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Synthesis, Biological Evaluation and in silico Computational Studies of 7-Chloro-4-(1H-1,2,3-triazol-1-yl)quinoline Derivatives. Search for new controlling agents against Spodoptera frugiperda (Lepidoptera: Noctuidae) larvae

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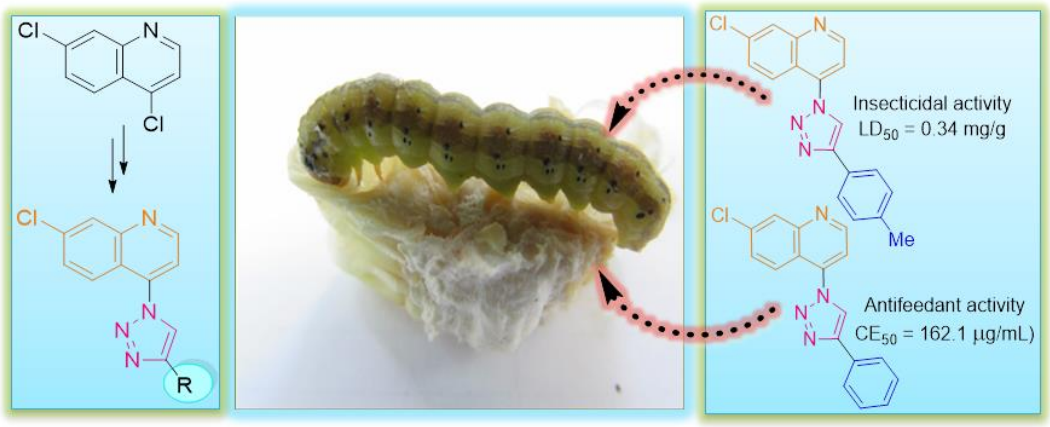

Synthesis, Biological Evaluation and *in silico* Computational Studies of 7-Chloro-4-(1*H*-1,2,3-triazol-1-yl)quinoline Derivatives. Search for new controlling agents against *Spodoptera frugiperda* (Lepidoptera: Noctuidae) larvae

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Abstract

The insecticidal and antifeedant activities of five 7-chloro-4-(1*H*-1,2,3-triazol-1-yl)quinoline derivatives were evaluated against the maize armyworm, *Spodoptera frugiperda* (J.E. Smith). These hybrids were prepared through a copper–catalyzed azide alkyne cycloaddition (CuAAC, known as a click reaction) and displayed larvicidal properties with LD_{50} values below 3 mg/g insect, and triazolyl-quinoline hybrid **6** showed an LD_{50} of 0.65 mg/g insect, making it two-fold less potent than methomyl, which was used as a reference insecticide (LD_{50} = 0.34 mg/g insect). Compound **4** was the most active antifeedant derivative (CE_{50} =162.1 μg/mL) with a good antifeedant index (56-79%) at concentrations of 250-1000 μg/mL. Additionally, triazolyl-quinoline hybrids **4-8** exhibited weak inhibitory activity against commercial acetylcholinesterase from *Electrophorus electricus* (*electric-eel* AChE) (IC_{50} = 27.7 μg/mL) as well as low anti-ChE activity on *S. frugiperda* larvae homogenate (IC_{50} = 68.4 μg/mL). Finally, molecular docking simulations suggested that hybrid **7** binds to the catalytic active site (CAS) of this enzyme and around the rim of the enzyme cavity, acting as a mixed (competitive and noncompetitive) inhibitor like methomyl. Triazolyl-quinolines **4-6** and **8** inhibit AChE by binding over the perimeter of the enzyme cavity, functioning as noncompetitive inhibitors. The results described in this work can help to identify lead triazole structures from click chemistry for the development of insecticide and deterrent products against *S. frugiperda* and related insect pests.

Keywords 1,2,3-triazolyl-quinoline hybrids; Spodoptera frugiperda; insecticidal activity; antifeedant activity; acetylcholinesterase inhibitory activity; molecular docking simulations, Lipinski' parameters; Tice's rule; "ag-likeness"

1. Introduction

Currently, most methods for pest control in agriculture are based on chemical insecticides derived from specific classes of organic compounds such as organophosphates, carbamates, pyrethroids and benzoylureas. These latter compounds still play an important role in modern agricultural pest management. The main disadvantages of their use are the progressive reduction of efficiency due to the increased resistance by pest insects and their high negative impact on the beneficial insect population and agricultural ecosystems.[1-5] To address these serious problems, scientists have been dedicated to developing novel potent and eco-friendly insecticides with new mechanisms of action. These innovative insecticides could be designed and developed using a molecular hybridization strategy based on the combination of the pharmacophores of different bioactive substances to produce a new hybrid molecule with improved biological profiles, similar to what is done in drug development.[6-8] Quinolines and triazoles are two important classes of small heterocyclic molecules that have a wide array of agricultural uses; both moieties can be found in herbicides, fungicides and insecticides.[9-15] Thus, the combination (fusion or conjugation) of these privileged rings in a single hybrid molecule could represent a substantial advance in agricultural chemistry, especially in insecticide research (Figure 1).

One of most destructive pests of many economically important small-grain crops (maize, cotton, rice and sorghum) is the fall armyworm moth *Spodoptera frugiperda* (J. E. Smith).[16] To date, the most common methods for controlling this pest depend on the use of conventional insecticides such as methomyl, carbaryl, and cypermethrin. Unfortunately, their efficacy is consistently decreasing due to the development of resistance.[17-20] In

addition, they exhibit a high general toxicity. Therefore, new tools, including novel active and nontoxic phosphate(carbamate)-free organic molecules for pest management are urgently required.[21,22]

Recently, 1,2,3-triazoles linked to quinoline derivatives have emerged as one of the most interesting and attractive biological models in medicinal and agricultural chemistry.[23,24] The design and development of triazolyl-quinolines could also be improved by pesticide-likeness analysis.[25-29] This approach is mainly based on Lipinski's "Rule–of–Five"[30] for pharmaceutical discovery and was adopted later by Tice for agrochemical applications.[31]

According to the statements described above and taking into account our current interest in developing new insecticide agents able to control *S. frugiperda*,[32] this research was focused on preparing five triazolyl-quinoline hybrids, 7-chloro-4-(1*H*-1,2,3-triazol-1-yl)quinolines, and determining their *in vivo* larvicidal, antifeedant and anti-acetylcholinesterase activities. Furthermore, the physicochemical profiles (Lipinski parameters and Tice criteria) and molecular docking simulations for the prepared molecules were determined and are discussed.

2. Materials and Methods

2.1 General Experimental Procedures. Melting points were determined with a Fisher–Johns melting point apparatus and are not corrected. Infrared spectra were recorded using KBr pellets on a Shimadzu, model IRA Affinity-1 FT-IR spectrophotometer. Nuclear magnetic resonance spectra were measured on a Bruker Avance-400 (400 MHz) spectrometer. Chemical shifts are reported in ppm with the solvent resonance as the internal

71 standard ($CDCl_3$: δ 7.26 ppm; DMSO-d_6: δ 2.50 ppm). Chemical shifts (δ) and coupling
72 constants (*J*) are reported in ppm and Hz, respectively. Mass spectra were recorded on an
73 ESI-IT Amazon X (Bruker Daltonics) with direct injection. Data were acquired in full scan
74 mode at 300°C and a capillary voltage of 4500 V using nitrogen as the nebulizer gas at a
75 flow rate of L/min at 30 psi. A Hewlett Packard 5890a Series II Gas Chromatograph
76 interfaced with an HP MS ChemStation Data System was also used for MS identification at
77 70 eV using a 60 m capillary column coated with HP-5 [5%
78 phenylpoly(dimethylsiloxane)]. Elemental analyses were performed on a Thermo Scientific
79 CHNS-O analyzer (Model Flash 2000), and the experimental results were within ± 0.4 of
80 the theoretical values. Thin-layer chromatography (TLC) separations were performed using
81 Merck silica gel 60 F254 precoated plates (0.25 mm). Column chromatography separations
82 were performed using silica gel 60 (0.063 – 0.200 mm) 70-230 mesh with mixtures of
83 hexane-ethyl acetate as the eluents.

84 Methomyl (analytical standard), cypermethrin (analytical standard), acetylthiocholine
85 iodide (ATCh) (≥ 98% purity), acetylcholinesterase from Electrophorus electricus (EC
86 3.1.1.7, Type VI-S), 5,5'-dithiobis-(2-nitro)benzoic acid (DTNB) (99%), sodium hydrogen
87 phosphate (98%), Tween® 20 for molecular biology, 4,7-dichloroquinoline (97%), sodium
88 azide (99%), phenylacetylene (98%), 1-ethynyl-4-methylbenzene (97%), 1-ethynyl-4-
89 methoxybenzene (97%), propargyl alcohol (99%), 2-methyl-3-butyn-2-ol (98%) and
90 tetrahydrofuran (99%) were obtained from Sigma-Aldrich (St. Louis, Missouri, USA). Di-
91 potassium hydrogen phosphate (98% purity) was acquired from Panreac (Barcelona,
92 Spain). Sodium chloride (99.5% purity) was obtained from Mallinckrodt (St. Louis,
93 Missouri, USA). Dimethyl sulfoxide, absolute ethanol, acetone, hexane, ethyl acetate, ethyl

ether, silica gel 60 (230-400 mesh), $CDCl_3$ and DMSO-d_6 were acquired from Merck (Darmstadt, Germany).

2.2 Recollection and mass-rearing of *S. frugiperda*. Field populations of S. *frugiperda* larvae were collected from a crop of corn during harvest time in the municipality of Girón, Santander, Colombia. Larvae were transported to the laboratory in a plastic container with enough plant material to prevent cannibalism. Subsequently, they were placed in plastic containers (one larva per vessel) with enough food corn. They were reared in an environmental chamber at 25 ± 1 °C with a 16:8 h light:dark photoperiod before they were used for experiments.

2.3 Synthetic Chemistry.

Preparation of 4-azido-7-chloroquinoline (**2**)[33]

4,7-Dichloroquinoline (10 g, 0.05 mol) was dissolved in 50 mL of DMSO, and sodium azide (4.88 g, 0.075 mol) was added in small portions at room temperature. The resulting mixture was stirred at room temperature for 12 h. The reaction mixture was treated with water and extracted with Et_2O (3 x 25 mL). The organic extracts were washed with water, dried over Na_2SO_4, concentrated, and then purified by column chromatography on silica gel using a hexane-ethyl acetate mixture (1:1) as the eluent to give colorless product **2**. Yield 95%, m.p.: 115-116 °C. IR (KBr): 2125, 1568, 1490, 1421, 1305, 1278, 1203, 815 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.11 (1H, d, J = 4.8 Hz; 3-H), 7.46 (1H, dd, J = 8.8, 2.0 Hz; 6-H), 7.97 (1H, d, J = 8.8 Hz; 5-H), 8.04 (1H, d, J = 2.0 Hz; 8-H), 8.81 (1H, d, J = 4.8 Hz; 2-H) ppm. ^{13}C NMR ($CDCl_3$): δ 108.7, 119.9, 123.7, 127.5, 128.1, 136.5, 146.3, 149.5, 151.2 ppm. GC-MS: t_R = 15.63 min, m/z (EI): 162 ($M^{+\cdot}$ - 42). Elemental analysis: calcd for $C_9H_5ClN_4$: C, 52.83; H, 2.46; N, 17.33; found: C, 52.55; H, 2.77; N, 17.18.

General procedure for the synthesis of triazolyl-quinolines **4-8**:

The alkyne (phenylacetylene, 1-ethynyl-4-methylbenzene, 1-ethynyl-4-methoxybenzene, propargyl alcohol, or 2-methyl-3-butyn-2-ol) (0.015 mol) was dissolved in methanol (12 mL), and 4-azido-7-cloroquinolina (0.01 mol), copper sulfate (0.25 mmol), sodium ascorbate (0.50 mmol), water (2 mL) and tetrahydrofuran (THF) (8 mL) were added. The resulting mixture was stirred at room temperature for 12 h and then treated with $NaHCO_3$ solution (5%, 10 mL). The products were extracted with ethyl acetate (3 x 20 mL). The organic extracts were washed with water, dried over Na_2SO_4, concentrated, and then purified by column chromatography on silica gel using hexane and ethyl acetate as the eluents to afford hybrids **4-8** as stable colorless powders.

7-Chloro-4-(4-phenyl-1H-1,2,3-triazol-1-yl)quinoline (**4**).[34] Yield 67%, colorless powder, m.p.: 157-158 °C. IR (KBr): 1606, 1597, 1562, 1483, 1234, 1022, 879, 767, 694 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.26 (1H, t, J = 7.4 Hz; 4-H_{Ar}), 7.34 (2H, t, J = 7.6 Hz; 3,5-H_{Ar}), 7.40 (1H, d, J = 4.6 Hz; 3-H), 7.45 (1H, dd, J = 9.1, 2.1 Hz; 6-H), 7.79 (2H, d, J = 7.8 Hz; 2,6-HAr), 7.91 (1H, d, J = 9.1 Hz; 5-H), 8.096 (1H, d, J = 2.4 Hz; 8-H), 8.099 (1H, s, 5-H_{TA}), 8.92 (1H, d, J = 4.6 Hz; 2-H) ppm. ^{13}C NMR ($CDCl_3$): δ 115.9, 120.6, 121.1, 124.6, 125.9 (2C), 128.8, 128.9, 129.1 (2C), 129.4, 129.4, 136.9, 140.9, 148.5, 150.2, 151.4 ppm. GC-MS: t_R = 11.33 min, m/z (EI): 306 ($M^{+\cdot}$), 280, 278, 243, 214, 162. Elemental analysis: calcd for $C_{17}H_{11}ClN_4$: C, 66.56; H, 3.61; N, 18.26; found: C, 66.43; H, 3.86; N, 18.09.

7-Chloro-4-(4-(4-methoxyphenyl)-1H-1,2,3-triazol-1-yl)quinoline (**5**). Yield 47%, colorless powder, m.p.: 160-161 °C. IR (KBr): 1612, 1562, 1494, 1452, 1440, 1252, 1020, 875, 786, 678 cm^{-1}. ^1H NMR ($CDCl_3$): δ 3.86 (3H, s, -OCH_3), 7.00 (2H, d, J = 8.8 Hz; 3,5-H_{Ar}), 7.53 (1H, d, J = 4.6 Hz; 3-H), 7.59 (1H, dd, J = 9.1, 2.1 Hz; 6-H), 7.86 (2H, d, J = 8.8 Hz; 2,6-H), 8.07 (1H, d, J = 9.1 Hz; 5-H), 8.15 (1H, s, 5-H_{TA}), 8.23 (1H, d, J = 2.1 Hz; 8-

H), 9.05 (1H, d, J = 4.6 Hz; 2-H) ppm. ^{13}C NMR ($CDCl_3$): δ 55.3, 114.4 (2C), 115.8, 120.3, 120.6, 122.0, 124.7, 127.3 (2C), 128.9, 129.4, 136.9, 141.0, 148.3, 150.1, 151.4, 160.1 ppm. GC-MS: t_R = 16.15 min, m/z (EI): 336 ($M^{+\cdot}$), 310, 308, 293, 237, 203, 175, 154. Elemental analysis: calcd for $C_{18}H_{13}ClN_4O$: C, 64.20; H, 3.89; N, 16.64; found: C, 64.32; H, 4.03; N, 16.38.

7-Chloro-4-(4-methylphenyl)-1H-1,2,3-triazol-1-yl)quinoline (**6**).[34] Yield 97%, white powder, m.p.: 169-170 °C. IR (KBr): 1597, 1562, 1436, 1230, 1029, 1016, 877, 817 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 2.37 (3H, s, -CH_3), 7.34 (2H, d, J = 7.9 Hz; 3,5-H_{Ar}), 7.80 (1H, dd, J = 9.1, 2.2 Hz; 6-H), 7.89 (2H, d, J = 8.1 Hz; 2,6-H_{Ar}), 7.92 (1H, d, J = 4.7 Hz; 3-H), 8.16 (1H, d, J = 9.1 Hz; 5-H), 8.30 (1H, d, J = 2.1 Hz; 8-H), 9.18 (1H, d, J = 4.7 Hz; 2-H), 9.26 (1H, s, 5-H_{TA}) ppm. ^{13}C NMR (DMSO-d_6): δ 20.8, 116.7, 120.1, 123.1, 125.4 (2C), 125.6, 126.9, 128.0, 128.9, 129.5 (2C), 135.5, 137.9, 140.4, 147.1, 149.4, 152.3 ppm. GC-MS: t_R = 16.15 min, m/z (EI): 336 ($M^{+\cdot}$), 310, 308, 293, 237, 203, 175, 154. Elemental analysis: calcd for $C_{18}H_{13}ClN_4O$: C, 64.20; H, 3.89; N, 16.64; found: C, 64.32; H, 4.03; N, 16.38.

1-(7-Chloroquinolin-4-yl)-1H-1,2,3-triazol-4-yl)methanol (**7**). Yield 55%, white powder, m.p.: 164-165 °C. IR (KBr): 3257, 1612, 1591, 1440, 1230, 1058, 1033, 879, 844 cm^{-1}. ^{1}H NMR (DMSO-d_6): δ 4.71 (2H, d, J = 5.4 Hz, -CH_2-), 5.44 (1H, t, J = 5.6 Hz; -OH), 7.77 (1H, dd, J = 9.1, 2.2 Hz; 6-H), 7.82 (1H, d, J = 4.7 Hz; 3-H), 8.02 (1H, d, J = 9.1 Hz; 5-H), 8.26 (1H, d, J = 2.2 Hz; 8-H), 8.69 (1H, s, 5-H_{TA}), 9.13 (1H, d, J = 4.7 Hz; 2-H) ppm. ^{13}C NMR (DMSO-d_6): δ 54.8, 116.8, 120.3, 124.9, 125.4, 128.0, 128.8, 135.2, 140.5, 148.8, 149.3, 152.3 ppm. GC-MS: t_R = 5.69 min, m/z (EI): 262 ($M^{+\cdot}$), 260, 217, 215, 203, 168, 162. Elemental analysis: calcd for $C_{12}H_9ClN_4O$: C, 55.29; H, 3.48; N, 21.49; found: C, 55.46; H, 3.64; N, 21.67.

164 *2-(1-(7-Chloroquinolin-4-yl)-1H-1,2,3-triazol-4-yl)propan-2-ol* (**8**).[34] Yield 91%, white
165 powder, m.p.: 148-149 °C. IR (KBr): 3356, 2978, 1612, 1595, 1564, 1438, 1238, 1168,
166 879, 821 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 1.78 (6H, s, $(-CH_3)_2$), 5.29 (1H, s, -OH), 7.47 (1H, d, *J*
167 = 4.6 Hz; 3-H), 7.56 (1H, d, *J* = 9.1, 2.0 Hz; 6-H), 7.96 (1H, s, 5-H_{TA}), 7.99 (1H, d, *J* = 9.1
168 Hz; 5-H), 8.21 (1H, d, *J* = 2.1 Hz; 8-H), 9.02 (1H, d, *J* = 4.6 Hz; 2-H) ppm. ^{13}C NMR
169 ($CDCl_3$): δ 30.5 (2C), 68.7, 115.9, 120.5, 121.2, 124.6, 128.9, 129.3, 136.9, 141.0, 150.1,
170 151.3, 156.5 ppm. GC-MS: t_R = 5.41 min, m/z (EI): 290 ($M^{+\cdot}$), 288, 247, 245, 205, 203,
171 167, 162. Elemental analysis: calcd for $C_{14}H_{13}ClN_4O$: C, 58.24; H, 4.54; N, 19.40; found:
172 C, 58.39; H, 4.76; N, 19.58.

173 **2.4 Insecticidal assay.** Compounds **4-8** were evaluated by topical application following
174 a previously described bioassay protocol.[35] First, groups of 10 larvae were selected and
175 weighed to determine the average weight of each group (500 ± 50 mg). The experimental
176 compounds and reference insecticide (methomyl) were each tested at doses of 1000, 500,
177 250, 100, 50 and 1 μg/larva using acetone as the solvent.

178 After selecting the group for each concentration of the test compounds **4-8**, the larvae
179 were placed in Petri dishes (one larva per box to avoid cannibalism). Then, 1 μL of solution
180 was applied to the 2nd and 3rd mesothoracic segments of the larvae, leaving a considerable
181 time between applications. Assays were performed in triplicate. The mortality rates (%)
182 were determined 2, 4, 6, 8, 24, 48 and 72 h after treatment, considering death as when the
183 larvae do not respond to mild twinges. Finally, the lethal concentration, the concentration at
184 which 50% of the population dies (LC_{50}), was calculated using the following equation:

185 **Mortality% = (Total dead larvae/Total larvae) x 100 (1)**

186

2.5 Antifeedant assay. The antifeedant effect was estimated through a no-choice assay.[35] *S. frugiperda* larvae with an average weight of 500 ± 50 mg and five acetone solutions with concentrations of 1000, 500, 250 100 and 50 µg/mL were used. Cypermethrin was employed as a reference compound. Solutions of experimental compounds **4-8** were prepared in 1 mL of acetone and 100 µL of these solutions were added to disks with an average weight of maize of 400 ± 50 mg, and 100 µL of an acetone solution of cypermethrin was used as the control.

Larvae selected for the bioassay were stored in individual containers without food for six hours before the bioassay. Then, a corn disk with the experimental compounds was given to the larvae. Ten larvae were used for each concentration. The food was weighed every 24 h for 72 h, and the antifeedant effect (% **AI**) was calculated with the following equation:

$$\%AI = [(C-T) / (C+T)] \times 100\% \qquad (2)$$

where **C** represents the weight of the diet consumed by the control larvae, and **T** is the weight of the diet consumed by the treatment larvae.

2.6 *In vitro* acetylcholinesterase activity. Acetylcholinesterase (AChE, EC 3.1.1.7, Type VI-S) inhibition was assessed by the Ellman method modifying by scaling microplates based on the reaction of released thiocholine to give a colored product with a chromogenic reagent.[36] The assay was performed in liquid medium in a 96-well microplate with a final volume of 200 µL. Solutions of the reference compound (methomyl) or experimental compounds **4-8** (at serial concentrations from $1x10^{-3}$ M to 4.88×10^{-7} M) prepared in phosphate–buffered saline (pH 7.5, 100 µL) and 50 µL of the AChE solution (0.25 U/mL) were placed in each well.

The plate was incubated at 25 °C for 30 min and then 100 µL of the substrate solution (DTNB and ATCh at pH 7.5) was added. After five minutes, the absorbance was determined at 405 nm using a Biochrom EZ 400 UV-Visible spectrophotometer. The IC_{50} is defined as the concentration of the studied compound corresponding to exactly 50% of the maximum inhibitory effect against AChE. This value (AChEI, %) was calculated from the data acquired using SoftMax Pro 5.2 software from Molecular Devices based on the following equation:

$$\textbf{AChEI (\%) = 100-[(AS-AB)/(AC-AB)] x 100} \qquad (\textbf{3})$$

where **AS** is the measured absorbance, **AB** is the absorbance of the blank, and **AC** is the absorbance of the control, and this was used to determine the enzyme activity without an inhibitor.

2.7 *Ex vivo* AChE activity on larvae homogenate. For the acetylcholinesterase assay, pools of twenty heads of *S. frugiperda* larvae (500 ± 5 mg) were quickly manually macerated in 10 mL of 0.1 M sodium phosphate buffer (pH 8.0, cold ice). The homogenate was immediately centrifuged (4500 rpm, 5 °C) for 45 min following the known protocol. The resulting supernatant was decanted and centrifuged for 15 min to better separate the mitochondrial enzyme solution. The final supernatant was used as the enzymatic source. To quantify the amount of acetylcholinesterase enzyme present in the obtained extract, the standard addition method was used. Briefly, 20 µL of the enzymatic extract was added to 0, 100, 200, 300 and 400 µL of an enzyme standard solution with a known concentration (0.50 U/mL) of electric-eel AChE and brought to 1 mL with 0.1 M sodium phosphate buffer (pH 8.0). To evaluate the *ex vivo* inhibitory capacity of the triazolyl-quinoline derivatives **4-8** for AChE, the procedure described in Section 2.6 was followed using the extract obtained from *S. frugiperda* heads as the enzymatic source.

2.8 eeAChE tertiary structure prediction. The structure of *Electrophorus electricus* acetylcholinesterase (*ee*AChE) was predicted by bioinformatic tools as indicated below. The sequence of *ee*AChE hosted on UniProt (code 042275) was submitted to the I-TASSER server (https://zhanglab.ccmb.med.umich.edu/I-TASSER/).[37-39] The predicted model of the tertiary structure with the highest score and serine hydrolase properties was selected as the preliminary tertiary structure for performing the simulations. The structure was optimized in aqueous media by energy minimization in a cubic simulation cell with side lengths of 14.559 nm. Explicit water molecules, such as TIP4P,[40] were added to simulate the solvation. Several optimizations were run with the *Steepest descent* method and the amber99sb force field[41] implemented in GROMACS.[42-44] Then, 20 ns molecular dynamics (MD) simulations were performed to determine the most conformation of the enzyme monomer under laboratory conditions. The MD simulations were run at 298 K and 1 atm with periodic boundary conditions with a leap-frog method implemented in GROMACS.[42-44] The enzyme structures with a RMSD of the backbone less than 0.09 nm were clustered together. All docking calculations were performed using the protein structure chosen from the largest cluster.

2.9 Docking calculations. Molecular docking calculations of the interactions between hybrids **4-8** and *ee*AChE were performed to elucidate the possible *in vitro* molecular inhibition mechanism of *ee*AChE by triazolquinolines. Additionally, a molecular docking simulation of *ee*AChE – *E*-methomyl binding was performed as a reference. The docking simulation was run with an amber94 force field[45] implemented in Autodock Vina.[46]

2.10 Statistical analysis. All experiments were carried out in triplicate. The concentration giving 50% inhibition (IC) was calculated by nonlinear regression using

Prism GraphPad Prism version 4.0 for Windows (GraphPad Software, San Diego, CA, USA). The dose–response curve was obtained by plotting the percentage inhibition versus the concentration. The lethal concentration (LC_{50}) is expressed as the standard error of the mean (SEM) of three different experiments in triplicate, and the analyses were performed using regression probit analysis with SPSS for Windows version 19.0.

3. Results and discussion

3.1 Chemistry. The desired 7-chloro-4-(1*H*-1,2,3-triazol-1-yl)quinoline derivatives (**4-8**) were obtained in moderate to good yields through a CuAAC click reaction between 4-azido-7-chloroquinoline (**2**)[33] and corresponding alkynes **3** (Scheme 1).[23-24]

The crude reaction products were generally purified by column chromatography. The yields for these click reactions were in the range of 50-97%. The final products were stable, colorless powders with well-defined melting points (see the SI, Table SI1). The structures and chemical purities of hybrids **4-8** were confirmed on the basis of spectrometric data (IR spectroscopy, GC-MS, and ^{1}H and ^{13}C NMR spectroscopy) and comparison with literature data.[34] The proton at the C-5 position of the triazole ring of these hybrids appeared as a singlet at δ 8.03-8.30 ppm indicating the 1,4-regioisomers were obtained (Scheme 1).

3.2 Biological evaluation of insecticidal and antifeedant properties. The insecticidal activities of prepared triazolyl-quinolines **4-8** against *S. frugiperda* larvae weighing 500 ± 50 mg indicated that the tested molecules were lethal to 50% of the population at concentrations below 3 mg/g insect (Table 1). The most active compound was 4-(4-methylphenyl)-1*H*-1,2,3-triazolyl-quinoline (hybrid **6**), which showed an LD_{50} of 0.65

mg/g insect making it two-fold less potent than methomyl, a reference insecticide (LD_{50} = 0.34 mg/g insect). 4-Methoxyphenyl-triazolyl quinoline (**5**) was less active as an insecticide with an LD_{50} value of 2.83 mg/g insect, while hybrid **7** was the most potent compound with an LD_{50} value of 1.68 mg/g insect. Within this range, 4-phenyl-1*H*-1,2,3-triazolyl quinoline (**4**, LD_{50} = 1.99 mg/g insect) and hybrid **8** (LD_{50} = 1.80 mg/g insect), with hydroxyalkyl chains at C-5 of the triazole moiety, showed good insecticidal activities (Table 1).

Compounds **4-8** were tested as potential antifeedant agents using a no-choice assay in which corn disks with compounds **4-8** preabsorbed were administered to *S. frugiperda larvae*. The results of this experiment (Table 1) showed that three of the hybrids, **4**, **5** and **8** had moderate deterrent effects on *S. frugiperda* larvae (CE_{50} < 300 µg/mL).

The antifeedant activity of cypermethrin, which is widely used to control armyworm, afforded better results (CE_{50} = 23.9 µg/mL). The least active compound in this test was hybrid **7** (CE_{50} = 557.4 µg/mL). Based on their antifeedant index values (% AI values) (Table 2), almost of the prepared hybrids (comp. **4-6** and **8**) (AI = 71-79%) showed excellent antifeedant activities at a concentration of 1000 µg/mL relative to the antifeedant index of cypermethrin (AI = 78%). Out of all derivatives, triazolyl-quinoline **4** was the best deterrent compound with an index of 79%, while hybrid **7** was the least active antifeedant molecule (AI = 61%). Notably, triazolyl-quinoline **4** was sufficiently active at a concentration of 250 µg/mL (AI = 56%). The antifeedant effects of this compound are shown in Figure 2.

Because AChE is the target enzyme for inhibition by different insecticides,[34-36] the inhibitory effects of hybrids **4-8** on AChE were determined based on *ee*AChE and the AChE of the insect crude extract. The results (Table 3) revealed that all the tested hybrids showed weak AChE inhibitory effects (27-34 µg/mL < IC_{50} < 68-75 µg/mL) and similar

304 structure-activity relationship (SAR) trends, *e.g*., compound **4** inhibited *ee*AChE and *S.*
305 *frugiperda* larvae ChE with IC_{50} values 52.1 and 73.9 μg/mL, respectively, and so on.
306 However, in general, the test compounds more efficiently inhibited *ee*AChE than they did
307 crude *S. frugiperda* larvae ChE homogenate, and the anti-*ee*AChE activity of quinoline
308 hybrids **4-8** tended to increase as follows: **7**>**6**>**8**>**4**>**5**, while their inhibitory effects on
309 crude *S. frugiperda* larvae ChE fell in the following order: **6**>**8**>**7**>**4**>**5**. Notably, the two
310 most active enzyme inhibitors, **6** and **7**, showed interesting behavior against both enzymes,
311 whereas hybrid **7** bearing a hydroxymethyl substituent (hydrophilic moiety) on the triazole
312 ring, presented the strongest inhibitory effect on *ee*AChE (IC_{50} = 27.7 μg/mL), hybrid **6**,
313 with a 4-methylphenyl fragment (lipophilic moiety) on the triazole ring, revealed the best
314 inhibition of crude *S. frugiperda* larvae AChE homogenate (IC_{50} = 34.6 μg/mL).

315 Considering that compound **6** possesses a good insecticidal activity (LD_{50} = 0.65 μg/g
316 insect) and that compound **7** is inactive (LD_{50} = 1.68 μg/g insect), the observed effects
317 seem to be interesting and may be, significant. Therefore, to better understand these results,
318 we performed *in silico* computational studies.

319 **3.3 *In silico* computational studies using the DataWarrior program.** Currently,
320 the initial assessment of a potential bioactive compound starts with the prediction of its
321 possible biological activities and with an understanding of its possible mechanism of
322 action.[47,48] For this purpose, we first determined whether compounds **4-8** have chemical and
323 physical properties that would make them potential pharmacological agents. Thus, using
324 the stand-alone DataWarrior program (Osiris),[49] we established that molecular hybrids **4-8**
325 fulfil Lipinski "rule–of–five"; they snow *i)* octanol/water partition coefficients (cLogP
326 values) ≤ 5; *ii)* molecular weights (MWs) ≤ 500 Da; *iii)* have ≤ 10 hydrogen bond acceptors

(HBA); *iv)* have ≤ 5 hydrogen bond donors (HBD); *v)* have ≤ 10 rotatable bonds (ROTB); and *vi)* have topological polar surface areas (TPSA values) ≤ 140 (Table 4).[50]

Nevertheless, due to the biodiversity of pests and the environmental conditions, another set of rules (physicochemical properties) beyond Lipinski's "rule–of–five" should be considered in agrochemical development and discovery including in the design of insecticides.[27,29] This rule is Tice's criteria, which established specific and primary parameters for agrochemical agents, and these include: *i)* a cLogP between 0 and 5; *ii)* an MW between 150 and 500 Da; *iii)* an HBA between 1 and 8; *iv)* an HBD ≤ 2; and *v)* a ROTB < 12.[31] Therefore, hybrids **4-8** and the reference compounds, methomyl (**Met**) and cypermethrin (**Cyp**), have good agrochemical-likeness ("ag-likeness") because none of these derivatives breaks or exceeds Tice's rules (Table 4). However, analyzing the main molecular descriptors of these hybrids and methomyl, it is clear that methomyl, a small molecule, potent insectcide (LD_{50} = 0.34 mg/g insect), is hydrophilic (clogP = 0.61) and highly water soluble (clogS = -1.35), while the most active hybrid **6**, LD_{50} = 0.65 mg/g insect, is the most lipophilic molecule of this series (clogP = 3.49) and is poorly water soluble (clogS = -4.90). Moreover, hybrid **7**, which showed weak insecticidal properties (LD_{50} = 1.68 mg/g insect), possesses a similar hydrophilicity (clogP = 0.85, clogS = -2.69) to that of methomyl.

Applied topically to the dorsal of the larvae, the molecules must penetrate the cuticle, an integument that protects the inner and outer surfaces of insects.[51] As the insect integument can be considered a two-phased structure, with lipophilic (epi- and exocuticles containing lipids, lipoproteins and proteins) and hydrophilic (endocuticle, a chitin-protein complex) layers,[52-54] both lipophilic molecules (hybrids **4-8**) and hydrophilic compounds (methomyl) can generally pass through this active biochemical barrier into the hemolymph

to reach specific targets. Indeed, earlier Gerolt's tests with methomyl showed this insecticide can pass through the integument of adult housefly at a high rate, *i.e.*, at 8-10% (depending on dose) over a period of 19 h.[55,56] Thus, the insecticidal activity of a molecule is the result of a series of multifarious interactions between the compound and the insect's tissues, and penetration is an important initial step, but is still poorly studied. Based on these factors, this situation results in highly complex structural activity relationships. In addition to Tice's parameters, the insecticidal activities of new compounds are also related to their size, shape, stability, and pKa values.[28,29]

Regarding the final step, *i.e.*, the interaction between the toxicant and its possible targets, *e.g.,* AChE enzyme, enzymatic inhibitory activity is more accurately predicted by molecular docking studies.

3.4 Molecular docking studies. To perform the docking simulations with the *ee*AChE, it was necessary to predict its tertiary structure, which was not reported in the Protein Data Bank (PDB, http://www.rcsb.org/pdb). Thus, using known protocols, the secondary and tertiary structures of this enzyme were predicted from its amino acid sequence (code 042275), which is available in the UniPro database.[37-39] The constructed tertiary structure of *ee*AChE displayed a globular shape with a catalytic active site (CAS) containing an acyl pocket (Phe312, Phe315 and Phe355), a choline binding side (Trp107) and a catalytic triad (Glu223, Ser224 and His493) (Figure 2A-C), and a peripheral anionic site (PAS) at the rim of the cavity, involving Tyr93, Asp95, Thr96, Ser97, Tyr98, Glu302, Ser364, Asp366 and Tyr358 residues; these are important properties of serine hydrolases.[57]

The docking experiments showed interesting results. First, the molecular docking of *ee*AChE – *E*-methomyl revealed spontaneous interactions of between this carbamate and

374 the CAS and the two binding zones (**I** and **II**) on the enzyme surface, and binding energies
375 between -5.2 and -4.5 kcal/mol were observed (Figure 3A, Table 5).

376 Our binding model indicates that methomyl may act as a competitive inhibitor of
377 *ee*AChE as it reaches the CAS (Figure 3B, C) and forms multiple dipole-dipole interactions
378 with the hydroxyl groups of Tyr145 and Tyr354, and the guanidine group of Arg258. At the
379 same time, however, this carbamate binds Val153 and Asp155 residues in the backbone of
380 binding zone **I** via dipole-dipole interactions, while it binds zone **II** via hydrogen bonds and
381 multiple dipole-dipole interactions (Data not shown). Thus, these results confirmed that *E*-
382 methomyl is a mixed inhibitor, as reported in the literature.[58] Regarding the analysis of
383 *ee*AChE – triazolyl-quinoline **4-8** interactions, we found that among the five hybrids, only
384 hybrid **7** could occupy the CAS of the enzyme with a binding energy of -7.1 kcal/mol
385 (Table 5). The π-π interactions between the choline binding site of the enzyme (indole
386 group of Trp107 and phenolic hydroxyl group of Tyr354) and the quinoline ring of this
387 hybrid, and the hydrogen bond of the guanidine group of Arg258 in the acyl pocket and the
388 hydroxyl group of triazolyl-quinoline **7** are important for the spontaneity of the binding.
389 Additionally, the conformation suggests a dipole-dipole interaction between the triazole
390 ring of the compound and the hydrogens of the benzyl groups of Phe312 and Phe355
391 (Figure 4). This result indicates that hybrid **7** may be a competitive inhibitor of
392 acetylcholine hydrolysis by AChE, as predicted by the *E*-methomyl – *ee*AChE molecular
393 docking results. No other hybrids reached the CAS in the molecular modeling studies,
394 which is consistent with the inhibitory potential of triazolyl-quinoline **7**, being the best
395 AChE inhibitor (LD_{50} = 27.7 μg/mL) (Table 3). The molecular volume data (Table 5) also
396 indicated that compounds **4-6** and **8** may be too large to fit in the CAS of the enzyme.

Overall, the molecular docking results with tested triazolyl-quinoline derivatives **4-8** suggested that all hybrids, including compound **7**, could bind to two zones outside the enzyme cavity (Figure 5), the same spots as *E*-methomyl (Figure 3), with multiple binding modes. The more spontaneous binding modes of hybrids **4-8** to zone **I** (Figure 5A, C) suggested a dipole-dipole interaction with the enzyme; moreover, these modes could be stabilized via π-π stacking interactions with several aromatic residues around the cavity. On the other hand, in binding zone **II**, two main interactions were observed, and they are important in triazoles recognition (Figure 5B, D). The π-π stacking interactions between the aromatic rings of hybrids **4** and **5** and the benzyl group of Phe312 formed a T-shape. Additionally, dipole-dipole interactions are formed between the triazole rings of compounds **7** and **8** and the guanidine groups of Arg268 and Arg313.

The proximity of binding zones **I** and **II** to the rim of the enzyme cavity and the spontaneous binding of the tested hybrids could stabilize an enzyme conformation and destroy the flexibility necessary to carry out its catalytic action, indicating that these hybrids could work as noncompetitive inhibitors of *ee*AChE.

4. Conclusions

The larvicidal, antifeedant, and anti-AChE activities of five triazolyl-quinoline hybrids **4-8** were evaluated against the maize armyworm, *Spodoptera frugiperda* (J.E. Smith). We found that 4-(4-methylphenyl)-1*H*-1,2,3-triazolyl-quinoline, hybrid **6**, was the most promising compound, and it exhibited insecticidal (LD_{50} = 0.65 mg/g insect), antifeedant (antifeedant index 56-79% at 250-1000 μg/mL) and AChE activities on both commercial *ee*AChE (27.7 μg/mL) and supernatant ChE enzyme from *S. frugiperda* larvae head homogenate (53.1 μg/mL).

Additionally, to understand these *in vivo* results, *in silico* agrochemical-likeness evaluations and molecular docking studies were performed. We established that an inactive insecticidal and hydrophilic hybrid, such as **7** (clogP = 0.85), binds to the CAS of AChE (-7.1 kcal/mol) in zones outside the enzyme cavity, working as a mixed (competitive and noncompetitive) inhibitor, while the most potent insecticidal and lipophilic hybrid (**6**, clogP = 3.49) did not reach the CAS of AChE. Therefore, the *in vivo* insecticidal activity of hybrid **6** could be attributed to its inhibition of AChE, while the insecticidal inactivity of hybrid **7**, the most active AChE inhibitor, can be explained by its strong hydrophilicity and relative large molecular volume, which prevents this compound from reaching the AChE target.

All this information can help to identify lead triazole structures derived from click chemistry for the development of insecticide and deterrent products against *S. frugiperda* and related insect pests.

ASSOCIATED CONTENT

Supporting Information

Physicochemical properties of hybrids **4-8**; copies of the NMR and IR spectra of triazolylquinolines **4-8** and azido-quinoline precursor (**2**); GC-MS data of triazolylquinolines **4-8**; biological information related to compounds **4-8**.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest, financial or otherwise.

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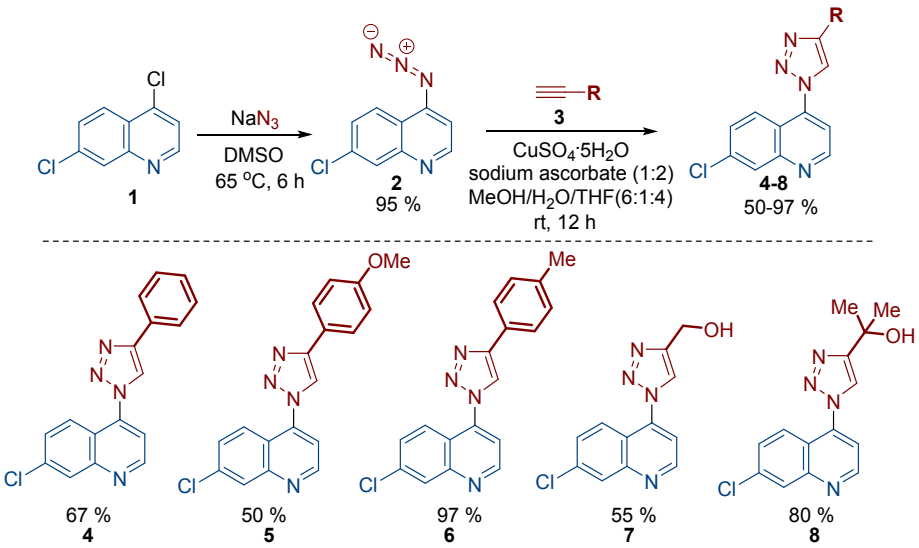
556 **SCHEMES**



Scheme 1. Straightforward preparation of 7-chloro-4-(1*H*-1,2,3-triazol-1-yl)quinolines **4-8** from 4,7-dichloroquinoline.

TABLES

Table 1. Insecticidal and antifeedant activities of triazolyl-quinoline molecules **4-8** and reference agrochemicals.[a,b]

Tested comp.	Insecticidal activity		Antifeedant activity	
	LD_{50}, mg/g insect (CI)	χ^2	CE_{50}, µg/mL (CI)	χ^2
4	1.99 (1.84 - 1.20)	0.98	162.1 (155.3 - 168.9)	0.77
5	2.83 (2.74 - 2.92)	0.97	296.3 (287.6 - 305.0)	0.89
6	0.65 (0.66 - 0.79)	0.92	356.0 (345.9 - 366.2)	0.90
7	1.68 (1.67 - 1.69)	0.99	557.4 (529.4 - 585.4)	0.92
8	1.80 (1.78 - 1.82)	0.99	286.5 (282.1 - 290.9)	0.79
Methomyl	0.34 (0.31 - 0.37)	0.85	nt[c]	--
Cypermethrin	nt[c]	--	23.9 (22.5 - 25.4)	0.77

[a] Expressed in LD_{50} values as well as CE_{50} values and shown as the mean (n > 3) values; 95% confidence interval (CI) values for the respective compounds; [b] χ^2 Chi-squared test; [c] Not tested.

Table 2. Antifeedant index (% AI) of triazolyl-quinolines **4-8** and cypermethrin, as a reference deterrent agrochemical.

	Concentration, µg/mL				
Tested comp	1000	500	250	100	50
4	79	70	56	44	33
5	75	58	44	32	15
6	71	53	41	31	15
7	61	49	33	20	13
8	73	63	49	30	12
Cypermethrin	78	72	66	61	56

Table 3. Inhibition potencies of tested molecules **4-8** against *ee*AChE and crude *S. frugiperda* larvae AChE homogenate.

	AChE inhibition properties[a]	
Tested comp.	*ee*AChE	*S. frugiperda* larvae ChE homogenate

	IC_{50}, µg/mL (CI)	χ^2	IC_{50}, µg/mL (CI)	χ^2
4	52.1 (51.3 - 52.9)	0.81	73.9 (71.5 - 76.2)	0.67
5	68.4 (65.3 - 71.4)	0.81	75.8 (75.6 - 76.1)	0.66
6	29.5 (28.5 - 30.5)	0.71	34.6 (33.8 - 35.4)	0.59
7	27.7 (27.5 - 27.9)	0.78	53.1 (52.8 - 53.5)	0.49
8	32.3 (31.8 - 32.9)	0.77	44.3 (43.3 - 45.2)	0.62
Met.[b]	0.31 (0.304 - 0.316)	0.73	0.41 (0.407 - 0.409)	0.59

[a] IC_{50} values are expressed in µg/mL and shown as the mean (n > 3) values; 95% confidence interval (CI) values for the respective compounds; χ^2 Chi-squared test; [b] Methomyl.

Table 4. Molecular descriptors calculated for molecules **4-8** according to the DataWarrior program and analysis of Tice's criteria.

Comp.	MW[a]	cLogP[b]	cLogS[c]	HBA[d]	HBD[e]	ROTB[f]	TPSA[g]	Tice's rule violations
4	306.755	3.1462	-4.560	4	0	2	43.60	0
5	336.781	3.0762	-4.578	5	0	3	52.83	0
6	320.782	3.4901	-4.904	4	0	2	43.60	0
7	260.683	0.8527	-2.690	5	1	2	63.83	0
8	288.370	1.7259	-3.175	5	1	2	63.83	0
Met.[h]	162.212	0.6131	-1.349	4	1	3	75.99	0
Cyp.[i]	416.303	5.3412	-6.688	4	0	7	59.32	0

[a] Molecular Weight (g/mol); [b] Logarithm of the partition coefficient between *n*-octanol and water; [c] Logarithm of the solubility measured in mol/L; [d] Number of hydrogen-bond acceptors; [e] Number of hydrogen-bond donors; [f] Number of rotatable bonds (calculated using the Molinspiration Cheminformatics software); [g] Polar surface area (Å^2); [h] Methomyl; [i] Cypermethrin.

Table 5. Binding energy of *ee*AChE – triazolyl-quinolines **4-8** interactions

Comp.	ΔG, kcal mol^{-1}			MV[a] (Å^3)
	Binding zone **I**	Active site	Binding zone **II**	

4	-9.1 (π-p[d], d-d[e])	n.f.[g]	-8.7 (π-π[d], π-p[d])	257.29
5	-8.9 (π-p[d], d-d[e])	n.f.[g]	-8.3 (π-π[c], π-p[d])	282.83
6	-9.2 (π-p[d], d-d[e])	n.f.[g]	-8.3 (d-d[e])	273.85
7	-7.7 (d-d[e])	-7.1 (HB[b], π-π[c], d-d[d])	-7.0 (d-d[e])	210.70
8	-7.9 (π-π[c], π-c[f])	n.f.[g]	-8.7 (d-d[e])	243.52
Met[h]	-4.5 (d-d[e])	-5.2 (d-d[e])	-4.5 (HB[b], d-d[e])	144.08

Interaction type in parenthesis. [a] MV: Molecular volume; [b] HB: Hydrogen bond; [c] π-π; [d] π-p: π-polar; [e] d-d: dipole-dipole; [f] π-c: π-cation; [g] Not found; [h] *E*-Methomyl.

FIGURES

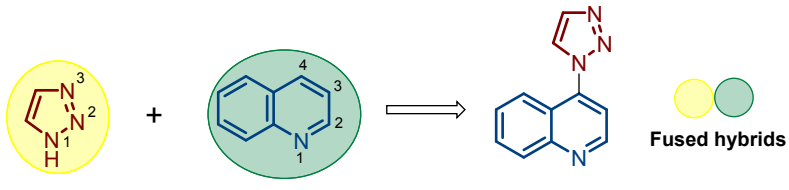
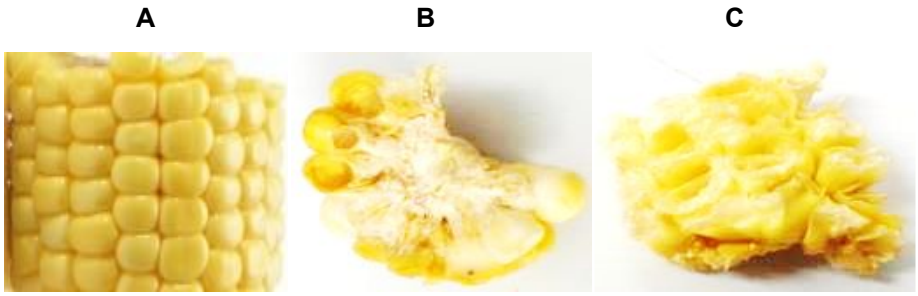


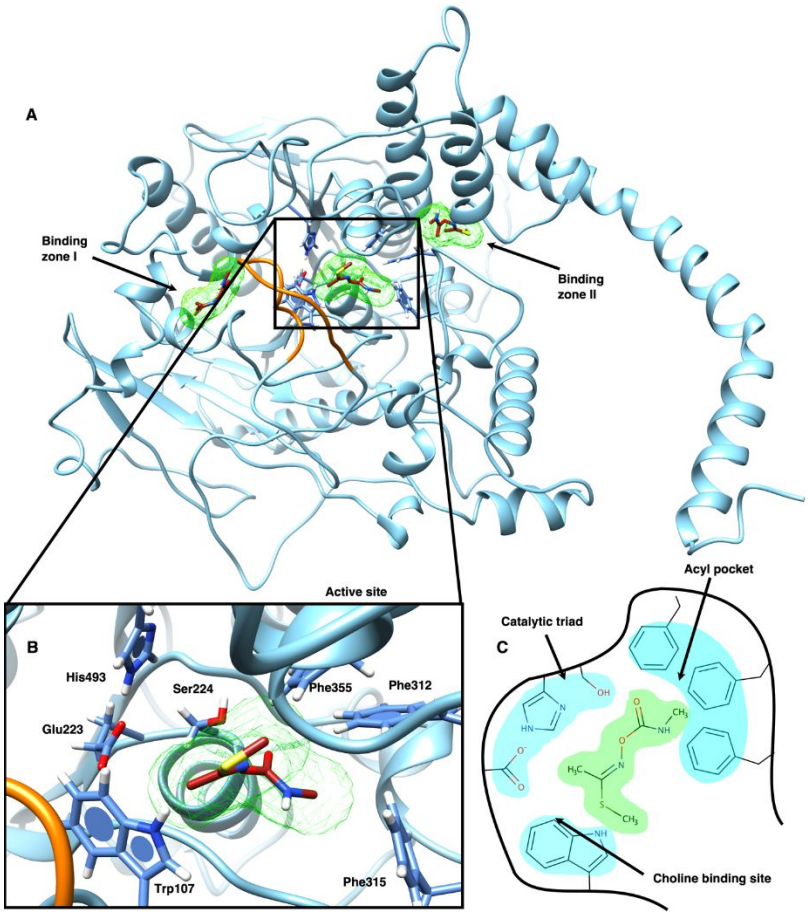
Figure 1. Design of triazolylquinolines using a hybridization strategy.



644

645 **Figure 2.** Images of food consumption in the antifeedant activity test. **A**) Healthy corn disk. **B**)
646 Control corn disk (solvent, acetone) consumed by the *S. frugiperda* larvae after 72 h treatment. **C**)
647 Corn disk treated with an acetone solution of triazolquinoline **4** in the presence of the *S. frugiperda*
648 larvae (72 h).

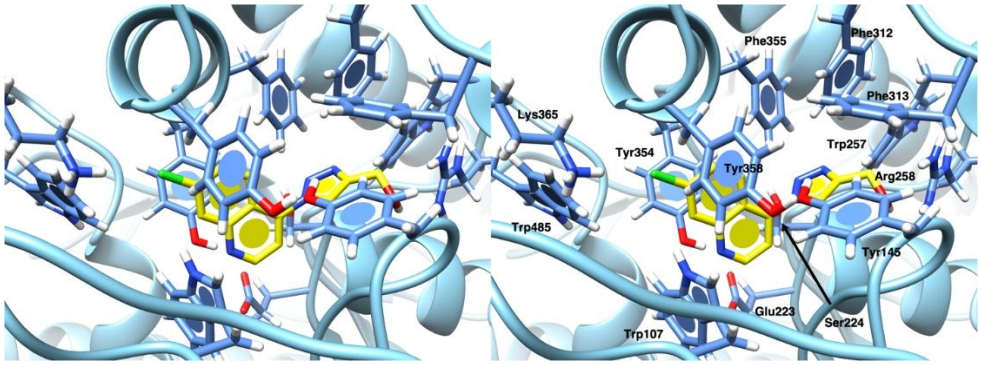
649



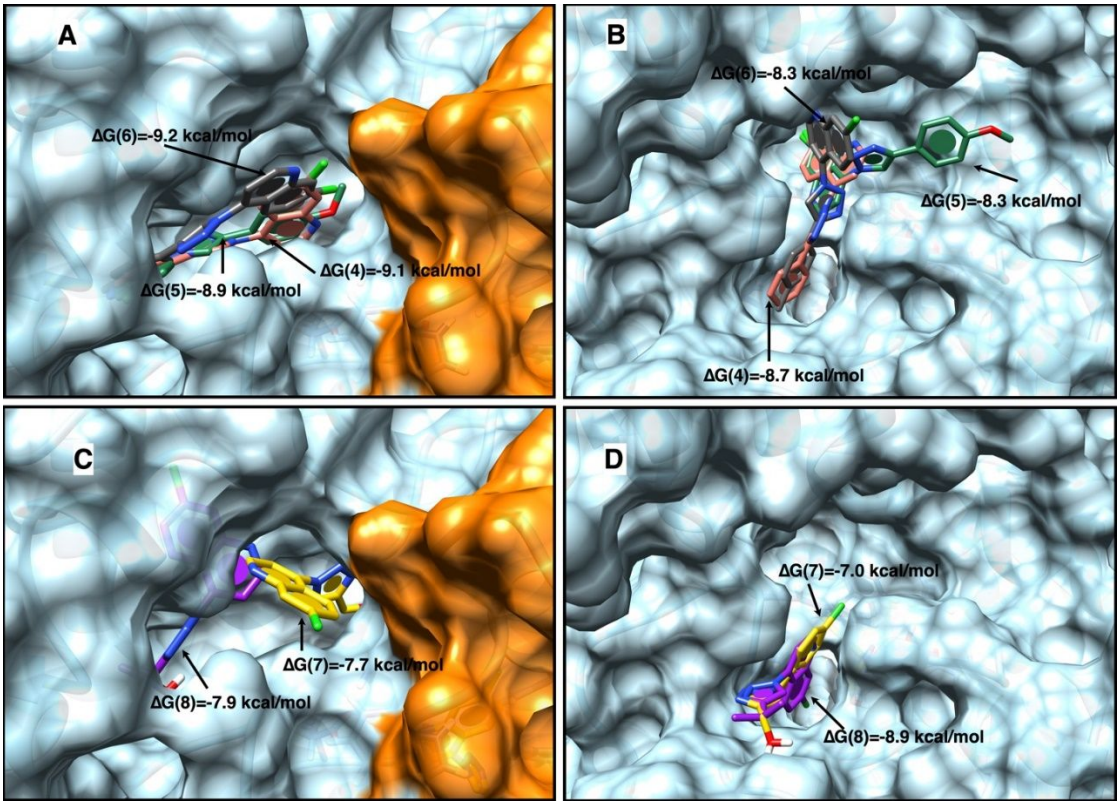
650

651 **Figure 3.** Molecular docking of *ee*AChE – *E*-methomyl interactions. **A**) *E*-Methomyl docking
652 locations over *ee*AChE (lid in orange). **B**) Active site of *ee*AChE and *E*-methomyl overlaid. **C**) 2D

653 schematic of the docking model of *E*-methomyl with *ee*AChE CAS.[51] *E*-Methomyl is depicted in
654 red with the surface in green.
655
656
657



658
659 **Figure 4.** Cross-eyed stereo image of triazolyl-quinoline **7** on the CAS of *ee*AChE. Hybrid **7** is
660 depicted in yellow, and the *ee*AChE residues are depicted in blue. These interactions are associated
661 with a binding energy of -7.1 kcal/mol.



662

663 **Figure 5.** Molecular docking of *ee*AChE – triazolquinolines **4-8** interactions with zones **I** and **II**. **A**)
664 Triazolquinolines **4-6** (compounds with phenyl groups at the end of the C-5 R group) with binding
665 zone **I**. **B**) Hybrids **4-6** with the binding zone **II**. **C**) Compounds **7** and **8** (those bearing
666 hydroxyalkyl chains at the C-5 position of the triazole ring with binding zone **I**. **D**)
667 Triazolquinolines **7** and **8** with binding zone **II**. Hybrid **4** is depicted in salmon, **5** in green, **6** in
668 gray, **7** in yellow and **8** in magenta. Methomyl is depicted in red. Binding energies are shown.