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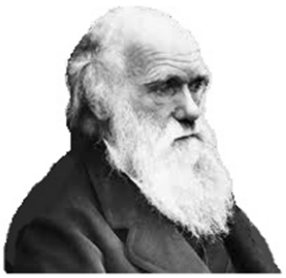

World News of Natural Sciences

An International Scientific Journal

WNOFNS 19 (2018) 58-64 EISSN 2543-5426

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*

(Received 10 June 2018; Accepted 20 June 2018; Date of Publication 21 June 2018)

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 importance from public health viewpoint. Filariasis is endemic in 17 States and six Union Territories, with about 553 million people at risk of infection [1]. Te 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].

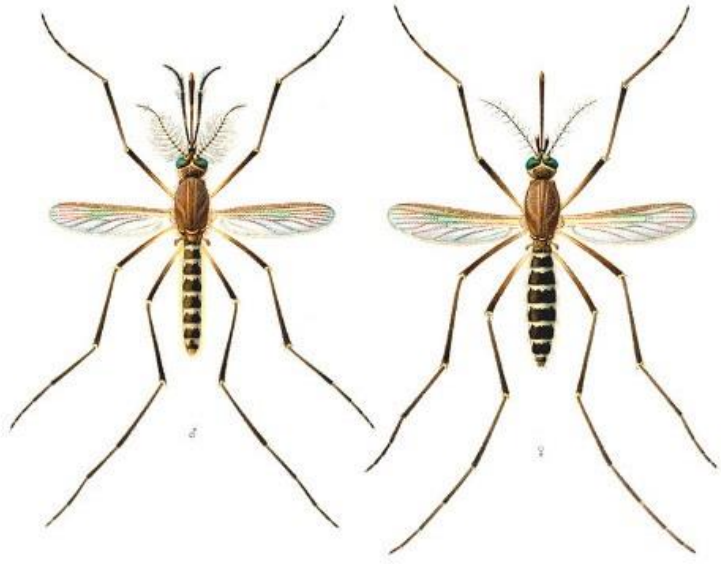


Figure 1. *Culex quinquefasciatus* Say, 1823.
Left: male; right: female

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.

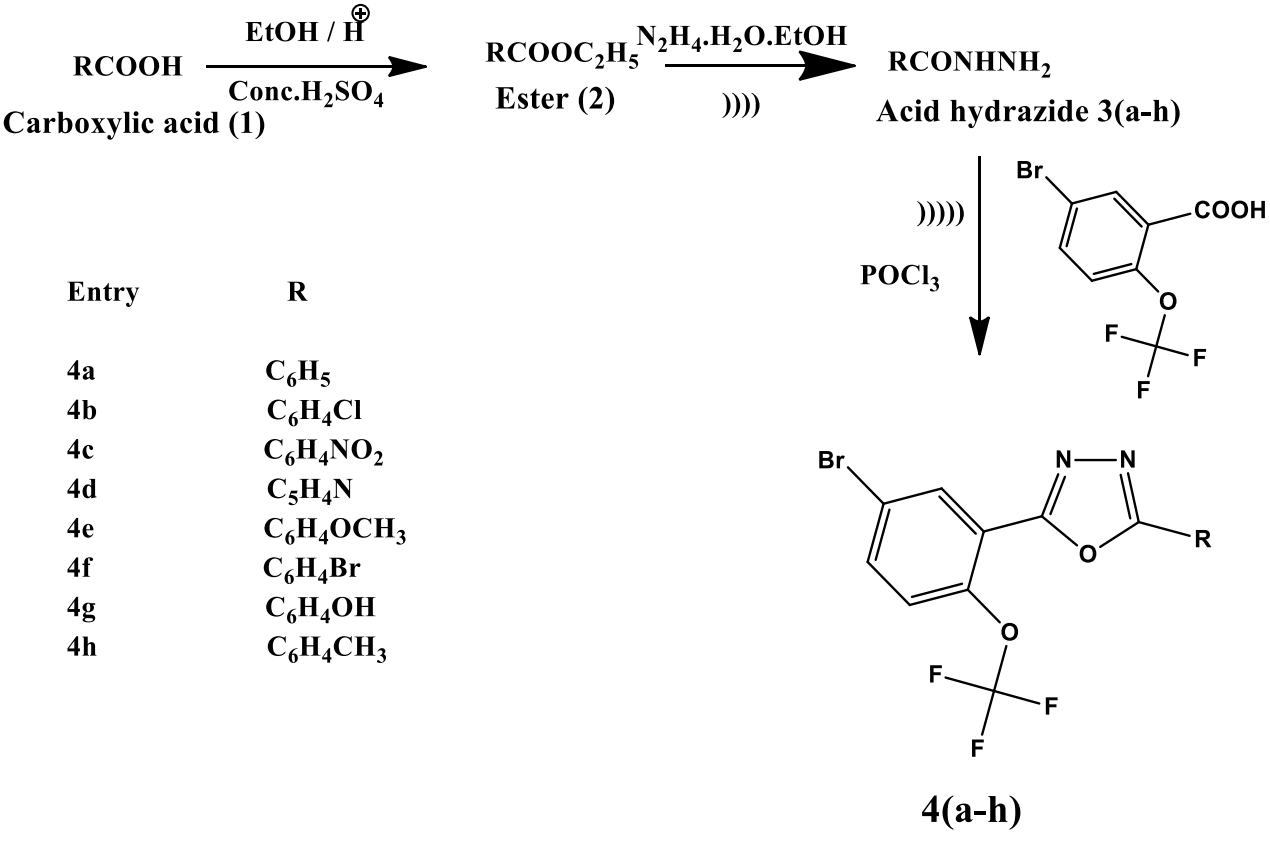
Synthesis of ester

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. H_2SO_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



Entry	R
4a	C_6H_5
4b	C_6H_4Cl
4c	$C_6H_4NO_2$
4d	C_5H_4N
4e	$C_6H_4OCH_3$
4f	C_6H_4Br
4g	C_6H_4OH
4h	$C_6H_4CH_3$

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 (0.01mol) in $POCl_3$ (5ml) 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: $C_{15}H_8BrF_3N_2O_2$; 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). ^{1}H-NMR (400 MHz, DMSO-d_6): 7.09-7.93 δ (8H, Aromatic protons); ^{13}C-NMR (400 MHz, DMSO-d_6): 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: $C_{15}H_7BrClF_3N_2O_2$; 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). ^{1}H-NMR (400 MHz, DMSO-d_6): 6.86-7.44 δ (7H, Aromatic protons); ^{13}C-NMR (400 MHz, DMSO-d_6): 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: $C_{15}H_7BrF_3N_3O_4$; 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). ^{1}H-NMR (400 MHz, DMSO-d_6): 7.10-7.94 δ (7H, Aromatic protons); ^{13}C-NMR (400 MHz, DMSO-d_6): 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: $C_{14}H_7BrF_3N_3O_2$; 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). ^{1}H-NMR (400 MHz, DMSO-d_6): 7.06-7.90 δ (7H, Aromatic protons); ^{13}C-NMR (400 MHz, DMSO-d_6): 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: $C_{16}H_{10}BrF_3N_2O_3$; 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). ^{1}H-NMR (400 MHz, DMSO-d_6): 7.04-7.91 δ (7H, Aromatic protons); 3.82 δ (3H, OCH_3 group). ^{13}C-NMR (400 MHz, DMSO-d_6): 113.65-139.95 δ (Aromatic carbon); 166.27 δ (C of 1,3,4-Oxadiazole ring); 55.32 δ (OCH_3 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: $C_{15}H_7Br_2F_3N_2O_2$; 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). ^{1}H-NMR (400 MHz, DMSO-d_6): 7.04-7.91 δ (7H, Aromatic protons); ^{13}C-NMR (400 MHz, DMSO-d_6): 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: $C_{15}H_8BrF_3N_2O_3$; 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). ^{1}H-NMR (400 MHz, DMSO-d_6): 7.03-7.84 δ (7H, Aromatic protons); 10.12 δ (1H, OH group) ^{13}C-NMR (400 MHz, DMSO-d_6): 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: $C_{16}H_{10}BrF_3N_2O_2$; 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). ^{1}H-NMR (400 MHz, DMSO-d_6): 7.16-7.98 δ (7H, Aromatic protons); 2.49 δ (3H, CH_3 group). ^{13}C-NMR (400 MHz, DMSO-d_6): 117.14-135.90 δ (Aromatic carbon); 164.77 δ (C of 1,3,4-Oxadiazole ring); 26.10 δ (CH_3 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 –CH_3 (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-NO_2, -OCH_3 and –OH in phenyl group with oxodiazole 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.

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