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Synthesis and biological evaluation of ursolic acid derivatives containing an aminoguanidine moiety

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Abstract

Three series of ursolic acid derivatives containing an aminoguanidine moiety were designed, synthesized, and evaluated for anti-bacterial and anti-inflammatory activity. Some compounds displayed potent anti-bacterial activity against Gram-positive bacterial strains (including multidrug-resistant clinical isolates) and Gram-negative bacterial strains, with minimum inhibitory concentration (MIC) values in the range of 2–64 μg/mL. Compounds **3a**, **5a**, and **7l** showed significant inhibitory activity against the Gram-positive bacterial strain *Staphylococcus aureus* RN 4220, the Gram-negative bacterial strain *Escherichia coli* 1924, and four multidrug-resistant Gram-positive bacterial strains, with MIC values of 2 and 4 μg/mL. In anti-inflammatory tests, most of the compounds exhibited potent activity, in particular compound **3a** displayed the most potent activity with 81.61% inhibition after intraperitoneal administration, which was more potent than ursolic acid and the reference drugs (ibuprofen and indomethacin). The cytotoxic activity of compound **3a** was assessed in HeLa, Hep3B, and A549 cells.

Keywords Ursolic acid · Aminoguanidine · Antibacterial activity · Anti-inflammatory activity · Cytotoxicity

Introduction

Emerging infectious diseases and the increasing number of multidrug-resistant microbial pathogens make bacterial infections a challenging therapeutic problem. Infectious diseases are responsible for a large number of deaths in the world population (Zafer et al. 2008; Nelson et al. 2014). In addition, there is a relationship between inflammation and bacterial infection (Sy et al. 2011). Inflammation is a very common and important basic pathological process, but exaggerated inflammation can cause a physiological imbalance in tissues and organs (Serhan and Savill 2005). Currently, nonsteroidal anti-inflammatory drugs, such as indomethacin and ibuprofen, are used to treat acute inflammation, but their long-term use can cause adverse reactions, including bone tissue damage, gastrointestinal injury, liver damage, and toxic kidney injury. As a result, there is a need for novel anti-bacterial and anti-inflammatory agents (Damodar et al. 2016; Tanaka et al. 2014).

Ursolic acid (UA) is a natural pentacyclic triterpene isolated from many foods and herbs, including apples, cranberries, rosemary, and oregano (Ikeda et al. 2008). UA has been reported to have antiviral (Chiang et al. 2005), anti-bacterial (Nascimento et al. 2014), anti-HIV (Ma et al. 2000), anticancer (Shanmugam et al. 2013), and anti-inflammatory (Chattopadhyay et al. 2002) properties. Key functionalities, including C_3-OH and C_{17}-COOH, make UA amenable to a variety of chemical transformations (Pathak et al. 2002). The guanidine moiety plays an important biological role in many compounds, and aminoguanidine derivatives have a diverse range of biological properties, including anti-bacterial, antifungal, and anti-inflammatory activities (Gadad et al. 2000; Xu and Wang 2010). In our previous work, we reported the development of a series of aminoguanidine derivatives that

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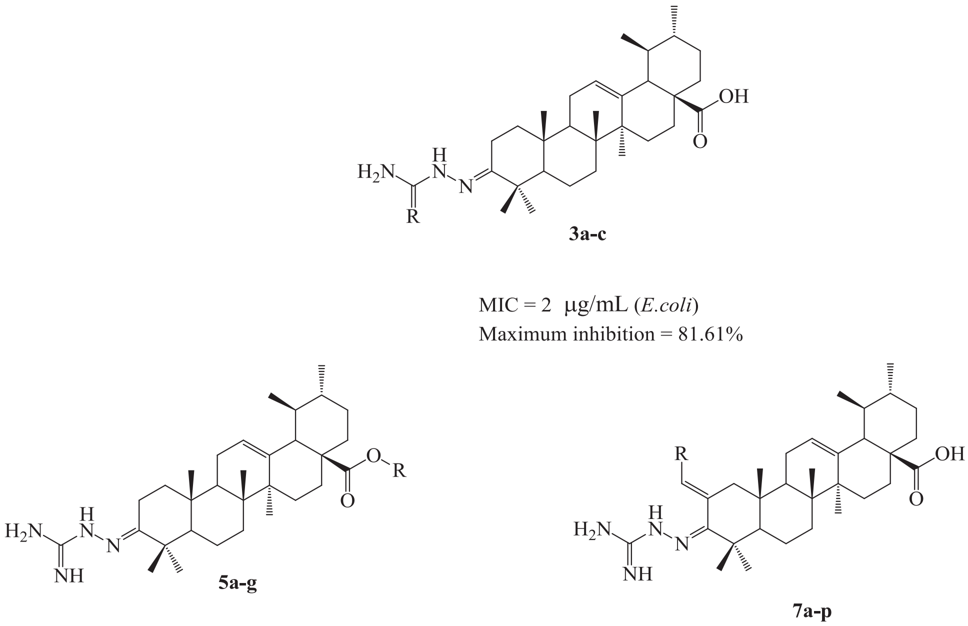


MIC = 2 µg/mL (*E.coli*)
Maximum inhibition = 81.61%

Fig. 1 The structures of the target compounds

exhibited good anti-bacterial and anti-inflammatory activities (Wei et al. 2016; Li et al. 2015), and Wei et al. (2018) reported that UA derivatives showed potent anti-inflammatory activitiy. Thus, as part of our ongoing research, we designed and synthesized three novel series of UA derivatives containing an aminoguanidine moiety via a structure-based design strategy (Fig. 1). The compounds synthesized were characterized and screened for anti-bacterial activity in vitro and anti-inflammatory activity in vivo.

Materials and methods

Chemistry

All commercially available reagents were obtained from Sigma-Aldrich (Saint Louis, MO, USA) and Fluka (Milwaukee, WI, USA). Melting points were determined in open capillary tubes and are uncorrected. Reaction courses were monitored by TLC on silica gel precoated F254 Merck plates and the developed plates were examined under UV lamps (254 nm). Column chromatography was undertaken with 200-mesh silica gel (Merck). ^{1}H NMR and ^{13}C NMR spectra were measured on Bruker AV-300 or 500 spectrometer, with TMS as internal standard. Mass spectra were obtained on an HP1100LC (Agilent Technologies, USA).

Synthesis

General procedure for the synthesis of the intermediates 2

To a solution of UA (10 mmol) in acetone (20 mL) at room temperature was added Jones reagent drop wise till yellow color persists. The reaction was monitored by TLC till its completion in 2 h. After quenching the reaction with cold water, there action mixture was filtered to remove insoluble residue. The filtrate was extracted with ethyl acetate (3 × 20 ml). The combined organic layer was dried over sodium sulfate, concentrated under vacuum and purified by column chromatography to give pure compound **2** (Dar et al. 2016).

General procedure for the preparation of target compounds 3a–c

To a solution of compound **2** (1 mmol) and aminoguanidine bicarbonate (semicarbazide hydrochloride or thiosemicarbazide) (1 mmol) in absolute ethanol (10 mL) was added drops of Con.HCl. The reaction mixture was refluxed for 6 h and further purified by the silica gel column chromatography (methanol /dichloromethane, 1: 30) to afford white compounds **3a**–**c**. The yield, melting point and spectral data of each compound are given below.

(1S,2R,4aS,6aS,6bR,10E,12aR)-10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylic acid (3a) $R_f = 0.30$, methanol/dichloromethane (v/v 1:8); White powder; Yield: 82%; m.p. 262–264 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.96 (s, 1H, COOH), 10.92 (s, 1H, C=NH), 7.45 (s, 3H, NH$_2$, C-NH), 5.16 (s, 1H), 3.50–3.40 (m, 1H), 2.58 (s, 1H), 2.37 (s, 1H), 2.12 (d, $J = 10.8$ Hz, 1H), 1.90 (s, 3H), 1.78 (d, $J = 13.6$ Hz, 2H), 1.47 (d, $J = 25.1$ Hz, 8H), 1.33 (s, 2H), 1.24 (s, 2H), 1.17 (s, 4H), 1.05 (s, 8H), 0.93 (d, $J = 7.6$ Hz, 6H), 0.81 (d, $J = 8.6$ Hz, 5H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 178.75 (C=O), 166.11 (C=NH), 156.51 (C=N), 138.71 ($\underline{C}$H=C), 125.00 (CH=$\underline{C}$), 56.49, 55.05, 52.94, 47.36, 46.58, 42.26, 41.61, 38.96, 38.14, 36.78, 36.66, 32.76, 30.67, 28.93, 27.98, 24.29, 23.99, 23.62, 23.43, 21.52, 21.36, 19.56, 19.02, 17.49, 17.35, 15.24. HRMS (ESI) *m/z* calcd for $C_{31}H_{51}N_4O_2^+$ (M+H)$^+$ 511.40065, found 511.40033. Purity: 99.37% by HPLC.

(1S,2R,4aS,6aS,6bR,10E,12aR)-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethyl-10-thiosemicarbazidopicene-4a-carboxylic acid (3b) $R_f = 0.57$, methanol/dichloromethane (v/v 1:20); White powder; Yield: 47%; m.p. 195–197 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.94 (s, 1H, COOH), 10.01 (s, 1H, C=NH), 8.06 (s, 1H, NH), 7.33 (s, 1H, NH), 5.15 (s, 1H), 3.44 (s, 1H), 2.63 (s, 1H), 2.32 (s, 1H), 2.12 (d, $J =$ 9.5 Hz, 1H), 1.88 (s, 4H), 1.67 (s, 1H), 1.51 (s, 8H), 1.27 (d, $J = 20.7$ Hz, 5H), 1.13 (s, 3H), 1.04 (s, 8H), 0.93 (s, 6H), 0.80 (s, 5H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 179.20 (C=S), 178.74 (C=O), 163.05 (C=N), 138.69 ($\underline{C}$H=C), 125.02 (CH=$\underline{C}$), 56.48, 55.18, 52.93, 47.35, 46.70, 42.24, 41.59, 38.96, 38.47, 36.77, 36.65, 32.80, 30.66, 28.76, 27.98, 24.28, 23.97, 23.62, 23.44, 21.52, 20.58, 19.60, 19.02, 17.49, 17.35, 15.30. HRMS (ESI) *m/z* calcd for $C_{31}H_{50}N_3O_2S^+$ (M+H)$^+$ 528.36182, found 528.36163.

(1S,2R,4aS,6aS,6bR,10E,12aR)-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethyl-10-semicarbazidopicene-4a-carboxylic acid (3c) $R_f = 0.41$, methanol/dichloromethane (v/v 1:20); White powder; Yield: 41%; 286–288 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.96 (s, 1H, COOH), 8.97 (s, 1H, C=NH), 6.14 (s, 2H, NH_2), 5.15 (s, 1H), 3.53–3.40 (m, 1H), 2.58 (s, 1H), 2.12 (d, $J = 10.6$ Hz, 2H), 1.85 (d, $J = 26.7$ Hz, 4H), 1.66 (s, 1H), 1.50 (dd, $J = 24.4$, 15.9 Hz, 6H), 1.28 (d, $J = 24.4$ Hz, 5H), 1.11 (s, 3H), 1.04 (s, 4H), 0.99 (s, 4H), 0.93 (d, $J = 6.4$ Hz, 6H), 0.81 (d, $J = 9.3$ Hz, 6H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 178.77 (COOH), 158.17 (C=O), 157.71 (C=N), 138.70 ($\underline{C}$H=C), 125.05 (CH=$\underline{C}$), 56.50, 55.32, 52.93, 47.36, 46.68, 42.24, 41.03, 38.97, 38.29, 36.78, 36.68, 32.84, 30.67, 28.91, 27.98, 24.29, 24.20, 23.62, 23.42, 21.53, 19.84, 19.63, 19.03, 17.51, 17.39, 15.10. HRMS (ESI) *m/z* calcd for $C_{31}H_{50}N_3O_3^+$ (M+H)$^+$ 512.38467, found 512.38440.

General Procedure for the synthesis of the target compound 4a

To a solution of compound **2** (1 mmol) and K_2CO_3 (1 mmol) in DMF (10 mL) was added methyl iodide (1 mmol), the resulting mixture was stirred with heating at room temperature for 6 h. The mixture was extracted with ethyl acetate, then combined the organic phases, dried ($MgSO_4$) and concentrated under pressure to give the crude product. The crude product was purified by the silica gel column chromatography (petroleum ether /ethyl acetate, 5:1) to give the desired product **4a**.

General procedure for the synthesis of the intermediates 4b–g

A mixture of compound **2** (1 mmol), catalytic amount of K_2CO_3 (1 mmol) and the different substituted benzyl chlorides(1 mmol) was stirred in refluxing acetone (10 mL) at 56 °C for 12 h. Upon completion of the reaction, as determined by TLC, the solvent was removed under reduced pressure to give a solid. The solid was dissolved in ethyl ether, dried ($MgSO_4$) and concentrated to give the crude product. The crude product was purified by the silica gel column chromatography (petroleum ether /ethyl acetate, 30:1) to give the desired products **4b**–**g**.

General procedure for the synthesis of the intermediates 6a–p

To a mixture of compound **2** (1 mmol) and sodium hydroxide (1 mmol) in absolute ethanol (10 mL) was added different aldehydes (1 mmol) at room temperature. Upon completion of the reaction, as determined by TLC, added water to the mixture, adjusted PH = 7 with 5% aqueous hydrochloric acid and filtered to give the crude product. The crude product was purified by the silica gel column chromatography (dichloromethane) to give the desired products **6a**–**p**.

General procedure for the preparation of target compounds 5a–g and 7a–p

To a solution of compounds **4a**–**g** or **7a**–**p** (1 mmol) and aminoguanidine bicarbonate (1 mmol) in absolute ethanol (10 mL) was added drops of Con.HCl. The reaction mixture was refluxed for 6 h and further purified by the silica gel column chromatography (methanol/dichloromethane, 1: 30) to afford white compounds **5a**–**g** and **7a**–**p**. The yield, melting point and spectral data of each compound are given below.

(1S,2R,4aS,6aS,6bR,10E,12aR)-methyl 10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylate (5a) $R_f = 0.40$, methanol/dichloromethane (v/v 1:10); White powder; Yield: 57%; 152–154 °C; ^{1}H NMR (DMSO-d_6, 500 MHz, ppm) δ 10.80 (s, 1H, C=NH), 7.38 (s, 3H, NH$_2$, C-NH), 5.18 (s, 1H), 3.52 (s, 3H, O-CH$_3$), 2.59 (dt, $J = 9.5$, 5.6 Hz, 1H), 2.41–2.32 (m, 1H), 2.16 (d, $J =$ 11.3 Hz, 1H), 2.00 (d, $J = 4.2$ Hz, 1H), 1.88 (dd, $J = 10.2$, 2.9 Hz, 2H), 1.76–1.67 (m, 2H), 1.58 (d, $J = 13.0$ Hz, 2H), 1.54–1.51 (m, 1H), 1.49 (s, 1H), 1.47 (s, 1H), 1.45–1.42 (m, 1H), 1.36 (dd, $J = 14.2$, 9.5 Hz, 2H), 1.32–1.27 (m, 2H), 1.24 (s, 1H), 1.17 (s, 4H), 1.09 (d, $J = 11.2$ Hz, 1H), 1.05 (s, 6H), 0.94 (s, 3H), 0.91 (d, $J = 5.1$ Hz, 3H), 0.82 (d, $J =$ 6.4 Hz, 3H), 0.72 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 177.32 (C=O), 166.13 (C=NH), 156.46 (C=N), 138.45 ($\underline{\text{C}}$H=C), 125.32 (CH=$\underline{\text{C}}$), 56.49, 55.04, 53.04, 51.78 (O-CH$_3$), 47.94, 46.54, 42.15, 41.62, 38.89, 38.76, 38.12, 36.64, 32.64, 30.48, 28.88, 27.91, 24.24, 23.97, 23.69, 23.44, 21.42, 21.29, 19.51, 19.03, 17.42, 17.03, 15.20. HRMS (ESI) *m/z* calcd for $C_{32}H_{53}N_4O_2^+$ (M+H)$^+$ 525.41630, found 525.41638.

(1S,2R,4aS,6aS,6bR,10E,12aR)-3-chlorobenzyl 10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylate (5b) $R_f = 0.50$, methanol/dichloromethane (v/v 1:10); White powder; Yield: 45%; 160–162 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 10.74 (s, 1H, C=NH), 7.41 (s, 2H, Ar-H), 7.39 (s, 3H, C-NH, NH$_2$), 7.33 (d, $J = 6.7$ Hz, 2H, Ar-H), 5.25–5.05 (m, 2H, O-CH$_2$), 4.94 (d, $J = 12.7$ Hz, 1H), 2.57 (s, 1H), 2.37 (d, $J = 5.8$ Hz, 1H), 2.20 (d, $J = 11.1$ Hz, 1H), 2.01 (s, 1H), 1.84 (d, $J =$ 12.0 Hz, 1H), 1.70 (s, 2H), 1.63 (s, 1H), 1.59 (s, 1H), 1.46 (d, $J = 7.1$ Hz, 3H), 1.39 (s, 1H), 1.34 (s, 1H), 1.24 (s, 5H), 1.16 (s, 4H), 1.04 (s, 7H), 0.91 (d, $J = 4.7$ Hz, 5H), 0.86 (s, 2H), 0.84–0.80 (m, 4H), 0.53 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 176.50 (C=O), 166.16 (C=NH), 156.44 (C=N), 139.18 (Ar-C), 138.34 ($\underline{\text{C}}$H=C), 133.47 (Ar-C), 130.75 (Ar-C), 128.32 (Ar-C), 128.30 (Ar-C), 127.15 (Ar-C), 125.55 (CH=$\underline{\text{C}}$), 64.97 (CH$_2$), 56.49, 55.00, 53.03, 48.02, 46.51, 42.19, 41.61, 38.85, 38.82, 38.11, 36.61, 32.71, 30.49, 28.87, 27.74, 24.30, 23.97, 23.60, 23.35, 21.41, 21.27, 19.47, 19.03, 17.37, 17.06, 15.16. HRMS (ESI) *m/z* calcd for $C_{38}H_{56}ClN_4O_2^+$ (M+H)$^+$ 635.40863, found 635.40894.

(1S,2R,4aS,6aS,6bR,10E,12aR)-3-chlorobenzyl 10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylate (5c) $R_f = 0.36$, methanol/dichloromethane (v/v 1:8); White powder; Yield: 40%; 150–152 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 10.93 (s, 1H, C=NH), 7.74–7.64 (m, 2H, Ar-H), 7.43 (d, $J = 8.1$ Hz, 3H, C-NH, NH$_2$), 7.35 (d, $J = 8.2$ Hz, 2H, Ar-H), 5.23–5.03 (m, 2H, O-CH$_2$), 4.93 (d, $J = 12.6$ Hz, 1H), 2.61 (d, $J =$ 16.0 Hz, 1H), 2.38 (d, $J = 10.8$ Hz, 1H), 2.18 (d, $J = 11.0$ Hz, 1H), 1.98 (d, $J = 11.7$ Hz, 1H), 1.82 (d, $J = 10.5$ Hz, 1H), 1.65 (dd, $J = 14.3$, 7.2 Hz, 4H), 1.58 (d, $J = 6.4$ Hz, 2H), 1.45 (d, $J = 10.3$ Hz, 4H), 1.39–1.33 (m, 3H), 1.23 (s, 2H), 1.16 (s, 3H), 1.08 (s, 1H), 1.04 (d, $J = 6.5$ Hz, 8H), 0.91 (s, 7H), 0.81 (d, $J = 6.0$ Hz, 3H), 0.49 (s, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm) δ 176.43 (C=O), 166.07 (C=NH), 156.50 (C=N), 138.22 ($\underline{\text{C}}$H=C), 135.70 (Ar-C), 133.10 (Ar-C), 131.96 (Ar-C), 130.52 (Ar-C), 129.11 (Ar-C), 128.80 (Ar-C), 125.55 (CH=$\underline{\text{C}}$), 65.47 (O-CH$_2$), 56.48, 55.00, 53.09, 47.93, 46.53, 42.15, 41.59, 38.86, 38.79, 38.14, 36.58, 30.47, 28.87, 27.73, 24.26, 23.94, 23.59, 23.37, 21.39, 19.45, 19.11, 19.01, 17.35, 17.04, 15.18, 14.00. HRMS (ESI) *m/z* calcd for $C_{38}H_{56}ClN_4O_2^+$ (M+H)$^+$ 635.40863, found 635.40869.

(1S,2R,4aS,6aS,6bR,10E,12aR)-2,4-dichlorobenzyl 10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylate (5d) $R_f = 0.54$, methanol/dichloromethane (v/v 1:8); White powder; Yield: 47%; 167–169 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 10.90 (s, 1H, C=NH), 7.65 (d, $J = 2.0$ Hz, 1H, Ar-H), 7.57–7.28 (m, 5H, NH$_2$, C-NH, Ar-H), 5.16 (dd, $J = 8.1$, 4.4 Hz, 2H, O-CH$_2$), 4.98 (d, $J = 12.7$ Hz, 1H), 2.60 (dd, $J = 10.7$, 4.1 Hz, 1H), 2.42–2.34 (m, 1H), 2.18 (d, $J = 11.2$ Hz, 1H), 1.99 (td, $J = 13.2$, 4.2 Hz, 1H), 1.90–1.82 (m, 1H), 1.71 (dd, $J = 11.5$, 6.7 Hz, 3H), 1.58 (d, $J = 11.7$ Hz, 3H), 1.50–1.42 (m, 4H), 1.36–1.27 (m, 3H), 1.22 (d, $J =$ 11.3 Hz, 2H), 1.15 (s, 4H), 1.07 (s, 1H), 1.04 (dd, $J = 11.6$, 7.0 Hz, 7H), 0.93–0.88 (m, 6H), 0.81 (d, $J = 6.4$ Hz, 3H), 0.44 (s, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm) δ 177.13 (C=O), 166.83 (C=NH), 157.34 (C=N), 138.86 ($\underline{\text{C}}$H=C), 135.48 (Ar-C), 135.11 (Ar-C), 133.98 (Ar-C), 135.77 (Ar-C), 130.12 (Ar-C), 128.70 (Ar-C), 126.43 (CH=$\underline{\text{C}}$), 63.59 (O-CH$_2$), 57.29, 55.81, 53.87, 48.91, 42.93, 42.39, 39.68, 37.37, 31.28, 29.68, 24.75, 24.39, 24.18, 22.19, 20.27, 19.82, 18.16, 15.97. HRMS (ESI) *m/z* calcd for $C_{38}H_{55}Cl_2N_4O_2^+$ (M+H)$^+$ 669.36966, found 669.36945.

(1S,2R,4aS,6aS,6bR,10E,12aR)-2,6-dichlorobenzyl 10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylate (5e) $R_f = 0.68$, methanol/dichloromethane (v/v 1:8); White powder; Yield: 46%; 158–160 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 10.89 (s, 1H, C=NH), 7.53 (d, $J = 7.9$ Hz, 3H, NH$_2$, C-NH), 7.49–7.35 (m, 3H, Ar-H), 5.19 (dd, $J = 24.3$, 12.1 Hz, 3H, O-CH$_2$, CH=C), 2.60 (d, $J = 16.1$ Hz, 1H), 2.38 (d, $J = 10.0$ Hz, 1H), 2.13 (d, $J = 11.6$ Hz, 1H), 1.96 (d, $J = 11.8$ Hz, 1H), 1.84 (s, 1H), 1.75 (s, 2H), 1.57 (d, $J = 9.5$ Hz, 3H), 1.49 (d, $J = 10.7$ Hz, 2H), 1.42 (s, 2H), 1.30 (dd, $J = 24.9$, 13.6 Hz, 5H), 1.16 (s, 3H), 1.08 (s, 1H), 1.04 (d, $J = 4.6$ Hz, 8H), 0.91 (s, 7H), 0.79 (d, $J = 6.1$ Hz, 3H), 0.67 (s, 3H). ^{13}C

NMR (DMSO-d_6, 75 MHz, ppm) δ 176.35 (C=O), 166.08 (C=NH), 156.49 (C=N), 138.06 ($\underline{\text{C}}$H=C), 136.48 (Ar-C), 136.48 (Ar-C), 131.95 (Ar-C), 131.63 (Ar-C), 129.19 (Ar-C), 129.19 (Ar-C), 125.60 (CH=$\underline{\text{C}}$), 61.15 (O-CH$_2$), 56.48, 55.03, 53.10, 48.30, 46.50, 42.17, 41.59, 38.89, 38.72, 38.11, 36.59, 32.76, 30.44, 28.89, 27.83, 24.23, 23.98, 23.61, 23.45, 21.35, 21.35, 19.49, 19.02, 17.34, 17.20, 15.19. HRMS (ESI) *m/z* calcd for $C_{38}H_{55}Cl_2N_4O_2^+$ (M+H)$^+$ 669.36966, found 669.36951.

(1S,2R,4aS,6aS,6bR,10E,12aR)-4-fluorobenzyl 10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylate (5f) R_f = 0.54, methanol/dichloromethane (v/v 1:8); White powder; Yield: 48%; 138–140 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 10.93 (s, 1H, C=NH), 7.45 (s, 3H, NH$_2$, C-NH), 7.41–7.34 (m, 2H, Ar-H), 7.19 (t, J = 8.8 Hz, 2H, Ar-H), 5.23–4.90 (m, 3H, O-CH$_2$, CH=C), 2.61 (d, J = 17.4 Hz, 1H), 2.38 (d, J = 6.4 Hz, 1H), 2.18 (d, J = 11.2 Hz, 1H), 1.98 (d, J = 12.2 Hz, 1H), 1.84 (s, 1H), 1.70 (s, 2H), 1.57 (s, 3H), 1.48 (s, 4H), 1.33 (s, 2H), 1.23 (s, 3H), 1.16 (s, 3H), 1.08 (s, 1H), 1.03 (s, 6H), 0.90 (s, 7H), 0.81 (d, J = 6.2 Hz, 5H), 0.53 (s, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm) δ 176.47 (C=O), 166.06 (C=NH), 163.92 (Ar-C), 156.51 (C=N), 138.25 ($\underline{\text{C}}$H=C), 132.98 (Ar-C), 130.91 (Ar-C), 130.80 (Ar-C), 125.50 (CH=$\underline{\text{C}}$), 115.76 (Ar-C), 115.47 (Ar-C), 65.07 (O-CH$_2$), 56.47, 55.02, 53.08, 47.91, 46.53, 42.15, 41.59, 38.87, 38.79, 38.14, 36.59, 32.70, 30.46, 28.87, 27.74, 24.24, 23.96, 23.59, 23.38, 21.39, 21.32, 19.48, 19.02, 17.36, 17.06, 15.15. HRMS (ESI) *m/z* calcd for $C_{38}H_{56}FN_4O_2^+$ (M+H)$^+$ 619.43818, found 619.43811.

(1S,2R,4aS,6aS,6bR,10E,12aR)-4-methylbenzyl 10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylate (5g) R_f = 0.43, methanol/dichloromethane (v/v 1:8); White powder; Yield: 47%; 153–155 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 10.92 (s, 1H, C=NH), 7.69 (d, J = 8.5 Hz, 1H, Ar-H), 7.46 (s, 3H, C-NH, NH$_2$), 7.19 (q, J = 8.0 Hz, 3H, Ar-H), 5.22–4.84 (m, 3H, O-CH$_2$, CH=C), 2.61 (d, J = 15.2 Hz, 1H), 2.37 (s, 1H), 2.29 (s, 3H), 2.18 (d, J = 11.3 Hz, 1H), 1.97 (d, J = 9.5 Hz, 1H), 1.82 (d, J = 12.4 Hz, 2H), 1.72 (s, 1H), 1.66 (d, J = 7.0 Hz, 1H), 1.58 (d, J = 10.1 Hz, 2H), 1.47 (s, 2H), 1.41–1.32 (m, 3H), 1.23 (s, 3H), 1.16 (s, 4H), 1.08 (s, 1H), 1.04 (s, 7H), 0.91 (s, 7H), 0.81 (d, J = 6.2 Hz, 4H), 0.57 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 176.52 (C=O), 166.08 (C=NH), 156.50 (C=N), 138.27 ($\underline{\text{C}}$H=C), 137.67 (Ar-C), 133.65 (Ar-C), 131.97 (Ar-C), 129.35 (Ar-C), 129.12 (Ar-C), 128.62 (Ar-C), 125.50 (CH=$\underline{\text{C}}$), 65.73 (O-CH$_2$), 56.48, 55.03, 53.09, 47.90, 46.56, 42.15, 41.60, 38.88, 38.79, 38.14, 36.60, 32.72, 30.47, 28.88, 27.76, 24.25, 23.95, 23.62, 23.38, 21.40, 21.22, 19.47, 19.02, 17.37, 17.10, 15.17. HRMS (ESI) *m/z* calcd for $C_{39}H_{59}N_4O_2^+$ (M+H)$^+$ 615.46325, found 615.46301.

(1S,2R,4aS,6aS,6bR,10Z,11E,12aR)-11-benzylidene-10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylic acid (7a) R_f = 0.34, methanol/dichloromethane (v/v 1:8); White powder; Yield: 54%; 143–145 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.95 (s, 1H, COOH), 10.19 (s, 1H, C=NH), 7.69 (d, J = 8.6 Hz, 5H, Ar-H, C-NH), 7.45 (d, J = 7.6 Hz, 3H, NH$_2$, Ar-H), 6.52 (s, 1H, C=CH), 5.17 (s, 1H), 2.89 (d, J = 15.9 Hz, 1H), 2.10 (t, J = 14.2 Hz, 1H), 1.90 (s, 2H), 1.79 (s, 1H), 1.68–1.61 (m, 5H), 1.55 (s, 2H), 1.37 (dd, J = 14.8, 7.3 Hz, 7H), 1.23 (s, 1H), 1.15 (d, J = 5.0 Hz, 3H), 1.09–1.03 (m, 3H), 1.00 (s, 2H), 0.91 (t, J = 7.3 Hz, 11H), 0.81 (d, J = 5.4 Hz, 2 H), 0.75 (s, 1H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 178.74 (C=O), 167.42 (C=NH), 156.32 (C=N), 138.77 ($\underline{\text{C}}$H=C), 135.90 (C=C), 133.12 (Ar-C), 132.18 (Ar-C), 131.98 (Ar-C), 130.18 (Ar-C), 129.13 (Ar-C), 128.77 (Ar-C), 128.21 (C=C), 124.66 (CH=$\underline{\text{C}}$), 65.48, 56.49, 52.94, 47.35, 46.77, 42.93, 42.23, 39.05, 38.95, 36.78, 32.21, 30.67, 30.48, 27.98, 24.26, 23.61, 23.43, 22.18, 21.49, 19.12, 19.03, 17.56, 16.88, 16.37, 14.01. HRMS (ESI) *m/z* calcd for $C_{38}H_{55}N_4O_2^+$ (M+H)$^+$ 599.43195, found 599.43202.

(1S,2R,4aS,6aS,6bR,10Z,11E,12aR)-11-(4-chlorobenzylidene)-10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylic acid (7b) R_f = 0.24, methanol/dichloromethane (v/v 1:8); White powder; Yield: 43%; 190–192 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.95 (s, 1H, COOH), 10.37 (s, 1H, C=NH), 7.69 (d, J = 8.6 Hz, 5H, C-NH, Ar-H), 7.48 (s, 2H, NH$_2$), 6.52 (s, 1H, C=CH), 5.16 (s, 1H), 2.83 (d, J = 14.9 Hz, 1H), 2.12 (d, J = 10.3 Hz, 1H), 2.03 (d, J = 16.1 Hz, 1H), 1.89 (s, 2H), 1.79 (s, 1H), 1.69–1.61 (m, 4H), 1.54 (s, 2H), 1.44–1.35 (m, 5H), 1.23 (s, 1H), 1.14 (d, J = 5.9 Hz, 5H), 1.08 (s, 1H), 1.00 (s, 3H), 0.90 (dd, J = 13.8, 6.8 Hz, 10H), 0.82 (s, 3H), 0.75 (s, 2H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 178.72 (C=O), 167.42 (C=NH), 165.34 (Ar-C), 156.36 (C=N), 138.74 ($\underline{\text{C}}$H=C), 134.82 (C=C), 132.65 (Ar-C), 132.18 (Ar-C), 131.98 (Ar-C), 131.59 (Ar-C), 129.13 (Ar-C), 128.71 (C=C), 124.67 (CH=$\underline{\text{C}}$), 65.48, 56.49, 52.93, 47.34, 46.72, 42.93, 42.23, 39.11, 38.95, 36.78, 32.20, 30.66, 30.48, 27.98, 24.25, 23.61, 23.42, 22.18, 21.50, 19.12, 19.03, 17.58, 16.88, 16.33, 14.01. HRMS

(ESI) m/z calcd for $C_{38}H_{54}ClN_4O_2^+$ $(M+H)^+$ 633.39298, found 633.39325.

(1S,2R,4aS,6aS,6bR,10Z,11E,12aR)-11-(2-chlorobenzylidene)-10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylic acid (7c) $R_f = 0.34$, methanol/dichloromethane (v/v 1:8); White powder; Yield: 57%; 207–209 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.93 (s, 1H, COOH), 10.44 (s, 1H, C=NH), 7.75–7.70 (m, 3H, Ar-H), 7.68 (s, 1H, C-NH), 7.52 (s, 1H, NH), 7.40 (dt, $J =$ 15.0, 7.3 Hz, 2H, NH, Ar-H), 6.52 (s, 1H, C=CH), 5.10 (s, 1H), 2.73 (d, $J = 15.4$ Hz, 1H), 2.09 (d, $J = 11.1$ Hz, 1H), 1.88 (d, $J = 13.8$ Hz, 2H), 1.73 (s, 1H), 1.70–1.59 (m, 5H), 1.55 (s, 1H), 1.50 (s, 1H), 1.45 (s, 1H), 1.37 (dd, $J = 15.1$, 7.5 Hz, 5H), 1.22 (s, 2H), 1.14 (s, 2H), 1.08 (s, 1H), 1.06 (s, 1H), 1.03 (s, 1H), 1.00 (s, 2H), 0.94 (s, 1H), 0.92–0.88 (m, 6H), 0.82–0.76 (m, 6H), 0.72 (s, 2H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 178.67 (C=O), 167.42 (C=NH), 165.13 (Ar-C), 156.52 (C=N), 138.76 ($\underline{C}$H=C), 133.75 (C=C), 133.20 (Ar-C), 132.99 (Ar-C), 132.18 (Ar-C), 131.98 (Ar-C), 129.13 (Ar-C), 127.24 (C=C), 124.51 (CH=$\underline{C}$), 65.48, 56.49, 52.89, 47.32, 46.72, 43.37, 42.23, 38.93, 38.90, 36.75, 32.39, 30.65, 30.48, 27.96, 24.23, 23.60, 23.37, 22.15, 21.49, 19.12, 19.03, 17.50, 16.97, 15.88, 14.01. HRMS (ESI) m/z calcd for $C_{38}H_{54}ClN_4O_2^+$ $(M+H)^+$ 633.39298, found 633.39355.

(1S,2R,4aS,6aS,6bR,10Z,11E,12aR)-11-(3-chlorobenzylidene)-10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylic acid (7d) $R_f = 0.24$, methanol/dichloromethane (v/v 1:8); White powder; Yield: 46%; 221–223 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.95 (s, 1H, COOH), 10.42 (s, 1H, C=NH), 7.78–7.61 (m, 3H, Ar-H, C-NH), 7.52 (s, 1H, NH), 7.46 (d, $J = 4.8$ Hz, 2H, NH, Ar-H), 7.39 (d, $J = 4.8$ Hz, 1H, Ar-H), 6.54 (s, 1H, C=CH), 5.16 (s, 1H), 2.82 (d, $J = 14.5$ Hz, 1H), 2.12 (d, $J = 11.4$ Hz, 1H), 2.02 (d, $J = 15.1$ Hz, 1H), 1.93 (s, 1H), 1.86 (d, $J = 9.3$ Hz, 2H), 1.78 (s, 1H), 1.65 (dd, $J =$ 14.0, 7.1 Hz, 2H), 1.53 (d, $J = 12.4$ Hz, 4H), 1.46 (s, 1H), 1.40 (d, $J = 7.7$ Hz, 2H), 1.35 (d, $J = 7.2$ Hz, 1H), 1.29 (d, $J = 8.0$ Hz, 2H), 1.23 (s, 1H), 1.17 (s, 3H), 1.13 (s, 2H), 1.01 (s, 4H), 0.94 (s, 1H), 0.91–0.86 (m, 6H), 0.80 (d, $J =$ 6.1 Hz, 3H), 0.75 (s, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm) δ 178.68 (C=O), 165.14 (C=NH), 156.46 (C=N), 138.75 ($\underline{C}$H=C), 133.29 (C=C), 132.45 (Ar-C), 131.96 (Ar-C), 130.49 (Ar-C), 129.89 (Ar-C), 129.11 (Ar-C), 128.56 (Ar-C), 127.84 (C=C), 124.61 (CH=$\underline{C}$), 65.46, 56.47, 52.89, 47.32, 46.73, 43.05, 42.21, 39.23, 38.93, 36.75, 32.23, 30.47, 27.97, 24.22, 23.60, 22.15, 21.50, 19.66, 19.11, 19.02, 17.53, 16.89, 16.33, 14.00. HRMS (ESI) m/z calcd for $C_{38}H_{54}ClN_4O_2^+$ $(M+H)^+$ 633.39298, found 633.39331.

(1S,2R,4aS,6aS,6bR,10Z,11E,12aR)-11-(4-fluorobenzylidene)-10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylic acid (7e) $R_f = 0.41$, methanol/dichloromethane (v/v 1:8); White powder; Yield: 41%; 279–281 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.95 (s, 1H, COOH), 10.36 (s, 1H, C = NH), 7.53 (dd, $J = 8.3$, 5.7 Hz, 5H, C-NH, NH$_2$, Ar-H), 7.26 (t, $J = 8.8$ Hz, 2H, Ar-H), 6.52 (s, 1H, C=CH), 5.16 (s, 1H), 2.83 (d, $J =$ 14.8 Hz, 1H), 2.12 (d, $J = 11.1$ Hz, 1H), 2.03 (d, $J =$ 16.1 Hz, 1H), 1.89 (s, 3H), 1.79 (s, 1H), 1.52 (d, $J = 9.1$ Hz, 5H), 1.46 (s, 1H), 1.41 (s, 2H), 1.30 (s, 3H), 1.23 (s, 1H), 1.15 (d, $J = 5.6$ Hz, 6H), 1.05 (s, 1H), 1.00 (s, 3H), 0.94 (s, 1H), 0.89 (d, $J = 11.7$ Hz, 6H), 0.81 (d, $J = 6.3$ Hz, 3H), 0.75 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 178.71 (C=O), 165.51 (C=NH), 160.87 (Ar-C), 156.45 (C=N), 138.74 ($\underline{C}$H=C), 132.47 (Ar-C), 132.35 (C=C), 132.29 (Ar-C), 132.04 (Ar-C), 130.68 (C=C), 124.68 (CH=$\underline{C}$), 115.66 (Ar-C), 115.49 (Ar-C), 56.48, 55.39, 52.93, 47.34, 46.75, 45.32, 42.89, 42.22, 39.05, 38.95, 36.77, 32.21, 30.66, 29.09, 27.98, 24.26, 23.62, 23.42, 22.18, 21.51, 19.77, 19.03, 17.58, 16.88, 16.34. HRMS (ESI) m/z calcd for $C_{38}H_{54}FN_4O_2^+$ $(M+H)^+$ 617.42253, found 617.42285.

(1S,2R,4aS,6aS,6bR,10Z,11E,12aR)-11-(2-fluorobenzylidene)-10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylic acid (7f) $R_f = 0.41$, methanol/dichloromethane (v/v 1:8); White powder; Yield: 51%; 274–276 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.94 (s, 1H, COOH), 10.46 (s, 1H, C=NH), 7.91–7.58 (m, 4H, C-NH, NH$_2$, Ar-H), 7.47–7.36 (m, 1H, Ar-H), 7.26 (dd, $J = 16.6$, 8.5 Hz, 2H, Ar-H), 6.49 (s, 1H, C=CH), 5.13 (s, 1H), 2.76 (d, $J = 15.0$ Hz, 1H), 2.10 (d, $J = 11.2$ Hz, 1H), 1.96 (d, $J = 14.7$ Hz, 2H), 1.87 (s, 1H), 1.80 (d, $J =$ 8.4 Hz, 2H), 1.52 (d, $J = 10.9$ Hz, 5H), 1.44 (d, $J =$ 12.7 Hz, 3H), 1.33 (s, 1H), 1.28 (d, $J = 9.7$ Hz, 2H), 1.23 (s, 1H), 1.18 (s, 3H), 1.13 (s, 3H), 1.07 (d, $J = 7.1$ Hz, 1H), 1.00 (s, 3H), 0.90 (s, 4H), 0.84 (s, 3H), 0.79 (d, $J = 6.2$ Hz, 3H), 0.73 (s, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm) δ 178.67 (C=O), 165.07 (C=NH), 156.55 (C=N), 138.74 ($\underline{C}$H=C), 133.32 (C=C), 132.22 (Ar-C), 130.33 (C=C), 125.46 (Ar-C), 124.56 (CH=$\underline{C}$), 123.51 (Ar-C), 123.33

(Ar-C), 115.78 (Ar-C), 115.50 (Ar-C), 56.47, 54.47, 52.89, 47.32, 46.72, 45.23, 43.10, 42.22, 38.92, 38.92, 36.75, 32.29, 30.64, 28.58, 27.97, 24.25, 23.60, 23.39, 22.12, 21.49, 19.66, 19.02, 17.51, 16.91, 16.10. HRMS (ESI) *m/z* calcd for $C_{38}H_{54}FN_4O_2^+$ $(M+H)^+$ 617.42253, found 617.42212.

(1S,2R,4aS,6aS,6bR,10Z,11E,12aR)-11-(3-fluorobenzylidene)-10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylic acid (7g) $R_f = 0.48$, methanol/dichloromethane (v/v 1:8); White powder; Yield: 52%; 279–281 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.95 (s, 1H, COOH), 10.44 (s, 1H, C=NH), 7.60 (s, 3H, C-NH, NH$_2$), 7.46 (dd, $J = 14.4$, 7.4 Hz, 1H, Ar-H), 7.31 (d, $J =$ 8.0 Hz, 2H, Ar-H), 7.16 (t, $J = 8.1$ Hz, 1H, Ar-H), 6.54 (s, 1H, C=CH), 5.16 (s, 1H), 2.84 (d, $J = 15.6$ Hz, 1H), 2.12 (d, $J = 11.5$ Hz, 1H), 2.03 (d, $J = 15.6$ Hz, 1H), 1.88 (s, 3H), 1.79 (s, 1H), 1.52 (d, $J = 11.6$ Hz, 5H), 1.43 (d, $J =$ 12.7 Hz, 3H), 1.29 (d, $J = 8.6$ Hz, 2H), 1.23 (s, 1H), 1.15 (d, $J = 9.5$ Hz, 7H), 1.07 (d, $J = 7.1$ Hz, 1H), 1.00 (s, 3H), 0.88 (d, $J = 11.5$ Hz, 7H), 0.80 (d, $J = 6.0$ Hz, 3H), 0.75 (s, 3H). ^{13}C NMR (DMSO-d_6, 75 MHz, ppm) δ 178.68 (C=O), 165.24 (C=NH), 160.71 (Ar-C), 156.52 (C=N), 138.70 ($\underline{\mathrm{C}}$H=C), 138.43 (C=C), 132.30 (Ar-C), 131.90 (Ar-C), 130.59 (Ar-C), 126.24 (C=C), 124.66 (CH=$\underline{\mathrm{C}}$), 116.91 (Ar-C), 116.63 (Ar-C), 56.46, 54.31, 52.90, 47.32, 46.73, 45.26, 43.00, 42.21, 38.93, 38.93,36.75, 32.23, 30.65, 28.91, 27.97, 24.25, 23.60, 23.41, 22.15, 21.49, 19.71, 19.01, 17.54, 16.88, 16.31. HRMS (ESI) *m/z* calcd for $C_{38}H_{54}FN_4O_2^+$ $(M+H)^+$ 617.42253, found 617.42224.

(1S,2R,4aS,6aS,6bR,10Z,11E,12aR)-11-(2-bromobenzylidene)-10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylic acid (7h) $R_f = 0.40$, methanol/dichloromethane (v/v 1:8); White powder; Yield: 44%; 278–279 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.93 (s, 1H, COOH), 10.51 (s, 1H, C=NH), 7.88–7.60 (m, 5H, C-NH, NH$_2$, Ar-H), 7.45 (s, 1H, Ar-H), 7.29 (s, 1H, Ar-H), 6.46 (s, 1H, C=CH), 5.10 (s, 1H), 2.71 (d, $J = 13.8$ Hz, 1H), 2.09 (d, $J = 11.1$ Hz, 1H), 1.85 (d, $J = 14.9$ Hz, 2H), 1.72 (s, 3H), 1.53 (d, $J = 8.1$ Hz, 5H), 1.44 (d, $J = 8.3$ Hz, 3H), 1.23 (s, 7H), 1.15 (s, 3H), 1.07 (d, $J = 7.1$ Hz, 1H), 1.00 (s, 3H), 0.90 (s, 4H), 0.83–0.75 (m, 6H), 0.72 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 178.67 (C=O), 165.06 (C=NH), 156.47 (C=N), 138.77 ($\underline{\mathrm{C}}$H=C), 135.45 (C=C), 132.82 (Ar-C), 132.67 (Ar-C), 132.61 (Ar-C), 132.36 (Ar-C), 130.21 (Ar-C), 127.79 (C=C), 124.50 (CH=$\underline{\mathrm{C}}$), 123.95 (Ar-C), 56.49, 55.38, 52.89, 47.33, 46.72, 44.57, 43.48, 42.23, 38.93, 38.90, 36.75, 32.41, 30.65, 28.13, 27.96, 24.23, 23.61, 23.36, 22.22, 21.50, 19.50, 19.03, 17.50, 16.99, 15.87. HRMS (ESI) *m/z* calcd for $C_{38}H_{54}BrN_4O_2^+$ $(M+H)^+$ 677.34247, found 677.34249.

(1S,2R,4aS,6aS,6bR,10Z,11E,12aR)-11-(3-bromobenzylidene)-10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylic acid (7i) $R_f = 0.30$, methanol/dichloromethane (v/v 1:8); White powder; Yield: 43%; 279–281 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.96 (s, 1H, COOH), 10.42 (s, 1H, C=NH), 7.65 (s, 3H, C-NH, NH$_2$), 7.49 (s, 3H, Ar-H), 7.39 (s, 1H, Ar-H), 6.53 (s, 1H, C=CH), 5.15 (s, 1H), 2.81 (d, $J = 14.4$ Hz, 1H), 2.12 (d, $J = 11.2$ Hz, 1H), 2.02 (d, $J = 16.1$ Hz, 1H), 1.85 (dd, $J = 23.4$, 15.8 Hz, 3H), 1.52 (d, $J = 12.3$ Hz, 5H), 1.43 (d, $J = 13.3$ Hz, 2H), 1.36–1.27 (m, 3H), 1.23 (s, 2H), 1.15 (d, $J = 11.7$ Hz, 6H), 1.09–1.03 (m, 2H), 1.00 (s, 3H), 0.88 (d, $J = 10.4$ Hz, 7H), 0.80 (d, $J = 6.2$ Hz, 3H), 0.74 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 178.70 (C=O), 165.16 (C=NH), 156.37 (C=N), 138.78 ($\underline{\mathrm{C}}$H=C), 138.38 (C=C), 132.77 (Ar-C), 132.40 (Ar-C), 131.65 (Ar-C), 130.82 (Ar-C), 130.76 (Ar-C), 128.88 (C=C), 124.61 (CH=$\underline{\mathrm{C}}$), 121.93 (Ar-C), 56.49, 55.39, 52.89, 47.34, 46.72, 45.12, 43.07, 42.23, 39.25, 38.94, 36.76, 32.24, 30.65, 28.88, 27.98, 24.25, 23.62, 23.39, 22.17, 21.51, 19.69, 19.03, 17.55, 16.90, 16.35. HRMS (ESI) *m/z* calcd for $C_{38}H_{54}BrN_4O_2^+$ $(M+H)^+$ 677.34247, found 677.34271.

(1S,2R,4aS,6aS,6bR,10Z,11E,12aR)-11-(2,4-dichlorobenzylidene)-10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylic acid (7j) $R_f = 0.33$, methanol/dichloromethane (v/v 1:8); White powder; Yield: 41%; 285–287 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.95 (s, 1H, COOH), 10.62 (s, 1H, C=NH), 7.79 (d, $J = 8.4$ Hz, 1H, Ar-H), 7.69 (d, $J = 2.0$ Hz, 4H, Ar-H, C-NH, NH$_2$), 7.51 (d, $J = 8.3$ Hz, 1H, Ar-H), 6.47 (s, 1H, C=CH), 5.11 (s, 1H), 2.67 (d, $J = 14.7$ Hz, 1H), 2.09 (d, $J = 11.1$ Hz, 1H), 1.95–1.81 (m, 2H), 1.76 (s, 2H), 1.52 (d, $J = 10.1$ Hz, 5H), 1.43 (d, $J = 11.9$ Hz, 2H), 1.22 (d, $J = 7.3$ Hz, 9H), 1.13 (s, 3H), 1.00 (s, 4H), 0.90 (s, 4H), 0.85 (s, 1H), 0.79 (d, $J = 9.7$ Hz, 5H), 0.72 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 178.68 (C=O), 164.86 (C=NH), 156.48 (C=N), 138.75 ($\underline{\mathrm{C}}$H=C), 134.16 (C=C), 133.91 (Ar-C), 133.85 (Ar-C), 133.52 (Ar-C), 132.81 (Ar-C), 129.04 (Ar-C), 128.99 (Ar-C), 127.51 (C=C), 124.52 (CH=$\underline{\mathrm{C}}$), 56.49, 52.89, 47.32, 46.68, 44.71, 43.37, 42.24, 38.93, 38.91, 36.75, 32.36, 30.65, 29.50, 27.97, 24.23, 23.61, 23.37, 22.14, 21.50, 19.54, 19.03, 17.53, 16.97, 15.88, 14.42. HRMS (ESI) *m/z* calcd for $C_{38}H_{53}Cl_2N_4O_2^+$ (M+H)$^+$ 667.35401, found 667.35425.

(1S,2R,4aS,6aS,6bR,10Z,11E,12aR)-11-(2,6-dichlorobenzylidene)-10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylic acid (7k) $R_f = 0.53$, methanol/dichloromethane (v/v 1:8); White powder; Yield: 57%; 297–299 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.93 (s, 1H, COOH), 9.53 (s, 1H, C=NH), 7.73 (s, 3H, C-NH, NH$_2$), 7.59 (d, $J = 8.1$ Hz, 2H, Ar-H), 7.48–7.41 (m, 1H, Ar-H), 6.46 (s, 1H, C=CH), 5.06 (s, 1H), 2.24–2.15 (m, 2H), 2.08 (d, $J = 11.1$ Hz, 1H), 1.90 (d, $J = 12.8$ Hz, 1H), 1.78 (d, $J = 13.1$ Hz, 3H), 1.60 (s, 4H), 1.55 (s, 3H), 1.49 (s, 2H), 1.45 (s, 1H), 1.39 (s, 1H), 1.33 (s, 2H), 1.30 (s, 2H), 1.12 (s, 3H), 1.02 (s, 3H), 0.90 (s, 4H), 0.85 (s, 1H), 0.81 (s, 3H), 0.77 (d, $J = 6.1$ Hz, 3H), 0.71 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 178.64 (C=O), 163.76 (C=NH), 156.44 (C=N), 138.82 ($\underline{C}$H=C), 134.57 (C=C), 134.52 (Ar-C), 132.96 (Ar-C), 132.96 (Ar-C), 130.95 (Ar-C), 128.86 (Ar-C), 128.86 (Ar-C), 127.51 (C=C), 124.35 (CH=$\underline{C}$), 56.48, 55.48, 52.84, 47.29, 46.62, 45.84, 44.06, 42.22, 39.34, 38.91, 36.74, 32.57, 30.64, 29.51, 27.97, 24.21, 23.58, 22.57, 21.50, 19.48, 19.03, 17.51, 17.07, 15.61, 14.42. HRMS (ESI) *m/z* calcd for $C_{38}H_{53}Cl_2N_4O_2^+$ (M+H)$^+$ 667.35401, found 667.35413.

(1S,2R,4aS,6aS,6bR,10Z,11E,12aR)-11-(4-methylbenzylidene)-10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylic acid (7l) $R_f = 0.33$, methanol/dichloromethane (v/v 1:8); White powder; Yield: 45%; 302–304 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.96 (s, 1H, COOH), 10.24 (s, 1H, C=NH), 7.52 (s, 3H, C-NH, NH$_2$), 7.36 (d, $J = 8.0$ Hz, 2H, Ar-H), 7.26–7.17 (m, 2H, Ar-H), 6.47 (s, 1H, C=CH), 5.17 (s, 1H), 2.87 (d, $J = 15.6$ Hz, 1H), 2.32 (s, 3H), 2.28 (s, 1H), 2.14 (s, 1H), 2.10 (s, 1H), 2.03 (s, 1H), 1.90 (s, 3H), 1.52 (s, 3H), 1.42 (s, 2H), 1.33 (s, 2H), 1.23 (s, 3H), 1.14 (d, $J = 3.0$ Hz, 3H), 1.05 (s, 3H), 1.00 (s, 2H), 0.89 (d, $J = 10.8$ Hz, 7H), 0.81 (d, $J = 7.7$ Hz, 5H), 0.75 (s, 3H), 0.65 (s, 1H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 178.71 (C=O), 165.99 (C=NH), 165.74 (Ar-C), 156.64 (Ar-C), 156.37 (C=N), 138.74 ($\underline{C}$H=C), 137.60 (C=C), 133.11 (Ar-C), 130.16 (Ar-C), 129.81 (Ar-C), 129.33 (Ar-C), 125.00 (C=C), 124.69 (CH=$\underline{C}$), 56.48, 55.06, 52.93, 47.35, 46.78, 42.85, 42.24, 39.32, 38.95, 36.77, 32.20, 30.67, 28.93, 27.98, 24.26, 23.62,23.42, 22.18, 21.51, 21.35, 19.56, 19.03, 17.59, 16.87, 16.39, 14.42. HRMS (ESI) *m/z* calcd for $C_{39}H_{57}N_4O_2^+$ (M+H)$^+$ 613.44760, found 613.44745.

(1S,2R,4aS,6aS,6bR,10Z,11E,12aR)-11-(4-nitrobenzylidene)-10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylic acid (7m) $R_f = 0.30$, methanol/dichloromethane (v/v 1:8); Yellow powder; Yield: 55%; 257–259 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.95 (s, 1H, COOH), 10.54 (s, 1H, C=NH), 8.27 (d, $J = 8.7$ Hz, 2H, Ar-H), 7.75 (d, $J = 8.7$ Hz, 2H, Ar-H), 7.60 (s, 3H, C-NH, NH$_2$), 6.69 (s, 1H, C=CH), 5.15 (s, 1H), 2.87 (d, $J = 15.0$ Hz, 1H), 2.11 (d, $J = 10.9$ Hz, 1H), 2.05 (s, 1H), 1.90 (d, $J = 7.7$ Hz, 2H), 1.84 (s, 1H), 1.79 (s, 1H), 1.52 (d, $J = 13.2$ Hz, 5H), 1.45 (s, 1H), 1.41 (s, 1H), 1.35–1.27 (m, 3H), 1.23 (s, 2H), 1.16 (d, $J = 9.9$ Hz, 6H), 1.09–1.03 (m, 2H), 1.00 (s, 2H), 0.88 (d, $J = 11.3$ Hz, 6H), 0.80 (d, $J = 6.2$ Hz, 3H), 0.74 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 178.71 (C=O), 164.87 (C=NH), 156.39 (C=N), 146.70 (Ar-C), 142.78 (Ar-C), 138.74 ($\underline{C}$H=C), 134.76 (C=C), 131.46 (Ar-C), 131.36 (Ar-C), 131.36 (C=C), 124.66 (CH=$\underline{C}$), 123.83 (Ar-C), 123.83 (Ar-C), 56.49, 52.91, 47.34, 46.69, 45.39, 43.10, 42.24, 39.34, 38.94, 36.77, 32.19, 30.64, 28.93, 27.99, 24.24, 23.62, 23.38, 22.20, 21.50, 19.74, 19.03, 17.60, 16.89, 16.32. HRMS (ESI) *m/z* calcd for $C_{38}H_{54}N_5O_4^+$ (M+H)$^+$ 644.41703, found 644.41724.

(1S,2R,4aS,6aS,6bR,10Z,11E,12aR)-10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethyl-11-((naphthalen-1-yl)methylene)picene-4a-carboxylic acid (7n) $R_f = 0.40$, methanol/dichloromethane (v/v 1:8); White powder; Yield: 40%; 293–295 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.95 (s, 1H, COOH), 10.35 (s, 1H, C=NH), 7.94 (s, 4H, Ar-H), 7.55 (s, 6H, C-NH, NH$_2$, Ar-H), 6.70 (s, 1H, C=CH), 5.15 (s, 1H), 3.01 (d, $J = 14.6$ Hz, 1H), 2.09 (s, 2H), 1.90 (s, 5H), 1.55 (s, 3H), 1.43 (d, $J = 15.5$ Hz, 3H), 1.22 (d, $J = 6.8$ Hz, 7H), 1.16 (s, 3H), 1.05 (dd, $J = 7.9$, 6.0 Hz, 4H), 0.99 (s, 3H), 0.94 (s, 1H), 0.89 (s, 4H), 0.83 (s, 1H), 0.78 (d, $J = 6.7$ Hz, 3H), 0.74 (s, 2H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 178.70 (C=O), 166.10 (Ar-C), 165.57 (C=NH), 156.53 (Ar-C), 156.33 (C=N), 138.73 ($\underline{C}$H=C), 133.55 (C=C), 133.20 (Ar-C), 132.62 (Ar-C), 131.17 (Ar-C), 129.27 (Ar-C), 128.57 (Ar-C), 128.08 (Ar-C), 128.02 (Ar-C), 126.93 (C=C), 124.68 (CH=$\underline{C}$), 56.49, 55.38, 52.89, 47.33, 46.76, 43.12, 42.21, 41.61, 39.27, 38.93, 36.77, 32.26, 30.63, 28.94, 27.98, 24.24, 23.61, 23.41, 22.26, 21.49, 19.74, 19.03, 17.56, 16.91, 16.35. HRMS (ESI) *m/z* calcd for $C_{42}H_{57}N_4O_2^+$ $(M+H)^+$ 649.44760, found 649.44733.

(1S,2R,4aS,6aS,6bR,10Z,11E,12aR)-11-((furan-2-yl)methylene)-10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethylpicene-4a-carboxylic acid (7o) $R_f = 0.45$, methanol/dichloromethane (v/v 1:8); Yellow powder; Yield: 34%; 290–292 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.93 (s, 1H, COOH), 10.10 (s, 1H, C=NH), 7.82 (s, 1H, Ar-H), 7.39 (s, 3H, C-NH, NH_2), 6.66 (d, $J = 18.5$ Hz, 2H, Ar-H), 6.39 (s, 1H, C=CH), 5.22 (s, 1H), 2.98 (d, $J = 17.1$ Hz, 1H), 2.17 (d, $J = 7.2$ Hz, 1H), 2.09 (s, 1H), 1.98 (d, $J =$ 9.4 Hz, 2H), 1.91 (s, 1H), 1.82 (s, 1H), 1.53 (s, 1H), 1.48 (s, 1H), 1.44 (s, 1H), 1.39 (s, 1H), 1.24 (s, 7H), 1.14 (s, 2H), 1.12 (s, 2H), 1.08 (s, 1H), 1.06 (s, 3H), 1.03 (s, 1H), 0.91 (s, 3H), 0.86 (s, 3H), 0.79 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 178.74 (C=O), 164.69 (C=NH), 156.18 (C=N), 151.39 (C-O), 144.14 (C-O), 138.78 ($\underline{C}$H=C), 127.59 (C=C), 124.78 (CH=$\underline{C}$), 120.98 (C=C), 113.72 (C=C), 112.68 (C=C), 56.49, 52.95, 47.37, 46.57, 45.89, 43.08, 42.26, 38.98, 38.81, 36.79, 34.14, 32.28, 30.67, 29.48, 24.28, 23.62, 22.56, 22.39, 21.52, 19.89, 19.03, 17.61, 16.93, 15.97, 14.42. HRMS (ESI) *m/z* calcd for $C_{36}H_{53}N_4O_3^+$ $(M+H)^+$ 589.41122, found 589.41144.

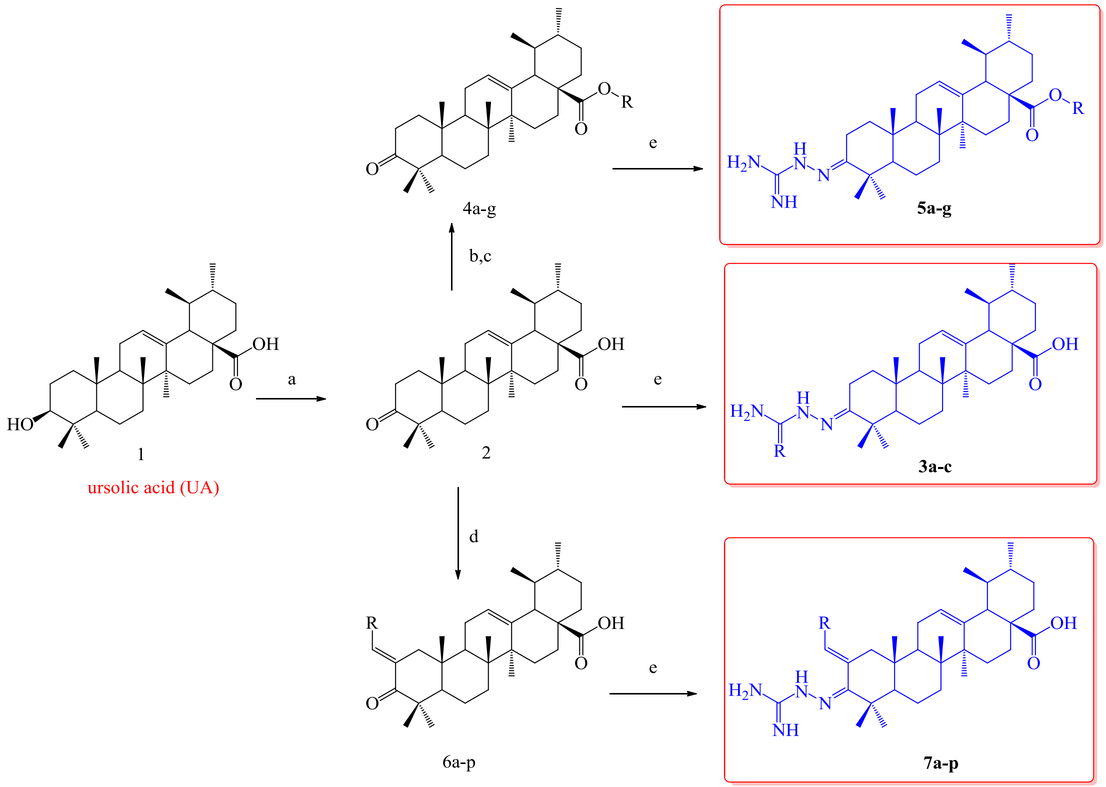
(1S,2R,4aS,6aS,6bR,10Z,11E,12aR)-10-guanidinoimino-1,2,3,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-icosahydro-1,2,6a,6b,9,9,12a-heptamethyl-11-((thiophen-2-yl)methylene)picene-4a-carboxylic acid (7p) $R_f = 0.45$, methanol/dichloromethane (v/v 1:8); White powder; Yield: 36%; 287–289 °C; ^{1}H NMR (DMSO-d_6, 300 MHz, ppm) δ 11.90 (s, 1H, COOH), 7.56 (d, $J = 4.7$ Hz, 4H, C-NH, NH_2, Ar-H), 7.14–7.05 (m, 1H, Ar-H), 6.87 (d, $J = 3.0$ Hz, 1H, Ar-H), 5.97 (d, $J = 11.1$ Hz, 1H), 5.08 (s, 1H, CH=C), 2.73 (s, 1H), 2.18 (t, $J = 7.1$ Hz, 1H), 2.09 (d, $J = 11.2$ Hz, 1H), 1.90 (d, $J = 13.9$ Hz, 1H), 1.78 (d, $J = 12.2$ Hz, 1H), 1.52 (s, 3H), 1.45 (s, 1H), 1.40 (d, $J = 6.0$ Hz, 2H), 1.29 (d, $J =$ 5.1 Hz, 3H), 1.24 (s, 7H), 1.20 (s, 2H), 1.08 (s, 2H), 1.05 (d, $J = 6.7$ Hz, 5H), 0.91 (s, 3H), 0.84 (d, $J = 6.8$ Hz, 2H), 0.78 (d, $J = 6.1$ Hz, 2H), 0.68 (s, 3H), 0.41 (s, 2H). ^{13}C NMR (DMSO-d_6, 126 MHz, ppm) δ 178.68 (C=O), 174.95 (C-S), 172.82 (C=NH), 153.24 (C=N), 138.64 ($\underline{C}$H=C), 138.57 (C-S), 127.79 (C=C), 127.56 (C=C), 126.69 (C=C), 126.69 (C=C), 124.68 (CH=$\underline{C}$), 56.48, 53.01, 51.08, 47.42, 46.37, 45.42, 42.39, 39.37, 38.95, 36.73, 34.16, 31.76, 31.34, 29.49, 24.26, 23.44, 22.74, 22.56, 21.49, 19.25, 19.03, 17.36, 16.83, 16.63, 14.42. HRMS (ESI) *m/z* calcd for $C_{36}H_{53}N_4O_2S^+$ $(M+H)^+$ 605.38837, found 605.38812.

Evaluation of anti-bacterial and anti-fungal activities in vitro

The microbes used in the present study were *S. aureus* RN 4220, *S.mutans* KTCT 3289, *Escherichia coli* KTCT 1924, *C. albicans* 7535. The strains of multidrug-resistant clinical isolates were methicillin-resistant *Staphylococcus aureus* (MRSA CCARM 3167 and MRSA CCARM 3506) and quinolone-resistant *Staphylococcus aureus* (QRSA CCARM 3505 and QRSA CCARM 3519). Clinical isolates were collected from various patients hospitalized in several clinics. In order to obtain final concentrations of 64-1 μg/mL, a twofold serial dilution technique was used. Test bacteria were diluted 1000-fold in Mueller-Hinton broth (MHB) when they grew to mid-log phase in the same medium. The bacteria of 10^5 CFU/mL were vaccinated into MHB and distributed at 0.2 mL/well in a 96-well microtiter plate. Oxacillin, norfloxacin, gatifloxacin, moxifloxacin, fluconazole and itraconazole were used as positive controls. The compounds which were tested were dissolved in DMSO, the final concentration did not surpass 0.05%. The MIC value was defined as the concentration of a compound used in test that entirely inhibited bacteria growth during 24 h cultivation at 37 °C. The absorption at 650 nm by which the Bacteria growth was determined was measured by using a microtiter enzyme-linked immunosorbent assay (ELISA) reader. All experiments were tested three times.

Evaluation of anti-inflammatory activity in vivo

Anti-inflammatory assay: Assay in the xylene-induced ear edema method via the intraperitoneal route: The anti-inflammatory activity was evaluated by an in vivo inhibition assay by monitoring xylene-induced ear edema in mice. In the primary screening, all tested compounds, ibuprofen and indomethacin were freshly prepared (dissolved with DMSO) prior to administered i.p. at a dose of 100 mg/kg to mice and at a concentration of 0.2 mL/20 g body weight. Control mice received the vehicle only (DMSO, 0.2 mL/20 g of body weight). Thirty minutes after administration i.p., animals were used in the xylene-induced ear edema test, 20 μL xylene was applied to the surface of the right ear of each mouse by a micropipette. After keeping them from struggling for 30 min, a cylindrical plug (diameter, 7 mm) was excised from each of the treated and untreated ears. Edema was quantified by the difference in weight between the two plugs. Anti-inflammatory activity was expressed as percent reduction in edema compared with the DMSO-administered control group. The NSAID ibuprofen and indomethacin were tested in parallel as reference. Assay in the xylene-induced ear edema method via the oral route: In thelatter evaluation, tested compounds and indometacin



Scheme 1 Synthetic scheme for the synthesis of compounds **3a–c**, **5a–g**, **7a–p**. Reagents and conditions: **a** Jones reagent, acetone, 0 °C, 5 h; **b** Methyl iodide, K_2CO_3, DMF, r.t., 6 h; **c** Benzyl chlorides, K_2CO_3, acetone, reflux, 8 h; **d** R-CHO, 5 % NaOH, absolute ethanol, r.t 2 h; **e** Concentrated HCl, absolute ethanol, reflux, 8 h

were homogenized with 0.5% sodium carboxymethylcellulose (CMC-Na) and administered via the oral route to mice at a concentration of 0.2 mL/20 g mice weight. Control mice received the vehicle only (0.5% CMC-Na, 0.2 mL/20 g). To explore the peak activity of the compound, edema was quantified at different intervals (1, 2, 3, 4, 5, and 24 h). Compounds **3a** and ibuprofen homogenized with 0.5% CMC-Na were administered orally to mice (lower doses of 50 and 25 mg/kg and 0.2 mL/20 g mice body weight). Control mice received 0.5% CMC-Na (0.2 mL/20 g body weight) and edema quantified at the peak interval of 3 h. Edema was quantified by the difference in weight between the two plugs. Edema values, expressed as mean standard deviation, were compared statistically using one-way-ANOVA followed by Dunnet's test. A level of $p < 0.05$ was adopted as the test of significance.

Evaluation of cytotoxicity in vitro

Cytotoxicity on human cancer cells: The cytotoxicity test of selected compounds was measured through the colorimetric MTT assay. Human cervical cancer cells (HeLa), liver cancer cells (Hep3B) and lung cancer cells (A549) suspension in DMEM medium supplemented with 10% FBS and antimycotic was added in 96-well microplates at 1.8 × 104 cells/well. A variety of concentrations of the test compounds (200, 100, 50, 25, 12.5, 6.25, 3.125, 1.625 μmol/L) dissolved by distilled 10% DMSO was added to each well. Incubation for 24 h at 37 °C under 5% CO_2, 2.5 mg/mL of MTT solution was added to each well. Further the plate was incubated for 4 h. Then, the incubation medium was aspirated, added DMSO (100 μL) to solubilize the MTT formazan product. The absorbance at 570 and 630 nm were measured after mixing. The difference between absorbance of 570 and 630 nm was used as an index of the cell viability. The morphology of the cells was observed by Giemsa stain under Phase contrast microscope. The selected compounds were used as positive control, whereas untreated cells were used as negative controls. The IC_{50} values were defined as the concentrations inhibiting 50% of cell growth. All experiments were performed in triplicate.

Results and discussion

Chemistry

The synthetic pathway for the target compounds **3a–c**, **5a–g**, and **7a–p** is presented in Scheme 1. Intermediate **2**

Table 1 Inhibitory activity of compounds **3a**–**c**, **5a**–**g**, and **7a**–**p** expressed as MIC[a] (μg/mL) against strains of Gram-positive (Staphylococcus aureus RN 4220, Streptococcus mutans 3289) bacteria, Gram-negative (Escherichia coli KCTC 1924) bacteria and Candida albicans 7535

Compound	R	Gram-positive strains		Gram-negative strains	Fungus
		4220[b]	3289[c]	1924[d]	7535[e]
UA	–	32	32	>64	>64
3a	NH	2	16	2	64
3b	S	>64	>64	>64	>64
3c	O	>64	>64	>64	>64
5a	CH3	8	16	2	16
5b	Phenyl(3-Cl)	>64	>64	>64	>64
5c	Phenyl(4-Cl)	>64	>64	>64	>64
5d	Phenyl(2,4-2Cl)	>64	>64	>64	>64
5e	Phenyl(2,6-2Cl)	>64	64	>64	>64
5f	Phenyl(4-F)	>64	>64	>64	>64
5g	Phenyl(4-CH$_3$)	>64	>64	>64	>64
7a	Phenyl	>64	>64	>64	>64
7b	Phenyl(4-Cl)	>64	>64	>64	>64
7c	Phenyl(2-Cl)	64	64	>64	>64
7d	Phenyl(3-Cl	64	64	>64	>64
7e	Phenyl(4-F)	16	64	8	>64
7f	Phenyl(2-F)	64	64	32	>64
7g	Phenyl(3-F)	16	64	32	>64
7h	Phenyl(2-Br)	64	64	>64	>64
7i	Phenyl(3-Br)	32	64	>64	>64
7j	Phenyl(2,4-2Cl)	64	32	>64	>64
7k	Phenyl(2,6-2Cl)	64	>64	>64	>64
7l	Phenyl(4-CH$_3$)	4	8	16	>64
7m	Phenyl(4-NO$_2$)	32	32	16	>64
7n	Naphthyl	32	32	>64	>64
7o	Furyl	32	64	32	>64
7p	Thienyl	16	32	16	16
Gatifloxacin		0.25	0.25	2	0.5
Moxifloxacin		0.25	0.25	2	0.5
Fluconazole		n.d.	n.d.	n.d.	1
Itraconazole		n.d.	n.d.	n.d.	0.6

S. aureus RN 4220 is a genotype of *S. aureus*. *KCTC* Korean Collection for Type Cultures, *CCARM* Culture Collection of Antimicrobial Resistant Microbes, *n.d.* not determined

[a] The antibacterial tests was carried out three times, and the average values were taken as the MICs

[b] *Staphylococcus aureus* 4220

[c] *Streptococcus mutans* 3289

[d] *Escherichia coli* 1924

[e] *Candida albicans*7535

was synthesized by reacting UA with Jones reagent in acetone. Compounds **3a**–**c** were prepared by reacting intermediate **2** with aminoguanidine bicarbonate, semicarbazide hydrochloride, and thiosemicarbazide in refluxing ethanol. Compound **2** was reacted with methyl iodide and different substituents of benzyl chloride to provide compounds **4a**–**g**. Compounds **6a**–**p** were prepared by Claisen–Schmidt condensations between compound **2** and different aldehydes. Finally, compounds **4a**–**g** and **6a**–**p** were reacted with aminoguanidine bicarbonate in refluxing ethanol to afford compounds **5a**–**g** and **7a**–**p**, respectively. The structures of the desired compounds were characterized by ^{1}H NMR, ^{13}C NMR, and HRMS spectra.

Anti-bacterial and anti-fungal activities

The minimum inhibitory concentration (MIC) is defined as the lowest concentration of anti-bacterial agent required to visibly inhibit the growth of bacteria. In vitro anti-bacterial activity was evaluated using a 96-well microtiter plate and the serial dilution method to obtain the MIC values for several different pathogenic strains, including four multidrug-resistant Gram-positive strains (methicillin-resistant *Staphylococcus aureus* CCARM 3167 and 3506, and quinolone-resistant *S. aureus* CCARM 3505 and 3519), two Gram-positive strains (*S. aureus* RN 4220 and *S. mutans* KTCT 3289), one Gram-negative strain (*Escherichia coli* KCTC 1924), and one fungus (*Candida albicans* 7535). The anti-bacterial agents gatifloxacin, moxifloxacin, norfloxacin, and oxacillin, and the antifungal agents fluconazole and itraconazole were included as positive controls.

The results for the anti-bacterial and antifungal activities are presented in Tables 1 and 2. As shown in Table 1, most of the compounds (except **5b**–**g**) showed potent inhibitory activities against the different bacteria, with MIC values in the range of 2–64 μg/mL. Compounds **3a** and **5a**, which showed high levels of inhibitory activity against *E. coli* KCTC 1924 with MIC values of 2 μg/mL, had the same potency as the standard drugs gatifloxacin (MIC = 2 μg/mL) and moxifloxacin (MIC = 2 μg/mL). Compounds **3a**, **5a**, and **7l** exhibited the highest activity of all the synthesized compounds against *S. aureus* RN 4220 and *S. mutans* KTCT 3289 with MIC values of 2–16 μg/mL, which was lower

Table 2 Inhibitory activity (MIC[a], μg/mL) of compounds **3a**–**c**, **5a**–**g**, and **7a-p** against clinical isolates of multidrug-resistant Gram-positive strains

Compound	R	Multidrug-resistant Gram-positive strains			
		MRSA		*QRSA*	
		3167[b]	3506[c]	3505[d]	3519[e]
UA	–	32	32	32	32
3a	NH	2	2	2	4
3b	S	>64	>64	>64	>64
3c	O	>64	64	64	>64
5a	CH3	4	4	4	4
5b	Phenyl(3-Cl)	>64	>64	>64	>64
5c	Phenyl(4-Cl)	>64	>64	>64	>64
5d	Phenyl(2,4-2Cl)	>64	>64	64	>64
5e	Phenyl(2,6-2Cl)	>64	>64	64	>64
5f	Phenyl(4-F)	64	>64	64	>64
5g	Phenyl(4-CH_3)	>64	>64	>64	>64
7a	Phenyl	16	>64	>64	>64
7b	Phenyl(4-Cl)	>64	>64	>64	>64
7c	Phenyl(2-Cl)	>64	>64	32	>64
7d	Phenyl(3-Cl	64	>64	32	>64
7e	Phenyl(4-F)	8	32	16	32
7f	Phenyl(2-F)	8	64	32	>64
7g	Phenyl(3-F)	8	16	32	>64
7h	Phenyl(2-Br)	16	>64	32	>64
7i	Phenyl(3-Br)	64	64	16	>64
7j	Phenyl(2,4-2Cl)	>64	>64	16	>64
7k	Phenyl(2,6-2Cl)	32	>64	64	>64
7l	Phenyl(4-CH_3)	2	8	8	8
7m	Phenyl(4-NO_2)	8	32	16	32
7n	Naphthyl	16	32	32	32
7o	Furyl	8	32	32	32
7p	Thienyl	8	8	16	16
Gatifloxacin		2	2	8	4
Moxifloxacin		1	1	4	4
Norfloxacin		8	4	>64	>64
Oxacillin		>64	>64	1	1

[a] The antibacterial tests were carried out three times, and the average values were taken as the MICs

[b] Methicillin-resistant *Staphylococcus aureus*3167

[c] Methicillin-resistant *Staphylococcus aureus* 3506

[d] Quinolone-resistant *Staphylococcus aureus* 3505

[e] Quinolone-resistant *Staphylococcus aureus* 3519

Table 3 Inhibitory activities (MIC_{50} and MIC_{90}, μg/mL) of compounds **3a**, **5a**, and **7l** against test bacteria

Compound	Gram-positive strains		Gram-negative strains	
	4220[a]		1924[b]	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
3a	1.7	3.3	1.4	2.7
5a	5.4	10.8	1.6	2.8
7l	2.3	5.2	5.5	13.9
Gatifloxacin	0.02	0.38	1.4	2.7

[a] Staphylococcus aureus 4220

[b] Escherichia coli 1924

activity than observed for the standard drugs gatifloxacin (MIC = 0.25 μg/mL) and moxifloxacin (MIC = 0.25 μg/mL). Furthermore, UA did not exhibit any inhibitory activity against the one fungus tested (*C. albicans* 7535), while compounds **5a** and **7p** showed moderate to good levels of activity with MIC values of 16 μg/mL. As shown in Table 2, against the four multidrug-resistant Gram-positive strains (methicillin-resistant *S. aureus* CCARM 3167 and 3506 and quinolone-resistant *S. aureus* CCARM 3505 and 3519), compounds **3a**, **5a**, and **7l** exhibited good activity with MIC values in the range of 2–8 μg/mL. Compounds **3a** and **7l** were the most potent against methicillin-resistant *S. aureus* CCARM 3167 with MIC values of 2 μg/mL, which was four times more potent than norfloxacin (MIC = 8 μg/mL) and equipotent with gatifloxacin (MIC = 2 μg/mL). Compound **3a** was equipotent or more potent than the standard drugs gatifloxacin and moxifloxacin against quinolone-resistant *S. aureus* CCARM 3505 and 3519 with MIC values of 2 and 4 μg/mL, respectively.

Compound **3a, 5a and 7l** were chosen to evaluate their MIC_{50} and MIC_{90}, gatifloxacin was used as positive control. As indicated in Tables 3 and 4, compound **3a** displayed great activity ($MIC_{50} = 1.4$ μg/mL, $MIC_{90} = 2.7$ μg/mL), equivalent to gatifloxacin against *E. coli* KCTC 1924, and exhibited more potent activity than gatifloxacin against *S. aureus* CCARM 3505.

From the analysis of these results, we obtained several structure–activity relationships (SAR). First, it was clear that the aminoguanidine moiety is critical for anti-bacterial activity, as determined from the activity results of compounds **3a**–**c**. Second, except for compound **5a**, compounds **5b**–**g** did not have any anti-bacterial activity, suggesting that the presence of a benzene ring on the ester group was detrimental for activity. Third, the introduction of different substituents (different substituted benzene rings, a naphthalene ring, and a heterocycle) to the C-2 of UA increased the anti-bacterial activity. For the compounds in series **7**, the naphthalene ring and heterocycle substituted derivatives (**7n**, **7o** and **7p**) the activity was in the order thienyl > furyl > naphthyl. In addition, the order of activity for the benzene rings substituted derivatives was 4-CH_3 > -F > -Br > -Cl. Overall, compound **3a** showed the most potent anti-bacterial activity, and is a promising lead compound for further investigation.

Table 4 Inhibitory activities (MIC_{50} and MIC_{90}, μg/mL) of compounds **3a**, **5a**, and **7l** against the clinical isolates of multidrug-resistant Gram-positive bacterial strains

Compound	Multidrug-resistant Gram-positive strains							
	3167[a]		3506[b]		3505[c]		3519[d]	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
3a	1.5	3.0	1.4	2.7	1.4	2.7	1.1	4.6
5a	3.0	7.1	3.3	6.2	2.9	5.4	2.9	5.7
7l	1.5	2.8	5.4	10.7	1.4	8.1	6.6	11.4
Gatifloxacin	1.1	2.6	0.7	2.4	5.1	10.7	1.4	3.1

[a]Methicillin-resistant *Staphylococcus aureus*3167

[b]Methicillin-resistant *Staphylococcus aureus* 3506

[c]Quinolone-resistant *Staphylococcus aureus* 3505

[d]Quinolone-resistant *Staphylococcus aureus* 3519

Table 5 Anti-inflammatory activity of compounds **3a–c**, **5a–g**, and **7a–p** administrated i.p.

Compound	R	Dose (mg/kg)	Number of mice	Edema mean ± S.D. (mg)	Inhibition rate (%)
DMSO	–	100	10	6.19 ± 0.66	–
Indometacin	–	100	10	3.56 ± 0.89*	42.42
Ibuprfen	–	100	10	3.35 ± 0.55*	45.86
UA	–	100	10	2.76 ± 0.41**	55.35
3a	NH	100	10	1.14 ± 0.31***	81.61
3b	S	100	10	2.03 ± 0.49***	67.28
3c	O	100	10	4.63 ± 0.86	25.26
5a	CH3	100	10	1.96 ± 0.64***	68.28
5b	Phenyl(3-Cl)	100	10	3.56 ± 0.55*	42.42
5c	Phenyl(4-Cl)	100	10	3.49 ± 0.56*	43.63
5d	Phenyl(2,4-2Cl)	100	10	3.08 ± 0.55**	50.31
5e	Phenyl(2,6-2Cl)	100	10	4.28 ± 0.79	30.91
5f	Phenyl(4-F)	100	10	5.00 ± 0.85	19.2
5g	Phenyl(4-CH_3)	100	10	3.11 ± 0.45**	49.69
7a	Phenyl	100	10	1.55 ± 0.36***	74.95
7b	Phenyl(4-Cl)	100	10	2.14 ± 0.43***	65.45
7c	Phenyl(2-Cl)	100	10	2.59 ± 0.67***	58.18
7d	Phenyl(3-Cl)	100	10	2.36 ± 0.29***	61.81
7e	Phenyl(4-F)	100	10	3.63 ± 0.59*	41.42
7f	Phenyl(2-F)	100	10	3.58 ± 0.79*	42.23
7l	Phenyl(4-CH_3)	100	10	1.63 ± 0.51***	73.67
7m	Phenyl(4-NO_2)	100	10	1.89 ± 0.66***	69.49
7n	Naphthyl	100	10	5.76 ± 1.22	6.87
7o	Furyl	100	10	3.41 ± 0.72**	44.84
7p	Thienyl	100	10	3.95 ± 1.22	36.17

*: $p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with vehicle group

–: no anti-inflammatory activity

Anti-inflammatory activity

Some of the synthesized compounds were screened for their anti-inflammatory activity using the xylene-induced ear-edema test in mice. The potency of the test compounds was determined relative to the ability to prevent edema. Dimethyl sulfoxide was used as the vehicle and ibuprofen and indomethacin were used as reference drugs. As shown in Table 5, most of the test compounds exhibited good anti-inflammatory activity at 100 mg/kg. Compounds **3a**, **3b**, **5a**,

Table 6 Anti-inflammatory activity of compound **3a** administered orally at different times before xylene application

Time (h)	Dose (mg/kg)	Number of mice	Inhibition (%)	
			3a	Ibuprfen
1	100	10	25.45	19.49
2	100	10	31.04^{*}	23.80^{*}
3	100	10	38.06^{**}	34.44^{**}
4	100	10	33.23^{**}	25.45^{*}
5	100	10	28.96^{*}	16.35
24	100	10	15.50	13.35

*$0.01 < p < 0.05$ compared with vehicle group, **$p < 0.01$ compared with vehicle group

Table 7 Anti-inflammatory activity of compound **3a** administered orally at different doses

Time (h)	Dose (mg/kg)	Number of mice	Inhibition (%)	
			3a	Ibuprfen
3	100	10	47.94^{***}	33.42^{**}
3	50	10	29.39^{*}	21.72
3	25	10	23.48	13.58

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ compared with vehicle group

Table 8 Cytotoxic activity (IC_{50}[a], μg/mL) of compound **3a** against human cell lines

Compound	Substituents	In vitro cytotoxicity IC_{50}[a] (μg/mL)		
	R	HeLa[b]	Hep3B[c]	A549[d]
3a	NH	>100	>100	>100

[a] IC_{50} is the concentration required to inhibit the cell growth by 50%. Data represent the average of three independent experiments running in triplicate. Variation was generally between 5–10%

[b] Human cervical cancer cells

[c] Human liver cancer cells

[d] Human lung cancer cells

7a, **7b**, **7c**, **7d**, **7l**, and **7m** showed significant activity with inhibition in the range of 58.18% to 81.61%, which was higher than for the reference drugs and UA. Compounds **3c**, **5e**, **5f**, **7n**, and **7p** did not display any anti-inflammatory activity at the same dose. SAR analysis revealed that the aminoguanidine moiety was important for anti-inflammatory activity (**3a** > **3b** and **3c**). Compound **3a** and the compounds in series **7** were more active than the compounds in series **5**, indicating that retaining the carboxylic acid at C-17 was beneficial for the activity.

According to the anti-inflammatory activity of the designed compounds, compound **3a** was chosen for further evaluation. A dose of 100 mg/kg was administered via the oral route at different intervals (1, 2, 3, 4, 5, and 24 h) after xylene application. As shown in Table 6, the activity of compound **3a** showed a regular increase as the time interval lengthened until a peak was reached at 3 h (38.06%), and compound **3a** displayed more potent activity than ibuprofen (34.44%). The activity of compound **3a** was also screened at concentrations of 100, 50, and 25 mg/kg at 3 h after oral administration. As shown in Table 7, compound **3a** showed the highest anti-inflammatory activity at 100 mg/kg with 47.94% inhibition.

To determine whether the synthesized compounds were selectively toxic toward bacteria, we evaluated the cytotoxicity of compound **3a** using a standard technique. As shown in Table 8, compound **3a** did not show any appreciable cytotoxic activity ($IC_{50} > 100$ μmol/L) against HeLa, Hep3B, and A549 cells. This result indicated that the promising anti-bacterial activity of these compounds is not because of cytotoxicity and compound **3a** is a promising anti-bacterial inhibitor for use in further work.

The mechanism of action for the antimicrobial and anti-inflammatory activities of these compounds is unknown. Wei et al. (2018) reported that the UA derivatives exhibited high affinity for the COX-2 active site and possibly exhibit their anti-inflammatory potency via inhibiting COX-2. We infer that a similar mechanism might be involved for our synthesized compounds. Further study on the possible mechanism of action is currently ongoing in our laboratory.

Conclusion

In summary, three series of compounds, a total of 26 UA derivatives containing an aminoguanidine moiety, were synthesized, characterized, and evaluated for anti-bacterial and anti-inflammatory activity. Some of these compounds exhibited high levels of anti-bacterial and anti-inflammatory activity. Compound **3a** showed the most potent anti-bacterial activity against the different bacteria tested with MIC values in the range of 2–16 μg/mL, and also exhibited the highest anti-inflammatory activity with inhibition of 81.61%. Compound **3a** was also evaluated for cytotoxicity and did not exhibit any appreciable cytotoxic activity in human cervical cancer HeLa, liver cancer Hep3B, and lung cancer A549 cell lines. This work provides an experimental basis for the development of UA derivatives with potent anti-bacterial and anti-inflammatory activities. Further work toward the design of more potent compounds and investigating the mechanism of action are currently underway in our laboratory.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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