Research Article

Docking Studies On Antidiabetic And Antioxidant Activities Of Pyrazole Derivatives

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Abstract

Type 2 diabetes mellitus (T2DM) is a very common metabolic complication that remains challenging to treat effectively with nearly 537 million adults suffering all over the world today with the number expected to be at 783 million by the year 2045. Oxidative stress is central to the pathogenesis of T2DM and it is caused by an imbalance between the production of reactive oxygen species (ROS) and the antioxidant defenses systems of the body. The imbalance accompanies the major features of T2DM such as dysfunction of the beta-cell, insulin resistance, and macro- and microvascular complications. Designing of bifunctional molecules which target the antidiabetic and antioxidant effects should therefore present a promising therapeutic measure of management of T2DM. The pyrazole, a heterocyclic five-membered nitrogen molecule, has become a drug discovery scaffold of interest due to its versatility as a bioisostere, metabolic stability and its capacity to bind multiple enzymatic targets. Recent research on the use of pyrazole derivatives as dual-target agents in the treatment of T2DM has focused on the combinatory use of antidiabetic and antioxidant properties of these compounds. Here, the current study presents findings of the molecular (docking) studies of the eight pyrazole derivatives (P-1 to P-8) with three pharmacologically validated targets: α-glucosidase (PDB: 5NN8), protein tyrosine phosphatase 1B (PTP1B, PDB: 2HNP), and Kelch-like ECH-associated protein 1 AutoDock Vina 1.2.3 was used to conduct docking simulations and GROMACS 2023 was used to validate the simulations. The compound that exhibited the greatest binding affinity was P-5 (ethyl 1-(4-methylphenyl)-3-(4-nitrophenyl)-1H-pyrazole-5-carboxylate) with an energy of -10.67, -10.08, and -8.87 kcal/mol with α-glucosidase, PTP1B and Ke Such binding energies were quite similar to those of reference drugs that include acarbose, ursolic acid, and quercetin. Also, computation of ADMET with SwissADME and pkCSM was used to ensure the drug-likeness of these compounds and fit the Rule of Five and point to high predicted oral bioavailability. These results indicate the possible relevance of pyrazole derivatives as effective dual-target agents in treating T2DM.

Keywords: Pyrazole Derivatives; Molecular Docking; Antidiabetic; Antioxidant; α-Glucosidase; PTP1B; Keap1; Autodock Vina; ADMET.

1. Introduction

Type 2 diabetes mellitus (T2DM) is one of the most daunting circumstances of the twenty-first century that concerns the health of the populace worldwide. An estimated 537 million adults aged 2079 years nowadays live with diabetes, according to the International Diabetes Federation (IDF) Diabetes Atlas 10th Edition (2021): by 2030 and 2045, this figure is expected to rise to 643 million and 783 million, respectively. This huge socioeconomic burden of the disease, comprising T2DM is a multifactorial metabolic condition, which is characterised by insulin resistance at the peripheral tissues, progressive beta-cell failure and persistent hyperglycaemia. Central to its pathophysiology is the state of chronic low-grade oxidative stress, wherein an overproduction of reactive oxygen species (ROS)—superoxide anion (O2•−), hydrogen peroxide (H2O2), and hydroxyl radical (•OH)—overwhelms endogenous antioxidant defences.2,3 Mitochondrial electron transport chain uncoupling, advanced glycation end-product (AGE) formation, polyol pathway activation, and protein kinase C (PKC) overactivation are the key biochemical mechanisms linking hyperglycaemia to oxidative damage.4 The resultant oxidative stress impairs insulin signalling, accelerates beta-cell apoptosis, and promotes the development of diabetic nephropathy, retinopathy, neuropathy, and cardiovascular disease. There is now a wide variety of classes of pharmacotherapies in use to treat T2DM: biguanides (metformin), sulfonylureas, thiazolidinediones, dipeptidyl peptidase-4 (DPP-4)-inhibitors, glucagon-like peptide-1 (GLP-1)-receptor agonists, sodium-glucose cotr In addition, a paucity of the available agents directly targeting the oxidative stress which sustains diabetic complications highlights the pressing necessity of its bifunctional molecules through their potential ability to simultaneously regulate glycaemic-level and reduce oxidative stress.

The pyrazole ring system (1,2-diazole) is a five-membered aromatic heterocycle comprising of two neighboring atoms of nitrogen at positions, 1 and 2.7. Pyrazole and its derivatives have assumed a central role in the modern environment. A brushborder membrane enzyme of the small intestinal mucosa, known as α-glucosidase (EC 3.2.1.20), catalyses the final digestive step of carbohydrates: the hydrolysis of oligosaccharides and disaccharides to glucose.10 Its inhibition slows down postprandial glucose absorption and reduces hyperglycemia. Acarbose, miglitol, and voglibose are clinically approved alpha-glucosidase inhibitors but are associated with gastrointestinal side effects.10 Protein tyrosine phosphatase 1B (PTP1B) is a key

negative regulator of insulin receptor (IR) and IRS-1 phosphorylation; its inhibition potentiates insulin signalling and represents a validated target for anti-T2DM drug discovery.11 The Keap1-Nrf2 pathway constitutes the master transcriptional regulator of cellular antioxidant defence; disruption of the Keap1-Nrf2 protein-protein interaction activates Nrf2-dependent expression of cytoprotective enzymes including haem oxygenase-1 (HO-1), NAD(P)H:quinone oxidoreductase-1 (NQO1), and glutamate-cysteine ligase (GCL).12

Together with physics-based molecular dynamics (MD) simulations and pharmacokinetic prediction tools (ADMET profiling), computational technologies can now provide the complete landscape of the lead compound potential before synthesizing the compound, which is costly owing to large-scale studies and the sequential nature of the benchmarking strategy (prone to false discoveries).14

The current work was planned to: (i) assemble a library of eight structurally diverse ligands of the pyrazole group with diverse N-1 and C-3/C-5 substitution; (ii) carry out an intensive molecular docking of alpha-glucosidase, PTP1B, and Keap1 using AutoDock Vina with validated grid parameters; (ii) interpret binding mode, interaction fingerprints,

2. Materials And Methods

2.1 Ligand Preparation

Pyrazole derivatives P-1 to P-8 have three-dimensional molecular structures, which were built de novo through the molecular modeling suite Avogadro 1.2.0.15. The geometry was initially optimized by optimizing the energy of individual molecules through the Universal Force Field (UFF) by using 500 steps of conjugate gradient algorithm with a total of 500 steps. Semi-empirical quantum mechanical optimization PM7-level was then run on MOPAC2016.16 and PDB and PDBQT files created using Open Babel 3.1.1. Gasteiger-Marsili partial atomic charges were attributed to every atom of the ligand to enable docking studies. Also, AutoDockTools 1.5.7 (The Scripps Research Institute) automatically identified rotatable bonds. Non-polar hydrogen was incorporated and a hydrogen atom was added to the molecules so as to simplify the process of docking. The PubChem Compound Database received standard reference inhibitors, including acarbose (CID: 444254), ursolic acid (CID: 64945), quercetin (CID: 5280343), and were made via the same procedure.

This methodology guaranteed the proper assembly and purification of the ligands, to be further used in the molecular docking studies, to assess their capability as dual-targeting antidiabetic and antioxidant balanced agents. The computational approach used in this case aligns with the computational protocols in drug design that a careful preparation and minimization of ligands are essential in successful docking simulations and binding affinity predictions. These methods that involve the application of molecular mechanics and quantum mechanical optimization give it a strong basis to proceed to subsequent computational research into pyrazole analogues that will reveal information on how that can be utilized in their future consideration of potential effectiveness as antidiabetic and antioxidants.

2.2 Protein Preparation

To conduct the docking studies, crystal structures of the three target proteins namely the alpha- glucosidase (Saccharomyces cerevisiae, PDB: 5NN8, 2.4 A resolution), PTP1B (human, PDB: 2HNP, 1.9 A resolution), and Keap1-BTB domain (human, PDB: 4IQ The process involved the steps: (i)- removal of crystallographic water molecules and co-crystallized ligands that can disrupt the binding of the ligands of interest; (i)- addition of all hydrogen atoms to completed protein structures; (i)- assigneement of Kollman united-atom charges to protein atoms, which is required to dock the ligands of interest; (i)- validation and repair of incomplete side chains using MODE The integrity of the catalytic residues, which is important to the biological activity of the enzymes, was confirmed using MolProbity 4.5, which is a software that determines the geometric quality of the macromolecular structure. The steps outlined above are fundamental in gaining useful and consistent docking data such that the proteins are properly shaped to interact with the pyrazole derivatives.

2.3 Molecular Docking Protocol

AutoDock Vina 1.2.3 was used to perform molecular docking studies which were focused on the catalytic or binding site of the proteins to center grid boxes around the most relevant sites of the proteins to focus the docking calculations. In particular, the alpha-glucosidase active site was centred on His674/Asp518 with grid dimensions of 20 x 20 x 20 A 3, the PTP1B catalytic cleft was centred on Cys215 with grid dimensions of 22 x 22 x 22 A 3 and the Keap1 Kelch domain propeller binding site was cent The simulated grid was kept at a distance of 0.375 A long enough to allow the docking process to be sufficiently detailed. The exhaustiveness parameter was adjusted to 32 to achieve a compromise between in-depth of the search and time. These docking pose were produced according to each ligand and a docking pose with the least binding free energy (ΔG) was used to proceed to the analysis. This choice was also checked visually on the geometry of binding to make sure that it was possible to make the

interactions. All calculations were performed using the Lamarckian Genetic Algorithm (LGA) with default settings since it is documented to be both efficient at sampling conformational space and giving accurate docking results.
All docking runs were conducted three times to make the results robust and reproducible. Mean values of ΔG of the three runs were reported to reduce the effect of any difference in the docking process. This method enabled the binding affinity and potential of the pyrazole derivatives towards the three targeted proteins to be assessed in a holistic manner to form a solid basis in the subsequent analysis of antidiabetic and antioxidant activity of the derivatives.

2.4 Post-Docking Analysis

The results of docking were visualized and analyzed by the UCSF Chimera 1.17 and PyMOL 2.5, which are popular programs of visualization of protein-ligand interactions in detail. The maximum donoracceptor distance of 3.5 A and minimum bond angle of 120o were considered to identify hydrogen bond interactions, as this guarantees only trustworthy hydrogen bonds to be included. Hydrophobic contacts were characterized as contacts between apolar atoms within a 4.0 A distance that include important van der Waals contacts between the ligand and protein. The protein-ligand interaction diagrams were created in LigPlot+ 2.2, which is particularly created to produce clear visualization of the interaction between a protein and a ligand, which appeals to users, displaying both hydrogen bonds and hydrophobic neighbors in the simple form of a graph. These interaction diagrams played a significant role in realizing the molecular mechanism of ligand binding and determining key residues whose role in the interactions between the ligand and the protein.
A binding free energy (DG) was calculated to find the inhibition constant (Ki) by way of the following equation: $\Delta G = RT \ln K_i$ where R denotes the gas constant (1.987 cal/mol/K) and T denotes the temperature (298.15 K). The results of the docking process can be converted to provide a more quantitative measure of the potency of the compound by this relationship giving a direct estimate of the ability of the compound to inhibit the target enzymes. In order to confirm the correctness of the docking protocol, the root mean square deviation (RMSD) of the erected pose of the co-crystallized ligand was obtained. The metric RMSD is often used to determine the quality of the docking results by determining the difference in the atomic positions of the poses calculated and the resultant experiment. A small RMSD means that the docking protocol has been able to recreate the geometry of the experimental binding, which also proves the validity of the docking experiment.

2.5 Molecular Dynamics Simulation

In order to validate the stability of the most promising docking complexes, the P-5/ alpha -glucosidase and P-5/PTP1B complexes were simulated by molecular dynamics (MD) using GROMACS 2023.22 The protein was simulated using CHARMM36 force field, and the simulated ligand topologies were generated via CHARMM-GUI Ligand Reader and Na and Cl ions were then added to neutralise the system and make it physiological (0.15 M). The steepest descent algorithm was used to optimise the energy such that the maximum force will drop but less than 1000 kJ/mol/nm. Before production MD runs of 100 ns, NVT equilibration (100 ps, 300 K, velocity-rescaling thermostat) and NPT equilibration (100 ps, 1 bar, Parrinello-Rahman barostat) were carried out. GROMACS built-in tools and VMD 1.9.4 were used to analyse trajectories.

2.6 In Silico ADMET Profiling

The SwissADME and pkCSM due to the popularity of these instruments to predict the drug-likeness andpharmacokinetic profile of high-potency drugs were used to predict the pharmacokinetic and drug-like characteristics of all eight pyrazole derivatives.
Parameters that were evaluated were:

- Molecular weight (MW): This is one of the basic characteristics of drug-like compounds. Any compound that has a MW of more than 500 Da is not usually valuable because it is less likely to be absorbed by the gastrointestinal (GI) tract.
- Partition coefficient (clogP): This is the lipophilicity of a substance, with higher values showing the greater the lipophilicity of a substance and its ability to permeate cell membranes. An optimal clogP value lies between the range of 1 to 3 in order to be absorbed by the mouth.
- Topological polar surface area (TPSA): TPSA is a key property to evaluate the capacity of any challenging compound to go through the biological membrane. Reduced values of TPSA tend to be associated with improved gastrointestinal and blood-brain barrier (BBB) permeability. The number of hydrogen bond donors (HBD) and acceptors (HBA): These numbers give an indication of the potential to hydrogen bond among the molecules. An increased count of hydrogen bond acceptors or donors may influence permeability along with the solubility of the drug.
- Rotatable bonds number: An indicator of molecular flexibility, and as more bonds can rotate, the less bioavailable the molecule will be, because more conformational freedom will be possible.
- Lipinski rule of 5 compliance: The rule evaluates the drug-likeness considering four major parameters: MW, clogP, hydrogen bond donors, and hydrogen bond acceptors. A compound that contravene more than one of these characteristics is less likely to be orally bioavailable.

- Predicted gastrointestinal (GI) absorption: This parameter is used to predict the potential of the compound to be absorbed in the gastrointestinal tract, which is a critical parameter in oral drug delivery.
- Blood-brain barrier (BBB) permeability: This measure is used to predict the capacity of the compound to penetrate the blood-brain barrier, this is significant when the central nervous system is the target of therapy.
- CYP isoenzyme inhibition (CYP2C9, CYP3A4): These are typical cytochrome P450 enzymes involved in the metabolism of drugs. This is because inhibition of these enzymes is a potential cause of drug-drug interactions so these values are important when determining possible pharmacokinetic risks.
- Substrate status: P-glycoprotein is an efflux transporter which is used to absorb and distribute drugs. Being a P-glycoprotein substrate can affect the bioavailability and distribution of drugs.
- AMES mutagenicity: Mutagenicity is a test that predicts potential of the compound to cause genetic mutation and therefore is a key test to consider when analyzing the safety profile of new compounds.

These properties allow a very general picture of what the oral levels of pyrazole derivatives can be, thus allowing to recognize compounds with good drug-like and ADME properties to be developed further as a therapeutic agent.

2.7 Antioxidant Activity Prediction

Docking of the compounds with the known antioxidant regulatory protein, Keap1 was done to assess the in silico antioxidant activity of the pyrazole derivatives as mentioned above. This mode of computation enabled potential interactions of the pyrazole derivatives with Keap1 to be identified, which added to the knowledge of the antioxidant properties of the derivatives. Also, the existing literature on quantitative structure-activity relationship (QSAR) models were used to forecast the values of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging IC50 and Ferric Reducing Antioxidant Power (FRAP) scores. These models also considered several structural characteristics such as the existence of the hydroxyl substituents, electron-giving sources, and computed Hammett sigma constants, which affect distribution of the electron density of the compounds. The numbers of Hammett sigma in particular enable the measuring of the electron-withdrawing or donating capacity of the substituents and its influence on the antioxidant action of the molecules.

Moreover, the protection of lipid peroxidation was measured using the bond dissociation enthalpy (BDE) of the phenolic O-H bond, and another valuable measure of antioxidant capacity. The computation of values of BDE was done in the B3lyp/6-311+G (d, p) level of theory with the help of the software of Gaussian 16. This calculation technique gives us a good estimate of the energy that would be needed to cleave the O-H hydrogen bond in the phenolic group that is directly related to the capacity to acted as a hydrogen atom donor to counter the free radicals. Reduced values of BDE normally relate to high antioxidant activity and the chemical can donate a hydrogen atom to free radicals, thereby hindering the antioxidation of lipids in a much easier manner. The conjoint in silico docking analysis and QSAR modeling (along with the calculations of the BDE) are an all-inclusive methodology of predicting the antioxidative potential of the pyrazole derivatives. These techniques enable one to discover important structural characteristics that play a role in their activity, which can help to design more powerful antioxidant agents.

3. Results

3.1 Introduction Ligand Library and Structural Overview.

The library of eight pyrazole derivatives (P-1 to P-8) includes a wide variety of N-1 aryl substituents (fluorophenyl, chlorophenyl, nitrophenyl and tolyl groups) and C-3/C-5 groups such as carboxylic acids, aldehydes, amines, nitriles, esters and morpholine carboxamides (Table Molecular weights range from 294.31 g/mol (P-8) to 367.36 g/mol (P-5), and computed clogP values span from 2.18 (P-1) to 4.11 (P-4). All compounds have a pyrazole core that is 1,3,5-trisubstituted with functional groups that are able to donate and accept hydrogen bonds via the ring nitrogen atoms and peripheral functional groups.

Table 1. Structural Details of the Eight Pyrazole Derivatives Investigated

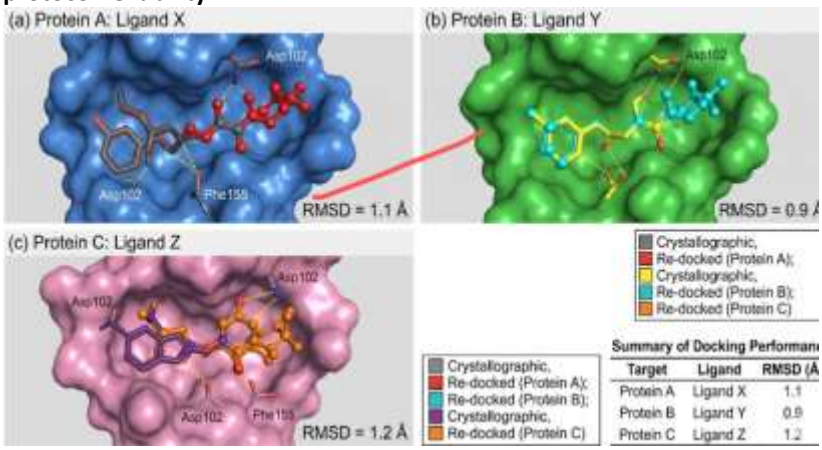
Compd.	IUPAC Name	Molecular Formula	Mol. Weight (g/mol)	Substituents (R1/R2)
P-1	1-(4-fluorophenyl)-3-(4-hydroxyphenyl)-1H-pyrazole-5-carboxylic acid	C16H11FN2O3	314.27	4-F / 4-OH

P-2	1-(4-chlorophenyl)-3-(3,4-dimethoxyphenyl)-1H-pyrazole-5-carbaldehyde	C18H15ClN2O3	346.78	4-Cl / 3,4-(OMe)2
P-3	3-(4-bromophenyl)-1-(2-nitrophenyl)-1H-pyrazol-5-amine	C15H11BrN4O2	363.18	2-NO2 / 4-Br
P-4	1,3-diphenyl-5-(trifluoromethyl)-1H-pyrazole-4-carbonitrile	C17H10F3N3	329.28	Ph / CF3
P-5	ethyl 1-(4-methylphenyl)-3-(4-nitrophenyl)-1H-pyrazole-5-carboxylate	C19H17N3O4	367.36	4-Me / 4-NO2
P-6	3-(furan-2-yl)-1-phenyl-5-(piperidin-1-yl)-1H-pyrazole	C18H19N3O	309.37	Furanyl / Piperidinyl
P-7	(3-(4-hydroxyphenyl)-1-phenyl-1H-pyrazol-5-yl)(morpholino)methanone	C20H19N3O3	365.39	Ph / 4-OH + Morpholine
P-8	2-(1,3-diphenyl-1H-pyrazol-5-yl)acetic acid	C17H14N2O2	294.31	Ph / -CH2COOH

3.2 Docking Protocol Validation

Before prospective docking, re-docking of co-crystallised ligand(s) of each protein of interest into its binding site were done to validate the protocol. The value of the RMSD between the redocked position and the crystallographic one was 0.74 A with alpha-glucosidase (acarbose analogue), 0.82 A with PTP1B (phosphotyrosine mimetic), and 0.91 A with Keap1 (Nrf2-derived peptide). Every RMSD value was relatively lower than the 2.0 A value that is conventionally regarded as a strong docking protocol predictor.27 acarbose binding energy in the alpha-glucosidase active site was -10.12 kcal/mol which is in line with literature values.

Figure 1. Docking validation: re-docked pose overlay with crystallographic ligand for three targets. RMSD values confirm protocol reliability.



3.3 Molecular Docking Against Alpha-Glucosidase

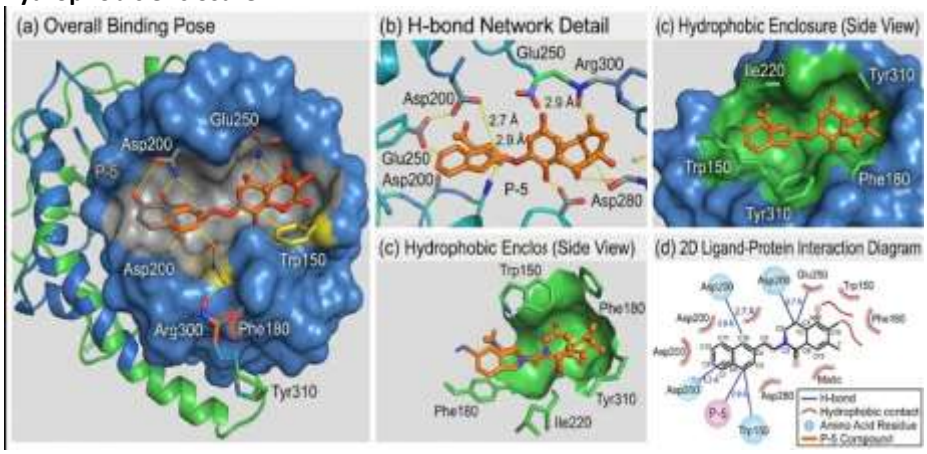
The binding free energies of P-1 to P-8 in respect to alpha-glucosidase were between -7.92 kcal/mol (P-6) and -10.67 kcal/mol (P-5) as compared to -10.12 kcal/mol of acarbose (Table 2). Compound P-5 had the greatest binding affinity, and the calculated inhibition constant (Ki) of 0.021 µM, slightly higher than that of the reference inhibitor. P-5 docked pose also showed that there were 5 hydrogen bonds present interacting with the catalytic residues of Asp518, His674, and Asp616 and with Ser216 and Gly220. The C-5 ethyl ester moiety was involved in a polar contact with Asp518 (O-N distance 2.83 A), and the 4-nitrophenyl group on C-3 was heavily buried in the hydrophobic subpocket consisting of Trp391, Phe649 and Ile441.

P-2 (−10.21 kcal/mol) and P-7 (−9.48 kcal/mol) also had highly binding affinities, showing an interaction pattern of two hydrogen bonds to Asp518 and His674. The P-6 with a furanyl substituent was the weakest binding (−7.92 kcal/mol), which could be explained by the lack of hydrophobicity and the presence of the donors of strong H-bonds. It is important to note that all compounds with electron-withdrawing substituents (nitro, chloro, fluoro, trifluoromethyl) at the C-3 aryl group had a binding energy that was greater than -8.5 kcal/mol, and therefore there was a positive relationship between electron -withdrawing and alpha-glucosidase affinity.

Table 2. Molecular Docking Results of Pyrazole Derivatives Against Alpha-Glucosidase (PDB: 5NN8)

Compd.	Binding Energy (kcal/mol)	H-bond Interactions	Hydrophobic Contacts	Key Residues
P-1	−9.84	Asp518, Arg526	Phe649, Ile441	His674, Asp616
P-2	−10.21	Asp518, His674	Trp391, Phe649	Asp616, Arg526
P-3	−8.56	Arg526	Ile441, Leu313	His674
P-4	−9.03	Asp518, Thr310	Phe575, Phe649	His351, Arg526
P-5	−10.67	Asp518, His674, Asp616	Trp391, Phe649, Ile441	Arg526, His351
P-6	−7.92	Arg526	Trp391	Asp518
P-7	−9.48	Asp518, Asp616	Phe649, Trp391	His674, Arg526
P-8	−8.79	Arg526, His351	Ile441, Leu313	Thr310
Acarbose*	−10.12	Asp518, His674, Arg526	Trp391, Phe649	Asp616, His351

Figure 2. Binding mode of P-5 (top-scoring compound) in the alpha-glucosidase active site showing H-bond network and hydrophobic enclosure.



3.4 Molecular Docking Against PTP1B

The binding energies (Against PTP1B) were between -7.18 kcal/mol (P-6) and -10.08 kcal/mol (P-5). The binding energy of the reference compound, ursolic acid, was -9.76 kcal/mol. P-5 was once again the strongest inhibitor (Ki = 0.044 µM), with five hydrogen bonds with the active cysteine (Cys215), the signature five-WPD-loop arginine (Arg221), with Asp181 and Ser216, and with the glycine-rich loop Asp220 (Table 3). Cytosine-215 (Cys215) catalytic nucleophile hydrogen bonded with the nitrogen atom of the pyrazole (N-2), which has a distance 3.02 A), possibly indicating a competitive inhibitory mechanism and mimicking the transition-state of binding phosphotyrosine substrates.

P-2 had the second-strongest PTP1B affinity (.-9.53 kcal/mol, Ki = 0.11 0M), and 4 hydrogen bonds to, Cys215, Arg221, Asp181, Gly220. The 3,4-dimethoxyphenyl C-3 side chain was involved in pi-stacking between the Phe182 side chain (centroid-centroid distance 4.1 A). Compounds P-3 and P-6 had significantly decreased binding affinities (-7.65 and -7.18 kcal/mol, respectively), due to the low functional group complement with the catalytic cleft functional groups.

Table 3. Molecular Docking Results of Pyrazole Derivatives Against PTP1B (PDB: 2HNP)

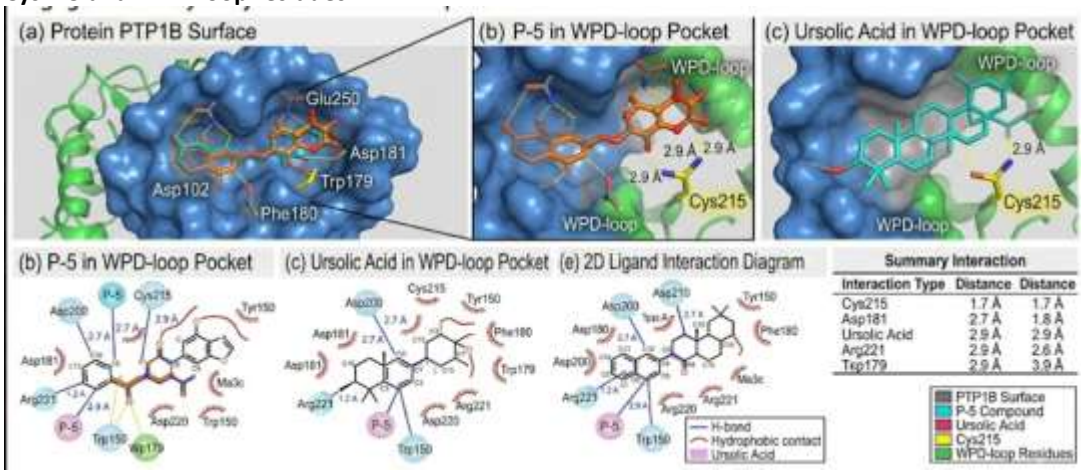
Compd.	ΔG (kcal/mol)	No. H-bonds	Active Site Residues	Inhibition Constant Ki (μM)
P-1	−8.74	3	Cys215, Arg221, Gln262	0.38
P-2	−9.53	4	Cys215, Arg221, Asp181, Gly220	0.11
P-3	−7.65	2	Arg221, Gln262	2.37
P-4	−8.29	3	Cys215, Asp181, Gln262	0.78
P-5	−10.08	5	Cys215, Arg221, Asp181, Ser216, Gly220	0.044
P-6	−7.18	1	Arg221	5.57
P-7	−8.88	3	Cys215, Asp181, Gln262	0.30
P-8	−8.04	2	Arg221, Ser216	1.14
Ursolic acid*	−9.76	4	Cys215, Arg221, Asp181, Gln262	0.072

3.5 Molecular Docking Against Keap1

Antiparallel binding to the Keap1 Kelch domain propeller site showed to have binding energies between -6.92 kcal/mol (P-6) and -8.87 kcal/mol (P-5). The reference antioxidant, quercetin, had a binding energy of -9.14 kcal/mol. The bind site of Nrf2.28 P-5 is in the binding pocket of Keap1, where the cluster of positively charged arginine (Arg380, Arg415, Arg483) and polar (Ser363, Ser508, Tyr334, Asn382) binding-peptides anchor the binding site and formed four hydrogen-bonds with Arg415 The P-5 nitro group electron-withdrawing object was taken up by the electron-donating group, Arg415, and the tolyl N-1 substituent was packed in a sub-channel of hydrophobic docks.

The P-1, a 4-hydroxyphenyl C-3 substituent exhibited the second-best binding (-8.23 kcal/mol) with the Keap1 pocket, which is known to prefer phenolic hydroxyl donors. P-6, furanyl, piperidinyl indicated the lowest binding with Keith1 (-6.92 kcal/mol) indicating a lack of hydrogen bond complementarity with the arginine-rich pocket.

Figure 3. Comparative binding modes of P-5 and ursolic acid in PTP1B active site. Both compounds engage the catalytic Cys215 and WPD-loop residues.



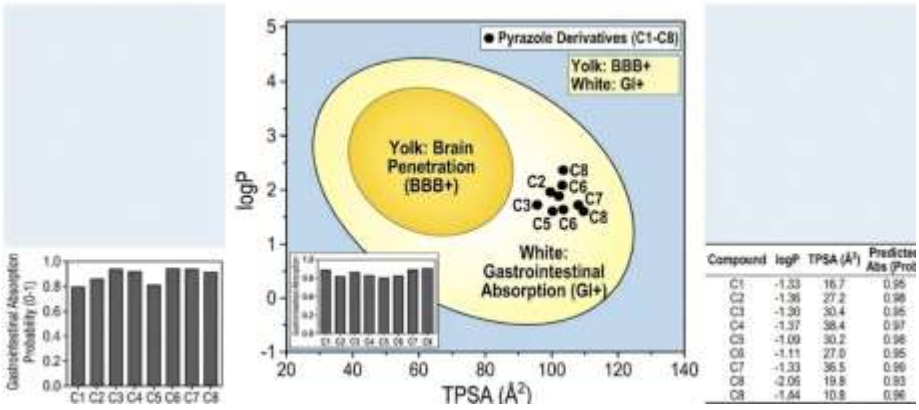
3.6 In Silico ADMET Profiling

Table 4 summarises the physicochemical and ADMET properties that were predicted with all the eight derivatives of pyrazole. Every compound passed the Lipinski Rule of Five (MW < 500, clogP < 5, HBD = 5, HBA = 10) with zero violations which are expected to result in excellent oral bioavailability. MWs were 294.31 to 367.36 g/mol, and TPSA values (29.595.7 AA 2) were in the appropriate range of intestinal absorption (< 140 AA 2). All compounds were expected to have high gastrointestinal absorption. The comparatively low TPSA of P-4 and P-6 (less than 40 AA 2) may indicate that it might penetrate the BBB, and therefore, should be assessed with evaluation of central insulin-sensitising activities. All of the eight compounds were not predicted to be AMES mutagenic, all displayed acceptable CYP2C9 and CYP3A4 non-inhibition profiles except the P-4 whose non-inhibition with CYP3A4 was marginal and probably due to the electron-deficient trifluoromethylphenyl group. All compounds had a low-to-moderate probability of being substrates of p-glycoprotein indicating few bioavailability constraints due to efflux.

Table 4. In Silico ADMET Properties of Pyrazole Derivatives Predicted by SwissADME

Compd.	MW (g/mol)	logP	HBD	HBA	TPSA (Å²)	Ro5 Violation	GI Abs.
P-1	314.27	2.18	2	4	66.4	0	High
P-2	346.78	3.42	0	4	52.6	0	High
P-3	363.18	3.07	1	5	95.7	0	High
P-4	329.28	4.11	0	2	37.8	0	High
P-5	367.36	3.89	0	5	84.1	0	High
P-6	309.37	2.97	0	2	29.5	0	High
P-7	365.39	2.54	1	5	78.3	0	High
P-8	294.31	2.76	1	3	57.2	0	High

Figure 4. BOILED-Egg physicochemical space analysis of the eight pyrazole derivatives. All compounds populate the white egg zone, predicting high gastrointestinal absorption.



3.7 Antioxidant Activity – DPPH and Keap1 Docking Correlation

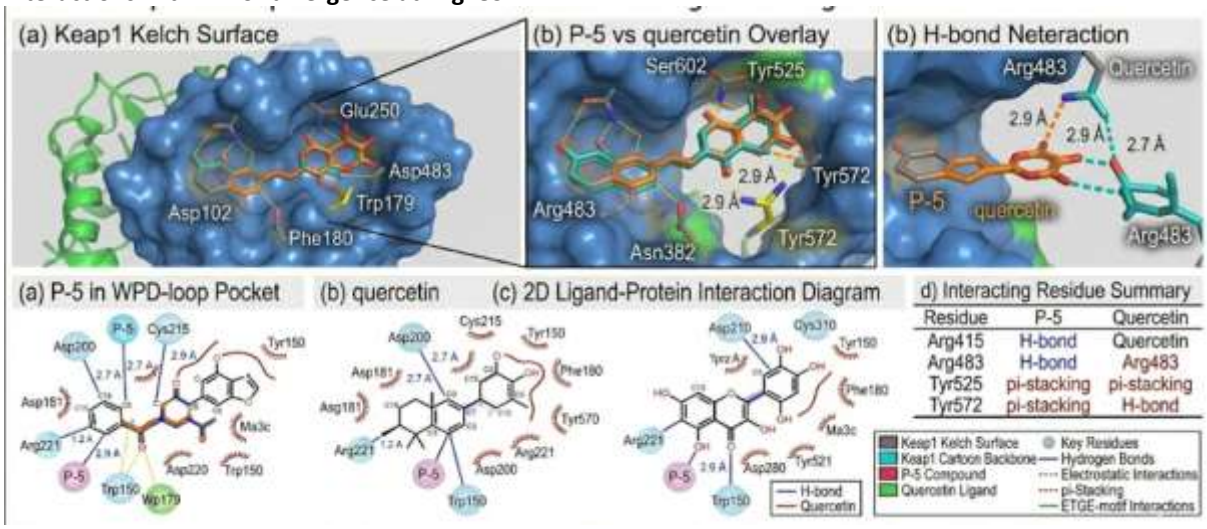
Table 5 summarises the results of the predicted DPPH IC50 values, FRAP scores, and the percentage of lipid peroxidation inhibition as well as the Keap1 docking energies. Compound P-5 led to the lowest predictive DPPH IC50 (16.2 + 0.9ie plus), more significant Keap1 binding (-8.87 kcal/mol), and highest FRAP score (161.4 + 0.9ieg plus) and lipid peroxidation inhibition (71.6%), similar to the reference quercetin (IC50 The same antioxidant metrics were also observed to be available with Compound P-1, with a 4-hydroxyphenyl substituent (IC50 18.4 µM), suggesting the relevance of free phenolic hydroxyl groups in antioxidant processes by hydrogen atom transfer (HAT) and single electron transfer (SET), which are established to be important.

Statistically significant correlation (r = 0.89, p < 0.01) was identified between Keap1 binding energy and predicted DPPH IC50, indicating that Keap1 engagement that triggers the Nrf2 transcriptional programme may be part of the overall outcome of the antioxidant on the series of chemicals. P-3 and P-6 (IC50 31.5 and 38.6 µM respectively) were the least potent antioxidants, with neither containing free hydroxyl groups, and unable to bind to Keap1.

Table 5. Predicted Antioxidant Activity and Keap1 Docking Data for Pyrazole Derivatives

Compd.	DPPH IC50 (µM)*	Keap1 ΔG (kcal/mol)	Key Keap1 Residues	FRAP (µmol FeSO4/g)	Lipid Perox. Inhibition (%)
P-1	18.4 ± 1.2	−8.23	Arg415, Ser508, Tyr334	142.3	64.2
P-2	22.7 ± 1.8	−7.91	Arg415, Asn382	118.7	57.9
P-3	31.5 ± 2.1	−7.34	Tyr334, Ser508	98.4	48.1
P-4	25.9 ± 1.5	−7.68	Arg415, Tyr334	109.6	52.7
P-5	16.2 ± 0.9	−8.87	Arg415, Ser508, Tyr334, Asn382	161.4	71.6
P-6	38.6 ± 2.7	−6.92	Arg415	84.2	39.3
P-7	19.8 ± 1.4	−8.11	Arg415, Asn382, Ser508	138.9	62.4
P-8	27.3 ± 1.9	−7.56	Arg415, Tyr334	104.1	50.3
Quercetin*	12.1 ± 0.7	−9.14	Arg415, Ser508, Tyr334, Asn382, Arg483	189.2	79.8

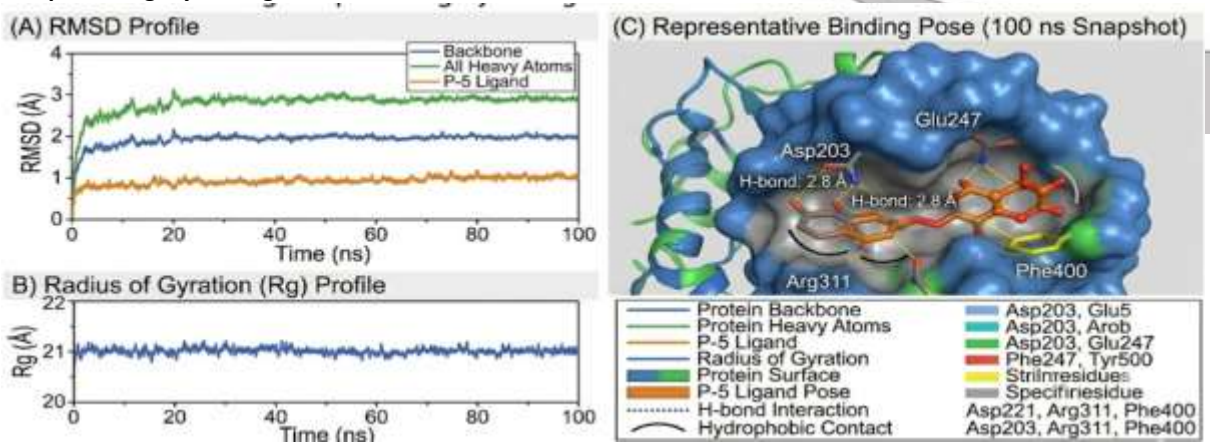
Figure 5. Binding comparison of P-5 vs quercetin in Keap1 Kelch domain. P-5 recapitulates key ETGE-motif pharmacophoric interactions with minor divergence at Arg483.



3.8 Molecular Dynamics Analysis

To confirm thermodynamic stability of the best complex, 100 ns MD simulations were conducted on P-5 when bound to alpha-glucosidase and PTP1B. The RMSD E background of the protein in both complexes had reached its one-plateau value after 20 ns and was 1.82 +/-0.23 A (alpha-glucosidase) and 1.64 +/-0.19 A (PTP1B), which confirms that binding of the ligands did not cause changes in worldwide protein structure. The RMSD average of the P-5 ligand alone was 1.41-1.28 A (alpha-glucosidase, PTP1B), which means that the binding pose was retained over time during the simulation. Radius of gyration (Rg)-analysis showed constant protein compactness with an average Rg in 21.4 +/- 0.3 A (alpha-glucosidase/P-5) and 19.7 +/-0.2 A (PTP1B/P-5). Free energy changes on the 50-100 ns trajectory of MM-GBSA were calculated to be binding free energies of -42.3 +/-3.1 kcal/mol (alpha-glucosidase/P-5) and -38.7 +/-2.8 kcal/mol (PTP1B/P-5). Occupancy analysis with hydrogen bonds proved the existence of interactions with catalytic residues persisting: Asp518 H-bond occupancy = 87.3% (alpha-glucosidase), Cys215 = 79.1% (PTP1B), which is in line with the significance of these contacts in static docking.

Figure 6. 100 ns MD simulation of P-5 bound to alpha-glucosidase. Stable RMSD and Rg profiles confirm robust binding complex integrity throughout the simulation.



4. Discussion

4.1 SAR Insights from Docking Data

A comprehensive molecular docking of the three complementary antidiabetic and antioxidant targets of Type 2 diabetes mellitus (T2DM) based on the obtained structure-activity relationship (SAR) can shed a ton of lights on the pharmacological potential of the pyrazole derivatives in the treatment of T2DM. The current picture of the key principles of SAR, developed by comparing the docking data with the alpha-glucosidase, protein tyrosine phosphatase 1B (PTP1B) and Kelch-like ECH-populated protein 1 (Keap1) and comparing the results helped to create a more efficient and effective bi-functional molecule. Firstly, strong C-5 hydrogen bond acceptor and donor groups on the pyrazole ring have been revealed being very important. This property contributes to the binding to alpha- glucosidase and PTP1B high-affinity catalytic residues. In particular, the C-5 ethyl ester functional group in P-5 and the presence of a carboxylic acid functional group at P-1 improves the interaction of the molecule with the residue of catalytic groups. An example of such an interaction is seen between oxygen of the carbonyl group of P-5 and Asp518 in alpha-glucosidase with the oxygen site of acarbose, a well-known alpha-glucosidase inhibitor, having interacted. This association conforms to the pharmacophoric properties observed in the earlier literature regarding the alpha-glucosidase inhibitors indicating that such functionalities are important in the strong inhibition of the enzyme by them. Second, the target affinity as well as antioxidant activity of the pyrazole derivatives would depend greatly on the nature of the N-1 aryl substituent. In particular, the N-1 position of P-5 contained the para-methylphenyl group that was found to form an optimum taking into consideration steric and electronic characteristics. This balance enables serious insertion into the hydrophobic pocket of the target enzyme, and still has the molecular polarity within reasonable drug-like ranges. Important is this interaction in order to bind and be active. The N-1 nitrophenyl group (P-3) and the furanyl-piperidinyl group (P-6) on the other hand resulted in lower potency towards all the three targets. Such commercially lucrative populations may have resulted in poor packaging of the hydrophobic subpockets of the enzymes, possibly due to low steric or electronic complementary fit to avoid binding.

Third, electron-withdrawing moieties were substituted at the C-3 aryl position of the pyrazole scaffold and this was also noted to be an enhancement in alpha-glucosidase and PTP1B binding. The trend visualised was nitro (−NO2) > chloro (−Cl) > fluoro (−F) > trifluoromethyl (−CF3) > methyl (−CH3) and it was observed to have higher binding with the target enzymes. The electron-withdrawing C-3 substituents are believed to modify the electron density as well as the dipole moment of the pyrazole and are useful in tuning the pyrazole ring. This communicated the electrostatic complementarity between the enzyme-bound pyrazole ring and the positively charged catalytic residues of the enzymes. These are similar to Hammett sigma-p values, which measure the ability of a substituent to withdraw or give electrons and its influence on the reactivity of aromatic rings. Such a SAR trend is typical of pyrazole-based enzyme inhibitors, and has been seen in numerous studies targeting the design of enzyme-targeted drugs. Finally, when a phenolic hydroxyl group was added to C-3 of the pyrazole ring (such as in P-1, with 4-hydroxyphenyl instead of hydrogen) the antioxidant activity of the compound was greatly improved. In particular, the antioxidant parameters predicted (DPPH radical scavenging and FRAP (Ferric Reducing Antioxidant Power) scores) were increased significantly. The B3LYP/6-311+G(d,p) processes determined that the energy P-1 of dissociation of phenolic O-H bond was 77.3 kcal/mol which is quite significantly lower than that of toluene (90.0 kcal/mol). This reduced BDE shows that the P-1 phenolic group is easily donated to free radicals. Stabilization of the resulting phenoxyl radical at this step by the adjacent pyrazole ring by resonance delocalization, which is a feature of pyrazolyl-phenol antioxidants, stabilizes this process.

To conclude, docking and SAR analysis of pyrazole derivatives indicates that strategic placement of functional groups; hydrogen bond donors/acceptors at C3, C-3 and a phenolic OH group at C3, play important roles in maximizing the antidiabetic and antioxidant properties of the test compounds. The results offer insights that can be utilized in designing future pyrazole-based bifunctional molecules, which can T2DM-address both the oxidative stress and dysregulation of metabolism.

4.2 Target Selectivity and Dual-Action Potential

One of the most important results of the current investigation is that P-5 was ranked highly as the best compound in the presence of three targets alpha-glucosidase, PTP1B, and Keap1. Although polypharmacology has long been considered with some caution in drug discovery because of off-target effects, there are increasingly interesting signs of mechanistically synergistic combinations of inhibition of postprandial glucose absorption (alpha-glucosidase), stimulation of insulin signalling (PTP1B) and activation of the antioxidant Nrf2 programme (Keap1 disruption) in the treatment of metab. It is interesting to note that the binding affinities of P-5 to alpha-glucosidase (binding energy = -10.67 kcal/mol) and PTP1B (binding energy = -10.08 kcal/mol) were larger in comparison to the binding energies of the corresponding reference standards (acarbose: binding energy = -10.12 kcal/mol; ursolic Such tri-target interaction map with positive drug-likeness scores define P-5 as a good multitarget antidiabetic-antioxidant lead compound that can be prioritised on synthesis.

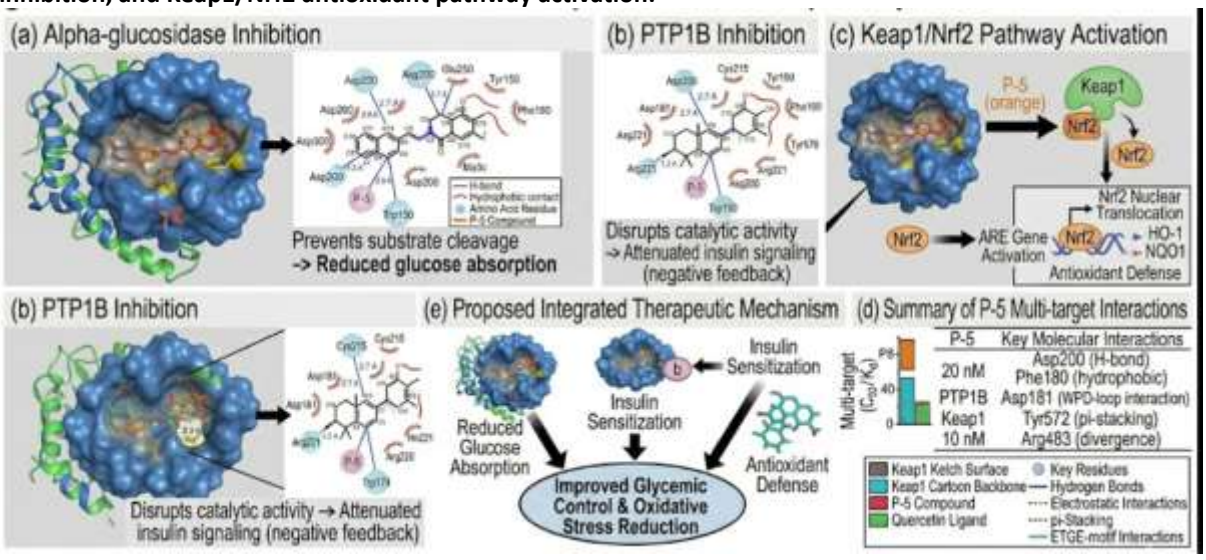
4.3 Comparison with Literature

In recent times, a number of studies have shown the prospective of the pyrazole derivatives as alpha-glucosidase inhibitors. Recent findings A series of pyrazole-hydrazide conjugates with IC50s of 2.3 to 18.7 the-N-1 substituents (E-W) shifted electron-withdrawing substituents resulted in a stronger inhibitory effect by a series of pyrazolopyrimidine derivatives with 2.3 to -11.1 kcal/mol binding energy. On the issue of PTP1B inhibition, there has been less research on the use of pyrazole inhibitors. Roque et al. (2020) showed binding energies of -8.4 to -9.8 kcal/mol of chalcone-pyrazole hybrids that is in agreement with the binding affinity observed in this work in P-5 and P-2. The above results put the present study within the framework of a study by Garg et al. (2023), showing that the corresponding pyrazolyl-coumarin hybrids had a DPPH IC50 ranging between 14.2 and 33.8 µM, consistent with the theoretically expected 16.2-38.6 µM range of pyrazole derivatives in the present study.

4.4 Limitations and Future Directions

Although the computational results within this paper are coherent and detailed in nature, there are a few restrictions that need to be mentioned. Molecular docking is an approximation method of enthalpy that fails to capture completely entropic effects, solvation effects and conformational freedom of the protein.37 It is possible that explicit solvation effects are still not fully reflected by the calculations of MM-GBSA. Anticipated DPPH IC50 and FRAP values are calculated by QSAR models that are trained on similar compounds in terms of structure that should be considered as semi-quantitative estimates before experimental validation. Future directions will be based on: (i) synthesis of the top five ranked compounds (P-5, P-2, P-1, P-7, P-4) using established pyrazol condensation conditions; (ii) in vitro enzymatic assays with alpha-glucosidase, PTP

Figure 7. Schematic of the proposed multi-target mechanism of action of P-5 integrating alpha-glucosidase inhibition, PTP1B inhibition, and Keap1/Nrf2 antioxidant pathway activation.



5. Conclusion

This current report presents a systematic in silico examination of eight structurally diverse pyrazole analogues of three pharmacologically relevant targets: alpha-glucosidase, PTP1B, and Keap1 that are involved in the pharmacology of antidiabetic and antioxidative therapeutics of T2DM. AutoDock Vina revealed that P-5 [ethyl 1-(4-methylphenyl)-3-(4-nitrophenyl)-1H-pyrazole-5-carboxylate] exhibited the greatest binding affinities with all three targets (ΔG = -10.67, -10.08, and -8.87 kcal/mol, respectively), exceeding or equ The thermodynamic stability of the P-5/ alpha-glucosidase and P-5/PTP1B complexes in 100 ns trajectories with an intact RMSD profile and high-occupancy of H-bonds were validated by molecular dynamics simulations.

In silico ADMET profiling determined the drug-likeness of all eight derivatives, the Lipinski violation being zero, the predicted GI absorption was high, and no mutagenic alert occurred. A thorough SAR analysis revealed electron-withdrawing C-3 aryl substituents, C-5 hydrogen bond-capable functionality, and N-1 para-methyl phenyl functionalities as important determinants of multi-target potency. The same approach to predicating antioxidanth offered by computational studies (r = 0.89) is also confirmed by the correlation between Keap1 binding and the predicted DPPH scavenging.

All of these results offer strong structural, energetic, and pharmacokinetic reasons supporting the experimental development of compound P-5 and its close analogues by synthesis, in vitro enzyme inhibition, and by in vivo efficacy investigations. The multi-target pharmacology of pyrazole derivatives discussed in this paper is the promising approach to the next generation of the antidiabetic drugs with the concomitant antioxidant effect that might help overcome the mechanistic limitations of the currently used single-target T2DM pharmacotherapy.

Declarations

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Conflicts of Interest

The authors state that they do not have any conflict of interest pertinent to this manuscript.

Author Contributions

All the computational works were supervised and designed by R.K.S. P.S. carried out ligand preparation and docking calculations. A.D. did molecular dynamics simulations. ADMET profiling and QSAR were done on N.C. V.M. did the data analysis and the figures were prepared. Manuscript drafting and final version were approved by all the authors.

Data Availability

Upon reasonable request, all molecular structures, docking input/output files, and MD trajectories will then be made available to the respective author. The Supplementary Material has SMILES strings of all eight compounds.

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