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In vitro cytotoxic evaluation of new indolo-triterpene derivatives, synthesized from serjanic acid

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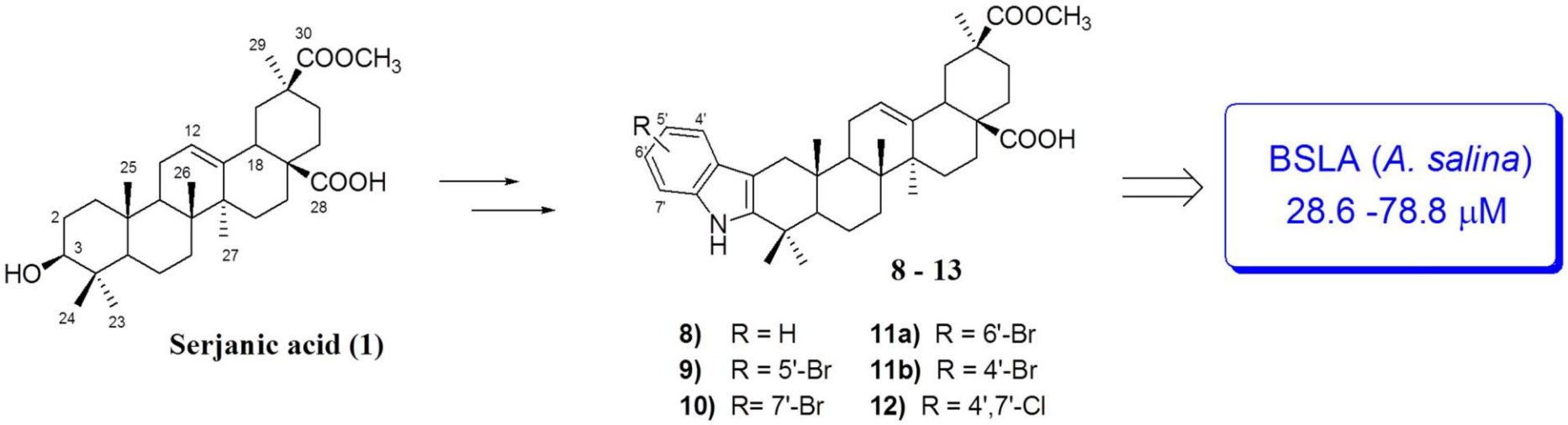
Graphical Abstract



Abstract

In this study we carried out the synthesis and structural characterization of six new indolotriterpene derivatives from the natural triterpenoid serjanic acid (**1**). The compounds were synthesized via indolization reactions between ketone **2** and phenylhidrazines **3**–**7**and elucidated by IR, ^{1}H-NMR, ^{13}C-NMR and HR-MS techniques as: Indolo[2,3-b]olean-12-en-28,30 dioicacid-30-methyl ester (**8**), 5-bromoindolo[2,3-b]olean-12-en-28-dioic acid-30-methyl ester (**9**), 7-bromoindolo [2,3-b]olean-12-en-28-dioic acid-30-methyl ester (**10**), 4-bromo-indolo[2,3-b] olean- 12-en-28-dioic acid-30-methyl ester (**11a**), 6-bromo-indolo[2,3-b]olean-12-en-28-dioic acid-30-methyl ester (**11b**) and 4,7-dichloro-indolo[2,3-b]olean-12-en-28-dioic acid -30-methyl ester (**12**).

All compounds were tested through the brine shrimp lethality assay to determinate their potential as cytotoxic agents. LC_{50} values of the new synthesized compounds were between 28.6 and 78.8 µM.

Keywords: Indolotriterpene, serjanic acid, $IC_{50,}$ Brine Shrimp Lethalithy Assay.

Introduction

Natural sources have been used for thousands of years by living beings to fight various diseases, this thanks to the organic compounds in them. One of the most important groups of natural compounds from a biological point of view is that of triterpenoids, within which are the triterpenes. Pentacyclic triterpenes represent a promising expansion and biologically active natural compounds platform, whose potential has begun to be exploited by the pharmaceutical industry.

The study of these metabolites has been focused primarily on assessing their potential as anticancer and antiviral drugs, and in many cases the increased activity is closely linked to the modification of the hydroxyl group or carboxylate, located in the position C-3 and C-28. Currently, most of the work concerning the modification of these compounds involves the insertion of heterocycles, either through links or by fusion in one of its rings.

Serjanic acid (**1**) is a natural pentacyclic triterpene found in the fruits of the species *Phytolacca icosandra* (Phytolaccaceae). Despite the structural similarities that this triterpene shares with other bioactive triterpenes such as oleanolic, boswellic and moronic acid, there are very few studies concerning the preparation of semi-synthetic derivatives.

Results and discussion

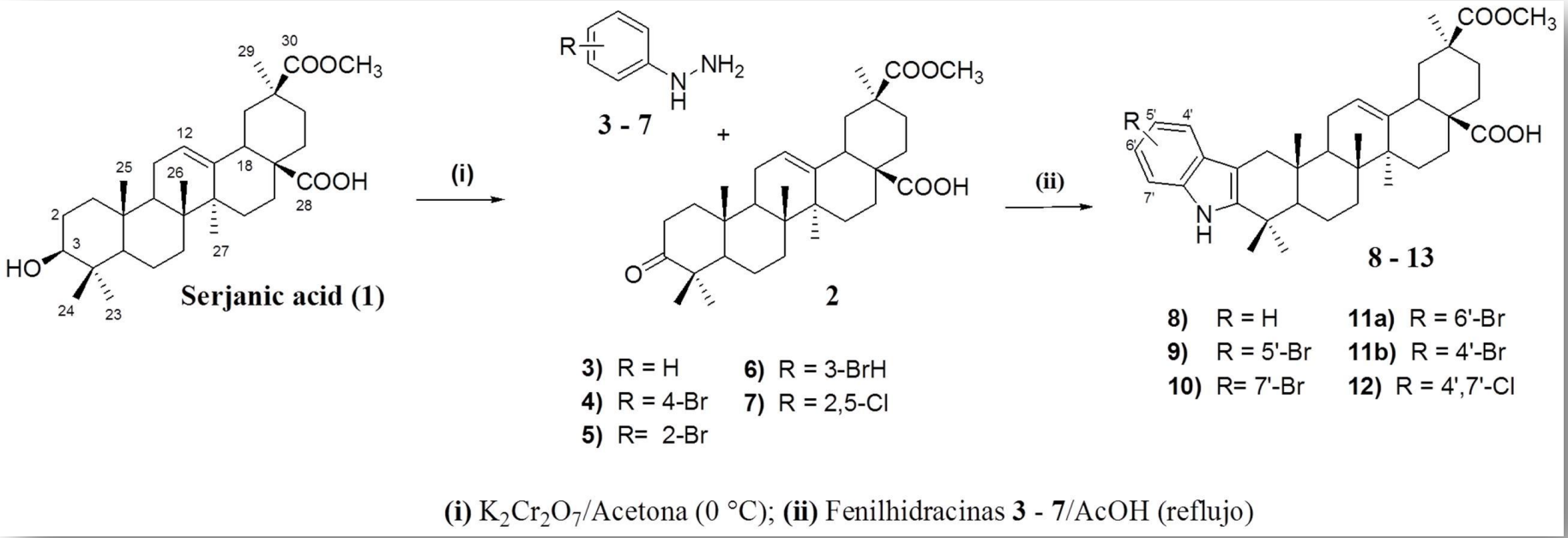
Synthesis of indolo-triterpenes 8-12

Compounds **8** – **12** were obtained by dissolving 0,12 mmol of 3-oxo-serjanic acid (previously obtained by a Jone's oxidation of serjanic acid) in glacial acetic acid (2 mL). A solution of hydrazines **3** – **7** (0,13 mmol) in glacial AcOH was added dropwise and the mixture was stirred and refluxed for 2-4 hours. The reaction was followed by TLC until completion of reaction.

Table 1. Experimental data related to the synthesized compounds **8** - **12**

Comp.	M.p. (°C)	M.F.	$M.W_{calc.}$	$HR\text{-}MS_{exp.}$	% Yield - time
8	224 - 226	$C_{37}H_{49}NO_4$	571,3662	571,3932	74,3% - 2h
9	215 - 217	$C_{37}H_{48}BrNO_4$	649,2767	649,2666	71,7% - 2h
10	189 - 191	$C_{37}H_{48}BrNO_4$	649,2767	649,2917	64,2% - 2h
11a + 11b	-	$C_{37}H_{48}BrNO_4$	649,2767	649,2251	75,5% - 3h
12	136 - 138	$C_{37}H_{47}Cl_2NO_4$	639,2882	639,2730	63,1% - 4h

Once cooled, the mixture was added 40 mL of cold distilled water and extracted with CH_2Cl_2 (10 mL x 3), the organic phase was then washed with 5% solutions of NaOH (20 mL) and NaCl (20 mL x2), dried with $MgSO_4$ and the solvent removed in a rota-vapor to obtain a reaction crude. Finally, the compounds were purified using preparative TLC on silica gel (Merck 15-40 μm, PF_{254}) and eluted with a mixture of Hexane/EtOAc (2:3 or 1:1 depending on the compound) **(Scheme 1)**. The reaction yields are summarized in **Table 1**.



Scheme 1. Reagents and conditions: **(i)** K2Cr2O7/H2SO4, acetone 0°C reflux (2h); **(ii)** AcOH/hydracines **3**-**7**, reflux (4h)

In-vitro cytotoxicity assay

All compounds were tested for their toxicity properties through the Brine Shrimp Lethality Assay (BSLA) to determinate their potential as cytotoxic agents. These results are summarized in **Table 2**.

Compound	IC_{50} (µM)	Compound	IC_{50} (µM)
1	85.3 ± 14	**10**	78.8 ± 7
2	159.3 ± 12	**11**	29.3 ± 4
8	54.1 ± 4	**12**	49.4 ± 6
9	28.6 ± 4		

Table 2. Inhibitory concentration at 50% values in µM, of compounds **1**, **2**, **8** – **12**.

As we observed the IC_{50} values for compounds **1** and **2**, it can be seen the importance of the hydroxyl group in the toxicity of the oleanane triterpenes, evidenced by the increase of the IC_{50} value of compound **2** compared to compound **1**.

We can also notice from **Table 2**, a decrease in the IC_{50} values of compounds **8** – **12** in comparison with 1 and 2, which could mean that the inclusion of the indole ring in positions C-2 and C-3 of the triterpene increased the cytotoxic potential of the molecule. The reasons for this increase are not totally clear, however, this fact is consistent with the reported data in the literature not only for indole rings but also for heterocyclic rings fused with triterpenes at positions C-2/C-3.

Also it can be observed for compounds **8**-**12** that the presence of halogen atoms within the indole ring increases the toxicity of the compounds, since the IC_{50} values of almost all halogenated derivatives (except for **10**) were better than compound **8**. The fact that compound 10 doesn't follow this behavior leads us to think that the toxicity is only due to the presence of halogens in the indole ring but there might be other factors such as the electronic or spatial effect that these atoms have in the molecule.

Out ff all synthesized indolo-triterpenes, compound **9** was the most effective, showing a toxicity three times higher than the starting compound. In a general manner , the values of preliminary toxicity are good, this means that the compounds could be submitted to more specific in vitro studies.

Materials & Methods:

General procedure for the isolation of Serjanic acid (1).

A fraction of the dry MeOH extract of the fruits of *Phytolacca icosandra* (34.2 g) was dissolved in a mixture of MeOH/H_2O (1:1) and hydrolyzed with HCl (32 mL) until pH = 2. The mixture was then refluxed for 6h and the precipitate was filtered and dried (23.46 g). This residue was submitted to column chromatography (CC) using mixtures of $CHCl_3$/Acetone as eluent. Compound **1** was obtained from fractions “C” and “D” of the chromatography as a white solid (4.71 g) (288 - 291 °C).

General procedure for the isolation of 3-oxo-serjanic acid (2).

Compound **2** was obtained adding little portions of Jone’s reagent ($K_2Cr_2O_7/H_2SO_4$) to a solution of **1** (1,3 mmol) dissolved in acetone at 0°C. The presence of a blue precipitate and persistence of orange color in the mixture, indicated the end of the reaction. The solution was filtered and the solvent concentrated to obtain the 3-oxo-serjanic acid (**2**) as a withe solid (276 – 278 °C).

Brine Shrimp Lethality Assay (BSLA)

The bioassay was performed in the similar way described in reference (Meyer et al., 1982) with some minor modifications. Brine shrimp eggs (Gulf Breeze) were hatched in artificial sea water prepared with commercial salt mixture (Instant Ocean ®) and oxygenated with an aquarium pump. After 48h incubation at 28 °C, nauplii were collected with pasteur pipette after attracting the organisms to one side of vessel with a light source. Ten shrimps were transferred to each sample vial, and artificial sea water was added to make 2.5 mL. Samples for testing were made up to 1, 25 and 100 mg/mL in artificial sea water (2.5 mL) which were dissolved in 50 µL DMSO prior to adding sea water. Sample solutions (2.5 mL) were added to each test vial (finally, total 5 mL). The vials were maintained under illumination.

Survivors were counted after 2 and 24 h, and the percent deaths at each dose and control were determined. DMSO in this concentration did not affect this bioassay. The LC_{50} values were determined from 24 h counts using the probit analysis. The results are summarized in **Table 2**.

Conclusions

- With this study, it has been has once again demonstrated the chemical relevance of pentacyclic triterpenoids of the oleanane series as biologically and/or pharmacologically active compounds.

- An efficient route for the synthesis of new indolo-triterpenes (**8**-**12**) from serjanic acid (**1**) was designed, and the compounds were obtained in good yields (63-76%).

- The cytotoxic potential of all compounds was measured by an in vitro assays against *Artemia salina*. The results of this test indicate that the derivatives **8** - **12** exhibited significant cytotoxic effect on the crustacean, with IC_{50} values between 28.6 and 78.8 uM.

- The indole ring fused to the C-2 and C-3 position and the presence of bulky atoms within the ring are factors likely to increase the potential toxicity of these compounds, however more specific tests are needed to corroborate this, including greater number and structural diversity of derivatives

Acknowledgments