

Synthesis and antioxidant evaluation of some novel *ortho*-hydroxypyridine-4-one iron chelators

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Abstract

A series of *ortho*-hydroxypyridine-4-ones were prepared in high yields and evaluated for antioxidant and iron chelating activities. N¹-H hydroxypyridinones **Va**, **Vb**, and **Ve** were the best radical scavengers in DPPH free radical scavenging assay. Compound **Vb** was proved to be the most potent compound in hydrogen peroxide scavenging assay. All of the synthesized compounds had very close chelating ability, compounds containing N¹-CH₃ hydroxypyridinone ring were stronger chelating agents.

Keywords: *Ortho*-Hydroxypyridine-4-one; Antioxidant activity; Iron chelating activity; Radical scavenging activity

INTRODUCTION

The importance of researches on the roles of free radical species in biological systems has been significantly increased in the last two decades. The production of these species in naturally occurring biological reactions is an inevitable phenomenon in aerobic metabolic processes. The presence of a singlet electron in free radicals renders them extreme reactivity which may cause severe oxidative damages to the biomolecules such as lipids, proteins and DNA (1). Various disorders such as cancer, atherosclerosis, diabetes, alzheimer, parkinson and disease related to aging process are long term manifestations of these damages (2). Since this kind of damages can exert irreversible adverse effects on cells, there are some antioxidant mechanisms and compounds with endogenous and exogenous origin to deactivate or neutralize these reactive species. Glutathion reductase (GSH), superoxide dismutase (SOD) and catalase are well-known enzymes built in these mechanisms. Radical scavenging compounds such as α -tocopherol, ascorbic acid, carotenoids, and natural polyphenols are some examples of exogenous

antioxidant compounds. These enzymes and antioxidant compounds help the body to equilibrate the generation and neutralization of free radicals. When these defensive mechanisms become insufficient or defective, design of potent and safe antioxidant molecules seems to be necessary to hold this balance again (3,4).

Oxidative reactions have multiple dimensions which make it possible to have different plans for designing antioxidant substances. Molecules with free radical scavenging abilities are the most-studied antioxidant agents. It is revealed that many different substances are involved in the process of free radical generation, among them metal ions with specific roles in biological reactions are especially important. Iron is the fundamental catalyst of Fenton and Haber-Weiss reactions which produce the hydroxyl radical which is the most destructive free radical (5). Substances which can chelate iron cations potentially suppress oxidative reactions to alleviate the destroying effects of free radicals. *Ortho*-Hydroxypyridinones comprise one of the well-known chelating moieties in medicinal chemistry. 3(5)-hydroxypyridin-4-ones

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have the most chelating ability among the three classes of *ortho*-hydroxypyridinones: 1-hydroxypyridin-2-ones, 3-hydroxypyridin-2-ones, and 3(5)-hydroxypyridin-4-ones. These compounds like other high affinity Fe^{3+} chelating agents bind both Fe^{3+} and Fe^{2+} under most physiological conditions (6). 3(5)-hydroxypyridin-4-one derivatives form five-membered chelate rings with iron in which the metal is coordinated by two vicinal oxygen atoms, thus these bidentate chelators form neutral and stable 3: 1 complexes with iron cations and inhibit their redox activity (3).

Co-administration of iron chelating compounds, to prevent Fenton chemistry induced by free iron, and radical scavenging agents, to neutralize the free radicals, has been one of the strategies to control oxidative stress conditions. Prevention of post-ischemic cardiac injury using a combination of 1,2-dimethyl-3-hydroxypyridin-4-one (deferiprone) and antioxidant flavonoid (+) cyanidanol-3 (7) and more recently, combined treatment with deferiprone and the antioxidant idebenone in individuals with Friedreich's ataxia (8), are some reported cases of this combination therapy. Design of compounds possessing the dual ability of iron chelation and radical scavenging has been another strategy to control oxidative conditions. Some chemical entities possess both iron chelating and antioxidant abilities. For example, the antioxidant activity of flavonoids is attributed to a combination of iron chelation and free radical scavenging activities (9). Iron-chelating-radical-scavenging 8-hydroxyquinoline derivatives with neuroprotective properties are under investigation for neurodegenerative disorders such as Alzheimer and Parkinson disease (10). Famous 3-hydroxypyridin-4-one derivatives (e.g. deferiprone, CP20, or CP08, Fig. 1) in contrast to many iron chelators lack a remarkable radical scavenging ability (11).

There are few reports on the design of small molecules with 3(5)-hydroxypyridin-4-one scaffold to render them this dual activity. A world patent in 1999 claimed that *ortho*-hydroxypyridinone derivatives possess iron chelating and antioxidant activities. The inventors provide compounds which may be

used in the treatment of oxidative stress, particularly oxidative damage to the central nervous system as neuroprotective agents (12). In a more recent article, functionalized *ortho*-hydroxypyridinones with the desired dual activity are provided to combat neurodegeneration associated with Alzheimer's disease (13).

Here, we report on the synthesis of some novel 5-hydroxypyridin-4-one derivatives. These compounds are chelating agents due to the presence of the *ortho*-hydroxypyridinone ring in their structure. All of these compounds have at least one labile hydrogen atom in the auxiliary moiety attached to the hydroxypyridin-4-one ring. This renders them a radical scavenging ability which is greater than what is expected for the main scaffold. These compounds are evaluated as free radical scavenging and Fe^{2+} chelating agents. The H_2O_2 scavenging power of these compounds is determined as well.

MATERIALS AND METHODS

Chemistry

All chemicals used for the synthesis of the compounds were supplied by Merck or Sigma. Melting points were determined on a Mettler capillary melting point apparatus and were uncorrected. The IR spectra were recorded with a WQF-510 Ratio Recording FT-IR spectrometer as a KBr disc (γ , cm^{-1}). The ^1H NMR spectra (DMSO-d_6) were recorded on a Bruker 400 MHz spectrometer. Chemical shifts (δ) are reported in ppm downfield from the internal standard tetramethylsilane (TMS). Electrospray mass spectra (ESI-MS) were obtained in negative and positive ion mode on a SHIMADZU LCMS-2010 EV spectrometer

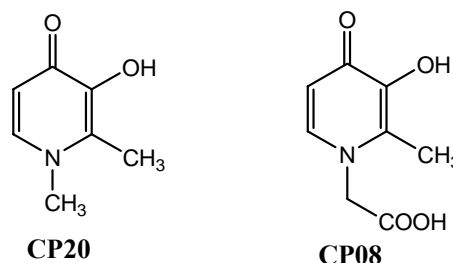


Fig. 1. CP20 and CP08: Two well-known 3-hydroxy pyridin-4-one derivatives

using methanol and dimethylsulfoxide as solvent, a capillary voltage of 4500 V and a cone voltage of 10 V. The purity of the compounds was checked by thin layer chromatography (TLC) on silica gel plate using chloroform and methanol. The procedure for the synthesis of the desired compounds is depicted in Scheme 1.

Synthesis of 5-(benzyloxy)-2-(hydroxymethyl)-4H-pyran-4-one (**I**)

This compound was prepared according to the instruction already published (14).

Synthesis of 5-Benzyloxy-2-(hydroxymethyl)-pyridin-4(1H)-one (**II_a**) and 5-(benzyloxy)-2-(hydroxymethyl)-1-methylpyridin-4(1H)-one (**II_b**)

These compounds were prepared according to the method reported by Ma and coworkers (15).

Synthesis of 5-(benzyloxy)-4-oxo-1,4-dihydro pyridine -2- carbaldehyde (**III_a**) and 5-(Benzyloxy) -1- methyl -4- oxo -1, 4-dihydro pyridine-2-carbaldehyde (**III_b**)

Aldehyde **III_a** was prepared by the oxidation of compound **II_a** using manganese dioxide as described by Becker (16). Aldehyde

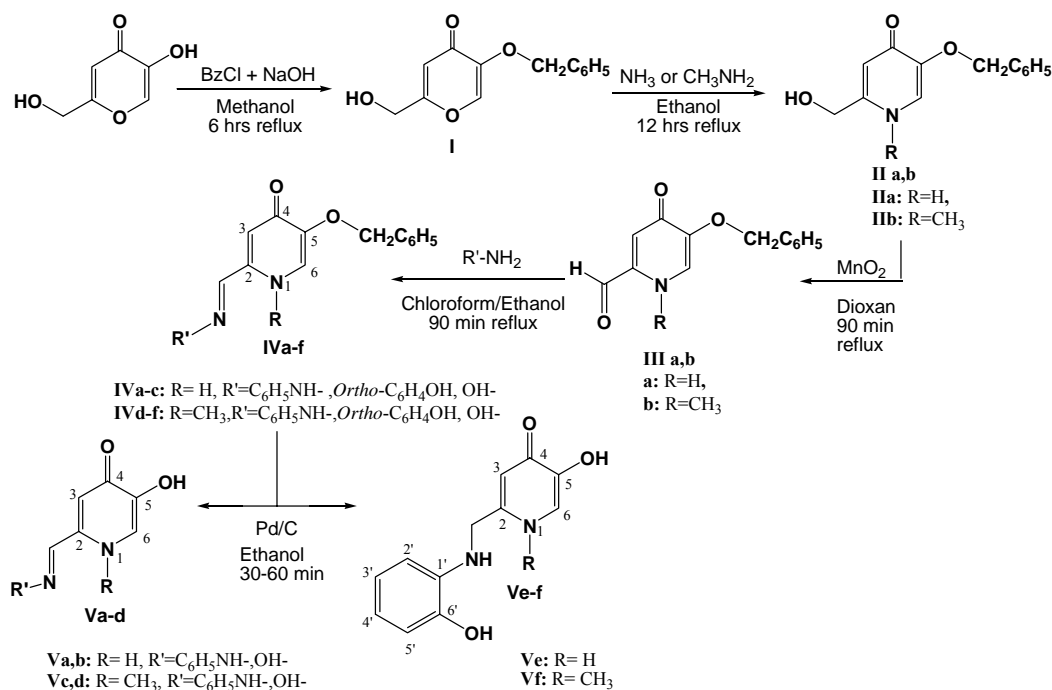
III_b was prepared by the same method as **III_a**.

General procedure for the synthesis of **IV_{a-f}** derivatives

2.063 g (8.7 mmol) of **III_a** or **III_b** was dissolved in 30 ml chloroform and 10 ml ethanol. 8.7 mmol of the proper -NH₂ containing compound was added to this solution. The mixture was then refluxed and the progression of the reaction was monitored by TLC. The reaction was completed within 90 min. The product was filtrated off, washed with chloroform and recrystallized in methanol. The obtained crystals were dried under vacuum.

General procedure for the synthesis of **V_{a-f}** derivatives

0.500 g of each of **IV_{a-f}** was dissolved in 100 ml of absolute ethanol. Then 5.00 g of 10% palladium/charcoal was added. Hydrogen was bubbled into the reaction mixture using a balloon connected to the reaction vessel. The mixture was shaken vigorously at room temperature for 30-60 min. When the reaction was completed, palladium/charcoal was filtrated off and washed 3 times by ethanol. The product was crystallized from the filtrate and dried under reduced pressure (Scheme 1).



Scheme 1. General procedure for the synthesis of the studied compounds

Antioxidant evaluation

DPPH free radical scavenging assay

This assay was performed according to the method described by Blois and coworkers (17). Briefly, 4 ml of different concentrations of methanolic solution of standard or test compounds (0.4 to 0.00625 mg/ml) were added to 2 ml of DPPH methanolic solution (60 mM). The mixture was shaken vigorously and allowed to stand for 30 min, and then the absorbance of the resulting solution was measured at 517 nm using a UV/Vis-spectrophotometer. Scavenging of DPPH free radicals was calculated as:

$$\text{DPPH scavenging activity (\%)} = [(Ac - At) / Ac] \times 100$$

where, Ac is the absorbance of the control tube (containing all reagents except the test compound), and At is the absorbance of the test tube. Ascorbic acid, gallic acid, BHT and BHA were used as standards.

Hydrogen peroxide scavenging assay

H₂O₂ scavenging power was determined according to the method of Ruch and coworkers (18). This method is based on the ability of a compound to convert hydrogen peroxide to water. A 40 mM solution of hydrogen peroxide was prepared in saline phosphate buffer (pH 7.4) and concentrations were determined spectrophotometrically at 230 nm. 100 µl of 0.312 mg/ml methanolic solutions of the test compounds or standards was added to 2 ml of hydrogen peroxide solution (40 mM) and the absorbance was determined at 230 nm after 20 min against a blank solution (100 µl of methanolic solutions of test compounds or standards and 2 ml of saline phosphate buffer). The hydrogen peroxide scavenging for compounds and standards was calculated using the following equation:

$$\text{H}_2\text{O}_2 \text{ scavenging activity (\%)} = [(Ac - At) / Ac] \times 100$$

Where Ac is the absorbance of the control and At is the absorbance of tested compounds or standards. Ascorbic acid and gallic acid were used as standards.

Iron chelating ability

The chelating effects were determined according to the method of Dinis and

coworkers (19). 500 µl of the standard or test compounds (12.5 µg/ml) was added to 50 µl of FeCl₂ solution (2 mM). The reaction was initiated by adding 20 ml of ferrozine methanolic solution (5 mM). The mixture was shaken vigorously and left at room temperature for 10 min. The absorbance of the solution was measured spectrophotometrically at 562 nm. The chelation ability of the prepared compounds was calculated based on the inhibition of ferrozine-Fe²⁺ complex formation using the following formula:

$$\text{Metal chelating activity (\%)} = [(Ac - As) / Ac] \times 100$$

where, Ac is the absorbance of the control (control contained 50 µl of FeCl₂, 20 µl of ferrozine and 500 µl of methanol), and at is the absorbance of the test compound. EDTA and gallic acid were used as controls.

RESULTS

Chemistry

5-Hydroxypyridine-4-one derivatives described here were synthesized following the synthetic routes outlined in scheme 1. Reaction of kojic acid (5-Hydroxy-2-(hydroxymethyl)-4H-pyran-4-one) with benzyl chloride resulted in the protected form of this molecule (**I**) which was then reacted with ammonia or methyl amine to produce the corresponding pyridin-4(1H)-one derivatives (**II_{a,b}**). Oxidation of these alcohols with activated manganese (IV) oxide in dioxane afforded the desired aldehydes (**III_{a,b}**). These aldehydes were prepared to be used as intermediates for the preparation of some 5-hydroxypyridine-4-one derivatives containing appropriate moieties for stabilizing the radical species which would be formed from the reaction of these compounds with free radicals. Thus, **III_a** and **III_b** were reacted with phenyl hydrazine, 2-aminophenol and hydroxylamine to afford the desired final compounds after debenylation of the 5-benzyloxy group of the obtained molecules. The structures of all compounds were confirmed by FTIR, ¹HNMR and mass spectroscopy. Carbon atoms are numbered sequentially to facilitate the assignment of protons in ¹HNMR (Scheme 1). Compounds **IV_{a-c}** were revealed to be as two isomers as shown in Fig. 2.

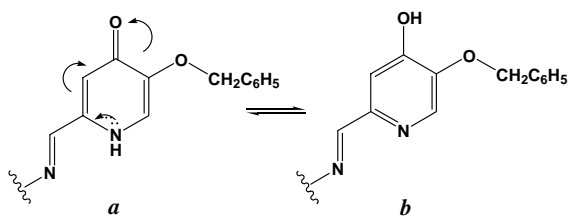


Fig. 2. The observed tautomerism in compounds IVa-c

5-(Benzyloxy)-1-methyl-4-oxo-1,4-dihydropyridine-2-carbaldehyde (III_b)

White crystals; yield 80%; m.p. 227-229°C; IR (KBr) cm^{-1} : 3047 (C-H, aromatic, str.), 2893 (C-H, aliphatic, str.), 1716 (C=O, aldehyde, str.), 1618 (C=O ketone, str.), 1583 (shouldered, C=C aromatic, C=C alkene), 1437 (C=C, aromatic, bend); ^1H NMR (DMSO- d_6): δ 9.68 (s, 1H, CHO), 7.44 (s, 1H, C6-H), 7.16-7.33 (m, 5H, $\text{CH}_2\text{-C}_6\text{H}_5$), 6.75 (s, 1H, C3-H), 5.07 (s, 2H, O- $\text{CH}_2\text{-C}_6\text{H}_5$), 3.85 (s, 3H, NCH_3).

5-Benzyloxy-2-[(phenylaminoimino)methyl]pyridin-4(1H)-one (IV_a)

Yellow crystals; yield 74%; m.p. 236-237°C; a,b tautomers: $a/b=100/40$. IR (KBr) cm^{-1} : 3381 (N-H, str.), 3222 (O-H, str.), 3051 and 3033 (C-H, aromatic, str.), 2968 and 2931 (C-H, aliphatic, str.), 1630 (C=O, str.), 1593 (C=C, aromatic, str.), 1529 (shouldered, C=C alkene, C=N), 1493 (C=C, aromatic, bend); ^1H NMR (DMSO- d_6): δ 11.15 [s, 1H, =N-NH- (*a*)], 10.87 [s, 1H, $\text{N}^1\text{-H}$ - (*a*)], 10.76-10.78 [m, 2H, =N-NH- (*b*), C4-OH (*b*)], 8.08 [s, 1H, -CH=N- (*b*)], 7.00-7.70 [m, 12H, - $\text{CH}_2\text{-C}_6\text{H}_5$ (*a,b*), -CH=N- (*a*) and C6-H (*b*)], 6.80 [bs, 2H, C6-H (*a*), C3-H (*b*)], 6.31 [1H, C3-H (*a*)], 5.171 [s, 2H, - $\text{CH}_2\text{-C}_6\text{H}_5$ (*b*)], 5.032 [s, 2H, - $\text{CH}_2\text{-C}_6\text{H}_5$ (*a*)]; ESI-MS (+) m/z (%): 320.1 ($\text{M} + \text{H}^+$) (95), 342.2 ($\text{M} + \text{Na}^+$) (100).

5-Benzyloxy-2-[(2-hydroxyphenylimino)methyl]pyridin-4(1H)-one (IV_b)

Pale yellow crystals; yield 79%; m.p. 235-236°C; a,b tautomers: $a/b=100/30$. IR (KBr) cm^{-1} : 3234 (O-H, str.), 3066 (N-H, str.), 3032 (C-H, aromatic, str.), 2866 (C-H, aliphatic, str.), 1633 (C=O, str.), 1603 (C=C, aromatic, str.), 1541 (shouldered, C=C alkene, C=N), 1487 (C=C, aromatic, bend); ^1H NMR (DMSO- d_6): δ 11.67 [s, 1H, $\text{N}^1\text{-H}$ (*a*)], 10.68

[s, 1H, C4-OH (*b*)], 9.10-9.30 [m, 2H, disubstituted phenyl-OH (*a,b*)], 8.65 [s, 1H, -CH=N- (*a*)], 8.45 [s, 1H, -CH=N- (*b*)], 6.87-8.30 [m, 20H, $\text{CH}_2\text{-C}_6\text{H}_5$ (*a,b*), C6-H (*b*), C3-H (*a*), disubstituted phenyl ring hydrogens (*a,b*)], 6.64 [s, 1H, C6-H (*a*)], 5.23 [s, 2H, - $\text{CH}_2\text{-C}_6\text{H}_5$ (*b*)], 5.08 [s, 2H, - $\text{CH}_2\text{-C}_6\text{H}_5$ (*a*)]; ESI-MS (-) m/z (%): 319.1 ($\text{M} - \text{H}^+$) (100).

5-Benzyloxy-4-oxo-1,4-dihydropyridine-2-carbaldehyde oxime (IV_c)

White crystals; yield 82%; m.p. 231-232°C; a,b tautomers: $a/b=100/50$. IR (KBr) cm^{-1} : 3278, 3168 (N-H, str.), 3064, 3035 (C-H, aromatic, str.), 2868 (C-H, aliphatic, str.), 2629 (O-H, str.), 1637 (C=O, str.), 1601 (C=C, aromatic, str.), 1537 (shouldered, C=C alkene, C=N), 1483 (C=C, aromatic, bend); ^1H NMR (DMSO- d_6): δ 12.57 [s, 1H, =N-OH (*a*)], 11.95 [s, 1H, $\text{N}^1\text{-H}$ (*a*)], 11.35 [s, 1H, =N-OH (*b*)], 10.65 [s, 1H, C4-OH (*b*)], 7.10-8.30 [m, 15H, CH=N- (*a,b*), $\text{CH}_2\text{-C}_6\text{H}_5$ (*a,b*), C3-H (*a*), C6-H (*a,b*)], 6.35 [s, 1H, C3-H (*b*)], 5.0-5.2 [m, 4H, $\text{CH}_2\text{-C}_6\text{H}_5$ (*a,b*)]; ESI-MS (-) m/z (%): 243.1 ($\text{M} - \text{H}^+$) (100).

5-Benzyloxy-2-[(phenylaminoimino)methyl]-1-methylpyridin-4(1H)-one (IV_d)

Yellow crystals; yield 72%; m.p. 241-242°C; IR (KBr) cm^{-1} : 3433 (O-H, str.), 3169 (N-H, str.), 3044, (C-H, aromatic, str.), 2936 (C-H, aliphatic, str.), 1619 (C=O, str.), 1570 (C=C, aromatic, str.), 1523 (C=C alkene), 1497 (C=N), (1448 (C=C, aromatic, bend); ^1H NMR (DMSO- d_6): δ 10.88 (s, 1H, =N-NH-), 7.61 (m, 1H, CH=N-), 7.34-7.45 (m, 5H, $\text{CH}_2\text{C}_6\text{H}_5$), 7.26 (t, 2H, C3'-H, C5'-H), 7.08 (d, 2H, C2'-H, C6'-H), 6.83 (t, 1H, C4'-H), 6.59 (s, 1H, C6-H), 5.03 (s, 2H, $\text{CH}_2\text{C}_6\text{H}_5$), 3.77 (s, 3H, NCH_3).

5-Benzyloxy-2-[(2-hydroxyphenylimino)methyl]-1-methylpyridin-4(1H)-one (IV_e)

Yellow crystals; yield 69%; m.p. 228-229°C; IR (KBr) cm^{-1} : 3434 (O-H, str.), 3066 (C-H, aromatic, str.), 2948 (C-H, aliphatic, str.), 1605 (shouldered, C=O, C=C, aromatic, str.), 1553 (C=N), 1453 (C=C, aromatic, bend); ^1H NMR (DMSO- d_6): δ 9.25 (bs, 1H, C2'-OH), 8.68 (s, 1H, -CH=N-), 7.71 (s, 1H, C6-H), 7.31-7.50 (m, 5H, $\text{CH}_2\text{-C}_6\text{H}_5$), 7.26 (d, J=7.50

Hz, 1H, C6'-H), 7.14 (t, J=7.50 Hz, 1H, C4'-H), 7.05 (s, 1H, C3-H), 6.92 (d, J= 8.00 Hz, 1H, C3'-H), 6.86 (t, J= 7.50 Hz, 1H, C5'-H), 5.09 (s, 2H, CH₂-C₆H₅), 3.95 (s, 3H, NCH₃).

5- Benzyloxy -1- methyl -4- oxo -1,4- dihydro pyridine-2-carbaldehyde oxime (IV_f)

White crystals; yield 83%; m.p. 239-240°C; IR (KBr) cm⁻¹: 3157 (N-H, str.), 3062 (C-H, aromatic, str.), 2998,2928,2867 (C-H, aliphatic, str.), 2692 (O-H, str.), 1620 (C=O, str.), 1564 (shouldered, C=C alkene, C=N), 1490 (C=C, aromatic, bend); ¹HNMR (DMSO-D₆): δ 12.36 (s, 1H, OH), 8.24 (s, 1H, -CH=N-), 7.68 (s, 1H, C6-H), 7.33-7.43 (m, 5H, CH₂-C₆H₅), 6.60 (s, 1H, C3-H), 5.01 (s, 2H, CH₂-C₆H₅), 3.72 (s, 3H, NCH₃).

5- Hydroxy -2- [(phenylaminoimino)methyl] pyridin-4(1H)-one (V_a)

Yellow crystals; yield 86%; m.p. 235-236°C; IR (KBr) cm⁻¹: 3311 (N-H, str.), 3290 (N-H, str.), 1639 (C=O, str.), 1593 (C=N, str.), 1520 (C=C, aromatic, str.), 1479 (C=C, aromatic, bend) ¹HNMR (DMSO-d₆): δ 11.2 (s, 1H, -NH=N-), 10.81 (s, 1H, N¹-H), 7.57 (s, 1H, CH=N-), 7.23 (bs, 5H, -C₆H₅), 6.80 (s, 1H, C6-H), 6.33 (s, 1H, C3-H), 4.0-6.0 (bs, 1H, OH). ESI-MS (-) m/z (%): 228.1 (M - H⁺) (100).

5- Hydroxy -4- oxo -1,4- dihydro pyridine-2-carbaldehyde oxime (V_b)

White crystals; yield 86%; m.p. 262-263°C; IR (KBr) cm⁻¹: 3402 (O-H, str.), 3261 (O-H, str.), 3132 (N-H, str.), 1624 (C=O, str.), 1537 (C=N, str.), 1473 (C=C, aromatic), ¹HNMR (DMSO-d₆): δ 8.22 (s, 1H, -CH=N-), 7.55 (s, 1H, C6-H), 6.74 (s, 1H, C3-H). ESI-MS (-) m/z (%): 153.1 (M - H⁺) (100).

5-Hydroxy-2-[(phenylaminoimino)methyl]-1-methylpyridin-4(1H)-one (V_c)

Yellow crystals; yield 72%; m.p. 241-242°C; IR (KBr) cm⁻¹: 3440 (O-H, str.), 3244 (N-H, str.), 3194 (C-H, aromatic, str.), 2966 and 2925 (C-H, aliphatic, str.), 1616 (C=O, str.), 1599 (C=C, aromatic, str.), 1525 (shouldered, C=C alkene, C=N), 1481 (C=C, aromatic, bend); ¹HNMR (DMSO-d₆): δ 10.86 (s, 1H, NH), 7.80 (s, 1H, -CH=N-), 7.44 (s,

1H, C3-H), 6.80-7.30 (m, 5H, CH₂-C₆H₅), 6.61 (s, 1H, C6-H), 6.05 (bs, 1H, OH), 3.75 (s, 3H, NCH₃).

5-Hydroxy-1-methyl-4-oxo-1,4-dihydropyridine-2-carbaldehyde oxime (V_d)

White crystals; yield 85%; m.p. 245-246°C; IR (KBr) cm⁻¹: 3446 (O-H, str.), 2956 (C-H, aliphatic, str.), 1541 (shouldered, C=C alkene, C=N); ¹HNMR (DMSO-d₆): δ 8.24 (s, 1H, -CH=N-), 7.49 (s, 1H, C6-H), 6.59 (s, 1H, C3-H), 4.20-5.50 (bs, 1H, OH), 3.71 (s, 3H, NCH₃).

5-Hydroxy-2-[(2-hydroxyphenylamino)methyl] pyridin-4(1H)-one (V_e)

Pale yellow crystals; yield 82%; m.p. 255-256°C; IR (KBr) cm⁻¹: 3396 (N-H, str.), 3286 (N-H, str.), 3070 (C-H, aromatic, str.), 2850, 2727 (C-H, aliphatic, str.), 1608 (C=O, str.), 1556, 1523 (C=C, aromatic); ¹HNMR (DMSO-d₆): δ 11.10 (s, 1H, N¹-H), 7.27 (s, 1H, C6-H), 6.67 (d, J= 7.60 Hz, 1H, C2'-H), 6.563 (t, J= 7.60 Hz, 1H, C4'-H), 6.423 (t, J= 7.60 Hz, 1H, C3'-H), 6.345 (d, J= 7.60 Hz, 1H, C5'-H), 6.16 (s, 1H, C3-H), 5.41 (bs, 1H, NH-CH₂), 4.15 (d, J= 5.20 Hz, 2H, NH-CH₂). ESI-MS (+) m/z (%): 231.1 (M + H⁺) (100).

5-Hydroxy -2-[(2-hydroxyphenylamino)methyl]-1-methylpyridin-4(1H)-one (V_f)

Pale yellow crystals; yield 85%; m.p. 244-245°C; IR (KBr) cm⁻¹: 3387 (O-H, str.), 3157 (N-H, str.), 3044 (C-H, aromatic, str.), 2950, 2930 (C-H, aliphatic, str.), 1597 (C=O, str.), 1556 (C=N), 1443 (C=C, aromatic, bend); ¹HNMR (DMSO-d₆): δ 7.42 (s, 1H, C6-H), 6.44-6.72 (m, 4H, NH-C₆H₄-OH), 6.14 (s, 1H, C3-H), 5.25 (bs, 1H, NH-CH₂), 4.27 (d, J= 2.80 Hz, 2H, NH-CH₂), 3.67 (s, 3H, NCH₃).

Antioxidant evaluation

The antioxidant potential of the synthesized compounds was assessed using two different methodologies, namely DPPH free radical and hydrogen peroxide scavenging assays.

DPPH free radical scavenging assay

DPPH scavenging evaluation is a standard assay in antioxidant activity studies and offers a rapid technique for screening the radical

scavenging activity of specific compounds. This is a common antioxidant assay method which determines the ability of the compound to react with a free radical to give hydrogen to it.

A freshly prepared methanolic solution of DPPH has a deep purple color with an absorption maximum at 517 nm. This color generally disappears when an antioxidant is present in the medium. Thus, antioxidant molecules can react with DPPH free radicals (by providing hydrogen atoms or by electron donation via a free radical attack on this molecule) and convert them to colorless/

bleached product (20,21).

The DPPH free radical scavenging activity of methanolic solutions of 5-hydroxy-pyridine-4-one derivatives **Va-f** were examined and compared in Table 1 and Fig. 3.

From analysis of Table 1 and Fig. 3, we can conclude that compounds with no substituent at N¹ atom of the pyridinone ring (**Va**, **Vb** and **Ve**) exhibited more scavenging effects on DPPH radicals compared with compounds having methyl substituent on this atom. Among them compound **Va** (IC₅₀=0.013 mg/ml) was the most potent one.

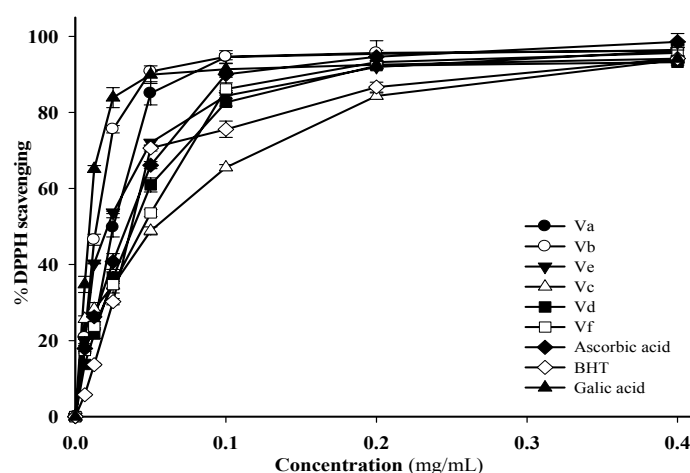


Fig. 3. % DPPH scavenging activity of the compounds **Va-f** (0.00625-0.4 mg/ml)

Table 1. IC₅₀ values for DPPH scavenging ability of the compounds **Va-f**

Compound	Va	Vb	Vc	Vd	Ve	Vf	Ascorbic acid	BHT	Galic acid
IC ₅₀ (mg/ml)	0.013	0.021	0.053	0.038	0.026	0.045	0.033	0.058	0.009

Table 2. H₂O₂ scavenging activity values for the compounds **Va-f**

Compound	Va	Vb	Vc	Vd	Ve	Vf	Ascorbic acid	Galic acid
H ₂ O ₂ scavenging (%) ± SD*	74.14±0.87	79.62±2.14	72.51±1.11	70.33±1.81	71.75±1.25	68.37±0.95	89.15±0.78	97.14±2.39

* These values are measured at 0.312 mg/ml concentration

Table 3. Fe²⁺ chelating power for the compounds **Va-f**

Compound	Va	Vb	Vc	Vd	Ve	Vf	EDTA	Galic acid
Chelation power (%) ± SD*	39.79±1.74	42.21±0.99	43.24±0.76	48.76±1.05	38.01±1.06	35.19±1.13	71.42±1.05	57.98±0.85

*These values are measured at 12.50 µg/ml concentration

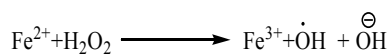
Hydrogen peroxide scavenging assay

The method provided by Ruch and coworkers which is based on the ability of a compound to convert hydrogen peroxide to water was used for determination of H₂O₂ scavenging power. Hydrogen peroxide is a weak oxidizing agent capable of oxidizing the essential thiol (-SH) groups of proteins, thus inactivating a few enzymes. It rapidly passes through the cell membranes and inside the cell reacts with Fe²⁺ to form hydroxyl radical which exerts several toxic effects (22). Thus, it is advantageous for cells to control the amount of hydrogen peroxide to prevent oxidative stress conditions.

The values for hydrogen peroxide scavenging power of compounds **Va-f** are provided in Table 2. It is understood from this table that **Vb** was the most potent compound in this assay.

Iron chelation ability

Ferrous ion can initiate biological damages by the Fenton reaction. Inasmuch as H₂O₂, by itself, is a poorly reactive oxidant, it participates in the direct formation of the hydroxyl radicals in the presence of free iron ions; as it can be explained by Fenton reaction as follows:



Ferrozine forms red colored complexes with ferrous ions. When chelating agents is present in the medium this complex formation is interrupted and this leads to the decrease of the red color. Measuring the amount of color reduction allows determining the chelating effect of the coexisting compounds. The formation of the ferrozine-Fe²⁺ complex was interrupted in the presence of **Va-f**, indicating their chelating activity. The results of this assay are provided in Table 3. Ferrous iron chelating agents form coordinate bonds with

Fe²⁺; which reduces the redox potential of this cation, and thereby stabilizes the oxidized form of Fe²⁺. This property renders iron chelators effective secondary antioxidant ability (23).

DISCUSSION

The antioxidant potency of the compounds can be explained according to the differences in their chemical structures after categorizing them in two chemical classes. Three of the synthesized compounds contain N¹-H hydroxypyridinone ring and the other ones have a methyl group substituted on the nitrogen atom of the ring. In both antioxidant assays performed in this research, there was a dominant difference between the results for these two groups. Compounds with N¹-H show more antioxidant potency than those with N¹-CH₃ moiety. The proofs to explain this phenomenon can be provided considering the mechanism of DPPH free radical scavenging assay provided in Fig. 4.

The hydrogen abstracted from R-H in Fig. 4 is a labile hydrogen and the most potent compounds in this assay (**Va,b,e**) possess several hydrogens of this type. A common hydrogen in these compounds is the N1-H of hydroxypyridinone ring which is absent in the less active compounds (**Vc, Vd, and Vf**). Compound **Vb** was proved to be the most potent compound in hydrogen peroxide scavenging assay. All the synthesized compounds proved to have very close chelating ability, and compounds containing N¹-CH₃ hydroxypyridinone ring were stronger chelating agents. The reason was the inductive effect of the methyl group which pushes more electrons to the oxygen attached to the C-4 position of the ring making it a good ligand for iron chelation. The other reason is the fact that

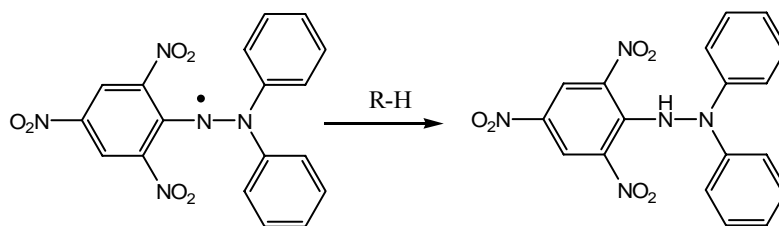


Fig. 4. DPPH reaction with an antioxidant

N^1 -H derivatives lose a proton which results in a neutral molecule with a hydroxyl group, instead of a negative oxygen, as it is formed in N^1 -CH₃ derivatives, in the C-4 position of the hydroxypyridinone ring. The latter case performs definitely better in iron chelation.

CONCLUSION

In sum, the *ortho*-hydroxypyridinones prepared in this research seem to be promising antioxidant iron chelator agents for *in vitro* studies of oxidative stress conditions.

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REFERENCES

- Guelman LR, Pagotto RM, Di Toro CG, Zieher LM. Deferoxamine antioxidant activity on cerebellar granule cells γ -irradiated *in vitro*. *Neurotoxicol Teratol*. 2004;26:477-483.
- Sjödin B, Hellsten-Westling Y, Apple FS. Biochemical mechanisms for oxygen free radical formation during exercise. *Sports Med*. 1990;10:236-254.
- Reeder BJ, Hider RC, Wilson MT. Iron chelators can protect against oxidative stress through ferryl heme reduction. *Free Radic Biol Med*. 2004;44:264-273.
- Loganathan JK, Gunasundari D, Hemalatha M, Shenbhagaram R, Kaviyaran V. Antioxidant and phytochemical potential of wild edible mushroom *Termitomyces reticulatus*: individual cap and stipe collected from eastern part of India. *IJPSR*. 2010;1:62-72.
- Palmer C. *Metals and Oxidative Damage in Neurological Disorders*. New York, Plenum Press, 1997. p. 205-236.
- Hider RC. Potential protection from toxicity by oral iron chelators. *Toxicol Lett*. 1995;82/83:961-967.
- van der Kraaij AMM, van Eijk HG, Koster JF. Prevention of postischemic cardiac injury by the orally active iron chelator 1,2-Dimethyl-3-Hydroxy-4-Pyridone (L1) and the antioxidant (+)-cyanidanol-3. *Circulation* 1989;80:158-164.
- Velasco-Sánchez D, Aracil A, Montero R, Mas A, Jiménez L, O'Callaghan M, *et al*. Combined therapy with idebenone and deferiprone in patients with Friedreich's ataxia. *Cerebellum*, 2011;10:1-8.
- van Acker SA, van Balen GP, van den Berg DJ, Bast A, van der Vijgh WJ. Influence of iron chelation on the antioxidant activity of flavonoids. *Biochem Pharmacol*, 1998;56:935-943.
- Zheng H, Weiner LM, Bar-Am O. Design, synthesis, and evaluation of novel bifunctional iron-chelators as potential agents for neuroprotection in Alzheimer's, Parkinson's, and other neurodegenerative diseases. *Bioorg Med Chem*. 2005;13:773-783.
- Kayyali R, Pannala AS, Khodr H, Hider RC. Comparative radical scavenging ability of bidentate iron (III) chelators. *Biochem Pharmacol*. 1998;55:1327-1332.
- David B, Nat M, Suneel G, Alan P, Richard P, Craig M. *Ortho*-hydroxypyridinone derivatives as iron chelating and antioxidant agents. *PAT-WO9923075*. 1999.
- Schugar H, Green DE, Bowen ML, Scott LE, Storr T, Böhmerle K, *et al*. Combating Alzheimer's disease with multifunctional molecules designed for metal passivation. *Angew Chem*. 2007;119:1746-1748.
- Streater M, Taylor PD, Hider RC, Porter J. Novel 3-hydroxy-2(1H)-pyridinones. Synthesis, iron (III)-chelating properties and biological activity. *J Med Chem*. 1990;33:1749-1755.
- Ma YM, Luo W, Quinn PJ, Zudong L, Hider RC. Design, synthesis, physicochemical properties, and evaluation of novel iron chelators with fluorescent sensors. *J Med Chem*. 2004;47:6349-6362.
- Becker HD. The conversion of kojic acid into comenaldehyde and comenic acid. *Acta Chem Scand*. 1962;16:78-82.
- Blois MS. Antioxidant determinations by the use of a stable free radical. *Nature*. 1958;181:1199-1200.
- Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*. 1989;10:1003-1008.
- Dinis TCP, Maderia VMC, Almeida LM. Action of phenolic derivatives (acetaminophen, salicylate and 5-Amino salicylate) as inhibitor of membrane lipid peroxidation and as peroxy radical scavengers. *Arch Biochem Biophys* 1994;315:161-169.
- Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA. Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chemistry*. 2004;84:551-562.
- Siddhuraju P, Becker K. The antioxidant and free radical scavenging activities of processed cowpea (*Vigna Unguiculata* (L.) Walp.) seed extracts. *Food Chemistry*. 2007;101:10-19.
- Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine*. 4th ed. Oxford, Clarendon Press. 2006. p. 419-420.
- Gordon MH. The mechanism of antioxidant action *in vitro*. In: B.J.F. Hudson. *Food Antioxidants*. London. Elsevier Applied Science. 1990. p. 1-18.