Algicidal effects of thiazolinedione derivatives against *Microcystis aeruginosa*

Don-Sang You*, Yeon Woong Lee**, Dubok Choi***, Young-Cheol Chang****, and Hoon Cho**,†

*Leaders in Industry-University Cooperation Foundation, Chosun University, Gwangju 61452, Korea
**Department of Polymer Science & Engineering, Chosun University, Gwangju 61452, Korea
***Biotechnology Laboratory, B-K Company Ltd., Jeonbuk 54008, Korea
****Biosystem Course, Division of Applied Sciences, Muroran Institute of Technology, 27-1 Mizumoto 050-8585, Japan

Abstract−Novel algicidal compounds against *Microcystis aeruginosa* were developed. A series of 64 thiazolidinedione (TD) derivatives were synthesized and analyzed for algicidal activity. Eleven compounds (2, 22, 38, 40, 49, 52, 54, 56, 58, 60, and 63) showed potent specific algicidal activity against *M. aeruginosa* with IC_{50} values <0.5 μM. An acute ecotoxicity test for these compounds was conducted using *Danio rerio* for ten days to verify their environmental impacts. Compounds 2 and 22 presented low ecotoxicity (EC_{50} 13.59 and 8.59 μM, respectively). To evaluate the ecotoxic effect of various concentrations of compound 2, *Daphnia magna* were treated with 2.0, 2.3, 2.7, or 3.0 μM compound 2 for 25 days. Survival rate was 100% after 25 days in the 2.0 μM group, but declined to 96% at 8 days and 30% at 17 days in the 2.3 μM group. Our results indicate that compound 2 could be a potential bio-agent for controlling harmful algal blooms.

Keywords: Thiazolidinedione, Algicidal Activity, *Microcystis aeruginosa*, Ecotoxicity

INTRODUCTION

Harmful algal blooms (HABs) have increasingly become an environmental and public problem globally, and have seriously undermined the sustainable development of coastal areas. It is estimated that a severe HAB event can cause the loss of millions of US dollars. Moreover, approximately 2,000 cases of human poisoning resulting from algal toxins are reported each year [1]. Many studies have been conducted regarding different chemical and biological techniques for the control of HABs. Several chemical-based methods have been used to mitigate HABs, but safer and more selective algicidal agents are needed to achieve better control of different fish-killing HABs. Copper sulfate, chelated copper compounds, and diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea) are currently approved for use by the US Environmental Protection Agency (EPA) [2]. Although the application of chemicals is one of the most common methods for controlling the development of noxious phytoplankton, their use has limitations, including toxicity toward non-targeted species [2,3]. Biological control techniques have also been developed to control the growth of HABs [4-6]. However, controlling targeted algal species using biological control agents has many logistical problems and is far from the application stage [7]. Yellow clay, because it potentially avoids these problems, has been used to control fish-killing HABs in many countries [8-11]. However, Shumway et al. [12] reported a negative effect of clay on filter-feeding invertebrates at high clay concentrations. Therefore, while yellow clay is very effective, its use remains controversial because

of the possibility of adverse effects on benthic organisms [13,14]. There thus remains a need for the development of safe and environmentally friendly selective algicidal control methods, as no satisfactory field application approaches have been reported.

Thiazolidinediones (TD), also called glitazones, were introduced as a class of oral medicines for treating type 2 diabetes mellitus in the late 1990s. TD bind and activate peroxisome proliferator-activated receptors (PPARs), whose activation reduces insulin resistance. PPARs bound by TD activate transcription of several genes related to lipid and glucose metabolism. In addition, some TD have been used as antibacterial and antifungal agents, as well as drugs [15]. These compounds have been investigated to search for specific algicidal chemicals with minimal effects on non-harmful organisms and the environment.

Recently, we reported the chemical synthesis of various TD derivatives, along with their algicidal activities against HAB-causing microalgae. Among the compounds tested, some TD derivatives showed effective algicidal activity against *Heterosigma akashiwo*, *Chattonella marina*, and *Cochlodinium polykrikoide*, while non-harmful algae were relatively tolerant to these compounds [16]. We also synthesized a novel TD derivative TD118, which showed selective algicidal effects for red tide control. TD118 showed specific algicidal activity on *Raphidophyceae* and *Dinophyceae*. The O_2 evolution and photosystem II efficiency of *C. marina, H. akashiwo*, and *C. polykrikoides* were also tested by TD118 treatment. These results imply that the relationship between species and TD structure may be attributable to structural and/or physiological differences among microalgal species [17]. To improve delivery, a liposomal delivery system was designed for TD53, a novel algicidal drug. Liposomal TD53 was evaluated for its algicidal effects as well as its selectivity against harmful and non-harmful algae. Harmful algae activity de-

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†To whom correspondence should be addressed.
E-mail: hcho@chosun.ac.kr

creased by approximately 50% after liposomal TD53 treatment versus that with non-liposomal TD53. Furthermore, the algicidal effect of liposomal TD53 was insignificant against non-harmful algae. These results suggested that liposomal delivery systems may be effective in enhancing the efficacy of TD53, while maintaining selectivity to harmful algal species [18]. Yim et al. [19] investigated the toxic effects of two newly designed algicides TD49 and TD53, which were synthesized to selectively control red tide in the aquatic ecosystem, by using *U. pertusa* Kjellman. In addition, Kim et al. [20] conducted acute toxicity assessments of TD49 and TD53 using *Skeletonema costatum*, *Daphnia magna*, and *Paralichthys olivaceus*. The results showed that a formulization study of algicides with high specificity should be conducted to reduce surrounding ecological toxicity. Baek et al. [21] evaluated the algicidal impact of TD49 on HAB species in aquatic coastal ecosystems by using the compound during the growth stages of *Rapidophyceae*. These strains could easily be destroyed during the lag phase owing to relatively weaker cell walls compared to the logarithmic and stationary phases. It has been thought that inoculation of TD49 into initial or developmental natural blooms with a threshold concentration ($2\,\mu M$) could maximize algicidal activity. Even though various TD compounds have been researched, there have been no reports regarding the effectiveness against *Microcystis aeruginosa* of thiazolidine-2,4-dione derivatives with introduced hydroxyl groups. Therefore, new substances for removing harmful algae species must be developed. Recently, we found an effective algicidal activity against *Microcystis aeruginosa* using new TD derivative compounds among various harmful algal blooms.

In this study, to develop novel algicidal compounds against *Microcystis aeruginosa*, we newly synthesized a series of 64 TD derivative compounds containing various introduced substituent groups. Compound efficacies and selectivities were examined by analyzing their structure-activity relationships. We also tested acute ecotoxicology using the water flea and zebrafish models to verify the environmental safety of the newly synthesized TD compounds.

MATERIAL AND METHODS

1. Chemistry

Thiazolidinedione derivatives were prepared by using various methods. The connection of cyclohexylalcohol to the central aryl aldehyde was accomplished via Mitsunobu coupling to produce an intermediate aldehyde with a yield of 69-98%. Knoevenagel condensation between the intermediate aldehyde and 2,4-thiazolidinedione in refluxing toluene containing a catalytic amount of piperidine and acetic acid produced 2,4-thiazolidinedione, which was crystalized from the reaction mixture with high purity. Hydroxybenzaldehyde, as a starting material, was reacted with various substituents to produce an intermediate substituted benzaldehyde, at good yields. This intermediate was then used in a coupling reaction with thiazolidine-2,4-dione to generate the appropriate thiazolidinedione derivative. All compounds were characterized by ^{1}H NMR.

2. General Procedure for the Synthesis of Compounds (1-64)

2-1. 5-(2-Hydroxybenzylidene)thiazolidine-2,4-dione (1)

2-Hydroxybenzaldehyde (1.043 g, 8.54 mmol) and 2,4-thiazolidinedione (1 g, 8.54 mmol) were dissolved in a round-bottom flask containing toluene (20 mL) fitted with a Dean-Stark trap. Piperidine (0.42 mL, 4.27 mmol) and acetic acid (0.18 mL, 4.27 mmol) were then added and the mixture was stirred under reflux for 18 h at 80 °C. After cooling to room temperature, the precipitate was washed with hexane. Compound 1 was obtained by re-crystallization as a yellow solid (1.67 g, 88%). ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.51 (s, 1H), δ 10.52 (s, 1H), δ 8.02 (s, 1H), δ 7.34 (m, 2H), δ 6.97 (m, 2H). ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.51 (s, 1H), δ 10.52 (s, 1H), δ 8.02 (s, 1H), δ 7.34 (m, 2H), δ 6.97 (m, 2H).

2-2. 5-(2-Hydroxy-3-methoxybenzylidene)thiazolidine-2,4-dione (2)

Compound 2 was prepared in a manner similar to that described for 1 at 92% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.52 (s, 1H), δ 9.72 (s, 1H), δ 8.04 (s, 1H), δ 7.12 (dd, *J*=6.96 and 2.58 Hz, 1H), δ 6.96 (m, *J*=2.58, 1H), δ 3.89 (s, 3H).

2-3. 5-(2-Hydroxy-4-methoxybenzylidene)thiazolidine-2,4-dione (3)

Compound 3 was prepared in a manner similar to that described for 1 at 91% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.38 (s, 1H), δ 10.64 (s, 1H), δ 7.97 (s, 1H), δ 7.27 (d, *J*=8.79 Hz, 1H), δ 6.59 (dd, *J*=8.79 and 2.19 Hz, 1H), δ 6.50 (d, 2.19 Hz, 1H), δ 3.76 (s, 3H).

2-4. 5-(2-Hydroxy-5-methoxybenzylidene)thiazolidine-2,4-dione (4)

Compound 4 was prepared in a manner similar to that described for 1 at 92% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 9.89 (s, 1H), δ 7.87 (s, 1H), δ 6.91 (m, 3H), δ 3.27 (s, 3H).

2-5. 5-(2-Hydroxy-6-methoxybenzylidene)thiazolidine-2,4-dione (5)

Compound 5 was prepared in a manner similar to that described for 1 at 89% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.22 (s, 1H), δ 10.56 (s, 1H), δ 7.92 (s, 1H), δ 7.27 (t, *J*=8.43 Hz, 1H), δ 6.54 (d, *J*=8.43 Hz, 2H), δ 3.19 (s, 3H).

2-6. 5-(2-Hydroxy-3,4-dimethoxybenzylidene)thiazolidine-2,4-dione (6)

Compound 6 was prepared in a manner similar to that described for 1 at 81% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.44 (s, 1H), δ 9.93 (s, 1H), δ 9.96 (s, 1H), δ 7.09 (d, *J*=8.79 Hz, 1H), δ 6.74 (d, *J*=8.79 Hz, 1H), δ 3.84 (s, 3H), δ 3.69 (s, 3H).

2-7. 5-(6-Bromo-2-hydroxy-3-methoxybenzylidene)thiazolidine-2,4-dione (7)

Compound 7 was prepared in a manner similar to that described for 1 at 85% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.45 (s, 1H), δ 10.26 (s, 1H), δ 7.69 (s, 1H), δ 7.16 (d, *J*=8.43 Hz, 1H), δ 7.01 (d, *J*=8.43 Hz, 1H), δ 3.83 (s, 3H).

2-8. 5-(3-Hydroxybenzylidene)thiazolidine-2,4-dione (8)

Compound 9 was prepared in a manner similar to that described for 1 at 90% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.59 (s, 1H), δ 9.83 (s, 1H), δ 7.68 (s, 1H), δ 7.34 (t, *J*=8.04 and 7.71 Hz, 1H), δ 7.04 (d, *J*=7.71 Hz, 1H), δ 6.97 (s, 1H), δ 6.89 (dd, *J*=8.04 Hz, 1H).

2-9. 5-(2-Chloro-3-hydroxybenzylidene)thiazolidine-2,4-dione (9)

Compound 9 was prepared in a manner similar to that described for 1 at 90% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.71 (s, 1H), δ 10.60 (s, 1H), δ 7.93 (s, 1H), δ 7.33 (t, *J*=8.07 and 7.68 Hz, 1H), δ 7.09 (dd, *J*=8.07 Hz, 1H), δ 7.02 (d, *J*=7.68 Hz, 1H).

2-10. 5-(2-Chloro-3-hydroxy-4-methoxybenzylidene)thiazolidine-2,4-dione (10)

Compound 10 was prepared in a manner similar to that described

for 1 at 79% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$d6$) δ 12.61 (s, 1H), δ 9.84 (s, 1H), δ 7.91 (s, 1H), δ 7.14 (d, J=8.79 Hz, 1H), δ 7.04 (d, J=8.79 Hz, 1H), δ 3.89 (s, 3H).

2-11. 5-(2-Bromo-5-hydroxy-4-methoxy-benzylidene)thiazolidine-2,4-dione (11)

Compound 11 was prepared in a manner similar to that described for 1 at 87% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$d6$) δ 12.64 (s, 1H), δ 9.91 (s, 1H), δ 7.81 (s, 1H), δ 7.32 (s, 1H), δ 7.03 (s, 1H), δ 3.84 (s, 3H).

2-12. 5-(4-Hydroxy-benzylidene)thiazolidine-2,4-dione (12)

Compound 12 was prepared in a manner similar to that described for 1 at 98% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$d6$) δ 12.46 (s, 1H), δ 10.32 (s, 1H), δ 7.69 (s, 1H), δ 7.46 (d, J=8.43 Hz, 2H), δ 6.92 (d, J=8.43 Hz, 2H).

2-13. 5-(4-Hydroxy-2-methoxy-benzylidene)thiazolidine-2,4-dione (13)

Compound 13 was prepared in a manner similar to that described for 1 at 85% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$d6$) δ 12.36 (s, 1H), δ 10.38 (s, 1H), δ 7.92 (s, 1H), δ 7.26 (d, J=8.43 Hz, 1H), δ 6.54 (m, 2H), δ 3.83 (s, 3H).

2-14. 5-(4-Hydroxy-3-methoxybenzylidene)thiazolidine-2,4-dione (14)

Compound 14 was prepared in a manner similar to that described for 1 at 87% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$d6$) δ 12.47 (s, 1H), δ 9.97 (s, 1H), δ 7.71 (s, 1H), δ 7.17 (d, J=1.83 Hz, 1H), δ 7.08 (dd, J=8.04 and 1.83 Hz, 1H), δ 6.93 (d, J=8.84 Hz, 1H), δ 3.81 (s, 3H).

2-15. 5-(3-Ethoxy-4-hydroxybenzylidene)thiazolidine-2,4-dione (15)

Compound 15 was prepared in a manner similar to that described for 1 at 77% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$d6$) δ 12.46 (s, 1H), δ 9.88 (s, 1H), δ 7.69 (s, 1H), δ 7.15 (d, J=1.83 Hz, 1H), δ 7.07 (dd, J=8.43 and 1.83 Hz, 1H), δ 6.94 (d, J=8.43 Hz, 1H), δ 4.10 (m, J=6.96 Hz, 2H), δ 1.37 (t, J=6.96 Hz, 3H).

2-16. 5-(4-Hydroxy-3-methylbenzylidene)thiazolidine-2,4-dione (16)

Compound 16 was prepared in a manner similar to that described for 1 at 76% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$d6$) δ 12.41 (s, 1H), δ 10.22 (s, 1H), δ 7.64 (s, 1H), δ 7.31 (s, 1H), δ 7.29 (dd, J=8.04 and 2.19 Hz, 1H), δ 6.93 (d, J=8.04 Hz, 1H), δ 2.15 (s, 3H).

2-17. 5-(3-Fluoro-4-hydroxybenzylidene)thiazolidine-2,4-dione (17)

Compound 17 was prepared in a manner similar to that described for 1 at 74% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$d6$) δ 12.67 (s, 1H), δ 10.91 (s, 1H), δ 7.67 (s, 1H), δ 7.43 (dd, J=1.83 Hz, 1H), δ 7.27 (dd, J=8.79 and 1.83 Hz, 1H), δ 7.11 (t, J=8.79 Hz, 1H).

2-18. 5-(3-Chloro-4-hydroxybenzylidene)thiazolidine-2,4-dione (18)

Compound 18 was prepared in a manner similar to that described for 1 at 74% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$d6$) δ 11.97 (s, 1H), δ 11.50 (s, 1H), δ 7.65 (s, 1H), δ 7.62 (d, J=2.22 Hz, 1H), δ 7.41 (d, J=8.43 and 2.22 Hz, 1H), δ 7.11 (d, J=8.43 Hz, 1H).

2-19. 5-(3-Bromo-4-hydroxybenzylidene)thiazolidine-2,4-dione (19)

Compound 19 was prepared in a manner similar to that described for 1 in 69% yield as a yellow solid.^{1}H NMR (300 MHz, DMSO-$d6$) δ 12.54 (s, 1H), δ 11.16 (s, 1H), δ 7.78 (d, J=2.19 Hz, 1H), δ 7.69 (s, 1H), δ 7.45 (dd, J=8.79 and 2.19 Hz, 1H), δ 7.10 (d, J=8.79 Hz, 1H).

2-20. 5-(4-Hydroxy-3-nitrobenzylidene)thiazolidine-2,4-dione (20)

Compound 20 was prepared in a manner similar to that described for 1 at 74% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$d6$) δ 12.28 (s, 1H), δ 8.15 (d, J=2.19 Hz, 1H), δ 7.78 (s, 1H), δ 7.76 (dd, J=8.79, 2.19 Hz, 1H), δ 7.27 (dd, J=8.79 Hz, 1H).

2-21. 5-(3-Bromo-4-hydroxy-5-methoxybenzylidene)thiazolidine-2,4-dione (21)

Compound 21 was prepared in a manner similar to that described for 1 at 75% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$d6$) δ 12.55 (s, 1H), δ 10.39 (s, 1H), δ 7.70 (s, 1H), δ 7.36 (d, J=1.83 Hz, 1H), δ 7.18 (d, J=1.83 Hz, 1H), δ 3.88 (s, 3H).

2-22. 5-(3-Bromo-5-chloro-4-hydroxybenzylidene)thiazolidine-2,4-dione (22)

Compound 22 was prepared in a manner similar to that described for 1 at 88% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$d6$) δ 12.59 (s, 1H), δ 11.09 (s, 1H), δ 7.74 (d, J=1.83 Hz, 1H), δ 7.69 (s, 1H), δ 7.63 (d, J=1.83 Hz, 1H).

2-23. 5-(3-Methoxybenzylidene)thiazolidine-2,4-dione (23)

Compound 23 was prepared in a manner similar to that described for 1 at 95% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$d6$) δ 12.64 (s, 1H), δ 7.77 (s, 1H), δ 7.48 (t, J=8.04 Hz, 1H), δ 7.17 (m, J=2.55 Hz, 2H), δ 7.08 (dd, J=8.04 and 2.55 Hz, 1H), δ 3.80 (s, 3H).

2-24. 5-(3,5-Dimethoxybenzylidene)thiazolidine-2,4-dione (24)

Compound 24 was prepared in a manner similar to that described for 1 at 93% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$d6$) δ 12.64 (s, 1H), δ 7.72 (s, 1H), δ 6.75 (d, J=1.83 Hz, 2H), δ 6.63 (t, J=1.83 Hz, 1H), δ 3.81 (s, 6H).

2-25. 5-(3,4,5-Trimethoxybenzylidene)thiazolidine-2,4-dione (25)

Compound 25 was prepared in a manner similar to that described for 1 at 97% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$\underline{d6}$) δ 12.62 (s, 1H), δ 7.73 (s, 1H), δ 6.92 (s, 2H), δ 3.82 (s, 6H), δ 3.71 (s, 3H).

2-26. 5-(4-Methoxybenzylidene)thiazolidine-2,4-dione (26)

Compound 26 was prepared in a manner similar to that described for 1 at 95% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$d6$) δ 12.52 (s, 1H), δ 7.75 (s, 1H), δ 7.58 (d, J=8.79 Hz, 2H), δ 7.11 (d, J=8.79 Hz, 2H), δ 3.82 (s, 3H).

2-27. 5-(4-Ethoxybenzylidene)thiazolidine-2,4-dione (27)

Compound 27 was prepared in a manner similar to that described for 1 at 90% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$d6$) δ 12.50 (s, 1H), δ 7.74 (s, 1H), δ 7.56 (d, J=8.79 Hz, 2H), δ 7.09 (d, J=8.79 Hz, 2H), δ 4.13 (m, J=6.60 Hz, 2H), δ 1.36 (t, J=6.60 Hz, 3H).

2-28. 5-(4-Propoxybenzylidene)thiazolidine-2,4-dione (28)

Compound 28 was prepared in a manner similar to that described for 1 at 92% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$d6$) δ 12.49 (s, 1H), δ 7.74 (s, 1H), δ 7.56 (d, J=8.76 Hz, 2H), δ 7.10 (d, J=8.76 Hz, 2H), δ 4.02 (t, J=6.60 Hz, 2H), δ 1.77 (m, J=6.96 and 7.35, 2H), δ (t, J=7.35 Hz, 3H).

2-29. 5-(4-Methylbenzylidene)thiazolidine-2,4-dione (29)

Compound 29 was prepared in a manner similar to that described for 1 at 98% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-$d6$) δ 12.57 (s, 1H), δ 7.74 (s, 1H), δ 7.49 (d, J=8.40 Hz, 2H), δ 7.35 (d, J=8.40 Hz, 2H), δ 2.35 (s 3H).

2-30. 5-(4-Fluorobenzylidene)thiazolidine-2,4-dione (30)

Compound 30 was prepared in a manner similar to that described for 1 at 71% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 13.02 (s, 1H), δ 8.02 (m, 2H), δ 7.41 (m, 2H).

2-31. 5-(3-Fluorobenzylidene)thiazolidine-2,4-dione (31)

Compound 31 was prepared in a manner similar to that described for 1 at 70% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.70 (s, 1H), δ 7.79 (s, 1H), δ 7.62 (m, *J*=8.43 Hz, 1H), δ 7.47 (m, *J*=8.43 Hz, 2H), δ 7.36 (m, *J*=8.43).

2-32. 5-(2-Fluorobenzylidene)thiazolidine-2,4-dione (32)

Compound 32 was prepared in a manner similar to that described for 1 at 73% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.73 (s, 1H), δ 7.78 (s, 1H), δ 7.59 (m, 2H), δ 7.41 (m, 2H).

2-33. 5-(3,4-Difluorobenzylidene)thiazolidine-2,4-dione (33)

Compound 33 was prepared in a manner similar to that described for 29 at 80% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.69 (s, 1H), δ 7.78 (s, 1H), δ 7.75 (m, 1H), δ 7.66 (m, 1H), δ 7.48 (m, 1H).

2-34. 5-(2,4-Difluorobenzylidene)thiazolidine-2,4-dione (34)

Compound 34 was prepared in a manner similar to that described for 1 at 80% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.74 (s, 1H), δ 7.71 (s, 1H), δ 7.63 (m, *J*=6.60 Hz, 1H), δ 7.51 (m, *J*=6.60 Hz, 1H), δ 7.31 (m, 1H).

2-35. 5-(2,6-Difluorobenzylidene)thiazolidine-2,4-dione (35)

Compound 35 was prepared in a manner similar to that described for 1 at 95% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.69 (s, 1H), δ 7.64 (s, 1H), δ 7.62 (m, *J*=8.76 Hz, 1H), δ 7.29 (m, *J*=8.76 Hz, 2H), δ 7.08 (dd, *J*=8.04 and 2.55 Hz, 1H), δ 3.80 (s, 3H).

2-36. 5-(3-Chlorobenzylidene)thiazolidine-2,4-dione (36)

Compound 36 was prepared in a manner similar to that described for 1 at 95% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.70 (s, 1H), δ 7.77 (s, 1H), δ 7.67 (s, 1H), δ 7.58 (m, 3H).

2-37. 5-(4-Chlorobenzylidene)thiazolidine-2,4-dione (37)

Compound 37 was prepared in a manner similar to that described for 1 at 95% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.67 (s, 1H), δ 7.79 (s, 1H), δ 7.65 (m, 4H).

2-38. 5-(3,4-Dichlorobenzylidene)thiazolidine-2,4-dione (38)

Compound 38 was prepared in a manner similar to that described for 1 at 81% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.76 (s, 1H), δ 7.91 (d, *J*=1.83 Hz, 1H), δ 7.81 (d, *J*=8.04 Hz, 2H), δ 7.56 (dd, *J*=8.04 and 1.83 Hz, 1H).

2-39. 5-(2-Bromobenzylidene)thiazolidine-2,4-dione (39)

Compound 39 was prepared in a manner similar to that described for 1 at 73% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.77 (s, 1H), δ 7.87 (s, 1H), δ 7.82 (d, *J*=7.71 Hz, 1H), δ 7.59 (m, *J*=7.71 and 4.02 Hz, 2H), δ 7.45 (m, *J*=4.02 Hz, 1H).

2-40. 5-(3-Bromobenzylidene)thiazolidine-2,4-dione (40)

Compound 40 was prepared in a manner similar to that described for 1 at 85% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.71 (s, 1H), δ 7.82 (s, 1H), δ 7.78 (s, 1H), δ 7.69 (d, *J*=7.68 Hz, 2H), δ 7.59 (d, *J*=7.68 Hz, 1H), δ 7.51 (t, *J*=7.68 Hz, 1H).

2-41. 5-(4-Bromobenzylidene)thiazolidine-2,4-dione (41)

Compound 41 was prepared in a manner similar to that described for 1 at 85% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.65 (s, 1H), δ 7.76 (s, 1H), δ 7.75 (m, *J*=8.79 Hz, 2H), δ 7.56 (m, *J*=8.79 Hz, 2H).

2-42. 5-(4-Iodobenzylidene)thiazolidine-2,4-dione (42)

Compound 42 was prepared in a manner similar to that described for 1 at 81% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.64 (s, 1H), δ 7.91 (d, *J*=8.43 Hz, 2H), δ 7.72 (s, 1H), δ 7.38 (d, *J*=8.43 Hz, 2H).

2-43. 5-(4-Nitrobenzylidene)thiazolidine-2,4-dione (43)

Compound 43 was prepared in a manner similar to that described for 1 at 72% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.81 (s, 1H), δ 8.35 (d, *J*=8.76 Hz, 2H), δ 7.88 (s, 1H), δ 7.86 (d, *J*=8.76 Hz, 2H).

2-44. 5-(4-Dimethylaminobenzylidene)thiazolidine-2,4-dione (44)

Compound 44 was prepared in a manner similar to that described for 1 at 79% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.33 (s, 1H), δ 7.66 (s, 1H), δ 7.43 (d, *J*=8.79 Hz, 2H), δ 6.82 (d, *J*=8.79 Hz, 2H), δ 3.01 (s, 6H).

2-45. 5-(2-Chloro-4-fluorobenzylidene)thiazolidine-2,4-dione (45)

Compound 45 was prepared in a manner similar to that described for 1 at 91% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.76 (s, 1H), δ 7.86 (s, 1H), δ 7.71 (dd, *J*=8.79 and 2.55 Hz, 1H), δ 7.65 (m, *J*=8.79 Hz, 1H), δ 7.45 (m, *J*=2.55 Hz, 1H).

2-46. 5-(4-Fluoro-3-methylbenzylidene)thiazolidine-2,4-dione (46)

Compound 46 was prepared in a manner similar to that described for 1 at 99% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.61 (s, 1H), δ 7.74 (s, 1H), δ 7.54 (m, 2H), δ 7.34 (t, 1H), δ 2.29 (s, 3H).

2-47. 5-(4-Nitro-2-trifluoromethylbenzylidene)thiazolidine-2,4-dione (47)

Compound 47 was prepared in a manner similar to that described for 1 at 71% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.82 (s, 1H), δ 7.85 (dd, *J*=9.15 and 2.55, 1H), δ 7.78 (m, 2H).

2-48. 5-(4-Fluoro-3-methoxybenzylidene)thiazolidine-2,4-dione (48)

Compound 48 was prepared in a manner similar to that described for 1 at 70% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.64 (s, 1H), δ 7.78 (s, 1H), δ 7.43 (m, 2H), δ 7.18 (m, 1H), δ 3.88 (s, 3H).

2-49. 5-(4-Bromo-2-fluorobenzylidene)thiazolidine-2,4-dione (49)

Compound 49 was prepared in a manner similar to that described for 1 at 75% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.77 (s, 1H), δ 7.79 (dd, *J*=8.43 and 1.83 Hz, 1H), δ 7.70 (s, 1H), δ 7.62 (dd, 1H), δ 7.51 (t, *J*=8.43 Hz, 1H).

2-50. 5-(4-Chloro-2-fluorobenzylidene)thiazolidine-2,4-dione (50)

Compound 50 was prepared in a manner similar to that described for 1 at 78% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.75 (s, 1H), δ 7.69 (s, 1H), δ 7.60 (m, *J*=8.43 and 1.47 Hz, 2H), δ 7.43 (m, *J*=8.43 and 1.47 Hz, 1H).

2-51. 5-(2-Fluoro-5-iodobenzylidene)thiazolidine-2,4-dione (51)

Compound 51 was prepared in a manner similar to that described for 1 at 89% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.79 (s, 1H), δ 7.89 (m, *J*=2.19 Hz, 1H), δ 7.77 (dd, *J*=6.96 and 2.19 Hz, 1H), δ 7.65 (s, 1H), δ 7.25 (t, *J*=6.96 Hz, 1H).

2-52. 5-(4-Chloro-3-fluorobenzylidene)thiazolidine-2,4-dione (52)

Compound 52 was prepared in a manner similar to that described for 1 at 95% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 12.73 (s, 1H), δ 7.78 (m, *J*=8.04, 3H), δ 7.45 (d, *J*=8.04 Hz, 1H).

2-53. 5-(3-Fluoro-4-methylbenzylidene)thiazolidine-2,4-dione (53)

Compound 53 was prepared in a manner similar to that described

for 1 at 99% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 12.74 (s, 1H), δ 7.75 (s, 1H), δ 7.48 (d, J=8.04 Hz, 1H), δ 7.42 (m, J=1.47 Hz, 1H), δ 7.33 (dd, J=8.04 and 1.47 Hz, 1H), δ 2.28 (s, 3H).

2-54. 5-(2-Bromo-4-methoxybenzylidene)thiazolidine-2,4-dione (54)

Compound 54 was prepared in a manner similar to that described for 1 at 71% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 12.58 (s, 1H), δ 7.84 (d, J=1.83, 1H), δ 7.73 (s, 1H), δ 7.60 (dd, J=8.79 and 1.83 Hz, 1H), δ 7.28 (d, J=8.79 Hz, 1H), δ 3.91 (s, 3H).

2-55. 5-(2-Bromo-5-methoxybenzylidene)thiazolidine-2,4-dione (55)

Compound 55 was prepared in a manner similar to that described for 1 at 80% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 12.78 (s, 1H), δ 7.80 (s, 1H), δ 7.70 (dd, J=6.60 Hz, 1H), δ 7.04 (m, J=6.60 Hz, 2H), δ 3.80 (s, 3H).

2-56. 5-(3-Chloro-4-methoxybenzylidene)thiazolidine-2,4-dione (56)

Compound 56 was prepared in a manner similar to that described for 1 at 88% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 12.60 (s, 1H), δ 7.74 (s, 1H), δ 7.71 (d, J=2.22 Hz, 1H), δ 7.57 (dd, J=8.79 and 2.22 Hz, 1H), δ 7.33 (d, J=8.79 Hz, 1H), δ 3.92 (s, 3H).

2-57. 5-(3-Iodo-4-methoxybenzylidene)thiazolidine-2,4-dione (57)

Compound 57 was prepared in a manner similar to that described for 1 at 70% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 12.55 (s, 1H), δ 8.01 (d, J=2.22, 1H), δ 7.70 (s, 1H), δ 7.61 (dd, J=8.79 and 2.22 Hz, 1H), δ 7.16 (d, J=8.79 Hz, 1H), δ 3.89 (s, 3H).

2-58. 5-(3-Chloro-4,5-dimethoxybenzylidene)thiazolidine-2,4-dione (58)

Compound 58 was prepared in a manner similar to that described for 1 in 94% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 12.67 (s, 1H), δ 7.73 (s, 1H), δ 7.27 (d, J=1.83 Hz, 1H), δ 7.24 (d, J=1.83 Hz, 1H), δ 3.88 (s, 3H), δ 3.81 (s, 3H).

2-59. 5-(2-Bromo-4,5-dimethoxybenzylidene)thiazolidine-2,4-dione (59)

Compound 59 was prepared in a manner similar to that described for 1 at 97% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 12.68 (s, 1H), δ 7.82 (s, 1H), δ 7.35 (s, 1H), δ 7.00 (s, 1H), δ 3.85 (s, 3H), δ 3.81 (s, 3H).

2-60. 5-(3-Iodo-4,5-dimethoxybenzylidene)thiazolidine-2,4-dione (60)

Compound 60 was prepared in a manner similar to that described for 1 at 91% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 12.65 (s, 1H), δ 7.71 (s, 1H), δ 7.56 (d, J=2.22 Hz, 1H), δ 7.28 (d, J=2.22 Hz, 1H), δ 3.86 (s, 3H), δ 3.76 (s, 3H).

2-61. 5-(5-Iodo-2,4-dimethoxybenzylidene)thiazolidine-2,4-dione (61)

Compound 61 was prepared in a manner similar to that described for 1 at 65% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 12.53 (s, 1H), δ 7.80 (s, 1H), δ 7.67 (s, 1H), δ 6.77 (s, 1H), δ 3.94 (s, 6H).

2-62. 5-(2-Bromo-4-fluoro-5-methoxybenzylidene)thiazolidine-2,4-dione (62)

Compound 62 was prepared in a manner similar to that described for 1 at 69% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 12.79 (s, 1H), δ 7.83 (m, 2H), δ 7.23 (d, J=8.79 Hz, 1H), δ 3.90

(s, 3H).

2-63. 5-(3-Chloro-2,6-difluorobenzylidene)thiazolidine-2,4-dione (63)

Compound 63 was prepared in a manner similar to that described for 1 at 91% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 12.78 (s, 1H), δ 7.82 (m, 1H), δ 7.61 (s, 1H), δ 7.35 (m, 1H).

2-64. 5-(4-Bromo-2,6-difluorobenzylidene)thiazolidine-2,4-dione (64)

Compound 64 was prepared in a manner similar to that described for 1 at 96% yield as a yellow solid. ^{1}H NMR (300 MHz, DMSO-d6) δ 12.74 (s, 1H), δ 7.71 (d, J=7.74 Hz, 1H), δ 7.67 (d, J=4.47 Hz, 1H), δ 7.55 (s, 1H).

3. Algicidal Activity

Algicidal activity of the various thiazolidinedione derivatives against *Microcystis aeruginosa* was examined at various concentrations. Experiments were conducted in 96-well tissue culture test plates with approximately 1 mL total volume per well. Various concentrations of the test compounds were introduced to the algal cultures during the exponential growth phase. All of the microalgae were exposed to the compounds at final concentrations of 0.1, 0.2, 0.5, 1.2, 5, 10, and 100 μM. The control cultures were not exposed to the TD derivatives. TD compounds were added to the microalgae investigated in this study, and the number of cells was counted at 120 h post-treatment using a light microscope (Olympus Co., Tokyo, Japan) with a Burker Turk hemocytometer and a Sedgwick-Rafter counting chamber. The number of cells was counted and transformed to reduction rate (%) to obtain LC_{50} values. The formula used is as follows:

Reduction rate $(\%)=(1-T_t/C_t)\times100$

where T_t is the cell density after treatment, C_t is the cell density of the untreated control, and t is incubation time.

The LC_{50} value (LC_{50}-120 h) was calculated with SigmaPlot version 11.2 software using a four parameter logistic curve. The formula used is as follows:

$$Y=D+\frac{(A-D)}{\left(1+\left(\frac{x}{C}\right)^B\right)}$$

where Y is algicidal activity, A is the maximum algicidal activity of the treatment concentration, D is the minimum algicidal activity of the treatment concentration, C is the value of LC_{50}-120 h of inoculation concentration range, and B is the Hill's slope of the curve.

4. Algal Culture

Microcystis aeruginosa (KMMCC-973) was obtained from the algal culture collection of the Korea Marine Microalgae Culture Center (KMMCC). Cultures of *M. aeruginosa* were grown in a 6-L culture flask (Becton Dickinson Labware, Franklin Lakes, NJ, USA, 45 rpm) at 25 ^{o}C under constant light (100 μmol m^{-2}s^{-1} (12L : 12D) in 3 L of BG11 medium (pH 7.0 or 9.0; Unipath Ltd., Basingstock, UK) without Si and with filtered seawater. BG11 media was prepared with sterile-filtered seawater using 0.20-μm filtration units (Nalgene, Rochester, NY, USA) and was enriched aseptically using nutrients and vitamins purchased from Sigma Aldrich (St. Louis, MO, USA).

5. Acute Ecotoxicity Test of TD Compound Using *Danio rerio*

Danio rerio were acclimatized to laboratory conditions in 6-L glass tanks for two weeks before the start of experimentation. During

the acclimatization period, the *D. rerio* were fed twice a day with a semi-synthetic fish food diet (tetramin). All of the animals were healthy, with an observed mortality rate of less than 3% in the stock during the acclimatization period. Four-day static acute toxicity tests were performed in our laboratory to determine the LC_{50} values of the TD compound in *D. rerio*. On the day of the experiment, 3 L glass aquaria containing a test or control solution (rearing water) were aerated to restore the concentration of dissolved oxygen to at least 70-80% of its air saturation value. TD compound concentrations of 0.5, 1, 3, 5, 10, 15, and 20 μM (the medium without TD were used as the control). After acclimatization to laboratory conditions, seven adult fish were randomly distributed to each of the test aquariums. The control *D. rerio* groups were kept in clean water. During the acute toxicity test, the animals were not fed. The number of dead fish was recorded at 24, 48, 72, and 96 h. The dead fish were removed from the tanks. The test was conducted at 25±1 $^{\circ}$C and pH 7.5±0.2 under a 12 : 12 h light/dark cycle. At the end of the tests, the overall mortality of the fish was recorded.

6. Acute Ecotoxicity Test of TD Compound Using *Daphnia magna*

Daphnia magna culture was maintained at 20±2 $^{\circ}$C in a 6-L polyester jar in the Water Environmental Ecology and Restoration Laboratory at Chosun University. *D. magna* were fed daily with *Selenastrum capricornutum* (KCTCAG-1009). The culture water was changed three times per week. The culture and experimental solutions were maintained at 20±2 $^{\circ}$C under a light/dark cycle of 12 : 12 h, and *D. magna* was cultured in-house in moderately hard water. Acute toxicity tests were performed to determine the 48-h EC_{50} for *D. magna*. Four replicates of five juveniles (<24-h old) were exposed to 0.5, 1, 2, 5, and 10 μM (the medium without TD was used as the control) concentrations of the TD compound. The test volume was set at 75 mL for five neonates with four replicates so that the loading density did not exceed 15 mL of medium per neonate. The temperature was kept at 20±1 $^{\circ}$C and 50 μM photon m^{-2}s^{-1} under a 12 : 12 h cycle (light:dark). Organisms were not fed during the acute toxicity tests. For the duration of the experiment, death or immobility after 24 to 48 h of exposure was interpreted as an adverse response. Immobilization was viewed as an endpoint and was noted if no movement was detected for 15 s after gentle shaking of the test vessel. The median effective and lethal concentrations (EC/LC_{50}) and associated confidence intervals were calculated using the US EPA probit analysis and the Spearman-Karber method in the computer program TOXSTAT.

7. Statistical Analysis

The experiments were performed a minimum of three times. The data are reported as the mean±SD. All statistical analyses were performed using SPSS 17.0 software (IBM, Armonk, NY, USA). The statistical significance of the differences between the mean values was determined by one-way variance analysis (ANOVA) followed by a Tukey's honest significant difference (HSD) *post hoc* test. A *p* value <0.05 was considered significant.

RESULTS AND DISCUSSION

Microcystis sp. is the most common bloom-forming cyanobacterium in the world's eutrophic and hypereutrophic waters [22,23].

Many strains of *Microcystis* are known to affect drinking water supplies and human health by producing a range of toxins, especially *M. aeruginosa* [23-25]. Therefore, an efficient method of controlling the *M. aeruginosa* blooms is needed. Heterocyclic compounds have been extensively studied owing to their important properties and applications in the pharmaceutical industry. Among these compounds, TD derivatives have received increasing attention in recent years. Thiazolidine-2,4-dione is an important example of the thiazolidine family, and is used industrially as a raw material for pharmaceuticals and other functional chemicals such as algicides. Previously, we reported that the introduction of a five-member ring at the hydroxyl group of 5-(4hydroxybenzylidene)thiazolidine-2,4-dione resulted in a significant increase in its inhibitory potency [1]. We also introduced a methylene group between the cyclohexyl ring and oxygen of 5-(3-chloro-4-hydroxybenzylidene)-thiazolidine-2,4-dione for the synthesis of TD derivatives as a novel class of algicides [26]. Based on our previous results, we synthesized 64 new derivatives of TD, and the algicidal activities of the various synthetic TD derivatives according to their structures were measured using the structure-activity relationship. All the synthesized compounds were assayed *in vitro* against *M. aeruginosa*. Furthermore, their acute ecotoxicity was tested using *D. rerio* and *Daphnia magna*. The algicidal effects of TD derivatives where a hydroxy group was substituted to the R_1 position and a methoxy group was substituted to the R_2, R_3, R_4, or R_5 positions were investigated. The activities of the synthesized TD derivatives (compounds 1-7) are shown in Table 1. When a methoxy group was introduced to the R_2 position, it resulted in the highest LC_{50} value (0.213 μM, compound 2). However, when the methoxy group was moved to R_3, R_4, or R_5, a decrease in inhibitory potency was noted (compounds 3, 4, and 5, respectively). The introduction of a methoxy group at the R_2 and R_3 positions, or a methoxy group at the R_2 position with a bromine group at the R_5 position, also decreased algicidal activity. These results indicate that the algicidal activity of 5-(2-hydroxybenzylidene)thiazolidine-2,4-dione was strongly affected by the positioning of the methoxy substituent.

Table 2 shows a comparison of the effects on algicidal activity

Table 1. Algicidal effects of compounds 1-7
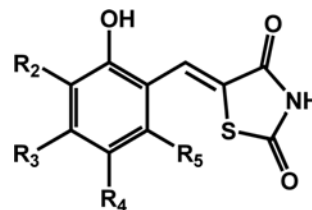
Compound no.	R_2	R_3	R_4	R_5	LC_{50} (μM)
1	-H	-H	-H	-H	2.769±0.112
2	-OCH$_3$	-H	-H	-H	0.213±0.003
3	-H	-OCH$_3$	-H	-H	0.852±0.008
4	-H	-H	-OCH$_3$	-H	5.333±0.316
5	-H	-H	-H	-OCH$_3$	13.256±0.332
6	-OCH$_3$	-OCH$_3$	-H	-H	15.789±0.689
7	-OCH$_3$	-H	-H	-Br	2.829±0.187

Table 2. Algicidal effects of compounds 8-11
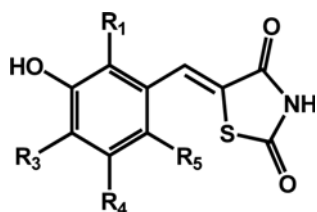
Compound no.	R_1	R_3	R_4	R_5	LC_{50} (μM)
8	-H	-H	-H	-H	4.189±0.268
9	-Cl	-H	-H	-H	5.623±0.216
10	-Cl	-OCH$_3$	-H	-H	12.126±0.625
11	-H	-OCH$_3$	-H	-Br	5.178±0.189

between chloride- and bromine-substituted TD derivatives with a hydroxy group at the R_2 position. When hydrogen and chlorine groups were introduced to the R_1 position, algicidal activity was similar (compounds 8 and 9, respectively). However, when a chlorine group was introduced at the R_2 position and a methoxy group at the R_3 position, algicidal activity decreased by about 54% (compound 10) compared to compound 9. When a methoxy group was introduced at the R_3 position and a bromo group at the R_5 position, algicidal activity was about twice as high as that of the compound containing a methoxy group at R_3 without a bromo group at R_5.

Table 3 shows the comparison of the effects on algicidal activity between halogen- and methoxy-substituted TD derivatives with a hydroxyl group at the R_3 position. When a hydroxy substituent was introduced to the phenyl moiety at the R_1 position, the maximal algicidal activity was obtained. However, algicidal activity decreased

Table 3. Algicidal effects of compounds 12-22
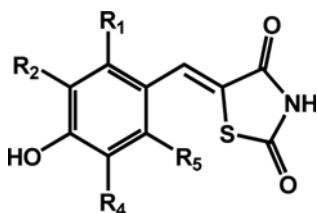
Compound no.	R_1	R_2	R_4	R_5	LC_{50} (μM)
12	-H	-H	-H	-H	11.557±0.525
13	-OCH$_3$	-H	-H	-H	3.573±0.123
14	-H	-OCH$_3$	-H	-H	120.237±6.217
15	-H	-OC$_2$H$_5$	-H	-H	125.669±7.220
16	-H	-CH$_3$	-H	-H	2.809±0.018
17	-H	-F	-H	-H	4.028±0.326
18	-H	-Cl	-H	-H	3.515±0.128
19	-H	-Br	-H	-H	1.777±0.010
20	-H	-NO$_2$	-H	-H	12.333±0.239
21	-H	-Br	-OCH$_3$	-H	3.494±0.027
22	-H	-Br	-Cl	-H	0.499±0.025

when it was moved to the R_2 or R_3 position. When a methoxy group was substituted at the R_1 position, algicidal activity was almost similar to when the hydroxy group was present. When the methoxy group was moved from the R_1 to the R_2 position, algicidal activity decreased (compounds 13 and 14). However, when a methyl group was introduced to R_2, the TD derivative presented an LC_{50} of 2.809 μM (compound 16), which was about four times higher than when hydrogen was present in that position. When halogen element (F, Cl, and Br) groups were introduced to the R_2 position, algicidal activities increased approximately 2.9 to 6.5 times compared with non-halogen substituent groups. Among the halogen groups, bromo group substitution at the R_2 position generated the highest algicidal activity with an LC_{50} value of 1.777 μM (compound 19). Furthermore, when bromine at the R_2 position and chlorine at the R_3 position were substituted simultaneously, an LC_{50} value of 0.499 μM was measured (compound 22), which was about 3.5- and 7.0-fold higher than that of bromo and chlorine group substitution alone, respectively. However, an LC_{50} of 3.494 μM was detected for a TD derivative with a bromo substitution at the R_2 position and a methoxy group at the R_3 position. These results suggest that algicidal activity was strongly affected by the presence of halogen elements in the structures of TD derivatives.

Table 4 shows a comparison of the effects on algicidal activity upon introducing alkoxyl, halogen, and nitro groups in TD derivatives without a hydroxyl substituent group. To examine the effects of an alkoxy group on algicidal activity, the effects of introducing methoxy, dimethoxy, and trimethoxy groups at the R_2, R_3, and R_4 positions were investigated. When methoxy groups were simultaneously substituted at the R_2, R_3, and R_4 positions, an LC_{50} of 118.556 μM was observed (compound 25). However, when a methoxy group was substituted at the R_2 position alone or at both the R_2 and R_4 positions, LC_{50} values of 1.697 and 2.535 μM, respectively, were determined (compound 23 and 24). When a trimethoxy group was substituted at the R_3 position, an LC_{50} of 3.534 μM was measured (compound 28), which was about 62.31% lower than when a methoxy group was present at that location. These results show that algicidal activity was strongly affected by carbon chain length and the number of alkoxy groups. To research the effects of halogen substituent groups on algicidal activity, TD derivatives with fluorine, chlorine, bromo, and iodine substitutions were investigated. When fluorine was substituted at R_3, an LC_{50} of 0.789 μM was obtained (compound 32). The substitution of fluorine at both R_3 and R_4 resulted in a derivative with an LC_{50} of 0.892 μM. When chlorine was substituted at R_2, an LC_{50} of 0.515 μM was obtained. However, substitution of chlorine at both R_2 and R_3 resulted in an increase in LC_{50} to 0.430 μM. Bromo substitution position was found to be important for the algicidal activity of TD derivatives. When bromo was substituted at R_2, an LC_{50} of 0.335 μM was obtained (compound 41). Conversely, iodine substitution at R_3 resulted in an LC_{50} value of 15.665 μM (compound 42). However, substitution of fluorine at R_1 or methoxy group at R_3 position increased algicidal activity in TD derivatives containing iodine substituents compared to compound 42. When a nitro group was introduced at R_3 as an electron withdrawing group, an LC_{50} value of 1.801 μM was detected. Conversely, when a dimethylamine group was introduced at R_3 as an electron donating group, the LC_{50} de-

Table 4. Algicidal effects of compounds 23-64
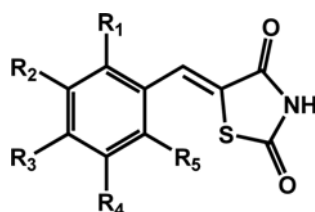
Compound no.	R_1	R_2	R_3	R_4	R_5	LC_{50} (μM)
23	-H	OCH$_3$	-H	-H	-H	1.697±0.036
24	-H	-OCH$_3$	-H	-OCH$_3$	-H	2.535±0.062
25	-H	-OCH$_3$	-OCH$_3$	-OCH$_3$	-H	118.556±61.25
26	-H	-H	-OCH$_3$	-H	-H	1.332±0.012
27	-H	-H	-OC$_2$H$_5$	-H	-H	2.211±0.052
28	-H	-H	-OC$_3$H$_8$	-H	-H	3.534±0.041
29	-H	-H	-CH$_3$	-H	-H	2.594±0.124
30	-H	-H	-F	-H	-H	12.447±0.632
31	-H	-H	-H	-F	-H	1.372±0.026
32	-H	-H	-H	-H	-F	0.789±0.032
33	-H	-H	-F	-F	-H	0.892±0.043
34	-F	-H	-F	-H	-H	1.565±0.525
35	-F	-H	-H	-H	-F	2.498±0.127
36	-H	-Cl	-H	-H	-H	0.515±0.268
37	-H	-H	-Cl	-H	-H	0.822±0.042
38	-H	-Cl	-Cl	-H	-H	0.430±0.024
39	-Br	-H	-H	-H	-H	1.031±0.045
40	-H	-Br	-H	-H	-H	0.335±0.017
41	-H	-H	-Br	-H	-H	0.764±0.035
42	-H	-H	-I	-H	-H	15.662±0.724
43	-H	-H	-NO$_2$	-H	-H	1.801±0.097
44	-H	-H	-N(CH$_3$)$_2$	-H	-H	118.651±5.926
45	-H	-H	-F	-H	-Cl	0.562±0.025
46	-H	-H	-F	-CH$_3$	-H	1.282±0.098
47	-H	-H	-F	-H	-CH$_3$	0.975±0.045
48	-H	-H	-F	-OCH$_3$	-H	2.756±0.057
49	-F	-H	-Br	-H	-H	0.344±0.015
50	-F	-H	-H	-H	-Cl	1.607±0.012
51	-F	-H	-H	-I	-H	1.932±0.235
52	-H	-F	-Cl	-H	-H	0.248±0.088
53	-H	-F	-CH$_3$	-H	-H	0.943±0.078
54	-Br	-H	-OCH$_3$	-H	-H	0.351±0.045
55	-Br	-H	-H	-OCH$_3$	-H	1.647±0.097
56	-H	-Cl	-OCH$_3$	-H	-H	0.399±0.014
57	-H	-I	-OCH$_3$	-H	-H	0.82±0.042
58	-H	-OCH$_3$	-OCH$_3$	-Cl	-H	0.465±0.034
59	-H	-OCH$_3$	-OCH$_3$	-H	-Br	3.494±0.145
60	-H	-I	-OCH$_3$	-OCH$_3$	-H	0.376±0.028
61	-H	-I	-OCH$_3$	-H	-OCH$_3$	130.114±1.55
62	-Br	-H	-F	-OCH$_3$	-H	0.878±0.041
63	-F	-Cl	-H	-H	-F	0.480±0.031
64	-F	-H	Br	-H	-F	0.748±0.034

creased to 118.651 µM. These results suggest that electron withdrawing groups of thiazolidine-2,4-dione are essential to orient the molecule more favorably toward the binding sites of harmful algae. When fluorine was introduced in conjunction with other substituent groups, algicidal activity increased. In particular, introduction of fluorine at R_1 with bromo at R_3, as well as fluorine at R_2 with chloride at R_3, resulted in LC_{50} values of 0.344 µM (compound 49) and 0.248 µM (compound 52), respectively. When bromo was introduced at R_1 with a methoxy group, algicidal activity increased. In particular, introduction of bromo at R_1 and a methoxy group at R_4 increased the IC_{50} value about 4.7-fold compared to the TD derivatives with bromine at R_1 and a methoxy group at R_3. In addition, when a methoxy group was introduced with an iodine group at the R_2 position, algicidal activity increased. Furthermore, algicidal activity significantly increased when the methoxy group position was changed from R_5 to R_4 in the presence of an iodine group at R_2. These results indicate that algicidal activity was affected by methoxy group position in the presence of a halogen substituent group. Therefore, these compounds may have structural competence to target *M. aeruginosa*, but the mechanisms of this selectivity need to be examined further.

Compounds 2, 22, 38, 40, 49, 52, 54, 56, 58, 60, and 63 were the most potent against *M. aeruginosa* with IC_{50} values <0.5 µM. To verify the ecosystem introduction feasibility of these eleven compounds, an acute ecotoxicity test using *D. rerio* was carried out over ten days. The results are shown in Table 5. Among compounds tested, the lowest ecotoxicities obtained were EC_{50} values of 13.59 and 8.59 µM for compounds 2 and 22, respectively. Overall, the methoxy and hydroxy substituent derivatives exhibited better acute ecotoxicity against *D. rerio* than halogen substituent derivatives.

To evaluate the concentration-dependent acute ecotoxic effects of compound 2 and 22 on *Danio rerio*, cultures were treated with compound 22 (1.0 or 3.0 µM) or compound 2 (10 and 15 µM) for 10 days. The results are shown in Fig. 1. When 1 µM of compound 22 was used, the mortality rate was 10% after one day of culture. However, mortality was 100% after one day of culture at 3 µM. When compound 2 was used, no mortality was observed at 10 µM. However, when 15 µM was used, the mortality rate was 70% after two days of culture. These results show that compound 2
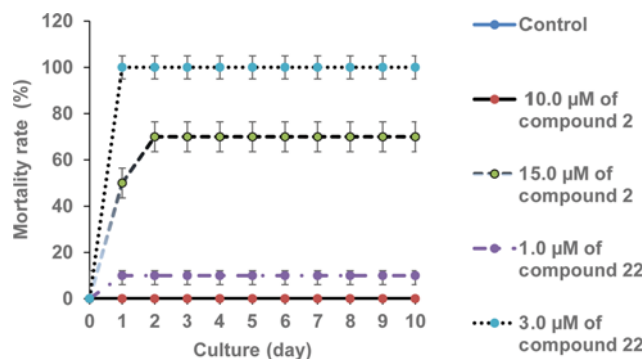

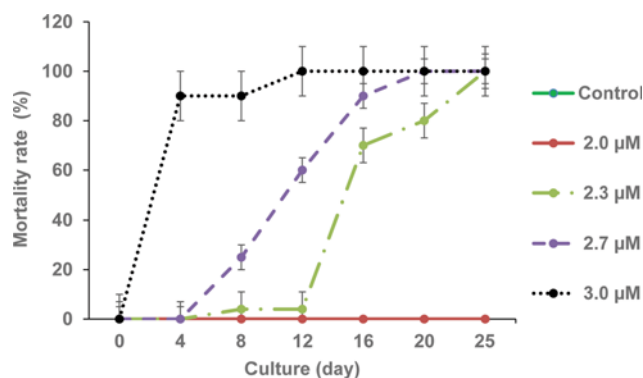Fig. 1. Acute ecotoxic effect of compounds 2 and 22 on *Danio rerio*.

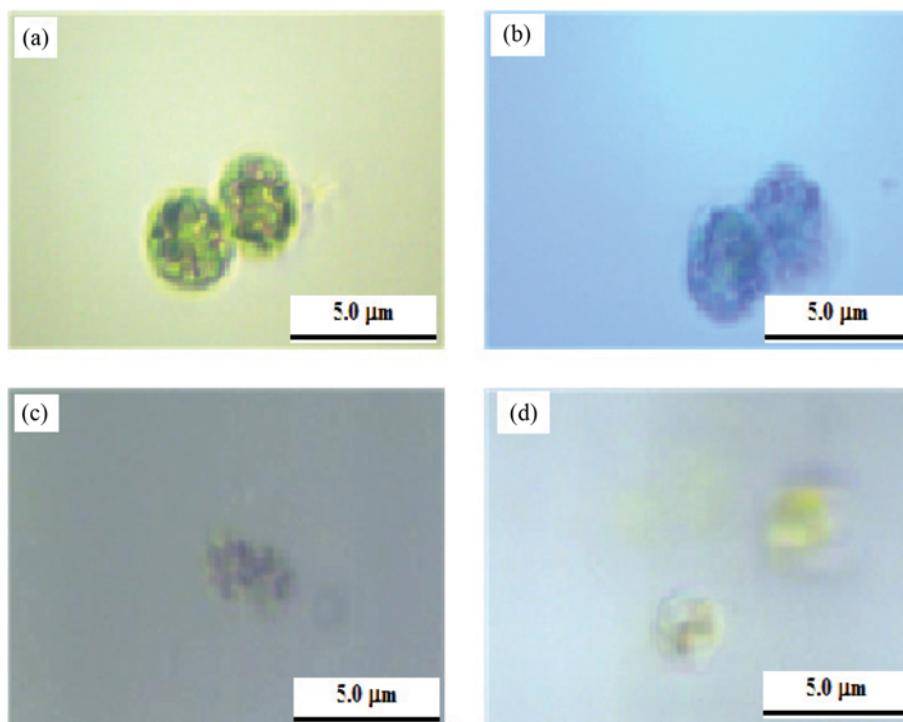Fig. 2. Acute ecotoxic effect of compound 2 on *Daphnia magna*.

should be selected as the final candidate as an ecofriendly algicide.

To evaluate the acute ecotoxic effect of compound 2 against *Daphnia magna*, 2.0, 2.3, 2.7, and 3.0 µM of compound 2 were used for 25 days. The results are shown in Fig. 2. When 2 µM was used, 100% survival rate was observed at 25 days. When *Daphnia magna* was treated with 2.3 µM of compound 2, 4% mortality was observed after 8 days and 70% mortality was shown after 17 days of culture. The EC_{50} value for 25 days was 2.15 µM. TD derivative compounds generally showed higher algicidal efficiency when exchange reagents were applied. However, compounds with halogen substituents showed high toxicity to both the zebrafish and the water flea relative to the compounds with the hydroxyl and alkoxy substituents. Therefore, compound 2, which showed both outstanding algicidal capability and much lower toxicity than compound 22, was ultimately selected.

To monitor the algicidal processes of compound 2 (1.0 µM) against red tide-forming algae, optical microscopy observations were performed with an Olympus camera at a magnification of 400× (Fig. 3). *M. aeruginosa* was cultured, and compound 2 was added to the cultures. *M. aeruginosa* cells were affected by 1 µM of compound 2. After addition of 1 µM compound 2, *M. aeruginosa* cells began to change in size starting at day three. After five days, cellular components began to leak and cells were lysed. However, no cell mortality was observed in *Anabaena flosaquae* or *Aphanizomenon flosaquae* (data not shown). Therefore, compound 2 developed in this study is judged to be an environmentally friendly algicide, which can effectively and selectively control *M. aeruginosa*.

Table 5. Acute ecotoxicity effects of various synthetic thiazolinedione compounds on *Danio rerio*

Compound no.	EC_{50} (µM)
2	13.59±0.623
22	8.59±0.327
38	2.89±0.125
40	0.48±0.0119
49	0.40±0.013
52	0.45±0.015
54	1.65±0.095
56	2.00±0.035
58	0.88±0.038
60	0.32±0.011
63	0.62±0.031

Fig. 3. Morphology change of *Microcystis aeruginosa* after addition of compound 2 (1 μM).
(a) 2 days, (b) 3 days, (c) 4 days, (d) 5 days

CONCLUSIONS

The development of HABs causes serious problems for public health and fishing industries worldwide. Therefore, we sought to synthesize an environmentally friendly algicide that is highly effective in eliminating algae and yet not toxic to other species. To select the most active compound among the synthesized derivatives, algicidal effects against harmful algal species were evaluated, and ecotoxicology tests using *Daphnia magna* and *D. rerio* were conducted to assess biological stability. Most new derivatives are highly active against *M. aeruginosa*, with algicidal effects being strongly affected by substituent group position in 2,4-thiazolidinedione. Activity level by substituent group was in the order of hydroxyl>methoxy>fluorine>chlorine>bromo. Among alkoxyl groups, the methoxy group produced the highest activity. For the hydroxy group and fluorine, the maximum activity was obtained when the group was at the R_2 position. In the case of the methoxy group, chlorine, and bromo, the maximum activity was observed at the R_3 position. Furthermore, the ecotoxicology effects, as determined by effect on *Danio rerio*, was in order of bromo>fluorine>chlorine>hydroxy>methoxy group. When hydroxy and methoxy groups were introduced simultaneously with a halogen substituent group, the algicidal activity was decreased. However, the ecotoxicity was decreased over 2.0 times. When dimethylamine was introduced as an electron-donating group (compound 44), a higher algicidal activity was obtained relative to when an electron-withdrawing group was introduced. Compound 2 was observed to exert algicidal effects starting from three days of culture. After 5 days, the algal bloom culture was almost eliminated. Compound 2 also demonstrated low ecotoxicity, with an EC_{50} value of 13.59 μM. Therefore, compound 2, which showed outstanding algicidal capability and yet much lower toxicity than compound 22, was finally selected. Compound 2, developed in this study, is therefore judged to be an environmentally friendly algicide that is less toxic to the ecosystem, which can effectively and selectively control *M. aeruginosa*. Currently, we are making efforts toward the elucidation of the modes of action of 2,4-thiazolidinedione derivatives, their stabilities, and their biodegradation properties.

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