

Full Length Research Paper

Synthesis and biological activities of substituted *N'*-benzoylhydrazone derivatives

Qinrong Wang¹, Ying Pan¹, Jinzhi Wang¹, Qing Peng², Hongjun Luo³ and Jinhong Zheng^{1*}

¹Department of Chemistry, Shantou University Medical College, Guangdong P. R. China.

²Department of Pharmacology, Shantou University Medical College, Guangdong P. R. China.

³Central Laboratories, Shantou University Medical College, Guangdong P. R. China.

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The present study describes the synthesis, antioxidant and antibacterial activities of substituted *N'*-benzoylhydrazone derivatives, to investigate activities in relation to their SAR. Compounds have been characterised by melting points, MS, ¹H NMR and IR spectra. Antioxidant activities were evaluated using hydroxyl radical (OH) and 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assays and the IC₅₀ values ranged from 0.68 to 23.66 mg/L and from 0.13 to 22.87 mg/L, respectively. Antibacterial activities of these derivatives were examined by a microdilution method. Compounds 4j and 4q possess significant antibacterial properties on Gram-positive bacteria based on the MIC. Our results indicate that some of these derivatives possess promising antioxidant and antibacterial activities, which can be explored for generating new leads of potential drug candidates.

Key words: Acylhydrazone derivatives, antioxidant activity, antibacterial activity, SAR.

INTRODUCTION

In recent years, there has been a growing interest in substances which exhibit antioxidant and antimicrobial properties that can find application in clinical conditions (Lima et al., 2009; Reddy et al., 2010; Vukovic et al., 2010; Zerbo et al., 2010).

Free radicals, essential elements in our body, may play important roles in the origin of life and biological evolution, implicated by their beneficial effects in many types of organisms (Demir et al., 2009). However, free radicals, containing one or more unpaired electrons that make them highly unstable and cause direct attack against cellular components, are found to be responsible for many diseases including ischaemia and reperfusion injury in many tissues, the central nervous system, cancer and AIDS (Ebrahimzadeh et al., 2008; Wang et al., 2010). Antioxidants, which can inhibit or delay the oxidation of an oxidizable substrate in a chain reaction, therefore, appear to be very effective in the prevention of many diseases by being beneficial to health (Pourmorad et al.,

2006; Krishnaiah et al., 2007; Zhang et al., 2009a). In addition, the dramatically rising prevalence of multidrug-resistant microbial infections has developed due to indiscriminate and frequent incorrect use of commercial antibacterial drugs and has forced scientists to search for safe and powerful antibacterial substances that can be used in clinical treatment (Ver-porte et al., 1982; Rollas et al., 2007; Mbata et al., 2008; Tajbakhsh et al., 2008).

Acylhydrazones are organic compounds characterized by the presence of a -CONHN=CH- group in their molecule. These compounds have been extensively investigated for their biological activities because of their versatile coordination chemistry characteristics and capability to generate varied molecular architecture and coordination geometry (Raparti et al., 2009; Mangalam et al., 2010; Naskar et al., 2010). This versatility ensures that acylhydrazones are good polydentate- chelating agents, which can form a variety of complexes with various transition and inner transition metals and therefore, have attracted the attention of many researchers (Said et al., 2003; Melnyk et al., 2006; Avaji et al., 2009; Li et al., 2009; Juliano et al., 2010; Patel and Patel., 2010). Thus, many vital enzymatic reactions that are catalyzed by these transition metals would not occur in the presence of acylhydrazones (Wang,

*Corresponding author. E-mail: jhzheng@yahoo.cn. Tel: +86 754 88900499. Fax: +86 754 88557562.

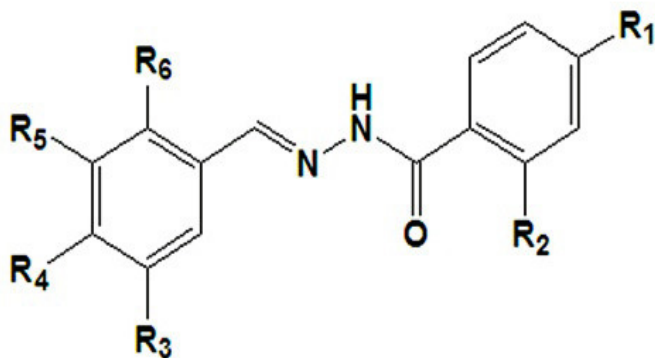


Figure 1. Structure of hydroxyl-substituted *N'*-benzoylhydrazone derivatives.

et al., 1997).

In the literature, many pharmacological activities, such as anticancer, anticonvulsant, anti-HIV, anti-inflammatory, antibacterial and antioxidant properties, have been associated with acylhydrazones (Ferreira et al., 2008; Vicini et al., 2009; Candéa et al., 2009; Liu et al., 2009).

In an effort to continue exploration of the structural and electronic effects of different substitutes in the benzene ring of acylhydrazones, we report, in this article, the synthesis of a series of hydroxyl-substituted *N'*-benzoylhydrazone derivatives and the evaluation of the *in vitro* antimicrobial activity of these compounds (Figure 1). We also present results of our investigation into the relationship between their physicochemical properties and microbiological effects. It is interesting to explore the connection, if any, between the antioxidant and antibacterial activities of these derivatives.

MATERIALS AND METHODS

General measures

Fourier transform infrared (FT-IR) data were acquired with a Perkin Elmer 2000-FTIR spectrophotometer in the frequency range of 4000 to 400 cm^{-1} with samples embedded in KBr discs. ^1H NMR spectra were determined in DMSO- d_6 containing ~1% TMS as an internal standard on a Bruker AMX 400 MHz spectrometer. Mass spectra were obtained with a GC/VG Micromass 12 at 70 eV. Melting points were determined with an XT4 apparatus and are presented as uncorrected values. The progress of all reactions was monitored by TLC, which was performed on 2 to 6 cm aluminum sheets that were precoated with silica gel 60 to a thickness of 0.25 mm. The developed chromatograms were then viewed under ultraviolet light (254 and 365 nm) and treated with iodine vapor. All reagents were purchased commercially and used without any further purification.

General procedure for the preparation of target derivatives

A mixture of hydrazide derivatives (1.0 equiv) and EtOH (8 ml) was stirred at room temperature for 10 min. A solution of aromatic aldehydes (1.5 equiv) in ethanol was added to this stirred solution.

This mixed solution was refluxed on an oil bath for 2.5 h with stirring. When this solution was cooled to room temperature, a large amount of precipitate appeared. The precipitate was separated from the solution using suction filtration, purified by washing several times with EtOH and recrystallized from Dimethyl sulfoxide (DMSO) or acetone to obtain specific compounds (Table 1), which were then dried in a vacuum.

Characterization of *N'*-benzoylhydrazone derivatives

N'-(2-hydroxybenzylidene) benzohydrazide (4a).

The title compound was obtained as a white solid. Yield: 83%. M.P.: 190 to 192°C. IR (KBr, cm^{-1}): 3307, 3270, 1675, 1612, 873. ^1H NMR (400 MHz, d_6 -DMSO): 12.11(s, 1H, N-H), 11.29(s, 1H, O-H), 8.65(s, 1H, CH=N), 6.91~7.95 (m, 9H, ArH). ESMS: m/z 241.5 (M+H) $^+$.

N'-(4-hydroxybenzylidene) benzohydrazide (4b)

The title compound was obtained as a white solid. Yield: 80%. M.P.: 246 to 248°C. IR (KBr, cm^{-1}): 3268, 3058, 1606, 1576, 838. ^1H NMR (400 MHz, d_6 -DMSO): 11.64(s, 1H, N-H), 9.93(s, 1H, O-H), 8.36(s, 1H, CH=N), 6.84~7.91 (m, 9H, ArH). ESMS: m/z 241.5 (M+H) $^+$.

N'-(4-hydroxy-3-methoxybenzylidene) benzohydrazide (4c)

The title compound was obtained as a white solid. Yield: 81%. M.P.: 220 to 221°C. IR (KBr, cm^{-1}): 3483, 3239, 1636, 1606, 874. ^1H NMR (400 MHz, d_6 -DMSO): 11.68(s, 1H, N-H), 9.55(s, 1H, OH), 8.35(s, 1H, CH=N), 6.84~7.91 (m, 8H, ArH), 3.84 (s, 3H, O-CH₃). ESMS: m/z 271.5 (M+H) $^+$.

N'-Benzylidenesalicylhydrazide (4d)

The title compound was obtained as a white solid. Yield: 84%. M.P.: 246 to 248°C. IR (KBr, cm^{-1}): 3240, 3027, 1657, 1629, 856. ^1H NMR (400 MHz, d_6 -DMSO): 11.84(s, 1H, N-H), 8.47(s, 1H, O-H), 7.89~7.91(s, 1H, CH=N), 6.95~7.76 (m, 9H, ArH). ESMS: m/z 241.5 (M+H) $^+$.

N'-(2-hydroxybenzylidene)-2-hydroxybenzohydrazide (4e)

The title compound was obtained as a white solid. Yield: 80%. M.P.: 235 to 237°C. IR (KBr, cm^{-1}): 3186, 3043, 1633, 1619, 880. ^1H NMR (400 MHz, d_6 -DMSO): 12.03(s, 1H, N-H), 11.77(s, 1H, O-H), 11.20 (s, 1H, O-H), 8.69(s, 1H, CH=N), 6.92~7.91 (m, 8H, ArH). ESMS: m/z 257.5 (M+H) $^+$.

N'-(4-hydroxybenzylidene)-2-hydroxybenzohydrazide (4f)

The title compound was obtained as a white solid. Yield: 81%. M.P.: 246 to 248°C. IR (KBr, cm^{-1}): 3340, 3269, 1627, 1606, 828. ^1H NMR (400 MHz, d_6 -DMSO): 12.00(s, 1H, N-H), 11.69(s, 1H, O-H), 9.97(s, 1H, O-H), 8.36(s, 1H, CH=N), 6.84~7.90 (m, 8H, ArH). ESMS: m/z 257.5 (M+H) $^+$.

N'-(4-methoxybenzylidene)-2-hydroxybenzohydrazide (4g)

The title compound was obtained as a white solid. Yield: 78%. M.P.: 226 to 228°C. IR (KBr, cm^{-1}): 3258, 3066, 1628, 1607, 827. ^1H

Table 1. Structure and physical data of target compounds.

Compound	R ₁	R ₂	R ₃	R ₄	R ₅	R ₆	Formula
4a	H	H	H	H	H	OH	C ₁₄ H ₁₂ N ₂ O ₂
4b	H	H	H	OH	H	H	C ₁₄ H ₁₂ N ₂ O ₂
4c	H	H	OCH ₃	OH	H	H	C ₁₅ H ₁₄ N ₂ O ₃
4d	H	OH	H	H	H	H	C ₁₄ H ₁₂ N ₂ O ₂
4e	H	OH	H	H	H	OH	C ₁₄ H ₁₂ N ₂ O ₃
4f	H	OH	H	OH	H	H	C ₁₄ H ₁₂ N ₂ O ₃
4g	H	OH	H	OCH ₃	H	H	C ₁₅ H ₁₄ N ₂ O ₃
4h	H	OH	OCH ₃	OH	H	H	C ₁₅ H ₁₄ N ₂ O ₄
4i	H	OH	OC ₂ H ₅	OH	H	H	C ₁₆ H ₁₆ N ₂ O ₄
4j	H	OH	OC ₂ H ₅	OH	NO ₂	H	C ₁₆ H ₁₅ N ₃ O ₆
4k	OH	H	H	H	H	H	C ₁₄ H ₁₂ N ₂ O ₂
4l	OH	H	H	H	H	OH	C ₁₄ H ₁₂ N ₂ O ₃
4m	OH	H	H	OH	H	H	C ₁₄ H ₁₂ N ₂ O ₃
4n	OH	H	H	OCH ₃	H	H	C ₁₅ H ₁₂ N ₂ O ₃
4o	OH	H	OCH ₃	OH	H	H	C ₁₅ H ₁₄ N ₂ O ₄
4p	OH	H	OC ₂ H ₅	OH	H	H	C ₁₆ H ₁₆ N ₂ O ₄
4q	OH	H	OC ₂ H ₅	OH	NO ₂	H	C ₁₆ H ₁₅ N ₃ O ₆

NMR (400 MHz, d₆-DMSO): 11.95 (s, 1H, N-H), 11.75 (s, 1H, O-H), 8.42 (s, 1H, CH=N), 6.95~7.91 (m, 8H, ArH), 3.83 (s, 3H, O-CH₃). ESMS: m/z 271.5 (M+H)⁺.

***N'*-(4-hydroxy-3-methoxybenzylidene)-2-hydroxybenzohydrazide (4h).**

The title compound was obtained as a white solid. Yield: 77%. M.P.: 132 to 134°C. IR (KBr, cm⁻¹): 3351, 3244, 1638, 1603, 860. ¹H NMR (400 MHz, d₆-DMSO): 11.96 (s, 1H, N-H), 11.70 (s, 1H, O-H), 9.59 (s, 1H, O-H), 8.35 (s, 1H, CH=N), 6.85~7.90 (m, 7H, ArH), 3.84 (s, 3H, O-CH₃). ESMS: m/z 287.5 (M+H)⁺.

***N'*-(3-ethoxy-4-hydroxybenzylidene)-2-hydroxybenzohydrazide (4i)**

The title compound was obtained as a yellow power. Yield: 78%. M.P.: 180 to 182°C. IR (KBr, cm⁻¹): 3415, 3248, 1631, 1595, 820. ¹H NMR (400 MHz, d₆-DMSO): 11.96 (s, 1H, N-H), 11.69 (s, 1H, O-H), 9.51 (s, 1H, O-H), 8.33 (s, 1H, CH=N), 6.85~7.89 (m, 7H, ArH), 4.05~4.10 (s, 2H, CH₂), 1.35~1.39 (s, 3H, CH₃). ESMS: m/z 301.1 (M+H)⁺.

***N'*-(3-ethoxy-4-hydroxy-5-nitrobenzylidene)-2-hydroxybenzohydrazide (4j).**

The title compound was obtained as a yellow power. Yield: 69%. M.P.: 224 to 225°C. IR (KBr, cm⁻¹): 3226, 3083, 1711, 1621, 824. ¹H NMR (400 MHz, d₆-DMSO): 11.88 (s, 1H, N-H), 11.78 (s, 1H, O-H), 10.77 (s, 1H, O-H), 8.40 (s, 1H, CH=N), 6.94~7.89 (m, 6H, ArH), 4.18~4.23 (s, 2H, CH₂), 1.40~1.43 (s, 3H, CH₃). ESMS: m/z 346.1 (M+H)⁺.

***N'*-benzylidene-4-hydroxybenzohydrazide (4k)**

The title compound was obtained as a white solid. Yield: 84%. M.P.: 236 to 238°C. IR (KBr, cm⁻¹): 3225, 3056, 1653, 1617, 850. ¹H

NMR (400 MHz, d₆-DMSO): 11.62 (s, 1H, N-H), 10.11 (s, 1H, O-H), 8.43 (s, 1H, CH=N), 6.85~7.82 (m, 9H, ArH). ESMS: m/z 241.5 (M+H)⁺.

***N'*-(2-hydroxybenzylidene)-4-hydroxybenzohydrazide (4l)**

The title compound was obtained as a white solid. Yield: 81%. M.P.: 227 to 229°C. IR (KBr, cm⁻¹): 3320, 3160, 1643, 1608, 843. ¹H NMR (400 MHz, d₆-DMSO): 11.91 (s, 1H, N-H), 11.42 (s, 1H, O-H), 10.17 (s, 1H, O-H), 8.60 (s, 1H, CH=N), 6.87~7.84 (m, 8H, ArH). ESMS: m/z 257.5 (M+H)⁺.

***N'*-(4-hydroxybenzylidene)-4-hydroxybenzohydrazide (4m)**

The title compound was obtained as a white power. Yield: 82%. M.P.: 226 to 227°C. IR (KBr, cm⁻¹): 3232, 2814, 1606, 1583, 831. ¹H NMR (400 MHz, d₆-DMSO): 11.41 (s, 1H, N-H), 10.06 (s, 1H, O-H), 9.88 (s, 1H, O-H), 8.32 (s, 1H, CH=N), 6.82~7.79 (m, 8H, ArH). ESMS: m/z 257.5 (M+H)⁺.

***N'*-(4-methoxybenzylidene)-4-hydroxybenzohydrazide (4n)**

The title compound was obtained as a white power. Yield: 76%. M.P.: 224 to 226°C. IR (KBr, cm⁻¹): 3170, 3045, 1600, 1590, 825. ¹H NMR (400 MHz, d₆-DMSO): 11.49 (s, 1H, N-H), 10.08 (s, 1H, O-H), 8.36 (s, 1H, CH=N), 7.01~7.80 (m, 8H, ArH), 3.81 (s, 3H, O-CH₃). ESMS: m/z 271.5 (M+H)⁺.

***N'*-(4-hydroxy-3-methoxybenzylidene)-4-hydroxybenzohydrazide (4o)**

The title compound was obtained as a white power. Yield: 73%. M.P.: 226 to 227°C. IR (KBr, cm⁻¹): 3258, 3053, 1641, 1604, 849. ¹H NMR (400 MHz, d₆-DMSO): 11.43 (s, 1H, N-H), 10.07 (s, 1H, O-H), 9.49 (s, 1H, O-H), 8.31 (s, 1H, CH=N), 6.82~7.79 (m, 7H, ArH), 3.41~3.47 (s, 3H, O-CH₃). ESMS: m/z 287.5 (M+H)⁺.

***N'*-(3-ethoxy-4-hydroxybenzylidene)-4-hydroxybenzohydrazide (4p)**

The title compound was obtained as a white power. Yield: 72%. M.P.: 200 to 202°C. IR (KBr, cm⁻¹): 3272, 3074, 1644, 1603, 851. ¹H NMR (400 MHz, d₆-DMSO): 11.43 (s, 1H, N-H), 10.07 (s, 1H, O-H), 9.41 (s, 1H, O-H), 8.29 (s, 1H, CH=N), 6.84~7.79 (m, 7H, ArH),

4.04~4.09 (s, 2H, CH₂), 1.34~1.38 (s, 3H, CH₃). ESMS: m/z 301.1 (M+H)⁺.

***N'*-(3-ethoxy-4-hydroxy-5-nitrobenzylidene)-4-hydroxybenzohydrazide (4q)**

The title compound was obtained as a yellow solid. Yield: 68%. M.P.: 256 to 258°C. IR (KBr, cm⁻¹): 3343, 3209, 1653, 1608, 855. ¹H NMR (400 MHz, d₆-DMSO): 11.70 (s, 1H, N-H), 10.69 (s, 1H, O-H), 10.02 (s, 1H, O-H), 8.36 (s, 1H, CH=N), 6.85~7.79 (m, 6H, ArH), 4.17~4.22 (s, 2H, CH₂), 1.39~1.43 (s, 3H, CH₃). ESMS: m/z 346.1 (M+H)⁺.

Hydroxyl radical (•OH) scavenging activity

The scavenging ability of the test derivative compounds from the test on OH was determined based on the deoxyribose method, as previously described (Oluwaseun et al., 2008; Li et al., 2010), but with a slight modification. A solution of the test compounds was prepared with DMSO. The reaction mixture, which contained freshly prepared FeSO₄ (10 mM) and H₂O₂ (10 mM), was mixed with the tested-compound solution and 1 ml of phosphate buffer (0.1 M, pH 7.4) was added to the mixture. This mixture was incubated at 37°C for 60 min in a water bath and then 1, 10-phenanthroline (10 mM) was added into it. Finally, the reaction mixture was cooled, centrifuged at 800 × g for 8 min and the absorbance of the supernatant was measured at 536 nm. All tests were carried out 8 times. Vitamin C was used as a positive control. The suppression ratio for •OH was calculated from the following equation:

$$\text{Hydroxyl radical scavenging activity (\%)} = [(A_0 - A_i)/A_0] \times 100$$

Where, A₀ is the absorbance of the control reaction (containing all reagents except the test compound) at 536 nm and A_i is the absorbance of the sample at 536 nm. The IC₅₀ value denotes the concentration of a sample that is required to scavenge 50% of hydroxyl free radicals.

DPPH free radical-scavenging activity

The antioxidant activity of the synthesized complexes estimated on the basis of the scavenging activity of the stable 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical was determined as previously described (Padmaja et al., 2009; Sawant et al., 2009), but with a slight modification. A solution of 0.1 mM DPPH in ethanol was prepared and 1.0 ml of solution of the test compounds was added to 3.0 ml of the solution in water at different concentrations (0.25 to 2.5 mg/L). The mixture was shaken vigorously and left to stand for 30 min at room temperature in the dark. Absorbance at 517 nm was determined using a spectrophotometer and all tests were performed 8 times. Vitamin C served as the positive control. Lower absorbance of the reaction mixture indicates higher free radical-scavenging activity. Inhibition of free radical by DPPH was calculated by the following formula:

$$\text{DPPH radical-Scavenging effect (\%)} = (A_b - A_s / A_b) \times 100$$

Where, A_b is the absorbance of the control reaction and A_s is the absorbance of the test compound. The IC₅₀ value denotes the concentration of a sample that is required to scavenge 50% of DPPH free radicals.

Microorganisms

Activities of the synthesized complexes were measured against clinical isolates of Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*) bacteria. The bacteria were obtained from the microorganism collection of the First Affiliated Hospital of the Shantou University Medical College.

Antibacterial susceptibility testing

Antibacterial activity was evaluated by mean inhibitory concentration (MIC) based on a microdilution method (Angelusiu et al., 2010) that was slightly modified. *E. coli*, *P. aeruginosa* and *S. aureus* cultures were subcultured for testing in the same medium and grown at 37°C. Thereafter, the cultured organisms were suspended in saline solution, in accordance with the McFarland protocol, to produce a suspension of about 10⁵ CFU/ml. Serial dilutions of the test compounds, previously dissolved in DMSO, were prepared in test tubes to obtain final concentrations of 1024 to 4 µg/L. The MIC, regarded as the lowest concentration of the test compounds that inhibits visible growth after 18 h, was determined visually after incubation for 24 h at 37°C. Streptomycin was used as the reference antibacterial agent. The inhibitory activity of DMSO was also employed as a negative control.

Statistical analysis

All data are expressed as mean ± S.E.M. The Student's unpaired *t*-test was used to compare differences between two groups. ANOVA followed by Student–Newman–Keul's test was used for comparisons of more than two groups. P < 0.05 was considered statistically significant. IC₅₀ values were determined by non-linear regression using the SPSS package for Windows (Version 17.0).

RESULTS AND DISCUSSION**Synthesis of the compounds**

All complexes were prepared by similar methods according to the reported procedure (Sacconi, 1954; Kotali et al., 1998; Xu et al., 2007; Forman and Yu, 2009). Compounds 2 and 3 were prepared according to methods described in literature (Windholz 1988; Figueiredo et al., 2000). The synthetic schema for the preparation of target compounds starts with benzyl acid derivatives (Figure 2). Benzyl acid derivatives were allowed to react with ethanol to obtain compound 2, which was made to react with an 80% hydrazine hydrate solution in ethanol to obtain compound 3. Finally, the target compounds 4a-q was obtained in good yields by condensing compound 3 with corresponding aromatic aldehydes (Ar-CHO) in ethanol.

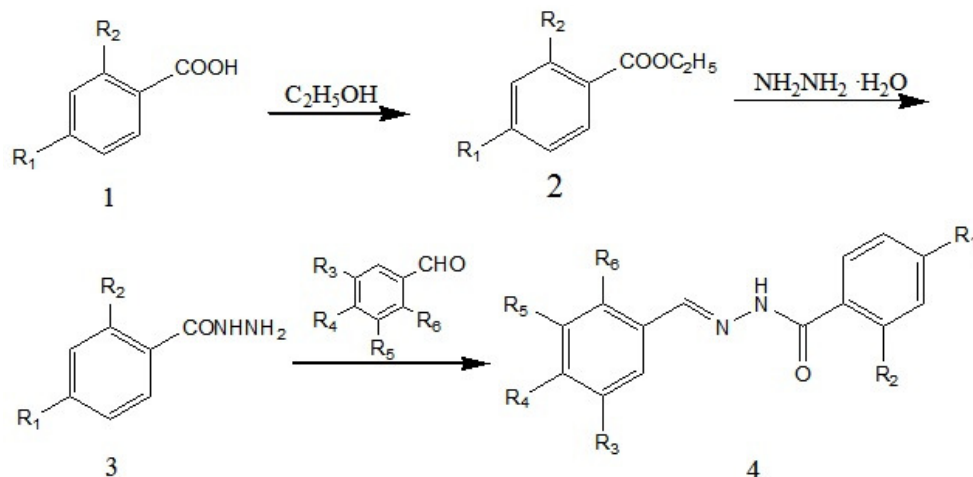


Figure 2. Synthesis schema of hydroxyl-substituted *N'*-benzoylhydrazone derivatives.

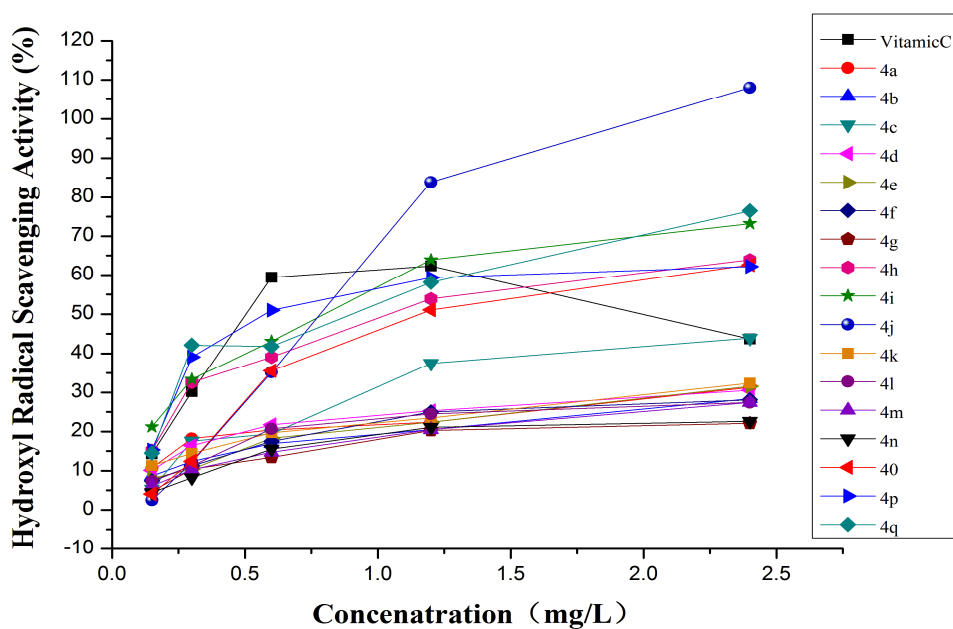


Figure 3. The elimination rate of $\cdot\text{OH}$ from compounds at different concentrations.

Pharmacology

Antioxidant activity of target derivatives

The formation of free radicals in biological systems is catalyzed by iron, which is, under normal conditions, normally non-reactive as it is bound to proteins mediating iron transport and storage due to various exogenous and endogenous factors. Some studies demonstrated that synthetic molecules, such as hydrazone compounds, may possess potential antioxidant activity due to their versatile coordination capability. In addition, the antioxidant potential of phenolic compounds is attributed

to their strong capability for electron transfer to ROS/free radicals, chelating metal ions; activate antioxidant enzymes and inhibitory oxidases (Hall and Cuppett, 1997; Park et al., 2006). It has been reported that this free radical-scavenging ability is optimally directed toward hydroxyl radical and superoxide radicals (Wang et al., 2009).

In antioxidant activity experiments, the $\cdot\text{OH}$ in aqueous media was generated through the Fenton Reaction (Figure 3) and the hydroxyl radical-scavenging activity also increased with increase in concentration. Only Vitamin C, 4c, 4h, 4i, 4j, 4p and 4q demonstrated a good scavenging activity on hydroxyl radicals. By contrast, other compounds

Table 2. IC₅₀ values for scavenging •OH by samples (mg/L).

Compound	IC ₅₀	Compound	IC ₅₀	Compound	IC ₅₀
Vc	0.48	4f	12.15*	4l	13.03*
4a	21.4*	4g	23.66*	4m	14.27*
4b	20.9*	4h	0.96	4n	16.62*
4c	2.87*	4i	0.71	4o	1.07*
4d	15.17*	4j	0.68	4p	0.81
4e	10.19*	4k	14.68*	4q	0.71

Asterisk indicates $p < 0.05$ as compared to the control group.

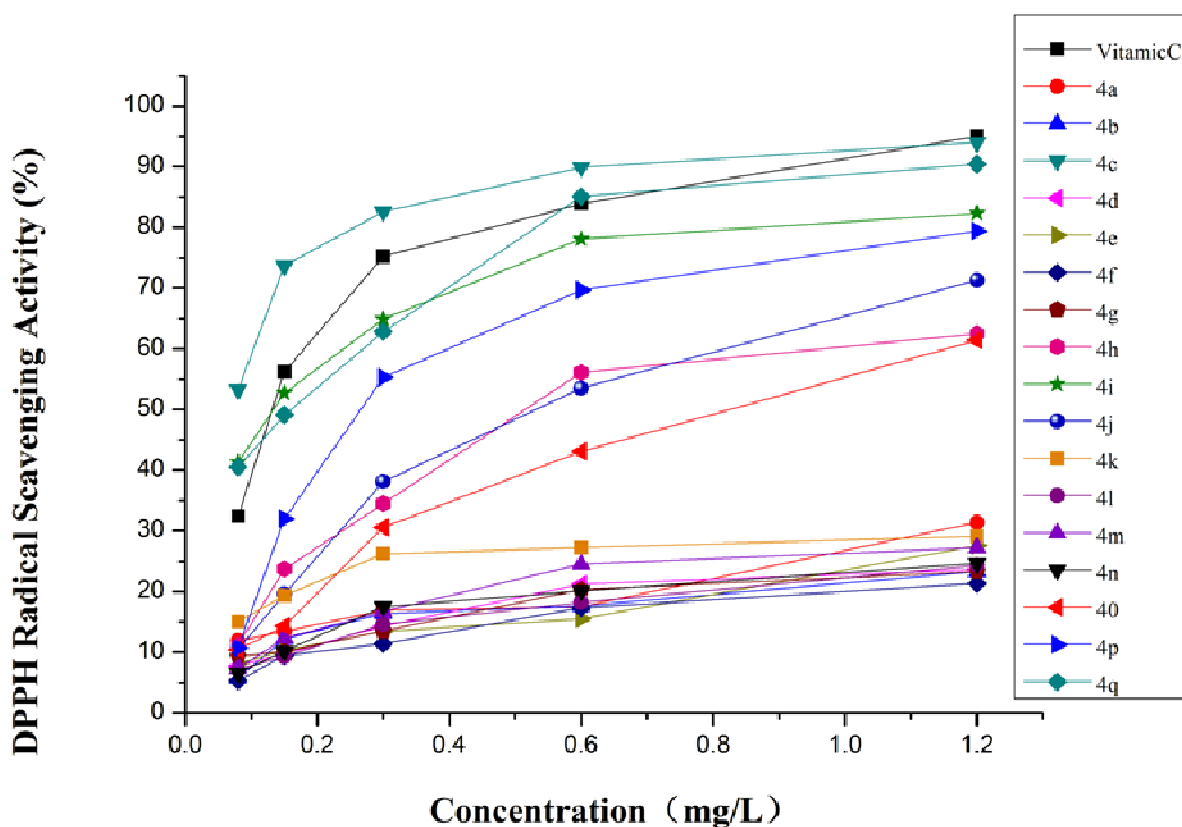


Figure 4. The elimination rate of DPPH from compounds at different concentrations.

revealed very low values for scavenging activities. Table 2 presents antioxidant activity with IC₅₀ values for different derivatives. The hydroxyl radical-scavenging activity in the samples decreased in the following order: Vitamin C > 4j, 4i, 4q > 4p > 4h > 4o > 4c > 4e > 4f, 4l, 4m > 4d, 4n > 4a, 4b.

The DPPH free radical-scavenging assay is an easy, rapid, reproducible and sensitive method for the screening of antioxidants. In addition, the assay is useful for screening a large number of samples with different polarity (Al-Zubairi et al., 2009). It is reported that the decrease in the absorbance of DPPH radical that is caused by phenolic compounds is attributable to the reaction between

antioxidant molecules and radicals this results in the scavenging of the radical by hydrogen donation and is visualized as a discoloration from deep-violet to light-yellow color (Meir et al., 1995; Ghimeray et al., 2009). From the analysis presented in Figure 4, it can be concluded that scavenging effects of all derivative compounds on DPPH increased with increase in the concentration and were excellent. Vitamin C, 4h, 4i, 4j, 4p and 4q exhibited significantly greater antioxidant properties than other compounds ($p < 0.05$) antioxidant properties in the DPPH assay. Table 3 presents data on antioxidant activity with IC₅₀ values of different derivatives. The DPPH-scavenging activity in the samples decreased in

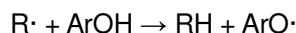
Table 3. IC₅₀ values for scavenging DPPH• by samples (mg/L).

Compound	IC ₅₀	Compound	IC ₅₀	Compound	IC ₅₀
Vc	0.13	4f	17.4*	4l	13.53*
4a	14.3*	4g	22.87*	4m	7.09*
4b	17.12*	4h	0.58	4n	10.62*
4c	0.06	4i	0.13	4o	0.76
4d	13.66*	4j	0.51	4p	0.31
4e	13.1*	4k	20.24*	4q	0.14

Asterisk | indicates $p < 0.05$ as compared to the control group.

the following order: 4c > Vitamin C, 4i, 4q > 4p > 4h, 4j, 4o > 4m > 4n > 4a, 4d, 4e, 4l > 4b, 4f > 4k, 4g.

The mechanism of the antioxidant activity of hydroxyl-substituted benzoyl acylhydrazones may involve scavenging of a radical (R) by abstracting a hydrogen atom from the aromatic hydroxyl group at the para position (ArOH), thus forming a radical (ArO), which is fairly stable due to delocalization of the unpaired electron (Arty, et al., 2000).



It is interesting that the derivatives can exhibit high antioxidant activity when electron-donating (-OCH₃, -OC₂H₅) and electron-withdrawing (-NO₂) groups of the Ar-ring are incorporated together into the B ring of the structure of derivative compounds. Further, the variation of the size, position, ability of hydrogen bonding as well as the structural changes of the acylhydrazone, will affect the alkaline and stability constants of chelates of these compounds. The fact that the compounds showed similar rankings by activity in clearing DPPH and •OH indicates that hydroxyl-substituted benzoyl acylhydrazones may clear these two types of free radicals by similar mechanisms.

Antibacterial activity of the derivatives

Antibacterial activity is attributed to the formation of stable chelates with transition metals that are present within the cell. By reacting with salts of transition metals, acylhydrazones can coordinate metal ions bidentate, through carbonyl oxygen and azomethinic nitrogen atoms. Structural changes of the segment attached to the acylhydrazone will affect the metal binding of the ligand and exhibit interesting coordination with transition metal ions that are present in living systems (Angelusiu et al., 2010). Therefore, factors that effectively influence antibacterial activity include the geometrical shape and the nature of central atoms.

A comparative study of MIC values indicates that the inhibitory activity of these compounds on Gram-negative bacteria is better than that on Gram positive bacteria. Thorough analysis of the results obtained indicate that 4j and 4q with -NO₂ unit showed comparatively high

antimicrobial activity (MIC= 64 to 128 µg/L) as compared with other derivatives. This indicates that the antibacterial activities of the derivatives are reduced by electron-donating groups, such as -OH, -OCH₃, -OC₂H₅ of the Ar-ring (Table 4).

However, compounds possessing electron-withdrawing groups, such as the -NO₂ of the Ar-ring, possess excellent activity. This finding accords with results reported in the recent work by Zhang et al. (2009b) with regard to the antibacterial activity of hydrazones.

The variation in the activity of the compounds against different microorganisms depends on the water solubility of these compounds and impermeability of the microorganism cells or differences between the ribosomes of microbial cells (Colak et al, 2010).

Further, chelation reduces the partial sharing of positive charge with donor groups and possibly, π-electron delocalization within the entire chelate ring system that is formed during the coordination (Angelusiu et al., 2010).

Conclusion

Acylhydrazone compounds are reported to have utility in a variety of applications, such as transporting agents in the pharmaceutical industry and as agents in pharmacotherapy. The results obtained in this study clearly demonstrate that some of the derivative compounds tested in this study exhibited different levels of antioxidant and antibacterial properties and structure-activity relationship influences on these two properties.

The high antioxidant and antibacterial property of compounds 4j and 4q may be due to the electron-withdrawing and electron-donating groups that exist in the chemical structure of hydroxyl-substituted *N*'-benzoylhydrazone compounds that can provide the necessary factor.

In summary, the observed activities with potential for positive benefits may provide support for their application as new leads in drug development.

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Table 4. MIC values for assayed bacteria ($\mu\text{g/L}$).

Compound	<i>E. coli</i>	<i>S. aureus</i>	<i>P. aeruginosa</i>
4a	128	256	128-256
4b	256	256	256
4c	128	256	128
4d	256	256	256
4e	128	256	256
4f	128-256	256	256
4g	128	128	128
4h	>256	256	>256
4i	128	128	128
4j	128	64	128
4k	128	256	256
4l	256	256	>256
4m	>256	>256	>256
4n	256	256	>256
4o	>256	256	>256
4p	256	256	256
4q	128	128	128
Streptomycin	16	16	8
Control (DMSO)	\	\	\

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REFERENCES

- Al-Zubairi AS, Abdul AB, Wahab SIA, Peng CY, Mohan S, Elhassan MM (2009). Eleusine indica possesses antioxidant, antibacterial and cytotoxic Properties. *Evid Based Complement Alternat Med*. In press.
- Angelusia MV, Barbuceanub SF, Draghici C, Almajan GL (2010). New Cu(II), Co(II), Ni(II) complexes with aroyl-acylhydrazone based ligand. Synthesis, spectroscopic characterization and in vitro antibacterial evaluation. *Eur. J. Med. Chem.* 45: 2055-2062.
- Arty IS, Timmerman H, Samhoedi M, Sastrohamidjojo, Sugiyanto, Van Der Goot H (2000). Synthesis of benzylideneacetophenones and their inhibition of lipid peroxidation. *Eur. J. Med. Chem.* 35: 449-57.
- Avaji PG, Vinod Kumar CH, Patil SA, Shivananda KN, Nagaraju C (2009). Synthesis, spectral characterization, in-vitro microbiological evaluation and cytotoxic activities of novel macrocyclic bis hydrazone. *Eur. J. Med. Chem.* 44: 3552-3559.
- Candéa ALP, Ferreira ML, Pais KC, Cardoso LNF, Kaiser CR, Henriques MGMO, Lourenço MCS, Bezerra FAFM and Souza MVN (2009). Synthesis and antitubercular activity of 7-chloro-4-quinolinylacyl- hydrazones derivatives. *Bioorg. Med. Chem. Lett.* 19: 6272-6274.
- Colak A, Terzi Ü, Col M, Karaoglu SA, Karaböcek S, Küçükdumlu A, Ayaz FA (2010). DNA binding, antioxidant and antimicrobial activities of homo- and heteronuclear copper (II) and nickel(II) complexes with new oxime-type ligands. *Eur. J. Med. Chem.* 45: 5169-5175
- Demir H, Açik L, Bali EB, Koç LY, Kaynak G (2009). Antioxidant and antimicrobial activities of *Solidago virgaurea* extracts. *Afr. J. Biotechnol.* 8: 274-279.
- Ebrahimzadeh MA, Pourmorad F, Hafezi S (2008). Antioxidant Activities of Iranian Corn Silk. *Turk. J. Biol.* 32: 43-49.
- Ferreira ML, Cardoso LNF, Gonçalves RSB, Silva DET, Lourenço MCS, Vicente FR, Souza De, Lett MVN (2008). Synthesis and Antitubercular Evaluation of N'-[(E)-(hydroxyl, methoxy and ethoxy-substituted-phenyl) Methylidene] isonicotinohydrazide Derivatives. *Lett. Drug Des. Discov.* 5: 137-140.
- Figueiredo JM, Camara CA, Amarante EG, Miranda ALP, Santos FM, Rodrigues CR, Fraga CAM, and Barreiro EJ (2000). Design and synthesis of novel potent antinociceptive agents: N-acetyl-imidazolyl N-acylacylhydrazone derivatives. *Bioorg. Med. Chem. Lett.* 8: 2243-2248.
- Forman B, Yu D (2009). Ligands for estrogen related receptors and methods for synthesis of said ligands. US Patent No. 7,544,838.
- Ghimeray AK, Jin CW, Ghimire BK, Cho DH (2009). Antioxidant activity and quantitative estimation of azadirachtin & nimbin in *Azadirachta indica* A. Juss grown in foothills of Nepal. *Afr. J. Biotechnol.* 8: 3084-3091.
- Hall CA, Cuppett SI (1997). Structure-activities of natural antioxidants, in *Antioxidant Methodology In Vivo and in Vitro Concepts*, Ed by Auroma OI and Cuppett SL, AOCS Pres, Champaign, IL, pp. 141-170.
- Juliano C, Mattana A, Solinas C (2010). Versatile coordinating behaviour of bis(acylhydrazone) ligands derived from imino- and methyl-iminodiacetic acid diethyl ester. Antimicrobial properties of their trinuclear copper(II) complexes. *Transition Met Chem.* 35: 253-261.
- Kotali A, Papapetrou M, Dimos V, Harris PA (1998). A novel and facile synthesis of o-acylbenzaldehydes. *Org. Prep. Proced. Int.* 30: 177-181.
- Krishnaiah D, Sarbatly R, Bono A (2007). Phytochemical antioxidants for health and medicine – A move towards nature. *Biotechnol. Mol. Biol. Rev.* 1: 97-104.
- Li B, Sun XZ, Cheng GZ, Ji ZP (2009). Synthesis, Crystal Structure and Bioactivities of a Novel Propeller Shaped Manganese Complex with the Ligand N'-Benzylidenesalicylhydrazone. *Chin. J. Chem.* 27: 1312-1316.
- Li Y, Yang ZY, Wang MF (2010). Synthesis, Characterization, DNA Binding Properties, Fluorescence Studies and Antioxidant Activity of Transition Metal Complexes with Hesperetin-2-hydroxy Benzoyl Acylhydrazone. *J. Fluoresc.* 20: 891-905.

- Lima TY, Lim YY, Yule CM (2009). Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four *Macaranga* species. *Food Chem.* 114: 594-599.
- Liu XH, Shi YX, Ma Y, He GR (2009). Synthesis of some N, N'-diacylhydrazine derivatives with radical scavenging and antifungal activity. *Chem. Biol. Drug. Des.* 73: 320-327.
- Mangalam NA, Sheeja SR, Kurup MRP (2010). Mn(II) complexes of some acylhydrazones with NNO donor sites: Syntheses, a spectroscopic view on their coordination possibilities and crystal structures. 29: 3318-3323.
- Mbata TI, Debiao LU, Saikia A (2008). Antibacterial activity of the crude extract of Chinese green tea (*Camellia sinensis*) on *Listeria monocytogenes*. *Afr. J. Biotechnol.* 7: 1571-1573.
- Meir SJ, Kanner B, Akiri SPH (1995). Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. *J. Agric. Food Chem.* 43: 1813-1817.
- Melnyk P, Leroux V, Sergheraert C, Grelhier P (2006). Design, synthesis and in vitro antimalarial activity of an acylhydrazone library. *Bioorg. Med. Chem. Lett.* 16: 31-35.
- Naskar S, Naskar S, Butcher RJ, Chattopadhyay SK (2010). Synthesis and spectroscopic properties of Ni(II) complexes of some aroyl acylhydrazone ligands with 2,6-diacetyl pyridine monooxime: X-ray crystal structure of the salicyloylacylhydrazone Ni(II) complex. *Inorg. Chim. Acta.* 363: 3641-3646
- Oluwaseun AA, Ganiyu O (2008). Antioxidant properties of methanolic extracts of mistletoes (*viscum album*) from cocoa and cashew trees in Nigeria. *Afr. J. Biotechnol.* 7: 3138-3142.
- Padmaja A, Payania T, Reddy GD, Padmavathi V (2009). Synthesis, antimicrobial and antioxidant activities of substituted pyrazoles, isoxazoles, pyrimidine and thioxopyrimidine derivatives. *Eur. J. Med. Chem.* 44: 4557-4566
- Park HR, Park E, Rim AR, Jeon KI, Hwang JH, Lee SC (2006). Antioxidant activity of extracts from *Acanthopanax senticosus*. *Afr. J. Biotechnol.* 5: 2388-2396.
- Patel HS, Patel SJ (2010). Synthesis and Biological Activity of 2-Hydroxy-N (5-methylene- 4-oxo-2-aryl-thiazolidin-3-yl)-benzamide. *Phosphorus Sulfur.* 185: 1632-1639.
- Pourmorad F, Hosseinimehr SJ, Shahabimajid N (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr. J. Biotechnol.* 5: 1142-1145.
- Raparti V, Chitre T, Bothara K, Kumar V, Dangre S, Khachane C, Gore S, Deshmane B (2009). Novel 4-(morpholin-4-yl)-N'-(arylidene)benzohydrazides: synthesis, antimycobacterial activity and QSAR investigations. *Eur. J. Med. Chem.* 44: 3954-3960.
- Reddy MVN, Reddy GCS, Kumar KS, Reddy CS, Raju CN (2010). Synthesis, antioxidant and antimicrobial activity of novel benzene-1, 4-diamine-bis-dioxaphosphine-6λ⁵ iminophosphoranes. *J. Heterocycl. Chem.* 47: 538-542.
- Rollas S, Küçükgülzel SG (2007). Biological Activities of Hydrazone Derivatives. *Molecules.* 12: 1910-1939.
- Sacconi L (1954). Komplexchemische Reaktionen. V. Mehrkernige Nickel-Innerkomplexe mit Acylhydrazonen von α- und β-Diketonen. *Z. Anorg. Allg. Chem.* 275: 249-256.
- Said SB, Elagamey AA, Khadr RE (2003). Facile oxidative conversion of aroyl hydrazones into 1, 3, 4-oxadiazoles. *Egypt. J. Chem.* 46: 881-888.
- Sawant O, Kadam VJ, Ghosh R (2009). In vitro free radical scavenging and antioxidant activity of *Adiantum lunulatum*. *J. Herb. Med. Toxicol.* 3: 39-44.
- Tajbakhsh S, Mohammadi K, Deilami I, Zandi K, Fouladvand M, Ramedani E, Asayesh G (2008). Antibacterial activity of indiumcurcumin and indium diacetylcurcumin. *Afr. J. Biotechnol.* 7: 3832-3835.
- Ver-porte R, Ijin A, Tsoui A, Vandourne H, Baerheim M (1982). Medicinal Plants of Suriname. I. Antimicrobial Activity of Some Medicinal Plants. *J. Ethnopharmacol.* 5: 221-226.
- Vicini P, Incerti M, La CP, Loddo R (2009). Anti-HIV evaluation of benzo [d] isothiazole acylhydrazones. *Eur. J. Med. Chem.* 44: 1801-1807.
- Vukovic N, Sukdolak S, Solujic S, Niciforovic N (2010). Substituted imino and amino derivatives of 4-hydroxycoumarins as novel antioxidant, antibacterial and antifungal agents: Synthesis and in vitro assessments. *Food Chem.* 120: 1011-1018.
- Wang DB, Chen BH, Ma YX (1997). Aroyl hydrazones containing triazole and their divalent nickel complexes. *Synth. React. Inorg. Met.-Org. Chem.* 27: 479-486.
- Wang H, Gan D, Zhang X, Pan Y (2010). Antioxidant capacity of the extracts from pulp of *Osmanthus fragrans* and its components. *LWT-Food Sci. Technol.* 43: 319-325.
- Wang Q, Yang ZY, Qi GF, Qin DD (2009). Synthesis, crystal structure, antioxidant activities and DNA-binding studies of the Ln (III) complexes with 7-methoxychromone-3-carbaldehyde-(4'- hydroxy) benzoyl acylhydrazone. *Eur. J. Med. Chem.* 44: 2425-2433.
- Windholz M (1988). The Merck Index. 11th ed . Rahway (New Jersey): Merck and Co., Inc. 6038.
- Xu G, Xia QH, Lu XH, Zhang Q, Zhan HJ (2007). Selectively catalytic epoxidation of styrene with dry air over the composite catalysts of Co-ZSM-5 coordinated with ligands. *J. Mol. Catal. A-chem.* 266: 180-187.
- Zerbo A, Koudou J, Ouédraogo N, Ouédraogo R, Guissou IP (2010). Antioxidant and antibacterial activities of *Piliostigma reticulatum* (DC.) Hochst extracts. *Afr. J. Biotechnol.* 9: 5407-5411.
- Zhang M, Lu JR, Xin CW, Liu F, Zhou CM (2009b). Synthesis, Characterization and Antibacterial Activities of 5-Bromo-2-hydroxybenzoyl Substituted Aromatic Aldehyde Hydrazones. *Chin. J. Appl. Chem.* 26: 1386-1390.
- Zhang WM, Li B, Han L, Zhang HD (2009a). Antioxidant activities of extracts from areca (*Areca catechu* L.) flower husk and seed. *Afr. J. Biotechnol.* 8: 3887-3892.