Synthesis of Schiff bases modified inulin derivatives for potential antifungal and antioxidant applications

Yuan Chen a,b,c, Yingqi Mi a,b,c, Qing Li a, Fang Dong a, Zhanyong Guo a,b,c,*

a Key Laboratory of Coastal Biology and Bioresource Utilization, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China
b Center for Ocean Mega-Science, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, China
c University of Chinese Academy of Sciences, Beijing 100049, China

A R T I C L E    I N F O

Article history:
Received 26 August 2019
Received in revised form 22 September 2019
Accepted 29 September 2019
Available online 11 November 2019

Keywords:
Inulin
Schiff bases
Chemical modification
Antifungal
Antioxidant

A B S T R A C T

In this study, the structure of inulin was chemically modified by Schiff bases in order to improve its biological activity. A total of 6 kinds of inulin derivatives were synthesized according to aza-Wittig reaction. Their structures were confirmed by FTIR, 1H NMR, and 13C NMR spectroscopy. The antioxidant activity of the inulin derivatives was evaluated in vitro. Their antifungal activities against three kinds of plant pathogenic fungi, including Botrytis cinerea, Fusarium oxysporum f. sp. cucumerium Owen, and Phomopsis asparagi, were also studied. The results showed that the biological activities of the derivatives were significantly improved compared to pure inulin. 3HBSAIL could completely scavenge hydroxyl radical and DPPH radical at 1.6 mg mL−1. 3,4DHBASAIL and 2,3,4THBSAIL exhibited strong antioxidant activity as far as the four tested antioxidant systems. Moreover, the scavenging rates of 3,4DHBASAIL and 2,3,4THBSAIL against DPHH radicals were both 100% even at the lowest test concentration (0.1 mg mL−1). The synthetic inulin derivatives showed a broad antifungal spectrum against the tested fungi. At 1.6 mg mL−1, the inhibitory rates of 3HBSAIL against Botrytis cinerea, Fusarium oxysporum f. sp. cucumerium Owen, and Phomopsis asparagi were 93%, 83%, and 82%, respectively. The biological activities of the inulin derivatives were closely related to the DS, the number of phenolic hydroxyl groups and their substitutive positions. The products described in this paper have great potential as biomaterials with good bioactivity and biocompatibility.

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1. Introduction

Natural polysaccharides play important roles in living organisms and have draw increasing attention in biomedical applications for their abundance, low toxicity, biocompatibility, and biodegradability [1,2]. Polysaccharides and their chemically modified derivatives as biomaterials have been widely applied in pharmaceuticals, biomedical use, food additives, and cosmetics [3–6]. Inulin is a potential candidate of biomaterial and has aroused great interests for its special construction, diverse physiological functions and physicochemical properties [7,8]. Inulin is a kind of plant storage polysaccharide, which is linked by β-2,1-glycosidic bonds. The terminal of inulin molecule is usually combined with a glucose residue [9]. Because of the β-configuration of anomeric carbon, inulin cannot be digested like a common carbohydrate in the human small intestine, which results in the wide utilization of inulin in food and pharmaceutical areas [2,10]. In food industry, inulin is mostly added to replace sugar or fat, and to improve the texture of some low-fat food [11]. Inulin is known as dietary fiber and prebiotic for its diverse physiological functions, such as hypoglycemic action, promoting mineral absorption, and reducing the risk of gastrointestinal diseases [10]. For pharmaceutical utilization, it reveals many therapeutic benefits of inulin on reduced mammary tumor risk, reduced constipation, mitigated inflammation, improved anti-biofilm behaviors, and protection against oxidative stress [12–15]. Inulin is also used as stabilizing excipient to protect the activity of proteins, enzymes, and vaccines [16]. Moreover, inulin is extensively studied to deliver drugs for targeted treatment, especially for colon and lung relevant diseases [17,18]. Inulin served as drug carrier exhibits advantageous properties, for instance, improved bioavailability, reduced toxicity of the drug to non-targeted organs, enhanced dissolution rate and controlled release.

Inulin has great potential for biomaterial applications for its biocompatible, biodegradable, and diverse physiological functions. However, the deficiency on biological activity of inulin would limit its further utilization, because the biological activity of polysaccharide-based biomaterials are essential properties to avoid infection and immunoreaction [19]. It is well known that biological activity of polysaccharides is closely related to their structures. It is assumed that by means of organic synthesis, the molecular structure of inulin could be changed and specific functional groups are accordingly introduced to improve its biological
activity. This is called chemical modification, which has been widely used as a targeted, convenient and high-efficient way to improve, even introduce new characteristics for specific applications [5,20,21]. Stevens et al concluded the derivatization of inulin [22]. However, most of the derivatization is referred to changes in physicochemical properties of inulin. The attempt to improve the biological activity of inulin is few in literatures. Inulin provides a mass of hydroxyl groups as modification sites and its solubility in water and organic solvent make it convenient to be modified [23]. In consideration of these unique properties and wide applications prospects, chemical modification of inulin is quite important not only to reveal its bioactivity (anticancer activities, immunomodulatory effects, etc.), but also to develop various inulin based advanced materials (drug carriers, synthetic vaccines, etc.).

Various groups such as Schiff bases, thioureas, sulfates, and quaternary ammonium salts, have been introduced into polysaccharides to improve their applied values [24–27]. Schiff bases, characterized by C=N, find applications in medicine, catalysis, analytical chemistry, preservatives, and photochromic materials [28–31]. In the past few decades, Schiff bases have been hot topics in the structural modifications of polysaccharides for their expanded biofunctional properties. These polysaccharides modified by Schiff bases are improved obviously on biological activities including antibacterial, antifungal, anticancer, and antioxidant activities thus developed in areas of drug and protein delivery, wound healing, self-healing and tissue engineering [32–34]. In our previous works, we succeed in grafting Schiff bases onto inulin backbone through the reaction between the inulin iminophosphorane ylides and aromatic aldehydes [35]. The Schiff bases with halogens brought notably antifungal activity for inulin. On this basis, a series of inulin derivatives were designed and synthesized using benzaldehydes containing hydroxyl as reaction reagents. The derivatives were expected to exhibit strong antioxidant activity, and still have strong antifungal activity. Their antifungal and antioxidant activities were evaluated for further biomaterial applications.

2. Experimental

2.1. Measurement and reagents

Inulin (from chicory, DP is around 20) was purchased from Hao-yuan Biological Technology Co., Ltd. (Xi’an, China). All chemical reagents were purchased from different companies and used without further purification. For example, N-bromosuccinimide (NBS), triphenylphosphine (Ph3P), cuprous iodide, and all the aromatic aldehydes such as benzaldehyde, salicylaldehyde, 3-hydroxybenzaldehyde, 4-hydroxybenzaldehyde, 3,4-dihydroxybenzaldehyde, and 2,3,4-trihydroxybenzaldehyde were purchased from the Sigma–Aldrich Chemical Co., Ltd (Shanghai, China). Other reagents such as N,N-dimethylethanolamine (DMF), pyridine, acetic anhydride, diethyl ether, triethylamine, tetrahydrofuran, and ethyl alcohol were purchased from the Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). FTIR spectra were recorded in the range of 4000–400 cm⁻¹ by a Jasco-4100 infrared spectrometer (provided by JASCO Co., Ltd., Shanghai, China), using transmittance modes at a resolution of 4.0 cm⁻¹. The samples were measured with KBr mixed tablet at room temperature (RT). ¹H NMR and ¹³C NMR were measured on a Bruker AVIII 500 spectrometer (Fällanden, Switzerland, provided by Bruker Tech. and Serv. Co., Ltd., Beijing, China), using DMSO-d₆ as solvents. Chemical shift values were recorded in the range of 0–9 ppm (¹H NMR spectra) and 0–180 ppm (¹³C NMR spectra) at room temperature. The ultraviolet–visible (UV–vis) spectrum was recorded on a UV–vis spectrometer (TU-1810, provided by Puxi General Instrument Co., Ltd., City, China).

2.2. The synthesis of inulin derivatives

The synthesis of 6-azido-6-deoxy-3,4-di-O-acetyl inulin (AAIL) were on the basis of our previous works [35]. Firstly, C₆-OH of inulin was activated by bromination with NBS/Ph₃P reagents. Then, other hydroxyl groups were protected by acetic anhydride to improve the solubility of inulin in organic solvents. Not only that, the acetylation of hydroxyl groups was conductive to the separation of products against deionized water. After that, AAIL was obtained by a nucleophilic substitution between 6-bromo-6-deoxy-3,4-di-O-acetyl inulin and sodium azide at a high temperature. Afterwards, a series of aromatic aldehyde compounds were reacted with inulin Staudinger ylide, which was derived from the Staudinger reaction between AAIL and Ph₃P. Thereout, the Schiff bases modified inulin derivatives were synthesized successfully. The specific synthesis steps were described as follows (Scheme 1).

![Scheme 1. Synthesis routes of inulin derivatives.](image-url)
2.2.1. The synthesis of 6-bromo-6-deoxy-3,4-di-O-acetyl inulin (BAIL)

In a 250 mL flask, 30 mmol inulin was stirred to dissolution. Then, NBS (90 mmol) and Ph₃P (90 mmol) were added separately at 0 °C. The reaction was carried out at 80 °C for 3 h under argon atmosphere. Amounts of precipitate were obtained and filtered by suction filtration after the solution was poured into excess acetone. The crude products were subsequently dissolved in 100 mL of pyridine. Then, acetic anhydride (25 mL) was added dropwise to the flask. After the solution was stirred overnight at room temperature, it was poured into excess ice water to form amounts of precipitate. The precipitate was separated by centrifugation and washed with deionized water for 3 times. The 6-bromo-6-deoxy-3,4-di-O-acetyl inulin was freeze dried at −53 °C in vacuum. Yield: 87%; ¹³C NMR/DMSO: δ 170 (C-O-acetyl), δ 162 (C-7=N), δ 128–135 (aromatic carbons), δ 21 (CH₃-acetyl); FTIR: ν 1742 (C=O of acetyl), ν 1632 (imine), ν 1670 (imine), ν 3057, 1595, 1540, 692, 727 (benzene ring).

2.2.2. The synthesis of 6-azido-6-deoxy-3,4-di-O-acetyl inulin (AAIL)

Above 6-bromo-6-deoxy-3,4-di-O-acetyl inulin (10 mmol) was mixed with sodium azide (30 mmol) in DMF (100 mL). The solution was reacted with magnetic stirring at 80 °C for 4 h under argon atmosphere. After that, the solution was cooled to room temperature and was reacted with magnetic stirring at 80 °C for 4 h under argon atmosphere. The reaction was carried out at 80 °C for 3 h under argon atmosphere. The 6-bromo-6-deoxy-3,4-di-O-acetyl inulin was freeze dried at 53 °C in vacuum. Yield: 87%; ¹³C NMR/DMSO: δ 170 (C-O-acetyl), δ 162 (C-7=N), δ 128–136 (aromatic carbons), δ 21 (CH₃-acetyl); FTIR: ν 1742 (C=O of acetyl), ν 170 (C=O of acetyl), ν 1670 (imine), ν 3061, 1595, 1540, 694, 757 (benzene ring).

2.2.3. The synthesis of inulin derivatives

A mixture of 6-azido-6-deoxy-3,4-di-O-acetyl inulin (2 mmol), triphenylphosphine (6 mmol), and aromatic aldehydes (60 mmol) in THF (15 mL) were stirred at 50 °C. The reaction progress was monitored by infrared spectroscopy, and was subsequently separated by suction filtration and washed with diethyl ether to form precipitates, which were subsequently separated by suction filtration and washed with diethyl ether and acetone for three times. The eventually product was obtained by freeze drying at −53 °C in vacuum after further purification in a Soxhlet apparatus with acetone for 48 h.

6-amino-(N-benzylidene)-6-deoxy-3,4-di-O-acetyl inulin (BAIL): Yield: 64%; DSₜₐₙₘᵢ𝑛ₑ 0.34; ¹³C NMR/DMSO: δ 162 (C-7=N), δ 128–136 (aromatic carbons), δ 21 (CH₃-acetyl); FTIR: ν 1742 (C=O of acetyl), ν 1670 (imine), ν 3061, 1595, 1540, 694, 757 (benzene ring).

6-amino-(N-2-hydroxybenzylidene)-6-deoxy-3,4-di-O-acetyl-inulin (2HBAIL): Yield: 48%; DSₜₐₙₘᵢ𝑛ₑ 0.61; ¹³C NMR/DMSO: δ 170 (C=O-acetyl), δ 162 (C-7=N), δ 128–133 (aromatic carbons), δ 21 (CH₃-acetyl); FTIR: ν 1742 (C=O of acetyl), ν 1670 (imine), ν 3057, 1591, 1540, 693, 727 (benzene ring).

6-amino-(N-3-hydroxybenzylidene)-6-deoxy-3,4-di-O-acetyl-inulin (3HBAIL): Yield: 85%; DSₜₐₙₘᵢ𝑛ₑ 0.90; ¹³C NMR/DMSO: δ 170 (C=O-acetyl), δ 160 (C-7=N), δ 129–135 (aromatic carbons), δ 21 (CH₃-acetyl); FTIR: ν 1742 (C=O of acetyl), ν 1669 (imine), ν 3061, 1586, 1540, 691, 756 (benzene ring).

6-amino-(N-4-hydroxybenzylidene)-6-deoxy-3,4-di-O-acetyl-inulin (4HBAIL): Yield: 88%; DSₜₐₙₘᵢ𝑛ₑ 0.88; ¹³C NMR/DMSO: δ 170 (C=O-acetyl), δ 162 (C-7=N), δ 128–133 (aromatic carbons), δ 21 (CH₃-acetyl); FTIR: ν 1742 (C=O of acetyl), ν 1671 (imine), ν 3057, 1581, 1504, 693, 727 (benzene ring).

6-amino-(N-3,4-dihydroxybenzylidene)-6-deoxy-3,4-di-O-acetyl-inulin (3,4DHBAIL): Yield: 62%; DSₜₐₙₘᵢɴₑ 0.65; ¹³C NMR/DMSO: δ 170 (C=O-acetyl), δ 162 (C-7=N), δ 128–133 (aromatic carbons), δ 21 (CH₃-acetyl); FTIR: ν 1742 (C=O of acetyl), ν 1671 (imine), ν 3061, 1589, 1492, 692, 727 (benzene ring).

2.3. Antioxidant assays

In general, antioxidant research is divided into in vitro and in vivo antioxidant activity. Antioxidant research in vivo is based on antioxidant activity in vitro. There are various spectrophotometric methods to assess the antioxidant activity in vitro, including hydroxyl radical scavenging assay, DPPH radical scavenging assay, superoxide anion radical scavenging assay, and reductive ability. These methods are widely applied to assess the polysaccharide antioxidant activity as well as its derivatives [36].

2.3.1. Hydroxyl radical scavenging assay

Hydroxyl radical scavenging assay is generally carried out on the basis of Fenton oxidation [37]. In brief, a total of 4.5 mL of solution containing inulin or inulin derivatives (1 mL, 0.45 mg mL⁻¹, 0.9 mg mL⁻¹, 1.8 mg mL⁻¹, 3.6 mg mL⁻¹, and 7.2 mg mL⁻¹), EDTA-Fe²⁺ (0.5 mL, 200 µmol L⁻¹), potassium phosphate buffer (1 mL, 150 mmol L⁻¹, pH 7.4), safranine T (1 mL, 0.23 µmol L⁻¹), and H₂O₂ (1 mL, 60 µmol L⁻¹) was reacted for 30 min at 37 °C avoiding light. Its absorbance at 520 nm was measured subsequently. The test was repeated for three times and the hydroxyl radicals scavenging ability was calculated by

![Fig. 1. FT-IR spectra of inulin, BAIL, AAIL and inulin derivatives.](image-url)
following equation:

\[
\text{Scavenging rate (\%)} = \frac{A_{\text{sample 520 nm}} - A_{\text{blank 520 nm}}}{A_{\text{control 520 nm}} - A_{\text{blank 520 nm}}} \times 100
\]

where \(A_{\text{blank 520 nm}}\) was the absorbance of the blank (distilled water instead of the samples), \(A_{\text{control 520 nm}}\) was the absorbance of the control (distilled water instead of H\(_2\)O\(_2\)).

2.3.2. DPPH radical scavenging assay

The DPPH radical scavenging assay was assessed on the basis of Tan’s methods [38]. In general, inulin or inulin derivatives (1 mL, 0.3 mg mL\(^{-1}\), 0.6 mg mL\(^{-1}\), 1.2 mg mL\(^{-1}\), 2.4 mg mL\(^{-1}\), and 4.8 mg mL\(^{-1}\)) mixed with DPPH solution (2 mL, 180 µmol L\(^{-1}\), dissolved in ethanol) was incubated for 30 min at 25 °C avoiding light. Then, the absorbance of the remained DPPH radical was measured at 517 nm against a blank. Three replicates for each sample were tested and the DPPH-radical scavenging effect was calculated according to the following equation:

\[
\text{Scavenging rate (\%)} = \left(1 - \frac{A_{\text{sample 517 nm}} - A_{\text{control 517 nm}}}{A_{\text{blank 517 nm}}} \right) \times 100
\]

where \(A_{\text{control 517 nm}}\) is the absorbance of the control (ethanol instead of DPPH solution for each concentration) and \(A_{\text{blank 517 nm}}\) is the absorbance of the blank (distilled water instead of the samples).

2.3.3. Superoxide anion radical scavenging assay

The superoxide radical scavenging ability was assessed by Zhang’s methods [39]. Inulin or inulin derivatives (1.5 mL, 0.3 mg mL\(^{-1}\),...
0.6 mg mL$^{-1}$, 1.2 mg mL$^{-1}$, 2.4 mg mL$^{-1}$, and 4.8 mg mL$^{-1}$) was mixed with 1.5 mL of Tris-HCl buffer (16 mmol L$^{-1}$, pH 8.0) containing NADH (0.5 mL, 456 μmol L$^{-1}$, dissolved in), NBT (0.5 mL, 300 μmol L$^{-1}$), and PMS (0.5 mL, 60 μmol L$^{-1}$). The mixed solution was incubated at room temperature for 5 min. Then the absorbance was measured at 560 nm against a blank. Three replicates for each sample concentration were tested and the superoxide radical scavenging ability was calculated using the following equation:

\[
\text{Scavenging rate} \, (\%) = \left(1 - \frac{A_{\text{sample} \, 560 \, \text{nm}} - A_{\text{control} \, 560 \, \text{nm}}}{A_{\text{blank} \, 560 \, \text{nm}}} \right) \times 100
\]

where $A_{\text{control} \, 560 \, \text{nm}}$ is the absorbance of the control (distilled water instead of NADH for each concentration) and $A_{\text{blank} \, 560 \, \text{nm}}$ is the absorbance of the blank (distilled water instead of the samples).

### 2.3.4. Reductive ability

The reductive ability was determined according to the methods of Luan [40]. In brief, 1.0 mL testing sample of different concentrations dissolved in phosphate buffer (200 μmol L$^{-1}$, pH 6.6) was mixed with 1 mL potassium ferricyanide. The mixed solution was incubated at 50 °C for 20 min followed by adding trichloroacetic acid (10%, w/v) to terminate the reaction. Then the solution was mixed with 3 mL of distilled water

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**Fig. 3.** $^{13}$C NMR spectra of inulin, AAIL and inulin derivatives.
and 0.6 mL of ferric chloride (0.1%, w/v), and the absorbance was measured at 700 nm.

2.4. Antifungal assay

We studied the antifungal activity of inulin derivatives according to previous methods [35]. Briefly, the three test fungi were rejuvenated twice then incubated on solid medium (PDA) to use. The aqueous solution of inulin or inulin derivatives were added to sterilized PDA medium and the mixture was shaken well to give final concentrations of 0.1, 0.5 and 1.0 mg mL\(^{-1}\). The culture media containing samples were transferred to Petri dishes (7 cm) and cooled to solid. Then a piece of prepared test fungi cake (5 mm) was inoculated to the medium of each Petri dish. It was allowed to incubated at 27 °C for 2–3 days until the mycelia of fungi reached the edges of the blank plate (without samples).

2.5. Statistical analysis

All data were expressed as means ± SD and used SPSS for analysis. Results with P < 0.05 were considered statistically significant and the means were separated by Duncan’s multiple range test.

3. Results and discussion

3.1. Structures of inulin derivatives

The structures of inulin derivatives were confirmed by FTIR, \(^1\)H NMR, and \(^{13}\)C NMR spectroscopy. The FTIR spectra of inulin, BAIL, AAIL, and inulin derivatives were shown in Fig. 1. The pure inulin was characterized by peaks at 3401, 2923, and 1025 cm\(^{-1}\) that belonged to O—H stretching, C—H stretching and C—O—C bending respectively [41]. In the FTIR spectrum of BAIL, the characteristic peak of acetyl group appeared at around 1743 cm\(^{-1}\). A new peak appeared at 539 cm\(^{-1}\) attributed to C-Br [42]. After azido grafted onto inulin, the corresponding absorption of azido appeared at 2107 cm\(^{-1}\) resulting the disappearance of C-Br [42]. Regarding the six inulin derivatives, compared with AAIL, the absorption of acetyl (1734 cm\(^{-1}\)) still existed, but the absorption of azido (2107 cm\(^{-1}\)) disappeared completely. Moreover, there were new peaks at around 1670–1680 cm\(^{-1}\), which belonged to the imine [35,43] Additionally, at around 3060 and 1450–1600 cm\(^{-1}\), there appeared the characteristic absorption of the benzene ring. At the fingerprint region, absorptions at 754, 727, and 693 cm\(^{-1}\) were also the evidence of the benzene conjunction [43].

\(^1\)H NMR (Fig. 2) and \(^{13}\)C NMR (Fig. 3) spectra were analyzed to further confirm the structure of these inulin derivatives. According to the literature, the signals of the inulin backbone, including the anhydroglucose unit, located at 3.0–5.4 ppm in \(^1\)H NMR spectra and 60–103 ppm in \(^{13}\)C NMR spectra, which always existed for each products [44]. Because of the acetylation and azidation, in the \(^1\)H NMR spectra of AAIL, it appeared new signals at around 2.0 ppm for the hydrogen of the acetyl (Ac) and 3.7 ppm for the H—C=N. Correspondingly, \(^{13}\)C NMR spectra of AAIL also showed new signals at 170 ppm for the C=O of the acetyl (Ac) and 52.6 ppm for the C=C=N [43]. After further modification, obvious changes could be observed in the NMR spectra of the inulin derivatives. For example, new signals appeared at around 8.4 ppm in \(^1\)H NMR spectra and at around 166 ppm in \(^{13}\)C NMR spectra, which were assigned to the hydrogen of the H—C=N and the carbon proton of the C=C=N, respectively. Moreover, signals of the aromatic rings in the inulin derivatives could be observed in the range of 6.5–8.0 ppm in the \(^1\)H NMR spectra and 120–140 ppm in the \(^{13}\)C NMR spectra [35,43].

All of the abovementioned data proved the successful preparation of inulin derivatives.

3.2. Degree of substitution (DS)

The DS for inulin derivatives was calculated by the ratio of the integral values of aromatic protons and H\(_7\) to that of inulin backbone protons (H\(_2\)-6/6) [43]. For example, the \(DS_{\text{amine}}\) value of BAIL is calculated according to the following equation by \(^1\)H NMR (Fig. 4). The results are summarized at Table 1.

\[
\text{DS}_{\text{amine}} = \frac{7 \times I_{\text{H, aromatic}} - H_7}{6 \times I_{\text{H, AGU}}}
\]

I = integral values; H, aromatic = aromatic protons; H,7 = the proton of —CH=N; H, AGU = inulin backbone protons. The coefficient 7 means the amount of hydrogen protons on inulin backbone, and the coefficient 6 means the amount of hydrogen protons on benzene ring and imine.

3.3. Water solubility

It is important to prepare aqueous solution of the test samples in order to accurately assess their antioxidant activity. Fig. 4 shows the aqueous solution of inulin and inulin derivatives at a concentration of

### Table 1

| Compound     | Yield | Degree of substitution |
|--------------|-------|------------------------|
| Inulin       | /     | /                      |
| BAIL         | 64%   | 0.34                   |
| 2HBSAIL      | 48%   | 0.61                   |
| 3HBSAIL      | 85%   | 0.90                   |
| 4HBSAIL      | 88%   | 0.88                   |
| 3,4DHBAIL    | 62%   | 0.65                   |
| 2,3,4THBSAIL | 74%   | 0.63                   |
3. All the solutions except AAIL are clear, which indicate a favorable water solubility of inulin and inulin derivatives.

3.4. Antioxidant activity

Reactive oxygen species (ROS), which mainly include the free radicals such as superoxide (O$_2^-$) and hydroxyl (·OH) as well as the non-radical molecules such as hydrogen peroxide (H$_2$O$_2$), are necessary for maintaining normal cell functions. However, when the level of ROS exceeds the defense mechanisms, they can affect many cellular functions by damaging the molecules that are essential to life, such as DNA, RNA, enzymes, and other biomolecules [45]. Regarding biomaterials, the sustainable release of antioxidant substances are advantageous to avoid the inflammatory processes caused by oxidative damage. For this reason, the antioxidant activity is very important for biomaterial.

3.4.1. Hydroxyl radical scavenging ability

Hydroxyl radical is an extremely strong oxidative free radical, which could lead to cell death or mutations by destruction of purine and pyrimidine in deoxyribonucleic acid (DNA) [46]. Safranine T, as a chromogenic agent, has the maximum UV absorption peak at 520 nm. The hydroxyl radical produced by Fenton reaction could oxidize the saffron T, leading to the reduction of the absorbance value. When the hydroxyl radical scavenging sample was added, it could protect the saffron T from oxidation by consuming the hydroxyl radical. As a result, the higher the UV absorbance at 520 nm, the stronger scavenging ability of the sample.

Fig. 6a shows the hydroxyl radical scavenging ability of inulin and inulin derivatives. Inulin shows the weakest hydroxyl radical scavenging ability, which is close to Vc. Compared to inulin, all the inulin derivatives exhibit hydroxyl radical scavenging ability in varying degrees and the results are discussed as follows. The inhibitory indexes of all the samples are positively correlated with their concentration. From the figure, all...
the inulin derivatives except 2HBSAIL are powerful than BSAIL, which indicate that phenolic hydroxyl groups at meta or para position of benzene ring are helpful to enhance the hydroxyl radicals scavenging ability of these inulin derivatives. Among them, 3HBSAIL is the most efficient inulin derivative, whose clearance rate reaches 84% at 0.4 mg mL\(^{-1}\) and it could completely scavenge hydroxyl radicals at 0.8 mg mL\(^{-1}\). By a further analysis, we find that increasing amounts of the phenolic hydroxyl groups on benzene exhibit negative effect on scavenging ability. For example, at 1.6 mg mL\(^{-1}\), the inulin derivative with three phenolic hydroxyl groups (2,3,4THBSAIL, scavenging rate is 79%) is weaker than that with two phenolic hydroxyl groups (3,4DHBSAIL, scavenging rate is 89%), and 3,4DHBSAIL is weaker than 3HBSAIL (scavenging rate is 100%) and 4HBSAIL (scavenging rate is 100%).

3.4.2. DPPH radical and superoxide radical scavenging ability

DPPH radical is a very stable commercial nitrogen-centered free radical due to the conjugation and steric hindrance effect. Its ethanol solution shows violet coloration and is absorbed at 517 nm. If the test sample is efficient, it could help to stabilize or reduce the DPPH radical, thus fade the color of the solution. In this way, the absorbance value at 517 nm is also reduced.

Superoxide anion free radical is a kind of free radical produced in the metabolic process of organisms. It could directly attack biological macromolecules, additionally act as precursor of hydroxyl radical and hydrogen peroxide causing damage to cell structure and function. Superoxide may be considered the ultimate danger for the aerobic life, because it is unavoidably produced and is potentially toxic [46,47]. Under a mild alkaline condition, NADH could be oxidized by PMS to generate superoxide anion free radical, which could further react with NBT to form blue substance, namely formazan. The maximum UV absorption wavelength of the formazan solution locates at 560 nm. When efficient sample was added, it could be preferentially combined with the superoxide anion free radical, thus prevented the generation of the colored substance. In this way, the sample with lighter color has stronger superoxide scavenging ability.

Fig. 6b and c respectively show the scavenging ability of inulin and inulin derivatives against DPPH radicals and superoxide radicals. In Fig. 5b, it is obvious that 3,4DHBSAIL and 2,3,4THBSAIL have great scavenging effect on DPPH radicals. They could completely scavenge the DPPH radicals even at the lowest test concentration (0.1 mg mL\(^{-1}\)). Regarding superoxide radicals, 2,3,4THBSAIL and 3,4DHBSAIL also exhibit the strongest scavenging effect among all the inulin derivatives. At 1.6 mg mL\(^{-1}\), the scavenging rate of 2,3,4THBSAIL is 100%, and the value for 3,4DHBSAIL is 90%. For both DPPH and superoxide radicals scavenging ability, inulin derivatives containing multiple phenolic hydroxyl groups are significantly improved than those containing single or none phenolic hydroxyl groups. The results indicate that more amounts of phenolic hydroxyl groups on benzene are the key to enhance the scavenging ability of DPPH and superoxide radicals. Except 3,4DHBSAIL and 2,3,4THBSAIL, the scavenging rate of other inulin derivatives against DPPH and superoxide radicals decrease in the order of 3HBSAIL > 4HBSAIL > 2HBSAIL > BSAIL. The result further proves the importance of phenolic hydroxyl groups on radicals scavenging ability. Moreover, the position of phenolic hydroxyl groups on benzene ring also make an influence on the scavenging ability. In general, meta-position is more advantageous.

3.4.3. Reductive ability

The reductive ability of polysaccharides is also a common method to compare the antioxidant ability as the reductive ability is closely related to antioxidant activity [46]. If the test sample has antioxidant activity, it could oxidize K\(_{2}\)Fe(CN)\(_{6}\) to form K\(_{3}\)Fe(CN)\(_{6}\). The K\(_{2}\)Fe(CN)\(_{6}\) is able to react with Fe (III) to generate Fe\(_{3}\)[Fe(CN)\(_{6}\)]. The resulting Fe\(_{3}\)[Fe(CN)\(_{6}\)] has a special ultraviolet absorption at 700 nm. Therefore, the reductive ability of inulin and derivatives could be determined by UV–visible spectrophotometry. The stronger the reductive ability of the sample, the more Fe\(_{3}\)[Fe(CN)\(_{6}\)] is generated, the greater the absorbance is at 700 nm.

The reductive ability of inulin and inulin derivatives are studied and the results are presented in Fig. 6d. Among all the samples, there are two inulin derivatives exhibiting strong reductive ability, which are derivatives containing more than one phenolic hydroxyl group, namely 3,4DHBSAIL and 2,3,4THBSAIL. At 1.6 mg mL\(^{-1}\), the reductive ability value of 2,3,4THBSAIL reaches 3.9, which is slightly higher than that of 3,4DHBSAIL (reductive ability value is 3.7). The reductive ability of other synthesized derivatives such as BSAIL, 2HBSAIL, 3HBSAIL, and 4HBSAIL are improved indistinguishably compared to inulin. As a result, the reductive ability of inulin derivatives are affected by the amounts of phenolic hydroxyl groups on benzene ring. Single phenolic hydroxyl group is not enough to bring the derivatives a distinct improvement on reductive ability. When the amounts of phenolic hydroxyl groups increase to two or three, the effect becomes obvious.

3.5. Antifungal activity

For crops and fruit plants, fungi cause more economic damage than any other kind of microorganisms, and annual losses are estimated at more than 200 billion dollars [48]. Traditional fungicides are unavoidable suffer from volatility, high toxicity, and fungicides resistance. Anti-fungal polymers are promising to overcome these problems. They are expected to have function of slow release as well [49]. In order to evaluate the antifungal activity of the synthesized inulin derivatives, three kinds of plant pathogens, namely, Botrytis cinerea, Fusarium oxysporum f. sp. cucumerium Owen, and Phomopsis asparagi, were selected to carry out the antifungal assay. The antifungal activity of AAIL and BSAIL are tested at the same time to analyze the structure-function relationship. Each sample was tested at 0.1, 0.5, and 1.0 mg mL\(^{-1}\). The results are shown in Fig. 7.

According to Fig. 7, inulin exhibits no inhibitory effect against all test fungi. The antifungal activity of AAIL is negligible compared to that of the inulin derivatives, as azide grafting had little effect on antifungal action. Regarding all the inulin derivatives, involving BSAIL, 2HBSAIL, 3HBSAIL, 4HBSAIL, 3,4DHBSAIL, and 2,3,4THBSAIL, show distinct antifungal activity, and the inhibitory indices of each derivatives are positively correlated with the concentration, which indicates the active effect of the Schiff bases on antifungal activity. Moreover, the synthetic inulin derivatives show a broad antifungal spectrum against the tested fungi.

Fig. 7a shows the antifungal activity of inulin, AAIL, and inulin derivatives against Botrytis cinerea. Rather strong antifungal activities of all the inulin derivatives could be observed from the figure. Different structures of the derivatives result in the differences of inhibitory indices. The inhibitory indices of BSAIL, 2HBSAIL, 3HBSAIL, 4HBSAIL, 3,4DHBSAIL, and 2,3,4THBSAIL are 65.3%, 86.5%, and 92.9%, 94.0%, 59.4%, and 81.5% at 1.0 mg mL\(^{-1}\), respectively. The obtained antifungal activity is benefit from the introduction of the aromatic Schiff bases. The permeability-based view is acceptable to explain the correlogram antimicrobial mechanism. As the oil film on the surface of the cell will only allow lipid soluble substances to get inside, and the lipophilic characteristics of the benzene ring as well as the acetyl make the derivatives are easier to get inside the cell, leading to the apoptosis. Besides, the inulin derivatives modified with the Schiff bases have an excellent metal binding property, which suppresses the growth of microbes by interacting with the cellular components. Among all the derivatives, the inhibitory indices of 2HBSAIL, 3HBSAIL, and 4HBSAIL locate the front rank and their inhibitory indices are near. Their inhibitory rates are all higher than BSAIL and the values are more than 85% at 1.6 mg mL\(^{-1}\). As indicated that single phenolic hydroxyl group on benzene ring could help to further improve the antifungal activity of the inulin derivatives on the basis of the Schiff bases. Then, the derivatives with multiple phenolic hydroxyl groups, namely 3,4DHBSAIL, and 2,3,4THBSAIL, exhibit lower inhibitory indices than the derivatives with single phenolic hydroxyl groups.
hydroxyl group. The result indicates that more amounts of phenolic hydroxyl groups would decrease the antifungal activity of the derivatives, which may be connected with the reduced hydrophobicity.

The antifungal assay results of the inulin derivatives against *Fusarium oxysporum* f. sp. cucumerium Owen and *Phomopsis asparagi* are shown in Fig. 7b and c, respectively. All the inulin derivatives show antifungal activity in varying degrees and their inhibitory rates are mounted up with an increasing concentration. The inhibitory indices of BSAIL, 2HBSAIL, 3HBSAIL, 4HBSAIL, and 2,3,4THBSAIL are 70.0%, 78.3%, 83.0%, 76.9%, and 80.6%, respectively at 1.0 mg mL\(^{-1}\) against *Fusarium oxysporum* f. sp. cucumerium Owen. The values are relatively high compared to inulin. 3HBSAIL is the most efficient sample against *Fusarium oxysporum* f. sp. cucumerium Owen. The highest inhibitory rate of 3HBSAIL is consistent with the highest degree of substitution. Besides, the inhibitory indices of BSAIL, 2HBSAIL, 3HBSAIL, 4HBSAIL, 3,4DHBSAIL, and 2,3,4THBSAIL are 70.0%, 78.3%, 83.0%, 76.9%, 68.1%, and 80.6%, respectively at 1.0 mg mL\(^{-1}\) against *Fusarium oxysporum* f. sp. cucumerium Owen. The values are relatively high compared to inulin. 3HBSAIL is the most efficient sample against *Fusarium oxysporum* f. sp. cucumerium Owen.

It is hard to summarize the regularity of different samples against the test fungi, because the antifungal activity of the samples is affected by many factors, including the DS, and the number of phenolic hydroxyl groups and their substitutive positions, rather than a single factor. But the distinct antifungal activity of all the inulin derivatives confirmed that it is feasible to chemically modify inulin with Schiff bases to confer high antifungal activity to inulin.

In the present work, the increased hydrophobicity of the inulin derivatives due to the aromatic substitution and acetyl, along with the enhanced metal binding ability arising due to the presence of imine groups, appears to be responsible for the observed antifungal property of the derivatives.

4. Conclusions

In this study, the structure of inulin was chemically modified by introducing Schiff bases to inulin backbone. A total of 6 kinds of inulin derivatives were conveniently obtained, and their structures were characterized by FTIR, \(^1\)H NMR, and \(^{13}\)C NMR spectroscopy, respectively. Their structures differed in the number and substitutive position of the phenolic hydroxyl groups on benzene ring. Subsequently, their biological activities, including antioxidant and antifungal activities, were emphatically studied. The antioxidant activity was evaluated by scavenging abilities of hydroxyl radical, DPPH radical, and superoxide radical, as well as reductive ability. The antioxidant activity of all the inulin derivatives was significantly improved compared to inulin. Furthermore, the antifungal activities against three kinds of plant pathogenic fungi (*Botrytis cinerea, Fusarium oxysporum* f. sp. cucumerium Owen, and *Phomopsis asparagi*) were evaluated in vitro by mycelium growth rate method. The inulin derivatives exhibited strong antifungal activity.
compared to pure inulin. The biological activity of the inulin derivatives was affected by composite factors, including the DS, and the number of phenolic hydroxyl groups and their substitutive positions. The products described in this paper have great potential as biomaterials with good bioactivity and good biocompatibility. Finally, the structure-activity relationship needs to be further investigated in the future.

Declaration of competing interest

The authors have declared no conflicts of interest.

Acknowledgements

The authors thank the National Natural Science Foundation of China, China, 41576156, Natural Science Foundation of Shandong Province, China, ZR2017BD015, Science and Technology Service Network Initiative of Chinese Academy of Sciences, China, KJ-STS-ZDTP-023, the Public Science and Technology Research Funds Projects of Ocean, China, 2015418022-3, and the Seed Project of Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, China, Y755031011, for financial support of this work.

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