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Novel triazolyl-functionalized chitosan derivatives with different chain lengths of aliphatic alcohol substituent: Design, synthesis, and antifungal activity

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ABSTRACT

Chemical modification of chitosan is increasingly studied for its potential of providing new application for chitosan. Here, we modify chitosan at its primary hydroxyl via 'click chemistry', and a group of novel water soluble chitosan derivatives with substituted 1,2,3-triazolyl group were designed and synthesized. Aliphatic alcohols with different lengths were used as functional dendrons to improve the antifungal activity of chitosan derivatives. Meanwhile, their antifungal activity against two kinds of phytopathogens was estimated by hypha measurement *in vitro*. All the chitosan derivatives exhibited excellent activity against tested fungi. It is found that the antifungal activity of chitosan derivatives against the tested fungi increases with augment in the chain length of straight aliphatic alcohols. And the hydrophobic moiety (alkyl) at the periphery of the synthesized chitosan derivatives tends to affect their antifungal activity.

1. Introduction

Chemical modification of polysaccharides is increasingly studied for its potential of providing new applications for such abundant polymers. Chitosan is one of the most abundant natural polysaccharides. The antifungal ability, coupled with its non-toxicity, biodegradability, and biocompatibility, facilitates its emerging applications in food science, agriculture, medicine, pharmaceuticals, and textile areas.[1–6] To promote its antifungal activity, a large number of chitosan derivatives have been prepared such as *N*,*O*-(acyl)chitosan, hydroxyethylacryl chitosan, and acyl thiourea chitosan by performing chemical modification.[7–11] However, the application of chitosan and many chitosan derivatives is limited due to their solubility mostly in diluted organic solutions such as formic, acetic, and succinic acid, as well as in a very few inorganic solvents, such as hydrochloric, phosphoric, and nitric acid at pH below 6.5.[12] Therefore, it is strongly desired that chitosan derivatives with high antifungal ability and good water solubility are developed.

Triazole and its derivatives represent an interesting class of heterocyclic compounds. They are known to possess many biological activities such as antimicrobial, anti-tubercular, anti-inflammatory, and anticancer activities.[13–17] In fact, some of their derivatives are active constituents of currently used drugs.[13] It is also reported that a series of triazole derivatives were discovered as a novel class of tubulin polymerization inhibitors that bind to the colchicine site on tubulin.[16] The copper(I)-mediated 1,3-dipolar cycloaddition of azides and terminal alkynes developed by Sharpless and Meldal is considered to be the most popular reaction of the "click chemistry" concept and it represents the most straightforward synthesis of 1,2,3-triazoles.[18] The most representative click reaction should be the Cu (I)-catalyzed [3 + 2] cycloaddition. In recent years, copper-free click reaction using strained cyclooctyne is also extensively investigated and utilized for the bioorthogonal and biocompatible labeling of a variety of biomolecules, living cells, and animals.[19–22] This reaction leads to the efficient formation of the corresponding 1,4-disubstituted-1,2,3-triazoles as a sole regioisomer using alkyl, aryl, or sulfonyl azides.[23] The reactions take place with high yields, under mild conditions, and use copper sources that have no impact on most of the other functional groups. The modification of carbohydrate polymers by "click chemistry" will help to overcome their disadvantages, such as low selectivity, complicated reaction condition, various side

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reactions, and low yield, and remarkably improve their substitution efficiency.[24]

On the basis of these observations, it was thought of synthesizing a new class of chitosan derivatives, wherein triazolyl was linked to chitosan as substituent via "click reaction". Application of "click reaction" allows us to design various chitosan based materials via a one-step reaction from azido-chitosan and terminal alkynes with different substituent groups. In our previous work, it was found that the length of alkyl substituent at the periphery of the polymer tended to affect the antifungal activity of chitosan derivatives.[25] Sajomsang and Badawy also reported that the antifungal activity of chitosan derivatives against the plant pathogenic fungi increased with an increase in the chain length of alkyl substituent.[26,27] These observations inspired us to modify chitosan with long alkyl chain substituted 1,2,3-triazolyl a at the periphery of the polymer to improve antifungal activity of chitosan derivatives.

Our aim is to develop water-soluble chitosan derivatives which possess good antifungal ability. In this paper, we report design, synthesis, and antifungal activity of a group of novel water-soluble chitosan derivatives. "Click reaction" is selected as the key step to synthesize 1,4-disubstituted-1,2,3-triazolyl. Straight aliphatic alcohols with different lengths were used as functional dendrons to improve the antifungal activity of chitosan derivatives. For comparison, chitosan derivatives with two branched aliphatic alcohols substituted 1,2,3-triazolyl were also synthesized and studied under identical conditions. The chemical structures of the derivatives were characterized by FT-IR and ^{13}C NMR (Table S1 in Supplementary material).

Plant pathogenic fungi negatively affect a large number of important fruits and vegetables, and limit crop production worldwide, especially in developing countries. For example, *Phomopsis asparagi* can cause stem blight of asparagus and lead to yield loss.[28] Control of these plant-threatening fungi could benefit the production of related vegetables, fruits, and crops. Therefore, two common plants-threatening fungi, *Colletotrichum lagenarium* and *P. asparagi* were selected to evaluate the antifungal property of the derivatives by hypha 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. The low molecular chitosan was degraded from the chitosan mentioned above. 3-Pyridinecarboxaldehyde was purchased from Aladin Chemical Corp. Terminal alkynes (propargyl alcohol, 3-butyn-1-ol, 3-butyn-2-ol, 5-hexyn-1-ol, 10-undecyn-1-ol, and 3-methyl-1-pentyn-3-ol) 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 were supplied by Sinopharm Chemical Reagent Co., Ltd., Shanghai, China.

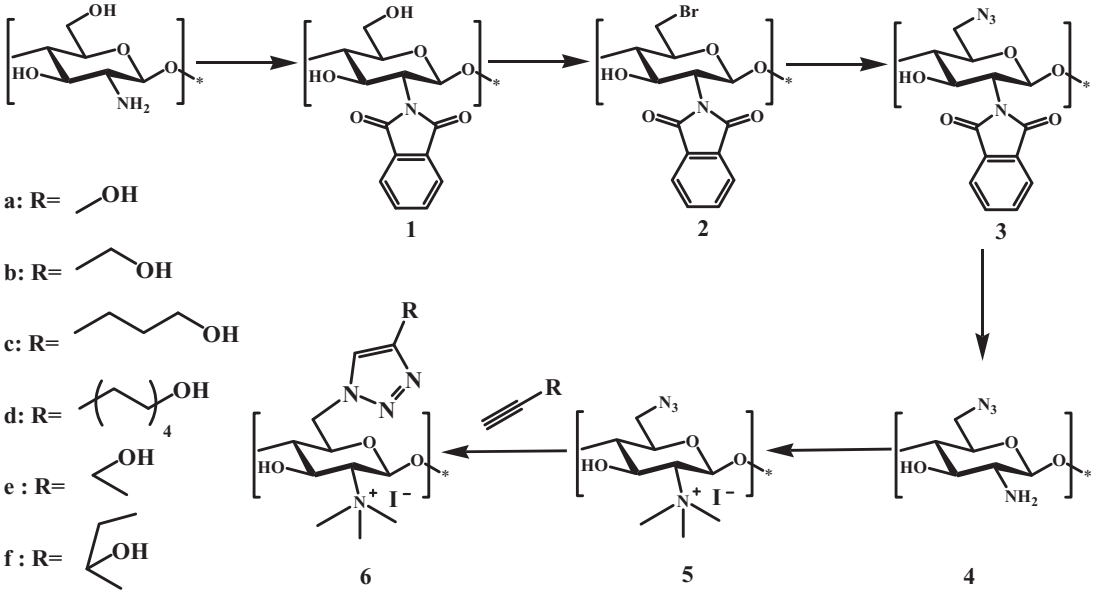
2.2. Analytical methods

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 Cryo Probe (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.

2.3. The synthesis of chitosan derivatives

The synthetic routes for the preparation of chitosan derivatives are shown in Scheme 1.

6-Azido-6-deoxy-*N*-phthaloyl-chitosan (**3**) was prepared according to the methods reported by Ifuku et al.[29] DS_{azide} 0.94; ^{13}C NMR/DMSO: δ 172.8 ppm (carbon of C═O in phthaloyl group);δ 139.8, 136.7, and 128.3 ppm (phthaloyl group); δ 102.4–55.2 ppm (pyranose rings); FT-IR (thin film): *v* 3463 (NH_2 and OH), *v* 2105 (C-6-azido), *v* 1774, 1716 (C═O in phthaloyl group), *v* 721 (arom).



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