# CHOLANE AND LANOSTANE DERIVATIVES: ANTIMICROBIAL EVALUATION

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## INTRODUCTION

Bile acids such as lithocholic acid (LCA) and ursodeoxycholic acid (UDCA) (Scheme 1) have been considered quite useful as starting points for a rich and different set of medicinal chemistry activities.[1] The literature describes a large amount of pharmacological applications for bile acids derivatives, in particular antimicrobial, antifungal and antitumor. Besides, the discovery of bioactive ingredients from plants and fungi is always the main target in medicinal chemistry. The lanostane-type triterpenoid 3β-hydroxylanosta-8,24-diene-21-oic acid (Trametenolic acid, TMA) (Scheme 2) was the main bioactive component of *Gloeophyllum odoratum*[2], which was reported to possess widely bioactivities, including tumor cell anti-proliferation effects (for example, human HL-60 leukemia, human KB epidermoid carcinoma, murine L1210 leukemia cells, Caski, HT-3, T-24, etc.), inhibition of enzyme activity (human thrombin, bovine trypsin and so on). Nevertheless, trametenolic acid was scarcely investigated as antimicrobial agent.

A new series of lithocholic, ursodeoxycholic and lanostane acid derivatives were synthesized through modification at the oxygenated carbon C-3 and/or C-7 and at the carboxyl carbon C-24 or C-21.

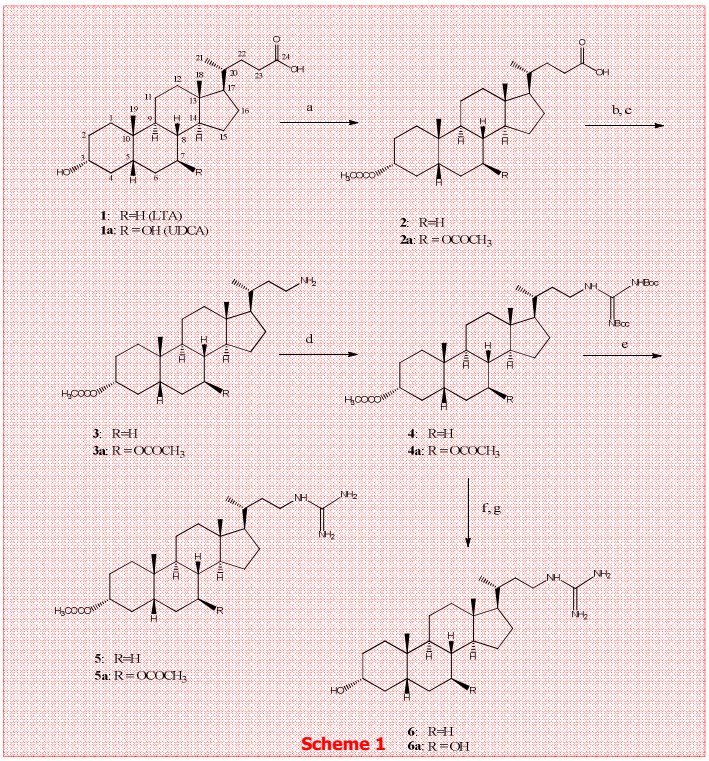
1. R. Sharma, A. Long, J. Gilmer, *Current Med. Chem.* 2011, *18*, 4029-4052.
2. F. Cateni, V. Lucchini, M. Anderluh, P. Martinuzzi, M. Zacchigna, A. Piltaver, B. Doljak, *Letters in Drug Design & Discovery* 2010, *7*, 521-527.

## RESULTS

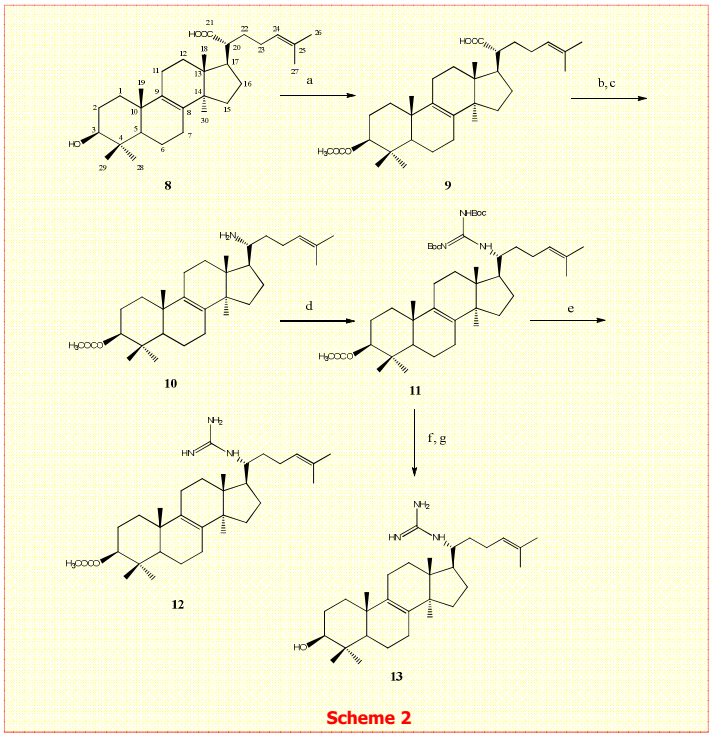
The aim of the present study is to obtain a new series of derivatives bearing a guanidine moiety in their side chain through modification at the carboxyl carbon C-24 or C-21 and an acetoxy group at the oxygenated carbon C-3 and/or C-7.

The activity of bile acids, trametenolic acid and derivatives was evaluated against the growth of *S. aureus*, *B. subtilis* and *M. smegmatis*. The derivative 3α-hydroxy-23-guanidino-5β-cholane (**6**) showed the best activity, with MIC values of 12.5 μM against *S. aureus*, 5 μM against *B. subtilis* and 50 μM against *M. smegmatis*. The cytotoxic activity of bile acids, trametenolic acid and derivatives was also evaluated against HT-29 cell line.

## SYNTHESIS OF CHOLANE AND LANOSTANE DERIVATIVES




Scheme 1




Scheme 2

**Scheme 1**: Reagents and conditions: (a) $Ac_2O$/pyridine, DMAP, rt, 24 h, 96% (**2**), 78% (**2a**); (b) $NEt_3$, DPPA, Toluene, 150° C, reflux, 3h; (c) NaOTMS/THF 1M, 0° C, 1h, 64% (**3**), 66% (**3a**); (d) $NEt_3$, $AgNO_3/CH_2Cl_2$, N,N'-Di-Boc-S-methylisothiourea, 40% (**4**), 41% (**4a**); (e) $TFA/CH_2Cl_2$, 86% (**5**), 89% (**5a**); (f) NaOH/MeOH, 150° C, 1h; (g) $TFA/CH_2Cl_2$, 82% (**6**), 76% (**6a**)

**Scheme 2**:Reagents and conditions: (a) $Ac_2O$/pyridine, DMAP, rt, 24 h, 87%; (b) $NEt_3$, DPPA, Toluene, 150ºC, reflux, 3h; (c) NaOTMS/THF 1M, 0° C, 1h, 67%; (d) $NEt_3$, $AgNO_3/CH_2Cl_2$, N,N'-Di-Boc-S-methylisothiourea, 44%; (e) $TFA/CH_2Cl_2$, 87%; (f) NaOH/MeOH, 150° C, 1h; (g) $TFA/CH_2Cl_2$, 87%

## IN VITRO STUDY

**Table 1**: Values of the minimal inhibitory concentration (MIC) of the synthesized compounds. Each value is the highest of three replicates.

| Compounds | Bacterial strains MIC [μM] | | | Bacterial strains MIC [μg·mL] | | |
|---|---|---|---|---|---|---|
| | *S. aureus* ATCC 6538P | *Bacillus subtilis* PY79 | *Mycobacterium smegmatis* mc² 155 | *S. aureus* ATCC 6538P | *Bacillus subtilis* PY79 | *Mycobacterium smegmatis* mc² 155 |
| 1 | > 100 | 25 | > 150 | > 37 | 9.5 | > 55.5 |
| 1a | > 100 | 100 | > 150 | > 39 | 39 | > 59 |
| 5 | 25 | 10 | 150 | 10 | 4.5 | 64.5 |
| 5a | 20 | 10 | 50 | 9 | 4.5 | 22.5 |
| 6 | 12.5 | 5 | 50 | 5 | 2 | 20 |
| 6a | 10 | 10 | 50 | 4 | 4 | 20 |
| 8 | > 100 | > 100 | > 150 | > 48 | > 48 | > 72 |
| 12 | n.d. | > 100 | n.d. | n.d. | > 48 | n.d. |
| 13 | > 100 | 50 | > 150 | > 46 | 46 | > 76 |

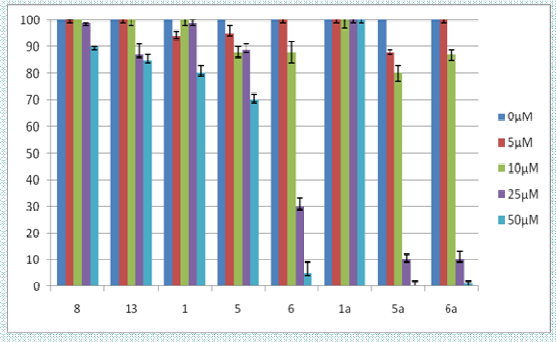



**Figure 1**: MTT assay. Effect of the different compounds on HT29 cells survival after 24 h of treatment, at different concentrations from 0 to 50 μM. Cell cytotoxicity determined by MTT assay is expressed as percentage of cell viability and indicated as mean ± SD (n=3), P<0.05. ANOVA test.