Biotechnology and Bioprocess Engineering 21: 463-476 (2016)
DOI 10.1007/s12257-016-0175-8

RESEARCH PAPER

Synthesis and Algicidal Activity of New Dichlorobenzylamine Derivatives against Harmful Red Tides

Dubok Choi, Sunjong Yu, Seung Ho Baek, Yoon-Ho Kang, Young-Cheol Chang, and Hoon Cho

Received: 18 February 2016 / Revised: 26 April 2016 / Accepted: 22 May 2016

Abstract In the present study, we synthesized 65 dichlorobenzylamine derivatives and investigated their algicidal activity against harmful red tides. The 3,4-dichlorobenzylamine derivatives showed relatively high activity against *Cochlodinium polykrikoides, Heterosigma akashiwo, Chattonella marina*, and *Heterocapsa circularisquama*, and the synthesized compounds 27, 28, 33, 34, 35, and 36 showed the highest algicidal activity after 24 h at 0.1 ~ 1.0 μM LC_{50} against the four harmful algae species. To verify the safety of the compounds, acute ecotoxicology tests using the water flea (*Daphnia magna*) and zebrafish (*Danio rerio*) were conducted, and the tests confirmed that compounds 33 and 34 were not harmful because the target organisms showed high survival rates at 15 μM. The results indicate that compounds 33 and 34 are suitable substances for use in controlling harmful algae species.

Keywords: harmful algae blooms, algicides, red tide, dichlorobenzylamine, ecotoxicology

Dubok Choi
Biotechnology Laboratory, B-K Company Ltd, Jeonbuk 573-879, Korea

Sunjong Yu, Hoon Cho*
Department of Polymer Science & Engineering, Chosun University, Gwangju 501-759, Korea
Tel: +82-62-230-7635; Fax: +82-62-230-2474
E-mail: hcho@chosun.ac.kr

Seung Ho Baek
South Sea Institute, Korea Institute of Ocean Science and Technology (KIOST), Geoje 656-830, Korea

Yoon-Ho Kang
Monitoring and Analysis Division, Wonju Regional Environmental Office, Wonju 220-947, Korea

Young-Cheol Chang
Biosystem Course, Division of Applied Sciences, Muroran Institute of Technology, 27-1 Mizumoto 050-8585, Japan

1. Introduction

Harmful algal blooms (HABs), known as red tides, affect marine coastal ecosystems around the world and are caused by microalgae and cyanobacteria [1]. To control HABs, many scientists have conducted physiological and ecological studies with the aim of reducing the damage caused by HABs to fisheries [2-4]. The application of clay can treat the environmental problems, but the toxins released from flocculated cells and the adverse effects on other organisms need to be considered [5]. Clay flocculants are effective in the treatment of *Cochlodinium*, which causes fish deaths in finfish cage culture in coastal Japan [6,7] and in Chinese mariculture ponds [8]. Yellow loess is effective in the sedimentation of dinoflagellates [9,10]. However, ruptured or damaged cells may release intracellular toxins into the surrounding water, which requires the use of expensive removal processes, such as activated carbon and/or oxidative ozone and chlorine [11]. Mechanical and physico-chemical methods have been devised in an attempt to manage HABs with limited success [12-14]. The application of chemicals is one of the most common methods of controlling the development of noxious phytoplankton, but their use has limitations, such as toxicity toward non-target species [15-17]. Some chemicals have been used to mitigate HABs, but safer and more selective algicidal agents are needed to better control harmful algal blooms. Copper sulfate, chelated copper compounds, and diuron are some of the chemicals currently approved by the U.S. Environmental Protection Agency [18]. The most direct control method involves the use of chemical treatments, such as algicides, including copper, reglone, anthraquinone, potassium permanganate, chlorine, simazine, and clotrimazole [19-21]. Unfortunately, these compounds have undesirable characteristics, including broad-spectrum toxicity towards

phytoplankton, subsequent water quality deterioration, and lengthy persistence that creates environmental safety concerns [19,22]. Natural antialgal compounds extracted from a range of bioresources have also been reported. These include furano-diterpenes [23] at low biosurfactant concentrations [24], alleochemicals [25], and barley straw. Other studies [25-29] have also attempted to manage red tide growth using controlling agents.

Previously, we reported the synthesis of various thiazolidinedione (TD) derivatives along with their algicidal activity against microalgae that cause harmful algal blooming. Among the various compounds tested, some TD derivatives showed effective algicidal activity against *Heterosigma akashiwo*, *Chattonella marina*, and *Cochlodinium polykrikoides*, while non-harmful algae were relatively tolerant of them [30]. Studies have also been conducted on the design of a liposomal delivery system for TD53, in order to improve its delivery properties, and on the evaluation of its algicidal effects as well as selectivity toward harmful algae and non-harmful algae *via* the liposomal delivery system [31]. In addition, we examined the acute toxicity to the marine ecosystem of two new algicides, thiazolidinedione derivatives TD49 and TD53, which were synthesized to control red tide selectively [32]. Acute toxicity assessments of the new algicides TD49 and TD53 were performed using the new International Organization for Standardization (ISO) standard method with *Ulva pertusa* and three representative species: an alga, a crustacean, and a fish. The toxicity levels assessed were the 50% effective concentration (EC_{50}), the 50% lethal concentration (LC_{50}), the no-observed-effect concentration (NOEC), and the predicted-no-effect concentration (PNEC) [33]. We have also investigated algicidal activity of thiazolidinedione derivatives against the harmful algal species *H. akashiwo, C. marina*, and *C. polykrikoides* and the non-harmful species *Amphidinium* sp., *Navicula pelliculosa, Nannochloropsis oculata*, and *Phaeodactylum* EPV [34]. A series of naphthoquinone-benzothiazole conjugates were also synthesized as algicides, and their efficacies against harmful algal blooming species, such as *C. marina*, *H. akashiwo*, and *C. polykrikoides*, were examined. The introduction of substituted benzothiazole at the C2 position of 1,4-naphthoquinone resulted in higher algicidal activity against *C. polykrikoides* than the C6 conjugates [35]. In addition, novel TD derivatives have been found to have algicidal effects on harmful algal bloom microalgae. Seventy-five TD derivatives were synthesized and analyzed for algicidal activity. Among these synthetic TDs, compound 17 showed specific algicidal activity on two strains belonging to Raphidophyceae and one strain belonging to Dinophyceae. The most reactive TD derivative, TD118, was selected and tested for morphological and physiological changes. These results imply that the species-specificity-TD structure relation might be due to the structural and/or physiological differences among microalgal species [36]. Even though various algicidal compounds were researched, there is no report about dichlorobenzylamine derivatives against harmful red tides. Therefore, new substances for removing harmful algae species must be developed.

In this study, in order to develop new algicidal compounds for harmful algal bloom control, the molecules were synthesized with the introduction of various substituent groups to benzylamine and benzamide derivatives and their algicidal activities against harmful red tides were examined at various concentrations. In addition, to verify the safety of compounds, acute ecotoxicology tests using the water flea (*Daphnia magna*) and zebrafish (*Danio rerio*) were conducted.

2. Materials and Methods

2.1. General procedure for the synthesis of compounds 1-56

2.1.1. (3,4-Dichlorobenzyl)ethylamine (1)

Ethylamine (0.257 g, 5.714 mmol) was added slowly to 3,4-dichlorobenzaldehyde (1.0 g, 5.714 mmol) dissolved in 10 mL of methanol. The mixture was then stirred for 1 h at room temperature, and sodium borohydride 0.32 g (8.45 mmol) was added slowly to the mixture until the starting materials (using thin layer chromatography (TLC) analysis) began to disappear. The resulting solution was extracted from the mixture using 40 mL of water and 90 mL of methylene chloride and dehydrated using anhydrous $MgSO_4$. The resulting solution was concentrated under reduced pressure and purified using column chromatography over silica gel (elution with n-hexane/ethyl acetate, 20:1) to produce (3,4-dichlorobenzyl)ethylamine (compound **1**): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, J = 2.1 Hz, 1H), δ 7.38 (d, J = 8.4 Hz, 1H), δ 7.17 (dd, J = 8.4 and 2.1 Hz, 1H), δ 3.74 (s, 2H), δ 2.69 (m, J = 6.9, 2H), δ 1.28 (b, 1H), δ 1.15 (t, J = 6.9 Hz, 3H).

2.1.2. (3,4-Dichlorobenzyl)propylamine (2)

Compound **2** was prepared as a colorless liquid in a manner similar to that described for **1** (94.3% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (d, J = 1.8 Hz, 1H), δ 7.39 (d, J = 8.4 Hz, 1H), δ 7.17 (dd, J = 8.4 and 1.8 Hz, 1H), δ 3.73 (s, 2H), δ 2.58 (t, J = 7.3 Hz, 2H), δ 1.58 (m, J = 7.3 Hz, 2H), δ 1.42 (b, 1H), δ 0.94 (t, J = 7.3 Hz, 3H).

2.1.3. Butyl-(3,4-dichlorobenzyl)amine (3)

Compound **3** was prepared as a colorless liquid in a manner

similar to that described for **1** (93.4% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, *J* = 2.1 Hz, 1H), δ 7.39 (d, *J* = 8.0 Hz, 1H), δ 7.17 (dd, *J* = 8.0 and 2.1 Hz, 1H), δ 3.74 (s, 2H), δ 2.62 (t, *J* = 6.9 and 7.3 Hz, 2H), δ 1.53 (m, *J* = 6.9 Hz, 4H), δ 1.25 (b, 1H), δ 0.93 (t, *J* = 7.32 Hz, 3H).

2.1.4. (3,4-Dichlorobenzyl)pentylamine (4)

Compound **4** was prepared as a colorless liquid in a manner similar to that described for **1** (68.2% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, *J* = 1.83 Hz, 1H), δ 7.39 (d, *J* = 8.43 Hz, 1H), δ 7.18 (dd, *J* = 1.83 and 8.43 Hz, 1H), δ 3.74 (s, 2H), δ 2.61 (t, *J* = 7.32 Hz, 2H), δ 1.55 (m, *J* = 6.96 Hz, 2H), δ 1.32 (m, 4H), δ 0.91 (t, *J* = 6.96 Hz, 3H).

2.1.5. (3,4-Dichlorobenzyl)hexylamine (5)

Compound **5** was prepared as a colorless liquid in a manner similar to that described for **1** (88% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, *J* = 2.19 Hz, 1H), δ 7.39 (d, *J* = 8.43 Hz, 1H), δ 7.17 (dd, *J* = 8.43 and 2.19 Hz, 1H), δ 3.74 (s, 2H), δ 2.61 (t, *J* = 7.32 and 6.96 Hz, 2H), δ 1.51 (m, 8H), δ 0.90 (t, *J* = 6.96 Hz, 3H).

2.1.6. (3,4-Dichlorobenzyl)pentylamine (6)

Compound **6** was prepared as a colorless liquid in a manner similar to that described for **1** (83% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, *J* = 2.22 Hz, 1H), δ 7.39 (d, *J* = 8.43 Hz, 1H), δ 7.17 (dd, *J* = 8.43 and 2.22 Hz, 1H), δ 3.73 (s, 2H), δ 2.61 (t, *J* = 6.96 and 7.32 Hz, 2H), δ 1.51 (m, 10H), δ 0.90 (t, *J* = 6.96 Hz, 3H).

2.1.7. (3,4-Dichlorobenzyl)isobutylamine (7)

Compound **7** was prepared as a colorless liquid in a manner similar to that described for **1** (90% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ (d, *J* = 1.83 Hz, 1H), δ 7.39 (d, *J* = 8.07 Hz, 1H), δ 7.18 (dd, *J* = 1.83 and 8.07 Hz, 1H), δ (d, *J* = Hz, 1H), δ 3.78 (s, 2H), δ 2.41 (d, *J* = 6.96 Hz, 2H), δ 1.79 (m, 1H), δ 0.92 (d, *J* = 6.6 Hz, 6H).

2.1.8. sec-Butyl-(3,4-dichlorobenzyl)amine (8)

Compound **8** was prepared as a colorless liquid in a manner similar to that described for **1** (91% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, *J* = 1.47 Hz, 1H), δ 7.38 (d, *J* = 8.07 Hz, 1H), δ 7.18 (dd, *J* = 8.07 and 1.47 Hz, 1H), δ 3.79 (m, *J* = 13.56, 10.23 Hz, 2H), δ 2.63 (m, *J* = 6.24 Hz, 1H), δ 1.57 (m, 1H), δ 1.06 (d, 3H), δ 0.92 (t, 3H).

2.1.9. 3,4-Dichlorobenzyl-(3-methylbutyl)amine (9)

Compound **9** was prepared as a colorless liquid in a manner similar to that described for **1** (94% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, *J* = 2.19 Hz, 1H), δ 7.38 (d, *J* = 8.0 Hz, 1H), δ 7.17 (dd, *J* = 8.0 and 2.19 Hz, 1H), δ 3.74 (s, 2H), δ 2.63 (t, 2H), δ 1.70 (m, *J* = 6.9 Hz, 1H), δ 1.42 (m, *J* = 6.9 Hz, 2H), δ 1.28 (b, 1H), δ 0.90 (d, 6H).

2.1.10. 3,4-Dichlorobenzyl-(2-methoxyethyl)amine (10)

Compound **10** was prepared as a colorless liquid in a manner similar to that described for **1** (92.1% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (d, *J* = 1.8 Hz, 1H), δ 7.39 (d, *J* = 8.0 Hz, 1H), δ 7.18 (dd, *J* = 8.0 and 1.8 Hz, 1H), δ 3.76 (s, 2H), δ 3.52 (t, *J* = 5.1 Hz, 2H), δ 3.35 (s, 3H), δ 2.79 (t, *J* = 5.3 Hz, 2H).

2.1.11. 3,4-Dichlorobenzyl-(3-methoxypropyl)-mine (11)

Compound **11** was prepared as a colorless liquid in a manner similar to that described for **1** (94% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, *J* = 1.83 Hz, 1H), δ 7.39 (d, *J* = 8.04 Hz, 1H), δ 7.17 (dd, *J* = 8.04 and 1.83 Hz, 1H), δ 3.74 (s, 2H), δ 3.47 (t, *J* = 6.21 Hz, 2H), δ 3.33 (s 3H), δ 2.71 (t, *J* = 6.21 Hz, 2H), δ 1.81 (m, *J* = 6.21, 2H).

2.1.12. 3,4-Dichlorobenzyl-(2,2-dimethoxyethyl)amine (12)

Compound **12** was prepared as a colorless liquid in a manner similar to that described for **1** (88.3% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, *J* = 1.8 Hz, 1H), δ 7.39 (d, *J* = 8.0 Hz, 1H), δ 7.17 (dd, *J* = 8.0 and 1.8 Hz, 1H), δ 4.48 (t, *J* = 5.5 Hz, 1H), δ 3.76 (s, 2H), δ 3.38 (s, 6H), δ 2.73 (d, *J* = 5.5 Hz, 2H).

2.1.13. 3,4-Dichlorobenzyl-(4,4-dimethoxybutyl)amine (13)

Compound **13** was prepared as a colorless liquid in a manner similar to that described for **1** (87.5% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, *J* = 1.8 Hz, 1H), δ 7.39 (d, *J* = 8.0 Hz, 1H), δ 7.17 (dd, *J* = 8.0 and 1.8 Hz, 1H), δ 4.38 (t, *J* = 5.49 Hz, 1H), δ 3.74 (s, 2H), δ 3.31 (s, 6H), δ 2.64 (t, 2H), δ 1.69 (m, 4H).

2.1.14. N'-(3,4-Dichlorobenzyl)-N,N-dimethylethane-1,2-diamine (14)

Compound **14** was prepared as a colorless liquid in a manner similar to that described for **1** (93.2% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, *J* = 2.2 Hz, 1H), δ 7.39 (d, *J* = 8.4 Hz, 1H), δ 7.18 (dd, *J* = 8.4 and 2.2 Hz, 1H), δ 3.76 (s, 2H), δ 2.68 (m, *J* = 5.8 Hz, 2H), δ 2.45 (t, *J* = 5.8 Hz, 2H), δ 2.21 (s, 6H), δ 2.12 (s, 1H).

2.1.15. N'-(3,4-Dichlorobenzyl)-N,N-diethylethane-1,2-diamine (15)

Compound **15** was prepared as a colorless liquid in a manner similar to that described for **1** (89.5% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, *J* = 1.8 Hz, 1H), δ 7.39 (d, *J* = 8.0 Hz, 1H), δ 7.18 (dd, *J* = 8.0 and 1.8 Hz, 1H), δ 3.76 (s, 1H), δ 2.68 (m, 8H), δ 1.06 (t, *J* = 6.9 Hz, 6H).

2.1.16. N'-(3,4-Dichlorobenzyl)-N,N-dimethylpropane-1,3-diamine (16)

Compound **16** was prepared as a colorless liquid in a manner similar to that described for **1** (89.9% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, J = 1.8 Hz, 1H), δ 7.39 (d, J = 8.4 Hz, 1H), δ 7.17 (dd, J = 8.4 and 1.8 Hz, 1H), δ 3.74 (s, 2H), δ 2.66 (t, J = 6.9 Hz, 2H), δ 2.34 (t, J = 6.9 Hz, 2H), δ 2.22 (s, 6H), δ 1.72 (m, J = 6.9 Hz, 3H).

2.1.17. N'-(3,4-Dichlorobenzyl)-N,N-diethylpropane-1,3-diamine (17)

Compound **17** was prepared as a colorless liquid in a manner similar to that described for **1** (90.7% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, J = 1.8 Hz, 1H), δ 7.39 (d, J = 8.0 Hz, 1H), δ 7.17 (dd, J = 8.0 and 1.8 Hz, 1H), δ 3.73 (s, 2H), δ 2.66 (t, J = 6.5 Hz, 2H), δ 2.57 (m, 6H), δ 1.72 (m, J = 6.5 Hz, 1H), δ 1.05 (t, J = 7.3 Hz, 6H).

2.1.18. (3,4-Dichlorobenzyl)phenylamine (18)

Compound **18** was prepared as a colorless liquid in a manner similar to that described for **1** (87% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.45 (d, J = 1.8 Hz, 1H), δ 7.39 (d, J = 8.0 Hz, 1H), δ 7.2 (m, 3H), δ 6.76 (tt, J = 7.3 and 1.0 Hz, 1H), δ 6.59 (dt, J = 7.3 and 1.0 Hz, 2H), δ 4.29 (s, 2H), δ 4.10 (b, 1H).

2.1.19. 3,4-Dichlorobenzyl-(3,4,5-trimethoxyphenyl)amine (19)

Compound **19** was prepared as a colorless liquid in a manner similar to that described for **1** (82.8% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.48 (d, J = 1.8 Hz, 1H), δ 7.42 (d, J = 8.4 Hz, 1H), δ 7.23 (dd, J = 8.4 and 1.8 Hz, 1H), δ 5.83 (s, 2H), δ 4.27 (s, 2H), δ 3.77 (d, 9H).

2.1.20. 4-Chlorophenyl-(3,4-dichlorobenzyl)amine (20)

Compound **20** was prepared as a colorless liquid in a manner similar to that described for **1** (89% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, J = 1.8 Hz, 1H), δ 7.41 (d, J = 8.0 Hz, 1H), δ 7.19 (dd, J = 8.0 and 1.8 Hz, 1H), δ 7.13 (dt, J = 9.8 and 2.2 Hz, 2H), δ 6.52 (dt, J = 9.8 and 2.2 Hz, 2H), δ 4.28 (s, 2H), δ 4.14 (b, 1H).

2.1.21. 4-Bromophenyl-(3,4-dichlorobenzyl)amine (21)

Compound **21** was prepared as a colorless liquid in a manner similar to that described for **1** (30.5% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, J = 1.8 Hz, 1H), δ 7.41 (d, J = 8.0 Hz, 1H), δ 7.26 (tt, 2H), δ 7.19 (dd, J = 8.0 and 1.8 Hz, 1H), δ 6.48 (tt, 2H), δ 4.29 (d, 2H), δ 4.16 (b, 1H).

2.1.22. 3,4-Dichlorobenzyl-(4-fluorophenyl)amine (22)

Compound **22** was prepared as a colorless liquid in a manner similar to that described for **1** (86% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (d, J = 1.8 Hz, 1H), δ 7.41 (d, J = 8.0 Hz, 1H), δ 7.21 (dd, J = 8.0 and 1.8 Hz 1H), δ 6.91 (m, 2H), δ 6.53 (m, 2H), δ 4.26 (s, 2H), δ 4.01 (b, 1H).

2.1.23. Benzyl-(3,4-dichlorobenzyl)amine (23)

Compound **23** was prepared as a colorless liquid in a manner similar to that described for **1** (89.1% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.47 (d, J = 2.2 Hz, 1H), δ 8.74 (d, J = 8.0 Hz, 1H), δ 8.73 (m, 5H), δ 7.20 (dd, J = 8.0 and 1.8 Hz, 1H), δ 3.79 (s, 2H), δ 3.76 (s, 2H), δ 1.60 (b, 1H).

2.1.24. (3,4-Dichlorobenzyl)pyridin-2-ylmethylamine (24)

Compound **24** was prepared as a colorless liquid in a manner similar to that described for **1** (73.3% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, 1H), δ 7.68 (td, J = 7.6 and 1.8 Hz, 1H), δ 7.48 (d, J = 1.8 Hz, 1H), δ 7.40 (d, J = 8.0 Hz, 1H), δ 7.30 (d, J = 8.0 Hz, 1H), δ 7.21 (m, 2H), δ 3.90 (s, 2H), δ 3.79 (s, 2H), δ 2.16 (b, 1H).

2.1.25. (3,4-Dichlorobenzyl)pyridin-3-ylmethylamine (25)

Compound **25** was prepared as a colorless liquid in a manner similar to that described for **1** (69.8% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (d, J = 1.8 Hz, 1H), δ 8.53 (dd, J = 4.7 and 1.8 Hz, 1H), δ 7.72 (d, J = 7.7 Hz, 1H), δ 7.47 (d, J = 1.8 Hz, 1H), δ 7.41 (d, J = 8.43 Hz, 1H), δ 7.30 (m, 1H), δ 7.20 (dd, J = 8.4 and 1.8 Hz, 1H), δ 3.80 (s, 2H), δ 3.77 (s, 2H), δ 2.04 (b, 1H).

2.1.26. (3,4-Dichlorobenzyl)pyridin-4-ylmethylamine (26)

Compound **26** was prepared as a colorless liquid in a manner similar to that described for **1** (72% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.57 (dd, 2H), δ 7.47 (d, J = 1.83 Hz, 1H), δ 7.42 (d, J = 8.0 Hz, 1H), δ 7.29 (dd, 2H), δ 7.20 (dd, J = 8.0 and 1.83 Hz, 1H), δ 3.81 (s, 2H), δ 3.76 (s, 2H), δ 1.75 (b, 1H).

2.1.27. (3,4-Dichlorobenzyl)phenethylamine (27)

Compound **27** was prepared as a colorless liquid in a manner similar to that described for **1** (87.4% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.38 (m, 7H), δ 7.12 (dd, J = 8.4 and 1.8 Hz, 1H), δ 3.74 (s, 2H), δ 2.89 (m, 4H).

2.1.28. 3,4-Dichlorobenzyl-(2-pyridin-2-yl-ethyl)amine (28)

Compound **28** was prepared as a colorless liquid in a manner similar to that described for **1** (37.3% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (d, 1H), δ 7.64 (td, J = 7.6 and 1.8 Hz, 1H), δ 7.41 (d, J = 1.8 Hz 1H), δ 7.37 (d, J = 8.0 Hz, 1H), δ 7.18 (m, 3H), δ 3.78 (s, 2H), δ 3.01 (m, 4H).

2.1.29. 3,4-Dichlorobenzyl-(2-pyridin-3-yl-ethyl)amine (29)

Compound **29** was prepared as a colorless liquid in a

manner similar to that described for **1** (62.2% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.48 (s, 2H), δ 7.54 (dt, 1H), δ 7.39 (m, 2H), δ 7.26 (m, 1H), δ 7.13 (dd, *J* = 8.4 and 2.1 Hz, 1H), δ 3.76 (s, 2H), δ 2.90 (m, 4H).

2.1.30. 3,4-Dichlorobenzyl-(2-pyridin-4-yl-ethyl)amine (30)
Compound **30** was prepared as a colorless liquid in a manner similar to that described for **1** (76.7% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (d, 2H), δ 7.39 (m, 2H), δ 7.15 (m, 3H), δ 3.76 (s, 2H), δ 2.91 (m, 2H), δ 2.83 (m, 2H), δ 2.37 (b, 1H).

2.1.31. Cyclopropyl-(3,4-dichlorobenzyl)amine (31)
Compound **31** was prepared as a colorless liquid in a manner similar to that described for **1** (48.7% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (d, *J* = 2.2 Hz, 1H), δ 7.38 (d, *J* = 8.4 Hz, 1H), δ 7.15 (dd, *J* = 8.4 and 2.2 Hz, 1H), δ 3.78 (s, 2H), δ 2.14 (m, 1H), δ 0.46 (m, 4H).

2.1.32. Cyclobutyl-(3,4-dichlorobenzyl)amine (32)
Compound **32** was prepared as a colorless liquid in a manner similar to that described for **1** (45.7% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, *J* = 2.19 Hz, 1H), δ 7.38 (d, *J* = 8.4 Hz, 1H), δ 7.16 (dd, *J* = 8.4 and 2.19 Hz, 1H), δ 3.65 (s, 2H), δ 3.30 (m, 1H), δ 2.25 (m, 2H), δ 1.73 (m, 4H).

2.1.33. Cyclopentyl-(3,4-dichlorobenzyl)amine (33)
Compound **33** was prepared as a colorless liquid in a manner similar to that described for **1** (96% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, *J* = 1.8 Hz, 1H), δ 7.39 (d, *J* = 8.0 Hz, 1H), δ 7.17 (dd, *J* = 8.0 and 1.8 Hz, 1H), δ 3.72 (s, 2H), δ 3.12 (m, *J* = 6.5 Hz, 1H), δ 1.87 (m, 8H).

2.1.34. Cyclohexyl-(3,4-dichlorobenzyl)amine (34)
Compound **34** was prepared as a colorless liquid in a manner similar to that described for **1** (90.2% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, *J* = 1.8 Hz, 1H), δ 7.38 (d, *J* = 8.0 Hz, 1H), δ 7.17 (dd, *J* = 8.0 and 1.8 Hz, 1H), δ 3.76 (s, 2H), δ 2.47 (m, 1H), δ 1.91 (m, 10H).

2.1.35. Cycloheptyl-(3,4-dichlorobenzyl)amine (35)
Compound **35** was prepared as a colorless liquid in a manner similar to that described for **1** (64% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, *J* = 1.8 Hz, 1H), δ 7.38 (d, *J* = 8.4 Hz, 1H), δ 7.17 (dd, *J* = 8.4 and 1.8 Hz, 1H), δ 3.76 (s, 2H), δ 2.47 (m, 1H), δ 1.91 (m, 12H).

2.1.36. Cyclooctyl-(3,4-dichlorobenzyl)amine (36)
Compound **36** was prepared as a colorless liquid in a manner similar to that described for **1** (73.5% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 (d, *J* = 1.8 Hz, 1H), δ 7.38 (d, *J* = 8.0 Hz, 1H), δ 7.17 (dd, *J* = 8.0 and 1.8 Hz, 1H), δ 3.72 (s, 2H), δ 2.70 (m, 1H), δ 1.80 (m, 14H).

2.1.37. (3,4-Dichlorobenzyl)indan-1-yl-amine (37)
Compound **37** was prepared as a colorless liquid in a manner similar to that described for **1** (61.2% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.52 (d, *J* = 1.8 Hz, 1H), δ 7.39 (m, 2H), δ 7.25 (m, 4H), δ 4.28 (t, *J* = 6.5 Hz, 1H), δ 3.91 (m, 2H), δ 3.00 (m, 1H), δ 2.87 (m, 1H), δ 2.46 (m, 1H), δ 1.87 (m, 1H).

2.1.38. (3,4-Dichlorobenzyl)indan-2-yl-amine (38)
Compound **38** was prepared as a colorless liquid in a manner similar to that described for **1** (63.1% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.46 (d, *J* = 1.83 Hz, 1H), δ 7.39 (d, *J* = 8.07 Hz, 1H), δ 7.21 (m, 5H), δ 3.81 (s, 2H), δ 3.68 (m, 1H), δ 3.02 (m, 2H), δ 2.83 (m, 2H).

2.1.39. Benzylcyclopentylamine (39)
Compound **39** was prepared as a colorless liquid in a manner similar to that described for **1** (95.4% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 5H), δ 3.77 (s, 2H), δ 3.16 (m, *J* = 6.57 Hz, 1H), δ 1.86 (m, 8H).

2.1.40. Benzylcyclohexylamine (40)
Compound **40** was prepared as a colorless liquid in a manner similar to that described for **1** (61.8% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (m, 5H), δ 3.77 (s, 2H), δ 3.16 (m, 1H), δ 1.90 (m, 10H).

2.1.41. Benzylcycloheptylamine (41)
Compound **41** was prepared as a colorless liquid in a manner similar to that described for **1** (73.2% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (m, 5H), δ 3.77 (s, 2H), δ 2.69 (m, 1H), δ 1.89 (m, 12H).

2.1.42. Benzyl-(2-pyridin-2-yl-ethyl)amine (42)
Compound **42** was prepared as a colorless liquid in a manner similar to that described for **1** (87% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (d, 1H), δ 7.61 (td, *J* = 7.6 and 1.8 Hz, 1H), δ 7.31 (m, 7H), δ 3.83 (s, 2H), δ 3.08 (m, 4H).

2.1.43. (2-Chloro-benzyl)cyclohexylamine (43)
Compound **43** was prepared as a colorless liquid in a manner similar to that described for **1** (95% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.40 (m, 2H), δ 7.26 (m, 2H), δ 3.89 (s, 2H), δ 2.49 (m, 1H), δ 1.94 (m, 10H).

2.1.44. (3-Chloro-benzyl)cyclohexylamine (44)
Compound **44** was prepared as a colorless liquid in a manner similar to that described for **1** (95% yield): ^{1}H

NMR (300 MHz, $CDCl_3$) δ 7.33 (s, 1H), δ 7.27 (m, 3H), δ 3.78 (s, 2H), δ 2.49 (m, 1H), δ 1.93 (m, 10H).

2.1.45. (4-Chloro-benzyl)cyclohexylamine (45)

Compound **45** was prepared as a colorless liquid in a manner similar to that described for **1** (85.5% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27 (d, J = 2.94 Hz, 4H), δ 3.77 (s, 2H), δ 2.48 (m, 1H), δ 1.91 (m, 10H).

2.1.46. Cyclohexyl-(2,3-dichlorobenzyl)amine (46)

Compound **46** was prepared as a colorless liquid in a manner similar to that described for **1** (96.4% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37 (dd, J = 1.47 and 7.68 Hz, 1H), δ 7.34 (dd, J = 1.47 and 7.68 Hz, 1H), δ 7.19 (t, J = 7.68 Hz, 1H), δ 3.92 (s, 2H), δ 2.48 (m, 1H), δ 1.93 (m, 10H).

2.1.47. Cychlohexyl-(2,4-dichlorobenzyl)amine (47)

Compound **47** was prepared as a colorless liquid in a manner similar to that described for **1** (86.3% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36 (m, 2H), δ 7.22 (dd, J = 1.83 and 8.04 Hz, 1H), δ 3.85 (s, 2H), δ 2.47 (m, 1H), δ 1.92 (m, 10H).

2.1.48. Cychlohexyl-(2,5-dichlorobenzyl)amine (48)

Compound **48** was prepared as a colorless liquid in a manner similar to that described for **1** (81.5% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.44 (d, J = 2.58 Hz, 1H), δ 7.27 (d, J = 8.43 Hz, 1H), δ 7.17 (dd, J = 2.58 and 8.43, Hz, 1H), δ 3.85 (s, 2H), δ 2.50 (m, 1H), δ 1.94 (m, 10H).

2.1.49. Cychlohexyl-(2,6-dichlorobenzyl)amine (49)

Compound **49** was prepared as a colorless liquid in a manner similar to that described for **1** (84% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30 (d, J = 8.04 Hz, 2H), δ 7.14 (t, J = 8.04 Hz, 2H), δ 4.07 (s, 2H), δ 2.48 (m, 1H), δ 1.95 (m, 10H).

2.1.50. Cychlohexyl-(3,5-dichlorobenzyl)amine (50)

Compound **50** was prepared as a colorless liquid in a manner similar to that described for **1** (91.2% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.26 (s, 3H), δ 3.76 (s, 2H), δ 2.44 (m, 1H), δ 1.91 (m, 10H).

2.1.51. 2-Bromobenzyl-(2-pyridin-2-yl-ethyl)amine (51)

Compound **51** was prepared as a colorless liquid in a manner similar to that described for **1** (31.8% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (m, 1H), δ 7.62 (td, J = 7.6 and 1.8 Hz, 1H), δ 7.53 (dd, J = 8.0 and 1.1 Hz, 1H), δ 7.38 (dd, J = 7.6 and 1.8 Hz, 1H), δ 7.29 (td, 1H), δ 7.1 (d, J = 7.6 Hz, 1H), δ 7.14 (m, 2H), δ 3.90 (s, 2H), δ 3.07 (m, 4H), δ 2.08 (b, 1H).

2.1.52. 3-Bromobenzyl-(2-pyridin-2-yl-ethyl)amine (52)

Compound **52** was prepared as a colorless liquid in a manner similar to that described for **1** (40.5% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.54 (d, J = 4.3 Hz, 1H), δ 7.63 (td, J = 7.7 and 1.8 Hz, 1H), δ 7.46 (s, 1H), δ 7.37 (d, J = 7.7 Hz, 1H), δ 7.23 (m, 4H), δ 3.79 (s, 2H), δ 3.06 (m, 4H).

2.1.53. 4-Bromobenzyl-(2-pyridin-2-yl-ethyl)amine (53)

Compound **53** was prepared as a colorless liquid in a manner similar to that described for **1** (68.3% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (d, J = 4.7 Hz, 1H), δ 7.62 (td, J = 7.7 and 1.8 Hz, 1H), δ 7.43 (d, 2H), δ 7.19 (m, 4H), δ 3.78 (s, 2H), δ 3.06 (m, 4H).

2.1.54. 2-Cychlohexylaminomethylphenol (54)

Compound **54** was prepared as a colorless liquid in a manner similar to that described for **1** (78.9% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.18 (td, J = 7.32 and 1.47 Hz, 1H), δ 6.98 (d, J = 7.32 Hz, 1H), δ 6.83 (m, 2H), δ 4.01 (s, 2H), δ 2.56 (m, 1H), δ 1.99 (m, 10H).

2.1.55. 3-Cyclohexylaminomethylphenol (55)

Compound **55** was prepared as a colorless liquid in a manner similar to that described for **1** (81% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.15 (t, J = 7.68 Hz, 1H), δ 6.77 (d, J = 7.68 Hz, 1H), δ 6.69 (m, 2H), δ 3.71 (s, 2H), δ 2.57 (m, 1H), δ 1.96 (m, 10H).

2.1.56. 4-Cyclohexylaminomethylphenol (56)

Compound **56** was prepared as a colorless liquid in a manner similar to that described for **1** (75.7% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.04 (d, J = 8.04 Hz, 2H), δ 6.55 (d, J = 8.04 Hz, 2H), δ 3.80 (b, 1H), δ 3.69 (s, 2H), δ 2.58 (m, 1H), δ 1.98 (m, 10H).

2.2. General procedure for the synthesis of compounds 57-63

2.2.1. 3,4-Dichloro-N-(3-methylbutyl)benzamide (57)

Isoamylamine (0.416 g, 4.774 mmol) and triethylamine (1 mL, 7.161 mmol) were added slowly to 1 g 3,4-dichlorobenzoyl chloride (4.774 mmol) melted in 20 mL of tetrahydrofuran (THF). The mixture was then stirred for 1 h at room temperature until the starting materials (TLC analysis) began to disappear. The resulting solution was extracted from the mixture using 40 mL of water and 90 mL of methylene chloride, and was dehydrated using anhydrous $MgSO_4$. The resulting solution was concentrated under reduced pressure and purified by column chromatography over silica gel (elution with n-hexane/ethyl acetate, 20:1) to produce 3,4-dichloro-N-(3-methylbutyl)benzamide.

Compound **57** was obtained by re-crystallization as a light red solid (90.7% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85 (d, *J* = 1.8 Hz, 1H), δ 7.59 (dd, *J* = 8.0 and 1.8 Hz, 1H), δ 7.51 (d, *J* = 8.0 Hz, 1H), δ 6.05 (b, 1H), δ 3.50 (m, 2H), δ 1.74 (m, *J* = 6.6 Hz, 1H), δ 1.54 (m, *J* = 6.6 Hz, 2H), δ 0.96 (d, *J* = 6.6 Hz, 6H).

2.2.2. 3,4-Dichloro-N-(2-methoxyethyl)benzamide (58)

Compound **58** was prepared as a light red solid in a manner similar to that described for **57** (88.9% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.89 (d, *J* = 2.1 Hz, 1H), δ 7.62 (dd, *J* = 8.4 and 2.1 Hz, 1H), δ 7.52 (d, *J* = 8.4 Hz, 1H), δ 6.47 (b, 1H), δ 3.67 (m, *J* = 4.7 Hz, 2H), δ 3.57 (t, *J* = 4.7 Hz, 2H), δ 3.40 (s, 3H).

2.2.3. 3,4-Dichloro-N-(2,2-dimethoxyethyl)benzamide (59)

Compound **59** was prepared as a light red solid in a manner similar to that described for **57** (98% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.88 (d, *J* = 1.8 Hz, 1H), δ 7.61 (dd, *J* = 8.0 and 1.8 Hz, 1H), δ 7.52 (d, *J* = 8.0 Hz, 1H), δ 6.31 (b, 1H), δ 4.50 (t, *J* = 5.1 Hz, 1H), δ 3.61 (t, *J* = 5.1 Hz, 2H), δ 3.44 (s, 6H).

2.2.4. 3,4-Dichloro-N-(2-dimethylaminoethyl)benzamide (60)

Compound **60** was prepared as a light red solid in a manner similar to that described for **57** (95% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.91 (d, *J* = 2.2 Hz, 1H), δ 7.65 (dd, *J* = 8.0 and 2.2 Hz, 1H), δ 7.52 (d, *J* = 8.0 Hz, 1H), δ 6.96 (b, 1H), δ 3.55 (m, *J* = 5.8 Hz, 2H), δ 2.58 (t, *J* = 5.8 Hz, 2H), δ 2.30 (s, 6H).

2.2.5. 3,4-Dichloro-N-(2-diethylaminoethyl)benzamide (61)

Compound **61** was prepared as a light red solid in a manner similar to that described for **57** (94.1% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.90 (d, *J* = 2.1 Hz, 1H), δ 7.62 (dd, *J* = 8.0 and 2.1 Hz, 1H), δ 7.51 (d, *J* = 8.0 Hz, 1H), δ 7.1 (b, 1H), δ 3.51 (m, *J* = 5.1 Hz, 2H), δ 2.69 (t, *J* = 5.1 Hz, 2H), δ 2.63 (m, *J* = 6.9 Hz, 4H), δ 1.08 (t, *J* = 6.9 Hz, 6H).

2.2.6. 3,4-Dichloro-N-(3-dimethylaminopropyl)benzamide (62)

Compound **62** was prepared as a light red solid in a manner similar to that described for **57** (97.6% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.02 (b, 1H), δ 7.88 (d, *J* = 1.8 Hz, 1H), δ 7.65 (dd, *J* = 8.4 and 1.8 Hz, 1H), δ 7.51 (d, *J* = 8.4 Hz, 1H), δ 3.59 (m, *J* = 5.8 Hz, 2H), δ 2.61 (t, *J* = 5.8 Hz, 2H), δ 2.37 (s, 6H), δ 1.84 (m, *J* = 5.8 Hz, 2H).

2.2.7. 3,4-Dichloro-N-(3-diethylaminopropyl)benzamide (63)

Compound **63** was prepared as a light red solid in a manner similar to that described for **57** (91.2% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.31 (b, 1H), δ 7.89 (d, *J* = 2.1 Hz, 1H), δ 7.68 (dd, *J* = 8.4 and 2.1 Hz, 1H), δ 7.51 (d, *J* = 8.4 Hz, 1H), δ 3.59 (m, *J* = 5.4 Hz, 2H), δ 2.68 (m, 6H), δ 1.81 (m, *J* = 5.4 Hz, 2H), δ 1.09 (t, *J* = 6.9 Hz, 6H).

2.3. General procedure for the synthesis of compounds 64 and 65

2.3.1. 3-(3,4-Dichlorophenyl)-1-phenylpropenone (64)

Acetophenone (0.686 g, 5.714 mmol) was added slowly to 3,4-dichlorobenzaldehyde (1 g, 5.741 mmol) melted in 20 mL of ethanol. The mixture was stirred at 0°C. NaOH (1.2 mL, 4M) was slowly added to the mixture, and it was stirred at room temperature for 3 h until the starting materials (TLC analysis) began to disappear. The resulting solution was filtered from the mixture. It was then concentrated under reduced pressure and purified by column chromatography over silica gel (elution with n-hexane/ethyl acetate, 20:1) to produce 3-(3,4-dichlorophenyl)-1-phenylpropenone. Compound **64** was obtained by re-crystallization as a white solid (73.2% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (dt, 2H), δ 7.74 (d, *J* = 1.83 Hz, 1H), δ 7.68 (s, 1H), δ 7.64 (tt, 1H), δ 7.55 (m, 5H).

2.3.2. 1-(4-Chlorophenyl)-3-(3,4-dichlorophenyl)propanone (65)

Compound **65** was prepared as a white solid in a manner similar to that described for **57** (75.8% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (dt, 2H), δ 7.74 (d, *J* = 1.83 Hz, 1H), δ 7.68 (s, 1H), δ 7.52 (m, 5H).

2.4. Algal cultures, medium, and culture conditions

The red tide-causing marine strains, *C. polykrikoides, Heterocapsa circularisquama, C. marina,* and *H. akashiwo* were obtained from the algal culture collection of the National Fisheries Research & Development Institute (NFRDI). Cultures of *C. polykrikoides, H. circularisquama, C. marina,* and *H. akashiwo* were grown in a culture flask (Becton Dickinson Labware, Franklin Lakes, NJ, USA, 45 rpm) at 23°C under constant light (100 μmol/m^2/sec (12L : 12D) in Guillard's f/2 medium (pH 8.0) without Si and with filtered seawater, as reported previously [37]. The f/2 medium was prepared by sterile-filtering seawater using 0.20-μm filtration units (Nalgene, Rochester, NY, USA) and was enriched aseptically using nutrients and vitamins purchased from Sigma (St. Louis, MO, USA). *Selenastrum gracile* and *D. rerio* were obtained from the algal culture collection of the National Fisheries Research & Development Institute (NFRDI). Cultures of *S. gracile* were grown in culture flasks (Becton Dickinson Labware, Franklin Lakes, NJ, USA, 45 rpm) at 20°C under constant light (60 μmol/m^2/sec (14 L : 10D) in BG11 medium (pH 7.0) without Si

and with filtered seawater. The BG11 medium was prepared by sterile-filtering seawater using 0.20 μm filtration units (Nalgene, Rochester, NY, USA) and was enriched aseptically using nutrients and vitamins purchased from Sigma (St. Louis, MO, USA). In the case of the *S. gracile* culture, it was cultured at 26°C for 2 weeks, and tests were then conducted. Feed (Tabia) was used once in two days.

2.5. Algicidal activities of dichlorobenzylamine derivatives

The algicidal activity of the dichlorobenzylamine derivatives against *C. polykrikoides, H. circularisquama, C. marina,* and *H. akashiwo* were examined at various concentrations. Each experiment was conducted in 24-well tissue culture test plates (SPL) with approximately 1 mL total volume per well. Various concentrations of the test compounds were introduced to the cultures during the exponential growth phase. All of the microalgae were exposed to the compounds at final concentrations of 5, 2, 1, 0.5, 0.2, and 0.1 μM. The control cultures were performed without the dichlorobenzylamine derivatives. The algal cell density was counted 3 days after inoculation with the compounds. The algal cells were counted using a Burker Turk hemocytometer with Sedgwick-Rafter counting chambers under an Olympus light microscope with 40X and 100X magnification (Olympus Co., Tokyo, Japan). Algicidal activity profiling of the dichlorobenzylamine derivatives growth rates were then calculated and expressed as the reduction ratio (%) from the number of cell divisions per day. The reduction ratio (%) was determined using the following equation: % Algicidal activity = $(1\text{-}T_t/C_t) \times 100$, where T (treatment) and C (control) are the cell densities with and without each dichlorobenzylamine derivative at different concentrations and t is the inoculation time (day).

2.6. Statistical data analysis

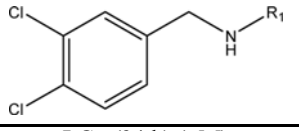
The experiments were carried out a minimum of three times. The data are reported as the mean ± SD. All statistical analyses were performed using SPSS 17.0 software (SPSS, USA). The statistical significance of the differences between the mean values was determined by one-way variance analysis (ANOVA)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

3. Results and Discussion

The main objective of this study is to design environmentally safe and selective algicides to manage HABs. Therefore, several derivatives of dichlorobenzylamine derivatives were newly synthesized and these efficacy and selectivity were examined with analyzing structure –activity relationship of various dichlorobenzylamine derivatives. Table 1 shows algicidal activities of the alkyl amino group-substituented dichlorobenzylamine derivatives (compounds **1-9**) against *C. polykrikoides, H. circularisquama, C. marina,* and *H. akashiwo*. The synthesized compounds were tested under the assay conditions at various micro molar concentrations, and the results and statistical significance were verified. The synthesized compounds resulted in an increase in their inhibitory potency against *H. akashiwo.* In particular, when the carbon number of R_1 was 4 or 5, the algicidal activities of the synthesized compounds **3, 4,** and **9** against *C. polykrikoides, H. circularisquama, C. marina,* and *H. akashiwo* were higher than those of other compounds. The results show that the algicidal activities against harmful red tides were affected by the carbon number of R_1 in 3,4-dichlorobenzyl amine derivatives.

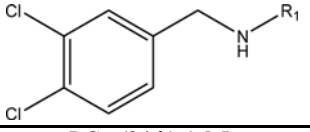
Table 2 shows algicidal activities of the methoxy-introduced dichlorobenzylamine derivatives (compounds **10-13**). When the alkyl group as a substituent was increased,

Table 1. Algicidal effects of synthetic compounds **1-9** against harmful red tides



	Compounds	LC_{50} (24 h) (μM)			
NO.	R_1	*C. M*	*H. A*	*H. C*	*C. P*
1	$(CH_2)_1CH_3$	5<	1.18	5<	5<
2	$(CH_2)_2CH_3$	2.67	0.73	5<	4.38
3	$(CH_2)_3CH_3$	3.16	0.44	2.86	3.51
4	$(CH_2)_4CH_3$	1.65	0.80	5<	1.71
5	$(CH_2)_5CH_3$	2.14	1.50	5<	3.29
6	$(CH_2)_6CH_3$	3.94	3.10	5<	5<
7	$CH_2CH(CH_2)_2$	1.14	0.30	5<	4.44
8	$CH(CH_3)CH_2CH_3$	0.52	0.32	4.45	5<
9	$CH_2CH(CH_3)_2$	2.00	0.28	1.50	1.53

Table 2. Algicidal effects of synthetic compounds **10-13** against harmful red tides



	Compounds	LC_{50} (24 h) (μM)			
NO.	R_1	*C. M*	*H. A*	*H. C*	*C. P*
10	$(CH_2)_2OCH_3$	5<	1.30	5<	5<
11	$(CH_2)_3OCH_3$	0.99	0.29	5<	4.82
12	$CH_2CH(OCH_3)_2$	5<	4.00	5<	5<
13	$(CH_2)_3CH(OCH_3)_2$	5<	1.3	4.8	4.41

the activities of compounds were increased. However, in the case of the methoxy group, they were decreased in general except for *C. marina* and *H. akashiwo*.

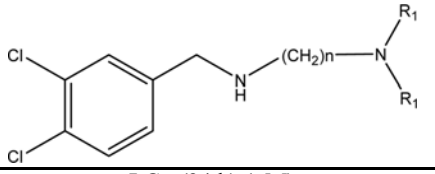
Table 3 shows algicidal activities of the nitrogen-introduced dichlorobenzylamine derivatives (compounds **14-17**) to the alkyl amino group. There was no effect of nitrogen substituent. However, when the carbon number was increased, the algicidal activities were increased. The activities of the synthesized compounds **16** and **17**, in particular, against *C. polykrikoides* were significantly increased to LC_{50} values of 0.29 and 0.30 μM, respectively, compared to those of other compounds.

Table 4 shows algicidal activities of the aniline- and halogen-substituted dichlorobenzylamine derivatives (compounds **18-22**) instead of the amino group. The algicidal activities of the aniline-substituted compounds were decreased in principle, compared to the amine group. In addition, when the methoxy group was introduced, the activity decreased. In the case of the halogen-substituted compounds, the activity against *H. circularisquama* increased. However, it decreased against *C. marina.* These results indicate that the algicidal activity against harmful red tides was negatively affected by the methoxy group.

The carbon chain length at the N position of pyridine was changed, and their activities were determined. Table 5 shows the effect of carbon chain length on the aniline structure of dichlorobenzylamine derivatives (compounds **23-30**) on algicidal activity. When the carbon chain length was increased from 1 to 2, the algicidal activity increased. Notably, the algicidal activities of the synthesized compounds **27** and **28** against *C. polykrikoides, H. circularisquama, C. marina,* and *H. akashiwo* were higher than other compounds. The results show that the carbon chain length at the aniline structure affected algicidal activity.

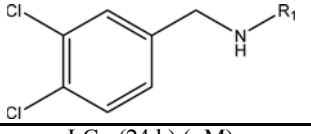
The cyclo alkyl groups were introduced at the R_1 position, and their activities were determined. Table 6 shows the effect of dichlorobenzylamine derivatives (compounds **31-38**) on algicidal activity. When the carbon number of monocyclo alkyl was increased from 3 to 7, the algicidal activity

Table 3. Algicidal effects of synthetic compounds **14-17** against harmful red tides



Compounds			LC_{50} (24 h) (μM)			
NO.	n	R_1	*C. M*	*H. A*	*H. C*	*C. P*
14	2	CH_3	5<	2.25	3.10	5<
15	2	CH_2CH_3	2.90	1.58	1.76	2.30
16	3	CH_3	0.81	1.26	3.30	0.29
17	3	CH_2CH_3	2.60	1.30	3.40	0.30

Table 4. Algicidal effects of synthetic compounds **18-22** against harmful red tides



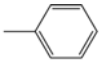
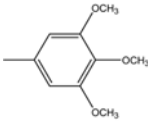
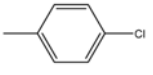
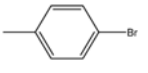
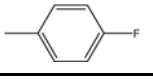
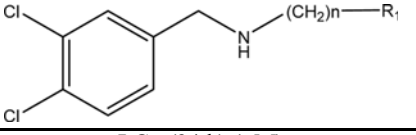
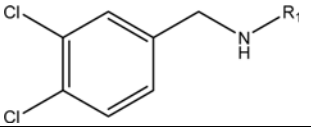
Compounds		LC_{50} (24 h) (μM)			
NO.	$-R_1$	*C. M*	*H. A*	*H. C*	*C. P*
18		3.50	1.27	5<	4.14
19		5<	5<	5<	5<
20		5<	1.28	3.30	3.60
21		5<	1.19	2.83	4.00
22		5<	1.54	5<	5<

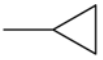
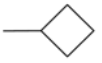
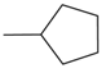
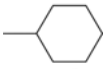
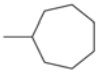
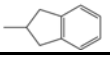
Table 5. Algicidal effects of synthetic compounds **23-30** against harmful red tides



Compounds			LC_{50} (24 h) (μM)			
NO.	n	R_1	*C. M*	*H. A*	*H. C*	*C. P*
23	1		3.46	0.6	3.23	2.75
24	1		3.40	0.72	5<	5<
25	1		5<	0.79	3.55	2.00
26	1		5<	2.28	5<	5<
27	2		0.46	0.12	1.50	0.87
28	2		0.31	0.18	1.05	0.50
29	2		3.50	0.59	1.60	0.42
30	2		3.47	0.42	1.91	2.70

Table 6. Algicidal effects of synthetic compounds **31-38** against harmful red tides



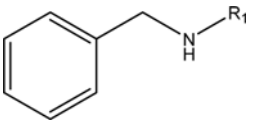
Compounds NO.	R_1	LC_{50} (24 h) (μM) *C. M*	*H. A*	*H. C*	*C. P*
31		5<	5<	5<	5<
32		1.13	0.48	3.08	1.66
33		0.32	0.24	1.04	0.36
34		0.29	0.21	0.97	0.15
35		0.25	0.26	0.69	0.10
36		0.50	0.34	1.19	0.10
37		1.44	1.14	2.00	0.31
38		1.69	0.71	5<	3.76

against *C. polykrikoides, H. circularisquama, C. marina,* and *H. akashiwo* increased. We found that among four kinds of harmful red tides, the algicidal activity of compound **35** against *C. polykrikoides* was 2.5 times higher than other harmful algae. However, with a carbon number greater than 8 of mono-cyclo alkyl, algicidal activity decreased. When the carbon number of di-cyclo alkyl was increased, algicidal activity also decreased compared to the mono-cyclo alkyl group. The results indicate that the algicidal activity was affected by the carbon number of the mono-cyclo alkyl group introduced at the R_1 position.

After removing 3,4-dichloro from 3,4-dichloro benzylamine, algicidal activities of the synthesized compounds were determined. The algicidal activities of benzylamine derivatives are shown in Table 7. The high activity of benzylamine derivatives (compounds **39-42**) against *C. marina* and *H. akashiwo* were obtained with an LC_{50} of 0.34 and 0.31 μM, respectively. Conversely, in the case of *C. polykrikoides* and *H. circularisquama,* there was no algicidal activity of benzylamine derivatives. The results indicate that algicidal activity against harmful red tides was strongly affected by the structure with 3,4-dichlorobenzene.

The effects of the number and position of chlorine on algicidal activity of chlorobenzylamine derivatives (compounds **43-50**) were investigated, and the results are shown in Table 8. Among various compounds, the maximum algicidal

Table 7. Algicidal effects of synthetic compounds **39-42** against harmful red tides



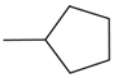
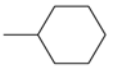
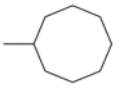
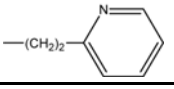
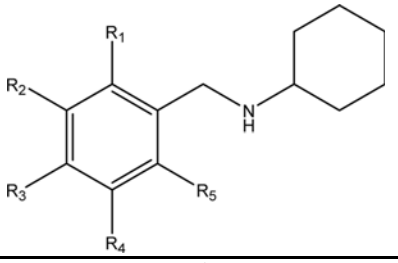
Compounds NO.	R_1	LC_{50} (24 h) (μM) *C. M*	*H. A*	*H. C*	*C. P*
39		1.69	0.66	5<	5<
40		5<	0.67	5<	5<
41		0.52	0.15	5<	5<
42		0.34	0.13	5<	5<

Table 8. Algicidal effects of synthetic compounds **43-50** against harmful red tides

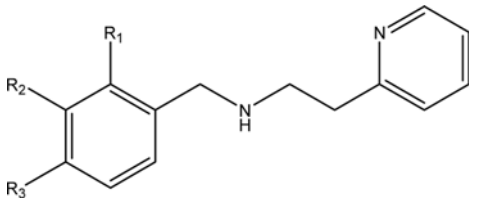


Compounds NO.	R_1	R_2	R_3	R_4	R_5	LC_{50} (24 h) (μM) *C. M*	*H. A*	*H. C*	*C. P*
43	Cl	-	-	-	-	0.79	1.43	5<	5<
44	-	Cl	-	-	-	0.47	0.44	4.18	3.45
45	-	-	Cl	-	-	0.31	0.14	3.65	1.93
46	Cl	Cl	-	-	-	0.97	1.47	5<	2.06
47	Cl	-	Cl	-	-	3.4	3.85	5<	4.1
48	Cl	-	-	Cl	-	5<	5<	5<	5<
49	Cl	-	-	-	Cl	3.78	5<	5<	5<
34[a]	-	Cl	Cl	-	-	0.29	0.21	1.14	0.15
50	-	Cl	-	Cl	-	4.02	5<	5<	3.7

activity of 4-chlorobenzylamine compound **45** located in R_3 was obtained. However, compared to 3,4-dichlorobenzylamine compound **34**, it was low.

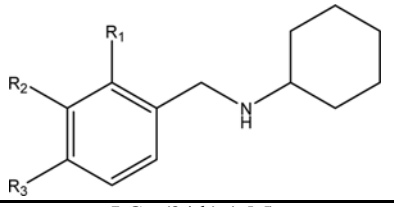
The effects of substituting bromine for chlorine at the R_1-R_3 position on the algicidal activity of dichlorobenzylamine derivatives (compounds **51-53**) were investigated, and the results are shown in Table 9. The activities of synthetic compounds **51-53** against *H. circularisquama*

Table 9. Algicidal effects of synthetic compounds **51-53** against harmful red tides



Compounds				LC_{50} (24 h) (μM)			
NO.	R_1	R_2	R_3	*C. M*	*H. A*	*H. C*	*C. P*
51	Br	-	-	0.92	0.28	5<	5<
52	-	Br	-	0.74	0.57	5<	3.5
53	-	-	Br	0.72	0.14	5<	5<

Table 10. Algicidal effects of synthetic compounds **54-56** against harmful red tides



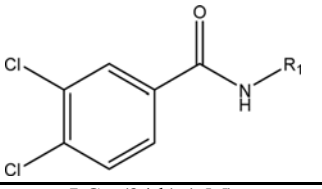
Compounds				LC_{50} (24 h) (μM)			
NO.	R_1	R_2	R_3	*C. M*	*H. A*	*H. C*	*C. P*
54	OH	-	-	0.49	0.36	5<	5
55	-	OH	-	5<	3.46	5<	1.92
56	-	-	OH	5<	5<	5<	5<

and *C. polykrikoides* were very low. However, in the case of *C. marina* and *H. akashiwo,* the LC_{50} was 0.28 and 0.92 μM, respectively. The results show that the algicidal activity against *C. marina* and *H. akashiwo* was affected by the bromo-substituted derivatives.

Effects of OH introduction instead of chlorine at the R_1-R_3 position on algicidal activity of dichlorobenzylamine derivatives (compounds **54-56)** were investigated, and the results are shown in Table 10. When OH was introduced at the R_1-position, the maximum activities of synthetic compound **54** against *C. marina* and *H. akashiwo* were obtained with LC_{50} of 0.49 and 0.36 μM, respectively. However, in the case of *H. circularisquama* and *C. polykrikoides*, algicidal activity was very low. The results show that the algicidal activity against harmful red tides was affected by the OH position.

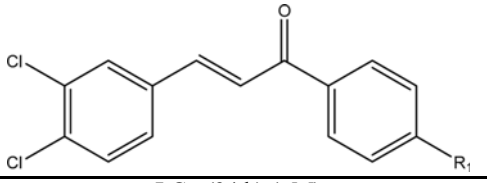
Table 11 shows algicidal activity of 3,4-dichlorobenzamide derivatives (compounds **57-63**). Algicidal activities of amid groups (compounds **60-63**) were higher than compounds **57-59**. On the other hand, algicidal activities of 3,4-

Table 11. Algicidal effects of synthetic compounds **57-63** against harmful red tides



Compounds		LC_{50} (24 h) (μM)			
NO.	R_1	C. M	H. A	H. C	C. P
57	$-(CH_2)CH(CH_3)_2$	5<	5<	5<	5<
58	$-(CH_2)OCH_3$	5<	5<	5<	5<
59	$-CH_2CH(OCH_3)_2$	5<	5<	5<	5<
60	$-(CH_2)_2N(CH_3)_2$	4.5<	1.63	4<	4<
61	$-(CH_2)_2N(CH_2CH_3)_2$	4.5<	1.85	1.97	4.55
62	$-(CH_2)_3N(CH_3)_2$	4.5<	1.55	4<	4<
63	$-(CH_2)_3N(CH_2CH_3)_2$	4.5	2.27	4<	2.22

Table 12. Algicidal effects of synthetic compounds **64-65** against harmful red tides



Compounds		LC_{50} (24 h) (μM)			
NO.	R_1	*C. M*	*H. A*	*H. C*	*C. P*
64	H	5<	3.67	5<	1.66
65	Cl	5<	3.7	5<	1.33

dichlorobenzamide derivatives (compounds **60-63**) using the same substituent group against *C. polykrikoides, H. circularisquama, C. marina,* and *H. akashiwo* were lower than those of 3,4-dichlorobenzylamine derivatives (compounds **14-17**). The results indicate that benzylamine can be used as a new algicide for harmful red tides.

Table 12 is algicidal activity of 3,4-dichlorobenzen derivatives (compounds **64** and **65**), with hydrogen and chlorine at the R_1 position against harmful red tides. Algicidal activities of compounds **64** and **65** against *H. circularisquama, C. marina,* and *H. akashiwo* were very low. However, in the case of *C. polykrikoides,* the LC_{50} results of compounds **64** and **65** were 1.33 ~ 1.66 μM, respectively.

Table 13 is summary of algicidal activity against harmful algae data and the ecotoxicological data of the compounds **33** and **34** used in the laboratory toxicity test. When treated with 1 μM, almost all cells were confirmed to be destroyed. To verify the safety of compounds **33** and **34**, acute ecotoxicology tests using the water flea (*D. magna*) and

Table 13. Summary of algicidal activity against harmful algae and the ecotoxicological data of the synthetic compounds **33** and **34**

Species	Classification	Duration	Compound/LC_{50}, EC_{50} (μM)	
			33	**34**
C. marina	Red tide	24 h/survival	0.32	0.29
H. akashiwo			0.24	0.21
H. circularisquama			1.04	0.97
C. polykrikoides			0.36	0.15
D. magna	Water flea	48 h/survival	>15	>15
D. rerio	Fish	330 h/survival	>15	>15

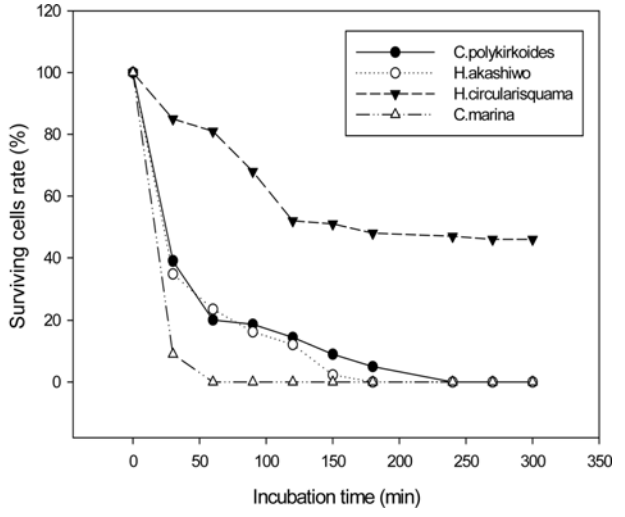


Fig. 1. Time-dependent algicidal activity of compound **34** (1 μM).

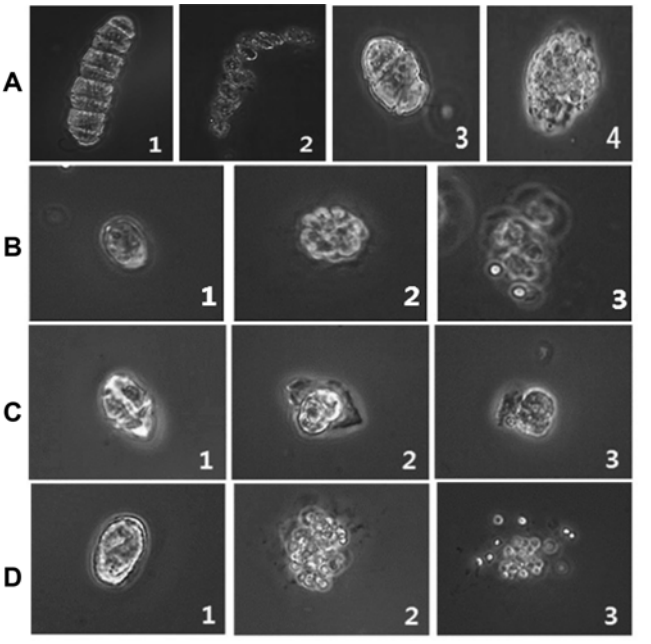


Fig. 2. Microscopic observations of time-dependent algicidal activity of compound **34** (1 μM). (A) *C. polykrikoides,* (B) *H. akashiwo,* (C) *H. circularisquama,* (D) *C. marina* ; (A)-1, (A)-3, (B)-1, (C)-1, and (D)-1 are controls without compound **34**.

zebrafish (*D. rerio*) were conducted. The test confirmed that the compounds **33** and **34** were safe in terms of ecotoxicity by showing high survival of *D. magna* and *D. rerio* for over 48 h and 330 h of incubation, respectively, at 15 μM, while *C. polykrikoides, H. circularisquama, C. marina,* and *H. akashiwo* survived for over 24 h of incubation. The results indicate that compounds **33** and **34** are suitable substances to control harmful algae species. Compound **34** was selected for further examination of the algicidal effect of dichlorobenzylamine derivatives due to its strong algicidal effects. When the time-dependent manner of the algicidal activity of compound **34** was monitored, 1 μM killed *H. akashiwo, C. marina, and C. polykrikoides* tested within 180 min of incubation, but did not kill *H. circularisquama* (Fig. 1). Fig. 2 depicts microscopic observations of *H. akashiwo, C. marina, C. polykrikoides,* and *H. circularisquama* and the time-dependent algicidal activity produced by compound **34** (1 μM). To monitor the algicidal processes of compound **34** against red tide-forming algae, optical microscopy observations were performed using an Olympus camera, model DP71, at a magnification of 600X for *H. akashiwo, H. circularisquama*, and *C. marina*, and with an Olympus camera, model IX71, at a magnification of 640X for *C. polykrikoides*. The four red tide-forming algal species were cultured, and compound **34** was added to the cultures. The control cultures did not receive compound **34,** and we observed them at the same time as the inoculated cultures. These microscopic observations were repeated twice, and similar observations were made. The *C. marina, H. akashiwo,* and *C. polykrikoides* cells were rapidly affected by 1 μM of compound **34**. After adding 1 μM of the compound, most of the cells lost their motility within 1 min. The cellular components began to leak, and cells were lysed 3 h after inoculation. When 1 μM of compound **34** was applied, the morphology of *H. circularisquama* changed within 30 min of incubation. The cell membranes were dissembled within 30 min of incubation and were disrupted within 2 h of incubation. The cellular organelles were released after 3 h of incubation.

4. Conclusion

We studied chemical methods of efficiently controlling harmful algae species by synthesizing 65 new chemicals known to be active against harmful algae species. We inoculated 5 ~ 0.1 μM of the synthesized chemical to harmful algae species and analyzed the activity change according to the structure-activity relationship. The results indicate that the 3,4-dichlorobenzylamine derivatives show relatively high activity against *C. polykrikoides, H. akashiwo, C. marina*, and *H. circularisquama.* Among the 65 compounds tested, several synthetic dichlorobenzylamine derivatives exhibited remarkable algicidal activity. Compounds **33** and **34** were found to be the most active candidates for significantly decreasing the growth of *H. akashiwo*, with IC_{50} levels of 0.24 and 0.21 μM, respectively, and for decreasing the growth of *H. circularisquama*, with LC_{50} levels of 1.04 and 0.97 μM, respectively. In the case of *C. marina* and *C. polykrikoides*, compounds **33** and **34** were found to have LC_{50} values of 0.15 and 0.35 μM, respectively. When treated with 1 μM, almost all cells were confirmed to be destroyed. To verify the safety of compounds, acute ecotoxicology tests using the water flea (*D. magna*) and zebrafish (*D. rerio*) were conducted, and the tests confirmed that the compounds **33** and **34** were safe by showing high survival rates at 15 μM. Our results suggest that the dichlorobenzylamine derivatives can be considered as promising lead structures for the development of algicides against harmful red tide algal species. In the future, risks to the marine ecosystem should be systematically assessed using the mesocosm to study the feasibility of field applications.

Acknowledgements

This work was supported by the Human Resource Training Program for Regional Innovation and Creativity through the Ministry of Education and National Research Foundation of Korea (NRF-2014H1C1A1067030).

References

1. Anderson, D. M (2009) Approaches to monitoring, control and management of harmful algal blooms (HABs). *Ocean Coastal Manag.* 52**:** 342-347.
2. Yanagi, T., T. Yamamoto, Y. Koizumi, T. Ikeda, M. Kamizono, and H. Tamori (1995) A numerical simulation of red tide formation. *J. Mar. Syst*. 6: 269-285.
3. Uchida, T., S. Toda, Y. Matsuyama, M. Yamaguchi, Y. Kotani, and T. Honjo (1999) Interactions between the red tide dinoflagellates *Heterocapsa circularisquama* and *Gymnodinium mikimotoi* in laboratory culture. *J. Exp. Mar. Biol. Ecol*. 241: 285-299.
4. Kim, C. S., S. G. Lee, and H. G. Kim (2000) Biochemical responses of fish exposed to a harmful dinoflagellate Cochlodinium polykrikoides. *J. Exp. Mar. Biol. Ecol*. 254: 131-141.
5. Boesch, D. F., D. M. Anderson, R. A. Horner, S. E. Shumway, P. A. Tester, and T. E. Whitledge (1997) *NOAA Coastal Ocean Program Decision Analysis Series*. p. 46. No. 10. NOAA Coastal Office, Sliver Spring, MD.
6. Shirota, A. (1989) Red tide problem and countermeasures. *Int. J. Aquat. Fish Technol*. 1: 195-223.
7. Pierce, R. H., M. S. Henry, C. J. Higham, P. Blum, M. R. Sengco, and D. M. Anderson (2004) Removal of harmful algal cells (Karenia brevis) and toxins from seawater culture by clay flocculation. *Harmful Algae* 3: 141-148.
8. Yu, Z., J. Zou, and X. Ma (1994) Applications of clays to removal of red tide organisms I. Coagulation of red tide organisms with clays. *Chin. J. Oceanol. Limnol*. 12: 193-200.
9. Noi, G. H., W. J. Choi, and Y. Y. Chun (1996) A study on red tide control with loess suspension. *Kor. J. Aquacult*. 9: 239-245.
10. Choi, H. G., P. J. Kim, W. C. Lee, S. J. Yun, H. G. Kim, and H. J. Lee (1998) Removal efficiency of cochiodinium polykrikoides by Yellow Loess. *J. Korean Fish Soc*. 31: 109-113.
11. Gumbo, R. J., G. Ross, and E. T. Cloete (2008) Biological control of microcystis dominated harmful algal blooms. *Afr. J. Biotechnol*. 7: 4765-4773.
12. Sengco, M. R. and D. M. Anderson (2004) Controlling harmful algal blooms through clay flocculation. *J. Eukaryot. Microbiol.* 51: 169-172.
13. Yu, Z., M. R. Sengco, and D. M. Anderson (2004) Flocculation and removal of the brown tide organism, *Aureococcus anophagefferens* (Chrysophyceae), using clays. *J. Appl. Phycol*. 16: 101-110.
14. Sengco, M. R. (2008) Prevention and control of *Karenia brevis* blooms. *Harmful Algae* 8: 623-628.
15. Liu, J., H. Zhang, W. Yang, J. Gao, and Q. Ke (2004) Studies on biquaternary ammonium salt algaecide for removing red tide algae. *Mar. Sci. Bull*. 6: 60-65.
16. Zhou, X., M. Bai, S. Deng, K. Dong, and L. Xing (2004) Study on killing *Gymnodinium mukimotoi* with hydroxyl radical. *Mar. Environ. Sci*. 23: 64-66.
17. Jancula, D., M. Drabkova, J. Cerny, M. Karaskova, R. Korýnkova, J. Rakusan, and B. Marsalek (2008) Algicidal activity of phthalocyanines—Screening of 31 compounds. *Environ. Toxicol*. 23: 218-223.
18. Poovey, A. G., K. D. Getsinger, J. G. Skogerboe, T. J. Koschnick, J. D. Madsen, and R. M. Stewart (2004) Small-plot, low-dose treatments of triclopyr for selective control of Eurasian watermilfoil. *Lake Reserv. Manage*. 20: 322-332.
19. Schrader, K**.** K., N. P. Nanayakkara, C. S. Tucker, A. M. Rimando, M. Ganzera, and B. T. Schaneberg (2003) Novel derivatives of 9,10-anthraquinone are selective algicides against the musty-odor cyanobacterium *Oscillatoria perornata. Appl. Environ. Microb.* 69: 5319-5327.
20. Lam, A. K. Y., E. E. Prepas, D. Spink, and S. E. Hrudey (1995) Chemical control of hepatotoxic phytoplankton blooms: implications for human health. *Water Res*. 29: 1845-1854.
21. Porsbring, T., H. Blanck, H. Tjellström, and T. Backhaus (2009) Toxicity of the pharmaceutical clotrimazole to marine microalgal communities. *Aqu. Toxicol*. 91: 203-211.
22. Tucker, C. S. (2000) Off-flavor problems in aquaculture. *Rev. Fish. Sci.* 8: 45-88.
23. DellaGreca, M., A. Fiorentino, M. Isidori, P. Monaco, F. Temussi, and A. Zarrelli (2001) Antialgal furano-diterpenes from *Potamogeton natans* L. *Phytochem*. 58: 299-304.
24. Gustafsson, S., M. Hultberg, R. I. Figueroa, and K. Rengefors (2009) On the control of HAB species using low biosurfactant concentrations. *Harmful Algae* 8: 857-863.
25. Sanaa, M. M. and Shanab (2007) Bioactive allelo-chemical com-

pounds from *Oscillatoria* Species. *Int. J. Agri. Biol*. 9: 617-621.
26. Terlizzi, D. E., M. D. Ferrier, E. A. Armbrester, and K. A. Anlauf (2002) Inhibition of dinoflagellate growth by extracts of barley straw. *J. Appl. Phycol*. 14: 275-280.
27. Li, F. M., and H. Y. Hu (2005) Isolation and characterization of a novel antialgal allelochemical from *Phragmites communis*. *Appl. Environ. Microbiol*. 71: 6545-6553.
28. Kim, J. S., J. C. Kim, S. Lee, B. H. Lee, and K. Y. Cho (2006) Biological activity of L-2-azetidinecarboxylic acid, isolated from *Polygonatum odoratum* var. *pluriflorum*, against several algae. *Aquat. Bot*. 85: 1-6.
29. Waybright, T. J., D. E. Terlizzi, and M. Drew (2009) Chemical characterization of the aqueous algistatic fraction of barley straw (*Hordeum vulgare*) inhibiting *Microcystis aeruginosa*. *J. Appl. Phycol*. 21: 333-340.
30. Kim, Y. M., Y. Wu, T. U. Duong, G. S. Ghodake, S. W. Kim, E. S. Jin, and H. Cho (2010) Thiazolidinediones as a novel class of algicides against red tide harmful algal species. *Appl. Biochem. Biotechnol.* 162: 2273-2283.
31. Han. H. K., Y. M. Kim, S. S. Lim, S. G. Hong, and H. Cho (2011) Enhanced efficacy of TD53, a novel algicidal agent, against the harmful algae *via* the liposomal delivery system. *Inter. J. Pharmaceutics*. 405: 137-141.
32. Yim, E. C., I. T. Park, H. K. Han, S.I. Kim, H. Cho, and S. J. Kim (2010) Acute toxicity assessment of new algicides of thiazolidnedionse derivatives, TD53 and TD49, using *Ulva pertusa Kjellman*. *Environ. Health Toxicol*. 25: 273-278.
33. Kim S. J., E. C. Yim, I. T. Park, S. W. Kim and H. Cho (2011) Comparison of the acute toxicities of novel algicides, thiazolidinedione derivatives TD49 and TD 53, to Various marine organisms. *Environ. Toxicol. Chem.* 30: 2810-2816.
34. Kim, Y. M., Y. Wu, T. U. Duong, S. G. Jung, S. W.S, Kim, H. Cho, and E. S. Jin (2012) Algicidal activity of thiazolidinedione derivatives against harmful algal blooming species. *Mar. Biotechnol*. 14: 312-322.
35. Kwon, H. L., J. H, Kim, D. H. Na, D. H. Byeun, Y. Wu, S. W. Kim, E. S. Jin, and H. Cho (2013) Combination of 1,4-Naphthoquinone with benzothiazoles had selective algicidal effects against harmful algae. *Biotech. Biopro. Eng*. 18: 932-941.
36. Wu Y., Y. Lee, S. G. Jung, C. Y. Eom, S. W Kim, H. Cho, and E. S. Jin (2014) A novel thiazolinedione derivative TD118 showing selective algicidal effects for red tide control. *World J. Microbiol. Biotechnol*. 30: 1603-1614.
37. Guillard, R. R. L. and M. D. Keller (1984) Culturing dinoflagellates. In: D. L. Spector (ed.), pp. 391-442. *Dinoflagellates*. Academic Press, NY, USA.