Anticonvulsant Activity of Phenylmethylenehydantoins: A Structure−Activity Relationship Study

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Phenylmethylenehydantoins (PMHs) and their *des*-phenyl analogues were synthesized and evaluated for anticonvulsant activity using the maximal electroshock seizure (MES) assay. The phenyl rings of PMHs were substituted with a wide spectrum of groups, and the selection of substituents was guided by Craig's plot. Phenylmethylenehydantoins substituted with alkyl (**2**, **3**, **5**, **6**, **12**, **14**), halogeno (**35**, **38**, **41**), trifluoromethyl (**11**), and alkoxyl (**23**) groups at the phenyl ring were found to exhibit good anticonvulsant activity with $ED_{MES(2.5)}$ ranging from 28 to 90 mg/kg. Substitution of polar groups such as $-NO_2$, −CN, and −OH was found to be less active or inactive on PMHs. Replacement of the phenyl ring with heteroaromatic rings reduced or caused the loss of anticonvulsant activity. The study identified two PMHs, **14** ($ED_{MES(2.5)} = 28 \pm 2$ mg/kg) and **12** ($ED_{MES(2.5)} = 39 \pm 4$ mg/kg), to be the most active candidates of the series, which are comparable to phenytoin (**55**, $ED_{MES(2.5)} = 30 \pm 2$ mg/kg) in their protection against seizure. Multivariate analysis performed on the whole series of 54 PMHs further supported the finding that the alkylated phenylmethylenehydantoins are the best acting compounds. The SAR model derived on the basis of 12 of the most active phenylmethylenehydantoins demonstrated good predicting ability (root-mean-square error of prediction (RMSEP) = 0.134; RMSEE = 0.057) and identified LUMO energy and the log *P* as critical parameters for their anticonvulsant activity.

Introduction

Epilepsy, one of the most frequent neurological afflictions in man characterized by excessive temporary neuronal discharges resulting in uncontrolled convulsion, requires special medical attention. Though several new anticonvulsants are introduced, some types of seizures are still not adequately treated with current therapy. Toxicity, intolerance, and lack of efficacy for certain types of seizure are some of the limitations of the current medications.[1]

Hydantoins, a class of cyclic imides, have been demonstrated to possess good anticonvulsant property.[2] Depending on the nature of substitution on the hydantoin ring, a wide range of other pharmacological properties,[3] e.g., fungicidal,[3a] herbicidal,[3b] antitumor,[3c] anti-inflammatory,[3c] anti-HIV,[3d] hypolipidemic,[3e] and antihypertensive[3f] activities, have also been identified. The anticonvulsant activity of 5,5-diphenylhydantoin, commonly known as phenytoin, has been known since 1938 as one of the most useful anticonvulsant drugs and is still regarded as the drug of choice for treating generalized tonic-clonic seizure (grandmal).[2] However, a number of side effects have limited its use.[2b,4] Structural modifications of the phenytoin molecule have led to several derivatives that exhibit different degrees of anticonvulsant activity.[5] Poupaert et al.[5c] observed that the anticonvulsant activity was reduced when the H-bonding groups (CO or NH) were removed from the phenytoin structure. This study was supported by that of Cortes et al.,[5b] when they observed the loss of activity on changing the hydantoin skeleton to imidazolone or imidazolidinone by removing or modifying the H-bonding groups. The alteration of the hydantoin ring in phenytoin to thiohydantoin or iminohydantoin also caused a drastic reduction in the anticonvulsant activity.[5g] Thus, any form of change in the hydantoin ring would diminish the activity of phenytoin. On the other hand, studies have shown that one phenyl ring attached at C5 of the hydantoin nucleus was sufficient to exhibit anticonvulsant activity.[5b] For example, 5-phenylhydantoin and 5-benzylhydantoins[5a] were found to be active against electrically induced seizures. This observation gained further support from the work of Brouillette et al.,[5e] which demonstrated that 5-pentyl-5-phenylhydantoin exhibited the same affinity as that of phenytoin for the neuronal voltage-dependent sodium channel. Therefore, in general, the structure−activity relationship studies of anticonvulsants revealed the need for the retention of at least one hydrophobic site and H-bond donors/acceptors together with certain relative orientation between these features to exhibit anticonvulsant activity.[5a−g] The hydantoin nucleus and the phenyl ring in phenylmethylenehydantoin (PMH) provided the basic structural requirement for activity. Previous studies from our laboratory have demonstrated a weak maximal electroshock seizure (MES) response for the unsubstituted PMH.[6] Thus, the main objective of the present study is to investigate the influence of substitutions in the phenyl ring of PMH on its anticonvulsant activity. On the basis of this investigation, a correlation was derived between the anticonvulsant

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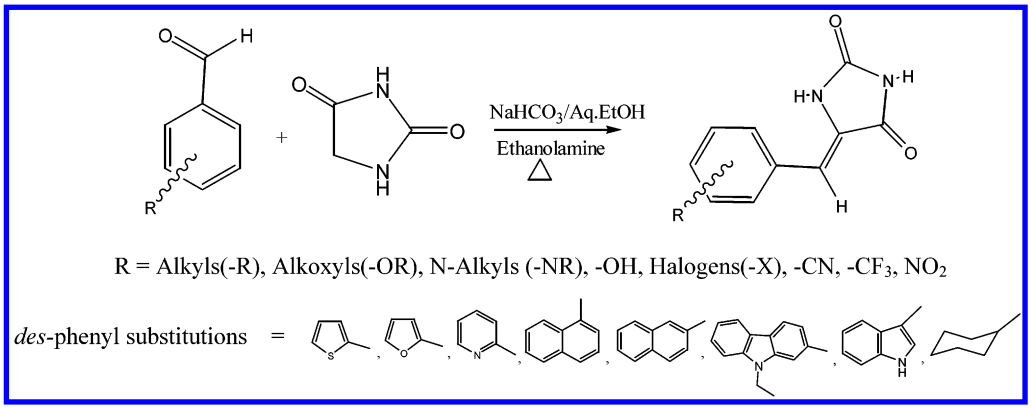
Scheme 1. General Method for the Synthesis of Substituted Phenylmethylenehydantoins and *des*-Phenylmethylenehydantoins



activity of PMHs and their relevant chosen physicochemical descriptors. A thorough review of literature to date (since 1967) showed no report that could provide a comprehensive correlation between the anticonvulsant activity and the physicochemical properties of phenylmethylenehydantoins. Thus, this first report on the PMHs would be of significant value in an effort to design the less toxic and more efficacious PMH analogues.

Chemistry and Molecular Design

Phenylmethylenehydantoin, which was first reported to possess anticonvulsant property by Wong et al. in 1989,[6a,c] was considered as the lead compound for molecular design. The importance of the phenyl ring was investigated by replacing it with (a) a π-rich (furan, thiophene) or a π-deficient (pyridine) aromatic heterocyclic ring, (b) a polycyclic aromatic ring (indole, naphthalene), or (c) a saturated carbocyclic ring (cyclohexane). Preliminary results indicated unsatisfactory anticonvulsant activities in these *des*-phenyl compounds. Thus, the phenyl ring was retained and further structural modification was concentrated mainly on the substitution at different positions of the phenyl ring. The selection of substituents was mainly based on the lipophilic (π) and electronic (σ) considerations as defined by Craig's plot.[7] Many of the earlier studies revealed the importance of the electronic and lipophilic parameters on the anticonvulsant activity.[8] In the present study, −F, −Cl, −Br, $-CF_3$, and $-NO_2$ were selected to represent electron-withdrawing ($+\sigma$) and lipophilic ($+\pi$) substituents; −CN was selected for its electron-withdrawing and less lipophilic ($+\sigma/-\pi$) property, while groups like −OH, −OMe, −OPr, and −OBu were representative of electron-donating and less lipophilic ($-\sigma/-\pi$) substituents; −Me, −Et, $-{}^{i}$Pr, $-{}^{n}$Bu, and -^{t}Bu represented electron-donating and more lipophilic ($-\sigma/+\pi$) substitution.

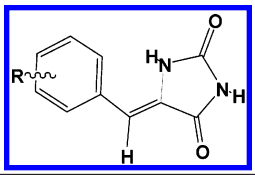
Phenylmethylenehydantoins were synthesized by a base-catalyzed condensation of hydantoins with appropriately substituted benzaldehydes (Scheme 1).

Although geometrical isomerism (*E*/*Z* isomers) was possible because of the restricted rotation about the exocyclic C═C double bond of the methylenehydantoins, in the quantity prepared for study, all the compounds were obtained exclusively in the *Z* form as confirmed by the analytical data (Supporting Information Table 1). In all the IR spectra of PMHs, the stretching of the C═C bond appeared in a higher frequency region (1660−1675 cm^{-1}) compared to those expected for the *E* form (1630−1640 cm^{-1}). The ^{1}H NMR spectra of PMHs showed that the most diagnostic olefinic proton, H6, was deshielded more in the *Z* form (6.40−7.00 ppm) than in the *E* form (6.20−6.30 ppm)[9] because of the anisotropic effect exerted by the nearby C4 carbonyl group in the former. A total of 54 (**1**−**54**) compounds were synthesized and evaluated for anticonvulsant activity using the MES assay. Eight of them were found to be new as indicated in Table 1. The structures of compounds synthesized are shown in Table 1 and their physical and analytical data are listed in the Supporting Information Table 1.

Multivariate analysis was carried out using SIMCA on all the PMHs (**1**−**54**) which were characterized by 13 physicochemical descriptors representing various size, electronic and lipophilic properties (Supporting Information Table 2). The lipophilicity of the phenylmethylenehydantoins was described by theoretically determined log *P* values and calculated lipophilicity factor (π values) for the nonionized molecules. The size parameters of PMHs were described by log *A* and log *V* (Connolly surface area and Connolly volume) and were obtained using the MOLCAD module within Sybyl (version 6.6). Other size parameters, such as molecular refractivity and bond counts, were computed from the software MedCache and used in the QSAR analysis. The Pearson correlation of these size parameters showed a sound relationship among the size parameters, revealing their physicochemical similarity (Supporting Information Table 3). The electronic properties of phenylmethylenehydantoins were described by the dipole moment, highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), and ionization potential (IP), and they were calculated in silico using MedCache. The difference in ^{13}C chemical shift ($\Delta\delta$), an additional electronic parameter, was experimentally determined by NMR studies. All these electronic parameters exhibited a good correlation among themselves, as illustrated by the Pearson correlation chart (Supporting Information Table 3).

Principal component analysis (PCA) was used to evaluate the molecular diversity of the PMHs selected for the QSAR study.[10] The partial least squares projection to latent structures (PLS), which is a regression extension of PCA, was later used to quantify and predict

Table 1. Anticonvulsant Activity of Substituted Methylenehydantoins (**1**−**54**)



compd	R	$ED_{MES(2.5)}$[a] (SEM), mg/kg	$\log(1/ED_{MES(2.5)})$
	Class 1 (▲), Alkyl Substitutent Category		
2	2Me	59(±6)	−1.77
3	3Me	74(±8)	−1.87
4	4Me	>200	−2.30
5	4Et	74(±9)	−1.87
6	4^{i}Pr	80(±6)	−1.90
7[d]	4^{n}Bu	121(±13)	−2.08
8	4^{t}Bu	>200	−2.30
12[d]	2,4-diMe	39(±4)	−1.59
13[d]	2,4,5-triMe	>200	−2.30
14	2,4,6-triMe	28(±2)	−1.45
	Class 2 (□), Halo/EWG[b] Substituent Category		
9[d]	2CF_3	>200	−2.30
10	3CF_3	115(±13)	−2.06
11	4CF_3	64(±7)	−1.81
31	2F	>200	−2.30
32	3F	172(±13)	−2.24
33	4F	>200	−2.30
34	2Cl	198(±18)	−2.29
35	3Cl	82(±7)	−1.91
36	4Cl	>200	−2.30
37	2Br	>200	−2.30
38	3Br	89(±9)	−1.95
39	4Br	>200	−2.30
40	2,4-diCl	>200	−2.30
41	2,6-diCl	72(±7)	−1.86
42	3,4-diF	121(±8)	−2.08
43	3NO_2	>200	−2.30
44	4NO_2	>200	−2.30
45	4CN	>200	−2.30
	Class 3 (+), Alkoxy Substituent Category		
15	2OH	>200	−2.30
16	3OH	>200	−2.30
17	4OH	>200	−2.30
18	2OMe	84(±9)	−1.92
19	3OMe	>200	−2.30
20	4OMe	>200	−2.30
21	2OEt	>200	−2.30
22	4OEt	107(±12)	−2.03
23[d]	4OPr	77(±8)	−1.89
24	4OBu	>200	−2.30
25[d]	3OPh	>200	−2.30
26[d]	2OCH_2Ph	>200	−2.30
27	4OCH_2Ph	157(±19)	−2.20
28	2,4-diOMe	175(±15)	−2.24
29	4NMe_2	>200	−2.30
30	4NEt_2	>200	−2.30
	Class 4 (∗), Heteroaryls and Other Aryls[c]		
1	H	125(±7)	−2.10
46	2-furyl	167(±11)	−2.22
47	2-thienyl	>200	−2.30
48	2-pyridyl	141(±16)	−2.15
49	3-indolyl	>200	−2.30
50[d]	3-Carbazolyl	>200	−2.30
51	1-naphthyl	>200	−2.30
52	2-naphthyl	>200	−2.30
53	4-phenyl	>200	−2.30
54	cyclohexyl	156(±17)	−2.19
	Class 5		
2	2Me	59(±6)	−1.77
3	3Me	74(±8)	−1.87
5	4Et	74(±9)	−1.87
6	4^{i}Pr	80(±6)	−1.90
11	4CF_3	64(±7)	−1.81
12	2,4-diMe	39(±4)	−1.59
14	2,4,6-triMe	28(±2)	−1.45
18	2OMe	84(±9)	−1.92
23	4OPr	77(±8)	−1.89
35	3Cl	82(±7)	−1.91
38	3Br	89(±9)	−1.95
41	2,6-diCl	72(±7)	−1.86
	Reference Compound		
55	phenytoin	30(±2)	−1.48

[a] Effective dose at an MES score of 2.5. The arbitrary scores of 1 and 4 indicate no protection and full protection, respectively. MES score at 2.5 is considered equivalent to the ED_{50} value for those compounds that attain the maximum score of 4, while for others it is not equivalent to ED_{50}. Hence, for the consistency of data for comparison, the table lists the effective dose at an MES score of 2.5. [b] EWG = electron-withdrawing groups. [c] The heteroaryls and aryls indicate the replacement of the phenyl ring with the other ring systems and not the substituent at the phenyl ring. [d] Compounds not listed in the Chemical Abstract Databases (1967 to present).

the relationship between activity and physicochemical descriptors.[10]

Results and Discussion

Anticonvulsant Activity. The anticonvulsant activity of the methylenehydantoins (**1**−**54**) was evaluated by using the MES assay on Swiss albino male mice.[6] The effective dose was considered as the dose that would produce a response score of 2.5 (i.e., $ED_{MES(2.5)}$ in mg/kg), and the logarithmic value was used in the QSAR study. The activity data indicated that the alkylated PMHs were found to produce the best protection against electrically induced seizure (Table 1) compared to the rest of the compounds in the series. Six of the 10 alkylated PMHs had $ED_{MES(2.5)}$ less than 80 mg/kg. The most active candidate was identified to be 2,4,6-trimethylphenylmethylenehydantoin (**14**, $ED_{MES(2.5)}$ = 28 mg/kg), which showed comparable protection as phenytoin ($ED_{MES(2.5)}$ = 30 mg/kg), as determined from the present study. The dialkylated derivative 2,4-dimethylphenylmethylenehydantoin (**12**) ($ED_{MES(2.5)}$ = 39 mg/kg), which is a new compound, was the second best active candidate in the PMH series tested, also comparable to phenytoin in its activity. 2-Methyl- and 3-methyl-substituted

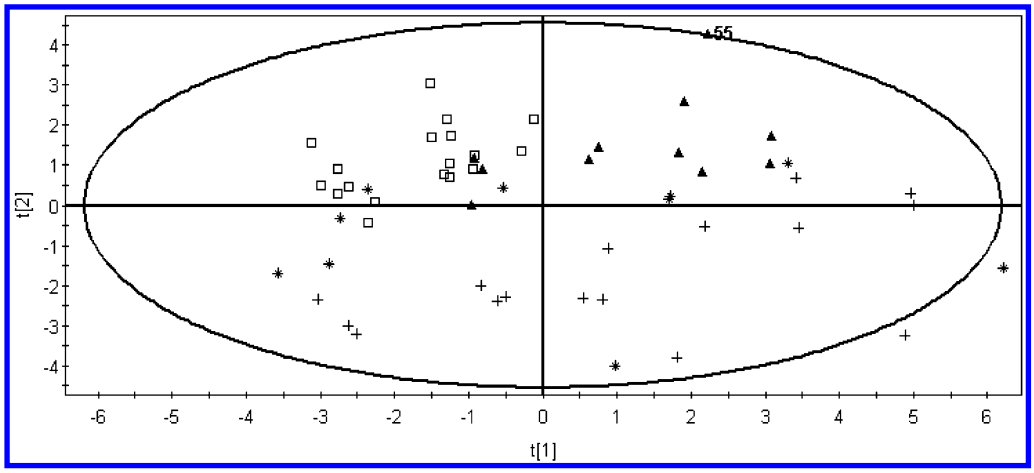


Figure 1. Score plot of principal components t_1 against t_2 for substituted phenylmethylenehydantoins: (class 1 consisting of alkyls, ▲) **2**−**8** and **12**−**14**; (class 2 consisting of halogens and other electron-withdrawing groups, □) **15**−**30**; (class 3 consisting of alkoxyls, +) **9**−**11** and **31**−**45**; (class 4 consisting of heteroaryls and aryls, ∗) **1** and **46**−**54**. **55** is the location of the reference compound phenytoin. The ellipse represents the confidence region based on Hotelling's T^2 (0.05).

PMHs were active while the 4-methyl derivative was inactive (Table 1), displaying the same trend as reported for the corresponding phenytoin analogues.[6c] Among the para-alkylated PMHs (**4**−**8**) tested, the PMH with ethyl group (**5**) appeared to be of optimal size for activity. In the case of halogenated PMHs (**31**−**42**), the best active candidate was 2,6-dichlorophenylmethylenehydantoin (**41**) ($ED_{MES(2.5)}$ = 72 mg/kg). Halogenation at the meta position of the phenyl ring showed protection against electrically induced seizure (3-Cl (**35**), $ED_{MES(2.5)}$ = 82 mg/kg; 3-Br (**38**), $ED_{MES(2.5)}$ = 89 mg/kg). Despite sharing the same lipophilicity, 2- and 4-halogenated PMHs (**31**, **33**, **34**, **36**, **37**, and **39**) showed poor seizure protection compared to 3-halogenated PMHs (**10**, **32**, **35**, and **38**). This could be attributed to the fact that the halo substitution at positions 2- and 4- of the phenyl ring might invoke conjugation between the halogen atom and the phenyl ring, resulting in a partial positive charge on the halogen and partial negative charge on the carbonyl oxygen of the hydantoin ring. Thus, the overall polarization of the molecule might be one of the reasons for its poor activity.

Among the alkoxy-substituted phenylmethylenehydantoins (**15**−**28**), 4-propoxyPMH (**23**, $ED_{MES(2.5)}$ = 77 mg/kg) was identified as the most active candidate followed by 2-methoxyPMH (**18**, $ED_{MES(2.5)}$ = 84 mg/kg). However, it is noteworthy that the 2-methoxyphenyl-substituted derivative of phenytoin was reported to be inactive.[6c] It is interesting to note that the hydroxyl group (electron-releasing/ hydrophilic function) and the nitro and cyano groups (electron-withdrawing/less lipophilic functions) behave similarly in this study by showing no protection against electrically induced seizure, while the 4-CF_3 group, which is also electron-withdrawing but relatively more lipophilic than hydroxyl, nitro, and cyano groups, exhibited good activity ($ED_{MES(2.5)}$ = 64 mg/kg). This observation indicated the importance of lipophilicity as well as electronic properties of the substituents on the activity of PMHs. The aromatic substituents at the C5 position were widely varied in order to probe the electronic and size requirements. Another noteworthy observation was that the introduction of an aryl group (**1** and **46**−**53**) other than phenyl in the methylenehydantoin structure had caused a significant reduction or the complete loss of activity (Table 1), while the exclusion of aryl ring by incorporating a cyclohexyl group (**54**) ($ED_{MES(2.5)}$ = 156 mg/kg) did not lead to the complete loss of activity, revealing that even a nonaryl group on methylenehydantoin could also protect the mice against seizure marginally.

A simple qualitative comparison of the structures of PMHs and their experimental data on seizure protection did not fully explain which of the physicochemical properties played a major role in contributing to the activity in this series of compounds. Hence, a systematic statistical analysis was considered necessary to derive a model that would describe the quantitative structure−activity relationship of PMHs and identify the role of critical physicochemical parameters responsible for the activity.

Multivariate Analysis. It was found that an overall stepwise linear single regression analysis between the descriptors and the anticonvulsant activity of all these compounds did not yield a significant correlation. Hence, all the 54 PMHs were classified into four classes based on the chemical nature of their substituents. Multivariate analysis was attempted on each class to explore potential correlation between the anticonvulsant activity of PMHs (**1**−**54**) and their 13 chosen physicochemical descriptors (Supporting Information Table 2).

In Figure 1, alkyl PMHs (▲; n = 10) were grouped as class 1 (**5**−**8**, **12**−**14**). All phenylmethylenehydantoins with electron-withdrawing substituents, such as halogens (**31**−**33**, **35**−**42**), nitro (**43**, **44**), trifluoromethyl (**9**−**11**), and cyano (**45**) groups, were considered as class 2 compounds (□; n = 18). The hydroxylated (**15**−**17**), O-alkylated (alkoxy) (**18**−**28**), and N-alkylated (**29**, **30**) phenylmethylenehydantoins (+; n = 16) formed class 3. Aryl (**1**, **51**−**53**), heteroaryl (**46**−**50**), and cyclohexyl (**54**) methylenehydantoins (∗; n = 10) were treated as class 4 in the analysis.

The individual PLS analyses on each class of these compounds did not yield a good correlation except for class 1, which showed a moderate correlation (q^2 = 0.574). Interestingly, the score plot (Figure 1) showed clustering of many active alkylated PMHs ($-\sigma/+\pi$) in the upper-right quadrant. The well-established anticonvulsant drug phenytoin (**55**), which was included in

Table 2. Summary of PLS Models Developed for Active PMHs in the SAR Analysis

model description	no. of components[a]	r^2	q^2	n^b	no. of descriptors	RMSEE[c]	RMSEP[d]
model 1	2	0.617	0.224	12	12		
model 2	2	0.838	0.642	11	11	0.062	0.087[e]
							0.053[f]
							0.248[i]
							0.283[j]
							0.134[e]
model 3	2	0.863	0.790	11	4	0.057	0.049[f]
							0.296[i]
							0.341[j]
							0.115[e]
model 4[l]	2	0.999	0.961	5	4	0.008	0.051[f]
							0.070[g]
							0.150[h]
							0.295[i]
							0.341[j]
							0.358[k]

[a] Number of significant components obtained in the PLS analysis. [b] Number of compounds used in the analysis. [c] Root-mean-square error of estimation in the analysis (predicted vs observed). [d] Root-mean-square error of prediction. [e] Root-mean-square error of prediction including all active compounds in the class. [f] RMSEP of all active compounds in the class 5 except outlier **11**. [g] RMSEP of all active compounds in the class 5 compounds inclusive of outlier excluding those in the training set. [h] RMSEP of all active compounds in the class 5 compounds except outlier **11** and those in the training set (**2**, **3**, **14**, **23**, **38**). [i] After excluding major outliers (**8**, **13**, **26**, **50**, **54**) from the series. [j] RMSEP of all 54 PMHs included in the study. [k] RMSEP of all 54 PMHs in the study without the training set. [l] Model obtained for the selected training set (**2**, **3**, **14**, **23**, **38**).

the analysis as a reference, was also clustered together with this group in the upper-right quadrant (▲; $n = 10$), suggesting that the class 1 compounds and phenytoin shared similar physicochemical properties. However, class 1 could not act as the representative model of the whole series because their physicochemical properties were different from those of compounds in the other classes. The other three classes, viz., 2, 3, and 4, did not yield a correlation probably because of the presence of many inactive compounds in them. Therefore, it was decided to group all the active members from each class and to have PLS analysis performed to investigate whether they shared any common physicochemical characteristics in exhibiting activity.

QSAR Modeling

Active Phenylmethylenehydantoins. Class 5 consisted of all active members, viz., **2**, **3**, **5**, **6**, **11**, **12**, **14**, **18**, **23**, **35**, **38**, and **41**. The selection of the active candidates was based on the cut off value at $ED_{MES(2.5)}$ of 90 mg/kg because the MES evaluation procedure required compounds to exhibit significant positive response at a dose of 100 mg/kg for inclusion as candidates for investigation. This classification was considered reasonable because the separate PLS analysis of all four classes yielded no PLS or poor PLS models on preliminary analysis when classified with respect to their physicochemical properties, thus leading to no conclusive result to represent all PMHs.

The *t*/*u* score plot of the PLS model displayed the observations in $x(t)$ space and activity in $y(u)$ space and identified the extent to which the "*y*" space (biological activity) correlated to the "*x*" space (descriptors).[10] The analysis of active compounds did not yield a reasonable model ($q^2 = 0.224$; model 1, Table 2), while the exclusion of the outlier, 4-trifluoromethylPMH (**11**), produced model 2 that could account for 83% and predict 64% of anticonvulsant activity of compounds in class 5. Earlier reports stated that models with q^2 greater than 0.5 were considered to have predictive capability better than chance, and with higher q^2, higher reliability could be attained.[10] Hence, the model was further refined to improve q^2 and in turn the predictive power. On the basis of the relative importance, out of 13 physicochemical descriptors, some might not significantly contribute to the activity, and hence, it was decided to exclude the less important ones from the analysis to improve the predictive power of the model. The variable importance projection (VIP) plot, one of the useful functions available in the SIMCA, is a measure of the relative importance of variables included in the analysis with respect to their contribution to the activity. Descriptors with a VIP value greater than 1 was considered to have an above-average influence on the activity.[10]

The careful inspection of the VIP plot showed little contribution from many descriptors. The bond count, log *V*, molecular refractivity, dipole moment, ionization potential, and ^{13}C chemical shift differences were found to be less important because their VIP values were less than 1 (Figure 2a). Nevertheless, omission of all of them might result in a better model with higher r^2 and q^2 values and a reduction or loss of predictive power, which would not be a wise approach. Hence, proper care was taken in removing the variables systematically so that predictive power was not reduced, for which the coefficient plot (Figure 2b) was also referred. However, in the present case, the exclusion of descriptors with VIP value less than 1 improved the model and accounted for 86% and predicted 79% of the anticonvulsant activity of active PMHs (model 3). The quality of model 3 was estimated from the root-mean-square error of prediction value (RMSEP) = 0.134. The predictive power of model 3 was further evaluated by selecting a few compounds from the work set of this model. By treatment of them as a "training set", the activity of the remaining unselected compounds in the "test set" was predicted. The selection of the compounds for the training set was based on of the score plot of model 3, where compounds exhibited a good spectrum of activity ranging from active to inactive compounds. The PLS analysis on the training set (**2**, **3**, **14**, **23**, **38**) yielded model 4, which could account for 99% and predict 96% of the anticon-

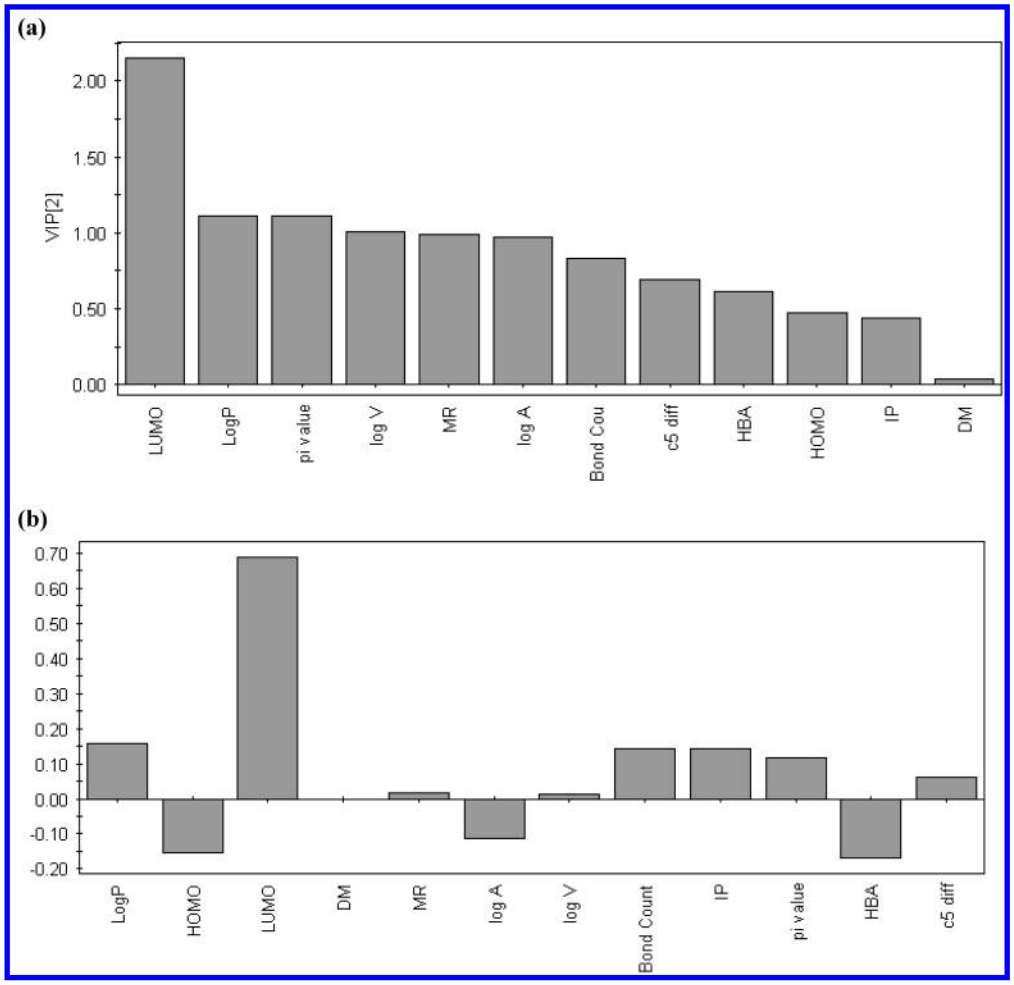


Figure 2. (a) VIP Plot from PLS analysis of data in model 2 (Table 2). (b) Coefficient plot from PLS analysis of data from model 2. Parameters with positive coefficients are directly proportional to the activity, while those with negative coefficients are inversely proportional to the activity. LUMO and log *P* are main descriptors directly related to the activity as shown in the plot.

vulsant activity (Table 2). The predictive power of this model for all the compounds in class 5 including the outlier (**11**) and excluding those in the training set (**2**, **3**, **14**, **23**, **38**) was found to be RMSEP = 0.150 (Figure 1b in Supporting Information), and its root-mean-square error of estimation (RMSEE) value, to compare with the estimated error in the observed and predicted values, was found to be 0.0082, showing the very good predictive power of the model.

Since the 12 active compounds were gathered from all four classes, the model (model 4) obtained for class 5 was expected to represent the whole series of all methylenehydantoins (**1**−**54**) under study. Hence, the overall predictive power of the model was evaluated and found to be within the reasonable range, RMSEP = 0.358 inclusive of all outliers and inactive PMHs in the study without including the training set of active PMHs in the prediction (Supporting Information Figure 3), while the exclusion of major outliers (**8**, **13**, **26**, **50**, and **54**) from the whole series yielded an RMSEP of 0.295, which was considered a good predictive ability attained by this representative model (model 4).

A careful observation of model 3 revealed the importance of both electronic and lipophilic parameters as main contributors of the anticonvulsant activity. The descriptors were found in the order of increasing importance: log *A* < π values < log *P* < LUMO. The LUMO, log *P*, and π values were directly related to the activity, while log *A* was inversely related (Supporting Information Figure 2). Thus, compounds in class 5 were expected to show enhanced anticonvulsant activity when they possessed high energy LUMO (poor acceptors) and high lipophilicity. All other size parameters such as log *V* and bond count were less contributing according to model 3, requiring the compounds to have less of them, while in the improved version, model 4, LUMO and log *P* were identified as main contributors.

From the VIP and coefficient plots (Figure 2 and Supporting Information Figure 2), the most influential descriptor was found to be LUMO, the best descriptor in contributing to the anticonvulsant activity. The LUMO energy is the inverse measure of the electron acceptor ability of a molecule. In other words, the higher the LUMO energy the lower will be the electron acceptor ability of the molecule and the better will be the anticonvulsant activity. This is expected because many of the active members of this series are better donors, bearing electron-donating groups in the phenyl ring (**2**, **3**, **5**, **6**, **12**, **14**, **18**, and **23**). The substitution of electron-donating groups at the phenyl ring makes the molecule rich in electron density, making the molecule poor acceptors and better donors. Thus, those compounds that satisfy the above conditions would be more active, whereas the electron-withdrawing substituents that favor the acceptor ability of PMHs were expected to disfavor seizure protection. This result derived from the

QSAR analysis was consistent with the experimental data shown in Table 1, where those with withdrawing nitro and cyano groups (**43**−**45**) did not exhibit activity. However, halogen-substituted PMHs (**35** and **38**), despite their electronegativity, exhibited lipophilicity that contributed to their anticonvulsant activity. These observations supported the importance of both electronic and lipophilic properties in exhibiting activity. Thus, compounds with higher LUMO energy and appropriate lipophilic properties would tend to be better active candidates of the series.

The reliability of model 4 was further confirmed by multiple linear regression (MLR) analysis using the statistical software SPSS (version 10), and the significant regression equation (eq 1) obtained demonstrated effectively the role of LUMO and log *P* in these compounds. Equation 1 indicates the significance of each of these parameters:

$$-\log \mathrm{ED}_{\mathrm{MES}(2.5)} = -1.247(\pm 0.149) + 0.795(\pm 0.152)\mathrm{LUMO} + 0.150(\pm 0.045)\log P \quad (1)$$

$$n = 11; \quad r^2 = 0.834; \quad r^2_{\mathrm{cv}} = 0.793; \quad \mathrm{SE} = 0.063; \quad F = 20.09; \ \mathrm{Sig} = 0.001$$

The numbers in parentheses are the 95% confidence intervals associated with the coefficients, *n* is the number of phenylmethylenehydantoins included in the study, r^2 is the squared correlation coefficient corresponding to the fraction of observed variance accounted for by the model, SE is the standard error of estimation, *F* is the *F*-test value, a statistic for assessing the overall significance of the derived equation, r^2_{cv} is the cross-validated (leave-one-out) correlation coefficient.

The positive correlation for log *P* and LUMO energy suggests that the activity improves with an increase in the values of these descriptors. The cross-correlation coefficients between the variables LUMO and log *P* were verified from the Pearson correlation data and found to be significantly lower (0.33) (Supporting Information Table 3), revealing their true independence from each other to make a meaningful contribution in the equation.

The results of the present study gained support from many previous reports.[8,11] Tasso et al., in their recent study on QSAR analysis of modified hydantoin derivatives and other chemical classes, reported the best regression expression ($-\log \mathrm{ED}_{50} = -11.669E_{\mathrm{LUMO}} + 1.206$; $r^2 = 0.931$), indicating a strong dependence of anticonvulsant activity on the LUMO energy (E_{LUMO}).[8a] The increase in LUMO energy resulted in an increase in the anticonvulsant activity. According to the study, the manifestation of activity implies an acceptor−donor electrostatic interaction between the ligand and the active site of the receptor. Another recent report on the QSAR of hydantoin analogues by Weaver et al. also revealed the determinant role of LUMO on the anticonvulsant activity of compounds in this class.[8b] Murray et al. have reported the key role of molecular surface electrostatic potentials on the anticonvulsant activity.[8c] Many other SAR studies have claimed various structural and physicochemical requirements in hydantoin derivatives, including log *P*,[8c,e] hydrogen-bond donor/acceptor abilities,[5b,c] and a nonpolar hydrophobic moiety in proper orientation and distance with respect to the polar functions.[5c,8g,h] It was observed that the structural modification that removed the NH function from the hydantoin ring in the phenytoin molecule caused significant reduction in the activity.[5] This could be correlated to the present observation on the anticonvulsant activity of PMHs that the high LUMO energy of molecule, which was found to be a favorable positive factor to exhibit activity, would disfavor the deprotonation of NH in PMHs. It could be related to the fact that deprotonation deprived the hydantoin ring of a proton donor (NH) for drug−receptor interaction and hence the reduction in activity. In addition to Brouillette et al.'s studies on the role of log *P* in the transport phenomena in vivo and through membranes,[5e,f] many other reports demonstrated the importance of log *P* in exhibiting anticonvulsant activity.[5] Although PMHs have the presence of a hydantoin ring bearing H-bonding groups and one phenyl ring as a hydrophobic site, their limited conformational flexibility due to its extended conjugation and a flat geometry might not exactly fulfill the stereochemical requirement[8g,h] as other conformationally flexible hydantoins do. Despite that limitation, PMHs revealed significant activity response in MES assays (Table 1), resulting in two promising candidates: **12** and **14**. This stereochemical limitation gained support from 3-hydroxy-3-acetonylindole;[8g] which exhibited anticonvulsant activity against MES-induced seizures with its geometrically restricted stereochemistry that could not fulfill the stereochemical requirement like phenytoin. Thus, the present study manifested the importance of electronic and hydrophobic properties rather than the restricted stereochemistry of the molecule. Although previous studies on the structure−activity relationship of anticonvulsant drugs of different chemical classes suggested electronic, lipophilic, and stereochemical requirements to achieve better activity,[5,8,11] a thorough literature search on chemical abstract databases to date showed no comprehensive report on the structure−activity relationship of phenylmethylenehydantoin. Hence, the present investigation is a new first report that systematically studied the structure−activity relationship of phenylmethylenehydantoins to identify the critical parameters that contribute to the seizure protection.

Conclusion

A series of 54 substituted methylenehydantoins, based on the electronic, steric, and lipophilic nature of their substituents, were designed, synthesized, and evaluated for their anticonvulsant activity using the MES assay. An attempt was made to develop a comprehensive model that would correlate the anticonvulsant activity of PMHs and their appropriately chosen physicochemical properties.

Two PMHs (**12** and **14**) were found to be the most active, with $\mathrm{ED}_{\mathrm{MES}(2.5)}$ less than 40 mg/kg, which interestingly turned out to be comparable to that of phenytoin, the standard anticonvulsant drug. QSAR study using multivariate analysis gave a significant PLS model for active candidates that could account for 99% and predict 96% of their anticonvulsant activity (class 5). The model demonstrated that the LUMO energy and the lipophilicity of the compounds played a determinant role in the activity. Multilinear regression analysis also

identified LUMO and log P as important descriptors with their quantitative contributions (eq 1) and was consistent with the experimental observation and previous reports.[5,8,11] From the QSAR study described above, it was clear that alkylated PMHs exhibited the best protective effect electrically against seizure.

Experimental Section

Chemistry. Melting points were determined using Gallenkamp melting points apparatus and were uncorrected. The elemental analysis and mass spectra were recorded with a Finnigan MAT-LCMS (San Jose, CA) spectrometer and VG Micromass 7035 E mass spectrometer. IR spectra were recorded using a Jasco-FTIR-430 instrument. ^{1}H NMR spectra were obtained from a DPX-300 MHz Bruker NMR spectrometer using DMSO-d_6 as the solvent and TMS as the internal reference. TLC of the final products was performed on silica gel aluminum sheets (with fluorescent indicator) using $CHCl_3$/MeOH (9.5:0.5) as eluants.

Chemical Synthesis. The general procedure for the synthesis of PMHs and the *des*-phenyl anlogues is as follows.[12] In a clean round-bottom flask, hydantoin (1.0 g, 10.0 mM) was dissolved in 10 mL of water at 70 °C with stirring. After complete dissolution, the pH of the mixture was adjusted to 7.0 with saturated $NaHCO_3$ solution. Ethanolamine (0.9 mL) was added to the reaction mixture, and the temperature was increased to 90 °C by use of an oil bath. To this, an equimolar quantity of the appropriate aldehyde (10.0 mM) solution in 10 mL of alcohol was added dropwise with continuous stirring. The temperature was raised to 120 °C and kept under reflux at that temperature for approximately 5−10 h depending on the nature of the aldehyde used. Progress of the reaction, as evident by the precipitation of solids, was monitored at regular intervals by TLC. When the mixture was cooled, the precipitate was filtered and washed with alcohol/water (1:5) in order to remove the soluble impurities before recrystallization using ethanol. The yields of all compounds, their melting points, and the spectral and elemental analyses data are given in Supporting Information Table 1.

Evaluation of Anticonvulsant Activity. The in vivo anticonvulsant activity of PMHs was evaluated using the maximal electroshock seizure (MES) test.[6] Maximal electroshock induced seizure is an established model for the generalized tonic-clonic seizures. Swiss albino male mice (25−30 g) were used for the tests. The mice were housed in an environmentally controlled room under 12 h light and dark cycles with proper food and water before testing. Prior to drug administration, the mice were subjected to the rotorod test, and those that could pass this test by staying on the rotating rod for the full 5 min of the testing period were used for the MES assay. The test compounds (**1**−**55**) were dissolved in DMSO and injected into the mice intraperitoneally at an initial dose of 100 mg/kg. Depending on the test scores from this initial dose, subsequent doses used were gradated higher or lower to obtain a complete dose−activity profile. At 30 min after drug administration, the mice were subjected to a current of 45 mA (100 MHz) using a UGO BASILE ECT unit 7801 instrument for 0.2 s via auricular electrodes to elicit maximal electroshock seizures. The anticonvulsant activity of each compound on the mice was assessed by an arbitrary scoring system (1, 2, 3, and 4) at various stages of seizure.[6] A score of 1 or 4 indicates no protection or full protection of seizure, respectively. At each dose level, the average MES score given for a test sample of five to six mice was recorded. The dose (mg/kg) that responded a score of 2.5 was used as the parameter to compare the anticonvulsant activity of different compounds. Table 1 gives the dosages that result in an MES score of 2.5 (i.e., $MES_{(2.5)}$ dosages) of the PMHs studied.

Determination of the Chemical Shift at the β Carbon (C5). The ^{13}C NMR chemical shift of the β carbon was used to identify the electronic influence of the substituents at the phenyl ring. The difference in the chemical shift $\Delta\delta$ was given by ($\delta_H - \delta_R$), where δ_R is the chemical shift of the substituted phenylmethylenehydantoin and δ_H is that of the unsubstituted compound (**1**), as in ref 13. The $\Delta\delta$ value is known to be sensitive to the electronic influence of the substituents on the aryl moieties.[13]

Molecular Modeling Methods To Calculate Descriptors. All the compounds under study were minimized using the MMFF94s force field in Sybyl, version 6.6 (software from Tripos Inc., St. Louis, MO before calculating their physicochemical properties. Connolly surfaces (log *V* and log *A*) were calculated from MOLCAD module within sybyl. The log *P*, molecular refractivity, bond counts, ionization potential, orbital energies HOMO and LUMO, and the dipole moment were obtained from the software MedCache, version 5.5 (Fujitsu) after minimizing the structures using MM/PM3 force fields.

Statistical Methods. Multiple linear regression analyses were carried out using SPSS 11 (SPSS, Inc., Chicago, IL). The measure of explained variance (r^2), the 95% confidence interval variables, the Fischer significance ratio (*F*) at $P = 0.05$, and the standard error (SE) were determined for the regression equation. Cross-validation of the r^2 and standard error was performed using the PLS-QSAR module of Sybyl.

The multivariate analyses were performed with SIMCA-P (version 8.0)[14] (Umetrics) using the default settings.

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Supporting Information Available: Tables 1 and 2 listing analytical data and the physicochemical properties of all PMHs , respectively, Table 3 listing correlation matrix descriptors, Figure 2 showing the VIP and coefficient plots, and Figures 1−3 showing PLS score plots and a plot of predicted versus observed activity response of principal components for active PMHs. This material is available free of charge via the Internet at http://pubs.acs.org.

Note Added after ASAP Posting. This manuscript was released ASAP on 2/13/2004 with errors in eq 1. The correct version was posted 2/19/2004.

References

(1) World Health Organization Report, 2001. http://www.who.int/inf/en/pr-2002−30.html.

(2) (a) Merritt, H. H.; Putnam, T. J. A New Series of Anticonvulsant Drugs Tested by Experiments on Animals. *Arch. Neurol. Psychiatry* **1938**, *39*, 1003−1015. (b) Hassell, T. M.; Johnson, M. C.; Dudley, K. H. In *Phenytoin Induced Teratology and Gingival Pathology*; Raven Press: New York, 1980.

(3) (a) Marton, J.; Enisz. J.; Hosztafi, S.; Timar, T. Preparation and Fungicidal Activity of 5-Substituted Hydantoins and Their 2-Thio Analogs. *J. Agric. Food. Chem*. **1993**, *41* (1), 148−152. (b) Hanessian, S.; Sanceau, J. Y.; Chemla, P. Synthesis of Surrogate Structures Related to the Herbicidal Agent Hydantocidin. *Tetrahedron* **1995**, *51* (24), 6669−6678. (c) Ahmed, K. I. Synthesis of Arylidenehydrazano- and Glycopyranosylhydrazinosulfonylbenzylidenediones as Potential Antiviral and Antitumoral Agents. *Carbohydr. Res.* **1998**, *306* (4), 567−573. (d) Comber, R. N.; Reynolds, R. C.; Friedrich, J. D.; Manguikian, R. A.; Buckheit, R. W.; Truss, J. J. W.; Shannon, W. M.; Secrist, J. A., III. 5,5-Disubstituted hydantoins: Syntheses and anti-HIV Activity. *J. Med. Chem*. **1992**, *35*, 3567−3572. (e) Tompkins, E. 5,5-Diaryl-2-thiohydantoins and 5,5-Diaryl-N-substituted-2-thiohydantoins as Potential Hypolipidemic Agents. *J. Med. Chem*. **1986**, *29* (5), 855−859. (f) Menendez, J. C.; Diaz, M. P.; Bellver, C.; Sollhuber, M. M. Synthesis, Anticonvulsant and Antihypertensive Activity of Diastereomeric 9,10-Dimethoxy-1,3,4,6,7,11b-hexahydrospiro-[benzo[*a*]quinolizin-2,4-imidazolidine]-2′,5′-diones. *Eur. J. Med. Chem*. **1992**, *27*, 61−66.

(4) (a) Rogawski, M. A.; Porter, R. J. Antiepileptic drugs: Pharmacological Mechanisms and Clinical Efficacy with Consideration of Promising Developmental Stage Compounds. *Pharmacol. Rev*,

1990, *42* (3), 223−286. (b) Keith, D. A. Side effects of Diphenylhydantoin: A Review. *J. Oral Surg.* **1978**, *36*, 206−209. (c) Finnell, R. H. Phenytoin-induced Teratogenesis: A Mouse Model. *Science* **1981**, 211, 483−484.

(5) (a) Mehta, N. B.; Diuguid, C. A. R.; Soroko, F. E. Potential Anticonvulsants. 1. 5-Benzylhydantoins. *J. Med. Chem.* **1981**, *24*, 465−468. (b) Cortes, S.; Liao, Z. K.; Watson, D.; Kohn, H. Effect of Structural Modification of the Hydantoin Ring on Anticonvulsant Activity. *J. Med. Chem.* **1985**, *28* (5), 601−606. (c) Poupaert, J. H.; Vandervorst, D.; Guiot, P.; Mustafa, M. M. M.; Dumont, P. Structure−Activity Relationships of Phenytoin-like Anticonvulsant Drugs. *J. Med. Chem.* **1984**, *27*, 76−78. (d) Krall, R. L.; Penry, J. K.; White, B. G.; Kupfergerg, H. J.; Swinyard, E. A. Antiepileptic drug development: II. Anticonvulsant Drug Screening. *Epilepsia* **1978**, *19*, 409−428. (e) Brouillette, W. J.; Jestkov, V. P.; Brown, M. L.; Akhtar, M. S.; DeLorey, T. M.; Brown, G. B. Bicyclic Hydantoins with a Bridgehead Nitrogen. Comparison of Anticonvulsant Activities with Binding to the Neuronal Voltage-Dependent Sodium Channel. *J. Med. Chem.* **1994**, *37*, 3289−3293. (f) Brown, M. L.; Brown, G. B. Brouillette, W. J. Effect of Phenyl Ring Conformation on the Binding of 5-Phenylhydantoins to the Voltage-Dependent Sodium Channel. *J. Med. Chem.* **1997**, *40*, 602−607. (g) Kwon, C. H.; Iqbal, M. T.; Wurpel, J. N. D. Synthesis and Anticonvulsant Activity of 2-Iminohydantoins. *J. Med. Chem.* **1991**, *34*, 1845−1849.

(6) (a) Wong, P. T. H.; Tan, S. F. Pharmacological Profiles of 5-Phenylmethylenehydantoin and Its Methoxy Substituted Derivatives. *Jpn. J. Pharmacol.* **1989**, *49* (3), 309−315. (b) Wong, P. T. H.; Tan, S. F. N-Demethylation of Methyl and Dimethyl Derivatives of Phenytoin and Their Anticonvulsant Activities in Mice. *Jpn. J. Pharmacol.* **1988**, *48* (4), 473−478. (c) Wong, P. T. H.; Tan, S. F.; Teo, W. L. Testing for Anticonvulsant Activities of Derivatives of Phenytoin and 5-Phenylmethylenehydantoin. *Asia Pac. J. Pharmacol.* **1989**, *4*, 107−112.

(7) van der Waterbeemd, H. Quantitative Approaches to Structure Activity Relationships. In *The Practice of Medicinal Chemistry*; Wermuth, C. G., Ed.; Academic Press: London, 1996; p 367.

(8) (a) Tasso, S. M.; Bruno-Blanch, L. E.; Moon, S. C.; Estiu, G. L. Pharmacophore Searching and Quantitative Analysis in the Design of Anticonvulsant Drugs. *J. Mol. Struct.: THEOCHEM* **2000**, *504*, 229−240. (b) Weaver, D. F.; Sutherland, J. F. Development of Quantitative Structure Activity Relationships and Classification Models for Anticonvulsant Activity of Hydantoin Analogues. *J. Chem. Inf. Comput. Sci.* **2003**, *43* (3), 1028−1036. (c) Murray, J. S.; Abu-Awwad, F.; Politzer, P.; Wilson, L. C.; Toupan, A. S.; Wall, R. E. Molecular Surface Electrostatic Potentials of Anticonvulsant Drugs. *Int. J. Quantum Chem.* **1998**, *70* (6), 1137−1143. (d) Hadjipavlou-Litina, D. Review, Reevaluation, and New Results in Quantitative Structure−Activity Studies of Anticonvulsants. *Med. Res. Rev.* **1998**, *18* (2), 91−119. (e) Scholl, S.; Koch, A.; Henning, D.; Kempter, G.; Kleinpeter, E. The Influence of Structure and Lipophilicity of Hydantoin Derivatives on Anticonvulsant Activity. *Struct. Chem.* **1999**, *10*, 355−366. (f) Lien, E. J. Structure Activity Correlations of Anticonvulsant Drugs. *J. Med. Chem.* **1970**, *13* (6), 1189−1191. (g) Codding, P. W.; Lee, T. A.; Richardson, J. F. Cyheptamide and Hydroxy-3-phenacyloxyindole: Structural Similarity to Diphenylhydantoin as the Basis for Anticonvulsant Activity. *J. Med. Chem.* **1984**, *27*, 649−654. (h) Camerman, A.; Camerman, N. Diphenylhydantoin and Diazepam: Molecular Structure Similarities and Steric Basis of Anticonvulsant Activity. *Science* **1970**, 1457−1458.

(9) (a) Tan, S. F.; Ang, K. P.; Fong, Y. F. (*Z*)- and (*E*)-5-Arylmethylenehydantoins: Spectroscopic Properties and Configuration Assignment. *J. Chem. Soc., Perkin Trans. 2* **1986**, 1941−1944. (b) Kleinpeter, K. The Structure of Hydantoins in Solution and in the Solid State. *Struct. Chem.* **1997**, *8* (2), 161−173.

(10) (a) Winiwarter, S.; Bonham, N. M.; Ax, F.; Anders, H.; Lennernas, H.; Karlen, A. Correlation of Human Jejunal Permeability (in Vivo) of Drugs with Experimentally and Theoretically Derived Parameters. A Multivariate Data Analysis Approach. *J. Med. Chem.* **1998**, *41*, 4939−4949. (b) Liu, M.; Wilairat, P.; Go, M. L. Antimalarial Alkoxylated and Hydroxylated Chalcones: Structure−Activity Relationship Analysis. *J. Med. Chem.* **2001**, *44*, 4443−4452.

(11) (a) Bardel, P.; Bolanos, A.; Kohn, H. Synthesis and Anticonvulsant Activities of α-Acetamido-*N*-benzylacetamide Derivatives Containing an Electron-Deficient α-Heteroaromatic Substituent. *J. Med. Chem.* **1994**, *37*, 4567−4571. (b) Lien, E. J.; Tong, G. L.; Chou, J. T.; Lien, L. L. Structural Requirements for Centrally Acting Drugs I. *J. Pharm. Sci.* **1973**, *62* (2), 246−250. (c) Farrar, V. A. Rutkowska, M. C.; Grochowski, J.; Serda, P.; Pilati, T.; Filippini, G.; Hinko, C. N.; El-Assadi, A.; Moore, J. A.; Edafiagho, I. O.; Andrews, C. W.; Cory, M.; Nicholson, J. M.; Scott, K. R. Synthesis and CLOGP Correlation of Imidooxy Anticonvulsants. *J. Med. Chem.* **1993**, *36* (23), 3517−3525. (d) Seydal, J. K.; Schaper, K. J.; Coats, E. A.; Cordes, H. P.; Emig, P.; Engel, J.; Kutscher, B.; Polymeropoulos, E. E. Synthesis and Quantitative Structure−Activity Relationships of Anticonvulsant 2,3,6-Triaminopyridines. *J. Med. Chem.*, **1994**, *37* (19), 3016−3022, 3289−3293. (e) Lapszewicz, D.; Lange, J.; Rump, S.; Walczyna, K.; Quantitative Structure−Activity Relationship in Anticonvulsants. A QSAR Analysis of New Phenylsuccinimide Derivatives. *Eur. J. Med. Chem. Chim. Ther.* **1978**, *13* (5), 465−468.

(12) (a) Tan, S. F.; Ang, K. P.; Jayachandran, H.; Fong, Y. F. Substituent Effects in ^{1}H and ^{13}C Nuclear Magnetic Resonance Correlations of Chemical Shifts in *para*-Substituted 5-Arylmethylenehydantoins. *J. Chem. Soc., Perkin Trans. 2* **1987**, *11*, 1043−1045. (b) Tan, S. F.; How, G. F.; Yeoh, P. T. Synthesis and NMR Study of 5-Furylmethylenehydantoins and 5-Thienylmethylenehydantoins. *J. Phys. Org. Chem.* **1991**, *4*, 681−688. (c) Tan, S. F.; Ang, K. P.; How, G. F. NMR Spectroscopic Study of Configurations and Conformations of 5-Pyridylmethylenehydantoins. *J. Phys. Org. Chem.* **1990**, *3*, 559−566. (d) Tan, S. F.; Yong, P. L. A.; Lee, L. T. *Z*−*E* Isomerism of 5-Cyclohexylmethylenehydantoins. *J. Phys. Org. Chem.* **1992**, *5*, 795−802. (e) Fresneau, P.; Thomasson, F.; Morand, J. M.; Cussac, M. Determination of the Stereochemistry of Substituted 5-Methylenehydantoins and Thiohydantoins by Carbon-13 NMR and Homonuclear NOE Experiments. *Spectrosc. Lett.* **1993**, *26* (8), 1511−1521. (f) Namjoshi, V. G.; Dutt, S. J. *Indian J. Chem. Soc.* **1931**, *8*, 241−246.

(13) (a) Wernly Chung, G. N.; Mayer, J. M.; Tsantili-Kakoulidou, A.; Testa, B. Structure−Reactivity Relationships in the Chemical Hydrolysis of Prodrug Ester of Nicotinic Acid. *Int. J. Pharm.* **1990**, *63*, 129−134. (b) Rong, X.; Go, M. L.; Structure−Hydrolyzability Relationships in a Series of Piperidinyl and Tropinyl Esters with Antimuscarinic Activity. *Chem. Pharm. Bull.* **1997**, *45*, 476−481.

(14) *SIMCA*; Umetrics AB (Box 7960, S-907 19): Umea, Sweden, 1999.

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