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Syntheses of New Antimicrobial Cellulose Materials Based 2-((2-aminoethyl)amino)-4-aryl-6-indolylnicotinonitriles

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2-((2-aminoethyl)amino)-4-aryl-6-indolylnicotinonitriles (1a-c), were grafted into two different cellulosic intermediates, where 6-deoxy tosyl cellulose that was prepared by the reaction of microcrystalline cellulose (MCC) with tosyl chloride in DMA/LiCl. Additionally, the dialdehyde carboxymethyl cellulose (2,3-DACMC) obtained by regioselective periodate oxidation of carboxymethyl cellulose (CMC) were also condensed with the same amine compounds (1a-c) via Schiff's base reaction aiming to obtain new functionalized amino cellulosic derivatives with predictable applications. The products were characterized by FT-IR, ^{13}C-NMR, elemental analysis, SEM, and X-ray diffraction. The antimicrobial activity of the new cellulose amine derivatives and Schiff's bases of (2,3-DACMC) were assayed on four different microorganisms. Among the tested compounds, (3a) and (3b) proved to be promising antimicrobial cellulosic material that could be used as a broad spectrum wound healing gels or manufacturing of packaging materials.

Keywords: Heterocyclic amino compounds, 6-deoxycellulose tosylate, Schiff`s base, CMC, Periodate oxidation, Antimicrobial activity..

Introduction

Nicotinonitrile skeletons especially those with an amino group at C_2 and/or 4,6-diaryl-substitutents have demonstrated a broad range of biological activities, such as antibacterial [1], antifungal[2] antituberculosis [3], antiviral[4], analgesic, and anti-inflammatory effects [5,6]. Furthermore, they have been used as inhibitors of adenosine kinase, topoisomerase [7], anti-proliferative agents that have been used for the treatment of a number of human cancer cell lines [8 - 10].

The chemical modification of cellulose is done in order to obtain different kinds of cellulosic derivatives that diverse in their physical and chemical features and could be used in several industrial and environmental applications. The poor solubility of cellulose in water and most common organic solvents is the major obstacle for cellulose reactivity and subsequent modification[11,12]. This is due to the intra and intermolecular hydrogen bonds along the polymer chains. The solubility of cellulose sulfonates particularly cellulose tosylate in organic solvents made them suitable key intermediate for selective nucleophilic substitution reactions on C-6 of anhydroglucose unit during cellulose functionalization [13,14]. The broad spectrum antimicrobial properties of amino cellulose as well as their good biocompatibility made them suitable candidate for the design of novel amino cellulose biopolymers through the chemical grafting of cellulose by amino compounds. Recently, there is an increasing interest for the preparation of new amino cellulose derivatives for their potential applications in different research fields, such as anion-exchange resin, selective membranes [12,13], anticoagulants, biosensors, enzyme immobilization in bioassays [14-16], flame retardants[17], and antimicrobial agents [18]. The antimicrobial activity of the modified natural polysaccharides, such as some chitosan

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and starch derivatives encouraged their use in the manufacturing of packaging materials. However, their water solubility and durability are the main drawbacks that hinder their applications in food packaging industries[19].

Moreover, carboxymethyl cellulose (CMC) is water-soluble anionic cellulose derivative. It widely used in many industrial pharmaceutics, cosmetics, drug delivery, and wound dressing because of their high water absorption property, gelation behavior, bio-degradability, and biocompatibility[20-22]. Additionally, Schiff bases has received great attention due to their reported biological activities[23,24]. In this report we described the synthesis, characterization and antimicrobial activity of new cellulose amine derivatives, and new CMC based Schiff's bases using 2-((2-aminoethyl)amino)-6-(1H-indol-3-yl)-4-aryl nicotinonitriles (1a-c) which have been synthesized by our group [10] and evaluated for their biological potency as antiproliferating and antimicrobial agents.

Experimental

Materials

Microcrystalline cellulose MCC (Merk, DP 225), Carboxymethyl cellulose sodium salt CMC medium viscosity, degree of substitution of 0.65–0.90 was purchased from Fluca, Sodium periodate ($NaIO_4$), p-toluenesulfonyl chloride, Dimethyl acetamide (DMA), anhydrous lithium chloride LiCl, triethyl amine (Et_3N) were purchased from sigma aldrich, the materials were used as received. IR spectra were recorded on JASCO FT/IR 6100 Japan spectrometer (NRC, Dokki, Giza, Egypt) using KBr disc. ^{13}C-NMR spectra were determined using JEOL ECA-500 run for 125 MHz spectrometer (NRC, Dokki, Giza, Egypt). Chemical shifts were expressed in part per million δ (ppm) against tetramethylsilane (TMS) as an internal standard. The samples of MCC, TsMCC and cellulose amines 2a-c were imaged using JEOL JXA-840A Scanning electron microscope (NRC, Dokki, Giza, Egypt).The X-ray diffraction profiles for CMC, 2,3-DACMC, and Schiff`s bases (3a-c) were obtained using a Philips 1730 X-ray diffractometer (National Research Center Cairo, Egypt). All reactions were followed up by thin layer chromatography (TLC) using aluminum sheets recoated with UV fluorescent silica gel (Merck Kieselgel 60 F245), UV lamp, and ninhydrine solution.

Methods

Tosylation of cellulose was carried out according to the method described by Rahn et al.[26] in two step.

Step 1: dissolution of MCC in DMA/LiCl

MCC was dried at 70°C for 72 h in oven. Then, (120 mL) of DMA was added to MCC (5.0 g, 30.8 mmol of anhydroglucose unit) in 500 mL round flask, and stirred at 130°C for 2 h where a colorless slurry is observed. The slurry had been allowed to cool down to 100°C, Then, (10 g) of anhydrous LiCl in (25 ml) of DMA were added under stirring. The stirring was continued overnight at room temperature until the complete dissolution of cellulose.

Step 2: tosylation of MCC

A mixture of TEA (18.6 mL, 185 mmol, 6 mol/AGU) in (10 mL) DMA was added to the solution of MCC under stirring at room temperature, the stirring was continued for another 30 min, followed by drop wise addition of a solution of p-toluenesulfonyl chloride (35.3 g, 184.8 mmol, 6mol/mol AGU dissolved in 25 mL of DMA) over a period of 30-60 min. at 3-8 °C. The stirring was continued for another 24 h at room temperature, then the mixture was poured slowly into (1L) of ethanol. The precipitate was filtered off, washed carefully with about (1L) of distilled water, suspended in 250 mL of boiling acetone and reprecipitated in distilled water (500 mL), and filtered off. Then washed with ethanol (250 mL) for three times, to remove the unreacted TsCl. The resulted TsMCC was dried at 50 °C in oven for 48h. Yield: 65%, elemental analysis: C= 45.63, H= 5.49, S = 6.11.

The degree of substitution (DS) was determined by sulfur analysis and calculated according to the equation:

$$DS = M_{AUG} * S(\%)/ M_S * 100(\%) - M_{Tos} * S(\%)[26]$$

where M_{AUG} denotes the molar mass of AGU (AGU = 162), MS is the molar mass of sulfur (S = 32), M_{Tos} is the molar mass of the tosyl group (Ts = 155).

$$DS_{Ts} = 162 * 0.0611/ 32 * 100(\%)-155* 0.0611= 0.44.$$

Characterization of TsMCC

IR (KBr, υ/cm^{-1}): 3442 (OH $_{str}$), 1383($SO_{2\ asym}$), 1177 ($SO_{2\ sym}$), 812 cm^{-1} (S-O-C). ^{13}C-NMR (DMSO-d_6) δ ppm: 21.65 (SP^3, p-CH_3 of Ts group), 56.57, 69.04, 71.07, 72.48, 75.37, 76.15,

81.02, 98.96, 101.24 (SP^3, C-6_{non-Ts}, C-6_{Ts}, C-5, C-4, C-3, C-2, and C-1), 128.30, 130.17, 137.35, and 145.98 (4 signals for 6 SP^2 carbon atoms of the substituted Ts group).

Substitution of tosyl group by 2-((2-Aminoethyl) amino)-6-(1H-indol-3-yl)-4-(aryl) nicotinonitrile (1a-c)

A series of cellulose amine derivatives (2a-c) were prepared by the substitution of tosyl group by 2-((2-Aminoethyl) amino)-6-(1H-indol-3-yl)-4-(aryl) nicotinonitrile (1a-c) according to the following method: TsMCC, DS_{Ts} = 0.44 (1 g, 2.1 mmol/AGU) was dissolved in DMF (1 mL). Then (75 mg) of the proper amine solution (1a-c) in (1 mL) of DMF containing (100 µL) of triethyl amine were added drop wise to the TsMCC solution. The mixture was stirred at 90 °C for 24 h. The progress of the reaction was monitored by TLC and the heterocyclic amine grafting was visualized by ninhydrine stain, untile the disappearance of the violet spot on the TLC which means the amine reacted completely. After completing the reaction, the product was separated by precipitation in ethanol (200 mL). The solid products were filtered off, purified by boiling in acetone (500 mL for 3 times), filtered off, and dried at 50 °C in oven for 48h. The structure of the new cellulose amine derivatives (2a-c) were confirmed by elemental analysis, IR, and ^{13}C-NMR.

Characterization of cellulose amine (2a)

Elemental analysis: C; 43.23, H; 5.79, N; 2.10, S; 1.72. IR (KBr, υ/cm^{-1}): 3423 (broad, $NH_{str.}$ and $OH_{str.}$), 2924 (C-$H_{str.}$), 2201 (CN), 1608 (C=$N_{aromatic}$), and 1589 (indolyl $NH_{bending}$): ^{13}C-NMR (DMSO-d_6) δ ppm: 21.98 (2 SP3, p-CH_3 of Ts group), 46.36, 47.64 (2 signals for 2 CH_2 of indolylnicotinonitrile 1a), 56.44 (OCH3), 59.86, 69.80, 71.74, 73.38, 75.12, 76.26, 79.25, 100.45, 101.16 (C-6 non-Ts, C-6 Ts, C-5, C-4, C-3, and C-2 and C-1 of AGU), 109.88 (CN), 86.55, 114.46, 115.31, 118.47, 118.58, 119.50, 122.52, 123.40, 124.87, 128.69, 129.05, 130.13, 135.80, 137.46, 146.15, 147.63, 155.94, 157.78, 163.06, and 166.43 (20 signal for different SP^2 aromatic carbons of substituted Ts groups, and the indolylnicotinonitrile 1a along the cellulose chain).

Characterization of cellulose amine (2b)

Elemental analysis: C; 43.83, H; 5.84, N; 1.42, S; 2.86. IR (KBr, υ/cm-1): 3419 (broad, $NH_{str.}$ and $OH_{str.}$), 2924 (C-$H_{str.}$), 2204 (CN), and 1602 (C=$N_{aromatic}$).^{13}C-NMR (DMSO-d_6) δ ppm: 21.65 (p-CH3), 46.21, 48.05 (2 signals for 2 CH_2 of indolylnicotinonitrile 1b), 63.64, 71.32, 73.48, 74.99, 78.23 99.66, and 101.21 (C-6_{non-Ts}, C-6_{Ts}, C-5, C-4, C-3 and C-1 of AGU), 107.88 (SP, CN), 85.20, 115.19, 115.68, 119.01, 119.33, 122.57, 123.35, 126.03, 126.57, 128.24, 128.63, 130.78, 131.07, 135.05, 135.37, 146.27, 146.56, 158.28, 159.19, 160.90, and 164.01 (20 signal for different SP^2 aromatic carbons of substituted Ts groups, and the indolylnicotinonitrile 1b along the cellulose chain).

Characterization of cellulose amine (2c)

Elemental analysis: C; 46.47, H; 5.78, N; 1.60, S; 3.42. IR (KBr, υ/cm^{-1}): 3413 (broad, $NH_{str.}$ and $OH_{str.}$), 2924 (C-$H_{str.}$), 2205 (CN), and 1596 (C=$N_{aromatic}$, and indolyl $NH_{bending}$). ^{13}C-NMR (DMSO-d_6) δ ppm: 21.75 (p-CH_3), 45.72, 46.33 (2 signals for 2 CH_2 of indolylnicotinonitrile 1c), 60.82, 69.63, 73.85, 75.46, 78.34, 102.74 (C-6_{non-Ts}, C-6_{Ts}, C-5, C-4, C-3, C-2, and C-1 of AGU), 107.29 (CN), 86.50, 113.65, 116.08, 119.00, 122.47, 123.56, 127.90, 128.79, 129.13, 130.96, 131.54, 135.44, 138.64, 145.38, 146.98, 151.75, 155.50, 157.98, 160.43, and 163.58 (20 signal for different SP^2 aromatic carbons of substituted tosyl groups, and the indolylnicotinonitrile 1c along the cellulose chain).

Scanning electron microscope SEM

The samples of MCC, TsMCC and 2a-c were imaged using JEOL JXA-840A Eletron probe microanalyzer, by sprinkling onto an aluminum stub that was covered with carbon.

Periodatate oxidation of carboxymethylcellulose CMC:

The oxidation of carboxymethyl cellulose was carried out according to the method described by E. Bordallo *et al.* [22] Where carboxymethyl cellulose (5 g, 24 mmol of AUG) was dissolved in (100 mL) of distilled water by stirring at room temperature for 24h until complete dissolution. Then, $NaIO_4$ (5g, 23.5 mmol) was dissolved in (10 mL) of water and added to the CMC solution drop wise, the pH was adjusted to 3 by sulfuric acid, and the mixture was stirred in the dark at 35°C for 48 h. The excess of periodate was decomposed by adding (20 mL) of ethylene glycol to the reaction mixture and further stirring for 1h to stop the oxidation reaction. Then (500 mL) of ethanol was added to precipitate the product of 2,3-dialdehyde carboxymethyl cellulose (2,3-DACMC), which is filtered off, and washed several times with ethanol/

water (90/10,v/v). 2,3-DACMC was suspended in another (500 mL) of acetone and stirred for 10 min, and filtered off. The white powder was dried under vacuum for 3 h, and at 50 °C for 24 h.

Elemental analysis for 2,3-DACMC: C; 46.76%, H; 5.52%, N; 0%, IR (KBr, υ/cm^{-1}): 3466 (br, OH $_{str.}$), 2930 (C-H $_{str}$), 1738 (CO $_{str.\ aldehydic}$), 1632 (CO $_{carboxyl}$), 889 (hemiacetal bond between the hydroxyl group and the aldehyde group).

Determination of the aldehyde content of 2,3-DACMC

The degree of oxidization of CMC was evaluated by the determination of the aldehyde content by the oxime formation method as described by Kim et. al [30]. Where 0.5 g 2,3-DACMC was dissolved in (5 mL) distilled water and the pH was adjusted to 5 by sodium hydroxide solution. Then, a solution of hydroxylamine hydrochloride (20 mL, 1g, 0.014 mol) was added to 2,3-DACMC solution. The mixture was stirred at 40 oC for 24 h. Then the reaction mixture was titrated against the resulted hydrochloric acid with (1N) NaOH. The volume used from NaOH solution was recorded as V_{sample}. The same procedure was applied on the blank sample of CMC and its consumption of the NaOH solution was expressed as $V_{blank.}$ Thus, the % of aldehyde content (AC) in 2,3-DCMC can be calculated by that equation:

$$AC = M_{NaOH} * (V_{sample} - V_{blank})/ m/211 \times 100.$$

where M_{NaOH} = 1.0 mol/L, m is dry weight of 2,3-DACMC sample in g, and 211 is the molecular weight of oxidized ahydroglucose unit in 2,3-DACMC. The experiment was repeated for three times to take the average.

Schiff`s base formation (3a-c):

(75 mg) of 2-((2-Aminoethyl)amino)-6-(1H-indol-3-yl)-4-aryl-nicotinonitrile (1a-c) was dissolved in (1 mL) of DMF containing (136 µL) of trimethylamine and stirred for 15 min. Then the amine solution was slowly added under stirring to a solution of 2,3-DACMC (1 g, 4.7 mmol) dissolved in (5-7 mL) of distilled H_2O, the ratio of grafting is 13 of 2,3-DACMC:1 of the proper amine wt: wt. the reaction mixture was stirred in darkness at 50 °C until the reaction was completed, where the progress of the reaction was monitored by TLC, and a solution of ninhydrine was used to visualize the unreacted amine. After completing the reaction that evidenced by the disappearance of the heterocyclic amine spot on the TLC, the mixture was poured into (250 mL) of acetone and the resulted precipitate was filtered off, washed with another (250 mL) of methanol, and vacuum dried. The structure of the Schiff base products was confirmed by elemental analysis, IR, and XRD. The solubility difficulties of Schiff`s bases hindered their NMR analysis.

Characterization of Schiff base (3a)

Elemental analysis: C; 43, H; 5.76, N; 1.29, IR (KBr, υ/cm^{-1}): 3376 (br, OH $_{str.}$, NH $_{str.)}$, 2927 (C-H $_{str}$), 2210 (CN), 1606 and 1578 (C=N $_{aromatic}$).

Characterization of Schiff `s base (3b)

Elemental analysis: C; 39.33, H; 5.38, N; 1.22, IR (KBr, υ/cm^{-1}): 3426 (br, OH $_{str.}$, NH $_{str.}$), 2937 (C-H $_{str}$), 2204 (CN), 1638 and (CO $_{carboxyl}$), and 1585 (C=N $_{aromatic}$).

Characterization of Schiff`s base (3c)

Elemental analysis: C; 42.41, H; 3.60, N; 1.55, IR (KBr, υ/cm^{-1}): 3418 (br, OH $_{str.}$, NH $_{str.}$), 2925 (C-H $_{str}$), 2202 (CN), and 1594 (C=N $_{aromatic}$).

X-ray diffraction measurements

The X-ray diffraction profiles for CMC, 2,3-DACMC, and Schiff bases (3a-c) were obtained. The diffraction patterns were recorded using nickel-filtered Cu as target with a secondary monochromator at 45 kV and 30 mA. The samples were scanned in the range of diffraction angle (2θ) from 5^0 to 75^0 with a scanning rate of 20/min at ambient temperature and humidity. The crystallinity index (CrI) was calculated via the equation[30].

$CrI = [(I_{002}-I_{am})/I_{002}]$ where I_{002} is the intensity in the diffraction profile at the position of the 002 peak, and Iam is the intensity at locations for the amorphous background.

The antimicrobial assay

The antimicrobial activity of compounds (1a-c) at concenrations of 20 µg/ ml, MCC-Ts, 2a-c, 2,3-DACMC, and Schiff`s bases (3a-c) at concentrations (200 mg/mL in 50% H_2O/ DMSO) were screened against four different microorganisms including the gram positive (Bacillus subtilis, Staphylococcus aureus), and the gram negative (Escherichia coli) and Candida as (yeast) by well diffusion method[32]. Where the prepared nutrient agar media were inoculated with the microorganism and wells were made on the agar surface with 1mm cork borer. Then (100 µL) of each compound was added to each well in the plates. The plates were incubated at 37C° for 24

hours, the inhibition zones around the wells were measured. Compounds 2,3-DACMC, 3a and 3b were selected as the most promising antimicrobial agents for further screening in order to determine the minimum inhibitory concentration. Each microorganism was seeded by (100 μL) from the different polymer concentrations (50, 100, and 300 mg/mL). The readings were taken in three different fixed directions and the average values were tabulated.

Results and Discussion

Tosylation of cellulose

In this study, we used microcrystaline cellulose MCC with (DP) 225, determined by viscosity measurement for a sample dissolved in copper-ammonium hydroxide solution[25]. 6-Deoxycellulose tosylate (TsMCC) as an active intermediate was prepared following the method described by Rahn et al.[26] Where 6 mole equivalent of p-toluenesulfonyl chloride per anhydroglucose unit was used as a tosylting agent, while trimethylamine was used as a base catalyst. The reaction was carried out under homogenous dissolution system using DMA/LiCl. The reaction was conducted at low temperature (3-8^{o}C) to avoid the formation of 6-chlorodeoxy cellulose which was reported to occur at elevated temperatures[27]. Based on the elemental analysis for the sulfur content, the DSTs value for the tosyl groups on cellulose chains was found to be 0.44. It is worth to mention that, the tosylation reaction usually takes place preferably on the primary hydroxyl group at C-6 of the AGU which is more reactive than the other secondary hydroxyls at C-3 and C-2[26].

The IR spectrum (KBr, υ/cm^{-1}) revealed the existence of characteristic vibrational bands at 1383 for ($SO_{2\ asym}$), and 812 for (S-O-C). The ^{13}C-NMR (DMSO-d_6), demonstrated typical peaks δ (ppm): 21.75 (SP_3, p-CH_3 of Ts group), 56.57, 69.04 for C-$6_{non\text{-}Ts}$, C-6_{Ts}, and 128.30, 130.17, 137.35, and 145.98 that are corresponds to the phenyl ring of the substituted Ts group. So far, the data obtained was compatible with that in literature(12-14,26). With the degree of tosyl substitution 0.44, the resulted TsMCC was soluble in organic solvents including acetone, DMF, dioxin, and DMSO.

Substitution of tosyl groups by 2-((2-aminoethyl) amino)-6-(1H-indol-3-yl)-4-aryl nicotinonitrile (1a-c):

The strategy involves the replacement of the Ts group which is known to be a good leaving and cellulose activating group by the heterocyclic amine (1a-c) using a catalytic amount of triethylamine (Scheme 1). The ratio of grafting between TsMCC: heterocyclic amine was 13: 1 Wt/Wt, 1. The products were confirmed by the elemental analysis, FT-IR, and ^{13}C-NMR.

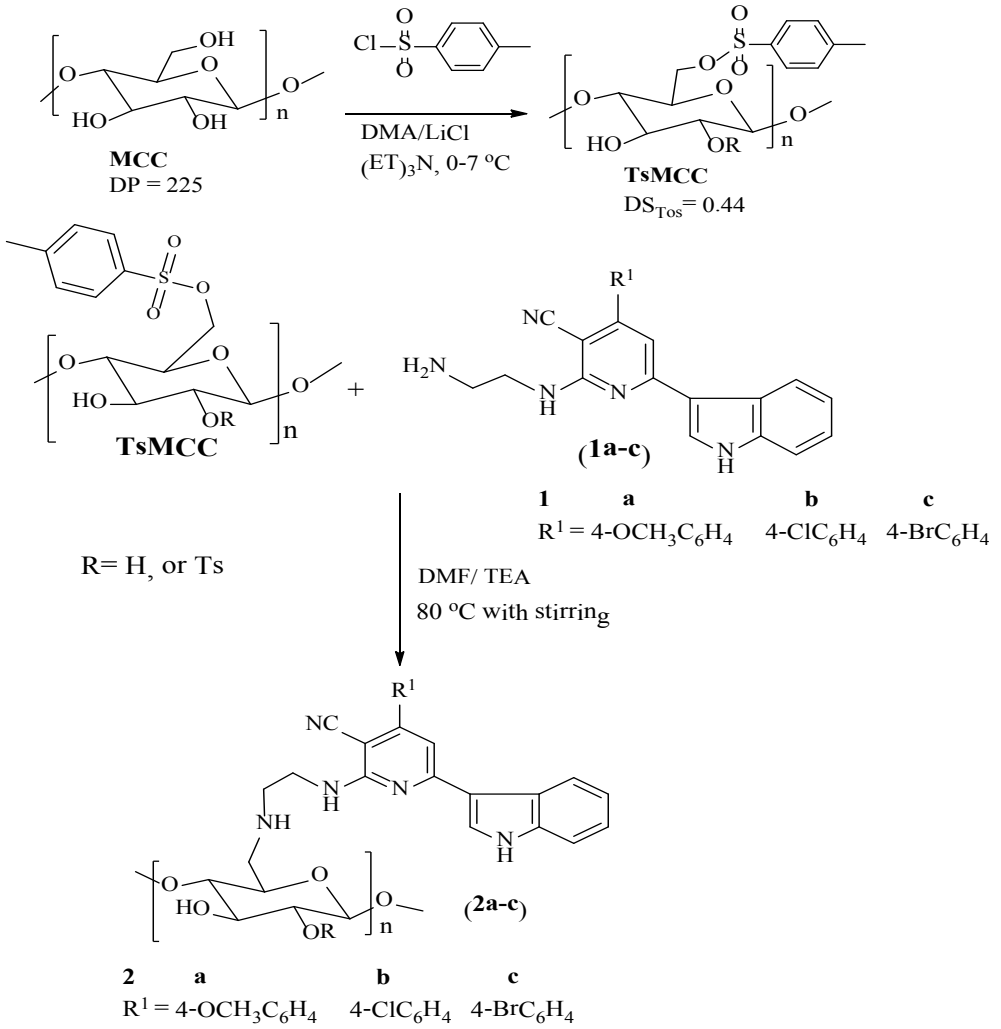
Taking compound (2c) as an example, the decrease of sulfur content and the increase in the nitrogen content reflects the partial substitution of the tosyl groups by the corresponding amine derivative. Additionally, the IR spectrum revealed the presence of stretching vibrational band at 3413 cm^{-1} for (OH, and NH), 2205 cm^{-1} for the (CN) group and the absorption band at 1596 cm^{-1} which was assigned to the ($C{=}N_{aromatic}$). The ^{13}C-NMR (DMSO-d_6) δ (ppm) showed the following signals 21.75 (p-CH_3), 45.72, 46.33 (2 signals for 2 CH_2 of indolylnicotinonitrile 1c), 107.29 for (CN), and the signals from 86.50-163.58 are attributed to 20 signal for different SP^2 aromatic carbons of substituted tosyl groups, and the indolylnicotinonitrile 2c along the cellulose chain).

Characterization by scanning electron microscopy (SEM)

The morphological characteristics of TsMCC, and cellulose amine derivatives (2a-c) were studied and compared to the unmodified MCC. The elongated fibrous structure of MCC changed to porous, interconnected, sheets like structure in TsMCC. In case of cellulose amine derivatives, no notable changes in the morphology in compounds (2a, and b) from TsMCC. However, amino cellulose derivative (2c) showed laminar porous pattern. The tosyl groups and aminoindolyl nicotinonitrile (1a-c) can be noticed on the surface of cellulose polymer chains (Fig. 1).

Oxidation of CMC by sodium periodate

The regioselective oxidation of CMC was carried out by using sodium periodate. It is known that, the periodate is a selective mild oxidizing agent that is usually used to oxidize the vicinal OH at C-2 and OH at C-3 in the AGU[29,30] of cellulose leading to ring


MCC
DP = 225
DMA/LiCl
(ET)3N, 0-7 ºC
TsMCC
DSTos= 0.44
TsMCC
(1a-c)
1 a b c
R1 = 4-OCH3C6H4 4-ClC6H4 4-BrC6H4
R= H, or Ts
DMF/ TEA
80 ºC with stirring
(2a-c)
2 a b c
R1 = 4-OCH3C6H4 4-ClC6H4 4-BrC6H4

Scheme 1


MCC
HV 20.00 kV
mag 2 000 x
spot 4.0
WD 10.8 mm
det BSED
3/1/2016 12:50:13 PM
HFW 207 µm
50 µm
National Research Center Quanta FEG250

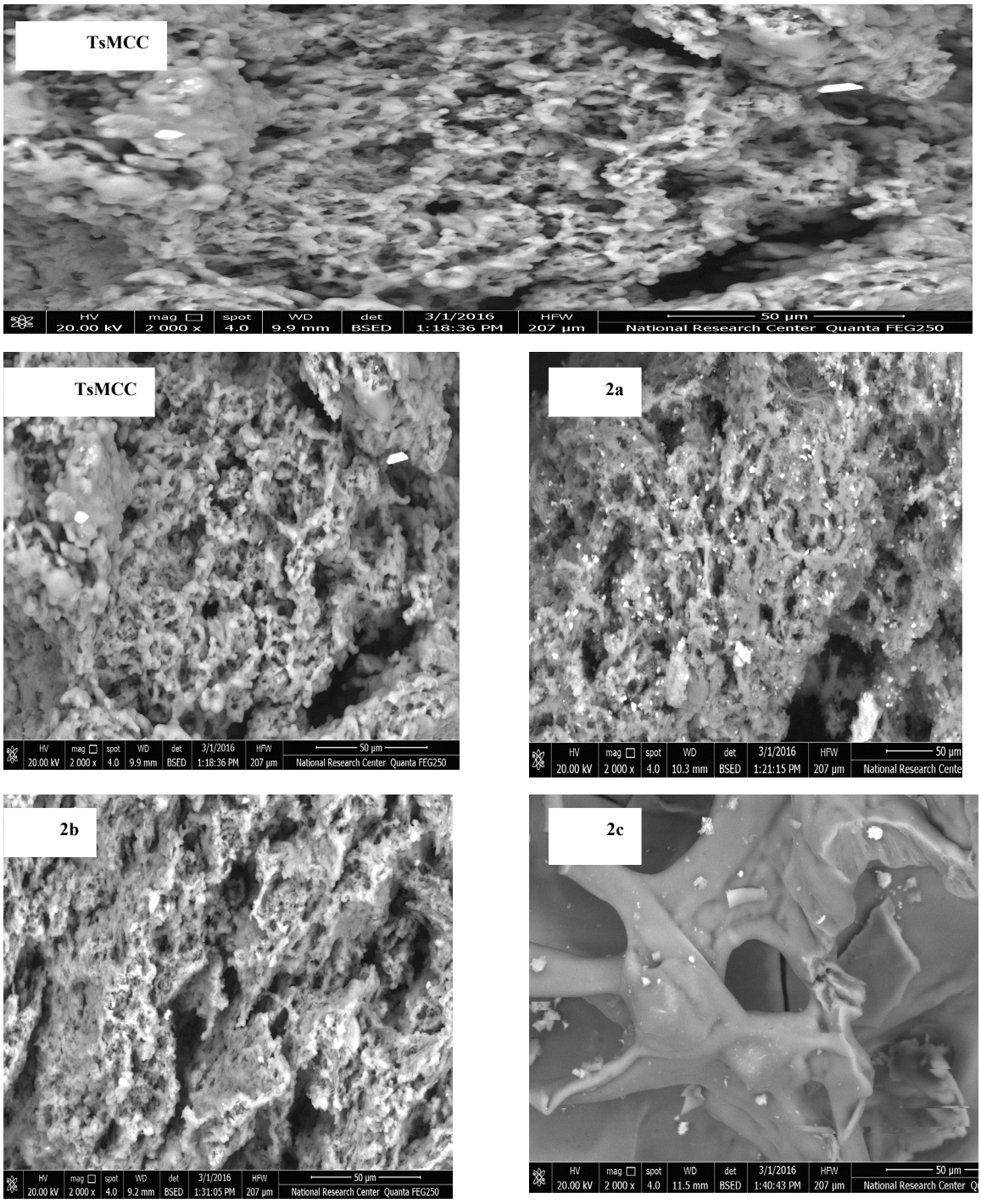
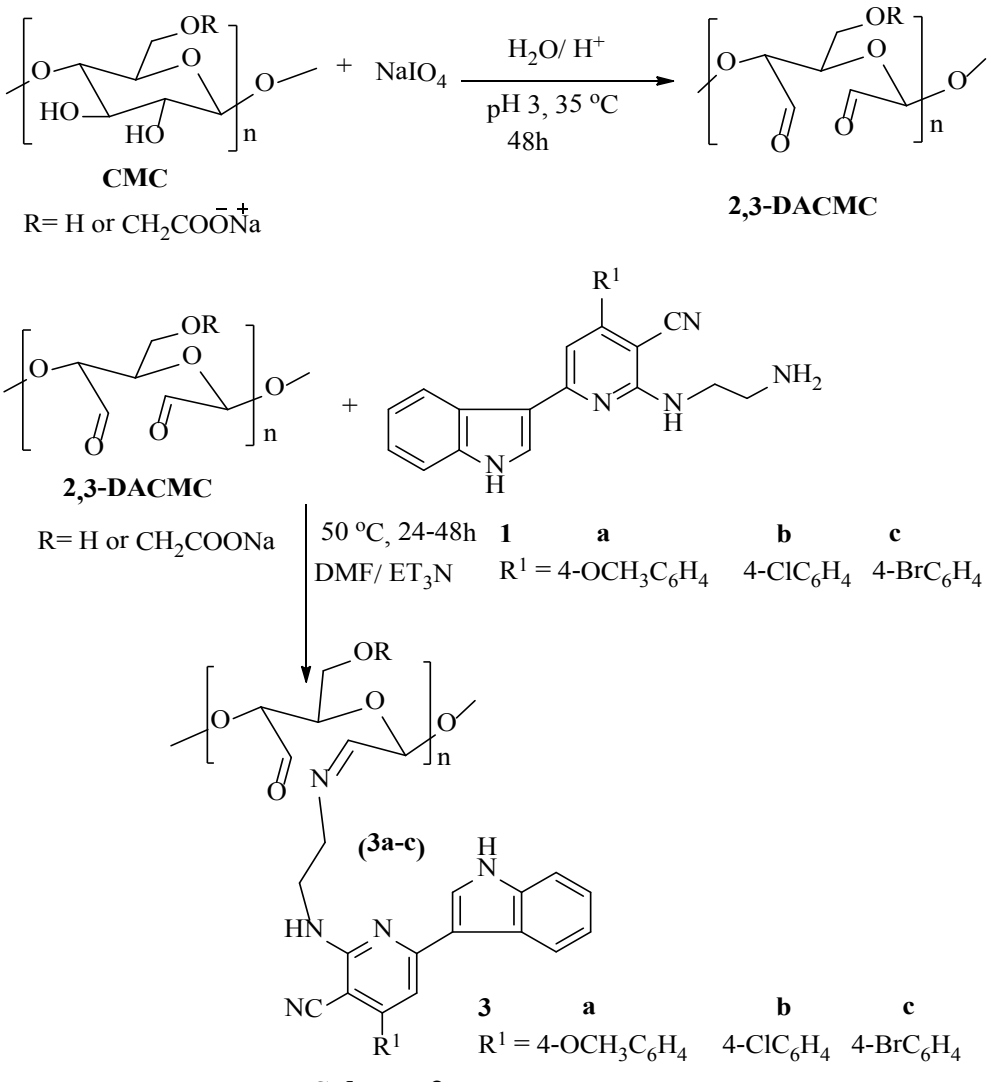


Fig.1. Characterization for MCC, TsMCC, and aminocellulose derivativesd (2a-c) .

opening, by breaking the bond between C-2 and C-3 to obtain the corresponding 2,3-dialdehyde derivative 2,3- DACMC (Scheme 2). Following the method described by E. Bordallo et al.[22] The molar ratio of AGU: periodate was1:1.2 and the oxidation time was continued for 48h at 35^{o}C, (Scheme 2).

The aldehyde content was determined by the oxime formation method described by U. J. Kim *et a1*.[30] and it was found to be 65 %. According to H. Li *et al.*[31] the calculated value for AC is not equal to the whole aldehyde content on the chain due to the formation of hemiacetal bonds between the aldehyde groups, and the neighboring



hydroxyl groups along the CMC backbone. The dialdehyde oxidation of CMC was confirmed by IR that displayed a stretching vibrational band at 1738 cm^{-1} corresponds to the aldehydic carbonyl groups while the band at 889 cm-1 is assigned to the formation of the hemiacetal bond. This was consistent with the reported IR spectra for 2,3-DACMC[22, 29-31].

Schiff`s base formation

2,3-DACMC was used as a precursor for the synthesis of some new antimicrobial hydrogels through the Schiff`s base reactions with the primary amino group of 2-aminoethylamino indolylnicotinonitrile derivatives (1a-c) TEA was used as a catalyst (Scheme 2). The structures of the products have been confirmed by FT-IR, elemental analysis, and XRD.

The IR for the Schiff`s base products (3a-c) revealed the existence of the characteristic vibrational band which is assigned to the (CN) group at 2202 cm^{-1} for (3a), 2204 cm^{-1} for (3b) and 2210 cm^{-1} for (3c). Additionally the appearance of new strong bending band for (N-H) at 1594 cm^{-1} for (3a), 1597 cm^{-1} for (3b) and 1578 cm^{-1} for (3c) .

The X-Ray diffraction study for Schiff's bases.

The effect of periodate oxidation on CMC, and Schiff bases formation (3a-c) on the degree of crystallinity was studied. The sharp peaks in the XRD diffractogram patterns of 2,3-DACMC, and its Schiff`s bases (3a-c) reveals their crystalline nature compared to CMC. Where the XRD pattern of CMC revealed the existence of three characteristic peaks at 2θ of 8.82 (73.79), 20.63o (100), 44.62 (33.20). The

XRD pattern for (2,3-DACMC) showed peaks 2θ of 12.66 (100.00), 15.04 (95.52), 18.42 (63.62), 22.21 (75.46), 25.82 (66.30), 27.83 (50.52), 29.67 (83.75), 30.83 (24.95). for Schiff`s base (3a), 20.71 (78.63), 21.78 (36.83), 27.83 (36.22), 30.27 (100). while Schiff`s base (3b) the sharp peaks at 2θ of 12.73 (23.98), 22.89 (100), 25.79 (30.86), indicated the high crystalline arrangement of the polymer chains. For compound (3c), 20.76 (100), 21.83 (50.96), 27.92 (60.18), 30.32 (85.78), (Fig. 4). The crystalline nature of the Schiff`s bases may be due to the ability of hydrophobically modified 2,3-DACMC chains to be arranged in a well-packed structure. These results indicated that (1a-c) condensed effectively with 2,3-DACMC. The projection of the Schiff bases along the C=N axis resulted in an increase in the interchain distance, improving the organization of cellulose Schiff base [29-31].

Evaluation of the antimicrobial activity:

The antimicrobial activity of (1a-c, at 2 mg/mL), TsMCC, amino cellulose derivatives (2a-c), 2,3-DACMC, and Schiff`s bases (3a-c) were evaluated at concentrations (200 mg/mL) on four different potentially pathogenic microbial

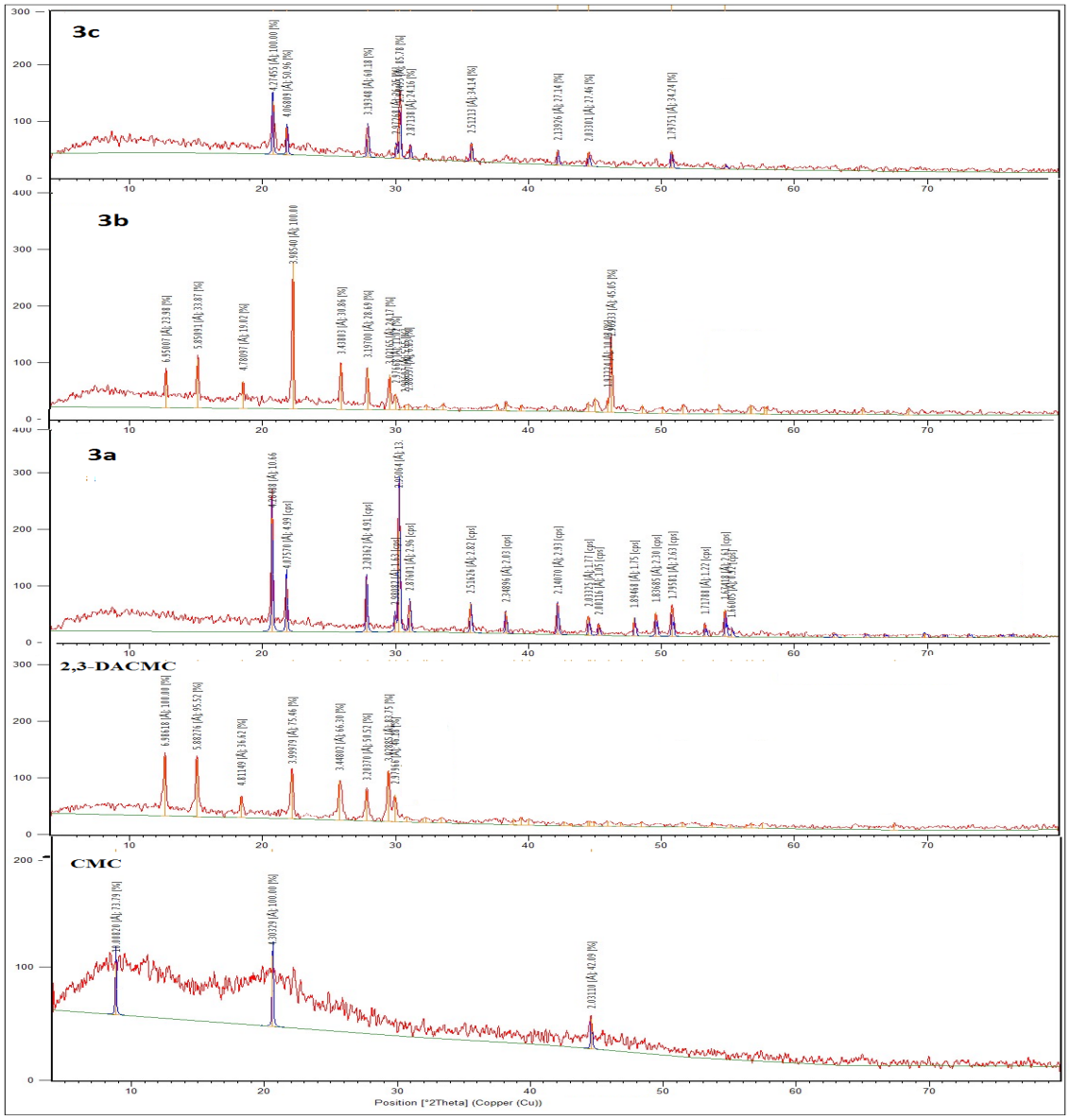


Fig.2. XRD for CMC, 2,3-DACMC, and Schiff`s bases (5-c).

strains namely; Staphylococcus, and *Bacillus subtilis* (gram positive), *Escherichia coli* (gram-negative), and *Candida* (yeast) using well diffusion method the plates were incubated at 37°C for 24 hours and the inhibition zones around the wells were measured.

As we can see that 2-aminoethylamino indolylnicotinonitrile (1a) showed minor activity against *Bacillus subtilis* and *Candida* but they were not active against *Staphylococcus aureus* or *E. coli.* Meanwhile, compounds (1b) and (1c) had mild activity against gram-positive bacteria and candida, Meanwhile, they did not show any antibacterial activity against the gram-negative bacteria *E.coli.* Meaning that their activity is selective to the gram positive bacterial strains. The immobilization of the active heterocyclic amines on the surface of cellulose tosylate retarded the antimicrobial performance of the novel cellulose amine derivatives even at high concentrations (300 mg/ml). On the other hand, 2,3-DACMC and Schiff`s bases 3a and 3b showed moderate, broad spectrum antimicrobial activity, while (3c) had low activity at (200 mg/mL), (Table 1). The minimum inhibitory concentrations were determined for compounds 2,3-DACMC, (3a) and (3b). Where, each microorganism was seeded by 100 µL of each compound at three different concentrations (50, 100, and 300 mg/mL). By increasing the concentration to 300 mg/mL the diameter of inhibition zone increased, decreasing the concentration to 100 mg/mL the activity decreased. There was no activity for the tested compounds at 50 mg/mL. In conclusion, Schiff`s bases (3a) and (3b) showed promising broad spectrum antimicrobial activity in particular at high polymer concentration therefore we can say that conjugating compounds (1a) and (1b) to 2,3-DACMC through Schiff`s base formation increased the antimicrobial spectrum for the conjugates (3a) and (3b), and the potency for Schif`sbases (3a) and (3b). On the other hand, conjugating (1c) to 2,3-DACMC diminished its activity.

Conclusion

In this study, we have introduced new heterocyclic grafted cellulose derivatives. Starting with microcrystalline cellulose, tosyl cellulose intermediates was prepared with DS 0.44 under homogeneous conditions in DMA\LiCl. The tosyl

TABLE 1. Inhibitory zone in (mm) at 2 mg/100µL of the polymer concentration and 200 µg/100µL of (1a-c)).

Compound	*Bacillus subtilis*	*Staphylococcus aureus*	*Escherichia coli*	*Candida*
50% DMSO	-	-	-	-
2,3-DACMC	5.66	3.3	2.5	5
1a	2	-	-	1.33
1b	4.3	3.67	-	3.67
1c	3.3	3.5	-	2.6
3a	5	3.67	5.5	6
3b	4.3	1.67	6.5	-
3c	-	-	1.33	-

TABLE 2. Inhibitory zone in (mm) at different polymer concentration.

Microorganism	Conc. mg/mL	2,3-DACMC	3a	3b
Bacillus subtilis	50	-	-	-
Bacillus subtilis	100	4.5	5.5	4
Bacillus subtilis	300	10	16	15
Staphylococcus aureus	50	-	-	-
Staphylococcus aureus	100	4.5	3.5	4
Staphylococcus aureus	300	5.5	6.5	12
Escherichia coli	50	-	-	-
Escherichia coli	100	1.5	4	5
Escherichia coli	300	6	6	10
Candida	50	-	-	-
Candida	100	4	4	3.5
Candida	300	6	8	5.5

group underwent further nucleophilic replacement with 2-((2-Aminoethyl)amino)-6-(1H-indol-3-yl)-4-aryl-nicotinonitriles derivatives (1a-c) which are reported to exhibit significant biological activity. Moreover, 2,3-DACMC was obtained by periodate oxidation of CMC and condensed with 2-((2-Aminoethyl)amino)-6-(1H-indol-3-yl)-4-aryl-nicotinonitrile, affording Schiff`s bases derivative. The antimicrobial results suggested that none of the prepared tosylated amino cellulose derivatives had antimicrobial activity, while out of the three Schiff`s bases we prepared two of them have shown a good broad spectrum antimicrobial activity. Concluding that, incorporating compound 1a, or b into 2,3-DACMC via the Schiff`s base reaction improved and increased the antimicrobial activity of CMC.

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تحضير مواد سليلوزيه جديده كمضادات ميكروبيه باستخدام مشتقات الأمينو إندوليل نيكوتينونيتريل

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استخدمت مشتقات الأمينو إندوليل نيكوتينونيتريل (١a-c) كقواعد امينيه لتطعيم التوصيل سليلوزالذى تم تحضيره بتفاعل الميكروكريستالين سيليلوز و مركب التولوييين سلفونيل كلوريد فى وجود الترای ايثيل امين عن طريق الإحلال الجزئى لمجموعة التوصيل (Ts) واستبدالها بمجموعة الأمينو إندوليل نيكوتينونيتريل معطيا مشتقات جديدة من الامينو سيليلوز(٢a-c).

و من ناحية اخرى تم اكسدة الكربوكسى ميثيل سليلوز الى ٢,٣-ثنائى ألديهيد كربوكسى ميثيل سليلوز باستخدام الصوديم ميتابيرايودات ($NaIO_4$) بدرجة أكسدة ٢٢٪. ثم تفاعل الـ٢,٣-ثنائى ألديهيد كربوكسى ميثيل سليلوز مع الـ٢-أمينو-٦-إندوليل نيكوتينونيتريل (١a-c) باستخدام الترای إيثل أمين كمحفز قاعدى معطيا مشتقات جديده من قواعد شيف (٣a-c). تم اثبات التركيب البنائى للمواد السليلوزيه المحضرة باستخدام الرنين النووى المغناطيسى , الاشعه تحت الحمراء, المسح الاكترونى الميكروسكوبى, و الاشعة السينيه.

اختبرت فاعلية المشتقات السليلوزيه المحضره (٢a-c) و (٣a-c) كمضادات لنمو البكتريا. مشتقات الامينو سليلوز (٢a-c) لم تكن لها نشاطا مضادا للبكتيريا. بينما أظهرت النتائج ان مركب ٣) a, b) لهما القدره على تثبيط نمو البكتريا (جرام موجب و جرام سالب) و كذالك الكنديدا عند تركيزات ٣٠٠-١٠٠) مج/ مل(و فقدت فاعليتها كمضادات بكتريه عند تركيز ٥٠ مج/ مل, على عكس مشتقات الـ٦-إندوليل نيكوتينونيتريل (١a-c) التى لم تكن لها فاعليه ضد بكتريا جرام سالب.