Saudi Journal of Biological Sciences 29 (2022) 2389–2395

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Mosquito larvicidal activity of pyrrolidine-2,4-dione derivatives: An investigation against *Culex quinquefasciatus* and molecular docking studies

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ARTICLE INFO

Article history:
Received 7 September 2021
Revised 15 November 2021
Accepted 4 December 2021
Available online 10 December 2021

Keywords:
Mannich base
Pyrrolidine-2
4-dione derivative
Grindstone chemistry
Larvicidal activity
Culex quinquefasciatus
Molecular docking

ABSTRACT

The pyrrolidine-2,4-dione derivatives were used to conduct a larvicidal test on *Culex quinquefasciatus* larvae of the second instar. Mannich base condensation method was used to synthesis the pyrrolidine-2,4-dione derivatives by grindstone method. The reaction conditions were mild, resulting in high yields. An analysis of the synthesized compounds was carried out using FTIR, ^{1}H NMR, ^{13}C NMR, mass spectrometry, and elemental analysis. Synthesized compounds (**1a-h**) were evaluated for larvicidal activities. Compound **1e** (LD_{50}: 26.06 μg/mL), and **1f** (LD_{50}: 26.89 μg/mL), and were notably more active against *Culex quinquefasciatus* than permethrin (LD_{50}: 26.14 μg/mL). The docking studies also demonstrated that **1e,** and **1f** are potent larvicides with higher binding energy (−12.6 kcal/mol) than the control in the mosquito odorant binding protein (PDB ID: 3OGN). The larvicidal properties of lead molecules have made them important for use as insecticides.

1. Introduction

Mosquitoes play an important role in the transmission of malaria (Georges et al., 2008, Govindarajan, 2010). This type of disease has a global impact both economically and socially. In several regions, *Culex quinquefasciatus* is particularly associated with vector-spread diseases. Larvicides are insecticides designed to kill larval insects. As larvae grow, methoprene prevents them from developing beyond pupa stage (Lawler, 2017). There are mosquitoes transmitted by *Culex quinquefasciatus* in both rural and urban environments (Alvarez et al., 2006; Bisset et al., 1998). Botanical insecticide studies have been conducted in recent years to come up with alternatives to synthetic insecticides. Their non-toxic, natural, low-toxicity and biodegradable characteristics allow them to be used for insecticides, larvicides, deterrents, repellents, growth inhibitors, and antifeedants (Isman, 2006). The continued use of synthetic insecticides in water, soil, and atmosphere is ecologically unacceptable, as they cause serious environmental problems and contaminate animals and food. The result is that insects, fungi, and bacteria become resistant to it. Diseases spread by insect vectors, such as leishmaniasis, yellow fever, malaria, dengue, and filariasis, also affect public health. In regions with unsanitary conditions, inadequate potable water supplies, and overcrowded areas, these diseases have a significant economic and social impact. A grant for odorant binding proteins (OBPs) was funded for this research. OBPs are small proteins (15 kDa) with six helices (α1-α6) and three disulfide bridges. A pheromone-binding protein (PBP) and a odorant-binding protein (GOBP) are two major classes of these proteins (GOBPs) (Pelosi and Maida, 1995; Zhou, 2010). OBP can be summarized in three groups such as long-chain, middle-chain, and short-chain amino acids.

OBPs act as carriers of chemical signals (semiochemicals) to the olfactory receptors (ORs) so that they can be processed before reaching the neuron. *Aedes aegypti, Culex quinquefasciatus*, and *Nopheles gambiae* are common vectors of malaria, filariasis, and

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.12.003
1319-562X/

cephalitis, respectively (Chandre et al., 1998; Barbosa aet al., 2010). Insects OBPs have an outstanding pH-dependent mechanism of odorant binding and release, which able to bind odorants at a basic pH (6.5), transport and release them at an acid pH (4.5) (Di Luccio et al., 2013). Focusing of this study, we examined *Culex quinquefasciatus* (CquiOBP1) (PDB code: 3OGN). *Culex quinquefasciatus* CquiOBP1 (3OGN) is unique because of its short C-terminus, which differs from Bombyx mori (BmorPBP) and Antheraea polyphemus (ApolPBP), which have their C-terminus integrated into the central cavity wall (Mao et al., 2010). The CquiOBP1 and oviposition pheromone (MOP, (5R,6S)-6-acetoxy-hexadec anolinide) complexes bind strongly at pH 7 but poorly at pH 5 (Leal et al., 2008). A mechanism analysis of CquiOBP1 may prove useful based on the close agreement between theoretical and experimental results.

There is a great need for greener reactions in organic chemistry because a large number of industrial processes use hazardous chemicals and solvents, causing severe environmental damage. Organic chemistry uses green techniques including reactions of C–H bond activation, asymmetric synthesis, the use of water as a solvent, or even without a solvent, microwaves, ultrasounds, and ultraviolet radiation (Wei and Berkeley, 2018).

The active compounds contained in natural products, including nicotine, toosentanin, pyrethrum, and the essential oil of Caesulia axillaris.

Fig. 1 shows that pyrrolidine-2,4-dione (also known as tetramic acid) natural compound such as tenuazonic acid, erythroskyrine, melophlin A, cryptocin, hazzianic acid (Schobert and Schlenk, 2008; Dixon et al., 1999; Aoki et al., 2000; Li et al., 2000). With regard to herbicides, a series of 3-(α-hydroxy-benzylidene)pyrroli dine-2,4-dione derivatives having high efficiency, and tenuazonic acid derivatives with oxime moieties showed herbicidal and antifungal activities (Zhu et al., 2009). The pyrrolidine-2,4-dione moiety is located on nitrogenous heterocyclic compounds, such as natural tetramic acids, which are commonly used as antitumors, antivirals, insecticides, fungicides, and herbicides (Mary et al., 1991; Singh et al., 1998; Chen et al., 2008; Cole and Rolinson,1972; Li et al., 2000). Some of the pyrrolidine-2,4-dione derivatives, and tetramic acid derivatives have impressive active core molecules in various biological system (Han et al., 2012; Si et al., 2011; Zheng et al., 2011; Zhu et al., 2007; Zhu et al., 2005). As noted earlier, in comparison with tetramic acid, and tenuazonic acid in the oxime ether family, the chemicals with appropriate groups added to the 3-position displayed superior activity against phytopathogenic fungi (Wang et al., 2010; Zhu et al., 2010a, Zhu et al., 2010b; Zhu et al., 2009). Strabilurin A is a chemical compound made up of reactive groups of methoxyacrylates that is used to make fungicides. Their unique mechanism of action as well as high activity, and outstanding environmental tolerance distinguish them from many other available pesticides (Yan et al., 2006). The most common tool for controlling mosquito populations is chemical insecticide (Bowman, et al., 2016), however, non-target organisms and their environmental toxicity limit their use (Nkya et al., 2013; Jayaraj et al., 2017), a number of larval active targets have been identified in previous studies, but chemical insecticides pose many more problems such as resistance to chemicals and disruption of biocontrol systems (Park et al., 2005; Yang et al., 2013). To solve these problems, new mosquito larvae inhibitors are needed, as well as greener methods that can be achieved using pyrrolidine-2,4-dione derivatives synthesized from Mannich condensation method.

2. Materials and methods

Nicolet iS5 from Thermo scientific was used to analyze all compounds using FTIR (4000–400 cm^{-1}). An Bruker DRX-300 MHz, 75 MHz NMR spectrometer was used to analyze the 1H and 13C NMR spectra. An elemental analyzer (the Vario EL III) was used to find the percentage (%) of assemblies (C, H, N, and S). GCMS model Clarus SQ8 (EI) from Perkin Elmer measured the mass spectra.

2.1. Synthesis of compound 1a-h: General procedure

In a mortar, a mixture of cinnamaldehyde (0.01 mol) was mixed with pyrrolidine-2,4-dione (0.01 mol) and substituted amine (0.01 mol) and ground at room temperature. Solid material was extracted by column chromatography (Ethyl acetate4:hexane6). Compounds **1b-1h** were prepared by following the above method.

2.1.1. 3-(1-hydrazinyl-3-phenylallyl)pyrrolidine-2,4-dione (1a)

Yield 87%; mp: 142–140 °C; IR(KBr) *v*: 3214.41, 3069.38, 3011.50, 1739.18, 1611.23 cm^{-1}; ^{1}H NMR (300 MHz): δ 8.61 (s, 2H, NH_2), 8.35 (s, 1H), 7.42–7.40 (dd, 2H, J = 7.36 Hz, J = 7.31 Hz), 7.33–7.0 (d, 1H, J = 7.31 Hz), 7.26–7.22 (d, 2H, J = 6.23 Hz, 1H), 6.51 (s, 1H), 6.19 (s, 1H), 4.54 (s, 2H), 3.76 (d, 1H, J = 7.42 Hz), 3.52 (d, 1H, J = 7.43 Hz), 2.28 (s, NH); ^{13}C NMR (75 MHz): 207.1, 176.5 (1C), 134.4, 133.1, 137.2, 128.8, 128.6 (6C, Ph ring), 127.2, 64.3, 56.8; EIMS (m/z): 245.30 (M^+, 28%).

2.1.2. 3-(1-(2-benzylidenehydrazinyl)-3-phenylallyl)pyrrolidine-2,4-dione (1b)

Yield 86%; mp: 167–164 °C; IR(KBr) *v*: 3212.38, 3078.31, 3018.32, 1721.10, 1610.29 cm^{-1}; ^{1}H NMR (300 MHz): δ 8.45 (s,

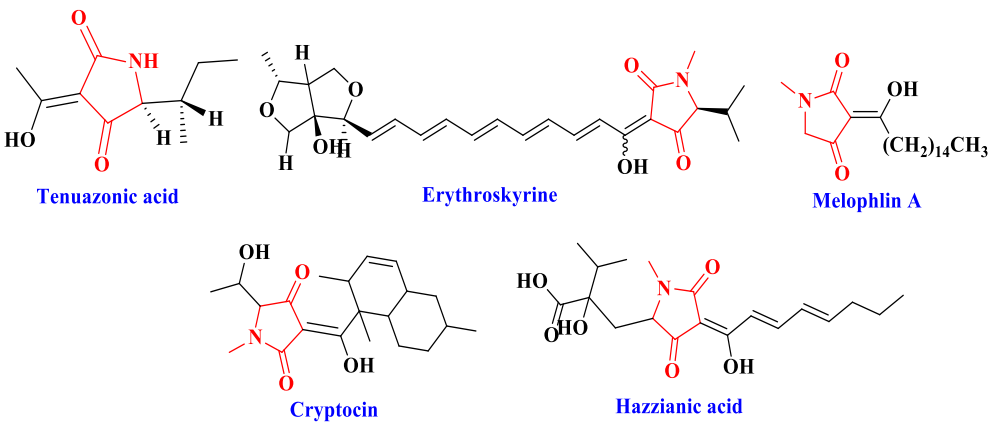


Fig. 1. Some bioactive naturally existing pyrrolidine-2,4-dione.

1H), 8.31 (s, 1H), 7.54 (d, 1H, *J* = 7.33 Hz), 7.87–7.80 (d, 2H, *J* = *7.12* Hz), 7.52–7.50 (dd, *J* = 7.35 Hz, *J* = 7.31 Hz, 2H, Ar-ring), 7.53–7.51 (dd, 2CH, *J* = *7.11* Hz), 7.50 (d, 1CH, *J* = *7.12* Hz), 7.33–7.30 (d, 1H, *J* = 7.31 Hz), 7.26–7.22 (d, 1H, *J* = 6.21 Hz), 6.54 (s, 1H), 6.18 (s, 1H), 4.41 (s, -CH_2-), 3.78 (d, 1H, *J* = 7.42 Hz), 3.53 (d, 1H, *J* = 7.43 Hz), 2.20 (s, NH); ^{13}C NMR (75 MHz): 206.3, 176.1 (1C), 143.1, 137.3, 129.0, 128.4, 128.3, 127.6, 136.2, 134.4, 133.9, 131.2 129.2, 128.5, 127.9, (6C, Ph ring), 65.3, 53.8, 52.3; EIMS(*m*/*z*) 333.36 (M^+, 36%).

2.1.3. 3-(3-phenyl-1-(phenylamino)allyl)pyrrolidine-2,4-dione (1c)

Yield 85%; mp: 147–142 °C; IR(KBr) *ν*: 3202.08, 3058.06, 3011.78, 1720.78, 1618.21 cm^{-1} ; ^{1}H NMR (300 MHz): *δ* 8.38 (s, 1H), 7.41–7.39 (dd, 2H, *J* = 7.36 Hz, *J* = 7.31 Hz), 7.33–7.31 (d, *J* = 7.31 Hz, 1H, Ar-ring), 7.25–7.23 (d, *J* = 6.21 Hz, 1H, Ar-ring), 7.24–7.21 (d, 2CH, *J* = *7.12* Hz, Phenyl ring), 6.82–6.80 (dd, 2CH, *J* = *7.12* Hz), 6.72 (d, 1H, 6.51 (s, 1H), 6.13–6.10 (d, 1H, *J* = *7.12* Hz), 6.17 (s, 1H, CH), 4.46 (2H, -CH_2), 4.21 (s, NH), 3.75 (d, 1H, *J* = 7.42 Hz), 3.57 (d, 1H, *J* = 7.43 Hz); ^{13}C NMR (75 MHz): 206.2, 176.8 (1C), 147.2, 136.6, 134.8, 129.6, 128.3,128.4, 126.2 (6C, Ph ring), 128.2, 120.8, 119.5, 67.5, 56.9, 52.4 (1C); EIMS(*m*/*z*): 306.17 (M^+, 25%).

2.1.4. 3-(3-phenyl-1-(p-tolylamino)allyl)pyrrolidine-2,4-dione (1d)

Yield 89%; mp: 147–142 °C; IR(KBr) *ν*: 3211.12, 3064.09, 3002.31, 1723.17, 1613.21 cm^{-1}; ^{1}H NMR (300 MHz): *δ* 8.23 (s, 1H), 7.40–7.38 (dd, 2H, *J* = 7.33 Hz), 7.35 (d, 1H, CH, *J* = 7.31 Hz), 7.33–7.29 (d, *J* = 7.21 Hz, 1H, Ar-ring), 7.24–7.21 (d, *J* = 7.30 Hz, 2H, Ar-ring), 7.50 (d, 1CH, *J* = *7.12* Hz), 7.26–7.21 (d, 1H, *J* = 6.21 Hz), 7.05–7.01 (d, 2CH, *J* = 7.02 Hz, Phenyl ring), 6.49–6.44 (d, 2CH, *J* = *6.31* Hz), 6.15 (s, 1H, CH), 6.51 (s, 1H), 4.56 (s, CH), 4.02 (NH), 3.79 (d, *J* = 6.32 Hz, 1H, CH), 3.49 (d, 1H, *J* = 7.41 Hz), 2.28 (s, NH); ^{13}C NMR (75 MHz): 208.6, 175.6, 136.9, 128.6, 128.5, 187.6, 127.0, 128.3, 113.6, 54.3, 52.6 (1C), 28.6, 21.2; EIMS(*m*/*z*): 320.19 (M^+, 15%).

2.1.5. N-(1-(2,4-dioxopyrrolidin-3-yl)-3-phenylallyl)acetamide (1e)

Yield 90%; mp: 147–142 °C; IR(KBr) *ν*: 3252.12, 3071.10, 3017.19, 1720.09, 1609.19 cm^{-1}; ^{1}H NMR (300 MHz): *δ* 8.35 (s, 1H), 8.03 (s,1H), 7.46–7.42 (dd, 2H, *J* = 7.28 Hz, *J* = 7.31 Hz), 7.36–7.34 (d, 1H, *J* = 6.21 Hz), 7.33–7.0 (d, 1H, *J* = 7.31 Hz), 7.29–7.27 (d, *J* = 6.25 Hz, 1H), 7.23–7.20 (dd, 2H, *J* = *6.12* Hz), 6.50 (s, 1H), 6.16 (s, 1H), 4.66 (d, 1H, *J* = *6.21* Hz), 4.48 (d, 2H), 3.98 (d, 1H, *J* = *6.11* Hz), 3.76 (d, *J* = 7.42 Hz, 1H, CH), 3.52 (d, 1H, *J* = 7.43 Hz), 2.28 (s, NH), 1.86 (s, 3H); ^{13}C NMR (75 MHz): 207.6, 176.1 (1C), 170.3, 142.2, 137.9, 129.1, 187.2, 131.2, 134.4, 137.2, 128.8, 128.6 (6C, Ph ring), 127.2, 67.3, 52.6, 45.6; EIMS(*m*/*z*): 272.30 (M^+, 29%).

2.1.6. N-(1-(2,4-dioxopyrrolidin-3-yl)-3-phenylallyl)benzamide (1f)

Yield 88%; mp: 147–142 °C; IR(KBr) *ν*: 3221.31, 3068.01, 3011.32, 1718.21, 1601.21 cm^{-1}; ^{1}H NMR (300 MHz): *δ* 8.38 (s, 1H), 8.45 (s, NH), 8.09–8.05 (d, 2H, *J* = 6.21 Hz),7.89–7.81 (d, 2CH, *J* = *7.12* Hz, Phenyl ring), 7.70 (d, 1H, *J* = 6.26 Hz), 7.65–7.61 (dd, 2H, *J* = 6.21 Hz), 7.53–7.51 (dd, 2CH, *J* = *7.11* Hz), 7.50 (d, 1CH, *J* = *7.12* Hz), 7.42–7.40 (dd, 2H, *J* = 7.21 Hz), 7.39–7.37 (dd, 2H, *J* = 7.36 Hz, *J* = 7.31 Hz), 7.36 (1H, -CH, *J* = *7.20* Hz), 7.33–7.30 (d, *J* = 7.31 Hz, 1H), 7.26–7.22 (d, *J* = 6.23 Hz, 1H), 7.23–7.20 (dd, 2H, *J* = *7.21* Hz), 6.51 (s, 1H), 6.17 (s, 1H), 4.76 (d, 1H, *J* = *6.31* Hz), 4.56 (s, 2H), 3.93 (d, 1H, *J* = *6.30* Hz, -CH); ^{13}C NMR (75 MHz): 206.1, 176.9 (1C), 134.6, 128.7, 136.5, 128.3, 128.1, 127.0, 167.2, 134.2, 127.4, 128.7, 132.0, 67.9, 52.9 (1C), 46.1; EIMS(*m*/*z*): 334.10 (M^+, 31%).

2.1.7. 1-((1-(2,4-dioxopyrrolidin-3-yl)-3-phenylallyl)-3-((3-phenylallylidene)urea (1 g)

Yield 85%; mp: 147–142 °C; IR(KBr) *ν*: 3254.08, 3065.15, 3021.30, 1742.13, 1641.20 cm^{-1}; ^{1}H NMR (300 MHz): *δ* 8.17 (s, NH), 7.50 (d, 1CH, *J* = *7.11* Hz), 7.48 (d, 1CH, *J* = *7.12* Hz), 7.43–7.40 (dd, 2H, *J* = 7.31 Hz, *J* = 7.30 Hz), 7.33 (s, 1H), 7.33–7.30 (d, 1CH, *J* = *7.32* Hz, Phenyl ring), 7.27–7.22 (d, 2H, *J* = 7.33 Hz), 7.21 (s, 1H), 6.55 (s, 1H), 6.12 (s, 1H), 7.26–7.22 (d, 1H, *J* = 6.21 Hz), 6.50 (s, 1H), 6.11 (s, 1H, CH), 4.47 (s, CH), 3.76 (d, 1H, *J* = 7.42 Hz), 3.52 (d, 1H, *J* = 7.43 Hz); ^{13}C NMR (75 MHz): 207.6, 176.9 (1C), 127.9, 128.6, 128.4,135.6, 134.9,126.9, 138.0, 128.8, 134.9, 136.2, 128.7, 129.6, 128.1, 65.9, 53.2, 51.3; EIMS(*m*/*z*): 387.47 (M^+, 18%).

2.1.8. 3-(1-(methylamino)-3-phenylallyl)pyrrolidine-2,4-dione (1h)

Yield 91%; mp: 147–142 °C; IR(KBr) *ν*: 3245.18, 3011.11, 3014.03, 1720.09, 1602.19 cm^{-1}; ^{1}H NMR (300 MHz): 8.31 (s, NH), 7.37–7.34 (dd, 2H, *J* = 7.21 Hz, *J* = 7.12 Hz), 7.35 (d, 1CH, *J* = *7.31* Hz), 7.26–7.21 (d, 2H, *J* = 7.29 Hz), 6.52 (s, 1H), 6.14 (s, 1H, CH), 4.51 (s, -CH_2), 3.78 (d, 1H, *J* = 7.42 Hz), 3.54 (d, 1H, *J* = 7.43 Hz), 3.29 (s, -CH_3), 2.28 (s, NH); ^{13}C NMR (75 MHz): 206.9, 177.3, 129.4, 135.2, 136.7, 127.9, 129.3, 127.2, 67.8, 54.9, 52.3, 33.9; EIMS(*m*/*z*): 244.39 (M^+, 24%).

2.2. Biological activities

2.2.1. Larvicidal activity

As part of the test, compounds (**1a-1h**) were tested at 10, 25, 50 and 100 μg/mL based on previously described methods (Sathish Kumar et al., 2020). By calculating the ratio (%) of dead larvae to live larvae, we can determine the level of mortality caused by the compounds. By using probit analysis, LD_{50} was calculated.

2.3. Molecular docking

2.3.1. Ligand preparation

The ligand (**1a-1h**) were draw the using of Chemdraw 12.0 and Chem3Dpro software. A Protein Data Bank (PDB) file of the ligand molecules was then created for use in docking studies.

2.3.2. Receptor preparation

Downloaded from the protein data bank, A mosquito odorant binding protein is represented by this structure (PDB ID: 3OGN). Discovery Studio 2019 is used to remove ligands and water molecules from the receptor. The SWISS PDB Viewer was used to minimize the receptor's energy. Molecular docking was then performed on the receptor.

2.3.3. Identification of binding pocket

The binding pocket of the target protein was identified using a co-crystallized ligand and Discovery Studio 2019 program. In the binding pocket, the residues His121 and Phe123 were present.

2.3.4. Docking

Molecular docking studies were conducted using Schrodinger Maestro 9.2 version to examine the interactions between compounds **1a-1h,** and **permethrin**, the mosquito odorant binding protein. In selecting the docking grid boxes, we considered the amino acid residues that occupied the binding pocket. It consisted of the following dimensions: x: 22, y: 20, and z: 22. A 1.0 Å spacing between x: 18.681, y: 49.66, and z: 11.409 formed the center of the chart. In the Discovery studio and Pymol programs, the interactions were analyzed visually using a value of 8 for exhaustiveness.

2.3.5. Statistical analysis

A minimum of three independent sites were used in the calculations, and The LD_{50} values were calculated by using Microsoft Excel to calculate the standard deviation (SD).

3. Results

Grindstone method was used to synthesize the compounds (**1a-h**) through pyrrolidine-2,4-dione ground together with cinnamaldehyde, and substituted amine. Following this, the samples were purified using column chromatography. Scheme 1 shows the outline of the synthetic route. Yields were between 88 and 92 % for the compounds. An analysis of the obtained compounds was carried out by FT-IR, ^{1}H, Rand ^{13}C NMR spectroscopy. The IR spectrum of compounds shows bands at 1601–1618, 1720–1742, 3202–3254, and 3014–3078 cm^{-1} conforming to the -C=O, -C=O, –NH, and –CH-NH– groups, respectively.

The protons presence in compounds (**1a-h**) were confirmed from ^{1}H NMR spectrum, which obtained peaks at δ 8.21–8.45, 3.75–3.79, 4.41–4.56, 6.51–6.58, and 6.13–6.18 ppm, matching with protons such as –NH, –4-CH, $–CH_{2,}$ –CH=CH–, and –CH=CH–, groups. The carbons presence in compounds (**1a-h**) were confirmed from^{13}C NMR spectrum, which obtained values at δ 53.2–54.9, 52.3–52.6, 135.2–136.6, 176.9–177.3, 206.9–207.6, and 127.9–128.4 ppm, matching with –CH, $–CH_2$, –CH=CH–, -C=O, -C=O and –CH=CH– groups. The mass spectrum and elemental analyses of all these compounds were used to determine their conformation. The second instar larvae of *Culex quinquefasciatus* were tested against eight compounds (**1a-1h**). Based on the relationship between structure and activity, the final compounds contained pyrrolidine-2,4-dione with substituted amines and thus exerted larvicidal and toxic effects.

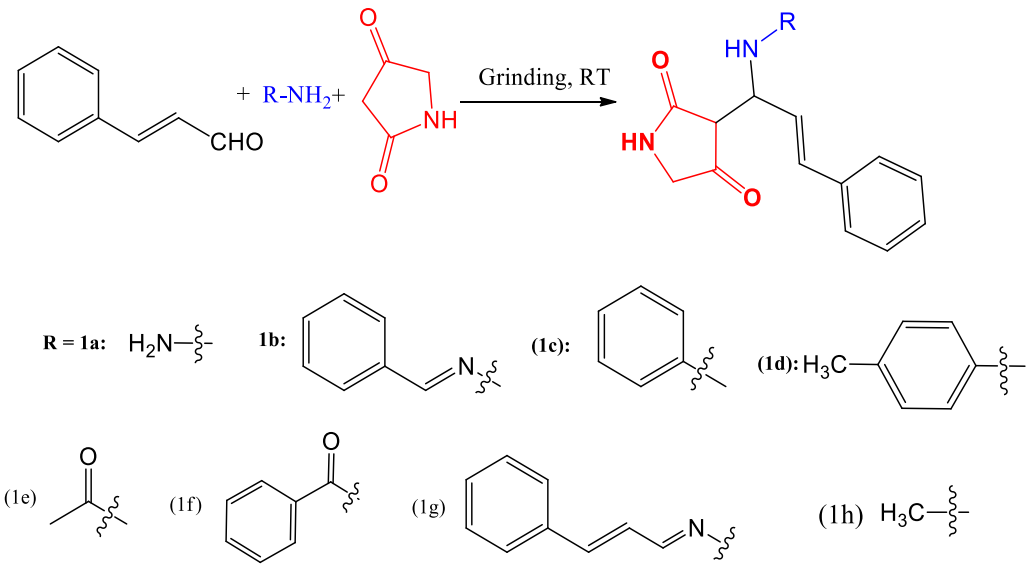
4. Discussion

The grindstone method is often more efficient than a traditional organic reaction and can even be more selective in some cases (Abdel Hameed, 2015). It has many other advantages, including low costs, high reproducibility, mild conditions, a short reaction time, less pollution, and simplicity in process and handling (Li et al., 2011). As a result, grindstone chemistry has gained increasing attention in greener organic transformations in the past decade.

We synthesize target compounds by grinding stone method using the reaction mixture of cinnamaldehyde (0.01 mol), substituted amine (0.01 mol), and pyrrolidine-2,4-dione (0.01 mol) at room temperature. The reaction mixture was ground for 30 min·H_2-O was added and EtOAc was used to extract the solution. The solvents were evaporated and the solution was purified using column chromatography on silica gel, filtered through Celite, and concentrated under vacuum. Solid material was extracted with ethyl acetate4:hexane6 column chromatography.

Compound **1e** (LD_{50}: 26.06 μg/mL) and **1f** (LD_{50}:26.89 μg/mL) showed a higher larvicidal activity than other compounds and then permethrin (LD_{50} of 26.14 μg/mL). At 100 μg/mL for compounds **1e** and **1j**, 100% of the mortality rates were achieved, and **1c** also reached 100% mortality at 100 μg/mL. However, The compound **1e** and **1f** shows highly active compared to **1c** (27.91 μg/mL) due to the amide group. This suggests that the presence of amide and pyrrolidin-2,4-dione moiety may be the reason for the observed biological effects of compounds **1e** and **1f** , respectively. In compound **1a**, mortality was 86% at 100 g/mL, since hydarazine hydazide, phenylallyl and pyrrolidine-2,4-dione were presented in compound **1a**. Low activity for compound **1d, 1g,** and **1h** are obtained with a mortality rate of 70–78% with LD_{50} values of 53. 06–54.22 μg/mL range. This suggests that the presence of the p-tolylamino, pyrrolidine-2,4-dione of compound **1d**, 3-phenylallyl, 3-phenylallylidene) urea, and methylamino, 3-phenylallyl, pyrrolidine-2,4-dione groups could be the reason for the respective observed biological effects. The presence of benzylidene hydrazine, phenylallyl, and pyrrolidine-2,4-dione groups in compound **1b** led to a 61% mortality rate at 100 μg/mL. Therefore, the above analysis indicates that compounds **le** and **1f** were significantly active in larvicidal screening. LD_{50} values and mortality percentages are shown in Table 1.

Schrodinger Maestro 9.2 is the software used to determine the docking behaviour of compounds **1e, 1f,** and **permethrin** with 3OGN protein. 2D representation molecule docked with receptor of compound **1a-1h** shown in Fig. 2(**a-h**). The compounds **1e** and **1f** have much lower binding affinity (−12.6 kcal/mol) than permethrin with (−8.5 kcal/mol) (Sathish Kumar et al., 2020) in 3OGN protein respectively. Permethrin was used as a standard, since it is a commercially available mosquito insecticide. The hydrogen



Scheme 1. Synthetic route of compound pyrrolidine-2,4-dione derivatives.

Table 1
Archived larvicidal activities (1**a–1h**).

Compounds	% of Mortality			LD_{50} (μg/mL)[a]
	25 μg/mL	50 μg/mL	100 μg/mL	
1a	35.1 ± 0.7	70.2 ± 0.2	86.2 ± 0.2	33.91
1b	21.2 ± 0.1	47.1 ± 0.1	61.0 ± 0.9	64.20
1c	41.3 ± 0.6	88.3 ± 0.2	100 ± 0.1	27.91
1d	21.6 ± 0.1	42.5 ± 0.1	78.1 ± 0.7	53.46
1e	48.2 ± 0.9	86.2 ± 0.1	100 ± 0.0	26.06
1f	47.1 ± 0.2	85.5 ± 0.2	100 ± 0.0	26.89
1g	22.1 ± 0.3	46.8 ± 0.3	74.8 ± 0.1	53.06
1h	28.9 ± 0.1	43.0 ± 0.4	70.9 ± 0.2	54.22
Permethrin	51.1 ± 0.1	76.3 ± 0.5	100 ± 0.0	26.14

[a] Values are mean ± SD (n = 3).

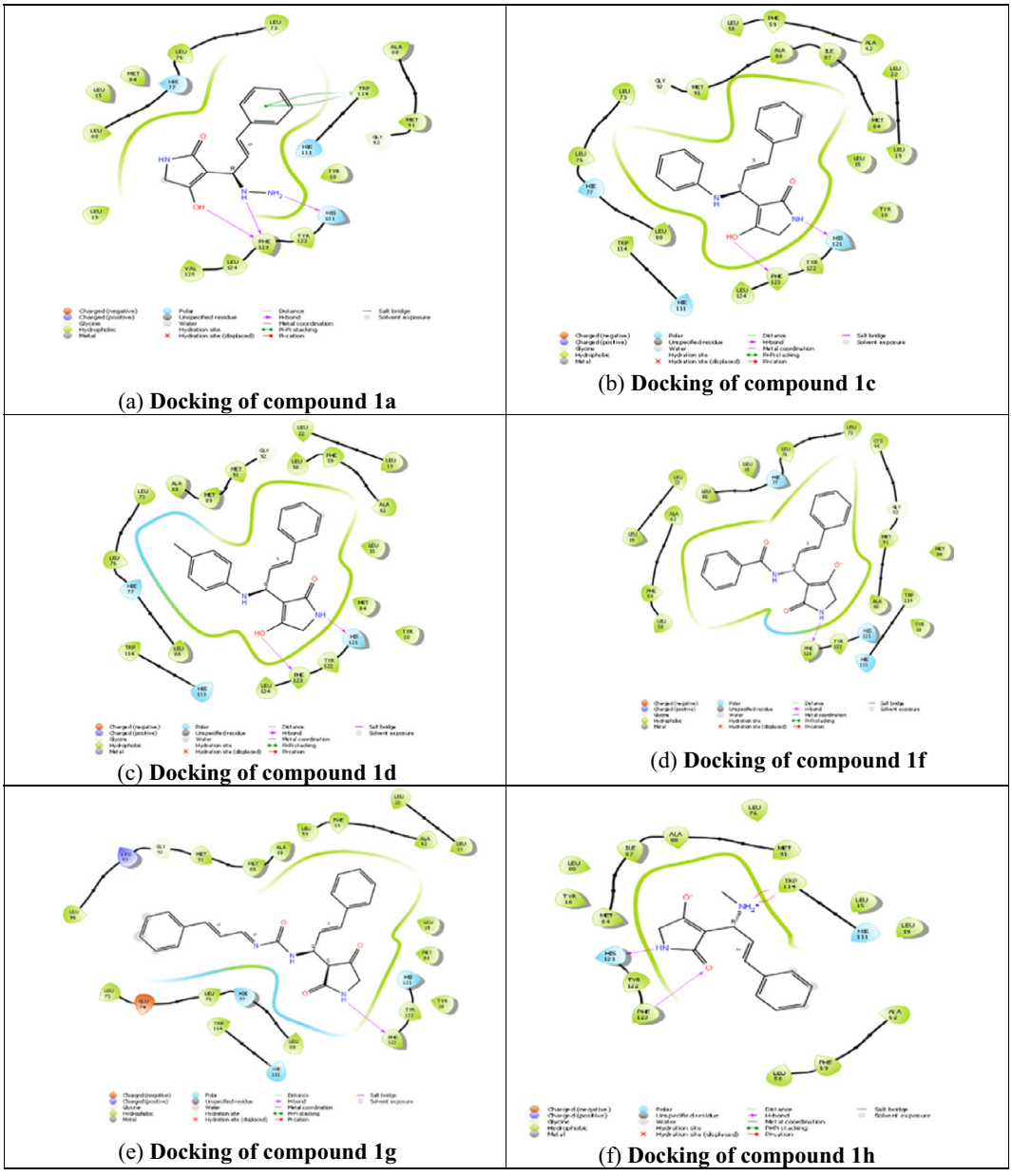


Fig. 2. 2D representation molecule docked with receptor of compound **1a-1h**.

bond plays a significant role in the stability of protein-ligand binding, and the favourable distance between the H-donor and the H-acceptor atoms is less than 3.5 Å. Hydrogen bond distances of less than 3.5 Å were found in compounds (**1a-h**) with strong hydrogen bonds with respective 3OGN proteins. Hydrogen bond interactions with receptor 3OGN were formed by compounds **1e** and **1f**. Phe123

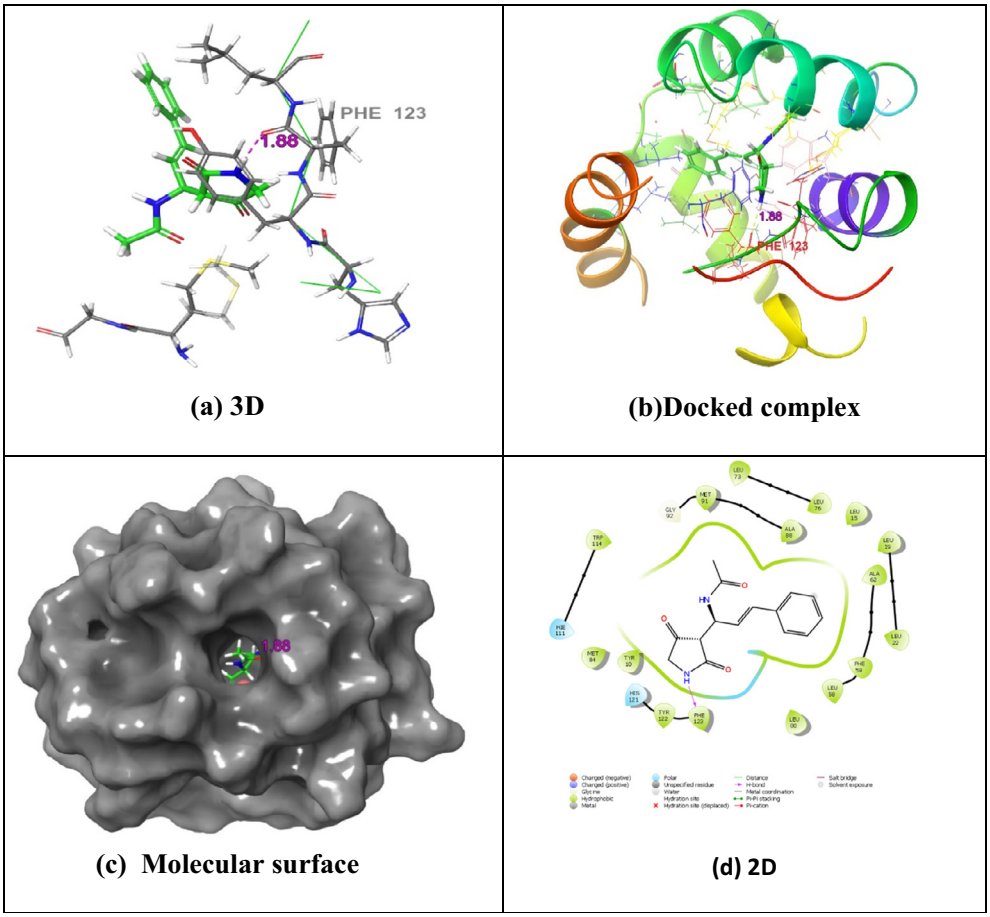


Fig. 3. Highly active compound 1e shows docking with mosquito odorant binding protein (3OGN).

is an amino acid residue that interacts with hydrogen and has a bond length of 1.94 Å. The amino acid residues Tyr10, Leu15, Leu22, Leu56, Phe59, Ala62, Leu73, Leu76, Hie77, Leu80, Met84, Ala88, Met91, Gly92, Cys95, Hie111, Trp114, His121, Tyr122 and Phe123 were involved in hydrophobic interactions. The interaction of the compound **1e** with the mosquito odorant binding protein (PDB ID: 3OGN) protein is shown in Fig. 3. The 3D representation of inhibitor molecule docked into the receptor was shown in Fig. 3a. The helix representation of inhibitor molecule docked into the receptor was shown in Fig. 3b. The inhibitor molecule docked into the binding pocket of the receptor was shown in Fig. 3c. The 2D representation molecule docked with receptor was shown in Fig. 3d. The permethrin receptor 3OGN does not form a hydrogen bond with the permethrin. The results of molecular docking were summarized in Table 2.

Table 2
Docking interactions between compounds (1a-1h).

Compounds	Dock Score	Interacting residues	Bond length
1a	−8.8	His 121, Phe 123(2)	2.38, 2.05, 2.18
1b	−11.4	Phe 123	2.30
1c	−10.9	His 121, Phe 123	2.32, 2.32
1d	−11.4	His 121, Phe 123	2.33, 2.14
1e	−12.6	Phe 123	1.94
1f	−12.6	Phe 123	1.94
1g	−11.8	Phe 123	2.29
1h	−10.7	His 121, Phe 123	1.96, 2.19
Permethrin	−8.5	–	–

5. Conclusions

A novel larvicidal active pyrrolidine-2,4-dione derivatives were synthesized by using a Mannich base and grindstone method in this study. It was investigated whether these compounds could be used as larvicides against *Culex quinquefasciatus*. We tested eight compounds and found compound **1e** to be the most active (LD_{50} = 26.06 g/mL) in contrast to *Culex quinquefasciatus*. The compounds (**1a-1h),** and permethrin were docked against the 3OGN protein, and compound **Ie** and **1f** achieved the best docking scores. In conclusion, our study suggests that compound is the most effective insecticide, and the chemicals described here could serve as a basis for developing eco-friendly insecticides and biopharmaceuticals.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors extend their appreciation to the Deanship of Scientific Research at Imam Mohammad Ibn Saud Islamic University for funding this work through Research Group no. RG-21-09-87.

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