

Original Research Article

Preparation of new Schiff base derivatives from chitosan grafted with polyvinylpyrrolidone and study of their antimicrobial activity

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Abstract

Purpose: To synthesize and characterize novel Schiff base-chitosan composites and determine their antimicrobial activity.

Methods: Novel Schiff-base chitosan composites were synthesized by grafting polyvinylpyrrolidone and then replacing it with different aldehydes. The resulting composites were characterized using advanced analytical techniques, including thermogravimetric analysis (TGA), x-ray diffraction (XRD) studies and Fourier transform infrared spectroscopy (FT-IR).

Results: The FT-IR results revealed that the Schiff-base chitosan was successfully produced during mixing and the crystallinity change of the samples was explained by the XRD patterns. Thermogravimetric analysis (TGA) of compounds A1, A3 and A4 showed weight loss of the compounds with the most significant weight loss observed in the fourth and final steps of the reaction. Analysis of the surface morphology of compounds A3, A5, and A6 by scanning electron microscopy (SEM), revealed multiple layers of sheets in the dark gray sections, as well as kinked and wrinkled portions. Compounds A2 – A6 exhibited biological activities against Gram-positive and Gram-negative bacteria as well as fungal species. Docking studies indicate that compound A6, with a binding affinity of -3.09, demonstrates antibacterial properties. Conversely, compound A2, with a binding affinity of -9.102, exhibited antifungal activity.

Conclusion: The synthesized compounds possess higher antifungal potential due to the presence of benzene ring which enhances the binding affinity for antifungal activity while diminishing their antibacterial activity.

Keywords: Chitosan, Molecular docking, Polymer, Polyvinylpyrrolidone, Scanning electron microscopy (SEM), Thermogravimetry

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INTRODUCTION

Polymers are used in pharmaceuticals as binders in tablets and as agents that control flow and viscosity in liquids, suspensions and emulsions.

They are also applied as film coatings to improve the flavor of drug or to cover up its disagreeable taste. In addition, pharmaceutical polymers are frequently used to improve stability, bioavailability and controlled release (such as

prolonged, pulsatile and targeted) in addition to flavor masking. Drugs that are distributed within a polymer matrix and released by diffusion are known as monolithic delivery devices. The initial drug concentration and relaxation of the polymer chains, which together exhibit a sustained release characteristic, determine the rate of drug release from a matrix product [1].

Schiff bases represent a vast group of compounds containing a double bond linking carbon and nitrogen atoms and they have several applications due to their antiviral, antibacterial, anticancer, antioxidant and medicinal properties [2]. As a result of their stability, chelating properties, simplicity of synthesis, and wide variety of biological applications, Schiff base compounds are a significant and well-researched class [3]. One of the most used biopolymers as immobilization carriers is chitosan, a poly-N-acetyl glucosamine which is an N-deacetylated derivative of chitin, the primary component of crab shells. Chitin is the second most prevalent biopolymer after cellulose [4] and recent studies have identified chitosan as a flexible raw material for the synthesis and production of numerous goods, having uses in the food, pharmaceutical, medical and healthcare industries, and protection against pollution in the environment, industry and agriculture. Interestingly, this compound is famous for its gel-producing, polyelectrolyte, metal ions neutralizing, antibacterial, and antioxidant properties as well as its ability to be readily processed. These functional properties are due to the presence of reactive hydroxyl and amino groups in chitosan [5]. Chitosan dissolves partially in diluted organic acids like acetic acid (HAc), formic acid and phosphoric acid (H_2PO_4), and partially in diluted mineral acids like nitric acid (HNO_3), hydrochloric acid (HCl), and phosphoric acid (H_2PO_4) [6].

Polyvinylpyrrolidone (PVP), a water-soluble polymer first used as a shellac resin in hair fixative agents, has found applications in the food, pharmaceutical, biomedical, cosmetics and plastics industries. Due to its special chemistry and features, advances have been made in its synthesis to produce a variety of PVP types, including copolymers, crosslinked PVP and homopolymers with varying molecular weights [7]. Therefore, the objective of this study is to synthesize low molecular weight polymers with known bioactive properties using the ring-opening polymerization of polyvinylpyrrolidone with chitosan. Low molecular weight polymers provide a number of benefits, such as reduced toxicity, increased specificity of action, and the

ability to dissolve in water, making them potentially useful therapeutic agents.

EXPERIMENTAL

Preparation of chitosan grafted with polyvinylpyrrolidone (A1)

Chitosan (1 g, 0.006 mol) was dissolved in 10 mL of an aqueous solution of sulfuric acid (2 M) in a round-bottom flask equipped with a reflux condenser and magnetic stirrer. The mixture was heated for 10 h at 70 °C. The white lumpy material polymer (the resulting material) was dissolved in 10 mL of dimethylformamide (DMF) and drops of NaOH (0.5 M) were added. Thereafter, 0.68 g (0.006 mol) of PVP, dissolved in DMF, was added to the mixture and heated for 10 h at 70 °C. The resulting compound was purified with diethyl ether. Sodium carbonate (10 M) was added to the mixture until it reached a pH of 7.0 [8].

Preparation of compound A2

One gram (1 g) of A1 was dispersed in 10 mL of ethanol and placed in a round-bottom flask provided with condenser and magnetic stirrer. The resulting solution was mixed with 4-chlorobenzaldehyde dissolved in absolute ethanol, few drops of glacial acetic acid were added and the mixture was refluxed for 10 h at 70 °C [9]. The resulting compound (A2), with a yield of 84 %, was purified with (10 mL) of diethyl ether. The same procedure was used to prepare different compounds from the reaction of compound (A1) with 4-nitrobenzaldehyde (A3), 4-hydroxy benzaldehyde (A4), Glutaraldehyde (A5), and Formaldehyde (A6) [10].

Biological efficacy

Muller Hinton agar (38 g in 1L distilled water) was completely dissolved by stirring over a hot plate device and then autoclaved at 121 °C and 15 atmospheric pressure. When slightly cooled, the slurry was aseptically transferred into Petri dishes and allowed to solidify. Once the prepared isolates were added to the dishes, they were distributed evenly across the medium's surface with a sterile spreader and left to dry overnight. The polymer was added at several doses (ppm) and incubated for 24 h at 37 °C. The diameter of inhibition was determined in millimeters using a ruler to record the results [11].

Molecular docking

The best binding pose was identified by sampling different ligand conformations and orientations.

After the compounds were prepared and grid generated employed, the docking process began. Synthesized compounds were docked into the active sites for fungi target (PDB code: 1IYK) and for bacteria target (PDB code: 6TOP). Binding rates and interactions were determined to ascertain possible leads by adding hydrogen atoms to the receptor structures, removing water molecules that are more than 5 Å from the hit group, and figuring out bonding orders and charges, which are steps in the protein manufacturing process. Following construction, the protein structures were reduced using the OPLS_2005 force field with a root mean square deviation (RMSD) of 0.30 adopted. The receptor grids were created using the Maestro Receptor Grid Generation tool, and the default parameters were kept. Finally, the built-up ligand and receptor grids were moved to the Glide module of the Maestro-Schrodinger molecular modeling program (version 2023-1). Molecular docking investigations were then performed using the extra-precision Glide docking method. The exact position and alignment of the produced molecules upon attachment to an enzyme or protein receptor were predicted using the Maestro software (version 12.5). The methodology used in this study is summarized in Figure 1 [12,13].

X-ray diffraction studies (XRD)

To verify if the medication was crystalline or amorphous, an x-ray diffractometer (XRD-6000, Shimadzu, Japan) was used to determine the x-ray diffractograms of SEM and the chosen

microcrystal formulae. The continuous scan range was between 10 and 80° (2 θ) with the operating voltage and current at 30 mA and 40 kV, respectively [14].

Scanning Electron Microscopy (SEM)

Three (resulting material) samples were examined using a scanning electron microscope (JEOL-JSM6460LV) attached to an INCA Micro-analyser system on a back-scattered electron (BSE) mode to gain insight into the prepared compounds and porosity values. The samples were analyzed using the Back-scatter Electron Image (BSEI) with some use of secondary electron image (SEI). The SEM and BSE images were analysed using the open-source software package, ImageJ [15].

Fourier Transform Infrared (FT-IR) Spectra

The synthesized compounds were analyzed using FT-IR spectroscopy to examine the structural characteristics of the chemical bonds (functional groups) separating the molecules. A Shimadzu FT-IR 8400S (Japan) with a wave number range of (400 – 4000 cm⁻¹) was used to determine this FT-IR. Samples were made by combining 1 % of compounds with 99 % KBr that had been compressed into a disk [16].

Proton-Nuclear magnetic resonance (¹H-NMR)

¹H-NMR spectra of some polymers were obtained using DMSO-d₆ as a solvent.

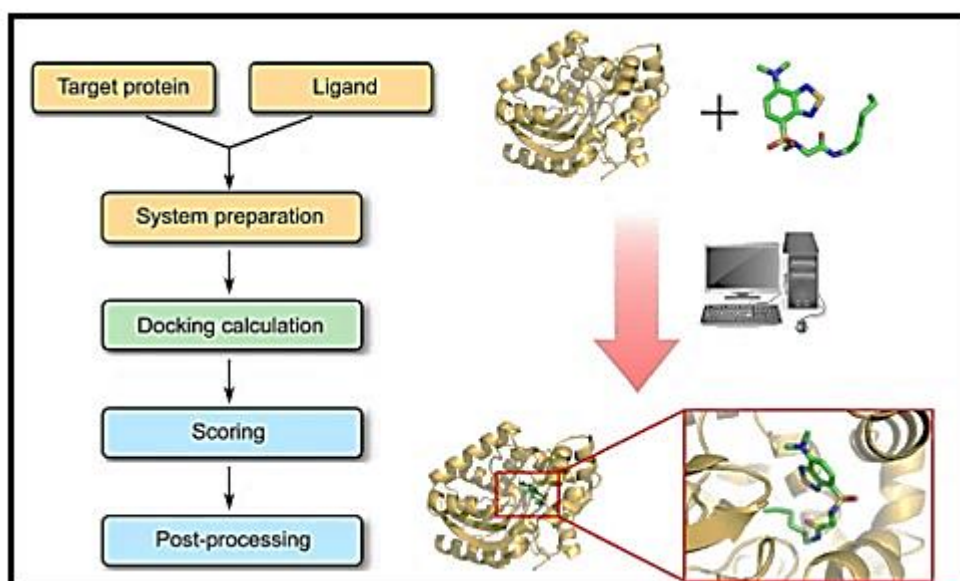


Figure 1: Docking methodology in Schrodinger software

RESULTS

Preparation of novel Schiff base-chitosan composites

Figure 2 depicts the reaction mechanism of five new poly Schiff compounds prepared by modification of PVP with chitosan. The carbonyl group in "Polyvinylpyrrolidone" reacted with chitosan by nucleophilic attacking one mole of hydroxyl group on chitosan.

Fourier transform infrared (FT-IR) Spectra

All of the polymers made by Schiff base had FT-IR spectra that revealed several absorption bands representing the stretching and bending vibrations of various groups. The results are shown in Table 1, and the spectra patterns in Figure 3.

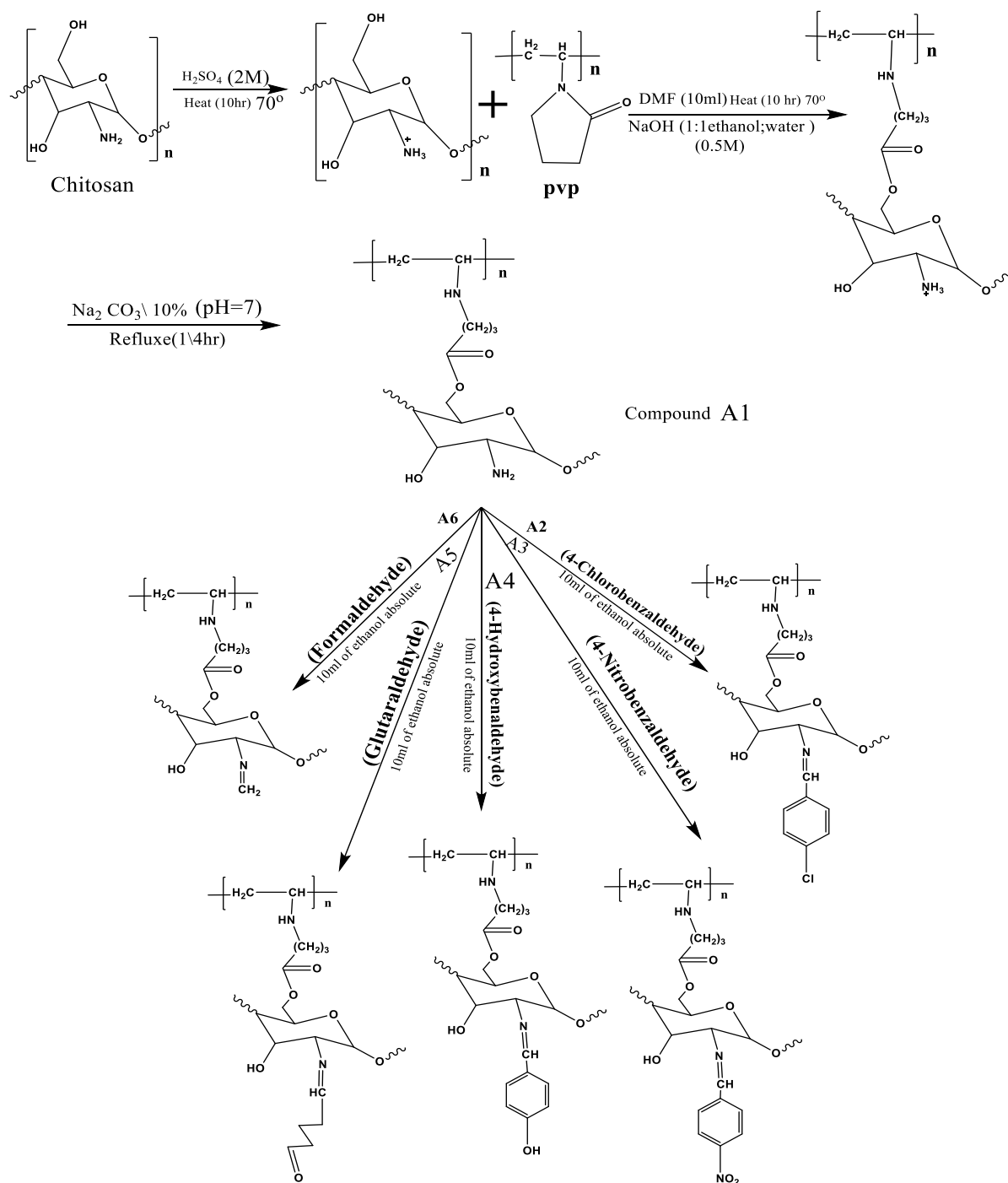


Figure 2: Synthesis of new Schiff bases from chitosan grafted with PVP (A1 - A6)

Table 1: Fourier Transform Infrared (FT-IR) Spectra of compounds (A2-A6)

Compound	$\nu(\text{N-H})$ cm^{-1}	$\nu(\text{C-H})$ cm^{-1} (aliphatic)	$\nu(\text{C=O})$ cm^{-1} ester	$\nu(\text{C=N})$ cm^{-1}	$\nu(\text{C-N})$ cm^{-1}	$\nu(\text{C-O})$ cm^{-1}
A2	3435	2958-2824	1714	1593	1462	1382
A3	3442	2930-2846	1705	1649	1419	1323
A4	3410	2955-2840	1715	1637	1460	1288
A5	3442	2945-2867	1699	1651	1467	1292
A6	3427	2933-2870	-	1645	1463	1292

Proton-nuclear magnetic resonance ($^1\text{H-NMR}$) data

The $^1\text{H-NMR}$ spectra of the synthesized polymers are displayed in Figure 4. Compound (A2) shows the following peaks: δ 1.58 – 2.25 ppm ($\text{CH}_2\text{-CO}$, 4H, t), δ 2.5 ppm ($\text{CH}_2\text{-CH}$ 2H, s), δ 2.75 – 3.18 ppm (CH-NH -3H,t), δ 3.2 – 4.1 ppm (NH, 1H,s), δ 7.95 – 8.37 ppm (CH arom, 6H, m) while polymer (A3) showed the following peaks in the $^1\text{H-NMR}$ spectrum viz δ 1.60-1.85 ppm ($\text{CH}_2\text{-CO}$, 4H, m), δ 2.02 – 2.13ppm ($\text{CH}_2\text{-O}$, 2H, t), δ 4.38 ppm (NH, 2H, s), δ 8.13 8.26 ppm (CH arom, 4H, m), δ 10 ppm (Ar-OH, 1H, s). On the other hand, compound A4 showed the following peaks in the $^1\text{H-NMR}$ spectrum as follows δ 1.83 ppm ($\text{CH}_2\text{-CO}$, 4H, s), δ 2.43 ppm ($\text{CH}_2\text{-O}$, 2H, t), δ 3.51-4.68 ppm (NH, 4H, s), 6.92 – 6.99 ppm (CH=N , 2H, t), δ 7.67 – 7.75ppm (CH arom, 4H, m), δ 9.69 – 10 ppm (Ar-OH, 1H, s).

Thermal properties of synthesized polymers

Stages of weight loss were identified by thermogravimetric examination of compounds A1, A3 and A4 as follows: In the first stage, the compounds showed a decrease in weight due to the evaporation of water contained in the form of moisture. In the second stage, weight loss was thought to be caused by the breakdown of chitosan bonds with benzaldehyde and partial benzaldehyde decomposition. This stage thus represents the percentage of reacted benzaldehyde because the percentage of weight loss was quite low. In addition, weight loss in the third stage was related to the breakdown of the bonds that produced chitosan and the structure of the remaining benzaldehyde. Finally, the most significant weight loss was seen in the fourth and final steps. Loss of weight in these steps indicated chitosan is still degrading and that carbon dioxide is still being emitted. The remaining weight percentage comprised carbon waste (Table 2).

Table 2: Thermogravimetric analysis (TGA) for A1, A3, A4

Code no.	Temperature ($^{\circ}\text{C}$)	Weight loss (%)
A1	100	21.03
	155	9.869
	210	10.28
	308	32.33
	600	25.15
A3	78	18.35
	150	10.40
	200	4.437
	315	12.98
	450	14.35
A4	600	6.396
	110	13.36
	175	4.359
	250	11.33
	314	10.66
	440	14.66
	600	7.838

X-ray diffractograms (XRD)

The XRD patterns of the samples, which were generated by the hydrothermal method and subsequently processed in a conventional oven, are displayed in Figure 5.

Biological activities of the compounds

Antibacterial and antifungal activities of the synthesized compounds were evaluated against Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus feacalis*), Gram-negative bacteria (*Escherichia coli*, *Acinetobacter baumannii*) and fungi (*Candida albicans*, *Candida tropocalis*) in different concentrations. The results are shown in Table 3.

Molecular docking studies of anti-bacterial and anti-fungal activities

The docking results of antibacterial activity showed that compound A6 (Figure 7) had the highest docking score of the synthesized compounds. On the other hand, docking results of antifungal activity showed that compound A2 (Figure 8) exhibited the highest docking score of synthesized compounds with comparable values to the reference native compound (Table 4).

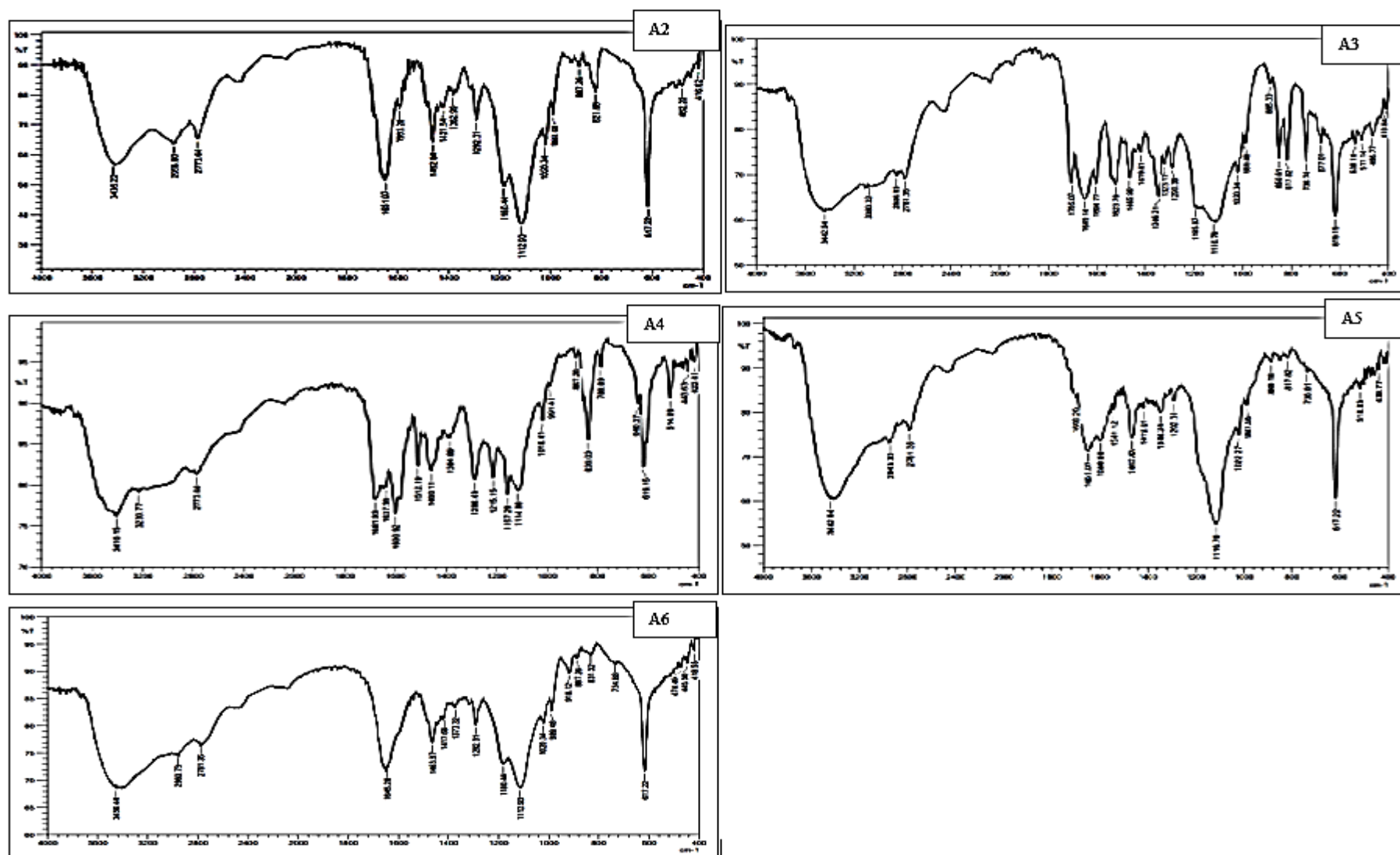
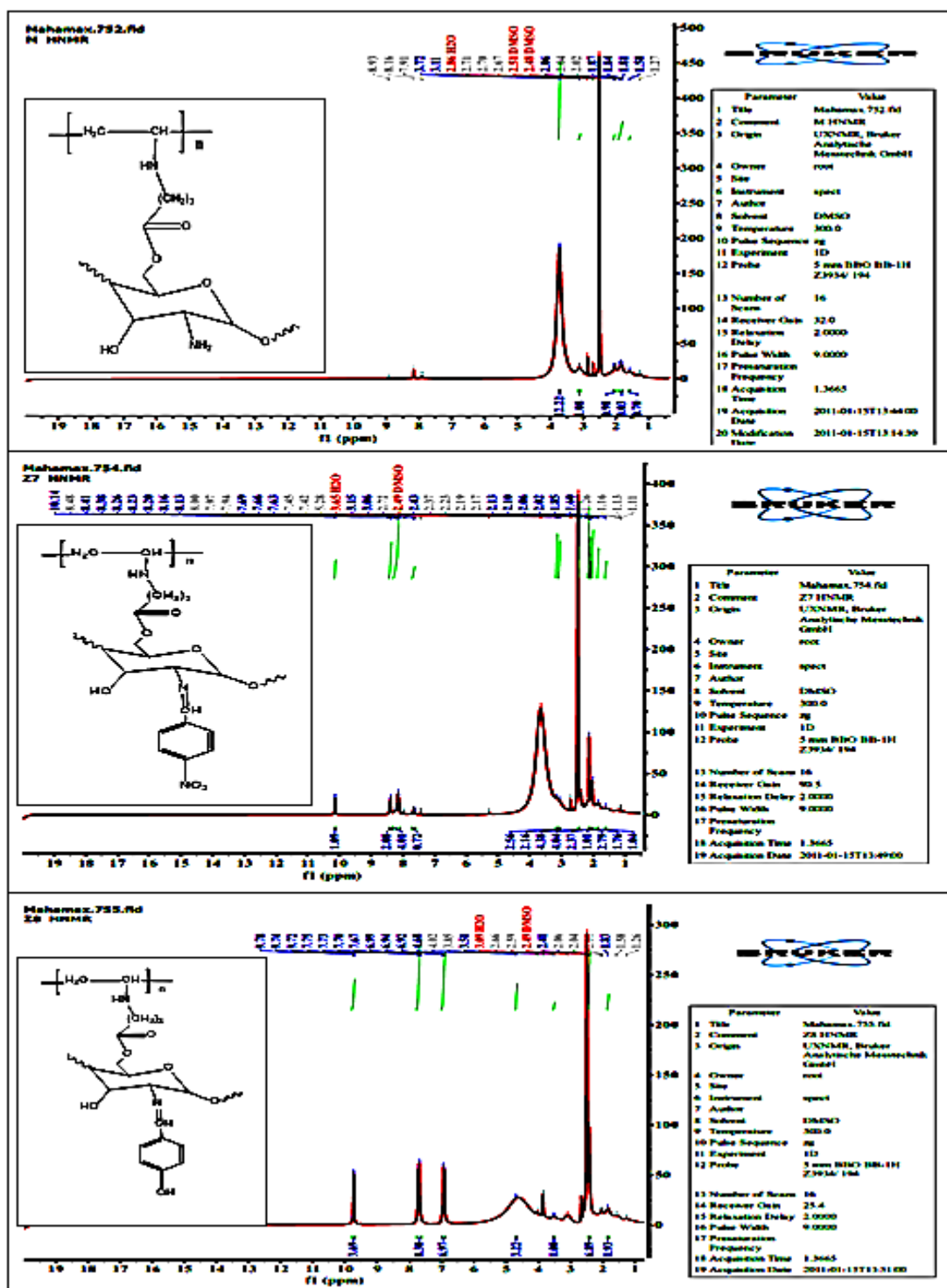


Figure 3: Fourier Transform Infrared (FT-IR) spectra of compounds A2 – A6



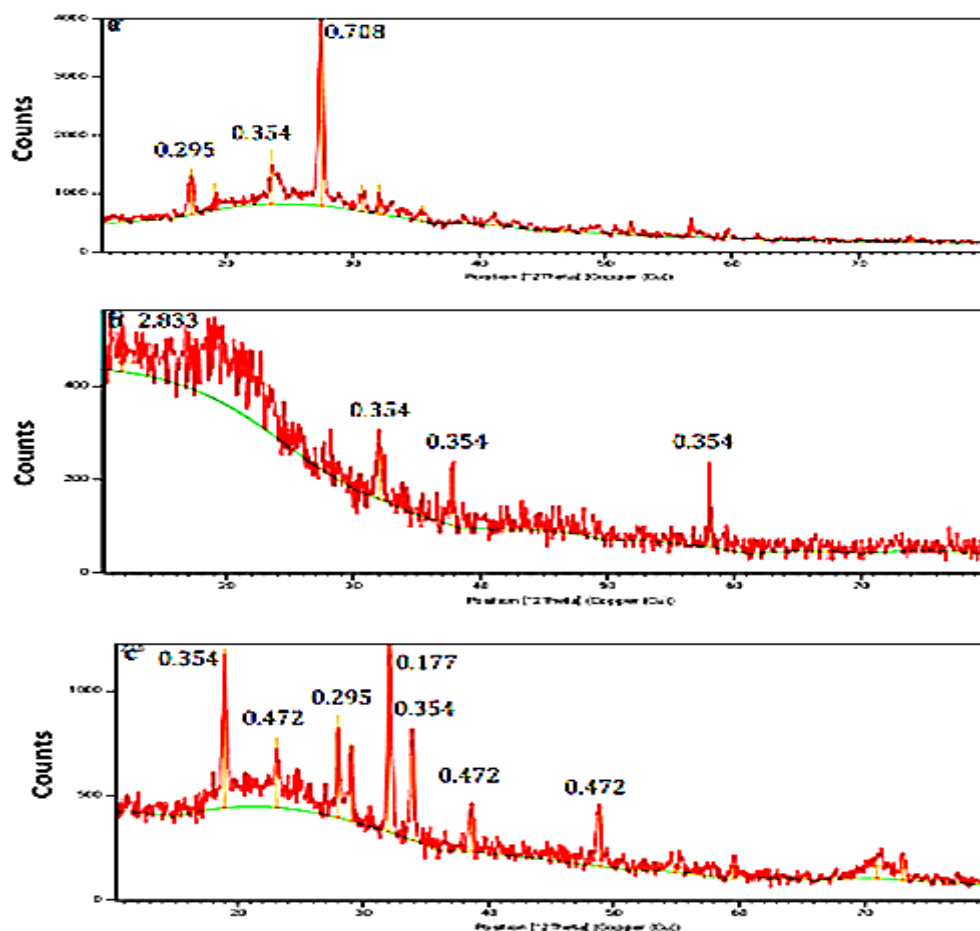


Figure 5: (a) X-ray diffraction patterns of Compound (A3), (b) X-ray diffraction patterns of Compound (A5), (c) X-ray diffraction patterns of Compound (A6)

Scanning Electron Microscopy (SEM)

The surface morphology of A3, A5, and A6 is shown in Figure 6. The majority of these sheets are reduced in size to include particles that have flat surfaces, sharp edges, and spherical or

semispherical shapes. There are multiple layers of sheets in the dark gray sections, as well as kinked and wrinkled portions. The compounds are evidently nano-sized and are effective against bacteria and fungi and also in the molecular docking study.

Table 3: Anti-bacterial and anti-fungal effectiveness of compounds (A2 – A6)

Conc.	25%	50%	100%	25%	50%	100%	25%	50%	100%
No.	<i>Staphylococcus aureus</i>			<i>Escherichia coli</i>			<i>Candida albicans</i>		
A2	11	14	17	12	15	20	13	15	20
A3	10	16	21	18	20	23	17	20	25
A4	0	5	14	0	0	19	0	5	15
A6	22	25	26	0	2	12	22	26	30
	<i>Streptococcus faecalis</i>			<i>Acinetobacter Baumanii</i>			<i>Candida tropicalis</i>		
A5	10	10	16	16	17	21	10	10	15

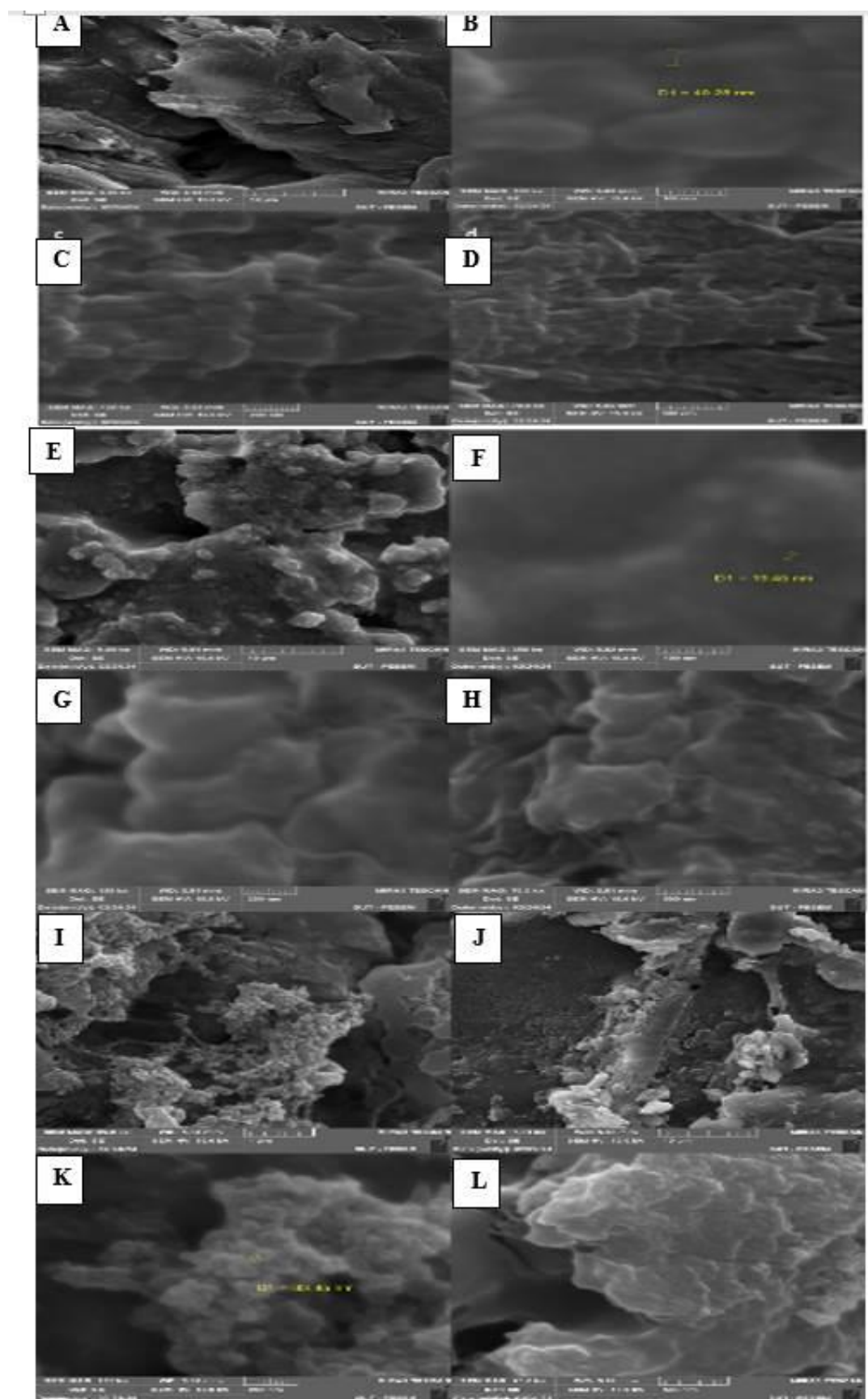
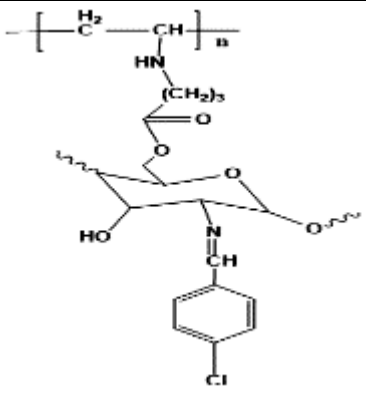
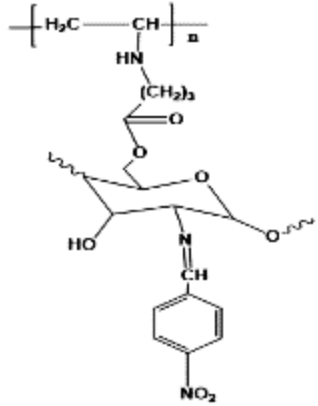
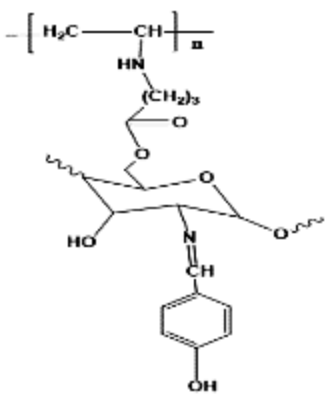
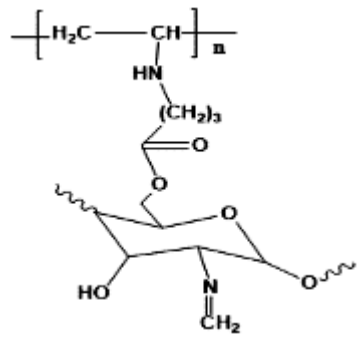


Figure 6: SEM of (A-D) Compound A3 (A)10 μm, (B)100 nm, (C) 200 nm (D)500 nm; (E-H) Compound A5 (E)10 μm, (F)100 nm, (G) 200 nm (H) 500 nm; (I – L) Compound A6 (I) 1 μm, (J) 10 μm, (K) 200 nm, (L) 500 nm

Table 4: Computational result of the targeted compounds against bacteria and fungi with native ligand (as reference for the computational run)

Name	Structure	Dock score interaction energy for bacteria	Dock score interaction energy for fungi
A2		-2.873	-9.102
A3		-2.888	-7.550
A4		-2.029	-7.509
A6		-3.099	-8.536

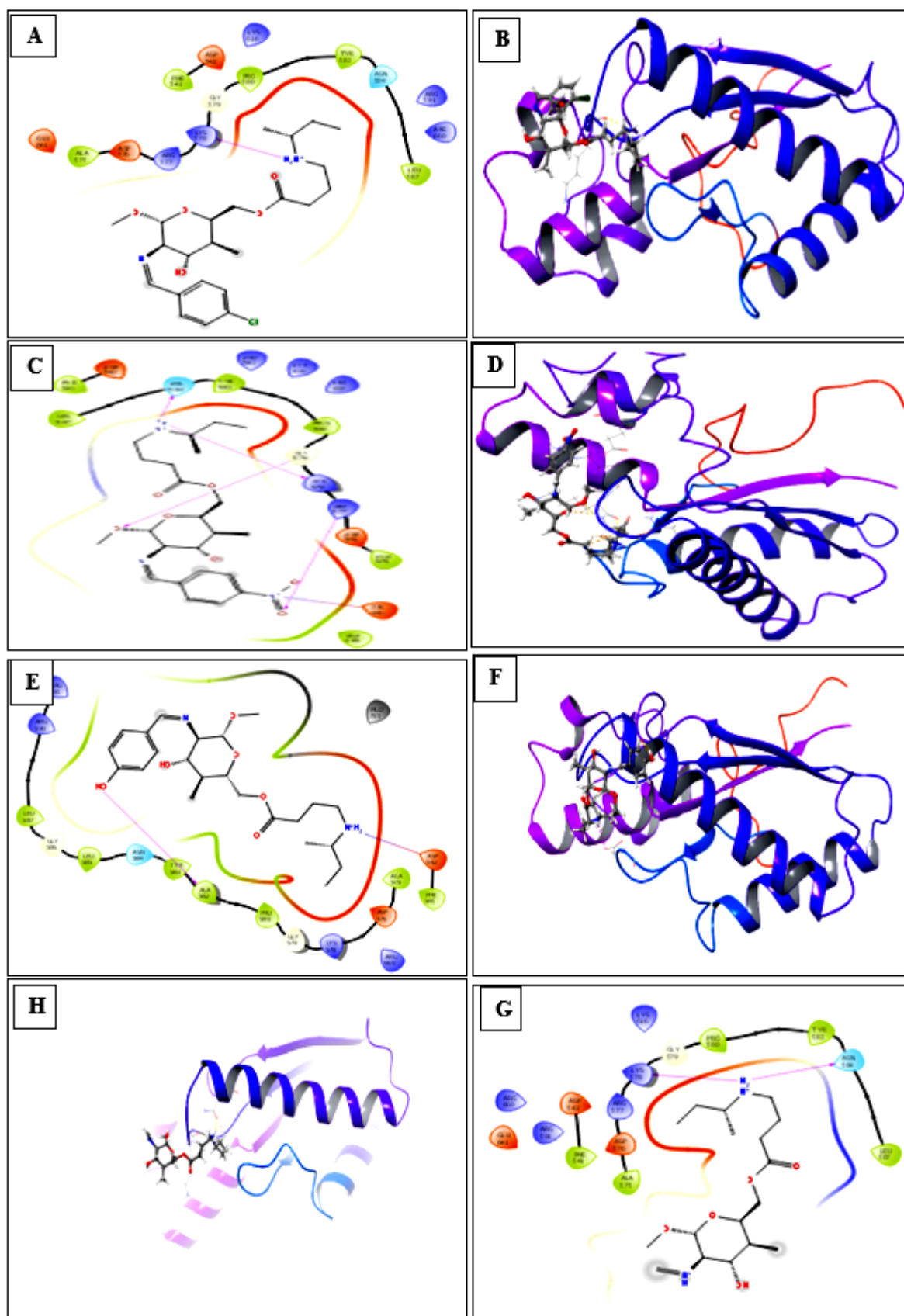


Figure 7: Docking results of antibacterial activity 2D and 3D of compounds A2 (A and B), A3 (C and D), A4 (E and F) and A6 (G and H)

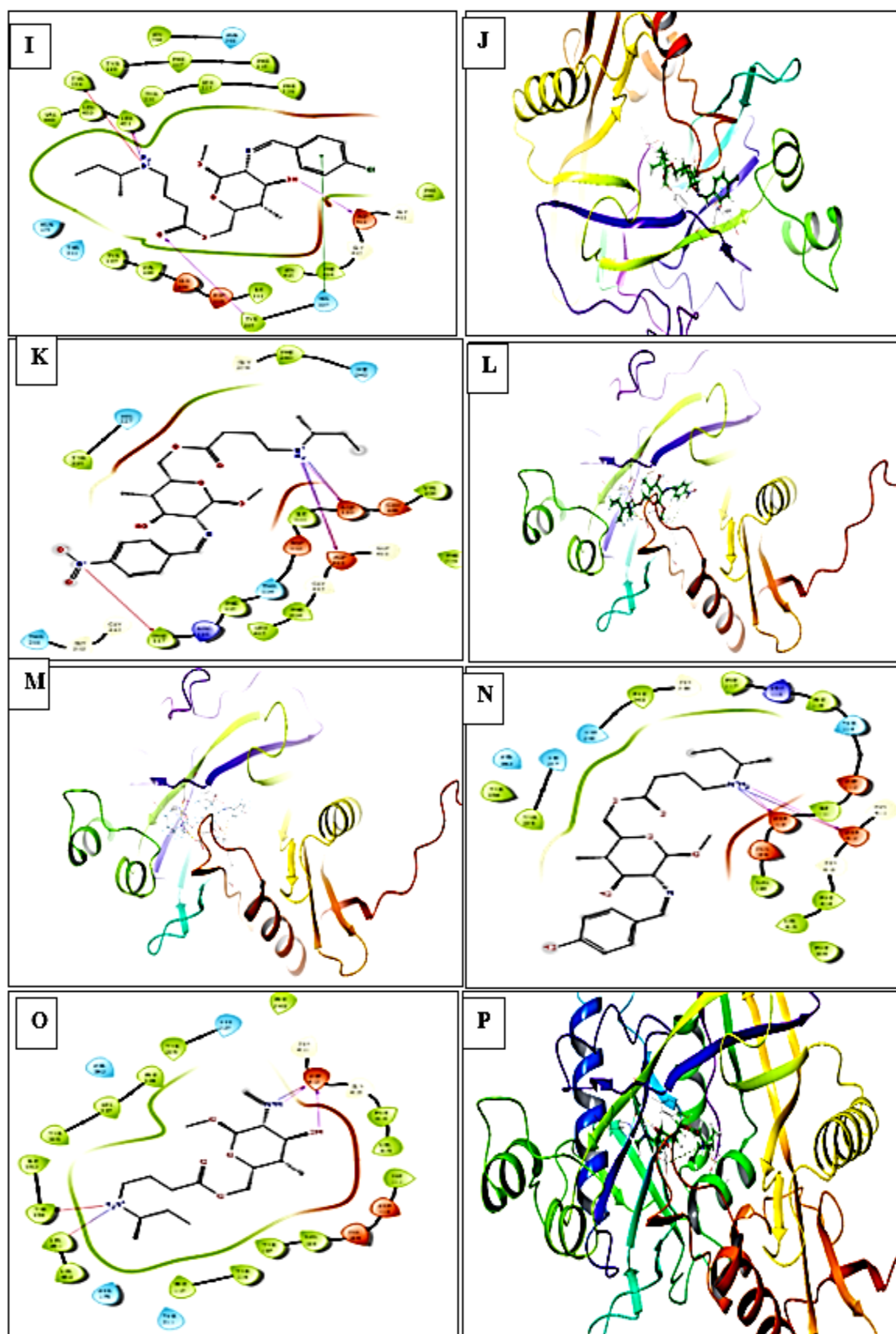


Figure 8: Docking results of antifungal activity 2D and 3D of compounds A2 (I and J), A3 (K and L), A4 (M and N) and A6 (O and P)

As presented in Table 4, compound A2 had the highest docking score of the designed compounds but less than the reference ligand 11YK. By using the view interactions tool within Maestro 12.5, many interactions were performed by compound A2.

DISCUSSION

In the past, the only method to ascertain a drug's possible activity was by performing wet lab experiments. With recent advances in Pharmacology and Molecular Biology, it is now feasible to determine how medications relate to the main biological targets of human diseases *in silico*. One of the bottlenecks in the drug-making process is the identification and confirmation of targets, which is the initial stage in contemporary drug development. Determining possible targets for therapeutic action is also crucial for assessing the safety of new drug compounds and clarifying their modes of action [17,18].

Results of this study revealed that compound A2 had a docking score of -2.873 of the designed compounds but less than the reference ligand 6TOP. By using the 'view interactions' tool within Maestro 12.5, only one interaction was performed by compound A2. These interactions with the active site were H-bond interaction between amine and LYS 578 (distance of 1.78), pi-cation interaction between secondary amine and TYR 354 (distance of 5.60) and ionic bond interaction with LEU 451 (distance of 3.42), while the carbonyl group of esters made H-bond with TYR255 (distance of 2.01). Hydroxyl makes an H-bond with ASP 412 with a distance of 2.55 and the last interaction is pi-pi stacking between the benzene ring and HIS 227, with a distance of 4.76.

When these compounds were tested on bacteria and fungi, compounds A2, A3, A4, and A6 gave biological activity against the two types of bacteria and fungi. In addition, the determination of the inhibition zone showed that compound A6 expresses higher activity against *S. aureus* and *C. albicans*. Furthermore, compound (A5) also showed activity on *S. faecalis*, *A. beumanii* and *C. tropicalis*.

CONCLUSION

Docking studies indicate that compound A6, with a binding affinity of -3.09, demonstrates antibacterial properties. Conversely, compound A2, with a binding affinity of -9.102, exhibits antifungal activity. Additionally, the presence of a benzene ring enhances the binding affinity for antifungal activity while diminishing its

antibacterial activity. Consequently, the synthesized compounds possess higher antifungal potentials.

DECLARATIONS

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Ethical approval

Not required.

Use of Artificial intelligence/Large language models

We also declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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