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Mendeleev Commun., 2022, **32**, 357–359

Mendeleev Communications

Synthesis and *in vitro* antifungal activity of selenium-containing chitin derivatives

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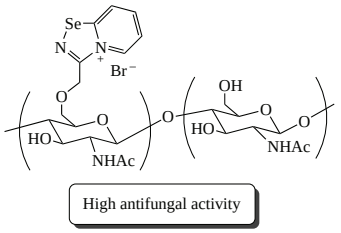
DOI: 10.1016/j.mencom.2022.05.022

Mild and 'green' ultrasound-assisted reaction of chitin with 3-(chloromethyl)[1,2,4]selenadiazolo[4,5-*a*]pyridin-4-ium bromide in water affords novel selenium-containing cationic chitin derivatives. The thus obtained chitin derivatives are water soluble and are characterized by high *in vitro* antifungal activity comparable with conventional antifungal drug Amphotericin B.

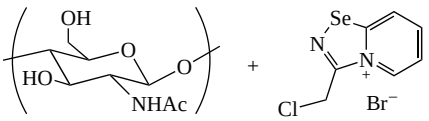


Keywords: organoselenium compounds, chitin, alkylation, [1,2,4]selenadiazolo[4,5-*a*]pyridin-4-ium salts, ultrasound, antifungal activity.

Chitin is a renewable biocompatible, biodegradable and non-toxic polymer being the second most common natural polymer after cellulose. Also, chitin is characterized by the absence of immunogenicity and carcinogenicity. These circumstances make chitin an attractive source for obtaining new compounds with useful physicochemical and biological properties. To impart new properties to chitin, its chemical modification is required. However, approaches to chemical modification of chitin are scarcely developed, especially in the preparation of water-soluble derivatives with biological and pharmacological activities. Until recently, only few examples of water-soluble derivatives of fully *N*-acetylated chitin were described, namely, tosyl chitin (with less than 30% of tosylated units),[1] diethyl aminoethyl chitin,[2] hydroxypropylated chitin,[3] 6-deoxy-6-amino chitin,[4] chitin phosphate,[5] propyltrimethylammonium chitin,[6,7] and succinylated chitin.[8] This may be explained by (1) low reactivity of chitin macromolecule and (2) difficulties in the chemical modification of chitin due to its insolubility in most common solvents.

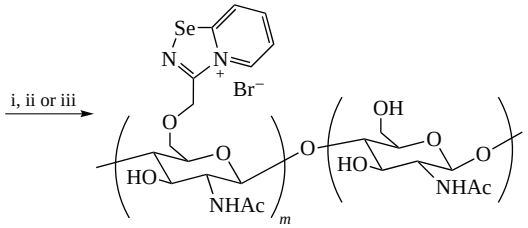
On the other hand, organoselenium compounds are characterized by a number of attractive biological activities, among which there is fungicidal effect.[9] The search for new highly effective fungicidal compounds is an important task, which is indicated by the increasing number of highly cited publications in this area.[10]

It should be noted that the literature does not describe chitin derivatives containing grafted selenium-containing substituents. Within the frame of this work, we made the first attempt to obtain chitin derivatives of this type. Herein, we prepared the first selenium-containing chitin derivatives by the O-alkylation of chitin **1a–c** with 3-(chloromethyl)[1,2,4]selenadiazolo[4,5-*a*]-pyridin-4-ium bromide **2** (Scheme 1), keeping in mind that



1a–c **2**

a *M* = 35000
b *M* = 71000
c *M* = 172000



3a–c, **3′a–c**, **3″a–c**

3a–c DS ~ 0.16 (for i)
3′a–c DS ~ 0.41 (for ii)
3″a–c DS ~ 0.64 (for iii)

Scheme 1 *Reagents and conditions*: i, **1**/**2** = 3 (equiv./equiv.), water, 25 °C, sonication (90 kHz, 250 W), 25 min; ii, the same, **1**/**2** = 5 (equiv./equiv.); iii, the same, **1**/**2** = 8 (equiv./equiv.).

selenadiazoles are promising antifungal agents.[11] In our experiments, we used crab shell chitin samples **1a–c** with a viscosity-average molecular weight M of 3.5×10^4, 7.1×10^4 and 17.2×10^4, respectively, and a degree of acetylation of 100% (Sigma Aldrich, USA).

The O-alkylation of chitin is usually performed in *N*,*N*-dimethylacetamide/LiCl solvent system. The dissolving of chitin in this system is laborious while such an alkylation requires anhydrous and often anaerobic conditions. However, in some cases, the O-alkylation of chitin can be performed in water under heterogeneous conditions when the nucleophilicity of chitin is improved by adding concentrated alkali. Therefore, we treated chitin samples **1a–c** with 3-(chloromethyl)[1,2,4]selenadiazolo[4,5-*a*]pyridin-4-ium bromide **2** in the presence of NaOH. However, we were able to achieve only low degree of substitution (*ca*. 0.16) even upon using large excess of chitin (6 equiv.). Raising the temperature from 25 to 70 °C did not lead to any noticeable increase in the degree of substitution but accelerated hydrolysis of reactant **2**.

The use of sonochemistry method often results in promotion of many polymer-analogous transformations of natural polysaccharides, including chitin and chitosan.[12–18] In sonochemistry, alkylation of alcohols leading to ethers is thoroughly studied, and acoustic intervals for this reaction are recommended as 15–150 kHz and 80–350 W.[19] In this work, we found that the optimum acoustic conditions for coupling between **1a–c** and **2** in water were at 90–100 kHz and 250–300 W. Importantly, the ultrasound-assisted reaction takes only 25 min and does not require addition of NaOH. The reaction results in high-, moderate- or low-substituted products **3** depending on the **1**/**2** molar ratio. Thus, starting from chitins **1a–c** of low (3.7×10^4 Da), medium (6.9×10^4 Da) and high (17.8×10^4 Da) molecular weight, respectively, we synthesized new chitin derivatives **3a–c**, **3′a–c** and **3′′a–c** with low (*ca*. 15), medium (*ca*. 40) and high (*ca*. 60) degree of substitution, respectively (see Scheme 1). The resultant polymers are summarized in Table 1.

The obtained derivatives **3** are soluble in water. Their ^{1}H NMR spectra (Figure 1) confirm that the obtained polymers are the chemoselective O-substituted chitin derivatives, hence no side partial deacetylation occurs.

The reported data provide only few examples for behavior of chitin under ultrasonic irradiation, *e.g.*, on partial chitin depolymerization and deacetylation.[20,21] However, other sources[13] state that ultrasonic treatment of chitin does not cause deacetylation or partial depolymerization of the polysaccharide backbone. These seeming contradictions indicate that the ultrasound-mediated degradation of chitin is a very complex and controversial problem, which depends on a great variety of factors and it should be studied in each case individually. To

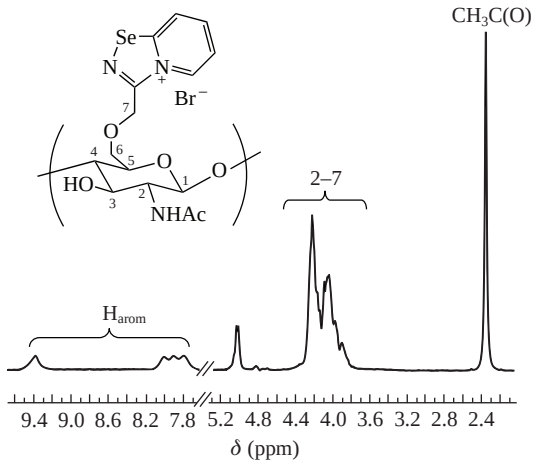


Figure 1 Typical ^{1}H NMR spectrum of the selenium containing derivative (**3′′**).

estimate the possibility of depolymerization or deacetylation of chitin under the conditions used for promotion of chitin alkylation, we treated chitin aqueous suspension by ultrasound (90 kHz, 250 W, 15 min). The viscosity-average molecular weight of thus obtained material did not essentially change. Moreover, ^{1}H NMR spectra of the synthesized new chitin derivatives demonstrated that the degree of acetylation was *ca*. 100%, additionally indicating that deacetylation of chitin did not proceed under these ultrasonic conditions.

It is important to note that the developed synthetic procedures of ultrasound-assisted preparation of the chitin derivatives are in accordance with the principles of green chemistry, since nature-friendly reactants and water as a solvent were used.

Next, we evaluated the *in vitro* antifungal effect of the synthesized selenium containing derivatives **3** against fungi *A. fumigatus* and *G. candidum* compared to the commercially available antifungal drug Amphotericin B using the conventional agar diffusion method. Diffusion of an antifungal compound into the agar inhibits the growth of the fungi inoculated on the agar which results in the appearance of inhibition zone around the site of application of the antifungal compound. All synthesized polymers **3** exhibit antifungal activity higher than the starting chitin (see Table 1). Antifungal activity of the derivatives is increased with the increase of their degree of substitution. This can be explained by increase in cationic density of the polymer. The fact is that polycations effectively bond to negatively charged moieties of the fungal cell wall provoking its damage, osmotic imbalance, and disruption of ion pumps. The cascade of the mentioned unfavorable events inevitably results in the death of fungi. Moreover, data in

Table 1 Characteristics, antifungal activity and toxicity of the prepared selenium-containing chitin derivatives.

Sample	M of the starting chitin /×10^4	DS of the derivative	Inhibition zone/mm		Cell viability (%)
			A. fumigatus	*G. candidum*	
Chitin	7.1	–	11.8 ± 0.1	9.9 ± 0.1	99
3a	3.5	0.16	15.2 ± 0.3	12.4 ± 0.1	97
3b	7.1	0.16	16.5 ± 0.1	15.1 ± 0.3	97
3c	17.2	0.15	14.3 ± 0.2	12.2 ± 0.1	97
3′a	3.5	0.41	22.4 ± 0.2	18.2 ± 0.3	96
3′b	7.1	0.41	23.8 ± 0.2	20.5 ± 0.1	97
3′c	17.2	0.43	21.1 ± 0.1	17.8 ± 0.1	97
3′′a	3.5	0.65	27.3 ± 0.3	24.3 ± 0.3	95
3′′b	7.1	0.64	28.5 ± 0.1	26.5 ± 0.3	95
3′′c	17.2	0.64	26.1 ± 0.4	22.1 ± 0.2	95
Amphotericin B	–	–	28.7 ± 0.3	26.4 ± 0.1	37

Table 1 clearly show that derivatives of moderate molecular weight are slightly more effective than those of high and low molecular weights.

The *in vitro* toxicity of the synthesized conjugates was evaluated using HEK-293 cell culture as a conventional model of mammalian cells using MTT-test, a colorimetric technique for evaluating the number of viable cells in culture. The essence of this technique is that NADPH-dependent dehydrogenase of viable cells reduces the colorless compound MTT, *viz*. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, converting it into the intensely violet-coloured formazan. Thus, the viability of the cells in their culture is proportional to the intensity of the violet colour. Cell viability in the case of prepared derivatives is more than 90%, while in the case of Amphotericin B this value is 37% (see Table 1). Consequently, the resulting polymers are significantly less toxic than Amphotericin B.

In conclusion, we developed the convenient and effective way to preparation of the novel selenium containing chitin derivatives, and this way corresponds to the concept of green chemistry. The study on antifungal activity of the prepared polymers showed that the most effective derivative had moderate molecular weight and high degree of substitution (sample **3′′b**), its antifungal activity was comparable with that of Amphotericin B. Moreover, unlike Amphotericin B, sample **3′′b** is characterized by low toxicity. Undoubtedly, substance **3′′b** can be of interest for the further *in vivo* studies, which are underway in our group.

This study was supported by the RUDN University Strategic Academic Leadership Program and by the Russian Science Foundation (grant no. 21-76-00002).

Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2022.05.022.

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Received: 27th September 2021; Com. 21/6705