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Acetophenone benzoylhydrazones as antioxidant agents: Synthesis, *in vitro* evaluation and structure-activity relationship studies

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ABSTRACT

Acetophenone and its analogues are naturally-occurring compounds found in many foods and plants. In this study, a series of acetophenone benzoylhydrazones **5a-o** were designed and synthesized as new potential antioxidant agents. Designed molecules contain hydrazone and phenolic hydroxyl moieties which possibly contribute to antioxidant activity. The antioxidant properties of compounds **5a-o** in terms of reducing ability and radical-scavenging activity were assessed by using FRAP and DPPH tests, respectively. While the unsubstituted compound **5a** had the superior capacity in the FRAP assay, the 2,4-dihydroxyacetophenone analogue **5g** was the most potent radical scavenger in the DPPH method. The antioxidant potential of representative compounds **5a** and **5g** was further confirmed by TEAC and ORAC assays. Cell viability assays revealed that while the promising compounds **5a** and **5g** had no significant toxicity against HepG2 and NIH3T3 cells, they potently protected HepG2 cells against H_2O_2-induced oxidative damage at low concentrations. Furthermore, spectroscopic studies with different biometals demonstrated that **5g** was able to interact with Cu^{2+} to form a 1:1 complex.

1. Introduction

Oxidative stress has been described as the unbalance of reactive oxygen species' (ROS) generation and the organism's capacity to counteract their action (Persson, Popescu, & Cedazo-Minguez, 2014). High concentrations of free radicals and ROS, including hydrogen peroxide, superoxide, hydroxyl radical and peroxynitrite may cause damage to lipids, proteins and DNA in biological systems (Sugamura & Keaney, 2011). It has been estimated that oxidative stress contributes to the pathogenesis and pathophysiology of over 100 diseases, such as cardiovascular and inflammatory diseases, cataracts, carcinogenesis, Parkinson's, Alzheimer's disease, Huntington's disease and amyotrophic lateral sclerosis (Sahebkar, Panahi, Yaribeygi, & Javadi, 2018; Varghese, Patel, & Yadav, 2018). Furthermore, oxidative stress plays critical roles in the impairment of physiological functions and in the biology of aging (Franco & Vargas, 2018; Maulik, McFadden, Otani, Thirunavukkarasu, & Parinandi, 2013).

Antioxidants are currently considered to be a potential treatment for oxidative stress-related diseases and are widely used as ingredients of functional foods to prevent chronic diseases, such as cancer, atherosclerosis and heart disease (Lee, Woo, Ahn, & Je, 2014; Varadharaj et al., 2017). Also, antioxidants are beneficial as supplements in foods for maintaining redox balance and avoiding oxidative damage to protect against lipid oxidation and off-flavour development (Lönn, Dennis, & Stocker, 2012). In particular, natural antioxidants, such as vitamin C, α-tocopherol, ubiquinol, and polyphenols, are widely used to scavenge free radicals and to combat the harmful effects of ROS (Choi, Lee, Hong, & Lee, 2012). Recently, many efforts have been focussed on designing antioxidants containing phenolic hydroxyl groups (Bandgar et al., 2013). The potential antioxidant effect of polyphenols and phenolic compounds is related to their reducing activity, hydrogen-donating, and singlet oxygen-quenching properties (Prior, Wu, & Schaich, 2005).

Acetophenone and its substituted analogues are naturally occurring compounds found in many foods, such as apples, apricots, bananas, beef, cheese, and cauliflowers (Müller-Schwarze, & Houlihan, 1991), as well as in many plants, such as *Camellia sinensis* (Dong et al., 2012). Also, acetophenone has been approved by FDA for use as a flavouring agent in non-alcoholic beverages, ice creams, candies, baked goods,

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gelatins, puddings, and chewing gums (Hazardous Substances Data Bank, http://toxnet.nlm.nih.gov/).

The quantum mechanical calculation studies of hydroxyacetophenone derivatives by Bentes, Borges, Monteiro, de Macedo, and Alves (2011) postulated that the presence of a carbonyl group in phenolic derivatives may stabilize the radical formed during oxidation, extending the conjugation via resonance effects (Bentes et al., 2011). Furthermore, Rezk, Haenen, van der Vijgh, and Bast (2002) have reported the potential antioxidant activity of 2,6-dihydroxyacetophenone that is possibly explained by stabilization of the radical that is formed after hydrogen abstraction (Rezk et al., 2002).

On the other hand, several hydrazones were reported as potent antioxidants due to their free radical-scavenging activity (Mohammed Khan et al., 2012). Structurally, the hydrazones are characterized by an azomethine group, which has a critical rule in antioxidant activity (Belkheiri et al., 2010).

Based on these findings and in continuation of our works on antioxidant agents (Emami, Hosseinimehr, Shahrbandi, Enayati, & Esmaeeli, 2012; Foroumadi et al., 2007; Khoobi et al., 2011, 2011), we report here the synthesis and antioxidant properties of acetophenone benzoylhydrazones containing a phenolic group and their structure-activity relationships (Fig. 1). As depicted in Fig. 1, designed molecules contain a phenolic hydroxyl group and hydrazone moiety which possibly contribute to radical-scavenging activity and the antioxidant property.

2. Materials and methods

2.1. Chemical reagents and instruments

The required starting materials and reagents were obtained from Sigma-Aldrich and Merck. The intermediate compounds **2a,b** and **3a,b** were prepared according to the reported methods (Bhole, & Bhusari, 2011; Masunari, & Tavares, 2007; Rando, Avery, Tekwani, Khan, & Ferreira, 2008). 4-Methoxy-2-hydroxyacetophenone (**4e**) was synthesized from 2,4-dihydroxyacetophenone as described previously (Safavi et al., 2010). All reactions were monitored by thin-layer chromatography (TLC) using pre-coated silica gel plates (Kieselgel 60 F_{254}). The spots on TLC were visualized and detected by UV lamp (254 nm). Melting points were determined in glass capillary tubes on a Stuart Scientific apparatus and are uncorrected. IR spectroscopy was carried out on a FT-IR Perkin Elmer spectrometer (KBr disks). All NMR spectra were recorded on a Bruker ultrashield avance III spectrometer, working at 400 MHz and chemical shifts are expressed as ppm in respect to the internal standard tetramethylsilane (TMS). Elemental analyses were carried out on a CHN-O-rapid elemental analyzer (GmbH-Germany) for C, H and N, and the results are within ± 0.4% of the theoretical values.

2.2. General procedure for the synthesis of compounds *5a-o*

To a solution of benzohydrazides **3a** or **3b** (1 mmol) and acetophenone derivative **4a-h** (1 mmol) in methanol (5 ml), a few drops of glacial acetic acid were added and the reaction mixture was refluxed at 70 °C for 6–8 h. After consumption of starting materials (monitoring with TLC), the reaction mixture was cooled to room temperature and left in the refrigerator overnight. The precipitated crystals were separated by filtration and washed with cooled methanol to give pure compounds **5a-o**.

*N′-(1-Phenylethylidene)benzohydrazide (**5a**)*. Yield: 42%; mp: 147–148 °C; IR (ν_{max}, cm^{-1}): 3467, 3054, 1611, 1541, 1488, 1316, 1284, 1133, 1026, 799, 758, 693, 564; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.38 (s, 3H, CH_3), 7.38–7.48 (m, 3H, H-3′, H-4′ and H-5′), 7.50–7.62 (m, 3H, H-3, H-4 and H-5), 7.78–7.95 (m, 4H, H-2′, H-6′, H-2 and H-6), 10.79 (br s, 1H, NH). Anal. Calcd for $C_{15}H_{14}N_2O$: C, 75.61; H, 5.92; N, 11.76. Found: C, 75.49; H, 5.89; N, 11.73.

*4-Hydroxy-N′-(1-phenylethylidene)benzohydrazide (**5b**)*. Yield: 81%; mp: 246–247 °C; IR (ν_{max}, cm^{-1}): 3500, 3150, 1670, 1583, 1436, 1323, 1222, 1174, 903, 846, 773, 699, 504; ^{1}H NMR (400 MHz, DMSO-d_6) δ: 2.36 (s, 3H, CH_3), 6.86 (d, 1H, J = 8.4 Hz, H-3 and H-5), 7.38–7.46 (m, 3H, H-3′, H-4′ and H-5′), 7.79 (d, 2H, J = 8.8 Hz, H-2 and H-6), 7.80–7.88 (m, 2H, H-2′ and H-6′), 10.09 (s, 1H, 4-OH), 10.52 (s, 1H, NH). Anal. Calcd for $C_{15}H_{14}N_2O_2$: C, 70.85; H, 5.55; N, 11.02. Found: C, 70.97; H, 5.53; N, 10.98.

*N′-(1-(2-Hydroxyphenyl)ethylidene)benzohydrazide (**5c**)*. Yield: 44%; mp: 183–184 °C; IR (ν_{max}, cm^{-1}): 3211, 1637, 1612, 1577, 1485, 1304, 1285, 1025, 931, 834, 744, 712, 690; ^{1}H NMR (400 MHz, DMSO-d_6) δ:

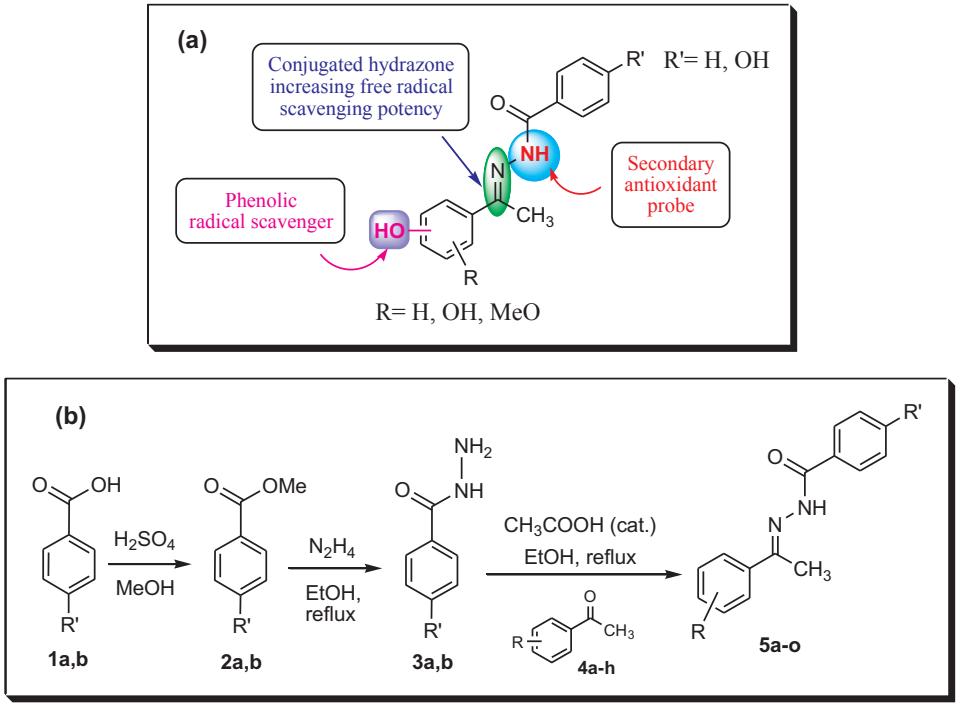

Fig. 1. (a) Design of acetophenone benzoylhydrazones as antioxidant agents; (b) Synthesis of compounds **5a-o**.