



Faculty of Pharmacy  
Ain Shams University

# **Computer Aided Drug Design and Synthesis of Some Triterpenoidal Carboxylic Acid Derivatives with Potential Biological Activity**

Thesis presented by:

**Mohamed Osman Ahmed**

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Under the supervision of

**Mohamed Abdel Hamid Ismail**

Professor of pharmaceutical chemistry  
Ain Shams University

**Atef Gobran Hanna**

Professor of natural products Chemistry,  
National Research Centre

**Nasser Saad Mohamed**

Lecturer of pharmaceutical Chemistry, Ain Shams  
University

Faculty of pharmacy  
Ain Shams University

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## List of abbreviations

**2D**: 2-Dimensional.  
**3D**: 3-Dimensional.  
**Å**: Angstroms.  
**Ala**: Alanine.  
**Asp**: Aspartic acid.  
**CHARMm**: Chemistry of Harvard Molecular Mechanics.  
**COX**: Cyclo-oxygenase.  
**Cys**: Cysteine.  
**CC**: Column Chromatography.  
**DMF**: Dimethyl Formamide.  
**EC**: Enzyme Commission number.  
**ESI/Ms**: Electrospray Ionization Mass Spectroscopy.  
**HRESI/MS**: High Resolution Electrospray Ionization Mass Spectroscopy.  
**Ile**: Isoleucine  
**Glu**: Glutamic Acid.  
**Gly**: Glycine.  
**GRAS**: Generally Recognized As Safe.  
**G.I.T**: Gastro-Intestinal Tract.  
**GTA**: Glycyrrhetic acid.  
**GZA**: Glycyrrhizic acid.  
**His**: Histidine.  
**Lys**: Lysine.  
**Leu**: Leucine.  
**MOE**: Molecular Operating Environment.  
**NMR**: Nuclear Magnetic Resonance.  
**NO**: Nitric Oxide.  
**NSAIDs**: Non-Steroidal Anti-inflammatory Drugs.  
**PDB**: Protein Data Bank.  
**Phe**: Phenylalanine.  
**RMSD**: Root Mean Square Deviation.  
**SAR**: Structure Activity Relationship.  
**Ser**: Serine.  
**SNMC**: Stronger Neo-Minophagen C.  
**sPLA<sub>2</sub>**: Secreted Phospholipase A<sub>2</sub>.  
**TEA**: Triethylamine.

**THF:** Tetrahydrofuran.  
**Tyr:** Tyrosine.  
**TLC:** Thin Layer Chromatography.  
**TNF:** Tumor Necrosis Factor.  
**UV:** Ultraviolet.  
**Val:** Valine.  
**XO:** Xanthine Oxidase

## **Abstract**

### **Title of Thesis:**

**Computer Aided drug design and synthesis of  
some triterpenoidal carboxylic acid derivatives  
with potential biological activities.**

### **Name of candidate:**

***Mohamed Osman Ahmed***

Researcher associate in chemistry of natural

compounds department

National Research Centre

### **Thesis supervised by:**

***Prof. Dr. Mohamed Abdel Hamid Ismail***

***Prof. Dr. Atef Gobran Hanna***

***Dr. Nasser Saad Mohamed***



## Abstract

This study involves the design and synthesis of some new derivatives of glycyrrhetic acid. The design of these agents was based on the direct molecular modeling simulation, comprising docking study on PLA<sub>2</sub> enzyme using Accelrys Discovery Studio software 2.5.

This thesis comprises the purification of glycyrrhetic acid from licorice roots and synthesis of the following reported intermediates **II**, **III** & **IX**.

Furthermore the study involves the synthesis of the following targeted compounds **V (a-e)**, **IV (a-m)**, **VI**, **VII**, **VIII**, **X**, **XI** & **XII**.

The structures of these compounds were confirmed by spectral data.

Detailed descriptions of the performed synthesis and molecular modeling were emphasized in the thesis.

Finally, *in vivo* anti-inflammatory test was carried out for evaluation of the synthesized compounds.

# **1-Introduction**

## Introduction

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In the last seven decades, development of triterpenoids' chemistry has led to the synthesis of a series of unique biologically active compounds that are definitely of interest as possible drugs<sup>1</sup>.

Pentacyclic triterpenoids, the compounds whose chemical structures are related to that of steroidal hormones, are the objects of numerous investigations of chemists engaged in organic synthesis and pharmacology.

Glycyrrhetic acid (**GTA**, **1**, sometimes called glycyrrhetic acid) is a readily available pentacyclic triterpenoid belonging to the  $\beta$ -amyrin series. This compound possesses a broad spectrum of pharmacological activities and serves as a base for highly active drug preparations<sup>2, 3</sup>. **GTA** is the aglycon of glycyrrhizic acid (**GZA**, **2**, sometimes called glycyrrhizinic acid), a saponin glycoside present in the roots of licorice.

Licorice (herbs of *Glycyrrhiza glabra*; F: Leguminosae), also known as sweet wood, has remarkable therapeutic properties that had paid ancient's attention for its use as a medicinal plant in the distant past. Licorice is native to central and south western Asia and the Mediterranean region. It is cultivated in the Mediterranean basin of Africa, South Europe and in India. Roots and rhizomes (**Figure 1**) are parts of medicinal importance of licorice<sup>4</sup>.



**Figure 1 : Licorice roots**

In the past two decades, there has been growing interest in the study of licorice. The renewed interest in licorice reflects the general trend observed in medicinal practice, where remedies of natural origin are finding increasing application despite considerable success in the use of many synthetic drugs. The drug preparations based on modified natural compounds frequently exceed the parent substances in activity or of lower adverse effects. In this connection, investigations into the chemistry of biologically active substances based on available natural substrates have become an important direction in modern organic chemistry<sup>5</sup>.

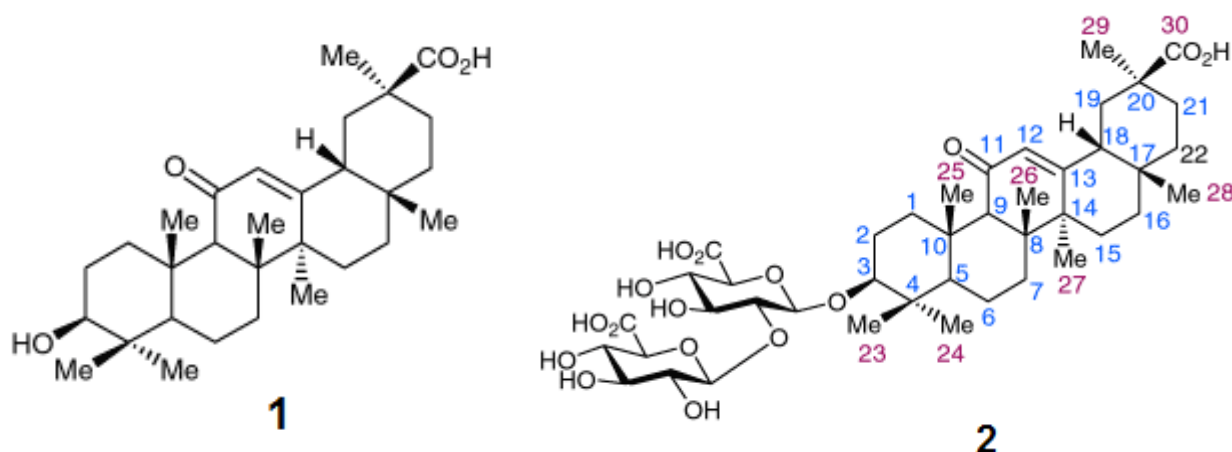
### 1.1. Composition and properties of licorice

A number of components have been isolated from licorice, including a water-soluble, biologically active complex that accounts for 40-50 percent of total dry material weight. This complex is composed of triterpene saponins (mostly glycyrrhizin), flavonoids (responsible for the yellow color of the roots), isoflavonoids, polysaccharides, pectins, simple sugars, amino acids, mineral salts, and various other substances<sup>6, 7</sup>.

Glycyrrhizin is a triterpenoidal compound that accounts for the sickly sweet taste of licorice root (50 times sweet as sucrose)<sup>8</sup>. It is responsible for the sweet taste and the foaming properties of the root. This compound represents a mixture of potassium-calcium-magnesium salts of a tribasic **GZA**. The content of glycyrrhizin varies from 2 to 25 % range depending on the particular species and the site of occurrence<sup>1</sup>. It represents the most important medicinal constituent of licorice due to similar biological activity of its main metabolite, **GTA**, to adrenocortical hormones.

### 1.2. Structure of glycyrrhizic acid

Among the natural saponins, glycyrrhizic acid is triterpenoidal saponin that is composed of two molecules of glucuronic acid as a hydrophilic part and glycyrrhetic acid representing a hydrophobic part<sup>6</sup>. Its structure was determined in 1937 as 3-O-(2'-(O- $\beta$ -D-glucuronopyranosyl)- $\beta$ -D-glucuronopyranoside) of 18 $\beta$ -glycyrrhetic acid. **GTA** was originally isolated by Tschirch and Gederberg<sup>9</sup> by the hydrolysis of **GZA**. Subsequent investigations performed by several groups of researchers, including Ruzicka et al.<sup>10</sup>, Djerassi and Foltz<sup>11</sup>, Beaton and Spring<sup>12</sup>, and Conrey and Cantrall<sup>13</sup>, allowed the **GTA** structure to be identified as 3 $\beta$ -hydroxy-11-oxo-18 $\beta$ -H, 20 $\beta$ -olean-12-en-30-oic acid. The data related to the **GTA** structure identification were reviewed in 1966<sup>14</sup>.



### 1.3. Pharmacokinetics of glycyrrhizic acid

GZA, after oral administration, is hydrolyzed to GTA by intestinal bacteria, possessing a specialized  $\beta$ -glucuronidase<sup>15,16</sup>. After oral administration of 100 mg glycyrrhizin in healthy volunteers, no glycyrrhizin was found in plasma but glycyrrhetic acid was found at < 200 ng/mL. Furthermore intravenously administered glycyrrhizin is metabolized in the

liver by lysosomal  $\beta$ -D-glucuronidase to 3-mono-glucuronide glycyrrhetic acid. This metabolite is excreted with bile into the intestine, where it is metabolized by bacteria into glycyrrhetic, which can be reabsorbed<sup>16</sup>.

### 1.4. Glycyrrhetic acid isolation

Isolation of **GTA** from licorice roots is a rather complicated process, including the stages of extraction and acid hydrolysis of the raw extract. The aglycone obtained upon the acid hydrolysis of the native glycoside requires purification by cumbersome methods<sup>17,18</sup>. To provide for a more complete extraction of **GTA** from the roots of licorice, the material is preliminarily comminuted (0.5-1.0 mm)<sup>19</sup>. The purification of **GTA** obtained from the licorice roots is performed by recrystallization from propionic acid<sup>20,21</sup> or by membrane ultrafiltration<sup>22</sup>. A method of pure **GTA** isolation from the licorice root extract, proposed in the Japan patent<sup>23</sup>, is based on the target product adsorption on a polymeric column containing amino and amide groups<sup>23</sup>.

Another method widely used for obtaining **GTA** is based on the hydrolysis of **GZA** and its salts with mineral acids<sup>24,25</sup>. Most frequently, **GZA** or its potassium or ammonium salts are hydrolyzed with 5 % aqueous sulfuric acid<sup>1</sup>. Murav'ev and Savchenko proposed a quantitative variant of the hydrolysis of **GZA** or its salts with 7 % aqueous hydrochloric acid<sup>24</sup>. **GTA** obtained by the acid hydrolysis of glycoside and its salts is purified by chromatography on  $\text{Al}_2\text{O}_3$  or activated charcoal, or by recrystallization from 40 % ethanol<sup>25</sup>.

As known, the acid hydrolysis of **GZA** yields, besides the major **GTA**, some minor impurities as 18 $\alpha$ -glycyrrhetic, licuritic, 11 deoxy- 18 $\beta$ -glycyrrhetic, and 18-dehydroglycyrrhetic acids<sup>26,27</sup>.

Because the acid hydrolysis of **GZA** leads both to the scission of glucouronide bonds and the aglycone transformations<sup>25,28</sup>, some more

attractive pathways based on the enzymatic hydrolysis under mild conditions were developed in recent years<sup>29,30</sup>. The enzymatic hydrolysis of **GZA** is performed with  $\beta$ -glucuronidase from bovine liver<sup>30</sup>.

### 1.5. Literature survey of biological activities of GTA

**GTA** and its precursor, **GZA**, have made licorice in a leading position among different medicinal plants, being a permanent pharmacopeial drug in (Egyptian, United States and British pharmacopeias (**EP**, **USP**, and **BP**)). In addition, glycyrrhizin is a well-known sweetening agent, and it is approved in the USA for the use as generally recognized as safe (**GRAS**)<sup>31</sup>.

Substances extracted from the licorice root extract, as well as glycyrrhetic acid and its derivatives, are used in the production of drugs offering anti-ulcerous and anti-inflammatory activity. Various **GTA** derivatives are also used in the treatment of skin diseases. The most pronounced and important therapeutic activities for **GTA** are illustrated below.

#### 1.5.1. Anti-inflammatory activity

The action of glucocorticoid hormones is known to depend on the presence of  $\alpha$  and  $\beta$ -unsaturated ketone groups and the  $\alpha$ -ketol group in the side chain. The first two structural elements of corticoid hormones are present in **GTA** acid<sup>Error! Bookmark not defined.</sup>.(see introduction 1.13)

**GTA** has shown anti-inflammatory properties in different animal models<sup>32,33,34</sup>. In *in vivo* study, ammonium glycyrrhizate (glycyrram) and sodium glycyrrhizate were capable of suppressing formalin-induced edema in both intact and adrenalectomized animals<sup>34</sup>. The effects of **GZA** and **GTA**, observed on the model of formalin arthritis in rats were similar to the action of hydrocortisone, while their effects on the model of carageenan-induced