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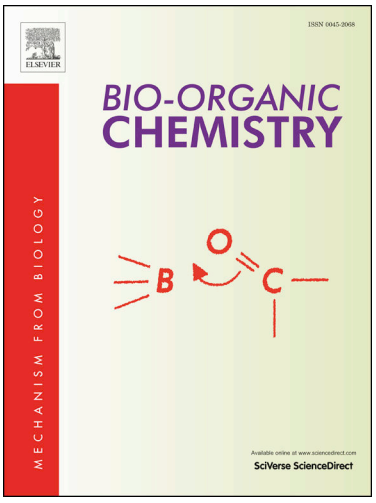

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The larvicidal activity of natural inspired piperine-based dienehydrazides against *Culex pipiens*

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Abstract

A series of piperine-based dienehydrazide derivatives were designed and synthesized to be used as insecticides *against Culex pipiens*. The chemical structure of compound **5n** was confirmed by single-crystal x-ray diffraction. Their insecticidal activities of synthesized compounds were tested against third-instar larval of *Cx. pipiens* at concentrations ranging from 0.1-1.2 mg/mL. Among all derivatives, compounds **5a**, **5b**, **5f**, **5g**, **5m**, **5n**, **5o**, **5p**, and **5u** displayed good activities. The final mortality rates at the concentration of 0.75 mg/mL after 48 h treatment, were found to be in the range from 80.00 to 83.33% and with LC_{50} values ranging from 0.221 to 0.094 mg/mL. These compounds demonstrated higher insecticidal activities than piperine and Deltamethrin (a commercial positive control). Molecular modelling reveals several molecular interactions between synthesized compounds and the substrate binding sits of acetylcholinesterase (AChE) that are predicted to be responsible for its binding and inhibition activity.

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Keywords: Piperine; Natural product; Molecular docking; Structural modification; *Culex pipens*; Larvicidal activity

1. Introduction

Although synthetic chemical pesticides have a significant role in modern agricultural pest management such as crops protection, repeat application of those agrochemicals over the last years has led to increase in the insect pest populations resistance and environmental problems[1–4]**.** Instead, plant secondary metabolites result from the interaction between plants and surrounding environment (life and non-life) during the long period of evolution, indicating that pesticides originated from plant secondary metabolites may lead to less or slower resistance development and lower pollution. Consequently, discovery of selective, low mammalian toxicity, effective and ecofriendly insecticides is of great interest to many research groups[5–7]**.** Natural products have been used for thousands of years as food preservatives, drugs and pesticidal agents[8,9]**.**

Black pepper extracts have drawn attention for their insecticidal constituents toward *Cx. Pipien*, *Aedesaegypti* larvae[10], rice weevils, cowpea weevils[11] and larvae of various mosquito vectors[12]. Piperine, an alkaloid from pepper (*Piper nigrum*) exhibited a wide range of biological activities including inhibition of liver CYP3A4 enzyme[13], and being used as antiadipogenic[14], anti-inflammatory[15] and antimicrobial functions[16]. In addition, modified structures of piperine also exhibited interesting insecticidal activity[17,18]**.**

Cx. pipiens, the common house mosquito, is a worldwide vector. It has been incriminated as a vector of serious diseases such as filarial worms, west Nile virus, St. Louis encephalitis virus and also avian malaria[10]**.** The unwisely usage of chemical insecticides led to several environmental problems[19]**.** The toxicity to non-target population[19], development of resistance phenomenon and the residual effects of these insecticides are main concerns of scientists[20]. With the emergence of *Cx. pipiens* resistance to many insecticides, control is becoming more difficult and that led to

increasing rates of diseases transmission[21]**.** There is a dire need to develop environmental friendly insecticides to protect the environment from hazards of chemical insecticides[22].

Several researchers investigated the potential of natural compounds as a model for the development of new insecticides. Many amides and hydrazones of piperine have been synthesized and been investigated against insects [23–25]**.** Inspired by these, a series of piperine-based dienehydrazide derivatives were designed and synthesized as insecticidal agents against 3rd instar larvae of *Cx. pipens*.

2. Experimental and methods

2.1. Chemicals and Instruments.

All utilized chemical reagents were obtained and used without further purification. Piperine and hydrazine (≥99.0%) were obtained from Aladdin Industrial Corporation (Shanghai, China). Oxalyl chloride (≥99.0%) was obtained from TCI (Tokyo Chemical Industry Co. Ltd). Other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd.

Proton (^{1}H NMR) and carbon nuclear magnetic resonance spectra (^{13}C NMR) were obtained in DMSO-d_6 or $CDCl_3$ on a Bruker Avance 600 MHz instrument using tetramethylsilane (TMS) as the internal standard. Coupling constants (J) were reported in Hz. Mass spectra were recorded on an Agilent 6530 accurate-mass Q-TOF spectrometer. X-Ray data were collected using a BRUKER SMART APEX-CCD diffractometer with Cu-Kα radiation (λ = 0.71073 Å). Gas sorption experiment was carried out with a Micrometrics ASAP 2020 instrument. The infrared spectra of the generated compounds were done on FT-IR spectra recorded in KBr on a thermo nicolet iS10 FTIR spectrophotometer. Measurements of melting points (mp) were done on a

XT-4 digital melting point apparatus (Beijing Tech Instrument Co., Ltd., Beijing, China) and were incorrected.

2.2. Maintenance of mosquito colony, *Cx. pipiens* L. (Diptera: Culicidae)

The egg rafts of common house mosquito, *Cx. pipiens* were obtained from the Research and Training Center on Vectors of Diseases (RTC), Faculty of Science, Ain Shams University, Egypt. The mosquitoes were reared for at least eight generations in insectary rooms, under controlled laboratory conditions at temperature 27±2 ºC, and relative humidity RH 70±10%, for photoperiods 14:10 (light:dark) hours. Egg rafts were placed in white enamel dishes 35-40 cm in diameter and 10 cm in depth filled with 1500 ml of distilled water. Newly hatched larvae were fed on fish food (Tetra-/Min, Germany) as a diet sprinkled twice daily over the water surface of the breeding pans[26]. Distilled water in each dish was stirred daily and changed every two days to avoid scum formation on the water surface or on the walls and bottoms of pans. Small air pump was used to aerate the breeding water gently every day for about 5 minutes. Pupae were collected routinely and separated in plastic containers filled with distilled water then introduced into screened wooden cages until emergence. Adults were reared in (24 x 24 x 24 cm) wooden cages. Adults were provided daily with cotton pads soaked in 10% sucrose solution for a period of four days. After this period the females were allowed to take a blood meal from a pigeon host. To obtain best blood feeding, sucrose was removed 24 hours prior to blood meal. Oviposition containers filled with distilled water were placed in adult cages 48 hours after the females had been provided with blood meals. Egg rafts were collected routinely and placed in white enamel dishes. When mosquito larvae developed to the 2nd instar, they were poured into clean pans (25 x 30 x 15 cm) containing three liters of tap water left for 24 h and observed daily[26,27]. Larvicidal toxicity of experimental compounds was tested toward early third larval instars of *Cx. pipiens*.

2.3. Statistical analysis

The results of the insecticidal activities were analyzed using one-way analysis of variance (ANOVA) by SPSS 19.0 program. Moreover, the LC_{50} values were calculated using probit analysis.

2.4. The Synthesis of target molecule

2.4.1. Piperic Acid (Compound 2)

The piperic acid was produced from the hydrolysis of piperine according literature[28]. The piperic acid was recrystallized from methyl alcohol to afford piperic acid as a pale-yellow solid (93.4% yield).

2.4.2. General Procedure for Preparation of Piperic Acid hydrazide (Compound 4)

According to literature[29] with some modification, oxalyl chloride (1.24 g, 10 mmol) in DCM (5 mL) and three drops of DMF as a catalyst were added to piperic acid (217 mg, 1.01 mmol) and stirred at room temperature for 4 h. After completing the reaction, the oxalyl chloride excess and solvent were then evaporated. The obtained acid chloride was dissolved in DCM (5 mL) and was added dropwise to hydrazine (40 mg, 1.2 mmol) in DCM (5 mL) at room temperature. The reaction mixture was then stirred for 7 h, and the solvent was evaporated under reduced pressure. Water was added to the mixture, which was subsequently extracted with DCM. The organic layer was separated and dried over anhydrous Na_2SO_4 and the solvent was evaporated. The crude product was purified by silica gel column chromatography (DCM:MeOH=20:1) to give the desired piperic acid hydrazide (210 mg, 87% yield).

2.4.3. Synthesis of piperine-based dienehydrazide derivatives (Compounds 5a-u)

A mixture of 4 (100 mg, 0.43 mmol), the corresponding carbonyl compounds (0.45 mmol), and four drops of glacial acetic acid in MeOH (10 mL) was heated to reflux.

The reaction was completed in 2-6 h as shown by TLC. The obtaining reaction mixture was cooled to precipitate the desired product. The crude solid product was collected via filtration and washed with cold methanol to give compounds **5a-u** in **55-85%** yield.

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)penta-2,4-dienoic acid (2): Pale yellow solid, m.p. 218°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.20 (s, 1H, -CO_2H), 7.28-7.33 (m, 1H), 7.24 (d, *J* = 1.4 Hz, 1H), 6.92-7.02 (m, 4H), 6.05 (s, 2H, -OCH_2O-), 5.92 (d, *J* = 15.44 Hz, 1H).

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)penta-2,4-dienehydrazide (4): Pale yellow solid, m.p. 180°C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 9.28 (s, 1H, NH), 7.25 (d, *J*= 1.29 Hz, 1H), 7.15-7.19 (m, 1H), 6.85(d, , *J*= 15.55 Hz, 1H), 6.92-7.02 (m, 4H), 6.04 (s, 2H, -OCH_2O-), 6.02 (d, *J*=15.01Hz, 1H), 4.41 (s, 2H, NH).

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N'-((Z)-2-hydroxybenzylidene)penta-2,4-dienehydrazide (5a): Pale brown solid, m.p. 99-101°C; IR: 3587, 3197, 3037, 2887, 1652, 1617, 1567, 1488, 1312, 1245. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.84 (s, 1H, NH), 11.20 (s, 1H, OH), 8.84 (s, 1H, CH=N), 7.52 (d, *J* = 7.8 Hz, 1H), 7.15-7.19 (m, 1H), 6.85(d, *J* = 15.55 Hz, 1H), 6.92-7.02 (m, 4H), 6.04 (s, 2H, -OCH_2O-), 6.02 (d, *J* = 15.01Hz, 1H), 4.41 (s, 2H, NH). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.51, 161.98, 159.81, 159.56, 148.45, 148.43, 148.41, 148.37, 147.18, 143.48, 142.80, 141.51, 139.57, 139.33, 131.29, 131.23, 129.30, 128.88, 126.26, 125.82, 125.67, 123.45, 123.41, 122.97, 120.39, 116.14, 108.95, 106.16, 106.11, 101.78.

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N'-((Z)-2-chlorobenzylidene)penta-2,4-dienehydrazide (5b): Yellow solid, m.p. 130-133°C; IR: 3283, 3189, 3027, 2903, 1642, 1596, 1569, 1497, 1443, 1374, 1259. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.30 (d, *J* = 131.9 Hz, 1H), 8.02 (d, *J* = 112.6 Hz, 1H), 7.54 (d, *J* = 8.6 Hz, 2H), 7.35 (ddd, *J* = 22.4, 15.0, 10.7 Hz, 1H), 7.29 (d, *J* = 1.4 Hz, 1H), 7.18 – 7.07 (m, 1H), 7.02 (dd, *J* =

18.3, 9.1 Hz, 1H), 6.97 (d, J = 11.8 Hz, 1H), 6.92 (dd, J = 8.0, 4.6 Hz, 1H), 6.83 (d, J = 8.5 Hz, 2H), 6.16 (d, J = 14.9 Hz, 1H), 6.06 (d, J = 3.0 Hz, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.51, 161.98, 159.81, 159.56, 148.45, 148.43, 148.41, 148.37, 147.18, 143.48, 142.80, 141.51, 139.57, 139.33, 131.29, 131.23, 129.30, 128.88, 126.26, 125.82, 125.67, 123.45, 123.41, 122.97, 120.39, 116.14, 108.95, 106.16, 106.11, 101.78.

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N'-((Z)-1-(benzo[d][1,3]dioxol-5-yl)ethylidene)penta-2,4-dienehydrazide (5c): Pale yellow solid, m.p. 150-160^{o}C; IR: 3212, 3153, 3015, 2885, 1642, 1616, 1547, 1444, 1285, 1244. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.46 (d, J = 29.3 Hz, 1H), 7.46–7.26 (m, 4H), 7.23–7.10 (m, 1H), 7.01 (d, J = 15.3 Hz, 1H), 7.00–6.90 (m, 3H), 6.46 (d, J = 14.9 Hz, 1H), 6.07 (d, J = 9.5 Hz, 4H), 2.24 (d, J = 16.7 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ: 167.38, 162.53, 148.42, 147.38, 138.44, 141.46, 139.76, 133.05, 131.25, 126.30, 125.58, 123.34, 123.20, 121.47, 121.14, 120.54, 109.17, 107.90, 106.35, 106.14, 101.38.

4-((Z)-(2-((2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)penta-2,4-dienoyl)hydrazono)methyl)phenyl 1,1,2,2,3,3,4,4,5,5,6,6,6-tridecafluorohexane-1-sulfonate (5d): Pale yellow solid, m.p. 185-190^{o}C; IR: 3204, 3041, 2904, 1652, 1601, 1488, 1426, 1303, 1211. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.64 (s, 1H), 8.17 (d, J = 128.5 Hz, 1H), 7.86 (s, 2H), 7.59 (s, 2H), 7.38 (s, 1H), 7.27 (s, 1H), 7.11 (s, 1H), 6.99 (s, 2H), 6.92 (s, 1H), 6.17 (s, 1H), 6.06 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 148.47, 144.85, 143.09, 141.90, 141.07, 139.88, 131.20, 129.56, 129.23, 126.10, 125.54, 123.55, 122.44, 119.63, 108.96, 106.19, 101.82.

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N'-((Z)-4-methoxybenzylidene)penta-2,4-dienehydrazide (5e): Pale yellow solid, m.p. 216-219^{o}C; IR: 3200, 3052, 2895, 1642, 1602, 1505, 1365, 1253. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.37 (d, J = 116.6 Hz, 1H),

8.08 (d, *J* = 118.3 Hz, 1H), 7.65 (d, *J* = 8.8 Hz, 2H), 7.42 – 7.26 (m, 2H), 7.20 – 7.07 (m, 1H), 6.99 (ddd, *J* = 40.2, 24.5, 18.2 Hz, 5H), 6.16 (d, *J* = 14.9 Hz, 1H), 6.06 (s, 2H), 3.81 (s, 3H).^{13}C NMR (151 MHz, DMSO-d_6) δ 166.63, 162.03, 161.10, 148.40, 146.63, 142.95, 141.51, 139.47, 131.21, 129.09, 128.73, 127.37, 126.20, 125.68, 123.56, 122.96, 120.19, 114.93, 108.74, 106.14, 101.72, 55.63.

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N'-((Z)-4-nitrobenzylidene)penta-2,4-dienehydrazide (5f): Yellow solid, m.p. 230-233^{o}C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.80 (d, *J* = 163.5 Hz, 1H), 8.37 – 8.20 (m, 2H), 8.13 (s, 1H), 7.98 (d, *J* = 8.8 Hz, 2H), 7.43 (d, *J* = 11.1 Hz, 1H), 7.28 (s, 1H), 7.14 (t, *J* = 14.0 Hz, 1H), 7.06 – 6.95 (m, 2H), 6.93 (s, 1H), 6.20 (d, *J* = 14.7 Hz, 1H), 6.06 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.22, 151.95, 150.75, 148.64, 148.49, 148.34, 147.13, 140.52, 131.36, 131.21, 128.93, 128.79, 128.54, 128.35, 123.43, 122.93, 122.24, 111.79, 111.53, 109.22, 108.27, 106.11, 106.16, 101.72.

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N'-((Z)-furan-2-ylmethylene)penta-2,4-dienehydrazide (5g): Pale yellow solid, m.p. 203-206^{o}C; IR: 3423, 3130, 2990, 2900, 1661, 1600, 1496, 1406, 1259. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.45 (d, *J* = 119.8 Hz, 1H), 8.03 (d, *J* = 129.5 Hz, 1H), 7.84 (s, 1H), 7.37 (ddd, *J* = 20.7, 15.1, 10.8 Hz, 1H), 7.31 (d, *J* = 11.3 Hz, 1H), 7.15 (dd, *J* = 15.4, 11.1 Hz, 1H), 7.00 (ddd, *J* = 15.7, 13.1, 6.1 Hz, 3H), 6.96 – 6.85 (m, 2H), 6.63 (dd, *J* = 3.3, 1.7 Hz, 1H), 6.14 (d, *J* = 14.9 Hz, 1H), 6.06 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.61, 162.18, 150.00, 149.78, 148.45, 145.56, 145.28, 143.37, 142.04, 141.88, 139.99, 139.63, 136.64, 131.24, 131.20, 126.08, 125.64, 123.55, 122.93, 122.30, 119.70, 113.91, 113.73, 113.54, 109.02, 108.88, 106.25, 106.10, 101.80.

4-((Z)-(2-((2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)penta-2,4-dienoyl)hydrazono) methyl)benzoic acid (5h): Pale yellow solid, m.p. 209-211^{o}C; IR: 3209, 3062, 2893,

2671, 2548, 1651, 1613, 1558, 1497, 1370, 1254. ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.10 (s, 1H), 11.66 (d, J = 125.3 Hz, 1H), 8.07 (d, J = 23.2 Hz, 1H), 7.97 (s, 2H), 7.82 (s, 2H), 7.39 (s, 1H), 7.30 (s, 1H), 7.15 (s, 1H), 7.02 (s, 2H), 6.98 (d, J = 10.2 Hz, 1H), 6.93 (s, 1H), 6.18 (s, 1H), 6.07 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.41, 166.94, 148.47, 142.28, 142.07, 138.94, 138.83, 132.03, 131.87, 131.23, 131.17, 130.21, 127.50, 127.18, 126.18, 125.61, 123.50, 108.92, 106.27, 106.11, 101.82.

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N'-((Z)-naphthalen-1-ylmethylene)penta-2,4-dienehydrazide (5i): Pale yellow solid, m.p. 215-218^{o}C; IR: 3211, 3185, 3038, 1645, 1612, 1546, 1503, 1337, 1255. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.59 (d, J = 156.6 Hz, 1H), 8.93 – 8.72 (m, 1H), 8.55 (d, J = 8.0 Hz, 1H), 8.11 – 7.84 (m, 3H), 7.81 – 7.51 (m, 3H), 7.42 (d, J = 11.8 Hz, 1H), 7.32 (s, 1H), 7.18 (dd, J = 27.1, 15.0 Hz, 1H), 7.04 (dd, J = 18.1, 12.7 Hz, 2H), 6.94 (d, J = 7.6 Hz, 1H), 6.23 (d, J = 14.8 Hz, 1H), 6.07 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.78, 161.21, 148.47, 146.83, 143.27, 143.16, 142.05, 141.92, 139.72, 139.61, 134.82, 134.81, 131.22, 130.29, 129.36, 129.12, 127.51, 127.18, 126.24, 125.65, 123.54, 122.41, 120.12, 109.02, 108.91, 106.25, 106.20, 106.10, 101.82, 101.11.

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N'-((Z)-pentylidene)penta-2,4-dienehydrazide (5j): Pale yellow solid, m.p. 170-175^{o}C; IR: 3206, 3066, 2929, 1648, 1611, 1557, 1495, 1388, 1254. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.04 (d, J = 125.9 Hz, 1H), 7.41 (ddd, J = 51.8, 10.0, 4.6 Hz, 1H), 7.33 – 7.24 (m, 2H), 7.07 (dt, J = 28.1, 14.1 Hz, 1H), 7.03–6.97 (m, 1H), 6.98 – 6.87 (m, 3H), 6.09–6.06 (m, 1H), 6.05 (s, 2H), 2.30 – 2.13 (m, 2H), 1.52 – 1.40 (m, 2H), 1.39 – 1.27 (m, 2H), 0.96 – 0.86 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.24, 161.78, 151.27, 148.47, 147.65, 142.73, 141.34, 139.52, 139.16, 131.17, 126.12, 125.65, 123.36, 123.07, 120.27, 109.10, 106.21, 101.77, 32.17, 28.31, 22.22, 13.97.

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N'-((Z)-2,3-dihydro-1H-inden-1-ylidene) penta-2,4-dienehydrazide (5k): Pale yellow solid, m.p. 212-215°C; IR: 3184, 3035, 2889, 1655, 1603, 1555, 1501, 1252. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.40 (d, J = 14.4 Hz, 1H), 7.70 (dd, J = 27.4, 7.6 Hz, 1H), 7.44 – 7.27 (m, 5H), 7.23 – 7.12 (m, 1H), 6.98 (ddd, J = 33.0, 20.3, 7.6 Hz, 3H), 6.46 (d, J = 15.0 Hz, 1H), 6.06 (s, 2H), 3.13 – 3.04 (m, 2H), 2.88–2.80 (m, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.19, 162.38, 161.14, 157.17, 149.14, 148.72, 148.36, 142.86, 141.38, 139.55, 139.27, 138.42, 131.31, 130.95, 130.73, 127.49, 127.36, 125.67, 123.48, 123.32, 121.88, 121.32, 120.65, 108.96, 106.23, 106.02, 101.87, 28.64, 28.60, 27.91, 27.41.

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N'-((1Z,2E)-3-phenylallylidene)penta-2,4-dienehydrazide (5l): Yellow solid, m.p. 179-184°C; IR: 3300, 3146, 3017, 2884, 1646, 1635, 1547, 1491, 1335, 1242. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.41 (d, J = 112.6 Hz, 1H), 8.10–7.76 (m, 1H), 7.61 (d, J = 7.6 Hz, 2H), 7.42–7.24 (m, 5H), 7.17 – 6.87 (m, 6H), 6.15 (d, J = 14.8 Hz, 1H), 6.06 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.53, 162.07, 148.78, 148.45, 141.97, 141.82, 139.56, 139.17, 138.84, 136.44, 131.20, 129.40, 129.23, 127.56, 127.50, 126.20, 125.67, 123.51, 123.05, 122.44, 109.03, 108.91, 106.26, 106.11, 101.80.

(2E,4E)-N'-((Z)-anthracen-9-ylmethylene)-5-(benzo[d][1,3]dioxol-5-yl)penta-2,4-dienehydrazide (5m): Yellow solid, m.p. 225-230°C; IR: 3443, 3184, 3048, 2900, 1651, 1607, 1558, 1496, 1335, 1248. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.76 (d, J = 169.7 Hz, 1H), 9.33 (s, 1H), 8.63 (s, 2H), 8.18 (s, 1H), 7.63 (d, J = 42.0 Hz, 3H), 7.44 (s, 1H), 7.31 (s, 1H), 7.00 (s, 3H), 6.27 (s, 1H), 6.06 (s, 2H). ESI-HRMS: Calcd for $C_{27}H_{20}N_2O_3$ ([M^+]), 420.27; Found, 420.26.

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N'-((Z)-4-(diethylamino)-2-hydroxy benzylidene)penta-2,4-dienehydrazide (5n): Yellow solid, m.p. 105-107°C; IR:

3208, 3034, 2972, 2884, 1629, 1547, 1518, 1443, 1371, 1246. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.52 (s, 1H), 11.34 (s, 1H), 8.20 (s, 1H), 7.36 – 7.28 (m, 2H), 7.20 (d, *J* = 11.6 Hz, 1H), 7.02 (d, *J* = 14.7 Hz, 2H), 6.98 – 6.90 (m, 2H), 6.13 (d, *J* = 14.9 Hz, 1H), 6.11 (d, *J* = 2.3 Hz, 1H), 6.06 (s, 2H), 3.35 (s, 4H), 1.11 (s, 6H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 161.26, 160.10, 150.52, 148.91, 148.34, 141.39, 139.36, 131.92, 131.69, 125.64, 123.41, 122.84, 122.23, 108.95, 107.01, 106.21, 106.21, 103.80, 101.78, 44.36, 12.86.

Crystallographic description of *5-(2H-1,3-benzodioxol-5-yl)-N'-((4-(diethylamino)-2-hydroxyphenyl)methylidene)penta-2,4-dienehydrazide methanol solvate* (5n): $C_{23}H_{25}N_3O_4.CH_3OH$, crystal dimensions 0.15 x 0.15 x 0.1 mm^3, M_r = 439.50, monoclinic, space group P 2_1/c (14), Cell: *a* = 11.298(3), *b* = 13.398(3), *c* = 15.678(4) Å, $\alpha = 90°$, $\beta = 107.247(4)°$, $\gamma = 90°$, *V* = 2266.5(10) Å^3, *Z* = 4, Density (calculated) = 1.288 Mg/m^3, μ = 0.091 mm^{-1}, *F*(000)= 936, reflection collected / unique = 17545 / 4630, refinement method = full-matrix least-squares on *F*2, Final R indices [I>2sigma(I)]: *R1* = 0.0475, *wR2* = 0.1263, R indices (all data): *R1* = 0.0948, *wR2* = 0.1535, goodness of fit on F^2 = 1.037. CCDC 1934954 for ***5-(2H-1,3-benzodioxol-5-yl)-N'-((4-(diethylamino)-2-hydroxyphenyl)methylidene)penta-2,4-dienehydrazide methanol solvate*** **(5n):** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N'-((Z)-1-(4-nitrophenyl)ethylidene)penta-2,4-dienehydrazide (5o): Deep yellow solid, m.p. 201-205^{o}C; IR: 3169, 3067, 2999, 1665, 1596, 1441, 1345, 1248. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.33-11.26 (d, *J* = 114.6 Hz, 1H), 7.66 (d, J= 8.77 Hz, 2H), 7.31-7.39 (m, 2H), 7.11-7.18 (m, 1H), 6.93-7.06 (m, 4H), 6.92 (m, 1H), 6.95(s, 1H), 6.48 (d, J = 15.90 Hz, 1H), 6.05(s, 2H), 2.32(s, 3H). ESI-HRMS: Calcd for $C_{20}H_{17}N_3O_5$ ([M^+]), 379.37; Found, 379.32.

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N'-((Z)-1-(benzo[d][1,3]dioxol-5-yl)propan-2-ylidene)penta-2,4-dienehydrazide (5p): Yellow solid, m.p. 107-110^{o}C; IR: 3146,

3017, 2884, 1649, 1635, 1547, 1491, 1335, 1242. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.13 (d, J = 43.0 Hz, 1H), 7.29 (s, 2H), 7.02 (s, 1H), 6.92 (d, J = 8.0 Hz, 3H), 6.86 (s, 1H), 6.80 (d, J = 6.8 Hz, 1H), 6.71 (s, 1H), 6.35 (d, J = 15.0 Hz, 1H), 6.05 (s, 2H), 5.95 (d, J = 4.8 Hz, 2H), 2.81 – 2.69 (m, 2H), 1.89 (d, J = 14.7 Hz, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.70, 162.35, 148.30, 147.17, 145.42, 140.78, 139.23, 135.72, 135.09, 130.73, 126.10, 123.22, 121.67, 109.67, 109.38, 108.74, 108.11, 106.14, 101.30, 100.59, 31.68, 16.78.

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N'-((Z)-2-hydroxy-3-methoxybenzylidene) penta-2,4-dienehydrazide (5q): Pale yellow solid, m.p. 178-180^{o}C; IR: 3449, 3229, 3016, 2992, 1673, 1609, 1502, 1490, 1340, 1254. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.82 (s, 1H), 10.92 (s, 1H), 8.42 (s, 1H), 7.37 (dd, J = 24.1, 10.8 Hz, 1H), 7.30 (s, 1H), 7.22 – 6.95 (m, 5H), 6.93 (d, J = 7.7 Hz, 1H), 6.85 (dd, J = 13.9, 7.0 Hz, 1H), 6.15 (d, J = 14.9 Hz, 1H), 6.06 (s, 2H), 3.82 (d, J = 8.9 Hz, 3H). ^{13}C NMR (150 MHz, DMSO-d_6) δ 166.46, 161.95, 149.39, 149.11, 148.48, 148.44, 148.37, 147.38, 143.74, 142.86, 141.50, 139.63, 139.35, 131.25, 126.26, 126.22, 125.68, 123.41, 123.01, 122.51, 121.41, 120.34, 116.06, 115.89, 110.25, 109.47, 108.94, 106.16, 101.79, 56.18.

4-((Z)-(2-((2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)penta-2,4-dienoyl)hydrazono) methyl)phenyl 1,1,2,2,3,3,4,4,5,5,6,6,7,7,8,8,8-heptadecafluorooctane-1-sulfonate (5r): Pale yellow solid, m.p. 227-231^{o}C; IR: 3448, 3060, 2939, 1661, 1598, 1577, 1339, 1252. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.68 (s, 1H), 8.17 (s, 1H), 7.89 (s, 2H), 7.60 (s, 2H), 7.41 (s, 1H), 7.33 (s, 1H), 7.09 (s, 1H), 7.01 (s, 3H), 6.23 (s, 1H), 6.01 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.41, 150.96, 148.57, 144.86, 143.93, 141.89, 139.97, 135.56, 131.30, 130.13, 128.75, 126.10, 125.26, 123.77, 122.53, 109.45, 105.93, 101.53.

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N'-((Z)-4-(trifluoromethyl)benzylidene) penta-2,4-dienehydrazide (5s): Deep yellow solid, m.p. 198-201°C; IR: 3443, 3164, 3012, 2899, 1663, 1601, 1500, 1448, 1403, 1261. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.84 (s, 1H), 8.77 (d, J = 128.5 Hz, 1H), 7.56 (s, 2H), 7.49 (s, 2H), 7.46 (s, 1H), 7.37 (s, 1H), 7.18 (s, 1H), 6.92 (s, 2H), 6.82 (s, 1H), 6.17 (s, 1H), 6.06 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 168.47, 164.85, 150.09, 148.90, 145.07, 140.88, 137.20, 133.56, 130.23, 128.10, 126.54, 124.55, 108.96, 106.19, 103.82. ^{19}F NMR (565 MHz, $CDCl_3$) δ -63.08.

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N'-((Z)-benzylidene)penta-2,4-dienehydrazide (5t): Pale yellow solid, m.p. 243-248 °C; IR: 3184, 3035, 2889, 1655, 1501, 1314, 1252. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.52 (d, J = 120.1 Hz, 1H), 8.14 (d, J = 121.7 Hz, 1H), 7.72 (d, J = 7.5 Hz, 2H), 7.51 – 7.34 (m, 4H), 7.30 (d, J = 1.4 Hz, 1H), 7.23 – 7.09 (m, 1H), 7.08 – 6.88 (m, 3H), 6.19 (d, J = 15.0 Hz, 1H), 6.07 (s, 2H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.78, 162.22, 148.47, 146.81, 143.27, 143.12, 142.05, 141.90, 139.79, 139.63, 134.88, 134.80, 131.21, 130.29, 129.36, 127.51, 127.18, 126.24, 125.65, 123.55, 120.02, 109.03, 108.90, 106.25, 106.20, 106.05, 101.81, 101.11.

(2E,4E)-5-(benzo[d][1,3]dioxol-5-yl)-N'-((Z)-(3,5a1-dihydropyren-1-yl)methylene) penta-2,4-dienehydrazide (5u): Deep yellow solid, m.p. 250-256°C, IR: 3184, 3035, 2889, 1655, 1603, 1555, 1501, 1445, 1374, 1252. ^{1}H NMR (600 MHz, DMSO-d_6) δ 11.70 (d, J = 179.5 Hz, 1H), 9.21 (d, J = 65.8 Hz, 1H), 8.84 (d, J = 9.3 Hz, 1H), 8.59 (dd, J = 45.4, 7.9 Hz, 1H), 8.43 – 8.31 (m, 3H), 8.31 – 8.20 (m, 2H), 8.14 (t, J = 7.6 Hz, 1H), 7.54 – 6.90 (m, 6H), 6.27 (d, J = 14.6 Hz, 1H), 6.08 (s, 2H). ESI-HRMS: Calcd for $C_{29}H_{20}N_2O_3$([M^+]), 444.29; Found, 444.20.

2.5. Biological assays

The larvicidal activity of the tested compounds against the third larval instar of *Cx. pipiens* was evaluated by immersion methods at concentrations ranging from 0.1-1.2 mg/mL for two days. For each compound at least 75 larvae (25 newly moulted larvae / group) were used. All compounds were dissolved in acetone solutions and deltamethrin was used as a positive control. The 3rd instar larvae of *Cx. pipiens* were immersed into the corresponding concentration for 24 and 48 hours. The untreated larvae were considered as blank control group. The experiment procedure was done at 25±2 °C and relative humidity (RH) 65–80%, and on 12 h/12 h (light/dark) photoperiod.

2.6. Molecular modelling:

We performed molecular modeling of compounds 5a,5o, 5g and 5n in a homology model of acetyl cholinesterase of the organism Cx. Pipien**.** The homology model was generated using I-TASSER [30,31]. The reference structure used for protein structure prediction is the crystal structure of Acetylcholinesterase Catalytic Subunit of the Malaria Vector Anopheles Gambiae (PDB:5X61).[32] Compounds were modeled based on the binding pose of the highly similar scaffold MR28926 in the X-ray structure of acetyl cholinesterase (PDB:6ezh). Predicting the ligand binding poses based on existing similar scaffolds is the recommended approach by the D3R Grand challenge and pose prediction [33]. Refinement of the protein-ligand complex was performed using Macromodel [34].

3. Results and discussion

3.1. Chemistry

Piperine, the main ingredients of *Piper nigrum*, and its amide derivatives have a variety of biological activities[35]. Piperine is consisted of three parts: benzodioxol group (A); a pentadienoic acid (B); the amine moiety (C) as shown in Figure **1**. Modulation of part A of piperine via introduction of an alkyl or halogen at phenyl ring, or replacing the whole benzodioxol substructure with other phenyl groups has been

achieved to give a new series of compounds[36]**.** Several of these molecules exhibited a good bioevaluation indices[37].

<<< Figure 1>>>>

In this work, the modifications of part C was carried out via substitution of the piperidine group (amine moiety) by aliphatic and aromatic hydrazones. We studied the impact of these substituents on the overall insecticidal activities of synthesized compounds against *Cx. pipens*. The synthesis of compounds **5a–u** is outlined in Figure **2**. The piperic acid (**2**) was obtained by alkaline hydrolysis of piperine. Then, piperic acid hydrazide (**4**) was easily prepared via the reaction of **2** with oxalyl chloride in the DCM as solvent and DMF as a catalyst, followed by reaction with hydrazine in DCM. The obtained hydrazide derivative was reacted with different corresponding carbonyl compounds in methanol in the presence acetic acid as a catalyst to afford piperine-based hydrazo-amide derivatives (**5a–u**) in 70-90% yield. All compounds were characterized by IR, ^{1}H, ^{13}C, ^{19}F NMR, ESI-MS and mp (**See supplementary data**). Moreover, the configuration of **5u** was elucidated by single-crystal X-ray diffraction analysis (Figure **3)**[38]**.**

<<< Figure 2>>>>

<<< Figure 3>>>>

3.2. Biological activity

The evaluation of larvicidal activity of **5a–u** was carried out against the 3rd instar larvae of *Cx. pipiens* by immersion method at concentrations ranging from 0.1-1.2 mg/mL for two days[39]. Deltamethrin, a conventional insecticide was utilized as the positive control at same concentrations. In this study, the work aimed to evaluate the toxicity of each synthesized compound and find out if the structural modification

carried out on piperine would result in a synthetic product more toxic than the natural product. The results of the bioassays are shown in Table **1**, Figures **4** and **S22.** The corresponding mortality after 48 hours was far higher than those after 24 hours. This phenomena indicated that the mechanism was different from neurotoxic insecticides such as pyrethroids, organophosphates, and carbamates, which exhibit delayed insecticidal activity[40,41]. Also, as noted in Table **1**, where the amide group of **1** was replaced by -COOH or hydrazide moiety, derivatives such as **2** and **4** did not improve the insecticidal activity than piperine. Interestingly, when **4** was reacted with different aldehydes and ketones to give piperine based dienehydrazide compounds **5a–u**, the all synthesized dienehydrazide derivatives gave more toxicity toward the investigated insect than deltamethrin as conventional insecticide. Eleven compounds (**5a-c**, **5f**, **5g**, **5m**, **5n**, **5o**, **5p**, **5u**, and **5q**) gave more insecticidal activity against 3rd instar larvae of *Cx. pipiens* than piperine as natural product insecticide. Their insecticidal activity was ranged from 70.66 to 83.33% after 48 h. Compounds **5a, 5b, 5p, 5g, 5u** and **5n** exhibited highest insecticidal activities compared to piperine and deltamethrin, and the mortality percentages were 81.33%, 81.33%, 81.33%, 82.66%, 82.66% and 83.33%, respectively compared to 26.25% as the final mortality rate of deltamethrin. The data shows that the presence of substituents groups at the C-2 position on the phenyl ring of the dienehydrazide derivatives was important to improve their insecticidal activity. For example, the final mortality rate of **5t** (not containing substituent group on the phenyl ring) was 52.50 %, whereas the final mortality rate of **5q** (containing 2-dihydroxy and 3-methoxy on the phenyl ring), **5b** (containing 4-chloro on the phenyl ring), and **5a** (containing 2-hydroxy on the phenyl ring) were 70.66, 81.33 and 81.33% respectively. It appears that there is a relationship between the chemical structure of phenolic moieties and its insecticidal activity.

<<< Table 1>>>>

<<< Figure 4>>>>

These compounds contain aromatic nucleus attached to a polar functional group (OH). It is well known that phenolic hydroxyl group is quiet reactive and easily forms hydrogen bonds with active sites of enzymes[42]**.** Similarly, the final mortality rate of **5f** and **5o** (containing 4-Nitro on the phenyl ring) were 80.00%; whereas the final mortality rate of **5n** (containing 2-OH and 4-*N,N*-diethylamino on the phenyl ring) was 83.33%. However, introduction of the substituent R as methoxy or carboxyl at 4-postion (e.g., **5e** and **5h**) did not exhibit more effective compounds compared to their precursor piperine. The introduction of OH group at position 2 with methoxy group at position 3 improved the final mortality to reach 70.66%. Interestingly, for compounds **5d**, **5s** and **5r** (final mortality was 61.33, 65.33 and 68.00%, respectively), it was found that the final mortality increased gradually as the number fluorinated carbons increased. Also, the results showed that the final mortalities of **5t**, **5i**, **5m** and **5u** were 52.50, 54.66, 80.00 and 82.66%, respectively, where the final mortality increased gradually with increasing the number of fused phenyl rings. This is may be due to the formation of more π-π stacking interactions with the active site of the enzyme, which make it more potent compound.

<<< Figures 5>>>>

As shown in Figures **5, S23** and **S24**, the 3rd instar larvae of *Cx. pipiens* treated with the synthesized compounds exhibited various morphological aberrations including the appearance of larval-pupal intermediate, malformed pupa and incomplete eclosions of adults. Similar results were described by Bosly who found that distinct malformations of larvae and pupae of the house fly were induced after treatment of the third larval instar with LC50 and LC75 of *Mentha piperita* and *Lavandula*

angustifoli[43]. These abnormalities could be attributed to hormonal disturbance that caused by **5n** and affect the insect metamorphosis. Mansour showed that Various ten morphological abnormalities as abnormal pupal size in addition to pupal adult intermediate were induced by using essential oils against *Cx. Pipiens* and *Musca domestica*, respectively[44].

3.3. Molecular docking studies

We performed molecular modelling followed by energy minimization of compounds **5a, 5f, 5n, 5g** in AChE of *Cx. Pipiens* to gain insights into the possible interactions between them and the binding site of AChE [32]. AChE is of interest because it is a critical nervous system enzyme responsible for synaptic transmission and is the target site for organophosphoate and carbamate insecticides [45,46]. Molecular modeling shows that pentadienoic acid is predicted to make π-π stacking interactions with Trp408. The phenol aromatic ring makes also π-π stacking interactions with Trp212. Molecular modeling of **5a** in the binding site of AchE shows that the hydroxyl group of the phenol group can form hydrogen bonding with His567. This explains why presence of substituents groups at the C-2 position on the phenyl ring of the dienehydrazide derivatives was important to improve their insecticidal activity (Figure **6**).

<<< Figure 6>>>>

Adding a nitro group as in compound **5f** results in formation of electrostatic interaction as a salt bridge between the nitrogen of the nitro group and the carboxyl group of Glu326 hence this modification is predicted to strengthen the binding (Figure **7**).

<<< Figure 7>>>>

Alternatively, replacing the phenol group with furan as in **5g** results in formation of π-π stacking interactions with His567 (Figure **8**).

<<< Figure 8>>>>

Adding 4-*N,N*-diethylamino to the phenol group in **5n** resulted in similar interactions as in **5a** and possibly enhanced fitting of the compound in the binding site (Figure **9**).

<<< Figure 9>>>>

The molecular docking analysis suggests that these compounds are predicted to be tight binders of AChE and most probably producing their insecticidal activities through inhibition of AChE enzyme.

4. Conclusion

A series of piperine-based dienehydrazide derivatives were designed and synthesized from piperine and their structures were elucidated by melting point, IR, ^{1}H, ^{13}C, ^{19}F NMR and ESI-HRMS. An x-ray single crystal of compound **5n** was obtained to further illustrate the structural indices of this series of synthesized compounds. The bioassays results showed that all these synthesized compounds showed better activities against pre-third-instar larval of *Cx. Pipiens* than commercial insecticide deltamethrin. And most of them exhibited stronger insecticidal activities than piperine. These kinds of compounds deserve further exploration to discover green pesticides.

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Supporting Information

Supporting information may be found in the online version of this article.

5. References:

[1] C. Lamberth, S. Jeanmart, T. Luksch, A. Plant, Current challenges and trends in the discovery of agrochemicals, Science (80-.). 341 (2013) 742–746. doi:10.1126/science.1237227.

[2] R. Pavela, Possibilities of botanical insecticide exploitation in plant protection.,

Pest Technol. 1 (2007) 47–52.

[3] J.D. Ríos-Díez, C.I. Saldamando-Benjumea, Susceptibility of Spodoptera frugiperda (Lepidoptera: Noctuidae) Strains From Central Colombia to Two Insecticides, Methomyl and Lambda-Cyhalothrin: A Study of the Genetic Basis of Resistance, J. Econ. Entomol. 104 (2011) 1698–1705. doi:10.1603/ec11079.

[4] D.G. Heckel, Insecticide Resistance After Silent Spring, Science (80-.). 337 (2012) 1612–1615.

[5] I.C. Yadav, N.L. Devi, J.H. Syed, Z. Cheng, J. Li, G. Zhang, K.C. Jones, Current status of persistent organic pesticides residues in air, water, and soil, and their possible effect on neighboring countries: A comprehensive review of India, Sci. Total Environ. 511 (2015) 123–137. doi:10.1016/j.scitotenv.2014.12.041.

[6] H.-R. Köhler, R. Triebskorn, Wildlife Ecotoxicology of Pesticides : Can We Track Effects to the, Science (80-.). 341 (2013) 759–765. doi:10.1126/science.1237591.

[7] F.E. Dayan, C.L. Cantrell, S.O. Duke, Natural products in crop protection, Bioorganic Med. Chem. 17 (2009) 4022–4034. doi:10.1016/j.bmc.2009.01.046.

[8] A.M.E. Palazzolo, C.L.W. Simons, M.D. Burke, The natural productome, Proc. Natl. Acad. Sci. 114 (2017) 5564–5566. doi:10.1073/pnas.1706266114.

[9] S. O Duke, S. B Powles, Glyphosate: a once-in-a-century herbicide, Pest Manag. Sci. 63 (2008) 1100–1106. doi:10.1002/ps.

[10] S. Il Kim, Y.J. Ahn, Larvicidal activity of lignans and alkaloid identified in Zanthoxylum piperitum bark toward insecticide-susceptible and wild Culex pipiens pallens and Aedes aegypti, Parasites and Vectors. 10 (2017) 1–10. doi:10.1186/s13071-017-2154-0.

[11] A.E. Hussein, A.E. H, R.A. Mohamed, A.E. Z, Toxicity of three chemical extracts of black pepper fruits against two stored grain insect pests, 6 (2017) 20–28.

[12] I.K. Park, S.G. Lee, S.C. Shin, J.D. Park, Y.J. Ahn, Larvicidal activity of isobutylamides identified in Piper nigrum fruits against three mosquito species, J. Agric. Food Chem. 50 (2002) 1866–1870. doi:10.1021/jf011457a.

[13] P. Makhov, K. Golovine, D. Canter, A. Kutikov, J. Simhan, M.M. Corlew, R.G. Uzzo, V.M. Kolenko, Co-administration of piperine and docetaxel results in improved anti-tumor efficacy via inhibition of CYP3A4 activity, Prostate. 72 (2012) 661–667. doi:10.1002/pros.21469.

[14] U.H. Park, H.S. Jeong, E.Y. Jo, T. Park, S.K. Yoon, E.J. Kim, J.C. Jeong, S.J. Um, Piperine, a component of black pepper, inhibits adipogenesis by antagonizing PPARγ activity in 3T3-L1 cells, J. Agric. Food Chem. 60 (2012) 3853–3860. doi:10.1021/jf204514a.

[15] G.S. Bae, M.S. Kim, W.S. Jung, S.W. Seo, S.W. Yun, S.G. Kim, R.K. Park, E.C. Kim, H.J. Song, S.J. Park, Inhibition of lipopolysaccharide-induced inflammatory responses by piperine, Eur. J. Pharmacol. 642 (2010) 154–162. doi:10.1016/j.ejphar.2010.05.026.

[16] Z.M. Mirza, A. Kumar, N.P. Kalia, A. Zargar, I.A. Khan, Piperine as an inhibitor of the MdeA efflux pump of Staphylococcus aureus, J. Med. Microbiol. 60 (2011) 1472–1478. doi:10.1099/jmm.0.033167-0.

[17] V.F. de Paula, L.C. de A. Barbosa, A.J. Demuner, D. Piló-Veloso, M.C. Picanço, Synthesis and insecticidal activity of new amide derivatives of piperine, Pest Manag. Sci. 56 (2002) 168–174. doi:10.1002/(sici)1526-4998(200002)56:2<168::aid-ps110>3.3.co;2-8.

[18] W.S. Tavares, I. Cruz, F. Petacci, S.S. Freitas, J.E. Serrão, J.C. Zanuncio, Insecticide activity of piperine: Toxicity to eggs of *Spodoptera frugiperda* (Lepidoptera : Noctuidae) and *Diatraea saccharalis* (Lepidoptera : Pyralidae) and phytotoxicity on several vegetables, J. Med. Plants Res. 5 (2011) 5301–5306.

[19] D. Grzywacz, P.C. Stevenson, W.L. Mushobozi, S. Belmain, K. Wilson, The use of indigenous ecological resources for pest control in Africa, Food Secur. 6 (2014) 71–86. doi:10.1007/s12571-013-0313-5.

[20] E.U. Asogwa, L.N. Dongo, Problems associated with pesticide usage and application in Nigerian cocoa production: A review, African J. Agric. Res. 4 (2009) 675–683.

[21] V. Hongoh, L. Berrang-Ford, M.E. Scott, L.R. Lindsay, Expanding geographical distribution of the mosquito, Culex pipiens, in Canada under climate change, Appl. Geogr. 33 (2012) 53–62. doi:10.1016/j.apgeog.2011.05.015.

[22] Y. Kebede, T. Gebre-Michael, M. Balkew, Laboratory and field evaluation of neem (Azadirachta indica A. Juss) and Chinaberry (Melia azedarach L.) oils as repellents against Phlebotomus orientalis and P. bergeroti (Diptera: Psychodidae) in Ethiopia, Acta Trop. 113 (2010) 145–150. doi:10.1016/j.actatropica.2009.10.009.

[23] M. Elliott, A.W. Farnham, N.F. Janes, D.M. Johnson, D.A. Pulman, Synthesis and Insecticidal Activity of Lipophilic Amides. Part 6: 6-(Disubstituted-phenyl)hexa-2,4-dienamides, Pestic. Sci. 18 (1987) 239–244.

[24] M. Elliott, A.W. Farnham, N.F. Janes, D.M. Johnson, D.A. Pulman, R.M. Sawicki, Insecticidal Amides with Selective Potency Against a Resistant (Super-Kdr) Strain of Houseflies Musca Domestica L, Agric. Biol. Chem. 50 (1986) 1347–1349. doi:10.1080/00021369.1986.10867574.

[25] Z. Latif, T.G. Hartley, M.J. Rice, R.D. Waigh, P.G. Waterman, G. St, G. Glasgow, F.M.T.G. Hartley, Novel and Insecticidal Isobutylamides from Dinosperma erythrococca Soxhlet extraction of the dried ground aerial parts, 3864 (1998) 614–619.

[26] M. Kasap and L. Demirhan, The effect of various larval foods on the rate of adult emergence and fecundity of mosquitoes, Turkiye Parazitol. Derg. 161 (1992) 87–97.

[27] E. Gerberg, Manual for mosquito rearing and experimental techniques, Bull. 5. Am. Mosq. Control Assoc. Lake Charles, Louisiana, USA. (1970).

[28] H. Qu, X. Yu, X. Zhi, M. Lv, H. Xu, Natural-product-based insecticidal agents 14 . Semisynthesis and insecticidal activity of new piperine-based hydrazone derivatives against Mythimna separata Walker in vivo, Bioorg. Med. Chem. Lett. 23 (2013) 5552–5557.

[29] J. Demuner, F. De Paula, L.C.D.A. Barbosa, Synthesis and insecticidal activity of new amide derivatives of piperine †, Pest Manag. Sci. 174 (2000) 168–174.

[30] A. Roy, A. Kucukural, Y. Zhang, I-TASSER: a unified platform for automated protein structure and function prediction, Nat. Protoc. 5 (2010) 725–738. doi:10.1038/nprot.2010.5.

[31] J. Yang, R. Yan, A. Roy, D. Xu, J. Poisson, Y. Zhang, The I-TASSER Suite: protein structure and function prediction, Nat. Methods. 12 (2015) 7–8. doi:10.1038/nmeth.3213.

[32] Q. Han, D.M. Wong, H. Robinson, H. Ding, P.C.H. Lam, M.M. Totrov, P.R. Carlier, J. Li, Crystal structure of acetylcholinesterase catalytic subunits of the malaria vector Anopheles gambiae, Insect Sci. 25 (2018) 721–724. doi:10.1111/1744-7917.12450.

[33] G. S., L. S., C. M., Y. H., S. J.A., K. Y.N., D. J., K. G., D. J.B., C. H.A., B. S.K., W. W.P., A. R.E., F. V.A., V.A.. O. http://orcid. org/000.-0001-9899-8304

Gilson M.K. AO - Feher, D3R grand challenge 2015: Evaluation of protein-ligand pose and affinity predictions, J. Comput. Aided. Mol. Des. (2016). doi:10.1007/s10551-013-1673-7.

[34] Schrödinger Release 2019-1: MacroModel, Schrödinger, LLC, New York, NY, 2019., (n.d.).

[35] F. Saczewski, A. Bułakowska, Synthesis, structure and anticancer activity of novel alkenyl-1,3,5-triazine derivatives, Eur. J. Med. Chem. 41 (2006) 611–615. doi:10.1016/j.ejmech.2005.12.012.

[36] A. Kumar, I.A. Khan, S. Koul, J.L. Koul, S.C. Taneja, I. Ali, F. Ali, S. Sharma, Z.M. Mirza, M. Kumar, P.L. Sangwan, P. Gupta, N. Thota, G.N. Qazi, Novel structural analogues of piperine as inhibitors of the NorA efflux pump of Staphylococcus aureus, J. Antimicrob. Chemother. 61 (2008) 1270–1276. doi:10.1093/jac/dkn088.

[37] E.A. Correa, E.D. Högestätt, O. Sterner, F. Echeverri, P.M. Zygmunt, In vitro TRPV1 activity of piperine derived amides, Bioorganic Med. Chem. 18 (2010) 3299–3306. doi:10.1016/j.bmc.2010.03.013.

[38] A.H. Tantawy, H. Jiang, M.Q. Wang, CCDC 1934954: Experimental Crystal Structure Determination, 2019, DOI: 10.5517/ccdc.csd.cc22ygwd, 2019.

[39] R. Toolabi, M. Reza Abai, M.M. Sedaghat, H. Vatandoost, M. Shayeghi, S. Tavakoli, M.S. Aghdam, Larviciding Activity of Acroptilon repens Extract against Anopheles stephensi, Culex pipiens and Culex quinquefaciatus under Laboratory Conditions, Pharmacogn. J. 10 (2018) 453–456. doi:10.5530/pj.2018.3.74.

[40] X.I.Y. Ao, X.I.N.G.Z. Hang, Semisynthesis and Quantitative Structure - Activity Relationship (QSAR) Study of Novel Aromatic Esters of 4 -Demethyl-4-deoxypodophyllotoxin as Insecticidal Agents, 67 (2009) 7919–7923. doi:10.1021/jf9020812.

[41] H. Xu, J. Wang, Bioorganic & Medicinal Chemistry Letters Natural products-based insecticidal agents 5 . Design , semisynthesis and insecticidal activity of novel 4 0 -substituted benzenesulfonate derivatives of 4-deoxypodophyllotoxin against Mythimna separata Walker in v, Bioorg. Med. Chem. Lett. 20 (2010) 2500–2502. doi:10.1016/j.bmcl.2010.02.108.

[42] D.A. Kraut, P.A. Sigala, B. Pybus, C.W. Liu, D. Ringe, G.A. Petsko, D. Herschlag, Testing electrostatic complementarity in enzyme catalysis: Hydrogen

652 bonding in the ketosteroid isomerase oxyanion hole, PLoS Biol. 4 (2006) 501–
653 519. doi:10.1371/journal.pbio.0040099.
654 [43] B. Bosly, Evaluation of insecticidal activities of Mentha piperita and Lavandula
655 angustifolia essential oils against house fly, Musca domestica L. (Diptera:
656 Muscidae), J. Entomol. Nematol. 5 (2013) 50–54. doi:10.5897/jen2013.0073.
657 [44] S. A. Mansour, Larvicidal Activity of Some Botanical Extracts, Commercial
658 Insecticides and their Binary Mixtures Against the Housefly, Musca Domestica
659 L., Open Toxinology J. 4 (2012) 1–13. doi:10.2174/1875414701104010001.
660 [45] K. Singh, D.K. Singh, Toxicity to the snail Limnaea acuminata of plant-derived
661 molluscicides in combination.pdf, 898 (2000) 889–898.
662 [46] D. Aygun, Z. Doganay, L. Altintop, H. Guven, M. Onar, T. Deniz, T. Sunter,
663 Serum acetylcholinesterase and prognosis of acute organophosphate poisoning,
664 J. Toxicol. - Clin. Toxicol. 40 (2002) 903–910. doi:10.1081/CLT-120016962.
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667 **LIST OF FIGURES**

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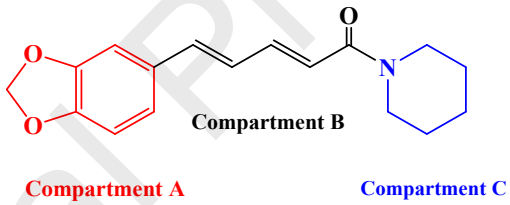
No.	Caption
Figure 1	Chemical structure of piperine.
Figure 2	General scheme for synthetic route for the synthesis of **5a–u**. Reagents and conditions: (a) KOH, 95% methanol, reflux, 48h, 93.4%; (b) $(COCl)_2$, DCM, three drops DMF, room temperature, 4 h; (c) NH_2NH_2/DCM, room temperature, 7 h, 87%; (d) corresponding carbonyl compounds, methanol, reflux, 2-6 h, 55-86%.
Figure 3	The X-ray single crystal structure of **5n**.
Figure 4	The corrected mortality rates of *Cx.* pipens caused by more active compounds with the increase of concentration after 48 h.
Figure 5	**A-** Normal 3rd instar larvae; **B-** 4th instar larva showing symptoms of dwarfism and body shrinkage; **C-** Malformed larvae with the appearance of neck between head and 1st thoracic segment; **D-** Larva has a swollen thorax, which finally lead to rupture of the larva; **E-** 4th instar larva with old exuvium of the 3rd instar larva on the posterior part of the body.
Figure 6	**A.** two- dimensional and **B.** three-dimensional figures demonstrating molecular interactions of compound **5a** in the binding site of AchE. π-π stacking interactions are colored green lines while hydrogen bonding interactions are shown as purple lines.
Figure 7	**A.** two- dimensional and **B.** three-dimensional figures demonstrating molecular interactions of compound **5f** in the binding site of AchE. π-π stacking interactions are colored green lines while hydrogen bonding interactions are shown as purple lines
Figure 8	**A.** two- dimensional and **B.** three-dimensional figures demonstrating molecular interactions of compound **5g** in the binding site of AchE. π-π stacking interactions are colored green lines while hydrogen bonding interactions are shown as purple lines.
Figure 9	**A.** two- dimensional and **B.** three-dimensional figures demonstrating molecular interactions of compound **5n** in the binding site of AchE. π-π stacking interactions are colored green lines while

	hydrogen bonding interactions are shown as purple lines

Figure 1



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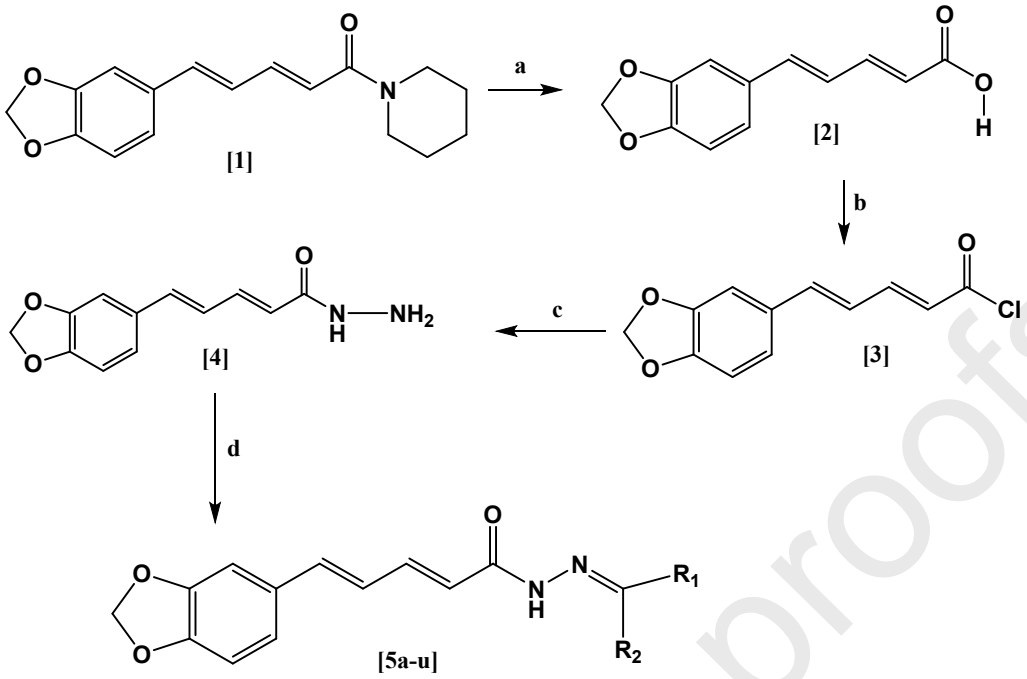

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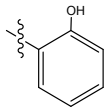
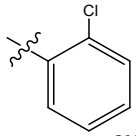
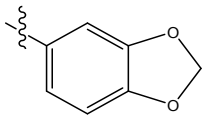
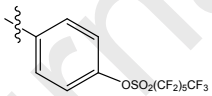
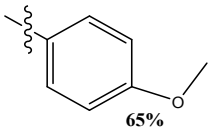
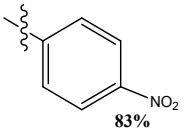
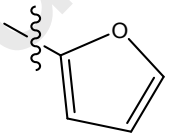
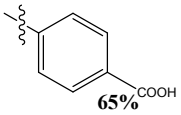
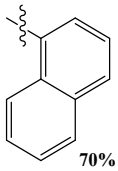
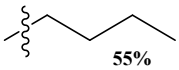
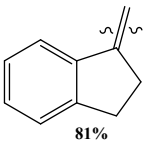
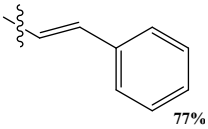
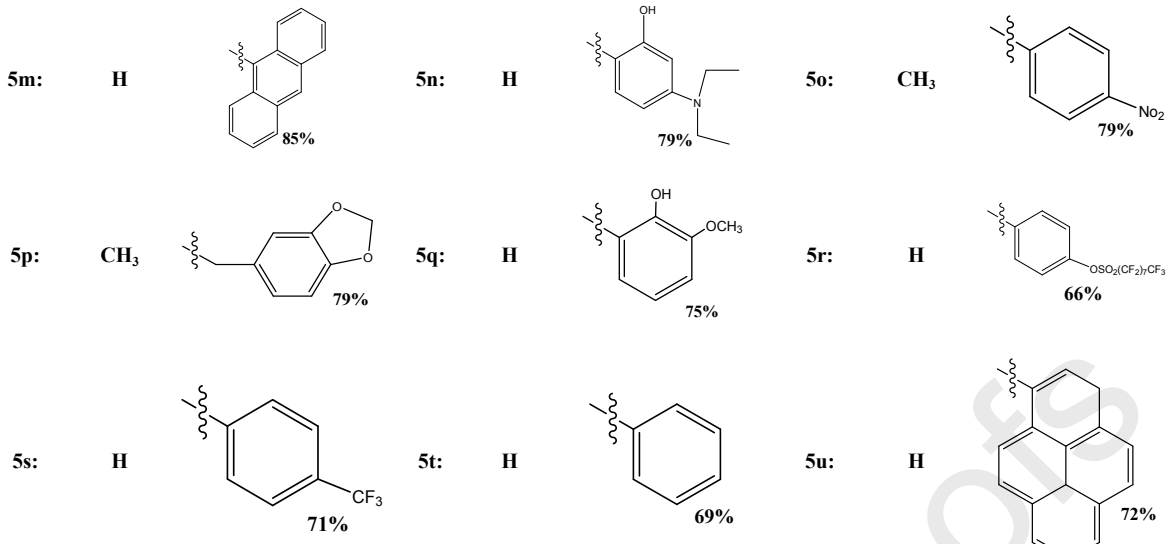
	R1	R2		R1	R2		R1	R2
5a:	H	 80%	5b:	H	 80%	5c:	CH_3	 78%
5d:	H	 69%	5e:	H	 65%	5f:	H	 83%
5g:	H	 77%	5h:	H	 65%	5i:	H	 70%
5j:	H	 55%	5k:		 81%	5l:	H	 77%



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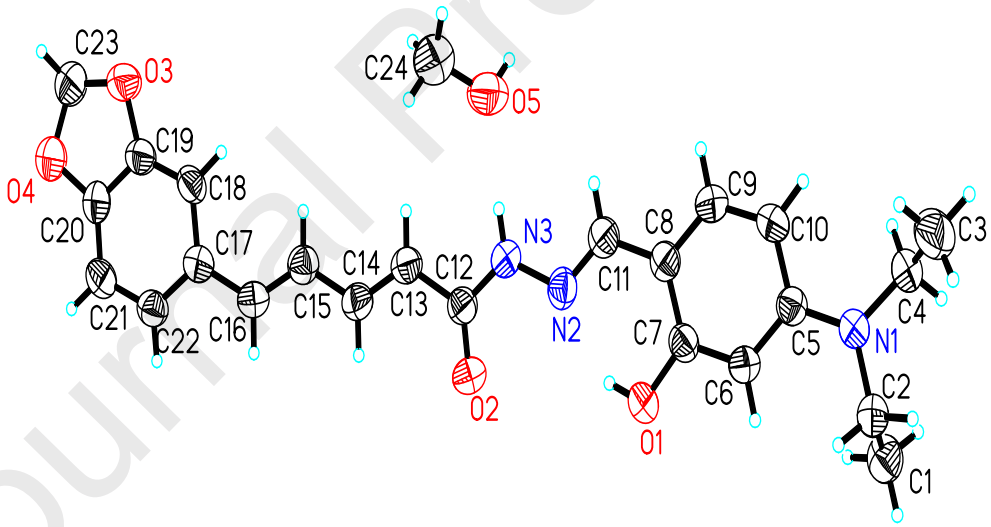

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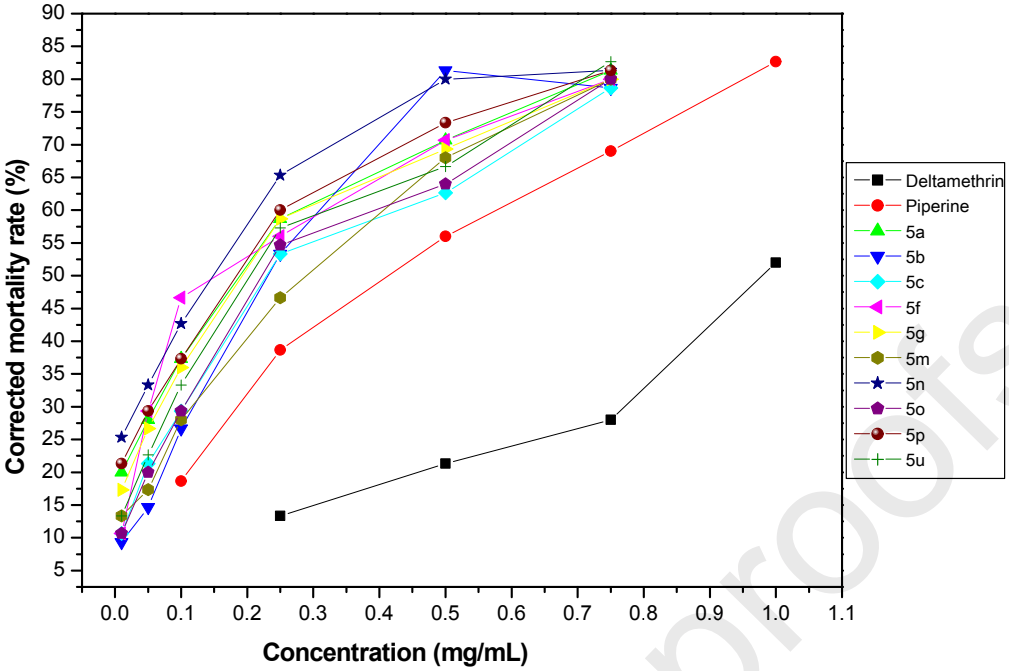
704 **Figure 4**



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735 **Figure 5**

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Figure 6

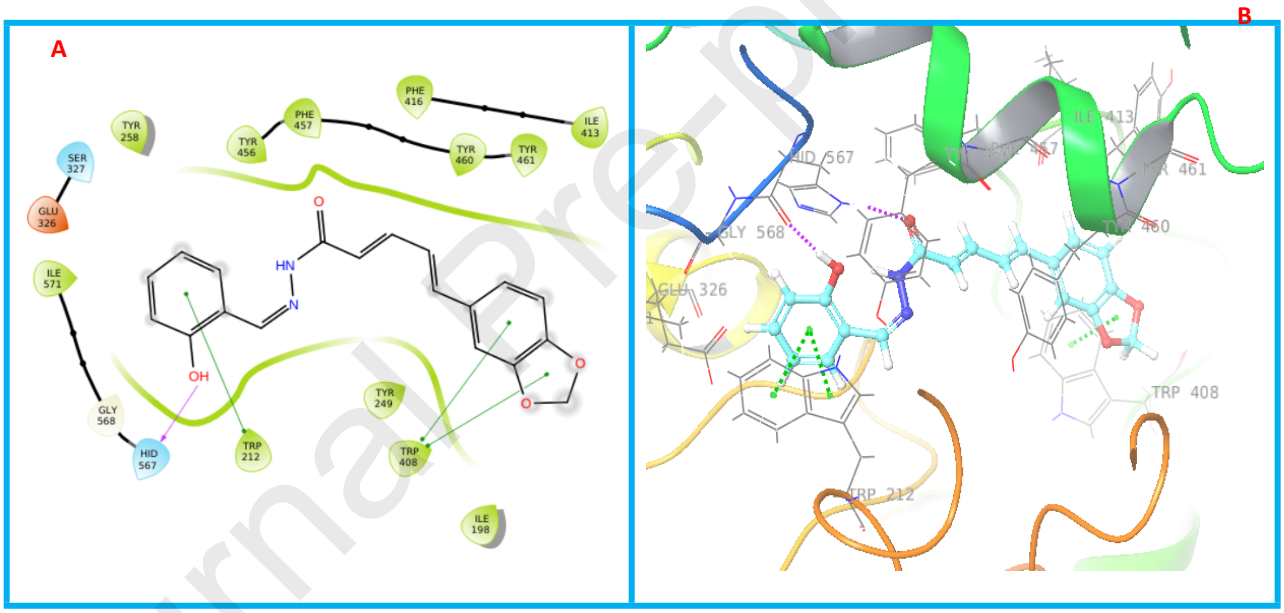


Figure 7

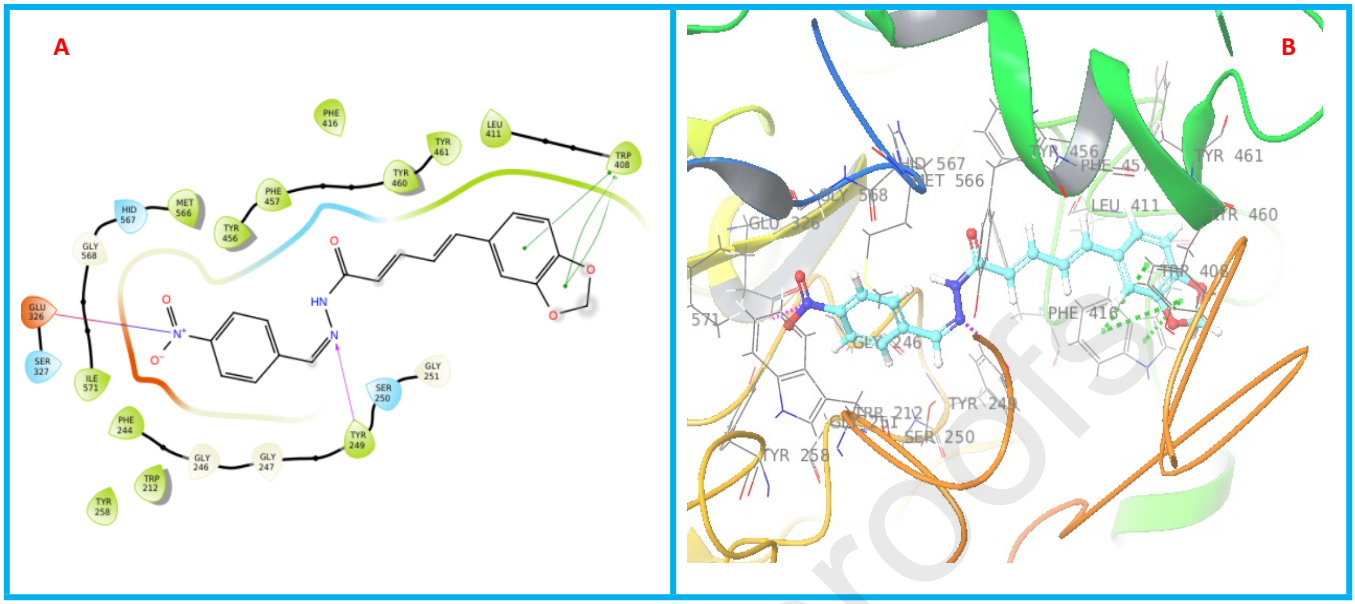
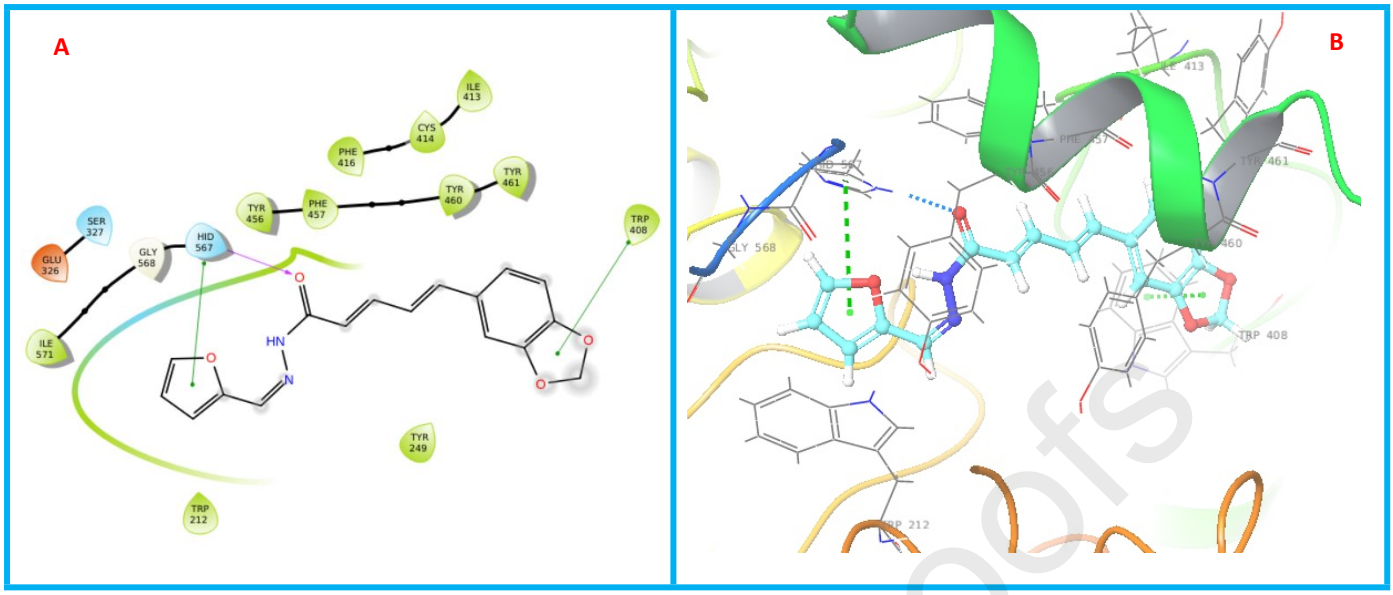


Figure 8



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792 **Figure 9**

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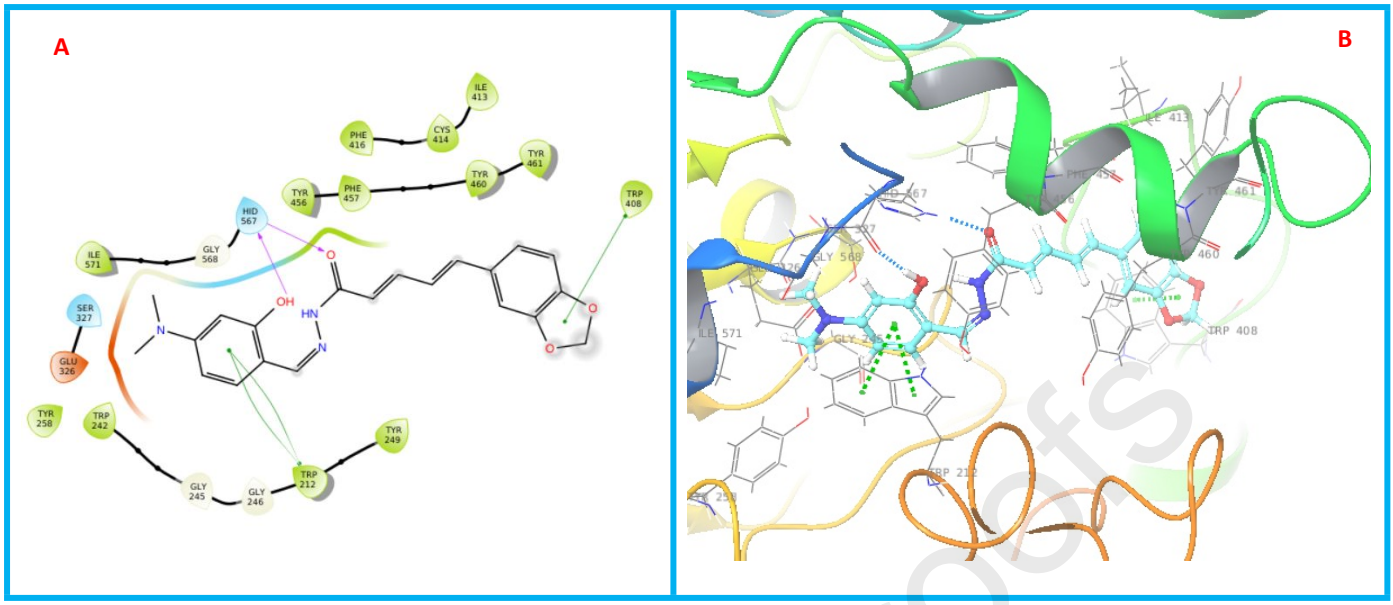

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List of Tables

Table 1	Insecticidal activity of the synthesized compounds **5a–u** against 3rd larval instar of *Cx. pipens* at a concentration of 0.75 mg/mL.

825 **Table 1**

compound	Corrected mortality rate (% ± SD)		LC_{50} (mg/mL ± SD)
	24 hours	48 hours	
2	61.33±0.577	64.00±0.00	0.398±0.02
4	60.00±0.00	62.66±0.57	0.421±0.03
5a	76.00±0.00	81.33±0.58	0.139±0.03
5b	78.66±0.58	81.33±0.58	0.216±0.05
5c	74.66±0.58	78.66±0.57	0.217±0.04
5d	58.66±0.57	61.33±0.58	0.447±0.03
5e	58.66±0.58	61.33±0.58	0.444±0.03
5f	70.66±0.58	80.00±0.00	0.146±0.03
5g	80.00±0.00	82.66±0.00	0.153±0.01
5h	56.33±0.58	59.33±0.58	0.612±0.06
5i	50.66±0.58	54.66±0.58	0.546±0.09
5j	61.33±0.58	65.33±0.58	0.393±0.04
5k	57.33±0.58	58.66±0.58	0.466±0.03
5l	54.66±0.57	57.33±0.58	0.476±0.05
5m	76.00±0.00	80.00±0.00	0.221±0.03
5n	80.00±0.00	83.33±0.58	0.094±0.03
5o	77.33±0.00	80.00±0.00	0.209±0.03
5p	78.66±0.57	81.33±0.58	0.128±0.09
5q	66.66±0.58	70.66±0.57	0.371±0.03
5r	62.66±0.58	68.00±0.00	0.363±0.02
5s	61.33±0.58	65.33±0.58	0.405±0.03
5t	50.33±0.58	52.50±0.00	0.514±0.04
5u	80.00±0.00	82.66±0.58	0.174±0.05
Piperine (1)	64.00±0.00	69.00±0.00	0.357±0.05

Deltamethrin (insecticide)	22.50±1.00	26.25±1.00	1.457±0.04

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The larvicidal activity of natural inspired piperine-based dienehydrazides against *Culex pipiens*

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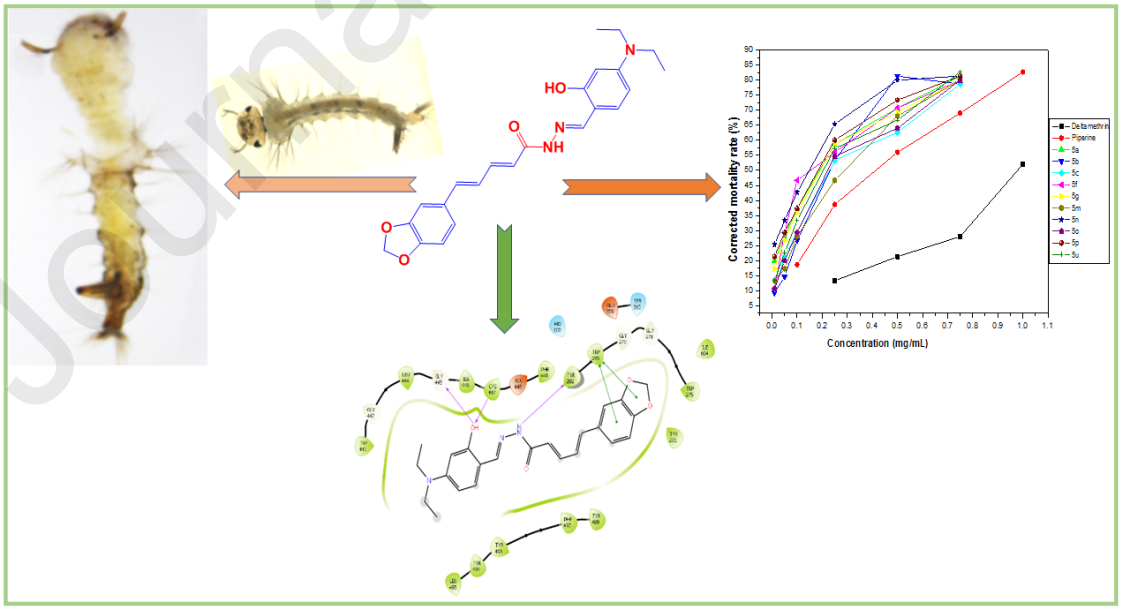
Graphical abstract:



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Highlights

- A series of piperine-based dienehydrazide derivatives were synthesized from piperine as a natural product.
- Chemical structures of synthesized compounds have been elucidated via various spectroscopic tools.
- The larvicidal activity of prepared compounds at different concentrations were evaluated against third-instar larval of *Cx. pipiens*.
- Molecular modelling of several molecular interactions between synthesized compounds and the substrate binding sits of acetylcholinesterase (AChE) was studied.