Sulfonylureas and Glinides Exhibit Peroxisome Proliferator-Activated Receptor \( \gamma \) Activity: A Combined Virtual Screening and Biological Assay Approach

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ABSTRACT

Most drugs currently employed in the treatment of type 2 diabetes either target the sulfonylurea receptor stimulating insulin release (sulfonylureas, glinides), or target the peroxisome proliferator-activated receptor (PPAR-\( \gamma \)) improving insulin resistance (thiazolidinediones). Our work shows that sulfonylureas and glinides additionally bind to PPAR-\( \gamma \) and exhibit PPAR-\( \gamma \) agonistic activity. This activity was predicted in silico by virtual screening and confirmed in vitro in a binding assay, a transcription assay, and by measuring the expression of PPAR-\( \gamma \) target genes. Among the measured compounds, gliquidone and glipizide (two sulfonylureas), as well as nateglinide (a glinide), exhibit PPAR-\( \gamma \) agonistic activity at concentrations comparable with those reached under pharmacological treatment. The most active of these compounds, gliquidone, is shown to be as potent as pioglitazone at inducing PPAR-\( \gamma \) target gene expression. This dual mode of action of sulfonylureas and glinides may open new perspectives for the molecular pharmacology of antidiabetic drugs, because it provides evidence that drugs can be designed that target both the sulfonylurea receptor and PPAR-\( \gamma \). Targeting both receptors could increase pancreatic insulin secretion and improve insulin resistance. Glinides, sulfonylureas, and other acidified sulfonamides may be promising leads in the development of new PPAR-\( \gamma \) agonists. In addition, we provide a unified concept of the PPAR-\( \gamma \) binding ability of seemingly disparate compound classes.

Poor eating habits and sedentary lifestyle have led to an increasing prevalence of metabolic disorders, such as type 2 diabetes mellitus, which is attaining epidemic proportions (World Health Organization, 2002). Primary prevention by dietary adjustments and increased exercise remains the most desirable and cost-effective strategy. Nevertheless, pharmacotherapeutic intervention to prevent severe immediate and long-term consequences of type 2 diabetes is often unavoidable.

The peroxisome proliferator-activated receptors (PPARs) are involved in the regulation of lipid and glucose metabolism (Willson et al., 2001). They are ligand-dependent transcription factors that contain an N-terminal activation domain, a DNA-binding domain, and a ligand-binding domain (Renaud and Moras, 2000). PPARs activate target genes by binding to response elements located within regulatory regions of these target genes (Laudet and Gronemeyer, 2002). Accessory proteins, termed coactivators and corepressors, associate with DNA-bound PPARs and modulate the expression of target genes through chromatin structure modifications and recruitment of the transcription machinery (Hermanson et al., 2002). Three subclasses of PPARs are known, called \( \alpha \), \( \gamma \), and \( \delta \), that are coded by different genes, exhibit tissue-specific expression patterns, and are associated with various functions. Of these, PPAR-\( \gamma \) is expressed mostly in adipose tissue, where it is essential in adipocyte differentiation and controls the storage of fatty acids, increasing triglyceride synthesis and storage within adipocytes. In addition, there is strong evidence that PPAR-\( \gamma \) regulates glucose homeostasis (Willson...
Sulfonylureas and Glinides Exhibit PPARγ Activity

The goal of the present study was to identify new PPARγ agonists among known drugs and biologically active compounds by combining virtual screening with experimental verification in biological assays. This strategy provides a detailed model of ligand-receptor complexes, together with an experimental confirmation of ligand-receptor binding and the consequent biological activity. We followed a three-step approach. First, a virtual screening search on two large databases of drugs and biologically active compounds allowed us to identify a few glinides and sulfonylureas as promising PPARγ ligands and prompted us to concentrate on these two drug classes, screening several more members thereof. Most of these compounds showed good affinities to PPARγ in silico.

In a second step, we found that selected sulfonylureas and glinides bind to PPARγ and enhance PPARγ-mediated gene expression in vitro. In a third step, encouraged by these results, we screened in silico a few new compounds related to the sulfonylureas (i.e., N-aclysulfonamides), resulting in the prediction that they would bind PPARγ with relatively high binding affinities.

Sulfonylureas and glinides are hypoglycemic drugs in clinical use for the treatment of type 2 diabetes, by virtue of their insulin secretagogue properties. These compounds bind to the sulfonylurea receptor SUR1 on the membrane of β-cells, triggering the closure of the nearby potassium channel, which in turns leads the β-cell to increase insulin secretion (Farret et al., 2005). Our discovery that some insulin secretagogue drugs activate PPARγ has attractive implications for the pharmacological treatment of type 2 diabetes. Moreover, sulfonylureas and N-aclysulfonamides are new classes of PPARγ agonists.

Materials and Methods

Virtual Screening Database. The TheraStrat AG inhouse database (Hug et al., 2003; Gut and Bagatto, 2005) contains most marketed drugs and many of their metabolites (approximately 8000 compounds). The freely available Chembank database contains approximately 6000 bioactive compounds (http://chembank.broad.harvard.edu).

Ligand Docking. Each compound was docked into the PPARγ binding site using the AutoDock 3.0.5 software (Morris et al., 1998). AutoDock finds several low-energy arrangements ("poses") of a given flexible ligand into a given receptor assumed to be rigid. For each pose, a pKᵢ value is calculated. The PPARγ three-dimensional structure was obtained from Protein Data Bank entry 1FM9. This is a 2.1Å resolution crystal structure of the heterodimer of the human retinoid X receptor α and PPARγ ligand binding domains, bound to 9 cis-retinoic acid and farglitazar, respectively, together with coactivator peptides (Game et al., 2000). The PPARγ-farglitazar complex was imported in MOE (Chemical Computing Group Inc., Montreal, QC, Canada) where hydrogens were added and energy-minimized. The resulting structure without farglitazar was imported in AutoDock, where the protonation state of acidic and basic groups was adjusted (His323 and His449 were protonated), and partial charges were assigned. The protonation state of ligands to be docked was adjusted to the species assumed predominant at physiological pH. In particular, carboxylic acid, thiazolidinedione, sulfonylurea, and N-aclysulfonamide moieties were deprotonated. Partial charges were assigned according to the MMFF94x force field (MOE). For method verification and calibration, we docked to PPARγ a set of 121 carboxylic acids that are PPARγ agonists with known experimental binding affinities, a collection detailed in our earlier work (Rücker et al., 2006). For the compounds whose best pose showed both carboxylate oxygen atoms within 2 Å of the corresponding atoms of farglitazar in the X-ray structure (85% of the total), the pKᵢ calculated by AutoDock and the experimental pKᵢ correlated with r² = 0.6 (slope and intercept of the linear regression were 0.9 and 3.5, respectively). Because in this test AutoDock consistently overestimated experimental pKᵢ values, all calculated pKᵢ values given in the following are linearly rescaled using the above numbers for slope and intercept.

Fig. 1. Structures of some endogenous and synthetic PPARγ agonists. 4-OH DHA, 4-hydroxydocosahexaenoic acid.
wells. IC50 values (concentrations at which 50% of the fluorescent activity) were determined for test compounds by measuring the fluorescence intensities of the S (parallel to excitation values for different competitors were determined in a ViewLux upon ligand displacement by nonlabeled competitors. Millipolarization of the complex between the ligand and the ligand-binding domain exhibits high fluorescence polarization, which is lost upon ligand displacement by nonlabeled competitors. Millipolarization values for different competitors were determined in a ViewLux reader (PerkinElmer Life and Analytical Sciences, Boston, MA) by measuring the fluorescence intensities of the S (parallel to excitation) and P (perpendicular to excitation) channels, and background fluorescence of the assay buffer was subtracted. Relative specific activity (Ar) was determined by scaling measured values according to Ar = (V - B)/(C - B), where V is the value measured, and B and C are the median millipolarization values of 0% and 100% control wells. IC50 values (concentrations at which 50% of the fluorescent ligand is displaced) and pIC50 values (negative decadic logarithm of IC50) were determined for test compounds by measuring Ar values for a series of concentrations. A four-parametric sigmoidal curve was fitted to each data set using DataFactory (BioFocus DPI, Allschwil, Switzerland) or Data Analysis Toolbox (Elsevier MDL, San Ramon, CA), and the IC50 value was determined from the fitted curve.

**Ligand Binding Assay.** The Green PolarScreen PPAR Competitor Assay (Invitrogen) was used according to the manufacturer's instructions. This cell-free assay is based on purified recombinant human PPARγ ligand-binding domain and a fluorescent fluorescent coactivator recruitment (Cronet et al., 2001), which justify the constraints above.

**Reagents and Plasmids.** Standard cell culture and transfection reagents were purchased from Invitrogen (Carlsbad, CA). Charcoal-stripped delipidated FBS was purchased from Sigma-Aldrich (Buchs, Switzerland). Gliquidone was provided by Apin Chemicals (Oxon, UK); glipizide, glimepiride, repaglinide, and linoleic acid were purchased from Sigma-Aldrich; nateglinide was purchased from Toronto Research Chemicals Inc. (North York, ON, Canada); and mitiglinide and pioglitazone were purchased from Molekula Ltd. (Dorset, UK). The 3xPPRE-Luc vector was kindly provided by Ron Evans (The Salk Institute for Biological Studies, San Diego, CA). The expression vector encoding human PPARγ was provided by Walter Wühl (Centre Intégratif De Génomique, Lausanne, Switzerland).

**Transactivation Assay.** CV-1 cells were cultured in DMEM (4500 mg/l glucose), supplemented with 10% FBS and 50 U of penicillin/streptomycin. Three days before transfection, cells were sterol-depleted by exchanging the culture medium to DMEM/Ham’s F12 medium without Phenol Red, supplemented with 10% charcoal-stripped FBS and 50 U of penicillin/streptomycin. Cells were plated in a 96-well dish with a density of 5 x 10^4 cells/ml (100 µl per well). DNA transfection was carried out in OptiMEM I (Invitrogen) without Phenol Red using Lipofectamine (Invitrogen). Each well received 8 ng of expression vector, 20 ng of reporter vector, and 60 ng of β-galactosidase vector. Twenty-four hours after transfection, drugs dissolved in dimethyl sulfoxide were added in DMEM/Ham’s F12 medium without Phenol Red, supplemented with 10% charcoal-stripped delipidated FBS (Sigma-Aldrich), and 50 U of penicillin/streptomycin. Sixteen hours after addition of drugs, cells were lysed in chloroform:isoamylalcohol:water (24:1:1). Supernatants were analyzed for luciferase activity by addition of luciferase reagent (Catalys AG/Promega, Wallisellen, Switzerland). Background normalization was conducted by transfection of β-galactosidase activity as described previously (Inagae-Lluhi et al., 1997). EC50 values (concentrations at which 50% of the maximal gene expression is induced) and pEC50 values (negative decadic logarithm of EC50) were determined for test compounds by measuring the increase of PPARγ target gene expression induced at different concentrations. A four-parametric sigmoidal curve was fitted to each data set using Prism software from GraphPad Software (San Diego, CA), and the EC50 value was determined from the fitted curve. Experiments were performed in quadruplicate, and error bars represent S.D.

**Measurement of PPARγ Target Gene Expression.** 3T3-L1 fibroblasts were purchased from European Collection of Cell Cultures (Porton Down, Wiltshire, UK). They were amplified in DMEM/10% calf serum and subsequently seeded into six-well plates. Two days after the cells reached confluence, the medium was changed to DMEM/10% fetal calf serum containing 0.5 M 3-isobutyl-1-methylxanthine, 2 µg/ml insulin (Actrapid), and 0.5 mM dexamethasone, to which the experimental compounds were added. Two days after induction of the cells, the medium was changed to DMEM/10% fetal calf serum containing 2 µg/ml insulin, to which the experimental compounds were freshly added. Compounds were tested at the following concentrations: rosiglitazone, 1 µM; pioglitazone, 10 µM; gliquidone, 10 µM; glipizide, 100 and 200 µM; nateglinide, 50 and 200 µM; and repaglinide, 50, 100, and 200 µM. The cells were harvested in TRIzol (Invitrogen, Breda, the Netherlands) 5 days after induction, and RNA was isolated by the standard procedure. RNA (1 µg) was used for cDNA synthesis using iScript (Bio-Rad Laboratories, Veenendaal, the Netherlands). cDNA was amplified with Platinum Taq polymerase using SYBR green on a Biorad MyQ RTqPCR (Invitrogen) was used according to the manufacturer’s instructions. This assay is based on purified recombinant human PPARγ ligand-binding domain and a selective fluorescent coactivator recruitment (Cronet et al., 2001), which justify the constraints above.

**Results**

In a first step, all compounds in the TheraSTrat and Chembank databases were docked to PPARγ. Among them, repaglinide (a carboxylic acid belonging to the glinide group of drugs), sulfadimidine (an acidified sulfonamide), and glimepiride (a sulfonylurea) were thereby assigned relatively high binding affinities (Scarsi et al., 2005). In a second step of virtual screening, we focused on these compound classes, docking several more members thereof, as well as structurally related compounds.

**Several Glinides and Sulfonylureas Are Docked to PPARγ with a High Binding Affinity.** Figure 2, A to C, depict repaglinide, nateglinide, and mitiglinide, respectively, docked into PPARγ and superimposed to the farglitazar complex X-ray structure (for structures, see Fig. 3). The predicted bound conformation of PPARγ are similar to that of farglitazar. The carboxylate group of the glinides superimposes well with that of farglitazar, forming hydrogen bonds with residues His323, His449, Ser289, and Tyr473 of PPARγ, respectively, with that of farglitazar, forming hydrogen bonds with residues His323, His449, Ser289, and Tyr473 of PPARγ, respectively.

Farglitazar is occupied by its long hydrophobic tail. The carboxylate group of the glinides superimposes well with that of farglitazar, forming hydrogen bonds with residues His323, His449, Ser289, and Tyr473 of PPARγ, respectively. In the case of farglitazar is occupied by its long hydrophobic tail. The carboxylate group of the glinides superimposes well with that of farglitazar, forming hydrogen bonds with residues His323, His449, Ser289, and Tyr473 of PPARγ, respectively. Interestingly, binding of farglitazar is occupied by its long hydrophobic tail.
Fig. 2. Three-dimensional structures of three glinides and three sulfonylureas docked to PPARγ. Repaglinide (A), nateglinide (B), mitiglinide (C), gliquidone (D), glimepiride (E), and glipizide (F) (gray ball and stick models) docked to PPARγ and superimposed to the farglitazar complex X-ray structure (green stick model). Of PPARγ, only the side chains of His323, His449, Ser289, and Tyr473 are shown (gray stick models). Compounds were docked into the PPARγ binding site using the AutoDock software. For each ligand, the figure reports the best docked pose. For details on pose selection, see Materials and Methods.
linide, and meglitinide (molecular mass, <350 Da) bind at a lower affinity compared with the larger repaglinide (molecular mass, 453 Da), because the latter forms more favorable contacts between the hydrophobic cavities of the PPARγ binding site and its large hydrophobic moieties.

Figure 2, D to F, show gliquidone, glimepiride, and glipizide, respectively, docked into PPARγ and superimposed to the farglitazar complex X-ray structure (for structures, see Fig. 3). The predicted binding mode of gliquidone and glimepiride in the polar part of the binding site exhibits interesting similarities to that of farglitazar. In analogy to the carboxylate oxygens of farglitazar, the sulfonamide oxygen atoms point toward the pocket built by the side chains of His323, His449, Ser289, and Tyr473 and form hydrogen bonds to the H donor atoms in these residues. It is noteworthy that in the case of gliquidone and glimepiride, there are two alternatives for H-bond formation to His449, either to a sulfonyle oxygen or to the urea oxygen atom. Glimepiride deviates considerably in the lower part of the binding cavity from the bound conformation of farglitazar. This is not unexpected, because this part of the binding cavity is wide, allowing some conformational freedom for the ligand (Nolte...
et al., 1998; Xu et al., 1999). Glipizide exhibits a slightly different binding mode compared with gliclizide and glimipiride, in that the sulfonyl group does not superpose to the carboxylate of farglitazar. In this case, two hydrogen bonds are formed by the urea oxygen atom with Tyr473 and with His449, whereas the deprotonated sulfonamide nitrogen forms a hydrogen bond with Ser289. Here, the deprotonated amide seems to be almost as good a mimic of farglitazar’s carboxylate as the sulfonamide group is in the other examples.

Many sulfonylureas are predicted to bind PPARγ, with pKi values ranging from 3.7 to 8.8 (Table 1). As in the case of the glinides, smaller sulfonylureas such as tolbutamide and chlorpropamide (molecular weight <300) are assigned lower binding affinities (pKi, ~4) than larger ones such as gliclizide, glipizide, and glisamuride (molecular weight >400, pKi, ~6–8). The larger molecules form a higher number of favorable contacts to the hydrophobic walls of the receptor’s binding cavity.

Sulfonylureas and Glinides Bind to PPARγ. The predicted PPARγ ligands gliclizide, glipizide, glimipiride, repaglinide, nateglinide, and mitoglinide, as well as two known ligands, linoleic acid, an endogenous agonist, and pioglitazone, a synthetic drug used in the treatment of type 2 diabetes (all selected for commercial availability), were tested in a PPARγ competitor binding assay. Gliquidone, glimepiride, repaglinide, nateglinide, pioglitazone, and linoleic acid bind to PPARγ and completely displace the reference ligand at different concentrations. The pIC50 values resulting from this experiment are 5.1 for gliclizide, 3.9 for glipizide, 2.8 for repaglinide, 3.5 for nateglinide, 6.5 for pioglitazone, and 6.6 for linoleic acid. Glipizide and mitoglinide partially displace the reference ligand at concentrations between 500 and 2000 µM (maximal concentration measured), but IC50 values could not be determined.

### Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Calculated pKi</th>
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<tbody>
<tr>
<td>Gliclizide</td>
<td>8.8</td>
</tr>
<tr>
<td>CS 476</td>
<td>8.3</td>
</tr>
<tr>
<td>Glicamamide</td>
<td>8.1</td>
</tr>
<tr>
<td>SPC 5002</td>
<td>8.0</td>
</tr>
<tr>
<td>Glisindamide</td>
<td>7.8</td>
</tr>
<tr>
<td>Glimipiride</td>
<td>7.7</td>
</tr>
<tr>
<td>Gliquidone</td>
<td>7.5</td>
</tr>
<tr>
<td>71 5w</td>
<td>7.5</td>
</tr>
<tr>
<td>Glisentide</td>
<td>7.1</td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>7.1</td>
</tr>
<tr>
<td>Gliseopido</td>
<td>6.8</td>
</tr>
<tr>
<td>Glipizide</td>
<td>6.5</td>
</tr>
<tr>
<td>Glisolamide</td>
<td>6.4</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>5.9</td>
</tr>
<tr>
<td>Glisondamide</td>
<td>5.6</td>
</tr>
<tr>
<td>Glibornuride</td>
<td>5.4</td>
</tr>
<tr>
<td>Acetoxyamide</td>
<td>5.0</td>
</tr>
<tr>
<td>Metahexamide</td>
<td>4.8</td>
</tr>
<tr>
<td>Tolazamide</td>
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</tr>
<tr>
<td>Tosifen</td>
<td>4.7</td>
</tr>
<tr>
<td>Tolbutamide</td>
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</tr>
<tr>
<td>Carbutamide</td>
<td>3.7</td>
</tr>
<tr>
<td>Chloropropamide</td>
<td>3.7</td>
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</table>

The effects of gliclizide, glipizide, nateglinide, repaglinide, pioglitazone, and rosiglitazone on the expression of PPARγ target genes were measured in 3T3-L1 fibroblasts. For the sulfonylureas and glinides, concentrations bracketing EC50 values from the activation study were chosen (see Materials and Methods). Three bona fide target genes of PPARγ (Knouff and Auwerx, 2004) were selected for analysis by quantitative reverse transcription-polymerase chain reaction: adiponectin, aP2, and GLUT4. Gliquidone, nateglinide, and glipizide significantly enhanced the expression of these genes. For these three compounds, maximal induction was observed at the lowest measured concentration (10 µM for gliclizide, 50 µM for nateglinide, and 50 µM for glipizide). In contrast, repaglinide showed no induction at concentrations ranging from 50 to 200 µM. Figure 4 shows the results for the selected sulfonylureas and glinides, together with pioglitazone as positive control. The induction of gene expression is reported relative to that observed in the presence of 1 µM rosiglitazone, a strong thiazolidinedione PPARγ agonist. Gliquidone is as potent as pioglitazone and at 10 µM causes approximately 80% of the induction observed in the presence of 1 µM rosiglitazone. Nateglide and glipizide show somewhat lower activities (between 30% and 70% compared with 1 µM rosiglitazone) at higher concentrations compared with gliclizide.

Acidified Sulfonamides Other Than Sulfonylureas Are Docked to PPARγ with a High Binding Affinity. Because the docking study did not reveal any significant role in PPARγ binding for the second N atom of the sulfonylureas, we replaced this terminal NH in silico with CH2, arriving at carbon analogs of gliclizide, glicamuride, and glibenclamide (N-acylsulfonamides; Fig. 5). These compounds were subjected to the docking procedure, which resulted in predicted pKi values of 7.2, 7.2, and 6.9, respectively.
For C-glimepiride and C-glibenclamide $p_K$ values are close to those obtained for the parent sulfonylureas, whereas C-glisamuride exhibits a somewhat lower $p_K$ than glisamuride. In these three cases, the binding mode of the polar moiety of analogs was very similar to that of the parent sulfonylureas (i.e., to Fig. 2, D and E).

**Discussion**

The major results of this work are that several sulfonylurea and glinue drugs bind to and activate PPARγ in vitro and that a detailed three-dimensional binding mode underlying this activation is proposed. Experimental evidence for direct binding to PPARγ has been provided in a competitor binding assay, whereas PPARγ agonistic activity was measured both in a transactivation assay and by observing target gene levels in 3T3-L1 cells. In all these experiments, gliquidone showed the strongest PPARγ agonistic activity among the measured sulfonylureas and glinides.

While this study was underway, two sulfonylureas, glimepiride and glibenclamide, were reported to activate PPARγ (Fukuen et al., 2005; Inukai et al., 2005). Our work provides strong evidence that additional sulfonylureas, as well as glinides (which equally target the sulfonylurea receptor), can bind and activate PPARγ and allows the interpretation of binding data based on docking results.

Sulfonylureas and glinides are standard treatments for type 2 diabetes. So far, members of these classes were presumed to act by a mechanism independent of PPARγ, suggested to act by a mechanism independent of PPARγ, leading to increased insulin production (Farret et al., 2005). Glimepiride $C_{\text{max}}$ values are approximately 40% higher in Chinese than in white patients (Jönsson et al., 2000). The suggested maximal single dose of glipizide is 15 mg (40 mg is the maximum daily dose; http://www.pfizer.com/pfizer/download/uspi_glucontrol.pdf). This may lead to glipizide concentrations in the plasma where PPARγ activation starts being significant.

$C_{\text{max}}$ values for glimepiride can reach 1 $\mu M$ after a single 8-mg dose (Langtry and Balfour, 1998), which is the suggested maximum single dose (http://www.fda.gov/cder/foi/label/2002/20496s7lbl.pdf). This is 2 orders of magnitude below the glimepiride concentration required for PPARγ activation according to our measurements (100 $\mu M$). However, in similar experiments, other authors reported glimepiride PPARγ agonistic activity at 1 and 10 $\mu M$ (Fukuen et al., 2005; Inukai et al., 2005).

For nateglinide, a $C_{\text{max}}$ value of 18 $\mu M$ has been reported in patients treated with a 120-mg dose (Luzio et al., 2001; Weaver et al., 2001; McLeod, 2004). The maximum recommended single dose of nateglinide is 180 mg (http://www.starlix.info/starlix/content/pages/basic.php). According to our measurements, nateglinide starts exhibiting PPARγ agonistic activity between 10 and 100 $\mu M$. Hence, phar-

![Fig. 4. Effect of selected compounds on the expression of PPARγ target genes. Mouse 3T3-L1 fibroblasts were induced to differentiate and simultaneously treated with two common PPARγ agonists (1 $\mu M$ rosiglitazone and 10 $\mu M$ pioglitazone), as well as with two sulfonylureas (glicluzide and glipizide) and two glinides (repaglinide and nateglinide) at increasing concentrations (see Materials and Methods). Five days after induction, cells were harvested and expression levels of three PPARγ target genes (adiponectin, aP2, and GLUT4) were measured using reverse transcription-polymerase chain reaction. For each treatment, the lowest effective dose is shown (10 $\mu M$ for glicluzide, 50 $\mu M$ for nateglinide and repaglinide, 100 $\mu M$ for glipizide). Data are normalized using cyclophilin expression and shown relative to the induction observed with 1 $\mu M$ rosiglitazone. Values are mean ± S.D. ($n = 3$).]
macological concentrations of nateglinide may be sufficient for activating PPARγ. Indeed, PPARγ activation might explain the beneficial effects on insulin resistance recently observed in patients with diabetes treated with nateglinide (Hazama et al., 2006).

Repaglinide levels in the plasma can be as high as 0.4 μM after a 4-mg dose, which is the highest recommended single dose (Owens et al., 2000; Culy and Jarvis, 2001; Hatorp, 2002). This concentration is below the minimal repaglinide concentration (around 10 μM) at which we observed PPARγ activation. Hence, repaglinide does not seem to show PPARγ activity under pharmacological treatment.

Mitiglinide starts exhibiting PPARγ agonistic activity at between 100 and 250 μM. This concentration is above the C_{max} value measured in a patient treated with an unspecified dose of mitiglinide (1.6 μM) (Anonymous, 2004).

To summarize, there is evidence that gliquidone, glipizide, and nateglinide may activate PPARγ at pharmacologically relevant concentrations, whereas glimepiride, repaglinide, and mitiglinide only activate PPARγ at concentrations higher than the those reached under clinical circumstances.

Our computational results strongly suggest to experimentally test members of the third compound class considered, N-acylsulfonamides (N-sulfonylcarboxamides), such as C-glimepiride, C-glisamuride, and C-glibenclamide, for PPARγ activity. These compounds are not yet synthesized. One recent publication, however, described a N-acylsulfonamide, FK614, as both an insulin sensitizer and PPARγ activator (Minoura et al., 2004). When subjected to our docking procedure, FK614 was assigned a pK_{i} value of 6.4.

As illustrated in Fig. 6, common properties of carboxylic acids (I) and thiazolidinediones (2), the major PPARγ ligand classes at present, are their acidity and the hydrogen bond acceptor potential of their deprotonated forms. According to X-ray analyses of PPARγ-ligand complexes, these properties are highly important for binding (Nolte et al., 1998; Gampe et al., 2000; Cronet et al., 2001; Xu et al., 2001; Sauerberg et al., 2002; Ebdrup et al., 2003). Sulfonamides (3) are not sufficiently acidic for binding unless acidified by a substituent such as an acyl group –C(=O)R’ that stabilizes the conjugate base by further delocalizing the negative charge. At the same time, such a substituent provides another H bond acceptor atom, the carbonyl oxygen. The compound classes shown or predicted here to be PPARγ ligands, sulfonylureas [4, R’ = (substituted) amino] and N-acylsulfonamides [4, R’ = (substituted) alkyl or aryl, pK_{a} values of ~5] smoothly fit into this picture. Other compound classes exhibiting similar or higher acidity and similar H acceptor ability of their anions were recently shown to be PPARγ ligands [oxazolidinediones (Momose et al., 2002b), tetrazoles (Momose et al., 2002a), phosphates, and thiophosphates (Durgam et al., 2005)]. Circumstantial evidence for our views is provided by the observation that the succinimide analog of an antihyperglycemic thiazolidinedione (2, but S replaced by CH_{2}, pK_{a} 9.7), as well as the corresponding N-methylthiazolidinedione, are both inactive (Cantello et al., 1994). Thus, a unified understanding of the PPARγ binding ability of seemingly disparate compound classes is emerging.

![Fig. 5. Structures of carbon analogs of glimepiride, glisamuride, and glibenclamide (N-acylsulfonamides).](image)

![Fig. 6. Acid strength and hydrogen bond acceptor ability of some PPARγ agonist compound classes. Analogy in acid strength and in hydrogen bond acceptor ability between carboxylic acids (1), thiazolidinediones (2), sulfonamides (3), sulfonylureas [4, R’ = (substituted) amino], and N-acylsulfonamides [4, R’ = (substituted) alkyl or aryl], or the respective anions. In the anions, oxygen and nitrogen atoms participating in charge delocalization as indicated are potential hydrogen bond acceptors. Both sulfonyl oxygen atoms are equivalent. For simplicity, charge delocalization onto the second sulfonyl oxygen atom is not shown.](image)
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