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# Novel Carboxamides as Potential Mosquito Repellents

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## Novel Carboxamides as Potential Mosquito Repellents

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**ABSTRACT** A model was developed using 167 carboxamide derivatives, from the United States Department of Agriculture archival database, that were tested as arthropod repellents over the past 60 yr. An artificial neural network employing CODESSA PRO descriptors was used to construct a quantitative structure-activity relationship model for prediction of novel mosquito repellents. By correlating the structure of these carboxamides with complete protection time, a measure of repellency based on duration, 34 carboxamides were predicted as candidate mosquito repellents. There were four additional compounds selected on the basis of their structural similarity to those predicted. The compounds were synthesized either by reaction of 1-acylbenzotriazoles with secondary amines or by reaction of acid chlorides with secondary amines in the presence of sodium hydride. The biological efficacy was assessed by duration of repellency on cloth at two dosages (25 and 2.5  $\mu\text{mol}/\text{cm}^2$ ) and by the minimum effective dosage to prevent *Aedes aegypti* (L.) (Diptera: Culicidae) bites. One compound, (E)-N-cyclohexyl-N-ethyl-2-hexenamide, was superior to N,N-diethyl-3-methylbenzamide (deet) at both the high dosage (22 d versus 7 d for deet) and low dosage (5 d versus 2.5 d for deet). Only one of the carboxamides, hexahydro-1-(1-oxohexyl)-1H-azepine, had a minimum effective dosage that was equivalent or slightly better than that of deet (0.033  $\mu\text{mol}/\text{cm}^2$  versus 0.047  $\mu\text{mol}/\text{cm}^2$ ).

**KEY WORDS** repellents, carboxamides, quantitative structure-activity relationship, CPT, *Aedes aegypti*

N,N-diethyl-3-methylbenzamide (deet) is the most effective and best-studied arthropod repellent currently on the market. However, it does not provide a long duration of protection from the bites of all mosquito species, particularly species of *Anopheles* that transmit malaria (Klun et al. 2004). An ideal mosquito repellent that provides this long-term duration of efficacy against all mosquito species has not yet been identified (Fradin 1998, Khan et al. 1969, Strauss et al. 1968) despite an extensive research program that was initiated over 60 yr ago (Travis et al. 1949). Although the safety profile of deet is remarkable after 53 yr of worldwide use, this repellent is not recommended for use on infants (Veltri et al. 1994, Osimitz and Grothaus 1995), and in rare cases has resulted in adverse effects when applied on adult human skin (Clem et al. 1993). The ideal repellent compound would prevent bites from a broad range of arthropod species, remain effective for at least 8 h, cause no irritation to the skin

or mucous membranes, possess no systemic toxicity or plasticizing effect, be resistant to abrasion and rub off, and be totally greaseless and odorless (Fradin 1998).

We recently achieved successful results from an interdisciplinary project that involved methodologies from medical entomology, and synthetic and theoretical chemistry to predict novel acylpiperidine repellents (Katritzky et al. 2008). Through structural modeling, a subset of acylpiperidines, were predicted as efficacious repellents, and 34 of these were synthesized. The model was validated by subsequent bioassays with female *Aedes aegypti* (L.) (Diptera: Culicidae) mosquitoes, wherein some of these compounds provided a complete protection time (CPT) up to 3 times longer than deet. We now present a continuation of our previous approach to model a data set of structurally related repellents and predict novel compounds to be tested as repellents. Reported in this study is the construction of models from previously examined carboxamides that have repellency class reported in the United States Department of Agriculture (USDA) archives. Carboxamides have been studied previously as mosquito repellents, and numerous compounds of this class have demonstrated efficacy (Tsakotellis et al. 1971, Gualtieri et al. 1972, Klun et al. 2003). Recently, carboxamides have also been shown

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**Table 1.** Class definitions according to the first bite occurring within the time ranges minimum and maximum (days)

Class	Minimum day	Maximum day
1	0	1
2	1	5
3	5	10
4	10	21
5	21	-

to be active as cockroach repellents (Gaudin et al. 2008).

A nonlinear quantitative structure-activity relationship approach employing CODESSA PRO descriptors (Katritzky et al. 2001) was used to identify suitable repellent candidates for synthesis and biotesting (Katritzky et al. 2008). The candidates were tested for both duration of repellency and the minimum effective dosage (MED) (USDA 1977).

### Materials and Methods

**Data Set.** A data set consisting of 167 carboxamides was subjected to a quantitative structure-activity relationship analysis using an artificial neural network (ANN) modeling. The original data on carboxamides were obtained from USDA archives (USDA 1954, 1967, 1977). In these records, the experimental protection times were defined according to the "time to first bite" and were measured by testing the effectiveness of the carboxamides on cloth or stocking against female *Ae. aegypti* mosquitoes. Repellency results were divided into five classes (Table 1).

**Optimization and Modeling Procedures.** The geometry of each structure was optimized using molecular mechanic force field, followed by final geometry refinement employing the AM1 semiempirical method as implemented in HyperChem 7.5 (HyperCube 2003). Depending on the structure of each carboxamide, between 529 and 1,557 descriptors were calculated.

The objective of the present calculations was to classify the time effectiveness of the repellents based on an ANN model. To avoid the ANN overfitting problem caused by the mathematical complexity when a large number of adjustable parameters are used, the data set was divided into training and validation subsets, comprised of 120 and 47 compounds, respectively.

To find relevant descriptors for the ANN model, scatter plots of the descriptor values versus the duration of protection were screened. The descriptors showing significant variation with the property under consideration were selected as candidates for building the ANN model.

The selected model is based on a back propagation learning algorithm for optimization of the ANN weights to reduce the error. The 120 compounds in the training set were used to develop the model. The root mean square error (rms) of the validation set and the corresponding  $R^2$  were monitored to avoid overtraining of the ANN and to stop the training process.

**Synthesis of Carboxamides.** A total of 38 carboxamides was synthesized. The 1-acylbenzotriazoles **2** (Fig. 1; Supporting Information [SI] Table S1, SI Text 1) were prepared by treatment of the corresponding carboxylic acids **1** at 20°C with thionyl chloride and benzotriazole in methylene chloride in 1:1:3 mol ratio based on a modified procedure (as described in Katritzky et al. 2006). Reaction of 1-acylbenzotriazoles **2a-2i** with one equivalent of secondary amines **4a-4j** either in toluene under reflux or in tetrahydrofuran at 20°C yielded carboxamides **5a-5u**, **5j'**, and **5k'** in 70–100% and **5i'** and **5l'** in 36 and 28% yields, respectively (Fig. 1, path A (Katritzky et al. 2000a); Table S1, SI Text 1). When reacted with a secondary amine under neutral conditions (path A, Fig. 1), nonblocked  $\alpha,\beta$ -unsaturated 1-acylbenzotriazoles **2** also gave undesired Michael-type addition of benzotriazole to carboxamides **5**. The resulting mixture of byproduct  $Bt^1$ -adduct **6b**, byproduct  $Bt^2$ -adduct **6a**, and the de-

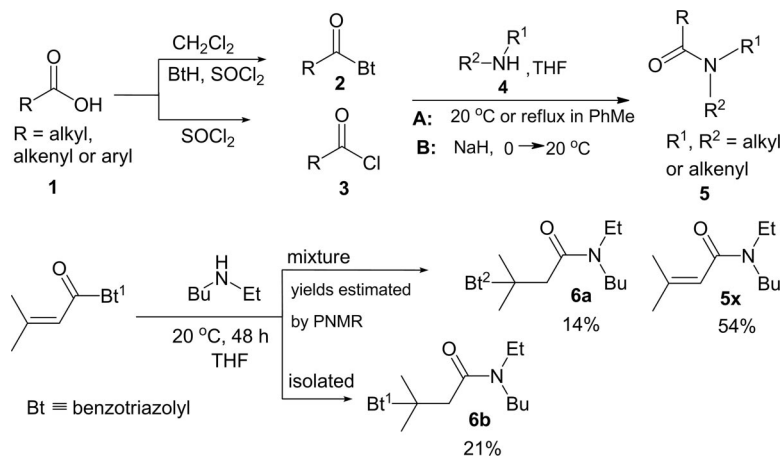


Fig. 1. Synthetic pathway for production of carboxamides 5a–5l'.



**Fig. 2.** The cloth patch assays involve insertion of an arm into a cage of mosquitoes. The hand is protected from bites by a glove, and a nylon stocking followed by plastic sleeve are affixed on the arm. The sleeve has a  $4 \times 8$ -cm window cut out of it to allow attractive human emanations to escape and draw mosquitoes to the opening that is located on the anterior surface of the arm. Cloth treated with a repellent is affixed over the window, and the ability of the treated cloth to repel mosquitoes from biting is evaluated. Photo credit: Greg Allen, USDA-Agricultural Research Service. (Online figure in color.)

sired product 5x could not be separated by column chromatography.

Therefore, path B was chosen for the preparation of the corresponding carboxamides 5v-h' (Fig. 1; Table S1, SI Text 1). Acid chlorides 3 were either commercially available or prepared in situ by treatment of the corresponding carboxylic acids 1 with 20–27% excess of thionyl chloride at 20°C overnight. Reaction of acid chlorides 3a–d (Table S1) with one equivalent of secondary amines 4a, 4b, 4d–4 h in tetrahydrofuran in the presence of 8% excess of sodium hydride at 0–20°C led to formation of carboxamides 5v–h' in 70–97% yield (Fig. 1, path B; Table S1, SI Text 1). For details about carboxamide rotamers' coalescence temperature study, see SI Text 2.

**Biological Testing.** The mosquitoes used for testing were female *Ae. aegypti* (Orlando strain, 1952) from the colony maintained at USDA-Agricultural Research Service-Center for Medical, Agricultural, and Veterinary Entomology (Gainesville, FL). Pupae were obtained from the colony, and newly emerged mosquitoes were maintained on 10% sugar water and kept in laboratory cages at an ambient temperature of  $28 \pm 1^\circ\text{C}$  and RH of 35–60%. Nulliparous female mosquitoes were preselected from stock cages using a hand-draw box and trapped in a collection trap (Posey and Schreck 1981). After 500 ( $\pm 10\%$ ) females were collected in the trap, they were transferred to a test

cage ( $\approx 59,000 \text{ cm}^3$  with dimensions  $45 \times 37.5 \times 35 \text{ cm}$ ) and allowed to acclimatize for  $17.5 (\pm 2.5) \text{ min}$  before testing was initiated (Barnard et al. 2007).

Appropriate masses of each carboxamide were dissolved in 1 ml of acetone in a 2-dram vial to produce 25 and  $2.5 \mu\text{mol}/\text{cm}^2$  of each carboxamide on a  $50\text{-cm}^2$  muslin cloth piece that had been inserted into the vial. Vials were kept sealed in a freezer at  $-4^\circ\text{C}$  until used for the tests, normally  $< 48 \text{ h}$ . Before the start of testing, the cloth was removed from the vial and affixed by staples onto two sections of card stock ( $5 \times 2.5 \text{ cm}$ ). Approximately 5 cm of masking tape was affixed to the edges of the card stock. The cloth and card stock were then placed on a drying rack and allowed to dry for at least 3 min before testing.

A test assay consisted of protecting the hand of each human volunteer with a soft-embossed long cuff poly glove (Atlantis Products, Mankato, MN), followed by a second layer of a powder-free latex glove (Diamond Grip; Microflex, Reno, NV) (see Fig. 2). The gloved hand and arm were then placed inside a knee-high stocking (Leggs Everyday Knee Highs, Winston-Salem, NC). A plastic sleeve constructed of polyvinyl was then placed over the arm and stocking. The sleeve has a lengthwise Velcro strip to allow tight sealing around the arm. There is a window cut into the sleeve ( $4 \times 8\text{-cm}$  opening) approximately halfway between the wrist and elbow, and the sleeve is worn so that the



**Table 2.** Predicted versus experimental time classes for the training set

ID	Exp. classes	Pred. classes	ID	Exp. classes	Pred. classes	ID	Exp. classes	Pred. classes
S0019612	1	1	S0039630	1	1	S0054939	2	3
S0020458	2	1	S0039631	3	2	S0054993	1	1
S0020597	1	1	S0039632	2	2	S0054994	3	3
S0020598	1	1	S0039633	1	3	S0054995	2	3
S0021740	2	3	S0039634	5	5	S0054996	2	3
S0023789	1	1	S0039638	1	1	S0054997	1	1
S0023796	1	1	S0039642	4	4	S0054998	4	3
S0023797	1	1	S0039643	1	2	S0055000	1	1
S0023798	1	1	S0039644	1	1	S0055003	2	3
S0026588	1	1	S0039671	4	2	S0055004	1	1
S0026782	1	2	S0039672	1	1	S0055005	1	2
S0032779	1	4	S0039673	1	2	S0055007	2	3
S0033022	2	2	S0039674	1	2	S0055014	4	4
S0033449	2	3	S0039675	5	4	S0055016	1	2
S0035767	3	3	S0039708	2	3	S0055019	2	3
S0036321	1	1	S0039709	1	1	S0055020	1	1
S0036322	4	4	S0039753	1	1	S0055022	1	1
S0036326	4	4	S0039773	4	4	S0055023	3	2
S0036327	3	4	S0039839	3	2	S0055025	3	3
S0036693	3	3	S0039892	1	1	S0055026	5	5
S0036701	1	1	S0054015	2	3	S0055027	2	3
S0036702	1	1	S0054108	3	2	S0055029	2	3
S0037682	1	1	S0054110	1	2	S0055032	3	4
S0037683	1	1	S0054218	3	2	S0055035	4	3
S0037988	3	3	S0054220	3	3	S0055037	1	1
S0037989	1	1	S0054221	1	1	S0055038	1	1
S0037990	1	1	S0054225	1	2	S0055042	3	3
S0038320	1	2	S0054229	3	4	S0055048	1	1
S0038818	1	1	S0054293	1	3	S0055049	1	1
S0038819	1	2	S0054376	3	3	S0055050	1	1
S0038820	1	2	S0054377	5	4	S0055051	2	3
S0039044	1	1	S0054420	1	2	S0055054	2	2
S0039045	2	2	S0054435	2	3	S0055055	1	2
S0039046	1	1	S0054511	1	2	S0055063	1	1
S0039047	1	2	S0054512	1	1	S0055065	1	1
S0039200	1	1	S0054554	3	3	S0055068	1	1
S0039295	1	1	S0054577	2	3	S0055073	1	1
S0039296	1	1	S0054915	2	3	S0055074	1	1
S0039628	5	3	S0054916	3	2	S0055093	1	1
S0039629	1	1	S0054934	3	3	S0055094	1	1

ID, identification; Exp., experimental; Pred., predicted.

opening is on the anterior portion of the arm. This window allows odors from the volunteer’s skin surface to escape from the sleeve through the opening, which is covered with the treated cloth. The lower dosage (2.5 μmol/cm<sup>2</sup>) samples were assayed before the higher dosage (25 μmol/cm<sup>2</sup>). The patch test sequence was randomized among volunteers daily.

The arm, sleeve, and cloth were inserted into the mosquito cage for 1 min to determine whether the compound and dosage on cloth were repellent to the mosquitoes. The number of blood-feeding mosquitoes was determined by shaking the arm briskly after 1 min and counting the number of mosquitoes that remained biting through the cloth. This procedure was repeated daily until the cloth failed to prevent a threshold level of bites. The failure point for these experiments was five bites (1% of the cage population obtained blood), rather than use of time to first bite to estimate the CPT. Confirmation was obtained by evaluating a cloth patch that failed on consecutive 2 d. The failure point was recorded as the first day that five mosquitoes bit through the treated cloth in 1 min or less. During the testing process, no more than 10

compounds were assayed in succession with a caged population of test mosquitoes before allowing a 15-min recovery period. This is necessary because after prolonged and repeated repellent exposure, mosquitoes exhibit fatigue and a decreased response to attractant (skin) odors (Fradin and Day 2002, Katritzky et al. 2008).

Two volunteers (male and female) participated in the study of repellency duration. The participants evaluated the remaining patches daily, at approximately the same time (1300 h) each day until all treated cloth patches failed twice. To evaluate the MED (USDA 1977), two series of dosages were used. The series of high dosages were as follows: 25,000, 12,500, 6,250, and 3,125 μmol/cm<sup>2</sup>. The lower consisted of cloth samples treated with 2,500, 1,250, 0,625, 0,313, 0,156, 0,078, 0,039, and 0,020 μmol/cm<sup>2</sup>. Testing for MED was initiated using the middle range (0.313 μmol/cm<sup>2</sup>)-treated cloth and evaluating higher or lower dosage treatments as necessary until all volunteers had evaluated the cloths and pinpointed the dosage at the 1% (five bite) failure point. If the 2,500 μmol/cm<sup>2</sup> cloth was not efficacious (>5 bites in 1

49	1			
15	4	6	1	
2	16	10	2	1
1		3	5	2
				2

Fig. 3. Confusion matrix representing the agreement between the predicted and experimental repellent classes for the training set of 120 carboxamides.

min), then the higher dosage series was used to determine the MED. There were five to six volunteers (four to five male, one female) who tested each cloth. During each test, all volunteers wore a particular patch and tested it for 1-min intervals. Patches were rotated among the volunteers, and no patch was evaluated beyond 10 min after the 3-min drying period to avoid any bias that may result from evaporative loss of treatment of the cloth throughout the test. All procedures were approved by the University of Florida Human Use Institutional Review Board-01, and informed consent was provided by all participants (Project 636-2005 entitled "Laboratory Evaluation of Repellents for Personal Protection from Mosquitoes and Biting Flies").

### Results and Discussion

The final ANN model possessed architecture 6-4-1 (i.e., six input neurons representing the selected descriptors, four hidden neurons, and one output for the time classes). The input descriptors for the ANN model were as follows: weighted partial positive surface area (partial positive surface area  $3 \times$  total molecular surface area/1000) (Zefirov PC), average valency for atom H, molecular volume/XYZ box, highest normal mode vibration frequency, highest normal mode vibration transition dipole, minimal resonance

energy for H-C bond. Based on the six descriptor ANN model, the predicted values for the training set of the repellents are shown in Table 2.

In this table, the identification number is listed according to the formerly used AI3 numbers (USDA 1954). The other columns indicate the experimental class compared with what the model predicted for the training set. The rms error of the model is 0.72. The coefficient of determination as a linear fit between the experimental and predicted classes is 0.622.

Fig. 3 shows the confusion matrix; each column represents the instances in the predicted class, whereas each row represents the instances in the actual (experimental) class. As can be seen, 70 of the 120 points lie on the main diagonal and 45 more lie just one diagonal off ( $R^2 = 0.622$ ). The probabilities to predict a certain class exactly (calculated as  $N/N_s$ , where "N" is the number of the repellents in certain class and "N<sub>s</sub>" is the number of successive 100% predictions) are as follows: class 1-0.73, class 2-0.20, class 3-0.53, class 4-0.62, and class 5-0.40. The reason that class 1 has the best chance to be predicted correctly is the result of the high number of repellents in this class and the smaller error interval of 12 h. Thus, the ANN model has provided a weighted prediction on class 1.

The results for the external validation set are shown in Table 3. Because the defined classes are represented by integer values, a rms error of 1.15 for the validation set indicates that most of the predictions lie just one diagonal off the main diagonal of the confusion matrix, which is comparable to that for the training set.

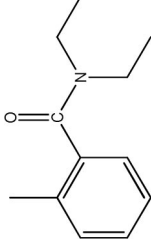
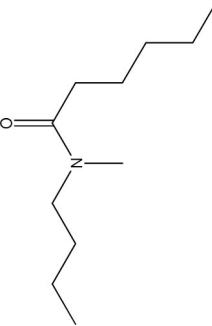
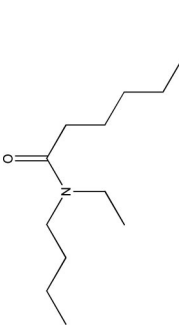
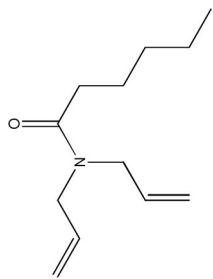
The validation set was used as a diagnostic tool in two ways. First, it provides an estimate of the predictive power of the model and, second, helpful information to avoid an overfitting of the model. Because of the nonlinear time scale used to define the classes, the higher classes that contain longer time intervals provide greater experimental error. Nevertheless, the ANN model is capable of predicting classes 4 and 5 with satisfactory accuracy.

Table 3. Predicted versus experimental time classes for the validation set

ID	Exp. classes	Pred. classes	ID	Exp. classes	Pred. classes	ID	Exp. classes	Pred. classes
S0055095	1	2	S0055138	2	1	S0055172	3	4
S0055099	1	2	S0055140	2	2	S0055173	3	4
S0055102	1	2	S0055142	1	3	S0055175	2	2
S0055104	1	1	S0055143	2	3	S0055178	5	3
S0055106	3	3	S0055144	4	4	S0065533	1	3
S0055107	1	1	S0055147	2	3	S0065534	2	3
S0055108	2	3	S0055149	3	3	S0070310	2	1
S0055111	1	1	S0055151	1	2	S0070311	5	5
S0055113	1	3	S0055152	2	1	S0070372	2	3
S0055118	3	2	S0055153	1	1	S0070373	2	3
S0055119	1	3	S0055155	3	3	S0070374	1	3
S0055120	1	1	S0055156	1	1	S0070375	1	1
S0055123	1	3	S0055157	1	1	S0070618	1	1
S0055124	4	3	S0055162	2	3	S0070619	1	1
S0055126	1	3	S0055163	1	1	S0070620	5	3
S0055133	2	4	S0055164	2	1			

ID, identification; Exp., experimental; Pred., predicted.

Table 4. Predicted and experimental repellency at two dosages for carboxamides 5a–5h' and minimum effective dosage for carboxamides 5a–5l'

Entry	Compound		Structure	ANN predicted class	Protection time (d)		MED $\pm$ SE $\mu\text{mol}/\text{cm}^2$
	ID	Name			25 $\mu\text{mol}/\text{cm}^2$	2.5 $\mu\text{mol}/\text{cm}^2$	
1	Deet	N,N-diethyl-3-methylbenzamide		-	7	2.5	0.047 $\pm$ 0.007
2	5a	N-butyl-N-methyl-hexanamide		4	1.5	1	0.117 $\pm$ 0.017
3	5b <sup>a</sup>	N-butyl-N-ethylhexanamide		3	2	1	0.156 $\pm$ 0.043
4	5c	N,N-diallylhexanamide		4	1	1	0.195 $\pm$ 0.039

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Table 4. Continued

Entry	Compound		ANN predicted class	Protection time (d)		MED $\pm$ SE $\mu$ mol/cm <sup>2</sup>
	ID	Name		25 $\mu$ mol/cm <sup>2</sup>	2.5 $\mu$ mol/cm <sup>2</sup>	
5	5d	Hexahydro-1-(1-oxohexyl)-1H-azepine	4	7	1	0.033 $\pm$ 0.004
6	5e	N-cyclohexyl-N-ethylhexanamide	5	12	1	0.266 $\pm$ 0.047
7	5f	N-ethyl-N-phenylhexanamide	4	7.5	2	0.625 $\pm$ 0.171

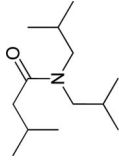
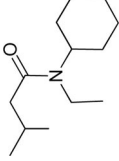
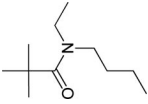
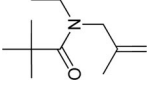
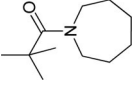
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Table 4. Continued

Entry	Compound		Structure	ANN predicted class	Protection time (d)		MED $\pm$ SE $\mu\text{mol}/\text{cm}^2$
	ID	Name			$25 \mu\text{mol}/\text{cm}^2$	$2.5 \mu\text{mol}/\text{cm}^2$	
8	5g <sup>d</sup>	N-butyl-N-ethyl-2-methylpentanamide		5	1	1	$0.104 \pm 0.016$
9	5h <sup>a</sup>	1-(1-azepanyl)-2-methyl-1-pentanone		5	3.5	1	$0.102 \pm 0.033$
10	5i <sup>a</sup>	N-butyl-N,2-diethylbutanamide		4	3	3	$0.125 \pm 0.019$
11	5j <sup>a</sup>	N,2-diethyl-N-(2-methyl-2-propenyl)butanamide		4	1	1	$0.375 \pm 0.062$
12	5k <sup>a</sup>	N-butyl-N-ethyl-3-methylbutanamide		5	1	1	$0.117 \pm 0.017$

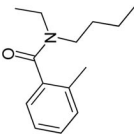
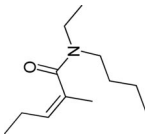
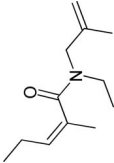
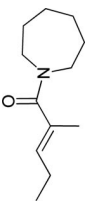
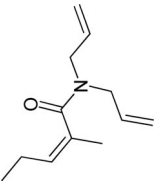
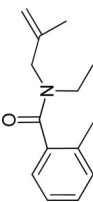
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Table 4. Continued

Entry	Compound		ANN predicted class	Protection time (d)		MED $\pm$ SE $\mu$ mol/cm <sup>2</sup>
	ID	Name		Structure	25 $\mu$ mol/cm <sup>2</sup>	
13	5l	N,N-diisobutyl-3-methylbutanamide		4	1	0.406 $\pm$ 0.094
14	5m <sup>a</sup>	N-cyclohexyl-N-ethyl-3-methylbutanamide		5	7	0.172 $\pm$ 0.058
15	5n <sup>a</sup>	N-butyl-N-ethyl-2,2-dimethylpropanamide		3	1	0.286 $\pm$ 0.108
16	5o <sup>a</sup>	N-ethyl-2,2-dimethyl-N-(2-methyl-2-propenyl)propanamide		5	1	0.469 $\pm$ 0.099
17	5p	1-(1-azepanyl)-2,2-dimethyl-1-propanone		5	1	0.313 $\pm$ 0.000

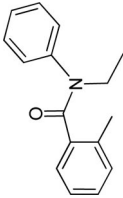
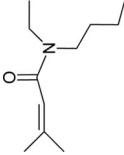
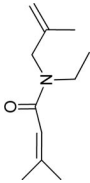
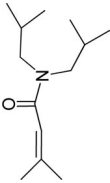
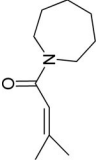
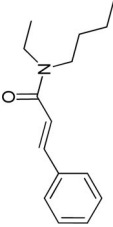
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Table 4. Continued

Entry	Compound		ANN predicted class	Protection time (d)		MED $\pm$ SE $\mu\text{mol}/\text{cm}^2$	
	ID	Name		Structure	$25 \mu\text{mol}/\text{cm}^2$		$2.5 \mu\text{mol}/\text{cm}^2$
18	5q <sup>a</sup>	N-butyl-N-ethyl-2-methylbenzamide		3	15	2	0.156 $\pm$ 0.043
19	5r <sup>a</sup>	(E)-N-butyl-N-ethyl-2-methyl-2-pentenamide		2	2	1	0.117 $\pm$ 0.017
20	5s <sup>a</sup>	(E)-N-ethyl-2-methyl-N-(2-methyl-2-propenyl)-2-pentenamide		4	2	1.5	0.182 $\pm$ 0.044
21	5t <sup>a</sup>	(E)-1-(1-azepanyl)-2-methyl-2-penten-1-one		3	4	1.5	0.098 $\pm$ 0.020
22	5u	(E)-2-methyl-N,N-di-2-propenyl-2-pentenamide		3	2	2	0.417 $\pm$ 0.066
23	5v <sup>a</sup>	N-ethyl-2-methyl-N-(2-methyl-2-propenyl)benzamide		5	13	2	0.145 $\pm$ 0.049

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Table 4. Continued

Entry	Compound		Structure	ANN predicted class	Protection time (d)		MED $\pm$ SE $\mu$ mol/cm <sup>2</sup>
	ID	Name			25 $\mu$ mol/cm <sup>2</sup>	2.5 $\mu$ mol/cm <sup>2</sup>	
24	5w	N-ethyl-2-methyl-N-phenyl-benzamide		4	3.5	1	5.160 $\pm$ 4.960
25	5x <sup>a</sup>	N-butyl-N-ethyl-3-methyl-2-butenamide		3	1	1	0.192 $\pm$ 0.055
26	5y <sup>a</sup>	N-ethyl-3-methyl-N-(2-methyl-2-propenyl)-2-butenamide		4	1	1	0.313 $\pm$ 0.086
27	5z	N,N-diisobutyl-3-methyl-crotonamide		2	2	2.5	0.219 $\pm$ 0.038
28	5a'	Hexahydro-1-(3-methylcrotonoyl)-1H-azepine		3	3	1	0.140 $\pm$ 0.016
29	5b'	N-butyl-N-ethyl-cinnamide		2	8.5	0.5	10.750 $\pm$ 5.828

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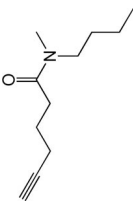
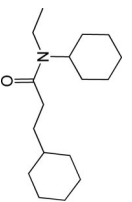
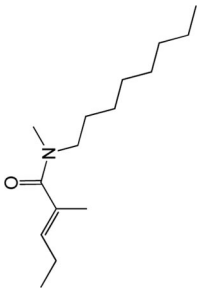
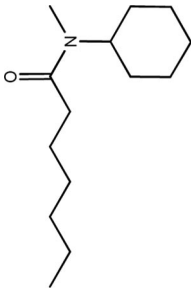
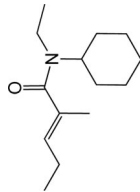
Table 4. Continued

Entry	Compound		Structure	ANN predicted class	Protection time (d)		MED $\pm$ SE $\mu$ mol/cm <sup>2</sup>
	ID	Name			25 $\mu$ mol/cm <sup>2</sup>	2.5 $\mu$ mol/cm <sup>2</sup>	
30	5c <sup>a</sup>	N,N-bis(2-methylpropyl)-3-phenyl-2-propenamide		4	2	1.5	20.125 $\pm$ 4.875
31	5d <sup>1</sup>	N-ethyl-N,3-diphenyl-2-propenamide		4	1	0	20.250 $\pm$ 4.750
32	5e <sup>a</sup>	(E)-N-n-butyl-N-ethyl-2-hexenamide		4	7.5	1.5	0.274 $\pm$ 0.039
33	5f <sup>a</sup>	(E)-N,N-di-(2-methylpropyl)-2-hexenamide		5	8	2	0.625 $\pm$ 0.140
34	5g <sup>a</sup>	(E)-N-cyclohexyl-N-ethyl-2-hexenamide		3	22	5	0.651 $\pm$ 0.377

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Table 4. Continued

Entry	Compound		Structure	ANN predicted class	Protection time (d)		MED $\pm$ SE $\mu\text{mol}/\text{cm}^2$
	ID	Name			25 $\mu\text{mol}/\text{cm}^2$	2.5 $\mu\text{mol}/\text{cm}^2$	
35	5h <sup>a</sup>	N-butyl-N-methyl-5-hexynamide		4	7	1	0.182 $\pm$ 0.026
36	5i <sup>a</sup>	N,3-dicyclohexyl-N-ethylpropanamide		s	nt	nt	20,500 $\pm$ 4,108
37	5j <sup>a</sup>	(E)-N,2-dimethyl-N-octylpent-2-enamide		s	nt	nt	0.125 $\pm$ 0.017
38	5k <sup>a</sup>	N-cyclohexyl-N-methylheptanamide		s	nt	nt	0.172 $\pm$ 0.035
39	5l <sup>a</sup>	(E)-N-cyclohexyl-N-ethyl-2-methylpent-2-enamide		s	nt	nt	0.140 $\pm$ 0.014

nt, not tested; s, were not predicted, but selected based on structural similarity to others in this set of compounds. For known compounds, see SI text, Table S1, and the following references: 5a, Mowafy 2004; 5c, Tsuruoka 1971; 5d, Mowafy 2004; 5e, McCovern and Burden 1984; 5f, Katritzky et al. 2000b; 5i, Bao et al. 1994; 5p, Li 2005; 5u, Koshino et al. 1990; 5w, Schickedantz 1967; 5z, 5a', Schickedantz 1967; 5b', Papa et al. 1950; 5d', El Ali et al. 2000.

<sup>a</sup> Novel compounds.

From the 120 compounds in the training set, the nine compounds having predicted and experimental repellency of class 4 or greater (Fig. 3 and Table 2) were selected as a basis for designing 144 structurally similar compounds, intentionally selected to be easy to synthesize. ANN modeling was used to predict the activities of the 144 compounds, and the best 34 of them were selected for synthesis. We then designed four additional similarly structured compounds based on visual inspection of the 34 best compounds. As shown in Table 4, 23 of the 38 carboxamides (i.e., 60.5%) were predicted to be highly active repellents (classes 4 and 5). Eight were predicted to be moderately active (class 3), and three compounds (5r, 5z, and 5b') were predicted to have relatively weak repellent activity (class 2). The complex nonlinear dependence encoded in the ANN model and the lack of regression coefficients and their corresponding signs do not allow us to provide a clear physicochemical interpretation of the interrelation between the descriptors and the property under investigation.

**Biological Testing.** Duration of repellency at two dosages was tested with two volunteers using the cloth patch assay (USDA 1977). The averaged protection time (in days) of each repellent compound and MED are reported in Table 4. The carboxamide (E)-N-cyclohexyl-N-ethyl-2-hexenamide (5g') provided the longest protection time (22 d) on cloth, i.e., over 3 times the protection time provided by deet (7 d) at the 25  $\mu\text{mol}/\text{cm}^2$  dose, and double that of deet at the lower 2.5  $\mu\text{mol}/\text{cm}^2$  dose (5 d compared with 2.5 d). The compounds N-butyl-N-ethyl-2-methylbenzamide (5q), N-ethyl-N-(2-methylallyl)-2-methyl-benzamide (5v), and N-cyclohexyl-N-ethylhexanamide (5e) all provided 1.5–2 times the protection time compared with the deet at the higher dosage, but were less effective than deet at the lower dosage. All other carboxamides were essentially equal to or less effective than deet with respect to repellency duration.

Because of the lack of a wide separation of repellent efficacy based on duration, a second statistic of repellency was used to examine the carboxamides. The MED is a measure of repellent potency that is used at times to evaluate repellents, especially natural compounds that may exhibit a higher volatility (USDA 1977, Bernier et al. 2005). The MED value is essentially the threshold (amount/surface area) dosage of a compound that no longer prevents bites (i.e., that is no longer repellent). Thus, MED does not take into account duration; however, with proper formulation, the surface duration of such a repellent with a low MED could be extended.

The most potent carboxamide (lowest MED) was 1-(1-azepanyl)-1-hexanone (5d), with a MED equal to that of deet ( $0.047 \pm 0.007 \mu\text{mol}/\text{cm}^2$ ) (Table 4). Later in potency were (E)-1-(1-azepanyl)-2-methyl-2-penten-1-one (5t) ( $0.098 \pm 0.020 \mu\text{mol}/\text{cm}^2$ ), 1-(1-azepanyl)-2-methyl-1-pentanone (5h) ( $0.102 \pm 0.033 \mu\text{mol}/\text{cm}^2$ ), and N-butyl-N-ethyl-2-methylpentanamide (5g) ( $0.104 \pm 0.016 \mu\text{mol}/\text{cm}^2$ ). Interestingly, these compounds were inferior to deet with respect to duration of repellency (Table 4). The longest last-

ing carboxamide, (E)-N-cyclohexyl-N-ethyl-2-hexenamide (5g'), with over 3 times the duration of repellency of deet, has a MED ( $0.625 \pm 0.377$ ) that is nearly 20 times higher than deet.

## Conclusions

Although the modeling of the carboxamide data set did not produce a predictive model with a clear physicochemical interpretation correlating compound structure and repellent efficacy against *Ae. aegypti*, there were still some promising leads from this study. Many of these predicted and synthesized compounds were repellent in some capacity, with four carboxamides exceeding the repellent duration of deet when tested on cloth at the (higher) 25  $\mu\text{mol}/\text{cm}^2$  dosage. Although duration of repellency measured by protection time (commonly CPT) is often used when evaluating repellents, the MED of a repellent needed to prevent arthropod bites minimizes the influence of evaporative loss of repellent and provides an indication of the relative potency level of a repellent. Whereas the predicted carboxamides were not chosen based on MED, the MED of 1-(1-azepanyl)-1-hexanone (5g') was similar to that of deet. In the future, we will examine the five best repellents (four based on CPT, one based on the MED) against other arthropods (ticks, stable flies) of medical and veterinary importance.

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