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Chroman Amide and Nicotinyl Amide Derivatives: Inhibition of Lipid Peroxidation and Protection Against Head Trauma

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A series of chroman amide and nicotinyl amide derivatives was designed and synthesized for the treatment of traumatic and ischemic CNS injury. Five compounds were significantly more potent inhibitors of lipid peroxidation *in vitro* than the reference antioxidant, trolox ($p < 0.01$). Quantitative structure activity studies demonstrated that the inhibitory action was related to the ability to donate electrons, charge on hydroxy group and E_{LUMO}, to scavenging radicals and to the lipophilicity log *P*, which determines penetration of membrane lipids. ESR study indicated the ability of **12** to scavenge the hydroxyl radicals. The most promising compound, [(3,4-dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2yl)carbonyl]-3′-(aminoethyl) indole (**12**), inhibited *ex vivo* lipid peroxidation in a head injury model and showed potent *in vivo* neuroprotective efficacy. Improvement of neurological recovery within 1 h of injury (grip test score) by as much as 200% was observed together with significant anti-anoxia activity. Compound **12** was a potent antagonist of methamphetamine-induced hypermotility resulting from dopamine release in the mouse brain. These results support the importance of cerebroprotective radical-scavenging agents for the treatment of traumatic injury and anoxia as well as provide additional evidence for the role of oxygen radicals and dopamine in brain damage.

Keywords: Antioxidant, trolox, cerebroprotective radical scavenger, brain trauma, anoxia, antioxidative vitamins, QSAR, ESR

INTRODUCTION

Lipid peroxidation is an important pathophysiological process in degenerative diseases,[1] atherosclerosis,[2,3] ischemic–reperfusion injury,[4,5] myocardial infarction[6] and cancer.[7] The major agents responsible for these phenomena are reactive oxygen species (ROS), namely superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($HO^{\bullet}$) and lipid radicals ($ROO^{\bullet}$, $RO^{\bullet}$). These radicals are generated *in vivo* under stress

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conditions and damage the organelles at the molecular level. Molecules with highly oxidizable structures, such as unsaturated fatty acids, are sensitive to radicals, therefore, membrane lipids, especially those in the spinal cord and brain which contain high levels of polyunsaturated fatty acids, are particularly affected.[8] Moreover, the brain contains considerable amounts of prooxidant transition metal ions and utilizes large quantities of oxygen. These properties set the stage for ROS generation and subsequent acute cellular injury following traumatic brain injury and cerebral ischemia and reperfusion.[9–12]

As ROS formation and the oxidation of macromolecules are implicated in acute and chronic CNS disorders, agents that can interfere with these processes may be useful therapeutically. Many antioxidants have been developed for the treatment of CNS trauma and ischemia by structural modification of anti-oxidative vitamins; for example, vitamin E derivatives such as trolox and its analog[13] and 2,3-dihydro-1-benzofuran-5-ols;[14] and vitamin C derivatives synthesized as antiradicals, including 2-O-alkylascorbic acid[15] and 3-O-alkylascorbic acid.[16] Recently, the 21-aminosteroid U74006F,[17] 1-(acylamino)-7-hydroxyindan derivatives,[18] the glutathione derivative Y-737,[19] indole carboxamide derivatives[20] and 5-aminocoumarans[21] were found to be promising antiradicals. Consideration of the structures of the newly developed compounds shows that most contain nuclei bearing an hydroxyl group and/or an amino group.

Chroman amides and nicotinyl amides having the general formulae given in Figure 1 were synthesized and evaluated in this study. The inhibitory action of antiradical agents against lipid peroxidation is related to the ability of the molecule to donate electrons to scavenging radicals and the lipophilicity of the molecule which determines penetration of the membrane lipid. Structural modification was therefore accomplished by varying the chemical functional groups on both sides of amides. The acid parts were the chromanol portion of vitamin E, nicotinic acid and 6-hydroxynicotinic acid; the amine parts were a heterocyclic system connected with an alkyl chain.

Antilipid peroxidation activity was evaluated and the structure/physicochemical properties of the synthesized compounds were determined in order to study the quantitative structure activity relationship (QSAR). The *in vivo* cerebroprotective effects against traumatic injury and anoxia were determined for the two most promising compounds. *Ex vivo* lipid peroxidation of **12** in the mouse brain after traumatic injury was measured to investigate the role of antiradicals in cerebroprotection. The ability of **12** to scavenge radicals was investigated using ESR.

In addition to oxygen radicals, dopamine is released after brain injury and ischemia. There are several possible mechanisms through which dopamine could exacerbate the cell damage produced by cerebral ischemia and trauma. The oxidation of dopamine by molecular oxygen results in the formation of superoxide anion.[22] The reaction between dopamine and hydroxyl radicals generates the neurotoxin, 6-hydroxydopamine.[23] Experimental evidence suggests that decrease in brain dopamine levels protects brain tissues from ischemic damage.[24] Oxygen radicals and dopamine are components of an interactive cascade leading to membrane damage and cell death. Consequently, compounds capable of regulating these factors might be able to limit post-traumatic tissue damage and enhance neurological recovery. Antagonism *in vivo* of the metamphetamine (MAP)-induced hypermotility resulting from dopamine release in the CNS by **12** was therefore investigated.

MATERIALS AND METHODS

Chemistry

Melting points of the compounds were determined on a Buchi capillary melting point apparatus and uncorrected. Infrared (IR) spectra were

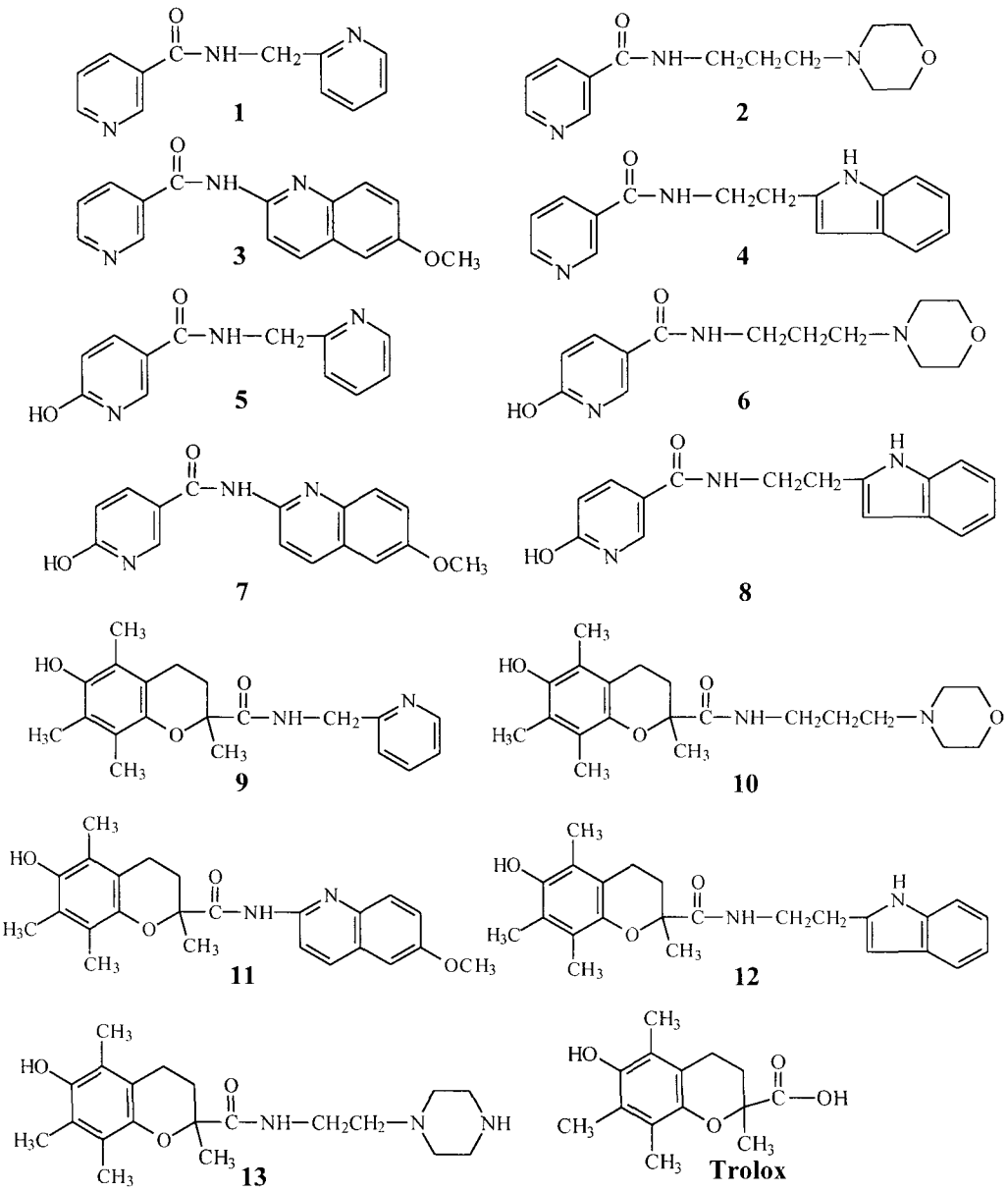

FIGURE 1 Structures of compounds **1–13** and trolox.

run on an FTIR Nicolet 500 as a potassium bromide pellet. Proton nuclear magnetic resonance (^{1}H NMR) spectra were obtained with a JEOL JNM-A-500 (500 MHz). Chemical shifts were reported in ppm related to the internal standard, tetramethylsilane. Mass spectrophotometry (MS) was done using a JEOL FX 900 (90 MHz). Thin-layer chromatography (TLC) was carried out on Merck Kieselgel 60 F_{254} plates and the purified compounds each showed a single spot. Chromatographic purification was performed on silica gel columns; the silica gel used for column chromatography was Merck Kieselgel 60 of 0.063–0.200 mm. Analytical results obtained

from an elemental analyzer (Perkin Elmer PE 400) were within ±0.4% of the theoretical value for all compounds. Spectral (IR, MS, NMR) data were compatible with the assigned structures in all cases.

The general procedures for the synthesis of the amides involved the reaction of selected carboxylic acids with the amino compounds by coupling with N,N′-carbonyldiimidazole (CDI). Compound **13** was synthesized by coupling with dicyclohexylcarbodiimide (DCC) and N-hydroxysuccinimide.[25] All starting materials were commercially available except 2-amino-6-methoxyquinoline which was prepared by the reported method.[26]

The reaction conditions varied according to the starting materials. Pyridine was used as reaction medium in the preparation of nicotinyl amides and hydroxynicotinyl amides while tetrahydrofuran (THF) was used for preparing trolox amides. For all amides except amides carrying 6-methoxyquinoline, the reaction was performed at room temperature for 24 h after adding the amine. For the preparation of amides containing 6-methoxyquinoline, the reaction was refluxed for 8 h. The products were isolated from the reaction mixture by extraction and/or column chromatography. The physical properties of the compounds synthesized are listed in Table I. Representive methods used to prepare the target amides are described in the following examples.

[*(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2yl) carbonyl*]-2′-*amino-6′-methoxyquinoline* (**11**) Carbonyldiimidazole (0.162 g, 1 mM) was added to a solution of trolox (0.250 g, 1 mM) in THF (1 ml) and the mixture was stirred for 2 h at room temperature. Then, 2-amino-6-methoxyquinoline (0.174 g, 1 mM) in THF (3 ml) was added dropwise to the stirring mixture. The reaction mixture was refluxed for 8 h and then evaporated to dryness under reduced pressure. The resulting residue was purified by column chromatography ($CHCl_3/CH_3OH$ 5 : 1). Recrystallization from ethanol/water gave **11**, 0.277 g (68.07% yield), m.p. 203–204°C. IR (KBr) (cm^{-1}): 3386 (O–H), 3070 (N–H), 2992–2970 (aromatic C–H), 2937–2842 (aliphatic C–H), 1697 (C=C), 1624–1584 (aromatic C=C, cyclic C=N, N–H), 1494 (N–CO), 1351–1321 (aromatic C–N), 1252–1233 (aliphatic C–N), 1127–1090 (C–O), 833–815 (aromatic C–H). ^1H NMR ($CDCl_3$): δ 1.63 (s, 3H, 2-C$\underline{H}_3$), 2.01 (m, 1H, H3$_a$), 2.10 (s, 3H, 8-C$\underline{H}_3$), 2.20 (s, 3H, 7-C$\underline{H}_3$), 2.35 (s, 3H, 5-C$\underline{H}_3$), 2.45 (m, H3$_b$), 2.69 (q, *J* 6.41 Hz, 2H, H4), 3.91 (s, 3H, 6′–OC$\underline{H}_3$), 4.33 (s, exchangeable with D_2O, 6-O$\underline{H}$), 7.05 (d, *J* 2.7 Hz, H5′), 7.30 (dd, *J* 2.74 and 9.16 Hz, H4′), 7.73 (d, *J* 9.15 Hz, H3′), 8.05 (d, *J* 8.85 Hz, H7′), 8.37 (d, *J* 8.85 Hz, H8′), 9.05

TABLE I Chroman, nicotinyl and hydroxynicotinyl amides

Cpd. no	Formula	Recrystallization solvent	m.p. (°C)	% Yield[a]
1	$C_{12}H_{11}N_3O_2 \cdot 1/2H_2O$	—	—[b]	57
2	$C_{13}H_{19}N_3O_2 \cdot H_2O$	Ethanol	123.5–124.5	50
3	$C_{16}H_{13}N_3O_2$	Ethanol	198–198.5	59
4	$C_{16}H_{15}N_3O$	Acetone–water	100–101	72
5	$C_{12}H_{11}N_3O_2 \cdot H_2O$	Ethanol–ether	124–125	50
6	$C_{13}H_{19}N_3O_3 \cdot H_2O$	—	103.5[c]	64
7	$C_{16}H_{13}N_3O_3 \cdot H_2O$	Ethanol	283–283.5	73
8	$C_{16}H_{15}N_3O_2$	Ethanol–water	229 (d)	74
9	$C_{20}H_{25}N_2O_3$	Ethyl acetate	142–144	52
10	$C_{21}H_{32}N_2O_4$	Ethyl acetate	122–124	57
11	$C_{24}H_{26}N_2O_4$	Ethanol–water	203–204	68
12	$C_{24}H_{28}N_2O_3$	Ethanol–water	173–175	74
13	$C_{20}H_{31}N_3O_3$	Ether	174–175	55

[a]No attempts were made to optimize yields.[b]Viscous brown liquid.[c]Boiling point.

(s, exchangeable with D_2O, CO–NH). Anal. – Calcd. for $C_{24}H_{26}N_2O_4$: C, 70.91; H, 6.46; N, 6.89. Found: C, 71.22; H, 6.71; N, 7.17.

[*(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2yl)carbonyl*]-*3′ (aminoethyl)indole* (**12**) Carbonyldiimidazole (0.162 g, 1 mM) was added to a solution of trolox (0.250 g, 1 mM) in THF (1 ml) and the mixture was stirred for 2 h at room temperature. Then, tryptamine (0.160 g, 1 mM) in THF (3 ml) was added dropwise to the stirring mixture. The reaction mixture was stirred at room temperature for 24 h and then evaporated to dryness under reduced pressure. The residue was acidified by addition of 1 M HCl to pH 6 and extracted with methylene chloride. The combined extract was washed (water) and evaporated under reduced pressure. Recrystallization from ethanol : water (9 : 1) gave **12**, 0.292 g (74.40% yield), m.p. 173–175°C. IR (KBr) (cm^{-1}): 3347–3338 (O–H), 3307 (N–H), 2983 (aromatic C–H), 2955–2859 (aliphatic C–H), 1642 (C=O), 1536 (aromatic C=C, cyclic C=N, N–H), 1460 (N–CO), 1357–1339 (aromatic C–N), 1290–1260 (aliphatic C–N), 1108 (C–O), 744–737 (aromatic C–H). ^1H NMR ($CDCl_3$): δ 1.47 (s, 3H, 2-C$\underline{H}_3$), 1.82 (m, 1H, H3$_a$), 1.89 (s, 3H, 8-C$\underline{H}_3$), 2.06 (s, 3H, 7-C$\underline{H}_3$), 2.13 (s, 3H, 5-C$\underline{H}_3$), 2.36 (m, 1H, H3$_b$), 2.50 (m, 1H, H4$_a$), 2.59 (m, 1H, H4$_b$), 2.83 (m, 1H, NH–CH$_2$–C$\underline{H}_a$H$_b$-indole), 2.91 (m, 1H, NH–CH$_2$–CH$_a\underline{H}_b$-indole), 3.52 (m, 1H, NH–C$\underline{H}_a$H$_b$–CH$_2$-indole), 3.64 (m, 1H, NH–CH$_a\underline{H}_b$–CH$_2$-indole), 4.33 (s, exchangeable with D_2O, 6-O$\underline{H}$, 6.51 (t, *J* 5.80 Hz, exchangeable with D_2O, CO–N$\underline{H}$), 6.61 (d, *J* 2.44 Hz, H2′), 7.09 (t, *J* 7.48 Hz, H5′), 7.18 (t, *J* 7.63 Hz, H6′), 7.34 (d, *J* 8.24 Hz, H4′), 7.55 (d, *J* 7.92 Hz, H7′), 7.90 (s, exchangeable with D_2O, NH1′). Anal. – Calcd. for $C_{24}H_{28}N_2O_3$: C, 73.43; H, 7.20; N, 7.14. Found: C, 73.08; H, 7.05; N, 7.18.

Evaluation of Antilipid Peroxidation Activity

In Vitro Antilipid Peroxidation

The supernatant fraction of pig brain homogenate was prepared according to the method of Stock *et al.*[27] The diluted homogenate was stored at −80°C for up to 8 weeks. Lipid peroxidation was assessed by the formation of thiobarbituric acid (TBA)-reactive substances (e.g. malondialdehyde) as described previously.[28] Most compounds were prepared in water or water : ethanol and were diluted serially. IC_{50} values of compounds showing inhibition greater than 65% were determined.

Ex Vivo Antilipid Peroxidation in Normal and Head Injury Model

Groups of 5 male Swiss albino mice (27–33 g) were subjected to a concussive head injury (force of 1500 g cm). Compound **12** (50 and 100 mg/kg) or vehicle was administered subcutaneously after the injury. The brain was excised 1 h post administration of test compound or vehicle and homogenized in ice-cold potassium phosphate buffer (1 : 19, w/v). The mixture was centrifuged after addition of perchloric acid (35%, v/v). Thiobarbituric acid (1%, v/v in 50% glacial acetic acid) was added to the supernatant and the mixture was heated to 100°C for 15 min. The fluorescence of TBA-reactive substances was measured at 515 nm (excitation wavelength) and 553 nm (emission wavelength).

Acute Toxicity

Swiss albino mice weighing 27–33 g were used in groups of 10 animals for each test compound. Survival was observed over 7 days after intraperitoneal (i.p.) administration of **11** and **12** at the dose of 500 mg/kg, 10 times the *in vivo* testing dose.

Cerebroprotective Effect

Effect Against Mouse Head Injury[17]

Groups of 8 male Swiss albino mice (27–33 g) were subjected to a concussive head injury (force of 1500 g cm) that resulted in immediate unconsciousness (loss of righting reflex). Each mouse received vehicle (DMSO) or test compound (**11**, **12**) at a dose of 50 mg/kg; i.p. within 5 min post

injury. At 1 h after injury, the sensorimotor status of the head-injured mice was tested using a grip test. The mice were individually picked up by the tail and placed on a taut string 60 cm in length suspended 40 cm above a padded table between two upright metal bars. Care was taken so that both front paws came in contact with the string. The tail was gently released, at which time the mouse either fell, due to inability to hold on, or maintained on the string. The mean grip test score (the mean time in seconds that mice could remain on a grip string in some manner i.e., one to four paws, tail or paws plus tail) was measured for each treatment group. Comparisons between vehicle and drug treated mice were made using Duncan's multiple range test.

Anti-Anoxic Activity[29]

Groups of 8 male Swiss albino mice (27–33 g) were used. Two mice were placed in a 1 l glass container into which a current of nitrogen gas was passed continuously at a flow rate of 2 l/min. Vehicle or test compound was administered 30 min before this treatment. Time before respiratory cessation was recorded as the survival time. Comparisons between vehicle and drug treated mice were made using Duncan's multiple range test.

Suppression of MAP-Induced Hypermotility in Mice

Groups of 6 male ddY mice (25–35 g) were used. One hour after injection of vehicle or **12** (50 mg/kg, i.p.), MAP at a dose of 1 mg/kg or normal saline was administered i.p. to increase the locomotor activity. The negative control group had vehicle alone. Three minutes after injection of MAP or normal saline, the locomotor activity of mice was monitored for 60 min using Animex Auto.

QSAR Analysis

Biological Data for QSAR Study

The *in vitro* percent inhibition of lipid peroxidation and trolox was used as the dependent variable.

QSAR Study

The selected structure and physicochemical properties used were molecular orbital energy (E_{LUMO} and E_{HOMO}), charge, lipophilicity (log P) and total refractivity (MR). The structures were minimized using the conjugation method and then subjected to full energy optimization using semi-empirical AM1 Halmitonian in MOPAC program (Interface/MOPAC option of Sybyl 6.3). The electronic descriptors were molecular orbital energy (E_{LUMO} and E_{HOMO}) and charge of hydroxyl group (C_{OH}). The hydrophobic or lipophilic descriptors were the log P calculated from program Molgen 4.0. MR was obtained from Apex-3D program (Biosym/MSI). The intercorrelations between these independent descriptors were determined to avoid colinearity. Regression analysis was performed on a Pentium-133 Mz PC using multiple linear regression (MLR) in SPSS for Windows Release 7.

Statistical Method

MLR was used to develop the QSAR model. The reliability and predictive ability of the model were considered by multiple correlation coefficients (r^2) standard deviation (S), F-test values and predictive r^2 or cross-validated r^2 or Q^2. The uncertainty of the prediction was expressed in terms of S_{PRESS}.

Electron Spin Resonance Study

For evaluation of $HO^{\bullet}$ trapping ability, **12** and trolox were examined as spin traps using ESR spectroscopy and compared to α-pheny-*tert*-butylnitrone (PBN). $CuSO_4/H_2O_2$ reaction was used to generate $HO^{\bullet}$. Compound **12** or the mixture of **12** (final concentration, 5–75 mM) and PBN (final concentration 25 mM) was added to a test tube containing the freshly solution mixture between $CuSO_4$ (final concentration, 0.1 mM) and H_2O_2 (final concentration, 10 mM), and phosphate buffer pH 7.4 was added to a final volume of 1.0 ml. As test compound dissolving in ethanol and ethanol itself is a $HO^{\bullet}$ trap, thus, controls

containing an equal volume of ethanol was used. The spectra were recorded at 5 min after mixing the solution on a JOEL (JES-RE2X) ESR spectrometer with the following parameters: magnetic field strength 335.10 mT, microwave power 20 mW, modulation amplitude 1×1000 mT, time constant 0.1, sweep width 5.0×1 mT, and modulation width 0.5×0.1 mT.

RESULTS

Chroman and nicotinyl amides **1–13**, whose structures are displayed in Figure 1, were proposed and synthesized as radical scavenging agents (Table I). The chroman amides and hydroxynicotinyl amides inhibited lipid peroxidation at a dose of 50 μM whereas nicotinyl amides were inactive. Chroman amides showed greater inhibitory action than hydroxynicotinyl amides, 58–67% versus 3–24% inhibition (Table II). Chroman amides also exhibited greater activity against lipid peroxidation than trolox, the reference compound and IC_{50} of **9**, **11** and **12** were 14.88, 3.74 and 4.51 μM, respectively.

The quantitative relation between activity and structure/physicochemical properties was determined. The QSAR model was generated from the data set of 13 synthesized compounds and trolox. The *in vitro* % inhibition of lipid peroxidation represented the activity (dependent variable) and the independent variables were steric, electronic and lipophilic descriptors (Table II).

The QSAR models were generated with all combinations of property descriptors: log *P*, MR, E_{LUMO}, E_{HOMO}, OH-indicator and C_{OH}. Although there was a large number of models obtained from the analysis models, some models were ruled out by MLR stepwise method owing to high intercorrelation between independent variables namely log *P* and total refractivity. The best model obtained from QSAR study was represented by the following equation:

$$\begin{aligned} \%\ \text{Inhibition} = {} & 9.387(\pm 2.98)\log P \\ & + 33.166(\pm 12.26)E_{LUMO} \\ & - 76.055(\pm 40.97)C_{OH} \\ & + 6.619(\pm 9.32) \\ (n = 14,\ r^2 = 0.828,\ & S = 13.735, \\ F = 15.994,\ Q^2 = 0.597,\ & S_{PRESS} = 21.00). \end{aligned}$$

The model was validated using statistical approaches which were the correlation coefficient

TABLE II The biological activity and values of steric, lipophilic and electrostatic descriptors

Cpd	% Inhibition[a]	MR[d]	OH-indicator	Lipophilicity log P[e]	Charge C_{OH}	Molecular orbital energy	
						E_{LUMO}	E_{HOMO}
1	0	59.462	0	0.66	0	−0.4027	−9.9176
2	0	69.225	0	−0.46	0	−0.3945	−9.2583
3	0	78.150	0	2.18	0	−0.8682	−8.7687
4	0	77.831	0	1.39	0	−0.3061	−8.3843
5	8.53[b]	61.470	1	0.98	−0.238	−0.3766	−9.8385
6	24.45[b]	71.233	1	−0.13	−0.238	−0.3698	−9.2712
7	3.25[b]	80.158	1	2.50	−0.238	−0.8805	−8.7810
8	21.15[b]	79.839	1	1.72	−0.238	−0.2825	−8.3940
9	65.50[b,c]	96.595	1	3.59	−0.278	−0.0551	−8.9809
10	57.96[b,c]	106.360	1	2.48	−0.258	0.1849	−8.5192
11	66.82[b,c]	115.280	1	4.89	−0.277	−0.7849	−8.8634
12	67.28[b,c]	114.960	1	4.33	−0.279	0.0384	−8.8011
13	64.65[b,c]	103.680	1	2.15	−0.279	0.0374	−8.9199
Trolox	38.10	67.793	1	3.19	−0.278	−0.0458	−9.0484

[a]% Inhibition of lipid peroxidation (*in vitro*), mean of three determinations and the variation between each determination was less than ± 5%. [b]$p < 0.01$ versus control and [c]$p < 0.01$ versus trolox. [d]Total molar refractivity. [e]Log *P* calculated from Molgen program using Crippen's method.

(r^2) characterizing the overall fit, and the predictive power or reliability of the model in terms of cross-validated correlation coefficient or Q^2 and predictive standard error (S_{PRESS}). The derived QSAR model yielded significant predictive cross-validated r^2 higher than the accepted value (0.3). The scatter plots of predicted values versus the experimental % inhibition were shown in Figure 2.

Compounds **11** and **12**, the most active compounds in the *in vitro* antilipid peroxidation test, were evaluated at a dose of 50 mg/kg, i.p., for *in vivo* cerebroprotective activity in two models. Significant neuroprotection against CNS trauma and anoxia was observed. The test compounds were found to enhance early neurological recovery in a mouse head injury model (Table III). In this assay, improvement in the 1 h-post head injury neurological status by as much as 200% was observed when compared with the control. Compound **12** was more active than trolox, the reference compound.

In an assay for cerebroprotective activity, compounds **11** and **12** at a dose of 50 mg/kg, i.p., significantly prolonged the survival time in mice under anoxic conditions as compared with the vehicle treated group (Table IV). No acute toxicity was seen; all mice survived for seven days after treatment with compound **12** at a dose of 50 mg/kg, i.p.

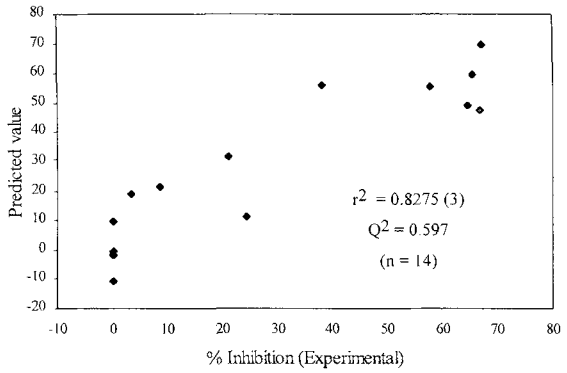

FIGURE 2 Plot of experimental versus predicted % inhibition of lipid peroxidation *in vitro* for 14 compounds. Predicted values were calculated from the derived QSAR model, cross-validated r^2 (Q^2) given along with number of components in parentheses.

The *ex vivo* antilipid peroxidation activity of **12** was tested to investigate the cerebroprotective effect of this antiradical agent. Compound **12** inhibited this lipid peroxidation as shown in Table V. In addition, compound 12 and trolox reduced the hypermotility induced by MAP, a dopamine releaser in mice (Table VI).

TABLE III Effect against mouse head injury

Treatment ($n = 8$)	1 h Post injury grip score(s) Mean (SEM)	% Increase
Vehicle	12.38 (2.95)	—
11	27.00 (5.45)[a]	118
12	37.13 (5.12)[a,b]	200
Trolox	22.38 (2.46)[a]	83

Mice received vehicle or test compound (50 mg/kg, i.p.) within 5 min post-concussive head injury (force of 1500 g cm). At 1 h after injury neurological function was evaluated by a mean grip test score (the mean time that mice could remain on a grip string). [a]$p < 0.05$ versus vehicle, [b]$p < 0.05$ versus trolox (Duncan's multiple range test).

TABLE IV Anti-anoxic activity in mice

Treatment ($n = 8$)	Survival time (s) Mean (SEM)	% Increase
Vehicle	58.13 (6.11)	—
11	111.25 (15.03)*	91
12	104.25 (3.69)*	79
Trolox	113.38 (13.69)*	95

Two mice were placed in a 1 l glass chamber into which nitrogen gas was passed continuously at a flow rate of 2 l/min. Vehicle or test compound (50 mg/kg) was administered 30 min before this treatment. Time before respiratory cessation was recorded as the survival time. *$p < 0.01$ versus vehicle (Duncan's multiple range test).

TABLE V *Ex vivo* antilipid peroxidation in mice

Compound **12** (mg/kg) ($n = 5$)	% Inhibition, Mean(SD)	
	Normal	Traumatic brain injury[a]
50	5.02 (1.36)	18.45 (5.89)*
100	16.30 (4.33)*	41.17 (7.84)*
150	27.12 (8.35)*	—

The inhibition of *ex vivo* lipid peroxidation was measured in brain homogenates 1 h post administration. *$p < 0.01$ versus vehicle treated controls. [a]Compound **12** or vehicle was administered subcutaneously after a concussive head injury (force of 1500 g cm).

TABLE VI Effect on the suppression of MAP-induced hypermotility in mice

Treatment ($n = 6$)	Inducer	Locomotor activity Mean (SEM)	% Suppression of hypermotility
DMSO	Saline	458.17 (56.13)	—
DMSO	MAP	1333.67 (101.20)	0.00
12	MAP	196.83 (61.29)*	85.24
Trolox	MAP	457.00 (36.89)*	65.73

The test compound at dose of 50 mg/kg in DMSO was injected i.p. 30 min before MAP injection (1 mg/kg in saline) and locomotor activity was measured for 30 min after administration of MAP. *$p < 0.001$ compared to the DMSO–MAP-treated control.

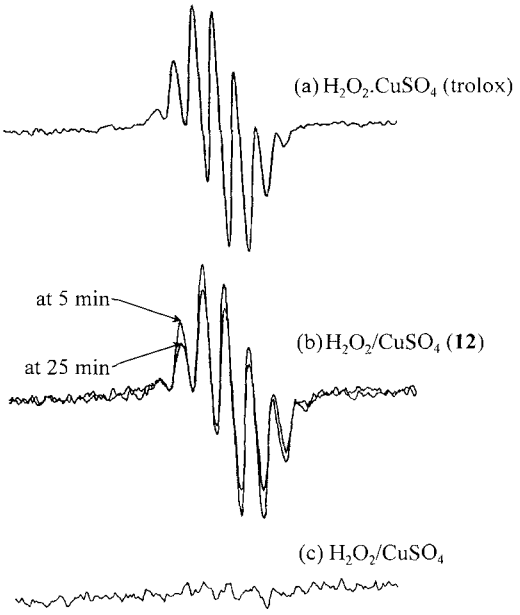

FIGURE 3 The ESR spectra after adding test compounds (5 mM) to HO• generated from $H_2O_2/CuSO_4$ system; (a) trolox, (b) chroman amide **12** and (c) control.

The ability of the most potent compound **12** to trap hydroxyl radical (HO•) was evaluated by using ESR spectroscopy. The ESR spectra of HO• generated by $H_2O_2/CuSO_4$ system in the presence of trolox and chroman amide **12** were shown in Figure 3. Chromanoxyl radical of **12** was found to decay over time, the area of its signal decreased by 21% after standing for 20 min (signal areas at 5 and 25 min were 343 and 271 Ms, respectively). By using PBN as a spin trap, the ability of test compounds to trap HO• were determined by ESR signals of PBN/HO• adduct. Effect of **12** on the ESR signals of the PBN/HO• adduct was shown in Table VII.

TABLE VII Effect of varying the concentration of test compounds on the ESR signal areas of the PBN/HO• adduct[a]

Compounds	Concentration (mM)	Peak area of PBN/HO• adduct (Ms)	% Reduction of PBN/HO• adduct
PBN	—	48.70[b]	—
12	5	25.80[b]	47.02
	25	17.10[b]	64.89
	75	7.62[b]	85.09

[a]The ESR spectra were recorded at 5 min after adding a mixture of PBN (25 mM) and different concentrations of tested compounds to $H_2O_2/CuSO_4$ system. [b]Partial peak areas of PBN/HO• adduct that were not overlapped with the HO• adduct of the test compounds.

DISCUSSION

Several new radical scavengers have been synthesized during the past few years for the treatment of radical-involving diseases and syndromes. We have synthesized new amides **1–13** with the aim of increasing the efficacy of natural antioxidants. Heterocyclic amines or long chain alkyl groups were added to provide greater lipophilicity to improve the accessibility to lipid membranes. Structural modification was manipulated by varying the acid and the amine portions of the amide. The selected acid was either the chromanol portion of vitamin E (trolox) or a heterocyclic system, nicotinic acid or 6-hydroxynicotinic acid. The amine portions differed in the length of the alkyl chain and the type of heterocyclic system, a quinoline, indole or piperazine ring.

The derived QSAR model indicated the high correlation between lipid peroxidation inhibitory action and the lipophilicity, log P, charge of hydroxy group and E_{LUMO}. These suggested that the hydroxyl group was the active functional group that reacted with radicals and suppressed the production of lipid radicals. Of the compounds containing a hydroxyl functional group, chroman amides were more potent than hydroxynicotinyl

amides, owing to the greater lipophilicity of the chromanol ring. These chroman amides should reach the site of peroxidation, the membrane lipid, more easily, leading to greater activity as radical scavengers than for the hydroxynicotinyl amides. According to the derived model, the activity (% inhibition of lipid peroxidation) could be improved by firstly, adding a bulky and/or lipophilic group to enhance the transportation of the drug to the site of action; secondly, increasing E_{LUMO} to more than zero to potentiate the ability to receive an unpaired electron from radicals and lastly, increasing the charge of the hydroxyl function in the aromatic ring to a more negative value to increase the reactivity of the functional group (OH) on free radicals.

The study of *ex vivo* antilipid peroxidation of **12** in normal and head injured mice suggested that head injury enhances the potential for tissue lipid peroxidation and that this is inhibited by prior administration of **12**. Beside radicals, the increased release of dopamine in brain injury and ischemia exacerbates membrane damage and cell death. The preliminary results showed that **12** significantly reduced MAP-induced hypermotility resulting from dopamine release. The antilipid peroxidation of **12** and its antagonistic activity on hypermotility resulting from dopamine release explains the *in vivo* cerebroprotective effect in traumatic head injury and anoxia.

As $^{\bullet}$OH radical is the predominant radical contributing to cellular damage in biological system, the ability of **12** to trap $^{\bullet}$OH was evaluated. Reaction of **12** and $^{\bullet}$OH was investigated by the ESR spin trapping method. The result showed that chroman amide **12** and trolox reacted with $^{\bullet}$OH yielding chromanoxyl radicals which decay over time as observed in Figure 3. ESR spin trapping studies of PBN/$^{\bullet}$OH adduct in the presence of **12** or trolox were conducted to support the hydroxy scavenging ability. It appeared that **12** suppressed the $^{\bullet}$OH generation resulting in the decrease of signal area of PBN/$^{\bullet}$OH adduct. Since ESR signals of chromanoxyl radical of **12** partly overlapped signals of PBN/$^{\bullet}$OH adduct, the area measurements were made only in the region of the unoverlapped signal. Chroman **12** were found to inhibit the PBN/$^{\bullet}$OH adduct signal area in a concentration dependent manner. The effectiveness in the suppression of $^{\bullet}$OH radical leading to decrease on PBN/$^{\bullet}$OH signal strongly suggested that **12** was an effective hydroxy radical scavenger.

In conclusion, the inhibitory action against lipid peroxidation of **12** is governed by at least two factors, namely the ability to trap the radicals and the lipophilicity of the molecule, which determines its ability to penetrate the membrane lipid. Further investigation on other factors such as metal chelating ability should be conducted since lipid peroxidation in brain is largely iron-dependent. The functional groups that facilitate antilipid peroxidation are groups that can donate electrons to the radicals i.e. the hydroxyl group. The log P value for the maximal activity of lipid peroxidation inhibitor was between 4.5 and 5, which agrees with the reported log P for the maximal activity of uric acid and urate derivatives.[30] The lipid-soluble chroman amides **11** and **12**, which are potent inhibitors of lipid peroxidation, were effective in *in vivo* models of traumatic brain injury and anti-anoxia. The *in vivo* and *ex vivo* cerebroprotection studies support the role of free radicals and dopamine in the pathophysiology of acute CNS trauma and anoxia, as well as a link between *in vitro* antilipid peroxidation activity and *in vivo* cerebroprotective activity. They also provide evidence for an important role of radical-induced lipid peroxidation in brain damage. Pharmacological studies of **11** and **12** are currently in progress and will be reported in due course.

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