Spectroscopic and Molecular Docking Techniques to Study the Biological Activity for the Synthesis of a Novel Organic Compound Derived from β-naphthol

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Abstract

A novel synthesized organic reagent compound, (E)-4-(2-hydroxynaphthalen-1-yl)diazenyl)-N-(pyrimidin-2-yl)benzenesulfinamide (4-HNPBS), derived from β-naphthol, was prepared by diazotization of sulphadiazine with 2-naphthol at a specific pH. 4-HNPBS was characterized by FT-IR, 1H-NMR, 13C-NMR, thermal analysis (DSC, DTA), CHNS, and melting point techniques. The biological activity towards the selection of antibacterial and fungal strains (E. coli, S. aureus, C. albicans, Proteus) was studied. 4-HNPBS (0.1 M) exhibited a good influence on the selected microorganisms. Molecular docking was studied using the AutoDock 4.2.6 program by taking 2D and 3D protein structures of the selected microorganisms and then docked with 4-HNPBS. The highest negative energy and affinity between the synthesized compound and the target microorganisms appeared in E. coli (Gram-negative bacteria) at -9.91 kcal/mol.

Keywords: *sulfapyrimidine, β-naphthol, biological activity, molecular docking.*

1. INTRODUCTION

In terms of manufacturing and quantity, azo dyes are the most common type of colorant, and they have a wide range of industrial uses for coloring various substrates [1-3]. Many high-tech applications for this material exist, including photo-optical media [4], photo switches [5], photo-mechanical systems [6], micro-patterning [7], data storage and non-linear optics [8], molecular shuttles [9], and nanotubes [10], in addition to the traditional uses of azo dyes for coloring paper, food, and cloth fibers [4].

Heterocyclic diazo components are utilized to create innovative disperse azo dyes [5] and to make filters and eye protection glasses [11] because they are more tinctorially potent and brighter than aromatic "analogues". Sulfonamides medicines are the active ingredient in a wide range of medications that are used to treat a wide range of diseases [12]. They have been used as antibacterial [13], anticancer [14], anti-obesity [15], carbonic anhydrase [16], and acetylcholinesterase inhibitor agents in clinical medicine for the treatment of Alzheimer's disease [17]. In light of the aforementioned advantages and in the progression of our interest in bio-active compounds [18-22], we present here the formulation of a novel (E)-4-(2-hydroxynaphthalen-1-yl)diazenyl)-N-

(pyrimidin-2-yl)benzenesulfinamide, (4-HNPBS). It contains a sulphonamido group to examine its biological activity practically and theoretically by molecular docking. A proposed ligand's binding to a receptor is simulated computationally by molecular docking, which determines the preferred orientation of one molecule to another when they are linked to one another to create a stable complex [23].

2. Materials, Methods, and Instruments

Chemicals used in this study include sulfapyrimidine, β-naphthol, hydrochloric acid, sodium nitrite, absolute ethanol, distilled water, sodium hydroxide, beakers, test tubes, pipettes, volumetric flasks, graduated cylinders, and various equipment such as UV-Visible spectrophotometer (single-double band), FT-IR and 1H-NMR-13C-NMR spectrophotometers, mass spectrometers, electro-thermal melting point apparatus, pH meter, thermal analysis spectrophotometer (DSC, TG/DTG), LECO CHNS-932 analyzer, and the AutoDock 4.2.6 program.

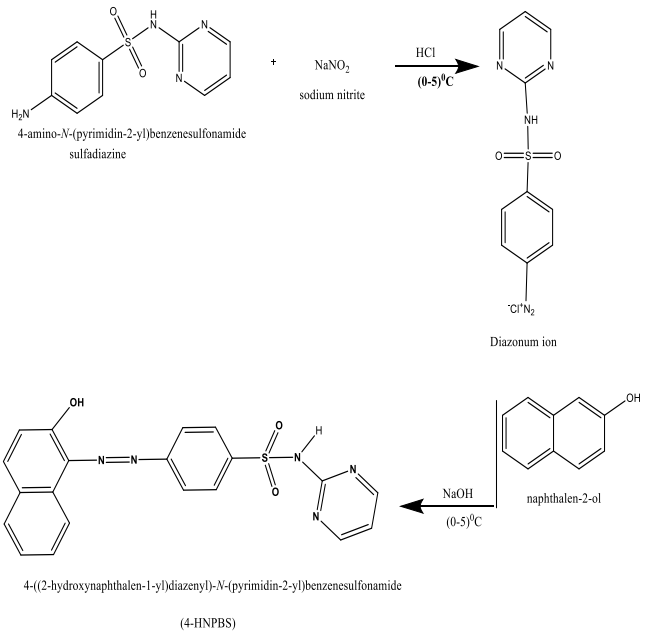
2.1 Experiment

2.1.1 The process of synthesizing (E)-4-((2-hydroxynaphthalen-1-yl)diazenyl)-N-(pyrimidine-2-yl)benzenesulfinamide ,(4-HNPBS).

The reagent was synthesized in Sketch-1, following the procedure mentioned in reference [24]. The solution of NaNO2 (0.05 mol), which had been dissolved in water D.W and cooled down to 0°C, was gently added and agitated for a few minutes. Sulfapyrimidine (3.4498 g, 0.05 mol) was dissolved in conc. HCl (15 mL, 36%) and cooled to 0-5°C in an ice bath. The Diazonium Ion was then added gradually to a solution of β-naphthol (7.2085 g, 0.05 mol) that had been dissolved in a sodium hydroxide (2.5 M) solution with good stirring at controlled pH <7. The product was left for a day, after which the precipitate was filtered, allowed to dry, and then recrystallized in absolute ethanol. The resulting product was 43% with a melting point of 136-138°C (see Figure 1).

The calculated analysis for C20H15N5SO3: C, 59.250; H, 3.729; N, 17.274; S, 7.907; O, 11.838. The analysis found was: C, 58.61; H, 3.429; N, 16.274; S, 7.007.

Figure 1. Sketch-1 ,Synthesis route of 4-HNPBS



2.1.2 Biological Activity

The bacteria and fungi under investigation were cultivated in Muller-Hinton (MH) Media at a temperature of 37 °C for a duration of 24 hours. To perform the Agar Plate diffusion method, 100uL of the reagent was injected into 7 mm diameter holes that were cut into the agar gel. All antimicrobial studies were conducted at Al-Ameen Center for Research and Advanced Biological Technologies in Iraq.

2.2. Molecular Docking Analysis

The protein data of E.coli, S.aureus, C.albicans, and Proteus (2Q85, 2XCT, 5TZ1, 6Y4F) were downloaded and their 2D and 3D structures were loaded and then analyzed with the synthesized reagent (4-HNPBS) in AutoDock 4.2.6 program. During the docking procedure, both the protein and ligands are considered rigid.

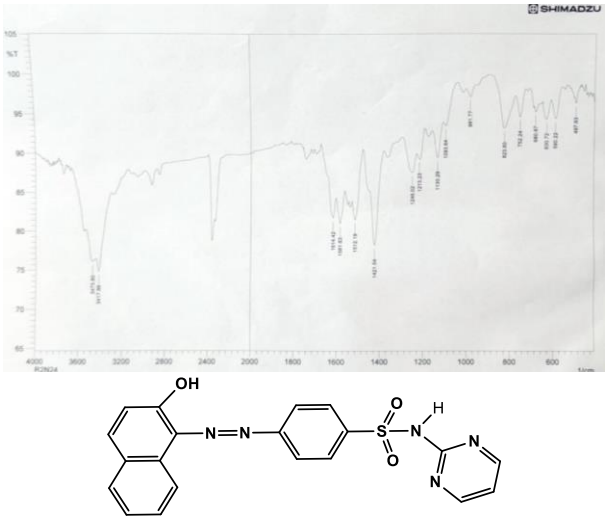
3. Results and Discussion

3.1 Reagent diagnoses

3.1.1 Fourier-Transform Infrared Spectroscopy (FTIR)

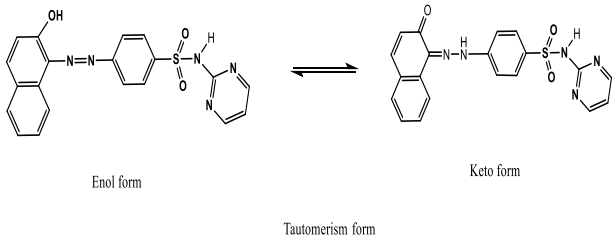
Several spectroscopic tools and elemental analyses were used to determine the chemical structure and purity of the prepared compound, which was obtained as a solid powder with a dark reddish-orange color and identified as an azo reagent. FT-IR for the synthesized reagent (4-HNPBS) exhibited broad band stretching vibration at 3473.80 cm-1 which belongs to (OH, naphthol), 3417.86 cm-1(NH, sulphadiazine), 1614.42 cm-1(C=N, pyrimidine), 1581.63 cm-1, 1512.19 cm-1 (C=C aromatic, naphthol-sulphadiazine), 1330, 1130.29 cm-1 (S=O) and 1412.54 cm-1 (sharp, cleavage) belong to N=N. Figure (2)

Figure 2 FT-IR spectrum of (4-HNPBS)



The infrared spectrum showed the absence of a carbonyl band and a strong hydroxyl naphthol band at 3473.80 cm-1 for the heterocyclic diazo component (crystalline phase) prepared in this work. The component was found in a controlled manner in the tautomeric state, represented in the hydrazone form. It was identified as azo hydrazone tautomerism, where an azo chromophore is combined with an azo dye. The transfer of protons between oxygen and nitrogen atoms is both theoretically and practically interesting, as reported in [25-26]. Figure (3)

Figure 3. Tautomerism form of 4-HNPBS.

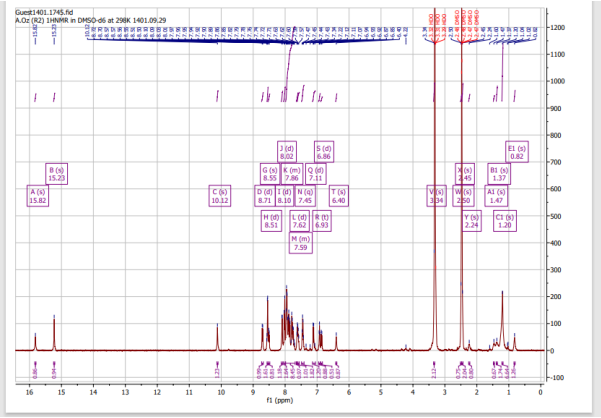


3.1.2 Nuclear Magnetic Resonance Spectroscopy (H-NMR,13CNMR)

H-NMR spectrum shown in Figure 4 for (4-HNPBS) shows a single peak at δ=11.2 ppm refer to the proton of NH, a singlet signal δ =8.5 ppm refers to HC=N, multiple peaks

from δ (6.9-8.1) ppm which are signals for protons aromatic rings in the reagent. Additionally, a single peak at δ=2.5 ppm belongs to the solvent (DMSO).

Figure 4. H-NMR spectrum of 4-HPBNS



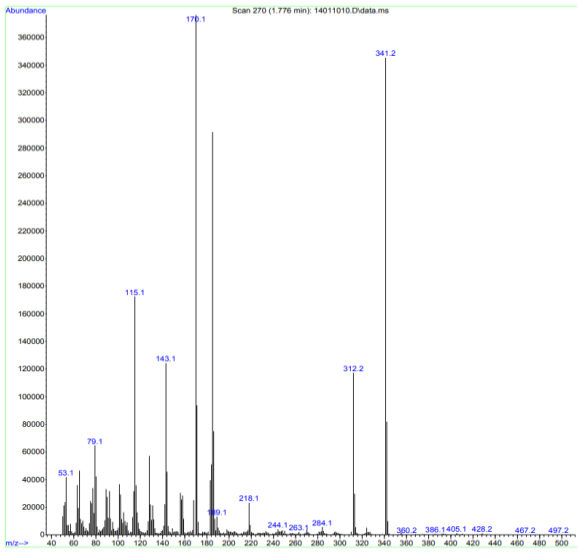
3.1.3: Mass characterization

Mass spectrometry is a contemporary analytical method that enables rapid and highly sensitive detection and identification of various chemical species [27].

The device used in mass spectrometry is designed to measure the mass-to-charge ratio (m/z) of one or more molecules present in a given sample. By conducting these tests, the exact molecular weight of the constituents within the sample can often be determined, as shown in Figure (5) below.

Based on the basic peaks, the category that the ion fragments fall under can be determined. The molecular weight of the prepared reagent (4-HPBNS) was found to be 405.4292, which appears as a peak at 405.1 m/z at the end of the spectrum. The ion fragments at peaks (143.1, 244.1) m/z belong to β-naphthol and sulphadiazine, respectively. These fragments lose small particles, which are represented in the mass spectrum.

Figure 5. The mass spectrum for the 4-HNPBS



3.1.4 Thermal analysis of 4-HNPBS, Thermogravimetry (T.G-D.T.A) and Differential Scanning Calorimetry (D.S.C)

The term "thermal analysis" generally refers to a technique used to identify the temperature and time at which a substance undergoes a physical transformation upon heating or cooling. According to the kinds of physical changes being examined, each technique is specified. The choice of techniques used for evaluating material properties depends on the specific objective and may involve the use of different procedures or a combination of multiple techniques. Differential Scanning Calorimetry (DSC) is a technique used to measure the fluctuation in the heat flow given out or taken in by a specimen when subjected to temperature scanning in a controlled environment. Each change in a material caused by heating or cooling involves an exchange of heat; DSC makes it possible to identify the temperature of this change and quantify the heat it produces. In Figure 6, the DSC thermogram shows an exothermic peak at (40.61-95.58) ºC for 4-HNPBS, which

refers to the melting point of the prepared reagent. In Figures 7 and 8, the thermograms for the reactants 2-naphthol and sulphadiazine exhibit exothermic peaks at (120.89-126.82) ºC and (256.3-266.4) ºC, respectively.

Figure 6. D.S.C thermogram for 4-HNPBS

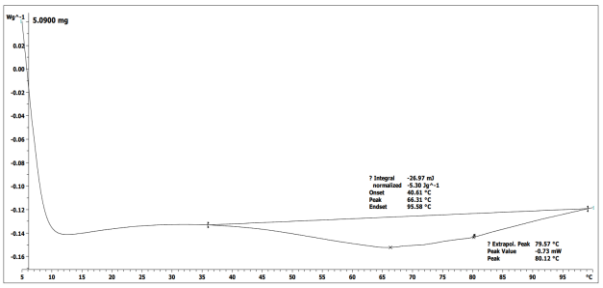


Figure 7. DSC thermogram for β-naphthol

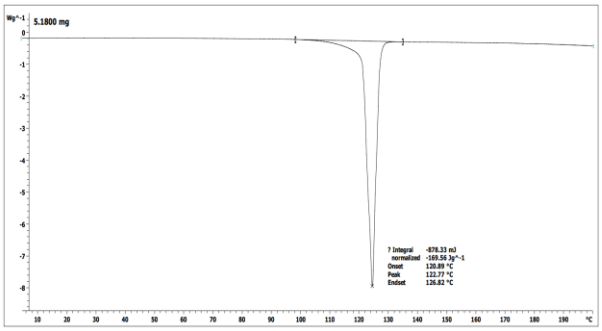


Figure 8. DSC thermogram for sulphadiazine

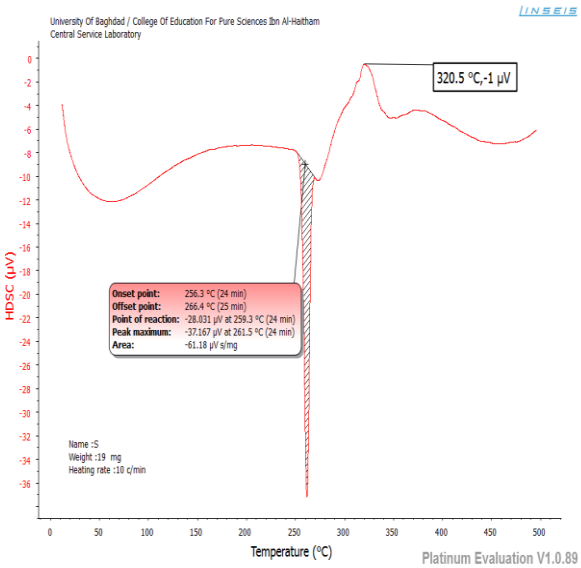


Table 1: DSC thermal values

Materials	1exo effect			2endo effect			3exo effect		
	Tonset (ºC)	Peak max	Toffset (ºC)	Tonset (ºC)	Peak max	Toffset (ºC)	Tonset (ºC)	Peak max	Toffset (ºC)
β-naphthol	120.89	-122.77	126.82	-	-	-	-	-	-
sulphadiazine	256.3	-37.167	266.4	-	-	-	275	-3.5	349
4-HNPBS	40.61	-66.31	95.58	-	-	-	-	-	-

Thermogravimetry is a method utilized in measuring the alteration in the mass of a given sample. Vapor emission or mass gain are both possible outcomes of this mass fluctuation (gas fixation). The measurement of the temperature difference between a sample and a reference (a thermally inert material) as a function of time or temperature when two items are subjected to temperature scanning in a controlled environment is known as differential thermal analysis. Every transformation can be found using the DTA approach for all types of materials. DTA also records changes in materials when no mass loss occurs, such as crystal structure changes, melting, glass transition, etc. On the other

hand, TG solely measures changes brought on by mass loss. The thermograph Figure (9) of (4-HNPBS) exhibited three stages of decreases in the mass of the material: the first regarded with de-hydration of water molecules on the surface of granular specimen powder, the second stage loss of a large amount of content about (-43.14%), and the final stage, at (445-600)ºC, involved the destruction of the organic reagent composition. Figures (10) and (11) showed the thermograms for reactants Table (2) shows TG-DTG data for the materials.

Table 2:TG-DTG thermal values

Materials	Step1			Step2			Step3			%loss of total mass
	Tonset (ºC)	Toffset (ºC)	Mass changes(%)	Tonset (ºC)	Toffset (ºC)	Mass changes(%)	Tonset (ºC)	Toffset (ºC)	Mass changes(%)	
β-naphthol	30	120	-0.84	120	200	-89.38	200	600	-2.86	-93.08
sulphadiazine	25	315.128	-28.6071	423.244	494.573	-24.0122	494.573	500	-3.98835	-56.6765
4-HNPBS	30	235	-2.2	235	445	-43.14	445	600	-5.26	-48.4

Figure 9. TG-DTA thermogram for 4-HNPBS

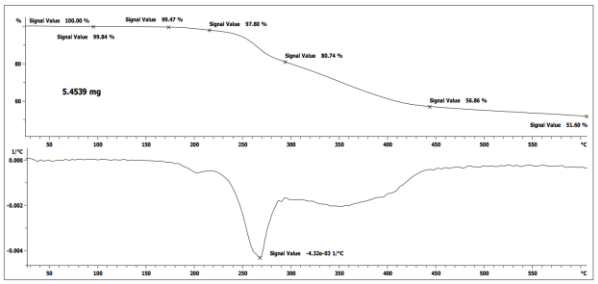


Figure 10. TG-DTA thermogram for β-naphthol

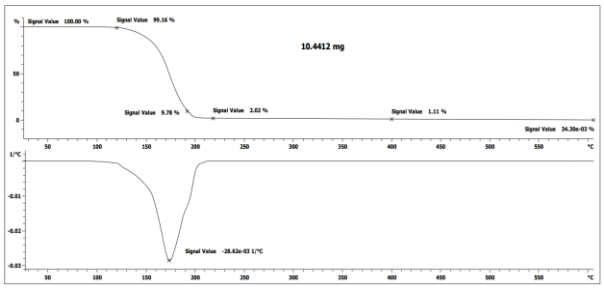
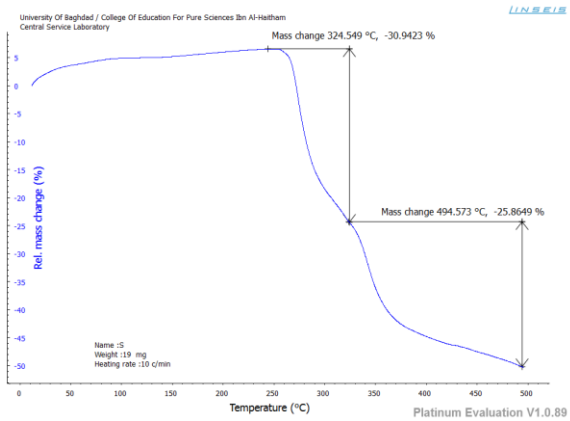


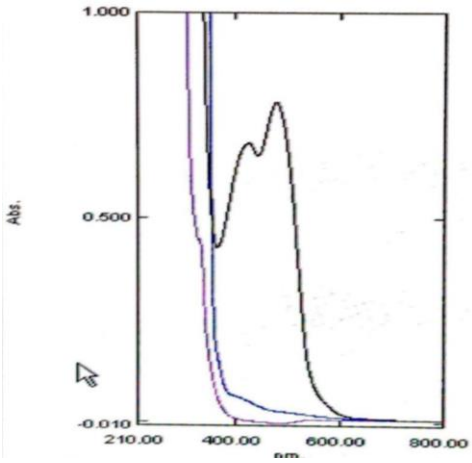
Figure 11.TG-DTA thermogram for sulphadiazine



3.1.5 Uv-vis spectra

The reddish-brown colored solution of 4-HNPBS (dissolved in ethanol) had a λmax at 477 nm, which was attributed to the N=N moiety. Figure 12 confirms that the compound was synthesized and that this band does not appear in any of the starting materials.

Figure 12. UV-VIS spectra for 4-HNPBS



3.2 Bio Activity

3.2.1 Antimicrobial and the Limits of inhibition concentration

The novel compound 4-HNPBS was tested for its in-vitro antibacterial activity against Staphylococcus aureus (a type of Gram-positive bacteria), Proteus vulgaris, and Escherichia coli (both types of Gram-negative bacteria). The antifungal potential of the compound was also evaluated against Candida albicans by comparing it to a control substance (ethanol) using the Agar Plate diffusion method. To perform the test, the bacteria and fungi were cultured in Muller-Hinton (MH) Media for 24 hours at 37 °C. Then, 100 µL of the reagent was injected into 7 mm diameter wells that were cut into the agar gel.

The activity of the reagent was tested at three different concentrations: 1*10-1 mole/L, 1*10-2 mole/L, and 1*10-3 mole/L using the inhibition zone diameter in millimeters as a measure of positive activity. The activity was also quantified using indexed values.

$$\%Activity\ index = \frac{zone\ of\ ihibition\ by\ test\ compound(diameter)}{zone\ of\ ihibition\ by\ standard(diameter)} \times 100$$

The percent of inhibition (%) was checked for the appointment microorganisms from maximum to minimum in Conc.1*10-1M S. Arureus> C.albicans, Protus vulgaris > E.coli, in 1*10-2M S. Arureus > C.albicans, E.coli> Protus vulgaris.

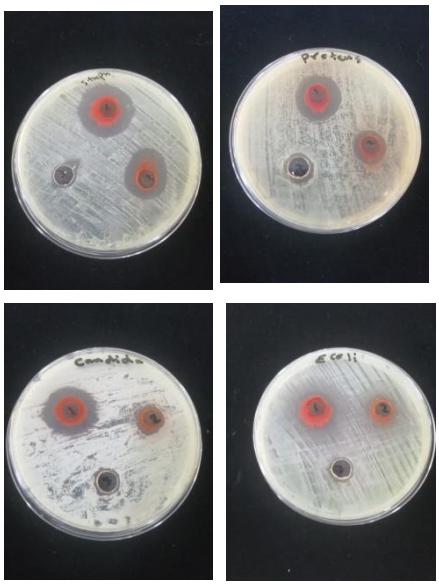
In 1*10-3M Protus vulgaris> C.albicans, S. Arureus> E.coli. The activity of the reagent belongs to the nitrogen of pyrimidine and oxygen, sulphur moieties in heterocyclic reagent compounds because of its specific biological and pharmacological activities [28].

All the results are shown in (Table 3) and pictures in Figure (13) are displayed below.

Table 3. The antibacterial and antifungal activities (%) of the (4-HNPBS) were expressed in three concentrations (mole/L).

	Concentration	$1*10^{-1}$M	$1*10^{-2}$M	$1*10^{-3}$M
	Inhibition zone, mm (activity indexed %)	Inhibition zone, mm (activity indexed %)	Inhibition zone, mm (activity indexed %)	Inhibition zone, mm (activity indexed %)
Fungi	*C.albicans*	26(173.3%)	15(100%)	13(86.7%)
Gram-positive bacteria	*S.Arureus*	30(200%)	24(160%)	13(86.7%)
Gram-negative bacteria	*Protus vulgaris*	26(173%)	12(80%)	15(100%)
	E.coli	20(133%)	15(100%)	12(80%)
Standard(ethanol)		15(100%)	15(100%)	15(100%)

Figure 13. Images for the antibacterial and antifungal affected by 4-HNPBS.



3.2.2 Molecular Docking Study

Docking analysis typically involves two main steps. First, the precise orientation of the conformers is predicted to identify the best active site pocket, which is referred to as the pose. Next, the strength of the binding interactions between the target and ligand is determined by scoring the results of the docking analysis [20]. The stability of molecular docking results obtained through the use of the molecular docking technique can vary depending on various factors. However, the binding affinity determined through the scoring function can serve as a reliable indicator of the strength of the binding interactions between the ligand and target. In general, a high number of hydrogen bonds and low binding energy values suggest high stability of the complex, while negative values obtained from the scoring function are indicative of strong binding interactions between the receptor and the investigated ligand (4-HNPBS). The antibacterial and antifungal activities of 4-HNPBS were ranked in Table 4 and illustrated in Figures 14-17 according to the calculated energy values obtained from the docking analysis.

Table: 4 Docking scores of 4-HNPBS towards antibacterial and antifungal proteins.

Type of ANTI-BA., ANTI-FUN.		proteins	ENERGY Kcal/mol
E.coli	Gram negative bacteria	2Q85	-9.91
S. aureus	Gram positive bacteria	2XCT	-9.68
C. albicans	fungi	5TZ1	-9.04
Proteus	Gram negative bacteria	6Y4F	-8.42

Figure 14. 2D and 3D molecular docking results for 4-HNPBS binding with C. albicans 5TZ1 protein, hydrogen bond interaction of 5TZI.

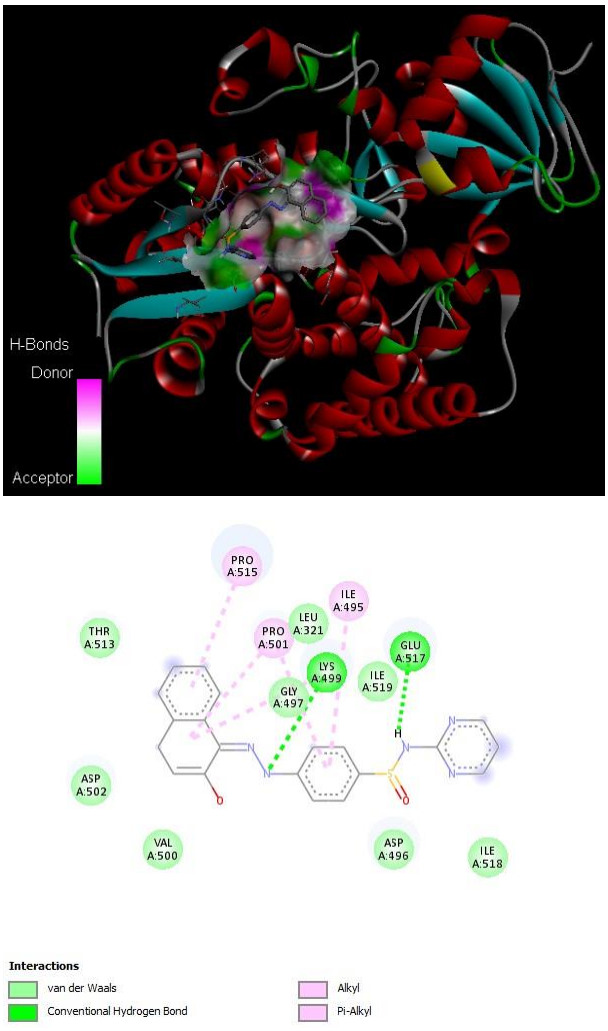


Figure 15. 2D and 3D molecular docking results for 4-HNPBS binding with E.coli 2Q85 protein, hydrogen bond interaction of 2Q85.

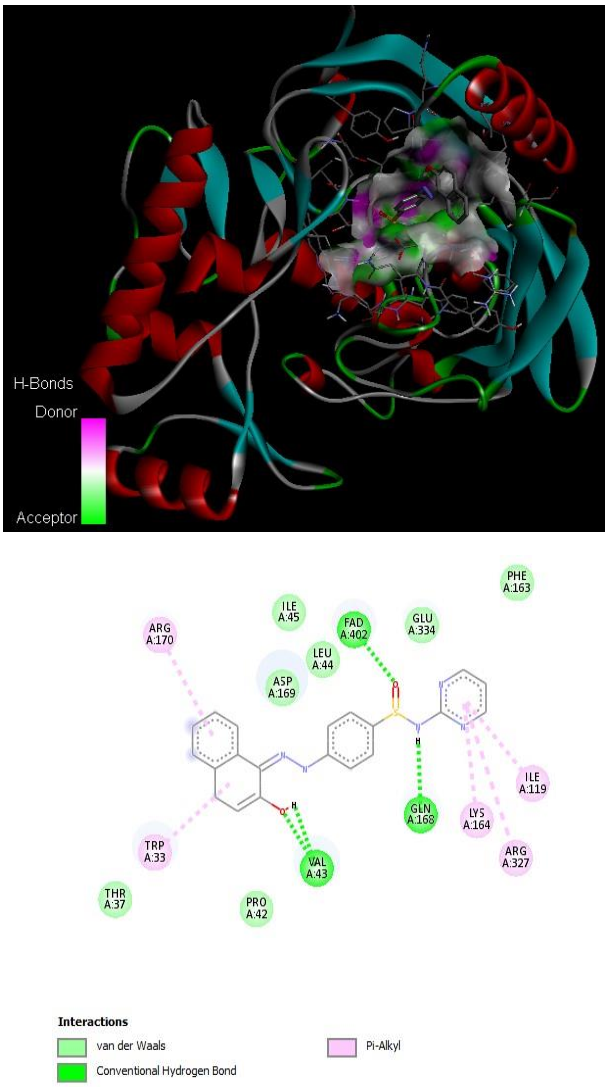


Figure 16. 2D and 3D molecular docking results for 4-HNPBS binding with Proteus 6Y4F protein, hydrogen bond interaction of 6Y4F.

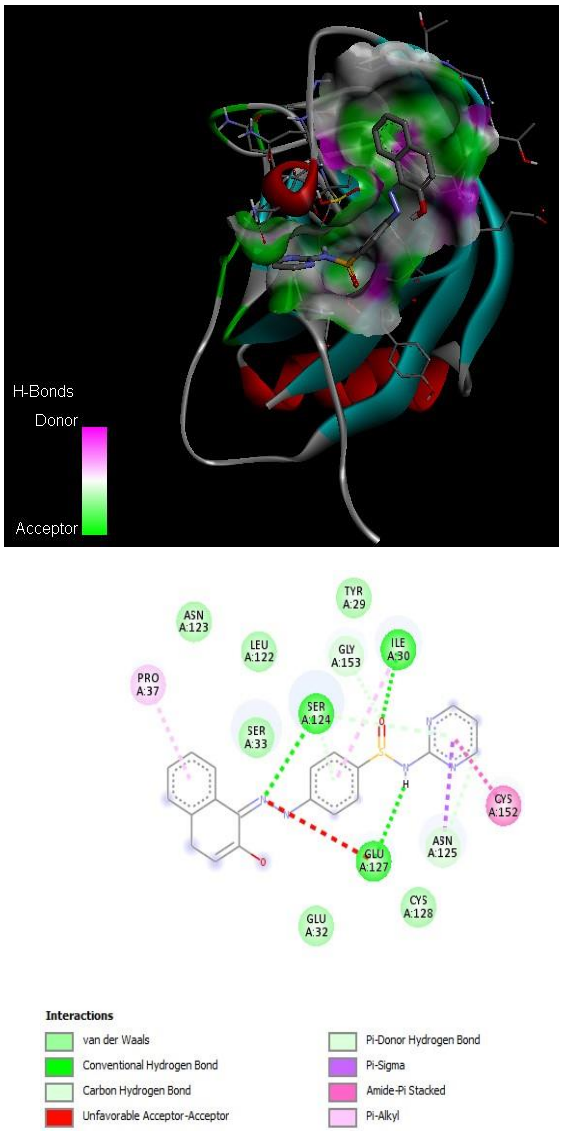
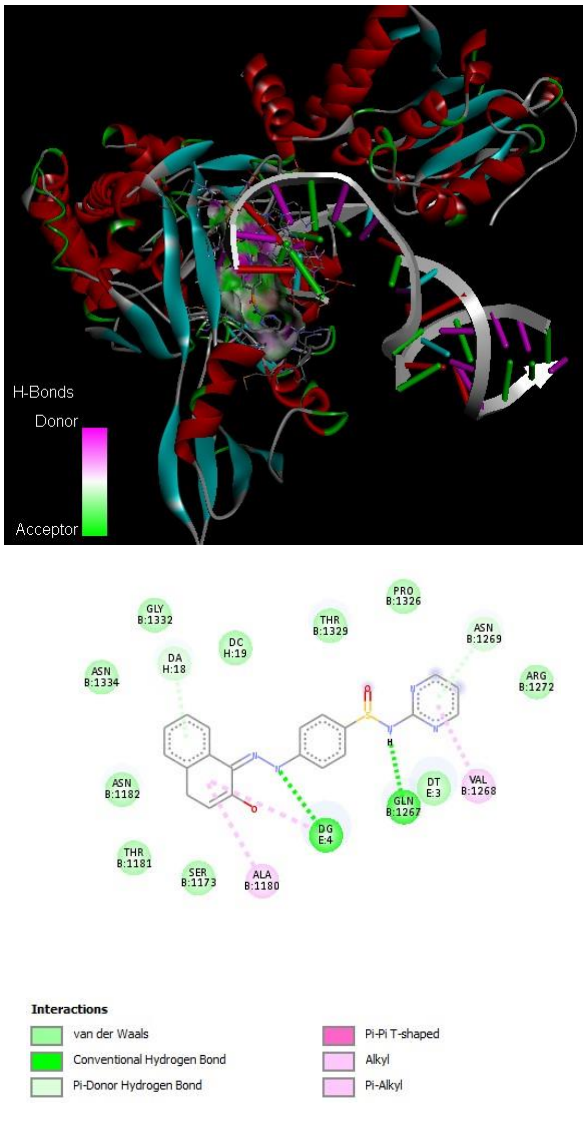


Figure 17. 2D and 3D molecular docking results for 4-HNPBS binding with S.aureus 2XCT protein, hydrogen bond interaction of 2XCT.



4. CONCLUSION

The diazotization reaction of sulphadiazine with 2-naphthol was coupled in a regulated pH range between 6.0 and 7.0 to create the novel reagent 4-HNPBS, which is produced from 2-naphthol. It was possible to purify and then characterized with more than one technique and established its correct form. The active reagent was further examined and its antimicrobial activity expressed the best

inhibitory concentration in 0.1 M. Using molecular docking and emulation study it was identified that 4-HNPBS could be an effective agent to inhibit the antibacterial and antifungal proteins.

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