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Carbamate and pyrethroid resistance in the akron strain of *Anopheles gambiae*

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ABSTRACT

Insecticide resistance in the malaria vector, *Anopheles gambiae*, is a serious problem, epitomized by the multi-resistant Akron strain, originally isolated in the country of Benin. Here we report resistance in this strain to pyrethroids and DDT (13-fold to 35-fold compared to the susceptible G3 strain), but surprisingly little resistance to etofenprox, a compound sometimes described as a "pseudo-pyrethroid." There was also strong resistance to topically-applied commercial carbamates (45-fold to 81-fold), except for the oximes aldicarb and methomyl. Biochemical assays showed enhanced cytochrome P450 monooxygenase and carboxylesterase activity, but not that of glutathione-*S*-transferase. A series of substituted α,α,α,-trifluoroacetophenone oxime methylcarbamates were evaluated for enzyme inhibition potency and toxicity against G3 and Akron mosquitoes. The compound bearing an unsubstituted phenyl ring showed the greatest toxicity to mosquitoes of both strains. Low cross resistance in Akron was retained by all analogs in the series. Kinetic analysis of acetylcholinesterase activity and its inhibition by insecticides in the G3 strain showed inactivation rate constants greater than that of propoxur, and against Akron enzyme inactivation rate constants similar to that of aldicarb. However, inactivation rate constants against recombinant human AChE were essentially identical to that of the G3 strain. Thus, the acetophenone oxime carbamates described here, though potent insecticides that control resistant Akron mosquitoes, require further structural modification to attain acceptable selectivity and human safety.

1. Introduction

Success in reducing malaria mortality and morbidity due to expansive use of pyrethroid-treated ITNs in Africa is well documented [1–3]. Although IRS has also been widely used [4], ITNs provide superior protection against malaria through reduced mosquito survival, feeding success and feeding frequency, all leading to reduced density of vector populations [5–8]. Because pyrethroids are the only insecticides approved by the WHO [9] and used for deployment in ITNs, continued use has led to the emergence of resistance, which is now widespread across Africa [10,11].

Carbamate-treated nets have shown great efficacy in controlling mosquitoes, even in pyrethroid-resistant populations [12–14]. However, the existing carbamates, though effective in killing mosquitoes, have generated concern regarding human toxicity [12]. Currently available carbamates are also compromised due to the existence of MACE resistant mosquitoes [15], which express a modified acetylcholinesterase having a G119S mutation [16]. There have been few chemical insecticides developed for wide-scale public health use in the last 30 years, because of reduced investment by industry, compared to agricultural pesticides [11]. Thus, there is a need to develop new insecticides that are less toxic to humans and effective on mosquitoes to be used as alternatives, in mixtures and/or rotations with pyrethroids.

In previous work, a series of structural modifications was undertaken to methylcarbamate insecticides in order to achieve high selectivity (up to 500-fold) for inhibition of WT AgAChE relative to hAChE [17]. This selective inhibition should imbue the compounds with low human toxicity, and indeed we found that two of the highly

Abbreviations: AChE, acetylcholinesterase; *Ag*AChE, wild type *Anopheles gambiae* acetylcholinesterase; G119S *Ag*AChE, MACE-resistant *Anopheles gambiae* acetylcholinesterase; *h*AChE, human recombinant acetylcholinesterase; IRS, indoor residual sprays; ITN, insecticide treated net; LC_{50}, concentration that killed 50% of treated subjects in the WHO paper assay; LD_{50}, dose that killed 50% of treated subjects; α-NA, α-naphthyl acetate; β-NA, β-naphthyl acetate; RR, resistance ratio.

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selective compounds possessed low mouse oral toxicity, with LD_{50} values >1500 mg/kg [18]. However these compounds were not toxic (RRs > 100) to the multiply resistant Akron strain of *Anopheles gambiae* in tarsal contact assays [19]. Further structural modifications of these carbamates demonstrated that those with a pyrazole core killed Akron strain mosquitoes with RR values that were typically less than 2-fold [19]. Enhanced efficacy was attributed to their reduced molecular volume compared to typical benzene-core carbamates, which allowed them to enter the more crowded G119S active site and effectively inhibit G119S *Ag*AChE [19].

In the present study, we document resistance ratios to pyrethroids, DDT, and carbamates in the Akron strain of *An. gambiae*, originally isolated in Benin, west Africa [20], and characterize metabolic resistance in this strain using biochemical assays. Knowing that the oxime carbamate aldicarb is toxic to the resistant Akron strain [19], we also prepared and evaluated experimental trifluoroacetophenone oxime carbamates **1**–**8** in enzyme assays and for tarsal contact toxicity to susceptible G3 and Akron strains of *An. gambiae*. Results presented here offer insights into the design of carbamates that could control carbamate-resistant mosquito populations, thereby guiding future discovery of public health mosquitocides.

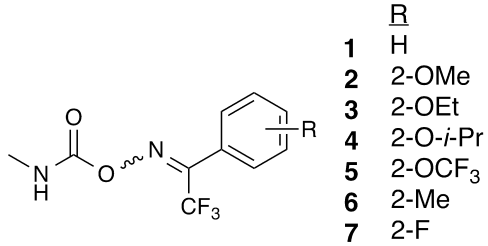
2. Materials and methods

2.1. Insects

Susceptible G3 (MRA-112) and Akron strains (MRA-913, isolated in Benin) were obtained from BEI Resources through the CDC-MR4 program [20]. As supplied by BEI, Akron mosquitoes have documented knockdown resistance (kdr) to pyrethroids (L1014F) and the G119S mutation (ace-1R), which confers resistance to carbamates. Mosquito colonies were maintained at the Fralin Life Science Center insectary, Virginia Tech, Blacksburg, VA 24061, USA, or at the Emerging Pathogens Institute, University of Florida, Gainesville, FL 32610, USA, under 75% relative humidity and 25 °C, with a 12/12 dark and light cycle.

2.2. Chemicals

Samples of DDT, pyrethroids, and established carbamate insecticides were obtained from commercial sources, except terbam, which was synthesized as previously described [17]. Experimental trifluoroacetophenone oxime carbamates (**1**–**8**, Fig. 1) were synthesized and purified to >95% purity as per published procedures [21,22]. These compounds were isolated as chromatographically inseparable mixtures of the (*E*)- and (*Z*)-oxime isomers. In all cases mass spectrometric and ^{1}H, ^{13}C, and ^{19}F NMR spectroscopic data were consistent with the assigned structures. Absolute ethanol used as a bioassay solvent was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO).



	R
1	H
2	2-OMe
3	2-OEt
4	2-O-*i*-Pr
5	2-OCF_3
6	2-Me
7	2-F
8	4-F

Fig. 1. Chemical structures of the experimental α,α,α-trifluoroacetophenone oxime carbamates (**1**–**8**) investigated in this study. These compounds were prepared and studied as mixtures of the (*E*)- and (*Z*)-isomers.

2.3. Topical toxicity assays

The method of Pridgeon et al. [23] was used for topical application assays, with slight modifications. Briefly, mosquitoes were chilled on ice for 3 min, during which 200 nL of chemical (dissolved in 95% Ethanol) was applied onto the pronotum using a handheld microapplicator (Hamilton Co., Reno, NV, USA). For each chemical, 5 doses with 10 mosquitoes per dose was repeated 2–3 times using different batches of mosquitoes. A solvent-only treatment was included in each experiment as a negative control. Mosquitoes were transferred into paper cups covered with netting and supplied with sugar water for 24 hours, after which mortality was recorded. Mortality data from the triplicate experiments were pooled and analyzed by log-probit using Poloplus (LeOra Software, Petaluma, CA, USA) or SAS (SAS Institute, Cary, NC, USA) software to determine 24 hr LD_{50} values.

2.4. WHO paper toxicity assays

The 2006 WHO protocol [24] was used to assess contact toxicity of the chemicals on 2–5 day old *An. gambiae* non-blood fed females, with some slight modifications. A range finding assay was performed with 1, 0.5 and 0.1 mg/mL single paper treatments after which a subsequent detailed assay followed to determine actual LC_{50} values. For each chemical, 5 concentrations were prepared, and 2 mL of each concentration was applied to a 12 cm × 15 cm paper, with 95% ethanol as solvent. Mosquitoes were chilled for 3 min on ice, after which 25 females were placed in the WHO cylindrical holding chamber to acclimatize for one hour. Mosquitoes were then moved to the treatment chamber wrapped on the inside with treated paper and left for 1 hr, after which they were transferred back to the holding chamber and maintained on 10% sugar solution for 24 hrs. Each concentration was repeated in triplicate using different batches of mosquitoes to minimize inter-batch variability, with an ethanol-only treated negative control. Mortality was recorded 24 hr after treatment and experiments having control mortality >20% were discarded. Mortality data were corrected for control mortality and analyzed by log-probit using Poloplus (LeOra Software, Petaluma, CA, USA) or SAS (SAS Institute, Cary, NC, USA) software to generate the 24 hr LC_{50} for each chemical.

2.5. Detoxication enzyme activity assays

General esterase activity was determined in adult mosquitoes according to the method described by Anderson and Zhu [25]. Each sample was homogenized in ice-cold 0.1 M sodium phosphate (pH 7.8) containing 0.3% (v/v) Triton X-100. After the homogenates were centrifuged at 10,000 × *g* for 10 min at 4 °C, the supernatants were used as the enzyme source for measuring the detoxication enzyme activities. General esterase activity was determined with α-NA and β-NA as substrates. The absorbance was read using a SpectraMax M2 multimode microplate reader (Molecular Devices, Inc., Sunnyvale, CA) at 600 and 560 nm for α-NA and β-NA, respectively. Glutathione *S*-transferase activity was determined using 1-chloro-2, 4-dinitrobenzene (CDNB) as a substrate. The conjugation of glutathione toward CDNB was determined by recording the change in absorbance at 340 nm for CDNB for 1 min at 10-sec intervals. Non-enzymatic controls were performed in parallel to correct for non-enzymatic conjugation. Cytochrome P450-dependent *O*-deethylation activity was determined according to the method of Anderson and Zhu [25] using 7-ethoxycoumarin as a substrate. The relative fluorescence units were measured using a multimode microplate reader at 465 nm emission wavelength, and excitation at 390 nm. Total protein in each sample preparation was determined using the method of Smith et al. [26] with bovine serum albumin as a standard. The measurement was performed on a multimode

microplate reader at 560 nm. The substrates and reagents were purchased from Sigma Aldrich Chemical Co.

2.6. Acetylcholinesterase inhibition studies

Crude enzyme preparations were obtained from homogenates of whole G3 or Akron mosquitoes as previously described [19]. Recombinant *h*AChE (product # C1682) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Inhibition of AChEs by carbamate insecticides was assessed by measuring apparent second-order rate constants k_i ($mM^{-1}\,min^{-1}$) for inactivation of the enzymes. A standard progressive inactivation approach was used [17,19] in which enzymes were incubated with different concentrations of carbamates for differing times before measuring residual enzyme activity (v/v_0). Enzymes were incubated with typically five concentrations of inhibitors (and a vehicle control) for up to 6 minutes at approximately 1 min intervals. For propoxur, the Akron homogenate enzyme inhibition was very low after 10 min at 10 μM, and thus measurements were extended to 60 min (10 min intervals). Residual activities v/v_0 are the ratio of the rate in the presence of an inhibitor to a time-matched solvent control, and were used to calculate pseudo first-order rate constants k_{obs} for inactivation at three or more inhibitor concentrations ([I]). Plots of k_{obs} vs [I] were then constructed, where the slope of the unconstrained linear fit is the apparent second-order rate constant k_i ($mM^{-1}\,min^{-1}$) for inactivation. The error in k_i is estimated as the standard error in the slope of this plot. Inhibitor concentrations [I] were chosen to be low enough to be within the domain where a plot of k_{obs} vs [I] was linear. For fast-inactivating carbamates, (such as **2** or **3** on G3 homogenate), signs of saturation behavior occurred above [I] = 0.2 μM. Saturation behavior is expected for a two-step mechanism of inhibition [27]. Enzymatic sensitivity ratios and *Ag*/*h* selectivity for inhibition were calculated from the measured k_i values; the error in these ratios was determined using a standard propagation of error method [28].

3. Results

3.1. Toxicity of pyrethroids and DDT to An. gambiae strains

The topical toxicities of DDT and three pyrethroids against the G3 and Akron strains of *An. gambiae* are shown in Table 1. For both strains, sensitivity to all insecticides was deltamethrin > permethrin > etofenprox > DDT. Compared to LD_{50} values in the susceptible strain (G3), deltamethrin was 13-fold more toxic than permethrin, which was 20-fold more active than etofenprox, which was in turn about 3-fold more active than DDT. The multiply resistant strain Akron conferred significant levels of resistance to DDT and pyrethroid insecticides. It was highly resistant to permethrin (>30-fold) and moderately resistant to deltamethrin and DDT (≥13-fold). In contrast, there was little or no resistance to etofenprox (RR = 1.4). Additional screens with mosquitoes reared from the same cohort of eggs confirmed these findings. Application of 1 ng doses of permethrin to Akron resulted in 70% and 50% mortality in two replicates of 10 females each. These levels of mortality corresponded closely with the calculated LD_{50} of permethrin in the Akron strain (Table 1).

3.2. Toxicity of carbamates to An. gambiae strains

Data for toxicity by topical application and resistance ratios of some selected commercial carbamates to the susceptible G3 strain and the resistant Akron strain are summarized in Table 2. The benzene and naphthalene core methylcarbamates (propoxur, bendiocarb, carbofuran, carbaryl, terbam) were all toxic to susceptible G3 strain *An. gambiae*. In this series, the most toxic were carbofuran and bendiocarb; propoxur, carbaryl, and terbam were less toxic, with LD_{50} values between 3 and 5 ng/female. The two commercial oxime carbamates evaluated (aldicarb and methomyl) were also the most toxic to the G3 strain, by factors of 6- to 10-fold. This pattern of activity was roughly maintained in studies of Akron strain of *An. gambiae*. The oxime carbamates aldicarb and methomyl were again the most toxic, and among the benzene/naphthalene-core carbamates, bendiocarb and methomyl proved more toxic than propoxur, terbam, and carbaryl. A most striking difference can be seen in these two chemical series, where the expected carbamate resistance occurred with the benzene/naphthalene-core carbamates (RR values of 45–81), but for the oxime carbamates, RR values were near unity.

3.3. Detoxication enzyme activities in An. gambiae strains

We compared the general esterase, glutathione *S*-transferase, and cytochrome P450 monooxygenase activities between the two strains of mosquitoes (Fig. 2). The general esterase activity of G3 mosquitoes was 19.2% and 20.0% less than that of the Akron mosquitoes when α-NA and β-NA were used as substrates, respectively ($P < 0.05$). The cytochrome P450 *O*-deethylation activity of the G3 mosquitoes was also 23.5% less than that of the G3 mosquitoes ($P < 0.05$). In contrast, the glutathione *S*-transferase activity of G3 did not differ from that of the Akron mosquitoes, although there was greater assay variability in these measurements.

3.4. AChE inhibition and toxicity of α,α,α- trifluoroacetophenone oxime methylcarbamates

As shown in Table 3, experimental methylcarbamates **1**–**8** potently inhibited *Ag*AChE present in G3 homogenates, with k_i values ranging from 44% (**8**) to 450% (**2**) of that of propoxur (Table 3). Unlike propoxur, however, experimental methylcarbamates **1**–**8** showed

Table 1
Synergism and resistance to topical treatments of pyrethroids and DDT in strains of *An. gambiae*. Superscript letters designate the results of a T-test comparison of synergized vs un-synergized LD_{50} values for each compound, where entries labeled by different letters are significantly different ($p < 0.05$).

Compound	G3[a]	Akron[a]		RR[b]
Permethrin	0.032 (0.019–0.045)	1.05	(0.82–1.3)	33
Deltamethrin	0.0025 (0.0008–0.004)	0.043	(0.03–0.05)	17
Etofenprox	0.20 (0.14–0.33)	0.39	(0.38–0.52)	1.4
DDT	2.1 (0.7–3.4)	27.8 (18–37)		13

[a] LD_{50}, ng/mg (95% confidence limits).
[b] RR: resistance ratio for G3 = LD_{50} Akron strain/LD_{50} G3 strain.

Table 2
Toxicity of commercialized *N*-methylcarbamates to susceptible (G3) and resistant (Akron) strains of *Anopheles gambiae*.

Carbamate	G3[a]	Akron[a]	RR[b]
Propoxur	3.2 (2.4–4.2)[c]	201 (138–295)	63
Terbam	4.5 (3.6–5.4)[c]	223 (202–245)	50
Carbaryl	4.1 (3–5)[c]	186 (131–253)	45
Bendiocarb	0.74 (0.52–0.97)[c]	35 (25–48)	47
Carbofuran	0.85 (0.7–1.1)[c]	69 (48–95)	81
Aldicarb	0.52 (0.4–0.74)	0.61 (0.41–1.2)	1.2
Methomyl	0.48 (0.35–0.64)	0.67 (0.52–0.97)	1.4

[a] Topical LD_{50} values are ng/insect (95% confidence limits), essentially ng/mg for the size insects reared for this study.
[b] RR is the resistance ratio: LD_{50} Akron/LD_{50} G3.
[c] Topical LD_{50} values taken from Hartsel et al. [17].

Table 3
AChE inhibition and toxicity of propoxur and experimental α, α, α-trifluoroacetophenone oxime carbamates (**1**–**8**) on G3 and Akron strain *Anopheles gambiae*. Comparative data for propoxur and aldicarb taken from Wong et al. [19]].

Compound (phenyl-R)[a]	G3 AChE[b]	Akron AChE[b]	*h*AChE[b]	G3 LC_{50}[c]	Akron LC_{50}[c]	RR[d]
Propoxur	323 ± 8 {19}[e]	0.040 ± 0.005 {8000}[f]	17.0 ± 0.4	39 (32–45)	>5000	>128
Aldicarb	13.6 ± 0.3 {4.7}	3.15 ± 0.1 {4.3}	2.9 ± 0.04	70 (66–74)	32 (30–55)	0.5
1 (H)	525 ± 32 {2.8}	9.87 ± 0.6 {53}	188 ± 8	91 (87–96)	332 (309–353)	4
2 (2-OMe)	1470 ± 20 {0.93}	18.9 ± 0.5 {78}	1,580 ± 40	131 (116–145)	643 (613–674)	5
3 (2-OEt)	957 ± 17 {0.75}	11.9 ± 0.5 {80}	1,280 ± 30	275 (207–338)	625 (558–700)	2.3
4 (2-O-*i*-Pr)	620 ± 12 {0.86}	15.6 ± 0.6 {40}	719 ± 35	586 (562–614)	40% @ 500 μg mL^{-1}	≈1
5 (2-OCF_3)	559 ± 39 {0.74}	18.9 ± 1.0 {30}	749 ± 35	457 (438–475)	939 (882–1061)	2.2
6 (2-Me)	1360 ± 60 {1.7}	27.0 ± 0.8 {50}	792 ± 40	131 (120–145)	242 (186–316)	1.8
7 (2-F)	271 ± 13 {2.5}	5.19 ± 0.6 {52}	107 ± 10	314 (284–345)	693 (653–733)	2.2
8 (4-F)	144 ± 4 {1.3}	2.97 ± 0.1 {48}	108 ± 8	334 (300–365)	580 (554–605)	1.7

[a] Compound tested, with R group defined for experimental oxime carbamates as shown in Fig. 1.
[b] AChE k_i values (mM^{-1} min^{-1}), where G3 AChE and Akron AChE represent enzymes in homogenate of G3 strain expressing *Ag*AChE; and Akron strain expressing G119S *Ag*AChE, respectively, and *h*AChE.
[c] LC_{50} values are μg/mL (95% confidence limits).
[d] RR is the lethality resistance ratio: Akron LC_{50}/G3 LC_{50}.
[e] {AChE selectivity ratio} in the G3 AChE column is calculated as G3 AChE k_i/rhAChE k_i.
[f] {AChE resistance ratio} in the r*Ag*G119S column is calculated as r*Ag*WT k_i/r*Ag*G119S k_i.

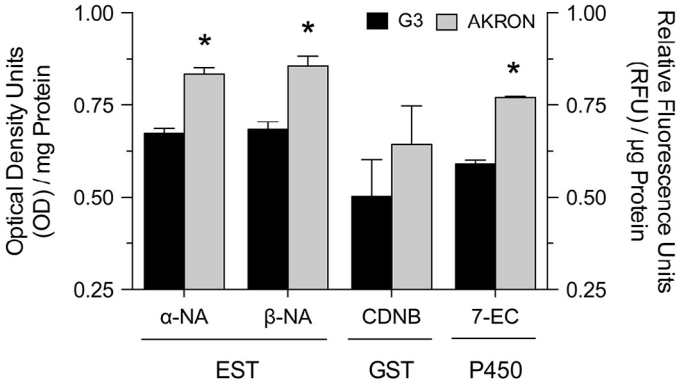


Fig. 2. Comparison of general esterase (EST), glutathione-*S*-transferase (GST), and cytochrome P450-dependent *O*-deethylation (P450) activities of insecticide-susceptible (G3) and -resistant (Akron) mosquitoes. Vertical bars indicate standard errors of the mean ($n = 4$), asterisks (*) denote means that are significantly different between the two mosquito strains (two-tailed *t*-test, $P < 0.05$). α-naphthyl acetate (α-NA), β-naphthyl acetate (β-NA), 1-chloro-2, 4-dinitrobenzene (CDNB), 7-ethoxycoumarin (7-EC).

measurable inhibition of G119S *Ag*AChE present in Akron homogenates. Unfortunately, like aldicarb itself, compounds **1**–**8** have very poor selectivity for inhibition of *Ag*AChE over *h*AChE (Table 3).

All of the experimental methylcarbamates **1**–**8** proved toxic to the G3 strain of *An. gambiae* in the WHO paper contact assay (Table 3), with LC_{50} values ranging from 91 μg/mL (**1**) to 586 μg/mL (**4**). These compounds thus have toxicities ranging from 43% to 7% of propoxur. Additionally, as anticipated from their inhibition of the G119S *Ag*AChE present in Akron homogenates, these compounds were toxic to the Akron strain. LC_{50} values varied over a narrow 4-fold range: the most toxic compound was **6**, with an LC_{50} value (242 μg/mL) roughly 8-fold higher than that of aldicarb. In contrast, propoxur had no toxicity to the Akron strain at the highest concentration tested (5000 μg/mL), and this test was used to re-confirm carbamate resistance in this strain in the present study. RR values for these compounds range from 1 (**4**) to 5 (**1**), as might be expected from the oxime methylcarbamate toxophore; for reference, the RR value of aldicarb was 0.5 (Table 3).

4. Discussion

To our knowledge, the present study is the first to report the LD_{50}s and associated resistance ratios of the G3 and Akron strains of *An. gambiae* to pyrethroid insecticides. The topical LD_{50} value for permethrin we observed for G3 *An. gambiae* was similar to that observed for susceptible *Aedes aegypti* of 0.049 ng/mg [23], but significantly less (32-fold) than the 1.02 ng/mg reported for the susceptible Kisumu strain of *An. gambiae* [29]. As documented previously, the Akron strain carries a *kdr* mutation [20] and mosquitoes more recently collected from the Akron region of Benin were shown to possess up-regulated P450 monooxygenase expression levels of about 2-fold [30], consistent with but greater in magnitude than the enhanced levels of model substrate metabolism we observed (Fig. 2). Accordingly, the resistance against deltamethrin and permethrin likely represents effects of both target site insensitivity and metabolism, although enhanced metabolism of model substrates is not synonymous with increased insecticide metabolism. Additional experiments are required to characterize the effect of elevated P450 and carboxylesterase activity on pyrethroid metabolism in the Akron strain, including the enzyme isoforms involved. The resistance observed with DDT would appear to be due to target site insensitivity or other mechanisms and not metabolic detoxication, given the apparent lack of elevated GST activity (Fig. 2). The absence of any cross resistance to etofenprox is puzzling, given that this compound usually shows less toxicity to strains having oxidase and kdr mechanisms [31]. Additional experiments are needed to clarify this discrepancy.

Compared to the pyrethroids, there were larger resistance ratios in Akron expressed against commercial carbamates, excluding oximes. This result is most likely due to the MACE mechanism. The G119S mutation is known to reduce the inactivation rate of AChE by standard carbamates [16]. Similarly, low levels of cross resistance were observed to aldicarb at the enzyme level [32], consistent with the low cross resistance observed to mosquitoes here. A previous study by Wong et al. [19] reported low cross resistance in Akron mosquitoes to aldicarb in the WHO paper assay. The high toxicity of aldicarb to Akron mosquitoes is probably due to better fit into the active site, and perhaps enhanced bioactivation to more toxic sulfone and sulfoxide derivatives, *in vivo*. However, this study also found high levels of resistance to methomyl in the WHO paper assay, which was a puzzling finding, given its structural similarity to aldicarb and now the lack of cross resistance observed in topical treatments (Table 2). To check the integrity of the Akron strain, a batch of mosquitoes was exposed as larvae to a selection concentration of 10 ppm, which is about 60× the LC_{50} in susceptible G3 larvae [33]. This treatment killed about half the larvae and the survivors were subsequently tested as adult females, at which point we observed 30% mortality at 800 μg/mL, which is substantial resistance, but less than that observed

previously [19]. Apparently, the paper transfer or other pharmacokinetic processes of methomyl delivery to the CNS in Akron mosquitoes prevents significant expression of toxicity via that route, and these factors are rendered moot by topical application. Note that methomyl has a reported log P value of 0.6, aldicarb 1.13, and the other commercial carbamates 1.5–2.4 [34], indicating that methomyl is by far the most polar compound studied. Perhaps additional carbamate selection of the Akron strain would restore the levels of resistance on paper observed by Wong et al. [19]. Otherwise, the high levels of cross resistance in the WHO paper assay previously observed for carbamates [19] were generally matched by those following topical treatment (Table 2).

Trifluorinated acetophenone oxime carbamates displayed enzyme inhibition and toxicities to G3 similar to that of propoxur. In a limited series of mostly *ortho* position substituents (Table 3), those having alkoxy groups gave increased enzyme potency, but did not improve toxicity compared to the un-substituted phenyl ring (**1**). To some extent, these observations of positional substitution at the phenyl ring of acetophenone oxime carbamates agree with previous findings that *ortho* alkoxy aryl substitutions are favorable for insecticidal activity [35,36]. Fluorination of the phenyl ring at ortho (**7**) or para positions (**8**) had a negative effect on enzyme potency, but less of a reduction in toxicity. Presumably, a fluorine-mediated protection of the phenyl ring from metabolic detoxication (P450) was therefore achieved with these compounds. This proposed effect was not operative in **5**, which displayed a k_i value equal to **1**, but about 5-fold less toxicity to G3, and the lack of good correlation between *in vitro* enzyme potency and *in vivo* toxicity was characteristic of these compounds. Correlation should be enhanced by using synergized toxicity measurements. Despite the loss in toxicity observed with phenyl substituents, the compounds in this series showed RR values in Akron near unity. The toxicity of aldicarb to Akron provides a useful benchmark for comparisons of enzyme inhibition and toxicity for the acetophenone oximes: k_i values ranged from 100% of aldicarb (**8**) to 900% of aldicarb (**6**). Thus, the oxime carbamate toxophore of compounds **1**–**8** allows them to successfully engage the catalytic serine in the crowded active site of G119S *Ag*AChE. Unfortunately these compounds also had poor selectivity with respect to *h*AchE. Thus, acetophenone oximes would require further structural modification to attain acceptable selectivity and human safety prior to scale up for mosquitocide development.

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