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In silico Study of Cardiac Troponin Inhibitors

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Running Title: Synthesis of anti-myocardial infarction inhibitors

Abstract

Troponin, is a complex regulatory protein involved in cardiac muscle contraction. Its concentration is increased in individuals with heart failure and myocardial infarction. The assessment of cardiac-specific troponins is widely used as indicator of muscular damage. The overexpression and increased level of troponin is a serious concern in heart failure. Therefore, in this study, we synthesized the molecules including 4-amino-5-(2-hydroxyphenyl)-1,2,4-triazol-3-thione (UI-1) and 4-(2-hydroxybenzalidine) amine-5-(2-hydroxy) phenyl-1,2,4-triazole-3-thiol (UI-2), at 160^{o}C by fusion method that showed effective inhibition of troponin. The UI-1 and UI-2 were structurally characterized by FTIR and NMR. We analyzed the binding interaction of these compounds with troponin by *in silico* molecular interaction. The binding energies were observed for UI-1: -4.4328 and UI-2 -5.2686 kcal/mol. This study would help to design new anti-myocardial infarction agents to modify the treatment against heart failure.

Keywords: troponin; ligan molecules; triazole derivatives; anti-MI compounds; computational analysis

1 Introduction

Myocardial infarction (MI), referred to as a heart attack, is a condition in which the heart muscle experiences infarction (tissue death) due to a reduction in or cessation of blood flow in one of the coronary arteries. Women tend to present with arm pain, fatigue, or neck pain rather than chest pain. Of people over 75, approximately 5% experienced a MI with little to no history of symptoms. Cardiogenic shock, arrhythmia, heart failure, and cardiac arrest can all result from a MI. Atypical symptoms are present in about 30% of individuals. About 15.9 million myocardial infarctions happened globally in 2015. In the US, almost a million people get a MI per year (Steg et al., 2012).

Coronary artery disease is the cause of the majority of MIs. High blood pressure, diabetes, smoking, obesity, low exercise, high blood cholesterol, poor diet, and excessive alcohol consumption are risk factors. The fundamental cause of a MI is typically the total blockage of a coronary artery brought on by the rupture of an atherosclerotic plaque. Older age, current smoking, high blood pressure, diabetes mellitus, total cholesterol, and high-density lipoprotein levels are the main risk factors for myocardial infarction. Myocardial infarction shares many risk factors with coronary artery disease, which is the main cause of the condition (Steg et al., 2012; O'Connor et al., 2010). These risk factors include alcohol consumption, male sex, low physical activity, previous family history, obesity, and low physical activity levels. Risk factor stratification scores, like the Framingham Risk Score, frequently include risk factors for myocardial disease. Men are more likely than women to develop cardiovascular disease at any given age. A recognized risk factor is having high blood cholesterol, especially when it comes to high triglycerides, low high-density lipoprotein, and high low-density lipoprotein levels (Vos et al., 2016).

The complex regulatory protein known as troponin, or the troponin complex, is essential to the contraction of cardiac and skeletal muscle, but not smooth muscle. In the treatment of myocardial infarction and acute coronary syndrome, measurements of cardiac-specific troponins are widely used as diagnostic and prognostic indicators. Although blood troponin levels have a low sensitivity, they can be used as a diagnostic marker for ongoing myocardial injury or stroke. In patients experiencing chest pain or acute coronary syndrome, troponin are measured in the blood to distinguish between unstable angina and myocardial infarction, or heart attack. A myocardial infarction victim has more damaged heart muscle in certain places and higher blood levels of cardiac troponin. A form of myocardial infarction characterized by severe constriction of the cardiac blood vessels, known as coronary vasospasm, can also cause

this. Heart troponins indicate damage to the heart muscle in its entirety, not just myocardial infarction, the most serious kind of heart disease. Currently, the WHO sets a threshold of 2 µg or higher for elevated troponin levels that indicate myocardial infarction in order to meet diagnostic criteria. Critical values of other cardiac biomarkers, like creatine kinase, are also important (Perk et al., 2012; Strandberg et al., 2021).

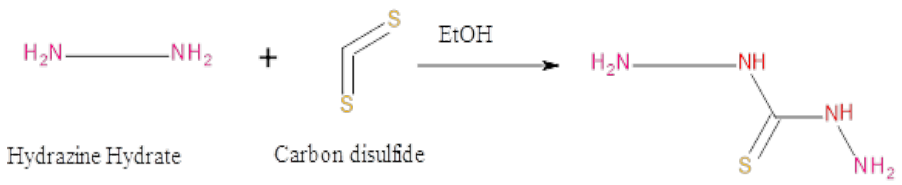
Time is crucial when treating a MI. Following a MI, it is usually advised to make lifestyle changes in addition to receiving long-term aspirin, beta blocker, and statin treatment (Smith et al., 2006). For effective treatment strategies, still there is a need of new molecules and new investigations. The development of drugs against MI is a slow process. Therefore, new drug development strategies are required to identify new therapeutic drugs for treatment of MI. The *in-silico* investigation is more economic and efficient technique to design targeted-structural drug, rather than conventional drug discovery through ligand (Muhammad et al., 2014; Mdluli et al., 2006). Such techniques have become the necessary component for the drug discovery and the drug-development investigations (Bajorath, 2002). MI inhibitors as triazole derivatives are projected through optimized binding affinity and minimum binding energy. Triazoles have been used in development of novel ligand which has attained much importance of pharmaceutical industry due to its biological as well as therapeutic activities (Bekircan et al., 2005). This analysis is designed to examine the anti-MI activity of the synthesized triazole molecules against troponin target.

2 Materials and Methods

2.1 Triazole synthesis

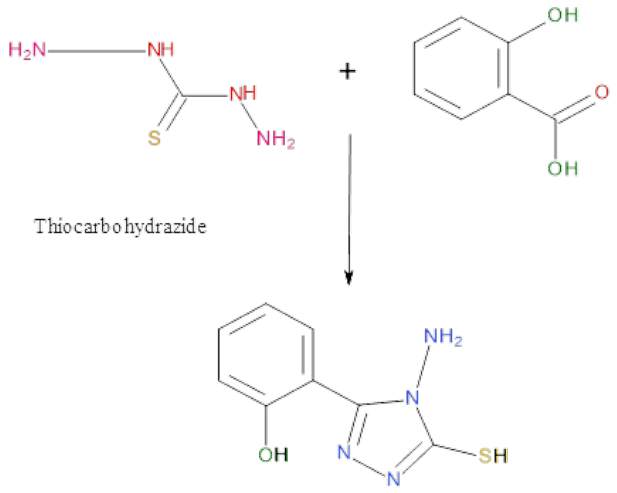
UI-1: 4-amino-5(2-hydroxyphenyl)-1, 2,4-triazol-3-Thione and UA2: 4-(2-hydroxybenzelidine) amine-5-(2-hydroxy) phenyl-1,2,4-trizole-3-thiol, was synthesized (Muhammad et al., 2014).

Stage 1. Hydrazine hydrate (30 ml) and carbon disulfide (15 ml) were heated at reflux condenser for approximately 4 hours in the presence of 150 ml of ethanol to create thiocarbohydrazide.



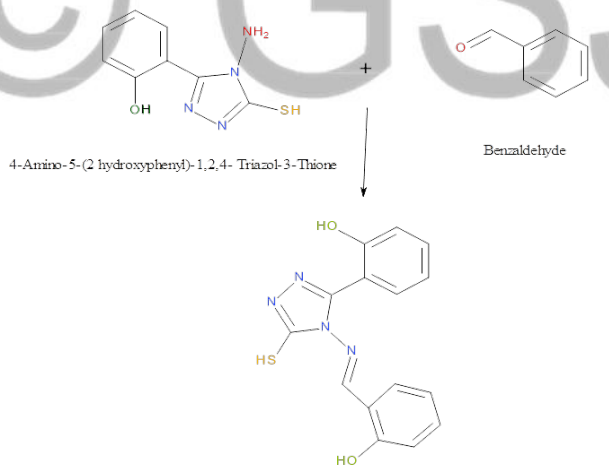
Thiocarbohydrazide

Stage 2. Salicylic acid and thiocarbohydrazide were combined in equimolar amounts (0.1 mol) to create UI-1. The mixture is continuously agitated in an oil bath at 160°C for two hours, and then Thin-Layer Chromatography is performed. After the reaction is finished, the mixture is cooled, filtered, and then crystallized again from 70% ethanol.



4-Amino-5-(2 hydroxyphenyl)-1,2,4- Triazol-3-Thione

Stage 3. One gram of UI-1 was dissolved in ethanol to create UI-2. An equimolar amount of benzaldehyde was then added, and the reaction was continuously stirred for about four hours before TLC indicated the reaction was finished.



4-(2-hydroxybenzalidine) amine-5-(2-hydroxy) phenyl-1,2,4-Triazol-3-thiol

2.2 Structural description

The structure and physical characteristics of these compounds were characterized using Fourier-transform infrared spectroscopy, or FTIR. The wavelength range in which the interferogram results were recorded was 3600-650 cm-1. NMR samples were prepared by dissolving 15 mg in 0.5 ml ddH2O. A 300 MHz Bruker spectrometer fitted with a 5 mm probe head was utilized for NMR spectral 1H analysis.

2.3 Drug likeness and pharmacokinetics

Using the Swiss ADME tool, drug likeness and pharmacokinetic properties of these compounds were predicted (Daina et al., 2017). These characteristics dictate that the molecule must meet specific requirements in terms of physicochemical properties, drug likeness, and kinetics with little to no deviations. A good drug would typically have molecules that are water soluble, exhibit characteristics similar to those of lead, and meet certain requirements, such as having hydrogen bond donors with a molecular weight of less than 500 Da, hydrogen bond acceptors with a molecular weight of less than 10 (NH and O atoms), and a log P coefficient (C log P) of less than 5 (Muhammad et al., 2014).

2.4 Preparation of Triazole Molecules and Accession of Target Protein

The chemical structures of the both ligand UI-1 and UI-2 were prepared through Accelrys Draw. During this analysis Mol SDF format was used for both ligands. The 3D-structure of troponin was modelled using Pyre tool (Kelley et al., 2015) and the quality of the model was assessed.

2.5 Active binding site and molecular interaction analysis

Using MOE, the targeted protein's active site was examined. It specifies the ligand coordinates for the original target protein's active site. Using the docking software, computational ligand-target docking was used to identify the structural complexes in troponin with triazole derivatives. Grid points were assigned to the target protein and these triazole derivatives based on their interaction energies. At each stage, the interaction energy between the ligand and the protein was examined.

3 Results

3.1 Synthesis of derivatives

The synthetic melting temperatures of UI-1 and UI-2, which are troponin inhibitors, were 1200C and 1700C, respectively. It was found that both of these compounds were soluble in ethanol, with Rf-values of 0.16 and 0.12, respectively (Table 1).

3.2 Spectral analysis

Peak values for the functional groups UI-1 and UI-2 were verified by FITR. OH, exhibited a spectral peak at 3137 cm-1 for UI-1 and 3061–3100 cm-1 for UI-2. For UI-1 and UI-2, the distinctive stretching vibrations were detected at 1591 and 1599 cm-1, respectively. The formation of these compounds is attributed to cyclo-condensation, as indicated by the lack of

N, H, and O absorption band spectra. As indicated by the aliphatic and aromatic group molecules' 1H NMR spectral signals. The H NMR (DMSO, 300 MHz, 5 ppm) values for UI-1 are as follows: 7.25–7.37 (m, 4H, Ar-H), 5.37 (s, 2H, NH2), 10.3 (bS, 1H, 0H), and 13.9 (S, 1H, SH). as well as [7.28–7.43 (m, 8H, Ar-H), 9.1 (S, 1H, CH), 10.5 (bS, 2H, OH), 13.8 (9S, 1H, SH)] for UI-2 information.

3.3 Predicting pharmacokinetics and likeness properties

The majority of the features are within the highlighted region, according to the graph, which displayed the summary of the UI-1 and UI-2 ligands. The intrinsic properties of these molecules, which exhibited lead-like characteristics, were revealed by their physicochemical properties. The pharmacokinetics parameters demonstrated that our ligands do not interfere with or inhibit any metabolic enzymes, particularly cytochrome p-450. All of our ligands—including Lipinski, Ghose, and Veber—passed the drug likeness regulations with no violations. The bioavailability score of these ligands was significant (0.55). The partition coefficient log P for UI-2 is 1.407, the molecular weight is 208.46, and the number of violations is 0.0. For UI-1, the molecular weight is 312.354, the log p-value is 3.397, and the number of violations is 0.0 (Tabe 2).

3.4 Protein model analysis

Based on multiple templates, 50% of our protein models could be modeled with 100% confidence, and 60% could be modeled with >90% confidence. According to the Ramachandran plot analysis, >90% of the predicted model's amino acid residues are found in the preferred region, while just 3% or fewer are found in the prohibited region. Although the target protein's backbone confirmation is displayed graphically, this analysis also reveals the lower energy conformations with regard to psi and phi (Figure 1).

3.5 Analysis of active site

Troponin's surface analysis of the active site identifies the likely locations. PHE982, PHE1075, VAL981, ASP985, ALA986, ASN988, LYS989, PHE995, LEU997, ASP1038, LYS1039, ILE1040VAL1076, ASN1077, THR1078, SER1081, ARG1082, SER1083, LEU1084 were observed (Figure 2).

3.6 Hydrophilic, hydrophobic and docking analysis

According to MOE docking results, hydrogen bonds, stacking interactions, or intermolecular interactions stabilize troponin lower energy complexes. As a result, in order to dock with

troponin, the ligand atoms OH, N, and S are tangled. Along with ligand confirmation, the hydrogen bond interactions between the ligand and targets determine the binding strength. As a result, the final dock energy for UI-1 and UI-2 was determined to be -4.2328 and -5.2686 kcal/mol, respectively. Tables 3 and 4 present the energy information and docking results. According to the docking results, the target protein troponin can be interacted with by the ligands UI-1 and UI-2 when they bind to the substrate through the active site (Figure 3).

4 Discussion

We used human troponin to perform a docking analysis of the triazoles derivatives (Muhammad et al., 2014) in this work. Troponin expression has been linked to cardiovascular disorders, particularly myocardial infarction, according to earlier research, which makes this enzyme a viable target for medication. In order to adjust the treatment strategies, molecular studies are necessary because of the dearth of comprehensive information. With the synthesis of anti-MI molecules that focus on novel treatments that will inhibit or decrease the activity of troponin overexpression, this study has identified troponin as an effective target for the development of this strategy. Salicylic acid and thiocarbohydrazide were used to create UI-1 (4-amino-5-(2-hydroxyphenyl)-1,2,4-triazol-3-thione) and UI-2 (4-(2-hydroxybenzalidine) amine-5-(2-hydroxy) phenyl-1,2,4-triazole-3-thiol), which are anti-MI molecules. This reaction was carried out at 160°C.

These compounds are known to have antimicrobial and anti-cancerous properties, which made them an important active ingredient. Consequently, the solubility of these triazole compounds in ethanol is known due to the relevant flow of 0.12 and 0.16. The RO5 (rule of 5) was passed with zero violations by other properties, such as molecular weight, partition coefficient, and other pharmacokinetic properties (Zaid et al., 2010).

Using the obtained spectral data, the peak values were observed from the functional group of the triazole molecules. N-H and C=O did not exhibit any absorption bands, indicating that they were formed by cyclocondensation. The >C =N, or C-N, FTIR peaks displayed characteristic absorption bands whose triazole ring was seen at 1562–1598cm-1 and 1313–1365cm-1, respectively (Prakash et al., 2004). Accordingly, the presence of aliphatic and aromatic groups was indicated by the 1H NMR data spectral signals. In a similar vein, the compounds' S-H presence, C=S absence, and absorption of N-H suggest that the triazole ring is exiting its thiol form (Prakash et al., 2004; Sztanke et al., 2006).

By employing these heterocyclic triazoles, the association of troponin in MI drug development may prove to be an efficacious treatment strategy. The effective binding energies between the ligand and the target protein, which are -4.4328 and -5.2686 kcal/mol

UI-1 and UI-2, respectively, are confirmed by the in-silico ligand-binding affinity for the target protein from troponin. As a result, these triazole derivatives' strong binding affinity demonstrated their anti-MI activity.

5 Conclusion

The remarkable method of in-silico screening and docking analysis of triazole molecules as drug ligands aids in the understanding of protein-ligand affinity. One promising treatment option for MI is the inhibition of troponin protein by triazole derivatives. The intermolecular interaction, which can be explored for future experimental applications, stabilizes the energy values of the triazole molecule and target protein complexes.

Conflicts of interest

No conflict of interest among authors

Declarations of interest

None

References

P.G. Steg, S.K. James, D. Atar, L.P. Badano, C. Blömstrom-Lundqvist, M.A. Borger, et al. "ESC Guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation," Euro. Heart J. 33 (20): 2569–619, 2012.

R.E. O'Connor, W. Brady, S.C. Brooks, D. Diercks, J. Egan, C. Ghaemmaghami, et al. "Part 10: acute coronary syndromes: 2010 American Heart Association Guidelines for Cardiopulmonary Resuscitation and Emergency Cardiovascular Care", Circulation. 122: S787–817, 2010.

T. Vos, C. Allen, M. Arora, R.M. Barber, Z.A. Bhutta, A. Brown, et al. "Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015", Lancet. 388: 1545–1602, 2016.

L.L. Coventry, J. Finn, A.P. Bremner, "Sex differences in symptom presentation in acute myocardial infarction: a systematic review and meta-analysis", Heart & Lung. 40 (6): 477–91, 2011.

E. Rubin, F. Gorstein, R. Rubin, R. Schwarting, D. Strayer. Rubin's Pathology — Clinicopathological Foundations of Medicine. Maryland: Lippincott Williams & Wilkins. p. 549. ISBN 978-0-7817-4733-2, 2001.

J. Perk, G. De Backer, H. Gohlke, I. Graham I, Z. Reiner, M. Verschuren, et al. "European Guidelines on cardiovascular disease prevention in clinical practice. The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts)", Europ. Heart J. 33 (13): 1635–701, 2012.

S.C. Smith, J. Allen, S.N. Blair, R.O. Bonow, L.M. Brass, G.C. Fonarow, et al. "AHA/ACC guidelines for secondary prevention for patients with coronary and other atherosclerotic vascular disease: 2006 update endorsed by the National Heart, Lung, and Blood Institute", J Amer Coll Cardiol. 47 (10): 2130–9, 2006.

L.S. Strandberg, A. Roos, M.J. Holzmann, "Stable high-sensitivity cardiac troponin T levels and the association with frailty and prognosis in patients with chest pain", Amer J Med Open. 1–6: 100001, 2021.

S. A. Muhammad, A. Ali, T. Ismail, R. Zafar, U. Ilyas, J. Ahmad, Insilico study of anti-carcinogenic lysyl oxidase-like 2 inhibitors, Comput Biol Chem, 51, 71-82, 2014.

K. Mdluli, M. Spigelman, Novel targets for tuberculosis drug discovery, Curr. opin. pharmacol. 6, 459-467, 2006.

J. Bajorath, Integration of virtual and high-throughput screening, Nat. Rev. Drug. Discov. 1, 882–894, 2002.

O. Bekircan, N. Gümrükçüoğlu, Synthesis of some 3, 5-diphenyl-4H-1, 2, 4-triazole derivatives as antitumor agents, Indian J. Chem. 44B, 2107-2113, 2005.

A. Daina, O. Michielin, V. Zoete, SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, Sci. Rep. 7, 1-13, 2017.

L. A. Kelley, S. Mezulis, C. M. Yates, M. N. Wass, M. J. E. Sternberg, The Phyre2 web portal for protein modeling, prediction and analysis, Nat. Protoc. 10, 845-858, 2015.

H. Zaid, J. Raiyn, A. Nasser, B. Saad, A. Rayan, Physicochemical properties of natural based products versus synthetic chemicals, Open Nutraceuticals J. 3, 194-202, 2010.

O. Prakash, V. Bhardwaj, R. Kumar, P. Tyagi, K. R. Aneja, Organoiodine (III) mediated synthesis of 3-aryl/hetryl-5,7-dimethyl-1,2,4-triazolo[4,3-a]pyrimidines as antibacterial agents, Eur. J. Med. Chem. 39, 1073-1077, 2004.

K. Sztanke, K. Pasternak, A. Sidor-Wójtowicz, J. Truchlińska, K. Jóźwiak, Synthesis of imidazoline and imidazo[2,1-c][1,2,4]triazole aryl derivatives containing the methylthio group as possible antibacterial agents, Bioorg. Med. Chem. 14, 3635-3642, 2006.

FIGURE LEGENDS

Figure 1 The conformational and helical structure of troponin protein model. Ramachandran plot troponin confirmed the quality of protein model constructed by Drug Discovery Studio version 3.0 indicated that the amino acid residues occur in the “favored region” of the plot.

Figure 2 Active binding sites of troponin target.

Figure 3 Molecular docking and binding interaction of UI-1 and UI-2 with target protein.

Table 1 Characteristic Data of triazole derivatives

Molecules	**UI-1**	**UI-2**
Molecular formula	$C_8H_8N_4OS$	$C_{15}H_{12}N_4O_2S$
Color	Yellow	Yellow
Solubility in water	Not soluble	Not soluble
Solubility in ethanol	Not soluble	Not soluble
melting point	120◦C	170◦C
Form	Crystalline	Crystalline
R_f value	0.16	0.2

Table 2. Physicochemical and drug likeness properties of UI-1 and UI-2 derivatives

Properties	**UI-1**	**UI-2**
Formula	C8H8N4OS	C15H12N4O2S
Molecular weight	208.24 g/mol	312.35 g/mol
Num. heavy atoms	14	22
Num. arom. heavy atoms	11	17
Fraction Csp3	0	0
Num. rotatable bonds	1	3
Num. H-bond acceptors	3	5
Num. H-bond donors	2	2
Molar Refractivity	54.88	86.19
TPSA	115.76 Å²	122.33 Å²

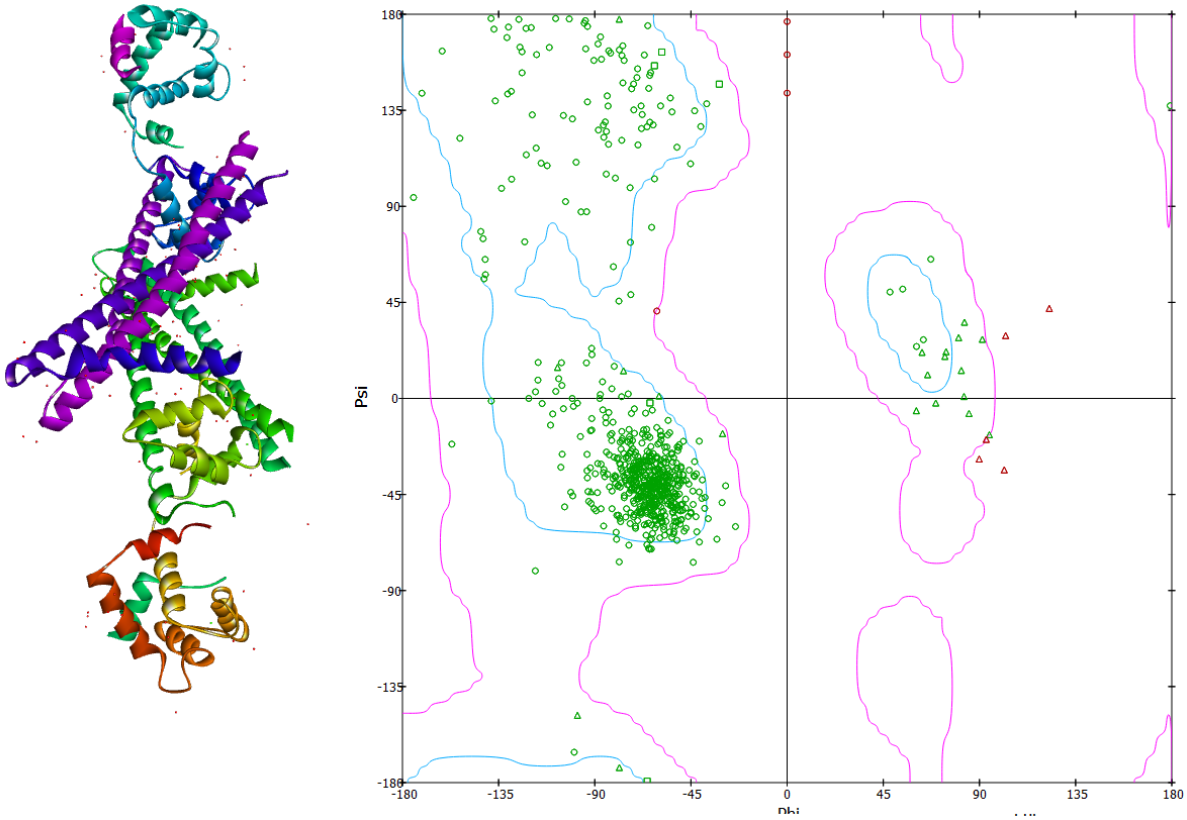


Figure 1. The conformational and helical structure of troponin protein model. Ramachandran plot troponin confirmed the quality of protein model constructed by Drug Discovery Studio version 3.0 indicated that the amino acid residues occur in the “favored region” of the plot.

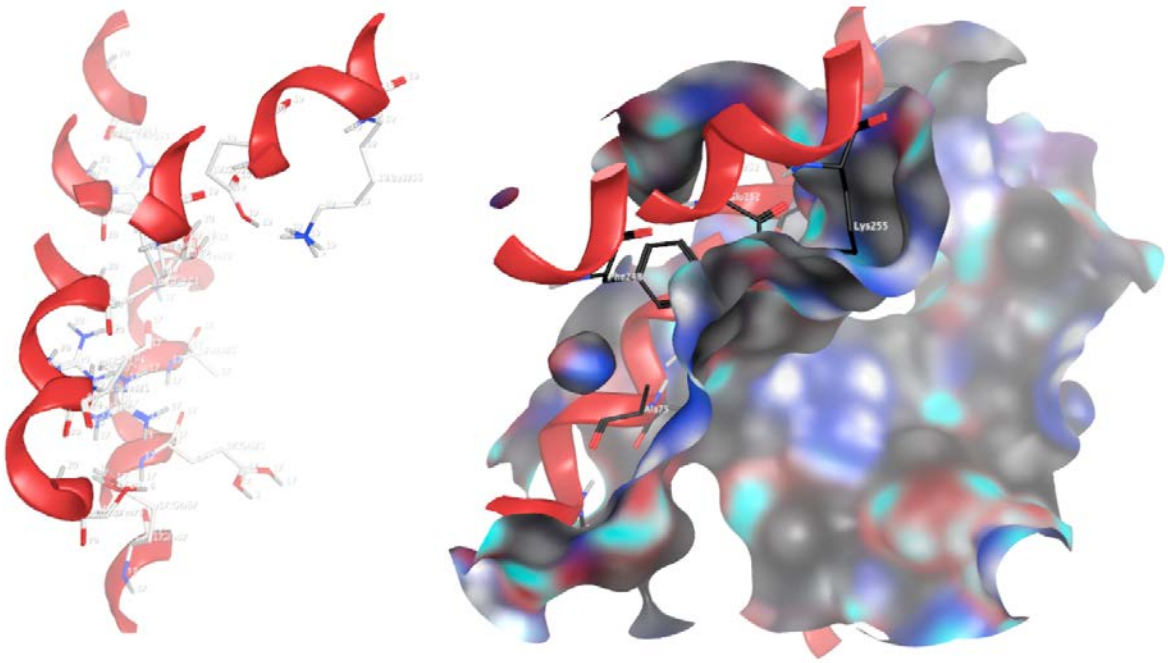


Figure 2. Active binding sites of troponin target.

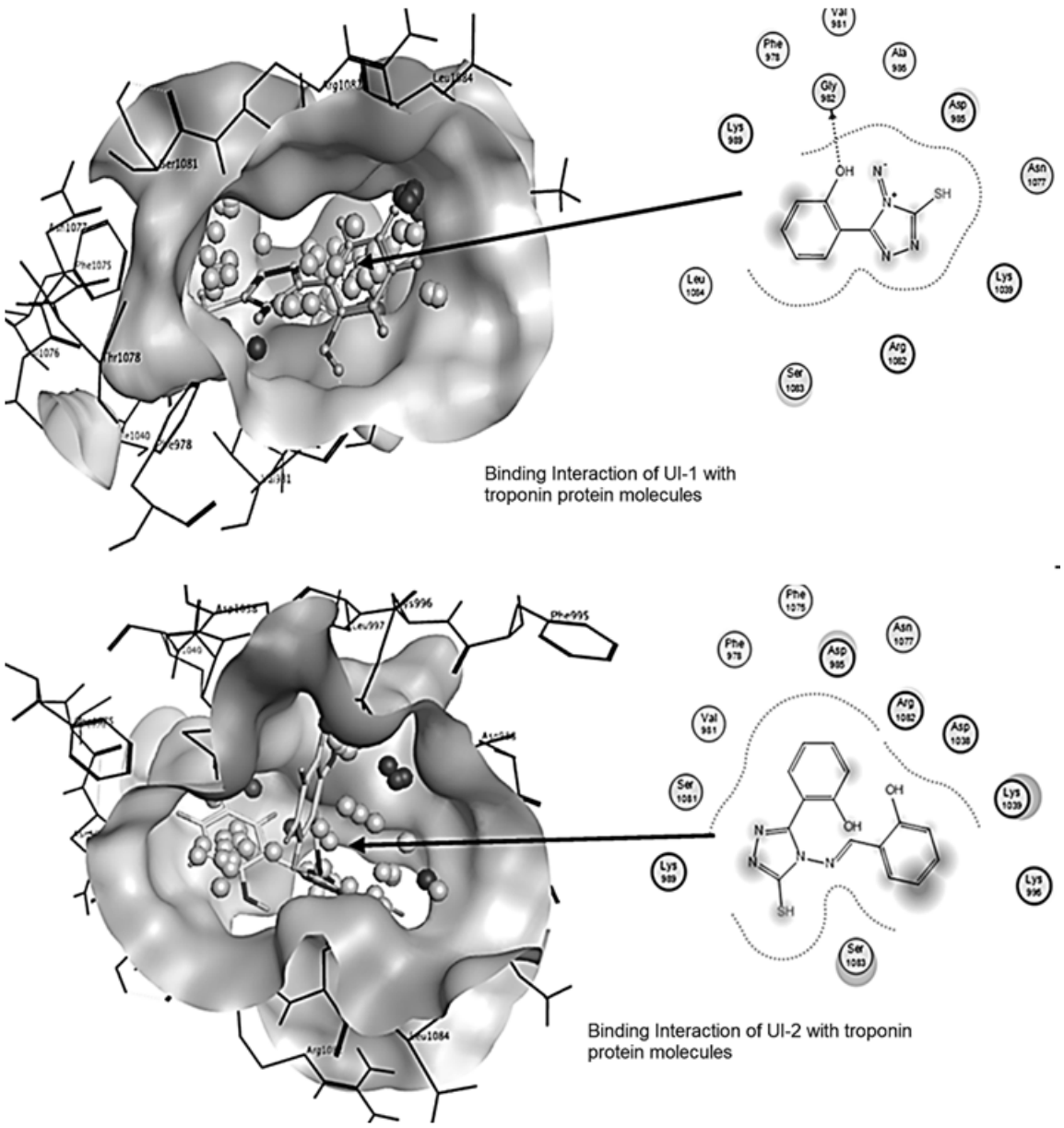


Figure 3. Molecular docking and binding interaction of UI-1 and UI-2 with target protein

Table 3 Energy Values Obtained During Docking Analysis of UI-1 and troponin protein

S. NO.	MOL	RSEQ	MSEQ	S	RMSD_REFINE	E_CONF	E_PLACE	E_SCORE1	E_REFINE	E_SCORE2
1	UI-1	1	1	-4.4328	1.6883	-19.5045	-57.6372	-8.65	-13.7489	-4.4328
2	UI-1	1	1	-4.1425	3.179	-20.3494	-55.7177	-8.7457	-13.6448	-4.1425
3	UI-1	1	1	-4.0777	4.3644	-16.9346	-68.9705	-8.6376	-14.452	-4.0777
4	UI-1	1	1	-4.0583	2.2313	-162,917	-66.9044	-9.0125	-12.7144	-4.0583
5	UI-1	1	1	-3.938	2.1873	-19.9674	-55.4801	-9.0106	-10.4294	-3.938
6	UI-1	1	1	-3.575	2.3434	-18.4323	-58.6611	-8.887	-6.9816	-3.575
7	UI-1	1	1	-3.2458	3.3934	-17.4727	-58.1451	-8.5958	-4.4361	-3.2458

Table 4 Energy Values Obtained During Docking Analysis of UI-2 And Target Protein troponin

S. No.	MOL	RSEQ	MSEQ	S	RMSD_REFINE	E_CONF	E_PLACE	E_SCORE1	E_REFINE	E_SCORE2
1	UI-2	1	1	-5.2686	1.5623	75.5717	-72.9299	-12.5211	-18.4309	-5.2686
2	UI-2	1	1	-5.2331	2.9356	75.0873	-66.3743	-11.1746	-14.9212	-5.2331
3	UI-2	1	1	-5.1066	1.4808	75.7112	-72.0299	-11.6525	-17.944	-5.1066
4	UI-2	1	1	-5.0353	1.7335	77.7083	-66.87	-11.6234	-16.2194	-5.0353
5	UI-2	1	1	-4.8253	2.0812	78.863	-67.3941	-10.9886	-17.6672	-4.8253
6	UI-2	1	1	-4.7986	0.6334	77.0574	-91.1087	-11.0898	-10.3277	-4.7986
7	UI-2	1	1	-4.6374	1.6071	84.0031	-96.1255	-11.9535	-11.0818	-4.6374
8	UI-2	1	1	-4.571	1.9367	79.4771	-68.6839	-10.8255	-15.5981	-4.571
9	UI-2	1	1	-4.3266	2.132	77.5481	-84.8722	-11.3369	-4.0335	-4.3266