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Design, Synthesis, and Characterization of Novel Azo Dyes Derivatives as Anticancer Agent Targeting Aurora kinase A

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Abstract

Some new azo dye derivatives have been designed for the development of novel kinase inhibitors. A molecular docking study of designed compounds against Aurora kinase A (PDB: 3K5u) was conducted to identify new drug candidates for cancer therapy. The binding free energy calculated by Molegro virtual docker (MVD) to select the most promising results. The corresponding docking score values in Aurora A kinase of compound 4 gave the best docking energy of -123.2 kcal/mol and for the compound 1 gave the docking energy of -88 kcal/mol. Compound 1 gaven bindings to the following amino acids Ala213, Ala273, Lys162, and Asn261 and compound 4 gaven bindings to the following amino acids Ala213, Ala273, Lys162, and Thr217. These bindings were similar to the primary ligand PFQ-1001, which is Ala213 and Ala273. A complex of azo dye derivatives containing sulfonamide with 8- hydroxyquinoline was synthesized by the conventional two-step method of formation of diazonium moiety and formation of azo dyes. Compound 1 was synthesized from a sulfonamide-containing azo dye derivatives with 8-hydroxyquinoline by a conventional two-step method. The first step is the formation of diazonium ion from a primary aromatic amine compound in acidic nitric acid medium at zero temperature. The second step is the reaction of diazonium ion with 8-hydroxyquinoline compound which has an aromatic ring with a electron donating group (OH) at the para position which facilitates the condensation of the two compounds and the formation of azo groups.The structure of the synthesized compound was well determined by mass spectrometry (MS), infrared spectroscopy (IR), 1H nuclear magnetic resonance (1H NMR), 13C nuclear magnetic resonance (13C NMR), and elemental analysis.

Keywords: cancer, anticancer, sulfonamide, 8- hydroxyl quinolone, azo dyes, Molegro virtual docker.

Full-length article;*Corresponding Author*, e-mail: *mf.chehna@gmail.com; pharmacist.nagham@gmail.com* Doi # https://doi.org/10.62877/120-IJCBS-24-25-19-120

1. Introduction

The genetic disorder of cancer originates from both genetic and epigenetic alterations in cells. It is one of the most complex diseases, characterized by abnormal cell growth that continues to multiply uncontrollably and spreads quickly into nearby tissues. Such cells often referred to as malignant, as they may invade or destroy surrounding tissues. The most common forms of cancer worldwide are prostate, breast, lung, stomach, colorectal cancers, and malignant non-melanoma skin tumors [1-2]. There are various ways to treat cancer, such as surgery, chemotherapy, hormonal therapy, immunotherapy [3-4], and phototherapy [5]. Today, anticancer chemotherapy remains one of the main treatment methods [6]. Examples of chemotherapy drugs include antitumor antibiotics, antimetabolites, mitotic inhibitors, and hormonal therapies. A significant advantage of cancer chemotherapy is that it treats the whole body, including cells that may have spread from the primary tumor [4-7]. The medical community currently faces several challenges in treating cancer due to the development of drug resistance and the unwanted off-target effects of anticancer drugs.

This creates a pressing need for medicinal chemists to synthesize more effective anticancer drugs with reduced toxicity, ensuring that new agents continuously lower toxicity

levels. Sulfonamide derivatives are one such class of compounds with various biological activities, including anticancer properties. Some sulfonamide derivatives, such as Belinostat, ABT-199, and Amsacrine, have marketed for cancer treatment. These derivatives have shown mutant anticancer effects on targets such as aromatase, carbonic anhydrase (CA), B-cell lymphoma, phosphatidylinositol-4-phosphate 5-kinase, and Aurora kinase A [8]. Quinolone is one of the most important drug nuclei in drug discovery in recent decades, especially in cancer control research. Numerous studies have shown that quinolone derivatives effectively reduce cancer cell growth, induce apoptosis, prevent blood vessel formation (thus inhibiting tumor growth), and inhibit protein kinases and other molecular targets [9]. Azo dyes are generally characterized by a nitrogen-nitrogen (–N=N–) double bond.

Which provides various properties useful in textile dyeing, coloring materials, biological and medical studies, and organic synthesis. It is essential for azo dyes to contain heterocyclic compounds with nitrogen, oxygen, or sulfur, which enhances their therapeutic effects as anticancer and antibacterial agents [10]. Protein kinase inhibitors have been among the most successful targeted anticancer drugs. These enzymes are responsible for protein phosphorylation, which is critical for basic processes such as cell cycle regulation, proliferation, differentiation, motility, and apoptosis. Our laboratory research has focused on the synthesis of compounds that inhibit several enzymes, including EGFR TK p [11], carbonic hydras ix [12], and aromatase enzyme [13]. This research will study the inhibition of the Aurora kinase enzyme using newly designed compounds resulting from the condensation of sulfonamides with quinolones to form azo dyes, using the Molegro Virtual Docker program.

2. Materials and Methods

Protein Data Bank (PDB), PubMed and software like ChemSketch version 14.01, Marvin sketch version 21.2, and Molegro Virtual Docker (MVD) version 2011.4.3 implemented within the current study. Melting points were determined in open capillary on a BÜCHI Melting Point B-540 apparatus (BÜCHI Labortechnik, Switzerland). IR spectra (KBr disc) were recorded using an ATR-FTIR Bruker spectrophotometer (Bruker, Billerica, Massachusetts). 1H-NMR spectra were scanned on JEOL-ECA NMR spectrophotometer (Joel, Tokyo, Japan), operating at 500 MHz for 1H and 13C. Chemical shifts are expressed in δ-values (ppm), using DMSO-d6 as a solvent. Mass spectra (MS) were scanned by the triple quadrupole mass spectrometer with positive ionization (Sciex, Framingham, USA). The m/z values of the more intense peaks are mentioned.

2.1. Receptors selected for this study

Receptors selected for this study the enzyme—required for the docking studies—has retrieved from the protein data bank (PDB). The Aurora Kinase A has (3K5u) a resolution factor of 2.35 Å [14]. We defined the active site of based on the X-ray complex structure of Aurora Kinase A and 2-[(5, 6-diphenylfuro [2, 3-d] pyrimidin-4-yl) amino] ethanol (Fig. 1).

2.2. Preparation of ligand

Chemical structures were obtained from PubChem [15]. Marvin Sketch was employed to draw and optimize structures of designed compounds and files saved as mol2. Compounds were prepared using the standard setting for generating structure, assigning bonds, creating hydrogen bonds, assigning bond orders, assigning hybridization, assigning charges (calculated by MVD), assigning atom types, and detecting flexible torsions in the ligands. The following table shows the sulfonamide compounds used in this study.

2.3. Docking

In this research, we use the Molegro software, which was used in previous research and it is available free. Docking Molegro software was used to dock the Aurora Kinase A with compounds. The following parameters used for docking in Aurora Kinase A.

2.4. Active pocket identification

The enzyme pockets were identified by MVD software. The selected pocket with a basic ligand was PFQ-10013 and selected pocket size was 484.352 Å3, while polar area was 1313.28 Å. The link energy according to the function MolDock is calculated from the following formula:

$$Etotal=Einter+Eintra$$

2.5. Synthesis of compound 1

In flask A with one neck 0.49 g of sulfanilamide, 0.13 g of sodium carbonate (Na_2CO_3), and 5 ml of water are placed in a hot water bath to dissolve the contents. In flask B, a solution containing 0.2 g of sodium nitrite ($NaNO_2$) and 1 ml of hydrochloric acid (HCl) is prepared to obtain the diazonium ion (N_2^+). The contents of flask B are immediately poured into flask, A which is placed in an ice water bath. 8-hydroxyquinoline (0.38 g) dissolved with 2 ml of 2.5 M NaOH and added to the previous mixture with continuous stirring. The solution is heated in a thermal water bath until it boils. Then, 1 g of sodium chloride (NaCl) is added and allowed to cool to room temperature. The resulting solid is filtered using a Büchner funnel and the precipitate is washed with a saturated sodium chloride solution [16]. The mechanism of the reaction is illustrated in fig 4 (as follows).

3. Results and discussion

3.1. Azo dyes derivatives

The core scaffold of sulfonamide and 8- hydroxy-quinolone was used to design several derivatives in the current study. The structure of designed azo dyes derivatives is shown in (Table 3). The Lipinski guidelines of five were also employed in the selection of compounds as well as polar surface area guidelines. The following table outlines the properties of the compounds designed under the Lipinski guideline of five and polar surface area.

3.2. Molecular docking results

These compounds were docked into Aurora Kinase A and Compared to the compound 2-[(5, 6-diphenylfuro [2, 3-D] pyrimidine-4-yl) amino] ethanol, which is known Aurora Kinase A inhibitor. It is worth mentioning that most of the compounds can work as Aurora Kinase A inhibitors as well as having a good energy docking.

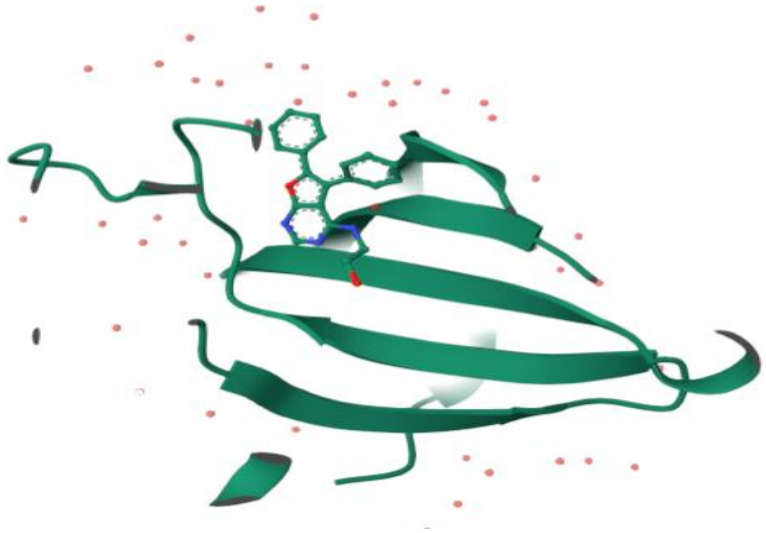


Figure 1. The X-ray complex structure of Aurora Kinase A and 2-[(5, 6-diphenylfuro [2, 3-d] pyrimidin-4-yl) amino] ethanol

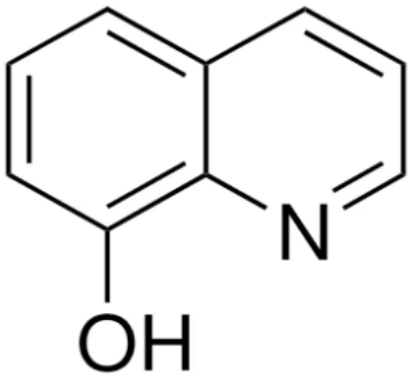


Figure 2. The structure of 8- hydroxy-quinolone

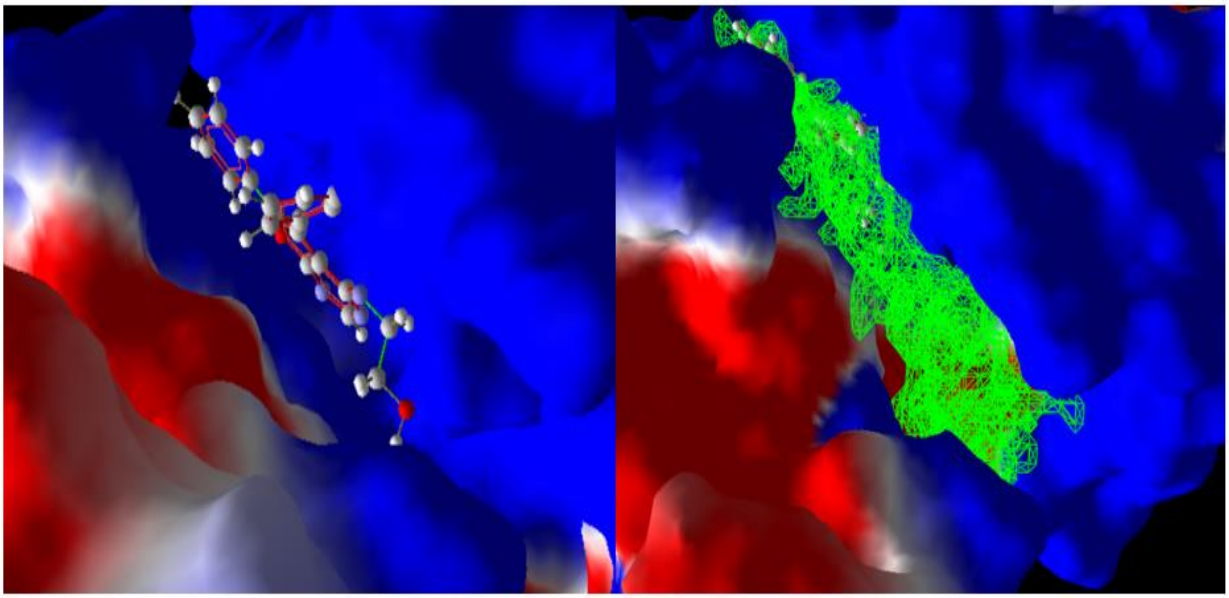


Figure 3. Active pocket inside basic ligand PFQ- 1001

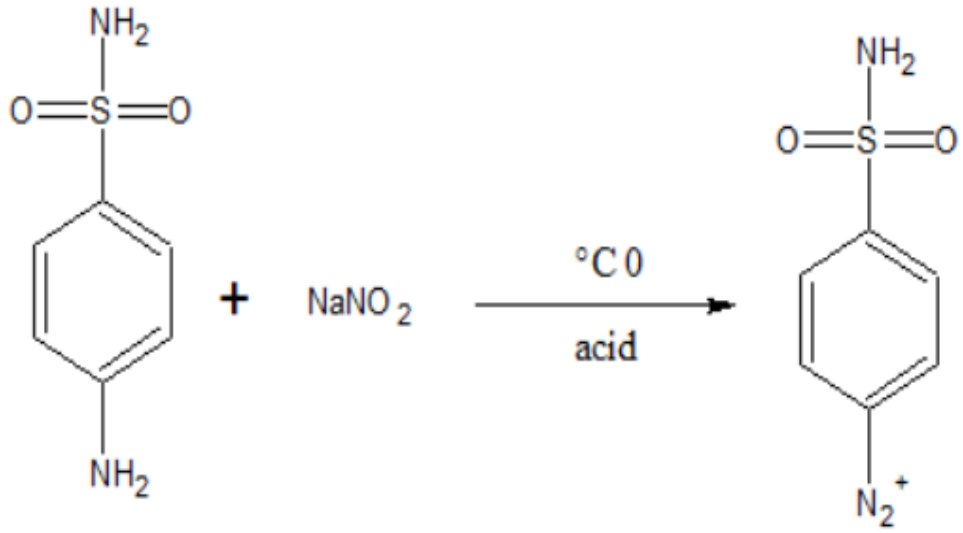


Figure 4. The mechanism of synthesis of 4-[(E)-(8-hydroxyquinolin-5 yl) diazenyl] benzene sulfonamide (Step 1: Formation of diazonium ion)

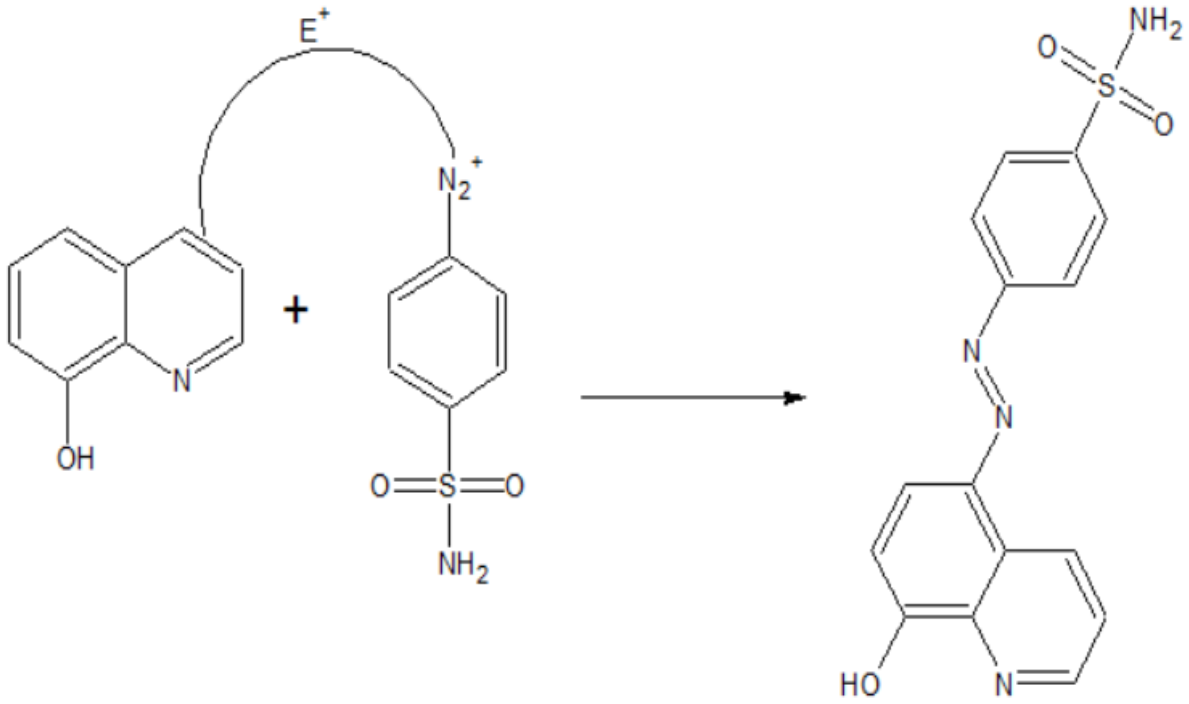


Figure 5. The mechanism of synthesis of 4-[(E)-(8-hydroxyquinolin-5 yl) diazenyl] benzene sulfonamide (Step 2: Formation of azo dyes)

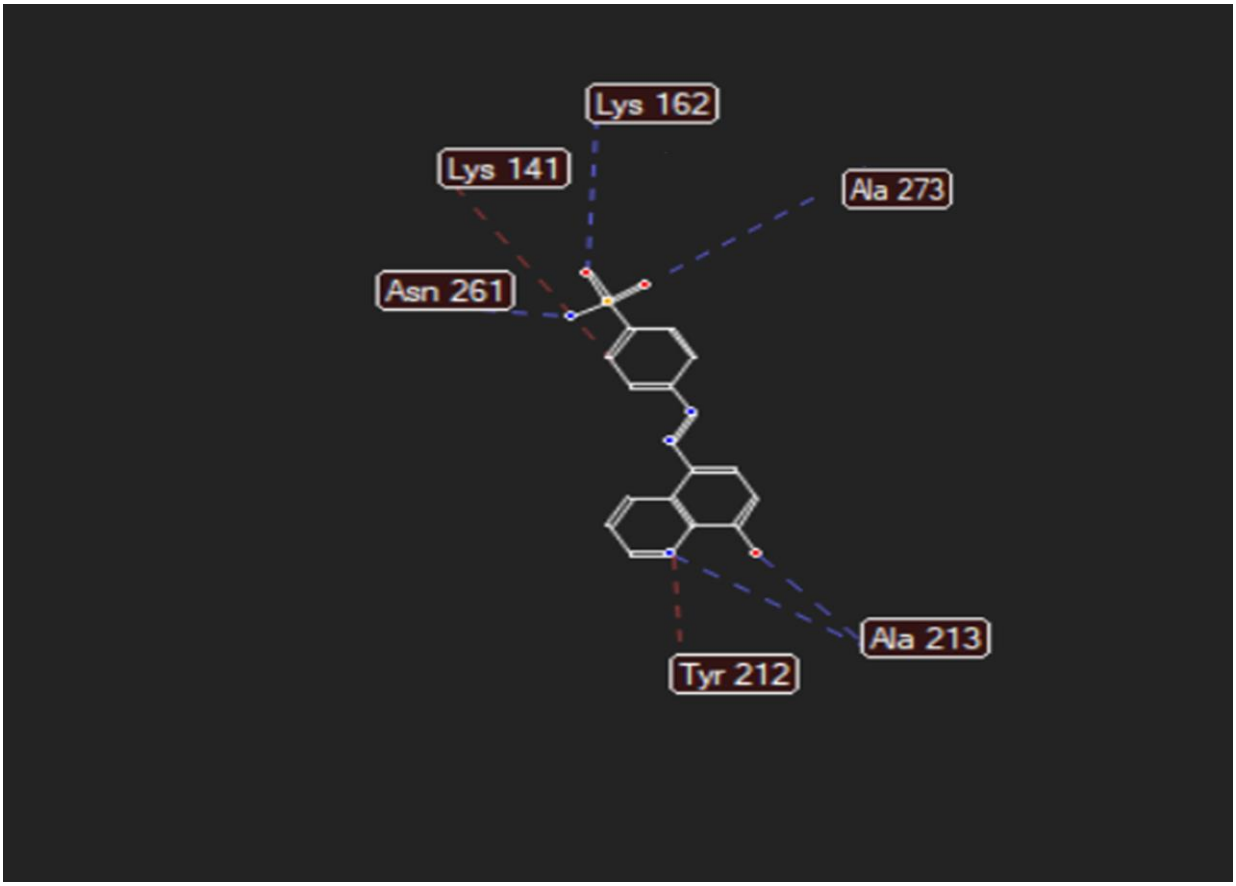


Figure 6. Associations with amino acids of compound 1 within the active pocket

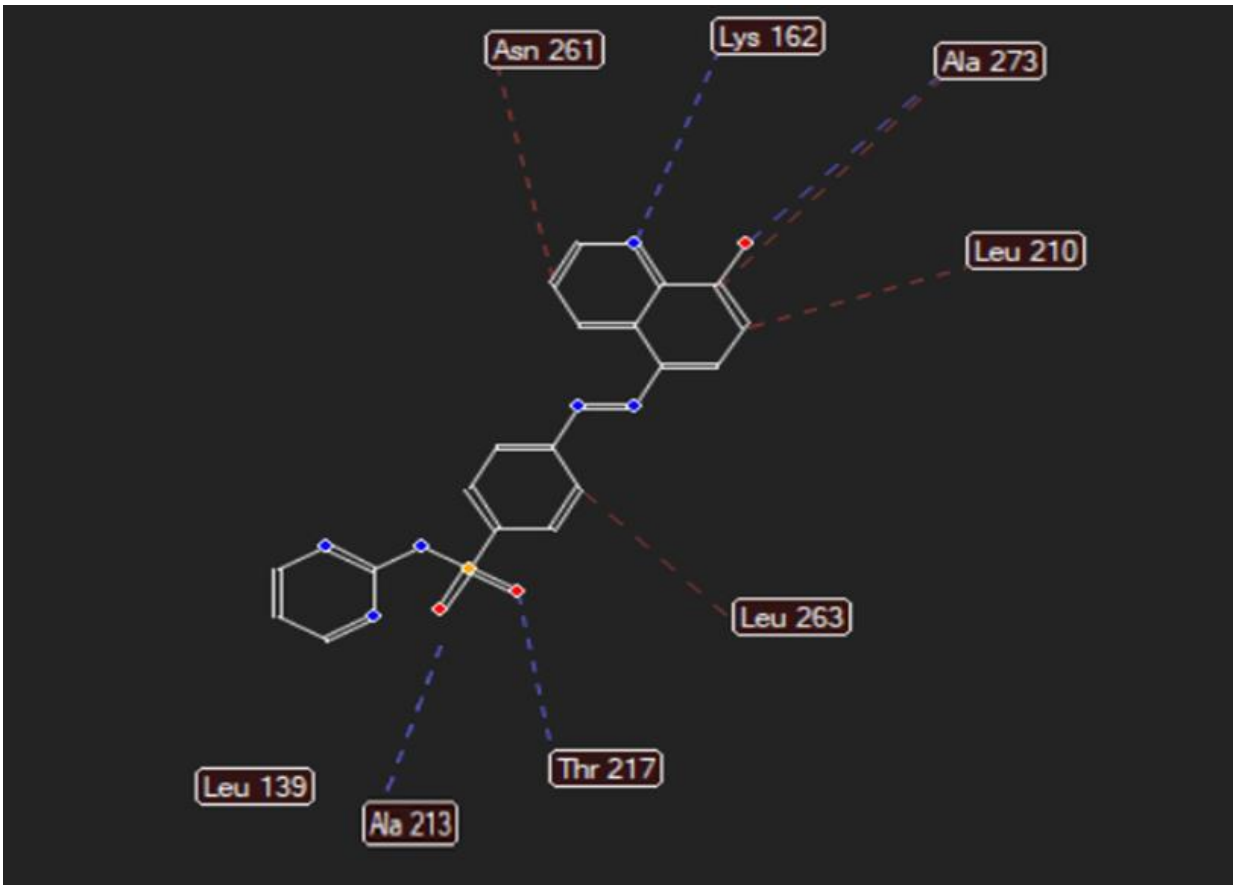


Figure 7. Associations with amino acids of compound 4 within the active pocket

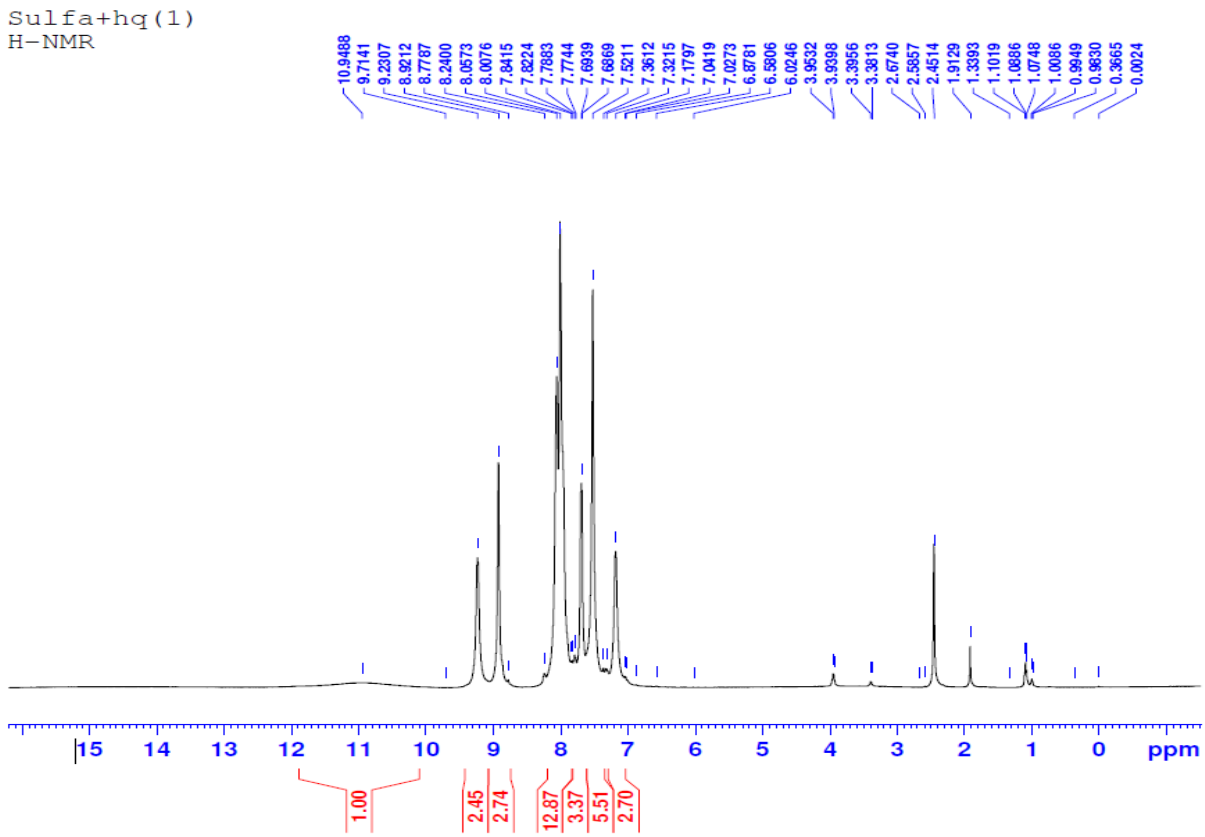


Figure 8. 1H-NMR spectrum for 1 compound.

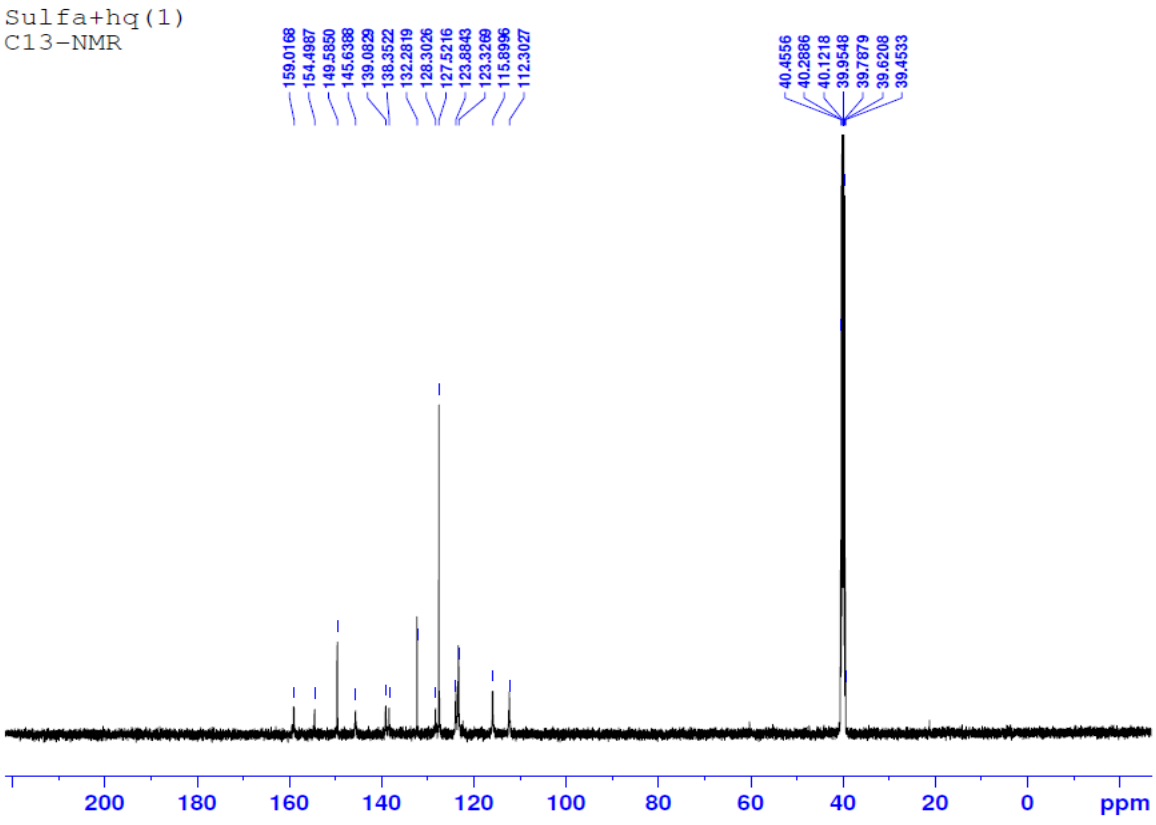


Figure 9. 13C-NMR spectrum for 1 compound.

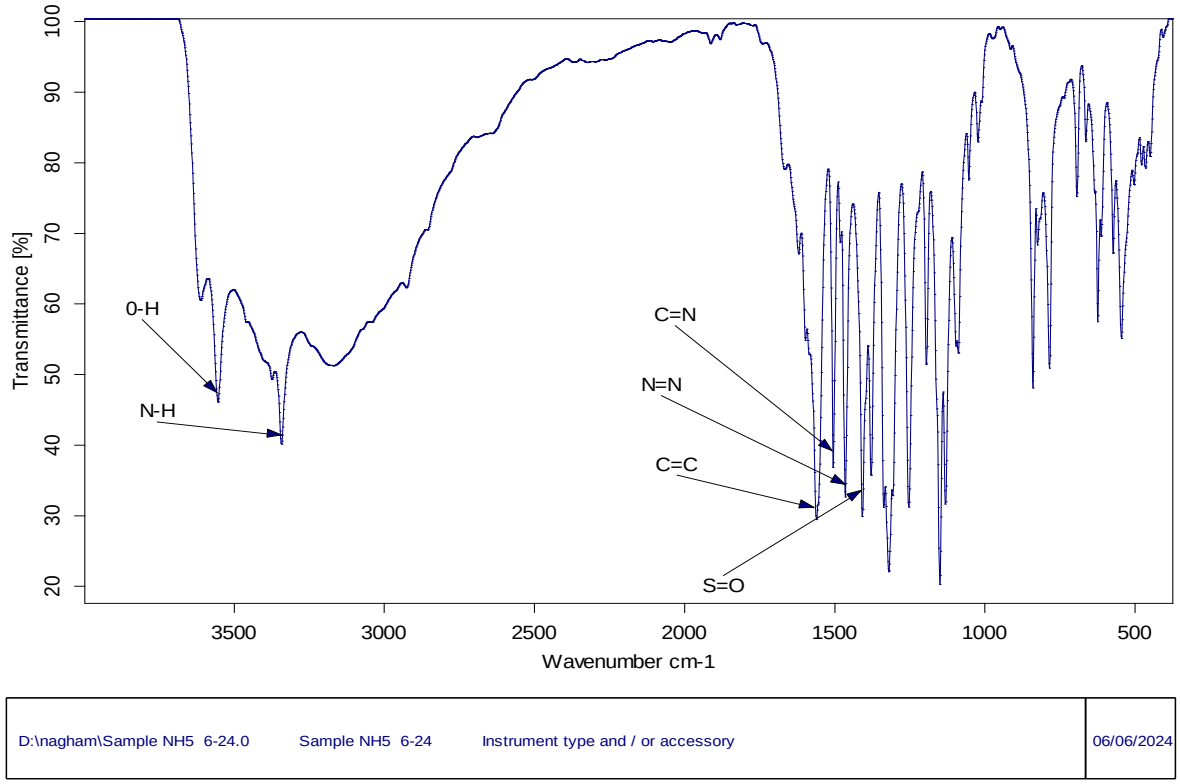


Figure 10. IR spectrum for 1 compound

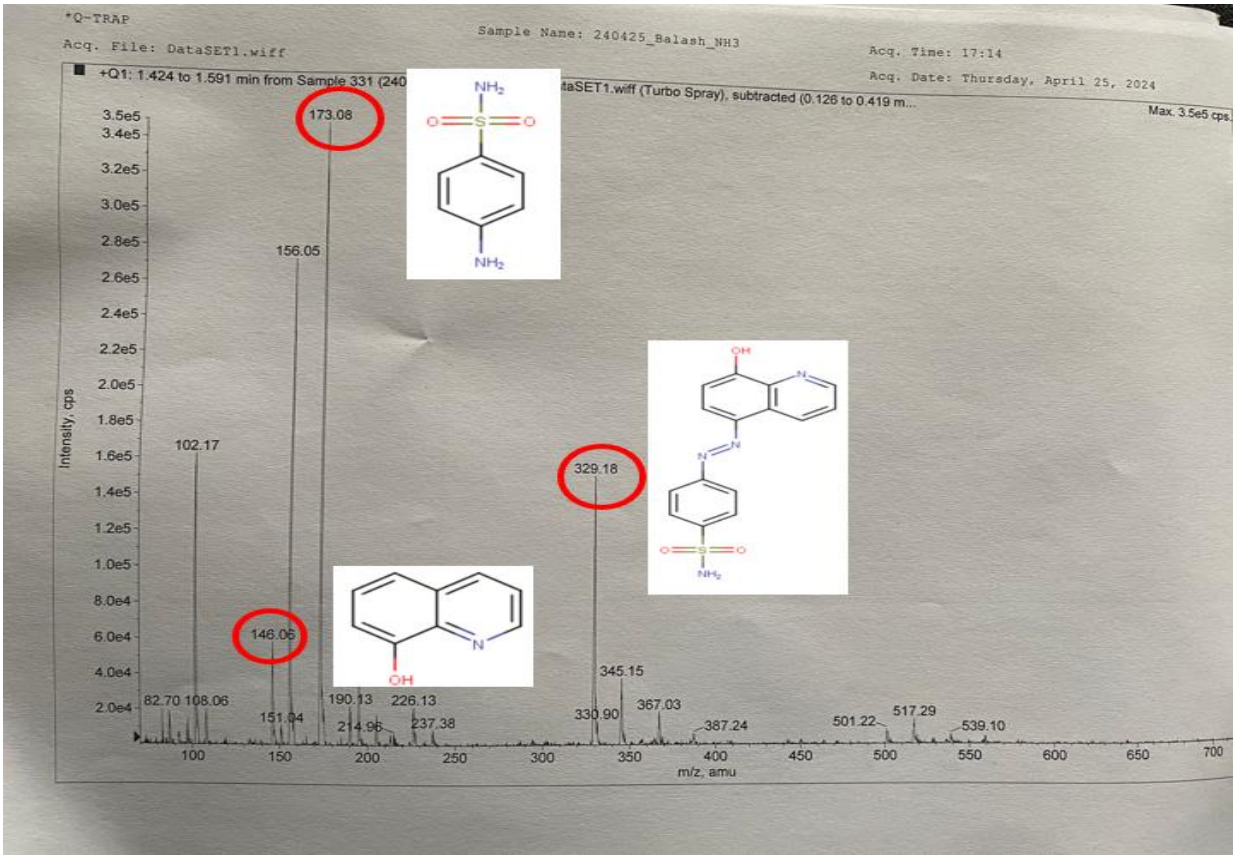


Figure 11. ESI-MS for 1 compound

Table 1. The structure of sulfonamide compounds used in this study

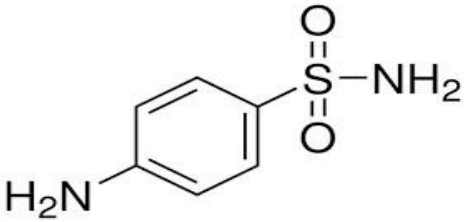
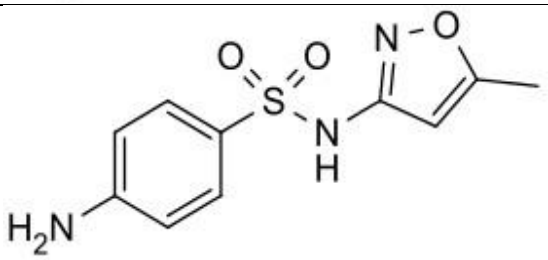
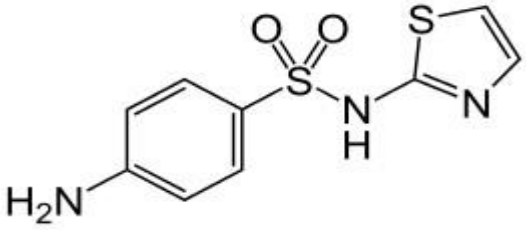
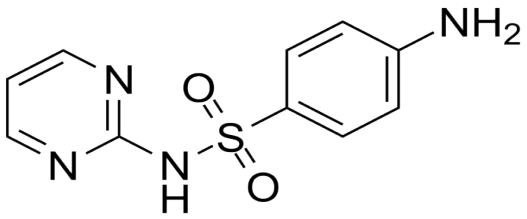
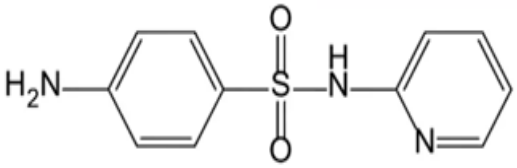
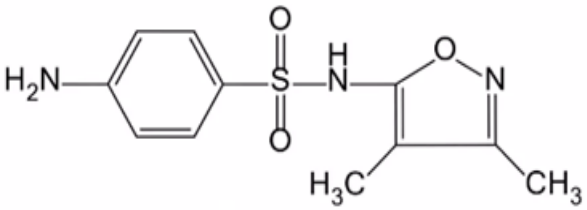
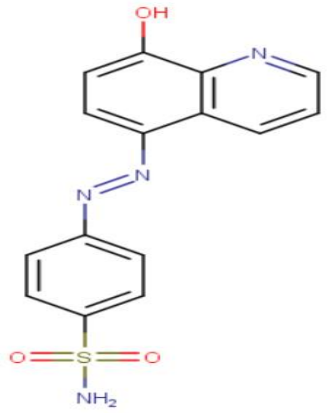
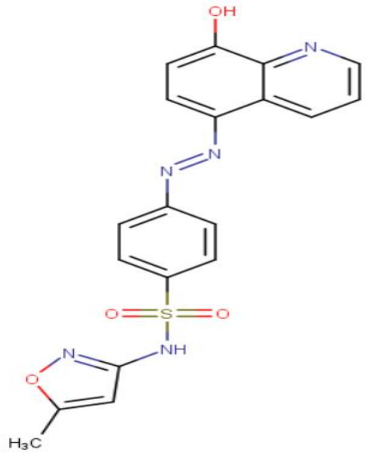
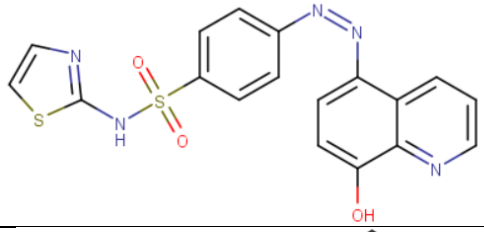
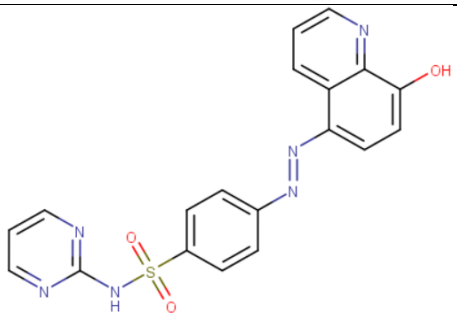
S. No.	Chemical name	Compound structure
1	Sulfanilamide	
2	Sulfamethoxazole	
3	Sulfathiazole	
4	Sulfadiazine	
5	Sulphapyridine	
6	Sulfisoxazole	

Table 2. Parameters of Molegro Virtual Docker

Parameters	Value
Scoring function	MolDock (GRID)
Grid resolution (A)	0.80
Binding site radius (A)	17
Searching algorithm	MolDock optimizer
Number of runs	10
Max iterations	2000
Max population size	50
Energy threshold	100
Simplex evaluation (max steps)	300
Neighbor distance factor	1

Table 3. shows the chemical structure of sulfonamide components with 8-hydroxy quinolone by formation of azo dyes in this study

S. No.	Compound structure
1	
2	
3	
4	

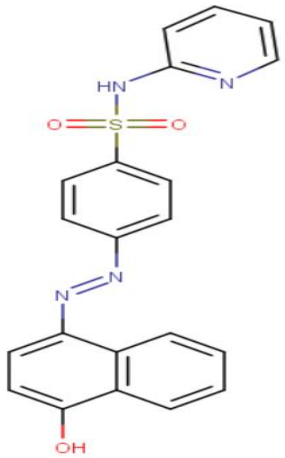
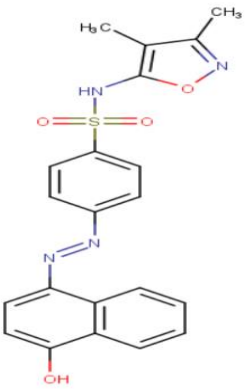
5	
6	

Table 4. Shows Lipinski's rule and PSA for the designed compounds.

Ligand	MW	Log p	Accepter	Donor	Lipinski's rules violation	PSA
1	328	2.2	7	2	Yes	126
2	409	3.78	9	2	Yes	138
3	411	3.18	8	2	Yes	153
4	406	2.7	9	2	Yes	138
5	404	3.7	9	2	Yes	104
6	422	4.7	8	2	Yes	106

Table 5. Amino acids associated with designer drugs, the doctrine of hydrogen bonds, Vanderwaals, and binding energy

Ligand	Van der Waals interaction	H-BOND	Energy docking
PFQ- 1001	Leu210, Gly216, leu139, Glu211	Ala213, Ala273	-133(Kcal/mol)
1	Lys141, Tyr 212	Ala213, Ala273, lys162, Asn261	-88(Kcal/mol)
2	Ala213, Asn261	Ala213, lys162, Asn261	-107(Kcal/mol)
3	Thr204, Ala167, Phe165	Glu170, Glu168, Arg205	-78(Kcal/mol)
4	Ala273, Asn261, Leu210, leu263	Ala213, Ala273, lys162, Thr217	-123.2(Kcal/mol)
5	GLU170,Ala203,Thr204, Ala167, Phe165, Gln168	Ale167, Arg205	-75(Kcal/mol)
6	Ala167, Thr204, Glu170	Ala203	-82.88(Kcal/mol)

Table 5 shows the docking results between the Aurora Kinase A binding pocket and the azo dye derivatives.

3.3. Validation docking method

Dock Validation was validated to confirm the positioning and orientation of the ligand binding obtained from docking studies by the MVD program. The parameters must, therefore, be validated by re-docking the native ligand into the crystal structure (PDB ID: 3K5u). The extracted ligand was then re-docked into the pocket to generate an X-ray binding mode. The capability of this algorithm to generate active binding modes of ligands was assessed by calculating the RMSD between the docking solution and the X-ray binding mode. The threshold in computer-aided drug design is the RMSD < 2 Å. The RMSD value for the top-ranked docking solution of the ligand was 1.02.Therefore, applied docking methods were able to generate X-ray binding mode of the ligand. Interactions of the residues of the binding pocket of Aurora kinase A with azo dyes derivatives. Only one compound showed higher docking scores toward the receptor than the reference ligand, Table 5 shows how the azo dye derivatives interact with the binding pocket parts of Aurora kinase A, along with the docking energy. Most of the compounds given hydrogen bonds and van der Waals bonds to receptors with different bond lengths and binding energies. The ligand displayed hydrogen bonds with amino acid residues of the active pocket of Aurora kinase A. The predicted binding modes of compounds 1 and 4 and their interactions with the residues in the active pocket of Aurora kinase A are shown in Fig 6 and Fig 7.

3.4. Analytical data

4-[(E)-(8-hydroxyquinolin-5-yl) diazenyl] benzene sulfonamide: Red Powder 88% m.p 204 C°. IR spectrum (νmax, cm−1): 3400(NHstr), 3450(NHstr), 1596(NHbend) 3257(OH), 1314.94 (S=Oasym str), 1150.18(S=Osym str), N=N (1500). 1H NMR (DMSO-d6, δ, ppm): δ 8.2 (M, Ar-H), 8 (d, J = 8.7 Hz, 2H, Ar-H), 7.42 (d, J = 8.8 Hz, 2H, Ar-H), 7.3 (s, 2H, NH2). 13C NMR (DMSO-d6, δ, ppm): δ-124 143.8(=CH), 167.18 (-CO). ESI-MS: *m*/*z* 329 [M+H]+, 173 m/z Molecular Weight of sulfonamide and m/z146 Molecular Weight of 8- hydroxy-quinolone [11-17].

4. Conclusion

Several azo dyes derivatives were docked into the aurora Kinase A binding pocket using the Molegro Virtual Docker software. The binding free energy was calculated to predict their affinity toward azo dyes to select novel candidates as aurora Kinase A inhibitors for treating cancer. The results showed that compound four gave the highest energy docking -123.2 Kcal/mol toward the aurora Kinase A. The obtained results suggested that these compounds may be novel candidates for cancer treatment by targeting aurora Kinase A. Then, compound one was synthesized with a simple procedure in two steps. The reaction condition easy and excellent yields of compound was obtained and their structure was confirmed by spectral and elemental analysis. Finally, more studies still needed to identify the mechanism of action of these derivatives and we are hoping that it will show significant anticancer activity.

Acknowledgements

The authors are thankful to University of Marburg, Institute of Pharmacy, Department of Pharmaceutical Chemistry, and Germany, for analyze our synthesized compounds by IR spectrum, mass spectrum, and H-NMR and 13C NMR spectrum.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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