(concentration of ligand which reduced maximal specific [^{3}H]PZ binding by 50%) were obtained by fitting the data to a one site binding model by nonlinear regression. The IC_{50} values were corrected for receptor occupancy by [^{3}H]PZ as described by Cheng and Prusoff[68] to give K_A values (concentration of ligand that causes half-maximal receptor occupancy in the absence of [^{3}H]PZ). For comparative purposes, unlabeled pirenzepine (M_1), AF-DX 116 (M_2) and 4-DAMP (M_3) were used to determine the rank order of potency of the subtype selective antagonists. All assays were performed in polyethylene tubes and equilibrated at 30 °C for 60 min. Bound radioactivity was trapped on Whatman GF/B glass fiber filters that were soaked in 50 mM sodium-potassium phosphate buffer containing 0.05% polyethylenimine (pH 7.4).

In separate studies, saturation experiments were conducted by incubating the tissue homogenate with [^{3}H]PZ (1-100 nM) in a total volume of 1 mL. Nonspecific binding was determined in the presence of 10 μM atropine. Protein was determined by the method of Lowry et al.[69] These studies showed that [^{3}H]PZ had a K_D = 15.5 ± 0.7 nM and a B_{max} = 580 ± 72 fmol/mg protein (N = 4). In addition, 11-point competition curves for pirenzepine (M_1), AF-DX 116 (M_2; 11-[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6*H*-pyrido[2,3-*b*][1,4]benzodiazepin-6-one) and 4-DAMP (M_3; 4-(diphenylacetoxy)-*N*-methylpiperidine methiodide) were constructed to confirm the muscarinic subtype associated with the rabbit ganglia. The affinities of these compounds are shown in Table II.

Acknowledgment. Support for this study was provided by grants from the Swedish Natural Science Research Council and United States Public Health Service (Grant No. GM-37816). The authors thank Dr. Chris Folkeson Welch for recording the ^{13}C-^{1}H correlation spectrum of compound **31** and Professor Kosta Steliou for kindly providing a personal copy of PCMODEL (-89).

Registry No. (±)-**8**, 141063-74-3; (*S*)-**8**, 141194-08-3; (*S*)-**8** oxalate, 141194-09-4; (*R*)-**8** oxalate, 141194-07-2; (±)-**9**, 141063-75-4; (*S*)-**9**, 141194-11-8; (*R*)-**9**, 141194-10-7; (±)-**10**, 141063-76-5; (*R*)-**10** oxalate, 141194-13-0; (*S*)-**10** oxalate, 141194-15-2; (±)-**11**, 141063-77-6; (*R*)-**11**, 141194-16-3; (*S*)-**11**, 141194-17-4; **12**, 141063-78-7; (±)-**13**, 141063-79-8; **14**, 141063-80-1; (±)-**15**, 141063-81-2; **16**, 141063-83-4; (±)-**17**, 141063-84-5; (±)-**17** oxalate, 141063-85-6; **18**, 141063-86-7; (±)-**19**, 141063-87-8; (±)-**20**, 65337-13-5; (*R*)-**21**, 141194-18-5; (*S*)-**21**, 141194-19-6; (±)-**21** oxalate, 141063-89-0; (*R*)-**21** oxalate, 141269-21-8; (*S*)-**21** oxalate, 141269-22-9; **22**, 141063-90-3; **22** oxalate, 141063-91-4; (±)-**23**, 141063-92-5; **24**, 75858-50-3; **24** oxalate, 141063-93-6; (±)-**25**, 141063-94-7; (±)-**25** oxalate, 141063-95-8; (±)-**36**, 61489-97-2; (±)-**27**, 141063-96-9; (*R*)-**28**, 141063-97-0; (*S*)-**28**, 141064-08-6; (±)-**29** (isomer 1), 141063-98-1; (±)-**29** (isomer 2), 141064-04-2; (*R*)-**30**, 3113-93-7; (*S*)-**30**, 100837-07-8; (*R,R'*)-**31**, 114351-86-9; (*S,S'*)-**31**, 100837-08-9; **32a**, 141063-99-2; **32b**, 141064-05-3; **33**, 3004-45-3; (*R*)-**34**, 141064-00-8; (*S*)-**34**, 141064-06-4; **35**, 25217-01-0; **36**, 69921-30-8; (*R*)-**37**, 141064-01-9; (*S*)-**37**, 141064-07-5; **38**, 141064-02-0; (*S*)-(-)-MTPA, 17257-71-5; (*R*)-MTPA chloride, 39637-99-5; $NHMe_2$, 124-40-3; $CF_3CON(Me)CH_2C{\equiv}CH$, 111903-30-1; $Me_2NCH_2NMe_2$, 51-80-9; (±)-$CF_3CON(Me)CH(Me)C{\equiv}CH$, 141064-03-1; ClC_6H_4-*m*-CH_2COCl, 41904-39-6; 3-chlorophenyl isocyanate, 2909-38-8; 3-chlorophenylacetic acid, 1878-65-5.

Supplementary Material Available: ^{1}H and ^{13}C NMR spectral data for compounds (±)-**10** (base), (±)-**11**, **12**, **14-16**, **18**, **19**, **27**, and ^{1}H NMR spectral data for (*R*)-**8**-oxalate, (*S*)-**10**-oxalate, (±)-**21** (base), and **24**-oxalate (2 pages). Ordering information is given on any current masthead page.

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Synthesis and Anticonvulsant Activity of Enaminones

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A new series of novel enaminones has been synthesized from cyclic β-dicarbonyl precursors which were condensed with morpholine, pyrrolidine, phenethylamine, hydrazines, substituted benzyl amines, and substituted anilines. These compounds were subsequently evaluated for anticonvulsant activity in a variety of anticonvulsant models by the National Institute of Neurological and Communicative Disorders and Stroke and in our laboratory. Several of these compounds exhibited potent anticonvulsant activity with a remarkable lack of neurotoxicity. The most active analog, methyl 4-[(*p*-chlorophenyl)amino]-6-methyl-2-oxo-cyclohex-3-en-1-oate (**27**), was protective in the maximal electroshock (MES) seizure test in the rat with an oral ED_{50} of 5.8 mg/kg with no toxicity noted at doses up to 380 mg/kg, thus providing a protective index (TD_{50}/ED_{50}) of >65.5. A similar protective index for **27** was noted upon intraperitoneal (ip) administration in mice. The anticonvulsant effect of **27** occurred within 15 min of administration and the compound remained active beyond 4 h. Compound **27** was also active in the rat corneal kindled model. The application of Free-Wilson analysis to structure-activity correlation in this series is discussed.

Enamines have been shown by previous workers[1-6] to be highly unstable in aqueous solution. Enaminones, enamines of β-dicarbonyl compounds, however are quite stable and have been employed as prodrugs with variable results.[7-12] In addition, two articles have reported the potential use of enaminones for biological purposes.[13,14] Scheone and co-workers[13] indicated that several enaminones prepared were evaluated for hypoglycemic effectiveness; however, they reported poor activity. Kase'

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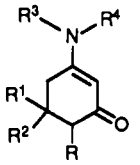
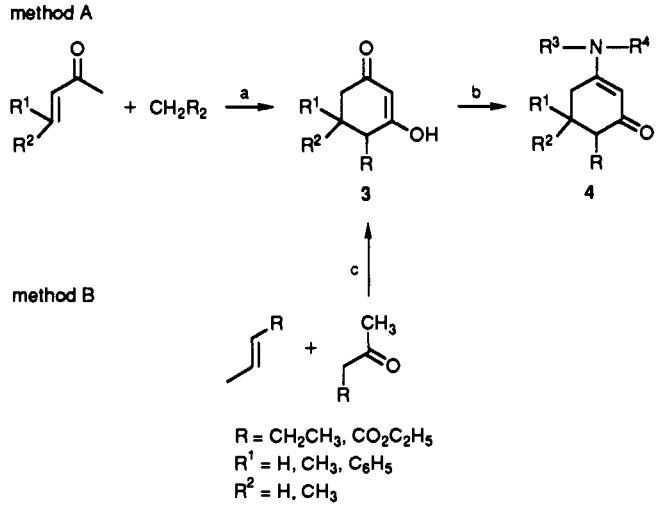
Table I. Physical Properties of Enaminones[a]



compd	R	R^1	R^2	R^3	R^4	% yield, method	mp, °C or bp, °C	formula	anal.[b]
5	CO_2CH_3	CH_3	CH_3		$-(CH_2)_4-$	76, C	112–113[c]	$C_{14}H_{21}NO_3$	C, H, N
6	CO_2CH_3	CH_3	CH_3		$-(CH_2)_2O(CH_2)_2-$	45, C	132–133[d]	$C_{14}H_{21}NO_4$	C, H, N
7	CO_2CH_3	CH_3	H		$-(CH_2)_2O(CH_2)_2-$	49, C	125–127[e]	$C_{13}H_{19}NO_4$	C, H, N
8	CO_2CH_3	C_6H_5	H		$-(CH_2)_2O(CH_2)_2-$	63, C	194–195[f]	$C_{18}H_{21}NO_4$	C, H, N
9	CO_2CH_3	CH_3	H		$-(CH_2)_4-$	76, C	138–139[d]	$C_{13}H_{19}NO_3$	C, H, N
10	$CO_2C_2H_5$	C_6H_5	H		$-(CH_2)_4-$	48, C	168–169[f]	$C_{19}H_{23}NO_3$	C, H, N
11	$CONHCH_2C_6H_5$	C_6H_5	H	H	$CH_2C_6H_5$	22, E	219–220[g]	$C_{27}H_{26}N_2O_2$	C, H, N
12	CO_2CH_3	CH_3	H	H	$CH_2C_6H_5$	90, C	154–155[d]	$C_{16}H_{19}NO_3$	C, H, N
13	CO_2CH_3	CH_3	CH_3	H	$CH_2C_6H_5$	79, C	138–139[d]	$C_{17}H_{21}NO_3$	C, H, N
14	CO_2CH_3	CH_3	CH_3	H	$(CH_2)_2C_6H_5$	46, C	130–131[f]	$C_{18}H_{23}NO_3$	C, H, N
15	$CONH(CH_2)_2C_6H_5$	CH_3	H	H	$(CH_2)_2C_6H_5$	55, E	171–172[d]	$C_{24}H_{28}N_2O_2$	C, H, N
16	CO_2CH_3	CH_3	H	H	$CH_2C_6H_4(p\text{-Cl})$	64, C	173–174[g]	$C_{16}H_{18}NO_3Cl$	C, H, N, Cl
17	CO_2CH_3	CH_3	H	H	$CH_2C_6H_4(p\text{-}CH_3)$	48, C	160–163[g]	$C_{17}H_{21}NO_3$	C, H, N
18	CO_2CH_3	CH_3	H	H	$CH_2C_6H_4(p\text{-}CO_2H)$	63, C	231–235 dec[g]	$C_{17}H_{19}NO_5$	C, H, N
19	H	CH_3	CH_3	H	$CH_2C_6H_5$	15, C	124–127[g,h]	$C_{15}H_{19}NO$	C, H, N
20	CO_2CH_3	CH_3	H	H	$CH_2C_6H_4(p\text{-}NO_2)$	66, C	174–176[i]	$C_{16}H_{18}N_2O_5$	C, H, N
21	CO_2CH_3	CH_3	H	H	$CH_2C_6H_4(p\text{-F})$	62, C	174–176[g]	$C_{16}H_{17}NO_3F$	C, H, N
22	$CONHCH_2C_6H_5$	CH_3	H	H	$CH_2C_6H_5$	81, E	219–220[g]	$C_{22}H_{24}N_2O_2$	C, H, N
23	$CONHC_6H_5$	CH_3	H	H	C_6H_5	34, F	243–244[i]	$C_{20}H_{20}N_2O_2$	C, H, N
24	CO_2CH_3	CH_3	H	H	C_6H_5	28, D	141–144[f]	$C_{15}H_{17}NO_3$	C, H, N
25	CO_2CH_3	CH_3	H	H	NHC_6H_5	71, D	167–167.5[k]	$C_{15}H_{18}N_2O_3$	C, H, N
26	CO_2CH_3	CH_3	H	H	$C_6H_4(p\text{-}C_2H_5)$	43, D	153.5–155[f]	$C_{17}H_{21}NO_3$	C, H, N
27	CO_2CH_3	CH_3	H	H	$C_6H_4(p\text{-Cl})$	61, D	178–180[f]	$C_{15}H_{16}NO_3Cl$	C, H, N, Cl
28	CO_2CH_3	CH_3	H	H	$C_6H_4(p\text{-}CH_3)$	67, D	144–146[f]	$C_{16}H_{19}NO_3\cdot H_2O$	C, H, N
29	CO_2CH_3	CH_3	H	H	$C_6H_4(p\text{-}NO_2)$	55, D	186–187[f]	$C_{15}H_{16}N_2O_5$	C, H, N
30	CO_2CH_3	CH_3	H	H	$C_6H_3(2'\text{-}OCH_3, 5'\text{-}CH_3)$	28, D	160.5–161.5[f]	$C_{17}H_{21}NO_4$	C, H, N
31	CO_2CH_3	CH_3	H	H	$C_6H_3(2',5'\text{-}(OCH_3)_2)$	88, D	134–135[f]	$C_{17}H_{21}NO_5$	C, H, N
32	CO_2CH_3	CH_3	H	H	$C_6H_4(p\text{-}CO_2H)$	89, D	226–228 dec[g]	$C_{16}H_{17}NO_5$	C, H, N
33	CO_2CH_3	CH_3	H	H	$N(CH_2)_2O(CH_2)_2$	52, D	200–202[k]	$C_{13}H_{20}N_2O_4$	C, H, N
34	CO_2CH_3	CH_3	H	H	$N(CH_2)_2O(CH_2)_2$	52, D	207–212[g]	$C_{13}H_{20}N_2O_4$	C, H, N
35	CO_2CH_3	CH_3	H	H	$C_6H_4(p\text{-OH})$	41, D	199.5–201[f]	$C_{15}H_{17}NO_3$	C, H, N
36	H	CH_3	CH_3	H	C_6H_5	89, D	181–183[f,m]	$C_{14}H_{17}NO$	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] EtOAc–petroleum ether (80–100 °C). [d] EtOAc. [e] EtOAc–ligroine (70–90 °C). [f] 2-Propanol. [g] Methanol. [h] Reference 17, mp 129–130 °C; reference 18, mp 129.2–130.0 °C. [i] Methanol–H_2O. [j] Toluene–methanol. [k] Toluene. [l] Total yield of the two isomers. [m] Reference 17, mp 174–177 °C.

Scheme I[a]



[a] Reagents: (a) NaOMe; (b) Δ, amine.

and co-workers[14] mentioned in an abstract that MK 1-203 (5,5-dimethyl-3-[(*o*-chlorophenyl)amino]-2-(*N*-piperidinylmethyl)-cyclohex-2-en-1-one, 1, and MK 1-907 (5,5-dimethyl-3-[(*m*-methoxyphenyl)amino]-2-(*N*-

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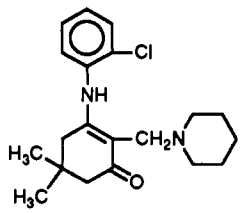
Table II. Anticonvulsant Screening Project (ASP): Phase I Test Results

compd	dose, mg/kg	activity: MES[a] 30 min	MES[a] 4 h	scMet[b] 30 min	scMet[b] 4 h	Tox[c] 30 min	Tox[c] 4 h	ASP classification[d]
12	100	2/3	0/3	0/1	0/1	0/8	0/4	1
	300	1/1	0.1	0/1	0/1	3/4	0/2	
13	300	1/1	0/1	0/1	0/1	1/4	0/2	2
14	100	1/3	0/3	0/1	0/1	0/8	0/4	1
	300	1/1	0/1	0/1	0/1	1/4	0/2	
15	300	0/1	1/1	0/1	0/1	0/4	0/2	2
16	300	1/1	1/1	1/1 (0/4)	0/1	0/4 (0/4)	0/2	2
19	100	1/3	0/3	0/1	0/1	0/8	0/4	1
	300	1/1	1/1	0/1	0/1	1/4	0/2	
21	300	0/1	1/1	0/1	0/1	0/4	0/2	2
25	300	1/1	0/1	1/1 (0/4)	0/1	0/4 (0/4)	0/2	2
26	100	2/3	0/3	0/1	0/1	0/8	0/4	1
27	100	3/3	0/3	0/1	0/1	1/8	0/4	1
	300	1/1	1/1	0/1	0/1	4/4	0/2	
28	30	0/1	0/1	1/1 (0/4)	0/1	0/4 (0/4)	0/2	1
	300	1/1	0/1	0/1	0/1	2/4	0/2	
29	100	0/3	0/3	1/1 (0/4)	0/1	0/8 (0/4)	0/4	1
31	100	1/3	0/3	0/1	0/1	0/8	0/4	1
	300	1/1	0/1	0/1	0/1	0/4	0/2	
36	100	2/3	0/3	0/1	0/1	0/8	0/4	1
	300	1/1	0/1	0/1	0/1	0/4	0/2	

[a] Maximal electroshock test (number of animals protected/number of animals tested). [b] Subcutaneous pentylenetetrazol test. [c] Rotorod toxicity (number of animals exhibiting toxicity/number of animals tested). [d] The classifications are as follows: 1, anticonvulsant activity at 100 mg/kg or less; 2, anticonvulsant activity at doses greater than 100 mg/kg. Values in parentheses are the results of a second trial. [e] Toxic at 30 min.

methyl-*N*-phenethylaminomethyl)cyclohex-2-en-1-one, **2**, possessed analgesic, papaverine-like, and anticonvulsant actions. We herein report our studies on certain enaminones as potential anticonvulsants.



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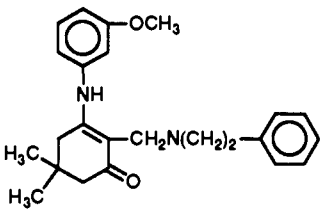

2

Results and Discussion

Chemistry. Cyclic enaminone esters **4** (Table I) were synthesized from β-hydroxy keto esters **3**, which in turn were synthesized by Michael addition of a vinyl ketone to a malonic ester, followed by a ring-closing Claisen condensation (method A), or by a base-catalyzed condensation of a crotonate ester and acetoacetate (method B)[15] as shown in Scheme I. In our hands, method B was preferred especially in the case of 3-penten-2-one, which was available in only 65% purity. 5-Methylcyclohexane-1,3-dione was formed by the acid-catalyzed decarboxylation of either methyl 4-hydroxy-6-methyl-2-oxocyclohex-3-en-1-oate or the ethyl ester. The β-hydroxy keto esters **3** were refluxed with 1 equiv of the appropriate amino compound under various conditions to provide the desired product. In the case of **5–10**, **12–14**, and **16–21**, the reaction proceeded effectively in the presence of toluene (method C). In the case of the hydrazines and anilines, a much lower temperature was employed, due most probably to the lower pK_a of the aniline (4.63 for aniline) derivatives compared to the benzylamine analogs (9.33 for benzylamine)[16] (method D). The enaminone esters can, in turn, be reacted with additional amine to yield enaminone amides, either through the β-hydroxy ketones (method E) or through the intermediate enaminone esters (method F). The NMR results at 300 MHz of the enaminones were consistent with the assigned structures. Of interest is the assignment of the methoxy groups in methyl 4-[(2,5-dimethoxyphenyl)-amino]-6-methyl-2-oxocyclohex-3-en-1-oate, **31**. The chemical shifts (δ ppm) for the carbomethoxy, 2′-methoxy, and 5′-methoxy protons occurred at δ 3.81, 3.84, and 3.78, respectively. The X-ray crystal structure of methyl 4-(benzylamino)-6-methyl-2-oxocyclohex-3-en-1-oate, **12**, indicated that **12** occurred as a single diastereiomer with a trans orientation of the chiral 1-carbomethoxy and 6-methyl groups.

Pharmacology. Preliminary pharmacological testing of the compounds listed in Table I has been provided by

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Table III. Phase II Quantification Data

compound	MES, ED_{50}[a,b]	TD_{50}[a,c]	PI, MES[d]	TPE[e] activity	TPE[e] toxicity
12	64.7 (41–89)	>500 (nd)	7.7	0.5	1
13	131.9 (92–189)	>500 (nd)	3.8	0.5	1
27	26.2 (17–40)	254.8 (202–322)	9.7	0.5	2
36	109.4 (88–124)	232.6 (200–273)	2.1	0.5	0.5
phenytoin	9.5 (8–10)	65.5 (55–72)	6.9	2	2
carbamazepine	8.8 (5–14)	71.6 (46–135)	8.1	0.25	0.25
valproate	272 (247–338)	426 (369–450)	1.6	0.25	0.25

[a] ED_{50} and TD_{50} values are in milligrams/kilogram of test drug delivered intraperitoneally (ip). Test compounds administered in a 0.5% methylcellulose suspension. [b] Measured at time of peak effect. [c] Measured at time of peak neurologic deficit on the rotorod. [d] PI = protective index (TD_{50}/ED_{50}). [e] Time of peak effect. Numbers in parentheses are 95% confidence interval. nd = not determined. [f] Values from ref 20.

the Antiepileptic Drug Development (ADD) Program, Epilepsy Branch, Neurological Disorders Program, National Institutes of Neurological and Communicative Disorders and Stroke, by testing procedures that have been described.[19] Phase I results of the active enaminone moieties in mice are shown in Table II. The three tests were maximal electroshock seizure (MES), subcutaneous pentylenetetrazol (scMet), and neurologic toxicity (Tox). Compounds **16**, **25**, **28**, and **29** gave spurious results in the scMet evaluation and were not advanced further. As a result of these tests, compounds **12**, **13**, **27**, **31**, and **36** were advanced to Phase II trials for quantification of their anticonvulsant activity and neurotoxicity in mice by determining the median effective dose (ED_{50}) and median toxic dose (TD_{50}). These data are shown in Table III. Also included are data for several currently marketed anticonvulsants for comparison. The protective indices for **12** (7.7) and **27** (9.7) compare favorably with phenytoin (6.9) and carbamazepine (8.1) and both far surpass valproate (1.6). Compounds **12**, **19**, and **21** were also evaluated in our laboratory for their ability to protect against MES and to elevate the threshold for electroshock-induced maximal (tonic extension) seizures in mice. In addition, the neurotoxicity of these compounds was assessed by measuring their effect on rotorod performance. Both phenobarbital and valproic acid were evaluated as reference standards. These data are shown in Table IV. It should be noted that the MES ED_{50} (42 mg/kg) for intraperitoneal (ip) administration of **12** was comparable to the MES ED_{50} (64 mg/kg) determined for this same compound in Phase II evaluation by the ADD Program. In addition, the TD_{50} values for ip administration of **12** were determined to be >500 mg/kg by both laboratories. Valproic acid also demonstrated similar activity against MES in both laboratories (ip MES ED_{50} = 272 mg/kg by ADD Program vs MES ED_{50} = 248 mg/kg by our laboratory) and similar neurotoxicity (ip TD_{50} = 426 mg/kg by ADD Program vs ip TD_{50} = 442 mg/kg by our laboratory). Compounds **19** and **21** were less effective than **12** in protecting against MES after ip administration; however, **12**, **19**, and **21** were all more active than valproic acid in this test. Upon oral administration (po), compounds **12** and **19** were still effective against MES. As compared to valproic acid, compounds **12**, **19**, and **21** were 6–10 times more effective in elevating electroshock-induced seizure threshold. This test, which measures the ability of the drug to alter the seizure threshold, is much more sensitive than the MES test, which uses a supramaximal stimulus to induce tonic limb extension.[21]

As a result of the favorable protective indices, compounds **12** and **27** were evaluated for oral activity in the rat by the ADD Program. Two additional compounds were included, the *p*-fluorobenzylamine derivative **21** and the aniline enaminone of dimedone **36**. These data are shown in Table V. As previously observed in mice, **12** and **27** displayed excellent protection (MES ED_{50}'s of 18.7 and 65.6 mg/kg, respectively). Also included in Table V are several commercial anticonvulsants for comparison. It should also be noted that the criteria for evaluation of toxicity for the three commercial compounds in the rat were less stringent than the rotorod test for the compounds synthesized herein. Whereas compounds **12**, **21**, **27**, and **36** were evaluated for the ability of the test animals to remain on the rotorod, the testing of the commercial products was based on the onset of ataxia. None of the enaminones in this report displayed ataxia. Compound **12** was subjected to a special intraperitoneal (ip) rat screening evaluation. This special screen attempts to determine whether toxicity of the compound changes when employing the ip route of administration versus oral intubation. The previous rat screen (Table V) in which **12** was administered orally, showed no toxicity up to doses of 500 mg/kg. The results from the ip screen of **12** reduce the possibility that lack of oral toxicity was due to an absorption phenomena. No toxicity was noted throughout the 4 h toxicity evaluation with the ip administration of 100 mg/kg of compound **12**. Corneal kindling studies were performed in our laboratories on **12**, **27**, phenobarbital, and valproic acid. The results are shown in Table VI. Comparative data for the commercial products was kindly provided by the ADD Program. It should be noted that we employed the modified Racine scoring system[22] while the ADD Program employed the Racine system.[23] The ED_{50} values calculated in our laboratory are slightly more conservative than those reported by the ADD Program in that the former is equal to the dose which reduced seizure severity by 50% and the latter is equal to the dose which reduced seizure severity from stage 5 to stage 4 in 50% of the animals. Seizures evoked in electroshock-kindled rats provide a suitable model consistent with complex partial seizures evolving into generalized motor seizures in hu-

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Table IV. Maximal Electroshock Seizure (MES), Threshold for Maximal Electroconvulsions, and Toxicity Testing in Mice

compound	route of administration	MES, ED_{50}[a] (95% confidence limits)	dose elevating electroshock-induced seizure threshold by 20%[b]	TD_{50}[c] (95% confidence limits)	PI, MES[d]	TPE[e] activity	TPE[e] toxicity
12	ip	42 (34–52)	13	>500	>11.9	0.5	0.25
	po	162 (140–189)	nd[f]	>500	>3.1	0.5	g
19	ip	53 (44–64)	20	148 (135–163)	2.8	0.5	0.25
	po	211 (163–207)	nd[f]	nd	nd[f]	0.5	nd[f]
21	ip	159 (122–207)	10	nd	nd[f]	0.5	nd[f]
phenobarbital	ip	18 (16–21)	3	92 (78–108)	5.1	1.0	1.0
valproate	ip	248 (227–271)	132	441 (229–653)	1.8	0.5	0.5

[a] Dose (mg/kg) protecting 50% of the mice from tonic hind limb extension at the time of peak anticonvulsant effect. [b] Dose (mg/kg) elevating threshold for maximal (tonic extension) electroconvulsions by 20% at the time of peak anticonvulsant effect. [c] Median neurotoxic dose (mg/kg) as determined by the rotorod test at the time of peak neurotoxic effect. [d] PI = protective index (TD_{50}/MES ED_{50}). [e] Time of peak effect. [f] nd = not determined. [g] No toxicity observed with 500 mg/kg during the first 120 min after po administration.

Table V. Phase VI Oral Rat Data

compound	dose, mg/kg	time, h	MES[a]	Tox[b]	ED_{50}[c]	TD_{50}[c]	PI[d]
12	50	0.25	2/4	0/4	26.8	>500	18.7
		0.50	4/4	0/4	(16–38)	(nd)	
		1.00	3/4	0/4			
		2.00	2/4	0/4			
		4.00	2/4	0/4			
21	50	0.25	2/4	0/4	49.3	>230	4.7
		0.50	2/4	0/4	(34–78)	(nd)	
		1.00	1/4	0/4			
		2.00	2/4	0/4			
		4.00	3/4	0/4			
27	10	0.25	3/4	0/4	5.8	>380	65.6
		0.50	4/4	0/4	(4–7)	(nd)	
		1.00	4/4	0/4			
		2.00	4/4	0/4			
		4.00	3/4	0/4			
	6	0.25	nd	nd			
		0.50	3/4	0/4			
		1.00	2/4	0/4			
		2.00	1/4	0/4			
		4.00	nd	nd			
36	60	0.25	3/4	0/4	30.1	>250	8.3
		0.50	3/4	0/4	(15–54)	(nd)	
		1.00	0/4	0/4			
		2.00	0/4	0/4			
		4.00	0/4	0/4			
phenytoin[e]					29.8 (22–39)	>3000[f]	>100
carbamazepine[e]					8.50 (3–11)	813[f] (489–1234)	95.7
valproate[e]					490 (351–728)	280[f] (191–353)	0.6

[a] Maximal electroshock test (refer to Table II for definition). [b] Rotorod toxicity (refer to Table II for definition). [c] ED_{50} and TD_{50} values are in milligrams/kilogram of test drug delivered orally. [d] Refer to Table III for definition. [e] Values from ref 20. [f] Tox data based on ataxia.

mans.[24] Compound 27 provided an ED_{50} of 34.2 mg/kg, whereas phenytoin was inactive in this test via the oral route. The ED_{50} value for 27 of 34.2 mg/kg compares favorably with that for carbamazepine (28.9 mg/kg). Both 12 and 27 were evaluated in Phase V of the ADD Program evaluation scheme. Phase V provides quantification against bicuculline, picrotoxin, pentylenetetrazol, and strychnine. This screen provides information on the mechanism of action of the anticonvulsant activity. Bicuculline, picrotoxin, and strychnine correspond to GABA inhibitory function, chloride channel inhibition, and inhibition by glycine, respectively. Compound 12 was uniformly inactive in all tests at doses of 12.5, 25, 50, and 100 mg/kg. While compound 27 was also inactive in these tests, it did not lower the seizure threshold to the chemostimulants. In addition, 27 was inactive in the benzodiazepine receptor binding evaluation, the GABA receptor binding test, and the adenosine uptake assay. In view of the recent reports that 5-fluoro-4-oxopentanoic acid[25] and 3,5-dioxocyclohexanecarboxylic acid[26] inhibit GABA am-

(24) Methods—Corneally kindled rats, Antiepileptic Drug Development (ADD Program Protocol).

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Table VI. Rat Corneal Kindling Data[a]

compound[b]	route and time of test, h	dose, mg/kg	% reduction	ave seizure score (SEM[c])	N[d]	ED_{50}, mg/kg[e] (95% confidence interval)
12	po; 0.5	0	0	5.0 (0.0)	29	
		50	29	3.6 (0.7)	9	78.3 (51–119)
		60	40	3.0 (0.7)	11	
		75	47	2.6 (0.7)	11	
27	po; 0.5	0	0	4.9 (0.0)	57	
		20	40	3.0 (0.7)	12	34.2 (18–64)
		30	40	3.0 (0.8)		
		40	49	2.6 (0.9)	7	
		50	67	1.7 (0.9)	9	
phenobarbital	ip; 0.5	0	0	5.0 (0.0)	40	
		15	47	2.7 (0.8)	9	15.0 (10–23)
		20	68	1.7 (0.7)	9	
		25	70	1.5 (0.5)	10	
		30	74	1.3 (0.5)	13	
valproic acid	ip; 0.5	0	0	5.0 (0.0)	35	
		100	49	2.6 (0.9)	7	96.0 (66–139)
		150	76	1.2 (0.6)	9	
		200	98	0.1 (0.1)	8	
		250	90	0.5 (0.3)	10	
clonazepam[f]	po; 1.0					0.7 (0.5–1)
carbamazepine[f]	po; 1.0					28.9 (8–76)
phenytoin[f]	po; 0.5					>100
phenytoin[f]	ip; 0.5					48.25 (25–78)
valproic acid[f]	po; 0.5					117.4 (68–189)

[a] Rats kindled to stage 5 seizures as described in Experimental Section. Seizures were scored according to the modified Racine scoring system:[22] 0 = no effect; 1 = immobility; 2 = facial clonus, head nodding; 3 = facial clonus and rearing; 4 = forelimb clonus and rearing; 5 = forelimb clonus and falling. [b] Compounds 12 and 27 administered in a 0.5% methylcellulose suspension. Sodium phenobarbital was administered in a 0.9% NaCl solution and valproic acid was administered in corn oil. [c] Standard error of the mean. [d] Number of animals tested. [e] ED_{50} = dose which reduced seizure severity by 50%. [f] Data provided by the ADD Program. Rats kindled to stage 5. Endpoint was reduction from stage 5 to stage 4 (ref 23).

inotransferase and were classified as "Class IV" inactivators by Silverman,[27] the starting β-hydroxy ketones were evaluated for anticonvulsant activity. All starting β-hydroxy ketones were inactive in Phase I evaluations. This fact, together with the rapid onset of action of **12** and **27**, would preclude their acting as prodrugs.[12,28] From the above data, a structure–activity relationship can be tentatively proposed.

(1) Amino substitution: For potential activity, primary amines should be employed, as pyrrolidine (**5**, **9** and **10**) and morpholine (**6–8**) analogs are uniformly inactive. The primary amine should be attached directly, or through a methylene, ethylene, or amino bridge, to an aromatic ring. The condensation of β-hydroxy keto ester **3** ($R{=}CO_2CH_3$; $R^1 = CH_3$; $R^2{=}H$) with *N*-aminomorpholine produced the two isomers, **33** and **34**; however, neither was active in Phase I evaluations. In addition, it was observed that, in proceeding through the homologous series from the highly active unsubstituted benzylamine analog **12** to the unsubstituted phenylamine compound **24**, activity was lost.

(2) Substitution on position 6: Substitution may accommodate a methyl or dimethyl functionality, while phenyl substitution (**8**, **10**, and **11**) produces inactive compounds.

(3) Ester functionality at position 1: This function may not be required as two dimedone analogs **19**[17,18] and **36**[17] have also proved to be potent anticonvulsants. Phase II (Table III) and VI (Table V) data for **36** indicate that the protective index in mice is modest, while in rats anticonvulsant activity is lost after 1 h. Thus, for maximum sustained activity and safety, the carbomethoxy function should be present.

(4) Carboxamide functionality at position 1: As with the ester function, the homologous carboxamides vary from the highly active phenethyl analog **15** to the proconvulsant benzyl analog **22** to the inactive phenyl compound **23**.

(5) Aromatic substitution: in view of the highly active **12**, we undertook a systematic evaluation of the electronic and steric effects involved in para substitution. We had previously employed a similar technique in our study of anticonvulsant spirosuccinimides.[29] While the Topliss[30,31] approach was used previously, we now employed the Free–Wilson analysis[32] successfully employed by Craig.[33,34] The substituted benzylamines **16–18**, **20**, and **21** provided only a slight modification in activity compared with **12**, with only **21** providing any significant activity in Phase II evaluation. The conclusion that activity may reside in the $+\sigma$, $+\pi$ quadrant was thus established. The fact that the enaminone of aniline, **24**, the starting compound in our analysis, was inactive in anticonvulsant evaluations was remarkable in our view due to the previous report.[14] Nevertheless, we prepared **26–32** and **35**. With the exceptions of **32** (comparable to inactive **18** in the benzylamine series) and **35**, anticonvulsant activity was observed.

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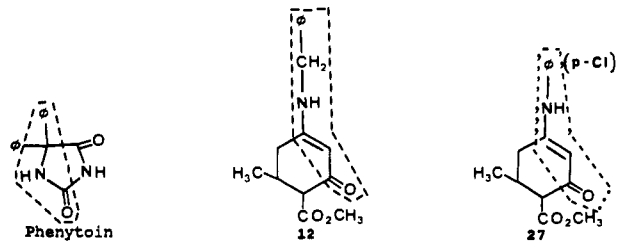

Figure 1. Proposed common "fingerprint" region of anticonvulsant activity (enclosed) for phenytoin, methyl 4-(benzylamino)-6-methyl-2-oxocyclohex-3-en-1-oate (**12**), and methyl 4-[(*p*-chlorophenyl)amino]-6-methyl-2-oxocyclohex-3-en-1-oate (**27**). Regions of commonality include (a) imide carbonyl of phenytoin and 2-oxo carbonyl of **12** and **27**, (b) 4-nitrogen of phenytoin and the 3-ene carbon of **12** and **27**, and (c) 5-phenyl of phenytoin and the 4-(benzylamino) or 4-[(*p*-chlorophenyl)-amino] function of **12** and **27**, respectively. Attention is drawn to the increased bond distance from a to c in **12** and **27**.

Thus, the conclusion that anticonvulsant activity can be enhanced by para substitution to the $+\sigma$, $+\pi$ quadrant as well as the $-\sigma$, $+\pi$ quadrant appears valid. Due to the similarity in anticonvulsant spectrum to phenytoin, it seemed logical to compare the structure of each of the active enaminones, **12** and **27**, to that of phenytoin. This is shown in Figure 1. Furthermore, the lack of enhanced anticonvulsant activity in the benzylamine series may be due to the lack of a –I effect with the para substituents due to the methylene bridge which effectively blocks this electronic contribution. As noted by Topliss,[30] *p*-fluoro substitution produces a minimal change in σ and π effects compared to the unsubstituted compound. This is borne out in the anticonvulsant activity of **21**. Steric effects in the benzylamine series cannot be overlooked. Figure 1 indicates an increased distance from the phenyl ring to the carbonyl group of **12** compared to phenytoin and **27**. The aniline series bears out the conclusion that a –I effect enhances activity, and that strong $+\pi$ groups enhance activity. Comparing *p*-chlorobenzylamine **16** to *p*-chlorophenylamine **27**, *p*-methylbenzylamine **17** to *p*-methylaniline **28**, and *p*-nitrobenzylamine **20** to *p*-nitroaniline **29**, it is clear that activity is enhanced in all cases. In addition, the *p*-hydroxy phenyl analog **35** is inactive, which parallels the metabolic inactivation of phenytoin. Thus, the active anticonvulsant region of the enaminones can tentatively be outlined as indicated in Figure 1. With the further evaluation of compound **27**, the synthesis of additional analogs, CLOGP[35] evaluation, further X-ray crystallographic analysis, and molecular modeling, a clearer picture of these enaminones should emerge. Further research in these areas is underway in our laboratories and will be reported shortly.

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. 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 Table I. Ethyl 4-hydroxy-6-methyl-2-oxocyclohex-3-en-1-oate was prepared by Method B.[15] Compounds **19**,[17,18] and **36**[17,28,36] were prepared by method D with comparable yields compared with those of method C employed in the literature. Typical experiments illustrating the general procedures for the preparation of the enaminones and intermediates are described below. X-ray crystallographic experiments were performed in the laboratory of Dr. Penelope W. Codding, University of Calgary, Department of Chemistry, Calgary, Alberta, Canada.

Methyl 4-Hydroxy-6-methyl-2-oxocyclohex-3-en-1-oate (3) (R = CH_3, R^1 = CH_3, R^2 = H). Method A. To a freshly prepared solution of sodium (7 g, 0.3 g-atom) in methanol (100 mL) was added dimethyl malonate (48 g, 0.36 mol) over 30 min and the mixture stirred on an ice bath for an additional 30 min. 3-Penten-2-one (65%, 21 g, 0.16 mol) was added and stirring continued in the cold for 1 h and then refluxed for an additional 2 h. The reaction mixture was cooled and the white precipitate which formed from the reddish-brown solution was collected, dissolved in a minimum amount of cold water (20 mL), acidified with 2 M sulfuric acid (120 mL), and extracted with dichloromethane (2 × 200 mL). After washing with water, the dichloromethane extract was dried over sodium sulfate. Additional **3** was obtained on evaporation of the methanolic solution and workup of the residue as previously indicated. Evaporation of the combined extract and recrystallization of the residue from toluene provided **3** (10.6 g, 39%): white plates; mp 122–123 °C; NMR ($CDCl_3$) δ 1.03 (3 H, d, J = 6 Hz, CH_3CH), 2.50 (2 H, m, CH_2), 3.20 (2 H, m, 2 × 2 H), 3.70 (3 H, s, OCH_3), 5.03 (1 H, s, =CH); IR ($CHCl_3$ solution), 3150 (br), 3020 (CH), 1730, 1659, 1610 cm^{-1}. Anal. ($C_9H_{12}O_4$) C, H.

Method B. To a freshly prepared solution of sodium (17.8 g, 0.77 g-atom) in methanol (220 mL) was added methyl acetoacetate (89.7 g, 0.77 mol) over 30 min and the mixture stirred on an ice bath for an additional 15 min. Ethyl crotonate (96%, 100 mL, 0.77 mol) was added dropwise and the mixture stirred at room temperature for an additional 30 min. After refluxing for 2 h, the mixture was cooled and the precipitate treated as indicated in method A to yield 57 g (40%) with identical constants as in method A.

5-Methylcyclohexane-1,3-dione (3) (R = R^1 = H, R^2 = CH_3). Ethyl 4-hydroxy-6-methyl-2-oxocyclohex-3-en-1-oate,[15] prepared by method B (129 g, 0.59 mol as the sodium enolate) was dissolved in 60 mL of water, 118 mL of a 5 M sodium hydroxide solution added, and the mixture refluxed for 1 h. After cooling to room temperature, the mixture was acidified with 90 mL of 5 M sulfuric acid and extracted with dichloromethane (2 × 100 mL). After washing with water, the organic layer was dried over magnesium sulfate, the solvent removed under reduced pressure, and the residue distilled [bp 60 °C (0.3 mm), 33.5 g, 44.9%] and crystallized on standing for 1 week. The solid was recrystallized from toluene, mp 139–141 °C. Anal. (C, H).

Methyl 4-(Benzylamino)-6-methyl-2-oxocyclohex-3-en-1-oate (12). Method C. To a solution of **3** (R = CO_2CH_3, R^1 = CH_3, R^2 = H) (2 g, 10.9 mmol) in 100 mL of toluene was added benzylamine (1.61 g, 15 mmol) and the mixture refluxed for 3 h using a Dean–Stark water separator. During the reaction, 0.25 mL of water was collected. Evaporation of the mixture to dryness and two crystallizations from ethyl acetate provided 1.6 g (81.3%) of the title compound, mp 154–155 °C. Anal. (C, H, N).

Methyl 4-[(*p*-Chlorophenyl)amino]-6-methyl-2-oxocyclohex-3-en-1-oate (27). Method D. A solution containing 60 mL of ethyl acetate and 140 mL of ether was employed to dissolve **3** (R = CO_2CH_3, R^1 = CH_3, R^2 = H) (5 g, 27 mmol) and 4-chloroaniline (4.16 g, 33 mmol) (**Caution**! *Possible mutagen*). The mixture was stirred and refluxed for 4 h. After evaporation of the solvents and refrigeration, the dark brown viscous liquid solidified. Two recrystallizations from toluene provided **27**, mp 178–180 °C. Anal. (C, H, N).

***N*-Benzyl-4-(benzylamino)-6-methyl-2-oxocyclohex-3-ene-1-carboxamide (22). Method E.** Compound **3** (R =

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CO_2CH_3, $R^1 = CH_3$, $R^2 = H$) (46 g, 0.25 mol) and benzylamine (80.4 g, 0.75 mol) in 350 mL of toluene was refluxed under a Dean–Stark water separator for 6 h. The solvent was evaporated, and the residue was crystallized from methanol to give 19.2 g (22%), mp 219–220 °C. Anal. (C, H, N).

Method F. Compound 12 (5.34 g, 19.5 mmol) was mixed with 2.09 g (19.5 mmol) of benzylamine in 100 mL of toluene and the mixture refluxed for 6 h. Workup as indicated in method E provided 11 with the identical constants as reported.

Pharmacology. Initial evaluation for anticonvulsant activity was done by the Antiepileptic Drug Development Program, Epilepsy Branch, Neurological Disorders Program, National Institute of Neurological and Communicative Disorders and Stroke and included Phases I, II, and V test procedures, which have previously been described.[19] These tests were performed in male Carworth Farms #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% polyethylene glycol 400 and were administered by intraperitoneal injection at three dosage levels (30, 100, and 300 mg/kg) with anticonvulsant activity and neurotoxicity noted 30 min and 4 h after administration. The compounds listed in Table II were those which displayed significant activity in Phase I evaluations. Phase II testing quantitated the anticonvulsant activity and neurotoxicity observed for the most promising compounds in Phase I. Thus, 12, 13, 27, and 36 were evaluated in this development scheme. These data are provided in Table III. Phase II determined the median effective dose (ED_{50}) and median toxic dose (TD_{50}). The ED_{50} and TD_{50} values and their confidence limits were determined at the time of peak effect of each compound by the method of Litchfield and Wilcoxon.[37] The MES and neurotoxicity tests done in our laboratory (Table III) were also carried out in CF1 male mice (ca. 24–34 g) by the same methods employed by the ADD Program. All compounds were administered in a 0.5% methylcellulose suspension by either ip or oral (po) administration. The elevation of maximal seizure threshold tests were performed according to the method previously described by Loscher and Schmidt.[21] Briefly, the current which induced hind limb extension in 50% of control mice (threshold current = CC_{50}) was determined according to the "up and down" method, i.e., the stimulus intensity for each mouse was dependent upon the response of the mouse previously tested. If the previous animal failed to exhibit a tonic seizure, then the stimulus intensity was increased by an increment, or if the animal failed to respond, decreased to the next lower increment. Each control group consisted of 15–25 mice treated with 0.5% methylcellulose and a control group was tested with each experimental group. Each experimental group of 15–25 mice was treated with a dose of the test drug and subjected to the same procedure as the controls. At least three doses of each test drug were evaluated. The dose of the test drug which elevated the control threshold current by 20% was calculated by plotting the doses of the test drug against the percentage threshold increases. Phase V of the ADD testing protocol measured the ability of the compounds to provide protection against seizures induced by subcutaneous injection of the CD_{97} of the following convulsant agents; Met (85 mg/kg), bicuculline (2.7 mg/kg), picrotoxin (3.15 mg/kg), and strychnine (1.2 mg/kg). Compounds 12 and 27 were uniformly inactive in this phase of testing. Phase VI provided oral rat data comparable to Phase II ip data in mice. Male Sprague–Dawley rats were employed in this evaluation. Table V details the results of this evaluation for compounds 12, 21, 27, and 36. A special ip toxicity evaluation was performed on compound 12 in Sprague–Dawley rats. This compound was nontoxic at 100 mg/kg at 15, and 30 min and 1, 2, and 4 h. Compounds 12 and 27, as well as phenobarbital and valproic acid, were tested in corneal kindled rats in our laboratory according to the procedure described by Skeen et al.[38] The results are shown in Table VI. In this procedure, Sprague–Dawley rats (85–95 g initial weight) were subjected to twice a day, 4 s, 8 mA stimulations via corneal electrodes for 5 to 6 days, followed by a single stimulation per day until a criterion of 10 consecutive stage 5 seizures was reached. A 1% solution of butacaine sulfate was applied to the cornea as a topical anesthetic. The seizure score according to a modified Racine scale,[22] seizure duration, and duration of forelimb clonus was recorded. The modified Racine scale was as follows: 0 = no effect; 1 = immobility; 2 = facial clonus, head nodding; 3 = facial clonus and rearing; 4 = forelimb clonus and rearing; 5 = forelimb clonus and falling. Following the establishment of kindling, rats received vehicle (0.5% methylcellulose, 0.9% saline, or corn oil) by either ip or po administration and were subjected to a single shock at the corresponding test drug's time of peak effect to assure a stage 5 response. Following a 2-day shock-free period, rats received either compound 12, 27, phenobarbital, or valproic acid by either ip or po administration and a single shock at the test drug's time of peak effect. The animal's response was recorded. The ED_{50} for each test drug was calculated by the method of Litchfield and Wilcoxon[37] and is equivalent to the dose which reduced seizure severity by 50%. Data for po administration of clonazepam, phenytoin, and valproic acid and for ip injection of phenytoin were provided by the ADD Program and the ED_{50} for each of these drugs was calculated to be the dose which reduced seizure severity to a stage 4 in 50% of the animals.[24] The methodology used was similar to what is described above, however the Racine scale[23] was used to score seizure severity: 0 = immobility; 1 = facial and mouth movements; 2 = head nodding; 3 = forelimb clonus; 4 = forelimb clonus and rearing; 5 = forelimb clonus and falling.

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