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Synthesis of 6-Hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid *N*-Alkyl Amides and their Antioxidant Activity

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A series of 6-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid *N*-alkyl amides (**3a** - **g**) were synthesized and their antioxidant activities were evaluated using rat brain homogenates.

Key words: Antioxidant Activity, Chromene-2-carboxylic acid *N*-alkyl amides

INTRODUCTION

Low levels of reactive oxygen species (ROS) are continuously generated in the cells of aerobic organisms, and are likely to play important physiological roles, particularly 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 neurological diseases, particularly neurodegenerative diseases, as well as cardiovascular diseases and cancer (Gilgun-Sherki *et al.*, 2001; Gilgun-Sherki, *et al.*, 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 cell damage (Braughler *et al.*, 1986; Halliwell *et al.* 1989; Roberfroid *et al.* 1995). The lipids from biological membranes, particularly those in the brain, contain polyunsaturated fatty acids that are particularly vulnerable to damage caused by ROS (Andersen *et al.*, 2004; Barnham *et al.*, 2004). Moreover, the brain contains considerable amounts of pro-oxidant transition metal ions and uses a significant amount of oxygen on account of its high metabolic activity. Lipid peroxidation is an important mediator of the pathophysiological events in several disorders of the central nervous system such as cerebral ischemia and trauma (Hall *et al.*, 1991). Lipid peroxidation is induced by free radicals, which are the major components of ROS. Many natural and synthetic antioxidants that inhibit lipid peroxidation have been reported to retard oxidative damage and disease progression (Maxwell *et al.*, 1995).

D-α-Tocopherol (vitamin E) is the most important and widely studied natural, lipid-soluble, chain-breaking antioxidant. Recent studies have highlighted the protective effects of vitamin E against atherosclerosis and reperfusion injury (Stephens *et al.*, 1996; Vendetti *et al.*, 2000). Trolox (6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid) (**1**) is a hydrophilic analog of vitamin E and a chain-breaking antioxidant that acts as a radical scavenger via the H-donating group in its chromanol nucleus (Tsuchiya *et al.*, 1992). Its protective effects against oxidative damage, particularly against lipid peroxidation, have been demonstrated both *in vitro* and *in vivo* (Wu *et al.*, 1990; Forrest *et al.*, 1994). The conversion of the natural carboxylic acids to the amide functionality was reorted to improve their antioxidant activity (Vairagupta *et al.,* 2000). Several 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 recently reported the synthesis and the antioxidant activities of chroman-2-carboxamide derivatives (**2**) (Lee *et al.*, 2005). In the present study, a series of 4-oxochromene-2-carboxylic acid *N*-alkyl amides (**3**) were synthesized (Fig. 1), and their antioxidant activities were evaluated. Hydroxy and methoxy substituents of the target compounds can be found in a number of natural antioxidants including curcumin (Priyadarsini *et al.*, 2003) and eugenol (Naderi *et al.*, 2004). Various alkyl substituents on the amide nitrogen were introduced to examine the structure-activity relationship. The antioxidant activity was determined by measuring the inhibition of lipid peroxidation initiated by

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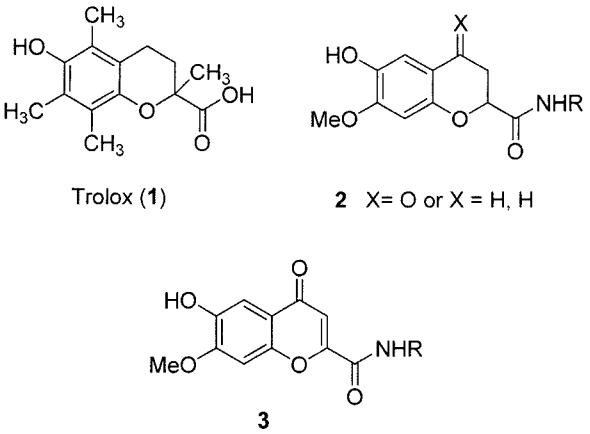


Fig. 1. Structures of compound **1-3**

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 those obtained from trolox (**1**) (Fig. 1).

MATERIALS AND METHODS

The melting points were recorded on a Electrothermal IA9100 digital melting point apparatus and were uncorrected. The IR spectra were determined using a Jasco FT/IR-300E spectrophotometer and the peak absorbances are reported as cm^{-1}. The ^{1}H-NMR spectra were recorded on Bruker DPS300 NMR spectrometer using TMS as an internal standard and the chemical shifts are reported as ppm (δ). Unless stated otherwise, all commercially available regents and solvents were used as received without further purification.

1-(2,5-Dihydroxy-4-methoxyphenyl)ethanone (5)

Acetyl chloride (1.65 g, 21.4 mmole) was added to a solution of 2-methoxybenzene-1,4-diol (**4**) (3 g, 21.4 mmole) in 1,2-dichloroethane (100 mL) in ice bath and treated with $AlCl_3$ (2.85 g, 21.4 mmole). The reaction mixture was stirred under reflux for 2 h under a nitrogen atmosphere. After cooling, the mixture was treated with H_2O (50 mL) for 5 min and extracted with CH_2Cl_2 (3 × 40 mL). The organic layer was concentrated in vacuo and the residue was purified by flash column chromatography (1 : 4 = EtOAc : Hexane) to give 1-(2,5-Dihydroxy-4-methoxyphenyl) ethanone (**5**) (1.84 g, 51%) as an oil: IR (KBr) 3446, 2999, 1705, 1644 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 6.89 (s, 1H, Ar-H), 6.72 (s, 1H, Ar-H), 3.84 (s, 3H, OCH_3), 2.29 (s, 3H, $COCH_3$).

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

Sodium (5.2 g, 230.4 mmole) was dissolved in abs EtOH (100 mL) and compound **5** (7 g, 38.4 mmole) and diethyloxalate (16.8 g, 115.2 mmole) were added. The reaction mixture was stirred under reflux for 2 h. After cooling, the mixture was treated with 6*N*-HCl (100 mL) for 10 min and extracted with CH_2Cl_2 (3 × 100 mL). The organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was dissolved in EtOH (70 mL) and treated with *c*-HCl (3 mL). The mixture was stirred under reflux for 24 h. The mixture was concentrated in vacuo and purified by flash column chromatography (1 : 2 = EtOAC : Hexane). Recrystallization of the residue from EtOAc and ether afforded 6-Hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid ethyl ester (**6**) (1.9 g, 19%) as a yellow solid: mp 160~162°C; IR (KBr) 3334, 2997, 1691, 1678 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.78 (s, 1H, COCH), 6.83 (s, 1H, Ar-H), 6.80 (s, 1H, Ar-H), 4.43 (q, J = 7.1 Hz, 2H, OCH_2CH_3), 3.97 (s, 3H, OCH_3), 1.41 (t, J = 7.1 Hz, 3H, OCH_2CH_3).

General procedure for 6-Hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid *N*-alkyl amides (3a-g)

To a solution of compound **6** (263 mg, 0.99 mmole) in toluene (20 mL) was added alkylamine (1 mL). The reaction mixture was stirred under reflux for 24 h. After cooling, the precipitate was filtered to give compound **3a-g**.

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

The product was obtained as a yellow solid in 37% yield: mp 210~213°C; IR (KBr) 3285, 3206, 1675, 1652 cm^{-1}; ^{1}H-NMR (CD_3OD, 300 MHz) δ 7.00 (s, 1H, COCH=C), 6.87 (s, 1H, Ar-H), 6.17 (s, 1H, Ar-H), 3.85 (s, 3H, OCH_3), 3.28-3.21 (m, 2H, $NHCH_2CH_2CH_3$), 1.59-1.52 (m, 2H, $NHCH_2CH_2CH_3$), 0.91 (t, J = 7.1 Hz, 3H, $NHCH_2CH_2CH_3$).

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

The product was obtained as a yellow solid in 37% yield: mp 206~209°C; IR (KBr) 3298, 3229, 1675, 1641 cm^{-1}; ^{1}H-NMR (CD_3OD, 300 MHz) δ 6.99 (s, 1H, COCH=C), 6.87 (s, 1H, Ar-H), 6.16 (s, 1H, Ar-H), 3.84 (s, 3H, OCH_3), 3.28 (t, 2H, J = 6.98 Hz, $NHCH_2CH_2$), 1.55-1.46 (m, 2H, $CH_2CH_2CH_2$), 1.39-1.26 (m, 2H, $CH_2CH_2CH_3$), 0.88 (t, J = 7.06 Hz, 3H, CH_2CH_3).

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

The product was obtained as a yellow solid in 38% yield:

mp 225~227°C; IR (KBr) 3295, 3217, 1676, 1641 cm^{-1}; ^{1}H-NMR (CD_3OD, 300 MHz) δ 6.96 (s, 1H, COCH=C), 6.86 (s, 1H, Ar-H), 6.13 (s, 1H, Ar-H), 3.83 (s, 3H, OCH_3), 3.31 (t, 2H, *J* = 7.2 Hz, $NHCH_2CH_2$), 1.61-1.53 (m, 1H, $CH_2CH(CH_3)_2$), 1.43-1.39 (m, 2H, $CH_2CH_2CH(CH_3)_2$), 0.86 (d, *J* = 6.5 Hz, 6H, $CH(CH_3)_2$).

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

The product was obtained as a yellow solid in 27% yield: mp 208~209°C; IR (KBr) 3296, 3217, 1675, 1646 cm^{-1}; ^{1}H NMR (CD_3OD, 300 MHz) δ 7.00 (s, 1H, COCH=C), 6.89 (s, 1H, Ar-H), 6.16 (s, 1H, Ar-H), 3.85 (s, 3H, OCH_3), 3.27 (t, 2H, *J* = 7.0 Hz, $NHCH_2CH_2$), 1.55-1.50 (m, 2H, $CH_2CH_2CH_2$), 1.29-1.12 (m, 8H, $CH_2(CH_2)_4CH_3$), 0.80 (t, *J* = 6.8 Hz, 3H, CH_2CH_3).

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

The product was obtained as a yellow solid in 40% yield: mp 204~206°C; IR (KBr) 3298, 3211, 1675, 1641 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 7.09 (s, 1H, COCH=C), 7.06 (s, 1H, Ar-H), 6.25 (s, 1H, Ar-H), 3.86 (s, 3H, OCH_3), 3.25-3.15 (m, 2H, $NHCH_2CH_2$), 1.50-1.48 (m, 2H, $CH_2CH_2CH_2$), 1.24 (m, 12H, $CH_2(CH_2)_6CH_3$), 0.80 (t, *J* = 6.8 Hz, 3H, CH_2CH_3).

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

The product was obtained as a yellow solid in 34% yield: mp 211~212°C; IR (KBr) 3305, 3217, 1675, 1641 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 7.10 (s, 1H, COCH=C), 7.08 (s, 1H, Ar-H), 6.27 (s, 1H, Ar-H), 3.87 (s, 3H, OCH_3), 3.23 (m, 2H, $NHCH_2CH_2$), 1.51 (m, 2H, $CH_2CH_2CH_2$), 1.25 (m, 14H, $CH_2(CH_2)_7CH_3$), 0.85 (t, *J* = 6.8 Hz, 3H, CH_2CH_3).

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

The product was obtained as a yellow solid in 38% yield: mp 210~211°C; IR (KBr) 3299, 3211, 1675, 1635 cm^{-1}; ^{1}H-NMR (DMSO, TMS) δ 7.11 (s, 1H, COCH=C), 7.07 (s, 1H, Ar-H), 6.27 (s, 1H, Ar-H), 3.87 (s, 3H, OCH_3), 3.25-3.23 (m, 2H, $NHCH_2CH_2$), 1.51 (m, 2H, $CH_2CH_2CH_2$), 1.24 (m, 16H, $CH_2(CH_2)_8CH_3$), 0.84 (t, *J* = 6.8 Hz, 3H, CH_2CH_3).

Assay of lipid peroxidation in the rat brain homogenates

Lipid peroxidation in the rat forebrain homogenates was initiated by Fe^{+2} (10 μM) and *L*-ascorbic acid (100 μM). The reaction mixture was incubated at 37°C for 1 h in the presence or absence of various concentrations of the compound. The reaction was quenched by adding trichloroacetic acid (28% w/v) and thiobarbituric acid (1% w/v) in succession. The mixture was then heated at 100°C for 15 min. After centrifugation to remove the precipitates, the absorbance was measured at 532 nm using a VERSA$_{max}$ microplate reader (Molecular Devices, U.S.A.). The percentage inhibition was calculated using the following formula:

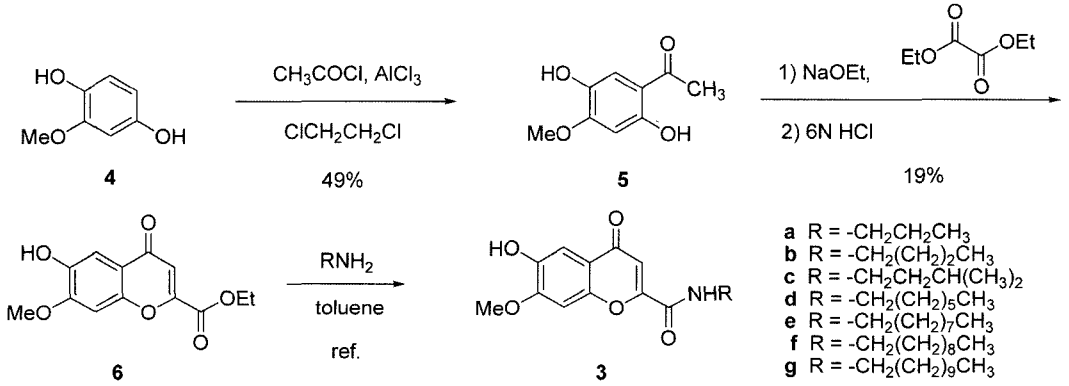
$$\text{Inhibition (\%)} = 100 \times (Abs_{control}\ Abs_{compound})/Abs_{control}.$$

Assay for DPPH radical scavenging activity

The reaction mixture containing various concentrations of the compounds and a methanolic DPPH solution (150 μM) was incubated at 37°C for 30 min and the absorbance was measured at 520 nm. The percent scavenging activity was calculated using the same formula used in the lipid peroxidation assay.

RESULTS AND DISCUSSION

Scheme 1 outlines the general synthetic strategy used to prepare the target compounds (**3a-g**), which was based on the Friedel-Crafts acylation of methoxyhydroquinone (**4**) with acetyl chloride. The starting hydroquinone **4** was treated with acetyl chloride in the presence of $AlCl_3$ in dichloroethane to give 1-(2,5-dihydroxy-4-methoxyphenyl)



Scheme 1. Synthesis of 6-Hydroxy-7-methoxy-4-oxo-4*H*-chromene-2- carboxylic acid *N*-alkylamides

ethanone (**5**) in 51% yield (Crombie *et al.*, 1985). Compound **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 19% yield. Compound **6** was treated with the corresponding alkyl amines (propylamine, *n*-butylamine, isopentylamine, heptylamine, nonylamine, decylamine and undecylamine) in toluene under reflux for 24 h to give the 6-hydroxy-7-methoxy-4-oxo-4*H*-chromene-2-carboxylic acid *N*-alkyl amides (**3a-g**) in 27-40% yield.

The antioxidant properties of the newly synthesized 4-oxochromene derivatives (**3a-g**) were evaluated by investigating their effects on lipid peroxidation in rat brain homogenates using a thiobarbituric acid reactive substances (TBARS) assay according to the method reported elsewhere (Cho and Lee, 2004). The test compounds marginally inhibited the formation of lipid peroxides at levels ranging from 10 to 54%. Table I lists the % inhibitory concentrations at 300 μM. The value for trolox is given for comparison.

It was previously reported that compounds containing the chroman skeleton were more active than those containing the chromanone skeleton (Lee *et al.*, 2005). This prompted us to prepare the 4-oxochromene derivatives (**3a-g**). The target compounds were designed to have an enone system instead of the previous chroman system (**2**). *N*-Alkyl side chain of the target compounds was introduced to examine the antioxidant activity according to the alkyl chain length. Various *N*-alkyl substituents were introduced on amide nitrogen in an attempt to delineate the structure activity relationship. The inhibition of lipid peroxidation is a multifactorial event. The propensity for radical formation and stabilization, the ability of metal complexation and lipophilicity are important factors for the inhibitory activity. The presence of an *o*-electron donating methoxy substituent in phenolic compounds is known to increase the stability of the radical and hence, the antioxidative activity (Rajan *et al.*, 2001), which contribute to the formation of a complex with iron. Therefore, the target compounds were designed to have both hydroxy and methoxy substituents on the 4-oxochromene ring.

Table I. *In vitro* antioxidant activity (% inhibition in 300 μM) of the 4-oxochromene derivatives

Compounds	LPO (%)	DPPH (%)
6	54.1	28.2
3a	21.5	17.1
3b	12.7	14.5
3c	26.6	17.5
3d	14.0	15.6
3e	17.2	14.7
3f	9.5	17.2
3g	12.2	16.6
Trolox	65.7	96.8

As shown in Table I, all the 4-oxochromene-2-carboxamides (**3a-g**) tested were poor inhibitors of lipid peroxidation compared with chroman-2-carboxamides (**2**) (Lee *et al.*, 2005). This indicates that the enone system of the 4-oxochromene derivatives is detrimental to the inhibitory activity of lipid peroxidation. Among the 4-oxochromene derivatives (**3a-g** and **6**), only 4-oxochromene-2-carboxylic acid ethyl ester (**6**) exhibited comparable inhibitory activity to that of trolox (Table I).

The radical scavenging effects were also examined using radicals generated by DPPH (Cho and Lee, 2004). Like the inhibitory effects on lipid peroxidation, the 4-oxochromene derivatives (**3a-g** and **6**) exhibited less potent radical scavenging activities than the chroman-2-carboxamides (**2**) reported previously (Lee *et al.*, 2005). This suggests that the saturated chroman ring system may be essential for good activity. Further studies aimed at designing, synthesizing, and evaluating additional compounds in this and related systems are currently underway.

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