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Antioxidant and antiproliferative activities of hydroxyl-substituted Schiff bases

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ABSTRACT

Eight hydroxyl-substituted Schiff bases with the different number and position of hydroxyl group on the two asymmetric aromatic rings (A and B rings) were prepared by the reaction between the corresponding aromatic aldehyde and aniline. Their antioxidant effects against the stable galvinoxyl radical (GO·) in ethyl acetate and methanol, and 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH)-induced DNA strand breakage, and their antiproliferative effects on human hepatoma HepG2 cells, were investigated. Structure–activity relationship analysis demonstrates that *o*-dihydroxyl groups on the aromatic A ring and 4-hydroxyl group attached to the aromatic B ring contribute critically to the antioxidant and antiproliferative activities.

Reactive oxygen species (ROS) and free radicals such as superoxide anion, hydrogen peroxide and hydroxyl radicals are considered to be implicated in degenerative processes related to aging, cancer and atherosclerosis, mainly because they can induce the oxidative damage of cell membranes, DNA, and proteins.[1] Thus, blocking the generation of ROS and free radicals by supplementation of antioxidants might have a beneficial role in preventing these free radical-related diseases.[2] The attachment of hydroxyl groups on the aromatic ring makes hydroxyl-substituted Schiff bases the effective antioxidants, and potential drugs to prevent disease related to free radical damage. Recently, Liu and co-workers have reported the protective effects of hydroxyl-substituted Schiff bases against free radical-induced peroxidation of triolein in micelles, haemolysis of human red cells, and oxidation of DNA.[3]

On the other hand, hydroxyl-substituted Schiff bases obtained from the reaction between the corresponding aromatic aldehyde and aniline, have a similar structure to *trans*-stilbene skeleton of resveratrol (3,5,4′-trihydroxy-*trans*-stilbene), a well-characterized antioxidant and cancer chemopreventive molecule found in grapes and a variety of medicinal plants.[4] Their structural differences exist only in the connection of two aromatic rings, one is carbon–nitrogen double bond, and the other is carbon–carbon double bond. Although many studies have investigated the antioxidant properties of resveratrol,[5] there have been only a few reports of antioxidant and antiproliferative effects of hydroxyl-substituted Schiff bases. On our ongoing research project on bioantioxidants, we previously found that simple structural modification of resveratrol could significantly enhance its antioxidative activity.[6] Encouraged by the aforementioned information and in an attempt to better understand the structure–activity relationship of hydroxyl-substituted Schiff bases as antioxidants and cancer chemopreventive agents, we synthesized herein eight hydroxyl-substituted Schiff bases (**1**–**8**) with the different number and position of hydroxyl group on the two asymmetric aromatic rings (A and B rings), and investigated their antioxidant effects against the stable galvinoxyl radical (GO·) in ethyl acetate and methanol, and 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH)-induced DNA strand breakage, and their antiproliferative effects on human hepatoma HepG2 cells.

Eight hydroxyl-substituted Schiff bases (**1**–**8**) were easily synthesized using the corresponding aromatic aldehyde and aniline in a small amount of water or methanol (Scheme 1).[7] The full details for synthesis, characterization (^{1}H NMR,^{13}C NMR and EI-MS) and the subsequent activity tests of compounds (**1**–**8**) have been described in the Supplementary data.

It is well-known that one of the main characteristic responsible for the antioxidant activity of a phenolic compound is its ability to scavenge free radicals. GO· is a relatively stable oxygen radical and has been widely used for evaluating antioxidant activities.[8] Consequently, a quantitative kinetic study of the scavenging reaction of **1**–**8** toward GO· at 25 °C was performed in ethyl acetate and methanol by UV–vis spectroscopy by recording the decay of the GO· visible absorbance (λ_{max} = 428 nm). If a large excess of compound **3**

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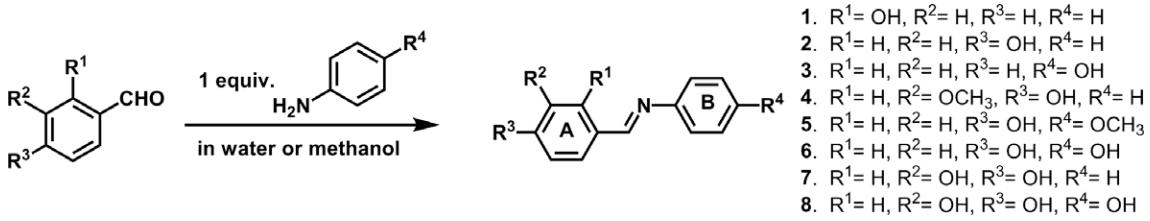

Scheme 1. Synthetic scheme for the production of hydroxyl-substituted Schiff bases (**1**–**8**) and chemical structures of compounds investigated.

was employed, the decay of GO· in ethyl acetate occurred with pseudo-first-order kinetics (Fig. 1). Plotting this pseudo-first-order rate constant (k_{obs}) versus the concentration of compound **3** gave a straight line (the inset of Fig. 1). The slope of the straight line readily gave access to the second-order rate constant (k) for the GO·-scavenging reaction by **3**. The values of k for compounds (**1**–**8**) in ethyl acetate and methanol were summarized in Table 1. It appeared that the rate constants for the reactions of GO· with these compounds in methanol were remarkably higher than that in ethyl acetate. Litwinienko and Ingold have observed previously an abnormal increase of rate constants of 2,2-diphenyl-1-picrylhydrazyl (DPPH·) radical scavenging reaction in alcoholic media which was attributed to partial ionization of the phenolic and a very fast electron transfer from phenolate anion to DPPH·.[9] These studies, together with our recent results in resveratrol and its analogues[6a] and α-pyridoin and its derivatives,[10] suggest that, in alcoholic media, the sequential proton loss electron transfer (SPLET mechanism) predominates over the direct hydrogen atom transfer (HAT mechanism) for hydroxyl-substituted Schiff bases. This kinetic results made apparent the influence of the different number and position of hydroxyl group on the two asymmetric aromatic rings, with the ranking activity order being **8** > **7** > **3** > **5** > **4** > **2** ∼ **6** > **1**. Compound **3** was more active in the GO·-scavenging reaction than compound **2**, indicating that the lower O–H bond dissociation enthalpy is obtained for 4-OH in B ring. Notably, compounds (**7** and **8**) bearing *o*-diphenolic groups exhibited remarkably higher GO·-scavenging activity than those bearing no such groups and resveratrol, a well-known antioxidant. It is well-established fact that the *o*-diphenolic group is one of the main contributors to the antioxidant activity of phenolic compounds, which is mainly due to its low oxidative potential[11] and resonance stabilization of the resulting phenoxyl radical intermediate with subsequent *o*-quinone formation.[12]

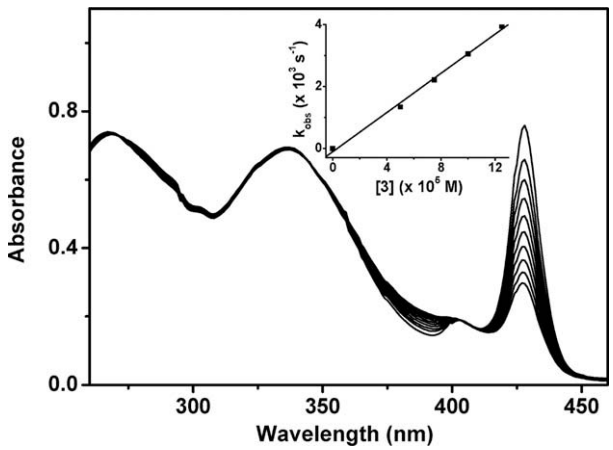

Figure 1. Spectral changes observed upon addition of compound **3** (50 μM) to an ethyl acetate solution of GO· (5 μM) at 298 K (interval: 10 min). Inset: plot of the pseudo-first-order rate constant (k_{obs}) versus the concentration of compound **3**.

Table 1
GO·-scavenging activity and antiproliferative activity against HepG2 cells of hydroxyl-substituted Schiff bases

Compounds	k[a] (M^{-1} s^{-1})		IC_{50}[c] (μM)
	Ethyl acetate	Methanol	
1	0.01 ± 0.001	0.04 ± 0.002	>300
2	0.11 ± 0.01	0.22 ± 0.02	>300
3	31.9 ± 2.8	120 ± 3	108 ± 1
4	0.64 ± 0.05	0.91 ± 0.053	>300
5	1.10 ± 0.10	2.48 ± 0.21	>300
6	0.19 ± 0.01	0.22 ± 0.02	>300
7	103 ± 3	2.19 ± 0.09 × 10^3[b]	6.8 ± 0.1
8	1.08 ± 0.07 × 10^3[b]	14.8 ± 0.09 × 10^3[b]	5.6 ± 0.1
Resveratrol	13.4 ± 1.2	42.0 ± 3.7	79.6 ± 8.1
VP-16			4.1 ± 0.2

[a] Data are expressed as the mean ± SD for three determinations.
[b] The rates were measured by the second-order kinetics with the ratio of [**7** or **8**]/[GO·] being 1/1.
[c] Antiproliferative activity is expressed as IC_{50} values, the concentration for the compound to cause 50% inhibition of the cell viability. Data are expressed as the mean ± SD for three determinations.

In addition, the ability of compounds (**1**–**8**) to inhibit AAPH-induced oxidative damage of DNA was also assessed in vitro by measuring the conversion of supercoiled pBR322 plasmid DNA to the open circular and linear forms using agarose gel electrophoresis analysis.[13] As shown in Figure 2A, the supercoiled DNA was gradually converted to open-circular DNA (indication of a single-strand breakage) with the increase of concentration of AAPH, and that open-circular DNA was gradually converted to linear DNA (indication of a double-strand breakage) with the further increase of concentration of AAPH. The inhibition effects produced by compounds (**1**–**8**) depended on the specific compound used as exemplified in Figure 2B. On the basis of the percentage of intact supercoiled DNA, the relative activity order in inhibiting the DNA strand breakage was as follows: **8** > **4** > **7** > **3** > **5** ∼ **6** > **2** > **1** (Fig. 2C), which is similar to that obtained from kinetic measurement in GO·-scavenging reaction.

Furthermore, the antiproliferative effect of compounds (**1**–**8**) on HepG2 cells was assessed by sulforhodamine B assay,[14] and the IC_{50} values and their stand deviations are reported in Table 1. The clinically used anticancer drug, etoposide (VP-16), and resveratrol were used reference drugs. Compounds are mainly divided into three different groups according to the IC_{50} values: compounds (**7** and **8**) bearing *o*-diphenolic groups were most active, followed by resveratrol and compound **4**, while the other compounds were inactive. Especially, the antiproliferative activity of **7** and **8** were comparable to that of VP-16, a positive control. Intriguingly, compounds (**7** and **8**) with higher antioxidant activity exhibited higher antiproliferative activity, which reinforces the idea of designing antioxidant-based cancer chemoprevention agents.

Because the most of Schiff bases can be hydrolyzed back to the corresponding amines and aldehydes depending on the reaction conditions, we also determined the stability of compound **8** in

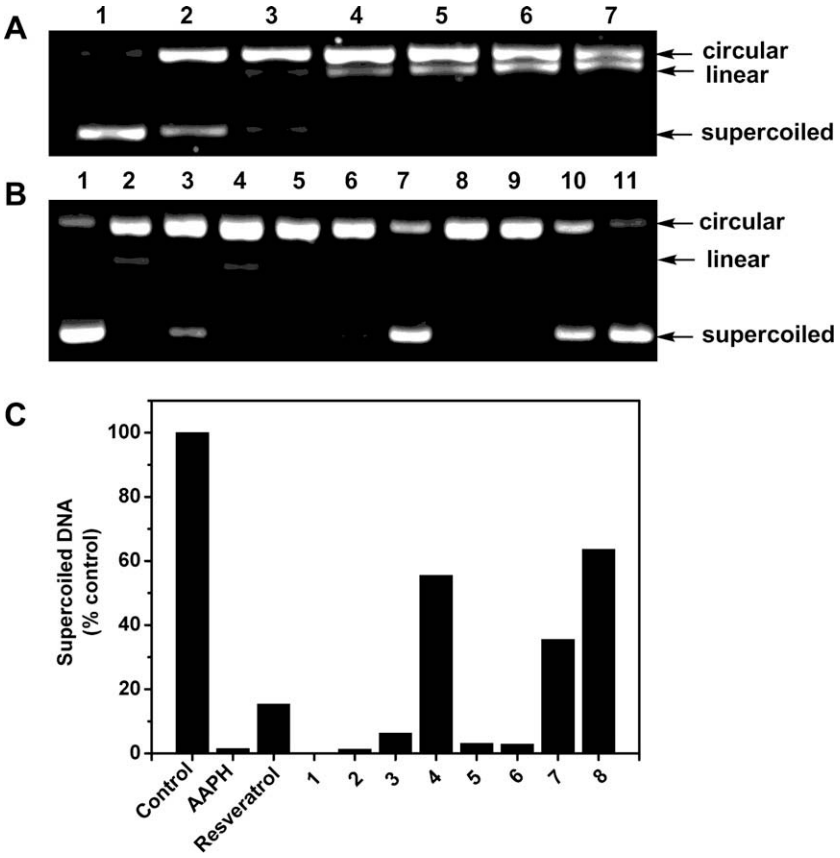

Figure 2. Agarose gel electrophoresis pattern of pBR322 DNA strand breakage induced by AAPH and inhibited by compounds (**1**–**8**). Supercoiled plasmid DNA (100 ng) was incubated with AAPH and/or compounds (**1**–**8**) in 25 μL phosphate-buffered saline (PBS, pH 7.4) at 37 °C for 60 min. (A) DNA strand breakage induced by the indicated concentration of AAPH: (lane 1) control; (lanes 2–7) 1.25, 2.5, 5, 10, 20, and 40 mM AAPH, respectively. (B) Inhibitory effects of compounds (**1**–**8**) (10 μM) against AAPH (10 mM)-induced DNA strand breakage: (lane 1) control; (lane 2) 10 mM AAPH alone; (lanes 3–11) resveratrol, **1**, **2**, **3**, **4**, **5**, **6**, **7**, and **8**, respectively. (C) Quantitative analysis of protective effects of compounds (**1**–**8**) against AAPH-induced DNA strand breakage. DNA damage is represented by the percentage of supercoiled DNA to native DNA.

phosphate-buffered saline (PBS) (pH 7.4) at 37 °C by UV–vis spectroscopy. As shown in Figure 3A, the rapid disappearance of the absorptions of **8** centered at 340 and 428 nm suggested that it undergoes degradation in PBS by hydrolysis. However, the degradation was considerably inhibited by the plasma protein, human serum albumin (HSA), and the UV–vis spectra of **8** bound to HSA had a broadening weak in the range of 400–475 nm (Fig. 3B). These results imply that HSA could stabilize compound **8** to maintain its biological activities in vivo. Similarly, curcumin, a well-known cancer preventive agent with low stability in aqueous solution, has also been confirmed to be well stabilized by the plasma proteins HSA and fibrinogen.[15]

In conclusion, eight hydroxyl-substituted Schiff bases were synthesized and bio-evaluated for their antioxidant and antiproliferative activities in pursuit of more active antioxidant and cancer chemopreventive agents. Structure–activity relationship analysis

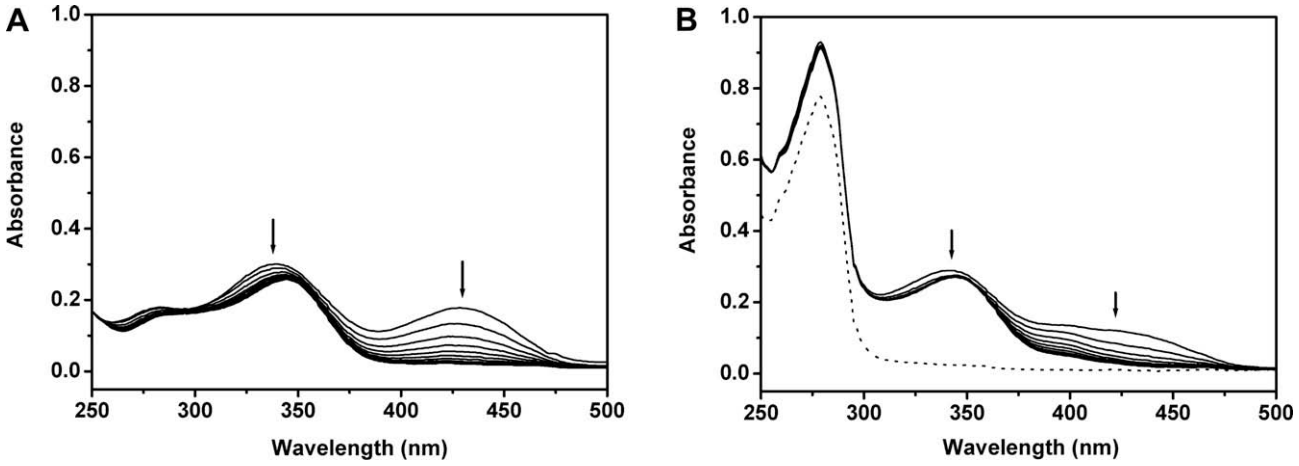

Figure 3. UV–vis absorption spectra of compound **8** (20 μM) at 37 °C in (A) pH 7.4 PBS (interval: 5 min) and (B) HSA (20 μM) (interval: 5 min). Dot line shows UV–vis spectrum of HSA, and arrows show the time-related absorbance changes.

indicates that *o*-diphenolic groups and 4-hydroxyl group attached to the aromatic B ring are vital for their antioxidant and antiproliferative activities.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.039.

References and notes

1. (a) Finkel, T. *Nat. Rev. Mol. Cell Biol.* **2005**, *6*, 971; (b) Hussain, P. S.; Hofseth, L. J.; Harris, C. C. *Nat. Rev. Cancer* **2003**, *3*, 276; (c) Finkel, T.; Holbrook, N. J. *Nature* **2000**, *408*, 239.
2. (a) Rice-Evans, C. A.; Diplock, A. T. *Free Radical Biol. Med.* **1993**, *15*, 77; (b) Surh, Y.-J. *Nat. Rev. Cancer* **2003**, *3*, 768; (c) Pan, M.-H.; Ghai, G.; Ho, C.-T. *Mol. Nutr. Food Res.* **2008**, *52*, 43.
3. (a) Liu, Z.-Q. *QSAR Comb. Sci.* **2007**, *26*, 488; (b) Tang, Y.-Z.; Liu, Z.-Q. *Cell Biochem. Funct.* **2009**, *26*, 185; (c) Zhao, F.; Liu, Z.-Q. *J. Phys. Org. Chem.* **2009**, *22*, 791.
4. (a) Saiko, P.; Szakmary, A.; Jaeger, W.; Szekere, T. *Mutat. Res.* **2008**, *658*, 68; (b) Baur, J. A.; Sinclair, D. A. *Nat. Rev. Drug Disc.* **2006**, *5*, 493.
5. (a) Ray, P. S.; Maulik, G.; Cordis, G. A.; Bertelli, A. A. E.; Bertelli, A.; Das, D. K. *Free Radical Biol. Med.* **1999**, *27*, 160; (b) Burkitt, M. J.; Duncan, J. *Arch. Biochem. Biophys.* **2000**, *381*, 253; (c) Stojanović, S.; Sprinz, H.; Brede, O. *Arch. Biochem. Biophys.* **2001**, *391*, 79; (d) Stojanović, S.; Brede, O. *Phys. Chem. Chem. Phys.* **2002**, *4*, 75; (e) Hung, L. M.; Su, M. J.; Chu, W. K.; Chiao, C. W.; Chan, W. F.; Chen, J. K. *Br. J. Pharmacol.* **2002**, *135*, 1627; (f) Amorati, R.; Lucarini, M.; Mugnaini, V.; Pedulli, G. F. *J. Org. Chem.* **2004**, *69*, 7101; (g) Murias, M.; Jäger, W.; Handler, N.; Erker, T.; Horvath, Z.; Szekeres, T.; Nohl, H.; Gille, L. *Biochem. Pharmacol.* **2005**, *69*, 903; (h) Nakanishi, I.; Shimada, T.; Ohkubo, K.; Manda, S.; Shimizu, T.; Urano, S.; Okuda, H.; Miyata, N.; Ozawa, T.; Anzai, K.; Fukuzumi, S.; Ikota, N.; Fukuhara, K. *Chem. Lett.* **2007**, *36*, 1276; (i) Fabris, S.; Momo, F.; Ravagnan, G.; Stevanato, R. *Biophys. Chem.* **2008**, *135*, 76; (j) Fukuhara, K.; Nakanishi, I.; Matsuoka, A.; Matsumura, T.; Honda, S.; Hayashi, M.; Ozawa, T.; Miyata, N.; Saito, S.; Ikota, N.; Okuda, H. *Chem. Res. Toxicol.* **2008**, *21*, 282.
6. (a) Shang, Y.-J.; Qian, Y.-P.; Liu, X.-D.; Dai, F.; Shang, X.-L.; Jia, W.-Q.; Liu, Q.; Fang, J.-G.; Zhou, B. *J. Org. Chem.* **2009**, *74*, 5025; (b) Fang, J.-G.; Zhou, B. *J. Agric. Food Chem.* **2008**, *56*, 11458; (c) Fan, G.-J.; Liu, X.-D.; Qian, Y.-P.; Shang, Y.-J.; Li, X.-Z.; Dai, F.; Fang, J.-G.; Jin, X.-L.; Zhou, B. *Bioorg. Med. Chem.* **2009**, *17*, 2360.
7. (a) Tanaka, K.; Shiraishi, R. *Green Chem.* **2000**, *2*, 272; (b) Blumenthal, T.; Dosen, M.; Gillis, R. G.; Porter, Q. N. *Aust. J. Chem.* **1993**, *46*, 895.
8. Watanabe, A.; Noguchi, N.; Fujisawa, A.; Kodama, T.; Tamura, K.; Cynshi, O.; Niki, E. *J. Am. Chem. Soc.* **2000**, *122*, 5438.
9. Litwinienko, G.; Ingold, K. U. *Acc. Chem. Res.* **2007**, *40*, 222. and references cited therein.
10. Cheng, L.-X.; Jin, X.-L.; Teng, Q.-F.; Chang, J.; Yao, X.-J.; Dai, F.; Qian, Y.-P.; Tang, J.-J.; Li, X.-Z.; Zhou, B. *Org. Biomol. Chem.* **2010**, *8*, 1058.
11. Gaspar, A.; Garrido, E. M.; Esteves, M.; Quezada, E.; Milhazes, N.; Garrido, J.; Borges, F. *Eur. J. Med. Chem.* **2009**, *44*, 2092.
12. Wright, J. S.; Johnson, E. R.; Dilabio, G. A. *J. Am. Chem. Soc.* **2001**, *123*, 1173.
13. Rahman, A.; Fazel, F.; Greensill, J.; Ainley, K.; Parish, J. H.; Hadi, S. M. *Mol. Cell. Pharmacol.* **1992**, *111*, 3.
14. Skehan, P.; Storeng, R.; Scudiero, D.; Monks, A.; McMahon, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyd, M. R. *J. Natl. Cancer Inst.* **1990**, *82*, 1107.
15. Leung, M. H. M.; Kee, T. W. *Langmuir* **2009**, *25*, 5773.