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ZINCPharmer-Based Identification of Multi-Target Inhibitors for Erectile Dysfunction: Pharmacophore Virtual Screening and Molecular Docking of Commercially Available Compounds

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Abstract

Erectile dysfunction (ED) affects approximately 300 million men globally, with current phosphodiesterase-5 (PDE-5) inhibitors associated with cardiovascular complications, headache, and addiction potential. This study employed ZINCPharmer pharmacophore-based virtual screening to identify novel multi-target inhibitors from commercially available compounds addressing multiple ED pathophysiological pathways. Using established PDE-5 inhibitors as query templates, four hit compounds were evaluated through molecular docking against PDE-5, arginase, protein kinase C (PKC), and O-GlcNAc transferase (OGT). Compound 4 demonstrated superior multi-target binding performance with strongest PDE-5 affinity (-9.11 kcal/mol versus -8.96 kcal/mol for sildenafil), exceptional O-GlcNAc transferase binding energy (-45.41 kcal/mol, surpassing sildenafil at -31.00 kcal/mol), and favorable PKC binding (-52.13 kcal/mol), indicating robust polypharmacological potential. Notably, its higher LD₅₀ value (1000 mg/kg) suggests wider therapeutic window compared to Compound 1 (380 mg/kg), despite two Lipinski violations and low gastrointestinal absorption requiring optimization. Compound 1 exhibited remarkably favorable arginase binding energy (-3.63 kcal/mol), zero Lipinski violations, high gastrointestinal absorption, non-blood-brain barrier permeability, and minimal CYP₄₅₀ inhibition. Toxicological predictions indicated hepatotoxicity for Compound 4 and nephrotoxicity for Compound 1, though both maintained favorable carcinogenicity, mutagenicity, and cytotoxicity profiles. These findings establish Compound 4 as a particularly promising multi-target candidate warranting optimization, while Compound 1 offers complementary drug-like advantages. Both compounds address multifactorial ED pathophysiology more comprehensively than current single-mechanism therapies, establishing foundation for experimental validation and medicinal chemistry optimization.

Keywords: Erectile dysfunction; ZINCPharmer; Pharmacophore screening; Multi-target inhibition; Phosphodiesterase-5; Molecular docking

1 Introduction

Sexual activity is a fundamental aspect of human life, playing crucial roles in reproduction, interpersonal relationships, and overall quality of life [1]. However, sexual dysfunction (SD), particularly erectile dysfunction (ED), represents a significant

global health challenge affecting millions of men worldwide. ED is characterized by the persistent inability to achieve or maintain penile erection sufficient for satisfactory sexual intercourse despite adequate sexual stimulation [2]. The prevalence of ED has been estimated to range between 14% and 48%, with the 2021 National Survey of Sexual Wellbeing reporting a prevalence of 24.2% in the United States alone, though this value may be underestimated due to male reservedness and privacy concerns [3]. Globally, ED is projected to affect approximately 300 million men by 2025, with prevalence increasing significantly with age and comorbidities such as diabetes mellitus, cardiovascular diseases, and metabolic disorders [2,4]. This substantial burden on men's health and quality of life necessitates immediate attention and the development of effective, accessible treatment options.

The pathophysiology of ED involves complex interactions among vascular, neurological, hormonal, and psychological factors [5]. At the molecular level, penile erection is achieved through relaxation of intracavernosal smooth muscle, mediated primarily by the nitric oxide (NO)/cyclic guanosine monophosphate (cGMP) pathway [2]. Nitric oxide released from endothelial cells and cavernous nerves stimulates guanylate cyclase to produce cGMP, which triggers smooth muscle relaxation, increased blood flow into the corpus cavernosum, and subsequent rigid erection [6]. However, several enzymes can disrupt this delicate process. Phosphodiesterase-5 (PDE-5) degrades cGMP, leading to vasoconstriction and penile flaccidity [2]. Arginase competes with nitric oxide synthase for L-arginine substrate, thereby reducing NO bioavailability [7]. Protein kinase C (PKC) overactivity impairs the NO/cGMP pathway in penile vascular tissues, particularly in diabetic ED [4]. Additionally, O-GlcNAc transferase (O-GlcNAc) has been implicated in ED pathogenesis through modulation of cellular signaling pathways [8].

Current pharmacological management of ED primarily relies on PDE-5 inhibitors such as sildenafil (Viagra®), tadalafil, and vardenafil, which enhance erectile function by preventing cGMP degradation [1]. While effective, these medications are associated with significant adverse effects including headache, dizziness, flushing, dyspepsia, nasal congestion, and potentially serious cardiovascular complications [3,4]. Furthermore, their short-term effects and potential for addiction necessitate the exploration of alternative therapeutic strategies [2]. The need for safer, more effective

treatments with fewer side effects has prompted researchers to investigate novel compounds and multi-target therapeutic approaches.

In silico analysis, particularly molecular docking, has emerged as a powerful computational tool in modern drug discovery, enabling researchers to predict and evaluate potential drug-target interactions before costly and time-consuming experimental validation [9]. This approach allows for rapid screening of large compound libraries to identify promising lead candidates with optimal binding affinity and specificity for target proteins. As demonstrated in a study exploring *Monodora myristica* seed oil compounds as inhibitors of sterol O-acyltransferase 1, computational methods can effectively predict molecular interactions and guide the development of novel therapeutics for complex metabolic disorders [10]. ZINCPharmer, a specialized pharmacophore-based virtual screening tool, facilitates the search of the ZINC database containing millions of commercially available compounds [11]. By utilizing three-dimensional pharmacophore models derived from known active compounds or protein-ligand complexes, ZINCPharmer enables rapid identification of structurally diverse molecules that share similar spatial arrangements of chemical features essential for biological activity. This approach significantly reduces the time and resources required for hit identification while expanding the chemical space explored beyond traditional similarity-based searches [11].

Despite the clinical success of PDE-5 inhibitors, their associated side effects and limitations underscore the need for novel therapeutic agents with alternative mechanisms of action. Given that ED involves multiple enzymatic pathways, targeting enzymes such as arginase, PKC, and OGT alongside or instead of PDE-5 may offer improved therapeutic outcomes with reduced adverse effects. Therefore, this study utilizes ZINCPharmer to identify potentially novel compounds from the ZINC database that could serve as multi-target inhibitors for enzymes implicated in ED pathogenesis. By exploring compounds with mechanisms extending beyond conventional PDE-5 inhibition, this research aims to contribute to the development of safer and more effective therapeutic alternatives for erectile dysfunction management.

2 Methods

2.1 Ligand Library Creation

Candidate ligands were identified through pharmacophore-based virtual screening using the ZINCPharmer database (<http://zincpharmer.csb.pitt.edu/pharmer.html>) [11]. The search protocol utilized a prepared PDE-5 crystal structure as the receptor template, with established PDE-5 inhibitors (sildenafil, tadalafil, vardenafil, and avanafil; Fig. 1) serving as reference compounds for pharmacophore generation. Pharmacophore features (hydrophobic, hydrogen bond donor/acceptor, positive/negative ions, and aromatic) were automatically identified from the reference compounds using SMARTS pattern matching via the OpenBabel toolkit. These features were stored in a spatial index to enable efficient database searching. The search algorithm evaluated conformers against the query pharmacophore, returning hits only when all pharmacophore features aligned within specified tolerance radii while minimizing the weighted root mean squared deviation (RMSD). The indexed search approach enabled rapid screening independent of library size [11].

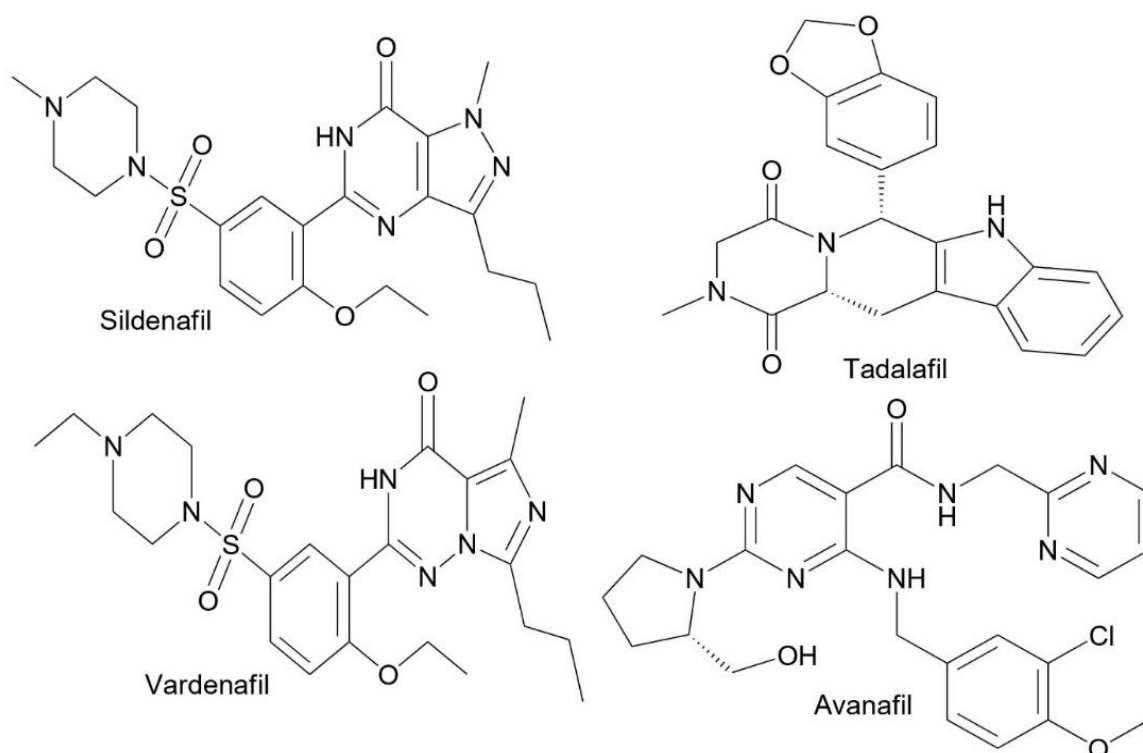
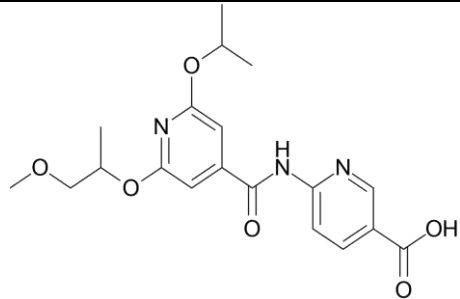
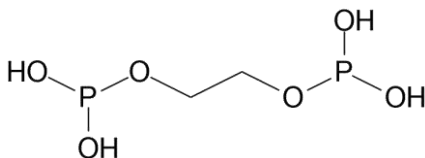
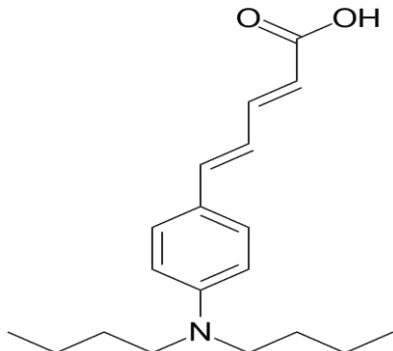


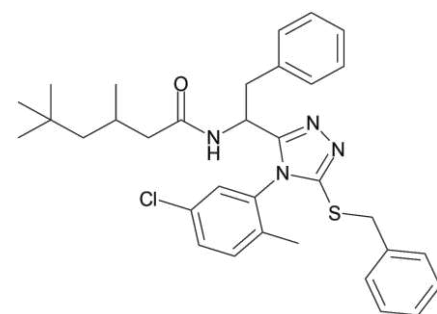
Fig. 1: Chemical structures of standard phosphodiesterase-5 (PDE-5) inhibitors used as reference compounds for pharmacophore-based virtual screening: sildenafil, tadalafil, vardenafil, and avanafil

The query parameters virtual screening yielded four hit compounds (Table 1). Notably, sildenafil and vardenafil generated identical hit compounds due to their structural homology. In contrast, avanafil did not produce any hit compounds under the specified screening conditions. Structural data for the identified compounds were retrieved from the PubChem database (<https://pubchem.ncbi.nlm.nih.gov/>, accessed 30 December 2025) taking into consideration their molecular weights and other properties in the database. Three-dimensional structures were downloaded in their native Structure Data File (SDF) format (.sdf) to preserve coordinate information for subsequent docking studies.

Table 1: Hit compounds identified from pharmacophore-based virtual screening against PDE-5 using standard erectile dysfunction therapeutics as query templates.

Standard drug	Compound identified	PubChem CID	Molecular formula	Structure
Sildenafil and vardenafil	6-[[2-(1-methoxypropan-2-yloxy)-6-propan-2-yloxypyridine-4-carbonyl]amino]pyridine-3-carboxylic acid (Compound 1)	77301337	C ₁₉ H ₂₃ N ₃ O ₆	
	2-dihydroxyphosphanyloxyethyl dihydrogen phosphite (Compound 2)	19796168	C ₂ H ₈ O ₆ P ₂	
	(2E,4E)-5-[4-(dibutylamino)phenyl]penta-2,4-dienoic acid (Compound 3)	18324776	C ₁₉ H ₂₇ NO ₂	

Tadalafil	N-[1-[5-benzylsulfanyl-4-(5-chloro-2-methylphenyl)-1,2,4-triazol-3-yl]-2-phenylethyl]-3,5,5-trimethylhexanamide (Compound 4)	3965416	C ₃₃ H ₃₉ ClN ₄ OS
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Avanafil No hit compound

2.2 Ligand Preparation

The retrieved 3D structure of the hit compounds were subjected to ligand preparation using the LigPrep module (Schrödinger, Maestro Version 12.5.139, MM share Version 5.1.139, Release 2020-3, Platform Windows-x64) using the force field of Optimized Potentials for Liquid Simulations (OPLS-3e) [12]. Prepared ligands underwent energy minimization using the default options on the Macro Model minimization wizard and were saved in standard database format for molecular docking analyses.

2.3 Protein Preparation

The three-dimensional (3D) X-ray crystal structures of human PDE-5 (PDB ID: 2H42) [13], arginase (PDB: 4I06) [14], PKC (PDB: 3IW4) [15], and O-GlcNAc (PDB: 6MA5) [16] in complexes with sildenafil, inhibitor 14, NVP-AEB071, and inhibitor 1a, respectively, were retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (<https://www.rcsb.org/>) (accessed on 30 December 2025). The crystallographic structures exhibited X-ray resolutions of 2.3 Å, 1.8 Å, 2.8 Å, and 2.0 Å, with R-free values of 0.246, 0.195, 0.277, and 0.210, respectively. All protein structures were imported into the Maestro protein preparation panel for preprocessing. Protein structures containing homotrimeric chains (PDB: 2H42; PDB: 3IW4; PDB: 4I06) were reduced to monomeric chains before initiating preprocessing. Using the Protein Preparation Wizard [17], the receptors were optimized, preprocessed, and refined by removing covalently bonded atoms and water molecules, adjusting bond orders, adding hydrogens, creating zero-order metal bonds and disulfide bonds, and generating het states using Epik at pH 7.0 ± 0.2. Protein refinement was performed by optimizing hydrogen bond assignments where necessary, followed by energy minimization using the OPLS-3e force field [18,19].

2.4 Creation of Receptor Grids

The Receptor Grid Generation tool was used to generate the protein grid on the binding site (Glide Grid), which defines the region of ligand-protein interaction. The coordinates of the co-crystallized ligands were used to specify and generate the receptor grid for docking. By selecting the co-crystallized ligand at the active site, the binding location was automatically mapped using a cubic grid box that encompassed all amino acid residues at the active site. The default Glide Grid settings were applied, and the three-dimensional grid center coordinates (X, Y, Z) for each protein are presented in Table 2. The generated grids were saved as zip files and subsequently imported into the Maestro workspace.

Table 2: Grid center coordinates (X, Y, Z) of the active sites for the selected protein targets involved in erectile dysfunction.

Protein/active site coordinate	PDE-5			Arginase			PKC			O-GlcNAc		
	X	Y	Z	X	Y	Z	X	Y	Z	X	Y	Z
Grid Center	31.3	119.91	10.41	33.92	85.7	71.95	5.42	29.98	52.16	-0.22	-45.63	15.49
Coordinates	10	10	10	10	10	10	10	10	10	10	10	10

2.5 Molecular Docking and Molecular Mechanics-Generalized Born Surface Area (MM-GBSA)

Molecular docking simulations were performed using Schrödinger-Maestro version 12.5.139 Grid-based Ligand Docking with Energetics (GLIDE) to evaluate the interactions between the prepared phytochemicals and receptor targets. To minimize overemphasis of steric repulsive interactions that could result in rejection of correct binding modes, the van der Waals radii of receptor atoms were scaled to 0.80 Å with a partial charge cut-off of 0.15 to reduce nonpolar receptor potential and post-docking minimization was enabled. GLIDE standard precision (SP) docking was employed with flexible ligand sampling to generate conformations and evaluate theoretical interactions of phytochemicals with amino acids within the generated grid boxes [18,20]. Binding poses were validated based on GLIDE docking scores, binding affinity, and interactions with experimentally proven important residues [21]. Phytochemicals demonstrating favorable scoring functions compared to the standards (sildenafil and native protein inhibitors) were selected for further analysis. The binding

free energy of receptor-ligand complexes was calculated using Prime MM-GBSA in the Schrödinger suite, with the solvent model and force field set to VSGB and OPLS3e, respectively, while other parameters remained at default settings.

2.6 Validation of Molecular Docking Analysis

To validate the molecular docking protocol, co-crystallized ligands were extracted and re-docked into their respective active sites. The native ligands and top-ranked compounds were optimally superimposed with each target, yielding root mean square deviation (RMSD) values of ≤ 0.5 Å (Figure S1 and Table S1) [22]. Discovery Studio v25.1.0 was used to validate each of the produced complexes [23].

2.7 Pharmacokinetics, Drug-Likeness, and Toxicity Studies

The absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties and drug-likeness characteristics of the highest-ranked HEMEL compounds were predicted using their SMILES notations obtained from PubChem. Predictions were performed using the Swiss ADME web server (<http://www.swissadme.ch/>) and the ProTox-II server (https://tox-new.charite.de/protox_II/) (accessed on 4 January 2026) [24].

3 Results

3.1 Molecular Docking of Hit Compounds Identified from Pharmacophore-Based Virtual Screening

Molecular docking utilizes the predicted binding site geometry to optimize ligand-receptor complementarity. Ligand orientation is critical for optimal interactions with active site residues and co-crystallized ligands. Among the tested compounds, Compounds **1** and **4** demonstrated the most favorable binding affinities (docking scores and MM-GBSA values) against PDE-5, arginase, PKC, and O-GlcNAc (Table 3 and Table S2).

Table 3: Docking scores (kcal/mol), MM-GBSA binding energies (kcal/mol), and interacting residues of PDE-5, Arginase, PKC, and O-GlcNAc transferase with their co-crystallized ligands, sildenafil, and test compounds

Target	Compound	Docking score (kcal/mol)	MM- GBSA (kcal/mol)	Interaction
2H42	Sildenafil (CCL)	-8.96	-65.77	27 bonds: ^a (LEU725, ILE768, GLN775, ALA779, ALA783, GLY819, ALA823), ^b (TYR612, GLN817), ^c (MET816), ^d (PHE820), ^e (ALA767, VAL782, ILE813), ^f (TYR612, HIS613, LEU765, VAL782, LEU804, MET816)
	Compound 1	-9.06	17.79	28 bonds: ^a (TYR612, HIS617, ASP654, HIS657, ASN661, ASN662, SER663, ILE665, GLU682, THR723, LEU725, ASP764, LEU765, ALA783, ILE813, MET816, GLN817, ILE824), ^c (HIS653), ^d (PHE820), ^e (LEU804), ^f (VAL782, PHE820), ^g (ZN501, MG502), ^h (MG502), ⁱ (HIS613, PHE786)
	Compound 4	-9.11	-55.98	27 bonds: ^a (ASN661, ASN662, TYR664, ALA779, VAL782, ALA783, THR802, ASP803, MET805, LYS812, GLN817, GLY819), ^b (SER663), ^c (SER663), ^d (PHE820), ^e (LEU725, MET816, PHE820), ^f (ILE665, PHE786, ILE813, ALA823, ILE824), ^j (PHE786, PHE820)
4I06	CCL	-7.46	77.68	35 bonds: ^a (HIS120, ASP143, ALA146, ASN149, SER155, SER156, ASN158, GLY161, VAL201, ASP251, ASP253, THR255, VAL267), ^b (ASP147, GLU296), ^c (ASP200, ASP202, GLU205), ^g (MN401, MN402), ^h (MN402), ^k (ASP200, ASP202, GLU205, ASP147), ^l (HIS160), ^m (HIS145), ⁿ (ASP202)
	Sildenafil	-2.99	7.82	26 bonds: (ARG39, HIS120, ASN149, SER155, SER156, ASN158, GLY161, ASP253, GLY264, VAL267, MN401, MN402), ^b (ASP200), ^c (ASP147, HIS160, ASP200, ASP202, ASP251, THR251, THR265, PRO266, GLU296), ^l (ASP200), ^m (HIS415)
	Compound 1	-4.10	-3.63	22 bonds: ^a (HIS120, ASP143, SER156, ASP202, PRO203, ASP253, THR265, GLU296), ^b (SER155), ^c (ASP147, HIS160, GLY161), ^d (HIS145, HIS160), ^g (MN401, MN402), ^l (HIS160), ^o (ASP251)

3IW4	Compound 4	-3.78	15.75	23 bonds: ^a (ARG39, HIS120, ASP147, SER156, ASN158, GLY161, ARG199, ASP200, VAL201, PRO203, ASP251, ASP253, THR265, VAL267, GLU296, MN401), ^b (SER155, ASP202), ^d (HIS145, HIS160), ^p (MN402)
	CCL	-8.06	-42.37	26 bonds: ^a (GLY346, PHE350, THR401, GLU418, TYR419, GLY423, ASP424), ^b (LEU345, VAL420, ASP467), ^c (PHE350, ASP467), ^f (LEU345, VAL353, ALA366, LYS368, MET417, VAL420, ALA480), ^j (MET470)
	Sildenafil	-6.90	-61.13	33 bonds: ^a (GLY346, PHE350, GLU387, THR401, ASN421, GLY423, MET426, TYR427, ASP481), ^b (VAL420), ^c (GLU418, TYR419, VAL420, ASP424, ASP467), ^e (VAL353, LYS368, MET417, ALA366, TYR419), ^f (LEU345, VAL353, ALA366, ALA480), ^g (ASP424, ASP467), ^j (MET470)
	Compound 1	-5.73	-34.58	22 bonds: ^a (SER349, ALA366, LEU391, PHE415, MET417, TYR419, VAL420, ASP467, ASN468, PHE482), ^b (ASP481), ^c (GLU387), ^d (PHE350), ^e (VAL353, LEU345, LYS368, MET470), ^f (VAL353, MET470, ALA480), ^k (LYS465)
6MA5	Compound 4	-5.74	-52.13	29 bonds: ^a (LEU345, GLY346, LYS368, THR401, MET417, GLU418, TYR419, ASP424, MET426, TYR427, GLN430, LYS465, ASN468, ASP481), ^b (ASP467), ^d (PHE350), ^e (VAL353, MET470), ^f (PHE350, VAL353, ALA366, VAL420, MET470, ALA480), ^l (ASP467), ^m (PHE350)
	CCL	-5.15	-25.08	23 bonds: ^a (HIS558, PRO559, THR560, LEU563, THR633, LYS634, LEU653, GLY654, PHE694, LEU866, PHE868, THR921, THR922), ^b (GLN839, TYR), ^c (GLN839, HIS920), ^e (HIS498, PRO656, VAL895), ^g (LYS842), ^k (LYS842), ^q (HIS920)
	Sildenafil	-4.96	-31.00	27 bonds: ^a (PRO656, TYR655, GLY654, THR560, LEU563, PHE694, TYR841, GLN839, PRO897, ASP925), ^b (LYS842, LYS898, THR921, THR922), ^c (LEU653, ALA896, HIS920, THR921), ^e (VAL895, LYS898), ^f (PRO559, PHE868, HIS901)

Compound 1	-4.87	-14.82	27 bonds: ^a (HIS461, HIS499, LEU502, THR560, HIS562, LEU563, THR633, LEU653, GLY654, TYR655, PRO656, PHE694, TYR841, VAL895, THR921, THR922), ^b (TYR841, GLN839), ^d (HIS901), ^f (HIS498, PRO559, HIS920), ^g (LYS898), ⁱ (HIS920), ^l (HIS901), ^p (LYS842)
Compound 4	-5.01	-45.41	23 bonds: ^a (ASN557, THR560, LEU563, LYS634, LEU653, PHE694, TYR841, GLN839, LYS842, LEU866, VAL895, THR921), ^d (HIS498), ^e (PRO559), ^f (HIS498, HIS558, PRO656), ⁱ (PHE868), ^j (HIS498, HIS920), ^r (GLY654)

^a:Van der Waal force; ^b:Hydrogen bond; ^c:Carbon-hydrogen bond; ^d:Pi-pi stacked interaction; ^e:Alkyl interaction; ^f:Pi-alkyl interaction; ^g:Attractive charge; ^h:Metal-acceptor bond; ⁱ:Pi-pi T-shaped interaction; ^j:Pi-sulfur interaction; ^k:Salt bridge; ^l:Pi-anion interaction; ^m:Pi-sigma interaction; ⁿ:Unfavorable negative-negative interaction; ^o:Unfavourable acceptor-acceptor interaction; ^p:Pi-cation interaction; ^q:Pi-donor hydrogen bond; ^r:Amide-pi stacked interaction

3.2 Compound Interactions with Amino Acid Residues on Target Proteins

3.2.1 PDE-5 (2H42) Interaction

For PDE-5, Compound 4 (-9.11 kcal/mol) demonstrated the strongest binding affinity, slightly surpassing the co-crystallized ligand (-8.96 kcal/mol), through 27 bonds including VDW forces, hydrogen bonds, carbon-hydrogen bond, pi-pi stacked interaction (PHE820), alkyl interactions, and pi-alkyl interactions (Fig. 2a, 2b; Table 3). However, the co-crystallized ligand sildenafil exhibited superior binding energy (MM-GBSA: -65.77 kcal/mol) compared to Compound 4 (-55.98 kcal/mol). Compound 1 (-9.06 kcal/mol) showed comparable docking scores with 28 bonds involving extensive interactions including metal coordination with ZN501 and MG502, and pi-pi T-shaped interactions, though its MM-GBSA value (17.79 kcal/mol) indicated unfavorable binding energy (Fig. 2a, 2b; Table 3).

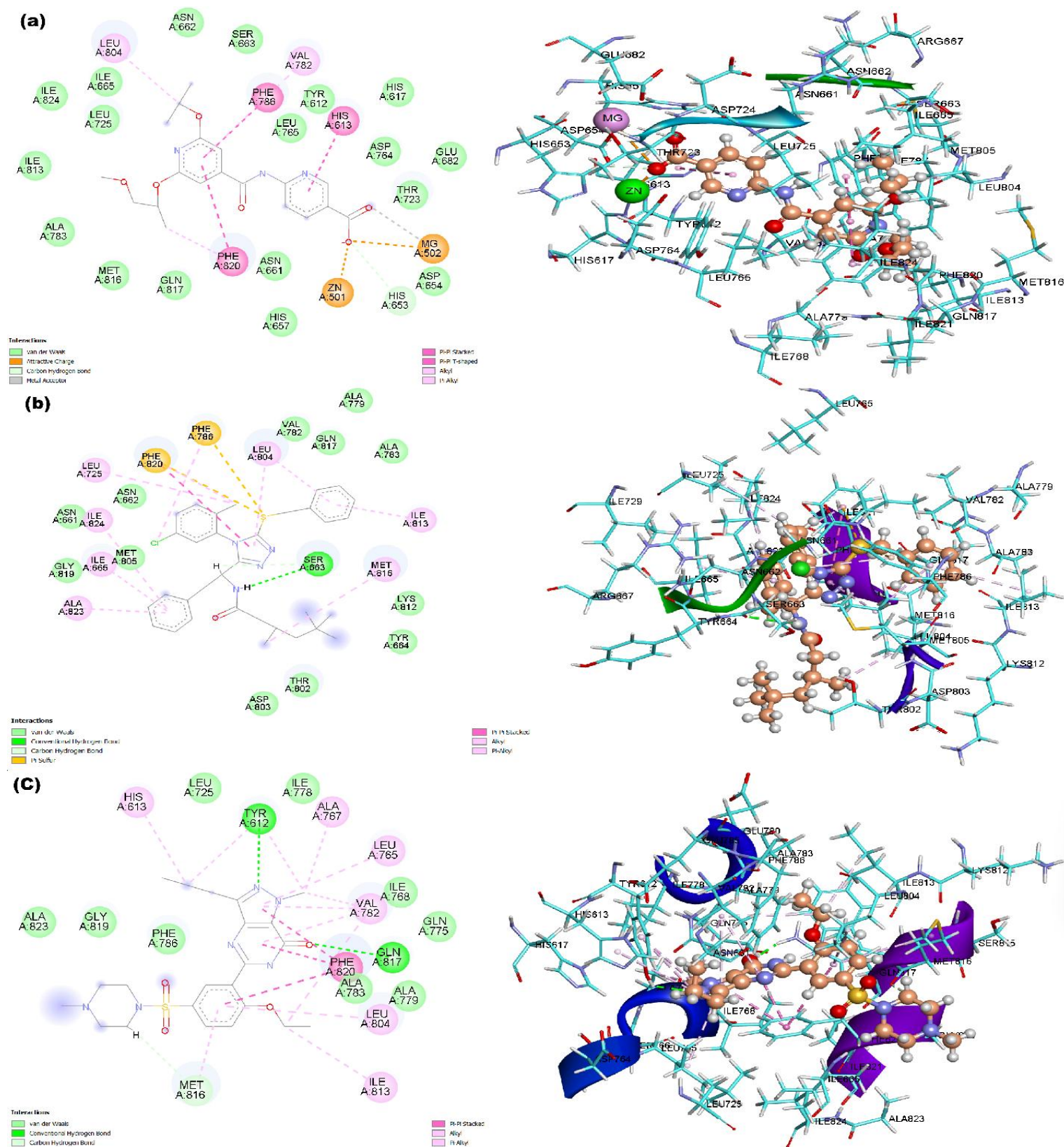


Fig. 2a: 2D and 3D interaction of PDE-5 (2H42) with (a) Compound 1; (b) Compound 4; (c) Sildenafil

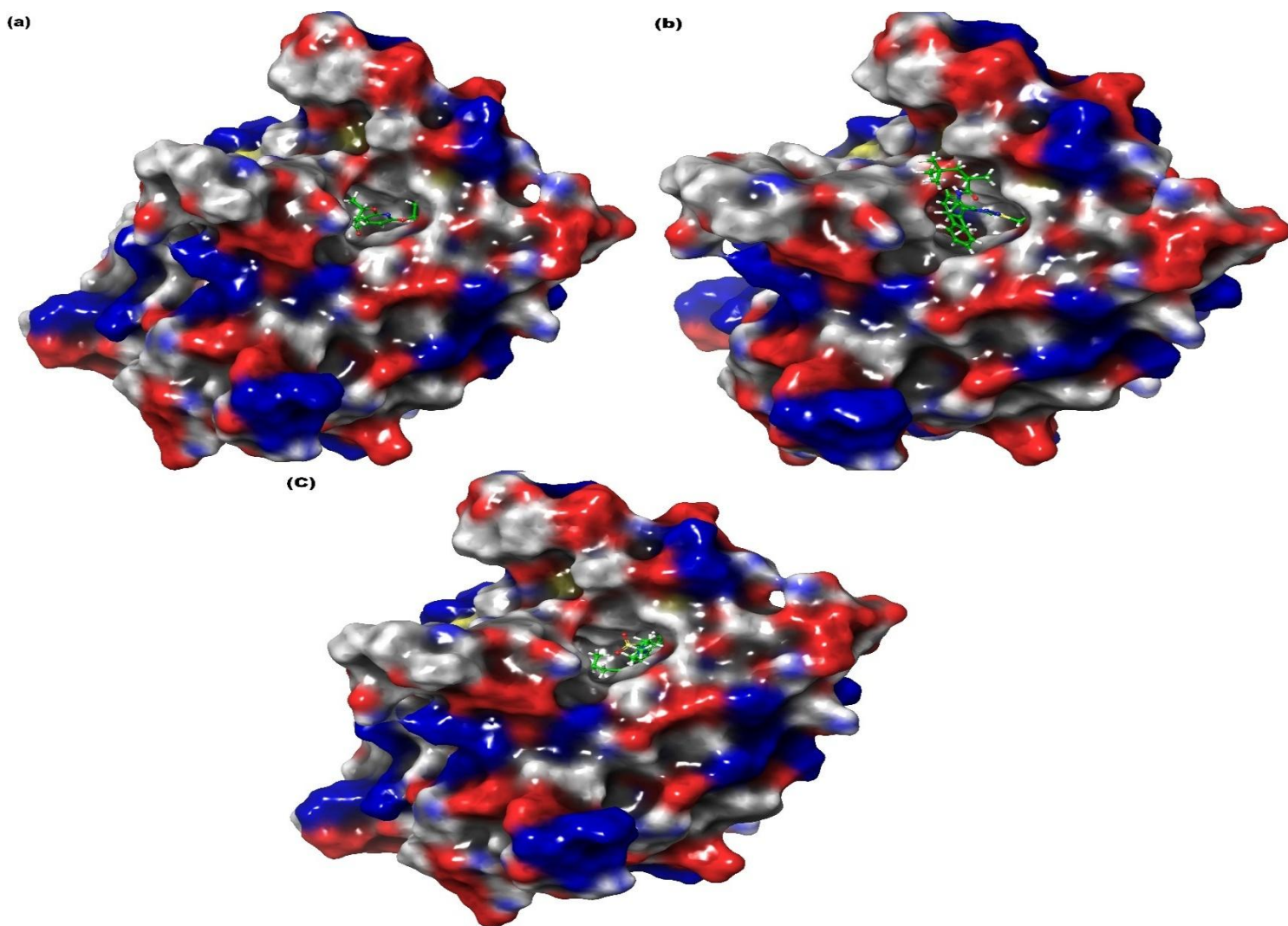


Fig. 2b: Molecular surface (generated based on residue charge) of (a) Compound 1; (b) Compound 4; (c) Sildenafil (co-crystallised ligand) at the binding pocket of PDE-5 (2H42)

3.2.2 Arginase (4I06) Interaction

For arginase, the co-crystallized ligand (-7.46 kcal/mol) exhibited the strongest docking affinity with 35 bonds comprising VDW forces, hydrogen bonds, carbon-hydrogen bonds, metal coordination (MN401, MN402), and salt bridge interactions (Fig. 3a, 3b; Table 3). Compound 1 (-4.10 kcal/mol) demonstrated superior binding energy (MM-GBSA: -3.63 kcal/mol) compared to the co-crystallized ligand (77.68 kcal/mol), forming 22 bonds with VDW forces, hydrogen bonds, pi-pi stacked interactions with HIS145 and HIS160, and metal coordination. Compound 4 (-3.78 kcal/mol) showed moderate interaction with 23 bonds, while sildenafil (-2.99 kcal/mol) exhibited the weakest docking score despite forming 26 bonds (Fig. 3a, 3b; Table 3).

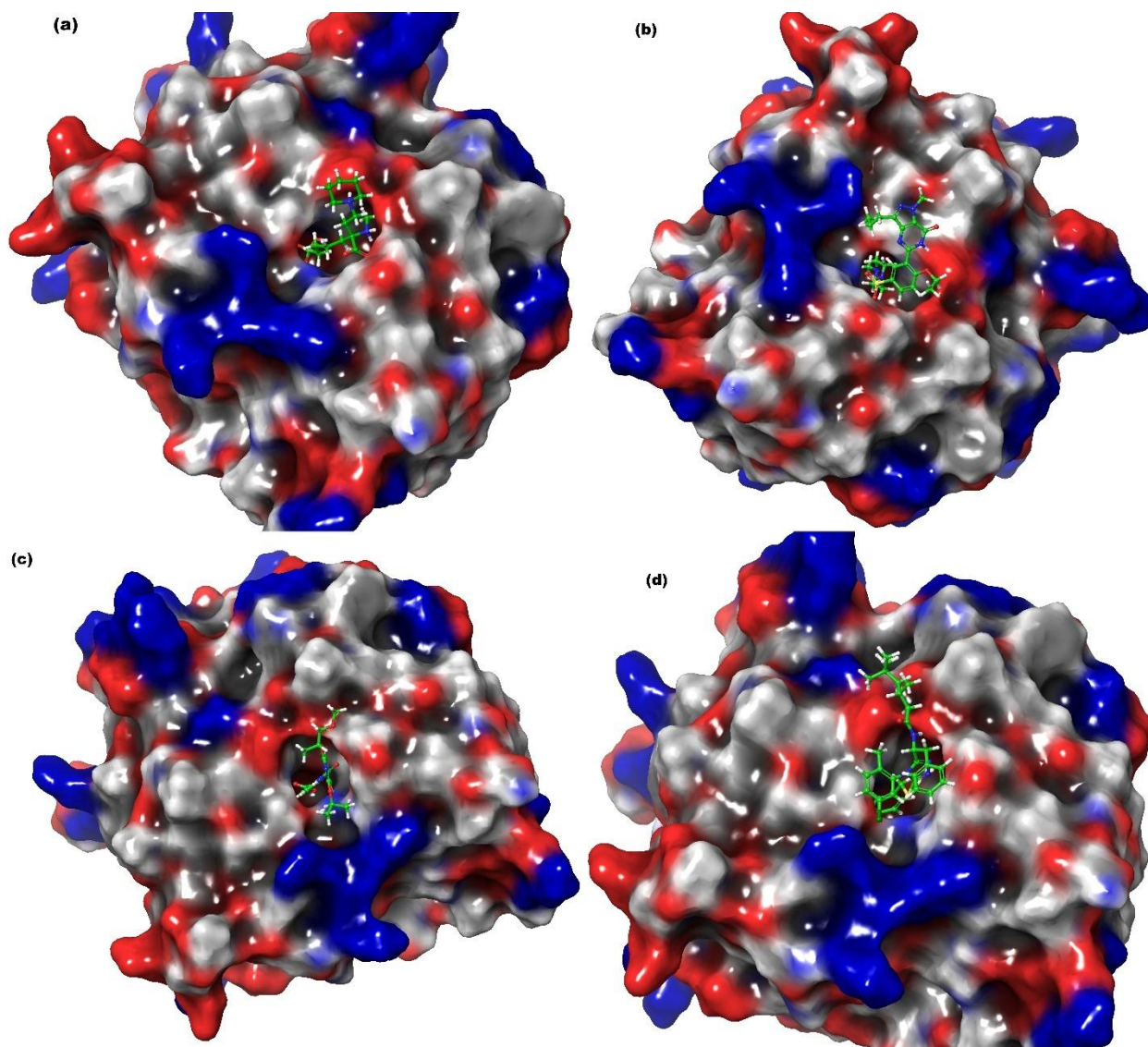


Fig. 3b: Molecular surface (generated based on residue charge) of (a) co-crystallized ligand; (b) Sildenafil; (c) Compound 1; (d) Compound 4 at the binding pocket of arginase (4I06)

3.2.3 Protein Kinase C (3IW4) Interaction

For PKC, the co-crystallized ligand (-8.06 kcal/mol) displayed the highest binding affinity with favorable binding energy (MM-GBSA: -42.37 kcal/mol) through 26 bonds including VDW forces, hydrogen bonds, carbon-hydrogen bonds, pi-alkyl interactions, and pi-sulfur interaction with MET470 (Fig. 4a, 4b; Table 3). Sildenafil (-6.90 kcal/mol) exhibited the most favorable binding energy (-61.13 kcal/mol) with extensive interactions comprising 33 bonds, including alkyl and pi-alkyl interactions (Fig. 4a, 4b; Table 3). Compounds 1 and 4 showed similar docking scores (-5.73 and -5.74 kcal/mol

respectively), with Compound 4 demonstrating better binding energy (-52.13 kcal/mol) through 29 bonds (Fig. 4a, 4b; Table 3).

3.2.4 O-GlcNAc Transferase (6MA5) Interaction

For O-GlcNAc transferase, the co-crystallized ligand (-5.15 kcal/mol) exhibited the strongest docking affinity with favorable binding energy (MM-GBSA: -25.08 kcal/mol) through 23 bonds involving VDW forces, hydrogen bonds, carbon-hydrogen bonds, alkyl interactions, and attractive charge with LYS842 (Fig. 5a, 5b; Table 3). Compound 4 (-5.01 kcal/mol) demonstrated the most favorable binding energy (-45.41 kcal/mol) among tested compounds with 23 bonds including pi-pi stacked and pi-pi T-shaped interactions (Fig. 5a, 5b; Table 3). Sildenafil (-4.96 kcal/mol) showed moderate binding energy (-31.00 kcal/mol) with 27 bonds, while Compound 1 (-4.87 kcal/mol) exhibited weaker binding energy (-14.82 kcal/mol) despite forming 27 interactions (Fig. 5a, 5b; Table 3).

3.2.5 Pharmacokinetics, Drug-Likeness, and Toxicity Assessment of Top-Ranked Compounds

The ADMET properties of the top-ranked compounds are presented in Tables 4 and 5. Compound 1 showed no Lipinski violations with favorable drug-like properties (MW: 389.40 g/mol, high GIA, non-BBB permeant), while Compound 4 exhibited 2 Lipinski violations with lower GIA and served as a P-glycoprotein substrate. Both test compounds showed minimal cytochrome P₄₅₀ inhibition profiles compared to sildenafil, which inhibited CYP_{2C9} and CYP_{3A4} (Table 4). Toxicological profiling revealed that sildenafil exhibited no active toxicity patterns across all parameters (LD₅₀: 1000 mg/kg, Class 4). Compound 1 showed nephrotoxicity activity with a lower LD₅₀ value (380 mg/kg, Class 4), while Compound 4 demonstrated hepatotoxicity activity (LD₅₀: 1000 mg/kg, Class 4). All other toxicity endpoints including carcinogenicity, immunogenicity, mutagenicity, and cytotoxicity remained inactive for both test compounds (Table 5).

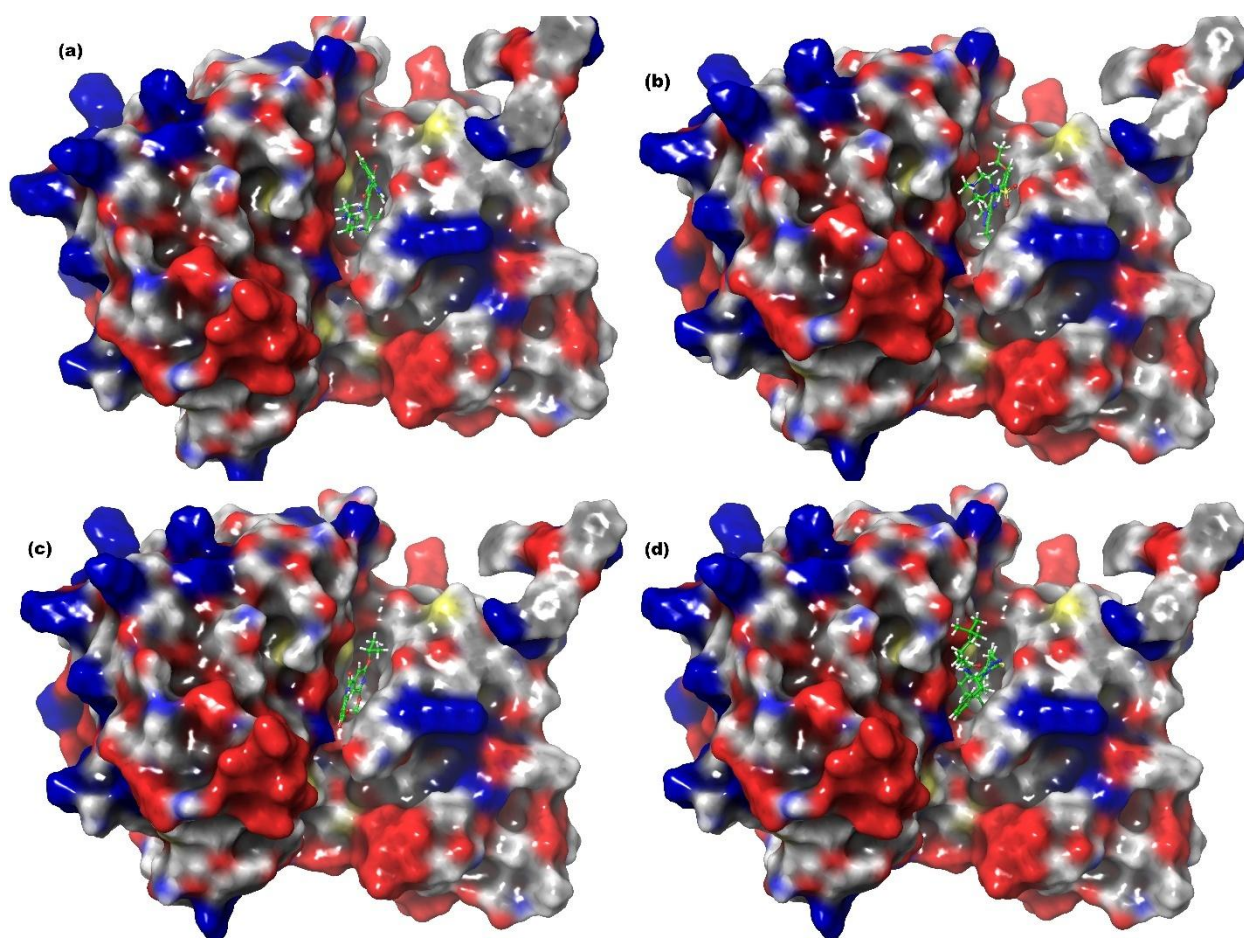


Fig. 4b: Molecular surface (generated based on residue charge) of (a) co-crystallized ligand; (b) Sildenafil; (c) Compound 1; (d) Compound 4 at the binding pocket of PKC (3IW4)

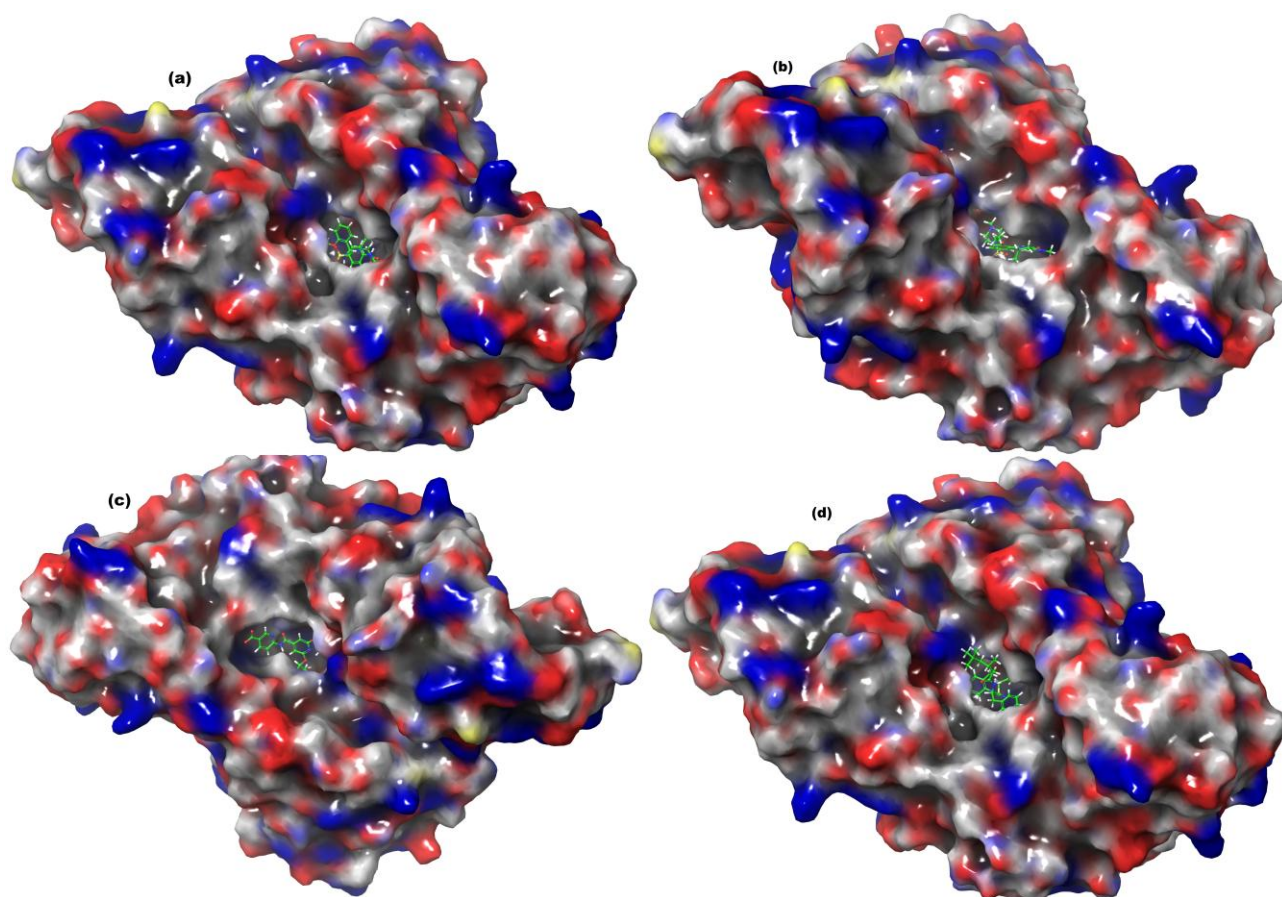


Fig. 5b: Molecular surface (generated based on residue charge) of (a) co-crystallized ligand; (b) Sildenafil; (c) Compound 1; (d) Compound 4 at the binding pocket of O-GlcNAc (6MA5)

3.2.6 Pharmacokinetics, Drug-Likeness, and Toxicity Assessment of Top-Ranked Compounds

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Table 4: Pharmacokinetic and drug-likeness properties of test compounds and reference ligands used in molecular docking analysis

Compounds	MW (g/mol)	NHA	NHD	LP	BS	LVN	GIA	BBP	PgpS	CYP1A2 Inb.	CYP2C19 Inb.	CYP2C9 Inb.	CYP2D6 Inb.	CYP3A4 Inb.	SA
Compound 1	389.40	8	2	-0.26	0.56	0	High	No	Yes	No	No	No	No	No	3.66
Compound 4	575.21	3	1	5.66	0.17	2	Low	No	Yes	No	Yes	No	Yes	Yes	5.31
Sildenafil (Standard drug)	474.58	8	1	1.20	0.55	0	High	No	Yes	No	No	Yes	No	Yes	3.95
Co-crystallized ligand															
Inhibitor 14	317.21	7	5	-3.09	0.55	0	High	No	Yes	No	No	No	No	No	3.30
NVP-AEB071	438.48	5	2	1.86	0.55	0	High	No	Yes	Yes	Yes	Yes	No	No	3.68
Inhibitor 1a	541.60	8	3	0.96	0.11	1	Low	Yes	No	No	No	Yes	No	Yes	4.03

MW: Molecular weight; NHA: Number hydrogen bond acceptor; NHD: Number hydrogen bond donor; LP: Lipophilicity (MLogP); BS: Bioavailability score; LVN: Lipinski number of violations; GIA: Gastrointestinal absorption; BBP: Blood barrier permeant; PgpS: Phosphoglycoprotein substrate; CYP: Cytochrome P450; Inb: inhibitor; SA: Synthetic accessibility

Table 5: Toxicity profiles of test compounds and reference ligands used in molecular docking analysis

Compounds	LD ₅₀ (mg/kg)	Class	Hepatotoxicity	Nephrotoxicity	Cardiotoxicity	Carcinogenicity	Immunogenicity	Mutagenicity	Cytotoxicity
Compound 1	380	4	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Inactive
Compound 4	1000	4	Active	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Sildenafil (Standard drug)	1000	4	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive
Co-crystallized ligand									

Inhibitor 14	5000	5	Inactive	Active	Inactive	Inactive	Inactive	Inactive	Inactive
NVP-AEB071	800	4	Inactive	Inactive	Inactive	Inactive	Inactive	Active	Inactive
Inhibitor 1a	5000	5	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive	Inactive

4 Discussion

Erectile dysfunction (ED), characterized by persistent inability to achieve or maintain penile erection sufficient for satisfactory sexual intercourse, affects an estimated 300 million men globally [2]. The pathophysiology involves complex vascular, neurological, hormonal, and psychological interactions, with penile erection achieved through intracavernosal smooth muscle relaxation mediated by the NO/cGMP pathway [5]. While PDE-5 inhibitors represent the current therapeutic gold standard, their association with cardiovascular complications, headache, dizziness, and addiction potential necessitates alternative strategies [4]. Moreover, ED involves multiple enzymatic pathways beyond PDE-5, including arginase competition with nitric oxide synthase for L-arginine substrate, PKC overactivity impairing NO/cGMP signaling particularly in diabetic ED, and O-GlcNAc transferase (OGT) modulation of cellular pathways [4,7]. This multi-factorial etiology supports multi-target therapeutic approaches potentially offering improved efficacy with reduced adverse effects. This study employed ZINCPharmer pharmacophore-based virtual screening to identify novel commercially available compounds capable of inhibiting multiple ED-implicated targets.

Compounds 1 and 4 emerged as lead candidates with favorable binding affinities across multiple targets. For PDE-5, Compound 4 exhibited superior docking scores (-9.11 kcal/mol) versus co-crystallized sildenafil (-8.96 kcal/mol), with critical interactions at PHE820, TYR612, and GLN817—residues essential for PDE-5 catalytic activity [13]. The pi-pi stacking with PHE820 is particularly significant, as this aromatic residue forms part of the hydrophobic pocket accommodating PDE-5 inhibitors and contributes substantially to binding affinity [25]. Compound 4 may effectively prevent cGMP degradation, thereby maintaining smooth muscle relaxation and penile blood flow. This mechanism parallels findings by Folawiyo et al. [3], where catechin from *Annona senegalensis* demonstrated comparable PDE-5 binding through similar residue interactions. However, MM-GBSA calculations revealed sildenafil's superior thermodynamic stability (-65.77 versus -55.98 kcal/mol for Compound 4), indicating that while Compound 4 achieves favorable geometric complementarity, the energetic profile may be less optimal for sustained target engagement. Compound 1's positive MM-GBSA value (17.79 kcal/mol) despite favorable docking scores highlights

the importance of employing multiple computational metrics, as docking scores alone may overestimate binding potential by neglecting solvation effects and entropic penalties [26]. The extensive interactions of Compound 1 including metal coordination with ZN501 and MG502 suggest potential allosteric modulation warranting molecular dynamics investigation.

Arginase inhibition represents a complementary strategy, as arginase competes with nitric oxide synthase for L-arginine, reducing NO bioavailability essential for erectile function [27]. Compound 1 demonstrated remarkably superior binding energy (-3.63 kcal/mol) compared to the co-crystallized ligand (77.68 kcal/mol), suggesting significant potential for enhancing NO production. Previous studies demonstrate that arginase inhibition in corpus cavernosum enhances erectile responses in diabetic models through restored NO/cGMP signaling [28]. The interactions with HIS145 and HIS160 through pi-pi stacking, coupled with metal coordination to MN401 and MN402, indicate engagement with the binuclear manganese cluster essential for arginase catalytic activity [29]. Multi-target inhibition of both PDE-5 and arginase could provide synergistic benefits by simultaneously preventing cGMP degradation and enhancing NO production.

PKC overactivity, particularly in diabetic ED, impairs endothelial NO/cGMP function through multiple mechanisms including increased reactive oxygen species, reduced eNOS expression, and enhanced contractile protein sensitivity [30]. Compound 4's PKC binding energy (-52.13 kcal/mol) approached the co-crystallized NVP-AEB071 (-42.37 kcal/mol), with 29 interactions including pi-alkyl interactions with VAL353 and MET470. The pi-alkyl and pi-sulfur interactions observed may confer selectivity advantages over less discriminate inhibitors, as demonstrated by El-Gamal et al. [31] where molecular docking analysis revealed that such weak interactions, including pi-sulfur and pi-alkyl contacts, contributed to securing stable binding networks within kinase active sites and enhanced target engagement. Interestingly, sildenafil demonstrated superior PKC binding (-61.13 kcal/mol), suggesting PDE-5 inhibitors may exert off-target PKC modulation contributing to therapeutic effects—a phenomenon warranting investigation.

O-GlcNAc transferase (OGT) catalyzes O-GlcNAcylation of cellular proteins, a post-translational modification implicated in ED pathogenesis through modulation of eNOS

activity and insulin signaling pathways [32]. Compound 4 exhibited binding affinity (-5.01 kcal/mol) comparable to the co-crystallized inhibitor (-5.15 kcal/mol), while demonstrating the most favorable binding energy (-45.41 kcal/mol) among all tested compounds, surpassing both sildenafil (-31.00 kcal/mol) and the co-crystallized ligand (-25.08 kcal/mol). The 23 interactions including pi-pi stacked and pi-pi T-shaped interactions with HIS498 and HIS920 suggest engagement with residues critical for UDP-GlcNAc substrate recognition and peptide substrate binding [33]. Given OGT's role in modulating cellular signaling pathways implicated in ED pathogenesis [34], this multi-target capacity positions Compound 4 as a polypharmacological candidate potentially superior to single-mechanism drugs. Such multi-target approaches increasingly represent rational strategies for complex diseases where single-target therapies demonstrate limited efficacy due to compensatory pathway activation and disease heterogeneity [35].

Pharmacokinetic profiles revealed critical distinctions. Compound 1's compliance with all Lipinski criteria (MW = 389.40 g/mol, HBD = 2, HBA = 8, LogP = -0.26) contrasts favorably with sildenafil's two violations, suggesting superior oral bioavailability [36]. High gastrointestinal absorption coupled with non-blood-brain barrier permeability indicates systemic availability without central nervous system penetration, potentially mitigating headache and dizziness associated with PDE-5 inhibitors [37]. Absence of CYP₄₅₀ inhibition, particularly CYP_{3A4} and CYP_{2C9}, suggests reduced drug-drug interaction potential versus sildenafil which inhibits both isoforms—presenting advantages in patients on polypharmacy. The favorable synthetic accessibility score (3.66) indicates more practical prospects for cost-effective production. However, Compound 4's two Lipinski violations (MW = 575.21 g/mol, low GI absorption) and P-glycoprotein substrate status, while potentially limiting oral bioavailability, may be addressable through structural optimization or formulation strategies without compromising its superior multi-target binding profile.

Toxicological predictions require careful interpretation. While Compound 1's nephrotoxicity prediction occurs at a lower LD₅₀ (380 mg/kg, Class 4), Compound 4 demonstrates a notably higher LD₅₀ value (1000 mg/kg, Class 4) with hepatotoxicity prediction. The higher LD₅₀ suggests a wider therapeutic window and potentially greater safety margin for Compound 4, particularly relevant given its exceptional binding energies across multiple ED targets. Both compounds maintain favorable

profiles regarding carcinogenicity, mutagenicity, and cytotoxicity, though computational toxicology predictions require experimental validation. The convergence of Compound 4's superior docking performance across PDE-5, PKC, and OGT, coupled with its higher LD₅₀ value, positions it as a particularly promising candidate despite pharmacokinetic challenges that may be surmountable through medicinal chemistry optimization.

Comparison with natural product-based ED research reveals convergent findings. Ejeje et al. [4] identified catechin and epicatechin from *Detarium senegalense* with PDE-5 binding affinities of -9.877 and -11.408 kcal/mol respectively—comparable to our Compound 4. Similarly, Folawiyo et al. [3] demonstrated catechin's polypharmacological potential against multiple ED targets, supporting the multi-target approach validated in this study. The structural diversity between natural flavonoids and our synthetic ZINC compounds suggests multiple viable chemical scaffolds for ED therapeutic development. Limitations include the computational nature of predictions, which require experimental validation through enzymatic assays, cellular studies, and animal models. The absence of molecular dynamics simulations for lead compounds limits understanding of binding stability over time. Future investigations should incorporate MD analysis, particularly for Compound 1 given the MM-GBSA discrepancy, to assess conformational dynamics and residence time at target sites. Additionally, the lack of avanafil-derived hits in ZINCPharmer screening warrants investigation into pharmacophore model sensitivity and potential for false negatives.

Conclusion

This study successfully employed ZINCPharmer pharmacophore-based virtual screening to identify structurally diverse, commercially available multi-target inhibitor candidates for erectile dysfunction from the ZINC database. Compounds 1 and 4 demonstrated favorable binding affinities across PDE-5, arginase, PKC, and OGT—enzymes critically implicated in ED pathophysiology. Compound 4 emerged as a particularly promising candidate, exhibiting superior binding performance across multiple targets including exceptional PDE-5 affinity (-9.11 kcal/mol), PKC binding energy (-52.13 kcal/mol), and the most favorable O-GlcNAc transferase binding energy (-45.41 kcal/mol) among all tested compounds. Additionally, its higher LD₅₀ value (1000 mg/kg) suggests a wider therapeutic window compared to Compound 1

(380 mg/kg), despite predicted hepatotoxicity requiring validation. While Compound 4's pharmacokinetic profile presents challenges including two Lipinski violations and low gastrointestinal absorption, these limitations may be addressable through structural optimization strategies that preserve its exceptional multi-target binding capabilities. Compound 1 offers complementary advantages with zero Lipinski violations, superior drug-likeness, minimal CYP450 inhibition, and favorable synthetic accessibility (3.66), though its lower LD50 and nephrotoxicity prediction warrant careful consideration. The multi-target inhibitory profiles of these compounds address the multifactorial pathophysiology of ED more comprehensively than conventional single-mechanism PDE-5 inhibitors, potentially offering enhanced therapeutic efficacy with reduced side effects. However, the MM-GBSA discrepancies and computational nature of predictions underscore the critical need for experimental validation through enzymatic assays, cellular studies, molecular dynamics simulations, and animal models. Future research should prioritize Compound 4 optimization to enhance bioavailability while maintaining its superior multi-target activity, alongside comprehensive toxicological evaluation and selectivity profiling against off-target phosphodiesterase isoforms. This work demonstrates the utility of structure-based pharmacophore screening in early-stage drug discovery and contributes to the evolving paradigm supporting polypharmacological approaches for complex diseases where single-target therapies demonstrate therapeutic limitations.

Abbreviations: ADMET: absorption, distribution, metabolism, excretion, and toxicity; BBB: blood-brain barrier; cGMP: cyclic guanosine monophosphate; CYP450: cytochrome P450; ED: erectile dysfunction; GLIDE: Grid-based Ligand Docking with Energetics; GIA: gastrointestinal absorption; LD50: median lethal dose; MM-GBSA: Molecular Mechanics-Generalized Born Surface Area; MW: molecular weight; NO: nitric oxide; OGT: O-GlcNAc transferase; OPLS-3e: Optimized Potentials for Liquid Simulations-3e; PDE-5: phosphodiesterase-5; PKC: protein kinase C; RCSB PDB: Research Collaboratory for Structural Bioinformatics Protein Data Bank; RMSD: root mean square deviation; SD: sexual dysfunction; VDW: van der Waals

Author contribution: Ibrahim Adeola Moronfolu: Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Software, Validation, Writing – original draft, Writing – review & editing; Abiodun Musliudeen Ibrahim: Formal analysis,

Investigation, Data curation, Software, Validation, Writing – original draft. All authors read and approved the final version of the manuscript.

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Declarations

Ethical Approval: Not applicable. This is an *in silico* study.

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