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Thiazolidinediones as a Novel Class of Algicides Against Red Tide Harmful Algal Species

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Abstract This paper reports the synthesis of 28 thiazolidinedione derivatives along with their algicidal activity against microalgae causing harmful algal blooming. Among the 28 compounds tested, most showed effective algicidal activity against *Heterosigma akashiwo*, *Chattonella marina*, and *Cochlodinium polykrikoides*, while non-harmful algae were relatively tolerant to these thiazolidinedione derivatives. Compounds **6**, **13**, and **22** were the most potent against *C. polykrikoides* with IC_{50} values <0.5 μM. Among the thiazolidinedione derivatives tested, compounds **7**, **13**, **27**, and **28** were extremely competent and selective to *C. polykrikoides* with IC_{50} values ranging from 0.1 to 2 μM, while *C. marina* and *H. akashiwo* showed an IC_{50} value ranging from 30 to 130 μM. These results show that some thiazolidinedione derivatives can act as potent algicides against harmful algal blooms.

Keywords Harmful algal blooms · Toxicity · Thiazolidinediones · Algicides · Red tide

Introduction

The impact of harmful algal blooms (HABs) on aquatic ecosystems, water resources, and human health is a major problem throughout the world. HABs occur regularly in eutrophic water bodies during spring, winter, and summer [1, 2]. Dense growths of red tide algae

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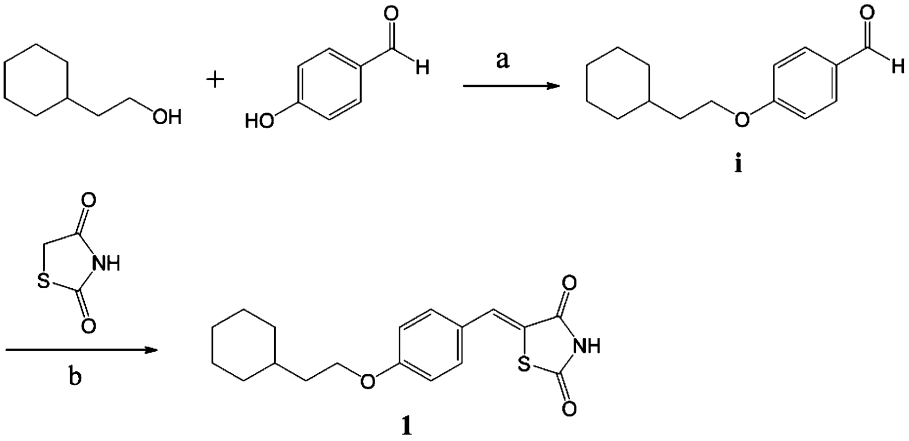
cause severe problems, such as hindering boat traffic, blocking approaches, obstructing wash processes, and catching cavities, creating unattractive foul-smelling loads and killing natural biota [2–4]. The growth of red tide algal inhabitants and increasing algal toxin levels are involved in causing severe diurnal instability in the dissolved oxygen levels, which can kill many aquatic animals [2, 3, 5].

Many scientists have conducted physiological and ecological studies in the hope of reducing the extent of damage to fisheries caused by HABs [6–8]. 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 [9]. Clay flocculants are effective in the treatment of *Cochlodinium*, which causes fish deaths in a finfish cage culture in coastal Japan [10, 11] and in Chinese mariculture ponds [12]. Yellow loess is effective in sedimenting dinoflagellates [13, 14]. However, ruptured or damaged cells may release intracellular toxins into the surrounding water, which require the use of expensive removal processes, such as activated carbon and/or oxidative ozone and chlorine [15]. Mechanical and physicochemical methods have been devised in an attempt to manage HABs with limited success [16–18].

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 towards non-target species [19–21]. Therefore, considerable effort has been made to identify new compounds that are selectively effective against red tide algae.

A number of processes of developing selective control techniques for aquatic herbicides have been evaluated [22]. Some chemicals have been used to mitigate HABs, but a search for safer and selective algicidal agents is needed to better control HABs. Copper sulfate, chelated copper compounds, and diuron (3-[3,4-dichlorophenyl]-1,1-dimethylurea), etc. are currently approved by the US Environmental Protection Agency [23]. The most direct control method involves the use of chemical treatments, such as algicides, including copper, reglone a (diquat, 1,1-ethylene-2,2-dipyridilium dibromide), 9,10-anthraquinone, potassium permanganate, chlorine and simazine (2-chloro-4,6-bis(ethylamino)-s-triazine), and clotrimazole [23–25]. Unfortunately, these compounds have undesirable characteristics, including broad-spectrum toxicity towards phytoplankton, subsequent water quality deterioration, and lengthy persistence that creates environmental safety concerns [23, 26]. Natural antialgal compounds extracted from a range of bioresources have also been reported. These include furano-diterpenes [27] at low biosurfactant concentrations [28], alleochemical [29], and barley straw. Other studies [29–33] have also attempted to manage red tide growth using controlling agents.

Thiazolidinedione (TD) was introduced in the late 1990s as an adjunctive therapy for diabetes mellitus (type 2) and related diseases. TDs act by binding to peroxisome proliferator-activated receptors, a group of receptor molecules inside the cell nucleus [34]. The design of environmentally safe, selective algicides to manage the growth of harmful algal species has been an ongoing research topic. In this preliminary structure activity relationship study, various substituents were introduced to the hydroxyl group of 5-(4-hydroxybenzylidene)thiazolidine-2,4-dione, and the effects of these derivatives on the growth of a number of harmful algal species were tested. The synthesis of compound **1** (Scheme 1) highlights the general route used to prepare the TDs described in Table 1. This paper describes the results of laboratory tests on the efficacy of the synthetic TD derivatives to control harmful algal species. The TDs were very competent and selective against the HABs studied and exhibited IC_{50}s in the nanomolar range.



Scheme 1 Reagents and conditions: (**a**) PPh_3, DEAD, THF, 25 °C, 18 h (88%); (**b**) Piperidine, AcOH, reflux, 12 h (90%)

Materials and Methods

General Procedure for the Synthesis of TD Compounds

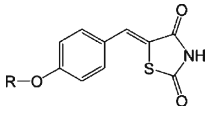
Diethyl azodicarboxylate (40% in toluene, 3.74 g, 8.58 mmol) was slowly added to a stirred solution of cyclohexane ethanol (1 g, 7.80 mmol), *p*-hydroxybenzaldehyde (953 mg, 7.80 mmol), and triphenylphosphine (2.25 g, 8.58 mmol) in THF (20 mL) over a 10-min period at 0 °C. The mixture was then stirred at room temperature until the starting materials had disappeared (TLC analysis). The resulting solution was concentrated under reduced pressure and purified by column chromatography over silica gel (elution with hexane/ethyl acetate, 20:1) to afford 1.60 g of the intermediate, 4-(2-cyclohexylethoxy)benzaldehyde (88.4%) as a yellow oil. A mixture of 4-(2-cyclohexylethoxy)benzaldehyde (1.50 g, 6.47 mmol), 2,4-thiazolidinedione (758.9 mg, 6.47 mmol), piperidine (0.28 ml, 3.24 mmol), and acetic acid (0.20 ml, 3.24 mmol) in toluene (20 mL) was then added to a round bottom flask fitted with a Dean–Stark water trap and stirred overnight under reflux. After cooling to room temperature, the precipitate was washed with hexane and dried to afford compound **1**.

*5-(4-(2-Cyclohexylethoxy)benzylidene)thiazolidine-2,4-dione (**1**)* Obtained by recrystallization as a yellow solid (1.28 g, 901% yield); mp 172–176 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.20 (s, 1H), 7.68 (s, 1H), 7.47 (d, J=14.7 Hz, 2H), 6.90 (d, J=14.7 Hz, 2H), 4.04 (t, J=11.7 Hz, 2H), 1.57–1.73 (m, 5H), 1.44–1.47 (m, 1H), 1.04–1.22 (m, 3H), and 0.81–1.03 (m, 2H).

*5-(4-((1-Methylcyclohexyl)methoxy)benzylidene)thiazolidine-2,4-dione (**2**)* Obtained by recrystallization as a yellow solid (1.6 g, 87% yield); mp 174–178 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.49 (s, 1H), 7.72 (s, 1H), 7.55 (d, J=8.7 Hz, 2H), 7.11 (d, J=8.7 Hz, 2H), 3.75 (s, 2H), 1.14–1.45 (m, 7H), 1.31–1.34 (m, 3H), and 0.99 (s, 3H).

*5-[4-(2-Thiomorpholine-1,1-dioxideethoxy)benzylidene]thiazolidine-2,4-dione (**3**)* Obtained by recrystallization as a yellow solid (1.21 g, 88% yield); mp 248–250 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.73 (s, 1H), 7.56 (d, J=8.7 Hz, 2H), 7.11 (d, J=8.7 Hz, 2H), 4.17 (t, J=10.8 Hz, 2H), 3.09 (t, J=10.2 Hz, 4H), 3.03 (t, J=10.2 Hz, 4H), and 2.95 (t, J=10.8 Hz, 2H).

Table 1 Algicidal effects of the various synthetic thiazolidinediones.



NO.	R	IC_{50}[a] (μM)					
		Harmful algal species				Non-harmful algal species	
		C. marina	*H. akashiwo*	*C. polyckrikoides*	*Amphidinium sp.*	*Phaeodactylum EPV*	*Navicula pelliculosa*
1	Cyclohexylethyl	2.224 ± 0.035	17.668 ± 0.113	5.821 ± 1.146	34.308 ± 1.005		
2	(1-Methylcyclohexyl)methyl	0.426 ± 4.250	3.775 ± 0.396	2.228 ± 0.055	100 >		
3	2-Thiomorpholine-1,1-dioxideethyl	122.001 ± 8.647	93.660 ± 9.818	118.373 ± 14.270	98.136 ± 2.731		
4	2-(Thiophen-2-yl)ethyl	1.650 ± 0.578	2.428 ± 0.928	0.712 ± 8.254	100 >		
5	2-Morpholinoethyl	70.580 ± 8.386	59.890 ± 1.372	33.038 ± 2.612	100 >		
6	2-(Thiophen-3-yl)ethyl	2.015 ± 0.653	2.838 ± 0.223	0.457 ± 9.280	69.306 ± 6.224		
7	2-Isopropoxyethyl	41.793 ± 2.030	39.928 ± 18.410	0.980 ± 8.604	95.723 ± 1.047		
8	2-(Pyridin-2-yl)ethyl	37.495 ± 1.949	17.151 ± 1.110	27.195 ± 8.011	100 >		
9	2-(Cyclohexylamino)ethyl	10.767 ± 1.729	30.187 ± 3.023	38.583 ± 8.609	100 >		
10	2-(Tetrahydro-2*H*-pyran-2-yl)ethyl	15.624 ± 4.642	39.735 ± 3.118	3.991 ± 1.229	80.528 ± 1.599		
11	2-(Piperidin-1-yl)ethyl	2.886 ± 0.185	6.565 ± 1.025	16.141 ± 2.445	82.282 ± 4.078		
12	2-(4-Methylthiazol-5-yl)ethyl	8.503 ± 0.821	10.216 ± 0.552	0.562 ± 6.738	86.910 ± 4.142		
13	3-Thiomorpholine-1,1-dioxidepropyl	90.497 ± 3.386	128.642 ± 6.611	0.133 ± 8.764	100 >		
14	Thiophen-2-ylmethyl	1.203 ± 1.601	1.927 ± 0.009	0.616 ± 0.000	5.904 ± 3.265	ND	ND
15	Thiophen-3-ylmethyl	3.658 ± 2.385	2.789 ± 0.016	0.570 ± 8.099	39.148 ± 6.851		
16	2-Cyclopentylethyl	1.515 ± 1.193	3.282 ± 0.426	0.846 ± 1.886	100 >		
17	Furan-2-ylmethyl	5.453 ± 0.130	7.642 ± 0.499	0.779 ± 5.922	5.016 ± 1.253		
18	Pyridin-2-ylmethyl	6.109 ± 0.605	13.100 ± 6.152	0.741 ± 0.000	7.214 ± 7.475		
19	4-Methoxybenzyl	11.920 ± 9.570	15.226 ± 4.244	0.793 ± 4.831	100 >		
20	4-Methylbenzyl	2.141 ± 1.391	2.022 ± 0.633	0.560 ± 0.001	52.124 ± 10.460		
21	Benzo[*d*][1,3]dioxol-5-ylmethyl	2.582 ± 0.263	3.057 ± 0.311	0.877 ± 0.000	98.788 ± 1.884		
22	Cyclopentylmethyl	4.221 ± 3.172	2.717 ± 0.002	0.254 ± 0.000	100 >		
23	4-(Chloromethyl)benzyl	6.168 ± 0.415	10.178 ± 1.357	2.267 ± 0.066	100 >		
24	(4-Methylcyclohexyl)methyl	1.754 ± 1.207	8.026 ± 0.987	2.799 ± 6.123	100 >		
25	2-(Cyclohexyloxy)ethyl	5.349 ± 1.293	3.294 ± 0.072	4.430 ± 0.002	13.618 ± 3.462		
26	(2,3-Dihydrobenzo[*b*][1,4]dioxin-2-yl)methyl	1.242 ± 0.677	8.806 ± 1.667	0.462 ± 0.000	100 >		
27	(4-Methyloxycarbonylcyclohexyl)methyl	70.618 ± 1.380	85.753 ± 0.064	1.385 ± 1.193	100 >		
28	Biphenyl-4-ylmethyl	29.537 ± 0.000	115.097 ± 5.390	2.046 ± 6.144	100 >		

[a] Values are the mean ± SD of three experiments. ND, no detectable activity

*5-(4-(2-(Thiophen-2-yl)ethoxy)benzylidene)thiazolidine-2,4-dione (**4**)* Obtained by recrystallization as a yellow solid (1.59 g, 88% yield); mp 176–180 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.12 (s, 1H), 7.73 (s, 1H), 7.56 (d, J=8.7 Hz, 2H), 7.35 (d, J=6.0 Hz, 1H), 7.12 (d, J=8.7 Hz, 2H), 6.94–6.97 (m, 2H), 4.28 (t, J=12.6 Hz, 2H), and 3.29 (t, J=12.6 Hz, 2H).

*5-(4-(2-Morpholinoethoxy)benzylidene)thiazolidine-2,4-dione (**5**)* Obtained by recrystallization as a yellow solid (1.16 g, 82% yield); mp 156–158 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.14 (s, 1H), 7.71 (s, 1H), 7.54 (d, J=8.7 Hz, 2H), 7.10 (d, J=8.7 Hz, 2H), 4.22 (t, J=11.4 Hz, 2H), 3.81 (t, J=9.6 Hz, 4H), 3.13 (t, J=11.4 Hz, 2H), and 2.76 (t, J=9.6 Hz, 4H).

*5-(4-(2-(Thiophen-3-yl)ethoxy)benzylidene)thiazolidine-2,4-dione (**6**)* Obtained by recrystallization as a yellow solid (1.24 g, 86% yield); mp 198–203 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.12 (s, 1H), 7.69 (s, 1H), 7.56 (d, J=11.7 Hz, 2H), 7.45 (d, J=7.8 Hz, 1H), 7.31 (d, J=7.8 Hz, 1H), 7.11 (d, J=11.7 Hz, 2H), 7.09 (s, 1H), 4.28 (t, J=13.8 Hz, 2H), and 3.07 (t, J=13.8 Hz, 2H).

*5-(4-(2-Isopropoxyethoxy)benzylidene)thiazolidine-2,4-dione (**7**)* Obtained by recrystallization as a yellow solid (1.26 g, 86% yield); mp 151–153 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (s, 1H), 7.81 (s, 1H), 7.43 (d, J=9.0 Hz, 2H), 7.02 (d, J=9.0 Hz, 2H), 4.19 (t, J=9.6 Hz, 2H), 3.84 (t, J=9.6 Hz, 2H), 3.65–3.76 (m, 1H), and 1.24 (d, J=6.0 Hz, 6H).

*5-(4-(2-(Pyridin-2-yl)ethoxy)benzylidene)thiazolidine-2,4-dione (**8**)* Obtained by recrystallization as a yellow solid (1.21 g, 85% yield); mp 205–208 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.36 (s, 1H), 8.45 (d, J=4.8 Hz, 1H), 7.78 (s, 1H), 7.72–7.28 (m, 1H), 7.488 (d, J=8.4 Hz, 2H), 7.28 (d, J=7.8 Hz, 1H), 7.19–7.23 (m, 1H), 6.92 (d, J=8.4 Hz, 2H), 4.17 (t, J=14.7 Hz, 2H), and 3.05 (t, J=14.7 Hz, 2H).

*5-(4-(2-(Cyclohexylamino)ethoxy)benzylidene)thiazolidine-2,4-dione (**9**)* Obtained by recrystallization as a yellow solid (1.11 g, 76% yield); mp 203–207 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.50 (d, J=8.7 Hz, 2H), 7.31 (s, 1H), 7.07 (d, J=8.7 Hz, 2H), 4.24 (t, J=9.9 Hz, 2H), 3.30 (t, J=9.9 Hz, 2H), 2.87–2.94 (m, 1H), 2.28 (s, 1H), 1.89–2.07 (m, 2H), 1.73–1.89 (m, 2H), 1.57–1.61 (m, 1H), and 1.09–1.30 (m, 4H).

*5-(4-(2-(Tetrahydro-2H-pyran-2-yl)ethoxy)benzylidene)thiazolidine-2,4-dione (**10**)* Obtained by recrystallization as a yellow solid (1.27 g, 88% yield); mp 196–202 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.29 (s, 1H), 7.73 (s, 1H), 7.55 (d, J=9.0 Hz, 2H), 7.10 (d, J=9.0 Hz, 2H), 3.98 (d, J=5.1 Hz, 2H), 3.85–3.89 (m, 2H), 3.60–3.64 (m, 1H), 1.97–2.12 (m, 4H), 1.60–1.86 (m, 2H), and 1.28–1.47 (m, 2H).

*5-(4-(2-(Piperidin-1-yl)ethoxy)benzylidene)thiazolidine-2,4-dione (**11**)* Obtained by recrystallization as a yellow solid (1.13 g, 80% yield); mp 182–190 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.52 (s, 1H), 7.41 (d, J=10.2 Hz, 2H), 6.97 (d, J=10.2 Hz, 2H), 4.10 (t, J=11.1 Hz, 2H), 2.81 (t, J=11.1 Hz, 2H), 2.38 (m, 4H), 1.41–1.51 (m, 4H), and 1.29–1.31 (m, 2H).

*5-(4-(2-(4-Methylthiazol-5-yl)ethoxy)benzylidene)thiazolidine-2,4-dione (**12**)* Obtained by recrystallization as a yellow solid (1.27 g, 88% yield); mp 258–265 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.49 (s, 1H), 8.82 (s, 1H), 7.72 (s, 1H), 7.56 (d, J=8.7 Hz, 2H), 7.10 (d, J=8.7 Hz, 1H), 4.24 (t, J=12.3 Hz, 2H), 3.25 (t, J=12.3 Hz, 2H), and 2.28 (s, 3H).

*5-[4-(3-Thiomorpholine-1,1-dioxidepropoxy)benzylidene]-thiazolidine-2,4-dione (***13***)* Obtained by recrystallization as a yellow solid (1.07 g, 81% yield); mp 218–220 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.19 (s, 1H), 7.71 (s, 1H), 7.54 (d, J=8.1 Hz, 2H), 7.09 (d, J=8.1 Hz, 2H), 4.09 (t, J=12 Hz, 2H), 3.06–3.89 (m, 8H), 2.62 (t, J=14.1 Hz, 2H), and 1.82–1.91 (m, 2H).

*5-(4-(Thiophen-2-ylmethoxy)benzylidene)thiazolidine-2,4-dione (***14***)* Obtained by recrystallization as a yellow solid (1.17 g, 81% yield); mp 215–222 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.51 (s, 1H), 7.75 (s, 1H), 7.57 (d, J=8.4 Hz, 2H), 7.56 (t, J=2.4 Hz, 1H), 7.25 (t, J=3.3 Hz, 1H), 7.19 (d, J=8.4 Hz, 2H), 7.05 (m, 1H), and 5.37 (s, 2H).

*5-(4-(Thiophen-3-ylmethoxy)benzylidene)thiazolidine-2,4-dione (***15***)* Obtained by recrystallization as a yellow solid (1.24 g, 86% yield); mp 208–216 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.50 (s, 1H), 8.59 (s, 1H), 7.86 (d, J=7.8 Hz, 1H), 7.73 (s, 1H), 7.57 (d, J=8.7 Hz, 2H), 7.52 (d, J=7.8 Hz, 1H), 7.20 (d, J=8.7 Hz, 2H), and 5.25 (s, 2H).

*5-(4-(2-Cyclopentylethoxy)benzylidene)thiazolidine-2,4-dione (***16***)* Obtained by recrystallization as a yellow solid (1.16 g, 81% yield); mp 169–172 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.50 (s, 1H), 7.74 (s, 1H), 7.55 (d, J=8.7 Hz, 2H), 7.09 (d, J=8.7 Hz, 2H), 4.14 (t, J=13.2 Hz, 2H), 1.81–1.98 (m, 1H), 1.70–1.77 (m, 4H), 1.46–1.61 (m, 4H), and 1.10–1.19 (m, 2H).

*5-(4-(Furan-2-ylmethoxy)benzylidene)thiazolidine-2,4-dione (***17***)* Obtained by recrystallization as a yellow solid (1.26 g, 85% yield); mp 210–213 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.52 (s, 1H), 7.75 (s, 1H), 7.71 (t, J=1.8 Hz, 1H), 7.57 (d, J=8.4 Hz, 1H), 7.20 (d, J=8.4 Hz, 2H), 6.63 (d, J=3.0 Hz, 1H), 6.48 (d, J=1.8 Hz, 1H), and 5.14 (s, 2H).

*5-(4-(Pyridin-2-ylmethoxy)benzylidene)thiazolidine-2,4-dione (***18***)* Obtained by recrystallization as a yellow solid (1.24 g, 85% yield); mp 232–235 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.56 (s, 1H), 8.59 (d, J=4.2 Hz, 1H), 7.87 (t, J=16.8 Hz, 1H), 7.74 (s, 1H), 7.58 (d, J=9.0 Hz, 2H), 7.53 (d, J=7.8 Hz, 1H), 7.37 (m, 1H), 7.20 (d, J=9.0 Hz, 2H), and 5.25 (s, 2H).

*5-(4-(4-Methoxybenzyloxy)benzylidene)thiazolidine-2,4-dione (***19***)* Obtained by recrystallization as a yellow solid (1.19 g, 86% yield); mp 185–190 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.52 (s, 1H), 7.90 (s, 1H) 7.47 (d, J=8.7 Hz, 2H), 7.25 (d, J=8.7 Hz, 2H), 7.31 (d, J=8.4 Hz, 2H), 7.17 (d, J=8.4 Hz, 2H), 5.06 (s, 2H), and 3.79 (s, 3H).

*5-(4-(4-Methylbenzyloxy)benzylidene)thiazolidine-2,4-dione (***20***)* Obtained by recrystallization as a yellow solid (1.15 g, 80% yield); mp 218–223 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.51 (s, 1H), 7.80 (s, 1H), 7.56 (d, J=8.7 Hz, 2H), 7.35 (d, J=7.8 Hz, 2H), 7.21 (d, J=7.8 Hz, 2H), 7.17 (d, J=8.7 Hz, 2H), 5.13 (s, 2H), and 2.30 (s, 3H).

*5-(4-(Benzo[d][1,3]dioxol-5-ylmethoxy)benzylidene)thiazolidine-2,4-dione (***21***)* Obtained by recrystallization as a yellow solid (1.22 g, 88% yield); mp 210–215 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.50 (s, 1H), 7.72 (s, 1H), 7.56 (d, J=9.0 Hz, 2H), 7.16 (d, J=9.0 Hz, 2H), 7.01 (s, 1H), 6.96 (d, J=8.1 Hz, 2H), 6.92 (d, J=8.1 Hz, 1H), 6.01 (s, 2H), and 5.06 (s, 2H).

*5-(4-(Cyclopentylmethoxy)benzylidene)thiazolidine-2,4-dione (***22***)* Obtained by recrystallization as a yellow solid (1.16 g, 78% yield); mp 174–176 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.50 (s, 1H), 7.73 (s, 1H), 7.55 (d, *J*=9.0 Hz, 2H), 7.09 (d, *J*=9.0 Hz, 2H), 3.92 (d, *J*=7.2 Hz, 2H), 2.25–2.35 (m, 1H), 1.75–1.77 (m, 2H), 1.53–1.60 (m, 4H), and 1.28–1.34 (m, 2H).

*5-(4-(4-(Chloromethyl)benzyloxy)benzylidene)thiazolidine-2,4-dione (***23***)* Obtained by recrystallization as a yellow solid (1.12 g, 81% yield); mp 187–190 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.55 (s, 1H), 7.75 (s, 1H), 7.58 (d, *J*=12.3 Hz, 2H), 7.45 (m, 4H), 7.19 (d, *J*= 12.3 Hz, 2H), 5.22 (s, 2H), and 4.76 (s, 2H).

*5-(4-((4-Methylcyclohexyl)methoxy)benzylidene)thiazolidine-2,4-dione (***24***)* Obtained by recrystallization as a yellow solid (1.17 g, 86% yield); mp 133–135 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.49 (s, 1H), 7.69 (s, 1H), 7.54 (d, *J*=9.0 Hz, 2H), 7.10 (d, *J*= 9.0 Hz, 2H), 3.96 (d, *J*=6.9 Hz, 1H), 3.85 (d, *J*=6.6 Hz, 1H), 1.66–1.98 (m, 4H), 1.19–1.52 (m, 4H), 0.98–1.16 (m, 2H), and 0.85–0.94 (m, 3H).

*5-(4-(2-(Cyclohexyloxy)ethoxy)benzylidene)thiazolidine-2,4-dione (***25***)* Obtained by recrystallization as a yellow solid (1.14 g, 82% yield); mp 115–118 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.65 (s, 1H), 7.73 (s, 1H), 7.44 (d, *J*=14.7 Hz, 2H), 7.02 (d, *J*=14.7 Hz, 2H), 4.20 (t, *J*=9.9 Hz, 2H), 3.30 (t, *J*=9.9 Hz, 2H), 3.32–3.40 (m, 1H), 1.96–2.18 (m, 2H), 1.75–1.77 (m, 2H), 1.55–1.59 (m, 1H), and 1.22–1.39 (m, 5H).

*5-(4-((2,3-Dihydrobenzo[b][1,4]dioxin-2-yl)methoxy)benzylidene)thiazolidine-2,4-dione (***26***)* Obtained by recrystallization as a yellow solid (1.12 g, 82% yield); mp 209–213 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.51 (s, 1H), 7.73 (s, 1H), 7.58 (d, *J*=8.7 Hz, 2H), 7.17 (d, *J*=8.7 Hz, 2H), 6.82–6.92 (m, 4H), 4.46–4.58 (m, 1H), 4.41–4.46 (m, 1H), 4.26–4.41 (m, 2H), and 4.11–4.17 (m, 1H).

*Methyl4-((4-((2,4-dioxothiazolidine-5-ylidene)methyl)phenoxy)methyl)cyclohexane carboxylate (***27***)* Obtained by recrystallization as a yellow solid (1.03 g, 74% yield); mp 176–178 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.48 (s, 1H), 7.74 (s, 1H), 7.55 (d, *J*= 15.9 Hz, 4H), 7.58 (t, *J*=9.0 Hz, 2H), 7.10 (d, *J*=9.0 Hz, 2H), 3.90 (d, *J*=6.6 Hz, 2H), 3.61 (s, 3H), 2.60–2.62 (m, 1H), 1.89–1.98 (m, 3H), 1.45–1.68 (m, 4H), and 1.27–1.34 (m, 2H).

*5-(4-(Biphenyl-4-ylmethoxy)benzylidene)thiazolidine-2,4-dione (***28***)* Obtained by recrystallization as a yellow solid (1.12 g, 84% yield); mp 232–237 °C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 12.51 (s, 1H), 7.74 (s, 1H), 7.70 (t, *J*=15.9 Hz, 4H), 7.58 (t, *J*=15.9 Hz, 4H), 7.49 (t, *J*=14.7 Hz, 2H), 7.38 (m, 1H), 7.21 (d, *J*=8.7 Hz, 2H), and 5.24 (s, 2H).

Algal Cultures, Mediums, and Culture Conditions

Heterosigma akashiwo (CCMP 452) was obtained from the Provasoli-Guillard Center for the Culture of Marine Phytoplankton (CCMP). The microalga, *Chattonella marina*, *Navicula pelliculosa*, and *Amphidinium* sp., were provided by Professor M.-S. Han from Life Science Department of Hanyang University in Korea.

The red tide-causing marine strain *Cochlodinium polykrikoides* was obtained from the algal culture collection National Fisheries Research and Development Institute (NFRDI). *Nanno-*

chloropsis oculata and *Phaeodactylum* EPV were kindly provided by Professor M.-K. Kim from Young Nam University in Korea.

Cultures of *H. akashiwo*, *C. marina*, and *C. polykrikoides* and non-harmful algae were grown in a culture flask (Becton Dickinson Labware, Franklin Lakes, NJ, USA) at 20 °C under constant light in Guillard's f/2 medium with filtered seawater, as reported previously [35]. The f/2 medium was prepared by sterile-filtering seawater with 0.22 μm filtration units (Nalgene, Rochester, NY, USA) and enriched aseptically using nutrients and vitamins purchased from Sigma (St. Louis, MO, USA).

Screening of Algicidal Activities of TD Compounds

The algicidal activity of the different TD compounds against *H. akashiwo*, *C. marina*, and *C. polykrikoides* were examined at various concentrations. Each experiment was carried out 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 the microalgae were exposed to the compounds at final concentrations of 100, 50, 20, 10, 5, 2, 1, 0.1, and 0.05 μM. As the non-harmful algal control, the TDs were applied at concentrations >500 μM. The control cultures were performed without the TD compounds. The algal cell density was counted 3 days after inoculation them with the compounds. The algal cells were counted using a Burker Turk hemacytometer with Sedgwick–Rafter counting chamber under an Olympus light microscope with ×40 and ×100 magnification (Olympus Co., Tokyo, Japan). Algicidal activity profiling of the TD compounds growth rates were then calculated and are expressed as the reduction ratio (percent) from the number of cell divisions per day. The reduction ratio (percent) was determined using the following equation: $\%\mathrm{Algicidalactivity} = (1 - Tt/Ct) \times 100$, where T (treatment) and C (control) are the cell densities with and without each TD compound at different concentrations, and t is the inoculation time (day).

Statistical Data Analysis

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

Results and Discussion

Scheme 1 shows the general synthetic routes for the TD derivatives. *p*-Hydroxybenzaldehyde, as a starting material, was reacted with various substituents to afford the substituted benzaldehyde, an intermediate, in good yield. The intermediate was then used in the coupling reaction with thiazolidine-2,4-dione to afford the appropriated TD derivatives.

The different TD derivatives were screened for their algicidal activity against harmful red tide algae. The specificity and potency of the 28 TD compounds against *H. akashiwo*, *C. marina*, and *C. polykrikoides* were determined. The synthesized compounds were tested under the assay conditions at various micromolar concentrations, and the results and statistical significance were verified. Their inhibitory activity (IC_{50}) values are listed in

Table 1. When algicidal activity of the TDs to *H. akashiwo, C. marina*, and *C. polykrikoides* were compared, some of the TDs required high concentrations to inhibit growth, whereas a number of TDs showed moderate algicidal activity at the lowest concentrations against the red tide-causing alga selected for this study.

TD compounds, such as compounds **2**, **4**, **6**, **14**, **15**, **16**, **20**, and **21**, exhibited IC_{50} values <4 μM for all HABs studied, which makes them possible candidates for algicidal application. In contrast, compounds **3** and **5** exhibited relatively high IC_{50} values ranging from 35 to 120 μM, which suggests that the HABs are tolerant to these TDs. Among the 28 compounds tested, compounds **14**, **17**, **18**, and **25** exhibited IC_{50} of approximately 5–20 μM for all dinoflagellates studied. These compounds were potent for both HABs and non-harmful dianoflagellates indicating that these compounds are unsuitable as algicidal compounds (Table 1).

Other non-harmful algal controls, *N. oculata* and *Phaeodactylum* EPV, used in animal feed in the aqua industry, were not inhibited by any of the TDs studied.

The growth inhibition patterns of the HABs, including non-harmful algae, were examined upon exposure to compounds **6** and **16** at different concentrations for example (Fig. 1). The growth patterns showed an exponential decrease with increasing concentration of the selective TDs in the medium (Fig. 1). On the other hand, the non-harmful algae were relatively tolerant to these selective TDs.

C. polykrikoides blooms have caused heavy damage to fish farms in the Republic of Korea, Japan, and other countries [36–38]. The use of non-specific chemical algicides has been reported to have a number of drawbacks, including broad-spectrum toxicity towards phytoplankton [23–26, 39–42].

Of the compounds examined, compounds **4**, **6**, **7**, **12**, **13**, **14**, **15**, **16**, **17**, **18**, **19**, **20**, **21**, **22**, **22**, and **26** were the most potent against *C. polykrikoides* exhibiting IC_{50} values <1 μM. In particular, compounds **7**, **13**, and **27** were extremely competent and selective against *C. polykrikoides* and exhibited an IC_{50} value ranging from 0.1 to 2 μM, while *C. marina* and *H. akashiwo* showed an IC_{50} value ranging from 30 to 130 μM. Therefore, these compounds may have structural competence to *C. polykrikoides*, but the mechanisms of this selectivity need to be examined further.

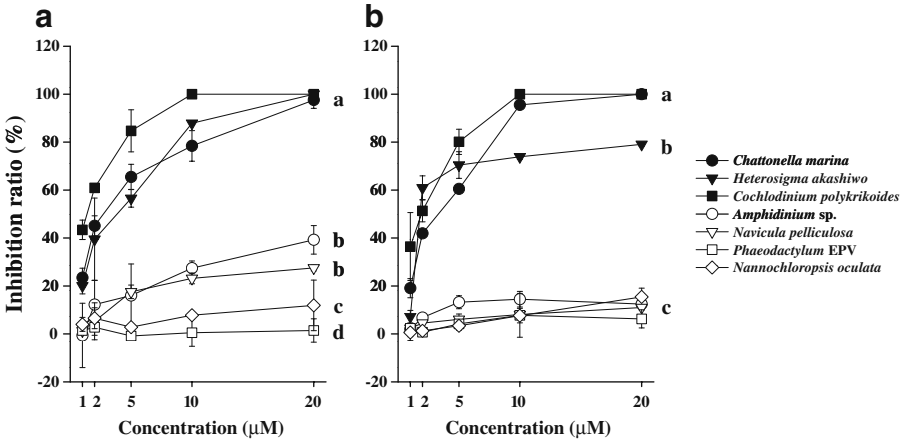


Fig. 1 Growth reduction ratio at different concentrations of the selected thiazolidinedione compounds against harmful and non-harmful algal species. **a** Compound **6**, (**b**) compound **16**, the *letters* in graphs **a** and **b** represent the means that are significantly different ($P<0.05$) using a Tukey's post hoc test

Red tide inhibitors made with Chinese herbs, such as golden thread and areca seed, had the merits of a low concentration and short reaction time [20]. Some natural materials were found to be selective against blue-green algae [43–45]. However, their large-scale use in the environment is still problematic. Biological control has many logistical problems and is far from the application stage [2]. The chemical structure of the TDs may be the reason for their selective and wide range of algicidal activity. The effective concentrations, potency, growth inhibition ratio, and their selective pattern to their corresponding functional groups of derivatives were studied (Table 1). The high activities of particular compounds, which are specific against only harmful algal species, might take advance of specific functional groups and their position. Their structural relation and importance in algicidal actions are discussed based on these results.

For the purpose of preliminary structure activity relationship studies, various substituents were introduced at the hydroxyl group of 5-(4-hydroxybenzylidene)thiazolidine-2,4-dione. The introduction of the 5-member ring at the hydroxyl group of 5-(4-hydroxybenzylidene) thiazolidine-2,4-dione resulted in a significant increase in its inhibitory potency against *C. polykrikoides*, as indicated for compounds **4**, **6**, **12**, **14**, **15**, **16**, **17**, and **22**. Interestingly, the 3-thiomorpholine-1,1-dioxidepropyl group as a substituent also increased the inhibitory potency against *C. polykrikoides* significantly, as indicated for compound **13**. However, a deletion of the methylene group of the 3-thiomorpholine-1,1-dioxidepropyl derivative (compound **3**) decreased the inhibitory potency of compound **13**. The most potent inhibitors of this series of compounds against *C. marina*, *H. akashiwo*, and *C. polykrikoides* are compounds 5-(4-((1-methylcyclohexyl)methoxy)benzylidene)thiazolidine-2,4-dione (compound **2**), 5-(4-(thiophen-2-ylmethoxy)benzylidene)thiazolidine-2,4-dione (compound **14**), and 5-(4-(3-thiomorpholine-1,1-dioxidepropoxy)benzylidene)-thiazolidine-2,4-dione (compound **13**) with a IC_{50} of 0.426, 1.927, and 0.133 μM, respectively. Table 1 gives a summary of the inhibitory potency of all these compounds.

In summary, this study examined TD derivatives as potential algicidal agents. This study provides strong evidence that several TD derivatives have effective algicidal activity against HABs but are safe to non-harmful algae. A few of the TDs exhibited remarkable algicidal activity against *H. akashiwo*, *C. marina*, and *C. polykrikoides* with low IC_{50} values (0.1 to 2 μM). Insights regarding the use of TD compounds may not limit or restrict the growth of other organisms due to their selectivity. A precise insight into the inhibitory action against the red tide algal remains to be determined. In addition, there are many aspects of potent or competent algal inhibitors in the treatment of HABs that need to be clarified, including safety, lifetime, water solubility, and stability.

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