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

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Abstract

To identify potential human-safe insecticides against the malaria mosquito we undertook an investigation of the structure activity relationship of aryl methylcarbamates inhibitors of acetylcholinesterase (AChE). Compounds bearing a β-branched 2-alkoxy or 2-thioalkyl group were found to possess good selectivity for inhibition of *Anopheles gambiae* AChE over human AChE; up to 530-fold selectivity was achieved with carbamate **11d**. A 3D QSAR model is presented that is reasonably consistent with log inhibition selectivity of 34 carbamates. Toxicity of these compounds to live *Anopheles gambiae* was demonstrated using both tarsal contact (filter paper) and topical application protocols.

Keywords

acetylcholinesterase; species-selectivity; malaria; mosquito; mechanism-based inactivator

Malaria exacts an enormous toll in the developing world, killing nearly 700,000 in 2010, most of whom were children under 5 years old.[1] Malaria therapeutic drugs directed at the parasite *Plasmodium sp.* play a critical role in reducing mortality,[2] but an important complementary approach involves controlling the vector of the malaria parasite, the

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Supplementary Material
Synthetic protocols and analytical characterization data for inhibitors; enzyme expression, assay, and mosquito toxicity protocols; and overlays of select inhibitors on the 3D QSAR lipophilic field visualization for *Ag*/*h* inhibition selectivity may be found in the online version, at doi.xxx.

mosquito *Anopheles gambiae*. Among vector control measures, the use of insecticide-treated nets (ITNs) has been particularly successful in reducing malaria mortality.[3] To date only two biological targets have been successfully exploited in the design of insecticides against adult vector mosquitoes: the voltage-gated Na^+ ion channel, and acetylcholinesterase (AChE).[4] Current generation ITNs rely on pyrethoids, which modulate the former target. However, widespread use of these nets has led to the emergence of pyrethroid-resistant mosquitoes, jeopardizing this important disease control measure.[5]

To confront this growing threat we sought to identify a class of insecticidal AChE inhibitors that would only weakly inhibit human AChE (*h*AChE). One intriguing strategy to achieve such selective inhibition relies upon the presence of a free cysteine in the active site gorge of *Anopheles gambiae* AChE (*Ag*AChE); human (*h*AChE) has no such free cysteine.[6] Recently, Pang and co-workers reported that the thiol-reactive compound **1** inhibited >95% of *Ag*AChE activity after a 1 h exposure at 6 μM; under these conditions *h*AChE activity was unchanged.[6d]

We chose to pursue a different strategy: namely, redesign of the classic insecticidal aryl methylcarbamate pharmacophore[7] (e.g. **2**–**5**, Figure 1) to achieve species-selectivity. This goal appeared feasible since *h*AChE is only 49% identical to *Ag*AChE.[6c] In addition, precedent for high species-selectivity for a carbamate is serendipitously provided by the Alzheimer's therapeutic drug rivastigmine **6**, which is 1,500-fold more potent towards *h*AChE than *Torpedo californica* AChE.[8] In previous work[6c] we assessed the inhibitory selectivity of several carbamates (including **2**) by measuring IC_{50} values following a 10 minute incubation. However since carbamates inhibit AChE by covalent modification of the catalytic serine, in this study we used the Ellman Assay[9] to monitor time-dependent inhibition of the enzyme, by measuring enzyme velocities as a function of incubation time at fixed inhibitor 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 constructed and the slope of the linear fit provided the apparent second-order rate constants k_i (mM^{-1} min^{-1}) for inactivation (See Supplementary Material for more detail).[8,10] Inhibition of both recombinant *Ag*AChE and *h*AChE by commercial carbamate insecticides **2**–**5** is described in Table 1.

As assessed by k_i ratio, **2** (propoxur) is 16-fold selective for inhibition of *Ag*AChE, greater than the 2-fold selectivity we reported previously based on IC_{50} values.[6c] However none of these carbamates **2**–**5** exhibits high (i.e. >100-fold) *Ag*/*h* selectivity, and thus it is not surprising that to date the World Health Organization has not authorized the use of any carbamate on ITNs.[11] We thus undertook the synthesis of a wide range of aryl carbamates to look for structural determinants of *Ag*/*h* selectivity. In this effort we focused on the use of methylcarbamates since they are known to have favorable insecticidal properties.[7a] In addition basic or quaternary nitrogen functionality were avoided, since these are known to reduce insecticidal potency.[7a,7b] One promising series to emerge featured β-branched 2-alkoxy and 2-thioalkyl substituents.[12] These compounds were trivially synthesized by alkylation of catechol (**7**) or 2-thiophenol (**8**), followed by carbamoylation (Scheme 1). *O*-Alkylated products **9a**–**f** were obtained in low to moderate yield due to competing dialkylation; *S*-alkylation to **10a**–**d**,**g** occurred in higher yield. Carbamoylation was achieved in moderate to excellent yield by deprotonation of the phenols **9** and **10** with KO*t*-Bu and trapping with MeNHC(O)Cl. New compounds **11a**–**f** and **12d** were recently described by the authors in a patent.[12] Inactivation rate constants for these compounds are given in Table 2.

As can be seen in Table 2, β-branched 2-alkoxyphenyl methylcarbamates **11a**–**d** are all more selective than **2**, which is an α-branched 2-alkoxyphenyl methylcarbamate. The

highest selectivity, 530-fold, is observed for **11d**, which bears a 2-(2-ethylbutoxy) group. To illustrate the high selectivity of this compound, Figure 2 provides plots of k_{obs} vs [**11d**] at both *Ag*AChE and *h*AChE.

Compounds **11e** and **11f** represent conformationally constrained analogs of **11d**; these modifications dramatically decreased *Ag*/*h* selectivity, by reducing *Ag*AChE k_i, without significantly affecting the *h*AChE k_i values. Thus on binding to *Ag*AChE, the ethyl groups in **11d** appear to be splayed apart, rather than adopting the *syn*-pentane-like conformation required by **11e**–**f**. 2-Thioalkylphenyl methylcarbamates **12a**–**d** also bear unconstrained β-branched alkyl groups, and offer 48- to 130-fold selectivity for inhibition of *Ag*AChE over *h*AChE. The highest selectivity in this series is seen for **12d**, which like **11d** bears a 2-ethylbutyl substituent on the heteroatom. To further assess the scope of isosteric substitution, novel carbamate **15d** was prepared, which features an X = CH_2 spacer unit (Scheme 1). As can be seen in Table 2, carbamate **15d** offers 100-fold selectivity for inhibition of *Ag*AChE over *h*AChE. Reviewing the performance of **11d** and **12d**, it is clear that the replacement of O by S renders **12d** significantly more inhibitory at both *Ag*AChE and *h*AChE. In contrast, for **11d** and **15d**, the replacement of O by CH_2 reduced the *Ag*AChE k_i value 3-fold, but left the *h*AChE k_i value relatively unchanged. But regardless of the spacer unit (O vs. S vs. CH_2) the 2-ethylbutyl group confers ≥ 100-fold selectivity for *Ag*AChE. Finally, to assess the effect of minimal 2-substitution on *Ag*/*h* inhibition potency and selectivity, compounds **12g**, **16** and **17** were prepared, that featured –SMe, -Me and –H in the *ortho*-position. As can be seen, *Ag*AChE k_i values of these compounds decrease dramatically relative to **11a**–**d**, **12a**–**d**, and **15d**, but *h*AChE k_i values remain largely unchanged.

In our 2008 report[6c] we also discussed the inhibition selectivity of 3-substituted aryl methylcarbamates, particularly those bearing *t*-Bu (**20m**) and trialkylsilyl substituents (**20s**,**t**). To ascertain whether the 3-position had potential to confer high selectivity for *Ag*AChE, a variety of 3-substituted aryl methylcarbamates were prepared, as shown in Scheme 2. 3-Alkylphenols **19l, n**–**r** were prepared by methylation of the corresponding 2° or 3° benzylic alcohols, using the Shishido protocol (**19n**, **p**)[13] or our recent modification of this method (**19l**,**o**,**q**,**r**).[14] These benzylic alcohols **18l,n**–**r** were in turn prepared from commercially available acetophenones or benzoate esters, by addition of the corresponding Grignard reagents or CF_3SiMe_3.[15] Phenols **19i,j,s,t** were prepared by Li/Br exchange on **21** and trapping with the appropriate electrophile. Phenol **19u** was prepared from **22** by a standard Weinreb amide/Wolff-Kishner approach. 3-Alkoxyphenol **19v** was prepared by alkylation of resorcinol **23**. Phenol **19w** was prepared from **21** by Li/Br exchange, trapping with the required Weinreb amide, and Wolff-Kishner reduction.

As can be seen in Table 3, most of the 3-substituted aryl methylcarbamates do not offer appreciable selectivity for inhibition of *Ag*AChE over *h*AChE. In particular **20s** and **20t** are only about 10-fold selective, as opposed to the previously reported 130-fold selectivity.[6c] Similarly **20m** is only 12-fold selective rather than the 38-fold figure reported earlier. We trace these variations, and that of **2** mentioned above, to an unanticipated but reproducible effect of our earlier inhibitor dilution protocol, which in this work has been replaced with a protocol delivering a constant final assay concentration of 0.1% (v/v) DMSO.[16] Compounds **20n**, **p**, **r**, **t**, **v**, and **w** have not been described previously. *Ag*AChE inactivation rate constants k_i are sensitive to the identity of the 3-substituent, varying from 17 (**20h**) to 10,000 mM^{-1} min^{-1} (**20l**).[17] In particular, α-branching of the substituent plays an important role: *Ag*AChE k_i increases significantly as the substituent R^3 is varied from methyl/1° alkyl (**20h**,**i**,**j**,**u**,**w**) to 2° alkyl (**20k**,**l**) or 3° alkyl (**20m**–**r**). It appears that 2° alkyl groups confer slightly faster inactivation than related 3° alkyl groups (cf. **20k** vs **20m**,**n; 20l** vs **20o**,**p**,**r**). However, in general a similar structural dependence is seen for *h*AChE k_i values, resulting

in lower *Ag*/*h* inhibition selectivities than those seen for select 2-substituted aryl methylcarbamates (e.g. **11c**, **11d**, **12d**, **15d**, Table 2). The highest *Ag*/*h* inhibition selectivity in this class is seen for 3-isopentylphenyl methylcarbamate **20w** (53-fold). We note that the γ-branched isopentyl group of **20u** bears a resemblance to the β-branched alkoxy/thioalkyl and γ-branched 2-ethylbutyl groups that confer selectivity in the 2-substituted aryl methylcarbamate class (Table 2, 11a–**d**, **12a**–**d**, **15d**). Yet interestingly, **20v**, which bears a 2-ethylbutoxy group at the C3 position, is poorly (6-fold) selective.

The high *Ag*/*h* inhibition selectivities demonstrated by **11c**, **11d**, **12d**, and **15d** (Table 2) must find their origin in differences in the primary sequences of *Ag*AChE and *h*AChE, and the possible role of particular amino acid substitutions is actively under study.

But at present we have constructed 3D QSAR models for Log (*Ag*AChE k_i), Log (*h*AChE k_i), and Log *Ag*AChE/*h*AChE selectivity. Multiple conformations of all compounds described in Tables 1–3 (except for **20w**) were generated and aligned in 3D using the Atomic Property Fields (APF) method[18] as implemented in ICM.[19] APF molecular alignment accuracy was favorably compared to other methods in a recent benchmarking study.[20] Following simplified APF 3D QSAR methodology, the pair-wise ligand APF scores were used to build a partial least square (PLS) models and derive property fields predictive of Log k_i values at both enzymes, as well as Log selectivity. Both enantiomers of chiral compounds **11c**, **12c**, **20l**, and **20p** were included in the initial modeling, and the worst fitting enantiomer in each pair was discarded. Thus the final 3D QSAR model presented below is chiral, and was selected arbitrarily from one of two equally valid enantiomeric solutions. The leave-one-out cross-validated R^2 values of PLS models of Log (*Ag*AChE k_i), Log (*h*AChE k_i), and Log *Ag*AChE/*h*AChE selectivity were 0.75, 0.81, and 0.66, respectively. The predicted value of **20w** was then used as an external test in the plot of predicted vs observed Log *Ag*AChE/*h*AChE selectivity in Figure 3. As can be seen, the prediction for **20w** falls fairly close to the regression line (predicted and actual Log selectivities are 1.35 and 1.72, respectively). Because the compounds in Tables 1–3 vary primarily in the structure of the lipophilic 2- or 3- substituents, the lipophilic atomic property fields of the 3D QSAR models are useful to illustrate regions of 3D space which, when occupied by a lipophilic moiety, enhance or reduce inhibition potency or selectivity. The 3D QSAR lipophilic property field visualization for Log selectivity is presented in Figure 4.

Overall, the 3D QSAR lipophilic field indicates that in the subsite where the aromatic ring docks, *Ag*AChE has a slightly larger ligand pocket than *h*AChE, so that steric bulk approximately two bonds away from the phenyl core is tolerated by *Ag*AChE but not *h*AChE, conferring selectivity. Regions in which lipophilic occupancy improves *Ag*/*h* inhibition selectivity are colored blue; regions in which lipophilic occupancy decreases inhibition selectivity are colored red. The aligned structure of **20w** is depicted in stick, and the placement of the terminal methyl groups in the selectivity-conferring distal blue region of the 3D QSAR lipophilicity field is evident; note that **20w** is moderately (53-fold) selective. Highly selective (100–500 fold) inhibitors bearing β-branched alkoxy/thioalkyl or γ-branched alkyl groups at the 2-position (e.g. **11c**–**d**, **12d, 15d**) were also examined in this way, and in each case the terminal methyl groups fell principally in the distal blue region and did not impinge the distal red region significantly (see Supplementary Material for Figures). In contrast, the cycloalkyl moieties of constrained analogs **11e** and **11f** were found to extend into the distal red region, consistent with the reduced selectivities (36- and 2-fold, respectively; see Supplementary Material). Finally, the low (6-fold) selectivity of **20v**, which bears a β-branched alkoxy substituent at the 3-position, is not well explained by the 3D QSAR lipophilicity field. The predicted selectivity of **20v** is relatively close to the regression line (predicted and actual log selectivies are 1.26 and 0.76, respectively) and the

accuracy of this prediction must be due to contribution from other property fields, likely penalizing the presence of an oxygen atom at C3.

Finally, toxicity of select carbamates to adult *Anopheles gambiae* was assessed using two standard protocols: tarsal contact of live mosquitoes with carbamate-treated filter papers,[21] and topical application[22] of ethanolic solutions of carbamates to the dorsal thorax of anesthetized mosquitoes (Table 4). With regard to the tarsal contact assay, commercial carbamates **2**–**5** gave LC_{50} values in the range of 16 – 42 ug/mL, and several of the low-selectivity aryl carbamates prepared in this work offered similar toxicities (**20i**,**l**,**m**,**n**,**p**). Compounds **20l**,**m** also proved comparable to **2**–**5** in the topical application assay. However, the highly species-selective carbamates (**11c**, **11d**, **12d**) were less toxic than commercial carbamates **2**–**5** in both tarsal contact and topical application assays.

Although there is no simple relationship between the measured *Ag*AChE k_i value and toxicity to live *Anopheles gambiae*, the expected negative correlations between LC_{50}/LD_{50} and k_i were seen (see Supplementary Material). In the 2-alkoxyphenyl series (**2**, **4**, **11b**–**d**), **4** (carbofuran) is the most rapid inactivator and has the lowest LC_{50}/LD_{50} values in the tarsal contact and topical application assays. Yet, **2** (propoxur) is 25-fold more toxic than **11d** in the topical application assay, despite their similar *Ag*AChE k_i values (266 ± 9 and 255 ± 12 mM^{-1} min^{-1}, respectively). Similarly, in the 3-alkylphenyl series (**20h**–**r**,**w**), **20l** is the most rapid *Ag*AChE inactivator (*Ag*AChE k_i = 10,000 ± 100 mM^{-1} min^{-1}), and in the tarsal contact assay it is among the most toxic inhibitors. However **20i**, which is 11-fold slower than **20l** for inactivation of *Ag*AChE, has very similar toxicity to **20l** in the tarsal contact assay. Thus *Ag*AChE inhibitory potency is not the only determinant of mosquito toxicity. Ability to penetrate the mosquito exoskeleton, as well as traditional ADME issues are likely at work. Work to address possible metabolic and transport liabilities of highly selective carbamates continues and will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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16. Previously, inhibitor stock solutions in DMSO were diluted consecutively with buffer, causing variable final assay DMSO concentrations that were proportionate to the final inhibitor concentration. How the previous dilution protocol led to different results is under study and will be reported in due course; it is noteworthy that the selectivities of some highly selective inhibitors (e.g. **11c**, **11d**) were relatively insensitive to DMSO concentration.
17. The inactivation rate of *Ag*AChE by 20l is very high, faster than the rate of inactivation of *Ag*AChE by chlorpyrifos oxon (Alout H, Djogbenou L, Berticat C, Chandre F, Weill M. Comp Biochem Physiol B-Biochem Mol Biol. 2008; 150:271. [PubMed: 18455457]) and similar to the rate of inactivation of *h*AChE by nerve agents (Bartling A, Worek F, Szinicz L, Thiermann H. Toxicology. 2007; 233:166. [PubMed: 16904809]).
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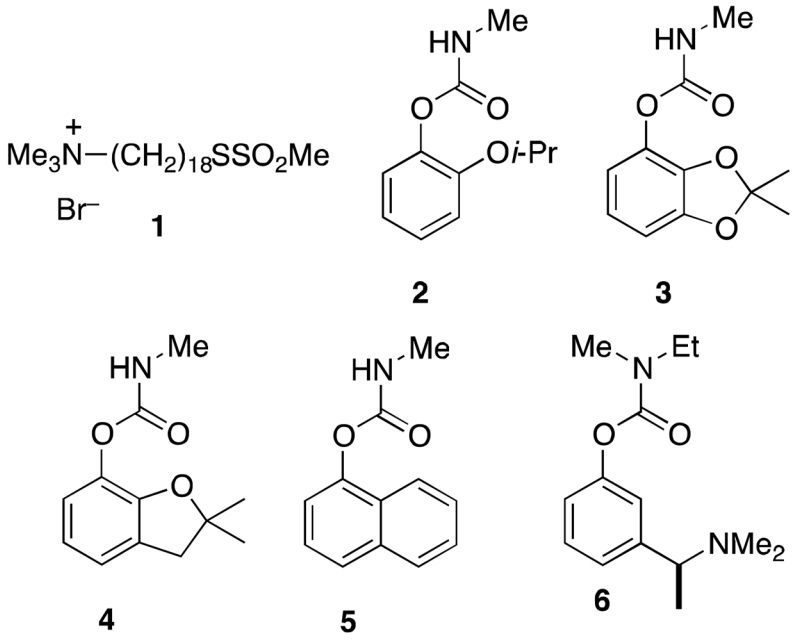


Figure 1.
Thiol-reactive *Ag*AChE-selective inhibitor **1**, insecticidal aryl methylcarbamates **2**–**5**, and *h*AChE-selective carbamate rivastigmine **6.**

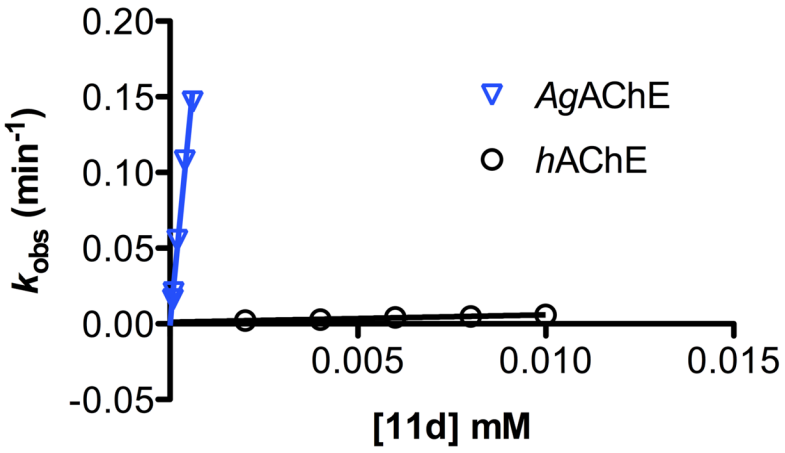


Figure 2.
Plot of k_{obs} vs [**11d**] at both *Ag*AChE and *h*AChE. Second-order rate constants for inactivation k_i (mM^{-1} min^{-1}) derive from the slope of each line.

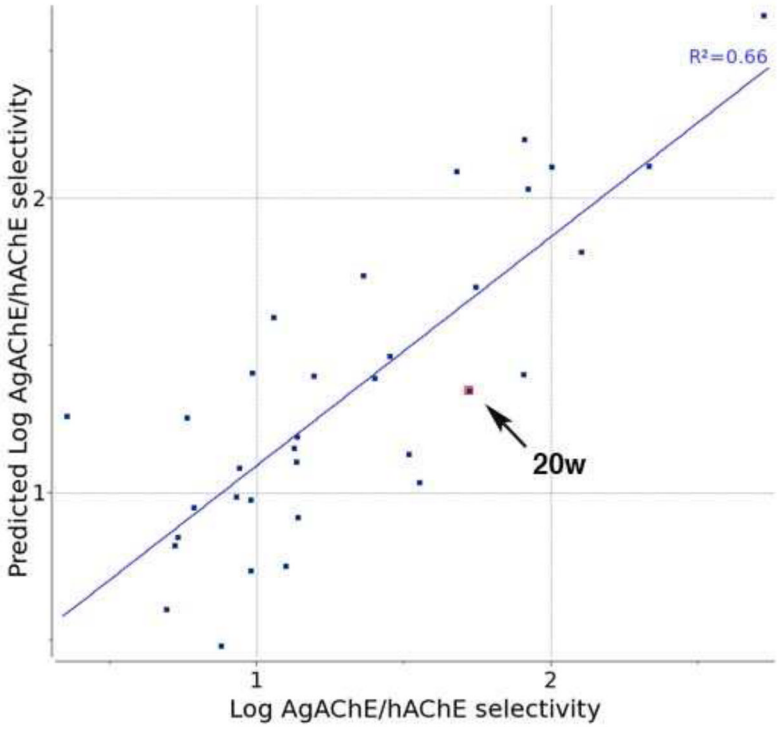


Figure 3.

Predicted vs observed Log *Ag*AChE/*h*AChE selectivity of inhibitors in Tables 1–3 except **20w** (33 points); an R^2 value of 0.66 is calculated. Note that prediction for each compound was made with a PLS model trained on a dataset excluding that particular compound, i.e. this is a leave-one-out prediction validation. Subsequently predicted vs. actual Log selectivity was determined for **20w** (indicated by arrow).

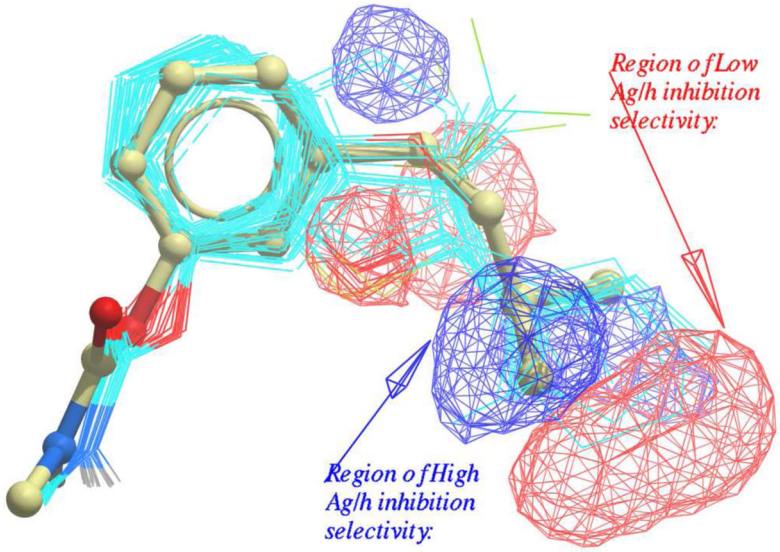
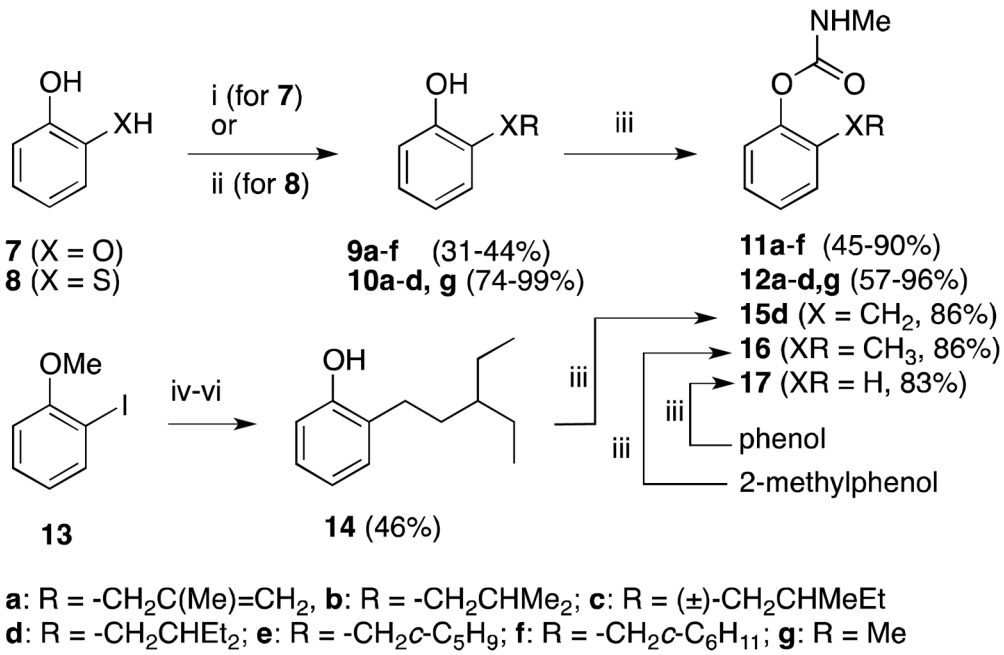


Figure 4.

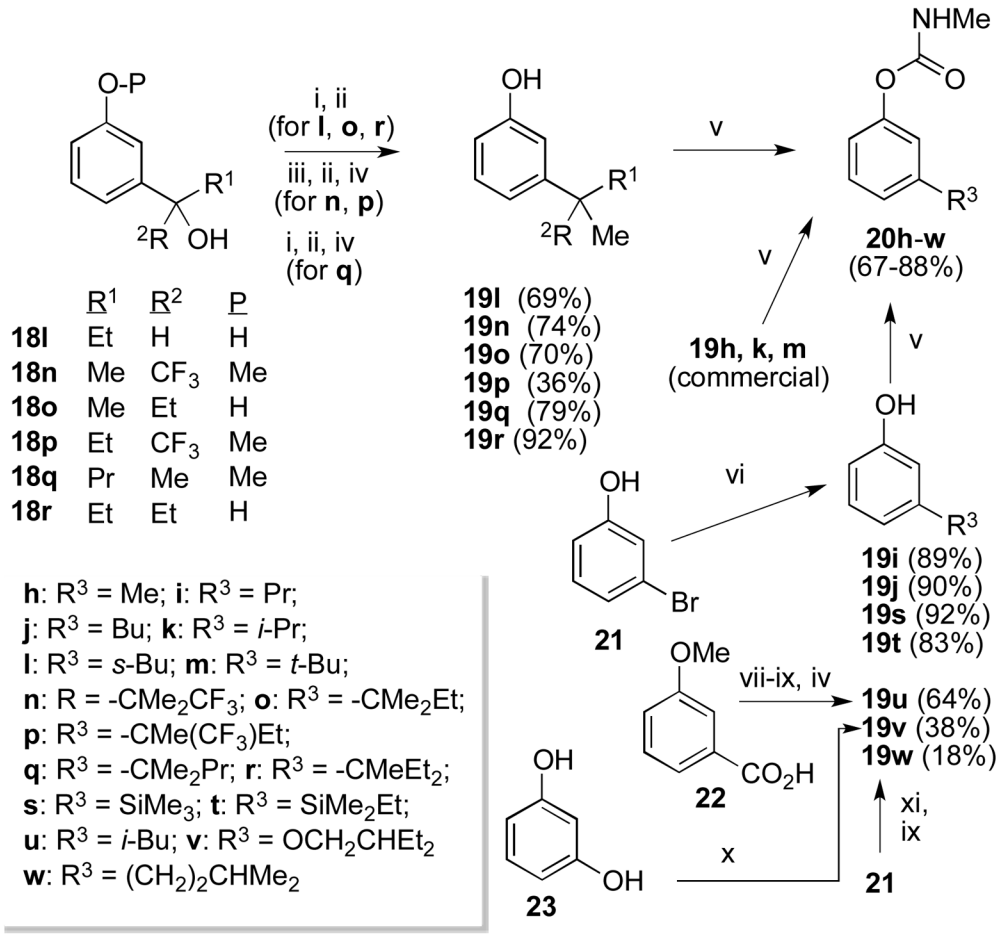
3D QSAR lipophilic field visualizations for the *Ag*/h inhibition selectivity of the compounds in Tables 1–3. Lipophilic occupancy of blue regions improves *Ag*/*h* inhibition selectivity, and lipophilic occupancy of red regions decreases selectivity. **20w** is shown in stick; other compounds are shown in CPK-colored wire (carbon is cyan).



a: R = -$CH_2C(Me)=CH_2$, **b**: R = -CH_2CHMe_2; **c**: R = (±)-$CH_2CHMeEt$
d: R = -CH_2CHEt_2; **e**: R = -CH_2*c*-C_5H_9; **f**: R = -CH_2*c*-C_6H_{11}; **g**: R = Me

Scheme 1.

Synthesis of 2-substituted aryl methylcarbamates and unsubstituted control **17**
i) Cs_2CO_3, R-Br (or Cl, I, OTs), DMF, 80 °C, 16 h. ii) $NaHCO_3$, R-Br (or Cl, I, OTs), DMF, 50 °C, 16 h. iii) KO*t*-Bu, THF; MeNHC(O)Cl. iv) *i*-PrMgCl, THF, −78 °C; $MeO(Me)NC(O)CH_2CH(Et)_2$. v) BBr_3, CH_2Cl_2, 0°C. vi) $NaBH_4$, CH_3OH; Pd/C, H_2, CH_3OH.



Scheme 2.

Synthesis of 3-substituted aryl methylcarbamates **20h**–**w**.

NHMe i) $SOCl_2$, 0°C. ii) $AlMe_3$, CH_2Cl_2, 0 °C. iii) NaH, THF, MsCl. iv) BBr_3, CH_2Cl_2.v) KO*t*-Bu, THF; $CH_3NHC(O)Cl$. vi) 2.1 equiv *n*-BuLi, THF, −78 to 0 °C; 2 equiv R^3-Br or R_3-Cl (for **19s**, **t**); aq. HCl. vii) DMAP, EDCI, HN(OMe)Me, CH_2Cl_2. viii) *i*-PrMgCl, Et_2O, reflux. ix) NH_2NH_2, EtOH; KOH, ethylene glycol, 160 °C. x) NaOMe, $BrCH_2CHEt_2$, MeOH. xi) 2.1 equiv *n*-BuLi, THF, −78 to 0 °C; $Me(MeO)NC(O)CH_2CHMe_2$.

Table 1

Ag AChE and *h*AChE inactivation rate constants k_i of commercial agricultural carbamates

Compound	*Ag*AChE[a] k_i (mM^{-1} min^{-1})	*h*AChE[b] k_i (mM^{-1} min^{-1})	*Ag*/*h* selectivity[c]
2 (propoxur)	266 ± 9	17.0 ± 0.4	16 ± 1
3 (bendiocarb)	839 ± 22	111 ± 5	7.6 ± 0.4
4 (carbofuran)	2,620 ± 150	428 ± 12	6.1 ± 0.4
5 (carbaryl)	386 ± 10	15.4 ± 0.4	25 ± 1

[a] Recombinant *Ag*AChE purified to 2,500 ± 100 U/mg (Supporting Information).

[b] Commercial recombinant *h*AChE (Sigma C1682).

[c] Defined as k_i(*Ag*)/k_i (*h*); error in ratio calculated by standard propagation of error.

Table 2

Ag AChE and *h*AChE inactivation rate constants k_i of 2-substituted aryl methylcarbamates **11a–f**, **12a–d**, **g 15d**, **16** and unsubstituted control **17**.

Cpd	X	R	*Ag*AChE[a] k_i (mM^{-1} min^{-1})	*h*AChE[b] k_i (mM^{-1} min^{-1})	*Ag*/*h* selectivity[c]
11a	O	-$CH_2CMe{=}CH_2$	62.2 ± 2.0	0.74 ± 0.05	84 ± 7
11b	O	-CH_2CHMe_2	52.8 ± 2.7	0.65 ± 0.20	81 ± 5
11c	O	(±)-$CH_2CHMeEt$	125 ± 2.8	0.58 ± 0.05	216 ± 18
11d	O	-CH_2CHEt_2	255 ± 12	0.48 ± 0.12	530 ± 130
11e	O	-CH_2*c*-C_5H_9	9.8 ± 0.3	0.28 ± 0.02	36 ± 3
11f	O	-CH_2*c*-C_6H_{11}	0.48 ± 0.02	0.21 ± 0.04	2.3 ± 0.4
12a	S	-$CH_2CMe{=}CH_2$	414 ± 18	7.5 ± 0.3	55 ± 3
12b	S	-CH_2CHMe_2	526 ± 11	6.5 ± 0.2	81 ± 3
12c	S	(±)-$CH_2CHMeEt$	806 ± 15	16.8 ± 0.4	48 ± 1
12d	S	-CH_2CHEt_2	1,850 ± 100	14.5 ± 1.5	130 ± 15
12g	S	Me	68.9 ± 1.6	5.1 ± 0.3	14 ± 1
15d	CH_2	-CH_2CHEt_2	75.3 ± 2.7	0.75 ± 0.03	100 ± 5
16	none	-CH_3	1.31 ± 0.07	0.24 ± 0.07	5.4 ± 1.5
17	none	-H	2.75 ± 0.26	0.20 ± 0.06	13 ± 4.4

[a] Recombinant *Ag*AChE purified to 2,500 ± 100 U/mg (Supporting Information).

[b] Commercial recombinant *h*AChE (Sigma C1682).

[c] Defined as k_i(*Ag*)/k_i (*h*); error in ratio calculated by standard propagation of error.

Table 3

Apparent bimolecular rate constants k_i for inactivation of *Ag*AChE and *h*AChE by 3-substituted aryl methylcarbamates

Cpd	R[3]	*Ag*AChE[a] k_i (mM^{-1} min^{-1})	*h*AChE[b] k_i (mM^{-1} min^{-1})	*Ag*/*h* selectivity[c]
20h	-CH_3	17.2 ± 0.3	0.52 ± 0.08	33 ± 5
20i	-Pr	881 ± 17	63.7 ± 1.9	14.0 ± 0.5
20j	-Bu	741 ± 5	32.1 ± 0.6	23.0 ± 0.5
20k	-*i*-Pr	3,270 ± 70	383 ± 19	8.5 ± 0.5
20l	-*s*-Bu (±)	10,000 ± 100	1,910 ± 110	5.3 ± 0.4
20m	-*t*-Bu	1,510 ± 110	126 ± 3	12 ± 1
20n	-CMe_2CF_3	1,580 ± 40	139 ± 6	11 ± 0.6
20o	-CMe_2Et	7,600 ± 300	784 ± 28	9.7 ± 0.5
20p	-$CMe(CF_3)Et$ (±)	3,270 ± 70	375 ± 6	8.7 ± 0.2
20q	-CMe_2Pr	6,690 ± 240	1,360 ± 40	4.9 ± 0.2
20r	-$CMeEt_2$	5,280 ± 170	553 ± 13	9.5 ± 0.4
20s	-$SiMe_3$	648 ± 29	67.8 ± 2.6	9.6 ± 0.6
20t	-$SiMe_2Et$	1,510 ± 70	110 ± 6	14 ± 1
20u	-CH_2CHMe_2	155 ± 6	5.5 ± 0.30	28 ± 2
20v	-OCH_2CHEt_2	28.8 ± 0.4	5.0 ± 0.6	5.8 ± 0.7
20w	-$(CH_2)_2CHMe_2$	272 ± 10	5.2 ± 2	53 ± 3

[a] Recombinant *Ag*AChE purified to 2,500 ± 100 U/mg (Supporting Information).

[b] Commercial recombinant *h*AChE (Sigma C1682).

[c] Defined as k_i(*Ag*)/k_i (*h*); error in ratio calculated by standard propagation of error.

Table 4

Toxicity of select carbamates to *Anopheles gambiae* using tarsal contact (filter paper) and topical application protocols.

Compound	Tarsal Contact to treated filter paper LC_{50} ug/mL (95% CI)	Topical application LD_{50} ng/mosquito (95% CI)
2 (propoxur)	39 (32–45)	3.2 (2.4–4.2)
3 (bendiocarb)	16 (14–17)	0.74 (0.52–0.97)
4 (carbofuran)	16 (11–25)	0.85 (0.7–1.1)
5 (carbaryl)	42 (32–55)	4.1 (3–5)
11b	290 (271–309)	19 (12–27)
11c	445 (396–503)	33 (24–43)
11d	27% @ 1,000 ug/mL	81 (64–94)
12a	317 (256–389)	12 (7–19)
12b	212 (145–279)	10 (7–14)
12d	27% @ 1,000 ug/mL	10 (8–12)
20h	712 (539–887)	nd
20i	41 (35–46)	nd
20j	237 (217–257)	nd
20l	31 (29–34)	1.6 (1.4–1.8)
20m	37 (14–60)	4.5 (3.6–5.4)
20n	61 (43–124)	nd
20o	115 (95–147)	nd
20p	68 (64–72)	nd
20q	236 (210–259)	nd
20r	72% @ 250 ug/mL	nd
20t	169 (162–176)	8 (6–10)
20w	342 (267–472)	nd

[a] Mosquitoes were exposed (1 h) to dried filter papers previously treated with ethanolic solutions of carbamates; mortality was recorded after 24 h. LC_{50} values derive from the concentrations of inhibitor used to treat the papers.

[b] Ethanolic solutions of the carbamate (0.2 uL) were applied to the dorsal thorax of anesthetized mosquitoes; mortality was recorded after 24 h.