Molecular Docking Analysis of N-[2-(3-Methylthio(1,2,4-Thiadiazol-5-Ylthio))Acetyl] Benzamide Molecule with Integrin and DNA

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Abstract - The most stable structure of the N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide molecule ($C_{12}H_{11}N_3O_2S_3$) have been determined by conformational analyses using semi-experimental AM1 calculations. The obtained most stable conformation has been used as the initial data of N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide in molecular docking analysis. Molecular docking studies of the title molecule with DNA and target protein $\alpha_5\beta_1$ integrin revealed the binding affinities as ΔG = -7.4 and -7.7 kcal/mol, respectively. The binding sites of the target protein integrin and DNA and the interactions of the ligand-integrin and ligand-DNA complexes have been determined, which play an important role in anticancer studies. The 3D docked structures of the investigated molecule in target molecules and the interacted groups of ligand receptor complexes were calculated and shown in figures.

Keywords: Benzamide molecule, Target protein integrin, Molecular docking, DNA, Anticancer properties

1. Introduction

Benzamide appears as an off-white crystals or powder [1]. It is a flammable substance, and is incompatible with strong oxidizing agents and strong bases and emits nitrogen oxides, carbon monoxide and carbon dioxide in combustion and thermal decomposition [2]. The benzamide molecule is not planar because it exhibits an angle of 26 degrees with the amide group and the benzene ring [3]. The amide group conformation may result from the repulsive interaction between the hydrogen atoms of the amide group and the atoms of the aromatic ring [4].

Kwolek et al. have produced PBA, a commercialized homopolymer with the simplest chemical structure among p-aramids [5]. PBA has p-aminobenzoic acid, which has condensed and reacted to generate benzamide on its own. PBA can be obtained directly by dissolving sulfinyl aminobenzoyl chlorine in an amide solvent such as tetramethylurea with equivalent water [6]. Benzamide is a carbonic acid amide of benzoic acid. It has the general formula $RCO\text{-}NH_2$ and belongs to the group of organic chemicals [7].

Benzamide and its derivatives have different pharmacological activities such as anticancer, analgesic, cardiovascular, antimicrobial and other biological activities [7]. For this reason, scientists have done many studies to develop different types of benzamide derivatives. For example, amisulpride and sulpiride, both from the benzamides family, have been employed in medicine and in medical materials [8]. Remoxipride, a new benzamide, has been introduced in 1993. Amidoxime derivatives, which are prodrug forms, have been established to solve bioavailability problems with

amide derivatives created before and amidine derivatives, which are thought to be physiologically beneficial [9].

Other study, radiotracer benzamide derivatives that target melanin for melanoma imaging to diagnose malignant melanoma have been designed [10]. Ren and coworkers have synthesized a benzamide derivative to use as 18F-labeled PET imaging probe that targets primary and metastatic melanotic-melanoma [11].

In a study, docking studies of several anticarcinogenic benziamide derivatives affecting human topoisomerase (I, II) enzymes have been carried out [12]. 4N6, 5N5 for Topo I compounds and 5N3, 5N7 for Topo IIa compounds have all been identified as potential anticancer chemicals [12].

The presence of compounds having pharmacological characteristics separates benzamides from other types. Benzamides have turned out to be more selective and more potent for certain HDACs than for all HDAC grades. Although it appears to inhibit HDAC1, HDAC2, and HDAC3 with high potency, benzamides have been shown to induce P21WAF1 expression, induce cell cycle arrest, activate several proapoptotic genes, and to be cytotoxic in a variety of tumor cell types at micromine concentrations. Additionally, specific benzamide analogues have been shown to induce breast cancer cell differentiation [13]. The HDACI family does not have natural products, however, specific synthetic benzamides play an important role in clinical trials on diseases such as breast cancer and lymphoma [13]. Benzamides such as entinostat and mocetinostat have been used in clinical studies on such diseases [14]. In clinical trials, it has so far passed through HDAC inhibitors and it has been approved by the Food and Drug Administration [15]. These inhibitors are panobinostat, vorinostat, romidepsin, and belinostat have been approved to treat different malignancies. Furthermore, a comprehensive investigation using compounds such as valproic acid, butyrates, givinostat, belinostat, entinostat, and mocetinostat has done, with varying results in clinics [16,17].

As a result, benzamide derivatives are evaluated in different fields, using different methods, and are especially important as bioactive molecules. There are great future prospects for compounds in this area. They play an important role in pioneering drug discovery [18,19]. Studies in this area are carried out by scientists to obtain more comprehensive and effective results.

In this study the most stable conformation of N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide was identified by conformational analysis. Molecular docking simulation was used to identify the interaction of the most stable conformer of the title molecule with DNA and $\alpha_5\beta_1$ integrin. The binding affinities and interacted sites have been determined.

2. Materials and Methods

The study was carried out with the help of the Spartan06 software [20] and the AM1 semiempirical quantum mechanical method [21]. The CAVER software [22] was used to determine the potential binding sites on the surface of the receptors. Molecular docking investigations were done using AutoDock-Vina software on the identified active sites [23].

3. Results and Discussions

3.1. Structure

The conformational preferences of the N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide molecule were investigated by performing conformational analysis and computational results revealed the three lowest energy conformations. The molecular models of the obtained the three lowest energy conformers of the N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide molecule are shown in Figure 1 and their calculated relative energies are tabulated in Table 1. The differences between the energies of the three conformers found are low, and therefore, the contribution of all these conformers to the molecular structure is expected at room temperature.

However, the lowest energy-optimized molecular structure (conformer I) was used in our further studies to determine the activity of the molecule.

Table 1. The energies of the three most stable conformers obtained by conformation analysis

Conformers	Relative energy (kj/mol)
(I)	0
(II)	0.24
(III)	2.38

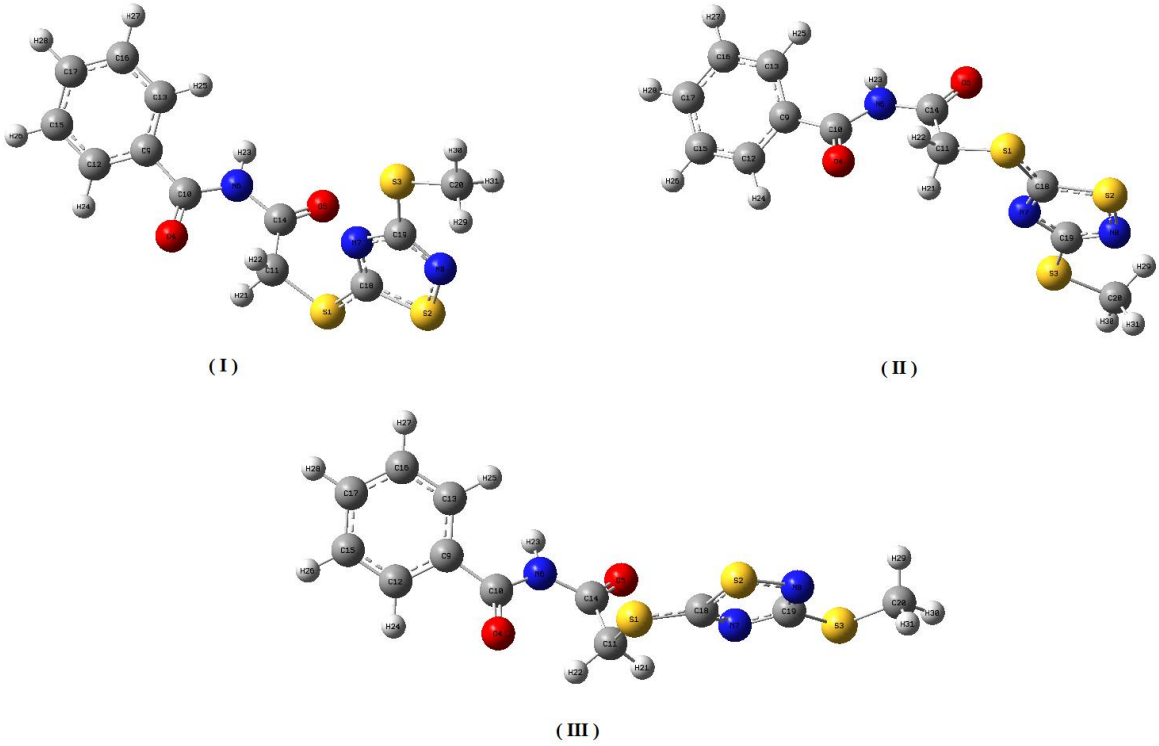

Figure 1. The three conformers with the lowest energy, obtained by conformational analysis of the N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide molecule.

3.2. Molecular Docking

Benzamide derivatives are known to have anticancer properties [24]. For this reason, the anticancer property of the title molecule, as benzamide derivative, was investigated by molecular docking simulations. The interactions of the title molecule with DNA and $\alpha_5\beta_1$ integrin were predicted by molecular docking calculations.

Docking analyses were performed by using AutoDockVina [23]. The crystal structure of DNA (PDB ID: 1BNA) was obtained from the protein database [25] and prepared for docking. The water molecules were removed, polar hydrogens were added and the DNA charges of Kollman were determined. The Geistenger technique was used to compute the partial charges of the N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide molecule and the active region of DNA was determined in grid size 40Å × 40Å × 40Å. The most stable conformer of the N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide molecule was shown to bind to the nucleic acids DC9, DG10, DC11, and DA17 of DNA through hydrogen bonds and pi-sulfur interactions (See

Figure 2). The binding affinity of the ligand to DNA was found as ΔG = -7.4 kcal/mol. It was revealed that the investigated ligand was docked in a similar active region of DNA given in the literature [26,27]. The interactions between the N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio)] benzamide molecule and the nucleic acids of DNA are given as follows:

Pi-sulfur interaction between DC9 and N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide molecule with length of 5.56 Å; Hydrogen bond interaction between DG10 and N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide molecule with length of 2.28 Å; Hydrogen bond interaction between DC11 and N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide molecule with length of 2.99 Å; Pi-sulfur interaction between DA17 and N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide molecule with length of 5.68 Å. In the molecular docking study on cyclo(Ala-His) and DNA, by Celik et al., it was reported that cyclic peptide interacted with DC9, DG10, DC11, DG16 and DA17 nucleic acids of DNA through H-bonds. The lengths of these hydrogen bonds were 3.1 Å with DC9, 2.39 Å with DG10, 2.96 Å with DC11, 2.53 and 2.39 Å with DG16, and 2.99 Å with DA17 [27]. In the molecular docking study between 5-chlorouracil (5-FU) molecule and DNA by Akalin et al., 5-FU was interacted with DNA through hydrogen bonds with DG10, DC15 and DG16 [28].

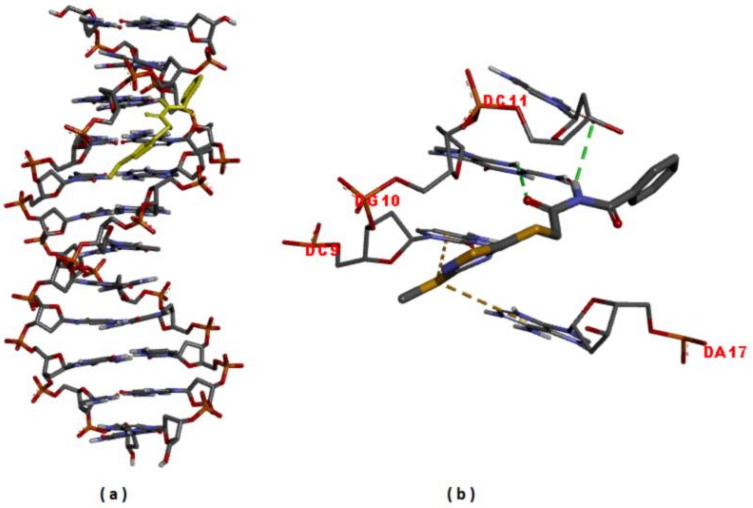

Figure 2. Molecular docked model of N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide with DNA (a) The interactions between the of N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide and DNA are labeled using coloured dashed lines (b) (ΔG =-7.4 kcal/mol).

Molecular docking studies in target protein $\alpha_5\beta_1$ integrin was performed to examine the anti-proliferation effect of the N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide for the anticancer activity.

Following the preparations of the $\alpha_5\beta_1$ integrin (PDB ID: 4WK0) and the ligand for the molecular docking analysis, docking simulation was performed on the active site of the target protein [29]. The binding affinity of the investigated ligand was found to be -7.7 kcal/mol. The 3D diagram of the docked N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide in the active site of $\alpha_5\beta_1$ integrin is shown in Figure 3. The figure also shows the interaction diagrams of the N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide and $\alpha_5\beta_1$ integrin complex.

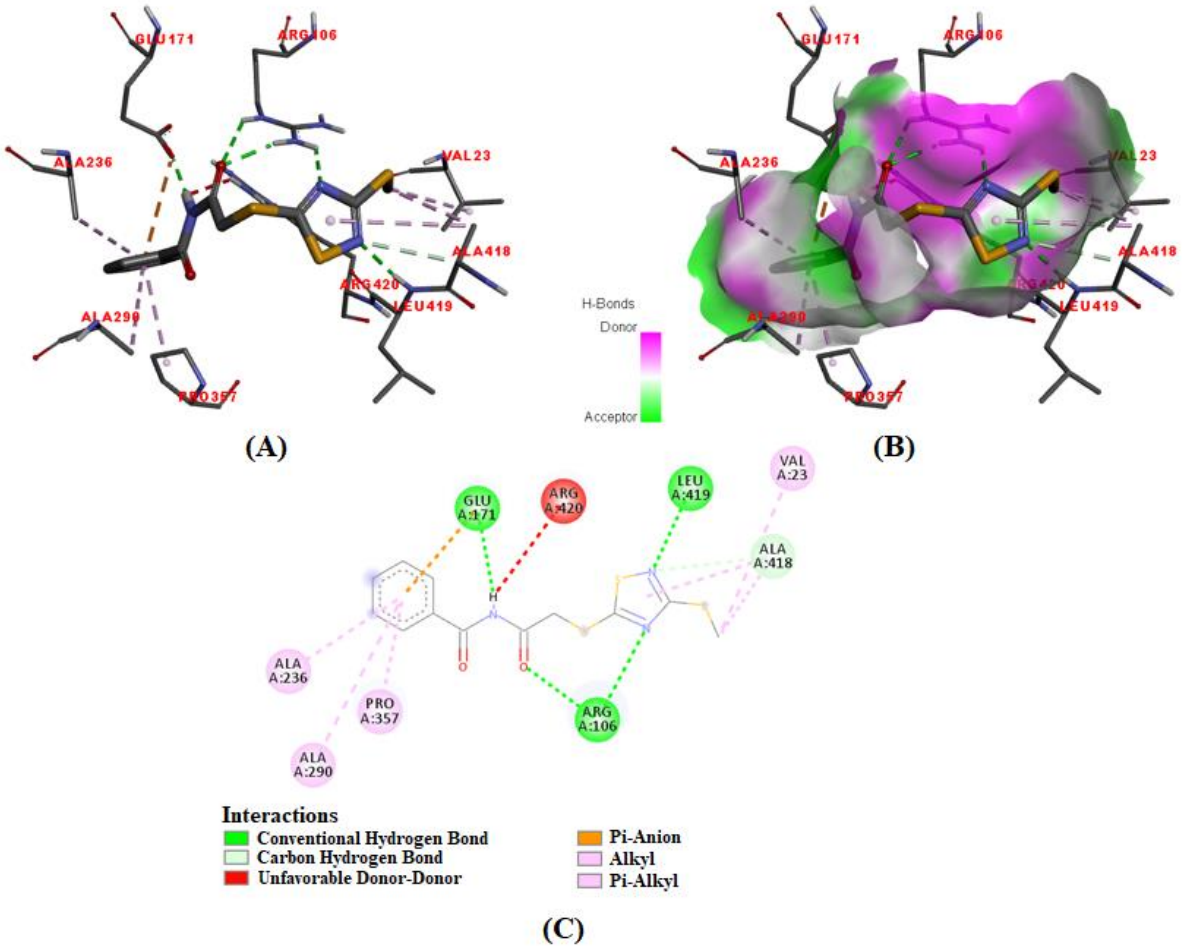

Figure 3. Visualization of docking analysis of N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide binding with $\alpha_5\beta_1$ integrin (-7.7 kcal/mol) (A-C) 3D and 2D representations describing bindings of N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide with active site of $\alpha_5\beta_1$ integrin (B) visualization of hydrogen bond.

The revealed interactions between N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide molecule and target protein integrin are as follows: 2.21, 2.48, and 2.98 Å long hydrogen bonds with Arg106; 4.51 Å long alkyl interaction between and Val23; 2.04 Å long hydrogen bond and 4.23 Å long pi-anion interaction with Glu171; 4.97 Å long pi-alkyl interaction with Ala236; 5.33 Å long pi-alkyl interaction with Ala290; pi-alkyl interaction of Pro357 with a length of 4.08 Å; 3.69 Å length alkyl interaction, 5.23 Å length pi-alkyl interaction, 3.61 Å length carbon-hydrogen bond with Ala418; 2.41 Å long hydrogen bond with Leu419; Unfavorable donor-donor interaction with Arg420 at 2.6 Å.

In the case of molecular docking studies between cationic Glu-Gln-Arg-Pro-Arg pentapeptide and the $\alpha_5\beta_1$ integrin, by Gasymov et al., it was found that the pentapeptide interacted with the Phe21, Ser22, Val23, Arg106, Phe168, Glu171, Ser234, Val235, Lys269, Tyr287, Leu355 , Ser417 and Arg420 amino acid residues of integrin, through salt bridge, attractive charge, hydrogen bond, carbon hydrogen bond, unfavorable positive-positive, unfavorable donor-donor interactions [30].

In our study some of the amino acid residues with which the title compound interacted with integrin have been found to be the same as the amino acids with which cationic Glu-Gln-Arg-Pro-Arg pentapeptide interacted. The result indicated that the title molecule bound to the target protein in its active site to which the cationic pentapeptide bound [30].

As a summary, the binding affinities and interactions of N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide ligand with the target DNA and $\alpha_5\beta_1$ integrin were tabulated in Table 2.

Table 2. The binding affinities and the molecular interactions between N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide and target DNA, and integrin, obtained by molecular docking simulations.

	DNA (PDB ID: 1BNA)	**$\alpha_5\beta_1$ (PDB ID: 4WK0)**
Docking score (kcal/mol)	-7.4	-7.7
H.Bonding interaction (distance; Å)	1- DG10 (2.28) 2- DC11 (2.99)	1- Arg106 (2.21; 2.48; 2.98) 2- Glu171 (2.04) 3- Leu419 (2.41)
Pi-sulfur (distance; Å)	1- DC9 (5.56) 2- DA17 (5.68)	
Alkyl (distance; Å)		1- Val23 (4.51) 2- Ala418 (3.69)
Pi-Anion (distance; Å)		1- Glu171 (4.23)
Pi-Alkyl (distance; Å)		1- Ala236 (4.97) 2- Ala290 (5.33) 3- Pro357 (4.08) 4- Ala418 (5.23)
Carbon-Hydrogen bond (distance; Å)		1- Ala418 (3.61)
Unfavorable donor-donor (distance; Å)		1- Arg420 (2.6)

The Molecular Mechanics Poisson-Boltzmann Surface Area (MM/PBSA) and the molecular mechanics generalized Born surface area (MM/GBSA) approaches are reliable binding free energy calculation methods, used to estimate the free energy of binding small ligands to biological macromolecules. Typically, they are based on molecular dynamics simulations of the receptor-ligand complex [31-36]. Due to the importance of both MM/PBSA and MM/GBSA approaches, the interaction free energies of the investigated ligand with DNA and $\alpha_5\beta_1$integrin were calculated using a program developed by Wang [31], which is based on MM/PB(GB)SA approach.

The predicted binding free energy of the N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide molecule with DNA and with $\alpha_5\beta_1$ integrin were obtained as -13.06 and -20.46 kcal/mol, respectively by using the MM/PB(GB)SA approaches with the GAFF2 and ff14SB force field combination and the GB6 procedure [31].

4. Conclusions

A semi-experimental method AM1 was used to analyze the conformational preferences of the N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide molecule. The docking simulations were performed to reveal the biological activity of the title molecule in its most stable conformer. Since the DNA and integrins are important targets for anticancer drugs, the docking simulations of N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide molecule were performed with DNA and $\alpha_5\beta_1$ integrin to predict the anticancer activity of the title molecule. The N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide had binding affinities of -7.4 and -7.7 kcal/mol to DNA and $\alpha_5\beta_1$ integrin, respectively. The ligand N-[2-(3-Methylthio(1,2,4-thiadiazol-5-ylthio))acetyl] benzamide predicted to have strong anti-tumor effects, according to molecular docking simulations.

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