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Review Article

Self-assembled Natural Product-based Carrier-free Nanoplatforms for Efficient Bioactivity

Xiao-Qing Xu[1], Xiao Xu[1], Yun Wang[1], Zhong-Rui Li[1,2*] and Chao Han[1*]

[1]*State Key Laboratory of Natural Medicines, Jiangsu Key Laboratory of Bioactive Natural Product Research, School of Traditional Chinese Pharmacy, China Pharmaceutical University, Nanjing, China;* [2]*Department of Medicinal Chemistry, School of Pharmacy, Nanjing Medical University, Nanjing, China*

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Abstract

Natural products featured by an abundant molecule skeleton and structural complexity exhibit extensive pharmacological or biological activities. Thus, natural active ingredients are an important source of drug research and development. However, the inherent defects, including low solubility, low bioavailability, and unacceptable off-target toxicity, affect their development into clinical drugs. Recently, carrier-free supermolecule nanodrugs have attracted considerable attention. These nanodrugs are self-assembled by pure drugs mainly through hydrophobicity, hydrogen bond, π-π stacking, and electrostatic interaction, which possess a high drug loading capability, enhanced water solubility of the drugs, and synergistic therapeutic efficacy. In this review, natural product-based carrier-free nanoplatforms with self-assembly for efficient bioactivity are examined. These self-assembled natural products include triterpenoids, alkaloids, flavonoids, and anthraquinones. Moreover, the morphology of the formed nanoplatforms can be a nanosphere, nanofiber, nanorod, or fibrillar network, and they can exhibit several bioactivities, such as antitumor, anti-inflammatory, immunoregulation, and liver protection. Briefly, we analyze the types and sources, formation mechanism, biological activity, and mode of action of nanomedicine, and discuss the future of this field. We believe this review would provide a landscape of natural product-based carrier-free nanoplatforms.

Introduction

Natural products possess extensive pharmacological or biological activities that could be the source of drug discovery.[1] Compared with synthetic small molecules, natural products have the advantage of an abundant molecule skeleton, structural complexity, and a high degree of stereochemistry. According to statistics,[2,3] approximately 50% of approved drugs among 1,881 agents over nearly four decades from 1981 to 2019 have either natural products or their derivatives. Studying natural products for treating diseases in humans has also always won a significant share of Nobel Prizes[4,5] and many famous drugs have been discovered in plants, including artemisinin, taxol, guanfu base A, and vincristine. Even, several natural products have been identified as effective in treating severe acute respiratory syndrome coronavirus 2.[6,7] Thus, natural products offer the best chance to discover novel effective structures to cure human illnesses. However, the inherent defects of natural products affect their development into clinical drugs, possibly owing to low solubility, low bioavailability, unacceptable off-target toxicity, and a narrow therapeutic window. Therefore, it is still a major challenge to exploit natural product-based drug discovery.

Drugs or small molecules are developed to be loaded in quantities of nanocarriers to improve the therapeutic properties, which have shown great success in the field of drug delivery.[8,9] The benefits of nanodrugs are owing to the range of properties and

Keywords: Natural products; Carrier-free nanoplatforms; Supermolecule self-assembly; Triterpenoid; Alkaloid.

Abbreviations: AA, aristolochic acid; ABBR, alkylated berberine; Asp, aspirin; BA, baicalin; BBR, berberine; CA, cinnamic acid; Ce6, chlorin e6; CPT, camptothecin; CST, celastrol; CTX, cabazitaxel; DAS, dasatinib; DOX, doxorubicin; DTA, dehydrotramenolic acid; EGCG, epigallocatechin gallate; Erg, ergosterol; GA, glycyrrhizic acid; GLA, glycyrrhetinic acid; GRb_1, ginsenoside Rb_1; GRg_1, ginsenoside Rg_1; GRo, ginsenoside Ro; HCPT, 10-hydroxycamptothecin; ICG, indocyanine green; IDM, indomethacin; LA, lactobionic acid; LAA, liquidambaric acid; MCA, 3,4,5-methoxycinnamic acid; PTX, paclitaxel; PUA, poly (ursolic acid); RHA, retinoic hydroxamic acid; Rhe, rhein; RHL, rhamnolipids; SSa, saikosaponin a; UA, ursolic acid; WOG, wogonoside.

***Correspondence to:** Chao Han, State Key Laboratory of Natural Medicines, Jiangsu Key Laboratory of Bioactive Natural Product Research, School of Traditional Chinese Pharmacy, China Pharmaceutical University, 24 Tong Jia Xiang, Nanjing 210009, China. ORCID: https://orcid.org/0000-0001-8580-4697. Tel: +86 159-5205-2127, E-mail: hanchao@cpu.edu.cn; Zhong-Rui Li, Department of Medicinal Chemistry, School of Pharmacy, Nanjing Medical University, 101 longmian Avenue, Nanjing 211166, China. ORCID: https://orcid.org/0000-0001-6830-4973. Tel: +86 135-9805-4657, E-mail: lizhongrui@njmu.edu.cn

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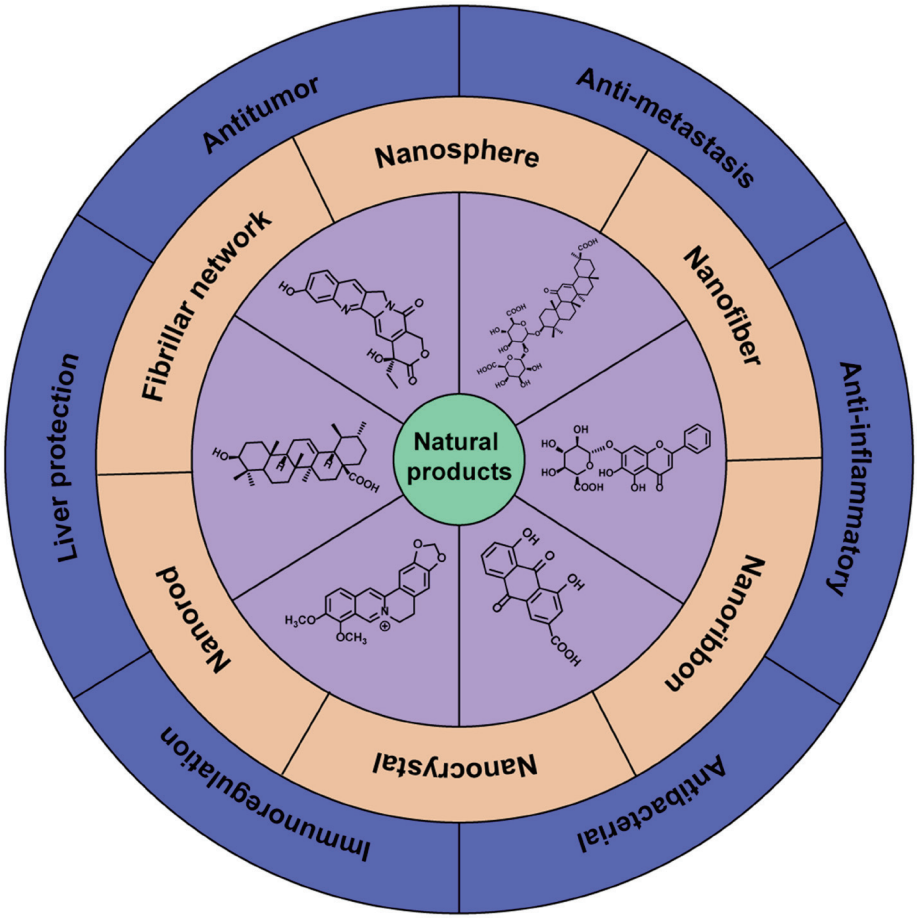

Fig. 1. Representative natural products, the self-assembled nanostructures and bioactivities.

interactions that are particular to the nanoscale structure with a size of 1–1,000 nm. Nanotechnology, such as polymer colloids, liposomes, micelles, carbon material, and a metal-organic framework, has been widely used in encapsulating natural products to increase the bioavailability, targeted delivery, and controlled release.[10,11] Nevertheless, most nanocarriers perhaps have the weaknesses of a low drug loading capacity, long-term materials toxicity, undesirable immune responses, and nearly all nanocarriers have no therapeutic effect. Fortunately, carrier-free supermolecule nanodrugs have been exploited in the nanodrug delivery system.[12–15] These nanodrugs are self-assembled by pure drugs without any accessories, which are probably formed through non-covalent interactions, such as Van der Waals' force, hydrophobicity, hydrogen bond, π-π stacking, and electrostatic interaction. More importantly, carrier-free nanodrugs could significantly improve the solubility and stability of drugs, and have almost non-toxicity, a high drug loading capability, and synergistic therapeutic efficacy. Additionally, carrier-free nanodrugs are deemed as promising candidates to be the next generation of drug formulations. As an emerging field, more and more natural products are being reported to form carrier-free nanostructures by self-assembly.[16] Therefore, it would be essential and of great interest to summarize and discuss natural product-based carrier-free nanoplatforms with self-assembly for efficient bioactivity. In this review, the emphasis would focus on the types and sources, formation mechanism, biological activity, and mode of action (Fig. 1). Finally, we would briefly outline the current existing problems and future development of this nanomedicine field.

Natural triterpenoids-based carrier-free nanoplatforms

Natural triterpenoids, widely distributed in nature, contain six isoprene units and usually exist in plants and animals in a free, ether, ester, or glycoside form.[17] Researchers[18,19] have recently discovered that triterpenoids act as an antitumor, antivirus, liver protector, bactericidal, anti-inflammatory, and other physiological activities, and some of them have been approved for the treatment of clinical conditions. Furthermore, with a unique stereostructure, multifunctional groups, and multi chiral centers, triterpenes are increasingly being used to assemble supramolecular gel systems that provide therapeutic or drug delivery strategies.[20,21]

Ursolic acid

Ursolic acid (UA), a pentacyclic triterpenoid, is found in many natural herbs and edible plants, including *Cornus officinalis*, *Prunella vulgaris*, and *Ligustrum lucidum*. UA has the advantages of low toxicity and high efficacy, which possesses a wide range of bioactivities, such as antitumor, anti-metastatic, anti-inflammatory, anti-angiogenic, and antidiabetic activities.[22] Fan *et al*.[23] designed

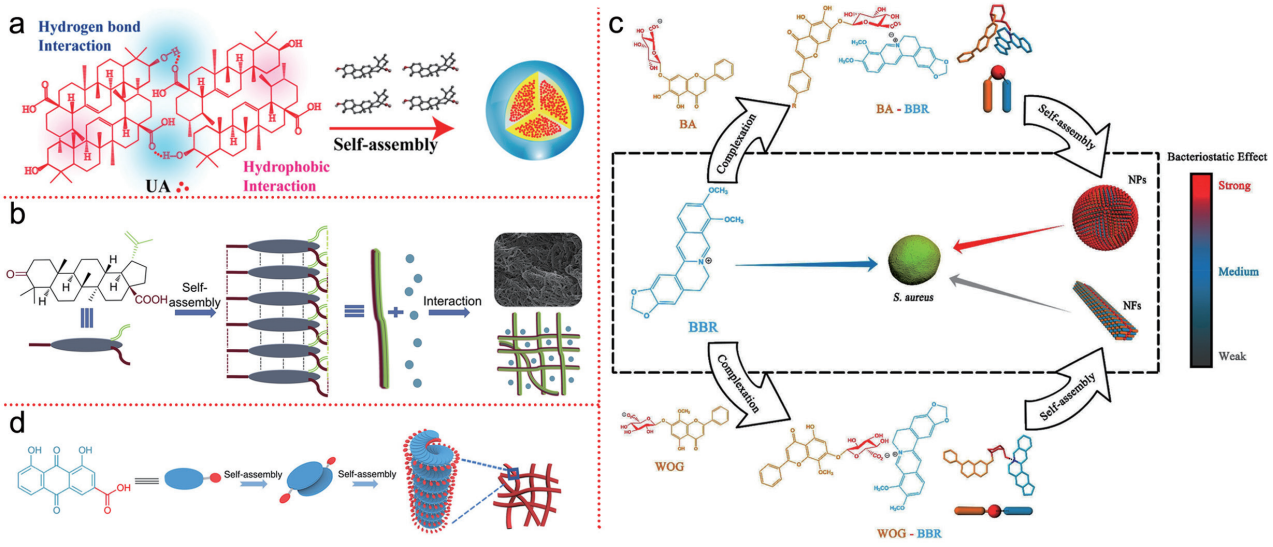

Fig. 2. Self-assembly and antibacterial activity of baicalin-berberine and wogonoside-berberine complexes. (a) Ursolic acid nanodrug formed by self-assembly for tumor immune therapy (Adapted from Ref. 23 with permission. Copyright © 2018 The American Chemical Society); (b) Formation mechanism by self-assembly of liquidambaric acid (Adapted from Ref. 53 with permission. Copyright © 2020 The Elsevier Publishing Group); (c) Self-assembling process of baicalin-berberine (BA-BBR) and wogonoside-berberine (WOG-BBR) and their antibacterial activity (Adapted from Ref. 66 with permission Copyright © 2019 The American Chemical Society); (d) Possible self-assembly diagram of rhein gel (Adapted from Ref. 87 with permission. Copyright © 2019 Springer Nature Publishing Group). UA, ursolic acid; BA, baicalin; BA-BBR, baicalin-berberine; BBR, berberine; WOG, wogonoside; WOG-BBR, wogonoside-berberine.

a carrier-free, pure nanodrug by self-assembly of UA (Fig. 2a). The UA nanoparticles exhibited a spherical shape with a size of 150 nm, which were formed based on the electrostatic and hydrophobic interaction between the UA molecules. More importantly, the UA nanoparticles proved to inhibit tumor growth and had the ability of liver protection and immunotherapy. Next, aspirin (Asp), a famous nonsteroidal anti-inflammatory drug,[24] could interact with UA to assemble Asp-UA nanoparticles.[25,26] As a pH-stimuli responsive nanodrug, the Asp-UA nanoparticles could accumulate in the tumor tissues via passive targeting, and release drugs in an acidic tumor micro-environment. Thereafter, the Asp-UA nanoparticles inhibited adhesion, migration, and invasion of breast cancer cells through upregulating the expression of E-cadherin, beta-catenin (β-catenin), and PTEN proteins, and downregulating the expression of integrin α6β1, CD44, MMP-2, COX-2, EGFR, and ERK proteins. Likewise, Zhang *et al*.[27] established a "core-shell" co-assembly carrier-free nanosystem based on UA and epigallocatechin gallate (EGCG) for hepatocellular carcinoma synergistic treatment. EGCG, the ingredient with antioxidant activity in green tea,[28] was used as a "shell" to self-polymerize to form a uniform layer, which could avoid the degradation of the UA "core". These nanodrugs showed low cytotoxicity, good biosafety, and efficient tumor accumulation. More importantly, UA not only led to tumor cell death and delivered tumor antigens, but also activated the immune system and boosted APC cell proliferation with EGCG to enhance the antitumor effect by the acquired immune cells.

UA could also interact with indocyanine green (ICG) and lactobionic acid (LA) to self-assemble UA-ICG-LA nanoparticles.[29] ICG is the only approved agent used in clinical imaging and detection,[30] and LA is an asialoglycoprotein receptor.[31] The UA-ICG-LA nanodrugs were capable of tumor imaging and specifically targeting the tumor tissue. In addition, the UA-ICG-LA nanodrugs with near-infrared irradiation displayed enhanced antitumor effects collaborated with photothermal therapy and photodynamic therapy. Furthermore, Jiang *et al*.[32] developed carrier-free nanodrugs for co-delivery of UA and chemotherapeutic doxorubicin (DOX). The coassembled dual nanodrugs exhibited a spherical shape with a size of 109 nm and a pH-triggered drug release manner. Additionally, UA could sensitize DOX for the enhanced antitumor effects and therefore significantly produced the synergistic treatment of human breast cancer BT474 cells. Likewise, Bag *et al*.[33] studied the self-assembly of UA in different liquids in detail. They discovered that UA could self-assemble into nanostructures with vesicles, tubes, fibers, and flowers in an organic and aqueous organic solvent. UA self-assemblies could be utilized for loading fluorophores and DOX. Next, a "self-contained bioactive nanocarrier" system based on UA and paclitaxel (PTX) was developed.[34] UA interacted with PTX to form a high drug loading nanoparticle via a hydrophobic interaction and hydrogen bonding. UA and PTX could significantly improve the synergistic therapeutic efficacy, while the nanodrugs were capable of eliminating the toxic side effects and risk of liver damage induced by the chemotherapy agents via the upregulation of key antioxidant proteins. Guo *et al*.[35] also developed a carrier-free theranostic nanodrug by the self-assembly of UA, PTX, and ICG on account of electrostatic, hydrophobic, and π-π stacking interactions. The UA-PTX-ICG nanoparticles exhibited long-term near-infrared fluorescence imaging, effective passive tumor targeting, and synergistic antitumor effect by chemotherapy, photodynamic therapy, and photothermal therapy. Finally, poly(ursolic acid) (PUA) was synthesized through the polycondensation of the hydroxyl group and carboxyl group of UA.[36] PUA could self-assemble into the nanoparticles with PTX. The assembled nanoparticles possessed prolonged blood circulation, enhanced tumor targeting, and significant antitumor efficacy against colorectal cancer CT26 cells.

Glycyrrhizic acid

Glycyrrhizic acid (GA), separated from *Glycyrrhiza uralensis*, *G.*

inflate, or *G. glabra*, is a triterpene saponin, which exhibits anti-inflammatory, antitumor, and other extensive biological activities.[37] GA consists of a hydrophilic di-glucuronic residue and a hydrophobic triterpenoid aglycon (glycyrrhetinic acid: GLA). Such an amphiphilic structure prompted GA to have a good self-assembly capability.[38] The GA molecules were forecasted to form stable aggregates with a size of about 10 nm through the simulation of molecular dynamics.[39] GA aggregation could also interact with the PTX molecules at a ratio of 3:1. Next, spherical micelles were observed in the GA aqueous solution with modest anisotropy.[40] The addition of metal ions affected the minimal micelle growth. In addition, Zhao *et al*.[41] found GA formed an injectable low-molecular-weight hydrogel with nanocluster morphology in the aqueous solution. This GA hydrogel selectively inhibited the growth of gram-positive *Staphylococcus aureus* and had good hemocompatibility and biocompatibility with mammalian cells. GA could assemble nanomicelles with baicalein to enhance the solubility of baicalein in the aqueous solution by more than 4,500 times.[42] The nanomicelles had a sustained release effect of baicalein, which was modulated by changing pH.

In terms of chemical structure, GLA was the triterpenoid aglycon of GA. GLA had a rigid, pentacyclic triterpenoid backbone with hydroxyl and carboxyl groups, and it could self-assemble thermoreversible gels with spherical and flower-like shapes consisting of fibrillar networks.[43] Wu *et al*.[44] considered GLA to form a hydrogel with a dipole-dipole interaction as the main driving force. The GLA hydrogel showed the properties of selective dye adsorption and sustainable release, which could be used in dye waste removal as an environmentally-friendly functional material and controlled drug delivery system. GLA interacted with PTX and oleanolic acid, a triterpene for treating hepatitis, to form a natural nanomedicine-cum-carrier delivery nanoplatform.[45] The nanoplatform had synergistic effects on the tumor treatment and an excellent hepatoprotective effect to reduce liver damage caused by PTX.

Ginsenosides

Ginseng is one of the most popular herbs worldwide and has been demonstrated to treat hypertension, stress, and different neurological disorders.[46] Ginsenoside Ro (GRo), the active ingredient of ginseng, could markedly increase the solubility of saikosaponin a (SSa), derived from *Bupleurum chinense*.[47] At low concentrations, GRo preferentially self-assembled into the vesicles to absorb SSa into themselves. At high concentrations, SSa first self-aggregated and then interacted with GRo to form mixed vesicles. Next, interactions between ginsenoside Rb_1 and Rg_1 (GRb_1 and GRg_1) with SSa were also explored.[48] GRb_1 could disperse the SSa solid in water, while no significant interaction was obtained between GRg_1 and SSa. Different from GRo, GRb_1 and GRg_1 formed spherical micelles in the aqueous medium. Compared to GRg_1, GRb_1 with greater sugar groups produced more binding sites with SSa, thus leading to stronger interaction. In order to expand the application of the GRo vesicles, different drug additives were examined in terms of their effect on the Ro vesicles' solubilization.[49] The hydrophobic molecules lacking hydrophilic groups (such as quercetin and coumarin) were mainly inserted in the hydrophobic layer of the GRo vesicle, while the amphipathic molecules, including the hydrophilic and hydrophobic groups (such as baicalin and SSa) were mainly located on the palisade layer of the GRo vesicle. These results were of great significance for the further development and application of ginsenosides.

Other triterpenoids

Celastrol (CST), a pentacyclic triterpenoid, exists in *Tripterygium wilfordii* for treatment of cancer. CST could interact with DOX to self-assemble into carrier-free nanoparticles for a synergistic antitumor.[50] CST-DOX nanoparticles could inhibit a P-gp expression to overcome DOX resistance through restraining NF-κB and activating heat shock factor 1. Thereafter, nanoparticles produced a synergistic combination chemotherapy via the ROS/JNK signaling pathway in the DOX resistant cells. Dai *et al*.[51] adopted the dissipative particle dynamics simulations method to study the micellization behavior of platycodin, which was derived from *Platycodon grandiflorum*. Numerous platycodin self-assemblies with spherical, ellipse, oblate, and multilamellar vesicles were observed, which had the potential to be used as biocarriers for the drug delivery. Poricoic acid A belonging to tricyclic triterpenoids and dehydrotrametenolic acid (DTA) belonging to tetracyclic triterpenoids were isolated in *Poria cocos*, and could self-assemble into a low molecular weight gelator, respectively.[52–54] DTA nanoparticles were also assembled based on intermolecular hydrogen bonding and could penetrate the gastrointestinal tract through an apical sodium-dependent bile transporter-based intestinal transport system for effective disease treatment. Liquidambaric acid (LAA, also known as betulonic acid) is isolated from *Liquidambar formosana* and possesses many biological activities.[55] Zhi *et al*.[53] prepared injectable LAA gel scaffolds loaded with DOX (Fig. 2b). LAA-DOX gel not only showed controlled gelation, sustained drug release, and less toxic side effects, but also achieved the synergistic treatment of tumors. LAA could also be loaded with PTX to assemble supramolecular nanoparticles via a hydrophobic interaction and hydrogen bond.[56] Except for antitumor activity, the LAA-PTX nanoparticles exhibited a series of advantages, such as excellent water solubility, efficient tumor targeting, high bioavailability, low toxicity, and biological safety. In addition, ibuprofen, the anti-inflammatory drug, was selected to be loaded on the LAA gel.[57] This gel could enhance the anti-inflammatory activity of ibuprofen by about two-thirds and its treatment even achieved about 140% of the OTC drugs. Hence, LAA gel provided a useful tool for a drug delivery strategy with enhanced anti-inflammatory activity. Natural sterols are a kind of important active ingredient, which exhibit the self-assembly ability to deliver drugs.[21,58] Ergosterol (Erg), β-sitosterol, and stigmasterol, the representative sterols, could self-assemble into carrier-free nanoparticles, respectively.[52,54,59] The photosensitizer chlorin e6 (Ce6) was loaded on Erg to assemble Erg-Ce6 nanoparticles through π-π stacking and hydrophobic interactions for a significantly combined antitumor. The Erg-Ce6 nanoparticles improved the water solubility and stability of Ce6 and possessed an excellent tumor targeting ability. In addition, the Erg-Ce6 nanoparticles induced reactive oxygen species generation due to the photodynamic therapy of Ce6 by promoting type I photoreactions, which resulted in a significant anticancer efficiency *in vitro* and *in vivo*. Natural sterols with better biocompatibility and biodegradability were expected to develop into nanomaterials for the drug delivery in the treatment of human illness.

Natural alkaloids-based carrier-free nanoplatforms

Natural alkaloids[60,61] are important nitrogen-based compounds, which are widely distributed in a large variety of plants with different genera, such as *Taxux*, *Picea*, *Ephedra*, *Cephalotaxus*, and *Stephania*. Alkaloids contain multiple biological activities, including antitumor, anti-inflammatory, antioxidant, and antimi-

crobial effects, thus making them an ideal source of drug discovery.

Berberine

Berberine (BBR) has been widely used clinically to treat bacterial diarrhea, which exists in the Chinese herb *Coptidis rhizoma*.[62] Lei *et al*.[63–65] found compound precipitation occurred when *C. rhizoma* was simultaneously decocted with another Chinese herb *Scutellaria baicalensis*. This compound precipitation was the complex of the intermolecular interaction between the BBR from *C. rhizoma* and baicalin (BA) from *S. baicalensis*, and exhibited a neuroprotective effect in cobalt chloride-induced PC12 cells. Furthermore, they discovered that BBR could self-assemble with BA to form carrier-free nanoparticles, and wogonoside (WOG), another active compound from *S. baicalensis*, assembled into nanofibers with BBR.[66] The BBR-BA nanoparticles and BBR-WOG nanofibers were formed through electrostatic and hydrophobic interactions. Compared with BBR, the BBR-BA nanoparticles exhibited enhanced bacteriostatic activity against *Staphylococcus aureus*, whereas the BBR-WOG nanofibers showed a weaker effect (Fig. 2c). The reason was that the BBR-BA nanoparticles with the hydrophilic groups toward the outside showed a stronger affinity to the bacteria population, thereby resulting in easily permeating into the biofilm. Next, the BBR-BA nanoparticles could also be used to treat diarrhea predominant irritable bowel syndrome.[67] The synergistic effect of the nanoparticles through microbiota–gut–brain axis was better rather than the simple mixing of BBR and BA, and the mechanism was concerned with the brain-gut peptides, immune inflammation, and intestinal flora. Moreover, Huang *et al*.[68] assembled the BBR nanoparticles with cinnamic acid (CA), a representative component from *Cinnamomum cassia*. The BBR molecule interacted with CA to form butterfly-like one-dimensional units, and then the units assembled three-dimensional spherical particles. The BBR-CA nanostructures showed enhanced inhibitory activity on multidrug-resistant *S. aureus*. 3,4,5-Methoxycinnamic acid (MCA) is the derivative of CA, which is extracted from *Polygala tenuifolia*. MCA could also self-assemble with BBR to form nanoparticles.[69] The formation of the BBR-MCA nanoparticles was mainly based on the π-π stacking interactions and intermolecular hydrogen bonds. More importantly, the BBR-MCA nanoparticles possessed inhibiting multidrug-resistant *S. aureus* through the binding on the surface of bacteria. Rhein (Rhe) is the active compound from *Rheum palmatum* and has an anthraquinone skeleton. Rhe could interact with BBR to assemble nanoparticles with Rhe acting as a stacked backbone and BBR inserting into it.[70] The antimicrobial activity against *S. aureus* of the BBR-Rhe nanoparticles significantly increased on account of the synergistic bacteriostasis of BBR and Rhe. The assembled nanostructures resulted in the death of the bacteria by adhering to the surface of the bacteria and increasing the drug concentration around the bacteria. Aristolochic acid (AA) is an active ingredient with a phenanthrene skeleton and exists in *Aristolochia debilis*. AA can cause a series of side effects, such as liver cancer, AA nephropathy, and acute kidney injury, which would seriously affect the use of herbs containing AA.[71] Fortunately, the BBR-based self-assemblies with AA could neutralize the acute nephrotoxicity of AA.[72] The BBR-AA self-assemblies with linear heterogenous supramolecules were formed based on the electrostatic attraction and π-π stacking. The BBR-AA supramolecules could block the toxic site of AA by activating the immune system and tumorigenesis-related pathways. These findings could offer a new strategy to eliminate the toxicity problems of herbs containing AA. Additionally, Shen *et al*.[73] synthesized a carrier-free supramolecule containing alkylated BBR (ABBR) and rhamnolipids (RHL) against *Helicobacter pylori*. The ABBR-RHL supramolecules with a negative charge and size of about 160 nm were derived by the electrostatic and hydrophobic interactions. More importantly, the assembled supramolecules could eradicate the *H. pylori* biofilms by breaking the extracellular polymeric substances. Moreover, the supramolecules inhibited adherence of the *H. pylori* to restrain the ability of the biofilm reformation, which could provide a foundation for treating biofilm-related infections.

Paclitaxel

Paclitaxel (PTX), a microtubule-interfering chemotherapy agent, has been widely employed in antitumors with an extensive spectrum, such as lung, breast, and gastric cancer.[74] Pei *et al*.[75] synthesized a glutathione-responsive PTX dimer (PTX-S-PTX) and then assembled the PTX-S-PTX nanovesicles. The nanovesicles showed a high drug loading, rapid GSH responsive release, and enhanced cancer theranostic by encapsulating the fluorescent molecule ICG. BBR with a positive charge could selectively target mitochondria to kill cancer cells, of which the mechanism was different from PTX. Therefore, the GSH-responsible dual drug conjugate (BBR-S-PTX) was obtained based on BBR and PTX through a disulfide bond.[76] Similarly, BBR-S-PTX could assemble into nanoparticles with a size of 165 nm, which was formed by the hydrophobic and π-π stacking interactions. The induced assembled nanoparticles enhanced the apoptosis of the cancer cells by targeting mitochondria and also exhibited better antimicrobial activity against *S. aureus* and *E. coli*. Zhang *et al*.[77] also prepared carrier-free nanocrystal aggregates based on PTX and indomethacin (IDM; a COX-2 inhibitor). PTX-IDM assemblies with a "brick-cement" architecture possessed synergetic antitumor effects of immunotherapy and chemotherapy. Cabazitaxel (CTX), the PTX derivative, could interact with dasatinib (DAS) to form nanoassemblies.[78] DAS with amphiphilicity drove the nano-assembly of the CTX-DAS nanoparticles without any exogenous excipients. The nano-assemblies exhibited 100% drug loading and aggregation-induced emission at 422 nm for tumor diagnosis. More importantly, the treatment of cancer with the CTX-DAS nanoparticles was significantly synergetic. This approach lays the groundwork for the combinatorial use of multiple drugs with different mechanisms of action.

Camptothecin and doxorubicin

Camptothecin (CPT), an isoquinoline alkaloid, is separated from *Camptotheca acuminate*. CPT is a broad-spectrum antitumor agent, which inhibits the DNA topoisomerase I enzyme to lead to DNA double-stranded breaks.[79] CPT was used to construct self-defensive nanostructures with the C-D-E rings of the planar construction.[80] 10-Hydroxycamptothecin (HCPT) with modification of hydroxyl on C-10 and carboxylic camptothecin with esterification of C-20 could also self-assemble into nanoparticles. However, due to the different molecular structures, CPT and HCPT were nanoribbons, and carboxylic CPT nanostructures were cylindric nanorods. Moreover, CPT-based assemblies could protect CPT from hydrolysis to enhance tumor therapy. Liang *et al*.[81,82] prepared carrier-free HCPT-DOX nanoparticles formulated by simple physical self-assembling HTCP and DOX. The HCPT uptake was improved by the CPT-DOX nanoparticles with a spherical morphology and positive charge. As expected, the nanoparticles exhibited enhanced a synergistic antitumor against the cancer cells. On the other hand,

the HCPT-DOX nanoparticles enhanced the antitumor in drug-resistant cancer cells. Nanosizing of HCPT and DOX showed a synergistic effect to improve the intracellular drug retention through inhibiting the P-gp efflux. Curcumin is a diarylheptanoid that exists in *Curcuma longa*, of which the structure is in enol-keto tautomeric equilibria with different chemical environments.[83] To achieve a better-targeted drug delivery, carrier-free nanostructures with self-assembling curcumin and CPT derivatives were constructed based on the molecular structures.[84] These nanoparticles with a stabilized size of 100 nm showed changeable surface charges of −10 mV with pH=7.4 and +40 mV in acidic environments. Additionally, the assembled nanoparticles showed the synergetic treatment of colorectal cancer with better lung and gallbladder targeting and macrophage-clearance escape. Likewise, Li *et al*.[85] synthesized a DOX dimer via a pH-triggered carbamate linker and then prepared carrier-free DOX conjugated nanoparticles. The conjugated nanoparticles exhibited a concentration-dependent acid-responsive drug release and enhanced antitumor efficacy, which provided a promising method for overcoming the multidrug resistance of the cancer cells and prevented tumor recurrence. In order to improve tumor drug delivery, a simple nanotransformer (DTIG) was utilized by assembling DOX, tannic acid, and ICG.[86] DTIG with hydrophilic particles exhibited prolonged blood circulation time, while DTIG became hydrophobic particles to be efficiently endocytosed by the tumor cells in the acidic micro-environment. These efficient instantaneous transformations promoted the lysosome escape of the drug and drugs release. Therefore, DTIG provided some references to the drug delivery process for cancer treatment.

Other natural product-based carrier-free nanoplatforms

Zheng *et al*.[87] successfully prepared rhein hydrogels through intermolecular π-π interactions and hydrogen bonds (Fig. 2d). Rhein hydrogels with a nanofiber network structure exerted better anti-neuroinflammation. Furthermore, the hydrogels could improve an intracellular drug uptake by binding and recognizing toll-like receptor 4, and thereby achieve optimal anti-inflammation by inhibiting a TLR4/NFκB signaling pathway. Moreover, Liu *et al*.[88] obtained robust nanoparticles by efficiently assembling ferric ion (Fe^{3+}) and luteolin, a natural flavonoid molecule. These nanoparticles could notably improve the solubility and stability of luteolin. In addition, the assembled nanoparticles broadened the absorption spectrum to the near-infrared region to produce a supramolecular photothermal effect, and the coordination assembly greatly enhanced the treatment of cancer through chemotherapeutic and photothermal effects. Retinoic hydroxamic acid (RHA) contained a hydrophobic all-trans retinoic acid backbone and a hydrosoluble hydroxamic group, of which the amphiphilic groups could induce the formation of nanoparticles by self-assembly.[89] Consequently, the RHA nanoparticles could result in tumor cell cycle arrest and apoptosis through inhibiting histone deacetylase and activating retinoic acid receptors. Thus, the RHA nanoparticles exhibited long-term anticancer effects with low toxicity, which could be a promising drug for melanoma therapy.

Future directions

In the field of nanobiology, natural product-based carrier-free nanoplatforms with self-assembly offer significant possibilities for drug research and development. The assembled nanodrugs have been extensively applied in targeted therapy, synergistic treatment, and theranostics. Their significant advantages include simple and "green" preparation methods, a high drug loading capacity, efficient accumulation of drugs, and effective co-delivery behavior, making them promising nanomedicine as a treatment strategy. Nevertheless, some drawbacks and challenges of carrier-free nanoplatforms still need to be solved. Self-assembled nanodrugs are slightly unstable, and it would be essential to embellish stabilizers on the surface of the nanodrugs to enhance the stability. In addition, the formation mechanism is still unclear. As such, it is confusing which kinds of natural products could self-assemble, and this should require preliminary experiments and lack basic empirical conclusions. In particular, it is difficult to precisely control the ratio of the natural products during self-assembly. Finally, the immunity function limited the therapeutic effect of the carrier-free nanodrugs. Thus, this is still a challenge for the research and development of natural product-based carrier-free nanoparticles.

Conclusions

In this review, we examined natural product-based carrier-free nanoplatforms with self-assembly for efficient bioactivity (Table 1 and Fig. 3).[23,25-27,29,32-35,39,41-45,47–50,52–54,56-59,63–66,68-70,72,77,78,81,82,84,86-88] Triterpenoids with a rigid skeleton structure and high degree of stereochemistry could self-assemble into organogels by themselves. These organogels could be formed into a nanosphere, nanofiber, nanorod, or fibrillar network through hydrophobicity, π-π stacking, hydrogen bond, and electrostatic interaction. Furthermore, the gels could be used to deliver drugs or fluorescent molecules, which would exhibit various physiological activities, such as antitumor, anti-inflammatory, immunoregulation, and liver protection. Berberine was an isoquinoline alkaloid with a positive charge, and thus it could interact with natural products containing the carboxyl group. Berberine-based nanostructures were mainly governed by electrostatic and hydrophobic interactions, and displayed good bacteriostatic activity. Next, the three clinical anticancer drugs (doxorubicin, paclitaxel, and 10-hydroxycamptothecin) could also self-assemble into nanoparticles with other small molecules mainly through hydrophobic interactions, and exhibit enhanced treatment of cancer. Therefore, this review provided a reference for the design of self-assemblies between natural products.

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Conflict of interest

The authors have no conflicts of interest related to this publication.

Table 1. Natural products-based carrier-free nanoplatforms with self-assembly

Molecule 1	Molecule 2	Morphology	Interaction force of self-assembly	Bioactivity	References
Ursolic acid	–	Nanosphere	Hydrophobic interactions/ Hydrogen bond	Antitumor	23
	Aspirin	Nanosphere		Antitumor metastasis	25,26
	Epigallocatechin gallate	Nanosphere		Antitumor	27
	Indocyanine green and lactobionic acid	Nanosphere	π-π stacking/hydrophobic interaction/electrostatic interaction	Antitumor	29
	Doxorubicin	Nanosphere		Antitumor	32,33
	Paclitaxel	Nanosphere		Antitumor	34,35
Glycyrrhizic acid	–	Nanosphere	Hydrophobic interactions/ Hydrogen bond	Antibacterial activity	39,41
	Baicalein	Nanosphere		–	42
Glycyrrhetinic acid	–	Nanogels with fibrillar networks		–	43,44
	Oleanolic acid and paclitaxel	Nanosphere		Antitumor	45
Ginsenoside Ro	Saikosaponin a	Nanosphere	Hydrogen bond/ Dipolar interaction	–	47–49
Celastrol	Doxorubicin	Nanosphere	Hydrophobic interactions/ Electrostatic adherence/ π-π stacking	Antitumor	50
Dehydrotrametenolic acid	Paclitaxel	Nanosphere	Hydrogen bond/π-π stacking	Antitumor	52–54
Liquidambaric acid	Paclitaxel or doxorubicin	Network nanofiber		Antitumor	56,57
	Ibuprofen	Network nanofiber		Anti-inflammatory	58
Ergosterol	Chlorin e6	Nanorod		Antitumor	52,54,59
Berberine	Baicalin	Nanosphere	Electrostatic interaction/ Hydrophobic interaction/ π-π stacking	Antibacterial activity	63–66
	Wogonoside	Nanofiber		Antibacterial activity	66
	Cinnamic acid	Nanosphere		Antibacterial activity	68
	3,4,5-Methoxycinnamic acid	Nanosphere		Antibacterial activity	69
	Rhein	Nanosphere		Antibacterial activity	70
	Aristolochic acid	Linear Supramolecule		Neutralizing nephrotoxicity	72
Paclitaxel	Indomethacin	Nanocrystal	Hydrophobic interaction/ π-π stacking	Antitumor	77
Cabazitaxel	Dasatinib	Nanosphere	Hydrogen bond/π-π stacking/ van der Waals interaction	Antitumor	78
10-Hydroxycamptothecin	Doxorubicin	Nanorod	Hydrophobic interaction/ π-π stacking	Antitumor	81,82
Camptothecin	Curcumin	Nanosphere	Hydrophobic interaction/ Hydrogen bond/π-π stacking	Antitumor	84
Doxorubicin	Indocyanine green and tannic acid	Nanosphere	π-π stacking/Electronic interaction	Antitumor	86
Rhein	–	Nanofiber	π-π stacking/Hydrogen bond	Anti-inflammatory	87
Luteolin	Ferric ion	Nanosphere	Coordinationinteraction/ van der Waals interaction	Antitumor	88

– Not applicable.

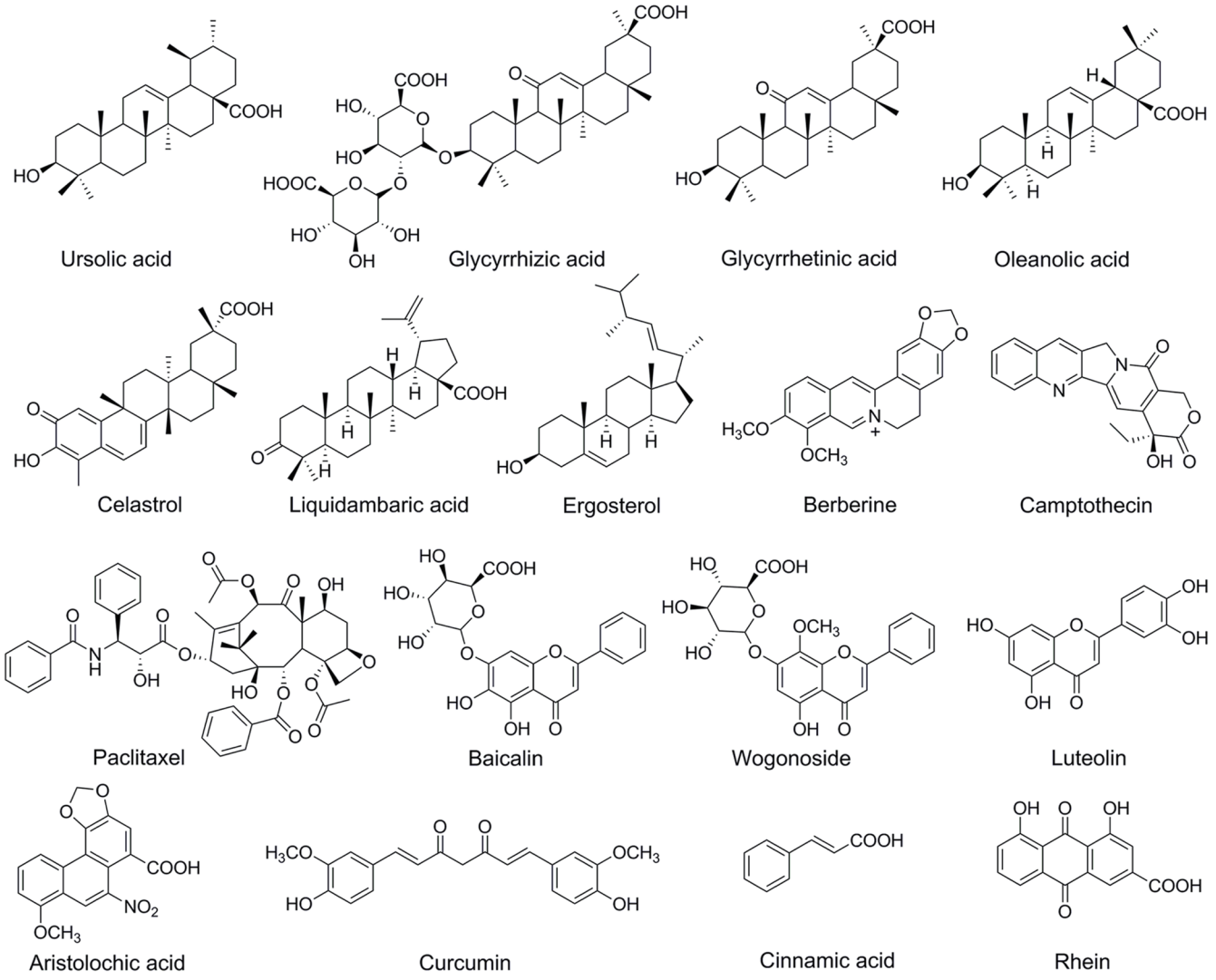

Fig. 3. Representative chemical structures of the natural products, including ursolic acid, glycyrrhizic acid, glycyrrhetic acid, oleanolic acid, celastrol, liquidambaric acid, ergosterol, berberine, camptothecin, paclitaxel, baicalein, wogonoside, luteolin, aristolochic acid, curcumin, cinnamic acid, and rhein.

Author contributions

Conceptualization (ZL and CH), writing-original draft preparation (XQX, XX and YW), writing-review and editing (CH), and supervision and funding acquisition (ZL and CH). All authors read and agreed to the published version of the manuscript.

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