Thermochimica Acta 476 (2008) 33–38

Contents lists available at ScienceDirect

Thermochimica Acta

journal homepage: www.elsevier.com/locate/tca

# Antibacterial activities of manganese(II) ebselen–porphyrin conjugate and its free components on *Staphylococcus aureus* investigated by microcalorimetry

Xu Xiang-Jiao[a,b], Xue Zhi[a], Qi Zu-De[a], Hou An-Xin[a], Li Chao-Hong[c], Liu Yi[a,*]

[a] State Key Laboratory of Virology, College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, PR China
[b] College of Chemical Engineering and Technology, Wuhan University of Science and Technology, Hubei, Wuhan 430081, PR China
[c] School and Hospital of Stomatology, Wuhan University, Wuhan 430072, PR China

ARTICLE INFO

*Article history:*
Received 15 May 2008
Received in revised form 11 July 2008
Accepted 14 July 2008
Available online 5 August 2008

*Keywords:*
Ebselen–porphyrin conjugate
*Staphylococcus aureus*
Antibacterial effects
Microcalorimetry
Thermokinetics

ABSTRACT

The antibacterial effect of Mn(II), tetraphenyl porphyrin (TPP), ebselen–porphyrin conjugate **1** and its manganese(II) complex **2** towards *Staphylococcus aureus* growth was studied by stop-flow microcalorimetry. By analyzing the power–time curves, crucial parameters such as rate constant of bacterial growth ($k$), half inhibitory concentration ($IC_{50}$), and generation time ($t_G$) were determined. The sequence of the antibacterial activities of these compounds tested was **2** > **1** > Mn(II) > TPP, with an $IC_{50}$ value of 100 μg/mL for complex **2**. Manganese(II) ebselen–porphyrin conjugate **2** is proposed to benefit from synergetic effects of Mn(II) and **1**.



## 1. Introduction

Multiple resistance to antibiotics is a growing public health issue that is compounded by the inability of the pharmaceutical industry to generate new strains of antibiotics to combat infections [1,2]. Identification of new antibacterial agents and exploitation of new approaches for the drug-resistant bacteria is in great demand [3–6]. Among the novel developed antibacterial agents, porphyrin complexes have attracted much attention [7–9] due to their ability to act as photosensitizers when irradiated by visible light. Because porphyrin analogous can take cytotoxic singlet oxygen directly in tumor cells causing cell death when irradiate, photosensitization can represent a useful approach to kill microbial. Several porphyrins and related compounds have displayed phototoxicity against bacteria and yeast [10–13].

In an effort to identify new antibacterial compounds and in connection with our research programme on porphyrins, we synthesized novel ebselen–porphyrin conjugates (**1** and **2**). Ebselen (2-phenyl-1, 2-benzisoselenazol-3-[2H]-one) is a lipid-soluble selenoorganic compound that has an array of pharmacological effects including anti-inflammatory, anti-oxidant and anti-tumor activities [14–21]. The combination of porphyrin with ebselen might provide stronger anti-tumor or antimicrobial activity due to the fact that porphyrins have specific affinity for microbial cells, which can enable the efficient accumulation of the ebselen moiety into the microbial cells. Manganese is an important trace element needed for normal physiological functions and development. It is also a cofactor or required metal ion for many enzymes, such as superoxide dismutase (SOD), glutamine synthetase and arginase [22]. Systematic study, herein, of the biological effect of manganese(II) ions, TPP, ebselen–porphyrin conjugates (**1** and **2**) may, thus, reveal interesting structure–activity relationships (SAR).

Microcalorimetry is a simple and straight-forward technique for the study of microorganisms, since it permits the online tests of bioactivity screening and can obtain a lot of important information about the process of inhibition, which cannot be obtained by the other technique [23–30]. Microcalorimetry can provide a continuous measurement of heat production and supply the power–time curves which can describe the growth process without disturbing the normal activity of the bio-system [31–33]. Therefore, it has been extensively used to study the interactions drugs with cells based on the thermodynamic and kinetic information [31,34–39].

In this paper, the microcalorimetric technique has been used to investigate the effects of Mn(II), TPP, **1** and **2** towards *Staphylococcus aureus* growth and the relationship between the rate of heat production and microbial growth. The results are very important for clinical applications of the antibiotics.

* Corresponding author. Tel.: +86 27 87218284; fax: +86 27 68754067.
*E-mail address:* prof.liuyi@263.net (Y. Liu).

0040-6031/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2008.07.007

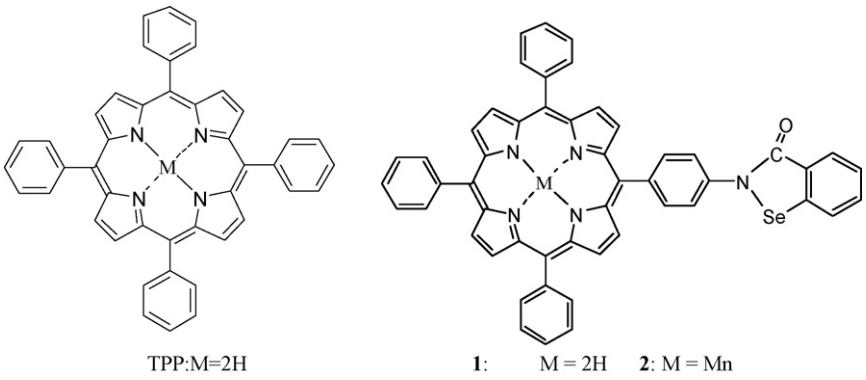


**Fig. 1.** Structures of TPP and ebselen–porphyrin conjugates (**1** and **2**).

## 2. Experimental

### 2.1. Materials

Ebselen–porphyrin conjugates (**1**, **2**) and TPP were synthesized and characterized by the Department of Chemistry, Wuhan University [40], PR China. The structure of them is shown in Fig. 1. Manganese acetate ($Mn(CH_3COO)_2{\cdot}4H_2O$) was obtained from *Chengdu Chemical corporation*.

All other chemicals used are of analytical-grade and are available locally.

### 2.2. S. aureus culture

*S. aureus* (CCTCC AB910393) was provided by China Center for Type Culture Collection, Wuhan University, PR China. Briefly the broth culture medium in a total volume of 1000 mL contained NaCl (5 g), beef extract (6 g), peptone (10 g), and pH 7.0. The volume of the container is 100 mL, and the volume of the culture medium was 25 mL. The culture medium was sterilized in high-pressure steam at 121–126 °C for 30 min. *S. aureus* were inoculated in 25 mL peptone culture medium and incubated in the shaker for 10 h at 37 °C. These cells were prepared for microcalorimetric measurements. The rotation speed of incubator shaker is 100 rpm. The flask is enveloped with cotton plug, so there is enough oxygen, which can be used by *S. aureus*.

### 2.3. Luria-Bertain (LB) medium

The bacteria were grown at pH 7.0–7.2 on a Luria-Bertain (LB) prepared from NaCl (5 g), peptone (10 g), and yeast extract (5 g). The medium was sterilized by autoclaving at 0.1 MPa and 120 °C for 30 min, and stored in a refrigerator.

### 2.4. Instruments

LKB-2277 Bioactivity Monitor manufactured by LKB corporation of Sweden, a heat-conduction isothermal microcalorimeter, was used to obtain the metabolic growth power–time curves of the bacteria. It is designed to monitor continuously a wide variety of processes and complex systems over temperatures ranging from 20 °C to 80 °C. Each measuring cylinder normally contains a sample and a reference in separate measuring cups (twin systems). The microcalorimeter was thermostated at 37 °C. The voltage signal was recorded by means of an LKB-2210 recorder (1000 mV range). This system is very sensitive; the detection limit is 0.15 μW and the baseline stability was 0.2 μW/24 h. For details of the performance and structure of the instrument (see reference [41]).

### 2.5. Methods

In the calorimetric experiment, the flow cell was completely cleaned and sterilized. The procedure was as follows: sterilized distilled water, NaOH (0.1 mol/L), alcohol solution (75%), HCl (0.1 mol/L), and sterilized distilled water were pumped in sequence through the system for 30 min by an LKB-2132 microperplex peristaltic pump at a flow rate of 50 mL/h. Once the system was cleaned and sterilized and the baseline had been stabilized, the bacterial suspension, initially containing $1 \times 10^6$ bacteria/mL and the appropriate compound (Mn(II), TPP, **1** or **2**), was pumped through the calorimetric cell with aid of the LKB-2132 pump at a flow rate of 50 mL/h. When the flow cell (volume 0.6 mL) was filled, the pump was stopped and the monitor was used to record the power–time curves of the bacterial growth [41]. In this type of experiment, the bacteria used were suspended in the peptone culture medium. The appropriate compound (Mn(II), TPP, **1** or **2**) was added from the beginning of the experiment, i.e., they were introduced as soon as the bacteria were inoculated in the peptone culture medium. All calorimetric experiments were conducted at 37 °C.

## 3. Results

### 3.1. Power–time curves of S. aureus

A typical power–time curve of microbes, which resembles the traditional growth curve, can be divided into a lag phase, a log (exponential) phase, a stationary phase, and a decline phase [41]. The power–time curve from the control experiment with no antibiotics was shown in Fig. 2.

Next, the corresponding power–time curves of the growth of *S. aureus* were recorded in the presence of different concentrations of the appropriate compound (Mn(II), TPP, **1** or **2**) and the corresponding plots are shown in Fig. 3. Good reproducibility was obtained under identical experimental conditions. As can be seen from the different profiles, the growth of *S. aureus* was significantly influenced by the above four chemicals.

### 3.2. Thermokinetics

The growth power–time curves of *S. aureus* show that the log phase of growth obeys the equation [41]:

$$\ln P_t = \ln P_0 + kt \quad (1)$$

Here, $P_0$ and $P_t$ are the heat output power at time 0 and $t$, respectively. Using this equation, the growth rate constant ($k$) of all the experiments is calculated by analyzing the date of the first peak. The generation times ($t_G$), which are $(\ln 2)/k$, were also obtained. The corresponding $k$ and $t_G$ are shown in Table 1.

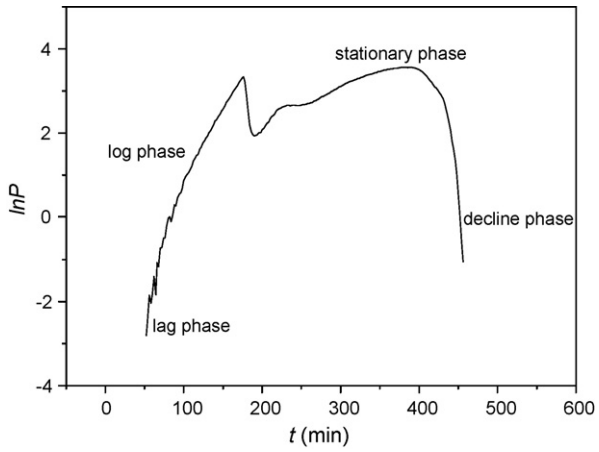


**Fig. 2.** The power–time curves of *S. aureus* growth at 37 °C in the absence of any substance.

The growth inhibition ratio is calculated on the basis of the growth rate constant.

Inhibitory ration can be defined as [41]:

$$I = \left[\frac{(k_0 - k_c)}{k_0}\right] \times 100\% \quad (2)$$

where $k_0$ is the growth rate constant in the absence of the appropriate compound (Mn(II), TPP, **1** or **2**), $k_c$ is the rate constant of *S. aureus* growth inhibited by an inhibitor at a certain concentration.

When the inhibitory ratio *I* is 50%, the corresponding concentration of inhibitor is called half inhibitory concentration ($IC_{50}$). The value of *I* and $IC_{50}$ of Mn(II), TPP, ebselen–porphyrin conjugates (**1** and **2**) acting on *S. aureus* are also shown in Table 1.

### 3.3. Relationship between k and c

To further compare the effect of Mn(II), TPP, **1** and **2**, the relationship between growth rate constant *k* and concentration *c* was studied. As can been seen from Table 1, an increase in compound concentration *c* caused a decrease in growth rate *k* of *S. aureus*. As for Mn(II), *k* at low concentrations was higher than or close to that of the control. This indicates that, at low concentration, Mn(II) stimulate the growth of *S. aureus*. For antibacterial assay, we, thus, focused on concentrations at which the growth rate was less than that of the control. The relationship between *k* and *c* could be established as follows:

Mn(II) : $k = 0.02774 - 3.844 \times 10^{-5} c(10 - 360\ \mu g/mL)$; $R = -0.9121$
TPP : $k = 0.03014 - 7.229 \times 10^{-5} c(0 - 1000\ \mu g/mL)$; $R = -0.9239$
**1** : $k = 0.03506 - 1.057 \times 10^{-4} c(0 - 136\ \mu g/mL)$; $R = -0.9836$
**2** : $k = 0.03372 - 1.640 \times 10^{-4} c(0 - 170\ \mu g/mL)$; $R = -0.9951$

### 3.4. Relationship between I and c

Usually, the inhibition ratio *I* increased with the increase of the appropriate compound (Mn(II), TPP, **1** or **2**) concentration, this can be seen from the date of Table 1. The variation tendency of inhibition ratio with concentration was varied with different complexes,

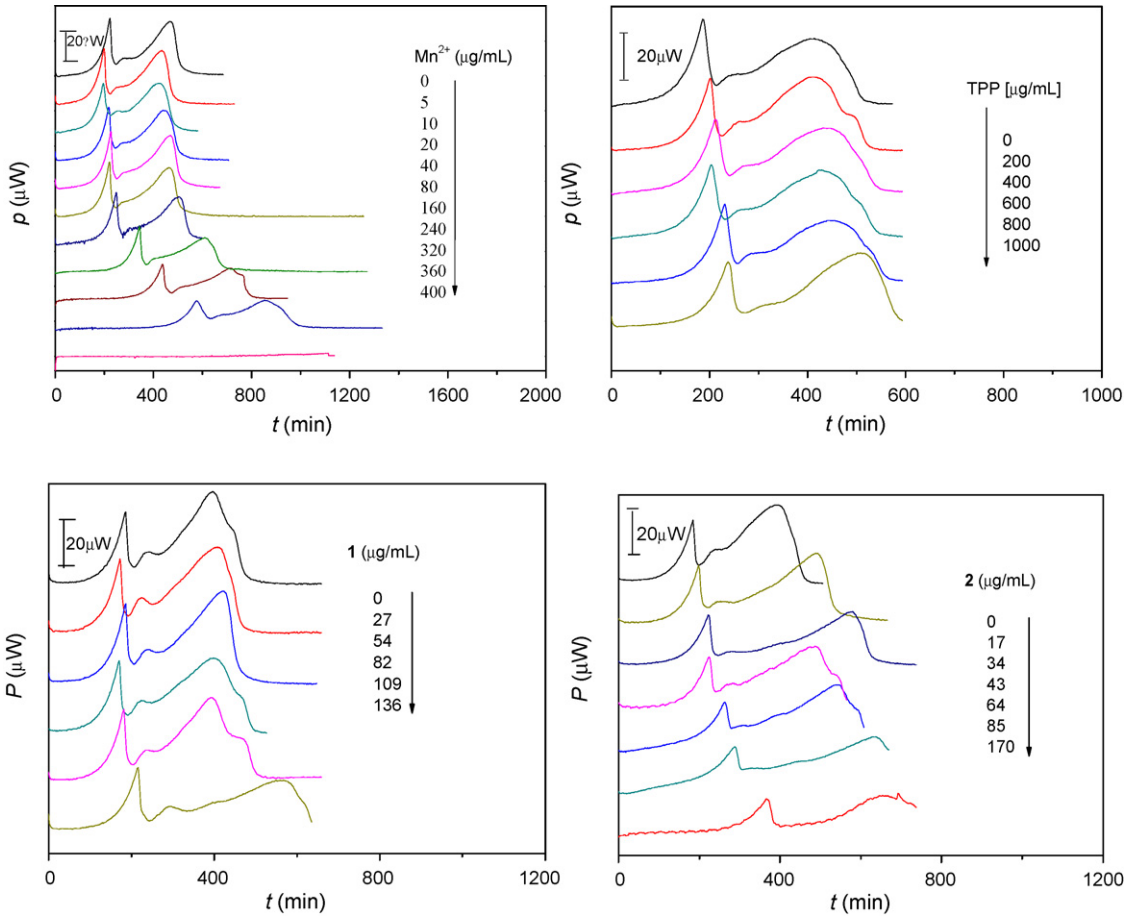


**Fig. 3.** The power–time curves of *S. aureus* growth in the presence of the appropriate compound (Mn(II), TPP, **1** or **2**). Concentration ranges: [Mn(II)] = 0–400 μg/mL; [TPP] = 0–1000 μg/mL; [**1**] = 0–136 μg/mL; [**2**] = 0–170 μg/mL.

**Table 1**
Thermokinetic data for the growth of *S. aureus* in different drugs at 37 °C

| Drug | $c$ (μg/mL) | $k$ (min$^{-1}$) | $R^a$ | $t_G$ (min) | $t_{max}$ (min) | $I$ (%) | $IC_{50}$ (μg/mL) |
|---|---|---|---|---|---|---|---|
| Mn(II) | 0 | 0.02613 | 0.9977 | 26.53 | 224.31 | 0.00 | 382 |
| | 5 | 0.02643 | 0.9970 | 26.22 | 196.83 | −1.15 | |
| | 10 | 0.02722 | 0.9987 | 25.46 | 195.72 | −4.17 | |
| | 20 | 0.02622 | 0.9994 | 26.44 | 218.01 | −0.34 | |
| | 40 | 0.02513 | 0.9962 | 27.58 | 226.36 | 3.83 | |
| | 80 | 0.02447 | 0.9945 | 28.33 | 222.53 | 6.35 | |
| | 160 | 0.02375 | 0.9959 | 29.19 | 248.32 | 9.11 | |
| | 240 | 0.02053 | 0.9942 | 33.37 | 342.50 | 20.51 | |
| | 320 | 0.01533 | 0.9935 | 41.21 | 433.97 | 41.33 | |
| | 360 | 0.01174 | 0.9913 | 51.19 | 576.54 | 55.07 | |
| | 400 | 0 | – | – | – | 100 | |
| TPP | 0 | 0.03099 | 0.9992 | 22.37 | 185.84 | 0.00 | >1000 |
| | 200 | 0.02850 | 0.9993 | 24.32 | 199.98 | 8.03 | |
| | 400 | 0.02617 | 0.9990 | 26.49 | 203.23 | 15.55 | |
| | 600 | 0.02545 | 0.9956 | 27.24 | 214.38 | 17.88 | |
| | 800 | 0.02484 | 0.9984 | 27.90 | 229.21 | 19.85 | |
| | 1000 | 0.02321 | 0.9987 | 29.86 | 237.12 | 25.10 | |
| **1** | 0 | 0.03581 | 0.9947 | 19.36 | 184.34 | 0.00 | 162 |
| | 27 | 0.03240 | 0.9994 | 21.39 | 174.14 | 9.52 | |
| | 54 | 0.02786 | 0.9994 | 24.88 | 183.61 | 22.20 | |
| | 82 | 0.02677 | 0.9984 | 25.89 | 175.23 | 25.24 | |
| | 109 | 0.02263 | 0.9993 | 30.63 | 179.88 | 36.81 | |
| | 136 | 0.02177 | 0.9986 | 31.84 | 215.24 | 39.21 | |
| **2** | 0 | 0.03440 | 0.9953 | 20.15 | 184.03 | 0.00 | 100 |
| | 17 | 0.03133 | 0.9978 | 22.12 | 197.93 | 8.92 | |
| | 34 | 0.02787 | 0.9975 | 24.87 | 221.68 | 18.98 | |
| | 43 | 0.02574 | 0.9995 | 26.93 | 223.03 | 25.17 | |
| | 64 | 0.02348 | 0.9969 | 29.52 | 247.45 | 31.74 | |
| | 85 | 0.01922 | 0.9927 | 36.06 | 263.19 | 44.13 | |
| | 170 | 0.00627 | 0.9978 | 103.15 | 288.56 | 81.76 | |

[a] Correlation coefficient.

which suggested that the mode of action, drug absorption, etc. was different from different drugs. Fig. 4 showed the relationship between $I$ and $c$.

The above experimental relationships show how the inhibition ratio $I$ changed as a function of the concentration of the compounds. Thus, the inhibition ratio $I$ increased most rapidly for **2**. This result demonstrates that **2** benefits in a synergetic manner from the inherent properties of its free components Mn(II) and **1**, as also reflected in the experimental $IC_{50}$ values.

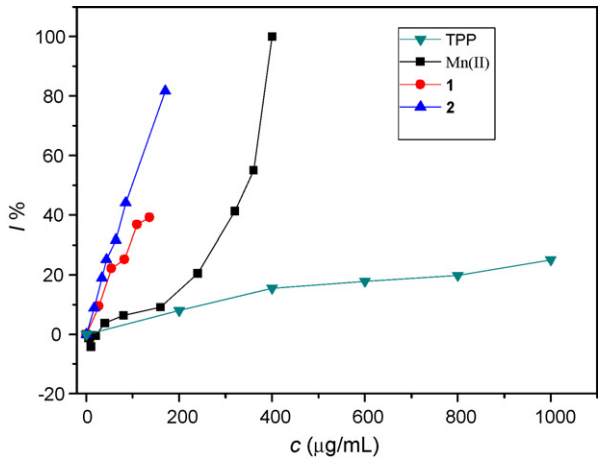


**Fig. 4.** Relationship between $I$ and $c$ for the appropriate compound (Mn (II), TPP, **1** or **2**). concentration ranges: [Mn(II)] = 0–400 μg/mL; [TPP] = 0–1000 μg/mL; [**1**] = 0–136 μg/mL; [**2**] = 0–170 μg/mL.

### 3.5. The relationship of $t_{max}$ and $c$

From Table 1, we can see that the time of the maximum heat power ($t_{max}$) for Mn(II) in the log phase of *S. aureus* was more shorter than that of the control, when the concentration was 0–10 μg/mL, and then increased in the concentration range 20–360 μg/mL. This phenomenon is in accordance with the relationship between $I$ and $c$. When the concentration reached the upper limit (400 μg/mL), the growth of *S. aureus* was completely inhibited by Mn(II). These results confirm that Mn(II) exhibits biphasic functions on *S. aureus* (see Fig. 5).

As for TPP and **1**, there was not a dose-dependence relationship between $t_{max}$ and concentration. However, in the case of **2**, $t_{max}$ increased linearly with increased concentration. Linear fitting then gave the relationship $t_{max} = 184.37 + 0.9509\,c$ (0–85 μg/mL); $R = 0.9948$. Thus, the observation of $t_{max}$ further confirms that **2** benefits from a synergetic effect in terms of its inhibitory action towards *S. aureus* (see Fig. 5).

## 4. Discussion

Analyses of the power–time curves of *S. aureus* under the action of four kinds of compounds (Mn(II), TPP, **1** or **2**) showed that with increasing concentrations of these compounds, the lag phase became longer, the time of the maximum heat power ($t_{max}$) increased and the generation time ($t_G$) delayed. All these phenomena suggest that all the four compounds have the capacity to inhibit the growth metabolism of *S. aureus* to different extents and the inhibitory extent varied with the different drugs.

In the case of Mn(II), the relationships of $t_{max}$ and $I$ *vs.* $c$ indicate that Mn(II) has a stimulating effect on the growth of *S. aureus*

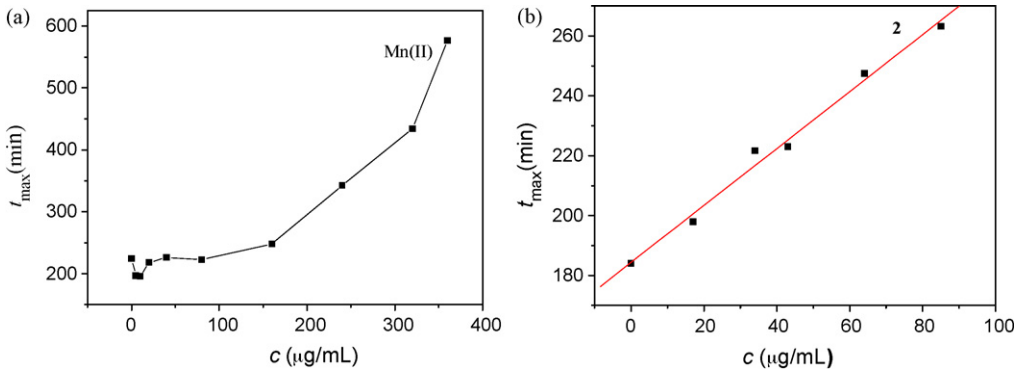


**Fig. 5.** Relationship between the times of peak of maximum power in the log phase ($t_{max}$) and $c$: (a) Mn(II): $t_{max}$–$c$ (0–400 μg/mL); (b) **2**: $t_{max}$–$c$ (0–85 μg/mL).

at low concentration, but an inhibitory one at high concentration. The biphasic function was interpreted as follows. At low concentration, Mn(II) can inhibit lipid peroxidation and GSH depletion against oxidative stress. While at the high concentration, Mn(II) has inhibitory action on growth. It might be explained that Mn(II) can activate cytochrome oxidase P450 and produce reactive oxygen species (ROS) and induce cell death.

In the case of TPP, the structure of cell membrane is most likely altered as a result of the affinity of porphyrins to biological organisms. An enhanced activity is observed for **1** porphyrin structure bearing ebselen group. Determining the characteristics of a dose *c*–response curve are the drug's mode of action in cells, its number of target sites, and its affinity for those target sites. Selenium containing in **1** can catalyze the production of reactive oxygen radical ($O_2^-$) resulting in the oxidative damage. In this study, the growth of *S. aureus* was inhibited by selenium excess probably through the catalysis of oxidation reactions of thiol groups (SH) to S–S or S–Se–S bonds. During this process, more active free radicals may be produced that further damage the membrane structure and functions of cells [42,43].

Finally, the synergetic function of **2** on *S. aureus* could be due to the unique structure of this complex carrying simultaneously Mn(II) unit and ebselen group, the porphyrin ring being compatible with the biological structure. All these factors rationalize why **2** has a higher affinity to the bacterial cell membrane than Mn(II) and **1** alone. To better understand this phenomenon from a mechanistic point, further studies are required, though.

In conclusion, microcalorimetry is a powerful tool for monitoring and controlling the growth process of microbes. It provides kinetic and thermodynamic information that cannot be obtained by conventional bacteriological techniques. The result shows that these compounds are potential antibacteria reagent and their inhibitory capacities are concentration-depended. The sequence of antibiotic activity of these compounds was **2** > **1** > Mn(II) > TPP, with an $IC_{50}$ value of 100 μg/mL for complex **2**. Furthermore, all of these informations are very significant for the synthesis of antibiotics and the studies of toxicology and pharmacology.

## Acknowledgements

We gratefully acknowledge financial support of project supported by the National Natural Science Foundation of China (No.: 30570015, 20621502); Science Research Foundation of Chinese Ministry of Education (No.: [2006]8IRT0543); and Natural Science Foundation of Hubei Province (2005ABC002). We thank the reviewers for their critical comments on the manuscript.

## References

[1] K.M. Overbye, J.F. Barrett, Drug Discov. Today 10 (2005) 45–52.
[2] S.B. Levy, Adv. Drug Deliv. Rev. 57 (2005) 1446–1450.
[3] A.J. Alanis, Arch. Med. Res. 36 (2005) 697–705.
[4] I. Chopra, Curr. Opin. Microbiol. 1 (1998) 495–501.
[5] B. Spellberg, J.H. Powers, E.P. Brass, L.G. Miller, J.E. Edwards Jr., Clin. Infect. Dis. 38 (2004) 1279–1286.
[6] D.M. Livermore, Lancet Infect. Dis. 5 (2005) 450–459.
[7] J.P. Tome, M.G. Neves, A.C. Tome, J.A. Cavaleiro, M. Soncin, M. Magaraggia, S. Ferro, G. Jori, J. Med. Chem. 47 (2004) 6649–6652.
[8] V.S.P. Branland, V. Chaleix, R. Granet, M. Guilloton, F. Lamarche, B. Verneuil, P. Krausz, Bioorg. Med. Chem. Lett. 14 (2004) 4207–4211.
[9] S. Banfi, E. Caruso, L. Buccafurni, V. Battini, S. Zazzaron, P. Barbieri, V. Orlandi, J. Photochem. Photobiol. Sect. B 85 (2006) 28–38.
[10] N.S. Soukos, L.A. Ximenez-Fyvie, M.R. Hamblin, S.S. Socransky, T. Hasan, Antimicrob. Agents Chemother. 42 (1998) 2595–2601.
[11] G. Bertoloni, F.M. Lauro, G. Cortella, M. Merchat, Biochim. Biophys. Acta 1475 (2000) 169–174.
[12] L. Polo, A. Segalla, G. Bertoloni, G. Jori, K. Schaffner, E. Reddi, J. Photochem. Photobiol. B: Biol. 59 (2000) 152–158.
[13] M.R. Hamblin, D.A. O'Donnell, N. Murthy, K. Rajagopalan, N. Michaud, M.E. Sherwood, T.J. Hasan, Antimicrob. Chemother. 49 (2002) 941–951.
[14] H. Sies, Free Radic. Biol. Med. 14 (1993) 313–323.
[15] G. Mugesh, H.B. Singh, Chem. Soc. Rev. 29 (2000) 347–357.
[16] C. Jacob, G.I. Giles, N.M. Giles, H. Sies, Angew. Chem. 115 (2003) 4890–4907.
[17] A. Muller, E. Cadenas, P. Graf, H. Sies, Biochem. Pharmacol. 33 (1984) 3235–3239.
[18] P.V. Jaquemin, L.E. Christiaens, M.J. Renson, M.J. Evers, N. Dereu, Tetrahedron Lett. 33 (1992) 3863–3866.
[19] T.G. Back, B.P. Dyck, J. Am. Chem. Soc. 119 (1997) 2079–2083.
[20] H. Wójtowicz, K. KIoc, I. Maliszewska, J. Młochowski, M. Pi□tka, E. Piasecki, IL FARMACO 59 (2004) 863–868.
[21] B. Mishra, K.I. Priyadarsini, H. Mohan, G. Mugesh, Bioorg. Med. Chem. Lett. 16 (2006) 5334–5338.
[22] V.V. Dukhande, G.H. Malthankar-Phatak, J.J. Hugus, C.K. Daniels, J.C.K. Lai, Neurochem. Res. 31 (2006) 1349–1357.
[23] L. Nunez-Regueira, J.A. Rodriguez-Anon, J. Proupin-Castineiras, O. Nunez-Fernandez, Soil Biol. Biochem. 38 (2006) 115–124.
[24] Z. Heng, Z. Congyi, W. Cunxin, W. Jinbin, G. Chaojiang, L. Jie, L. Yuwen, J. Therm. Anal. Cal. 79 (2005) 45–50.
[25] L. Wadsö, F. Gomez, I. Sjöolm, P. Rocculi, Thermochim. Acta 422 (2004) 89–93.
[26] F. Postollec, W. Norde, H.C. van der Mei, H.J. Busscher, J. Microbiol. Methods 55 (2003) 241–247.
[27] I. Wadsö, Thermochim. Acta 394 (2002) 305–311.
[28] A. Abderrahmane, L. Yi, G.W. Ying, S. Ping, Q.S. Sheng, J. Therm. Anal. Cal. 68 (2002) 909–916.
[29] A.X. Hou, Y. Liu, K.W. Huang, Z. Xue, S.S. Qu, Acta Physico-Chimica Sinica 19 (2003) 134–135.
[30] C.L. Xie, H.K. Tang, Z.H. Song, S.S. Qu, Thermochim. Acta 123 (1988) 33–41.
[31] A.X. Hou, Z. Xue, Y. Liu, S.S. Qu, K.W. Huang, Chem. Biodivers 4 (2007) 1492–1500.
[32] Y. Liu, F.J. Deng, R.M. Zhou, X.S. Shen, C.X. Wang, S.S. Qu, Chemsphere 40 (2000) 851–854.
[33] M.L.C. Montanari, A.E. Beezer, C.A. Montanari, Thermochim. Acta 328 (1999) 91–97.
[34] C.N. Yan, Y. Liu, T.Z. Wang, Z.Q. Tan, S.S. Qu, P. Shen, Chemosphere 38 (1999) 891–898.
[35] E.A. Boling, G.C. Blanchar, W.J. Russed, Nature 241 (1973) 472–473.
[36] Y. Liu, C.N. Yan, T.Z. Wang, R.M. Zhao, S.S. Qu, P. Shen, Thermochim. Acta 333 (1999) 103–108.
[37] L. Ruan, Y. Wang, L. Wai, Y. Hoi-fu, J. Anal. Cal. 89 (2007) 953–956.
[38] L.X. Zhang, Y. Liu, L.H. Cia, Y.J. Hu, J. Yin, P.Z. Hu, Thermochim. Acta 440 (2006) 51–56.

[39] Y. Yang, Y. Liu, J. Zhu, M.J. Li, P. Shen, J. Therm. Anal. Cal. 79 (2005) 645–648.
[40] Z. Xue, A.X. Hou, D.W.J. Kwong, W.K. Wong, Bioorg. Med. Chem. Lett. 17 (2007) 4266–4270.
[41] C.L. Xie, G.D. Xu, S.S. Qu, Acta Physico-Chimica Sinica 2 (1986) 363–370.
[42] J.T. Rotruck, A.L. Pope, H.E. Ganther, A.B. Swanson, D.G. Hafeman, W.G. Hoekstra, Science 179 (1973) 588–590.
[43] R. Landenstein, O. Epp, K. Bartels, A. Jones, R. Huber, A. Wendel, J. Mol. Biol. 134 (1979) 199–218.