Synthesis and *in vitro* activity of oleanane type derivatives against *Chlamydia trachomatis*

Oxana B. Kazakova¹ ¹*, Liudmila V. Rubanik², Irina E. Smirnova¹, Olga V. Savinova², Anastasiya V. Petrova¹, Nikolay N. Poleschuk², Elmira F. Khusnutdinova¹, Eugene I. Boreko² and Yuliya M. Kapustina²

¹Ufa Institute of Chemistry of the Ufa Federal Research Centre of the Russian Academy of Sciences, Ufa, 450054, Russian Federation
²Republican Research and Practical Center for Epidemiology and Microbiology, Minsk, 220114, Belarus

(Received July 29, 2019; Revised August 20, 2019; Accepted August 25, 2019)

**Abstract:** Modified synthesis of 3β-nicotinoyloxy-olean-12(13)-ene-28-oic acid and 3-deoxy-3α-homo-3α-aza-28-hydroxy-olean-12(13)-ene from natural occurring oleanolic acid is suggested. These compounds and two others of ursane and lupane type triterpenoids (3-oximino-urs-12-en-28-oic acid and 3-deoxy-3α-homo-3α-aza-28-hydroxy-lup-12(13)-ene) were screened *in vitro* against *Chlamydia trachomatis* strain F-3271/Belarus/2015. Oleanane triterpenoids became the leading compounds with chemotherapeutic index > 8 and were chosen for further research.

**Keywords:** Synthesis; triterpenoids; oleanane; ursane; *Chlamydia trachomatis*. © 2019 ACG Publications. All rights reserved.

1. **Introduction**

Triterpenoids are distributed widely in higher plants and are of interest because of their different types of biological activities.¹ For example, betulinic acid had a great effect against dysplastic nevus,² betulin is currently in phase III clinical trials for the treatment of burns and for its impregnation into medicinal bandages.³ Triterpenoids also exhibit a wide spectrum of antimicrobial activity. Different types of pentacyclic triterpenoids have significant antistaphylococcal activity on at least one strain, and it is postulated that the primary target for their antimicrobial activity is the cell membrane which could also explain one of their other effects.⁴

*Chlamydia trachomatis* is a common sexually transmitted pathogen that can cause infertility.⁵ This infection is treated with broad-spectrum antibiotics which affect pathogens as well as the...

* Corresponding author: E-mail: obf@anrb.ru
normal endogenous microflora. With conventional antibiotic treatments, there is difficulty in achieving the complete eradication of chronic chlamydial infections, the possibility of developing drug resistance, and the unintended creation of other antibiotic-resistant pathogens. For this reason, it is important to consider new compounds exhibiting various means of antimicrobial activity and finding novel non-toxic compounds for the treatment of chlamydial infections remains an important goal.\(^6\)

2. Background

The properties of different terpenoids as inhibitors of the bacteria family Chlamydiaceae, particularly \textit{C. trachomatis}, are poorly studied. For diterpene lactone Andrographolide IC\(_{50}\) values were found to be 46 and 50 \(\mu\)M for two different strains of \textit{C. trachomatis}.\(^7\) Some betulin derivatives showed high (>70\% growth inhibition) antichlamydial activity against \textit{C. pneumoniae} at 1 \(\mu\)M.\(^8\) To the best of our knowledge, there is no data about the inhibition of \textit{Chlamydia trachomatis} by triterpenoids and their derivatives.

3. Experimental

\textit{General}: The spectra recorded at the Center for the Collective Use ‘Chemistry’ of the Ufa Institute of Chemistry of the UFRC RAS. \(^1\)H and \(^13\)C-NMR spectra were recorded on a “Bruker AM-500” (Bruker, Billerica, MA, USA, 500 and 125.5 MHz, \(\delta\), ppm, Hz) in CDCl\(_3\), internal standard tetramethylsilane. Mass spectra were obtained on a liquid chromatograph–mass spectrometer LCMS-2010 EV (Shimadzu, Kyoto, Japan). Melting points were detected on a micro table “Rapido PHMK05” (Nagema, Dresden, Germany). Optical rotations were measured on a polarimeter “Perkin-Elmer 241 MC” (PerkinElmer, Waltham, MA, USA) in a tube length of 1 dm. Elemental analysis was performed on a Euro EA-3000 CHNS analyzer (Eurovector, Milan, Italy); the main standard is acetylalide. Thin-layer chromatography analyses were performed on Sorbil plates (Sorpolimer, Krasnodar, Russian Federation), using the solvent system chloroform-ethyl acetate, 40:1. Substances were detected by a 10\% solution of a sulfuric acid solution with subsequent heating at 100–120 \(^\circ\)C for 2–3 min. Oleaneolic and nicotinic acids were purchased from Sigma – Aldrich.

\textit{Synthesis of 3\(\beta\)-nicotinoyloxy-olean-12(13)-en-28-oic acid I}: A mixture of oleanolic acid (229 mg, 0.5 mmol), N,N\'-dicyclohexylcarbodiimide (103 mg, 1 mmol), DMAP (cat.) and nicotinic acid (160 mg, 1.3 mmol) in CH\(_2\)Cl\(_2\) (15 mL) was stirred for 6 h at 5 \(^\circ\)C, then poured into 5\% HCl solution (50 mL) and the precipitate was filtered off, washed with H\(_2\)O. The product was isolated by crystallization from ethanol with yield of 95\% (267 mg) as beige powder. \(R_f\) 0.15. M.p. 221 \(^\circ\)C. \([\alpha]_D^{20} + 97\) (c 0.5, CHCl\(_3\)). \([\text{Lit.}^5]: M.p. 220-222 \(^\circ\)C, \([\alpha]_D^{20} + 98\) (c 0.5, CHCl\(_3\))). \(^1\)H NMR (\(\delta\), ppm, 500 MHz, CDCl\(_3\)): 9.20 (s, 1H, H-6\'), 8.75 (d, \(J = 4\) Hz, 1H, H-5\'), 8.30 (d, \(J = 10\), 1H, H-3\'), 7.39 (t, \(J = 5\) Hz, 1H, H-4\'), 5.30 (s, 1H, H-12), 4.75 (t, \(J = 3.8\) Hz, 1H, H-3), 2.85 (t, \(J = 3.8\) Hz, 1H, H-18), 2.00–1.20 (m, 22H), 1.15 (s, 3H), 1.05 (s, 3H), 1.00 (s, 3H), 0.98 (s, 3H), 0.90 (s, 3H), 0.88 (s, 3H), 0.80 (s, 3H). \(^13\)C NMR (\(\delta\), ppm, 125.5 MHz, CDCl\(_3\)): 183.6 (C-28), 164.9 (C-1'), 152.9 (C-6'), 150.6 (C-5'), 143.8 (C-13), 137.3 (C-3), 126.9 (C-2'), 123.4 (C-4'), 122.4 (C-12), 82.4 (C-3), 55.4, 54.8, 47.6, 46.5, 45.9, 41.6, 40.9, 39.3, 38.1, 37.0, 34.6, 33.8, 33.1, 32.6, 32.5, 30.7, 28.2, 27.7, 25.9, 23.6, 23.4, 22.9, 18.2, 17.2, 16.9, 15.4 Anal. calc. for C\textsubscript{36}H\textsubscript{51}NO<sub>7</sub>: C, 76.97; H, 9.15; N, 2.49. Found: C, 76.69; H, 9.13; N, 2.52. MS(APCI): \(m/z\) 562.78 [M + H]\(^+\) (calc. 561.81).

\textit{Synthesis of 3-deoxy-3a-homo-3a-aza-28-hydroxy-olean-12(13)-ene 3}. A mixture of compound 2\textsuperscript{10} (235 mg, 0.5 mmol) and LiAlH\(_4\) (19 mg, 0.5 mmol) in anhydrous THF was refluxed for 30 min and then poured into a 5\% HCl solution (70 mL). The crude product was extracted with CHCl\(_3\) (3x50), the organic layer was washed with H\(_2\)O, dried under CaCl\(_2\) and evaporated in \textit{vacuo}. The product was isolated by crystallization from ethanol with yield of 88\% (194 mg) \(R_f\) 0.11. M.p. 174 \(^\circ\)C. \([\alpha]_D^{20} + 2\) (c 0.5, CHCl\(_3\)). \([\text{Lit.}^6]: M.p. 175 \(^\circ\)C, \([\alpha]_D^{20} + 1.7\) (c 0.5, CHCl\(_3\))). \(^1\)H NMR (\(\delta\), ppm, 500
MHZ, CDCl3): 5.20 (s, 1H, H-12), 3.50 and 3.28 (both d, J = 10.8 Hz 2H, H-28), 3.00–2.92 (m, 2H, H-3), 2.05–1.52 (m, 25H), 1.50 (s, 3H), 1.41 (s, 3H), 1.20 (s, 3H), 1.15 (s, 3H), 1.00 (s, 3H), 0.88 (s, 3H), 0.85 (s, 3H). 1H NMR (δ, ppm, 125.5 MHz, CDCl3): 143.8 (C-13), 122.7 (C-12), 69.5 (C-28), 62.3 (C-3), 54.6 (C-5), 46.2, 44.2, 42.5, 42.4, 41.1, 40.9, 40.1, 39.6, 37.0, 34.1, 33.2, 32.3, 31.0, 30.9, 28.3, 25.4, 25.3, 24.3, 23.7, 23.6, 22.3, 22.1, 22.0, 17.2, 16.6. Anal. calc. for C38H35NO: C, 81.57; H, 11.64; N, 3.17. Found: C, 81.49; H, 11.51; N, 3.11. MS(APCI): m/z 442.64 [M + H]+, (calcd. 441.74).

Reagents and organisms: Strain C. trachomatis F-3271/Belarus/2015 specialized collection of viruses and bacteria pathogenic for humans [http://www.belriem.by/about//collection](http://www.belriem.by/about//collection) (Research Center for Epidemiology and Microbiology, Minsk, Belarus) were used. Sequencing of the ompA gene using primers P1/OMP2, NL-F/NL-R, CT6F/OMP2, P1/CT6R showed that this strain corresponds to the serovar F and has 100% homology with the sequence of the strain F/IC-CAL3 [https://www.ncbi.nlm.nih.gov/nuccore/DQ064287.1](https://www.ncbi.nlm.nih.gov/nuccore/DQ064287.1). The nucleotide sequence of the ompA gene of strain C. trachomatis F-3271/Belarus/2015 is deposited in the GenBank database [https://www.ncbi.nlm.nih.gov/nuccore/MG733343](https://www.ncbi.nlm.nih.gov/nuccore/MG733343).

Cell Culture: C. trachomatis were propagated in McCoy cells grown in DMEM with 10% heat-inactivated fetal bovine serum (HyClone, USA) supplemented with 2 mM L-glutamine, 10 μg/mL gentamicin, 5 mg/mL amphotericin B and 1 μg/mL cycloheximide (AppiChem, Germany). McCoy cells were cultured in glass flasks for 72 h. The cells were suspended after the addition of 0.02% ethylenediamine tetraacetate (Lonza, USA). 1 mL of the cell suspension, containing 10⁵ cells, was transferred to flat–bottomed plastic tubes containing a coverslip (13 mm in diameter). The cells were incubated at 37 °C for 24 h to obtain a confluent cell layer. Then 80-90% confluent McCoy cell monolayer was infected stock suspension of C. trachomatis at a multiplicity rate of 1:1. The plate was centrifuged for 1 h at 1500 x g (relative centrifugal force) to synchronize the infection. After centrifugation, the tubes were incubated for 2 h at 37 °C, and thereafter a wide range of concentrations of compounds 1, 3-5 additions to the culture medium was performed. Tested compounds were preliminarily dissolved in 10% ethanol. Each test was performed in triplicate. All experiments were supplemented with negative (intact McCoy cells) and positive controls (McCoy cells infected by strain C. trachomatis). As quantitative criteria of the observable antichlamydial action, a decrease in the titer C. trachomatis was calculated in comparison with the control, TCID₅₀ (Median Tissue Culture Infectious Dose) of the compounds and chemotherapeutic index (CTI) were determined.

Assessment of Infective Progeny: The infection rate in the McCoy cells and infective progeny formation were estimated 72 h after pathogen inoculation. Titters were determined by infecting cell monolayers with 10-fold dilutions of the thawed stock suspension. All monolayers were stained using FITC-conjugated monoclonal antibody against chlamydial lipopolysaccharide (NearMedic Plus, Russian Federation). Inclusion-containing cells were visualized using the Nikon Eclipse 50i microscope at x1000 magnification.

4. Present Study

The aim of this study was to suggest the most convenient synthesis of two oleanane triterpenoids 1 and 3 from natural occurring oleanic acid. It was shown that 3β-nicotinoxyloxyolean-12(13)-en-28-oic acid 1 inhibited the influenza type A H1N1 and the papillomavirus HPV-11⁹ and 3-deoxy-3a-homo-3a-aza-28-hydroxy-olean-12(13)-en 3 showed activity against M. tuberculosis.¹¹ Environmentally friendly and economical synthesis of drugs and biologically active agents is one of the important aspects for the pharmaceutical industry, which is achieved by reducing the number of stages, using non-toxic solvents, etc. For this purpose, we acylated oleanolic acid by nicotinic acid in CH₂Cl₂ using as a dehydrating agent DCC and a catalytic amount DMAP with the formation of acylate 1 with a yield of 95% after crystallization from
Synthesis and *in vitro* activity of oleanane type derivatives

ethanol (Scheme 1). Previously this compound was synthesized by a two-step way using preliminary obtained nicotinic acid chloride with the following reaction with oleanolic acid in pyridine.\(^9\) Thus, we reduced the number of stages from two to one, explored nontoxic reagents and increased the yield of target compound 1 by 10%. The synthesis of compound 3 was modified in comparison with the earlier described method\(^{11}\) by reducing of the number of stages (from 5 to 4) and, respectively, by increasing the total yield. We have excluded the stage of protecting the COOH-group of oleanolic acid by methylation as described in\(^{11}\) and used A-azepanono-oleanolic acid 2\(^{10}\) as a starting material to obtain azepane 3.

Scheme 1. Synthesis of oleanane type triterpenoid derivatives

The second aim of this research was to evaluate the potential of triterpenoid derivatives against *C. trachomatis*. We decided to take the compounds, which have already shown any anti-infective activity (antiviral\(^9\) for compound 1, antitubercular\(^{11}\) for compounds 3, 5 and antimalarial\(^{13}\) for compound 4) (Figure 1).

Figure 1. Synthetic derivatives of ursane 4 and lupane 5 type

Compounds 1, 3–5 were screened *in vitro* toward *Chlamydia trachomatis* strain F-3271/Belarus/2015. TCID\(_{50}\) assay method to determine the viability of Chlamydia under compounds action was used. The degree of the pathogen sensitivity to tested compounds was determined on the basis of the chemotherapeutic index (CTI) (Table 1). It was revealed that triterpenoids showed different potential. Compounds 1 and 3 demonstrated a high *Chlamydia* inhibitory activity (chemotherapeutic index was > 8). The range of active non-toxic concentrations
for compounds 1 and 3 is quite wide (the lower limit is not reached). The minimum active concentration of compound 4 that reduces the Chlamydia titer by at least 1.25 lg was less than 100 µg/mL. Azepanobetulin 5 has a chemotherapeutic index of 2 and was classified as low active.

5. Conclusion

As a result of this screening study oleanane type derivatives 1 and 3 were found to be promising for further research and we have developed the most convenient synthesis of these biologically active triterpenoids.

Table 1. An activity of compounds 1, 3-5 against Chlamydia trachomatis in McCoy cells test

| Compound | Concentration, µg/mL | Titer strain, lg TCID_{50} | Difference with positive control, lg TCID_{50} | Chemotherapeutic index (CTI) |
|----------|----------------------|----------------------------|-----------------------------------------------|-----------------------------|
| 1        | 800                  | <2                         | >3.8                                          |                             |
|          | 400                  | <2                         | >3.8                                          |                             |
|          | 200                  | <2                         | >3.8                                          |                             |
|          | 100                  | <2                         | >3.8                                          |                             |
|          | 50                   | <2                         | >3.8                                          | >8                          |
|          | 25                   | <2                         | >3.8                                          |                             |
|          | 12.5                 | <2                         | >3.8                                          |                             |
|          | 6.25                 | <2                         | >3.8                                          |                             |
| 3        | 800                  | <2                         | >3.8                                          |                             |
|          | 400                  | <2                         | >3.8                                          |                             |
|          | 200                  | <2                         | >3.8                                          |                             |
|          | 100                  | <2                         | >3.8                                          | >8                          |
|          | 50                   | <2                         | >3.8                                          |                             |
|          | 25                   | <2                         | >3.8                                          |                             |
|          | 12.5                 | <2                         | >3.8                                          |                             |
|          | 6.25                 | <2                         | >3.8                                          |                             |
| 4        | 800                  | <2                         | >3.8                                          |                             |
|          | 400                  | <2                         | >3.8                                          |                             |
|          | 200                  | <2                         | >3.8                                          |                             |
|          | 100                  | <2                         | >3.8                                          |                             |
|          | 50                   | 4.70±0.05                  | 1.1                                           | 8                           |
|          | 25                   | 5.60±0.08                  | 0.2                                           |                             |
|          | 12.5                 | 5.8±0.1                    | 0                                             |                             |
|          | 6.25                 | 5.8±0.1                    | 0                                             |                             |
| 5        | 800                  | <2                         | >3.8                                          |                             |
|          | 400                  | 3.6±0.2                    | 2.2                                           |                             |
|          | 200                  | 5.8±0.1                    | 0                                             |                             |
|          | 100                  | 5.8±0.1                    | 0                                             |                             |
|          | 50                   | 4.90±0.15                  | 0.9                                           | 2                            |
|          | 25                   | 5.8±0.1                    | 0                                             |                             |
|          | 12.5                 | 5.10±0.07                  | 0.7                                           |                             |
|          | 6.25                 | 5.8±0.1                    | 0                                             |                             |
| Positive control | Culture cells infected with strain C. trachomatis CT-3271/Belarus/2015 | 5.8±0.1 | – |
Synthesis and in vitro activity of oleanane type derivatives

Acknowledgements

This work was supported by the State task projects no. AAAA-A17-117011910023-2 and AAAA-A19-119020890014-7.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/organic-communications

ORCID

Oxana B. Kazakova 0000-0002-5606-1588
Liudmila V. Rubanik: 0000-0002-7963-0026
Irina E. Smirnova 0000-0001-7176-505X
Olga V. Savinova 0000-0002-1835-7724
Anastasiya V. Petrova 0000-0003-2910-6805
Nikolay N. Poleschuk 0000-0003-1083-6680
Elmira F. Khusnutdinova 0000-0001-6769-6063
Eugene I. Boreko 0000-0001-9158-298X
Yuliya M. Kapustsina 0000-0002-6400-7186

References

[1] Bouberte, M.Y.; Krohn, K.; Hussain, H.; Dongo, E.; Schulz, B.; Hu, Q. Tithoniumamarin and Tithoniumamide: A new isocoumarin dimer and a new ceramide from Tithonia diversifolia. Nat. Prod. Lett. 2006, 20, 842–849.
[2] Bouberte, M.Y.; Krohn, K.; Hussain, H.; Dongo, E.; Schulz, B.; Hu, Q. Tithoniaquinone a and Tithoniumamide B: A new anthraquinone and a new ceramide from the leaves of Tithonia diversifolia. Z. Naturforsch. 2006, 61B, 78–82.
[3] Barret, J.P.; Podmelle, F.; Lipovy, B.; Renneckampff, H.O.; Schumann, H.; Schwieger-Briel, A.; Zahn, T.R.; Metelmann, H.R. Accelerated re-epithelialization of partial-thickness skin wounds by a topical betulin gel: results of a randomized phase III clinical trials program. Burns. 2017, 43, 1284–1294.
[4] a) Cunha, W.R.; Matos, G.X.; Souza, M.G.; Tzatti, M.G.; Silva, M.L.A.; Martins, C.H.; Silva, R.; Da Silva Filho, A.A. Evaluation of the antibacterial activity of the methylene chloride extract of Miconia ligustroides, isolated triterpene acids, and ursoic acid derivatives. Pharm. Biol. 2009, 47, 166–169. b) Catteau, G.L.; Zhu, L.; Bambeke, F.V.; Quenin-Leclercq, J. Natural and semi-synthetic pentacyclic triterpenes as antimicrobials and resistance modifying agents against Staphylococcus aureus: a review. Phytochem. Rev. 2018, 17, 1129–1163.
[5] a) Tazooa, D.; Krohn, K.; Hussain, H.; Kouam, S.F.; Dongoa, E. Laportoside A and laportomide A: A new cerebroside and a new ceramide from Leaves of Laportea ovalifolia. Z. Naturforsch. 2007, 62B, 1208-1212. b) Antoine, K.Z.; Hussain, H.; Dongo, E.; Kouam, S.F.; Schulz, B.; Krohn, K. Cameroonnemide A: A new ceramide from Helichrysum cameroonense. J. Asian Nat. Prod. Res. 2010, 12, 629–633.
[6] Eyong, K.O.; Krohn, K.; Hussain, H.; Folefoc, G.N.; Nkengfack, A.E.; Schulz B.; Hu, Q. Newbouldiaquinone and Newbouldiamide: A new naphthoquinone-anthraquinone coupled pigment and a new ceramide from Newbouldia laevis. Chem. Pharm. Bull. 2005, 53, 616–619.
[7] Hua, Z.; Frohlich, K.M.; Zhang, Y.; Feng, X.; Zhang, J.; Shen, L. Andrographolide inhibits intracellular Chlamydia trachomatis multiplication and reduces secretion of proinflammatory mediators produced by human epithelial cells. FEMS Pat. Dis. 2015, 73, 1–11.
[8] Salin, O.; Alakurtti, S.; Pohjala, L.; Siiskonen, A.; Maass, V.; Maass, M.; Yli-Kauhaluoma, J.; Vuorela, P. Inhibitory effect of the natural product betulin and its derivatives against the intracellular bacterium Chlamydia pneumonia. Biochem. Pharmacol. 2010, 80, 1141–1151.
[9] Kazakova, O.B.; Medvedeva, N.I.; Baikova, I.P.; Tolstikov, G.A.; Lopatina, T.V.; Yunusov, M.S.; Zaprutko L. Synthesis of triterpenoid acylates – an effective reproduction inhibitors of influenza A (H1N1) and papilloma viruses. Russ. J. Bioorg. Chem. 2010, 36, 841–848.
Kazakova et al., Org. Commun. (2019) 12:3 169-175

[10] Finlay, H.J.; Honda, T.; Gribble, G.W.; Danielpour, D.; Benoit, N.E.; Suh, N.; Williams, C.; Sporn, M.B. Novel A-ring cleaved analogs of oleanolic and ursolic acids which affect growth regulation in NRP.152 prostate cells. Bioorg. Med. Chem. Lett. 1997, 7, 1769–1772.

[11] Medvedeva, N.I.; Kazakova, O.B.; Lopatina, T.V.; Smirnova, I.E.; Giniyatullina, G.V.; Baikova, I.P.; Kataev, V.E. Synthesis and antimycobacterial activity of triterpenic A-ring azepanes. Eur. J. Med. Chem. 2018, 143, 464–472.

[12] Jurstrand, M.; Falk, L.; Fredlund, H.; Lindberg, M.; Olcen, P.; Andersson, S.; Persson, K.; Albert, J.; Backman, A. Characterization of Chlamydia trachomatis omp1 genotypes among sexually transmitted disease patients in Sweden. J. Clin. Microbiol. 2001, 39, 3915–3919.

[13] Dalla-Vechia, L.; Dassonville-Klimpt, A.; Grellier, P.; Sonnet, P.; Gosmann, G.; Gnoatto, S.C.B. The Beckmann rearrangement applied to ursolic acid with antimalarial activity in medicinal chemistry studies. Lett. Org. Chem. 2012, 9, 92–95.