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Synthesis and Biological Evaluation of New Imine- and Amino-Chitosan Derivatives

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Abstract: *N*-substituted chitosan derivatives were synthesized through condensation with a number of selected aryl and heteroaryl aldehydes. The synthesis of the amino-derivatives has been carried out by reductive amination with sodium borohydride as reducing agent. Their structures were characterized by (FT-IR, ^{1}HNMR, and XRD). The antimicrobial activity of Chitosan Schiff's base (CSB) derivatives were investigated against four types of bacteria and two crop-threatening pathogenic fungi, and the results indicated that the antibacterial and antifungal activities of the investigated derivatives are very promising. Additionally, different concentrations of the triazolo-Schiff's base derivative **3c** were used for cytotoxicity screening against Human Breast Adenocarcinoma Cells (MCF-7), Human Colon Carcinoma Cells (HCT-116), and Human Hepatocellular Liver Carcinoma Cells (HepG-2), and the obtained data revealed that the examined compounds have an excellent cell growth inhibitory effects on the cell lines as compared to standard.

Keywords: chitosan; imine; antimicrobial

1. Introduction

Natural polysaccharides such as chitosan (CS) comprise a class of very important polymers that have been widely utilized in a variety of fields [1]. The most important feature of chitosan is its low toxicity compared with other natural polysaccharides. It is safety in terms of inertness, and low or no toxicity has been demonstrated by *in vivo* toxicity studies, in which it's oral lethal dose 50 (LD_{50}) in mice was found to be in excess of 16 g/day/kg body weight, which is higher than that of sucrose [2,3]. Additionally, chitosan is well tolerated by living tissues, including the skin, ocular membranes, as well as the nasal epithelium. For these reasons, chitosan is very valuable for a wide range of biomedical applications [4–6].

Chitosan has a variety of applications in pharmaceutical, medicinal, and agricultural fields as well as wastewater treatment, food, cosmetics, and so on [7–10]. Also, being a natural polymer, chitosan can be used in nucleic acid delivery and tissue engineering applications. Chitosan is a biocompatible material that interacts with living cells without being cytotoxic [11]. Chitosan has various biological properties including antimicrobial properties [12], antioxidant properties [13], and anti-inflammatory properties [14]. Chitosan is also mucoadhesive, making it highly suitable for gene delivery to epithelium including the lungs and gastrointestinal tract [15–17]. Chitosan has found use in novel applications such as vaccine and peptide delivery, in addition to its

use in tissue engineering [2,6,18]. In fact, a number of commercial applications of chitosan benefit from its antimicrobial properties, including its use in food preservation [19,20], in dentistry and ophthalmology, in the manufacture of wound dressings, and antimicrobial finished textiles. Therefore, investigations of the. antimicrobial potential of chitosan and its derivatives has recently gained momentum. However, the unsatisfactory performance of naturally available polymers usually fails to meet the needs of different fields. In order to expand the range of applications, structure modification is considered to be the effective ways in improving the performance of natural polymers [21].

Accordingly, in this work we try to synthesize some new derivatives of chitosan by its reaction with a number of aromatic aldehydes and study their structures using different physical and chemical methods, as well as their antimicrobial and anticancer properties hoping to be more active.

2. Materials and Methods

2.1. Materials

Chitosan was purchased from Acros Organics, Morris Plains, NJ, USA. Its deacetylation degree is 88% and its average molecular weight is 100,000–300,000 Da. Acetic acid, methanol, were of analytical grade from Aldrich and were used as received. Dimethyl sulfoxide (DMSO), crystal violet and trypan blue dye were purchased from Sigma (St. Louis, MO, USA). Fetal Bovine serum, DMEM (Dulbecco Modified Eagle's Medium), RPMI-1640, HEPES buffer solution, L-glutamine, gentamycin, and 0.25% Trypsin-EDAT were purchased from Lonza (Basel, Switzerland). Crystal violet (1%) was composed of 0.5% (*w*/*v*) crystal violet and 50% methanol, then made up to volume dd H_2O and filtered through a whatmann No. 1 filter paper. Antimicrobial analysis and anti-cancer activity screening were done by the regional center for mycology and biotechnology, Al-Azhar University.

2.2. Characterization of Chitosan

Fourier transforms infrared spectroscopy (FT-IR) analysis: FT-IR spectra were recorded using KBr discs on Perkin Elmer- USA Spectrometer at room temperature within the wave number range of 4000–400 cm^{-1}; Proton Nuclear Magnetic Resonance (^{1}H NMR): ^{1}H NMR spectra were recorded using a Gemini-300 MHz instrument in DMSO–d6 as a solvent at 25 °C. Chemical shifts (δ) are expressed in part per million (ppm) using tetramethylsilane as an internal standard; X-ray diffraction (XRD) analysis: In X-ray diffraction technique (XRD), X-ray diffraction profiles of chitosan and chitosan derivatives were recorded by Bruker, Germany powder X-ray diffractometer, model D8 Advance, source 2.2 kW Cu anode. The relative intensities were recorded within the range of 10°–90° (2θ) at a scanning rate of $5^\circ \cdot min^{-1}$.

2.3. General Procedures for Chitosan Schiff-Base Synthesis

A solution of the aldehyde (20 mmol) in ethanol (20 mL) was added to chitosan (20 mmol) in 10% AcOH (50 mL). The mixture was stirred for 6–10 h at 70 °C, and then left overnight. After cooling, the homogenous hydrogels which formed were dried at 60 °C for dewatering to constant weight to give the product.

Chitosan furan Schiff-base (CFSB) **3a**. Obtained from ethyl 5-formyl-2-methylfuran-3-carboxylate **2a** in 91% yield as white powder; the mixture was stirred for 6 h; IR (KBr): 1642 (C=N), 1687 (COOEt), 3440 cm^{-1} (OH); Anal. Found: C, 52.18; H, 5.31; N, 4.13; O, 37.51.

Chitosan pyrrole Schiff-base (CPSB) **3b**. Obtained from 5-formyl-2-methyl-1*H*-pyrrole-3-carboxylate **2b** in 89.3% yield as white powder; The mixture was stirred for 8 h; IR (KBr): 1599 (C=N), 1655 (COOEt), 3322 cm^{-1} (OH); ^{1}H NMR (300 MHz, DMSO): δ = 1.93 (q, 2H, CH_2-ester; $J_{1,2}$ = 2.3 Hz, $J_{1,3}$ = 6.9 Hz), 2.38 (m, 1H, H-1′), 2.41(m, 2H, H-2′, H-3′), 2.61 (d, 2H, H-4′, H-5′; $J_{1,2}$ = 2.3 Hz), 2.75 (m, 2H, H-6a′, H-6b′), 3.17 (t, 3H, CH_3-ester; $J_{1,2}$ = 2.3 Hz, $J_{1,3}$ = 6.9 Hz), 3.25 (s, 3H, CH_3-pyrrole),

4.53 (bs, 2H, 3′-OH, 6′-OH; exchangeable with D_2O), 6.51 (s, 1H, CH=N), 6.55 (s, 1H, H-pyrrole), 10.33 (bs, 1H, NH; exchangeable with D_2O), Anal. Found: C, 52.84; H, 5.94; N, 8.43; O, 32.86.

Chitosan triazole Schiff-base (CTSB) **3c**. Obtained from 2-phenyl-2*H*-1,2,3-triazole-4-carbaldehyde **2c** in 95.1% yield as faint gray powder; The mixture was stirred for 10 h; IR (KBr): 1639 (C=N), 3455 cm^{-1} (OH); Anal. Found: C, 59.58; H, 5.17; N, 18.34; O, 15.82.

Chitosan nitrophenyl Schiff-base (CNPSB) **3d**. Obtained from *o*-nitrobenzaldehyde **2d** in 94.3% yield as yellow powder; The mixture was stirred for 6 h; IR (KBr): 1638 (C=N), 3480 cm^{-1} (OH); ^1H NMR (300 MHz, DMSO): δ = 3.56 (m, 1H, H-1′), 3.68 (m, 1H, H-2′), 3.83 (d, 2H, H-3′, H-4′; $J_{1,2}$ = 2.4 Hz), 3.90 (m, 1H, H-5′), 4.07 (m, 2H, H-6a′, H-6b′), 5.44 (bs, 2H, 3′-OH, 6′-OH; exchangeable with D_2O), 6.77 (s, 1H, CH=N), 6.98 (d, 2H, *o*-H; $J_{1,2}$ = 2.9 Hz), 7.83 (d, 2H, *m*-H; $J_{1,2}$ = 2.9 Hz), Anal. Found: C, 56.01; H, 5.11; N, 10.03; O, 28.65.

Chitosan bromophenyl Schiff-base (CBPSB) **3e**. Obtained from *p*-bromobenzaldehyde **2e** in 96.7% yield as white powder; The mixture was stirred for 7 h; IR (KBr): 1637 (C=N), 3466 cm^{-1} (OH); ^1H NMR (300 MHz, DMSO): δ = 3.27 (m, 1H, H-1′), 3.38 (m, 1H, H-2′), 3.54 (d, 1H, H-3′; $J_{1,2}$ = 1.8 Hz), 3.61 (m, 2H, H-4′, H-5′), 3.73 (m, 2H, H-6a′, H-6b′), 5.73 (bs, 2H, 3′-OH, 6′-OH; exchangeable with D_2O), 7.42 (s, 1H, CH=N), 7.53 (d, 1H, *o*-H; $J_{1,2}$ = 2.8 Hz), 7.84 (d, 2H, *m*-H; $J_{1,2}$ = 2.8 Hz), Anal. Found: C, 45.03; H, 4.26; N, 4.05; O, 23.14.

2.4. General Procedures for Reduction of Imine by $NaBH_4$

A solution of imine (20 mmol) in methanol (20 mL) was added to 10% AcOH (20 mL). The mixture was stirred for 10 min, and then 0.1 g of sodium borohydride was slowly added to the mixture with continuous stirring in ice bath for 24 h. After that the product was dried.

Chitosan-furan amine derivative **4a**. Obtained from imine **3a** in 84% yield as white powder; IR (KBr): 1647(COOEt), 3254, 3322 cm^{-1} (NH), (OH); Anal. Found: C, 61.07; H, 7.08; N, 4.64; O, 27.18.

Chitosan-pyrrole amine derivative **4b**. Obtained from imine **3b** in 86.2% yield as white powder; IR (KBr): 1659 (COOEt), 3334, 3387 cm^{-1} (NH), (OH); ^1H NMR (300 MHz, DMSO): δ = 1.83 (q, 2H, CH_2-ester; $J_{1,2}$ = 2.3 Hz, $J_{1,3}$ = 6.9 Hz), 2.41 (m, 1H, H-1′), 2.41 (m, 2H, H-2′, H-3′), 2.61 (d, 2H, H-4′, H-5′; $J_{1,2}$ = 2.3 Hz), 2.73 (m, 2H, H-6a′, H-6b′), 3.27 (t, 3H, CH_3-ester; $J_{1,2}$ = 2.3 Hz, $J_{1,3}$ = 6.9 Hz), 3.29 (s, 3H, CH_3-pyrrole), 4.61 (bs, 2H, 3′-OH, 6′-OH; exchangeable with D_2O), 6.51 (s, 2H, CH_2), 6.53 (s, 1H, H-pyrrole), 9.53 (bs, 1H, NH; exchangeable with D_2O), 10.35 (bs, 1H, NH; exchangeable with D_2O), Anal. Found: C, 57.95; H, 7.05; N, 9.02; O, 25.68.

Chitosan-triazole derivative **4c**. Obtained from imine **3c** in 84% yield as faint green powder; IR (KBr): 3260, 3321 cm^{-1} (NH), (OH); Anal. Found: C, 58.79; H, 6.04; N, 18.33; O, 15.58.

Chitosan-nitrophenyl amine derivative **4d**. Obtained from imine **3d** in 91% yield as faint yellow powder; IR (KBr): 3331, 3340 cm^{-1} (NH), (OH); ^1H NMR (300 MHz, DMSO): δ = 3.80 (d, 2H, H-1′, H-2′ $J_{1,2}$ = 2.2 Hz), 3.90 (m, 2H, H-3′, H-4′), 4.14 (m, 3H, H-5′, H-6a′, H-6b′), 5.46 (bs, 2H, 3′-OH, 6′-OH; exchangeable with D_2O), 6.97 (s, 2H, CH_2), 7.01 (d, 2H, *o*-H; $J_{1,2}$ = 2.4 Hz), 7.83 (d, 2H, *m*-H; $J_{1,2}$ = 2.4 Hz), 9.14 (bs, 1H, NH; exchangeable with D_2O), Anal. Found: C, 55.61; H, 5.47; N, 9.89; O, 28.14.

Chitosan-bromophenyl amine derivative **4e**. Obtained from imine **3e** in 91% yield as white powder; IR (KBr): 3264, 3398 cm^{-1} (NH), (OH); ^1H NMR (300 MHz, DMSO): δ = 3.37 (m, 2H, H-2′, H-1′), 3.48 (m, 3H, H-3′, H-4′, H-5′), 3.83 (m, 2H, H-6a′, H-6b′), 5.81 (bs, 2H, 3′-OH, 6′-OH; exchangeable with D_2O), 7.41 (s, 2H, CH_2), 7.53 (d, 2H, *o*-H; $J_{1,2}$ = 2.0 Hz), 7.83 (d, 2H, *m*-H; $J_{1,2}$ = 2.0 Hz), 9.31 (bs, 1H, NH; exchangeable with D_2O), Anal. Found: C, 44.76; H, 5.25; N, 4.12; O, 22.78.

2.5. Antimicrobial Activity

The antimicrobial activity of CSB derivatives were evaluated against Staphylococcus aureus (RCMBA 2004) and Bacillissubtilis (RCMBA 6005) as Gram-positive bacteria and against Pseudomonas aeruginosa and *Escherichia coli* (RCMBA 5003) as Gram-negative bacteria and against Aspergillus fumigates (RCMBA 06002), Syncephalastrum racemosum (RCMB 05098), as fungi. Agar disk diffusion method was used for the determination of the antibacterial and antifungal

activity, the well diameter was 6 mm (100 µL was tested), and the concentration of the tested sample was 5 mg/mL.

The susceptibility tests were performed according to the NCCLS recommendations (National Committee For Clinical Laboratory Standards, 1993). Screening tests regarding the inhibition zone were carried out by the well diffusion method [22].

The inoculums suspension was prepared from colonies grown overnight on an agar plate, and inoculated into Mular Hinton broth (Merk, Darmstadt, Germany). A sterile swab was immersed in the bacterial suspension and used to inoculate Mueller-Hinton agar plates. Amphotericin B, Ampicillin and Gentamicin were used as references for anti-fungi, anti-Gram positive bacteria, and anti-Gram negative bacteria, respectively. The compounds were dissolved in dimethylsulfoxide (DMSO). The inhibition zone was measured around each well after 24 h incubation at 37 °C; controls using DMSO were adequately done.

MIC determinations were performed in the same way using agar disc diffusion method, but by using different concentrations from the testing compound.

2.6. Antiproliferative Activity Screening

Regarding cell line propagation, the cells were propagated in (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50 µg/mL gentamycin. All cells were maintained at 37 °C in humidified atmosphere with 5% CO_2 and were sub cultured two times a week. Cell toxicity was monitored by determining the effect of the examined compound on cell morphology and cell viability.

For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1×10^4 cell per well in 100 µL of growth medium. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers that were then dispensed into 96-well flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37 °C in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of each tested sample. Control cells were incubated without test samples and with or without DMSO. After incubation of the cells for 24 h at 37 °C, various concentrations of each sample (50, 25, 12.5, 6.25, 3.125 and 1.56 µg) were added separately. Then the incubation was continued for 48 h.

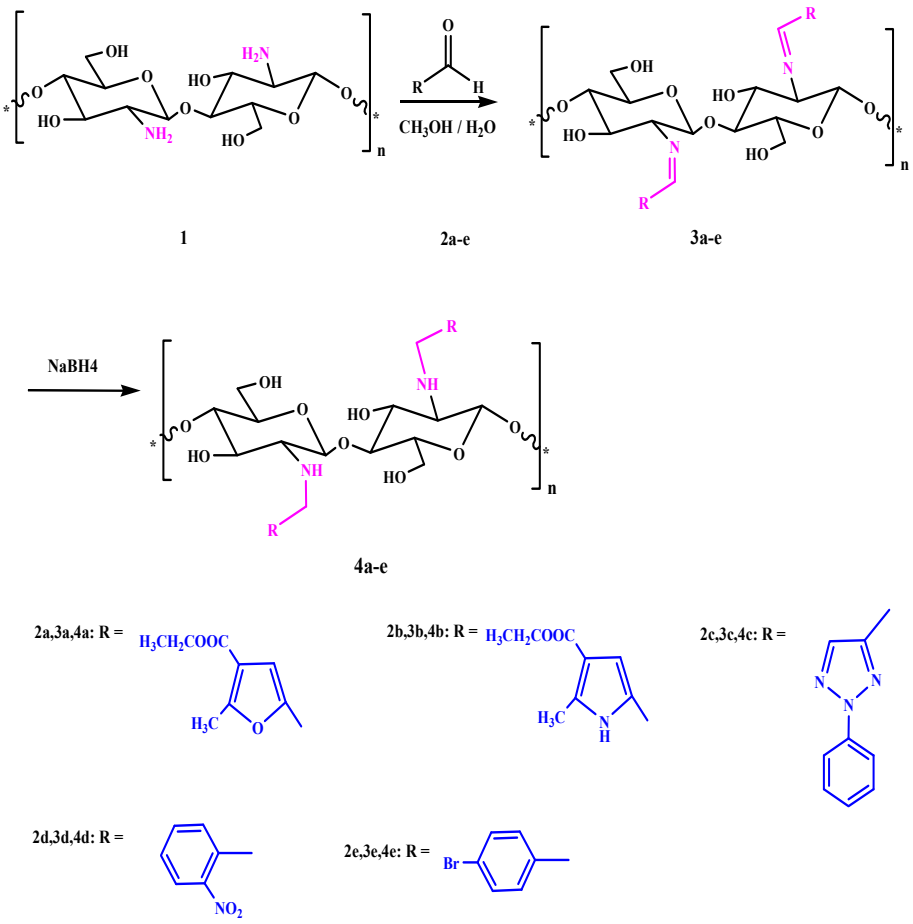
The viable cells yield was determined colorimetrically using MTTB (3,4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide). The water insoluble tetrazolium salt is converted to purple formazon by the mitochondrial dehydrogenase of viable cells. After the end of incubation period, media were aspirated, and the crystal violet solution (1%) was added to each well for at least 30 min. The stain was removed and plates were rinsed using tap water until all excess stain was removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, then the absorbance of the plates were measured after gently being shaken on Micro plate Reader (TECAN, Inc., Olympus Europa Holding GmbH, Männedorf, Switzerland), at 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compound. All experiments were carried out in the triplicate. The cell cytotoxicity effect of the tested compound was calculated [16,17].

3. Results and Discussion

3.1. Synthesis of Chitosan Schiff's Base (CSB) and Chitosan Amine Derivatives

Aryl and heteroaryl aldehydes were selectively grafted onto the primary amino groups of chitosan with formation of the corresponding Schiff bases **3a–e**. The imine group converted into the more stable amine with formation of the corresponding *N*-substituted amino-chitosan derivatives **4a–e** using sodium borohydride as a reducing agent.

Five different aldehydes were employed: (ethyl-2-formyl-5-methyl-4-furate **2a**, ethyl-5-formyl-2-methyl-1*H*-pyrrole-3-carboxylate **2b**, 1-(2-phenyl-2*H*-1,2,3-triazole-4-yl) ethanone **2c**, 2-nitrobenzaldehyde **2d**, and 4-bromobenzaldehyde **2e** in this reaction (Scheme 1).



Scheme 1. Synthesis of chitosan Schiff's base (CSB) and chitosan amine derivatives.

3.2. Characterization of Chitosan-Imine and Chitosan-Amine Derivatives

The infrared spectra of compounds **3a–e** showed the (OH) band at 3480–3322 cm^{-1}, and (C=N) at 1599–1642 cm^{-1}. On the other hand the infrared spectra of compounds **4a–e** showed the (OH) band at 3398–3321 cm^{-1}, and (NH) at 3254–3334 cm^{-1} (Table 1).

Table 1. The infrared data of compounds **3a–e**, **4a–e**.

Compound Numner	γ KBr Max cm^{-1}			
	C=N	COOEt	NH	OH
3a	1642	1687	-	3440
3b	1599	1655	2976	3322
3c	1639	-	-	3455
3d	1638	-	-	3480
3e	1637	-	-	3466
4a	-	1647	3254	3322
4b	-	1659	3334	3387
4c	-	-	3260	3321
4d	-	-	3331	3340
4e	-	-	3264	3398

The structure of imines **3a–e** is further proved by ^{1}H NMR spectra, which showed the 1′-OH proton at 5.53–5.73 ppm. The rest of the sugar protons are at the range 2.61–4.07 ppm. The methyl protons at position-2 in the pyrrole ring appeared as a singlet at δ 3.25 ppm; as well as the disappearance of the two protons of (NH_2), (Figure 1, Table 2). (OH) protons are D_2O exchangeable in compounds **3a–e**. Additionally, the structure of amines **4a–e** was proved by ^{1}HNMR spectra, which showed the 1′-OH proton at 5.06–5.05 ppm, the rest of the sugar protons at the range 4.58–4.28 ppm. As well as the appearance of the (NH) proton at 9.14–9.53 ppm, after shaking of compounds **4a–e** with D_2O, their ^{1}H NMR spectra, showed the disappearance of the (NH) proton, as well as (OH) protons (Table 3). In addition C^{13} NMR of compounds **3e** and **4e** showed the expected peaks.

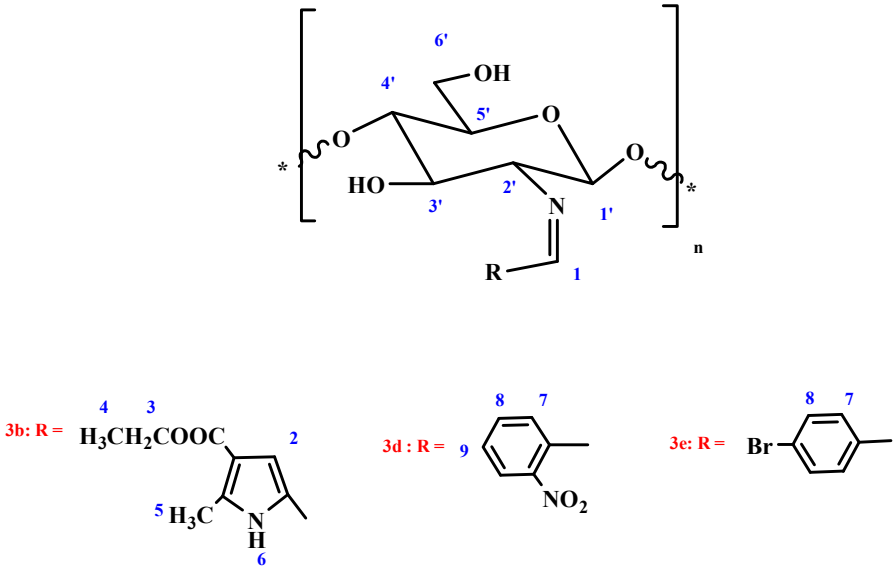


Figure 1. Chitosan-imine structure.

Table 2. The ^{1}H NMR data of chitosan-imine derivatives.

Compound Numner	δ (ppm)								
	H-1	**H-2**	**H-3**	**H-4**	**H-5**	**H-6**	**H-7**	**H-8**	**H-9**
3b	6.51 (s)	6.55 (s)	1.93 (s)	3.17 (s)	3.25 (d)	10.33 (bs)	-	-	-
3d	6.77 (s)	-	-	-	-	-	6.98 (d)	7.83 (d)	7.83 (d)
3e	7.42 (s)	-	-	-	-	-	7.53 (d)	7.84 (d)	-
Compound Numner	**δ (ppm)**								
	H-1′	**H-2′**	**H-3′**	**H-4′**	**H-5′**	**H-6a′, H-6b′**	**3′-OH**	**6′-OH**	
3b	2.38 (m)	2.41 (m)	2.41 (m)	2.61 (d)	2.61 (d)	2.75 (m)	4.53 (bs)	4.53 (bs)	
3d	3.56 (m)	3.68 (m)	3.83 (d)	3.83 (d)	3.90 (m)	4.07 (m)	5.44 (bs)	5.44 (bs)	
3e	3.27 (m)	3.38 (m)	3.54 (d)	3.61 (m)	3.61 (m)	3.73 (m)	5.73 (bs)	5.73 (bs)	

Decreasing the crystal structure of chitosan after condensation with aldehydes was appearing in XRD patterns of chitosan Schiff's base (CSB), which showed in case of compound **3e** one broad peak around 2θ = 25°. On the other hand, XRD patterns of compound **3d** showed two broad peaks around 2θ = 16° and 25° indicating a shift from the normal chitosan peaks a broad peak around 20° showing increasing in its amorphous nature (Figure 2).

Table 3. Inhibition indices of chitosan Schiff's base (CSB) against *S. aureus*, *B. subtilis*, *P. aeruginosa* and *E. coli*.

Sample	Tested Microorganisms			
	Gram Positive Bacteria		Gram Negative Bacteria	
	S. aureus	*B. subtilis*	*P. aeruginosa*	*E. coli*
3a	22.2 ± 0.58	24.6 ± 0.25	NA	21.4 ± 0.63
3b	18.2 ± 0.63	20.4 ± 0.58	NA	18.3 ± 0.72
3c	16.7 ± 0.36	19.2 ± 0.27	13.3 ± 0.36	13.6 ± 0.36
3d	11.3 ± 0.63	14.2 ± 0.58	NA	11.1 ± 0.63
3e	21.4 ± 0.63	22.3 ± 0.72	NA	21.2 ± 0.63
Ampicillin	23.8 ± 0.2	32.4 ± 0.3	NA	NA
Gentamicin	NA	NA	17.3 ± 0.1	19.9 ± 0.3

NA: No Activity.

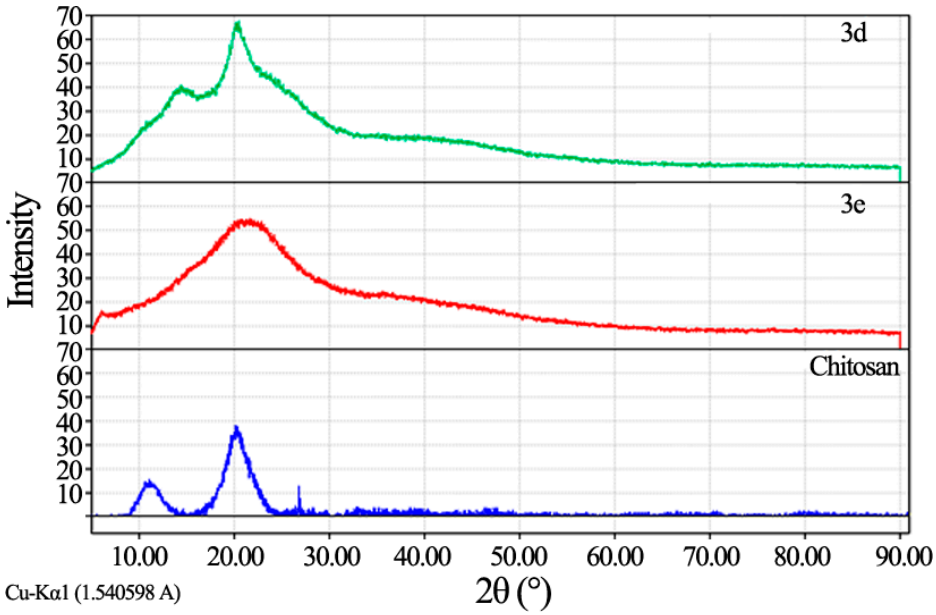


Figure 2. X-ray diffraction spectrum of chitosan derivatives.

3.3. Antimicrobial Activity

All of the synthesized substituted derivatives under investigation showed *in vitro* antimicrobial activity against the tested microorganisms. The results of antibacterial activity of the chitosan Schiff's base (CSB) derivatives using inhibition zone method are listed in Table 3. The obtained data revealed that all the tested compounds **3a**–**e** had no effect on *P. aeruginosa* (Gram positive bacteria), except the triazolo-derivative **3c**, which showed excellent inhibitory effect on both Gram-positive and Gram-negative bacteria as compared to Ampicillin and Gentamicin (Table 3). Our data displayed that compound **3a** is the most antimicrobially effective compound, which has an excellent anti-Gram positive bacteria, effect on both Straphylococcusaureus and Bacillissubtilis, and also on anti-Gram negative bacteria (*E. coli*). This effect may be due to the presence of the furan ring. On the other hand the bromo-derivative showed higher antibacterial effect than the nitro derivative on both Gram-positive and Gram-negative bacteria.

The antimicrobial activity of chitosan has been explained by several mechanisms. The first mechanism is mediated by the electrostatic forces between the protonated $–NH_3^+$ groups of chitosan and the electronegative charges on the microbial cell surface [23]. It suggests that the greater the number of cationized amines, the higher the antimicrobial activity.

Another proposed mechanism is the binding of chitosan with microbial DNA, which leads to the inhibition of the mRNA and protein synthesis via penetration of chitosan into the nuclei of the microorganisms [24]. The third mechanism is the chelating of metals, suppression of spore elements, and binding to essential nutrients to microbial growth.

The mean zone of inhibition in mm ± standard deviation beyond well diameter (6 mm) produced on a range of environmental and clinically pathogenic microorganisms using (5 mg/mL) concentration of tested samples. Results are depicted in the following table.

Generally, chitosan has been reported as being very effective in inhibiting spore germination, germ tube elongation, and radial growth [25]. The antifungal mechanism of chitosan involves cell wall morphogenesis with chitosan molecules interfering directly with fungal growth, similar to the effects observed in bacteria cells. The microscopic observation reported that chitosan molecules diffuse inside hyphae interfering on the enzymes activity responsible for the fungus growth [26].

The antifungal activities of CSB derivatives against *A. fumigatus* (RCMBA 06002), *Syncephalastrum racemosum* (RCMB 05098) are shown in Table 4, which shows that all the derivatives had effective activities against the *Aspergillus fumigates*, compared with that of the stander, with inhibitory indices ranging from 13.2 ± 0.72 to 21.3 ± 0.63 mm inhibition zone (Table 4). On the other hand, only one of the tested compounds showed an effect on *Syncephalastrum racemosum*, this activity of compound 3c may be due to the presence of the triazole ring. Again, the results also demonstrate how the antifungal activities are affected by the nature of the substituent in the aryl ring of the CSB derivatives, in which the bromo-derivative showed a greater effect compared to the nitro derivative (Table 4).

Table 4. Inhibition indices of CSB against *A. fumigatus*, *Syncephalast rumracemosum*.

Sample	Tested Microorganisms	
	A. fumigates	***S. rumracemosum***
3a	21.3 ± 0.63	NA
3b	16.3 ± 0.72	NA
3c	16.8 ± 0.39	13.4 ± 0.58
3d	13.2 ± 0.72	NA
3e	19.6 ± 0.58	NA
Amphotericin B	23.7 ± 0.1	19.7 ± 0.2

NA: means No Activity.

The results obtained in Tables 3 and 4 showed that all samples have promising results with mean zone of inhibition values less than the used standards. Amphotericin B as anti-fungi, Ampicillin as anti-Gram positive bacteria, and Gentamicin as anti-Gram negative bacteria.

Minimum inhibitory concentration (MIC) is the lowest concentration of an antimicrobial that will inhibit the visible growth of microorganisms after incubation for suitable time. The antimicrobial activity as MIC (µg/mL) of tested compounds **3a**, **3b**, **3c**, **3d** and **3e** against tested microorganisms showed that all these compounds are effective for Aspergillus fumigates with MIC 1.95, 31.25, 62.5, 62.5 and 3.9, respectively, meanwhile compound 3a has the lowest MIC compared to Amphotericin B which used as standard with MIC 0.98.

In addition, these examined compounds showed MIC values against *Staphylococcus aureus* 1.95, 7.81, 125, 125 and 1.95 respectively, in which compound **3a** and **3e** are the most active compounds compared to the Ampicillin standard with MIC value 0.98 (Table 5).

Additionally, these compounds are effective against other Gram-positive bacteria *Bacillus subtilis* with MIC 0.98, 3.9, 62.5, 125 and 1.95 indicating that compound **3a** is the most active one compared to Ampicillin MIC 0.49. Additionally, all these tested compounds are effective against *Escherichia coli* with MIC 1.95, 7.81, 125, 125 and 1.95 showed that compound **3a** is the most effective one compared to Gentamicin with MIC equal to 3.9. The obtained data confirmed that compound **3a** is the most active agent as antimicrobial, especially against Gram-negative bacteria (Table 5).

Table 5. Antimicrobial activity as MICS (μg/mL) of CSB against tested microorganisms.

Tested Microorganism	Samples					Standard
	3a	3b	3c	3d	3e	
Fungi			MIC (μg/mL)			AmphotericinB
Aspergillus Fumigates	1.95	31.25	62.5	62.5	3.9	0.98
Gram positive bacteria			-			Ampicillin
Staphilococcus aureus	1.95	7.81	125	125	1.95	0.98
Bacillis subtilis	0.98	3.9	62.5	125	1.95	0.49
Gram negative bacteria			-			Gentamicin
Escherichia coli	1.95	7.81	125	125	1.95	3.9

3.4. Antiproliferative Activity Screening

Cytotoxicity was tested against three cancer cell lines: HepG-2, Human Hepatocellular Liver Carcinoma Cells; HCT-116, Human Colon Carcinoma Cells; MCF-7, Human Breast Adenocarcinoma Cells.

Since triazole derivative showed strong reactivity against all the tested kinds of Gram-positive, and Gram-negative bacteria and fungi, it was chosen for cytotoxicity screening against cancer cells. The results show that the examined compound **3c** had an excellent inhibitory effect on the cell lines growth compared to standard.

The reactivity of the examined compound **3c** was tested against breast cancer (MCF-7), colon cancer (HCT-116) and hepatocellular cancer (HepG-2). The effect of this compound on cancer cell viability was tested using different concentrations (50–1.56 μg/mL) of the compound **3c**. See Table 6.

Table 6. Inhibitory activity of compound 3c against HepG-2, MCF-7 and HCT-116 cell line compared to doxorubicin as reference drug.

Sample conc. (μg/mL)	Viability %					
	3c			Doxorubicin (std.)		
	HepG-2	MCF-7	HCT-116	HepG-2	MCF-7	HCT-116
0	1.00000	1.00000	1.00000	1.00000	1.00000	1.00000
1	0.58716	0.652078	0.83326	0.5451	0.4256	0.3902
2.5	0.19456	0342510	0.54419	0.3903	0.2989	0.2900
5	0.25484	0.330447	0.29740	0.2500	0.2000	0.1990
10	0.20469	0.320388	0.25028	0.1959	0.1443	0.1510
IC_{50}	1.21	1.54	2.89	1.2	0.44	0.47

The maximum cell growth inhibitory effect was obtained on HepG-2 with IC_{50} equal to 1.21 μg compared to IC_{50} of the used standard 1.2 μg.

4. Conclusions

Some of new Chitosan Schiff's bases have been synthesized. Their structures were approved by standard methods. Evaluations of their anti-bacterial, anti-fungal, and cytotoxicity properties have been studied.

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