Supporting Information for the Article entitled

Re-engineering aryl methylcarbamates to confer high selectivity for inhibition of *Anopheles gambiae* vs human acetylcholinesterase

Joshua A. Hartsel[a], Dawn M. Wong[a], James M. Mutunga[b], Ming Ma[a], Troy D. Anderson[b], Ania Wysinski[b], Rafique Islam,[c] Eric A. Wong[d], Sally L. Paulson[b], Jianyong Li[e], Polo C. H. Lam[f], Maxim M. Totrov[f], Jeffrey R. Bloomquist[b,c], Paul R. Carlier[a,*]

[a]*Department of Chemistry, Virginia Tech, Blacksburg, VA, 24061, USA*
[b]*Department of Entomology, Virginia Tech, Blacksburg, VA, 24061, USA*
[c]*Departments of Entomology and Nematology, Emerging Pathogens Institute, University of Florida, Gainesville, FL 32610, USA*
[d]*Department of Animal and Poultry Science, Virginia Tech, Blacksburg, VA, 24061, USA*
[e]*Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061, USA*
[f]*Molsoft LLC, 11199 Sorrento Valley Road, San Diego, CA, 92121, USA*

Table of Contents

A. Synthetic procedures and analytical characterization data for new compounds

General:
Carbamates **2-5** were purchased from commercial sources. Carbamates **12a**, **12b**, **12g 16**, **17**, **20h-m,o,q,s, u** and their phenol precursors are previously known. The syntheses of **9d, 9f, 10c, 10d**, **11a-b**, **11d-f**, **12c-d**, **19t**, and **20t** are described in a patent application.[1] The syntheses of **19o** and **19r** are described in a recent paper.[2]

2-(2-Methylbutoxy)phenol (9c). A dry 50 mL round-bottom flask was charged with catechol (2.0 g, 18 mmol), *N,N*-dimethylformamide (18 mL), cesium carbonate (5.9 g, 18 mmol), and purged with nitrogen. Next, 1-chloro-2-methylbutane (3.3 mL, 2.9 g, 27 mmol) was added to the reaction and the contents were heated to 80 °C overnight. The reaction was cooled to room

temperature and quenched with 1 M hydrochloric acid (50 mL) and extracted with ethyl acetate (3 x 30 mL). The organic layers were combined, washed with brine, dried with sodium sulfate, and filtered. The solvent was evaporated in vacuo and the residue purified by silica gel chromatography (5:1 hexane:ethyl acetate) to yield a colorless oil weighing 1.1 g (34%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.96 – 6.92 (m, 1H), 6.89 – 6.81 (m, 3H), 3.91 (dd, *J* = 9.1, 5.9 Hz, 1H), 3.83 (dd, *J* = 9.1, 5.9 Hz, 1H), 1.92 (8-let, *J* = 5.9 Hz, 1H), 1.63 – 1.52 (m, 1H), 1.35 – 1.25 (m, 1H), 1.04 (d, *J* = 5.9 Hz, 3H), 0.97 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 146.20, 145.94, 121.40, 120.22, 114.54, 111.70, 73.74, 34.81, 26.29, 16.71, 11.44; HRMS (FAB): 180.11503 calcd for $C_{11}H_{16}O_2$ $[M]^+$ found 180.11354 (-8.5 ppm, -1.5 mmu).

2-(2-Methylbutoxy)phenyl *N*-methylcarbamate (11c). A dry 100 mL round-bottom flask was charged with **9c** (1.1 g, 6.2 mmol) and dry tetrahydrofuran (6 mL) prior to cooling to 0 °C. Next, the reaction flask was charged with potassium *tert*-butoxide (1 M in THF, 6.5 mL, 6.5 mmol), purged with nitrogen, and allowed to stir for 20 min. The reaction flask was then charged with *N*-methylcarbamyl chloride (1.2 g, 13 mmol), the ice-bath was removed, and the reaction was allowed to stir at room temperature for 3 h. The solvent was concentrated in vacuo and the residue taken up in dichloromethane (50 mL) and washed with 0.25 M hydrochloric acid (50 mL), and then brine (50 mL), and dried with sodium sulfate. The solution was filtered and concentrated in vacuo and the residue was purified by flash chromatography on silica gel (5:1 hexane : ethyl acetate) to afford a white solid weighing 1.08 g (74% yield); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 (td, *J* = 8.1, 1.6 Hz, 1H), 7.08 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.93 (dd, *J* = 8.1, 1.6 Hz, 1H), 6.90 (td, *J* = 7.8, 1.3 Hz, 1H), 4.99 (br s, 1H), 3.85 (dd, *J* = 8.8, 6.5 Hz, 1H), 3.76 (dd, *J* = 8.8, 6.5 Hz, 1H), 2.88 (d, *J* = 4.9 Hz, 3H), 1.85 (s-8, *J* = 6.5 Hz, 1H), 1.58 – 1.49 (m, 1H), 1.31 – 1.20 (m, 1H), 1.00 (d, *J* = 6.5 Hz, 3H), 0.94 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.21, 151.50, 140.53, 126.47, 123.23, 120.61, 113.45, 73.45, 34.92, 27.91, 26.17, 16.55, 11.50; HRMS (FAB): 238.1443 calcd for $C_{13}H_{19}NO_3$ $[M]^+$ found 238.1440 (-1.3 ppm, -0.3 mmu).

3-ethyl-1-(2-hydroxyphenyl)pentan-1-one (14)

Mg/I exchange and coupling with Weinreb amide: A dry 50 mL Schlenk flask purged with nitrogen was charged with 2-iodoanisole (1.78 g. 7.63 mmol) and dry THF (8 mL) prior to cooling to -78 °C in a dry-ice acetone bath. Isopropyl-magnesium chloride (2 M in THF, 4.2 mL, 8.4 mmol) was cautiously added over 10 min. To this mixture a solution of 3-ethyl-N-methoxy-N-methylpentanamide (1.47 g, 8.49 mmol) in dry tetrahydrofuran (8 mL) was cautiously added over 10 min. The reaction was allowed to stir for 5 h at -78 °C and then overnight at room temperature. Next, the reaction was cooled to 0 °C and cautiously quenched with 3 M hydrochloric acid (100 mL). The aqueous layer was extracted with diethyl ether (3 x 50 mL) and the combined organic layers were washed with brine and dried with sodium sulfate. The solution was filtered and then evaporated prior to purifying the residue with flash chromatography (1:1 hexane:dichloromethane). Evaporation of the solvent in vacuo yielded 3-ethyl-1-(2-methoxyphenyl)pentan-1-one as a colorless oil (1.37 g, 81%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.59 (dd, *J* = 7.6, 1.8 Hz, 1H), 7.43 (dd, *J* = 7.6, 1.8 Hz, 1H), 6.99 (td, *J* = 7.6, 1.8 Hz, 1H), 6.94 (d, *J* = 7.6 Hz, 1H), 3.89 (s, 3H), 2.88 (d, *J* = 6.8 Hz, 2H), 1.88 (7-let, *J* = 4.0 Hz, 1H), 1.43 – 1.22 (m, 4H), 0.85 (t, *J* = 7.4 Hz, 6H); HRMS (ESI): 221.1542 calcd for $C_{14}H_{21}O_2$ [M+H]+ found 221.1526 (-7.15 ppm).

Demethylation: Treatment of 3-ethyl-1-(2-methoxyphenyl)pentan-1-one with BBr_3 as described below in the synthesis of **19n** gave the corresponding phenol 3-ethyl-1-(2-hydroxyphenyl)pentan-1-one as a yellow oil (90%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.49 (s, 1H), 7.78 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.46 (ddd, *J* = 8.0, 7.3, 1.6 Hz, 1H), 6.98 (dd, *J* = 8.0, 0.9 Hz, 1H), 6.89 (ddd, *J* = 8.0, 7.3, 0.9 Hz, 1H), 2.89 (d, *J* = 6.8 Hz, 2H), 2.04 – 1.92 (m, 1H), 1.50 – 1.28 (m, 4H), 0.91 (t, *J* = 7.4 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 207.41, 162.76, 136.33, 130.24, 119.86, 118.92, 118.72, 42.45, 37.76, 26.07, 11.03. HRMS (ESI): 207.1380 calcd for $C_{13}H_{19}O_2$ [M+H]+ found 207.1362 (-8.54 ppm).

Two step reduction of the keto group: a 50 mL round-bottom flask was charged with 3-ethyl-1-(2-hydroxyphenyl)pentan-1-one (314 mg, 1.52 mmol), methanol (15 mL), 2 M sodium hydroxide (1.5 mL), and portion-wise addition of sodium borohydride (190 mg, 5.03 mmol). This was allowed to stir at room temperature overnight prior to evaporating the solvent and diluting in 1 M hydrochloric acid (50 mL). The aqueous layer was extracted with diethyl ether (3 x 30 mL) and the organic layers were combined, washed with brine, dried with sodium sulfate, and filtered. The solvent was evaporated and the residue was purified with flash chromatography (dichloromethane) to yield 2-(3-ethyl-1-hydroxypentyl)phenol as a yellow oil (292 mg, 1.40 mmol, 92%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.91 (s, 1H), 7.21 – 7.11 (m, 1H), 6.95 (dd, *J* = 7.5, 1.7 Hz, 1H), 6.87 (d, *J* = 7.5 Hz, 1H), 6.83 (td, *J* = 7.5, 1.7 Hz, 1H), 4.92 (dt, *J* = 8.8, 4.3 Hz, 1H), 2.48 (s, 1H), 1.95 – 1.86 (m, 1H), 1.64 (ddd, *J* = 14.2, 7.0, 5.3 Hz, 1H), 1.47 – 1.28 (m, 5H), 0.88 (t, *J* = 7.2 Hz, 3H), 0.84 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.75, 129.00, 128.07, 127.09, 119.86, 117.38, 74.55, 40.65, 36.75, 25.83, 24.89, 10.89, 10.43;

A 50 mL Schlenk flask was charged with 2-(3-ethyl-1-hydroxypentyl)phenol (248 mg, 1.19 mmol), methanol (12 mL), 10% palladium on carbon, and then a balloon filled with hydrogen was placed atop the reaction vessel. The contents were allowed to stir overnight at room temperature before filtering the reaction through a plug of celite. The filter bed was washed with excess methanol (50 mL) and the organic layers were combined and evaporated in vacuo. The residue was purified on silica gel chromatography using a gradient from 100% hexane to 100% dichloromethane to afford a yellow oil weighing 141 mg (0.733 mmol, 62%); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.13 (dd, *J* = 7.5, 1.1 Hz, 1H), 7.08 (td, *J* = 7.5, 1.1 Hz, 1H), 6.87 (td, *J* = 7.5, 1.1 Hz, 1H), 6.76 (dd, *J* = 7.5, 1.1 Hz, 1H), 4.65 (s, 1H), 2.61 – 2.54 (m, 2H), 1.59 – 1.52 (m, 2H), 1.42 – 1.33 (m, 4H), 1.31 – 1.24 (m, 1H), 0.88 (t, *J* = 7.4 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 153.49, 130.17, 129.17, 127.08, 120.97, 115.30, 40.48, 33.01, 27.33, 25.45, 10.99.

2-(3-ethylpentyl)phenyl methylcarbamate 15d

This compound was prepared from **14** as a colorless oil (107 mg, 86% yield), according to the procedure described above for **11c**; ^{1}H NMR (400 MHz, CDCl3) δ 7.25 – 7.03 (m, 1H), 4.96 (br s, 1H), 2.90 (d, *J* = 4.9 Hz, 3H), 2.58 – 2.44 (m, 2H), 1.55 – 1.46 (m, 2H), 1.40 – 1.29 (m, 4H), 1.28 – 1.19 (m, 1H), 0.86 (t, *J* = 7.4 Hz, 6H); ^{13}C NMR (126 MHz, CDCl3) δ 155.48, 149.20, 135.62, 130.20, 126.84, 125.80, 122.66, 40.54, 33.41, 27.93, 27.61, 25.42, 10.99. HRMS (ESI): 250.1802 calcd for $C_{15}H_{24}NO_2$ [M+H]+ found 250.1786 (-6.21 ppm).

3-(1,1,1-trifluoro-2-methylpropan-2-yl)phenol (19n)

Mesylation of **18n**: A dry 10 mL round-bottomed flask was charged with a 60% dispersion of sodium hydride in mineral oil (74 mg, 1.83 mmol) and placed in an ice-bath prior to the dropwise addition of a solution containing **18n** (1,1,1-trifluoro-2-(3-methoxyphenyl)propan-2-ol,

203 mg, 0.922 mmol) in of tetrahydrofuran (2.2 mL). After stirring for 30 min, the ice-bath was removed and mesyl chloride (143 μL, 1.84 mmol) was added dropwise. The reaction was allowed to stir for 18 h at room temperature before concentrating the solvent in vacuo. The residue was quenched by the dropwise addition of 5 mL of saturated sodium bicarbonate solution at 0 ^{o}C and extracted with ethyl acetate (3 x 25 mL). The organic layers were combined and washed with brine, dried with sodium sulfate, filtered, and concentrated in vacuo. The resulting residue was purified using flash chromatography (3:1 hexane:ethyl acetate) to yield the intermediate mesylate as a colorless oil weighing 270 mg (0.905 mmol, 98%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.36 (t, J = 8.1 Hz, 1H), 7.17 – 7.10 (m, 2H), 6.97 (dd, J = 8.1, 2.4 Hz, 1H), 3.83 (s, 3H), 3.12 (s, 3H), 2.28 (q, J = 0.9 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.63 (s), 136.08 (s), 129.70 (s), 123.35 (q, $^{1}J_{CF}$ = 284 Hz), 119.31 (s), 114.82 (s), 113.81 (q, $^{3}J_{CF}$ = 1.0 Hz), 87.45 (q, $^{2}J_{CF}$ = 31 Hz), 55.47 (s), 41.37 (s), 19.15 (s); HRMS (APCI): 298.0487 calcd for $C_{11}H_{13}O_4F_3S$ $[M]^+$ found 298.0485 (-0.53 ppm).

Reaction with Rupert-Prakash Reagent**:** A dry 50 mL round-bottom was charged with 1,1,1-trifluoro-2-(3-methoxyphenyl)propan-2-yl methanesulfonate (intermediate mesylate; 300 mg, 1.00 mmol) and dichloromethane (3 mL) while purging with nitrogen. The reaction was cooled in an ice-bath prior to the dropwise addition of 2.0 M trimethylaluminum in hexane (0.50 mL, 1.0 mmol). The ice-bath was removed and the reaction was allowed to stir at room temperature for 18 h. The colorless reaction was quenched with a solution containing 3 mL sodium bicarbonate and 1 mL brine and filtered through a plug of Celite and washed with ethyl acetate. The layers were separated and the aqueous layer was extracted with dichloromethane (3 x 10 mL). The organic layers were combined, washed with brine, dried with sodium sulfate, filtered, and concentrated in vacuo. The residue is purified with flash chromatography (12 : 1 hexane : ethyl acetate) to yield the protected anisole as a colorless oil weighing 168 mg (0.77 mmol, 77%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.30 (t, J = 8.1 Hz, 1H), 7.12 – 7.08 (m, 1H), 7.06 (s, 1H), 6.86 (ddd, J = 8.1, 2.5, 0.7 Hz, 1H), 3.83 (s, 3H), 1.57 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.50 (s), 141.74 (s), 129.25 (s), 128.46 (q, $^{1}J_{CF}$ = 283 Hz), 119.93 (s), 114.47 (s), 112.25 (s), 55.36 (s), 44.00 (q, $^{2}J_{CF}$ = 25 Hz), 22.81 (q, $^{3}J_{CF}$ = 2.4 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ -76.49 (s); HRMS (FAB): 218.0919 calcd for $C_{11}H_{13}F_3O$ $[M]^+$ found 218.0924 (2.44 ppm).

Demethylation: A dry 50 mL round-bottom flask was charged with 1-Methoxy-3-(1,1,1-trifluoro-2-methylpropan-2-yl)benzene (583 mg, 2.67 mmol) and dichloromethane (6 mL) while purging with nitrogen. The reaction was cooled to -78 ^{o}C in a dry ice-acetone bath prior to the addition of 1 M BBr_3 in dichloromethane (5.3 mL, 5.3 mmol). The reaction was allowed to stir overnight as the reaction warmed to room temperature. The reaction was quenched with water (x mL) and the aqueous layer was extracted with diethyl ether (3 x 10 mL). The organic layers were combined and washed with brine, dried with sodium sulfate. The solution was filtered, concentrated in vacuo, and purified with flash chromatography (5:1 hexane:ethyl acetate) to afford **19n** as a yellow oil (518 mg 95% yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (t, J = 8.1 Hz, 1H), 7.06 (ddt, J = 8.1, 2.5, 0.9 Hz, 1H), 6.99 (s, 1H), 6.79 (ddd, J = 8.1, 2.5, 0.9 Hz, 1H), 5.64 (s, 1H), 1.55 (q, J = 0.7 Hz, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.56 (s), 142.00 (s), 129.44 (s), 128.42 (q, $^{1}J_{CF}$ = 283 Hz), 119.88 (s), 114.99 (s), 114.68 (s), 43.91 (q, $^{2}J_{CF}$ = 25 Hz), 22.73 (q, $^{3}J_{CF}$ = 2.4 Hz); ^{19}F NMR (376 MHz, $CDCl_3$) δ -76.49 (s); HRMS (APCI): 203.0689 calcd for $C_{10}H_{10}F_3O$ $[M\text{-}OH]^-$ found 203.0697 (3.81 ppm).

3-(1,1,1-Trifluoro-2-methylbutan-2-yl)phenol (19p). This compound was prepared from **18p** as a yellow oil (158 mg, 36% over 3 steps) according to the procedure for **19n**. Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.23 (t, *J* = 8.0 Hz, 1H), 7.01 (dd, *J* = 8.0, 0.8 Hz, 1H), 6.93 (s, 1H), 6.78 (dd, *J* = 8.0, 2.5 Hz, 1H), 4.88 (s, 1H), 2.20 (dq, *J* = 14.7, 7.5 Hz, 1H), 1.89 – 1.78 (m, 1H), 1.51 (s, 3H), 0.74 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.49 (s), 139.82 (s), 129.45 (s), 128.52 (q, $^{1}J_{CF}$ = 283 Hz), 120.77 (s), 115.55 (s), 114.57 (s), 47.79 (q, $^{2}J_{CF}$ = 24 Hz), 27.45 (s), 18.09 (s), 7.83 (s); ^{19}F NMR (376 MHz, $CDCl_3$) δ -75.51 (s); HRMS (APCI): 217.0846 calcd for $C_{11}H_{12}OF_3$ $[M-H]^-$ found 217.0830 (-7.43 ppm).

3-(2-ethylbutoxy)phenol (19v). A 10 mL round-bottomed flask equipped with a stirbar was charged with resorcinol (301 mg, 2.73 mmol), sodium methoxide (289 mg, 5.35 mmol), capped with a septum, and purged with nitrogen. Methanol (1.4 mL) was added and after 10 minutes, 1-bromo-2-ethylbutane (380 uL, 450 mg, 2.72 mmol) was added via syringe. After 18 h, 1 M HCl (6 mL, 6 mmol) was added, and the reaction was extracted with dichloromethane (3 x 10 mL). The combined organic fractions were dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography (2:1 ethyl acetate:hexane), affording **11c** as a colorless oil (204 mg, 38%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.14 – 7.09 (m, 1H), 6.52 – 6.46 (m, 1H), 6.43 – 6.37 (m, 2H), 4.77 (s, 1H), 3.82 (d, *J* = 5.9 Hz, 2H), 1.71 – 1.61 (m, 1H), 1.54 – 1.38 (m, 4H), 0.92 (t, *J* = 7.5 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 160.94, 156.75, 130.19, 107.56, 107.26, 102.17, 70.28, 40.95, 23.47, 11.25. HRMS (APCI): calculated for $C_{12}H_{18}O_2$ 194.1307 $[M]^+$, found 194.1302(-2.5ppm).

3-(1,1,1-Trifluoro-2-methylpropan-2-yl)phenyl methylcarbamate (20n). This compound was prepared as a white solid according to the carbamoylation method for **11c** (62% yield); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.31 (m, 2H), 7.26 – 7.22 (m, 1H), 7.12 – 7.08 (m, 1H), 4.99 (br s, 1H), 2.90 (d, *J* = 5.3 Hz, 3H), 1.56 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.26 (s), 151.05 (s), 141.58 (s), 129.05 (s), 128.31 (q, $^{1}J_{CF}$ = 283.0 Hz), 124.46 (s), 121.05 (s), 43.97 (q, $^{2}J_{CF}$ = 26 Hz), 27.85 (s), 22.74 (q, $^{3}J_{CF}$ = 2.4 Hz); HRMS (APCI): 262.1049 calcd for $C_{12}H_{15}NO_2F_3$ $[M+H]^+$ found 262.1056 (2.55 ppm).

3-(1,1,1-Trifluoro-2-methylbutan-2-yl)phenyl *N*-methylcarbamate (20p). This compound was prepared as a white solid according to the carbamoylation method for **11c** (97 mg, 69% yield); NMR (400 MHz, $CDCl_3$) δ 7.34 (t, *J* = 8.0 Hz, 1H), 7.30 – 7.23 (m, 1H), 7.18 (s, 1H), 7.14 – 7.06 (m, 1H), 4.99 (br s, 1H), 2.89 (d, *J* = 4.9 Hz, 3H), 2.21 (dq, *J* = 14.8, 7.5 Hz, 1H), 1.93 – 1.76 (m, 1H), 1.52 (s, 3H), 0.75 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 155.24 (s), 151.16 (s), 139.36 (s), 129.58 (q, $^{1}J_{CF}$ = ND), 129.04 (s), 120.89 (s), 47.81 (q, $^{2}J_{CF}$ = 24 Hz), 27.85 (s), 27.42 (s), 18.14 (s), 7.82 (s); ^{19}F NMR (376 MHz, $CDCl_3$) δ -75.55 (s); HRMS (APCI): 276.1206 calcd for $C_{13}H_{17}NO_2F_3$ $[M+H]^+$ found 276.1216 (3.64 ppm).

3-(3-Methylpentan-3-yl)phenyl *N*-methylcarbamate (20r). This compound was prepared as a white solid from **19r** according to the carbamoylation method for **11c** (88% yield); ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.27 (t, *J* = 8.0 Hz, 1H), 7.11 (d, *J* = 8.0 Hz, 1H), 7.01 (t, *J* = 2.0 Hz, 1H), 6.94 (dd, *J* = 8.0, 2.0 Hz, 1H), 4.95 (br s, 1H), 2.89 (d, *J* = 4.9 Hz, 3H), 1.71 (dq, *J* = 14.8, 7.4 Hz, 2H), 1.54 (dq, *J* = 14.8, 7.4 Hz, 2H), 1.22 (s, 3H), 0.67 (t, *J* = 7.4 Hz, 6H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.53, 151.10, 149.68, 128.67, 123.68, 120.09, 118.47, 41.49, 35.26, 27.84, 22.94, 8.79; HRMS (APCI): 236.1645 calcd for $C_{14}H_{22}NO_2$ $[M+H]^+$ found 236.1650 (1.88 ppm).

3-(2-ethylbutoxy)phenyl methylcarbamate (20v)

This compound was prepared as a pale oil from **19v** (185 mg, 0.95 mmol) according to the procedure given above for **11c** (150 mg, 63% yield). ^{1}H NMR (500 MHz, CDCl3) δ 7.22 (t, *J* = 8.5 Hz, 1H), 6.74 (dd, *J* = 8.3, 1.5 Hz, 1H), 6.71 – 6.62 (m, 2H), 4.95 (s, 1H), 3.82 (d, *J* = 5.8 Hz, 2H), 2.89 (d, *J* = 4.9 Hz, 3H), 1.65 (7-let, *J* = 6.2 Hz, 1H), 1.53 – 1.37 (m, 4H), 0.92 (t, *J* = 7.5 Hz, 6H); ^{13}C NMR (126 MHz, CDCl3) δ 160.36, 152.13, 129.69, 113.62, 111.90, 108.25, 100.05, 70.32, 40.99, 27.86, 23.47, 11.26. HRMS (APCI): calculated for C14H21NO3 251.1521 [M]+, found 251.1505(-6.6ppm).

3-isopentylphenyl methylcarbamate (20w)

Under nitrogen, a two-neck dry 100 mL Schlenk flask equipped with a magnetic stir bar was charged with 3-bromophenol (600 mg, 3.47 mmol) and 20 ml dry THF, and cooled to -78 °C. *n*BuLi (2.5 M in hexanes, 2.9 ml, 7.3 mmol) was added. The reaction was stirred at -78 °C for 30 minutes. The solution of *N*-methoxy-*N*,3-dimethylbutanamide (1.32 g, 7.28 mmol) in 15 ml dry THF was added. After 5 hours the acetone and dry ice bath was removed and the reaction was allowed to stir at room temperature overnight. The reaction was cooled to 0 °C and quenched with 1N HCl solution and extracted with ethyl acetate (3 x 15 mL). The organic layers were combined, dried with sodium sulfate, filtered, and the solvent evaporated under reduced pressure. The residue was purified on silica gel (10:1 hexane:ethyl acetate) to yield 1-(3-hydroxyphenyl)-3-methylbutan-1-one as a colorless oil (283 mg, 46% yield).

Under nitrogen, a 10 mL Schlenk flask equipped with a magnetic stir bar was charged with 1-(3-hydroxyphenyl)-3-methylbutan-1-one (94.5 mg, 0.53 mmol), hydrazine hydrate (55%, 0.09 mL, 1.59 mmol) and 2.5 ml ethanol, and then placed in an oil bath to heat up to 100 °C. Reaction refluxed for 6 hours. Ethanol was evaporated by rotary evaporation and 6 ml of ethylene glycol was added to the flask containing crude hydrazone. To this potassium hydroxide (148 mg, 2.65 mmol) was added and the mixture was heated at 160 ºC under nitrogen for 12 hours. After completion of the reaction it was allowed to cool and quenched with 1N HCl solution. The resultant mixture was extracted with ether (3 x 15 ml). The organic layers were combined, dried with sodium sulfate, filtered, and the solvent evaporated under reduced pressure. The residue was purified on silica gel (10:1 hexane:ethyl acetate) to yield 3-isopentylphenol as a colorless oil (33 mg, 39%).

Under Nitrogen, to a 10 mL dry Schlenk flask equipped with a magnetic stir bar was charged with 3-isopentylphenol (33 mg, 0.21 mmol) and 5 ml dry THF, and then NaH (60%, 25 mg, 0.63 mmol) was added. Reaction stirred at room temperature for 30 minutes. Methylcarbamic chloride (68 mg, 0.72 mmol) was added. The reaction was allowed to stir overnight and quenched with water and extracted with ethyl acetate (3x 15mL). The organic layers were combined, dried with sodium sulfate, filtered, and the solvent evaporated under reduced pressure. The residue was purified on silica gel (10:1 hexane:ethyl acetate) to yield 3-isopentylphenyl methylcarbamate as a white solid (30.1 mg, 67% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25-7.22 (m, 1H), 7.01-6.99 (m, 1H), 6.95-6.91 (m, 2H), 5.00 (br, 1H), 2.86 (d, *J* = 4.0 Hz, 3H), 2.60 (t, $J_1 = J_2 = 4.0$ Hz, 2H), 1.64-1.54 (m, 1H), 1.54-1.47 (m, 2H), 0.92 (d, *J* = 8.0 Hz, 6 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.38, 151.08, 144.65, 128.98, 125.32, 121.43, 118.68, 40.50, 33.58, 27.69, 22.51; HRMS (APCI): calculated for $C_{13}H_{20}NO_2$ 222.1489 $[M+H]^+$, found 222.1475 (-5.96 ppm).

B. Expression and purification of recombinant *An. gambiae* AChE

A forward primer (CTCGAGAAAAGAGAGGCTGACAACGATCCGCTGGTGGTCAA) containing an XhoI restriction site (underlined nucleotides) and a reverse primer (TCTAGA GCTGCGCTGCTTTCGCACGGTT) containing an XbaI restriction site (underlined nucleotides) were designed and used for the amplification of the *Ag*AChE-WT (*ace-1*) catalytic domain. The amplified cDNA product was ligated into TA-cloning vector. After propagation in *Escherichia coli*, the cDNA was sub-cloned into a yeast protein expression vector (pPICZα A). The frame of the *Ag*AChE catalytic domain was verified by DNA sequencing. The pPICZα A, containing an α-factor signal sequence and the AChE catalytic domain, was arranged right after the signal sequence. After expression, the signal fragment should be cleaved by an endogenous protease. A recombinant vector containing WT *Ag*AChE catalytic domains was linearized by BstXI and used to transform competent *Pichia pastoris* cells, based on the manufacturer's chemical transformation protocol (Invitrogen). Individual positive colonies were selected and tested for *Ag*AChE expression based on enzyme activity assays. The selected colony (showing high *Ag*AChE activity after methanol induction) was selected for large scale *Ag*AChE expression. These cells were cultured at 37 °C and induced by methanol. After induction, the cells were cultured at 30 °C for 48 hrs, broken down with glass beads, and centrifuged at 34,500 g (4 °C, 30 min). [3] The soluble proteins in the supernatant were applied to a column packed with nickel-chelating resin. After thorough washing with buffer, the recombinant proteins were eluted using a buffer containing 250 mM imidazole, 300 mM NaCl, and 50 mM sodium phosphate, pH 8.0. The affinity purification resulted in the isolation of the recombinant protein at about 70% purity. Further purifications of the recombinant protein was achieved by Mono-Q and gel-filtration chromatographies. The purified protein was concentrated to 5 mg protein/mL in 10 mM phosphate buffer (pH 7) using a Centricon YM-50 concentrator (Millipore). SDS-Page analysis indicated >90% purity and a molecular mass between 60 and 70 kDa, consistent with the expected 64 kDa sequence. The concentration of the purified recombinant proteins was determined by a Bio-Rad protein assay kit (Hercules, CA) using BSA as a standard.

C. Enzyme assay and determination of k_i values

Recombinant human AChE was purchased from Sigma (C1682). Enzyme activity was measured using the Ellman Assay,[4] in a microtiter format, as described below. Inhibition potency of carbamate insecticides was assessed by measuring apparent bimolecular rate constants k_i (mM^{-1} min^{-1}) for inactivation of the enzymes. We adopted a progressive inactivation approach,[5] in which enzymes were incubated with different concentrations of carbamates for differing times before measuring enzyme residual activity (v/v_0). Enzymes were diluted in a 0.1 M sodium phosphate buffer (pH 7.7) containing 0.02% NaN_3 (w/v), 0.3% (v/v) Triton X-100, and 1 mg/mL bovine serum albumin (BSA), and kept over ice prior to use. Inhibitor solutions were prepared in 0.1 M sodium phosphate buffer containing 0.02% NaN_3 (w/v) with a DMSO concentration of 1% (v/v); following addition to the microtiter plate (see below) the final assay concentration of DMSO was thus 0.1% (v/v). Aliquots of the diluted enzymes (10 μl) incubated in triplicate, in a 96-well microplate with buffer (170 μl) and inhibitor solution (20 μL) for the desired incubation time *t*. Note that inhibitor-free controls substituted the inhibitor solution with 1% DMSO in buffer (20 μL). Thereafter, a freshly prepared solution of ATCh and DTNB (4 and 3 mM respectively, in buffer, 20 μl), was added and mixed manually to start the enzymatic reaction, which was monitored at 405 nm. Thus, a total volume of 200 μL and optical pathlength of 0.60 cm was achieved in each cell, with the following final concentrations: 0.015% (v/v) Triton X-

100, 0.05 mg/ml BSA, 0.4 mM ATCh, 0.3 mM DTNB. Enzymes were incubated with typically five concentrations of inhibitors (and an inhibitor-free control) for up to 6 minutes at approximately 1 min intervals. Each concentration was present in the microplate in duplicate, and each experiment was repeated. Residual activities v/v_0 are the ratio of the rate in the presence of inhibitor to a time-matched inhibitor-free control. This method therefore corrects for slow thermal inactivation of the enzyme. These residual activities were used to calculate pseudo first-order rate constants k_{obs} (min^{-1}, in duplicate) for by plotting $\ln(v/v_0)$ vs incubation time t. For each inhibitor these pseudo first-order rate constants were determined at three or more inhibitor concentrations ([I]). Plots of k_{obs} vs [I] were then constructed; note that at high [I] a departure from linearity is seen due to saturation (an expected consequence of the reversible association that proceeds carbamoylation). Thus [I] and associated k_{obs} values were chosen so as to remain in the linear portion, and the slope of the unconstrained linear fit of these data provides the apparent bimolecular rate constant k_i (mM^{-1} min^{-1}) for inactivation. In this analysis we follow the approach of Bar-On et al 2002 (see Figure 2 in this paper).[5a] As this paper shows, a small non-zero intercept (0.005 min^{-1}) can sometimes be observed, which we believe may be attributed to decarbamoylation. For the 68 rate constant determinations in this work, 75% of the plots gave calculated intercepts that were zero within the 95% confidence limits. Of the remaining 25%, cases, we examined cases where the calculated intercept exceeded 0.005 min^{-1}, and in each case the R^2 value of the k_{obs} vs. [I] plots was found to be greater than or equal to 0.98. The error in k_i is estimated as the standard error in the slope of this plot. Selectivity for inhibition was calculated from the measured k_i values; the error in these ratios was determined using a standard propagation of error method .[6]

D. Mosquito Toxicity Assays

The G3 strain of *An. gambiae* was obtained from MR4 (www.mr4.org) and has been in colony at Virginia Tech since 2005. The G3 strain (MRA-112, genotype 2La/+, 2r+/+, TEP1 s/s; phenotype red stripe, polymorphic for c+ (*collarless*)) is a "mongrel" strain and is reported to be sensitive to all insecticides [7]. Mosquitoes were reared in an environmental chamber (G3 28 ± 1 °C, 55-65% relative humidity (RH); 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.

Adult female non-blood fed *An. gambiae* 3-5 days old, were used for toxicity assay. Tarsal contact (filter paper) assays were performed in exposure tubes according to the 2006 World Health Organization recommendations, with slight modification [8]. In brief, filter papers (15 x 12 cm) were treated with 2.0 mL of various concentrations of the carbamate in ethanol, and allowed to dry overnight. For the G3 strain, batches of 20-25 mosquitoes (in triplicate) were transferred to a holding tube and allowed to adapt for one hour. Mosquitoes were then transferred to the exposure tube (held horizontally) that contained a treated filter paper. Knockdown was noted after 1 h, and all mosquitoes were transferred back to the holding tube (held upright), and given free access to 10% (w/v) sugar water. Mortality was recorded at 24 h. Both during exposure and the 23 h period following, mosquito tubes were kept in an environmental chamber at 24 ± 1 °C and 75% RH. To determine LC_{50} values, typically 5-8 concentrations were examined, and mortality data were used for probit analysis using PoloPlus (PoloPlus version 1.0, LeOra Software, 2002-2003). Topical application assays were performed according to the literature procedure, which involved anesthetizing mosquitoes on ice, and application of 0.2 uL of an ethanolic solution of the carbamate to the dorsal thorax.[9] Mosquitoes (10 per dose, 3-5

doses) were transferred to a holding tube, and given free access to 10% (w/v) sugar water at 24 ± 1 °C and 75% RH. Mortality was recorded at 24 h. The lethal dose (ng/mosquito) was calculated from mortality data using PoloPlus.

E. Overlay of select inhibitors on 3D QSAR lipophilicity field visualization for *Ag*/*h* inhibition selectivity

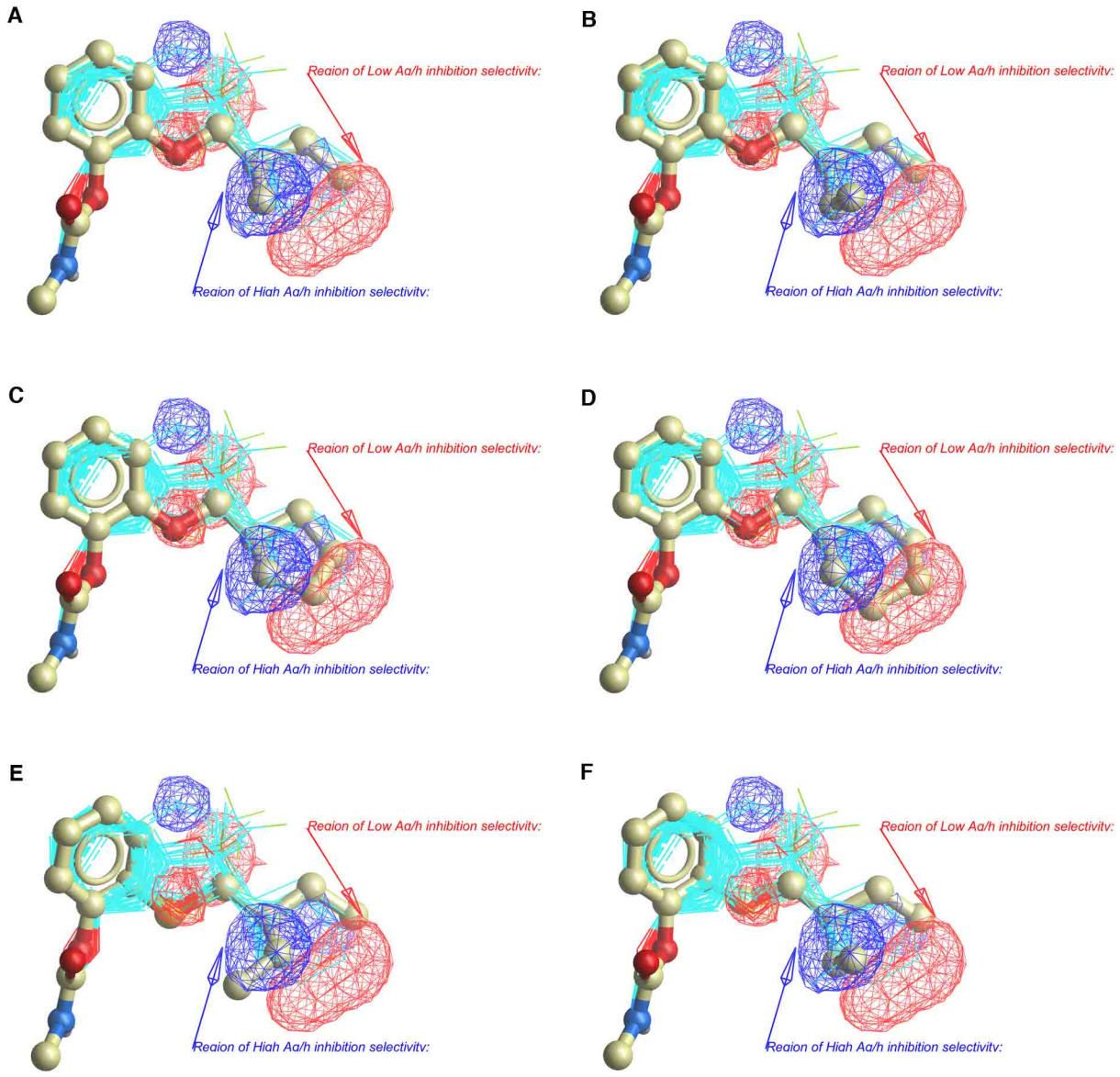

Figure S1. Overlays of select carbamates (CPK stick, carbon = white) on the 3D QSAR lipophilicity field visualization for *Ag*/*h* inhibition selectivity. A) **11c**, 216-fold selective; B) **11d**, 530-fold selective; C) **11e**, 36-fold selective; D) **11f**, 2-fold selective; E) **12d**, 130-fold selective; F) **15d**, 100-fold selective.

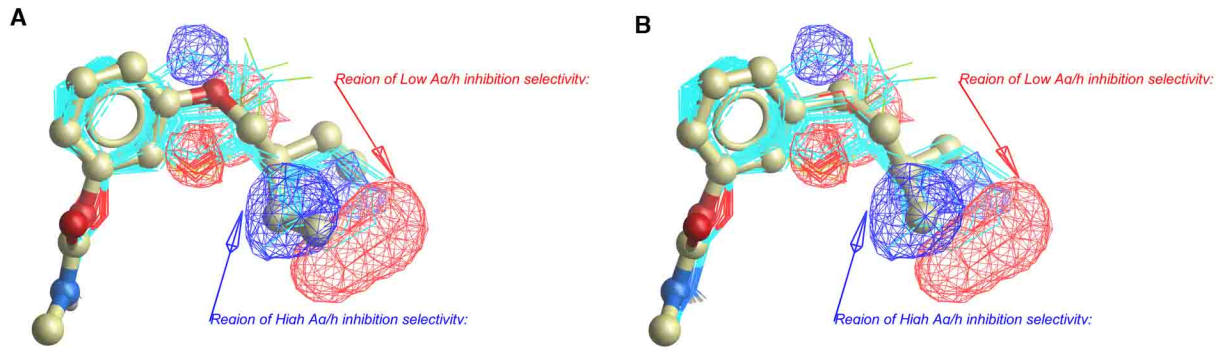

Figure S2. Overlays of select carbamates (CPK stick, carbon = white) on the 3D QSAR lipophilicity field visualization for *Ag*/*h* inhibition selectivity. A) **20v**, 6-fold selective (see discussion in paper); B) **20w**, 53-fold selective.

F. Correlation of *Ag*AChE k_i values to *An. gambiae* LC_{50} and LD_{50} values

Figure S3 depicts a plot of the 19 log (LC_{50} (ug/mL) values vs. the corresponding log (k_i (mM^{-1} min^{-1})) values (Tables 1-4). As expected, a negative correlation coefficient (-0.53) is observed, since as k_i increases, LC_{50} should decrease. However, considerable scatter is observed, suggesting an important role for ADME in determining toxicity in the tarsal contact assay.

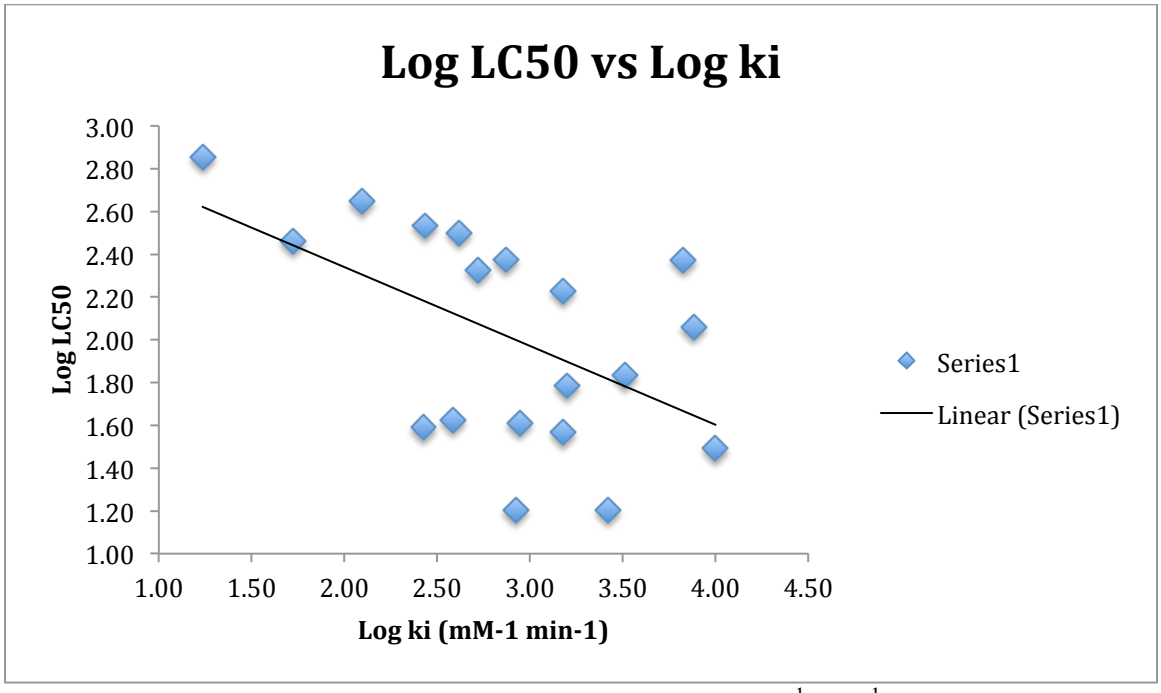

Figure S3. Relation of log (LC_{50} (ug/mL)) to log (k_i (mM^{-1} min^{-1})). Correlation coefficient (19 points) is -0.53. Measured rate constants pertain to *Ag*AChE.

Figure S4 depicts a plot of the 13 log (LD_{50} (ng/mosquito) values vs. the corresponding log (k_i (mM^{-1} min^{-1})) values (Tables 1-4). As expected, a negative correlation coefficient (-0.42) is observed, since as k_i increases, LD_{50} should decrease. However, considerable scatter is observed, suggesting an important role for ADME in determining toxicity in the topical application assay.

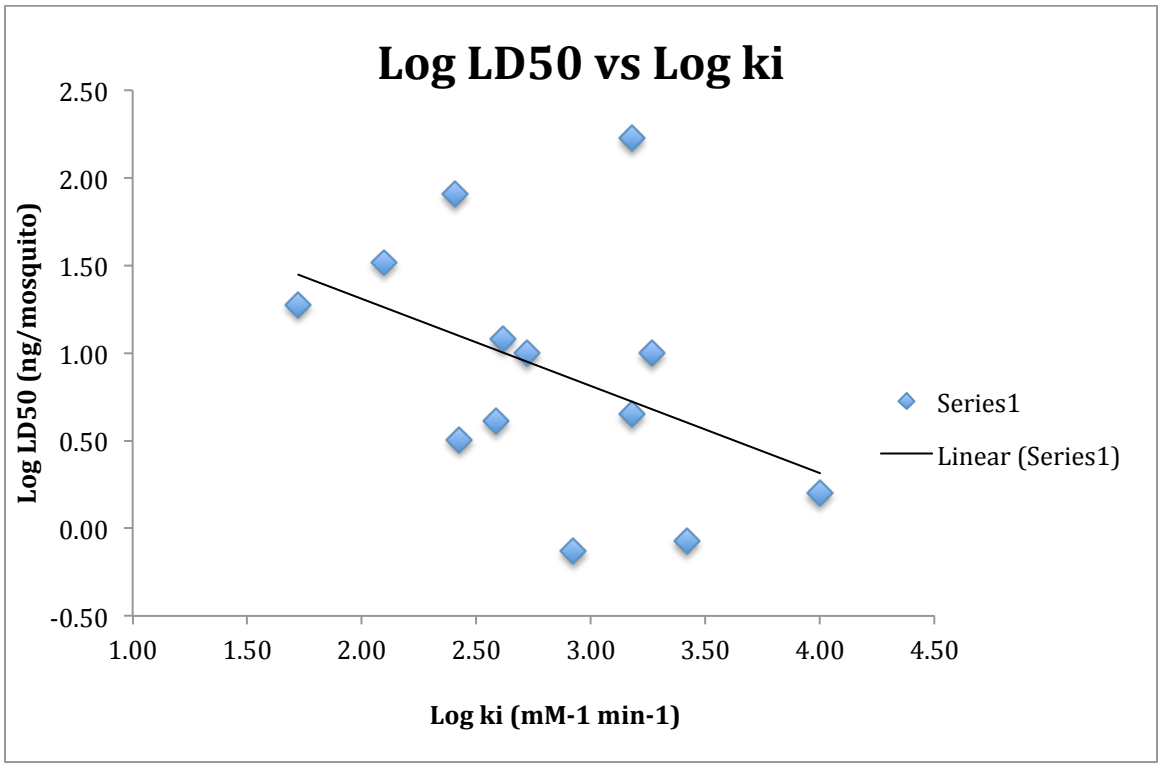

Figure S4. Relation of log (LD_{50} (ng/mosquito)) to log (k_i (mM^{-1} min^{-1})). Correlation coefficient (13 points) is -0.42. Measured rate constants pertain to *Ag*AChE.

G. References

1. Carlier, P.R.; Bloomquist, J. R.; Paulson, S. L.; Wong, E. A. "Species-Selective Insecticidal Carbamates for Mosquito Control" U.S. Patent Application No.: 12/209,301. Date Filed: September 12, 2008
2. Hartsel, J. A.; Craft, D. T.; Chen, Q.-H.; Ma, M.; Carlier, P. R. *J. Org. Chem.* **2012**, *77*, 3127.
3. Canales, M.; Buxado, J. A.; Heynngnezz, L.; Enriquez, A. *Enzyme Microb. Technol.* **1998**, *23*, 58.
4. Ellman, G. L.; Courtney, K. D.; Andres, V. J.; Featherstone, R. M. *Biochem. Pharm.* **1961**, *7*, 88.
5. (a) Bar-On, P.; Millard, C. B.; Harel, M.; Dvir, H.; Enz, A.; Sussman, J. L.; Silman, I. *Biochemistry* **2002**, *41*, 3555; (b) Reiner, E.; Aldridge, W. N. *Biochem. J.* **1967**, *105*, 171.
6. Andraos, J. *J. Chem. Educ.* **1996**, *73*, 150.
7. Benedict, M. Q.; McNitt, L. M.; Collins, F. H. *J. Hered.* **2003**, *94*, 227.
8. World Health Organization 2006. Guidelines for testing mosquito adulticides for indoor residual spraying and treatment of mosquito nets. Document WHO/CDS/NTD/WHOPES/GCDPP/2006.3, Geneva.
9. Pridgeon, J. W.; Pereira, R. M.; Becnel, J. J.; Allan, S. A.; Clark, G. C.; Linthicum, K. J. *J. Med. Entomol.* **2008**, *45*, 82.