Class III Antiarrhythmic Activity of Novel Substituted 4-[(Methylsulfonyl)amino]benzamides and Sulfonamides

John W. Ellingboe,*,† Walter Spinelli,§ Michael W. Winkley,‡ Thomas T. Nguyen,† Roderick W. Parsons,§ Issam F. Moubarak,§ Jan M. Kitzen,∥ Donna Von Engen,⊥ and Jehan F. Bagli†

Division of Exploratory Chemistry and Division of Cardiovascular and Metabolic Disorders, Wyeth-Ayerst Research, CN 8000, Princeton, New Jersey 08543-8000, Division of Chemical Development, Wyeth-Ayerst Research, 64 Maple Street, Rouses Point, New York 12979, and Department of Chemistry, Princeton University, Princeton, New Jersey 08540. *Received August 5, 1991*

The synthesis and Class III antiarrhythmic activity of a series of 4-[(methylsulfonyl)amino]benzamides and sulfonamides are described. Selected compounds show a potent Class III activity and are devoid of effects on conduction both in vitro (dog Purkinje fibers) and in vivo (anesthetized dogs). Compounds having a 2-aminobenzimidazole group were found to be the most potent, and one compound having this heterocycle (5, WAY-123,398) was selected for further characterization. Compound 5 was shown to have good oral bioavailability and a favorable hemodynamic profile to produce a 3-fold increase of the ventricular fibrillation threshold and to terminate ventricular fibrillation, restoring sinus rhythm in anesthetized dogs. Voltage-clamp studies in isolated myocytes show that 5 is a potent and specific blocker of the delayed rectifier potassium current (I_K) at concentrations that cause significant prolongation of action potential duration.

Sudden cardiac death (SCD) is a leading cause of mortality in Western society. Ventricular arrhythmias are thought to play a major role in SCD,[1] thus the prevention and control of life-threatening ventricular tachycardias/ventricular fibrillation has a great therapeutic significance. The majority of these arrhythmias occur in patients suffering from ischemic heart disease or congestive heart failure.[2] Clinical evidence and many studies in animal models have also indicated that most life-threatening arrhythmias in these patients are due to reentry.[3] Currently, the most widely prescribed drugs are those that slow conduction and increase refractoriness by blocking the fast sodium channel. Although these Class I antiarrhythmic agents (Vaughan Williams classification[4]) are very effective in reducing the frequency of premature ventricular contraction, they are not very effective in controlling or preventing life-threatening ventricular arrhythmias. Furthermore, the therapeutic usefulness of Class I agents is severely limited by adverse side effects, the most important of which are the negative inotropic and proarrhythmic effects.[5] Excessive depression of conduction may actually promote reentry and result in proarrhythmic effects. Similarly, a significant block of the fast inward Na current has been shown to decrease myocardial contractility. Both of these effects are thought to be exacerbated in the diseased myocardium. The results of the Cardiac Arrhythmia Suppression Trial (CAST) have clearly shown that potential negative side effects of potent Class I agents can outweigh their clinical benefits.[6]

One therapeutic alternative of current interest is the development of agents that prolong refractoriness (Class III antiarrhythmic effect) without depressing conduction of the cardiac impulse (Class I antiarrhythmic effect). Such an agent should be effective in reentrant arrhythmias: a large and selective prolongation of refractoriness would cause the reentrant impulse to enter tissue that has not yet recovered excitability and thus terminate the arrhythmia. An agent with this pharmacological profile should be devoid of both proarrhythmic effects resulting from excessive depression of conduction and negative inotropism resulting from the blockade of sodium channels. Furthermore, agents prolonging the action potential might cause a limited positive inotropic effect, which could be beneficial in the treatment of arrhythmias in congestive heart failure.[7] A number of Class III antiarrhythmics have been disclosed recently. These include sotalol[8] (1), which also has β-blocking activity, E-4031[9] (2), UK-68,798[10] (3),

† Division of Exploratory Chemistry.
§ Division of Cardiovascular and Metabolic Disorders.
‡ Division of Chemical Development.
∥ Present address: Rhone-Poulenc Rorer Central Research, Department of Cardiovascular Biology, King of Prussia, PA 19406.
⊥ Princeton University.

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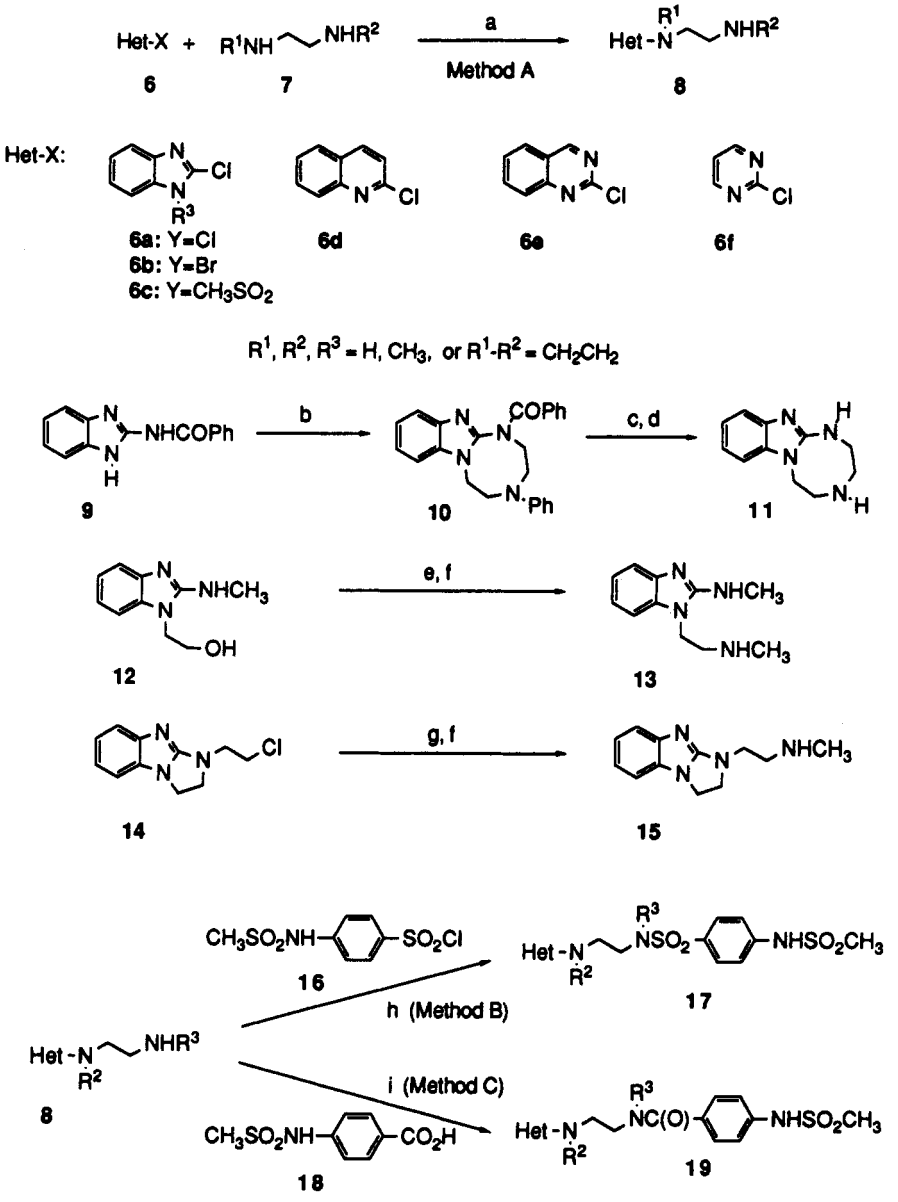
Scheme I



[a] (a) Reflux; (b) *N,N*-bis(chloroethyl)aniline, KOtBu, DMF; (c) NH_3, MeOH; (d) H_2, 10% Pd/C, 1 N HCl, dioxane; (e) MsCl, Et_3N, CH_2Cl_2; (f) CH_3NH_2, EtOH; (g) NaI, acetone; (h) Et_3N, CH_2Cl_2; (i) DCC, HOBT, THF, DMF.

and risotilide[11] (**4**) (Chart I). A common structural feature of these compounds is the 4-[(methylsulfonyl)amino]-phenyl group, a pharmacophore that appears to be associated with selective Class III activity. Also, **2** and **3** are "bis-aryl" compounds: two aromatic rings or an aromatic and a heteroaromatic ring are separated by an alkylamino tether. Compound **2** has a pyridine ring, suggesting that other heteroaromatic rings would also be tolerated. Thus, utilizing risotilide as a template, a novel series of specific Class III antiarrhythmics incorporating a heteroaromatic ring were synthesized, exemplified by WAY-123,398 (**5**, Chart I) and described herein.

Chemistry

The general approach utilized for the synthesis of the target compounds is illustrated in Scheme I. A heterocycle with a leaving group in the 2-position, such as 2-chloro-1*H*-benzimidazole (**6a**), 2-chloroquinoline (**6d**), 2-chloroquinazoline (**6e**), 2-chloropyrimidine (**6f**), 2-bromo-1*H*-benzimidazole (**6b**), or 2-(methylsulfonyl)-1*H*-benzimidazole (**6c**), was heated under reflux with an excess of an ethylenediamine (**7**) to yield a substituted ethylenediamine (**8**) (method A). For example, 2-chloro-1-methyl-1*H*-benzimidazole[12] (**6a**, $R^3 = CH_3$) was heated with excess *N,N'*-dimethylethylenediamine (**7**, R^1 and $R^2 = CH_3$) to give an 80% yield of **8** (Het = 2-benzimidazolyl, R^1, R^2, $R^3 = CH_3$). The triazocinobenzimidazole **11**[34] was prepared by reaction of 2-benzamidobenzimidazole (**9**)[13] with bis(2-chloroethyl)aniline to give **10** and subsequent deprotection. The methylamino compound **13** was prepared in two steps from the known alcohol **12**[14] by mesy-

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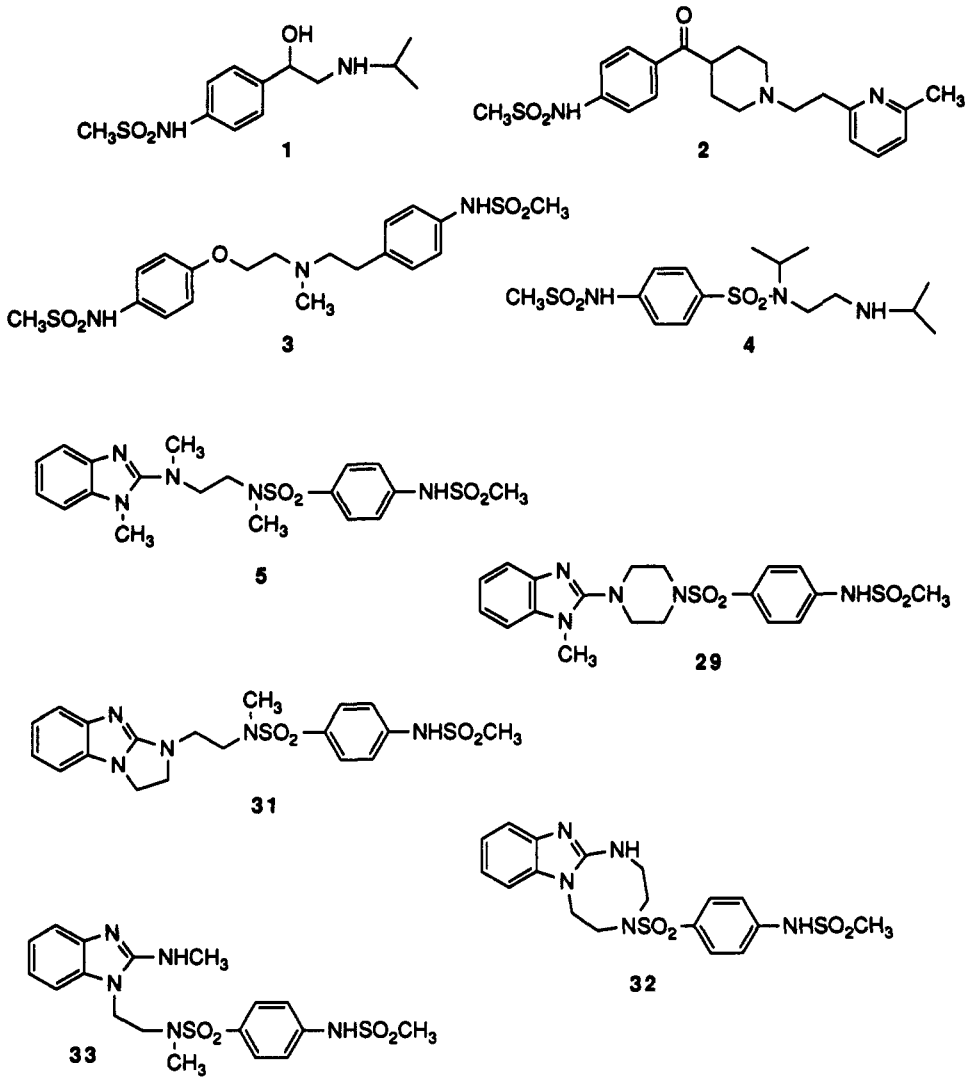
Chart I



lation and subsequent displacement with methylamine. Compound **15** was prepared from the chloro compound **14**[15] by conversion to an iodide with sodium iodide and displacement with methylamine. The amino compounds **8**, **11**, **13**, and **15** were then converted to the target compounds **17** and **19** by reaction with either 4-[(methylsulfonyl)amino]benzenesulfonyl chloride[16] (**16**) (method B) or 4-[(methylsulfonyl)amino]benzoic acid[17] (**18**) (method C).

A second synthetic approach (Scheme II, method D) was designed to produce the larger quantities of compound **5** needed for more extensive pharmacological evaluation. In particular, an alternative to the use of *N,N'*-dimethylethylenediamine was required. This reagent is expensive, and an excess must be used to supress the formation of a 2:1 benzimidazole–ethylenediamine adduct. Thus, compound **16** was treated with 2-methylaminoethanol to give alcohol **20**. Conversion to a mesylate (**21**) and displacement with methylamine yielded amino compound **22**. Finally, reaction with 2-bromo-1-methyl-1*H*-benzimidazole[18] (**23**) gave **5** in an overall yield of 58%. Compound **23** was prepared in a yield of 50% by bromination of 2-mercaptobenzimidazole (**24**) according to a method devised for the bromination of mercaptopurines,[19] and by methylation of the resulting 2-bromo-1*H*-benzimidazole[20]

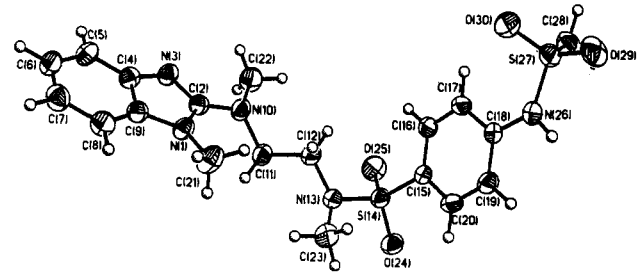


Figure 1. X-ray structure of **5**.

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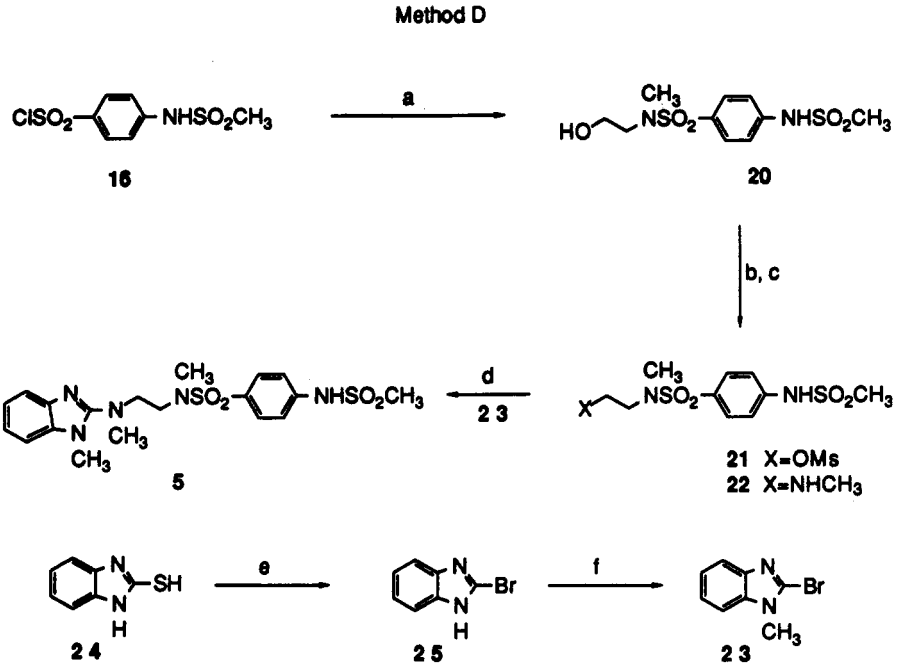
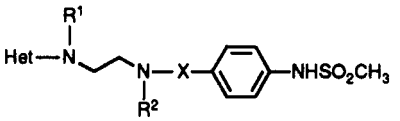
Scheme II



[a] (a) $CH_3NHCH_2CH_2OH$, Et_3N, THF; (b) MsCl, pyridine, THF; (c) CH_3NH_2, EtOH; (d) **23**, iPr_2NEt, nBuOH; (e) Br_2, concentrated HBr, HOAc; (f) 1 N NaOH, $(CH_3)_2SO_4$.

Table I. Physical Characteristics of Substituted Aryl Sulfonamides and Benzamides



compd	Het	R^1	R^2	X	method of preparation	mp, °C	formula[a]
5[b]	1-methylbenzimidazol-2-yl	CH_3	CH_3	SO_2	A, B, or D	212–214	$C_{19}H_{25}N_5O_4S_2$·HCl
26	1-methylbenzimidazol-2-yl	H	CH_3	SO_2	A, B	155–157	$C_{18}H_{23}N_5O_4S_2$·HCl·H_2O
27	1-methylbenzimidazol-2-yl	CH_3	H	SO_2	D	196–197	$C_{18}H_{23}N_5O_4S_2$·HCl
28	1-methylbenzimidazol-2-yl	CH_3	CH_3	CO	A, C	209–212	$C_{20}H_{25}N_5O_3S$·HCl
29[b]	1-methylbenzimidazol-2-yl	CH_2–CH_2		SO_2	A, B	220–221	$C_{19}H_{25}N_5O_4S_2$·HCl
30	1-methylbenzimidazol-2-yl	CH_2–CH_2		CO	A, C	255–256	$C_{20}H_{23}N_5O_3S$·HCl·0.5H_2O
31[b]	2,3-dihydro-1*H*-imidazo-[1,2-*a*]benzimidazol-1-yl		CH_3	SO_2	*c*, B	215–217	$C_{19}H_{23}N_5O_4S_2$·HCl
32[b]	2,3,5,6-tetrahydro[1,3,6]triazocino-[1,2-*a*]benzimidazol-4(1*H*)-yl			SO_2	*c*, B	192–200	$C_{18}H_{20}N_5O_4S_2$·HCl·$2H_2O$
33[b]	2-(methylamino)-1*H*-benzimidazol-1-ylethyl		CH_3	SO_2	*c*, B	291–292	$C_{18}H_{23}N_5O_4S_2$·HCl
34	quinolin-2-yl	CH_3	CH_3	SO_2	A, B	268–269	$C_{20}H_{24}N_4O_4S_2$·HCl
35	quinolin-2-yl	CH_3	CH_3	CO	A, C	154–156	$C_{21}H_{24}N_4O_3S$
36	quinazolin-2-yl	CH_3	CH_3	SO_2	A, B	238–239	$C_{19}H_{23}N_5O_4S_2$·HCl
37	pyrimidin-2-yl	H	H	SO_2	A, B	150–152	$C_{13}H_{17}N_5O_4S_2$

[a] Satisfactory, C, H, and N elemental analyses (±0.4%) were obtained, except as noted. [b] See Chart I for structures. [c] See text for method of preparation.

25 with dimethyl sulfate. Listed in Table I are the target compounds prepared (**5**, **26–37**).

Confirmation of the structure of **5** was obtained through single crystal X-ray analysis, as illustrated in Figure 1. One feature revealed by the X-ray analysis is the anti relationship of the benzimidazole 1-methyl and 2-*N*-methyl groups.

Results and Discussion

Compounds **5** and **26–37** were first evaluated using standard microelectrode techniques in canine Purkinje fibers. The actions of the compounds on the transmembrane potentials were studied during stimulation at cycle lengths of 300 and 1000 ms. The longer cycle length provides information on the drug effects at "normal" heart rates (60 beats/min), while the shorter cycle length (200 beats/min) gives a more useful indication of activity at a rate of beating closer to an episode of tachycardia. Compounds prolonging the duration of repolarization to −60 mV (APD_{-60}) with little or no depressant action on $\dot{V}_{max}$, the maximum rate of depolarization of the upstroke of the action potential, have a Class III profile. A depression of $\dot{V}_{max}$ indicates a blockade of the fast Na channel (I_{Na}). Because the depolarizing current flowing during the upstroke is a major determinant of the propagation of the

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Table II. Effects of Compounds on the Transmembrane Potential of Dog Purkinje Fibers Paced at a Cycle Length of 300 and 1000 ms [Results expressed as % change from predrug values ($\bar{X} \pm$ SE)]

compd	concn, μM	BCL = 300 ms[a]			BCL = 1000 ms		
		n[b]	APD_{-60}[c]	$\dot{V}_{max}$[d]	n	APD_{-60}	$\dot{V}_{max}$
5	0.3	7	24 ± 2	−3 ± 6	7	46 ± 9	0 ± 15
	3.0	4	40 ± 7	11 ± 6	7	57 ± 5	13 ± 4
26	3.0	3	19 ± 7	1 ± 13	3	67 ± 18	6 ± 8
27	3.0	2[e]	16 ± 4	9 ± 3	3	50 ± 10	−1 ± 6
28	3.0	4	17 ± 3	−5 ± 11	4	65 ± 34	−3 ± 5
29	3.0	3	36 ± 9	0 ± 4	3	57 ± 7	−5 ± 4
30	0.3	2	6 ± 2	14 ± 24	2	17 ± 1	−1 ± 4
	3.0	3	33 ± 4	−3 ± 9	3	75 ± 19	−2 ± 10
31	0.3	2	16 ± 1	−5 ± 2	2	30 ± 4	−4 ± 11
	3.0	2	*f*	*f*	2	110 ± 26	−12 ± 1
32	3.0	2	24	7	2	42	−2
33	3.0	2	28 ± 12	8 ± 1	3	47 ± 9	15 ± 12
34	3.0	1[e]	20	6	2	46 ± 7	−12 ± 15
35	3.0	2	17 ± 1	−18 ± 13	1[e]	36	7
36	3.0	2	18 ± 1	12 ± 12	2	29 ± 3	1 ± 3
37	3.0	2	−4 ± 5	−2 ± 3	2	24 ± 14	6 ± 10
1 (*dl*-sotalol)	30.0	4	18 ± 1	−12 ± 8	4	56 ± 8	2 ± 8
	300.0	4	36 ± 2	−20 ± 4	4	86 ± 11	−10 ± 5
2 (E-4031)	0.03	3	21 ± 7	8 ± 10	3	53 ± 14	7 ± 8
	0.3	4	27 ± 4	7 ± 10	4	74 ± 2	−6 ± 4
3 (UK-68798)	0.03	4	30 ± 5	7 ± 9	5	67 ± 14	2 ± 4
	0.3	2[e]	42	17 ± 9	4	101 ± 15	−7 ± 3

[a] BCL: basic cycle length of stimulation. [b] n: number of fibers. [c] APD_{-60}: repolarization time to −60 mV. [d] $\dot{V}_{max}$: maximum rate of depolarization of the upstroke of the action potential. [e] Additional fiber(s) did not pace. [f] Fibers did not pace at BCL = 300 ms.

cardiac impulse, a depression of $\dot{V}_{max}$ corresponds to a decrease of conduction velocity "in vivo" (Class I effect). Previous experience in this experimental model has shown that alterations of $\dot{V}_{max}$ by 10% or less are of little biological significance, and are probably due to random variability in a small sample.

Reported in Table II are the percent changes in APD_{-60} and $\dot{V}_{max}$ in canine Purkinje fibers at cycle lengths of 300 and 1000 ms. At a concentration of 3 μM, compounds **5**, **29**, and **30** increased the APD_{-60} by greater than 30% at a cycle length of 300 ms. With compound **31**, at 3 μM the prolongation of repolarization was so large that the fibers could not be paced at BCL = 300 ms (BCL is base cycle length of stimulation). At the longer cycle length of 1000 ms the above compounds increased the APD_{-60} by greater than 50%, as did **26**, **27**, **28**, and **31**. Also, compounds **32** and **33** approached the efficacy of the above compounds with increases in APD_{-60} of 24% and 28%, respectively, at 300 ms. The remaining compounds, **34–37**, as well as **26–28**, were less efficacious, increasing APD_{-60} at 300 ms by 20% or less. No indication of Class I activity was seen in Purkinje fibers with these compounds, except for **35**. At 300 ms, **35** decreased $\dot{V}_{max}$ by 18%.

A key structural feature that emerges from these results is that the most efficacious compounds (**5**, **29**, **30**, and **33**) contain a benzimidazole group, while in **34–37** other heterocycles replace the benzimidazole: quinoline (**34**, **35**), quinazoline (**36**), and pyrimidine (**37**). One difference among these heterocycles is their basicity. Reported[21] pK_a values for 2-aminoheterocycles are as follows: 2-aminobenzimidazole, 7.54; 2-aminoquinoline, 7.34; 2-aminoquinazoline, 4.43; and 2-aminopyrimidine, 3.54. The pK_a for compound **5** was found to be 7.1 (UV shift method). Other Class III agents (e.g. **1**, **2**, and **3**) generally have a secondary or tertiary amino group (pK_a 10–11) located several atoms away from the 4-[(methylsulfonyl)amino]-phenyl group while in **5** this position is occupied by the aminobenzimidazole. However, a pK_a of 7.0 has been reported for the tertiary amino group of compound **3**.[10] Thus, it appears that a pK_a of 7 may be a minimum requirement for good Class III antiarrhythmic activity. The quinoline compounds **34** and **35** appear to meet this requirement but are not as active as **5**. Some other factor such as lipophilicity may also be important in this case.

However, the inclusion of a benzimidazole group does not necessarily lead to efficacy at the shorter cycle length of 300 ms, as seen with **26–28**. Compounds **26** and **27** are desmethyl analogues of **5**, thus fully alkylated nitrogens appear to be required for optimal activity. Compounds **26** and **27** have been identified as metabolites occurring in minor amounts in dog plasma following oral dosing of **5** at 5 mg/kg. In **28**, replacement of the aryl sulfonyl group of **5** with a carbonyl group decreased efficacy. However, this result was not observed in the piperazine compounds **29** and **30**, where the same sulfonyl to carbonyl change was made.

Among the benzimidazole analogues **5**, **29**, **31**, and **32**, it can be seen that efficacy in the Purkinje fiber is not lost upon constraint of the methyl groups of **5** to form a piperazine (**29**), imidazobenzimidazole (**31**), or triazocinobenzimidazole system (**32**). In contrast to the anti relationship of the benzimidazole methyl groups of **5** revealed by the X-ray analysis, they are constrained in a "syn" type relationship in **31** by formation of a bond between them.

Several standards were also examined in Purkinje fibers: *dl*-sotalol (**1**), E-4031 (**2**), and UK-68,798 (**3**). Compound **1** is considerably less potent than the active compounds of the aryl sulfonamide and benzamide series described in this paper. A concentration of 300 μM of **1** is required to attain an increase in the APD_{-60} of 36% at 300 ms. At this concentration Class I activity is also evident as shown by a 20% decrease of $\dot{V}_{max}$. Compounds **2** and **3** are more potent than **5**, the best compound in the aryl sulfonamide series. However, **5** did not cause as large a difference in the percent increase in APD_{-60} at the two cycle lengths. At 3 μM, the ratio of the APD_{-60} increase at 1000 and 300 ms is 1.4. The ratios for **1** (300 μM), **2** (0.3 μM), and **3** (0.3 μM) are 2.4, 2.7, and 2.4, respectively. All of the above ratios were determined for concentrations at which the maximal effect is observed. The lower ratio for **5** is favorable in terms of safety. By producing a more limited

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Table III. Effects of Compounds following Intravenous Administration in Open-Chest Anesthetized Dogs[d]

compd	n[a]	dose, mg/kg	AERP[b]	ACT[b]	VERP[b]	VCT[b]	HR[c]	MBP[c]
5	5	1.0	40 ± 6	−6 ± 2	20 ± 5	−3 ± 1	−20 ± 3	−3 ± 3
	5	2.5	53 ± 6	−5 ± 1	24 ± 3	−4 ± 2	−24 ± 3	−4 ± 3
28	2	1.0	18 ± 9	−1 ± 2	10 ± 0	−1 ± 2	−6 ± 5	5 ± 6
	2	5.0	44 ± 14	−3 ± 1	24 ± 3	−6 ± 0	−27 ± 6	−1 ± 9
29	2	1.0	22 ± 3	3 ± 3	13 ± 1	−4 ± 4	−17 ± 0	2 ± 3
	2	5.0	40 ± 7	−3 ± 6	25 ± 1	2 ± 10	−37 ± 5	3 ± 3
30	2	1.0	33 ± 1	−10 ± 4	19 ± 1	−8 ± 5	−17 ± 7	−10 ± 3
	2	5.0	63 ± 14	−10 ± 7	28 ± 13	−13 ± 7	−37 ± 18	−23 ± 13
31	3	5.0	54 ± 8	6 ± 8	18 ± 10	−2 ± 5	−15 ± 7	−9 ± 10
	3	10.0	66 ± 8	3 ± 6	19 ± 9	−5 ± 3	−24 ± 6	−8 ± 11
1 (*dl*-sotalol)	5	2.5	42 ± 4	−5 ± 5	19 ± 2	−1 ± 2	−27 ± 3	−16 ± 4
	5	5.0	57 ± 6	−7 ± 5	25 ± 4	−1 ± 3	−28 ± 6	−17 ± 4
2 (E-4031)	5	0.1	34 ± 7	1 ± 5	20 ± 3	−3 ± 1	−18 ± 3	−6 ± 3
	5	1.0	55 ± 4	1 ± 5	26 ± 4	−2 ± 3	−25 ± 2	−19 ± 9
3 (UK-68798)	5	0.05	46 ± 6	−4 ± 2	23 ± 2	−4 ± 1	−14 ± 1	1 ± 2
	5	0.5	65 ± 10	−9 ± 2	27 ± 6	−4 ± 1	−24 ± 2	−2 ± 6

[a] Number of animals. [b] AERP: atrial effective refractory period. ACT: atrial conduction time. VERP: ventricular effective refractory period. VCT: ventricular conduction time (all parameters measured during pacing at BCL = 300 ms). [c] HR: heart rate. MBP: mean arterial pressure. [d] Results expressed as % change from predrug values ($\bar{X}$ ± SE).

prolongation of APD during an episode of bradycardia, **5** would be less likely to have a proarrhythmic effect.[22]

Compounds that showed a greater than 30% increase in APD_{-60} at 300 ms in Purkinje fibers (**5**, **29**, and **30**) were selected for examination in a pentobarbital-anesthetized, open-chest dog model following iv administration. Compound **31** was selected because its effects at both 300 ms (did not pace) and 1000 ms (110% increase in APD_{-60}) suggested good efficacy. Finally, **28** was examined in the anesthetized dog model, despite its lower efficacy in Purkinje fibers, so that additional in vivo data could be obtained for aryl benzamide as well as aryl sulfonamide type compounds (**17** and **19**). The effects of the compounds (percent changes) on atrial and ventricular effective refractory periods (AERP and VERP) and atrial and ventricular conduction times (ACT and VCT) during pacing at 300 ms, heart rate (HR), and mean arterial pressure (MBP) are reported in Table III. The two doses of each compound that are reported were selected from dose-response experiments to produce a 20% increase (lower dose) and a near maximum increase of the VERP (higher dose).

All of the compounds (**5**, **28–31**) caused at least a 40% prolongation of the AERP at the higher dose (2.5, 5.0, or 10.0 mg/kg). Compounds **5**, **28–30**, but not **31**, brought about a greater than 20% prolongation of the VERP at the higher dose. With **31**, higher doses of 5.0 and 10.0 mg/kg were required to produce significant activity in the atrium, suggesting rapid drug metabolism. In the ventricle, a lower effect was still seen. All compounds showed higher efficacy in the atrium than in the ventricle. The much larger effect on atrial refractoriness caused by compounds **5** and **28–31** suggests that these compounds should be efficacious against atrial arrhythmias at doses that cause minimal alteration of ventricular refractoriness or hemodynamic parameters.

At the lower doses of 1.0 or 5.0 mg/kg, **5**, **30**, and **31** caused a greater than 30% prolongation of the AERP, but only **5** and **30** prolonged the VERP by approximately 20%. At the doses examined, none of the compounds increased ACT or VCT significantly, indicating a lack of any effect on cardiac conduction (Class I action) and a specific Class III antiarrhythmic activity. A moderate decrease in HR was seen with all compounds, but only **30** and **31** had any effect on BP.

The standards **1**, **2**, and **3** were also examined in the open-chest dog. They increased the AERP by 35–65% and the VERP by 20–30% at the doses reported. None of the standards affected ACT or VCT, but all three caused a moderate decrease in HR. Finally, **1** and **2**, but not **3**, lowered MBP significantly.

Compounds **5** and **30** are equal in efficacy to the standards in the open-chest dog model. Compound **5** is more potent than **1** but less potent than **2** or **3**, while **30** is comparable to **1** in potency. Both **5** and **30** were examined in conscious dogs for behavioral side effects. The compounds were injected intravenously over a 45-min period to a final cumulative dose of 20 mg/kg. Compound **30** was found to induce agitation, salivation, and tremors, but **5** lacked any apparent behavioral effects. The difference in behavioral effects may arise from the difference in the lipophilicities of the two compounds. It has been reported that a log *P* of about 2 is optimal for penetration into the central nervous system (CNS).[23] The calculated log *P* (CLOGP) values[24] for **5** and **30** are 3.02 and 2.57, respectively, so **5** would be less likely to penetrate into the CNS than **30**. Compound **5** was selected for more extensive evaluation of oral bioavailability and duration of action.

Compound **5** was administered intravenously and intragastrically at a dose of 5 mg/kg to conscious instrumented dogs (Figures 2A and 2B). Following iv administration, the peak increase of VERP (+22%) occurred after 30 min; the VERP increase declined to 13% after 2 h. The peak response (18% increase of VERP) after ig administration occurred after about 1.5 h. The VERP increase subsequently declined to a plateau level about 10% above the baseline. Thus **5** shows good oral bioavailability. In the conscious animal, compound **5** (5 mg/kg iv) decreased HR by 11% at the time of peak increase of VERP. This effect contrasts with the 20–24% reduction observed in the pentobarbital-anesthetized dog after iv injection of lower doses (1.0–2.5 mg/kg). These results suggest that the much larger reduction of HR observed in anesthetized dogs might be in part due to the presence of anesthesia.

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(24) Pomona Medchem CLOGP.

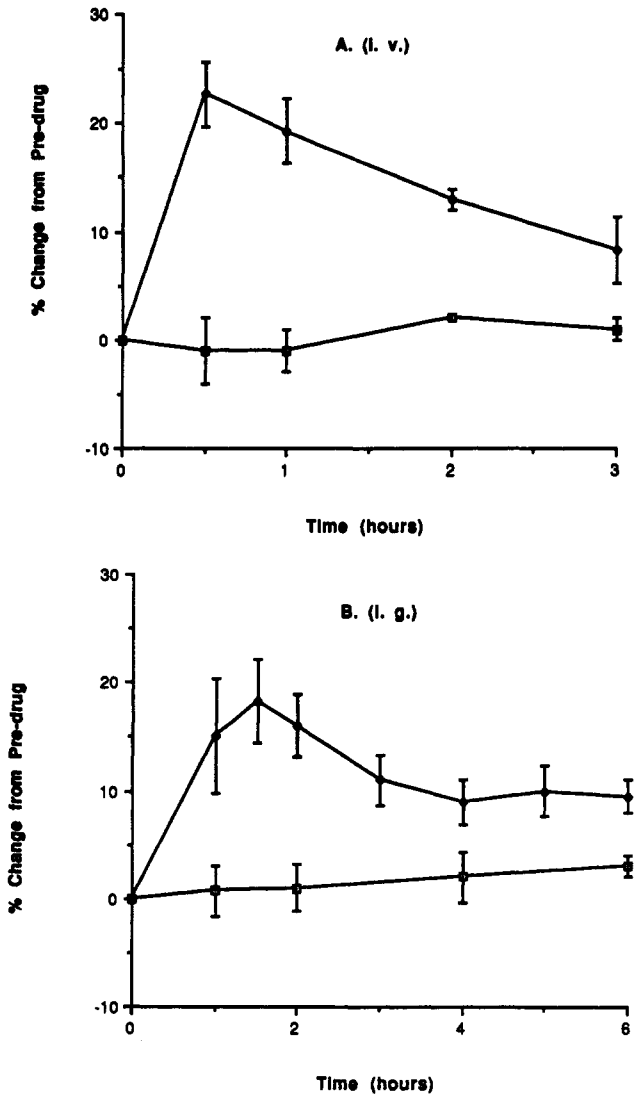

Figure 2. Effect of **5** (5 mg/kg) on ventricular effective refractory period (VERP) in conscious dogs after intravenous (iv; Figure 2A) and intragastric (ig; Figure 2B) administration. Results are expressed as mean ± SE (n = 3). Vehicle (□), compound **5** (●).

Drugs prolonging the duration of the action potential are known to have positive inotropic effects.[5] Although the mechanism of action is not clear, most results indicate that positive inotropism is independent from adrenergic stimulation and correlates with the prolongation of the ventricular action potential.[7] The inotropic effect of **5** in anesthetized dogs is shown in Figure 3. Intravenous infusion of **5** (5 mg/kg over 30 min) produced a sustained increase in cardiac contractility which, although not statistically significant, was clear and maintained for 2.5 h after termination of the infusion. Depression of contractility is an important toxic side effect of potent Class I agents which can produce heart failure in patients whose cardiac function is already compromised. The modest but sustained increase in contractility observed with **5** may provide further safety in the treatment of arrhythmias in congestive heart failure.

The antiarrhythmic efficacy of **5** was studied in a model of electrically-induced ventricular fibrillation (VF) in open-chest anesthetized dogs. The intensity of current necessary to produce an episode of sustained VF was measured under control conditions and after iv administration of **5** (Table IV). Experimental evidence suggests that local reentry in the vicinity of the stimulating electrode is responsible for the initiation of the arrhythmia.[25]

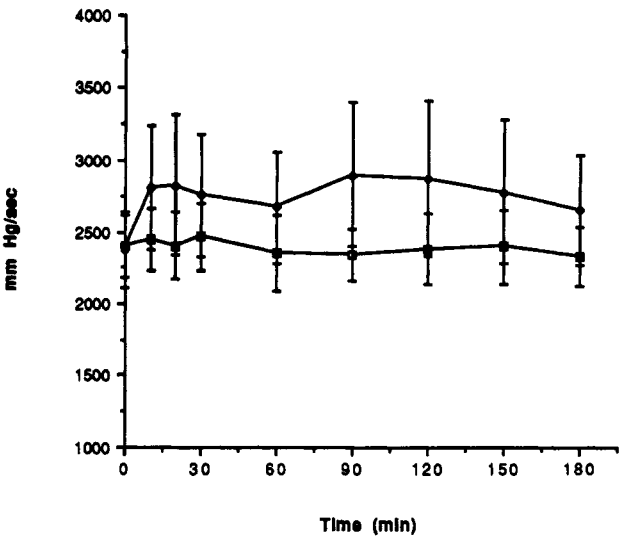

Figure 3. Effects of **5** on cardiac contractility. Intravenous infusion of 5 mg/kg over 30 min produced a modest but long-lasting increase of dP/dT. Results are expressed as mean ±SE (n = 5). Vehicle (□), compound **5** (●).

Table IV. Ventricular Fibrillation Threshold (VFT) of **5** in Open-Chest Anesthetized Dogs

		VFT, mA[b]	
	n[a]	predrug	postdrug
control	6	8 ± 1	8 ± 1
5 (2.5 mg/kg iv)	6	8 ± 2	24 ● 10[c]

[a] Number of animals. [b] $\bar{X}$ ● SE. [c] $p < 0.05$ vs predrug.

Compounds producing a large increase in the current threshold necessary to induce ventricular fibrillation (VFT) have been shown to be effective against life-threatening ventricular arrhythmias caused by reentry.[26] Compound **5** produced a 3-fold increase in VFT, from 8 to 24 mA. In two of the six dogs treated with **5**, the large increase of VFT was accompanied by episodes of reversion to sinus rhythm without electric countershock. An episode is shown in Figure 4: in part A the upper figure shows a lead II ECG trace and the lower figure shows the arterial pressure trace. A train of stimuli delivered to the ventricle (marked by arrow) produced a run of polymorphic ventricular tachycardia–ventricular fibrillation. After about 10 s, during which time arterial pressure fell rapidly toward zero, the arrhythmia reversed to sinus rhythm and the arterial pressure quickly recovered. "Spontaneous defibrillation" was observed repeatedly in the same two animals. No reversion to sinus rhythm without electrical countershock was observed in dogs treated with vehicle (Figure 4, part B).

Finally, to elucidate the mechanism of the Class III activity of compound **5**, its effects on membrane currents in isolated cat myocytes were studied. A Class III effect can result from an increase of inward currents or from a decrease of outward, repolarizing currents. Our goal was to develop a specific Class III antiarrhythmic agent that prolonged repolarization by inhibiting a specific outward current, the delayed rectifier K current (I_K). Such a compound should specifically block the tail currents associated with deactivation of the delayed rectifier current, without affecting other membrane currents, such as the

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(26) Moore, E. N.; Spear, J. F. Ventricular Fibrillation Threshold. *Archiv. Intern. Med.* **1975**, *135*, 446–453.

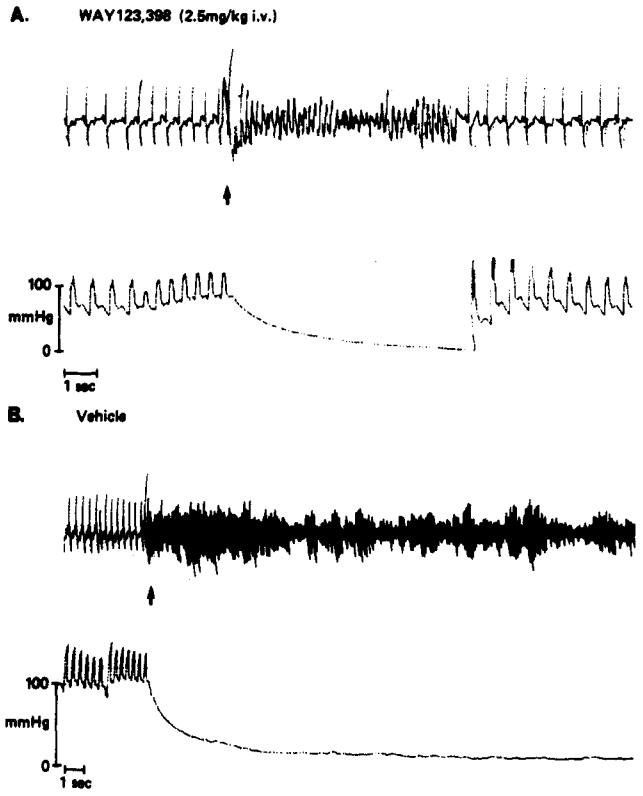

Figure 4. Restoration of sinus rhythm without electrical cardioversion by **5** (WAY-123,398) in open-chest dog. In Figure 4B electrical stimulation of the ventricle during the vulnerable period (marked by arrow) produces ventricular fibrillation (top trace, ECG) and hemodynamic collapse (bottom trace, arterial pressure). In Figure 4A, after administration of **5**, ventricular fibrillation terminates spontaneously after about 10 s with restoration of sinus rhythm.

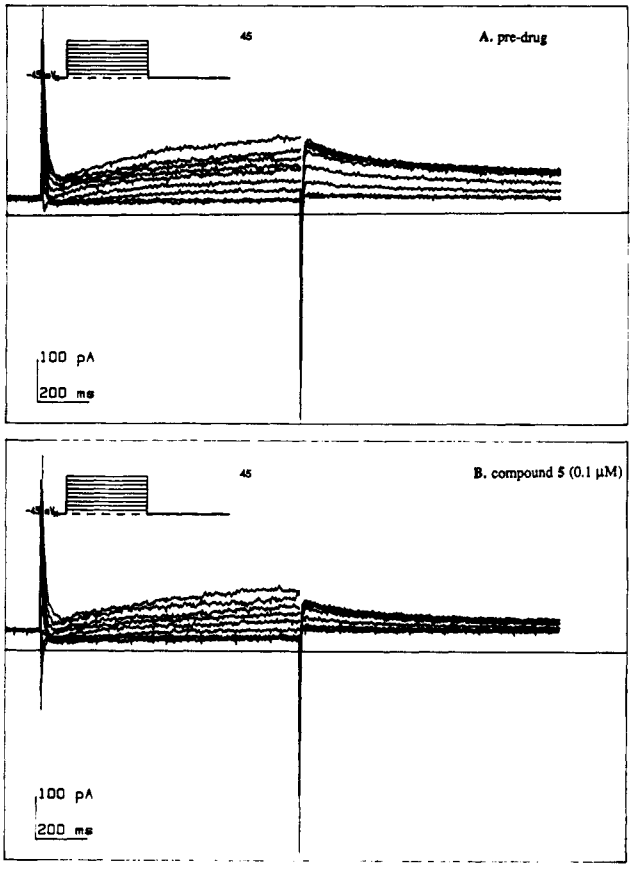

Figure 5. Effect of **5** on the delayed rectifier potassium current (I_K). Compound **5** (0.1 μM) decreased peak tail currents by 54% at +45 mV. The voltage protocol employed is shown in the top left corner of each figure; $I_{Ca\text{-}L}$ was blocked by nisoldipine (300 nM) present in Tyrode's solution. The continuous horizontal line represents the 0 current level.

inward rectifier K current (I_{K1}) or the inward Ca current flowing during plateau ($I_{Ca\text{-}L}$). As shown in Figure 5A, depolarizing voltage-steps at voltages more positive than −45 mV produce a family of outward current traces which are followed, on repolarization to −45 mV, by outward decaying tail currents. These tail currents represent the deactivation of I_K. After 8 min of superfusion, **5** (0.1 μM) produced a 50% block of the tails (Figure 5B), which was promptly reversable on washout. Concentration–response studies (ranging from 0.03 μM to 3.0 μM) showed that **5** blocks tail currents with an IC_{50} of 0.1 μM (data not shown). Concentrations of 3 μM caused a total block of tail currents. The lack of effects of **5** on $I_{Ca\text{-}L}$ and I_{K1} is shown in Figure 6. In part A, voltage-steps from −45 mV activate $I_{Ca\text{-}L}$: at 10 μM, a concentration 2 orders of magnitude higher than the IC_{50} for blocking I_K, **5** did not affect $I_{Ca\text{-}L}$ (n = 3) (Figure 6B). In these experiments, K currents were blocked by adding 2 mM CsCl in Tyrode's solution and substituting Cs for K in the microelectrode solution. In part C, voltage-steps from −35 to −105 mV activated a large inward current which reversed after a voltage-step to −80 mV. This current is I_{K1} and shows its characteristic inward rectification (i.e. little current is activated in the outward direction). Again, **5** (10 μM) did not affect I_{K1} (n = 6) (Figure 6D).

Conclusion

We have described a novel series of 4-[(methylsulfonyl)amino]benzamides and sulfonamides having specific Class III antiarrhythmic activity. In this series, compounds having a 2-aminobenzimidazole group were found to be the most potent in preliminary screens. Compound **5** (WAY-123,398) was selected for further characterization due to its efficacy in dog Purkinje fibers and anesthetized open-chest dogs. Compound **5** was shown to have good oral bioavailability in conscious dogs and to cause a 3-fold increase in the ventricular fibrillation threshold in anesthetized open-chest dogs. The mechanism by which **5** increases action potential duration and ventricular-effective refractory periods (Class III effects) was explored in voltage-clamp studies in isolated cat myocytes. At concentrations comparable to those producing APD prolongation, compound **5** blocked the delayed rectifier potassium current (I_K). At much higher concentrations **5** did not have any effect on the inward rectifier current (I_{K1}) or the L-type Ca current ($I_{Ca\text{-}L}$). These results show that **5** is a potent and specific Class III antiarrhythmic agent.

Experimental Section

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. The NMR spectra were recorded on a Varian VXR300 or a Bruker AM-400 instrument. The infrared spectra were recorded on a Perkin-Elmer diffraction grating or a Perkin-Elmer 784 spectrophotometer. The mass spectra were recorded on a LKB-9000S or a Finigan 8230 high-resolution mass spectrometer. Merck silica gel (70–230 mesh) or neutral alumina were used for column chromatography. Organic extracts were dried over $MgSO_4$.

Method A. 2-[*N*-Methyl-*N*-[2-(methylamino)ethyl]amino]-1-methyl-1*H*-benzimidazole (38). A solution of 2-chloro-1-methyl-1*H*-benzimidazole[12] (**6a**, $R^3 = CH_3$) (5.00 g, 0.030 mol) in *N,N'*-dimethylethylenediamine (**7**, R^1, $R^2 = CH_3$) (25 mL, 0.235 mol) was heated under reflux for 16 h. The solution was concentrated, dissolved in 10% aqueous acetic acid (100 mL), and

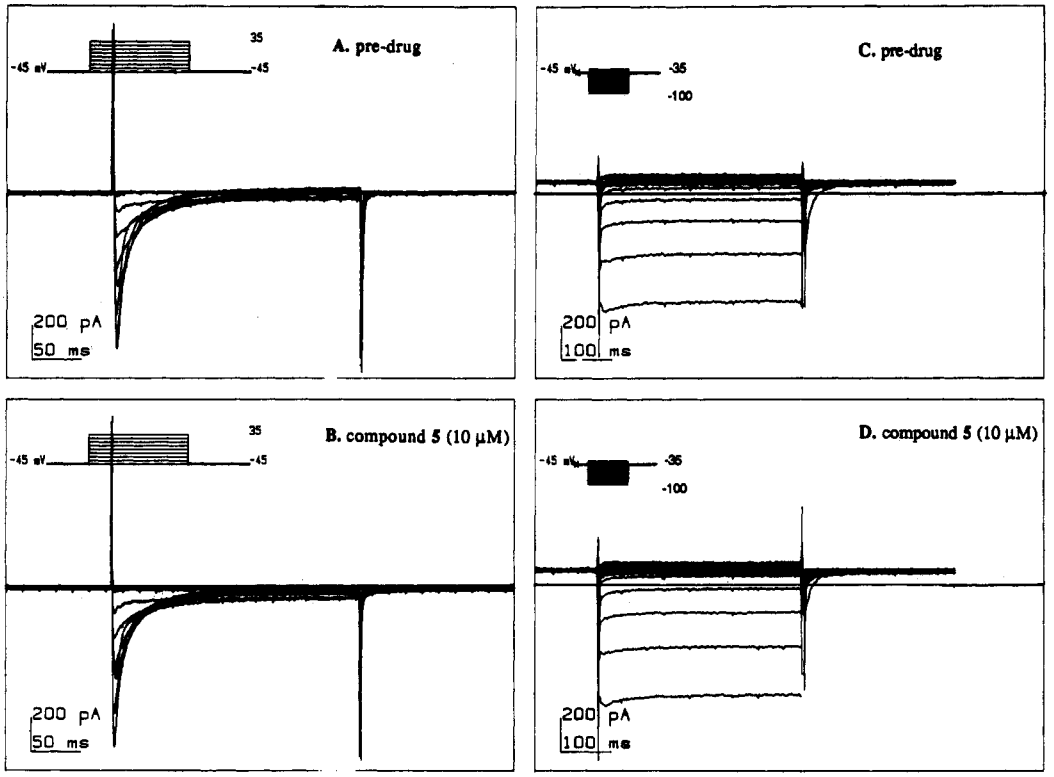

Figure 6. Effects of 5 on calcium current ($I_{Ca\text{-}L}$) and inward rectifier potassium current (I_{K1}) in cat ventricular myocytes. Compound 5 (10 μM) does not affect $I_{Ca\text{-}L}$ (Figure 6A,B) or I_{K1} (Figure 6C,D). The voltage protocol employed is shown in the top left corner of each figure. The continuous horizontal line shows the 0 current level; in 6C and 6D, $I_{Ca\text{-}L}$ was blocked by nisoldipine (300 nM).

extracted with EtOAc (discarded). The aqueous phase was made basic (pH 9) with solid KOH and extracted with EtOAc. The extracts were dried and concentrated to give 5.39 g (82%) of **38** as a yellow oil. An analytical sample was distilled bulb-to-bulb (oven temperature 220 °C/1 mm) to give a colorless oil. ^{1}H NMR (DMSO-d_6): δ 2.29 (s, 3 H), 2.72 (t, J = 6.7 Hz, 2 H), 2.93 (s, 3 H), 3.30 (t, J = 6.7 Hz, 2 H), 3.62 (s, 3 H), 7.04 (m, 2 H), 7.28 (m, 1 H), 7.34 (m, 1 H). Anal. ($C_{12}H_{18}N_4$) C, H, N.

1,2,3,4,5,6-Hexahydro[1,3,6]triazocino[1,2-*a*]benzimidazole Dihydrochloride (11). A mechanically stirred mixture of *N*-benzimidazol-2-ylbenzamide[13] (**9**, 19.0 g, 0.080 mol), DMF (800 mL), and toluene (100 mL) was azeotropically distilled until 20 mL of distillate had been collected. KOtBu (17.5 g, 0.156 mol) was added and 80 mL of additional distillate was collected. *N*,*N*-Bis(chloroethyl)aniline (17.8 g, 0.082 mol) was added, and the mixture was heated under reflux for 6 h. The cooled mixture was filtered, and the filtrate was concentrated to give an oil. Purification by column chromatography (neutral alumina, eluant 1:1 toluene/chloroform) gave 6.8 g (22%) of 1-benzoyl-1,2,3,4,5,6-hexahydro-4-phenyl[1,3,6]triazocino[1,2-*a*]benzimidazole (**10**). Recrystallization from $CHCl_3$/heptane gave colorless crystals, mp 219–220 °C. Anal. ($C_{24}H_{22}N_4O$) C, H, N.

A mixture of **10** (10.0 g, 0.026 mol) and MeOH (1150 mL) presaturated with NH_3 was sealed in a steel bomb and heated at 100 °C for 4 days. The mixture was concentrated and crystallized twice from MeOH to give 5.5 g (76%) of 1,2,3,4,5,6-hexahydro-4-phenyl[1,3,6]triazocino[1,2-*a*]benzimidazole (**39**), mp 159–160 °C. Anal. ($C_{17}H_{18}N_4$) C, H, N.

A mixture of **39** (5.8 g, 0.021 mol), 10% Pd/C (0.5 g), 1 N HCl (75 mL), and dioxane (250 mL) was hydrogenated at atmospheric pressure. The mixture was filtered, and the filtrate was concentrated. Recrystallization from MeOH gave 4.2 g (72%) of **11**,[34] mp 221–223 °C. ^{1}H NMR (DMSO-d_6): δ 3.41 (t, J = 5.3 Hz, 2 H), 3.52 (t, J = 5.0 Hz, 2 H), 4.01 (dt, J = 5.0, 5.3 Hz, 2 H), 4.83 (t, J = 5.0 Hz, 2 H), 7.27 (m, 2 H), 7.41 (dd, J = 3.1, 6.3 Hz, 1 H), 7.64 (dd, J = 3.1, 5.9 Hz, 1 H), 9.65 (t, J = 5.0 Hz, 1 H). Anal. ($C_{11}H_{14}N_4$·2HCl) C, H, N.

1-[2-(Methylamino)ethyl]-2-(methylamino)-1*H*-benzimidazole (13). To a cooled (0 °C), stirred mixture of 1-(2-hydroxyethyl)-2-(methylamino)-1*H*-benzimidazole[14] (**12**) (8.05 g, 0.042 mol), Et_3N (4.26 g, 0.042 mol), and CH_2Cl_2 (85 mL) was added a solution of MsCl (4.83 g, 0.042 mol) in CH_2Cl_2 (10 mL). After 2 h, H_2O was added and the layers were separated. The aqueous phase was extracted with CH_2Cl_2, and the combined extracts were washed with brine, dried, and concentrated to give 8.00 g (71%) of 2-[2-(methylamino)benzimidazol-1-yl]ethyl methanesulfonate (**40**) as a white solid, mp 48–50 °C. ^{1}H NMR (DMSO-d_6): δ 2.95 (d, J = 4.7 Hz, 3 H), 3.00 (s, 3 H), 4.32 (t, J = 5.5 Hz, 2 H), 5.41 (t, J = 5.5 Hz, 2 H), 6.70 (q, J = 4.7 Hz, 1 H), 6.95 (m, 2 H), 7.10 (m, 2 H).

A solution of **40** (8.00 g, 0.030 mol) and 33% CH_3NH_2/EtOH (60 mL) in EtOH (40 mL) was stirred at room temperature for 4 days. The mixture was concentrated, made basic (pH 10) with 2.5 N NaOH, and extracted with EtOAc. The combined extracts were dried and concentrated to give an oil. Purification by flash chromatography (CH_2Cl_2/MeOH/Et_3N 84:10:1) gave 4.20 g (70%) of **13** as a colorless oil. ^{1}H NMR (DMSO-d_6): δ 2.32 (s, 3 H), 2.75 (t, J = 5.5 Hz, 2 H), 2.93 (s, 3 H), 3.37 (s, 1 H), 3.98 (t, J = 5.5 Hz, 2 H), 6.78 (s, 1 H), 6.90 (m, 2 H), 7.18 (m, 2 H).

1-[2-(Methylamino)ethyl]-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole (15). A mixture of 1-(2-chloroethyl)-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole[15] (**14**) (1.70 g, 7.71 mmol), sodium iodide (1.73 g, 11.57 mmol), and 2-butanone (25 mL) was heated under reflux for 5 h. EtOAc (50 mL) was added, and the mixture was filtered. The filtrate was washed with H_2O, 10% sodium bisulfite (25 mL), and saturated aqueous $NaHCO_3$, and dried and concentrated to give 2.20 g (92%) of 1-(2-iodoethyl)-2,3-dihydro-1*H*-imidazo[1,2-*a*]benzimidazole (**41**) as a light brown solid, mp 101–103 °C. ^{1}H NMR (DMSO-d_6): δ 3.50 (t, J = 6.3 Hz, 2 H), 3.64 (t, J = 6.3 Hz, 2 H), 3.98 (m, 2 H), 4.14 (m, 2 H), 6.94 (m, 2 H), 7.13 (m, 1 H), 7.21 (m, 1 H).

A mixture of **41** (1.36 g, 4.34 mmol) and 33% methylamine in EtOH (8.03 M; 45 mL, 0.361 mol) was stirred at room temperature for 2 days. The mixture was concentrated, taken up in 10% aqueous acetic acid (20 mL), and extracted with EtOAc (discarded). The aqueous phase was made basic (pH 9) with solid NaOH and extracted with EtOAc. The extracts were dried and concentrated to give 738 mg (79%) of **15** as an off-white solid, mp 60–63 °C. ^{1}H NMR (DMSO-d_6): δ 2.31 (s, 3 H), 2.75 (t, J = 6.4 Hz, 2 H), 3.36 (t, J = 6.4 Hz, 2 H), 3.94 (m, 2 H), 4.10 (m, 2 H), 6.91 (m, 2 H), 7.10 (dd, J = 1.5, 6.9 Hz, 1 H), 7.17 (dd, J = 2.1, 7.9 Hz, 1 H).

Method B. *N*-Methyl-*N*-[2-(methylquinolin-2-ylamino)ethyl]-4-[(methylsulfonyl)amino]benzenesulfonamide Hydrochloride (34). To a stirred solution of 2-[*N*-methyl-*N*-[2-(methylamino)ethyl]amino]qunioline (**42**) (3.00 g, 13.9 mmol) and Et_3N (1.94 mL, 13.9 mmol) in CH_2Cl_2 (350 mL) was added **16**[16] (3.76 g, 13.9 mmol). After 20 h, the mixture was washed with brine, dried, and concentrated. Purification by flash chromatography (5% MeOH/CH_2Cl_2) gave 5.50 g of a white foam. Saturated methanolic HCl (70 mL) was added and the resulting solution was concentrated. Trituration with hot MeOH gave 5.01 g (74%) of **34** as a white solid, mp 268–269 °C. ^{1}H NMR (DMSO-d_6): δ 2.77 (s, 3 H), 3.11 (s, 3 H), 3.27 (m, 2 H), 3.39 (s, 3 H), 3.44 (m, 2 H), 4.06 (br s, 1 H), 7.33 (d, J = 8.7 Hz, 2 H), 7.51 (m, 2 H), 7.67 (d, J = 8.7 Hz, 2 H), 7.79 (m, 1 H), 7.94 (m, 1 H), 8.43 (m, 1 H). IR (KBr, cm^{-1}): 3400 (NH), 1640 (C=N). MS (*m/e*): 449 (MH^+). Anal. ($C_{20}H_{25}ClN_4O_4S_2$) C, H, N.

Method C. *N*-Methyl-*N*-[2-[methyl(1-methyl-1*H*-benzimidazol-2-yl)amino]ethyl]-4-[(methylsulfonyl)amino]benzamide Hydrochloride (28). A mixture of **38** (3.14 g, 14.4 mmol), **18**[17] (3.10 g, 14.4 mmol), DCC (2.97 g, 14.4 mmol), 1-hydroxybenztriazole hydrate (1.94 g, 14.4 mmol), DMF (4 mL), and THF (42 mL) was stirred at room temperature for 2.5 days. The mixture was filtered, and the filtrate was concentrated. $CHCl_3$ was added, and the mixture was washed with brine, saturated aqueous $NaHCO_3$, and brine, and dried and concentrated to give a brown oil. Purification by flash chromatography (5% MeOH/$CHCl_3$) gave 5.00 g of a white foam. Saturated methanolic HCl was added, and the solution was concentrated. Trituration with hot iPrOH and recrystallization from EtOH gave 2.20 g (34%) of **28** as a white solid, mp 209–212 °C. ^{1}H NMR (DMSO-d_6): δ 2.96 (s, 3 H), 2.99 (s, 3 H), 3.38 (br s, 3 H), 3.79 (br s, 5 H), 3.94 (br s, 2 H), 7.09 (m, 4 H), 7.48 (m, 1 H), 10.02 (s, 1 H), 13.87 (br s, 1 H). IR (KBr, cm^{-1}): 3440 (NH), 1635 (C=O). MS (*m/e*): 416 (MH^+). Anal. ($C_{20}H_{26}ClN_5O_3S$) C, H, N.

2-Bromo-1-methyl-1*H*-benzimidazole (23). According to a modification of a procedure of Beaman et al.,[19] to a cooled (water bath), mechanically stirred mixture of 2-mercapto-1*H*-benzimidazole (**24**, 10.0 g, 0.066 mol), 48% aqueous HBr (10 mL, 0.089 mol), and acetic acid (100 mL) was added bromine (12 mL, 0.239 mol) dropwise over 25 min. The mixture warmed slightly (40–45 °C) during the addition, and additional acetic acid (50 mL) was added to aid the stirring of the thick mixture. After the addition was complete, stirring was continued at room temperature for 4 h. Water (200 mL) was added, and the resulting solution was cooled in an ice bath. The pH was adjusted to 4 with solid NaOH (40 g), and the precipitate was collected by filtration to give 9.3 g (70%) of 2-bromo-1*H*-benzimidazole (**25**).[20] An analytical sample was recrystallized from acetone, mp 190–192 °C. ^{1}H NMR (DMSO-d_6): δ 7.19 (m, 2 H), 7.50 (br s, 2 H), 13.21 (br s, 1 H).

To a stirred mixture of **25** (9.0 g, 0.046 mol) and 1 N NaOH (105 mL, 0.105 mol) was added dimethyl sulfate (7.8 mL, 0.082 mol) all at once. Within 5 min, a precipitate formed, and the mixture warmed to 40 °C. Stirring was continued for 2 h, and the mixture was then left standing for 1 h. The solid was collected by filtration to give 7.2 g (75%) of **23**.[18] An analytical sample was recrystallized from ether, mp 103–105 °C. ^{1}H NMR (DMSO-d_6): δ 3.78 (s, 3 H), 7.21 (ddd, J = 1.2, 7.1, 7.3 Hz, 1 H), 7.28 (ddd, J = 1.2, 7.1, 7.3 Hz, 1 H), 7.59 (d, J = 7.3 Hz, 2 H).

Method D. *N*-Methyl-*N*-[2-[methyl(1-methyl-1*H*-benzimidazol-2-yl)amino]ethyl]-4-[(methylsulfonyl)amino]benzenesulfonamide Hydrochloride (5). To a stirred, cooled (0 °C) mixture of 2-(methylamino)ethanol (2.80 g, 0.037 mol) and triethylamine (3.75 g, 0.037 mol) in THF (40 mL) was added **16** (10.00 g, 0.037 mol) in several portions. The reaction mixture was stirred at 0 °C for 4 h. H_2O was added, and the mixture was made acidic (pH 2) with 2 N HCl. The mixture was extracted with EtOAc, and the combined extracts were dried and concentrated to give 10.30 g (90%) of *N*-[4-[[*N*-methyl-*N*-(2-hydroxyethyl)amino]sulfonyl]phenyl]methanesulfonamide (**20**) as a white solid. An analytical sample was recrystallized from EtOH/Et_2O, mp 106–107 °C. ^{1}H NMR (DMSO-d_6): δ 2.67 (s, 3 H), 2.96 (t, J = 4.9 Hz, 2 H), 3.10 (s, 3 H), 3.47 (t, J = 4.9 Hz, 2 H), 4.79 (s, 1 H), 7.34 (dd, J = 1.9, 6.9 Hz, 2 H), 7.70 (dd, J = 1.9, 6.9 Hz, 2 H), 10.45 (s, 1 H). Anal. ($C_{10}H_{16}N_2O_5S_2$) C, H, N.

To a stirred, cooled (0 °C) mixture of **20** (17.2 g, 0.056 mol) and pyridine (16.0 g, 0.140 mol) in THF (60 mL) was added a solution of methanesulfonyl chloride (11.1 g, 0.140 mol) in THF (10 mL). The reaction mixture was stirred at room temperature for 4 h. Cold water was added, and the mixture was made acidic (pH 2) with 2 N HCl. The resulting white precipitate was collected by filtration to give 21.5 g (99%) of 2-[*N*-methyl[[4-[(methylsulfonyl)amino]phenyl]sulfonyl]amino]ethyl methanesulfonate (**21**). An analytical sample was triturated with hot EtOH, mp 151–152 °C. ^{1}H NMR (DMSO-d_6): δ 2.73 (s, 3 H), 3.14 (s, 3 H), 3.20 (s, 3 H), 3.31 (t, J = 4.9 Hz, 2 H), 4.31 (t, J = 4.9 Hz, 2 H), 7.38 (dd, J = 1.9, 6.9 Hz, 2 H), 7.79 (dd, J = 1.9, 6.9 Hz, 2 H), 10.40 (s, 1 H). Anal. ($C_{11}H_{18}N_2O_7S_3$) C, H, N.

A mixture of **21** (41.6 g, 0.108 mol) and 33% methylamine in EtOH (8.03 M; 215 mL, 1.73 mol) was stirred at room temperature in a sealed flask for 24 h. The solution was concentrated, and the residue was coevaporated with EtOH to give 46.0 g (100%) *N*-[4-[[[*N*-methyl-*N*-2-(methylamino)ethyl]amino]sulfonyl]-phenyl]methanesulfonamide methanesulfonate (**22**) as a hygroscopic foam. ^{1}H NMR (DMSO-d_6): δ 2.35 (s, 3 H), 2.45 (s, 3 H), 2.68 (s, 3 H), 2.88 (t, J = 5.8 Hz, 2 H), 3.08 (t, J = 5.8 Hz, 2 H), 3.10 (s, 3 H), 7.33 (dd, J = 1.9, 6.9 Hz, 2 H), 7.70 (dd, J = 1.9, 6.9 Hz, 2 H), 10.45 (s, 1 H).

A mixture of **22** (1.00 g, 2.40 mmol), **23** (0.506 g, 2.40 mmol), and diisopropylethylamine (0.636 g, 4.90 mmol) in nBuOH (8 mL) was heated under reflux for 72 h. The mixture was cooled, and cold water was added. An off-white solid was collected by filtration and triturated with hot EtOH to give 0.704 g (65%) of the free base of **5** as a white solid, mp 193–194 °C. ^{1}H NMR (DMSO-d_6): δ 2.70 (s, 3 H), 2.97 (s, 3 H), 3.24 (t, J = 6.6 Hz, 2 H), 3.44 (t, J = 6.6 Hz, 2 H), 3.61 (s, 3 H), 7.05 (m, 2 H), 7.30 (m, 2 H), 7.36 (dd, J = 1.9, 6.9 Hz, 2 H), 7.73 (dd, J = 1.9, 6.9 Hz, 2 H), 10.40 (s, 1 H). IR (KBr, cm^{-1}): 1600 (C=N). MS (*m/e*): 451 (M^+). Anal. ($C_{19}H_{25}N_5O_4S_2$) C, H, N.

A similarily prepared batch of the free base of **5** (9.0 g) was suspended in MeOH (40 mL) and saturated methanolic HCl (100 mL) was added. All material went into solution, and then a precipitate formed and was collected by filtration (7.6 g). Recrystallization from EtOH/H_2O gave 6.1 g of **5** as colorless needles, mp 210–213 °C. ^{1}H NMR (DMSO-d_6): δ 2.67 (s, 3 H), 3.12 (s, 3 H), 3.29 (t, J = 5.6 Hz, 2 H), 3.36 (s, 3 H), 3.83 (s, 3 H), 3.87 (t, J = 5.6 Hz, 2 H), 7.35 (d, J = 8.8 Hz, 2 H), 7.50 (m, 2 H), 7.64 (m, 2 H), 7.71 (d, J = 8.8 Hz, 2 H), 10.51 (s, 1 H), 13.90 (br s, 1 H). IR (KBr, cm^{-1}): 1625 (C=N). MS (*m/e*): 452 (MH^+). Anal. ($C_{19}H_{25}N_5O_4S_2$·HCl) C, H, N.

Single Crystal X-Ray Analysis of 5. Colorless plates were obtained from ethanol/lactic acid (1 equiv). Intensity data were obtained at room temperature with graphite monochromated Cu Kα (λ = 1.5418 Å) radiation on a Nicolet R3m diffractometer, using $\omega/2\theta$ scans with variable speeds and a range of 1.20°. The standard reflections (three measured every 100 reflections) used to monitor data collection did not show significant deviations within the period of data collection. Reflections (3098 collected) having $-10 \le h \le 10$, $0 \le k \le 26$, $0 \le l \le 9$ were measured. There were 2816 independent reflections and 2625 ($|F| \ge 3.0\sigma(F)$) observed reflections. Unit cell constants were determined by a least-squares fit of the 2θ values of 25 reflections having $45° \le 2\theta \le 50°$. Raw intensities were reduced to structure factor amplitudes by correction for scan speed, background, and Lorentz and polarization effects. Absorption corrections were not applied.

The structure was solved by direct methods and refined using the SHELXTL package of programs.[27] All hydrogen atoms were located on a difference Fourier map. In the final cycles of blocked-cascade least-squares refinement, the non-hydrogen atoms were refined with anisotropic thermal parameters, the N–H hydrogen positional parameters were refined, and all other hydrogens were allowed to ride the carbon to which they are attached (C–H 0.96 Å, X–C–H 109° or 120°, B(H) = 1.2 × B(C)). The analytical scattering factors for the neutral atoms were used,[28] and all non-hydrogen scattering factors were corrected for both the real

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Table V. Crystal Data and Selected Details of the Refinement Calculations for 5

formula	$C_{19}H_{25}N_5O_4S_2$
formula weight	451.6
crystal size, mm	0.12 × 0.35 × 0.45
crystal system	monoclinic
space group	$P2_1$
a, Å	10.0655 (7)
b, Å	24.117 (3)
c, Å	8.6590 (7)
α, deg	90.0
β, deg	95.185 (7)
γ, deg	90.0
volume, Å^3	2093.4 (3)
Z	4
density (calc), g cm^{-3}	1.43
absorption coefficient, cm^{-1}	25.5
range, deg	$3 < 2\theta < 114$
$R = \sum\|F_o - F_c\|/\sum F_o$	0.053
$R_w = \sum w^{1/2}\|F_o - F_c\|/\sum w^{1/2}F_o$	0.061

and imaginary components of anomalous dispersion.[29] Crystal data and selected details of the refinement calculations are listed in Table V. An intermolecular hydrogen bond is present with bond distances and angles as follows: H(26)–N(3): 2.18 Å, N(26)–N(3): 2.86 Å, N(26)–H(26)–N(3): 154°.

In Vitro Pharmacology: Purkinje Fibers. The methods employed have been described.[30] Briefly, Purkinje fiber bundles were harvested from mongrel dogs anesthetized with pentobarbital. The fibers were superfused at 37.5–38.0 °C with Tyrode's solution containing the following (mM): NaCl (138), KCl (4), $CaCl_2$ (2), $NaHCO_3$ (24), $MgCl_2$ (0.5), Na_2HPO_4 (1.8), dextrose (5). The Tyrode's solution was equilibrated with 95% O_2–5% CO_2; the pH was 7.3–7.4. The fiber bundles were stimulated with square-wave current pulses 2.0 ms in duration and 2 times threshold. The preparations were allowed to equilibrate at least 1 h or until the action potential parameters had reached steady state before drug superfusion. In each fiber, action potentials were recorded from four to six sites during control and after drug superfusion. The action potential parameters were then averaged to provide mean values for the experimental intervention in each fiber. Because action potential parameters vary significantly in different locations of the fiber bundle, all impalements were made in the same location to minimize variability. When the same impalement was maintained throughout the experiment, the results were identical to those obtained from multiple impalements. Each compound was superfused for 45–60 min before the measurements of the action potential were taken. This time interval was sufficient to reach a steady state of effects for each concentration of compound.

In Vitro Pharmacology: Voltage-Clamp Experiments. Single ventricular myocytes were isolated from cat hearts by enzymatic isolation.[31] Ionic currents were measured under voltage-clamp conditions using the "perforated-patch" technique.[32] In brief, fire-polished microelectrodes were filled with the following solution (in mM): potassium aspartate (120), KCl (20), NaCl (5), Hepes (5), $MgCl_2$ (1), EGTA (0.05). The pH was adjusted to 7.4 with KOH. Nystatin, a polyene antibiotic, previously dissolved in DMSO (5 mg in 0.1 mL) was diluted in the electrode-filling solution to a final concentration of 100 μg/mL. After the formation of a high-resistance seal between the microelectrode and the cell membrane, nystatin acts as an ionophore forming small pores which allow the passage of small monovalent ions but prevents the movement of larger molecules. Thus, the nystatin-produced pores allow electrical continuity between the cell cytoplasm and the inside of the patch microelectrode while reducing the dialysis of the cytoplasmic constituents. For studies of K^+ currents, the cells were superfused with standard Tyrode's solution (same composition as the solution used for voltage recordings in Purkinje fibers) containing nisoldipine (300 nM) to block the Ca^{2+} current ($I_{Ca\text{-}L}$). For studies of $I_{Ca\text{-}L}$, the external Tyrode's solution contained CsCl (2 mM); for these studies the internal solution had the following composition (in mM): CsCl (20), Cs_2SO_4 (120), NaCl (5), $MgCl_2$ (1), EGTA (0.05), Hepes (5). The pH was adjusted to 7.4 with CsOH. Cs^+ was added to the external solution and substituted for K^+ in the internal solution to block K^+ currents. All studies were conducted at 35 °C using myocytes that were quiescent, rod-shaped, free of blebs, and with clear regular striations.

In Vivo Pharmacology: Open-Chest Anesthetized Dogs. The method used to measure atrial and ventricular refractoriness has been reported.[33] Briefly, mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg iv supplemented with 5 mg/kg per h). Epicardial electrodes were sutured on the free wall of the lower right atrium and near the base of the right ventricle. Each electrode set contained a linear array of electrodes consisting of one bipolar stimulating electrode and two bipolar recording electrodes embedded in a rigid acrylic matrix. Each electrode array was oriented to be parallel to the epicardial fiber axis. Atrial and ventricular refractory periods were determined by introducing an extra stimulus (S_2) every eight paced beats (S_1) at BCL = 300 ms. Both S_1 and S_2 were of identical duration (2 ms) and intensity (twice threshold). The S_1–S_2 interval was gradually decreased until the extra stimulus failed to induce a propagated response. This interval was considered to define the effective refractory period. Atrial and ventricular conduction times (ACT and VCT) were measured as the time interval between the two electrograms recorded at the proximal and distal sites of the recording electrode array.

In Vivo Pharmacology: Conscious Dogs. Effective refractory periods in the conscious dog were measured using the same methods as in the anesthetized dogs. Under pentobarbital anesthesia and using sterile techniques, bipolar electrodes were sutured to the ventricular epicardium to measure refractoriness; two more electrodes used to record the ECG were implanted subcutaneously. A catheter was implanted in the superior vena cava through the azygos vein. The leads from the electrodes and the catheter were exteriorized in the interscapular area. Two weeks after surgery, the dogs were studied while resting unsedated in a sling.

In Vivo Pharmacology: Cardiac Contractility. The effects on cardiac contractility were studied by monitoring the rate of development of the left ventricular pressure (LVP) in pentobarbital-anesthetized dogs. A Millar Mikro-Tip pressure transducer was advanced in the right carotid artery and positioned in the left ventricle to measure LVP; the rate of change in the left ventricular pressure (dP/dT), an index of cardiac contractility, was derived by electronic differentiation of LVP.

In Vivo Pharmacology: Ventricular Fibrillation Threshold. For ventricular fibrillation threshold (VFT) measurements, the surgical preparation was similar to the one used to measure refractoriness. Ventricular fibrillation was induced by a ventricular bipolar electrode during atrial pacing (BCL = 300 ms). Trains of square-wave current pulses (pulse duration: 4 ms) lasting 200 ms with a pulse frequency of 50 Hz were delivered to the right ventricle every 12 paced beats. The beginning of the train of pulses was timed to initiate approximately 50 ms after the peak of the R-wave of the electrocardiogram. The smallest current intensity producing sustained ventricular fibrillation (VF) was defined as the ventricular fibrillation threshold (VFT). When VF was induced, the heart was promptly cardioverted by a capacitor-discharge direct current defibrillator. Drugs

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were administered iv 30 min after the predrug determination of VFT. Control studies show that 15-30 min after an episode of VF arterial pressure and ECG parameters recover to control values. A first determination of VFT was performed 15 min after dosing and the dog was promptly defibrillated; as soon as arterial pressure and ECG parameters had recovered, a second VFT determination was performed. This time defibrillation was withheld in order to observe an eventual drug-induced termination of fibrillation.

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Supplementary Material Available: A listing of atomic coordinates, bond lengths and angles, isotropic and anisotropic thermal parameters, and H-atom coordinates for compound 5 (five pages). Ordering information is given on any current masthead page.

Phenothiazines as Lipid Peroxidation Inhibitors and Cytoprotective Agents

Melvin J. Yu,* Jefferson R. McCowan, K. Jeff Thrasher, Priscilla T. Keith, Charlotte A. Luttman, Peter P. K. Ho, Richard D. Towner, Barbara Bertsch, J. S. Horng, Suzane L. Um, Lee A. Phebus, and Royal D. Saunders

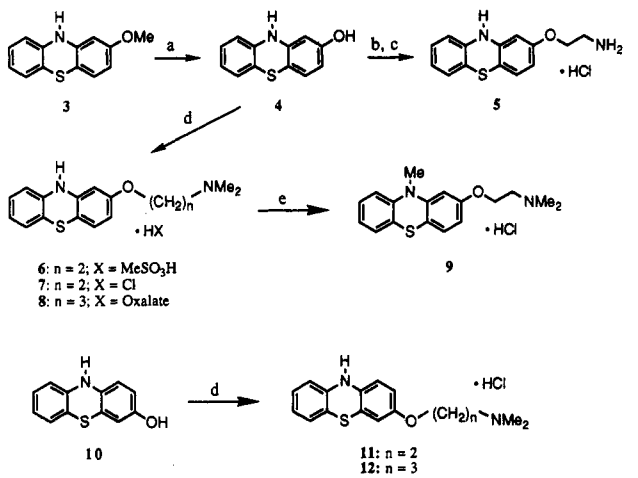
Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, Indiana 46285. *Received August 12, 1991*

A series of phenothiazines was synthesized and evaluated as in vitro inhibitors of iron-dependent lipid peroxidation. The MIC (minimum tested concentration that gave ≥50% inhibition) for 2-(10*H*-phenothiazin-2-yloxy)-*N*,*N*-dimethylethanamine methanesulfonate (6) was 0.26 μM. Whereas methyl substitution at N-10 diminished activity nearly 100-fold, other structural modifications such as varying the amine group, the distance separating the amine substituent from the phenothiazine nucleus, and the linking group had little effect. Compound 6 was more effective than probucol, a known antioxidant, in blocking Cu^{2+} catalyzed oxidation of low-density lipoprotein (LDL) as measured by competitive scavenger receptor mediated degradation of ^{125}I-labeled acetyl-LDL by mouse peritoneal macrophage cells in vitro. At a concentration of 5 μM, compound 6 also protected primary cultures of rat hippocampal neurons exposed to hydrogen peroxide (50 μM) when assessed 18 h later by fluorescein diacetate and propidium iodide uptake.

Toxic oxygen metabolites such as oxygen-derived free radicals and hydrogen peroxide may play an important role in a number of human disease states.[1] For example, oxidants have been associated with postischemic tissue damage in the myocardium[2] and brain[3] and in the pathogenesis of atherosclerotic lesions through oxidative modification of native low-density lipoprotein.[4,5] Since cell membrane phospholipids are particularly vulnerable to free radical induced damage, lipid peroxidation may represent a potentially important mechanism of oxygen-mediated cellular injury.[6]

The phenothiazines chlorpromazine (1) and promethazine at relatively high concentrations have previously been

Scheme I[a]



[a] (a) Pyridine hydrochloride; (b) NaH, $ClCH_2CN$; (c) LAH; (d) NaH, $Br(CH_2)_nNMe_2$; (e) KH, MeI.

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