Substituted 2-Benzothiazolamines as Sodium Flux Inhibitors: Quantitative Structure–Activity Relationships and Anticonvulsant Activity

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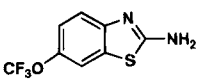
Abstract □ Thirty-two aryl-substituted 2-benzothiazolamines have been tested for their ability to modulate sodium flux in rat cortical slices. A QSAR analysis, applied to these derivatives, showed a trend toward increasing potency as sodium flux inhibitors with increasing lipophilicity, decreasing size, and increasing electron withdrawal of the benzo ring substitutents. Additionally, 4- or 5-substitution of the benzo ring was found to decrease potency. The combination of increased lipophilicity, small size, and electron withdrawal severely limited which groups were tolerated on the benzo ring, thus suggesting that the optimal substitution patterns have been prepared within this series. Nine of these compounds were potent inhibitors of veratridine-induced sodium flux (NaFl). These nine compounds also proved to be anticonvulsant in the maximal electroshock (MES) assay. Fourteen additional 2-benzothiazolamines demonstrated activity in the MES screen, yet exhibited no activity in the NaFl assay. These derivatives may be interacting at the sodium channel in a manner not discernible by the flux paradigm, or they may be acting by an alternative mechanism in vivo.

Introduction

There is currently a need for improved agents for the treatment of seizure disorders, since available drugs are effective in only 60–80% of epileptic patients.[1] Absence (petit mal) seizures are well controlled in most cases, but significant therapeutic advancement is required for the treatment of partial–complex (focal) seizures and generalized tonic–clonic (grand mal) epilepsy.[2] Most marketed anticonvulsant agents suffer from a wide range of side effects, including sedation, teratogenicity, cognitive dulling, blood dyscrasia, and liver toxicity.[3] Frequently, the failure to achieve control of seizures is a function of use-limiting side effects.

Clinically available anticonvulsants act by a variety of mechanisms that are not well-defined. Current theories suggest that several mechanisms function in normal neuronal tissues to control neuronal discharge.[4] Loss of these mechanisms causes synchronized and spreading waves of excitation, resulting in seizures. Potentially, anticonvulsant agents could exert their action by any number of mechanisms that would limit uncontrolled discharges. Many of these mechanisms ultimately result in the control of the flow of ions, particularly sodium and calcium, across neuronal cell membranes.[4]

In the past decade, the excitatory amino acid neurotransmitters L-glutamate and L-aspartate have been determined to play an important role in the etiology of certain seizure disorders.[5] If an imbalance exists between the release of these excitatory amino acids and inhibitory amino acids (GABA), then this might explain the synchronous and sustained neuronal discharges characteristic of epileptic activity. In 1985, riluzole[6-(trifluoromethoxy)-2-benzothiazolamine, PK 26124] was reported to be a potent anticonvulsant agent that functioned via a glutamatergic mechanism.[6] More recent evidence suggests that riluzole is not exerting its activity by an inhibitory action on glutamate-mediated ion channels but rather by a direct action on voltage-dependent sodium channels.[7] Modulation of sodium channels is a mechanism shared by the clinically useful anticonvulsants phenytoin and carbamazepine.[8]



Riluzole

In an effort to understand the potential of 2-benzothiazolamines to function as clinically useful anticonvulsants, 32 aryl-substituted 2-benzothiazolamines have been tested for their ability to modulate sodium flux in rat cortical slices. To investigate the usefulness of this sodium flux assay as an in vitro screen for anticonvulsant activity, these compounds were also evaluated in a maximal electroshock (MES) assay, as well as for their ataxic liability. This paper describes the synthesis, biological activity, and analysis via quantitative structure–activity relationship (QSAR) techniques of 2-benzothiazolamines as potential anticonvulsants.

Results and Discussion

Chemistry—Many of the substituted 2-benzothiazolamines were commercially available or were synthesized from the corresponding anilines via a one-pot procedure (Scheme 1, method A). In this route, the thiourea is produced in situ and then oxidatively cyclized to the desired heterocycle. While this one-step procedure was successful for a majority of the required benzothiazoles, the phenyl derivative **26** and the 4-methyl-5-fluoro compound **27** could not be prepared by this method. For these examples, the thiourea was synthesized as an intermediate, which was subsequently cyclized to the 2-benzothiazolamine (Scheme 1, method B). Methods A and B failed for anilines containing an electron-withdrawing substituent in the meta position. For example, when 3-(trifluoromethyl)aniline was treated with potassium thiocyanate and bromine in glacial acetic acid, the expected intramolecularly cyclized benzothiazole product was not isolated. Instead, the dimerized thiadiazole shown in Scheme 2 was the only product identified. Therefore, the requisite 5-(trifluoromethyl)-2-benzothiazolamine (**20**) was synthesized by treatment of 2-nitro-4-(trifluoromethyl)benzamine with sodium nitrite and copper thiocyanate. The intermediate aryl thiocyanate **33** was then cyclized following the nitro group reduction by tin in concentrated hydrochloric acid (Scheme 3).

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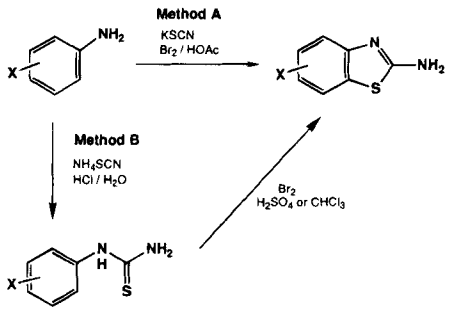
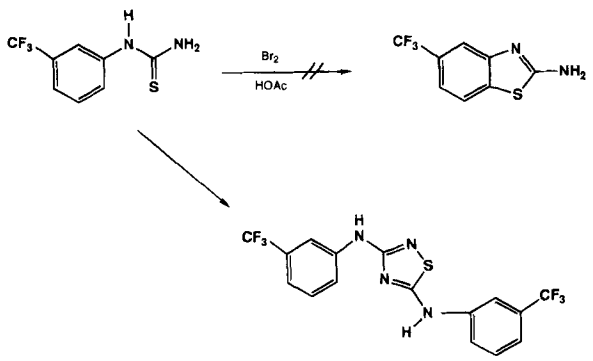
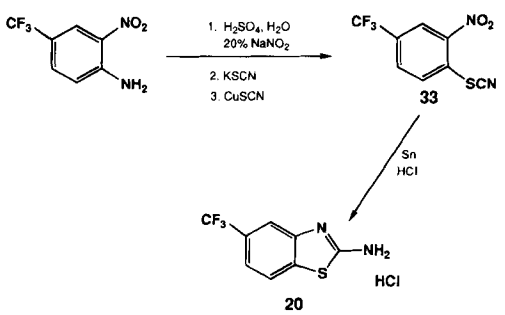


Scheme 1



Scheme 2



Scheme 3

The 6-benzothiazolol **16** was synthesized from the commercially available 6-methoxy derivative by treatment with refluxing hydrobromic acid. The diamino analog **17** was prepared by catalytic reduction of 6-nitrobenzothiazole. The physical properties of the 2-benzothiazolamines appear in Table 1.

Biological Testing—All aryl-substituted 2-benzothiazolamines including riluzole, a potent anticonvulsant agent, were tested in a sodium flux paridigm. This assay (NaFl) uses rat cortical slices and substitutes [^{14}C]guanidinium for sodium ions as previously described.[10] The NaFl results are shown in Table 2.

Twenty-five of the analogs were also evaluated for anticonvulsant activity and behavioral side effects, as described in the Experimental Section. The side effect profile for these compounds was of interest since it has been reported that a small group of substituted 2-benzothiazolamines possessed potent paralyzing effects following intravenous administration.[11] Of the compounds tested, 23 exhibited some protection against MES.

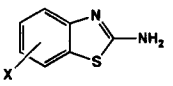
Compounds found to be active as sodium channel modulators (**1**, **2**, **3**, **5**, **8**, **12**, **14**, **24** and **26**) ($IC_{50} \leq 26$ μM) also exhibited anticonvulsant activity when administered intraperitoneally (ip) at doses of 10 or 30 mg/kg. There was no obvious relationship between potency as a sodium flux inhibitor and in vivo protection from seizures; however, because these compounds had relatively similar potencies in the NaFl assay (IC_{50} = 4.1–26 μM), one would expect their in vivo activities to be similar, if the data from the two assays were correlated. Since drugs were administered ip, metabolism may affect the in vivo activity and weaken the correlation with the in vitro data. Nonetheless, these results suggest that the anticonvulsant activity of these nine compounds is the result of modulation of sodium channels.[9]

Conversely, 14 of the compounds possessed some degree of anticonvulsant activity, but were inactive in the sodium flux assay. These compounds may still be interacting with the sodium channel, but this interaction may not be detected by the sodium flux paradigm. For example, if the drug bound exclusively to the inactivated state of the channel, or the drug interaction was highly voltage dependent, the flux of sodium ions may not be altered, even though the drug is interacting with sodium channels in a physiologically relevant manner. Alternatively, some of these compounds may be acting by an anticonvulsant mechanism independent of sodium channel modulation. Although an interaction of riluzole with known ligand recognition sites on either the AMPA/kainate or NMDA receptor could not be demonstrated, it has recently been reported that riluzole has a direct, but noncompetitive, action on ionotropic glutamate receptors.[12] Such an interaction with excitatory amino acid synapses may provide an alternative mechanism for anticonvulsant activity.

In general, anticonvulsant activity was seen at a dose lower than that producing ataxia. However, there was little separation between efficacious doses and those producing ataxia, since at the next highest dose most of the compounds produced ataxia and/or lethality. This indicates that the benzothiazolamines have a low therapeutic ratio (<3) and would probably not be very effective anticonvulsants in man. Of the 14 analogs that exhibited anticonvulsant activity but were inactive in the flux paradigm (NaFl assay), eight produced ataxia at the same dose at which anticonvulsant activity was observed. This is a significantly different pattern from the benzothiazolamines that were active in the NaFl assay, and supports the concept that at least these eight compounds (**9**, **18**, **22**, **23**, **27**, **28**, **29**, and **32**) may have other effects on the central nervous system that contribute to both their anticonvulsant activity and their ataxic liability. The remaining six compounds (**4**, **15**, **19**, **20**, **25**, and **31**) have a therapeutic ratio similar to that seen with the benzothiazolamines that were active sodium flux inhibitors. Therefore, it is difficult to conclude that the inhibition of sodium flux can predict anticonvulsant activity. Although in this series, all the compounds with IC_{50}s < 30 μM in the NaFl assay were effective in the MES assay, it cannot be concluded that the in vivo activity resulted solely from this action.

QSAR Analysis—To better understand the effect of benzo substitution on sodium flux inhibition, toward the design of potentially more potent congeners, the present 2-benzothiazolamines were analyzed via QSAR techniques. The compounds included, the biological activities, and the physicochemical parameters employed in the analysis are shown in Table 3. The $\log(1/IC_{50})$ in the NaFl screen was used as the dependent variable in the regressions; values ranged from 5.39 to 3.66 (IC_{50}s from 4.1 to 220 μM) with standard error of replicate determinations of 0.77. Inactive analogs were assigned an IC_{50} of 500 μM [$\log(1/IC_{50}) = 3.3$] so they could be included in the regressions. Physicochemical parameters considered included benzene π,[13] π^2, Hammett σ,[13] F (field), and R (resonance) components of σ,[14] and MR (molar refractivity; related to size); all were summed for the substituents on the benzo ring. MR was multiplied by 0.1 to place it on a scale similar to that of the other parameters. Reliable F and R values were unavailable for substituents on **6** and **26**; these analogs were dropped from regressions that employed these parameters. Examination of the structure–activity relation-

Table 1—Physical Properties of Substituted 2-Benzothiazolamines



No.	X	mp (°C)	% yield	Method or Source	Formula	Anal.	Recryst Solvent
1	6-OCF_3 (Riluzole)	215–216		A	$C_8H_5F_3N_2OS$·HCl	C,H,N,Cl	
2	H			*a*			
3	6-SMe			*a*			
4	6-Me			*a*			
5	6-iPr	123–124	49	A	$C_{10}H_{12}N_2S$	C,H,N,S	Toluene
6	6-COOH			*a*			
7	6-SO_2Me			*a*			
8	6-Cl			*a*			
9	6-OMe			*a*			
10	6-COPh	247–250	56	A	$C_{14}H_{10}N_2OS$	C,H,N	EtOH/DMF
11	6-SO_2NH_2			*a*			
12	6-Br			*a*			
13	6-NO_2			*a*			
14	6-CF_3	118–119	20	A	$C_8H_5F_3N_2S$	C,H,N,S	Toluene
15	6-F	180–181	53	A	$C_7H_5FN_2S$	C,H,N,H_2O	Diisopropyl ether
16	6-OH	255–256	69	C	$C_7H_6N_2OS$	C,H,N,S	Water
17	6-NH_2	202–203	51	D	$C_7H_7N_3S$	C,H,N,S	Toluene
18	5-OMe			*a*			
19	5-Me		6	A	$C_8H_8N_2S$	C,H,N,S	Diisopropyl ether
20	5-CF_3	255–257	28	E	$C_8H_5F_3N_2S$·HCl	C,H,N,Cl	Diisopropyl ether
21	5-COC_6H_5			*a*			
22	4-OMe			*a*			
23	4-Me			*a*			
24	4-Cl			*a*			
25	4-CF_3	154–155	31	B	$C_8H_5F_3N_2S$	C,H,N,S	Toluene/EtOH
26	4-Ph	134–136	18*	B	$C_{13}H_{10}N_2S$·HCl·0.55H_2O	C,H,N,Cl	MeOH/Water
27	4-Me, 5-F	174–175	35*	B	$C_8H_7FN_2S$	C,H,N	Hexane/Diisopropyl ether
28	4,5-benzofused			*a*			
29	4,6-F_2	233–234	9	A	$C_7H_4F_2N_2S$·HCl	C,H,N,Cl	EtOH/EtOAc
30	4,6-Cl_2	251–252	55	A	$C_7H_4Cl_2N_2S$·0.2H_2O	C,H,N	EtOH/EtOAc
31	4,6-Me_2			*a*			
32	5,6-Me_2			*a*			

[a] Commercially available or a gift.

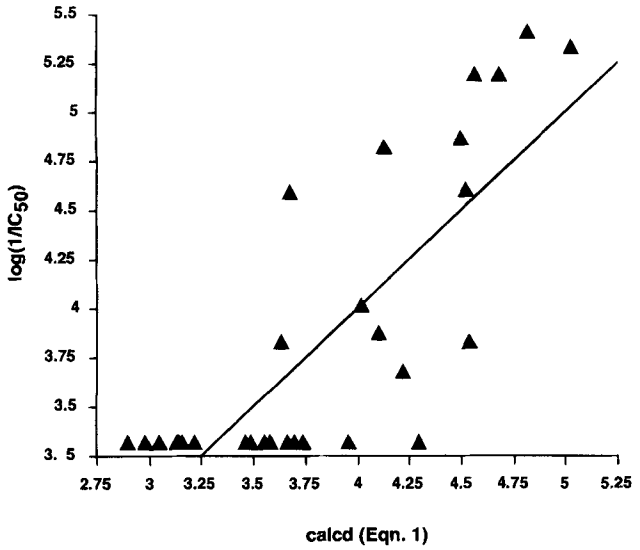


Figure 1—Observed versus calculated (eq 1) potency. Inactive compounds with assigned potencies of 500 μM [log(1/IC_{50}) = 3.3) appear at the bottom of the plot.

ships revealed that 4- and/or 5-substituted derivatives were uniformly less potent in the NaFl screen. Therefore, an indicator variable (Table 3, _45SUBST) was employed to denote those compounds with substitution at these positions.

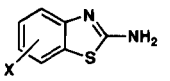
For the QSAR analysis, the 4- and 6-substituents were parameterized as "ortho" and "para" substituents to the thiazole nitrogen, the presumed reactive center in this molecule. This assumption was tested by rerunning the correlations considering the sulfur as the reactive center (the 4- and 6-substituents were parameterized as being "meta" substitutents). In all cases, the magnitudes of the correlations were reduced. A correlation matrix of the parameters employed in the regressions is shown in Table 4.

Using the resulting set of 27 2-aminobenzothiazoles, the following correlation was found:

$$\log(1/IC_{50}) = 0.57(\pm0.12)\pi - 0.90(\pm0.19)\_45SUBST$$
$$-0.45(\pm0.14)MR + 0.92(\pm0.38)R + 4.57 \quad (1)$$
$$n = 30 \quad R = 0.63 \quad F = 10.8 \quad s = 0.48$$

Potencies calculated using eq 1 appear in Table 3. Observed versus calculated potencies are shown in Figure 1. The results show a trend toward increased potency with increasing lipophilicity (π value), decreasing size (MR), increasing electron withdrawal in a resonance sense (R), and the absence of 4- or 5-substitution of the benzothiazolamine ring system. The potency/lipophilicity correlation is illustrated by Figure 2. By moving the open boxes upward 0.9 log units to make up for the loss in potency due to 4- or 5-substitution (the value of the coefficient on _45SUBST in eq 1), a trend toward increasing potency with increasing lipophilicity is apparent. This, however, may be a threshold effect. For example, a π value

Table 2—Sodium Flux (NaFl), Anticonvulsant (MES), and Ataxia (Ataxia) Results for Substituted 2-Benzothiazolamines



		NaFl			MES and Ataxia[b]			
						No. Protected:No. Ataxic		
No.	X	Concn (μM)	% Inhibition	$IC_{50} \pm SEM^a$ (μM)	Dose (mg/kg, ip)	0.5 h	2 h	4 h
Phenytoin				22.8	3	0:0	0:0	0:0
					10	1:0	2:0	2:0
					30	5:1	5:0	5:0
1	6-OCF_3			4.1 ± 0.35	3	0:0	0:0	0:0
(Riluzole)					10	5:0	0:0	0:0
					30	5:5	5:5	2[c]:4[c]
2	H			26 ($n = 1$)	10	1:0	0:0	0:0
					30	4:1	0:0	0:0
					100	5:5	5:2	1:0
3	6-SMe			16 ± 8.5	10	0:0	0:0	0:0
					30	2:0	0:0	0:0
					100	4[c]:3[d]	4[c]:0	5:0
4	6-Me			150	3	0:0	0:0	0:0
					10	0:0	0:0	0:0
					30	5:0	5:0	3:0
5	6-iPr			6.8 ± 0.7	10	3:0	0:0	0:0
					30	5:5	3:0[d]	0:0
					100	lethal	2[c]:2[c]	1[c]:0[d]
6	6-COOH			130	10			
					30		Inactive at all doses	
					100			
7	6-SO_2Me	10	4				Not tested	
		100	−70					
8	6-Cl			6.8 ± 6.6	3	0:0	0:0	0:0
					10	3:0	0:0	0:0
					30	5:5	5:0	0:0
9	6-OMe	10	3		10	0:0	0:0	0:0
		100	29		30	0:0	0:0	0:0
					100	5:5	5:5	4:2
10	6-COC_6H_5	10	−19				Not tested	
		100	39					
11	6-SO_2NH_2	10	10				Not tested	
		100	14					
12	6-Br			14 ± 11	3	0:0	0:0	0:0
					10	3:0	0:0	0:0
					30	5:0	2:0	0:0
13	6-NO_2			220	3			
					10		Inactive at all doses	
					30			
14	6-CF_3			4.9 ± 3.5	1	0:0	0:0	0:0
					3	0:0	0:4	0:0
					10	5:2	5:0	0:0
15	6-F	10	−23		3	0:0	0:0	0:0
		100	8		10	3:0	0:0	0:0
					30	5:5	5:0	0:0
16	6-OH	10	−46				Not tested	
		100	−12					
17	6-NH_2	10	−1				Not tested	
		100	13					
18	5-OMe	10	−11		10	0:0	0:0	0:0
		100	−1		30	0:0	0:0	0:0
					100	5:5	0:0	0:0
19	5-Me	10	23		3	0:0	0:0	0:0
		100	41		10	0:0	0:0	2:0
					30	4:5	4:0	2:0
20	5-CF_3			100	10	0:0	0:0	0:0
					30	2:0	2:0	0:0
					100	5:5	5:5	5:5
21	5-COC_6H_5	10	−2				Not tested	
		100	10					
22	4-OMe	10	−23		3	0:0	0:0	0:0
		100	11		10	0:0	0:0	0:0
					30	5:5	0:0	0:0

Table 2 (Continued)

No.	X	NaFl			MES and Ataxia[b]			
		Concn (μM)	% Inhibition	IC_{50} ± SEM[a] (μM)	Dose (mg/kg, ip)	No. Protected:No. Ataxic 0.5 h	2 h	4 h
23	4-Me			154	10	0:0	0:0	0:0
					30	5:2	0:0	0:0
					100		lethal	
24	4-Cl			26 ± 14 ($n = 3$)	3	0:0	0:0	0:0
					10	0:0	0:0	0:0
					30	2:0	0:0	0:0
25	4-CF_3			140	10	0:0	0:0	0:0
					30	2:0	0:0	0:0
					100	5:5	5:4	4:0
26	4-Ph			9.1 ± 3.9	3	0:0	0:0	0:0
					10	0:0	0:0	0:0
					30	0:2	2:0	0:0
27	4-Me, 5-F	10	−8		10	0:0	0:0	0:0
		100	33		30	5:5	0:0	0:0
					100	2[c]:2[c]	1[c]:1[c]	2[c]:2[c]
28	4,5-benzo fused	10	36		10	0:0	0:0	0:0
		100	58		30	0:0	0:0	0:0
					100	4:3	0:0	0:0
29	4,6-F_2	10	−21		3	0:0	0:0	0:0
		100	−2		10	0:0	0:0	0:0
					30	5:4	3:0	0:0
30	4,6-Cl_2	10	−16				Not tested	
		100	3					
31	4,6-Me_2	10	−1		10	0:0	2:0	2:0
		100	−7		30	0:3	2:0	3:0
					100	0:0	2:0	0:0
32	5,6-Me_2	10	3		10	0:0	0:0	0:0
		100	35		30	0:0	5:3	0:0
					100	5:5	5:5	5:5

[a] $n = 2$ unless otherwise indicated. [b] Five animals were tested in each group. Results are presented as the number protected:number ataxic. [c] Indicates that both the number of animals tested and the number protected (or ataxic) died. [d] Indicates number ataxic (number tested equal number protected).

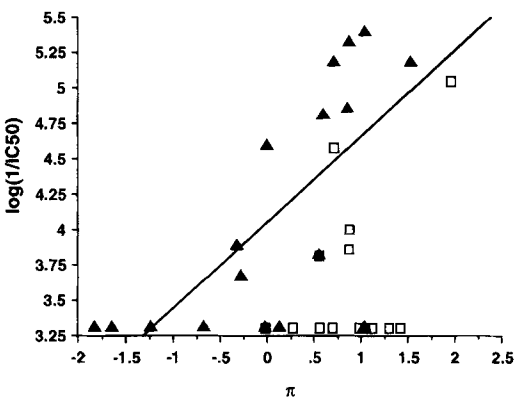


Figure 2—IC_{50} for sodium flux inhibition versus π of the benzo ring substituents. Compounds containing 4- or 5-substitution appear as open boxes.

of greater than 0.3 may be required for significant potency [$\log(1/IC_{50}) > 3.7$], and once this condition is met, π is no longer correlated with potency. It should be noted that the potency range for this compound set is only about 2 log units, while a log range of 4–5 log units is optimal for a QSAR study. This may explain why the correlation is lower and the standard error higher than expected.

Several specific compounds deserve comment. The inactive 6-fluoro analog (**15**) was poorly fit, having a calculated (eq 1) IC_{50} of 82 μM. It is not clear why this analog is inactive, while the 6-chloro (**8**) and 6-bromo (**12**) derivatives have IC_{50}s of 6.6 and 14.1 μM, respectively. Perhaps the correlation on π is stronger than eq 1 reveals, as eq 2 below suggests (compare the coefficients on the π term in eqs 1 and 2). The 4-chloro was also poorly fit, being significantly more active than predicted (Table 3, residual = 0.90). Summing physicochemical parameters for substituents on the benzo ring may be masking specific effects conferred by 4-substitution. However, there were too few compounds to adequately test the effect of parameterizing each position separately without introducing chance correlations.[15] Therefore additional syntheses is necessary to test this theory.

Since a substantial fraction (17/32) of the compounds were inactive (no measureable IC_{50}) and thus had their IC_{50} values arbitrarily set to 500 μM, it was of interest to rerun the regressions on only those analogs with measured IC_{50} values. Using this reduced set of 15 compounds (Table 3, IC_{50}s < 500 μM), the following correlation was found:

$$\log(1/IC_{50}) = 0.83(\pm 0.19)\pi - 0.77(\pm 0.23)\_45SUBST + 4.20 \quad (2)$$

$$n = 15 \quad r = 0.66 \quad F = 11.5 \quad s = 0.40$$

R and MR were no longer statistically significant, presumably because a number of analogs containing substituents that provided a spread in R and MR values were dropped from the analysis. For example, the 6-OCH_3 (**9**, Table 3), 6-OH (**16**), 6-NH_2 (**17**), and 4,6-F_2 (**29**) derivatives, all of which provided large (< -0.50) R values, were dropped. Similarly, inactive compounds containing relatively large groups, such as the 4,5-benzo-fused [naphthyl] (**28**), 6- and 5-COC_6H_5 (**10** and **21**), 6-SO_2CH_3 (**7**), and 6-SO_2NH_2 (**11**) derivatives, were not considered. These compounds undoubtedly contributed to the positive R and negative MR terms in eq 1. The coefficients of the π and _4SUBST terms in eq 2 are of the same sign as the comparable terms in eq 1, thus the conclusions for these properties remain unaltered. A plot of $\log(1/IC_{50})$ versus π

Table 3—Parameters Used in the QSAR Study

No.	X	-45SUBST	π	π^2	σ	F	R	MR[a]	IC_{50} (μM)[b]	log(1/IC_{50})	Calcd[c]	Residual
1	6-OCF_3	0	1.04	1.08	0.35	0.38	0.00	0.79	4.1	5.39	4.82	0.57
2	H	0	0.00	0.00	0.00	0.00	0.00	0.10	26	4.59	4.53	0.06
3	6-SCH_3	0	0.61	0.37	0.00	0.33	−0.19	1.38	16	4.80	4.13	0.67
4	6-CH_3	0	0.56	0.31	−0.17	−0.07	−0.11	0.56	150	3.82	4.54	−0.72
5	6-$CH(CH_3)_2$	0	1.53	2.34	−0.15	−0.07	−0.11	1.50	6.8	5.17	4.68	0.49
6	6-CO_2H	0	−0.32	0.10	0.45			0.69	130	3.88		
7	6-SO_2CH_3	0	−1.63	2.66	0.72	0.90	0.22	1.35	500	3.30	3.22	0.08
8	6-Cl	0	0.71	0.50	0.23	0.69	−0.16	0.60	6.8	5.17	4.57	0.60
9	6-OCH_3	0	−0.02	0.00	−0.27	0.41	−0.50	0.79	500	3.30	3.74	−0.44
10	6-COC_6H_5	0	1.05	1.10	0.43	0.30	0.16	3.03	500	3.30	3.96	−0.66
11	6-SO_2NH_2	0	−1.82	3.31	0.57	0.68	0.19	1.23	500	3.30	3.14	0.16
12	6-Br	0	0.86	0.74	0.23	0.73	−0.18	0.89	14	4.85	4.50	0.35
13	6-NO_2	0	−0.28	0.08	0.78	1.11	0.16	0.74	220	3.66	4.23	−0.57
14	6-CF_3	0	0.88	0.77	0.54	0.63	0.19	0.50	4.9	5.31	5.04	0.27
15	6-F	0	0.14	0.02	0.06	0.71	−0.34	0.09	500	3.30	4.30	−1.00
16	6-OH	0	−0.67	0.45	−0.37	0.49	−0.64	0.28	500	3.30	3.47	−0.17
17	6-NH_2	0	−1.23	1.51	−0.66	0.04	−0.68	0.54	500	3.30	2.99	0.31
18	5-OCH_3	1	−0.02	0.00	0.12	0.41	−0.17	0.79	500	3.30	3.14	0.16
19	5-CH_3	1	0.56	0.31	−0.07	−0.06	−0.04	0.56	500	3.30	3.70	−0.40
20	5-CF_3	1	0.88	0.77	0.43	0.62	0.07	0.50	100	4.00	4.02	−0.02
21	5-COC_6H_5	1	1.05	1.10	0.34	0.30	0.16	3.03	500	3.30	3.06	0.24
22	4-OCH_3	1	−0.02	0.00	−0.27	0.52	−0.43	0.79	500	3.30	2.90	0.40
23	4-CH_3	1	0.56	0.31	−0.17	−0.07	0.12	0.56	150	3.82	3.63	0.19
24	4-Cl	1	0.71	0.50	0.23	0.86	−0.14	0.60	26	4.58	3.68	0.90
25	4-CF_3	1	0.88	0.77	0.54	0.79	0.16	0.50	140	3.86	4.10	−0.24
26	4-C_6H_5	1	1.96	3.84	−0.01			2.54	9.1	5.04		
27	4-CH_3, 5-F	1	0.70	0.49	0.17	0.62	−0.24	0.66	500	3.30	3.55	−0.25
28	4,5-$(CHCH)_2$	1	1.30	1.69	0.00	0.20	−0.15	1.75	500	3.30	3.49	−0.19
29	4,6-F_2	1	0.28	0.08	0.12	1.59	−0.63	0.18	500	3.30	3.17	0.13
30	4,6-Cl_2	1	1.42	2.02	0.46	1.55	−0.30	1.21	500	3.30	3.67	−0.37
31	4,6-$(CH_3)_2$	1	1.12	1.25	−0.34	−0.14	−0.24	1.13	500	3.30	3.59	−0.29
32	5,6-$(CH_3)_2$	1	0.99	0.98	−0.30	−0.10	−0.19	1.13	4.9	3.30	3.56	−0.26

[a] Values are multiplied by 0.1 [b] Inactive compounds were assigned an IC_{50} of 500 μM so they could be included in the regression analyses. [c] Using eq 1.

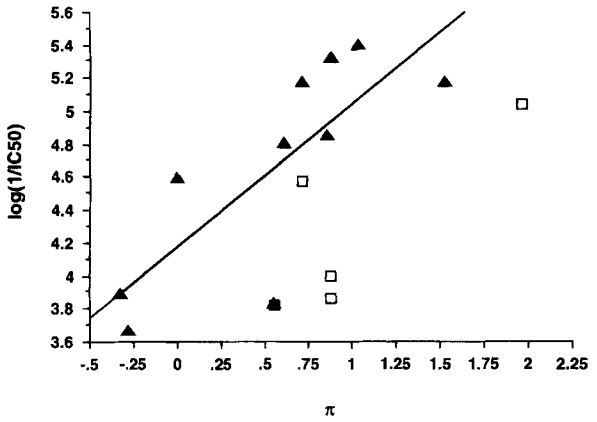


Figure 3—IC_{50} for sodium flux inhibition versus π of the benzo ring substituents. Only compounds with measured IC_{50} values are plotted. Compounds containing 4- or 5-substitution appear as open boxes.

(Figure 3) on this 15 compound subset reveals the stronger correlation with this parameter demonstrated by eq 2.

Conclusions

Thirty-two aryl-substituted 2-benzothiazolamines have been tested for their ability to modulate sodium flux in rat cortical slices. From the QSAR analysis, eq 1 is preferred, since it explains the variation in potency of a larger and more varied compound set. Because the parameters used to derive this equation are reasonably widely and independently varied, it appears that all four properties are required for an adequate explanation of the variation in potency in this series. The

Table 4—Correlation Matrix for Parameters Employed in the Regression Analyses

	log-(1/IC_{50})	-45SUBST	π	π^2	σ	F	R	MR
log(1/IC_{50})	1	−0.41	0.35	−0.05	0.18	−0.06	0.27	−0.16
-45SUBST		1	0.38	−0.13	−0.08	0.06	−0.07	0.04
π			1	−0.19	−0.01	−0.14	0.11	0.25
π^2				1	0.26	0.03	0.32	0.45
σ					1	0.62	0.75	0.21
F						1	−0.04	−0.16
R							1	0.39
MR								1

consequence is that substitution of the benzo ring seems to be restricted to small, neutral or electron-withdrawing, lipophilic 6-substituents. The combination of a requirement for increased lipophilicity and small size for increased potency sharply limits which substituents are tolerated on the benzo ring. Electron-withdrawing groups also tend to be polar [reduced lipophilicity], which further limits acceptable substituents. Given these circumstances, the QSAR predicted that, in general, large increases in potency over riluzole (**1**) would not be possible with further changes in benzo ring substitution.

The sodium flux assay suggested that nine of these compounds (**1**–**3**, **5**, **8**, **12**, **14**, **24**, and **26**) were potent modulators of voltage-dependent sodium channels. Fourteen additional compounds within this series demonstrated activity in MES, yet no activity in NaFl. Anticonvulsant activity was seen at a dose lower than that which produced ataxia, but the benzothiazolamines have a low therapeutic ratio and would probably not be useful anticonvulsant agents in man.

Experimental Section

Chemistry—Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were obtained on a Nicolet MX-1 FT spectrometer and ^1H NMR spectra were recorded on an IBM W-P100SY NMR spectrometer (100 MHz), a Varian XL200 NMR spectrometer (200 MHz), or a Varian XL 300 equipped with a 5-mm broadband switchable probe. IR and NMR spectra are not reported, but all spectra were consistent with the proposed structures. The mass spectra were obtained on a Finnigan 4500 mass spectrometer or a VG Analytical 7070E/HF mass spectrometer. Where analyses are indicated by the symbols of the elements, the results are within 0.4% of the theoretical values; values outside the limits are indicated. TLC was carried out with 0.25-mm silica gel F254 (E. Merck) glass plates. Some intermediate products were used directly without further purification or characterization.

*Method A. 6-(Trifluoromethyl)-2-benzothiazolamine (**14**)*—To a stirred solution of 4-(trifluoromethyl)aniline (12.6 g, 0.078 mol) in glacial acetic acid (150 mL) was added in one portion potassium thiocyanate (15.2 g, 0.156 mol). The reaction was vigorously stirred and a solution of bromine (4.0 mL, 0.078 mol) in glacial acetic acid (100 mL) was added dropwise over 30 min. The reaction was stirred vigorously for 24 h and poured into water (2000 mL). The aqueous mixture was neutralized over a 3-h period with concentrated ammonium hydroxide to pH 10. The reaction was stirred for 18 h and the precipitated product was collected by filtration. The product was recrystallized from toluene to produce a beige solid (3.21 g, 20% yield, mp 118–119 °C). Anal. ($C_8H_5F_3N_2S$) C, H, N.

Additional examples **5**, **10**, **14**, **15**, **19**, **30**, and **31** were prepared as described in method A.

*Method B. 4-(Trifluoromethyl)-2-benzothiazolamine (**25**)*—To a stirred solution of 2-(trifluoromethyl)benzeamine (20.0 g, 0.12 mol) in water (80 mL) was added ammonium thiocyanate (19.0 g, 0.25 mol). Concentrated hydrochloric acid (25 mL) was added in one portion and the reaction was heated to reflux, stirred vigorously for 4 h, and cooled to room temperature. The crude product was collected by filtration and washed with water. The product was recrystallized from toluene to yield the thiourea as a pale yellow solid (7.48 g, 27% yield). Anal. ($C_8H_7F_3N_2S$) C, H, N. To a stirred solution of the thiourea (7.4 g, 0.034 mol) in concentrated sulfuric acid (33 g) was added a catalytic amount of bromine (0.09 mL, 1.7 mmol). The reaction mixture was heated to 90 °C and stirred vigorously for 5 h. The reaction was cooled while constant stirring was maintained. The reaction was poured into water (10 volumes) and made basic by the dropwise addition of concentrated ammonium hydroxide. The suspension of crude material was stirred for 3 h, filtered, and washed with water to yield a solid (5.35 g). The product was chromatographed on a silica gel column eluted with hexane/ethyl acetate (7:3) and then recrystallized from toluene to produce the benzothiazole as a white fluffy solid (2.29 g, 31% yield, mp 154–155 °C). Anal. ($C_8H_5F_3N_2S$) C, H, N. S.

Additional examples, **26** and **27**, were prepared as described in method B. The intermediate thioureas for these compounds were characterized as follows:

*N-[1,1'-Biphenyl]-2-ylthiourea (Precursor for Compound **26**)*—The product was recrystallized from EtOH to afford a white solid in 40% yield, mp 182–183 °C. Anal. ($C_{13}H_{12}N_2S_1$) C, H, N.

*N-(3-Fluoro-2-methylphenyl)thiourea (Precursor for Compound **27**)*—The product was recrystallized from CH_2Cl_2/MeOH/hexane to afford a white solid in 34% yield, mp 165–166 °C. Anal. ($C_8H_9FN_2S$) C, H, N, S.

*Method C. 5-(Trifluoromethyl)-2-benzothiazolamine (**20**)*—To a stirred solution of 2-nitro-4-(trifluoromethyl)benzamine (20.6 g, 0.10 mol) in water/sulfuric acid (1:1, 60 mL) was added dropwise 20% sodium nitrite (37.5 mL). The reaction was maintained at between 0 and 5 °C for 90 min. Potassium thiocyanate (10 g. 0.103 mol in 20 mL water) was added dropwise and stirred for 15 min. The solution was poured into a vigorously stirred slurry of copper thiocyanate (18 g, 0.148 mol in 60 mL water). Gas evolution began and the reaction was stirred an additional 2 h at 3 °C and then heated to 70 °C for 20 min. The reaction was cooled and the solution was filtered. The aqueous filtrate was extracted with toluene (3 × 75 mL) and the toluene layer was dried (Na_2SO_4), filtered, and concentrated to produce the intermediate thiocyanate **33** as a purple oil. The crude material was placed on a silica gel column eluted initially with hexane followed by hexane/dichloromethane (7:3). The product **33** was isolated as a solid (8.9 g, 36%, mp 72–73 °C). Anal. ($C_8H_3N32O_2S$) C, H, N. The 2-nitrothiocyanate derivative **33** (4.0 g, 0.016 mol) was dissolved in concentrated hydrochloric acid (50 mL). Granulated tin (16 g, 0.13 mol) was added slowly over a 1-h period. The solution went from orange to white and the reaction exothermed and foamed during the addition. The reaction was stirred vigorously for 20 h and poured onto water. Concentrated ammonium hydroxide was added and the product and tin salts precipitated out. The solid was filtered and washed with hot chloroform (3 × 200 mL). The aqueous layer was extracted with chloroform. The chloroform washings were combined and then washed with concentrated ammonium hydroxide (200 mL). The chloroform layer was dried ($MgSO_4$) and concentrated to yield the product **20** as a dark brown solid. The crude solid (2.35 g) was converted to the hydrochloride salt by the addition of diethyl ether/hydrogen chloride to a diethyl ether solution of the crude benzothiazole. The salt was filtered and washed with additional diethyl ether to yield the salt **20** as a solid (1.16 g, 28.6% yield, mp 255–257 °C). Anal. ($C_8H_5F_3N_2S$·HCl) C, H, N, Cl.

*Method D. 2-Amino-6-benzothiazolol (**16**)*—6-Methoxy-2-benzothiazolamine (15.0 g, 0.083 mol) was dissolved in 48% hydrobromic acid (300 mL) and heated to reflux for 18 h. The reaction was cooled and the crude hydrobromide salt precipitated from the reaction solution. The salt was collected by filtration, redissolved in water, and neutralized with saturated sodium bicarbonate. The product precipitated as a white solid (9.5 g. 69% yield, mp 255–256 °C). Anal. ($C_7H_6N_2OS$) C, H, N, S.

*Method E. 2,6-Benzothiazoldiamine (**17**)*—6-Nitro-2-benzothiazolamine (10.0 g, 0.05 mol) was suspended in methanol (100 mL)/tetrahydrofuran (100 mL), and 10% Pd/C (1.5 g) and Raney nickel (3.0 g) were added. The mixture was hydrogenated at an initial pressure of 53 psi for 30 h. The mixture was filtered and the solvent was removed in vacuo. The crude brown solid was recrystallized from toluene to produce the diamine (4.29 g, 51% yield, mp 202–203 °C). Anal. ($C_7H_7N_3S$) C, H, N, S.

Biological Methods—*Uptake of [^{14}C]Guanidine into Rat Cortical Slices (NaFl Assay)*—Male Long-Evans rats (Blue Spruce Fams) weighing 200–300 g were sacrificed by decapitation, the brains were quickly removed, and the cortex was dissected out. Slices were cut (0.1 × 0.1 mm) on a McIlwain tissue chopper and dispersed in ice-chilled sodium-free Krebs–Ringer HEPES-buffered medium (pH 7.2). This medium was composed of the following: KCl (4.74 mM), $CaCl_2$ (1.25 mM), KH_2PO_4 (1.20 mM), $MgSO_4$ (1.18 mM), HEPES (22 mM), glucose (11 mM), and choline chloride (130 mM) to maintain osmolarity. Following three washes with the above medium, 10 mg of tissue was incubated in a 3-mL volume of medium at 25 °C for 5 min with 250 μM guanidine. Either 0.2 μCi [^{14}C]guanidine hydrochloride (Moravek Biochemicals, 48 mCi/mmol) in distilled water (for control conditions) or 0.2 μCi [^{14}C]guanidine hydrochloride plus 10 μM veratridine and 3.1 μg/mL α-scorpion venom (for stimulated conditions) was added and the reaction allowed to proceed for 3 min. Guanidinium ions substitute for sodium ions in this assay. At the end of the uptake period, samples were rapidly filtered through Whatman GF/F filters and washed twice with 5 mL of ice chilled 0.9% saline. The filters were placed in scintillation vials with 0.5 mL of 0.01 M KOH and allowed to stand at least 20 min before scintillation fluid was added (Beckman Ready-Solv HP). The samples were individually vortexed for 5 s and counted in a Beckman LS 2800 scintillation counter. Nonspecific activity was determined in a manner similar to other samples, but flasks were left on ice throughout the experiment. Results are expressed as IC_{50} values or percent inhibition. These values for compounds **1**–**32** are shown in the Table 2.

Anticonvulsant and Ataxia Screening in Mice—All experiments were conducted on male CF-1 mice (Charles River Laboratory, Portage Michigan). The mice weighed 20–35 g and were allowed free access to food and water prior to testing. The compounds were dissolved in various solutions of up to 50% organic solvents and 50% buffered solutions. All doses were injected intraperitoneally (ip) in a volume of 1.0 mL/100 g of body weight using a 25-gauge needle and a 1-mL syringe. Doses of the drugs were calculated as the free acid or base.

Thirty minutes and 2 and 4 h postdose, the mice were subjected to the inverted screen test, a measurement of ataxia. Ataxia was scored in this test if a mouse could neither cling to the bottom nor climb to the top of a 4-in. square of wire mesh within 60 s of its inversion in one trial. Ataxia was recorded as the number of mice that fell off the inverted screen out of five. The compound was considered active if two of the five mice fell off the inverted screen.

Maximal electroshock (MES) was administered via corneal electrodes with an electroshock apparatus (Wahlquist Instrument Co.) immediately following the inverted screen test. The stimulus consisted of a 50-mA current (100 mA peak-to-peak) of 0.2-s duration. The protocol for the MES assay involved groups of five mice per dose at each time period postdose. Typically, compounds were tested at three doses (Table 2). The mice were sacrificed with carbon dioxide immediately after MES. Anticonvulsant data were recorded as the number of mice protected from MES out of five. A result of two out of five was considered active.

Data Processing—Correlations, regressions, and plots were run on an IBM 3090 machine using the SAS program package.[16] In eqs 1 and 2, the figures in parentheses are the standard errors of the regression coefficients. For a given equation, n is the number of compounds, r is the correlation coefficient, F is a significance test, and s is the standard error.

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