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Determination of Potential Main Binding Sites of Apixaban in p38 MAPK Target Protein by Molecular Docking Analysis

Apiksabanın p38 MAPK Hedef Proteininde Potansiyel Ana Bağlanma Bölgelerinin Moleküler Kenetlenme Analizi ile Belirlenmesi

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ABSTRACT

Apixaban ($C_{25}H_{25}N_5O_4$), as an anticoagulant has been shown to be superior to warfarin in preventing stroke and systemic embolism and causes significantly less major bleeding based on large randomized trials. In this study, in the first stage, the conformational preferences of Apixaban molecule were examined by conformational analysis, using PM3. The most stable conformer of the Apixaban, which has the lowest energy among all possible conformations, was used as initial data in molecular docking analysis of the molecule into the target protein. Since p38 Mitogen-activated protein kinase protein (p38 MAPK) is an important player in the post-ischemic myocardial apoptotic signal transduction pathway, the interaction mechanisms of Apixaban docked into the p38 MAPK target protein were investigated. The interacting amino acid residues of the target protein with Apixaban, interaction modes, as well as the binding affinity were calculated.

Keywords: *Apixaban, Anticoagulant, Conformation, Molecular Docking*

ÖZET

Apiksaban ($C_{25}H_{25}N_5O_4$), bir antikoagülan olarak inme ve sistemik emboliyi önlemede varfarinden üstün olduğu ve büyük randomize çalışmalara dayanarak önemli ölçüde daha az majör kanamaya neden olduğu gösterilmiştir. Bu çalışmada, ilk aşamada, Apixaban molekülünün konformasyonları PM3 kullanılarak konformasyonel analiz ile incelenmiştir. Tüm olası konformasyonlar arasında en düşük enerjiye sahip olan Apiksaban'ın en kararlı konformeri, molekülün hedef proteine moleküler kenetlenme analizinde başlangıç verisi olarak kullanılmıştır. p38 Mitojenle aktive olan protein kinaz proteini (p38 MAPK) iskemi sonrası miyokardiyal apoptotik sinyal iletim yolağında önemli bir rolü olduğundan, Apiksaban'ın p38 MAPK hedef proteinine kenetlenerek etkileşim mekanizmaları araştırılmıştır. Hedef proteinin Apiksaban ile etkileşen amino asit kalıntıları, etkileşim modları ve bağlanma afinitesi hesaplanmıştır.

Anahtar kelimeler: *Apiksaban, antikoagülan, Konformasyon, Moleküler Kenetlenme*

INTRODUCTION

Apixaban is a substance that activates the conversion of prothrombin to thrombin during clotting in the vessel and prevents blood clotting (Deeks, 2012). In addition to being an anticoagulant,

Apixaban, which inhibits Factor Xa, is used clinically against stroke risk (Byon et al., 2019). A blood clot in heart arteries leads to heart attacks, and fibrinogen -a glycoprotein synthesized in the liver- is linked to thrombotic and cardiovascular diseases when levels increase (Lefevre et al., 2004). Apixaban, a Factor Xa inhibitor, prevents blood clotting by hindering prothrombin-thrombin conversion (Lassen et al., 2010; Wang et al., 2016). Clinically, it reduces stroke and embolism risks, particularly in patients with cardiac arrhythmias and during cardioversion (Flaker et al., 2014). It also provides thromboprophylaxis in cancer patients and post-surgery for hip/knee replacement (Carrier et al., 2019).

Apixaban, a 1-(4-methoxyphenyl)-7-oxo-6-(4-(2-oxopiperidin-1-yl)phenyl)-4, 5, 6, 7-tetrahydro -1H-pyrazole(Byon et al., 2019; Watson et al., 2011) pyridine-3-carboxamide molecule, has a molecular weight of 459.5 g/mol, a water solubility of 40–50 μg/mL and a cocoa-2 permeability of 0.9×10^{-6} cm/s (Byon et al., 2019). Apixaban has 50% bioavailability and is unaffected by food intake (Watson et al., 2011).

In cardiovascular diseases, the inhibition of p38 MAPK pathway plays therapeutic roles (Fisk et el.,2014). P38 MAPKs is a family that cansists of four isoforms: p38a, p38b, p38g and p38d. The p38a is the first identified isoform of the p38 MAKs family and is often denoted as p38 MAPK (Fisk et al., 2014). P38 MAPK is involved in inflammation regulation and insulin resistance, thus, represents a potential target for treating cardiovascular conditions (Liu, Z., & Cao 2009). Apixaban suppresses MAPK phosphorylation, reduces NO-mediated inflammation, and enhances p16 tumor suppressor expression (Jin et al., 2022). It is also beneficial in atrial fibrillation treatment, addressing tissue architecture changes and long-term management (Jin et al., 2022).

In this study, the lowest-energy stable conformer of apixaban was determined and utilized as the initial dataset for molecular docking analysis targeting the P38 mitogen-activated protein kinase (MAPK) (PDB ID 3E92) (Baldwin et al., 2008). The interactions between apixaban and p38 MAPK were investigated. The results of this study may provide valuable information on the mechanism of action of apaxiban as p38 MAPK inhibitor.

1. MATERIALS AND METHODS

Conformational analysis of the Apixaban molecule was conducted using Spartan06 software (Shao et al., 2006), employing the semi-empirical PM3 method (Stewart, J. J. 1989a; Stewart, J. J. 1989b; Stewart, J. J. 1991; Stewart, J. J. 2004) to achieve accurate determination of its potential conformations. The most energetically stable conformer, identified from this analysis, was selected for subsequent docking studies. Docking studies were conducted using the AutoDock-Vina software (Trott & Olson, 2010). The CAVER software (Jurcik et al., 2018) was utilized to identify potential binding sites during the docking process. As target protein, the crystal Structure of P38 Kinase in Complex with A Biaryl Amide Inhibitor (PDB ID: 3E92) (Baldwin et al., 2008) was retrieved from protein data bank and prepared for docking, by extracting ligand and water molecules, and adding polar hydrogens. The Kollman charges of target were determined. Partial

charges for the Apixaban molecule were calculated using the Gasteiger approach. The active site of the p38 (p38 MAPK) protein was defined using a grid size of 40Å×40Å×40Å.

2. RESULTS AND DISCUSIONS

2.1. Structure

In the first stage of this study, the most stable conformers of the Apixaban molecule were identified through conformational analysis using the PM3 method. The geometry of the most stable conformer is presented in **Figure 1.**

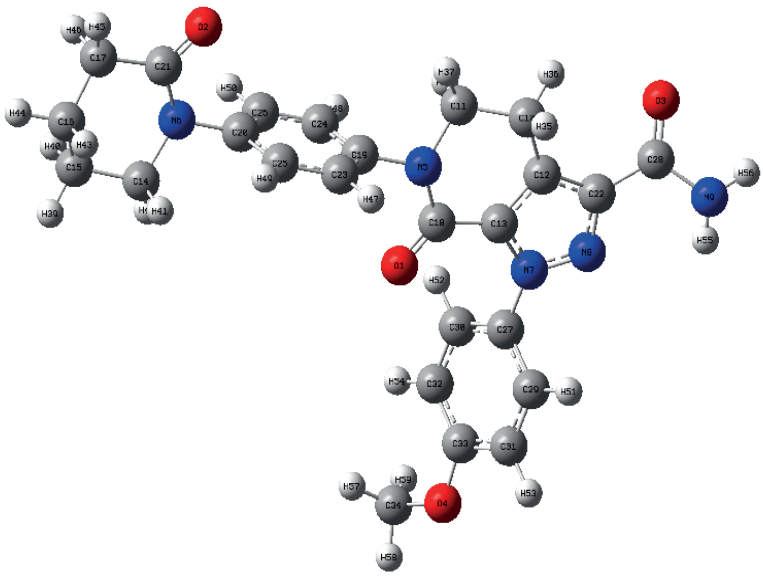

Figure 1. The most stable conformer of Apixaban molecule.

2.2. Molecular Docking

Apixaban, recognized for its bioactivity against cardiovascular disorders, was investigated for its interaction with the mitogen-activated protein kinase p38 (p38 MAPK), a key mediator in the post-ischemic myocardial apoptotic signaling pathway. Molecular docking is an important computational method to reveal drug-target protein interactions. In this study to evaluate binding modes, binding affinities of Apixaban-3E92 complex, and to determine specific amino acid residues involved in the interaction between Apixaban and the p38 MAPK target protein, Apixaban was docked into 3E92. **Figures 2** and **3** illustrate the docking results of Apixaban-3E92 complex. The binding affinity (ΔG) of Apixaban to p38 (p38 MAPK) was found to be -7.4 kcal/mol.

The detailed interactions between Apixaban and p38 (p38 MAPK) are as follows:

Apixaban forms three conventional hydrogen bonds with ARG67 (2.74 Å), GLU71 (2.71 Å) and LYS152(2.31 Å) and three carbon hydrogen bonds with ALA34 (3.26 Å), SER37 (3.51 Å) and HIS174 (3.38 Å) amino acid residues of p38 MAPK. Apixaban also realized an alkyl interaction with ALA34 (4.04 Å), a pi-cation interaction with LYS53 (4.28 Å), pi-anion interaction with GLU71 (4.37 Å) and alkyl interactions with ALA34 (4.04 Å) LEU171(4.74 Å).

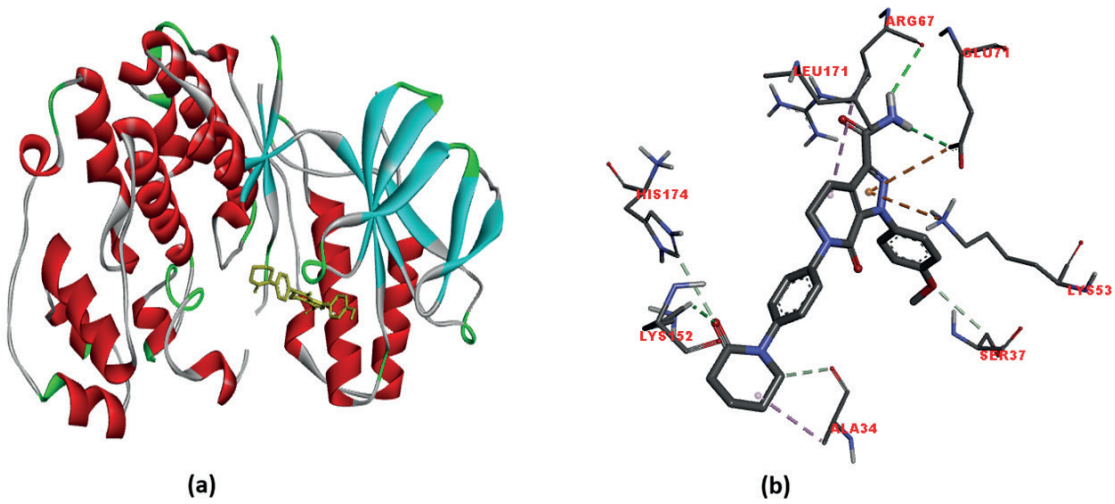

Figure 2. (a) Docking of Apixaban with p38 MAPK (b) Dashed lines indicate interactions.

Ambure et al. conducted molecular docking studies on a novel series of biphenyl amides to design p38 MAPK inhibitors (Ambure et al., 2012), and identified active site interactions between biphenyl amide derivatives and p38 MAPK. It was reported that the highly active compound **70** made two hydrogen conventional bonds with GLU71 and ASP168 (Ambure et al., 2012). Similarly, in our study Apixaban was also found to interacted with GLU71 via hydrogen bonding, indicating that Apixaban docked in the same active site of p38 MAPK with that of active compound **70**.

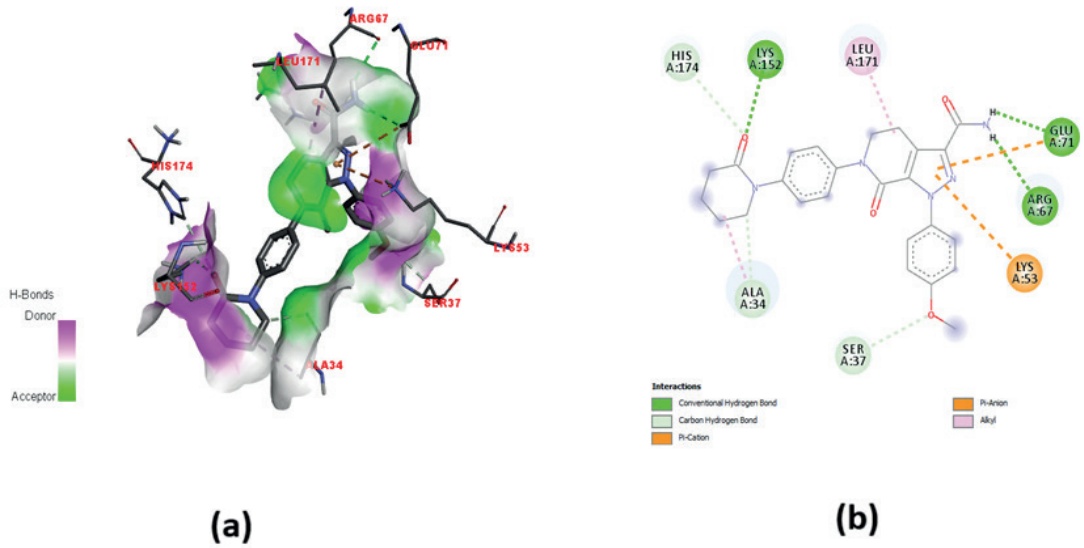

Figure 3. (a) 3D Hydrogen bonding surfaces around Apixaban, (b) Illustration of the isolated compound docked to the p38 MAPK active site.

CONCLUSIONS

In this study, the interaction of Apixaban with p38 MAK has been investigated by molecular docking technique and the binding mode and binding affinity was determined. It was found that H-bonding interactions play important role in stabilizing the ligand in the active site of receptor. The results revealed strong interactions between Apixaban and p38 MAPK target protein. The binding affinity was found as -7.4 kcal/mol. These data are believed to make a vital contribution to future drug design studies.

REFERENCES

Ambure, P. S., Gangwal, R. P., & Sangamwar, A. T. *(2012). 3D-QSAR and molecular docking analysis of biphenyl amide derivatives as p38 α mitogen-activated protein kinase inhibitors. Molecular diversity, 16(2), 377-388.*

Baldwin, I., Bamborough, P., Haslam, C. G., Hunjan, S. S., Longstaff, T., Mooney, C. J., ... & Somers, D. O. *(2008). Kinase array design, back to front: biaryl amides. Bioorganic & medicinal chemistry letters, 18(19), 5285-5289.*

Byon, W., Garonzik, S., Boyd, R. A., & Frost, C. E. *(2019). Apixaban: a clinical pharmacokinetic and*

pharmacodynamic review. Clinical pharmacokinetics, 58(10), 1265-1279.

Carrier, M., Abou-Nassar, K., Mallick, R., Tagalakis, V., Shivakumar, S., Schattner, A., ... & Wells, P. S. *(2019). Apixaban to prevent venous thromboembolism in patients with cancer. New England Journal of Medicine, 380(8), 711-719.*

Deeks, E. D. *(2012). Apixaban. Drugs, 72(9), 1271-1291.*

Fisk, M., Gajendragadkar, P. R., Mäki-Petäjä, K. M., Wilkinson, I. B., & Cheriyan, J. *(2014). Therapeutic potential of p38 MAP kinase inhibition in the management of cardiovascular disease. American Journal of Cardiovascular Drugs, 14, 155-165.*

Flaker, G., Lopes, R. D., Al-Khatib, S. M., Hermosillo, A. G., Hohnloser, S. H., Tinga, B., ... *& ARISTOTLE Committees and Investigators. (2014). Efficacy and safety of Apixaban in patients after cardioversion for atrial fibrillation: insights from the ARISTOTLE Trial.*

Jin, H. G., Kim, K. W., Li, J., Lee, D. Y., Yoon, D., Jeong, J. T., ... & Kim, Y. C. *(2022). Anti-inflammatory components isolated from Atractylodes macrocephala in LPS-induced RAW264. 7 macrophages and BV2 microglial cells. Applied Biological Chemistry, 65(1), 1-13.*

Jurcik, A., Bednar, D., Byska, J., Marques, S. M., Furmanova, K., Daniel, L., Kokkonen, P., Brezovsky, J., Strnad, O., Stourac, J., Pavelka, A., Manak, M., Damborsky,J., Kozlikova, B. *2018. CAVER Analyst 2.0: analysis and visualization of channels and tunnels in protein structures and molecular dynamics trajectories, Bioinformatics 34(20), 3586-3588.*

Lassen, M. R., Gallus, A., Raskob, G. E., Pineo, G., Chen, D., & Ramirez, L. M. *(2010). Apixaban versus enoxaparin for thromboprophylaxis after hip replacement. New England Journal of Medicine, 363(26), 2487-2498.*

Lefevre, M., Kris-Etherton, P. M., Zhao, G., & Tracy, R. P. *(2004). Dietary fatty acids, hemostasis, and cardiovascular disease risk. Journal of the American Dietetic Association, 104(3), 410-419.*

Liu, Z., & Cao, W. *(2009). p38 mitogen-activated protein kinase: a critical node linking insulin resistance and cardiovascular diseases in type 2 diabetes mellitus. Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders), 9(1), 38-46.*

Shao, Y., Molnar, L. F., Jung, Y., Kussmann, J., Ochsenfeld, C., Brown, S. T., Gilbert, A.T.B., Slipchenko, L.V., Levchenko, S.V., O'Neill, D.P., DiStasio Jr, R.A., Lochan, R.C., Wang, T., Beran, G.J.O, Besley, N.A., Herbert, J.M., Lin, C.Y., Voorhis, T.V., Chien, S.H., Sodt, A., Steele, R.P., Rassolov,V.A., Maslen, P.E., Korambath, P.P., Adamson, R. D., Austin, B., Baker, J., Byrd, E.F.C., Dachsel, H., Doerksen, R.J., Dreuw, A., Dunietz, B.D., Dutoi, A. D., Furlani,T. R., Gwaltney, S. R., Heyden, A., Hirata, S., Hsu, C.P., Kedziora, G., Khalliulin, R. Z., Klunzinger, P., Lee, A. M., Lee, M.S., Liang, W. Z., Lotan, I., Nair, N., Peters, B., Proynov, E. I., Pieniazek, P. A., Rhee, Y. M., Ritchie, J., Rosta, E., Sherrill, C. D., Simmonett, A. C., Subotnik, J. E., Woodcock, H. L., Zhang, W., Bell, A. T., Chakraborty, A. K., Chipman, D. M., Keil, F.J., Warshel, A., Hehre, W.J., Schaefer, H.F., Kong, J., Krylov, A.I., Gill, P.M.W., Head-Gordon, M. 2006. *Advances in methods and algorithms in a modern quantum chemistry Stewart, J. J. 1989a program package, Physical Chemistry Chemical Physics 8(27), 3172-3191.*

Stewart, J. J. *1989a. Optimization of parameters for semiempirical methods I. Method, Journal of computational chemistry 10(2), 209-220.*

Stewart, J. J.*1989b. Optimization of parameters for semiempirical methods II. Applications, Journal of computational chemistry 10(2), 221-264.*

Stewart, J. J.*1991. Optimization of parameters for semiempirical methods. III Extension of PM3 to Be, Mg, Zn, Ga, Ge, As, Se, Cd, In, Sn, Sb, Te, Hg, Tl, Pb, and Bi, Journal of computational chemistry 12(3), 320-341.*

Stewart, J. J. *2004. Optimization of parameters for semiempirical methods IV: extension of MNDO, AM1, and PM3 to more main group elements, Journal of Molecular Modeling 10(2), 155-164.*

Trott, O., & Olson, A. J. *2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading, Journal of computational chemistry 31(2), 455-461.*

Wang, X., Tirucherai, G., Marbury, T. C., Wang, J., Chang, M., Zhang, D., ... & Frost, C. *(2016). Pharmacokinetics, pharmacodynamics, and safety of Apixaban in subjects with end-stage renal disease on hemodialysis. The journal of clinical Pharmacology, 56(5), 628-636.*

Watson, J., Whiteside, G., & Perry, C. *(2011). Apixaban. Drugs, 71(15), 2079-2089.*