Synthesis and antitumor activities of novel α-aminophosphonate derivatives containing an alizarin moiety

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Original article

Synthesis and biological evaluation of novel aniline-derived asiatic acid derivatives as potential anticancer agents

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ABSTRACT

Asiatic acid (AA) derivatives **4** and **5** modified at the C-11 and C-28 positions were designed and synthesized, their structures were confirmed using HRMS, ^{1}H NMR and ^{13}C NMR. *In vitro* antitumor activities of all compounds against MGC-803, NCI-H460, HepG2, Hela and 7404 cancer cell lines were evaluated and compared with commercial anticancer drug 5-fluorouracil (5-FU), employing standard MTT assay. The new compounds **5a**–**5t** showed stronger anti-proliferative activity than AA, especially compound **5b** was found to be the best inhibition activity on HepG2 cell line. In addition, the mechanism of compound **5b** was preliminarily investigated by acridine orange/ethidium bromide staining, Hoechst 33258 staining, JC-1 mitochondrial membrane potential staining, flow cytometric, qRT-PCR (quantitative real-time PCR) and Western blot. Compound **5b** induced the productions of ROS, and altered anti- and pro-apoptotic proteins, leading to mitochondrial dysfunction and activations of caspase-9 and caspase-3 for causing cell apoptosis. Moreover, the cell cycle analysis showed that compound **5b** mainly arrested HepG2 cells in G1 stage.

1. Introduction

Natural products have provided a major source of therapeutic agents for human disease over the past century [1,2]. In recent years, a large number of terpenes has marked anticancer effects toward various types of cancer cell lines *in vitro*, some of them had been successfully developed for clinical use to treatment human cancers diseases in some therapeutic areas [3–12], especially the pentacyclic triterpenes including oleanolic acid (OA), glycyrrhetic acid, and carbenoxolone [13,14]. Therefore, development of novel pentacyclic triterpenes derivatives with better antitumor activities have greatly gained bioorganic chemists' interest.

Asiatic acid (AA, 2α, 3β, 23-trihydroxyurs-12-ene-28-oic acid), a well-known pentacyclic triterpene acid, exists abundantly in *Centalla asiatica*, which was used as a medicinal herb in Ayurvedic and traditional Chinese medicine in China [15]. AA has been primarily used to treatment skin diseases and leprosy disease [16–19]. Afterwards, the effects of AA have attracted the attention of the pioneers who aim to develop novel pharmacological reagents. It has been found to possess a wide range of pharmaceutical properties, including anti-alzheimer's disease [20,21], hepatoprotective [22], antiinflammation [23], antidiabetics [24], and protection of bloodvessel [25], especially antitumor activities [3,5]. Moreover, previous studies had reviewed that modified AA has properties of increasing the inhibition activity of anticancer drugs in various cells [26,27]. However, in most previous studies, the derivatives of AA have not been thoroughly explored for their anticancer activity. Thus, in order to find potentially important anticancer drug candidates, further study needed to investigate the mechanism of anti-proliferative activity.

It is well known that anilines could effectively improve the antitumor activity and the cells selectively by introducing to pentacyclic triterpenes [28–33]. In addition, it has been reported that polar group at C-28 position is more essential for the pharmacological activities of AA [10,26]. However, to the best of our knowledge, the structure activity relationships at the C-2, C-3, and C-23 position have not been clear. Thus, anilines group was rationally designed and introduced to the AA structure. Our present work in this paper is to design and synthesize AA derivatives, and to evaluate their *in vitro* antitumor activities. Results exhibited that the target products could inhibit proliferation of these selected tumor

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cell lines at moderate to high rates. Preliminary investigation on the mode of action of representative compound **5b** is also investigated.

2. Results and discussion

2.1. Chemistry

Asiatic acid derivatives bearing amine structure (compounds **5**) were synthesized as outlined in Scheme 1.

Asiatic acid was acetylated to give 2α, 3β, 23-Triacetoxyurs-12-ene-28-oic acid (**1**). 2α, 3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oic (**2**) was synthesized by the treatment of **1** in the presence of glacial acetic acid [34]. Asiatic acid chloride was obtained by the condensation of compound **2** in the presence of triethylamine with oxalyl chloride, this intermediate was then reacted with a variety of substituted phenyl amine gave the desired **4a**–**4t**, and it was then dealt with NaOH (aq) to offer **5a**–**5t** in overall good yields. All the new compounds were confirmed by various spectroscopic methods, including ^{1}H NMR, ^{13}C NMR and high resolution mass spectrum (HRMS).

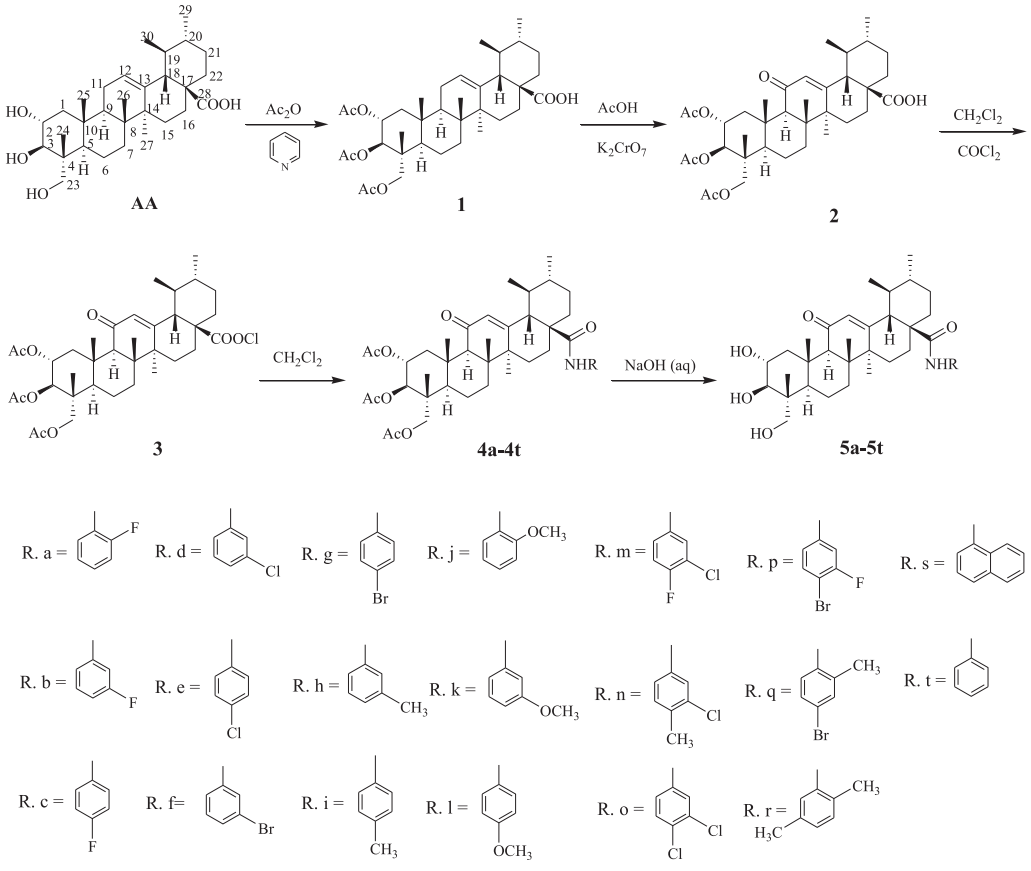
2.2. In vitro cytotoxicity

The *in vitro* cytotoxic potency of asiatic acid derivatives (**4** and **5**) were evaluated by MTT assay against MGC-803, NCI-H460, HepG2, Hela, 7404 cell lines (with 5-FU as the positive control). The inhibitory rates of cell viability with 10 μM concentration of all compounds for 48 h are shown in Table 1 and Table 1S.

As shown in Table 1S (See supporting Information), most of the acetylated derivatives **4** had no obvious effects. This cytotoxic inhibition screening results showed that the introduction of acetyl on AA should markedly weaken the antitumor activity against tumor cell line. Whereas, most of the title compounds of **5** displayed higher inhibitory activity than AA, and even better than the commercial anticancer drug 5-FU. It may be attributed to the water-soluble of **5** contained OH-group. These dates were indicated that the incorporation of an acyl at C-28, while retaining the polar group at C-2, C-3, C-23 significantly improved the antitumor bioactivities of the compounds.

As shown in Table 1, in all human cancer cell lines, most of compounds exhibited better inhibition than AA, and some of them even showed preferable cytotoxic activities than 5-FU. In HepG2 assay, except the compound **5u**, all the compounds exhibit better cytotoxicity than AA. It was suggested that the introduction of amido bond in C-28 and carbonyl in C-11 on AA could improve the antitumor activity against HepG2 cell line. Compounds **5b** and **5k** showed better inhibition on HepG2 cell line than the other compounds, with IC_{50} of 5.97 μM and 8.89 μM, respectively. For Hela, except the derivative **5l**, all the derivatives exhibit better cytotoxicity than AA. It was indicated that the introduction of amido bond in C-28 and carbonyl in C-11 on AA can also improve the antitumor activity against Hela cell lines. Therefore, it can be suggested that the introduction of amido bond in C-28 and carbonyl in C-11 significantly improved the antitumor activities of these derivatives.

It was important to note that the compounds of **5b** showed better inhibition on HepG2 cell line than other compounds, with IC_{50} of 5.97 μM. Meanwhile, compounds **5b**, **5d**, **5f**, **5h**, and **5k** were found to have the corresponding values of 5.97 μM, 11.60 μM, 20.43 μM, 19.38 μM, 8.89 μM, respectively. The results indicated compounds with meta- position substitutions of the acyl phenyl



Scheme 1. General synthetic route for compound **5a**–**5t**.

Table 1
IC_{50}[a] values (μM) of asiatic acid and complexes **5a**–**5t** and **AA** towards five selected tumor cell lines and normal cell lines for 48 h.

Compounds	IC_{50}					
	MGC-803	NCI-H460	HepG2	Hela	7404	HUVEC
5a	19.91 ± 0.13	61.63 ± 2.43	>100	32.55 ± 0.6	23.24 ± 0.32	>100
5b	14.33 ± 0.25	23.58 ± 0.31	5.97 ± 0.34	28.18 ± 0.083	14.13 ± 0.16	>100
5c	26.59 ± 0.87	31.94 ± 0.45	>100	20.76 ± 0.46	>100	>100
5d	19.44 ± 0.39	24.41 ± 0.19	11.60 ± 0.41	14.2 ± 0.055	34.43 ± 0.24	>100
5e	22.50 ± 0.29	12.8 ± 0.19	29.83 ± 1.01	13.1 ± 0.033	17.20 ± 0.1	>100
5f	15.52 ± 0.09	24.84 ± 0.03	20.43 ± 0.44	16.23 ± 0.12	22.44 ± 0.95	>100
5g	20.59 ± 0.59	31.18 ± 0.33	21.27 ± 0.26	18.46 ± 0.35	>100	>100
5h	21.18 ± 0.13	21.78 ± 0.44	19.38 ± 0.11	14.58 ± 0.15	16.11 ± 0.16	>100
5i	14.61 ± 0.63	30.16 ± 0.44	22.62 ± 0.17	32.45 ± 0.92	>100	>100
5j	18.96 ± 0.37	18.44 ± 0.6	10.91 ± 0.51	34.5 ± 1.34	16.25 ± 0.51	>100
5k	24.31 ± 2.03	23.05 ± 0.16	8.89 ± 0.47	17.81 ± 0.17	23.47 ± 0.92	>100
5l	30.07 ± 1.84	>100	16.62 ± 0.29	>100	>100	>100
5m	14.09 ± 0.06	24.21 ± 0.28	19.38 ± 0.07	14.25 ± 0.059	19.07 ± 0.38	>100
5n	20.08 ± 0.23	19.05 ± 0.36	18.42 ± 0.15	16.04 ± 0.072	20.57 ± 0.04	>100
5o	13.94 ± 0.44	21.45 ± 0.23	13.75 ± 0.13	12.4 ± 0.1	17.20 ± 0.08	>100
5p	16.03 ± 0.32	16.45 ± 0.65	28.36 ± 0.86	9.71 ± 0.26	17.39 ± 0.25	>100
5q	14.63 ± 0.21	23.82 ± 0.14	22.79 ± 0.17	15.69 ± 0.47	17.03 ± 0.19	>100
5r	20.66 ± 0.47	27.52 ± 0.13	25.47 ± 0.17	16.03 ± 0.047	15.72 ± 0.11	>100
5s	19.88 ± 0.16	16.59 ± 0.09	21.31 ± 0.45	14.01 ± 0.29	20.44 ± 0.16	>100
5t	>100	12.16 ± 0.45	>100	26.88 ± 0.79	>100	>100
AA	22.57 ± 0.17	39.55 ± 0.23	34.9 ± 0.25	37.26 ± 0.37	40.78 ± 0.21	>100
5-FU	46.93 ± 2.09	44.04 ± 0.54	29.98 ± 0.37	35.34 ± 2.72	40.21 ± 1.98	56.00 ± 4.40

[a] IC_{50} values are presented as the mean ± SD (standard error of the mean) from three separated experiments.

ring against HepG2 favored the antitumor bioactivities than other position. On the other hand, electron-donating group affected the antitumor activity superior than electron withdrawing group, such as **5b** and **5k**. Meanwhile, the activity of most compounds with halogen, methyl and methoxyl disubstituted of the acyl phenyl ring against five cancer lines had no obvious change than monosubstitution.

In addition, the inhibition activities of compounds **5** against HUVEC normal cell lines were also estimated. The data of MTT assay against HUVEC cell lines were listed in Table 1. The results indicated that the cytotoxicity of most of compounds against cancer cells was much higher than HUVEC normal cells, making them good candidates as antitumor drugs. These results showed that these derivatives have selective and significant effect on the cell lines.

2.3. *Preliminary investigation of the apoptosis-inducing effect of title compounds **5b***

Apoptosis is an ordered and orchestrated cellular process that occurs in physiological and pathological conditions. However, the mechanism of cell apoptosis in cancer cells is disrupted, thus results in the overgrowth of malignant cells [35]. It is therefore necessary to consider cell apoptosis as another effective approach in cancer treatment. Moreover, whether its derivative **5b** can induce apoptosis in certain cancer cell lines have not yet been reported so far. Therefore, compound **5b** which exhibited good cytotoxic inhibition in tumor cell lines was selected and its mechanism of growth inhibition of HepG2 cells was evaluated.

2.3.1. *Fluorescence staining*

Changes in the morphological character of HepG-2 cells were studied using acridine orange (AO)/ethidium bromide (EB), Hoechst 33258, JC-1 mitochondrial membrane potential staining and under fluorescence microscopy to estimate whether the growth inhibitory activity of the selected compound was related to the induction of apoptosis.

2.3.1.1. AO/EB staining. AO, which is a vital dye, can stain nuclear DNA across an intact cell membrane, whereas EB can only stains cells that had lost their membrane integrity. Hence, after simultaneous deal with AO and EB, live cells will be uniformly stained as green (in the web version) and early apoptotic cells will be densely stained as green yellow or show green yellow fragments (in the web version), whereas late apoptotic cells will be densely stained as orange or display orange fragments and necrotic cells will be stained as orange with no condensed chromatin.

The cytotoxicity of compound **5b** at the concentration of 20 μM against HepG2 cells from 12 to 24 h was detected by AO/EB staining, and HepG2 cells not dealt with **5b** was used as control at for 24 h. The results are given in Fig. 1. The results showed that the HepG2 cells dealt with **5b** from 12 to 24 h had obviously changed. The nuclei stained as yellow green or orange, and the morphology showed pycnosis, membrane blebbing and cell budding. These phenomena are associated with cell apoptosis.

In summary, the cells presented with apoptotic morphology. The nearly complete absence of red cells in compound **5b** showed that it was associated with very low cytotoxicity. These findings demonstrate that compound **5b** could induce apoptosis with low cytotoxicity.

2.3.1.2. Hoechst 33258 staining. Hoechst 33258, which stains the cell nucleus, is a membrane permeable dye with blue fluorescence. Live cells with uniformly light blue nuclei were observed under fluorescence microscope after treatment with Hoechst 33258, while apoptotic cells had bright blue nuclei on account of karyopyknosis and chromatin condensation; whereas, the nuclei of dead cells could not be stained. HepG2 cells dealt with compound **5b** at 20 μM from 12 to 24 h were stained with Hoechst 33258. HepG2 cells were not dealt with the **5b** was used as control at for 24 h. The results were given in Fig. 2.

Fig. 2 shows that cells were not dealt with compound **5b** had no obvious morphological changes (in the web version), but most cell nuclei appeared to be highly condensed (brightly stained). On the contrary, for **5b** treatment, the cells exhibited strong blue fluorescence and revealed typical apoptotic morphology after 12 h and 24 h, respectively. The observation demonstrates that compound **5b** induced apoptosis against HepG2 cell lines, consistent with the results for AO/EB double staining.

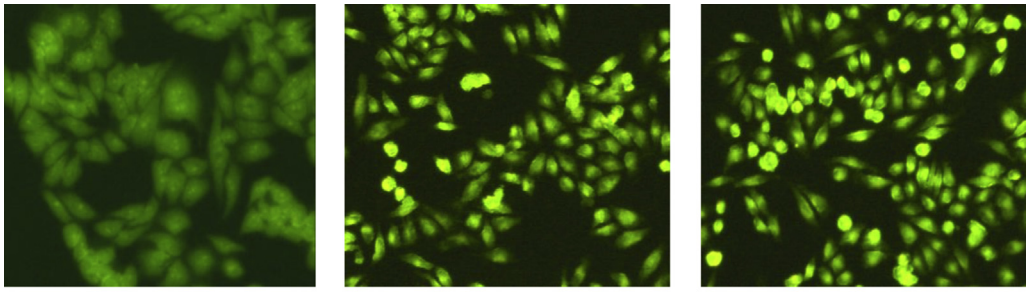


Fig. 1. AO/EB staining of compound **5b** in HepG2 cells. (a) Not dealt with compound **5b** was used as control at for 24 h, (b, c) dealt with compound **5b** (20 μM) for 12 h and 24 h, respectively.

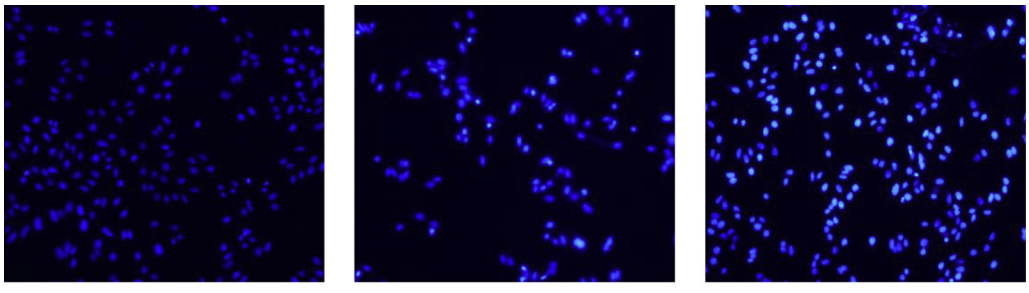


Fig. 2. Hoechst 33258 staining of compound **5b** in HepG2 cells. (a) Not dealt with the **5b** was used as control at for 24 h, (b, c) dealt with compound **5b** (20 μM) for 12 h and 24 h, respectively.

2.3.1.3. Mitochondrial membrane potential staining. In order to further research the apoptosis-inducing effect of target compounds **5b**, using the fluorescent probe JC-1 to designed and detected the changes of mitochondrial membrane potential. JC-1, which is a kind of lipophilic cationic dye, can easily pass through the plasma membrane into cells and accumulates in mitochondria [36]. Meanwhile, membrane potential and degree of accumulation of JC-1 in mitochondria exists intimate connection. Therefore, the dye of the accumulation degree in mitochondria as increase with the increase of the mitochondrial membrane potential, when at higher concentrations, the dye forms "J-aggregates". With the dye aggregation, fluorescence emission gradually changes from green to red. When the monomer of JC-1 is formed, can very easily as a sensitive tool by changing the color of the fluorescence to detect the changes of mitochondrial membrane potential. HepG2 cells dealt with compound **5b** at 20 μM from 12 to 24 h were stained with JC-1 and not dealt with the compound **5b** was used as control at for 24 h. The results were shown in Fig. 3.

The maximum excitation wavelength of JC-1monomers and J-aggregates were excited at 514 nm and 585 nm respectively, and then light emissions were collected at 515–545 nm (green) and 570–600 nm (red). Fig. 3 indicated that cells dealt with the compound **5b**, exhibit strong green fluorescence and show typical apoptotic morphology after 12 h and 24 h, respectively, whereas cells not dealt with the compound **5b** were normally red (in the web version). From the above discussions we may safely draw the conclusion that compound **5b** induced apoptosis against HepG2 cell line. The experimental results were the same as the previous, once again proved that the **5b** induced apoptosis against HepG2 cell line.

2.3.2. Flow cytometry

The apoptosis ratios induced by compounds **5b** in HepG2 tumor cells were quantitatively analyzed by flow cytometry. In all panels, cells in the upper left quadrant (Q1: AV/PI+) were damaged, cells in the upper right quadrant (Q2: AV+/PI+) were in late apoptosis/necrosis, cells in the lower left quadrant (Q3: AV−/PI−) were alive, and cells in the lower right quadrant (Q4: AV+/PI) were in early apoptosis appearing in the process of cell collection. Percentage of total signal within the quadrant was indicated. The results were given in Fig. 4.

Fig. 4 shows that compound **5b** (20 μM) could induce apoptosis in HepG2 cells. Apoptosis ratios (including the early and late apoptosis ratios) for compound **5b** were obtained after 12 h of treatment at a concentration of 10 μM and 20 μM, with the highest apoptosis ratio being 25.0%. In addition, the apoptosis of HepG2 cells treated with compound **5b** increased gradually in a concentration manner; Simultaneously, the apoptosis ratios of compound **5b** measured at different concentration points were found to 7.2% (10 μM) and 25% (20 μM), respectively, were higher than that of control (2.1%). The results further proved that the induced apoptosis by compound **5b** inhibition of cell proliferation.

2.4. Effect on cell cycle

To confirm the possible role of cell cycle arrest in AA derivative-induced growth inhibition, HepG2 cells were dealt with compound **5b** (20 μM) from 12 to 24 h. Cell cycle distribution was observed by flow cytometric analysis after staining of the DNA with propidium iodide (PI). As shown in Fig. 5, the dealt of HepG2 cells with compound **5b** increase cell cycle arrest at the G1 phase at different time, resulting in concomitant population increase in the G1 phase (55.29% and 65.58%) compare with the control cells (41.51%) and the population of the G2 phase decrease at a certain extent (6.09% and 5.56%) compared with the control cells (6.20%).

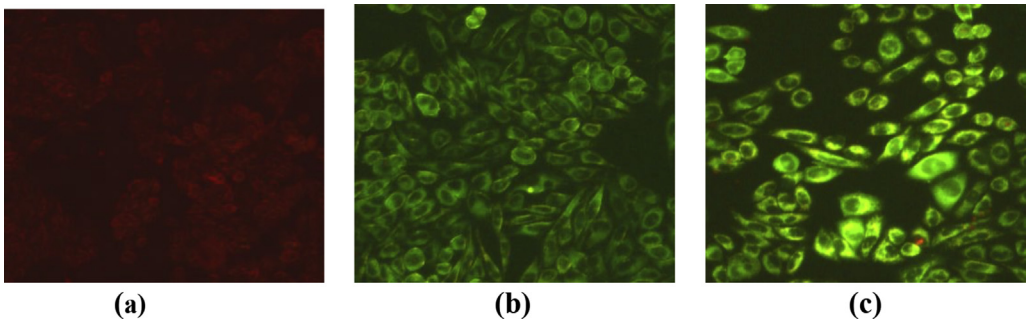


Fig. 3. JC-1 mitochondrial membrane potential staining of compound **5b** in HepG2 cells. (a) Not dealt with the **5b** was used as control at for 24 h, (b, c) dealt with compound **5b** (20 μM) for 12 h and 24 h, respectively.

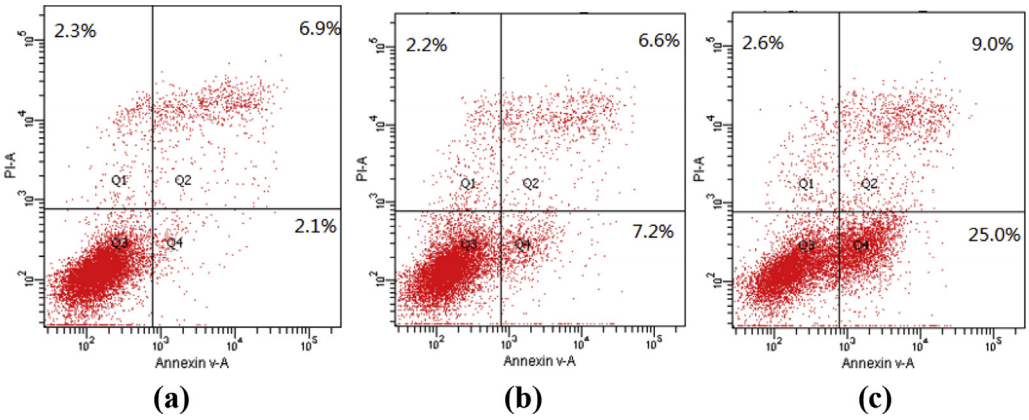


Fig. 4. Effect of compound **5b** on apoptosis of HepG2 cells. Apoptotic cells were analyzed by flow cytometry, after being stained with Annexin V-FITC together with PI. The percentage of cells positive for PI and/or Annexin V-FITC are reported inside the quadrants.

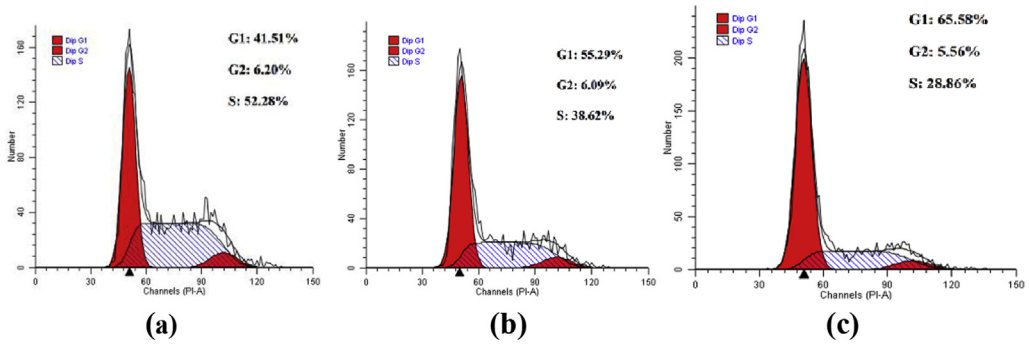


Fig. 5. Fluorescence-activated cell sorting analyses based on DNA content and stained by propidium iodide, (a) Not dealt with compound **5b** was used as control at for 24 h, (b, c) dealt with compound **5b** (20 μM) for 12 h and 24 h, respectively.

2.5. Measurement of reactive oxygen species (ROS)

The generation of intracellular ROS may be related to the induction of apoptosis [37,38], therefore, we investigated the induction of apoptosis by **5b** to confirm if it was associated with ROS generation. In this paper, *in vivo* assessment of intracellular ROS levels was measured by 2′, 7′-dichlorofluorescein (DCF) fluorescence. 2′, 7′-dichlorofluorescin diacetate (DCFH-DA) passively diffuses into cells and is hydrolyzed by cellular esterases to 2′, 7′-dichlorofluorescin (DCFH), a nonfluorescent molecule that can be oxidized to the fluorescent DCF in the presence of peroxides. ROS generation in HepG2 cells was visualized under fluorescence microscopy, HepG2 cells were dealt with compound **5b** (20 μM) from 12 to 24 h, and HepG2 cells not dealt with **5b** was used as control at for 24 h. As shown in Fig. 6, these finding indicated that cells not dealt with the compound **5b** were normally green (in the web version), For **5b** dealt, the cells showed strong green fluorescence. Hence, it could be concluded that compounds **5b** significantly increased the intracellular level of ROS.

2.6. Induces caspase-dependent apoptosis in HepG2 cells

Apoptosis can be caused by several stimuli and is controlled by two major manners, namely the mitochondrial pathway and membrane death receptor pathway. The membrane death receptor pathway is characterized by the binding between cell death ligands

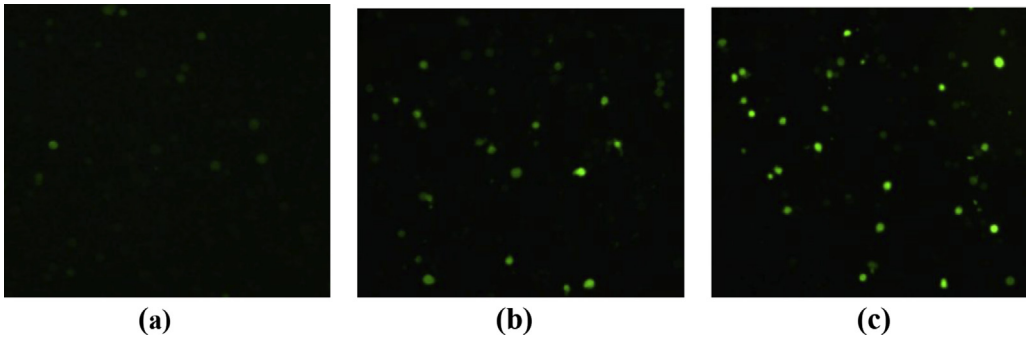


Fig. 6. ROS generation of compound **5b** in HepG2 cells was visualized under fluorescence microscopy. (a) Not dealt with compound **5b** was used as control at for 24 h, (b, c) dealt with compound **5b** (20 μM) for 12 h and 24 h, respectively.

and cell death receptors and the subsequent activation of caspase-8 and caspase-3. The compound of **5b** induced apoptotic pathway was investigated by qRT-PCR analysis of caspase expressions. The result was showed in Fig. 7, it indicated that the effects of compound **5b** treatment in caspases-3 and caspases-8 was time-dependent. Therefore, we preliminarily concluded that **5b** induced the apoptosis of HepG2 cells via the membrane death receptor pathways.

2.7. Compound **5b** induce apoptosis via a Mitochondria-Mediated Pathway

To explore the possible role of a mitochondrial-related pathway in compound **5b**-induced apoptosis, the effects of **5b** on the expression of Bax, Bcl-2, caspase-9, -3, PARP and cytochrome *c* were examined by Western blot analysis. Mitochondria play an essential role in cell death signal transduction. The mitochondria-dependent apoptotic pathway is regulated by the Bcl-2 family of pro- and anti-apoptotic proteins, which induce the permeabilization of the mitochondrial outer membrane and cytochrome *c* released into the cytosol, resulting in the activation of the caspase cascade and the induction of apoptotic cell death [39,40]. The effects of compound **5b** on the constitutive levels of Bax and Bcl-2 in HepG2 cells are shown in Fig. 8. In comparison with the control cells, **5b** induced a marked increase in the levels of Bax and a reduction in the levels of Bcl-2, in a dose-dependent fashion. Compound **5b** treatment caused an accumulation of cytochrome *c* in the cytosol, most probably due to the release of mitochondrial cytochrome *c* (Fig. 8). These results indicated an involvement of caspases in the apoptotic process downstream of mitochondria. Then, the roles of specific caspases (caspase-9 and caspase-3) were investigated. As shown in Fig. 8, treatment of HepG2 cells with **5b** caused a significant increase in the levels of caspase-9 and caspase-3 proteins compared to the control. These results revealed an involvement of caspases in the intrinsic apoptotic process downstream of mitochondria. A mitochondrial pathway plays an important role in compound **5b** induced apoptosis.

3. Conclusion

In summary, it is illustrated that retain the polar substituent at C-2, C-3, C-23 position is essential for the pharmacological activities of pentacyclic triterpenes, and introduction anilines formed amide bond at C-28 position significantly improved the antitumor bioactivities of the compounds. The apoptosis-inducing activity of representative compound **5b** in HepG2 cells was investigated by specific mechanisms. These results suggest that **5b** induced apoptosis may be mediated through generation of ROS, alteration of

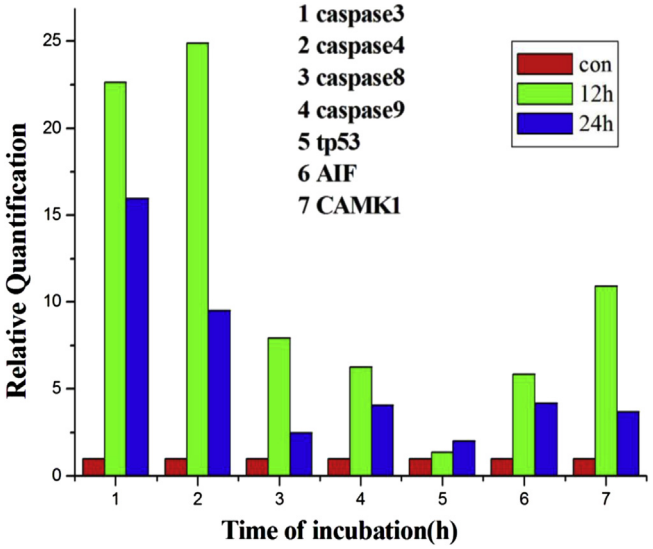


Fig. 7. Effects of **5b** after dealt for 12 h and 24 h on the apoptosis cascade by qRT-PCR.

Bax/Bcl-2 ratio and activation of caspase-3 and -9. Cell-cycle analysis proved that HepG2 cells treated with **5b** grow inhibition to undergo G1 phase arrest. Therefore, compound of **5b** may be considered valuable molecules for use as anti-proliferative agents.

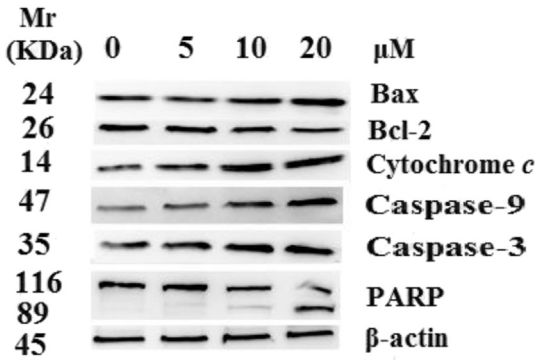


Fig. 8. Effect of compound **5b** on cytochrome *c* release and levels of Bax, Bcl-2, caspase-9, -3, PARP. HepG2 cells were treated with compound **5b** at 5 μM, 10 μM and 20 μM for 24 h. The cell lysates were collected and expression levels of cytochrome *c*, Bax, Bcl-2, caspase-9, -3 and cleaved PARP were determined by western blot analysis. β-actin was used as internal control.

4. Experimental section

4.1. General

Asiatic acid purchase from biological technology of wuhan, China. All the chemical reagents and solvents used were of analytical grade.

4.2. Instrumentation

NMR spectra were recorded on a Bruker AV-400 or 500 NMR spectrometer. Mass spectra were determined on an FTMS ESI spectrometer.

4.3. Synthesis

The syntheses of the aniline-derived asiatic acid conjugates **5a**–**5t** were achieved by a convenient procedure shown in Scheme 1.

4.3.1. General procedure for compound **1** (2α, 3β, 23-triacetoxyurs-12-ene-28-oic acid)

To a solution of **AA** (200 mg, 0.4 mmol) in pyridine (10 mL) was added acetic anhydride (0.5 mL, 5.0 mmol). The mixture was stirred at 20 °C for 8 h. After dilution with ethyl acetate (25 mL), the mixture was washed with aqueous 1 M HCl (10 mL × 5) and saturated $CuSO_4$ (15 mL × 2) and saturated NaCl solution (20 mL). The organic phase was dried over anhydrous sodium sulfate. Filtration and evaporation of solvent at reduced pressure, crude product was purified by column chromatography on silica gel eluted with petroleum ether/ethyl acetate (V:V = 3:1) to give **1**as a white solid.

Yield 85.5%. m.p. 151.2–154.6 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.19 (t, J = 3.2 Hz, 1H, H-12), 5.11 (td, J = 10.9, 4.6 Hz, 1H, H-2), 5.04 (d, J = 10.3 Hz, 1H, H-3), 3.80 (d, J = 11.8 Hz, 1H, H-23), 3.54 (d, J = 11.9 Hz, 1H, H-23), 2.14 (d, J = 11.3 Hz, 1H, H-9), 2.04 (s, 3H, CH_3CO), 1.98 (s, 3H, CH_3CO), 1.94 (s, 3H, CH_3CO), 1.89–1.11 (triterpene's H, 19H), 1.05 (s, 3H, CH_3-27), 1.03 (s, 3H, CH_3-24), 0.90 (s, 3H, CH_3-25), 0.83 (s, 3H, CH_3-26), 0.80 (d, J = 6.4 Hz, 3H, CH_3-29), 0.71 (d, J = 6.4 Hz, 3H, CH_3-30). ^{13}C NMR (126 MHz, $CDCl_3$) δ 184.04, 170.89, 170.54, 170.43, 138.03, 125.19, 74.77, 69.91, 65.21, 60.41, 52.39, 47.88, 47.53, 47.43, 43.66, 41.87, 39.45, 38.94, 38.75, 37.77, 36.61, 32.37, 30.54, 27.83, 23.90, 23.43, 23.29, 21.17, 21.09, 20.89, 20.79, 17.81, 17.01, 16.93, 14.19, 13.90; ESI-HRMS m/z Calc for $C_{36}H_{54}O_8$ $[M-H]^-$: 613.37459, found: 613.37483.

4.3.2. General procedure for compound **2** (2α,3β,23-triacetoxyurs-11-oxo-12-ene-28-oic acid)

A solution of **1** (100 mg, 0.16 mmol) and $K_2Cr_2O_7 \cdot 2H_2O$ (150 g, 0.5 mmol) in 20 mL of acetic acid was refluxed for 5 h. The mixture was cooled to 20 °C and neutralized with 10% $NaHCO_3$ solution to pH 7–8. Then, the mixture was diluted with ethyl acetate (20 mL) and washed with water (10 mL × 5). The organic phase was dried over anhydrous sodium sulfate. Filtration and evaporation of solvent at reduced pressure, The crude product was purified by silica gel chromatography with a gradient elution of CH_2Cl_2/MeOH (V:V = 30:1) to yield a right solid (125 mg).

Yield 85.5%. m.p. 191.2–194.8 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.72 (s, 1H, H-12), 5.27–5.21 (m, 1H, H-2), 5.00 (d, J = 10.2 Hz, 1H, H-3), 3.78 (d, J = 11.8 Hz, 1H, H-23), 3.55 (d, J = 11.9 Hz, 1H, H-23), 3.16 (dd, J = 12.8, 4.5 Hz, 1H, H-19), 2.39 (s, 1H, H-9), 2.21–1.06 (triterpene's H, 17H), 2.05 (s, 3H, CH_3CO), 1.98 (s, 3H, CH_3CO), 1.93 (s, 3H, CH_3CO) (3 × CH_3CO), 1.31 (s, 3H, CH_3-27), 1.22 (s, 3H, CH_3-24), 0.97 (d, J = 6.1 Hz, 3H, CH_3-29), 0.89 (d, J = 6.4 Hz, 3H, CH_3-30), 0.85 (s, 6H, CH_3-25/26). ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.05, 182.78, 170.84, 170.54, 170.25, 163.15, 130.58, 74.89, 69.03, 65.25, 61.12, 52.52, 47.50, 47.38, 44.64, 44.18, 43.76, 41.96, 38.60, 38.53, 37.71, 36.01, 32.48, 30.25, 28.33, 23.56, 21.04, 20.99, 20.93, 20.70, 20.80, 19.07, 17.80, 17.04, 16.97, 13.90; ESI-HRMS m/z Calc for $C_{42}H_{56}O_8$ $[M+H]^+$: 629.36841, found: 629.36667.

4.3.3. General procedure for the preparation of compounds **4**

Compound **2** (100.00 mg, 0.14 mmol) added to dry CH_2Cl_2 was stirred at 0 °C, oxalyl chloride was dripped into the mixture and stirred at room temperature for 8 h. After the reaction the solvent and excess oxalyl chloride was evaporated under reduced pressure. Then anilines (0.56 mmol) were added to the mixture and stirred at room temperature for 4 h. After dilution with ethyl acetate (25 mL), the mixture was washed with water (20 mL × 3). The organic phase was dried over anhydrous sodium sulfate. Filtration and evaporation of solvent at reduced pressure. The residue was purified by column chromatography on silica gel eluted with petroleum ether/ethyl acetate (V:V = 4:1) to give a white solid **4**.

4.3.3.1. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-o-fluoroaniline (**4a**).

Yield 86.5%. m.p. 169–174 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (t, J = 8.0 Hz, 1H, NH), 7.54 (d, J = 3.0 Hz, 1H, Ar–H), 7.09–6.98 (m, 3H, Ar–H), 5.72 (s, 1H, H-12), 5.21–5.28 (m, 1H, H-2), 5.01 (d, J = 10.2 Hz, 1H, H-3), 3.79 (d, J = 11.9 Hz, 1H, H-23), 3.55 (d, J = 11.9 Hz, 1H, H-23), 3.17 (dd, J = 12.8, 4.5 Hz, 1H, H-19), 2.39 (s, 1H, H-9), 2.25–1.09 (triterpene's H, 17H), 2.06 (s, 3H, CH_3CO), 1.98 (s, 3H, CH_3CO), 1.93 (s, 3H, CH_3CO) (3 × CH_3CO), 1.31 (s, 3H, CH_3-27), 1.22 (s, 3H, CH_3-24), 0.98 (d, J = 6.1 Hz, 3H, CH_3-29), 0.90 (d, J = 6.3 Hz, 3H, CH_3-30), 0.85 (s, 6H, CH_3-24/25) (9 × CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.29, 174.53, 170.68, 170.37, 170.01, 162.40, 130.60, 126.02, 124.51, 124.38, 121.83, 114.85, 114.66, 74.81, 68.91, 65.16, 61.20, 53.34, 48.52, 47.27, 44.52, 44.10, 43.86, 41.83, 39.09, 38.64, 37.61, 36.74, 32.28, 30.39, 28.10, 24.67, 20.97, 20.90, 20.89, 20.79, 20.66, 18.70, 17.61, 17.05, 16.97, 13.74; ESI-HRMS m/z Calc for $C_{42}H_{56}O_8$ $[M+Na]^+$: 744.38822, found: 744.38611.

4.3.3.2. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-m-fluoroaniline (**4b**).

Yield 86.5%. m.p. 185.7–188.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.51 (s, 1H, NH), 7.38 (d, J = 10.9 Hz, 1H, Ar–H), 7.19–7.14 (m, 1H, Ar–H), 7.00 (d, J = 8.1 Hz, 1H, Ar–H), 6.74 (m, 1H, Ar–H), 5.71 (s, 1H, H-12), 5.26–5.20 (m, 1H, H-2), 5.01 (d, J = 10.2 Hz, 1H, H-3), 3.78 (d, J = 11.9 Hz, 1H, H-23), 3.54 (d, J = 11.9 Hz, 1H, H-23), 3.15 (dd, J = 12.8, 4.5 Hz, 1H, H-19), 2.39 (s, 1H, H-9), 2.20–1.05 (triterpene's H, 17H), 2.04 (s, 3H, CH_3CO),1.97 (s, 3H, CH_3CO), 1.92 (s, 3H, CH_3CO) (3 × CH_3CO), 1.30 (s, 3H, CH_3-27), 1.21 (s, 3H, CH_3-24), 0.97 (d, J = 6.2 Hz, 3H, CH_3-29), 0.88 (d, J = 6.3 Hz, 3H, CH_3-30), 0.85 (s, 6H, CH_3-24/25) (9 × CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.54, 174.74, 170.76, 170.45, 170.12, 163.24, 139.28, 130.29, 129.99, 115.28, 111.12, 107.75, 107.49, 74.86, 69.00, 65.23, 61.27, 53.30, 48.20, 47.33, 44.65, 44.13, 43.99, 41.88, 39.13, 38.68, 37.67, 36.55, 32.34, 30.41, 28.15, 24.49, 21.00, 20.97, 20.94, 20.84, 20.71, 18.91, 17.66, 17.11, 17.01, 13.79; ESI-HRMS m/z Calc for $C_{42}H_{56}O_8$ $[M+Na]^+$: 744.38822, found: 744.38568.

4.3.3.3. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-p-fluoroaniline (**4c**).

Yield 84.2%. m.p. 194.6–196.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (s, 1H, NH), 7.35 (d, J = 8.8 Hz, 2H, Ar–H), 7.29 (d, J = 8.9 Hz, 2H, Ar–H), 5.71 (s, 1H, H-12), 5.26–5.20 (m, 1H, H-2), 5.01 (d, J = 10.3 Hz, 1H, H-3), 3.78 (d, J = 11.9 Hz, 1H, H-23), 3.55 (d, J = 11.9 Hz, 1H, H-23), 3.15 (dd, J = 12.8, 4.6 Hz, 1H, H-19), 2.39 (s, 1H, H-9), 2.15–1.03 (triterpene's H, 17H), 2.05 (s, 3H, CH_3CO), 1.98 (s, 3H, CH_3CO), 1.93 (s, 3H, CH_3CO) (3 × CH_3CO), 1.31 (s, 3H, CH_3-27), 1.21 (s, 3H, CH_3-24), 0.97 (d, J = 6.2 Hz, 3H, CH_3-29), 0.88 (d, J = 6.4 Hz, 3H, CH_3-30), 0.85 (s, 3H, CH_3-25), 0.83 (s, 3H, CH_3-26) (9 × CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.53, 174.65, 170.75,

170.44, 170.12, 163.26, 136.77, 131.93 (2 × Ar–C), 130.26, 121.70 (2 × Ar–C), 117.03, 74.84, 68.99, 65.23, 61.27, 53.39, 48.19, 47.32, 44.66, 44.13, 44.01, 41.88, 39.16, 38.70, 37.66, 36.58, 32.34, 30.41, 28.16, 24.50, 21.02, 20.98, 20.95, 20.85, 20.73, 18.95, 17.66, 17.13, 17.01, 13.81; ESI-HRMS *m/z* Calc for $C_{42}H_{56}O_8$ $[M+Na]^+$: 744.38822, found: 744.38690.

*4.3.3.4. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-3-chloroaniline (**4d**).* Yield 87.1%. m.p. 183.0–186.2 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.54 (s, 1H, Ar–H), 7.51 (s, 1H, NH), 7.17 (m, 2H, Ar–H), 7.01 (d, *J* = 7.6 Hz, 1H, Ar–H), 5.70 (s, 1H, H-12), 5.26–5.20 (m, 1H, H-2), 5.00 (d, *J* = 10.2 Hz, 1H, H-3), 3.78 (d, *J* = 11.8 Hz, 1H, H-23), 3.54 (d, *J* = 11.9 Hz, 1H, H-23), 3.15 (dd, *J* = 12.8, 4.5 Hz, 1H, H-19), 2.38 (s, 1H, H-9), 2.20–1.05 (triterpene's H, 17H), 2.04 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 1.91 (s, 3H, CH_3CO) (3 × CH_3CO), 1.30 (s, 3H, CH_3-27), 1.21 (s, 3H, CH_3-24), 0.96 (d, *J* = 6.1 Hz, 3H, CH_3-29), 0.87 (d, *J* = 6.4 Hz, 3H, CH_3-30), 0.84 (s, 6H, CH_3-25/26) (9 × CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.44, 174.68, 170.67, 170.34, 170.02, 163.13, 138.81, 134.45, 130.20, 129.81, 124.33, 120.20, 118.06, 74.76, 68.91, 65.15, 61.16, 53.17, 48.09, 47.24, 44.56, 44.04, 43.89, 41.79, 39.01, 38.57, 37.58, 36.46, 32.26, 30.32, 28.05, 24.38, 20.91, 20.87, 20.85, 20.75, 20.62, 18.85, 17.58, 17.02, 16.92, 13.70; ESI-HRMS *m/z* Calc for $C_{42}H_{56}ClNO_8$ $[M+Na]^+$: 760.35866, found: 760.35742.

*4.3.3.5. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-4-chloroaniline (**4e**).* Yield 85.4%. m.p. 173.6–176.4 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 (s, 1H, Ar–H), 7.36 (d, *J* = 2.1 Hz, 1H, Ar–H), 7.28 (s, 1H, Ar–H), 7.24 (s, 1H, Ar–H), 7.23 (s, 1H, NH), 5.73 (s, 1H, H-12), 5.28–5.22 (m, 1H, H-2), 5.03 (d, *J* = 10.3 Hz, 1H, H-3), 3.81 (d, *J* = 11.9 Hz, 1H, H-23), 3.56 (d, *J* = 11.9 Hz, 1H, H-23), 3.17 (dd, *J* = 12.8, 4.7 Hz, 1H, H-19), 2.41 (s, 1H, H-9), 2.30–1.08 (triterpene's H, 17H), 2.07 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO) (3 × CH_3CO), 1.33 (s, 3H, CH_3-27), 1.23 (s, 3H, CH_3-24), 0.99 (d, *J* = 6.4 Hz, 3H, CH_3-29), 0.91 (d, *J* = 6.4 Hz, 3H, CH_3-30), 0.87 (s, 3H, CH_3-25), 0.85 (s, 3H, CH_3-26) (9 × CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 198.48, 174.29, 170.72, 170.42, 170.07, 163.27, 134.98, 134.11, 130.25, 129.47 (2 × Ar–C), 120.07 (2 × Ar–C), 74.86, 68.96, 65.21, 61.32, 53.62, 48.04, 47.32, 44.65, 44.15, 44.04, 41.88, 39.24, 38.76, 37.67, 36.71, 32.32, 30.46, 28.19, 24.60, 21.00, 20.95, 20.82, 20.78, 20.70, 19.01, 17.64, 17.14, 17.01, 13.78. ESI-HRMS *m/z* Calc for $C_{42}H_{56}ClNO_8$ $[M+Na]^+$: 760.35866, found: 760.35632.

*4.3.3.6. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-3-bromoaniline (**4f**).* Yield 84.7%. m.p. 174.6–181.9 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (s, 1H, NH), 7.40 (s, 1H, Ar–H), 7.26 (d, *J* = 8.0 Hz, 1H, Ar–H), 7.18 (d, *J* = 8.0 Hz, 1H, Ar–H), 7.11 (t, *J* = 8.0 Hz, 1H, Ar–H), 5.72 (s, 1H, H-12), 5.21–5.28 (m, 1H, H-2), 5.02 (d, *J* = 10.3 Hz, 1H, H-3), 3.80 (d, *J* = 11.9 Hz, 1H, H-23), 3.55 (d, *J* = 11.9 Hz, 1H, H-23), 3.16 (dd, *J* = 12.8, 4.6 Hz, 1H, H-19), 2.39 (s, 1H, H-9), 2.22–1.07 (triterpene's H, 17H), 2.06 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 1.93 (s, 3H, CH_3CO) (3 × CH_3CO), 1.31 (s, 3H, CH_3-27), 1.23 (s, 3H, CH_3-24), 0.98 (d, *J* = 6.1 Hz, 3H, CH_3-29), 0.89 (d, *J* = 6.4 Hz, 3H, CH_3-30), 0.86 (s, 6H, CH_3-26) (9 × CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.42, 174.63, 170.69, 170.38, 170.04, 163.03, 138.90, 130.25, 130.18, 127.35, 122.98, 122.54, 118.48, 74.79, 68.92, 65.18, 61.22, 53.30, 48.16, 47.28, 44.59, 44.09, 43.94, 41.83, 39.09, 38.63, 37.62, 36.55, 32.29, 30.35, 28.10, 24.48, 20.95, 20.91, 20.88, 20.79, 20.67, 18.90, 17.61, 17.06, 16.96, 13.75. ESI-HRMS *m/z* Calc for $C_{42}H_{56}NO_8$ $[M+Na]^+$: 804.30815, found: 804.30579.

*4.3.3.7. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-4-bromoaniline (**4g**).* Yield 85.4%. m.p. 188.1–192.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.42 (s, 1H, NH), 7.35 (d, *J* = 8.8 Hz, 2H, Ar–H), 7.29 (d, *J* = 8.9 Hz, 2H), 5.71 (s, 1H, H-12), 5.26–5.20 (m, 1H, H-2), 5.01 (d, *J* = 10.3 Hz, 1H, H-3), 3.78 (d, *J* = 11.9 Hz, 1H, H-23), 3.55 (d, *J* = 11.9 Hz, 1H, H-23), 3.15 (dd, *J* = 12.8, 4.6 Hz, 1H, H-19), 2.39 (s, 1H, H-9), 2.16–1.06 (triterpene's H, 17H), 2.05 (s, 3H, CH_3CO), 1.98 (s, 3H, CH_3CO), 1.93 (s, 3H, CH_3CO) (3 × CH_3CO), 1.31 (s, 3H, CH_3-27), 1.21 (s, 3H, CH_3-24), 0.97 (d, *J* = 6.2 Hz, 3H, CH_3-29), 0.88 (d, *J* = 6.4 Hz, 3H, CH_3-30), 0.85 (s, 3H, CH_3-24), 0.83 (s, 3H, CH_3-25) (9 × CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.44, 174.57, 170.67, 170.36, 170.04, 163.18, 136.68, 131.84 (2 × Ar–C), 130.18, 121.62 (2 × Ar–C), 116.94, 74.75, 68.91, 65.14, 61.19, 53.31, 48.10, 47.24, 44.58, 44.04, 43.93, 41.80, 39.08, 38.62, 37.58, 36.50, 32.25, 30.32, 28.07, 24.42, 20.94, 20.90, 20.86, 20.77, 20.65, 18.87, 17.58, 17.04, 16.93, 13.73. ESI-HRMS *m/z* Calc for $C_{42}H_{56}BrNO_8$ $[M+Na]^+$: 804.30815, found: 804.30719.

*4.3.3.8. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-3-toluidine (**4h**).* Yield 88.3%. m.p. 172.1–174.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (s, 1H, NH), 7.24 (d, *J* = 8.2 Hz, 2H, Ar–H), 7.02 (d, *J* = 7.7 Hz, 2H, Ar–H), 5.70 (s, 1H, H-12), 5.26–5.19 (m, 1H, H-2), 5.00 (d, *J* = 10.2 Hz, 1H, H-3), 3.77 (d, *J* = 11.8 Hz, 1H, H-23), 3.54 (d, *J* = 11.9 Hz, 1H, H-23), 3.15 (dd, *J* = 12.7, 4.4 Hz, 1H, H-19), 2.38 (s, 1H, H-9), 2.24 (s, 3H, Ar-CH_3), 2.17-1.04 (triterpene's H, 17H), 2.04 (s, 3H, CH_3CO), 1.96 (s, 3H, CH_3CO), 1.91 (s, 3H, CH_3CO) (3 × CH_3CO), 1.29 (s, 3H, CH_3-27), 1.20 (s, 3H, CH_3-24), 0.96 (d, *J* = 5.8 Hz, 3H, CH_3-29), 0.87 (s, 3H, CH_3-30), 0.85 (s, 3H, CH_3-25), 0.84 (s, 3H, CH_3-26). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.44, 174.57, 170.67, 170.36, 170.04, 163.18, 136.68, 131.84 (2 × Ar–C), 130.18, 121.62 (2 × Ar–C), 116.94, 74.75, 68.91, 65.14, 61.19, 53.31, 48.10, 47.24, 44.58, 44.04, 43.93, 41.80, 39.08, 38.62, 37.58, 36.50, 32.25, 30.32, 28.07, 24.42, 20.94, 20.90, 20.86, 20.77, 20.65, 18.87, 17.58, 17.04, 16.93, 13.73. ESI-HRMS *m/z* Calc for $C_{43}H_{59}NO_8$ $[M+Na]^+$: 740.41329, found: 740.41522.

*4.3.3.9. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-4-toluidine (**4i**).* Yield 87.2%. m.p. 178.9–182.0 °C. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.28 (d, *J* = 8.4 Hz, 2H, Ar–H), 7.20 (s, 1H, NH), 7.08 (d, *J* = 8.2 Hz, 2H, Ar–H), 5.74 (s, 1H, H-12), 5.29–5.23 (m, 1H, H-2), 5.03 (d, *J* = 10.3 Hz, 1H, H-3), 3.80 (d, *J* = 11.9 Hz, 1H, H-23), 3.56 (d, *J* = 11.9 Hz, 1H, H-23), 3.17 (dd, *J* = 12.8, 4.7 Hz, 1H, H-19), 2.41 (s, 1H, H-9), 2.28 (s, 3H, Ar–CH_3), 2.19–1.05 (triterpene's H, 17H), 2.07 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO) (3 × CH_3CO), 1.32 (s, 3H, CH_3-27), 1.23 (s, 3H, CH_3-24), 0.99 (s, 3H, CH_3-25), 0.91 (s, 3H, CH_3-26), 0.87 (d, *J* = 3.7 Hz, 6H, CH_3-29/30) (9 × CH_3). ^{13}C NMR (126 MHz, $CDCl_3$) δ 198.48, 174.29, 170.72, 170.42, 170.07, 163.27, 134.98, 134.11, 130.25, 129.47 (2 × Ar–C), 120.07 (2 × Ar–C), 74.86, 68.96, 65.21, 61.32, 53.62, 48.04, 47.32, 44.65, 44.15, 44.04, 41.88, 39.24, 38.76, 37.67, 36.71, 32.32, 30.46, 28.19, 24.60, 21.00, 20.95, 20.82, 20.78, 20.70, 19.01, 17.64, 17.14, 17.01, 13.78. ESI-HRMS *m/z* Calc for $C_{43}H_{59}NO_8$ $[M+Na]^+$: 740.41329, found: 740.41229.

*4.3.3.10. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-2-methoxyaniline (**4j**).* Yield 85.2%. m.p. 170.9–173.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.31 (d, *J* = 7.9 Hz, 1H, Ar–H), 8.08 (s, 1H, NH), 6.99 (t, *J* = 7.7 Hz, 1H, Ar–H), 6.89 (t, *J* = 7.7 Hz, 1H, Ar–H), 6.82 (d, *J* = 8.0 Hz, 1H, Ar–H), 5.76 (s, 1H, H-12), 5.21–5.28 (m, 1H, H-2), 5.02 (d, *J* = 10.3 Hz, 1H, H-3), 3.84 (s, 3H, Ar–CH_3), 3.79 (d, *J* = 11.9 Hz, 1H, H-23), 3.56 (d, *J* = 11.9 Hz, 1H, H-23), 3.17 (dd, *J* = 12.7, 4.6 Hz, 1H, H-19), 2.42 (s, 1H, H-9), 2.27–1.07 (triterpene's H, 17H), 2.06 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 1.94 (s, 3H, CH_3CO), 1.32 (s, 3H, CH_3-27), 1.21 (s, 3H, CH_3-24), 0.99 (d, *J* = 6.1 Hz, 3H, CH_3-29), 0.92 (d, *J* = 6.4 Hz, 3H, CH_3-30), 0.85 (s, 3H, CH_3-25), 0.79 (s, 3H, CH_3-26) (9 × CH_3). ^{13}C NMR (101 MHz, CDCl3) δ 198.54, 174.54, 170.67, 170.36, 170.03, 162.50, 148.07, 130.86, 127.31, 123.65, 120.93, 119.69, 109.68, 74.76, 68.95, 65.13, 61.31, 55.62, 53.59, 48.74, 47.25, 44.70, 44.09, 43.89, 41.84, 39.35, 38.78, 37.58, 36.71, 32.14, 30.50, 28.10, 24.59, 20.97, 20.91, 20.79, 20.67, 18.32, 17.64, 17.14, 16.97,

13.74. ESI-HRMS *m/z* Calc for $C_{43}H_{59}NO_9$ $[M-H]^-$: 732.41171, found: 732.41238.

*4.3.3.11. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-3-methoxyaniline (**4k**).* Yield 84.2%. m.p. 175.8–179.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.34 (s, 1H, NH), 7.19 (s, 1H, Ar–H), 7.12 (d, J = 8.2 Hz, 1H, Ar–H), 6.82 (d, J = 8.0 Hz, 1H, Ar–H), 6.61 (dd, J = 8.2, 2.1 Hz, 1H, Ar–H), 5.72 (s, 1H, H-12), 5.27–5.20 (m, 1H, H-2), 5.01 (d, J = 10.3 Hz, 1H, H-3), 3.79 (d, J = 11.9 Hz, 1H, H-23), 3.74 (s, 3H, Ar–CH_3), 3.55 (d, J = 11.9 Hz, 1H, H-23), 3.16 (dd, J = 12.8, 4.6 Hz, 1H, H-19), 2.39 (s, 1H, H-9), 2.27–1.07 (triterpene's H, 17H), 2.05 (s, 3H, CH_3CO), 1.98 (s, 3H, CH_3CO), 1.93 (s, 3H, CH_3CO) (3 × CH_3CO), 1.31 (s, 3H, CH_3-27), 1.21 (s, 3H, CH_3-24), 0.97 (d, J = 6.2 Hz, 3H, CH_3-29), 0.89 (d, J = 6.4 Hz, 3H, CH_3-30), 0.86 (s, 3H, CH_3-25), 0.85 (s, 3H, CH_3-25) (9 × CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.47, 174.45, 170.68, 170.37, 170.03, 163.22, 160.04, 138.79, 130.20, 129.52, 112.04, 110.29, 105.68, 74.77, 68.91, 65.13, 61.20, 55.19, 53.36, 48.07, 47.24, 44.59, 44.05, 43.93, 41.79, 39.11, 38.63, 37.58, 36.55, 32.25, 30.36, 28.09, 24.49, 20.93, 20.89, 20.77, 20.64, 18.85, 17.59, 17.05, 16.93, 13.72. ESI-HRMS *m/z* Calc for $C_{43}H_{59}NO_9$ $[M+Na]^+$: 756.40820, found: 756.40729.

*4.3.3.12. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-4-methoxyaniline (**4l**).* Yield 87.0%. m.p. 186.1–189.0 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29 (s, 1H, NH), 7.27 (d, J = 4.0 Hz, 2H, Ar–H), 6.80 (d, J = 8.9 Hz, 2H, Ar–H), 5.72 (s, 1H, H-12), 5.26–5.20 (m, 1H, H-2), 5.02 (d, J = 10.3 Hz, 1H, H-3), 3.80 (d, J = 11.9 Hz, 1H, H-23), 3.75 (s, 3H, Ar–CH_3), 3.56 (d, J = 11.9 Hz, 1H, H-23), 3.17 (dd, J = 12.8, 4.6 Hz, 1H, H-19), 2.41 (s, 1H, H-9), 2.18–1.07 (triterpene's H, 17H), 2.07 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 1.94 (s, 3H, CH_3CO) (3 × CH_3CO), 1.32 (s, 3H, CH_3-27), 1.23 (s, 3H, CH_3-24), 0.99 (d, J = 6.2 Hz, 3H, CH_3-29), 0.91 (s, 3H, CH_3-30), 0.89 (s, 3H, CH_3-25), 0.87 (s, 3H, CH_3-26) (9 × CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.58, 174.37, 170.77, 170.46, 170.13, 163.45, 156.54, 130.66, 130.24, 122.02 (2 × Ar–C), 114.14 (2 × Ar–C), 74.86, 69.00, 65.22, 61.30, 55.44, 53.54, 47.93, 47.32, 44.68, 44.14, 44.06, 41.89, 39.22, 38.75, 37.68, 36.74, 32.36, 30.48, 28.20, 24.52, 21.01, 20.98, 20.86, 20.74, 19.08, 17.67, 17.16, 17.03, 13.82. ESI-HRMS *m/z* Calc for $C_{43}H_{59}NO_9$ $[M+Na]^+$: 756.40820, found: 756.40796.

*4.3.3.13. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-3-chloro-4-fluoroaniline (**4m**).* Yield 85.7%. m.p. 188.1–191.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, J = 6.5 Hz, 1H, Ar–H), 7.35 (s, 1H, NH), 7.17 (m, 1H, Ar–H), 7.03 (t, J = 8.7 Hz, 1H, Ar–H), 5.72 (s, 1H, H-12), 5.28–5.21 (m, 1H, H-2), 5.03 (d, J = 10.3 Hz, 1H, H-3), 3.80 (d, J = 11.9 Hz, 1H, H-23), 3.56 (d, J = 11.9 Hz, 1H, H-23), 3.16 (dd, J = 12.8, 4.7 Hz, 1H, H-19), 2.40 (s, 1H, H-9), 2.30–1.07 (triterpene's H, 17H), 2.07 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 1.94 (s, 3H, CH_3CO) (3 × CH_3CO), 1.32 (s, 3H, CH_3-27), 1.23 (s, 3H, CH_3-24), 0.99 (d, J = 6.2 Hz, 3H, CH_3-29), 0.90 (d, J = 6.4 Hz, 3H, CH_3-30), 0.87 (s, 3H, CH_3-25), 0.85 (s, 3H, CH_3-26) (9 × CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.43, 174.67, 170.73, 170.43, 170.08, 163.00, 134.23, 130.29, 122.37 (2 × Ar–C), 119.72, 116.69, 116.47, 74.83, 68.96, 65.22, 61.29, 53.45, 48.19, 47.33, 44.66, 44.14, 44.02, 41.89, 39.17, 38.72, 37.68, 36.66, 32.35, 30.38, 29.66, 28.14, 24.54, 21.01, 20.97, 20.93, 20.85, 20.72, 18.98, 17.66, 17.12, 17.02, 13.80. ESI-HRMS *m/z* Calc for $C_{42}H_{55}ClO_8$ $[M+Na]^+$: 778.34924, found: 778.34692.

*4.3.3.14. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-3-chloro-4-methylaniline (**4n**).* Yield 83.7%. m.p. 188.1–191.3 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (s, 1H, NH), 7.35 (s, 1H, Ar–H), 7.14–7.05 (m, 2H, Ar–H), 5.71 (s, 1H, H-12), 5.27–5.20 (m, 1H, H-2), 5.02 (d, J = 10.2 Hz, 1H, H-3), 3.79 (d, J = 11.9 Hz, 1H, H-23), 3.55 (d, J = 11.9 Hz, 1H, H-23), 3.16 (dd, J = 12.8, 4.6 Hz, 1H, H-19), 2.39 (s, 1H, H-9), 2.31–1.04 (triterpene's H, 17H), 2.27 (s, 3H, Ar–CH_3), 2.06 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 1.93 (s, 3H, CH_3CO) (3 × CH_3CO), 1.31 (s, 3H, CH_3-27), 1.22 (s, 3H, CH_3-24), 0.97 (d, J = 6.1 Hz, 3H, CH_3-29), 0.88 (d, J = 6.2 Hz, 3H, CH_3-30), 0.85 (s, 6H, CH_3-25/26) (9 × CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.44, 174.50, 170.72, 170.39, 170.04, 163.16, 136.30, 134.32, 131.92, 130.84, 130.18, 120.66, 118.31, 74.73, 68.89, 65.11, 61.18, 53.28, 48.02, 47.19, 44.55, 44.03, 43.91, 41.78, 39.07, 38.61, 37.58, 36.53, 32.24, 30.34, 29.60, 28.06, 24.43, 20.94, 20.90, 20.82, 20.69, 19.36, 18.89, 17.62, 17.07, 16.92, 13.75. ESI-HRMS *m/z* Calc for $C_{43}H_{58}ClNO_8$ $[M+Na]^+$: 774.37431, found: 774.37244.

*4.3.3.15. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-3,4-dichloroaniline (**4o**).* Yield 84.7%. m.p. 181.9–193.6 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (s, 1H, NH), 7.60 (s, 1H, Ar–H), 7.34–7.29 (m, 1H, Ar–H), 7.23 (dd, J = 8.7, 2.3 Hz, 1H, Ar–H), 5.74 (s, 1H, H-12), 5.30–5.23 (m, 1H, H-2), 5.04 (d, J = 10.2 Hz, 1H, H-3), 3.82 (d, J = 11.8 Hz, 1H, H-23), 3.58 (d, J = 11.9 Hz, 1H, H-23), 3.18 (dd, J = 12.8, 4.5 Hz, 1H, H-19), 2.42 (s, 1H, H-9), 2.37–1.08 (triterpene's H, 17H), 2.09 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 1.96 (s, 3H, CH_3CO) (3 × CH_3CO), 1.34 (s, 3H, CH_3-27), 1.25 (s, 3H, CH_3-24), 1.00 (d, J = 6.0 Hz, 3H, CH_3-29), 0.91 (d, J = 6.2 Hz, 3H, CH_3-30), 0.89 (s, 3H, CH_3-25), 0.87 (s, 3H, CH_3-26) (9 × CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.49, 174.80, 170.71, 170.38, 170.07, 163.13, 137.16, 132.58, 130.33, 130.18, 127.47, 121.77, 119.33, 74.73, 68.92, 65.14, 61.15, 53.13, 48.15, 47.23, 44.58, 44.01, 43.88, 41.79, 39.00, 38.57, 37.58, 36.43, 32.25, 30.28, 28.04, 24.34, 20.93, 20.90, 20.84, 20.78, 20.65, 18.85, 17.59, 17.01, 16.91, 13.72. ESI-HRMS *m/z* Calc for $C_{42}H_{55}Cl_2NO_8$ $[M+Na]^+$: 794.31969, found: 794.31830.

*4.3.3.16. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-4-bromine-3-fluoroaniline (**4p**).* Yield 88.9%. m.p. 173.9–178.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.18 (s, 1H, NH), 8.16 (s, 1H, Ar–H), 7.65–7.62 (m, 2H, Ar–H), 5.76 (s, 1H, H-12), 5.22–5.28 (m, 1H, H-2), 5.03 (d, J = 10.2 Hz, 1H, H-3), 3.81 (d, J = 11.7 Hz, 1H, H-23), 3.58 (s, 1H, H-23), 3.16 (dd, J = 12.8, 4.5 Hz, 1H, H-19), 2.41 (s, 1H, H-9), 2.35–1.14 (triterpene's H, 17H), 2.07 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO) (3 × CH_3CO), 1.34 (s, 4H, CH_3-27), 1.23 (s, 3H, CH_3-24), 1.01 (d, J = 5.9 Hz, 3H, CH_3-29), 0.92 (d, J = 6.4 Hz, 3H, CH_3-30), 0.87 (s, 3H, CH_3-25), 0.82 (s, 3H, CH_3-26) (9 × CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.42, 175.10, 170.75, 170.46, 170.11, 162.80, 143.70, 143.51, 130.37, 125.10 (2 × Ar–C), 119.26 (2 × Ar–C), 74.81, 68.95, 65.22, 61.30, 53.42, 48.62, 47.31, 44.68, 44.13, 44.02, 41.90, 39.19, 38.69, 37.78, 37.67, 36.53, 32.32, 30.35, 28.15, 24.63, 20.99, 20.93, 20.87, 20.74, 18.87, 17.68, 17.12, 17.00, 13.82. ESI-HRMS *m/z* Calc for $C_{42}H_{55}BrO_8$ $[M-H]^-$: 798.30223, found: 798.30305.

*4.3.3.17. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-2-methyl-4-bromineaniline (**4q**).* Yield 85.9%. m.p. 193.9–196.5 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.62 (d, J = 8.5 Hz, 1H, NH), 7.30 (s, 1H, Ar–H), 7.28–7.25 (m, 1H, Ar–H), 7.21 (s, 1H, Ar–H), 5.68 (s, 1H, H-12), 5.30–5.24 (m, 1H, H-2), 5.04 (d, J = 10.3 Hz, 1H, H-3), 3.83 (d, J = 11.9 Hz, 1H, H-23), 3.58 (d, J = 11.9 Hz, 1H, H-23), 3.18 (dd, J = 12.8, 4.6 Hz, 1H, H-19), 2.41 (s, 1H, H-9), 2.33–1.09 (triterpene's H, 17H), 2.19 (s, 3H, Ar–CH_3), 2.09 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO) (3 × CH_3CO), 1.34 (s, 3H, CH_3-27), 1.25 (s, 3H, CH_3-24), 1.01 (d, J = 6.2 Hz, 3H, CH_3-29), 0.91 (d, J = 5.5 Hz, 6H, CH_3-25/30), 0.89 (s, 3H, CH_3-26) (9 × CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.29, 174.43, 170.69, 170.38, 170.01, 162.76, 134.67, 133.13, 130.94, 130.53, 129.69, 124.28, 117.81, 74.81, 68.91, 65.19, 61.18, 53.51, 48.38, 47.31, 44.62, 44.09, 43.97, 41.85, 39.11, 38.69, 37.65, 37.15, 32.45, 30.42, 28.09, 24.70, 21.03, 20.98, 20.90, 20.81, 20.68, 19.20, 17.64, 17.55, 17.06, 17.00, 13.77. ESI-HRMS *m/z* Calc for $C_{43}H_{58}BrNO_8$ $[M+Na]^+$: 818.32380, found: 818.32220.

*4.3.3.18. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-2–5-dimethylaniline (**4r**).* Yield 87.9%. m.p. 173.8–176.7 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.17 (s, 1H, NH), 7.04 (s, 2H, Ar–H), 6.71 (s, 1H, Ar–H), 5.73 (s, 1H, H-12), 5.25–5.20 (m, 1H, H-2), 5.02 (d, J = 10.3 Hz, 1H, H-3), 3.80 (d, J = 11.9 Hz, 1H, H-23), 3.56 (d, J = 11.9 Hz, 1H, H-23), 3.18 (dd, J = 12.8, 4.6 Hz, 1H, H-19), 2.40 (s, 1H, H-9), 2.25 (s, 6H, Ar–CH_3 × 2), 2.16–1.03 (triterpene's H, 17H), 2.07 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 1.94 (s, 3H, CH_3CO) (3 × CH_3CO), 1.32 (s, 3H, CH_3-27), 1.23 (s, 3H, CH_3-24), 0.98 (d, J = 6.2 Hz, 3H, CH_3-29), 0.90 (d, J = 6.4 Hz, 3H, CH_3-30), 0.88 (s, 3H, CH_3-25), 0.86 (s, 3H, CH_3-26) (9 × CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.47, 174.32, 170.70, 170.40, 170.04, 163.19, 138.64 (2 × Ar–C), 137.39, 130.23, 126.16, 117.66 (2 × Ar–C), 74.80, 68.93, 65.16, 61.26, 53.53, 48.07, 47.27, 44.59, 44.11, 43.99, 41.83, 39.18, 38.71, 37.63, 36.70, 32.30, 30.42, 29.61, 28.14, 24.56, 21.28, 21.13, 20.97, 20.93, 20.81, 20.68, 18.98, 17.62, 17.11, 16.98, 13.76. ESI-HRMS *m/z* Calc for $C_{44}H_{61}NO_8$ $[M+Na]^+$: 754.42894, found: 754.42688.

*4.3.3.19. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-1-naphthylamine (**4s**).* Yield 84.9%. m.p. 178.8–183.7 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86–7.82 (m, 1H, Ar–H), 7.79 (s, 1H, NH), 7.77 (s, 1H, Ar–H), 7.72 (d, J = 7.9 Hz, 1H, Ar–H), 7.66 (d, J = 8.2 Hz, 1H, Ar–H), 7.51–7.40 (m, 3H, Ar–H), 5.78 (s, 1H, H-12), 5.29–5.22 (m, 1H, H-2), 5.03 (d, J = 10.3 Hz, 1H, H-3), 3.80 (d, J = 11.9 Hz, 1H, H-23), 3.56 (d, J = 11.9 Hz, 1H, H-23), 3.18 (dd, J = 12.8, 4.6 Hz, 1H, H-19), 2.40 (s, 1H, H-9), 2.26–1.13(triterpene's H, 17H), 2.08 (s, 3H, CH_3CO), 1.99 (s, 3H, CH_3CO), 1.94 (s, 3H, CH_3CO) (3 × CH_3CO), 1.34 (s, 3H, CH_3-27), 1.20 (s, 3H, CH_3-24), 1.00 (d, J = 6.2 Hz, 3H, CH_3-29), 0.92 (s, 3H, CH_3-30), 0.90 (s, 3H, CH_3-25), 0.85 (s, 3H, CH_3-26) (9 × CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 198.35, 174.93, 170.72, 170.40, 170.03, 163.04, 134.09, 132.08, 130.59, 128.80, 127.31, 126.36, 125.97, 125.70, 125.65, 120.92, 120.27, 74.85, 68.95, 65.21, 61.19, 53.50, 48.49, 47.34, 44.67, 44.12, 44.04, 41.85, 39.19, 38.69, 37.67, 37.24, 32.53, 30.53, 29.63, 28.18, 24.70, 21.01, 20.94, 20.82, 20.69, 19.31, 17.65, 17.08, 17.00, 13.77. ESI-HRMS *m/z* Calc for $C_{46}H_{59}NO_8$ $[M+Na]^+$: 776.41329, found: 776.41241.

*4.3.3.20. N-[2α,3β, 23-Triacetoxyurs-11-oxo-12-ene-28-oyl]-phenylamine (**4t**).* Yield 85.9%. m.p. 177.9–182.8 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, J = 7.6 Hz, 2H, Ar–H), 7.28 (m, 3H, Ar–H, NH), 7.08 (t, J = 7.4 Hz, 1H, Ar–H), 5.75 (s, 1H, H-12), 5.29–5.23 (m, 1H, H-2), 5.03 (d, J = 10.3 Hz, 1H, H-3), 3.81 (d, J = 11.9 Hz, 1H, H-23), 3.57 (d, J = 11.9 Hz, 1H, H-23), 3.18 (dd, J = 12.8, 4.6 Hz, 1H, H-19), 2.41 (s, 1H, H-9), 2.17–1.03 (triterpene's H, 17H), 2.08 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO) (3 × CH_3CO), 1.33 (s, 3H, CH_3-27), 1.24 (s, 3H, CH_3-24), 1.00 (d, J = 6.3 Hz, 3H, CH_3-29), 0.92 (d, J = 6.4 Hz, 3H, CH_3-30), 0.88 (d, J = 5.1 Hz, 6H, CH_3-25/26). ^{13}C NMR (101 MHz, $CDCl_3$) δ 199.93, 175.89, 172.17, 171.87, 171.52, 164.66, 139.00, 131.73, 130.43 (2 × Ar–C), 125.91, 121.49 (2 × Ar–C), 76.29, 70.41, 66.65, 62.75, 55.00, 49.56, 48.76, 46.09, 45.59, 45.48, 43.32, 40.67, 40.18, 39.11, 38.14, 33.77, 31.89, 29.62, 26.06, 22.45, 22.40, 22.28, 22.16, 20.42, 19.09, 18.59, 18.45, 15.24. ESI-HRMS *m/z* Calc for $C_{42}H_{57}NO_8$ $[M+Na]^+$: 726.39764, found: 726.40063.

*4.3.4. General procedure for the preparation of compounds **5***

To a solution of **4** (0.12 mmol) in MeOH (4 mL) and THF (6 mL), 4 N NaOH (1.2 mL) was added dropwise, and the resulting mixture was stirred room temperature for 2 h. The mixture was acidified with hydrochloric acid and extracted with ethyl acetate. The organic phase was washed with sodium bicarbonate solution and brine in sequence, and concentrated under reduced pressure. The crude product was purified column chromatography with a gradient elution of CH_2Cl_2/MeOH (V:V = 40:1) to give a white solid of **5**.

*4.3.4.1. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-2-fluoroaniline (**5a**).* Yield 65.9%. m.p. 197.5–201.8 °C. ^{1}H NMR (400 MHz, MeOD) δ 7.50 (t, J = 7.8 Hz, 1H, Ar–H), 7.18–7.10 (m, 3H, Ar–H), 5.66 (s, 1H, H-12), 3.75 (td, J = 11.2, 4.4 Hz, 1H, H-2), 3.52 (d, J = 11.1 Hz, 1H, H-, H-33), 3.37 (d, J = 9.6 Hz, 1H, H-23), 3.26 (d, J = 11.1 Hz, 1H, H-23), 3.06 (dd, J = 12.7, 4.3 Hz, 1H, H-19), 2.56 (d, J = 10.8 Hz, 1H, H-16), 2.50 (s, 1H, H-9), 2.34–2.27 (m, 1H, H-19), 1.99–1.29 (m, 15H), 1.40 (s, 3H, CH_3-27), 1.20 (s, 3H, CH_3-24), 1.02 (d, J = 6.3 Hz, 3H, CH_3-29), 0.95 (s, 3H, CH_3-30), 0.93 (d, J = 6.5 Hz, 3H, CH_3-25), 0.69 (s, 3H, CH_3-26). ^{13}C NMR (101 MHz, MeOD) δ 201.90, 178.01, 166.10, 158.56, 131.54, 127.81, 125.30, 125.26, 116.76, 116.56, 77.76, 69.39, 65.97, 62.66, 54.12, 49.44, 48.29, 47.84, 46.08, 45.40, 44.27, 40.07, 40.01, 39.09, 37.94, 33.57, 31.62, 29.39, 24.89, 21.53, 21.38, 19.97, 18.39, 18.19, 17.67, 13.95. ESI-HRMS *m/z* Calc for $C_{36}H_{50}O_5$ $[M+Na]^+$: 618.35652, found: 618.35524.

*4.3.4.2. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-3-fluoroaniline (**5b**).* Yield 64.3%. m.p. 215.5–218.2 °C. ^{1}H NMR (500 MHz, MeOD) δ 7.62 (t, J = 2.0 Hz, 1H, Ar–H), 7.39–7.32 (m, 1H, Ar–H), 7.23 (t, J = 8.1 Hz, 1H, Ar–H), 7.09–7.02 (m, 1H, 1H), 5.65 (s, 1H, H-12), 3.73 (td, J = 11.3, 4.5 Hz, 1H, H-2), 3.50 (d, J = 11.2 Hz, 1H, H-3), 3.34 (d, J = 9.6 Hz, 1H, H-23), 3.23 (d, J = 11.1 Hz, 1H, H-23), 3.04 (dd, J = 12.7, 4.4 Hz, 1H, H-19), 2.58 (d, J = 10.9 Hz, 1H, H-16), 2.48 (s, 1H, H-9), 2.33–2.24 (m, 1H, H-19), 1.95–1.23(m, 15H), 1.38 (s, 3H, CH_3-27), 1.17 (s, 3H, CH_3-24), 1.00 (d, J = 6.4 Hz, 3H, CH_3-29), 0.92 (d, J = 6.5 Hz, 3H, CH_3-30), 0.88 (s, 3H, CH_3-25), 0.67 (s, 3H, CH_3-26). ^{13}C NMR (126 MHz, MeOD) δ 202.04, 177.68, 166.60, 141.66, 131.36, 131.07, 117.56, 111.62, 109.96, 109.05, 77.75, 69.40, 65.95, 62.67, 54.01, 49.49, 48.28, 47.83, 46.18, 45.35, 44.27, 40.05, 40.01, 39.08, 37.40, 33.47, 31.55, 29.41, 24.68, 21.66, 21.35, 19.85, 18.38, 18.17, 17.68, 13.94. ESI-HRMS *m/z* Calc for $C_{36}H_{50}O_5$ $[M+Na]^+$: 618.35652, found: 618.35466.

*4.3.4.3. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-4-fluoroaniline (**5c**).* Yield 66.7%. m.p. 203.2–206.1 °C. ^{1}H NMR (400 MHz, MeOD) δ 7.45–7.38 (m, 2H, Ar–H), 7.01 (m, 2H, Ar–H), 5.66 (s, 1H, H-12), 3.74 (td, J = 11.3, 4.4 Hz, 1H, H-2), 3.51 (d, J = 11.2 Hz, 1H, H-3), 3.36 (d, J = 9.6 Hz, 1H, H-23), 3.24 (d, J = 11.1 Hz, 1H, H-23), 3.05 (dd, J = 12.7, 4.4 Hz, 1H, H-19), 2.59 (d, J = 10.9 Hz, 1H, H-16), 2.50 (s, 1H, H-9), 2.33–2.25 (m, 1H, H-19), 1.95–1.25 (m, 15H), 1.40 (s, 3H, CH_3-27), 1.18 (s, 3H, CH_3-24), 1.02 (d, J = 6.3 Hz, 3H, CH_3-29), 0.93 (d, J = 6.4 Hz, 3H, CH_3-30), 0.92 (s, 3H, CH_3-25), 0.68 (s, 3H, CH_3-26). ^{13}C NMR (101 MHz, MeOD) δ 202.42, 178.04, 166.99, 138.95, 131.81, 130.82, 130.13 (2 × Ar–C), 124.28 (2 × Ar–C), 78.20, 69.83, 66.41, 63.10, 54.47, 49.82, 48.71, 48.28, 46.62, 45.79, 44.74, 40.70, 40.46, 39.52, 37.94, 33.93, 32.00, 29.86, 25.14, 22.09, 21.79, 20.37, 18.83, 18.61, 18.12, 14.38. ESI-HRMS *m/z* Calc for $C_{36}H_{50}O_5$ $[M+Na]^+$: 618.35652, found: 618.35452.

*4.3.4.4. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-3-chloroaniline (**5d**).* Yield 62.4%. m.p. 215.6–218.2 °C. ^{1}H NMR (500 MHz, MeOD) δ 7.63 (t, J = 2.0 Hz, 1H, Ar–H), 7.40–7.34 (m, 1H, Ar–H), 7.24 (t, J = 8.1 Hz, 1H, Ar–H), 7.09–7.03 (m, 1H), 5.66 (s, 1H, H-12), 3.74 (td, J = 11.3, 4.5 Hz, 1H, H-2), 3.51 (d, J = 11.2 Hz, 1H, H-3), 3.36 (d, J = 9.6 Hz, 1H, H-23), 3.24 (d, J = 11.1 Hz, 1H, H-23), 3.05 (dd, J = 12.7, 4.4 Hz, 1H, H-19), 2.58 (d, J = 10.9 Hz, 1H, H-16), 2.49 (s, 1H, H-9), 2.34–2.25 (m, 1H, H-19), 1.96–1.22 (m, 15H), 1.39 (s, 3H, CH_3-27), 1.18 (s, 3H, CH_3-24), 1.01 (d, J = 6.4 Hz, 3H, CH_3-29), 0.93 (d, J = 6.5 Hz, 3H, CH_3-30), 0.90 (s, 3H, CH_3-25), 0.68 (s, 3H, CH_3-26). ^{13}C NMR (126 MHz, MeOD) δ 202.00, 177.66, 166.53, 141.15, 135.23, 131.34, 130.95, 125.18, 122.13, 120.32, 77.75, 69.36, 65.97, 62.63, 53.98, 49.44, 48.25, 47.82, 46.16, 45.32, 44.24, 40.01, 39.96, 39.05, 37.39, 33.45, 31.52, 29.38, 24.67, 21.63, 21.33, 19.86, 18.37, 18.15, 17.65, 13.91. ESI-HRMS *m/z* Calc for $C_{36}H_{50}ClNO_5$ $[M+Na]^+$: 634.32697, found: 634.32498.

*4.3.4.5. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-4-chloroaniline (**5e**).* Yield 62.4%. m.p. 208.4–211.2 °C. ^{1}H NMR (400 MHz, MeOD) δ 7.46 (d, J = 8.8 Hz, 2H, Ar–H), 7.26 (d, J = 8.8 Hz, 2H, Ar–H), 5.66 (s, 1H, H-12), 3.74 (td, J = 11.2, 4.4 Hz, 1H, H-2), 3.51 (d, J = 11.1 Hz, 1H, H-3), 3.36 (d, J = 9.6 Hz, 1H, H-23), 3.24 (d, J = 11.1 Hz, 1H, H-23), 3.05 (dd, J = 12.7, 4.3 Hz, 1H, H-19), 2.58 (d, J = 10.9 Hz, 1H, H-16), 2.49 (s, 1H, H-9), 2.33–2.25 (m, 1H, H-19), 1.95–1.27 (m, 15H), 1.39 (s, 3H, CH_3-27), 1.18 (s, 3H, CH_3-24), 1.01 (d, J = 6.2 Hz, 3H, CH_3-29), 0.93 (d, J = 6.2 Hz, 3H, CH_3-30), 0.90 (s, 3H, CH_3-25), 0.68 (s, 3H, CH_3-26). ^{13}C NMR (101 MHz, MeOD) δ 201.97, 177.59, 166.54, 138.51, 131.36, 130.37, 129.68 (2 × Ar–C), 123.84 (2 × Ar–C), 77.75, 69.38, 65.97, 62.65, 54.02, 49.37, 48.26, 47.83, 46.17, 45.34, 44.25, 40.03, 40.01, 39.07, 37.49, 33.48, 31.55, 29.41, 24.70, 21.65, 21.35, 19.93, 18.38, 18.17, 17.67, 13.93. ESI-HRMS *m/z* Calc for $C_{36}H_{50}ClNO_5$ $[M+Na]^+$: 634.32697, found: 634.32449.

*4.3.4.6. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-3-bromaniline (**5f**).* Yield 66.7%. m.p. 204.6–207.8 °C. ^{1}H NMR (400 MHz, MeOD) δ 7.78 (t, J = 1.8 Hz, 1H, Ar–H), 7.43 - 7.40 (m, 1H, Ar–H), 7.23–7.16 (m, 2H, Ar–H), 5.66 (s, 1H, H-12), 3.74 (td, J = 11.3, 4.4 Hz, 1H, H-2), 3.51 (d, J = 11.2 Hz, 1H, H-3), 3.36 (d, J = 9.6 Hz, 1H, H-23), 3.24 (d, J = 11.1 Hz, 1H, H-23), 3.05 (dd, J = 12.7, 4.4 Hz, 1H, H-19), 2.58 (d, J = 10.9 Hz, 1H, H-16), 2.49 (s, 1H, H-9), 2.33–2.25 (m, 1H, H-19), 1.95–1.24 (m, 15H), 1.39 (s, 3H, CH_3-27), 1.18 (s, 3H, CH_3-24), 1.01 (d, J = 6.3 Hz, 3H, CH_3-29), 0.94 (s, 3H, CH_3-30), 0.90 (s, 3H, CH_3-25), 0.68 (s, 3H, CH_3-26). ^{13}C NMR (101 MHz, MeOD) δ 202.00, 177.67, 166.52, 141.30, 131.36, 131.25, 128.18, 125.08, 123.14, 120.78, 77.75, 69.38, 65.98, 62.66, 54.00, 49.70, 48.3, 47.8, 46.18, 45.34, 44.25, 40.02, 39.99, 39.07, 37.41, 33.47, 31.54, 29.40, 24.69, 21.65, 21.35, 19.88, 18.39, 18.17, 17.67, 13.93. ESI-HRMS *m/z* Calc for $C_{36}H_{50}BrNO_5$ $[M+Na]^+$: 678.27645, found: 678.27655.

*4.3.4.7. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-4-bromaniline (**5g**).* Yield 62.9%. m.p. 222.2–225.0 °C. ^{1}H NMR (400 MHz, MeOD) δ 7.45 (d, J = 8.8 Hz, 2H, Ar–H), 7.24 (d, J = 8.8 Hz, 2H, Ar–H), 5.65 (s, 1H, H-12), 3.73 (td, J = 11.2, 4.4 Hz, 1H, H-2), 3.49 (d, J = 11.1 Hz, 1H, H-3), 3.37 (d, J = 9.6 Hz, 1H, H-23), 3.23 (d, J = 11.1 Hz, 1H, H-23), 3.04 (dd, J = 12.7, 4.3 Hz, 1H, H-19), 2.57 (d, J = 10.9 Hz, 1H, H-16), 2.48 (s, 1H, H-9), 2.32–2.24 (m, 1H, H-19), 1.94–1.26 (m, 15H), 1.38 (s, 3H, CH_3-27), 1.17 (s, 3H, CH_3-24), 1.02 (d, J = 6.2 Hz, 3H, CH_3-29), 0.92 (d, J = 6.2 Hz, 3H, CH_3-30), 0.89 (s, 3H, CH_3-25), 0.67 (s, 3H, CH_3-26). ^{13}C NMR (126 MHz, MeOD) δ 201.95, 177.54, 166.52, 138.97, 132.69 (2 × Ar–C), 131.33, 124.13 (2 × Ar–C), 117.88, 77.75, 69.36, 65.98, 62.62, 53.99, 49.38, 48.24, 47.82, 46.15, 45.32, 44.23, 40.00, 39.98, 39.05, 37.44, 33.47, 31.53, 29.40, 24.68, 21.65, 21.36, 19.92, 18.40, 18.16, 17.68, 13.94. ESI-HRMS *m/z* Calc for $C_{36}H_{50}BrNO_5$ $[M+Na]^+$: 678.27645, found: 678.27449.

*4.3.4.8. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-3-methylaniline (**5h**).* Yield 68.9%. m.p. 216.3–219.0 °C. ^{1}H NMR (500 MHz, MeOD) δ 7.29 (d, J = 8.4 Hz, 2H, Ar–H), 7.09 (d, J = 8.3 Hz, 2H, Ar–H), 5.67 (s, 1H, H-12), 3.74 (m, 1H, H-2), 3.51 (d, J = 11.2 Hz, 1H, H-3), 3.36 (d, J = 9.6 Hz, 1H, H-23), 3.24 (d, J = 11.1 Hz, 1H, H-23), 3.05 (dd, J = 12.7, 4.5 Hz, 1H, H-19), 2.59 (d, J = 10.9 Hz, 1H, H-16), 2.50 (s, 1H, H-9), 2.32–2.25 (m, 4H, H-19 and Ar–CH_3), 1.96–1.24 (m, 15H), 1.40 (s, 3H, CH_3-27), 1.18 (s, 3H, CH_3-24), 1.02 (d, J = 6.4 Hz, 3H, CH_3-29), 0.94 (d, J = 6.4 Hz, 3H, CH_3-30), 0.92 (s, 3H, CH_3-25), 0.68 (s, 3H, CH_3-26). ^{13}C NMR (126 MHz, MeOD) δ 202.06, 177.53, 166.72, 136.89, 135.35, 131.34, 130.16 (2 × Ar–C), 122.80 (2 × Ar–C), 77.74, 69.38, 65.93, 62.67, 54.05, 48.26, 47.82, 46.18, 45.37, 44.25, 40.09, 40.02, 39.07, 37.65, 33.49, 31.59, 29.43, 28.00, 24.74, 21.62, 21.33, 20.94, 20.01, 18.33, 18.15, 17.65, 13.90. ESI-HRMS *m/z* Calc for $C_{37}H_{53}NO_5$ $[M+Na]^+$: 614.38159, found: 614.37995.

*4.3.4.9. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-4-methylaniline (**5i**).* Yield 66.5%. m.p. 192.6–196.7 °C. ^{1}H NMR (500 MHz, MeOD) δ 7.29 (d, J = 8.4 Hz, 2H, Ar–H), 7.09 (d, J = 8.3 Hz, 2H, Ar–H), 5.66 (s, 1H, H-12), 3.77–3.72 (m, 1H, H-2), 3.51 (d, J = 11.2 Hz, 1H, H-3), 3.36 (d, J = 9.6 Hz, 1H, H-23), 3.24 (d, J = 11.1 Hz, 1H, H-23), 3.05 (dd, J = 12.7, 4.4 Hz, 1H, H-19), 2.60 (d, J = 11.0 Hz, 1H, H-16), 2.50 (s, 1H, H-9), 2.29 (d, J = 6.9 Hz, 4H, H-19 and Ar–CH_3), 1.96–1.27 (m, 15H), 1.40 (s, 3H, CH_3-27), 1.18 (s, 3H, CH_3-24), 1.02 (d, J = 6.3 Hz, 3H, CH_3-29), 0.94 (d, J = 6.5 Hz, 3H, CH_3-30), 0.92 (s, 3H, CH_3-25), 0.68 (s, 3H, CH_3-26). ^{13}C NMR (126 MHz, MeOD) δ 202.02, 177.51, 166.67, 136.87, 135.32, 131.32, 130.12 (2 × Ar–C), 122.77 (2 × Ar–C), 77.71, 69.35, 65.91, 62.65, 54.02, 49.26, 48.24, 47.79, 46.15, 45.34, 44.22, 40.06, 40.00, 39.04, 37.62, 33.46, 31.56, 29.40, 24.71, 21.58, 21.52, 21.29, 20.90, 19.97, 19.94, 18.30, 18.12, 17.61, 13.86. ESI-HRMS *m/z* Calc for $C_{37}H_{53}NO_5$ $[M+Na]^+$: 614.38159, found: 614.38049.

*4.3.4.10. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-2-methoxyaniline (**5j**).* Yield 64.5%. m.p. 193.8–196.0 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.00 (dd, J = 8.0, 1.5 Hz, 1H, Ar–H), 7.08–7.04 (m, 1H, Ar–H), 6.97–6.95 (d, J = 8.0 Hz, 1H, Ar–H), 6.90–6.86 (m, 1H, Ar–H), 5.75 (s, 1H, H-12), 3.85 (s, 3H, OCH_3), 3.77–3.71 (m, 1H, H-2), 3.51 (d, J = 11.2 Hz, 1H, H-3), 3.36 (d, J = 9.6 Hz, 1H, H-23), 3.23 (d, J = 11.1 Hz, 1H, H-23), 3.06 (dd, J = 12.7, 4.4 Hz, 1H, H-19), 2.51 (s, 1H, H-9), 2.43 (d, J = 10.5 Hz, 1H, H-16), 2.25–2.29 (m, 1H, H-19), 1.96–1.26 (m, 15H), 1.40 (s, 3H, CH_3-27), 1.16 (s, 3H, CH_3-24), 1.01 (d, J = 6.3 Hz, 3H, CH_3-29), 0.95 (d, J = 6.3 Hz, 3H, CH_3-30), 0.81 (s, 3H, CH_3-25), 0.67 (s, 3H, CH_3-26). ^{13}C NMR (101 MHz, MeOD) δ 201.52, 177.15, 165.04, 151.12, 132.03, 128.14, 126.00, 122.50, 121.62, 111.53, 77.75, 69.38, 65.96, 62.78, 56.40, 54.48, 50.00, 48.20, 47.81, 46.19, 45.34, 44.26, 40.40, 40.02, 39.05, 38.03, 33.31, 31.64, 29.33, 25.45, 21.65, 21.43, 19.43, 18.41, 18.17, 17.70, 13.95. ESI-HRMS *m/z* Calc for $C_{37}H_{53}NO_6$ $[M+Na]^+$: 630.37651, found: 630.37507.

*4.3.4.11. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-3-methoxyaniline (**5k**).* Yield 68.2%. m.p. 199.8–203.8 °C. ^{1}H NMR (500 MHz, MeOD) δ 7.19–7.14 (m, 2H, Ar–H), 7.01–6.98 (m, 1H, Ar–H), 6.67–6.63 (m, 1H, Ar–H), 5.67 (s, 1H, H-12), 3.76–3.72 (m, 4H, H-2 and Ar–CH_3O), 3.51 (d, J = 11.2 Hz, 1H, H-3), 3.36 (d, J = 9.6 Hz, 1H, H-23), 3.24 (d, J = 11.1 Hz, 1H, H-23), 3.05 (dd, J = 12.7, 4.4 Hz, 1H, H-19), 2.59 (d, J = 10.9 Hz, 1H, H-16), 2.49 (s, 1H, H-9), 2.32–2.24 (m, 1H, H-19), 1.96–1.24 (m, 15H), 1.39 (s, 3H, CH_3-27), 1.18 (s, 3H, CH_3-24), 1.01 (d, J = 6.4 Hz, 3H, CH_3-29), 0.93 (d, J = 6.4 Hz, 3H, CH_3-30), 0.92 (s, 3H, CH_3-25), 0.67 (s, 3H, CH_3-26). ^{13}C NMR (126 MHz, MeOD) δ 202.03, 177.56, 166.69, 161.38, 140.74, 131.32, 130.39, 114.64, 111.11, 108.27, 77.73, 69.37, 65.94, 62.65, 55.71, 54.03, 49.34, 48.25, 47.81, 46.18, 45.34, 44.24, 40.06, 39.98, 39.05, 37.48, 33.46, 31.57, 29.40, 24.73, 21.63, 21.35, 19.95, 18.37, 18.15, 17.66, 13.92. ESI-HRMS *m/z* Calc for $C_{37}H_{53}NO_6$ $[M+Na]^+$: 630.37651, found: 630.37446.

*4.3.4.12. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-4-methoxyaniline (**5l**).* Yield 64.6%. m.p. 214.3–217.0 °C. ^{1}H NMR (400 MHz, MeOD) δ 7.30 (d, J = 9.0 Hz, 2H, Ar–H), 6.84 (d, J = 9.0 Hz, 2H, Ar–H), 5.66 (s, 1H, H-12), 3.74 (m, 4H, H-2 and Ar–OCH_3), 3.52 (d, J = 11.2 Hz, 1H, H-3), 3.36 (d, J = 9.6 Hz, 1H, H-23), 3.25 (d, J = 11.1 Hz, 1H, H-23), 3.05 (dd, J = 12.7, 4.4 Hz, 1H, H-19), 2.58 (d, J = 10.9 Hz, 1H, H-16), 2.49 (s, 1H, H-9), 2.32–2.24 (m, 1H, H-19), 1.95–1.25 (m, 15H), 1.39 (s, 3H, CH_3-27), 1.19 (s, 3H, CH_3-24), 1.02 (d, J = 6.3 Hz, 3H, CH_3-29), 0.93 (d, J = 5.6 Hz, 6H, CH_3-30/25), 0.69 (s, 3H, CH_3-26). ^{13}C NMR (101 MHz, MeOD) δ 202.00, 177.49, 166.67, 158.24, 132.38, 131.36, 124.60 (2 × Ar–C), 114.90 (2 × Ar–C), 77.75, 69.38, 65.97, 62.67, 55.91, 54.05, 49.08, 48.27, 47.83, 46.18, 45.37, 44.26, 40.08, 40.04, 39.08, 37.75, 33.52, 31.61, 29.45, 24.75, 21.65,

21.37, 20.09, 18.38, 18.18, 17.68, 13.94. ESI-HRMS *m/z* Calc for $C_{37}H_{53}NO_6$ $[M+Na]^+$: 630.37651, found: 630.37432.

*4.3.4.13. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-3-chloro-4-fluoroaniline (**5m**).* Yield 67.6%. m.p. 224.4–228.2 °C. ^{1}H NMR (400 MHz, MeOD) δ 7.68 (dd, *J* = 6.7, 2.6 Hz, 1H, Ar–H), 7.37 (m, 1H, Ar–H), 7.14 (t, *J* = 9.0 Hz, 1H, Ar–H), 5.65 (s, 1H, H-12), 3.75 (td, *J* = 11.2, 4.4 Hz, 1H, H-2), 3.51 (d, *J* = 11.2 Hz, 1H, H-3), 3.36 (d, *J* = 9.6 Hz, 1H, H-23), 3.24 (d, *J* = 11.1 Hz, 1H, H-23), 3.05 (dd, *J* = 12.7, 4.3 Hz, 1H, H-19), 2.56 (d, *J* = 10.9 Hz, 1H, H-16), 2.49 (s, 1H, H-9), 2.33–2.25 (m, 1H, H-19), 1.94–1.23 (m, 15H), 1.38 (s, 3H, CH_3-27), 1.18 (s, 3H, CH_3-24), 1.01 (d, *J* = 6.2 Hz, 3H, CH_3-29), 0.92 (d, *J* = 6.4 Hz, 3H, CH_3-30), 0.90 (s, 3H, CH_3-25), 0.68 (s, 3H, CH_3-26). ^{13}C NMR (101 MHz, MeOD) δ 201.96, 177.62, 166.46, 136.79, 131.37, 124.36, 122.44, 122.37, 117.55, 117.33, 77.77, 69.38, 66.00, 62.64, 54.01, 49.38, 48.27, 47.85, 46.19, 45.34, 44.26, 40.07, 40.00, 39.08, 37.46, 33.49, 31.54, 29.40, 24.69, 21.66, 21.37, 19.90, 18.42, 18.18, 17.70, 13.96. ESI-HRMS *m/z* Calc for $C_{36}H_{49}ClO_5$ $[M+Na]^+$: 652.31755, found: 652.31590.

*4.3.4.14. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-3-chloro-4- methylaniline (**5n**).* Yield 64.9%. m.p. 210.5–213.5 °C. ^{1}H NMR (400 MHz, MeOD) δ 7.68 (dd, *J* = 9.0 Hz, 1H, Ar–H), 7.39–7.35 (m, 1H, Ar–H), 7.16–7.11 (t, *J* = 10.0 Hz, 1H, Ar–H), 5.65 (s, 1H, H-12), 3.75 (td, *J* = 11.2, 4.4 Hz, 1H, H-2), 3.51 (d, *J* = 11.2 Hz, 1H, H-3), 3.36 (d, *J* = 9.6 Hz, 1H, H-23), 3.24 (d, *J* = 11.1 Hz, 1H, H-23), 3.05 (dd, *J* = 12.7, 4.3 Hz, 1H, H-19), 2.56 (d, *J* = 10.9 Hz, 1H, H-16), 2.49 (s, 1H, H-9), 2.32–2.25 (m, 1H, H-19), 1.94–1.23 (m, 15H), 1.38 (s, 3H, CH_3-27), 1.18 (s, 3H, CH_3-24), 1.01 (d, *J* = 6.2 Hz, 3H, CH_3-29), 0.92 (d, *J* = 6.4 Hz, 3H, CH_3-30), 0.90 (s, 3H, CH_3-25), 0.68 (s, 3H, CH_3-26). ^{13}C NMR (101 MHz, MeOD) δ 202.03, 177.65, 166.60, 138.56, 131.42, 130.43, 129.74 (2 × Ar–C), 123.89 (2 × Ar–C), 77.81, 69.44, 66.03, 62.71, 54.08, 49.43, 48.32, 47.89, 46.23, 45.40, 44.31, 40.09, 40.07, 39.13, 37.55, 33.54, 31.61, 29.47, 24.75, 21.70, 21.40, 19.99, 18.44, 18.23, 17.73, 13.99. ESI-HRMS *m/z* Calc for $C_{37}H_{52}ClNO_5$ $[M+Na]^+$: 648.34262, found: 648.34095.

*4.3.4.15. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-3-4-dichloroaniline (**5o**).* Yield 68.9%. m.p. 210.1–214.0 °C. ^{1}H NMR (400 MHz, MeOD) δ 7.45–7.38 (m, 2H, Ar–H), 7.01 (t, *J* = 8.8 Hz, 2H, Ar–H), 5.66 (s, 1H, H-12), 3.74 (td, *J* = 11.3, 4.4 Hz, 1H, H-2), 3.51 (d, *J* = 11.2 Hz, 1H, H-3), 3.36 (d, *J* = 9.6 Hz, 1H, H-23), 3.24 (d, *J* = 11.1 Hz, 1H, H-23), 3.05 (dd, *J* = 12.7, 4.4 Hz, 1H, H-19), 2.59 (d, *J* = 10.9 Hz, 1H, H-16), 2.50 (s, 1H, H-9), 2.33–2.25 (m, 1H, H-19), 1.99–1.25 (m, 15H), 1.40 (s, 3H, CH_3-27), 1.18 (s, 3H, CH_3-24), 1.02 (d, *J* = 6.3 Hz, 3H, CH_3-29), 0.94 (s, 3H, CH_3-30), 0.92 (s, 3H, CH_3-25), 0.68 (s, 3H, CH_3-26). ^{13}C NMR (101 MHz, MeOD) δ 202.04, 177.50, 166.71, 139.38, 139.30, 131.35, 127.13 (2 × Ar–C), 120.42 (2 × Ar–C), 77.75, 69.39, 65.96, 62.67, 54.04, 48.28, 47.83, 46.18, 45.37, 44.26, 40.09, 40.00, 39.07, 37.61, 33.51, 31.61, 29.43, 24.75, 21.62, 21.54, 21.37, 19.99, 18.37, 18.17, 17.67, 13.93. ESI-HRMS *m/z* Calc for $C_{36}H_{49}Cl_2NO_5$ $[M+Na]^+$: 668.28800, found: 668.28652.

*4.3.4.16. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-3-fluoro-4-bromineaniline (**5p**).* Yield 63.9%. m.p. 233.4–236.9 °C. ^{1}H NMR (500 MHz, MeOD) δ 7.57 (dd, *J* = 11.2, 2.4 Hz, 1H, Ar–H), 7.51–7.45 (m, 1H, Ar–H), 7.22 (dd, *J* = 8.7, 1.8 Hz, 1H, Ar–H), 5.65 (s, 1H, H-12), 3.72–3.77 (m, 1H, H-2), 3.51 (d, *J* = 11.2 Hz, 1H, H-3), 3.35 (d, *J* = 9.6 Hz, 1H, H-23), 3.24 (d, *J* = 11.1 Hz, 1H, H-23), 3.05 (dd, *J* = 12.7, 4.4 Hz, 1H, H-19), 2.57 (d, *J* = 10.4 Hz, 1H, H-16), 2.49 (s, 1H, H-9), 2.33–2.26 (m, 1H, H-19), 1.96–1.24 (m, 15H), 1.39 (s, 3H, CH_3-27), 1.18 (s, 3H, CH_3-24), 1.01 (d, *J* = 6.4 Hz, 3H, CH_3-29), 0.93 (d, *J* = 6.4 Hz, 3H, CH_3-30), 0.87 (s, 3H, CH_3-25), 0.67 (s, 3H, CH_3-26). ^{13}C NMR (126 MHz, MeOD) δ 202.01, 177.68, 166.49, 141.14, 134.33, 131.41, 118.74, 110.02, 103.58, 103.42, 77.73, 69.38, 65.93, 62.64, 53.98, 49.56, 48.25, 47.81, 46.16, 45.31, 44.25, 40.05, 39.98, 39.05, 37.28, 33.44, 31.49, 29.37, 24.62, 21.63, 21.31, 19.78, 18.37, 18.15, 17.65, 13.91. ESI-HRMS *m/z* Calc for $C_{36}H_{49}BrO_5$ $[M+Na]^+$: 696.26703, found: 696.26492.

*4.3.4.17. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-2-methyl-4-bromineaniline (**5q**).* Yield 68.8%. m.p. 219.3–223.8 °C. ^{1}H NMR (400 MHz, MeOD) δ 7.38 (d, *J* = 1.7 Hz, 1H, Ar–H), 7.29 (dd, *J* = 8.4, 2.1 Hz, 1H, Ar–H), 7.03 (d, *J* = 8.4 Hz, 1H, Ar–H), 5.61 (s, 1H, H-12), 3.72–3.78 (m, 1H, H-2), 3.53 (d, *J* = 11.1 Hz, 1H, H-3), 3.36 (d, *J* = 9.6 Hz, 1H, H-23), 3.26 (d, *J* = 11.1 Hz, 1H, H-23), 3.05 (dd, *J* = 12.7, 4.3 Hz, 1H, H-19), 2.52 (d, *J* = 12 Hz, 1H, H-16), 2.49 (s, 1H, H-9), 2.31–2.24 (m, 1H, H-19), 2.18 (s, 3H, Ar–CH_3), 1.99–1.29 (m, 15H), 1.40 (s, 3H, CH_3-27), 1.21 (s, 3H, CH_3-24), 1.02 (d, *J* = 6.1 Hz, 3H, CH_3-29), 0.99 (s, 3H, CH_3-30), 0.91 (d, *J* = 6.3 Hz, 3H, CH_3-25), 0.70 (s, 3H, CH_3-26). ^{13}C NMR (101 MHz, MeOD) δ 201.82, 178.09, 166.27, 138.01, 136.49, 134.38, 131.49, 130.39, 129.74, 120.70, 77.74, 69.38, 65.96, 62.61, 54.13, 49.21, 48.26, 47.84, 46.12, 45.45, 44.27, 40.03, 40.01, 39.12, 38.33, 33.73, 31.66, 29.46, 24.78, 21.46, 21.37, 20.56, 18.40, 18.21, 18.16, 17.65, 13.97. ESI-HRMS *m/z* Calc for $C_{37}H_{52}BrNO_5$ $[M+Na]^+$: 692.29210, found: 692.29060.

*4.3.4.18. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-2-5-dimethylaniline (**5r**).* Yield 64.8%. m.p. 205.3–208.1 °C. ^{1}H NMR (400 MHz, MeOD) δ 7.40 (d, *J* = 1.7 Hz, 1H, Ar–H), 7.31 (dd, *J* = 8.4, 2.1 Hz, 1H, Ar–H), 7.05 (d, *J* = 8.4 Hz, 1H, Ar–H), 5.63 (s, 1H, H-12), 3.74–3.80 (m, 1H, H-2), 3.54 (d, *J* = 11.1 Hz, 1H, H-3), 3.38 (d, *J* = 9.6 Hz, 1H, H-23), 3.28 (d, *J* = 11.1 Hz, 1H, H-23), 3.07 (dd, *J* = 12.7, 4.3 Hz, 1H, H-19), 2.54 (d, *J* = 12 Hz, 1H, H-16), 2.51 (s, 1H, H-9), 2.33–2.20 (m, 1H, H-19), 2.20 (s, 3H, Ar–CH_3), 2.10 (s, 3H, Ar–CH_3), 2.01–1.31 (m, 15H), 1.42 (s, 3H, CH_3-27), 1.23 (s, 3H, CH_3-24), 1.04 (d, *J* = 6.1 Hz, 3H, CH_3-29), 1.01 (s, 3H, CH_3-30), 0.94 (d, *J* = 6.3 Hz, 3H, CH_3-25), 0.72 (s, 3H, CH_3-26). ^{13}C NMR (101 MHz, MeOD) δ 201.97, 177.68, 166.44, 139.81, 133.18, 131.42, 131.36, 128.05, 123.63, 121.65, 77.74, 69.38, 65.95, 62.64, 53.99, 49.52, 48.26, 47.82, 46.17, 45.32, 44.25, 40.04, 39.99, 39.06, 37.34, 33.45, 31.50, 29.38, 24.65, 21.63, 21.31, 20.41, 19.82, 19.06, 18.38, 18.16, 17.65, 13.92. ESI-HRMS *m/z* Calc for $C_{38}H_{55}NO_5$ $[M+Na]^+$: 628.39724, found: 628.39571.

*4.3.4.19. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-1-naphthylaniline (**5s**).* Yield 63.9%. m.p. 226.4–228.8 °C. ^{1}H NMR (400 MHz, MeOD) δ 7.91–7.85 (m, 2H, Ar–H), 7.78 (d, *J* = 8.2 Hz, 1H, Ar–H), 7.53–7.48 (m, 2H, Ar–H), 7.45 (d, *J* = 7.9 Hz, 1H, Ar–H), 7.35 (d, *J* = 7.2 Hz, 1H, Ar–H), 5.65 (s, 1H, H-12), 3.78–3.72 (m, 1H), 3.53 (d, *J* = 11.2 Hz, 1H, H-3), 3.37 (d, *J* = 9.6 Hz, 1H, H-23), 3.26 (d, *J* = 11.1 Hz, 1H, H-23), 3.06 (dd, *J* = 12.7, 4.3 Hz, 1H, H-19), 2.49 (d, *J* = 13.1 Hz, 2H, H-16 and H-9), 2.12–2.18 (m, 1H, H-19), 2.01–1.24 (m, 15H), 1.36 (s, 3H, CH_3-27), 1.18 (s, 3H, CH_3-24), 0.98 (d, *J* = 5.7 Hz, 3H, CH_3-29), 0.93 (s, 3H, CH_3-30), 0.84 (d, *J* = 6.3 Hz, 3H, CH_3-25), 0.69 (s, 3H, CH_3-26). ^{13}C NMR (101 MHz, MeOD) δ 201.87, 178.76, 166.65, 135.87, 134.58, 131.46, 131.29, 129.43, 127.99, 127.24, 127.20, 126.56, 125.52, 123.91, 77.76, 69.40, 65.98, 62.62, 54.08, 49.32, 48.29, 47.85, 46.14, 45.45, 44.27, 40.03, 39.83, 39.11, 38.19, 33.79, 31.71, 29.41, 24.67, 21.44, 21.37, 20.55, 18.41, 18.20, 17.58, 13.97. ESI-HRMS *m/z* Calc for $C_{40}H_{53}NO_5$ $[M+Na]^+$: 650.38159, found: 650.37968.

*4.3.4.20. N-[2α,3β, 23-Trihydroxyurs-11-oxo-12-ene-28-oyl]-phenylamine (**5t**).* Yield 67.3%. m.p. 214.5–219.5 °C. ^{1}H NMR (500 MHz, MeOD) δ 7.44 (d, *J* = 7.7 Hz, 2H, Ar–H), 7.27 (t, *J* = 7.9 Hz, 2H, Ar–H), 7.08 (t, *J* = 7.4 Hz, 1H, Ar–H), 5.67 (s, 1H, H-12), 3.75 (td, *J* = 11.3, 4.4 Hz, 1H, H-2), 3.51 (d, *J* = 11.2 Hz, 1H, H-3), 3.36 (d, *J* = 9.6 Hz, 1H, H-23), 3.24 (d, *J* = 11.1 Hz, 1H, H-23), 3.06 (dd, *J* = 12.7, 4.4 Hz, 1H, H-19), 2.60 (d, *J* = 10.9 Hz, 1H, H-16), 2.49 (s, 1H, H-9), 2.34–2.24 (m,

1H, H-19), 1.99–1.25 (m, 15H), 1.39 (s, 3H, CH_3-27), 1.18 (s, 3H, CH_3-24), 1.02 (d, $J = 6.4$ Hz, 3H, CH_3-29), 0.93 (d, $J = 6.4$ Hz, 3H, CH_3-30), 0.92 (s, 3H, CH_3-25), 0.67 (s, 3H, CH_3-26). ^{13}C NMR (126 MHz, MeOD) δ 202.00, 177.55, 166.66, 139.56, 131.33, 129.69 (2 × Ar–C), 125.54, 122.64 (2 × Ar–C), 77.71, 69.36, 65.92, 62.64, 54.01, 49.26, 48.25, 47.79, 46.16, 45.34, 44.24, 40.05, 39.99, 39.05, 37.56, 33.47, 31.58, 29.42, 24.72, 21.65, 21.38, 19.97, 18.38, 18.15, 17.69, 13.94. ESI-HRMS *m/z* Calc for C36H51NO5 $[M+Na]^+$: 600.36594, found: 600.36403.

4.4. In vitro cytotoxicity

The NCI-H460, 7404, HepG2, MGC-803, Hela, cell lines used in this study were all obtained from the Institute of Biochemistry and Cell Biology, China Academy of Sciences. All were supplemented with 10% heat-inactivated fetal bovine serum in a humidified atmosphere of 5% CO_2/95% air at 37 °C. In order to investigate the potential of compounds **4**, **5** and 5-FU, a commercial classical anticancer drug was used as a reference organic drug. Assays of cytotoxicity were determined in 96-well, flat bottomed microtiter plates. The supplemented culture medium with cell lines was added to the wells. Compounds **4**, **5**, and 5-FU were dissolved in the culture medium with 1% DMSO to give various concentrations (1.25, 2.5, 5, 10, 20 mg/mL, respectively). The resulted solutions were subsequently added to a set of wells. Control wells contained supplemented media with 1% DMSO. The microtiter plates were incubated at 37 °C in a humidified atmosphere of 5% CO_2/95% air for a further 3 day. Cytotoxic screening by 3-(4, 5-dimethylthiazol-2-yl)-2,5-di -phenyltetrazolium bromide (MTT) assay was conducted. At the end of each incubation period, the MTT solution (10 mL, 5 mg/mL) was added into each well and the cultures were incubated further for 48 h (for the time-dependent cytotoxic effects studies, the treatment time is 24, 48, 72 h, respectively) at 37 °C in a humidified atmosphere of 5% CO_2/95% air. After removal of the supernatant, DMSO (150 mL) was added to dissolve the formazan crystals. The absorbance was read by enzyme labeling instrument with 570/630 nm double wavelength measurement. The cytotoxicity was estimated based on the percentage cell survival in a dose dependent manner relative to the negative control. The final IC_{50} values were calculated by the Bliss method ($n = 5$). All the tests were repeated in at least three independent experiments.

4.5. AO/EB staining

Cells were seeded at a concentration of 5×10^4 cell/mL in a volume of 2 mL on a sterile cover slip in six-well tissue culture plates. Following incubation, the medium was removed and replaced with fresh medium plus 10% fetal bovine serum and supplemented with compound **5b** (20 μM). After the treatment period, the cover slip with monolayer cells was inverted on a glass slide with 20 μM of AO/EB stain (100 mg/mL). Fluorescence was read on a Nikon ECLIPSETE2000-S fluorescence microscope (OLYMPUS Co., Japan).

4.6. Hoechst 333258 staining

Cells grown on a sterile cover slip in six-well plates were treated with compounds for a certain range of time. The culture medium containing compounds was removed, and the cells were fixed in 4% paraformaldehyde for 10 min. After being washed twice with PBS, the cells were stained with 0.5 mL of Hoechst 33258 (Beyotime, Haimen, China) for 5 min and then again washed twice with PBS. The stained nuclei were observed under an Nikon ECLIPSETE2000-S fluorescence microscope using 350 nm excitation and 460 nm emission.

4.7. Mitochondrial membrane potential staining

JC-1 (Beyotime, Haimen, China) probe was employed to measure mitochondrial depolarization in NCI-H460 cells. Briefly, cells cultured in six-well plates after indicated treatments were incubated with an equal volume of JC-1 staining solution (5 μg/ml) at 37 °C for 20 min and rinsed twice with PBS. Mitochondrial membrane potentials were monitored by determining the relative amounts of dual emissions from mitochondrial JC-1 monomers or aggregates using an Nikon ECLIPSETE2000-S fluorescent microscope. Mitochondrial depolarization is indicated by an increase in the green/red fluorescence intensity ratio.

4.8. Flow cytometry

4.8.1. Apoptosis analysis

Apoptosis was discriminated with the annexin V-FITC/propidium iodide test. Cells were seeded at 2×10^6/well in 10% FBS-DMEM into 6-well plates, and treated with compounds for 24 h. The cells were washed twice with cold Phosphate Buffered Saline (PBS) and then resuspend cells in 1× Binding Buffer (0.1 M Hepes/NaOH (pH 7.4), 1.4 M NaCl, 25 mM $CaCl_2$) at a concentration of 1×10^6 cells/ml. Transfer 100 μL of the solution (1×10^5 cells) to a 5 mL culture tube, and add 5 μL of FITC Annexin V (BD, Pharmingen) and 5 μL propidium iodide (PI) to each tube. Gently vortex the cells and incubate for 30 min at RT (25 °C) in the dark. Add 200 μL PBS to each tube. Analysis was performed with the system software (Cell Quest; BD Biosciences). Lower left quadrant, viable cells (annexin V−/PI−); lower right quadrant, early apoptotic cells (annexin V+/PI−); upper right quadrant, late apoptotic cells (annexin V+/PI+); upper left quadrant, necrotic cells (annexin V−/PI+). The percentage of cells positive for PI and/or Annexin V-FITC was reported inside the quadrants.

4.8.2. Cell cycle analysis

The cells lines were treated with indicated concentrations of compounds. After incubated for 48 h, cells were washed twice with ice-cold PBS, fixed and permeabilized with ice-cold 70% ethanol at −20 °C overnight. The cells were treated with 100 μg/ml RNase A at 37 °C for 30 min after washed with ice-cold PBS, and finally stained with 1 mg/ml propidium iodide (PI) in the dark at 4 °C for 30 min. Analysis was performed with the system software (Cell Quest; BD Biosciences).

4.9. ROS assay

HepG2 cells were seeded into six-well plates and subjected to various treatments. Following treatment, cells were incubated with 20 μM DCFH-DA (Beyotime, Haimen, China) dissolved in cell-free medium at 37 °C for 30 min in dark, and then washed three times with PBS. Cellular fluorescence was quantified using Nikon ECLIPSETE2000-S fluorescence microscope at an excitation of 485 nm and an emission of 538 nm.

4.10. Immunoblotting and real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted from the HepG2 cells after treatment with 10 μM, 20 μM, 40 μM aniline-derived asiatic acid derivatives for 24 h using the RNA pure Kit (Aidlab, RN0302, China) as described previously. RNA samples were reverse-transcribed for 30 min at 42 °C with the High Capacity cDNA Reverse Transcription Kit (TaKaRa, Biotechnology, Dalian). The SYBR® Green PCR Master Mix (Fermentas, K0251, Lithuania) and specific primer pairs were used for selected genes, and the primer pair for actin was used as

the reference gene. RT-PCR was performed according to the following conditions: 2 min at 50 °C, 10 min at 95 °C, and 40 cycles of 15 s at 95 °C and 1 min at 60 °C using 0.5 μl of complementary (c) DNA, 2 × SYBR Green PCR Master Mix, and 500 nM of the forward and reverse primers on a 7500 real-time PCR System (Applied Biosysterms). The threshold cycle number (Ct) was calculated with the 7500 ABI software. Relative transcript quantities were calculated using the △Ct method with actin as the reference gene amplified from the same samples. Ct is the difference in the threshold cycles of messenger (m)RNA for selected genes relative to those of actin mRNA. The real-time RT-PCR was performed in triplicate for each experimental group. The PCR primers used here are given in Table 2S (See supporting information).

4.11. Western blot

HepG2 cells (3×10^5) were cultured in each well of 6-well plates to 80–90% confluence. The cell culture medium was replaced with 0.1% FCS RPMI 1640 and incubated for 12 h. The cells were exposed to **5b** (0–20 μM) for 24 h (for Bax, Bcl-2, PARP, cytochrome *c* and caspase-9, -3), and then washed once with ice-cold PBS and extracted with the sample buffer. The cell extracts were separated on polyacrylamide-SDS gels, transferred to nitrocellulose membrane and probed with primary antibodies as indicated. The membrane was incubated with anti-rabbit IgG (AP-linked) and developed by an ECL Western blot system (Kodak, USA).

4.12. Statistics

The data were processed by the Student's *t*-test with the significance level $P \geq 0.05$ using SPSS.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.08.003.

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