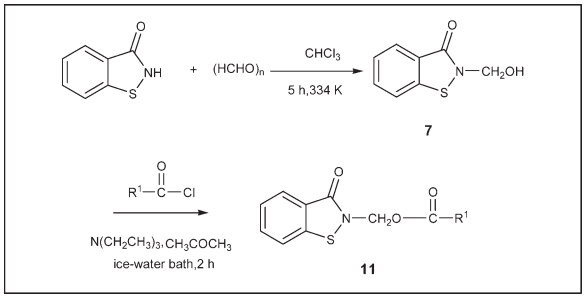
Synthesis and Bioactivity of Novel Benzisothiazolone Derivatives as Potential Microbiocides

Fengling Xu,[a,b] Qiang Lin,[c]* and Baorong Hou[a]

[a]Key Laboratory of Corrosion Science of Shandong, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China
[b]Graduate School of Chinese Academy of Science, Beijing 100039, China
[c]Key Laboratory for Tropic Biological Resources, Hainan University, Haikou 570228, China
*E-mail: lingqiang@haino.edu.cn

Received April 8, 2008
DOI 10.1002/jhet.65
Published online 13 April 2009 in Wiley InterScience (www.interscience.wiley.com).



Novel microbiocides 2-(hydroxymethyl)benzo[*d*]isothiazol-3(2*H*)-one (**7**) and (3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)methyl benzencarboxylates (**11a-c**) were synthesized in good yields, and their structures were characterized by means of ^{1}H NMR, MS, and elemental analysis. The new compounds were tested preliminarily in laboratory assays against the aquicolous bacteria including *Escherichia coli, Staphyloccus aurueus, Vibrio alginolyticus, Aeromonas hydrophila*, and *Bacillus subtilis*. The results show all the synthesized compounds have good antimicrobial activity. The antimicrobial activity of all the tested compounds against all test bacteria is >96.6% at the concentration of 10^{-2} mg mL^{-1}. These compounds can be further developed for effective microbiocides in the future.

J. Heterocyclic Chem., **46**, 320 (2009).

INTRODUCTION

Marine biological fouling, usually termed marine biofouling, can be defined as the undesirable accumulation of microorganisms, plants, and animals on artificial surfaces immersed in sea water. The first phase of the fouling is the attachment of microorganisms. So it is important to inhibit the microorganism for antifouling. After the definitive ban on tin-based antifouling substances, the most important issue for paint manufacturers is to develop an alternative antifouling paint that achieves the same performance as the organic tin-based antifouling paints without significant cost increase.

As a class of antimicrobial agents against bacteria and fungi isothiazole derivatives have many advantages such as highly efficient, low poisonous; they are environmentally friendly [1]. Isothiazole-3(2*H*)-ones and heterocyclic bioisomeric derivatives such as **1-10** shown in Figure 1 are potent industrial and medicinal microbiocides with antifungal and antibacterial activities [2–4]. Okachi *et al*. synthesized 2-(hydroxymethyl)benzo[*d*]isothiazol-3(2*H*)-one **7** and its derivatives (**8** and **9**) and tested for their *in vitro* antibacterial activity against *Mycobacterium tuberculosis* H37Rv including resistant strains against streptomycin, kanamycin, or isonicotinic acid hydrazide. All the compounds showed good antibacterial activity and no crossresistance between the current antitubercular agents [5]. Vicini *et al*. described a series of 2-aminobenzo[*d*]isothiazol-3(2*H*)-one derivatives **10**, which were synthesized and screened *in vitro* for inhibition of platelet aggregation and for their spasmolytic activity, with the awareness that the development of antiplatelet agents with additional vasodilation activity could be beneficial in the treatment of various vaso-occlusive disorders. The tested compounds show a powerful antiplatelet activity and various modifications resulted in molecules possessing antiaggregating effects as well as spasmolytic actions [6].

According to the earlier-mentioned facts and the principle of combination of bioactive substructure, we herein designed and synthesized benzisothiazolone derivatives 2-(hydroxymethyl)benzo[*d*]isothiazol-3(2*H*)-one (**7**) and (3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)methyl benzencarboxylates (**11a-c**) in good yields, and their structures as shown in Scheme 1. The structures of **11** were confirmed by means of ^{1}H NMR, MS, and

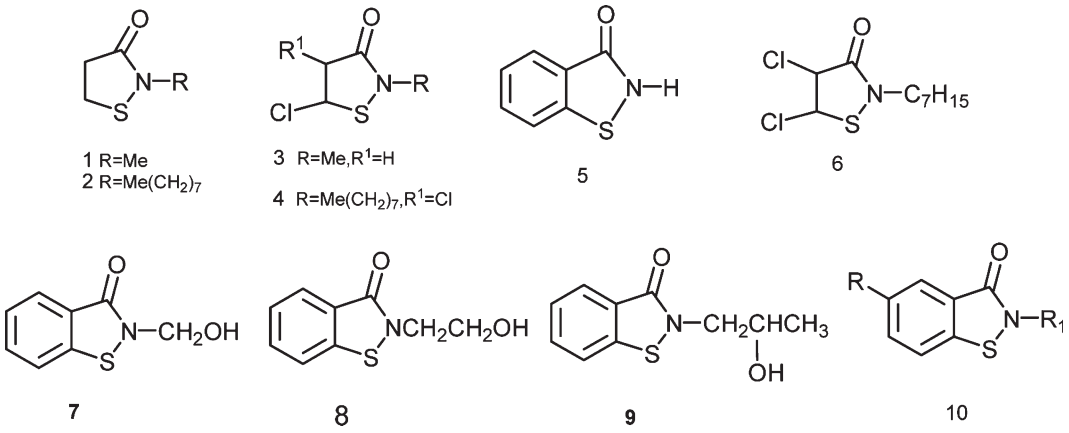


Figure 1. Previously reported bioactive compounds **1-10**.

elemental analysis. Their bioactivities were also tested preliminarily.

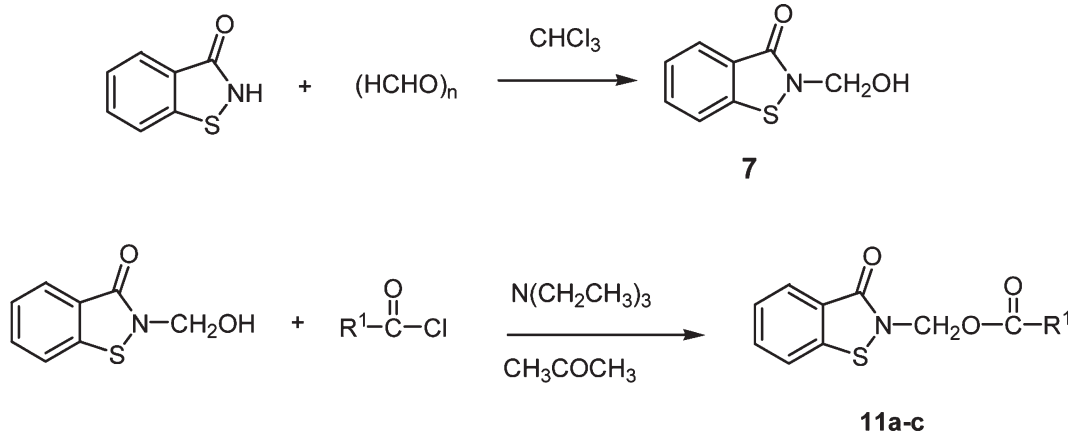
RESULTS AND DISCUSSION

We synthesized novel compounds (3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)methyl benzencarboxylates (**11a-c**) by the reaction of 2-(hydroxymethyl)benzo[*d*]isothiazol-3(2*H*)-one (**7**) and benzoyl chloride derivatives (see Scheme 1) in good yield (66–72%). Their structures were characterized by means of ^{1}H NMR, MS, and elemental analysis.

The compounds **7** and **11a-c** were preliminarily tested in laboratory *in vitro* to evaluate their antimicrobial effect. The five bacterium used here include *Escherichia coli, Staphyloccus aurueus, Vibrio alginolyticus, Aeromonas hydrophila*, and *Bacillus subtilis*. We tested the antimicrobial activity using the viable cells plate count method as described in the literature [7,8].

Plate cultures were prepared as follows (g L^{-1}): 3 g beef, 5 g peptone, 5 g sodium chloride, 1 g yeast extract, and 18 g agar. The medium was dissolved completely and the pH value of the culture medium was adjusted to 7.2 with NaOH. And then the medium was autoclaved at 121°C and 20 psi for 15 min. The compounds synthesized in this article were dissolved in DMSO concentration in different concentration and then absolutely mixed with cultures (same as plate cultures except agar) containing bacteria. The mixture was homogenized in a laboratory homogenizer (IKA, MS2, minishaker, China) and serial dilutions were prepared, then 0.1 mL of each dilution was spread with a bent sterile glass rod on duplicate prepoured plates. After

Scheme 1. Synthesis route to the title compounds **7** and **11**.



11a, R^1=C_6H_5, **11b**, R^1= 4-FC_6H_4, **11c**, R^1= 3,5-$Me_2C_6H_3$

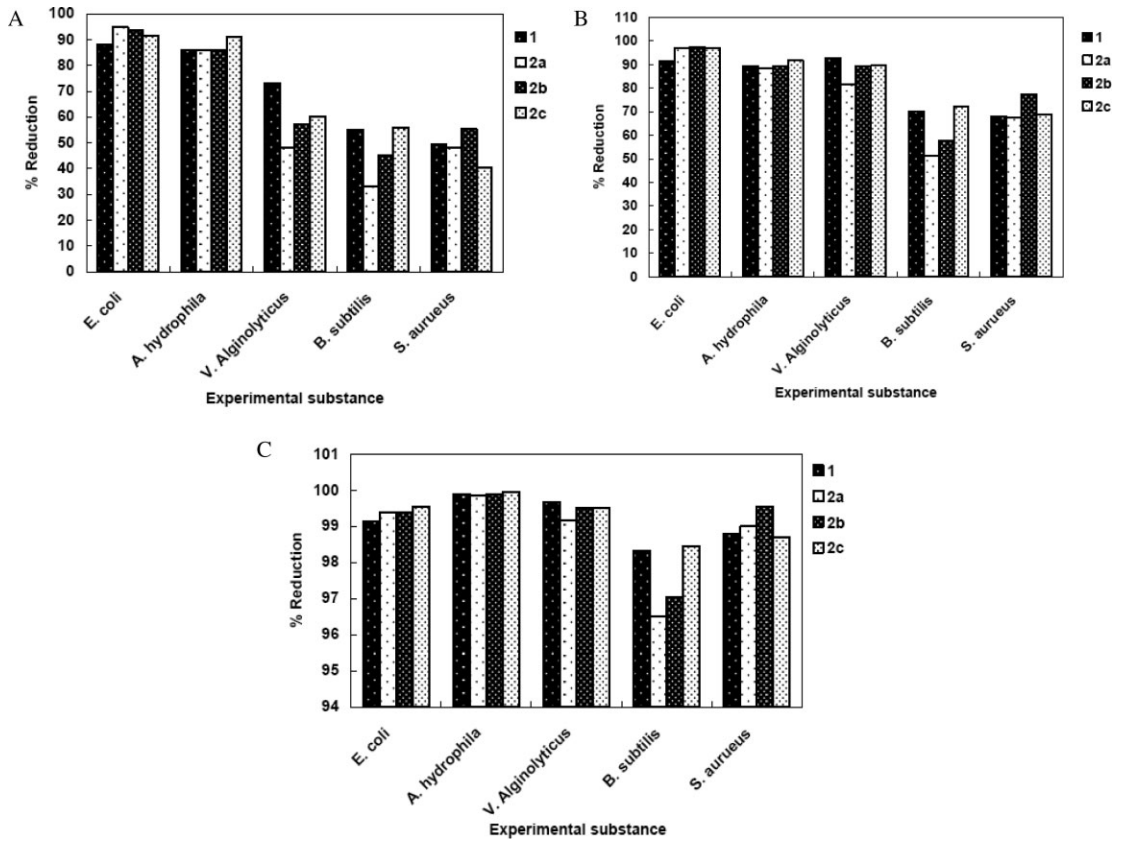


Figure 2. Microbial activity data of compounds **7** and **11a-c** in different concentration: 10^{-4} mg mL^{-1} (A), 10^{-3} mg mL^{-1} (B), 10^{-2} mg mL^{-1} (C), expressed as percent viable counting reduction.

24 h incubation at 28°C, colonies were counted. Pure DMSO (17 μL, 8.5% *v*/*v*) was used as negative control. The antimicrobial activity of the tested compounds (**11a-c**) was compared with compound **7** under similar culture conditions and has proved to be a good antimicrobial compound. Percent viable counting reduction was calculated using the following formula [9]:

$$100\% - \frac{\text{Experimental viable count}}{\text{DMSO viable count}} \times 100\% \qquad (1)$$

The antimicrobial activity of the synthesized compounds is shown in Figure 2. All the synthesized compounds showed certain antimicrobial activities against the tested bacterium. It is shown in Figure 2 that the microbial activity increased obviously with the increasing of the concentration of the compounds. When the concentration of the compounds is 10^{-4} mg mL^{-1} [Fig. 2(A)], the compounds showed good microbial activity against *Escherichia coli* and *Aeromonas hydrophila* with the viable cell reduction about 90%. When the compounds concentration reached 10^{-2} mg mL^{-1} [Fig. 2(C)], all the tested compounds showed excellent microbial activity against all the tested bacterium with the viable cell reduction >96.6%. Compared with compound **7** under similar culture conditions, the tested compounds (**11a-c**) showed equivalent or better antimicrobial activity. So the novel synthesized compounds should be potential microbial inhibitors.

CONCLUSION

In conclusion, we have achieved a short total synthesis of (3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)methyl benzencarboxylates (**11a-c**) in good yield and their microbial activities were evaluated against the aquicolous bacteria *Escherichia coli, Staphyloccus aurueus, Vibrio alginolyticus, Aeromonas hydrophila*, and *Bacillus subtilis*. The synthesized **11a-c** exhibited high-antimicrobial activity. These results will allow us to investigate the structure-activity relationships in more detail by using more various derivatives of 2-(hydroxymethyl)benzo[*d*]isothiazol-3(2*H*)-one (**7**) in the future.

EXPERIMENTAL

Melting points were determined with an Electrothermal-XT4-100 digital melting point apparatus. The ^{1}H NMR spectra were recorded on a Bruker AV-400 FT NMR spectrometer using

TMS as internal standard and DMSO-d_6 as solvent. The elemental analyses were performed with a Bruker DALTONICS analyzer. The MS spectra were taken on a HP-5988A spectrometer.

Procedure for preparation of 7. A mixture of benzoisothiazolone (3.02 g, 0.02 mol commercial from National Medicine Corporation, Shanghai of China), polyoxymethylene (5.28 g, 0.022 mol) in 10 mL of chloroform was refluxed for 5 h at 60°C under stirring. Compound **7** (3.13 g, 87%) was obtained by the usual work-up of the reaction mixture. The physical and spectral data of compound **7** were in accordance with the data reported by Morley *et al*. [4].

General procedure for the synthesis of (3-oxobenzo[*d*]isothiazol-2(3*H*)-yl)methyl benzencarboxylates 11a-c. To the stirred and cooled solution of compound **7** (1.81 g, 10 mmol), triethylamine (1.22 g, 11 mmol) in 20 mL of acetone (dried before used) with ice-water bath, benzoyl chloride (1.41 g, 10 mmol) was added dropwise. The colorless precipitates were obtained as soon as by addition of benzoyl chloride. When benzoyl chloride was added completely, the ice-water bath was removed and the resulting mixture was continuously refluxed for 2 h with stirring. After the reaction quenched, the reaction mixture was concentrated under reduced pressure, and purified by column chromatography on silica gel (petroleum ether:ethyl acetate = 3:1) to gave pure crystals of **11a**, mp 114–116°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 6.15 (s, 2H, CH_2), 7.27∼7.87 (m, 9H, Ar-H); GC-MS (ESI) *m*/*z*: 285 (M^+), 256, 164, 136, 105, 77; *Anal*. Calcd. for $C_{15}H_{11}NO_3S$: C, 63.14; H, 3.89; N, 4.91; Found: C, 62.87; H, 3.46; N 4.88.

(3-Oxobenzo[d]isothiazol-2(3H)-yl)methyl 4-fluorobenzencarboxylate (11b). This compound (2.18 g, 72%) was obtained as crystals, mp 95–96°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 6.14 (s, 2H, CH_2), 7.07∼7.85 (m, 9H, Ar-H); MS (ESI) *m*/*z*: 303.3(M^+); *Anal*. Calcd. for $C_{15}H_{10}FNO_3S$: C, 59.40; H, 3.32; N, 4.62; Found: C, 59.10; H, 3.39; N, 4.57.

(3-Oxobenzo[d]isothiazol-2(3H)-yl)methyl 3,5-dimethylbenzencarboxylate (11c). This compound (2.16 g, 69%) was obtained as crystals, mp 109–111°C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 1.27 (m, 6H, $2CH_3$), 6.10 (s, 2H, CH_2), 7.07∼7.79 (m, 7H, Ar-H); MS (ESI) *m*/*z*: 313.4 (M^+); *Anal*. Calcd. for $C_{17}H_{15}NO_3S$: C, 65.16; H, 4.82; N, 4.47; Found: C, 64.96; H, 4.45; N 4.41.

Acknowledgments. This work was supported by Natural Science Foundation of Hainan Province of China (No. 20503) and Key Laboratory Open Foundation of Chinese Ministry of Education. The authors thank Professor Yongcan Zhou of Hainan University for his help.

REFERENCES AND NOTES

[1] Ronald, R. P.; Joel, M. S. J Coating Technol 2003, 75, 53.

[2] Khalaj, A.; Adibpour, N.; Shahverdi, A. R. Eur J Med Chem 2004, 39, 699.

[3] Adibpour, N.; Khalaj, A.; Rezaee, S.; Daneshtalab, M. Folia Microbiol 2007, 52, 573.

[4] Morley, J. O.; Oliver, A. J.; Charlton, M. H. J Mol Struct: Theochem 1998, 429, 103.

[5] Okachi, R.; Niino, H.; Kitaura, K.; Mineura, K.; Nakamizo, Y.; Murayama, Y.; Ono, T.; Nakamizo, A. J Med Chem 1985, 28, 1772.

[6] Vicini, P.; Amoretti, L.; Ballabeni, V.; Tognolini, M.; Barocelli, E. Bioorg Med Chem 2000, 8, 2355.

[7] Zambuchini, B.; Fiorini, D.; Verdenelli, M. C.; Orpianesi, C.; Ballini, R. LWT - Food Sci Technol 2008, 41, 1733.

[8] Xie, Y.-S.; Pan, X.-H.; Zhao, B.-X.; Liu, J.-T.; Shin, D.-S.; Zhang, J.-H.; Zheng, L.-W.; Zhao, J.; Miao, J.-Y. J Organomet Chem 2008, 693, 1367.

[9] Al-Bakri, A. G.; Afifi, F. U. J Microbiol Meth 2007, 68, 19.