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PPAR α / γ , adiponectin and GLUT4 overexpression induced by moronic acid methyl ester influenced on glucose and triglycerides levels of experimental diabetic mice

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Abstract: The current study aimed to determine the antidiabetic and antidyslipidemic activities of moronic acid methyl ester (**1**) by *in vivo*, *in vitro*, *in silico* and molecular biology studies. Compound **1** was evaluated to establish its dose-dependent antidiabetic and antihyperglycemic (50 mg/kg) activities, in diabetic and normoglycemic male CD1 mice, respectively. Also, compound **1** was subjected to a sub-acute study (50 mg/kg/day for eight days) to determine blood biochemical profiles and the expression of PTP-1B, GLUT4, PPAR- α , PPAR- γ , adiponectin, IL-1 β , and MCP1 in adipose tissue of animals after treatment. Different doses in acute administration of **1** decreased glycemia ($p < 0.05$), compared with vehicle, showing greater effectiveness in the range 50-160 mg/kg. Also, the oral glucose tolerance test (OGTT) showed that **1** induced a significant antihyperglycemic action by opposing the hyperglycemic peak ($p < 0.05$). Moreover, **1** subacute administration decrease glucose and triglycerides levels after treatment ($p < 0.05$); while the expression of PPAR- α and γ , adiponectin and GLUT4 displayed an increase ($p < 0.05$) compared with the diabetic control group. In conclusion, compound **1** showed antihyperglycemic, antidiabetic and antidyslipidemic effects in normal and diabetic mice, probably due to insulin sensitization through increase mRNA expression of GLUT4, PPAR- α , PPAR- γ and adiponectin genes.

Key words: Moronic acid methyl ester; antidiabetic effect; adiponectin; GLUT4; PPAR's; PTP1B.

45 **Introduction**

46 Diabetes is a chronic metabolic disease that occurs when the body cannot
47 produce any or enough insulin or cannot use it, in consequence hyperglycemia
48 occurs (Alberti et al, 1997; Trisha Dunning, 2013; IDF, 2019). Currently, diabetes
49 is one of the largest global health emergencies of the 21st century. The World
50 Health Organization (WHO) estimates that globally, high blood glucose is the third
51 highest risk factor for premature mortality, after high blood pressure and tobacco
52 (IDF, 2019). It is estimated that 463 million people are living with diabetes; in
53 addition, there are 379 million adults with impaired glucose tolerance, which puts
54 them at high risk of developing the disease (IDF, 2019). Type 2 Diabetes (T2D) is
55 the most prevalent form of diabetes and it has increased alongside cultural and
56 societal changes, and accounts for 90–95% of all diabetic patients. Due to its
57 prevalence, therapy for T2D relies mainly on several approaches intended to
58 reduce the hyperglycemic status, such as: biguanides, sulfonylureas, meglitinides,
59 TZDs, α -glucosidase inhibitors, DPP-4 inhibitors, etc (ADA, 2015). Among the
60 strategies for finding new chemical entities with pharmaceutical potential, plants-
61 derived natural compounds are the major source to discover these molecules,
62 which serve as prototypes to design and synthesize new drugs, either by improving
63 their biological and/or physicochemical characteristics. Therefore, there is a
64 continuum interest to develop new drugs, focused on treatment of metabolic
65 disorders such as T2D and dyslipidemia. In this regard, the pentacyclic triterpenic
66 acids show plenty of biological activities, such as antidiabetic, antiinflammatory,
67 antimicrobial, antiviral, cardioprotective, etc (Guzmán-Ávila et al, 2018; Liu, 2005;

Loza-Rodriguez et al, 2020; Ramírez-Espinosa et al, 2011; Ramírez-Espinosa et al, 2013; Ramírez-Espinosa et al, 2014; Somova et al, 2003; Jung et al, 2007). Thus, structural modifications on these pentacyclic triterpenic acids allowed to obtain semisynthetic derivatives as bioactive compounds with antidiabetic effect, acting by selective inhibition of PTP-1B; besides, they show binding interaction in extended sites of union, useful for design new inhibitors for this enzyme (Ramírez-Espinosa et al, 2014; Cerón-Romero et al, 2016). In this regard, it has been reported 3-oxo-6- β -hydroxyolean-18-en-28-oic acid, a semisynthetic derivative of moronic acid (Fig. 1), which is a competitive inhibitor of PTP-1B with *in silico* affinity on the two binding sites, the catalytic and the extended binding sites (Bialy and Waldmann, 2005; Puius et al, 1997).

Thus, the aim of current work is to determine the acute dose-dependent and subacute antidiabetic actions of **1** (Figure 1) on non-insulin dependent diabetic mice model (NIDDM), and after treatment, to establish the blood glucose and lipid profiles, and the genic expression in adipose tissue for several genes related with diabetes, insulin sensitization and pro-inflammatory cytokines.

Materials and methods

Chemicals

Substances used [glucose, tween 80, nicotinamide, streptozotocin (STZ)] were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Other reagents (glibenclamide, acarbose, metformin, among others) were acquired from local distributors. Compound **1** (moronic acid methyl ester) was obtained as described

elsewhere.¹³ Sodium pentobarbital (Pisabental®) was bought from PISA® (Mexico City, Mexico). Test kit for enzymatic end-point evaluations for glucose (GLU), total cholesterol (CHOL), triglycerides (TG) and high-density lipoprotein (HDL) were purchased from Wiener Labs (Mexico City, Mexico).

Animals

CD1 mice were acquired from Faculty of Medicine animal facilities of the Universidad Autónoma del Estado de Morelos. Animals (20-25 g) were allotted in groups of six ($n = 6$) under laboratory conditions (12-h light/dark cycle, $25 \pm 2^\circ\text{C}$ and 45-65% of humidity). Before acute experimentation, all animals were fasted for 13 h with water ad libitum. The study was conducted in accordance with the policy for experimental and clinical studies following local regulations (SAGARPA, NOM-062-ZOO-1999, Mexico), ratified by the Institutional Animal Care and Use Committee (UNAM, Protocol 1368), based on Canadian Council on Animal Care (2nd ed) and NRC Guide for the Care and Use of Laboratory Animals (8th ed).

Induction of diabetes

Diabetes was induced in overnight fasted mice by a single intraperitoneal injection of 100 mg/kg STZ (dissolved in citrate buffer pH 4.5), 15 min after the i.p. administration of 40 mg/kg of nicotinamide (suspended in normal physiological saline solution). Hyperglycemia was confirmed by the elevated glucose concentration in plasma, determined after 2 weeks with a glucometer (AccuCheck Performa, Roche®, Mexico City, Mexico). Mice with $200 > \text{mg/dL}$ of glycemia were

used for the antidiabetic testing (Hayashi et al, 2006; Goyal et al, 2016; Verspohl, 2002).

Acute antidiabetic assay at different doses

Mice of experimental groups (n = 6) were administered with a suspension of compound **1** (prepared in 10% tween 80) at 5, 16, 50, 160 and 500 mg/kg body weight. Control group was treated with vehicle (tween 80 at 10%, 0.1 mL), and positive control group was administered with glibenclamide (3 mg/kg). Blood samples were collected from the caudal vein at 0, 1, 3, 5 and 7 h after vehicle, test sample and drug administration (Cerón-Romero et al, 2016; Giles-Rivas et al, 2020). Blood glucose concentration was estimated using a commercial glucometer (AccuCheck Performa).

Oral glucose tolerance tests (OGTT)

A load of 2 g/kg of glucose solution was administered to mice 30 min after test samples administration. Then, blood samples were obtained at 0 time (before oral administration), 0.5, 1, 1.5, 2 and 3 h after vehicle, positive control, and extract administrations from caudal vein. Glycemia was estimated using a commercial glucometer (Accu-Chek). Moronic acid (50 mg/kg), compound **1** (50 mg/kg), glibenclamide (3 mg/kg), and vehicle (tween 80 at 10%, 0.1 mL) were administered to groups (n = 6) in the same volume of solution (Giles-Rivas et al, 2020).

Sub-acute antidiabetic assay

Animals were allotted in four groups (n = 6). Diabetic animals were daily administered with 1 (50 mg/kg), metformin (120 mg/kg, reference drug) and vehicle for 8 days; meanwhile, normoglycemic group was treated with vehicle. Glucose was monitored at 5 and 24 h after test samples administration, at days 1, 3, 5 and 8 to identify the antidiabetic effect, body weight was determined before administration.

At the end of treatments, all animals were anesthetized with sodium pentobarbital (3.5 mg/kg, i.p.), blood samples were obtained by direct cardiac puncture for biochemical parameters' determination. Epididymal fat was isolated, rinsed with 0.9 % NaCl, frozen immediately in dry ice and stored at -70°C until RNA isolation.

Blood parameters' determination

Blood samples from sub-acute evaluation were centrifuged at 3500 rpm for 15 min at room temperature. Next, plasma was obtained for analytical determination of GLU, CHO, and TG using enzymatic colorimetric methods (Wiener Labs, Mexico).

Relative expression of PTP-1B, GLUT-4, PPAR- α , PPAR- γ , adiponectin, IL-1 β and MCP-1 in epididymal tissue

Total RNA was isolated from epididymal fat tissue (500 mg) by Chomczynski's method using the TriPure isolation reagent (Roche®). The total

RNA obtained was resuspended in 20 μ L of RNA secure resuspension solution (Ambion). The absorbance of each sample was measured in the equipment EPOCH, at 260 nm to calculate the concentration of total RNA, whereas the quality (O.D. = 1.8-2.0) was evaluated with the ratio 260/280 nm. To confirm RNA integrity, 1 μ g of total RNA was observed by electrophoresis in 1% agarose gel. Two major ribosomal bands (28S and 18S rRNA) were visualized by an Image Analyzer (BioRad, USA, data not shown).

Reverse transcription (RT) was catalyzed by the reverse transcriptase from the ImProm II kit (Promega) using 2 μ g of total RNA following the manufacturer's instructions. The reaction was incubated in a thermo-cycler (Applied Biosystems GeneAmp PCR System 2700), following the Reverse transcription program: 25°C for 5 min, followed by extension at 42°C for 55 min. The reaction was stopped by heat at 70°C for 15 min and cooled to 4°C for 5 min. The PCR assay was performed with 1 μ L of cDNA per sample using the Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific) and 0.5 mM of the customized primers (Table 1) at final volume of 5 μ L (384 well plate).

The process was carried out using the 7900HT Fast Real-Time PCR System with 384-Well Block Module (Applied Biosystems), which continuously measures the products of PCR amplification in each cycle. All the reactions were processed by duplicated with the following amplification conditions: 10 min at 95°C for pre-incubation of the enzyme, a cycling phase of 35 or 40 cycles, with each cycle consisting of 95°C for 15s, and 60°C for 60s. At the end, a dissociation curve stage was added to verify only one amplification product. To ensure the quality of

the measurements, each assay included a negative control. The β -actin gene was used as a reference gene. Relative mRNA levels were determined using the $2^{-\Delta\Delta C_t}$ method.

Molecular docking studies

The crystal structure of PPAR α/γ were retrieved from the PDB with the accession codes: 5HYK and 2XKW, respectively. Docking calculations were conducted with Autodock Tools version 1.5 6rc3. The program performs several runs in each docking experiment, besides each experiment provides one predicted binding mode of molecules assayed. All water molecules and cocrystal ligands were remove from the crystallographic structure. The grid maps were generated using Autogrid4. Each grid was centered at the crystallographic coordinates of the cocrystal ligands. The grid dimensions were 50 x 50 x 50 points separated by 0.375 Å. Also, the protein file was selected as the rigid part meanwhile the ligand file as the flexible one, allowing its torsions to rotate during docking. On the Autodock Tools program, it was added charges to the protein and merged the non-polar hydrogens to perform scoring calculation. The Genetic Algorithm were selected for the search using default parameters. The number of docking runs was 20. The resulting poses were visualized on Pymol and compared against the cocrystalized ligand over the protein.

The experiments of molecular docking were validated by redocking the corresponding ligand into the binding site of its protein. For the PPAR α model the root mean square deviation (RMSD) between the cocrystal ligand [2-methyl-2-[4-

(naphthalen-1-yl)phenoxy]propanoic acid] and the docked structure was 0.60 Å and for the PPAR γ model and 5(R)-5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzyl}-1,3-thiazolidine-2,4-dione, the RMSD was 1.6 Å. Those values indicate that the protocol used to reproduce orientation and conformation docking simulations is accurate. Pymol version 2.4 and MOE were used for visualization.

Statistical analysis

Data showed in plots are expressed as mean $\pm$ S.E.M. Plots were constructed with Microcal™ Origin 6.0 software (Microcal Software Inc., USA). All values were expressed as mean $\pm$ S.E.M. Statistical significance was estimated by analysis of variance (ANOVA), $p < 0.05$ implies significance.

Results and discussion

Acute antidiabetic action of different doses of compound 1

In a previous study, it was described the acute antidiabetic action of compound **1** (50 mg/kg) in NIDD rat model, which induces its effect by PTP-1B enzymatic inhibition established through *in vitro* ($IC_{50} = 10.8 \pm 0.5 \mu M$), and *in silico* studies (Cerón-Romero et al, 2016). Moreover, compound **1** acts as competitive inhibitor of PTP-1B and was more selective to PTP-1B than other assayed PTPs. In current work, compound **1** was evaluated for *in vivo* antidiabetic activity on diabetic CD1 mice. Metformin (120 mg/kg) and glibenclamide (3 mg/kg) were taken as positive controls. Acute administration of compound **1** at different doses, confirmed its antidiabetic activity after 3 h of treatment, in comparison with vehicle

($p < 0.05$). In addition, the effect was sustained during the period of the experiment, showing greater effectiveness with 50 and 160 mg/kg doses, and displaying major potency than its precursor moronic acid (Figure 2). The fact that the antidiabetic action occurred after 3 h of dosage, correlates with our previous findings where we hypothesized that compound **1** is a pro-drug, since it is a methyl ester that can be metabolized immediately in bloodstream by abundant plasma esterases (Augustijns et al, 1998; Zhou et al, 2017). Drug-like properties of pentacyclic acid triterpenoids are often not favorable. Pro-drug strategy is an effective method to improve the biopharmaceutical and pharmacokinetic pattern as well as the efficacy, and selectivity of this class of secondary metabolites. This structural modification favored its permeability and absorption in the organism, showing a higher bioavailability joined with increased half-life, facilitating transfer to different organs and tissues, as observed by lowering blood glucose during the test associated with a time-dependent decrease (Zhou et al, 2017). Thus, the structural modification along with inhibition of PTP-1B previously described (Cerón-Romero et al, 2016) allowed to propose that compound **1** is a potential co-drug, since both moronic acid and its methyl ester derivative showed *in vitro* and *in vivo* activities; suggesting that the presence of both compounds in plasma could synergize the antidiabetic action.

Antihyperglycemic effect of compound 1

After antidiabetic determination of different doses of compound **1**, we used 50 mg/kg as the most efficient dose to produce the antidiabetic action. In this context, the antihyperglycemic action of compound **1** was determined in

normoglycemic mice using glucose tolerance test by loading glucose at 2 mg/kg. As described in Figure 3, the administration of compound **1** significantly decreased hyperglycemic peak at 0.5 hour, which remained for three hours, showing the efficiency of **1** to control high glucose levels. Latter evidence suggests that compound **1** could induce its effects by two general mechanisms of action, one could be the avoidance of intestinal glucose absorption through Na⁺-glucose and/or GLUT2 transporters interaction, or by extrapancreatic mechanisms that enhance insulin sensitizing by possible PPAR- γ activation and/or overexpression, as some pentacyclic acid triterpenes do (Loza-Rodriguez et al, 2020), besides its PTP-1B inhibition (Cerón-Romero et al, 2016). Additional experiments will be necessary to establish whether compound **1** is able to inhibit the above-mentioned carriers.

Sub-acute antidiabetic assay

Compound **1** administration (50 mg/kg) for 8 days induced a significant antidiabetic action from day 1, and this was preserved along the end of the treatment, compared with the untreated diabetic group ($p < 0.05$); whereas the intragastric administration of metformin (120 mg/kg) to diabetic mice, showed a similar decrease than the observed for compound **1** (Figure 4). Also, the antidiabetic activity of **1** and metformin were significant when glucose was measured after 5 (Figure 4a), and 24 h (Figure 4b) treatment. Twenty-four hours after compound **1** treatment revealed a similar decrease of glucose to that induced by metformin treated group, showing a significant decrease at 1, 3 and 8 days

276 compared to vehicle group ($p < 0.05$). On the other hand, glucose measured 5 h
277 after daily treatment significantly lowered glycemia in diabetic mice treated with
278 compound **1** and metformin, which indicate its effectiveness in experimental
279 diabetes treatment, i.e., it might be by insulin sensitization in tissues (Loza-
280 Rodriguez et al, 2020; García-Díaz et al, 2016). It is important to mention that along
281 the treatment, mice weight was monitored; however, we did not find any significant
282 change between diabetic groups (data do not show).

283 After 8 days of treatment some blood biochemical parameters were
284 analyzed (Figure 5). It was clear that compound **1** in diabetic mice significantly
285 restored glucose to normal value (about 120 mg/dL), compared to untreated
286 diabetic mice (about 430 mg/dL), and this effect was even better than that induced
287 by metformin. Also, triglycerides and total cholesterol were significantly diminished
288 by compound **1**, while HDL and VLDL were not modified. The fact that compound
289 **1** produced an important antidiabetic action could be attributable to inhibition of
290 PTP-1B, and also to a possible increase in the expression of PPAR- γ , inducing
291 insulin sensitization (Loza-Rodriguez et al, 2020; Thangavel et al, 2017). In this
292 context, insulin action in adipose tissue involves the stimulation of glucose uptake
293 and the inhibition of lipolysis, two processes classically considered to be the
294 earliest defects detected in insulin resistance (Fernández-Veledo et al, 2008).
295 Thus, results showed that short-term compound **1** treatment had a significant
296 influence on glucose metabolism; in addition, compound **1** also produced favorable
297 changes in plasma lipid profile. These observations suggest that compound **1** may
298 influence energy metabolism via a peripheral site of action.

299 After treatment, we obtained the epididymal fat pad in order to analyze gene
300 expression of some proteins related with glucose and lipid metabolism, as well as
301 the pro-inflammatory profile. As mentioned, adipocytes are important in the body
302 energetic metabolism; adipose tissue is also a highly active endocrine organ,
303 based on its ability to secrete a wide variety of biologically active adipokines, such
304 as leptin, adiponectin, tumor necrosis factor- α (TNF- α), or interleukin-6 (IL-6),
305 which are involved in different physiological processes (Frühbeck et al, 2019).
306 Also, it is well known that T2D is a metabolic disease existing on a background of
307 inflammatory mediators, such that interleukin 1 β (IL-1 β), IL-6, IL-18, IL-8, TNF- α ,
308 intracellular cell adhesion molecules, vascular cell adhesion molecules, matrix
309 metalloproteinases, and monocyte chemoattractant protein 1, are upregulated in
310 diabetes, and are associated with diabetes-related complications. In Figure 6 it is
311 shown that adiponectin was significantly reduced in diabetic mice without
312 treatment compared with normoglycemic mice (Figure 6a), similar to diabetic and
313 obese patients that show low plasma adiponectin values as well as expression
314 (Palomer et al, 2005; Caselli, 2014). However, in diabetic animals compound **1**
315 induced about 45-fold overexpression of adiponectin than diabetic without
316 treatment, and about 5-fold than metformin (Figure 6b, $p < 0.001$). These results
317 are exceptional since they indicate that compound **1** counteracted the observed
318 insulin resistance to insulin sensitive diabetic mice, as described *in vivo*
319 antihyperglycemic and antidiabetic actions. On the other hand, compound **1** was
320 able to increase the expression of PPAR- γ in a significant way (about 50-fold
321 compared to diabetic control, Figure 7), that confirm its potential antidiabetic action

as insulin sensitizer. Activated and augmented expression of PPAR- γ regulate the secretion of adiponectin, leptin, and others which have a positive effect on insulin sensitivity, and it also maintained the secretion of negative modulators of insulin sensitivity, like resistin and TNF- α . PPAR- γ provided protection to liver and skeletal muscle and maintained their normal function by prevention of lipid overload. Besides the adipogenic activity, PPAR- γ modulates genes important for the release, transport, and storage of fatty acids. PPAR- γ also upregulates the expression of genes, such as glucose transporter type 4 (GLUT4), and c-Cbl-associated protein (CAP) involved in glucose homeostasis. Due to participation of PPAR- γ in lipid and glucose metabolism, it is a target for the PPAR- γ agonists used in the treatment of T2D (Thangavel et al, 2017). Thus, drugs that activate PPAR- γ are named insulin sensitizers.

At this moment, we can suggest that compound **1** acts as a multitarget drug by inducing a PTP-1B inhibition (Cerón-Romero et al, 2016), lowered the PTP-1B expression (not significantly, Figure 8) that promoted increase in the phosphorylation state of the insulin receptor, and therefore, an increase in the translocation of GLUT4 as observed in the expression by compound **1**. Moreover, the overexpression of the adiponectin and PPAR- γ genes, also confluent on insulin sensitive by GLUT4 translocation and overexpression (Thangavel et al, 2017). To corroborate last asseveration, mRNA expression of GLUT4 was determined in adipose tissue from compound **1**-treated and -untreated animals. As shown in Figure 9, untreated diabetic mice showed significantly low GLUT4 expression compared to normoglycemic mice, which is a common feature in diabetes.

345 However, compound **1**-treated diabetic animals displayed 5-fold expression than
346 untreated diabetic mice, which corroborated the significant *in vivo* acute and sub-
347 acute antidiabetic action mentioned before.

348 As observed, adiponectin, PPAR γ and GLUT4 were highly expressed,
349 which are directly related with insulin sensitization. As it is known, adiponectin may
350 bind to three receptors: AdipoR1, AdipoR2, and T-cadherin to exert its actions;
351 bound adiponectin regulates glucose and lipid homeostasis by improving insulin-
352 sensitizing effects, such as fatty acid oxidation, mitochondrial biogenesis, and by
353 mediating anti-oxidative and anti-inflammatory actions (Yaribeygi et al, 2019). The
354 primary targets activated by bound adiponectin to AdipoR1 and AdipoR2, are
355 AMPK and PPAR- α , respectively. While AMPK glucose uptake very similar to
356 insulin, i.e., it promotes GLUT4 translocation to the cell membrane and upregulates
357 hexokinase II expression. AMPK also modulates lipid metabolism by fatty acid
358 oxidation and cholesterol synthesis regulation; in addition, AMPK phosphorylation
359 decreases lipogenesis-associated enzymes activity, like: sterol regulatory
360 element-binding protein-1 (SREBP-1), acetyl CoA carboxylase-1 (ACC-1), and
361 hydroxymethylglutaryl CoA reductase (HMGCR), which in turn limits malonyl CoA
362 synthesis, then attenuating the inhibition of carnitine palmitoyl transferase-1 (CPT-
363 1) activity to favor transport of fatty acids into the mitochondria for β -oxidation,
364 interrupting fatty acid synthesis as well (Kim and Park, 2019). Moreover, AdipoR2-
365 induced activation of PPAR- α that upregulates genes involved in fatty acid
366 transport, binding and activation and peroxisomal and mitochondrial fatty acid β -
367 oxidation. PPAR- α -mediated gene transcription upregulates the PGC1 α and

ERR1 α axis, enhancing mitochondrial oxidative leading to reduce oxidative stress, contributing to decrease fatty liver (Kim and Park, 2019). As mentioned, adiponectin and PPAR- α expression increase could be related to diminished triglyceridemia in mice treated with compound **1** (Figure 10). Finally, IL-1 and MCP-1 were not significantly modified (Figure 11), although they showed propensity to increase in diabetic animals and to decrease in diabetic animals treated with compound **1**.

Docking Analysis

Based on the *in vitro* assays of relative expression of PPAR- α and PPAR- γ , compound **1** and its phase I metabolite (Moronic acid) were employed for docking calculations. The experiments performed on the PPAR α showed that both, the ester molecule **1** and the carboxylic metabolite, internalize into the binding pocket, nevertheless these molecules interact by electrostatic bonds. The carboxylic form presents a score of -11.25 kcal/mol, meanwhile the ester form **1** had a score of -7.37 kcal/mol, showing less affinity than its metabolized form (Figure 12). In the case of PPAR- γ both molecules cannot fully enter the binding site (Figure 13). The result of the score for the carboxylate form is -6.59 kcal/mol and for the ester form **1** the score is -7.37 kcal/mol. Despite the differences between the conformation of the co-crystal ligand and compound **1**, the *in vitro* results suggest that the affinity for the receptor is due to a probable extended recognition site. Both molecules present lipophilic and weak polar interactions with PPAR- α/γ , showing a ligand exposure out of the pocket of binding site. This fact agrees with previous report

where lupeol and β -amyrin (Giacoman-Martínez et al, 2019), both of them closely related to pentacyclic triterpenes, showed interactions through a hydrogen bond between its hydroxyl groups and Glu259 residue. Collectively, these results are consistent with previously findings that report the dual PPAR action of several triterpenes such as oleanolic acid (Loza-Rodríguez et al, 2020), lupeol and β -amyrin (Giacoman-Martínez et al, 2019).

Predicted Pharmacokinetic profile

In order to evaluate the pharmacokinetic profile of compound **1** and moronic acid, we used the platform ADMETLab 2.0 (<https://admetmesh.scbdd.com/>) (Xiong et al, 2021) to calculate the ADME profile (Table 2), starting with absorption parameters: GI absorption, Blood-brain barrier permeability and the bioavailability. Compound **1** present high values of absorption and it is able to cross the BBB, whereas moronic acid did not. The distribution parameters calculated were plasma protein binding and volume of distribution. Optimal plasma protein binding is less than 95% and compound **1** met this parameter. However, compounds with high protein bound may have a low therapeutic index, such as Moronic acid. Metabolic stability was also predicted, and both compounds are substrates of CYP450, in particular of CYP2C19 isoform, which implies that this enzyme is the main metabolizing protein for these two compounds. To complete the ADME predictions, it was calculated the excretion parameters, where both compounds showed satisfactory values of clearance and only moronic acid

showed a predicted long half-life ($> 3\text{h}$), while compound **1** showed a short $T_{1/2}$, presumably for being a prodrug of moronic acid.

Conclusion

Compound **1** shows antihyperglycemic, antidiabetic and antidisypidemic effects in normal and diabetic mice, probably due to its potential insulin sensitization through GLUT4, PPAR- α , PPAR- γ and adiponectin gene expression, coupled with PTP-1B inhibition previously described.

Conflict of interest

The authors report no conflicts of interest.

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Author contributions

L. Cerón Romero: blood biochemical parameter acquisition, data validation, *in vivo* studies and RT-PCR experiments; **S. Estrada-Soto:** Study design, *in vivo* studies, manuscript preparation, supervision and funding acquisition; **G. Navarrete-Vázquez:** *in silico* studies and manuscript preparation; **E. Rosales-Ortega:** *in silico* studies; **J. Gómez-Zamudio:** RT-PCR experiments, manuscript preparation and validation; **M. Cruz:** RT-PCR experiments and manuscript preparation; **R. Villalobos-Molina:** blood biochemical parameter acquisition, data, validation and manuscript preparation.

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Table 1. Primers sequences. Primers were designed from the mRNA sequence reported in the NCBI GeneBank using the Primer3-Blast tool (<https://www.ncbi.nlm.nih.gov/tools/primer-blast>).

Gene	Primer sequence (5'-3')
PTP-1B	Forward-GGCTATTTACCAGGACATTCGAC; Reverse-RCTCGGTACCTGTTCCGGTTT
GLUT-4	Forward -GCTCTGACGATGGGGAACC; Reverse - AAAGTGAAGGGAGCCAAGCA
PAR- α	Forward -ATGCCAGTACTGCCGTTTTTC; Reverse -GCCACCTCTTTGCTCTGCTC
PPAR- γ	Forward -CCAGAGTCTGCTGATCTGCG; Reverse -GCCACCTCCTTTGCTCTGCTC
adiponectin	Forward -CCAATGTTCCCATTTCGCTT; Reverse -TAGTAGAGCCCCGGAATGTT
IL-1 β	Forward -TATGTCTTGCCCGTGGAGCTT; Reverse -GTCACAGAGGACGGGCTCTT
MCP1	Forward - ATCCCAATGAGTAGGCTGG; Reverse -CTCTTGAGCTTGGTGACAA
β-actin	Forward-CCTGAGCGCAAGTACTCTGTGT; Reverse-GCTGATCCACATCTGCTGGAA

Table 2. Pharmacokinetic predictive values calculated with ADMETLab 2.0 (<https://admetmesh.scbdd.com/>) for compound **1** and moronic acid

Model	Compounds	
	1	Moronic acid
Gastrointestinal Absorption	(+) High	(-) Low
Blood-Brain Barrier permeability	(+) Yes	(-) No
Bioavailability (F)	>30%	<20%
Plasma Protein Binding	92.87%	97.95%
Volume distribution	0.980 L/kg	0.904 L/kg
CYP3A4 substrate	(+) Yes	(-) No
CYP2D6 substrate	(+) Yes	(+) Yes
CYP2C19 substrate	(+++) Yes	(+++) Yes
Clearance (Cl)	5.542 mL/min/kg	1.516 mL/min/kg
Half Life($T_{1/2}$)	0.107 h	>3 h

583 **List of figures.**

584 **Figure 1.** Chemical structures of moronic acid and compound 1.

585 **Figure 2:** Antidiabetic dose-response effect of compound 1 on streptozotocin-
586 nicotinamide diabetic CD1 mice. a) 5, 16 and 50 mg/kg, b) 160 and 500 mg/kg,
587 vehicle (tween 80 at 10%, 0.1 mL), glibenclamide (3 mg/kg) and metformin (120
588 mg/kg). Each plot represents the means $\pm$ S.E.M. n = 6; P.O. p < 0.05 compound
589 1 vs. vehicle.

590 **Figure 3.** Glucose tolerance test of normoglycemic CD1 mice treated with moronic
591 acid (50 mg/kg), compound 1 (50 mg/kg), vehicle (tween 80 at 10%, 0.1 mL) and
592 glibenclamide (3 mg/kg) after a single oral administration of 2 g/kg of glucose. Each
593 plot represents the means $\pm$ S.E.M. n = 8; P.O. p < 0.05 compound 1 vs. vehicle.

594 **Figure 4:** Subacute antidiabetic effect of normoglycemic and streptozotocin-
595 nicotinamide diabetic CD1 male mice treated with compound 1 (50 mg/kg/day),
596 metformin (120 mg/kg), and vehicle (tween 80 at 10%, 0.1 mL) for every 8 days.
597 A) Glycemia at 5 h and b) 24 h after administration of test samples. Each plot
598 represents the means $\pm$ S.E.M. n = 6; P.O. p < 0.05 compound 1 vs. vehicle.

599 **Figure 5:** Glucose and lipids blood profiles of normoglycemic and streptozotocin-
600 nicotinamide diabetic CD1 male mice treated with compound 1 (50 mg/Kg),
601 metformin (120 mg/kg), and vehicle (tween 80 at 10%, 0.1 mL) after 8 days of
602 treatment. Glucose (GLU), triglycerides (TG), total cholesterol (CHO), HDL
603 cholesterol and VLDL cholesterol. Each plot represents the means $\pm$ S.E.M. n = 6;
604 p < 0.05 compound 1 vs. vehicle.

Figure 6: RT-PCR analysis of adiponectin expression on adipose tissue after 8 days treatment; a) normoglycemic and streptozotocin-nicotinamide diabetic CD1 male mice treated with vehicle (tween 80 at 10%, 0.1 mL); b) streptozotocin-nicotinamide diabetic CD1 male mice treated with compound **1** (50 mg/kg), metformin (120 mg/kg), and vehicle (tween 80 at 10%, 0.1 mL). The results are the means $\pm$ SEM for four independent experiments.

Figure 7: RT-PCR analysis of PPAR- γ expression on adipose tissue after 8 days treatment a) normoglycemic and streptozotocin-nicotinamide diabetic CD1 male mice treated with vehicle (tween 80 at 10%, 0.1 mL); b) streptozotocin-nicotinamide diabetic CD1 male mice treated with compound **1** (50 mg/kg), metformin (120 mg/kg) and vehicle (tween 80 at 10%, 0.1 mL). The results are the means $\pm$ SEM for four independent experiments.

Figure 8: RT-PCR analysis of PTP-1B expression on adipose tissue after 8 days treatment a) normoglycemic and streptozotocin-nicotinamide diabetic CD1 male mice treated with vehicle (tween 80 at 10%, 0.1 mL); b) streptozotocin-nicotinamide diabetic CD1 male mice treated with compound **1** (50 mg/kg), metformin (120 mg/kg) and vehicle (tween 80 at 10%, 0.1 mL). The results are the means $\pm$ SEM for four independent experiments.

Figure 9: RT-PCR analysis of GLUT4 expression on adipose tissue after 8 days treatment a) normoglycemic and streptozotocin-nicotinamide diabetic CD1 male mice treated with vehicle (tween 80 at 10%, 0.1 mL); b) streptozotocin-nicotinamide diabetic CD1 male mice treated with compound **1** (50 mg/kg),

metformin (120 mg/kg) and vehicle (tween 80 at 10%, 0.1 mL). The results are the means $\pm$ SEM for four independent experiments.

Figure 10: RT-PCR analysis of PPAR- α expression on adipose tissue after 8 days treatment a) normoglycemic and streptozotocin-nicotinamide diabetic CD1 male mice treated with vehicle (tween 80 at 10%, 0.1 mL); b) streptozotocin-nicotinamide diabetic CD1 male mice treated with compound **1** (50 mg/kg), metformin (120 mg/kg), and vehicle (tween 80 at 10%, 0.1 mL). The results are the means $\pm$ SEM for four independent experiments.

Figure 11: A) RT-PCR analysis of IL-1 β expression on adipose tissue after 8 days treatment a) normoglycemic and streptozotocin-nicotinamide diabetic CD1 male mice treated with vehicle (tween 80 at 10%, 0.1 mL); b) streptozotocin-nicotinamide diabetic CD1 male mice treated with compound **1** (50 mg/kg), metformin (120 mg/kg), and vehicle (tween 80 at 10%, 0.1 mL). The results are the means $\pm$ SEM for four independent experiments. B) RT-PCR analysis MCP1 expression of adipose tissue; a) normoglycemic and streptozotocin-nicotinamide diabetic male CD1 mice treated with vehicle (tween 80 at 10%, 0.1 mL); b) streptozotocin-nicotinamide diabetic male CD1 mice treated with compound **1** (50 mg/Kg), metformin (120 mg/kg), and vehicle (tween 80 at 10%, 0.1 mL). The results are the means $\pm$ SEM for four independent experiments.

Figure 12: 3-D (above) and 2-D (below) binding model of Compound **1** (left) and its carboxylate form (right) into the binding site of PPAR α .

Figure 13: 3-D (above) and 2-D (below) binding model of Compound **1** (left) and its carboxylate form (right) into the binding site of PPAR γ .

Figure 1.

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Figure 2.

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662 **Figure 4.**

Figure 5.

Figure 6.

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Figure 8.

Figure 9.

Figure 10.

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Figure 11.

Figure 12.

Figure 13