Synthesis and Biological Evaluation of Pentacyclic Triterpenoid Derivatives as Potential Novel Antibacterial Agents

Panpan Wu [a, b], Borong Tu [a], Jinfeng Liang [a], Shengzhu Guo [a], Nana Cao [a], Silin Chen [a], Zhujun Luo [a], Jiahao Li [a], Wende Zheng,[a], Xiaowen Tang [a], Dongli Li [a], Xuetao Xu [a], Wenfeng Liu [a], Xi Zheng [a], Zhaojun Sheng [a], Adam P. Roberts [c], Kun Zhang [a, b*], Weiqian David Hong [a, d*]

[a]School of Biotechnology and Health Sciences, Wuyi University, Jiangmen 529020, P. R. China

[b]Department of Pharmaceutical Engineering, Faculty of Chemical Engineering and Light Industry, Guangdong University of Technology, Guangzhou, 510006 P.R. China

[c]Centre for Drugs and Diagnostics, Department of Tropical Disease Biology, Liverpool School of Tropical Medicine, Liverpool, L3 5QA, United Kingdom

[d]Department of Chemistry, University of Liverpool, Liverpool, L69 7ZD, United Kingdom

***Corresponding Authors:**

Kun Zhang, Telephone: +86 13822330019, Email: kzhang@gdut.edu.cn

Weiqian David Hong, Telephone: +44 7863354263, Email: davidhwq@liverpool.ac.uk

Email addresses for other authors:

wyuchemwpp@126.com (P.-P. Wu), tuborong@163.com (B.-R. Tu), jinfengliang96@126.com (J.-F. Liang), shengzhuguo@163.com (S.-Z. Guo), caonana611@126.com (N.-N. Cao), chensilin2430@163.com (S.-L. Chen), 15826692097@163.com (Z.-J. Luo), 18676125540@163.com (J.-H. Li), 15875045599@163.com (W.-D. Zheng), 15575386250@163.com (X. -W. Tang), wyuchemldl@126.com (D.-L. Li), wyuchemxxt@126.com (X.-T. Xu), wyuchemlwf@126.com (W.-F. Liu), xizheng@pharmacy.rutgers.edu (X. Zheng), wyuchemszj@126.com (Z.-J. Sheng),

Adam.Roberts@lstmed.ac.uk (Adam P. Roberts), kzhang@gdut.edu.cn (K. Zhang), davidhwq@liverpool.ac.uk (W. David Hong).

Abstract

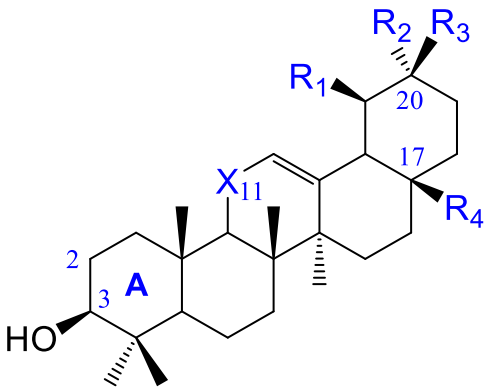
A series of ursolic acid (UA), oleanolic acid (OA) and 18*β*-glycyrrhetinic acid (GA) derivatives were synthesized by introducing a range of substituted aromatic side-chains at the C-2 position after the hydroxyl group at C-3 position was oxidized. Their antibacterial activities were evaluated *in vitro* against a panel of four *Staphylococcus* strains. The results revealed that the introduction of aromatic side-chains at the C-2 position of GA led to the discovery of potent triterpenoid derivatives for inhibition of both drug sensitive and resistant *S. aureus*, while the other two series derivatives of UA and OA showed no significant antibacterial activity even at high concentrations. In particular, GA derivative **33** showed good potency against all four strains of *Staphylococcus* (MIC = 1.25 - 5 μmol/L) with acceptable pharmacokinetics properties and low cytotoxicity *in vitro*. Molecular docking was also performed using *S. aureus* DNA gyrase structure to rationalize the observed antibacterial activity. Therefore, this series of GA derivatives have strong potential for the development of a new type of triterpenoid antibacterial agent.

Key words: Pentacyclic triterpenes; 18*β*-glycyrrhetinic acid; natural product derivatives; Gram-positive bacteria; antibacterial

1. Introduction

Many of the medical achievements of the last century could be lost through the spread of antimicrobial resistance[1-3]. Previously curable infectious diseases may

become untreatable and spread throughout the world, which has already started to happen[4, 5]. In particular, antibiotic resistant *Staphylococcus aureus* remains a serious clinical problem. Normal treatments become less effective as resistance develops[6, 7]. Herein, the development of new antibiotics is an urgent issue, meaning that the development of new classes of antibiotics to circumvent existing antimicrobial resistance is constantly needed[8-11].



GA: R_1=H, R_2=CH_3, R_3=COOH, R_4=CH_3, **X**=CO

UA: R_1=CH_3, R_2=CH_3, R_3=H, R_4=COOH, **X**=CH_2

OA: R_1=H, R_2=CH_3, R_3=CH_3, R_4=COOH, **X**=CH_2

Figure 1. The chemical structure of GA, UA and OA.

The natural products of ursolic acid (UA), oleanolic acid (OA) and 18β-glycyrrhetinic acid (GA) (Figure 1), are biologically active pentacyclic triterpenoids which are produced by secondary metabolism of plants and prevalent in various plants[12, 13]. Potent pharmacological activities of these triterpenes have been demonstrated including their ability to inhibit the growth of various pathogens [14, 15], against some infectious viruses[13, 16, 17], induce cancer cells differentiation and apoptosis[18, 19], and prevent herbivore infections in the host[20, 21]. Some pentacyclic triterpenoids have already emerged as new series of

chemotherapeutics and some of them are currently in clinical trials[22, 23]. Moreover, with these pronounced pharmacological activities, medicinal chemists were attracted by the safety characteristics of pentacyclic triterpenoids while compared with other clinically available chemotherapeutic agents that often suffer serious side effects[24, 25]. However, the antibacterial activity of pentacyclic triterpenoids is relatively weak[26]. In a recent report by Huang and co-workers, tri-hydroxyl groups were introduced in ring A while an ester moiety was formed at C-20 of the oleanane-type triterpene GA to enhance their antimicrobial property[14]. Previous structure-activity relationship (SAR) studies of GA have suggested that the carboxylic acid group at C-20 and ring A are involved in various biological activities [27-29].

We have focused on the modifications at the C-2 and C-3 positions of UA, OA and GA and report a series of GA derivatives displaying *in vitro* antibacterial activity against both antibiotic sensitive and resistant *Staphylococcus spp.* which are significantly higher than that of the parent compound and provide a basis for onward development of triterpenoids as antibacterial agents.

2. Results and Discussion

2.1 Derivatives design

Previously, multiple series of pentacyclic triterpenoids derivatives were obtained by modification at positions of C-3 and C-28 in our group to evaluate their potential for α-glucosidase inhibition[30-33], such as compounds **1** - **9** (Figure 2). To our knowledge, their activity against bacteria were not evaluated or reported yet, so these derivatives of UA were assessed for their *in vitro* antibacterial activities in this study

initially.

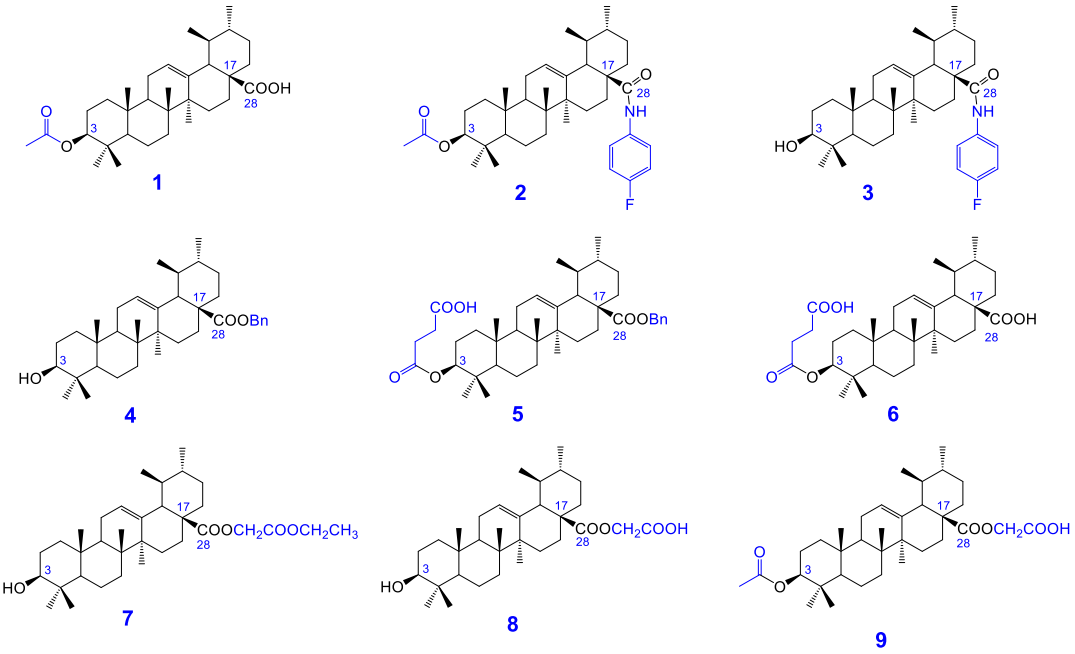
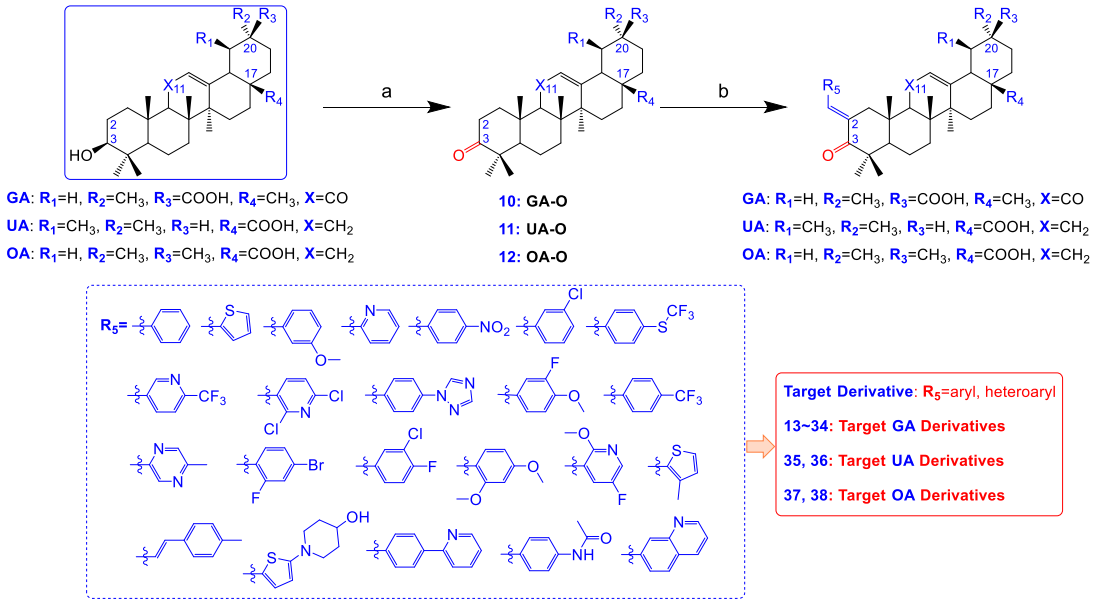


Figure 2. Structures of ursolic acid derivative **1** - **9**. The fragments in blue are the introduced groups; the numbers within the structures are the crucial modification sites.

Three series of novel UA, OA and GA derivatives were prepared with modifications at C-2 and C-3 positions of selected pentacyclic triterpenoids in two high yielding steps as detailed in Scheme 1[34]. Jones reagent was used to oxidize the three pentacyclic triterpenoids to give the ketone intermediates **10**, **11**, and **12**. Three series target derivatives were then produced by Claisen Schmidt condensation at C-2 position of the ketone intermediates of UA, OA and GA, in which derivatives **13** - **34** were obtained from parent compound GA, derivatives **35** and **36** were obtained from UA and derivatives **37** and **38** were obtained from OA. They were also evaluated for the *in vitro* antibacterial activities in this study as showed in Table 1 and Table 2.



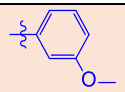
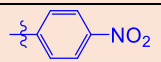
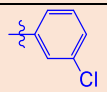
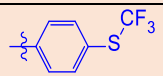
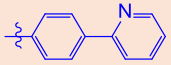
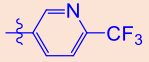
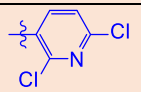
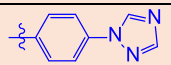
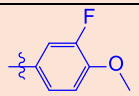
Scheme 1 Synthesis of three series of pentacyclic triterpenoids derivatives at positions of C-2 and C-3. Reagents and conditions: (a) Jones reagent, acetone, 0 ℃ to rt, 2 h, 91-96%; (b) R_5-CHO, KOH, EtOH, rt, 3h, 66-93%.

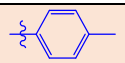
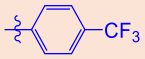
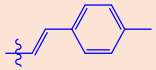
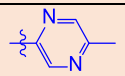
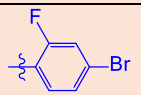
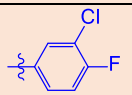
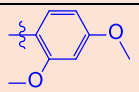
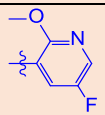
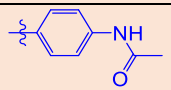
2.2 Antibacterial activity

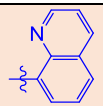
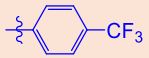
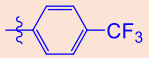
The antibacterial activity of all the pentacyclic triterpenoids derivatives were assayed against four Gram-positive bacteria strains. All bacterial strains were cultured in Muller Hinton agar at 37 ℃ overnight.

Table 1. Biological evaluation of series pentacyclic triterpenoids derivatives expressed in the inhibition zone (mm)[a]

Compound code	**R_5**	**Bacterium and Inhibition Zone (mm) Dosage: 80 nmol**		
		Staphylococcus aureus (ATCC 6538)	*Staphylococcus aureus* (ATCC 29213)	*Staphylococcus epidermidis* (ATCC 12228)
GA	-	6.78 ±0.22	6.85 ±0.20	7.46 ±0.18
UA	-	6.76 ±0.33	6.55 ±0.27	7.24 ±0.19
OA	-	6.29 ±0.43	6.81 ±0.22	7.08 ±0.25
1~12	-	<6[b]	<6	<6
13		9.85 ±0.22	9.66 ±0.18	9.09 ±0.18
14		8.25 ±0.22	8.39 ±0.88	7.92 ±0.22

15		8.96 ±0.36	9.00 ±0.57	8.56 ±0.08
16		9.31 ±0.23	9.58 ±0.39	8.99 ±0.75
17		10.01 ±0.09	10.08 ±0.27	11.21 ±0.07
18		7.34 ±0.05	6.91 ±0.41	6.89 ±0.78
19		9.86 ±1.24	10.74 ±0.38	9.09 ±0.73
20		9.55 ±0.08	9.68 ±0.55	9.88 ±0.03
21		10.03 ±0.17	10.39 ±0.36	9.90 ±0.77
22		10.01 ±0.06	10.23 ±0.21	10.59 ±0.17
23		10.19 ±0.25	9.69 ±0.53	9.89 ±0.56
24		8.55 ±0.29	8.47 ±0.30	9.09 ±0.23

25		9.37 ±0.38	9.38 ±0.26	9.68 ±0.41
26		8.99 ±1.01	9.55 ±0.19	9.39 ±0.70
27		7.01 ±0.18	8.43 ±0.81	6.99 ±0.93
28		10.25 ±0.99	10.68 ±1.14	11.20 ±0.22
29		7.25 ±0.28	7.18 ±0.37	7.20 ±0.33
30		11.21 ±1.33	13.23 ±0.88	12.39 ±0.11
31		7.66 ±0.28	7.54 ±0.50	7.89 ±0.03
32		8.90 ±0.09	8.75 ±0.17	9.11 ±0.20
33		14.83 ±0.55	15.60 ±0.46	15.93 ±0.12

34		7.91 ±0.28	8.11 ±0.23	8.37 ±0.88
35		<6	<6	<6
36		<6	<6	<6
37		<6	<6	<6
38		<6	<6	<6
Gatifloxacin[c]	-	19.12 ±0.73	17.13 ±0.64	18.67 ±0.25

[a]Results are expressed as the diameter of inhibition zone (mm), values represent the means of three independent replicates ± SD.

[b]<6, no measureable inhibition zone.

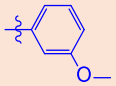
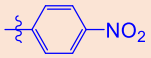
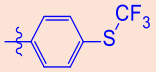
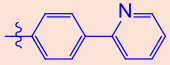
[c] The dosage of gatifloxacin used in the inhibition zone assay was 1 nmol.

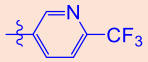
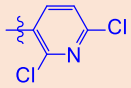
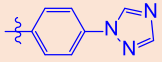
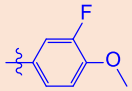
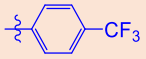
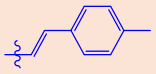
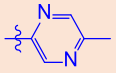
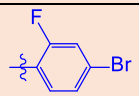
The antimicrobial activity of the pentacyclic triterpenoid derivatives against three sensitive strains of Gram-positive bacteria were firstly assessed by a Kirby–Bauer assay and summarized in Table 1. The dosage of each examined derivative was 80 nmol in this assay. The sizes of the inhibition zone (IZ) diameter showed that the GA derivatives (**13** - **34**) were more potent than the parent compound of GA, the oxidized intermediates (**GA-O**, **UA-O** and **OA-O**) and all others derivatives of UA (**1 – 9**, **35** and **36**) and OA (**37** and **38**), in which the IZ diameter was in the range from 6.89 ±0.78 to 15.93 ±0.12 mm of three examined Gram-positive strains. However, all the tested derivatives exhibited no

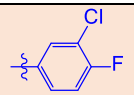
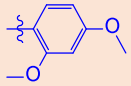
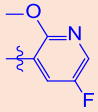
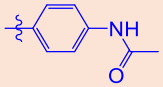
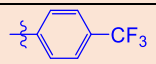
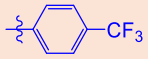
obviously inhibitory activity against the two Gram-negative strains，*Salmonella typhimurium* (CMCC 50115) and *Escherichia coli* (CMCC 44102) (data not showed). The difference of antibacterial activities among the series of GA derivatives (**13 - 34**) during this agar disk diffusion assay were not fully demonstrated, so a microtiter plate dilution method was conducted to determine the minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) in 96-well plates. After incubation for 24 hours, the plates were evaluated for the presence or absence of bacterial growth. Each sample concentration was repeated four times and Gatifloxacin was employed as positive control in the assay. The final concentration of DMSO in the 96-well plate had no effect on bacterial growth.

Table 2. Biological evaluation of pentacyclic triterpenoids derivatives expressed in MIC[a] and MBC[b] (μmol/L)

Compound code	**R_5**	**MICs and MBCs of Selected Bacterium (μmol/L)**							
		Staphylococcus aureus (ATCC 6538)		*Staphylococcus aureus* (ATCC 29213)		*Staphylococcus epidermidis* (ATCC 12228)		*Methicillin-resistant Staphylococcus aureus* (MRSA)	
		MIC[a]	MBC[b]	MIC	MBC	MIC	MBC	MIC	MBC
GA	-	200	NT[c]	200	NT	200	NT	＞200	NT

UA	-	＞200	NT	＞200	NT	＞200	NT	＞200	NT
OA	-	＞200	NT	＞200	NT	＞200	NT	＞200	NT
1~12	-	＞200	NT	＞200	NT	＞200	NT	＞200	NT
13		6.25	12.5	12.5	12.5	12.5	25	50	100
14		12.5	12.2	6.25	12.5	25	25	100	100
15		12.5	25	6.25	12.5	12.5	25	50	50
16		12.5	12.5	6.25	12.5	12.5	12.5	50	100
17		12.5	25	12.5	25	12.5	25	50	100
18		12.5	25	12.5	25	25	50	100	100
19		6.25	12.5	6.25	12.5	6.25	12.5	50	50
20		6.25	12.5	3.125	12.5	12.5	25	50	50

21		6.25	12.5	3.125	6.25	3.125	6.25	50	50
22		25	50	25	50	25	50	50	100
23		6.25	12.5	6.25	12.5	12.5	12.5	25	50
24		12.5	25	12.5	25	12.5	12.5	50	100
25		6.25	12.5	3.125	6.25	6.25	12.5	12.5	25
26		12.5	25	12.5	25	25	50	50	50
27		12.5	12.5	6.25	12.5	12.5	12.5	25	50
28		6.25	12.5	6.25	12.5	6.25	12.5	25	25
29		6.25	12.5	6.25	12.5	12.5	25	25	50

30		12.5	25	12.5	25	12.5	25	50	50
31		12.5	12.5	6.25	12.5	12.5	25	50	50
32		3.125	6.25	1.5625	3.125	6.25	12.5	25	25
33		1.25	2.5	1.25	2.5	1.25	2.5	5	5
34		5	5	5	10	5	5	5	10
35		＞200	NT	＞200	NT	＞200	NT	＞200	NT
36		＞200	NT	＞200	NT	＞200	NT	＞200	NT
37		＞200	NT	＞200	NT	＞200	NT	＞200	NT
38		＞200	NT	＞200	NT	＞200	NT	＞200	NT
Gatifloxacin	-	0.2	0.2	0.2	0.2	0.2	0.2	NT	NT

[a]MIC (μmol/mL), minimum inhibitory concentration, i.e., the lowest concentration of the compound that completely inhibits the growth of bacteria.

[b]MBC (μmol/mL), minimum bactericidal concentration, i.e., the lowest concentration of the compound that completely kills the bacteria.

[c]NT, not tested.

The MIC and MBC results of the derivatives determined by the micro-dilution method were presented in Table 2. The results suggested that GA derivatives **13** - **34** (MIC=1.25 - 100 μmol/L, MBC=2.5 - 100 μmol/L) had inhibitory activity against all four strains of *Staphylococcus*, which was in accordance with the agar disk diffusion study results (Table 1). These assays of GA derivatives displayed considerable effect on inhibition of Methicillin-resistant *Staphylococcus aureus* (MRSA) with MIC range from 5 to 100 μmol/L (Table 2). The results also confirmed that there is no improvement to the inhibitory of the tested bacterial strains by introducing exocyclic *α*, *β*-unsaturated ketone group at the similar (C-2) position of OA and UA (**35** - **38**). Since GA derivatives are structurally different from OA and UA derivatives in terms of their natural product cores, it suggests that the difference in antibacterial activity of three series derivatives might be related to the structural differences in ring C and ring E, such as the position of the carboxylic acid and/or the carbonyl group. Amongst the GA derivatives, different sizes of the aromatic side-chains are well tolerated at the C-2 position e.g. phenyl ring (**13**, MIC = 6.25 - 12.5 μmol/L) vs. quinolone ring (**34**, MIC = 5 μmol/L) vs. biaryl rings (**20** & **23**, MIC = 6.25 - 12.5 μmol/L). A number of mono- or disubstituted phenyl or other heterocyclic aryl side-chains also promoted reasonable activities against the tested Gram-positive bacteria. In general, for this series of GA derivatives, the potency differences between the sensitive strain and resistant strains of *S. aureus* are small (≤ 2 fold) and the differences between corresponding MIC

values and MBC values are also quite small (≤ 2 fold). Overall, this series of GA derivatives showed consistent activity against all four tested strains of *Staphylococcus,* which were significantly higher than both the parent compound (GA) and the ketone intermediate (**10**). In particular, compound **33** demonstrated the highest activity (MIC = 1.25 μmol/L) against all four strains within this series.

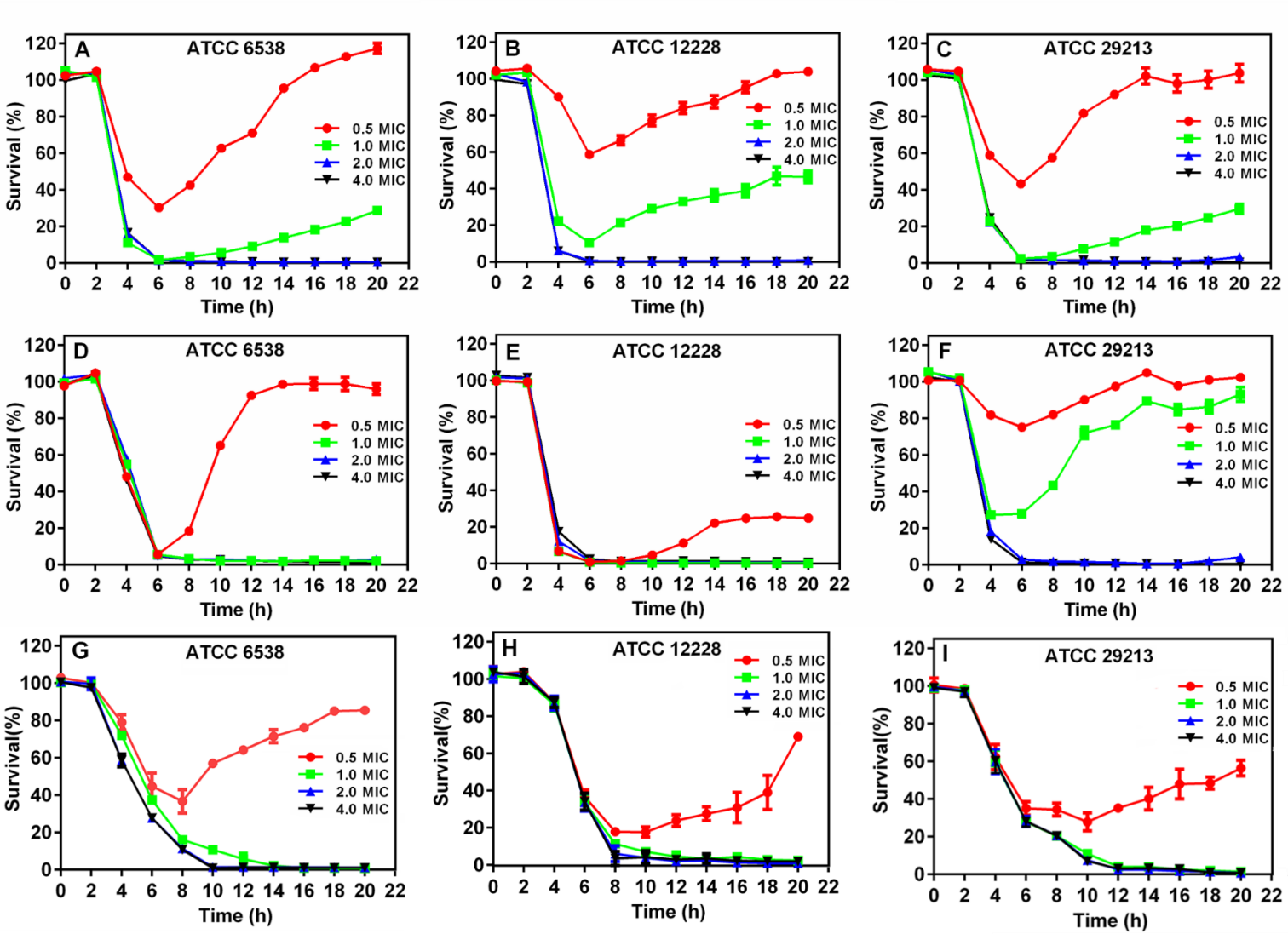

A
ATCC 6538
B
ATCC 12228
C
ATCC 29213
D
ATCC 6538
E
ATCC 12228
F
ATCC 29213
G
ATCC 6538
H
ATCC 12228
I
ATCC 29213
Survival (%)
Survival(%)
Time (h)
0.5 MIC
1.0 MIC
2.0 MIC
4.0 MIC

Figure 3. The time killing kinetic studies of GA derivatives **21**, **32** and **33** against three Gram-positive bacteria strains of *Staphylococcus*. Including two strains of *Staphylococcus aureus* (ATCC 6538 and ATCC 29213) and *Staphylococcus epidermidis* (ATCC 12228), exposed to four different concentrations of derivative **21** (Figure 3A, 3B, 3C), **32** (Figure 3D, 3E, 3F) and **33** (Figure 3G, 3H, 3I) according to their respective MICs (n=4).

The time killing kinetic studies were performed over a period of 20 hours' assay at 37 ℃ according to previously reported study with a slightly modification [35-38]. Figure 3 displayed the time-kill curves of selected GA derivatives of **21**, **32** and **33** against two strains of drug sensitive *Staphylococcus aureus* (ATCC 6538 and ATCC 29213) and *Staphylococcus epidermidis* (ATCC 12228). As presented in Figure 3, all tested strains of bacteria were effectively inhibited at the MICs of derivatives **21**, **32** and **33** with a slight growth close to the end of the kinetic study. The bacterial growth was totally inhibited at higher concentrations of 2×MIC and 4×MIC at the end of the assay, which were also in accordance with the biological assay of MBCs. While the bacteria strains were incubated with 0.5×MIC of **21**, **32** and **33**, the number of bacteria initially decreased at a rapid rate, then gradually raised, and the bacteria inhibition was maintained for 6-8 hours. Furthermore, similar growth inhibition patterns were observed for all three strains of *Staphylococcus*.

2.3 Molecular docking

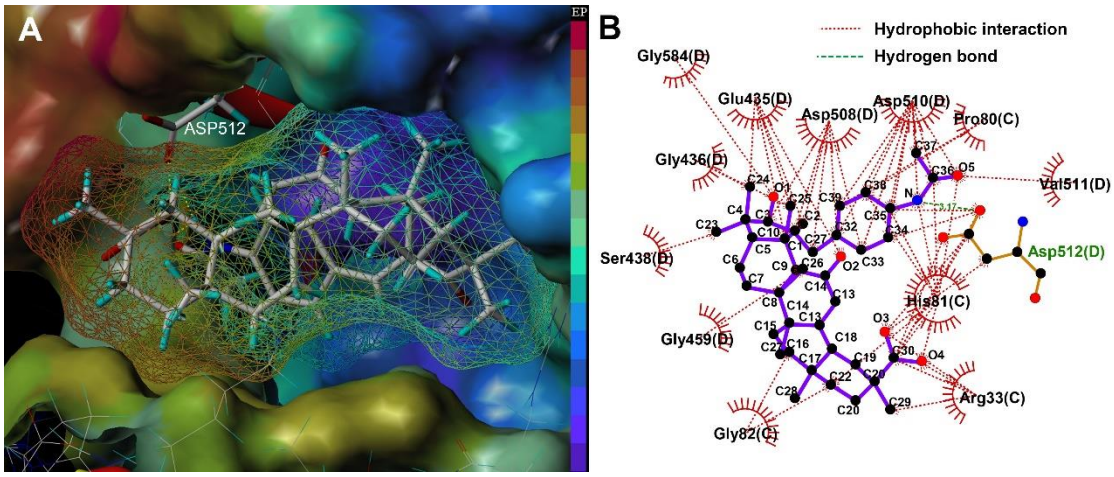


Figure 4. The poses of compound **33** docked in the cleavage site of *S. aureus* DNA gyrase with surface of electrostatic potential (A) and hydrophobic interaction (B).

In order to rationalize the observed antibacterial activity and to investigate the interactions of the newly prepared compounds in the DNA gyrase catalytic site, compounds with significant antibacterial activity and target protein from *S. aureus* DNA gyrase (PDB code: 5cdq) were selected for molecular docking with the SYBYL-X 2.0 program. The binding model of compound **33** and gyrase-DNA is depicted in Figure 4, which revealed that compound is well filled in the binding pocket[39]. As show in Figure 4A, in this binding mode, the molecular structure of the compound exhibits a large bended shape and the carboxyl amide group in compound **33** is in close proximity (3.17 Å) with amino acid residue ASP512 and has the potentially of hydrogen bonding interaction. It can be seen from the molecular surface of the compound and protein that the high electrostatic potential position of the compound structure was located in the corresponding high electrostatic potential region of the protein and *vice versa*, which is propitious to form more stable ligand-protein complexes. The 2D hydrophobic interaction diagram (Figure 4B) showed that **33** accommodated in the hydrophobic sub-pocket of the active site surrounded by the hydrophobic site chains of the amino acids Gly82, Gly459, Ser438, Gly436, Gly584,

Glu435, Asp508, Asp510, Pro80, Val511, Hls81, Arg33, which enhanced the bonding force between the compound and the protein. Noticeably, the vast majority of the hydrophobic forces were concentrated in the site substituted with aromatic rings.

2.4 Pharmacokinetics and cytotoxicity

Table 3. DMPK and cytotoxicity data for selected GA derivatives **33** and **34**

	33	**34**
LogD[a]	3.40	3.40
Solubility at pH 7.4[b]	533	75
Human PPB (% Free)[c]	0.28	0.05
Rat Heps. Cl_{int}[d]	21.6	26.2
Human Mics. Cl_{int}[e]	<3.00	54.40
CC_{50}[f]	>64	>64

[a]Octanol/water partitioning, pH 7.4, measured value.

[b]Aqueous solubility in pH 7.4 PBS buffer (μM).

[c]Human plasma protein binding (%free).

[d]Rat hepatocytes intrinsic clearance ($\mu L \cdot min^{-1}$ 1 $\times 10^6$ $cells^{-1}$).

[e]Human microsome intrinsic clearance ($\mu L \cdot min^{-1}$ $\cdot mg^{-1}$).

[f]The concentration of the compound that reduced mammalian cell viability to 50% (μM), cycloheximide as positive control (CC_{50} = 0.25±0.03).

Two GA derivatives, **33** and **34** were assessed for their DMPK properties (Table 3). In terms of physiochemical properties, both compounds have similar lipophilicity and were highly bound to plasma protein, but **33** was noticeably more soluble than **34** in aqueous medium. For metabolic stability, although their turnover rates by rat hepatocytes were both reasonably low, **33** showed much lower clearance than **34** by human microsome *in vitro*. In addition, from the *in vitro* toxicity assessment, both **33** and **34** showed low cytotoxicity (CC_{50} > 64 μM) against BV2 microglial cells that

demonstrated sufficient safety margin (> 50 folds) in comparison to the antibacterial activity *in vitro*. The *in vitro* DMPK and safety profiles of both compounds indicated they were suitable for further optimization as early leads for either an oral or IV administrative antibiotic series.

3. Conclusion

In summary, a number of derivatives of pentacyclic triterpenoids: UA, OA and GA, were synthesized and tested for their antibacterial activity. Amongst this group of natural product derivatives, those modified from GA showed significantly higher potency than their parent and other analogues derived from UA or OA cores. The modification in this work was mainly focused on the C-2 position of the pentacyclic triterpenoid scaffolds. With a wide range of side-chains substituted at the C-2 position of the GA scaffold, it indicated that this position can tolerate different sized and types of aromatic rings as substitutions, maintaining reasonable antibacterial activities. This finding lays a solid foundation for future optimization, and the SAR at this position, and indeed other positions of the GA scaffold, are being investigated in more detail in ongoing studies. Using molecular docking study, the selected lead compound **33** can fit in well with the binding site of the *S. aureus* DNA gyrase structure, although further computational and experiment studies are still required to investigate this preliminary observation. Preliminary assessments of DMPK and safety properties suggested that the two selected lead compounds were well positioned for further optimization and development. Other key aspects of the next stage optimization/development are to broaden the antibacterial spectrum of the GA derivatives against Gram-negative bacteria and to further understand the mechanism of action and resistance potential of this novel series of semi-synthetic compounds.

4. Experimental Section

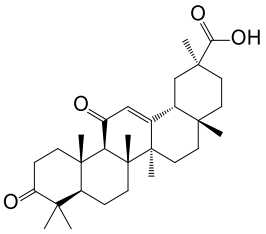
214 4.1 Chemistry materials and methods

215 All reagents were purchased from Adamas Reagent Ltd. (Shanghai China) in
216 analytical reagent grade and were used directly without further purification. Flash
217 chromatography was carried out using silica gel (200-300 mesh) which was supplied
218 by Inno-chem Co., Ltd. (Beijing China). Analytical TLC was performed on pre-coated
219 silica gel F254 plates (0.25 mm; E. Merck), and the products were visualized under
220 UV (254 nm) or by treated with an ethanolic solution of *p*-anisaldehyde spray
221 followed by heating. All derivatives of GA, UA and OA were characterized by ^{1}H
222 NMR, ^{13}C NMR and HRMS. The antimicrobial activity was assayed by using a
223 Multi-model Plate Reader (Infinite 200). The purities of all tested compounds were
224 confirmed by analytical HPLC with a dual pump Shimadzu LC 20A system equipped
225 with a C18 column (250 mm x 4.6 mm, 5 μM YMC). Analytical method conditions:
226 flow rate = 0.5 mL/min, injection volume = 10 μL, isocratic elution system = 80%
227 solvent A (70% water, 20% acetonitrile, 5% glacial acetic acid, 5% tetrahydrofuran)
228 and 20% solvent B (acetonitrile) at room temperature and run time = 15 min. The
229 purities of all compounds are over 95% and R_t are between 7.6~9.2 min.

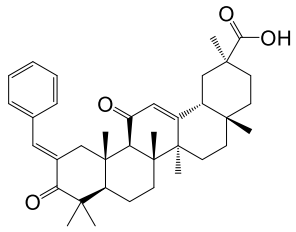
230 4.1.1 General procedure for the synthesis of GA derivatives (**13**~**34**)

231 GA derivatives **13 - 34** were obtained according to Scheme 1. GA was dissolved
232 in acetone at 0 ℃; Jones reagent was added to the reaction mixture drop-wisely until
233 the solution colour was stable in light brown, which implied that the Jones reagent
234 was in slight excess to oxidize the C-3 hydroxyl group into ketone to produce the
235 intermediate **10**. Purification of compound **10** by flash column chromatography was
236 carried out using eluent (petroleum ether/ ethyl acetate, 3 : 1, containing 0.5% formic
237 acid). Derivatives **13 - 34** could be prepared by Claisen Schmidt condensation of
238 intermediate **10** with corresponding aldehydes in the presence of ethanolic potassium

hydroxide in good yield at room temperature. All the results were detailed below.

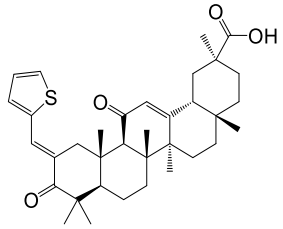


(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR)-2,4a,6a,6b,9,9,12a-heptamethyl-10,13-dioxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (GA-O, **10**, $C_{30}H_{44}O_4$). Yield: 96%; white solid; mp: 291-292 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.75 (s, 1H), 3.03 – 2.88 (m, 1H), 2.71 – 2.55 (m, 1H), 2.45 (s, 1H), 2.42 – 2.31 (m, 1H), 2.22 (d, *J* = 10.7 Hz, 1H), 2.11 – 1.98 (m, 2H), 1.94 (d, *J* = 13.5 Hz, 1H), 1.91 – 1.79 (m, 1H), 1.77 – 1.50 (m, 4H), 1.50 – 1.35 (m, 7H), 1.35 – 1.30 (m, 1H), 1.30 – 1.20 (m, 8H), 1.18 (s, 3H), 1.11 (s, 3H), 1.07 (s, 3H), 1.05 – 0.99 (m, 1H), 0.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 217.3, 199.8, 181.2, 169.9, 128.6, 61.2, 55.7, 48.4, 47.9, 45.5, 43.9, 43.5, 41.2, 39.9, 37.9, 36.9, 34.4, 32.3, 32.1, 31.1, 28.7, 28.6, 26.7, 26.6, 23.5, 21.6, 19.0, 18.7, 15.8. HRMS (ESI): $C_{30}H_{45}O_4$ (469.3312) $[M+H]^+$=469.3314.



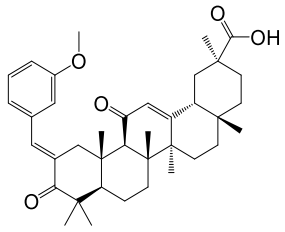
(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR)-11-((Z)-benzylidene)-2,4a,6a,6b,9,9,12a-heptamethyl-10,13-dioxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (**13**, $C_{37}H_{48}O_4$). According to the general procedure, derivative **13** was prepared by Claisen Schmidt condensation of intermediate **10** with benzaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 6 : 1, containing 0.5% formic acid). Yield: 88%; white

solid; mp: 262-263 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (d, *J* = 7.5 Hz, 2H), 7.49 (s, 1H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.35 – 7.30 (m, 1H), 5.83 (s, 1H), 4.28 (d, *J* = 16.7 Hz, 1H), 2.58 (s, 1H), 2.29 (t, *J* = 16.5 Hz, 2H), 2.14 – 1.96 (m, 3H), 1.90 (t, *J* = 12.0 Hz, 1H), 1.81 – 1.64 (m, 2H), 1.63 – 1.49 (m, 3H), 1.49 – 1.41 (m, 5H), 1.40 – 1.29 (m, 2H), 1.25 (d, *J* = 9.5 Hz, 6H), 1.21 (s, 3H), 1.20 – 1.14 (m, 6H), 1.13 – 1.03 (m, 2H), 0.89 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.8, 199.5, 181.9, 170.0, 137.2, 135.9, 134.0, 130.5, 128.6, 128.5, 128.4, 59.4, 53.3, 48.3, 45.4, 45.0, 44.6, 43.8, 43.38, 41.0, 37.7, 36.2, 31.9, 31.5, 30.8, 29.7, 28.6, 28.4, 26.5, 26.4, 23.2, 22.5, 19.6, 18.0, 15.4. HRMS (ESI): $C_{37}H_{49}O_4$ (557.3625) $[M+H]^+$=557.3632.

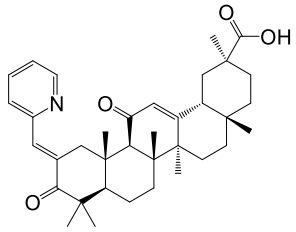


(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR,Z)-2,4a,6a,6b,9,9,12a-heptamethyl-10,13-dioxo-11-(thiophen-2-ylmethylene)-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (**14**, $C_{35}H_{46}O_4S$). According to the general procedure, derivative **14** was prepared by Claisen Schmidt condensation of intermediate **10** with formylthiophene in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 4 : 1, containing 0.5% formic acid). Yield: 85%; white solid; mp: 294-295 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.73 (s, 1H), 7.47 (d, *J* = 5.0 Hz, 1H), 7.33 (d, *J* = 3.4 Hz, 1H), 7.10 (dd, *J* = 4.9, 3.9 Hz, 1H), 5.86 (s, 1H), 4.37 – 4.25 (m, 1H), 2.62 (s, 1H), 2.24 (t, *J* = 14.6 Hz, 2H), 2.13 – 1.96 (m, 3H), 1.89 (td, *J* = 13.4, 4.1 Hz, 1H), 1.77 – 1.62 (m, 1H), 1.60 – 1.48 (m, 4H), 1.48 – 1.40 (m, 5H), 1.28 – 1.23 (m, 8H), 1.23 – 1.19 (m, 6H), 1.17 (s, 3H), 1.13 (s, 2H), 0.88 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.8,

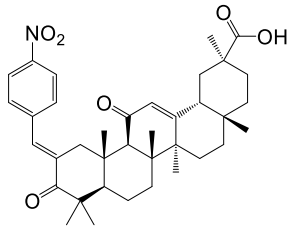
199.6, 181.1, 170.0, 139.7, 132.7, 130.8, 130.5, 130.1, 128.9, 127.6, 59.7, 53.2, 48.5, 45.2, 44.0, 43.6, 41.3, 37.9, 36.2, 32.1, 31.7, 31.1, 30.1, 29.9, 28.8, 28.6, 26.8, 26.6, 23.4, 22.7, 19.9, 18.2, 16.0. HRMS (ESI): $C_{35}H_{47}O_4S$ (563.3190) $[M+H]^+$=563.3187.



(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR)-11-((Z)-3-methoxybenzylidene)-2,4a,6a,6b,9,9,12a-heptamethyl-10,13-dioxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (**15**, $C_{38}H_{50}O_5$). According to the general procedure, derivative **15** was prepared by Claisen Schmidt condensation of intermediate **10** with 3-methoxybenzaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 6 : 1, containing 0.5% formic acid). Yield: 83%; white solid; mp: 183-184 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, *J* = 4.3 Hz, 1H), 7.69 (td, *J* = 7.7, 1.4 Hz, 1H), 7.45 (d, *J* = 7.9 Hz, 1H), 7.42 (s, 1H), 7.14 (dt, *J* = 42.3, 21.1 Hz, 1H), 5.79 (s, 1H), 4.38 (d, *J* = 18.1 Hz, 1H), 2.59 (s, 1H), 2.46 (d, *J* = 18.2 Hz, 1H), 2.24 (d, *J* = 10.8 Hz, 2H), 2.11 – 1.93 (m, 3H), 1.86 (td, *J* = 13.4, 3.7 Hz, 2H), 1.78 – 1.59 (m, 3H), 1.59 – 1.46 (m, 4H), 1.46 – 1.39 (m, 5H), 1.27 – 1.19 (m, 8H), 1.19 – 1.13 (m, 8H), 1.11 – 0.97 (m, 2H), 0.83 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.83, 199.32, 181.64, 169.81, 159.42, 137.16, 137.10, 134.33, 129.41, 128.62, 122.78, 115.70, 114.48, 59.38, 55.35, 53.32, 48.27, 45.46, 45.06, 44.48, 43.82, 43.35, 40.99, 37.71, 36.26, 31.91, 31.53, 30.89, 29.65, 28.61, 28.45, 26.56, 26.40, 23.27, 22.58, 19.61, 18.05, 15.52. HRMS (ESI): $C_{38}H_{51}O_5$ (587.3731) $[M+H]^+$=587.3734.

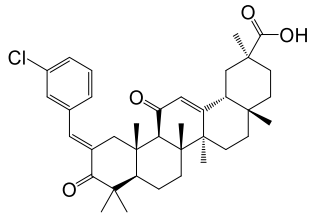


(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR,Z)-2,4a,6a,6b,9,9,12a-heptamethyl-10,13-diox o-11-(pyridin-2-ylmethylene)-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-i cosahydropicene-2-carboxylic acid (**16**, $C_{36}H_{47}NO_4$). According to the general procedure, derivative **16** was prepared by Claisen Schmidt condensation of intermediate **10** with 2-pyridinecarboxaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 5 : 1, containing 0.5% formic acid). Yield: 79%; white solid; mp: 202-203 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (s, 1H), 7.30 (t, *J* = 8.0 Hz, 1H), 7.09 (d, *J* = 7.7 Hz, 1H), 7.03 (s, 1H), 6.86 (dd, *J* = 8.2, 2.3 Hz, 1H), 5.79 (s, 1H), 4.27 (d, *J* = 17.0 Hz, 1H), 3.83 (s, 3H), 2.54 (s, 1H), 2.34 – 2.16 (m, 2H), 2.10 – 1.94 (m, 3H), 1.87 (td, *J* = 13.4, 3.8 Hz, 2H), 1.79 – 1.60 (m, 2H), 1.52 (dd, *J* = 19.4, 11.4 Hz, 4H), 1.45 – 1.38 (m, 5H), 1.25 (d, *J* = 6.8 Hz, 9H), 1.21 (s, 3H), 1.19 – 1.13 (m, 2H), 0.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.9, 199.4, 182.0, 169.9, 159.6, 137.4, 137.3, 134.5, 129.5, 128.8, 115.8, 114.7, 59.6, 55.5, 53.6, 48.4, 45.6, 45.2, 44.0, 43.5, 41.2, 37.9, 36.5, 32.1, 31.7, 31.1, 29.8, 28.7, 28.6, 26.7, 26.6, 23.4, 22.7, 19.8, 18.2, 15.6. HRMS (ESI): $C_{36}H_{48}NO_4$ (558.3578) $[M+H]^+$=558.3585.



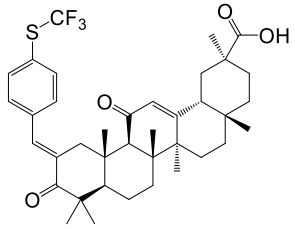
(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR)-2,4a,6a,6b,9,9,12a-heptamethyl-11-((Z)-4-nit robenzylidene)-10,13-dioxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-ico

sahydropicene-2-carboxylic acid (**17**, $C_{37}H_{47}NO_6$). According to the general procedure, derivative **17** was prepared by Claisen Schmidt condensation of intermediate **10** with 4-nitrobenzaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 6 : 1, containing 0.5% formic acid). Yield: 89%; white solid; mp: 230-231 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.26 (d, *J* = 8.7 Hz, 2H), 7.65 (d, *J* = 8.7 Hz, 2H), 7.53 – 7.43 (m, 1H), 5.74 (s, 1H), 4.44 (s, 3H), 4.25 – 4.16 (m, 1H), 3.38 – 3.24 (m, 1H), 2.62 (s, 1H), 2.41 – 2.18 (m, 2H), 2.18 – 2.04 (m, 1H), 2.04 – 1.84 (m, 3H), 1.84 – 1.72 (m, 1H), 1.70 – 1.51 (m, 5H), 1.48 – 1.38 (m, 5H), 1.29 (d, *J* = 16.3 Hz, 3H), 1.24 – 1.17 (m, 6H), 1.17 – 1.14 (m, 3H), 1.12 – 1.05 (m, 2H), 0.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.9, 200.0, 178.9, 171.8, 146.9, 142.15, 137.52, 134.19, 130.5, 129.5, 128.8, 127.8, 123.3, 59.0, 53.2, 45.5, 44.8, 43.9, 43.4, 43.3, 41.0, 37.4, 36.1, 31.6, 31.5, 31.1, 30.7, 28.9, 28.2, 27.9, 26.2, 26.0, 22.7, 22.1, 19.2, 17.6, 14.9. HRMS (ESI): $C_{37}H_{48}NO_6$ (602.3476) $[M+H]^+$=602.3478.



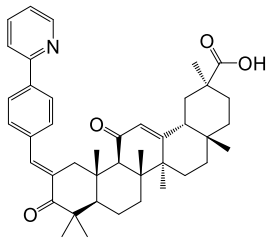
(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR)-11-((Z)-3-chlorobenzylidene)-2,4a,6a,6b,9,9, 12a-heptamethyl-10,13-dioxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a, 9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (**18**, $C_{37}H_{47}ClO_4$). According to the general procedure, derivative **18** was prepared by Claisen Schmidt condensation of intermediate **10** with 3-chlorobenzaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate,

6 : 1, containing 0.5% formic acid). Yield: 81%; white solid; mp: 235-236 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43 (s, 1H), 7.37 (s, 2H), 7.32 (t, *J* = 7.7 Hz, 1H), 7.29 – 7.25 (m, 2H), 5.80 (s, 1H), 4.68 (s, 1H), 4.20 (d, *J* = 16.7 Hz, 1H), 2.54 (s, 1H), 2.23 (d, *J* = 15.3 Hz, 2H), 2.11 – 1.94 (m, 3H), 1.94 – 1.81 (m, 1H), 1.80 – 1.60 (m, 1H), 1.60 – 1.47 (m, 3H), 1.46 – 1.32 (m, 6H), 1.25 (d, *J* = 9.2 Hz, 3H), 1.20 (s, 3H), 1.18 (s, 3H), 1.16 (s, 6H), 1.07 (d, *J* = 16.0 Hz, 2H), 0.86 (s, 3H).^{13}C NMR (100 MHz, $CDCl_3$) δ 207.6, 199.4, 181.8, 170.0, 137.9, 135.7, 135.6, 134.5, 130.5, 129.8, 128.8, 128.5, 128.2, 59.5, 53.7, 48.5, 45.7, 45.2, 44.4, 44.0, 43.6, 41.2, 37.9, 36.5, 32.1, 31.7, 31.1, 29.7, 28.8, 28.6, 26.8, 26.6, 23.4, 22.7, 19.7, 18.2, 15.6. HRMS (ESI): $C_{37}H_{48}ClO_4$ (591.3236) $[M+H]^+$=591.3235.



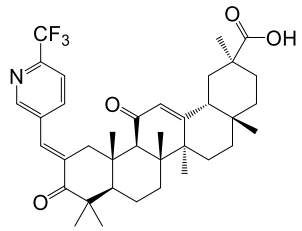
(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR)-2,4a,6a,6b,9,9,12a-heptamethyl-10,13-dioxo-11-((Z)-4-((trifluoromethyl)thio)benzylidene)-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene -2-carboxylic acid (**19**, $C_{38}H_{47}F_3O_4S$). According to the general procedure, derivative **19** was prepared by Claisen Schmidt condensation of intermediate **10** with 4-(trifluoromethylthio)benzaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 6 : 1, containing 0.5% formic acid). Yield: 82%; white solid; mp: 258-259 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.66 (d, *J* = 8.2 Hz, 2H), 7.53 (d, *J* = 8.3 Hz, 2H), 7.42 (s, 1H), 5.83 (s, 1H), 4.24 (d, *J* = 17.0 Hz, 1H), 2.56 (s, 1H), 2.33 – 2.18 (m, 2H), 2.13 – 1.94 (m, 3H), 1.88 (td, *J* = 13.3, 3.6 Hz, 1H), 1.79 – 1.69 (m, 1H), 1.65 (t, *J* = 13.6 Hz, 1H), 1.60 – 1.48 (m, 2H), 1.48 – 1.33 (m, 6H), 1.33 – 1.25 (m, 2H), 1.24 (s, 3H), 1.21

(s, 3H), 1.19 (s, 3H), 1.16 (d, *J* = 2.4 Hz, 6H), 1.07 (d, *J* = 14.0 Hz, 2H), 0.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.6, 199.7, 181.8, 170.6, 138.5, 136.3, 136.2, 135.4, 131.3, 131.2, 128.7, 128.1, 124.4, 59.5, 53.6, 48.5, 45.7, 45.2, 44.6, 44.0, 43.6, 41.2, 37.9, 36.5, 32.1, 31.7, 31.1, 30.3, 29.7, 28.8, 28.6, 26.7, 26.6, 23.4, 22.7, 19.7, 18.2, 15.6. HRMS (ESI): $C_{38}H_{48}F_3O_4S$ (657.3220) $[M+H]^+$=657.3222.

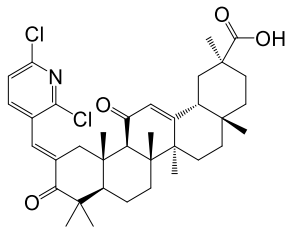


(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR)-2,4a,6a,6b,9,9,12a-heptamethyl-10,13-dioxo-11-((Z)-4-(pyridin-2-yl)benzylidene)-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (**20**, $C_{42}H_{51}NO_4$). According to the general procedure, derivative **20** was prepared by Claisen Schmidt condensation of intermediate **10** with 4-(2-Pyridinyl)benzaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 6 : 1, containing 0.5% formic acid). Yield: 89%; white solid; mp: 239-240 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (d, *J* = 4.3 Hz, 1H), 8.00 (d, *J* = 8.2 Hz, 2H), 7.81 – 7.66 (m, 2H), 7.60 (d, *J* = 8.3 Hz, 2H), 7.55 – 7.46 (m, 1H), 7.21 (t, *J* = 4.9 Hz, 1H), 5.79 (d, *J* = 9.9 Hz, 1H), 4.36 – 4.25 (m, 1H), 2.56 (d, *J* = 14.7 Hz, 1H), 2.38 – 2.18 (m, 2H), 2.11 – 1.95 (m, 3H), 1.93 – 1.81 (m, 1H), 1.80 – 1.49 (m, 7H), 1.45 – 1.40 (m, 5H), 1.26 (s, 3H), 1.23 (s, 3H), 1.20 – 1.15 (m, 9H), 1.06 (d, *J* = 13.1 Hz, 2H), 0.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.7, 199.3, 181.2, 169.9, 156.7, 149.5, 138.9, 137.0, 136.6, 136.5, 134.7, 130.9, 129.1, 128.7, 127.0, 126.9, 125.4, 122.3, 120.8, 59.5, 53.4, 48.3, 45.5, 45.1, 44.8, 43.8, 43.4, 41.1, 37.7, 36.3, 31.9, 31.6, 29.7, 29.7, 28.6, 28.4, 26.6, 26.5, 22.6, 19.6, 18.1, 15.5. HRMS (ESI): $C_{42}H_{52}NO_4$ (634.3891)

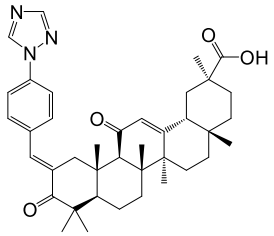
$[M+H]^+$=634.3903.



(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR,Z)-2,4a,6a,6b,9,9,12a-heptamethyl-10,13-dioxo-11-((6-(trifluoromethyl)pyridin-3-yl)methylene)-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (**21**, $C_{37}H_{46}F_3NO_4$). According to the general procedure, derivative **21** was prepared by Claisen Schmidt condensation of intermediate **10** with 2-trifluoromethyl-pyridine-5-carbaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 4 : 1, containing 0.5% formic acid). Yield: 84%; white solid; mp: 174-175 ℃; ^{1}H NMR (500 MHz, Chloroform-*d*) δ 8.88 – 8.66 (m, 1H), 8.01 (dd, *J* = 8.2, 1.6 Hz, 1H), 7.71 (d, *J* = 8.2 Hz, 1H), 7.43 (s, 1H), 5.80 (s, 1H), 4.21 (d, *J* = 17.9 Hz, 1H), 2.54 (s, 1H), 2.30 – 2.21 (m, 2H), 2.08 – 1.94 (m, 3H), 1.86 (td, *J* = 13.5, 4.1 Hz, 1H), 1.79 – 1.69 (m, 1H), 1.63 (t, *J* = 13.6 Hz, 1H), 1.59 – 1.47 (m, 4H), 1.45 – 1.39 (m, 5H), 1.36 – 1.31 (m, 1H), 1.30 – 1.20 (m, 8H), 1.19 – 1.13 (m, 8H), 1.10 – 1.03 (m, 1H), 0.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.9, 199.6, 181.6, 170.9, 151.7, 147.1, 138.7, 137.6, 134.8, 131.5, 128.6, 123.0, 120.3, 59.4, 53.8, 48.5, 45.9, 45.2, 44.5, 43.9, 43.6, 41.2, 37.8, 36.6, 32.1, 31.7, 31.1, 29.6, 28.8, 28.5, 26.7, 26.6, 23.4, 22.8, 19.7, 18.2, 15.6. HRMS (ESI): $C_{37}H_{47}F_3NO_4$ (626.3452) $[M+H]^+$=626.3455.

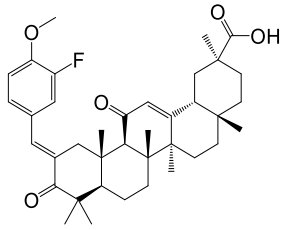


(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR,Z)-11-((2,6-dichloropyridin-3-yl)methylene)-2,4a,6a,6b,9,9,12a-heptamethyl-10,13-dioxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (**22**, $C_{36}H_{45}Cl_2NO_4$). According to the general procedure, derivative **22** was prepared by Claisen Schmidt condensation of intermediate **10** with 2,6-dichloropyridine-3-carbaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 4 : 1, containing 0.5% formic acid). Yield: 76%; white solid; mp: 232-233 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 8.5 Hz, 1H), 7.54 (s, 1H), 6.69 (d, *J* = 8.4 Hz, 1H), 5.77 (s, 1H), 4.03 (dd, *J* = 16.3, 7.6 Hz, 1H), 2.50 (d, *J* = 4.4 Hz, 1H), 2.29 – 2.19 (m, 1H), 2.14 – 1.98 (m, 3H), 1.98 – 1.79 (m, 2H), 1.78 – 1.68 (m, 1H), 1.68 – 1.59 (m, 1H), 1.59 – 1.54 (m, 2H), 1.54 – 1.47 (m, 2H), 1.46 – 1.32 (m, 9H), 1.23 (s, 3H), 1.21 – 1.16 (m, 9H), 1.07 (d, *J* = 13.6 Hz, 2H), 0.86 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 206.7, 199.8, 181.7, 171.1, 150.5, 149.4, 140.7, 138.1, 130.6, 129.7, 128.3, 122.9, 59.2, 53.8, 48.3, 46.1, 45.1, 43.8, 43.5, 43.4, 41.0, 37.7, 36.7, 31.9, 31.6, 30.9, 29.1, 28.6, 28.4, 26.5, 26.4, 23.2, 22.8, 19.4, 18.2, 15.51.



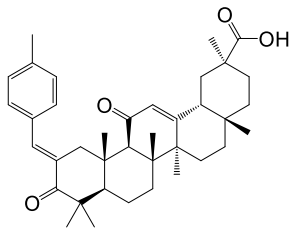
(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR)-11-((Z)-4-(1H-1,2,4-triazol-1-yl)benzylidene)-2,4a,6a,6b,9,9,12a-heptamethyl-10,13-dioxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (**23**, $C_{39}H_{49}N_3O_4$). According to

the general procedure, derivative **23** was prepared by Claisen Schmidt condensation of intermediate **10** with 4-(1H-1,2,4-triazol-1-yl) benzaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 4 : 1, containing 0.5% formic acid). Yield: 75%; white solid; mp: 204-205 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (s, 1H), 8.15 (s, 1H), 7.73 (d, *J* = 8.6 Hz, 2H), 7.64 (d, *J* = 8.6 Hz, 2H), 7.49 (s, 1H), 5.82 (s, 1H), 4.30 (d, *J* = 16.8 Hz, 1H), 2.57 (s, 1H), 2.35 – 2.19 (m, 2H), 2.12 – 1.95 (m, 3H), 1.94 – 1.82 (m, 1H), 1.81 – 1.70 (m, 1H), 1.65 (t, *J* = 13.5 Hz, 1H), 1.61 – 1.49 (m, 4H), 1.49 – 1.40 (m, 3H), 1.40 – 1.30 (m, 2H), 1.27 – 1.21 (m, 7H), 1.21 – 1.15 (m, 9H), 1.07 (d, *J* = 15.1 Hz, 2H), 0.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.4, 199.7, 180.7, 170.7, 152.3, 140.8, 136.4, 136.1, 135.5, 135.3, 131.8, 128.6, 128.6, 123.0, 119.9, 59.5, 53.6, 48.5, 45.7, 45.1, 44.5, 43.8, 43.5, 41.3, 37.8, 36.5, 32.0, 31.7, 31.1, 29.7, 28.7, 28.6, 26.7, 26.6, 23.4, 22.8, 19.7, 18.2, 15.6. HRMS (ESI): $C_{39}H_{50}N_3O_4$ (624.3796) $[M+H]^+$=624.3793.



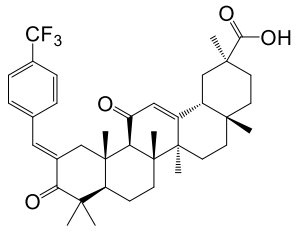
(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR)-11-((Z)-3-fluoro-4-methoxybenzylidene)-2,4a,6a,6b,9,9,12a-heptamethyl-10,13-dioxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (**24**, $C_{38}H_{49}FO_5$). According to the general procedure, derivative **24** was prepared by Claisen Schmidt condensation of intermediate **10** with 3-fluoro-4-methoxybenzaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 6 : 1, containing 0.5% formic acid). Yield: 85%; white solid; mp: 188-189 ℃; ^{1}H NMR

(400 MHz, $CDCl_3$) δ 7.37 (s, 1H), 7.28 – 7.22 (m, 2H), 6.98 (t, *J* = 8.8 Hz, 1H), 5.82 (s, 1H), 4.21 (d, *J* = 16.8 Hz, 1H), 3.89 (s, 3H), 2.57 (s, 1H), 2.25 (d, *J* = 15.8 Hz, 2H), 2.13 – 1.96 (m, 3H), 1.93 – 1.81 (m, 1H), 1.79 – 1.61 (m, 2H), 1.60 – 1.48 (m, 4H), 1.48 – 1.34 (m, 6H), 1.31 – 1.22 (m, 3H), 1.20 (d, *J* = 8.4 Hz, 6H), 1.15 (s, 6H), 1.07 (d, *J* = 13.7 Hz, 2H), 0.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.6, 199.6, 182.3, 170.1, 152.0, 148.1, 136.03, 133.12, 129.3, 128.8, 127.3, 118.2, 113.2, 59.5, 56.3, 53.4, 48.4, 45.4, 45.2, 44.7, 43.9, 43.5, 41.1, 37.8, 36.3, 32.0, 31.6, 31.0, 29.9, 28.7, 28.5, 26.7, 26.6, 23.3, 22.7, 19.7, 18.2, 15.5. HRMS (ESI): $C_{38}H_{50}FO_5$ (605.3637) $[M+H]^+$=605.3642.

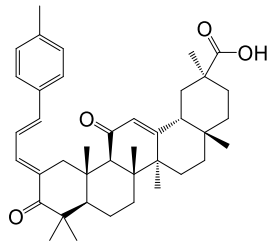


(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR)-2,4a,6a,6b,9,9,12a-heptamethyl-11-((Z)-4-methylbenzylidene)-10,13-dioxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (**25**, $C_{38}H_{50}O_4$). According to the general procedure, derivative **25** was prepared by Claisen Schmidt condensation of intermediate **10** with p-tolualdehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 6 : 1, containing 0.5% formic acid). Yield: 79%; white solid; mp: 292-293 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 7.45 (s, 1H), 7.40 (d, *J* = 8.1 Hz, 2H), 7.18 (t, *J* = 7.4 Hz, 2H), 5.81 (s, 1H), 2.56 (s, 1H), 2.34 (s, 3H), 2.31 – 2.18 (m, 2H), 2.11 – 1.95 (m, 3H), 1.87 (td, *J* = 13.4, 3.9 Hz, 1H), 1.78 – 1.60 (m, 2H), 1.55 (s, 4H), 1.47 – 1.39 (m, 5H), 1.40 – 1.29 (m, 2H), 1.29 – 1.22 (m, 3H), 1.21 (s, 3H), 1.19 (s, 3H), 1.15 (d, *J* = 2.6 Hz, 6H), 1.07 (d, *J* = 13.7 Hz, 2H), 0.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.9, 199.6, 181.4, 169.9, 138.8, 137.5,

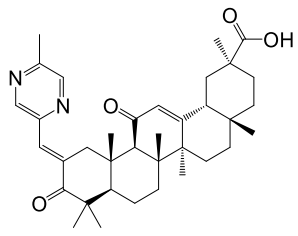
133.3, 133.3, 130.7, 129.4, 128.8, 59.6, 53.5, 48.5, 45.5, 45.2, 44.9, 44.0, 43.5, 41.2, 37.9, 36.4, 32.1, 31.7, 31.1, 29.9, 28.8, 28.6, 26.7, 26.6, 23.4, 22.7, 21.5, 19.8, 18.2, 15.6. HRMS (ESI): $C_{38}H_{51}O_4$ (571.3782) $[M+H]^+$=571.3784.



(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR)-2,4a,6a,6b,9,9,12a-heptamethyl-10,13-dioxo-11-((Z)-4-(trifluoromethyl)benzylidene)-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (**26**, $C_{38}H_{47}F_3O_4$). According to the general procedure, derivative **26** was prepared by Claisen Schmidt condensation of intermediate **26** with 4-trifluoromethylbenzaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 6 : 1, containing 0.5% formic acid). Yield: 87%; white solid; mp: 280-281 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 8.3 Hz, 2H), 7.57 (d, *J* = 8.3 Hz, 2H), 7.46 (s, 1H), 5.81 (s, 1H), 4.24 (d, *J* = 16.8 Hz, 1H), 2.55 (s, 1H), 2.25 (d, *J* = 16.3 Hz, 2H), 2.12 – 1.93 (m, 3H), 1.93 – 1.81 (m, 1H), 1.79 – 1.69 (m, 1H), 1.65 (t, *J* = 13.5 Hz, 1H), 1.60 – 1.47 (m, 4H), 1.47 – 1.38 (m, 3H), 1.38 – 1.30 (m, 1H), 1.24 (dd, *J* = 16.6, 7.9 Hz, 8H), 1.19 (s, 3H), 1.17 (d, *J* = 1.9 Hz, 6H), 1.07 (d, *J* = 13.3 Hz, 2H), 0.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.3, 199.5, 180.6, 170.3, 139.4, 139.4, 136.2, 135.3, 130.3, 128.6, 125.4, 59.3, 53.6, 48.4, 45.7, 45.1, 44.3, 43.7, 43.4, 41.1, 37.7, 36.4, 31.9, 31.6, 30.9, 29.5, 28.6, 28.4, 26.6, 26.4, 23.2, 22.6, 19.6, 18.1, 15.4. HRMS (ESI): $C_{38}H_{48}F_3O_4$ (625.3499) $[M+H]^+$=625.3501.

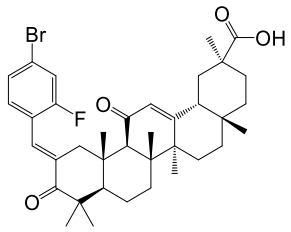


(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR,Z)-2,4a,6a,6b,9,9,12a-heptamethyl-10,13-dioxo-11-((E)-3-(p-tolyl)allylidene)-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (**27**, $C_{40}H_{52}O_4$). According to the general procedure, derivative **27** was prepared by Claisen Schmidt condensation of intermediate **10** with trans-4-methylcinnamaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 6 : 1, containing 0.5% formic acid). Yield: 83%; white solid; mp: 294-296 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 (d, *J* = 8.0 Hz, 2H), 7.27 (d, *J* = 7.0 Hz, 2H), 7.11 (d, *J* = 7.9 Hz, 2H), 7.03 – 6.93 (m, 1H), 6.88 (d, *J* = 15.4 Hz, 1H), 5.85 (s, 1H), 2.57 (s, 1H), 2.34 – 2.22 (m, 4H), 2.13 (d, *J* = 17.4 Hz, 1H), 2.08 – 1.94 (m, 3H), 1.94 – 1.83 (m, 1H), 1.79 – 1.61 (m, 2H), 1.59 – 1.48 (m, 3H), 1.48 – 1.40 (m, 3H), 1.39 – 1.33 (m, 1H), 1.30 – 1.23 (m, 6H), 1.20 (s, 3H), 1.17 (d, *J* = 6.0 Hz, 6H), 1.13 (s, 3H), 1.07 (d, *J* = 13.5 Hz, 2H), 0.88 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.6, 199.9, 181.5, 170.2, 140.9, 139.1, 137.9, 134.2, 132.4, 129.6, 128.9, 127.4, 123.0, 59.5, 53.7, 48.5, 45.4, 45.2, 44.0, 43.6, 43.0, 41.2, 37.9, 36.4, 32.1, 31.8, 31.1, 29.8, 28.8, 28.6, 26.7, 26.6, 23.4, 22.8, 21.5, 19.8, 18.3, 15.5. HRMS (ESI): $C_{40}H_{53}O_4$ (597.3938) $[M+H]^+$=597.3939.



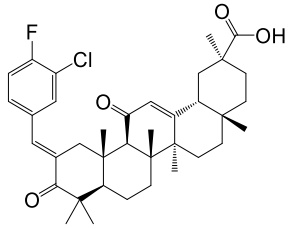
(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR,Z)-2,4a,6a,6b,9,9,12a-heptamethyl-11-((5-met

hylpyrazin-2-yl)methylene)-10,13-dioxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (**28**, $C_{36}H_{48}N_2O_4$). According to the general procedure, derivative **28** was prepared by Claisen Schmidt condensation of intermediate **10** with 2-pyrazinecarboxaldehyde,5-methyl- in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 4 : 1, containing 0.5% formic acid). Yield: 88%; white solid; mp: 213-214 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (d, *J* = 7.1 Hz, 2H), 7.40 (s, 1H), 5.80 (s, 1H), 4.48 (d, *J* = 18.4 Hz, 1H), 2.60 (s, 1H), 2.56 (s, 3H), 2.50 (d, *J* = 19.7 Hz, 1H), 2.29 – 2.20 (m, 1H), 2.13 – 1.95 (m, 3H), 1.87 (td, *J* = 13.3, 3.6 Hz, 1H), 1.80 – 1.61 (m, 2H), 1.59 – 1.50 (m, 3H), 1.50 – 1.31 (m, 5H), 1.28 – 1.21 (m, 8H), 1.20 – 1.11 (m, 9H), 1.07 (d, *J* = 13.4 Hz, 2H), 0.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.6, 199.4, 181.6, 169.8, 152.0, 148.5, 146.2, 144.3, 139.9, 130.8, 128.8, 59.3, 53.6, 48.5, 45.6, 45.3, 45.2, 44.0, 43.6, 41.2, 37.9, 36.1, 32.1, 31.7, 31.1, 29.9, 28.8, 28.6, 26.7, 26.6, 23.4, 22.6, 21.5, 19.8, 18.2, 15.6. HRMS (ESI): $C_{36}H_{49}N_2O_4$ (573.3687) $[M+H]^+$=573.3692.



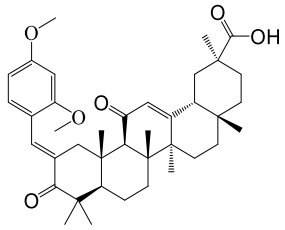
(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR)-11-((Z)-4-bromo-2-fluorobenzylidene)-2,4a,6a,6b,9,9,12a-heptamethyl-10,13-dioxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (**29**, $C_{37}H_{46}BrFO_4$). According to the general procedure, derivative **29** was prepared by Claisen Schmidt condensation of intermediate **10** with 2-bromo-4-fluorobenzaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 6 : 1,

547 containing 0.5% formic acid). Yield: 68%; white solid; mp: 282-283 ℃; ^{1}H NMR
548 (400 MHz, DMSO-d_6) δ 12.12 (s, 1H), 7.69 – 7.59 (m, 1H), 7.52 – 7.39 (m, 2H), 7.36
549 (s, 1H), 5.45 (s, 1H), 3.80 (d, *J* = 16.9 Hz, 1H), 2.64 (s, 1H), 2.45 (d, *J* = 18.7 Hz, 1H),
550 2.17 – 2.03 (m, 2H), 1.86 – 1.66 (m, 5H), 1.66 – 1.48 (m, 3H), 1.44 – 1.34 (m, 6H),
551 1.31 – 1.17 (m, 5H), 1.12 – 1.07 (m, 9H), 0.98 (s, 3H), 0.89 – 0.82 (m, 1H), 0.77 (s,
552 3H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 206.0, 199.0, 178.1, 171.0, 162.0, 156.2,
553 137.4, 132.2, 128.0, 127.8, 127.3, 123.0, 119.7, 58.6, 52.6, 48.7, 45.5, 45.0, 43.7, 43.5,
554 43.2, 41.2, 38.0, 36.2, 35.6, 32.1, 29.5, 28.9, 28.3, 26.6, 26.3, 23.2, 23.0, 19.4, 18.2,
555 16.8, 15.4. HRMS (ESI): $C_{37}H_{46}{}^{79}BrFNaO_4$ (675.2456) $[M+Na]^+$=675.2460,
556 $C_{37}H_{46}{}^{81}BrFNaO_4$ (677.3442) $[M+Na]^+$=677.2448.

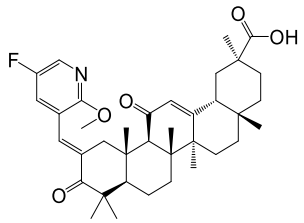


557
558 *(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR)-11-((Z)-3-chloro-4-fluorobenzylidene)-2,4a,6*
559 *a,6b,9,9,12a-heptamethyl-10,13-dioxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b*
560 *,13,14b-icosahydropicene-2-carboxylic acid* (**30**, $C_{37}H_{46}ClFO_4$). According to the
561 general procedure, derivative **30** was prepared by Claisen Schmidt condensation of
562 intermediate **10** with 3-chloro-4-fluorobenzaldehyde in the presence of ethanolic
563 potassium hydroxide at room temperature. Purification of product by flash column
564 chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 6 : 1,
565 containing 0.5% formic acid). Yield: 66%; mp: 133-134 ℃; ^{1}H NMR (400 MHz,
566 $CDCl_3$) δ 7.42 (dd, *J* = 7.1, 2.0 Hz, 1H), 7.40 – 7.33 (m, 1H), 7.03 (t, *J* = 8.7 Hz, 1H),
567 6.45 (s, 1H), 5.78 (s, 1H), 3.73 (d, *J* = 14.8 Hz, 1H), 2.58 – 2.49 (m, 1H), 2.29 – 2.20
568 (m, 2H), 2.12 – 1.81 (m, 5H), 1.79 – 1.60 (m, 3H), 1.60 – 1.54 (m, 2H), 1.46 – 1.39
569 (m, 6H), 1.29 (s, 3H), 1.28 – 1.23 (m, 6H), 1.20 – 1.18 (m, 3H), 1.16 – 1.13 (m, 3H),

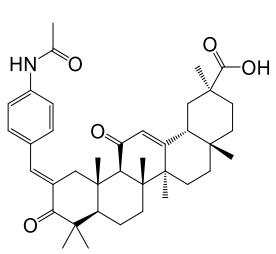
1.07 (s, 2H), 0.87 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 207.3, 199.5, 182.1, 170.3, 157.8, 135.0, 134.8, 133.2, 133.0, 130.0, 128.6, 121.1, 116.6, 59.3, 53.4, 48.31, 45.6, 45.1, 44.1, 43.8, 43.4, 41.0, 37.7, 36.3, 31.9, 31.5, 30.9, 29.6, 28.6, 28.4, 26.6, 26.4, 23.2, 22.6, 19.6, 18.1, 15.4. HRMS (ESI): $C_{37}H_{47}ClFO_4$ (631.2960) $[M+Na]^+$=631.2933.



(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR)-11-((Z)-2,4-dimethoxybenzylidene)-2,4a,6a,6b,9,9,12a-heptamethyl-10,13-dioxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (**31**, $C_{39}H_{52}O_6$). According to the general procedure, derivative **31** was prepared by Claisen Schmidt condensation of intermediate **10** with 2,4-dimethoxybenzaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 6 : 1, containing 0.5% formic acid). Yield: 85%; mp: 209-210 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (s, 1H), 7.41 (d, *J* = 8.6 Hz, 1H), 6.51 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.44 (d, *J* = 2.3 Hz, 1H), 5.79 (s, 1H), 4.17 (d, *J* = 16.6 Hz, 1H), 3.82 (s, 3H), 3.80 (s, 3H), 2.54 (s, 1H), 2.27 – 2.14 (m, 2H), 2.12 – 1.92 (m, 3H), 1.87 (td, *J* = 13.4, 3.9 Hz, 1H), 1.78 – 1.59 (m, 2H), 1.59 – 1.46 (m, 4H), 1.46 – 1.38 (m, 5H), 1.38 – 1.30 (m, 1H), 1.29 – 1.21 (m, 3H), 1.21 – 1.11 (m, 12H), 1.06 (d, *J* = 13.8 Hz, 2H), 0.86 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.4, 199.6, 181.0, 169.8, 161.6, 160.2, 132.7, 131.6, 131.2, 128.9, 118.2, 104.4, 98.6, 59.7, 55.7, 55.5, 53.6, 48.5, 45.5, 45.2, 44.7, 43.9, 43.5, 41.2, 37.9, 36.6, 32.1, 31.8, 31.1, 29.9, 28.7, 28.6, 26.7, 26.6, 23.4, 22.9, 19.8, 18.3, 15.6. HRMS (ESI): $C_{39}H_{53}O_6$ (617.3837) $[M+H]^+$=617.3842.

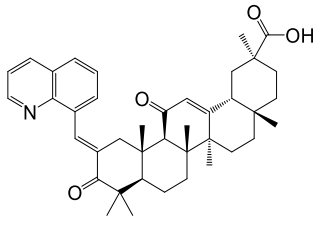


(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR,Z)-11-((5-fluoro-2-methoxypyridin-3-yl)methyl ene)-2,4a,6a,6b,9,9,12a-heptamethyl-10,13-dioxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11, 12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (**32**, $C_{37}H_{48}FNO_5$). According to the general procedure, derivative **32** was prepared by Claisen Schmidt condensation of intermediate **10** with 5-fluoro-2-methoxynicotinaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 5 : 1, containing 0.5% formic acid). Yield: 72%; white solid; mp: 194-195 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, *J* = 2.9 Hz, 1H), 7.59 – 7.52 (m, 1H), 7.50 (dd, *J* = 8.6, 2.8 Hz, 1H), 5.79 (s, 1H), 4.17 – 4.04 (m, 1H), 3.96 (s, 2H), 2.53 (s, 1H), 2.35 – 2.21 (m, 1H), 2.16 (dd, *J* = 16.6, 1.5 Hz, 1H), 2.12 – 1.93 (m, 3H), 1.87 (td, *J* = 13.3, 3.7 Hz, 1H), 1.78 – 1.60 (m, 2H), 1.60 – 1.47 (m, 4H), 1.47 – 1.40 (m, 5H), 1.40 – 1.26 (m, 3H), 1.27 – 1.22 (m, 4H), 1.21 – 1.12 (m, 10H), 1.12 – 1.03 (m, 2H), 0.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 206.9, 199.4, 182.0, 170.2, 158.6, 154.9, 136.5, 133.0, 129.9, 128.7, 125.8, 120.0, 59.4, 54.2, 53.8, 48.4, 45.8, 45.2, 44.1, 43.9, 43.5, 41.1, 37.8, 36.6, 32.0, 31.7, 31.0, 29.6, 28.7, 28.5, 26.7, 26.6, 23.3, 22.8, 19.7, 18.2, 15.5. HRMS (ESI): $C_{37}H_{49}FNO_5$ (606.3589) $[M+H]^+$=606.3590.



(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR)-11-((Z)-4-acetamidobenzylidene)-2,4a,6a,6b,

9,9,12a-heptamethyl-10,13-dioxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (**33**, $C_{39}H_{51}NO_5$). According to the general procedure, derivative 31 was prepared by Claisen Schmidt condensation of intermediate **10** with 4-Acetamidobenzaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 2 : 3, containing 0.5% formic acid). Yield 82.3%; yellow solid; mp: 214-216 ℃; ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.21 (s, 1H), 10.13 (s, 1H), 7.63 (d, J = 8.2 Hz, 2H), 7.45 (d, J = 8.2 Hz, 2H), 7.30 (s, 1H), 5.47 (s, 1H), 3.93 (d, J = 17.1 Hz, 1H), 2.67 (s, 1H), 2.48 (s, 4H), 2.10 (d, J = 13.0 Hz, 2H), 1.87 – 1.61 (m, 6H), 1.59 – 1.45 (m, 3H), 1.41 (d, J = 21.5 Hz, 5H), 1.35 (d, J = 13.4 Hz, 2H), 1.29 – 1.12 (m, 4H), 1.11 – 1.06 (m, 8H), 1.04 (s, 2H), 0.96 (s, 3H), 0.76 (s, 2H). ^{13}C NMR (126 MHz, DMSO-d_6) δ 206.2, 199.1, 178.1, 170.9, 169.0, 140.3, 136.3, 132.8, 131.7, 130.4, 128.0, 119.1, 58.6, 52.4, 48.6, 45.0, 44.9, 43.8, 43.6, 43.5, 41.2, 38.0, 36.0, 32.0, 31.3, 30.8, 29.9, 29.0, 28.2, 26.6, 26.3, 24.6, 23.2, 22.9, 19.6, 18.1, 15.5. HRMS (ESI): $C_{39}H_{51}NNaO_5$ (636.3659) $[M+Na]^+$=636.3649.

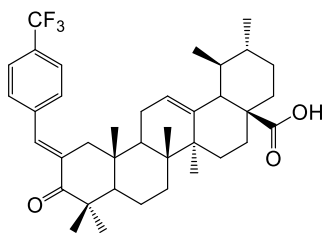


(2S,4aS,6aS,6bR,8aR,12aS,12bR,14bR,Z)-2,4a,6a,6b,9,9,12a-heptamethyl-10,13-dioxo-11-(quinolin-8-ylmethylene)-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydropicene-2-carboxylic acid (**34**, $C_{40}H_{49}NO_4$). According to the general procedure, derivative 31 was prepared by Claisen Schmidt condensation of intermediate **10** with 8-quinolinecarboxaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column

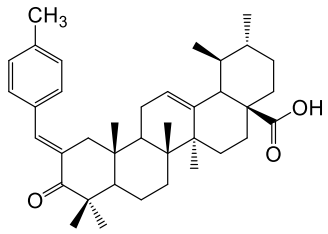
chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 2 : 1, containing 0.5% formic acid). Yield 89%; white solid; mp: 210-211 ℃; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.99 (dd, *J* = 4.2, 1.8 Hz, 1H), 8.65 (s, 1H), 8.16 (dd, *J* = 8.3, 1.7 Hz, 1H), 7.87 (d, *J* = 7.2 Hz, 1H), 7.80 (d, *J* = 8.0 Hz, 1H), 7.61 (t, *J* = 7.7 Hz, 1H), 7.45 (dd, *J* = 8.2, 4.2 Hz, 1H), 5.77 (s, 1H), 4.29 (d, *J* = 17.1 Hz, 1H), 2.55 (s, 1H), 2.32 – 2.25 (m, 1H), 2.22 (dd, *J* = 13.2, 3.5 Hz, 1H), 2.09 – 2.00 (m, 2H), 1.98 – 1.92 (m, 1H), 1.87 (td, *J* = 13.6, 4.1 Hz, 1H), 1.75 (t, *J* = 10.5 Hz, 1H), 1.66 (d, *J* = 13.6 Hz, 1H), 1.58 (td, *J* = 16.3, 14.0, 6.3 Hz, 3H), 1.51 (d, *J* = 13.3 Hz, 2H), 1.43 (d, *J* = 14.9 Hz, 6H), 1.27 (s, 3H), 1.26 (d, *J* = 1.8 Hz, 6H), 1.24 (s, 6H), 1.19 (s, 2H), 0.87 (s, 3H). 13C NMR (126 MHz, $CDCl_3$) δ 207.2, 199.6, 181.8, 170.1, 149.8, 147.1, 136.4, 134.8, 134.8, 134.3, 130.2, 128.5, 128.5, 128.3, 126.2, 121.3, 59.3, 53.7, 48.2, 45.7, 45.1, 44.1, 43.8, 43.4, 40.9, 37.7, 36.6, 31.9, 31.6, 30.9, 29.5, 28.6, 28.5, 26.5, 26.4, 23.3, 22.9, 19.5, 18.1, 15.5. HRMS (ESI): $C_{40}H_{49}NNaO_4$ (630.3554) $[M+Na]^+$=630.3556.

4.1.2 General procedure for the synthesis of UA derivatives **35**, **36**.

UA derivatives **35**, **36** were obtained according to Scheme 1. UA was dissolved in acetone at 0 ℃; Jones reagent was added to the reaction system drop-wisely until the solution colour was stable in light brown, implied that the Jones reagent was in slight excess to oxidize the C-3 hydroxyl group into ketone to provide intermediate **11** without further purification. Derivatives **35**, **36** were prepared by Claisen Schmidt condensation of intermediate **11** with corresponding aldehydes in the presence of ethanolic potassium hydroxide in good yield at room temperature.



(1S,2R,4R,6aS,6bR,12aR)-1,2,6a,6b,9,9,12a-heptamethyl-10-oxo-11-((Z)-4-(trifluoro methyl)benzylidene)-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10, 11,12,12a,12b,13,14b-icosahydropicene-4-carboxylic acid (**35**, $C_{38}H_{49}F_3O_3$). According to the general procedure, derivative **35** was prepared by Claisen Schmidt condensation of intermediate **11** with 4-trifluoromethylbenzaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 8 : 1, containing 0.5% formic acid). Yield: 91%; white solid; mp: 171-172 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.67 (d, *J* = 8.2 Hz, 2H), 7.51 (d, *J* = 8.5 Hz, 3H), 5.27 (t, *J* = 3.2 Hz, 1H), 2.98 (d, *J* = 16.3 Hz, 1H), 2.34 – 2.14 (m, 2H), 2.09 – 1.97 (m, 1H), 1.94 (dd, *J* = 8.6, 3.2 Hz, 2H), 1.86 (td, *J* = 13.7, 4.0 Hz, 1H), 1.78 – 1.61 (m, 4H), 1.59 – 1.46 (m, 3H), 1.46 – 1.18 (m, 6H), 1.14 (d, *J* = 7.9 Hz, 9H), 1.08 – 0.99 (m, 1H), 0.96 (d, *J* = 6.1 Hz, 3H), 0.90 (d, *J* = 5.7 Hz, 3H), 0.87 (s, 3H), 0.81 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.6, 183.7, 139.6, 139.6, 138.3, 136.0, 135.8, 130.4, 125.6, 125.50, 125.46, 53.4, 52.8, 48.2, 45.5, 45.4, 44.1, 42.4, 39.6, 39.3, 39.0, 36.8, 36.6, 32.2, 30.8, 29.7, 28.1, 24.2, 23.7, 23.6, 22.9, 21.3, 20.4, 17.2, 16.9, 15.6. ESI-MS *m/z* 609.4 $[M-H]^-$. HRMS (ESI): $C_{38}H_{49}F_3NaO_3$ (633.3526) $[M+Na]^+$=633.3525.

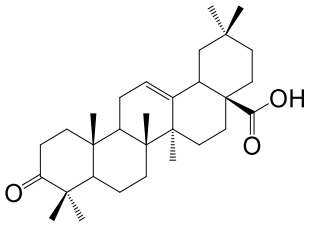


(1S,2R,4R,6aS,6bR,12aR)-1,2,6a,6b,9,9,12a-heptamethyl-11-((Z)-4-methylbenzyliden e)-10-oxo-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a, 12b,13,14b-icosahydropicene-4-carboxylic acid (**36**, $C_{38}H_{52}O_3$). According to the general procedure, derivative **36** was prepared by Claisen Schmidt condensation of

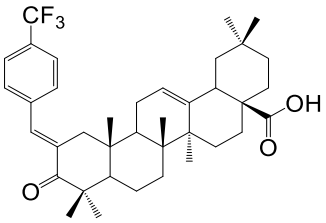
intermediate **12** with 4-methylbenzaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 8 : 1, containing 0.5% formic acid). Yield: 87%; white solid: mp: 142-143 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.53 (s, 1H), 7.35 (d, *J* = 8.0 Hz, 2H), 7.23 (d, *J* = 7.9 Hz, 2H), 5.28 (s, 1H), 3.03 (d, *J* = 16.2 Hz, 1H), 2.38 (s, 3H), 2.31 – 2.24 (m, 1H), 2.22 (d, *J* = 11.4 Hz, 1H), 2.09 – 1.93 (m, 3H), 1.86 (td, *J* = 13.4, 3.8 Hz, 1H), 1.78 – 1.61 (m, 3H), 1.59 – 1.45 (m, 4H), 1.45 – 1.33 (m, 4H), 1.26 (s, 3H), 1.14 (d, *J* = 3.3 Hz, 9H), 0.96 (d, *J* = 6.0 Hz, 3H), 0.91 (d, *J* = 6.4 Hz, 3H), 0.87 (s, 3H), 0.81 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.9, 183.8, 138.8, 138.2, 137.8, 133.3, 133.1, 130.6, 129.4, 125.8, 53.3, 52.9, 48.2, 45.5, 45.3, 44.3, 42.4, 39.6, 39.3, 39.0, 36.9, 36.4, 32.3, 30.8, 29.8, 28.2, 24.3, 23.8, 23.6, 22.8, 21.5, 21.3, 20.5, 17.2, 16.9, 15.6. ESI-MS *m/z* 555.4 $[M-H]^-$. HRMS (ESI): $C_{38}H_{52}NaO_3$ (579.3809) $[M+Na]^+$=579.3812.

4.1.3 General procedure for the synthesis of UA derivatives **37**, **38**

OA derivatives **37**, **38** were obtained according to Scheme 1. OA was dissolved in acetone at 0 ℃; Jones reagent was added to the reaction system drop-wisely until the solution colour was stable in light brown, implied that the Jones reagent was in slight excess to oxidize the C-3 hydroxyl group into ketone to provide the intermediate **12**. Purification of compound **12** by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 1 : 1, containing 0.5% formic acid). Derivatives **37**, **38** were prepared by Claisen Schmidt condensation of intermediate 16 with corresponding aldehydes in the presence of ethanolic potassium hydroxide in good yield at room temperature.

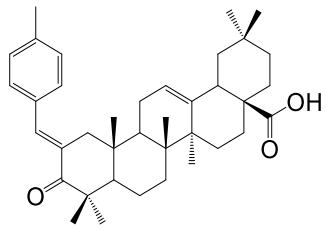


(4aS,6aS,6bR,12aR)-2,2,6a,6b,9,9,12a-heptamethyl-10-oxo-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylic acid (OA-O, **12**, $C_{30}H_{46}O_3$). Yield: 91%; white solid; mp: 209-210 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.31 (t, *J* = 3.3 Hz, 1H), 2.84 (dd, *J* = 13.8, 4.1 Hz, 1H), 2.63 – 2.47 (m, 1H), 2.42 – 2.32 (m, 1H), 2.07 – 1.80 (m, 4H), 1.80 – 1.68 (m, 2H), 1.68 – 1.55 (m, 4H), 1.52 – 1.45 (m, 3H), 1.43 (s, 1H), 1.41 – 1.17 (m, 6H), 1.15 (s, 3H), 1.09 (s, 3H), 1.04 (d, *J* = 6.0 Hz, 5H), 0.99 – 0.85 (m, 7H), 0.81 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 217.7, 183.2, 143.8, 122.6, 55.6, 47.6, 47.1, 46.8, 46.0, 42.0, 41.3, 39.5, 39.3, 37.0, 34.3, 34.0, 33.2, 32.6, 32.4, 30.8, 27.9, 26.7, 26.0, 23.7, 23.7, 23.2, 21.6, 19.8, 17.2, 15.2. HRMS (ESI): $C_{30}H_{46}NaO_3$ (477.3339) $[M+Na]^+$=477.3342.



(4aS,6aS,6bR,12aR)-2,2,6a,6b,9,9,12a-heptamethyl-10-oxo-11-((Z)-4-(trifluoromethyl)benzylidene)-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylic acid (**37**, $C_{38}H_{49}F_3O_3$). According to the general procedure, derivative **37** was prepared by Claisen Schmidt condensation of intermediate **12** with 4-trifluoromethylbenzaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 3 : 1, containing 0.5% formic acid). Yield: 90%; white solid; mp: 268-269 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.64 (d, *J* = 8.2 Hz, 2H), 7.50 (d, *J* = 8.5 Hz, 3H), 5.33 (t, *J* = 3.1 Hz, 1H), 2.95 (d, *J* = 16.4

Hz, 1H), 2.86 (dd, *J* = 13.5, 4.0 Hz, 1H), 2.28 (d, *J* = 16.3 Hz, 1H), 2.06 – 1.88 (m, 3H), 1.85 – 1.55 (m, 7H), 1.55 – 1.31 (m, 7H), 1.20 (s, 4H), 1.17 (s, 3H), 1.15 (s, 3H), 1.00 – 0.89 (m, 6H), 0.86 (s, 3H), 0.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.7, 182.9, 143.9, 139.6, 136.0, 135.7, 130.4, 125.5, 125.5, 125.4, 122.4, 53.3, 46.8, 46.1, 45.6, 45.5, 44.2, 42.2, 41.4, 39.4, 36.5, 34.0, 33.2, 32.5, 32.0, 30.8, 29.9, 29.8, 27.8, 25.9, 23.8, 23.7, 23.2, 22.8, 20.5, 16.8, 15.4. HRMS (ESI): $C_{38}H_{49}F_3NaO_3$ (633.3526) $[M+Na]^+$=633.3522.



(4aS,6aS,6bR,12aR)-2,2,6a,6b,9,9,12a-heptamethyl-11-((Z)-4-methylbenzylidene)-10-oxo-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylic acid (**38**, $C_{38}H_{52}O_3$). According to the general procedure, derivative **38** was prepared by Claisen Schmidt condensation of intermediate **12** with 4-methylbenzaldehyde in the presence of ethanolic potassium hydroxide at room temperature. Purification of product by flash column chromatography was carried out using eluent (petroleum ether/ ethyl acetate, 3 : 1, containing 0.5% formic acid). Yield: 93%; white solid; mp: 154-155 ℃; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.52 (s, 1H), 7.33 (d, *J* = 8.1 Hz, 2H), 7.20 (d, *J* = 8.0 Hz, 2H), 5.33 (t, *J* = 3.1 Hz, 1H), 3.00 (d, *J* = 16.3 Hz, 1H), 2.85 (dd, *J* = 13.6, 3.8 Hz, 1H), 2.38 (s, 3H), 2.28 (d, *J* = 15.9 Hz, 1H), 2.07 – 1.88 (m, 3H), 1.83 – 1.69 (m, 3H), 1.69 – 1.54 (m, 3H), 1.54 – 1.44 (m, 3H), 1.44 – 1.30 (m, 3H), 1.28 – 1.16 (m, 6H), 1.14 (d, *J* = 8.7 Hz, 6H), 0.92 (d, *J* = 7.2 Hz, 6H), 0.85 (s, 3H), 0.81 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 207.9, 183.9, 143.9, 138.8, 137.7, 133.3, 133.0, 130.6, 129.3, 122.6, 53.2, 46.8, 46.1, 45.6, 45.3, 44.4, 42.1, 41.3, 39.4, 36.4, 34.0, 33.2, 32.5, 32.0, 30.8, 29.9, 27.9, 25.9, 23.8, 23.7, 23.2, 22.8, 21.5,

20.5, 16.8, 15.4. HRMS (ESI): $C_{38}H_{52}NaO_3$ (579.3809) $[M+Na]^+$=579.3803.

4.2 Methods for biological assessments

4.2.1 Microorganisms and Culture Media

The bacterial strains of *Staphylococcus aureus* (ATCC 6538), *Staphylococcus aureus* (ATCC 29213), *Staphylococcus epidermidis* (ATCC 12228), *Methicillin*-resistant *Staphylococcus aureus* (MRSA), *Salmonella typhimurium* (CMCC 50115) and *Escherichia coli* (CMCC 44102) were obtained from Guangdong Culture Collection Center (Guangdong, People's Republic of China). All the six strains were cultured in Mueller-Hinton Agar (MHA) and Mueller-Hinton broth (MHB).

4.2.2 Agar disk diffusion method

The antimicrobial activity of were determined according to the standard agar disk diffusion method with a slight modification [38, 40-42]. A 0.5 McFarland (1×10^7 to 1×10^8 CFU/mL) concentration of the bacterial suspension was uniformly inoculated onto MHA solidified in 120 mm petri dishes. Once the dishes were prepared, 6 mm-diameter discs of filter paper containing 5 μL of the triterpenoids derivatives, which had been diluted ten times with dimethyl sulfoxide (DMSO), were pressed gently against the surface of the agar. Discs containing gatifloxacin was used as positive control, while DMSO was used as the negative control. The dishes were incubated in a constant temperature incubator at 37 ℃ for 24 h. The inhibition zone (IZ) diameter was measured by a vernier caliper. All experiments were performed in triplicate.

4.2.3 Broth microdilution method

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined by a microdilution method in 96-well plates

according to Clinical and Laboratory Standards Institute (CLSI), with a slight modification [37, 38, 43]. A dilution series of the triterpenoids derivatives were obtained with DMSO as the solvent by two-fold serial dilution. Each well received 5 μL of a specific concentration of the triterpenoids derivative and 195 μL of MHB inoculated with the test microorganism (1.5×10^5 CFU/mL); the final concentration of the examined derivative was reached. Gatifloxacin was used as positive control and DMSO was used as negative control. The microplates were incubated in a bacteriological oven for 24 h at 37 ℃, and the antibacterial results of the tested derivatives were monitored by measuring the absorbance at 600 nm using a Multimodel Plate Reader (Infinite 200). The lowest concentration without visible growth was defined as the MIC.

The minimum bactericidal concentrations (MBCs) were determined based on the MIC results [38, 44, 45]: serial sub-cultivation of a 5 μL aliquot near the MIC in microtiter plates containing 195 μL of Mueller Hinton broth per well; incubation for 24 h at 37 ℃. The lowest concentration of antimicrobial agent that killed at least 99.9% of the starting inoculum was defined as the MBC endpoint, which was determined by measuring the absorbance at 600 nm using a Multimodel Plate Reader (Infinite 200). All experiments were conducted in triplicate.

4.2.4 Killing kinetic studies

The killing kinetic assay on the Gram-positive strains [35-38], including *Staphylococcus aureus* (ATCC 6538), *Staphylococcus aureus* (ATCC 29213) and *Staphylococcus epidermidis* (ATCC 12228), was performed against three selected derivatives **21, 32** and **33** in 96-well plates and four different concentrations (0.5×MIC, 1×MIC, 2×MIC, 4×MIC) of each derivative were assayed. The microplates were incubated for 20 h at 37 ℃, and the growth of bacteria was

monitored by measuring the absorbance at 600 nm using a Multimodel Plate Reader (Infinite 200) every 2 h.

4.2.4 Molecular docking

Molecular docking was carried out using the Surflex-Dock GeomX module of SYBYL-X 2.0. Briefly, potential ligand binding sites (ProtoMol) were defined for the protein-ligand complex based on the ligand bound in the original crystal structure. The top pose and protein were loaded into work area and the MOLCAD (Molecular Computer Aided Design) program was employed to visualize the binding mode between the protein and the ligand. MOLCAD calculates and exhibits the surfaces of channels and cavities. And the protein-ligand complexes were moved to LigPlus program to determine the hydrophobic interaction.

4.2.5 Pharmacokinetic properties assays

The DMPK results showed in the Table 3 were assessed through a high through-put platform kindly provided by AstraZeneca U.K. The methods of the five assays, including $LogD_{7.4}$, aqueous solubility, plasma protein binding, microsome and hepatocyte clearance measurements have been reported previously[46, 47].

4.2.6 Cytotoxicity test

BV2 microglial cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) and 1% (v/v) penicillin-streptomycin (Gibco, CA, USA), and incubated at 37 ℃ under humidified atmosphere containing 5% CO_2.

The cytotoxicity of GA derivatives (**33** and **34**) was tested on BV2 cells by MTT assay. Briefly, BV2 cells were seeded in 96-well plates at a density of 5×10^3 cells/well. After incubation overnight, the medium was replaced with fresh medium containing various concentrations of **33** and **34** (0, 1, 2, 4, 8, 16, 32, 64 μM). After

incubating for another 24 h, the cells were washed with PBS, and then incubated with fresh medium containing MTT (0.5 mg/mL) for 4 h. Subsequently, 200 μL of DMSO was added to each well, and the optical density was recorded at 550 nm by a Multiskan GO microplate reader (Thermo Fisher Scientific, MA, USA). The cell viability was calculated from: cell viability = (OD sample/OD control) × 100%, where the sample represents the cells treated with **33** and **34** solution and the control means non-treated cells.

Author Contributions

Performing the experimental work and drafting the manuscript: (PPW, BRT, NNC, SLC, XTX, WDH). Performing the bioactivity test: (PPW, WDZ, JHL, SZG, WFL). Performing the experimental statistical analysis (PPW, ZJS, XWT). The director as well as the designer of the manuscript: (WDH, APR, HM, XZ). The project coordinator: (WDH, DLL, KZ).

Declaration of Competing Interest

The authors declare no competing financial interests.

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Abbreviations

UA, ursolic acid; OA, oleanolic acid; GA, 18β-glycyrrhetinic acid; MIC, minimal inhibitory concentration; MBC, minimal bactericidal concentration; IZ, inhibition zone; SAR, structure-activity relationship.

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