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New hydrophilic grafting cellulose based on amino-carboxylic acid: Synthesis and antibacterial evaluation

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ABSTRACT

Background: There is a growing interest in developing the antibacterial properties of hydrophilic polymers. **Methods:** In this research, new grafting cellulose based on amino-carboxylic acids are obtained, and their biological activities are evaluated. The sequences are started by treating cellulose with tosylate chloride to get 2, which further reacts with sodium azide. **Results:** To produce compound 3. Azide reduction to amino analog by sodium sulfide produces compound 4. The alkylation with chloroacetic acid compound 5 is obtained with amino-acid residue 5. **Conclusions:** Finally, extensive antibacterial studies have been performed against two types, *Staphylococcus aureus* and *Escherichia coli*. The results demonstrated that the novel derivatives may influence bacteria at low concentrations, and their water solubility could lead to them being biocompatible agents.

Keywords: Antibacterial agents, Antimicrobial Activity, Carbohydrates, Escherichia coli, Staphylococcus aureus.

Introduction

Due to the widespread use of antibiotics in treating infectious diseases caused by microbes, researchers have become interested in the Synthesis of substances that are of natural origin and biologically effective (1). Studies and applications on synthetic and carbohydrate polymers have expanded in the pharmaceutical and biomedical fields. Due to their properties, such as being inexpensive, non-toxic, biodegradable, and biocompatible, as they originate from natural sources (2). Cellulose as a biopolymer is the most abundant natural product that is always renewable from plants. Based on its structure, it consists of glucose units that are attached by glycoside bonds, i.e., β-1, 4-glycosides (3). In addition to the functional groups in the structure of cellulose, its structure can be modified chemically, it can be used in various activation processes to produce more biologically effective molecules (4). However, the large surface area of cellulose fibers and the difficulty of removing moisture makes them a suitable medium for growing microorganisms, so cellulose has been modified by different chemicals for the transfer of antimicrobial activity to cellulosic materials (5).

Cellulose has been chemically modified by replacing the hydroxyl groups with different functional groups (6). In this context, increasing

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resistance of bacterial strains to anti-bacterial agents is an increasing risk of chronic infections and difficulties in their treatments, antimicrobial polymers are used to solve this problem (7). cellulose with triazolyl aldehyde has a high ability to treat infectious diseases because of the link between chemical components of cell bacteria with factional groups of polymers (8). Indeed, the triazole ring showed reasonable biological activities (9).

To bacteria, mainly, *Staphylococcus aureus (S. aureus)* and *Escherichia coli (E. coli)*. These kinds are the greatest risks (10-12). That is the reason for numerous infectious diseases in almost all target cells of the human (13-16)**.** Hence, our attention has been paid to the preparation of cellulose cellulose-modified polymers that are functionalized with triazolyl aldehyde to study the effects of these groups upon attaching to the starch. We chose cellulose as a starting material due to its biocompatibility, low cost, availability, and simple modification methodologies. Furthermore, extensive investigations grafting cellulose polymers on the two bacteria discussed above have been performed.

2.1. Materials and Methods:

All solvents and chemicals were commercially available and, unless otherwise stated, were used as received. Cellulose powder, pyridine, tosyl chloride, acetone, DMF, NaN_3, hydroxybenzaldehyde, sodium carbonate, propargyl chloride, ethanol, sodium ascorbate, and CuCl were provided by Sigma Aldrich Company. All of these chemicals were analytical grade and used without any treatment. The anti-bacterial study was estimated by the agar well diffusion method (17).

2.2. Synthesis of 4-(2-propynyloxy) benzaldehyde:

P-Hydroxy benzaldehyde (5g, 62.5 mmol) was dissolved in acetone (50ml), propargyl chloride (5g, 67.1 mmol), sodium carbonate (5g, 47.16 mmol) was added, and the solution was refluxed for 72 hours at room temperature and monitored by TLC. The residue was dissolved in a mixture of chloroform and washed extensively with water. The organic layer was separated. The mixture was dried with (Na_2SO_4) and filtered, the solvent was removed under a vacuum to obtain the final product as a white powder (3.54g, yield 54%).

2.3. Synthesis of tosyl cellulose:

In a 250 mL round bottom flask, a mixture of cellulose (5g, 30.8 mmol), p-Toluene sulfonyl chloride (TsCl) (7.0 g, 36.71 mmol), and dry pyridine (50 ml) were added. The mixture was stirred for 24 hours at room temperature. The precipitate was filtered off and washed with acetone to remove the remaining organic species. The polymer was washed extensively with distilled water. The air drying of the polymer is weighed to give (3.43 g) (18).

2.3. Synthesis of cellulose azide:

Tosyl Cellulose (5.59 g, 17.14 mmol), and NaN3 (5 g, 76.92 mmol) were dissolved in DMF (50 mL), The mixture was stirred for 72 hours at room temperature, The precipitate was filtered off and washed with acetone to remove the remaining organic species. The polymer was washed extensively with distilled water. Producing white powder (3.2 g) (19).

2.4. Synthesis of cellulose 6-N-ethyl carboxylic acid:

Cellulose-6-amine (1 g) was suspended in acetonitrile, and potassium carbonate was added. The mixture was stirred for 30 minutes. Chloroacetic acid (0.5 g, 1.5 mmol) was added, and the mixture was refluxed for 48 hours. The mixture was cooled down, and the solid was washed with diluted acetic acid and acetone in a filtration apparatus to furnish polymer 5 as a white solid (1.2 g) (19).

Antibacterial activity:

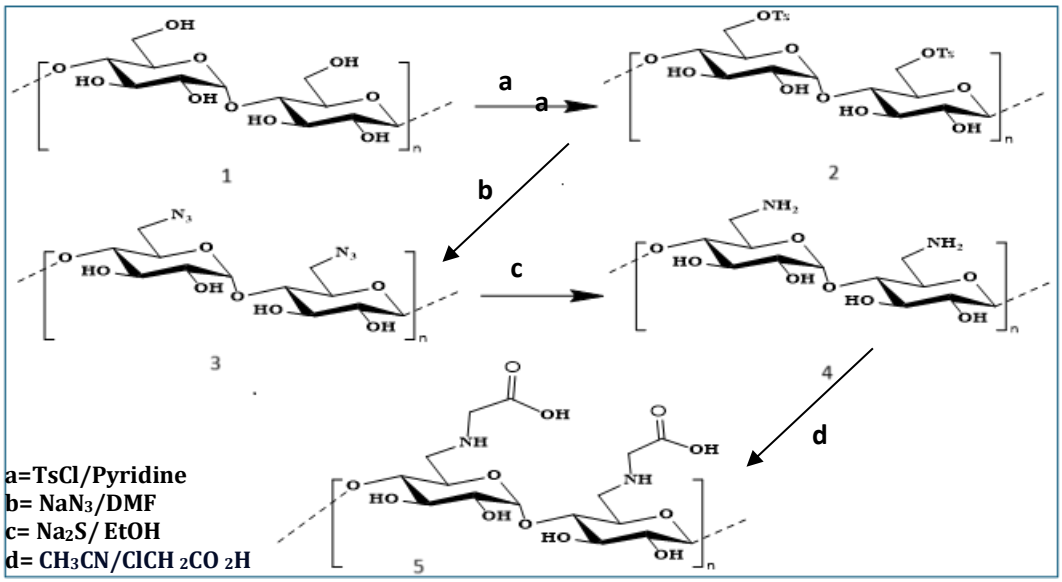
The antibacterial activities of compounds 2,3, and 4 were studied using agar well diffusion methods against bacteria (*E. coli* and *S. aureus*). Five pure isolated colonies of fresh culture were suspended in five milliliters of brain heart infusion broth on the agar plate's surface of Mueller Hinton agar and left at 37°C for (4-8) hours **(20)**. The turbidity produced by growth culture was calibrated with sterile broth to get an optical density Comply with requirements to the 0.5 McFarland. A sterile piece of cotton was dipped in the suspension and then used to streak the entire surface. Then, using a sterile cork Porer, Pores (7 mm diameter) were created and filled with 2,3,4(100 uL) in four concentrations (125, 250,500, and 1000 µg/ml), and the five walls were filled with Amoxycillin as a control. The Petri dishes were then incubated at 37°C for 24 hours. A metric ruler measured the diameter of the growth inhibition zones in millimeters to determine antimicrobial activity **(21)**.

Results and Discussion:

4.1. Synthesis:

The sequences of synthesis of amino groups on cellulose (1) followed the same steps that were applied to the starch analog. The selective tosylation of primary hydroxyls on the cellulose pyranoside glucose units to get (2) is a smooth reaction and produces the tosylation on the specific position. The reaction of 2 with sodium azide under S_N2 reaction using DMF as a solvent at (80 ^oC) furnished the starch polymer with azide groups (3). Furthermore, the reaction of CeN_3 polymer with $Na_2S.9H_2O$ in ethanol is furnished amino-cellulose (4) (Scheme-1).

Finally, the alkylation of amine follows the S_N2 reaction mechanism, and it is accomplished by the reaction of chloroacetic acid with the amino-cellulose (4) in the presence of sodium carbonate, producing the desired polymer 5.



Scheme (1): sequences of synthesis polymer **5**

The FT-IR spectra (Fig.1) depict the entire polymer sequences involved in synthesizing polymer **5** starting from cellulose. The cellulose bands overlap with the tosylate derivative (**2**) bands, making it challenging to distinguish them significantly. However, the creation of cellulose-azide (**3**) by replacing the azide group instead of tosylate is visible with a new band at 2111 cm^{-1}, indicating the stretching vibration of the azide group. Additionally, azide reduction to an amine group is evident from the disappearance of the peak at 2111 cm^{-1}. The N-H band is not visible as it overlaps with the O-H groups in the entire polymer (**4**). Finally, the confirmation of the acetic acid residue grafting onto polymer **5** is supported by the bands at 1638 and 1549 cm^{-1}, representing the stretching vibration of the C=C double bonds of the trityl group

4. 1. SEM of grafting polymer 5.

The SEM image of the polymer **5** sample is represented in Fig (2) The type of aggregation of the polymeric chains resembles the thick sticks that bent in the middle sometimes. As the cellulose itself is a linear polymer that exhibits a stick shape when the chains of the polymer aggregate, the grafting with the chloroacetic acid could cause curved aggregations

4.2. Anti-bacterial study:

The anti-bacterial study was performed on *E. coli* and *S. aureus*, where new materials derived from cellulose were tested due to resistance to antibiotics, which are developed by the two bacteria strains. However, the new derivatives that only differ in the Functional group in the same position gave promising candidates for overcoming the resistance towards other antibiotics. Indeed, the cellulose polymer is compatible with bacterial components of carbohydrates which could assist the interaction with the cell membrane. From Table 1, compounds 2,3, and 4 have a wide spectrum activity in that they inhibited the growth of both Gram-positive and Gram-negative bacteria when compared with standard antibiotics. The inhibition zones increased with increasing the concentration of these compounds.

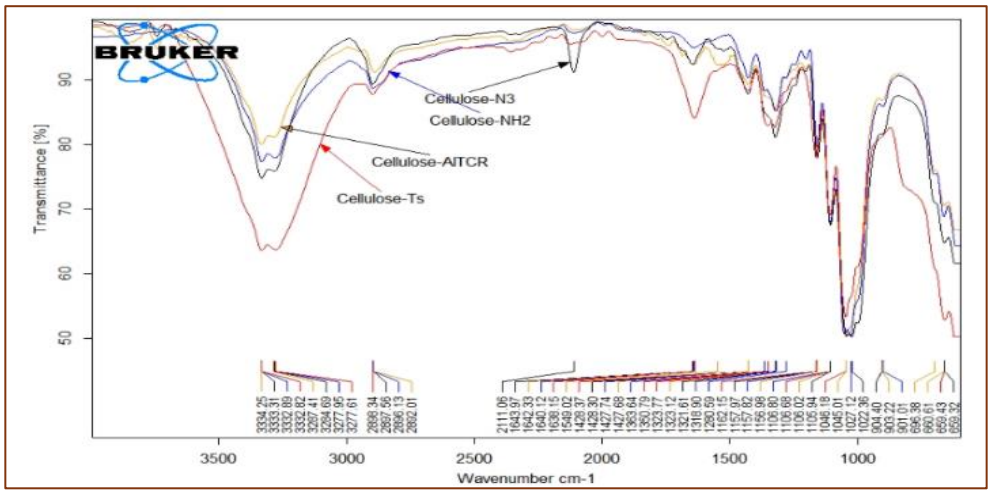


Fig (1): FT-IR spectra of compounds **2**, **3**, **4** and **5**

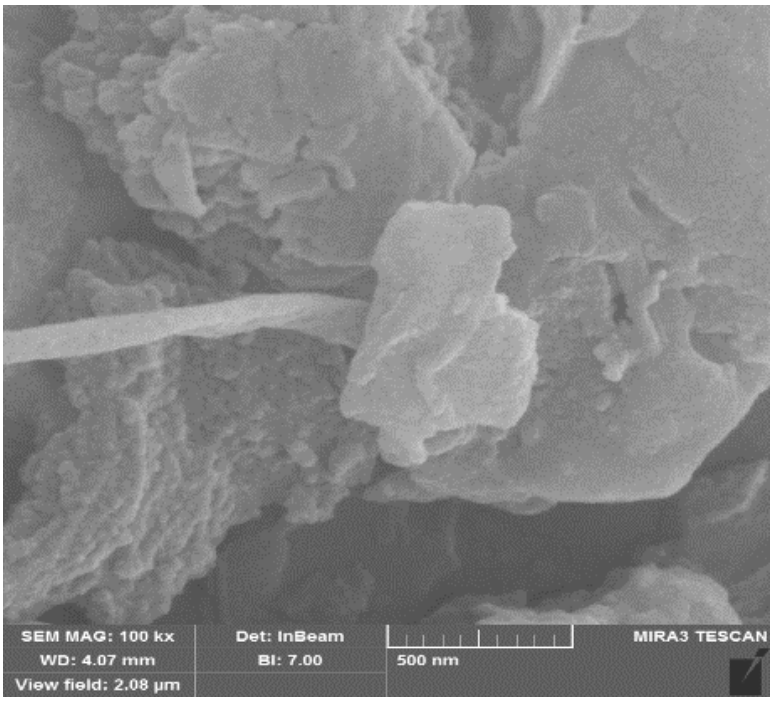


Fig (2): SEM of compound **5**

Table (1) shows the inhibition of the growth of the bacteria (Inhibition Zone) by 2,3,5 recorded in millimeter units.

Compounds & control	**Concentration (µg/ml)**	***Staphylococcus aureus***	***Escherichia coli***
2	125	0	0
2	250	0	0
2	500	7	6
2	1000	13	10
Amoxycillin	1000	20	18
3	125	0	0
3	250	7	0
3	500	11	8
3	1000	17	13
Amoxycillin	1000	19	18
5	125	0	0
5	250	11	7
5	500	16	11
5	1000	22	20
Amoxicillin	1000	20	19

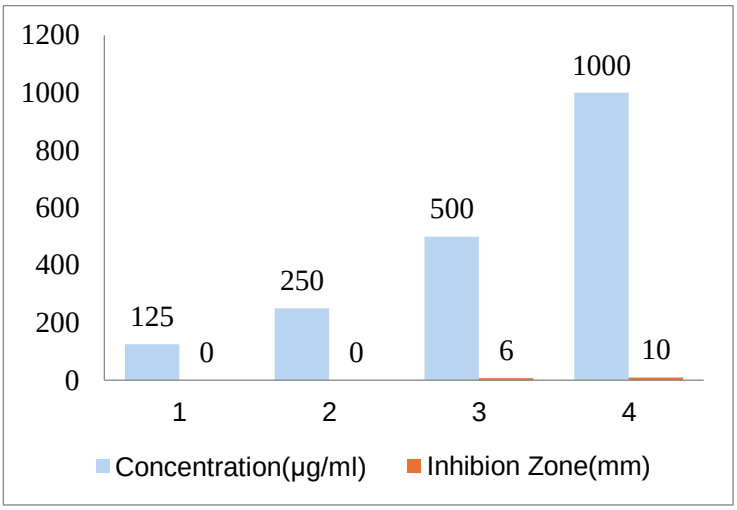


Figure (3): Relationship between Concentration with Inhibition Zone in Bacteria (E. coli) by compound (2)

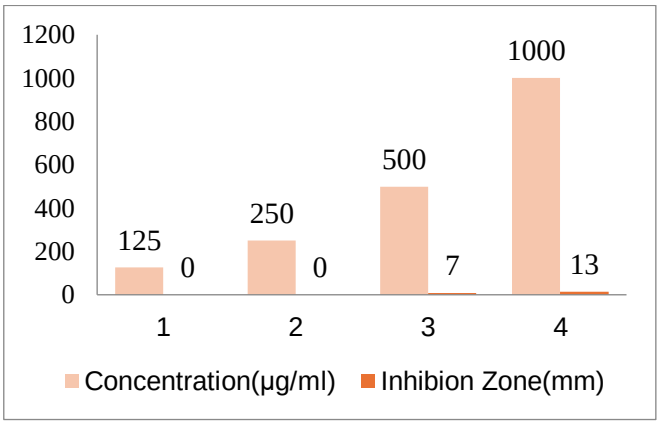


Figure (4): Relationship between Concentration with Inhibition Zone in Bacteria (Staph. aureus) by compound **(2)**

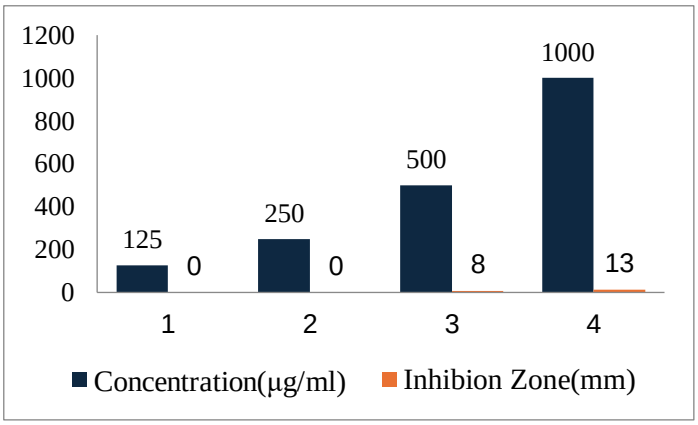


Figure (5): Relationship between Concentration with Inhibition Zone in Bacteria (E. coli) by compound (3)

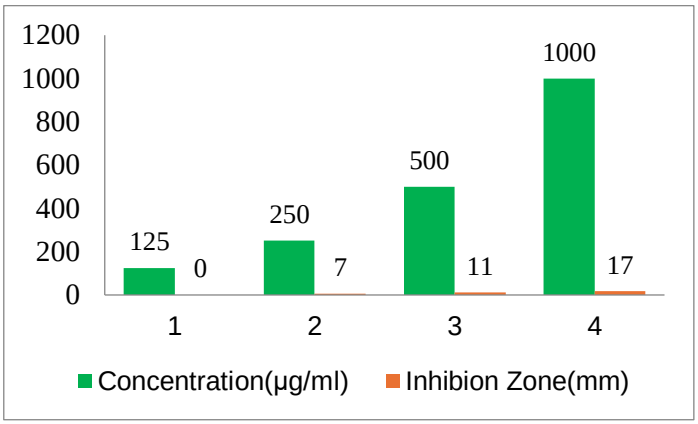


Figure (6): Relationship between Concentration with Inhibition Zone in Bacteria (Staph. aureus) by compound (3)

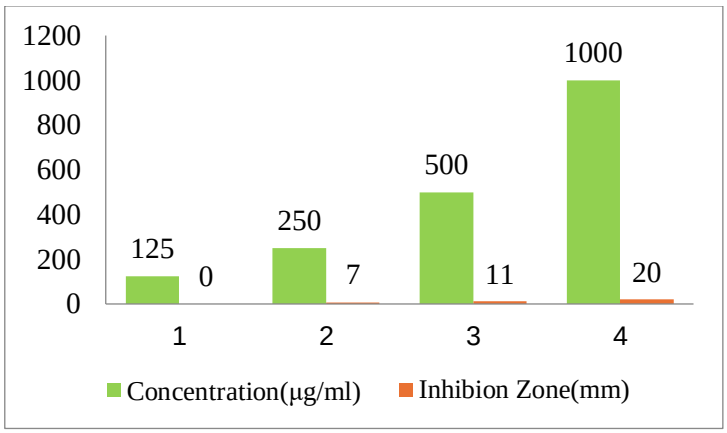


Figure (7): Relationship between Concentration with Inhibition Zone in Bacteria (*E. coli*) by compound (5)

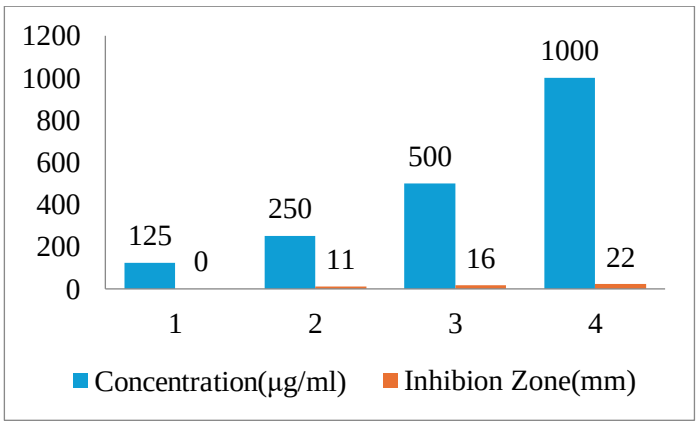


Figure (8): Relationship between Concentration with Inhibition Zone in Bacteria (Staph. aureus) by compound (5)

Ethical disclosures:

In this research, the procedures followed complied with the regulations of the Clinical Research Ethics Committee and the Code of Ethics of the World Medical Association (Declaration of Helsinki). The authors declare that they have followed the protocols of their work center regarding the publication of patient data. The authors declare that they have not used any type of generative artificial intelligence to write this paper.

Conclusion:

In conclusion, the synthesis of newly derivative cellulose with an azide group could be combined directly with propargyl derivative to form triazole for improving anti-bacterial activity; our approach is converting the azide cellulose to the corresponding amine by simple reduction, which will enhance the solubility as well. For that, the resulting grafting polymer with carboxylate exhibits a high ability to inhibit the growth of Gram-negative and Gram-positive bacteria in comparison with amoxicillin in the same dose.

Conflict of interest: NIL

Funding: NIL

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