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Potential for *Aedes aegypti* Larval Control and Environmental Friendliness of the Compounds Containing 2-Methyl-3,4-dihydroquinazolin-4-one Heterocycle

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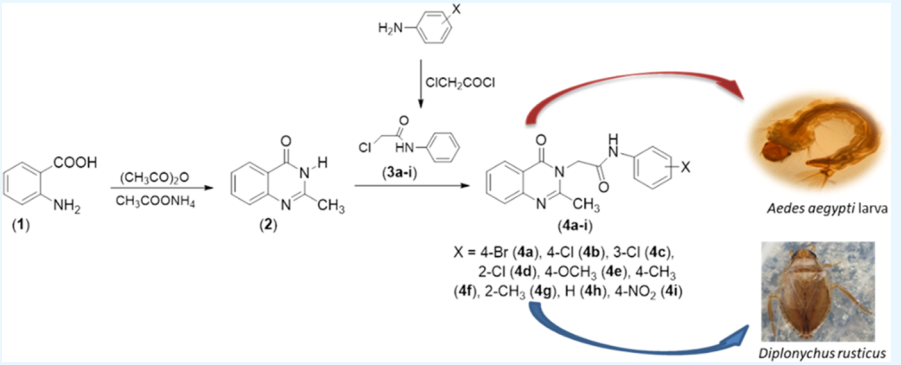

ABSTRACT: 2-Methylquinazolin-4(3*H*)-one was prepared by the reaction of anthranilic acid, acetic anhydride, and ammonium acetate. The reaction of 2-methylquinazolin-4(3*H*)-one with *N*-aryl-2-chloroacetamides in acetone in the presence of potassium carbonate gave nine *N*-aryl-2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetamide compounds. The structures of these compounds were elucidated on the basis of their IR, ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, and high-resolution mass spectrometry (HR-MS) spectral data. These synthesized compounds containing the 2-methyl-3,4-dihydroquinazolin-4-one moiety exhibited activity against *Aedes aegypti* mosquito larvae with LC_{50} values of 2.085−4.201 μg/mL after 72 h exposure, which is also confirmed using a quantitative structure−activity relationship (QSAR) model. Interestingly, these compounds did not exhibit toxicity to the nontarget organism *Diplonychus rusticus*. In silico molecular docking revealed acetylcholine binding protein (AChBP) and acetylcholinesterase (AChE) to be potential molecular targets. These data indicated the larvicidal potential and environmental friendliness of these *N*-aryl-2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetamide derivatives.

INTRODUCTION

Quinazolin-4(3*H*)-one is the basic skeleton found in many compounds having biological activity. Studies have shown that some compounds containing 2-methylquinazolin-4(3*H*)-one heterocycle possess various biological effects such as antimicrobial,[1] antifungal,[2] anti-inflammatory,[3] analgesic,[4] and anticancer activities.[5] Along with the quinazolin-4(3*H*)-one nucleus, the acetamide component is also present in the structure of many biologically active compounds. A wide range of biological activities such as antimicrobial, antifungal,[6] antioxidant, anti-inflammatory,[7] and enzyme-inhibiting[8] have been attributed to the compounds containing the acetamide component.

Many studies have shown the insecticide potential of quinazoline derivatives against *Chironomus tentans*, *Periplanata americana*, *Chrysornyia albiceps*, *Mythimna separata*, *Plutella xylostella*, *Prodenia litura*, and *Anopheles arabiensis*.[9−16]

Notably, many quinazolin-4(3*H*)-one-containing derivatives have shown potential AChE inhibitory activity. The 2-substituted quinazolin-4(3*H*)-one derivatives have shown promising AChE and BChE inhibitory activities, with the 4-Cl substituent and the substituent (3-OCH_3, 4-OH) exhibiting promising inhibitory activity comparable to standard drug galantamine.[17] In addition, quinazolinone derivatives exhibit potential AChE inhibitory activities.[18,19] Combining quinazolin-4(3*H*)-one nucleus and acetamide group thus promises to produce organic molecules with effective larvicidal activity against mosquito species.

The *Aedes aegypti* mosquito is the principal vector of dengue, Zika, yellow fever, and chikungunya viruses, and it has spread to

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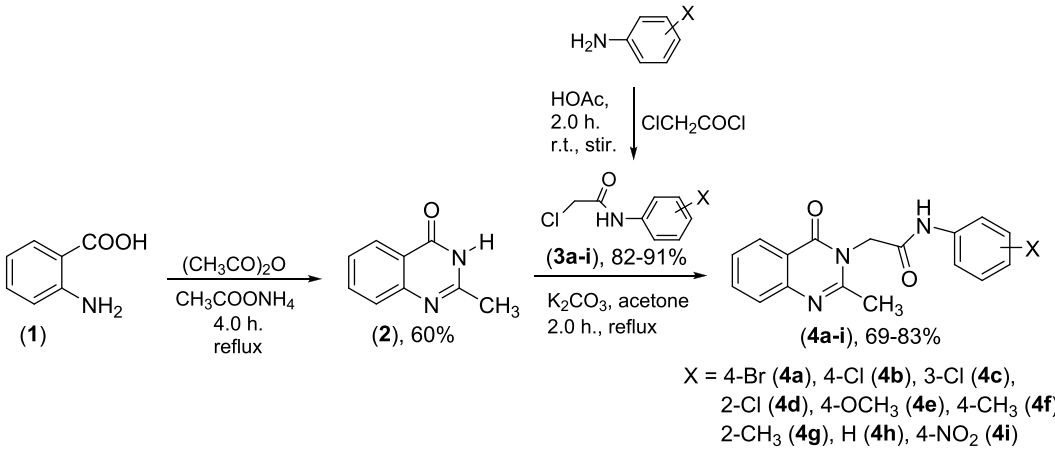
Scheme 1. Synthetic Pathway of the Acetamides Containing 2-Methyl-3,4-dihydroquinazolin-4-one Heterocycle



most tropical and subtropical towns and cities.[20] According to WHO,[21] dengue incidence has been rising; the number of cases reported annually is 96 million cases, including 1.9 million critical cases and 9110 deaths. Urbanization[22] and global warming[23] are facilitating the territorial expansion of *Aedes aegypti*. The global population of *Aedes aegypti* has developed resistance due to the prolonged and consistent use of popular insecticides such as organochlorides, carbamates, pyrethroids, and organophosphates over numerous years.[24]

Furthermore, these insecticides can present disadvantages such as toxicity to humans and other nontarget species, degradation of the aquatic environment, high annual costs, and the development of target-resistant populations. In response to the challenges of controlling *Aedes aegypti* and other mosquito species, it is clear and urgent to find new insecticides that overcome the adverse problems of currently used insecticides. So, the present study reports the synthesis of some *N*-aryl 2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetamide compounds, evaluates their *Aedes aegypti* mosquito larvicidal activity, and investigates their toxicity to nontarget organisms.

MATERIALS AND METHODS

Materials. All starting materials were sourced from Acros Organics or Xilong (distributed by Thinh Phat Scientific Equipment Co., Ltd., Hanoi City, Vietnam) and used without purification. Melting points were measured in open capillary tubes on a Gallenkamp melting point apparatus without calibration. The structures of all compounds were confirmed by their IR, ^{1}H NMR, ^{13}C NMR, and HR-MS spectral data. IR spectra (ν, cm^{-1}) were recorded on an FTIR-8400S−SHIMADZU spectrometer using KBr pellets. The NMR spectra were recorded on a Bruker Avance III spectrometer (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) using residual solvent DMSO-d_6 signals (δ_H 2.50, δ_C 39.52) as internal references. The spin−spin coupling constants (J) are given in hertz. Peak multiplicity is reported as b (broad), s (singlet), d (doublet), dd (doublet-doublet), ddd (doublet-doublet-doublet), and *m* (multiplet), respectively. The HR-ESI-MS spectra were recorded on an Agilent 6200 Q-TOF B.06.01 spectrometer in positive mode.

Synthesis of Derivatives of 2-Methylquinazolin-4(3*H*)-one. The target compounds were synthesized according to the synthetic route in Scheme 1.

*2-Methylquinazolin-4(3H)-one (**2**).* The mixture of anthranilic acid (13.7 g, 0.1 mol) and acetic anhydride (5 mL) was refluxed for 1.0 h. After cooling, ammonium acetate (7.7 g, 0.1 mol) was added and then the reaction mixture was refluxed with stirring for 3.0 h. Finally, the mixture was cooled to room temperature and poured into ice-cold water. The precipitate was filtered and recrystallized from ethanol and water to give white needle crystals. Yield: 60.0%. Mp. 239−241 °C;[25] ^{1}H NMR (DMSO-d_6) δ: 12.19 (1H, b), 8.07 (1H, dd, J_1 = 8.0 Hz, J_2 = 1.0 Hz), 7.75 (1H, ddd, $J_1 = J_2$ = 7.5 Hz, J_3 = 1.5 Hz), 7.56 (1H, d, J = 8.0 Hz), 7.44 (1H, d, J = 8.0 Hz), 2.35 (3H, s); IR (KBr) cm^{-1}: 3404, 3034, 2868, 1655, 1607, 1468. HR-ESI-MS *m/z*: 161.0722 ([M + H]$^+$, Calcd for $C_9H_9N_2O$: 161.0714).

General Procedure for the Synthesis of *N*-Aryl-2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetamide Compounds (4a−i). An equimolar mixture of 2-methylquinazolin-4(3*H*)-one (0.16 g, 1 mmol) and anhydrous potassium carbonate (0.138 g, 1 mmol) in acetone (20 mL) was stirred for 30 min; then, 1 mmol of a definite *N*-aryl-2-chloroacetamide compound (**3a−i**) was added. The reaction mixture was refluxed for 2 h with stirring, then cooled to room temperature, and poured into ice-cold water. The white precipitate was filtered off and purified by crystallization from ethanol to afford pure product **4a−i**, respectively.

*N-(4-Bromophenyl)-2-(2-methyl-4-oxoquinazolin-3(4H)-yl)acetamide (**4a**).* Yield: 82.0%. Mp. 288−289 °C; ^{1}H NMR (DMSO) δ: 10.60 (1H, s), 8.10 (1H, dd, J_1 = 8.0 Hz, J_2 = 1.5 Hz), 7.83 (1H, dd, $J_1 = J_2$ = 7.5 Hz), 7.64 (1H, d, J = 8.5 Hz), 7.58 (2H, d, J = 9.0 Hz), 7.52 (2H, d, J = 8.5 Hz), 7.51 (1H, dd, $J_1 = J_2$ = 8.0 Hz), 4.98 (2H, s), 2.56 (3H, s); ^{13}C NMR (DMSO) δ: 166.2 (s), 161.7 (s), 155.8 (s), 147.6 (s), 138.4 (s), 135.0 (s), 132.2 (s), 127.1 (s), 126.9 (s), 126.6 (s), 121.6 (s), 120.1 (s), 115.7 (s), 47.5 (s), 23.5 (s); IR (KBr) cm^{-1}: 3310, 3281, 3065, 2918, 1686, 1647, 1595, 1543, 656; HR-ESI-MS *m/z*: 372.0353 ([M + H]$^+$, Calcd for $C_{17}H_{15}BrN_3O_2$: 372.0348).

*N-(4-Chlorophenyl)-2-(2-methyl-4-oxoquinazolin-3(4H)-yl)acetamide (**4b**).* Yield: 78.0%. Mp. 256−257 °C; ^{1}H NMR (DMSO) δ: 10.61 (1H, s), 8.11 (1H, d, J = 8.0 Hz), 7.83 (1H, dd, $J_1 = J_2$ = 7.5 Hz), 7.64 (3H, d, J = 9.0 Hz), 7.51 (1H, dd, $J_1 = J_2$ = 7.5 Hz), 7.39 (2H, d, J = 9.0 Hz), 4.98 (2H, s), 2.56 (3H, s); ^{13}C NMR (DMSO) δ: 166.2 (s), 161.7 (s), 155.8 (s), 147.6 (s), 138.0 (s), 135.0 (s), 129.2 (s), 127.7 (s), 127.1 (s), 126.8 (s), 126.6 (s), 121.2 (s), 120.1 (s), 47.5 (s), 23.5 (s); IR (KBr) cm^{-1}: 3312, 3283, 3067, 2972, 1686, 1647, 1591, 1547, 1470, 818; HR-ESI-MS *m/z*: 328.0865 ([M + H]$^+$, Calcd for $C_{17}H_{15}ClN_3O_2$: 328.0853).

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*N-(3-Chlorophenyl)-2-(2-methyl-4-oxoquinazolin-3(4H)-yl)acetamide (**4c**).* Yield: 69.0%. Mp. 258−259 °C; ^{1}H NMR (DMSO) δ: 10.67 (1H, s), 8.09 (1H, dd, J_1 = 7.5 Hz, J_2 = 1.5 Hz), 7.83 (1H, ddd, J_1 = J_2 = 7.0 Hz, J = 1.5 Hz), 7.80 (1H, dd, J_1 = J_2 = 1.5 Hz), 7.63 (1H, d, J = 8.0 Hz), 7.51 (1H, dd, J_1 = J_2 = 7.5 Hz), 7.45 (1H, d, J = 8.0 Hz), 7.37 (1H, dd, J_1 = J_2 = 8.0 Hz), 7.15 (1H, dd, J_1 = 8.0 Hz, J_2 = 1.0 Hz), 4.97 (2H, s), 2.55 (3H, s); ^{13}C NMR (DMSO) δ: 166.5 (s), 161.7 (s), 155.8 (s), 147.6 (s), 140.5 (s), 135.1 (s), 133.6 (s), 131.1 (s), 127.1 (s), 126.9 (s), 126.6 (s), 123.8 (s), 120.1 (s), 119.2 (s), 118.0 (s), 47.6 (s), 23.5 (s); IR (KBr) cm^{-1}: 3318, 3285, 3007, 2916, 1692, 1647, 1593, 1553, 772; HR-ESI-MS *m/z*: 328.0866 ([M + H]$^+$, Calcd for $C_{17}H_{15}ClN_3O_2$: 328.0853).

*N-(2-Chlorophenyl)-2-(2-methyl-4-oxoquinazolin-3(4H)-yl)acetamide (**4d**).* Yield: 75.0%. Mp. 266−267 °C; ^{1}H NMR (DMSO) δ: 10.11 (1H, s), 8.12 (1H, dd, J_1 = 8.0 Hz, J_2 = 1.0 Hz), 7.83 (1H, ddd, J_1 = J_2 = 7.5 Hz, J_3 = 1.5 Hz), 7.71 (1H, dd, J_1 = 8.0 Hz, J_2 = 1.5 Hz), 7.63 (1H, d, J = 8.0 Hz), 7.53 (1H, d, J = 7.5 Hz), 7.51 (1H, dd, J_1 = J_2 = 7.5 Hz), 7.34 (1H, dd, J_1 = J_2 = 7.5 Hz), 7.23 (1H, dd, J_1 = J_2 = 7.5 Hz), 5.08 (2H, s), 2.57 (3H, s); ^{13}C NMR (DMSO) δ: 166.7 (s), 161.7 (s), 155.8 (s), 147.6 (s), 135.0 (s), 134.8 (s), 130.1 (s), 128.0 (s), 127.2 (s), 127.1 (s), 126.9 (s), 126.8 (s), 126.7 (s), 120.2 (s), 47.0 (s), 23.4 (s); IR (KBr) cm^{-1}: 3204, 3038, 1682, 1665, 1605, 1541, 781; HR-ESI-MS *m/z*: 328.0867 ([M + H]$^+$, Calcd for $C_{17}H_{15}ClN_3O_2$: 328.0853).

*N-(4-Methoxyphenyl)-2-(2-methyl-4-oxoquinazolin-3(4H)-yl)acetamide (**4e**).* Yield: 78%. Mp: 234−235 °C; ^{1}H NMR (DMSO) δ: 10.31 (1H, s), 8.10 (1H, d, J = 8.0 Hz), 7.83 (1H, dd, J_1 = J_2 = 7.5 Hz), 7.63 (1H, d, J = 8.0 Hz), 7.51 (1H, dd, J_1 = J_2 = 7.5 Hz), 7.50 (2H, d, J = 8.0 Hz), 6.90 (2H, d, J = 9.0 Hz), 4.95 (2H, s), 3.73 (3H, s), 2.55 (3H, s); ^{13}C NMR (DMSO) δ: 165.4 (s), 161.7 (s), 160.0 (s), 155.9 (s), 147.6 (s), 135.0 (s), 132.2 (s), 127.1 (s), 126.8 (s), 126.6 (s), 121.2 (s), 120.1 (s), 114.4 (s), 55.7 (s), 47.3 (s), 23.4 (s); IR (KBr) cm^{-1}: 3277, 3017, 2957, 1676, 1643, 1597, 1533; HR-ESI-MS *m/z*: 324.1361 ([M + H]$^+$, Calcd for $C_{18}H_{18}N_3O_3$: 324.1348).

*2-(2-Methyl-4-oxoquinazolin-3(4H)-yl)-N-(p-tolyl)-acetamide (**4f**).* Yield: 83%. Mp: 252−253 °C; ^{1}H NMR (DMSO) δ: 10.38 (1H, s), 8.10 (1H, d, J = 8.0 Hz), 7.83 (1H, dd, J_1 = J_2 = 7.5 Hz), 7.63 (1H, d, J = 8.5 Hz), 7.51 (1H, dd, J_1 = J_2 = 7.0 Hz), 7.48 (2H, d, J = 8.5 Hz), 7.13 (2H, d, J = 8.5 Hz), 4.96 (2H, s), 2.55 (3H, s), 2.26 (3H, s); ^{13}C NMR (DMSO) δ: 165.7 (s), 161.7 (s), 155.9 (s), 147.6 (s), 136.6 (s), 135.0 (s), 133.0 (s), 129.7 (s), 127.1 (s), 126.8 (s), 126.6 (s), 120.1 (s), 119.6 (s), 47.4 (s), 23.5 (s), 20.9 (s); IR (KBr) cm^{-1}: 3312, 3061, 2918, 1667, 1595, 1537; HR-ESI-MS *m/z*: 308.1412 ([M + H]$^+$, Calcd for $C_{18}H_{18}N_3O_2$: 308.1399).

*2-(2-Methyl-4-oxoquinazolin-3(4H)-yl)-N-(o-tolyl)-acetamide (**4g**).* Yield: 77%. Mp: 258−259 °C; ^{1}H NMR (DMSO) δ: 9.83 (1H, s), 8.12 (1H, d, J = 7.5 Hz), 7.83 (1H, dd, J_1 = J_2 = 8.0 Hz), 7.63 (1H, d, J = 8.5 Hz), 7.51 (1H, dd, J_1 = J_2 = 8.0 Hz), 7.40 (1H, d, J = 7.5 Hz), 7.24 (1H, d, J = 7.5 Hz), 7.18 (1H, dd, J_1 = J_2 = 7.5 Hz), 7.11 (1H, dd, J_1 = J_2 = 7.5 Hz), 5.03 (2H, s), 2.57 (3H, s), 2.26 (3H, s); ^{13}C NMR (DMSO) δ: 166.1 (s), 161.7 (s), 155.9 (s), 147.6 (s), 136.2 (s), 135.0 (s), 132.4 (s), 130.9 (s), 127.1 (s), 126.8 (s), 126.7 (s), 126.5 (s), 126.0 (s), 125.5 (s), 120.2 (s), 47.0 (s), 23.4 (s), 18.3 (s); IR (ν, cm^{-1}): 3246 (NH), 3059 (C−H aromatic), 2947 (C−H aliphatic), 1682, 1653 (C═O), 1599, 1539 (C═N, C═C aromatic); HR-ESI-MS *m/z*: 330.1218 ([M + Na]$^+$, Calcd for $C_{18}H_{17}N_3NaO_2$: 330.1218).

*2-(2-Methyl-4-oxoquinazolin-3(4H)-yl)-N-phenylacetamide (**4h**).* Yield: 80%. Mp: 261−262 °C; ^{1}H NMR (DMSO) δ: 10.48 (1H, s), 8.10 (1H, d, J = 8.0 Hz), 7.83 (1H, dd, J_1 = J_2 = 8.0 Hz), 7.64 (1H, d, J = 8.0 Hz), 7.60 (2H, d, J = 7.5 Hz), 7.51 (1H, dd, J_1 = J_2 = 7.5 Hz), 7.33 (2H, dd, J_1 = J_2 = 7.0 Hz), 7.08 (1H, dd, J_1 = J_2 = 8.0 Hz), 4.98 (2H, s), 2.56 (3H, s); ^{13}C NMR (DMSO) δ: 166.0 (s), 161.7 (s), 155.9 (s), 147.6 (s), 139.1 (s), 135.0 (s), 129.3 (s), 127.1 (s), 126.9 (s), 126.6 (s), 124.1 (s), 120.1 (s), 119.6 (s), 47.4 (s), 23.4 (s); IR (ν, cm^{-1}): 3279 (NH), 2970 (C−H aliphatic), 1684, 1651 (C═O), 1595, 1549 (C═N, C═C aromatic); HR-ESI-MS *m/z*: 294.1269 ([M + H]$^+$, Calcd for $C_{17}H_{16}N_3O_2$: 294.1243).

*2-(2-Methyl-4-oxoquinazolin-3(4H)-yl)-N-(4-nitrophenyl)-acetamide (**4i**).* Yield: 76%. Mp: 297−298 °C. ^{1}H NMR (DMSO) δ: 11.09 (1H, s), 8.25 (2H, d, J = 9.0 Hz), 8.10 (1H, d, J = 9.0 Hz), 7.85 (2H, d, J = 9.0 Hz), 7.84 (1H, dd, J_1 = J_2 = 9.0 Hz), 7.64 (1H, d, J = 8.5 Hz), 7.52 (1H, dd, J_1 = J_2 = 7.5 Hz), 5.04 (2H, s), 2.57 (3H, s); ^{13}C NMR (DMSO) δ: 167.2 (s), 161.7 (s), 155.8 (s), 147.6 (s), 145.2 (s), 143.0 (s), 135.1 (s), 127.1 (s), 126.9 (s), 126.6 (s), 125.6 (s), 120.0 (s), 119.5 (s), 47.8 (s), 23.5 (s); IR (ν, cm^{-1}): 3283 (NH), 3092 (C−H aromatic), 1694, 1641 (C═O), 1589, 1570 (C═N, C═C aromatic), 1497, 1470 (NO_2); HR-ESI-MS *m/z*: 339.1107 ([M + H]$^+$, Calcd for $C_{17}H_{15}N_4O_4$: 339.1093).

Biological Evaluation. *Mosquito Larvicidal Assay.* Adults reared from wild *Aedes aegypti* larvae were maintained under laboratory conditions (at 25 ± 2 °C, 65−75% relative humidity, and a 12:12 h light/dark cycle) at Duy Tan University. The larvae of subsequent generations were used to evaluate larvicidal activity. The larvicidal activities were performed according to our previous descriptions.[26] Twenty larvae were transferred into 250 mL beakers containing 150 mL of the test solution at 100, 75, 50, 25, 12.5, 6.25, 3.125, and 1.5625 μg/mL. DMSO (Merck) was used to dissolve the pure compounds; permethrin was used as a positive control, and a 150 mL solution containing 1 mL of DMSO was used as a negative control. The concentration of compounds, permethrin (positive control), and DMSO (negative control) was measured four times (i.e., four independent experiments). Mortality was recorded after 24, 48, 72, and 96 h of exposure.

Diplonychus rusticus Insecticidal Assay. Adults of *Diplonychus rusticus* were collected and identified in our previous report.[27] Common water hyacinth plants (*Eichhornia crassipes* (Mart.) Solms) were released into tanks to provide shelter for *Diplonychus rusticus*. The 20 insects were screened against pure compounds at a concentration of 25 μg/mL, four independent experiments were conducted, and mortality was recorded after 24 h and 48 h exposure.

Acetylcholinesterase (AChE) Inhibition Assay. Acetylcholinesterase (AChE) inhibitory activity of compounds was performed according to the method described by Ellman and our previous study.[28] The stock solution was obtained by dissolving the compounds in DMSO (Merck), which was then diluted with H_2O (deionized distilled water) to obtain different experimental concentrations. Each solution mixture consisted of 140 μL of phosphate buffer solution (pH 8); 20 μL of test compound solutions at concentrations of 500, 100, 20, and 4 μg/mL; and 20 μL of the electric eel (*Electrophorus electricus*) AChE (0.25 IU/mL). The reaction mixtures were transferred to the test wells of a 96-well microtiter plate and incubated at 25 °C for 15 min. Then, 10 μL of dithiobisnitrobenzoic acid (DTNB, 2.5 mM) and 10 μL of acetylthiocholine iodide (ACTI, 2.5 mM) were added to each of the test wells and incubation was

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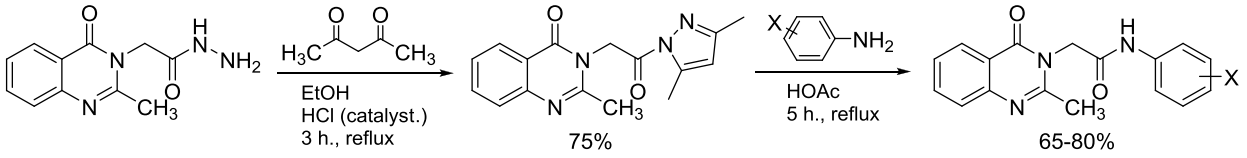
Table 1. *Aedes aegypti* Protein Target Structures from Homology Modeling

Aedes aegypti protein target	target sequence (UniProt ID)	template PDB structure	sequence identity (%)	global model quality estimation (GMQE)
acetylcholinesterase (AChE)	Q9TX11	1QO9	68.96	0.78
	Q9TX11	1DX4	69.96	0.79
acetylcholine receptor (AChR)	A0A6I8T9N7	7EKP	42.17	0.60
angiotensin-converting enzyme 2 (ACE2)	A0A1S4G6D0	6S1Y	71.43	0.93
carboxylesterase 5A (CBEB5A)	Q17G39	4FNM	31.26	0.64

Scheme 2. Synthetic Pathway of the *N*-Aryl-2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetamide



(X = H, 4-Br, 2-Cl, 2-CH_3, 3-CH_3, 4-CH_3, 2-OCH_3, 3-OCH_3, 4-OCH_3, 2-OC_2H_5, 3-OC_2H_5, 4-OC_2H_5, 2-NO_2, 3-NO_2, 4-NO_2)

continued for 10 min at 25 °C. At the experiment's end, each solution's absorbance was measured at 405 nm. Galantamine was used as a positive control. The negative control well did not contain the test sample. Each test was carried out in triplicate (i.e., three different plates).

Molecular Modeling Density Functional Theory (DFT) Calculation. The DFT method was employed to investigate the ground-state geometry of the studied compounds. The B3LYP function and the 6-311++g(d,p) set of basis functions were used in the optimization process using the G09 software.[29,30] The optimized structures were analyzed to determine the compound's energy, charge properties, and vibrational frequencies. The optimization is successful if the optimized structure contains no imaginary frequencies and meets the convergence criteria of the G09 program.

Molecular Docking. Molecular docking of the *N*-aryl-2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetamide compounds was carried out according to Alamzeb et al.[31] The structures of the compounds were assembled using Spartan 18 for Windows, v 1.4.4 (Wavefunction, Inc.). Molecular docking was employed using Molegro Virtual Docker v 6.0.1 (Molegro ApS). A total of 17 relevant *Aedes Aegytpi* protein targets were used for molecular docking. Twelve protein targets were obtained from the Protein Data Bank (PDB): arylalkylamine *N*-acetyltransferase, 4FD4, 4FD5, and 4FD6; carboxylesterase, 5W1U; D7 salivary protein, 3BKS, and 3DXL; glutathione *S*-transferase, 5FT3; odorant binding protein, 3K1E, 3OGN, 6OII, 6OMW, 6OPB, and 6P2E; and sterol carrier protein-2, 2QZT. Five *Aedes aegypti* target proteins were prepared by homology modeling (see below): acetylcholinesterase (AChE, based on PDB structures 1DX4 and 1QO9), acetylcholine receptor (AChR, based on 7EKP), angiotensin-converting enzyme 2 (ACE2, based on 6S1Y), and carboxylesterase 5A (CBEB5A, based on 4FNM). The orientations of the ligands with the target proteins were ranked based on the MolDock "rerank" energy values (E_{dock}) and then corrected to account for the bias due to molecular weight to give normalized docking scores (DS_{norm}).[32]

Homology Modeling. Homology models of the *Aedes aegypti* proteins AChE, AChR, ACE2, and CBEB5A were created using the SWISS-MODEL server (https://swissmodel.expasy.org/). Appropriate protein target sequences were obtained from UniProt Knowledgebase (UniProtKB, https://β.uniprot.org/). Three-dimensional structural models were obtained based on multiple-threading alignments; the global model quality estimation was used to rank models (Table 1).

Data Analysis. Lethality data were subjected to log-probit analysis[33] to obtain LC_{50} values, LC_{90} values, and 95% confidence limits using Minitab version 19.2020.1 (Minitab, LLC, State College, PA).

RESULTS AND DISCUSSION

Chemical Experiment Results. 2-Methylquinazolin-4(3*H*)-one (**2**) was prepared according to the reported methods.[21] The affording products showed similarity in melting point and spectral characteristics, including IR[25] and ^{1}H NMR,[34] with those reported for the corresponding compounds in the literature (Table S1 and Figure S1).

The procedure described in the literature was applied to the synthesis of *N*-aryl-2-chloroacetamide compounds (**3a−i**).[35] Accordingly, a defined aromatic amine reacted with chloroacetyl chloride in the presence of sodium acetate to obtain the corresponding acetamides. The products' structures were confirmed by comparing their melting point with our previous study for the corresponding *N*-aryl-2-chloroacetamides.[33]

In alkaline media, (**2**) in the role of a nucleophilic agent, attacks the 2-chloroacetamide molecules (**3a−i**) and substitutes the chlorine atom to form the corresponding *N*-aryl-2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetamide compounds (**4a−i**). Acetone is used as an aprotic solvent to facilitate these S_N2 reactions. The HR-MS spectra of the products (**4a−i**) showed pseudomolecular ion peaks $[M + H]^+$($[M + Na]^+$ for compound **4g**) in agreement with their molecular formulas. In the IR spectra of compounds (**4a−i**), in addition to the absorption peak of the C=O group in the quinazolin-4-one ring around 1641−1667 cm^{-1}, the appearance of a new band around 1670−1694 cm^{-1} indicated the presence of the C=O group in the acetamide group. The N−H bond in the acetamide group absorbs at a lower frequency (3204−3318 cm^{-1}) than that of the N−H bond in the original 2-methylquinazolin-4(3*H*)-one molecule (3404 cm^{-1}). In the ^{1}H NMR spectra, the signal of the N−H proton in the acetamide group of the compounds (**4a−i**) is also upfield shifted (9.83−11.09 ppm) in comparison to the signal of the N−H proton in the molecule of the original compound (**2**) (12.19 ppm). Compared with the ^{1}H NMR spectrum and the ^{13}C NMR spectrum of compound (**2**), the remarkableness in the ^{1}H NMR and ^{13}C NMR spectra of compounds (**4a−i**) is an appearance of a signal in the aliphatic

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Table 2. *Aedes aegypti* Larvicidal Activity (μg/mL) of Oxoquinazolines[e]

compound	LC_{50}	LC_{90}	χ^2	p
4a		24 h		
	5.819 (5.358−6.391)	8.528 (7.729−9.730)	1.57	0.456
		48 h		
	3.663 (3.271−4.078)	6.396 (5.755−7.334)	0.00144	0.999
		72 h		
	2.261 (1.723−2.693)	5.273 (4.620−6.340)	4.02	0.134
		96 h		
	1.486 (0.704−1.984)	4.470 (3.864−5.530)	0.953	0.621
4b		24 h		
	12.68 (11.32−14.16)	31.11 (26.90−37.20)	19.71	0.001
		48 h		
	3.642 (3.270−4.042)	7.610 (6.607−9.160)	5.76	0.218
		72 h		
	3.476 (3.134−3.846)	6.945 (6.055−8.327)	4.21	0.379
		96 h		
	[a]	[a]		
4c		24 h		
	15.06 (12.70−18.02)	[b]	10.20	0.017
		48 h		
	10.11 (8.10−12.38)	[b]	7.37	0.061
		72 h		
	4.423 (2.770−6.030)	[b]	3.78	0.286
		96 h		
	1.799 (0.574−3.188)	[b]	2.59	0.459
4d		24 h		
	52.38 (36.87−88.24)	[b]	14.79	0.005
		48 h		
	22.73 (16.39−35.22)	[b]	11.40	0.022
		72 h		
	4.201 (1.867−6.900)	[b]	5.59	0.232
		96 h		
	[c]	[c]		
4e		24 h		
	9.252 (8.156−10.562)	25.81 (21.16−33.50)	5.66	0.129
		48 h		
	4.274 (3.643−4.966)	16.26 (12.95−22.06)	7.96	0.047
		72 h		
	2.756 (2.219−3.294)	12.27 (9.67−17.09)	7.77	0.051
		96 h		
	1.280 (0.801−1.729)	7.414 (5.780−10.598)	1.83	0.609
4f		24 h		
	8.671 (7.863−9.617)	14.67 (13.22−16.69)	4.22	0.239
		48 h		
	5.921 (4.944−6.924)	14.33 (12.51−17.09)	1.03	0.795
		72 h		
	2.085 (0.418−3.258)	11.29 (9.56−14.17)	0.816	0.846
		96 h		
	[a]	[a]		
4g	not soluble			
4h	>50 (96 h)	>50 (96 h)	[d]	[d]
4i	not soluble			
permethrin	24 h	24 h	4.64	0.031
	0.000643 (0.000551−0.000753)	0.00246 (0.00192−0.00344)		

[a]LC_{50} and LC_{90} not reliable; >50% lethality at the lowest concentration. [b]LC_{90} values not reliable; <90% lethality at the highest concentration. [c]Lethality too flat across the different concentrations, not dose-dependent. [d]Not defined. [e]χ^2: goodness of fit. p: determined by comparison between the probabilities based on the experimental data and the probabilities from the model.

region (around 4.95−5.08 ppm in the ^{1}H NMR spectra and around 47.0−47.8 ppm in the ^{13}C NMR spectra). These signals correspond to those of protons and carbon in the methylene group. Moreover, additional signals corresponding to the protons and carbons in the *N*-aryl groups also appear in the aromatic region of the spectra (Figures S2−S10).

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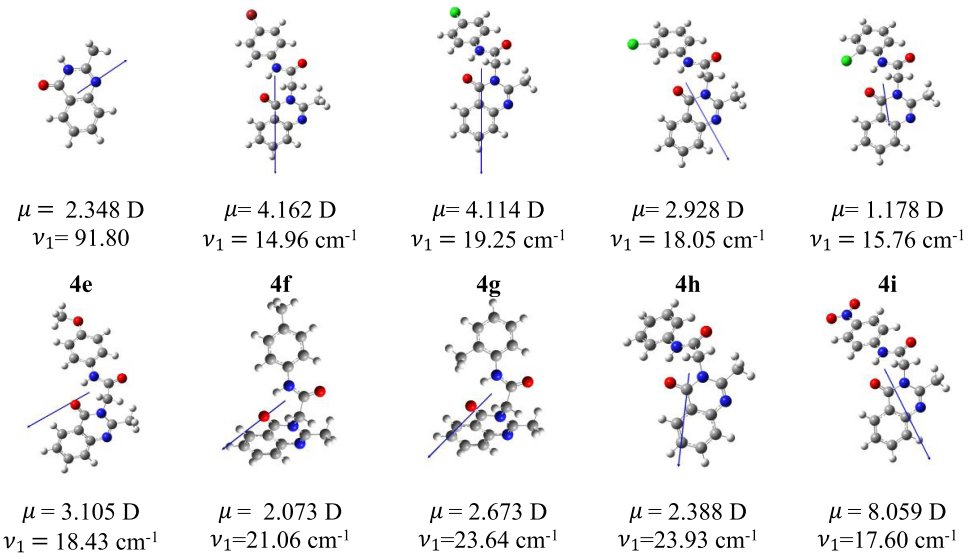

Figure 1. Optimized structures, magnitude and orientation of electric dipole moment, and the lowest infrared vibrational frequency of the compounds **4a−4i** determined by B3LYP/6-311++g(d,p).

N-Aryl-2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetamide compounds were synthesized by Zotta et al.[36] according to the synthetic pathway in Scheme 2.

Obviously, this presented method presented by Zotta et al.[36] is more complicated than the synthetic procedure introduced in this work. The Zotta method requires preparation of a hydrazide intermediate, which is unnecessary in the procedure described herein. Besides that, a few characteristics of *N*-aryl-2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetamides (NMR and MS) were mentioned by Zotta et al.,[37] and these produces should be further investigated.

Biological Activity. All of the acetamide compounds (**4a−i**) containing heterocycle were evaluated for their potential activity against *Aedes aegypti* mosquito larvae and adults of *Diplonychus rusticus*. Except for the two compounds **4g** and **4i** which were insoluble, compound **4h** showed less activity; meanwhile, all other compounds showed greater larvicidal activities with LC_{50} values at 72 h of exposure varying between 2.085 and 4.201 μg/mL. (Table 2).

The 4X substituent derivatives, at 24 h of exposure, tend to show efficacy against mosquito larvae in the order of **4i** (4-NO_2) < **4b** (4-Cl) < **4e** (4-OCH_3) ≤ **4f** (4-CH_3) < **4a** (4-Br). However, the solubility of the compounds in DMSO may have influenced their biological activity. When changing the position of the Cl substituent, derivatives with different mosquito larval activity were created, specifically 24 h LC_{50} values in the order of **4b** (4-Cl) ≤ **4c** (3-Cl) < **4d** (2-Cl).

Several previous studies have shown that quinazolin-4(3*H*)-one derivatives exhibit potent insecticide activity. The 3-[(2-chloroquinolin-3-yl)methyl]quinazolin-4(3*H*)-ones have shown promising larvicidal activity against *Chironomus tentans* with LC_{50} values between 60 and 90 μg/mL.[9] All quinazolin-4(3*H*)-one derivatives synthesized by Anil exhibited potent insecticidal activity against *Periplanata americana* with knock-down time in the range of 6.5−22 h at 5 g/L.[10] Four 3-[4(3*H*)-quinazolinone-2-yl-thiomethyl]-1,2,4-triazole-5-thiol compounds were active against adults of *Chrysornyia albiceps* equivalent to malathion with LD_{50} from 2.20 to 4.20 μg/mL for males and from 4.20 to 5.20 μg/mL for females, but they did show weak activity against the larval state.[11] The 2-(substituted phenyl)-2,3-dihydroquinazolin-4(1*H*)-ones derivatives showed larvicidal activity comparable to temephos against *Anopheles arabiensis* and mortality rates ranging from 43 to 93% at 4 μg/mL after 48 h of exposure.[12] Most of the 2,3-dihydroquinazolin-4(1*H*)-one derivatives demonstrated moderate to high insecticidal activity against *M. separata*, particularly 2-(3-bromo-1-(3-chloropyridine-)-2-yl)-1*H*-pyrazol-5-yl-6-chloro-3,8-dimethyl-2,3-dihydroquinazolin-4(1*H*)-one showed 80% mortality at 5 μg/mL concentration.[13] A series of 6,8-dichloroquinazoline derivatives bearing a sulfide group showed good insecticidal activities against *Plutella xylostella*; among them, compound 6,8-dichloro-4-(((6-chloropyridine-3-yl)-methyl)thio)quinazoline has shown a mortality rate of 85% at 500 μg/mL.[14] The quinazoline derivatives containing 1,1-dichloropropene moiety displayed good insecticidal activities against *Prodenia litura* equivalent to positive control pyridalyl.[15] The compound methyl 4-(4-chlorophenyl)-8-iodo-2-methyl-6-oxo-1,6-dihydro-4*H*-pyrimido[2,1-*b*]quinazoline-3-carboxylate exhibiting larvicidal activity against *Anopheles arabiensis* was significantly stronger than temephos.[16]

The mechanism of the mosquito larvicidal activities of 2-methylquinazolin-4(3*H*)-one derivatives may be due to their effects on the central nervous system. Quinazolinone derivatives have been reported to exhibit anticonvulsant property.[38] Several thiadiazolyl and thiazolidinonyl quinazolin-4(3*H*)-one derivatives exhibited anticonvulsant activity compared with positive controls.[37] Several 3-substituted-2-(substituted-phenoxymethyl) quinazolin-4(3*H*)-one derivatives have demonstrated significant anticonvulsant activity.[39] Previously, 2-methyl-3-(*o*-tolyl)-4(3*H*)-quinazolone (methaqualone) was used for sedation and sleep induction. Derivatives bearing a substituted 1,3,4-thiadiazole may show inhibitory activity on AChE enzyme.[40] However, screening of compounds **4a−i** for inhibition of electric eel (*Electrophorus electricus*) AChE only displayed the median inhibitory activities (IC_{50}) ranging from 57 to 266 μg/mL. It may be that *Aedes aegypti* AChE will show better inhibition, but this enzyme was unavailable.

DFT Calculations. The analysis results of the electric dipole moment values determined by the B3LYP/6-311++G(d,p) method were consistent with previous studies on organic

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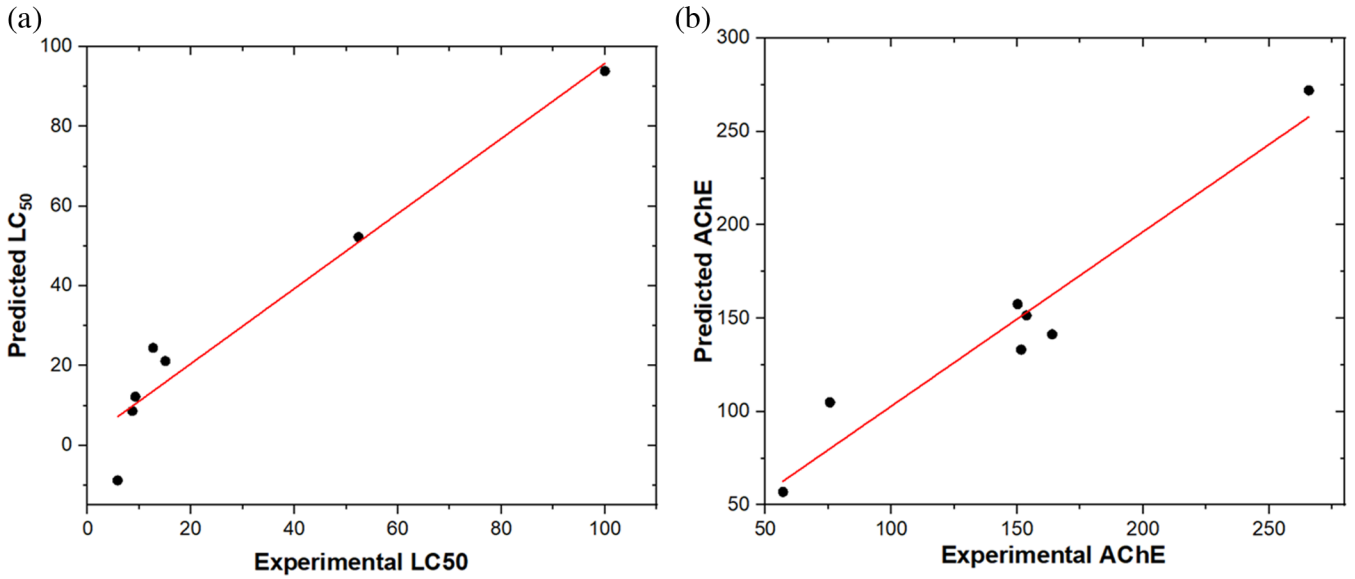

Figure 2. QSAR plots: (Left) Predicted versus experimental activities of LC_{50} (24 h) and (right) predicted versus experimental activities of AChE.

Table 3. AChE Inhibitory Activities of Oxoquinazolines[a]

concentration (μg/mL)	4a	4b	4c	4d	4e	4f	4g	4h	4i
250	48.46	72.94	97.84	70.27	nt	98.92	73.11	nt	75.60
100	28.64	34.72	56.54	36.55	nt	62.78	34.64	nt	35.55
20	13.99	26.64	29.31	19.23	nt	34.22	13.41	nt	6.08
4	3.75	18.15	15.40	7.58	nt	17.65	−1.33	nt	2.50
IC_{50}	265.77	163.89	75.64	151.50	nt	57.03	150.14	nt	153.64

[a]nt: not tested.

compounds with mosquito-repellent activity.[29,30] The calculated values of the electric dipole moment, HOMO and LUMO energies, and Mulliken charge on the N atoms and ring, logP: octanol−water partition coefficient are presented in Table S2. The results showed that the electric dipole moment values of compounds **4a**−**4i** ranged from 1.178 to 8.059 D, with the highest value observed for compound **4i**, which contains the *para*-substituted NO_2 group with the lowest HOMO energy value of −7.026 eV. Similarly, compounds **4a** and **4b**, containing the *para*-substituted Br and Cl groups, respectively, exhibited an increase in electric dipole moment values. In contrast, compound 4f with the CH_3 group substitution had a lower value than compound **4h**. The study of Silva[41] found that some compounds with high dipole moments did not show good larvicidal activity. This is consistent with the results of *Aedes aegypti* larvae activity testing, which showed that the compound with a Cl group at the *ortho* position had the lowest μ value of 1.178 D and the strongest activity with a value of 52.38 μg/mL at 24 h, and this also occurred at 48 h with a value of 22.73 μg/mL, while at 72 h, its value was quite large at 4.201 μg/mL. Log *P* is also a parameter related to the evaluation of the insecticidal activity of compounds. According to da Silva et al.,[42] substituting groups can enhance their activity while reducing the dipole moment values and increasing the log *P* values. The Cl group at the *ortho* position in **4d** (2-Cl) performed very well with a sizeable log *P* value of 1.52 compared to compound **4h**. The results of dipole moment values and log *P* values are consistent with the calculated structural parameters of the molecules (Figure 1).

The present study aims to develop a quantitative structure−activity relationship (QSAR) model to establish a relationship between the structural parameters and larvicidal activity of compounds.[30,41,42] The indicator variable of activity LC_{50} (24 h) and AChE was used to develop the QSAR model. The best relationship was obtained by a linear combination of five descriptors, namely, electric dipole moment (μ), energy of the HOMO (ε_{HOMO}), energy of the LUMO (ε_{LUMO}), HOMO−LUMO energy difference (Δ_ε), and octanol−water partition coefficient (log *P*). The multiple linear regression equation obtained from this model was tested for its reliability by comparing predicted and observed larvicide and inhibitory activities (Figure 2). The index of the parameter deviation of the statistical equation, R^2, showed that the results of the regression were reliable. The QSAR model revealed a positive correlation between the values of ε_{LUMO} and electric dipole moment (μ) with LC_{50}, indicating that an increase in these factors leads to an increase in LC_{50} values. On the other hand, a negative correlation was found between the values of ε_{HOMO}, Δ_ε, and log *P* with LC_{50}, indicating that an increase in these factors results in a decrease in LC_{50} values. This is the opposite when considering the correlation of AChE (eq 2) with structural parameters.

$$LC_{50} = 422.005 + 67.564 \times \mu - 232\,52.443 \times \varepsilon_{HOMO} + 24\,180.103 \times \varepsilon_{LUMO} - 174.222 \times \log P - 22\,943.270 \times \Delta_\varepsilon \quad (1)$$

where $n = 7$, $R = 0.970$ and $R^2 = 0.941$.

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Table 4. Lowest-Energy Docking Scores and Key Intermolecular Contacts of *N*-Aryl-2-(2-methyl-4-oxoquinazolin-3(4*H*)-yl)acetamide Derivatives with *Aedes aegypti* Acetylcholine Receptor, Acetylcholinesterase, and Odorant Binding Protein

compound	DS_{norm}	interacting protein residues
		Aedes aegypti AChR PDB (7EKP)
4a	−109.4	Tyr200 (face-to-face $\pi-\pi$), Phe147 (hydrophobic), Asn114 (hydrophobic), Glu149 (hydrophobic), Phe163 (hydrophobic), Gln59 (H-bond), Cys148 (hydrophobic), Tyr113 (edge-to-face $\pi-\pi$)
4b	−113.0	Tyr200 (face-to-face $\pi-\pi$), Asn114 (H-bond), Phe147 (hydrophobic), Trp75 (face-to-face $\pi-\pi$), Asp198 (hydrophobic), Gln59 (H-bond)
4c	−112.2	Tyr200 (face-to-face $\pi-\pi$), Asn114 (H-bond), Trp75 (face-to-face $\pi-\pi$), Phe147 (hydrophobic), Asp198 (hydrophobic), Gln59 (H-bond)
4d	−115.9	Tyr200 (face-to-face $\pi-\pi$), Trp75 (face-to-face $\pi-\pi$), Asp198 (hydrophobic), Asn114 (hydrophobic), Tyr113 (edge-to-face $\pi-\pi$), Phe147 (hydrophobic), Lys165 (H-bond)
4e	−118.3	Tyr200 (face-to-face $\pi-\pi$), Phe147 (edge-to-face $\pi-\pi$), Trp75 (face-to-face $\pi-\pi$), Asp198 (hydrophobic), Asn114 (hydrophobic), Gln59 (H-bond)
4f	−118.4	Tyr200 (face-to-face $\pi-\pi$), Phe147 (hydrophobic, H-bond), Asn114 (hydrophobic), Glu149 (hydrophobic), Phe163 (hydrophobic), Cys148 (hydrophobic), Gln59 (H-bond)
4g	−115.2	Tyr200 (face-to-face $\pi-\pi$), Phe147 (hydrophobic), Asn114 (hydrophobic), Trp75 (face-to-face $\pi-\pi$), Tyr113 (hydrophobic), Asp198 (hydrophobic), Ser58 (H-bond)
4h	−106.9	Tyr200 (face-to-face $\pi-\pi$), Asn114 (hydrophobic), Glu149 (hydrophobic), Phe147 (hydrophobic), Gln59 (H-bond)
4i	−119.4	Tyr200 (face-to-face $\pi-\pi$), Glu149 (H-bond), Trp75 (face-to-face $\pi-\pi$), Asp198 (hydrophobic), Phe163 (hydrophobic), Tyr113 (hydrophobic), Phe147 (hydrophobic), Cys148 (hydrophobic), Lys165 (H-bond)
		Aedes aegypti AChE PDB (1DX4)
4a	−106.9	Trp111 (face-to-face $\pi-\pi$), Tyr390 (face-to-face $\pi-\pi$), His501 (hydrophobic), Ser258 (H-bond)
4b	−110.1	Tyr390 (face-to-face $\pi-\pi$), Trp111 (face-to-face $\pi-\pi$), His501 (hydrophobic), Phe391 (edge-to-face $\pi-\pi$), Gly173 (hydrophobic), Trp291 (edge-to-face $\pi-\pi$), Ser258 (H-bond)
4c	−111.1	Tyr390 (face-to-face $\pi-\pi$), Trp111 (face-to-face $\pi-\pi$), His501 (hydrophobic), Trp291 (edge-to-face $\pi-\pi$), Phe391 (edge-to-face $\pi-\pi$), Ser258 (H-bond)
4d	−112.8	Tyr390 (face-to-face $\pi-\pi$), Trp111 (face-to-face $\pi-\pi$), His501 (hydrophobic), Trp291 (edge-to-face $\pi-\pi$), Phe391 (edge-to-face $\pi-\pi$), Ser258 (H-bond)
4e	−116.2	Trp111 (face-to-face $\pi-\pi$), Tyr390 (face-to-face $\pi-\pi$), His501 (hydrophobic, H-bond), Phe391 (edge-to-face $\pi-\pi$), Ser258 (H-bond)
4f	−114.4	Tyr390 (face-to-face $\pi-\pi$), Trp111 (face-to-face $\pi-\pi$), His501 (hydrophobic), Phe391 (edge-to-face $\pi-\pi$), Gly173 (hydrophobic), Ser258 (H-bond)
4g	−117.1	Trp111 (face-to-face $\pi-\pi$), Tyr390 (face-to-face $\pi-\pi$), Gly173 (hydrophobic), Glu108 (hydrophobic), Phe391 (edge-to-face $\pi-\pi$), Ser258 (H-bond)
4h	−114.8	Trp111 (face-to-face $\pi-\pi$), Tyr390 (face-to-face $\pi-\pi$), Gly173 (hydrophobic), Phe391 (edge-to-face $\pi-\pi$), Glu108 (hydrophobic), Ser258 (H-bond)
4i	−121.8	Tyr390 (face-to-face $\pi-\pi$), Trp111 (face-to-face $\pi-\pi$), His501 (hydrophobic), Phe391 (edge-to-face $\pi-\pi$), Gly173 (hydrophobic), Ser258 (H-bond)
9-(3-phenylmethylamino)-1,2,3,4-tetrahydroacridine[a]	−118.7	Phe391 (edge-to-face $\pi-\pi$), Gly173 (hydrophobic), Phe350 (edge-to-face $\pi-\pi$), Trp291 (edge-to-face $\pi-\pi$), Tyr390 (edge-to-face $\pi-\pi$)
		Aedes aegypti OBP PDB 6OMW
4a	−105.4	Phe108 (hydrophobic), Leu68 (hydrophobic), Phe105 (hydrophobic), Phe51 (hydrophobic), Ile116 (hydrophobic), Gly104 (hydrophobic)
4b	−111.0	Phe108 (hydrophobic), Leu68 (hydrophobic), Phe105 (hydrophobic), Phe51 (hydrophobic), Ile116 (hydrophobic), Gly104 (hydrophobic)
4c	−110.6	Phe108 (hydrophobic), Leu68 (hydrophobic), Phe105 (hydrophobic), Gly104 (hydrophobic)
4d	−106.4	Phe108 (hydrophobic), Leu68 (hydrophobic), Phe105 (hydrophobic), Pro63 (hydrophobic), Phe51 (hydrophobic), Ile116 (hydrophobic)
4e	−113.2	Phe108 (hydrophobic), Leu68 (hydrophobic), Phe105 (hydrophobic), Phe51 (hydrophobic), Gly104 (hydrophobic), Ile116 (hydrophobic)
4f	−114.8	Phe108 (hydrophobic), Leu68 (hydrophobic), Phe105 (hydrophobic), Phe51 (hydrophobic), Ile116 (hydrophobic), Gly104 (hydrophobic)
4g	−111.5	Leu68 (hydrophobic), Phe108 (hydrophobic), Gly104 (hydrophobic), Phe105 (hydrophobic)
4h	−116.3	Phe108 (hydrophobic), Leu68 (hydrophobic), Phe105 (hydrophobic), Gly104 (hydrophobic), Phe51 (hydrophobic)
4i	−116.4	Leu68 (hydrophobic), Phe108 (hydrophobic), Gly104 (hydrophobic), Ile116 (hydrophobic), Phe105 (hydrophobic), Leu72 (hydrophobic)
palmitoleic acid[a]	−91.8	Arg15 (electrostatic), Phe51 (hydrophobic), Phe105 (hydrophobic), Phe108 (hydrophobic), Ile116 (hydrophobic)

[a]Co-crystallized ligand.

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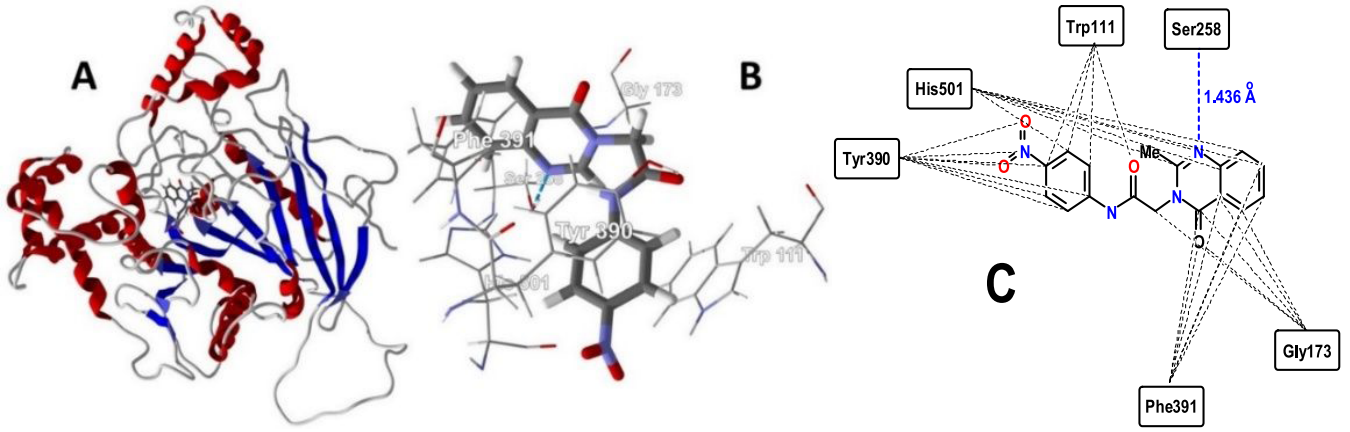

Figure 3. Lowest-energy docked pose of **4i** with *Aedes aegypti* acetylcholinesterase (homology model based on *Drosophila melanogaster* AChE, PDB 1DX4). (A) Ribbon structure of the protein with the docked ligand (CPK stick figure). (B) Key intermolecular interactions between **4i** and amino acid residues in the binding site; the hydrogen bond is shown as a blue dashed line. (C) Two-dimensional interaction diagram showing key interactions of **4i** in the hydrophobic binding site of *Aedes aegypti* acetylcholinesterase.

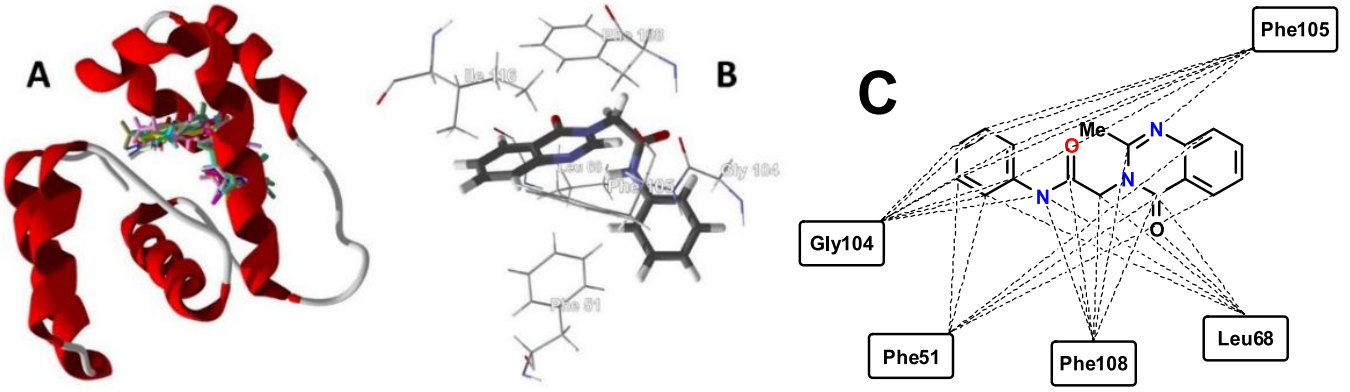

Figure 4. Lowest-energy docked pose of **4h** with *Aedes aegypti* odorant binding protein (PDB 6OMW). (A) Ribbon structure of the protein with the docked ligands (colored stick figures). (B) Key intermolecular interactions between **4h** and amino acid residues in the hydrophobic binding site. (C) Two-dimensional interaction diagram showing fundamental interactions of **4h** in the hydrophobic binding site of *Aedes aegypti* odorant binding protein.

$$\begin{aligned} \text{AChE} = {} & 548.607 - 172.099 \times \mu + 200\,989.960 \\ & \times \varepsilon_{\text{HOMO}} - 202\,692.387 \times \varepsilon_{\text{LUMO}} \\ & + 860.912 \times \log P + 20\,0031.759 \times \Delta_{\varepsilon} \end{aligned} \quad (2)$$

where $n = 7$, $R = 0.067$ and $R^2 = 0.935$.

Molecular Docking. In order to provide some insight into the possible mechanism of activity of the 2-methylquinazolin-4(3*H*)-one derivative, an *in silico* molecular docking analysis was carried out using relevant *Aedes aegypti* protein targets available from the Protein Data Bank (PDB) or prepared by homology modeling (see Table 3). These protein targets include acetylcholinesterase (AChE, homology models based on *Drosophila melanogaster* AChE, PDB 1DX4 and 1QO9); acetylcholine receptor (AChR, homology model based on human α 7 nicotinic acetylcholine receptor PDB 7EKP); angiotensin-converting enzyme (ACE2, homology model based on *Anopheles gambiae* ACE2, PDB 6S1Y); arylalkylamine *N*-acetyltransferase (aaNAT, PDB 4FD4, 4FD5, and 4FD6); carboxylesterase (CBEB5A, homology model based on *Lucilia cuprina* α esterase 7, PDB 4FNM); D7 salivary protein (D7SP, PDB 3BKS and 3DXL); glutathione *S*-transferase (GSTe2, PDB 5FT3); odorant binding protein (OBP, PDB 3K1E, 6OII, 6OMW, 6OPB, and 6P2E); and sterol carrier protein-2 (SCP2, PDB 2QZT). The docking scores are listed in Table S3.

Three protein targets showed preferential docking scores, acetylcholine receptor (AChR), acetylcholinesterase (AChE), and odorant binding protein (OBP). The lowest docking scores (most exothermic) and key intermolecular contacts are summarized in Table 4. In the acetylcholine receptor, the ligands preferentially dock into a binding site formed between two adjacent monomeric proteins of the pentameric structure. The binding site is a hydrophobic pocket flanked by Tyr200, Trp75, and Asp198 of one monomer and Glu149, Phe163, Phe147, and Cys148 of the adjacent monomer. Additional hydrogen bonding is provided by Gln59 of one monomer and Asn114 and Lys165 of the adjacent monomer (see Table 3).

In the acetylcholinesterase, the ligands dock into the enzyme's binding site, with the *N*-arylacetamide groups sandwiched in a π-π sandwich formed by Tyr390 and Trp11 and the quinazolinone moiety in a hydrophobic pocket formed by His501, Phe391, and Gly173 (Figure 3).

The binding pocket of *Aedes aegypti* OBP is surrounded by the hydrophobic residues Phe108, Leu68, Phe105, Phe51, and Ile116, and the *N*-aryl-2-(2-methylquinazolin-4(3*H*)-yl)-acetamides all docked in this hydrophobic pocket (Figure 4).

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CONCLUSIONS

In this present work, nine compounds *N*-aryl-2-(2-methylquinazolin-4(3*H*)-yl)acetamides (**4a−i**) were successfully synthesized, and their structures were determined by IR, ^{1}H NMR, ^{13}C NMR, and HR-MS spectral analysis. This investigation also indicated that the acetamide compounds (**4a−i**) exhibited larvicidal activities against *Aedes aegypti* mosquito. Furthermore, none of the compounds showed toxicity to the nontarget organism *Diplonychus rusticus* at 25 μg/mL, with mortality ranging from 0 to 3.75%. Our results showed that the 2-methyl-3,4-dihydroquinazolin-4-one heterocyclic derivatives exhibited significant activity at 72 h of exposure. Additionally, molecular docking studies suggested that the acetylcholine receptor (AChR), acetylcholinesterase (AChE), and odorant binding protein (OBP) were potential targets of these compounds. The promising results of these compounds on *Aedes aegypti* mosquito larvae are also supported by bioactivity mechanisms.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c01686.

^{1}H NMR, ^{13}C NMR, and HR-MS spectra of compounds (Figures S1−S10) (PDF)

Synthesized compounds' IR, ^{1}H NMR, ^{13}C NMR, and HR-MS spectra, electric dipole moment, electronic and steric descriptors, and in silico ModDock molecular docking scores (Tables S1−S3) (PDF)

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Notes

The authors declare no competing financial interest.

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After this paper was published ASAP on July 5, 2023, a correction was made to the name of author Ping-Chung Kuo. The corrected version was reposted July 6, 2023.

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