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The antioxidant effect of imine resveratrol analogues

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ABSTRACT

Twenty five Imine resveratrol analogues (IRAs) were synthesized, replacing the C=C bond in resveratrol with C=N bond, as well as substitution modifications on aromatic rings. Radical scavenging activities against DPPH, along with singlet oxygen quenching capacities were evaluated, and further confirmed using density functional theory calculations (DFT). It was found that IRAs bearing *ortho*-OH on B ring have better radical scavenging activities against DPPH than resveratrol, these compounds were also found to be effective $^1O_2$ quenchers. Theoretical studies on the reaction mechanism of these compounds with $^1O_2$ suggest that the 1,3-addition to a double bond with a –OH group with the formation of allylic hydroperoxide is the most probable reaction route.

Resveratrol, a well-known stilbene phytoalexin present in grapes, peanuts, mulberries and various medicinal plants, has attracted a great deal of attention in the past decades mainly because of its simple structure and significant potential in treating ageing[1] and various human diseases, such as cancer,[2,3] neurodegenerative diseases,[4] cardiovascular diseases[5] and stroke.[1] Its multiple activities may be traced back to its antioxidant activity, mainly on eliminating reactive oxygen species (ROS, including hydroxyl radical ·OH, superoxide anion $O_2^{\cdot-}$ and singlet oxygen $^1O_2$). Excessive ROS are considered to be important causative agents to induce the peroxidation of lipids and oxidative damage of DNA and proteins in human body[6] which result in ageing[7] and various disease above mentioned.[8,9] Therefore, the past few years has witnessed many researches focus on the synthesis of new resveratrol analogues and derivatives aiming to find more potent antioxidants.[10–12]

Considerable previous researchers found that simple modification on resveratrol such as introduction of electron-rich group,[13,14] incorporated hybrid moiety[15] and elongated the conjugated links[16] could significantly enhance its radical scavenging activity. In the present study, the concept of resveratrol analogue was expanded by replacing one carbon atom in the C=C bond by one nitrogen atom, 25 so-called imine resveratrol analogues (IRAs) were synthesized with different substitutions (majorly hydroxyl groups) on both aromatic rings. Some semblable structures have been studied in sort of Schiff base. Liu group reported the protective effects of hydroxyl-substituted Schiff bases against radical-induced oxidation of DNA,[17] hemolysis of human erythrocytes,[18] and peroxidation of triolein in micelles.[19] Zhou and co-workers studied the scavenging activity against galvinoxyl radical and antiprolifeactive activity.[20] Herein, radical scavenging activities against DPPH, along with singlet oxygen quenching capacities of these IRAs were investigated, while the mechanism was further deduced using DFT calculations.

The IRAs were prepared as described by Tanaka and Shiraishi with some modification (Scheme 1)[21]. Synthetic details, characterization (^{1}H NMR, ^{13}C NMR and ESI-MS) and experimental details of biological evaluation of all IRAs, as well as subsequent DFT calculation details, are available in the Supplementary data.

DPPH is a relative stable azo radical widely used in antioxidant activity evaluation. In this study, 25 IRAs' scavenging activities against DPPH were performed following our previously reported cases.[22,23] The results presented in Table 1 show that compounds **1–7** have much better scavenging activities compared with resveratrol, while compounds **8–14** have lower radical scavenging activities than resveratrol and others have negligible effect on DPPH-scavenging. Compounds **1–14** which show more or less antioxidant activities all have a OH group on B ring, therefore, it is concluded that OH group on B ring is crucial for these compounds' radical scavenging activity. Among them, compounds **8–14** have much lower scavenging activities shows that *meta*- and *para*-OH groups on B ring contribute relatively less to the scavenging activity. Compounds **1–7** with a same structure moiety scored best, which indicates that *ortho*-OH group on B ring is critical in determination of scavenging activities. The most conceivable reason for this is that the lone electron pair of the N atom overlap with the phenolic radical at *ortho*- position and in this way provide supplementation of an electron to the phenolic radical.[19] On the other hand, only attachment of OH group on A ring cannot make the

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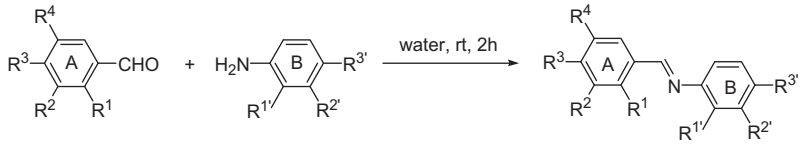


Compounds	R^1	R^2	R^3	R^4	$R^{1'}$	$R^{2'}$	$R^{3'}$
1	H	H	H	H	OH	H	H
2	OH	H	H	H	OH	H	H
3	H	OH	H	H	OH	H	H
4	H	H	OH	H	OH	H	H
5	H	OH	OH	H	OH	H	H
6	H	OCH_3	H	H	OH	H	H
7	H	H	OCH_3	H	OH	H	H
8	OH	H	H	H	H	OH	H
9	H	H	OH	H	H	OH	H
10	H	H	H	H	H	H	OH
11	OH	H	H	H	H	H	OH
12	H	H	OH	H	H	H	OH
13	H	OH	H	OH	H	H	OH
14	H	OCH_3	H	OCH_3	H	H	OH
15	OH	H	H	H	H	H	H
16	H	OH	H	H	H	H	H
17	H	OH	H	H	H	H	CH_3
18	H	H	OH	H	H	H	H
19	H	H	OH	H	H	H	OCH_3
20	H	H	OH	H	H	CH_3	H
21	H	H	OH	H	H	H	CH_3
22	H	H	OCH_3	H	H	H	OCH_3
23	H	OCH_3	H	H	H	H	CH_3
24	H	OCH_3	H	H	CH_3	H	H
25	H	H	H	H	H	H	H

Scheme 1. Synthetic scheme of imine resveratrol analogues (IRAs) and chemical structures of compounds investigated.

Table 1
DPPH scavenging activities of imine resveratrol analogues (IRAs)

Compounds	IC_{50} (μM) [a]	Compounds	IC_{50} (μM) [a]
1	23.9 ± 0.5	**10**	142.5 ± 1.9
2	27.4 ± 0.9	**11**	561.3 ± 4.7
3	23.1 ± 0.7	**12**	94.3 ± 2.0
4	24.3 ± 1.0	**13**	163.6 ± 2.7
5	12.1 ± 0.3	**14**	413.4 ± 4.6
6	24.9 ± 0.8	Other	>1000
7	28.1 ± 0.9	Resveratrol [b]	71.2 ± 2.3
8	468.2 ± 5.8	Vitamin C [b]	19.3 ± 0.6
9	362.1 ± 3.5		

[a] IC_{50} values were expressed as means ± standard deviation for three determinations.
[b] Resveratrol and vitamin C were used as positive control.

compounds radical scavenger, but it can affect the scavenging ability of compounds bearing OH group on B ring. IC_{50} values of compounds **2–4** indicate that *meta*- or *para*-OH groups on A ring have more positive effect than *ortho*-OH, while methoxy group is negative on corresponding OH-substituted compounds on scavenging activity. Notably, compound **5** bearing *ortho*-OH group on B ring and the *o*-diphenolic group on A ring is the most effective one, which may due to the stabilization effect of the intramolecular hydrogen bond on resulting *o*-hydroxyphenoxyl radical.[24,25] In conclusion, these results reveal that the higher radical scavenging activity of compound **5** is not only related to the the *ortho*-OH group on B ring, but also the *o*-diphenolic group which can stabilize the formed radical.

Based on these encouraging phenomena, further investigation on IRAs' effect on singlet oxygen was also carried out. Singlet oxygen $^1O_2$, a reactive form of molecular oxygen usually generated in the photosensitized oxidations process, can cause severe damage

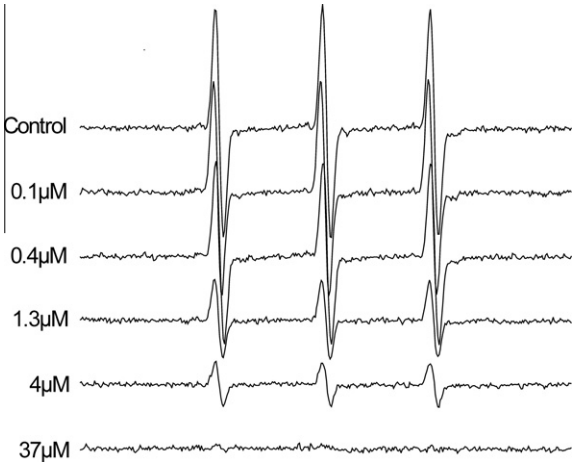
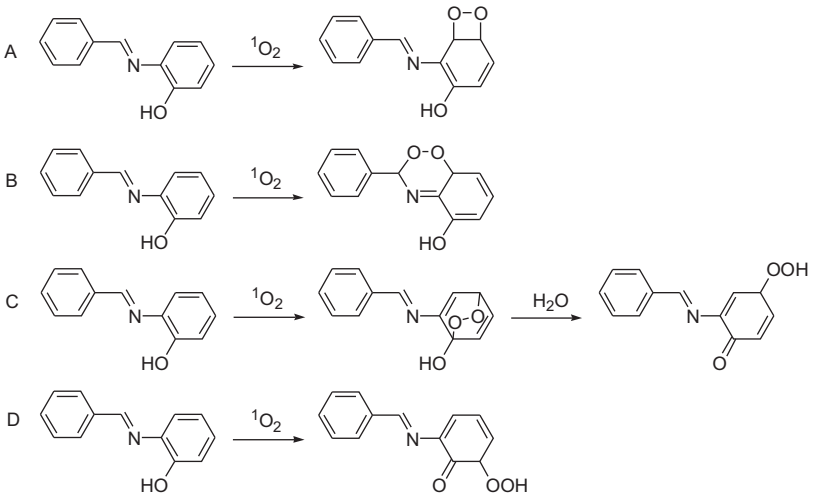


Figure 1. Quenching effect of compound **4** on TEMP-$^1O_2$ adduct formation.

Table 2
Quenching capacities of imine resveratrol analogues on singlet oxygen $^1O_2$

Compounds	IC_{50}(μM)[a]
1	3.47 ± 0.13
2	2.92 ± 0.12
3	2.18 ± 0.10
4	0.99 ± 0.06
5	2.26 ± 0.15
6	1.42 ± 0.04
7	1.30 ± 0.06
Resveratrol[b]	16.94 ± 0.73

[a] IC_{50} values were expressed as means ± standard deviation for three determinations.
[b] Resveratrol was used as positive control.



Scheme 2. Four possible reaction routes between compound **1** and $^1O_2$.

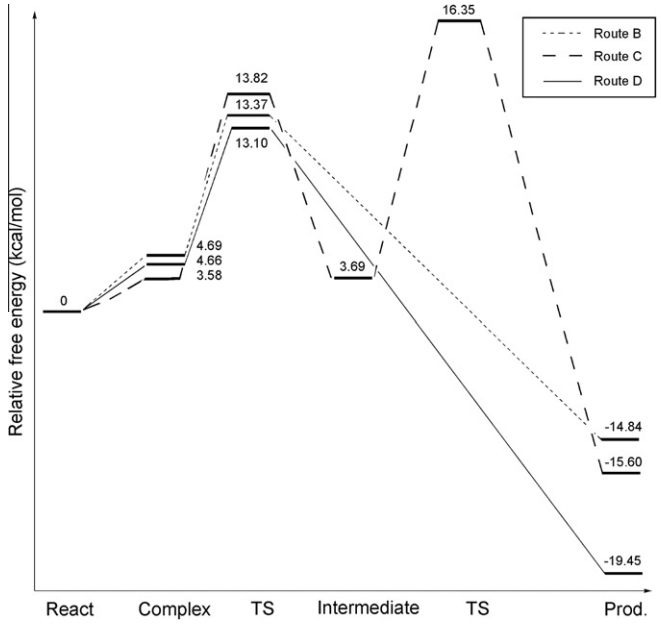


Figure 2. Reaction profile for the three most exergonic reaction routes between compound **1** and $^1O_2$ using DFT at the B3LYP/6–31 + G(d) level.

to a large number of biological targets including lipids, [26] DNA,[27] proteins,[26,28] and other important biological molecules.[29] Our group has previously found that resveratrol and its oligomers were selective $^1O_2$ quenchers but not effective ·OH and $O_2^{\cdot-}$ scavengers.[23,30,31] Herein, EPR spin-trapping technique was applied to determine the quenching activities of selected IRAs as previously described.[31] Compounds **1–7** which show better DPPH scavenging activities were chosen as the objects of study. The EPR spectra in Figure 1 reveals that compound **4**, as an example, inhibited the formation of TEMP-$^1O_2$ adduct in a dose-dependent manner. Only 1.3 μM compound **4** can obviously inhibit the generation of $^1O_2$, and the addition of 37 μM caused complete inhibition. The results presented in Table 2 demonstrate that all these seven compounds are effective $^1O_2$ quenchers, which have much better quenching activities compared with resveratrol. The activity order is like **4** > **7** > **6** > **3** > **5** > **2** > **1** > resveratrol. The introduction of OH group on A ring and methoxy group on *meta*- or *para*-position all increased the quenching activities. Compound **4** have the minimum IC $_{50}$ value, which is only 0.99 μM, more than 15-fold higher than that of resveratrol, and even better than curcumin (a well-known $^1O_2$ quencher, IC_{50} = 2.75 μM).[32]

Furthermore, compound **1** was representatively studied by DFT calculations, aiming to give deeper insight into the mechanism of these compounds' effect on $^1O_2$. According to some well-accepted results,[31,33–35] four possible oxygen-addition reaction types were proposed and each type have similar reaction sites, so four corresponding possible reactions are take as examples shown in Scheme 2. (A 1,2-cycloaddition to an isolated double bond; B 1,4-cycloaddition to two conjugated double bonds; C 1,4-addition to phenols; D 1,3-addition to a double bond connected to a OH group). All these possible reaction products were examined to determine the thermodynamically preferred reaction routes. Ten products are thermodynamically permitted with energies ranging from −0.014 to −19.45 kcal/mol. On the basis of their degree of ΔG, the full path of three most exergonic reactions (Scheme 2. B, C, D) with energies ranging from −14.84 to −19.45 kcal/mol were further investigated. Comparing the three possible competing routes (Fig. 2), route D has the lowest activation energy, with an energy barrier of 13.10 kcal/mol relative to the reactants, and the most negative free energy with Δ*G* −19.45 kcal/mol, therefore it is predicted to be the most likely route for these compounds reacting with $^1O_2$.

In conclusion, 25 IRAs were synthesized for radical scavenging activity research. It was found that seven compounds bearing *ortho*-OH on B ring have much better radical scavenging activity against DPPH than resveratrol. These compounds are also found to be effective $^1O_2$ quenchers by using the EPR spin-trapping technique. Theoretical study of the reaction mechanism suggests that 1,3-addition to a double bond with OH group to form of an allylic hydroperoxide is the most probable reaction route. These results may provide useful information for designing new drug for treating singlet oxygen mediated diseases such as cataractogenesis,[36] pellagra, erythropoietic protoporphyria, porphyries,[37] ageing and cancer.[38]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.06.026.

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