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Synthesis of Selenium-Containing Chitosan Derivatives and Their Antibacterial Activity

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Abstract—The interaction of chitosan with 3-(chloromethyl)-[1,2,4]selendiazole[4,5-a]pyridin-4 bromide results in water-soluble, selenium-containing, cationic chitosan derivatives. Derivatives of chitosan with degrees of substitution of 0.15, 0.45, and 0.65 were obtained. These derivatives are characterized by a pronounced in vitro antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, and the antibacterial activity of the derivatives increases with an increase in their degree of substitution. The antibacterial activity of the highly substituted derivative is comparable to that of the conventional antibiotics ampicillin and gentamicin.

Keywords: chitosan, selenium, heterocycles, antibacterial activity
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INTRODUCTION

Due to the steady increase in the resistance of pathogens of nosocomial and community-acquired infections to antimicrobial drugs, the development and synthesis of new, effective antibacterial drugs is a high-priority task of modern pharmacology. Chitosan is favorable among the various compounds with antibacterial activity due to its biocompatibility, hypoallergenicity, biodegradability, and lack of toxicity.

Chitosan is a linear, natural polymer composed of glucosamine units that alternate with *N*-acetylglucosamine units. Chitosan derivatives are often characterized by pronounced antibacterial activity [1]. It is known that chitosan derivatives have several mechanisms of antibacterial action at once, which are, in essence, universal physicochemical mechanisms: the electrostatic interaction of a polycation with a negatively charged surface of a bacterial cell, the chelation and binding of ions and nutrients important for a microbial cell, etc. Due to the complex antibacterial effect of chitosan derivatives, the manifestation of bacterial resistance to these polymers in the near future is unlikely, if not impossible [2].

In the complex mechanism of the antibacterial action of chitosan, a key point is the interaction of the chitosan polycation with the negatively charged surface of the bacterial cell. The chitosan polycation forms due to the basic properties of the primary amino groups of the chitosan macromolecule. The electrostatic interaction of such a polycation with a bacterial cell leads to at least two unfavorable consequences for the cell: a sharp change in membrane permeability, which causes an internal osmotic imbalance and, therefore, inhibits microorganism growth. In addition, peptidoglycans are hydrolytically cleaved in the wall of the microorganism, which leads to leakage of the intracellular electrolytes, such as potassium ions, as well as important components of organic nature, such as proteins, nucleic acids, glucose, lactate dehydrogenase, etc. [3]. These processes, which are unfavorable for the bacterial cell, ultimately lead to its death.

Characteristics of chitosan that severely limit its antibacterial effect are its poor solubility in water and low cationic density. The introduction of a cationic substituent can overcome these limitations [4–6]. Usually, chemical modification of chitosan with a substituent containing a quaternized nitrogen atom is used for these purposes.

It is also known that a number of selenium-containing heterocycles are characterized by a pro-

Table 1. Synthesis conditions and degrees of substitution of derivatives

DS	Derivative cipher	T,°C	pH	DS_N	DS_O	MS *	Time
0.15	X-Se-15	60°C	3	0.15	0	1 : 2.2	7 h
0.45	X-Se-45	60°C	3	0.45	0	1 : 6.0	7 h
0.65	X-Se-65	60°C	3	0.50	0.15	1 : 13.5	7 h

* MC is the molar ratio of chitosan: 3-(chloromethyl)-[1, 2, 4]selendiazole[4,5-a]pyridine-4 bromide.

nounced antibacterial effect and pharmacological activity [7]. In this regard, selenium-containing derivatives of chitosan are of undoubted interest as potential antibacterial agents; however, they have not been described in the literature until this study.

The goal of this work is to chemically modify chitosan with a selenium-containing cationic heterocyclic fragment and to study the antibacterial activity of the obtained chitosan derivatives in comparison with the initial chitosan and the antibiotics gentamicin and ampicillin against gram-positive and gram-negative bacteria (*Staphylococcus aureus* and *Escherichia coli*).

METHODOLOGY

Crab chitosan (OOO Bioprogress, Russia) with an average molecular weight of 3.6×10^4, a degree of acetylation of 0.26, a humidity of 8.8%, and 3-(chloromethyl)-[1, 2, 4]selendiazole[4,5-a]pyridine-4 bromide kindly provided by A.G. Tskhovrebov (Peoples' Friendship University of Russia, Russia) was used in the study. The dialysis membranes (MWCO 12000–14000) were purchased from Orange Scientific (Braine-l'Alleud, Belgium). The nuclear magnetic resonance (NMR) spectra of ^1H was recorded on a Bruker Avance instrument (Bruker, United States) II + 400 MHz in a solution of D_2O/CF_3COOH 100/1 at 70°C. The integral intensity of H-1 signals from glucosamine fragments of chitosan and its derivatives was taken as 1.

Selenium-containing derivatives of chitosan were synthesized as follows: 0.1 g of chitosan was dissolved in 10 mL of 1% acetic acid (pH of 3.0), and 2.2, 6.0, or 13.5 equivalents of 3-(chloromethyl)-[1, 2, 4]selendiazole[4,5-a]pyridine-4 bromide. The reaction mixture was sealed, bubbled with argon, and stirred at 60°C for 7 h on a magnetic stirrer. The resulting polymers were precipitated with acetone, washed free of impurities from low-molecular compounds with methanol and diethyl ether, dissolved in distilled water, dialyzed against distilled water for 3 days, and then freeze-dried.

The antibacterial activity of the obtained chitosan derivatives against the strains *S. aureus* RCMB 010027 and *E. coli* RCMB 010051 (collection of microorganisms of Vitebsk State Medical University, Belarus) was studied via diffusion in agar. The commercially available antibiotics used in the comparison were ampicillin (for *S. aureus*) and gentamicin (for *E. coli*) (Aldrich, United States). The activity was determined from measurements of the diameter of the zone of inhibition (in mm). Each zone of inhibition was measured after overnight cultivation in an incubator at 37°C. The experiments were repeated at least three times [8].

RESULTS AND DISCUSSION

Selenium-containing derivatives of chitosan were obtained via chitosan treatment with 3-(chloromethyl)-[1, 2, 4]selendiazole[4,5-a]pyridine-4 bromide (Fig. 1). The reaction was carried out at 60°C (pH of 3) for 7 h.

Variation of the excess of 3-(chloromethyl)-[1, 2, 4]selendiazole[4,5-a]pyridine-4 bromide made it possible to obtain chitosan derivatives with low (0.15), medium (0.45), and high (0.65) degrees of substitution (DS). Upon a slight excess of reagent in the synthesis of low- and medium-substituted derivatives, the reaction proceeded selectively at the amino group of chitosan with the formation *N*-substituted polymers (Fig. 1, reaction I). With the use of a large excess of regent (13.5-fold), highly substituted derivatives formed (DS = 0.65), while the proportion of *N* substitution (DS_N) was 0.50, and the proportion of *O* substitution (DS_O) was 0.15 (Fig. 1, reaction II). The structure of the obtained compounds was confirmed via NMR spectroscopy on ^1N nuclei. Figure 2 shows a typical spectrum.

The antibacterial activity of the obtained derivatives was studied in vitro with the agar-diffusion method. The research results are presented in Table 2.

Table 2. Antibacterial activity of the obtained derivatives

Sample	Microorganism	
	S. aureus	*E. coli*
	zone of inhibition, mm*	
Chitosan	14.3 ± 0.1	11.4 ± 0.3
Ampicillin	30.3 ± 0.1	–
Gentamicin	–	22.2 ± 0.3
B	30.8 ± 0.3	21.4 ± 0.3
C	30.4 ± 0.1	21.6 ± 0.2
X-Se-15	20.6 ± 0.1	12.4 ± 0.3
X-Se-45	26.8 ± 0.3	17.6 ± 0.2
X-Se-65	34.4 ± 0.2	26.3 ± 0.2

* Mean value ± standard deviation ($n = 3$)

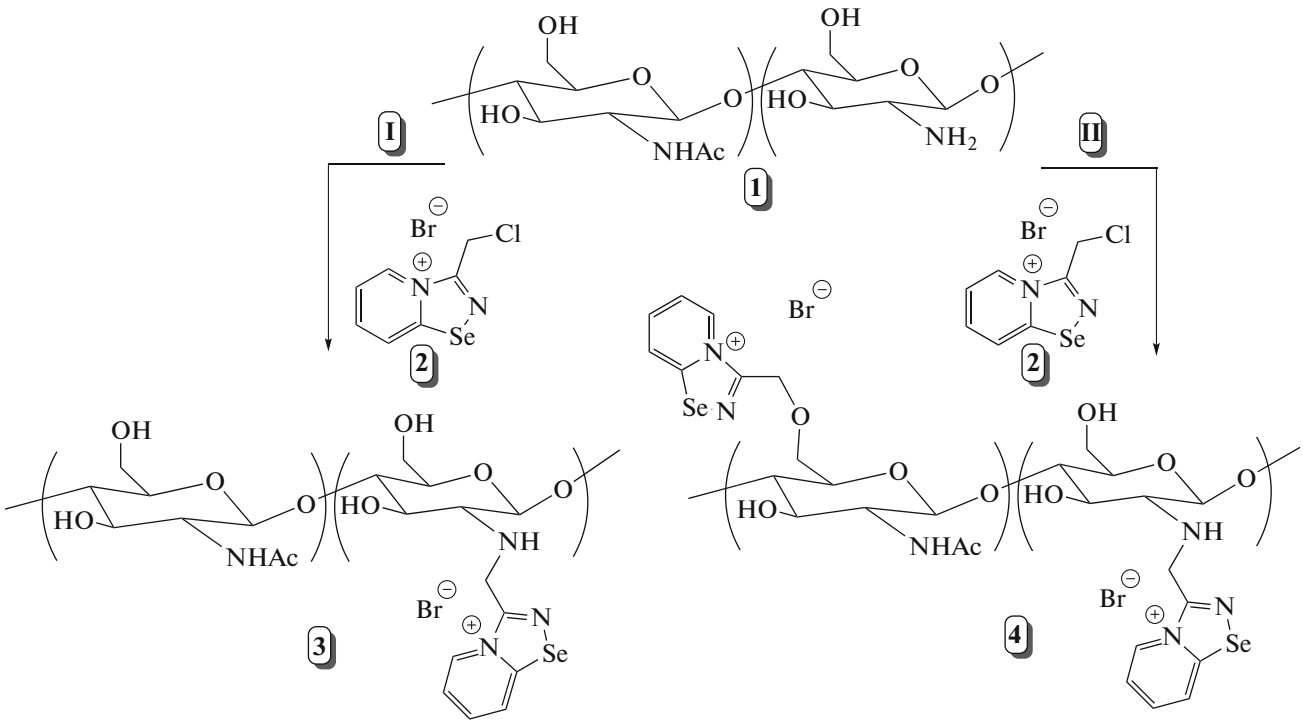

Fig. 1. Synthesis of selenium-containing derivatives of chitosan: 1, chitosan; 2, (chloromethyl)-[1, 2, 4]selenadiazole[4,5-a]pyridinium bromide; 3, *N*-([1, 2, 4]selenadiazole[4,5-a]pyridin-3-ylmethyl) chitosan; 4, *N, O*-([1, 2, 4]selenadiazole[4,5-a]pyridin-3-ylmethyl) chitosan; I, reaction with a slight excess of the reagent 3-(chloromethyl)-[1, 2, 4]selendiazole[4,5-a]pyridine-4 bromide; II, reaction with a large excess of the reagent 3-(chloromethyl)-[1, 2, 4]selendiazole[4,5-a]pyridine-4 bromide.

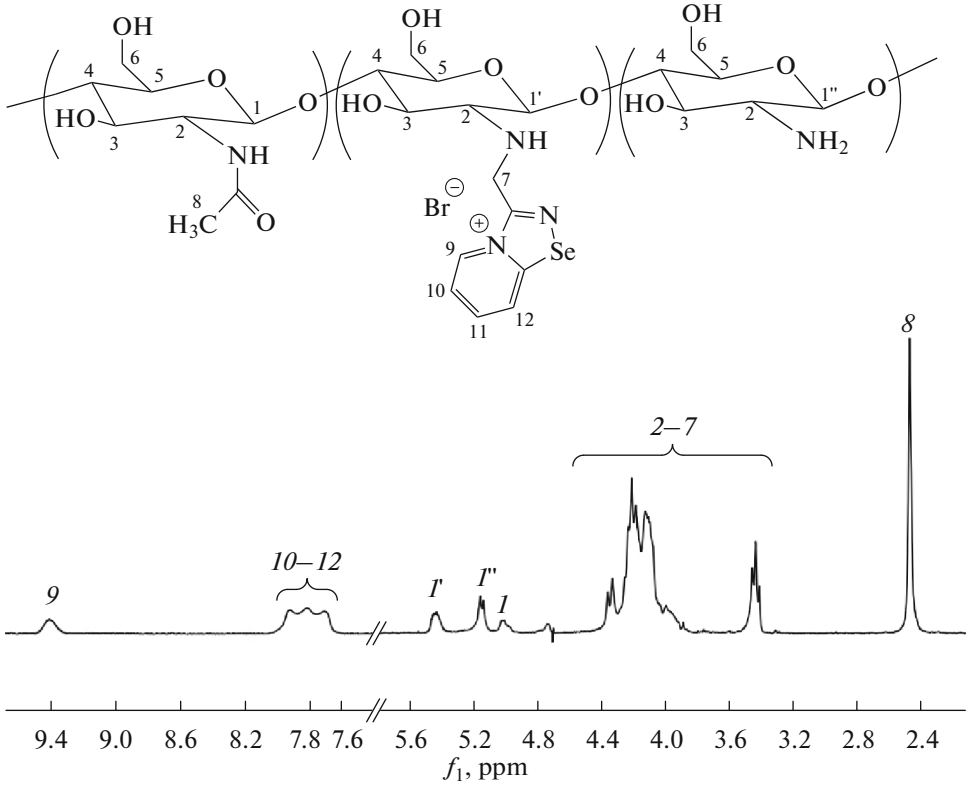

Fig. 2. Nuclear magnetic resonance spectrum of *N*-([1, 2, 4]selenadiazole[4,5-a]pyridin-3-ylmethyl) chitosan with a degree of substitution of 0.45 (X-Se-45).

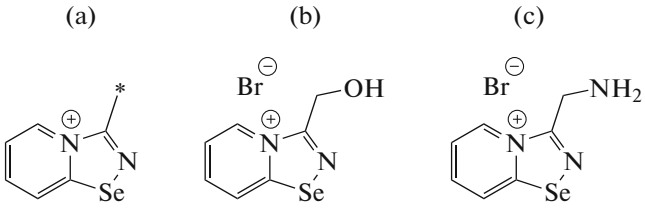

Fig. 3. Substituent and the corresponding low molecular weight compounds: a, 3-methylene-[1, 2, 4]selenadiazole[4,5-a]pyridinium; b, 3-(hydroxymethyl)-[1, 2, 4]selenadiazole[4,5-a]pyridinium bromide; c, 3-(aminomethyl)-[1, 2, 4]selenadiazole[4,5-a]pyridinium bromide.

The data given in Table 2 indicate that the antibacterial activity of all of the obtained chitosan derivatives exceeded that of the initial chitosan. In this case, the antibacterial effect of the derivatives increased with an increase in the degree of substitution. On the one hand, this can be explained by the increase in cationic density upon an increase in the proportion of the cationic substituent in the macromolecule. On the other hand, it is possible that the introduced heterocyclic, selenium-containing substituent is itself characterized by the presence of a pronounced antibacterial effect. To test this assumption, the antibacterial activity of low molecular weight compounds **b** and **c**, which correspond to the introduce substituents, was studied (Fig. 3).

CONCLUSIONS

It has been shown that the low molecular weight compounds **b** and **c**, which are characterized by pronounced antibacterial activity against both *S. aureus* and *E. coli*, and their antibacterial activity is comparable to that of the antibiotics ampicillin and gentamicin. It should also be noted that the highly substituted selenium-containing chitosan derivative X-Se-65 was characterized by an antibacterial effect exceeding that of ampicillin and gentamicin. This fact can be explained by the symbatic effect of the introduction of the antibacterial, selenium-containing pharmacophore and polymer chain into the chitosan matrix. Apparently, this is due to the fact that the polymer chain is capable of assuming a conformation that ensures its strongest binding to the membrane of a microbial cell in comparison with a low-molecular-weight compound and, therefore, leads to a more pronounced dysfunction of the cell membrane.

Thus, as a result of the work, a highly active, antibacterial, cationic, selenium-containing chitosan derivative was obtained, and it is of undoubted interest for further in vivo research.

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COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflicts of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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REFERENCES

1. Seidi, F., Yazdi, M.K., Jouyandeh, M., Dominic, M., Naeim, H., Nezhad, M.N., Bagheri, B., Habibzadeh, S., Zarrintaj, P., Saeb, M.R., and Mozafari, M., *Int. J. Biol. Macromol.*, 2021, vol. 183, pp. 1818–1850.
2. Tien, N.D., Lyngstadaas, S.P., Mano, J.F., Blaker, J.J., and Haugen, H.J., *Molecules*, 2021, vol. 26, no. 9. https://doi.org/10.3390/molecules26092683
3. Ke, C.L., Deng, F.S., Chuang, C.Y., and Lin, C.H., *Polymers*, 2021, vol. 13, no. 6. https://doi.org/10.3390/polym13060904
4. Kritchenkov, A.S., Egorov, A.R., Dysin, A.P., Volkova, O.V., Zabodalova, L.A., Suchkova, E.P., Kurliuk, A.V., and Shakola, T.V., *Int. J. Biol. Macromol.*, vol. 137, pp. 592–603.
5. Kritchenkov, A.S., Egorov, A.R., Artemjev, A.A., Kritchenkov, I.S., Volkova, O.V., Kurliuk, A.V., Shakola, T.V., Rubanik, V.V., Tskhovrebov, A.G., Yagafarov, N.Z., and Khrustalev, V.N., *Int. J. Biol. Macromol.*, 2020, vol. 143, pp. 143–152.
6. Kritchenkov, A.S., Egorov, A.R., Volkova, O.V., Kritchenkov, I.S., Kurliuk, A.V., Shakola, T.V., and Khrustalev, V.N., *Int. J. Biol. Macromol.*, 2019, vol. 139, pp. 103–113.
7. Semenov, K.N., Charykov, N.A., Keskinov, V.A., Kritchenkov, A.S., and Murin, I.V., *Industr. Eng. Chem. Res.*, 2013, vol. 52, no. 46, pp. 16095–16100.
8. *Bioassay Techniques for Drug Development,* Rahman, A.-U., Choudhary, M.I., and Thomson, W.J., Eds., Harwood Acad. Publ., 2005.