Performance of Marine Bioactivities of N-Acylxyethyl-1,2-Benzisothiazol-3(2H)-one Antifouling Paint

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Abstract: A series of N-acloxyethyl-1,2-Benzisothiazol-3(2H)-ones were synthesized. Their inhibitory potency the growth of Isochrysis galbana and Platymonas subcordiformis and Navicula, bioactivities on barnacle nauplii, and marine antifouling application were studied. The results obtained here showed that the target compounds had favorable inhibitory activities on marine alga. The bioactivities on barnacle larvae were obvious when the concentration of target compounds was 1.0 mg/L. The studies of marine antifouling showed that the target compounds had a better antifouling performance of in the seawater.

1. Introduction

Marine biological fouling, a process known as biofouling, can be defined as an undesirable accumulation of animals, plants, and microorganisms on solid surfaces immersed into sea water.¹,² Surfaces such as ships, staff floats and various artificial facilities submerged in marine environments often naturally are colonized rapidly by marine organisms. The buildup of biofouling on marine vessels may cause a serious problem. For instances, the hull surface structure and driving systems can be damaged.³ The accumulation of fouling organisms on hulls can increase both the hydrodynamic friction and the hydrodynamic volume of a vessel, causing drag to be increased up to 60%.⁴ It is reported that decreasing speeds by up to 10% requires increase of fuel consumption up to 40%.⁵ It is predicted that the increased fuel use due to biofouling contributed to adverse environmental effects and the increase of emissions of carbon dioxide and sulfur dioxide between 38% and 72% by 2020.⁶ In addition, the negative effects caused by this biological settlement includes not only the high frictional resistance, but also the increase of the operation frequency of dry-docking, deterioration of the surface coating, and in draught of alien species.⁷⁻⁹

The common and effective methods for combating biofouling were coated with an anti-fouling paint containing toxic ingredient which was often detrimental to non-target environmental organisms. The traditional organotin and cuprous oxide were added to the coatings for preventing biofouling, which were harmful to marine fish.⁵ Therefore, foul-release coatings, which minimize attachment and adhesion of fouling organisms (rather than killing them) were promising alternatives. Development of a green, efficient and non-toxic antifouling coating for combating marine biofouling
is imperative. \cite{6} Benzo[d]isothiazol-3(2H)-one was a kind of broad-spectrum anti-bacterial material with high efficiency and low toxicity and environmental safety. \cite{7} In our previous work, derivatives of benzo[d]isothiazol-3(2H)-one bearing an aliphatic ester group are designed and synthesized, those compounds had an excellent inhibitory activity for bacteria and fungi. \cite{10,12} In this paper, a further research on their inhibitory properties on the growth of Isochrysis galbana, Platymonas subcordiformis and Navicula, the bioactivities on barnacle nauplii, and marine antifouling application were reported.

2. Result and Discussion

2.1. Chemistry

N-Acyloxyethyl-1,2-Benzisothiazol-3-ones 2 were prepared as shown in Scheme 1. Firstly, commercially available 1,2-benzisothiazol-3-one (BIT) was alkylated by treatment with 2-chloroethanol and sodium carbonate in water to produce, the N-hydroxyethyl-benzisothiazol-3-one 1, and the resulting compound 1 further reacted with aliphatic acid at room temperature to generate N-acyloxyethyl-benzisothiazol-3-one derivatives 2 in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethyl-aminopyridine (DMAP).

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\begin{align*}
\text{CICH}_2\text{CH}_2\text{OH} & \quad \text{NaOH} , 100 \quad \text{Microwave} \\
\text{CICH}_2\text{CH}_2\text{OH} & \quad \text{NaOH} , 100 \quad \text{Microwave} \\
\text{CICH}_2\text{CH}_2\text{OH} & \quad \text{NaOH} , 100 \quad \text{Microwave} \\
\end{align*}
\]

Scheme 1. Synthesis of N-Acyloxyethyl-1,2-Benzisothiazol-3(2H)-ones.

2.2. Biological activity

The inhibition ability of N-acyloxyethyl-benzisothiazol-3-one derivatives 2 for the growth of seaweed were examined (Fig. 1).

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\text{Fig. 1 The inhibition ability of compound 2 for the Isochrysis galbana}
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The target compounds 2 displayed a significant inhibitory potency on the growth of Isochrysis galbana (Fig. 1). In the beginning, the Isochrysis galbana showed a little survivability, growing slowly in the solution of the target compound within 24 hours. With the development of the cultivation, the compounds exhibited obvious inhibitory activities on the growth of Isochrysis galbana after 24 h. Compounds with long chain alkyl 2d, 2e, 2f, 2g had a good growth inhibitory activity against Isochrysis galbana, and it almost stopped growing after 70 h. The compounds 2e with a n-amyl group, 2f with a n-undecyl group, and 2g with a tetradecyl group showed the strongest inhibitory activities, leading growth of the Isochrysis galbana to almost stop. The inhibitory activity of compound 2a with a methyl group is lower than that of BIT, but is higher than that of control group. The inhibitory
activity of 2b with an ethyl group and 2c with a propyl group is comparable to that of the BIT. Those results showed that the inhibitory properties on the growth of Isochrysis galbana were associated with the structure: the inhibition efficiency would be enhanced with the increase of carbon chain in the substituents.

Fig. 2 The impact of compounds on Platymonas subcordiformis

Compounds 2 showed remarkable potency inhibitory activities on Platymonas subcordiformis (Fig. 2). With the prolongation of incubation, the compounds showed different inhibitory activities against Platymonas subcordiformis. Compared with BIT, compounds 2d, 2e, 2f and 2g displayed stronger inhibitory activities against the algae after 25h, resulting in complete stop of the growth of Platymonas subcordiformis. The inhibitory activities of compounds 2a, 2b and 2c were similar to that of BIT, but stronger than that of blank control group. The results indicated that the inhibitory effects of the target compounds on the marine alga were enhanced with the increase of the carbon chain. Compounds 2 have obvious inhibitory activities on the growth of Navicula (Fig. 3). The inhibition on Navicula is less than that on Isochrysis galbana and Platymonas subcordiformis, but can compound 2 can slow the growth of Navicula. There were no outstanding differences of the inhibition between compound 2 on Navicula in 30 h, but all the inhibitions were better than BIT. However, compounds 2b, 2d, 2f and 2g had stronger activities than those of 2a, 2c, 2c on the growth inhibition properties in 72 h.

Fig. 3 The impact of compounds on Navicula

2.3 Biological activities on barnacle larvae

The acute toxicity effects of compounds 2 on barnacle larvae were measured in Fig. 4 and Table 1. The mortality rate of the barnacles larvae caused by compounds 2a-2c could be up to 100% when the concentration of the compounds was 5.0 mg/L at 12 h. As shown in the Fig.4, the mortality rate of barnacle larvae increased slowly with the increase of concentration of BIT from 1 mg/L to 5mg/L, while, the mortality rate of barnacle larvae caused compounds 2 substituted by a fatty acid ethyl ester
increased faster with the increase of concentration, indicating that the sensitivity of the barnacle larvae to the test compounds was stronger. The lethal rate of barnacle larvae with compound 2f was only 7.8%, while the lethal rate of barnacle larvae reached 51.1% with compound 2a when the concentration of compound was 1.0 mg/L at 12 h. When the concentration of 2f was increased to 5.0 mg/L, the lethal rate of barnacle larvae was up to 91.1%. The result showed that the larvae were highly sensitive to compound 2f, indicating that 2f possessed high anti-fouling performance: the compounds showed a significant lethal effect at a very low concentration.

Table 1 The mortality of barnacle larvae of (12h)

| Compd. | 1.0 mg/L | 2.0 mg/L | 3.0 mg/L | 4.0 mg/L | 5.0 mg/L |
|--------|---------|---------|---------|---------|---------|
| a      | 51.1%   | 64.4%   | 85.6%   | 98.9%   | 100.0%  |
| b      | 32.2%   | 61.1%   | 81.1%   | 97.5%   | 100.0%  |
| c      | 35.6%   | 54.4%   | 71.1%   | 93.3%   | 100.0%  |
| d      | 37.8%   | 50.0%   | 72.2%   | 93.3%   | 98.9%   |
| e      | 11.1%   | 32.2%   | 53.3%   | 81.1%   | 98.9%   |
| f      | 7.8%    | 25.6%   | 46.7%   | 75.6%   | 91.1%   |
| g      | 6.7%    | 27.8%   | 45.6%   | 71.1%   | 82.2%   |
| BIT    | 24.4%   | 33.3%   | 38.9%   | 44.4%   | 53.3%   |

Fig. 4 Chemical toxicity effect on barnacle larvae

Table 2 The mortality of barnacle larvae of (24h)

| Compd. | 1.0 mg/L | 2.0 mg/L | 3.0 mg/L | 4.0 mg/L | 5.0 mg/L |
|--------|---------|---------|---------|---------|---------|
| a      | 55.56%  | 80.00%  | 94.44%  | 100.00% | 100.00% |
| b      | 41.11%  | 68.89%  | 87.78%  | 100.00% | 100.00% |
When the concentration of compounds 2 increased to 5 mg/L, the mortality rate of barnacle larvae reached 91%, while the value of BIT was 64.44% after 24 h (Figure 5 and Table 2). Those results showed that compound 2 had a good inhibitory effect on the barnacle larvae at high concentrations, and the toxic effect was better than that of BIT. It's shown in Table 2 when increasing the concentration of compounds 2, the mortality of barnacle larvae will be increased. Moreover, when the concentration of the compounds was in the range of 1.0 mg/L to 4.0 mg/L, the desirable mortality trend of barnacle larvae could be obtained.

All benzisothiazol-3-ones 2 substituted by fatty groups displayed a low LC$_{50}$ value with a concentration of µg/L against Balanus reticulates naupliar larva II (Table 3). The LC$_{50}$ values of 2 in 12 h and 24 h were lower than that of BIT, implying better inhibitory potency on barnacle larvae. Compound 2a with a methyl group showed the highest, followed by 2b, 2c, 2d, 2e, 2f and 2g.

### Table 3 The LC$_{50}$ of organic compounds on barnacle larvae:

| No. | LC$_{50}$ c / (µg·L$^{-1}$)  |
|-----|----------------------------|
|     | 12 h | 24 h |
| a   | 1182  | 933  |
| b   | 1470  | 1236 |
| c   | 1538  | 1277 |
| d   | 1545  | 1165 |
| e   | 2220  | 1632 |
| f   | 2640  | 1780 |
| g   | 2868  | 1938 |
| BIT | 4468  | 2937 |

#### 3. Experimental

**3.1 Chemistry: general procedures**

All chemicals and solvents were purchased from commercial suppliers and used without further purification. Melting points were determined using a digital melting point apparatus and uncorrected. Shanghai sanpont GF$_{254}$ plates were used as TLC analytical; flash column chromatography was performed on shanghai sanpont Gel (100-200 mesh). $^1$H NMR spectra were recorded on a 400 MHz Bruker Avance. Chemical shifts for $^1$H NMR spectra are quoted in ppm downfield from TMS. Coupling constants are referred to as J values in hertz. FT-IR spectra were recorded at room temperature in the range of 4000-400 cm$^{-1}$ with a PerkinElmer spectrometer 100 FT-IR spectrometer using KBr pellets.

**3.2. General procedure for acyloxyethyl-1,2-benzisothiazol-3(2H)-one**

In 150 mL three-necked, round-bottomed flask, 1.6 g of sodium hydroxide and 50 mL water was mixed until the sodium hydroxide dissolved completely, and then 3.0 g of BIT and 8 ml of chloroethanol were added. The mixtures was placed in a microwave reactor under 75-80°C and stirred magnetically for 2 h. After completion of the reaction, the solvent was distilled under reduced pressure,
and 40 mL of dichloromethane was added, dried over MgSO₄. The solution was filtered to remove the solids, and the solvent was distilled under reduced pressure to yield the crude product. The crude products were recrystallized with ethyl acetate and petroleum ether mixture and the compd 1 obtained with pale yellow needles, yield 85.0%, mp 111-113°C;

In 100 mL round bottom flask equipped with magnetic stirring was charged with 0.02 mol 2-hydroxyethyl benzo[d]isothiazole-3(2H)-one, 0.025 mol fatty acid, a catalytic amount of DMAP and 20 mL dichloromethane, until the solid dissolved completely, the solution cooled in ice-bath, and added 0.026 Mol of DCC for reaction of 6-10 h. After completion of the reaction, the white precipitate was filtered, and the filtrate was concentrated, washed twice with water, products 2 were separated and purified by flash column chromatography on silica gel eluting with petroleum ether and ethyl acetate mixtures.

N-Acloyxethyl-1,2-Benzisothiazol-3(2H)-one (2a), light yellow liquid with a yield of 63.8%, ¹H NHR: (400MHz, DMSO-d₆) δ: 2.01 (s, 3H), 4.07-4.10 (t, 2H), 4.27-4.30 (t, 2H), 7.43-7.99 (m, 4H); IR (KBr, cm⁻¹): 3000, 1740, 1658, 1447, 1338, 1228, 1042, 741, 674.

N-Propionylxethy-1,2-Benzisothiazol-3(2H)-one (2b), light yellow liquid with a yield of 90%. ¹H NHR: (400MHz, DMSO-d₆) δ: 0.95-0.99 (t, 3H), 2.24-2.30 (m, 2H), 4.07-4.10 (t, 2H), 4.28-4.30 (t, 2H), 7.38-7.96 (m, 4H); IR (KBr, cm⁻¹): 2890, 1738, 1657, 1448, 1337, 1177, 1082, 741, 674.

N-Butanoyloxyethyl-1,2-Benzisothiazol-3(2H)-one (2c), light pink liquid with a yield of 87.0%. ¹H NHR: (400MHz, DMSO-d₆) δ: 0.82-0.85 (t, 3H), 1.53-1.57 (m, 2H), 2.27-2.31 (t, 2H), 4.17-4.17 (t, 2H), 4.35-4.38 (t, 2H), 7.45-8.03 (m, 4H); IR (KBr, cm⁻¹): 3963, 1737, 1660, 1449, 1337, 1173, 1081, 741, 674.

N-Valeryloxyethyl-1,2-Benzisothiazol-3(2H)-one (2d), light yellow viscous liquid with a yield of 55.6%. ¹H NHR: (400MHz, DMSO-d₆) δ: 0.79-0.74 (t, 3H), 1.10-1.19 (m, 2H), 1.37-1.45 (m, 2H), 4.08-4.10 (t, 2H), 4.28-4.31 (t, 2H), 7.38-7.96 (m, 4H); IR (KBr, cm⁻¹): 2958, 1738, 1664, 1449, 1337, 1170, 1107, 742, 674.

N-Hexanoyloxyethyl-1,2-Benzisothiazol-3(2H)-one (2e), light yellow viscous liquid with a yield of 65.0%. ¹H NHR: (400MHz, DMSO-d₆) δ: 0.79-0.82 (t, 3H), 1.14-1.21 (m, 2H), 1.46-1.53 (m, 2H), 2.27-2.31 (t, 2H), 4.16-4.18 (t, 2H), 4.36-4.39 (t, 2H), 7.44-8.02 (m, 4H); IR (KBr, cm⁻¹): 2955, 2866, 1738, 1664, 1449, 1337, 1167, 1104, 741, 674.

N-Dodecanoyloxyethyl-1,2-Benzisothiazol-3(2H)-one (2f), light yellow viscous liquid with a yield of 62.6%. ¹H NHR: (400MHz, DMSO-d₆) δ: 0.83-0.86 (t, 3H), 1.15-1.27 (m, 16H), 1.44-1.50 (t, 2H), 4.11-4.13 (t, 2H), 4.31-4.34 (t, 2H), 7.42-7.99 (m, 4H); IR (KBr, cm⁻¹): 2925, 2740, 1667, 1450, 1336, 1162, 1114, 741, 673.

N-Tetradecanoyloxyethyl-1,2-Benzisothiazol-3(2H)-one (2g), pale yellow waxy solid with a yield of 56.5%. m.p. 40-42°C; ¹H NHR: (400MHz, DMSO-d₆) δ: 0.83-0.86 (t, 3H), 1.14-1.27 (m, 2H), 1.45-1.48 (m, 2H), 2.25-2.28 (t, 2H), 4.09-4.11 (t, 2H), 4.29-4.32 (t, 2H), 7.41-8.00 (m, 4H); IR (KBr, cm⁻¹): 2924, 2853, 2740, 1667, 1449, 1335, 1163, 1114, 740, 673.

3.3 Inhibition of algae growth

Through ultraviolet wavelength scanning by spectrophotometer, to obtained the maximum absorption wavelength. The maximum absorption wavelength of Navicula, Isochrysis galbana and Platymonas subcordiformis were 342 nm, 428 nm and 680 nm respectively. The solution concentration of algae was determined by counting the blood cell count plate, and the absorption value of each diluent was measured at the maximum absorption wavelength. The standard curve was drawn with the light absorption value as the abscissa and the concentration of the algae liquid as the ordinate.

According to the absorbance & concentration curve, 50 mL of algae solution with absorbance value between 0.05-0.1 was placed in 100 mL conical flask. Preparing a solution of tested compounds with concentration of 0.2 mg/mL, then draw 1 mL into the conical flask, each group set up three parallel samples, selects BIT and the same concentration of blank as control group, and then measuring the
absorbance of each conical flask every 12 hours. In accordance with algae solution absorbance-concentration curve, calculating the corresponding concentration of algae, and drawing concentration-time curves of algae. \[13\]

3.4 Biological activity tests of barnacles
Dissolving 10 mg tested compound in 10 mL DMSO and then preparing list of solution with concentration 1, 2, 3, 4 and 5 mg/L respectively, employing BIT and blank as control groups. Each group was divided into 3 parallel experiments, and repeated the procedure 3 times. Draw quantitative barnacle larvae carefully with dropper and put it into petri dish, mixed it with tested compounds solution at the same concentration. In each dish the number of barnacle larvae is 30 and the survival of barnacle larvae be observed by stereomicroscope at 12 h and 24 h, if it was found that the barnacle larvae sink to the bottom of petri dish and stop swimming for 15 seconds or more, then judged it had been killed. \[14\]

4. Conclusions
In summary, fatty acid ethyl ester substituted 1, 2-benzisothiazol-3-one was identified as a novel and potent marine fouling organisms inhibitor. Through the research procedures of seaweed growth inhibition, inhibitory activities of barnacle larvae and the immersing sheet metal experiment, the comprehensive antifouling performance of the N-substituted-1,2- benzisothiazol-3-one compounds were evaluated. The result showed that the target compounds had obvious inhibitory effects on the growth of three marine algae. Among which compounds 2f and 2g had the strongest inhibitory on the growth of Isochrysis galbana and platymonas subcordiformis. The result of barnacle growth inhibition showed that the tested compounds have strong inhibitory effect on the growth of the barnacle, and with the increase of the concentration of the compounds and the prolongation of time and the inhibitory effect increased. The LC50 data showed that the compound with fewer carbon atoms substituent group had better inhibitory effects on barnacle larvae, and the compound with fewer carbon atoms substituent group showed relatively better antifouling effects.

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Reference
[1] Yan T, Yan W X, Dong Y , et al. Investigation biofouling in offshore areas east of Hainan Island, the South China Sea[J]. Oceanologia et Limnologia Sinica, 1998, 29 (4): 374-380.
[2] Bernardo A.P. da Gama, Erwan Plouguerné, Renato C. Pereira. Chapter Fourteen-The Antifouling Defence Mechanisms of Marine Macroalgae. Adv. Bot. Res, 2014, 71, 413-440.
[3] Ren R T, Liang J. Marine Antifouling Coatings: Development and Trends [J]. Development & Application of Materials, 2014, 29 (01): 1-8.
[4] Nikita K. Biofouling and design of a biomimetic hull-grooming tool [J]. Ship Syst. Int. Des. Dep. Tech. Rep., 2007: NSWCCD-CISD-2007/002.
[5] Wilson L H, Callow M E. Engineered antifouling micro topographies-correlating wet-ability with cell attachment [J]. Bio-fouling, 2006, 22(1): 11-21.
[6] Xu Y, Xue S J. Synthesis and Biological Activities of Alpha-Amino Acylamines Derivatives Containing Furan and Pyridine Ring [J]. Chem Res Chin Univ, 2009, 25(6): 846-850.
[7] Dou D, Alex D, Du B, Tiew KC, Aravapalli S, Mandadapu SR, Calderone R, Groutas WC. Antifungal activity of a series of 1,2-benzisothiazol-3(2H)-one derivatives. Bioorg. Med. Chem. 2011, 19, 5782–5787.
[8] Zheng H H. Bioactivity and marine antifouling performance of four kinds of Benzisothiazolones [D]. Hainan University, 2011:1-80.
[9] Qin S, Lin H Z, Jiang P. New frontiers of phycology[J]. Journal of Biology, 2010, 27(1): 64-60.
[10] Yang J X, You C H, Wang X H, Lin Q. The Synthesis and Bioactivities of 2-Hydroxy ethyl Benzoisothiazole-3(2H)-one Marine Antifouling Paints[J]. Adv. Mater. Res. 2013, 646, 24-29.
[11] You C H, Wang X H, Yin X Q, et al. Synthesis of Novel 2-Hydroxyethylbenzo[d] isothiazol-3(2H)-one Aliphatic Acid Esters and Their Anti-bacterial Activities[J]. Chinese Journal of Synthetic Chemistry, 2013, 21(2):125-128.
[12] You C H, Wang X H, Yin X Q, Yang J X, Lin Q. Synthesis of Novel 2-Hydroxyethylbenzo[d]isothiazol- 3(2H)-one Aliphatic Acid Esters and Their Anti-bacterial Activities. Chinese Journal of Synthetic Chemistry. 2013, 21(2): 125-128.
[13] Zheng H H, Wang X H, Yang J X, et al. Synthesis and biological activity of benzothiazole porphyrin ketones[J]. Journal of Biology, 2011.28(4): 7-9.
[14] Zhou Y X, Zhang Z S. Aquatic toxicity test method. First edition. Beijing: Agricultural Press, 1989, 109-143, 157-165.
[15] Xie H B, Chen Y B, Cai-Tong K E, et al. Analysis of Retaining Wall Earth Pressure under Seismic Role[J]. Natural Science Journal of Hainan University, 2014.