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Design, synthesis of novel starch derivative bearing 1,2,3-triazolium and pyridinium and evaluation of its antifungal activity

Wenqiang Tan[a,b], Qing Li[a], Zhenpeng Gao[c], Shuai Qiu[d], Fang Dong[a], Zhanyong Guo[a,*]

[a] Key Laboratory of Coastal Biology and Bioresource Utilization, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China
[c] School of Marine Science and Technology, Harbin Institute of Technology at Weihai, Weihai 264209, China
[d] College of Life Sciences, Yantai University, Yantai 264005, China

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ABSTRACT

Based on cuprous-catalyzed azide-alkyne cycloaddition (CuAAC), starch derivative bearing 1,2,3-triazole and pyridine (II) was prepared and subsequently followed by alkylation with iodomethane to synthesize starch derivative bearing 1,2,3-triazolium and pyridinium (III). The antifungal activities of starch derivatives against *Colletotrichum lagenarium*, *Watermelon fusarium*, and *Phomopsis asparagi*, were then assayed by hypha measurement in vitro. Apparently, starch derivatives showed enhanced antifungal activity against three fungi at the tested concentrations compared with starch. Especially, the best inhibitory index of starch derivative (III) against *Colletotrichum lagenarium* attained 97% above at 1.0 mg/mL. Meanwhile, starch derivative (III) had stronger antifungal activity than starch derivative (II), which was reasonable to propose that the alkylation of 1,2,3-triazole and pyridine was significant for enhanced antifungal activity. As this novel starch derivative bearing 1,2,3-triazolium and pyridinium could be prepared efficiently and exhibited superduper antifungal activity, this material might provide an effective way and notion to prepare novel antifungal agents.

1. Introduction

Starch, the natural polysaccharide derived from a large variety of higher green plants, such as cereals, legumes, and tubers (Fan et al., 2016), is composed of anhydroglucose units (AGU) linked together by α-glucosidic bonds (Nep, Ngwuluka, Kemas, & Ochekpe, 2016). Due to its interesting properties such as abundant, cheap, nontoxic, biodegradable, and biocompatible (Ubeyitogullari & Ciftci, 2016; Verma, Le Bras, Jain, & Muzart, 2013), starch has been developed and used in some fields, including in the food, pharmaceutical, beverages, papermaking, packaging, and textiles (Li et al., 2016; Shi & Gao, 2016). However, native starch can not meet the demands for further industrial applications on account of lacking active groups such as carboxyl, sulfate ester, and amino (Tan, Li, Wang et al., 2016). In order to effectively broaden the industrial applications of new valuable products based on starch in sustainable chemical research field and bioactive material, one valid solution is the chemical modification via introduction of the individual functional moieties to native starch molecules (Kumar, Verma, & Jain, 2015; Sukhija, Singh, & Riar, 2016; Verma, Jain & Sain, 2011; Verma, Jain, & Sain, 2011; Verma, Le Bras, Jain, & Muzart, 2013; Verma et al., 2013c).

The outstanding features of the 1,3-dipolar cycloaddition of azide and alkyne using catalytic amounts of Cu(I), such as versatility, high efficiency, and robustness (Singh et al., 2015; Sood et al., 2014), have promoted the development of an extremely broad palette of polysaccharide materials containing 1,2,3-triazole units. The many interesting biological properties of 1,2,3-triazoles, such as anti-HIV (Pribut, Veale, Basson, van Otterlo, & Pelly, 2016), antimicrobial (Garudachari, Isloor, Satyanarayana, Fun, & Hegde,

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

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2014; Zhang, Wei, Vijaya Kumar, Rasheed, & Zhou, 2014), anticancer (Kumar et al., 2011), antimalarial (Pereira et al., 2014), and antioxidant (Tan, Li, Li, Dong & Guo, 2016), have also facilitated the chemical modification of polysaccharide with 1,2,3-triazoles. Meanwhile, alkylation of 1,2,3-triazoles can provide the 1,2,3-triazolium cations, which have been prepared for novel ionic liquids (Mudraboyina, Obadia, Abdelhedi-Miladi, Allaoua, & Drockenmuller, 2015; Obadia, Crépet, Serghei, Montarnal, & Drockenmuller, 2015; Obadia et al., 2014) and catalysts (Aizpurua et al., 2014; Jha & Jain, 2013; Ohmatsu, Hamajima, & Ooi, 2012) because of high thermal stability, tunable solubility, and low flammability (Liu et al., 2016). However, although the 1,2,3-triazole-linked starch derivatives have been reported and described, to our knowledge there are no reports on synthesis and bioactivity of starch derivatives bearing 1,2,3-triazolium cations so far. Moreover, pyridine group has been also regarded as an excellent reactive precursor, which can synthesize pyridinium group by the alkylation reaction (Jia, Duan, Fang, Wang, & Huang, 2016). But the effect of alkylation of 1,2,3-triazole and pyridine groups on the bioactivity of starch derivatives was still unknown.

This study aimed to investigate the effect of 1,2,3-triazolium and pyridinium groups on biological activity of starch derivative. Herein, we presented the synthesis, characterization, and antifungal activity of starch derivative bearing 1,2,3-triazolium and pyridinium (III) obtained by alkylation of starch derivative bearing 1,2,3-triazole and pyridine (II) issued from CuAAC reaction. The chemical structures of the starch derivatives were characterized by FTIR, ^{1}H NMR, and ^{13}C NMR. Three plant-threatening fungi, including *Colletotrichum lagenarium* (*C. lagenarium*), *Watermelon fusarium* (*W. fusarium*), and *Phomopsis asparagi* (*P. asparagi*), were selected to evaluate the antifungal property of starch and starch derivatives (II) and (III) by hypha measurement *in vitro*.

2. Experimental

2.1. Materials

Soluble starch from potato (granules) with weight-average molecular weight of 9.8×10^4 Da, was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). *N*-bromosuccinimide (NBS), triphenylphosphine (TPP), nicotinoyl chloride hydrochloride, propargyl amine, and iodomethane were purchased from the Sigma-Aldrich Chemical Corp (Shanghai, China). Triadimefon (20% emulsifiable concentrates) was obtained from Hebei Shenhua Pharmaceutical Co., Ltd. (Hebei, China). The other reagents were all analytical grade and used as received.

2.2. Analytical methods

Fourier transform infrared (FTIR) spectra were recorded on a Jasco-4100 Fourier Transform Infrared Spectrometer (Japan, provided by JASCO Co., Ltd. Shanghai, China) at 25 °C in the transmittance mode. About 1 mg of sample with 100 mg of KBr was fully grinded and mixed. The mixed samples were pressed into pills with a compressor and prepared pellets were used for studies. All spectra were scanned against a blank KBr pellet back-ground in the range of 4000–400 cm^{-1} with resolution of 4.0 cm^{-1}. ^{13}C Nuclear magnetic resonance (^{13}C NMR) and ^{1}H Nuclear magnetic resonance (^{1}H NMR) spectra were all recorded on a Bruker AVIII-500 Spectrometer (Switzerland, provided by Bruker Tech. and Serv. Co., Ltd., Beijing, China) at 25 °C using DMSO-d6 or D_2O as solvent. Chemical shifts (δ ppm) were referenced to tetramethylsilane (TMS). The elemental analyses (C, H, and N) were performed on a Vario EL III (Elementar, Germany). The degrees of substitution (DS) of starch derivatives were calculated on the basis of the percentages of carbon and nitrogen. X-ray diffraction (XRD) analyses of the samples were performed using an X-ray diffractometer (Rigaku Ultima IV, Rigaku Corporation, Japan) using Cu Kα radiation (λ = 1.5418 Å) set at 40 kV and 30 mA. All samples were scanned at diffraction angle (2θ) from 5 to 50° at a rate of 1.20°/min and with a step size of 0.02°. The morphology of the samples was examined through a Scanning electron microscope (SEM) (S-4800, Hitachi, Japan). Each sample was coated with gold in an ion sputter (E-1045, Hitachi, Japan) before being scanned and photographed at the magnifications (1000×). An accelerating potential of 3 kV was used during image acquisition.

2.3. The synthesis of the starch derivatives

2.3.1. Synthesis of N-prop-2-ynylnicotinamide (I)

A stirred solution of propargyl amine (0.65 mL, 10 mmol), triethyl amine (1.4 mL, 10 mmol), and DMAP (24 mg, 0.2 mmol) in 20 mL of CH_2Cl_2 was cooled to 0 °C. The nicotinoyl chloride hydrochloride (1.78 g, 10 mmol) was then added in batches. The reaction mixture was then stirred at 0 °C for 0.5 h and overnight at room temperature. The mixture was then extracted with 0.1 M aqueous solutions of HCl (2 × 10 mL) and NaOH (2 × 10 mL), washed with water (3 × 20 mL), dried over $MgSO_4$, filtrated and the solvent evaporated under vacuum. The resulting *N*-prop-2-ynylnicotinamide (I) was sufficiently pure to be used without further purification. *N*-prop-2-ynylnicotinamide (I), Yield: 35.89%. FTIR: ν 3224, 3046, 2958, 2113, 1658, 1596, 1550, 713, 640 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-*d*6): δ 9.02 (m, 1H, Py-2-**H**), 8.73 (m, 1H, Py-6-**H**), 8.21 (m, 1H, Py-4-**H**), 8.19 (m, 1H, N**H**CH_2C≡CH), 7.53 (m, 1H, Py-5-**H**), 4.09 (dd, *J* = 1.5, 3.9 Hz, 2H, NHC**H**$_2$C≡CH), 2.51 (dt, *J* = 1.8, 3.6 Hz, 1H, NHCH_2C≡C**H**) ppm. ^{13}C NMR (125 MHz, DMSO-*d*6): δ 164.99 (1C, **C**=O), 152.57 (1C, Py-2-**C**), 148.88 (1C, Py-6-**C**), 135.49 (1C, Py-4-**C**), 129.75 (1C, Py-3-**C**), 123.98 (1C, Py-5-**C**), 81.41 (1C, NHCH_2**C**≡CH), 73.60 (1C, NHCH_2C≡**C**H), 28.92 (1C, NH**C**H_2C≡CH) ppm. MS [ESI]: *m*/*z* $[M+H]^+$ calcd for $C_9H_8N_2O$ 161.06; found 161.17.

2.3.2. The dissolution of starch

Soluble starch (3.24 g, 20 mmol) was stirred in 80 mL of anhydrous *N*,*N*-dimethylformamide (DMF), while the mixture was heated to 120 °C for 1 h. The slurry was then allowed to cool to 90 °C, at which point LiBr (3.47 g, 40 mmol) was added. The starch could dissolve within 5 min to form a transparent solution. The contents of the flask were allowed to cool further to room temperature while stirring.

2.3.3. Synthesis of 6-bromo-6-deoxy starch (BDST)

When transparent solution above-mentioned was cooled to 0 °C, *N*-bromosuccinimide (NBS) (14.24 g, 80 mmol) and triphenylphosphine (TPP) (20.99 g, 80 mmol) were added. The reaction solution was heated to 80 °C for 3 h under an argon atmosphere. The product was isolated by adding the reaction mixture slowly to 400 mL of 95:5 (v/v) mixture of absolute ethanol and deionized water, followed by filtration. The unreacted NBS, TPP, and other outgrowth, were extracted in a Soxhlet apparatus with ethanol and acetone for 48 h, respectively. The 6-bromo-6-deoxy starch was obtained by freeze-drying overnight in vaccum. Yield: 89.31%. FTIR: ν 3405.67, 2923.56, 1029.80, 682.68 cm^{-1}. ^{1}H NMR (500 MHz, DMSO-*d*6): δ 5.85-3.30 (pyranose rings), 3.44 (C**H**$_2$Br) ppm. ^{13}C NMR (125 MHz, DMSO-*d*6): δ 100.22–70.08 (pyranose rings), 34.78 (**C**H_2Br) ppm.

2.3.4. Synthesis of 6-azido-6-deoxy starch (ADST)

In a 100 mL three-necked round-bottom flask, 6-bromo-6-deoxy starch (2.25 g, 10 mmol) was weighed and dissolved in 40 mL of anhydrous dimethylsulfoxide (DMSO). Then, NaN_3 (1.3 g, 20 mmol) was added to the flask and dissolved. The solution was