2-(Aminomethyl)chromans That Inhibit Iron-Dependent Lipid Peroxidation and Protect against Central Nervous System Trauma and Ischemia

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Received March 16, 1992

A series of 2-(aminomethyl)chromans was developed as potent inhibitors of iron-dependent lipid peroxidation. Compounds within this class are extremely effective at inhibiting lipid peroxidation with IC_{50}'s as low as 0.2 μM. Selected members were found to enhance early neurological recovery and survival in a mouse head injury model. In this assay, improvement in the 1-h post-head-injury neurological status (grip test score) by as much as 230% of control was observed. One of the most efficacious compounds (**35**) was evaluated in two models of cerebral ischemia where significant neuroprotection was observed. These results provide further support for the importance of cerebroprotective antioxidants for the treatment of traumatic and ischemic injury as well as additional evidence for the role of oxygen radicals in postischemic brain damage.

Lipid peroxidation is recognized as an important pathophysiological process in many disease states, drug toxicities, and ischemic events. For instance, oxygen free radicals and lipid peroxidation are reported to be involved in atherosclerosis, cancer, asthma, arthritis, myocardial infarction, burn, and inflammation.[1] In the central nervous system (CNS) area, peroxidative processes are implicated in the irreversible loss of neuronal tissue after traumatic injury to the head and spinal cord, stroke, subarachnoid hemmorrhage, neurotoxic damage, Parkinson's disease, and perhaps dementia.[1-6] Many factors initiate the formation of oxygen free radicals and the lipid peroxidation process. Iron plays a key role in both the initiation[7] and propagation[8] of oxygen free radicals during conditions of oxidative stress following traumatic injury. Other sources of oxygen free radicals include the activation of the arachidonic acid cascade, xanthine oxidase, monoamine oxidase, mitochondrial leak, and phagocytosis.[9,10]

Some of the most potent antioxidants known have structures based on hindered phenols. Of these, vitamin

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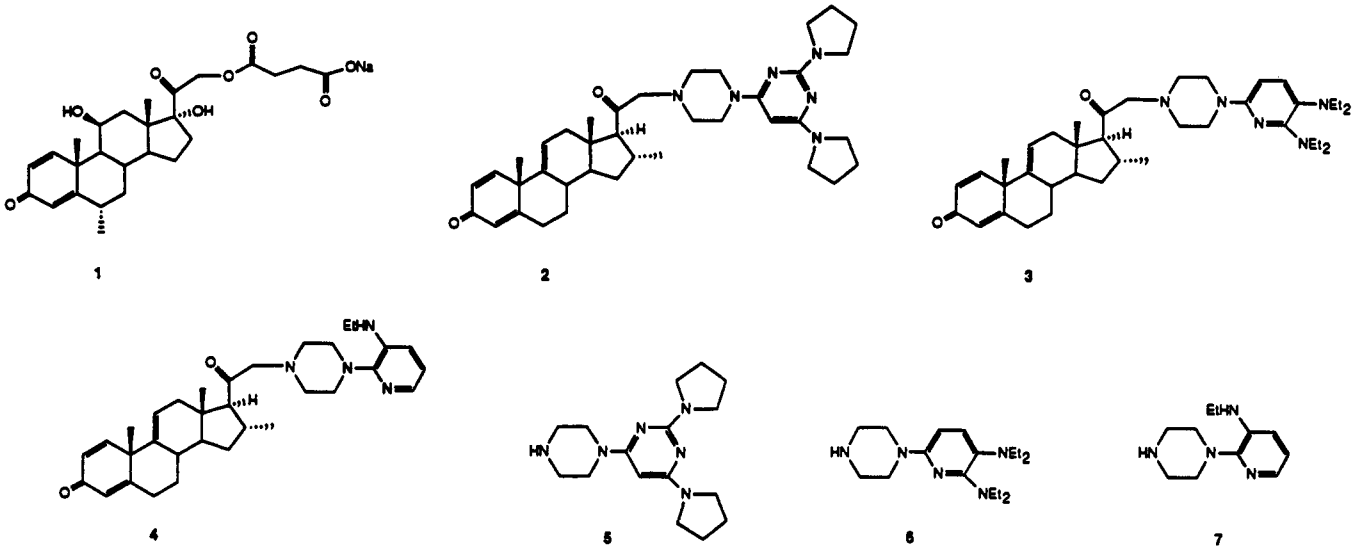
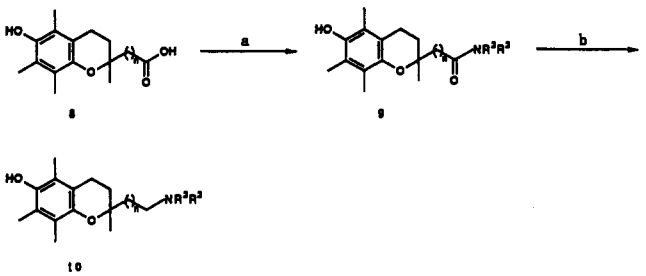
Chart I



Scheme I [a]



[a] (a) (1) CDI, THF, (2) HNR^2R^3, THF or CH_2Cl_2; (b) BH_3–DMS or LAH, THF.

E is the most important and widely studied. This endogenous chain-breaking antioxidant is an efficient scavenger of peroxyl, hydroxyl, and alkoxyl radicals.[11,12] The chromanol portion of vitamin E (trolox) is also an effective scavenger of superoxide anion.[13] The free radical scavenging actions of vitamin E are thought to involve the loss of $H^{\bullet}$ to form the tocopherol radical.[14] It is one of natures first lines of defense against peroxidative damage on a cellular level.[15] Additionally, several in vivo models of cerebral ischemia have shown that pretreatment with vitamin E exerts a beneficial effect on neurological recovery.[3b,16]

Methylprednisolone (1), a weak inhibitor of lipid peroxidation,[17] was recently shown to be effective for the treatment of spinal cord injury in a multicenter study.[18] High-dose treatment with 1 has also proven effective in animal models of head and spinal trauma.[19] In relation to methylprednisolone we recently reported on a class of 21-aminosteroids which are potent inhibitors of lipid peroxidation.[20,21] Selected members of this class are extremely effective in animal models of brain and spinal trauma,[22] stroke,[23] and subarachnoid hemorrhage.[24] In particular, compounds 2–4 (Chart I) were found to be much more potent and efficacious than methylprednisolone in inhibiting lipid peroxidation and in the aforementioned models of CNS damage. Aminosteroid 2 was selected for clinical trials for the treatment of head injury.

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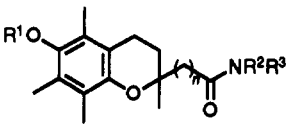
Table I. Physical Data of Trolox Amides



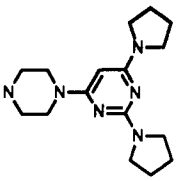
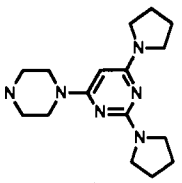
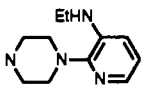
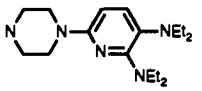
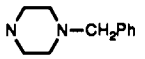
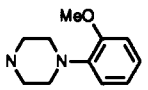
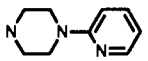
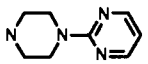
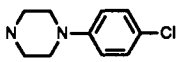
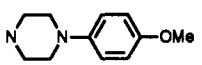
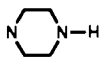
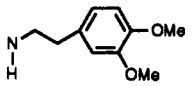
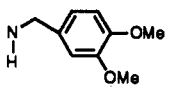
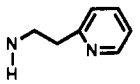
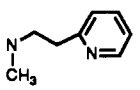
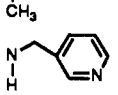
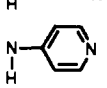
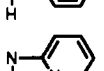
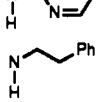
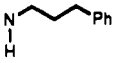
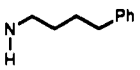
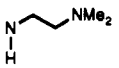
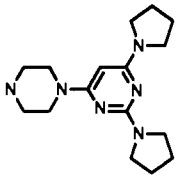
	R^1	NR^2R^3	n	mp, °C	% yield	formula	analysis
11	H		0	123–127	80	$C_{30}H_{42}N_6O_3$	C,H,N
12	Ac		0	191–192	62[a]	$C_{32}H_{44}N_6O_4$	C,H,N
13	H		0	200–201	90	$C_{25}H_{34}N_4O_3$	C,H,N
14	H		0	138–140 dec	72	$C_{31}H_{47}N_5O_3 \cdot (HCl)_{3/2} \cdot (H_2O)_{3/2}$	C,H,N,Cl
15	H		0	183–186	82	$C_{25}H_{32}N_2O_3 \cdot HCl \cdot (H_2O)_{3/4}$	C,H,N,Cl
16	H		0	150–151	94	$C_{25}H_{32}N_2O_4 \cdot (H_2O)_{1/4}$	C,H,N
17	H		0	153–155	81	$C_{25}H_{28}N_3O_3$	C,H,N
18	H		0	166–169	78	$C_{22}H_{28}N_4O_3$	C,H,N
19	H		0	164–165	98	$C_{24}H_{29}N_2O_3Cl$	C,H,N,Cl
20	H		0	157–159	63	$C_{25}H_{32}N_2O_4$	C,H,N
21	H		0	153–155	67	$C_{18}H_{28}N_2O_3$	C,H,N
22	H		0	oil	98	$C_{18}H_{25}NO_3$	–
23	H		0	127–127.5	77	$C_{24}H_{31}NO_5$	C,H,N
24	H		0	156–157	63	$C_{23}H_{29}NO_5$	C,H,N
25	H		0	105–107	78	$C_{21}H_{28}N_2O_3$	C,H,N
26	H		0	oil	70	$C_{22}H_{28}N_2O_3$	–
27	H		0	137–138.5	82	$C_{20}H_{34}N_2O_3$	C,H,N
28	H		0	217–219	14	$C_{18}H_{22}N_2O_3 \cdot (H_2O)_{1/8}$	C,H,N
29	H		0	173–174	37	$C_{18}H_{22}N_2O_3$	C,H,N
30	H		0	90–91	71	$C_{22}H_{27}NO_3 \cdot (H_2O)_{1/3}$	C,H,N

Table I (Continued)

	R^1	NR^2R^3	n	mp, °C	% yield	formula	analysis
31	H		0	91–92	41	$C_{23}H_{29}NO_3 \cdot (H_2O)_{1/6}$	C,H,N
32	H		0	73–74	49	$C_{24}H_{31}NO_3 \cdot (H_2O)_{1/8}$	C,H,N
33	H		0	127–128	96	$C_{18}H_{28}N_2O_3$	C,H,N
34	H		1	60–71	17	$C_{31}H_{44}N_6O_3 \cdot (CH_4SO_3)_{7/4} \cdot (H_2O)_{3/2}$	C,H,N

[a] Prepared by acylation of the phenol.

One of the most important roles of the steroid group in our aminosteroid series involves its interaction with membrane lipids and the targeting of the desired amine to the site of oxidative stress.[20] In considering possible steroid replacements, the chromanol portion of vitamin E was intriguing as it may not only interact with the lipid layer but may also provide antioxidant activity in its own right. While several laboratories have attached simple amines[25] or amino acids[26] to vitamin E derived templates, there are no reports of the combination of amines having potent antioxidant abilities with chroman moieties. We would now like to report on a combination of these two approaches to form a class of 2-(aminomethyl)chromans. Compounds within this group join the antioxidant chroman portion of vitamin E with the heterocyclic amine portion of the 21-aminosteroids. The result of this approach is a series of potent inhibitors of lipid peroxidation. Furthermore, selected members are extremely effective in models of CNS traumatic and ischemic insult.

Chemistry

The 2-(aminomethyl)chromans were prepared as shown in Scheme I. Either commercially available amines or the complex heterocyclic amines that were previously described[20] (**5–7**, Chart I) were coupled to trolox (**8**; $n = 0$) or chroman-2-acetic acid[27] (**8**; $n = 1$) using 1,1'-carbonyldiimidazole to form the appropriate amides (**9**). Reduction with either lithium aluminum hydride or borane–methyl sulfide complex provided the desired amines (**10**). Table I highlights the physical data of the amides prepared (**11–34**), while Table II presents data for the amines (**35–60**) which were often characterized as their acid salts. In order to determine the effect of chirality upon the compounds reported herein, trolox was resolved with (*S*)-α-methylbenzylamine following the procedure of Scott.[28] Coupling of the *R* and *S* enantiomers of trolox with amine **5** and subsequent reduction provided *R*-(−)-**59** and *S*-(+)-**60**, respectively.

Biological Results and Discussion

The compounds listed in Tables I and II were initially screened in vitro for their ability to inhibit iron-dependent lipid peroxidation (MDA assay). Homogenized rat brain in Krebs buffer was exposed to 200 μM $FeCl_2$. The resultant lipid peroxidation was evaluated by the formation of thiobarbituric acid reactive products[7a] (TBAR, e.g., malondialdehyde). The compounds were tested at doses up to 300 μM with IC_{50} values provided in Table III.

Almost all compounds tested were extremely effective at inhibiting iron-catalyzed lipid peroxidation. Typically, IC_{50} values of approximately 1–10 μM were observed regardless of the amine or amide side chain. Apparently the vitamin E portion completely dominates the activity as compounds containing amines with demonstrated antioxidant abilities while quite potent, were no more so than many of the analogs with simpler side chains. For example, **35**, which contains the same amine as found on **2**, was comparable in activity to the *N*-benzyl derivative **39** (0.6 μM vs 1.0 μM). Chain extension as with the homotrolox analogs (**34** and **58**) or acetylation of the phenol (**12** and **36**) had little effect on in vitro activity, except for

(22) (a) Hall, E. D.; Yonkers, P. A.; McCall, J. M.; Braughler, J. M. Effects of the 21-Aminosteroid U-74006F on Experimental Head Injury in Mice. *J. Neurosurg.* 1988, *68*, 456–461. (b) McCall, J. M.; Braughler, J. M.; Hall, E. D. A New Class of Compounds for Stroke and Trauma: Effects of 21-Aminosteroids on Lipid Peroxidation. *Acta. Anaesthesiol. Belg.* 1987, *38*, 417–420. (c) Hall, E. D. Effects of the 21-Aminosteroid U-74006F on Posttraumatic Spinal Cord Ischemia in Cats. *J. Neurosurg.* 1988, *68*, 462–465. (d) Hall, E. D. Beneficial Effects of the 21-Aminosteroid U-74006F in Acute CNS Trauma and Hypovolemic Shock. *Acta. Anaesthesiol. Belg.* 1987, *38*, 421–425. (e) Anderson, D. K.; Braughler, J. M.; Hall, E. D.; Waters, T. R.; McCall, J. M.; Means, E. D. Effects of Treatment with U-74006F on Neurological Outcome Following Experimental Spinal Cord Injury. *J. Neurosurg.* 1988, *69*, 562–567.

(23) Hall, E. D.; Pazara, K. E.; Braughler, J. M. 21-Aminosteroid Lipid Peroxidation Inhibitor U-74006F Protects Against Cerebral Ischemia in Gerbils. *Stroke* 1988, *19*, 997–1002.

(24) (a) Hall, E. D.; Travis, M. A. Effects of the Nonglucocorticoid 21-Aminosteroid U-74006F on Acute Cerebral Hypoperfusion Following Experimental Subarachnoid Hemorrhage. *Exp. Neurol.* 1988, *102*, 244–248. (b) Steinke, D. E.; Weir, B. K. A.; Findlay, J. M.; Tanabe, T.; Grace, M.; Krushelnycky, B. W. A Trial of the 21-Aminosteroid U-74006F in a Primate Model of Chronic Cerebral Vasospasm. *Neurosurg.* 1989, *24*, 179–186. (c) Kanamaru, K.; Weir, B. K. A.; Simpson, I.; Witbeck, T.; Grace, M. Effect of 21-Aminosteroid U-74006F on Lipid Peroxidation in Subarachnoid Clot. *J. Neurosurg.* 1991, *74*, 454–459.

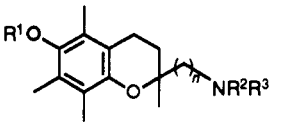
(25) (a) Kurrary EP 202,580-A. (b) Scott, J. W.; Cort, W. M.; Harley, H.; Parrish, D. R.; Saucy, G. 6-Hydroxychroman-2-carboxylic Acids: Novel Antioxidants. *J. Am. Oil Chem. Soc.* 1974, *51*, 200–203. (c) Bolkenius, F. N.; Grisar, J. M.; De Jong, W. A Water-Soluble Quaternary Ammonium Analog of α-Tocopherol, That Scavenges Lipoperoxyl, Superoxyl and Hydroxyl Radicals. *Free Radical Res. Commun.* 1991, *14*, 363–372.

(26) Taylor, M. J.; Richardson, T.; Jasensky, R. D. Antioxidant Activity of Amino Acids Bound to Trolox-C. *J. Am. Oil Chem. Soc.* 1981, *58*, 622–626.

(27) Cohen, N.; Banner, B. L.; Neukom, C. Improvements in the Synthesis of a Key α-Tocopherol Intermediate-(S)-(-)-3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2H-1-benzopyran-2-acetic Acid. *Synth. Commun.* 1982, *12*, 57–65.

(28) Scott, J. W.; Parrish, D. R. U.S. Patent 4,003,919.

Table II. Physical Data of Trolox Amines



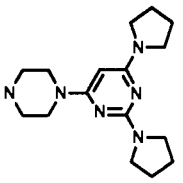
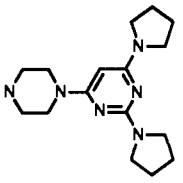
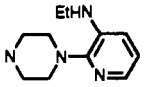
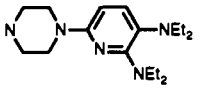
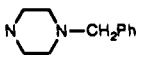
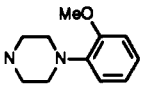
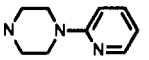
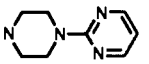
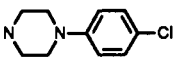
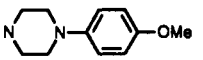
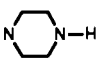
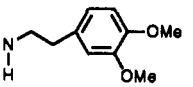
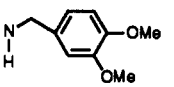
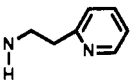
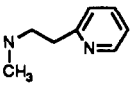
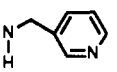
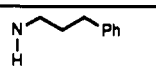
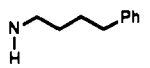
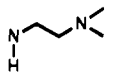
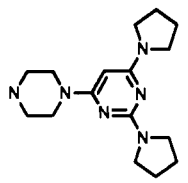
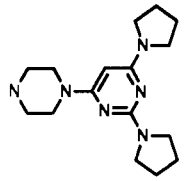
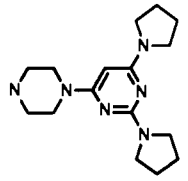
	R^1	NR^2R^3	n	mp, °C	method	% yield	formula	analysis
35	H		1	235–237	B	82	$C_{30}H_{44}N_6O_2 \cdot (HCl)_2 \cdot (H_2O)_{3/2}$	C,H,N,Cl
36	Ac		1	171–172.5	–	36[a]	$C_{32}H_{46}N_6O_3$	C,H,N
37	H		1	208–211	B	82	$C_{25}H_{36}N_4O_2 \cdot (HCl)_2 \cdot (H_2O)_{3/4}$	C,H,N,Cl
38	H		1	215–220 dec	C	88	$C_{31}H_{49}N_5O_2 \cdot (HCl)_{2.75} \cdot H_2O$	C,H,N,Cl
39	H		1	248–250 dec	C	64	$C_{25}H_{34}N_2O_2 \cdot (HCl)_2$	C,H,N,Cl[b]
40	H		1	213–216	C	47	$C_{25}H_{34}N_2O_3 \cdot (HCl)_2 \cdot (H_2O)_{1/3}$	C,H,N,Cl
41	H		1	277–278	C	36	$C_{23}H_{31}N_3O_2 \cdot (HCl)_2 \cdot (H_2O)_{5/4}$	C,H,N,Cl
42	H		1	106–109	C	31	$C_{22}H_{30}N_4O_2$	C,H,N
43	H		1	141–142	C	27	$C_{24}H_{31}N_2O_2Cl$	C,H,N
44	H		1	109–110	C	61	$C_{25}H_{34}N_2O_3$	C,H,N
45	H		1	210–212	C	32	$C_{18}H_{28}N_2O_2 \cdot (HCl)_2 \cdot (H_2O)_{1/2}$	C,H,N,Cl
46	H		1	99–100	C	70	$C_{18}H_{27}NO_2$	C,H,N
47	H		1	187–189	C	98	$C_{24}H_{33}NO_4 \cdot HCl$	C,H,N[c],Cl
48	H		1	224–225	C	24	$C_{23}H_{31}NO_4 \cdot C_2H_2O_4$	C,H,N
49	H		1	167–168	B	39	$C_{21}H_{28}N_2O_2 \cdot (C_2H_2O_4)_{3/2} \cdot (H_2O)_{1/2}$	C,H,N
50	H		1	229–230	B	95	$C_{22}H_{30}N_2O_2 \cdot (C_2H_2O_4)_{3/2} \cdot H_2O$	C,H,N[d]
51	H		1	229–231	B	48	$C_{20}H_{28}N_2O_2 \cdot (C_2H_2O_4)_{3/2}$	C,H,N
52	H		1	304–305	C	31	$C_{19}H_{24}N_2O_2 \cdot HCl$	C,H,N,Cl
53	H		1	140–143	C	28	$C_{19}H_{24}N_2O_2$	C,H,N
54	H		1	219–220	C	25	$C_{22}H_{29}NO_2 \cdot C_2H_2O_4$	C,H,N

Table II (Continued)

	R^1	NR^2R^3	n	mp, °C	method	% yield	formula	analysis
55	H		1	201–202	C	35	$C_{23}H_{31}NO_2 \cdot C_2H_2O_4$	C,H,N
56	H		1	179–180	C	17	$C_{24}H_{33}NO_2 \cdot HCl$	C,H,N,Cl
57	H		1	104–106	C	61	$C_{18}H_{30}N_2O_2$	C,H[e],N
58	H		2	148–152	C	49	$C_{31}H_{46}N_6O_2 \cdot (CH_4SO_3)_{1.33} \cdot (H_2O)_{1/2}$	C,H,N,S
59	H		1	251–253	B	93	$C_{30}H_{44}N_6O_2 \cdot (HCl)_2$	C,H,N,Cl
60	H		1	251–253	B	68	$C_{30}H_{44}N_6O_2 \cdot (HCl)_2$	C,H,N,Cl

[a] Prepared by acylation of the phenol. [b] Cl: Calcd 15.17; found, 14.56. [c] N: Calcd 3.21; found, 2.70. [d] N: Calcd 5.52; found, 4.90. [e] H: Calcd 9.87; found, 10.37.

Table III. Malondialdehyde (MDA) Formation Assay

compd	MDA formation: IC_{50}[a] (μM) or % inhibn at 300 μM	compd	MDA formation: IC_{50}[a] (μM) or % inhibn at 300 μM
11	0.5	38	1.0
12	17	39	1.0
13	12	40	1.0
14	0.2	41	1.0
15	1.0	42	1.0
16	15	43	0.9
17	1.0	44	0.2
18	23	45	1.0
19	0.8	46	1.4
20	0.7	47	1.0
21		48	11
22		49	23
23	4.1	50	177
24	5.5	51	48
25	11	52	16
26	5.4	53	0.2
27	47	54	0.9
28	2.3	55	1.0
29	1.8	56	0.8
30	1.3	57	1.7
31	1.0	58	1.3
32	0.7	59	1.5
33	8.7	60	1.1
34	1.3	1	0%
35	0.6	2	18
36	1.5	3	3
37	1.0	α-tocopherol	28
		trolox	43

[a] Each result represents the mean of three determinations. Variation between each experiment was less than ±5.0%.

12 where roughly a 10-fold decrease in potency was noted. In contrast to the excellent activity observed for most compounds within this series, two chromanols which contain an alkylpyridine side chain (50, 51) were much less effective antioxidants for reasons that are not clear at this time.

The effectiveness of the (aminomethyl)chromans in the MDA assay is readily apparent when compared to known standards. For example, potent inhibitors of lipid peroxidation such as α-tocopherol[21a,c] (vitamin E) or trolox were weaker antioxidants with IC_{50} values of 28 and 43 μM, respectively. The 21-aminosteroids were as a class less potent with only compound 3 comparable in activity (3.0 μM). The amine portions by themselves were also ineffective in this screen.[21c] Apparently the combination of the amine functionality from the 21-aminosteroid series (or in many cases much simpler amines) with the antioxidant chromanol portion of vitamin E results in a dramatic improvement in the ability to inhibit lipid peroxidation. One of the most potent aminochromans (35) in this assay was evaluated for its maximal efficacy.[35] The formation of TBAR was nearly completely inhibited by 35 at dose ranges of 10–30 μM. In comparison, a ceiling of approximately 70% was observed for vitamin E and 2. The enantiomers of 35 (59 and 60) were also equieffective to each other and the racemate in terms of efficacy and potency.[35]

Many of the compounds that were effective inhibitors of lipid peroxidation were evaluated for in vivo neuroprotectant activity in a mouse head injury (MHI) model.[22a,29] Unanesthetized male mice were subjected to a concussive head injury (approximate force of 900 g-cm) that was produced by a 50-g weight dropped from a height of 18 cm. This concussive injury resulted in immediate

(29) Hall, E. D. Glucocorticoid Effects on Central Nervous Excitability and Synaptic Transmission. *Int. Rev. Neurobiol.* **1982**, *23*, 165–195.

Table IV. Mouse Head Injury Assay

compd	1-h postinjury grip score, mean ± SEM (best dose, mg/kg)	lowest effective dose (mg/kg)
vehicle[a]	5.3 ± 3.3	
11	17.5 ± 2.4 (1.0)	0.01
14	IA[b]	
16	12.8 ± 1.5 (1.0)	0.01
17	8.2 ± 1.4 (0.01)	0.01
18	12.0 ± 3.2 (1.0)	1.0
28	11.5 ± 1.9 (0.01)	0.01
29	14.4 ± 2.1 (0.01)	0.01
33	IA	
34	IA	
35	13.7 ± 1.8 (10.0)	0.001
35[c]	14.7 ± 1.7 (10.0)	0.01
36	9.2 ± 2.5 (1.0)	1.0
37	10.4 ± 1.3 (0.01)	0.01
37[c]	11.9 ± 1.7 (10.0)	0.01
38	11.9 ± 2.2 (0.01)	0.01
39	13.0 ± 1.7 (1.0)	0.01
40	IA	
41	10.7 ± 2.0 (1.0)	0.01
42	10.3 ± 2.2 (0.01)	0.01
47	8.4 ± 2.3 (1.0)	0.01
53	12.3 ± 1.9 (0.01)	0.01
54	8.7 ± 1.7 (1.0)	1.0
57	IA	
58	IA	
59	14.4 ± 2.1 (1.0)	0.001
60	11.5 ± 1.9 (1.0)	0.001
1	13.5 ± 3.6 (60.0)	15.0
2	14.5 ± 3.0 (10.0)	0.003
3	14.1 ± 3.5 (0.1)	0.003

[a] The vehicle has either 0.05 N HCl or 0.5% Tween 80, depending on the test compound which was administered by intravenous dosing unless otherwise indicated. [b] Inactive: no dose (≤60 mg/kg iv) produced 50% increase in grip score compared to that of vehicle-treated animals. [c] Oral dosing.

unconsciousness (loss of righting reflex). The compounds of interest were tested at a dose range from 0.001 to 30.0 mg/kg administered intravenously at 5 min after injury. At 1 h after injury, the sensorimotor status of the mice was evaluated by using a grip test.[22a]

The results for the mouse head injury assay are shown in Table IV. While many of the compounds tested in this assay were active, the most efficacious compounds contained the electron-rich heterocyclic amines (**5** and **7**) that were among the most effective in the 21-aminosteroid series. In comparison to aminosteroid **2**, aminochromans **11**, **35**, and **37** were equal or better in terms of efficacy and potency. However, in contrast to **2**, both **35** and **37** were also found to be very effective in the MHI assay following oral administration. The enantiomers of **35** (**59** and **60**) were also equieffective in this assay, indicating that the chiral center has no significant effect on the biological activity. Little difference in activity between compounds containing amide or amine side chains was observed as evidenced for **11** and **35**. Interestingly, the chain-extended analogs (**34** and **58**) which contain the same amine portion were completely inactive. Acylation of the phenol did result in a loss of activity as noted for **36**.

Several other compounds were also quite efficacious at certain doses in the MHI model. Of particular note are **16**, **28**, **29**, **39**, and **53** which contain relatively simple amine side chains. However, these compounds (except for **16**) had a much narrower dose–response curve than standards **2** or **35**. The rest of the aminochromans, while potent inhibitors of iron-dependent lipid peroxidation in vitro, were relatively ineffective neuroprotectants in this assay.

Table V. Cytotoxic Hypoxia Assay[a]

compd	dose (mg/kg)	LD_{50} (% of LD_{50} in vehicle-treated mice)
11	10	99.3
11	20	115.2*
35	10	114.0*
35	20	134.2*
39	10	130.0*
39	20	181.4*
2	10	103.6
2	20	128.4*

[a] (*) $p < 0.05$, Spearman-Karber method.

Most likely, the superior activity observed for **11**, **35**, and **37** across a wide dose range is a reflection of the dual antioxidant nature of these compounds where both the amine and the chromanol portions contribute to the overall activity. Compounds lacking amine–amide side chains with inherent antioxidant abilities, while still often active, were found not to have the overall potency and efficacy of the heteroaromatic piperazinylchromans.

(Aminomethyl)chromans **11**, **35**, and **39** were also found to antagonize potassium cyanide-induced cytotoxic hypoxia. In this assay[30] male CF-1 mice were pretreated with drug or vehicle (0.9% saline) and challenged with increasing doses of potassium cyanide. The antihypoxic effect of the test compound was evaluated by comparing LD_{50}'s with vehicle LD_{50}'s. As shown in Table V the chromanol compounds are more effective as antagonists of KCN lethality than the related 21-aminosteroids. This finding is consistent with the enhanced antioxidant ability of the aminochromans over the aminosteroids as cyanide toxicity in mice is known to be associated with the induction of CNS lipid peroxidation.[31]

The most promising compound within this series (**35**) was evaluated in several other models of ischemic CNS injury. For instance, in a model of cerebral ischemia[21,32,35] (3-h unilateral carotid occlusion in gerbils) **35** significantly improved neuronal survival at 24 h after ischemia as compared to the vehicle-treated group with data illustrated in Figure 1. In the medial cortical area, **35**-treated gerbil brain had neuronal densities of 86.3% (of normal) compared to only 34.2% in the control group.[35] A similar protective effect was also apparent in the lateral cortical area (48.2% vs 3.3%) where the injury is more severe. The neuroprotective ability of **35** in this assay closely parallels that of **2** as previously reported.[23]

The decline of cerebral cortical extracellular Ca^{2+} following periods of ischemia was first observed by Harris.[33]

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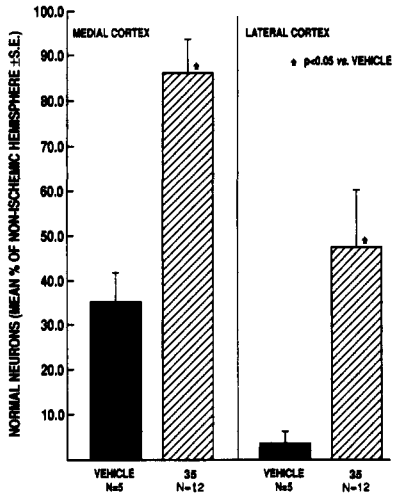

Figure 1. Bar graphs of attenuation of 24-h postischemic (3-h unilateral carotid occlusion [UCO]) cortical neuronal necrosis in male Mongolian gerbils by **35** 10 mg/kg ip at 10 min before and again immediately after the 3-h UCO. Values are given as mean ± SEM neuronal densities expressing ischemic hemisphere as a percentage of contralateral nonischemic hemisphere. Statistical comparison between vehicle- and drug-treated animals was carried out using one-way analysis of variance.

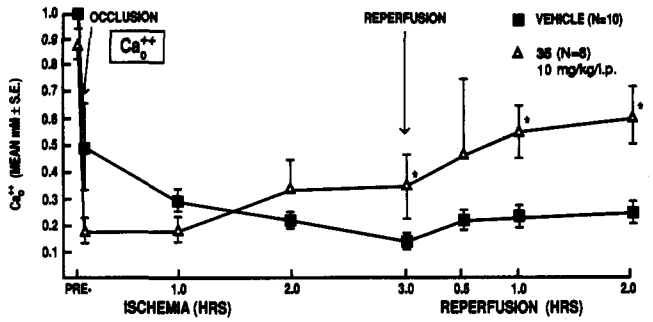

Figure 2. Dose–response curves showing enhancement of postischemic (3-h unilateral carotid occlusion [UCO]) recovery of cerebral cortical extracellular calcium measured via ion-sensitive microelectrode in male Mongolian gerbils by **35** 10 mg/kg ip 10 min before and again immediately after the 3-h UCO.

In the aforementioned ischemia model a rapid drop (within 5 min of the occlusion) in cortical extracellular Ca^{2+} was observed for both the vehicle- and **35**-treated gerbils. In contrast to the control group, **35** produced a partial recovery of Ca^{2+} which reached statistical significance (compared to vehicle) 3 h after the occlusion as illustrated in Figure 2. After reperfusion, further significant recovery of the cortical Ca^{2+} was observed for the **35**-treated group.[32] In contrast, only a modest recovery of extracellular Ca^{2+} occurred for the control group over the same time course.

The ability of **35** to facilitate the postischemic recovery of cerebral Ca^{2+} is nearly equivalent to that demonstrated[34] for **2**. This enhanced recovery of cortical Ca^{2+} in the **2**- and **35**-treated gerbils may be attributed to a protection of membrane Ca^{2+} extrusion mechanisms (e.g., Na^+–Ca^{2+} exchanger, Ca^{2+}–ATPase) from lipid peroxidative damage. Additionally, the recovery of extracellular Ca^{2+} in gerbil cerebral cortex as effected by **2** has been correlated with the attenuation of brain vitamin E depletion over the same time frame.[34] Presumably, **2** quenches the postischemic lipid peroxidation maintaining the endogenous levels of vitamin E. While the measurement of brain vitamin E has not been carried out with **35**, it is conceivable that the levels of vitamin E would be protected in a similar fashion after ischemic insult. This is especially likely given that **35** shares the same chromanol moiety as vitamin E.

Table VI. Attenuation of Postischemic (1 Week) Loss of Hippocampal CA1 Neurons in Gerbils Subjected to a 15-Minute Period of Bilateral Carotid Occlusion by **35**: Comparison of 1-Day versus 7-Day Dosing

group	*n*	CA1 neurons/ 315 μm
sham-operated	7	94.6 ± 1.6
vehicle-treated	18	26.6 ± 4.2
10 mg/kg (1 day)[a]	8	27.5 ± 10.6
1 mg/kg (7 day)[b]	10	53.4 ± 11.2[c]
10 mg/kg (7 day)	9	51.4 ± 11.5[c]

[a] First intraperitoneal dose given 30 min before ischemia, and second dose given 2 h after ischemia. [b] Same as 10 mg/kg group plus additional intraperitoneal dose once daily. [c] $p < 0.05$ vs vehicle (analysis of variance). Values are given as mean ± SEM.

(Aminomethyl)chroman **35** was also evaluated in a global forebrain ischemia model.[32] The ischemic event was induced by a 15-min bilateral carotid occlusion. Seven days after ischemia, the hippocampal CA1 neuronal population was evaluated. As shown in Table VI there was a 72% loss of CA1 pyramidal neurons for the vehicle-treated gerbils. A similar neuronal loss was observed for animals receiving two 10 mg/kg ip doses of **35** on the same day of the ischemic insult. However, if additional daily doses (1 or 10 mg/kg) of **35** were administered for the seven days following ischemia, a significant reduction in CA1 necrosis was observed for both dose groups. The necessity of a sustained dose regimen was also required for aminosteroid **2** to show efficacy in this model.

The combination of the amine functionality of **2** with the chromanol portion of vitamin E has provided one of the most potent inhibitors of iron-catalyzed lipid peroxidation observed to date. Both the hindered phenol and the amine work together in a synergistic fashion to scavenge lipid peroxyl, hydroxyl, and alkoxyl free radicals. Given the considerable evidence for the important role of oxygen free radicals in the pathophysiology of acute CNS trauma and ischemia, it is not surprising that a potent inhibitor of lipid peroxidation has excellent activity in animal models of brain injury and stroke. In support of the link between anti-peroxidative activity and in vivo cerebral protective efficacy, brain levels of **35** in mice were found to correlate with the in vitro doses.[35] Other possible cerebral protective mechanisms[35] such as hypothermia, antagonism of excitatory amino acids, and neurotransmitter receptor interaction appear not to play a role for **35**. In further support for the lack of any receptor interaction, the stereoisomers of **35** (**59** and **60**) were identical in all aspects.

In conclusion, the (aminomethyl)chromans are novel, lipid-soluble antioxidants with a potency of up to 100-fold greater than vitamin E against iron-dependent lipid peroxidation. Many compounds were effective in in vivo models of trauma and ischemia. Detailed studies of several compounds have shown that their in vitro antioxidant activity closely parallels their cerebroprotective potency in in vivo models of cerebral ischemia and thus provides further evidence for an important role of iron-catalyzed oxygen radical-induced lipid peroxidation in postischemic and traumatic brain damage.

Experimental Section

Chemistry. Thin-layer and flash chromatography utilized E. Merck silica gel (230–400 mesh). Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are

uncorrected. The final products were analyzed for purity by HPLC using a Perkin-Elmer Series 4 liquid chromatograph with a Kratos Spectroflow 757 detector (254 nm). Mass spectra, infrared spectra, and combustion analysis were obtained by the Physical and Analytical Chemistry Department of The Upjohn Company. ^{1}H NMR spectra were recorded at 300 MHz with a Brucker Model AM-300 spectrometer.

In cases where synthetic intermediates or products were isolated by "aqueous workup" (organic solvent, drying agent), the procedure was to quench the reaction mixture with H_2O, dilute with the indicated organic solvent, separate the organic layer, extract the aqueous layer several times with the organic solvent, dry the combined organic layers with the indicated drying agent, and remove the solvent with a rotary evaporator at reduced pressure. When "basic workup" (organic solvent, aqueous basic solvent, drying agent) is indicated, the procedure was similar to the aqueous workup, except the indicated aqueous base was used instead of H_2O. When "acid workup" (organic solvent, organic solvent, drying agent) is indicated, the procedure was to dilute the reaction mixture with the first indicated organic solvent, extract the organic solution several times with 1 N HCl, basify the combined acidic layers with solid KOH or NaOH, extract the basic layers with the second indicated organic solvent several times, dry the organic layers with the indicated drying agent, and remove the solvent with a rotary evaporator under reduced pressure. Tetrahydrofuran (THF) and ether were distilled from sodium and benzophenone. Dichloromethane, triethylamine, and diisopropylamine were distilled from calcium hydride. All other solvents were Burdick and Jackson or Fisher reagent grade, distilled in glass.

Method A. Typical Procedure for Amide Formation. 1-[(3,4-Dihydro-6-hydroxy-2,5,7,8-tetramethyl-2*H*-1-benzopyran-2-yl)carbonyl]-4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-piperazine (11). 1,1'-Carbonyldiimidazole (10.7 g, 66.0 mmol) was added to a solution of trolox (15.0 g, 59.9 mmol) and THF (300 mL). After stirring for 1 h at room temperature, a solution of 18.0 g (59.5 mmol) of 4-(1-piperazinyl)-2,6-di-1-pyrrolidinylpyrimidine (5) and CH_2Cl_2 (200 mL) was added dropwise over 30 min. The mixture was allowed to stir for 20 h at room temperature. Aqueous workup (CH_2Cl_2, brine wash, $MgSO_4$) and purification by flash chromatography (1:1 cyclohexane–ethyl acetate) gave 25.3 g (80%) of amide 11 as an off-white powder. Recrystallization from ethyl acetate–hexane provided an analytical sample of 11 as a white powder (mp 123–127 °C): IR (Nujol) 3337, 1632, 1566, 1445, 1345, 1246, 1223, 1207 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) 4.81 (s, ArH), 4.32 (s, OH), 4.06 (br s, 2 H), 3.3–3.7 (m, 14 H), 2.7–2.9 (m, 1 H), 2.5–2.7 (m, 2 H), 2.17 (s, $ArCH_3$), 2.16 (s, $ArCH_3$), 2.08 (s, $ArCH_3$), 1.8–2.0 (m, 8 H), 1.65–1.8 (m, 1 H), 1.61 (s, CCH_3); MS (EI) *m/e* 534, 371.

Method B. Typical Procedure for Amide Reduction. 2-[[4-[3-(Ethylamino)-2-pyridinyl]-1-piperazinyl]methyl]-3,4-dihydro-2,5,7,8-tetramethyl-2*H*-1-benzopyran-6-ol (37). Borane–methyl sulfide complex (71.4 mL, 0.714 mol, 10.0 M) was added dropwise over 10 min to a solution of amide 13 (13.0 g, 29.6 mmol) and THF (120 mL). The solution was stirred for 72 h at room temperature, and then excess hydride was decomposed with 10% HCl. Basic workup (CH_2Cl_2, $NaHCO_3$, brine wash, $MgSO_4$) and purification by flash chromatography (1:1 ethyl acetate–cyclohexane) gave the amine which was converted directly to the dihydrochloride salt (ethereal HCl). Recrystallization from MeOH–ether provided 12.4 g (84%) of 37 as a colorless solid (mp 203–205 °C): IR (Nujol) 3246, 3211, 3199, 2434, 1561, 1457, 1254, 1086, 945 cm^{-1}; MS (EI) *m/e* 424, 219. Spectral data for the free base: ^{1}H NMR (300 MHz, $CDCl_3$) 7.70 (dd, J = 4.8, 1.7 Hz, ArH), 6.89 (dd, J = 7.9, 4.8 Hz, ArH), 6.78 (dd, J = 7.9, 1.5 Hz, ArH), 4.05–4.4 (m, OH, NH), 2.95–3.2 (m, 6 H), 2.5–2.9 (m, 6 H), 2.56 (s, CCH_2N), 2.16 (s, $ArCH_3$), 2.12 (s, $ArCH_3$), 2.09 (s, $ArCH_3$), 1.9–2.2 (m, 1 H), 1.6–1.8 (m, 1 H), 1.15–1.4 (m, CCH_3, CH_2CH_3).

Method C. Typical Procedure for Amide Reduction. 3,4-Dihydro-2,5,7,8-tetramethyl-2-[[4-(phenylmethyl)-1-piperazinyl]methyl]-2*H*-1-benzopyran-6-ol (39). A solution of amide 15 (2.00 g, 4.90 mmol) and THF (10.0 mL) was added dropwise over 10 min to a suspension of lithium aluminum hydride (370 mg, 9.75 mmol) and THF (10.0 mL) at 0 °C. After 15 min the mixture was allowed to warm to room temperature and was stirred for 18 h. Excess hydride was quenched at 0 °C with ethyl acetate (2.0 mL), and then water (0.40 mL), 15% NaOH (0.40 mL), and water (1.2 mL) were added successively. After warming to room temperature, the mixture was filtered through Celite, and the solids were washed with ethyl acetate several times. Acidic workup (ethyl acetate, $CHCl_3$, NaOH, Na_2SO_4) and purification by flash chromatography (1:1 ethyl acetate–hexane) gave 1.24 g (64%) of the amine. The hydrochloride salt was prepared in ether and recrystallized from MeOH–water to provide 35 as a white powder (mp 248–250 °C): IR (Nujol) 3436, 2412, 1462, 1424, 1377, 1260, 1086, 757, 703 cm^{-1}; MS (EI) *m/e* 394, 189, 91. Spectral data for the free base: ^{1}H NMR (300 MHz, $CDCl_3$) 7.1–7.4 (m, ArH), 4.36 (br s, OH), 3.48 (s, NCH_2Ar), 2.3–2.8 (m, NCH_2), 2.14 (s, $ArCH_3$), 2.10 (s, $ArCH_3$), 2.05 (s, $ArCH_3$), 1.8–2.2 (m, 2 H), 1.6–1.8 (m, 1 H), 1.21 (s, CCH_3).

Lipid Peroxidation Studies. Details for this assay were previously reported.[13a,30] In brief, rat brain homogenates (1:10, w/v) were prepared in Krebs buffer [15 mM *N*-(2-hydroxyethyl)-piperazine-*N'*-2-ethanesulfonic acid (HEPES), pH 7.4, 10 mM glucose, 140 mM NaCl, 3.6 mM KCl, 1.5 mM $CaCl_2$, 1.4 mM KH_2PO_4, 0.7 mM $MgSO_4$] and used immediately. Incubations were initiated by the addition of 200 μM $FeCl_2$ prepared as described elsewhere.[13a] After 20 min, the reaction was stopped by the addition of 12.5% trichloroacetic acid in 0.8 N HCl. Lipid peroxidation was assessed by the formation of thiobarbituric acid reactive products (e.g. malondialdehyde) as described previously.[13a] Most compounds were prepared in H_2O or H_2O–ethanol and were diluted serially.

Mouse Head Injury. The details of the mouse head injury model have been published in detail previously.[32a,39] As in the past studies, male CF-1 mice weighing 17–20 g were utilized. Each mouse that survived the injury received vehicle (0.05 N HCl) or test drug in a 0.1 mL volume as an iv bolus within 5 min postinjury. Groups of 20–25 mice were injured in rapid succession. Each trial was carried out blind with a vehicle group and four groups receiving various doses of test compound. At 1 h postinjury the neurological status of the injured mice was blindly evaluated using a grip test. The mean time that the injured mice could remain on the grip string was measured and comparisons between vehicle and drug treated mice made using Duncan's multiple range test.

Cytotoxic Hypoxia: KCN Lethality. Upjohn Male CF-1 mice weighing between 18–22 g were pretreated with vehicle (0.9% saline or 0.5% Tween-80) or test drug via single dose tail-vein injections given in a volume of 0.2 mL/mouse. After 15 min, the mice were weighed and challenged with a rapid tail-vein injection of KCN 0.01 mL/g, dissolved in 0.9% saline. After another 15 min, the number of deaths was assessed. Ascending doses (0.5 mg/kg interval) of KCN were administered to groups of six mice, and the LD_{50}'s and 95% confidence intervals were determined with the Spearman-Karber program in EZSTATS. The antihypoxic effect of the various test drugs was evaluated by comparing the resulting LD_{50}'s of the same day and using the 95% confidence intervals to determine significance.

Acknowledgment. We thank Physical and Analytical Chemistry for their support.