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Synthesis of water soluble chitosan derivatives with halogeno-1,2,3-triazole and their antifungal activity

Qing Li [a], Wenqiang Tan [a,b], Caili Zhang [c], Guodong Gu [d], Zhanyong Guo [a,*]

[a] Key Laboratory of Coastal Biology and Bioresource Utilization, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, Shangdong 264003, China
[b] Graduate School of Chinese Academy of Sciences, Beijing 100039, China
[c] School of Chemistry and Chemical Engineering, Yantai University, Yantai 264005, China
[d] Alliance Pharma, Inc. 17 Lee Boulevard Malvern, PA 19355, USA

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ABSTRACT

Chitosan is an abundant and renewable polysaccharide, which exhibits attractive bioactivities and natural properties. Improvement such as chemical modification of chitosan is often performed prior to further utilization. Three novel water soluble chitosan derivatives containing 1,2,3- triazole with or without halogen was designed and synthesized. Their antifungal activity against three kinds of phytopathogens was estimated by hyphal measurement *in vitro*. The inhibitory property and water solubility of the synthesized chitosan derivatives exhibited a remarkable improvement over chitosan. It is hypothesized that thiazolyl groups enable the synthesized chitosan to possess obviously better antifungal activity. Moreover, **CTCTS** and **BTCTS**, which have halogens at the periphery of polymers, inhibited the growth of tested phytopathogens more effectively with inhibitory indices of 81–93% at 1.0 mg/mL. The halogens could have a synergistic effect with triazole as they exhibited antifungal activity and electron-withdrawing capacity, which improve the antifungal activity of chitosan derivatives.

1. Introduction

Chitosan is one of the most abundant natural polysaccharides and has attracted broad attention due to its antifungal activity against various groups of pathogenic fungi [1–6]. The antifungal ability, coupled with its non-toxicity, biodegradability, and biocompatibility, facilitates emerging applications of chitosan in food science, agriculture, medicine, and textile areas [7]. Another advantage of chitosan, which makes it highly desirable, is the capability of being chemically modified [8]. Through chemical modification, a great deal of chitosan derivatives have been prepared to promote their biological activity [9–13]. However, the application of chitosan is restricted to only acidic conditions where the NH_2 group becomes protonated [14,15]. The further enhancement of the antimicrobial activity of chitosan over a broader pH range will promote the better application of chitosan in many areas.

Triazole derivatives represent an interesting class of heterocyclic compounds; they possess many biological activities such as antimicrobial, anti-tubercular, anti-inflammatory and anticancer activities [16–20]. As an important type of nitrogen-containing aromatic heterocyclic compounds, 1,2,3-triazole was regarded as the bioisosteric replacement of itsbioisoster 1,2,4-triazole, amide, or even other nitrogen-containingaromatic heterocycles [21,22]. 1,2,3-Triazole was stable to metabolic degradation and capable of forming hydrogen bonds, which could favor binding to biomolecular targets and improve solubility [23]. The team of Ifuku prepared new chitosan derivatives containing triazolyl moieties at the C_6 position of glucosamine units by coupling between azide and propargyl groups of chitosan via a 1,3-dipolar cycloaddition [24–26]. Novel 1,2,3-triazole-linked starch derivatives synthesized via 'click chemistry' exhibited improved antibacterial property and antioxidant property [21,27]. These observations inspired us to modify chitosan with triazole as a substituent to improve antifungal activity of chitosan derivatives. Moreover, 1,2,3-triazole as an attractive bridge group could connect pharmacophores to give an innovative bioactive compound. This synthetic strategy enables further chemical modification at the residual positions of chitosan, which leads to the creation of finely designed novel chitosan derivatives having several different functional groups at each position.

In this paper, we reported the synthesis and antifungal properties of a group of chitosan derivatives with 1,2,3- triazole as substituent including **TCTS**, **CTCTS**, and **BTCTS** (Scheme 1). After the

* Corresponding author.
E-mail address: zhanyongguo@hotmail.com (Z. Guo).

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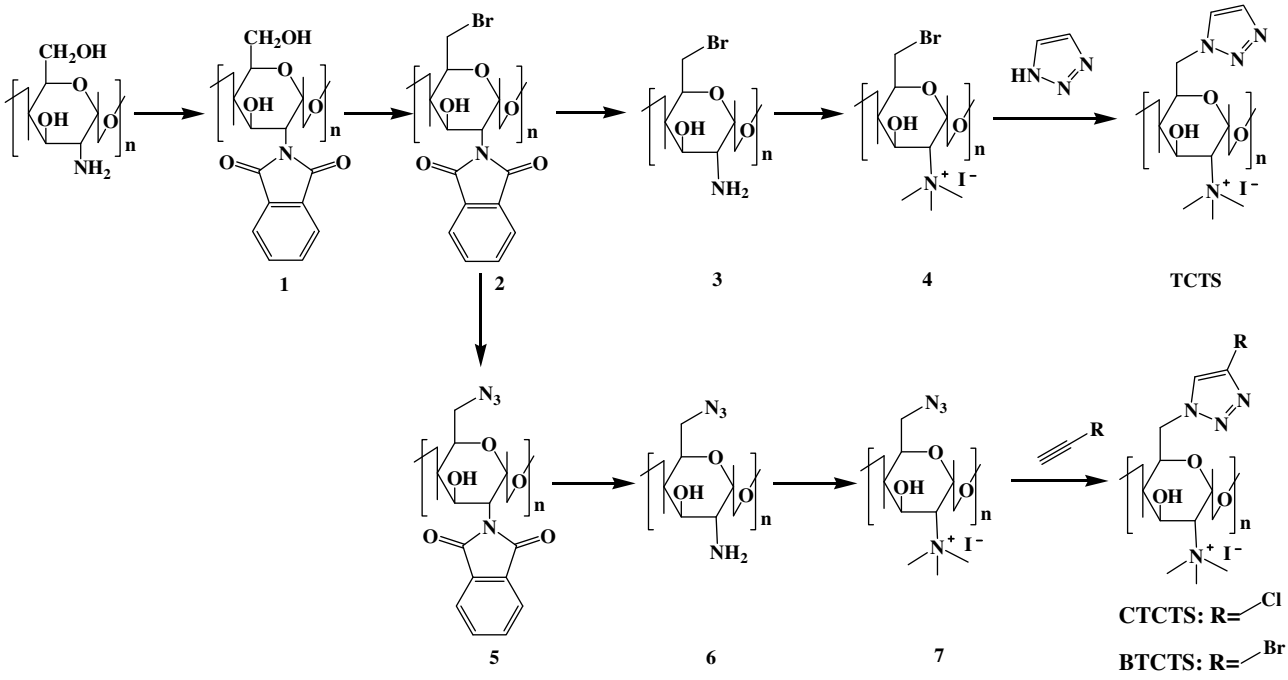

Scheme 1. Synthetic scheme for the preparation of chitosan derivatives.

protection of C_2—NH_2 by phthaloyl, we activated C_6—OH and prepared a 6-azido-6-deoxy-*N*-phthaloyl-chitosan firstly. [24] Then 6-azido-6-deoxy-*N*-phthaloyl-chitosan was treated with aqueous hydrazine monohydrate to remove the phthaloyl protecting group and the C_2—NH_2 was modified as quaternary ammonium salt. That quaternary ammonium salt was selected by virtue of water solubility, which can enlarge the application of chitosan as a food preservative or bioactive matrix. Subsequently, **CTCTS** and **BTCTS** were synthesized via "click reaction" using a 6-azido-6-deoxy-*N*-quaternary ammonium chitosan derivative with two halogenated terminal alkynes. For comparison, a chitosan derivative without halogen, **TCTS** was also synthesized and studied under identical conditions. **TCTS** was synthesized through the nucleophile substitution reaction between 6-bromo-6-deoxy-*N*-quaternary ammonium chitosan and 1,2,3-triazole. The target chitosan derivatives designed in this way were expected to have advantageous features, namely high antifungal activity and good water solubility. The chemical structures of the derivatives were characterized by FT-IR and ^{13}C NMR. Three common plant-threatening fungi, *Colletotrichum lagenarium* (Pass) Ell.et halst (ATCC30016), *Fusarium oxysporum f.sp.niveum* (ATCC36116), *and Fusarium oxysporum.f.sp.cucumebrium* Owen (ATCC42357) were selected to evaluate the antifungal properties of the derivatives by hyphal measurement *in vitro*.

2. Experimental

2.1. Materials

Chitosan was purchased from Qingdao Baicheng biochemical Corp. (China). Its degree of deacetylation is 97% and the viscosity-average molecular weight is 7.0×10^4 Da. 3-pyridinecarboxaldehyde was purchased from Aladin Chemical Corp. Halogenated terminal alkynes (3-chloropropyn and 3-bromopropyne) and 1,2,3-1*H*-triazole were purchased from Sigma-Aldrich with a minimum purity of 98%. The other reagents such as hydrazine monohydrate, iodomethane, sodium iodide, sodium hydroxide, cuprous iodide, potassium iodide and solvents are analytical grade and are supplied by Sinopharm Chemical Reagent Co., Ltd., Shanghai, China.

2.2. Analytical methods

FT-IR spectra were measured on a Jasco-4100 Fourier Transform Infrared Spectroscopy (Japan, provided by JASCO Co., Ltd. Shanghai, China) with KBr disks. ^{13}C Nuclear Magnetic Resonance (^{13}C NMR) spectra were measured with a Bruker AVIII-850 Spectroscopy with TCI CryoProbe (Switzerland, provided by Bruker Tech. and Serv. Co., Ltd. Beijing, China.). The elemental analyses (C, H, and N) were performed on a Vario Micro Elemental Analyzer (Elementar, Germany). The Degree of Substitution (DS) was calculated based on elemental analysis results. [28]

$$DS_x = \frac{100}{n_{C,R} - n_{C,P}} \left(n_{C,R} - \frac{M_N}{M_C} \times n_{N,R} \times w_{C/N} \right)$$

where $n_{C,R}$ and $n_{N,R}$ are the number of carbon and nitrogen moles per mole of reactant unit respectively; $n_{C,P}$ is the number of nitrogen moles per mole of product unit; M_C and M_N are the molar mass of carbon and nitrogen; $w_{C/N}$ is the mass ratio between carbon and nitrogen.

2.3. The synthesis of chitosan derivatives

2.3.1. Synthesis of 6-azido-6-deoxy-N-trimethyl quaternary ammonium chitosan (7)

6-Azido-6-deoxy-***N***-phthaloyl-chitosan (5) was prepared according to the methods reported by Ifuku [24]. DS_{azide} 0.94; ^{13}C NMR/DMSO: δ172.8 ppm (carbon of C=O in phthaloyl group); δ139.8, 136.7, and 128.3 ppm (phthaloyl group); δ102.4–55.2 ppm (pyranose rings); FT-IR (thin film): ν 3463 (NH_2 and OH), ν 2105 (C-6-azido), ν 1774, 1716 (C=O in phthaloyl group), ν 721 (arom).

6-Azido-6-deoxy-chitosan (6) and compound 7 were prepared according to the methods of literatures [29,30].

Compound 6: 6-azido-6-deoxy-***N***-phthaloyl-chitosan (5) (2 g, 6.3 mmol) was dissolved in 60 mL *N*-methyl-2-pyrrolidone, 50 mL of 4 M aqueous hydrazine monohydrate was added afterward, and