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Discovery of FZU-03,010 as a self-assembling anticancer amphiphile for acute myeloid leukemia

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ABSTRACT

Recently various drug candidates with excellent anticancer potency have been demonstrated, whereas their clinical application largely suffers from several limitations especially poor solubility. Ursolic acid (UA) as one of ubiquitous pentacyclic triterpenes in plant kingdom exhibited versatile antiproliferative effects in various cancer cell lines. However, the unfavorable pharmaceutical properties became the main obstacle for its clinical development. With the aim of development of novel derivatives with enhanced potency, a series of diversified UA amphiphiles have been designed, synthesized, and pharmacologically evaluated. Amphiphile **10** (**FZU-03,010**) with significant improved antiproliferative effect can self-assemble into stable nanoparticles in water, which may serve as a promising candidate for further development.

Recently, natural products attract increasing attention in academic institutions and pharmaceutical industry due to their unparalleled resources, and diverse interesting biological characteristics.[1–3] Among them, pentacyclic triterpene compounds widely exist throughout nature, possessing considerable pharmacological activities, especially anticancer potency.[4,5] Therefore, they are expected to serve as a pivotal resource to develop novel promising drug candidates targeting diverse signaling pathways.[6,7] Ursolic acid (UA, Fig. 1) as one of the pentacyclic triterpenes is ubiquitous in plant kingdom.[8] UA is particularly valuable for its versatile bioactivities including anti-inflammatory, antioxidative, anticancer, antibacterial, and sedative activities.[9] Its significant anticancer and fascinating chemoprevention effects have been intensively studied. Recent studies revealed that UA is involved in a series of the activities of anticancer, such as induction of cancer cell apoptosis, prevention of tumorigenesis, and inhibition of cancer cell proliferation.[10] In addition, UA has been demonstrated to significantly enhance immune function of human body.[11] All these findings indicate that UA has great potential for clinical application. However, UA suffers from poor water solubility, rapid metabolism and low bioavailability, limiting its further clinical development.[12,13]

Despite these limitations, UA possesses the common structural characteristic of the plant-derived triterpenoids bearing two polar groups separated by a non-polar spacer of appropriate length.[14] This inherent amphiphilic feature renders it as one of the ideal building blocks for development of self-delivering amphiphiles with improved anticancer potency through self-assembly. Due to the diversity spacers and unique spatial structure arrangements, Bag, Ju and Hu, as well as Mezzenga studied the assembly behaviors of triterpenoids especially focusing on supramolecular recognition, assembly and diverse supramolecular architectures (including fibers, vesicles, flowers, helices, and spheres).[15–17] For example, Ju reported a novel uracil-appended glycyrrhetinic acid conjugate and fully investigated its gelation characteristics as well as the gel-to-sol phase transition process.[17]

Recently, construction of complex amphiphilic drug–drug conjugates (ADDCs) is considered as an emerging strategy for the enhancement of therapeutic efficacy *via* simple conjugation of a hydrophilic anticancer drug with a hydrophobic one through a biodegradable bond.[18] For example, Yan and Zhu synthesized Ir–Cb conjugate consisting of the hydrophilic anticancer drug irinotecan (Ir) and the hydrophobic anticancer drug chlorambucil (Cb) via a hydrolyzable ester linkage.[19] This complex conjugate could self-assemble into nanoparticles in water and facilitated the accumulation of drugs in tumor tissues, eventually resulting in significant improvement of therapeutic efficacy.[19]

Owing to the unique properties of self-assembled nanoparticles and higher accumulation in tumors *via* the enhanced permeation

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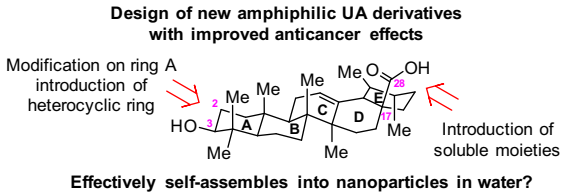


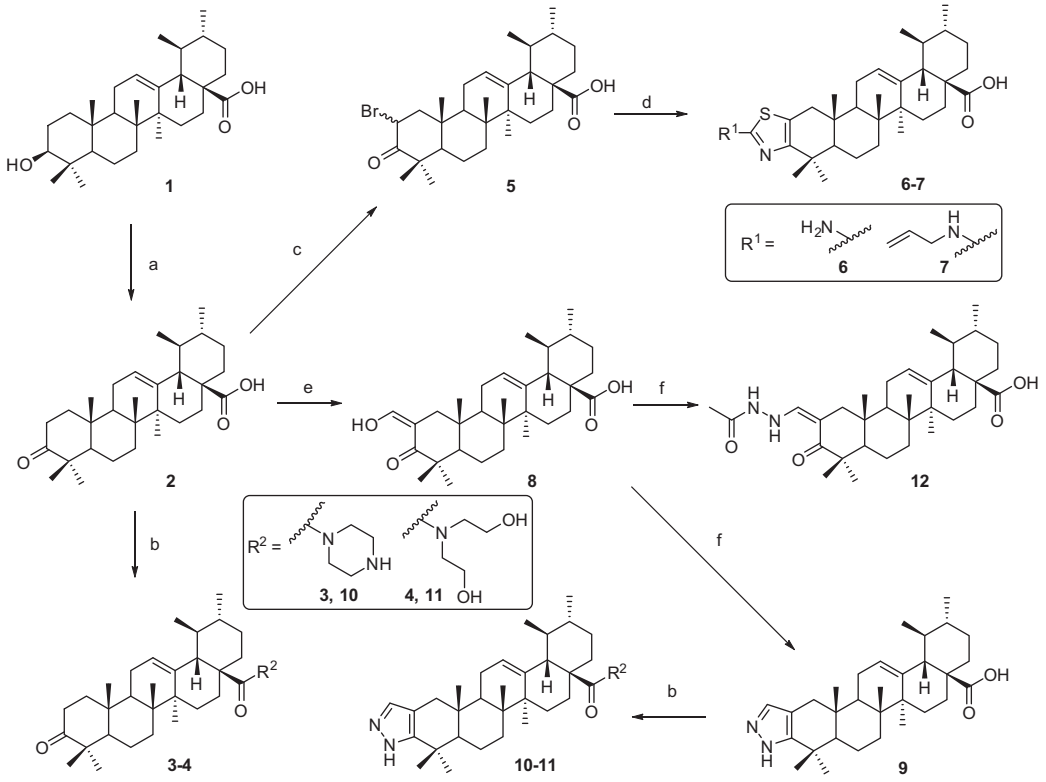
Fig. 1. Drug design self-delivery strategy for new amphiphilic UA derivatives with improved anticancer effects.

and retention (EPR) effect, amphiphiles with improved anticancer potency can enhance permeability and reduce side effects.[18] The skeleton of UA is lined with readily accessible A ring and carboxyl group at the position 28, which allows for the facile introduction of functional moieties to promote reversible and directional interactions and engender supramolecular assemblies. With these considerations in mind, we hypothesized that amphiphilic UA derivatives consisting of the hydrophilic privilege fragments around A-ring and hydrophobic tail at the 28-position might solve the limitation of poor solubility of UA.

In this work, we designed and synthesized a series of novel amphiphilic UA derivatives with favorable physicochemical properties which were associated with incorporating nitrogen into the core scaffold and evaluated their anticancer activities. Among them, UA derivative **10** (**FZU-03,010**) could be a promising anticancer agent for its highly inhibitory activity against human leukemia cells. To our knowledge, such a promising strategy through development of amphiphilic UA derivatives to self-assemble into its own nanostructures for the enhancement of anticancer efficacy has not been reported to date.

Intensive researches demonstrated that various biological properties of pentacyclic triterpenes are related to the C-3 hydroxyl group.[20–23] In this respect, a variety of structure modifications of UA on C-3 position have been conducted to improve the biological activity.[24–26] The heterocyclic ring derivatives have been proved possess highly anticancer effects. To date, increasing studies have demonstrated that a variety of UA derivatives with structural modifications on A ring possess effective improvement of anticancer effects.[27,28] Accumulating investigation revealed that pentacyclic triterpenes with thiazole and pyrazole moiety at A-ring are expected to possess enhanced anticancer potency.[29,30] In addition, accumulating evidence indicated that the introduction of suitable moieties at the 28-position of UA would significantly enhance the anticancer activity.[31,32] Therefore, our efforts first focused on modification at C-2 and C-3 positions followed by introduction of the privileged fragments at the 28-position (Fig. 1) to synthesize amphiphilic UA derivatives.

The synthetic routes to new UA derivatives reported in this work are outlined in Scheme 1. Following the literature procedures, the key intermediate 3-keto UA (**2**) was prepared by the oxidation of UA (**1**) with Jones reagent in 90% yield. The compounds **3** and **4** were obtained in a two-step synthesis starting from 3-keto UA (**2**). The C-28 chloride of UA analogues was easily prepared from 3-keto UA (**2**) in oxalyl chloride in CH_2Cl_2, and then treated with piperazine or diethanolamine to give the corresponding analogues **3** and **4**, respectively. Notably, the moieties of piperazine and diethanolamine have been demonstrated to enhance potency and drug-like properties.[24,33] Bromination of **2** with $PyHBr_3$ in THF led to obtain the intermediate **5** in 88% yield. Hantzsch reaction of **5** with thiourea or allyl thiourea in the refluxing ethanol directly afforded corresponding derivatives **6** and **7** in the yield of 62% and 60%, respectively. Compound **8** was obtained by Claisen



Scheme 1. Reagents and conditions: (a) Jones reagent, acetone, rt, 2 h, 90%; (b) (i) oxalyl chloride, CH_2Cl_2, rt, 24 h; (ii) corresponding amines, EtOAc, rt, 3 h, 38–67%; (c) $PyHBr_3$, THF, rt, 16 h, 88%; (d) corresponding thioureas, EtOH, reflux, 5 h, 60–62%; (e) CH_3ONa, ethyl formate, benzene, rt, 12 h, 80%; (f) corresponding hydrazines, EtOH, reflux, 6 h, 80–85%.

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