Synthesis and Anticonvulsant Activity of Enaminones. 2. Further Structure–Activity Correlations[1,2]

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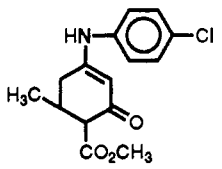
This report continues the in-depth evaluation of methyl 4-[(*p*-chlorophenyl)amino]-6-methyl-2-oxocyclohex-3-en-1-oate, 1 (ADD 196022), and methyl 4-(benzylamino)-6-methyl-2-oxocyclohex-3-en-1-oate, **2**, two potent anticonvulsant enaminones. These compounds were evaluated employing the amygdala kindling model. Neither 1 nor **2** was active against amygdala kindled seizures, further supporting the corneal kindled model as a definitive tool for antielectroshock seizure evaluation as previously reported. Additional intraperitoneal (ip) data on 1 revealed toxicity at 24 h at 100 mg/kg. Several active analogs have been prepared with the view to minimizing toxicity. In a special ip rat screen developed by the Antiepileptic Drug Development (ADD) Program, these newer analogs were evaluated for protection against maximal electroshock seizures (MES) at 10 mg/kg and neurotoxicity at 100 mg/kg. From this screen, several compounds were shown to be safer alternatives, the most notable was methyl 4-[(*p*-bromophenyl)amino]-6-methyl-2-oxocyclohex-3-en-1-oate, **13**. Compound **13** had an ip ED50 of 4 mg/kg in the rat and a TD50 of 269 mg/kg, providing a protective index (TD50/ED50) of >67. By variation in the ring size, additional aromatic substitutions and the synthesis of acyclic analogs, these newer compounds provide a more definitive insight into the structure–activity correlation. CLOGP evaluation and molecular modeling studies are also provided to further elaborate the molecular characteristics of potential anticonvulsant enaminones.

As part of a continuing study of potential anticonvulsant agents,[2-9] an initial study of enaminones was undertaken.[2] Previously, only a few enaminones had been reported to possess demonstrated biological activity.[10,11] The discovery of a series of potent anticonvulsant enaminones in our laboratory, notably methyl 4-[(*p*-chlorophenyl)amino]-6-methyl-2-oxocyclohex-3-en-1-oate, 1 (ADD 196022), and methyl 4-(benzylamino)-6-methyl-2-oxocyclohex-3-en-1-oate, **2**, with oral ED50s of 5.8 and 26.8 mg/kg, respectively, in the rat has prompted further studies. The active



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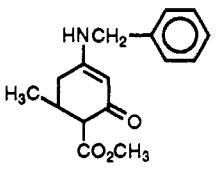

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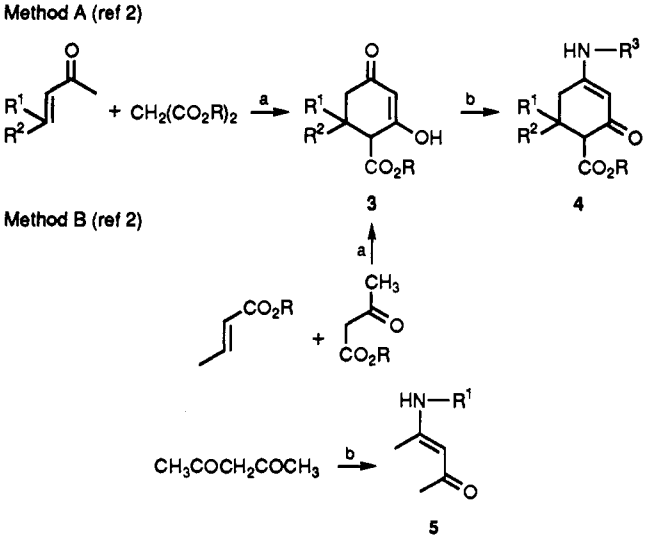
anticonvulsant enaminones were, for the most part, specific against maximal electroshock seizures (MES) and the corneal kindling model, and they possessed weak or no activity against chemostimulants (pentylenetetrazol, bicuculline, strychnine, or picrotoxin). However, the threshold level to these convulsant agents was not lowered. These compounds were inactive in the benzodiazepine receptor binding evaluation, the GABA receptor binding test, and the adenosine uptake assay. These activities were also species specific, with the rat being more sensitive than the mouse (ED50 of 26.2 and 64.7 mg/kg for 1 and 2 ip, respectively, in the mouse). This report provides further insight into the characteristics of active enaminones by evaluation of CLOGP,[12] molecular modeling studies,[13] and the synthesis of additional analogs.

Scheme I[a]



[a] (a) NaOMe; (b) Δ, amine. R = CH_3, C_2H_5; R^1 = H, CH_3, C_6H_5; R^2 = H, CH_3.

Results and Discussion

Chemistry. Cyclic enaminone esters, **1**, **2**, **7–33**, **55**, **56**, **58–64**, and enaminone amides **34** and **57** (Table I) were synthesized from β-hydroxy keto esters, **3**, as previously reported (Scheme I).[2] The various conditions for con-

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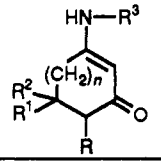
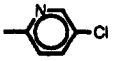
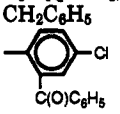
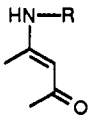
Table I. Physical Properties of Cyclic Enaminones[a]



compd	n	R	R^1	R^2	R^3	% yield, method	mp (°C) or bp (°C (mm))	formula	anal.[b]
1[c]	1	CO_2CH_3	CH_3	H	C_6H_4(*p*-Cl)	61, D	178–180	$C_{16}H_{16}NO_3Cl$	C, H, N, Cl
2[c]	1	CO_2CH_3	CH_3	H	$CH_2C_6H_5$	90, C	154–155	$C_{16}H_{16}NO_3$	C, H, N
7[c]	1	CO_2CH_3	CH_3	H	C_6H_4(*p*-C_2H_5)	43, D	153.5–155	$C_{17}H_{21}NO_3$	C, H, N
8	1	CO_2CH_3	CH_3	H	$CH(CH_3)C_6H_5$	24, D[d]	190–193	$C_{17}H_{21}NO_3$	C, H, N
9	1	CO_2CH_3	CH_3	H	$(CH_2)_2C_6H_5$	73, C[d]	116–118	$C_{17}H_{21}NO_3$	C, H, N
10	1	CO_2CH_3	CH_3	H	$(CH_2)_3C_6H_5$	82, C[e]	163–164	$C_{16}H_{23}NO_3$	C, H, N
11	1	CO_2CH_3	CH_3	H	$(CH_2)_4C_6H_5$	41, C[f]	90–92	$C_{16}H_{25}NO_3$	C, H, N
12	1	CO_2CH_3	CH_3	H	C_6H_4(*p*-F)	75, D[g]	161–164	$C_{16}H_{16}NO_3F$	C, H, N, F
13	1	CO_2CH_3	CH_3	H	C_6H_4(*p*-Br)	91, D[h]	188–190	$C_{16}H_{16}NO_3Br$	C, H, N, Br
14	1	CO_2CH_3	CH_3	H	C_6H_4(*p*-$C(CH_3)_3$)	68, D[g]	221–222	$C_{16}H_{25}NO_3$	C, H, N
15	1	CO_2CH_3	CH_3	H	C_6H_4(*p*-CF_3)	57, D[f]	169–170	$C_{16}H_{16}NO_3F_3$	C, H, N, F
16	1	CO_2CH_3	CH_3	H		43, C[d]	206–208	$C_{14}H_{15}N_2O_3$	C, H, N
17	1	CO_2CH_3	CH_3	CH_3	C_6H_5	66, D[i]	168–169	$C_{16}H_{16}NO_3$	C, H, N
18	1	CO_2CH_3	CH_3	CH_3	C_6H_4(*p*-NO_2)	41, D[f]	178–179	$C_{16}H_{16}N_2O_5$	C, H, N
19	1	CO_2CH_3	CH_3	CH_3	C_6H_4(*p*-Cl)	53, D[i]	141–143	$C_{16}H_{16}NO_3Cl$	C, H, N, Cl
20	1	CO_2CH_3	CH_3	CH_3	C_6H_4(*p*-$C(CH_3)_3$)	44, D[f]	170–171	$C_{20}H_{27}NO_3$	C, H, N
21	1	CO_2CH_3	CH_3	CH_3	C_6H_4(*p*-CF_3)	46, D[f]	158–159	$C_{17}H_{18}NO_3F_3$	C, H, N, F
22	1	$CO_2C_2H_5$	CH_3	H	C_6H_4(*p*-Cl)	69, D[i]	161–163	$C_{16}H_{16}NO_3Cl$	C, H, N, Cl
23	1	$CO_2C_2H_5$	CH_3	H	C_6H_4(*p*-Br)	63, D[i]	151–154	$C_{16}H_{16}NO_3Br$	C, H, N, Br
24	1	$CO_2C_2H_5$	CH_3	H	C_6H_4(*p*-F)	60, D[i]	150–151.5	$C_{16}H_{16}NO_3F$	C, H, N, F
25	1	$CO_2C_2H_5$	CH_3	H	C_6H_4(*p*-CH_3)	76, D[f]	134.5–135.5	$C_{17}H_{21}NO_3$	C, H, N
26	1	$CO_2C_2H_5$	CH_3	H	C_6H_4(*p*-C_2H_5)	74, D[f]	148.5–150	$C_{16}H_{23}NO_3$	C, H, N
27	1	$CO_2C_2H_5$	CH_3	H	C_6H_4(*p*-NO_2)	17, D[i]	173–175	$C_{16}H_{16}N_2O_5$	C, H, N
28	1	$CO_2C_2H_5$	CH_3	H	C_6H_3(2′,5′-$(OCH_3)_2$)	77, D[i]	137–138	$C_{16}H_{23}NO_5$	C, H, N
29	1	$CO_2C_2H_5$	CH_3	H	C_6H_5	74, D[g]	155–158	$C_{16}H_{16}NO_3$	C, H, N
30	1	$CO_2C_2H_5$	CH_3	H	C_6H_4(*p*-$C(CH_3)_3$)	54, D[i]	165.5–166	$C_{20}H_{27}NO_3$	C, H, N
31	1	$CO_2C_2H_5$	CH_3	H	C_6H_4(*p*-CF_3)	72, D[i]	184.5–186	$C_{17}H_{16}NO_3F_3$	C, H, N, F
32	1	$CO_2C_2H_5$	CH_3	H	$CH_2C_6H_5$	77, D[f]	134.5–135.5	$C_{17}H_{21}NO_3$	C, H, N
33	1	$CO_2C_2H_5$	CH_3	H		24, D[f]	154–156	$C_{23}H_{22}NO_4Cl$	C, H, N, Cl
34	1	$CONHCH_2C_6H_4$(*p*-F)	CH_3	H	$CH_2C_6H_4$(*p*-F)	8, D[i]	183–184	$C_{22}H_{22}N_2O_2F$	C, H, N, F
35[c]	1	H	CH_3	CH_3	$CH_2C_6H_5$	15, C	124–127	$C_{16}H_{16}NO$	C, H, N
36[c]	1	H	CH_3	CH_3	C_6H_5	89, D	181–183	$C_{14}H_{17}NO$	C, H, N
37	1	H	CH_3	CH_3	C_6H_4(*p*-Cl)	75, D[j,k]	208–210	$C_{14}H_{16}NOCl$	C, H, N, Cl
38	1	H	CH_3	CH_3	C_6H_4(*p*-NO_2)	15, D[j,l]	242–244	$C_{14}H_{16}N_2O_3$	C, H, N
39	1	H	CH_3	CH_3	C_6H_4(*p*-CH_3)	57, D[m,n]	203–204	$C_{16}H_{16}NO$	C, H, N
40	1	H	CH_3	CH_3	C_6H_4(*p*-C_2H_5)	68, D[m]	201–202.5	$C_{16}H_{21}NO$	C, H, N
41	1	H	CH_3	CH_3	C_6H_4(*p*-OCH_3)	66, D[i,o]	189–191	$C_{16}H_{16}NO_2$	C, H, N
42	1	H	CH_3	CH_3	C_6H_4(*p*-NH_2)	58, D[m,p]	211.5–212.5	$C_{14}H_{16}N_2O$	C, H, N
43	1	H	CH_3	CH_3	C_6H_4(*p*-$C(CH_3)_3$)	44, D[g]	206–208	$C_{16}H_{23}NO$	C, H, N
44	1	H	CH_3	CH_3	C_6H_4(*p*-CF_3)	46, D[g]	240.5–241.5	$C_{16}H_{16}NOF_3$	C, H, N, F
45	1	H	CH_3	CH_3	$(CH_2)_2C_6H_5$	75, D[i,q]	121–123	$C_{16}H_{21}NO$	C, H, N
46	1	H	CH_3	H	C_6H_4(*p*-Cl)	88, D[r]	198–199.5	$C_{16}H_{14}NOCl$	C, H, N, Cl
47	1	H	H	H	C_6H_4(*p*-Cl)	67, D[e]	190–191.5	$C_{12}H_{12}NOCl$	C, H, N, Cl
48	1	H	H	H	$CH_2C_6H_5$	25, D[e,t]	125–127	$C_{16}H_{15}NO$	C, H, N
49	1	H	H	H	$(CH_2)_2C_6H_5$	70, D[u,v]	93.5–95	$C_{14}H_{17}NO$	C, H, N
50	1	H	H	H	C_6H_4(*p*-$C(CH_3)_3$)	59, D[g]	185–187	$C_{16}H_{21}NO$	C, H, N
51	1	H	H	H	C_6H_4(*p*-CF_3)	62, D[i]	203–204	$C_{16}H_{12}NOF_3$	C, H, N, F
52	0	H	H	H	C_6H_4(*p*-Cl)	84, C[r]	216–217	$C_{12}H_{11}NOCl$	C, H, N, Cl
53	0	H	H	H	$CH_2C_6H_5$	25, D[w]	139–141.5	$C_{16}H_{15}NO$	C, H, N
54	1	H	H	H	$(CH_2)_2C_6H_5$	70, D[i]	136–138	$C_{14}H_{17}NO$	C, H, N
55[c]	1	CO_2CH_3	CH_3	CH_3	$CH_2C_6H_5$	79, C	138–139	$C_{17}H_{21}NO_3$	C, H, N
56[c]	1	CO_2CH_3	CH_3	CH_3	$(CH_2)_2C_6H_5$	46, C	130–131	$C_{16}H_{23}NO_3$	C, H, N
57[c]	1	$CONH(CH_2)_2C_6H_5$	CH_3	H	$(CH_2)_2C_6H_5$	55, E	171–172	$C_{24}H_{28}N_2O_2$	C, H, N
58[c]	1	CO_2CH_3	CH_3	H	$CH_2C_6H_4$(*p*-Cl)	64, C	173–174	$C_{16}H_{16}NO_3Cl$	C, H, N, Cl
59[c]	1	CO_2CH_3	CH_3	H	$CH_2C_6H_4$(*p*-F)	62, C	174–176	$C_{16}H_{16}NO_3F$	C, H, N, F
60[c]	1	CO_2CH_3	CH_3	H	NHC_6H_5	71, D	167–167.5	$C_{16}H_{16}N_2O_3$	C, H, N
61[c]	1	CO_2CH_3	CH_3	H	C_6H_4(*p*-CH_3)	67, D	144–146	$C_{16}H_{16}NO_3 \cdot H_2O$	C, H, N
62[c]	1	CO_2CH_3	CH_3	H	C_6H_4(*p*-NO_2)	55, D	186–187	$C_{16}H_{16}N_2O_5$	C, H, N
63[c]	1	CO_2CH_3	CH_3	H	C_6H_3(2′-OCH_3,5′-CH_3)	28, D	160.5–161.5	$C_{17}H_{21}NO_4$	C, H, N
64[c]	1	CO_2CH_3	CH_3	H	C_6H_3(2′,5′-$(OCH_3)_2$)	88, D	134–135	$C_{17}H_{21}NO_5$	C, H, N

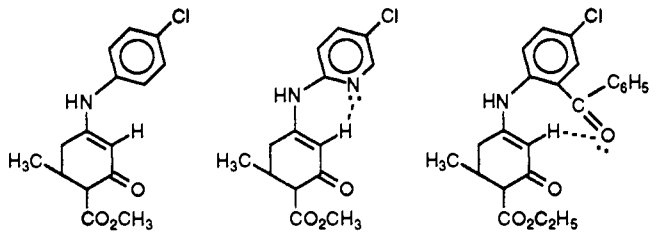
[a] The infrared and ^1H NMR spectra were consistent with assigned structures. Recrystallization solvents as indicated. [b] All compounds gave satisfactory C, H, N, and halogen (when required) analyses (±0.4%). [c] Reference 2. [d] Toluene. [e] EtOAc/EtOH. [f] EtOAc/petroleum ether (bp 38–54 °C). [g] EtOAc/Me_2CO. [h] 2-PrOH/Me_2CO. [i] EtOAc. [j] MeOH/Me_2CO. [k] Reference 51, mp 210 °C, yield 22%. [l] Reference 51, mp 246–247 °C. [m] EtOH. [n] Reference 51, mp 204 °C. [o] Reference 51, mp 191–192 °C. [p] Reference 51, mp 212–213 °C. [q] Reference 47, mp 126 °C. [r] EtOAc–Me_2CO–MeOH (2:2:1). [s] 2-PrOH. [t] Reference 52, mp 122–124 °C. [u] EtOAc/ligroine (bp 70–90 °C). [v] Reference 47, mp 104–105 °C; reference 52, mp 98–99 °C. [w] MeOH.

Table II. Physical Properties of Acyclic Enaminones[a]



compd	R	% yield, method	mp (°C) or bp (°C (mm))	formula	anal.[b]
65	C_6H_4(*p*-Cl)	85, D	113–115 (0.25); 57–59[c,d]	$C_{11}H_{12}NOCl$	C, H, N, Cl
66	C_6H_4(*p*-Br)	88, D	125–130 (0.25); 58–60.5[c]	$C_{11}H_{12}NOBr$	C, H, N, Br
67	C_6H_4(*p*-NO_2)	38, D	144–145[e,f]	$C_{11}H_{12}N_2O_3$	C, H, N
68	C_6H_4(*p*-OCH_3)	83, D	118–120 (0.20); 41–43[c,g]	$C_{12}H_{15}NO_2$	C, H, N
69	$CH_2C_6H_5$	92, D	107–110 (0.50)[h]	$C_{12}H_{15}NO$	C, H, N
70	$(CH_2)_2C_6H_5$	81, D	138 (0.45)[i]	$C_{13}H_{17}NO$	C, H, N
71	C_6H_4(*p*-C_2H_5)	63, D	109 (0.25)	$C_{16}H_{17}NO$	C, H, N
72	C_6H_4(*p*-CH_3)	83, D	100–110 (0.20); 65–67[c,j]	$C_{12}H_{15}NO$	C, H, N

[a] The infrared and ^1H NMR spectra were consistent with assigned structures. Recrystallization solvents as indicated. [b] All compounds gave satisfactory C, H, N, and halogen (when required) analyses (±0.4%). [c] Petroleum ether (bp 49–54 °C). [d] Reference 48, mp 61–62 °C; reference 49, mp 60–61 °C. [e] Methanol. [f] Reference 48, mp 145.5–146 °C. [g] Reference 48, mp 50–52 °C; reference 49, mp 51–52.5 °C. [h] Reference 53, mp 24 °C; reference 54, mp 23–24.5 °C. [i] Reference 50, bp 125–130 °C (5 mm). [j] Reference 48, mp 67–68 °C; reference 50, mp 68–69 °C.



1 16 33

Figure 1. NMR structural correlation for compounds 1, 16, and 33.

densation of the esters with the appropriate amino compound varied slightly. Wherever possible, toluene (method C) was replaced with benzene which lowered the reaction temperature and improved yields. As noted in the initial study,[2] the synthesis of the diastereoisomers **1**, **2**, **7–16**, **22–34**, and **57–64** and enantiomers **17–21**, **46**, **55**, and **56** yielded a single isomer. Acidification of the reaction mixture from the base-catalyzed saponification of either the methyl or ethyl esters, **3**, provided the decarboxylation product, 5-methylcyclohexane-1,3-dione which was condensed to form **46**. Dimedone, 5,5-dimethylcyclohexane-1,3-dione, was used to form **35–45**, while cyclohexane-1,3-dione was condensed to form **47–54**. Acyclic enaminones, **65–72**, were synthesized from acetylacetone and are shown in Table II.

The NMR results at 300 MHz of the enaminones were consistent with the assigned structures. Of interest is the difference in spectrum of the 2-pyridine analog, **16**, and **1**. The chemical shifts for methyl (δ ~1.00) and methoxy (δ ~3.70) protons were similar for **1** and **16**; however, in enaminone ester **16**, the vinyl proton (δ 6.82) was deshielded relative to the vinyl proton in **1** (δ 5.25 ppm). The analogous downfield shift occurred in **33**, indicating the involvement of the vinyl proton in intramolecular hydrogen bonding. This is shown in Figure 1. In addition, the acidic NH proton for **16** (δ 9.57) was more deshielded than that for **1** (δ 7.96 in a saturated solution).

Pharmacology. Preliminary pharmacological testing of the compounds listed in Tables I and II has been provided by the Antiepileptic Drug Development (ADD) Program, Epilepsy Branch, Neurological Disorders Program, National Institutes of Neurological and Communicative Disorders and Stroke (NINCDS), by testing procedures that have been described.[14] Phase I studies of the enaminones involved three tests: maximal electroshock seizure (MES), subcutaneous pentylenetetrazol (scMet), and neurologic toxicity (Tox). Intraperitoneal (ip) administration of the test compounds was either as a solution or a suspension in 0.5% methylcellulose. Due to the species specificity of this series, all class 1 and 2 MES-active analogs were evaluated for oral (po) activity (phase VIA) in the rat at 50 mg/kg. This latter concentration provided a rapid comparison to the activity of lead compound **1**. Additionally, several analogs were also evaluated in the mouse by ip administration. The results indicated that several safer, orally effective alternatives to **1** emerged. Compounds **12**, **13**, **15**, **16**, **19**, **21–25**, **30**, and **31** were found to be safe and effective in this screening procedure, affording complete anti-MES protection to the mouse without displaying any motor impairment at 50 mg/kg for periods up to 4 h. Consistent with our original report, none of the enaminones elicited an ataxic response in the rat. A special ip MES time of peak effect and motor impairment screen was also performed on **16**, **22**, **23**, **25**, **30**, and **31** in the rat. The MES dosage evaluations were at 10 mg/kg and motor impairment at 100 mg/kg. As an indication of species specificity, compound **22** was 25% effective in mice (10 mg/kg ip) at 30 min, while the same dose given ip in rats provided 75% MES protection and no toxicity at 15 and 30 min and 100% MES protection of the animals at 1 h. Compound **23** proved the safest in this evaluation. Compounds **13** and **15** were screened in a more selective (ip) evaluation in the rat. As shown in Table III, compound **13** displayed a TD50 of 270 mg/kg and an ED50 of 4 mg/kg while **15** had a TD50 >64 mg/kg and an ED50 of 2.95 mg/kg. The protective indices for **13** and **15** were >67 and >21, respectively.

Due to the remarkable activity of **1**, a special ip rat screening evaluation was performed. This screen attempts to determine whether toxicity of the compound changes on changing the route of administration. As previously reported **1** showed no oral toxicity up to doses of 380 mg/kg. The results of the ip evaluation are shown in Table IV. Also included are **7** and **36**. Compound **7** had demonstrated a MES ED50 of 114.1 mg/kg at 15 min and no toxicity observed at doses >750 mg/kg ip in mice, providing a protective index (TD50/ED50) >6.6, while **36** had a MES ED50 of 36.4 mg/kg at 15 min and no toxicity noted at doses >500 mg/kg po in rats (PI >13.7). As noted in Table IV, all compounds displayed toxicity in this evaluation in contrast to compound **2** which displayed no toxicity for periods up to 4 h.[2] The delayed toxicity of **1** was investigated. Although hydrolysis of **1** was initially ruled out by virtue of its rapid onset of action,[2] the delayed

Table III. Special Intraperitoneal (ip) Quantitation Data for Compounds 13 and 15 in the Rat[a]

	MES				Tox				
compd	time (h)	dose (mg/kg)	a	ED50[c]	time (h)	dose (mg/kg)	b	TD50[c]	PI,[d] MES
13	0.25	1.2	0/8	4.0	2.0	110	0/8	269.9	67.4
		3.6	5/8	(2.4–5.6)		180	2/8	(198.3–451.4)	
		4.5	3/8			220	4/8		
		7.0	7/8			440	6/8		
15	0.50	1.0	0/8	2.95	0.25–24	8	0/2	>64	>21.7
		2.0	3/8	(2.2–4.1)		16	0/2		
		3.0	3/8			32	0/2		
		3.5	4/8			64	0/2		
		4.0	7/8						

[a] Number of animals protected from electroshock seizures/number of animals tested. [b] Number of animals exhibiting neurotoxicity/number of animals tested. [c] ED50 and TD50 values are in milligrams/kilogram of test drug delivered intraperitoneally. [d] PI = protective index (TD50/MES ED50). Numbers in parentheses are 95% confidence interval.

Table IV. Intraperitoneal (ip) Toxicity of Compounds 1, 7, and 36 in the Rat (100 mg/kg)

	ip toxicity[a]		
time (h)	1	7	36
0.25	0/8	1/8	5/8
0.50	3/8	5/8	7/8
1.00	6/8	0/8	6/8
2.00	7/8	0/8	6/8
4.00	2/8	0/8	3/8
6.00	1/8	0/8	3/8
8.00	0/8	nd[b]	nd[b]
24.00	2/8[c]	0/8	0/8

[a] Number of animals displaying neurotoxicity/number of animals tested. [b] nd = not determined. [c] Two animals died.

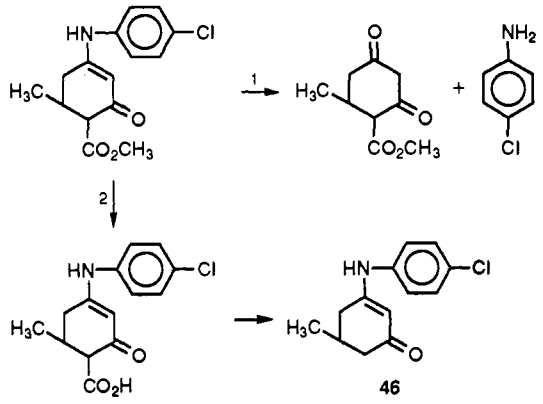


Figure 2. Potential biotransformation pathways of compound 1.

toxicity could be due to two pathways as shown in Figure 2. Pathway 1 leads to *p*-chloroaniline, a known mutagen, while pathway 2 leads to the β-keto acid which would subsequently decarboxylate to form 3-[(*p*-chlorophenyl)-amino]-5-methyl-2-cyclohexenone, **46**. While the po LD50 of *p*-chloroaniline is 310 mg/kg,[16] Rankin and co-workers[17] noted toxicity of this compound at 1.5 mmol/kg ip (equivalent to 440 mg/kg of 1). Incubation of 1 at pH 7.0 and 7.4 over 24 h at 37 °C and monitoring by ultraviolet and TLC analyses did not indicate any hydrolysis of 1 to the potential mutagen. Treatment of 1 with esterase at pH 8 at 37 °C produced a 20% conversion to **46** at 24 h. Phase I evaluation of **46** revealed hyperactivity in all of the animals tested at 100 mg/kg and motor impairment at 300 mg/kg in the MES evaluation at 30 min and at 4 h.

Previously, it was shown that the MES-active enaminones 1 and 2 were also active in the corneal kindled rat model.[2] In this report, compounds 1 (350 mg/kg, po) and 2 (500 mg/kg, po) were evaluated in the amygdala kindling model, a frequently used animal model of complex partial seizures with secondary generalization.[18] The results of anticonvulsant testing in amygdala kindled rats are shown in Table V. Neither 1 nor 2 had significant anticonvulsant action in suppressing amygdala kindled seizures (MANOVA; 1, $p = 0.12$; 2, $p = 0.67$). There was no evidence of toxicity (ataxia, muscle relaxation, screen test) of 1 (350 mg/kg, po) and 2 (500 mg/kg, po) at the doses tested. Anticonvulsants, such as sodium valproate, which are active in the corneal kindling model are also effective against amygdala kindled seizures.[2,19] The lack of anticonvulsant activity of enaminones 1 and 2 in amygdala kindled rats (Table V) and their demonstrated activity in corneal kindled rats[2] may suggest that the newly developed corneal kindling model[20] is able to detect a broad spectrum of anticonvulsant agents. Although the exact neurophysiological and/or neurochemical mechanism for kindling is not known,[21] several abnormalities are found in the brain of kindled rats which relate to GABA-mediated inhibitory synaptic transmission.[22–27] Since both 1 and 2 were inactive in two phase V evaluations related to the GABA system, namely benzodiazepine and GABA receptor binding displacement,[2] their inactivity with the amygdala kindling model might be expected.

Table V. Effect of Oral (po) Administration of Compounds 1 and 2 on Amygdala Kindled Seizures in the Rat[a]

	baseline (0.5% MC)[b,c]	1 (350 mg/kg)	baseline (0.5% MC)[b,c]	2 (500 mg/kg)
		Afterdischarge		
Amygdala:				
Duration (s)	76.7 (±6.6)	79.8 (±6.7)	78.6 (±5.4)	73.9 (±6.3)
Cortex:				
Latency (s)	2.1 (±0.2)	2.7 (±0.2)	1.3 (±0.1)	2.8 (±0.5)
Duration (s)	83.4 (±5.4)	79.6 (±6.3)	92.0 (±6.6)	78.9 (±3.9)
		Behavioral Seizure		
Latency (s)	2.1 (±0.1)	2.1 (±0.0)	2.0 (±0.1)	2.1 (±0.0)
Duration (s)	90.0 (±4.1)	82.3 (±6.0)	100.0 (±7.4)	79.5 (±4.4)
		Forelimb Clonus		
Latency (s)	5.6 (±1.1)	9.2 (±2.4)	8.7 (±1.7)	9.3 (±1.7)
Duration (s)	54.8 (±2.9)	45.3 (±2.6)	41.7 (±7.0)	44.2 (±5.4)
		Seizure Stage		
	5.0 (±0.0)	5.0 (±0.0)	5.0 (±0.0)	5.0 (±0.0)

[a] See the Experimental Section for details. [b] MC = methylcellulose. [c] Data in parentheses represents the standard error based on $N = 8$ animals for 1 and $N = 6$ animals for 2.

CLOGP Evaluation. The dependence of biological activity in a set of congeneric agents on lipophilic character has been shown in many types of drug action.[28] In particular, the reports by Lien and co-workers indicated that anticonvulsant activity of different types of compounds was correlated with lipophilicity (log *P*, where *P* is the octanol–water partition coefficient).[29,30] Also Loscher and Frey have shown that a set of antiepileptic drugs penetrate the cerebrospinal fluid at a rate which parallels the benzene–water partition coefficient.[31] However, it was observed that the maximum potency of drugs which act on the central nervous system are obtained with congeners having an optimum lipophilicity near 2.[28] A computer program is available which provides calculated log *P* values, CLOGP.[12] CLOGP values for the active enaminone compounds varied from 1.734 for **42** to 4.573 for **30**, while lead compound, **1**, had a value of 3.233. In comparing the 1-substituted esters **1–33**, **55**, **56**, and **58–64** with a the unsubstituted analogs **35–54**, a consistent trend was noted. The unsubstituted analogs had a higher CLOGP value and lower activity (compound **2** had a CLOGP of 2.716 while **35**, the less active dimedone analog had a CLOGP of 3.459; **1** had a CLOGP value of 3.233, while **37**, the inactive dimedone analog, had a CLOGP value of 3.976). This trend was further noted by comparing active *p*-methyl **25** (CLOGP 3.246) with the inactive dimedone **39** (CLOGP 3.460) and active *p*-ethyl **7** (CLOGP 3.246) with the less active dimedone **40** (CLOGP 3.989).

While the CLOGP values of 1-carbethoxy analogs exceeded the comparable 1-carbomethoxy analogs by 0.529, an absolute correlation with activity could not be obtained. No difference in activity was observed with the highly active analog pairs which included *p*-chloro analogs **1** and **19**, *p*-fluoro analogs **12** and **24**, *p*-bromo analogs **13** and **23**, and *p*-trifluoromethyl analogs **15** and **31**. The trend to more active hydrophilic compounds was also observed with the highly active 1-carbomethoxybenzylamine **2** compared to inactive 1-carbethoxy **32** (CLOGP 3.245) and the highly active 1-carbomethoxy *p*-ethyl **7** compared the less active 1-carbethoxy **26**, while the opposite was observed with inactive 1-carbomethoxy *p*-*tert*-butyl **14** (CLOGP 4.044) with the highly active 1-carbethoxy **30** (CLOGP 4.573). In the latter case, the dimensions of the bulky *tert*-butyl group may have a more profound effect than lipophilicity. While each of the CLOGP values of the active analogs are higher than that postulated for maximum activity, the single exception being **42**, a second factor which should be borne in mind is the presence of polar moieties.[32,33] This factor will be discussed more fully in the structure–activity correlation section.

Molecular Modeling Studies. The report of the ADD Program on lead compound **1**[34] indicated that its anticonvulsant profile was almost identical to that of phenytoin, their primary effect being to prevent seizure spread. Thus, we hypothesized regions of molecular complementarity between these compounds.[2] To verify our initial hypothesis, a novel SYBYL program was employed.[35] Compound **1**, as a single diasteriomer with a trans orientation of the chiral centers as described for the X-ray crystal structure of **2**,[2] and phenytoin were individually minimized and their lowest energy conformers superimposed onto each other. This minimization verified our hypothesis that the 2-oxo carbonyl of **1** and the 2-oxo carbonyl of phenytoin were regions of commonality with the highest interactive potential. This superimposed

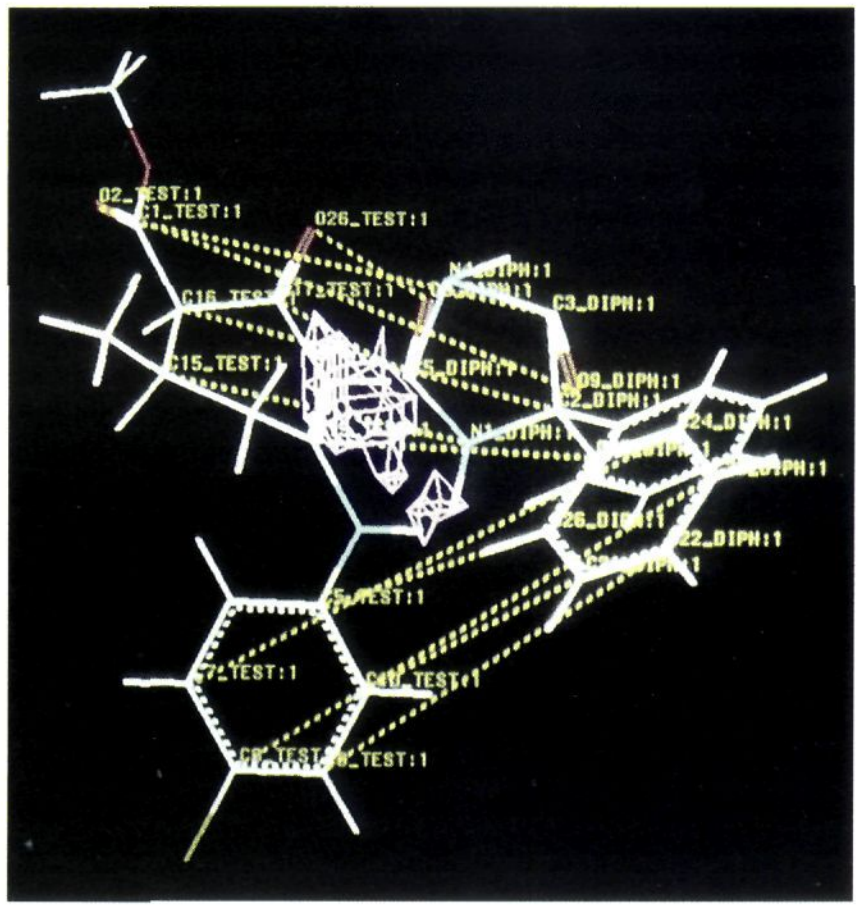


Figure 3. Superimposition of compound **1** (test) and phenytoin (Diph). Carbonyl O2, O26 (Test) and O9, O8 (Diph) were determined to have a high probability of coincidence as indicated by minimization analysis (see the Experimental Section).

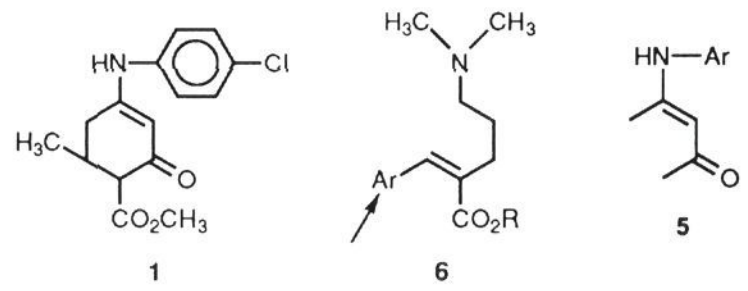


Figure 4. Structural correlation of cyclic enaminone **1**, with the Dimmock (ref 37) template **6**, and the acyclic enaminone of the acetylacetone series, **5**.

minimization is shown in Figure 3. All enaminones in Tables I and II were subsequently evaluated by this procedure. Quanta and CHARMM software were then employed to analyze each structure by classical molecular mechanics. As with **1**, all 1,6-substituents were depicted as a single diastereomer with a trans orientation of the chiral centers. Each structure was minimized and the lowest energy conformer superimposed onto phenytoin using the common carbonyl(s) as shown in Figure 3. These superimposed conformers were rigidly fitted, and the van der Waals volume was determined by two methods: the union volume, which displays all van der Waals volumes for both structures, and the intersection volume, that area where the structures overlap. All of the calculations had a grid resolution of 0.500 Å. The average union volume for these active analogs is 338.84 Å, while the intersection volume is 90.88 Å, the difference being 247.96 Å. The values for the inactive analogs are 369.92 and 110.37 Å, respectively, the difference in this group being 259.55 Å. The difference in fit is related to the degree of complementarity to phenytoin. The smaller difference with the active analogs would indicate a greater degree of agreement to the prototype anticonvulsant.[36]

Structure–Activity Correlation. Using **6** (Figure 4) as the template for the anticonvulsant Mannich bases, Dimmock[32] synthesized a series of 30 compounds, the most potent of which was active in the mouse MES assay and

inactive to benzodiazepine and GABA receptor binding displacement as well as the adenosine uptake evaluation similarly noted in our anticonvulsant enaminones. Template 6 also bears a remarkable resemblance to our lead compound 1. The lack of oral activity in the rat with compounds of template 6 may possibly be due to a combination of any of the following structural characteristics: (a) the lack of the missing fragment which provides the rigid configuration as in 1; (b) the aryl function (arrow in 6), which when placed in position 6 of the enaminone system[2] resulted in the abolition of activity; or (c) the tertiary amino (pyrrolidine, morpholine, aminomorpholine) functionality which was inactive in our studies, the requirement being a secondary arylamino function. To determine whether a rigid structural requirement was essential, a series of acyclic enaminones, 65–72 (Table II), using acetylacetone was employed. As noted, this series lacks position 6 in the cyclic system and has increased flexibility as observed by Dimmock.[37] Those active acyclic analogs in the phase I evaluation (compounds 65, 66, 68–72) uniformly displayed motor impairment at the higher dosages and were inactive in the phase VIA oral rat evaluation at 50 mg/kg. Thus, the cyclic enaminones were found to be more active than the acyclic analogs. The restricted conformation of the cyclic enaminones provides a more rigid interaction for anticonvulsant activity, affording a greater margin of safety, as well as providing oral activity. With regard to the cyclic analogs, several correlations were noted. Previously, our study was based on the Free–Wilson analysis[38] as modified by Craig.[39,40] It was found that anticonvulsant activity may be enhanced by para substitution in the $+\sigma$, $+\pi$ quadrant, and this finding was borne out in this continuing series, the only exception being the *p*-amino enaminone, 42.

1. **Increasing Bond Distance.** Comparing compounds 2, 9, 10, and 11, it should be noted that for maximum activity the distance of the secondary amine to the aromatic ring should not exceed two methylene groups as the phenylpropyl analog 10 and the phenylbutyl analog 11 were inactive. Also of interest was the fact that the branched-chain isomer of the active phenethyl analog 9, i.e. compound 8, was inactive. The phenethylamine analogs of dimedone 45[42] and cyclopentenone 54 were active, however, to a lesser degree, thus fortifying our initial hypothesis that for maximum activity, the 1-carbomethoxy group should be present. Compounds 49 and 54, the phenethyl analogs of cyclohexane and cyclopentane, respectively, and the comparable benzylamine analogs 48 and 53 were also active in phase I analysis at 100 mg/kg; however, in comparison to other more potent analogs herein reported, no further studies on these compounds were undertaken.

2. **Anilines.** Consistent with our initial findings, unsubstituted anilines 17 and 29 were inactive.

3. **$+\sigma$, $+\pi$ Quadrant.** Analogs synthesized are exemplified by *p*-chloro (1, 16, 19, 22, 33, 46, and 47), *p*-nitro (18, 27, and 38), *p*-fluoro (12, 24, and 34), *p*-bromo (13 and 23), and *p*-trifluoromethyl (15, 21, 31, 44, and 51). Of these 20 compounds, all but one possessed activity including the hindered 2-pyridinyl analog 16 previously discussed. The inactivity of compound 33, the 2-benzoyl homolog of 1, would indicate that there are steric constraints to aromatic substitution, while the inactivity of compound 34, a carboxamide, would indicate that this substituent produced unpredictable results as reported earlier. It has now been demonstrated that *p*-bromo and *p*-trifluoromethyl analogs possessed high anticonvulsant activity with a higher margin of safety than the comparable chloro compounds, all of whom exhibited toxicity at equivalent dosages.

3. **$-\sigma$, $+\pi$ Quadrant.** Analogs synthesized are exemplified by *p*-methyl (25 and 39), *p*-ethyl (7, 26, and 40), and *p*-*tert*-butyl (14, 20, and 30) substitutions. Of the eight compounds synthesized in this quadrant, five were active, quantitatively to a lesser degree than the previous $+\sigma$, $+\pi$ quadrant. In addition, the structure–activity correlation was less clear. *p*-Methyl substitution leads to an active 25 as well as inactive 39, *p*-ethyl substitution yields a highly active 7, less active 26 and an inactive 40, while *p*-*tert* butyl substitution produces inactive 14 and 20 but a highly active 30.

4. **$-\sigma$, $-\pi$ Quadrant.** Two analogs synthesized were the inactive *p*-methoxy 41 and the active *p*-amino analog 42. Of interest is the activity of the 2′,5′-dimethoxy analog 28. It should be noted that the *m*-methoxy group appears in the highly active $+\sigma$, $+\pi$ quadrant.[39] As noted from the above data, the synthetic thrust concentrated on the $+\sigma$, $+\pi$ quadrant. The assessment of the enaminones into a linear regression equation would, of necessity, involve the synthesis of all para (and meta) substituents in the Craig plot[39] from a single β-hydroxy ketone, and the ED50 and TD50 determination of all analogs.[41]

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. Kinetic hydrolysis studies were conducted on a Milton Roy 1201 UV spectrophotometer. TLC analysis employed a butanol–acetic acid–water (5:4:1) elution solvent mixture and 5- × 10-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 I and II. Ethyl 4-hydroxy-6-methyl-2-oxocyclohex-3-en-1-oate was prepared by method B[42] and methyl 4-hydroxy-6-methyl-2-oxocyclohex-3-en-1-oate was prepared by methods A and B.[2] Typical experiments illustrating the general procedures for the preparation of the enaminones are described below.

Methyl 6,6-Dimethyl-4-hydroxy-2-oxocyclohex-3-en-1-oate (3) ($R = CH_3$, $R^1 = R^2 = CH_3$). Method A. To a freshly prepared solution of sodium (23 g, 1 mol) in methanol (300 mL) was added dimethyl malonate (132 g, 1 mol), and the mixture was stirred on an ice bath for 15 min after the addition. Mesityl oxide (100 g, 1 mol) was added and the mixture stirred at room temperature for an additional 30 min. After the mixture was refluxed for 12 h, the white precipitate which separated was dissolved in a minimum amount of cold water and the aqueous solution was acidified with hydrochloric acid (400 mL of a 2.5 M solution) and extracted with dichloromethane (4 × 200 mL). The organic phase was dried (Na_2SO_4) and evaporated and the residue crystallized from toluene to give the title compound. The methanolic filtrate was evaporated to dryness, and the residue was dissolved in cold water, acidified with hydrochloric acid, and extracted with dichloromethane. The residue was recrystallized from toluene: total yield 122.3 g (62%); mp 99–101 °C; ^{1}H NMR ($CDCl_3$) δ 1.14 (6H, d, 2 × CH_3), 2.20 (2H, AB system, diastereotropic CH_2), 3.14 (1H, s, CH of cyclohexene ring), 3.70 (3H, s, OCH_3), 5.41 (1H, s, =CH), 9.35 (1H, br s, OH). Anal. (C, H).

Methyl 4′-[(4′-Chloro-2′-pyridinyl)amino]-6-methyl-2-oxocyclohex-3-en-1-oate (16). Method C. To a solution of 3

(R = CH_3, R^1 = CH_3, R^2 = H) (5 g, 27 mmol) in 140 mL of benzene was added 2-amino-5-chloropyridine (3.86 g, 30 mmol), and the mixture was refluxed for 5 h using a Dean–Stark water separator. During the reaction, 0.50 mL of water was collected. A light yellow precipitate formed on cooling to room temperature (1.5 g, mp 197–200 °C). The filtrate was evaporated under reduced pressure to yield an orange residue which was recrystallized from toluene to yield a second crop (1.95 g, mp 197–198 °C). Further recrystallization of both crops from toluene gave 16, in a total yield of 43% (3.45 g, mp 206–208 °C): ^{1}H NMR (DMSO-d_6) δ 0.96 (3H, d, J = 4.89 Hz, CH_3), 2.24–3.58 (4H, m, CH_2 + 2CH of cyclohexene ring), 3.61 (3H, s, OCH_3), 6.82 (1H, s, =CH), 7.03–8.32 (3H, m, pyridine ring), 9.57 (1H, s, NH). Anal. (C, H, N, Cl).

Ethyl 4-[(4′-Chloro-2′-benzoylphenyl)amino]-6-methyl-2-oxocyclohex-3-en-1-oate (33). Method D. Compound **3** (R = C_2H_5, R^1 = CH_3, R^2 = H) (1.98 g, 10 mmol)[2] was dissolved in absolute ethanol (150 mL), 2-amino-5-chlorobenzophenone (2.6 g, 11 mmol) was added, and the mixture was refluxed for 4 h. After the solution was evaporated under reduced pressure, the residual red oil was triturated with ethyl acetate–petroleum ether (bp 37–54 °C) and refrigerated until crystals were formed. The crystals were collected and recrystallized from ethyl acetate to yield **33** (1.0 g, 24%, mp 154–156 °C): ^{1}H NMR ($CDCl_3$) δ 1.24 (3H, d, J = 7.36 Hz, CH_3), 1.25 (3H, t, J = 6.25 Hz, CH_3), 1.63–3.54 (3H, m, CH_2 + CH of cyclohexene ring), 4.19 (3H, m, CH_2 of ethyl group + CH of cyclohexene ring), 7.16–7.56 (7H, m, C_6H_5 + NH + vinyl H), 7.68–8.03 (3H, m, C_6H_3 ring). Anal. (C, H, N, Cl).

3-[(4′-Chlorophenyl)amino]-5-methyl-2-cyclohexen-1-one (46). Method D. 5-Methyl-1,3-cyclohexanedione (5 g, 38.8 mmol) was added to *p*-chloroaniline (5.5 g, 42.7 mmole) (*CAUTION*! Possible mutagen), dissolved in a mixture of benzene (100 mL) and absolute ethanol (75 mL), and the mixture was refluxed for 4 h. Evaporation of the solvents produced a yellow solid (2.43 g, 31%) which was recrystallized successively from toluene (mp 195–196 °C) and a mixture of ethyl acetate–acetone–methanol (2:2:1) and provided an analytical sample (mp 198–199.5 °C). Workup of the mother liquors produced additional **46** (5.80 g), providing an overall yield of 88%: ^{1}H NMR (DMSO-d_6) δ 1.02 (3H, d, J = 6.29 Hz, CH_3), 1.84–2.54 (5H, m, cyclohexene ring), 5.32 (1H, s, =CH), 7.16–7.48 (4H, m, C_6H_4), 8.91 (1H, s, NH). Anal. (C, H, N, Cl).

3-[(4′-Chlorophenyl)amino]-2-cyclopentenone (52). Method D. Cyclopentane-1,3-dione (5 g, 51 mmol) and *p*-chloroaniline (7.15 g, 56 mmol) (*CAUTION*! Possible mutagen) were dissolved in a mixture of 50 mL of benzene and 75 mL of absolute ethanol, and the mixture was refluxed for 4 h. On cooling and evaporation under reduced pressure, a solid residue formed. This residue was recrystallized from acetone–methanol–ethyl acetate: yield 8.83 g (84%); mp 216–217 °C; ^{1}H NMR. (DMSO-d_6) δ 2.21–2.76 (4H, m, cyclopentene ring), 5.45 (1H, s, =CH), 7.21–7.43 (4H, m, C_6H_4), 9.69 (1H, s, NH). Anal. (C, H, N, Cl).

Kinetic Hydrolysis. Compound **1** (5.9 mg) was dissolved in 95% ethanol and diluted to 50 mL with ethanol. This solution was 4.0×10^{-5} M. Phosphate buffer, pH 7.4, and water were used in this study. All experiments were performed in triplicate. A volume of the stock ethanol solution of **1** (0.2 mL) was diluted with the appropriate solvent to make 1 mL of solution. Each mixture was sealed in a Teflon-capped reaction vial and incubated at 37 °C for the same time periods as indicated in the ip rat toxicity study (0.25, 0.5, 1.0, 2.0, 4.0, 6.0, 8.0, and 24.0 h). After each time period, the vials were removed from the incubator and quenched at −80 °C until ready for analysis. Initial scan (t = 0 h) indicated a λ_{max} at 311 nm for **1**. The potential hydrolysis products, methyl 4-hydroxy-6-methyl-2-oxocyclohex-3-en-1-oate had a λ_{max} of 277 nm and *p*-chloroaniline had a λ_{max} of 232 nm under the same conditions. After all samples were incubated, the UV spectra was taken from 190 to 500 nm using the respective solvent as a blank. The results were plotted in terms of absorbance vs time of incubation. The average absorbance value for **1** in aqueous media was 0.929 (range 0.789–1.069), while in pH 7.4 buffer the average value was 1.112 (range 0.983–1.240), however the wavelength did not change from 311 nm. Statistical analysis revealed that these individual differences were not significant (p <0.05).

Enzymatic Hydrolysis. A solution of **1** (4×10^{-5} M), prepared as indicated above, Tris buffer (0.01 M, pH 8.0), and esterase (EC 3.1.1.1, 200 units/mg, 19 mg dissolved in Tris buffer and diluted to 10 mL) was employed. The above assay was repeated in triplicate using 0.2 mL of stock solution, 1 mL buffer, and 0.1 mL of enzyme solution, and the incubation was carried out at 37 °C for each time period reported in the ip rat toxicity evaluation (i.e. 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h). The blank (time = 0 h) contained 0.2 mL of stock solution and 1.1 mL of buffer but no enzyme. After each time period, the samples were quenched with 1 mL of methylene chloride and frozen at −80 °C until ready for assay. In a preliminary experiment, **1** and **46**, each 4×10^{-5} M in methylene chloride, were analyzed by UV spectroscopy. The λ_{max} values for **1** were 301.5 and 226.7 nm, while the λ_{max} values for **46** were 301.5 and 229.4 nm. The latter wavelength differentiation was employed in the subsequent assay. Each sample was thawed, and the methylene chloride layer scanned in the UV region. Analysis at 24 h indicated a 20% decrease in concentration compared to the blank.

CLOGP. CLOGP computations for the enaminones listed in Tables I and II were performed on a Silicon Graphics Personal Iris 4D/35 workstation running PCmodels application of the 4.2 version of the Daylight software.[12]

Molecular Modeling. Computations were performed on a Silicon Graphics Personal Iris 4D/35 workstation running Tripos SYBYL/Advanced Computation software. Compound **1** and phenytoin were minimized employing steepest descents (100 minimization steps) and subsequently by adopted-basis Newton Raphson (ABNR, 1000 steps). Each minima were superimposed as described.[36] Molecular Simulations Quanta and CHARMM molecular mechanics software[13] was employed for all other compounds. A principal structure file (PSF) was derived for the C=CNC fragment. All other fragments were available in the residual topology file (RTP). Each structure in Tables I and II was initially minimized employing steepest descents (100 minimization steps) and subsequently by adopted-basis Newton Raphson (ABNR, 1000 steps). These individual minima were saved for a conformational search employing the torsional angles for carbons C3, C4, and N5 (torsion 1) and C4, N5, and aryl C7 (torsion 2). All torsions were restricted to 180° ± 20° as noted by previous studies.[43,44] These analogs were minimized employing ABNR (100 steps), and the lowest energy conformer was retained for molecular similarity studies with phenytoin. The minimized analog and phenytoin were matched manually using the carbonyl groups as the initial regions of best fit.[36] The molecular volumes were calculated within grid point range of 0.500 Å. A typical superimposition is shown with phenytoin and **1** in Figure 3.

Pharmacology. Initial evaluations for anticonvulsant activity were done by the Antiepileptic Drug Development (ADD) Program, Epilepsy Branch, Neurological Disorders Program, National Institute of Neurological and Communicative Disorders and Stroke and included phases I, II, and VIA test procedures which have previously been described.[14] These tests were performed in male Carworth Farms no. 1 (CF1) mice. Phase I of the evaluation included three tests: maximal electroshock (MES), subcutaneous pentylenetetrazol (scMet), and the rotorod test for neurological toxicity (Tox). Compounds were either dissolved or suspended in 30% poly(ethylene glycol) 400 or 0.5% aqueous methyl cellulose and were administered by intraperitoneal injection 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 VIA 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 VIA 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 III and IV.

Compounds **1** and **2** were also evaluated in the amygdala kindling model. The methods for amygdala kindling including procedures for stereotaxic surgery and scoring of electroencephalographic (EEG) and behavioral variables have been detailed previously.[45] Briefly, male Sprague–Dawley rats (260–300 g) were surgically implanted with electrodes in the amygdala and in the skull over the frontal cortex. After 7–10 days recovery from

surgery, rats were stimulated twice daily in the amygdala with a 400-μA constant current stimulation (60 Hz, biphasic symmetrical square wave, 2.0-ms total pulse duration) until a stage 5 amygdala kindled seizure[45] was elicited. Rats were then kindled once daily until a criterion of 10 stage 5 seizures was achieved. The effects of compound 1 (350 mg/kg, po; $n = 8$) and 2 (500 mg/kg, po; $n = 6$) were evaluated in a group of amygdala kindled rats which had met this criterion. Drugs were administered in 0.5% methyl cellulose by gastric intubation 0.5 h prior to amygdala stimulation, the time of peak anticonvulsant effect as determined in mouse anticonvulsant studies.[2] Baseline seizure responses for comparison of drug effects, determined prior to the evaluation of each compound, were measured on 2 consecutive days 1 h after po administration of the vehicle. Toxicity of the compounds was evaluated 5 min before anticonvulsant testing by evaluating gross motor impairment (ataxia), muscle relaxation, and ability to stay on a vertical screen.[46] These data are shown in Table V.

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Supplementary Material Available: Tables VI–VIII giving phase II mouse (ip) data and phase VIA rat (po) data, intraperitoneal time of peak effect and toxicity of compounds **16**, **22**, **23**, **25**, **30**, and **31** in the rat, and intraperitoneal evaluation of compound **13** and **15** in the rat (11 pages). Ordering information is given on any current masthead page.

References

(1) Presented in part at the Fourteenth Annual Undergraduate Research Seminar, University of West Virginia, 1992, by E. L. Richardson.
(2) The previous paper in this series: Edafiogho, I. O.; Hinko, C. N.; Chang, H.; Moore, J. A.; Mulzac, D.; Nicholson, J. M.; Scott, K. R. Synthesis and anticonvulsant activity of enaminones. *J. Med. Chem.* **1992**, *35*, 2798–2805.
(3) Scott, K. R.; Moore, J. A.; Zalucky, T. B.; Nicholson, J. M.; Lee, J. M.; Hinko, C. N. Spiro[4.5] and spiro[4.6]carboxylic acids: cyclic analogues of valproic acid. Synthesis and anticonvulsant evaluation. *J. Med. Chem.* **1985**, *28*, 413–417.
(4) Tarver, M. L.; Nicholson, J. M.; Scott, K. R. Spirosuccinimides as potential anticonvulsants. *J. Pharm. Sci.* **1985**, *74*, 785–787.
(5) Scott, K. R.; Sethi, M. L.; Lee, J. M.; Nicholson, J. M.; Warner, P. E.; Acheampong, A. A. Spiranes III. Structure-activity correlation on selected anticonvulsant spirocarboxylic acids. *Trans. Pharm. Sci.* **1987**, *I*, 23–37.
(6) Sethi, M. L.; Scott, K. R.; Acheampong, A. A. Effect of cyclic analogues of valproic acid on glutamic decarboxylase activity as determined by different methods. *Phytochemistry* **1987**, *26*, 3141–3144.
(7) Edafiogho, I. O.; Scott, K. R.; Moore, J. A.; Farrar, V. A.; Nicholson, J. M. Synthesis and anticonvulsant activity of imidooxy derivatives. *J. Med. Chem.* **1991**, *34*, 387–392.
(8) Scott, K. R.; Edafiogho, I. O.; Moore, J. A.; Farrar, V. A.; Nicholson, J. M. Synthesis and anticonvulsant activity of a spirosuccinimide. *Pharmacy World J.* **1991**, *8*, 44–50.
(9) Edafiogho, I. O.; Hinko, C. N.; Farrar, V. A.; Moore, J. A.; Nicholson, J. M.; Scott, K. R. Imidooxy anticonvulsants: Structural analogs with special emphasis on N-(benzyloxy)-2-azaspiro[4.4]nonane-1,3-dione. *Drugs Future* **1992**, *17*, 395–408.
(10) Romussi, G.; Parodi, B.; Bignardi, G.; Menozzi, G.; Scheone, P. Reaction of N,N-disubstituted α-aminomethyleneketones with tosyl isocyanate. *Il Farmaco Ed. Sc.* **1986**, *41*, 539–547.
(11) Kase', Y.; Saita, M.; Takahama, K.; Masaki, K.; Miyata, T. Pharmacological studies on centrally-acting drugs belonging to enaminone Mannich bases. II. Pharmacology of MK 1-203 and 1-907, new potent analgesics. *Jpn. J. Pharmacol.* **1974**, *24* (Suppl.), 86.
(12) Leo, A.; Weninger, D.; Weninger, A. CLOGP, CMR Medicinal Chemistry Project, Pomona College: Claremont, CA 91711; version 4.2, distributed by Daylight Information Systems, 1992.
(13) Quanta Release 3.2.3, Molecular Simulations Corporation, Waltham, MA, 1992.
(14) (a) Anticonvulsant Screening Project, Antiepileptic Drug Development Program, National Institutes of Health, DHEW Publ (NIH)(U.S.) 1978, NIH 78-1093. (b) Porter, R. J.; Cereghino, J. J.; Gladding, G. D.; Hessie, B. J.; Kupferberg, H. J.; Scoville, B.; White, B. G. Antiepileptic drug development program. *Cleveland Clin. Q.* **1984**, *51*, 293–305. (c) Krall, R. L.; Penry, J. K.; White, B. G.; Kupferberg, H. J.; Swinyard, E. A. Antiepileptic drug development: II. Anticonvulsant drug screening. *Epilepsia* **1978**, *19*, 400–428.
(15) Swinyard, E. A.; Woodhead, J. H.; White, H. S.; Franklin, M. R. General Principles-Experimental selection, quantification and evaluation of anticonvulsants. In *Antiepileptic* Drugs, 3rd ed.; Levy, R. H., Dreifuss, F. E., Mattson, R. H., Meldrum, B. S., Penry, J. K., Eds.; Raven Press: New York, 1989; p 98.
(16) *The Merck Index*, 10th ed.; Windholz, M., Budavari, S., Blumetti, R. R., Otterbein, E. S., Eds.; Merck and Company: Rahway, NJ, 1983; p 2088.
(17) Rankin, G. O.; Yang, D. J.; Cressey-Veneziano, K.; Casto, S.; Wang, R. T.; Brown, P. I. In vivo and in vitro nephrotoxicity of aniline and its monochlorophenyl derivatives in the Fischer 344 rat. *Toxicology* **1986**, 269–283.
(18) Loscher, W.; Schmidt, D. Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. *Epilepsy Res.* **1988**, *2*, 145–181.
(19) Kupferberg, H. Antiepileptic drug development program: A cooperative effort of government and industry. *Epilepsia* **1989**, *30* (Suppl. 1), S51–56.
(20) Skeen, G.; Woodhead, J.; Wolf, H.; Swinyard, E.; Tietz, E.; White, S. Development of kindled seizures following electrical stimulation via the cornea. *Soc. Neurosci. Abs.* **1990**, *16*, Abs. 138.1.
(21) Schmitz, E.; Loscher, W.; Honack, D.; Reichelt, R.; Hebebrand, J. Kindling does not induce persistent changes in fluorographic labeling patterns of benzodiazepine binding proteins in various rat brain regions. *Epilepsy Res.* **1991**, *9*, 105–112.
(22) Kalichman, M. W. Neurochemical correlates of the kindling model of epilepsy. *Neurosci. Behav. Rev.* **1982**, *6*, 165–181.
(23) McNamara, J. O. Kindling model of epilepsy. In *Basic Mechanisms of the Epilepsies, Molecular and Cellular Approaches*; Delgado-Escueta, A. V., Ward, A. A., Jr., Woodbury, D. M., Porter, R. J., Eds.; Raven Press: New York, 1986; pp 308–318.
(24) Burnham, W. M.; Niznik, H. B.; Kish, S. J. Biochemical changes in the limbic system following kindling: assay studies. In *The Limbic System: Functional Organization and Clinical Disorders*; Doane, B. K., Livingston, K. E., Eds.; Raven Press: New York, 1986; pp 123–128.
(25) McNamara, J. O. Pursuit of the mechanisms of kindling. *Trends Neurosci.* **1988**, *11*, 33–36.
(26) Bureau, M.; Olsen, R. W. $GABA_A$/benzodiazepine receptor carries binding sites on both two major peptide subunits. *Biochem. Biophys. Res. Commun.* **1988**, *153*, 1006–1011.
(27) Peterson, S. L.; Albertson, T. E. Neurotransmitter and neuromodulator function in the kindled seizure and state. *Prog. Neurobiol.* **1990**, *19*, 237–270.
(28) Hansch, C.; Bjorkroth, J. P.; Leo, A. Hydrophobicity and central nervous system agents: On the principle of minimal hydrophobicity in drug design. *J. Pharm. Sci.* **1987**, *76*, 663–687.
(29) Lien, E. J.; Tong, G. L.; Chou, J. T.; Lien, L. L. Structural requirement for centrally acting drugs. I. *J. Pharm. Sci.* **1973**, *62*, 246–250.
(30) Lien, E. J.; Liao, R. C. H.; Shinouda, H. G. Quantitative structure-activity relationships and dipole moments of anticonvulsant and CNS depressants. *J. Pharm. Sci.* **1979**, *68*, 463–465.
(31) Loscher, W.; Frey, H. H. Kinetics of penetration of common antiepileptic drugs into cerebrospinal fluid. *Epilepsia* **1984**, *25*, 346–352.
(32) Hansch, C.; Steward, A. R.; Anderson, S. M.; Bentley, D. The parabolic dependence of drug action upon lipophilic character as revealed by a study of hypnotics. *J. Med. Chem.* **1967**, *11*, 1–11.
(33) Glave, W. R.; Hansch, C. Relationship between lipophilic character and anesthetic activity. *J. Pharm. Sci.* **1972**, *61*, 589–591.

(34) (a) Swinyard, E. A.; Wolf, H. H.; Franklin, M. R.; White, H. S.; Woodhead, J. H.; Kupferberg, H. J.; Stables, J. P. The profile of anticonvulsant activity and minimal toxicity of 196022 and some prototype antiepileptic drugs in mice and rats, Epilepsy Branch, Neurological Disorders Program, NINDS, 1992. (b) Mulzac, D.; Scott, K. R. The profile of anticonvulsant activity and minimal toxicity of methyl 4-[(p-chlorophenyl)amino]-6-methyl-2-oxocyclohex-3-en-1-oate and some prototype antiepileptic drugs in mice and rats. *Epilepsia*, in press.
(35) Andrews, C. W. Program for conformational minimization, Tripos Associates Users Group Meeting, 1992. Used by permission.
(36) Molecular Simulations, personal communication.
(37) Dimmock, J. R.; Jonnalagadda, S. S.; Phillips, O. A.; Erciyas, E.; Shyam, K.; Semple, H. A. Anticonvulsant properties of some Mannich bases of conjugated arylidene ketones. *J. Pharm. Sci.* **1992**, *81*, 436–440.
(38) Free, S. M., Jr.; Wilson, J. W. A mathematical contribution to structure-activity studies. *J. Med. Chem.* **1964**, *7*, 395–399.
(39) Craig, P. N. Interdependence between physical parameters and selection of substituent groups for correlation studies. *J. Med. Chem.* **1971**, *14*, 680–684.
(40) Craig, P. N. Structure-activity correlations of antimalarial compounds. 1. Free-Wilson analysis of 2-phenylquinoline-4-carbinols. *J. Med. Chem.* **1972**, *15*, 144–148.
(41) Dr. Paul N. Craig, personal communication.
(42) Spencer, T. A.; Newton, M. D.; Baldwin, S. W. Condensation of diethyl malonate with methyl vinyl ketone. *J. Org. Chem.* **1964**, *29*, 787–789.
(43) Friedinger, R. M.; Whitter, W. L.; Gould, N. P.; Holloway, M. K.; Chang, R. S. L.; Lotti, V. J. Novel glutamic acid derived cholecystokinin receptor ligands. *J. Med. Chem.* **1990**, *33*, 591–595.
(44) Oberlender, R.; Pfaff, R. C.; Johnson, M. P.; Huang, X.; Nichols, D. E. Stereoselective LSD-like activity in d-lysergic acid amides of (R)- and (S)-2-aminobutane. *J. Med. Chem.* **1992**, *35*, 203–211.
(45) Tietz, E. I.; Rosenberg, H. C.; Chiu, T. H. A comparison of the anticonvulsant effects of 1,4 and 1,5 benzodiazepines in amygdala kindled rat and their effects on motor function. *Epilepsy Res.* **1989**, *3*, 31–40.
(46) Racine, R. J. Modification of seizure activity by electrical stimulation. II. Motor seizure. *Electroenceph. Clin. Neurophysiol.* **1972**, *32*, 281–294.
(47) Kotera, K. Infrared absorption spectra of cyclic diketones. IV. Studies on keto-amine derivatives. *Yakugaku Zasshi* **1960**, *80*, 1275–1278.
(48) Martin, D. F.; Janusonis, G. A.; Martin, B. B. Stabilities of bivalent metal complexes of some β-ketoimines. *J. Am. Chem. Soc.* **1961**, *83*, 73–75.
(49) Roberts, E.; Turner, E. E. The factors controlling the formation of some derivatives of quinoline, and a new aspect of the problem of substitution in the quinoline series. *J. Chem. Soc.* **1927**, 1832–1857.
(50) Naringrekar, V. H.; Stella, V. J. Mechanism of hydrolysis and structure-stability relationship of enaminones as potential prodrugs of model primary amines. *J. Pharm. Sci.* **1990**, *79*, 138–146.
(51) Greenhill, J. V. Aromatic Enaminones. Part 1. Ultraviolet absorption of N-aryl enaminones derived from dimedone. *J. Chem. Soc., Perkin Trans. 1* **1976**, 2207–2210.
(52) Greenhill, J. A. Determination of the tautomeric ratio in certain enaminones. *J. Chem. Soc. B* **1969**, 299–300.
(53) Rugheimer, L.; Ritter, G. On (β-benzyliminopropyl)-methyl ketone: A contribution to the knowledge of keto–enol isomerism. *Ber.* **1912**, *45*, 1332–1340.
(54) Dudek, G. O.; Holm, R. H. Nuclear magnetic resonance studies of keto-enol equilibria. III. α,β-Unsaturated-β-ketoamines. *J. Am. Chem. Soc.* **1962**, *84*, 2691–2696.