RJC

RASĀYAN *J. Chem.*
Vol. 15 | No. 4 |2922-2927| October - December | 2022
ISSN: 0974-1496 | e-ISSN: 0976-0083 | CODEN: RJCABP
http://www.rasayanjournal.com
http://www.rasayanjournal.co.in

THE INFLUENCE OF DIFFERENT PHENYL MOIETY SUBSTITUTIONS OF SILDENAFIL DERIVATIVES ON THEIR POTENTIAL INTERACTION TOWARD PHOSPHODIESTERASE TYPE-5

T. Yulianti✉, R. Emran Kartasasmita, S. Ibrahim and F. Kurniawan
School of Pharmacy, Bandung Institute of Technology, Jl. Ganesha No. 10. Bandung, West Java, Indonesia
✉Corresponding Author: tantiyulianti2018@gmail.com

ABSTRACT

Sildenafil has FDA approval as the first agent for erectile dysfunction oral therapy. It works by inhibiting the phosphodiesterase type 5 enzyme and preventing the breakdown of cyclic guanosine monophosphate. Therefore, this study aims to modify the structure of sildenafil to obtain a safer derivative with better phosphodiesterase type-5 inhibition activity. An interaction assessment using molecular docking and dynamic simulation (in silico) was carried out to evaluate the interaction affinity of the derivatives with the phenyl moiety, which has different substituents for PDE5. The results showed that the removal of 4-methylpiperazine-sulfonyl and methoxy from the moiety as well as the addition of propyl ether in the para position led to significant changes in pharmacokinetic properties and affinity toward PDE5. This substituent enables two hydrogen interactions between the Gln817 amino acid residue and the pyrazolopyrimidine ring with a binding energy of -115.65 kJ/mol. Through the reduction of competitive interference for H-bonding with water, these interactions indicate the presence of a higher affinity protein-ligand association. Furthermore, the root-mean-square deviation profile of this sildenafil derivative-PDE5 complex was significantly lower compared to the other five ligand-enzyme complexes, and this indicates a stable conformation.

Keywords: In-silico, Sildenafil, PDE5, Sildenafil Analogs.

RASĀYAN *J. Chem.*, Vol. 15, No. 4, 2022

INTRODUCTION

Erectile dysfunction (ED) has affected more than one million men globally, and it is one of the important factors causing a low quality of life. [1] Several studies revealed that its incidence is associated with aging and several illnesses, such as heart disease, diabetes, hypertension, and neurological conditions. [2] Sildenafil, tadalafil, vardenafil, or avanafil, which are phosphodiesterase type 5 enzyme (PDE5) inhibitors, were the oral medications used to treat it. Furthermore, the PDE5 enzyme plays a key role in intracellular cyclic guanosine monophosphate (cGMP) homeostasis by hydrolyzing cGMP into its inactive form, namely 5-GMP. Inhibitor PDE5's mechanism of action also involves the induction of intracellular cGMP levels. [3] cGMP triggers cGMP-dependent protein kinase (PKG), which decreases calcium levels, trabecular smooth muscle relaxation, venous constriction, and arterial dilatation, leading to the erection state. [4,5] Sildenafil is the most studied pharmacological PDE5 inhibitor among other compounds, and several studies have stated that it is safe and effective for erectile dysfunction treatment. [6] However, some adverse effects, such as headaches, flushing, rhinitis, dizziness, hypotension, dyspepsia, blurred vision, and unexplained myalgia, were also reported. [7] In this study, four new PDE5-I model compounds derived through sildenafil structure modification of the phenyl moiety were studied using docking and molecular dynamic simulation toward PDE5. Combining these techniques resulted in a more accurate prediction of each ligand's proximity to human system environments, as indicated by the free binding energies value and type of interaction within each ligand's active site. Figure-1 shows the two-dimensional structure of four sildenafil derivatives. The pharmacokinetic parameters of the compounds were predicted using the Absorption, Distribution, Metabolism, and Excretion (ADME) predictor application. Assessment of a ligand's molecular interaction in a complex with PDE5 and energy binding value calculations were also carried out. Additionally, the protein conformation changes were measured and expressed by the value of the root mean square deviation (RMSD). Therefore, this study aims to obtain preliminary data related to

http://doi.org/10.31788/RJC.2022.1547078

the variation of phenyl moiety substituents in sildenafil structure by altering its potential interaction with PDE5.

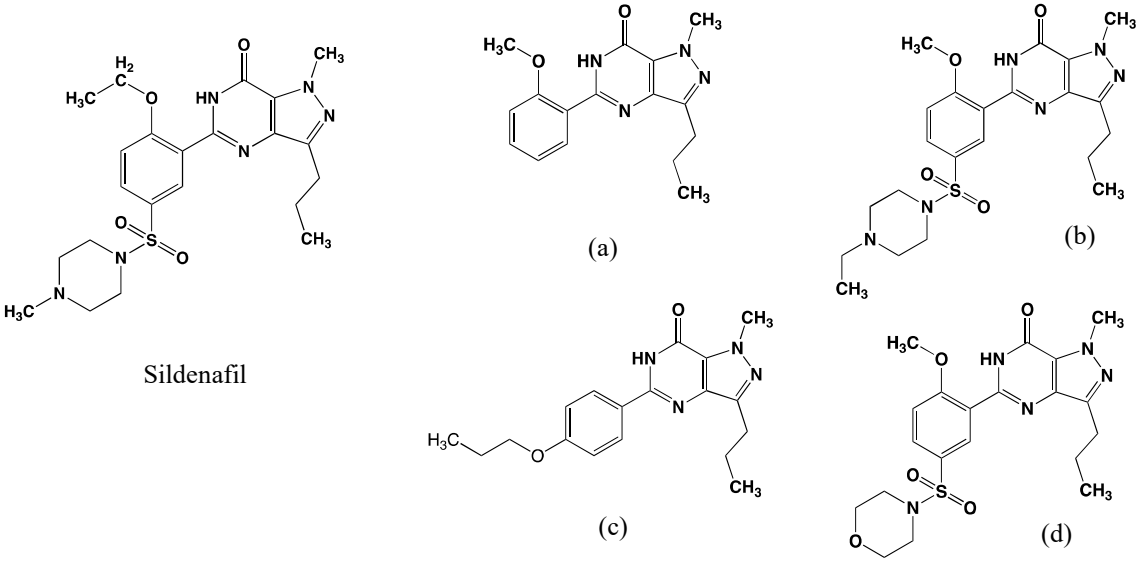


Fig.-1:Two-dimensional structures of sildenafil and its derivatives: (a) 5-(2-methoxyphenyl)-1-methyl-3-propyl-1,6-dihydro-7H-pyrazolo[4,3-d] pyrimidin-7-one (NSA-1); (b) 5-(2-methoxy-5- ((4-methylpiperazin-1-yl) sulfonyl) phenyl) -1-methyl-3-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*] pyrimidin-7-one (NSA-2); (c) 1-methyl-5- (4-propoxyphenyl) -3-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (NSA-3); (d) 5-(2-methoxy-5-(morpholinosulfonyl)phenyl)-1-methyl-3-propyl-1,6-dihydro-7*H*-pyrazolo[4,3-*d*] pyrimidin-7-one (NSA-4).

EXPERIMENTAL

Absorption, Distribution, Metabolism, and Excretion (ADME) Analysis

A web-based Swiss ADME predictor was used to predict the pharmacokinetic behaviors of sildenafil derivatives, with sildenafil as a reference.[8]

Ligands Preparation

The three dimensional structure of the compounds was built using GaussView 5.0.8 software and then applied to the quantum chemistry model using the Gaussian09 software, leading to the most optimum conformation structures.[9]

Macromolecule Preparation

The three-dimensional PDE5 enzyme's structure is derived from the protein data bank, with ID: 2H42. The free PDE5 enzyme molecule was prepared by eliminating other molecules, such as water, metal, and sildenafil. Subsequently, adding the polar hydrogen atoms was carried out using the Discovery Studio Visualizer 2017 software.[10]

In Silico Analysis

Docking has been run using Auto Dock 4.2.3 software. The docking location coordinates were 30.790Å; 119.320Å; and 11.038Å, with a volume box of 50x50x50. The simulation was carried out based on the principles of the Lamarckian Genetic Algorithm.[11] Furthermore, the simulation of molecular dynamics (MD) has been carried out using Amber 16. MD simulation was carried out at 200 ns without restraint on any specified atom, and the temperature was adjusted to 310 K along with the application of the constant pressure dynamic parameter. A descent step method was then performed in terms of energy minimization for stable conformation.[12,13] The enzyme-ligand complexes' binding free energies were calculated using the MM/PBSA method.[14.15] Visualization and evaluation of trajectory were then performed using Biovia Discovery Studio 2017 and Chimera-1.15.[16]

RESULTS AND DISCUSSION

Absorption, Distribution, Metabolism, and Excretion (ADME) Analysis

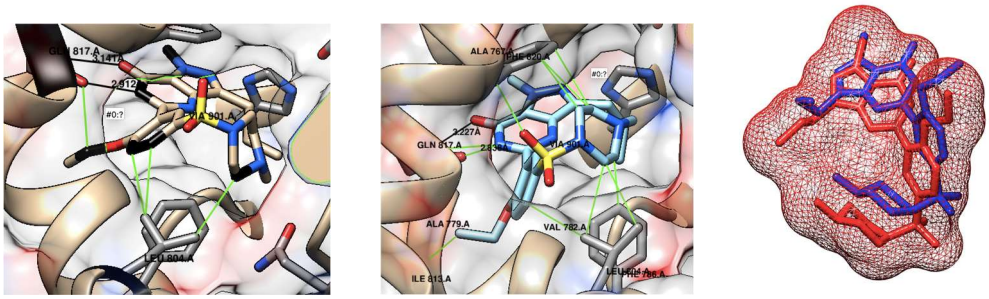
Table-1 shows the pharmacokinetic prediction data of sildenafil and its four derivatives with different substituents in the phenyl moiety. Generally, its predicted pharmacokinetic behavior involves significant changes in the propyl ether substituent in the para position (NSA-3). NSA-3 showed high-level absorption on the gastrointestinal, with the ability to distribute or cross the blood-brain barrier, and its coefficient value of skin permeability (Log Kp) was less negative than sildenafil, namely -6.13 m/s. Its metabolism and excretion were observed from the interaction with five important cytochrome P450 enzymes, namely CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. NS-3 inhibited the activity of those enzymes. Differently from other derivative compound results (NSA-1, NSA-2, and NSA-4), the results revealed that the structural modifications in NSA-1, NSA-2, and NSA-4 did not alter the ADME behavior of sildenafil significantly.

Table-1: Pharmacokinetics Prediction from Swiss ADME Protocol

Name	GI Abs.	BBB	P-gp Subs.	CYP1-A2inh	CYP2-C19inh	CYP2-C9 inh	CYP2-D6 inh	CYP3-A4 inh	Log Kp (cm/s)
Sildenafil	High	No	Yes	No	No	Yes	No	Yes	-8.14
NSA-1	High	No	Yes	No	No	Yes	No	Yes	-8.14
NSA-2	High	No	Yes	No	No	Yes	No	Yes	-8.13
NSA-3	High	Yes	No	Yes	Yes	Yes	Yes	Yes	-6.13
NSA-4	High	No	Yes	No	No	Yes	No	Yes	-8.37

In silico Analysis

The molecular docking parameter setup was validated with the redocking process. The conformation redocking result displayed an RMSD value of 1.5Å. The result also suggests that there is a sustainable hydrogen interaction between sildenafil and Gln817 of the amino acid residue as well as some other chemical interactions, as shown in Fig.-2. All four new substances—NSA-1, NSA-2, NSA-3, and NSA-4—were subjected to a docking simulation procedure toward PDE-5. The result revealed that the most negative score among all the ligands of -52.22 kJ/mol was obtained from NSA-2, followed by NSA-4 with -40.04 kJ/mol. Sildenafil, NSA-1, and NSA-3 had the closest docking scores to each other, which were in the range of -38.32 to -39.20 kJ/mol. Table-2 shows all the simulation results for the compounds assessed. All of the derivatives developed one type of hydrogen interaction with the Gln817 amino acid residue, with distances ranging from 2.201 to 2.576, which are shorter than those of sildenafil. The hydrogen bond with the Gln817 residue plays a key role in the interaction between the compound and its derivative, PDE5.[17] Meanwhile, the variation of interacting amino acid residues that contributed to hydrophobic interaction was observed with Asp764, Ala783, His613, Asp764, Phe786, Asp654, Val782, Leu765, His617, Met816, Phe820, Ile813, and Ala779. The results revealed that the derivatives were still in the catalytic area of residue numbers 613–820, and this concurs with a prior study, which was within the range of 537–860.[18] The overall results of the docking simulation indicate that sildenafil and its four derivatives can interact in a similar way with PDE5.



(A) (B) (C)

Fig.-2: Visualization of (A) 2H42 Crystal of Sildenafil-PDE5 complex; (B) Redocking Result of Sildenafil on PDE-5; (C) Superimposed of Conformation Structure of Sildenafil After Redocking (blue) and that of Initial Sildenafil in PDB ID:2H42 (red). All Figures Are Visualized in A Zone of Less Than 2 Å.

Table-2: Docking Result of Sildenafil and Its Analogues Towards PDE5

Name	Docking score (kJ/mol)	Hydrogen binding			Hydrophobic binding
		No. of H-bond	PDE5 interacting residue	Bond length Å	PDE5 interacting residue
Re-docking (sildenafil)	-39.20	1	Gln817	3.127	Ile813, Ala767, Leu804, Val782, Phe820, Phe786, Ala779
NSA-1	-38.32	1	Gln817	2.576	Ala779, Ile813, Ala783, Leu804, Ala779, Ile813, Val782, Asp803, Ala767
NSA-2	-52.22	1	Gln817.	2.476	Asp764, Ala783, His613, Asp764, Phe786, Asp654, Val782, Leu765, His617, Met816, Phe820, Ile813, Ala779
NSA-3	-38.70	1	Gln817	2.201	Ile813, Phe787, Val782, Phe786, Met816, Asp803, Leu804, Lys809, Phe786
NSA-4	-40.04	1	Gln817	2.336	Phe820, Met816, Ala767, Val782, Leu804

As an effort to simulate the experimental condition, all the compound-PDE5 enzyme complexes' behavior was further explored using MD simulation. The poses of sildenafil analogs obtained through the docking process were used for MD simulation. Subsequently, the stability of the interaction between sildenafil analogs and the PDE5 enzyme was analyzed in a thermodynamic environment that was closer to humans' physiological conditions. [19] The results showed that the introduction of the four different substituents in all derivatives did not change their orientation in the active cavity of PDE5. However, the substitution variation of the phenyl moiety led to a decrease in the energy binding value compared to that of the original compound. The methoxy substituent on the phenyl moiety of NSA-1 significantly decreased its binding energy to -100.47 kJ/mol compared to that of sildenafil, namely -139.26 kJ/mol. The different substituents in NSA-2, NSA-3, and NSA-4 decreased the binding energy values in the range of -115.65 kJ/mol to -125.87 kJ/mol. Despite the decrease in binding energy value, changes in substituents also altered their interaction with PDE5. NSA-1 and NSA-2 caused a conformational alteration in a complex with PDE5, hence the conventional hydrogen binding with Gln817 became a weak hydrogen-carbon bond. Meanwhile, NSA-3 formed two hydrogen bonds with Gln817 of PDE5. It was assumed that the prophoxy substituent on the phenyl moiety, which was attached in paraposition to the pyrazolopyrimidine ring in its structure, favored the interaction with PDE-5. Sildenafil's association with PDE5 was not altered in NSA-4. In biological complexes, hydrogen bonds are the most common directional intermolecular association, and they contribute more to molecular recognition specificity.[17] Table-3 and Fig.-4 show the energy binding values, interaction type, ligand orientation, and conformation structure of the compound and its derivatives in the complex after a 200 ns MD simulation. The similarity metric RMSD is often used in macromolecular structure and dynamics studies.[20]

Table 3. Ligand-PDE5 Interaction Type by MD Simulation

Substances	ΔG_{Bind} (kJ/mol)	Hydrogen binding			Hydrophobic binding
		No. of H-bond	PDE5 interacting residue	Bond length Å	PDE5 interacting residue
Sildenafil	-139.26	1	Gln817	1.695	Phe820, Val782, Leu765, Leu825, Ile813
NSA-1	-100.47	-	-	-	Phe820, Phe786, Leu604, Ala813, Ala783, Val782, Ile768, Ala767
NSA-2	-125.87	-	-	-	Ala823, phe820, Ala767, Ala779, Val782, Ile813, Ala783, Leu604
NSA-3	-115.65	2	Gln817	1.877; 1.892	Ile778, Val782, Phe820, Phe786, Leu604
NSA-4	-118.15	1	Gln817	1.815	Ile813, Leu804, Phe786, Leu825, Phe820, Leu765, Tyr612

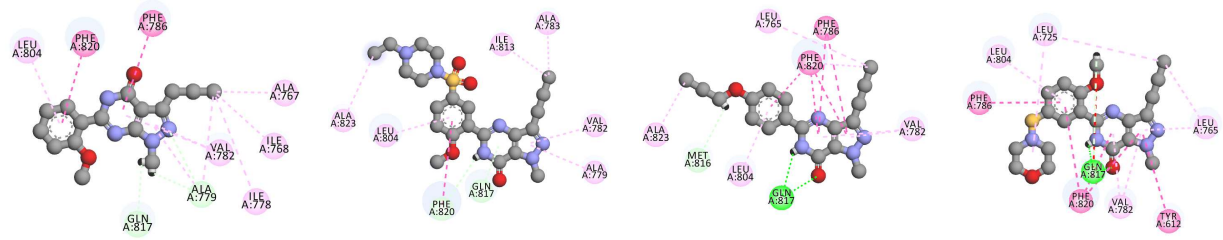


Fig.-4: Three-Dimensional Visualization of Ligand's Orientation Conformation in the Active Site of PDE5 from MD Simulation.

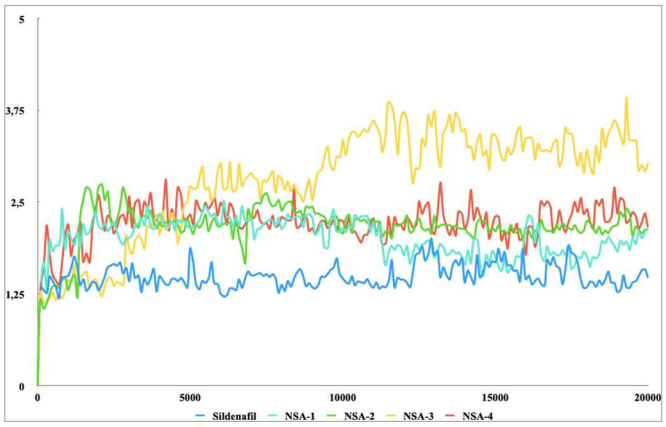


Fig.-5: RMSD Graphic of Sildenafil, a New Derivative Substance in a Complex with PDE5 Enzyme

The values obtained are shown in the graphic between RMSD and time of the production run, as in Fig.-5. Sildenafil, NSA-1, and NSA-3 can stabilize the PDE5 enzyme structure, but the graphic shows that NSA-2 and NS-4 RMSD initiated stability in the last 100 ns.

CONCLUSION

Different substituents in the phenyl moiety of four sildenafil derivatives can significantly change their association with PDE5. Straight orientation and conformation of the NSA-3 structure in the catalytic site of PDE5 cause favorable changes, such as the formation of more hydrogen bonds with Gln817, an important amino acid residue for selective activity.

REFERENCES

1. D.S. Elterman, S.K. Bhattacharyya, M. Mafilios, E. Woodward, K. Nitschelm, and A.L. Burnett, *Research and Reports in Urology*, **13**, 79(2003), https://doi.org/10.2147/RRU.S283097
2. O. Shaeer, and K. Shaeer, *Journal of Sexual Medicine*, **9(12)**, 3018(2012), https://doi.org/10.1111/j.1743-6109.2012.02976.x
3. J.D. Corbin, *International Journal of Impotence Research*, **16(1)**, Jun(2004), https://doi.org/10.1038/sj.ijir.3901205
4. K.E. Andersson, *British Journal of Pharmacology*, **175(13)**, 2554(2018), https://doi.org/10.1111/bph.14205
5. K.E. Andersson, and G. Wagner, *Physiological Reviews*, **75(1)**, 191(1995), https://doi.org/10.1152/physrev.1995.75.1.191
6. U. Gresser, and C.H. Gleiter, *European Journal of Medical Research*, **7(10)**, 435(2002)
7. M.D. Cheitlin, A.M. Hutter, R.G. Brindis, P. Ganz, S. Kaul, R.O. Russell, and R.M. Zusman, *Journal of The American College of Cardiology*, **33(1)**, 273(1999), https://doi.org/10.1016/S0735-1097(98)00656-1
8. A. Daina, O. Michielin, and V. Zoete, *Scientific Reports*, **7**, 42717(2017), https://doi.org/10.1038/srep472717

9. G. Al-Dahash, H.I. Abood, G.A.H. Jaber, and B.A. Ghalib, *International Journal of ChemTech Research*, **9(10)**, 83(2016)
10. S.R.S Nayara, K.S.G. Luana, L.D. Jonatas, S.S. Juliane, F.S. Cesar, S.B. Francinaldo, C.S. Rai, S.C. Josivan, I.S.H. Loran, and B.R.D.S. Cleydon, *Computational Molecular Bioscience*, **4**, 47(2014), https://doi.org/10.4236/cmb.2014.44006
11. G.M. Morris, D.S. Goodsell, R.S. Halliday, R. Hueyh, W. E. Hart, R.K. Belew, A. J. Olson, *Journal of Computational Chemistry*, **19(14)**, 1639 (1998)
12. D.A. Case, T. Cheatham, T. Darden, R.E. Duke, *Journal of Computational Chem*istry, **26(16)**, 1668(2005), https://doi.org/10.1002/jcc.20290
13. B.R. Miller, T.D. McGee, J.M. Swails, N. Homeyer, H. Gohlke, and A.E. Roitberg, *Journal of Chemical Theory and Computation*, **8(9)**, 3314 (2012), https://doi.org/10.1021/ct300418h
14. C. Wang, D. Greene, L. Xiao, R. Qi, and R. Luo, *Frontiers in Molecular Biosciences*, **4**, 87(2018), https://doi.org/10.3389/fmolb.2017.00087
15. E.F. Pettersen, T.D. Goddard, C.C. Huang, G.S. Couch, D.M. Greenblatt, E.C. Meng, and T.E. Ferrin, *Journal of Computational Chemistry*, **25(13)**, 1605(2004), https://doi.org/10.1002/jcc.20084
16. G. Kayık, N. Tüzün, and S. Durdagi, *Journal of Enzyme Inhibition and Medicinal Chemistry*, **32(1)**, 311(2017), https://doi.org/10.1080/14756366.2016. 1250756
17. B.J. Sung, H.K. Yeon, J.Y. Ho, J. Lee, J.H. Kim, J. Moon, Y.L. Hyun, E. Kim, S.J. Eum, S.Y. Park, J.O. Lee, T.G. Lee, S. Ro, and J.M. Cho, *Nature*, **425(6953)**, 98(2003), https://doi.org/10.1038/nature01914
18. S. Purawat, P.U. Ieong, R.D. Malmstrom, J.C. Garret, A.K. Yeung, R.C. Walker, I. Altintas, and R.E. Amaro, *Biophysical Journal*, **112(12)**, 2469 (2017), https://doi.org/10.1016/j.bpj.2017.04.055
19. R.F. De Freitas, and M. Schapira, *MedChemComm,* **8(10)**, 1970(2017), https://doi.org/10.1016/c7md00381a
20. K. Sargsyan, C. Grauffel, and C. Lim, *Journal of Chemical Theory and Computation*, **13(4)**, 1518(2017), https://doi.org/10.1021/acs.etc.7b00028

[RJC-7078/2022]