European Journal of Medicinal Chemistry 68 (2013) 385–393

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Antioxidant effectiveness generated by one or two phenolic hydroxyl groups in coumarin-substituted dihydropyrazoles

Gao-Lei Xi, Zai-Qun Liu*

Department of Organic Chemistry, College of Chemistry, Jilin University, Changchun 130021, China

ARTICLE INFO

Article history:
Received 20 March 2013
Received in revised form
20 June 2013
Accepted 26 June 2013
Available online 13 August 2013

Keywords:
Coumarin
Dihydropyrazole
Antioxidant
Oxidation of DNA
Free radical

ABSTRACT

A cascade operation was designed to synthesize nine coumarin-substituted dihydropyrazoles with only one or two phenolic hydroxyl groups contained. Antioxidant abilities of the obtained compounds were evaluated by inhibiting 2,2′-azobis(2-amidinopropanehydrochloride) (AAPH)-, Cu^{2+}/glutathione (GSH)-, and ·OH-induced oxidation of DNA. It was found that less phenolic hydroxyl groups can enhance the abilities of coumarin-substituted dihydropyrazoles to protect DNA against the oxidation. Moreover, these coumarin-substituted dihydropyrazoles were employed to scavenge 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) cationic radical ($ABTS^{+\cdot}$), 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH), and galvinoxyl radical, respectively. It was found that double phenolic hydroxyl groups were more beneficial for enhancing the abilities of coumarin-substituted dihydropyrazoles to quench the aforementioned radicals. Therefore, dihydropyrazole linked with coumarin exhibited powerful antioxidant effectiveness even in the case of less phenolic hydroxyl groups involved.

1. Introduction

The *in vivo* oxidative stress of DNA is regarded as the pathogenesis of many fatal diseases [1]. The free radicals from the metabolism [2] or the environment [3] may abstract hydrogen atom from membrane, lipid, protein, and DNA, and consequently, lead to damages of many biological species [4]. The supplementation of antioxidants is beneficial for avoiding oxidative damages [5], keeping redox balance *in vivo* [6], and designing novel medicines [7]. The natural antioxidants attract much research attentions because of their high capacities and low toxicities. Natural polyphenols are antioxidants widely used to scavenge radicals [8]. On the other hand, many efforts are contributed to synthesize antioxidants containing phenolic hydroxyl groups [9]. Increasing the amount of phenolic hydroxyl groups may enhance the antioxidant effectiveness according to traditional concept. However, it was recently found that more phenolic hydroxyl groups contained may cause prooxidative effect and improve the risk in the biological systems. For example, hydroxycinnamic acids containing more than two phenolic hydroxyl groups accelerated the oxidation of DNA in the presence of Cu(II) ions. This was because phenolic hydroxyl groups can reduce Cu(II) to form Cu(I), and Cu(I) can cause the decomposition of DNA [10]. Hence, it is worthy to explore whether a compound containing less than two phenolic hydroxyl groups can also function as an antioxidant.

2. Chemistry

The modification on the structure of natural antioxidants [11] and the recombination of known antioxidative structures [12] are the efficient ways to increase the antioxidant ability. For example, polyphenols were used to link with 4-amino methylbenzylamine or tris(2-aminoethyl)amine to form dendritic structure. As a result, the abilities of the obtained antioxidants to scavenge 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH) [13] and to inhibit 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH, R–N=N–R, R = $-CMe_2C(=NH)NH_2$)- and Cu^{2+}-induced oxidations of low-density lipoprotein and DNA were improved markedly [14]. Moreover, we applied pyrazole [15] and 1,2,4-oxadiazole [16] to bridge with phenols. It has been found that single phenolic hydroxyl group in these compounds exhibited high abilities to inhibit AAPH-induced oxidation of DNA and to scavenge radicals. This result reveals that a suitable arrangement of every structural feature can lead to high antioxidant effectiveness even in the case of less phenolic hydroxyl group involved. On the other hand, coumarin is an important skeleton widely used to construct anticancer [17], antimicrobial [18], antiviral [19], and anti-inflammatory drugs [20]. The aim of this work is to synthesize coumarin-related compounds with less than two phenolic

* Corresponding author. Tel.: +86 431 88499174; fax: +86 431 88499159.
E-mail address: zaiqun-liu@jlu.edu.cn (Z.-Q. Liu).

0223-5234/$ – see front matter © 2013 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.ejmech.2013.06.059

hydroxyl groups contained, and to screen the antioxidant effectiveness. Thus, as shown in Scheme 1, we herein design a series of compounds with dihydropyrazole linking with coumarin and two benzene rings for comparing the antioxidant effectiveness.

3. Pharmacology

The antioxidant effectiveness can be estimated by many methods [21]. We herein evaluated the abilities of the obtained compounds to inhibit the oxidation of DNA induced by AAPH [22], Cu^{2+}/glutathione (GSH) [23], and hydroxyl radical (·OH) [24] and to scavenge 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) cation ic radical ($ABTS^{+\cdot}$), DPPH, and galvinoxyl radical, respectively.

4. Results and discussion

4.1. Synthesis and spectral interpretation

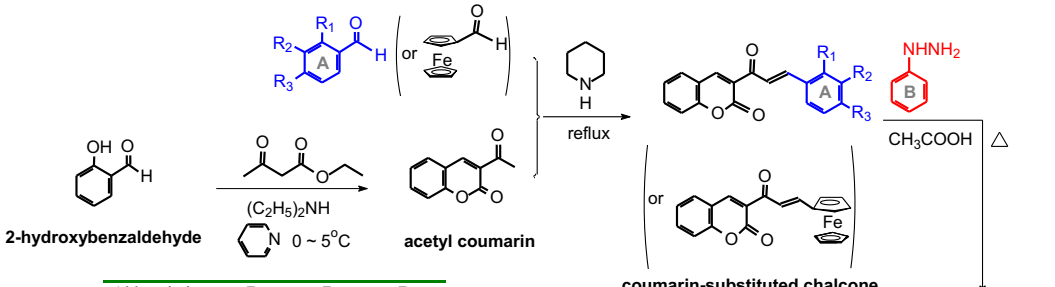
Acetyl coumarin was prepared by the reaction of 2-hydroxybenzaldehyde (10.0 mmol) with the same amount of ethyl acetoacetate (10.0 mmol) in the presence of pyridine and diethylamine as the catalysts. This step was carried out at 0–10 °C for 3 h, and the yield was >95%. The following reaction was Claisen–Schmidt condensation taking place between acetyl coumarin (2.0 mmol) and benzaldehyde (2.2 mmol) in the presence of piperidine (0.25 mL) as the catalyst and ethanol as the solvent. The catalyst used in the condensation between acetyl coumarin and benzaldehyde was the same as in the synthesis of acetyl coumarin. The difference was only on the operation. The preparation of acetyl coumarin was performed at 0–10 °C, and the Claisen–Schmidt condensation was carried out under refluxing for 6 h. The yield was also >95%. Finally, excess phenylhydrazine was added, followed by the addition of the catalyst (acetic acid) and refluxing for 3 h. Thus, the isolation after the final step was to remove phenylhydrazine. The cascade operation was just to change the catalyst from bases (pyridine, diethylamine, and piperidine) to acid (acetic acid). The yields of every step were so high that all the reagents were almost exhausted. Thus, the amount of excess reagents in the first and the second step was too little to influence the following reaction.

The peaks in ^{1}H NMR spectra of these compounds can be generally divided into three parts. The first part located around 8–10 ppm, which can be assigned to the hydrogen atom in –OH. The second part ranged from 6 to 8 ppm, which was contributed from the hydrogen atoms in the aromatic rings. The last part ranged from 3 to 6 ppm, which was donated to CH_2 and CH in dihydropyrazole. Accordingly, the peaks in ^{13}C NMR spectra of these compounds can be divided into two parts. The first part ranged from 100 to 160 ppm, which can be assigned to the carbon atoms at aromatic rings. Other peaks were lower than 100 ppm, which was contributed from CH_2 and CH in dihydropyrazole.

4.2. Effects of coumarin-substituted dihydropyrazoles on Cu^{2+}/GSH-induced oxidation of DNA

The Cu(II) may oxidize GSH to form GSH radical ($GS^{\cdot}$), while the produced Cu(I) combines with DNA to form a complex, DNA-Cu(I)–OOH. The complex can be degraded in the presence of $GS^{\cdot}$, leading to the formation of the oxidative products of DNA [23].

The oxidative products of DNA can be detected after colorized by thiobarbituric acid [25]. As shown as the control line in Fig. 1 (the upper line), the continuous increase of the absorbance reveals much more oxidative products were produced from the mixture of DNA, Cu^{2+}, and GSH with the increase of the reaction period. However, all the absorbance lines locate below that of the control experiment, indicating that the addition of all these coumarin-substituted dihydropyrazoles can decrease the amount of oxidative products of DNA, and thus, these dihydropyrazoles act as antioxidants to protect DNA against Cu^{2+}/GSH-induced oxidation. It is worthy to note that **1**, **2**, and **3** also function as antioxidants even without phenolic hydroxyl group attaching. This finding is in agreement with our previous result that 1,3,5-triphenyl-1*H*-pyrazole, a pyrazole derivative without phenolic hydroxyl group involved, can inhibit Cu^{2+}/GSH-induced oxidation of DNA [15]. Moreover, the absorbance lines in the presence of other coumarin-substituted dihydropyrazoles locate at lower positions than those of non-hydroxyl-involving dihydropyrazoles, demonstrating that the phenolic hydroxyl group enhances the antioxidant effects of coumarin-substituted dihydropyrazoles. Especially, the additions of



Abbreviation	R_1	R_2	R_3
1	H	H	H
2	H	H	CH_3O
4	OH	H	H
5	H	OH	H
6	H	H	OH
7	H	CH_3O	OH
8	H	OH	OH
9	OH	H	OH

Scheme 1. Synthetic routine of coumarin-substituted dihydropyrazoles.