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Synthesis and Antibacterial Activity of Ursolic Acid Derivatives

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Abstract: To obtained natural products with remarkable antibacterial activities, three 3-amino substituted (UA) derivatives 2, 3 and 4 were designed and synthesized, and their antibacterial activity were assayed using broth microdilution method. Compared to UA, there three derivatives present higher antimicrobial activities against methicillin-resistant *Staphylococcus aureus* (MRSA) with the minimum inhibitory concentrations of 16 to 32 μg/mL. Furthermore, their cytotoxicities to human hepatoma cell (BEL-7402) were evaluated using MTT method, and the results indicated that their derivatives had no toxicity (IC_{50} value was larger than 10 μM), while UA presented a certain cytotoxicity.

Keywords: Ursolic Acid, Antibacterial Activity, Synthesis, Structural Modification, Mic, Mtt

1. Introduction

Ursolic acid (UA), a pentacyclic triterpene, is widely distributed in various plants (Chen L 2011). It had significant bioactivities such as anti-tumor (Simone 2008), anti-nociceptive effect (Ana et al. 2012), anti-inflammatory, anti-oxidant (Isabel et al. 2014), anti-HIV (Ma C et al. 1999) and antibacterial activities (Fumiko et al. 2002). To obtain more efficient bioactivities, many derivatives at C-3 and C-28 positions of UA were designed and synthesized. Ma C et al. (1998,1999) found that the esterification of UA at the C-3 hydroxyl group would increase its anti-HIV activities. Moreover, the longer the fatty acyl chain, the stronger the anti-HIV activity. Meng et al. (2009) discovered the inhibitory effect of three UA derivatives at C-3 hydroxyl and C-28 carboxyl groups on cervical cancer Hela cells would be significantly improved, while that on gastric cancer BGC cells was slightly increased. Deng et al. (2007) designed and synthesized a series of *α*-aminophosphonate conjugates of 3-O-*β*-acetyl UA, some of them have significant anti-HIV activity and no cytotoxicity on HT-29 cells (human colon adenocarcinoma cell line). A new derivative (Zhang et al. 2006) of phenylalanine on the carboxyl group of C-28 was synthesized, and presented remarkable inhibitory activity against PTP1B enzyme compared with UA. Li W et al. (2018) synthesized a novel UA derivative using a nitrogen-containing heterocyclic scaffold and a privileged fragment at the C-28 position the derivative significantly repressed the proliferation of the breast cancer (BC) SUM149PT and HCC1937 cells in a dose-dependent manner, and exhibited decreased cytotoxicity compared with vehicle-treated cell lines. Jiang et al. (2018) designed and synthesized a series of inhibitors of NF-κB based on UA derivatives containing long-chain diamine moieties. These compounds exhibited significant inhibitory activity to the NF-κB with IC_{50} values at micromolar concentrations in A549 lung cancer cell line.

As so far, 3-amino substituted UA derivatives have no report, three 3-amino substituted UA derivatives (**2-4**) were designed and synthesized for discovering UA derivatives with more efficient anti methicillin-resistant *Staphylococcus aureus* (MRSA) activity based on our previous work (Li J 2019), and these compounds presented improved water solubility and less toxicity and more remarkable anti-MRSA activity.

2. Experimental

2.1. Chemistry

In the experiment, ^{13}C-NMR, ^{13}C-DEPT 135°, ^{1}H-^{1}H COSY and ^{1}H NMR were recorded on an AV-400 NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shifts are given in ppm, using tetramethylsilane (TMS) as an internal standard. Column chromatography was performed using silica gel (200–300 mesh, Qingdao Marine Chemical, Inc., China), GF254-silica gel plates (Qingdao Marine Chemical, Inc., China). Ursolic acid (Xi'an Virgin Biotechnology Co., Ltd).

2.1.1. C-3 oxidation

To a round-bottom flask (100 mL) were added UA (0.5 g, 1 mM) and dichloromethane. Stirring were till

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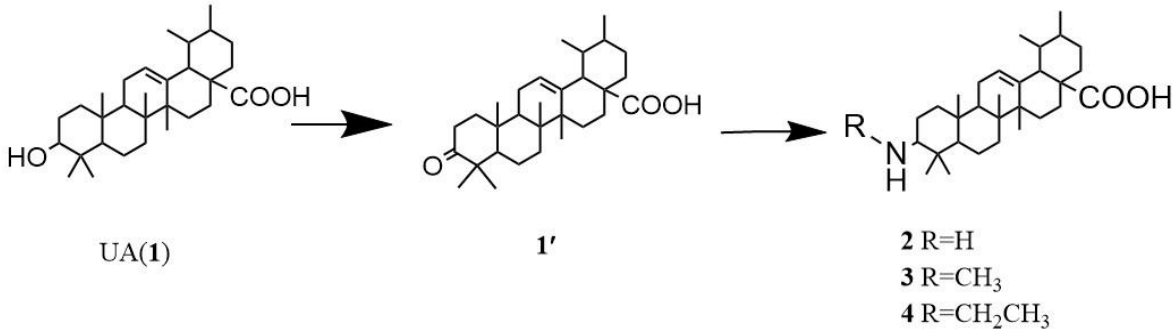
dissolved. Sarrett reagent (0.538 g, 2.5 mM) were added and were Stirred at 10-40℃ for 15 to 240 min. Reactions were monitored by TLC analysis, and spots were visualized by spraying with 5% H_2SO_4 in EtOH (*v*/*v*) and subsequently heating. Distilled water was added to system to quench after the reaction finished. The derivative **1′** was obtained via silica gel chromatography. The experimental route is shown in Figure 1.

2.1.2. Reductive amination

This reaction opted one-pot synthesis which was firstly catalyzed using Titanium tetraisopropanolate (Alinezhad et al. 2009) and then reduced by a reducing agent (sodium triacetoxyborohydride) (Huang et al. 2008) to obtain the final product. Reaction precursor was derivative **1′** which UA was oxidated. The reaction was shown in Figure 1.



UA(**1**) **1′** **2** R=H, **3** R=CH_3, **4** R=CH_2CH_3

Figure 1. Synthesis of derivative **1′** and derivatives **2**, **3**, **4**.

2.1.2.1. Synthesis of derivative 2.

To a flask-three-neck (50 mL) were added derivative **1′** (0.136 g, 0.3 mM) and ammonia ethanol saturated solution. Stirring were till dissolved. Titanium tetraisopropanolate (0.5 mL) were added under Argon atmosphere with stirring and refluxing at 25-50°C for 4 to 6 h. After cooling to room temperature, sodium triacetoxyborohydride (0.063 g, 0.3 mM) were added for 2 to 3 h. The system was quenched with distilled water. The residue was purified by column chromatography using petroleum and ethyl acetate (5:1, *v*/*v*) as eluent to afford **2**.

2.1.2.2. Synthesis of derivatives 3 and 4.

To a flask-three-neck (50 mL) were added derivative **1′** (0.136 g, 0.3 mM) in EtOH. Methylamine alcohol solution (0.9 mM) or alcohol solution of ethylamine (0.9 mM) were added under argon atmosphere. Titanium tetraisopropanolate (0.5 mL) were added in the system with stirring and refluxing at 40°C for 4 h. After cooling to room temperature, sodium triacetoxyborohydride (0.063 g, 0.3 mM) were added for 2 h. The system was quenched with distilled water. The residue was purified by column chromatography using petroleum and ethyl acetate (5:1, *v*/*v*) as eluent to afford **3** and **4**.

2.2. Antibacterial activity

The bacterial strains included methicillin-resistant Staphylococcus aureus (MRSA) ATCC 33592 were purchased from American Type Culture Collection, a clinic isolate MRSA HK01 were friendly presented by Hainan General Hospital, Haikou, China. Escherichia coli (S002). Levofloxacin and vancomycin were purchased from was used as a positive control.

All the compounds were evaluated with reference to the CLIS 2012 standard for micro-broth dilution (Xu et al. 2016). UA previously dissolved in dimethyl sulfoxide (DMSO), were prepared to final concentrations of 1024, 512, 256, 128, 64, 32, 16, 8, 4 μg/mL (100 μL). Levofloxacin and vancomycin were also prepared to final concentrations of 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 μg/mL (100 μL). Mueller-Hinton Broth Medium (MHB) was used as a blank control. Negative control was the 5% DMSO-MHB (*v*/*v*). The MHB contains UA (100 μL) were added to the bacterial suspension (100 μL) and were incubated at 35°C for 24 h. When the microbial growth in the well of blank controls was sufficient, the lowest concentration visibly inhibited the microbial growth was determined as the MIC of each sample. The MIC of UA against MRSA HK01 to HK03 were also determined by the above procedure. Moreover, The MRSA HK01 and MRSA ATCC 33592 were selected to determine the MICs of the derivatives **2**, **3** and **4**. Each experiment was performed in triplicate.

2.3. Cytotoxicity test

MTT method was used to determine the cytotoxicity of the derivatives **2**, **3** and **4**. The bacterial strain was human hepatoma cell (BEL-7402). The cell in logarithmic growth phase were digested with 0.01% trypsin followed inoculate on a 96-well plate. After culturing overnight, the derivatives of different concentrations were added to incubate for 72 h. CCK-8 were added at 35°C for 2-4 h followed measured using Spectra Max 190 with the wavelength at 450 nm. Then, the wavelength was plotted against the compounds of different concentrations obtain the regression equation, and their IC_{50} were calculated according to this

equation. According to above method, the cytotoxicity of UA and the derivatives **2-4** were evaluated. An IC_{50} $>10^{-5}$ mol was considered to be non-toxic, and an IC_{50} $<10^{-5}$ mol was considered cytotoxic. And the smaller the value, the greater the toxicity.

3. Results and discussion

Three 3-amino substituted UA derivatives (**2**, **3**, **4**) were first designed and synthesized by oxidation - reductive amination according to the route shown on Figure 1.

1′, white powder, 41.49, 28.35, 218.43 (C-3, **C=O**), 39.63, 55.27, 20.08, 35.86, 38.54, 48.15, 37.84, 18.61, 121.19 , 142.84 , 45.82, 29.63, 25.54, 48.15, 53.29, 40.79, 40.18, 31.42, 38.26, 28.35, 14.91, 15.90, 18.35, 24.51, 181.33 (C-28), 25.54, 21.68.

2, white powder, ^{13}C-NMR (100MHz, CD_3OD) δ 38.98, 27.82, 60.36 (C-3, **CH**-NH_2), 42.00, 55.05, 18.86, 33.71, 39.35, 47.08, 39.85, 18.88, 125.97 (C-12), 133.08 (C-12), 42.06, 28.75, 23.95, 48.19, 50.00, 39.34, 38.91, 30.40, 36.69, 25.75, 16.27, 16.34, 17.70, 22.66, 180.21, 22.30, 20.55.

3, white powder, ^{13}C-NMR (100MHz, CD_3OD) δ 34.93, 27.68, 58.34 (C-3, C_3-NH**CH_3**), 41.65, 56.18, 19.35, 32.36, 39.37, 48.05, (C-10), 18.51, 125.71, 138.28, 42.17, 29.40, 23.97, 48.23, 53.22, 39.13, 39.01, 30.45, 36.69, 28.05, 14.36, 14.97, 22.55, 180.24, 22.65, 21.69, 47.63.

4, white powder, ^{13}C-NMR (100MHz, CD_3OD) δ 35.14, 26.70, 57.40, 42.25, 55.05, 19.34, 33.71, 41.98, 46.21, (C-10) 18.85, 124.89, 138.67, 43.09, 29.43, 25.53, 48.17, 53.68, 39.13, 39.01, 30.45, 36.73, 26.80, 16.29, 16.91, 22.61, 180.75, 22.67, 20.86, 14.33 (C_3-$NHCH_2$**CH_3**), 42.73 (C_3-NH**CH_2**CH_3).

The bacteriostatic activity of **2**, **3**, **4** and UA against *Staphylococcus aureus* (MRSA) ATCC 33592, MRSA HK01, *Escherichia coli* was evaluated using broth microdilution method. The antibacterial assays indicated that the anti-MRSA activity of these compounds against two MRSA strains increased 4 to 8 folds and the results are shown in Table 1. Their

cytotoxicity against human hepatoma cell BEL-7402 was reduced compared to that of UA by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) assay, and 3-alkylamino substituted UA derivatives were predicted to have lower toxicity [Table 2 near here]. Furthermore, these three compounds presented better solubility in water and aqueous ethanol than UA. Thereby, the amino UA derivatives at 3-position were worthy of researching on their antibacterial or other biological activities.

Table 1. Minimum inhibitory concentrations (μg/mL) of UA and its derivatives against two MRSA strains[a].

Strains	UA	Vancomycin	**2**	**3**	**4**
MRSA ATCC 33592	128.0	1.0	16.0	16.0	32.0
MRSA HK01	64.0	1.0	16.0	16.0	16.0

[a]: Broth microdilution method; [b]: ND: No determination.

Table 2. Cytotoxic activities of UA derivatives on human hepatoma cell BEL-7402.

Compounds	Concentration (mol)					Evaluation[b]
	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	
UA	80.3	76.4	11.2	0.3	0.0	cytotoxity
2	73.3	7.5	18.4	15.7	0.0	non-toxic
3	84.4	22.6	21.9	0.0	0.0	non-toxic
4	78.7	4.6	4.0	2.4	5.3	no-toxic
Doxorubicin	100.0	84.8	83.8	35.2	0.0	cytotoxity

[a]: CCK-8 method; [b]: 10^{-5} mol<50%: non-toxic, 10^{-5} mol>50%: cytotoxity.

Disclosure statement

The authors declare no conflict of interest, financial or otherwise.

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