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Synthesis and evaluation of 6-hydroxy-7-methoxy-4-chromanone- and chroman-2-carboxamides as antioxidants

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Abstract—A series of 6-hydroxy-7-methoxy-4-chromanone- (**2a**–**e**) and chroman-2-carboxamides (**3a**–**e**) were synthesized and their antioxidant activities were evaluated. While compounds **2a**–**e** were less active, compounds **3a**–**e** exhibited more potent inhibition of lipid peroxidation initiated by Fe^{2+} and ascorbic acid in rat brain homogenates. Among them, *N*-arylsubstituted-chroman-2-carboxamides (**3d** and **3e**) exhibited 25–40 times more potent inhibition than trolox (**1**). The DPPH radical scavenging activity of compound **3d** was comparable to that of trolox.

Lipid peroxidation is an important mediator of pathophysiological events in central nervous system disorders such as cerebral ischemia and trauma.[1] Lipid peroxidation is induced by free radicals with the major species of reactive oxygen species (ROS). ROS are by-products of a variety of pathways of aerobic metabolism. They are unstable and react readily with a wide range of biological substrates such as lipids, DNA, and protein, resulting in cell damage.[2–4] Lipids of biological membranes, especially those in the brain contain highly oxidizable polyunsaturated fatty acids and are particularly vulnerable to free-radical-induced damage.[5] Moreover, the brain contains considerable amounts of prooxidant transition metal ions and utilizes a lot of oxygen. Many natural and synthetic antioxidants inhibiting lipid peroxidation have been reported to retard oxidative damage and disease progression.[6]

Tocopherol (vitamin E) is the most important and widely studied natural, lipid-soluble, chain-breaking antioxidant. Recent studies provide evidence of protective effects of vitamin E against atherosclerosis[7] and reperfusion injury.[8] Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid) (**1**), a hydrophilic analog of vitamin E, is also a chain-breaking antioxidant that acts as a scavenger of radicals via the H-donating group in its chromanol nucleus.[9] Its protective effects against oxidative damages, particularly against lipid peroxidation, have been demonstrated in vitro and in vivo.[10,11] It has been shown that the conversion of the natural carboxylic acid to the amides functionality improves their antioxidant activity.[12] A number of chroman carboxamides have been reported based on the structure of trolox.[13]

In an effort to develop a novel antioxidant, we synthesized a series of 4-chromanone-2-carboxamide (**2a**–**e**) and chroman-2-carboxamide derivatives (**3a**–**e**) and evaluated their antioxidant activities. Chroman nucleus of the target compounds were taken from the structure of trolox. Hydroxy and methoxy substituents of the target compounds can be found in a number of natural antioxidants such as curcumin[14] and eugenol.[15] Alkyl or aryl substituents on amide nitrogen were introduced to explore the structure–activity relationship. The antioxidant activity was determined measuring the inhibition of lipid peroxidation initiated by Fe^{2+} and L-ascorbic acid in rat brain homogenates.[16,17] Their radical scavenging activities were also evaluated using a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH).[16,18] The results were compared with that of trolox (**1**) (Fig. 1).

Keywords: Antioxidant; Lipid peroxidation; Chroman-2-carboxamide.
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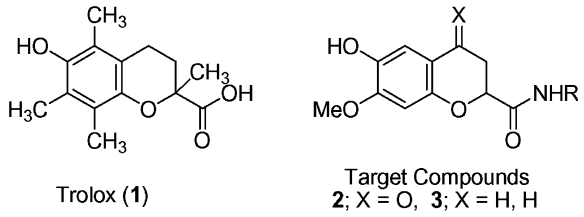


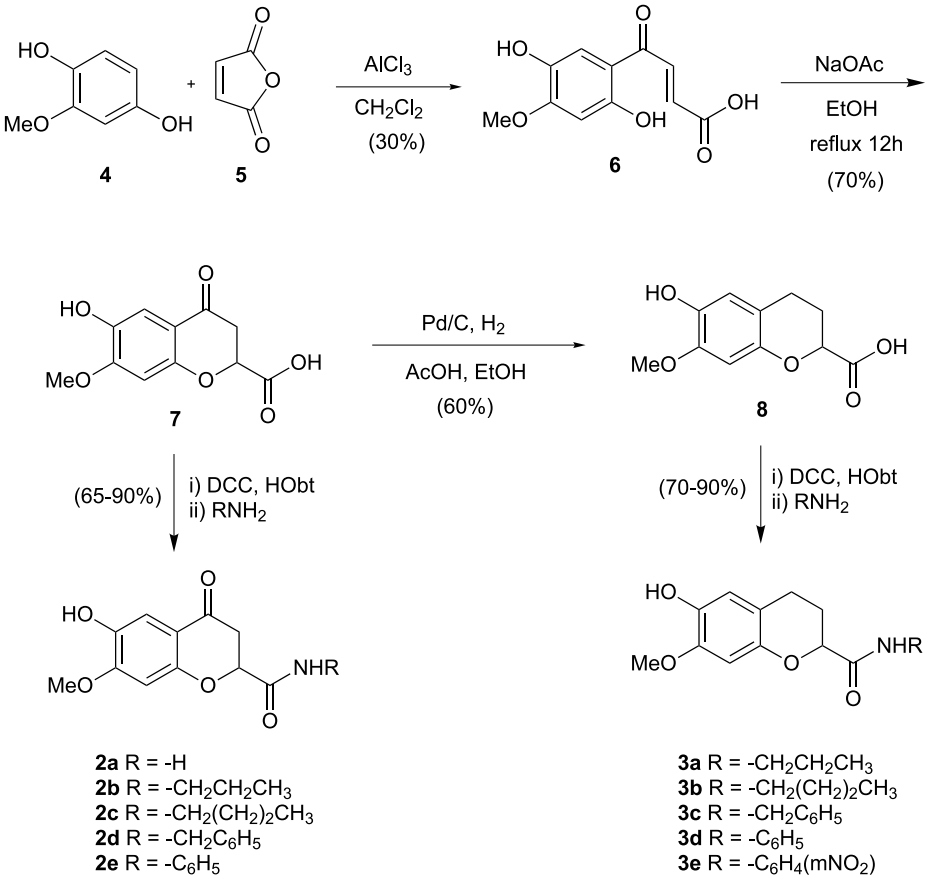
Figure 1. Structure of trolox (**1**) and target compounds **2** and **3**.

The general synthetic strategy employed to prepare the target compounds was based on the Friedel–Crafts acylation of methoxyhydroquinone (**4**) with maleic anhydride (**5**) and is outlined in Scheme 1. The starting hydroquinone **4** was treated with maleic anhydride (**5**) in the presence of $AlCl_3$ in dichloromethane to give 4-(2,5-dihydroxy-4-methoxyphenyl)-4-oxobut-2-enoic acid (**6**) in 30% yield. The treatment of the intermediate **6** with NaOAc in ethanol at reflux for 12 h afforded 6-hydroxy-7-methoxy-4-chromanone-2-carboxylic acid (**7**) in 70% yield. Coupling of the 4-chromanone-2-carboxylic acid **7** with various amines (ammonia, propylamine, butylamine, benzylamine, and aniline) using 1,3-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBT) in THF provided the 6-hydroxy-7-methoxy-4-chromanone-2-carboxamides (**2a**–**e**) in 65–90% yield after flash column chromatography.

In order to synthesize the target compounds, **3a**–**e**, catalytic hydrogenation of the 4-chromanone-2-carboxylic acid **7** was carried out with 10% Pd–C in ethanol in the presence of a catalytic amount of acetic acid to give the chroman-2-carboxylic acid **8** in 60% yield. The same coupling reaction of the acid **8** with various amines (propylamine, butylamine, benzylamine, aniline, and 3-nitroaniline) using DCC and HOBT in THF provided the 6-hydroxy-7-methoxychroman-2-carboxamides (**3a**–**e**) in 70–90% yield after flash column chromatography.

In order to evaluate antioxidant properties of the newly synthesized chroman derivatives,[19] the effects on lipid peroxidation in rat brain homogenates were examined using thiobarbituric acid reactive substances (TBARS) assay according to the method reported previously.[16,17] The formation of lipid peroxides was significantly inhibited by the test compounds. The IC_{50} values, 50% inhibitory concentrations, were determined by non-linear regression of the mean values using Prism (Graph Pad Software Inc., USA) (Table 1). The IC_{50} value for trolox is given for comparison.

Inhibition of lipid peroxidation is a multifactorial event. Propensity of radical formation and stabilization, ability of metal complexation, and lipophilicity are important factors for the inhibitory activity. The presence of *o*-electron donating methoxy substituent of the phenolic compounds is known to increase the stability of the rad-



Scheme 1. Synthesis of chromanone (**2**) and chroman (**3**) derivatives.

Table 1. Antioxidant activity of the 6-hydroxy-7-methoxy-4-chromanone- and chroman-2-carboxamides

Compound	Inhibition of lipid peroxidation IC_{50} (μM)[a]	DPPH scavenging activity IC_{50} (μM)[b]
7	>300.0	104.5
2a	>300.0	213.9
2b	>300.0	80.8
2c	176.8	66.4
2d	>300.0	118.5
2e	293.3	119.0
8	>300.0	16.8
3a	118.9	42.9
3b	51.3	24.8
3c	16.6	21.7
3d	6.9	24.9
3e	3.8	40.1
Trolox	165.8	18.2

[a] IC_{50} = the concentration (μM) exhibiting 50% inhibition of lipid peroxidation.

[b] IC_{50} = the concentration (μM) exhibiting 50% DPPH radical scavenging activity. Each experiment was performed at least three times in duplicate. The results are presented as an average value.

ical and hence, the antioxidative activity.[20] This would also contribute to the formation of the complex with iron. Considering these possibilities, the target compounds were designed to have both hydroxy and methoxy substituents on the phenyl ring.

As shown in Table 1, all of the chroman-2-carboxamides (**3a**–**e**) tested in this study exhibited more potent inhibition of lipid peroxidation than 4-chromanone-2-carboxamides (**2a**–**e**). This result indicates that keto group of the 4-chromanone derivatives is detrimental to the inhibitory activity of lipid peroxidation. Among the 4-chromanone derivatives (**2a**–**e** and **7**), only compound **2c** bearing an *N*-butyl substituent exhibited comparable inhibitory activity to that of trolox. Among the chroman derivatives (**3a**–**e** and **8**), compounds **3d** and **3e** bearing *N*-aryl substituent were more potent than compounds **3a**–**c** bearing *N*-alkyl or benzyl substituents. They exhibited 25–40 times more potent inhibition than trolox. These findings suggest that *N*-aryl substituents might be more important for the inhibition of lipid peroxidation than *N*-alkyl chain. Compounds **3a**–**e** were shown to be more potent than the corresponding carboxylic acid **8**, implying the importance of the amide functionality for the inhibition of lipid peroxidation.

The radical scavenging effects were also examined in the present study using radicals generated by DPPH.[16,18] In consistence with the inhibitory effects on lipid peroxidation, the chroman-2-carboxamides (**3a**–**e**) exhibited more potent radical scavenging activities than 4-chromanone-2-carboxamides (**2a**–**e**) (Table 1). The radical scavenging activities of compounds **3a**–**e** were comparable to or slightly less potent than that of trolox. However, no significant difference was observed between the compounds bearing *N*-alkyl and *N*-aryl substituents (**3a**,**b** vs **3d**,**e**). The radical scavenging activities of the carboxamides **2a**–**e** and **3a**–**e** were comparable to those of the corresponding carboxylic acids **7** and **8**, respectively. Based on this observation, unlike the inhibition of lipid peroxidation, the amide functionality appeared not to be a major contributor for the radical scavenging activity.

In summary, we have synthesized a series of 6-hydroxy-7-methoxy-4-chromanone- (**2a**–**e**) and chroman-2-carboxamides (**3a**–**e**), and evaluated their antioxidant activities. Chroman-2-carboxamides (**3a**–**e**) exhibited more potent inhibition of lipid peroxidation and DPPH radical scavenging activities than 4-chromanone-2-carboxamides (**2a**–**e**). The most active compounds **3d** and **3e** were 25–40 times more potent than trolox in the inhibition of lipid peroxidation. This may warrant further in-depth biological evaluations. Work is in progress to design, synthesize, and evaluate additional compounds in this and related systems.

Acknowledgments

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17. Assay of lipid peroxidation in the rat brain homogenates: Lipid peroxidation was initiated by Fe^{2+} (10 μM) and L-ascorbic acid (100 μM) in the rat forebrain homogenates. The reaction mixture was incubated at 37 °C for 1 h in the absence or presence of various concentrations of the compound. The reaction was stopped by the addition of trichloroacetic acid (28% w/v) and thiobarbituric acid (1% w/v) in succession, and the mixture was then heated at 100 °C for 15 min. After centrifugation to remove precipitates, absorbance was measured at 532 nm using VERSA$_{max}$ microplate reader (Molecular Devices, USA). The percent inhibition was calculated using the following formula: Inhibition (%) = $100 \times (Abs_{control} - Abs_{compound})/Abs_{control}$.
18. Assay for DPPH radical scavenging activity: The reaction mixture containing various concentrations of the compounds and DPPH methanolic solution (150 μM) was incubated at 37 °C for 30 min and absorbance was measured at 520 nm. The percent scavenging activity was calculated using the same formula given in Ref. 17.
19. All new compounds gave analytical and spectroscopic results consistent with the assigned structure.
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