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Synthesis, Characterization, Molecular Docking, Synthesis and biological activity of Schiff bases derivatives derived from Ceftriaxone as Anti-PC3

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ABSTRACT: Schiff bases are formed by reacting aromatic or aliphatic ketones or aldehydes with heterocyclic amines, yielding azo methine derivatives. The ceftriaxone reacts with aldehydes, such as 4-(N, N-dimethylamino) benzaldehyde and 4-heptoxybenzaldehyde, giving new schiff bases derivatives and characterization by FTIR. These derivatives (A and B) tested for in vitro biological activity against Bacillus subtilis, Streptococcus pneumonia, E. coli, Bacillus subtilis, Candida, and Aspergillus nigaer. The biological properties of derivatives A and B have been evaluated against different bacteria and fungi. Docking is crucial for examining binding mechanisms. The function of GP6 synthase in the formation of microbial cell walls. Both compounds had similar binding affinities, with ceftriaxone measuring -6.9 kcal/mol. Experiments confirm that both substances possess antibacterial properties. The derivative A has a more pronounced influence on suppressing bacterial growth in petri dish zones. The most substantial effect on fungus was seen in the case of Aspergillus niger due to derivative A. The cytotoxic effect of the prepared Ceftriaxone derivatives against human prostate cancer cells (PC3) was investigated using the MTT colorimetric assay. The cytotoxicity assay reveals that the B (heptoxybenzaldehyde Ceftriaxone derivative) is more active to inhibit the growth of PC3 cells compared to the A (N, N-dimethylamino) benzaldehyde Ceftriaxone derivative).

Keywords: Antimicrobial, Bacterial resistance, Docking analysis, Schiff base, Prostate cancer

1. INTRODUCTION

Schiff bases are formed by the mixture of aromatic aldehydes with aromatic amines, yielding azomethine derivatives. These derivatives have received approval for use in chemistry owing to the existence of a lone pair of electrons on the nitrogen atom and the inherent electron-donating nature of the double bond [1]. Schiff bases constitute a significant family of chemicals in the medical and medicine domains, exhibiting various physiological actions involving antifungal, antibacterial, anti-inflammatory, cancer prevention, antimicrobial, and antioxidant properties [2]. They are employed as anti-corrosion pigments and catalysts [3]. Prostate cancer (PC) is defined a cancerous growth develops in the region of prostate in men. The PC is a little gland in males that has a form resembling a walnut [4]. Its primary function is to generate the seminal fluid that nourishes and conveys sperm. Prostate cancer is a highly prevalent form of cancer [5]. Prostate cancer is a prevalent disease, ranking second only to skin cancer in terms of its frequency among males and individuals assigned male at birth (AMAB) [6]. The majority of individuals will lead typical lives and ultimately pass away from factors that are not related to prostate cancer. Specific individuals may not require any form of medical intervention [7]. The FDA-approved third-generation cephalosporin drug ceftriaxone is mainly employed for the treatment of mild-to-moderate or pneumonia acquired in the community [8]. Ceftriaxone suppresses lung cancer growth by targeting Aurora B [9].

The progress in modern medicine is being compromised by the emergence of antibiotic resistance [10], leading to a resurgence of infectious diseases that are now a significant global threat due to the widespread presence of antibiotic-resistant organisms [11]. Antimicrobial agents, such as antibiotics, are available in various forms to hinder bacteria growth, reproduction, or eradication. Certain chemicals can profoundly alter physiological and metabolic systems. Antimicrobial resistance (AMR) refers to the capacity of microorganisms to persist and survive in the presence of

antimicrobial medicines. The persistent and widespread use of antibiotics has led to the emergence of antimicrobial resistance (AMR) in bacteria [12,13]. Antimicrobial medications are employed to treat microbial infections, although the efficacy of this form of infection therapy is significantly impeded by the emergence of antibiotic resistance [14]. Hence, identifying and advancing novel antimicrobial medications are imperative for efficiently managing microbial diseases. It is essential to develop new therapeutic strategies to combat germs resistant to antimicrobial agents [15].

The inability to administer therapeutic drugs in a way that ensures the majority of drug molecules selectively reach the intended targets at ideal concentrations while causing little unintended harm hinders the ability to treat many human diseases [16]. Treating diseases like cancer, inflammatory conditions, or degenerative disorders is difficult due to the disruption of natural and necessary biological processes [17]. Human diseases typically comprise diverse cell types, including sick cells, standard cell groups from which the disease originated, vascular cells, fibroblasts, and immune cells. Abnormal cells inside a tissue have distinct biological traits compared to their healthy counterparts [18]. This research aimed to the efficacy of ceftriaxone and Schiff bases in eradicating bacteria that exhibit resistance to ceftriaxone using in vitro procedures.

2. PROCEDURES

2.1 AZOMETHINE DERIVATIVES (A AND B) SYNTHESIS

A 4-(N, N-dimethylamino) benzaldehyde (1.49 gm, 0.01 mole) with 4-heptoxybenzaldehyde (2.20 gm, 0.01 mole) separately dissolve in 15 mL of 100% ethanol until both of them completely dissolve. Ligand ceftriaxone (5.54 gm, 0.01 mole) was added to each solution. The mixtures were then heated at 75 °C and stirred for 2 hours. The solutions were allowed to cool to room temperature for 3 hours. The yellow precipitates undergo filtration, ethanol washing, and vacuum drying [3]. The structure of these derivatives is shown in Figure 1.

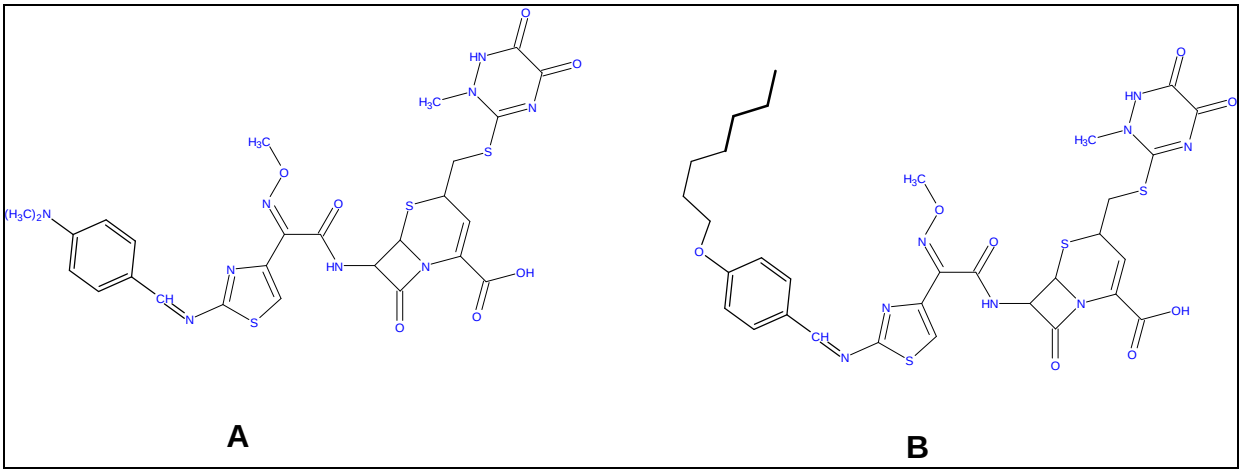


FIGURE 1. - Structures of products from modification of ceftriaxone

2.2 INVESTIGATION OF THE ANTIMICROBIAL ACTIVITY OF CEFTRIAXONE AND SCHIFF BASE DERIVATIVES A AND B

The different bacterial strains, such as Bacillus subtilis, Streptococcus pneumonia, E. coli, and Bacillus subtilis, used Muller-Hinton agar to grow by streaked technique, initiating the broth technique [19]. A single well formed in the agar. Everyone enjoyed 100 µL of the appropriate dilution of azomethine derivatives (A and B), which were efficiently digested. The container was airtight and kept at 37 ºC for 24 h.[20].

2.3 DOCKING ANALYSIS

The crystallographic three-dimensional model of glucose-6-phosphate synthase (GP6 synthase) was acquired from the Protein Data Bank (PDB code: 1MOQ). The protein model underwent energy reduction with Swiss Protein Viewer (version 4.1). Multiple preliminary phases were executed, eliminating a water molecule and co-crystallized substances. After that, the structure underwent essential repairs, and polar hydrogen was included. The synthesized compounds were shown with ChemDraw Ultra (version 12.0) and then saved as a PDF file [21]. Energy reduction was conducted with Chem3D (version 12.0) followed by saved to pdb file format with OpenBabel application. A docking technique was performed using Autodock Tools (version 1.5.7) as outlined by Ermakova et al. in 2020. The results were stored in pdbqt files after the energy minimization for both enzymes and ligands. The molecular docking procedure was performed with Autodock Tools and the AutoGrid software. The grid box was specified with dimensions of 40 × 40 ×

40, and its center was located at coordinates 18.770 (x), 21.038 (y), and 6.366 (z). Applying the docking parameters to their default settings produced 9 forms. Discovery Studio software was employed to generate illustrations.

3. RESULTS AND DISCUSSION

3.1 CHARACTERIZATION BY FTIR

The hydroxyl group exhibited at 3429 cm-1, the aromatic C-H bond at 3022 cm-1, the aliphatic C-H bond at 2941 and 2881 cm-1, and the carbonyl group of the amide and azomethine were observed at 1685 and 1627 cm-1, respectively [22]. The FTIR analysis of the derivative revealed the presence of a C-C aromatic bond at 1594 cm-1, as indicated by the A peak, as shown in Figure 2.

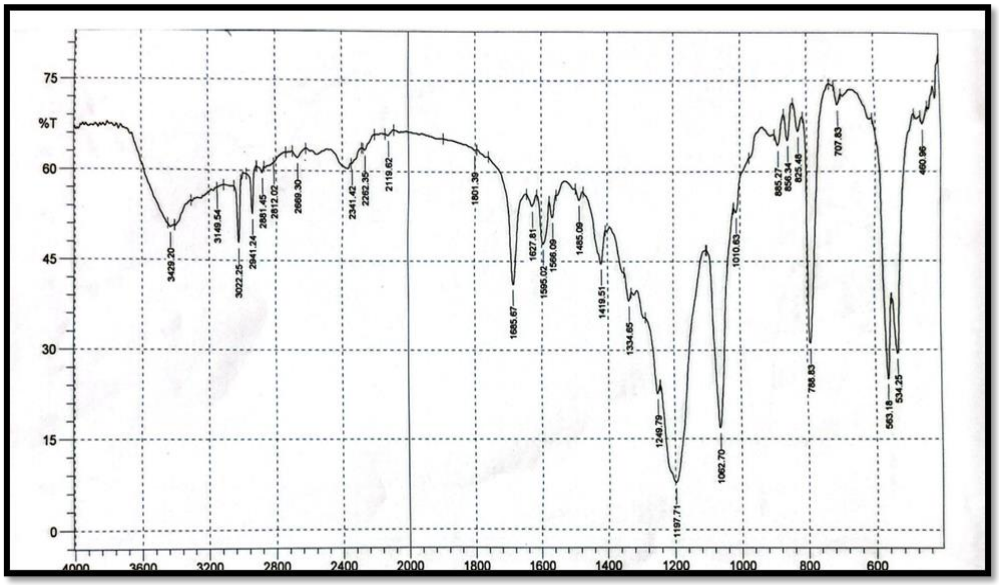


FIGURE 2. - FTIR of derivative A

The broad band of the -OH appeared 3433 cm-1, while aliphatic group C-H at 2852 and 2802 cm-1, and the C=O of the amide and imine shows at 1660 and 1635 cm-1, respectively [23]. The C-C aromatic at 1612 cm-1, in the FTIR (cm-1) of the derivative established B, as shown in Figure 3.

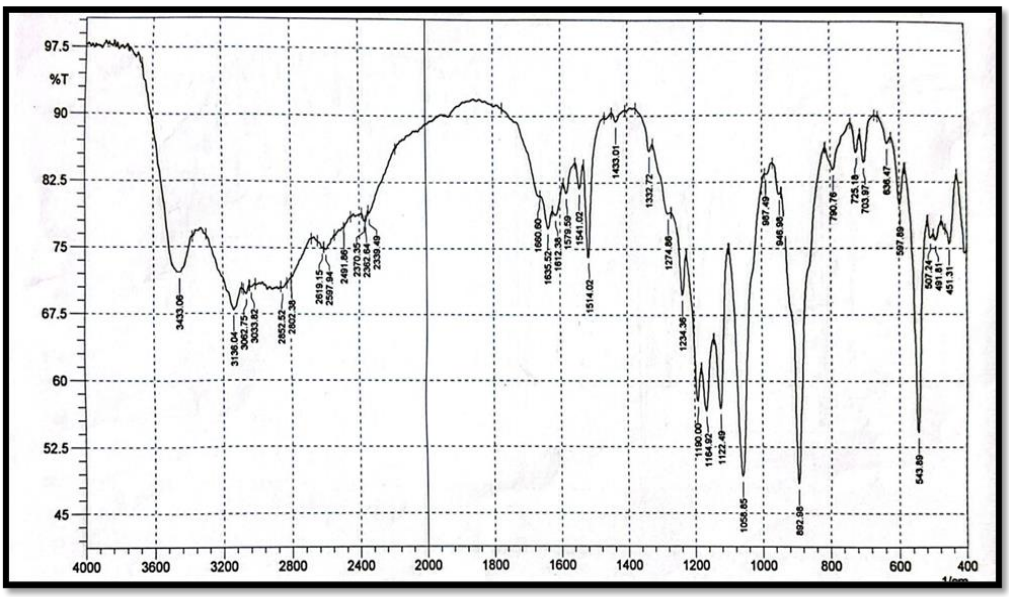


FIGURE 3. - FTIR of derivative B

3.2 STUDY OF DOCKING

Molecular docking is a computational method employed in drug discovery and molecular biology to forecast the interaction between a small molecule (ligand) and a target protein (receptor). It assists researchers in comprehending the binding interactions between a drug and its target, which is essential for the development of novel pharmaceuticals and the enhancement of current formulations. Docking models are essential for studying the binding mechanisms that bring ligands to target the molecular structure. The importance of glucosamine-6-phosphate synthase (GP6 synthase) in microbial cell wall production has attracted considerable interest from many researchers [24]. During the early phases of hexosamine, the process of biosynthesis enzymes catalyzes the transformation of fructose-6-phosphate to glucosamine-6-phosphate, thereby expediting the process. GlcN-6-P functions as a chemical precursor for uridine diphosphate N-acetyl glucosamine (UDP-NAG), an essential constituent of the peptidoglycan layer in microbial cell walls [25]. Figures 4 and 5 illustrate the docking characteristics of ceftriaxone. The three-dimensional configurations of ligands display several interactions, notably hydrogen bonding, in addition to van der Waals and pi-alkyl interactions. The binding affinities of both compounds were comparable, with a predicted value of -6.9 kcal/mol for ceftriaxone. The results of experiments further corroborate the evidence that both molecules demonstrate antibacterial action. As shown in (figures 4 and 5).

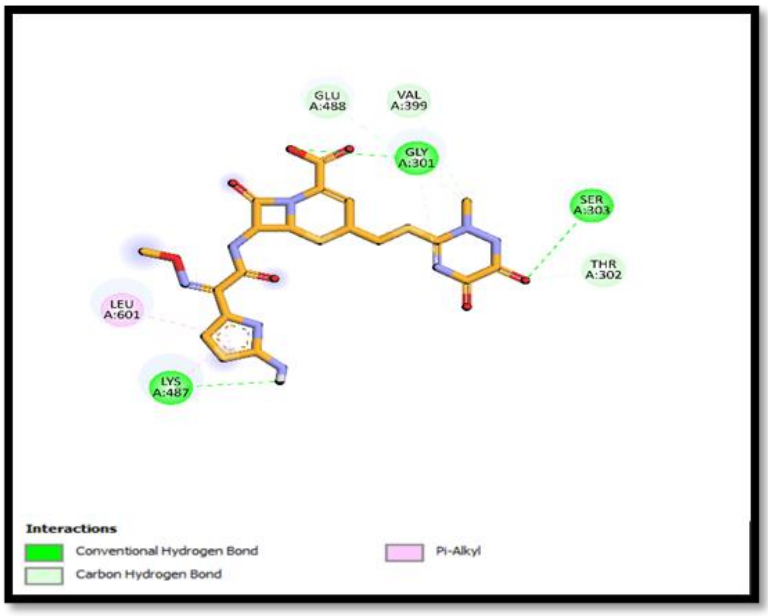


FIGURE 4. - 2D Docking model of ceftriaxone with enzyme GP6 synthase

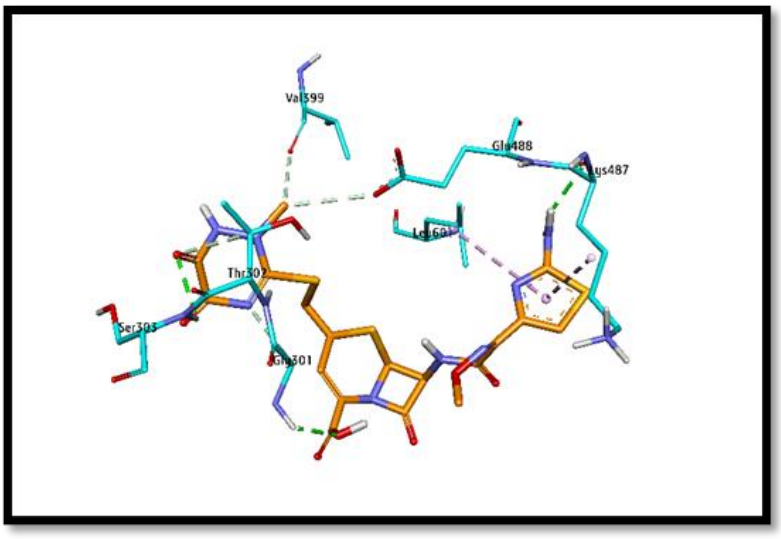


FIGURE 5. - 3D Docking model of ceftriaxone with enzyme GP6 synthase

S (Kcal/mol)	RMSD (Å)
-7.7943	1.9289

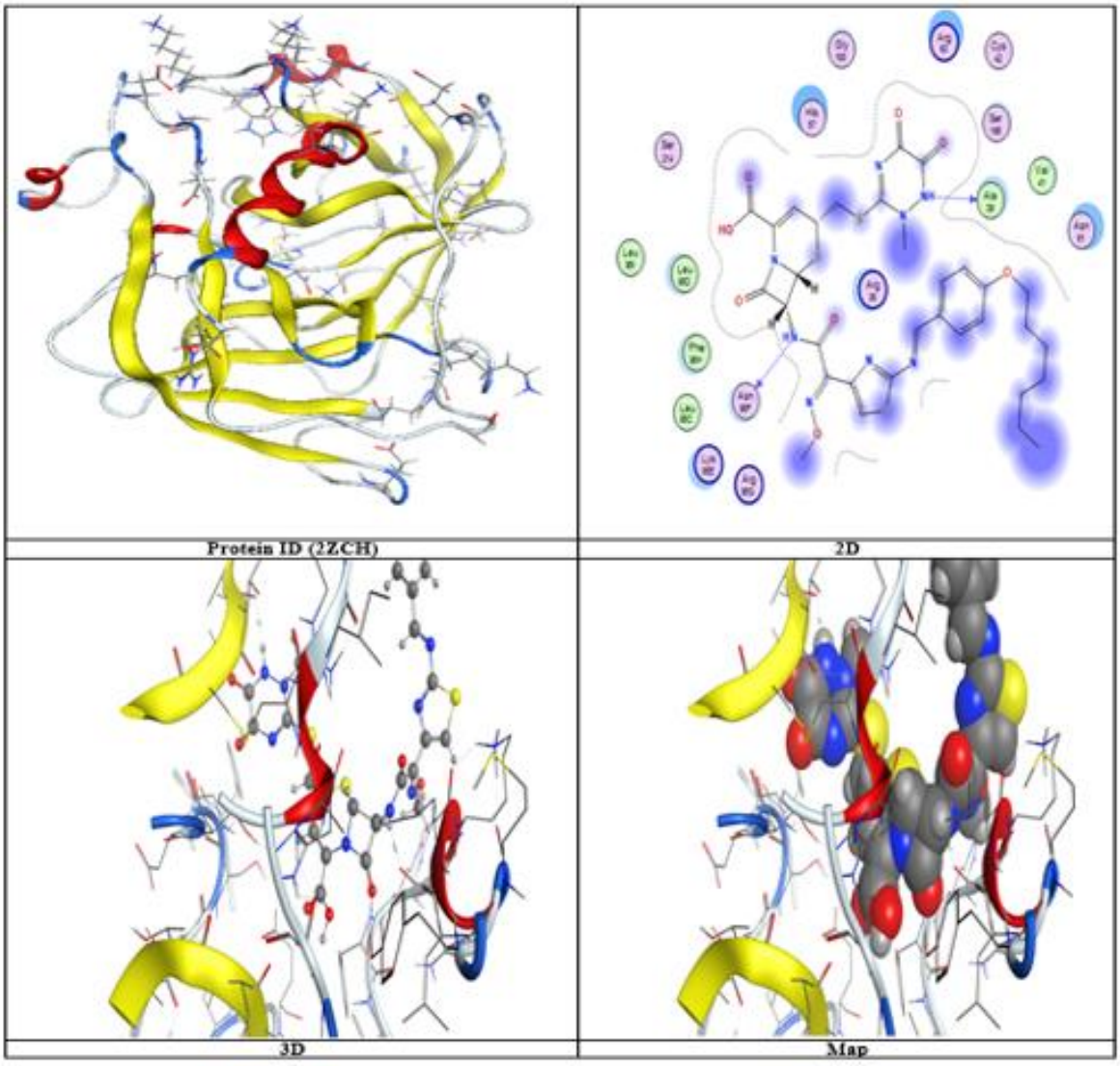
Ligand Interactions Report

Sat May 04 14:07:19 2024 (MOE 2015.10)

2ZCH: IMMUNE SYSTEM / 2ZCH

Ligand		Receptor				Interaction	Distance	E (kcal/mol)
N	15	O	ASN	95	(P)	H-donor	3.11	-2.8
N	39	O	ALA	39	(P)	H-donor	3.01	-2.3

Interactions



Protein ID (2ZCH) | 2D

3D | Map

FIGURE 6. - 2D, 3D, and map docking interactions of the studied Schiff base (B) synthesized with 3SHS protein of E.coli bacteria

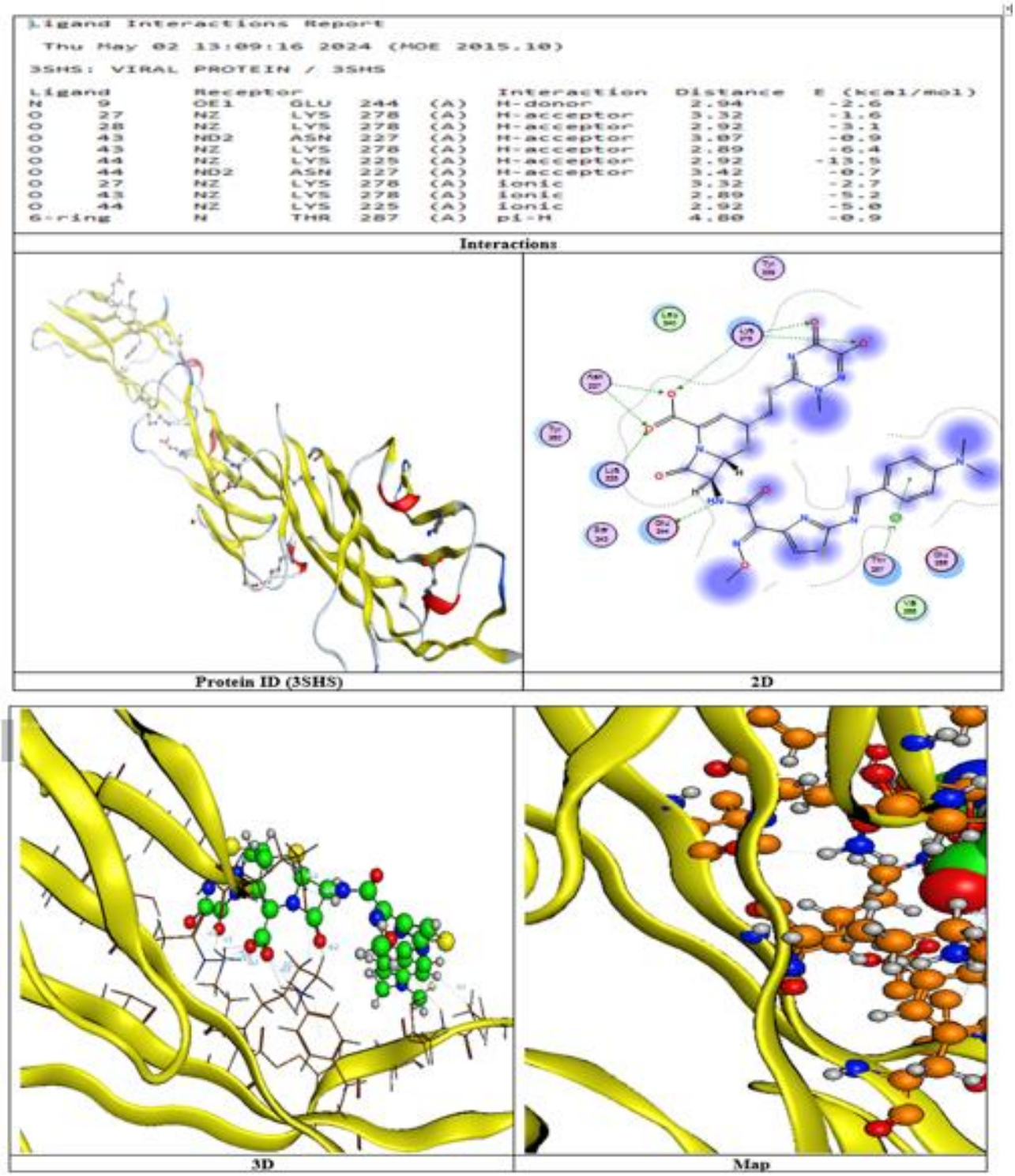


FIGURE 7. - 2D, 3D, and map docking interactions of the studied Schiff base (A) synthesized with 3SHS protein of E. coli bacteria

S (Kcal/mol)	RMSD (Å)
-7.3735	1.1423

Ligand Interactions Report

Sat May 04 17:14:35 2024 (MOE 2015.10)

4AG9: TRANSFERASE / 4AG9

Ligand		Receptor				Interaction	Distance	E (kcal/mol)
O	13	NZ	LYS	123	(A)	H-acceptor	3.03	-1.3
O	28	N	ILE	137	(A)	H-acceptor	3.45	-1.5

Interactions

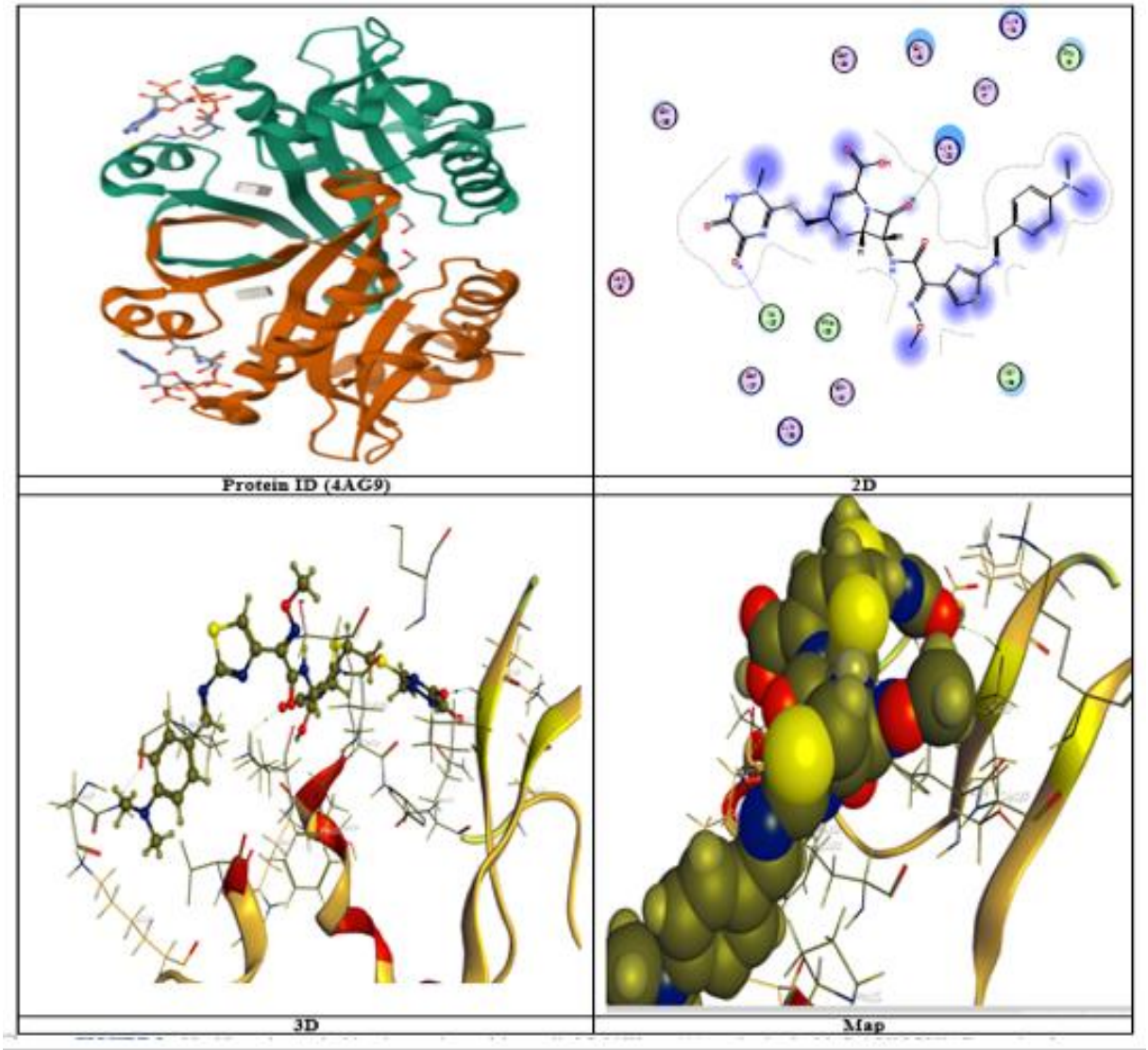


FIGURE 8. - 2D, 3D, and map docking interactions of the studied Schiff base (A) synthesized with G-6 PHOSPHAT protein of E. coli bacteria

3.3 BIOLOGICAL ACTIVITY

The derivative A has a more significant effect on the zone inhibition of growth bacteria in petri dishes. The most crucial effect on bacteria was on E. Coli by derivative B. The less impact on bacteria was on S. aureus by derivative B, as listed in Table 1. The groups of two-methyl and a lone pair of electrons on the nitrogen atom increase derivative A more than derivative B, which contains a chain of epoxy groups in its structure. As shown in docking analysis, the derivative's effect on G-6 Phosphate protein and 3SHS protein in E. Coli bacteria.

Table 1. - Anti-bacterial activities for Schiff base derivatives (A and B)

Derivative	Zone inhibition (mm)			
	Bacillus subtilis	Streptococcus penumonia	E. coli	Bacillus subtilis
A	17	14	23	11
B	15	11	9	13
Ligand	17	15	19	11

The most significant effect on fungi was on Candida by derivative A. Derivate A has more biological activity than derivate B, which has more activity than derivative B, as shown in Table 2.

Table 2. - Activities as anti-fungal derivatives (A and B)

Derivatives	Inhibition of zone (mm)	
	Candida fungal	Aspergillus nigaer fungal
A	19	13
B	12	14

3.4 VIABILITY ASSAY MTT

The MTT assay is a prevalent colorimetric technique employed to evaluate cell viability and cytotoxicity in PC3 prostate cancer cells, relying on mitochondrial activity. In this assay, MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), a yellow tetrazolium dye, is reduced by metabolically active cells to form formazan, an insoluble purple crystal. The PC3 cells are generally inoculated in 96-well plates, permitted to adhere, and subsequently exposed to test compounds for 24 to 72 hours. Absorbance is subsequently quantified at 570 nm, with increased absorbance signifying enhanced cell viability. This method of assay is frequently employed in drug screening, cytotoxicity assessment, and cancer research to investigate the impact of diverse compounds on PC3 cell proliferation and apoptosis. Using the MTT technique, the Schiff base derivative (A and B) was cytotoxic to the cell lines PC3. After 24 and 48 hours, the cell viability was evaluated and treated with various doses of each produced derivative [0 to 320 g/ml]. Figure 9 shows the outcome of Schiff base derivative (A) (11.3886 – 100) at 24 hours and (4.0412 – 100) at 48 hours. The research results suggested the effect on the PC3 cell line was sensitive to the dosage. These compounds were chosen based on their inhibitory efficacy against the Human androgen receptor (PDB ID: 1E3G) and may be regarded as the most promising candidates for anti-cancer medicines.

The impact of Schiff base derivative (A) on the vitality of PC3 cells was evaluated, as seen in Figure 9; after 48 hours, an increasing concentration (PPM) reduced PC3 viability percentage by more than 24 hours.

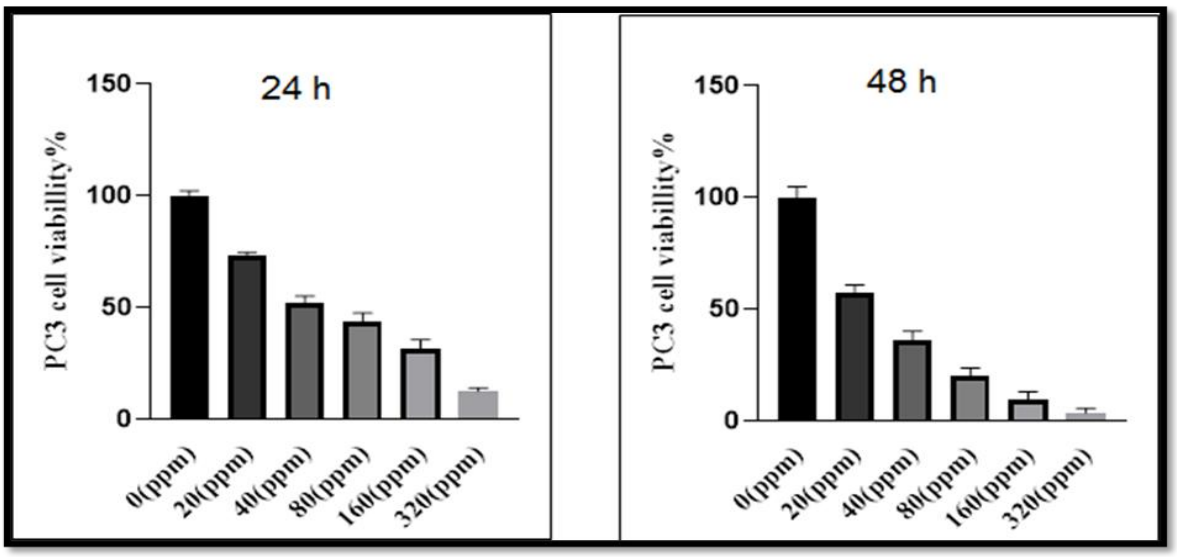


FIGURE 9. - Azomethine compounds (A) effects on PC3 cell lin

Table 3. - IC50 rates of Schiff base compounds (A) induced PC-3 cell

Concentration	At 24 hours		At 48 hours	
	Mean and SD		Mean and SD	
0 ppm	100	2.897928	100	3.764662
20 ppm	70.8652	3.183313	55.4532	2.420856
40 ppm	53.8991	3.167733	38.0013	2.464329
80 ppm	41.0873	2.785346	21.1536	2.238765
160 ppm	34.0136	2.855375	11.2352	1.644538
320 ppm	11.3886	1.523526	4.0412	1.865872

The analysis of Schiff base derivative (B) shown in Figure 10 indicates that after 48 hours, a rising concentration (PPM) resulted in a greater reduction in PC3 viability percentage compared to 24 hours. Nonetheless, the cell viability at 24 hours is 10.4391, exceeding 3.0086 in 48 hours, as seen in Figure 10. All findings acquired are shown in Table 4.

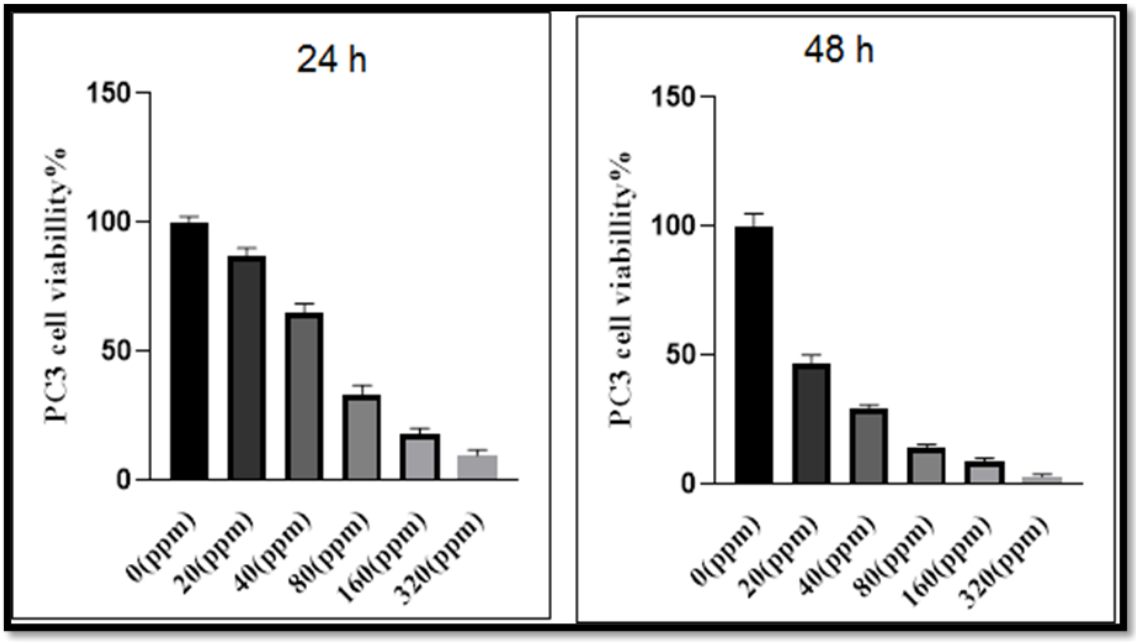


FIGURE 10. - Azomethine compounds (B) effects on PC3 cell line

Table 4. - IC50 rates of Schiff base derivative (B) induced PC-3 cell

Concentration	At 24 h		At 48 h	
	Mean and SD		Mean and SD	
0 ppm	100	2.890620	100	3.798892
20 ppm	86.5355	3.245234	43.8819	3.018624
40 ppm	62.7321	3.234538	33.0684	2.169924
80 ppm	35.0876	2.463669	14.2491	2.268812
160 ppm	16.5678	2.003452	10.6551	1.975439
320 ppm	10.4391	1.865932	3.0086	1.198596

4. CONCLUSION

These derivatives of azomethine synthesis can be done in one step and in less time with a good percentage of the product. The derivatives synthesized A have more biological activity than derivative B as antibacterial, but derivative B has less activity than derivative A. We will synthesize new derivatives in the future. As shown in docking analysis, the derivative's effect on G-6 Phosphate protein and 3SHS protein in bacteria affects the role in microbial cell wall synthesis.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

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