4,5-Dihydro-3-(methanesulfonamidophenyl)-1-phenyl-1*H*-2,4-benzodiazepines: A Novel Class III Antiarrhythmic Agents

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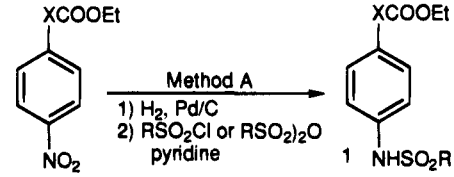
A series of 4,5-dihydro-3-[2-(methanesulfonamidophenyl)ethyl]-1-phenyl-1*H*-2,4-benzodiazepines has been identified as potential antiarrhythmic agents that interact at the delayed rectifier myocardial potassium channels (I_{Kr}) and prolong the ventricular effective refractory period (ERP) in rabbit isolated Langendorff heart preparations. Structure–activity relationship (SAR) studies based upon prolongation of ERP indicate that placement of the sulfonamido group is important for potent activity in this model. Furthermore, methanesulfonamido has enhanced activity over its ethyl or trifluoromethyl analogs. Slightly greater activity was observed in compounds that had a heteroatom in the ethyl bridge that connects the methanesulfonamidophenyl to the benzodiazepine. Further incremental improvements in activity were noted when the 1-phenyl ring was substituted with a variety of substituents. Chirality of the compounds of interest in this series does not appear to influence activity in this model. Several of these compounds were chosen for advanced evaluation, and all possess high selectivity for blockade of potassium current in hearts relative to other ion channels. In addition, these compounds prolong cardiac refractoriness in dogs following oral dosing. Thus, these agents may represent potential new class III agents, but with the potential liability of myocardial I_{Kr} blockers.

Following the outcome of the cardiac arrhythmia suppression trial (CAST),[1,2] a perception has emerged that the negative dromotropic and inotropic influences associated with antiarrhythmic agents which interact with cardiac sodium channels may limit their therapeutic use. As such, development of new antiarrhythmic agents has focused upon identifying compounds devoid of an interaction with cardiac sodium channels,[3–5] thereby potentially limiting conduction slowing and cardiac dysfunction attributed to class I antiarrhythmic drugs. Efforts in this area have focused upon the development of cardiac I_{Kr} K^+ channel blockers[5–8] designed to prolong ventricular refractoriness and prevent the development of lethal tachyarrhythmias. We have previously reported that **3** was identified as a class I/III antiarrhythmic.[9] In this report, we describe the synthesis and biological activity of a related novel subseries of 4,5-dihydro-3-(methanesulfonamidophenyl)-1-phenyl-1*H*-2,4-benzodiazepines that (a) possess nanomolar potency for blocking cardiac I_{Kr} K^+ channels and prolonging effective refractory period in isolated hearts, (b) are at least 1000× selective for blocking I_{Kr} K^+ relative to expressed human cardiac Na^+ channels, and (c) are orally active in dogs at dosages of 0.3 mg/kg.

Chemistry

Esters **1** were prepared by the route outlined in Scheme 1 (method A) which involved catalytic reduction

Scheme 1



followed by alkylsulfonation. The terminal step for the preparation of benzodiazepines **4, 5, 8, 9,** and **13** also utilized the same method (Scheme 2).

The syntheses of pure class III antiarrhythmic agents **4**–**20** were similar to those reported previously (Scheme 3).[9] The key intermediate benzenedimethanediamines **2** were prepared by borane reduction of appropriately substituted phthalazinones.[10] These compounds were then converted to 4,5-dihydro-1-phenyl-1*H*-2,4-benzodiazepines **7** and intermediates for **4, 5, 8, 9,** and **13** by orthoester condensation in acetic acid (method B) or by condensing esters **1** and diamines **2** with either trimethyl- or triisobutylaluminum to give **6, 10**–**12**, and **14**–**20** (method C).

The route to prepare chiral materials was circuitous but gave high yields (method D). The condensation products of **2a,e**–**g** with triethyl orthoacetate gave high yields of 3-methyl-2,4-benzodiazepines **21** which could be readily separated into their enantiomeric salts with either D- or L-dibenzoyltartaric acid (DBT). After isolating enantiomers, each was separated from its DBT salt, and the chiral diamines ***R*-2** and ***S*-2** were generated by reacting them with ethylenediamine (Scheme 4). Treatment of these diamines under the conditions of method C gave the desired chiral benzodiazepines ***R*-14, *S*-14, *R*-16, *S*-16, *R*-17, *S*-17, *R*-18,** and ***S*-18**.

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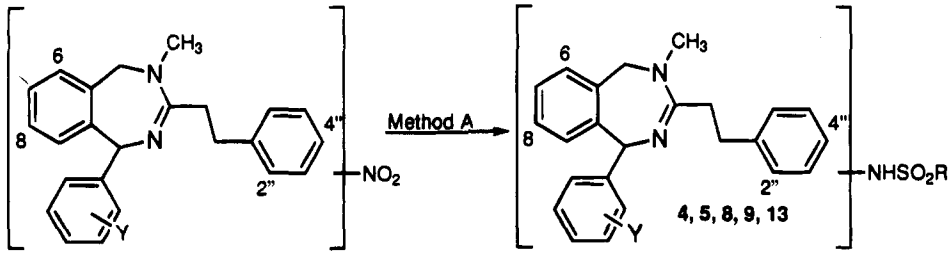
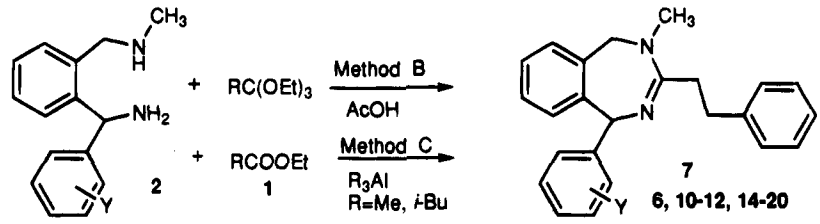
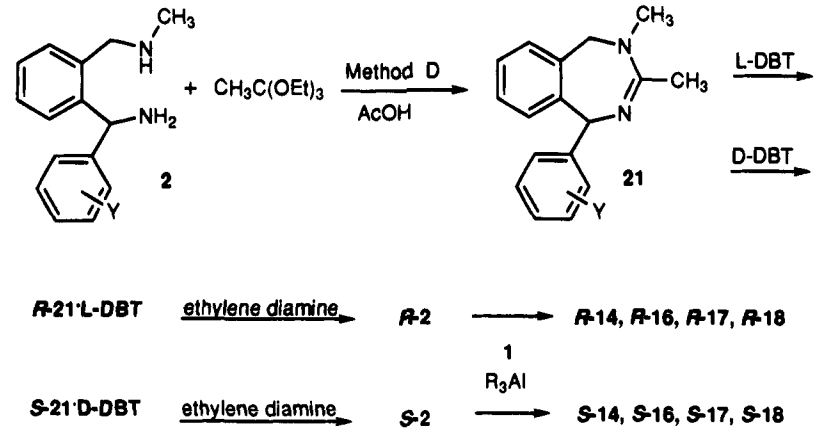
Scheme 2



Scheme 3



Scheme 4



Results and Discussion

For the purposes of developing structure–activity relationships (SAR) for this subseries of pure class III antiarrhythmics, we found that the rabbit Langendorff heart model in vitro gave reliable and meaningful results. The measurement that was used to quantitate class III antiarrhythmic activity was defined as the EC_{20}, the concentration of test compound that resulted in an increase of cardiac refractoriness of 20 ms. Cardiac function was measured in the same model and was defined as dP/dt_{20}, the dose at which a 20% reduction in the rate of pressure development in the left ventricle was noted. The SAR discussion that follows deals almost exclusively with differences in EC_{20} (Table 1). All compounds in this subseries had no effect on dP/dt, and these data are not reported. The results of testing our previously described[9] mixed class I/III antiarrhythmic **3** are included in Table 1 to show the importance of methanesulfonamido in improving class III activity while eliminating negative inotropic effects. The structure of **4** differs from **3** by only the methanesulfonamido substitution and is devoid of cardiac depressant activity (dP/dt) while expressing potent effects on cardiac refractoriness.

The placement of the methanesulfonamido is also important. Thus, compounds **5**, **6**, **7**, and **8** all have the methanesulfonamido group; however, all are less potent than **4**. It should be noted that although these compounds are less active than **4**, all show no effect on cardiac function.

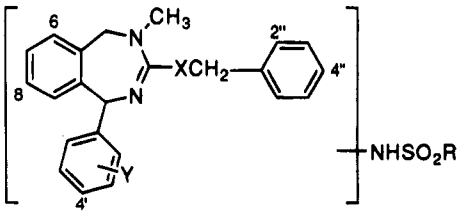
Further evidence for the importance of the methanesulfonamide is found in the comparison of **4** to the weakly active ethanesulfonamide **9** and the inconsistantly active trifluoromethanesulfonamide **10**.

Compounds **11** and **12** were prepared with the expectation that the second methanesulfonamido group would improve activity as observed for the potent class III antiarrhythmic dofetilide.[8] The lack of improved class III activity suggests that the benzodiazepines disclosed here may act at a different binding site than dofetilide in or associated with rapidly-activating delayed rectifier (I_{Kr}) channels.

Although less important than the substitutions already discussed, the placement of heteroatoms in the ethyl linker normally improves activity. As seen in the three compounds **13**, **14**, and **15**, activity can be ranked $O \geq S \geq CH_2$. Another contribution to the SAR is the increase of activity seen when the CH_2 of **4** is replaced by an oxygen to give **16**.

Chirality appears to be unimportant for antiarrhythmic activity in this series. Four sets of enantiomers were prepared (***R*-14/*S*-14**, ***R*-16/*S*-16**, ***R*-17/*S*-17**, ***R*-18/*S*-18**) and tested in the rabbit Langendorff model. In all cases, there were no significant differences between enantiomers.

Table 1. 4,5-Dihydro-3-(methanesulfonamidophenyl)-1-phenyl-1*H*-2,4-benzodiazepines: Structure, Activity in Rabbit Langendorff Assay, Method of Synthesis, and Physical Properties



compd	*X*	*Y*	$NHSO_2R$	R	in vitro (nM) rabbit Langendorff EC_{20}[a]	in vitro (nM) rabbit Langendorff dP/dt_{20}[b]	method[c]	mp, °C	yield, %[d]	recrystn solvent	formula[e]
3	CH_2	H			60 ± 30	700 ± 17					
4	CH_2	H	4''	CH_3	20 ± 10		B,A	153–159	64	$EtOH/Et_2O$	$C_{15}H_{27}N_3O_2S{\cdot}HC{\cdot}EtOH$
5	CH_2	H	2''	CH_3	500 ± 400		B,A	245–246	52	$EtOH/Et_2O$	$C_{25}H_{27}N_3O_2S{\cdot}HCl$
6	CH_2	4'-$NHSO_2CH_3$		CH_3	780 ± 270		C	121–123	27	EtOH	$C_{25}H_{27}N_3O_2S{\cdot}^1/_3EtOH{\cdot}^1/_2H_2O$
7	CH_2	H	6	CH_3	inactive at 1000		B	270–270.5	68	$MeOH/Et_2O$	$C_{25}H_{27}N_3O_2S{\cdot}HCl$
8	CH_2	H	8	CH_3	655 (n = 1)		B,A	170–182	40	CH_3CN/Et_2O	$C_{25}H_{27}N_3O_2S{\cdot}HCl$
9	CH_2	H	4''	C_2H_5	100 ± 20		B,A	240–241	55	MeOH/MtBE	$C_{26}H_{29}N_3O_2S{\cdot}HCl$
10	CH_2	H	4''	CF_3	520 ± 510		C	287–287.5	69	HOAc/MeOH	$C_{25}H_{24}F_3N_3O_2S$
11	O	4'-$NHSO_2CH_3$	4''	CH_3	140 ± 90		C	198–200	16	EtOH	$C_{25}H_{28}N_4O_5S_2{\cdot}C_4H_4O_4{\cdot}^3/_2EtOH$
12	CH_2	4'-$NHSO_2CH_3$	4''	CH_3	inactive at 1000		C	274–275	41	EtOH	$C_{26}H_{30}N_4O_4S_2{\cdot}HCl{\cdot}^6/_5H_2O$
13	CH_2	4'-Cl	4''	CH_3	10 ± 2		B,A	168–172	58	$EtOH/Et_2O$	$C_{25}H_{26}ClN_3O_2S{\cdot}HCl$
14	O	4'-Cl	4''	CH_3	3 ± 1		C	257–258	54	$MeOH/Et_2O$	$C_{24}H_{24}ClN_3O_3S{\cdot}HCl$
R-14					2 ± 1		D	243–243.5	31	EtOH/Ace	$C_{24}H_{24}ClN_3O_3S{\cdot}HCl$
S-14					4 ± 1		D	244–244.5	37	EtOH/Ace	$C_{24}H_{24}ClN_3O_3S{\cdot}HCl$
15	S	4'-Cl	4''	CH_3	5.5 ± 1		C	237–239	21	$EtOH/Et_2O$	$C_{24}H_{24}ClN_3O_2S_2{\cdot}HCl$
16	O	H	4''	CH_3	6 ± 1		C	183–184	62	$MeOH/Et_2O$	$C_{24}H_{25}N_3O_3S{\cdot}C_4H_4O_4$
R-16					1.5 ± 0.5		D	234–235	13	$MeOH/Et_2O$	$C_{24}H_{25}N_3O_3S{\cdot}C_4H_4O_4$
S-16					4 ± 1		D	234–235	9	$MeOH/Et_2O$	$C_{24}H_{25}N_3O_3S{\cdot}C_4H_4O_4$
17	O	4'-SCH_3	4''	CH_3	4 ± 1		C	206.5–209.5	63	$MeOH/Et_2O$	$C_{25}H_{27}N_3O_3S_2{\cdot}HCl{\cdot}^4/_5H_2O$
R-17					7 ± 1		D	184–186	11	CH_3CN/Et_2O	$C_{25}H_{27}N_3O_3S_2{\cdot}HCl$
S-17					8 ± 4		D	221–222.5	9	CH_3CN/Et_2O	$C_{25}H_{27}N_3O_3S_2{\cdot}HCl$
18	CH_2	2',4'-F_2	4''	CH_3	4 ± 2		C	198–200	41	acetone	$C_{25}H_{25}F_2N_3O_2S{\cdot}HCl$
R-18					7 ± 3		D	134–143	54	CH_2Cl_2/EtOAc/Et_2	$C_{25}H_{25}F_2N_3O_2{\cdot}HCl{\cdot}^1/_2H_2O$
S-18					8 ± 6		D	138–148	8	EtOAc	$C_{25}H_{25}F_2N_3O_2S{\cdot}HCl{\cdot}^1/_2H_2O$
19	O	4'-OCH_3	4''	CH_3	4 ± 1		C	157–161	71	EtOH/MeOH	$C_{25}H_{27}N_3O_4S{\cdot}HCl{\cdot}H_2O$
20	O	3',4''-Cl_2	4''	CH_3	2 ± 0.5		C	181–186	41	$MeOH/Et_2O$	$C_{24}H_{23}Cl_2N_3O_3S{\cdot}HCl{\cdot}H_2O$
dofetilide					2 ± 0.1						

[a] EC_{20}, reported in nanomolars, is the concentration estimated from linear regression of dose response data that increases ERP by 20 ms. [b] dP/dt_{20} is the concentration (nM) at which the rate of pressure developoment in the left ventricle is reduced by 20%. Both ERP and dP/dt were evaluated in the same hearts (n = 3–5/compound). Data were analyzed using an ANOVA for repeated measures and expressed as mean change from control data ± SEM. Statistical significance was established at a probabililty of error less than 0.05. [c] Refers to the general method used and is described in the Experimental Section. [d] Yields were not optimized. [e] Analyses within ± 0.4% for C, H, and N were obtained for all indicated formulas.

Optimization of the activity in this class of compounds was accomplished by preparing **17–20** with a variety of substitutions on the 1-phenyl but maintaining equiactivity. Compounds **16–18** were evaluated further in more advanced cellular models and in vivo, and these activities are shown in Tables 2 and 3.

For the most part, there was not a significant difference among pairs of enantiomers in their potency for blocking I_{Kr} (Table 2). In one case, the (+) enantiomer ***R*-18** was about half as potent as the (−) enantiomer ***S*-18.** In general, all compounds had IC_{50} values between 20 and 100 nM and were roughly equipotent with the class III agent dofetilide.

Selectivity was evaluated by comparing effects on Na^+ channel blockade (human HH1 clone), displacement of BTX from site 2 of the sodium channel, and displacement of a verapamil analog (D888) from cardiac Ca^{2+} channels (Table 2). Effects on blockade of HH1 were observed at 30–100 μM concentrations; displacement of BTX occurred in the 1–7 μM range. No apparent differences among enantiomers were noted. Displacement of D888 binding occurred in the low to submicromolar range with no apparent difference between enantiomers. Similar results were evident with displacement of ligands for the dihydropyridine binding (data not shown). Although direct blockade of myocardial Ca^{2+} current was not measured, the well-known correlation between potency for displacement of radiolabeled ligands that bind to the Ca^{2+} channel with blockade of Ca^{2+}current suggests that these compounds will have low micromolar potency for Ca^{2+} entry blockade.

Table 2. Effects of Selected Enantiomeric Pairs of 4,5-Dihydro-3-(methanesulfonamidophenyl)-1-phenyl-1*H*-2,4-benzodiazepines on K^+, Na^+, and Ca^{2+} Channels

	IC_{50}			
	ion channel blockade		ion channel–ligand displacement	
compd	I_{Kr}[a]	I_{Na}-HH1[a]	BTX[b]	D888[b]
R-16	38	126	5.2	2.8
S-16	29	>30	6.9	0.9
R-17	21	30	1.0	0.7
S-17	56% at 100	>30	3.0	1.1
R-18	85	>100	3.6	0.5
S-18	38	>100	2.5	1.1
dofetilide	44	>300	NT[c]	NT[c]

[a] Values are the IC_{50} for blockade of the delayed rectifier K^+ current from guinea pig isolated myocytes (I_{Kr}, nM) or Na^+ current expressed in frog oocytes from a human (HH1) cardiac sodium channel clone (I_{Na}-HH1, μM). [b] Values are the IC_{50} (μM) for displacement of [^{3}H]BTX (Na^+ channel ligand) or [^{3}H]D888 (Ca^{2+} channel ligand). [c] NT = not tested.

Table 3. Oral Activity of Selected Enantiomeric Pairs of 4,5-Dihydro-3-(methanesulfonamidophenyl)-1-phenyl-1*H*-2,4-benzodiazepines in Mongrel Dogs

compd	maximum increase in ERP (ms)[a]		
	500 ms[b]	400 ms	250 ms
R-16	40 ± 9	36 ± 9	31 ± 11
S-16	18 ± 6	20 ± 3	18 ± 1
R-17	33 ± 6	33 ± 1	28 ± 2
S-17	28 ± 3	20 ± 3	11 ± 1
R-18	28 ± 4	27 ± 3	21 ± 11
S-18	33 ± 4	33 ± 8	22 ± 3
dofetilide	30 ± 4	28 ± 3	21 ± 4

[a] Values are the $\bar{X} \pm$ SEM for n = animals. Animals were dosed orally at 0.3 mg/kg; values represent the maximum increase in ERP observed over a 3 h interval. [b] Pacing was done at basic cycle lengths of 500, 400, and 250 ms for each compound.

Prolongation of ERP, an index of biological activity, of all enantiomers was also evident in mongrel dogs following oral dosing at 0.3 mg/kg (Table 3). There was a trend for two of the (+) enantiomers (***R*-16** and ***R*-17**) to produce greater increases in ERP relative to the (−) enantiomers. Since these agents were relatively equiactive at blocking I_{Kr} and increasing ERP in vitro (Tables 1 and 2), this difference may be reflective of a selective biodistribution of the (+) enantiomer. All enantiomers increased ERP at the three different pacing rates used in this study (equivalent to 120, 150, and 240 beats/min). While there was a tendency for Δ ERP to be lower at the faster rate, there was no clear significant difference.

Experimental Section

Reactions involving organometallic reagents were run under a N_2 or an Ar atmosphere. Solvents and reagents from commercial sources were used without further purification. Melting points (Pyrex capillary) are uncorrected. For ^{1}H NMR spectra, multiplicity is denoted by s (singlet), d (doublet), t (triplet), q (quartet), quin (quintet), sep (septet), m (multiplet), and br (broad). Coupling constants are in hertz. ^{1}H NMR spectra were run on either a 200, 270, or 300 MHz instrument. Infrared spectra (IR) were measured as KBr pellets. If the requisite orthoesters for general method B were not available commercially, they were prepared by literature procedures.[11]

General Method A. Ethyl 3-(4-methanesulfonamidophenyl)propionate (1a). This procedure illustrates the general method for the preparation of **1a, 1b,** ethyl 3-[4-(trifluoromethanesulfonamido)phenyl]propionate (**1c**), and ethyl (4-methanesulfonamidophenyl)thioacetates **1d, 4, 5, 8, 9,** and **13**. Ethyl (4-aminophenyl)thioacetate was the starting material for **1b**, and therefore the reduction step was omitted in the preparation of **1b**. Ethyl 4-nitrocinnamate (124 g, 0.55 mol, 1 equiv) was suspended in 200 mL of EtOH, and 0.2 g of 10% Pd/C was cautiously added. The mixture was then hydrogenated at 50 psi for 4 h. An additional 0.5 g of 10% Pd/C was then added, and the mixture was hydrogenated a further 2 h at 50 psi. EtOH was then removed in vacuo, and the residue was azeotroped with PhMe (3 × 100 mL) to give ethyl 3-(4-aminophenyl)propionate (108 g, 99%) as a yellow oil that was used immediately without further purification. The oil was dissolved in 400 mL of dry CH_2Cl_2 under Ar at room temperature, and 150 mL of pyridine was added. This solution was cooled to 0 °C and treated dropwise with (48.2 mL, 0.61 mol, 1.1 equiv) of methanesulfonyl chloride. The reaction mixture was allowed to warm to room temperature and was stirred for 16 h. The volatiles were then removed in vacuo, azeotroping with H_2O. The residue was then partitioned between 0.1 N HCl and Et_2O, and the organic phase was washed with saturated NaCl. The solution was dried (Na_2SO_4), and solvents were removed in vacuo to give 151 g of crude product as a yellow oil. The oil was distilled (bp 140 °C/0.4 mm) to give 138 g of pure **1a** (92%) as a colorless oil which crystallized on standing to provide a white crystalline solid: mp 88−89 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.19 (t, J = 7.1 Hz, 3H), 2.56 (t, J = 7.8 Hz, 2H), 2.88 (t, J = 7.6 Hz, 2H), 2.93 (s, 3H), 4.08 (q, 2H), 7.15 (m, 4H), 7.42 (s, 1H); ^{13}C NMR ($CDCl_3$, 75.4 MHz) δ 14.0 (q), 30.0 (t), 35.5 (t), 38.8 (q), 60.3 (t), 121.2 (d), 129.2 (d), 135.0 (s), 137.6 (s), 172.8 (t).

General Method B. (*RS*)-4,5-Dihydro-6-methanesulfonamido-1-phenyl-3-(phenylethyl)-1*H*-2,4-benzodiazepine Monohydrochloride (7). This procedure illustrates the general method for the preparation of **4, 5, 7−9,** and **13**. To a solution of *N*-methyl-α′-phenyl-4-methanesulfonamidobenzenedimethanamine dihydrochloride (**2a**)[10] (0.70 g, 2.6 mmol) and trimethyl orthophenylpropionoate (2.18 g, 10.4 mmol) in 8 mL of MeOH were added acetic acid (0.62 g, 10.4 mmol) and 2 drops of methanolic HCl. After stirring at room temperature for 1 day, 2 drops more of methanolic HCl was added, followed by another drop of methanolic HCl after another day. After 4 days of stirring, methanolic HCl were added until the reaction mixture was acidic. The mixture was diluted with 7 mL of ether and cooled in ice, and the precipitate was collected and washed with first 1:1 ether:MeOH and then ether to give 0.75 g (68%) of (***RS*-7**).

General Method C. (*RS*)-1-(2,4-Difluorophenyl)-4,5-dihydro-3-[2-(4-methanesulfonamidophenyl)ethyl]-1*H*-2,4-benzodiazepine Monohydrochloride (18). This procedure illustrates the general method for the preparation of **6, 10−12,** and **14−20**. *N*-Methyl-α′-(2,4-difluorophenyl)benzenedimethanamine dihydrochloride (**2b**)[10] (14.0 g, 41.8 mmol, 1 equiv) was suspended in 400 mL of toluene at room temperature under Ar and treated dropwise with 145 mL of 1 M $(iBu)_3Al$ (145 mmol, 3.5 equiv) over 10 min. The mixture was then heated to 100 °C, and ester **1a** (11.9 g, 43.8 mmol, 1.05 equiv) was added, causing a strong exotherm. The reaction was refluxed 4 h, cooled to room temperature, and added to 400 mL of Rochelle's salt solution. Saturated Na_2CO_3 (100 mL) was then added followed by 500 mL of EtOAc. The mixture was stirred vigorously at room temperature, the phases were separated, and the aqueous phase was reextracted with EtOAc. The organic solution was dried (Na_2SO_4), and solvents were removed in vacuo to furnish a yellow solid. Recrystallization (EtOH) provided 14.2 g (71%) of **18** as an EtOH-solvated crystalline solid, mp 202−204 °C. This free base was then dissolved in a minimum amount of EtOH, acidified with EtOH/HCl, and added to 500 mL of Et_2O to furnish the amorphous hydrochloride salt as a white solid. Crystallization (acetone/MeOH) and drying under high vacuum furnished 8.17 g (41% from **2b**) of **18** as a colorless crystalline hydrochloride salt: mp 198−200 °C; ^{1}H NMR (300 MHz, DMSO) δ 2.78−2.95 (m, 4H), 2.92 (s, 3H), 3.24 (s, 3H), 4.61 (d, J = 15.0 Hz, 1H), 4.79 (d, J = 15.0 Hz, 1H), 6.32 (s, 3H), 7.03−7.46 (m, 11H), 9.73 (s, 1H), 10.4 (s, 1H); ^{13}C NMR (75.4 MHz, DMSO) δ 31.9 (t), 37.9 (t), 38.8 (q), 40.0 (q), 53.7 (d), 55.0 (t), 103.1 (C3′, t, $^2J(^{19}F-^{13}C)$ = 25.2 Hz), 110.9 (C5′, dd, $^2J(^{19}F-^{13}C)$ = 20.1 Hz, $^4J(^{19}F-^{13}C)$ = 3.0 Hz), 120.3 (d), 123.6 (d), 126.5 (C1′, dd, $^2J(^{19}F-^{13}C)$ = 13.1 Hz, $^4J(^{19}F-^{13}C)$ = 3.0 Hz), 127.0 (d), 127.8 (d), 128.1 (d), 129.1 (d), 131.4 (C6′, t, $^3J(^{19}F-^{13}C)$ = 9.1 Hz), 135.2 (s), 136.1 (s), 137.4 (s), 143.0 (s), 156.9 (C-2, s), 158.7 (C4′, dd, $^1J(^{19}F-^{13}C)$ = 128 Hz, $^3J(^{19}F-^{13}C)$ = 12.1 Hz), 162.0 (C2′, dd, $^1J(^{19}F-^{13}C)$ = 125 Hz, $^3J(^{19}F-^{13}C)$ = 12.1 Hz).

General Method D. Separation of the Enantiomers of (*RS*)-1-(2,4-Difluorophenyl)-4,5-dihydro-3-[2-(4-methanesulfonamidophenyl)ethyl]-1*H*-2,4-benzodiazepine Monohydrochloride (18). This procedure illustrates the general method for the preparation of the enantiomers of **14,** and **16−18**. *N*-Methyl-α′-(2,4-difluorophenyl)benzenedimethanamine dihydrochloride[10] (**2b**) (47.0 g, 0.140 mol, 1 equiv) was dissolved in 275 mL of MeOH, and 36.1 mL of trimethyl orthoacetate (0.281 mol, 2 equiv) was added followed by 22.1 g of NaOAc (0.267 mol, 1.9 equiv). The mixture was heated at 50 °C for 2 h, then refluxed 1 h, cooled, and filtered. The filtrate was concentrated in vacuo and the residue partitioned between Et_2O and 0.5 N HCl. The aqueous phase was then basified with 35% NaOH and extracted with 1:1 CH_2Cl_2:MTBE (2 × 200 mL), and the aqueous phase was saturated with NaCl and again extracted with 1:1 CH_2Cl_2:MTBE. The combined

organic extracts were washed with saturated NaCl, dried (Na_2SO_4), and concentrated in vacuo to furnish a white solid. Crystallization (EtOAc/MTBE) provided 28.9 g (72%) of 1-(2,4-difluorophenyl)-4,5-dihydro-3,4-dimethyl-1*H*-2,4-benzodiazepine (**21**) as a white solid: mp 161−161.5 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.01 (s, 3H), 3.05 (s, 3H), 3.77 (d, J = 14.3 Hz, 1H), 5.39 (d, J = 14.3 Hz, 1H), 6.56 (d, J = 7.1 Hz, 1H), 6.63 (s, 1H), 6.79 (dt, J = 2.4, 10.8 Hz, 1H), 7.00 (dt, J = 2.7, 8.5 Hz, 1H), 7.14−7.27 (m, 3H), 7.99 (m, 1H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 25.9 (q), 41.3 (q), 54.5 (d), 56.2 (t), 103.1 (C-3′, t, $^2J(^{19}F-^{13}C)$ = 25.2 Hz), 111.0 (C-5′, dd, $^2J(^{19}F-^{13}C)$ = 20.1 Hz, $^4J(^{19}F-^{13}C)$ = 4.0 Hz), 124.7 (d), 126.3 (C-1′, dd, $^2J(^{19}F-^{13}C)$ = 14.1 Hz, $^4J(^{19}F-^{13}C)$ = 4.0 Hz), 127.1 (d), 127.8 (d), 128.3 (d), 131.3 (C-6′, dd, $^3J(^{19}F-^{13}C)$ = 6.0, 9.1 Hz), 134.9 (s), 143.5 (s), 156.1 (s), 159.3 (C-2′, dd, $^1J(^{19}F-^{13}C)$ = 138 Hz, $^3J(^{19}F-^{13}C)$ = 11.1 Hz), 162.7 (C-4′, dd, $^1J(^{19}F-^{13}C)$ = 137 Hz, $^3J(^{19}F-^{13}C)$ = 12.1 Hz).

A solution of **21** (25.7 g, 0.090 mol, 1 equiv) in 1 L of warm MeOH and a warm solution of dibenzoyl-L-tartaric acid (L-DBT, 33.5 g, 0.092 mol, 1.03 equiv) in 500 mL of MeOH was added. The resulting solution was allowed to cool slowly. The white needles thus obtained were recrystallized from 2.3 L of hot MeOH to give 18.1 g (63%) of ***R*-21·L-DBT** as white needles: mp 159−161 °C; $[\alpha]_D$ = +32.8° (c = 0.119, MeOH); ^{1}H NMR (300 MHz, DMSO) δ 2.24 (s, 3H), 3.21 (s, 3H), 4.68 (m, 2H), 5.66 (s, 2H), 6.30 (s, 1H), 7.10−7.26 (m, 3H), 7.35−7.47 (m, 7H), 7.57−7.64 (m, 3H), 7.90 (m, 4H); ^{13}C NMR (75.4 MHz, $CDCl_3$) δ 21.0 (q), 42.1 (q), 53.9 (t), 55.7 (d), 72.5 (d), 104.7 (t, $^2J(^{19}F-^{13}C)$ = 26.2 Hz), 111.5 (d, $^2J(^{19}F-^{13}C)$ = 21.2 Hz), 123.1 (d, $^2J(^{19}F-^{13}C)$ = 9.1 Hz), 128.5 (d), 129.0 (d), 129.2 (d), 129.7 (s), 130.35 (C-2′, m), 133.2 (s), 133.7 (s), 136.7 (s), 159.0 (C-4′, dd, $^1J(^{19}F-^{13}C)$ = 224 Hz, $^3J(^{19}F-^{13}C)$ = 12.1 Hz), 162.6 (C-2′, dd, $^1J(^{19}F-^{13}C)$ = 220 Hz, $^3J(^{19}F-^{13}C)$ = 12.0 Hz), 163.9 (s), 164.9 (s), 168.0 (s).

The ***R*-21·L-DBT** (18.0 g, 27.9 mmol, 1 equiv) was converted to the free base with 2 N NaOH, extracted with CH_2Cl_2, and concentrated to give ***R*-21** as a crystalline solid, 7.94 g (99%): mp 137−138 °C; $[\alpha]_D$ = +236° (c = 1.025, EtOH). This solid (7.80 g, 27.2 mmol, 1 equiv) was dissolved in 80 mL of PhMe under N_2, and 1.86 mL of ethylenediamine (27.5 mmol, 1.01 equiv) was added. The mixture was refluxed for 24 h, cooled, and concentrated in vacuo. The residue was partitioned between 0.5 N NaOH and MTBE, the aqueous phase was reextracted with MTBE, and the combined organic phases were washed with saturated NaCl (2×), dried (Na_2SO_4), and again concentrated in vacuo to give 6.96 g of a yellow oil. The amorphous HCl salt was then prepared from MeOH/HCl and Et_2O to furnish (after drying under high vacuum) 8.76 g of ***R*-2·2HCl** (96%) as a white solid: ^{1}H NMR (300 MHz, DMSO) δ 2.64 (s, 3H), 3.47 (br, 2H), 4.18 (d, J = 13.4 Hz, 1H), 4.50 (d, J = 13.4 Hz, 1H), 6.25 (s, 1H), 7.20−7.45 (m, 4H), 7.67−7.74 (m, 2H), 7.93-8.01 (m, 1H), 9.65 (br, 3H); ^{13}C NMR (75.4 MHz, DMSO) δ 32.5 (q), 47.1 (d), 47.4 (t), 104.4 (C-3′, t, $^2J(^{19}F-^{13}C)$ = 25.2 Hz), 111.7 (C-5′, d, $^2J(^{19}F-^{13}C)$ = 19.1 Hz), 121.8 (C-1′, dd, $^2J(^{19}F-^{13}C)$ = 14.1 Hz, $^4J(^{19}F-^{13}C)$ = 4.0 Hz), 128.7 (d), 128.9 (d), 129.4 (C-6′, dd, $^3J(^{19}F-^{13}C)$ = 10.3, 4.0 Hz), 129.6 (d), 130.2 (s), 131.6 (d), 136.0 (s), 159.0 (C-4′, dd, $^1J(^{19}F-^{13}C)$ = 238 Hz, $^3J(^{19}F-^{13}C)$ = 13.1 Hz), 162.2 (C-2′, dd, $^1J(^{19}F-^{13}C)$ = 236 Hz, $^3J(^{19}F-^{13}C)$ = 12.1 Hz).

The MeOH mother liquors from L-DBT salt formation (vide infra) were evaporated, basified (2 N NaOH), and treated with D-DBT. Two recrystallizations from MeOH provided optically pure ***S*-21·D-DBT**(−). Ethylenediamine hydrolysis and dihydrochloride salt formation as described above furnished ***S*-2·2HCl**. The ^{1}H NMR and ^{13}C NMR spectra of **(*S*-2·2HCl)** were identical to those of ***R*-2·2HCl**.

Using general method C, ***R*-2 and *S*-2** were condensed with ester **1a** to give ***R*-18** (58%) (mp 111−115 °C; $[\alpha]_D$ = +90° (c = 0.50, $CHCl_3$)) and ***S*-18** (31%) (mp 114−118 °C, $[\alpha]_D$ = −89° (c = 0.51, $CHCl_3$)), respectively.

Biological Studies. All research involving animals described was performed in accord with the Sterling Winthrop Pharmaceuticals Research Division's (SWPRD) Policy on Animal Use and all national and federal legislation. All SWPRD animal facilities and programs are accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

Isolation of Canine Cardiac Sarcolemma. Canine cardiac sarcolemma vesicles were isolated as described by Jones.[12]

Radioligand Binding Studies: A. [^{3}H]Desmethoxyverapamil Binding to the Canine Cardiac L-Type Ca^{2+} Channel. Canine cardiac sarcolemma (50 μg of protein) was incubated with [^{3}H]desmethoxyverapamil (D888, NEN, specific activity = 87 Ci/mmol) in buffer containing 50 mM Tris-HCl (pH 7.4) and 0.1% BSA for 90 min at 25 °C in a volume of 0.25 mL. All incubations were performed in triplicate, and nonspecific binding was determined in the presence of 31.6 μM (*S*-(−)-verapamil (Research Biochemicals Inc.). In competitive binding studies the incubations contained 5 nM [^{3}H]D888. For Scatchard determinations, [^{3}H]D888 concentrations of 1−32 nM were utilized. Bound radioactivity was determined following filtration on Whatman GFC filters using a Brandel-48 Harvester. Prior to use, filters were preincubated for 60 min at room temperature in 20 mM Tris-HCl (pH 7.4) containing 0.5% polyethylenimine. Filters were washed with 2000 mL of 10 mM HEPES-Tris (pH 7.4) containing 10% ethanol (vol/vol).

B. [^{3}H]Batrachotoxin Benzoate Binding to the Canine Cardiac Na^+ Channel. Canine cardiac sarcolemma (50 μg of protein) was incubated with [^{3}H]batrachotoxin benzoate (BTX-B, NEN, specific activity = 52.7 Ci/mmol) in 0.1 mL of buffer containing 130 mM choline chloride, 50 mM HEPES-Tris (pH 7.4), 5.4 mM KCl, 0.5 mM EDTA, 0.4 mM $MgCl_2$, 0.1% BSA, and 0.12 mg/mL of Leiurus quinquestriatus venom (Sigma V-5251). All incubations were performed in triplicate for 60 min at 37 °C. Nonspecific binding was determined in the presence of 0.3 mM veratridine HCl (Sigma). In competitive binding studies, the incubations contained 5 nM [^{3}H]BTX-B. In Scatchard analyses, [^{3}H]BTX-B concentrations of 1−32 nM were used. Filtration was performed as described for [^{3}H]-D888 binding except that filters were preincubated for 60 min in 20 mM Tris-HCl (pH 7.4) containing 0.25% polyethylenimine.

[^{3}H]Batrachotoxin A 20 α-benzoate ([^{3}H]BTX-B; NET-876, 52.7 Ci/mmol) was from NEN. [^{3}H]Desmethoxyverapamil ([^{3}H]-D888; TRK.834, 84 Ci/mmol) was from Amersham.

Electrophysiological Methods: A. Delayed Rectifier Potassium Current. Methods for cell preparation and current recording were as previously described.[13] Briefly, cardiac myocytes were isolated by collagenase dispersion from adult guinea pig ventricular tissue. Calcium-tolerant cells were placed on a microscope slide and continuously perfused during recording with a bath solution consisting of the following (mM): NaCl 145, KCl 4.5, $MgCl_2$ 1, $CaCl_2$ 0.1, HEPES 10, glucose 12, $NiCl_2$ 2, pH 7.4. Recording was performed at 33−35 °C using standard whole-cell patch clamp techniques with pipette solutions containing the following (mM): NaCl 10, KCl 112, EGTA 10, HEPES 10, K_2ATP 5, Mg_2ATP 5, pH 7.4. Membrane potential was held at −40 mV, and tail current amplitudes were measured following depolarizing voltage steps to +60 mV before and after exposure to test agent.

B. Human Cardiac Sodium Current. Human cardiac sodium currents (HH1) were expressed and recorded in Xenopus oocytes as previously described.[14] Briefly, Xenopus laevis oocytes were isolated, and in vitro transcripts of the HH1 gene were injected. Oocytes were allowed to incubate at room temperature for 24−72 h prior to recording. Sodium currents were measured by standard two-microelectrode voltage clamp using electrodes filled with 3 M KCl and a bath solution of the following (mM): NaCl 140, KCl 2, $CaCl_2$ 1.8, $MgCl_2$ 1, HEPES 5, pH 7.5. Experiments were performed at 21−23 °C. Membrane potential was held at −100 mV, and current amplitudes were measured at the peak of the current−voltage relationship before and after exposure to test agent.

Measurement of Refractoriness and Contractility in Isolated Rabbit Hearts. New Zealand White male rabbits (2.5−3.5 kg) were anesthetized with sodium pentobarbital (35 mg/kg iv). Hearts were isolated and perfused (30 mL/min) in a Langendorff mode with Kreb's solution (mM): 118.0 NaCl, 4.5 KCl, 1.3 $CaCl_2$, 1.16 $MgSO_4$, 11 dextrose, 25.0 $NaHCO_3$,

equilibrated with 95% O_2 and 5% CO_2 and maintained at 37 °C. Left ventricular pressure and its first derivative were recorded after establishing a stable end diastolic pressure of approximately 5 mmHg. Hearts were stimulated, after right atrial removal and AV nodal ablation, at 120 stimuli/min using bipolar 2 ms constant current pulses. Stimuli were delivered at twice the threshold with a 1 mA minimum intensity. Hearts were allowed to equilibrate for 45 min prior to obtaining control data. The effective refractory period (ERP; mean of 200 ± 10 ms) was determined, following a 15 min exposure to test agent or vehicle using standard stimulation techniques scanning diastole at twice the threshold with a single premature stimuli. Cumulative concentration responses were recorded for five concentrations of each test agent. The concentration at which ERP increased by 20 ms and d*P*/d*t* decreased by 220 mmHg were derived from the concentration response curves. Data were analyzed using an ANOVA for repeated measures and expressed as mean change from control data ± SEM. Statistical significance was established at a probability of error less than 0.05.

Conscious Instrumented Dog. Male colony-bred mongrel dogs (10–15 kg) obtained from Hazelton Research Products, Cumberland, VA, were sedated with atropine (0.027 mg/kg im) and acepromazine (1.0 mg/kg im) and anesthetized with surital (2.5%) iv until consciousness was lost. Anesthesia was maintained with isoflurane (1%) and oxygen administered with an Ohmeda V.M.C. (West Yorkshire, England) anesthesia machine and a North American Drager (Telford, PA) model SAU respirator. A left thoracotomy was performed at the fourth intercostal space, and the ribs were retracted. The superior lobes of the left lung were retracted and the pericardium incised. A Konigsberg transducer (Konigsberg Instruments Inc., Pasadena, CA) was placed into the left ventricle through a stab wound in the apex of the heart and purse string sutured into place. Two pair of epicardial electrodes were sutured to the left ventricle. The Konigsberg cable and the electrode wires were exited subcutaneously to skin buttons in the midscapular region. The lungs were overinflated to minimize atelectasis, and the thoracotomy was closed with umbilical tape. The muscle layers were sutured with 2-0 polyvicryl and the skin closed with 3-0 nylon monofiliment. The pneumothorax was reduced by evacuation of air from the chest using an 18 gauge needle and a 60 cm^3 syringe. Talwin was administered (2 mg/kg im), and a 5 day regimen of Combiotic (0.1 mL/kg) was begun.

After at least 7 days recovery from the surgical procedure, the dogs were acclimated to testing slings. On the day of the experiment, dogs were suspended in a sling and left ventricular pressure, the first derivative of the left ventricular pressure (d*P*/d*t*), the lead II ECG, and the cardiac electrogram were continuously monitored on a Grass model 7 polygraph. The cardiac electrogram and pacing stimuli were digitized by an 8 bit A/D converter (R. C. Electronics, Santa Barbara, CA) and displayed on an Apple IIe computer. This system was used to monitor the cardiac response to synchronous pacing for ERP determinations. Pacing at basic cycle lengths of 500, 400, and 250 ms was performed through the second pair of electrodes using a Bloom DTU-201 stimulator (Bloom Electronics, Inc., Reading, PA). Ventricular refractoriness was determined by premature stimulation (S2) following an 8 beat S1 train. The interval between the last S1 and and the premature S2 pulse was reduced in 10 ms increments until a ventricular response was not initiated. ERP was defined as the longest S1-S2 interval that failed to produce a ventricular response. Hemodynamic function was evaluated during normal sinus rhythm. Following three baseline measurements of ERP and cardiac function, the dogs were orally medicated by gastric lavage. Measurements were taken at 15 min intervals for 3 h. Values represent the maximum increase in ERP observed over this interval.

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