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Synthesis of 7-Hydroxy-4-Oxo-4*H*-Chromene- and 7-Hydroxychroman-2-Carboxylic Acid *N*-Alkyl Amides and Their Antioxidant Activities

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A series of 7-hydroxy-4-oxo-4*H*-chromene- (**3a** - **h**) and 7-hydroxychroman-2-carboxylic acid *N*-alkyl amides (**4a** - **g**) were synthesized and their antioxidant activities were evaluated. While compounds **3a** - **h** were less active, compounds **4a** - **g** exhibited more potent inhibition of lipid peroxidation initiated by Fe^{2+} and ascorbic acid in rat brain homogenates. Among them, 7-hydroxychroman-2-carboxylic acid *N*-alkylamides (**4e** - **g**) bearing nonyl, decyl, and undecyl side chain exhibited 3 times more potent inhibition than trolox (**1**).

Key words: Antioxidant activity, 7-Hydroxychroman-2-carboxylic acid *N*-alkyl amides

INTRODUCTION

Reactive oxygen species (ROS) are constantly produced in the course of aerobic metabolism, and they are likely to play important physiological roles, especially in signal transduction processes (Forman *et al.*, 2001; Suzuki *et al.*, 1997). However, elevated levels of these species have been implicated in the initiation or progression of various pathological conditions including neurological diseases, particularly neurodegenerative diseases, as well as cardiovascular diseases and cancer (Gilgun-Sherki *et al.*, 2001, 2002; Kohen *et al.*, 2002; Griendling *et al.*, 2003; Cash *et al.*, 2004). ROS are unstable and react readily with a wide variety of biological substrates such as lipids, DNA, and proteins causing extensive cell damage (Braughler *et al.*, 1986; Halliwell *et al.*, 1989; Roberfroid *et al.*, 1995).

Lipids of biological membranes, especially those in the brain contain highly oxidizable polyunsaturated fatty acids and are particularly vulnerable to damage by ROS (Andersen *et al.*, 2004; Barnham *et al.*, 2004). Moreover, the brain contains considerable amounts of prooxidant transition metal ions and utilizes a lot of oxygen because of the high metabolic activities of the brain. Lipid peroxidation is an important mediator of pathophysiological events in central nervous system disorders such as cerebral ischemia and trauma (Hall *et al.*, 1991). Lipid peroxidation is induced by free radicals with the major species of ROS. Many natural and synthetic antioxidants inhibiting lipid peroxidation have been reported to retard oxidative damage and disease progression (Maxwell *et al.*, 1995).

Tocopherol (vitamin E) has been reported to exert protective effects against ischemic events, such as cerebral infarction, cardiac ischemia, and renal ischemia (van der Worp *et al.*, 1998; Mishima *et al.*, 2003; Sagach *et al.*, 2002; Rego *et al.*, 1999). Its protective effect has been widely believed to be due to its anti-oxidant activity. Tocopherol (vitamin E) is the most important and widely studied natural, lipid-soluble, chain-breaking antioxidant. 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 scavenger of radicals via the H-donating group in its chromanol nucleus (Tsuchiya *et al.*, 1992). Its protective effects against oxidative damages, particularly against lipid peroxidation, have been demonstrated *in vitro* and *in vivo* (Sagach *et al.*, 2002; Wu *et al.*, 1990; Forrest *et al.*, 1994). It has been shown that the conversion of the natural carboxylic acid to the amides functionality improves their antioxidant activity (Vairagupta

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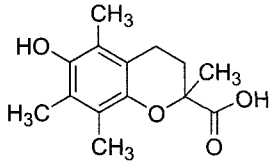
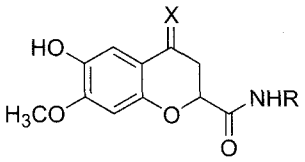
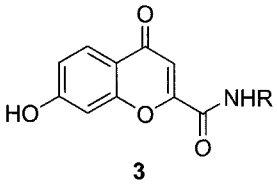


Trolox (**1**)



2; X=O or X=H,H



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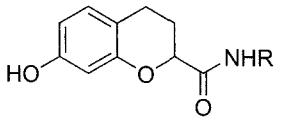

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et al., 2000). A number of chroman carboxamides have been reported based on the structure of trolox (Charbier *et al.*, 19990; Koufaki *et al.*, 2004).

In an effort to develop a novel antioxidant, we reported synthesis and antioxidant activities of chroman-2-carboxamide derivatives (**2**) (Lee *et al.*, 2005). In the present study, we synthesized a series of 7-hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid *N*-alkyl amides (**3**) and 7-hydroxychroman-2-carboxylic acid *N*-alkyl amides (**4**) (Fig. 1) and evaluated their antioxidant activities. The position of hydroxy substituent was varied from 6 to 7. Various alkyl 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 (Cho and Lee, 2004). Their radical scavenging activities were also evaluated using a stable free radical, 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Cho and Lee, 2004). The results were compared with that of trolox (**1**).

MATERIALS AND METHODS

Melting points were recorded on a Electrothermal IA9100 digital melting point apparatus and are uncorrected. IR spectra were determined with a Jasco FT/IR-300E spectrophotometer and reported in cm^{-1}. ^1H-NMR spectra were recorded on Bruker DPS300 NMR spectrometer using TMS as an internal standard and chemical shifts are reported as δ ppm units. Commercially available regents and solvents were used without additional purification unless otherwise stated.

7-Hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid ethyl ester (6)

Sodium (9.0 g, 394.3 mmole) was dissolved in abs EtOH (150 mL) and 2',4'-dihydroxyacetophenone (**5**) (10.0 g, 65.8 mmole) and diethyloxalate (28.0 g, 197.2 mmole) were added. The reaction mixture was stirred at reflux for 2 h under nitrogen atmosphere. After cooling, the mixture was treated with 6*N*-HCl (100 mL) for 10 min and was extracted with CH_2Cl_2 (3x150 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was dissolved in EtOH (100 mL) and treated with c-HCl (3 mL). The mixture was stirred at reflux for 24 h. The mixture was concentrated *in vacuo* and purified by flash column chromatography (EtOAC: Hexane=1: 2). Recrystallization of the residue from EtOAc and ether afforded compound **6** (15 g, 95%) as a light yellow solid: mp 219~222°C; IR (KBr) 3299, 2997, 1670, 1606cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.86 (d, *J* = 8.76 Hz, 1H, Ar-H), 6.86 (s, 1H, COCH), 6.82 (d, *J* = 8.4 Hz, 1H, Ar-H), 6.80 (s, *J* = 8.4 Hz, 1H, Ar-H), 4.33 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3), 1.30 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3).

General procedure for 7-Hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid *N*-alkyl amides (3a - h).

To a sloution of 7-hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid ethyl ester (**6**) (460 mg, 1.96 mmole) in xylene (25 mL) was added alkylamine (1 mL). The reaction mixture was stirred at refulx for 24 h. After cooling, the reaction mixture was treated with 1*N*-HCl (10 mL) and extracted with EtOAc (3x50 mL). The organic layer was washed with water (3x30 mL) and brine (3x30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (1:2 = EtOAc : Hexane to 5 % MeOH / CH_2Cl_2) to give compounds **3a - h**.

7-Hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid *N*-propylamide (3a)

The product was obtained in 41% yield as a cream colored solid: mp 235~237°C; IR (KBr) 3299, 3094, 1632, 1576cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 7.82 (d, 1H, *J* = 8.8 Hz, Ar-H), 6.87 (s, 1H, COCH), 6.82 (d, *J* = 8.7 Hz, 1H, Ar-H), 6.74 (s, 1H, Ar-H), 3.26 (m, 2H, $NHCH_2CH_2$), 1.56 (m, 2H, $CH_2CH_2CH_3$), 0.84 (t, *J* = 7.5 Hz, 3H, CH_2CH_3).

7-Hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid *N*-butylamide (3b)

The product was obtained in 39% yield as a cream colored solid: mp 243~246°C; IR (KBr) 3330, 3192, 1619, 1588cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 7.91 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.95 (s, 1H, COCH), 6.89 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.81 (s, 1H, Ar-H), 3.34 (t, 2H, *J* = 7.1 Hz, $NHCH_2CH_2$), 1.61-1.51 (m, 2H, $CH_2CH_2CH_2$), 1.41-1.31 (m, 2H, $CH_2CH_2CH_3$), 0.91 (t, *J* = 7.3 Hz, 3H, CH_2CH_3).

7-Hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid *N*-isopentylamide (3c)

The product was obtained in 38 % yield as a cream

colored solid: mp 249~252°C; IR (KBr) 3315, 3194, 1622, 1582cm^{-1}; ^{1}H NMR (DMSO, 300 MHz) δ 7.91 (d, 1H, *J* = 8.8 Hz, Ar-H), 6.98 (s, 1H, COCH), 6.95 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.67 (s, 1H, Ar-H), 3.35-3.28 (m, 2H, NHCH_2CH_2), 1.67-1.56 (m, 1H, CH_2CH(CH_3)$_2$), 1.49-1.42 (m, 2H, CH_2CH_2CH), 0.91 (d, *J* = 6.5 Hz, 6H, CH(CH_3)$_2$).

7-Hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid *N*-heptylamide (3d)

The product was obtained in 52 % yield as a light brown solid: mp 217~219°C; IR (KBr) 3331, 3194, 1617, 1588 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 7.92 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.96 (s, 1H, COCH), 6.91 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.81 (s, 1H, Ar-H), 3.32 (m, 2H, NHCH_2CH_2), 1.56 (m, 2H, CH_2CH_2CH_2), 1.38-1.21 (m, 8H, CH_2(CH_2)$_4$CH_3), 0.82 (t, *J* = 7.3 Hz, 3H, CH_2CH_3).

7-Hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid *N*-octylamide (3e)

The product was obtained in 41% yield as a brown solid: mp 208~210°C; IR (KBr) 3253, 3142, 1629, 1588 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 7.92 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.96 (s, 1H, COCH), 6.91 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.81 (s, 1H, Ar-H), 3.29 (m, 2H, NHCH_2CH_2), 1.52 (m, 2H, CH_2CH_2CH_2), 1.38-1.21 (m, 8H, CH_2(CH_2)$_4$CH_3), 0.82 (t, *J* = 7.3 Hz, 3H, CH_2CH_3).

7-Hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid *N*-nonylamide (3f)

The product was obtained in 39 % yield as a light brown solid: mp 209~212°C; IR (KBr) 3332, 3194, 1617, 1588 cm^{-1}; ^{1}H NMR (DMSO, 300 MHz) δ 7.89 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.99 (s, 1H, COCH), 6.96 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.88 (s, 1H, Ar-H), 3.33-3.24 (m, 2H, NHCH_2CH_2), 1.53-1.51 (m, 2H, CH_2CH_2CH_2), 1.24-1.20 (m, 10H, CH_2(CH_2)$_5$CH_3), 0.83 (t, *J* = 7.3 Hz, 3H, CH_2CH_3).

7-Hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid *N*-decylamide (3g)

The product was obtained in 32% yield as a yellow solid: mp 207~212°C; IR (KBr) 3334, 3194, 1617, 1588 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 7.94 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.97 (s, 1H, COCH), 6.92 (d, *J* = 8.8 Hz, 1H, Ar-H), 6.83 (s, 1H, Ar-H), 3.34 (m, 2H, NHCH_2CH_2), 1.65-1.56 (m, 2H, CH_2CH_2CH_2), 1.30-1.23 (m, 12H, CH_2(CH_2)$_6$ CH_3), 0.86 (t, *J* = 7.3 Hz, 3H, CH_2CH_3).

7-Hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid *N*-undecylamide (3h)

The product was obtained in 40% yield as a yellow solid: mp 197~200°C; IR (KBr) 3333, 3194, 1616, 1582 cm^{-1}; ^{1}H NMR (DMSO, 300 MHz) δ 7.89 (d, *J* = 8.7 Hz, 1H, Ar-H), 6.99 (s, 1H, COCH), 6.96 (d, *J* = 8.7 Hz, 1H, Ar-H), 6.69 (s, 1H, Ar-H), 3.33-3.24 (m, 2H, NHCH_2CH_2), 1.53-1.51 (m, 2H, CH_2CH_2CH_2), 1.27-1.23 (m, 14H, CH_2 (CH_2)$_7$CH_3), 0.83 (t, *J* = 7.3 Hz, 3H, CH_2CH_3).

7-Hydroxychroman-2-carboxylic acid ethyl ester (7)

To a solution of 7-hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid ethyl ester (**6**) (500 mg, 2.1 mmole) in EtOH (25 mL) was added 10% Pd/C (100 mg) and AcOH (1 mL). The reaction mixture was stirred for 24 h at ambient temperature under hydrogen atmosphere. The mixture was filtered through a celite pad and concentrated. The residue was purified by flash column chromatography (1:1=EtOAc:Hexane) to give compound **7**; (360 mg, 85 %) as a light yellow solid: mp 73~75°C; IR (KBr) 3419, 2993, 1740cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.86 (d, 1H, *J* = 8.2 Hz, Ar-H), 6.47 (s, 1H, Ar-H), 6.40 (d, *J* = 8.2 Hz, 1H,Ar-H), 4.68 (m, 1H, OCH(CH_2)CO), 4.26 (q, *J* = 7.1 Hz, 2H, OCH_2CH$_3$), 2.81-2.61 (m, 2H, CH_2), 2.30-2.09 (m, 2H, CH_2), 1.29 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3).

General procedure for 7-Hydroxychroman-2-carboxylic acid *N*-alkyl amides (4a-g).

To a sloution of 7-hydroxychroman-2-carboxylic acid ethyl ester (**7**) (300 mg, 1.35 mmole) in xylene (10 mL) was added alkylamine (1 mL). The reaction mixture was stirred at refulx for 24 h. After cooling, the reaction mixture was treated with 1*N*-HCl (10 mL) and extracted with EtOAc (3x50 mL). The organic layer was washed with water (3x30 mL) and brine (3x30 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (EtOAc:Hexane=1:2) to give compound **4a-g**.

7-Hydroxychroman-2-carboxylic acid *N*-propylamide (4a)

The product was obtained in 30% yield as a cream colored solid: mp 110~111°C; IR (KBr) 3369, 3218, 1656 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.90 (d, *J* = 8.2 Hz, 1H, Ar-H), 6.45 (d, *J* = 8.2 Hz, 1H, Ar-H), 6.44 (s, 1H, Ar-H), 4.52-4.48 (m, 1H, OCH(CH_2)CO), 3.33-3.26 (m, 2H, NHCH_2CH_2), 2.83-2.62 (m, 2H, PhCH_2CH_2CH), 2.41-2.36 (m, 1H, PhCH_2CH_2CH), 2.04-1.91 (m, 1H, PhCH_2CH_2CH), 1.62-1.50 (m, 2H, CH_2CH_2CH_3), 0.92 (t, 3H, *J* = 7.3 Hz, CH_2CH_3).

7-Hydroxychroman-2-carboxylic acid *N*-butylamide (4b)

The product was obtained in 59% yield as a yellow solid: mp 111~113°C; IR (KBr) 3322, 3166, 1620cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.90 (d, *J* = 7.9 Hz, 1H, Ar-H), 6.48 (d, *J* = 7.9 Hz, 1H, Ar-H), 6.44 (s, 1H, Ar-H), 4.52-

4.48 (m, 1H, OC<u>H</u>(CH$_2$)CO), 3.37-3.29 (m, 2H, NHC<u>H$_2$</u>CH$_2$), 2.83-2.62 (m, 2H, PhC<u>H$_2$</u>CH$_2$CH), 2.42-2.36 (m, 1H, PhCH$_2$C<u>H$_2$</u>CH), 2.04-1.93 (m, 1H, PhCH$_2$C<u>H$_2$</u>CH), 1.57-1.48 (m, 2H, CH$_2$C<u>H$_2$</u>CH$_2$), 1.40-1.27 (m, 2H, CH$_2$C<u>H$_2$</u>CH$_3$), 0.92 (t, *J* = 7.3 Hz, 3H, CH$_2$C<u>H$_3$</u>).

7-Hydroxychroman-2-carboxylic acid *N*-isopentylamide (4c)

The product was obtained in 68% yield as a cream colored solid: mp 116~118°C; IR (KBr) 3361, 3255, 1657 cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 6.89 (d, *J* = 8.0 Hz, 1H, Ar-<u>H</u>), 6.49 (d, *J* = 8.0 Hz, 1H, Ar-<u>H</u>), 6.43 (s, 1H, Ar-<u>H</u>), 4.51-4.47 (m, 1H, OC<u>H</u>(CH$_2$)CO), 3.38-3.31 (m, 2H, NHC<u>H$_2$</u>CH$_2$), 2.81-2.62 (m, 2H, PhC<u>H$_2$</u>CH$_2$CH), 2.42-2.34 (m, 1H, PhCH$_2$C<u>H$_2$</u>CH), 2.04-1.93 (m, 1H, PhCH$_2$C<u>H$_2$</u>CH), 1.64-1.55 (m, 1H, CH$_2$C<u>H</u>(CH$_3$)$_2$), 1.47-1.40 (m, 2H, CH$_2$C<u>H$_2$</u>CH), 0.91 (d, *J* = 6.6 Hz, 6H, CH(C<u>H$_3$</u>)$_2$).

7-Hydroxychroman-2-carboxylic acid *N*-heptylamide (4d)

The product was obtained in 51% yield as a yellow solid: mp 72~73°C; IR (KBr) 3374, 3273, 1665cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 6.91 (d, *J* = 8.0 Hz, 1H, Ar-<u>H</u>), 6.47 (d, *J* = 8.0 Hz, 1H, Ar-<u>H</u>), 6.42 (s, 1H, Ar-<u>H</u>), 4.52-4.48 (m, 1H, OC<u>H</u>(CH$_2$)CO), 3.35-3.28 (m, 2H, NHC<u>H$_2$</u>CH$_2$), 2.83-2.63 (m, 2H, PhC<u>H$_2$</u>CH$_2$CH), 2.43-2.34 (m, 1H, PhCH$_2$C<u>H$_2$</u>CH), 2.05-1.93 (m, 1H, PhCH$_2$C<u>H$_2$</u>CH), 1.55-1.51 (m, 2H, CH$_2$C<u>H$_2$</u>CH$_2$), 1.28-1.27 (m, 8H, CH$_2$(C<u>H$_2$</u>)$_4$CH$_3$), 0.88 (t, *J* = 6.9 Hz, 3H, CH$_2$C<u>H$_3$</u>).

7-Hydroxychroman-2-carboxylic acid *N*-nonylamide (4e)

The product was obtained in 55% yield as a brown solid: mp 65~68°C; IR (KBr) 3361, 3290, 1624cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 6.91 (d, *J* = 8.0 Hz, 1H, Ar-<u>H</u>), 6.46 (d, *J* = 8.0 Hz, 1H, Ar-<u>H</u>), 6.42 (s, 1H, Ar-<u>H</u>), 4.52-4.48 (m, 1H, OC<u>H</u>(CH$_2$)CO), 3.34-3.28 (m, 2H, NHC<u>H$_2$</u>CH$_2$), 2.84-2.63 (m, 2H, PhC<u>H$_2$</u>CH$_2$CH), 2.42-2.36 (m, 1H, PhCH$_2$C<u>H$_2$</u>CH), 2.05-1.92 (m, 1H, PhCH$_2$C<u>H$_2$</u>CH), 1.53 (m, 2H, CH$_2$C<u>H$_2$</u>CH$_2$), 1.26 (m, 12H, CH$_2$(C<u>H$_2$</u>)$_6$CH$_3$), 0.88 (t, *J* = 6.9 Hz, 3H, CH$_2$C<u>H$_3$</u>).

7-Hydroxychroman-2-carboxylic acid *N*-decylamide (4f)

The product was obtained in 52% yield as a brown solid: mp 66~69°C; IR (KBr) 3348, 3295, 1625cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 6.91 (d, *J* = 8.1 Hz, 1H, Ar-<u>H</u>), 6.46 (d, *J* = 8.1 Hz, 1H, Ar-<u>H</u>), 6.42 (s, 1H, Ar-<u>H</u>), 4.52-4.48 (m, 1H, OC<u>H</u>(CH$_2$)CO), 3.34-3.28 (m, 2H, NHC<u>H$_2$</u>CH$_2$), 2.80-2.64 (m, 2H, PhC<u>H$_2$</u>CH$_2$CH), 2.43-2.34 (m, 1H, PhCH$_2$C<u>H$_2$</u>CH), 2.05-1.93 (m, 1H, PhCH$_2$C<u>H$_2$</u>CH), 1.53-1.51 (m, 2H, CH$_2$C<u>H$_2$</u>CH$_2$), 1.26 (m, 14H, CH$_2$(C<u>H$_2$</u>)$_7$CH$_3$), 0.88 (t, *J* = 7.8 Hz, 3H, CH$_2$C<u>H$_3$</u>).

7-Hydroxychroman-2-carboxylic acid *N*-undecylamide (4g)

The product was obtained in 36 % yield as a brown solid: mp 65~68°C; IR (KBr) 3305, 3295, 1623cm^{-1}; ^{1}H NMR (CDCl$_3$, 300 MHz) δ 6.90 (d, *J* = 8.1 Hz, 1H, Ar-<u>H</u>), 6.46 (d, *J* = 8.0 Hz, 1H, Ar-<u>H</u>), 6.42 (s, 1H, Ar-<u>H</u>), 4.52-4.48 (m, 1H, OC<u>H</u>(CH$_2$)CO), 3.35-3.28 (m, 2H, NHC<u>H$_2$</u>CH$_2$), 2.83-2.62 (m, 2H, PhC<u>H$_2$</u>CH$_2$CH), 2.43-2.36 (m, 1H, PhCH$_2$C<u>H$_2$</u>CH), 2.05-1.92 (m, 1H, PhCH$_2$C<u>H$_2$</u>CH), 1.53-1.51 (m, 2H, CH$_2$C<u>H$_2$</u>CH$_2$), 1.25 (m, 14H, CH$_2$(C<u>H$_2$</u>)$_7$CH$_3$), 0.88 (t, *J* = 6.9 Hz, 3H, CH$_2$C<u>H$_3$</u>).

Assay of lipid peroxidation in the rat brain homogenates

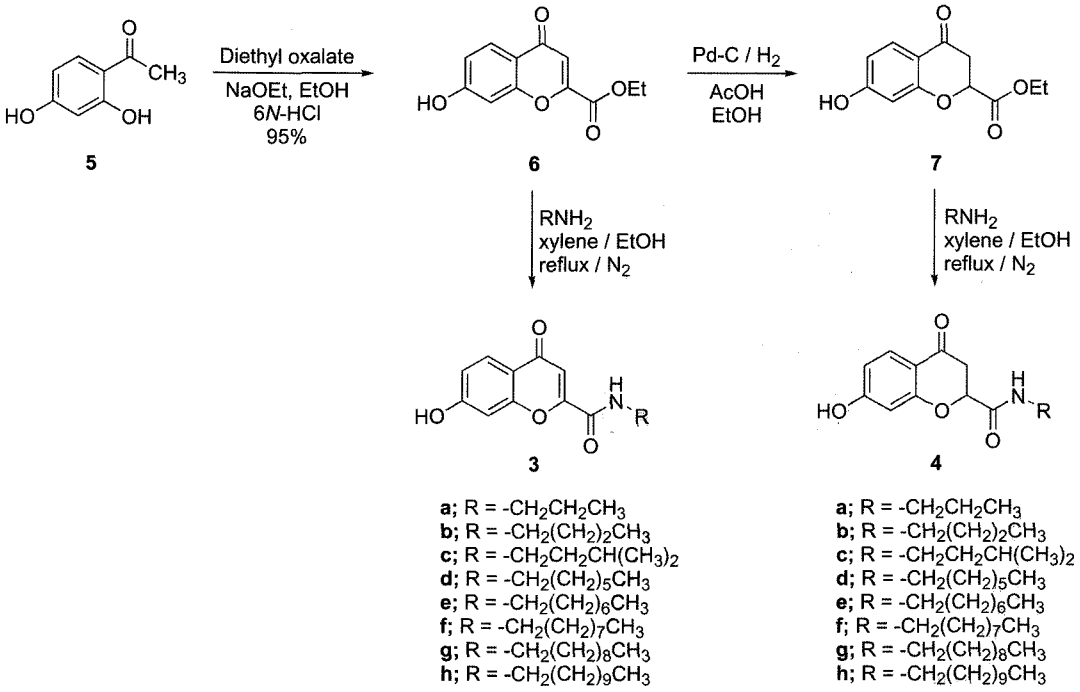
Lipid peroxidation was initiated by Fe^{+2} (10 μM) and *L*-ascorbic acid (100 μM) in the rat forebrain homogenates as described previously (Cho and Lee, 2004). The reaction mixture was incubated at 37°C for 1 h in the absence or presence of various concentrations of compound. The reaction was stopped 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 (%) = 100x(Abs$_{control}$ Abs$_{compound}$)/Abs$_{control}$.

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 as described previously (Cho and Lee, 2004). The percent scavenging activity was calculated using the same formula given in the assay of lipid peroxidation.

RESULTS AND DISCUSSION

The general synthetic strategy employed to prepare the target compounds (**3a-h**) and (**4a-g**) is outlined in Scheme 1. The starting dihydroxyacetophenone (**5**) was treated with sodium ethoxide and diethyl oxalate followed by 6*N*-HCl to give a cyclized 4-oxochromene-2-carboxylic acid ethyl ester (**6**) in 95% yield. The treatment of compound **6** with a series of corresponding alkyl amines (propylamine, n-butylamine, isopentylamine, heptylamine, octylamine, nonylamine, decylamine and undecylamine) in xylene at reflux for 24 h afforded 7-hydroxy-4-oxo-4*H*-chromene-2-carboxylic acid *N*-alkyl amides (**3a-h**) in 32 - 52 % yield. In order to make the target compounds (**4a-g**), compound **6** was reduced to give a chroman intermediate **7**. The reduction was carried out with catalytic hydrogen-



Scheme 1. Synthesis of 7-Hydroxy-4-oxo-4*H*-chromene- (**3a-h**) and 7-Hydroxychroman-2-carboxylic acid *N*-alkylamides (**4a-g**)

ation (10% Pd/C) in EtOH-AcOH at atmospheric pressure. The treatment of compound **7** with a series of corresponding alkyl amines (propylamine, n-butylamine, isopentylamine, heptylamine, nonylamine, decylamine and undecylamine) in xylene at reflux for 24 h afforded 7-hydroxychroman-2-carboxylic acid *N*-alkyl amides (**4a-g**) in 30-68% yield.

In order to evaluate antioxidant properties of the newly synthesized compounds (**3a-h**) and (**4a-g**), the effects on lipid peroxidation in rat brain homogenates were examined using thiobarbituric acid reactive substances (TBARS) assay. The formation of lipid peroxides was significantly inhibited by several test compounds. The % inhibition at the maximal concentration tested in this study (300 μM) and 50% inhibitory concentrations (IC_{50} values) calculated by non-linear regression of the mean values using Prism (Graph Pad Software INC., U.S.A.) are listed in Table I. The value for trolox is also given for comparison.

The previous study revealed that the compounds containing chroman skeleton were more active than those with chromanone skeleton (Lee *et al.*, 2005). This prompted us to prepare the 7-hydroxy-4-oxo-4*H*-chromene (**3a-h**) and 7-hydroxychroman derivatives (**4a-g**). The target compounds were designed to have an enone system and saturated chroman system. A series of *N*-alkyl substituents were introduced on amide nitrogen to explore the antioxidant activity based on the alkyl chain length.

As shown in Table 1, all of the 4-oxochromene-2-

Table I. *In vitro* antioxidant activities of 7-hydroxychroman derivatives

Compounds	Inhibition of lipid peroxidation		DPPH radical scavenging activity (% control)[c]
	% inhibition[a]	IC_{50}[b]	
6	3.6	-	3.5
3a	1.9	-	5.3
3b	5.4	-	4.3
3c	4.7	-	1.0
3d	6.8	-	6.2
3e	3.8		5.1
3f	5.7	-	3.3
3g	9.9	-	29.7
3h	5.0	-	3.7
7	9.3	-	8.5
4a	9.4	-	7.5
4b	15.5	-	3.8
4c	38.0	-	6.0
4d	70.2	112.0	4.6
4e	71.3	58.3	7.6
4f	73.9	57.9	5.5
4g	69.7	60.2	5.2
Trolox	65.7	154.2	96.8

[a] % inhibition of lipid peroxidation at the concentration of 300 μM. [b] IC_{50} = the concentration (μM) exhibiting 50% inhibition of lipid peroxidation. [c] % control of DPPH radical scavenging activity at 300 μM. Each experiment was performed at least three times in duplicate. The results are presented as an average value.

carboxamides (**3a-h**) tested in this study exhibited poor inhibition of lipid peroxidation compared to chroman-2-carboxamides (**4a-g**). This result indicates that enone system of the 4-oxochromene derivatives is detrimental to the inhibitory activity of lipid peroxidation. Among the chroman derivatives (**4a-g** and **7**), compounds **4e**, **4f**, and **4g** bearing *N*-nonyl, -decyl, and -undecyl amide functionality exhibited 3 times more potent inhibition than trolox. Compound **4d** showed comparable inhibitory activity to trolox.

The radical scavenging effects were also examined in the present study using radicals generated by DPPH (Cho and Lee, 2004). None of compounds exhibited significant radical scavenging activities. They were much less potent than our previous chroman-2-carboxamides (**2**) (Lee *et al.*, 2005). This result suggests that the position of the hydroxy substituent of chroman nucleus may be necessary to be para to the chroman ring oxygen atom. The poor activity of the target compounds might be ascribed to the lack of *o*-electron donating methoxy substituent of the phenolic compounds which is known to increase the stability of the radical and hence, the antioxidative activity (Rajan *et al.*, 2001). Work is in progress to design, synthesize, and evaluate additional compounds in this and related systems.

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