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Original Scientific Paper

Quantitative Structure-activity Relationship Modeling of Mosquito Repellents Using Calculated Descriptors

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Quantitative structure-activity relationship (QSAR) modeling of 40 DEET-related mosquito repellents was carried out using calculated molecular descriptors. When the four different classes of descriptors (topochemical, topostructural, geometrical and quantum chemical) were used in a hierarchical fashion, topochemical descriptors were found to explain much of the variance in the data and this indicated the importance of the chemical nature of the atoms and groups towards repellency of these compounds. Ridge regression (RR) outperformed partial least square regression (PLS) and principal component regression (PCR). We also used descriptor thinning *via* a modified Gram-Schmidt algorithm prior to ridge regression. This resulted in a four-parameter model with a 20-fold cross-validated R^2 of 0.734. The q^2 (R^2_{cv}) reported here is the "true-q^2" because the descriptor thinning was embedded within the cross-validation step. Inclusion of any calculated physicochemical property (secondary descriptor) did not result in improvement of the models built with the calculated molecular descriptors (primary descriptors). This result has great implications in the QSAR assisted design of new mosquito repellents because calculation of the primary descriptors does not require any input other than the molecular structure.

Keywords
DEET
mosquito repellents
QSAR
topological indices

INTRODUCTION

Since the report of the most popular mosquito repellent DEET (*N,N*-diethyl-3-methylbenzamide) in 1954,[1] (Table I, Seq # 18), several attempts have been made to synthesize repellents better than DEET and these studies were mostly on amides. McGovern *et al*, synthesized and tested quite a large number of amides and these include cyclic carboxamides of secondary amines,[2] *N*-acyl and *N*-alkylsulfonyl derivatives of heterocyclic amines, and *N,N*-dialkylalkanesulfonamides.[3] Though McGovern could draw some qualitative conclusions on the effect of structure on the repellency of chemicals, no quantitative structure-activity modeling was done by his group. Basak *et al.*[4] carried out hierarchical quantitative structure-activity relationship (HiQSAR) modeling of McGovern's data set of 30 cyclic carboxamides for two species of mosquitoes namely, *Aedes aegypti* and *Anopheles quadrimaculatus. Aedes aegypti* repellency data were modeled better than those of *Anopheles quadrimaculatus*. In the case of re-

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pellency towards *Aedes aegypti*, only size and shape of the amide molecules appeared to play an important role, as indicated by the fact that the best regression model utilized the topostructural (TS) parameters alone. On the other hand, repellency of the cyclic carboxamides towards *Anopheles quadrimaculatus* appeared to depend not only on shape and size but also on the chemical nature, namely the presence of a double bond. This was indicated by the improvement in predictability of the regression models on adding topochemical descriptors (TC) to topostructural descriptors. A comprehensive study on these data sets and others on insect repellency will be published elsewhere.

Suryanarayana *et al*.[5] synthesized and tested 40 amides of aromatic and cyclohexyl carboxylic acids. They also developed QSAR models using lipophilicity (log*P*), vapor pressure (VP) and molecular length (ML). They came up with the following regression equation to model protection time (PT):

$$\mathrm{PT} = a\ \log P + b\ \log\mathrm{VP} + c\ \mathrm{ML} + d \qquad (1)$$

$$n = 40; \quad r = 0.551$$

It should be noted the lipophilicity parameter (log*P*) was obtained from the HPLC capacity factor, while the vapor pressure at 303 K was calculated from Antoine equation by measuring VP at three or more higher temperatures. The above model obtained for the complete data set had a low correlation coefficient ($r = 0.551$). However, when the data set was split into 8 smaller sets each containing only 5 compounds that are structurally very similar, the correlation improved significantly. However, in the model for classified data they replaced the molecular length (ML) by molar refraction (MR). The individual models can be expressed by the generic equation:

$$\mathrm{PT} = a\ \log P + b\ \log\mathrm{VP} + c\ \mathrm{MR} + d \qquad (2)$$

This regression model requires experimentally determined physicochemical properties and moreover uses three parameters to model five observations. These problems and the absence of cross-validation are some of the major limitations of the work.

Bhattacharjee *et al.*[6] assessed the similarity of 15 of the DEET analogs from Suryanarayana's data set with a juvenile hormone mimic to indicate the importance of the amide moiety for bioactivity. The importance of the amide moiety and the putative pharmacophore for repellency of amides was very well demonstrated by the biophore overlay study[7] on DEET and the diastereomers of AI3-37220 and Picaridin. This study also brought out the importance of chiral center(s) in the piperidine amides towards their insect repellent property. Ma *et al.* [8] compared electronic properties, such as molecular electrostatic potential, dipole moment and atomic charges, of 31 of the 40 DEET related compounds but did not develop any quantitative prediction model.

Recently Katritzky *et al*.[9] modeled the truncated set of 31 compounds from Suryanarayana's data using Codessa Pro software[10] to develop QSAR models with calculated parameters. We observe the following problems in their methodology:

1. They used variable selection from a large pool of descriptors, and in such a situation, variable selection[11,12] must be included within the cross-validation step. In the absence of embedding variable selection in the cross-validation, the q^2 reported (naïve-q^2) is generally higher than the true-q^2. The problem of naïve-q^2 and over-fitting in chemometric data had been addressed recently by Basak *et al*.[13] and Kraker *et al*.[14]

2. The authors developed a model to predict vapor pressures of the DEET analogs but by oversight they seem to have missed the fact that the vapor pressure data collected from SciFinder Scholar[15] are calculated, rather than experimental, vapor pressures.

We report in this paper QSAR models developed on the complete set of 40 compounds originally reported by Suryanarayana *et al.* The QSAR models discussed were developed using the following three different approaches:

1. Hierarchical QSAR (HiQSAR): In this approach, linear regression models are developed by adding the four classes of descriptors (topochemical, topostructural, 3-dimensional and quantum chemical) in a hierarchical fashion based on their complexity and demand for computational resources.

2. Descriptor thinning: Model building is performed using a reduced number of descriptors after variable selection/descriptor thinning in which validation had been applied at the proper stage.

3. Adding secondary descriptors to the models initially built with primary descriptors: Primary and secondary descriptors are combined in model building, where primary descriptors are molecular descriptors calculated only from the structure and secondary descriptors are physicochemical properties that are calculated.

A brief account of these approaches is given below.

Hierarchical QSAR (HiQSAR)

A large number of molecular descriptors are available for QSAR modeling and they can be computed from the molecular structure as the only required input. These descriptors are classified into four categories namely,

1. Topostructural Indices (TS)
2. Topochemical Indices (TC)
3. 3D or geometrical parameters (3D)
4. Quantum chemical descriptors (QC)

Computation of topostructural descriptors does not include the nature of bonds or atom types while topoche-

mical indices do encode these features. However, only the 3-D parameters take into account the geometry of the molecules. Electronic properties such as energy of the highest occupied molecular orbital (E_{HOMO}), energy of the lowest unoccupied molecular orbital (E_{LUMO}), atomic charges, dipole moment *etc.*, are classified as quantum chemical descriptors. Thus, the complexity, as well as the time required for computation of the indices, increase in the order TS < TC< 3D < QC. In the hierarchical quantitative structure-activity relationship (HiQSAR) approach, descriptor classes are used in increasing order (hierarchy) of their complexity. The relative importance of a class of descriptors is brought out by examining the increase in model quality, or lack thereof, upon inclusion of that class of descriptors. Several HiQSAR studies have been carried out by Basak *et al.*[16–20]

Descriptor Thinning and Proper Cross-validation

In QSAR modeling of property or bioactivity of chemicals using calculated molecular descriptors, generally there are few observations (compounds) but many descriptors. In order to address this problem, descriptor-thinning/variable selection methods are used to select a proper subset of descriptors. It is vital to incorporate the descriptor selection, as well as any parameter selection, as part of the modeling procedure to be cross-validated for assessment of the model. When the cross-validation step does not include such elements of the modeling procedure, the "naïve-q^2" thus estimated suffers from an upward bias. Application of proper cross-validation that includes descriptor thinning is necessary for developing QSAR models with reliable predictive ability.[14] It is important to embed descriptor selection as well as parameter selection inside the cross-validation step, resulting in calculation of the "true-q^2".

Combining Primary and Secondary Descriptors in QSAR Modeling

The molecular descriptors that are calculated from the chemical structure as the only input are called primary descriptors, while the calculated physicochemical properties such as Clog*P*, logK_{oc}, vapor pressure, *etc.*, are called secondary descriptors because they are calculated by group contribution methods or atom/substituent contribution methods. Hence, the classification of these properties as secondary descriptors does not reflect their application, but rather the method of calculation. We hypothesize that after developing QSAR models with molecular descriptors (primary descriptors), inclusion of the secondary descriptors will not make a significant improvement due to the redundancy of information. On the other hand, inclusion of experimental physicochemical property might improve the regression model because it has information much closer to the real world situation. In this paper we tested the above hypothesis, and any possible improvement in the model on including an additional parameter was determined from the *t*-test and the *P*-value.

MATERIALS AND METHODS

Biological Activity Data

This study utilizes the repellency of each of the 40 DEET-related compounds applied to the human arm (~150 cm^2) measured against 200 female (5 to 7 days old) *Aedes aegypti* mosquitoes and reported as protection time (PT) by Suryanarayana *et al.*[5] Protection time for all the compounds are given in Table I. The natural log (ln) transformation of the protection time was the response modeled.

Calculation of Molecular Descriptors

A large set of topological descriptors was calculated using POLLY v2.3[21] and Triplet.[22] Electrotopological state indices, hydrogen bonding parameters, and kappa shape indices were obtained using Molconn-Z v3.5.[23] Quantum chemical descriptors such as E_{HOMO}, E_{LUMO}, solvent accessible surface area, molecular surface area, solvent excluded volume, dipole moment, *etc*., were calculated using Chem3D Ultra 8.0.[24] A list of theoretical descriptors along with brief descriptions and hierarchical classification, is provided in one of our recent publications.[20] It is also available as supporting information which can be downloaded from the journal website.

Data Preparation and Statistical Analysis

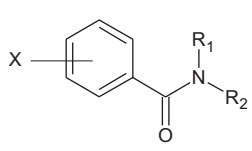
In order to handle the large differences in the magnitudes of the various descriptors, the descriptors were transformed as $\log_e(N + x)$, where N is the numerical value of the descriptor, and $x = 1$ when $N > -1$, which is true for most of the descriptors. However, some of the Molconn-Z parameters have values of ≤ -1. For those descriptors, the value of x is the smallest whole number which results in a positive sum for $(N + x)$. The CORR procedure of the SAS statistical package[25] was used to identify pairs of perfectly correlated descriptors ($R = 1$), and only one descriptor of each such pair was retained. In addition, any descriptors possessing a constant value for all compounds within the data set were omitted. The final descriptor set contained 261 molecular descriptors (inclusive of all topological, geometrical, and quantum chemical descriptors). Linear regression models were developed using the program LinMods[26] which is capable of performing the three linear fitting techniques namely, ridge regression (RR), principal component regression (PCR) and partial least square regression (PLS).

RESULTS AND DISCUSSION

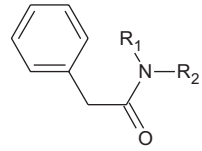
Hierarchical QSAR

Results of HiQSAR are given in Table II and they indicate that topochemical indices were able to explain

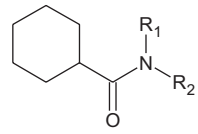
TABLE I. Independent parameters and biological responses of repellents



Seq # 1–30



Seq # 31–35



Seq # 36–40

Seq#	X	R_1	R_2	E*	ASV_2*	E_{HOMO}*	SHssNH*	C$\log P^a$	$\log P^{(a)}$	LogVP$^{(a)}$ (exptl)	$\log P^{(b)}$ (exptl)	$\log_e$PT (exptl)	$\log_e$PT (predict)
1	4-OCH_3	C_2H_5	H	1.932	0.00114	–0.422	1.004	0.111	0.723	–5.083	1.140	–2.526	–2.344
2	4-OCH_3	CH_3	CH_3	1.946	0.00171	0.087	0.000	–0.142	0.620	–5.547	1.600	0.000	0.564
3	4-OCH_3	C_2H_5	C_2H_5	2.081	0.00092	0.114	0.000	0.916	1.296	–5.599	2.500	0.000	0.529
4	4-OCH_3	i-C_3H_7	i-C_3H_7	2.217	0.00085	0.097	0.000	1.534	1.933	–4.167	2.800	0.157	0.089
5	4-OCH_3	C_5H_{10}		2.134	0.00160	0.127	0.000	1.725	1.353	–1.906	2.400	–0.288	–0.176
6	4-CH_3	C_2H_5	H	1.665	0.00155	–0.406	0.995	2.001	1.812	–5.067	1.380	–2.526	–1.589
7	4-CH_3	CH_3	CH_3	1.764	0.00236	0.074	0.000	1.748	1.711	–4.510	1.720	1.386	0.847
8	4-CH_3	C_2H_5	C_2H_5	1.925	0.00121	0.111	0.000	2.806	2.817	–3.713	2.380	1.040	0.938
9	4-CH_3	i-C_3H_7	i-C_3H_7	2.265	0.00109	0.059	0.000	3.424	3.131	–4.141	2.500	–0.693	–0.179
10	4-CH_3	C_5H_{10}		1.941	0.00200	0.130	0.000	2.531	2.923	–3.464	2.800	0.000	0.287
11	H	C_2H_5	H	1.440	0.00209	–0.411	0.993	1.482	1.608	–6.502	0.900	–0.545	–1.072
12	H	CH_3	CH_3	1.564	0.00325	0.073	0.000	1.229	1.507	–6.502	1.200	0.513	1.007
13	H	C_2H_5	C_2H_5	1.716	0.00160	–0.011	0.000	2.287	2.613	–2.288	1.800	1.386	1.748
14	H	i-C_3H_7	i-C_3H_7	1.942	0.00140	0.021	0.000	2.905	2.801	–4.457	2.500	1.099	0.940
15	H	C_5H_{10}		1.860	0.00221	0.141	0.000	2.012	2.719	–2.884	2.200	1.099	0.431
16	3-CH_3	C_2H_5	H	1.483	0.00163	–0.412	0.996	2.001	1.812	–6.645	1.320	–0.400	–0.931
17	3-CH_3	CH_3	CH_3	1.601	0.00248	0.072	0.000	1.748	1.711	–5.203	1.600	1.099	1.399
18	**3-CH_3**	**C_2H_5**	**C_2H_5**	**1.783**	**0.00126**	**0.108**	**0.000**	**2.806**	**2.817**	**–3.650**	**2.340**	**1.609**	**1.460**
19	3-CH_3	i-C_3H_7	i-C_3H_7	2.167	0.00113	0.055	0.000	3.424	3.131	–4.193	3.000	0.982	0.181
20	3-CH_3	C_5H_{10}		1.890	0.00224	0.143	0.000	2.531	2.923	–9.210	2.600	0.351	0.290
21	2-Cl	C_2H_5	H	1.702	0.00171	–0.859	1.006	1.665	1.786	–7.419	0.950	–0.545	–0.889
22	2-Cl	CH_3	CH_3	1.786	0.00262	–0.122	0.000	1.412	1.685	–4.880	1.540	1.609	1.016
23	2-Cl	C_2H_5	C_2H_5	1.935	0.00131	–0.083	0.000	2.470	2.791	–2.810	2.100	1.099	1.260
24	2-Cl	i-C_3H_7	i-C_3H_7	2.285	0.00117	–0.123	0.000	3.088	2.936	–0.258	3.000	0.000	0.091
25	2-Cl	C_5H_{10}		1.991	0.00195	0.051	0.000	2.195	2.897	–3.572	2.800	0.000	0.303
26	2-OC_2H_5	C_2H_5	H	1.922	0.00104	–0.482	1.015	1.678	1.198	–8.112	2.400	–2.526	–2.152
27	2-OC_2H_5	CH_3	CH_3	1.999	0.00156	0.030	0.000	1.550	1.096	–3.634	3.800	1.040	0.589
28	2-OC_2H_5	C_2H_5	C_2H_5	2.097	0.00084	–0.059	0.000	2.608	1.772	–6.725	2.500	1.253	0.906
29	2-OC_2H_5	i-C_3H_7	i-C_3H_7	2.253	0.00077	–0.026	0.000	3.226	2.409	–4.241	3.000	0.077	0.277
30	2-OC_2H_5	C_5H_{10}		2.087	0.00138	0.149	0.000	2.439	1.829	–5.809	2.600	0.285	0.109
31	–	C_2H_5	H	1.159	0.00320	–0.823	0.981	2.101	2.064	–5.150	1.000	0.000	0.213
32	–	CH_3	CH_3	1.449	0.00337	0.052	0.000	1.848	1.963	–6.215	1.480	0.775	1.416
33	–	C_2H_5	C_2H_5	1.741	0.00197	0.039	0.000	2.906	3.069	–2.260	2.400	1.792	1.284
34	–	i-C_3H_7	i-C_3H_7	2.175	0.00130	0.027	0.000	3.524	2.982	–6.571	3.100	0.000	0.095
35	–	C_5H_{10}		1.951	0.00154	0.098	0.000	2.631	3.175	–1.707	2.440	0.948	0.638
36	–	C_2H_5	H	1.440	0.00209	–0.411	0.961	1.482	1.608	–4.086	2.280	–0.693	–0.929
37	–	CH_3	CH_3	1.564	0.00325	0.073	0.000	1.229	1.507	–4.298	2.190	1.099	1.007
38	–	C_2H_5	C_2H_5	1.716	0.00160	–0.011	0.000	2.287	2.613	–1.809	3.120	1.386	1.748
39	–	i-C_3H_7	i-C_3H_7	1.942	0.00140	0.021	0.000	2.905	2.801	–1.258	1.780	0.693	0.940
40	–	C_5H_{10}		1.860	0.00221	0.141	0.000	2.012	2.719	–3.458	3.200	0.693	0.431

* Transformed according to the scaling procedure $\log_e(N + x)$, where N is the numerical value of the descriptor, and $x = 1$ when $N > -1$.
(a) Calculated using Chem 3D Ultra 8.0.
(b) Experimental values reported by Suryanarayana *et al.*
(c) DEET (Seq # 18) is shown in bold faces.
(d) Seq # 5, 15, 20, 25, 30, 35 and 40 are *N*-acyl piperidine.

TABLE II. Results of HiQSAR

Model	R^2_{cv}			PRESS		
	RR	PCR	PLS	RR	PCR	PLS
TS	–0.114	0.051	0.174	49.8	42.4	36.9
TS+TC	0.603	0.283	0.601	17.7	32.1	17.8
TS+TC+3D	0.603	0.248	0.582	17.8	33.6	18.7
TS+TC+3D+QC*	0.588	0.277	0.555	18.4	32.3	19.9
TS	–0.114	0.051	0.174	49.8	42.4	36.9
TC	0.619	0.318	0.585	17.0	30.5	18.6
3D	–0.052	–0.121	–0.052	47.0	50.1	47.0
QC*	0.378	–1.015	0.039	27.8	90.1	42.9

* QC parameters were calculated using Chem3D Ultra 8.0.

much of the variance in the data. The addition of 3D and quantum chemical descriptors did not improve the model quality. This indicates that the chemical nature of atoms plays a significant role in determining the repellency of the amides investigated, contrary to the earlier[4] HiQSAR modeling of cyclic carboxamides where shape and size of the molecules encoded by TS accounted for most of the variance in the data. In the case of cyclic carboxamides, the major structural variation was the size of the alkyl substituent and the only variation with respect to the chemical nature was the introduction of unsaturation in the cyclohexane ring, and TS parameters were sufficient to account for most of the variance in the data. On the other hand, the 40 compounds (Table I) that form the data set for the present study have structural variation with respect to shape and size, atom types and, in addition, electronic properties must also be different due the presence of an aromatic ring or the saturated cyclohexane ring with different substituents. This not only explains the reason for the TC parameters being picked by HiQSAR as the major class of parameters, but also explained why the QC parameters are the second important class of parameters. In the development of HiQSAR models, we used the leave-one-out (LOO) cross-validation procedure and did not split the data set into training and test sets. Recent studies with chemical data sets and data simulation have shown that utilizing the complete data set for model building yields a more robust model than setting aside part of the data as test set.[27]

Variable Selection and Two-deep Cross-validation

Though HiQSAR did shed some light on the class of parameters to be used and the necessity to use higher level quantum chemical descriptors, it suffers from the general criticism that model interpretability is poor because the RR model uses more descriptors than there are observations, a common phenomenon encountered in chemometrics data. Variable selection (descriptor thinning) performed before building RR models is an alternative procedure to improve the model interpretability and reduce computation time. We used a modified Gram-Schmidt algorithm[28] for the purpose of variable selection which, in its original form, is typically used to numerically stabilize the calculation of regression coefficients for ordinary least-squares regressions. The modified version consecutively selects the descriptor most highly correlated with the response, after the residuals have been adjusted to include the effects of previously selected descriptors. Cross-validation was used to select the number of predictors and the ridge parameter value to be used to fit the model. A computer program was developed in the R-language[29] to perform variable selection to select the parameter values, and to assess using two-deep cross-validation; RR models were then fit using the selected descriptors. Assessment of model predictive ability implemented 20-fold cross-validation; that is, the data were randomly split into 20 groups of approximately equal size; then, each group of size $w \approx n / 20$ is left out in turn, and the model, with all inherent steps, is fit to the remaining (n-w) observations. The left-out group is predicted using this model and the predicted sum of squares is calculated. This results in the commonly referenced predicted sum of squares (PRESS) statistic. To ensure that the results are not dependent on this one particular random split, the average of the PRESS statistic over several such splits should be used. For this dataset, the PRESS statistic was averaged over 3 random splits of the data into 20 groups.

$$R^2 = \frac{1 - \mathrm{PRESS}}{\mathrm{SS_{total}}} \tag{3}$$

where the PRESS statistic is the sum of the squared prediction errors and the SS_{total} is the sum of the squared deviations of the observed responses from the overall response mean. When the predictions are made *via* a cross-validation procedure, we conform to the usual notation of q^2 to represent the (20-fold) cross-validated R^2.

The descriptor thinning procedure as described above resulted in a four-predictor parameter model with a true-q^2 of 0.734. The ridge regression model is:

$$\ln(\mathrm{PT}) = 9.4735 - 4.4665*\mathrm{SHssNH} - 3.8707*E - 692.60*\mathrm{ASV_2} - 2.2157*E_{\mathrm{HOMO}} \tag{4}$$

It should be noted that all compounds were used to fit the model. The predictive ability of the model was assessed by the "true" q^2 value; since the predictor selection was included within the cross-validation procedure, the RR-model does not suffer from overfitting and the q^2 value should not overestimate model predictive ability. The log-transformed (natural logarithm) values of the response are given in Table I. The four predictors are:

1. *E:* Total Energy (thermodynamic calculated using MM2).
2. ASV_2: Triplet index from adjacency matrix, distance sum, and vertex degree; operation $y = 2$.

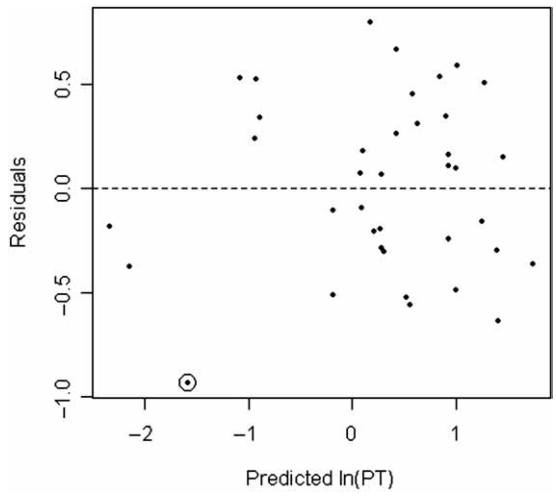


Figure 1. Residual plot for 4-predictor RR model.

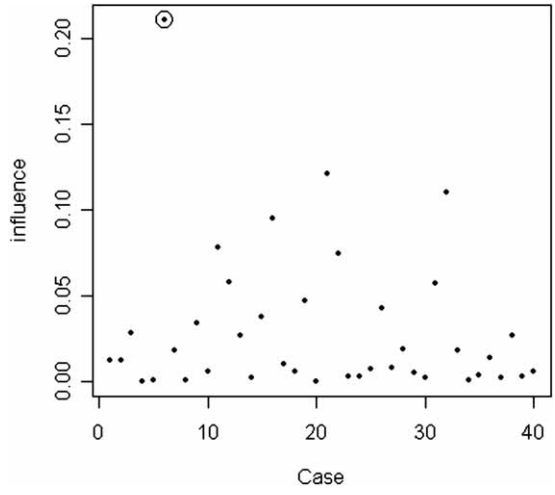


Figure 2. Plot of influence measure for 4-predictor RR model.

3. E_{HOMO}: Energy of the highest occupied molecular orbital.
4. SHssNH: Sum of atom-type H, electrotopological state index for –NH–.

Of these four parameters, ASV_2 is topostructural; SHssNH is topochemical; while E_{HOMO} and E are quantum chemical. This is in conformity with the results of the HiQSAR approach. The class of parameter that gave the best correlation in HiQSAR is topochemical and the second best is the quantum chemical descriptors. One possible reason for picking up ASV_2, a topostructural descriptor in the final model is that it may be strongly correlated to some of the topochemical or quantum chemical parameters in the final descriptor set of 261.Though, the descriptor SHssNH had zero value for all but the eight primary amides (See Table I, Seq # 1, 6, 11, 16, 21, 26, 31 and 36), it accounted for the poor repellency of the primary amides. It is interesting to note that the coefficients of all the predictors are negative. Picking up of

TABLE III. Results of adding calculated physicochemical properties (secondary descriptors) to the four-parameter model with molecular descriptors (primary descriptors)

For the four-parameter model: Ridge parameter = 0.1; Residual SS = 7.805; DF* = 35.01

	Secondary descriptors added			
	logVP	*C*log*P*	log*P*	log*P*exp
t statistic	0.225	1.061	0.929	1.110
DF*	34.1	34.1	34.1	34.1
P-value	0.823	0.296	0.359	0.275

DF*: degrees of freedom.

E_{HOMO} as one of the predictors in the final model indicates the dependence of the response (PT) on the nucleophilicity of the repellent molecules.

A residual plot (see Figure 1) shows that the three observations with log(PT) = –2.526 are all over-predicted. Figure 2 shows a plot of Cook's influence, suggesting that the most influential compound (circled on the figure) is case 6. While other transformations were considered to address this, the log transformation was the only one which properly transformed the response in order to attain consistent variance across predicted values. If this point is deleted, however, predictions change only minimally. Thus, the above model is used as the final fitted model. However, these observations suggest that these three cases might need some closer examination. There are several reasons such as uniqueness of structure, experimental error, purity of chemical *etc*., for an observation to be an outlier.

Combining Primary and Secondary Descriptors

The four-predictor RR model obtained as mentioned above was taken as the best "subset" model based on the calculated molecular descriptors, primary descriptors. We added each secondary descriptor individually to the model developed with the primary descriptors. For this operation we used the same ridge parameter k as for the "subset" model, and the improvement in the model quality was judged from the p-value. The secondary descriptor is determined to contribute significantly to the overall fit of the model only if the p-value is less than some significance level; here we used 0.05. The secondary descriptors used were Clog*P* and log*P* computed using Chem 3D Ultra 8.0 and we also used the lipophilicity parameter (log*P*) and the vapor pressure (VP) reported by Suryanarayana *et al.* The results of the statistical analyses (Table III) indicate that the addition of any of the secondary descriptor does not result in significant improvement in the overall quality of the model developed with the primary descriptors; in fact, all the p-values are quite large. This indicates that the information encoded by the secondary descriptors is redundant and already

accounted for by the primary descriptors. Inclusion of the secondary descriptors and assessment merely by correlation coefficient (r^2) value would lead to overfitting instead of providing any insight into the mechanism. It may be reiterated that information encoded by the calculated physicochemical properties are usually redundant in the calculated molecular descriptors.

CONCLUSIONS

Developing novel repellents that are superior to DEET in protection and less deleterious is of great importance in the first line of defense against mosquitoes, the vectors of many life threatening diseases such as malaria and West Nile Virus. This can be achieved by understanding of the olfactory system (sense of smell) and the biochemistry[30] of interaction of the odorant/repellents with enzymes and proteins associated with olfaction. This underlines the importance of molecular modeling in a concerted effort to protect human beings against the vector-borne diseases. The results of the study indicate that the repellent activities of DEET related compounds could be predicted by calculated molecular descriptors. Addition of either calculated or experimental vapor pressure or lipophilicity parameters did not improve the predictive ability of the models. Hence, computer assisted design of new repellents can be carried out using calculated structural descriptors which require no *a priori* knowledge other than the structure of the chemicals to be tested. If the repellency of a set of related compounds is known, a virtual library of structurally related compounds can be screened. The results of the study support the statistically robust methodology for variable selection from a large pool of molecular descriptors.

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Supporting Information Available. – A list of theoretical descriptors along with brief descriptions and hierarchical classification, and the calculated values of the molecular descriptors (261) for the 40 DEET-related compounds are given as supporting information.

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SAŽETAK

Modeliranje kvantitativnog odnosa između strukture i aktivnosti repelenata za komarce pomoću računskih deskriptora

Ramanathan Natarajan, Subhash C. Basak, Denise Mills, Jessica J. Kraker i Douglas M. Hawkins

U radu je prikazano modeliranje kvantitativnog odnosa strukture i aktivnosti (QSAR) 40 repelenata za komarce, pomoću izračunatih molekulskih deskriptora. Hijerarhijska primjena četiriju različitih klasa deskriptora (topokemijskih, topostrukturnih, geometrijskih i kvantnokemijskih) pokazala je da topokemijski deskriptori objašnjavaju velik dio varijance podataka. Stoga su kemijska priroda atoma i grupa važni za repelentnu aktivnost ovih spojeva. Regresija po hrptu (eng. ridge-regression) nadmašila je metodu parcijalnih najmanjih kvadrata (eng. partial least squares) i regresiju s glavnim komponentama. Prije regresije po hrptu broj deskriptora smanjen je modificiranim Gram-Schmidtovim postupkom. Za tako dobiven četvero-parametarski model, 20-struka unakrsna validacija dala je $R^2 = 0.734$. U radu prikazane vrijednosti za q^2 (R^2_{cv}) su prave q^2-vrijednosti jer je smanjenje broja deskriptora ugrađeno u unakrsnu validaciju. Proširenje skupa deskriptora izračunatim fizičko-kemijskim svojstvima (sekundarnim deskriptorima) nije dovelo do poboljšanja modela s izračunatim molekulskim deskriptorima (primarnim deskriptorima). Ovaj rezultat vrlo je važan za dizajn novih repelenata za komarce pomoću modela QSAR jer je za račun primarnih deskriptora potrebna samo molekulska struktura.

SUPPLEMENT

Table A. List of molecular descriptors and their explanation

	Topostructural (TS)
I^W_D	Information index for the magnitudes of distances between all possible pairs of vertices of a graph
$\bar{I}^W_D$	Mean information index for the magnitude of distance
W	Wiener index = half-sum of the off-diagonal elements of the distance matrix of a graph
I^D	Degree complexity
H^V	Graph vertex complexity
H^D	Graph distance complexity
$\overline{IC}$	Information content of the distance matrix partitioned by frequency of occurrences of distance h
M_1	A Zagreb group parameter = sum of square of degree over all vertices
M_2	A Zagreb group parameter = sum of cross-product of degrees over all neighboring (connected) vertices
${}^h\chi$	Path connectivity index of order h = 0-10
${}^h\chi_C$	Cluster connectivity index of order h = 3-6
${}^h\chi_{PC}$	Path-cluster connectivity index of order h = 4-6
${}^h\chi_{Ch}$	Chain connectivity index of order h = 3-10
P_h	Number of paths of length h = 0-10
J	Balaban's index based on topological distance
nrings	Number of rings in a graph
ncirc	Number of circuits in a graph
DN^2S_y	Triplet index from distance matrix, square of graph order, and distance sum; operation y = 1-5
DN^21_y	Triplet index from distance matrix, square of graph order, and number 1; operation y = 1-5
$AS1_y$	Triplet index from adjacency matrix, distance sum, and number 1; operation y = 1-5
$DS1_y$	Triplet index from distance matrix, distance sum, and number 1; operation y = 1-5
ASN_y	Triplet index from adjacency matrix, distance sum, and graph order; operation y = 1-5
DSN_y	Triplet index from distance matrix, distance sum, and graph order; operation y = 1-5
DN^2N_y	Triplet index from distance matrix, square of graph order, and graph order; operation y = 1-5
ANS_y	Triplet index from adjacency matrix, graph order, and distance sum; operation y = 1-5
$AN1_y$	Triplet index from adjacency matrix, graph order, and number 1; operation y = 1-5
ANN_y	Triplet index from adjacency matrix, graph order, and graph order again; operation y = 1-5
ASV_y	Triplet index from adjacency matrix, distance sum, and vertex degree; operation y = 1-5
DSV_y	Triplet index from distance matrix, distance sum, and vertex degree; operation y = 1-5
ANV_y	Triplet index from adjacency matrix, graph order, and vertex degree; operation y = 1-5
	Topochemical (TC)
O	Order of neighborhood when IC_r reaches its maximum value for the hydrogen-filled graph
O_{orb}	Order of neighborhood when IC_r reaches its maximum value for the hydrogen-suppressed graph
I_{ORB}	Information content or complexity of the hydrogen-suppressed graph at its maximum neighborhood of vertices
IC_r	Mean information content or complexity of a graph based on the r^{th} (r = 0-6) order neighborhood of vertices in a hydrogen-filled graph

SIC_r	Structural information content for r^{th} (r = 0-6) order neighborhood of vertices in a hydrogen-filled graph
CIC_r	Complementary information content for r^{th} (r = 0-6) order neighborhood of vertices in a hydrogen-filled graph
${}^h\chi^b$	Bond path connectivity index of order h = 0-6
${}^h\chi^b{}_C$	Bond cluster connectivity index of order h = 3-6
${}^h\chi^b{}_{Ch}$	Bond chain connectivity index of order h = 3- 6
${}^h\chi^b{}_{PC}$	Bond path-cluster connectivity index of order h = 4-6
${}^h\chi^v$	Valence path connectivity index of order h = 0-10
${}^h\chi^v{}_C$	Valence cluster connectivity index of order h = 3-6
${}^h\chi^v{}_{Ch}$	Valence chain connectivity index of order h = 3-10
${}^h\chi^v{}_{PC}$	Valence path-cluster connectivity index of order h = 4-6
J^B	Balaban's index based on bond types
J^X	Balaban's index based on relative electronegativities
J^Y	Balaban's index based on relative covalent radii
AZV_y	Triplet index from adjacency matrix, atomic number, and vertex degree; operation y = 1-5
AZS_y	Triplet index from adjacency matrix, atomic number, and distance sum; operation y = 1-5
ASZ_y	Triplet index from adjacency matrix, distance sum, and atomic number; operation y = 1-5
AZN_y	Triplet index from adjacency matrix, atomic number, and graph order; operation y = 1-5
ANZ_y	Triplet index from adjacency matrix, graph order, and atomic number; operation y = 1-5
DSZ_y	Triplet index from distance matrix, distance sum, and atomic number; operation y = 1-5
DN^2Z_y	Triplet index from distance matrix, square of graph order, and atomic number; operation y = 1-5
nvx	Number of non-hydrogen atoms in a molecule
nelem	Number of elements in a molecule
fw	Molecular weight
si	Shannon information index
totop	Total Topological Index *t*
sumI	Sum of the intrinsic state values *I*
sumdelI	Sum of delta-*I* values
tets2	Total topological state index based on electrotopological state indices
phia	Flexibility index (kp_1* kp_2/*nvx*)
Idcbar	Bonchev-Trinajstić information index
IdC	Bonchev-Trinajstić information index
Wp	Wienerp
Pf	Plattf
Wt	Total Wiener number
knotp	Difference of chi-cluster-3 and path/cluster-4
knotpv	Valence difference of chi-cluster-3 and path/cluster-4
nclass	Number of classes of topologically (symmetry) equivalent graph vertices
NumHBd	Number of hydrogen bond donors
NumHBa	Number of hydrogen bond acceptors
SHCsats	E-State of C sp^3 bonded to other saturated C atoms
SHCsatu	E-State of C sp^3 bonded to unsaturated C atoms
SHvin	E-State of C atoms in the vinyl group, =CH-
SHtvin	E-State of C atoms in the terminal vinyl group, $=CH_2$
SHavin	E-State of C atoms in the vinyl group, =CH-, bonded to an aromatic C

SHarom	E-State of C sp^2 which are part of an aromatic system
SHHBd	Hydrogen bond donor index, sum of Hydrogen E-State values for –OH, =NH, -NH_2, -NH-, -SH, and #CH
SHwHBd	Weak hydrogen bond donor index, sum of C-H Hydrogen E-State values for hydrogen atoms on a C to which a F and/or Cl are also bonded
SHHBa	Hydrogen bond acceptor index, sum of the E-State values for –OH, =NH, -NH_2, -NH-, >N-, -O-, -S-, along with –F and –Cl
Qv	General Polarity descriptor
$NHBint_y$	Count of potential internal hydrogen bonders (y = 2–10)
$SHBint_y$	E-State descriptors of potential internal hydrogen bond strength (y = 2–10)
	Electrotopological State index values for atoms types: *SHsOH, SHdNH, SHsSH, SHsNH2, SHssNH, SHtCH, SHother, SHCHnX, Hmax Gmax, Hmin, Gmin, Hmaxpos, Hminneg, SsLi, SssBe, Sssss, Bem, SssBH,SsssB, SssssBm, SsCH3, SdCH2, SssCH2, StCH, SdsCH, SaaCH, SsssCH, SddC, StsC, SdssC, SaasC, SaaaC, SssssC, SsNH3p, SsNH2, SssNH2p, SdNH, SssNH, SaaNH, StN, SsssNHp, SdsN, SaaN, SsssN, SddsN, SaasN, SssssNp, SsOH, SdO, SssO, SaaO, SsF, SsSiH3, SssSiH2, SssssSiH, SssssSi, SsPH2, SssPH, SsssP, SdsssP, SssssssP, SsSH, SdS, SssS, SaaS, SdssS, SddssS, Ssssssss, SsCl, SsGeH3, SssGeH2, SsssGeH, SssssGe, SsAsH2, SssAsH, SsssAs, SdsssAs, SssssssAs, SsSeH, SdSe, SssSe, SaaSe, SdssSe, SddssSe, SsBr, SsSnH3, SssSnH2, SsssSnH, SssssSn, SsI, SsPbH3, SssPbH2, SsssPbH, SssssPb*
	Geometrical (3D)/Shape
kp_0	Kappa zero
kp_1-kp_3	Kappa simple indices
ka_1-ka_3	Kappa alpha indices
V_W	Van der Waals volume
^{3D}W	3D Wiener number based on the hydrogen-suppressed geometric distance matrix
$^{3D}W_H$	3D Wiener number based on the hydrogen-filled geometric distance matrix
	Quantum Chemical (QC)
E_{HOMO}	Energy of the highest occupied molecular orbital
E_{HOMO-1}	Energy of the second highest occupied molecular
E_{LUMO}	Energy of the lowest unoccupied molecular orbital
E_{LUMO+1}	Energy of the second lowest unoccupied molecular orbital
ΔH_f	Heat of formation
μ	Dipole moment
E_b	Bend energy
SAS	Solvent accessible surface area
MS	Molecular surface area
SEV	Sovent excluded volume
ElcE	Electrionic energy
NRE	Repulsion enery
Ev	Non 1,4-VDW energy
E_{14}	1,4-VDW energy
NRE	Repulsion energy
E_s	Stretch energy
E_b	Bend energy
E_{sb}	Stretch-bend energy
E	Total energy (MM2)

TABLE B. Standard descriptor labels and corresponding POLLY descriptor labels. These labels are used in the descriptor set provided in the supporting information

Connectivity indices

Std	POLLY	Std	POLLY
$I^W{}_D$	idw	O	max_ic
$\bar{I}^W{}_D$	midw	O_{orb}	I_orbmax
W	w	I_{ORB}	i_orb
I^D	id	IC_r	ic0 to ic6
H^V	hv	SIC_r	sic1-sic6
H^D	hd	CIC_r	cic1-cic6
$\overline{IC}$	ic_bar	${}^h\chi^b$	b0-b6
M_1	m1	${}^h\chi^b{}_C$	bc3-bc6
M_2	m2	${}^h\chi^b{}_{Ch}$	bch3-bc6
${}^h\chi$	s0-s6	${}^h\chi^b{}_{PC}$	bpc3-bpc6
${}^h\chi_C$	sc3-sc6	${}^h\chi^v$	v0-v6
${}^h\chi_{PC}$	spc3-spc6	${}^h\chi^v{}_C$	vc3-vc6
${}^h\chi_{Ch}$	sch3-sch6	${}^h\chi^v{}_{Ch}$	vch3-vc6
P_h	k1-k10	${}^h\chi^v{}_{PC}$	vpc3-vpc6
J	j	*nrings*	*nrings*
J^B	jb	*ncirc*	*ncirc*
J^y	jy	*nelem*	nelem
J^x	jx	*nvx*	nvx

Triplet Indices

Std	POLLY	Std	POLLY
DN^2S_y	dn2s1-dn2s5	ASZ_y	asz1-asz5
DN^21_y	dn211-dn215	AZN_y	azn1-azn5
$AS1_y$	as11- as15	ANZ_y	anz1-anz5
$DS1_y$	ds11- ds15	DSZ_y	dsz1-dsz5
ASN_y	asn1- asn5	DN^2Z_y	dn2z1-dn2z5
DSN_y	dsny-dsn5	ANV_y	anv1-anv5
DN^2N_y	dn2n1-dn2n5	AZV_y	azv1- azv5
ANS_y	ans1- ans5	AZS_y	azs1-azs5
$AN1_y$	an11-an15	ASZ_y	asz1-asz5
ANN_y	Ann1-ann5	AZN_y	azn1-azn5
ASV_y	asv1-asv5	ANZ_y	anz1-anz5
DSV_y	Dsv1-dsv5	DSZ_y	dsz1-dsz5
AZV_y	azv1- azv5	DN^2Z_y	dn2z1-dn2z5
AZS_y	azs1-azs5		

FINAL DESCRIPTOR SET (261) USED IN THE STUDY

seq,idw,midw,w,id,hv,hd,ic_bar,max_ic,i_orb,max_orb,m1,m2,ic0,ic1,ic2,ic3,ic4,ic5,ic6,sic0,sic1,sic2,sic3,sic4,sic5,sic6,cic0,cic1,cic2,cic3,cic4,cic5,cic6,s0,s1,s2,s3,s4,s5,s6,sc3,sc5,scy6,spc4,spc5,spc6,b0,b1,b2,b3,b4,b5,b6,bc3,bc5,bcy6,bpc4,bpc5,bpc6,v0,v1,v2,v3,v4,v5,v6,vc3,vc5,vcy6,vpc4,vpc5,vpc6,k0,k1,k2,k3,k4,k5,k6,k7,k8,k9,k10,j,jb,jx,jy,azv1,azv2,azv3,azv4,azv5,asv1,asv2,asv3,asv4,asv5,dsv1,dsv2,dsv3,dsv4,dsv5,azs1,azs2,asz1,asz2,asz3,asz4,asz5,dn2s1,dn2s2,dn211,dn212,dn213,dn214,dn215,as11,as12,as13,as14,as15,ds11,ds12,ds13,ds14,ds15,asn1,asn2,asn3,asn4,asn5,dsn1,dsn2,dsn3,dsn4,dsn5,dn2n1,dn2n2,dn2n3,dn2n4,dn2n5,ans1,ans2,ans3,ans4,ans5,anv1,anv2,anv3,anv4,anv5,azn1,azn2,azn3,azn4,azn5,anz1,anz2,anz3,anz4,anz5,an11,an12,an13,an14,an15,dsz1,dsz2,dsz3,dsz4,dsz5,ann1,ann2,ann3,ann4,ann5,dn2z1,dn2z2,dn2z3,dn2z4,dn2z5,nvx,nrings,ncirc,nelem,fw,xp7,xp8,xp9,xp10,xvp7,xvp8,xvp9,xvp10,kp0,kp1,kp2,kp3,ka1,ka2,ka3,si,totop,sumI,sumDELI,tets2,phia,SHssNH,SHother,Hmax,Gmax,Hmin,Gmin,Hmaxpos,SsCH3,SssCH2,SaaCH,SsssCH,SdssC,SaasC,SssNH,SsssN,SdO,SssO,SsCl,idc,idcbar,Wp,pf,Wt,knotp,knotpv,nclass,numHBd,numHBa,SHCsats,SHCsatu,SHarom,SHHBD,SHHBA,Qv,NHBint2,NHBint4,SHBint2,SHBint4,Eb,SAS,MS,SEV,DPL,ElcE,Homo,Lumo,Hlgap,HF,Ev,NRE,Es,Esb,E,E14,PT,VP,L_PT,L_VP,_OBSTAT_

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