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Antidiabetic, antidyslipidemic and toxicity profile of ENV-2: A potent pyrazole derivative against diabetes and related diseases

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ABSTRACT

Diabetes is a major health problem and a predisposition factor for further degenerative complications and, therefore, novel therapies are urgently needed. Currently, cannabinoid receptor 1 (CB_1 receptor) antagonists have been considered as promissory entities for metabolic disorders treatment. Accordingly, the purpose of this work was the evaluation of the sub-acute antidiabetic, anti-hyperglycemic, antidyslipidemic and toxicological profile of **ENV-2**, a potent hypoglycemic and antioxidant CB_1 receptor antagonist. In this study, **ENV-2** showed a pronounced anti-hyperglycemic effect even at a dose of 5 mg/kg ($P < 0.05$) in a glucose tolerance test on normoglycemic rats. Moreover, after administration of **ENV-2** (16 mg/kg) to diabetic rats, a prominent antidiabetic activity was observed ($P < 0.05$), which was higher than glibenclamide. Sub-acute treatment (10 days) of **ENV-2** resulted in a significant reduction of plasma glucose ($P < 0.05$). Also, the levels of peripheral lipids were improved; blood triacylglycerols (TG) and cholesterol (CHOL) were diminished ($P < 0.05$). In addition, it was found that **ENV-2** reduced IL-1β and IL-18 mRNA expression in adipose tissue ($P < 0.05$). Due to the satisfactory outcomes, we were interested in evaluating the toxicity of **ENV-2** in both acute and sub-chronic approaches. Regarding the acute administration, the compound resulted to be non-toxic and was grouped in category 5 according to OECD. It was also found that sub-chronic administration did not increase the size of the studied organs, while no structural damage was observed in heart, lung, liver and kidney tissues. Finally, neither AST nor ALT damage hepatic markers were augmented.

1. Introduction

Metabolic illnesses are of the most important degenerative health problems worldwide. Among those pathologies, diabetes mellitus (DM) is a major endocrine disorder that affects millions of people in the world. The international Diabetes Federation (IDF) estimates that approximately 422 million people were diagnosed with diabetes in 2014, and alarmingly, the number of people suffering this disease will raise to over 642 million by 2040 (Guariguata et al., 2014). Although the pandemic of DM is increasing, the lack of an ideal treatment is still a challenge for modern medicinal chemistry.

In this context, 1,5-diarylpyrazole derivatives, such as rimonabant (**1**) (Fig. 1), have attracted the attention of several research groups due to their metabolic-related pharmacological effects, attributed to the cannabinoid receptor 1 (CB_1 receptor) blockade (Crespo et al., 2008). Amongst those properties, the antidiabetic-related constitute an alternative for DM treatment. For example, the rimonabant analogue AM251 (**2**) (Fig. 1) showed plasma glucose reduction (Irwin et al., 2008), as well as ameliorated the glomerular hypertrophy in diabetic nephropathy (Barutta et al., 2010). In other work, rimonabant was able to improve insulin resistance and lipid metabolism (Nam et al., 2012). On the other hand, CB_1 receptor activation is associated with the interruption of insulin signaling (Kim et al., 2011). The last findings highlight the potential application of CB_1 receptor antagonists in the

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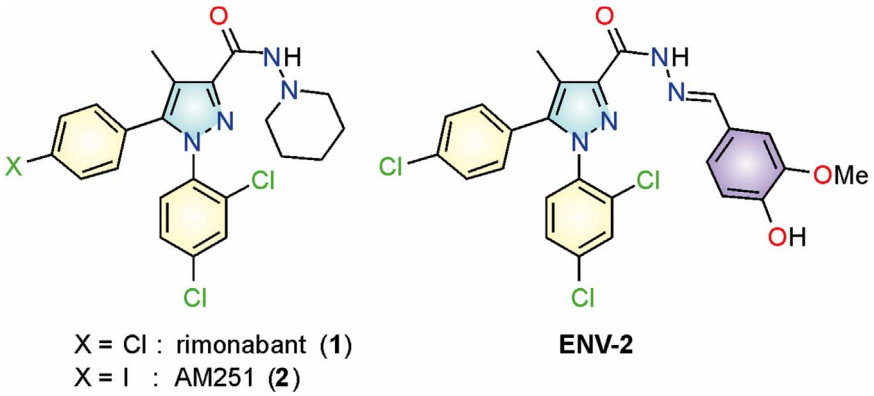

Fig. 1. Structures of the 1,5-diarylpyrazole derivatives rimonabant (**1**), AM251 (**2**) and **ENV-2**.

control and treatment of DM, which, among the well-known anti-obesity properties of rimonabant (Kang and Park, 2012) and its analogues (Méndez-Díaz et al., 2015), make them excellent candidates for further optimization against metabolic syndrome.

During our current investigation concerning the synthesis of novel 1,5-diarylpyrazole derivatives as antidiabetic entities, we synthesized the hybrid **ENV-2** ([(5-(4-chlorophenyl)−1-(2,4-dichlorophenyl)-*N*′-[(*Z*)-(4-hydroxy-3-methoxyphenyl)methylidene]−4-methyl-1*H*-pyrazole-3-carbohydrazide, Fig. 1), a novel dual compound that exhibited both antidiabetic and *in vitro* antioxidant effects (Hernández-Vázquez et al., 2015). **ENV-2** is capable to decrease the plasma glucose levels of diabetic rats in 70%, while the reduction for rimonabant in the same model was about 31% (Hernández-Vázquez et al., 2013). Moreover, the antioxidant properties of **ENV-2** were confirmed by the DPPH and ORAC assays. The above results are relevant since it is well reported the participation of oxidative stress in diabetes and its complications (Giacco and Brownlee, 2010; Rains and Jain, 2011) due to its capability to oxidize and damage lipids, DNA and proteins implicated in insulin resistance development (Evans et al., 2003). Owing to the excellent biological activity of the 1,5-diarylpyrazole **ENV-2**, the purpose of this manuscript was the evaluation of the sub-acute treatment of **ENV-2** in a non-insulin-dependent diabetic rat model to determine its antidiabetic, antidyslipidemic and toxicology effects.

2. Materials and methods

2.1. Materials

Glucose, nicotinamide, streptozotocin, glibenclamide and tween 80 were acquired from Sigma–Aldrich Co. (St. Louis, MO, USA). Sodium pentobarbital was purchased from Pisa Laboratory (Mexico City, Mexico). Metformin and others reagents were purchased from local distributors. Compound **ENV-2** was previously synthetized, purified and characterized (Hernández-Vázquez et al., 2015).

2.2. Animals

Male Wistar rats (200–250 g) and male ICR mice (25–30 g) were provided by FES Iztacala animal facilities, from Universidad Nacional Autónoma de México. Animals were housed in groups of six (n=6) under laboratory conditions (12-h light/dark cycle, 25 ± 2 °C and 45–65% of humidity). Before acute 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), ratified by the Institutional Animal Care and Use Committee (UNAM), based on US National Institutes of Health Publication #85-23, revised 1985.

2.3. Glucose tolerance test

The normoglycemic rats were divided into groups of six animals each (n=6). Thirty min after administration of test samples, a dose of 2 g/kg of glucose solution was administered to each animal. **ENV-2** (5, 16, 28, 50 and 160 mg/kg), metformin (50 mg/kg), and vehicle (tween 80, 10%) were administered to rats in the same volume of solution. Blood samples were collected from the tail tip at 0 (before oral administration), 1, 1.5, 2, 2.5 and 3 h after vehicle, positive control and test sample administration.

2.4. Induction of diabetes

Streptozotocin (STZ) was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in normal physiological saline solution. Sixteen h before diabetes induction all rats were deprived of food, but had access to water *ad libitum*. Non-insulin-dependent diabetes rat model was induced by intraperitoneal administration of 110 mg/kg of nicotinamide 15 min before i.p. injection of 65 mg/kg of streptozotocin (Ortiz-Andrade et al., 2008). Diabetes was confirmed by raised plasma glucose over 200 mg/dl measured by a glucometer (Roche AccuCheck Performa, Mexico City, Mexico).

2.5. Acute antidiabetic assay

The diabetic animals were divided into groups of six animals each (n=6). Rats of experimental groups were i.g. administered with **ENV-2** (prepared in 10% tween 80) (16, 50 and 160 mg/kg body weight). Control group animals were also fed with vehicle. Glibenclamide (5 mg/kg) was used as hypoglycemic reference drug. Blood samples were collected from the caudal vein at 0, 1, 3, 5 and 7 h after vehicle, sample and drug administration. Blood glucose concentration was estimated by enzymatic glucose oxidase method using a commercial glucometer (Accutrend GCT, Roche). The percentage variation of glycemia for each group was calculated in relation to initial (0 h) level, according to: % variation of glycemia=[(Gx-G0)/G0]x100, where G0 were initial glycemia values and Gx were the glycemia values at +1, +3, +5 and +7 h, respectively.

2.6. Sub-acute antidiabetic assay

Animals were randomly divided into 4 groups (n=6): 1) non-diabetic (vehicle, 1 ml/kg), 2) diabetic (vehicle, 1 ml/kg), 3) diabetic reference (metformin was given at a dose of 50 mg/kg), and 4) diabetic treated (**ENV-2** was given at a dose of 50 mg/kg) by intragastric route during ten days. At the end of the treatment, all rats were anesthetized with sodium pentobarbital by i.p. route, and blood samples were obtained by direct cardiac puncture for blood parameters determination. Epididymal fat as a representative of visceral fat was isolated,