Novel 21-Aminosteroids That Inhibit Iron-Dependent Lipid Peroxidation and Protect against Central Nervous System Trauma

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A novel class of 21-aminosteroids has been developed. Compounds within this series are potent inhibitors of iron-dependent lipid peroxidation in rat brain homogenates with IC_{50}'s as low as 3 μM. Furthermore, selected members enhance early neurological recovery and survival in a mouse head injury model. Significant improvement in the 1 h post-head-injury neurological status (grip test score) by as much as 168.6% of the control has been observed. The most efficacious compound in this assay (**30**) showed an increase in the 1-week survival of 78.6% as compared to 27.3% for the vehicle-treated mice in the head-injury model. Based on its biological profile, 21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-16α-methylpregna-1,4,9(11)-triene-3,20-dione monomethanesulfonate (**30**) was selected for further evaluation and is currently entering phase I clinical trials for the treatment of head and spinal trauma.

Lipid peroxidation is an important pathophysiological event in many illnesses, drug toxicities, and traumatic or ischemic injuries. For example, in the central nervous system, oxygen-radical generation and lipid peroxidation have been recognized as important degradative processes in the irreversible loss of neuronal tissue following brain or spinal cord injury,[1] stroke,[2] and possibly degenerative neurological disorders such as Parkinson's disease.[2a,3] It is becoming increasingly apparent that iron is involved in both the initiation[4] and propagation[5] of oxygen radicals in this peroxidative process. Thus, compounds that inhibit iron-dependent lipid peroxidation and prevent oxygen-radical-mediated tissue damage could be of great therapeutic importance.

Methylprednisolone (1) and related steroids inhibit lipid peroxidation.[6] More importantly, rigorous high-dose treatment with methylprednisolone enhances neurological

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recovery after head injury in mice,[7] spinal injury in cats,[8] and improves survival and speech recovery in severe head injury victims.[9] Because of the high doses required in these studies[7–9] for cerebroprotective effects, the beneficial action observed should be unrelated to glucocorticoid receptor activation, which typically is affected at much lower doses.[10]

A series of 21-aminosteroids has been developed that inhibit iron-dependent lipid peroxidation[11] (in vitro). This novel class of compounds has also shown excellent activity in in vivo models of experimental central nervous system (CNS) trauma and ischemia.[12] Compounds within this series lack glucocorticoid activity and in most cases have efficacy and potency which equal or surpass that of methylprednisolone. One compound, 21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-16α-methylpregna-1,4,9(11)-triene-3,20-dione monomethanesulfonate

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(1) (a) Hall, E. D.; Braughler, J. M. *Surg. Neurol.* 1982, *18*, 320. (b) Hall, E. D.; Braughler, J. M. *Cent. Nerv. Syst. Trauma* 1987, *3*, 281. (c) Anderson, D. K.; Means, E. D. *Neurochem. Pathol.* 1983, *1*, 249. (d) Saunders, R. D.; Dugan, L. L.; Demediuk, P.; Means, E. D.; Horrocks, L. A.; Anderson, D. K. *J. Neurochem.* 1987, *49*, 24. (e) Kontos, H. A.; Wei, E. P. *J. Neurosurg.* 1986, *64*, 803.
(2) (a) Halliwell, B.; Gutteridge, J. M. C. *Trends Neurosci.* 1985, *8*, 22. (b) Yamamoto, M.; Shima, T.; Uozumi, T.; Sogabe, T.; Yamada, K.; Kawasaki, T. *Stroke* 1983, *14*, 977. (c) Watson, B. D.; Busto, R.; Goldberg, W. J.; Santiso, M.; Yoshida, S.; Ginsberg, M. D. *J. Neurochem.* 1984, *42*, 268.
(3) (a) Slivka, A.; Cohen, G. *J. Biol. Chem.* 1985, *260*, 15466. (b) Cohen, G. *Pathology of Oxygen*; Autor, A. P., Ed.; Academic Press: New York, 1982; pp 115–126.
(4) (a) Braughler, J. M.; Duncan, L. A.; Chase, R. L. *J. Biol. Chem.* 1986, *261*, 10282. (b) Girotti, A. W.; Thomas, J. P. *J. Biol. Chem.* 1984, *259*, 1744. (c) Thomas, C. E.; Morehouse, L. A.; Aust, S. D. *J. Biol. Chem.* 1985, *260*, 3275.
(5) (a) O'Brien, P. J. *Can. J. Biochem.* 1969, *47*, 485. (b) Piette, L. H.; Baxely, L. H.; Grova, T. A.; Harwood, P. J. *Oxygen Radicals in Chemistry and Biology*; Walter de Gruyter & Co.: Berlin, 1984; pp 137–145.
(6) Demopoulos, H. B.; Flamm, E. S.; Seligman, M. L.; et al. *Can. J. Physiol. Pharmacol.* 1982, *60*, 1415.
(7) Hall, E. D. *J. Neurosurg.* 1985, *62*, 882.
(8) Hall, E. D.; Braughler, J. M. *J. Neurosurg.* 1982, *57*, 247.
(9) Giannotta, S. L.; Weiss, M. H.; Apuzzo, M. L. J.; et al. *Neurosurg.* 1984, *15*, 497.
(10) Haynes, R. C.; Murad, F. *The Pharmacological Basis of Therapeutics*, 6th ed.; Gilman, A. G., Goodman, L. S., Gilman, A., Ed.; Macmillan Publishing Co.: New York, 1980; pp 1466–1496.
(11) (a) Braughler, J. M.; Pregenzer, J. F.; Chase, R. L.; Duncan, L. A.; Jacobsen, E. J.; McCall, J. M. *J. Biol. Chem.* 1987, *262*, 10438. (b) Braughler, J. M.; Chase, R. L.; Neff, G. L.; Yonkers, P. A.; Day, J. S.; Hall, E. D.; Sethy, V. H.; Lahti, R. A. *J. Pharmacol. Exp. Ther.* 1988, *244*, 423. (c) Braughler, J. M.; Burton, P. S.; Chase, R. L.; Pregenzer, J. F; Jacobsen, E. J.; VanDoornik, F. J.; Tustin, J. M.; Ayer, D. E.; Bundy, G. L. *Biochem. Pharmacol.* 1988, *37*, 3853.
(12) (a) Hall, E. D.; Yonkers, P. A.; McCall, J. M.; Braughler, J. M. *J. Neurosurg.* 1988, *68*, 456. (b) McCall, J. M.; Braughler, J. M.; Hall, E. D. *Acta Anaesthesiol. Belg.* 1987, *38*, 417. (c) Hall, E. D. *J. Neurosurg.* 1988, *68*, 462. (d) Hall, E. D. *Acta Anaesthesiol. Belg.* 1987, *38*, 421. (e) Hall, E. D.; Berry, K. P.; Braughler, J. M. *Stroke* 1988, *19*, 997. (f) Braughler, J. M; Hall, E. D.; Jacobsen, E. J.; McCall, J. M.; Means, E. D. *Drugs Future* 1989, *14*, 143.

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Scheme I

Scheme II

(30), has been selected for further evaluation in the treatment of head and spinal cord injuries.

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Chemistry

The 21-aminosteroids were prepared as illustrated in Scheme I. Reaction of readily available[13] 21-halo- or 21-sulfonylpregnanes with the appropriately substituted piperazines in acetonitrile provided coupled products 11–16 and 18–32. In some cases (24, 30) the 21-*O*-mesylate was generated (from the corresponding alcohol) and reacted with the desired amine (CH_3CN, potassium carbonate) in situ. Acylation of 16 with *tert*-butylacetyl chloride provided 17. Table I highlights the physical data of the compounds prepared, which were typically characterized as their acid salts.

The novel heterocyclic piperazines utilized in this study were prepared by the routes illustrated in Schemes II and III. Treatment of 2,4,6-trichloropyrimidine with excess pyrrolidine gave 2 (Scheme II). Reaction of this intermediate with piperazine in hot pyridine provided triaminopyrimidine 3. Diaminopyridine 4 was prepared in an analogous fashion.

The synthesis of triaminopyridine 9 was carried out by the route shown in Scheme III. Reaction of 2,6-dichloro-3-nitropyridine with diethylamine provided chloropyridines 5A and 5B as a 5.8:1 mixture[15] of regiochemical isomers.[16] The major isomer (5A) was reacted with piperazine to provide 6. Hydrogenation, protection with di-*tert*-butyl dicarbonate and reductive amination[17] with acetaldehyde gave 8. Acidic deprotection provided 9 in good overall yield. Related diaminopyridine 10 was prepared by an analogous route starting with 2-chloro-3-nitropyridine.

Biological Results and Discussion

The compounds listed in Table I were initially screened in vitro for their ability to inhibit iron-dependent lipid peroxidation. Homogenized rat brain in Krebs buffer was exposed to 200 μM Fe^{2+}. The resulting lipid peroxidation was evaluated by the formation of thiobarbituric acid reactive products.[4a] Several of the compounds tested were found to bind avidly to glass and plastic. In these cases, the assays were run in 20% ethanol, which eliminated the glass-binding properties. The compounds were tested at doses up to 300 μM with IC_{50} values determined for the more active analogues as shown in Table II.

The amine substituent is required for effective biological activity in this assay. Simple steroidal piperazine analogues (11 or 12) were virtually inactive. Heteroaromatic piperazine substituents, however, inhibited lipid peroxidation reasonably well. The most effective compounds contain electron-rich piperazinylpyrimidines (3) or piperazinylpyridines (9, 10). In these cases, IC_{50} values as low as 3 μM were observed (Table II). For instance, in this assay, α-tocopherol (vitamin E), which is an extremely potent inhibitor of lipid peroxidation,[11a,c] has an IC_{50} of 2 μM. Desferrioxamine,[11a,c] a strong iron binder and inhibitor of lipid peroxidation, was relatively weak with an IC_{50} of over 200 μM, while methylprednisolone (1) was virtually inactive (<10% inhibition at 1 mM).

The effect of the steroid portion on the inhibition of lipid peroxidation was also evaluated by utilizing a common amine [1-(2-pyridyl)piperazine] as shown in Table II

(13) The 21-halosteroids utilized were prepared from the corresponding hydroxyl or hydrocarbon precursors by known methods.[14]

(14) For general synthesis of steroidal intermediates, see: (a) Fried, J.; Edwards, J. A. *Organic Reactions in Steroid Chemistry*, Van Nostrand Reinhold Co.: New York, 1972; Vols. I and II. (b) *Steroid Reactions: An Outline for Organic Chemists*; Djerassi, C., Ed.; Holden-Day Inc.: San Francisco, 1963.

(15) Based on chromatographically isolated products.

(16) The regiochemical assignments of 5A and 5B were based on literature precedent: Van Bebenburg, W.; Steinmetz, G.; Theile, K. *Chem.-Ztg.* 1979, *103*, 387.

(17) Borch, R. F.; Bernstein, M. D.; Dupont Durst, H. *J. Am. Chem. Soc.* 1971, *93*, 2897.

Table I. Physical Data of 21-Aminosteroids

compd	unsatn	R[1]	R[2]	R[3]	R[4]	R[5]	mp, °C	% yield	formula	analysis
11	$\Delta^{4,9(11)}$	H	H	OH	H	H	172–174	86	$C_{25}H_{36}N_2O_3 \cdot CH_4SO_3 \cdot (H_2O)_{1/3}$	C, H, N
12	$\Delta^{4,9(11)}$	H	H	OH	H	CH_2CH_2OH	151–152	82	$C_{27}H_{40}N_2O_4 \cdot (H_2O)_{1/5}$	C, H, N
13	$\Delta^{4,9(11)}$	H	H	OH	H	2-pyridine	213–215	74	$C_{30}H_{39}N_3O_3 \cdot CH_4SO_4$	C, H, N
14	Δ^{4}	O=	H	OH	H	2-pyridine	154–157	54	$C_{30}H_{39}N_3O_4$	C, H, N
15	$\Delta^{1,4}$	β-OH, H	CH_3	OH	H	2-pyridine	145–148	66	$C_{31}H_{41}N_3O_4 \cdot (H_2O)_{1/2}$	C, H, N
16	Δ^{4}	α-OH, H	H	OH	H	2-pyridine	245 (dec)	67	$C_{30}H_{41}N_3O_4 \cdot (HCl)_2 \cdot (H_2O)_{1/3}$	C, H, N, Cl
17	Δ^{4}	α-CO_2-CH_2 + Bu	H	OH	H	2-pyridine	179 (dec)	86	$C_{36}H_{51}N_3O_5 \cdot (HCl)_{1.4} \cdot (H_2O)_{1.5}$	C, H, N, Cl
18	Δ^{4}	H	H	H	H	2-pyridine	201 (dec)	61	$C_{30}H_{41}N_3O_2 \cdot (HCl)_2 \cdot H_2O$	C, H, N, Cl
19	Δ^{4}	H	H	OH	H	2-pyridine	219 (dec)	31	$C_{30}H_{41}N_3O_3 \cdot (HCl)_{1.75} \cdot (H_2O)_{1.5}$	C, H, N, Cl
20	$\Delta^{9(11)}$	H	H	OH	CH_3	2-pyridine	204 (dec)	88	$C_{31}H_{43}N_3O_3 \cdot (HCl)_2 \cdot (H_2O)_{1/4}$	C, H, N, Cl
21	Δ^{4}	O=	H	H	H	2-pyridine	202 (dec)	88	$C_{30}H_{39}N_3O_3 \cdot (HCl)_{1.75} \cdot (H_2O)_{1.5}$	C, H, N, Cl
22	3α-OH	O=	H	OH	H	2-pyridine	195 (dec)	88	$C_{30}H_{43}N_3O_4 \cdot (HCl)_2 \cdot (H_2O)_{1.5}$	C, H, N, Cl
23	$\Delta^{1,4}$	α-OH, H	H	OH	H	2-pyridine	141–142	39	$C_{30}H_{39}N_3O_4 \cdot C_4H_4O_4 \cdot H_2O$	C, H, N
24	$\Delta^{1,4,9(11)}$	H	H	H	CH_3	2-pyridine	174–177	84	$C_{31}H_{39}N_3O_2 \cdot (CH_4SO_3)_{4/3} \cdot (H_2O)_2$	C, H, N,[a] S
25	$\Delta^{4,9(11)}$	H	H	OH	H	2-anisole	164–166	83	$C_{32}H_{42}N_2O_4$	C, H, N
26	$\Delta^{4,9(11)}$	H	H	OH	H	OMe	228–231 dec	82	$C_{31}H_{41}N_3O_4 \cdot CH_4SO_3 \cdot H_2O$	C, H, N
27	$\Delta^{4,9(11)}$	H	H	OH	H	CH_2, HO	177–180	42	$C_{31}H_{41}N_3O_4 \cdot C_4H_4O_4 \cdot (H_2O)_{1.25}$	C, H, N
28	$\Delta^{4,9(11)}$	H	H	OH	H		190–195	36	$C_{34}H_{46}N_4O_3 \cdot C_4H_4O_4 \cdot (H_2O)_{3/4}$	C, H, N
29	$\Delta^{4,9(11)}$	H	H	OH	H		156–158	79	$C_{37}H_{52}N_6O_3 \cdot C_4H_4O_4$	C, H, N
30	$\Delta^{1,4,9(11)}$	H	H	H	CH_3		173–176 dec	80	$C_{38}H_{52}N_6O_2 \cdot CH_4SO_3$	C, H, N, S
31	$\Delta^{1,4,9(11)}$	H	H	H	CH_3		125 dec	84	$C_{39}H_{57}N_5O_2 \cdot (C_4H_4O_4)_2 \cdot (H_2O)_{1/2}$	C, H, N
32	$\Delta^{1,4,9(11)}$	H	H	H	CH_3	EtHN	170–171	68	$C_{33}H_{44}N_4O_2 \cdot C_4H_4O_4 \cdot (H_2O)_{1/4}$	C, H, N

[a] N: calcd, 6.47; found, 6.06.

Table II. Malonyldialdehyde (MDA) Formation Assay

compd	MDA formation: IC_{50}[a] or % inhibn at 300 μM	compd	MDA formation: IC_{50}[a] or % inhibn at 300 μM
11	15%	24	4%
12	0%	25	37%
13	23%	26	101 μM
14	3%	27	5%
15	0%	28	57 μM
16	20%	29	25%
17	6%	30	18 μM
18	9%	31	3 μM
19	26%	32	15 μM
20	31%	1	0%
21	14%	α-tocopherol	30 μM
22	3%	desferrioxamine	112 μM
23	0%		

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

(13–23). This amine is weak enough as a lipid peroxidation inhibitor that it does not dominate the biological activity of the steroidal portion. In this series, the steroidal substituents had only a modest effect on biological activity with a range of only 0–31% inhibition even at 300 μM. In general, while some structure–activity relationships (SAR) of the steroid portion are evident, the particular amine substituent overwhelms these effects with the more potent amines. Thus, in selecting the steroid, other factors such as chemical stability,[18] diminished glucocorticoid activity,[10,11b] and metabolic stability were taken into consideration.

(18) Some of the 17-hydroxy-21-aminosteroids were found to undergo a facile amino–Mattox rearrangement. See: Mattox, V. *J. Am. Chem. Soc.* **1952**, *74*, 4340. Also see ref 14a, Vol. II, pp 214–217.

Scheme III

Table III. Mouse Head Injury Assay

compd	1 h postinjury grip score mean, s ± SEM (best dose, mg/kg)	lowest effective dose, mg/kg
vehicle[a]	5.3 ± 3.3	
1	13.5 ± 3.6 (60.0)	15.0
13	12.7 ± 3.0 (30.0)	10.0
14	inactive[b]	
15	inactive	
24	14.7 ± 4.2 (30.0)	10.0
29	13.4 ± 2.9 (3.0)	1.0
30	14.5 ± 3.0 (10.0)	0.003
31	14.1 ± 3.5 (0.1)	0.003
32	10.7 ± 2.8 (0.3)	0.03

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

The compounds that were the most effective in inhibiting lipid peroxidation were also evaluated for in vivo neuroprotective activity in a mouse head injury model.[12a,19] Unanesthetized male mice were subjected to a concussive head injury (approximated 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 unconciousness (loss of righting reflex). The compounds of interest were tested at a dose range from 0.01 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.[12a]

Results for the compounds tested in the mouse head injury assay are shown in Table III. The most effective compounds are also among the most potent inhibitors of lipid peroxidation. While some of the simpler (2-pyridinylpiperazinyl)steroids do display acceptable in vivo activity, the more electron-rich triaminopyrimidines (**29** and **30**) and polyaminopyridines (**31** and **32**) are clearly superior in potency and efficacy. Long-term survival was also determined for **30**. At 1 week, 78.6% of the drug-treated mice had survived in comparison to 27.3% of the vehicle-treated mice ($p < 0.02$).

(19) Hall, E. D. *Int. Rev. Neurobiol.* **1982**, *23*, 165.

Table IV. Cytotoxic Hypoxia Assay[a]

compd	% increase in KCN LD_{50} 30 mg/kg iv	% increase in KCN LD_{50} 100 mg/kg iv
1	0	20*
29	0	33*
30	14*	20*
31	15*	46*

[a] * $p < 0.05$.

Several compounds were also evaluated in a potassium cyanide lethality (cytotoxic hypoxia) model.[20] Male CF-1 mice were pretreated with drug or vehicle (0.9% saline) and challenged with increasing doses of potassium cyanide. The antihypoxic effect for **29**–**31** was evaluated by comparing LD_{50}'s with vehicle LD_{50}'s. The results of this study are shown in Table IV. Both **30** and **31** were found to be active at doses as low as 30 mg/kg.

The 21-aminosteroids represent a unique class of antioxidants.[21] As noted above, we believe that the inhibition of lipid peroxidation and oxygen-radical generation represents a primary mechanism of action[22a,b] for compounds in this series in experimental models of CNS trauma and

(20) Nauquier, A.; Ashton, D.; Clincke, G.; Niemegeers, C. J. E. *Arch. Int. Pharmacodyn.* **1981**, *249*, 330.

(21) Braughler, J. M.; Pregenzer, J. F. *Free Radical Biol. Med.* In press.

(22) (a) Braughler, J. M.; Hall, E. D. *Free Radical Biol. Med.* **1989**, *6*, 289. (b) Hall, E. D.; Braughler, J. M. *Free Radical Biol. Med.* **1989**, *6*, 303.

ischemic-reperfusion injury. These compounds clearly have other activities, however, that distinguish them from other simple antioxidants.[23] A considerable amount of information has been obtained concerning the mechanisms by which the 21-aminosteroids inhibit lipid peroxidation and scavenge radicals. We have selected one compound, **30**, for clinical trials.

The mechanism of action of **30** has been examined in detail. This aminosteroid is an antioxidant and not an iron chelator.[11a,21] It inhibits lipid peroxidation by scavenging lipid peroxyl radicals and by blocking lipid free radical chain reactions.[21] During this process **30** was consumed (to provide as yet unidentified products) and, in this regard, acted like vitamin E. Not unexpectedly, **30** slowed the inactivation of vitamin E and enhanced vitamin E's radical-scavenging capacity.[21] This latter effect of **30** to spare vitamin E may be an important in vivo action of the compound since vitamin E is the major naturally occurring lipid-soluble antioxidant found in cell and organelle membranes.[24-26] While **30** and other 21-aminosteroids have been found to react readily with peroxyl radicals, their reactivity toward alkoxyl radicals is minimal.[21]

The 21-aminosteroids interact physically with membrane lipids. Concentrations of **30** in the range of 1–20 μM profoundly increase the order of membrane lipids and result in a reduction in membrane fluidity.[27] This may in part underlie their lipid peroxidation inhibitory capacity as they probably physically confine lipid radicals near their site of generation and prevent lipid radical diffusion through the membrane.

The 21-aminosteroids also block oxygen-radical-stimulated proteolysis.[28] While these compounds do not directly inhibit protease activity, they protect cellular proteins from proteolytic attack apparently because of their antioxidant activity. This activity appears to be independent of the ability of these compounds to inhibit lipid peroxidation. This is important because it points to an extramembrane activity of the 21-aminosteroids that would not have been predicted because of their high lipophilicity. Compound **30** is particularly protective in these systems and has been examined in detail for its ability to protect functional processes within membranes from damage caused by oxygen radicals. Aminosteroid **30** protected the neurotransmitter uptake system for GABA in purified rat brain synaptosomes from damage caused by exposure to superoxide radical or iodoacetate. The finding that **30** could protect GABA uptake from superoxide-mediated damage is consistent with its apparent ability to scavenge superoxide radical under certain conditions.

Additional studies with **30** in models of CNS trauma and ischemia have provided further evidence of its therapeutic utility. For instance, in a spinal cord compression injury model,[29] treatment with **30** resulted in a significant improvement in the neurological recovery of cats over a broad range of doses. In a model of cerebral ischemia[12e] in gerbils (the right carotid artery was occluded for 3 h), aminosteroid **30** improved long-term survival as compared to vehicle-treated animals. Furthermore, a histological examination of the brains after 24 h showed a significant preservation of neurons in the hippocampus and lateral cortex. In contrast, the vehicle-treated animals suffered as much as a 90% neuronal loss as compared to that of uninjured animals. In these and other[12] in vivo studies, **30** preserved plasma vitamin E levels after neurological injury, maintained caudate blood flow after subarachnoid hemorrhage, inhibited microvascular permeability increase after stroke, and enhanced the reversal of intracellular calcium accumulation after ischemia. Further studies on **30** and related 21-aminosteroids as inhibitors of lipid peroxidation with neuroprotectant abilities will be reported in due course.

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 analyses were obtained by the Physical and Analytical Chemistry Department of The Upjohn Company. ^{1}H NMR spectra were recorded at 80 MHz with a Varian Model CFT-20 spectrometer, at 200 MHz with a Varian Model XL 200 spectrometer, and 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. 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.

2,4-Di-1-pyrrolidinyl-6-chloropyrimidine (2). A solution of 80.0 g (1.12 mol) of pyrrolidine and 500 mL of THF was cooled to 0 °C and stirred mechanically. With a syringe pump, 50.0 g (0.273 mol) of 2,4,6-trichloropyrimidine was added over 35 min. The reaction was stirred at 0 °C for 1 h and was then allowed to warm to room temperature over 4 h. Pyridine (100 mL) was added to the reaction and the mixture was stirred at room temperature overnight. The reaction was concentrated. Basic workup (CH_2Cl_2, 3 × 200 mL; saturated $NaHCO_3$, 200 mL; $MgSO_4$) and purification by flash chromatography (10% ethyl acetate/hexane) gave 51.0 g (74%) of **2** as a crystalline solid (mp 77–79 °C): IR (Nujol) 2925, 1583, 1507, 1455, 1346, 1002, 775 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.64 (s, ArH), 3.2–3.7 (m, NCH_2), 1.8–2.1 (m, CH_2CH_2N); MS (EI) m/e 252, 224, 196, 121, 70.

4-(1-Piperazinyl)-2,6-di-1-pyrrolidinylpyrimidine (3). A solution of **2** (51.0 g, 202 mmol), piperazine (40.0 g, 464 mmol), and pyridine (100 mL) was heated at 100 °C for 50 h. Concentration, basic workup (CH_2Cl_2, 3 × 200 mL; saturated $NaHCO_3$, 200 mL; $MgSO_4$) and purification by flash chromatography (CH_2Cl_2 → 90:10:1 CH_2Cl_2–MeOH–NH_4OH) provided 51.0 g (83%) of **3** as a light yellow powder (mp 177–178 °C): IR (Nujol) 2954, 2925, 1564, 1444, 1346, 787 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) 4.83 (s, ArH), 3.2–3.7 (m, NCH_2), 2.97 (apparent s, NCH_2), 1.7–2.0 (m, CH_2CH_2N); MS (EI) m/e 302, 246, 233.

2-(1-Piperazinyl)-6-(1-pyrrolidinyl)pyridine (4): IR (Nujol) 3191, 2925, 2856, 1590, 1493, 1481, 1435, 1396, 1352, 1237, 1157, 769, 698 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) 7.30 (apparent t, J = 8.1 Hz, ArH), 5.88 (d, J = 8.0 Hz, ArH), 5.75 (d, J = 8.0 Hz,

(23) The oxidation potential of **30** is +1.02 V while that of **31** is +0.35 V (Kuwana, T.; Lunte, S. Unpublished observations). Vitamin E and **32** fall within this range with oxidation potentials of +0.51 and +0.72 V, respectively. Clearly based on their similar abilities as inhibitors of lipid peroxidation, but rather dissimilar oxidation potentials, they are not classical antioxidants related to vitamin E.

(24) Machlin, L. J.; Bendich, A. *FASEB J.* 1987, *1*, 441.
(25) McCay, P. B. *Annu. Rev. Nutr.* 1985, *5*, 323.
(26) Ingold, K. U.; Webb, A. C.; Witter, D.; Burton, G. W.; Metcalf, T. A.; Muller, D. P. R. *Arch. Biochem. Biophys.* 1987, *259*, 224.
(27) Braughler, J. M.; Audus, K. L. Unpublished observations.
(28) Braughler, J. M. Unpublished observations.
(29) Anderson, D. K.; Braughler, J. M.; Hall, E. D.; Waters, T. R.; McCall, J. M.; Means, E. D. *J. Neurosurg.* 1988, *69*, 562.

ArH), 3.49 (t, J = 5.1 Hz, CH_2N), 3.42 (t, J = 6.6 Hz, CH_2N), 2.98 (t, J = 5.1 Hz, CH_2N), 2.30 (s, NH), 1.85–2.05 (m, CH_2CH_2N); MS (EI) *m/e* 232, 190, 176.

6-Chloro-2-(*N*,*N*-diethylamino)-3-nitropyridine (5A). A solution of diethylamine (0.540 mL, 5.22 mmol) and acetonitrile (4.0 mL) was added dropwise over 40 min to a mixture of 2,6-dichloro-3-nitropyridine (1.00 g, 5.18 mmol), acetonitrile (16.3 mL), and potassium carbonate (0.848 g, 6.14 mmol) at 0 °C. After an additional 40 min at 0 °C, the reaction was allowed to warm to ambient temperature and stir overnight. Filtration, aqueous workup (CH_2Cl_2, 3 × 25 mL; $MgSO_4$) and purification by flash chromatography (5:1 hexane–ethyl acetate) provided 0.891 g (75%) of **5A** as an orange oil. Further elution gave 0.152 g (13%) of the regioisomer **5B**. Spectral data for **5A**: IR (neat) 2979, 1593, 1550, 1498, 1435, 1334, 1256, 1142, 854 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 8.00 (d, J = 8.3 Hz, ArH), 6.60 (d, J = 8.3 Hz, ArH), 3.43 (q, J = 7.1 Hz, $(CH_3CH_2)_2N$), 1.22 (t, J = 7.0 Hz, $(CH_3CH_2)_2N$); MS (EI) *m/e* 229, 214, 212, 168.

Spectral data for **5B**: IR (Nujol) 2926, 1600, 1528, 1317, 1309, 1291, 1041 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 8.20 (d, J = 9.2 Hz, ArH), 6.37 (d, J = 9.2 Hz, ArH), 3.60 (br s, $(CH_3CH_2)_2N$), 1.24 (t, J = 7.1 Hz, $(CH_3CH_2)_2N$); MS (EI) *m/e* 229, 214, 200, 186.

2-(*N*,*N*-Diethylamino)-3-nitro-6-(1-piperazinyl)pyridine (6). A mixture of **5A** (3.72 g, 16.2 mmol), acetonitrile (56.0 mL), piperazine (8.61 g, 100.0 mmol), and potassium carbonate (2.76 g, 20.0 mmol) was heated at reflux for 3 h. Upon cooling to room temperature, aqueous workup ($CHCl_3$, 3 × 150 mL; water wash of organic layers, 2 × 150 mL; K_2CO_3) provided 4.35 g (96%) of **6** as a thick, orange oil, which solidified upon standing: IR (Nujol) 2950, 2924, 1595, 1564, 1506, 1342, 1278 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 8.17 (d, J = 9.2 Hz, ArH), 6.02 (d, J = 9.2 Hz, ArH), 3.68 (apparent t, J = 5.3 Hz, CH_2N), 3.45 (q, J = 6.9 Hz, $(CH_3CH_2)_2N$), 2.97 (apparent t, J = 5.0 Hz, CH_2N), 1.78 (br s, NH), 1.25 (t, J = 7.0 Hz, $(CH_3CH_2)_2N$); MS (EI) *m/e* 279, 262, 175, 56.

3-Amino-2-(*N*,*N*-diethylamino)-6-(1-piperazinyl)pyridine (7). A mixture of 2-(*N*,*N*-diethylamino)-3-nitro-6-(1-piperazinyl)pyridine (1.00 g, 3.58 mmol), ethanol (24.0 mL), 1.2 N HCl (1.3 mL), and 10% palladium on carbon (0.216 g) was exposed to hydrogen at 46 psi in a Parr flask. After 16 h the residue was filtered through celite and concentrated. Basic workup ($CHCl_3$, 3 × 50 mL; saturated K_2CO_3, 100 mL; K_2CO_3) and purification of the residue through a plug of silica gel (4:1:0.25 $CHCl_3$–MeOH–NH_4OH) gave 0.623 g (70%) of aniline derivative **7** as a green black oil: IR (neat) 3309, 2967, 2828, 1581, 1474, 1451, 1258, 803 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 6.94 (d, J = 8.2 Hz, ArH), 6.25 (d, J = 8.8 Hz, ArH), 3.20–3.40 (m, NCH_2), 3.12 (q, J = 7.1 Hz, $(CH_3CH_2)_2N$), 2.9–3.1 (m, NCH_2), 1.05 (t, J = 6.9 Hz, $(CH_3CH_2)_2N$); MS (EI) *m/e* 249, 220, 207, 193, 177, 163.

2,3-Bis(*N*,*N*-diethylamino)-6-[4-(*tert*-butyloxycarbonyl)piperazin-1-yl]pyridine (8). To a solution of pyridine **7** (13.5 g, 54.1 mmol), triethylamine (8.33 mL, 59.8 mmol) and dichloromethane (400 mL) precooled to 0 °C was added a solution of di-*tert*-butyl dicarbonate (11.8 g, 54.1 mmol) and dichloromethane (25 mL) dropwise over 30 min. The resultant solution was allowed to slowly warm to room temperature. After 16 h, basic workup (CH_2Cl_2, 2 × 100 mL; saturated $NaHCO_3$, 250 mL; K_2CO_3) gave 18.0 g (95%) of the *tert*-butyl carbamate as a purple solid, sufficiently pure to be carried on crude: IR (Nujol) 3420, 3321, 2964, 2925, 1675, 1477, 1451, 1224, 982 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 6.95 (d, J = 8.2 Hz, ArH), 6.27 (d, J = 8.2 Hz, ArH), 3.5–3.7 (m, CH_2N), 3.25–3.4 (m, CH_2N), 3.13 (q, J = 7.1 Hz, $(CH_3CH_2)_2N$), 1.50 (s, NCO_2tBu), 1.06 (t, J = 7.2 Hz, $(CH_3CH_2)_2N$); MS (EI) *m/e* 349, 293, 264.

To a solution of the crude carbamate (4.00 g, 11.4 mmol), acetaldehyde (12.8 mL, 229 mmol), and acetonitrile (80 mL) was added sodium cyanoborohydride (1.73 g, 27.5 mmol). The resultant solution was stirred for 48 h at room temperature. At 24 h another 500 mg (7.96 mmol) of sodium cyanoborohydride and 5.0 mL (89.4 mmol) of acetaldehyde were added. Basic workup ($CHCl_3$, 3 × 50 mL; saturated K_2CO_3, 50 mL; K_2CO_3) and purification by flash chromatography (5:1 hexane–ethyl acetate) gave 2.40 g (52%) of the dialkylated material as a light yellow oil: IR (neat) 2971, 1700, 1566, 1451, 1419, 1240, 1172, cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 7.08 (d, J = 8.2 Hz, ArH), 6.05 (d, J = 8.2 Hz, ArH), 3.54 (q, J = 7.0 Hz, $(CH_3CH_2)_2N$), 3.45–3.6 (m, NCH_2), 3.3–3.45 (m, NCH_2), 2.95 (q, J = 7.1 Hz, $(CH_3CH_2)_2N$), 1.48 (s, tBuO), 1.07 (t, J = 7.0 Hz, $(CH_3CH_2)_2N$), 0.94 (t, J = 6.9 Hz, $(CH_3CH_2)_2N$); MS (EI) *m/e* 405, 349, 320, 276.

2,3-Bis(*N*,*N*-diethylamino)-6-(1-piperazinyl)pyridine (9). A solution of carbamate **8** (2.36 g, 5.82 mmol), ethyl acetate (50 mL), and 3.0 N HCl (37.5 mL) was stirred for 16 h at room temperature. Basic workup ($CHCl_3$, 3 × 100 mL; 10% NaOH, 140 mL; K_2CO_3) provided 1.77 g (100%) of the desired product as a dark green oil, homogenous by TLC analysis: IR (neat) 2967, 2930, 2819, 1587, 1565, 1476, 1467, 1449, 1373, 1244, 783 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) 7.08 (d, J = 8.2 Hz, ArH), 6.04 (d, J = 8.2 Hz, ArH), 3.4–3.7 (m, NCH_2), 3.53 (q, J = 6.9 Hz, $(CH_3CH_2)_2N$), 2.8–3.1 (m, CH_2N), 2.95 (q, J = 7.2 Hz, $(CH_3CH_2)_2N$), 1.07 (t, J = 7.0 Hz, $(CH_3CH_2)_2N$), 0.94 (t, J = 6.9 Hz, $(CH_3CH_2)_2N$); MS (EI) *m/e* 305, 276, 247, 232.

3-(*N*-Ethylamino)-2-(1-piperazinyl)pyridine (10): mp 46–48 °C; IR (Nujol) 3369, 2925, 1579, 1485, 1446, 1235, 1150 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) 7.69 (dd, J = 4.8, 1.7 Hz, ArH), 6.94 (dd, J = 7.9, 4.8 Hz, ArH), 6.83 (dd, J = 7.9, 1.5 Hz, ArH), 4.0–4.1 (m, NH), 3.3–3.5 (m, NCH_2), 3.0–3.2 (m, CH_3CH_2N), 1.30 (t, J = 7.1 Hz, CH_3CH_2N); MS (EI) *m/e* 206, 176, 162, 150, 134, 120.

Method A. Typical Procedure for Coupling of 21-Halosteroids with Substituted Piperazines. 17-Hydroxy-21-[4-(2-methoxyphenyl)-1-piperazinyl]pregna-4,9(11)-diene-3,20-dione (25). A mixture of the 21-bromosteroid (5.00 g, 12.3 mmol), 1-(2-methoxyphenyl)piperazine (2.60 g, 13.5 mmol), potassium carbonate (3.40 g, 24.6 mmol), and acetonitrile (100 mL) was heated at reflux for 18 h. After cooling to room temperature, the mixture was concentrated at reduced pressure. Aqueous workup (ethyl acetate, 250 mL; Na_2SO_4) and purification by flash chromatography (3:1 ethyl acetate–hexane) gave 5.30 g (83%) of **25** as a pale yellow powder. An analytical sample was crystallized from hexane–ethyl acetate (mp 164–166 °C).

Method B. Typical Procedure for Coupling of 21-Hydroxysteroids with Substituted Piperazines. 21-[4-[3-(Ethylamino)-2-pyridinyl]-1-piperazinyl]-16α-methylpregna-1,4,9(11)-triene-3,20-dione (32). Methanesulfonyl chloride (1.03 mL, 13.3 mmol) was added to a solution of the steroidal alcohol (4.43 g, 13.0 mmol), acetonitrile (18.8 mL), and triethylamine (2.10 mL, 15.1 mmol) at 0 °C. The mixture was stirred for 2 h at 0 °C and filtered, and the precipitate was washed with acetonitrile (2 × 6 mL).

The combined filtrates were added dropwise over 30 min to a mixture of **10** (2.89 g, 14.0 mmol), acetonitrile (14 mL), sodium iodide (150 mg, 1.00 mmol), and potassium carbonate (2.21 g, 16.0 mmol). After stirring for 42 h at room temperature, basic workup ($CHCl_3$, 3 × 100 mL; saturated K_2CO_3, 200 mL; K_2CO_3) and purification by flash chromatography (EtOAc) provided 4.70 g (68%) of the aminosteroid as a white foam. The grey maleic acid salt was prepared in methanol and recrystallized from ethyl acetate–petroleum ether (mp 170–171 °C).

11α-(3,3-Dimethyl-1-oxobutoxy)-17-hydroxy-21-[4-(2-pyridinyl)-1-piperazinyl]pregn-4-ene-3,20-dione (17). To a solution of 4-(dimethylamino)pyridine (42 mg, 0.34 mmol), *tert*-butylacetyl chloride (48 μL, 0.35 mmol), and chloroform (1.0 mL) were added alcohol **16** (0.15 g, 0.30 mmol) and triethylamine (48 μL, 0.34 mmol). After stirring for 72 h at room temperature, basic workup ($CHCl_3$, 3 × 5 mL; 5% NaOH, 5 mL; Na_2SO_4) and purification by flash chromatography (10:1 $CHCl_3$–MeOH) resulted in 156 mg (86%) of the desired ester: IR (Nujol) 3427, 2952, 2923, 2913, 2855, 1731, 1714, 1656, 1596, 1463, 1438, 1378, 1245 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) 8.0–8.3 (m, ArH), 7.3–7.8 (m, ArH), 6.5–6.75 (m, ArH), 5.75 (s, CH=C), 3.2–3.8 (m, CH_2N, $C(O)CH_2N$), 2.20 (s, $tBuCH_2C(O)$), 1.28 (s, CH_3C), 1.07 (s, tBu), 0.79 (s, CH_3C); MS (EI) *m/e* 605, 176, 147, 121, 95.

Lipid Peroxidation Assay. 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 (100 μL) in Krebs buffer were at 37 °C and contained 10 μL of brain homogenate, 20% ethanol, and the concentration of compound indicated. Reactions were initiated by the addition of 200 μM Fe^{2+} freshly prepared as described elsewhere.[4a] After 20 min, the reaction was stopped by the addition of 500 μL of ice-cold 12.5% trichloroacetic acid in 0.8 N HCl. Lipid peroxidation was assessed by the formation of thiobarbituric acid reactive products

(TBAR) as also described previously.[4a] Most compounds were prepared in H_2O. Although **30** is soluble in 1 equiv of HCl, at concentrations below 100 μM the compound binds avidly to glass and plastic. Therefore, all stock solutions and dilutions of **30** were prepared in ethanol, in which glass binding was not a problem.

Mouse Head-Injury Assay. Male CF-1 mice (Charles River plant, Portage, MI) weighing 16–22 g were utilized in this study. A concussive head injury was inflicted in each mouse. A 900 g cm injury force was used in mice weighing 16–20 g, while a 950 g cm force was used in mice weighing 20–22 g. Each trial consisted of four groups of 20 mice (a vehicle group and three doses of test compound). Each mouse was injured and within 3–5 min post-injury was administered either vehicle or test compounds iv, 0.1 mL bolus, blindly. The four groups were injured and treated 5–7 min apart. Various aqueous vehicles were used to obtain satisfactory solubility of each compound tested. The most commonly employed vehicle was 0.5% Tween 80. Anesthesia was not required since this injury consistently caused immediate unconsciousness as judged from the loss of righting reflex and the loss of any pain response.

At 1 h after injury, the sensorimotor status of the head-injured mice was tested by 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, allowing each mouse an equal chance to grasp the string. The tail was gently released, at which time the mouse either fell, due to inability to hold on, or remained on the string. The length of time the mice could remain on the string in some manner (i.e., one to four paws, tail, or paws plus tail) was measured with a 30-s maximum. The 1-h neurological recovery data was evaluated by a mean grip test score for each treatment group. This test consisted of an average time that all mice in the group could remain on the string.

Cytotoxic Hypoxia: KCN Lethality. Upjohn Male CF-1 mice weighing 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 LD50'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.

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Registry No. **2**, 111669-15-9; **3**, 111641-17-9; **4**, 111641-07-7; **5A**, 125173-52-6; **5B**, 125173-53-7; **6**, 125173-54-8; **7**, 125173-55-9; **7** *tert*-butyl carbamate derivative, 125173-56-0; **8**, 125173-57-1; **9**, 125173-58-2; **10**, 111669-24-0; **11**, 125173-59-3; **11** free base, 111669-45-5; **12**, 125173-60-6; **13**, 125173-61-7; **13** free base, 111640-37-0; **14**, 111640-53-0; **15**, 111640-54-1; **16**, 125173-62-8; **16** free base, 111640-56-3; **17**, 125173-63-9; **17** free base, 116894-86-1; **18**, 111668-42-9; **18** free base, 111640-69-8; **19**, 125173-64-0; **19** free base, 111640-72-3; **20**, 125276-12-2; **20** free base, 125276-13-3; **21**, 111668-47-4; **21** free base, 111640-73-4; **22**, 125173-65-1; **22** free base, 125173-66-2; **23**, 125173-67-3; **23** free base, 111667-63-1; **24**, 125197-00-4; **24** free base, 111667-71-1; **25**, 111640-44-9; **26**, 111668-30-5; **26** free base, 111640-30-5; **27**, 125173-68-4; **27** free base, 111667-58-4; **28**, 125173-69-5; **28** free base, 111667-59-5; **29**, 125173-70-8; **29** free base, 111667-61-9; **30**, 110101-67-2; **30** free base, 110101-66-1; **31**, 125173-72-0; **31** free base, 125173-71-9; **32**, 125197-01-5; **32** free base, 125173-73-1; Et_2NH, 109-89-7; 2,4,6-trichloropyrimidine, 3764-01-0; pyrrolidine, 123-75-1; piperazine, 110-85-0; 2,6-dichloro-3-nitropyridine, 16013-85-7; acetaldehyde, 75-07-0; 21-bromo-17-hydroxypregna-4,9(11)-diene-3,20-dione, 63973-98-8; 1-(2-methoxyphenyl)piperazine, 35386-24-4; 21-hydroxy-16α-methylpregna-1,4,9(11)-triene-3,20-dione, 56016-90-1.

Selective Class III Antiarrhythmic Agents. 1. Bis(arylalkyl)amines

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A series of bis(arylalkyl)amines is described and their effects on prolonging effective refractory period in isolated cardiac tissue listed. Most compounds prolonged the cardiac action potential without significantly altering the maximum rate of depolarization and may be defined as selective class III antiarrhythmic agents. It was found that a particularly advantageous structural feature was to have a methanesulfonamido moiety on both of the aryl rings. Thus, compound **16** [1-(4-methanesulfonamidophenoxy)-2-[*N*-(4-methanesulfonamidophenethyl)-*N*-methylamine]ethane] was selected for further investigations. The compound is highly potent and selective class III agent which acts by blockade of cardiac potassium channels.

Sudden cardiac death (SCD) is a leading cause of mortality among the adult population of the world's industrialized nations. SCD results from electrical instability of the heart muscle leading to a loss of regular cardiac rhythm, and it is accepted that ventricular arrhythmias such as sustained tachycardia (VT) and fibrillation (VF) play the major role in these deaths. Supraventricular arrhythmias cause life-threatening hemodynamic disturbances and thrombotic events such as strokes. Serious arrhythmias occur in two major settings, the first is acute myocardial infarction (AMI), where 45% of those who die generally do so within the first hour of the ischemic event. The second is a chronic situation where a patient may show a disposition to arrhythmias as a result of ischemic damage post-MI or from congestive heart failure (CHF). Prophylactic treatment of these high-risk patients with a safe antiarrhythmic agent represents a major therapeutic challenge.[1]

The Vaughan Williams classification[2] of antiarrhythmic drugs recognizes four distinct categories. The largest group are the class I drugs, whose mechanism of action is that of interference with the fast sodium channels in cell membranes. These drugs are effective against simple ventricular and, in some cases, supraventricular arrhythmias, but they are ineffective as long-term prophylactics for prevention of sudden cardiac death caused by VF.[1] Moreover, they generally have a low therapeutic ratio, their

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(1) Patterson, E.; Lucchesi, B. R. *Am. Heart J.* 1983, *106*, 426.
(2) Vaughan Williams, E. M. *J. Clin. Pharmacol.* 1984, *24*, 129.

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