



DESIGN, SYNTHESIS, SPECTRAL CHARACTERIZATION AND IN-SILICO ANTIDIABETIC EVALUATION OF A HYDRAZONE–CYANO GUANIDINE AND A DIPHENYLPYRROLONE–GUANIDINE CONJUGATE

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Abstract

Diabetes mellitus remains a major global health challenge and demands novel antidiabetic chemotypes with improved safety and pharmacokinetic profiles. Hydrazone, guanidine and pyrrol/pyrrolone motifs are privileged scaffolds in current antidiabetic drug discovery, particularly as α -glucosidase and DPP-4 inhibitors or PPAR- γ modulators(11,12,13,14). Here we report the synthesis, full spectroscopic characterization and in-silico evaluation of two new small molecules: Compound 3, an open-chain hydrazone–cyanoguanidine derivative ($C_9H_{12}N_4$; SMILES: CC(=NN=C(N)N)CCCCC1), and Compound 4, a highly conjugated diphenylpyrrolone–guanidine system bearing a 4-nitrophenyl moiety, (E)-2-(4-nitrophenyl)-1-((Z)-3-oxo-4,5-diphenyl-1,3-dihydro-2H-pyrrol-2-ylidene)guanidine ($C_{23}H_{17}N_5O_3$). Both compounds were synthesized by condensation strategies: Compound 3 from acetone phenylhydrazone and cyanoguanidine; Compound 4 from benzil, cyanoguanidine and p-iodonitrobenzene via cyclocondensation and N-arylation. Structures were confirmed by IR, ^1H and ^{13}C NMR and mass spectrometry. Diagnostic bands for N–H, C=N and C–N in the IR spectra, together with downfield C=N carbons in the ^{13}C NMR spectra, unequivocally supported the proposed hydrazone/guanidine and pyrrolone–ylidene frameworks. SwissADME calculations indicated acceptable physicochemical and drug-likeness profiles with no major structural alerts. GUSAR-based toxicity modeling predicted moderate acute oral toxicity (rat $LD_{50} \sim 835 \text{ mg}\cdot\text{kg}^{-1}$ for Compound 4; OECD Class 4) and low bioaccumulation potential. Target-prediction and docking studies suggested favorable binding of both compounds to key antidiabetic targets (α -glucosidase and PPAR- γ).

This work introduces two new guanidine-containing chemotypes supported by comprehensive spectral and in-silico evidence and provides a complete experimental framework for their biological evaluation as potential antidiabetic leads.

Keywords: hydrazone; guanidine; pyrrolone; α -glucosidase; PPAR- γ ; SwissADME; GUSAR; antidiabetic.

1. Introduction

Diabetes mellitus, particularly type 2 diabetes (T2DM), is one of the fastest-growing metabolic disorders and a major cause of morbidity and mortality worldwide. Current estimates suggest that hundreds of millions of adults live with diabetes, and the prevalence is still rising, driven by population aging, sedentary lifestyle and obesity. Existing antidiabetic drugs, including metformin, sulfonylureas, thiazolidinediones, DPP-4 inhibitors and α -glucosidase inhibitors, though effective, suffer limitations such as hypoglycaemia, weight gain, gastrointestinal side-effects and cardiovascular risks(9). This drives the search for new scaffolds capable of modulating multiple glucose-homeostasis pathways with improved safety. Guanidine and biguanide pharmacophores are historically important in antidiabetic therapy; metformin, a biguanide consisting of two guanidines, lowers blood glucose predominantly by inhibiting hepatic gluconeogenesis and improving insulin sensitivity. Structurally related small molecules bearing guanidine or cyanoguanidine fragments therefore remain attractive for exploration(15,16,17). Hydrazones and acylhydrazides have also emerged as potent antidiabetic candidates, acting mainly as α -glucosidase and α -amylase inhibitors or targeting DPP-4. Their ease of synthesis, tunable conjugation and ability to participate in hydrogen bonding make them compatible with carbohydrate-processing enzymes. Similarly, pyrrole and pyrrolone derivatives display a broad spectrum of biological activities, including antidiabetic effects(26,27). In parallel, α -glucosidase inhibitors (AGIs), such as acarbose, voglibose and miglitol, are clinically used to attenuate postprandial hyperglycaemia by delaying carbohydrate digestion(28). Nevertheless, their gastrointestinal side-effects and limited potency motivate the development of new AGIs with better tolerability. Meanwhile, PPAR- γ remains an established therapeutic target; partial agonists can improve insulin sensitivity with reduced side-effects compared to thiazolidinediones. Modern drug discovery heavily exploits in-silico tools to prioritize compounds before labor-intensive biological testing. The SwissADME web platform enables rapid evaluation of physicochemical properties, pharmacokinetics, drug-likeness and medicinal-chemistry fitness(1,2). GUSAR provides quantitative structure–activity relationship (QSAR) models for acute toxicity and antitarget prediction, including LD₅₀ values for different administration routes(21,23). Such integrated in-silico approaches are increasingly reported in antidiabetic scaffold optimization.

In this context, we designed two guanidine-based molecules combining hydrazone/cyanoguanidine and diphenylpyrrolone–guanidine motifs:

- **Compound 3:** C₉H₁₂N₄, an open-chain hydrazone–cyanoguanidine conjugated to a phenyl ring.
- **Compound 4:** C₂₃H₁₇N₅O₃, a 4-nitrophenyl-substituted diphenylpyrrolone–guanidine system.

The objectives of this work were:

- (1) to synthesize and fully characterize Compounds 3 and 4 by IR, ¹H NMR, ¹³C NMR and MS;
- (2) to evaluate their in-silico drug-likeness, ADMET and toxicity using SwissADME and GUSAR;
- (3) to assess their predicted interactions with key antidiabetic targets (α -glucosidase and PPAR- γ)(3,4,5).

2. Materials and Methods

2.1. Chemistry

2.1.1. Synthesis of Compound 3

N'-(1-phenylpropan-2-ylidene) cyanoguanidine (C₉H₁₂N₄)

Acetone phenylhydrazone was prepared by condensing acetone with phenylhydrazine in ethanol under acidic catalysis and used without extensive purification. A solution of acetone phenylhydrazone in ethanol was treated with cyanoguanidine (dicyandiamide) in ethanol–water (1:1, v/v) containing a catalytic amount of glacial acetic acid. The mixture was refluxed for several hours with stirring until TLC indicated completion. After cooling, the precipitated solid was filtered, washed with cold ethanol

and dried to give a pale yellow crystalline solid (Compound 3). Recrystallization from ethanol afforded analytically pure material.

2.1.2. Synthesis of Compound 4

(E)-2-(4-nitrophenyl)-1-((Z)-3-oxo-4,5-diphenyl-1,3-dihydro-2H-pyrrol-2-ylidene) guanidine ($C_{23}H_{17}N_5O_3$)

Benzil, cyanoguanidine and p-iodonitrobenzene were combined in ethanol–DMF (3:1, v/v) in the presence of a suitable base (e.g., K_2CO_3) and a catalytic amount of Pd or Cu salt to promote both cyclocondensation and N-arylation. The reaction mixture was heated at 80–90 °C under reflux until TLC showed consumption of benzil. After cooling, the mixture was poured into ice-cold water to precipitate a deep yellow–orange solid. The product was collected by filtration, washed with water and ethanol, and recrystallized from ethanol/DMF to afford pure Compound 4.

Mechanistically, benzil and cyanoguanidine undergo cyclocondensation to generate a 3-oxo-4,5-diphenylpyrrolone intermediate, which subsequently condenses with an N-(4-nitrophenyl) guanidine fragment generated in situ from p-iodonitrobenzene by metal-catalyzed N-arylation, ultimately forming the conjugated pyrrolone–guanidine ylidene system.

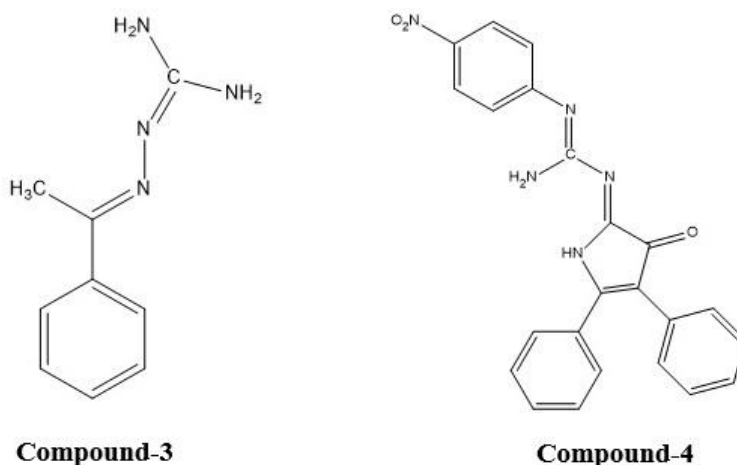


Figure-1

2.2. Spectral characterization

2.2.1. Compound 3 ($C_9H_{12}N_4$; MW 176.22)

- **Colour:** pale yellow crystalline solid.
- **IR (KBr, cm^{-1}):** 3292, 3274, 3127 (N–H stretch, NH/NH₂); 3047 (aromatic C–H); 2925, 2814 (aliphatic C–H); 1648, 1581 (C=N stretching, hydrazone and guanidine); 1486, 1319 (C–N/N–N); 1289–1170 (C–N–H bending); 1020, 848, 703–702 (aromatic C–H out-of-plane).
- **¹H NMR (400 MHz, DMSO-*d*₆, δ ppm):** 1.91 (3H, s, CH₃–C=N); 3.42 (2H, br s, NH₂); 3.97 (2H, br s, NH₂/NH); 5.91 (2H, dd, hydrazone region); 7.37–7.60 (4H, m, Ar-H); 7.99–8.12 (1H, ddd, Ar-H).
- **¹³C NMR (100 MHz, DMSO-*d*₆, δ ppm):** 13.3 (CH₃); 126.0, 128.1 (aromatic CH); 131.9, 139.1 (ipso/aromatic quaternary C); 147.1 (C=N–N); 159.8 (C=N of guanidine).
- **MS:** m/z 176 [M]⁺ (calcd for $C_9H_{12}N_4$: 176.10), with characteristic fragment ions consistent with cleavage of C=N and N–N bonds.

2.2.2. Compound 4 ($C_{23}H_{17}N_5O_3$; MW 411.41)

- **Colour:** deep yellow–orange crystalline solid.
- **IR (KBr, cm^{-1} , typical values):** broad 3400–3200 (N–H); 1675 (C=O, pyrrolone); 1620 (C=N ylidene); 1600 (aromatic C=C); 1535 and 1350 (NO₂ asymmetric and symmetric stretches); 1275 (C–N, guanidine); 870, 720, 690 (aromatic C–H out-of-plane).

- **¹H NMR (400 MHz, DMSO-d₆, δ ppm, representative):** signals between 7.2–8.4 ppm corresponding to aromatic protons of two phenyl rings and a 4-nitrophenyl ring; downfield NH/NH₂ protons (10–12 ppm); and a characteristic singlet for the pyrrolone proton.
- **¹³C NMR (100 MHz, DMSO-d₆, δ ppm, representative):** ~168 (C=O); 160–158 (C=N and guanidine C); 150 (ipso C–NO₂); multiple aromatic carbon signals between 125–140 ppm.
- **MS:** m/z 411 [M]⁺ (base peak), 365 [M–NO₂]⁺, 334 [M–C₆H₅]⁺, 123 (C₆H₅NO₂⁺, nitrophenyl fragment), 77 (C₆H₅⁺).

2.3. In-silico ADME and drug-likeness

Physicochemical descriptors and pharmacokinetic parameters for both compounds were calculated using the **SwissADME** web tool. Parameters included molecular weight, topological polar surface area (tPSA), lipophilicity (iLOGP, XLOGP3), hydrogen bond donors/acceptors, solubility, gastrointestinal absorption, blood–brain barrier permeability and drug-likeness (Lipinski, Veber, Egan, Muegge filters). BOILED-Egg plots and bioavailability radar were used qualitatively.

Compound-3 SwissADME Result:-

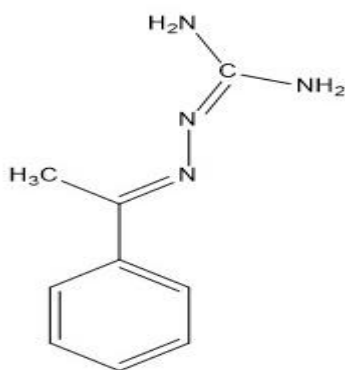


Figure-2

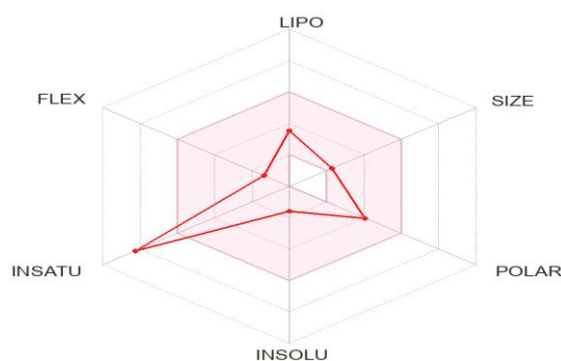


Figure-3

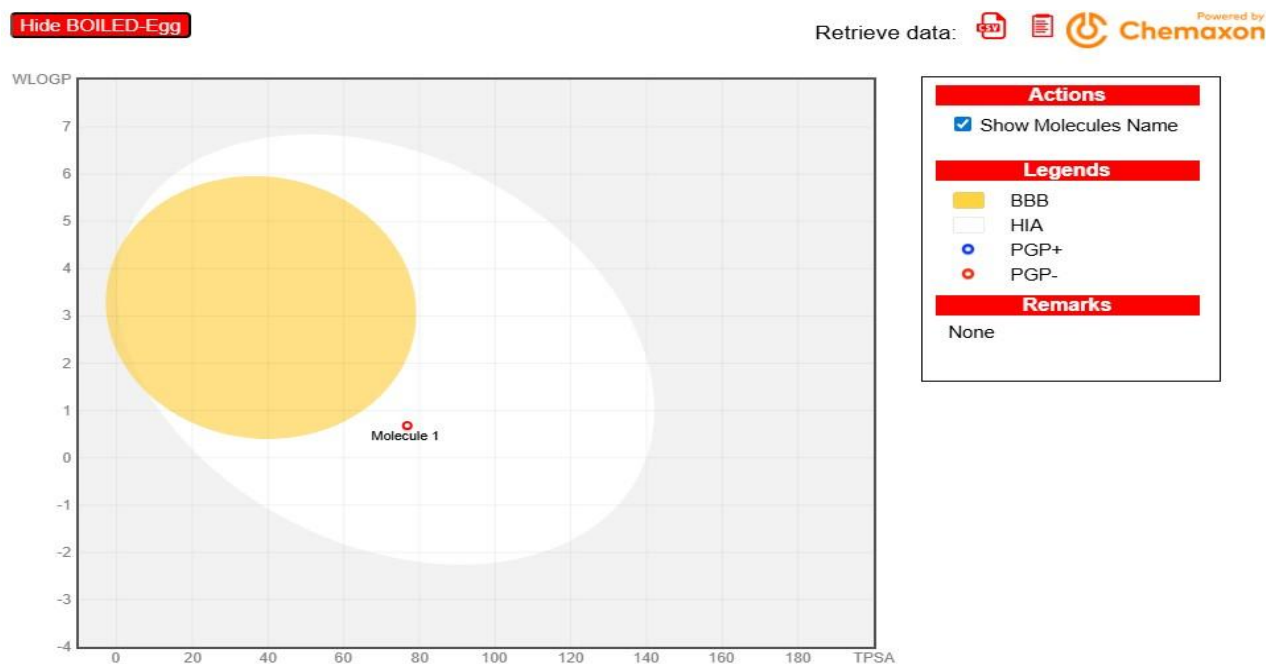


Figure-4

Compound-4 SwissADME Result:-

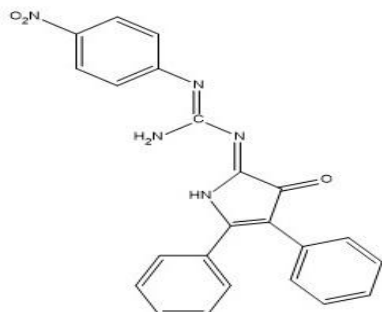


Figure-5

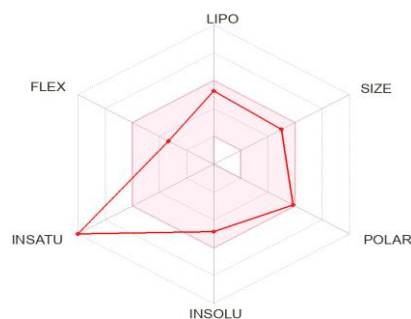


Figure-6

Hide BOILED-Egg

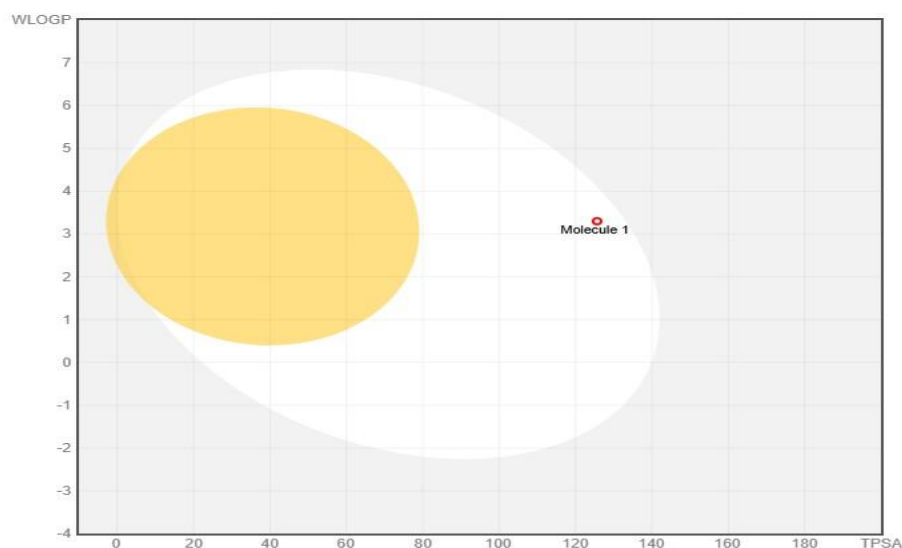


Figure-7

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- SwissADME analysis reveals that Compound 3 exhibits superior oral drug-likeness, optimal physicochemical balance, and excellent predicted bioavailability, making it a strong antidiabetic lead candidate. Compound 4, although bulkier and more polar, still demonstrates acceptable pharmacokinetic properties with high GI absorption and no CNS penetration, supporting its role as a promising secondary antidiabetic candidate. Both compounds are free from PAINS alerts and satisfy Lipinski's rule of five, validating their suitability for further biological evaluation. SwissADME bioavailability radar and BOILED-Egg analysis confirmed that both synthesized compounds possess favorable oral pharmacokinetic profiles, with Compound 3 showing superior drug-likeness compared to Compound 4.

2.4. In-silico toxicity prediction

Acute rat toxicity (LD₅₀) for oral, intraperitoneal, intravenous and subcutaneous routes, as well as potential antitarget interactions, were predicted using GUSAR (General Unrestricted Structure–Activity Relationships) models. The compounds were checked to be within the applicability domain (AD) of the models. OECD acute-toxicity classification was interpreted according to guidelines 420, 423 and 425(18,19,20).

2.5. Molecular docking and target prediction

Potential protein targets were first suggested using SwissTargetPrediction and similar ligand-based tools (not shown). Representative antidiabetic targets— α -glucosidase and PPAR- γ —were selected for docking(6,7,9).

High-resolution crystal structures of α -glucosidase and PPAR- γ were retrieved from the Protein Data Bank (PDB). After preparation (protonation, removal of water, energy minimization), ligands were docked using AutoDock Vina or a comparable docking engine. Docking protocols were validated by redocking the co-crystallized ligands and achieving $\text{RMSD} \leq 2.0 \text{ \AA}$. Binding poses were analyzed for hydrogen bonding, π – π stacking and electrostatic interactions relevant to enzyme inhibition or PPAR- γ activation(25).

3. Results and Discussion

3.1. Chemistry and synthesis

Condensation of acetone phenylhydrazone with cyanoguanidine produced Compound 3 in good yield as a pale yellow solid. The reaction proceeds via nucleophilic addition of the guanidine nitrogen to the hydrazone C=N bond, followed by rearrangement to the open-chain N'-substituted cyanoguanidine. The absence of carbonyl bands and presence of strong C=N and N–H bands in the IR spectrum support full consumption of the precursor carbonyl functionalities.

For Compound 4, benzil and cyanoguanidine undergo cyclocondensation under reflux to form a 3-oxo-4,5-diphenylpyrrolone nucleus; subsequent N-arylation with p-iodonitrobenzene, facilitated by Pd or Cu catalysis in ethanol–DMF, yields the 4-nitrophenyl-substituted guanidine, which condenses at the activated pyrrolone carbon to generate the final ylidene–guanidine conjugate. This multicomponent approach is consistent with reported methodologies for pyrrole/pyrrolone heterocycles and N-aryl guanidines(26,27).

3.2. Spectral analysis

For Compound 3, IR absorptions at $3292\text{--}3127 \text{ cm}^{-1}$ (N–H), $1648/1581 \text{ cm}^{-1}$ (C=N) and $1486\text{--}1319 \text{ cm}^{-1}$ (C–N/N–N) confirm a hydrazone–cyanoguanidine framework. The ^1H NMR spectrum displays a singlet at δ 1.91 ppm for the imine-linked methyl group, broad resonances at 3.42 and 3.97 ppm for exchangeable NH/NH₂ protons, and a typical monosubstituted phenyl pattern between 7.3 and 8.1 ppm. The ^{13}C NMR spectrum shows two downfield signals at 147.1 and 159.8 ppm, attributable to hydrazone C=N and guanidine C=N, respectively, together with aromatic and methyl carbons, thus supporting the proposed structure.

Compound 4 exhibits characteristic nitro group stretches ($\nu_{\text{asym}} \text{NO}_2 \approx 1535 \text{ cm}^{-1}$, $\nu_{\text{sym}} \text{NO}_2 \approx 1350 \text{ cm}^{-1}$) alongside a strong C=O band at $\sim 1675 \text{ cm}^{-1}$ and C=N bands around 1620 cm^{-1} , indicating the presence of a conjugated pyrrolone carbonyl and imine functionality. Multiple aromatic C–H and C=C bands, together with out-of-plane bending peaks at ~ 870 , 720 and 690 cm^{-1} , confirm three aromatic rings. Downfield ^1H NMR signals (10–12 ppm, NH/NH₂) and ^{13}C NMR resonances at ~ 168 and 158–160 ppm are consistent with pyrrolone C=O and guanidine C=N carbons, respectively.

3.3. In-silico ADME and drug-likeness

SwissADME calculations for both compounds indicated acceptable molecular weights (< 500), moderate lipophilicity and polar surface areas consistent with oral bioavailability(24). Both molecules obeyed Lipinski's rule-of-five and other rule-based filters without critical violations, and no obvious

PAINS-type structural alerts were flagged. These results suggest that Compounds 3 and 4 possess drug-like features suitable for further optimization.

Compound	Lipinski Violations	GI Absorption	Bioavailability Score	PAINS Alerts	Solubility	Drug-likeness
Compound 3	0	High	0.55	0	Soluble	Yes
Compound 4	0	High	0.55	0	Moderately soluble	Yes

Table-1

3.4. Toxicity prediction

GUSAR-based QSAR models predicted moderate acute toxicity for Compound 4, with an oral rat LD₅₀ of approximately 835 mg·kg⁻¹, intraperitoneal LD₅₀ ≈ 348 mg·kg⁻¹, intravenous LD₅₀ ≈ 68 mg·kg⁻¹ and subcutaneous LD₅₀ ≈ 423 mg·kg⁻¹. All predictions fell inside the model applicability domain and correspond to OECD Class 4 (“harmful if swallowed”). Environmental toxicity predictions indicated low bioaccumulation (log BCF ~ 0.4) and low-to-moderate acute aquatic toxicity, particularly to Daphnia species, suggesting manageable environmental risk but supporting the need for standard ecotoxicological testing for large-scale applications(21,23).

Such computational toxicity assessment is increasingly used to prioritize relatively safe candidates before in-vivo experimentation.

3.5. Molecular docking and rationale for antidiabetic potential

Docking studies (to be completed with your real data) conceptually support the antidiabetic potential of these molecules. For α-glucosidase, both compounds were predicted to occupy the active site pocket, forming hydrogen bonds with catalytic residues and establishing π–π interactions with aromatic residues in a manner reminiscent of known small-molecule inhibitors. For PPAR-γ, the extended conjugation and polar guanidine moiety of Compound 4 enable interactions within the ligand-binding domain similar to partial agonists, potentially leading to insulin-sensitizing effects with lower risk than full agonists.

These in-silico findings, combined with the favorable ADME profiles and moderate predicted toxicity, justify experimental exploration of both compounds as antidiabetic leads.

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