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Inhibitory study of some novel Schiff base derivatives on *Staphylococcus aureus* by microcalorimetry

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Abstract

The effect of a series of novel Schiff base compounds on *Staphylococcus aureus* was studied by microcalorimetric method at 37 °C The results showed that all of the organic compounds had the capacity to inhibit the growth of *S. aureus* in different extent. And the extent and duration of the inhibitory effect on the growth of *S. aureus*, judged from the rate constant (*k*), varied with the different structure of the Schiff base compounds. According to the power–time curves, the multiplication rate constant and inhibition ratio were calculated. The growth rate constant of *S. aureus* (in log phase) in the presence of Schiff base compounds decreased with the increasing of the concentrations of these compounds regularly. The experimental results revealed that the hydrophilicity of Schiff bases had a great influence on their antibacterial activity. Of these Schiff bases, the greater their hydrophilicity, the higher their antibacterial activity. The antibacterial structure–activity relationship (SAR) of Schiff base derivatives was also briefly discussed.

Keywords: Schiff base; Microcalorimetry; Antibacterial activity; *S. aureus*; Structure–activity relationship

1. Introduction

Compounds with the structure of AC = NB are known as Schiff base, which can be synthesized from the condensation of amino and active carbonyl. Some Schiff base and their metal complexes have antibacterial and antitumor activity, so it is always an interesting topic. Since the first Schiff base metal complex was synthesized by H. Schiff in 1869. The study of antibacterial and antitumor activity of Schiff base compounds and their metal complexes has been widely discussed. In 1960s, Popp et al., studied several Schiff bases of benzaldehyde N mustards and found them active enough in an experiment tumor system to merit clinical trials [1]. Following this lead, Modi et al., [2] reported the synthesis and study of Schiff base from substituted benzaldehyde N mustards and various arylamines. After Modi, lots of researchers studied the synthesis and characterization of Schiff bases and their metal complexes [3–6]. At the same time, the modes of Schiff base and their metal complexes, structure–activity relationship (SAR) of Schiff base compounds have been studied by many investigators [7–10].

Microcalorimetry provides a general analytical tool for the characterization of the microbial growth process. It has been extensively used in the study of the interactions between drugs and microbes with a great deal of useful information already obtained [11,12], and it is of great significance in drug designing, reveals the nature and mechanism of the interaction between drugs and microbes and further studying the nature of cell membrane. The power–time curves of bacterial growth can be determined by means of microcalorimetry, and by analyzing the exponential growth phase of the power–time curves, kinetic parameters, such as rate constant, for bacterial growth can be evaluated [13]. In this paper, LKB-2277 Bioactivity Monitor, a kind of heat conduction microcalorimeter, was used to investigate the inhibitory effects of Schiff bases on *Staphylococcus aureus* at 37 °C. A deeper understanding of the mechanisms of action of Schiff bases is highly desirable; the results of this study may be useful to researchers attempting to gain more understanding of the mechanisms of action of Schiff base compounds.

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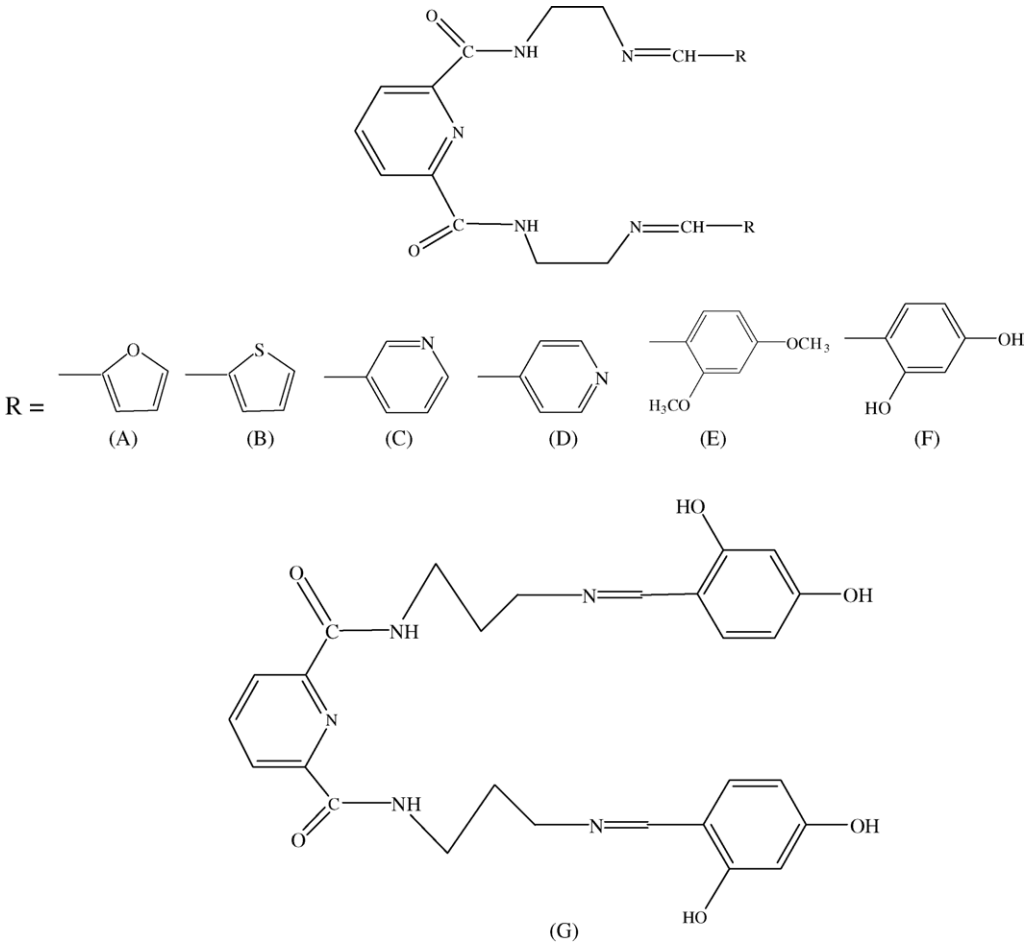

Fig. 1. Structures of Schiff base derivatives.

2. Materials and methods

2.1. Materials

Schiff base compounds were synthesized and characterized by the College of Chemistry and Molecular Science, Wuhan University, PR China. The structures of Schiff base derivatives discussed in this study are shown in Fig. 1.

All other chemicals used are of analytical grade and 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 5 g NaCl, 6 g beef extract, 10 g peptone, and pH 7.0. 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 volume of the container is 100 mL. The rotate 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. Microcalorimetric studies

The experiments were performed at 37 °C with a microcalorimeter, LKB-2277 Bioactivity Monitor manufactured by LKB (Bromma, Sweden). Luria-Bertain (LB) medium contained per 1000 mL (pH 7.0): 5 g NaCl, 5 g yeast extract, 10 g peptone. It was sterilized in high-pressure steam at 121–126 °C for 30 min. *S. aureus* were inoculated in 5 mL LB medium; initially the density of *S. aureus* was 1×10^6 bacteria/mL. The Schiff base compound was added at the beginning of the experiment, i.e. it was introduced as soon as the *S. aureus* were inoculated in LB medium. *N*,*N*-Dimthyrformamide was used as a solvent for preparing the original solution of the Schiff base compounds. Eventually, the LB medium contained *S. aureus* and Schiff base compound was pumped into the microcalorimetric cell with an LKB-2132 perplex peristaltic pump at a flow rate of 50 mL h^{-1}. 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. The remained bacterial suspension was discarded. Generally, the experiments were stopped, when the second peak appeared.

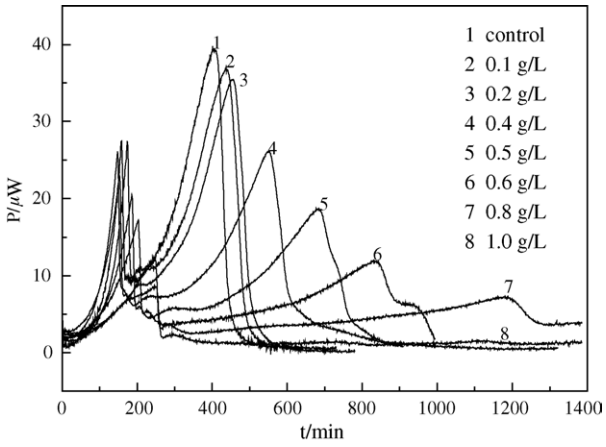

Fig. 2. Power–time curves of *S. aureus* at different concentrations of Schiff base compound D.

3. Results and discussion

3.1. Growth power–time curves

The power–time curves of *S. aureus* growth under the action of Schiff base derivatives at different concentrations are shown in Fig. 2. The power–time curves show that the shapes of the metabolic thermogenesis curves changed little when the Schiff base compounds at low concentration were added into the suspension of the bacterium. But when high concentration of Schiff base compounds was added, the shapes changed obviously. From Fig. 2, it can be seen that Schiff base compounds have an obvious inhibitory action on *S. aureus* growth. The times till the appearance of both peaks increase and their heights decrease with increasing concentration of Schiff base derivatives. The heights of the second peak are larger than the first peak in the range of 0–0.4 g L^{-1}. When the concentration of compound D is in the range of 0.5–0.8 g L^{-1}, the heights of second peak are smaller than the first peak. The second peak disappeared when the concentration of compound D is 1.0 g L^{-1}.

3.2. Growth rate constant of S. aureus and inhibition ratio

The growth power–time curves of *S. aureus* show that the log phase of growth obeys the equation [13]:

$$\ln P_t = \mathrm{In}\ P_0 + kt \quad (1)$$

where 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 data of the first peak. The growth inhibition ratio is also calculated on the basis of the growth rate constant.

Inhibitory ration can be defined as:

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

where k_0 is the growth rate constant of the control, k_c the rate constant for *S. aureus* growth inhibited by an inhibitor with concentration c. When the inhibitory ratio I is 50%, the corresponding concentration of inhibitor is called half inhibitory concentration (IC_{50}). The value of IC_{50} can be regarded as the inhibiting concentration of causing a 50% decrease of the growth rate constant. The values of k, I and IC_{50} of different Schiff base compounds acting on *S. aureus* are shown in Table 1.

3.3. Relationship between the growth rate constant (k) and concentration of Schiff base derivatives

The values of the growth rate constant (k) in Table 1 show that all of these Schiff base compounds have potent antibacterial activity against *S. aureus*. The growth rate constant (k) decreased with an increase in the concentration of Schiff base compounds. Fig. 3 shows the relationship between the growth rate constant and concentration of compound D. From it, we can see that the concentration rate constant relationship is nearly linear.

3.4. Relationship between the inhibition ratio (I) and concentration of Schiff base derivatives

Usually, the inhibition ratio increased with the increase of Schiff base compounds concentration, this can be seen from the data of Table 1. The variation tendency of inhibition ratio with concentration is varied with different compound, which suggested that the mode of action, drug absorption, etc. is different from different drugs. Fig. 4 shows the relationship between the inhibition ratio and concentration of compound D.

3.5. Influence of Schiff base compounds on S. aureus growth

The growth curve of *S. aureus* has two typical peaks. The first peak may suggest *S. aureus* to adopt one way of metabolism, and the second peak suggests *S. aureus* to adopt another way of metabolism. The presence or absence of oxygen (O_2) can be very important for the growth of bacterial, and it can influence the ways of metabolism of microbe. Under the condition of stop-flow method, the volume of measuring cell is 0.6 mL, and there is only a little oxygen, which can be used by *S. aureus* in the system. At the beginning of the experiment, there is a little oxygen available for *S. aureus*, and *S. aureus* adopts one way of metabolism. When the oxygen is consumed, microbe may adjust themselves and adopt another way of metabolism. This may explain why there are two typical peaks. The curve in Fig. 5 demonstrated the relationship between the time of the second peak and the concentration of compound D. It is clear that the addition of concentration of Schiff bases delays the t_{max}, which also suggests that the Schiff bases have inhibitory effect on *S. aureus*. At the same time, the power decreases correspondingly (as can be seen from the heights of peaks in Fig. 2). It is worth noting that the heights of the second peaks decrease greater than the first at high concentration of Schiff base compounds, so we may draw the conclusion that the second way of metabolism has been influenced greater than the first at high concentration of Schiff base compounds.

Table 1
Parameters of *S. aureus* growth in solutions with different drugs at 37.00 °C

Drug	C (g L^{-1})	k (min^{-1})	I (%)	IC_{50} (g L^{-1})
	0	0.02743	–	0.506
	0.1	0.02684	2.0	
	0.2	0.02474	9.8	
Compound	0.4	0.01717	37.4	
A	0.6	0.01047	61.8	
	0.8	0.00831	69.7	
	1.0	0.00683	75.1	
	1.2	0.00461	83.2	
	0	0.02790	–	0.474
	0.1	0.02640	5.4	
	0.2	0.02146	23.1	
Compound	0.4	0.01673	40.0	
B	0.6	0.00986	64.7	
	0.8	0.00915	68.0	
	0.9	0.01080	61.3	
	1.2	0.00991	64.5	
	0	0.02743	–	0.864
	0.1	0.02689	2.0	
	0.2	0.02553	6.9	
Compound	0.4	0.02284	16.7	
C	0.6	0.02058	25.0	
	0.9	0.01396	49.1	
	1.3	0.00408	85.1	
	0	0.02743	–	0.579
	0.1	0.02437	11.2	
	0.2	0.02380	13.2	
Compound	0.4	0.01885	31.3	
D	0.5	0.01656	39.6	
	0.6	0.01277	53.5	
	0.8	0.00867	68.4	
	1.0	0.00261	90.5	
	0	0.02743	–	0.980
	0.1	0.02674	2.5	
	0.2	0.02555	6.9	
Compound	0.4	0.02421	11.7	
E	0.6	0.02334	14.9	
	0.8	0.01595	41.9	
	1.0	0.01253	54.3	
	1.2	0.00916	66.6	
	0	0.02790	–	0.535
	0.1	0.02689	3.6	
	0.2	0.02267	18.8	
	0.3	0.02050	26.5	
Compound	0.4	0.01588	43.1	
F	0.5	0.01583	43.3	
	0.6	0.01280	54.1	
	0.7	0.00896	67.9	
	0.85	0.01032	63.0	
	1.0	0.00931	66.6	
	0	0.02838	–	0.841
	0.1	0.02754	3.0	
	0.2	0.02387	15.9	
	0.3	0.02172	23.5	
Compound	0.4	0.01926	32.1	
G	0.5	0.01843	35.1	
	0.6	0.01595	43.8	
	0.75	0.01501	47.1	
	0.9	0.01370	51.7	
	1.2	0.00990	65.1	

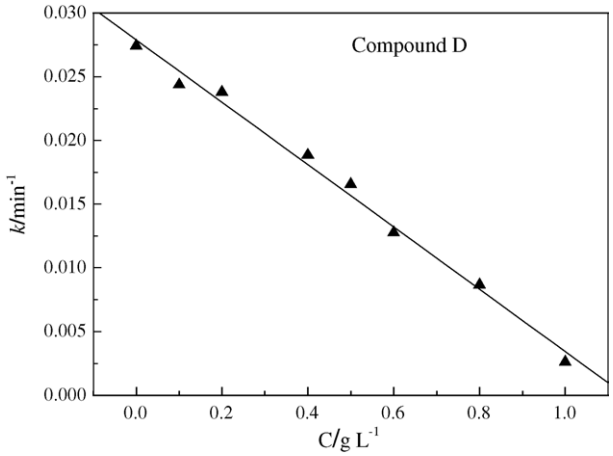

Fig. 3. Relationship between rate constant k and concentration c for compound D.

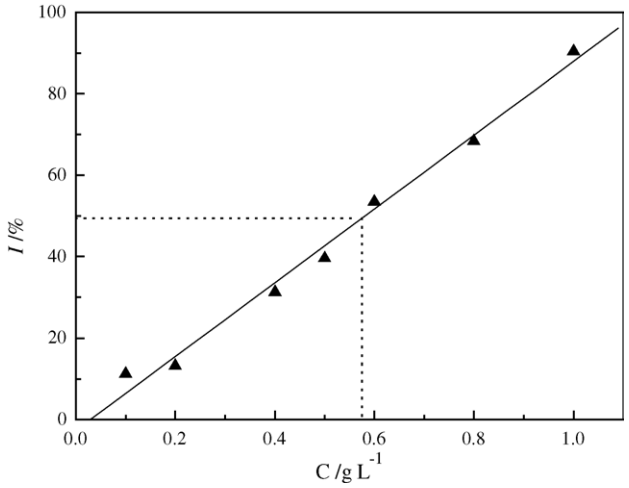

Fig. 4. Relationship between inhibition ratio I and concentration c for compound D.

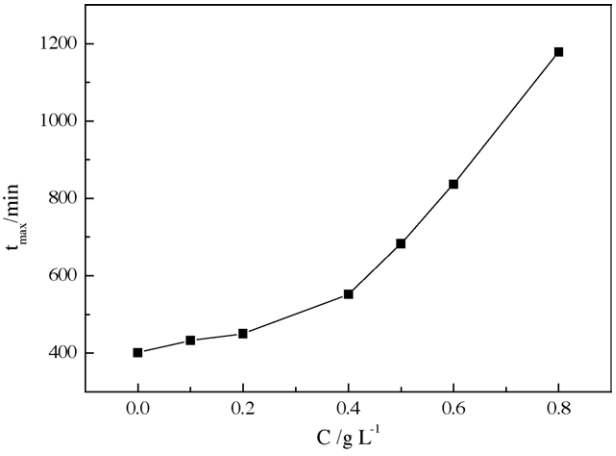

Fig. 5. Relationship between the time of the second peak and concentration for compound D.

3.6. Structure–activity relationships

The ways in which different Schiff base compounds react with *S. aureus* vary due to the difference in the structure. Analysis of Schiff base compounds may provide some explanation for the structure–activity relationships. Such an analysis might be helpful in the design of better inhibitors. The biological activity of a particular substance depends on a complex sum of individual properties including compound structure, affinity for the target site, and survival in the medium of application, survival within the biological system, transport properties, and state of the target organism [14]. In this study, we focus our attention on the structure–activity relationships.

Of the Schiff base derivatives whose inhibitory properties have been studied in detail, inhibition data of Table 1 shows that all compounds exhibit antibacterial activity against *S. aureus*. The inhibitory activity of compound A, B and F are better than that of the other compounds. Compound B with residue thiophenic shows the highest anti-microbial activity, and the IC_{50} value is about $0.474\,g\,L^{-1}$. Compound A and compound F have similar inhibitory activity, they contain the subset of furanyl and 2,4-dihydroxyphenyl, their IC_{50} value are $0.506\,g\,L^{-1}$ and $0.535\,g\,L^{-1}$, respectively.

It is interesting to note that compound C and D, their structures can be seen from Fig. 1, have a slight difference in structure, but their antibacterial activity is very different; their IC_{50} values are $0.864\,g\,L^{-1}$ and $0.579\,g\,L^{-1}$, respectively. Ligand–protein interaction plays a key role in the distribution and transport of small molecules in biological systems. The electric property of the compounds has close relations with biological activity. If the distribution of charge density of drug is just suited with the specific receptor, the interaction between drug and receptor would increase, and then drug and receptor are apt to form complexes and increase the activity. The weak antibacterial activity of compound C compared to compound D may be explained by their charge density distribution.

The anti-microbial activity of compound F is almost 2 times higher than that of compound E. We can know from Fig. 1, two hydrogens at the position *ortho* and *meta* of the phenyl ring of compound E and compound F are substituted by anisyl and hydroxyl, respectively. The methoxyl group is generally hydrophobic in character, whereas the hydroxyl group is hydrophilic, so the lipophilicity of compound E is higher than that of compound F. The enzyme inside the cell undoubtedly is the target for many antibacterial agents. If drugs are difficult to cross the lipid bilayer, they may fail in therapy. In the simplest version, to cross the lipid bilayer, the small molecular drugs must diffuse up to the membrane interface, absorb, cross the membrane core, desorb and diffuse away on the other sides. Many facts suggested that drug absorption is influenced by the hydrophilic/lipophilic properties. Drugs are usually absorbed by passive diffusion; the motive force of passive diffusion is the concentration gradient of drugs between both sides of lipid bilayers. Thus, drugs require a certain degree of solubility in water. Biological membranes are bilayers mainly made up of lipid; if drugs are to cross the lipid bilayers must have certain lipophilic properties. Drugs, which are insoluble or easily soluble in water but insoluble in lipid, could not be absorbed. Generally speaking, the activity of drugs is increased with the increase of lipophilicity of drugs. But the only increase in lipophilicity dose does not result in an enhance anti-microbial activity for the homologous family [15]. Some highly lipophilic compounds may have low antibacterial activity because the time needed for the diffusion into the bacterial cell might be too long compared with their biological half-life [16]. So, we think that the difference in antibacterial activity of compound F and E may be due, in part, to differences in lipophilicity of the compounds. What is more, the steric effect may be another reason for their different antibacterial activity. The anisyl is larger than hydroxyl group, they attach to the *ortho* and *meta* position of the phenyl ring, producing different steric effect.

Comparison of the structures of Schiff base compound F and G, and correlation with the IC_{50} values provides the explanation for the reduction in antibacterial activity of compound F and G. Compound G contains one more CH_2 unit compared to compound F, the hydrophobicity and steric dimension of alkyl increases with the increasing number of carbon atoms. If the compound contains one more CH_2 unit, their partition coefficient will be two to four times higher than that of the previous compound [17]. So, the lipophilicity of compound G is different from compound F resulting in different antibacterial activity.

4. Conclusions

In this research, anti-microbial activity and the structure–activity relationships of a series of novel Schiff base derivatives were described. The result showed that all these Schiff base compounds, compound B was found to be the most active derivative at an IC_{50} value of $0.474\,g\,L^{-1}$ against *S. aureus*. Compound A, C, D, E, F, and G exhibited moderate antibacterial activity with IC_{50} values of 0.506, 0.864, 0.579, 0.980, 0.535, and $0.841\,g\,L^{-1}$, respectively. The structure–activity relationships of these Schiff base derivatives indicated that furan ring was essential for the antibacterial activity. The different hydrogen of pyridine ring was substituted resulting in different antibacterial activity. Furthermore, the hydrophilicity seemed to be important for the antibacterial activity, it may be possible to produce other more potent inhibitors.

Acknowledgements

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