Synthesis, characterization and evaluation of mosquito larvicidal effect of 1,3,4-oxadiazole derivatives against *Culex quinquefasciatus*

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ABSTRACT

A new series of 1,3,4-oxadiazole derivatives were prepared from the condensation of acid hydrazide, 5-bromo-2-(trifluoromethoxy)benzoic acid in POCl\(_3\) by ultrasonic irradiation method. The new synthetic method furnished the desired products in shorter time and good yields. The chemical structures of compounds 4a-h were confirmed by Fourier transform infrared spectroscopy (IR), proton nuclear magnetic resonance (^1\(H\)-NMR), carbon nuclear magnetic resonance (^13\(C\)-NMR) and elemental analyses. Larvicidal bioassay tests were performed using several 2-(5-bromo-2-(trifluoromethoxy) phenyl)-5-aryl-1,3,4-oxadiazole. These synthesized compounds presented strong larvicidal activity against urban mosquitoes *Culex quinquefasciatus*. The results suggest that larvicidal activity might be correlated with the presence of electron-withdrawing substituents in the para position of the phenyl ring. The alterations observed in the larvae spiracular valves of the siphon and anal papillae by 1,3,4-oxadiazoles in the larvicidal bioassay are responsible for larvae’s death.

**Keywords**: 1,3,4-oxadiazole, ultrasonic irradiation, Larvicidal bioassay, *Culex Quinquefasciatus*

1. INTRODUCTION

Mosquitoes are important vectors of diseases, especially in the tropics. Mosquitoes are vectors for a large number of diseases, including filariasis (transmitted by *Culex*...
quinquefasciatus Say, 1823, Figure 1), malaria (transmitted by Anopheles gambiae) dengue fever and yellow fever (transmitted by Aedes aegypti). Regulation of mosquito populations to reduce the incidence of disease like malaria, filariasis and several arboviruses are important from public health viewpoint. Filariasis is endemic in 17 States and six Union Territories, with about 553 million people at risk of infection [1]. The key to control these diseases is based on the management of the larval population using the larvicidal agents [2]. The literature has described the resistance of mosquitoes to temephos chemical compound. Thus, the search for new larval insecticides has been the focus of a large number of publications addressing the use of essential oils and plant extracts [3-6].

Many 1,3,4-oxadiazole derivatives have been reported to possess broad spectrum insecticidal activity and are used as active ingredients for controlling agricultural and horticultural pests. 1,3,4-oxadiazole are the imperative heterocyclic nucleus which is effective against mosquito diseases [7]. Many molecules containing a 1,3,4-oxadiazole moiety exhibit interesting pharmacological properties, such as analgesic [8] anti-inflammatory [9] anti-convulsant [10] antitumor [11] anti-kinetoplastid [12] HPTPb inhibitors [13] human bII-tryptase inhibitors [14] and selective avb3 receptor antagonists [15]. The aim of the present study was to improve the synthesis of 2-(5-bromo-2-(trifluoromethoxy)phenyl)-5-aryl-1,3,4-oxadiazole by employing focused ultrasonic irradiation with a quick, eco-friendly and low-cost method of purification and to determine their larvicidal properties [16,17].

![Figure 1. Culex quinquefasciatus Say, 1823. Left: male; right: female](image)

2. EXPERIMENTAL

Materials and Methods

All solvents and chemicals were purchased from commercial sources (Sigma–Aldrich and Fisher Scientific) and were used without additional purification. The melting point of oxadiazoles was calculated in open capillaries and is uncorrected. FT-IR spectrum was
obtained by using an SHIMADZU Fourier transformed infrared (FT-IR) spectrometer using KBr (pellet form). The NMR spectra were measured on a Bruker instrument in DMSO-\(d_6\) solution. The chemical shifts were measured relative to TMS.

**Synthesis of ester**

The compounds 2a-h was prepared according to the procedure given in literature with a little modification [18]. Carboxylic acid (0.1mol), ethanol (60 ml) and conc. \(\text{H}_2\text{SO}_4\) (1.4 ml) were placed in a 250 ml round-bottom and were irradiated for 1 hr on a ultrasonic cleaning bath. The reaction mixture was concentrated on a rotatory evaporator. It was filtered and collected.

**Synthesis of acid hydrazide 3a-h**

The compounds 3a-h was prepared according to the procedure given in literature with a little modification [19]. Ester and hydrazine hydrate in 1:1 portion and ethanol (30 ml) was placed in a round-bottom flask. The mixture was irradiated for 30 min. The reaction mixture was concentrated on a rotatory evaporator. It was filtered and collected.

**Synthesis of 2-(5-bromo-2-(trifluoromethoxy)phenyl)-5-aryl-1,3,4-oxadiazole 4a-h**

Scheme 1. Synthesis of 1,3,4-oxadiazoles 4a-h
A mixture of acid hydrazide (0.01 mol) and 5-bromo-2-(trifluoromethoxy)benzoic acid (0.01 mol) in POCl₃ (5 ml) was irradiated on ultrasonic cleaning bath for 2 hrs. The reaction mixture was cooled and poured into crushed ice. It was neutralized with sodium bicarbonate solution and the resulting solid was filtered, dried and washed with water and recrystallized from ethanol to give 2,5-disubstituted-1,3,4-Oxadiazole 4(a-h). The Synthetic procedure is shown in Scheme 1.

2-(5-bromo-2-(trifluoromethoxy)phenyl)-5-phenyl-1,3,4-oxadiazole (4a)

Pale Yellow solid; Yield 69%, M.P: 193–195 °C, MF: C₁₅H₈BrF₃N₂O₂; IR (KBr): 3078 cm⁻¹ (C-H Ar str); 1598 cm⁻¹ (C=N str); 503 cm⁻¹ (C-Br str); 1165 cm⁻¹ (C-F str); 1080 cm⁻¹ (N-N str). ¹H-NMR (400 MHz, DMSO-d₆): 7.09-7.93 δ (8H, Aromatic protons); ¹³C-NMR (400 MHz, DMSO-d₆): 114.02-133.35 δ (Aromatic carbon); 163.11 δ (C of 1,3,4-Oxadiazole ring); 151.66 δ (C-O).

2-(5-bromo-2-(trifluoromethoxy)phenyl)-5-(4-chlorophenyl)-1,3,4-oxadiazole (4b)

Pale Yellow solid; Yield 72%, M.P: 132–135 °C, MF: C₁₅H₇BrClF₂N₂O₂; IR (KBr): 3076 cm⁻¹ (C-H Ar str); 1602 cm⁻¹ (C=N str); 516 cm⁻¹ (C-Br str); 1165 cm⁻¹ (C-F str); 1062 cm⁻¹ (N-N str); 736 cm⁻¹ (C-Cl str). ¹H-NMR (400 MHz, DMSO-d₆): 6.86-7.44 δ (7H, Aromatic protons); ¹³C-NMR (400 MHz, DMSO-d₆): 117.16-138.77 δ (Aromatic carbon); 161.72 δ (C of 1,3,4-Oxadiazole ring); 152.18 δ (C-O).

2-(5-bromo-2-(trifluoromethoxy)phenyl)-5-(4-nitrophenyl)-1,3,4-oxadiazole (4c)

Dark brown solid; Yield 65%, M.P: 152–155 °C, MF: C₁₅H₇BrF₂N₂O₃; IR (KBr): 3066 cm⁻¹ (C-H Ar str); 1602 cm⁻¹ (C=N str); 505 cm⁻¹ (C-Br str); 1166 cm⁻¹ (C-F str); 1060 cm⁻¹ (N-N str). ¹H-NMR (400 MHz, DMSO-d₆): 7.10-7.94 δ (7H, Aromatic protons); ¹³C-NMR (400 MHz, DMSO-d₆): 114.11-134.58 δ (Aromatic carbon); 166.76 δ (C of 1,3,4-Oxadiazole ring); 152.11 δ (C-O).

4-(5-(5-bromo-2-(trifluoromethoxy)phenyl)-1,3,4-oxadiazol-2-yl)pyridine (4d)

Pale Yellow solid; Yield 66%, M.P: 146–147 °C, MF: C₁₄H₂BrF₂N₂O; IR (KBr): 3064 cm⁻¹ (C-H Ar str); 1612 cm⁻¹ (C=N str); 513 cm⁻¹ (C-Br str); 1161 cm⁻¹ (C-F str); 1060 cm⁻¹ (N-N str). ¹H-NMR (400 MHz, DMSO-d₆): 7.06-7.90 δ (7H, Aromatic protons); ¹³C-NMR (400 MHz, DMSO-d₆): 119.03-134.86 δ (Aromatic carbon); 156.95 δ (C of 1,3,4-Oxadiazole ring); 156.08 δ (C-O).

2-(5-bromo-2-(trifluoromethoxy)phenyl)-5-(4-methoxyphenyl)-1,3,4-oxadiazole (4e)

Pale Yellow solid; Yield 75%, M.P: 124–126 °C, MF: C₁₆H₁₀BrF₃N₂O₃; IR (KBr): 3080 cm⁻¹ (C-H Ar str); 2941 cm⁻¹ (C-H Aliphatic str); 1579 cm⁻¹ (C=N str); 536 cm⁻¹ (C-Br str); 1166 cm⁻¹ (C-F str); 1064 cm⁻¹ (N-N str). ¹H-NMR (400 MHz, DMSO-d₆): 7.04-7.91 δ (7H, Aromatic protons); 3.82 δ (3H, OCH₃ group). ¹³C-NMR (400 MHz, DMSO-d₆): 113.65-139.95 δ (Aromatic carbon); 166.27 δ (C of 1,3,4-Oxadiazole ring); 55.32 δ (OCH₃ group); 151.65 δ (C-O).
2-(5-bromo-2-(trifluoromethoxy)phenyl)-5-(4-bromophenyl)-1,3,4-oxadiazole (4f)

Pale Yellow solid; Yield 63%, M.P: 162-165 °C, MF: C15H1BrF3N2O2; IR (KBr): 3070 cm⁻¹ (C-H Ar str); 1606 cm⁻¹ (C≡N str); 528 cm⁻¹ (C-Br str); 1062 cm⁻¹ (C-F str); 1178 cm⁻¹ (N-N str). ¹H-NMR (400 MHz, DMSO-d₆): 7.04-7.91 δ (7H, Aromatic protons); ¹³C-NMR (400 MHz, DMSO-d₆): 114.56-135.88 δ (Aromatic carbon); 166.76 δ (C of 1,3,4-Oxadiazole ring); 152.15 δ (C-O).

4-(5-(5-bromo-2-(trifluoromethoxy)phenyl)-1,3,4-oxadiazol-2-yl)phenol (4g)

Pale Yellow solid; Yield 71%, M.P: 122-124 °C, MF: C16H9BrF3N2O2; IR (KBr): 3083 cm⁻¹ (C-H Ar str); 1598 cm⁻¹ (C≡N str); 503 cm⁻¹ (C-Br str); 1168 cm⁻¹ (C-F str); 1062 cm⁻¹ (N-N str). ¹H-NMR (400 MHz, DMSO-d₆): 7.03-7.84 δ (7H, Aromatic protons); 10.12 δ (1H, OH group) ¹³C-NMR (400 MHz, DMSO-d₆): 111.94-136.36 δ (Aromatic carbon); 165.31 δ (C of 1,3,4-Oxadiazole ring); 158.36 δ (C-O).

2-(5-bromo-2-(trifluoromethoxy)phenyl)-5-p-tolyl-1,3,4-oxadiazole (4h)

Pale Yellow solid; Yield 67%, M.P: 116-119 °C, MF: C16H10BrF3N2O2; IR (KBr): 3072 cm⁻¹ (C-H Ar str); 2945 cm⁻¹ (C-H Aliphatic str); 1608 cm⁻¹ (C≡N str); 495 cm⁻¹ (C-Br str); 1168 cm⁻¹ (C-F str); 1060 cm⁻¹ (N-N str). ¹H-NMR (400 MHz, DMSO-d₆): 7.16-7.98 δ (7H, Aromatic protons); 2.49 δ (3H, CH₃ group). ¹³C-NMR (400 MHz, DMSO-d₆): 117.14-135.90 δ (Aromatic carbon); 164.77 δ (C of 1,3,4-Oxadiazole ring); 26.10 δ (CH₃ group); 152.17 δ (C-O).

Evaluation of larvicidal activity

The assessment of larvicidal activity of synthesized test compounds 4a-h was tested against the urban mosquitoes Culex quinquefasciatus using standard bioassay protocol [19]. Egg rafts of mosquito were obtained from a drainage system. The eggs were reared under standard insectary conditions at ambient temperature (29 ± 3 °C) and relative humidity (80 ± 5 %), 12:12 light: dark photoperiod and fed with ground shrimp feed daily. Larval development was monitored for 7 days. The second and third stage larvae were collected at the tip of a pasture pipette and placed in cotton bud to remove excess water and transferred gently to the test vial (10/vial) by tapping. The larval mortality was observed using various concentrations of synthesized compounds 4a-h (10, 20, 30, and 40 µg/mL).

3. RESULTS AND DISCUSSION

All compounds reported in the present work were tested against second instar Culex quinquefasciatus larvae and exhibited larvicidal activity shown in table 1. The introduction of a substituent group in the phenyl ring positions of these 1,3,4-oxadiazoles led to a increase in LC50 values in Culex quinquefasciatus larvicidal bioassays. The synthesized compounds containing –CH₃ (para, 4h) achieved a lower LC50 (15.78) value when compared to compounds containing the pyridine ring (4d) (LC50 16.29). The synthesized oxadiazole derivatives were evaluated in terms of their larvicidal activities. Consistent with their expected structure - activity relationship, compounds 4a-h were biologically active.
The presence of oxadiazole nucleus and the para substitution of the phenyl ring contribute to the observed activities.

**Table 1.** Larvicidal profile of compounds 4a-h on against second instar larvae of Culex sp

| Compounds | Mortality (%) at room temp concentration (μg/mL) |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| | 10 | 20 | 30 | 40 | LD50 (μg/mL) |
| 4a | 27 | 53 | 82 | 100 | 18.75 |
| 4b | 21 | 72 | 86 | 100 | 17.13 |
| 4c | 35 | 57 | 100 | - | 15.33 |
| 4d | 19 | 49 | 73 | 100 | 16.29 |
| 4e | 39 | 88 | 100 | - | 8.71 |
| 4f | 29 | 61 | 83 | 100 | 17.23 |
| 4g | 51 | 74 | 100 | - | 6.93 |
| 4h | 10 | 31 | 60 | 100 | 15.78 |

The larvicidal activity of the test compounds is listed in Table 4. Larvicidal activity was determined for compounds 4a-h by exposing second instar larvae for 24 h at room temperature. All the synthesized compounds exhibited moderate larvicidal activity against mosquito. Compounds 4c, 4e and 4g which contains a 4-NO₂, -OCH₃ and –OH in phenyl group with oxadiazole moieties, was a potent larvicide shows 100 % mortality at 30 μg/mL.

4. CONCLUSION

All the tested 1,3,4-oxadiazole derivatives possessed different range of larvicidal property which may be used as a traditional mosquito control agent. On the basis of the present investigation results we conclude that 4-nitro, 4-methoxy and 4-hydroxyl group containing derivatives shows maximum larvicidal bioactivity.

References
