

Synthesis and bioassay of improved mosquito repellents predicted from chemical structure

Alan R. Katritzky^{†‡}, Zuoquan Wang[†], Svetoslav Slavov[†], Maia Tsikolia[†], Dimitar Dobchev[†], Novruz G. Akhmedov[†], C. Dennis Hall[†], Ulrich R. Bernier[¶], Gary G. Clark[¶], and Kenneth J. Linthicum[¶]

[†]Center for Heterocyclic Compounds, Department of Chemistry, 127 Chemistry Research Building, University of Florida, Gainesville, FL 32611-7200; and [¶]United States Department of Agriculture, Agricultural Research Service, Center for Medical, Agricultural and Veterinary Entomology, Gainesville, FL 32608

Edited by Barry J. Beaty, Colorado State University, Fort Collins, CO, and approved April 1, 2008 (received for review January 18, 2008)

Mosquito repellency data on acylpiperidines derived from the U.S. Department of Agriculture archives were modeled by using molecular descriptors calculated by CODESSA PRO software. An artificial neural network model was developed for the correlation of these archival results and used to predict the repellent activity of novel compounds of similar structures. A series of 34 promising *N*-acylpiperidine mosquito repellent candidates (4a–4q') were synthesized by reactions of acylbenzotriazoles 2a–2p with piperidines 3a–3f. Compounds (4a–4q') were screened as topically applied mosquito repellents by measuring the duration of repellency after application to cloth patches worn on the arms of human volunteers. Some compounds that were evaluated repelled mosquitoes as much as three times longer than *N,N*-diethyl-*m*-toluamide (DEET), the most widely used repellent throughout the world. The newly measured durations of repellency were used to obtain a superior correlation equation relating mosquito repellency to molecular structure.

N-acylpiperidine | quantitative structure–activity relationship | CODESSA PRO | artificial neural network | *Aedes aegypti*

Mosquito-borne diseases such as malaria, arboviral encephalitis, dengue fever, Rift Valley fever, and yellow fever produce significant morbidity and mortality in humans and livestock in many parts of the world (1). Pathogens that cause these diseases are transmitted by injection of saliva into susceptible hosts by female mosquitoes needing protein from a blood meal to develop their eggs. Repellents play a vital role in interrupting this mosquito/human interaction by serving as a means of personal protection by reducing bites from mosquitoes. Although vaccines and genetically modified mosquitoes are under development for prevention of mosquito-borne diseases, new and improved topical repellents are needed to provide alternatives that are safer, effective for a longer duration, and more efficacious against mosquitoes and a wide range of arthropods.

Some repellents that are applied to the skin or clothing are highly effective but only of limited duration because of evaporative loss, dermal absorption, abrasive loss, or dissolution in water and perspiration (2, 3). Furthermore, some compounds cause skin irritation or a stinging sensation when they contact eyelids or lips. Finally, several are successful at preventing bites only when they are present on the skin or clothing in relatively large quantities. Thus, the current research is driven by the need to discover a new generation of repellents that overcome these limitations and supplement or replace today's standard repellent (*N,N*-diethyl-*m*-toluamide, DEET).

Insects are believed to detect repellents through receptor uptake of molecules with specific chemical characteristics (4–7). Therefore, we have conducted extensive studies of the relationship between molecular structure and the observed biological property of repellency. Our approach is based on a rational design similar to that used by the pharmaceutical industry for drug development but, in this case, is specifically aimed at products that interfere with mosquito olfaction and/or deter

biting of a host. The use of quantitative structure–activity relationship (QSAR) approaches to repellent discovery is relatively uncommon (8). In one of the most recent studies, three-dimensional (3D)-QSAR was used successfully with CATALYST software to develop models for repellents based on pharmacophores (8). We have chosen to use artificial neural network (ANN) modeling because it is one of the most efficient QSAR approaches. Neural networks have been applied in many diverse scientific endeavors, including economics, engineering, physics, chemistry, and medical science (9). A particular advantage of ANNs is their inherent ability to incorporate nonlinear dependencies between the dependent and independent variables without using an explicit mathematical function.

Methodology for a general QSAR approach has been developed and coded as the CODESSA PRO software package (10). We previously examined 31 repellents by using CODESSA PRO (11) and here extend this research by examining available data on *N*-acylpiperidines, calculating their structure–activity relationships, synthesizing novel compounds, and performing repellency assays with human volunteers (12).

Results and Discussion

Correlation of Existing Mosquito Repellency Data for *N*-acylpiperidines with Chemical Structures. Dataset. Early investigations on the synthesis of insect repellents and their practical tests on skin and cloth against mosquitoes and other biting Diptera have been well documented (13–24). In the present work, nonlinear QSAR modeling based on the ANN approach was performed using available data for 200 *N*-acylpiperidines. The efficacy of these compounds was determined by a “time point of failure” defined as a specified number of bites (usually a low number) by female *Aedes aegypti* (L.) mosquitoes through cloth treated with each candidate piperidine and fixed on the arm of a volunteer (12). The original data on piperidines were obtained from U.S. Department of Agriculture (USDA) records collected and compiled over the past 50 years (25–27). Historically, the level of repellency was divided into five classes, as defined by “the time to first bite.” This classification system placed all repellents that were efficacious for >21 d into class 5, the top tier. The remaining classes were divided as class 4 for 10- to 21-d protection, class 3 for 5- to 10-d protection, class 2 for 1- to 5-d protection, and class 1 as ineffective (<1-d protection).

Nonlinear QSAR modeling. The objective of these calculations was to build a predictive ANN model able to classify the duration of

Author contributions: A.R.K., S.S., D.D., U.R.B., G.G.C., and K.J.L. designed research; Z.W., M.T., N.G.A., U.R.B., G.G.C., and K.J.L. performed research; S.S., D.D., and C.D.H. analyzed data; and A.R.K., Z.W., S.S., M.T., C.D.H., U.R.B., G.G.C., and K.J.L. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[§]To whom correspondence should be addressed. E-mail: katritzky@chem.ufl.edu.

This article contains supporting information online at www.pnas.org/cgi/content/full/0800571105/DCSupplemental.

© 2008 by The National Academy of Sciences of the USA

repellent efficacy and use this information to predict structures of novel repellents. The data set was divided into training (150 repellents) and validation (50 repellents) subsets. The main model is based on the back propagation learning algorithm for optimization of the ANN (28, 29). To obtain good generalization of the ANN, the following factors were taken into account:

(i) To build a general model, 150 of the 200 compounds [see supporting information (SI) Table S1 for details] were randomly selected to form the training set.

(ii) The preferred architecture of the ANN model (by rms error) was 8-7-1 (i.e., 8 neurons in the input layer, 7 neurons in the hidden layer, and 1 neuron in the output) representing the time class of the repellent.

(iii) A sensitivity analysis was done by building 1-1-1 ANN models, and the descriptors that showed lowest error at the output were selected. In addition, scatter plot analysis between the descriptors and the time classes was carried out to reveal the variability of the classes with respect to the descriptors. Finally, a combination of eight descriptors was selected as inputs for the ANN: total number of bonds, molecular weight, Kier and Hall index (order 3), molecular surface area, total dipole of the molecule, total molecular electrostatic interaction, surface area for atom C, and surface area for atom N.

The training procedure of the ANN was stopped when the rms error of the validation set started to increase. The final results for the coefficient of determination and rms error for the training set were 0.73 and 0.87, respectively. The calculated rms error of the validation set was 1.4. As expected, the rms error of the validation set was bigger than that of the training subset.

Utilization of the ANN Model to Design and Select New Compounds for Synthesis. Discussion of the ANN model. Based on the results obtained by the ANN model, the repellent time classes for additional compounds were predicted. The expected accuracy of prediction of the repellency class for a new compound by using the results on measured compounds is given by N_s/N , where N_s is the number of the repellents predicted by the network at this particular class and N is the total number of the repellents in a given experimental class. Thus, the accuracies of each class are as follows (see Fig. S1 for more information): class 1, 0.76; class 2, 0.69; class 3, 0.50; class 4, 0.25; and class 5, 0.35. If classes 4 and 5 are combined into a single class, the exact number of compounds predicted from class 4 and class 5 combined is 39. Therefore, the predicted accuracy for class 4 and class 5 compounds is 71% (39/55). This prediction accuracy is quite high, considering the distribution of the class values. Therefore, this ANN model can be used with some confidence to predict novel candidate repellents.

Selecting compounds for synthesis. Good correlation existed between the experimental and predicted values of the repellent efficacy. Of the 34 compounds selected for synthesis, 11 of these (see Table 1 and Table S2 for more details and refs. 20 and 30–33) were chosen from those examined previously. The repellency of these 11 compounds was reassessed to (i) compare results obtained from the biological test procedure to experimental results of those conducted and reported previously (26); (ii) conduct a rigorous assessment of the testing protocol using new analogs and thereby to gain confidence in the utility of the output data; and (iii) increase predictive ability and accuracy by reexamination of the most effective repellent candidates identified as class 4 and class 5 from previous assays and then use the protocols to further divide the repellency of these compounds into additional subclasses.

At present, the activities of $\approx 2,000$ untested analogs have been predicted. A total of 23 were selected from this set for synthesis. Using the ANN model, we predicted these compounds to be principally members of repellent classes 3, 4, and 5 (see Table 1 and Table S3 for details, and refs. 15, 20, and 33–38). Thus,

together with the 11 compounds previously examined (see Table 1) with activity predicted by the ANN as classes 4 and 5, we synthesized a total of 34 compounds of general structure 4 as shown in Fig. 1.

Synthesis of Additional N-acylpiperidines. Introduction. N-acylpiperidines have generally been synthesized by reaction of acid chlorides with amines (either the amine itself or pyridine was used as a hydrochloric acid scavenger). The product amides are then isolated by routine extraction procedures and purified by crystallization, chromatography, or distillation under high vacuum.

The high reactivity of acid halides and their incompatibility with acid-sensitive functionalities prompted efforts to find alternative methods. A benzotriazolyl group is easily introduced, activates molecules toward numerous transformations, and can be removed easily at the end of each reaction sequence (39–44). 1-Acylbenzotriazoles are advantageous carboxylic acid derivatives because they are stable and readily available in one step from carboxylic acids even where an acid-sensitive functionality is present (45–47). We report herein the use of this method in the synthesis of N-acylpiperidines as candidates for mosquito repellents.

Synthetic results and discussion. 1-Acylbenzotriazoles 2 (Fig. 1) were produced by treatment of the corresponding carboxylic acids 1 at 25°C with thionyl chloride and benzotriazole in methylene chloride in 1:1:3 mole ratio (48).

Reaction of 1-acylbenzotriazoles 2 with 1 eq of piperidines 3 in THF at 20°C or in toluene under reflux resulted in formation of N-acylpiperidines 4 by our modified procedure (46). Subsequent evaporation of the solvent, addition of methylene chloride, and washing with aqueous sodium carbonate solution gave N-acylpiperidines 4a–4q' in 71–100% yield (Tables S2 and S3).

Experimental (if available) and predicted classes of repellency according to the ANN model are given in Table S1. From these data, the 11 candidates reselected for synthesis are listed in Table 1 and Table S2. These compounds have the highest measured activity (i.e., class 5) and also possess high activities predicted by the ANN model.

Another 23 compounds were synthesized as repellent candidates (Table 1 and Table S3). Of these, 17 (74%) were predicted by ANN as highly active repellents falling in classes 4 and 5, which in theory provide protection from mosquito bites for >10 d when applied to cloth worn over the skin. For controls, we also synthesized six compounds with relatively poor (classes 1 and 2) predicted repellency (4a–4f, Table 1, Table S3). Measurements of the biological activity of all these compounds are described in the section on biological testing.

NMR Spectra of N-acylpiperidines. The proton spectra of the unsymmetrical amides 4a, 4c, 4h, 4i, 4l, 4p, 4q, 4c', 4h'–k', 4n', and 4o' all revealed hindered rotation about the N–CO bond on the NMR time scale. Detailed variable temperature NMR spectra of 4c, 4p', and 4q' gave $\Delta G^\ddagger$ values of 16.2 ± 0.3 kcal·mol^{−1} for interconversion of the rotamers, corresponding to a first-order rate constant of ≈ 30 s^{−1} at ambient temperature. Thus, hindered rotation is not expected to influence the biological activity (see SI Text, Figs. S2–S5, and Table S4 for more details).

Biological Testing. Initially, mass concentrations (12) were used at the start of experiments, but a modification of the initial experimental setting was necessary so that the current modeling scheme would meet one of the following QSAR conditions (i) measurement of the induced biological effect at a constant concentration or (ii) measurement of the concentration that causes a constant biological effect. The first condition is closer to the actual application rate of repellents in the field and thus was chosen for further study. The experimental determination of repellency was based on

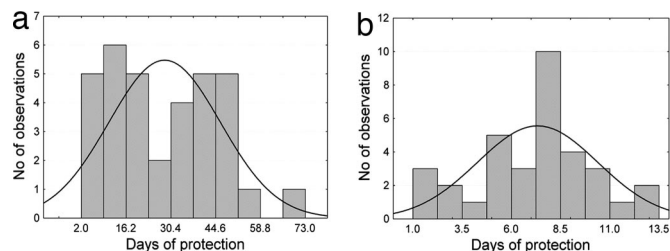


Fig. 2. Histograms and probability density functions of the averaged protection time (PT) at 25 $\mu\text{mol}/\text{cm}^2$ (a) and 2.5 $\mu\text{mol}/\text{cm}^2$ (b).

tration of 2.5 $\mu\text{mol}/\text{cm}^2$ are more precise than those at 25 $\mu\text{mol}/\text{cm}^2$. Hence, the QSAR modeling results at the lower concentration are expected to be more reliable.

Comparison of the Present Bioassay Values with the Archival Data.

The dataset of 34 *N*-acylperidines with averaged PT transformed into classes was used to estimate the predictive power of ANN. No significant correlation was found between the experimental PT values and those predicted by ANN at either 25 or 2.5 $\mu\text{mol}/\text{cm}^2$ (columns 5 vs. 10 and 5 vs. 15 produced $R^2 = 0.007$ and $R^2 = 0.06$, respectively). The possible mismatch between the ANN-predicted classes (based on archival data) and those measured in the present work could be due to the significant difference in the experimental settings: mass instead of molar concentrations used, fewer mosquitoes tested (500 instead of 2,000–4,000), different cage size, and use of a new PT definition (see *Methods*).

We conclude that the use of classes as such is not appropriate and does not provide sufficient levels to clearly discriminate the repellency effectiveness of these compounds. We now use the average days of protection (duration) as a more precise basis for QSAR modeling.

New QSAR Modeling. By using the Best Multilinear Regression (BMLR) algorithm integrated into CODESSA PRO [CODESSA PRO Software, University of Florida (2002), www.codessa-pro.com], QSAR models with up to seven descriptors [the maximum allowed by “5-to-1” rule of thumb (50)] were generated for both concentrations of the candidate repellents. As can be seen from Fig. 3, the models with four and more descriptors are characterized by very close statistical parameters. Following the “Occam’s Razor” rule of simplicity, QSAR models with four descriptors were preferred and considered further (Table 2 and Fig. 4), thus making the comparison of the two models easier. Using four descriptors, we obtain superior R^2 values of 0.729 and 0.689 for the 25 $\mu\text{mol}/\text{cm}^2$ and 2.5 $\mu\text{mol}/\text{cm}^2$ concentration data sets, respectively.

As can be seen from Table 2, the statistical parameters obtained in both cases are close, whereas the descriptors that appear in the two models are quite different. The reasons for such behavior may be as follows:

(i) Data distribution: a subtle distinction in the data distribution could lead to selection of a different descriptor set that fits the experimental data best.

(ii) High intercorrelation: at each consecutive step the BLMR algorithm selects only one of a pair or a set of highly intercorrelated descriptors, which is considered further. Thus, depending on the data set, different but physically similar and highly intercorrelated descriptors (Table S5) may appear in the different models.

The descriptors introduced into the two models (Table 2) may be classified as (i) electrostatic interaction-related descriptors: RNCG relative negative charge (QMNEG/QTMINUS), minimum e–n attraction for bond C–O, WPSA-2 weighted PPSA

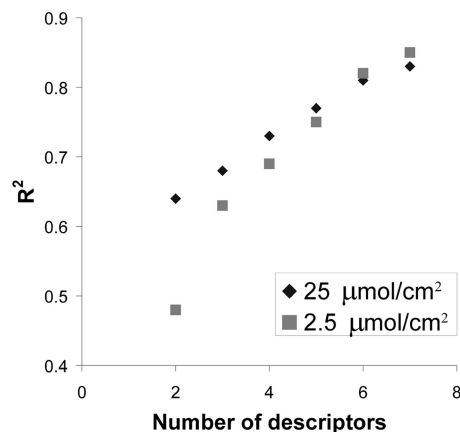


Fig. 3. Plot of R^2 vs. number of descriptors used.

(PPSA2* $\text{TMSA}/1000$), maximum 1-electron reactivity index for atom C, and maximum e–e repulsion for bond C–C; and (ii) steric interactions-related descriptors: YZ shadow/YZ rectangle, molecular volume/XYZ box, principal moment of inertia Z.

The mechanism of repellent action is known to be receptor-based, possibly involving the GPRor7 receptor (until recently, it was designated as AgOR7) on mosquito antennae as a target for the bioactive molecules (51). The first class of descriptors could be related to orientation effects and to the possibility of ligand–receptor noncovalent bonds. Most of the electrostatic descriptors depict the distribution of negative charge within the molecule and are probably connected to the heteroatoms present, such as oxygen and nitrogen. Especially the “Minimum e–n attraction for bond C–O” descriptor shows how important the presence of a carbonyl group is for the repellent effect and indicates that the stronger the $\text{C}=\text{O}$ bond the longer the protection time.

The second set of descriptors encoding the mass distribution and the size of the molecules probably depict the steric interactions that are responsible for the surface recognition between the ligand and receptor. The structures identified on the basis of our models as highly active at both concentrations (4k, 4j, 4i, 4o, 4n, 4o', 4f, 4l, 4j', and 4h) (Table 1 and Table S6) probably include all the structural features essential for repellency.

Conclusion

This report documents significant findings in the area of repellent research through the application of a combination of techniques and methods from the disciplines of medical entomology and synthetic and theoretical chemistry. Models were constructed by using a subset of 30,000 chemicals accumulated in the USDA archives over the last 60 years. The repellency assays originally used to study these archived chemicals were the

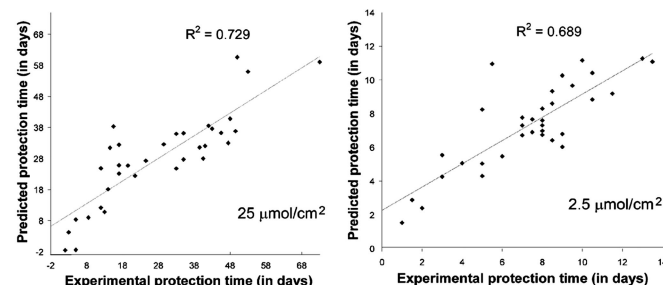


Fig. 4. Predicted vs. observed protection time values at concentrations 25 and 2.5 $\mu\text{mol}/\text{cm}^2$.

Table 2. Best four descriptors models and their statistical parameters

Conc., $\mu\text{mol}/\text{cm}^2$	No. of descriptors	B	S	t	IC	Name of descriptor
25 [†]	0	−188.8	84.08	−2.246		Intercept
	1	−2686	461.3	−5.823	0.09647	Maximum 1-electron reactivity index for atom C
	2	−2616	488.2	−5.359	0.7253	Principal moment of inertia C
	3	2.040	0.6920	2.948	0.3632	Maximum e–e repulsion for bond C–C
	4	−0.02195	0.009215	−2.382	0.7759	WPSA-2 weighted PPSA (PPSA2*TMSA/1000)
2.5 [‡]	0	−726.1	329.3	−2.205		Intercept
	1	−68.13	9.393	−7.254	0.5248	YZ shadow/YZ rectangle
	2	58.50	13.22	4.426	0.7120	Molecular volume/XYZ box
	3	−71.37	16.41	−4.350	0.5696	RNCG relative negative charge (QMNEG/QTMINUS)
	4	1.870	0.8053	2.321	0.2822	Minimum e–n attraction for bond C–O

B, regression coefficient; S, regression coefficient error; t, Student criterion; IC, partial intercorrelation; PPSA, partial positively charged molecular surface area; WPSA, weighted PPSA; RNCG relative negative charge, ratio between the maximum atomic negative charge and sum of the negative atomic charges in the molecule.

[†]N = 4; n = 34; $R^2 = 0.729$; $R_{\text{CV}00}^2 = 0.638$; $R_{\text{CV}00}^2 = 0.628$; $F = 19.50$; $s = 9.769$.

[‡]N = 4; n = 34; $R^2 = 0.689$; $R_{\text{CV}00}^2 = 0.608$; $R_{\text{CV}00}^2 = 0.582$; $F = 16.05$; $s = 1.815$.

same as those that led to the discovery of DEET in 1953, the most widely used repellent in the United States. The appeal of DEET as the “gold standard” is based on its excellent human-use safety record, its ability to protect humans from bites of a wide range of hematophagous arthropods, and its duration of protection on the skin—the measure of merit for the original repellency assays and for those used in the current study. While some newer commercial repellents are nearly as efficacious as DEET-based repellents with respect to their protection from insect bites, to the best of our knowledge, none protect users for significantly longer than equivalent stoichiometric or gravimetric amounts of DEET.

In this study, we performed a successful closing of the “QSAR–synthesis–bioassay” cycle. Using the original data, linear and nonlinear QSAR approaches were applied consecutively to two series of 200 and 34 compounds, respectively. On the basis of this model, we identified 23 compounds for synthesis and study that we expected to be as efficacious as DEET. Modification of the original repellency assays, including application of chemicals in stoichiometrically equivalent amounts and converting from a class system to recording of actual days of protection, confirmed that most of these novel acylpiperidines were equivalent to or better than DEET in duration of protection. Astonishingly, a number of these protected >3 times as long as DEET. Finally, the new repellency data were incorporated into structure–activity models and produced exceptional correlation coefficients. Modifications and retraining of the model will be expected as we assess the chemical-structural impact of new classes of compounds on mosquito repellent efficacy.

Methods

Synthesis. See [SI Text](#).

Biological Testing. Laboratory-reared *Aedes aegypti* mosquitoes (Orlando strain, 1952) were obtained from the USDA-Agricultural Research Service Gainesville colony. Adults were provided with sugar and water and maintained nulliparous in laboratory cages at an ambient temperature of $28 \pm 1^\circ\text{C}$ and relative humidity of 35–60%. Repellency assays were conducted in $45\text{ cm} \times 37.5\text{ cm} \times 35\text{ cm}$ ($\approx 59,000\text{ cm}^3$) cages containing 500 ± 50 mosquitoes (5–10 d old). Female mosquitoes were preselected from those that did not display host-seeking behavior and from males by capture in a trap after anemotaxis toward odors from a human hand in a draw box (52) and then were transferred to a test cage for acclimatization for 15–20 min before bioassays.

Two concentrations of each candidate repellent were prepared in a 2-dram vial with acetone (1 ml) as the solvent. An appropriate amount of each

piperidine was added to the vials to produce a deposition of 25 μmol and 2.5 μmol of each piperidine, respectively, per cm^2 on a 50 cm^2 -section of muslin cloth that was inserted into the vial. Each cloth and solution were kept sealed in vials and stored in a freezer at -4°C until used on “day 0.” On day 0, each cloth was removed from its vial and mounted on two sections of 5 $\text{cm} \times 2.5\text{ cm}$ card stock. Masking tape was applied to the card stock edges and the assembly was hung from a rack and allowed to dry for at least 1 h.

Two volunteers (one male and one female) participated in this study. The protocol was approved as project 636-2005 by the University of Florida Human Use Institutional Review Board-01. Informed consent was obtained for subjects before participation in this study. Bioassays were conducted by covering the volunteer’s hand with a soft-embossed long cuff poly glove (Atlantis Products) and then covering the same hand with a powder-free latex glove (Diamond Grip, Microflex). A stocking (Leggs everyday knee highs) was then pulled over the hand and arm to cover the skin surface of the arm. A polyvinyl plastic sleeve, with a Velcro seam to seal the edges around the arm, and with a $3 \times 8\text{ cm}$ opening (i.e., window) cut into the plastic approximately half way between the wrist and elbow end of the sleeve, was fastened around the arm. Each cloth patch assembly was affixed, one at a time, over the open window with masking tape to hold it in place on the sleeve. This allowed volatile kairomones to pass from the skin surface through the window opening and attract mosquitoes which might, if the treatment was not sufficiently repellent, bite through the cloth in the open window. All 2.5 $\mu\text{mol}/\text{cm}^2$ samples were assayed before the 25 $\mu\text{mol}/\text{cm}^2$ -treated patches; however, the order within each concentration group was random, differing not only for the individual volunteers but also day-to-day for a single individual.

The arm and affixed cloth patch were inserted into the cage of mosquitoes and held stationary for 1 min to determine whether the cloth patch was repellent. The number of feeding mosquitoes was counted before removal of the arm with a quick, brisk shaking movement. This procedure was repeated daily until the failure threshold was reached. Feeding mosquitoes that remained on the window were considered to be biting. A maximum of 10 different repellents were assayed with the same group of preselected mosquitoes. Each additional set of 10 candidate repellents was run in a separate cage of ≈ 500 mosquitoes. This procedure minimizes the fatigue and attenuated response of mosquitoes subjected to repeated exposures to repellents, it and avoids depletion of sufficient numbers of fresh mosquitoes to conduct bioassays with all compounds at both concentrations from day 0 onward.

The failure threshold for repellency for these experiments was established as 1% biting (5 bites) and confirmed by achievement of two consecutive days of 5 or more bites. The failure point was ultimately recorded as the first day, rather than the second day, that 5 bites were achieved through a repellent-treated cloth.

ACKNOWLEDGMENTS. We thank Drs. Graham White, David Carlson, and Robert Vander Meer for support and helpful discussions with this endeavor and Natasha Elejalde and Nathan Newlon for laboratory technical support with the biological testing of candidate repellents. This study was partly supported by the Deployed War-Fighter Protection Research Program, funded by the U.S. Department of Defense through the Armed Forces Pest Management Board.

1. Eldridge BF, Edman JD (2004) Introduction to medical entomology. *Medical Entomology: A Textbook on Public Health and Veterinary Problems Caused by Arthropods*, eds Eldridge BF, Edman JD (Kluwer, London), pp 1–12.
2. Rueda LM, Rutledge LC, Gupta RK (1998) Effect of skin abrasions on the efficacy of the repellent deet against *Aedes aegypti*. *J Am Mosq Control Assoc* 14:178–182.
3. Dethier VG (1947) *Chemical Insect Attractants and Repellents* (Blakiston, Philadelphia).
4. Bowen MF, Davis EE, Romo J, Haggart D (1994) Lactic acid sensitive receptors in the autogenous mosquito *Aedes atropalpus*. *J Insect Physiol* 40:611–615.
5. Klun JA, Schmidt WF, Debboun M (2001) Stereochemical effects in an insect repellent. *J Med Entomol* 38:809–812.
6. Nolen JA, Bedoukian RH, Maloney RE, Kline DL (2002) Method, apparatus and compositions for inhibiting the human scent tracking ability of mosquitoes in environmentally defined three dimensional spaces. US Patent 6,362,235.
7. Carlson DA, Smith N, Gouck HK, Godwin DR (1973) Yellow fever mosquitoes: Compounds related to lactic acid that attract females. *J Econ Entomol* 66:329–331.
8. Gupta RK, Bhattacharjee AK (2007) Discovery and design of new arthropod/insect repellents by computer-aided molecular modeling. *Insect Repellents: Principles, Methods, and Uses*, eds Debboun M, Frances SP, Strickman D (CRC, Boca Raton, FL), pp 195–228.
9. Hopfield JJ (1984) Neurons with graded response have collective computational properties like those of two-state neurons. *Proc Natl Acad Sci USA* 81:3088–3092.
10. Katritzky AR, Karelson M, Petruchin R (2001) Comprehensive descriptors for structural and statistical analysis. www.codessa-pro.com.
11. Katritzky AR, Dobchev DA, Tulp I, Karelson M, Carlson DA (2006) QSAR study of mosquito repellents using CODESSA PRO. *Bioorg Med Chem Lett* 16:2306–2311.
12. Barnard DR, Bernier UR, Xue RD, Debboun M (2007) Standard methods for testing mosquito repellents. *Insect Repellents: Principles, Methods, and Uses*, eds Debboun M, Frances SP, Strickman D (CRC, Boca Raton, FL), pp 103–110.
13. McGovern TP, Beroza M, Gouck HK (1967) Chemicals tested as space repellents for yellow-fever mosquitoes. II. Carbanilates, benzamides, aliphatic amides, and imides. *J Econ Entomol* 60:1591–1594.
14. McGovern TP, et al. (1974) *N,N*-substituted *n*-alkanesulfonamides as repellents for the yellow fever mosquito and the German cockroach. *J Econ Entomol* 67:71–73.
15. McGovern TP, Schreck CE, Jackson J, Beroza M (1975) *n*-Acylamides and *n*-alkylsulfonamides from heterocyclic amines as repellents for yellow fever mosquitoes. *Mosq News* 35:204–210.
16. McGovern TP, Schreck CE, Jackson J (1978) Alicyclic carboxamides from heterocyclic amines as repellents for *Aedes aegypti* and *Anopheles quadrimaculatus*. *Mosq News* 38:510–514.
17. Schreck CE, McGovern TP, Smith N (1978) Repellency of selected esters and amides of four alicyclic acids against the stable fly, *Stomoxys calcitrans* (Diptera: Muscidae). *J Med Entomol* 14:589–591.
18. Schreck CE, Smith N, McGovern TP (1979) Repellency of selected compounds against two species of biting midges (Diptera: Ceratopogonidae: *Culicoides*). *J Med Entomol* 16:524–527.
19. McGovern TP, Dame DA, Focks DA, Cameron AL (1980) Laboratory and field evaluation and structure-activity relationships of some aryl terpenoid ethers as insect growth regulators against *Anopheles quadrimaculatus*, *Anopheles albimanus*, and *Aedes taeniorhynchus*. *J Econ Entomol* 73:443–448.
20. McGovern TP, Schreck CE (1981) Alicyclic piperidine derivatives as insect repellents. US Patent 4,291,041.
21. McGovern TP, Schreck CE, Jackson J (1984) Mosquito repellents: *N,N*-dimethylbenzamides, *N,N*-dimethylbenzeneacetamides, and other selected *N,N*-dimethylcarboxamides as repellents for *Aedes aegypti*, *Anopheles quadrimaculatus* and *Anopheles albimanus*. *Mosq News* 44(1):11–16.
22. Schreck CE, McGovern TP (1985) Repellent tests in the field and laboratory against wild populations of *Mansonia titillans* (Diptera: Culicidae). *J Med Entomol* 22:658–662.
23. McGovern TP, Schreck CE (1988) Mosquito repellents: monocarboxylic esters of aliphatic diols. *J Am Mosq Control Assoc* 4:314–321.
24. Schreck CE, McGovern TP (1989) Repellents and other personal protection strategies against *Aedes albopictus*. *J Am Mosq Control Assoc* 5:247–250.
25. US Department of Agriculture (1954) *Chemicals Evaluated as Insecticides and Repellents at Orlando, Fla.*, compiled by King WV (US Dept Agriculture, Washington, DC), Agriculture Handbook No 69.
26. US Department of Agriculture (1967) *Materials Evaluated as Insecticides, Repellents, and Chemosterilants at Orlando and Gainesville, Fla, 1952–1964* (US Dept Agriculture, Washington, DC), Agriculture Handbook No 340.
27. US Department of Agriculture (1977) *Repellent activity of compounds submitted by Walter Reed Army Institute of Research Part I. Protection time and minimum effective dosage against Aedes aegypti mosquitoes* (US Dept Agriculture, Washington, DC), Technical Bulletin No 1549.
28. Haykin S (1998) *Neural Networks: A Comprehensive Foundation* (Prentice-Hall, Englewood Cliffs, NJ), 2nd Ed, pp 1–842.
29. Katritzky AR, et al. (2006) QSAR modeling of anti-invasive activity of organic compounds using structural descriptors. *Bioorg Med Chem* 14:6933–6939.
30. Coan SB, Papa D (1955) Antifungal agents. *J Am Chem Soc* 77:2402–2404.
31. Lindsay CM, Widdowson DA (1988) Organic synthesis with carbon monoxide: synthesis of carbamoylstannanes and aromatic carbamoylation. *J Chem Soc Perkin Trans 1*, 569–573.
32. Montaudo G, Finocchiaro P, Maravigna P, Overberger CG (1972) Conformational properties of structurally rigid polyamides. Conformation of model diamides. *Macromolecules* 5:197–202.
33. McGovern TP, Burden GS (1984) Cockroach repellents. US patent 6,625,329; (1985) *Chem Abstr* 102:144859.
34. House HO, Lee LF (1976) A new synthesis of 2-alkylpyrrolidines and 2-alkylpiperidines. *J Org Chem* 41:863–869.
35. Oare DA, Henderson MA, Sanner MA, Heathcock CH (1990) Stereochemistry of the Michael addition of *N,N*-disubstituted amide and thioamide enolates to α,β -unsaturated ketones. *J Org Chem* 55:132–157.
36. Yoshifuji S, Arakawa Y, Nitta Y (1985) Ruthenium tetroxide oxidation of 1-azabicycloalkane-2-ones. *Chem Pharm Bull* 33:5042–5047.
37. Skau EL, Mod RR, Magne FC (1967) *N*-Acyl derivatives of cyclic imines as poly(vinyl chloride) polymer plasticizer. US Patent 3,309,332.
38. Nambu H, Hata K, Matsugi M, Kita Y (2005) Efficient synthesis of thioesters and amides from aldehydes by using an intermolecular radical reaction in water. *Chem Eur J* 11:719–727.
39. Katritzky AR, Rachwal S, Hitchings GJ (1991) Benzotriazole: a novel synthetic auxiliary. *Tetrahedron* 4:2683–2732.
40. Katritzky AR, Lan X, Fan WQ (1994) Benzotriazole as a synthetic auxiliary: benzotriazolylalkylations and benzotriazole-mediated heteroalkylation. *Synthesis* 445–456.
41. Katritzky AR, Lan X, Yang JZ, Denisko OV (1998) Properties and synthetic utility of *N*-substituted benzotriazoles. *Chem Rev* 98:409–548.
42. Katritzky AR, Rogovoy BV (2003) Benzotriazole: an ideal synthetic auxiliary. *Chem Eur J* 9:4586–4593.
43. Katritzky AR, Manju K, Singh SK, Meher NK (2005) Benzotriazole mediated amino-, amido-, alkoxy- and alkylthio-alkylation. *Tetrahedron* 61:2555–2581.
44. Katritzky AR, Suzuki K, Wang Z (2005) Acylbenzotriazoles as advantageous *N*-, *C*-, *S*-, and *O*-acylating agents. *Synlett*, 1656–1665.
45. Katritzky AR, Shobana N, Pernak J, Afridi AS, Fan WQ (1992) Sulfonyl derivatives of benzotriazole. Part 1. A novel approach to the activation of carboxylic acids. *Tetrahedron* 48:7817–7822.
46. Katritzky AR, He HY, Suzuki K (2000) *N*-Acylbenzotriazoles: Neutral acylating reagents for the preparation of primary, secondary, and tertiary amides. *J Org Chem* 65:8210–8213.
47. Katritzky AR, Zhang Y, Singh SK (2003) Efficient conversion of carboxylic acids into *N*-acylbenzotriazoles. *Synthesis*, 2795–2798.
48. Katritzky AR, Cai C, Singh SK (2006) Efficient microwave access to polysubstituted amidines from imidoylbenzotriazoles. *J Org Chem* 71:3375–3380.
49. Pridgeon JW, et al. (2007) Structure-activity relationships of 33 piperidines as toxicants against female adults of *Aedes aegypti* (Diptera: Culicidae). *J Med Entomol* 44:263–269.
50. Crown H, ed (1990) *Comprehensive Drug Design* (Pergamon, New York), pp 1–19.
51. Jones WD, Nguyen TT, Kloss B, Lee KJ, Vossall LB (2005) Functional conservation of an insect odorant receptor gene across 250 million years of evolution. *Curr Biol* 15:119–121.
52. Posey KH, Schreck CE (1981) An airflow apparatus for selecting female mosquitoes for use in repellent and attraction studies. *Mosq News* 41:566–568.