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Anticancer and antimicrobial activity of new C-28 guanidine-functionalized triterpenoic acid derivatives

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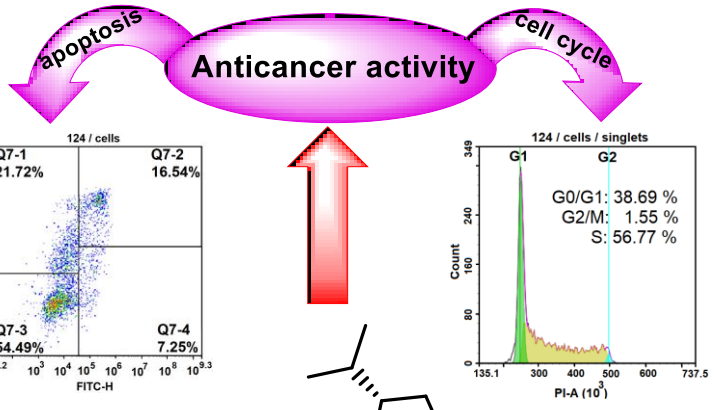
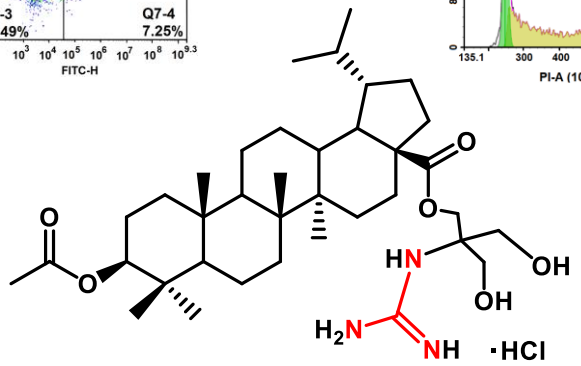
Russian Academy of Sciences

Anticancer and antimicrobial activity of new C-28 guanidine-functionalized triterpenoic acid derivatives

Graphical Abstract





Jurkat: IC_{50} = 3.1 μM

Fibroblasts: IC_{50} = 27 μM

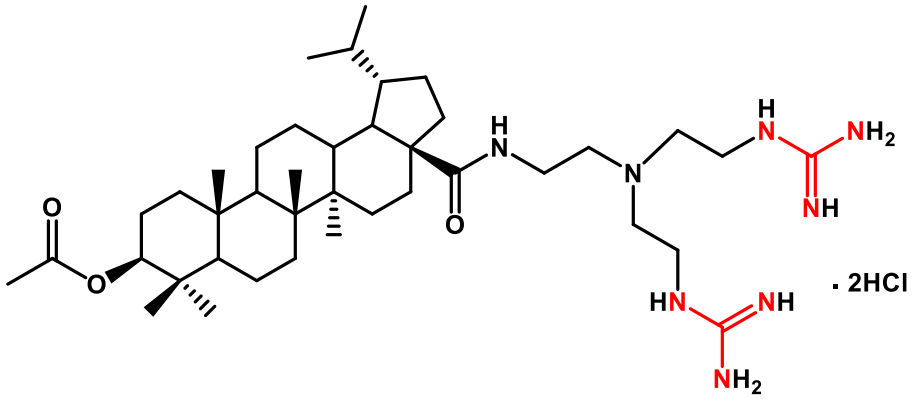
Antimicrobial activity

MIC (IC_{50}, μg/mL)

***Staphylococcus aureus*:** 0.5

***Candida albicans*:** 8.0

***Cryptococcus neoformans*:** < 0.25



Cytotoxicity (CC_{50}, μg/mL)

HEK 293: > 32

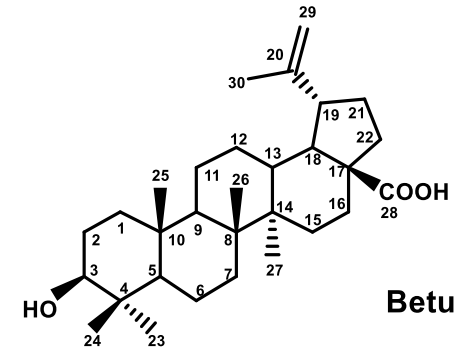
Abstract:

Novel betulinic, ursolic, and oleanolic acid derivatives, containing a guanidine moiety have been designed and synthesized in an attempt to develop potent antitumor, antibacterial and antifungal agents. Triterpenoic acids were converted into C-28-aminotriterpenoids in which polyamine moieties were bound with C-28 carboxylic group through an amide or ester bonds. These compounds served as precursors for the synthesis of novel guanidine-functionalized triterpenoic acids derivatives. The cytotoxicity was tested on five human tumor cell lines (Jurkat, K562, U937, HEK, and Hela) and compared with the tests on normal human fibroblasts. The antitumor activities of the most tested guanidine derivatives was lower than that of corresponding amines, but triterpenoids with the guanidine group were less toxic to human fibroblasts. The identified lead molecules with the highest antitumor characteristics were selected for extensive biological testing according to flow cytometry data, which showed that the antitumor activity of these compounds is caused by apoptotic processes and induction of cell cycle arrest in the S-phase. Most of the tested guanidine derivatives showed a good antibacterial effect against Gram-positive bacteria *Staphylococcus aureus* (MICs values 0.5-4.0 μg/mL) and expressed significant antifungal activity against *Candida albicans* (4.0 μg/mL) and *Cryptococcus neoformans* (0.25-4.0 μg/mL), higher than the standard fluconazole (8.0 μg/mL).

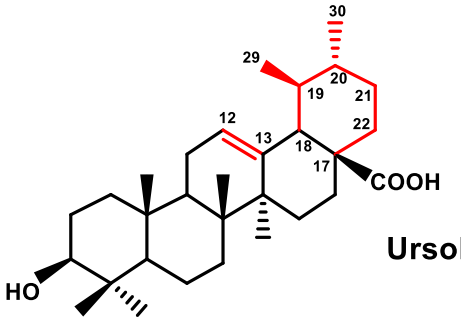
Keywords: triterpenoic acids, guanidine moiety, antitumor activity, antibacterial activity, antifungal activity

Introduction

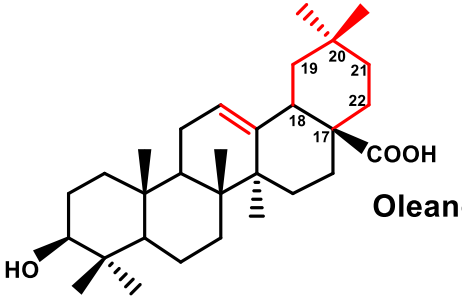
Triterpene acids (betulinic, ursolic, and oleanolic acids) are of interest for pharmacological research, as they exhibit a variety of biological activities including antimicrobial, antiparasitic, antitumor, and antiviral, in particular, anti-HIV, types of activity. Among these properties of triterpenoids, of special interest is their anticancer activity and the ability to trigger the mitochondrial apoptosis pathway in various types of human cancer cells. The useful pharmacological properties of triterpene acids are successfully combined with their acceptable systemic toxicity towards animals. However, the relatively low anticancer potential and high hydrophobicity, of these secondary metabolites markedly hamper their advancement as anticancer drug candidates.



Betulinic acid (BA)



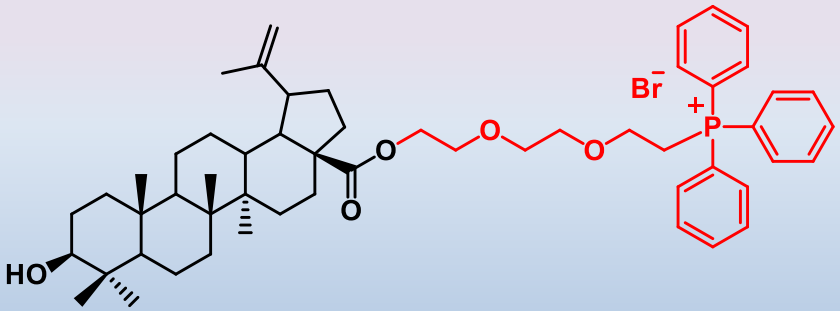
Ursolic acid (UA)



Oleanolic acid (OA)

Introduction

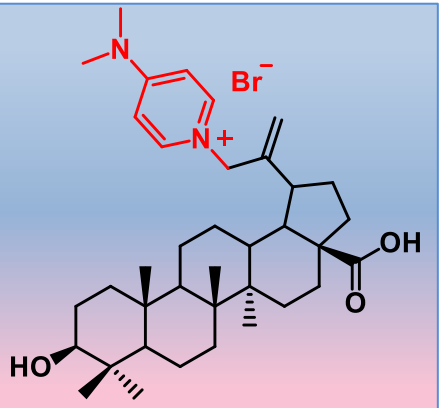
It has been shown that conversion of triterpene compounds to cationic derivatives such as quaternary ammonium, pyridinium or triphenylphosphonium salts may serve as an efficient approach to improving bioavailability and selectivity of their biological action.



Conjugation of betulinic acid with lypophilic triphenylphosphonium cation led to the dramatic enhancement of ability to trigger the mitochondrial apoptosis pathway in various types of cancer cells.

Nedopekina D.A., Gubaidullin R.R., Odinokov V.N., Maximchik P.V., Zhivotovsky B., Bel'skii Yu.P., Khazanov V.A., Manuylova A.V., Gogvadze V., Spivak A.Yu. *Med. Chem. Commun.*, **2017**, 8, 1934–1945

Dimethylaminopyridine derivatives of betulinic acid cause mitochondrial disruption and induce the permeability transition at cancer cells.



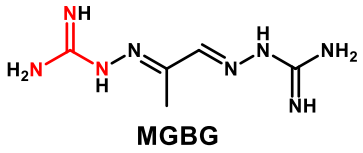
Bernardo T.C., Cunha-Oliveira T., Serafim T.L., Holy J., Krasutsky D., Kolomitsyna O., Krasutsky P., Moreno A.M., Oliveira P.J. *Bioorganic & Medicinal Chemistry*, **2013**, 21, 7239–7249

Introduction

We investigated antitumor and antimicrobial activities of novel cationic derivatives of ursolic, oleanolic and betulinic acids, containing guanidine groups whish are readily protonated at a physiological pH level.

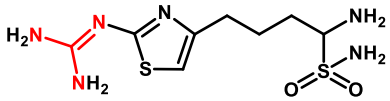
- The effect of introduction of the guanidine group into triterpenoid molecules has not been studied so far
- The introduction of hydrophilic guanidine groups into hydrophobic triterpene acid molecules may enhance their transmembrane transport and physicochemical characteristics
- The guanidine group is a common key unit in various natural and synthetic compounds demonstrating antimicrobial, antiviral, and antitumor activities
- Guanidine derivatives can be accumulated in the mitochondria of tumor cells, thus destroying the mitochondrial potential and inhibiting the mitochondrial respiratory chain

Examples of guanidines as fragments within drug molecules.

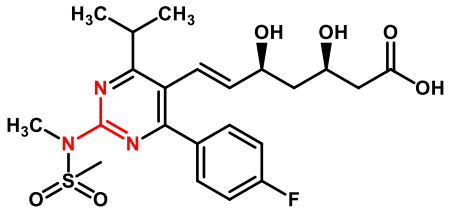


MGBG

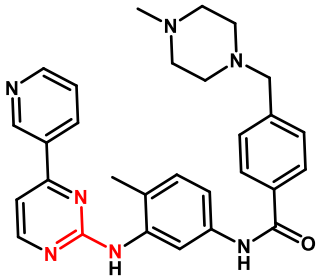
Metformin



Famotidine



Rosuvastatin

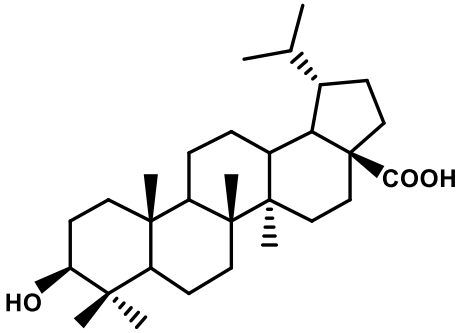


Imatinib

Bravo I., Alonso-Moreno C., I. Posadas, Albaladejo J., Carrillo-Hermosilla F., Ceña V., Garzón A., López-Solerae I., Romero-Castillo L. *RSC Advances*, **2016**, 10, 1–12

Results and discussion

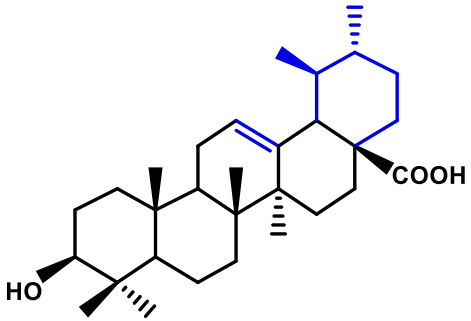
The cytotoxic activity of triterpene acids (dihydrobetulinic, ursolic and oleanolic acids), guanidinium salts, and some of their precursors, primary amines were tested *in vitro* on five human tumor cell lines: Jurkat (T-lymphoblastic leukemia), K562 (chronic myeloid leukemia), U937 (histiocytic lymphoma), HEK 293 (embryonic kidney), and HeLa (cervical cancer). The possible cell toxicity was assessed against normal human fibroblasts. Most of the tested compounds showed moderate or significant activity as compared to triterpenoic acids.



Dihydrobetulinic acid

Jurkat: IC_{50} = 59 μM

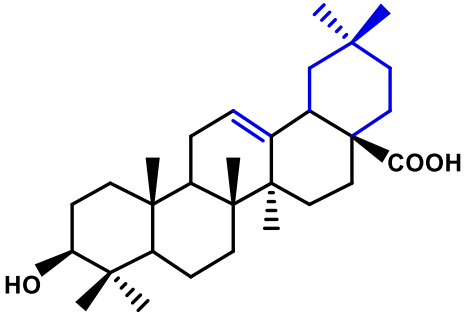
Fibroblasts: IC_{50} = 517 μM



Ursolic acid

Jurkat: IC_{50} = 23 μM

Fibroblasts: IC_{50} = 324 μM



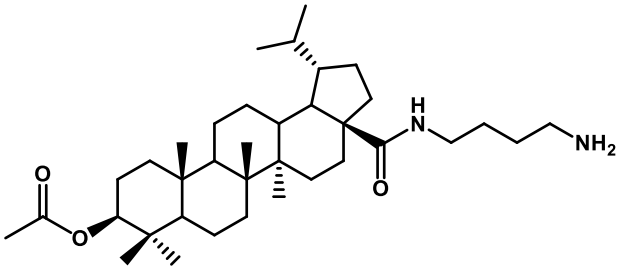
Oleanolic acid

Jurkat: IC_{50} = 271 μM

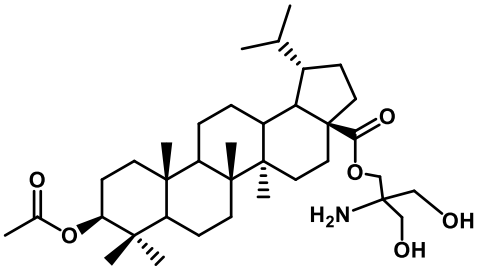
Fibroblasts: IC_{50} = 694 μM

Results and discussion

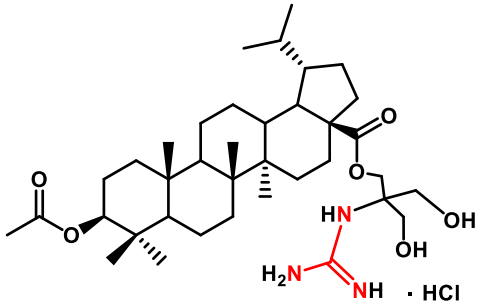
Anticancer activities of novel C-28 guanidine-functionalized triterpene acid derivatives



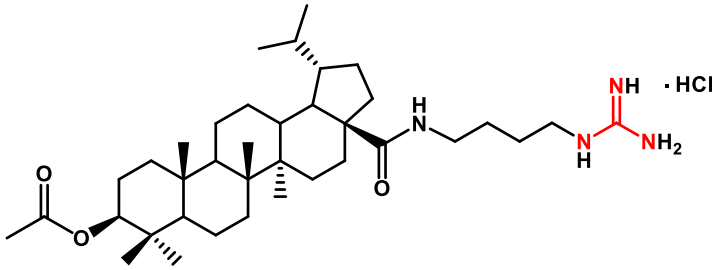
Jurkat: IC_{50} = 7.7 μM
Fibroblasts: IC_{50} = 8.3 μM



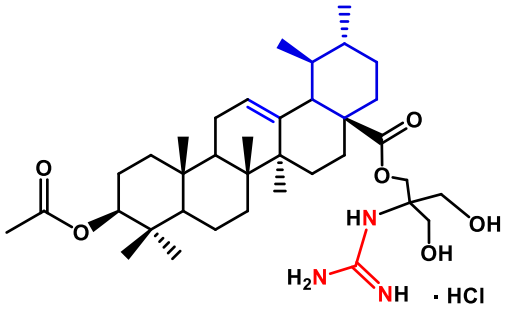
Jurkat: IC_{50} = 3.3 μM
Fibroblasts: IC_{50} = 31 μM



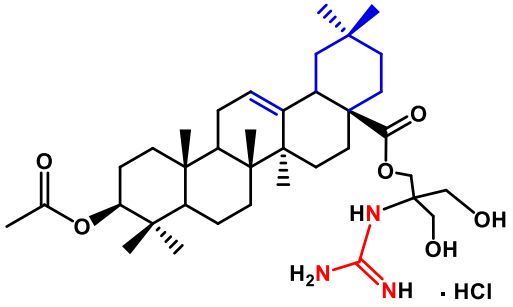
Jurkat: IC_{50} = 3.1 μM
Fibroblasts: IC_{50} = 27 μM



Jurkat: IC_{50} = 16 μM
Fibroblasts: IC_{50} = 117 μM



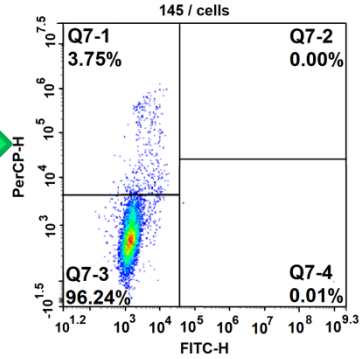
Jurkat: IC_{50} = 3.8 μM
Fibroblasts: IC_{50} = 51 μM



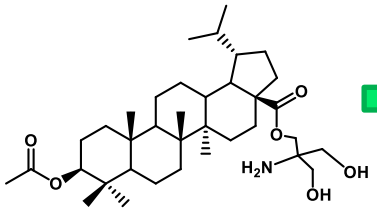
Jurkat: IC_{50} = 7.6 μM
Fibroblasts: IC_{50} = 54 μM

Results and discussion

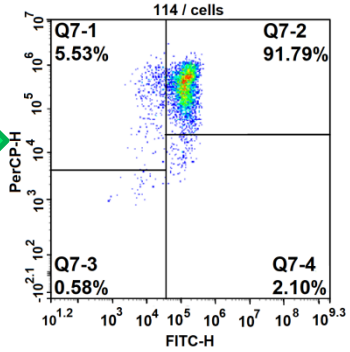
an untreated cell sample

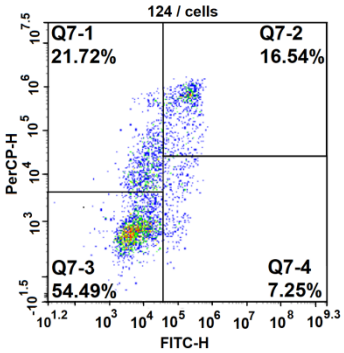


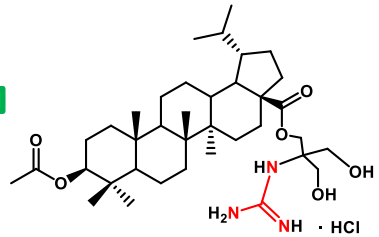
The identified lead compounds **15**, **15c**, **18c**, and **20c**, were evaluated for the possible apoptosis induction in Jurkat cells using Annexin V / 7-AAD staining. The highest percentage of late apoptosis (91.7%) was detected upon the addition of compound **15** (0.5 µM) to cells followed by 48 hour incubation. After treatment of Jurkat cells with compound **15c** apoptotic cells population was 23.7% (7.2% and 16.5% of early and late apoptotic cells, respectively). Comparable results were obtained with the guanidine derivative of ursolic acid **18c** (6.8% and 14.3% of early and late apoptotic cells, respectively). The apoptotic effect of the guanidine derivative of oleanolic acid **20c** was much weaker. After 48 hours, the normal cell population was 78.4% and the apoptotic cell population was 15.5 %



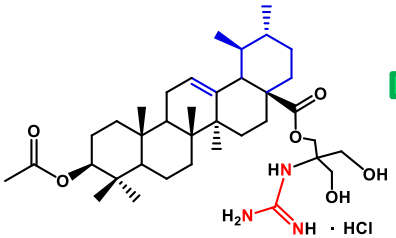
15



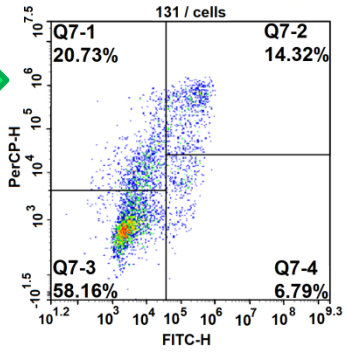


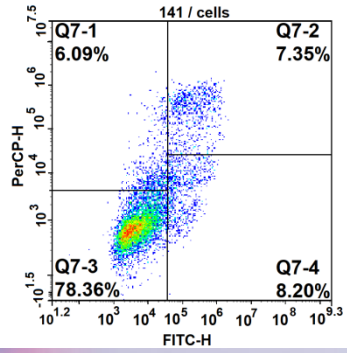


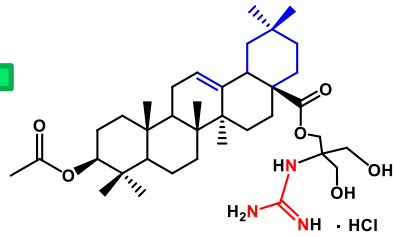
15c



18c



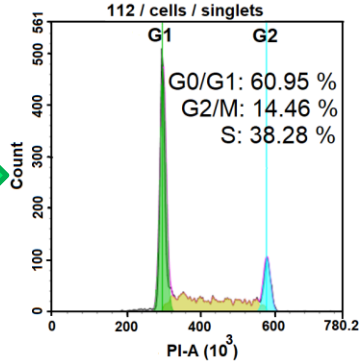




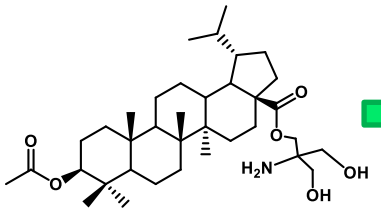
20c

Results and discussion

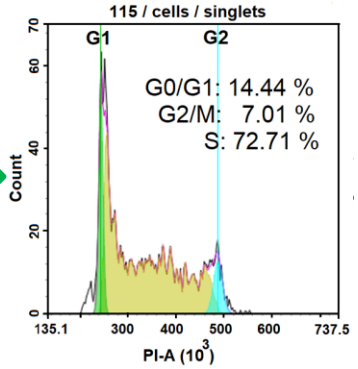
an untreated cell sample

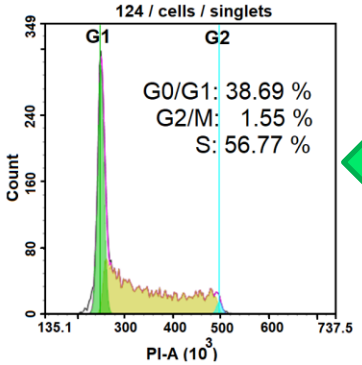


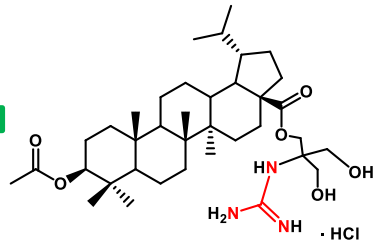
DNA flow cytometry was also used to analyze the cell cycle kinetics for Jurkat cells pre-incubated with compounds **15**, **15c**, **18c**, and **20c** (0.5 µM) for 48 hours. Simultaneously, the number of cells in the G0/G1 phase decreased, while the blockage of proliferation increased and the proliferation index decreased due to decreasing number of G2/M phase cells. These results may indicate that the cytotoxic activity of compounds **15** and **15c**, **18c** and **20c** against T-cell leukemia cells is due to the ability of these compounds to induce cell cycle arrest particularly in the S-phase.



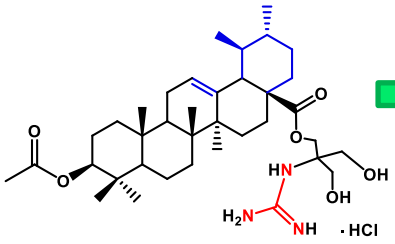
15



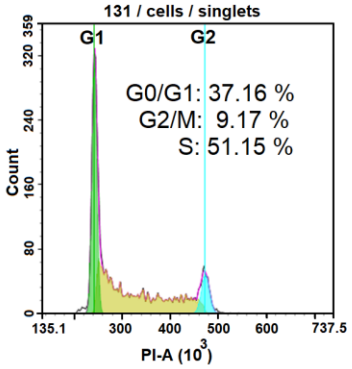


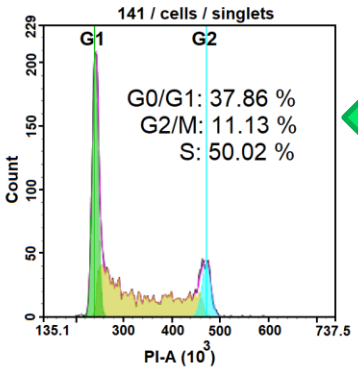


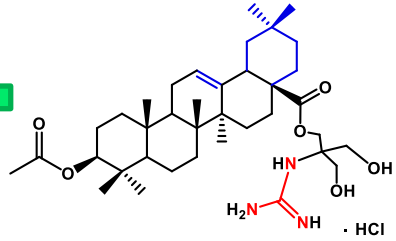
15c



18c



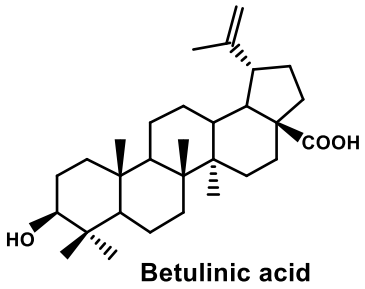




20c

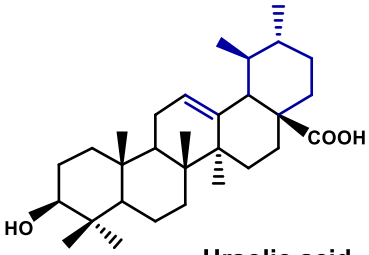
Results and discussion

Antibacterial activities of novel C-28 guanidine-functionalized triterpene acid derivatives



Betulinic acid

***S. aureus*: MIC > 256 µg/mL**

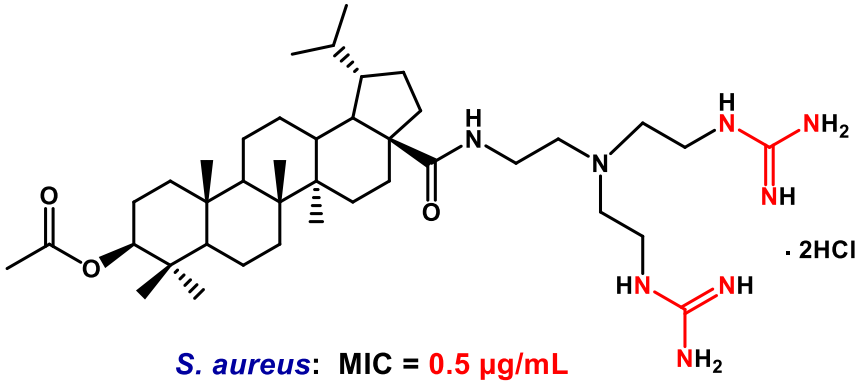


Ursolic acid

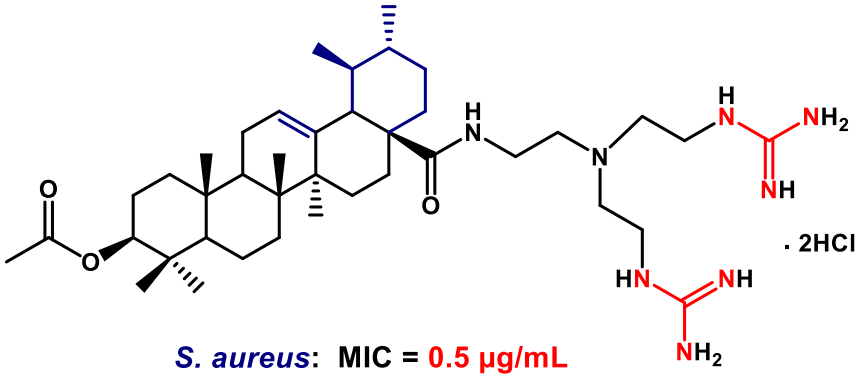
***S. aureus*: MIC = 8 µg/mL**

S. Fontanay et al. *Journal of Ethnopharmacology*, **2008**, 120, 272-276

In addition to the anticancer activities C-28 amine- and guanidine- functionalized triterpene acid derivatives showed antimicrobial effect. Our compounds were screened on cultures of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, as well as two fungi, *Candida albicans* and *Cryptococcus neoformans*. Guanidine-functionalized betulinic acid and ursolic acid derivatives demonstrated antibacterial activity, showing MIC in the range of 0.5 – 4.0 µg/mL against gram-positive bacteria *S. aureus.*



***S. aureus*: MIC = 0.5 µg/mL**



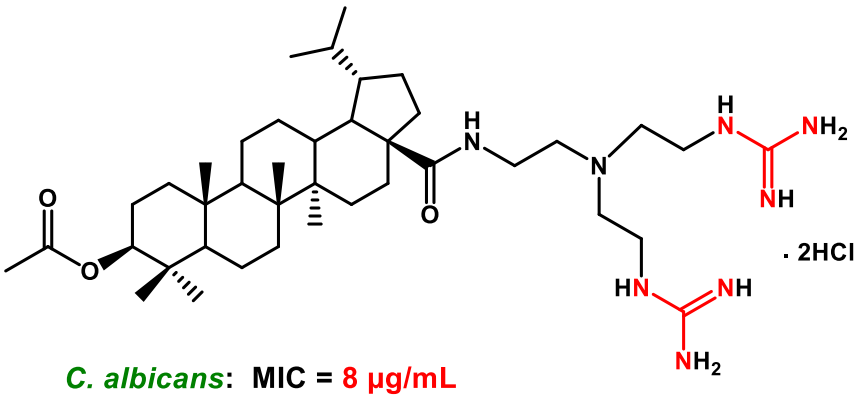
***S. aureus*: MIC = 0.5 µg/mL**

Antibiotic standard – **Vancomycin · HCl**: MIC = 1 µg/mL

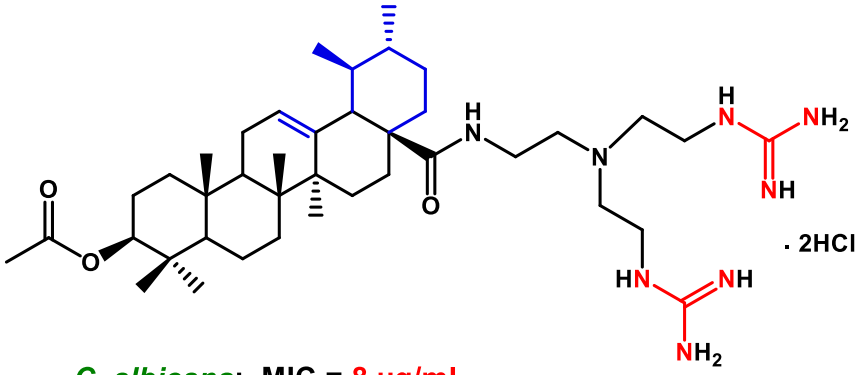
Results and discussion

Antifungal activities of novel C-28 guanidine-functionalized triterpene acid derivatives

These compounds displayed the excellent antifungal activity against *C. albicans* (MIC = 8 μg/mL) and *C. neoformans* with MIC values of 0.25 – 4.0 μg/mL.



***C. albicans*: MIC = 8 μg/mL**
***C. neoformans*: MIC < 0.25 μg/mL**
***CC_{50} > 32 μg/mL**



***C. albicans*: MIC = 8 μg/mL**
***C. neoformans*: MIC < 0.25 μg/mL**
***CC_{50} = 18.39 μg/mL**

Antifungal standards:
Fluconazole: *C. albicans* MIC = 0.125 μg/mL
C. neoformans MIC = 8 μg/mL

*CC_{50} cytotoxicity against a human embryonic kidney cell line, HEK293

Conclusions

- Here we describe the synthesis, cytotoxicity and apoptosis-inducing activities of novel pentacyclic lupane, ursane, and oleanane type triterpenoid derivatives containing guanidine groups. The introduction of hydrophilic guanidine groups into hydrophobic triterpene acid molecules may enhance their transmembrane transport and physicochemical characteristics
- The antitumor activities of the most tested guanidine-containing triterpene acids was lower, than that of corresponding amines, but triterpenoids with the guanidine moiety were less toxic to human fibroblasts
- The mechanism of the antitumor action of the more active compounds was investigated by using flow cytometry analysis, which revealed that compounds can induce cell apoptosis and cell cycle arrest in the S-phase in Jurkat cells
- Because guanidine-derivatives were the most active among the tested compounds, the guanidinyl substituent C-28 appears to be important for the antibacterial and antifungal activity of these compounds
- **The guanidine chain might be a pharmacophore involved in the antitumor and antimicrobial activities of these series of compounds**

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