# Mosquito Larvicidal Activity of Triazole Type Brassinosteroid Biosynthesis Inhibitors

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**Abstract:** The larval stage of the mosquitoes, harmful human diseases vectors, are attractive targets for insecticides. Because ecdysteroids show striking similarities in chemical structure with plant growth hormone of brassinosteroids (BR), we proposed that BR biosynthesis inhibitors may interfere the ecdysteroid biosynthesis thereby killing the mosquitoes at the larvae stage. To verify this hypothesis, we screening the larvicidal activity of BR biosynthesis inhibitors developed in our laboratory. The larvicidal activity of BR biosynthesis inhibitors were studied by using a laboratory bioassays against 3rd to 4th in star larvae of Aedesaegypti. Among the 28 test compounds, we found that 1-[4-(4-chlorophenoxymethyl)-2-(4-chloro-phenyl)-[1,3]dioxolan-2-ylmethyl]-1H-[1,2,4]triazole(compound 22) displayed potent larvicidal activity against Aedesaegypti with an LD50 value approximately 17.8±2.0 μM. In conclusion, we found a triazole type new lead compound which displays promising larvicidal activity against Aedesaegypti. Further structure-activity relationships studies may lead to the discovery of new class of insecticide for mosquito control.

**Key words:** Brassinosteroid biosynthesis inhibitor, larvicidal activity, mosquito larvae, *Aedesaegypti.*

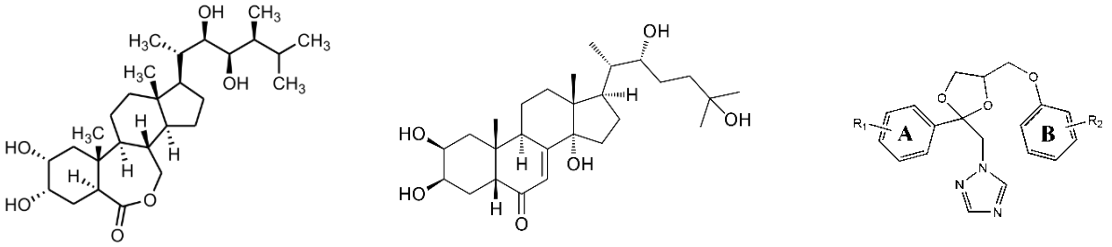
## 1. Introduction

Mosquitoes are harmful insects which transmit many dreadful diseases such as malaria, dengue fever, yellow fever. etc. [1]. It is estimated that mosquitoes are responsible for the death of millions of people worldwide [2]. Great efforts have been made to develop new technologies for mosquito management. An efficient way to control mosquitoes is the use of insecticides. In the past fifty years, mosquitoes have mainly been controlled by the application use of synthetic insecticides. Currently, many synthetic insecticides have been developed and employed in the field of mosquito control with great success. Larval stage of the mosquitoes are attractive targets for mosquito control because larvicide target mosquito larvae in the breeding habitat before they can mature into adult mosquitoes and disperse [3].

The life cycle of mosquitoes comprises four separate stages: Egg, Larva, Pupa, and Adult. Like other insects, mosquito larva shed (molt) their skins four times and growing larger after each molt. Thus, insect molting hormones (ecdysteroids) are key regulators that involve in the growth of mosquito larvae. Available evidences indicated that the biosynthesis and/or the signal transduction pathways of the ecdysteroid are potential targets for insecticides [4].

Ecdysteroids are a group of polyhydroxysteroids which show striking similarities in their chemical structure with plant growth hormone brassinosteroids (BR) (Fig. 1). Also, the enzymes involved in the biosynthesis of

both steroid hormones are quite similar. The structural diversity of BRs is generated by the action of several cytochromes P450. CYP90s and CYP85s are thought to catalyze the different steps of hydroxylation of campesterol [5]. Similarly, molecular functional analysis the enzymes of ecdysteroids biosynthesis indicated that cytochrome P450 enzymes play key roles in biosynthesis. CYP314A1 has been shown to involve in the biochemical conversion of ecdysone to 20-hydroxyecdysone [6]. CYP306A1 was shown to catalyze the C25 hydroxylation of ketodiol to ketotriol in ecdysteroid biosynthesis [7]. CYP315A1 is a 2-hydroxylase and CYP302A1 is a C-22 hydroxylase of ecdysteroid biosynthesis [8], [9]. Thus, the BR biosynthesis inhibitors targeting P450s are potential inhibitors for ecdysteroid biosynthesis thereby interfering the biosynthesis and function of the ecdysteroid.




Brassinolide ecdysone general structure of

BR biosynthesis inhibitor

Fig. 1. Chemical structure of brassinosteroid, ecdysteroid and BR biosynthesis inhibitors.

We have previously reported the synthesis of triazole type BR biosynthesis inhibitors. Based on the molecular scaffold of ketoconazole, a widely used P450 inhibitor, we discovered a series of new BR biosynthesis inhibitors [10]. Structure-activity relationship studies of triazole type BR biosynthesis inhibitor revealed a series of potent inhibitor of BR biosynthesis [11]-[13]. To determine the binding affinity of this synthetic series to CYP90D1, a BR biosynthesis enzyme, by using an analogue of YCZ-18 gave evidence that YCZ-18 binds to CYP90D1 with a Kd value of 0.79 μM [14]. Thus, it is likely that, inhibitors targeting P450s in BR biosynthesis may display inhibitory activity against ecdysteroid biosynthesis thereby interfering the growth of mosquito larvae. To verify this hypothesis, we report herein the biological evaluation the larvicidal activity of BR biosynthesis inhibitors against mosquito larvae.

## 2. Experimental Section

### 2.1. Chemicals

The BR biosynthesis inhibitors used in this study were synthesized and the chemical structures of the test compounds were characterized by a method that has been described previously [11]-[13]. Stock solutions of the test compounds were dissolved in DMSO at a concentration of 100 mM and stocked at −30 °C before use. Other reagents were of the highest grade and purchased from Wako, Pure Chemical Industries, Ltd. (Tokyo, Japan).

### 2.2. Maintenance of Mosquito Larvae

The eggs of Aedesaegypti were purchased from Sumitomo Service Inc. Ltd. (Hyogo, Japan) Eggs were maintained in the laboratory without any exposure to any known insecticide and transferred to 18×13×4 cm enamel trays containing 500 ml of water for hatching containing 50 mg of sterilized diet (40-mesh chick chow powder/yeast). Mosquitoes were reared at 28-30°C, 65-75% relative humidity. The feeding was

continued until the mosquito larvae transformed into the pupal stage.

### 2.3. Larval Toxicity Test

A laboratory colony of Aedesaegypti larvae was used for the determination of the larvicidal activity of triazole type BR biosynthesis inhibitors. 10 individuals of early-fourth instar larvae were kept in 3×3×2 cm polyethylene trays containing 10 ml of dechlorinated water and 10 μl of desired concentration of chemical stock solutions were added. Larval food was given for the test larvae. At each tested concentration, three trials were made and each trial consists of three replicates. The control was setup by mixing 10 μl of DMSO with 10 ml of dechlorinated water.

### 2.4. Statistical Analysis

All measurements were carried out at least in triplicate. Data analysis (t-test and analysis of variance) was applied to determine the significant difference with the use of significance throughout the manuscript being based upon $P<0.05$ unless stated otherwise.

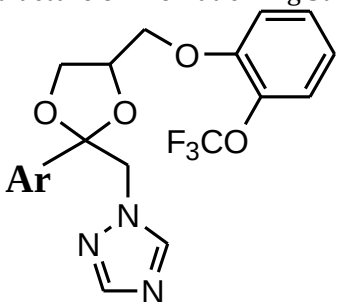
## 3. Results

The general chemical structure of BR biosynthesis inhibitors used in the present study was shown in Fig. 1. In the structure-activity relationship studies of this synthetic series on the inhibition of BRbiosynthesis, we found that introducing phenyl moieties with different substituent (Shown as ring A in Fig. 1) to the position 2 of the 1, 3-dioxolane displayed significant effect on promoting or reducing the activity [10]. To test the effect of chemical structure of ring A on larvicidal activity, 8 compounds with different substituents were subjected for the biological evaluation. The final concentration of the test compounds were 100μM and 3 to4instar mosquito larvae were used in all the experiment. Among 30 mosquito larvae, the number of the death of mosquito larvae in the presence of chemicals was counted and expressed as percentage of the larvicidal activity of the test compounds. As shown in Table 1, introducing a phenyl moiety without any substituent (compound 1) displayed a larvicidal activity approximately 57±29%. Introducing a fluoring atom (compound 2) or a phenyl substituent at the position 4 of the phenyl moiety (compound 3) did not promoted the larvicidal activity of this synthetic series with the activity approximately 52±19 and 50±21%, respectively. Introducing a trifluoromethyl substituent at the position 4 of the phenyl moiety (compound 4) significantly reduced the larvicidal activity from 57±29% to 21±7 %. In contrast, introducing a chlorine atom at the position 4 of the phenyl moiety (Compound 5) significantly promoted the larvicidal activity of this synthetic series (79±9%). Instead of phenyl moiety, introducing a naphthalene-2-yl to the position 2 of the 1,3-dioxolane (Compound 6) significantly reduced the larvicidal activity of this synthetic series with an activity approximately 24±10%. Introducing two chlorine atoms at position 2 and 4 (compound 7) or 3 and 5 (compound 8) to the phenyl moiety displayed different effect on promoting the larvicidal activity (Table 1). Data obtained from present work indicated that the compounds listed in table 1 exhibited larvicidal activity against mosquito larvae. Among the test compounds, compound 5 exhibited the most potent larvicidal activity.

Another factor that may influence the larvicidal activity of this synthetic series is the phenoxy moiety (Shown as ring B in Fig. 1). We next determine the larvicidal activity of the test compounds with different substituents of phenoxy moiety at ring B (As shown in Fig. 1). 20 compounds were subjected for biologicalevaluation (Table 2). Introduction of different alkyloxy substituent at position 2 or a fluorine atom at position 2 of the phenoxy moiety (compound 9 to 20) did not promote the activity of this synthetic series on larvicidal activity with a degree ranging from 5±5 to 40±13%. Introduction of two chloring atoms at different position of phenoxy moiety (Compound 23 to 28) also did not promote the larvicidal activity (Table 2). Introducing a chlorine atom at position 3 or 4 of the phenoxy moiety (compound 21 and 22)

enhanced the larvicidal activity with a degree of 76±10 and 100±0%, respectively. This result indicated that compound 22 display potent larvicidal activity against mosquito larvae. Next, we determine the does-dependent effect of compound 22 on larvicidal activity against mosquito larvae. As shown in Fig. 2, Compound 22 displayed a larvicidal activity in a does-dependent manner and the LD50 value was found approximately 17.8±2.0 μM.

Table 1. Effect of Chemical Structure of Aromatic Ring Structure on Larvicidal Activity



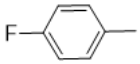
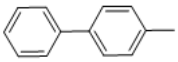
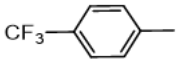
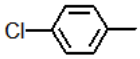
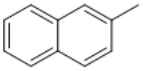
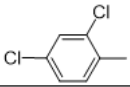
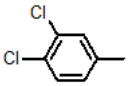
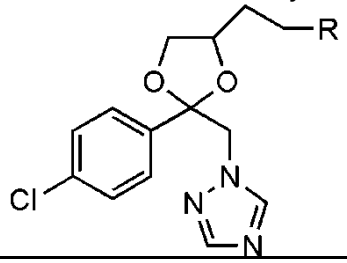

| No. | Ar | Dead larvae (%) |
|---|---|---|
| 1 |  | 57±29 |
| 2 |  | 52±19 |
| 3 |  | 50±21 |
| 4 |  | 21±7 |
| 5 |  | 79±9 |
| 6 |  | 24±10 |
| 7 |  | 67±10 |
| 8 |  | 43±8 |
| **Control** | | 7±4 |

Table 2. Effect of the Chemical Structure of Phenoxy Moiety on Larvicidal Activity




| Com. No. | —R | Dead larvae (%) |
|---|---|---|
| 9 | 2-methylphenoxy | 27±8 |
| 10 | 2-methoxyphenoxy | 26±6 |

| | | |
|---|---|---|
| 11 | 2-trifluoromethoxyphenoxy | 43±8 |
| 12 | 2-propoxyphenoxy | 29±14 |
| 13 | 2-allyloxyphenoxy | 40±13 |
| 14 | 2-butoxyphenoxy | 5±5 |
| 15 | 2-but-3-enyloxyphenoxy | 24±5 |
| 16 | 2-(3-methylbutoxy)phenoxy | 33±19 |
| 17 | 2-(3-methyl-but-2-enyloxy)phenoxy | 13±7 |
| 18 | 2-tert-butoxyphenoxy | 29±8 |
| 19 | 2-cyclopentyloxyphenoxy | 32±21 |
| 20 | 2-fluoro | 37±18 |
| 21 | 3-chlorophenoxy | 76±10 |
| 22 | 4-chlorophenoxy | **100±0** |
| 23 | 2,3-dichlorophenoxy | 33±10 |
| 24 | 2,4-dichlorophenoxy | 19±13 |
| 25 | 2,5-dichlorophenoxy | 19±15 |
| 26 | 2,6-dichlorophenoxy | 24±5 |
| 27 | 3,4-dichlorophenoxy | 33±19 |
| 28 | 3,5-dichlorophenoxy | 33±5 |
| Control | | 7±4 |

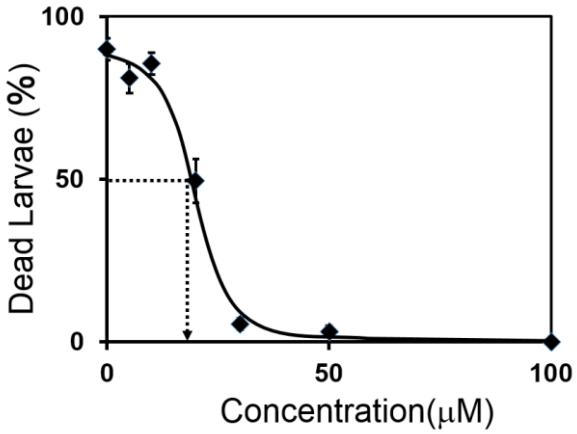



Fig. 2. Does-dependent effect of compound 22 on larvicidal activity against mosquito larvae.

Compound 22 was applied in 0, 5, 10, 20, 40, 50 100 μM. The percent of dead larvae was expressed as larvicidal activity. All the experiments were taken three times independently to establish the repeatability

## 4. Discussion

In the present work, we screened the larvicidal activity of a series of triazole-type BR biosynthesis inhibitors against mosquito larvae. Data obtained from present work revealed that 1-[4-(4-chlorophenoxymethyl)-2-(4-chloro-phenyl)-[1, 3] dioxolan-2-ylmethyl]-1H-[1, 2, 4] triazole(compound 22) displays potent lavicidal activity against mosquito larvae with an LD50 value approximately 17.8±2.0 □ M. Although the action mechanism of compound 22 is still remained to be determined, considering compound 22 is a triazole derivative which has been demonstrated to inhibit BR biosynthesis enzyme of CYP90D1 [13], we propose that the larvicidal activity of this synthetic series may due to the inhibition of ecdysteroid biosynthesis through interfering cytochrome P450 such as CYP314A1and/or CYP306A1 which are responsible to the side chain hydroxylation of the ecdysteroid biosynthesis [6], [7]. Since the compounds found in the present work is a new synthetic series which is

different from those steroid analogues with inhibitory activity against ecdysteroid biosynthesis as reported previously, we expect further experiment use of compound 22 to determine the action mechanism of the larvicidal activity may lead to the discovery of new class insectcide for mosquito control.

## 5. Conclusions

Overall, we discovered a new lead compound with larvicidal activity against mosquito larvae. The LD50 value of 1-[4-(4-chlorophenoxymethyl)-2-(4-chloro-phenyl)-[1,3]dioxolan-2-ylmethyl]-1H-[1,2,4]triazole (compound 22) was found approximately 17.8±2.0μM. Further studies on the structure-activity relationships of this synthetic series may lead to the discovery of new triazole type larvicide for mosquito control. Moreover, studies on the mode of action of the synthetic series found in the present work is a straight forward approach for search new insectcides for mosquito control against those exhibitinginsecticesresistance strain of insect pest.

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Author Contributions: Keimei Oh contributed to writing and preparing the manuscript, as well as experimental design and statistical analyses. Haruka.Kamada. contributed to perform the bioassay; Kazuhiro Yamada and Yuko.Yoshizawa contributed reagents.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript: RH: Relative Humidity, YCZ: Yucaizol, BR: Brassinosteroid

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