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Research paper

Correlation of antioxidant activities with theoretical studies for new hydrazone compounds bearing a 3,4,5-trimethoxy benzyl moiety

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ABSTRACT

A new series of antioxidants, namely imines bearing the well-known free radical scavenger group 3,4,5-trimethoxybenzyloxy, was designed and synthesized. Theoretical calculations based on density functional theory (DFT) were performed to understand the antioxidant activities. Experimental studies evaluating the antioxidant activities of the compounds using DPPH and FRAP assays verified the predictions obtained by DMOL3 based on DFT.1. The DPPH radical scavenging activities depended on the substitution pattern of the aromatic aldehyde, with both the substitution type and position showing significant effects. Compounds **7b, 7c** and **7d**, which contain a phenolic hydroxyl group at the para position to the imine as well as, additional electron donating groups at the ortho-position to this hydroxyl group, exhibited IC_{50} values of 62, 75 and 106 μg/mL, respectively, and potent antioxidant activities against DPPH, which were better than that of the reference compound BHT. With the exception of compounds **7a** and **7h** with a phenolic hydroxyl group at the ortho position, all of the investigated compounds exhibited ferric reducing activities above 1000 μM. Correlation analysis between the two antioxidant assays revealed moderate positive correlation ($r = 0.59$), indicating differing antioxidant activities based on the reaction mechanism. Therefore, imines bearing a 3,4,5-trimethoxybenzyloxy group can be proposed as potential antioxidants for tackling oxidative stress.

1. Introduction

Reactive oxygen species (ROS) such as hydroxyls ($HO^{\bullet}$), superoxides ($O2^{\bullet -}$), peroxyls ($ROO^{\bullet}$), alkoxyls ($RO^{\bullet}$), and nitric oxides ($NO^{\bullet}$) can lead to cellular injury in the form of damaged DNA, lipids and proteins. Therefore, the human body is able to counterbalance ROS by a variety of antioxidant defence mechanisms and immediately eliminate an excess of ROS from the cell by cellular antioxidant enzymes and other redox molecules [1]. Natural and synthetic antioxidants may protect against cells damage by virtue of their ability to scavenge free radicals.

Scientists from various disciplines are interested in new antioxidants compounds, of both synthetic and natural origin, to prevent or reduce the impact of oxidative stress on cells [2,3]. Various compounds have been designed and chemically synthesized based on data for the structural requirements of potent antioxidants. These compounds are only required in minute amounts. Generally, the free radical scavenging capacity of phenols is attributed to the hydrogen atom on the hydroxyl group [4], while nitrogen and sulphur analogues may provide alternatives for labile hydrogen atoms [5–7].

Hydrazones are well-known compounds with a wide range of pharmacological activities, including antitumoral [8,9], antifungal [10–12], and antibacterial behaviour [13,14]. They have also shown potent antioxidant activities with regards to scavenging free radicals [15,16]. Typically, the hydrazones are characterized by an imine (azomethine) group, which is critical for their antioxidant activities [4,17].

Methoxy groups on aromatic systems have been extensively investigated for their well-known antioxidant effects [18]. These

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groups are important in cytotoxic and microtubule-binding agents used for cancer chemotherapy [19,20]. For instance, structure activity relationship studies on combretastatin A-4, which is an anti-tumour drug from the combretastatin group [21], have shown that the 3,4,5-trimethoxy phenyl groups are important for its cytotoxic activity [22,23]. Moreover, recently 2,4,5-trimethoxy chalcones and their analogues 2,4,5-trimethoxy-2′,5′-dihydroxychalcone, have shown superior DPPH radical scavenging activities [24].

In the present study, SAR and rational design strategies were used to create molecules with multiple functions, which include a radical scavenging ability and other biological activities. This strategy was performed with and tested based on computational studies using DMOL3 based on DFT.1. The synthesized compounds have been characterized by IR, NMR and mass spectral analyses. The antioxidant activities of the synthesized compounds were experimentally verified by DPPH and FRAP assays.

2. Results and discussion

2.1. SAR and the rational design of antioxidant hydrazones

The generic structure of the new hydrazones, as illustrated in Fig. 1, consists of a well-known free radical scavenger, the 3,4,5-trimethoxybenzyl group (ring A). The active group –CO–NHN=C – enables resonance to the two adjacent aromatic rings B and C, leading to multiple resonance structures, which allows this functional group to act as an electron donating group to enhance the radical scavenging activity. The imine group of the hydrazone that contains a lone pair of electrons might be used to form a covalent bond with a biological target [25].

It has been reported that electron-donating substituents, such as alkyl or alkoxyl groups at the 2,4,6-positions, increase the primary antioxidant activities of phenols [26]. This is due to the lowering of the bond dissociation enthalpy (BDE) of the phenol O–H group [27] and the stabilization of the phenoxyl radical by inductive and hyperconjugative effects. Thus, hydrazones with a phenolic hydroxyl group at the para position on ring C with additional EDGs at the ortho-position can exhibit potent antioxidant activities, due to resonance-based stabilizing effects.

2.2. Synthesis

The starting material, 3,4,5-trimethoxy benzyl bromide (**2**), was prepared according to a procedure found in the literature [28]. Refluxing 3,4,5-trimethoxybenzyl bromide **2** with ethyl 4-hydroxybenzoate (**4**) in acetone in the presence of anhydrous K_2CO_3 resulted in ethyl 4-(3,4,5-trimethoxybenzyloxy)benzoate (**5**). The ester was converted to the corresponding acid hydrazide (**6**) by reaction with hydrazine hydrate in absolute ethanol. The synthetic process is summarized in Scheme 1. Treatment of the latter with various aromatic aldehydes in anhydrous ethanol, as shown in Scheme 2, provided the hydrazone derivatives **7a**–**i** in good yields. Their structures were confirmed by IR, NMR and mass spectral analyses.

The ^{1}H NMR spectrum of compound **5** exhibited signals originating from the ethyl ester and methoxy groups at 1.30 & 4.27 ppm and 3.67 & 3.79 ppm, respectively. Conversion of ester **5** into the key intermediate **6** led to the disappearance of signals belonging to the ethoxy group. Instead, new signals reflecting the hydrazide structure appeared at 4.43 ppm (2H) and 9.61 ppm (1H). All of the hydrazones **7a**–**i** showed a singlet peak in the region between 11.40 and 12.40 ppm due to the amide NH as well as, another singlet peak between 8.20 and 8.90 ppm reflecting an up field shift of the aldehyde due to the formation of the hydrazone. At the same time, the signal for the amine group of hydrazide **6** disappeared, thus confirming the presence of the imine in the hydrazone compounds. Protons of phenolic groups for compounds **7b, 7c, 7d, 7g**, and **7i** appear as a singlet peak in the region between 7.39 and 9.90 ppm. Whereas proton of phenolic groups for compound **7a** and **7h** were shifted further to lower field at 12.80 and 12.02 respectively. The shifted in chemical for **7a** and **7h** is believed due to hydrogen bonding between the OH group and the N atom of the imine, as shown in Fig. 2. Base on this observation we believed that all of the hydrazones, **7a**–**7i**, have trans (*E*) configurations at the imine double bonds. This observations are in agreement with the data reported by *Levrand* et al. [29].

2.3. In vitro antioxidant activities

In the present study, the antioxidant activities of synthesized compounds **5** and **6** and their hydrazone derivatives **7a**–**i** were tested *in vitro* by using DPPH and FRAP, the two most common antioxidant assays [30,31].

The DPPH radical scavenging assay is often used as a quick and reliable parameter to investigate the antioxidant activities of phenolic compounds [32–35]. DPPH is a stable free radical that can accept a hydrogen radical or an electron to become a stable molecule [36]. In the methanolic medium, it is odd electron configuration shows a strong absorption band at 515 nm. The absorbance decreases in the presence of free radical scavengers resulting in a colour change from deep purple to yellow [37,38]. The FRAP assay, on the other hand, is a simple, versatile and low-cost test that is commonly used to assess the antioxidant activities of extracts and pure natural products [39,40].

2.3.1. DPPH free radical scavenging activities

The DPPH radical scavenging ability strongly depends on the geometric accessibility of the radical trapping site. The presence of steric hindrance may prevent a test compound from reaching the radical site of DPPH, resulting in a low activity [41]. The DPPH assay is based on either a hydrogen atom transfer (HAT) or a single electron transfer (SET) mechanism.

In general, the two proposed mechanisms, by which the

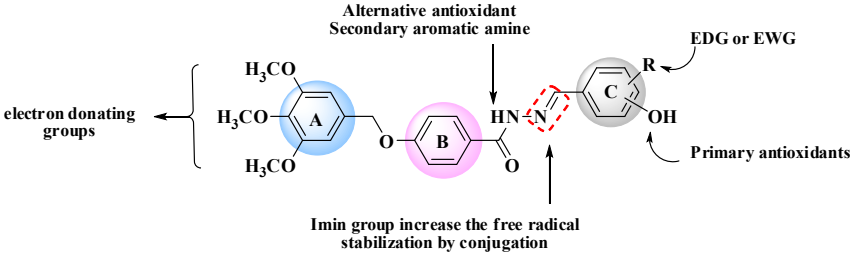

Fig. 1. SAR analysis of synthesized hydrazones.