Triterpenoid Building Blocks for Functional Nanoscale Assemblies: A Review

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ABSTRACT: Naturally abundant, renewable, and sustainable plant triterpenoids have gained considerable attention in biological application, including antitumor activities. However, due to their lipophilic nature, natural triterpenoids display limited solubility in aqueous media. Notably, the rigid backbone and the presence of functional groups also offer the possibilities towards a strategic design of a class of conformationally rigid amphiphiles. Such derivatives may improve

their bioavailability, and they display unique self-assembly properties relevant for soft functional materials. In recent years, a formation of nano-assemblies have been studied with plant triterpenoids and their semi-synthetic derivatives to investigate ways of their applications in material science. For example, naturally amphiphilic glycyrrhizic acid has been shown to form hydrogels. Similarly, other natural triterpenoid-based phosphates or heterocyclic-based quaternary conjugates have also been studied for supramolecular gelation. Triterpenoid-based supramolecular gels are important types of soft materials, displaying rapid self-healing, thixotropic behaviors, aging induced transition, and self-assembly induced anticancer and antimicrobial properties. In this review, we will provide a summary of (i) unique structural and functional properties of natural triterpenoids, (ii) self-assembly and gelation properties of natural triterpenoids, (iii) gelation properties of rationally designed triterpenoid derivatives, (iv) structural transition in aqueous media, and (v) their biological effects.

1. Introduction

Self-assembly of amphiphilic molecules allow structurally and functionally diverse morphologies across different length scales.[1] The classical surfactants have been studied for their self-assemblies into various morphologies including spherical micelles, rod-like micelles, tubular structures, vesicles and bilayers.[2] Diverse structures including double chain amphiphiles, Gemini surfactants and bolaamphiphiles have been prepared and studied for several decades in the literature.[3] Self-assembly of conventional surfactants is controlled by a balance between attractive and repulsive intermolecular interactions.[4-6] Based on the literature and available solid state structures, it is evident that natural steroids, sterols, triterpenoids, and their derivatives are structurally different from conventional surfactants. Therefore, their self-assembly characteristics

also significantly differ from conventional surfactans.[7-9] Importantly, it has been demonstrated that appropriate functionalization of steroids affects the molecular shape and amphiphilicity, producing spherical micelles, supermicelles,[10] and structural transition from micelles to fibers.[11]

Under the appropriate conditions, certain low molecular weight amphiphilic molecules self-assembled into three-dimensional (3D) fibrillar networks leading to a gelation. The resulting gels are known as molecular gels. Various non-covalent interactions, including hydrogen bonding,[12] electrostatic interactions,[13] hydrophobic effect,[14] London dispersion forces,[15] van der Waals interactions,[16] charge-transfer complexation,[17] metal coordination,[18-20] halogen bonding,[21] and fluorine-fluorine interactions,[22] have been explored effectively for designing functional molecular gels. It has been postulated that, molecular gels are metastable and kinetically trapped structures.[23,24] However, by tuning the supramolecular interactions and self-assembly conditions, the gelation process can be controlled to attain different material characteristics.[23,24] A delicate balance between gelation and crystallization has also been demonstrated.[25,26] Gelation has often been considered as failed crystallization.[25,26] A formation of three-dimensional (3D) structures, such as crystals, allows maximum stabilizing interactions compared to that of anisotropic one-dimensional (1D) structures found in the gels.[27] More importantly, a delicate balance exists between crystallization and gelation in molecular gels.[28] However, in some cases, such as peptide amphiphiles, the formation of 1D structures is inherently thermodynamically stable.[29] Molecular gels resulting from the nano-assembly of low molecular mass organic compounds have represented an important class of functional materials for application in catalysis, sensors and optoelectronics.[30-34] Low molecular weight gelators (LMWGs), undergo hierarchical assembly into highly entangled three-dimensional (3D) fibrillar networks, and macroscopically immobilize a large number of solvent molecules.[35] Notably, majority of the gels contain large amount of

solvent and extremely small amount of solid. However, molecular gels show solid-like viscoelastic properties under rheological experiments, and, importantly, they offer stimuli-responsive, dynamic and reversible control over their properties.[32] Various stimuli, including temperature,[36] light,[37] electric field,[38] pH,[39] oxidation-reduction,[40] and mechanical stress.[41] The reversible mechanical properties are consequences of a structural reorganization of gelators at the microscopic level.[33] The microscopic changes occurring in molecular gels can be attributed to weak non-covalent intermolecular interactions as mentioned above. Extensive research pursued over the last two decades on structurally and functionally different molecules have provided on-demand design of gelators with unique mechanical and material characteristics. The building blocks ranging from long chain hydrocarbons,[14] peptides,[42] polyaromatics,[17] heteroaromatics,[43] steroids,[44,45] carbohydrates,[46-48] phosphates,[49] have been studied extensively in the literature. Even if not all amphiphilic molecules are gelators, amphiphilicity seems to be a common characteristics of a large number of LMWGs, regardless the nature of the gels and the diversity of building blocks.[34] Another important property of a certain class of gelators is inherent rigidity in their structure. Such a conformational rigidity allows a formation of the self-assembled structures with reduced entropic losses.[50] Examples for conformationally rigid gelators include the derivatives of bile acids,[44] cholesterol,[45] plant sterols,[51,52] and sorbitol.[53] In the literature, it has been shown that conformationally rigid structures either lack hydrogen bonding interactions or the contribution of hydrogen bonding towards self-assembly is limited.[34]

It has been found that several natural triterpenoids spontaneously self-assembled in different solvents even without any chemical modification. They have been shown to form nano-architectures, such as vesicles, fibers, flower-like structures and spherical assemblies.[54] A number of triterpenoid derivatives were synthesized, and their self-assembly characteristics were

investigated in organic and aqueous media. Furthermore, self-assembly studies have also been coupled with their pharmacological effects. Interestingly, most of natural plant triterpenoids and their derivatives formed gels by a formation of fibrillary networks, vesicles or charge transfer complexes. A majority of the plant triterpenoids has been known to display pharmacologically and medicinally important characteristics. Therefore, varied self-assembled materials in different media have a great ability to become drug delivery systems showing synergistic effects. For example, nano-assemblies have been applied in a formation of gel-metal nanoparticle hybrid material, entrapment and release of drug molecules, removal of toxic or thermochromic chemical materials, and in the processes of heterogeneous catalysis. Despite many experimental studies on self-assembled nanostructures in different media, the self-assembly pathway of natural triterpenoids and their derivatives still cannot be predicted reliably.[54] We discuss some of the recent examples in the self-assembly of selected triterpenoids and their derivatives in this review.

2. An approach to the pentacyclic triterpene scaffolds as potential gelators

2.1. Natural triterpenoid molecules

Phytochemicals is a general term describing plant products having varied structures and functions (secondary metabolites).[55] The most representative group of phytochemicals are naturally abundant triterpenes comprising more than 20 000 recognized compounds.[56,57] They can be categorized as acyclic, mono-, bi-, tri-, tetra- and pentacyclic plant products according to the diverse features of their skeletons.[58] Pentacyclic triterpenes (PTs) are generally biosynthesized from six isoprene units that undergo to a cyclization to 2,3-oxidosqualene, which is a 30-carbon atoms precursor, and, therefore, they have a basic formula $C_{30}H_{48}$. A complex series of reactions catalyzed by oxidosqualene cyclases or triterpene synthases produce either

sterol or triterpene scaffolds, depending on the biosynthetic pathway followed. Determination of the biosynthetic pathway results in a determination of the scaffold biosynthesized. It is dependent on the initial substrate folding step. Folding 2,3-oxidosqualene through the chair–boat–chair conformation leads to a protosteryl cation intermediate, and gives rise to sterols *via* a formation of cycloartenol in plants. Folding 2,3-oxidosqualene through the chair–chair–chair conformation directs cyclization into the dammarenyl carbocation, which subsequently gives rise to diverse PT skeletons, namely those of the most widely studied ursane, oleanane and lupane series.[59] In the nature, PTs appear in fruit peel, leaves and stem bark. In contrast to the steroids, PTs are not involved in the major metabolic pathways. Their evolutionary role is to protect the host, most often a plant, from insect pests and pathogens.[60] Both natural and semisynthetic PTs have received emerging attention due to their broad range of pharmacologically important characteristics.[61,62]

In the field of soft materials, plant triterpenoids (natural PT derivatives bearing at least one substituent containing the oxygen atom) represent a class of naturally abundant, renewable, and sustainable resources that display sufficient conformational rigidity but have been so far much less explored than numbers of other plant products. Self-assembly of triterpenoid derivatives into functional nanostructured materials has not been investigated extensively, in spite of their unique molecular structure capable of forming various nanoscale systems.[63] Studies on the self-assembly of natural triterpenoids with no additional functionalization have been reported in the literature, but are limited to arjunolic acid (**AA**),[64] ursolic acid (**UA**),[65,66] maslinic acid (**MA**),[67] corosolic acid (**CA**),[68] betulin (**BT**),[69] glycyrrhetinic acid (**GA**),[70] betulinic acid (**BA**),[71] erythrodiol (**ED**),[72] and oleanolic acid (**OA**),[73] (Figure 1).

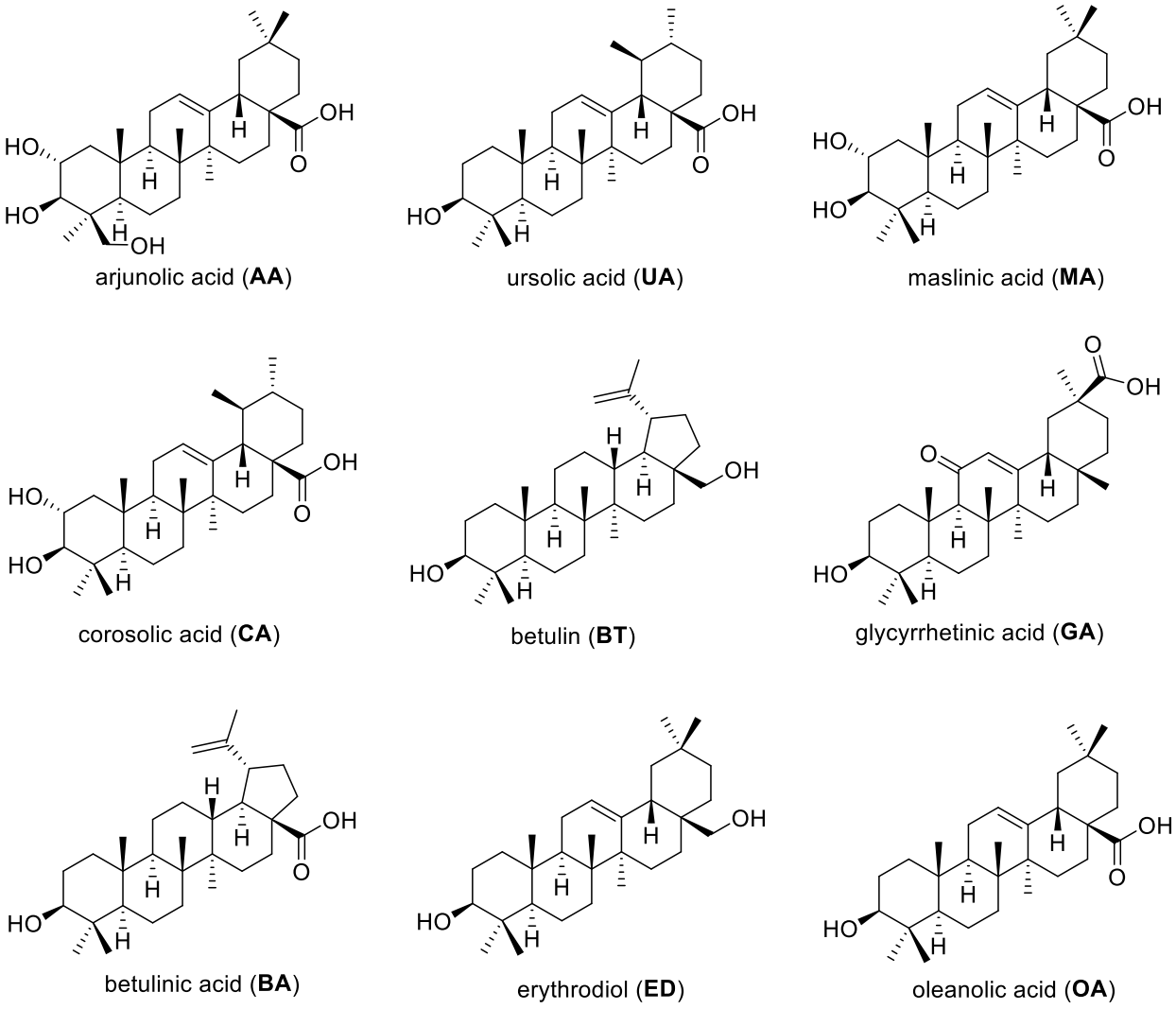

Figure 1. Chemical structures of the natural triterpenoids.

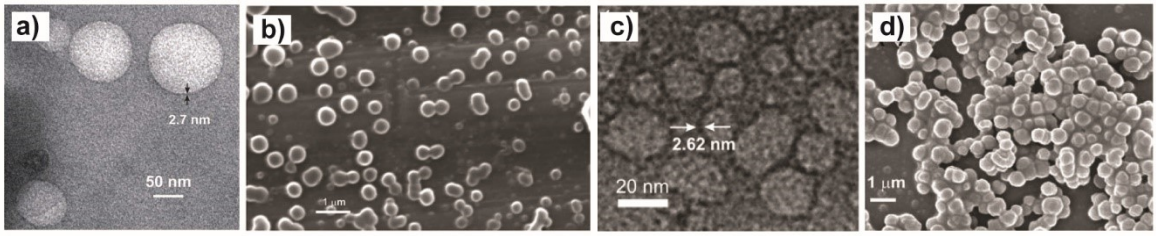

Figure 2. (a) TEM images of nano-assembled **AA** in DMSO–water (0.022%, 1:1, w/v); (b) SEM micrographs of the dried nano-assemblies of **UA** prepared from a colloidal suspension in ethanol–water (0.23%, 3:1, w/v); (c) HRTEM images of nano-assemblies prepared from **MA** in DMF−water (3.51 mM, 2:1, v/v); (d) FESEM images of a dry nano-assemblies prepared from **CA** in EtOH–water (4.23 mM, 3:1, v/v). Reproduced from Bag, B. G.; Majumdar, R. Vesicular Self-Assembly of a Natural Triterpenoid Arjunolic Acid in Aqueous Medium: Study of Entrapment Properties and *in situ* Generation of Gel–Gold Nanoparticle Hybrid Material. *RSC Adv.* **2014**, *4*, 53327–53334.[64] Copyright [2014] Royal Society of Chemistry. Reproduced from Bag, B. G.; Das, S.; Hasan, S. N.; Barai, A. C. Nanoarchitectures by Hierarchical Self-Assembly of Ursolic acid: Entrapment and Release of Fluorophores Including Anticancer Drug Doxorubicin. *RSC Adv.* **2017**, *7*, 18136–18143.[66] Copyright [2017] Royal Society of Chemistry. Reproduced from Bag, B. G.; Hasan, S. N.; Ghorai, S.; Panja, S. K. First Self-Assembly of Dihydroxy Triterpenoid Maslinic Acid Yielding Vesicles. *ACS Omega* **2019**, *4*, 7684–7690.[67] Copyright [2019] American Chemical Society. Reproduced from Bag, B. G.; Garai, C.; Ghorai, S. Vesicular Self-Assembly of a Natural Ursane-Type Dihydroxy-Triterpenoid Corosolic Acid. *RSC Adv.* **2019**, *9*, 15190–15195.[68] Copyright [2019] Royal Society of Chemistry.

A formation of bilayer vesicular self-assemblies was reported for mono-, di- and tri-hydroxy triterpenoids, **AA**,[64] **UA**,[66] **MA**,[67] and **CA**[68] (Figure 2), in aqueous binary liquid mixtures. It has been suggested that the H-bonding between hydroxyl and carboxyl groups (intramolecular and head-to-tail) and the dispersive interaction by the non-polar triterpenoid backbone to be major driving forces for the vesicular self-assembly. The vesicular self-assemblies obtained from these triterpenoids have been shown to entrap fluorophores and/or doxorubicin (DOX).[64-66,71] Such properties offer potential applications in removal dyes from contaminated water and has the potential to act as a controlled drug delivery system.[66]

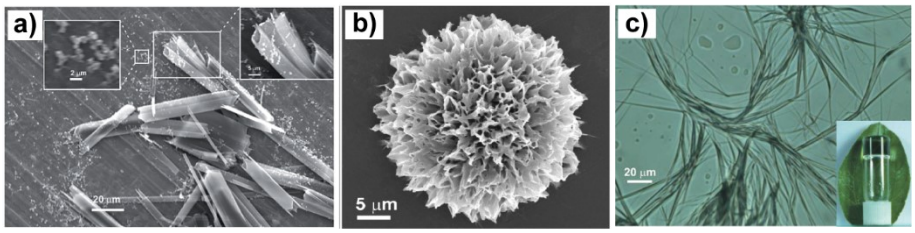

Figure 3. (a) Scanning electron micrographs of the dried self-assemblies of **UA** prepared from colloidal suspension in ethanol–water (0.23%, 3:1, w/v); (b) FESEM images of **BT** in *m*-xylene (1%, w/v); (c) OPM image (50× magnification) of **BA** gel in *o*-dichlorobenzene (0.41%, w/v) showing a fibrillar network. Inserted: an inverted vial containing a transparent *o*-dichlorobenzene gel with a *Ziziphus jujuba* leaf as the background. Reproduced from Bag, B. G.; Das, S.; Hasan, S. N.; Barai, A. C. Nanoarchitectures by Hierarchical Self-Assembly of Ursolic acid: Entrapment and Release of Fluorophores Including Anticancer Drug Doxorubicin. *RSC Adv.* **2017**, *7*, 18136–18143.[66] Copyright [2017] Royal Society of Chemistry. Reproduced from Bag, B. G.; Dash, S. S. Hierarchical Self-Assembly of a Renewable Nanosized Pentacyclic Dihydroxy-triterpenoid Betulin Yielding Flower-Like Architectures. *Langmuir* **2015**, *31*, 13664–13672.[69] Copyright [2015] American Chemical Society. Reproduced from Bag, B. G.; Majumdar, R. Self-assembly

of Renewable Nano-sized Triterpenoids. *Chem. Rec.* **2017**, *17*, 841–873.[54] Copyright [2017] Wiley-VCH Verlag, Germany.

Interestingly, **UA** self-assembled into tubular structures in an ethanol-water mixture, (Figure 3a). This finding suggests the possibility of extension from a 2D circular bilayer membrane to a 3D tubular structure.[66] In contrast to the triterpenoids mentioned above, **BT** yielded flower-like morphologies in organic solvents (Figure 3b). The results of a detailed investigation showed that 1D fibers formed by intermolecular H-bonding and dispersion interactions among the **BT** molecules resulted in the 3D flower-like structures *via* the 2D petals.[69] Such flower-like structures have also been observed for **GA**.[70] On the other hand, gels obtained from **BA** in organic liquids yielded densely packed fibrous networks (Figure 3c).[54,74]

Erythrodiol [**ED**; (3β)-3-olean-12-ene-3,28-diol; Figure 1] is a nanosized oleanane-type pentacyclic triterpenoid extractable from the dried leaves of olive (*Olea europea*) or obtained by a chemical reduction of **OA**. The self-assembly of **ED** nanosized fibrils (Figure 4a), microsized flowers, and grass-like architectures (Figure 4b) by a formation of densely assembled fibrils and petals or 2D sheets have been studied in different organic solvents and mixtures of aqueous and organic solvents.[72] The resulting porous self-assemblies possessed large surface areas, and showed their capability of adsorbing toxic fluorophores, e.g., rhodamine-B, rhodamine-6G, methylene blue, and crystal violet. Their ability in removing toxic pigments from aqueous solutions has been demonstrated by using UV-Vis spectrophotometry and epifluorescence microscopy.[72] Rhodamine-B was also used to prepare triterpenoid conjugates by covalently binding the dye; however, the self-assembly of the target products has not yet been studied.[75]

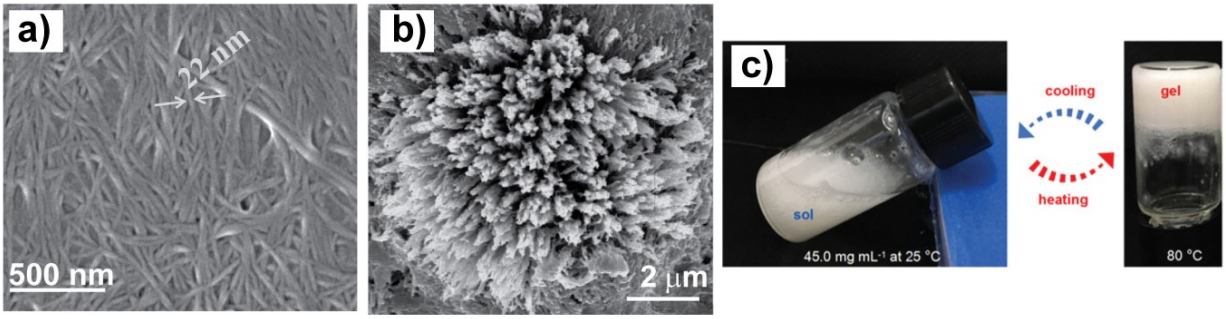

Figure 4. FESEM micrographs of dried nano-assemblies of **ED**. (a) nanofibers in chlorobenzene (1.5%, w/v); (b) grass-like architecture in *o*-dichlorobenzene (2%, w/v); (c) sol ⇔ gel transitions of [**OA**]-[choline] salt. Reproduced from Panja, S. K.; Bag, B. G. Flower- and Grass-like Self-Assemblies of an Oleanane-Type Triterpenoid Erythrodiol: Application in the Removal of Toxic Dye from Water. *ACS Omega*, **2020**, *5*, 30488–30494.[72] Copyright [2020] American Chemical Society. Reproduced from Fan, J.-P.; Zhong, H.; Zhang, X.-H.; Yuan, T.-T.; Chen, H.-P.; Peng, H.-L. Preparation and Characterization of Oleanolic Acid-Based Low-Molecular-Weight Supramolecular Hydrogels Induced by Heating. *ACS Appl. Mater. Interfaces* **2021**, *13*, 29130–29136.[79] Copyright [2021] American Chemical Society.

Gelation ability of salts obtained from triterpenoids was also investigated.[76-78] A simple [**OA**]-[choline] salt (structure is not shown) was prepared and studied as a hydrogelator to form a novel LMWG.[79] The authors found that the [**OA**]-[choline] salt showed unique characteristics in contrast to the common sol ⇔ gel transitions. This system undergoes a phase transition from the sol to the gel state upon heating (Figure 4c). Moreover, the phase separation was observed both, in the sol and the gel states, when the temperature increased, resulting in the irreversible transparent to turbid transitions. The gels showed excellent stability and injectability. They

displayed ability to become a drug delivery system for sustained release of drugs. In this regard, this work provided an efficient approach to designing the **OA**-based gelator for making heat-induced LMWGs.[79] The above studies suggest that small variations in the structure of triterpenoid molecules can alter their self-assembly properties.

Self-assembly of triterpenoids may also amplify their biological activities compared to the individual molecules. A recently published study revealed that self-assembled arjunolic acid (**AA**) can enter the cells and trigger the pathways that enhance production of reactive oxygen species (ROS), and cause cancer cell death.[80] In another study selective anticancer activity of self-assembled betulinic acid (**SA-BA**) in ethanol-water mixtures has been studied against acute (KG-1A) and chronic myeloid (K562) leukemia cell lines. The results revealed that **SA-BA** exhibits better anti-leukemic activity than **BA** itself.[81] The same group showed that a pre-treatment of peripheral blood lymphocytes with **SA-BA** prior to DOX treatment can alleviate the DOX induced inflammation, which occurs during a DOX-based chemotherapy in cancer patients.[82] In another study, ability of **SA-BA** to trigger both, the humoral and cellular immune responses were studied, and the results enriched its biomedical application as a potent immune stimulating agent.[83]

2.2. Synthetic derivatives of natural triterpenoids

Attempts to synthesize triterpenoid derivatives have mostly been towards their biological effects. Limited number of derivatives of triterpenoids have been studied in detail for functional organic nanomaterials. Existing studies dealing with derivatives of triterpenoids will be presented below.

Bag *et al.*[84] reported the synthesis and gelation of alkyl esters (**1a**–**1h**) and *p*-nitrophenyl methyl ester (**1i**) of **AA** (Figure 5) in several organic solvents. All derivatives were less polar than the parent **AA** that was not soluble in a majority of common organic solvents, with the exception involving polar protic solvents. Morphological characterization of self-assembled molecules using electron microscopy (EM), in native forms or as xerogels, revealed the formation of fibrous network and spherulitic type organizations. Importantly, amplification of chirality was also observed, and electron microscopy images displayed right-handed helicities (Figure 6a).[84] Bag *et al.*[85] also prepared aliphatic and aromatic ketals of **AA** (Figure 5), from which the only representative structures (**2a**–**2f**) are shown in Figure 5. The morphological studies carried out using optical, electron and atomic-force microscopies showed the presence of vesicles (Figure 6b) and fibers (Figure 6c) in various organic solvents at lower concentrations. It was deduced that the morphology of self-assembled structures depends on the ketal structures and the concentration of the gelator molecules. Rheological experiments showed that materials obtained are viscoelastic dispersions rather than strong gels. X-ray diffractograms revealed the crystalline structure of fibers.[85]

Inspired by the results obtained from esters and ketals of **AA**, Bag *et al.*[86,87] studied self-assembly characteristics of the ketal derivatives of methyl arjunolate (Figure 5). While benzylidene derivative **3b** exhibited poor gelation characteristics, the derivative **3a**, in which the C(2)-OH group was converted into the 2,4-dinitrobenzoate ester, was found to be an excellent gelator working at low concentration in a variety of solvents that include alcohols and various mixed solvents. Fibrous network along with cabbage leaf-like structures were detected by scanning electron microscopy (SEM).[86,87]

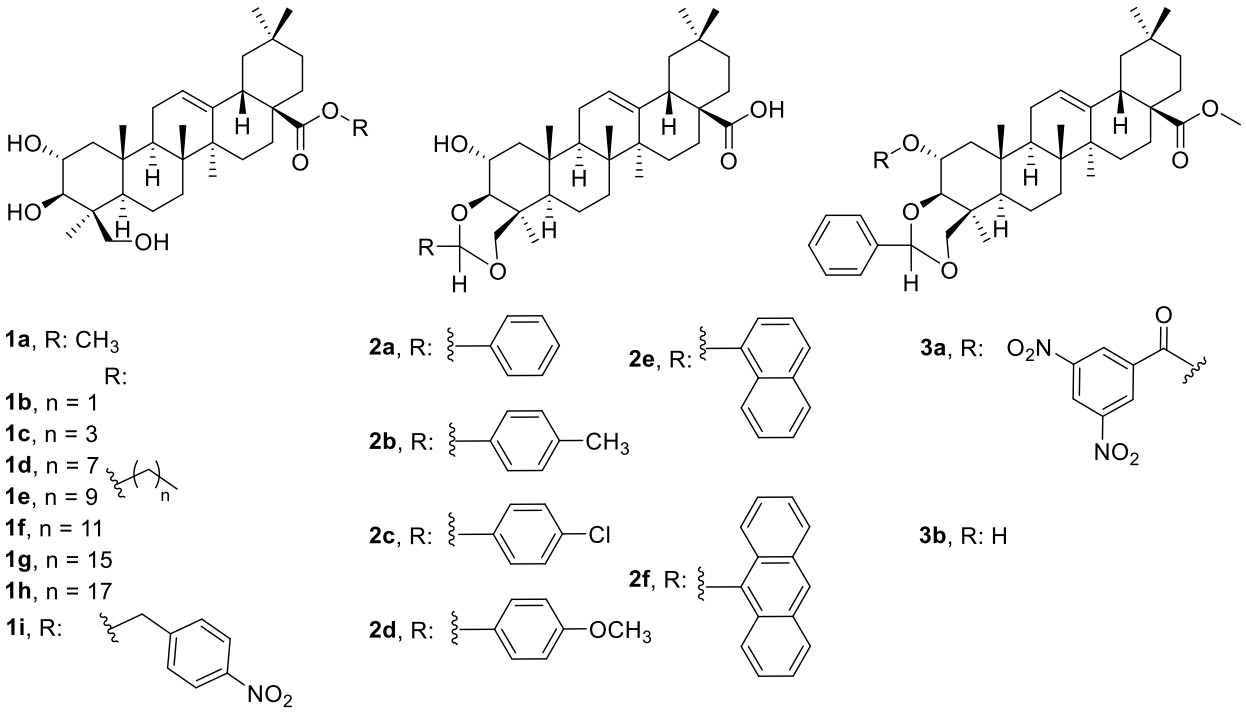

Figure 5. Chemical structures of arjunolic acid (**AA**) derivatives as potential gelators.

In addition to the **AA** derivatives, several reports about supramolecular properties of the **GA** derivatives (Figure 7) have also been reported in the literature. A fan-shaped C(3)-substituted molecule with three **GA** units (**4**) was synthesized and the self-assembly studies revealed that, while **4** can form organogel in a THF-methanol mixture through a formation of fibrous structures, solid spheres (Figure 6d) were observed in a THF-water mixture.[88]

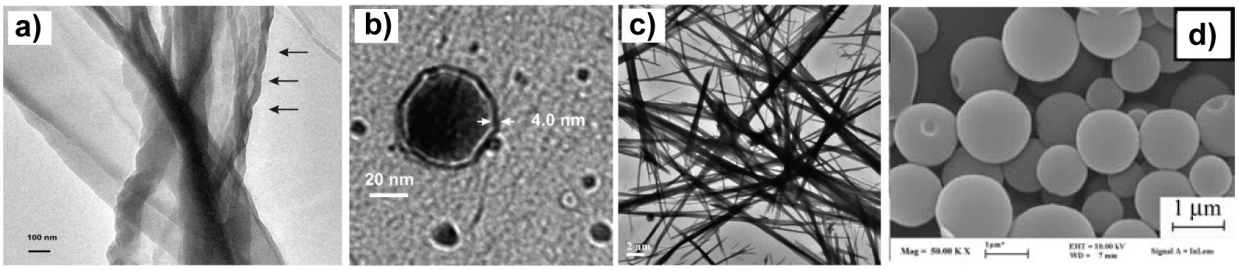

Figure 6. (a) TEM micrographs of xerogels of (a) **1i** (Figure 1) in toluene (2.1%, w/v); (b) giant vesicle of **2a** (Figure 1) in *o*-xylene (0.34%, w/v) without staining but showing the membrane thickness; (c) **2e** (Figure 1) in *m*-xylene (2.5% w/v); (d) SEM image of **4** (Figure 7) in THF-water (3:1, v/v). Reproduced from Bag, B. G.; Dinda, S. K.; Dey, P. P.; Mallia, V. A.; Weiss, R. G. Self-Assembly of Esters of Arjunolic Acid into Fibrous Networks and the Properties of their Organogels. *Langmuir* **2009**, *25*, 8663–8671.[84] Copyright [2009] American Chemical Society. Reproduced from Bag, B. G.; Majumdar, R.; Dinda, S. K.; Dey, P. P.; Maity, G. C.; Mallia, V. A.; Weiss, R. G. Self-Assembly of Ketals of Arjunolic Acid into Vesicles and Fibers Yielding Gel-Like Dispersions. *Langmuir* **2013**, *29*, 1766–1778.[85] Copyright [2013] American Chemical Society. Reproduced from Hu, J.; Yu, L.; Zhang, M.; Ju, Y. Synthesis of Fan-Shaped C3 Molecule with Three Glycyrrhetinic Acid Units and Self-Assembly Properties. *Chinese J. Chem.* **2011**, *29*, 1139–1142.[88] Copyright [2011] Wiley-VCH Verlag, Germany.

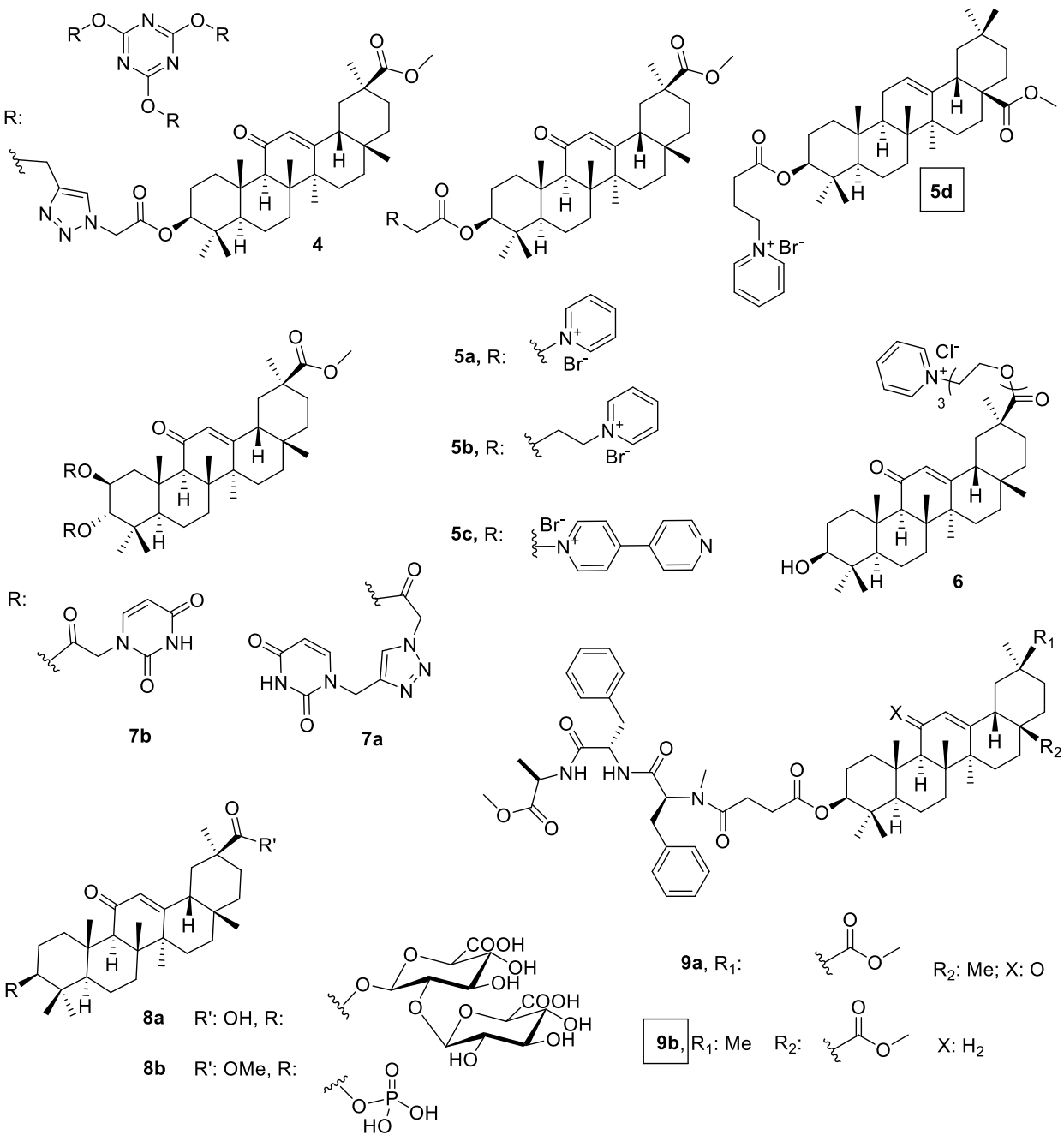

Figure 7. Chemical structures of **GA** and **OA** (numbers in squares) derivatives as potential gelators.

Assemblies of pyridinium functionalized methyl glycyrrhetate amphiphiles (**5a**–**5c**) (Figure 7) and methyl oleanolate amphiphile (**5d**) (Figure 7) were investigated by Gao *et al.*[89] Compound **5a** formed a transparent gel, by entanglement of nanofibers, in mixtures of chloroform and aromatic solvents. It was revealed by using many techniques that a gel formation was controlled by a combination of π–π stacking, van der Waals forces, and hydrophobic interactions. The handedness of helices were different in each solvent system for **5a**. Right-handed helices were formed by **5b** in chloroform/*o*-xylene mixture (Figure 8a). When the linker was elongated, **5c** assembled into well-defined left-handed helical nanofibers in a chloroform/toluene mixture (Figure 8b), whereas **5d** (**OA** derivative) formed left-handed helical ribbons in the methanol/water mixtures (Figure 8c).[89] A formation of chiral helical ribbons in water and ability of these ribbons to act as a droplet rebound inhibitor on a hydrophobic surface, regardless of incline angle of substrate were also demonstrated by the same group.[43] In addition to this work, Gao *et al.*[90] presented a preparation of a triterpenoid-based hydrogel in a physiological phosphate buffered saline (PBS). The analysis carried out indicated that π–π stacking, electrostatic interactions, van der Waals forces, and hydrophobic interactions led to a hydrogel formation of **6** (Figure 7) in PBS. The gelator **6** did not show cytotoxicity to the 3T3-L1 fibroblast cells and was able to encapsulate and release DOX under physiological conditions without altering packing pattern of the hydrogel.[90]

Compound **7a** (Figure 7) was proven to form gels in various solvents through a formation of fibrous structures. On the contrary to **7a**, compound **7b** (without 1,4-disubstituted 1,2,3-triazole ring; Figure 7) displayed no gelation abilities showing a role of π-π interaction between 1,2,3-triazoles in a formation of the gel besides intermolecular hydrogen bonding. Upon addition of

Hg^{2+} or F^- ions, a gel ⇔ sol phase transition was observed, which led to a destruction of the π-π interaction between 1,4-disubstituted 1,2,3-triazole rings or intermolecular hydrogen bonding.[91]

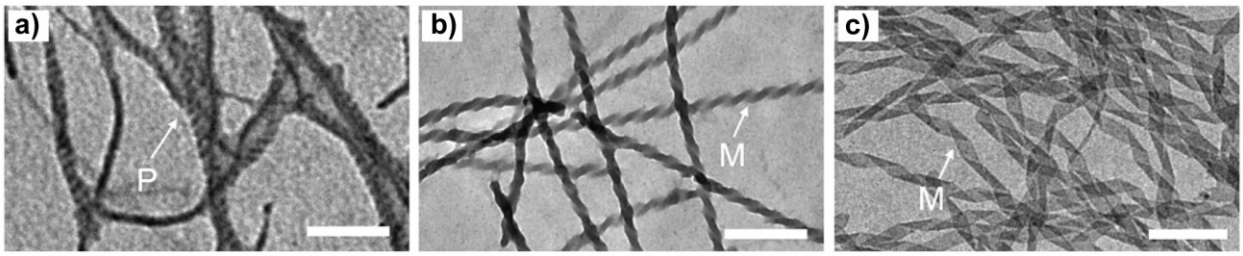

Figure 8. TEM images of triterpenoid-tailored pyridinium amphiphiles in different solvents. (a) **5b** chloroform/*o*-xylene (13 mM, 1:4, v/v); (b) **5c** chloroform/toluene (11 mM, 5:8, v/v); (c) **5d** methanol/water (7 mM, 1:2, v/v). Scale bars are 200 nm for (a), 300 nm for (b), and 2 μm for (c). Reproduced from Gao, Y.; Hao, J.; Wu, J.; Zhang, X.; Hu, J.; Ju, Y. Supramolecular Helical Nanofibers Assembled from a Pyridinium-Functionalized Methyl Glycyrrhetate Amphiphile. *Nanoscale* **2015**, *7*, 13568–13575.[89] Copyright [2015] Royal Society of Chemistry.

In 2015, the gelation and ultralong chiral nanofibril formation properties of glycyrrhizic acid was reported (**8a**, Figure 7).[92] The nanofibrils were also investigated as scaffolds for functional hybrids by incorporating graphene oxide and *in situ* formed gold nanoparticles. The resulting hybrid nanomaterial was studied for heterogeneous catalysis for the reduction of *p*-nitrophenol to *p*-aminophenol.[92] It was observed that the compound **8a** formed a stable and transparent hydrogel through nanoclusters in physiological phosphate buffered saline at 37 °C (Figure 9a and 9b). Moreover, this hydrogel showed acceptable injectability and moldability. The investigation

of antibacterial activity showed that **8a** inhibited completely the growth of Gram-positive *Staphylococcus aureus*, whereas, in turn, the same **8a** showed no effect on Gram-negative *Escherichia coli* (Figure 9c). In addition, cell viability and hemolysis assay resulted in a finding that **8a** displayed high biocompatibility and hemocompatibility to mammalian cells because of its natural origin. A simple and biocompatible, injectable and moldable hydrogel with inherent antibacterial ability has always a great impact in the area of biomaterials and 3D bioprinting.[93]

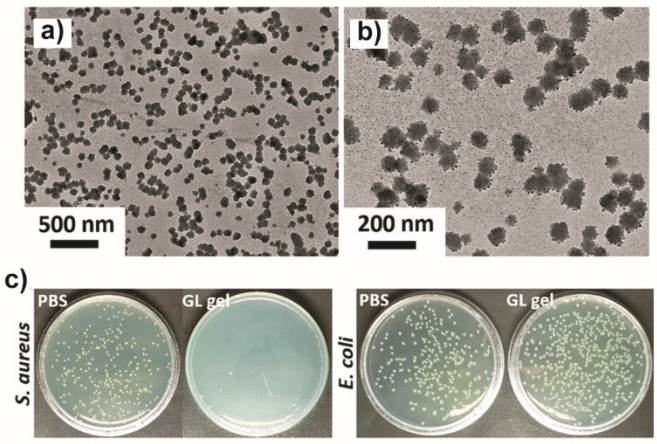

Figure 9. (a) and (b) TEM images of hydrogel of **8a** in PBS (10 mM, pH 7.4); (c) photographs of bacterial colonies formed by *S. aureus* and *E. coli* after treatment with **8a** at 1.5 mM (0.13 wt %). Reproduced from Zhao, X.; Zhang, H.; Gao, Y.; Lin, Y.; Hu, J. A Simple Injectable Moldable Hydrogel Assembled from Natural Glycyrrhizic Acid with Inherent Antibacterial Activity. *ACS Appl. Bio Mater.* **2020**, *3*, 648–653.[93] Copyright [2020] American Chemical Society.

Another derivative of **8a**, methyl glycyrrhetate phosphate (**8b**, Figure 7) has been designed and synthesized.[94] The results showed that **8b** forms stable hydrogel capable of removing gold (Au)

salt from water (cf. Figure 10a), followed by a spontaneous formation of gold nanoparticle (AuNP) *in situ*, nucleated by nanofibers without external reductants. The AuNPs were mainly found on the surface of the gel fibers (Figure 10b and 10c). The resulting hybrid gel exhibited emerging electrocatalytic and conductive properties. In addition, **8b** showed higher affinity towards heavy metals over other common metals as an efficient leaching extraction agent. Moreover, the compound displayed no obvious toxicity, which indicates its ability in converting heavy metals into electrochemical hybrid gels in a single step. Due to the existence of reductive diglucuronic unit in the molecule of **8b**, the compound is capable of spontaneous reducing heavy metal ions to metal nanoparticles without any additional external reducing agent.[94]

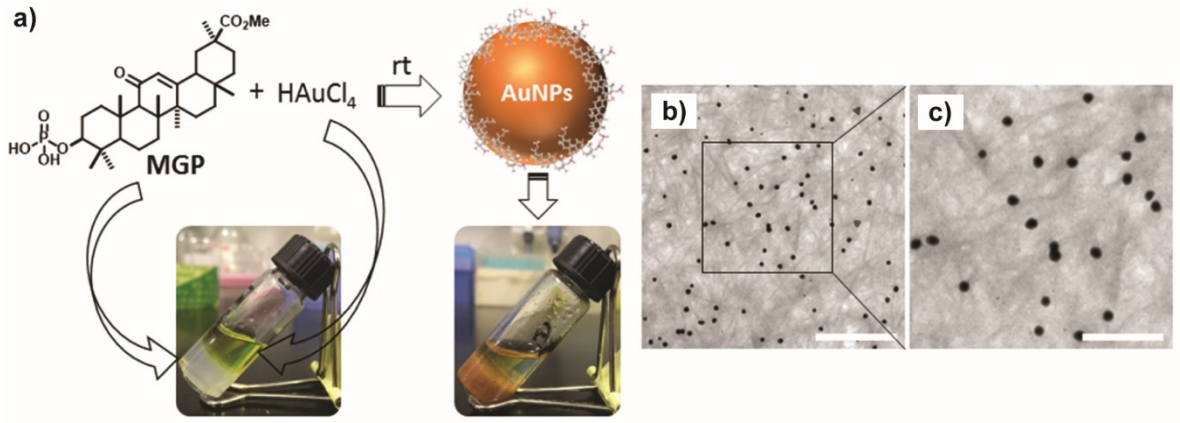

Figure 10. (a) Schematic illustration of the spontaneous reduction of gold metal ions *in situ* on hydrogel of **8b**, along with representative photographs of gels; (b) TEM images of **8b**−AuNPs hybrid gel; (c) enlarged area of TEM image displayed in (b). Scale bars are 500 nm for (b), and 200 nm for (c). Reproduced from Gao, Y.; Hao, J.; Yan, Q.; Du, F.; Ju, Y.; Hu, J., Natural Triterpenoid-Tailored Phosphate: In Situ Reduction of Heavy Metals Spontaneously to Generate Electrochemical Hybrid Gels. *ACS Appl. Mater. Interfaces* **2018**, *10*, 17352–17358.[94] Copyright [2018] American Chemical Society.

The **OA** and **GA** peptide conjugates (**9a** and **9b**; Figure 7) have been synthesized and have shown to form organogels in several aromatic solvents by the hydrogen bonding and by the van der Waals interactions. They are capable of gelating selectively aromatic solvents from water (phase selective gelation). The prepared xerogels were able to absorb the cationic dyes from water (Figure 11).[95]

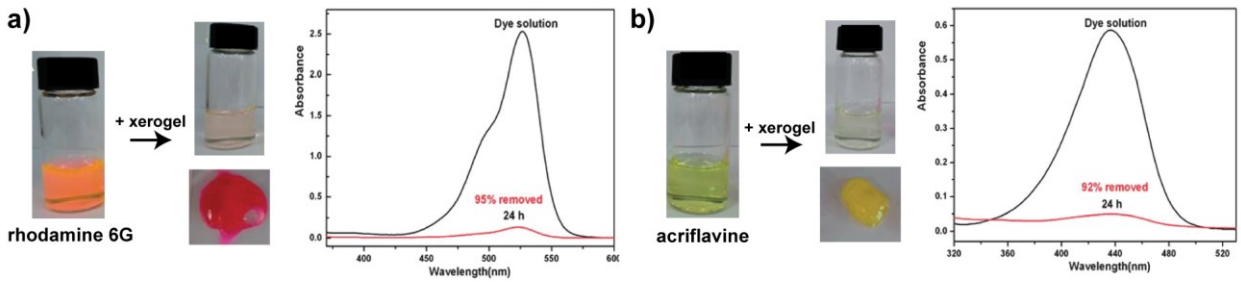

Figure 11. Photographs and the UV spectra of (a) rhodamine 6G and (b) acriflavine solutions before and after dye absorptions with xerogel of **9b**. Reproduced from Lu, J.; Gao, Y.; Wu, J.; Ju, Y. Organogels of Triterpenoid–Tripeptide Conjugates: Encapsulation of Dye Molecules and Basicity Increase Associated with Aggregation. *RSC Adv.* **2013**, *3*, 23548–23552.[95] Copyright [2013] Royal Society of Chemistry.

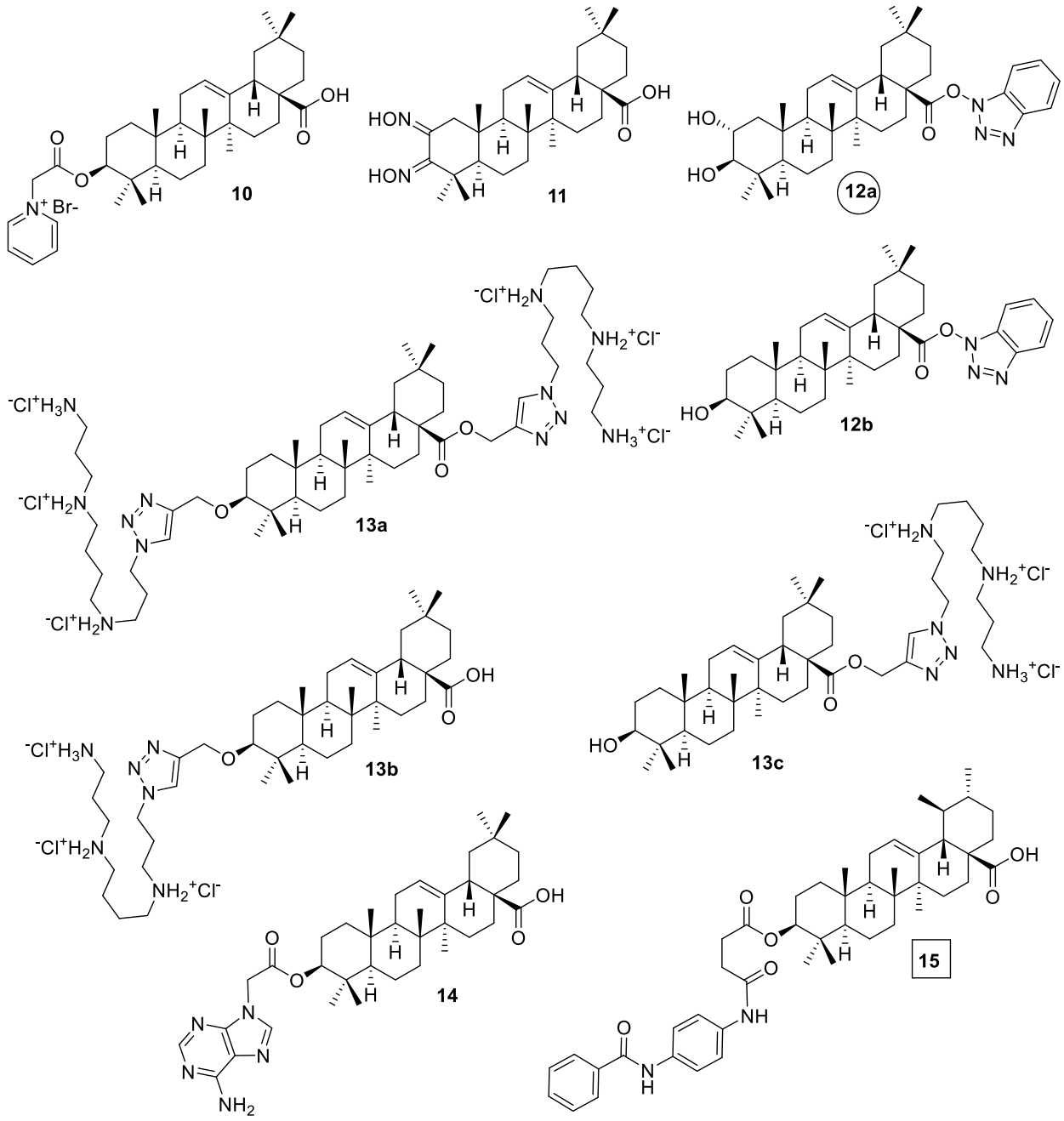

Figure 12. Chemical structures of **OA, MA** (number in circle) and **UA** (number in square) derivatives as potential gelators.

The effect of solvent polarity and solvent composition on morphology of self-assembled structures was studied with pyridinium-tailored oleanolate amphiphile (**10**; Figure 12). The stepwise assembly of **10** from spherical nanoparticles (in chloroform) to an opaque gel with rigid microrods (in non-polar solvents; chloroform/*p*-xylene mixtures; Figure 13a-13d), as well as from irregular nanoparticles (in methanol) to a transparent gel with flexible nanofibers in polar solvents (methanol/water mixtures; Figure 13e-13h) were achieved.[96]

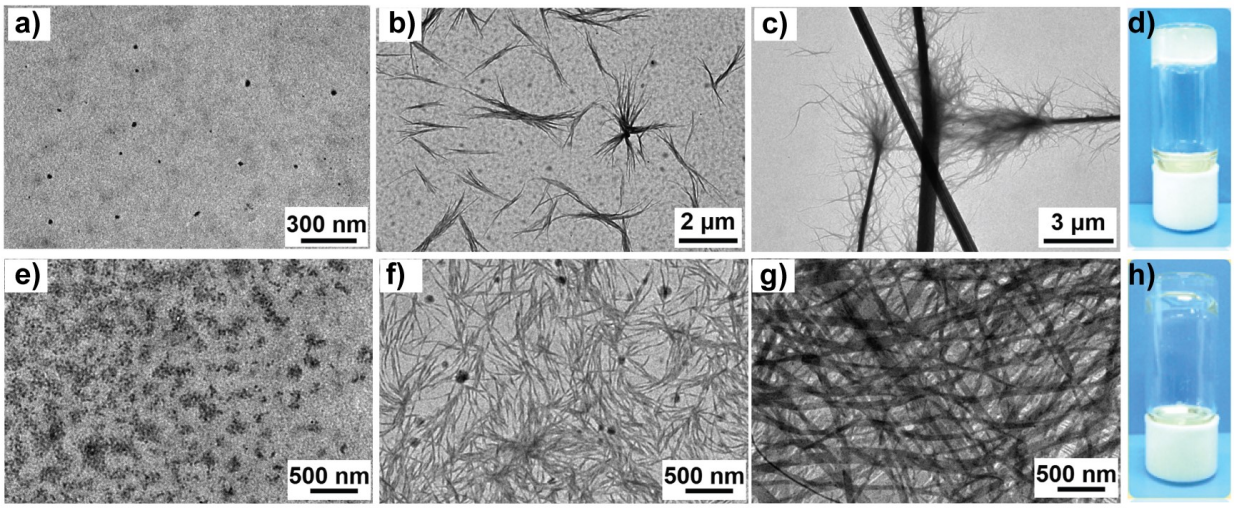

Figure 13. TEM images of assemblies of **10** (7 mM) at different volume ratios of chloroform/*p*-xylene mixtures: (a) 2:0; (b) 2:1; (c) 2:3 and (d) a picture of the prepared gel. TEM images of assemblies of **10** (7 mM) at different volume ratios of methanol and water mixtures: (e) 2:0; (f) 2:1; (g) 2:4 and (h) a picture of prepared gel. Reproduced from Gao, Y.; Hao, J.; Wu, J.; Zhang, X.; Hu, J.; Ju, Y. Solvent-Directed Assembly of a Pyridinium-Tailored Methyl Oleanolate Amphiphile: Stepwise Growth of Microrods and Nanofibers. *Langmuir* **2016**, *32*, 1685–1692.[96] Copyright [2016] American Chemical Society.

Compound **11** (Figure 12) has been reported to form organogels in various solvent. Conjugates obtained by methylation of carboxylic acid group or acetylation of oximes formed no gel, thus proved the necessity of a presence of both -COOH and -OH groups in the structure of the gelator to keep the gelation ability of the compound.[97]

In another study, two new **MA** and **OA** derivatives **12a** and **12b** (Figure 12) have been studied for their ability to develop nano-assemblies in the forms of long fibers, finally resulting in obtaining homogeneous gels.[98] Quite remarkably, these molecules were able to form gels by a slow catching of atmospheric water from their DMSO and DMF solutions. This mechanism of a gel formation has not been previously reported for other LMWGs, and it points out the generality of these compounds to form gels in a response to different external stimuli. The microscopic and macroscopic characteristics of the resulting gels were studied in detail by TEM, SEM, NMR, VCD, FTIR, XRD and rheology.[98] This investigation demonstrated that gels prepared by catching atmospheric water in DMSO were more homogeneous and more hardened than those obtained by a direct addition of water to the organic solution.[98]

Recently, we reported the rational design of three **OA**−triazole−spermine conjugates (**13a**–**13c**; Figure 12). The conjugates bear either one or two spermine units in the target molecules.[99] The Cu(I)-catalyzed Huisgen 1,3-dipolar cycloaddition reaction was applied during their synthesis to introduce 1,2,3-triazole rings to the molecules.[99] The resulting amphiphile-like molecules **13b** and **13c**, bearing just one spermine unit in each respective molecule, were found to self-assemble into highly entangled fibrous networks. Gelation was observed at a concentration as low as 0.5 % in alcoholic solvents (Figure 14a-14c). Using step-strain rheological measurements, rapid self-recovery, up to 96% of the initial storage modulus, and sol ⇔ gel transition under several cycles was described (Figure 14d). An important finding is that rheological flow curves revealed the

thixotropic behavior of the gels (Figure 14e). This kind of behavior had not been shown in the literature before, neither with any natural triterpenoid nor with the semi-synthetic triterpenoid derivatives. The conjugate **13a** (Figure 12) showed a bolaamphiphile-like structure, and it was found to be a non-gelator. The results indicated that the position and number of spermine units modify the gelation characteristics, gel strength, and their self-assembly behavior. Preliminary cytotoxicity studies of the target compounds **13a**–**13c** in four human cancer cell lines indicated that the position and number of spermine units affected the biological activity of the target compounds.[99]

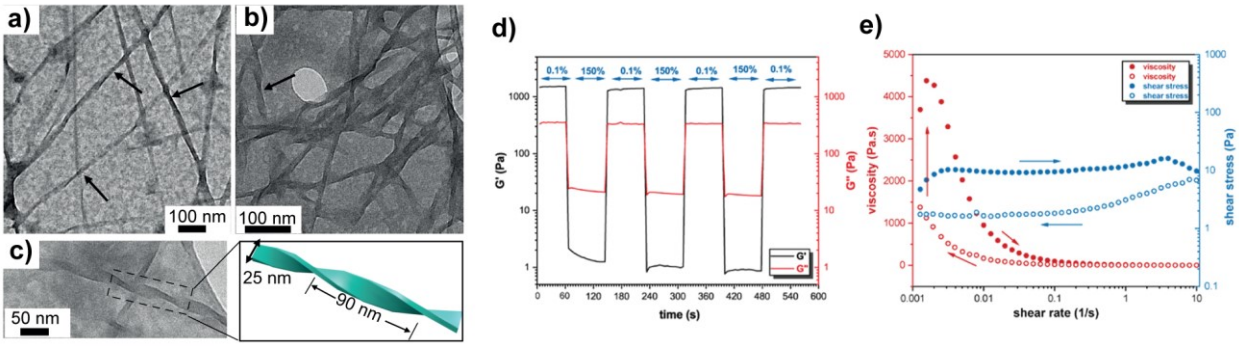

Figure 14. Transmission electron microscopy. (a) TEM micrographs of 1.0 % 1-propanol gel of **13b**; (b) TEM micrographs of 1.0 % 1-heptanol gel of **13c**; (c) a single fiber with helical twist is shown (left) together with its graphical representation (right); (d) step-strain experiments showing self-healing properties of the 1-butanol gel of **13b**; (e) flow tests showing viscosity *vs.* the shear rate, and shear stress *vs.* the shear rate for the 2-propanol gel of **13b**. Reproduced from Özdemir, Z.; Šaman, D.; Bertula, K.; Lahtinen, M.; Bednárová, L.; Pazderková, M.; Rárová, L.; Nonappa; Wimmer, Z. Rapid Self-Healing and Thixotropic Organogelation of Amphiphilic

Oleanolic Acid−Spermine Conjugates. *Langmuir* **2021**, *37*, 2693–2706.[99] Copyright [2021] American Chemical Society.

Even more recently, we have studied self-assembly of compounds **13a**–**13c** in aqueous media.[100] Using cryogenic transmission electron microscopy (cryo-TEM) imaging, we showed that the arrangement of lipophilic core and polar side chains leads to the multifaceted self-aggregated structures. The conjugates **13b** and **13c** self-assembled in water initially into kinetically favored metastable micellar nanoparticles (d ~ 6–10 nm). The nanoparticles further reorganized to form thermodynamically stable helical nanofibers (Figure 15b and 15c). Notably, cryo-TEM imaging also suggested the presence of the 2D sheet-like structures and intermediate spherulites. Micelles formed by **13a** did not transfer into any other supramolecular structure (Figure 15a). Infrared (IR) spectroscopy suggested that initial micelle-like structures underwent hydration and their aging induced structural transition into thermodynamically stable nanofibers was accompanied by dehydration. The self-assembly–induced amplification of chirality in a solution was monitored using electronic and vibrational circular dichroism (ECD and VCD) spectroscopy. The variable concentration (VC) and variable temperature (VT) ^{1}H and DOSY NMR spectroscopy provided further evidence on the aggregation of amphiphiles in D_2O.[100] In addition, all three investigated compounds exhibited high antimicrobial activity against a number of G^+ bacteria (MIC < 1.54 $\mu g \cdot mL^{-1}$),[100] encouraging their possible incorporation in high touch surface coatings to reduce expanding of the infectious agents.

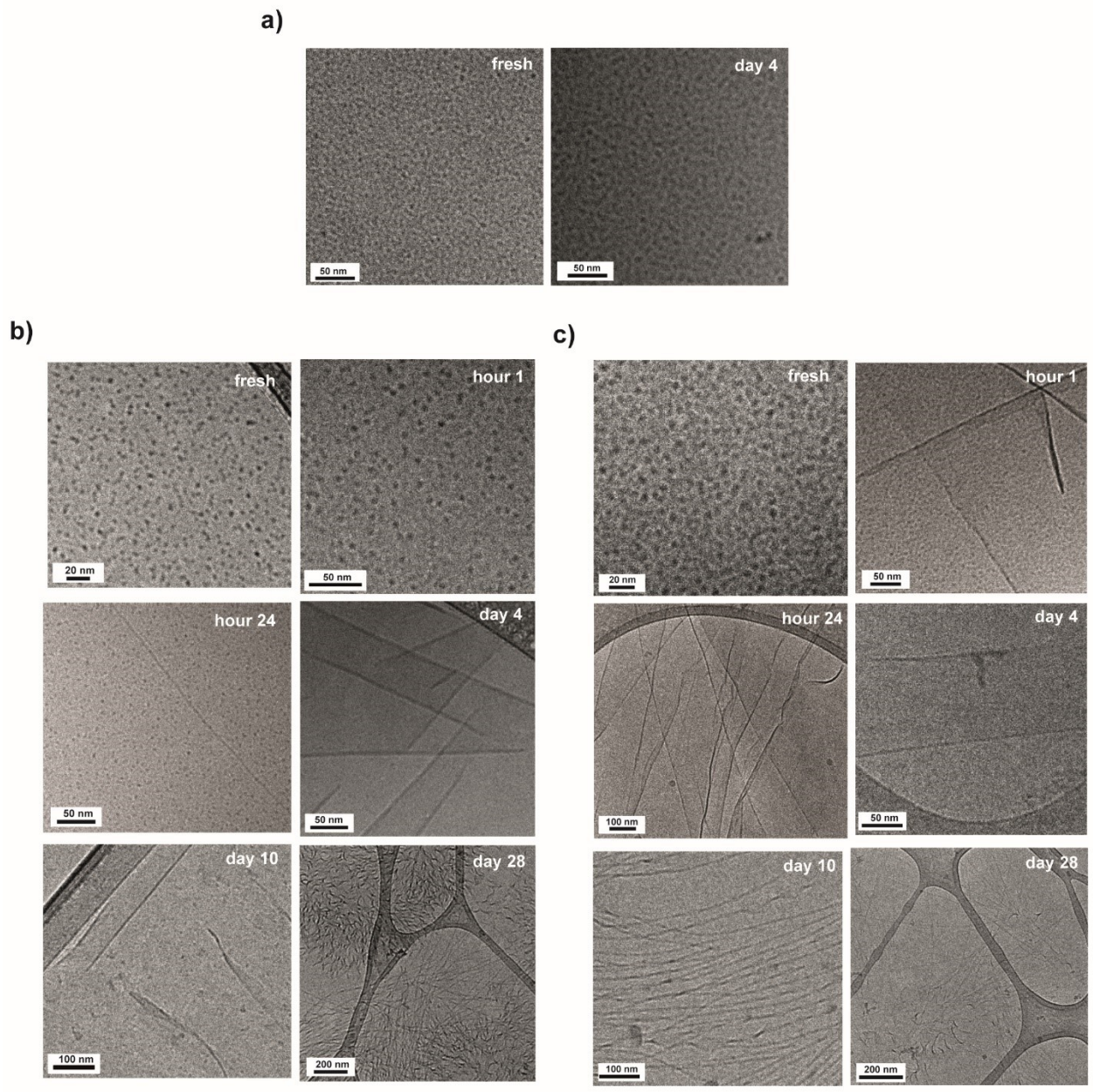

Figure 15: Cryo-TEM imaging and structural transition. (a) freshly prepared aqueous dispersion of **13a** (1.0 %) and after 4 days; (b) Freshly prepared aqueous dispersion of **13b** (1.0 %), after 1 h, after 24 h, after 4 days, after 10 days and after 28 days; (c) freshly prepared aqueous dispersion of **13c** (1.0 %) after 1 h, after 24 h, after 4 days, after 10 days and after 28 days. Reproduced from Özdemir, Z.; Šaman, D.; Bednárová, L.; Pazderková, M.; Janovská, L.; Nonappa; Wimmer, Z. Aging-Induced Structural Transition of Nanoscale Oleanolic Acid

Amphiphiles and Selectivity Against Gram-Positive Bacteria. *ACS Appl. Nano Mater.* **2022**, *5*, 3799–3810.[100] Copyright [2022] American Chemical Society.

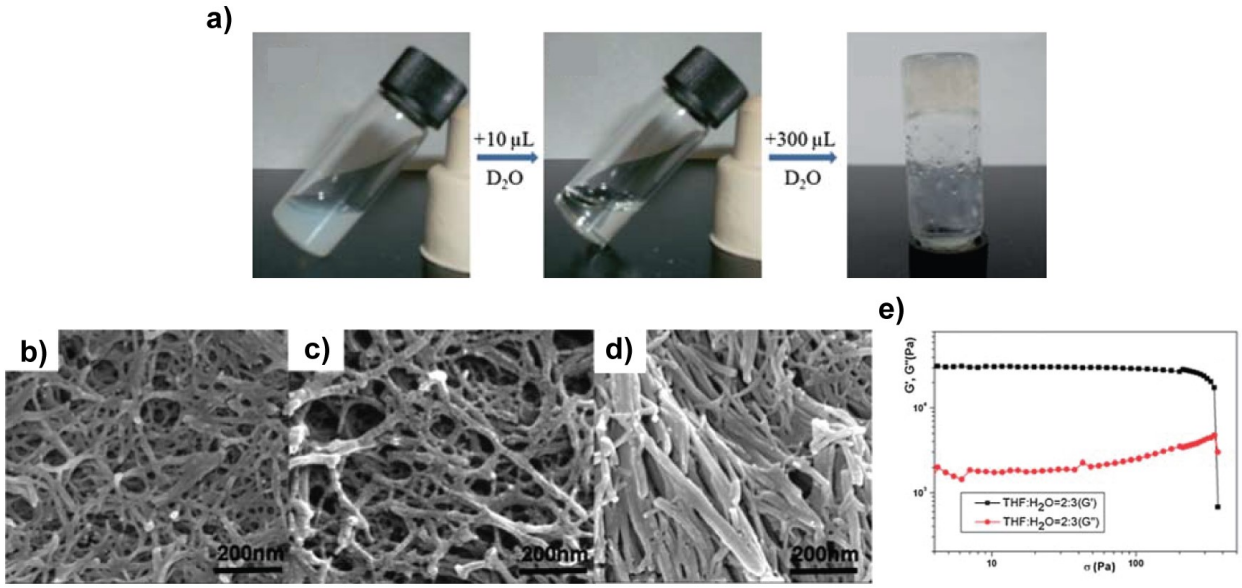

Figure 16. (a) The process of a gel formation in THF after addition of water, picture at left: Turbid solution of **14** in THF-*d8* (10 mg / 0.5 mL), picture at center: clear solution of **14** in THF-*d8* with 10 µL D_2O, picture at right: opaque gel in THF-*d8* with 300 µL D_2O. SEM micrographs of gels from **14** in (b) THF/H_2O = 2/1, (c) THF/H_2O = 1/1 and (d) THF/H_2O = 2/3; (e) Evolution of G' and G" of the gel as a function of the applied shear stress. Reproduced from Lu, J.; Hu, J.; Liu, C.; Gao, H.; Ju, Y. Water-Induced Gel Formation of an Oleanolic Acid–Adenine Conjugate and the Effects of Uracil Derivative on the Gel Stability. *Soft Matter* **2012**, *8*, 9576–9580.[101] Copyright [2012] Royal Society of Chemistry.

A comparison of investigations made with the compounds **13a**–**13c** in organic and aqueous media clearly indicated the differences in the found characteristics of the self-assembled systems.[99,100] Amphiphilic structures display enormous potential for the future investigation of their characteristics and for their potential application namely in pharmacology and medicine.

The **OA**-adenine conjugate (**14**; Figure 12) self-assembled into the fibrous networks to form gel in THF by addition of water (Figure 16a), and a ratio of THF/D_2O affected stiffness of the obtained gel (Figure 16a-16e).[101]

Self-assembly study of the **UA** derivative (**15**; Figure 12), in which benzene groups were conjugated to the **UA** through the amide bonds showed a gelation in *p*-xylene through a combination of hydrogen bonding, π-π stacking and van der Waals forces.[102]

3. Conclusion

In this review, we summarized the recent results and advance in investigation of triterpenoid-based supramolecular gels that displayed high biocompatibility, outstanding loading capacity, and long intracellular retention. The gels showed advantages in enhancing the effect of the drugs. The monomeric drugs were either included into the gels, or the monomeric structures formed the gels themselves and displayed biological effects. The gelators can be smartly activated by certain environmental stimuli, and spontaneously self-assemble into nanofibers or other nano-assemblies, and then form gels (Table 1). Advances achieved in recent years in the area of creation of supramolecular materials have been challenging for developing controlled release depots, which display ability in designing agents capable of responding to a specific biomarker

or disease indicator. The specific drug molecule may itself represent a useful supramolecular motif provided that the chemical structure is able to contribute to the intermolecular interactions that enable a formation of a supramolecular material. These materials may evoke drug-like functions, either through therapeutic biomimicry or through bioactivity associated with their structure. Finally, there are important challenges in using modular design to enrich targeting units onto the drug carriers. Thus, another important challenge in investigation of supramolecular design in creating new therapeutics has been envisaged for the continuing emergence of technologies based on these design principles in the coming years.

Table 1. Nano-assemblies formed by natural triterpenoids and their synthetic derivatives reviewed in this paper.

Natural triterpenoids				
Compound	Solvent	Observed morphology	Applications	Reference
UA, **MA**, **CA**, **AA**	aqueous binary mixtures	bilayer vesicular self-assemblies	removal of dye, drug delivery,	64, 66, 67, 68
UA	EtOH-H_2O mixtures	tubular self-assemblies		66
BT, **GA**, **ED**	organic solvents	flower-like assemblies		69, 70, 72
BA	organic solvents	fibrous assemblies		74
Synthetic derivatives of natural triterpenoids				
1i	organic solvents	helical nano-assemblies		84
2a	organic solvents	giant vesicular self-assemblies		85
2e	organic solvents	fibrous		85

		assemblies		
2d	organic solvents	giant vesicular self-assemblies		85
4	THF / water mixture	micelles		88
5b, **5c**, **5d**	organic solvents	helical nano-assemblies		89
8a, 8b	water	bilayer vesicular self-assemblies	coordination of metals	93
10	organic solvents	fibrous assemblies		96
13b, **13c**	organic solvents	helical nano-assemblies		99
13a, **13b**	water	aging, from micelles to fibrous assemblies and to plates		100
14	organic solvents	fibrous assemblies		101

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The manuscript was written through the contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

DOX, doxorubicin; LMWG, low-molecular-weight gelator; SEM, scanning electron microscopy; TEM, transmission electron microscopy; VCD, vibrational circular dichroism; XRD, X-ray diffraction.

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