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Synthesis of New Aminoguanidinium and Biguanidinium Derivatives of Ursolic and Corosolic Acids as Potential Antimicrobial Agents †

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Abstract: The antibiotics and antifungal agents currently used in practical medicine are losing their effectiveness as a result of the rapid development of multidrug resistance in pathogenic microorganisms. In the context of this problem, modern medicine and veterinary medicine urgently need to discover new drugs and new medical technologies for the treatment of bacterial infections. Biological studies of pentacyclic triterpene acids, including ursolic and corosolic acids, over the last few decades have shown that these available secondary metabolites are active against many human bacterial and fungal pathogens. However, their antibacterial activity is much weaker compared to known anibiotics produced by bacteria and fungi. In order to enhance the antibacterial potential of natural tritepenes, we synthesised a series of new derivatives of ursolic and corosolic acids bearing aminoguanidinium and biguanidinium end fragments in the C-28 side chain. Guanidinium and biguanidinium groups are known to determine the chemical and physicochemical properties of biologically active substances. Many compounds containing guanidinium and biguanidinium moieties constitute an important class of therapeutic agents, including antibacterial and antifungal drugs. The introduction of guanidinium and biguanidinium moieties into triterpene acid molecules was carried out by guanylation and biguanylation of pre-synthesised carboxyamides of ursolic and corosolic acids containing terminal primary amino groups at C-28 of the alkane side chain. We found optimal guanylating and biguanylating reagents, which provided relatively mild reaction conditions and high yields of the target products.

Keywords: pentacyclic triterpenoids; ursolic acid; corosolic acid; guanidines; biguanidines; antibacterial activity

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1. Introduction

Over the past decade, bacterial resistance to antibiotics has increased at an alarming rate and many commonly used antibiotics are becoming ineffective [1]. In the context of this problem, modern medicine and veterinary medicine urgently need to discover a new class of drugs and new medical technologies for the treatment of bacterial infections. Among the investigated compounds of natural origin, the available secondary plant metabolites pentacyclic triterpene acids such as ursolic and corosolic acids are of great interest. Ursolic and corosolic acids have a variety of biological properties, including antibacterial activity [2–5]. The bacteriostatic activity of ursolic and corosolic acids has been demonstrated against many bacterial species, both Gram-positive *Staphylococcus aureus* (methicillin-sensitive and resistant strains) and Gram-negative Escherichia coli [6]. The antibacterial effects of triterpene acids are much weaker than those of known antibiotics produced by bacteria and fungi. However, their low toxicity to eukaryotic cells makes

them a promising molecular platform for the discovery of new antibiotics, as well as for use in combination antibacterial therapy with known antimicrobial agents such as ampicillin or tetracycline [3]. We have previously published work where functionalisation of triterpene acids with a guanidinium group showed high activity against Gram-positive and Gram-negative bacteria as well as antimicrobial activity. For example, a guanidinium derivative of ursolic acid with a diaminobutane linker was highly active against S. aureus and C. neoformans [7]. Compounds containing guanidinium and biguanidinium groups are widely distributed in nature and are involved in many biochemical processes in cells [8]. Biguanide derivatives such as chlorhexidine, polyhexanide or polyaminopropyl biguanides are used as antibacterial agents. Alkyl biguanidinium salts have antimicrobial activity, low haemolytic activity and low toxicity [9]. However, there is no information in the literature about guanidinium derivatives of corosolic acid and biguanidinium derivatives of ursolic acid. Therefore, in this work we have synthesised new guanidinium and biguanidinium derivatives of these triterpene acids of the ursane series, in which the guanidinium and biguanidinium moieties are attached to the C-28 carboxyl function of the triterpene core via different linkers (ethylenediamine, diaminooctane).

2. Materials and Methods

2.1. Chemistry

All reagents and solvents were of the purest grade available, and generally were used without further treatment. The starting compounds ursolic acid and reagents: acetyl chloride, oxalyl chloride, 1,2-diaminoethane, 1,8-diaminooctane, triethylamine (Et_3N), dimethylaminopyridine (DMAP), *N*,*N*-diisopropylethylamine (DIPEA), 1*H*-pyrazole-1-carboxamidine hydrochloride, dicyandiamide, iron (III) chloride were purchased from Acros Organics (Geel, Belgium). Corosolic acid and *N*-amidinopyrazole-1-carboxamidine hydrochloride were prepared according to the procedures described in the literature [10,11]. The acetate of ursolic and corosolic acids were synthesized according to typical procedure [12]. The spectral data of compounds **5**, **6** corresponded to published data [13]. IR spectra (thin film) were obtained on a Vertex 70v spectrometer (Bruker, Karlsruhe, Germany). ^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or in MeOD with Me_4Si as internal standard on an AVANCE-500 instrument (500.13 (^1H), 125.78 MHz (^{13}C)) (Bruker). The mass spectrum was recorded on a high-resolution quadrupole mass spectrometer Bruker maXis Impact. Optical rotation was determined on a 141 polarimeter (Perkin–Elmer, Beaconsfield, UK). Specific rotation $[\alpha]_D$ is expressed in $(deg \cdot mL)/(g \cdot dm)^{-1}$; the concentration of the solution *c* is expressed in g/100 mL. TLC was performed on Sorbfil plates (Sorbpolymer, Krasnodar, Russia).

2.1.1. General Procedure for the Guanilation of Amines **5–8**

The amine (0.5 mmol) was dissolved in dry DMF (1 mL) and DIPEA (0.2 mL, 1.5 mmol) and 1*H*-pyrazole-1-carboxamidine hydrochloride (0.09 g, 0.6 mmol) was added while stirring vigorously. The mixture was stirred for 20-24 h. The mixture was diluted with H_2O and the precipitate formed was filtered off and washed with water to give pure compounds **9–12**. **(3*β*)-*N*-(2-ethylgyanidine)-3-acetyloxy-urs-12-en-28-amide hydrochloride (9)** White powder, 62% yield; m.p. 198–200°C (EtOH); $[\alpha]_D^{21}$ +39.1° (*c* 0.46, C_2H_5OH); IR (film) ν_{max} 1640 (C=O), 3338 (NH); ^1H-NMR (500 MHz, MeOD) δ: 0.82, 0.89, 0.92, 0.94, 0.99, 1.01, 1.16 (3H each, all s, H-23–H-27, 29 and H-30), 2.05 (3H, s, CH_3), 0.79-2.11 (24H, m, CH, CH_2 in pentacyclic skeleton), 2.17 (1H, d, *J* = 10.0 Hz, H-18), 3.23-3.30 (4H, m, H-1′, H- 2′), 4.47-4.50 (1H, m, H-3), 5.37 (1H, br s, H-12) ppm; MS (HRMS): m/z 583.4187 $[M+H]^+$ (calcd for $C_{35}H_{58}N_4O_3$, 582.451). **(3*β*)-*N*-(8-octylgyanidine)-3-acetyloxy-urs-12-en-28-amide hydrochloride (10)** White powder, 72% yield; m.p. 168–170°C (EtOH); $[\alpha]_D^{23}$ +27.8° (*c* 0.32, CH_2Cl_2); IR (film) ν_{max} 1650, 1734 (C=O), 3328 (NH); ^1H-NMR (500 MHz, MeOD) δ: 0.83, 0.90, 0.91, 0.98, 0.99, 1.01, 1.16 (3H each, all s, H-23–H-27, 29 and H-30), 0.82-1.99 (27H, m, CH, CH_2 in pentacyclic skeleton, 12H, H-2′, H- 3′, H-4′, H- 5′, H-6′, H- 7′), 2.03 (3H, s,

CH_3), 2.05 (1H, d, H-18), 3.13-3.20 (4H, m, H-1′, H- 4′), 4.47-4.49 (1H, m, H-3), 5.35 (1H, br s, H-12) ppm; MS (HRMS): m/z 667.5472 $[M+H]^+$ (calcd for $C_{41}H_{70}N_4O_3$, 666.545). **(2*α*, 3*β*)-N-(2-ethylgyanidine)-2, 3-acetyloxy-urs-12-en-28-amide hydrochloride (11)** White powder, 67% yield; m.p. 202–204°C (EtOH); $[\alpha]_D^{21}$ +3.7° (*c* 1.05, C_2H_5OH); IR (film) ν_{max} 1644, 1742 (C=O), 3338 (NH); ^{1}H-NMR (500 MHz, MeOD) *δ*: 0.82, 0.93, 0.95, 0.98, 0.99, 1.12, 1.17 (3H each, all s, H-23–H-27, 29 and H-30), 1.99 (3H, s, CH_3), 2.06 (3H, s, CH_3), 0.90-2.12 (21H, m, CH, CH_2 in pentacyclic skeleton), 2.19 (1H, d, *J* = 10 Hz, H-18), 3.26-3.39 (4H, m, H-1′. H-2′), 4.75 (1H, d, *J* = 10 Hz, H-3), 5.11-5.13 (1H, m, H-2), 5.38 (1H, br s, H-12) ppm; MS (HRMS): m/z 641.4478 $[M+H]^+$ (calcd for $C_{37}H_{60}N_4O_5$, 640.456). **(2*α*,3*β*)-N-(8-octyl-gyanidine)-2, 3-acetyloxy-urs-12-en-28-amide hydrochloride (12)** White powder, 69% yield; m.p. 152–154°C (EtOH); $[\alpha]_D^{21}$ +3.2° (*c* 0.56, C_2H_5OH); IR (film) ν_{max} 1650, 1742 (C=O), 3338 (NH); ^{1}H-NMR (500 MHz, MeOD) *δ*: 0.83, 0.92, 0.94, 0.95, 1.00, 1.11, 1.17 (3H each, all s, H-23–H-27, 29 and H-30), 0.91-1.86 (24H, m, CH, CH_2 in pentacyclic skeleton, 12H, H-2′, H- 3′, H-4′, H- 5′, H-6′, H- 7′), 1.99 (3H, s, CH_3), 2.06 (3H, s, CH_3), 2.18 (1H, d, *J* = 10 Hz, H-18), 3.12-3.20 (4H, m, H-1′, H-8′), 4.75 (1H, d, *J* = 10 Hz, H-3), 5.12 (1H m, H-2), 5.35 (1H, br s, H-12) ppm; MS (HRMS): m/z 725.5352 $[M+H]^+$ (calcd for $C_{43}H_{72}N_4O_5$, 724.550).

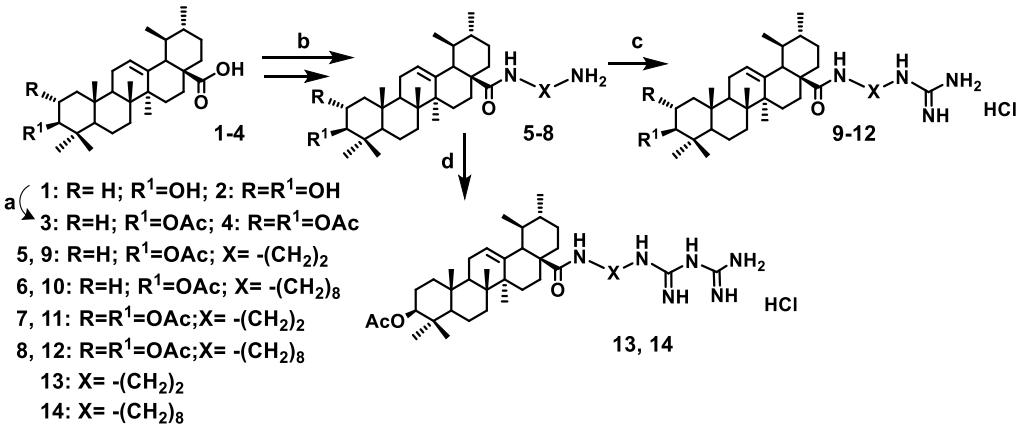
2.1.2. General Procedure for the Synthesis of Biguanides **13** and **14**

Method A. The corresponding amine (0.5 mmol) was dissolved in dry 1,4-dioxane (0.5 mL) and under vigorous stirring were added dicyandiamide (0.042 g, 0.5 mmol) and iron (III) chloride (0.08 g, 0.5 mmol). The mixture was stirred at 100 °C for 1.5 h. The mixture was then cooled down to room temperature, 0.13 *mL* of concentrated hydrochloric acid (12 M, 1.5 mmol) was added and the mixture was stirred for 5-10 min. The brown suspension became a yellow solution and a precipitate appeared. The precipitate which formed was filtered off and washed with ethyl acetate to give the pure products as a white powders **13** and **14** (41–56%).

Method B. N-Carbamimidoyl-1H-pyrazole-1-carboximidamide (0.1 g, 0.5 mmol) and the corresponding amine (0.55 mmol) were dissolved in pyridine (0.5 mL), and the solution was stirred at 40 °C for 24 h. The solution was cooled to r.t., to promote precipitation, pyridine was evaporated with toluene and triturated with diethyl ether to give a solid product, which was then chromatographed on silica gel, using CH_2Cl_2/MeOH 50:1→5:1, to give pure compounds **13** and **14** (47–54%). **(3β)-N-(2-ethylbiguanidine)-3-acetyloxy-urs-12-en-28-amide hydrochloride (13)** White powder, 54% yield; m.p. 180–182°C (EtOH); $[\alpha]_D^{21}$ +21° (*c* 0.41, CH_2Cl_2); IR (film) ν_{max} 1635, 1734(C=O), 3208 (NH), 3335 (C-N) cm^{-1}; ^{1}H-NMR (500 MHz, MeOD) *δ*: 0.82, 0.90, 0.93, 0.94, 0.99, 1.01, 1.16 (3H each, all s, H-23–H-27, H-29 and H-30), 0.87-2.10 (24H, m, CH, CH_2 in pentacyclic skeleton), 2.05 (3H, s, CH_3), 2.18 (1H, d, *J* = 10.5 Hz, H-18), 3.23-3.33 (4H, m, H-1′, H-2′), 4.50-4.46 (1H, m, H-3), 5.38 (1H, br s, H-12) ppm; MS (HRMS): m/z 625.4721 $[M+H]^+$ (calcd for $C_{36}H_{60}N_6O_3$, 624.473). **(3β)-N-(8-octylbiguanidine)-3-acetyloxy-urs-12-en-28-amide hydrochloride (14)** White powder, 56% yield; m.p. 148–150°C (EtOH); $[\alpha]_D^{21}$ +19.8° (*c* 0.48, CH_2Cl_2); IR (film) ν_{max} 1633, 1732(C=O), 3213 (NH), 3336 (C-N) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) *δ*: 0.73, 0.80, 0.85, 0.89, 0.92, 1.05, 1.24 (3H each, all s, H-23–H-27, H-29 and H-30), 0.78-1.93 (35H, m, CH, CH_2 in pentacyclic skeleton, H-2′-H-7′), 2.01 (3H, s, CH_3), 2.94-3.25 (4H, m, H-1′. H- 4′.), 4.45-4.48 (1H, m, H-3), 5.28 (1H, br s, H-12), 6.08, 6.65, 6.82, 7.44 (7H, m, NH) ppm; MS (HRMS): m/z 709.571 $[M+H]^+$ (calcd for $C_{42}H_{72}N_6O_3$, 708.567).

3. Results and Discussion

To synthesise the target conjugates, the hydroxyl functions in ursolic and corosolic acids **1**, **2** were acetylated. The resulting acetates **3** and **4** were converted to unstable acyl chlorides, which were reacted with linear diamines (1,2-diaminoethane, 1,8-diaminooctane) (Scheme 1).



Scheme 1. Synthesis of compounds **9–14**: (a) AcCl, THF, Py, DMAP, r.t.; (b) *1.* $(COCl)_2$, CH_2Cl_2; *2.* 1,2-diaminoethane or 1,8-diaminooctane, Et_3N, CH_2Cl_2, r.t.; (c) 1*H*-Pyrazole-1-carboxamidine hydrochloride, DIPEA, DMF, r.t., 24 h; (d) dicyandiamide, $FeCl_3$, 1,4-dioxane, 100 °C or *N*-amidinopyrazole-1-carboxamidine hydrochloride, Py, 40 °C.

The reaction was carried out with a 3-fold molar excess of amines relative to triterpene acetates to avoid the formation of dimeric products. Guanylation of the amines of triterpene acids **5–8** was performed using 1*H*-pyrazole-1-carboxamidine hydrochloride [14]. Different methods of biguanylation of various biologically active substances are known [15–17]. When choosing the reaction conditions, we tried to introduce the biguanidinium group using dicyandiamide in the presence of trimethylchlorosilane in acetonitrile or in the presence of iron (III) chloride in dioxane, and *N*-amidinopyrazole-1-carboxamidine hydrochloride in pyridine, but not all reagents were effective. Target compounds **13** and **14** were obtained in good yield by interaction of amines **7** and **8** with dicyandiamide in the presence of iron (III) chloride in 1.4-dioxane at 100 °C for 1.5 h or by interaction with *N*-amidinopyrazole-1-carboxamidine hydrochloride in pyridine at 40 °C for 24 h. Synthesis of *N*-amidinopyrazole-1-carboxamidine hydrochloride from pyrazole in the presence of diisopropylethylamine in different solvents (DMSO, DMF, EtOH) did not lead to the desired result [11,17]. *N*-amidinopyrazole-1-carboxamidine hydrochloride was obtained in high yield using dicyandiamide and hydrochloric acid in water [17].

The structures of all products were confirmed by 1D (^{1}H, ^{13}C) and 2D (HSQC, HMBC, COSY) NMR experiments and MS spectra.

4. Conclusions

We have synthesised new derivatives of ursolic and corosolic acids containing guanidinium and biguanidinium groups at the C-28 position via different linkers (ethylenediamine, diamino-octane). We hypothesise that the modification of ursolic and corosolic acids will increase their bioavailability and antimicrobial activity.

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