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Preparation of substituted quaternized arylfuran chitosan derivatives and their antimicrobial activity

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ABSTRACT

Heterocyclic modification of chitosan has been achieved through the formation of a Schiff base intermediate by the reaction of chitosan with substituted arylfurfural. The Schiff bases were further reacted with 10% sodium borohydride followed by reaction with methyl iodide to get the quaternized products. The formation of the Schiff bases and quaternized derivatives has been confirmed by elemental analysis, FTIR, ^{1}H NMR and UV–vis spectroscopy. The compounds are also characterized by thermo-gravimetric analysis. The parent compound and quaternized derivatives were compared for their antibacterial and antifungal activity. The results indicated that quaternized derivatives possess better inhibitory property than chitosan. Further this study confirms that heterocyclic aromatic substituent containing 'Cl' and 'NO_2' are effective in enhancing the antimicrobial activity of Chitosan.

1. Introduction

Chitosan, a polysaccharide composed of glucosamine and N-acetyl glucosamine units, is mainly obtained by deacetylation of chitin, a natural structural component present in crustaceans. This aminopolysaccharide has attracted much attention due to its physicochemical properties and biological activities [1,2]. Hydrophilicity, non-toxicity, biodegradability, biocompatibility and antibacterial activity are some of the advantageous characteristics which make chitosan an attractive material for extensive use in pharmaceutical been well documented [3,4]. Studies on the effect of molecular weight, degree of acetylation and polycationic nature on the antibacterial activity have been reported [5]. The antibacterial activity is proposed to be due to the formation of a protective layer on microbial cell surface. The ammonium groups on chitosan, formed on protonation at physiological pH interact with the anionic groups on the microbial surface resulting in an impermeable coat on the bacterial surface preventing its growth [6]. However, the exact mechanism of antibacterial action of Chitosan is still unknown.

Recently, much attention is being focused on the chemical modification of chitosan, making use of the reactive amino and hydroxyl groups [7]. The derivation is intended to improve the solubility of chitosan in common solvents; to enhance its biological activity or metal adsorption capacity; or to impart photosensitivity [2,5–10]. The Schiff bases of chitosan (R—C═N—) have been the intermediates for the preparation of N-substituted chitosan, chitosan ester and quaternised chitosan derivatives [2,5,7,10]. The antimicrobial activity of these compounds has been evaluated [2,5]. It is observed that the modification not only improves the antimicrobial activity but also expands the antimicrobial spectrum of chitosan.

Arylfurans are constituents of a variety of important class of pharmacologically active compounds [11]. Many compounds containing arylfuran moiety are found to have potent antibacterial, antifungal, anticonvulsant, anticancer and antioxidant properties [12–14].

Prompted by the above reports, we have attempted to modify chitosan with arylfuran moiety. We report herein the preparation, characterization and biological activity studies of Schiff bases of chitosan and the quaternized derivatives by the reaction with five 5-arylfurfural derivatives. The compounds have been screened for their biological activity. The antimicrobial assays against '*Psuedomonas aeroginosa*' (PA), a Gram negative bacterium, '*Mycobacterium smegmatis*' (MS), a Gram positive bacterium and a yeast '*Candida albican*' (CA), were investigated and the data have been reported. The results have been compared with that of the parent compound, Chitosan.

2. Methods

2.1. Materials

Chitosan (CS) (with specification: 75–85% deacetylated, molecular weight: 310 kDa based on viscosity) and furfural were

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purchased from Sigma Aldrich (India). 4-Chloroaniline, 2,4-dichloroanilne, 2,4,5-trichloroaniline, 3-chloro-4-fluroaniline, 4-nitroaniline, methyliodide (CH_3I), dimethylformamide (DMF), sodium borohydride ($NaBH_4$), sodium iodide (NaI) were purchased from Spectrochem (India). Copper chloride, N-methylpyrrolidone (NMP) and all other reagents were purchased from SRL Chemicals (India). Furfural was distilled and used. All other chemicals were used as received. The microbial cultures required for the antibacterial activity were purchased from IMTech, Chandigarh (India).

2.2. Synthesis of 5-aryl-furan-2-carbaldehyde derivatives (ArFC)

5-Aryl-furan-2-carbaldehyde derivatives were synthesized according to previous method [15]. The synthesis of 5-aryl-furan-2-carbaldehyde derivatives has already been reported in our previous work [16]. A mixture containing substituted aniline (2.56 g) and dilute hydrochloric acid was gently heated to get a clear solution. The solution was cooled to 0 °C and diazotized by the addition of 6 mL of 30% aqueous sodium nitrite solution. The diazonium salt was filtered and to the filtrate added water and furfural (2.8 g) followed by 2.5 mL of aqueous solution of copper chloride (1.125 g). The solid was collected by filtration. The crude compound was recrystallized from a mixture of dimethylformamide and ethanol to obtain pure ArFC.

2.2.1. Synthesis of Schiff's bases of chitosan (BCS)

0.2 g Chitosan was stirred in the aqueous solution of acetic acid for 30 min. To this solution, 0.25 mole of ArFC dissolved in DMF was added with stirring. The mixture was reacted at 60 °C for 5 h. The solution was then poured into substantial ethanol to remove the unreacted ArFC. The product was washed three times with anhydrous ethanol and dried at 50 °C for 24 h to obtain BCS.

2.2.2. Synthesis of N-substituted chitosan (NCS)

The N-substituted chitosan derivatives were synthesized according to the previous method [5]. To 0.1 g BCS was added 2 mL of 10% aqueous $NaBH_4$ and the reaction mixture was stirred at room temperature for 2 h. The product was precipitated in acetone, filtered and dried at 60 °C for 24 h to obtain NCS.

2.2.3. Synthesis of quaternized arylfuran chitosan derivatives [5] (QCS)

To prepare the quaternized chitosan derivatives, NCS (0.1 g) was dispersed in NMP (5 mL) by stirring at room temperature for 12 h. To this mixture, 0.01 M NaOH, NaI (0.15 g) and CH_3I (0.4 mL) were added and the reaction was carried out with stirring at 50 °C for 20 h. The products were precipitated by the addition of excess acetone and filtered. The QCS was obtained on drying at 60 °C for 24 h.

2.3. Characterization of the derivatives

2.3.1. Elemental analysis

The % of C, H and N content of the samples were analyzed using Flash EA1112 CHNS analyzer.

2.3.2. FTIR spectroscopy

The infrared spectra of the compounds were recorded on a IR-Prestige-21, FTIR spectrometer (Shimadzu, Japan). Samples were prepared as KBr pellet and scanned in the range 4000–400 cm^{-1}.

2.3.3. ^1H NMR spectroscopy

The ^1H NMR spectra was recorded on a 400 MHz Varian mercury VX-300 spectrometer using D_2O as solvent.

2.3.4. UV–vis spectroscopy

UV absorption spectra were obtained using a Shimadzu UV-2550 spectrophotometer in the range 200–400 nm.

2.3.5. Thermogravimetric analysis (TGA)

TGA was performed with a Shimadzu DTG-60 thermogravimetric analyzer. The samples were heated from 0 to 600 °C at a constant rate of 10 °C/min under nitrogen atmosphere during the analysis.

2.4. Biological screening tests

2.4.1. Preparation of microorganisms

The cultures of representative microbes were grown in nutrient agar before testing against the compounds. Gram-positive *Mycobacterium smegmatis* (MTCC 943) was grown at 35 ± 2 °C and Gram-negative *Pseudomonas aeroginosa* (MTCC4676) was grown at 30 ± 2 °C for 12 h. Antifungal study was carried out against *Candida albicans* (MTCC 183) grown in yeast extract peptone dextrose (YEPD) agar media at 30 ± 2 °C for 12 h.

2.4.2. Antimicrobial assays

The inhibition rate of CS and QCS samples against *M. smegmatis* (MS), *P. aeroginosa* (PA) *and C. albicans* (CA) were determined using suspension cultures in broth. The test solutions were made by dissolving different concentrations (0.2, 1 or 2 mg/mL) of CS in acetic acid and QCS in distilled water. 50 μL of the test samples were mixed with 50 μL of microbial culture at a concentration of 3.6×10^7 CFU/mL of PA, 3.58×10^8 CFU/mL of MS and 4.2×10^7 CFU/mL of CA and spread over the agar plat with the help of a spreader. Similarly, the agar plates with 50 μL of microbial culture and 50 μL of water or 1% acetic acid were prepared and considered as control. The test plates and control plates were incubated at 30 ± 2/35 ± 2 °C for 12 h and number of colonies was counted using quadrant method. Based on the number of colonies in the control and test plates, the percent inhibition was determined using the relation,

$$\%\,\text{inhibition} = \left[\frac{N_1 - N_2}{N_1}\right] \times 100$$

where N_1 and N_2 are the mean number of colonies in the control and test plates respectively.

3. Results and discussion

3.1. Synthesis

The synthesis of QCS has been accomplished by modification of CS through the Schiff base intermediate in three reaction steps following the traditional synthetic methodology adopted for derivatisation of chitosan [5]. As shown in Scheme 1, five different QCS derivatives have been made using the corresponding substituted anilines for the preparation of ArFC.

3.2. FTIR spectra

The FTIR spectra of NO_2-ArFc and Cl-ArFc are displayed in Fig. 1a and b respectively. Several common bands are observed in the spectra of these two compounds. The weak peaks around 3100 cm^{-1} correspond to aromatic C—H stretching, and around 2800 cm^{-1} correspond to aldhehyde —C—H; peaks at 1680 cm^{-1} correspond to aryl conjugated C=C strtching; at 1747 cm^{-1} correspond to C=O stretching of aldehyde group; peaks at 1143 and 1051 cm^{-1} correspond to C—O—C streching of furan ring. Apart from these common peaks, the peaks at 1518 and 1338 cm^{-1} observed in Fig. 1a are attributed to the asymmetrical and symmetrical streching of the aryl N—O of