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Antihyperglycemic and sub-chronic antidiabetic actions of morolic and moronic acids, *in vitro* and *in silico* inhibition of 11β-HSD 1

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ABSTRACT

Morolic (**1**) and moronic (**2**) acids are the main constituents of acetonic extract from *Phoradendron reichenbachianum* (Loranthaceae), a medicinal plant used in Mexico for the treatment of diabetes. The aim of the current study was to establish the sub-acute antidiabetic and antihyperlipidemic effects of compounds **1** and **2** over non insulin-dependent diabetic rat model. Also, to determine the antihyperglycemic action on normoglycemic rats by oral glucose tolerance test. Daily-administered morolic (**1**) and moronic (**2**) acids (50 mg/kg) significantly lowered the blood glucose levels at 60% since first day until tenth day after treatment than untreated group ($p < 0.05$). Moreover, analyzed blood samples obtained from diabetic rats indicated that both compounds diminished plasmatic concentration of cholesterol (CHO) and triglycerides (TG), returning them to normal levels ($p < 0.05$). Also, pretreatment with 50 mg/kg of each compound induced significant antihyperglycemic effect after glucose and sucrose loading (2 g/kg) compared with control group ($p < 0.05$). *In vitro* studies showed that compounds **1** and **2** induced inhibition of 11β-HSD 1 activity at 10 μM. However, *in silico* analysis of the pentaclyclic triterpenic acids on 11β-HSD 1 revealed that all compounds had high docking scores and important interactions with the catalytic site allowing them to inhibit 11β-HSD 1 enzyme. In conclusion, morolic and moronic acids have shown sustained antidiabetic and antihyperglycemic action possibly mediated by an insulin sensitization with consequent changes of glucose, cholesterol and triglycerides, in part mediated by inhibition of 11β-HSD 1 as indicated by *in vitro* and *in silico* studies.

Introduction

The type 2 diabetes mellitus (T2DM) is related to a loss of insulin sensitivity in target tissues such as muscle, liver and adipose tissue, which is associated with glucose intolerance (Stulning and Waldhäusl 2004). 11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD 1) mainly catalyzes the intracellular regeneration of active GCs (cortisol, corticosterone) from inert inactive 11-keto forms (cortisone). Multiple lines of evidence have indicated that 11β-HSD 1-mediated intracellular cortisol production may have a pathogenic role in type 2 diabetes and its co-morbidities. Accordingly, the 11β-HSD 1 becomes a novel target for anti-type 2 diabetes drug developments, and inhibition of 11β-HSD 1 offers a potential therapy to attenuate the T2DM (Morgan et al. 2009). Moreover, several studies have shown that enhanced enzymatic activity of 11β-HSD 1 is related to hypertension, dyslipidemic states and metabolic syndrome (Chiodini et al. 2007). Transgenic rodent models which over express 11β-HSD 1 have indicated a significant rise on visceral obesity, metabolic syndrome, and high levels of cortisol along visceral region instead of normal plasmatic levels, enhanced hunger and decreased leptin receptor expression (Dötsch and Rascher 2002).

Abbreviations: 11β-HSD, 11β-hydroxy steroid dehydrogenase type 1; MOE, Molecular Operating Environment; PDB, Protein Data Bank; RMS, Root Mean Square; GLU, glucose; CHO, cholesterol; TG, triglycerides; CBO, carbenoxolone; T2DM, type 2 diabetes.

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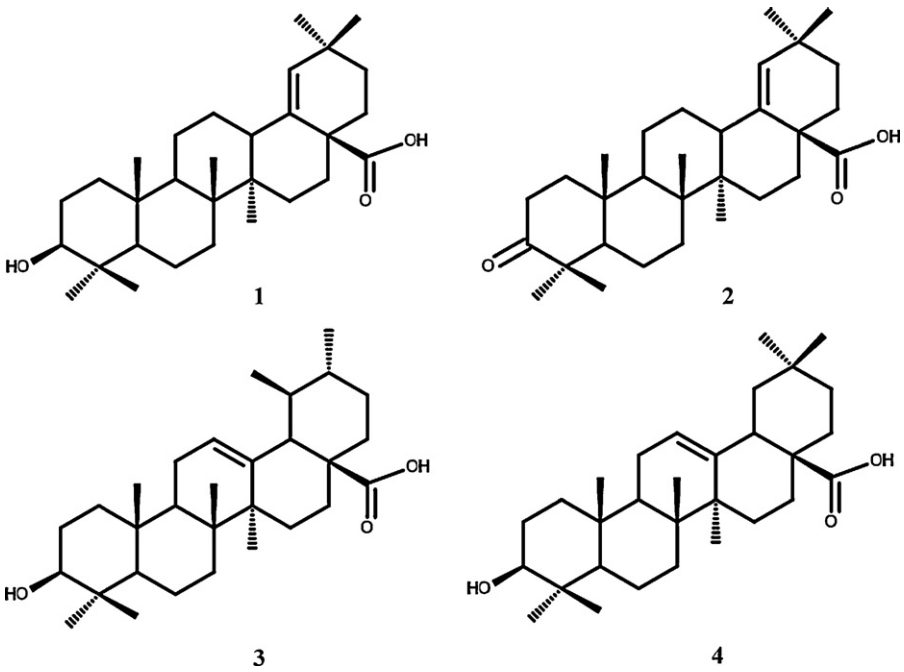

Fig. 1. Structure of triterpenic acids evaluated morolic (**1**), moronic (**2**), ursolic (**3**), and oleanolic (**4**) acids.

Recent reports reveal that a total of 15 applications have been registered by the US Patent office for 11β-HSD 1 inhibitors. From these, 10 bioactive compounds against 11β-HSD 1 are in development for the treatment of T2DM, most of them are in phase I clinical trials (Researchandmarkets.com, 2012); in this context, compound INCB013739 by Incyte Corporation has been completed a clinical trial for its safety and efficacy in T2DM (Clinicaltrials.gov., 2012). Among the 11β-HSD 1 inhibitors, carbenoxolone (CBO) is one of the most commonly used, which is a semisynthetic derivative of 18β-glycyrrethinic acid a type of triterpene found in several plants (Classen-Houben et al., 2009). Other triterpenic acids, particularly those with ursane or oleanane skeleton, are good inhibitors of this enzyme but also inhibit the 11β-HSD 2 isoform, the one that perform reverse reaction (Blum et al., 2009). However, small changes to the structures of the inhibitors could generate compounds with high affinity for type 1 isoform, such as corosolic acid which has an ursane structure and two hydroxyl groups on positions 2 and 3 that results on determinant factors for its inhibitory and specific activity (Rollinger et al., 2010). These compounds are examples of the so-called selectivity cliffs, because they show a closely related structural similarity but large changes in biological activity (Medina-Franco, 2012).

Previous investigations found that compounds **1** and **2** (Fig. 1) have an important antidiabetic effect over a non-insulin dependent Diabetes mellitus model and were proposed as potent, reversible and specific PTP-1B inhibitors (Ramírez-Espinosa et al. 2011).

Materials and methods

Chemical and drugs

Glucose, sucrose and tween 80 were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Acarbose, CBO, metformin, saxagliptin and others reagents were purchased from local distributors. Morolic (**1**) and moronic (**2**) acids were isolated from acetone extract of *Phoradendron reichenbachianum* as previously described (Rios et al., 2001). Ursolic (**3**) and oleanolic (**4**) acids were purchased from Sigma–Aldrich Co.

Animals

Wistar rats were provided by FES Iztacala animal facilities, from Universidad Nacional Autónoma de México. Animals (200–250 g) were housed in groups of six ($n = 6$) under laboratory conditions (12-h light/dark cycle, $25 \pm 2\,^{\circ}C$ and 45–65% of humidity). Before experimentation, all animals were fasted for 16 h with water *ad libitum*. All animal procedures were conducted in accordance with the Mexican Federal Regulations for Animal Experimentation and Care (SAGARPA, NOM-062-ZOO-1999, Mexico), and approved by the Institutional Animal Care and Use Committee (UNAM) based on US National Institute of Health publication (No. 85-23, revised 1985).

Oral glucose and sucrose tolerance tests

Thirty minutes after administration of test samples, a dose of 2 g/kg of substrate (glucose or sucrose) solution was administered to each rat. Compounds **1** and **2** (50 mg/kg), saxagliptine (10 mg/kg), acarbose (3 mg/kg) and vehicle were administered to rats in the same volume of solution. Blood samples were collected from the tail tip at 0 (before oral administration), 0.5, 1, 1.5, 2, 2.5, 3, and 4 h after vehicle, positive control and test sample administration (Ortiz-Andrade et al. 2007).

Sub-chronic antidiabetic assay

Four groups of non-insulin dependent diabetic rats (Ortiz-Andrade et al. 2008) with fasting glucose levels above 200 mg/dl, were administered daily with **1** or **2** (50 mg/kg), metformin (120 mg/kg) and vehicle (tween 80 10%, 1 ml) during 10 days; plasmatic glucose concentrations was monitored 24 h after administration at days 1, 3, 5, 8, and 10 to establish antidiabetic effect.