Design, synthesis and in combo antidiabetic bioevaluation of multitarget phenylpropanoic acids


Abstract: We synthesized a small series of five 3-[4-arylmethoxy)phenyl]propanoic acids using an easy and short step synthetic route. All compounds were tested in vitro against a set of four protein targets identified as key elements in diabetes: GPR40, aldose reductase (AKR1B1), PPARγ and GLUT-4. Compound 1 displayed an EC50 value of 0.075 μM against GPR40 and was an AKR1B1 inhibitor, showing IC50 = 7.4 μM. Compounds 2 and 3 behave as AKR1B1 inhibitors, GPR40 agonists and showed an increase of 2 to 4-times in the mRNA expression of PPARγ, as well as the GLUT-4 levels. Docking studies were conducted in order to explain the polypharmacological mode of action and the interaction binding mode of the most active compounds on these targets. Compounds 1-3 were tested in vivo at 100 mg/kg dose, being 2 and 3 orally actives, reducing glucose levels in a non insulin-dependent diabetes mellitus mice model. Compounds 2 and 3 showed robust in vitro and in vivo efficacy, and could be considered as promising multitarget antidiabetic drug candidates. This is the first report of a single molecule with these four polypharmacological target action.

Keywords: Diabetes; GPR40; AKR1B1; PPARγ; GLUT-4
### 1. Introduction

Diabetes Mellitus (DM) is a multifactorial metabolic disease that occurs with fasting blood glucose concentrations above 120 mg/dL (>7 mM), due to the abnormal pancreatic β-cells and/or insulin resistance. The susceptibility to environmental influences, and immune dysregulation or inflammation, lead to the variety of hyperglycemic phenotypes within the spectrum of DM [1]. Currently, experimental drug discovery for Type 2 Diabetes Mellitus (T2DM) is focused on developing drugs for insulin sensitizing and/or releasing effect by several mechanisms. One of them is mediated by the G protein-coupled receptor 40 (GPR40, also known as FFA1), which is primarily expressed in pancreatic β-cells and enteroendocrine cells of the gut [2, 3]. The GPR40 activation by medium to long chain fatty acids, stimulates insulin secretion only in the presence of elevated glucose concentration but does not affect insulin exocytosis at low glucose levels [4, 5]. This intriguing mechanism was suggestive in treating T2DM by small molecules agonists for GPR40, thus playing as novel insulin secretagogues with little or no risk of hypoglycemia [6]. An alternative mechanism for the control of glucose levels refers to the peroxisome proliferator-activated receptors (PPARs) action. PPARs, members of the nuclear hormone receptor superfamily, belonging to the ligand activated transcription factors that occur in three subtypes: PPARα, PPARγ, and PPARβ/δ [7]. Each subtype regulates tissue-specific target genes acting as lipid sensors and regulators of glucose homeostasis, such as the solute carrier family 2 (facilitated glucose transporter), member 4 (GLUT-4) [8]. Thiazolidine-2,4-diones function as insulin-sensitizing drugs, through the activation of PPARγ [9, 10], leading to enforced expression of target genes involved in glucose-sensing ability of pancreatic β-cells in diabetic subjects [11]. On the other hand, prolonged hyperglycemia plays a significant role in the development of diabetic complications such as atherosclerosis, neuropathy, end stage renal failure and blindness. The NADPH-dependent reduction of elevated levels of D-glucose to sorbitol catalyzed by aldose reductase (AKR1B1) is considered as one of the phenomena leading to the onset of long term diabetic complications [12]. Stressful and damaging cell conditions are produced by several factors such as the osmotic imbalance due to sorbitol accumulation, the loss of antioxidant power related with NADPH oxidation and the induction of additional advantageous conditions for protein glycation [13-15]. Inhibition of AKR1B1 represents a potential target of antidiabetic drug action. The purpose of the present work was to design, synthesize and screen in vitro, in vivo and in silico (in combo screens) a small series of five 3-[4-arylmethoxy)phenyl]propanoic acids to achieve the treatment of diabetes with a single molecule with multitarget action: activation of PPARγ, GLUT-4 and GPR40 and inhibition of AKR1B1 (Figure 1). This multitarget therapy may be an attractive option for the therapeutic treatment of diabetes that will not only control the glucose level but also reduce acute complications linked to hyperglycemic conditions.
Figure 1. Drug design of multitarget compounds 1–5, which were designed from pharmacophore extraction and reorganization from known modulators of four targets: GPR40, PPAR\(\gamma\), GLUT-4, and AKR1B1.

2. Results and Discussion

2.1. Drug design of derivatives 1–5

Compounds 1–5 were designed on the basis of the structure of the compounds GW9508 [16] and SHF-1 [17], maintaining the typical 4-point pharmacophore of synthetic GPR40 agonists [6], that mimic the fatty acid structure of endogenous agonists (Figure 1): (I) An acidic group (carboxylic acid or related bioisosteres); (II) A central aromatic ring; (III) An extra-lipophilic region and (IV) a flexible spacer that connects regions (I) and (IV), and tolerating the structure to adopt diverse conformations. The insulin-sensitizer targets PPAR\(\gamma\) and GPR40 which are activated by long chain fatty acids and thiazolidine-2,4-diones are described to function as an integrated two-receptor signal transduction pathway [18] being sensitive to closely related pharmacophores, as represented by GW9508 and SHF-1 [17]. Then, it is reasonable to assume that also compounds 1-5 may act as agonists on these targets in a similar fashion.
On the other hand, carboxylic acid derivatives comprise the majority of reported AKR1B1 inhibitors: epalrestat, tolrestat and zopolrestat. The other two chemical groups that function as inhibitors are: spirohydantoins (Sorbinil) and flavonoids such as quercetin [19]. Based on the relevance of the phenylpropanoic scaffold (Fig. 1) in determining the inhibitory ability of zopolrestat towards AKR1B1, we expect that also compounds 1-5 may act as inhibitors of the enzyme. Due to the multifactorial complexity of chronic diseases, the current therapeutic arsenal and the old-fashioned “one-molecule, one-target” model seem not so effective [20]. The design of new chemical entities (NCE) with two or more complementary bioactivities for the treatment of complex diseases would be very advantageous. Based on the above considerations, our work was focused on the rational design, synthesis and evaluation of these novel multitarget molecules to be active against Diabetes. Although compound 1 was previously synthesized [21, 22], in the current work the effectiveness as GPR40 agonist of this compound and of the accompanying molecules is extended and explored in vivo. A parallel investigation on the potential antidiabetic action of these compounds: the activation of PPARγ and GLUT-4 and the inhibition of AKR1B1, is also reported for first time. To our knowledge, this is the first study reporting the potential of the simultaneous targeting of these four relevant processes linked to the diabetic pathology by a single molecule.

2.2. Chemistry

Compounds 1-5 were prepared starting from 4-hydroxybenzaldehyde (6), which was condensed with malonic acid (7), following by microwave Knoevenagel reaction and decarboxylation conditions to give compound 8. This compound was reduced under catalytic hydrogenation with 10% Pd/C affording 3-(4-hydroxyphenyl)propanoic acid (9), which was reacted via esterification with thionyl chloride and ethanol as solvent, obtaining ethyl 3-(4-hydroxyphenyl)propanoate (10). Subsequently, 10 was reacted with the appropriately substituted methylaryl halide in polar aprotic solvents and potassium carbonate to give compounds 11-14. All were treated separately by basic hydrolysis with potassium hydroxide, to give 1-4 (Scheme 1). Compound 5 was obtained by direct SN2 reaction between acid 9 and N-1,3-benzothiazol-2-yl-2-chloroacetamide (15). Title compounds were recovered with 51–95% yields and purified by recrystallization or by column chromatography. Their chemical structures were confirmed by spectroscopic (1D and 2D NMR) and spectrometric data, and their purity determined by microanalysis.
2.3. In vitro GPR40 activity

Compounds 1–5 were tested in vitro as GPR40 agonist. This activity was measured with calcium flux assay in GPR40-transfected HEK293 cells [2, 23]. The preliminary screening results are summarized in Table 1. Linoleic acid (LA), one of the endogenous ligands for GPR40, was selected as positive control.

Compounds were tested in quadruplicates at 100 μM. After preliminary screening, compounds 1–5 were further tested in a concentration-dependent manner (Table 1).

Table 1. Preliminary screening (Efficacy) and GPR40 agonistic activities (Potency) of compounds 1–5 in calcium assay for hGPR40/Gα16/HEK293

<table>
<thead>
<tr>
<th>Comp.</th>
<th>Max Response % (100 μM)</th>
<th>EC50 (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>98±10</td>
<td>0.075±0.02</td>
</tr>
<tr>
<td>2</td>
<td>103±3</td>
<td>0.648±0.10</td>
</tr>
<tr>
<td>3</td>
<td>105±15</td>
<td>0.797±0.17</td>
</tr>
<tr>
<td>4</td>
<td>84</td>
<td>≈100</td>
</tr>
<tr>
<td>5</td>
<td>9.9</td>
<td>&gt;100</td>
</tr>
<tr>
<td>LA</td>
<td>100</td>
<td>8.30±1.7</td>
</tr>
</tbody>
</table>

*a Calcium flux assay in GPR40-transfected HEK293 cells. Means of two experiments.

As we expected, the results indicated that compound 1 was the most potent agonist, in accordance with the literature [21, 22], followed by compounds 2 and 3, with naphthyl and biphenylcarbonitrile substituents, respectively. All of them showed potencies in the nanomolar order. Compound 4, with a
quinolynyl substituent, provided a significant decrease in agonistic activity. Conversely, benzothiazole derivative 5 was inactive. It is noteworthy that the potency of compounds 1-3 is orders of magnitude greater than that obtained by endogenous fatty acid ligand LA.

2.4. In vitro aldose reductase (AKR1B1) inhibition

The in vitro AKR1B1 inhibitory activity of compounds 1-5 (Table 2) was evaluated by using a highly purified human recombinant enzyme preparation [24, 25]. Sorbinil was employed as reference drug.

Table 2. Inhibition of human recombinant AKR1B1 by compounds 1-5

<table>
<thead>
<tr>
<th>Comp.</th>
<th>IC₅₀(μM) ± 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8.9 (6.5 - 12.1)</td>
</tr>
<tr>
<td>2</td>
<td>17.4 (14.3 - 21.2)</td>
</tr>
<tr>
<td>3</td>
<td>21.0 (12.8 – 34.3)</td>
</tr>
<tr>
<td>4</td>
<td>31.7 (22.3 - 44.9)</td>
</tr>
<tr>
<td>5</td>
<td>23.3 (19.0 - 25.5)</td>
</tr>
<tr>
<td>Sorbinil</td>
<td>1.2 (0.9 - 1.5)</td>
</tr>
</tbody>
</table>

Compounds tested were shown to be generally interesting AKR1B1 inhibitors reaching up to low micromolar IC₅₀ values. Once again, compound 1, previously identified as GPR40 agonist, was the most active inhibitor of the series, finding this new activity for this known compound. Compound 2 was the second most active, followed by compound 3 and benzothiazole 5. Compound 4 was the least active. None of them were more active than sorbinil, a well-known AKR1B1 inhibitor. As we described previously, AKR1B1 plays a critical role in the development of chronic diabetic complications, such as retinopathy, nephropathy, neuropathy and also cardiovascular risk. Consequently, this enzyme has been presumed as an attractive target to prevent or even treat these pathologies which are a serious health hazard for diabetic patients.

2.5. Relative expression of PPARγ and GLUT-4

To determine the effect of compounds on PPARγ and GLUT-4 expression, 3T3-L1 fibroblasts were differentiated to the adipocyte phenotype. After that, cells were treated during 24 h with compounds 1-5 and pioglitazone (10 μM) as reference drug and the mRNA expression level evaluated. Results presented in Figure 2 shows that only compounds 2 and 3 increase significantly the levels of relative expression for PPARγ (about three to four-times) and GLUT-4 mRNA’s in the same way as pioglitazone. The activation of PPARγ could decrease the glucose serum levels in diabetic patients’ due to the reduction in insulin resistance. Our data also suggest that compounds 2 and 3 induces GLUT-4 expression in the same way than pioglitazone does. Several evidences indicate that the levels of GLUT-4 expression in skeletal muscle are crucial for the regulation of total body glucose homeostasis [7, 17, 26].
2.6. Molecular docking studies

Based on the in vitro biological assays and the preliminary enzyme inhibition evaluations, the most active compounds were selected to explain the experimental activities on these relevant targets. On this basis, a preliminary molecular docking study was conducted to evaluate the putative binding mode of compounds 1-5 into the receptors GPR40, PPARγ and the enzyme AKR1B1. A pilot in silico calculation was done using DIA-DB [27], a web server for the prediction of antidiabetic drugs via inverse virtual screening of the input molecules 1-5 against a set of 18 protein targets identified as key elements in diabetes, within which are included PPARγ, GPR40 and AKR1B1, among others [28]. Subsequently, a more specific and refined analysis was carried out for the most active compounds (1-3).

Refined molecular docking reveals that compounds 2 and 3 internalize into the ligand binding site of PPARγ and interact by electrostatic and hydrogen bonds with Ser-289, His-323, His-449 and Tyr-473, all of them essential for the activation of this receptor. However, compound 3 (the most active in vitro) showed an additional interaction with Ser-342 (Figure 3). For didactic purpose, the binding mode analysis of compound 1 was analyzed and it shows interactions with residues that are outside from the ligand binding site, which could explain its in vitro inactivity.
Figure 3. (A) 3D binding model of compounds 1-3 into the ligand binding site of PPAR\(\gamma\). Compounds 1 (green), 2 (cyan) and 3 (magenta) are shown as stick models, whereas aminoacids are depicted as lines. A dashed line represents polar interactions. (B) 2D interaction map of the most active compound 3 and PPAR\(\gamma\).

For GPR40, binding poses depicted in Figure 4 suggest that the \textit{in vitro} active compounds 1, 2 and 3 interact through electrostatic bonds with residues of Arg-183 and Arg-2258, and by hydrogen bonds with Tyr-91, Asn-2244, Tyr-2240, all of them showed by well-known GPR40 allosteric agonists (such as TAK-875). On the other hand, the disposition of the biphenyl ring in 1, which was the most potent in the \textit{in vitro} screening, fits into the GPR40 ligand-binding \textit{-pocket} better than the other compounds generating \(\pi-\pi\) interactions with Phe-142 (Figure 4B).

Figure 4. 3D binding model of compounds 1-3 into the allosteric ligand binding site of GPR40. Compounds 1 (green), 2 (cyan) and 3 (magenta) are shown as stick models, whereas aminoacids are depicted as lines. A dashed line represents polar interactions. (B) 2D interaction map of the most active compound 1 and GPR40.

In the case of AKR1B1, solutions of molecular docking into the catalytic site of this enzyme show that acid moieties of compounds 1, 2 and 3 interact with Tyr-48, His-110 and Trp-111 showed in several currently inhibitors of this enzyme, such as zopolrestat and tolrestat. Also, the naphthyl ring of 2...
conserves an interaction with Trp-111 through π–π stacking (Figure 5). All compounds showed moderate
in vitro inhibition of this enzyme.

Figure 5. 3D binding model of compounds 1-3 into the active site of Aldose reductase (AKR1B1). Compounds 1 (green), 2 (cyan) and 3 (magenta) are shown as stick models. (B) 2D interaction map of the second most active compound 2 and AKR1B1.

2.7. In vivo antidiabetic effect of compounds 1-3

Compounds 1-3 were the most active against some of targets identified as key elements in diabetes in this work, and they were selected in order to evaluate their in vivo activity over Streptozotocin and Nicotinamide diabetic induced mice, a well-known non insulin-dependent diabetes mellitus murine model [7, 17]. Glibenclamide was used as a positive control, in order to ensure that the damage over β-cells was partial and mice pancreas still produces insulin, that responds to a secretagogue such as sulfonylurea drug used in this work. The antidiabetic activity of compounds 1-3 were determined using a 100 mg/kg single dose, by intragastric route (Figure 6).

Figure 6. Effect of a single dose (100 mg/kg) of compounds 1-3 and glibenclamide (5 mg/kg) in streptozotocin-nicotinamide-induced diabetes mice model (Intragastric, n = 6). *p < 0.05 versus Tween 80 (10%) group.
In the in vivo assay, compound 1 exhibited erratic pharmacokinetic behavior and poor antidiabetic effect (Figure 6), and it could be explained by probable high degree of ionization at physiological pH, poor permeability due to high ClogP (5.47), elevated protein binding, or susceptibility to phase 1 (hydroxylation) or phase 2 metabolism (glucuronidation). In particular, compounds 2 and 3 showed the best in vivo activity, compared with 1 and control group (Tween 80). Compound 2 producing a sustained decrease of blood glucose levels, close to 80% less, 7 h after compound administration (Figure 6). This compound shown a statistically significant hypoglycemic effect like glibenclamide during the time that the experiment lasted. The glucose lowering effect of compound 3 was increasing after the first hour of administration (-20% at 3 h, -50% at 5 h, and -60% at 7 h). Besides, statistically significant difference is observed between 3 and tween 80 group, and the maximum effect was detected in the last hours of post-intragastric administration (Figure 6).

4. Materials and Methods

4.1. Chemistry

All compounds and solvents were purchased from Sigma-Aldrich and were used without any further purification. Melting points were determined using an EZ-Melt MPA120 automated apparatus from Stanford Research Systems and are uncorrected. Reactions were monitored by TLC on 0.2 mm precoated silica gel 60 F254 Merck plates. 1H NMR spectra were recorded on Varian Oxford (600 MHz) and 13C NMR (150 MHz) instruments. Chemical shifts are given in ppm relative to Me4Si, δ = 0 in DMSO-d6 and CDCl3; J values are given in Hz. The next abbreviations are used: s, singlet; d, doublet; q, quartet; dd, doublet of doublet; t, triplet; m, multiplet; bs, broad signal. MS were recorded on a JEOL JMS-700 spectrometer by electronic impact.

4.2. General procedure for the synthesis of compounds 1-4.

Ethyl esters 11-14 (0.70 mmol) were treated with a mixture of EtOH/H2O (1:1, v/v) and KOH (2 equiv). The mixture was stirred at reflux for 3-5 h. Subsequently, the ethanol was removed under vacuum and HCl solution (10%, v/v) was added until reach pH 5, to obtain a precipitate which was filtered and washed with cold water to give a solid. The crude products were recrystallized from ethanol or methanol affording title compounds.

4.2.1. 3-[4-(Biphenyl-3-ylmethoxy)phenyl]propanoic acid (1): Yield 83%, pearly flakes Mp 123.7-126°C; 1H NMR (600 MHz, DMSO-d6) δ: 2.43 (t, 2H, CH2), 2.74 (t, 2H, CH2), 5.13 (s, 2H, CH2), 6.94 (d, 2H, H-3A, H-5A, jo=7.74 Hz), 7.14 (d, 2H, H-2A, H-6A, jo= 8.04 Hz), 7.4 (t, 1H, H-4C), 7.43 (d, 1H, H-4B, jo=7.2 Hz), 7.48-7.46 (m, 3H, H-6B, H-3C, H-5C), 7.61 (t, 1H, H-5B, jo=7.5 6Hz), 7.66 (d, 2H, H-2C, H-6C, jo=8.22 Hz), 7.72 (s, 1H, H-2C) ppm. 13C NMR (150 MHz, DMSO-d6) δ: 30.4 (CH2), 36.9 (CH2), 69.6 (O-CH2), 115.1 (C-3A), 126.4 (C-6B), 126.6 (C-4B), 127.1 (C-2C, C-6C), 128 (C-4C), 129.4 (C-3C, C-5C), 129.5 (C-5B), 129.7 (C-2A, C-6A), 134 (C-1A), 138.5 (C-1C), 140.4 (C-3B), 157.0 (C-4A), 174.7 (C=O) ppm. MS/EI: m/z (% int. rel). 353 (M+Na), 178 (M+Na -175) 100%. Anal. Calcd for C22H20O3: C 79.50, H 6.06; Found C 79.54, H 6.04.

4.2.2. 3-[4-(1-Naphthylmethoxy)phenyl]propanoic acid (2): Yield 51%, white powder Mp 124.8-127.8°C; 1H NMR (600 MHz, DMSO-d6) δ: 2.49 (t, 2H, CH2), 2.76 (t, 2H, CH2), 5.50 (s, 2H, CH2), 7.0 (d, 2H, H-3A,
subsequent decarboxylation. The flask was shaken well and heated under microwave irradiation system.

1.1 equiv, 2.06 mmol) were suspended in pyridine (5 mL), following Knoevenagel reaction with 4-Hydroxybenzaldehyde 4.4.

Yield 27%, low melting point solid; 1H NMR (600 MHz, CDCl3) δ: 2.66 (t, 2H, CH2), 2.91 (t, 2H, CH2), 5.11 (s, 2H, CH2), 6.93 (d, 2H, H-3A, H-5A, J=8.52 Hz), 7.15 (d, 2H, H-2A, H-6A, J=8.46 Hz), 7.44 (d, 1H, H-4C, J=7.68 Hz), 7.51 (d, 1H, H-6C, J=7.86 Hz), 7.55 (2H, H-3B, H-5B, J=8.22 Hz), 7.58 (2H, H-2B, H-6B, J=8.16 Hz), 7.63 (t, 1H, H-5C, J=7.62 Hz), 7.76 (d, 1H, H-3C, J=7.74 Hz), 12.10 (s, 1H, OH) ppm. 13C NMR (150 MHz, CDCl3) δ: 29.8 (CH3), 35.9 (CH2), 69.6 (CH2), 111.3 (C-2C), 118.6 (CN), 114.9 (C-3A, C-5A), 127.6 (C-4C), 127.7 (C-3B, C-5B), 129 (C-2B, C-6B), 129.3 (C-2A, C-6A), 130.0 (C-6C), 132.8 (C-5C), 133.8 (C-3C), 137.7 (C-1B), 137.8 (C-1C), 145.1 (C-1C), 157.2 (C-O) ppm. MS/El: m/z (% int. rel). 307 (M+) 100%, 142 (M-165) 75%. Anal. Calcd for C19H17NO3: C 77.29, H 5.36, N 3.92; Found C 77.31, H 5.34, N 3.94.

2.3. Synthesis of 3-{4-[2-(1,3-benzothiazol-2-ylamino)-2-oxoethoxy]phenyl}propanoic acid (5).

Yield 95%, yellow crystals m.p 125.8-128.4°C; 1H NMR (600 MHz, CDCl3) δ: 2.49 (t, 2H, CH2), 2.74 (t, 2H, CH2), 5.33 (s, 2H, CH2), 6.97 (d, 2H, H-3A, H-5A, J=8.82 Hz), 7.15 (d, 2H, H-2A, H-6A, J=8.34 Hz), 7.62 (t, 1H, H-6′, J=7.8, J=7.14 Hz), 7.66 (d, 1H, H-3′, J=8.4 Hz), 7.79 (t, 1H, H-7′, J=6.96, J=8.34 Hz), 7.99, d, 1H, H-5′, J=8.16 Hz), 8.02 (d, 1H, H-4′, J=8.46 Hz), 8.41, d, 1H, H-8′, J=8.52 Hz), 12.1 (s, 1H, OH) ppm. 13C NMR (150 MHz, CDCl3) δ: 29.9 (CH2), 35.9 (CH2), 71.2 (CH2), 115.1 (C-3A, C-5A), 119.9 (C-3′, C-5′), 127.01 (C-6′), 128.4 (C-4a), 129.0 (C-7′), 129.8 (C-2A, C-6A), 130.3 (C-5′), 133.8 (C-1A), 137.5 (C-4′), 147.4 (C-8a), 156.9 (C-4A), 158.2 (C-2′), 174.2 (C=O). MS/EI: m/z (% int. rel). 306 (M+), 192 (M-165) 100%. Anal. Calcd for C20H18O3: C 78.41, H 5.92; Found C 78.43, H 5.91.

4.3. Synthesis of 3-[4-[2-(1,3-benzothiazol-2-ylamino)-2-chloroacetamide (9)]

Yield 27%, low melting point solid; 1H NMR (600 MHz, DMSO-d6) δ: 2.41 (t, CH2, H-2), 2.70 (t, CH2, H-3), 4.78 (s, CH2, H-4), 7.00 (t, H-5A, J=7.74 Hz), 7.20 (t, H-6A, J=7.68 Hz), 7.32 (d, H-2, H-6B, J=8.64 Hz), 7.45 (s, H-3B, H-5B), 7.64 (d, H-4A, H-7A, J=7.80 Hz). 13C NMR (150 MHz, DMSO-d6) δ: 19.24 (C-3), 35.09 (C-2), 65.00 (C-4), 119.47 (C-4B), 120.16 (C-1B), 122.86 (C-4A, C-7A), 125.98 (C-3B, C-5B), 125.99 (C-2B, C-6B), 130.55 (C-5A, C-6A), 134.17 (C-7A), 134.29 (C-3A), 136.06 (C-2 A), 157.95 (C-5), 171.56 (C-1); MS-ESI: m/z 357 (M + H+). Anal. Calcd for C30H26N4O8S: C 60.66, H 4.53, N 7.86; Found C 60.71, H 4.54, N 7.84.

4.4. Synthesis of (2E)-3-(4-hydroxyphenyl)acrylic acid (8).

4-Hydroxybenzaldehyde 6 (0.25 g, 2.04 mmol), β-alanine (0.1 equiv, 0.20 mmol) and malonic acid 7 (0.21g, 1.1 equiv, 2.06 mmol) were suspended in pyridine (5 mL), following Knoevenagel reaction with subsequent decarboxylation. The flask was shaken well and heated under microwave irradiation system.
(CEM Discovery Microwave System apparatus, 2450 MHz, 300 W) fitted with reflux condenser for 10 min at 70 °C. After irradiation, the mixture was poured onto cold water and acidified until pH 5. The precipitate was collected by filtration, washed with water, dried and recrystallized from ethanol. Yield 80 %, yellow crystals Mp 214°C (dec); 1H NMR (600 MHz, DMSO-d6) δ: 6.35 (d, 1H, =CH trans, J= 16 Hz), 6.86 (d, 2H, H-3’, H-5’, J_o=8.4 Hz), 7.55 (d, 2H, H-2’, H-6’, J_o= 8.4 Hz), 7.58 (d, 1H, =CH  trans, J= 16 Hz), 8.87 (bs, 2H, OH, COOH) ppm. 13C NMR (150 MHz, DMSO-d6) δ: 115.4 (C-2), 116.2 (C-3’, C-5’), 126.1 (C-1’), 130.4 (C-2’, C-6’), 145.3 (C-3), 158.3 (C-4’), 168.6 (C=O) ppm. MS/EI: m/z (% int. rel). 164 (M+).

4.5. Synthesis of 3-(4-hydroxyphenyl)propanoic acid (9).

A mixture of acid 8 (0.2 g, 1.20 mmol) and Pd/C 10% (0.02 g) in ethanol (40 mL) was reduced with H2 at 60 lb/in2 in a hydrogenation apparatus for 30 min. After filtration and evaporation of the solvent under reduce pressure a yellow oil was obtained. After cooling, a solid compound (0.2 g, 99 % yield) was obtained which purificated by recrystallization in ethanol. Yield 99 %, yellow crystals Mp 129-131°C; 1H NMR (600 MHz, DMSO-d6) δ: 2.45 (t, 2H, CH2), 2.72 (t, 2H, CH2), 6.65 (d, 2H, H-3´, H-5´, J_o=8.01 Hz), 7.05 (d, 2H, H-2´, H-6´, J_o= 8.02 Hz), 9.54 (bs, 2H, OH, COOH) ppm. 13C NMR (150 MHz, DMSO-d6) δ: 30.1 (CH2), 36.9 (CH2), 115.5 (C-3´, C-5´), 129.5 (C-2´, C-6´), 131.8 (C-1´), 155.5 (C-4´), 175.1 (C=O) ppm. MS/EI: m/z (% int. rel). 166 (M+).

4.6. Synthesis of ethyl 3-(4-hydroxyphenyl)propanoate (10).

To a stirred solution of acid 9 (3 g, 18 mmol) in 30 mL of ethanol, under ice-cooling, was added thionyl chloride (1.1 eq, 1.44 mL, 19 mmol) dropwise over 20 minutes. After stirring the reaction mixture for 3 h at room temperature, methanol is distilled out and 25 mL of water is added. The separated ester is extracted with ethyl acetate and washed with 10 mL of saturated sodium bicarbonate solution. Evaporation of the ethyl acetate gave the ester in pure form. Yield 94 %, yellow liquid; 1H NMR (600 MHz, CDCl3) δ: 1.22 (d, 3H, CH3), 2.59 (t, 2H, CH2), 2.87 (t, 2H, CH2), 4.12, q, 2H, CH2), 6.74 (d, 2.09, 6.48, H-3A-H-5A), 7.03 (d, 2H, 6.54, H-2A, H-6A). 13C NMR (150 MHz, CDCl3) δ: 14.2 (CH3), 30.2 (CH 2), 36.3 (CH2), 60.6 (CH2), 115.4 (C-3A, C-5A), 129.4 (C-2A, C-6A), 154.3 (C-4A), 173.6 (C=O). MS/EI: m/z (% int. rel). 194 (M+) 49 %, 142 (M-74) 100%.

4.7. General procedure for the synthesis of compounds 11-14

A solution of ethyl 3-(4-hydroxyphenyl)propanoate 10 (0.5 g, 2.5 mmol) and K2CO3 (0.71 g, 5.1 mmol, 2 equiv) in appropriate polar aprotic solvent (5 mL acetonitrile or acetone) was heated at 40°C for 30 minutes. Adequately substituted methylaryl halides (1.1 equiv.) were added dropwise. The mixture was heated to reflux for 8 h. The solvent was removed under reduced pressure. Cold water (10 mL) was added and the resulting emulsion was extracted with ethyl acetate (3 x 15 mL). The solvent was removed in vacuo to give an oily product, which were purified by column chromatography.

4.7.1. Ethyl 3-[4-(biphenyl-3-ylmethoxy)phenyl] propanoate (11). Yield 65 %, colorless liquid; 1H NMR (600 MHz, CDCl3) δ: 1.21 (t, 3H, CH3), 2.58 (t, 2H, CH2), 2.88 (t, 2H, CH2), 4.11 (c, 2H, CH2), 6.74 (d, 2H, 6.48, H-3A-H-5A), 7.11 (d, 2H, 6.54, H-2A, H-6A). 13C NMR (150 MHz, CDCl3) δ: 14.2 (CH3), 30.2 (CH2), 36.3 (CH2), 60.6 (CH2), 115.4 (C-3A, C-5A), 129.4 (C-2A, C-6A), 154.3 (C-4A), 173.6 (C=O). MS/EI: m/z (% int. rel). 194 (M+) 49 %, 142 (M-74) 100%.
60.3 (CH\textsubscript{2}'), 70.1 (CH\textsubscript{2}), 114.9 (C-3A, C-5A), 126.2 (C-4B), 126.3 (C-2B), 126.7 (C-BB), 127.2 (C-2C, C-6C), 127.4 (C-5B), 128.9 (C-3C, C-5C), 129 (C-4C), 129.3 (C-2A, C-6A), 133.0 (C-1A), 137.7 (C-1C), 140.9 (C-1B), 141.5 (C-3B), 157.2 (C-6A), 172.9 (C=O) ppm.  MS/EI: m/z (% int. rel). 360(M +) 31 %, 167(M-193) 100%.

4.7.2. Ethyl 3-[4-(1-naphthylmethoxy)phenyl]propanoate (12). Yield 78 %, colorless liquid; \(\delta\) ppm: 1.23 (t, 3H, CH\textsubscript{3}), 2.59 (t, 2H, CH\textsubscript{2}) 2.90 (t, 2H, CH\textsubscript{2}) 4.12 (c, 2H, CH\textsubscript{2}'), 5.44 (s, 2H, CH\textsubscript{2}), 6.97 (d, 2H, 7.14, H-3A, H-5A) 7.14 (d, 2H, \(J_o= 7.68\), H-2A, H-6A) 7.44 (t, \(J_o= 8.22\), H-3') 7.52-7.49 (m, 2H, H-6', H-7') 7.57 (d, 1H, \(J_o= 6.9\), H-2') 7.83 (d, 1H, \(J_o= 8.22\), H-4') 7.85 (d, 1H, \(J_o= 7.5\), H-5') 8.03 (d, 1H, \(J_o= 8.64\)) ppm.  13C NMR (150 MHz, CDCl\textsubscript{3}) \(\delta\): 14.2 (CH\textsubscript{3}), 30.2 (CH\textsubscript{2}), 36.2 (CH\textsubscript{2}), 60.4 (CH\textsubscript{2}'), 68.7 (CH\textsubscript{2}), 114.9 (C-3A, C-5A), 123.7 (C-8'), 125.3 (C-3'), 125.9 (C-6'), 126.5 (C-7'), 128.7 (C-2'), 128.9 (C-5'), 129.3 (C-2A, C-6A), 131.5 (C-4'), 132.4 (C-8a), 133.1 (C-4a), 133.7 (C-1'), 157.4 (C-4a), 172.9 (C=O) ppm.  MS/EI: m/z (% int. rel). 334 (M+) 11 %, 141(M-193) 100%.

4.7.3. Ethyl 3-{4-[(2'-cyanobiphenyl-4-yl)methoxy] phenyl}propanoate (13). Yield 64%, white crystals; \(\delta\) ppm: 1.14 (t, 3H, CH\textsubscript{3}), 2.56 (t, 2H, CH\textsubscript{2}) 2.78 (t, 2H, CH\textsubscript{2}) 4.03 (c, 2H, CH\textsubscript{2}'), 5.16 (s, 2H, CH\textsubscript{2}) 6.96 (d, 2H, \(J_o= 8.04\), H-3A, H-5A) 7.15 (d, 2H, \(J_o= 8.04\), H-2A, H-6A), 7.64-7.60 (m, 6H, H-2A, H-3A, H-5A, H-6A, H-4C, H-6C), 7.79 (t, 1H, \(J_o= 7.56\), H-5C) 7.95 (d, 1H, \(J_o= 6.9\), H-2') 7.83 (d, 1H, 7.5, H-4') 7.85 (d, 1H, 7.5, H-5') 8.03 (d, 1H, 8.55, H-8') ppm.  13C NMR (150 MHz, CDCl\textsubscript{3}) \(\delta\): 14.5 (CH\textsubscript{3}), 29.1 (CH\textsubscript{2}), 35.8 (CH\textsubscript{2}), 60.2 (CH\textsubscript{2}'), 69.1 (CH\textsubscript{2}), 110.6 (C-2C), 115 (H-3A, H-5A), 119 (CN), 128.7 (C-4C), 129.2 (C-2B, C-6B), 129.7 (C-2A, C-6A), 130.5 (C-6C), 133.2 (C-1A), 134 (C-5C), 134.3 (C-3C), 137.7 (C-4B), 138.3 (C-1B), 144.7 (C-1C), 157.2 (C-4A), 172.5 (C=O) ppm.  MS/EI: m/z (% int. rel). 385 (M+) 11%, 192(M-193) 100%.

4.7.4. Ethyl 3-[4-(quinolin-2-ylmethoxy)phenyl]propanoate (14). Yield 72%, yellow liquid; \(\delta\) ppm: 1.47 (t, 3H, CH\textsubscript{3}), 2.50 (t, 2H, CH\textsubscript{2}) 2.75 (t, 2H, CH\textsubscript{2}) 4.01 (c, 2H, CH\textsubscript{2}'), 5.32 (s, 2H, CH\textsubscript{2}), 6.96 (d, 2H, \(J_o= 6.66\), H-3A, H-5A) 7.13 (d, 2H, \(J_o= 8.58\), H-2A, H-6A), 7.62, (d, 1H, \(J_o= 7.35\), H-6'), 7.65 (d, 1H, \(J_o= 8.49\), H-3'), 7.79 (dd, 1H, \(J_o= 8.28\), H-7'), 7.97 (dd, 1H, \(J_o= 8.85\), H-5'), 8.01 (dd, 1H, \(J_o= 8.64\), H-4'), 8.39 (d, 1H, \(J_o= 8.55\), H-8') ppm.  13C NMR (75 MHz, CDCl\textsubscript{3}) \(\delta\): 14.0 (CH\textsubscript{3}), 29.4 (CH\textsubscript{2}), 35.4 (CH\textsubscript{2}), 59.7 (CH\textsubscript{2}'), 70.7 (CH\textsubscript{2}), 114.6 (C-3A, C-5A), 119.5 (C-3'), 127.1 (C-6'), 128.1 (C-2B, C-6B), 128.7 (C-2A, C-6A), 130.5 (C-6C), 133.2 (C-1A), 134 (C-5C), 134.3 (C-3C), 137.7 (C-4B), 138.3 (C-1B), 144.7 (C-1C), 157.2 (C-4A), 172.5 (C=O) ppm.  MS/EI: m/z (% int. rel). 335 (M+) 11%, 192(M-193) 10%.

A mixture 2-amino-1,3-benzothiazol (0.100 g) and sodium bicarbonate (3 equiv.) was dissolved in acetone (10 mL) and was stirred at room temperature. After 30 min, chloroacetyl chloride (1.5 equiv.) was added dropwise using a drop funnel apparatus and was stirred for 8 h. After the reaction was completed, the solvent was removed under reduced pressure, and the precipitated white solid was washed with cold water. Yield: 90 %, mp: 156.5-159.3°C; \(\delta\) ppm: 4.32 (s, CH\textsubscript{2}), 7.36 (t, H-6, \(J_o=8.10\) Hz), 7.48 (t, H-5, \(J_o=8.08\) Hz), 7.84 (q, H-4, H-7, \(J_o=7.92\) Hz); MS-EI: m/z 225.75 (M+)40%, 149.61(M-77) 100%.

4.3. Biological assays.
4.3.1. GPR40 agonistic activities of compounds 1-5
This bioassay was previously described [2]. Briefly: About 4×10\textsuperscript{4} hGPR40/HEK293 per well were seeded in 96-well plate. After culturing for 24 h, cells were loaded with 2 \(\mu\)mol/L Fluo-4 AM in Hanks’ balanced salt solution at 37°C for 40 minutes. After thorough washing with 50 \(\mu\)L assay buffer, 25 \(\mu\)L assay buffer...
containing compound was dispensed into the well, using a FlexStation III microplate reader and intracellular calcium change was recorded with an excitation wavelength of 485 nm and emission wavelength of 525 nm.

4.3.2. Aldose reductase (AKR1B1) inhibition assay

Human recombinant AKR1B1 was purified to electrophoretic homogeneity as previously described [24]. The enzymatic activity was measured at 37 °C following the decrease of absorbance at 340 nm. The assay mixture (final volume 0.7 mL) contained, in 0.25 M sodium phosphate buffer pH 6.8, 4.67 mM D,L-glyceraldehyde, 0.11 mM NADPH, 0.38 M (NH₄)₂SO₄ and 0.5 mM EDTA. One Unit of enzyme activity refers to the amount of enzyme that catalyses the oxidation of 1 μmol/min of NADPH in the above conditions. Compounds 1-5, tested as AKR1B1 inhibitors were dissolved at proper concentrations in a mixture DMSO/methanol (40:60, v/v) and added to the above described assay mixture containing 5 mU of purified AKR1B1; the concentrations of DMSO and methanol in all the assays were kept constant at 0.2 and 0.3% (v/v), respectively. IC₅₀ values was determined by nonlinear regression analysis by fitting the data to the equation describing one site competition in a log concentration-inhibition curve. For each curve, at least five different concentrations of inhibitor were analyzed [25].

4.3.3. In vitro PPARγ and GLUT-4 assay

Both assays were performed as previously described [7, 17, 29-31]. Briefly: 3T3-L1 fibroblasts (9×10⁵ cells per well) were cultured in 6-well plates in Dulbecco’s modified Eagle’s medium. After 2 days of confluence, the cells were differentiated to the adipocyte phenotype with 0.5 mM 3-isobutyl-1-methylxanthine, 0.25 μM dexamethasone acetate, and 0.8 μM insulin, for 48 h, followed by insulin alone for other 48 h. To determine the effect of compounds on PPARγ and GLUT-4 expression, the cells were treated by 24 h. RNA was isolated from cultured cells and two μg of total RNA were reverse-transcripted in a thermocycler. The enzyme was inactivated and finally samples were cooled. Then 1/10 volume of each reverse-transcripted reaction was amplified with SYBR Green master mix containing 0.5 mM of customized primers for PPAR-γ and GLUT-4. PCR was conducted and the threshold cycles (Ct) were measured in separate tubes and in duplicate. The ΔCt values were calculated in every sample for each gene of interest. Relative changes in the expression level of one specific gene (ΔΔCt) were calculated [31].

4.4. In vivo Assay

CD1 mice (20-25 g) were housed in groups of six (n = 6) under laboratory conditions. 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. Acute antidiabetic assay: Non-insulin-dependent diabetes mice model was induced in overnight fasted mice by a single i.p. injection of 100 mg/kg STZ, 15 min before the i.p. administration of 40 mg/kg of nicotinamide. Hyperglycemia was confirmed by the higher glucose concentration in plasma, determined at 2 weeks by glucometer. The animals with blood glucose concentration higher than 200 mg/dL were used for the screening [32-35]. The diabetic animals were divided into three groups of six animals each (n=6). Mice of experimental groups were administered a suspension of the compounds 1-3 (100 mg/kg, prepared in 10% tween 80). Control group animals were also treated with 10 % tween 80. 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 administration. Blood glucose concentration was estimated using a commercial
The percentage variation of glycemia for each group was calculated. All values were expressed as mean ± S.E.M. Statistical significance was estimated by analysis of variance (ANOVA).

4.5. In silico docking studies

The crystal structures of PPARγ, GPR40 and aldose reductase were retrieved from the PDB with the accession codes: 1I7I, 4PHU and 1Z3N respectively. Docking calculations were conducted with AutoDock, version 4.2. The program performs several runs in each docking experiment. Each run provides one predicted binding mode. All water molecules and every co-crystall ligands were removed from the crystallographic structure and all hydrogen atoms were added. For all ligands and proteins, Gasteiger charges were assigned and non-polar hydrogen atoms were merged. All torsions could rotate during docking. The auxiliary program AutoGrid generated the grid maps. Each grid was centered at the crystallographic coordinates of the crystallographic compound with dimensions of 60 x 60 x 60 Å with points separated by 0.375 Å. The number of docking runs was 100. After docking, all solutions were clustered into groups with RMSD lower than 2.0 Å. Discovery Studio version 3.5, Pymol version 1.0 and MOE [38] were used for visualization.

4.5.1. Docking Validation

The molecular docking protocols were validated by re-docking of co-crystal ligands into the active site of the structure of each protein target: PPARγ (Tesaglitazar), GPR40 (TAK-875) and aldose reductase (Lidorestat). The RMSD between the co-crystal ligand and the docked structure was less than 2.0 Å in all cases. This value indicates that the parameters for docking simulations are good in reproducing orientation and conformation in the X-ray crystal structure of enzyme and receptors.

5. Conclusions

Five phenylpropanoic acid derivatives active on different relevant targets involved in the control of glucose level and in the cell damage linked to hyperglycaemic conditions, are presented as promising antidiabetic compounds. In fact, compounds 1 to 3 are able with different effectiveness to combine a significantly increase in the mRNA expression of PPARγ, GLUT-4, with a GPR40 agonist action and a well detectable inhibition of the AKR1B1 activity. Compounds 2 and 3 also revealed their antidiabetic effectiveness in vivo, showing a robust hypoglycemic effect similar to glibenclamide. The in vitro satisfactory multtargeting anti-diabetic profile and the in vivo hypoglycemic activity make them suitable leads in developing new chemical entities with polypharmacological ability to be used for treatment of diabetes and its complications. To our knowledge, this is the first study reporting a multiple combined action of a single molecule facing towards four relevant targets involved into cellular glucose handling.

Supplementary Materials: Spectra are available online.

Acknowledgments: This work was supported in part by the Consejo Nacional de Ciencia y Tecnología (CONACyT) under grant No. 253814 (Ciencia Básica 2015) given to G. Navarrete-Vázquez. B. Colín-Lozano acknowledges the fellowship awarded by CONACyT (378373) to carry out Doctoral studies. This article is dedicated to all the people, mainly Pharmacist, who helped in the medication management and classification after the recent Mexico’s earthquake.
Author Contributions: B. Colín-Lozano: performed the chemical synthesis of all compounds, did the acquisition of antidiabetic in vitro and in vivo data, and analyzed the chemical and biological results. S. Estrada-Soto, F. Chavez Silva and L. Cerón Romero: Carried out and interpreted the antidiabetic assays. A. Gutierrez-Hernandez: performed and analyzed the in silico refined molecular docking into the PPARγ, GPR40 and AKR1B1. A. Giacoman-Martinez and J. C. Almanza-Perez: Conducted and interpreted the in vitro relative expression of PPARγ and GLUT-4 experiments. E. Hernández-Núñez: performed and interpreted the spectroscopic analysis using nuclear magnetic resonance in 1 and 2 dimensions. He also wrote the Supplementary Information. Z. Wang and X. Xie: performed and supervised the in vitro GPR40 experiments and edited the manuscript. M. Capiello, F. Balestri and U. Mura: performed the in vitro aldose reductase inhibition assay, interpreted the data for SAR analysis, contributed with reagents and analysis tools, drafted some parts of the manuscript and did a critical revision. G. Navarrete-Vázquez: Developed the concept, designed the compounds, analyzed and interpreted data, prepared and wrote the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

References


27. http://bio-hpc.ucam.edu/dia-db/


38. Molecular Operating Environment (MOE), 2016.08; Chemical Computing group ULC, 1010 Sherbooke St. West, Site #910, Montreal, QC, Canada, 2018. http://www.chemcomp.com

**Sample Availability:** Samples of the compounds are available from the authors.