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Synthesis and bioactivity of lignin related high-added-value 2*H*,4*H*-dihydro-pyrano[2,3-*c*]pyrazoles and 1*H*,4*H*-dihydro-pyrano[2,3-*c*]pyrazoles

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ABSTRACT

A series of novel 2*H*,4*H*-dihydro-pyrano[2,3-*c*]pyrazoles and 1*H*,4*H*-dihydro-pyrano-[2,3-*c*]pyrazoles were synthesized with aromatic aldehydes obtained from lignin and in vitro antioxidant using microwave-assisted technology and cytotoxic activities of these compounds were evaluated. The structure activity relationship (SAR) studies showed that the introduction of methoxy group in aromatic groups of dihydro-pyrano[2,3-*c*]pyrazoles could significantly increase their radical scavenging activities and the substituted moieties at N or C-3 position of dihydro-pyrano[2,3-*c*]pyrazoles could potentially influence on their antioxidant activities. Compared to positive drug control, syringyl (4-hydroxy-3,5-dimethoxyphenyl) substituted 2*H*,4*H*-dihydro-pyrano-[2,3-*c*]-pyrazoles **6a**, **6d**, **6g**, **6j** and 1*H*,4*H*-dihydro-pyrano-[2,3-*c*]pyrazoles **7a**, **7d**, **7g** have much better antioxidant activity. In addition, all of those compounds showed low cytotoxicity through cytotoxicity evaluation. Thus, these compounds might have potential as promising agents for curing some free radical-related diseases or food additives.

1. Introduction

Because of the current shortage of fossil fuels, lignocellulosic biomass has been recognized as a potential feedstock for energy production and the synthesis of high-value chemicals (Fermor, 1993; Bull, 1994; Sun and Cheng, 2002; Kim and Dale, 2004; Balata et al., 2008; Luo et al., 2009; Balat, 2011; Zhu and Zhuang, 2012). Lignin is, next to cellulose, the main constituent of lignocellulosic biomass existing widely in wood pulp and papermaking waste. At present, most of this technical lignin is often burned to produce energy (Luo et al., 2009; Rodrigues Pinto et al., 2011). However, burning lignin is just a resource-wasting model because of lignin's relative low heating value. Therefore, converting lignin into added-value applications is a key factor for creating economically feasible biorefinery processes (Rodrigues Pinto et al., 2011). Recently, producing added-value aromatic monomers (typically vanillin and syringaldehyde) (Rodrigues Pinto et al., 2010) and their related pharmaceutical chemicals (Bjørsvik and Liguori, 2002; Belkheiri et al., 2010; Makni et al., 2011; Menezes et al., 2011; Yang et al., 2011; Egüés et al., 2012) from lignin have been the topic of many publications. A number of studies showed that lignin, aromatic monomers degraded from lignin and their derivatives afforded good antioxidant (Ugartondo et al., 2009; Belkheiri et al., 2010; Yang et al., 2011; Menezes et al., 2011; Egüés et al., 2012), antimicrobial (Dong et al., 2011), anti-inflammatory, hepatoprotective (Makni et al., 2011), antihypertensive (Yang et al., 2012b) properties, etc. Therefore, there is considerable interest in converting lignin to these high-value potential drugs while, at the same time, disposing of a high-volume, low-value waste.

Many previous papers have confirmed that pyrano[2,3-*c*]pyrazoles and their related derivatives are an important class of pharmaceutical compounds and exhibit a broad spectrum of biological activities. In particular, these compounds have been confirmed possessing antifungal, antibacterial, anticancer, anti-inflammatory, analgesic, antiplatelet and antioxidant activities (Kuo et al., 1984; Abdel-Rahman et al., 2004; Pavlik et al.,

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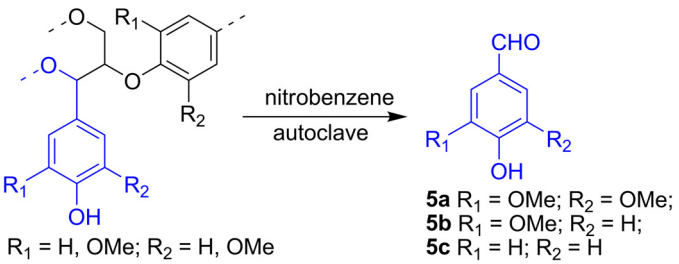


Scheme 1. Synthesis of aromatic aldehydes from lignin.

2009; Saundane and Manjunatha, 2012). Furthermore, these compounds also have been identified as vasodilators, hypotensive, hypoglycemic (Kuo et al., 1984; Huang et al., 1992; Abdelrazek et al., 2007; Pavlik et al., 2009), and some compounds have been demonstrated an affinity toward A1 and A2a adenosine receptors (Catarzi et al., 1995; Colotta et al., 1998). Therefore, our group remain high interest in these compounds and are focusing on synthesizing some novel pyrano[2,3-*c*]pyrazoles-like compounds with promising biological potential. In the present paper, some novel pyrano[2,3-*c*]pyrazoles were synthesized from lignin (Schemes 1 and 2) and their biological activities (antioxidant ability and cytotoxicity) were evaluated in view of lignin, aromatic monomers degraded from lignin, pyrano[2,3-*c*]pyrazoles and their derivatives possessing good antioxidant property.

2. Experimental

2.1. General

Vanillin, syringaldehyde and *p*-hydroxybenzaldehyde were synthesized according to the previously reported methods (Yang et al., 2011, 2012a, 2012b). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and Trolox were bought from Alfa Aesar. 2,2′-Azino-bis(3-ethylenzothiazoline-sulphonic acid) diammonium salt (ABTS$^+$) and $K_2S_2O_8$ were bought from Sigma–Aldrich Co. LLC. Other reagents were obtained from Sinopharm Chemical Reagent Co, Ltd. All reactions were conducted in MAS (II) Sineo microwave reactors using external surface sensors. All synthesized compounds were characterized by infra-red (IR), ^{1}H NMR, ^{13}C NMR, mass spectrometry (MS) and elemental analysis (EA). IR spectra were recorded with a Nicolet Magna-IR 550 spectrometer. Mass spectra were recorded on WATERS Q-TOF Premier Mass Spectrometer using electrospray ionization (ESI). ^{1}H and ^{13}C NMR spectra were recorded with a Bruker DRX-300 Advance spectrometer at 300 MHz and 75 MHz, respectively. Elemental analyses (C, H, N, S) were conducted using a PE-2400(II) Elemental Analyser, and results were found to be in good agreement (±0.2%) with the calculated values. The UV absorbance was measured by Perkin Elmer Lambda 2 Spectrophotometer.

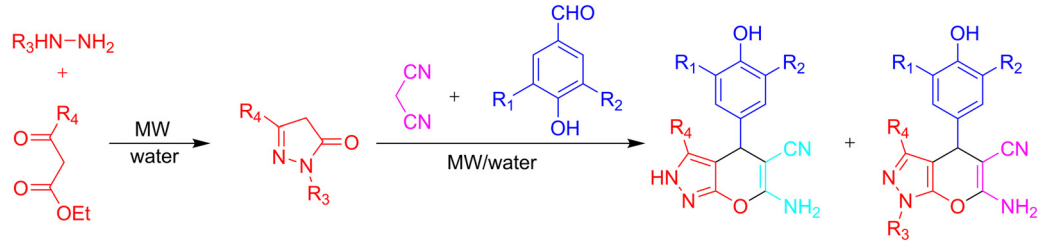
2.2. Synthesis of aromatic aldehydes from lignin

The aromatic aldehydes were synthesized according to our reported procedure (Yang et al., 2012b). Briefly, alkali lignin (100 g), 500 mL 2 mol L^{-1} sodium hydroxide and 112 mL nitrobenzene were placed in a 2 L stainless steel autoclave. The mixture was heated at 170 °C at 1 MPa for 2 h. After the autoclave was cooled to room temperature, the mixture was then transferred to a liquid–liquid extractor for continuous extraction with chloroform (3× 500 mL) to remove any nitrobenzene reduction product and excess of nitrobenzene. The oxidation mixture was acidified with concentrated HCl to pH 4 and further extracted with chloroform (3× 500 mL). The solvent from the second chloroform solution was removed by using a rotary evaporator at 40 °C under reduced pressure to obtain the mixture of aromatic aldehydes. The residue was isolated by column chromatography to obtain syringaldehyde, vanillin and *p*-hydroxybenzaldehyde with total yield 12–18%.

2.3. Typical procedure to synthesize dihydropyrano[2,3-c]pyrazoles

A stirred aqueous mixture of hydrazines **1** (5 mmol) and β-ketoesters **2** (5 mmol) was added to 10 mL water. The mixture was placed into an open microwave oven (300 W) at 80 °C in 2 min. Malononitrile **4** (5 mmol) and aromatic aldehydes **5** (5 mmol) were added swiftly and irradiated successively at 80 °C for another 3 min. The reaction mixture was cooled to room temperature to afford the product as a precipitate. The solid residue was filtered, washed with water and 5 mL of 50% ethanol, and then recrystallized from ethyl acetate/ethanol (80:20, v/v) to give the following products:

6-Amino-5-cyano-4-(4-hydroxy-3,5-dimethoxyphenyl)-3-methyl-2,4-dihydropyrano[2,3-*c*]pyrazole (**6a**).
6-Amino-5-cyano-4-(4-hydroxy-3-methoxyphenyl)-3-methyl-2,4-dihydropyrano[2,3-*c*]pyrazole (**6b**).
6-Amino-5-cyano-4-(4-hydroxyphenyl)-3-methyl-2,4-dihydropyrano[2,3-*c*]pyrazole (**6c**).
6-Amino-5-cyano-4-(4-hydroxy-3,5-dimethoxyphenyl)-3-ethyl-2,4-dihydropyrano[2,3-*c*]pyrazole (**6d**).
6-Amino-5-cyano-4-(4-hydroxy-3-methoxyphenyl)-3-ethyl-2,4-dihydropyrano[2,3-*c*]pyrazole (**6e**).
6-Amino-5-cyano-4-(4-hydroxyphenyl)-3-ethyl-2,4-dihydropyrano[2,3-*c*]pyrazole (**6f**).
6-Amino-5-cyano-4-(4-hydroxy-3,5-dimethoxyphenyl)-3-*tert*-butyl-2,4-dihydropyrano[2,3-*c*] pyrazole (**6g**).
6-Amino-5-cyano-4-(4-hydroxy-3-methoxyphenyl)-3-*tert*-butyl-2,4-dihydropyrano[2,3-*c*]pyrazole (**6h**).
6-Amino-5-cyano-4-(4-hydroxyphenyl)-3-*tert*-butyl-2,4-dihydropyrano[2,3-*c*]pyrazole (**6i**).
6-Amino-5-cyano-4-(4-hydroxy-3,5-dimethoxyphenyl)-3-phenyl-2,4-dihydropyrano[2,3-*c*] pyrazole (**6j**).
6-Amino-5-cyano-4-(4-hydroxy-3-methoxyphenyl)-3-phenyl-2,4-dihydropyrano[2,3-*c*]pyrazole (**6k**).



Scheme 2. Microwave-assisted synthesis of dihydro-pyrano[2,3-*c*]pyrazoles.