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Carla Correia, Cláudia Leite, M. Fernanda Proença and M. Alice Carvalho*

Centro de Química, Departamento de Química, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Corresponding author: M. Alice Carvalho
*Fax: +351 (253) 604 382.
*E-mail: mac@quimica.uminho.pt.

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Abstract

Novel hydroxylated benzylideneamino imidazole derivatives were synthesized and their radical scavenging activity was assessed against DPPH and hydroxyl radicals. In the DPPH assay, most of the synthesized compounds showed an IC_{50} in the range 3.2 μM ≤ IC_{50} ≤ 8.4 μM, lower than the reference compound trolox (IC_{50} = 9.5 μM) or the parent aldehydes (5.4 μM ≤ IC_{50} ≤ 11.6 μM). The activity depends mainly on the phenolic subunit (number and position of the hydroxyl groups) and the extent of conjugation with the imidazole ring. In the deoxyribose assay, all the compounds, including parent imidazoles and aldehydes, showed high activity against the hydroxyl radical and the ability to chelate iron ions. At 5 μM concentration, the compounds protected the deoxyribose from degradation by hydroxyl radical between 62-38%.

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Novel hydroxylated benzylideneamino imidazole derivatives were synthesized and their radical scavenging activity was assessed against DPPH and hydroxyl radicals. In the DPPH assay, most of the synthesized compounds showed an IC_{50} in the range 3.2 μM ≤ IC_{50} ≤ 8.4 μM, lower than the reference compound trolox (IC_{50} = 9.5 μM) or the parent aldehydes (5.4 μM ≤ IC_{50} ≤ 11.6 μM). The activity depends mainly on the phenolic subunit (number and position of the hydroxyl groups) and the extent of conjugation with the imidazole ring. In the deoxyribose assay, all the compounds, including parent imidazoles and aldehydes, showed high activity against the hydroxyl radical and the ability to chelate iron ions. At 5 μM concentration, the compounds protected the deoxyribose from degradation by hydroxyl radical between 62-38%.

Oxidative stress (OS) has been associated with a wide range of diseases such as atherosclerosis,[1] cancer,[2,3] diabetes,[4] acute lung injury,[5,6] as well as neurodegenerative disorders[7] including Alzheimer's[8] and Parkinson's disease[9].

OS occurs when intracellular oxidizing species, such as reactive oxygen species (ROS), increase abnormally and is often accompanied by the simultaneous loss of antioxidant capacity. ROS are generated in living organisms by the normal oxidative metabolism that is essential for cell survival.[10] Moreover, ROS may be generated via Fenton chemical reactions between free ions such as copper or iron ions with oxygen and in the presence of a biological reducing agent such as ascorbate.[11,12] Therefore, compounds that are able to scavenge ROS or prevent their formation, via chelation with free metal ions, are important in disease prevention and therapy.

In recent years, the imidazole nucleus has attracted much attention of medicinal chemists because of its potential to generate new chemotherapeutic agents. Imidazole-containing compounds showed to be active as anticancer,[13] antimicrobial,[14,15] antibacterial,[16] antifungal[17]

and antioxidant[18] agents. On the other hand, phenolic compounds have become a topic of interest mainly due to their application in the food industry and medicine, as antioxidants. They have been under very close scrutiny as potential therapeutic agents against a wide range of ailments including neurodegenerative diseases, cancer, diabetes, cardiovascular dysfunctions and inflammatory diseases.[19-22] Some synthetic phenolic compounds reported in the literature,[23,24] revealed high scavenging activity when they incorporate at least, a phenolic unit with two hydroxyl groups. It is well known that the excellent scavenging properties of polyphenols are attributed to the hydroxyl groups present.[25]

As part of a research program aiming to obtain new radical scavengers we combined an imidazole ring with electron withdrawing or electron donating substituents in N1, with phenolic subunits having one, two or three hydroxyl groups in different positions of the ring. As far as we know the combination of these moieties through an imine linker was never reported in literature. An imine was used as linker in order to extend resonance through the molecule, allowing an efficient electron transfer between imidazole and the phenolic ring. This was expected to improve the stability of the transient radicals and the ability of compounds to chelate active ions. The radical scavenging activity of the starting imidazoles, aldehydes and the new compounds was accessed using the DPPH and the 2-deoxy-D-ribose methods.

The phenol-imidazole conjugates **6a-p** were synthesized according to scheme 1. Diaminomaleonitrile **1** was refluxed with triethyl ortoformate, in dioxane, to generate compound **2.**[26] This compound was combined with one equivalent of amine, in ethanol, followed by treatment with an aqueous solution of potassium hydroxide to generate compounds of structure **4**.[27] The target compounds **6** were obtained by reacting compounds **4** with phenolic aldehydes in the presence of trifluoroacetic acid.[28] The structure of compounds **6** was confirmed by IR, NMR and elemental analysis.

In vitro radical scavenging activities were assayed against 2,2-diphenyl-1-picrylhydrazyl (DPPH)[29] and hydroxyl[30] radicals, according to the literature with a minor modification. A freshly prepared DPPH radical solution exhibits a deep purple colour with an absorption maximum at 517 nm. In the presence of an antioxidant, the DPPH free radical is quenched, generating colourless products. The reduction of absorbance at 517 nm is a measure of the free DPPH radical scavenged by the antioxidant. For each compound, in concentrations varying from 100 μM and 1 μM, the percentage of remaining DPPH was determined after 60 min. The IC_{50} values, the effective concentration at which 50% of the DPPH radicals were scavenged, were obtained for compounds having higher scavenging activities (Table 1).

Trolox was used as a reference compound together with the parent imidazoles **4** and aldehydes **5**.

The deoxyribose degradation assay introduced by Gutteridge[31-33] uses the Fenton reaction to generate hydroxyl radicals that degrade the 2-deoxy-D-ribose to malonyldialdehyde (MDA). In the presence of 2-thiobarbituric acid, MDA gives a pink pigment that absorbs at 532 nm. If a compound added to the reaction mixture reduces the absorption at 532 nm this means that the compound behaves as a hydroxyl radical scavenger. The method also allows assessing the ability of the tested compounds to chelate iron ions. Compounds with ligand properties compete with the 2-deoxy-D-ribose molecules for the iron ions, decreasing 2-deoxy-D-ribose degradation that is caused by iron-catalyzed hydroxyl radical attack.

According to the results shown in Table 1, most of the conjugates **6** have radical scavenging activity against DPPH radical higher than that of trolox, the reference compound, and that of parent aldehydes **5a**-**e**. The parent imidazoles are very weak radical scavengers. The substituent R has only marginal influence in the radical scavenging activity of compounds **6** against DPPH radical. Compounds having different groups R and the same group R^1 have similar IC_{50}. For example the IC_{50} of **6a**, **6e**, **6i** and **6n** varies between 3.2 μM and 4.4 μM. However the substituent R^1 has a remarkable influence on the radical scavenging ability of the compounds. The higher activity is observed for compounds having R^1 = 3,4-$(HO)_2C_6H_3$ (3.2 μM ≤ IC_{50} ≤ 4.4 μM) or R^1 = 3,4,5-$(HO)_3C_6H_2$ (4.0 μM ≤ IC_{50} ≤ 5.8 μM). Compounds with R^1= 2,3,4-$(HO)_3C_6H_2$ have radical scavenging activity similar to the reference compound, trolox, (7.0 μM ≤ IC_{50} ≤ 8.4 μM) and compounds with R^1 = 2,4,6-$(HO)_3C_6H_2$ or R^1= 4-HO-3-$MeOC_6H_3$ are very weak DPPH radical scavengers. The percentage of remaining DPPH radical in reaction mixtures using 100 μM solutions of compounds **6d**, **6h** and **6l** (R^1 = 2,4,6-$(HO)_3C_6H_2$) was about 50% and for **6m** was about 90%. These results show that the radical scavenging activity of the compounds increases when at least two vicinal hydroxyl groups are present in the molecule. In the literature, similar results have been reported in studies of the antioxidant activity of flavonoids[35] and phenolic oximes[36]. The literature also reports that the antioxidant activity of poliphenolic compounds increases when the bond dissociation enthalpy (BDE) of the H-O bond decreases and the BDE is lower when the number of vicinal hydroxyl groups increases.[37,38] Furthermore, the BDE of O-H bond in phenols can be modulated by substituents present around the ring. Electron-donating substituents in the *para* position relative to the most reactive OH function usually decrease BDE. Considering that compounds **6b**, **6c**, **6f, 6g, 6j,** 6**k, 6o** and **6p** only differ in the relative position of the

methyleneaminoimidazole unit, the difference observed in the IC_{50} of these compounds can only be attributed to this substituent. The kinetics of the reaction between compounds **6** and the DPPH radical was followed for 60 minutes using a 10 μM solution of each compound. For the less active compounds, a 100 μM solution was used. Figure 1 shows a selection of examples (**6e**, **6f**, **6g** and **6h**) that typically illustrate the behaviour of all the compounds studied. Compounds with two or three vicinal hydroxyl groups show high efficiency as DPPH radical scavengers as the percentage of the remaining DPPH, after 5 minutes, is lower than 50%. The most efficient compounds have R^1=3,4-$(HO)_2C_6H_3$ or 3,4,5-$(HO)_3C_6H_2$ and show a percentage of remaining DPPH lower than 50% after only 2 minutes of reaction. Compounds having only one hydroxyl group (R^1 = 4-HO-3-$MeOC_6H_3$) or compounds having multiple non-vicinal hydroxyl groups (R^1 = 2,4,6-$(HO)_3C_6H_2$) behave as very slow DPPH radical scavengers.

The results of radical scavenger activity of the tested compounds against hydroxyl radical are also shown in table 1. Considering that the IC_{50} for the DPPH radical scavenger activity of the tested compounds varies between 3,2 μM and 8,4 μM, the 2-deoxy-D-ribose assays were performed using DMSO solutions in the range of the DPPH IC_{50} in order to compare the antioxidant activity of the compounds at the same concentration.

All the compounds protect the 2-deoxy-D-ribose from degradation by hydroxyl radical at a concentration of 5 μM (Table 1, method A). The higher protection ($57 \leq$ % protection ≤ 62) was observed for derivatives **6e**, **6i**, **6k**, **6l** and **6n** that showed superior activity than the reference compound, trolox (53% protection), the parent aldehydes **5a**-**e** ($37 \leq$ % protection $\leq$ 51) and the imidazoles **4a**-**d** ($46 \leq$ % protection ≤ 54). The lower protection of 2-deoxy-D-ribose degradation was observed for derivatives **6f, 6g** and **6m** with percentages of protection of 49, 38 and 47%, respectively. It should be stressed that compounds **6d**, **6h** and **6l** having R^1 = 2,4,6-$(HO)_3C_6H_2$, **6m** (R^1=4-HO-3-$MeOC_6H_3$) aldehydes **5d,e** and imidazoles **4a**-**d** showed a low DPPH radical scavenging activity, however in the 2-deoxy-D-ribose assay these compounds present enhanced activity.

In the absence of EDTA (Table 1, method B) all the compounds protect 2-deoxy-D-ribose from degradation by the hydroxyl radical. The parent imidazoles **4a**-**d** show considerable protection of deoxyribose degradation by complexation with iron, that depends on the substituent R. The higher protection (57%) was observed for compound **4b** (R=4-HOC_6H_4). The parent aldehydes **5a**-**e** show similar ability to coordinate with iron having a percentage of protection of deoxyribose degradation between 30-38%. The conjugates **6a**-**p**, resulting from

the combination of imidazoles **4a-d** with aldehydes **5a-e**, show remarkable changes in the capacity to coordinate iron. The protection seems to depend mainly on the phenolic subunit. Best protection was obtained from compounds **6b**, **6f** and **6j** that have R^1 = 3,4,5-$(HO)_3C_6H_2$. High protection was also observed for compounds **6a** and **6e** having R^1 = 3,4-$(HO)_2C_6H_3$ and for compounds **6c**, **6g**, **6k** and **6p** (R^1 = 2,3,4-$(HO)_3C_6H_2$). Compound **6m** (R^1 = 4-HO-3-$MeOC_6H_3$) and compounds **6d**, **6h** and **6l**, with R^1 = 2,4,6-$(HO)_3C_6H_2$ show low protection of 2-deoxy-D-ribose from degradation by the hydroxyl radical. The parent aldehyde **5d** (R^1 = 2,4,6-$(HO)_3C_6H_2$) show higher ability to chelate iron (% protection of deoxyribose degradation = 36%) than that of the corresponding conjugates **6d**, **6h**, **6l** (16 % ≤ % protection of deoxyribose degradation ≤ 18 %). In the literature there are some examples of complexes formed between metal ions and imidazole analogues of structure **6**[39] that supported our prediction on the importance of the imine linker for coordination. However in the new derivatives reported in this work, the group R present in N1 of the imidazole subunit is close to the imine function hindering the coordination as a result of steric interference between ligands in the complex formation.

In conclusion, a series of new phenolic imidazole derivatives were synthesized and their antiradical activity was assessed against DPPH and hydroxyl radicals. The antiradical activity of the parent imidazoles and phenolic aldehydes was also assessed. In the DPPH assay, the parent imidazoles showed a marginal ability to scavenge DPPH radical however most of the conjugates have an IC_{50} lower than that of the parent aldehydes and trolox, used as reference. These results indicate that our synthetic molecules exhibit enhanced DPPH radical scavenger activity. The radical scavenging activity against DPPH radical depends strongly on the phenolic subunit and of conjugation with the imidazole ring, supporting the relevance of the linker. In the 2-deoxy-D-ribose degradation assay most of the compounds are comparable or better hydroxyl radical scavengers than the parent imidazoles, aldehydes or the reference compound. All the compounds are also able to chelate iron ions and the chelation ability is mainly dependent on the phenolic subunit. The novel imidazole derivatives are new potent antioxidants acting as radical scavengers or/and preventing radical formation. Studies are in progress in order to further understand the antioxidant behaviour of the compounds.

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28. General procedure for preparation of compounds **6**: Compounds **2-4** were synthesized according previously reported procedures, compound **2** is described in[26], compounds **3** and **4** are described in[27] . TFA (1 equiv) was added to a mixture of compound **4** (0.40 g, 1 equiv) and aldehyde **5** (1.1 equiv) in ethanol (5 mL), at room temperature under stirring. When TLC showed the absence of the reagent, the solid was filtered and washed with ethanol and was identified as **5**. *Compound **6a***: Yield 70%; mp: 236-238 ^{o}C; IR (Nujol mull) (ν_{max}/cm^{-1}): 3299, 2229, 1586, 1519. ^{1}H NMR (DMSO-*d6*, 400 MHz): δ_H (ppm) 10.01 (s, 1H, OH), 9.49 (s, 1H, OH), 8.71 (s, 1H, N=CH), 7.78 (s, 1H, H-2), 7.44 (d, *J* = 1.8 Hz, 1H, ArH), 7.26 (dd, *J* = 8.3/1.8 Hz, 1H, ArH), 6.88 (d, *J* = 8.4 Hz, 1H, ArH), 3.59 (s, 3H, Me). ^{13}C NMR (DMSO-*d6*, 100 MHz): δ_C (ppm) 164(C_{imin}), 151, 148(C-5), 146, 138(C-2), 127, 124, 117, 116(CN), 114, 97(C-4), 31.

Anal. Calcd for $C_{12}H_{10}N_4O_2$: C, 59.50; H, 4.13; N, 23.14%. Found: C, 59.55; H, 4.34; N, 22.98%. *Compound* ***6b***: Yield 80%; mp: 242-244 ^oC; IR (Nujol mull) (ν_{max}/cm^{-1}): 3555, 3270, 2215, 1586, 1533, 1515. ^1H NMR (DMSO-*d6*, 400 MHz): δ_H (ppm) 9.39 (br. s, 2H, OH), 9.19 (br. s, 1H, OH), 8.63 (s, 1H, N=CH), 7.78 (s, 1H, H-2), 6.95 (s, 2H, ArH), 3.59 (s, 3H, Me). ^{13}C NMR (DMSO-*d6*, 100 MHz): δ_C (ppm) 165(C_{imin}), 148(C-5), 146, 139, 138(C-2), 125, 117(CN), 109, 97(C-4), 31. Anal. Calcd for $C_{12}H_{10}N_4O_3.0.9H_2O$: C, 52.65; H, 4.43; N, 20.72%. Found: C, 52.52; H, 4.30; N, 20.42%. *Compound* ***6c***: Yield 70%; mp: > 275 (dec.) ^oC; IR (Nujol mull) (ν_{max}/cm^{-1}): 3453, 3120, 2227, 1636, 1601, 1525. ^1H NMR (DMSO-*d6*, 400 MHz): δ_H (ppm) 11.25 (s, 1H, OH), 10.18 (s, 1H, OH), 8.71 (br. s, 1H, OH), 8.98 (s, 1H, N=CH), 7.79 (s, 1H, H-2), 7.14 (d, *J* = 8.7 Hz, 1H, ArH), 6.48 (d, *J* = 8.7 Hz, 1H, ArH), 3.60 (s, 3H, Me). ^{13}C NMR (DMSO-*d6*, 100 MHz): δ_C (ppm) 164(C_{imin}), 152, 150, 147(C-5), 138(C-2), 133, 123, 116(CN), 113, 109, 98(C-4), 31. Anal. Calcd for $C_{12}H_{10}N_4O_3$: C, 55.81; H, 3.87; N, 21.70%. Found: C, 55.74; H, 4.05; N, 21.58%. *Compound* ***6d***: Yield 68%; mp: > 260 (dec.) ^oC; IR (Nujol mull) (ν_{max}/cm^{-1}): 3364, 3108, 2227, 1648, 1609, 1577, 1564. ^1H NMR (DMSO-*d6*, 400 MHz): δ_H (ppm) 11.62 (s, 2H, OH), 10.51 (s, 1H, OH), 9.24 (s, 1H, N=CH), 7.76 (s, 1H, H-2), 5.87 (s, 2H, ArH), 3.56 (s, 3H, Me). ^{13}C NMR (DMSO-*d6*, 100 MHz): δ_C (ppm) 166(C_{imin}), 163, 161, 147(C-5), 138(C-2), 117(CN), 102, 98(C-4), 95, 31. Anal. Calcd for $C_{12}H_{10}N_4O_3.0.1H_2O$: C, 55.43; H, 3.93; N, 21.56%. Found: C, 55.47; H, 4.26; N, 21.18%. *Compound* ***6e***: Yield 55%; mp: 285-287 ^oC; IR (Nujol mull) (ν_{max}/cm^{-1}): 3433, 3127, 2231, 1611, 1591, 1521. ^1H NMR (DMSO-*d6*, 400 MHz): δ_H (ppm) 9.97 (s, 1H, OH), 9.90 (s, 1H, OH), 9.47 (s, 1H, OH), 8.72 (s, 1H, N=CH), 8.01 (s, 1H, H-2), 7.30 (d, *J* = 7.0 Hz, 2H, ArH), 7.27 (d, *J* = 2.1 Hz, 1H, ArH), 7.17 (dd, *J* = 8.4/2.1 Hz, 1H, ArH), 6.88 (d, *J* = 7.0 Hz, 2H, ArH), 6.85 (d, *J* = 8.4 Hz, 1H, ArH). ^{13}C NMR (DMSO-*d6*, 100 MHz): δ_C (ppm) 165(C_{imin}), 158, 151, 148(C-5), 146, 137(C-2), 127, 126.6, 126, 124, 117(CN), 116, 114, 115.7, 98(C-4). Anal. Calcd for $C_{17}H_{12}N_4O_3.0.4H_2O$: C, 62.52; H, 3.89; N, 17.16%. Found: C, 62.39; H, 3.96; N, 16.92%. *Compound* ***6f***: Yield 81%; mp: 285-287 ^oC; IR (Nujol mull) (ν_{max}/cm^{-1}): 3309, 2238, 1605, 1586, 1519. ^1H NMR (DMSO-*d6*, 400 MHz): δ_H (ppm) δ 9.91 (s, 1H, OH), 9.37 (br s, 2H, OH), 9.16 (br s, 1H, OH), 8.64 (s, 1H, N=CH), 7.99 (s, 1H, H-2), 7.29 (d, *J* = 9.0 Hz, 2H, ArH), 6.88 (d, *J* = 9.0 Hz, 2H, ArH), 6.82 (s, 2H, ArH). ^{13}C NMR (DMSO-*d6*, 100 MHz): δ_C (ppm) 165(C_{imin}), 158, 149(C-5), 146, 139, 137(C-2), 127, 126, 125, 117(CN), 116, 109, 98(C-4). Anal. Calcd for $C_{17}H_{12}N_4O_4$: C,

60.71; H, 3.57; N, 16.67%. Found: C, 60.55; H, 3.71; N, 16.69%. *Compound* ***6g***: Yield 98%; mp: 289-291 ^{o}C; IR (Nujol mull) (ν_{max}/cm^{-1}): 3355, 3126, 2218, 1633, 1606, 1524, 1501. ^{1}H NMR (DMSO-*d6*, 400 MHz): δ_H (ppm) 11.24 (s, 1H, OH), 10,15 (s, 1H, OH), 8.99 (s, 1H, N=CH),9.97 (s, 1H, OH), 8.68 (s, 1H, OH), 8.01 (s, 1H, H-2), 7.32 (d, *J* = 8.7 Hz, 2H, ArH), 7.01 (d, *J* = 9.0 Hz, 1H, ArH), 6.89 (d, *J* = 8.7 Hz, 2H, ArH), 6.45 (d, *J* = 9.0 Hz, 1H, ArH). ^{13}C NMR (DMSO-*d6*, 100 MHz): δ_C (ppm) 165(C_{imin}), 158, 152, 150, 147(C-5), 138(C-2), 132, 127, 125, 124, 117(CN), 116, 112, 109, 99(C-4). Anal. Calcd for $C_{17}H_{12}N_4O_4.1.8H_2O$: C, 55.37; H, 4.23; N, 15.20%. Found: C, 55.10; H, 4.34; N, 15.08%. *Compound* ***6h***: Yield 86%; mp: 300 ^{o}C (dec.); IR (Nujol mull) (ν_{max}/cm^{-1}): 3368, 3302, 3141, 2227, 1652, 1609, 1582, 1568, 1522. ^{1}H NMR (DMSO-*d6*, 400 MHz): δ_H (ppm) 11.43 (s, 2H, OH), 10.48 (s, 1H, OH), 9.96 (s, 1H, OH), 9.31 (s, 1H, N=CH), 7.96 (s, 1H, H-2), 7.29 (d, *J* = 9.0 Hz, 2H, ArH), 6.88 (d, *J* = 9.0 Hz, 2H, ArH), 5.79 (s, 2H, ArH). ^{13}C NMR (DMSO-*d6*, 100 MHz): δ_C (ppm) 166(C_{imin}), 163, 161, 158, 147(C-5), 137(C-2), 127, 125, 117(CN), 116, 102, 98(C-4), 95. Anal. Calcd for $C_{17}H_{12}N_4O_4.0.6H_2O$: C, 58.82; H, 3.81; N, 16.15%. Found: C, 58.74; H, 3.91; N, 16.15%. *Compound* ***6i***: Yield 42%; mp: 253-255 ^{o}C; IR (Nujol mull) (ν_{max}/cm^{-1}): 3475, 3133, 2223, 1608, 1584. ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ_H (ppm) 9.70 (br s, 2H, OH), 8.73 (s, 1H, N=CH), 8.05 (s, 1H, H-2), 7.44 (d, *J* = 6.9 Hz, 2H, ArH), 7.26 (d, *J* = 1.8 Hz, 1H, ArH), 7.17 (dd, *J* = 8.1/1.8 Hz, 1H, ArH), 7.08 (d, *J* = 6.9 Hz, 2H, ArH), 6.84 (d, *J* = 8.1 Hz, 1H, ArH), 3.81 (s, 3H, Me). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ_C (ppm) 165(C_{imin}), 159, 151, 149(C-5), 146, 138(C-2), 127, 126.7, 126.5, 124, 116(CN), 115.7, 115, 114, 98(C-4), 56. Anal. Calcd for $C_{18}H_{14}N_4O_3$: C, 64.67; H, 4.19; N, 16.77%. Found: C, 64.52; H, 4.18; N, 16.90%. *Compound* ***6j***: Yield 91%; mp: 265-267 ^{o}C; IR (Nujol mull) (ν_{max}/cm^{-1}): 3306, 2238, 1606, 1584, 1538, 1519. ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ_H (ppm) 9.00 (br s, 3H, OH), 8.66 (s, 1H, N=CH), 8.04 (s, 1H, H-2), 7.43 (d, *J* = 8.7 Hz, 2H, ArH), 7.08 (d, *J* = 9.0 Hz, 2H, ArH), 6.82 (s, 2H, ArH), 3.81 (s, 3H, Me). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ_C (ppm) 166(C_{imin}), 159, 149(C-5), 146, 139, 137(C-2), 127, 126.7, 125, 116(CN), 114, 109, 98(C-4), 56. Anal. Calcd for $C_{18}H_{14}N_4O_4$: C, 61.71; H, 4.00; N, 16.00%. Found: C, 61.70; H, 4.25; N, 16.15%. *Compound* ***6k***: Yield 80%; mp: > 232 ^{o}C (dec.); IR (Nujol mull) (ν_{max}/cm^{-1}): 3310, 3125, 2112, 2221, 1633, 1602, 1585, 1531, 1519. ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ_H (ppm) 10.80 (br s, 3H, OH), 8.99 (s, 1H, N=CH), 8.04 (s, 1H, H-2), 7.46 (d, *J* = 8.7 Hz, 2H, ArH), 7.09 (d, *J* = 9.0 Hz, 2H,

ArH), 7.02 (d, *J* = 8.7 Hz, 1H, ArH), 6.45 (d, *J* = 8.7 Hz, 1H, ArH), 3.81 (s, 3H, Me). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ_C (ppm) 165(C_{imin}), 159, 152, 150, 147(C-5), 138(C-2), 133, 128, 127, 124, 116(CN), 115, 112, 109, 99(C-4), 56. Anal. Calcd for $C_{18}H_{14}N_4O_4$: C, 61.71; H, 4.00; N, 16.00%. Found: C, 61.51; H, 4.11; N, 15.97%. *Compound* ***6l***: Yield 98%; mp: 269-270 ^oC; IR (Nujol mull) (ν_{max}/cm^{-1}): 3331, 3125, 3112, 2225, 1633, 1602, 1585, 1531, 1519. ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ_H (ppm) 11.42 (s, 2H, OH), 10.49 (s, 1H, OH), 9.31 (s, 1H, N=CH), 7.99 (s, 1H, H-2), 7.44 (d, *J* = 9.0 Hz, 2H, ArH), 7.09 (d, *J* = 9.0 Hz, 2H, ArH), 5.79 (s, 2H, ArH), 3.81 (s, 3H, Me). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ_C (ppm) 166(C_{imin}), 163, 161, 160, 147(C-5), 137(C-2) 127, 126, 116(CN), 115, 102, 98(C-4), 95, 56. Anal. Calcd for $C_{18}H_{14}N_4O_4.H_2O$: C, 58.69; H, 4.35; N, 15.21%. Found: C, 58.63; H, 4.41; N, 15.06%. *Compound* ***6m***: Yield 98%; mp: 234-236 ^oC; IR (Nujol mull) (ν_{max}/cm^{-1}): 3491, 3120, 2219, 1614, 1586, 1514. ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ_H (ppm) 10.22 (s, 1H, OH), 8.80 (s, 1H, N=CH), 8.08 (s, 1H, H-2), 7.47 (d, *J* = 9.0 Hz, 2H, ArH), 7.40 (d, *J* = 1.8 Hz, 1H, ArH), 7.34 (dd, *J* = 8.3/2.1 Hz, 1H, ArH), 7.08 (d, *J* = 9.0 Hz, 2H, ArH), 6.89 (d, *J* = 8.3 Hz, 1H, ArH), 3.80 (s, 3H, MeO), 3.78 (s, 3H, MeO),. ^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ_C (ppm) 165(C_{imin}), 159, 152, 149(C-5), 148, 137(C-2), 127, 126.6, 126.5, 125, 116(CN), 115.7, 114, 111, 98(C-4), 56, 55.6. Anal. Calcd for $C_{19}H_{16}N_4O_3$: C, 65.52; H, 4.60; N, 16.09%. Found: C, 65.54; H, 4.52; N, 16.17%. *Compound* **6n**: Yield 80%; mp: 271-273 ^oC; IR (Nujol mull) (ν_{max}/cm^{-1}): 3549, 3132, 2222, 1605, 1586, 1515, 1529, 1519. ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ_H (ppm) 9.48 (br s, 2H, OH), 8.76 (s, 1H, N=CH), 8.12 (s, 1H, H-2), 7.60 (dd, *J* = 8.0/4.8 Hz, 2H, ArH), 7.41 (t, *J* = 9.0 Hz, 2H, ArH), 7.28 (d, *J* = 2.1 Hz, 1H, ArH), 7.19 (dd, *J* = 8.3/1.8 Hz, 2H, ArH), 6.86 (d, *J* = 8.3 Hz, 1H, ArH). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ_C (ppm) 165(C_{imin}), 162, 151, 149(C-5), 146, 137(C-2), 131, 128, 127, 125, 117(CN), 116, 115.7, 114, 98(C-4). Anal. Calcd for $C_{17}H_{11}N_4O_2F.0.3H_2O$: C, 62.31; H, 3.54; N, 17.10%. Found: C, 62.34; H, 3.41; N, 17.17%. *Compound* ***6o***: Yield 93%; mp: 260-262 ^oC; IR (Nujol mull) (ν_{max}/cm^{-1}): 3525, 3225, 2224, 1605, 1582, 1512. ^{1}H NMR (DMSO-*d*$_6$, 400 MHz): δ_H (ppm) 9.38 (br s, 2H, OH), 9.21 (s, 1H, OH), 8.68 (s, 1H, N=CH), 8.11 (s, 1H, H-2), 7.60 (dd, *J* = 9.0/4.8 Hz, 2H, ArH), 7.41 (t, *J* = 9.0 Hz, 2H, ArH), 6.83 (s, 2H, ArH). ^{13}C NMR (DMSO-*d*$_6$, 100 MHz): δ_C (ppm) 166(C_{imin}), 162, 149(C-5), 146, 140, 137(C-2), 131, 128, 125, 117(CN), 116, 108.8, 98(C-4). Anal. Calcd for $C_{17}H_{11}N_4O_3F.1.7H_2O$: C, 55.34; H, 3.91; N, 15.19%. Found: C, 55.35; H, 4.39; N,

15.12%. *Compound* ***6p***: Yield 97%; mp: 266-268 ^{o}C; IR (Nujol mull) (ν_{max}/cm^{-1}): 3377, 3145, 2219, 1633, 1605, 1583, 1528. ^{1}H NMR (DMSO-d_6, 400 MHz): δ_H (ppm) 11.04 (s, 1H, OH), 10.19 (s, 1H, OH), 9.02 (s, 1H, N=CH), 8.68 (s, 1H, OH), 8.11 (s, 1H, H-2), 7.64 (dd, *J* = 9.0/4.8 Hz, 2H, ArH), 7.43 (t, *J* = 9.0 Hz, 2H, ArH), 7.04 (d, *J* = 9.0 Hz, 1H, ArH), 6.46 (d, *J* = 9.0 Hz, 1H, ArH). ^{13}C NMR (DMSO-d_6, 100 MHz): δ_C (ppm) 165(C_{imin}), 162, 153, 150, 147(C-5), 138(C-2), 133, 130, 128, 124, 117, 116(CN), 113, 109, 99(C-4). Anal. Calcd for $C_{17}H_{11}N_4O_3F.0.05H_2O$: C, 60.19; H, 3.27; N, 16.52%. Found: C, 60.19; H, 3.54; N, 16.72%.

29. (a) Silva J. P., Areias F. M., Proença M. F., Coutinho O. P. Life Sci 2006, 78, 1256.; (b) *General procedure for evaluation of DPPH radical activity*: A volume of 20 µL of different concentrations (1 µM – 100 µM) of the compound in ethanol was placed in a 96-well plate and combined with 180 µL of a 0.002% (w/v) ethanolic DPPH solution. The absorbance was read at 517 nm after 60 minutes, in a Spectra Max 340PC microplate reader, versus a control containing ethanol instead of the compound in study. All measurements were performed in triplicate and mean were centered. The inhibition of discoloration was expressed as a percentage, towards the control, and the IC_{50} were then obtained from the inhibition curve.

30. (a) Halliwell, B.; Aeschbach, R.; Loliger, J.; Aruoma, O. I. Food Chem. Toxicol. 1995, 33, 601; (b) Method A: A reaction mixture (1 mL) was prepared by adding 780 µL of KH_2PO_4-KOH buffer 10 mM, pH 7.4, 100 µL of deoxyribose 2.8 mM (prepared in buffer solution), 50 µL of $FeCl_3$ 20 µM (prepared in 2 mM Na_2EDTA), 50 µL of H_2O_2 1.42 mM (prepared in buffer solution), 10 µL of ascorbic acid 50 µM (prepared in buffer solution), and 10 µL of a dimethylsulfoxide solution of the tested compound 5 µM (a concentration approximate to that registered as IC_{50} in DPPH method). After 1 h incubation at 37^{0}C, the reaction was stopped by adding 1 mL of 1% thiobarbituric acid in 50 mM NaOH and 1 mL of 2.8% trichloroacetic acid. The reaction mixture was then heated at 100 ^{0}C for 15 min. After cooling, absorbance values were determined at 535 nm in a microplate reader (SpectraMax 340PC). Assays were performed in triplicate. Reaction mixtures lacking the test compound served as positive control (100% MDA). The blank contained the full reaction mixture except 2-deoxy-D-ribose (negative

control); (c) Method B: As described in method A, but the $FeCl_3$ 20 μM solution was prepared in the buffer solution.

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39. a) Kuzniarska-Biernacka, I.; Carvalho, M. A.; Rasmussen, S. B.; Bañares, M. A.; Biernacki, K.; Magalhães, A. L.; Rolo, A.; Fonseca, A. M.; Neves, I. C. Eur. J. Inorg Chem., 2013, 5408. b) Kuźniarska-Biernacka, I.; Rodrigues, O.; Carvalho, M. A.; Neves, I. C.; Fonseca, A. M. Appl. Organometal. Chem. 2012, 26, 44.

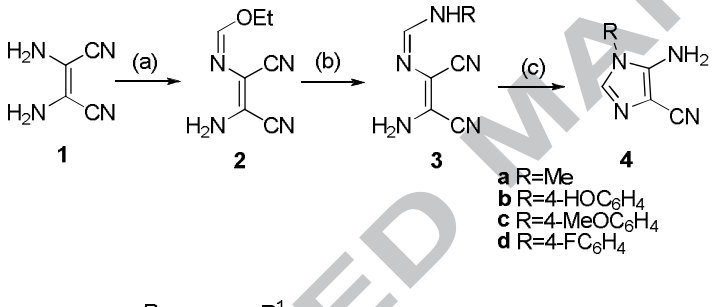
Captions to Scheme 1 and Figure 1

Scheme 1. Synthetic approach to phenol-imidazole conjugates.

Figure 1. Percentage of remaining DPPH in the reactions between compounds **6** or trolox with DPPH radical during 60 minutes, using a 10 μM solution of compounds **6e-g** and a 100 μM solution of compound **6h.**

Scheme 1 and Figure 1:

Scheme 1.



6	R	R^1
a	Me	3,4-$(HO)_2C_6H_3$
b	Me	3,4,5-$(HO)_3C_6H_2$
c	Me	2,3,4-$(HO)_3C_6H_2$
d	Me	2,4,6-$(HO)_3C_6H_2$
e	4-HOC_6H_4	3,4-$(HO)_2C_6H_3$
f	4-HOC_6H_4	3,4,5-$(HO)_3C_6H_2$
g	4-HOC_6H_4	2,3,4-$(HO)_3C_6H_2$
h	4-HOC_6H_4	2,4,6-$(HO)_3C_6H_2$
i	4-$MeOC_6H_4$	3,4-$(HO)_2C_6H_3$
j	4-$MeOC_6H_4$	3,4,5-$(HO)_3C_6H_2$
k	4-$MeOC_6H_4$	2,3,4-$(HO)_3C_6H_2$
l	4-$MeOC_6H_4$	2,4,6-$(HO)_3C_6H_2$
m	4-$MeOC_6H_4$	4-HO-3-$MeOC_6H_3$
n	4-FC_6H_4	3,4-$(HO)_2C_6H_3$
o	4-FC_6H_4	3,4,5-$(HO)_3C_6H_2$
p	4-FC_6H_4	2,3,4-$(HO)_3C_6H_2$

Reagents and conditions: (a) TEOF (1equiv.), dioxane, reflux, 30 min.;(b) RNH_2 (1 equiv.), EtOH, rt; (c) **3** (3 g), 15 mL KOH (aq., 1 M), rt; (d) **4** (0.3 g), **5** (R^1CHO; 1.1 equiv.), TFA (2 equiv.), EtOH, rt.

Figure 1.

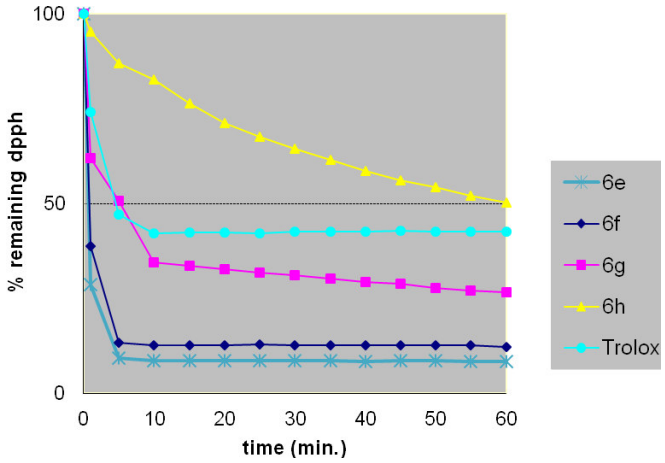


Table 1. Radical scavenging activity of imidazoles **4**, aldehydes **5** and phenol-imidazole conjugates **6** expressed as IC_{50}, in the DPPH assay, or expressed as percentage of protection of 2-deoxy-D-ribose degradation, in the 2-deoxy-D-ribose degradation assay.

Compound	R	R^1	IC_{50} **µM** (DPPH)	% protection Method A[b)]	% protection Method B[c)]
6a	CH_3	3,4-$(HO)_2C_6H_3$	3,8 ± 0,2	52 ± 3	27 ± 2
6b		3,4,5-$(HO)_3C_6H_2$	4,7 ± 0,4	54 ± 5	44 ± 7
6c		2,3,4-$(HO)_3C_6H_2$	7,0 ± 0,3	54 ± 7	37 ± 5
6d		2,4,6-$(HO)_3C_6H_2$	49,9% ± 1,5[a)]	54 ± 8	18 ± 5
6e	4-HOC_6H_4	3,4-$(HO)_2C_6H_3$	3,2 ± 0,6	60 ± 5	38 ± 1
6f		3,4,5-$(HO)_3C_6H_2$	4,8 ± 0,1	49 ± 4	51 ± 2
6g		2,3,4-$(HO)_3C_6H_2$	7,2 ± 0,2	38 ± 4	28 ± 7
6h		2,4,6-$(HO)_3C_6H_2$	45,9% ± 0,8[a)]	55 ± 9	17 ± 7
6i	4-$MeOC_6H_4$	3,4-$(HO)_2C_6H_3$	4,1 ± 0,1	61 ± 4	17 ± 8
6j		3,4,5-$(HO)_3C_6H_2$	4,0 ± 0,4	51 ± 2	42 ± 3
6k		2,3,4-$(HO)_3C_6H_2$	7,3 ± 0,2	57 ± 6	25 ± 7
6l		2,4,6-$(HO)_3C_6H_2$	48,3% ± 3,4[a)]	62 ± 2	16 ± 2
6m		4-HO-3-$MeOC_6H_3$	88,6% ± 2[a)]	47 ± 1	12 ± 2
6n	4-FC_6H_4	3,4-$(HO)_2C_6H_3$	4,4 ± 0,1	58 ± 2	11 ± 2
6o		3,4,5-$(HO)_3C_6H_2$	5,8 ± 0,7	54 ± 6	30 ± 5
6p		2,3,4-$(HO)_3C_6H_2$	8,4 ± 0,7	53 ± 3	34 ± 7
5a	----	3,4-$(HO)_2C_6H_3$	5,4 ± 0,1	51 ±7	35 ± 3
5b	----	3,4,5-$(HO)_3C_6H_2$	6,0 ± 0,4	50 ± 6	38 ± 1
5c	----	2,3,4-$(HO)_3C_6H_2$	11,6 ±0,2	46 ± 6	34 ± 1
5d	----	2,4,6-$(HO)_3C_6H_2$	91,2 ± 2,0[a)]	42 ± 7	36 ± 8
5e	----	4-HO-3-$MeOC_6H_3$	97,6 ± 0,3[a)]	37 ± 7	30 ± 7
4a	CH_3		94,2 ± 1,8[a)]	54 ± 6	23 ± 5
4b	4-HOC_6H_4		94,8 ± 1,5[a)]	47 ± 4	57 ± 1
4c	4-$MeOC_6H_4$		94,2 ± 3,4[a)]	46 ± 8	29 ± 8
4d	4-FC_6H_4		94,1 ± 1,3[a)]	50 ± 6	19 ± 1
Trolox			9,5 ± 0,4	53 ± 2	31 ± 8

Values are expressed as mean ± standard deviation, where $2 \leq n \leq 9$.

[a)] % of remaining DPPH using a 100 µM solution of antioxidant.

[b)] 2-deoxy-D-ribose degradation assay.[30b]

[c)] 2-deoxy-D-ribose degradation assay.[30c]

Graphical abstract