Synthesis and Anticonvulsant Activity of Enaminones. 3. Investigations on 4′-, 3′-, and 2′-Substituted and Polysubstituted Anilino Compounds, Sodium Channel Binding Studies, and Toxicity Evaluations[1,2]

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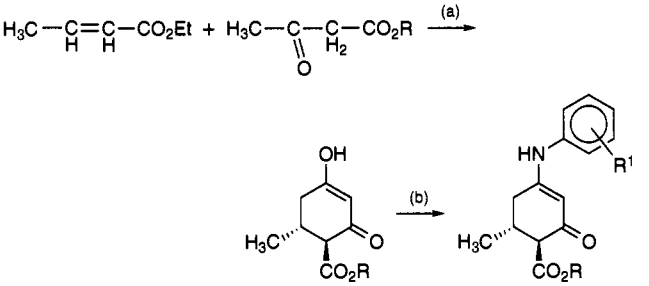
In a continuing evaluation of the aniline-substituted enaminones, the synthesis of additional para-substituted analogs has been made in an attempt to further quantify the electronic (σ) and lipophilic (π) requirements for anticonvulsant activity in this series. In addition, meta- and ortho-substituted and polysubstituted compounds have been synthesized and evaluated for anticonvulsant activity. In the para-substituted series, 4-cyano analogs (**32** and **33**) ($+\sigma$, $-\pi$), which were highly active via intraperitoneal (ip) injection in mice, were inactive on oral (po) administration in rats. The para-substituted trifluoromethoxy ($+\sigma$, $+\pi$) analog (**8**) had significant potency by both routes. Meta substitution limited the activity due to steric factors. Bromo and iodo substituents produced active para-substituted analogs (**5** and **17**) but were inactive when substituted in the meta position (**37** and **41**, respectively). Ortho substitution provided no clear relationship due to nonparametric deviations. Neither **1**, the prototype enaminone, nor **2**, the putative metabolite, produced significant nephrotoxicity or hepatotoxicity. Sodium channel binding of **1** and **8** indicated that **8** displayed relatively potent sodium channel binding but **1** showed weaker effects with IC_{50} values of 489 and 170 μM respectively against [^{3}H]batrachotoxinin A 20α-benzoate ([^{3}H]BTX-B).

We had previously reported[2] on a series of enaminone esters with potent oral anticonvulsant activity in the rat. In these reports, we employed the Craig plot,[3] molecular mechanics,[4] and CLOGP[5] in the analysis of para-substituted aniline derivatives, the most active amino moiety. This report continues from these initial investigations and includes an extensive evaluation of additional compounds. It includes the synthesis and anticonvulsant evaluation of additional para-, meta-, and ortho-substituted analogs, di- and trisubstituted derivatives, modification of the ester functionality, and *in vitro* sodium channel binding of two promising analogs.

It was previously reported that methyl 4-[(*p*-chlorophenyl)amino]-6-methyl-2-oxocyclohex-3-en-1-oate, (ADD 196022),[2b] **1** (Table 1), the most potent compound in the aniline series, was toxic in the intraperitoneal (ip) rat screen, with two of the eight animals dying at 24 h. We have reinvestigated **1** and its putative metabolite, 3-[(*p*-chlorophenyl)amino]-5-methylcyclohex-2-en-1-one, **2**, for nephrotoxicity and hepatotoxicity by repeating the 100 mg/kg ip toxicity study originally undertaken by the Anticonvulsant Drug Development (ADD) Program of NIH and in our laboratories.[2b] Additionally, the *in vivo* metabolic pathway of **1** was determined at 24 h after ip administration of 100 mg/kg. The additional analogs in this series have served to provide useful predictive quantitative structure–activity relationship (QSAR) information.

Scheme 1. Synthesis of the Enaminones[a]



[a] Conditions: (a) NaOEt, Δ; (b) substituted aniline, methods A–D (see the Experimental Section).

Results and Discussion

Chemistry. Cyclic enaminone esters of the aniline series (Table 1) as well as various miscellaneous heterocycles (Table 2) were synthesized from β-hydroxy keto esters, as previously reported.[2a,b,6] The synthetic pathway is shown in Scheme 1. The condensation reaction of ethyl crotonate with the respective acetoacetic ester was modified as reported by Friary and co-workers for the synthesis of the 4-carbo-*tert*-butoxy-5-methylcyclohexane-1,3-dione.[7] The subsequent condensation of the β-keto esters with the appropriate amino compound was varied to maximize overall yields. Wherever possible, a 1:1 ratio of absolute ethanol:ether (method A) or absolute ethanol:ethyl acetate (method B) was employed as solvent; however, in the case of the condensation of 2,4,6-trichloroaniline, forcing conditions,

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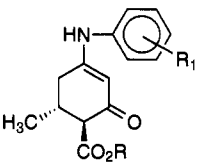
Table 1. Physical Properties of Cyclic Enaminones[a]



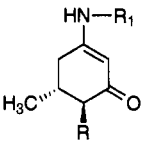
compd	R	R_1	method,[b] % yield	mp (°C), solvent	formula	anal.[c]
1	CO_2CH_3	4′-Cl	*d*	*d*	$C_{15}H_{16}NO_3Cl$	C,H,N,Cl
2	H	4′-Cl	*d*	*d*	$C_{13}H_{14}NOCl$	C,H,N,Cl
3	$CO_2C_2H_5$	4′-Cl	*d*	*d*	$C_{16}H_{18}NO_3Cl$	C,H,N,Cl
4	$CO_2C(CH_3)_3$	4′-Cl	B, 43	190–192[e]	$C_{18}H_{22}NO_3Cl$	C,H,N,Cl
5	CO_2CH_3	4′-Br	*d*	*d*	$C_{15}H_{16}NO_3Br$	C,H,N,Br
6	$CO_2C_2H_5$	4′-Br	*d*	*d*	$C_{16}H_{18}NO_3Br$	C,H,N,Br
7	$CO_2C(CH_3)_3$	4′-Br	B, 31	192–195[e]	$C_{18}H_{22}NO_3Br$	C,H,N,Br
8	CO_2CH_3	4′-OCF_3	B, 31	158–160[f]	$C_{16}H_{16}NO_4F_3$	C,H,N,F
9	$CO_2C_2H_5$	4′-OCF_3	B, 76	162–164[g]	$C_{17}H_{18}NO_4F_3$	C,H,N,F
10	$CO_2C(CH_3)_3$	4′-OCF_3	B, 47	168–171[e]	$C_{19}H_{22}NO_4F_3$	C,H,N,F
11	CO_2CH_3	4′-CF_3	*h*	*h*	$C_{16}H_{16}NO_3F_3$	C,H,N,F
12	$CO_2C_2H_5$	4′-CF_3	*h*	*h*	$C_{17}H_{18}NO_3F_3$	C,H,N,F
13	CO_2CH_3	4′-$C(CH_3)_3$	*h*	*h*	$C_{19}H_{25}NO_3$	C,H,N
14	$CO_2C_2H_5$	4′-$C(CH_3)_3$	*h*	*h*	$C_{20}H_{27}NO_3$	C,H,N
15	CO_2CH_3	4′-F	*h*	*h*	$C_{15}H_{16}NO_3F$	C,H,N,F
16	$CO_2C_2H_5$	4′-F	*h*	*h*	$C_{16}H_{18}NO_3F$	C,H,N,F
17	CO_2CH_3	4′-I	B, 78	193–194[i]	$C_{15}H_{16}NO_3I$	C,H,N,I
18	$CO_2C_2H_5$	4′-I	B, 80	160.5–162.5[j]	$C_{16}H_{18}NO_3I$	C,H,N,I
19	$CO_2C(CH_3)_3$	4′-I	B, 39	186–187[e]	$C_{19}H_{22}NO_3I$	C,H,N,I
20	CO_2CH_3	4′-CO_2H	*h*	*h*	$C_{16}H_{17}NO_5$	C,H,N
21	$CO_2C_2H_5$	4′-CO_2H	D, 71	230–231[k]	$C_{17}H_{19}NO_5$	C,H,N
22	CO_2CH_3	4′-OH	*h*	*h*	$C_{15}H_{17}NO_3$	C,H,N
23	$CO_2C_2H_5$	4′-OH	A, 73	160–162[e]	$C_{16}H_{19}NO_3$	C,H,N
24	CO_2CH_3	4′-$CONH_2$	*h*	*h*	$C_{16}H_{18}N_2O_4$	C,H,N
25	$CO_2C_2H_5$	4′-$CONH_2$	C, 87	228–229[k]	$C_{17}H_{20}N_2O_4$	C,H,N
26	CO_2CH_3	4′-NH_2	85	164–167	$C_{15}H_{18}N_2O_3$	C,H,N
27	$CO_2C_2H_5$	4′-NH_2	D, 69	187–190[k]	$C_{16}H_{20}N_2O_3$	C,H,N
28	CO_2CH_3	4′-SO_2NH_2	D, 50	210–214[e]	$C_{15}H_{18}N_2SO_5$	C,H,N
29	$CO_2C_2H_5$	4′-SO_2NH_2	D, 73	171–174[l]	$C_{16}H_{20}N_2SO_5$	C,H,N
30	CO_2CH_3	4′-$CO_2C_2H_5$	B, 52	180–183[e]	$C_{18}H_{21}NO_5$	C,H,N
31	$CO_2C_2H_5$	4′-$CO_2C_2H_5$	B, 72	195–197[e]	$C_{19}H_{23}NO_5$	C,H,N
32	CO_2CH_3	4′-CN	B, 92	210–213[e]	$C_{16}H_{16}N_2O_3$	C,H,N
33	$CO_2C_2H_5$	4′-CN	B, 65	190–193[k]	$C_{17}H_{18}N_2O_3$	C,H,N
34	CO_2CH_3	4′-OCH_3	A, 91	177–178[e]	$C_{17}H_{21}NO_4$	C,H,N
35	CO_2CH_3	3′-Cl	B, 49	137–138[g]	$C_{15}H_{16}NO_3Cl$	C,H,N,Cl
36	$CO_2C_2H_5$	3′-Cl	B, 72	137–138[g]	$C_{16}H_{18}NO_3Cl$	C,H,N,Cl
37	CO_2CH_3	3′-Br	B, 59	159–163[f]	$C_{15}H_{16}NO_3Br$	C,H,N,Br
38	$CO_2C_2H_5$	3′-Br	B, 86	135–137[g]	$C_{16}H_{18}NO_3Br$	C,H,N,Br
39	CO_2CH_3	3′-OCF_3	A, 44	173–176[f]	$C_{16}H_{16}NO_4F_3$	C,H,N,F
40	$CO_2C_2H_5$	3′-OCF_3	B, 80	151–152[g]	$C_{17}H_{18}NO_4F_3$	C,H,N,F
41	CO_2CH_3	3′-I	A, 52	163–166[f]	$C_{15}H_{16}NO_3I$	C,H,N,I
42	$CO_2C_2H_5$	3′-I	B, 83	120–124[l]	$C_{16}H_{18}NO_3I$	C,H,N,I
43	CO_2CH_3	3′-CO_2H	D, 55	164–168[e,f]	$C_{16}H_{17}NO_5$	C,H,N
44	$CO_2C_2H_5$	3′-CO_2H	D, 83	201–203[e,m]	$C_{17}H_{19}NO_5$	C,H,N
45	CO_2CH_3	3′-OH	B, 55	188–191[j]	$C_{15}H_{17}NO_4$	C,H,N
46	$CO_2C_2H_5$	3′-OH	B, 73	165–168[g]	$C_{16}H_{19}NO_4$	C,H,N
47	CO_2CH_3	3′-$CONH_2$	C, 11	200–203[e,f]	$C_{16}H_{18}N_2O_4$	C,H,N
48	CO_2CH_3	3′-CN	B, 22	65–68[n]	$C_{16}H_{16}N_2O_3$	C,H,N
49	$CO_2C_2H_5$	3′-CN	B, 75	165–167[g]	$C_{17}H_{18}N_2O_3$	C,H,N
50	CO_2CH_3	3′-OCH_3	B, 89	178–181[f]	$C_{16}H_{19}NO_4$	C,H,N
51	$CO_2C_2H_5$	3′-OCH_3	B, 67	111–113[l]	$C_{17}H_{21}NO_4$	C,H,N
52	CO_2CH_3	3′-NO_2	A, 63	166.5–168[f]	$C_{15}H_{16}N_2O_5$	C,H,N
53	$CO_2C_2H_5$	3′-NO_2	A, 58	155–157[i]	$C_{16}H_{18}N_2O_5$	C,H,N
54	CO_2CH_3	3′-CH_3	A, 34	165–166[f]	$C_{16}H_{19}NO_3$	C,H,N
55	$CO_2C_2H_5$	3′-CH_3	A, 59	125–127[l]	$C_{17}H_{21}NO_3$	C,H,N
56	CO_2CH_3	3′-CF_3	B, 9	167–169[f]	$C_{16}H_{16}NO_3F_3$	C,H,N,F
57	$CO_2C_2H_5$	3′-CF_3	B, 75	164–166[g]	$C_{17}H_{18}NO_3F_3$	C,H,N,F
58	CO_2CH_3	3′-C_2H_5	D, 10	146–149[f]	$C_{17}H_{21}NO_3$	C,H,N
59	$CO_2C_2H_5$	3′-C_2H_5	D, 44	116–118[l]	$C_{18}H_{23}NO_3$	C,H,N
60	CO_2CH_3	3′-F	B, 31	151–155[f]	$C_{15}H_{16}NO_3F$	C,H,N,F
61	$CO_2C_2H_5$	3′-F	B, 52	138–140[l]	$C_{16}H_{18}NO_3F$	C,H,N,F
62	CO_2CH_3	2′-Cl	B, 55	157–159[o]	$C_{15}H_{16}NO_3Cl$	C,H,N,Cl
63	$CO_2C_2H_5$	2′-Cl	B, 72	152–154[g]	$C_{16}H_{18}NO_3Cl$	C,H,N,Cl
64	$CO_2C_2H_5$	2′-NH_2	D, 49	151–153[g]	$C_{16}H_{20}N_2O_3$	C,H,N
65	CO_2CH_3	2′-Br	B, 33	157–160[e]	$C_{15}H_{16}NO_3Br$	C,H,N,Br
66	CO_2CH_3	2′-I	B, 7	148–150[e]	$C_{15}H_{16}NO_3I$	C,H,N,I
67	CO_2CH_3	2′-OH	D, 21	164–166[e]	$C_{15}H_{17}NO_4$	C,H,N
68	CO_2CH_3	2′-$CONH_2$	D, 17	165–167[e]	$C_{16}H_{18}N_2O_4$	C,H,N
69	CO_2CH_3	2′-SO_2NH_2	D, 32	197–199[e]	$C_{15}H_{18}N_2SO_5$	C,H,N

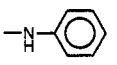
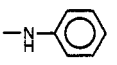
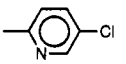
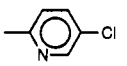
Table 1. (Continued)

compd	R	R_1	method,[b] % yield	mp (°C), solvent	formula	anal.[c]
70	CO_2CH_3	2′-CN	B, 14	155–157[e]	$C_{15}H_{16}N_2O_3$	C,H,N
71	CO_2CH_3	2′-OCH_3	B, 46	159–151[e]	$C_{16}H_{17}NO_4$	C,H,N
72	CO_2CH_3	2′-NO_2	B, 60	154–157[e]	$C_{15}H_{18}N_2O_5$	C,H,N
73	CO_2CH_3	2′-CH_3	B, 6	138–140[e]	$C_{16}H_{17}NO_3$	C,H,N
74	CO_2CH_3	2′-CF_3	B, 29	166–169[e]	$C_{15}H_{16}NO_3F_3$	C,H,N,F
75	CO_2CH_3	2′-C_2H_5	A, 37	194–196[e]	$C_{15}H_{21}NO_3$	C,H,N
76	CO_2CH_3	2′-F	B, 69	132–134[e]	$C_{15}H_{16}NO_3F$	C,H,N,F
77	CO_2CH_3	2′-OCH_3, 5′-CH_3	*h*	*h*	$C_{17}H_{21}NO_4$	
78	$CO_2C_2H_5$	2′-OCH_3, 5′-CH_3	D, 74	110–113[l]	$C_{18}H_{23}NO_4$	C,H,N
79	CO_2CH_3	3′-Cl, 4′-OCH_3	B, 55	175–177[f]	$C_{16}H_{18}NO_4Cl$	C,H,N,Cl
80	$CO_2C_2H_5$	3′-Cl, 4′-OCH_3	D, 66	133–135[l]	$C_{17}H_{20}NO_4Cl$	C,H,N,Cl
81	CO_2CH_3	2′-Cl, 5′-OCH_3	D, 12	138–140[e]	$C_{16}H_{18}NO_4Cl$	C,H,N,Cl
82	CO_2CH_3	2′,4′-Cl_2	D, 72	153–155[g]	$C_{15}H_{15}NO_3Cl_2$	C,H,N,Cl
83	$CO_2C_2H_5$	2′,4′-Cl_2	D, 80	153–155[l]	$C_{16}H_{17}NO_3Cl_2$	C,H,N,Cl
84	CO_2CH_3	2′,5′-Cl_2	D, 43	190–192[n]	$C_{15}H_{15}NO_3Cl_2$	C,H,N,Cl
85	$CO_2C_2H_5$	2′,5′-Cl_2	D, 47	160–162[g]	$C_{16}H_{17}NO_3Cl_2$	C,H,N,Cl
86	CO_2CH_3	3′,4′-Cl_2	D, 44	160–161[j]	$C_{15}H_{15}NO_3Cl_2$	C,H,N,Cl
87	$CO_2C_2H_5$	3′,4′-Cl_2	D, 53	171–173[m]	$C_{16}H_{17}NO_3Cl_2$	C,H,N,Cl
88	CO_2CH_3	2′,6′-Cl_2	D, 19	205–210[l]	$C_{15}H_{15}NO_3Cl_2$	C,H,N,Cl
89	$CO_2C_2H_5$	2′,6′-Cl_2	D, 18	187–190[p]	$C_{16}H_{17}NO_3Cl_2$	C,H,N,Cl
90	CO_2CH_3	3′,5′-Cl_2	D, 25	212–215[j]	$C_{15}H_{15}NO_3Cl_2$	C,H,N,Cl
91	$CO_2C_2H_5$	3′,5′-Cl_2	D, 54	191–193[g]	$C_{16}H_{17}NO_3Cl_2$	C,H,N,Cl
92	CO_2CH_3	2′,3′,4′-Cl_3	D, 65	205–207[q]	$C_{15}H_{14}NO_3Cl_3$	C,H,N,Cl
93	$CO_2C_2H_5$	2′,3′,4′-Cl_3	D, 82	165–167[g]	$C_{16}H_{16}NO_3Cl_3$	C,H,N,Cl
94	CO_2CH_3	2′,4′,5′-Cl_3	D, 33	200–201[g]	$C_{15}H_{14}NO_3Cl_3$	C,H,N,Cl
95	$CO_2C_2H_5$	2′,4′,5′-Cl_3	D, 83	183–184[k]	$C_{16}H_{16}NO_3Cl_3$	C,H,N,Cl
96	CO_2CH_3	3′,4′,5′-Cl_3	D, 57	181–183[g]	$C_{15}H_{14}NO_3Cl_3$	C,H,N,Cl

[a] The infrared and ^1H NMR spectra were consistent with assigned structures. Recrystallization solvents as indicated. [b] Method A, EtOH:Et_2O (1:1); method B, EtOH:EtOAc (1:1); method C, toluene; method D, C_6H_6:EtOH (1:1). [c] All compounds gave satisfactory C, H, N, and halogen (when required) analyses (±0.4%). [d] Reference 2b. [e] 2-PrOH. [f] MeOH. [g] EtOAc. [h] Reference 2a. [i] EtOAc:Me_2CO:MeOH. [j] EtOAc:Me_2CO. [k] EtOH. [l] EtOAc:petroleum ether (bp 45–54 °C). [m] EtOH:EtOAc:petroleum ether (bp 45–54 °C). [n] Et_2O. [o] Toluene. [p] EtOH:EtOAc. [q] MeOH:2-PrOH.

Table 2. Physical Properties of Miscellaneous Cyclic Enaminones[a]



compd	R	R_1	method,[b] % yield	mp (°C), solvent	formula	anal.[c]
98	CO_2CH_3		*d*	*d*	$C_{15}H_{18}N_2O_3$	C,H,N
99	$CO_2C_2H_5$		A, 76	148–150[e]	$C_{16}H_{20}N_2O_3$	C,H,N
100	CO_2CH_3		*f*	*f*	$C_{14}H_{15}N_2O_3Cl$	C,H,N,Cl
101	$CO_2C_2H_5$		D, 22	174–178[g]	$C_{15}H_{17}N_2O_3Cl$	C,H,N,Cl
102	CO_2CH_3		*d*	*d*	$C_{15}H_{23}NO_3$	C,H,N
103	$CO_2C_2H_5$		D, 21	141–144[e]	$C_{16}H_{25}NO_3$	C,H,N

[a] The infrared and ^1H NMR spectra were consistent with assigned structures. Recrystallization solvents as indicated. [b] See footnote *b* of Table 1. [c] All compounds gave satisfactory C, H, N, and halogen (when required) analyses (±0.4%). [d] Reference 2a. [e] EtOAc. [f] Reference 2b. [g] EtOH:EtOAc:petroleum ether (bp 45–54 °C).

e.g. refluxing toluene for 24 h, were tried but only the starting material was recovered. This latter procedure (method C) was employed also with the *p*- (**24**, **25**) and *m*-carboxamido (**47**) analogs with good results. A summary of these synthetic procedures is shown in Tables 1 and 2.

We have previously reported that the synthesis of para-substituted diastereomeric enaminone esters yielded a single enantiomer,[2a,b] in accord with the earlier work of Friary;[7] however, in the present work, enantiomeric purity was more difficult to determine with the meta-, and most particularly, with the ortho-substituted compounds (see the Experimental Section). Of interest was the greater reactivity of the carbethoxy compounds when compared with the carbomethoxy analogs. Yields of the former analogs were consistently higher than the comparable carbomethoxy derivatives. High-field NMR analyses on the reported compounds were consistent with the assigned structures. High-field NMR characteristics of the enaminones were used as an assessment of enantiomeric purity and were correlated to anticonvulsant activity.[8]

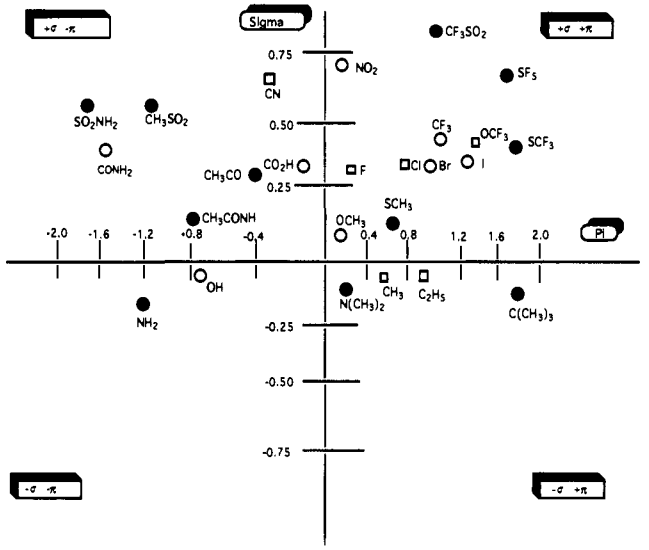


Figure 1. Craig plot of para-substituted analogs from 4-carbomethoxy-5-methylcyclohexane-1,3-dione. Legend: (□) class 1 active; (■) class 2 active; (○) inactive; (●) not synthesized.

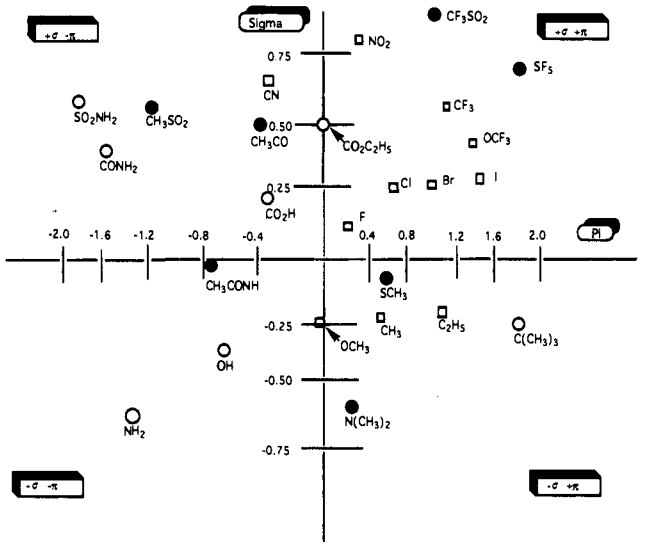


Figure 2. Craig plot of meta-substituted analogs from 4-carbomethoxy-5-methylcyclohexane-1,3-dione. Legend: (□) class 1 active; (■) class 2 active; (○) inactive; (●) not synthesized.

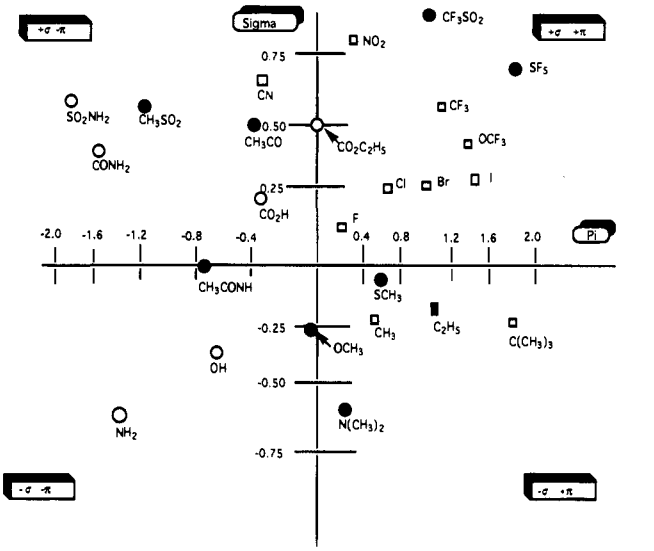


Figure 3. Craig plot of para-substituted analogs from 4-carbethoxy-5-methylcyclohexane-1,3-dione. Legend: (□) class 1 active; (■) class 2 active; (○) inactive; (●) not synthesized.

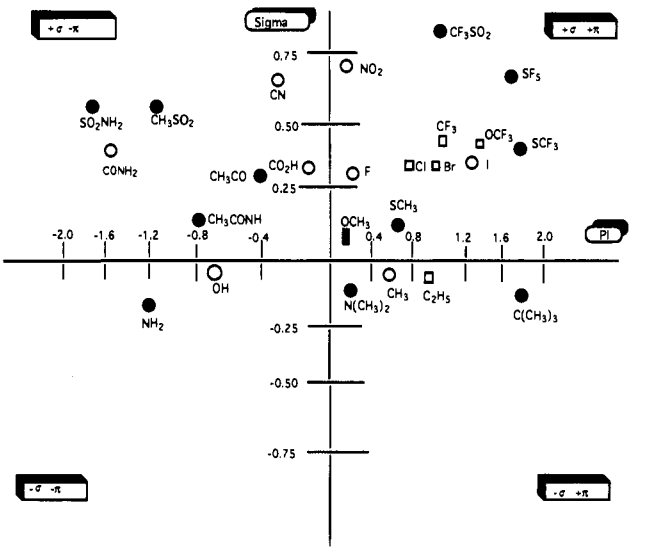


Figure 4. Craig plot of meta-substituted analogs from 4-carbethoxy-5-methylcyclohexane-1,3-dione. Legend: (□) class 1 active; (■) class 2 active; (○) inactive; (●) not synthesized.

While we had previously reported that the reaction of dimedone and 1,4-phenylenediamine provided the desired *p*-amino analog,[2b,9] we herein report mixed results with this direct synthetic procedure for the synthesis of amino-substituted analogs. Whereas the carbethoxy analog **27** reacted with 1,4-phenylenediamine to produce the desired product, the carbomethoxy analog **26** produced a compound that, while analytically correct, increased in melting point on subsequent recrystallizations and exhibited multiple spots with TLC analysis. Thus, we employed the catalytic reduction of the appropriate nitro compound according to the method of Clark and co-workers[10,11] to produce the para (**26**) analog. The syntheses of the carbomethoxy *p-O-* and *-N-*acetyl derivatives were attempted by refluxing the corresponding phenol or amino compound with acetic anhydride and, after suitable workup, provided liquids which resisted further purification.

Pharmacology. Preliminary pharmacological testing of the compounds listed in Tables 1 and 2 has been provided by the Antiepileptic Drug Development (ADD) Program, Epilepsy Branch, Neurological Disorders Program, National Institute of Neurological Disorders and Stroke (NINDS). These testing procedures have been described.[12] Phase I studies of the enaminones involved three tests: maximal electroshock seizure (MES), subcutaneous pentylenetetrazol (scMet), and neurologic toxicity (Tox) in mice. Intraperitoneal administration of the test compounds was as a suspension in 0.5% methylcellulose. This data is shown in Figures 1 and 2 for the 1-carbomethoxy compound, Figures 3 and 4 for the 1-carbethoxy compound, and Figure 5 for the 1-carbo-*tert*-butoxy compounds. As previously reported,[2b] to differentiate the results between different rodent species, the class 1 and 2 MES-active analogs were subsequently evaluated for oral (po) activity (phase VIA) in the rat.

Structure–Activity Correlations. Our initial study of the enaminone series as potential anticonvulsants[2a] utilized the Craig plot[3] to develop the proposed nuclear substitution into rational physicochemical parameters of lipophilicity (π) and electron-withdrawing (σ) char-

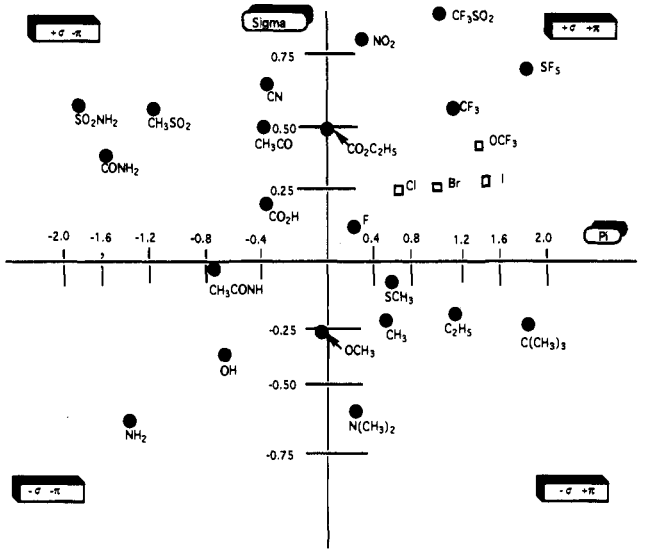


Figure 5. Craig plot of para-substituted analogs from 4-carbo-*tert*-butoxy-5-methylcyclohexane-1,3-dione. Legend: (□) class 1 active; (■) class 2 active; (○) inactive; (●) not synthesized.

acter. These substituents and the respective positions in the Craig plot are shown in Figures 1–5.

(A) Carbomethoxy Series. The synthesis of the reported meta-substituted anilines proved a more selective method of characterizing the anticonvulsant receptor.

(1) $+\sigma$, $+\pi$ Quadrant. As noted in Figures 1 and 2, the halides provided a clearer size restriction as the *p*-bromo (**5**)[2b] and *p*-iodo (**17**) analogs were active, but the meta analogs (**37** and **41**, respectively) were inactive. Chloro- (para, **1**[2a,b] meta, **35**) and fluoro-substituted compounds (para **15**,[2a] meta, **60**) were active. This isomeric change in position was also noted with the highly active *p*-nitro[2b] compared to the inactive *m*-nitro, **52**, and the active *p*-trifluoromethyl (**11**)[2b] compared to the inactive *m*-trifluoromethyl **56**. An anomaly, however, existed with the trifluoromethoxy analogs which retained activity as either the para (**8**) or meta (**39**) isomer.

(2) $+\sigma$, $-\pi$ Quadrant. In addition to the previously reported inactive acid (**20**)[2a] and carboxamide (**24**),[2a] the highly active 4′-cyano (**32**) and the inactive sulfonamido (**28**), and carbethoxy (**30**) compounds are included. Thus, of the six synthesized analogs in this quadrant, only one was active. While the results from evaluation of the cyano compound **32** in the mouse were encouraging, the rat evaluation results were disappointing. The inactivity in the po rat evaluation was most probably due to its insolubility in aqueous media. Upon comparison of the 4′-analogs with the comparable 3′-derivatives, the anticonvulsant results were similar, with activity residing only with the meta-substituted cyano compound (**48**), and inactivity was noted with the 3′-carboxamido (**47**), and the 3′-carboxylic acid (**43**) analogs.

(3) $-\sigma$, $-\pi$ Quadrant. In addition to the inactive 4′-hydroxy analog (**22**), previously reported,[2a] the 4′-amino (**26**) compound was also inactive. As noted in the previous quadrant, the comparable meta-substituted 3′-hydroxy (**45**) analog was inactive. Of further interest was the high anticonvulsant activity shown with the 4′-methoxy analog (**34**). However, the isomeric 3′-analog (**50**) was inactive. A possible explanation of this phenomenon might be the change in electronic character of this group when its position on the aromatic ring is changed. In the para position, the methoxy group is electron-donating (σ_p −0.268)[3a] while in the meta position it becomes electron-withdrawing (σ_m 0.115).[3a] This explanation is inconsistent with our original hypothesis that anticonvulsant activity was due to the electron-withdrawing ability of the substituent.

(4) $-\sigma$, $+\pi$ Quadrant. Both methyl (para,[2a] meta, **54**) and ethyl (para,[2a] meta, **58**) analogs retained activity in either position; thus in this quadrant, size plays a less critical role in determining activity than in the $+\sigma$, $+\pi$ quadrant. This further strengthens the hypothesis[2a,b] that lipophilicity is the predominant parameter in evaluating anticonvulsant activity in this series of compounds. Further, any anomaly in results may be due to unique structural differences explainable by differences in the theoretical CLOGP[5] algorithm and actual experimental log *P* evaluations. These evaluations are currently underway.

(B) Carbethoxy Series. With few exceptions, this series paralleled the carbomethoxy series.

(1) $+\sigma$, $+\pi$ Quadrant. When the para-substituted carbethoxy analogs in Figure 3 are compared to the carbomethoxy compounds in Figure 1, no differences in activity were observed in this quadrant. However, when the meta-substituted carbethoxy compounds in Figure 4 are compared to the carbomethoxy analogs in Figure 2, the following differences were noted: (a) enhanced activity was observed for the carbethoxy moiety when the meta-substitution was methoxy (**51**), bromo (**38**), or trifluoromethyl (**57**) and (b) inactivity was observed for the carbethoxy moiety when the meta substitution was fluoro (**61**). This latter finding was in contrast to the potent carbomethoxy *m*-fluoro analog (**60**) and indicates that in the carbethoxy series, the size restriction of the halides is a lesser constraint than the enhanced lipophilicity of the carbethoxy group. The activity of the *m*-methoxy analog **51** was in conformity with Kase's original report on the anticonvulsant dimedone, 5,5-dimethyl-3-[(3′-methoxyphenyl)amino]-2-[(*N*-methyl-*N*-phenethylamino)methyl]cyclohex-2-en-1-one[13] and differed from that of the carbomethoxy analog **50**.

(2) $+\sigma$, $-\pi$ Quadrant. When Figures 1 and 3 are compared, identical results were noted for the highly active 4′-cyano (**33**), the inactive 4′-acid (**21**), 4′-carboxamide (**25**), 4′-sulfonamide (**29**), and 4′-carbethoxy (**31**) substitutions. The cyano derivative, **33**, was also active in the pentylenetetrazole test as well and is currently undergoing additional evaluations. When Figures 2 and 4 are compared, the only significant difference to be noted is the inactivity of the 3′-cyano compound **49** when compared to the highly active carbomethoxy 3′-cyano compound **48**. Of additional interest was the fact that the carbethoxy 4′-cyano analog, **33**, was active against both MES as well as scMet evaluations.

(3) $-\sigma$, $-\pi$ Quadrant. Figures 1 and 3 represent parallel results in evaluating the inactive 4′-hydroxy (**23**) and 4′-amino (**27**) analogs, which are in confluence with carbomethoxy derivatives **22**[2b] and **26**, respectively. In comparing Figures 2 and 4, one notes a similar trend, with both substituents remaining inactive.

(4) $-\sigma$, $+\pi$ Quadrant. Figures 1 and 3 reveal a difference in activity as the 4′-*tert*-butyl derivative **14**[2b] was highly active, while the comparable carbomethoxy

analog **13**[2b] was inactive. Activity was comparable with the active 4′-methyl[2b] and 4′-ethyl[2b] compounds.

During the pharmacological evaluation of the reported compounds, the ADD Program initiated a new screening procedure, the threshold tonic extension (TTE) test.[14] This test is a clinically nonselective, electroconvulsive seizure model that identifies compounds that raise seizure threshold as well as those that prevent seizure spread. In addition, this test can identify certain compounds that are inactive by both the MES and the scMet tests. The test is similar to the MES screen but uses a lower level of electrical current. The lower current makes the TTE test more sensitive, but less discriminate than the MES screen. This ability makes the model attractive because it allows for the identification of compounds that may have been omitted by the standard identification screen. If a compound was found to possess significant activity in the TTE test while remaining inactive in the MES rescreen, it becomes a candidate for more advanced testing. These TTE active compounds may represent compounds acting by novel mechanisms and will be evaluated further. Our compounds were first selected for their evaluation. The MES-inactive *m*-iodo (**42**) and the *m*-hydroxy (**46**) compounds were found to be active at 100 mg/kg up to 4 h postdose. These compounds are undergoing further evaluation.

(C) Carbo-*tert*-butoxy Series. An additional series of carbo-*tert*-butoxy analogs (**4**, **7**, **10**, and **19**, Table 1) were synthesized to evaluate the effect of added bulk and lipophilicity to the ester functionality. As noted from our previous work, these compounds were from the highly active $+\sigma$, $+\pi$ quadrant. All compounds showed significant anticonvulsive activity. Comparative ED_{50} and TD_{50} values for these compounds are currently being determined. Thus, the initial conclusion[2b] that in the para substituted series the highly active analogs reside principally in the $+\sigma$, $+\pi$ quadrant has been conclusively shown with the synthesis and evaluation of representative analogs from each quadrant as well as by varying the ester functionality.

(D) Ortho-Substituted Analogs. The ortho-substituted analogs (**62**–**76**, Table 5) were synthesized for comparative purposes. Craig plot analysis does not include ortho-substituted analogs due to unpredictable variations in lipohilicity.[15] As previously noted by Taft,[16] σ values of the ortho substituents were fairly near those for the corresponding para substituents; thus it was of interest to evaluate these compounds on the basis of the single physicochemical parameter, σ. In the carbomethoxy series, the chloro (**62**, $\sigma = +0.20$),[16] iodo (**66**, $\sigma = +0.21$),[16] and ethyl (**75**) were highly active (class 1), while the cyano (**70**), nitro (**72**, $\sigma = +1.22$),[16] trifluoromethyl (**74**), and fluoro (**76**, $\sigma = +0.24$),[17a,b] were moderately active (class 2). The comparison of these active analogs parallels that of the para analogs. Further, the inactive bromo (**65**, $\sigma = +0.20$),[17c] not unlike the active chloro and iodo cited above, the hydroxy (**67**), sulfonamido (**69**), and methoxy (**71**, $\sigma = +0.39$)[17c] analogs parallel the meta derivatives. Only the inactive *o*-tolyl (**73**, $\sigma = -0.17$)[16] varied from both the highly active para[2a] and meta[2b,d] derivatives. The activity of this latter compound could not be explained solely on the basis of steric interactions as the ethyl analog (**75**) should also have been inactive as well. Clearly, quantitative structure–activity analysis of the ortho substituents in this series is highly complicated and would include polar factors as well as steric interactions as was reported earlier.[16,17] Thus, a combination of steric and electronic effects, lipophilicity, and hydrogen bonding appear to be necessary for the anticonvulsant activity of the enaminones.

(E) Multisubstitution. The three carbomethoxy monochloro analogs (para, **1**, meta, **35**, ortho, **62**) were all highly active anti-MES compounds; thus in this series, positional changes of the chlorine atom did not selectively discriminate between these analogs. As stated previously, the carbethoxy series also produced highly active *p*-chloro (**3**)[2b] and *m*-chloro compounds (**36**); however, the *o*-chloro compound (**63**) was inactive. Thus it was of interest to evaluate the effect of multichloro substitution on anticonvulsant activity. The disubstituted chloro analogs provided further insight into the structural requirements for activity. As noted in Table 5, the 2′,4′-dichloro (**82** and **83**) and 3′,4′-dichloro (**86** and **87**) analogs were highly active, while the isomeric 2′,6′-dichloro (**88** and **89**) and the 3′,5′-dichloro (**90** and **91**) analogs were inactive. Anomalous results were obtained with the 2′,5′-dichloro analogs; the carbomethoxy compound (**84**) was highly active, while the homologous carbethoxy compound (**85**) was inactive. An explanation for the consistent data may reside in the fact that a steric requirement may be active.[8] The proof will lie with the 2′,3′-dichloro analog. Its activity would indicate that the anticonvulsant receptor could accommodate disubstituted derivatives as long as they were on the same plane of the benzene ring. Its inactivity would indicate that the strong electron-withdrawing character of the 2′,3′-dichloro, 2′,5′-dichloro, 2′,6′-dichloro, and 3′,5′-dichloro analogs deactivate the para position where the interaction with the receptor is most favorable. The attempted synthesis of the 2′,3′-dichloro analog in either the carbomethoxy or carbethoxy series has led to the formation of intractable oils. Work on these aniline enaminones are continuing.

(F) Miscellaneous Cyclic Enaminones. These compounds were synthesized in order to determine the effect of altering the ester group on previously reported compounds. The physical properties are found in Table 2 and the comparative anticonvulsant results are shown in Table 6. Data on the hydrazino compounds (**98** and **99**) indicated that activity is lost with the carbethoxy moiety, while the reverse is true with the cyclohexyl compounds (**102** and **103**). Activity is retained with the potent 4′-chloro-2′-pyridinyl compounds (**100** and **101**).

Toxicology. Previous studies have demonstrated that some anticonvulsants can induce hepatotoxicity and nephrotoxicity in humans and/or animal models.[18–24] Therefore, to further evaluate the potential for organ-directed toxicity, the nephrotoxic and hepatotoxic potentials of **1** and its putative metabolite **2** were evaluated in male Fischer 344 rats. A dose of 100 mg/kg was used in these studies. This dose is greater than 15 times the ED_{50} value of the parent compound in the MES tests, but still at a dose where motor impairment was evident.[2b] Compound **1** administration resulted in only minor changes in kidney and liver function (Table 3). No increase in proteinuria, glucosuria, or hematuria was observed in the treated group. In addition, no change in blood urea nitrogen (BUN) concentration, kidney

Table 3. Effect of Administration of Compounds 1 and 2 on Renal and Hepatic Function[a,b]

parameter	time (day)	compound 1 (100 mg/kg)		compound 2 (100 mg/kg)	
		control	treated	control	treated
urine volume (mL)	0	12.4 ± 0.8	13.8 ± 0.6	12.6 ± 0.7	9.7 ± 0.6
	1	14.0 ± 2.4	9.6 ± 0.4[c]	9.7 ± 0.9[c]	8.8 ± 0.7
	2	12.0 ± 1.7	10.2 ± 1.2[c]	10.2 ± 1.3[c]	7.1 ± 0.7[c]
BUN concentration (mg %)	0	15.0 ± 1.0	14.0 ± 1.0	19.0 ± 1.0	20.0 ± 1.0
	2	17.0 ± 1.0[c,d]	14.0 ± 1.0	13.0 ± 1.0[c]	14.0 ± 1.0[c]
kidney weight (g/100 g of body weight)	2	0.35 ± 0.01	0.35 ± 0.01	0.38 ± 0.01	0.37 ± 0.01
ALT/GPT (units/L)	2	21.0 ± 4.0	25.0 ± 5.0	29.0 ± 2.0	30.0 ± 5.0
liver weight (g/100 g of body weight)	2	3.81 ± 0.08	4.16 ± 0.03[d]	4.00 ± 0.05	4.35 ±0.07[d]

[a] Values are means ±SE for $n = 4$ rats per group. [b] Animals received a single ip injection of the test compound (treated) or vehicle (control) and liver and kidney function monitored for 48 h. [c] Significantly different from the day 0 value within a group, $p < 0.05$. [d] Significantly different from the corresponding pair-fed control group value, $p < 0.05$

weight, or ALT/GPT activity was seen following **1** administration. Organic anion accumulation (*p*-aminohippurate; PAH) was decreased at 48 h (control S/M ratio, 4.15 ± 0.18; treated S/M ratio 3.17 ± 0.10), but organic cation uptake (tetraethylammonium, TEA) was not altered (data not shown). Liver weight was increased slightly at 48 h, Table 3). However, in the absence of elevated ALT/GPT activity, it is unlikely that the change in liver weight is a direct result of toxicity. In addition, no changes in renal or hepatic morphology were observed when tissues from treated rats were compared to tissues from control animals. Therefore, **1** is not a significant nephrotoxicant or hepatotoxicant.

Compound **2**, at a dose of 100 mg/kg, also did not induce marked changes in liver or kidney function (Table 3). Also, no increase in BUN concentration, kidney weight, ALT/GPT activity (Table 3), urine contents, or organic ion accumulation by renal cortical slices (data not shown) or change in renal or hepatic morphology was observed in the treatment group. Thus, like **1**, **2** is not a significant nephrotoxicant or hepatotoxicant. Further support for the safety of each compound was recently provided by the ADD Program. As previously reported,[2b] **1** displayed an oral ED_{50} of 5.8 mg/kg and a TD_{50} >380 mg/kg in the rat, thus providing a protective index (PI = TD_{50}/ED_{50}) > 65.5. Data for **2** in the rat provided an oral ED_{50} of 14.7 mg/kg and a TD_{50} > 186 mg/kg and a PI > 12.6. It should also be noted that the TD_{50} evaluation is still undergoing further testing; thus the PI may change. Further, no rats died or experienced toxicity during the special ip toxicity evaluation of **2** at 100 mg/kg for a period of 24 h.

Metabolism. We had previously postulated the biotransformation of **1** to **2** via a nonspecific esterase.[2b] An *in vitro* determination in our laboratory indicated that this transformation did occur at 37 °C at 24 h.[2b] Thus, the metabolism of **1** was evaluated in our laboratories employing Fisher 344 rats. A 100 mg/kg ip dose of **1** was given to two rats, and the urine samples were collected at 6 and 24 h. Urine samples for pair-fed control rats were also collected. Standard curves for compounds **1** and **2** in control urine were developed using methylene chloride extraction and UV analysis as previously reported.[2b] The results of this *in vivo* study indicate that **1** was not significantly metabolized over the 24 h study period.

Sodium Channel Binding. Compounds **1** and **8** were evaluated in synaptoneurosomal preparations from rat cerebral cortex. This assay was similar to literature procedures[25] and measured the ability of the test compound to inhibit the specific binding of [^{3}H]batrachotoxinin A 20α-benzoate ([^{3}H]BTX-B) to neurotoxin site 2 of the voltage-dependent sodium channel. [^{3}H]-BTX-B binds strongly to neurotoxin site 2 of the neuronal voltage-dependent sodium channel. Local anesthetics, class I antiarrhythmics, and class I anticonvulsants bind at pharmacologically relevant concentrations to a site (or sites) on the sodium channel that is allosterically linked to neurotoxin site 2, resulting in the inhibition of binding of [^{3}H]BTX-B. Electrophysiological studies for many of these drugs also reveal a frequency and voltage-dependent block of sodium channel conductance, allowing for an explanation of the selective effects of class I anticonvulsants on hyperactive versus normal neurons.[26] Among the commonly used anticonvulsants, only three (which are designated class I anticonvulsants)[26-28] appear to cause their anticonvulsant effects by binding to the voltage-dependent sodium channel. These are phenytoin (IC_{50} = 40 μM),[26,27] carbamazepine (IC_{50} = 131 μM),[26,27] and lamotrigine (IC_{50} = 114 μM)[28] which are relatively narrow spectrum anticonvulsants that exhibit activities against partial and generalized tonic-clonic seizures.

In order to further evaluate sodium channel binding and anticonvulsant effects, we conducted the study that is summarized in Table 4. The stimulus for the study was the similarity in anticonvulsant profile of **1** to phenytoin[2c] and the relative safety of the 4′-trifluoromethoxy analog, **8**, as shown in Table 4. In addition, a 24 h ip toxicity evaluation at 100 mg/kg in rats, **8** did not cause the death or motor impairment noted with **1** at this same dosage. An in-depth pharmacologic evaluation of **1**[2c] indicated this enaminone was inactive in all chemical tests, e.g. subcutaneous (scMet) and intravenous (ivMet) pentylenetetrazole, sc bicuculline (scBic), sc picrotoxin (scPic), and sc strychnine (sc Strych). The ivMet evaluation was used to assess whether **1** would lower the threshold for seizures ("proconvulsant") or raise the threshold ("anticonvulsant"). Compound **1** had no significant effect in this evaluation. Negative results were also noted with scBic, scPic, and scStrych and indicate that the anticonvulsant effect of **1** does not operate through the GABA, chloride channel, or glycine mechanisms, respectively.[2c] Further evaluations indicated that **1** displayed slight *in vitro* inhibition of [^{3}H]-flunitrazepam and [^{3}H]GABA binding and [^{3}H]adenosine uptake, at a few isolated and unrelated concentrations, and exhibited no consistent inhibitory properties.[2c] Like phenytoin, **1** was effective in the corneal kindling[2a] model but ineffective in the amygdala

Table 4. Biological Results

compound	Na^+ channel: IC_{50} (μM)[a]	phase II, mice [time of peak effect]			phase VIA, rats [time of peak effect]		
		MES ED_{50}[b,c]	MES TD_{50}[b,d]	scMet ED_{50}[b,c]	MES ED_{50}[c,e]	MES TD_{50}[c,e]	scMet ED_{50}[c,e]
1	489	26.2[f] (17–40) [0.5 h]	254.8[f] (202–322) [2.0 h]	>254[f] (nd) [0.5 h]	5.8[f] (4–7) [0.5 h]	>380[f] (nd) [0.25–24 h]	>190[f] (nd) [0.5 h]
8	170	22.4 (20–25) [0.5 h]	122.3 (118–132) [0.25 h]	113.7 (80–146) [1.0 h]	9.3 (6–13) [0.5 h]	>158 (nd) [0.25–24 h]	>79 (nd) [0.5 h]
Phenytoin	40[g]	9.5[h] (8–10) [2.0 h]	65.5[h] (55–72) [2.0 h]	no protection	29.8[h] (22–39) [4.0 h]	>3000[h,i] (nd) 0.5 h]	no protection
carbamazepine	131[g]	8.8[h] (5–14) [0.25 h]	71.6[h] (46–135) [0.25 h]	potentiates	8.5[h] (3–11) [0.5 h]	813[h,i] (489–1234) [0.5 h]	no protection
lamotrigine	114[j]	2.6[k] (1.7–3.8) [1.0 h]	nd	nd	nd	nd	nd

[a] The concentration necessary to inhibit 50% of the specific binding of [^{3}H]BTX-B (See the Experimental Section). [b] ED_{50} and TD_{50} values are in milligrams/kilogram of test drug delivered intraperitoneally (ip). Test compounds administered in a 0.5% methycellulose suspension. [c] Measured at time of peak effect. [d] Measured at time of peak neurologic deficit on the rotorod. Numbers in parentheses are 95% confidence interval. nd = not determined. [e] ED_{50} and TD_{50} values are in milligrams/kilogram of test drug delivered orally (po). Test compounds administered in a 0.5% methylcellulose suspension. [f] Values from refs 2b and 2c. [g] Values from refs 26 and 27. [h] Values from ref 30. [i] Minimal neurotoxicity based on ataxia. [j] Values from ref 28. [k] Values from ref 31.

kindling model;[2b] the latter model is related to the GABA system.

The preliminary sodium channel binding results on these compounds indicated that at 500 μM **1** and **8** inhibited [^{3}H]BTX-B by 85% and 89%, respectively, while at 250 μM **1** and **8** provided inhibition of 63% and 68%, respectively.[29] The phenytoin control provided 44% inhibition at 40 μM. As noted in Table 4, **8** displayed relatively potent sodium channel binding effects, providing an IC_{50} of 170 μM, while **1** showed weaker effects (IC_{50} 489 μM). While the potency of **8** did not approach the effectiveness of phenytoin, it was comparable to carbamazepine (IC_{50} 131 μM) and diazepam (IC_{50} 152 μM) and is greater than phenobarbital (IC_{50} 2.50 mM),[27] the former two agents useful in the treatment of generalized tonic-clonic convulsions.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Observed boiling points were also uncorrected. IR spectra were recorded on samples in Nujol, as diluted chloroform solutions in matched sodium chloride cells, or neat with a Perkin-Elmer 1330 spectrophotometer. ^{1}H NMR spectra were recorded on a General-Electric QE 300-MHz spectrometer in deuterated solvents using tetramethylsilane as an internal reference. TLC analysis employed a *n*-BuOH–HOAc–H_2O (5:4:1) or (5:1:4, upper layer) elution solvent mixture and 5- × 10-cm or 5- × 20-cm fluorescent plates (Whatman silica gel 60A). Elemental analyses (C, H, N, and halogen) were performed by Schwarzkopf Microanalytical Laboratory, Woodside, NY. Where analyses are indicated only by the symbols of the elements, analytical results for the elements were within 0.4% of the theoretical values. Experimental data for all of the enaminone compounds are provided in Tables 1 and 2. 4-Carbo-*tert*-butoxy-5-methylcyclohexane-1,3-dione,[7] 4-carbomethoxy-5-methylcyclohexane-1,3-dione,[2a] and carbethoxy-5-methylcyclohexane-1,3-dione[6] were prepared by literature methods. Typical experiments illustrating the general procedures for the preparation of the enaminones are described below.

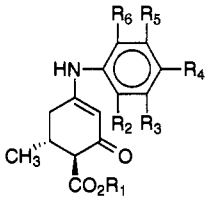
***tert*-Butyl 4-[(4′-Chlorophenyl)amino]-6-methyl-2-oxocyclohex-3-en-1-oate (4). Method B.** 4-Carbo-*tert*-butoxy-5-methylcyclohexane-1,3-dione (6.11 g, 27 mmol), mp 145–146 °C (lit. mp 130–131.5 °C),[7] and 4-chloroaniline (4.21 g, 33 mmol) were added to a mixture of absolute EtOH (100 mL) and EtOAc (100 mL), and the solution was refluxed and stirred for 6 h. Evaporation under reduced pressure yielded a yellow solid which was recrystallized from 2-PrOH: yield 3.96 g (43%); mp 190–192 °C; ^{1}H NMR ($CDCl_3$) δ 1.10 (3H, d, J = 6.3 Hz, CH_3), 1.48 (9H, s, 3 × CH_3 of *tert*-butyl group), 2.22–2.63 (3H, m, CH_2 + CH of cyclohexene ring), 2.90 (1H, d, J = 11.0 Hz, CH), 5.45 (1H, s, =CH), 6.90 (1H, bs, NH), 7.05–7.30 (4H, m, C_6H_4). Anal. (C, H, N, Cl).

Ethyl 4-[(4′-Aminophenyl)amino]-6-methyl-2-oxocyclohex-3-en-1-oate (27). Method D. To a solution of 4-carbethoxy-5-methylcyclohexane-1,3-dione (5.00 g, 25 mmol),[6] dissolved in a 100 mL mixture of C_6H_6:EtOH (1:1), was added *p*-phenylenediamine (97%, 2.79 g, 25 mmol) and the solution refluxed for 5 h. The reaction mixture was evaporated *in vacuo*, and the residue was recrystallized twice from EtOH:EtOAc to yield **27**: 4.95 g (69%); mp 187–190 °C; ^{1}H NMR (DMSO-d_6) δ 0.96 (3H, d, J = 5.1 Hz, CH_3), 1.18 (3H, t, J = 7.0 Hz, CH_3 of ethyl group), 2.27–2.54 (3H, m, CH_2 + CH of cyclohexene ring), 2.98 (1H, d, J = 11.0 Hz, CH), 4.09 (2H, q, J = 7.0 Hz, CH_2 of ethyl group), 4.96 (1H, s, =CH), 5.15 (2H, s, NH_2), 6.55–6.85 (4H, m, C_6H_4 ring), 8.70 (1H, bs, NH). Anal. (C, H, N).

Methyl 4-[(4′-Sulfonamidophenyl)amino]-6-methyl-2-oxocyclohex-3-en-1-oate (28). Method D. 4-Carbomethoxy-5-methylcyclohexane-1,3-dione (5.00 g, 27 mmol)[2a] and sulfanilamide (5.68 g, 33 mmol) were added to 250 mL of C_6H_6, the flask was attached to a Dean–Stark trap, and the reaction mixture was stirred and refluxed for 6 h. Only a fraction of the theoretical amount of water was collected due to partial solubility of sulfanilamide. After cooling, the contents were transferred to a 500 mL flask with the aid of 175 mL of absolute EtOH, and the mixture was refluxed an additional 6 h. Evaporation under reduced pressure yielded a gummy mass. Trituration with Et_2O yielded a hydrous mass. After air-drying at room temperature overnight, the residue was recrystallized from 2-PrOH. The solution formed a gel after 24 h. Filtration and drying provided fine light yellow crystals, which were recrystallized from 2-PrOH to yield **28**: 4.57 g (50%); mp 210–214 °C; ^{1}H NMR (DMSO-d_6) δ 0.99 (3H, d, J = 5.9 Hz, CH_3), 2.29–2.77 (3H, m, CH_2 + CH of cyclohexene ring), 3.12 (1H, d, J = 11.0 Hz, CH), 3.64 (3H, s, OCH_3), 5.47 (1H, s, =CH), 7.33 (2H, s, SO_2NH_2), 7.33–7.82 (4H, m, C_6H_4), 9.32 (1H, s, NH). Anal. (C, H, N, S).

Attempted Synthesis of Methyl 4-[(4′-Acetoxyphenyl)amino]-6-methyl-2-oxocyclohex-3-en-1-oate. Methyl 4-[(4′-hydroxyphenyl)amino]-6-methyl-2-oxocyclohex-3-en-1-oate (**22**) (2.00 g, 7.3 mmol)[2a] was added to 50 mL of Ac_2O and the mixture refluxed for 2 h. Distillation of the excess Ac_2O provided a dark brown oil which provided one spot on chromatography (R_f 0.87 vs R_f 0.58 for **22**). Preparative TLC and treatment of the fraction with methylene chloride purified the

Table 5. Pharmacological Evaluation of Ortho-Substituted and Multisubstituted Enaminones



compd	R_1	R_2	R_3	R_4	R_5	R_6	ASP classification[a]
62	CH_3	Cl	H	H	H	H	1
63	C_2H_5	Cl	H	H	H	H	3
64	C_2H_5	NH_2	H	H	H	H	3
65	CH_3	Br	H	H	H	H	3
66	CH_3	I	H	H	H	H	1
67	CH_3	OH	H	H	H	H	3
69	CH_3	SO_2NH_2	H	H	H	H	3
70	CH_3	CN	H	H	H	H	2
71	CH_3	OCH_3	H	H	H	H	3
72	CH_3	NO_2	H	H	H	H	2
73	CH_3	CH_3	H	H	H	H	3
74	CH_3	CF_3	H	H	H	H	2
75	CH_3	C_2H_5	H	H	H	H	1
76	CH_3	F	H	H	H	H	2
77	CH_3	OCH_3	H	H	CH_3	H	2
78	C_2H_5	OCH_3	H	H	CH_3	H	1
79	CH_3	H	Cl	OCH_3	H	H	3
80	C_2H_5	H	Cl	OCH_3	H	H	3
81	CH_3	Cl	H	H	OCH_3	H	2
82	CH_3	Cl	H	Cl	H	H	1
83	C_2H_5	Cl	H	Cl	H	H	1
84	CH_3	Cl	H	H	Cl	H	1
85	C_2H_5	Cl	H	H	Cl	H	3
86	CH_3	H	Cl	Cl	H	H	1
87	C_2H_5	H	Cl	Cl	H	H	2
88	CH_3	Cl	H	H	H	Cl	3
89	C_2H_5	Cl	H	H	H	Cl	3
90	CH_3	H	Cl	H	Cl	H	3
91	C_2H_5	H	Cl	H	Cl	H	3
92	CH_3	Cl	Cl	Cl	H	H	3
93	C_2H_5	Cl	Cl	Cl	H	H	2
94	CH_3	Cl	H	Cl	Cl	H	1
95	C_2H_5	Cl	H	Cl	Cl	H	3
96	CH_3	H	Cl	Cl	Cl	H	1
97	C_2H_5	H	Cl	Cl	Cl	H	2

[a] Anticonvulsant Screening Project (ASP), phase I ip MES results. The classifications are as follows: 1, anticonvulsant activity at 100 mg/kg or less; 2, anticonvulsant activity at doses greater than 100 mg/kg; 3, no anticonvulsant activity at doses up to and including 300 mg/kg.

residue; however, it resisted crystallization even after evaporation of the solvent under high vacuum for 24 h. Attempted distillation resulted in extensive decomposition of the product. No further purification was attempted.

Methyl 4-[(4′-Aminophenyl)amino]-6-methyl-2-oxocyclohex-3-en-1-oate (26). Methyl 4-[(4′-nitrophenyl)amino]-6-methyl-2-oxocyclohex-3-en-1-oate[2a] was hydrogenated as follows. A solution of the enaminone (5.00 g, 16.4 mmol) dissolved in 150 mL of absolute EtOH was added to a Paar hydrogenation bottle and 250 mg of 5% Pd on C. The mixture was subjected to low-pressure hydrogenation (45 psi) for 3 h, and the contents of the bottle were filtered through Celite. The filtrate was evaporated, and the resulting residue was purified by recrystallization from MeOH: yield 3.82 g (85%); mp 164–167 °C; ^{1}H NMR (DMSO-d_6) δ 1.10 (3H, d, J = 5.2 Hz, CH_3), 2.24–2.50 (3H, m, CH_2 + CH of cyclohexene ring), 2.98 (1H, d, J = 11.0 Hz, CH), 3.80 (3H, s, OCH_3), 4.96 (1H, s, =CH), 5.18 (2H, s, NH_2), 6.52–6.83 (4H, m, C_6H_4 ring), 8.67 (1H, bs, NH). Anal. (C, H, N).

Methyl 4-[(2′-Chloro-5′-methoxyphenyl)amino]-6-methyl-2-oxocyclohex-3-en-1-oate (81). Method D. 6-Chloro-*m*-anisidine hydrochloride (5 g, 26 mmol) was converted into the free base as follows. The compound was dissolved in 100 mL of H_2O, and 20 mL of 6 N NaOH solution was added. The precipitated free base was extracted successively with Et_2O (2 × 50 mL) and $CHCl_3$ (2 × 50 mL) and combined with the Et_2O extract, filtered through anhydrous Na_2SO_4 into a 250 mL flask, and evaporated under reduced pressure to dryness. 4-Carbomethoxy-5-methylcyclohexane-1,3-dione (3.90 g, 21 mmol),[2a] 100 mL of C_6H_6, and 100 mL absolute EtOH were added, and the resultant solution was stirred and refluxed for 6 h. Evaporation yielded an amorphous light green semisolid which spontaneously crystallized on standing at room temperature for 6 weeks. Recrystallization from 2-PrOH three times yielded an analytically pure sample of the product: yield 0.85 g (12%); mp 172–174 °C; ^{1}H NMR ($CDCl_3$) δ 1.12 (3H, d, J = 6.6 Hz, CH_3), 2.21–2.75 (3H, m, CH_2 + CH of cyclohexene ring), 3.11 (1H, d, J = 11.0 Hz, CH), 3.78 (3H, s, OCH_3), 3.79 (3H, s, OCH_3), 5.61 (1H, s, =CH), 6.14 (1H, s, NH), 6.67–7.33 (3H, m, C_6H_3). Anal. (C, H, N, Cl).

Methyl 4-[(2′,4′-Dichlorophenyl)amino]-6-methyl-2-oxocyclohex-3-en-1-oate (82). Method D. 4-Carbomethoxy-5-methylcyclohexane-1,3-dione (5.00 g, 27 mmol)[2a] and 2,4-dichloroaniline (5.35 g, 33 mmol) were dissolved in a mixture of 50 mL of C_6H_6 and 75 mL of absolute EtOH, and the stirred mixture was refluxed for 8 h. Evaporation *in vacuo* yielded a dark brown gel, which on trituration with ether solidified overnight. Recrystallization from EtOAc provided **88**: yield 6.40 g (72%); mp 153–155 °C; ^{1}H NMR ($CDCl_3$) δ 1.12 (3H, d, J = 6.6 Hz, CH_3), 2.27–2.76 (3H, m, CH_2 + CH of cyclohexene ring), 3.10 (1H d, J = 10.7 Hz, CH), 3.78 (3H, s, OCH_3), 5.50 (1H, s, =CH), 6.09 (1H, s, NH), 7.27–7.46 (3H, m, C_6H_3). Anal. (C, H, N, Cl).

Ethyl 4-(Phenylhydrazino)-6-methyl-2-oxocyclohex-3-en-1-oate (99). Method A. To a solution of 4-carbethoxy-5-methylcyclohexane-1,3-dione (5.00 g, 25 mmol),[6] dissolved in a 125 mL mixture of EtOAc:Et_2O (2:3), was added phenylhydrazine (3.57 g, 33 mmol), and the mixture was refluxed for 2 h, during which the reaction mixture bumped with the crystals of the product. The reaction was cooled overnight, and the crystals were collected and washed with Et_2O until the filtrate showed no orange color. Recrystallization of the crude product with EtOAc yielded **99** with the following properties: 5.50 g (76%); mp 148–150 °C; ^{1}H NMR (DMSO-d_6) δ 0.98 (3H, d, J = 5.2 Hz, CH_3), 1.17 (3H, t, J = 7.0 Hz, CH_3 of ethyl group), 2.24–2.53 (3H, m, CH_2 + CH of cyclohexene ring), 2.99 (1H, J = 10.7 Hz, CH), 4.09 (2H, q, J = 7.0 Hz, CH_2 of ethyl group), 5.00 (1H, s, =CH), 6.66–7.21 (5H, m, C_6H_5), 7.92 (1H, s, NH), 8.98 (1H, s, NH). Anal. (C, H, N).

Table 6. Anticonvulsant Evaluation of Miscellaneous Cyclic Enaminones

compd	ASP classification[a]	phase VIA[b]	comments
98	2	nd[c]	
99	3		
100	1	active at 50 mg/kg	special ip screen performed: Tox (100 mg/kg) and MES (10 mg/kg) in rats
101	1	active at 50 mg/kg; ED_{50} 12.1 (7.4–21.9);[d] TD_{50} >344 (nd);[c] PI[e] >28.4	
102	3		
103	1	active at 30 mg/kg	

[a] ASP classification, refer to Table 5. [b] Oral rat data. [c] Not determined. [d] Data in parentheses is the 95% confidence interval. [e] PI = protective index (TD_{50}/ED_{50}).

Ethyl 4-(Cyclohexylamino)-6-methyl-2-oxocyclohex-3-en-1-oate (103). Method D. To a solution of 4-carbethoxy-5-methylcyclohexane-1,3-dione (5.00 g, 25 mmol),[6] dissolved in a 125 mL mixture of C_6H_6:EtOH (2:3), was added cyclohexylamine (99%, 2.98 g, 30 mmol), and the mixture was refluxed for 5 h. The reaction mixture was evaporated, and the residue was triturated with Et_2O in an ice bath. After cooling in the freezer for an additional 2 h, the crystals were collected and recrystallized from EtOAc to yield **109**: 1.45 g (21%); mp 141–144 °C; ^{1}H NMR ($CDCl_3$) δ 1.06 (3H, d, J = 6.6 Hz, CH_3), 1.29 (3H, t, J = 7.4 Hz, CH_3 of ethyl group), 1.10–3.32 (11H, m, C_6H_{11}), 2.17–2.66 (3H, m, CH_2 + CH of cyclohexene ring), 2.98 (1H, d, J = 10.7 Hz, CH), 4.24 (2H, q, J = 7.4 Hz, CH_2 of ethyl group), 5.15 (1H, s, =CH), 4.72 (1H, s, NH). Anal. (C, H, N).

Pharmacology. Initial evaluations for anticonvulsant activity were performed by the Antiepileptic Drug Development (ADD) Program, Epilepsy Branch, Neurological Disorders Program, National Institute of Neurological Disorders and Stroke, and included phases I, II, and VIA test procedures which have been described.[12] These tests were performed in male Carworth Farms no. 1 (CF1) mice. Phase I of the evaluation included three tests: maximal electroshock (MES), subcutaneous (scMet), and the rotorod test for neurological toxicity (Tox). Compounds were suspended in 0.5% aqueous methylcellulose and were administered at three dosage levels (30, 100, and 300 mg/kg) with anticonvulsant activity and motor impairment noted 30 min and 4 h after administration. Phase II and phase VIB testing quantitated the anticonvulsant activity and motor impairment observed for the most promising compounds in phase I. Phase II quantified data in CF1 mice by intraperitoneal (ip) administration, while phase VIB provided oral rat data comparable to phase II ip data in mice. Male Sprague–Dawley rats were employed in this evaluation. Subsequent special ip evaluations in rats are shown in Tables 4 and 6. The TTE test[14] is described as follows. Twenty mice were pretreated with 100 mg/kg of the test compound. At several time intervals (15 min, 30 min, 1 h, 2 h, and 4 h) posttreatment with the test compound, four mice at each time point were challenged with 12.5 mA of electrical current for 0.2 s via corneal electrodes. This stimulation produced a TTE seizure in the animals. For each time interval, results were expressed as a ratio of the number of animals protected over the number of animals tested.

Toxicology. Compounds **1** and **2** were also evaluated for hepatotoxicity and nephrotoxicity in our laboratories. Male Fischer 344 rats (200–245 g) were purchased from Hilltop Lab Animals, Inc. (Scottsdale, PA) and placed in standard plastic animal cages prior to use. All animal holding and experimental rooms had a controlled light period (on 0600 h, off 1800 h), temperature (21–23 °C), and humidity (40–55%). At least one week was allowed to acclimate to the animal facilities prior to initiation of experiments. Following the acclimatization period, rats (four/group) were placed singly in stainless steel metabolism cages to allow for the separation of urine from feces. Following one day to allow the rats to acclimatize to the metabolism cages, control (day 0) values were obtained. On the following day, rats were administered a single ip injection of **1** or **2** (100 mg/kg) or vehicle (25% dimethyl sulfoxide (DMSO) in corn oil, 2.5 mL/kg), and renal function was monitored at 24 and 48 h as previously described.[24] Urine volume, food and water intake, and body weight were measured at 24 h intervals. Urine was analyzed semiquantitatively for the presence of glucose, ketones, and blood using Labstix (Ames Division, Miles Laboratory, Inc.). Urinary protein concentration was quantitated spectrophotometrically at 595 nm using Coomassie blue. At 48 h posttreatment, rats were anesthetized with diethyl ether and laparotomized. Blood was withdrawn from the dorsal aorta into heparinized syringes, and plasma was obtained following centrifugation (3000g, 5 min). One plasma aliquot from each rat was refrigerated until assayed for alanine aminotransferase (ALT/GPT) activity (Sigma Kit No. 59), while a second plasma aliquot was stored at −20 °C for blood urea nitrogen (BUN) concentration determinations (Sigma Kit No. 640, Berthelot reaction). A sample of blood also was obtained from the tail of each rat prior to placement in a metabolism cage to allow for the determination of day 0 BUN concentration. After obtaining the aortic blood sample, the liver and kidneys were rapidly removed, weighed, and placed in ice-cold buffer. The spleen from each animal was also removed and weighed. The left kidney was quartered, renal cortical slices prepared freehand, and the accumulation of [^{14}C]-p-aminohippurate (PAH) and [^{14}C]tetraethylammonium (TEA) by these slices was determined.[24] Lactate (10 mM) was added in some PAH experiments to determine lactate-stimulated PAH accumulation. Renal accumulation of PAH and TEA was expressed as the slice-to-medium (S/M) ratio where S equaled the radioactivity (dpm) per gram of tissue and M equaled the radioactivity (dpm) per mL of medium. The right kidney was quartered and placed in 10% neutral buffered formalin along with a section of liver. Tissue from treated and control rats was examined using light microscopy for evidence of chemically-induced morphological changes. In all experiments, control rats were pair fed to the appropriate treatment group to ensure that the observed effects were chemically induced and not due to altered food intake. Values in Table 3 were expressed as means ± SE. The data were analyzed using a one-way analysis of variance (ANOVA) test followed by a Dunnett's or Newman–Keuls analysis. All statistical tests were run at a 95% confidence interval and significance was denoted at $p < 0.05$.

A 24 h metabolism study of **1** and **2** was conducted on a Milton Roy 1201 UV spectrophotometer. A dose of 100 mg/kg of **1** was administered intraperitoneally to Fisher 344 rats (average weight: 200–245 g), and the urine samples were collected at 6 and 24 h. Urine sample for pair-fed control rats were collected also at 6 and 24 h. To determine the control values for the urinalysis, 1 mL of water (for the aqueous control) or 1 mL of rat urine (for the urine control) was added to a 5 mL React-A-Vial. Then 0.2 mL of 4×10^{-4} M solution of **1** (or **2**) was added. This was followed by 0.8 mL of 0.5 M potassium chloride solution. The React-A-Vial was sealed, vortexed and incubated in a water bath at 37 °C for 0, 6, and 24 h. After quenching with 1 mL of dichloromethane, the React-A-Vial was resealed, vortexed and kept in the freezer (−80 °C). When required, the sample was thawed, and the dichloromethane layer was withdrawn and assayed by UV spectroscopy between 190 and 500 nm as previously reported.[2b] The UV analysis of the urine collected from Fisher rats which had been treated with **1** (100 mg/kg) was similar to the control procedure. The urine sample was defrosted, and to 0.5–1.0 mL of urine was added an equal volume of dichloromethane in a 5 mL React-A-Vial. The React-A-Vial was sealed, vortexed, and kept in the freezer (−80 °C) until required. Then the sample was thawed, the dichloromethane layer was withdrawn and diluted appropriately (1:16 to 1:32 dilutions) with dichloromethane, and the UV spectrum was determined.

Sodium Channel Binding. Sodium channel binding assays on compounds **1** and **8** were performed by NovaScreen, Hanover, MD. [^{3}H]Batrachotoxinin A 20α-benzoate was prepared as described and assayed to contain a final ligand concentration of 2.0 nM.[25] The sodium channel binding assay was similar to reported methods.[25] Reactions were carried out in 50 nM HEPES (pH 7.4 at 25 °C) containing 130 mM choline chloride at 37 °C for 45 min. The reaction was terminated by rapid vacuum filtration of the reaction mixture onto glass fiber filters. Radioactivity trapped onto the filters was determined and compared to control values in order to ascertain any interactions of test compounds with the sodium site 2 binding site. The IC_{50} values (concentration of compound required to inhibit 50% of specific neurotoxin binding) were determined from a dose–response curve generated by plotting the log of anticonvulsant concentration (over a range of 10–800 μM) versus percent of specifically bound [^{3}H]BTX-B.

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