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Potential antioxidants and tyrosinase inhibitors from synthetic polyphenolic deoxybenzoins

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ABSTRACT

Deoxybenzoins (DOBs) are one-pot synthetic precursors of isoflavones with feature analogous to those beneficial polyphenols such as resveratrol (stilbene) and phloretin (dihydrochalcone). In this study, seventeen polyphenolic DOBs were synthesized and evaluated by various antioxidant assays and tyrosinase inhibitory effect in vitro. Results displayed that these DOBs are powerful antioxidants; for example, 2,3,4-trihydroxy-3′,4′-dimethoxydeoxybenzoin possesses an excellent anti-lipid peroxidation activity (IC_{50} = 0.72 ± 0.16 μM), whilst 2,4,4′,5-tetrahydroxydeoxybenzoin showed good DPPH radical scavenging activity (IC_{50} = 0.69 ± 0.04 μM), which were better than Trolox and vitamin C. Besides exhibiting a weak metal chelating effect, these DOBs were effective in scavenging $ABTS^{\cdot+}$ and superoxide anion ($O_2^{\cdot-}$) radicals. DOBs also exhibited potent mushroom tyrosinase inhibitory activity; for example 2,3,4′-trihydroxy-4-methoxydeoxybenzoin displayed stable and significant inhibitory effect on tyrosinase activity, with IC_{50} values 43.37, 43.10 and 46.10 μM at incubation intervals of 0.5, 1.5, and 2.5 h, respectively. These results suggest that, with the advantage of being readily synthesizable small molecules, DOBs can be potentially developed into clinical and industrial antioxidants.

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1. Introduction

Deoxybenzoins (DOBs) are precursors for one-pot synthesis of isoflavones[1–3] and can be recovered from the later by alkali hydrolysis.[4] These compounds closely related to angolensinoids (α-methyldeoxybenzoins), which are known to be reductive metabolites of isoflavones by intestinal microflora both in vitro and in vivo.[5–9] Structurally, DOBs can be described as the abridged dihydrochalcone (keto form) and α-hydroxystilbene (*enol* form) (Fig. 1).

Soy isoflavones and resveratrol (stilbene) are well known phytoestrogens with antioxidant, cardiovascular protection, cancer chemopreventive and other properties.[10,11] Furthermore, dihydrochalcones, such as phloretin[12,13] and synthetic 2′,5′-dihydroxydihydrochalcone,[14] and synthetic polyphenolic chalcones[15] have been demonstrated to be potential antioxidants and/or anti-inflammatory agents. Although DOBs have been reported to have various interesting pharmacological properties, they have long been neglected until recently. The 2,4,4′-trihydroxydeoxybenzoin (**2**), synthetic precursor of daidzein, has been reported as a phytoestrogen with more potent estrogenic receptor (ER) binding capability than daidzein, and was shown to be a selective ERβ agonist.[16] Another notable compound, 2,4,6-trihydroxy-4′-methoxydeoxybenzoin (**15**), was reported to be a potential agent for treating severe allergic and inflammatory disorders, as it exhibited potent inhibitory effects on tyrosine kinases SYK and BTK, IgE receptor/FcεRI-mediated mast cell degranulation and leukotriene C_4 release, and UVB-induced human keratinocyte prostaglandin E_2 release.[17] Other related derivatives of DOBs have also recently been studied for anti-bacterial activities.[18,19]

Antioxidant therapies have been increasingly recognized to be a potential strategy for preventing acute CNS injury,[20] cardiovascular diseases[21] and asthma.[22] In addition, applications of antioxidants as preservative in food industry[23] and skin-protective ingredients in cosmetics are also receiving increasing attention and interests.[24] DOBs are small molecules with great potential for development as antioxidants due to their structural analogousness to known natural products with therapeutic benefits (Fig. 1).

In this study, 17 polyphenolic DOBs were synthesized and evaluated for their antioxidant activities by various assays including anti-lipid peroxidation, metal chelating, reducing power, and free radical scavenging such as $ABTS^{\cdot+}$, DPPH and superoxide anion. In addition, selected DOBs were also evaluated for mushroom anti-tyrosinase inhibitory activity since antioxidants with whitening effect can be potentially used as cosmetic ingredients.

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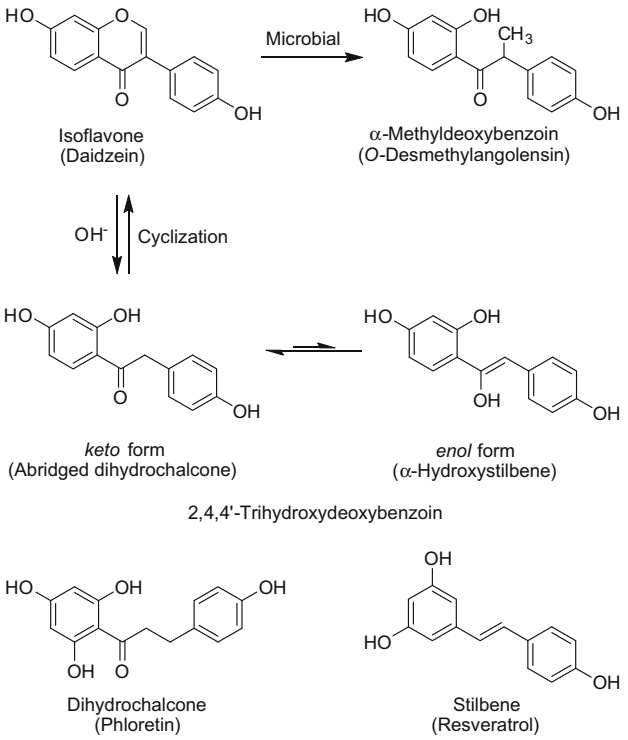


Figure 1. Analogous structures of the DOBs.

2. Results and discussion

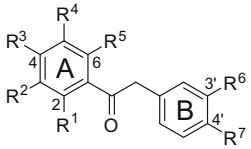
2.1. Chemistry

The synthesis of DOBs was convenient and facile by following the methods of Wähälä and Hase[1] and/or Balasubramanian et al.[2,3] as shown in Scheme 1. Seventeen including four newly synthesized (**4**–**6** and **13**) poly-oxygenated DOBs were obtained and isolated in gram quantity (yields: 43.4–92.4%) as depicted (Table 1).

2.2. Biological assays

DOBs were evaluated for antioxidant activities including anti-lipid peroxidation,[25] metal chelating,[26] reducing power,[27] and several free radical scavenging assays such as $ABTS^{\cdot+}$,[28] $DPPH^{\cdot}$,[26] and $O_2^{\cdot-}$.[29] These assays are commonly applied in the preliminary evaluation of natural product antioxidants, food additives, and cosmetic ingredients. Based on their structural analogousness to resveratrol, which is an effective tyrosinase inhibitor,[30] selected DOBs were subjected to the mushroom tyrosinase inhibitory assay.

Table 1
Substitution pattern of polyphenolic DOBs **1**–**17**



Entry	Formula	R^1	R^2	R^3	R^4	R^5	R^6	R^7
1	$C_{15}H_{14}O_4$	OH	H	OH	H	H	H	OMe
2	$C_{14}H_{12}O_4$	OH	H	OH	H	H	H	OH
3	$C_{16}H_{16}O_5$	OH	H	OH	H	H	OMe	OMe
4[a]	$C_{15}H_{14}O_4$	H	OH	OH	H	H	H	OMe
5[a]	$C_{16}H_{16}O_5$	H	OH	OH	H	H	OMe	OMe
6[a]	$C_{17}H_{18}O_4$	OH	Me	Me	OH	H	H	OMe
7	$C_{15}H_{14}O_4$	OH	H	OMe	OH	H	H	H
8	$C_{15}H_{14}O_5$	OH	H	OH	OH	H	H	OMe
9	$C_{14}H_{12}O_5$	OH	H	OH	OH	H	H	OH
10	$C_{15}H_{14}O_5$	OH	OH	OH	H	H	H	OMe
11	$C_{15}H_{12}O_5$	OH	OH	OH	H	H	H	OH
12	$C_{16}H_{16}O_6$	OH	OH	OH	H	H	OMe	OMe
13[a]	$C_{15}H_{14}O_5$	OH	OH	OMe	H	H	H	OH
14	$C_{14}H_{12}O_4$	OH	H	OH	H	OH	H	H
15	$C_{15}H_{14}O_5$	OH	H	OH	H	OH	H	OMe
16	$C_{14}H_{12}O_5$	OH	H	OH	H	OH	H	OH
17	$C_{16}H_{16}O_6$	OH	H	OH	H	OH	OMe	OMe

[a] New compounds.

2.2.1. Anti-lipid peroxidation activity of DOBs

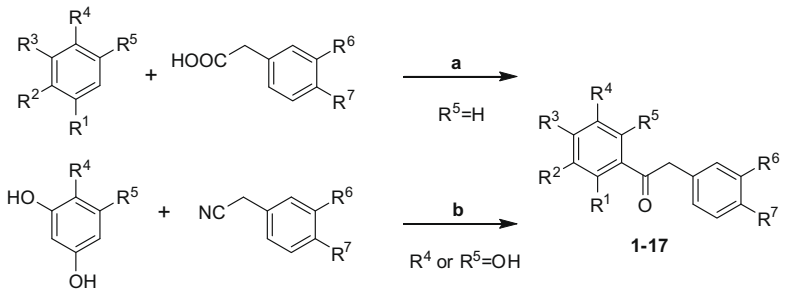
Table 2 (column 2) showed the anti-lipid peroxidation activity of various DOBs. It was found that DOB **12** possessed the most potent activity (IC_{50} = 0.72 ± 0.16 μM), which was about five times more potent than Trolox (IC_{50} = 3.68 ± 0.64 μM). This result explains that the poly-hydroxylated ring A of DOB can be an active anti-lipid peroxidation skeleton. However, the substitution of ring B favored the more lipophilic 4′-methoxylated, 3′,4′-dimethoxylated or unsubstituted phenyl, than the hydrophilic 4′-hydroxyphenyl. It is possible that the lipophilic ring B can enhance the contact of these antioxidants with lipid, and consequently resulting in an efficient termination of the chain reaction.

2.2.2. Metal ion chelating activity of DOBs

All DOBs tested showed a weak metal chelating effect (IC_{50} values > 1500 μM), with the exception of DOB **10**, of which the IC_{50} value was 1246.79 ± 5.66 μM (Table 2, column 6). This suggests that the anti-lipid peroxidation effects of these compounds were achieved via radical scavenging rather than the metal chelating property.

2.2.3. Reducing power of DOBs

Results showed that DOBs **5**, **7**–**11** and **13** possessed the greatest reducing power, especially DOB **13** (IC_{50} = 43.72 ± 2.66 μM)



Scheme 1. Synthesis of DOBs **1**–**17**. Reagents and conditions: (a) BF_3/Et_2O, 80–90 °C, 2–3 h (N_2 conditioning); (b) (1) $ZnCl_2/Et_2O$, 0 °C satd HCl, stirred 2 h; rt overnight; (2) $MeOH/H_2O$ (1:1); refluxing 3–5 h.

Table 2
IC_{50} values of DOBs in antioxidant activities

DOBs	IC_{50} (μM)					
	POV[a]	ABTS	DPPH	SOD-like	Metal chelation	Reducing power
1	7.33 ± 0.31	62.64 ± 4.19	57.40 ± 5.08	35.19 ± 1.32	>1500	>1000
2	25.53 ± 2.87	30.49 ± 3.52	25.20 ± 2.21	113.65 ± 2.58	>1500	499.55 ± 74.30
3	1.84 ± 0.59	41.94 ± 1.49	35.10 ± 1.11	89.34 ± 1.98	>1500	742.99 ± 14.20
4	6.47 ± 0.54	17.83 ± 2.02	75.93 ± 6.36	121.43 ± 3.72	>1500	>1000
5	24.93 ± 2.40	42.67 ± 1.04	2.53 ± 0.17	110.59 ± 4.79	>1500	112.95 ± 1.02
6	20.84 ± 1.68	41.68 ± 2.48	21.92 ± 1.40	39.02 ± 2.03	>1500	147.45 ± 3.29
7	6.20 ± 0.10	36.01 ± 1.90	20.27 ± 1.24	54.57 ± 3.14	>1500	81.16 ± 6.05
8	10.69 ± 0.55	39.60 ± 1.35	21.46 ± 1.17	68.18 ± 4.45	>1500	102.88 ± 10.29
9	88.38 ± 5.15	46.42 ± 1.35	0.69 ± 0.04	58.62 ± 1.77	>1500	68.54 ± 2.38
10	13.28±1.82	50.69 ± 2.26	21.64 ± 1.53	28.25 ± 0.58	1246.79 ± 5.66	120.22 ± 6.13
11	178.38 ± 10.02	12.88 ± 1.69	5.69 ± 0.69	115.46 ± 3.81	>1500	115.27 ± 3.96
12	0.72 ± 0.16	41.58 ± 1.12	4.34 ± 0.95	82.83 ± 3.62	>1500	320.95 ± 5.36
13	85.00 ± 7.52	50.51 ± 0.55	25.66 ± 1.82	96.82 ± 3.28	>1500	43.72 ± 2.66
14	13.36 ± 0.70	46.43 ± 1.48	12.25 ± 0.90	106.15 ± 6.43	>1500	275.34 ± 21.14
15	2.12 ± 0.15	61.61 ± 2.81	10.84 ± 1.57	31.61 ± 0.99	>1500	231.93 ± 9.53
16	37.42 ± 2.54	27.35 ± 2.62	22.77 ± 1.46	109.15 ± 5.08	>1500	146.77 ± 3.23
17	27.04 ± 1.32	87.24 ± 2.11	1.35 ± 0.13	80.72 ± 3.65	>1500	147.66 ± 6.25
Trolox[b]	3.68 ± 0.64	116.28 ± 0.72	9.20 ± 0.44	27.76 ± 2.36	—	240.56 ± 4.80
Ascorbic acid[c]	48.52 ± 1.42	—	73.69 ± 1.76	24.15 ± 0.85	—	131.65 ± 0.97
EDTA[d]	—	—	—	—	185.64 ± 0.34	

Value are presented as mean ± SD (n = 3).
[a] POV: anti-lipid peroxidation activity.
[b,c,d] Positive controls; 50% inhibition (IC_{50}) values are expressed as μM.

which was about three times more potent than ascorbic acid (IC_{50} = 131.65 ± 0.97 μM, Table 2 column 7). It is found that DOBs with *ortho* dihydroxyl ring A, except DOB **4**, exert an excellent ferric reducing power. It was also observed that DOBs with the same ring A displayed a better reducing activity when ring B was substituted with C-4′ OH.

2.2.4. $ABTS^{\cdot+}$ radical scavenging activity of DOBs

It is surprising to note that all DOBs **1**–**17** (IC_{50} ranging from 12.88 ± 1.69 μM to 87.24 ± 2.11 μM) exhibited a more efficient $ABTS^{\cdot+}$ radical scavenging than Trolox (IC_{50} = 116.3 ± 0.72 μM) (Table 2, column 3). DOB **11** with an IC_{50} value of 12.88 ± 1.69 μM was shown to be nearly 10 times more potent than the positive control. This result suggests that DOBs with C-4′ hydroxylated ring B such as DOBs **2**, **11**, and **16**, would exert a greater potency than those DOBs with the same substitution in ring A. It was shown that DOBs with polyphenol feature possessed promising $ABTS^{\cdot+}$ radical scavenging activity. Though $ABTS^{\cdot+}$ was a suitable assay for both hydrophilic and lipophilic antioxidants,[26] the more hydrophilic (polyhydroxylated) DOBs are likely to be better $ABTS^{\cdot+}$ radical scavengers. Since phenol is known to be an active functional group for free radical scavenging, suggesting that its property can be one of the main determinants in this activity. It is found that the existence of electron withdrawing ketyl of DOB leading to a more acidic C-4 phenol than that of Trolox. The better free radical scavenging activity of DOBs can be attributed to the capability of ion-pairing that enhanced the contact of phenolic anion with $ABTS^{\cdot+}$. Though Trolox can be dissociated into a carboxylic anion (COO^-), it was shown to be a lesser effective radical scavenger (Fig. 2).

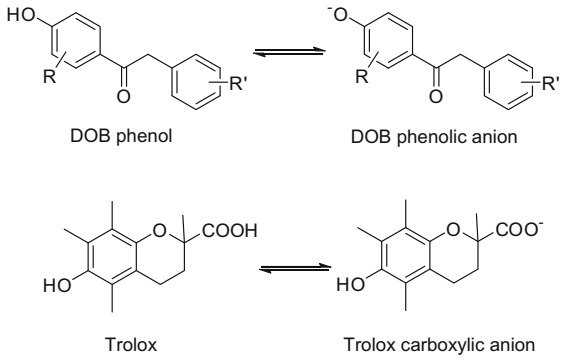


Figure 2. Dissociation of DOB and trolox.

2.2.5. DPPH radical scavenging activity of DOBs

Table 2 (column 4) showed the DPPH radical scavenging activity of DOBs. Besides DOB **4**, all other DOBs showed a more active DPPH radical scavenging than ascorbic acid (IC_{50} = 73.69 ± 1.76 μM). Generally, when the catechol, pyrogallol or phloroglucinol ring A of DOBs was substituted by a 3′,4′-dimethoxylated ring B (i.e., DOBs **5**, **12** and **17**), they would exert a greater potency in DPPH radical scavenging power. Among the different DOBs tested, DOB **9** exhibited the most potent DPPH radical scavenging activity (IC_{50} = 0.69 ± 0.04 μM), which was about 13 times more potent than Trolox (IC_{50} = 9.20 ± 0.44 μM).

2.2.6. Superoxide radical scavenging activity of DOBs

As shown in Table 2 (column 5), DOBs displayed a potent superoxide anion radical scavenging activity. Based on the IC_{50} values,

Table 3
IC_{50} values of selected DOBs in mushroom tyrosinase inhibitory activity

Treatment	IC_{50} (μM)		
	30 min	90 min	150 min
1	78.88	160.37	181.45
2	178.46	135.91	172.41
3	112.06	209.14	239.70
4	>300	167.97	177.68
8	84.35	144.19	197.36
10	>300	203.32	211.92
11	>300	>300	>500
12	228.79	131.53	139.99
13	43.37	43.10	46.10
15	71.05	213.86	230.24
16	>300	>300	261.26
Kojic acid[a]	132.83	225.14	438.35

Values are the average of three analyses (n = 3).
[a] Positive control; 50% inhibition (IC_{50}) values are expressed as μM.

DOBs **1**, **10** and **15** exhibited a comparable activity as Trolox and vitamin C. This result suggests that DOBs with a *p*-methoxylated ring B coupled with resorcinol, pyrogallol, or phloroglucinol ring A (DOBs **1**, **10**, and **15**) could be an effective superoxide anion radical scavenger. However, the anionic expulsion effect of DOBs with $O_2^{\cdot-}$ can limit the radical scavenging power, which might contribute to the contrasting results from that of the $ABTS^{\cdot+}$ assay.

2.2.7. Anti-tyrosinase activity of DOBs

Mushroom tyrosinase is the common inhibitory target for the evaluation of new whitening agents of cosmetics. Results revealed that most DOBs possessed a greater anti-tyrosinase activity than kojic acid (a commercial whitening ingredient) (Table 3). DOBs **1**, **8**, **13** and **15** showed a significant and immediate inhibition at first 30 min of incubation, of which were found to have a *meta*-dihydroxylated ring A with the exception of DOB **13**. This phenomenon is consistent with the demonstrated structural inhibition of resorcinol-containing 1,3-dihydroxyphenylpropionic acid derivatives.[31–33] In this study, the most active structure was DOB **13**, 2,3,4′-tihydroxy-4-methoxydeoxybenzoin, which showed an almost constant inhibitory effect on tyrosinase at all three incubation intervals. Though 4′-hydroxylated ring B of DOB possesses a similar feature to that of the common enzyme substrate, that is, L-tyrosine, a similar inhibition manner was not observed in DOBs **2**, **11** and **16**. It is therefore suggested that lipophilic C-4 methoxyl of DOB **13** may play an important role in the inhibition of mushroom tyrosinase activity.

3. Conclusion

Taken together, the present study concludes that DOBs with feature analogous to isoflavone, dihydrochalcone and stilbene displayed powerful antioxidant and anti-tyrosinase activities, suggesting that they possess potentials to be developed into clinical therapeutic antioxidants and as new ingredients of cosmetics. Furthermore, the fact that DOBs could be synthesized readily and easily would be another advantage of these compounds in the antioxidant market.

4. Experimental

4.1. Synthesis of DOBs

The synthesis of DOBs with resorcinol (**1**–**3**), catechol (**4**–**5**), 2,3-dimethylhydroquinone (**6**), 2-methoxyhydroquinone (**7**), pyrogallol (**10**–**12**) or 3-methoxycatechol (**13**) ring A (each 0.01 mole) was performed by condensing respective phenols with equivalent quantities of various phenylacetic acids in BF_3–Et_2O at 80–90 °C under nitrogen conditioning for 2–3 h (Scheme 1, method **a**). The reaction mixtures were poured into a large quantity of icy NaOAc solution, and then extracted by EtOAc according to Wähälä et al.[1] However, DOBs with 1,2,4-trihydroxybenzene (**8**–**9**) and/or phloroglucinol (**14**–**17**) ring A were prepared according to the method as described by Balasubramanian et al.[2,3] with modification. These phenols (0.02 mol each) and related phenylacetonitriles (0.01 mol) together with $ZnCl_2$ (0.01 mol) were pre-suspended in 40 mL of ethyl ether at 0 °C, then saturated by HCl (gas, caution!) and stirred for 2 h. After stirring, the reaction mixtures were left to stand over night at ambient temperature. The coagulated products (imines, not isolated) were hydrolyzed by refluxing in 100 mL MeOH–H_2O (1:1; v/v) for 3–5 h. DOBs (**8**–**9** and **14**–**17**) can be readily crystallized after cooling (Scheme 1, method **b**). Seventeen polyphenolic DOBs (**1**–**17**) were obtained (Table 1) after purifying with column chromatography and/or recrystallized to have satisfying yields (**8**–**9** and **14**–**17** scaled by respective phenylacetonitriles).

4.2. General procedures

Phenylacetic acids, phenylacetonitriles, boron trifluoride etherate (BF_3–Et_2O) and related phenols were purchased from Tokyo Chemical Industry (Tokyo, Japan) or Lancaster Synthesis (Morecambe, England). Melting point (uncorrected) was determined by Electrothermal 9100 melting point apparatus (Electrothermal Engineering Ltd, Landon, UK). Mass spectra were recorded on a Bruker Ft Mass Spectrometer (Bruker Daltonics, Bremen, Germany). ^1H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Varian Mercury-400 spectrometer (Varian Inc., Palo Alto, USA). Mushroom tyrosinase, 2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), 1,1-diphenyl-2-picryl-hydrazyl (DPPH), nitro-blue tetrazolium (NBT), dihydronicotinamide adenine dinucleotide (NADH), ethylenediaminetetraacetic acid (EDTA), Tween-20, ascorbic acid, kojic acid and Trolox were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

4.2.1. 2,4-Dihydroxy-4′-methoxydeoxybenzoin (1)

2.27 g, 88% (scale: 8.80 mmol). R_f = 0.42 (EtOAc–*n*-hexane–acetone = 2.5:7:0.5). Colorless needles (acetone–*n*-hexane), mp 162 °C. ^1H NMR (400 MHz, acetone-d_6): δ = 3.76 (3H, s, OMe), 4. 12 (2H, s, CH_2), 6.33 (1H, d, J = 2.8 Hz, H-3), 6.45 (1H, dd, J = 2.4, 8.8 Hz, H-5), 6.88 (2H, d, J = 8.8 Hz, H-3′, 5′), 7.26 (2H, d, J = 8.8 Hz, H-2′, 6′), 7.96 (1H, J = 8.8 Hz, H-6), 9.48 (1H, s, C-4 OH), 12.74 (1H, s, C-2 OH). ^{13}C NMR (100 MHz, acetone-d_6): δ = 44.4 (CH_2), 55.6 (OMe), 103.8 (C-3), 109.1 (C-5), 113.6 (C-1), 114.9 (C-3′, 5′), 128.1 (C-1′), 131.5 (C-2′, 6′), 159.8 (C-4′), 165.9 (C-2), 166.9 (C-4), 203.9 (C=O). EIMS *m/z*: 258 $[M]^+$, 137 [ring A+C=O]$^+$.[1,16]

4.2.2. 2,4,4′-Trihydroxydeoxybenzoin (2)

1.32 g, 54.1% (scale: 5.41 mmol). R_f = 0.38 (EtOAc–*n*-hexane–acetone = 2.5:7:0.5). Colorless powders (MeOH-H_2O), mp 194 °C. ^1H NMR (400 MHz, acetone-d_6): δ = 4.16 (2H, s, CH_2), 6.32 (1H, d, J = 2.4 Hz, H-3), 6.43 (1H, dd, J = 2.4, 8.8 Hz, H-5), 6.79 (2H, d, J = 8.8 Hz, H-3′, 5′), 7.16 (2H, d, J = 8.8 Hz, H-2′, 6′), 7.94 (1H, d, J = 8.8 Hz, H-6), 12.75 (1H, br s, C-2 OH). ^{13}C NMR (100 MHz, acetone-d_6): δ = 45.0 (CH_2), 104.3 (C-3), 109.5 (C-5), 114.1 (C-1), 116.9 (C-3′, 5′), 127.4 (C-1′), 131.9 (C-2′, 6′), 135.0 (C-6), 157.9 (C-4′), 166.4 (C-2), 167.4 (C-4), 204.5 (C=O). EIMS *m/z*: 244 $[M]^+$, 137 [ring A+C=O]$^+$.[1,16]

4.2.3. 2,4-Dihydroxy-3′,4′-dimethoxydeoxybenzoin (3)

2.05 g, 71.2% (scale, 7.12 mmol). R_f = 0.29 (EtOAc–*n*-hexane–acetone = 2.5:7:0.5). Colorless needles (MeOH), mp 177–178 °C. ^1H NMR (400 MHz, $CDCl_3$): δ = 3.85 (3H, s, OMe), 3.86 (3H, s, OMe), 4.16 (2H, s, CH_2), 6.37 (1H, d, J = 2.0 Hz, H-3), 6.39 (1H, dd, J = 2.4, 8.8 Hz, H-5), 6.78 (1H, d, J = 2.0 Hz, H-2′), 6.80 (1H, dd, J = 2.0, 8.4 Hz, H-6′), 6.84 (1H, d, J = 8.4 Hz, H-5′), 7.76 (1H, d, J = 8.4 Hz, H-6), 12.67 (1H, s, C-2 OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 44.7 (CH_2), 56.1 (OMe × 2), 103.9 (C-3), 108.1 (C-5), 111.6 (C-5′), 112.6 (C-2′), 113.7 (C-1), 121.8 (C-6′), 126.9 (C-1′), 133.0 (C-6), 148.3 (C-4′), 149.3 (C-3′), 163.0 (C-2), 165.9 (C-4), 202.5 (C=O). EIMS *m/z*: 288 $[M]^+$, 137 [ring A+C=O]$^+$.[3]

4.2.4. 3,4-Dihydroxy-4′-methoxydeoxybenzoin (4)

1.72 g, 66.7% (scale: 6.67 mmol). R_f = 0.66 (EtOAc–*n*-hexane–acetone = 3:6.5:0.5). Colorless to yellowish needles (MeOH–H_2O), mp 164-165 °C. ^1H NMR (400 MHz, acetone-d_6): δ = 3.75 (3H, s, OMe), 4.15 (2H, s, CH_2), 6.85 (2H, d, J = 8.8 Hz, H-3′, 5′), 6.90 (1H, d, J = 8.4 Hz, H-5), 7.20 (2H, d, J = 8.8 Hz, H-2′, 6′), 7.54 (1H, d, J = 1.2 Hz, H-2), 7.55 (1H, dd, J = 1.2, 8.0 Hz, H-6), 8.36 (1H, br s, phenolic OH), 8.71 (1H, br, OH). ^{13}C NMR (100 MHz, acetone-d_6): δ = 45.2 (CH_2), 56.1 (OMe), 115.3 (C-3′, 5′), 116.3 (C-2), 116.8 (C-5), 123.8 (C-6), 129.2 (C-1), 131.0 (C-1′), 132.0 (C-2′, 6′), 146.5 (C-3), 151.7 (C-4), 160.0 (C-4′), 197.3 (C=O). ESIMS (+) *m/z*: 281

[M+Na]$^+$; HRESIMS (+) *m/z*: 281.0792 (calcd for $C_{15}H_{14}O_4Na$ = 281.0790).

4.2.5. 3,4-Dihydroxy-3′,4′-dimethoxydeoxybenzoin (5)

2.66 g, 92.4% (scale: 9.24 mmol). R_f = 0.51 (EtOAc–*n*-hexane–acetone = 2.5:7:0.5). Yellowish needles (MeOH-H_2O), mp 256-257 °C. ^{1}H NMR (400 MHz, acetone-d_6): δ = 3.76, 3.77 (each 3H, s, OMe), 4.14 (2H, s, CH_2), 6.80 (2H, d, *J* = 2.0, 8.8 Hz, H-6′), 6.85 (1H, d, *J* = 8.0 Hz, H-5′), 6.90 (2H, d, *J* = 8.0 Hz, H-5), 6.91 (1H, d, *J* = 2.0 Hz, H-2′), 7.54 (1H, d, *J* = 2.0 Hz, H-2), 7.56 (1H, dd, *J* = 2.0, 8.0 Hz, H-6), 8.49 (2H, br, OH). ^{13}C NMR (100 MHz, acetone-d_6): δ = 44.3 (CH_2), 55.4 (OMe), 55.4 (OMe), 112.2 (C-5′), 113.8 (C-2′), 115.0 (C-2), 115.5 (C-5), 121.8 (C-6′), 122.6 (C-6), 128.5 (C-1), 129.8 (C-1′), 132.0 (C-2′, 6′), 145.2 (C-4), 148.5 (C-4′), 149.6 (C-3′), 150.3 (C-3), 195.9 (C=O). ESIMS (+) *m/z*: 311 (M+Na); HRESMS (+) *m/z*: 311.0893 (calcd for $C_{16}H_{16}O_5Na$ = 311.0895).

4.2.6. 2,5-Dihydroxy-4′-methoxy-3,4-dimethyldeoxybenzoin (6)

1.24 g, 43.4% (scale: 4.34 mmol). R_f = 0.60 (EtOAc–*n*-hexane–acetone = 2.5:7:0.5). Colorless needles (MeOH), mp 177–178 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.19 (3H, s, Ar-Me), 2.22 (3H, s, Ar-Me), 3.78 (3H, s, OMe), 4.11 (2H, s, CH_2), 6.86 (2H, d, *J* = 8.4 Hz, H-3′, 5′), 7.07 (1H, s, H-6), 7.14 (2H, d, *J* = 8.4 Hz, H-2′, 6′), 12.32 (1H, s, C-2 OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 11.5 (Ar-Me), 13.0 (Ar-Me), 44.1 (CH_2), 55.3 (OMe), 111.6 (C-6), 114.2 (C-3′, 5′), 115.6 (C-1), 126.2 (C-3), 127.1 (C-1′), 130.4 (C-2′, 6′), 134.5 (C-4), 145.5 (C-5), 155.7 (C-2), 158.6 (C-4′), 203.4 (C=O). EIMS *m/z*: 286 [M]$^+$, 165 [ring A + C=O]$^+$; HRESIMS (+) *m/z*: 309.1101 (calcd for $C_{15}H_{14}O_5Na$ = 309.1103).

4.2.7. 2,5-Dihydroxy-4-methoxydeoxybenzoin (7)

2.24 g, 86.8% (scale: 8.68 mmol). R_f = 0.45 (EtOAc–*n*-hexane–acetone = 2.5:7:0.5). Yellowish powders (MeOH–H_2O), mp 154–155 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.91 (3H, s, OMe), 4.19 (2H, s, CH_2), 6.44 (1H, s, H-3), 7.26–7.34 (total 5H, m, H-2′–6′), 7.33 (1H, s, H-6), 12.47 (1H, s, C-2 OH). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 45.2 (CH_2), 56.4 (OMe), 100.2 (C-3), 112.0 (C-1), 113.8 (C-6), 127.3 (C-4′), 129.0 (C-3′,5′), 129.6 (C-2′, 6′), 134.5 (C-1′), 138.3 (C-5), 154.1 (C-2), 159.8 (C-4), 202.2 (C=O). EIMS *m/z*: 258 [M]$^+$, 167 [ring A + C=O]$^+$.[34]

4.2.8. 2,4,5-Trihydroxy-4′-methoxydeoxybenzoin (8)

2.18 g, 79.6% (scale: 7.96 mmol). R_f = 0.24 (EtOAc–*n*-hexane–acetone = 3:6.5:0.5). Pinkish needles (MeOH–H_2O), mp 188–189 °C. ^{1}H NMR (400 MHz, acetone-d_6): δ = 3.77 (3H, s, OMe), 4.15 (2H, s, CH_2), 6.36 (1H, s, H-3), 6.88 (2H, d, *J* = 8.8 Hz, H-3′, 5′), 7.24 (2H, d, *J* = 8.8 Hz, H-2′, 6′), 7.42 (1H, s, H-6), 12.42 (1H, s, C-2 OH). ^{13}C NMR (100 MHz, acetone-d_6): δ = 43.8 (CH_2), 54.8 (OMe), 103.3 (C-3), 111.4(C-1), 114.1 (C-3′, 5′), 115.8(C-6), 127.4 (C-1′), 130.6 (C-2′, 6′), 138.1 (C-5), 154.3 (C-4), 158.9 (C-2), 159.8 (C-4′), 202.6 (C=O). EIMS *m/z* 274 [M]$^+$, 153 [ring A + C=O]$^+$.[34]

4.2.9. 2,4,5,4′-Tetrahydroxydeoxybenzoin (9)

1.96 g, 75.4% (scale: 7.54 mmol). R_f = 0.26 (EtOAc–*n*-hexane–acetone = 4:5.5:0.5). Colorless needles (MeOH–H_2O), mp 297–299 °C. ^{1}H NMR (400 MHz, acetone-d_6): δ = 4.10 (2H, s, CH_2), 6.35 (1H, s, H-3), 6.79 (2H, d, *J* = 8.8 Hz, H-3′, 5′), 7.15 (2H, d, *J* = 8.8 Hz, H-2′, 6′), 7.41 (1H, s,, H-6), 8.40 (3H, br, OH), 12.44 (1H, s, C-2 OH). ^{13}C NMR (100 MHz, acetone-d_6): δ = 43.9 (CH_2), 103.3 (C-3), 111.4 (C-1), 115.6 (C-3′,5′), 115.8 (C-6), 126.2 (C-1′), 130.6 (C-2′,6′), 138.1 (C-5), 154.4 (C-4), 156.5 (C-2), 159.8 (C-4′), 202.7 (C=O). EIMS *m/z*: 260 [M]$^+$, 153 [ring A + C=O]$^+$.[34]

4.2.10. 2,3,4-Trihydroxy-4′-methoxydeoxybenzoin (10)

2.40 g, 87.6% (scale: 8.76 mmol). R_f = 0.46 (EtOAc–*n*-hexane–acetone = 3:6.5:0.5). Colorless needles (MeOH–H_2O), mp 191–192 °C. ^{1}H NMR (400 MHz, acetone-d_6): δ = 3.76 (3H, s, OMe), 4.22 (2H, s, CH_2), 6.48 (1H, d, *J* = 8.8 Hz, H-5), 6.87 (2H, d, *J* = 8.8 Hz, H-3′, 5′), 7.25 (2H, d, *J* = 8.8 Hz, H-2′, 6′), 7.55 (1H, d, *J* = 8.4 Hz, H-6), 7.80 (1H, s, OH), 8.66 (1H, s, OH), 12.74 (1H, s, C-2 OH). ^{13}C NMR (100 MHz, acetone-d_6): δ = 44.9 (CH_2), 56.1 (OMe), 109.1 (C-5), 114.4 (C-1), 115.4 (C-3′, 5′), 124.8 (C-6), 128.7 (C-1′), 132.0 (C-2′, 6′), 133.8 (C-3), 153.4 (C-2), 154.1 (C-4), 160.3 (C-4′), 205.1 (C=O). ESIMS (+) *m/z*: 275 [M+H]$^+$.[1,2]

4.2.11. 2,3,4,4′-Tetrahydroxydeoxybenzoin (11)

1.42 g, 5.46% (scale: 54.6 mmol). R_f = 0.35 (EtOAc–*n*-hexane–acetone = 4:5.5:0.5). Colorless needles (MeOH–H_2O), mp 281–282 °C. ^{1}H NMR (400 MHz, acetone-d_6): δ = 4.18 (2H, s, CH_2), 6.48 (1H, d, *J* = 8.8 Hz, H-5), 6.78 (2H, d, *J* = 8.8 Hz, H-3′, 5′), 7.16 (2H, d, *J* = 8.8 Hz, H-2′, 6′), 7.55 (1H, d, *J* = 8.8 Hz, H-6), 8.27 (3H, br, OH), 12.77 (1H, br s, C-2 OH). ^{13}C NMR (100 MHz, acetone-d_6): δ = 43.6 (CH_2), 107.7 (C-5), 113.0 (C-1), 115.5 (C-3′, 5′), 123.5 (C-6), 126.1 (C-1′), 130.7 (C-2′, 6′), 132.5 (C-3), 152.0 (C-2), 152.8 (C-4), 156.5 (C-4′), 203.9 (C=O). EIMS *m/z*: 260 [M]$^+$, 153 [ring A+C=O]$^+$.[1,2,16]

4.2.12. 2,3,4-Trihydroxy-3′,4′-dimethoxydeoxybenzoin (12)

2.42 g, 79.6% (scale: 7.96 mmol). R_f = 0.43 (EtOAc–*n*-hexane–acetone = 4:5.5:0.5). Colorless needles (MeOH–H_2O), mp 166–167 °C. ^{1}H NMR (400 MHz, acetone-d_6): δ = 3.76 (3H, s, OMe), 3.77 (3H, s, OMe), 4.20 (2H, s, CH_2), 6.48 (1H, d, *J* = 8.8 Hz, H-5), 6.84 (2H, dd, *J* = 2.0, 8.4 Hz, H-6′), 6.87 (2H, d, *J* = 8.4 Hz, H-5′), 6.96 (1H, d, *J* = 2.0 Hz, H-2′), 7.55 (1H, d, *J* = 8.8 Hz, H-6), 7.96 (1H, br, OH), 8.78 (1H, br, OH), 12.74 (1H, s, C-2 OH). ^{13}C NMR (100 MHz, acetone-d_6): δ = 45.3 (CH_2), 56.7 (OMe × 2), 109.1 (C-5), 113.5 (C-5′), 114.4 (C-1), 115.0 (C-2′), 123.1 (C-6′), 124.7 (C-6), 129.2 (C-1′), 133.9 (C-3), 150.0 (C-2), 151.0 (C-4′), 153.4 (C-41), 154.1 (C-4), 205.0 (C=O). ESIMS (+) *m/z*: 305 [M+H]$^+$.[35]

4.2.13. 2,3,4′-Trihydroxy-4-methoxydeoxybenzoin (13)

2.15 g, 78.5% (scale: 7.85 mmol). R_f = 0.23 (EtOAc–*n*-hexane–acetone = 3:6.5:0.5). Colorless powders (MeOH–H_2O), mp 249–250 °C. ^{1}H NMR (400 MHz, acetone-d_6): δ = 3.91 (3H, s, OMe), 4.21 (2H, s, CH_2), 6.65 (1H, d, *J* = 8.8 Hz, H-5), 6.79 (2H, d, *J* = 8.4 Hz, H-3′, 5′), 7.16 (2H, d, *J* = 8.4 Hz, H-2′, 6′), 7.63 (1H, d, *J* = 8.4 Hz, H-6), 8.24 (1H, br, OH), 12.46 (1H, br, C-2 OH). ^{13}C NMR (100 MHz, acetone-d_6): δ = 43.9 (CH_2), 55.8 (OMe), 103.5 (C-5), 114.2 (C-1), 115.5 (C-3′, 5′), 122.9 (C-6), 126.1 (C-1′), 130.6 (C-2′, 6′), 134.5 (C-3), 151.9 (C-2), 153.2 (C-4), 156.5 (C-4′), 204.2 (C=O). ESIMS (+) *m/z*: 297 [M+Na]$^+$; HRESIMS (+) *m/z*: 297.0741 (calcd for $C_{15}H_{14}O_5Na$ = 297.0739).

4.2.14. 2,4,6-Trihydroxydeoxybenzoin (14)

1.77 g, 72.5% (scale: 7.25 mmol). R_f = 0.26 (EtOAc–*n*-hexane–acetone = 2.5:7:0.5). Yellowish granules (MeOH-H_2O), mp 164 °C. ^{1}H NMR (400 MHz, acetone-d_6): δ = 4.42 (2H, s, CH_2), 5.95 (2H, s, H-3, 5), 7.21 (1H, m, *J* = 8.4 Hz, H-4′), 7.28 (4H, m, H-2′, 3′, 5′, 6′), 9.28 (1H, br s, phenolic OH), 11.74 (2H, br s, C-2, 6OH). ^{13}C NMR (100 MHz, acetone-d_6): δ = 49.5 (CH_2), 95.3 (C-3, 5), 104.5 (C-1), 126.5 (C-4′), 128.3 (C-3′, 5′), 130.0 (C-2′, 6′), 136.4 (C-1′), 164.8(C-2, 6), 164.9 (C-4), 203.0 (C=O). EIMS *m/z*: 244 [M]$^+$, 153 [ring A + C=O]$^+$.[34]

4.2.15. 2,4,6-Trihydroxy-4′-methoxydeoxybenzoin (15)

2.02 g, 73.7% (scale: 7.37 mmol). R_f = 0.38 (EtOAc–*n*-hexane–acetone = 3:6.5:0.5). Reddish needles (MeOH–H_2O), mp 184–185 °C. ^{1}H NMR^{1}H NMR (400 MHz, CD_3OD): δ = 3.73, (3H, s, OMe), 7.30 (2H, s, CH_2), 5.82 (2H, s, H-3, 5), 6.81 (2H, d, *J* = 8.8 Hz, H-3′, 5′), 7.13 (2H, d, *J* = 8.8 Hz, H-2′, 6′). ^{13}C NMR (100 MHz, CD_3OD): δ = 49.6 (CH_2), 55.6 (OMe), 95.8 (C-3, 5), 105.2 (C-1), 114.6 (C-3′, 5′), 129.3 (C-1′), 131.7 (C-2′, 6′), 159.7

(C-4′), 165.8 (C-4, 166.3 (C-2, 6), 204.9 (C=O). EIMS *m/z*: 274 $[M]^+$, 153 [ring A + C=O]$^+$.[2,34]

4.2.16. 2,4,4′,6-Tetrahydroxydeoxybenzoin (16)

1.61 g, 61.9% (scale: 6.19 mmol). *R*f= 0.34 (EtOAc–*n*-hexane–acetone = 4:5.5:0.5).Yellowish powders (MeOH–H_2O), mp 272 °C. ^{1}H NMR (400 MHz, acetone-d_6): δ = 4.31 (2H, s, CH_2), 5.93 (2H, s, H-3, 5), 6.75 (2H, d, *J* = 8.4 Hz, H-3′, 5′), 7.11 (2H, d, *J* = 8.4 Hz, H-2′, 6′), 8.13 (1H, br s, phenolic OH), 9.25 (1H, br, phenolic OH), 11.74(1H, br s, C-2 OH). ^{13}C NMR (100 MHz, acetone-d_6): δ = 48.6 (CH_2), 95.2 (C-3, 5), 104.4 (C-1), 115.1 (C-3′, 5′), 127.0 (C-4′), 130.9 (C-2′, 6′), 156.2 (C-4′), 164.8 (C-4), 164.8 (C-2, 4), 203.6 (C=O). EIMS *m/z* 260 $[M]^+$, 153 [ring A + C=O]$^+$.[2,16]

4.2.17. 2,4,6-Trihydroxy-3′,4′-dimethoxydeoxybenzoin (17)

2.03 g, 66.8% (scale: 6.68 mmol). R_f = 0.31 (EtOAc–*n*-hexane–acetone = 4:5.5:0.5). Colorless needles (H_2O–MeOH), mp 188–190 °C; ^{1}H NMR (400 MHz, pyridine-d_5): δ = 3.76, (3H, s, OMe), 3.77 (3H, s, OMe), 4.37 (2H, s, CH_2), 5.34 (2H, s, H-3, 5), 6.79 (1H, dd, *J* = 2.0, 8.0 Hz, H-6′), 6.84 (2H, d, *J* = 8.0 Hz, H-5′), 6.91 (1H, d, *J* = 2.0 Hz, H-2′), 9.29 (1H, br s, phenolic OH), 11.75 (2H, br s, C-2, 6 OH). ^{13}C NMR (100 MHz, pyridine-d_5): δ = 49.0 (CH_2), 55.4 (OMe), 55.5 (OMe), 95.2 (C-3, 5), 104.4 (C-1), 112.1 (C-5′), 114.3 (C-2′), 122.1 (C-6′), 128.8 (C-1′), 148.5 (C-4′), 149.4 (C-3′), 164.8 (C-2, 6), 164.8 (C-4), 203.3 (C=O). EIMS *m/z*: 304 $[M]^+$, 153 [ring A + C=O]$^+$.[34]

4.3. Bilogical assays

4.3.1. Anti-lipid peroxidation assay

This assay was determined by the thiocyanate method as described by Mitsuda et al.[25] In brief, 2 mL of different concentrations of DOBs and positive controls (Trolox: a water soluble form of tocopherol; vitamin C: ascorbic acid) were taken and well mixed with 3 mL of linoleic acid emulsion consisting of 2.5 g Tween-20, 2.5 g linoleic acid, and 0.04 M of potassium phosphate buffer (pH 7.0), followed by incubating at 37 °C. After reacting with $FeCl_2$ and thiocyanate at several time intervals, the peroxide value was measured at wavelength 500 nm.

4.3.2. Metal chelating assay

The chelating effect of ferrous ions by the DOBs was determined as described previously.[27] In brief, 1 mL of DOBs at different concentrations was mixed with 3.7 mL of methanol and 0.1 mL of 2 mM $FeCl_2$. The reaction was initiated by the addition of 0.2 mL of 5 mM ferrozine, followed by shaking vigorously and left to react at room temperature for 10 min. The absorbance was measured at 562 nm. EDTA, a strong metal chelator, was used as a standard metal chelating agent.

4.3.3. Reducing power assay

The reducing power of DOBs was determined according to the method of Oyaizu.[29] In brief, 2.5 mL of various concentrations of DOBs or positive controls (i.e., Trolox and vitamin C) was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferric cyanide. After incubating the mixture at 50 °C for 20 min, 2.5 mL of 10% trichloroacetic acid was added, followed by centrifuging at 3000 rpm for 10 min. Five (5) milliliters of the upper layer solution were taken and mixed with 5 mL distilled water and 1 mL of 0.1% $FeCl_3$. The mixture was then measured at absorbance 700 nm.

4.3.4. ABTS$^{\cdot+}$ cation radical scavenging assay

The scavenging activity of ABTS$^{\cdot+}$ was measured according to the method described by Re et al.[26] with some modifications. Briefly, ABTS was dissolved in deionized water to 7 mM in concentration, which was then mixed with 2.45 mM potassium persulfate. The scavenging activity was determined by mixing with 180 μL of ABTS and 40 μL of DOBs or negative control (PBS; phosphate buffered saline) or positive controls (i.e., Trolox and vitamin C), followed by measuring at absorbance 734 nm.

4.3.5. DPPH radical scavenging assay

This assay was conducted according to the method as described by Wu and Ng.[27] Briefly, 1 mL of 0.1 mM DPPH radical solution was mixed with 3 ml of various concentrations of DOBs or Trolox or vitamin C dissolving in methanol. The mixture was then vortexed vigorously and left for 30 min at 40 °C in the dark. For the baseline control, 3 mL of methanol was used. The absorbance was measured at 517 nm.

4.3.6. Superoxide anion radical scavenging assay

The assay of DOBs was conducted according to the method described by Gülçin.[28] In brief, superoxide radicals were generated in 3 mL of phosphate buffer (0.1 M, pH 7.4) containing 1 mL of nitroblue tetrazolium (NBT, 300 μM), 1 mL of PMS (120 μM), 1 mL of NADH (968 μM) and 1 mL of DOBs at various concentrations. The mixture was spectrophotometrically measured at 560 nm. Trolox and vitamin C were used as positive controls.

4.3.7. Mushroom tyrosinase inhibitory assay

DOBs selected for this assay are presented in Table 3 and were determined according to the method reported previously.[36] DOBs and kojic acid (positive control) were dissolved in DMSO/methanol and diluted to various concentrations using potassium phosphate buffer (pH 6.8). The 96-well plate containing 80 μL of L-tyrosine (2.0 mM) and 20 μL of various concentrations of DOBs or kojic acid were diluted with buffer to 200 μL after adding 20 μL of mushroom tyrosinase (1000 U/mL, EC 1.14.18.1). Inhibitory percentage of the test compounds was determined at wavelength 490 nm after 0.5, 1.5 and 2.5 h of incubation.

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Supplementary data

Supplementary data (synthetic procedures and the NMR spectra of all the DOBs) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.05.019.

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