QSAR of the Anticonvulsant Enaminones; Molecular Modeling Aspects and other Assessments

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Abstract: The enaminones represent potentially useful agents for the clinical treatment in generalized tonic-clonic seizures (*Epilepsia,* **1993**, *34(6)*, 1141-1145, *Biopharm. Drug Disp.* **2003**, 397-407). A regression analysis was performed to provide a quantitative structure-activity relationship (QSAR) correlation model for prediction of activity for the anticonvulsant enaminones. Molecular modeling was performed to determine the molecular confluence of the Unverferth model (*J. Med. Chem.* **1998**, *41*, 63-73) to the enaminones. Conclusions related to the sodium channel model were assessed.

Key Words: Epilepsy, sodium channel, anticonvulsants, molecular modeling, regression analysis.

INTRODUCTION

Epilepsy is currently the most prevalent neurological disorder worldwide [1]. Many patients with epilepsy fail to experience adequate control of their seizures, despite the optimal use of available antiepileptic agents. Other patients do so only at the expense of significant toxic side effects [2]. There is thus a need for new drugs with a greater benefit as related to side effects and tolerability, even at the expense of efficacy, when compared to the existing antiepileptic agents [3,4]. Several new drugs have entered the therapeutic armamentarium, e.g. felbamate, lamotrigine, vigabatrin, gabapentin, tiagabine, topiramate, oxcarbazine, fosphenytoin, and levetiracetam [5]. In addition, several new entities are in the pipeline [6,7]. The search for antiepileptic compounds with more effective activity and lower toxicity continues to be an area of investigation in medicinal chemistry [8].

PHARMACOPHORE MODEL FOR ANTICONVULSANTS ACTING AS SODIUM CHANNEL BLOCKING AGENTS

In view of the broad etiology of the syndrome, epilepsy involves more than one mechanism that may be responsible for the various seizures, thus its response to different agents would be expected [9]. Voltage-gated sodium channels (VGSCs) are responsible for the initial inward current during the depolarization phase of the action potential in excitable cells [10]. The anticonvulsants phenytoin (**1**) and carbamazepine (**2**) prolong the inactive state of the VGSCs, and more recently, lamotrigine (**3**), zonisamide (**4**) and rufinamide (**5**), Fig. (**1**), have appeared and their modes of action suggest an involvement of sodium channel blockade [11].

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A new series of 3-aminopyrroles (**6**), Fig. (**1**), provided anticonvulsant activity and appear to act by inhibition of the sodium channel in a frequency-dependent manner as well [12]. Employing various theoretical and x-ray studies, [13-17] this group postulated an interesting pharmacophore model for anticonvulsant activity for the inhibitors of the VGSCs which included the following: (a) at least one aryl unit; (b) one or two electron donor atoms; and/or (c) an NH group in a steric arrangement Fig. (**2**). As noted in Fig. (**2**), R signifies an aryl ring, D represents a donor atom and a second donor atom in close proximity to the NH group forming a hydrogen bond acceptor/donor unit (HAD).

Employing this model, Unverferth and coworkers measured the distances between the three groups in five different anticonvulsants in Fig. (**1**) by five different computational methods (Table **1**) [12]. It was of note that the HA-HD distance of 2.4-2.6 Å in Fig. (**2**) was not used as this parameter was missing in several of the model compounds. Thus, there was good agreement with their model and the calculated measurements of the anticonvulsants. We have repeated their measurements with three different computer simulations (ChemDraw, SYBYL, and Spartan), (Table **1**) and found good agreement with compounds **1**-**5**. Comparing our Spartan data with the average values derived by Unverferth and colleagues [12], we found percent errors of 2.6, 3.7, and 6.9 for the R HAD, R D and D HAD parameters, respectively.

Extending this model to the most active aminopyrrole in their series (**6a**, R_1=H, R_2=CO_2CH_3, R_3= $N(CH_2CH_2)_2O$), R_4=4-Br), as well as more structurally diverse anticonvulsant compounds which act as inhibitors of the VGSCs, namely, vinpocetine (**7**), remacemide (**8**), and dezinamide (**9**), in Fig. (**3**), Unverferth and coworkers obtained similar results (Table **1**) for the R-HAD and D-HAD measurements, but found a wider difference in the R-D measurement than with the compounds **1**-**5** in Fig. (**1**) [12]. Our calculations for the

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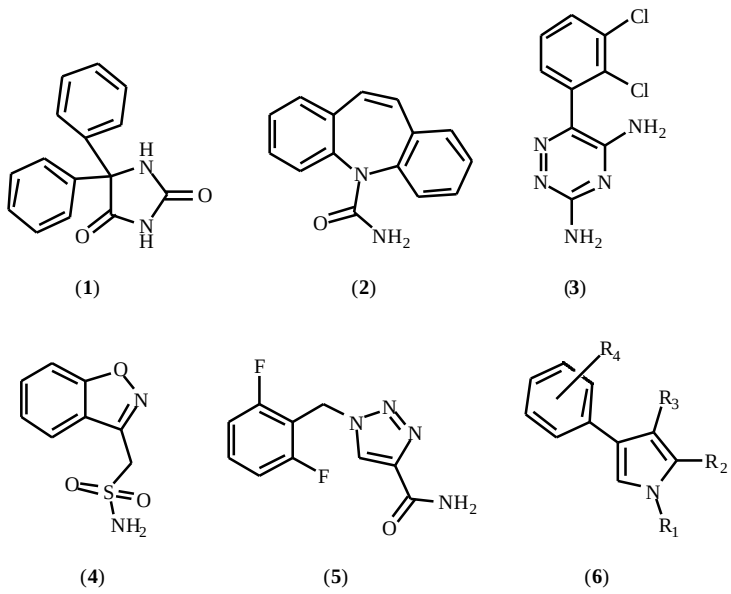


Fig. (1). Structures of anticonvulsants acting *via* sodium channel blockade.

latter series, however, differed significantly from those of the authors. We found percent errors of 16.5, 14.1 and 2.5 for the R HAD, R D and D HAD parameters, respectively. These differences may be explained, in part, by the multiple sites of interaction of these molecules as well as for the rigidity of the synthetic vincamine derivative, vinpocetine (**7**) [18].

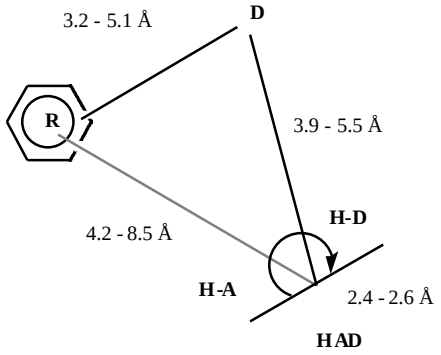


Fig. (2). Suggested pharmacophore model for anticonvulsant activity [12].

We have studied the anticonvulsant activity of the enaminones [19-31] and found that they act as inhibitors of the VGSCs as well [24]. A structural correlation was proposed by Carson and colleagues [32] who observed in their report on their highly active anticonvulsant aroyl-(aminoacyl) pyrroles (**6b**), that there existed a structural similarity to the *N*-benzyl enaminones (**10**), synthesized in our laboratories Fig. (**4**) [26]. Further, Carson indicated that the extensive charge delocalization involving the nitrogen atom and the carbonyl oxygen was similar in both the acyl pyrroles [33] and the enaminones [34]. It was thus of interest to compare this model with our compounds.

The results of the measurements for the enaminones (**11)**, are provided in Table **1** and show a clear relationship with the anticonvulsant pharmacophore.

REGRESSION ANALYSIS

With accumulated biological and physical data derived from our studies on the enaminones, a regression model would be a definitive method to predict potential activity and provide a direction for our synthetic efforts. We had earlier [27] developed a regression analysis model on a limited number of analogues of the general structure, **12** (Table **2**).

The reported regression equation was {log (1/A) = 2.017 σ_{para} – 0.337π + 3.825}.

When plotting the calculated versus the actual log 1/A values, there was good agreement in the data (r^2 = 0.80). We concluded that anticonvulsant activity was directly related to the electron withdrawing activity of the para substituent. This conclusion verified our earlier empirical inference [19]. It was of interest to extend the conclusion to an augmented series as noted in Table **3** that included the non-ester analogues (**11a-11ff**), which included: (a) various sites of substitution and/or non-substitution; (b) increasing the carbon chain separating the secondary amino from the aromatic ring; (c) inclusion/non-inclusion of the 5-methyl group; and (d) inclusion of a heterocyclic ring. ClogP [37] values were calculated which were shown previously to agree closely to the experimental $LogK_{IAM}$ values [27]. Experimental animal data was derived either from the Antiepileptic Drug Development (ADD) Program or from NovaScreen Laboratories.

RESULTS AND DISCUSSION

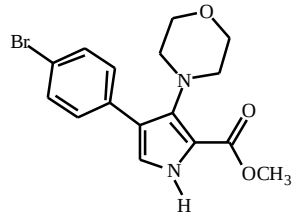
Chemistry

All enaminones reported in Tables **2** and **3** have been synthesized in our laboratories and most have been

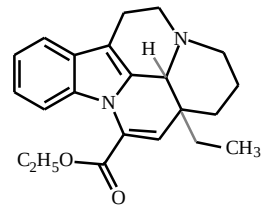
Table 1. Distance Ranges between the Essential Structural Elements R, D, and HAD of Selected Anticonvulsants 1-9 and 11, Figs. (1) and (2)

	Theoretical value (Å)[12]	Average reported value (Å)[12]	Calculated Value (Å)[a]	Calculated Value (Å)[b]	Calculated Value (Å)[c]
Compounds 1-5					
R HAD	8.5 ± 4.3	5.830 ± 1.094	5.639 ± 0.245	5.604 ± 0.885	5.673 ± 0.820
R D	5.1 ± 1.9	3.898 ± 0.350	3.890 ± 0.113	4.082 ± 0.283	4.132 ± 0.265
D HAD	5.5 ± 1.6	4.660 ± 0.290	4.336 ± 0.184	4.316 ± 0.258	4.335 ± 0.442
Compounds 6a-9					
R HAD	---	5.680 ± 0.283	5.854 ± 0.425[d]	6.948 ± 0.918	6.619 ± 0.990
R D	---	4.578 ± 0.265	5.008 ± 0.421[d]	5.230 ± 0.461	5.067 ± 0.604
D HAD	---	4.328 ± 0.088	3.868 ± 0.339	4.049 ± 0.795	3.854 ± 0.924
Compounds Series 11[e]					
R HAD	---	---	7.145 ± 0.500	7.687 ± 0.917	6.502 ± 0.115
R D	---	---	3.119 ± 0.213	4.757 ± 0.009	3.120 ± 0.096
D HAD	---	---	4.851 ± 0.132	4.010 ± 0.803	4.770 ± 0.000
Compounds Series 11[f]					
R HAD	---	---	6.821 ± 0.503	6.360 ± 0.010	6.310 ± 0.009
R D	---	---	2.986 ± 0.115	3.00 ± 0.000	2.99 ± 0.017
D HAD	---	---	4.881 ± 0.156	4.76 ± 0.002	4.77 ± 0.001

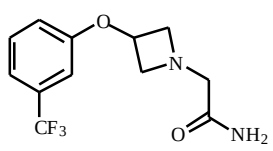
[a]ChemDraw (v. 8.0); [b]SYBYL (v. 6.9); [c]Spartan (v. 2.0); [d]Includes the median values (2) for remacemide (**9**). [e]Data only for those compounds found to be active (class 1 or 2), see Table 3 and text (*Experimental*) for definitions. [f]Data for those compounds found to be inactive (class 3, see Table 3).



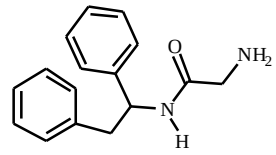
(**6a**)



(**7**)

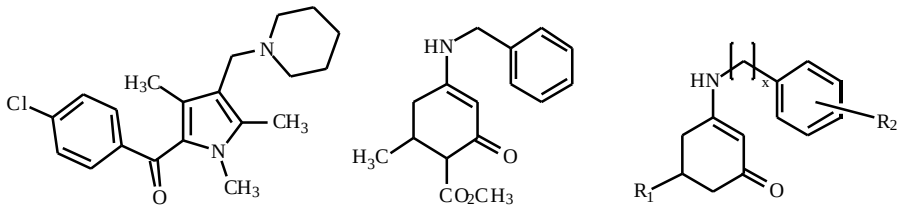


(**8**)



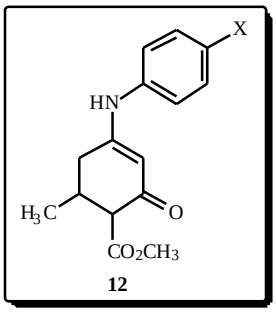
(**9**)

Fig. (3). Additional diverse anticonvulsants acting at the VGSCs [12].



6b **10** **11**

Fig. (4). Carson's pyrrole, **6b**, [32] Scott's benzylamine, **10**, [26] and the general pharmacophore model, **11**.



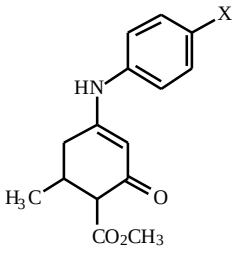
12

previously reported [24,28]. ^{1}H-NMR spectra were recorded on a General Electric QE 300-MHz spectrometer in deuterated solvents using tetramethylsilane as an internal reference. Coupling patterns are described as follows: s, singlet; bs, broad singlet; d, doublet; q, quartet; m, multiplet and 1H, 2H, etc. as the number of hydrogens integrated within a given coupling pattern. The chemical shifts were measured to two decimal points, while the coupling constants were rounded off to one decimal place. All enaminone compounds gave elemental analyses (C, H, N and halogen, where reported) values within $\pm$ 0.4% of theory and were performed by Schwarzkopf Microanalytical Laboratory, Woodside, NY 11377 USA. Chiral compounds (compounds **12a-i** and **11f-11ff**) were not resolved and evaluated as a racemic mixture. Typical synthesis of previously unreported compounds are provided in Scheme **1**.

Molecular Modeling

The enaminones were minimized and the bond distances between R, D and HAD parameters were measured to determine if they fit the Unverferth model [12]. As noted in Table **1**, analysis of the compounds found that their bond distances were within the range of the reference model. A

Table 2. Initial regression Analysis [27]



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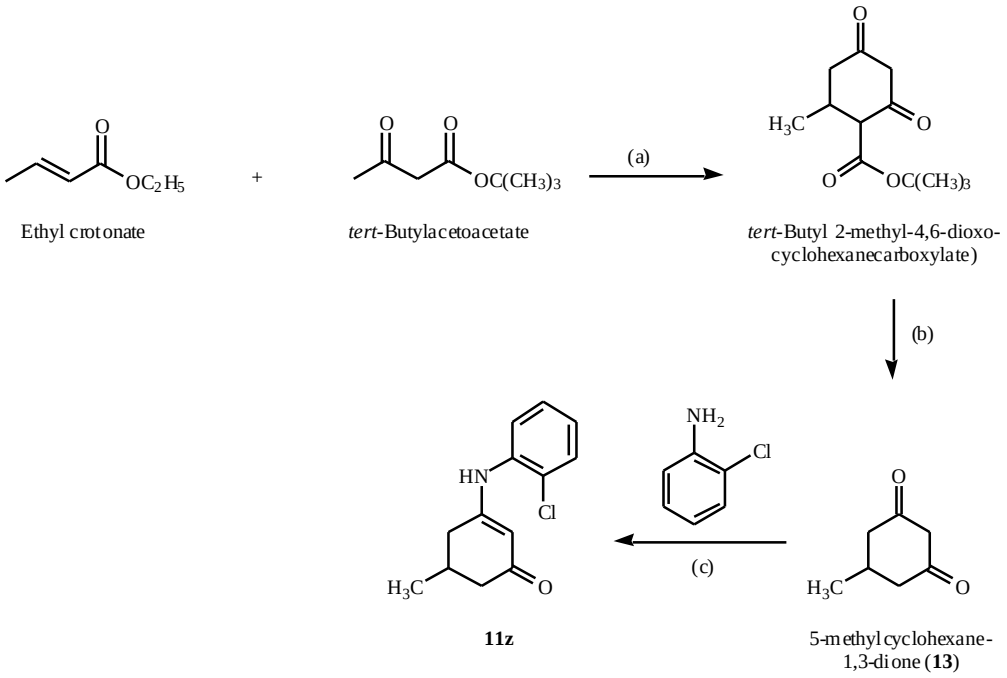
Compound	X	M.W.	ED_{50} (mg kg^{-1}), mice	ED_{50} (mmole kg^{-1}), mice	log (1/A)	σ_{para} [a]	π_{para} [a]
12a	Cl	293.77	26.20	8.919×10^{-5}	4.050	0.23	0.71
12b	CF_3	327.31	20.32	5.692×10^{-5}	4.245	0.54	0.88
12c	OCF_3	343.32	22.38	6.519×10^{-5}	4.186	0.35	1.04
12d	F	277.30	21.34	7.704×10^{-5}	4.113	0.06	0.14
12e	OCH_3	328.21	172.34	5.251×10^{-4}	3.280	-0.27	-0.02
12f	C_2H_5	287.39	114.39	3.980×10^{-4}	3.400	-0.15	1.02
12g	CH_3	273.36	279.17	1.021×10^{-3}	2.991	-0.17	0.56
12h	Br	338.20	55.68	1.652×10^{-4}	3.782	0.23	0.86
12i	I	385.22	49.25	1.278×10^{-4}	3.893	0.18	1.12

[a]Values[35,36]



[a]Conditions: (a) Na; C_2H_5OH (abs.); Δ; (b) 0.05 N H_2SO_4, Δ [36]; (c) EtOAc, C_2H_5OH (abs.); Δ

Scheme 1.[a]

superimposition of all of the class 1 compounds was generated by a fit atoms procedure using SYBYL v. 6.9 shown in Fig. (**5**). This model shows the overlap of the R, D, and HAD parameters of the enaminone analogues.

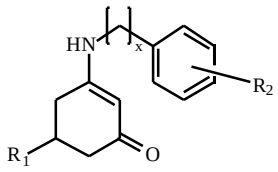
Regression Analysis

Table **4** shows the enaminone compounds synthesized and their anticonvulsant activity. A linear regression model was built to assess the relationship between anticonvulsant activity and the electron withdrawing activity.

As shown in Table **5**, the fitted regression equation for para substituents is $\{\log(1/A) = 3.127 + 1.082\sigma_{para} + 0.537\pi_{para}\}$.

Both σ and π are positively correlated with the anticonvulsant activity. Given a fixed π, one unit increase in the σ is associated with 1.082 unit increase in log(1/A)

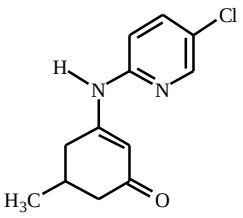
Table 3. Enaminones Under Study



11

Compound	R_1	R_2	x	Clog P[a]	Nova Screen$_1$[b]	Nova Screen$_2$[c]	ADD results[d]
11a	H	4-Cl	0	2.44	89	89	Class 1
11b	H	H	1	2.10	>150	136	Class 1
11c	H	H	2	2.48	>150	136	Class 1
11d	H	4-$C(CH_3)_3$	0	3.55	67	>150	Class 1
11e	H	4-CF_3	0	4.08	112.5	>150	Class 1
11f	CH_3	H	0	2.24			Class 1
11g	CH_3	4-Cl	0	2.96			M 40.41; R 14.72

(Table 3. Contd….)

Compound	R_1	R_2	x	Clog P[a]	Nova Screen$_1$[b]	Nova Screen$_2$[c]	ADD results[d]
11h	CH_3	4-Br	0	3.11			R 7.6
11i	CH_3	4-I	0	3.37			M 77; R 17.6
11j	CH_3	4-F	0	2.39			Class 3
11k	CH_3	4-CF_3	0	4.59			R 10.3
11l	CH_3	4-CN	0	1.68			Class 3
11m	CH_3	4-NO_2	0	-1.05			R 18.4
11n	CH_3	4-CH_3	0	2.74			Class 2
11o	CH_3	4-OCF_3	0	3.27			R 6.5
11p	CH_3	3-Cl	0	2.96			Class 1
11q	CH_3	3-Br	0	3.11			Class 3
11r	CH_3	3-I	0	3.37			Class 3
11s	CH_3	3-F	0	2.39			Class 2
11t	CH_3	3-CF_3	0	4.59			Class 3
11u	CH_3	3-OCF_3	0	3.27			Class 1
11v	CH_3	3-CN	0	1.68			Class 3
11w	CH_3	3-NO_2	0	-1.05			Class 3
11x	CH_3	3-CH_3	0	2.74			Class 2
11y	CH_3	3- OCH_3	0	2.16			Class 2
11z	CH_3	2-Cl	0	2.96			Class 1
11aa	CH_3	2,4-Cl_2	0	3.67			Class 1
11bb	CH_3	2,5-Cl_2	0	3.67			Class 3
11cc	CH_3	3,4-Cl_2	0	3.55			Class 3
11dd	CH_3	3,5-Cl_2	0	3.67			ND
11ee	CH_3	2,3-Cl_2	0	3.55			Class 1
11ff			0	1.54			Class 1

[a]Calculated;[37] [b]NovaScreen$_1$=tonic phase of seizures (mg kg^{-1}); [c]NovaScreen$_2$=clonic phase of seizures (mg kg^{-1}). [d]Preliminary ADD results, see text (*Experimental*) for definitions; M=ED_{50} in mice (mg kg^{-1}); R=ED_{50} in rats (mg kg^{-1}). ND=not determined.

(p=0.044); however, given a fixed σ, one unit increase in π is associated with 0.537 unit increase in log(1/A) (p=0.027). The plot of the calculated versus the actual log(1/A) shows that there is a relatively good agreement in the data. However, a linear regression model does not fit well with all substituents (p=0.359). More specifically, we did not find a significant model for meta and disubstituents (p=0.364, 0.525 respectively) (Table **6**).

Figure **6** shows the scatter plot of log(1/A) versus sigma and pi, respectively. The para substituents (LS-mean=3.672)

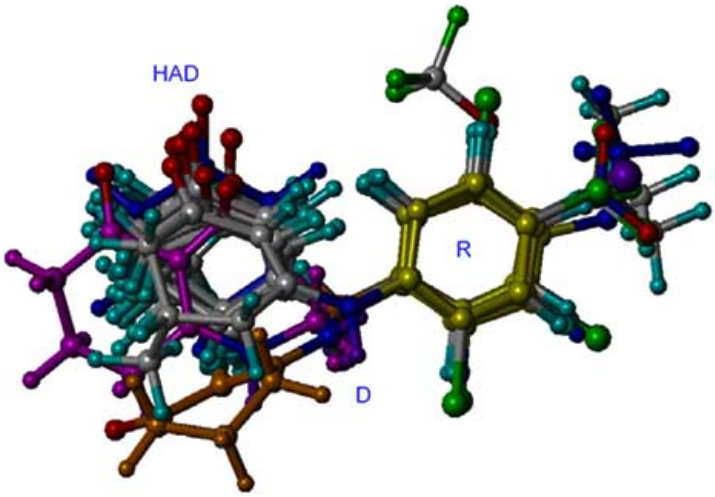


Fig. (5). Superimposition of the class 1 compounds as a result of a fit atoms method to reach maximum overlap between the R, D and HAD units. Compounds were fitted to compound (**11o**) (blue) the most active analogue. Compounds (**11b**) (orange) and (**11c**) (magenta) had D and HAD units that were not superimposable due to branching between the R and D areas of the molecule.

have significantly higher activity than meta (LS-mean= 3.081) and both (LS-mean=3.181) substituents ($p<0.05$).

EXPERIMENTAL

Chemistry

Although we had earlier proposed a "disubstitution hypothesis", [22,27] the difficulty lay in the synthesis of these chloro-substituted analogues, (**11z, 11aa-11ee**). It was found that forcing conditions, e.g. extended refluxing with high-boiling toluene, yielded mixed results and low yields. Success was achieved by modifying our original synthesis using a mixture of anhydrous ethanol and ethyl acetate and slowly removing the solvent during the reaction. Slow addition of anhydrous ether, with one exception (**11ee**), provided product in good yield and in a pure form. The

Table 4. Data Set for Enaminones

Compound	Mol. Wt.	ED_{50} (mg kg^{-1}) rat	ED_{50} (mol kg^{-1})rat	log (1/A)	σ_{para}[a]	π_{para}[a]
11a	221.06	100	4.52E-04	3.345	0.23	0.71
11b	201.29	100	4.97E-04	3.304	0	0
11c	215.29	100	4.64E-04	3.333	0	0
11d	243.34	100	4.11E-04	3.386	-0.2	1.98
11e	255.24	100	3.92E-04	3.407	0.54	0.88
11f	201.26	100	4.97E-04	3.304	0	0
11g	235.71	14.72	6.24E-05	4.204	0.23	0.71
11h	280.16	7.6	2.71E-05	4.567	0.23	0.86
11i	327.16	17.6	5.38E-05	4.269	0.18	1.12
11j	219.25	300	1.37E-03	2.864	0.06	0.14
11k	269.26	10.3	3.83E-05	4.417	0.54	1.16
11l	226.27	300	1.33E-03	2.878	0.66	-0.57
11m	246.26	18.4	7.47E-05	4.127	0.78	-0.28
11n	215.29	200	9.29E-04	3.032	-0.17	0.56
11o	285.26	6.5	2.28E-05	4.642	0.35	1.04
					σ_{meta}[a]	π_{meta}[a]

(Table 4. Contd….)

Compound	Mol. Wt.	ED_{50} (mg kg^{-1}) rat	ED_{50} (mol kg^{-1})rat	log (1/A)	σ_{para} [a]	π_{para} [a]
11p	235.71	81.13	3.44E-04	3.463	0.37	0.71
11q	280.16	300	1.07E-03	2.970	0.39	0.86
11r	327.16	300	9.17E-04	3.038	0.35	1.12
11s	219.25	200	9.12E-04	3.040	0.34	0.14
11t	269.26	300	1.11E-03	2.953	0.43	0.8
11u	285.26	100	3.51E-04	3.455	0.38	1.04
11v	226.27	300	1.33E-03	2.878	0.56	-0.57
11w	246.26	300	1.22E-03	2.914	0.71	-0.28
11x	215.29	200	9.29E-04	3.032	-0.07	0.56
11y	231.29	200	8.65E-04	3.063	0.12	-0.02
					σ_{ortho} [a]	π_{ortho} [a]
11z	235.71	39.4	1.67E-04	3.777	-	0.71
					σ^{-}	π^{-}
11aa	270.15	100	3.70E-04	3.432	0.75	1.25
11bb	270.15	300	1.11E-03	2.954	0.75	1.25
11cc	270.15	200	7.40E-04	3.131	0.52	1.25
11dd	270.15	300	1.11E-03	2.954	0.75	1.25
11ee	270.15	100	3.70E-04	3.432	0.52	1.25
11ff	236.7	100	4.22E-04	3.374	-	-

[a]Values[36,37,39].

critical analogue in the hypothesis was the 2,3-dichloro analogue, (**11ee**), which was isolated with difficulty (see *Experimental*). Its active (class 1) anticonvulsant evaluation verified our hypothesis and proved that steric factors do play a critical role in the QSAR evaluation of the enaminones.

3-(2-Chlorophenylamino)-5-methylcyclohex-2-enone (11z)

A mixture of 5-methyl-1,3-cyclohexanedione (**13**), [39] (3.41 g, 27 mmol) and 2-chloroaniline (4.21 g, 33 mmol), were added to a mixture of ethyl acetate (100 mL) and absolute ethanol (100 mL) in a 250 mL single neck flask connected to a Dean-Stark trap and the mixture stirred and refluxed for 6 hours, during which time the solvents were removed until the volume was reduced to ~ 50 mL over the 6 hour period. As the solution cooled to room temperature, an equal volume of anhydrous ether was slowly added through the trap, maintaining a constant reflux. The solution became cloudy and on stirring at room temperature overnight, yellow crystals appeared. These crystals were separated, dried at room temperature and recrystallized three times from ethyl acetate, an analytical sample was obtained, orange crystals, m.p. 168.5-170° C (3.5 g, 55%). There was negligible recovery from the original mother liquor. ^{1}H-NMR (DMSO-$_{d6}$): δ 1.06 (3H, d, *J*=6.7 Hz, CH_3), 1.70 (2H, s, CH_2 at C_4), 2.05-5.55 (3H, m, cyclohexene ring), 5.60 (1H, s, =CH), 6.10 (1H, bs, NH), 7.10-7.75 (4H, m, C_6H_5). Anal. Calc. for ($C_{13}H_{14}ClNO$) C, H, Cl, N.

3-(2,4-Dichlorophenylamino)-5-methylcyclohex-2-enone (11aa)

In a similar procedure, ketone **13** and 2,4-dichloroaniline produced the 2,4-dichloro enaminone (**11aa**). After three recrystallizations from ethyl acetate, the product occurred as tan crystals, m.p. 199-200° C (1.75 g, 24%). ^{1}H-NMR (DMSO-$_{d6}$): δ 1.06 (3H, d, *J*=6.7 Hz, CH_3), 1.90-2.92 (5H, m, cyclohexene ring), 5.60 (1H, s, =CH), 6.95 (1H, bs, NH), 7.20-7.54 (3H, m, C_6H_5). Anal. Calc. for ($C_{13}H_{13}Cl_2NO$) C, H, Cl, N.

3-(2,5-Dichlorophenylamino)-5-methylcyclohex-2-enone (11bb)

In a similar procedure, ketone **13** and 2,5-dichloroaniline produced the 2,5-dichloro enaminone (**11bb**). After three recrystallizations from ethyl acetate, the product occurred as orange plates, m.p. 209-210° C (2.63 g, 36%). ^{1}H-NMR (DMSO-$_{d6}$): δ 1.06 (3H, d, *J*=6.7 Hz, CH_3), 1.95-3.02 (5H, m, cyclohexene ring), 5.54 (1H, s, =CH), 5.65 (1H, bs, NH), 6.55-7.05 (3H, m, C_6H_5). Anal. Calc. for ($C_{13}H_{13}Cl_2NO$) C, H, Cl, N.

Table 5. Regression Analysis for Para Substituted Enaminones

	df	Sum of Square	Mean Square	F	p-value
Model	2	2.332	1.166	4.44	0.036
Error	12	3.149	0.262		
Corrected Total	14	5.481			

Parameters	Coefficients	Standard Error	t value	p-value
Intercept	3.126	0.226	13.84	<0.001
Sigma	1.082	0.482	2.25	0.044
Pi	0.537	0.214	2.52	0.027

Table 6. Regression Analysis for all Substitutions

	df	Sum of Square	Mean Square	F	p-value
Model	2	0.619	0.309	1.06	0.359
Error	27	7.852	0.291		
Corrected Total	29	8.471			

Parameters	Coefficients	Standard Error	t value	p-value
Intercept	3.266	0.185	17.63	<0.001
Sigma	-0.025	0.349	-0.07	0.943
Pi	0.230	0.158	1.46	0.157

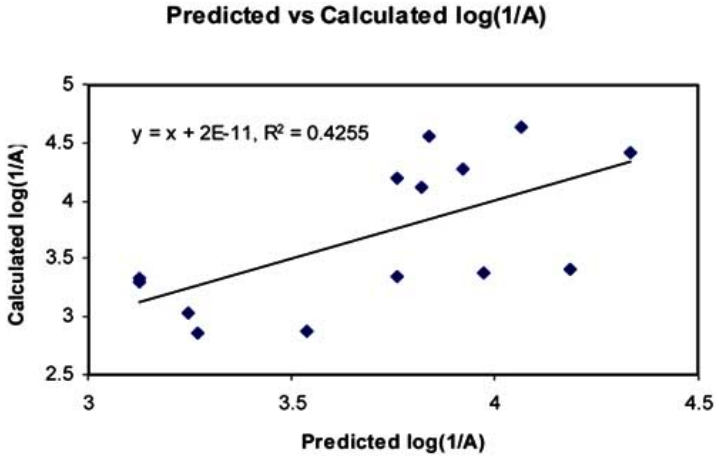


Fig. (6). Plot of predicted *vs* calculated log(1/A).

3-(3,4-Dichlorophenylamino)-5-methylcyclohex-2-enone (11cc)

In a similar procedure, ketone **13** and 2,4-dichloroaniline produced the 2,4-dichloro enaminone (**11cc**). After three recrystallizations from ethyl acetate, the product occurred as white crystals, m.p. 209-210° C (2.92 g, 40%). ^{1}H-NMR (DMSO-$_{d6}$): δ 1.06 (3H, d, *J*=6.7 Hz, CH_3), 1.90-3.08 (5H, m, cyclohexene ring), 5.50 (1H, s, =CH), 6.18-6.51 (3H, m, C_6H_5), 8.91 (1H, bs, NH). Anal. Calc. for ($C_{13}H_{13}Cl_2NO$) C, H, Cl, N.

3-(3,5-Dichlorophenylamino)-5-methylcyclohex-2-enone (11dd)

In a similar procedure, ketone **13** and 3,5-dichloroaniline produced the 3,5-dichloro enaminone (**11dd**). After three recrystallizations from ethyl acetate, the product occurred as yellow crystals, m.p. 214-215° C (1.20 g, 16%). ^{1}H-NMR (DMSO-$_{d6}$): δ 1.06 (3H, d, *J*=6.7 Hz, CH_3), 1.95-3.12 (5H, m, cyclohexene ring), 5.54 (1H, s, =CH), 5.90 (1H, bs, NH), 6.55-6.75 (3H, m, C_6H_5). Anal. Calc. for ($C_{13}H_{13}Cl_2NO$) C, H, Cl, N.

3-(2,3-Dichlorophenylamino)-5-methylcyclohex-2-enone (11ee)

In a similar procedure, ketone **13** and 2,3-dichloroaniline produced an oil that resisted crystallization. The slurry as taken up in 25 mL of chloroform and chromatographed on a 250 mL silica gel column, eluting with ethyl acetate: cyclohexane (3:1), collecting the eluate in 50 mL fractions. Evaporation of fractions 8-11 produced a brown residue that solidified on standing. After one recrystallization from ethyl acetate-benzene (2:1), the emanimone (**11ee**), occurred as a fine brown powder, m.p. 148-154° C (0.88 g, 12%). ^{1}H-NMR (DMSO-$_{d6}$): δ 1.06 (3H, d, *J*=6.8 Hz, CH_3), 1.92-2.78 (5H, m, cyclohexene ring), 5.63 (1H, s, =CH), 6.22-6.80 (3H, m, C_6H_5), 8.71 (1H, bs, NH). Anal. Calc. for ($C_{13}H_{13}Cl_2NO$) C, H, Cl, N.

3-(3-Chloropyridin-2-ylamino)-5-methylcyclohex-2-enone (11ff)

In a similar procedure to **11z**, ketone **13** and 2-amino-5-chloropyridine produced the enaminone, (**11ff**). After three recrystallizations from ethyl acetate, the product occurred as bright yellow crystals, m.p. 212-214° C (2.63 g, 36%). ^{1}H-NMR (DMSO-$_{d6}$): δ 1.06 (3H, d, *J*=6.7 Hz, CH_3), 1.92-3.02 (5H, m, cyclohexene ring), 5.54 (1H, s, =CH), 5.75 (1H, bs, NH), 6.90-8.45 (3H, m, pyridine ring). Anal. Calc. for ($C_{13}H_{13}Cl_2NO$) C, H, Cl, N.

Pharmacology

Initial evaluations for anticonvulsant activity were performed by the ADD program, Epilepsy Branch, National Institute of Neurological Disorders and Stroke (NINDS) Program, and included Phases I, II, VIA and VIB test procedures which have been described [40,41]. These test procedures were performed either in male Carworth Farms No. 1 (CF1) mice (weighing 18-25 g) or male Sprague-Dawley rats (weighing 100-150 g). Phase I, a qualitative anticonvulsant intraperitoneal (i.p.) evaluation in mice included three tests: maximal electroshock seizure (MES) test, subcutaneous pentylenetetrazol (scPTZ) seizure test and the rotorod test for neurological toxicity (Tox). Compounds were suspended in 0.5% methylcellulose and were administered at three dosage levels (30, 100, and 300 mg kg^{-1}) with anticonvulsant and motor impairment noted 30 min and 4 h after administration. Phase I classification is as follows: class 1 (active at 100 mg kg^{-1} or less); class 2 (active at > 100 mg kg^{-1} but less than 300 mg kg^{-1}); class 3 (no activity up to and including 300 mg kg^{-1}). Phase VIA evaluation was a similar qualitative evaluation to the Phase I evaluation, however, the test drug was administered orally in rats utilizing the three tests noted previously. Phase II test quantitated the anticonvulsant activity and motor impairment observed for the most promising compounds in Phase I and VIA, respectively. An i.p. test in rats was performed at 30 mg kg^{-1} for activity and toxicity. The time of peak effect i.p. in mice and rats was also noted for the determination of ED_{50} and TD_{50} values. Five compounds that were found to be class 1 by the ADD program were submitted to NovaScreen Laboratories, Hanover, MD, to generate quantitative ED_{50} data. The same MES procedure as that of the ADD program was employed using Swiss-Hla Outbred mice weighing 30 ± 3 mg. Enaminones were administered in doses of 30, 75 and 150 mg kg^{-1}. Differences in the ED_{50} data between the ADD program and NovaScreen are due, in part, to differences in the strains of mice used by each testing laboratory. All test data are listed in Table **3**.

Molecular Modeling

Computations were performed on the following:

(a) Bond Distances in Table 1

(i) ChemDraw - Power Mac G5 1 GB memory running ChemDraw Ultra v. 8; ChemDraw 3D Pro v. 5. After drawing the structures using ChemDraw, the structures were exported to ChemDraw 3D and minimized using the default settings in molecular mechanics 2 (MM2). The minimized structures were subjected to molecular dynamics analysis in MM2 modifying the default settings to terminate the run after 20,000 steps; (ii) iMac G4, 512-MB memory running Spartan v. 2; compounds were drawn and minimized using Spartan default settings in the molecular mechanics force field (MMFF). Bond distances were then measured between selected atoms; (iii) Octane2, Onyx2-IRIS 6.5 workstation running SYBYL, v. 6.9. Compounds were drawn in SYBYL v. 6.9 using the following: method, (Broyden, Fletcher, Goldfarb and Shanno) BFGS; initial optimization, Simplex; termination, gradient 0.05kcal/mol*A; maximum iterations, 5,000; energy force field and charge, (molecular mechanics force field 94) MMFF94. Bond distances were then measured between selected atoms.

(b) Superimposition

A superimposition of all class 1 compounds was generated by a fit atoms procedure using SYBYL v. 6.9. Molecules that had been previously minimized were used for a maximum overlap of the R, HAD and D. All class 1 compounds were superimposed to (**11o**), the most active analogue. After the fit atoms procedure, bond distances between R, D and HAD were measured again to determine if there was a difference between the bond distances of the initially minimized structures and the structures orientation after the fit atoms method was used. We noted that even in this conformation, R, D and HAD bond distances remained in the range of the suggested pharmacophore.

Regression Analysis

Data was analyzed in SAS v. 9.1 (SAS Institute, Cary, NC) using an IBM Net Vista (Win XP professional).

CONCLUSION

We have conclusively shown that the enaminones as well as the reported sodium channel blocking agents can indeed be modeled under the Unverferth hypothesis [12]. Further results on the enaminones will be reported.

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ABBREVIATIONS

QSAR	=	Quantitative Structure-activity Relationship
VGSCS	=	Voltage-gated sodium channels
ADD	=	Antiepileptic Drug Development
NINDS	=	National Institute of Neurological Disorders and Stroke
CF1	=	Carworth Farms No. 1
i.p.	=	Intraperitoneal
MES	=	Maximal electroshock seizure
ScPTZ	=	Subcutaneous pentylenetetrazol
Tox.	=	Toxicity
MM2	=	Molecular mechanics 2
MMFF	=	Molecular mechanics force field
MMFF94	=	Molecular mechanics force field 94
BFGS	=	Broyden, Fletcher, Goldfarb and Shanno

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