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

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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,

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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

heated to 80 °C and stirred for 24 h under an argon atmosphere. The product was isolated by pouring the reaction solution into 200 mL of absolute ethanol. The precipitate was collected by filtration, and washed with acetone. After being extracting in a Soxhlet apparatus with ethanol for 48 h and being dialyzed against deionized water for 48 h to remove the probable remained sodium azide, the 6-azido-6-deoxy starch was obtained by freeze-drying. Yield: 71.10%. DS_{azido}: 95.57%. FTIR: ν 3405.67, 2923.56, 2105.89, 1041.37 cm^{-1}. ^1H NMR (500 MHz, DMSO-*d*6): δ 5.72–3.30 (pyranose rings), 3.77 ($C\mathbf{H}_2N_3$) ppm. ^{13}C NMR (125 MHz, DMSO-*d*6): δ 100.22–70.26 (pyranose rings), 51.69 ($\mathbf{C}H_2N_3$) ppm.

2.3.5. Synthesis of starch derivative bearing 1,2,3-triazole and pyridine (II)

6-Azido-6-deoxy starch (187 mg, 1 mmol) was dissolved in 20 mL of DMSO, cuprous iodide (19 mg, 0.1 mmol), triethylamine (0.14 mL, 1 mmol), and *N*-prop-2-ynylnicotinamide (I) (322 mg, 2 mmol) were added, and the solution was stirred at 75 °C for 24 h under an argon atmosphere. The mixture was precipitated in 100 mL of acetone, and collected by filtration. The probable remained reagents were extracted in a Soxhlet apparatus with acetone for 48 h. After being dialyzed against deionized water for 48 h, the starch derivative (II) was obtained by lyophilization of their aqueous solutions. Yield: 90.48%. $DS_{triazole}$: 97.46%. FTIR: ν 3351.68, 3081.69, 2923.56, 1650.77, 1596.77, 1542.77, 1469.49, 1041.37, 809.96 cm^{-1}. ^1H NMR (500 MHz, DMSO-*d*6): δ 9.13 (s, 1H, Py-2-**H**), 8.76 (s, 1H, Py-6-**H**), 8.08 (2H, Py-4-**H** and CON**H**CH_2), 7.75 (m, 1H, Py-5-**H**), 7.49 (m, 1H, triazole-5-**H**), 5.72-3.02 (pyranose rings), 4.31 (s, NHC$\mathbf{H}_2$C), 3.84 (s, 1H, NC$\mathbf{H}_2$CH) ppm. ^{13}C NMR (125 MHz, DMSO-*d*6): δ 165.35 (1C, **C**=O), 152.17 (1C, Py-2-**C**), 148.74 (1C, Py-6-**C**), 144.45 (triazole-4-**C**), 124.47 (triazole-5-**C**), 135.45 (1C, Py-4-**C**), 129.95 (1C, Py-3-**C**), 124.47 (1C, Py-5-**C**), 99.90-69.66 (pyranose rings), 50.19 (1C, N**C**H_2CH), 35.06 (1C, NH**C**H_2C) ppm.

2.3.6. Synthesis of starch derivative bearing 1,2,3-triazolium and pyridinium (III)

A solution of 1,2,3-triazole-functionalized starch derivative (II) (347 mg, 1 mmol of 1,2,3-triazole groups) and iodomethane (0.187 mL, 3 mmol) in 15 mL of DMSO was stirred at 60 °C for 24 h. Afterwards, the remaining iodomethane was evaporated, and the reaction mixture was precipitated into 100 mL of acetone. The solid product was filtered, washed with acetone three times. After being dialyzed against deionized water for 48 h, the starch derivative (III) was obtained by lyophilization of their aqueous solutions. Yield: 76.35%. $DS_{quaternization}$: 93.02%. FTIR: ν 3397.96, 3062.41, 2919.70, 1670.05, 1592.91, 1542.77, 1500.35, 1041.37, 794.53 cm^{-1}. ^1H NMR (500 MHz, D_2O): δ 9.24 (d, J = 77.3 Hz, 1H, Py-2-**H**), 8.88 (m, 4H, Py-4,5,6-**H** and triazole-5-**H**), 8.20 (s, 1H, CON**H**CH_2), 5.52-3.14 (pyranose rings), 4.94 (s, NHC$\mathbf{H}_2$C), 4.42 (d, J = 41.3 Hz, 6H, $N^+C\mathbf{H}_3$), 4.09 (s, 1H, NC$\mathbf{H}_2$CH) ppm. ^{13}C NMR (125 MHz, D_2O): δ 164.02 (1C, **C**=O), 147.63 (1C, Py-4-**C**), 145.45 (1C, Py-2-**C**), 143.97 (1C, Py-6-**C**), 140.06 (triazolium-4-**C**), 128.30 (triazolium-5-**C**), 133.09 (2C, Py-3,5-**C**), 99.90-62.55 (pyranose rings), 49.11 (1C, N**C**H_2CH), 39.27, 32.93 (2C, $N^+\mathbf{C}H_3$), 36.73 (1C, NH**C**H_2C) ppm.

2.4. Antifungal assay

Antifungal assay was performed based on the method of Guo et al. with minor modification (Guo et al., 2014). Briefly, the compounds (starch and starch derivatives (II) and (III)) were dissolved in distilled water containing 2% (w/w) DMSO at a concentration of 5 mg/mL. Then, to each solution, sterilized potato dextrose agar was added to give a final concentration of 0.1, 0.5, and 1.0 mg/mL. After the mixture was cooled in the plate, the mycelium of fungi was transferred to the test plate and incubated at 27 °C for 2–3 days. When the mycelium of fungi reached the edges of the control plate

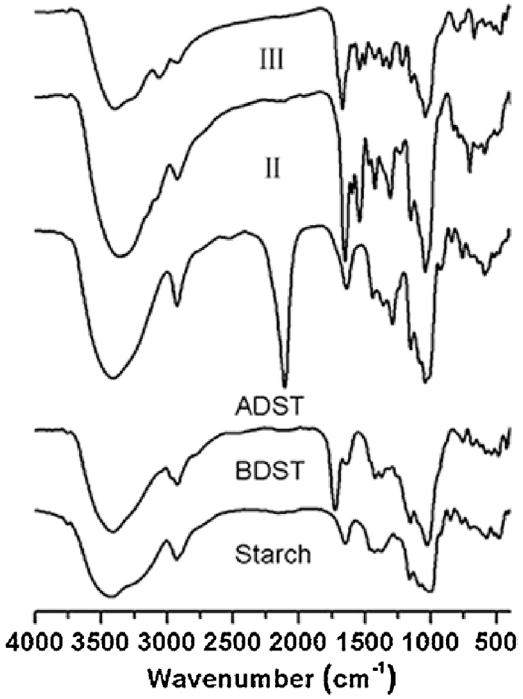


Fig. 1. FTIR spectra of starch and starch derivatives.

(without the presence of samples), the inhibitory index was calculated as follows:

$$\text{Inhibitoryindex}(\%) = (1 - D_a/D_b) \times 100$$

where D_a is the diameter of the growth zone in the test plates and D_b is the diameter of the growth zone in the control plate.

2.5. Statistical analysis

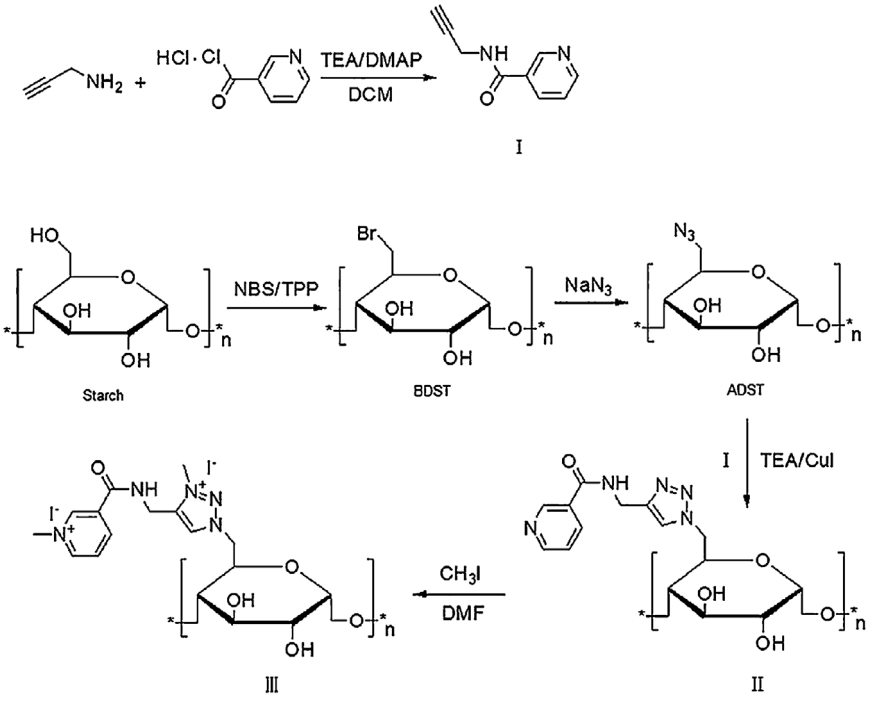
Each experiment was performed three times. All data were expressed as means ± SD. Data were analyzed by an analysis of variance ($P < 0.05$) and the Scheffe's method was used to evaluate the differences in inhibitory indices in antifungal tests. The results were processed by the computer programs: Origin and SPSS.

3. Results and discussion

3.1. Chemical synthesis and characterization

The synthetic strategy for the preparation of starch derivatives via CuAAC reaction is shown in Scheme 1. Each step of synthesis is followed by FTIR, ^1H NMR, and ^{13}C NMR spectroscopy shown in Figs. 1–3, respectively.

Firstly, 6-bromo-6-deoxy starch was first regioselectivity synthesized in *N,N*-dimethylformamide (DMF)/LiBr as a useful intermediate for synthesis of 6-azido-6-deoxy starch by S_N2 displacement of the bromide. This highly regioselectivity should be given the credit to alkoxyphosphonium salt intermediate at the primary hydroxyl group attached to the 6-carbon due to the steric bulk of three phenyl rings (Fox & Edgar, 2011). A bromide anion, supplied by the NBS, then attacked *C*-6 via S_N2 mechanism with triphenylphosphine oxide as the leaving group (Fox & Edgar, 2012). In FTIR spectrum of BDST, a new peak at 682.68 cm^{-1} is assigned to C-6-Br group (Tan, Li, Wang et al., 2016). In ^1H NMR spectrum of BDST, hydrogens of —C$\mathbf{H}_2$Br are observed at 3.44 ppm. Moreover, carbon of —**C**H_2Br is clearly observed at 34.78 ppm in ^{13}C NMR spectrum (Zhang & Edgar, 2015). However, there are many disturbances of BDST, which indicate the presence of a low DS of acetate ester



Scheme 1. Synthetic routes for starch derivatives.

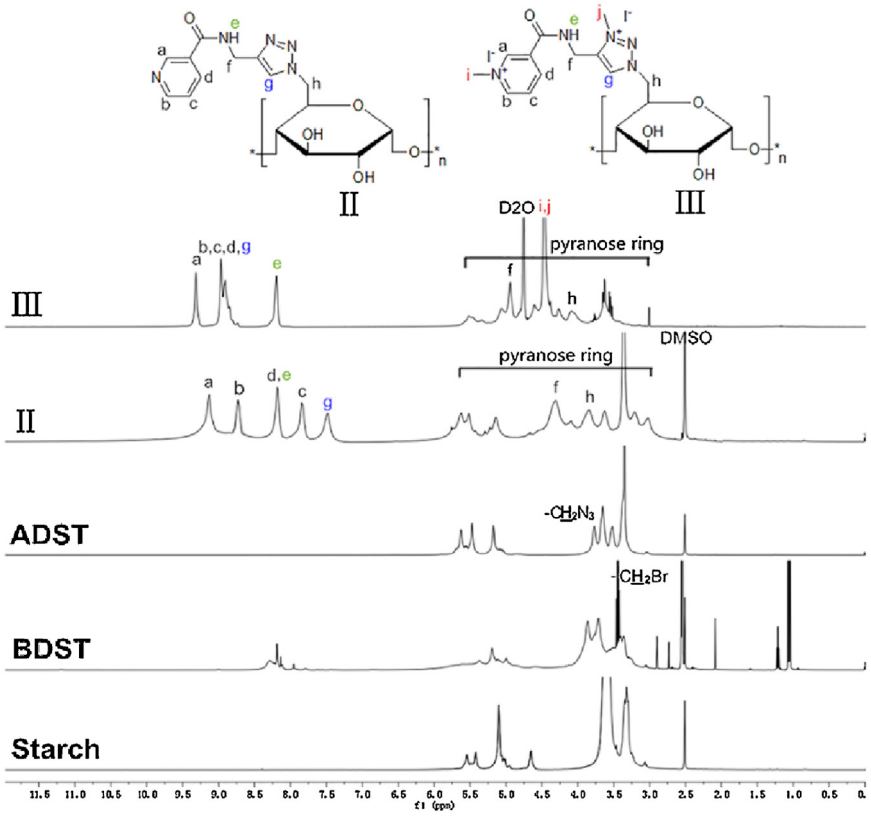


Fig. 2. ^{1}H NMR spectra of starch and starch derivatives.

groups attached to C-6-OH of starch. The reasonable interpretation was that during the S_N2 reaction with starch alkoxyphosphonium salt intermediate, the acetate group was a product of the DMF solvent sometimes acting as a nucleophile instead of bromide (Fox & Edgar, 2011). Fortunately, after azidation of BDST, these disturbances are disappeared absolutely and characteristic peak of C-6-N_3 is observed at 2105 cm^{-1} in FTIR spectrum (Li, Tan, Zhang, Gu, & Guo, 2015). As shown in Figs. 2 and 3, the peak of $-CH_2N_3$ shifts to 3.77 and 51.69 ppm in ^{1}H NMR and ^{13}C NMR respectively

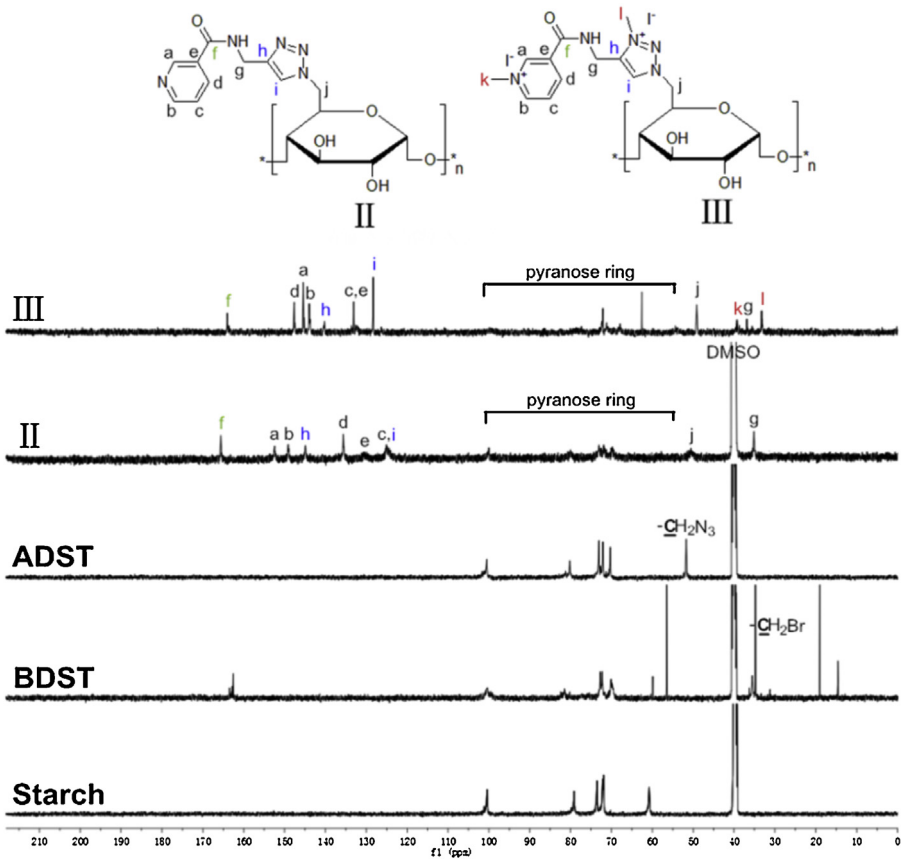


Fig. 3. ^{13}C NMR spectra of starch and starch derivatives.

(Zhang & Edgar, 2014), supporting regioselective and complete C-6 substitution of the azido moiety.

Subsequently, the click chemistry could be performed in an elegant way with *N*-prop-2-ynylnicotinamide (I) to synthesize the starch derivative bearing 1,2,3-triazole and pyridine (II). The peak at 2105 cm^{-1} in ADST disappears completely when the C-6-N_3 in ADST is transformed to 1,2,3-triazole and a new absorption band at about 1542.77 cm^{-1} appears (Fig. 1) (Tan, Li, Li et al., 2016). The appearance of the proton in 1,2,3-triazole at 7.49 ppm and the additional signals of the pyridyl and acylamino protons linked to the 1,2,3-triazole unit at 9.13, 8.76, 8.19, and 7.84 ppm further prove the successful CuAAC reaction. Moreover, the 1,2,3-triazole linker is clearly observed at 124.92 and 144.72 ppm as two new peaks in the ^{13}C NMR spectrum of starch derivative (II) (Tan, Li, Li et al., 2016), and other new signals for acylamino and pyridyl carbons appear at 165.35, 152.22, 148.90, 135.44, 130.32, and 124.92 ppm, respectively.

1,2,3-Triazole-functionalized starch derivative (II) was used as an important precursor for the preparation of starch derivative (III) by reacted with iodomethane in DMSO. The ^{1}H NMR spectrum of starch derivative (III) (Fig. 2) corroborates the alkylation reaction through the disappearance of the peak corresponding to the proton of the 1,2,3-triazole group at a signature value of 7.49 ppm and the appearance of new signals for the 1,2,3-triazolium proton at 8.97 ppm (Sood et al., 2014). Indeed, besides significant shift for the chemical displacement of the 1,2,3-triazolium proton compared with the 1,2,3-triazole proton, completion of the alkylation reaction is also corroborated by the appearance of new signals at 4.47 ppm for the pendant methyl group of 1,2,3-triazolium and pyridinium (Dimitrov-Raytchev, Beghdadi, Serghei, & Drockenmuller, 2013). In addition, all signals of the pyridinium and adjacent methylene groups are shifted towards down field compared to those initially neighboring the 1,2,3-triazole and pyridine groups, which leads to the partial overlap between 1,2,3-triazolium proton and pyridinium protons. ^{13}C NMR spectrum of starch derivative (III) (Fig. 3) also corroborates the alkylation since signals corresponding to 1,2,3-triazolium carbons at 140.23 and 128.30 ppm and the methyl group at 33.20 ppm are observed together with the total disappearance of the 1,2,3-triazole carbon signals initially located at 144.72 and 124.92 ppm (Abdelhedi-Miladi et al., 2014). Most other carbon signals are shifted after alkylation of the 1,2,3-triazole and pyridine rings.

3.2. Morphology analysis

The crystalline nature of starch and starch derivatives is confirmed by X-ray diffraction (XRD) as shown in Fig. 4(A). In the XRD of native starch, the peaks at around 2θ value of 5.68° and 22.10° are characteristic of B-type pattern (Lopez-Rubio, Flanagan, Gilbert, & Gidley, 2008; Nguyen Vu & Lumdubwong, 2016), while those at 14.96°, 17.08°, 19.58°, 23.58°, and 26.10° are indicative of the A-type pattern (Adak & Banerjee, 2016), which show that native potato starch is a typical C-type crystalline polymorph (Ahmed, Thomas, Taher, & Joseph, 2016). However, in starch derivatives no sharp peaks are observed, only broad peaks at 21.38°, 20.02°, 21.50°, and 23.64° are obtained which indicates that chemical modification fully transforms the starch into an amorphous material due to the rupture of intra and intermolecular hydrogen bonding (Li et al., 2017). Moreover, the morphology change of starch and starch derivatives is analyzed by scanning electron micrographs (SEM) as shown in Fig. 4(B). The native starch granules have large oval shapes of varying sizes with a smooth but not compact surface. Similar results for starch were earlier reported (Ahmed, Thomas, Taher, & Joseph, 2016). Compared to native starch, the surface of

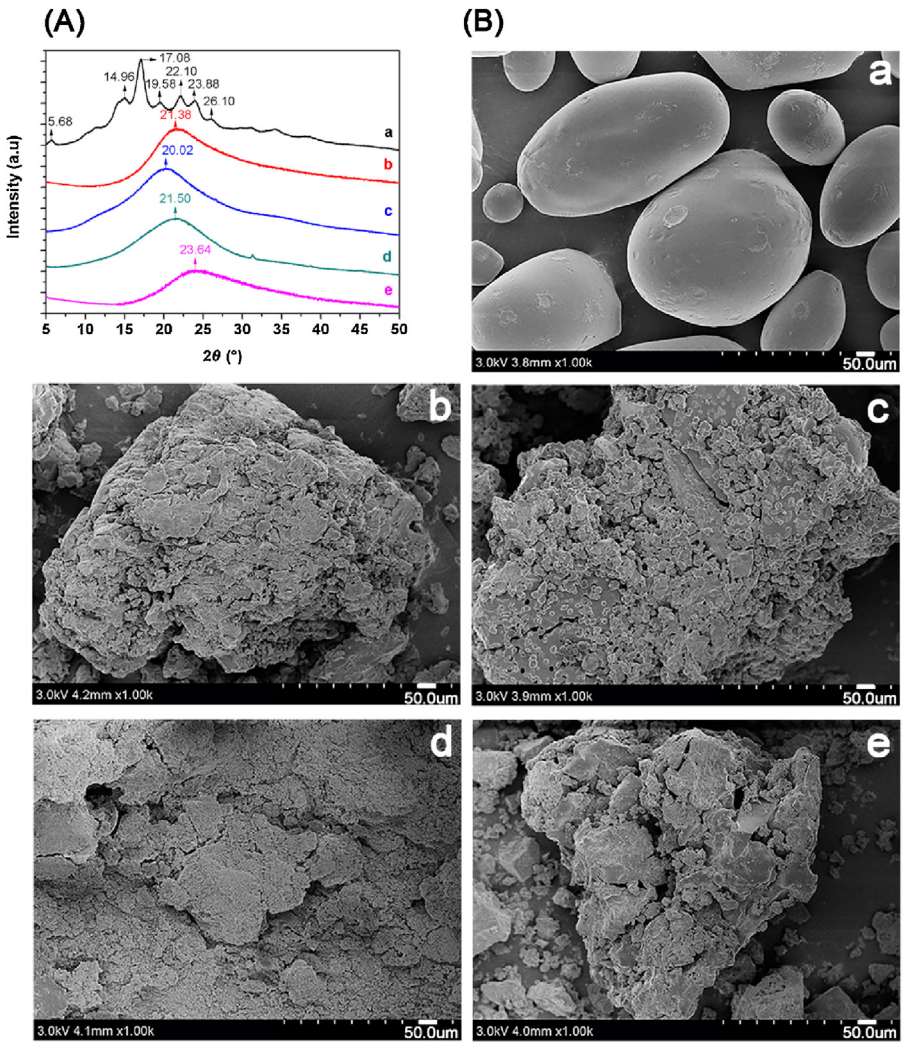


Fig. 4. (A) X-ray diffraction patterns and (B) SEM images of starch and starch derivatives (a) starch, (b) BDST, (c) ADST, (d) starch derivative (II), (e) starch derivative (III).

starch derivatives is found to be altered to a greater extent and has no granular appearance. The intact granules are predominantly disrupted and are fused together forming the rough masses. It was speculated that chemical modification significantly reduced inter-molecular hydrogen bonding of starch backbone and radically changed the granular structure (Adak & Banerjee, 2016).

3.3. Antifungal activity of starch derivatives (II) and (III)

The capabilities of starch and starch derivatives (II) and (III) to inhibit the growth of the tested three plant-threatening fungi, including *C. lagenarium*, *W. fusarium*, and *P. asparagi*, are shown in Fig. 5–7, respectively. The growth-inhibiting effect is quantitatively determined by the ratio of diameter of the growth zones in the medium with starch and starch derivatives (II) and (III) to those with the deionized water instead of starch derivatives (control).

Fig. 5 shows the antifungal activity of starch and starch derivatives (II) and (III) against *C. lagenarium*. The inhibitory indices of all the samples mount up with increasing concentration, and the strongest antifungal activity is observed at 1.0 mg/mL. Compared with starch with the inhibitory index 4.78%, the inhibitory indices of starch derivatives (II) and (III) are 27.07 and 97.73%, respectively. Evidently, the starch derivative bearing 1,2,3-triazole and pyridine (II) show stronger antifungal activity than starch, which suggests that 1,2,3-triazole and pyridine should be the antifungal function groups. Moreover, the starch derivative (III) exhibits higher inhibitory indices than that of starch derivative (II), which suggests that stronger antifungal activity of starch derivative (III) may mainly benefit from the alkylation of 1,2,3-triazole and pyridine and 1,2,3-triazolium and pyridinium should be the stronger antifungal active groups than 1,2,3-triazole and pyridine. Besides,

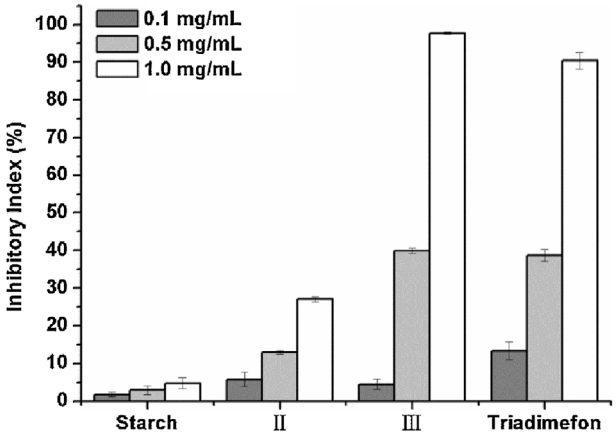


Fig. 5. The antifungal activity of starch and starch derivatives (II) and (III) against *C. lagenarium*.

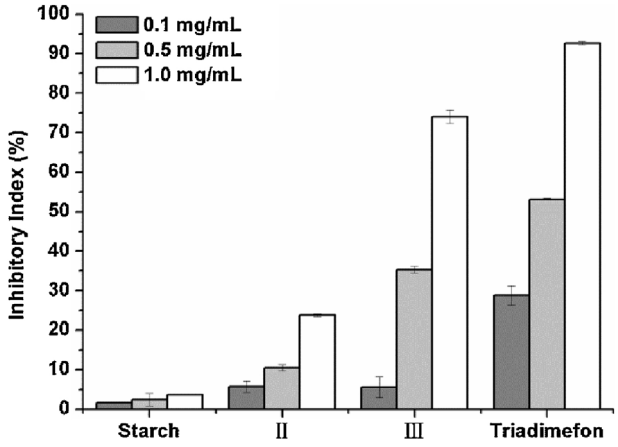


Fig. 6. The antifungal activity of starch and starch derivatives (II) and (III) against *W. fusarium*.

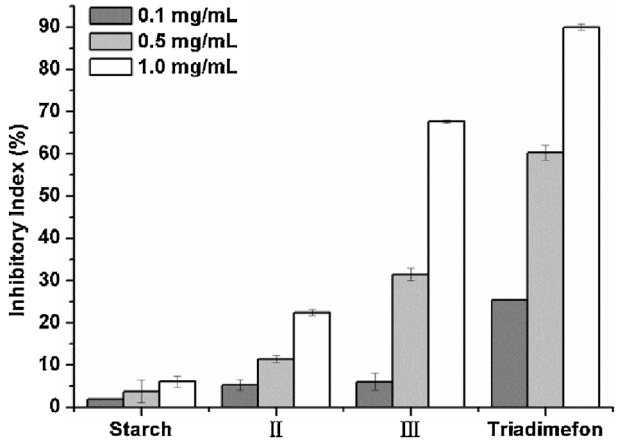


Fig. 7. The antifungal activity of starch and starch derivatives (II) and (III) against *P. asparagi*.

it is worthwhile to note that the antifungal activity of starch derivative (III) against *C. lagenarium* is comparative to that of triadimefon.

As shown in Fig. 6, the inhibitory indices of all the compounds enhance with the increase of the concentration. Starch derivatives (II) and (III) have better ability of inhibiting the growth of *W. fusarium* than pristine starch. The inhibitory indices of starch, starch derivatives (II) and (III) against *W. fusarium* are 3.67, 23.81, and 74.05% at 1.0 mg/mL, respectively. The results further confirm that 1,2,3-triazole and pyridine grafted into the synthesized starch derivatives contribute a lot to the antifungal action and consequently increase the antifungal activity. Meanwhile, compared with starch derivative (II), significantly stronger antifungal activity of starch derivative (III) should be ascribed to the alkylation of 1,2,3-triazole and pyridine. However, starch derivative (III) exhibits weaker antifungal property than triadimefon against *W. fusarium*.

The antifungal test results reveal that all the samples show antifungal activity against *P. asparagi*, and the antifungal activity of starch derivatives is concentration-dependent. Especially, starch derivative (III) possesses a most powerful antifungal activity. The inhibitory indices of starch and starch derivatives (II) and (III) against *P. asparagi* are 6.10, 22.45, and 67.61% at 1.0 mg/mL, respectively. It is obvious that starch derivative (II) shows much better antifungal activity than starch due to the introduction of functional groups – 1,2,3-triazole and pyridine groups. Besides, the results further reconfirm that the significant contribution of 1,2,3-triazolium and pyridinium groups to antifungal activity of the synthesized starch derivatives.

Based on the results mentioned above, starch derivative (II) presented evidently enhanced antifungal activity against three fungi at the tested concentrations compared with starch. The antifungal activity exhibited by the synthesized starch derivative (II) was due to 1,2,3-triazole and pyridine groups. Liking other nitrogen-containing aromatic heterocycles, 1,2,3-triazole and pyridine groups were stable to metabolic degradation and capable of forming hydrogen bonds to effectively bind to biomolecular targets, so that they could inhibit synthesis of the cell membrane and cell wall to exhibit antimicrobial activity (Tan, Li, Wang et al., 2016). Moreover, the starch derivative (III) exhibited higher inhibitory indices than that of starch derivative (II). The significant antifungal property should be ascribed to the alkylation of 1,2,3-triazole and pyridine moieties. As kinds of important quaternary ammonium salts, 1,2,3-triazolium and pyridinium groups possessed all the advantages of quaternary ammonium salts, such as strong antimicrobial activity (Guo et al., 2007a). The disruptive effect of them on the microorganism was probably based on the adsorption of the amphiphile molecules on the outer cellular membranes (Chang, Yang, & Liang, 2010). The positively charged moiety of the cationic molecules interacted with the negative charged components on fungal cell walls or cytomembranes, such as glucan, mannan, proteins, and lipids (Guo et al., 2007b). Once this electrostatic contact was accomplished by the hydrophilic region, the hydrophobic region proceeded to penetrate the hydrophobic bilayer to cause cell leakage and lysis (Anthierens, Billiet, Devlieghere, & Du Prez, 2012; Sajomsang, Gonil, & Tantayanon, 2009). This cascade could lead to the release of K^+ and cytoplasmic components, and finally caused the death of the cells (Fan et al., 2015). And the adhesion between biomolecular targets and pharmaceutical molecules generated by electrostatic and hydrophobic interactions might be much stronger than that produced by hydrogen bond interaction, which caused the higher inhibitory indices of starch derivative bearing 1,2,3-triazolium and pyridinium (III) compared with starch derivative bearing 1,2,3-triazole and pyridine (II).

4. Conclusion

In summary, we have recently proposed a straightforward synthetic route to novel starch derivative possessing 1,2,3-triazolium and pyridinium charged units by associating CuAAC step with efficient alkylation of 1,2,3-triazole and pyridine. The antifungal activity against three kinds of plant threatening fungi was estimated by hypha measurement *in vitro*. The synthesized starch derivative bearing 1,2,3-triazolium and pyridinium showed more significant antifungal activity than starch derivative with 1,2,3-triazole and pyridine and starch. The results indicated that 1,2,3-triazolium and pyridinium groups should be excellent antifungal function groups. The mechanism of the obtained antifungal activity was also discussed. The positive charged moiety of the cationic molecules could directly interfere with the fungal cell surface to change the permeability of the plasma membrane, thereby inhibiting the growth of plant-threatening fungi. These electrostatic and hydrophobic interactions might exhibit greater impact on antifungal activity than the hydrogen bond interaction. And the product described in this paper might serve as a new leading structure for further design of antifungal agents.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2016.09.093.

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