield, M. Fittall, A. Grigoriadis, M. Figini, *et al.*, Targeting folate receptor alpha for cancer treatment, *Oncotarget*, 2016, **7**(32), 52553–52574, DOI: 10.18632/oncotarget.9651.

27 P. Khongorzul, C. J. Ling, F. U. Khan, A. U. Ihsan and J. Zhang, Antibody-Drug Conjugates: A Comprehensive Review, *Mol. Cancer Res.*, 2020, **18**(1), 3–19, DOI: 10.1158/1541-7786.Mcr-19-0582.

28 Y. Luo, Z. Zeng, T. Shan, X. Xu, J. Chen, Y. He, T. Zhang, Z. Huang, G. Chai, Y. Huang, *et al.*, Fibroblast activation protein α activatable theranostic pro-photosensitizer for accurate tumor imaging and highly-specific photodynamic therapy, *Theranostics*, 2022, **12**(8), 3610–3627, DOI: 10.7150/thno.70308.

29 C. S. Sevier and C. A. Kaiser, Formation and transfer of disulphide bonds in living cells, *Nat. Rev. Mol. Cell Biol.*, 2002, **3**(11), 836–847, DOI: 10.1038/nrm954.

30 Y. Mu, L. Gong, T. Peng, J. Yao and Z. Lin, Advances in pH-responsive drug delivery systems, *OpenNano*, 2021, **5**, DOI: 10.1016/j.onano.2021.100031.

31 S. Dasari and P. B. Tchounwou, Cisplatin in cancer therapy: molecular mechanisms of action, *Eur. J. Pharmacol.*, 2014, **740**, 364–378, DOI: 10.1016/j.ejphar.2014.07.025.

32 S. Ghosh, Cisplatin: The first metal based anticancer drug, *Bioorg. Chem.*, 2019, **88**, 102925, DOI: 10.1016/j.bioorg.2019.102925.

33 P. Štarha and R. Křikavová, Platinum(IV) and platinum(II) anticancer complexes with biologically active releasable ligands, *Coord. Chem. Rev.*, 2024, **501**(15), DOI: 10.1016/j.ccr.2023.215578.

34 H. Song, H. Xiao, Y. Zhang, H. Cai, R. Wang, Y. Zheng, Y. Huang, Y. Li, Z. Xie, T. Liu, *et al.*, Multifunctional Pt(iv) pro-drug and its micellar platform: to kill two birds with one stone, *J. Mater. Chem. B*, 2013, **1**(6), 762–772, DOI: 10.1039/c2tb00206j.

35 S. Jin, Y. Guo, D. Song, Z. Zhu, Z. Zhang, Y. Sun, T. Yang, Z. Guo and X. Wang, Targeting Energy Metabolism by a Platinum(IV) Prodrug as an Alternative Pathway for Cancer Suppression, *Inorg. Chem.*, 2019, **58**(9), 6507–6516, DOI: 10.1021/acs.inorgchem.9b00708.

36 X. Wang, Y. Wang, S. Gou and F. Chen, A trifunctional Pt(II) complex alleviates the NHEJ/HR-related DSBs repairs to evade cisplatin-resistance in NSCLC, *Bioorg. Chem.*, 2020, **104**, 104210, DOI: 10.1016/j.bioorg.2020.104210.

37 R. G. Kenny and C. J. Marmion, Toward Multi-Targeted Platinum and Ruthenium Drugs-A New Paradigm in Cancer Drug Treatment Regimens?, *Chem. Rev.*, 2019, **119**(2), 1058–1137, DOI: 10.1021/acs.chemrev.8b00271.

38 I. N. Stepanenko, M. S. Novak, G. Mühlgassner, A. Roller, M. Hejl, V. B. Arion, M. A. Jakupec and B. K. Keppler, Organometallic 3-(1H-benzimidazol-2-yl)-1H-pyrazolo[3,4-b]pyridines as potential anticancer agents, *Inorg. Chem.*, 2011, **50**(22), 11715–11728, DOI: 10.1021/ic201704u.

39 L. Corte-Real, B. Karas, A. R. Bras, A. Pilon, F. Avecilla, F. Marques, A. Preto, B. T. Buckley, K. R. Cooper, C. Doherty, *et al.*, Ruthenium-Cyclopentadienyl Bipyridine-Biotin Based Compounds: Synthesis and Biological Effect, *Inorg. Chem.*, 2019, **58**(14), 9135–9149, DOI: 10.1021/acs.inorgchem.9b00735.

40 J. J. Champoux, DNA Topoisomerases: Structure, Function, and Mechanism, *Annu. Rev. Biochem.*, 2001, **70**(1), 369–413, DOI: 10.1146/annurev.biochem.70.1.369.

41 G. Capranico, J. Marinello and G. Chillemi, Type I DNA Topoisomerases, *J. Med. Chem.*, 2017, **60**(6), 2169–2192, DOI: 10.1021/acs.jmedchem.6b00966.

42 N. Khaiwa, N. R. Maarouf, M. H. Darwish, D. W. M. Alhamad, A. Sebastian, M. Hamad, H. A. Omar, G. Orive and T. H. Al-Tel, Camptothecin's journey from discovery to WHO Essential Medicine: Fifty years of promise, *Eur. J. Med. Chem.*, 2021, **223**, 113639, DOI: 10.1016/j.ejmech.2021.113639.

43 P. Botella and E. Rivero-Buceta, Safe approaches for camptothecin delivery: Structural analogues and nanomedicines, *J. Controlled Release*, 2017, **247**, 28–54, DOI: 10.1016/j.jconrel.2016.12.023.

44 R. Garcia-Carbonero and J. G. Supko, Current perspectives on the clinical experience, pharmacology, and continued development of the camptothecins, *Clin. Cancer Res.*, 2002, **8**(3), 641–661.

45 Y. Liang, W. Huang, D. Zeng, X. Huang, L. Chan, C. Mei, P. Feng, C. H. Tan and T. Chen, Cancer-targeted design of bioresponsive prodrug with enhanced cellular uptake to achieve precise cancer therapy, *Drug Delivery*, 2018, **25**(1), 1350–1361, DOI: 10.1080/10717544.2018.1477862.

46 V. J. Venditto and E. E. Simanek, Cancer therapies utilizing the camptothecins: a review of the in vivo literature, *Mol. Pharm.*, 2010, **7**(2), 307–349, DOI: 10.1021/mp900243b.

47 L. Liu, F. Liu, D. Liu, W. Yuan, M. Zhang, P. Wei and T. Yi, A Smart Theranostic Prodrug System Activated by Reactive Oxygen Species for Regional Chemotherapy of Metastatic Cancer, *Angew. Chem., Int. Ed.*, 2022, **61**(12), e202116807, DOI: 10.1002/anie.202116807.

48 J. H. Lee and J. M. Berger, Cell Cycle-Dependent Control and Roles of DNA Topoisomerase II, *Genes (Basel)*, 2019, **10**(11), 859, DOI: 10.3390/genes10110859.

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

49 S. Rivankar, An overview of doxorubicin formulations in cancer therapy, *J. Cancer Res. Ther.*, 2014, **10**(4), 853–858, DOI: 10.4103/0973-1482.139267.

50 W. G. Choi, D. K. Kim, Y. Shin, R. Park, Y. Y. Cho, J. Y. Lee, H. C. Kang and H. S. Lee, Liquid Chromatography-Tandem Mass Spectrometry for the Simultaneous Determination of Doxorubicin and its Metabolites Doxorubicinol, Doxorubicinone, Doxorubicinolone, and 7-Deoxydoxorubicinone in Mouse Plasma, *Molecules (Basel, Switzerland)*, 2020, **25**(5), 1254, DOI: 10.3390/molecules25051254.

51 M. Yao, X. Ma, X. Zhang, L. Shi, T. Liu, X. Liang, H. Zhao, X. Li, L. Li, H. Gao, *et al.*, Lectin-Mediated pH-Sensitive Doxorubicin Prodrug for Pre-Targeted Chemotherapy of Colorectal Cancer with Enhanced Efficacy and Reduced Side Effects, *Theranostics*, 2019, **9**(3), 747–760, DOI: 10.7150/thno.29989.

52 M. P. Nguyen, V. T. T. Thuy and D. Kim, Integration of iron oxide nanoparticles and polyaspartamide biopolymer for MRI image contrast enhancement and an efficient drug-delivery system in cancer therapy, *Nanotechnology*, 2020, **31**(33), 335712, DOI: 10.1088/1361-6528/ab8f49.

53 X. Q. Yu, X. Y. Shang, X. X. Huang, G. D. Yao and S. J. Song, Brusatol: A potential anti-tumor quassinoid from Brucea javanica, *Chin. Herb. Med.*, 2020, **12**(4), 359–366, DOI: 10.1016/j.chmed.2020.05.007.

54 S. J. Cai, Y. Liu, S. Han and C. Yang, Brusatol, an NRF2 inhibitor for future cancer therapeutic, *Cell Biosci.*, 2019, **9**, 45, DOI: 10.1186/s13578-019-0309-8.

55 S. Xing, F. Nong, Y. Wang, D. Huang, J. Qin, Y. F. Chen, D. H. He, P. E. Wu, H. Huang, R. Zhan, *et al.*, Brusatol has therapeutic efficacy in non-small cell lung cancer by targeting Skp1 to inhibit cancer growth and metastasis, *Pharmacol. Res.*, 2022, **176**, 106059, DOI: 10.1016/j.phrs.2022.106059.

56 S. Kallus, L. Uhlik, S. van Schoonhoven, K. Pelivan, W. Berger, E. A. Enyedy, T. Hofmann, P. Heffeter, C. R. Kowol and B. K. Keppler, Synthesis and biological evaluation of biotin-conjugated anticancer thiosemicarbazones and their iron(III) and copper(II) complexes, *J. Inorg. Biochem.*, 2019, **190**, 85–97, DOI: 10.1016/j.jinorgbio.2018.10.006.

57 F. Pellegrini and D. R. Budman, Review: tubulin function, action of antitubulin drugs, and new drug development, *Cancer Invest.*, 2005, **23**(3), 264–273, DOI: 10.1081/cnv-200055970.

58 R. Kaur, G. Kaur, R. K. Gill, R. Soni and J. Bariwal, Recent developments in tubulin polymerization inhibitors: An overview, *Eur. J. Med. Chem.*, 2014, **87**, 89–124, DOI: 10.1016/j.ejmech.2014.09.051.

59 Y. Lu, J. Chen, M. Xiao, W. Li and D. D. Miller, An overview of tubulin inhibitors that interact with the colchicine binding site, *Pharm. Res.*, 2012, **29**(11), 2943–2971, DOI: 10.1007/s11095-012-0828-z.

60 A. A. Ghawanmeh, K. F. Chong, S. M. Sarkar, M. A. Bakar, R. Othaman and R. M. Khalid, Colchicine prodrugs and codrugs: Chemistry and bioactivities, *Eur. J. Med. Chem.*, 2018, **144**, 229–242, DOI: 10.1016/j.ejmech.2017.12.029.

61 I. A. Gracheva, E. S. Shchegravina, H. G. Schmalz, I. P. Beletskaya and A. Y. Fedorov, Colchicine Alkaloids and Synthetic Analogues: Current Progress and Perspectives, *J. Med. Chem.*, 2020, **63**(19), 10618–10651, DOI: 10.1021/acs.jmedchem.0c00222.

62 C. Wang, Y. Zhang, Z. Wang, Y. Li, Q. Guan, D. Xing and W. Zhang, Design, synthesis, and biological evaluation of biotinylated colchicine derivatives as potential antitumor agents, *J. Enzyme Inhib. Med. Chem.*, 2022, **37**(1), 411–420, DOI: 10.1080/14756366.2021.2013832.

63 B. A. Weaver, How Taxol/paclitaxel kills cancer cells, *Mol. Biol. Cell*, 2014, **25**(18), 2677–2681, DOI: 10.1091/mbc.E14-04-0916.

64 J. Verweij, M. Clavel and B. Chevalier, Paclitaxel (Taxol) and docetaxel (Taxotere): not simply two of a kind, *Ann. Oncol.*, 1994, **5**(6), 495–505, DOI: 10.1093/oxfordjournals.annonc.a058903.

65 J. G. Vineberg, E. S. Zuniga, A. Kamath, Y. J. Chen, J. D. Seitz and I. Ojima, Design, synthesis, and biological evaluations of tumor-targeting dual-warhead conjugates for a taxoid-camptothecin combination chemotherapy, *J. Med. Chem.*, 2014, **57**(13), 5777–5791, DOI: 10.1021/jm500631u.

66 J. G. Vineberg, T. Wang, E. S. Zuniga and I. Ojima, Design, synthesis, and biological evaluation of theranostic vitamin-linker-taxoid conjugates, *J. Med. Chem.*, 2015, **58**(5), 2406–2416, DOI: 10.1021/jm5019115.

67 T. Wang, J. G. Vineberg, T. Honda and I. Ojima, Design and synthesis of tumor-targeting theranostic drug conjugates for SPECT and PET imaging studies, *Bioorg. Chem.*, 2018, **76**, 458–467, DOI: 10.1016/j.bioorg.2017.12.018.

68 J. L. Alberini, V. Edeline, A. L. Giraudet, L. Champion, B. Paulmier, O. Madar, A. Poinsignon, D. Bellet and A. P. Pecking, Single photon emission tomography/computed tomography (SPET/CT) and positron emission tomography/computed tomography (PET/CT) to image cancer, *J. Surg. Oncol.*, 2011, **103**(6), 602–606, DOI: 10.1002/jso.21695.

69 B. M. Sahoo, B. K. Banik, P. Borah and A. Jain, Reactive Oxygen Species (ROS): Key Components in Cancer Therapies, *Anti-Cancer Agents Med. Chem.*, 2022, **22**(2), 215–222, DOI: 10.2174/1871520621666210608095512.

70 K. Sugiura, Y. Yokochi, N. Fu, Y. Fukaya, K. Yoshida, S. Mihara and T. Hisabori, The thioredoxin (Trx) redox state sensor protein can visualize Trx activities in the light/dark response in chloroplasts, *J. Biol. Chem.*, 2019, **294**(32), 12091–12098, DOI: 10.1074/jbc.RA119.007616.

71 K. M. Hindi, M. J. Panzner, C. A. Tessier, C. L. Cannon and W. J. Youngs, The medicinal applications of imidazolium carbene-metal complexes, *Chem. Rev.*, 2009, **109**(8), 3859–3884, DOI: 10.1021/cr800500u.

72 I. Ott, On the medicinal chemistry of gold complexes as anticancer drugs, *Coord. Chem. Rev.*, 2009, **253**(11–12), 1670–1681, DOI: 10.1016/j.ccr.2009.02.019.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

73 Z. Yang, S. Huang, Y. Liu, X. Chang, Y. Liang, X. Li, Z. Xu, S. Wang, Y. Lu, Y. Liu, *et al.*, Biotin-Targeted Au(I) Radiosensitizer for Cancer Synergistic Therapy by Intervening with Redox Homeostasis and Inducing Ferroptosis, *J. Med. Chem.*, 2022, **65**(12), 8401–8415, DOI: 10.1021/acs.jmedchem.2c00300.
74 R. Kawasaki, R. Ohdake, K. Yamana, T. Eto, K. Sugikawa and A. Ikeda, Photodynamic therapy using self-assembled nanogels comprising chlorin e6-bearing pullulan, *J. Mater. Chem. B*, 2021, **9**(32), 6357–6363, DOI: 10.1039/d1tb00377a.
75 S. Karuppusamy, K. Hyejin and H. W. Kang, Nanoengineered chlorin e6 conjugated with hydrogel for photodynamic therapy on cancer, *Colloids Surf., B*, 2019, **181**, 778–788, DOI: 10.1016/j.colsurfb.2019.06.040.
76 K. Deng, C. Li, S. Huang, B. Xing, D. Jin, Q. Zeng, Z. Hou and J. Lin, Recent Progress in Near Infrared Light Triggered Photodynamic Therapy, *Small*, 2017, **13**(44), DOI: 10.1002/smll.201702299.
77 X. Li, S. Lee and J. Yoon, Supramolecular photosensitizers rejuvenate photodynamic therapy, *Chem. Soc. Rev.*, 2018, **47**(4), 1174–1188, DOI: 10.1039/c7cs00594f.
78 W. Liu, X. Ma, Y. Jin, J. Zhang, Y. Li, Y. Tang, Y. Song and S. Wang, Chlorin e6-Biotin Conjugates for Tumor-Targeting Photodynamic Therapy, *Molecules*, 2021, **26**(23), 7342, DOI: 10.3390/molecules26237342.
79 D. Perrone, F. Ardito, G. Giannatempo, M. Dioguardi, G. Troiano, L. Lo Russo, A. De Lillo, L. Laino and L. Lo Muzio, Biological and therapeutic activities, and anti-cancer properties of curcumin, *Exp. Ther. Med.*, 2015, **10**(5), 1615–1623, DOI: 10.3892/etm.2015.2749.
80 U. Bildziukevich, Z. Ozdemir, D. Saman, M. Vlk, M. Slouf, L. Rarova and Z. Wimmer, Novel cytotoxic 1,10-phenanthroline-triterpenoid amphiphiles with supramolecular characteristics capable of coordinating (64)Cu(II) labels, *Org. Biomol. Chem.*, 2022, **20**(41), 8157–8163, DOI: 10.1039/$d_2$ob01172g.
81 A. Upadhyay, S. Gautam, V. Ramu, P. Kondaiah and A. R. Chakravarty, Photocytotoxic cancer cell-targeting platinum(II) complexes of glucose-appended curcumin and biotinylated 1,10-phenanthroline, *Dalton Trans.*, 2019, **48**(47), 17556–17565, DOI: 10.1039/c9dt03490k.
82 S. Y. Y. Ha, Y. Zhou, W. P. Fong and D. K. P. Ng, Multifunctional Molecular Therapeutic Agent for Targeted and Controlled Dual Chemo- and Photodynamic Therapy, *J. Med. Chem.*, 2020, **63**(15), 8512–8523, DOI: 10.1021/acs.jmedchem.0c00893.
83 L. P. Roguin, N. Chiarante, M. C. Garcia Vior and J. Marino, Zinc(II) phthalocyanines as photosensitizers for antitumor photodynamic therapy, *Int. J. Biochem. Cell Biol.*, 2019, **114**, 105575, DOI: 10.1016/j.biocel.2019.105575.
84 J. Karges, J. Li, L. Zeng, H. Chao and G. Gasser, Polymeric Encapsulation of a Ruthenium Polypyridine Complex for Tumor Targeted One- and Two-Photon Photodynamic Therapy, *ACS Appl. Mater. Interfaces*, 2020, **12**(49), 54433–54444, DOI: 10.1021/acsami.0c16119.
85 L. Nagy, M. Mezősi-Csaplár, I. Rebenku, G. Vereb and Á. Szőőr, Universal CAR T cells targeted to HER2 with a biotin-trastuzumab soluble linker penetrate spheroids and large tumor xenografts that are inherently resistant to trastuzumab mediated ADCC, *Front. Immunol.*, 2024, **15**, 1365172, DOI: 10.3389/fimmu.2024.1365172.
86 Z. Ding, S. Wang, Y. Shi, X. Fei, B. Cheng, Y. Lu and J. Chen, Discovery of Novel d-(+)-Biotin-Conjugated Resorcinol Dibenzyl Ether-Based PD-L1 Inhibitors for Targeted Cancer Immunotherapy, *J. Med. Chem.*, 2023, **66**(15), 10364–10380, DOI: 10.1021/acs.jmedchem.3c00479.
87 Y. Li, X. Zhang, W. Luo, D. Wang, L. Yang, J. Wang, L. Zhang, S. Zhang, S. Luo and Y. Wang, Dual-functionalized nanoparticles loaded microbubbles for enhancement of drug uptake, *Ultrasonics*, 2018, **87**, 82–90, DOI: 10.1016/j.ultras.2018.02.003.
88 N. Wang, X. Cheng, N. Li, H. Wang and H. Chen, Nanocarriers and Their Loading Strategies, *Adv. Healthcare Mater.*, 2019, **8**(6), e1801002, DOI: 10.1002/adhm.201801002.
89 V. Weissig, Liposomes Came First: The Early History of Liposomology, *Methods Mol. Biol.*, 2017, **1522**, 1–15, DOI: 10.1007/978-1-4939-6591-5_1.
90 R. Ding, Z. Li, J. Wang, X. Zhu, Z. Zhao and M. Wang, Design and Synthesis of Galactose-Biotin Lipid Materials for Liposomes to Promote the Hepatoma Cell-Targeting Effect, *J. Pharm. Sci.*, 2019, **108**(9), 3074–3081, DOI: 10.1016/j.xphs.2019.04.007.
91 D. Roggenbuck, M. G. Mytilinaiou, S. V. Lapin, D. Reinhold and K. Conrad, Asialoglycoprotein receptor (ASGPR): a peculiar target of liver-specific autoimmunity, *Autoimmun. Highlights*, 2012, **3**(3), 119–125, DOI: 10.1007/s13317-012-0041-4.
92 S. L. Tsai, F. M. Suk, C. I. Wang, D. Z. Liu, W. C. Hou, P. J. Lin, L. F. Hung and Y. C. Liang, Anti-tumor potential of 15,16-dihydrotanshinone I against breast adenocarcinoma through inducing G1 arrest and apoptosis, *Biochem. Pharmacol.*, 2007, **74**(11), 1575–1586, DOI: 10.1016/j.bcp.2007.08.009.
93 F. M. Suk, W. J. Jou, R. J. Lin, S. Y. Lin, F. Y. Tzeng and Y. C. Liang, 15,16-Dihydrotanshinone I-induced apoptosis in human colorectal cancer cells: involvement of ATF3, *Anticancer Res.*, 2013, **33**(8), 3225–3231.
94 J. Luo, X. Meng, J. Su, H. Ma, W. Wang, L. Fang, H. Zheng, Y. Qin and T. Chen, Biotin-Modified Polylactic-co-Glycolic Acid Nanoparticles with Improved Antiproliferative Activity of 15,16-Dihydrotanshinone I in Human Cervical Cancer Cells, *J. Agric. Food Chem.*, 2018, **66**(35), 9219–9230, DOI: 10.1021/acs.jafc.8b02698.
95 N. Hashemi Goradel, M. Najafi, E. Salehi, B. Farhood and K. Mortezaee, Cyclooxygenase-2 in cancer: A review, *J. Cell. Physiol.*, 2019, **234**(5), 5683–5699, DOI: 10.1002/jcp.27411.
96 M. Mahboubi-Rabbani, M. Abbasi and A. Zarghi, Natural-Derived COX-2 Inhibitors as Anticancer Drugs: A Review of their Structural Diversity and Mechanism of Action,

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

Anti-Cancer Agents Med. Chem., 2023, **23**(1), 15–36, DOI: 10.2174/1389450123666220516153915.

97 L. Uram, M. Szuster, A. Filipowicz, K. Gargasz, S. Wolowiec and E. Walajtys-Rode, Different patterns of nuclear and mitochondrial penetration by the G3 PAMAM dendrimer and its biotin-pyridoxal bioconjugate BC-PAMAM in normal and cancer cells in vitro, *Int. J. Nanomed.*, 2015, **10**, 5647–5661, DOI: 10.2147/IJN.S87307.

98 D. Mukherjee, S. E. Nissen and E. J. Topol, Risk of cardiovascular events associated with selective COX-2 inhibitors, *J. Am. Med. Assoc.*, 2001, **286**(8), 954–959, DOI: 10.1001/jama.286.8.954.

99 L. Uram, A. Filipowicz, M. Misiorek, N. Pienkowska, J. Markowicz, E. Walajtys-Rode and S. Wolowiec, Biotinylated PAMAM G3 dendrimer conjugated with celecoxib and/or Fmoc-l-Leucine and its cytotoxicity for normal and cancer human cell lines, *Eur. J. Pharm. Sci.*, 2018, **124**, 1–9, DOI: 10.1016/j.ejps.2018.08.019.

100 T. Takahashi and M. Miyazawa, N-Caffeoyl, serotonin as selective COX-2 inhibitor, *Bioorg. Med. Chem. Lett.*, 2012, **22**(7), 2494–2496, DOI: 10.1016/j.bmcl.2012.02.002.

101 A. Malaviya and P. W. Sylvester, Synergistic Antiproliferative Effects of Combined gamma-Tocotrienol and PPAR gamma Antagonist Treatment Are Mediated through PPAR gamma-Independent Mechanisms in Breast Cancer Cells, *PPAR Res.*, 2014, **2014**, 439146, DOI: 10.1155/2014/439146.

102 J. Yu, X. He, Q. Zhang, D. Zhou, Z. Wang and Y. Huang, Iodine Conjugated Pt(IV) Nanoparticles for Precise Chemotherapy with Iodine-Pt Guided Computed Tomography Imaging and Biotin-Mediated Tumor-Targeting, *ACS Nano*, 2022, **16**(4), 6835–6846, DOI: 10.1021/acsnano.2c01764.

103 D. Patra, S. Kumar, P. Kumar, I. Chakraborty, B. Basheer and R. Shunmugam, Iron(III) Coordinated Theranostic Polyprodrug with Sequential Receptor-Mitochondria Dual Targeting and T(1)-Weighted Magnetic Resonance Imaging Potency for Effective and Precise Chemotherapy, *Biomacromolecules*, 2022, **23**(8), 3198–3212, DOI: 10.1021/acs.biomac.2c00302.

104 J. Kantapan, N. Anukul, N. Leetrakool, G. Rolin, J. Vergote and N. Dechsupa, Iron-Quercetin Complex Preconditioning of Human Peripheral Blood Mononuclear Cells Accelerates Angiogenic and Fibroblast Migration: Implications for Wound Healing, *Int. J. Mol. Sci.*, 2021, **22**(16), 8851, DOI: 10.3390/ijms22168851.

105 K. A. Thuomas, Aspects of image intensity and relaxation time assessment in magnetic resonance imaging. An experimental and clinical study, *Acta Radiol., Suppl.*, 1991, **375**(Pt 2), 49–90.

106 C. Scialabba, A. Sciortino, F. Messina, G. Buscarino, M. Cannas, G. Roscigno, G. Condorelli, G. Cavallaro, G. Giammona and N. Mauro, Highly Homogeneous Biotinylated Carbon Nanodots: Red-Emitting Nanoheaters as Theranostic Agents toward Precision Cancer Medicine, *ACS Appl. Mater. Interfaces*, 2019, **11**(22), 19854–19866, DOI: 10.1021/acsami.9b04925.

107 N. Soni, S. Singh, S. Sharma, G. Batra, K. Kaushik, C. Rao, N. C. Verma, B. Mondal, A. Yadav and C. K. Nandi, Absorption and emission of light in red emissive carbon nanodots, *Chem. Sci.*, 2021, **12**(10), 3615–3626, DOI: 10.1039/d0sc05879c.

108 M. Kciuk, B. Marciniak and R. Kontek, Irinotecan-Still an Important Player in Cancer Chemotherapy: A Comprehensive Overview, *Int. J. Mol. Sci.*, 2020, **21**(14), 4919, DOI: 10.3390/ijms21144919.

109 C. Daglioglu, Environmentally Responsive Dual-Targeting Nanoparticles: Improving Drug Accumulation in Cancer Cells as a Way of Preventing Anticancer Drug Efflux, *J. Pharm. Sci.*, 2018, **107**(3), 934–941, DOI: 10.1016/j.xphs.2017.10.029.

110 Z. Li, Q. Chen, Y. Qi, Z. Liu, T. Hao, X. Sun, M. Qiao, X. Ma, T. Xu, X. Zhao, *et al.*, Rational Design of Multifunctional Polymeric Nanoparticles Based on Poly(l-histidine) and d-alpha-Vitamin E Succinate for Reversing Tumor Multidrug Resistance, *Biomacromolecules*, 2018, **19**(7), 2595–2609, DOI: 10.1021/acs.biomac.8b00213.

111 C. B. Tripathi, P. Parashar, M. Arya, M. Singh, J. Kanoujia, G. Kaithwas and S. A. Saraf, Biotin anchored nanostructured lipid carriers for targeted delivery of doxorubicin in management of mammary gland carcinoma through regulation of apoptotic modulator, *J. Liposome Res.*, 2020, **30**(1), 21–36, DOI: 10.1080/08982104.2019.1579839.

112 S. Roy, A. K. Rawat, S. R. Sammi, U. Devi, M. Singh, S. Gautam, R. K. Yadav, J. K. Rawat, L. Singh, M. N. Ansari, *et al.*, Alpha-linolenic acid stabilizes HIF-1 α and downregulates FASN to promote mitochondrial apoptosis for mammary gland chemoprevention, *Oncotarget*, 2017, **8**(41), 70049–70071, DOI: 10.18632/oncotarget.19551.

113 W. Yang, Y. Xue, X. Cui, H. Tang and H. Li, Targeted delivery of doxorubicin to liver used a novel biotinylated beta-cyclodextrin grafted pullulan nanocarrier, *Colloids Surf., B*, 2022, **220**, 112934, DOI: 10.1016/j.colsurfb.2022.112934.

114 S. E. Glassford, B. Byrne and S. G. Kazarian, Recent applications of ATR FTIR spectroscopy and imaging to proteins, *Biochim. Biophys. Acta*, 2013, **1834**(12), 2849–2858, DOI: 10.1016/j.bbapap.2013.07.015.

115 A. Singh, R. K. Sharma, M. Chagtoo, G. Agarwal, N. George, N. Sinha and M. M. Godbole, ^{1}H NMR Metabolomics Reveals Association of High Expression of Inositol 1, 4, 5 Trisphosphate Receptor and Metabolites in Breast Cancer Patients, *PLoS One*, 2017, **12**(1), e0169330, DOI: 10.1371/journal.pone.0169330.

116 B. Purushothaman, J. Choi, S. Park, J. Lee, A. A. S. Samson, S. Hong and J. M. Song, Biotin-conjugated PEGylated porphyrin self-assembled nanoparticles co-targeting mitochondria and lysosomes for advanced chemo-photodynamic combination therapy, *J. Mater. Chem. B*, 2019, **7**(1), 65–79, DOI: 10.1039/c8tb01923a.

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

117 T. A. Debele, S. Peng and H. C. Tsai, Drug Carrier for Photodynamic Cancer Therapy, *Int. J. Mol. Sci.*, 2015, **16**(9), 22094–22136, DOI: 10.3390/ijms160922094.
118 M. Liu, B. Wang, C. Guo, X. Hou, Z. Cheng and D. Chen, Novel multifunctional triple folic acid, biotin and CD44 targeting pH-sensitive nano-actiniaes for breast cancer combinational therapy, *Drug Delivery*, 2019, **26**(1), 1002–1016, DOI: 10.1080/10717544.2019.1669734.
119 J. Cai, J. Fu, R. Li, F. Zhang, G. Ling and P. Zhang, A potential carrier for anti-tumor targeted delivery-hyaluronic acid nanoparticles, *Carbohydr. Polym.*, 2019, **208**, 356–364, DOI: 10.1016/j.carbpol.2018.12.074.
120 S. Handali, E. Moghimipour, M. Rezaei, Z. Ramezani, M. Kouchak, M. Amini, K. A. Angali, S. Saremy and F. A. Dorkoosh, A novel 5-Fluorouracil targeted delivery to colon cancer using folic acid conjugated liposomes, *Biomed. Pharmacother.*, 2018, **108**, 1259–1273, DOI: 10.1016/j.biopha.2018.09.128.
121 P. Yuan, D. Tan and F. Annabi-Bergaya, Properties and applications of halloysite nanotubes: recent research advances and future prospects, *Appl. Clay Sci.*, 2015, **112–113**, 75–93, DOI: 10.1016/j.clay.2015.05.001.
122 W. Jiang, Y. Huang, N. Han, F. He, M. Li, Z. Bian, J. Liu, T. Sun and L. Zhu, Quercetin suppresses NLRP3 inflammasome activation and attenuates histopathology in a rat model of spinal cord injury, *Spinal Cord*, 2016, **54**(8), 592–596, DOI: 10.1038/sc.2015.227.
123 A. M. Yamina, M. Fizir, A. Itatahine, H. He and P. Dramou, Preparation of multifunctional PEG-graft-Halloysite Nanotubes for Controlled Drug Release, Tumor Cell Targeting, and Bio-imaging, *Colloids Surf., B*, 2018, **170**, 322–329, DOI: 10.1016/j.colsurfb.2018.06.042.
124 K. Li, X. Zang, X. Meng, Y. Li, Y. Xie and X. Chen, Targeted delivery of quercetin by biotinylated mixed micelles for non-small cell lung cancer treatment, *Drug Delivery*, 2022, **29**(1), 970–985, DOI: 10.1080/10717544.2022.2055225.
125 L. L. Sun, D. Ellerman, M. Mathieu, M. Hristopoulos, X. Chen, Y. Li, X. Yan, R. Clark, A. Reyes, E. Stefanich, *et al.*, Anti-CD20/CD3 T cell-dependent bispecific antibody for the treatment of B cell malignancies, *Sci. Transl. Med.*, 2015, **7**(287), 287ra270, DOI: 10.1126/scitranslmed.aaa4802.
126 D. Xiong, Y. Xu, H. Liu, H. Peng, X. Shao, Z. Lai, D. Fan, M. Yang, J. Han, Y. Xie, *et al.*, Efficient inhibition of human B-cell lymphoma xenografts with an anti-CD20×anti-CD3 bispecific diabody, *Cancer Lett.*, 2002, **177**(1), 29–39, DOI: 10.1016/S0304-3835(01)00758-3.
127 C. Bai, P. Hu, W. Zhang, L. Song, D. Liu, Y. Chen, M. Ma, N. Gu and Y. Zhang, Modular design of Bi-specific nanoplatform engaged in malignant lymphoma immunotherapy, *Nanoscale*, 2020, **12**(35), 18418–18428, DOI: 10.1039/d0nr04450d.
128 Y. Wang, R. Song, K. Guo, Q. Meng, R. Zhang, X. Kong and Z. Zhang, A gadolinium(III) complex based dual-modal probe for MRI and fluorescence sensing of fluoride ions in aqueous medium and in vivo, *Dalton Trans.*, 2016, **45**(44), 17616–17623, DOI: 10.1039/c6dt02229d.
129 J. Chen, S. Chen, X. Zhao, L. V. Kuznetsova, S. S. Wong and I. Ojima, Functionalized single-walled carbon nanotubes as rationally designed vehicles for tumor-targeted drug delivery, *J. Am. Chem. Soc.*, 2008, **130**(49), 16778–16785, DOI: 10.1021/ja805570f.
130 T. Wang, Y. Zhang, L. Wei, Y. G. Teng, T. Honda and I. Ojima, Design, Synthesis, and Biological Evaluations of Asymmetric Bow-Tie PAMAM Dendrimer-Based Conjugates for Tumor-Targeted Drug Delivery, *ACS Omega*, 2018, **3**(4), 3717–3736, DOI: 10.1021/acsomega.8b00409.
131 S. V. K. Rompicharla, P. Kumari, H. Bhatt, B. Ghosh and S. Biswas, Biotin functionalized PEGylated poly(amidoamine) dendrimer conjugate for active targeting of paclitaxel in cancer, *Int. J. Pharm.*, 2019, **557**, 329–341, DOI: 10.1016/j.ijpharm.2018.12.069.
132 A. L. Givan, Flow cytometry: an introduction, *Methods Mol. Biol.*, 2011, **699**, 1–29, DOI: 10.1007/978-1-61737-950-5_1.
133 I. Poudel, R. Ahiwale, A. Pawar, K. Mahadik and C. Bothiraja, Development of novel biotinylated chitosan-decorated docetaxel-loaded nanocochleates for breast cancer targeting, *Artif. Cells, Nanomed., Biotechnol.*, 2018, **46**(sup2), 229–240, DOI: 10.1080/21691401.2018.1453831.
134 B. Tang, Y. Peng, Q. Yue, Y. Pu, R. Li, Y. Zhao, L. Hai, L. Guo and Y. Wu, Design, preparation and evaluation of different branched biotin modified liposomes for targeting breast cancer, *Eur. J. Med. Chem.*, 2020, **193**, 112204, DOI: 10.1016/j.ejmech.2020.112204.
135 M. Huang, Y. Pu, Y. Peng, Q. Fu, L. Guo, Y. Wu and Y. Zheng, Biotin and glucose dual-targeting, ligand-modified liposomes promote breast tumor-specific drug delivery, *Bioorg. Med. Chem. Lett.*, 2020, **30**(12), 127151, DOI: 10.1016/j.bmcl.2020.127151.
136 J. H. Park, H. J. Cho and D. D. Kim, Poly((D,L)lactic-glycolic) acid-star glucose nanoparticles for glucose transporter and hypoglycemia-mediated tumor targeting, *Int. J. Nanomed.*, 2017, **12**, 7453–7467, DOI: 10.2147/IJN.S147668.
137 Q. Liu, L. Zhou, R. Lu, C. Yang, S. Wang, L. Hai and Y. Wu, Biotin and glucose co-modified multi-targeting liposomes for efficient delivery of chemotherapeutics for the treatment of glioma, *Bioorg. Med. Chem.*, 2021, **29**, 115852, DOI: 10.1016/j.bmc.2020.115852.
138 O. Hanrahan, J. Harris and C. Egan, Advanced microscopy: laser scanning confocal microscopy, *Methods Mol. Biol.*, 2011, **784**, 169–180, DOI: 10.1007/978-1-61779-289-2_12.
139 A. D. Vadlapudi, R. K. Vadlapatla, D. Pal and A. K. Mitra, Biotin uptake by T47D breast cancer cells: functional and molecular evidence of sodium-dependent multivitamin transporter (SMVT), *Int. J. Pharm.*, 2013, **441**(1–2), 535–543, DOI: 10.1016/j.ijpharm.2012.10.047.
140 M. Chowdhury and P. Kumar Das, Paclitaxel-Loaded, Biotinylated Fe(2+)-Doped Carbon Dot: Combination

 This journal is © The Royal Society of Chemistry 2025

Published on 05 1446. Downloaded on 14/11/47 06:12:58 .

Therapy in Cancer Treatment, *ACS Appl. Bio Mater.*, 2021, **4**(6), 5132–5144, DOI: 10.1021/acsabm.1c00348.

141 L. Ndlwana, N. Raleie, K. M. Dimpe, H. F. Ogutu, E. O. Oseghe, M. M. Motsa, T. A. M. Msagati and B. B. Mamba, Sustainable Hydrothermal and Solvothermal Synthesis of Advanced Carbon Materials in Multidimensional Applications. A Review, *Materials (Basel, Switzerland)*, 2021, **14**(17), 5094, DOI: 10.3390/ma14175094.

142 R. Lu, L. Zhou, Q. Liu, S. Wang, C. Yang, L. Hai, L. Guo and Y. Wu, Skillfully collaborating chemosynthesis with GOx-enabled tumor survival microenvironment deteriorating strategy for amplified chemotherapy and enhanced tumor ablation, *Biomater. Sci.*, 2021, **9**(5), 1855–1871, DOI: 10.1039/d0bm01950j.

143 C. Yan, N. Liang, Q. Li, P. Yan and S. Sun, Biotin and arginine modified hydroxypropyl-beta-cyclodextrin nanoparticles as novel drug delivery systems for paclitaxel, *Carbohydr. Polym.*, 2019, **216**, 129–139, DOI: 10.1016/j.carbpol.2019.04.024.

144 H. W. Sung, W. H. Chang, C. Y. Ma and M. H. Lee, Crosslinking of biological tissues using genipin and/or carbodiimide, *J. Biomed. Mater. Res., Part A*, 2003, **64**(3), 427–438, DOI: 10.1002/jbm.a.10346.

145 R. A. Praphakar, M. A. Munusamy, A. A. Alarfaj, S. S. Kumar and M. Rajan, Zn^{2+} cross-linked sodium alginate-g-allylamine-mannose polymeric carrier of rifampicin for macrophage targeting tuberculosis nanotherapy, *New J. Chem.*, 2017, **41**(19), 11324–11334, DOI: 10.1039/C7NJ01808H.

146 X. Sun, P. Pradeepkumar, N. K. Rajendran, H. Shakila, N. N. Houreld, D. A. Al Farraj, Y. M. Elnahas, N. Elumalai and M. Rajan, Natural deep eutectic solvent supported targeted solid-liquid polymer carrier for breast cancer therapy, *RSC Adv.*, 2020, **10**(61), 36989–37004, DOI: 10.1039/d0ra03790g.

147 H. Shi, N. Liang, J. Liu, S. Li, X. Gong, P. Yan and S. Sun, AIE-active polymeric micelles based on modified chitosan for bioimaging-guided targeted delivery and controlled release of paclitaxel, *Carbohydr. Polym.*, 2021, **269**, 118327, DOI: 10.1016/j.carbpol.2021.118327.

148 Z. He, C. Ke and B. Z. Tang, Journey of Aggregation-Induced Emission Research, *ACS Omega*, 2018, **3**(3), 3267–3277, DOI: 10.1021/acsomega.8b00062.

149 D. Gao, S. Asghar, J. Ye, M. Zhang, R. Hu, Y. Wang, L. Huang, C. Yuan, Z. Chen and Y. Xiao, Dual-targeted enzyme-sensitive hyaluronic acid nanogels loading paclitaxel for the therapy of breast cancer, *Carbohydr. Polym.*, 2022, **294**, 119785, DOI: 10.1016/j.carbpol.2022.119785.

150 C. Yang, X. Wang, X. Yao, Y. Zhang, W. Wu and X. Jiang, Hyaluronic acid nanogels with enzyme-sensitive cross-linking group for drug delivery, *J. Controlled Release*, 2015, **205**, 206–217, DOI: 10.1016/j.jconrel.2015.02.008.

151 H. Wang, X. Shan, M. Ren, M. Shang and C. Zhou, Nucleosomes enter cells by clathrin- and caveolin-dependent endocytosis, *Nucleic Acids Res.*, 2021, **49**(21), 12306–12319, DOI: 10.1093/nar/gkab1121.

152 K. Ghazal and M. L. Piketty, Comment on biotin-induced thyroid stimulating hormone aberrations in the setting of immunotherapy, *J. Oncol. Pharm. Pract.*, 2021, **27**(6), 1562–1563, DOI: 10.1177/10781552211029706.

153 I. Mrosewski, I. Neumann and R. Switkowski, Interference of High Dose Biotin Supplementation with Thyroid Parameters in Immunoassays Utilizing the Interaction between Streptavidin and Biotin: a Case Report and Review of Current Literature, *Clin. Lab.*, 2019, **65**(1), DOI: 10.7754/Clin.Lab.2018.180637.

154 B. Mumma, D. Diercks, R. Twerenbold, A. Valcour, A. Ziegler, A. Schützenmeister, D. Kasapic and N. Tran, Clinical risk assessment of biotin interference with a high-sensitivity cardiac troponin T assay, *Clin. Chem. Lab. Med.*, 2020, **58**(11), 1931–1940, DOI: 10.1515/cclm-2019-0962.

155 Y. Lee, S. Lee and S. Jon, Biotinylated Bilirubin Nanoparticles as a Tumor Microenvironment-Responsive Drug Delivery System for Targeted Cancer Therapy, *Adv. Sci.*, 2018, **5**(6), 1800017, DOI: 10.1002/advs.201800017.