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Preparation of two sets of 5,6,7-trioxygenated dihydroflavonol derivatives as free radical scavengers and neuronal cell protectors to oxidative damage

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This paper is dedicated to Professor Zhong Jian JIA at Lanzhou University on the occasion of her 80s birthday celebrations

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ABSTRACT

An unusual class of 5,6,7-trioxygenated dihydroflavonols (**3a–e** and **4a–j**) were designed and prepared. Their antioxidative properties were assessed by examining their capacities in several in vitro models, including superoxide anion and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, rat liver homogenate lipid peroxidation inhibition, PC12 cells protection from oxidative damage, and xanthine oxidase inhibition. These dihydroflavonols displayed positive quenching abilities towards $O_2^{\cdot-}$ and DPPH free radicals, in which the majority exhibited superior antioxidant properties to Vitamin C. *cis*-Configurated compound (±)-**3e** demonstrated remarkable inhibition to LPO with an IC_{50} value of 1.9 ± 0.3 μM, which was apparently stronger than that of quercetin (IC_{50} = 6.0 ± 0.4 μM). *trans*-Configurated dihydroflavonol (±)-**4h** exhibited significant protective effect on PC12 cells against oxidative damage with an EC_{50} value of 41.5 ± 5.3 μM, more effective compared to that of quercetin (EC_{50} = 81.8 ± 8.7 μM). The 6-OH-5,7-dimethoxy analogue (±)-**3d** showed significant inhibition of xanthine oxidase with an IC_{50} value of 16.0 ± 0.8 μM, which is superior to that of allopurinol (IC_{50} = 23.5 ± 2.0 μM). In addition to the hypothesized action mechanism of the bio-active compounds, 3D modeling was used to analyze the relationship between the minimized-energy structures and antioxidant activities.

1. Introduction

The reduction of molecular oxygen leads to the formation of reactive oxygen species (ROS), including superoxide anion radicals and hydroxyl radicals. ROS has physiological functions such as bacterial ingestion. However, when there is an imbalance between cellular production of free radicals and the ability of cells to withstand them, excessive ROS will cause oxidative damage to cellular components. Oxidative damage may contribute to a variety of neurological diseases including stroke,[1,2] Parkinson's[3] and Alzheimer's disease (AD).[4,5] It also results in other pathologies, including cardiac disease, atherosclerosis, hypertension, autoimmune rheumatic disease, cancer, viral disease (such as AIDS) and aging.[6–10]

Xanthine oxidase (XO) is an enzyme involved in purine metabolism. There is evidence that the XO-catalysed reaction, which transfers electrons from hypoxanthine to uric acid, is coupled to the reduction of oxygen into superoxide radicals ($O_2^{\cdot-}$) or hydrogen peroxide.[11] Consequently, XO is considered to be associated with the pathogenesis of oxidant-induced microvascular changes, inflammation, aging, arteriosclerosis and designated gout.[12,13] In order to prevent and treat various diseases related to free radicals, considerable efforts have thus been made in the discovery and development of efficient synthetic or natural antioxidants acting as neuronal protector and XO inhibitors.

Dihydroflavonols such as taxifolin (**1**), which belongs to the flavonoids family, are an important class of secondary plant metabolites and exhibit a wide spectrum of pharmacological properties, including antioxidative, anti-fungal, hepato- and gastro-protective, and antineoplastic activities.[14–17] The potential health benefits attributable to the antioxidant activity of these compounds have received much attention recently. It is thought that the antioxidant properties of these polyphenolic compounds are the results of their high propensity to transfer electrons, to chelate ferrous ions and to scavenge reactive oxygen species.[18–20] Most of the reported dihydroflavonols possess a

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5,7-dihydroxy-2,3-dihydroflavonol skeleton. The predominant 2,3-dihydroflavonols were reported as 2,3-*trans*-configurated ones. To our knowledge, little antioxidant activity data has been reported for 2,3-*cis*-configurated dihydroflavonols.

It is noteworthy that another flavonoid, baicalein (**2**), has been the recent subject of considerable attention. This 5,6,7-trioxygenated flavone is capable of attenuating oxidative stress in various in vitro models, suggesting that it may possess significant antioxidant activity.[21] However, due to its adjacent trihydroxyl groups located on the A ring, baicalein (**2**) is somewhat unstable. A few derivatives were therefore produced by converting the hydroxyl groups to the alkoxy groups on the A-ring, resulting in increased activity on P-gp 170 inhibition.[22] Fernández et al. analyzed inhibition of aldose reductase by 33 baicalein derivatives with modifications on the A and B rings, leading to the conclusion that compounds with either three hydroxyl or methoxyl functionalities on the A ring are effective inhibitors of aldose reductase.[23] Furthermore, Morita et al. showed that the increased lipophilicity of phenolic hydroxyls via alkylation may enhance the therapeutic potential to X-ALD.[24] Increasing the lipophilicity of drug molecules is especially advantageous to the treatment of Alzheimer's Disease (AD), as this may enhance their ability to pass through the blood–brain barrier.[25] Thus multi-alkylation of baicalein may not only improve the stability of the highly-oxidized parent molecule, but also increase its chances of permeating cerebral tissue. It is therefore reasonable to suggest that methylated analogues of baicalein may constitute the pro-drug form of antioxidant lead compounds, which may more easily target diseased sites of the central nervous system whilst conferring protection against oxidative damage.

Based on the aforementioned information, the combination of the biologically effective 5,6,7-trioxygenated A ring moiety of baicalein (**2**) with an dihydroflavonol skeleton of taxifolin (**1**) may produce a new class of antioxidants, while introduction of methoxy groups to replace the sensitive phenolic hydroxyls in the A-ring of molecules confers much more stability to the 5,6,7-trioxygenated dihydroflavonols designed. However, examples of dihydroflavonols containing a tri-oxygenized pattern at C-5, C-6 and C-7 positions in ring A are rarely found in nature compared with dihydroflavonols di-oxygenated at C-5 and C-7. Systematic comparisons of SAR principles on di-oxygenated and trioxygenated dihydroflavonols are not available due to a lack of information relating to the trioxygenated ones. To overcome this shortfall, the paper presented here describes the design, preparation and antioxidative evaluation of assembled 5,6,7-trioxygenated dihydroflavonols (Fig. 1).

Two sets of a total of 15 new compounds as 5,6,7-trioxygenated dihydroflavonols possessing diverse groups introduced into ring B were synthesized. The antioxidant properties of these dihydroflavonols were assessed by way of various experimental pharmacological models in vitro. The preliminary structure–activity relationship was also analyzed and discussed.

2. Results and discussion

2.1. Synthesis

The two designed series of compounds include 5,7-dimethoxy-6-hydroxy-dihydroflavonols **3** and 5,6,7-trimethoxy-dihydroflavonols **4**. The synthetic routes employed for the syntheses of **3** and **4** are illustrated in Scheme 1 and Scheme 2, respectively.

Commercially available 2,4,6-trihydroxy acetophenone **5** served as starting material, and was selectively protected with Me_2SO_4 to provide an excellent yield of dimethyl ether **6**. *Elbs* oxidation of 2-hydroxy-4,6-dimethoxyacetophenone **6** followed by methoxymethylation of the resulting compound **7** in diisopropylethylamine ($(i\text{-Pr})_2$EtN) gave rise to **8**, which was then treated with methoxymethyl chloride (MOMCl) in a CH_2Cl_2-aqueous NaOH solution in the presence of tetrabutylammonium bromide (n-Bu_4NBr), to afford the key intermediate **9**, as previously reported.[26] Condensation of **9** with different benzaldehydes using ethanolic potassium hydroxide resulted in the chalcone **10**, which was further oxidized with alkaline hydrogen peroxide to the corresponding chalcone epoxides **11**. Although R_f values of **10** and **11** were similar, the reaction could be easily monitored by the variation of UV spectra of the two compounds, in which the λ_{max} changed from ca. 330 nm of the former (**10**) to ca. 280 nm in the case of the latter (**11**). Treatment of the epoxide **11** with hydrochloric acid in methanol furnished 5,7-dimethoxy-6-hydroxy-dihydroflavonols, (±)-**3**.

The 5,6,7-trimethoxy-dihydroflavonols **4** were synthesized as shown in Scheme 2. 1,2,3-Trimethoxy-5-nitro-benzene **13** was prepared by nitration of easily accessible 3,4,5-trimethoxybenzoic acid **12** with HNO_3/AcOH in 69% yield. Reduction of the nitro group was accomplished in 85% yield using palladium charcoal in hydrazine-ethanol solution. The resulting **14** was transformed into its diazonium salt, which was further hydrolyzed to yield 3,4,5-trimethoxy-phenol **15**.[27] The *Friedel–Crafts* reaction of **15** with acetic anhydride (Ac_2O) resulted in product **16** in a satisfactory yield, which was then protected with MOMCl to produce the key intermediate **17**. In a similar way to Scheme 1, condensation of **17** with different benzaldehydes followed by epoxidation smoothly led to **19**, and the epoxidation of **18** to produce **19** could be determined by the change of their UV spectra: the λ_{max} altered from ca. 328 nm of **18** to ca. 285 nm of **19**. Compound **19** were then susceptible to cyclization with HCl/MeOH to procure target compounds (±)-**4**. This synthetic protocol afforded an neat approach and satisfactory yield of 5,6,7-trimethoxylated dihydroflavonols.

Whereas epoxides **11** and **19** contain two centers of prochirality at C(α) and C(β), and the method for cyclization is non-enantioselective, thus the purified compounds (±)-**3** and (±)-**4** might be either (+)-2*R*,3*R*/(+)-2*S*,3*R* or (−)-2*S*,3*S*/(−)-2*R*,3*S* enantiomers. The absolute stereochemistries of the chromatographic pure compounds **3** and **4** were not determined yet; therefore the symbol of (±) was adopted for naming these dihydroflavonols. In the ^{1}H NMR spectra of (±)-**3a**, (±)-**3b**, (±)-**4a**, (±)-**4e–f**, (±)-**4h**, a doublet signal of

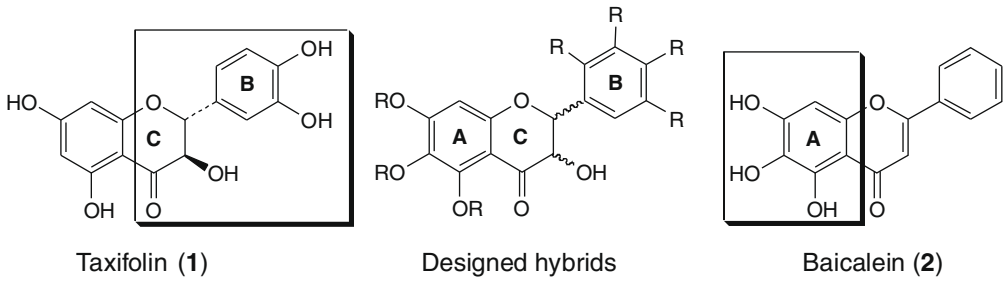
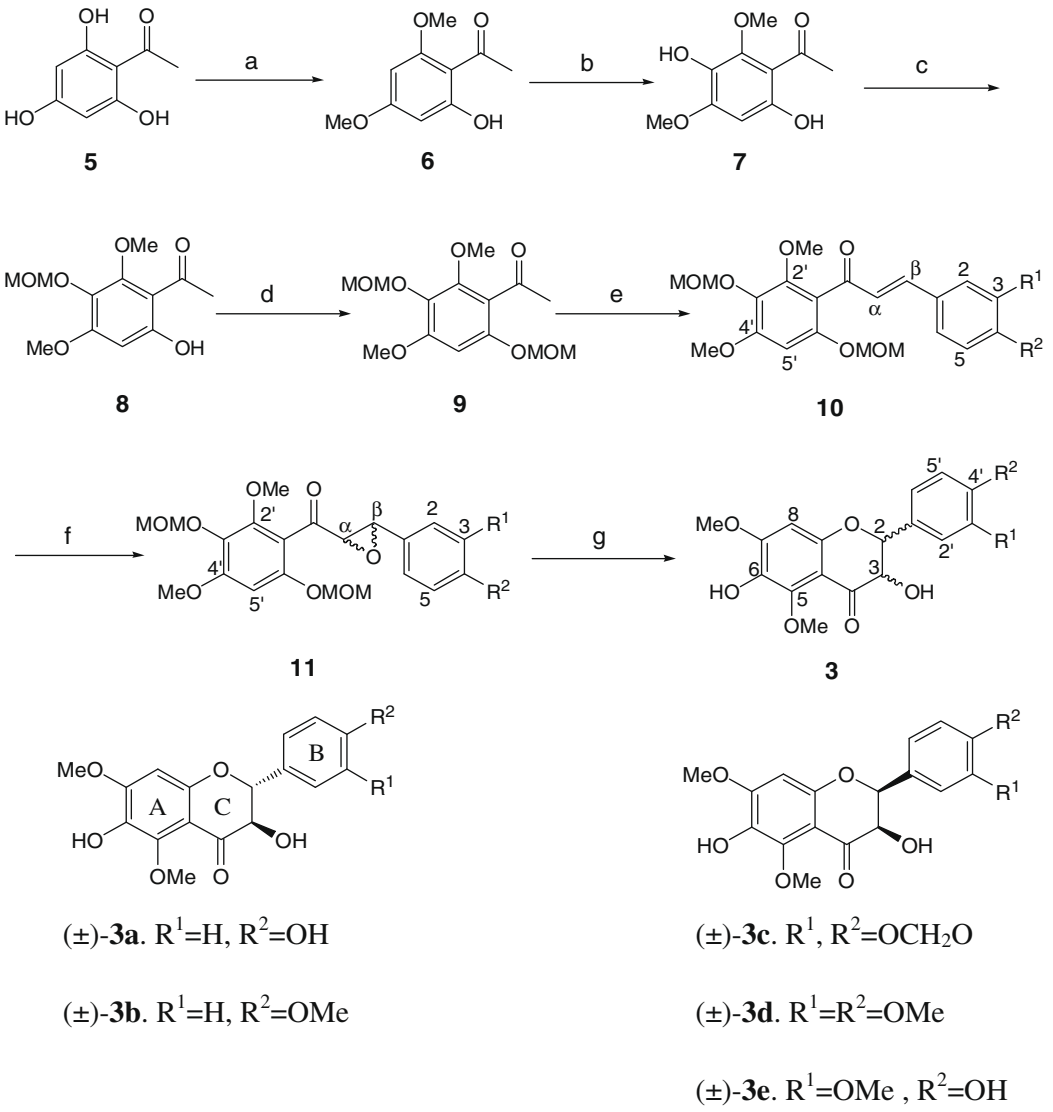
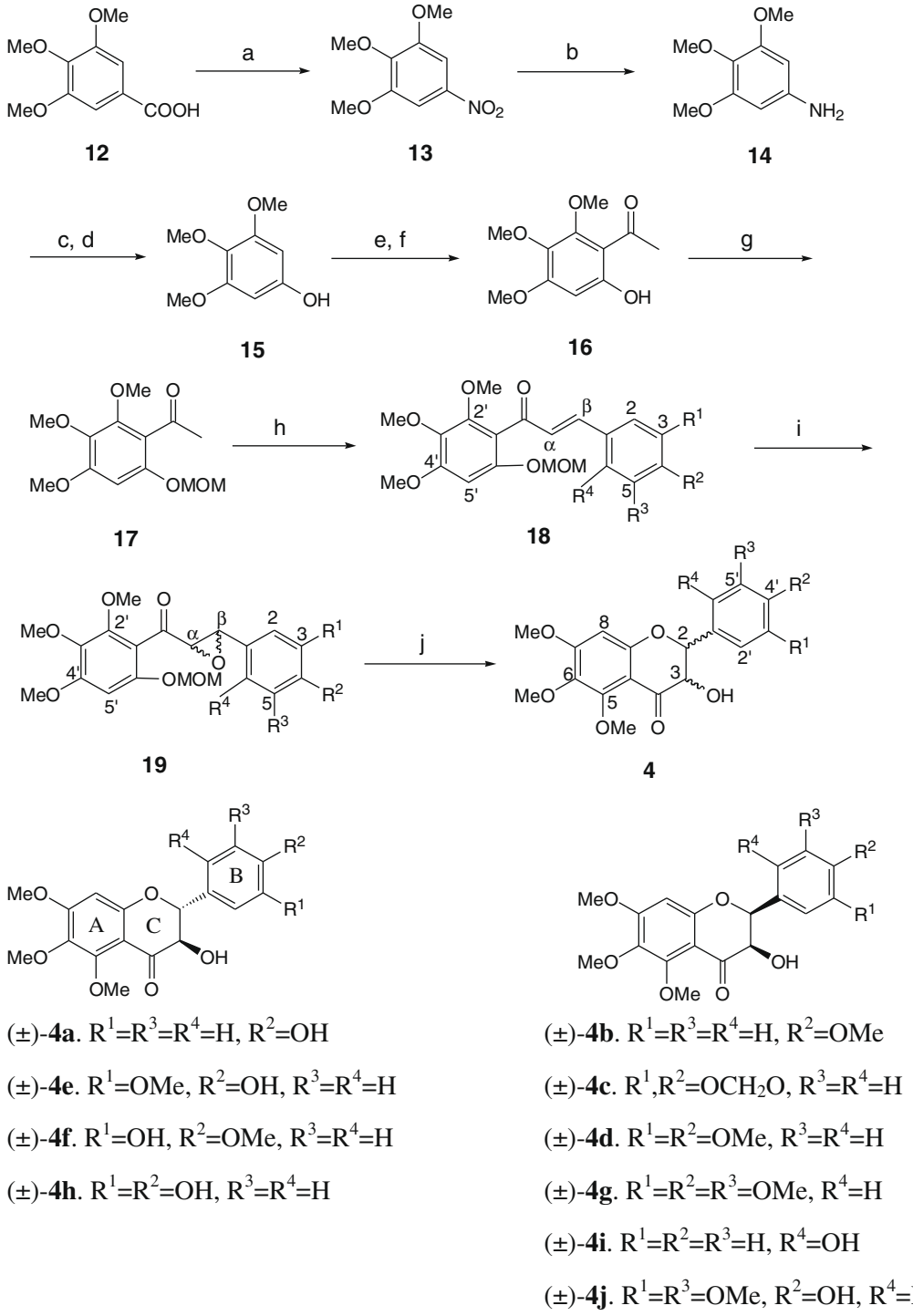


Figure 1. Structures of antioxidative flavonoids and designed hybridized molecules.



Scheme 1. Synthesis of 5,7-dimethoxy-6-hydroxy-dihydroflavonols **3a–e**. Reagents and conditions: (a) Me_2SO_4, K_2CO_3, acetone, rt; 78%; (b) $K_2S_2O_8$, KOH, H_2O, rt; 44%; (c) MOMCl, $(i\text{-Pr})_2$EtN, $CHCl_3$, reflux; 75%; (d) MOMCl, NaOH, Bu_4NBr, CH_2Cl_2, H_2O, rt; 80%; (e) Benzaldehydes, KOH, EtOH, rt; (f) 30% H_2O_2, NaOH, MeOH, rt; (g) HCl, MeOH, THF, 55 °C.

H-2 appears at ca. 4.95 ppm with a coupling constant J = 12.0 Hz, which indicated the relative configuration of these compounds is *trans*. Compounds (±)-**3c–e**, (±)-**4b–d**, (±)-**4g**, (±)-**4i–j** were assigned a *cis* orientation according to their diagnostic coupling constants of H-2 (ca. $J_{2,3}$ = 2.0 Hz).[28,29] Additionally, the coupling constants of H-3 of the synthetic compounds are also in agreement with above deduction in terms of their orientations. The 2,3-*cis* dihydroflavonols are rarely found as natural products, however, the formation of these *cis*-oriented natural flavonoids was substantiated by both biosynthetic background and phytochemical evidence.[28–30] Marais et al. described that the acid-catalysed cyclization of the chalcone epoxide will lead to the formation of either (2*R*,3*R*)-2,3-*trans* or (2*S*,3*R*)-2,3-*cis* dihydroflavonols, while the latter is thermodynamically less stable, and the epimerization/racemization may occur to the *cis* epimer upon elevated temperature and consequently yield another (2*S*,3*S*)-2,3-*trans* epimer.[31] However, in the presence of special chemical reagent, the *cis* dihydroflavonols could be asymmetrically synthesized.[31] There are several 2,3-*cis* dihydroflavonols obtained in this reported investigation, which might be attributable either to the mild reaction condition including the rather short reaction time, or to the multi-oxygenated A ring which may stabilize the cation transition state by a stronger σ-effect. The adjacent aromatic B ring also afforded the potency of stabilizing the cation, which make both the α-attack (lower attack) and the β-attack (upper attack) becomes available. These factors, including the acid environment as well as the polar protic solvent utilized in the reaction, made the transition state much favor to SN_1 characteristics leading to yield both 2,3-*trans* and 2,3-*cis* products.[31] Figure 2 hypothetically demonstrated the formation procedure of (±)-2,3-*trans* and (±)-2,3-*cis* compounds. It could be inferred that four kinds of conformers could be formed due to the non-enantioselective epoxidation and the following acidic cyclization procedures. Meanwhile, 2,3-*cis* analogues were obtained only by SN_1 pathway, whilst 2,3-*trans* conformers could be produced by two pathways: one is directly obtained by SN_2 pathway, the other is from either α-attack of the cation intermediate 2*R* or β-attack of the cation intermediate 2*S* (Fig. 2). However, increasing reaction temperature or prolonging reaction time could noticeably elevate the forming proportion of 2,3-*trans* to 2,3-*cis* conformers, which might be attributable to



Scheme 2. Synthesis of 5,6,7-trimethoxy-dihydroflavonols **4a–j**. Reagents and conditions: (a) HNO_3, CH_3COOH, rt; 69%; (b) 10% Pd/C, $N_2H_4{\cdot}H_2O$, EtOH, reflux; 85%; (c) 40% HBF_4, $NaNO_2$, THF, H_2O, 0 °C; 80%; (d) H_2SO_4, Na_2SO_4, H_2O, 70 °C; 70%; (e) Ac_2O, $BF_3{\cdot}Et_2O$, CH_2Cl_2, reflux; 84%; (f) $N(CH_2CH_2OH)_3$, H_2O, rt; 86%; (g) MOMCl, NaOH, Bu_4NBr, CH_2Cl_2, H_2O, rt; 85%; (h) Benzaldehydes, KOH, EtOH, rt; (i) 30% H_2O_2, NaOH, MeOH, rt; (j) HCl, MeOH, THF, 55 °C.

the reinforced epimerization or racemization of 2,3-*trans* conformers to 2,3-*cis* analogues by the increased temperature or reaction time.

2.2. Biological evaluation

The compounds synthesized were subjected to pharmacological screening via various in vitro models relating to free radicals, enzymes and cultured cell lines. The antioxidant properties of test compounds were assessed by examining their capacity to scavenge superoxide anion and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, as well as their ability to inhibit rat liver homogenate lipid peroxidation (LPO). Furthermore, the neuroprotective effects of the compounds prepared were performed on rat pheochromocytoma (PC12) cells subjected to hydrogen peroxide-induced damage. The inhibitory effects of the synthesized dihydroflavonols on xanthine oxidase were also measured.

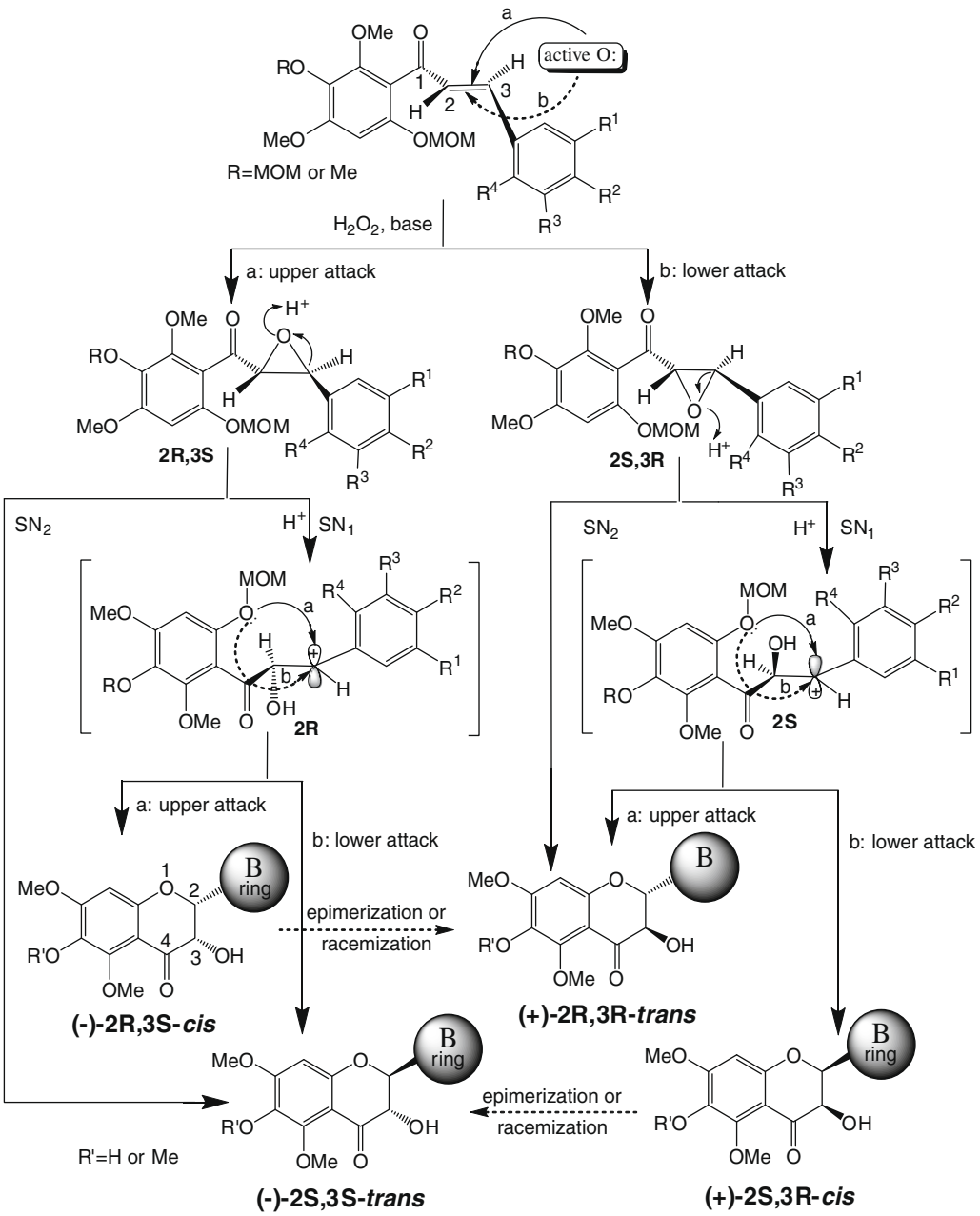


Figure 2. Possible forming and transforming mechanisms of the 5,6,7-trioxygenated-2,3-dihydroflavonols, SN_2 pathway produced merely 2,3-*trans* conformers, therefore 2,3-*cis* analogues were obtained only by SN_1 pathway (Shaded circles represent the B rings of (±)-**3** and (±)-**4** with different substituents).

2.2.1. Superoxide anion and DPPH radical scavenging

Due to the presence of an unpaired electron, the superoxide anion radical ($O_2^{\cdot-}$) is a highly active type of reactive oxygen species which can rapidly capture electrons from other molecules and lead to the generation of a hydroxide radical, the most harmful free radical to human beings known to date. All of the 15 compounds synthesized were subjected to $O_2^{\cdot-}$ scavenging experiments and the results are shown in Table 1. The most active $O_2^{\cdot-}$ scavenger among the synthesized series **3** was found to be (±)-**3d,** with an EC_{50} value of 50.0 ± 6.4 μM, comparable to that of the positive control quercetin (EC_{50} = 40.6 ± 4.4 μM). Among series **4**, (±)-**4j** was found to be the strongest $O_2^{\cdot-}$ scavenger, with an EC_{50} value of 55.5 ± 4.9 μM. These results imply that the 4′-OH substituent in the B ring has a positive impact on the $O_2^{\cdot-}$ scavenging capacity of the *cis*-set of compounds **4**. In general, the *cis*-oriented 5,6,7-trimethoxydihydroflavonols exhibited stronger $O_2^{\cdot-}$ quenching abilities than those of the *trans*-orientated analogues. Furthermore, the mono-oxygenated analogues at C-4′ in the B ring such as (±)-**3a**, (±)-**3b**, (±)-**4a** and (±)-**4b** did not exhibit a detectable scavenging effect of $O_2^{\cdot-}$ (Table 1). However, the other mono-oxygenated analogue substituted at C-2′ in the B ring, (±)-**4i**, showed a weak but distinct capacity to quench $O_2^{\cdot-}$, with an EC_{50} value of 164.5 ± 12.6 μM. In addition, dihydroflavonols (±)-**4b**, (±)-**4d** and (±)-**4g**, which share the same structural features of B ring being substituted purely by one or more methoxy groups, did not show any $O_2^{\cdot-}$ scavenging activity. It was observed that the majority of the designed hybrid dihydroflavonols, except for (±)-**3a**, (±)-**3b**, (±)-**4a**, (±)-**4b**, (±)-**4d** and (±)-**4g**, exhibited stronger scavenging ability towards $O_2^{\cdot-}$ radicals than Vitamin C whose EC_{50} value is 255.6 ± 28.2 μM (Table 1).

DPPH is a stable free radical existing in vitro, and bleaching of DPPH absorption is representative of the capacity of test compound to scavenge free aryl radicals with stable properties,

Table 1
Free radicals scavenging activities and inhibitory ability on rat liver homogenates lipid peroxidation of compounds **3** and **4**[a]

Compound	EC_{50} (μM)		IC_{50} of LPO (μM)
	Superoxide anion	DPPH	
(±)-**3a**	—[b]	35.3 ± 2.6	—
(±)-**3b**	—	94.9 ± 5.8	11.9 ± 0.8
(±)-**3c**	93.6 ± 8.7	18.4 ± 0.8	6.4 ± 0.7
(±)-**3d**	50.0 ± 6.4	35.9 ± 2.5	—
(±)-**3e**	97.2 ± 6.9	42.8 ± 2.6	1.9 ± 0.3
(±)-**4c**	80.3 ± 6.5	—	—
(±)-**4d**	—	—	28.6 ± 2.1
(±)-**4e**	97.1 ± 7.6	27.8 ± 2.5	5.5 ± 0.4
(±)-**4f**	112.2 ± 8.9	123.8 ± 9.6	—
(±)-**4g**	—	—	19.6 ± 1.7
(±)-**4h**	100.3 ± 10.5	7.3 ± 1.2	10.4 ± 0.7
(±)-**4i**	164.5 ± 12.6	82.4 ± 7.0	26.7 ± 2.3
(±)-**4j**	55.5 ± 4.9	54.1 ± 4.9	10.4 ± 0.9
Vitamin C	255.6 ± 28.2	154.5 ± 27.9	n.d.[c]
Quercetin	40.6 ± 4.4	5.5 ± 1.2	6.0 ± 0.4

[a] Data are expressed as the mean ± SD, $n = 3$.
[b] '—' indicates that EC_{50} or IC_{50} value is unable to be generated due to low quenching or inhibition rates at test concentrations. (±)-**4a** and (±)-**4b** are omitted herein due to no concrete EC_{50} and IC_{50} values could be generated.
[c] n.d. = not detected.

independent of any enzyme activity. The DPPH test results are shown in Table 1. Though the 4′-OH substituted compound (±)-**4a** did not exhibit scavenging activity against DPPH, the other 4′-OH substituted analogues (±)-**3a**, (±)-**3e**, (±)-**4e**, (±)-**4h** and (±)-**4j** displayed preferential quenching activity towards DPPH, compared to $O_2^{\cdot-}$. The *cis*-orientated derivatives of **4** possessing mono- or multi-methoxylated substituents alone at B ring such as (±)-**4b**, (±)-**4d** and (±)-**4g** again failed to show free radical scavenging activity with the DPPH model. This is in agreement with their deficiency towards $O_2^{\cdot-}$ (Table 1). It is therefore suggested that the single variety of substituent of mono- or multi-methoxylated B ring is unfavorable to compound's radical scavenging. It is noteworthy that compound (±)-**4h**, the only one possessing 3′,4′-*ortho*-diphenolic hydroxyls at the B ring, predictably enhanced the quenching activity of DPPH radicals with an EC_{50} value of 7.3 ± 1.2 μM, almost attained the efficacy of the reference standard quercetin (EC_{50} = 5.5 ± 1.2 μM). In a parallel comparison between the 6-OH series compounds **3** and its 6-OMe analogue **4**, compounds **3** were predominantly found to scavenge free radicals, especially DPPH radicals. This suggests that 5,7-dimethoxy-6-hydroxyl dihydroflavonols may possess higher quenching efficacy towards free radicals than their 5,6,7-trimethoxy substituted dihydroflavonol analogues. Furthermore, the designed dihydroflanonols demonstrated superior quenching ability towards DPPH radicals to that of Vitamin C (EC_{50} = 154.5 ± 27.9 μM), with exception of (±)-**4a**, (±)-**4b**, (±)-**4c**, (±)-**4d** and (±)-**4g** (Table 1).

2.2.2. Inhibition of lipid peroxidation

Hydroxyl and hydroperoxyl radicals are able to attack polyunsaturated fatty acids of phospholipids and other lipidic constituents of cell membrane, consequently resulting in lipid peroxidation (LPO). LPO-induced cell membrane damage alters its fluidity and ability to function correctly. Therefore, lipid peroxidation inhibition is used as an index of membrane antioxidant capacity. The results of LPO inhibition bioassay for compounds **3** and **4** are shown in Table 1. Though not every synthesized dihydrofalvonol showed significant inhibitory effect on LPO, the overall efficacy of active compounds was found to be satisfactory (Table 1). Among them, the 3′-methoxy-4′-hydroxy compound (±)-**3e** demonstrated the most impressive inhibition against LPO (IC_{50} = 1.9 ± 0.3 μM). Interestingly, its 6-OMe analogue (±)-**4e** also showed potent inhibitory effect (IC_{50} = 5.5 ± 2.1 μM) comparable to that of the positive control quercetin (IC_{50} = 6.0 ± 0.4 μM). As shown in Table 1, the multi-methoxylated derivatives (±)-**4d** and (±)-**4g** exhibited observable activities toward LPO inhibition alone, while dihydroflavonols (±)-**3d** and (±)-**4f** displayed clear radical scavenging activity but no inhibitory effects on LPO (Table 1). The diverse results suggest that 5,6,7-trioxygenated dihydroflavonols likely display different mechanisms of action towards $O_2^{\cdot-}$ and DPPH free radicals scavenging compared to LPO inhibition.

Furthermore, it was observed that (±)-**3e** and (±)-**4e** inhibited the lipid peroxidation in a concentration-dependent manner (Fig. 3), suggesting that (±)-**3e** and (±)-**4e** possess strong affinities for lipid peroxide radical ($LOO^{\cdot}$). (±)-**3e** was found to be superior to the positive control, quercetin, in all of the five concentrations tested (Fig. 3). However, (±)-**4e** failed to exhibit better inhibition of LPO at lower concentrations (<5.0 μM) while proving superior to quercetin against LPO at higher concentrations (6–100 μM), whilst remaining inferior to (±)-**3e** across the whole concentration range. Nevertheless, though (±)-**3e** possesses two radical scavenging phenolic sites, (±)-**4e** should have an improved lipid-binding affinity due to its higher hydrophobicity, conferred by the presence of a higher number of methyl groups.

The possible explanation for the best performance of (±)-**3e** observed in the anti-LPO experiment was addressed (Supplementary data 1). The free radicals may be trapped in both the delocalized A ring (conjugated by the adjacent 4-oxo functionality which may further stabilize the radical) and the delocalized B ring (Supplementary data 1), based on the attacking probabilities of the free radicals targeting the 5-OH (A-ring) or at 3′-OH (B-ring). This resulted in the significant inhibitory capacity of (±)-**3e** against LPO mediated by the Fe^{2+}–Vitamin C system.

A recent study showed that 5-methoxy flavonoids are easily metabolized into their 5-demethylated derivatives, the latter possessing superior anti-inflammatory properties.[32] This suggests that the methyloxy functionalities at C-5 on the A ring of dihydroflavonols reported here are also prone to demethylation and formation of a 5-OH metabolite, which together with the 4-oxo group forms an improved bi-ligand and should be beneficial to chelating ferrous ions. In the Fe^{2+}-mediated lipid peroxidation bioassay, the significant activity of (±)-**3e** on LPO inhibition may be attributable to the hypothesized metabolism of the 5-OMe becoming a 5-OH under the experimental conditions of the LPO test (Supplementary data 2). The exposed 5-hydroxyl metabolite subsequently chelates Fe^{2+} with the help of the adjacent 4-oxo group, which forms a stable six-membered ring, thus preventing free radicals from binding

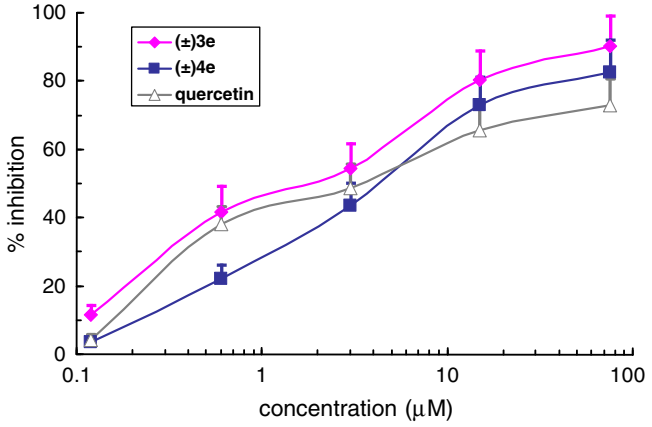


Figure 3. Concentration-dependent inhibitory activities of (±)-**3e**, (±)-**4e** and quercetin against Fe^{2+}-mediated peroxidation of rat liver homogenates: ♦ = (±)-**3e**, ■ = (±)-**4e** and △ = quercetin. Values are means ± SD ($n = 3$).

to the lipid membrane and polyunsaturated fatty acids of the phospholipid bilayer (Supplementary data 2). This assumption leads to the rationale that compound (±)-**3e** may also exhibit significant activity in vivo. Due to the known association of ferrous ions with the pathology of Alzheimer's disease (AD),[33] the synthesized dihydroflavonols, especially those with clear LPO inhibiting activity, may be developed as a new potential chemical entity for the treatment of AD.

2.2.3. Protection on PC12 cells

Though a conclusive pathogeny of AD has not yet been elucidated, recent studies suggest that oxidative stress is involved in apoptotic mechanisms in which excessive production of reactive oxygen species can lead to neuronal apoptosis, as seen for neurodegenerative disorders such as AD. Therefore, the therapeutic efforts aiming at removing or preventing free radical formation are beneficial to AD patients.[34] Tissue culture of rat pheochromocytoma (PC12) cells is commonly used as a screening model for testing the prevention of ROS-induced neuronal death.[35] The protective effect on neuronal cells against ROS hazard can be conveniently evaluated by the cell viability via MTT assay. The screening results in Table 2 show that 11 out of 15 synthesized compounds showed clear protective effect towards PC12 cells at the highest test concentration. Meanwhile, only dihydroflavonols (±)-**3b** and (±)-**4h** exhibited sufficient efficacy to enable the calculation of EC_{50} values. Compared to the reference substance quercetin (EC_{50} = 68.3 ± 5.3 μM), (±)-**3b** and (±)-**4h** possessed remarkable PC12 protective capacities, with EC_{50} values of 82.4 ± 9.9 μM and 33.1 ± 3.2 μM, respectively.

It is interesting to note that the pair (±)-**3d** and (±)-**4d** seemed to possess opposite antioxidant effects on the test models: (±)-**4d** displayed effective activity only on LPO inhibition (its protective effect on PC12 cell line was almost negligible) while its 6-OH analogue (±)-**3d** showed a broader antioxidant spectrum except for LPO inhibition. This is most likely caused by the different mechanisms of action conferred by the minor variance of molecular structures; additional studies should shed light on this phenomenon.

Table 2
Increased cell viability (at a gradient of test concentrations) and inhibition of XO (at 16 μg/mL) by compounds **3a–e** and **4a–d**, **4g** and **4h**[a]

Compound	Increased percentage of PC12 cell viability[b,c]			XO Inhibition rate[d,e]
	8 μg/mL	16 μg/mL	32 μg/mL	
(±)-**3b**	19.5 ± 1.2%	37.2 ± 3.5%	53.6 ± 5.9%	21.1 ± 1.6%
(±)-**3d**	2.9 ± 0.5%	19.8 ± 2.3%	22.0 ± 3.0%	74.3 ± 8.5%
(±)-**4d**	−2.5 ± 1.3%	1.9 ± 1.2%	2.4 ± 1.1%	46.6 ± 5.2%
(±)-**4h**	40.5 ± 4.1%	56.1 ± 5.0%	74.5 ± 4.6%	58.9 ± 3.3%
Quercetin	28.9 ± 2.7%	42.6 ± 5.6%	61.7 ± 7.2%	n.d.[f]
Allopurinol	n.d.	n.d.	n.d.	94.0 ± 5.9%

[a] Data are expressed as the mean ± SD, n = 3.

[b] The increased percentages of PC12 cell viability, respectively at 8, 16 and 32 μg/mL by other compounds are: −3.8 ± 0.4%, 13.4 ± 0.9%, 19.1 ± 0.9% for (±)-**3a**; 0.1 ± 0.2%, 0.3 ± 0.2%, 0.5 ± 0.3% for (±)-**3c**; −0.2 ± 0.2%, 0.5 ± 0.4%, 1.6 ± 0.6% for (±)-**3e**; −1.4 ± 0.4%, 5.1 ± 0.8%, 17.1 ± 1.2% for (±)-**4a**; 0.6 ± 0.4%, 3.4 ± 0.8%, 2.1 ± 0.6% for (±)-**4b**; 1.4 ± 0.2%, 5.4 ± 2.0%, 4.5 ± 1.8% for (±)-**4c**; 2.1 ± 0.6%, 9.2 ± 2.3%, 15.1 ± 1.2% for (±)-**4g**.

[c] EC_{50} means the effective concentration of compound for 50% increase of the PC12 cell viability. EC_{50} values for (±)-**3b**, (±)-**4h** and quercetin are 82.4 ± 9.9 μM, 33.1 ± 3.2 μM and 68.3 ± 5.3 μM, respectively.

[d] The inhibition rates of XO by other compounds at 16 μg/mL are: 19.8 ± 1.1% for (±)-**3a**; 12.5 ± 0.9% for (±)-**3c**; 15.4 ± 0.7% for (±)-**3e**; 0.2 ± 0.1% for (±)-**4a**; 24.9 ± 1.8% for (±)-**4b**; 0.8 ± 0.4% for (±)-**4c**; 28.9 ± 2.1% for (±)-**4g**.

[e] IC_{50} denotes the concentration that inhibits the xanthine oxidase by 50%. IC_{50} values for (±)-**3d**, (±)-**4h** and allopurinol are 16.0 ± 0.8 μM, 96.7 ± 6.8 μM and 23.5 ± 2.0 μM, respectively.

[f] n.d. = not detected.

2.2.4. Xanthine oxidase inhibition

Xanthine oxidase (XO) is a cytosolic enzyme that is an important source of oxygen free radicals. To explore the antioxidative activities of the various dihydroflavonols designed, selected compounds were subjected to XO inhibition assay. Compared to the experimental results listed in Table 1 and Table 2, the test compounds were found to behave differently in $O_2^{\cdot-}$ scavenging and XO enzyme inhibiting. Although XO inhibition could be closely associated with $O_2^{\cdot-}$ radicals, the XO inhibiting efficacies of the synthesized dihydroflavonols were independent of their $O_2^{\cdot-}$ scavenging action, according to the lack of correlation observed between these two assays (Table 1 and Table 2). It is worthy to point out that the B-ring-*ortho*-phenolic hydroxyl dihydroflavonol (±)-**4h**, possessing satisfactory antioxidative activity towards free radical scavenging, LPO inhibition and PC12 cell protection, displayed XO enzyme inhibition with an IC_{50} value of 96.7 ± 6.8 μM. More importantly, the best XO inhibitor among the test compounds, (±)-**3d** (IC_{50} = 16.0 ± 0.8 μM), showed an even better activity than that of the positive drug, allopurinol (IC_{50} = 23.5 ± 2.0 μM). Since allopurinol is currently the front-line chemotherapy agent used for the treatment of gout, the 5,6,7-trioxygenated dihydroflavonols designed, in particular (±)-**3d**, thus deserves to be further developed as a new XO-inhibiting lead for XO-related diseases, including gout.

Parallel comparison of the 6-OH dihydroflavonols (series **3**) and their 6-OMe analogues (series **4**) shows that the former set (**3**) possesses stronger inhibitory activity towards the XO enzyme than the latter (**4**) (Table 2). This result is consistent with those obtained in the $O_2^{\cdot-}$ and DPPH scavenging, LPO inhibition and PC12 cell protection experiments.

2.3. Energy-minimized analysis of selected 3D structures

In order to carry out preliminary exploration of the potential structural-related mechanisms which confer the antioxidant properties of dihydroflavonols investigated, the energy-minimized 3D models for selected compounds were established and analyzed. It could be observed that both baicalein and quercetin, which possess 2,3-double bond moieties, possessed more planar A/C ring systems than that of taxifolin (Fig. 4). The constructed 3D drawings of 2*R*,3*R*-**4e** has the similar stereochemistry to that of 2*R*,3*R*-taxifolin described by Trouillas et al.[36] Both 2*R*,3*R*-**4e** and 2*S*,3*S*-**4e** conformers have, as described for 2*R*,3*R*-taxifolin,[36] the axial H-2 and H-3 which results in the large coupling constants of these two protons (Fig. 4 and Section 4). This conformation favors 3-OH's forming the hydrogen bond with the adjacent 4-oxo group (Fig. 4A and Fig. 4B). In either stereochemistry, 2*S*,3*S*-*trans* and 2*R*,3*R*-*trans*, the sterically smaller H-2 and H-3 of (±)-**4e** always occupies the axial configuration positions (Fig. 4B). This configuration therefore allows the 3-OH to form a hydrogen bond with C-4 carbonyl groups. However, by comparison of the enantiomers of (±)-**3e** possessing 2*R*,3*S*-*cis* and 2*S*,3*R*-*cis* configurations, it could be observed that the pair of molecules had to sacrifice the hydrogen bonding possibility between 4-oxo and 3-OH (for both conformers take axial 3-OHs) in order to keep the larger C-2 aromatic substituent in an equatorial orientation (Fig. 4C).

Scrutiny of the minimized-energy figures of the molecules suggests that, in all cases, the B-ring did not demonstrate a co-planary status with the A/C ring plane, as also noted for the 2,3-unsaturated baicalein and quercetin. Furthermore, previous SAR calculations suggest that *cis*- or *trans*-configurations on the C ring do not result in major differences in scavenging free radicals, but variance could be found between these two configurations in the experiments carried out in enzyme models.[36] In the present study, among the compounds tested, the *cis*-configurated (±)-**3d** was found to be the best inhibitor of XO. Therefore, it may be that its

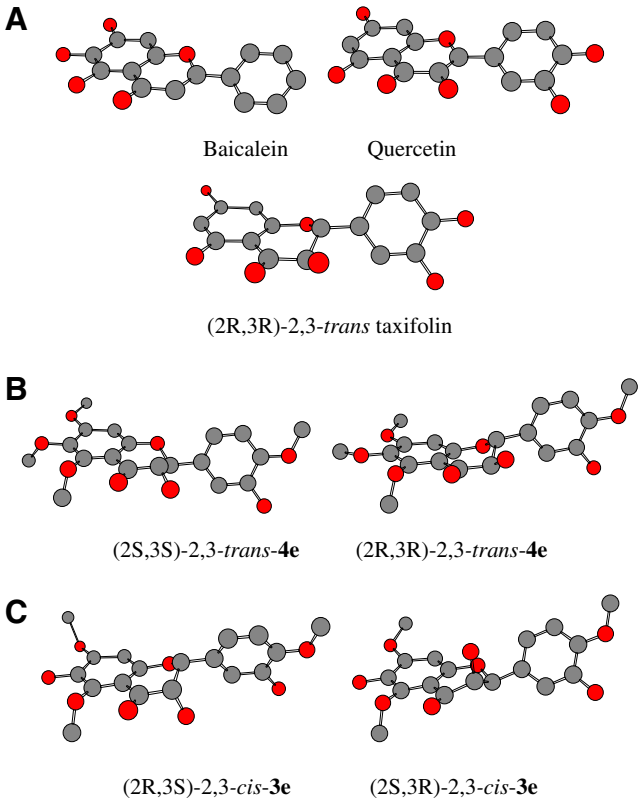


Figure 4. Minimized-energy analyses of 3D structures of the reference flavone, flavonol and dihydroflavonols.

specific B-ring orientation might afford a favorable chelating status to selectively block the active site of the XO enzyme, in addition to its ability to quench free radicals during purine metabolism.[37]

Furthermore, the reported SAR calculations suggest that taxifolin possesses an inferior antioxidant activity to that of quercetin which possesses a 2,3-double bond,[36] since the latter may stabilize the captured free radicals by more efficient electron delocalization. Surprisingly, as seen from the LPO inhibition results, both the 6-OH dihydroflavonol (±)-**3e** and the 6-OMe dihydroflavonol (±)-**4e** exhibited their superior inhibitory efficacy against LPO compared to quercetin, implying that the specific antioxidative mechanisms of 5,6,7-trioxygenated dihydroflavonols differ from that of quercetin. Therefore, it is clear that introduction of an adjacent trioxygenated moiety to the A ring can positively influence the activity of dihydroflavonols as antioxidants.

In addition, from the finding that (±)-**4h** possesses higher PC12 cells protective activity than that of quercetin, it is further surmised that the antioxidant efficiency of a molecule does not solely depend on the amount of its free phenol groups or quantum properties. Additional inhibitory factors associated with enzymes and/or receptors may also be involved in the antioxidative protection of the cells, if they are not the major factors. Enhanced electron density of the A ring in the synthesized compounds may also induce free radical attack, which elevates the ability of these highly-oxidized dihydroflavonols to consume free radicals and thus execute satisfactory antioxidant activities.

3. Conclusions

Two series of hybridized compounds composed of both active pharmacophores of 5,6,7-trioxygenated A ring (from baicalein) and 2,3-dihydroflavonol moiety (from taxifolin) were synthesized and their antioxidant properties were evaluated. The hybrids showed differential suppression of $O_2^{\cdot-}$ and DPPH free radical formation. A large portion of the designed target molecules exhibited better quenching ability towards these radicals when compared to Vitamin C. Comparison of the activities of the compounds, respectively belonging to series **3** and **4**, lead to the conclusion that 6-hydroxy substitution on the A ring is important to the antioxidant activities. The dihydroflavonol owning an *ortho*-dihydroxy substitution pattern on the B ring exhibited strong free radical scavenging activity, which might be attributable to its polyphenolic character. Some of the active compounds exhibited neuroprotective properties and/or XO inhibitory activity in some cases superior to those observed for the positive controls. The implication is that 5-O-methylated dihydroflavonols may serve as the suitable prodrug of their 5-hydroxyl metabolites. The present study may help to further comprehend the mechanisms underlying the antioxidative activity of dihydroflavonols and may therefore be useful for further optimizing their pharmaceutical application. The pharmacological efficacy observed suggests that these 5,6,7-trioxygenated dihydroflavonols hybridized by a taxifolin-like dihydroflavonol with a baicalein-like 5,6,7-trioxygenated A ring skeleton are worthy of further investigation including in vivo experiments to identify their potential in the application of treating ROS-related diseases such as AD, gout, aging and cardiovascular disorders resulting from oxidative damage. In addition, we afford series of naturally rare *cis*-configurated dihydroflavones, not only enriched the family of dihydroflavonoids, bust also broaden the pharmacological applicability of these *cis*-configurated flavonoids.

4. Experimental

4.1. Materials

Melting points were measured on a Perkin-Taike X-4 apparatus; uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AM-400 (400 MHz) instrument; chemical shifts δ in ppm with Me_4Si as internal standard, coupling constants J in Hertz. ESI-MS data were recorded on a Bruker Esquire 3000+ spectrometer and EI-MS was performed on a Varian MAT-95 MS instrument. HRES-I-MS data were recorded on a Micromass Q-Tof MS spectrometer. All reactions were carried out in an oven or flame-dried glassware with magnetic stirring. Solvents were purified by standard procedures. Yields refer to chromatographically and spectroscopically homogeneous materials unless otherwise stated. Thin-layer chromatography was performed on silica gel GF_{254}; detection by UV light (254 nm). Column chromatography (CC) was carried out on silica gel (200−300 mesh; Qingdao Ocean Chemical Plant, China).

The optical density (OD) was measured spectrophotometrically on an enzyme-linked immunosorbent assay reader (Bio-Tek Synergy HT, Bio-Tek Instruments Inc, Winooski, VT, USA). 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH), phenazine methosulfate (PMS), nitroblue tetrazolium (NBT), hydrogen peroxide (H_2O_2), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), thiobarbituric acid (TBA), and xanthine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Tris base, Trixon-100 and DMEM medium were obtained from Gibco (Grand Island, NY, USA). Reduced form disodium salt (NADH) was supplied from Amresco (Solon, OH, USA). Quercetin was prepared in our laboratory (HPLC purity of 99%). Vitamin C was purchased from Zhejiang Xianju Pharmaceutical Co. Ltd (Tiantai, Zhejiang, China). All other reagents were of the highest purity commercially available. The rat pheochromocytoma (PC12) cell line was obtained from the Shanghai Institute of Cell Biology, Chinese Academy of Sciences. Sprague-Dawley rats were obtained from the Zhejiang Center of Laboratory Animals, China. The use of animals was in accordance

with Guideline for the Care and Use of Laboratory Animals of Zhejiang University.

4.2. Synthesis[38]

4.2.1. Synthesis of Compounds 3a–e

4.2.1.1. (±)-*trans*-2,3-Dihydro-3,6-dihydroxy-5,7-dimethoxy-2-(4-hydroxyphenyl)-4H-1-benzopyran-4-one [(±)-3a]. To a solution of **11a** (95.0 mg, 0.21 mmol, see Supplementary data 3) in MeOH (3.0 mL) and THF (1.5 mL) was added, a mixture of concentrated HCl (0.2 mL) and MeOH (0.5 mL) dropwise. The mixture was then stirred at 55 °C for 30 min. After cooling, the reaction mixture was concentrated in vacuo. The residue was poured into ice-water (10.0 g) and extracted with EtOAc (3 × 10 mL). The organic phase was combined and dried over $MgSO_4$, and the filtered solvent was evaporated to give a yellow oil, which was subjected to CC (Si gel 10.0 g, EtOAc/petroleum ether 1:2) to afford 51.0 mg of **3a** (the major product). Yield: 75%. Yellowish solid. R_f (EtOAc/petroleum ether 1:2) 0.11. ^{1}H NMR (400 MHz, CD_3COCD_3) δ 3.81 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 4.29 (d, 1H, J = 2.4 Hz, 3-OH), 4.44 (dd, 1H, J = 12.0, 2.4 Hz, H-3), 4.93 (d, 1H, J = 12.0 Hz, H-2), 6.36 (s, 1H, H-8), 6.86 (d, 2H, J = 8.4 Hz, H-3′, H-5′), 7.37 (d, 2H, J = 8.4 Hz, H-2′, H-6′); ESI-MS m/z: 333 $[M+H]^+$. Anal. Calcd for $C_{17}H_{16}O_7$: C, 61.44; H, 4.85. Found: C, 61.36; H, 4.79.

4.2.1.2. (±)-*trans*-2,3-Dihydro-3,6-dihydroxy-5,7-dimethoxy-2-(4-methoxyphenyl)-4H-1-benzopyran-4-one [(±)-3b]. To a solution of **11b** (130.0 mg, 0.3 mmol, see Supplementary data 3) in MeOH (4.0 mL) and THF (2.0 mL) was added, a mixture of concentrated HCl (0.2 mL) and MeOH (0.5 mL) dropwise. The mixture was then stirred at 45 °C for 30 min. After cooling, the reaction mixture was concentrated in vacuo. The residue was poured into ice-water (10.0 g) and extracted with EtOAc (3 × 10 mL). The organic phase was combined and dried over $MgSO_4$, and the filtered solvent was evaporated to give a yellow oil, which was subjected to CC (Si gel 12.0 g, EtOAc/petroleum ether 1:3) to afford 68.2 mg of **3b**. Yield: 66%. Yellowish solid. R_f (EtOAc/petroleum ether 1:3) 0.15. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.84 (s, 3H, OCH_3), 3.91 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.49 (d, 1H, J = 12.0 Hz, H-3), 4.99 (d, 1H, J = 12.0 Hz, H-2), 6.35 (s, 1H, H-8), 6.98 (d, 1H, J = 8.4 Hz, H-3′, H-5′), 7.48 (d, 1H, J = 8.4 Hz, H-2′, H-6′); ESI-MS m/z: 347 $[M+H]^+$; HRESI-MS m/z: 345.0977 $[M-H]^+$ (calcd for $C_{18}H_{17}O_7$: 345.0969).

4.2.1.3. (±)-*cis*-2,3-Dihydro-3,6-dihydroxy-5,7-dimethoxy-2-(1,3-benzodioxol-5-yl)-4*H*-1-benzopyran-4-one [(±)-3c]. To a solution of **11c** (90.0 mg, 0.2 mmol, see Supplementary data 3) in MeOH (3.0 mL) and THF (1.5 mL) was added, a mixture of concentrated HCl (0.3 mL) and MeOH (0.4 mL) dropwise. The mixture was then stirred at 35 °C for 15 min. After cooling, the reaction mixture was concentrated in vacuo. The residue was poured into ice-water (10.0 g) and extracted with EtOAc (3 × 10 mL). The organic phase was combined and dried over $MgSO_4$, and the filtered solvent was evaporated to give a yellow oil, which was subjected to CC (Si gel 10.0 g, EtOAc/petroleum ether 1:3) to afford 41.9 mg of **3c** (the major product). Yield: 58%. Yellowish solid. R_f (EtOAc/petroleum ether 1:3) 0.25. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.94 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 4.57 (d, 1H, J = 2.0 Hz, H-3), 5.26 (d, 1H, J = 2.0 Hz, H-2), 5.95 (s, 2H, OCH_2O), 6.35 (s, 1H, H-8), 6.81 (d, 1H, J = 8.0 Hz, H-5′), 6.92 (dd, 1H, J = 8.0, 1.6 Hz, H-6′), 7.01 (d, 1H, J = 1.6, H-2′); ESI-MS m/z: 361 $[M+H]^+$. HRESI-MS m/z: 359.0726 $[M-H]^+$ (calcd for $C_{18}H_{15}O_8$: 359.0761).

4.2.1.4. (±)-*cis*-2,3-Dihydro-3,6-dihydroxy-5,7-dimethoxy-2-(3,4-dimethoxyphenyl)-4*H*-1-benzopyran-4-one [(±)-3d]. To a solution of **11d** (93.0 mg, 0.2 mmol, see Supplementary data 3) in MeOH (3.0 mL) and THF (1.5 mL) was added, a mixture of concentrated HCl (0.3 mL) and MeOH (0.4 mL) dropwise. Then the mixture was stirred at 35 °C for 15 min. After cooling, the reaction mixture was concentrated in vacuo. The residue was poured into ice-water (10.0 g) and extracted with EtOAc (3 × 10 mL). The organic phase was combined and dried over $MgSO_4$, and the filtered solvent was evaporated to give a yellow oil, which was subjected to CC (Si gel 10.0 g, EtOAc/petroleum ether 1:2) to afford 42.2 mg of **3d** (the major product). Yield: 56%. Yellowish solid. R_f (EtOAc/petroleum ether 1:2) 0.12. ^{1}H NMR (400 MHz, CD_3COCD_3) δ 3.80 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.57 (d, 1H, J = 2.4 Hz, H-3), 5.09 (d, 1H, J = 2.4 Hz, H-2), 6.37 (s, 1H, H-8), 6.92 (d, 1H, J = 8.4 Hz, H-5′), 6.96 (dd, 1H, J = 8.4, 1.6 Hz, H-6′), 7.06 (d, 1H, J = 1.6 Hz, H-2′); ESI-MS m/z: 377 $[M+H]^+$. Anal. Calcd for $C_{19}H_{20}O_8$: C, 60.64; H, 5.36. Found: C, 60.55; H, 5.18.

4.2.1.5. (±)-*cis*-2,3-Dihydro-3,6-dihydroxy-5,7-dimethoxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one[(±)-3e]. To a solution of **11e** (100.0 mg, 0.2 mmol, see Supplementary data 3) in MeOH (3.6 mL) and THF (1.8 mL) was added, a mixture of concentrated HCl (0.3 mL) and MeOH (0.4 mL) dropwise. The mixture was then stirred at 30 °C for 15 min. After cooling, the reaction mixture was concentrated in vacuo. The residue was poured into ice-water (10.0 g) and extracted with EtOAc (3 × 10 mL). The organic phase was combined and dried over Na_2SO_4, and the filtered solvent was evaporated to give a yellow oil, which was subjected to CC (Si gel 10.0 g, EtOAc/petroleum ether 1:2) to afford 45.4 mg of **3e** (the major product). Yield: 62%. Yellowish solid. R_f (EtOAc/petroleum ether 1:2) 0.16. ^{1}H NMR (400 MHz, CD_3COCD_3) δ 3.86 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 4.55 (d, 1H, J = 2.4 Hz, H-3), 5.08 (d, 1H, J = 2.4 Hz, H-2), 6.37 (s, 1H, H-8), 6.81 (d, 1H, J = 8.0 Hz, H-5′), 6.89 (dd, 1H, J = 8.0, 1.6 Hz, H-6′), 7.05 (d, 1H, J = 1.6 Hz, H-2′); ESI-MS m/z: 363 $[M+H]^+$. Anal. Calcd for $C_{18}H_{18}O_8$: C, 59.67; H, 5.08. Found: C, 59.57; H, 4.92.

4.2.2. Synthesis of Compounds 4a–j

4.2.2.1. (±)-*trans*-2,3-Dihydro-3-hydroxy-5,6,7-trimethoxy-2-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one [(±)-4a]. To a solution of **19a** (130.0 mg, 0.30 mmol, see Supplementary data 3) in MeOH (4.0 mL) and THF (2.0 mL) was added, a mixture of concentrated HCl (0.3 mL) and MeOH (0.5 mL) dropwise. The mixture was then stirred at 55 °C for 30 min. The reaction mixture was concentrated in vacuo. The residue was poured into ice-water (12.0 g) and extracted with EtOAc (3 × 10 mL). The organic phase was combined and dried over $MgSO_4$, and the filtered solvent was evaporated to give a yellow oil, which was subjected to CC (Si gel 12.0 g, EtOAc/petroleum ether 1:3) to afford 62.2 mg of **4a** (the major product). Yield: 60%. Yellowish solid. R_f (EtOAc/petroleum ether 1:3) 0.20. ^{1}H NMR (400 MHz, CD_3COCD_3) δ 3.70 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 4.47 (d, 1H, J = 12.0 Hz, H-3), 4.95 (d, 1H, J = 12.0 Hz, H-2), 6.35 (s, 1H, H-8), 6.86 (d, 2H, J = 8.0 Hz, H-3′, H-5′), 7.38 (d, 2H, J = 8.0 Hz, H-2′, H-6′); ^{13}C NMR (100 MHz, CD_3COCD_3) δ 56.6 (OCH_3), 61.2 (OCH_3), 61.9 (OCH_3), 73.6 (C-2), 84.2 (C-3), 97.2 (C-8), 107.5 (C-4a), 115.7 (C-3′, C-5′), 128.8 (C-1′), 130.1 (C-2′, C-6′), 138.3 (C-6), 154.4 (C-5), 158.8 (C-8a), 160.4 (C-7), 160.8 (C-4′), 192.0 (C-4); ESI-MS m/z: 347 $[M+H]^+$; HRESI-MS m/z: 345.0977 $[M-H]^+$ (calcd for $C_{18}H_{17}O_7$: 345.0969).

4.2.2.2. (±)-*cis*-2,3-Dihydro-3-hydroxy-5,6,7-trimethoxy-2-(4-methoxyphenyl)-4*H*-1-benzopyran-4-one [(±)-4b]. To a solution of **19b** (161.0 mg, 0.40 mmol, see Supplementary data 3) in MeOH (4.6 mL) and THF (2.3 mL) was added, a mixture of concentrated HCl (0.3 mL) and MeOH (0.5 mL) dropwise, ice was added to the water bath to lower the reaction temperature under 35 °C. The reaction mixture was stirred for 15 min and was concentrated in vacuo. The residue was poured into ice-water (12.0 g) and ex-

tracted with EtOAc (3 × 10 mL). The organic phase was combined and dried over $MgSO_4$, and the filtered solvent was evaporated to give a yellow oil, which was subjected to CC (Si gel 15.0 g, EtOAc/petroleum ether 1:2) to afford 86.0 mg of **4b** (the major product). Yield: 60%. Yellowish solid. R_f (EtOAc/petroleum ether 1:2) 0.39. ^{1}H NMR (400 MHz, CD_3COCD_3) δ 3.80 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 4.57 (d, 1H, J = 2.4 Hz, H-3), 5.06 (d, 1H, J = 2.4 Hz, H-2), 6.38 (s, 1H, H-8), 6.93 (d, 2H, J = 8.0 Hz, H-3′, H-5′), 7.38 (d, 2H, J = 8.0 Hz, H-2′, H-6′); ESI-MS m/z: 361 $[M+H]^+$. Anal. Calcd for $C_{19}H_{20}O_7$: C, 63.33; H, 5.59. Found: C, 62.24; H, 5.51.

4.2.2.3. (±)-*cis*-2,3-Dihydro-3-hydroxy-5,6,7-trimethoxy-2-(1,3-benzodioxol-5-yl)-4*H*-1-benzopyran-4-one [(±)-4c]. To a solution of **19c** (84.0 mg, 0.20 mmol, see Supplementary data 3) in MeOH (4.0 mL) and THF (2.0 mL) was added, a mixture of concentrated HCl (0.3 mL) and MeOH (0.5 mL) dropwise, ice was added to the water bath to lower the reaction temperature under 32 °C. The reaction mixture was stirred for 15 min and was concentrated in vacuo. The residue was poured into ice-water (10.0 g) and extracted with EtOAc (3 × 10 mL). The organic phase was combined and dried over $MgSO_4$, and the filtered solvent was evaporated to give a yellow oil, which was subjected to CC (Si gel 10.0 g, EtOAc/petroleum ether 1:3) to afford 41.1 mg of **4c** (the major product). Yield: 55%. Yellowish solid. R_f (EtOAc/petroleum ether 1:3) 0.28. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.86 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 4.51 (br s, 1H, H-3), 5.09 (br s, H-2), 5.90 (s, 2H, OCH_2O), 6.32 (s, 1H, H-8), 6.82 (d, 1H, J = 8.0 Hz, H-5′), 6.88 (dd, 1H, J = 8.0, 1.2 Hz, H-6′), 7.00 (d, 1H, J = 1.2 Hz, H-2′); ESI-MS m/z: 375 $[M+H]^+$. Anal. Calcd for $C_{19}H_{18}O_8$: C, 60.96; H, 4.84. Found: C, 60.88; H, 4.86.

4.2.2.4. (±)-*cis*-2,3-Dihydro-3-hydroxy-5,6,7-trimethoxy-2-(3,4-dimethoxyphenyl)-4H-1-benzopyran-4-one [(±)-4d]. To a solution of **19d** (130.0 mg, 0.30 mmol, see Supplementary data 3) in MeOH (5.0 mL) and THF (2.5 mL) was added, a mixture of concentrated HCl (0.4 mL) and MeOH (0.5 mL) dropwise, ice was added to the water bath to lower the reaction temperature under 32 °C. The reaction mixture was stirred for 15 min and was concentrated in vacuo. The residue was poured into ice-water (13.0 g) and extracted with EtOAc (3 × 10 mL). The organic phase was combined and dried over $MgSO_4$, and the filtered solvent was evaporated to give a yellow oil, which was subjected to CC (Si gel 15.0 g, EtOAc/petroleum ether 1:3) to afford 52.7 mg of **4c** (the major product). Yield: 45%. Yellowish solid. R_f (EtOAc/petroleum ether 1:3) 0.30. ^{1}H NMR (400 MHz, CD_3COCD_3) δ 3.80 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 4.02 (s, 3H, OCH_3), 4.54 (d, 1H, J = 2.0 Hz, H-3), 5.09 (d, 1H, J = 2.0 Hz, H-2), 6.36 (s, 1H, H-8), 6.92 (d, 1H, J = 8.0 Hz, H-5′), 6.96 (dd, 1H, J = 8.0, 1.6 Hz, H-6′), 7.05 (d, 1H, J = 1.6 Hz, H-2′); ESI-MS m/z: 391 $[M+H]^+$. Anal. Calcd for $C_{20}H_{22}O_8$: C, 61.53; H, 5.68. Found: C, 61.44; H, 5.59.

4.2.2.5. (±)-*trans*-2,3-Dihydro-3-hydroxy-5,6,7-trimethoxy-2-(4-hydroxy-3-methoxyphenyl)-4*H*-1-benzopyran-4-one [(±)-4e]. To a solution of **19e** (140.0 mg, 0.30 mmol, see Supplementary data 3) in MeOH (4.8 mL) and THF (2.4 mL) was added, a mixture of concentrated HCl (0.3 mL) and MeOH (0.5 mL) dropwise. The mixture was then stirred at 45 °C for 30 min. The reaction mixture was concentrated in vacuo. The residue was poured into ice-water (12.0 g) and extracted with EtOAc (3 × 10 mL). The organic phase was combined and dried over $MgSO_4$, and the filtered solvent was evaporated to give a yellow oil, which was subjected to CC (Si gel 15.0 g, EtOAc/petroleum ether 1:2) to afford 79.4 mg of **4e** (the major product). Yield: 70%. Yellowish solid. R_f (EtOAc/petroleum ether 1:3) 0.14. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.73 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 4.38 (d, 1H, J = 12.0 Hz, H-3), 5.00 (d, J = 12.0 Hz, H-2), 6.50 (s, 1H, H-8), 6.82 (d, J = 8.0 Hz, H-5′), 6.93 (s, H-2′), 6.98 (d, J = 8.0 Hz, H-6′); ESI-MS m/z: 377 $[M+H]^+$. Anal. Calcd for $C_{19}H_{20}O_8$: C, 60.63; H, 5.36. Found: C, 60.51; H, 5.32.

4.2.2.6. (±)-*trans*-2,3-Dihydro-3-hydroxy-5,6,7-trimethoxy-2-(3-hydroxy-4-methoxyphenyl)-4*H*-1-benzopyran-4-one [(±)-4f]. To a solution of **19f** (163.0 mg, 0.35 mmol, see Supplementary data 3) in MeOH (5.4 mL) and THF (2.7 mL) was added, a mixture of concentrated HCl (0.4 mL) and MeOH (0.6 mL) dropwise. The mixture was then stirred at 45 °C for 30 min. The reaction mixture was concentrated in vacuo. The residue was poured into ice-water (14.0 g) and extracted with EtOAc (3 × 10 mL). The organic phase was combined and dried over $MgSO_4$, and the filtered solvent was evaporated to give a yellow oil, which was subjected to CC (Si gel 15.0 g, EtOAc/petroleum ether 1:2) to afford 92.5 mg of **4f** (the major product). Yield: 70%. Yellowish solid. R_f (EtOAc/petroleum ether 1:2) 0.24. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.83 (s, 3H, OCH_3), 3.87 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 4.46 (d, 1H, J = 12.0 Hz, H-3), 4.95 (d, 1H, J = 12.0 Hz, H-2), 6.32 (s, 1H, H-8), 6.92 (d, 1H, J = 8.0 Hz, H-5′), 7.03 (dd, 1H, J = 8.0, 1.6 Hz, H-6′), 7.16 (d, 1H, J = 1.6 Hz, H-2′); ^{13}C NMR (100 MHz, CD_3COCD_3) δ 56.0 (OCH_3), 56.2 (OCH_3), 61.3 (OCH_3), 61.8 (OCH_3), 72.8 (C-2), 83.3 (C-3), 96.4 (C-8), 106.2 (C-4a), 113.5 (C-5′), 119.7 (C-6′), 129.2 (C-1′), 137.6 (C-6), 145.8 (C-3′), 147.3 (C-4′), 153.8 (C-5), 159.6 (C-8a), 160.3 (C-7), 191.2 (C-4); ESI-MS m/z: 377 $[M+H]^+$. Anal. Calcd for $C_{19}H_{20}O_8$: C, 60.63; H, 5.36. Found: C, 60.58; H, 5.41.

4.2.2.7. (±)-*cis*-2,3-Dihydro-3-hydroxy-5,6,7-trimethoxy-2-(3,4,5-trimethoxyphenyl)-4*H*-1-benzopyran-4-one [(±)-4g]. To a solution of **19g** (162.0 mg, 0.35 mmol, see Supplementary data 3) in MeOH (5.6 mL) and THF (2.8 mL) was added, a mixture of concentrated HCl (0.5 mL) and MeOH (0.5 mL) dropwise, ice was added to the water bath to lower the reaction temperature under 35 °C. The reaction mixture was kept stirring for 15 min and was concentrated in vacuo. The residue was poured into ice-water (12.0 g) and extracted with EtOAc (3 × 10 mL). The organic phase was combined and dried over $MgSO_4$, and the filtered solvent was evaporated to give a yellow oil, which was subjected to CC (Si gel 15.0 g, EtOAc/petroleum ether 1:2) to afford 52.5 mg of **4g** (the major product). Yield: 36%. Yellowish solid. R_f (EtOAc/petroleum ether 1:2) 0.36. ^{1}H NMR (400 MHz, CD_3COCD_3) δ 3.70 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.94 (s, 6H, 2 × OCH_3), 4.06 (s, 3H, OCH_3), 4.51 (d, 1H, J = 2.4 Hz, H-3), 5.15 (d, 1H, J = 2.4 Hz, H-2), 6.35 (s, 1H, H-8), 6.72 (s, 2H, H-2′, H-6′); ESI-MS m/z: 421 $[M+H]^+$. Anal. Calcd for $C_{21}H_{24}O_9$: C, 60.00; H, 5.75. Found: C, 59.85; H, 5.67.

4.2.2.8. (±)-*trans*-2,3-Dihydro-3-hydroxy-5,6,7-trimethoxy-2-(3,4-dihydroxyphenyl)-4*H*-1-benzopyran-4-one [(±)-4h]. To a solution of **19h** (150.0 mg, 0.30 mmol, see Supplementary data 3) in MeOH (5.6 mL) and THF (2.8 mL) was added, a mixture of concentrated HCl (0.4 mL) and MeOH (0.6 mL) dropwise. The mixture was then stirred at 55 °C for 30 min. The reaction mixture was concentrated in vacuo. The residue was poured into ice-water (13.0 g) and extracted with EtOAc (3 × 10 mL). The organic phase was combined and dried over $MgSO_4$, and the filtered solvent was evaporated to give a yellow oil, which was subjected to CC (Si gel 12.0 g, EtOAc/petroleum ether 2:3) to afford 58.3 mg of **4h** (the major product). Yield: 53%. Yellowish solid. R_f (EtOAc/petroleum ether 1:2) 0.12. ^{1}H NMR (400 MHz, DMSO-d_6) δ 3.73 (s, 3H, OCH_3), 3.84 (s, 3H, OCH_3), 3.88 (s, 3H, OCH_3), 4.38 (d, 1H, J = 12.0 Hz, H-3), 5.00 (d, 1H, J = 12.0 Hz, H-2), 6.50 (s, 1H, H-8), 6.82 (d, 1H, J = 8.0 Hz, H-5′), 6.93 (s, 1H, H-2′), 6.99 (d, 1H, J = 8.0 Hz, H-6′); ^{13}C NMR (100 MHz, CD_3COCD_3) δ 56.6 (OCH_3), 60.8 (OCH_3), 61.4 (OCH_3), 72.7 (C-2), 82.9 (C-3), 96.7 (C-8), 107.0

(C-4a), 115.1(C-2′), 115.3 (C-5′), 119.3 (C-6′), 128.1 (C-1′), 136.9 (C-6), 144.9 (C-4′), 145.7 (C-3′), 153.1 (C-5), 158.8 (C-8a), 159.1 (C-7), 191.1 (C-4); ESI-MS *m/z*: 363 $[M+H]^+$; HRESI-MS *m/z*: 361.0930 $[M-H]^+$ (calcd for $C_{18}H_{17}O_8$: 361.0918).

4.2.2.9. (±)-*cis*-2,3-Dihydro-3-hydroxy-5,6,7-trimethoxy-2-(2-hydroxyphenyl)-4*H*-1-benzopyran-4-one [(±)-4i]. To a solution of **19b** (152.0 mg, 0.35 mmol, see Supplementary data 3) in MeOH (4.6 mL) and THF (2.3 mL) was added, a mixture of concentrated HCl (0.4 mL) and MeOH (0.5 mL) dropwise, ice was added to the water bath to lower the reaction temperature under 35 °C. The reaction mixture was kept stirring for 15 min and was concentrated in vacuo. The residue was poured into ice-water (12.0 g) and extracted with EtOAc (3 × 10 mL). The organic phase was combined and dried over $MgSO_4$, and the filtered solvent was evaporated to give a yellow oil, which was subjected to CC (Si gel 12.0 g, EtOAc/petroleum ether 1:3) to afford 61.8 mg of **4i** (the major product). Yield: 53%. Yellowish solid. R_f (EtOAc/petroleum ether 1:3) 0.22. ^1H NMR (400 MHz, $CDCl_3$) δ 3.88 (s, 3H, OCH_3), 3.94 (s, 3H, OCH_3), 3.96 (s, 3H, OCH_3), 4.72 (d, 1H, J = 1.2 Hz, H-3), 5.24 (d, 1H, J = 1.2 Hz, H-2), 6.29 (s, 1H, H-8), 6.86–7.25 (m, 4 arom. H); ESI-MS *m/z*: 347 $[M+H]^+$. Anal. Calcd for $C_{18}H_{18}O_7$: C, 62.42; H, 5.24. Found: C, 62.32; H, 5.18.

4.2.2.10. (±)-*cis*-2,3-Dihydro-3-hydroxy-5,6,7-trimethoxy-2-(4-hydroxy-3,5-dimethoxyphenyl)-4*H*-1-benzopyran-4-one [(±)-4j]. To a solution of **19j** (100.0 mg, 0.20 mmol, see Supplementary data 3) in MeOH (7.0 mL) was added, a mixture of concentrated HCl (0.25 mL) and MeOH (0.5 mL) dropwise. Ice was added to the water bath to keep the reaction temperature under 35 °C. The reaction mixture was stirred for 15 min and was concentrated in vacuo. The residue was poured into ice-water (10.0 g) and extracted with EtOAc (3 × 10 mL). The organic phase was combined and dried over $MgSO_4$, and the filtered solvent was evaporated to give a yellow oil, which was subjected to CC (Si gel 10.0 g, EtOAc/petroleum ether 1:3) to afford 46.0 mg of **4j** (the major product). Yield: 56%. Yellowish solid. R_f (EtOAc/petroleum ether 1:3) 0.27. ^1H NMR (400 MHz, CD_3COCD_3) δ 3.85 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.92 (s, 6H, 2 × OCH_3), 3.98 (s, 3H, OCH_3), 4.47 (d, 1H, J = 1.2 Hz, H-3), 5.16 (d, J = 1.2 Hz, H-2), 6.31 (s, 1H, H-8), 6.68 (s, 2H, H-2′, H-6′); ESI-MS *m/z*: 407 $[M+H]^+$. Anal. Calcd for $C_{20}H_{22}O_9$: C, 59.11; H, 5.46. Found: C, 58.98; H, 5.41.

4.3. Superoxide anion scavenging activity

The superoxide anion scavenging activities of synthesized compounds were assayed spectrophotometrically as reported with a slight modification.[39] Superoxide anion radicals were generated in a non-enzymic phenazine methosulfate-NADH system by following of the reduction of nitroblue tetrazolium. In this assay, the superoxide anion radicals were measured in plates, which contained 78 μM of NADH, 50 μM of nitroblue tetrazolium, 5 μM of phenazine methosulfate and the tested samples with different concentrations in 16 mM Tris–HCl buffer at pH 8.0. The luminosity was monitored at 560 nm after 5 min of the incubation at room temperature. The blank samples did not contain phenazine methosulfate. Quercetin and Vitamin C were used as reference inhibitors.

4.4. DPPH free radical scavenging activity

Quenching of free radicals by the synthesized compounds was assayed spectrophotometrically at 517 nm against the absorbance of the stable radical DPPH radical.[40] The effect of test compound on free radical scavenging was reflected by the discoloration of DPPH radical. In brief, reaction mixtures dissolved in methanol containing various concentrations of the test compounds dissolved in DMSO and DPPH (0.4 mg/mL). The methanolic solution of DPPH served as a control while quercetin and Vitamin C were used as reference free radical scavengers. The absorbance was measured at 517 nm after the mixture was incubated at 37 °C for 30 min.

4.5. Inhibition of lipid peroxidation

The formation of malondialdehyde (MDA) was used as a measure of lipid peroxidation and was determined by the thiobarbituric acid (TBA) assay on rat liver homogenate in ice-cold PBS, using colorimetry.[41] The reaction mixture was composed of 200 μL of reaction solution, containing an aqueous solution of $FeSO_4$ (4 μM), Vitamin C (50 μM) and rat liver homogenate, 4 μL of synthesized compounds (concentration from 0.1 to 100 μM) was incubated at 37 °C in capped tubes for 1 h, then 100 μL of trichloroacetic acid (20%, v/v) was added to the mixture and reacted at room temperature for 30 min. Finally, 200 μL of HCl (0.1 M) and 100 μL of TBA (1%, w/v) were added to each tube and were incubated at 99 °C for 1 h. Centrifugation was carried out at 1000 rpm for 5 min, and the absorbance was measured at 532 nm. Quercetin was used as a standard.

4.6. Protection of PC12 against H_2O_2 induced cell injury

The rat pheochromocytoma (PC12) cells were maintained at 37 °C in a humidified atmosphere containing 5% CO_2. PC12 cells were seeded into multiwall plates (96) at a density of 6×10^3 per well in DMEM (high sucrose) medium, supplemented with 10% heat-inactivated bovine calf serum, 100 units/mL penicillin, and 100 units/mL of streptomycin. All experiments were carried out 36 h after cells were seeded. The PC12 cells were preincubated with samples which were dissolved in DMSO and diluted with medium to the final concentrations of 32, 16, 8 μg/mL, respectively. After 2 h, H_2O_2 (diluted by PBS, the final concentration was 500 μM) was added. Assays for cell viability were performed 4 h after H_2O_2 was added. Cell survivals were evaluated by two methods: morphological observation with phase-contrast microscope and MTT assay. A fresh solution of MTT (5 mg/mL) prepared in NaCl (0.9%) solution was added to each well, and the plates were incubated in a CO_2 incubator at 37 °C for additional 3 h. The MTT solution was aspirated off. 200 μL of DMSO was added to each well to solubilize the formazan crystals in viable cells. The absorbance was measured at 570 nm. Compared with the normal cells, the viability of cells treated with drugs is calculated by OD (drug-treated)/OD (normal cells) × 100%, while the negative control is calculated as OD (DMSO, which is used to exclude the solvent effect)/OD (normal cells) × 100%. Thus the increased cell viability could be calculated as the following formula:

$$[\mathrm{OD(drug\text{-}treated)/OD(normal\ cells)} \times 100\%] - [\mathrm{OD(DMSO)/OD(normal\ cells)} \times 100\%].$$

4.7. Inhibition of xanthine oxidase

The XO activity was evaluated by spectrophotometric measurement of the formation of formazan.[42] The reaction mixture in the sample wells consisted of xanthine oxidase (obtained from rat liver, 600 μL, 540 μM final concentration) in phosphate buffer 0.01 M, pH 8.75 (30 μL), NBT (30 μL, 100 μM, final concentration), PMS (30 μL, 100 μM final concentration), Triton X-100 (10 μL, 0.4%) and the test compounds (30 μL, at an original concentration

of 16 μg/mL). After 2 h of incubation at 37 °C in water bath, the absorbance was read at 550 nm. Xanthine was omitted in the blank samples. Allopurinol was used as positive control for the assay.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2009.03.032.

References and notes

1. Alexandrova, M. L.; Bochev, P. G. *Free Radical Biol. Med.* **2005**, *39*, 297.
2. Kossi, M. M. H. E.; Zakhary, M. M. *Stroke* **2000**, *31*, 1889.
3. Everse, J.; Coates, P. W. *Free Radical Biol. Med.* **2005**, *38*, 1296.
4. Perry, G.; Cash, A. D.; Smith, M. A. *J. Biomed. Biotechnol.* **2002**, *2*, 120.
5. Markesbery, W. R. *Free Radical Biol. Med.* **1997**, *23*, 134.
6. Lefer, D. J.; Granger, D. N. *Am. J. Med.* **2000**, *109*, 315.
7. Romero, J. C.; Reckelhoff, J. F. *Hypertension* **1999**, *34*, 943.
8. Sukkar, S. G.; Rossi, E. *Autoimmun. Rev.* **2004**, *3*, 199.
9. Toyokuni, S.; Okamoto, K.; Yodoi, J.; Hiai, H. *FEBS Lett.* **1995**, *358*, 1.
10. Schwarz, K. B. *Free Radical Biol. Med.* **1996**, *21*, 641.
11. Harris, C. M.; Sanders, S. A.; Massey, V. *J. Biol. Chem.* **1999**, *274*, 4561.
12. Hogg, N. *Semin. Reprod. Endocrinol.* **1998**, *16*, 241.
13. Pascual, E. *Curr. Opin. Rheumatol.* **2000**, *12*, 213.
14. Jovanovic, S. V.; Steenken, S.; Tosic, M.; Marjanovic, B.; Simic, M. G. *J. Am. Chem. Soc.* **1994**, *116*, 4846.
15. Ramanathan, R.; Das, N. P.; Tan, C. H. *Int. J. Oncol.* **1993**, *3*, 115.
16. Matsumoto, T.; Tahara, S. *Nippon Nogeik. Kaishi* **2001**, *75*, 659.
17. Haraguchi, H.; Mochida, Y.; Sakai, S.; Masuda, H.; Tamura, Y.; Mizutani, K.; Tanaka, O.; Chou, W. H. *Biosci. Biotechnol. Biochem.* **1996**, *60*, 945.
18. Montoro, P.; Braca, A.; Pizza, C.; De Tommasi, N. *Food Chem.* **2005**, *92*, 349.
19. Zhang, Q. -F.; Zhang, Z. -R.; Cheung, H. -Y. *Food Chem.* **2008**. doi: 10.1016/j.foodchem.2008.11.053.
20. Hernández, V.; Recio, M. C.; Máñez, S.; Giner, R. M.; Ríos, J.-L. *Life Sci.* **2007**, *81*, 480.
21. Lapchak, P. A.; Maher, P.; Schubert, D.; Zivin, J. A. *Neuroscience* **2007**, *150*, 585.
22. Lee, Y.; Yeo, H.; Liu, S. H.; Jiang, Z.; Savizky, R. M.; Austin, D. J.; Cheng, Y. C. *J. Med. Chem.* **2004**, *47*, 5555.
23. Fernández, M.; Caballero, J.; Helguera, A. M.; Castro, E. A.; González, M. P. *Bioorg. Med. Chem.* **2005**, *13*, 3269.
24. Morita, M.; Takahashi, I.; Kanai, M.; Okafuji, F.; Iwashima, M.; Hayashi, T.; Watanabe, S.; Hamazaki, T.; Shimozawa, N.; Suzuki, Y.; Furuya, H.; Yamada, T.; Imanaka, T. *FEBS Lett.* **2005**, *579*, 409.
25. Kavvadias, D.; Sand, P.; Youdim, K. A.; Qaiser, M. Z.; Rice-Evans, C.; Baur, R.; Sigel, E.; Rausch, W. D.; Riederer, P.; Schreier, P. *Br. J. Pharmacol.* **2004**, *142*, 811.
26. Gong, J. X.; Feng, Y. B.; Wang, F.; Wang, Y. G.; Li, H. B.; Wu, Y. H.; Hao, X. J.; Wu, X. M.; Bai, H.; Stöckigt, J.; Zhao, Y. *Chin. Chem. Lett.* **2006**, *17*, 449.
27. Duan, X. F.; Zhang, Z. B.; Duan, X. H. *Chin. J. Org. Chem.* **2003**, *23*, 353.
28. Prescott, A. G.; Stamford, N. P. J.; Wheeler, G.; Firmin, J. L. *Phytochemistry* **2002**, *60*, 589.
29. Markham, K. R.; Geiger, H. In *The Flavonoids: Advances in Research Since 1986*; Harborne, J. B., Ed.; Chapman and Hall: London, 1994; p 441.
30. Islam, Md. T.; Tahara, S. *Phytochemistry* **2000**, *54*, 901.
31. Marais, J. P. J.; Ferreira, D.; Slade, D. *Phytochemistry* **2005**, *66*, 2145.
32. Li, S.; Pan, M.-H.; Lo, C.-Y.; Tan, D.; Wang, Y.; Shahidi, C.; Ho, C.-T. *J. Funct. Foods* **2009**, *1*, 2.
33. Jayasena, T.; Grant, R. S.; Keerthisinghe, N.; Solaja, I.; Smythe, G. A. *Neurosci. Res.* **2007**, *57*, 454.
34. Sultana, R.; Newman, S.; Mohmmad-Abdul, H.; Keller, J. N.; Butterfield, D. A. *Free Radical Res.* **2004**, *38*, 449.
35. Luo, J.; Robinson, J. P.; Shi, R. *Neurochem. Int.* **2005**, *47*, 449.
36. Trouillas, P.; Fagnère, C.; Lazzaroni, R.; Calliste, C.; Marfak, A.; Duroux, J.-L. *Food Chem.* **2004**, *88*, 571.
37. Wang, F.; Yang, L.; Huang, K.; Li, X.; Hao, X.; Stöckigt, J.; Zhao, Y. *Nat. Prod. Res.* **2007**, *21*, 196.
38. Detailed synthesis of the intermediates and their physical data are reported in Supplementary data 3.
39. Robak, J.; Gryglewski, R. J. *Biochem. Pharmacol.* **1988**, *37*, 837.
40. Tapia, A.; Rodriguez, J.; Theoduloz, C.; Lopez, S.; Feresin, G. E.; Schmeda-Hirschmann, G. *J. Ethnopharmacol.* **2004**, *95*, 155.
41. Lee, S. E.; Ju, E. M.; Kim, J. H. *Exp. Mol. Med.* **2002**, *34*, 100.
42. Majkić-Singh, N.; Bogavac, L.; Kalimanovska, V.; Jelić, Z.; Spasić, S. *Clin. Chim. Acta* **1987**, *162*, 29.