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Discovery, synthesis and in combo studies of a tetrazole analogue of clofibric acid as a potent hypoglycemic agent

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ABSTRACT

A tetrazole isosteric analogue of clofibric acid (**1**) was prepared using a short synthetic route and was characterized by elemental analysis, NMR (^{1}H, ^{13}C) spectroscopy, and single-crystal X-ray diffraction. The in vitro inhibitory activity of **1** against 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) was evaluated, showing a moderate inhibitory enzyme activity (51.17% of inhibition at 10 μM), being more active than clofibrate and clofibric acid. The antidiabetic activity of compound **1** was determined at 50 mg/Kg single dose using a non insulin dependent diabetes mellitus rat model. The results indicated a significant decrease of plasma glucose levels, during the 7 h post-administration. Additionally, we performed a molecular docking of **1** into the ligand binding pocket of one subunit of human 11β-HSD1. In this model, compound **1** binds into the catalytic site of 11β-HSD1 in two different orientations. Both of them, show important short contacts with the catalytic residues Ser 170, Tyr 183, Asp 259 and also with the nicotinamide ring of $NADP^{+}$.

Fibrates, such as bezafibrate, clofibrate and fenofibrate are used as therapeutic agents in the treatment of dyslipidemia, heart disease and diabetic complications in humans, due to the fact that they are agonists of the nuclear receptor PPAR α (Peroxisome Proliferator-Activated Receptor).[1,2] The fibrates are a widely used class of lipid-modifying agents that decrease plasma triglycerides.[3,4] The fibrate pharmacophore has been of interest to medicinal chemists ever since the initial discovery that ethyl chlorophenoxyisobutyrate (clofibrate) possessed hypolipidemic properties.[5] Clofibrate is a prodrug which is biotransformed into clofibric acid, the active metabolite which binds to PPARα.

Recent studies showed that fenofibrate but not fenofibric acid, exerted a potent inhibitory activity against the enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1),[6] as well as negatively modulated the expression of 11β-HSD1 mRNA in hepatocytes.[7]

Several lines of evidence have implicated glucocorticoids and 11β-HSD1 activity in the etiology and/or maintenance of type 2 diabetes mellitus and metabolic syndrome. The glucocorticoids, such as cortisol, are potent antagonists of insulin action and promoters of gluconeogenesis in liver, leading to an increase in blood glucose concentration.[8] 11β-HSD1 catalyzes the conversion of inactive cortisone into the active hormone cortisol (in rodents 11-dehydrocorticosterone to corticosterone). 11β-HSD1 inhibitors are therefore of considerable interest as potential treatments for a number of diseases including type 2 diabetes, obesity and metabolic syndrome.[9]

The majority of fibrates are prodrugs, which are extensively metabolized by hydrolysis, leading to the free carboxylic acid form. Medicinal chemists have frequently faced the problem of developing surrogates for carboxylic acid groups. Planar acidic heterocycles, such as tetrazole, are commonly used as carboxylic acid bioisosteres.[10] This replacement improves the bioavailability, increases the potency and improves chemical stability.[11]

In our ongoing research on fibrate derivatives with antihyperlipidemic and antidiabetic activities, we have synthesized in this work the compound 2-(4-chlorophenoxy)-2-methyl-*N*-(1*H*-tetrazol-5-yl) propanamide (**1**), which is an isostere of clofibric acid, with a tetrazolamide group instead of carboxylic acid (Fig. 1).

The design of the compound **1** was based on the bioisosteric replacement of the carboxylic acid moiety by a tetrazole ring. In order to search for new compounds with improved biological activity, the present investigation deals with the synthesis of compound **1**, as outlined in Scheme 1, which might have useful biological and therapeutic activities. The crystal structure of title

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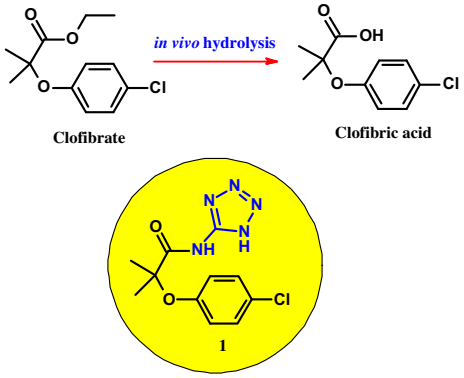
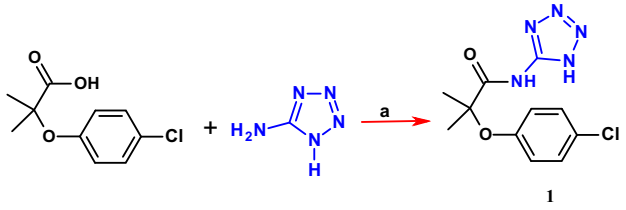


Figure 1. Metabolism of fibrates leading to carboxylic acid form, and the tetrazole isostere prepared in this work (**1**).



Scheme 1. Preparation of **1**, (a) DCC, DMAP (catalyst), methylene chloride, room temperature.

compound determined by single-crystal X-ray diffraction, the in vitro inhibition of 11β-HSD1, the in vivo hypoglycemic effect, as well as a theoretical and predictive mode of binding of compound with the enzyme is also reported here.

In vitro screens combined with in silico and in vivo assessments leading to the concept of in combo screening in drug discovery.[12]

Compound **1** was prepared in a single-step reaction, starting from the 2-(4-chlorophenoxy)-2-methylpropionic acid and 1*H*-tetrazol-5-amine, using dicyclohexylcarbodiimide as a coupling agent (Scheme 1).

Compound **1** was purified by recrystallization. The chemical structure was confirmed on the basis of its NMR (^{1}H, ^{13}C) spectroscopic data,[13] as well as mass spectrometry, the crystal structure was determined by single-crystal X-ray diffraction, and their purity ascertained by microanalysis.

A single crystal of **1** was grown at room temperature by slow evaporation of ethanol solution. Figure 2 shows the supramolecular synthon **I** with the atom labelling system. The most relevant crystallographic data and hydrogen bond geometry are summarized in the Supplementary data. [14]

The X-ray structure revealed that in compound **1** the unit cell contains two independent molecules with different conformation. The dihedral angles involving atoms O1–C7–C8–N1 and O3–C18–C19–N6 are −40.3 (2) and −10.3 (2)°. The conformers are coupled through bifurcated N–H N and N–H O hydrogen bonds. In a recent paper, we reported some other amide derivatives from clofibric acid and 2-aminobenzothiazole and all of them crystallized showing hypervalent contacts (C═O···S, S···S), hydrogen bonds Y–H X (Y = O, N, C; X = O, N, Cl, π) and van der Waals contacts (Cl···π, S···π, H···H).[15]

A preliminary in vitro compound screen to identify inhibitors of human 11β-HSD1 was performed. Inhibition of 11β-HSD1 was determined using a human embryonic kidney (HEK293) cell-based assay.[9,14,16] Compound **1**, clofibric acid and clofibrate were tested

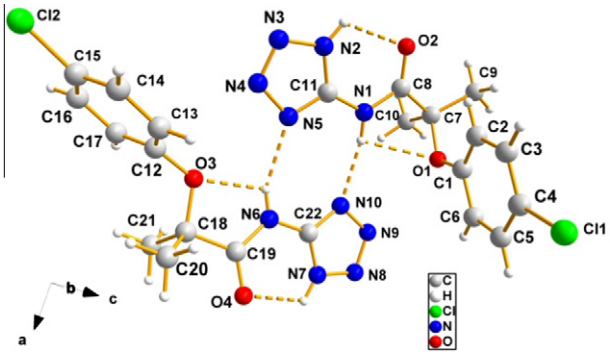


Figure 2. A view of the supramolecular synthon **I**, showing the atom labelling scheme. Bifurcated N–H···N and N-H···O hydrogen bonds are represented by dashed lines.

Table 1
Experimental in vitro inhibition effect of compounds against 11β-HSD1

Compound	% of Inhibition 11β-HSD1 @ [10 μM]
1	51.17
Clofibric acid	19.14
Clofibrate	18.11
Carbenoxolone	78.32

and showed 51.17%, 19.14% and 18% of inhibition at 10 μM, respectively (Table 1).

Results presented in Table 1 show that **1** was 2.5 times more effective inhibiting 11β-HSD1 than clofibric acid and clofibrate. The formation of the amide and the replacement of carboxylic acid by a tetrazole scaffold increased the potency against 11β-HSD1. The inhibition of this enzyme could reduce the glucose serum levels in diabetic patients due to the low production of cortisol.

In order to gain an insight into the binding mode of **1**, it was docked into the catalytic site of the human 11β-HSD1 (PDB code: 2-BEL) using the program AutoDock 4.0.[14] According to the results obtained from LigPrep, four tautomeric forms and one ionized state coexist at pH 7.0 ± 2.0. The p*K*a value calculated for the neutral form of the tetrazole ring of compound **1** is 2.89, which means that only the ionized state is possible at the pH experimental value (7.0). Even though only the ionized state is possible at pH 7.0, docking calculations were performed also on the tautomeric forms. The lowest binding energies calculated by AutoDock for the tautomeric forms were −7.14, −7.62, −7.83, and −7.79 kcal/mol and for the ionized state was −7.97 kcal/mol. Taken together, these results suggest that the ionized state is the most favorable form that binds into the catalytic site of 11β-HSD1. Analysis of the binding mode of **1** hereafter will be focused on the ionized state. Figure 3 shows the two top ranked binding modes of **1** found by AutoDock with docking energies of −7.97 and −7.62 kcal/mol, respectively. The first binding mode (Fig. 3A) is characterized by the ligand making contacts with the catalytic residue Ser170; as well as the polar residues Tyr177, Gly216, His232, Gln234, Tyr258, and Asp259, which makes a hydrogen bond with the tetrazole ring (Fig. 3A). Other hydrophobic contacts are made with Leu171, Ala172, Leu217, Ile218, Met233, Ala235, Ala236, and Leu262. In this binding model, **1** is blocking the entrance of the catalytic site. It is important to mention that only 14.5% of the solutions found by AutoDock fell into this orientation. The second binding mode in Figure 3B is differentiated from the first one in that compound **1** is located at the opposite side of the binding cavity making contacts with the catalytic residues Ser170 and Tyr183; other short contacts are made with the polar residues Tyr177, Gly216, Thr222 and hydrophobic

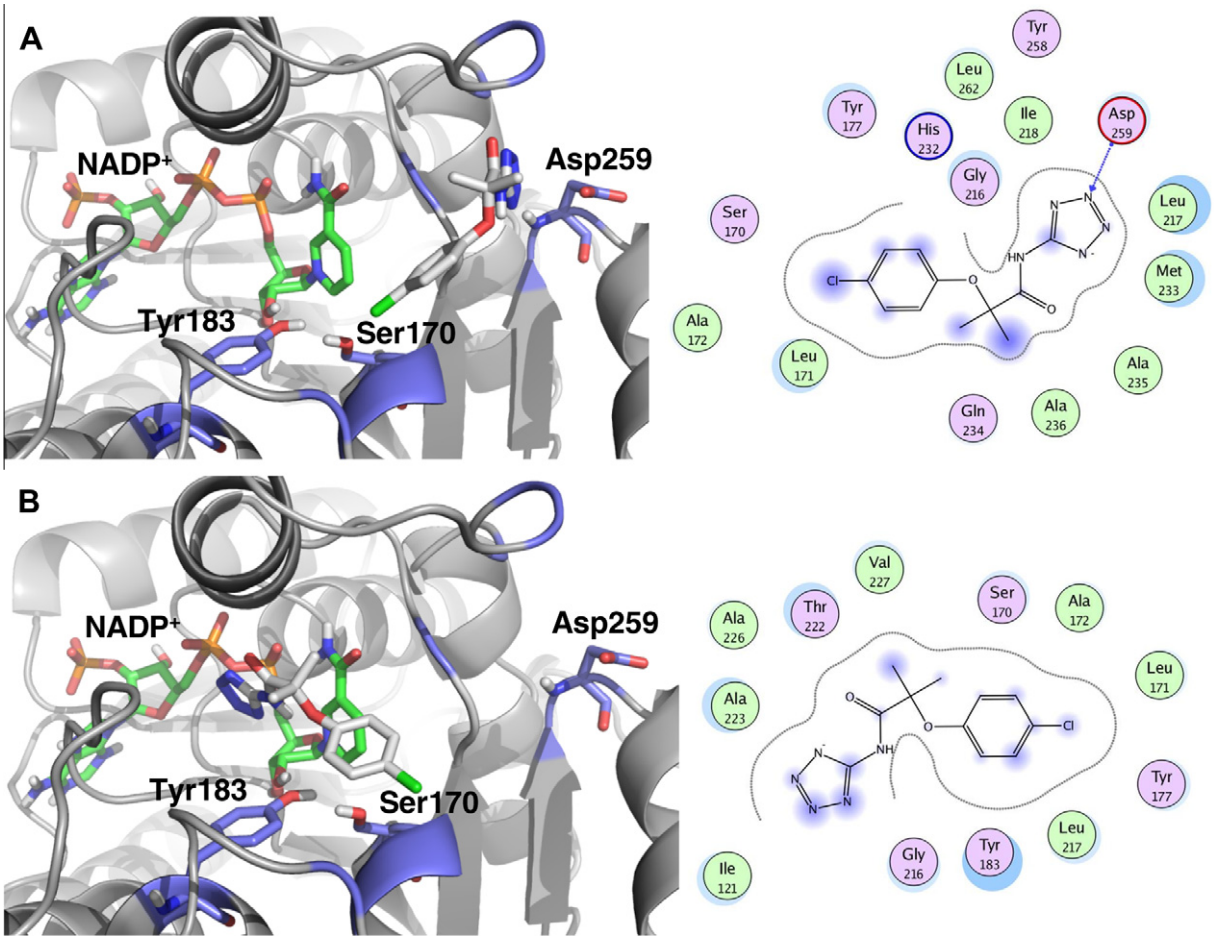


Figure 3. The two top ranked binding modes and ligand interaction diagrams of compound **1** into the catalytic site of 11β-HSD1. (A) Binding mode and ligand interaction diagram of compound **1** with the lowest binding energy (−7.97 kcal/mol). (B) Binding mode of compound **1** with second lowest binding energy (−7.62 kcal/mol). The active residues Ser170 and Tyr183 are represented in cobalt blue, $NADP^+$ in green and **1** in silver. The ligand interaction diagrams are represented as follow: The ligand proximity contour is depicted with a dotted line. The ligand solvent exposure is represented with blue circles; larger and darker circles on ligand atoms indicate more solvent exposure. The receptor solvent exposure differences, in the presence and absence of the ligand, are represented by the size and intensity of the turquoise discs surrounding the residues; larger and darker discs indicate residues highly exposed to solvent in the active site when the ligand is absent.

contacts with Ile121, Leu171, Ala172, Leu217, Ala223, Ala226, and Val227. Also an important short contact is made with the nicotinamide ring of $NADP^+$ (Fig. 3B). Interestingly, in the second binding mode the tetrazole ring is occupying a similar position as compared with the carboxylic group of carbenoxolone at position C30. The oxygen atom of the ether group of compound **1** is 3.4 Å away from C4 of the nicotinamide ring (where the proton transfer takes place) and 3.9 Å away from the hydroxyl group of the catalytic residue Tyr183. It should be noted that 80% of the solutions found by AutoDock fell into this orientation.

Compound **1** was evaluated for in vivo hypoglycemic activity using a STZ–nicotinamide rat model of non insulin dependent diabetes mellitus rat model.[14,16,17] Glibenclamide (5 mg/Kg, p.o.) was taken as positive control. The hypoglycemic activity of **1** was determined using a 50 mg/Kg intragastric single dose. Compound **1** demonstrated important hypoglycemic activity, by lowering glycemia ranging from 16% to 77%. The effect was sustained during the 7 h of experiment and it was more pronounced than the hypoglycemic action showed by glibenclamide (Fig. 4). It is important to mention that clofibric acid did not show any hypoglycemic effect. These results agree with those reported by Kim et al, where fenofibrate but not fenofibric acid, exerted a potent inhibitory activity against 11β-HSD1.[6]

In silico prediction of the acute toxicity is performed during drug design and development in order to avoid the experimental study of potentially harmful substances. The dose that is lethal to 50% of the treated animals (LD_{50}) of the compound **1**, clofibric acid and clofibrate was calculated through the ACD/ToxSuite software, v. 2.95 (Table 2).

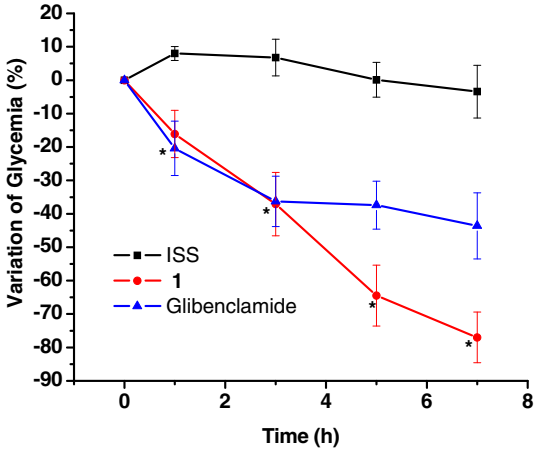


Figure 4. Effect of a single dose of **1** (50 mg/Kg; intragastric, $n = 5$) in streptozotocin–nicotinamide-induced diabetes rat model. ISS: Isotonic saline solution. $^*p < 0.05$ versus ISS group.

Table 2
Toxicity profiles predicted for compound **1** and selected fibrates

Compd	LD_{50} (mg/Kg)			
	Mouse		Rat	
	ip	po	ip	po
1	830	1900	1700	2200
Clofibric acid	290	1170	727	897
Clofibrate	540	1220	910	940

In these predictions, compound **1** was 2-times less toxic than the other fibrates, showing calculated LD_{50} by different administration routes ranging from 830 to 2200 mg/Kg.

In conclusion, we discovered a tetrazole isosteric analogue (**1**) of clofibric acid as a promising antidiabetic compound using in combo studies (in vitro, in silico and in vivo). Compound **1** shows significant 11β-HSD1 inhibitory activity, being 2.5-times more active than clofibrate. The formation of the amide and the replacement of carboxylic acid by the tetrazole scaffold in compound **1**, increased the potency against 11β-HSD1 and also improved the in vivo hypoglycemic effect.

Even though compound **1** has four stable tautomeric forms, it is suggested that the ionized state is the only one present at the experimental conditions. This is in agreement with the calculated binding energy for the ionized state. According to the docking models, **1** may bind into the catalytic site of 11β-HSD1 in two different orientations. In one of them, **1** shows important short contacts with catalytic residues and also with the nicotinamide ring of $NADP^+$. The high in vivo hypoglycemic activity showed by this compound makes it a suitable lead to develop new chemical entities for potential use in the treatment of diabetes and metabolic syndrome.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.03.122.

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13. 2-(4-chlorophenoxy)-2-methyl-*N*-(1*H*-tetrazol-5-yl)propanamide (**1**). Recrystallized from ethanol. Yield: 74.5%, mp: 151.2–152.5 °C. Mp: IR (KBr): ν_{max}/cm^{-1} 3250 (N–H), 1690 (C=O), 1591 (N=C). ^1H NMR (400 MHz, DMSO-d_6): δ_H 1.30 (s, 6H, *gem*-$(CH_3)_2$), 6.56–6.67 (dd, 2H, H-2, H-6, J = 8.5, J = 2.5 Hz), 6.88–6.94 (dd, 2H, H-3, H-5, J = 8.5, J = 2.5 Hz), 11.2 (s, 2H, NH_{amide},$NH_{tetrazole}$) ppm. ^{13}C NMR (100 MHz, DMSO-d_6): δ 24.3 (*gem*-$(CH_3)_2$), 80.8 (C–O), 121.9 (C-2, C-6), 127.9 (C-4), 128.7 (C-3, C-5), 152.2 (C-1, C-1″), 173.1 (C=O) ppm. MS/FAB$^+$: m/z 282 ($M+H^+$). HRMS (FAB$^+$): m/z 282.0749 $[M+H]^+$ (Calcd for $C_{11}H_{12}ClN_5O_2$: 282.0752). Anal. Calcd for $C_{11}H_{12}ClN_5O_2$: C, 46.90; H, 4.29; N, 24.86. Found: C, 46.97; H, 4.23; N, 24.89.
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