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Aryl methylcarbamates: potency and selectivity towards wild-type and carbamate-insensitive (G119S) *Anopheles gambiae* acetylcholinesterase, and toxicity to G3 Strain *An. gambiae*

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Abstract

New carbamates that are highly selective for inhibition of *Anopheles gambiae* acetylcholinesterase (AChE) over the human enzyme might be useful in continuing efforts to limit malaria transmission. In this report we assessed 34 synthesized and commercial carbamates for their selectivity to inhibit the AChEs found in carbamate-susceptible (G3) and carbamate-resistant (Akron) *An. gambiae* relative to human AChE. Excellent correspondence is seen between inhibition potencies measured with carbamate-susceptible mosquito homogenate and purified recombinant wild-type (WT) *An. gambiae* AChE (AgAChE). Similarly, excellent correspondence is seen between inhibition potencies measured with carbamate-resistant mosquito homogenate and purified recombinant G119S AgAChE, consistent with our earlier finding that the Akron strain carries the G119S mutation. Although high (100- to 500-fold) WT *An. gambiae* vs human selectivity is observed for several compounds, none of the carbamates tested potently inhibits the G119S mutant enzyme. Finally, we describe a predictive model for WT *An. gambiae* tarsal contact toxicity of the carbamates that relies on inhibition potency, molecular volume, and polar surface area.

Keywords

acetylcholinesterase; *Anopheles gambiae*; carbamate; malaria; resistance; toxicity

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Supplementary Data Available: Comparison of sequences of recombinant enzymes to deduced sequences of native enzymes; comparison of N-terminal domains of four mosquito acetylcholinesterases; details on correlation of log LC₅₀ to *k_i*(rAgAChE-WT) and select physicochemical descriptors.

1. Introduction

According to the World Malaria Report 2011, malaria killed nearly 700,000 people in 2010, most of whom were children under 5 years old [1]. Over the past decade, control of the mosquito vector *Anopheles gambiae* using insecticide-treated nets (ITNs) has been particularly successful in reducing malaria transmission and mortality [1, 2]. Current ITNs are impregnated with pyrethroid modulators of the voltage-gated sodium ion channel, and these nets have been widely distributed in malaria-endemic regions. However, emerging pyrethroid-resistant strains of *An. gambiae* jeopardize the future usefulness of these nets [3, 4]. As pointed out by Nauen in 2006, only one other biological target has been successfully exploited to control adult mosquitoes: acetylcholinesterase (AChE, EC 3.1.1.7) [5]. At present the World Health Organization Pesticide Evaluation Scheme (WHOPES, <http://www.who.int/whopes/en/>) has not approved any AChE inhibitors for use on ITNs, though five AChE inhibitors have been approved for indoor residual spraying (IRS).

To be useful for deployment on ITNs, we judged that insecticidal AChE inhibitors would need to show high selectivity for *An. gambiae* AChE (*AgAChE*) over human AChE (*hAChE*). Since the catalytic subunit of *AgAChE* is only 49% identical to that of *hAChE* [6], the goal of achieving significant inhibition selectivity appears feasible. We recently studied a series of experimental and commercial aryl methylcarbamates (Figure 1) and found that novel compounds bearing a β -branched alkoxy or thioalkyl substituent at the 2-position (e.g. **1d**) exhibit up to 500-fold selectivity for recombinant wild-type *AgAChE* (*rAgAChE*-WT) over recombinant *hAChE* (*rhAChE*) [7, 8]. In this paper we examine the same series of carbamates, using G3 Strain *An. gambiae* homogenate (*Ag* G3 hmg) as the source of WT enzyme, to ascertain the degree of correspondence of the native enzyme to our recombinant construct (*rAgAChE*-WT). As before, apparent second order inactivation rate constants k_i ($\text{mM}^{-1} \text{min}^{-1}$) were measured. Because a single mutation in *AgAChE* (G119S) confers high levels of resistance to carbamate insecticides [9, 10], we also determined k_i values for these compounds against recombinant G119S enzyme (*rAgAChE*-G119S) and the enzyme present in Akron strain *An. gambiae* homogenate (*Ag* Akron hmg). Finally, the relationship of G3 strain *An. gambiae* tarsal contact toxicity (LC_{50}) of 23 aryl methylcarbamates to their k_i values and physicochemical properties was explored.

2. Materials and Methods

2.1. Reagents and recombinant enzymes

Carbamates **1a-f**, **2a-d,g**, **3d**, **4h**, **5**, **6h-w** were prepared as described previously [7]; carbamates **7–10** and *rhAChE* (Sigma C1682) were purchased from commercial suppliers. Recombinant constructs of WT and G119S *AgAChE* (*rAgAChE*-WT and *rAgACh*-G119S, respectively) were expressed in yeast as described earlier [11]; the two recombinant enzymes differ only at residue 119.

2.2. Mosquito rearing, preparation of homogenates, and toxicity testing

G3 Strain (susceptible) and Akron strain (carbamate-resistant) *An. gambiae* were obtained from MR4 (www.mr4.org). To prevent cross-contamination, these strains were reared in separate environmental chambers (G3 28 ± 1 °C, 55–65% relative humidity (RH); Akron 28 ± 1 °C, 70% RH; both 14 h light, 10 h dark) using standard techniques. Pupae were removed daily to hatch in separate cages at 27 ± 1 °C and 80% RH, and adult mosquitoes were given free access to 10% (w/v) sugar water. Mosquito homogenates for enzyme assay were prepared from non-blood-fed mosquitoes, as described previously [11]. Tarsal contact LC_{50} values to G3 strain *An. gambiae* were determined using the standard WHO filter paper protocol, as described previously [7, 11, 12]

2.3. Enzyme kinetic methods and software used

Enzyme activity was measured using the Ellman assay [13] in a microtiter plate format, using acetylthiocholine (ATCh) as substrate. Apparent second-order inactivation rate constants k_i were determined by measuring enzyme velocities as a function of incubation time at fixed carbamate concentrations. These velocities (v/v_0) were used to calculate pseudo first-order rate constants k_{obs} (min^{-1}) for inactivation by plotting $\ln(v/v_0)$ vs incubation time t . For each inhibitor k_{obs} values were determined at three or more inhibitor concentrations ([I]). Plots of k_{obs} vs [I] were then constructed and the slope of the linear fit provided the apparent second-order rate constants k_i ($\text{mM}^{-1} \text{min}^{-1}$) for inactivation [7, 11, 14]. All kinetic data were evaluated using Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA). Correlations of the tarsal contact toxicity of the carbamates to G3 strain *An. gambiae* (Log LC_{50}) to the corresponding k_i values and select physicochemical properties were evaluated using ICM software [15].

3. Results and Discussion

3.1. Carbamate inhibition of WT AgAChE (recombinant and G3 homogenate)

Since G3 strain *An. gambiae* are carbamate-susceptible, the primary ATCh-hydrolyzing enzyme in the mosquito should be WT AgAChE. Our recombinant WT AgAChE construct (rAgAChE-WT) comprises the catalytic subunit (540 amino acids) and only the first 12 aa of the native 36 aa hydrophobic C-terminal domain; it does not include the 123 aa N-terminal region found in the native enzyme (Supplementary Data). Yet as shown in Table 1, for all 34 carbamates there is excellent correspondence between k_i values determined using rAgAChE-WT, and the homogenate of G3 strain *An. gambiae* (Ag G3 hmg). This close correspondence is highlighted graphically in Figure 2A, that plots $\log k_i(\text{Ag G3 hmg})$ vs $\log k_i(\text{rAgAChE-WT})$. As can be seen, the R^2 value for the correlation is 0.996, the slope is near unity (1.02) and the intercept is near zero (−0.02). Thus the large N-terminal domain in native AgAChE does not appear to affect the susceptibility of AgAChE to inhibition by carbamates. Consequently the high (100- to 500-fold) *An. gambiae* vs human inhibition selectivities reported previously for **1c**, **1d**, **2d** and **3d** based on rAgAChE-WT k_i values [7] are replicated when Ag G3 hmg k_i values are used to calculate the ratios (Table 1). The high selectivity of compound **1d** for inhibition of AgAChE over hAChE is also illustrated in Figure 3. The respective k_i values ($\text{mM}^{-1} \text{min}^{-1}$) derive from the slopes of plot of pseudo-first-order inactivation rate constants k_{obs} (min^{-1}) vs [**1d**]. As can be seen, the lines for rAgAChE-WT and Ag G3 hmg overlap and have a much higher slope than that of r hAChE. As discussed previously, **1c-d**, **2d** and **3d** share a common structural motif that confers high inhibition selectivity: a spacer atom at C2- of the benzene ring (O, S, CH_2), attached to a β -branched alkyl group. Although the key residue substitutions in AgAChE relative to hAChE responsible for this selectivity have not yet been identified, we did develop a 3D QSAR model that offers reasonable predictive power for $\log(\text{AgAChE}/\text{hAChE})$ selectivity [7].

3.2 Carbamate Inhibition of G119S mutant of AgAChE (recombinant and Akron homogenate)

Since the ideal insecticidal carbamate mosquitocide would also have good activity against *An. gambiae* bearing the G119S resistance mutation, we measured inactivation rate constants of both recombinant G119S AgAChE (rAgAChE-G119S) and the enzyme present in the homogenate of carbamate-resistant Akron strain *An. gambiae* (Ag Akron hmg). We have previously used PCR-RFLP analysis to verify the presence of the G119S mutation within the AChE of Akron strain *An. gambiae* [11]. Although the two recombinant enzymes differ only at residue 119, k_i values measured with rAgAChE-G119S are much (100- to 1000-fold) lower than those measured with rAgAChE-WT (Table 1). Only carbamate **6l** which very rapidly inactivates the WT construct (rAgAChE-WT, $k_i = 10,000 \pm 500 \text{ mM}^{-1}$

min⁻¹) inactivates the G119S mutant at a rate greater than 5 mM⁻¹ min⁻¹. Glycine119 is a key residue in the oxyanion hole, and replacement of its small side chain (H) with the hydroxymethyl side chain of serine appears to effectively hinder carbamoylation of the active site serine (S199 in *AgAChE*) [6, 10, 11]. It should be noted that k_i values measured with r*AgAChE*-G119S were very similar to those measured with Akron homogenate. A plot of log k_i (*Ag* Akron hmg) vs. log k_i (r*AgAChE*-G119S) gave an R² value of 0.984 (17 points, Figure 2B). Since the native enzyme present in Akron homogenate contains the 123 aa N-terminal domain, and our construct r*AgAChE*-G119S does not, these results again demonstrate again that large N-terminal domain in native *AgAChE* does not affect carbamate inhibition. At present the native function of this domain is not known, and we speculate that it may be involved in regulation of enzyme expression and/or localization. Sequence alignment reveals that three other vector mosquito AChEs (*Aedes albopictus*, *Aedes aegypti*, *Culex quinquefasciatus*) feature highly homologous, but slightly shorter N-terminal domains (93 vs 123 aa, Supplementary Data). Finally, the very small r*AgAChE*-G119S and Akron hmg k_i values seen for the aryl methylcarbamates in Table 1 suggest that this class of inhibitor is not well-suited to target carbamate-resistant *An. gambiae*. This point is illustrated for selective carbamate **1d** in Figure 3; inactivation of r*AgAChE*-G119S and *Ag* Akron hmg is barely perceptible, and is even slower than that of *hAChE* (see inset). Our recent report on pyrazol-4-yl methylcarbamates [11] indicates that small-core carbamates represent a viable strategy to rapidly inactivate r*AgAChE*-G119S and thus target AChE-resistant strain *An. gambiae* (e.g. Akron).

3.3. Correlation of carbamate *An. gambiae* (G3 strain) tarsal contact toxicity to WT inhibition potency and physicochemical parameters

We previously reported the tarsal contact toxicities of G3 strain *An. gambiae* for the carbamates listed in Figure 1, and noted that there is no simple correlation to r*AgAChE*-WT k_i values [7]. For example, in the 2-alkoxyphenyl series (**1b–d**, **7**, **9**), **9** (carbofuran) is the most rapid inactivator and has the lowest LC₅₀ value in the tarsal contact assays (16 ug/mL, cf. Tables 1 and 2). However, **7** (propoxur) is approximately 40-fold more toxic than **1d** despite their similar *AgAChE* k_i values (266 ± 9 and 255 ± 12 mM⁻¹ min⁻¹ respectively at r*AgAChE*-WT). Similarly, in the 3-alkylphenyl series (**6b,h–v**), **6l** is the most rapid *AgAChE* inactivator (k_i = 10,000 ± 500 mM⁻¹ min⁻¹ at r*AgAChE*-WT), and in the tarsal contact assay it is among the most toxic carbamates. However **6i** which is 11-fold slower than **6l** for inactivation of *AgAChE*, has very similar toxicity to **6l**. Overall, a plot of Log (LC₅₀) vs Log k_i (r*AgAChE*-WT, 23 points) gives a very poor correlation (R² = 0.16, Supplementary Data).

This finding indicates that factors other than enzyme inhibition potency strongly influence in vivo insecticidal efficacy of the carbamates. The role of metabolism in modulating insecticidal potency is well known [16], and must be partly responsible for the observed scatter. Metcalf noted in 1971 that housefly AChE IC₅₀ values of carbamate inhibitors did not correlate well to housefly LD₅₀ values, but exhibited improved correlation to LD₅₀ values measured in the presence of the insecticidal synergist piperonyl butoxide [17]. Other factors influencing insecticidal potency include adsorption, distribution, and excretion, and these are often correlated with basic physicochemical properties. We thus attempted to build a partial least square (PLS) prediction model for the Log (LC₅₀) based on the Log k_i (r*AgAChE*-WT) and a number of macroscopic and microscopic physicochemical descriptors that could be readily calculated or estimated. These included molecular weight, molecular volume, partition coefficient (LogP), solubility (LogS), and topological polar surface area (PSA); estimation of the latter four descriptors was performed in ICM (Table 2) [15]. Leave-one-out cross-validation was used to evaluate the models and avoid overtraining. The best model was obtained using only three terms: Log k_i (r*AgAChE*-WT), the molecular volume

(molVol), and polar surface area (molPSA). This model features an overall correlation R^2 value of 0.82, and a leave-one-out cross-validated R^2 value of 0.78 (Figure 4 and Supplementary Data). Further addition of physicochemical descriptors did not improve the model's performance. This model has the expected *negative* weighting for Log (k_i), i.e. increasing Log (k_i) decreases Log (LC_{50}). PSA also shows a negative weighting; as PSA increases, Log (LC_{50}) decreases. Finally molecular volume shows a positive weighting; the lower the volume, the lower the Log (LC_{50}) value. Thus in addition to having a high k_i value, toxicity is improved by lower molecular volume and higher PSA, at least within the limited ranges represented in this series. We speculate that these latter two properties affect exoskeleton and/or CNS barrier penetration by the carbamate, as they are known to play important roles in CNS drug ADME [18, 19].

4. Conclusion

The collection of inhibitors described herein (Figure 1) provides an excellent platform on which to characterize the principal ATCh-hydrolyzing enzymes in carbamate-susceptible (G3) and carbamate-resistant (Akron) strain *An. gambiae*. Measured k_i values for the susceptible G3 strain (*Ag* G3 hmg) exhibit an excellent correlation to those measured with our recombinant WT construct (r*Ag*AChE-WT, Table 1, Figure 2A), indicating that the large (123 aa) N-terminal domain present in native WT *Ag*AChE does not affect susceptibility to carbamates. Similarly, k_i values for the resistant Akron strain (*Ag* Akron hmg) exhibit a good correlation to those measured with our recombinant G119S construct (r*Ag*AChE-G119S, Table 1, Figure 2B), consistent with the presence of the G119S mutation in Akron strain *An. gambiae*. Finally, although G3 strain tarsal contact toxicities of the carbamates are not directly correlated to inhibition potency, a predictive model based on k_i , molecular volume, and polar surface area is presented (Figure 4).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

aa	amino acid
AChE	acetylcholinesterase
<i>Ag</i>AChE	<i>Anopheles gambiae</i> AChE
ATCh	acetylthiocholine
<i>h</i>AChE	human AChE
hmg	homogenate
IRS	indoor residual spraying
ITN	insecticide-treated net

PSA polar surface area

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Highlights

34 aryl methylcarbamates are examined for inhibition of AgAChE

Both WT and G119S carbamate-insensitive enzymes are examined.

Recombinant enzyme results are compared to those from mosquito homogenates

We show that the 123 aa N-terminal domain of AgAChE does not affect inhibition

A predictive model for carbamate toxicity to *An. gambiae* is presented.

a: $\text{R} = -\text{CH}_2\text{C}(\text{Me})=\text{CH}_2$; **b**: $\text{R} = -\text{CH}_2\text{CHMe}_2$; **c**: $\text{R} = (\pm)\text{-CH}_2\text{CHMeEt}$
d: $\text{R} = -\text{CH}_2\text{CHEt}_2$; **e**: $\text{R} = -\text{CH}_2\text{C-C}_5\text{H}_9$; **f**: $\text{R} = -\text{CH}_2\text{C-C}_6\text{H}_{11}$; **g**: $\text{R} = \text{Me}$
h: $\text{R} = \text{Me}$; **i**: $\text{R} = \text{Pr}$; **j**: $\text{R} = \text{Bu}$; **k**: $\text{R} = i\text{-Pr}$; **l**: $\text{R} = s\text{-Bu}$; **m**: $\text{R} = t\text{-Bu}$;
n: $\text{R} = -\text{CMe}_2\text{CF}_3$; **o**: $\text{R} = -\text{CMe}_2\text{Et}$; **p**: $\text{R} = -\text{CMe}(\text{CF}_3)\text{Et}$;
q: $\text{R} = -\text{CMe}_2\text{Pr}$; **r**: $\text{R} = -\text{CMeEt}_2$; **s**: $\text{R} = \text{SiMe}_3$; **t**: $\text{R} = \text{SiMe}_2\text{Et}$;
u: $\text{R} = \text{OCH}_2\text{CHEt}_2$; **v**: $\text{R} = (\text{CH}_2)_2\text{CHMe}_2$

Figure 1.
 Structures of synthesized (**1a-f**, **2a-d,g**, **3d**, **4h**, **5**, **6b,h-v**) and commercial (**7-10**) carbamates assayed.

Figure 2.

Comparison of Log k_i values of carbamates using mosquito homogenates and corresponding purified recombinant enzymes. A) log k_i (Ag G3 hmg) vs log k_i (rAgAChE-WT) B) log k_i (Ag Akron hmg) vs log k_i (rAgAChE-G119S). Data taken from Table 1.

Figure 3.

Plot of k_{obs} vs [carbamate] plot for compound **1d** at *AgAChE* (WT and G119S; recombinant and homogenate) and *rhAChE*. For clarity the data for **1d** at *rhAChE* and *AgAChE*-G119S (recombinant and Akron homogenate) are also plotted on expanded axes (inset).

Figure 4.

Plot of “leave-one-out cross-validated” predicted Log(LC₅₀) vs experimental Log(LC₅₀) for 23 carbamates listed in Table 2.

Table 1

Carbamate inactivation rate constants k_i for rAgAChE (WT & G119S), *An. gambiae* homogenates (G3 & Akron), and r*hAChE*.^a

Carbamate	rAgAChE-WT k_i (mM ⁻¹ min ⁻¹)	Ag G3 hmg k_i (mM ⁻¹ min ⁻¹) ^b	rAgAChE-G119S k_i (mM ⁻¹ min ⁻¹)	Ag Akron hmg k_i (mM ⁻¹ min ⁻¹) ^d	r <i>hAChE</i> k_i (mM ⁻¹ min ⁻¹)	WT selectivity ^e
1a	62.2 ± 2.0	76.6 ± 1.7	nd	nd	0.74 ± 0.05	84 ± 6
1b	52.8 ± 2.7	58.7 ± 1.3	<0.054 ± 0.015	<0.054 ± 0.033	0.65 ± 0.20	81 ± 5
1c	125 ± 2.7	136 ± 5	nd	nd	0.58 ± 0.05	220 ± 20 (230 ± 20)
1d	255 ± 12	311 ± 15	<0.060 ± 0.015	<0.039 ± 0.011	0.48 ± 0.12	530 ± 130 (640 ± 160)
1e	9.83 ± 0.33	11.2 ± 0.3	nd	nd	0.28 ± 0.02	35 ± 3
1f	0.48 ± 0.02	0.51 ± 0.03	nd	nd	0.21 ± 0.04	2.3 ± 0.4
2a	414 ± 18	515 ± 8	nd	nd	7.46 ± 0.27	55 ± 3
2b	526 ± 11	620 ± 12	<0.031 ± 0.016	<0.031 ± 0.007	6.53 ± 0.24	81 ± 3
2c	806 ± 15	1,040 ± 40	nd	nd	16.8 ± 0.4	48 ± 1
2d	1,850 ± 100	1,740 ± 35	<0.046 ± 0.019	<0.086 ± 0.015	14.5 ± 1.5	130 ± 15 (120 ± 13)
2g	68.9 ± 1.6	66.7 ± 1.1	nd	nd	5.05 ± 0.26	14 ± 1
3d	75.3 ± 2.7	75.3 ± 3.6	nd	nd	0.75 ± 0.03	100 ± 5 (100 ± 6)
4h	1.31 ± 0.07	0.99 ± 0.02	<0.018 ± 0.007	nd	0.24 ± 0.06	5.4 ± 1.5
5	2.75 ± 0.26	1.74 ± 0.07	<0.029 ± 0.008	nd	0.20 ± 0.06	14 ± 4
6b	155 ± 6	187 ± 3	<0.19 ± 0.04	<0.20 ± 0.02	5.47 ± 0.30	28 ± 2
6h	17.2 ± 0.3	15.4 ± 0.5	<0.139 ± 0.006	<0.165 ± 0.012	0.52 ± 0.08	33 ± 5
6i	881 ± 17	1020 ± 30	1.37 ± 0.03	2.02 ± 0.06	63.7 ± 1.9	14 ± 1
6j	741 ± 5	701 ± 16	1.68 ± 0.05	2.22 ± 0.09	32.1 ± 0.6	23 ± 1
6k	3,270 ± 70	2,900 ± 60	1.87 ± 0.07	2.58 ± 0.15	383 ± 19	8.5 ± 0.5
6l	10,000 ± 500	13,600 ± 600	15.7 ± 0.4	18.4 ± 1.3	1,910 ± 110	5.3 ± 0.4
6m	1,510 ± 110	1,710 ± 20 ^c	0.4 ± 0.03 ^c	0.65 ± 0.06 ^c	126 ± 3	12 ± 1
6n	1,580 ± 40	1,870 ± 30	nd	nd	139 ± 6	11 ± 1
6o	7,600 ± 300	8,520 ± 230	nd	nd	784 ± 28	9.7 ± 0.5
6p	3,270 ± 70	3,620 ± 60	nd	nd	375 ± 6	8.7 ± 0.2
6q	6,690 ± 240	6,930 ± 80	nd	nd	1,360 ± 40	4.9 ± 0.2
6r	5,280 ± 170	6,090 ± 170	nd	nd	553 ± 13	9.5 ± 0.4
6s	648 ± 29	924 ± 30	nd	nd	67.8 ± 2.6	9.6 ± 0.6

Carbamate	rAgAChE-WT k_i ($\text{mM}^{-1} \text{min}^{-1}$)	Ag G3 hmg k_i ($\text{mM}^{-1} \text{min}^{-1}$) ^b	rAgAChE-G119S k_i ($\text{mM}^{-1} \text{min}^{-1}$)	Ag Akron hmg k_i ($\text{mM}^{-1} \text{min}^{-1}$) ^d	rhAChE k_i ($\text{mM}^{-1} \text{min}^{-1}$)	WT selectivity ^e
6t	1,510 ± 70	1,610 ± 30	nd	nd	110 ± 6	14 ± 1
6u	28.8 ± 0.4	43.9 ± 1.0	0.529 ± 0.025	0.754 ± 0.027	4.96 ± 0.58	5.8 ± 0.7
6v	272 ± 10	318 ± 10	<0.122 ± 0.005	<0.163 ± 0.004	5.15 ± 0.20	53 ± 3
7 (propoxur)	266 ± 9	323 ± 8 ^c	<0.037 ± 0.007 ^c	<0.040 ± 0.005 ^c	17.0 ± 0.4	16 ± 1
8 (bendiocarb)	839 ± 22	865 ± 41 ^c	<0.055 ± 0.007 ^c	<0.053 ± 0.008 ^c	111 ± 5	7.6 ± 0.4
9 (carbofuran)	2,620 ± 150	2,760 ± 110 ^c	<0.044 ± 0.020 ^c	<0.069 ± 0.010 ^c	428 ± 12	6.1 ± 0.4
10 (carbaryl)	386 ± 10	343 ± 8 ^c	<0.037 ± 0.014 ^c	<0.049 ± 0.015 ^c	15.4 ± 0.4	25 ± 1

^aMeasured at 23 ± 1 °C, pH 7.7, 0.1% (v/v) DMSO. Values at rAgAChE-WT and rhAChE were reported previously [7].

^bG3 strain *An. gambiae* homogenate; these mosquitoes carry WT AgAChE and possess a carbamate-susceptible phenotype.

^cThis k_i value reported previously [11].

^dAkron strain *An. gambiae* homogenate; these mosquitoes carry G119S mutant AgAChE and possess a carbamate-resistant phenotype.

^eCalculated as $k_i(\text{rAgAChE-WT})$ divided by $k_i(\text{rhAChE})$; values in parenthesis are calculated using the corresponding $k_i(\text{An. gambiae G3 homogenate})$ values. Error in the selectivity is determined by standard propagation of error [20].

G3 strain *Anopheles gambiae* tarsal contact toxicity and physicochemical parameters of select aryl methylcarbamates.

Table 2

Carbamate	<i>An. gambiae</i> G3 LC ₅₀ μg/mL (95% CI) ^a	MW	molLogP ^e	molLogS ^e	molPSA ^e (Å ²)	molVol ^e (Å ³)
1b	290 (271–309)	223.1	2.47	–2.62	40.7	230
1c	445 (396–503)	237.1	3.05	–3.00	40.7	248
1d	1,500 ^b	251.2	3.62	–3.15	40.7	266
2a	317 (256–389)	237.1	2.86	–3.36	33.1	245
2b	212 (145–279)	239.1	2.92	–3.53	33.1	238
2c	800 ^c	253.1	3.50	–3.96	33.1	255
2d	1,500 ^b	267.1	4.08	–4.15	33.1	274
4h	712 (539–887)	165.1	1.50	–2.30	33.1	167
6i	41 (35–46)	193.1	2.60	–3.22	33.1	204
6j	237 (217–257)	207.1	3.08	–3.59	33.1	222
6l	31 (29–34)	207.1	2.92	–3.48	33.1	219
6m	37 (14–60)	207.1	2.83	–3.69	33.1	232
6n	61 (43–124)	261.1	3.47	–3.76	33.1	244
6o	68 (64–72)	221.1	3.35	–4.20	33.1	250
6p	115 (95–147)	275.1	3.99	–4.16	33.1	264
6q	236 (210–259)	235.2	3.84	–4.45	33.1	268
6r	200 ^d	235.2	3.87	–4.40	33.1	268
6s	169 (162–176)	223.1	1.65	–2.35	33.1	223
6v	342 (267–472)	221.1	3.40	–4.24	33.1	237
7 (propoxur)	39 (32–45)	209.1	1.93	–2.04	40.0	212
8 (bendiocarb)	16 (14–17)	223.1	2.66	–3.28	46.7	231
9 (carbofuran)	16 (11–25)	221.1	2.41	–3.21	39.5	241
10 (carbaryl)	42 (32–55)	201.1	2.55	–3.48	32.9	197

^aMosquitoes were exposed (1 h) to dried filter papers previously treated with ethanolic solutions of carbamates; mortality was recorded after 24 h. LC₅₀ values derive from the concentrations of inhibitor used to treat the paper and were reported previously [7].

^bEstimated, based on 27% mortality @ 1,000 ug/mL.

^cEstimated, based on 70% mortality @ 1,000 ug/mL.

d_p Estimated, based on 72% mortality @ 250 ug/mL.

e_{ICM} [15] was used to calculate Log partition coefficient (molLogP), Log solubility (molLogS), topological polar surface area (molPSA), and molecular volume (molVol).