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Study on synthesis, characterization and process, and biological activity of a melamine schiff base

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Abstract. This paper takes melamine and salicylaldehyde as raw materials to synthesis Schiff base (SM). The Schiff base is characterized by IR, ^{13}CNMR, MS and elemental analysis, and the structure of the product is analyzed. The OD600 value of bacterial liquid is measured by spectrophotometer, and the inhibitory activity of SM on Escherichia coli, Staphylococcus aureus and Bacillus subtilis is studied. The synthetic process of SM is discussed by single factor experimental method. The suitable process conditions for SM are as follows: The ratio of the quality of salicylaldehyde to melamine is 4:1, the solvent is DMF, the reaction temperature is 120℃,and the reaction time is 4H.

Key words. Melamine, Salicylaldehyde, Schiff base, Characterization, Bacteriostatic activity
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1. Introduction

Schiff base is a kind of substance containing the C=N group, andgenerated by the condensation reaction of amine and carbonyl compounds. Schiff base has excellent bacteriostatic and anticancer physiological activity. In addition, Schiff base has good coordination chemical properties, and its complexes have better properties. Melamine is a triazine nitrogen-containing heterocyclic organic compound, which is used to synthesize melamine resin, and widely used in coatings of industrial production, anti-fold ant-shrinking agent and other production[1−3].Salicylaldehyde is a commonly used spice material and an intermediate in organic synthesis,which is widely used in the synthesis of Schiff base compounds. According to the literature,

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the salicylaldehyde Schiff base has good bacteriostatic activity,so the research field of salicylic Schiff base is becoming more and more extensive[4]. It has obviously become one of the main trends of Schiff base research field to study one or one type of Schiff base which has good bacteriostatic activity to some or several kinds of bacteria[5−7].

Melamine and salicylaldehydeare dissolved in DMF in this experiment, and after condensation reflux reaction in oil bath, the pale-yellow melamine salicylaldehyde Schiff base is precipitated in toluene solution[8]. OD600 refers to the absorption of a solution at the length of the 600nm wave, the value of whichis direct proportion tothe concentration of the absorbable substance in the solution. The OD600 value of the bacterial liquidis direct proportion to the concentration of the bacteria. The bacteriostatic activity of SM isstudied by the method of measuring OD600 value, and this paper uses single factor experimental method to explore the best SM synthesis conditions[9−10].

2. Experimental

2.1. Main experimental instruments and reagents

Main reagents: Melamine (analytical reagent, purity≥99.5%, Tianjin Da Mao chemical reagents factory).Salicylaldehyde (analytical reagent, purity≥99%, Tianjin Da Mao chemical reagents factory).DMF (N,N-dimethylformamide) (analytical reagent, Tianjin Hengxing Chemical Reagent Co. Ltd.).Methylbenzene (analytical reagent, Tianjin Hengxing Chemical Reagent Co. Ltd.).Carbinol (analytical reagent, Tianjin Hengxing Chemical Reagent Co. Ltd.).Ethanediol (analytical reagent, Tianjin Hengxing Chemical Reagent Co. Ltd.).DMSO (dimethyl sulfoxide) (analytical reagent, Tianjin Hengxing Chemical Reagent Co. Ltd.).Peptone (GB/T4789.2-2010, Shanghai Bo Microbiology Technology Co., Ltd.).Beef extract powder (GB/T4789.2-2010, Shanghai Bo Microbiology Technology Co., Ltd.) and so on.

Major equipment:DF-1A heat collector Magnetism Msier, GZX-9070MBEAir dry oven, KQ3200Ultrasonic cleaner, 5700Fourier transform infrared spectrometer,Bruker Avance600NMR spectrometer,UV754UV spectrophotometer, AB SCIEX TripleTOF 5600+Time-of-flight mass spectrometer, Euro Vector EA3000Automatic elemental analyzer, SW-CJ-2FDClean Bench, YM50Vertical mode steam sterilizers.

2.2. Synthesis of Schiff Base

The computational method of the productivityof SM:

$$\text{Productivity}\,(\%) = \frac{(m+s)ZM}{mxN}100\%$$

Where, m is the mass of melamine, g; s is the mass of Salicylaldehyde, g; zisthe percentage of the mass of the Schiff base after reaction, %; M is the relative molecular mass of melamine;x is the purity of melamine; N isthe relative molecular mass of Schiff base.

Weighing0.9469g melamine (0.0075mol) place in flask with three necks,DFM is-dropped until melamine is completely dissolved in the 120 ℃ oil bath environment, and slowly dripping 3.664g salicylaldehyde (0.03mol) in the above solution. The solution gradually changed to pale-yellow, reflux reaction 4H at 120 ℃, and natural cooling to room temperature.The solution is slowly poured into a beaker containing 300ml toluene,and a large amount of precipitate produced.The pale-yellow solid is obtained by filtration, which is washed three times with a mixed solution of toluene and methanol(1:1), and the product is dried and preserved in a vacuum.The productivity of SM is 30.12%.The synthesis of Schiff base is shown in Figure 1.

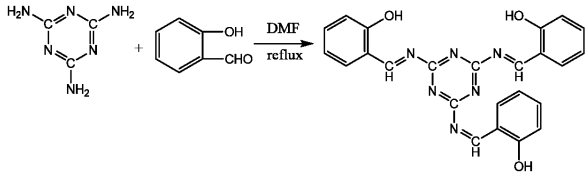

Fig. 1. The synthesis of Schiff base

3. Results and discussion

3.1. Analysis of mass spectrum characterization

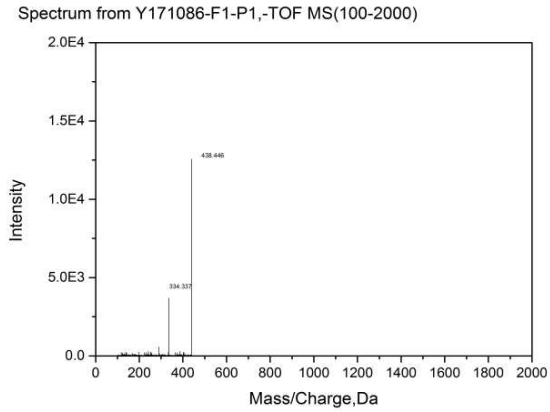

Fig. 2. Mass spectrogram of SM

The abscissa of first-order mass spectrogram represents mass charge ratio, and the maximum peak is the molecular mass of the product.The first-order mass spectrogramshows thatthe maximum peak of the mass spectrum detection of the product appears at 438.446, and the molecular mass of the product is 438.446. The molecular mass of SM is equal to the molecular mass (438-446)of the target product.

3.2. Elemental analysis

The element analysis data measured by the automatic elemental analyzer is shown in the following table.

Table 1. The elemental analysis of SM

	C %	H %??	N %	O %
Theoretical value	65.75	4.14	19.17	10.94
Detection value	65.68	4.15	19.57	10.60

The theoretical values of the target Schiff base C, H, O, N are 65.75%, 4.14%, 19.17%, 10.94%,respectively. The actual values of SM measured by the automatic elemental analyzer are 65.68%, 4.15%, 19.75%, 10.60%,respectively. The actual value is similar to the theoretical value, and it is basically in accordance with the ratio of C, H, N and O of the target Schiff base.

3.3. Analysis of NMR characterization

Using CDCl3 as solvent to detect CNMR spectra of SM, and the result of its characterization is as shown in the following figure:

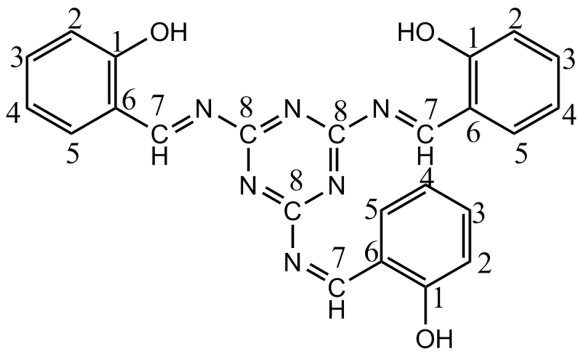

Fig. 3. 13CNMR spectrum of SM

Table 2. SM 13CNMR spectrum data(δ,ppm)

(δ,ppm)	The position of carbon
167.45	C8
161.01	C7
159.74	C1
134.99	C6
134.13	C4
121.00	C5
119.87	C3
118.76	C2
77.36	Solvent $CDCl_3$

Analysis is based on the data of 13CNMR in Table 2, 167.45, 161.01,159.74, 134.99, 134.13, 121, 119.87, 118.76. The deuterated solvent peak appears at 77.36ppm. There are 8 different carbon environments in the product, which are in accordance with the 8 different carbon environments of the target product structure.

3.4. Analysis of infrared characterization

The obtained product and melamineuses KBr pressed-disk technique to make infrared spectrum testin the wavelength range of 400-4000cm-1, and the obtained infrared spectra are shown as follows.

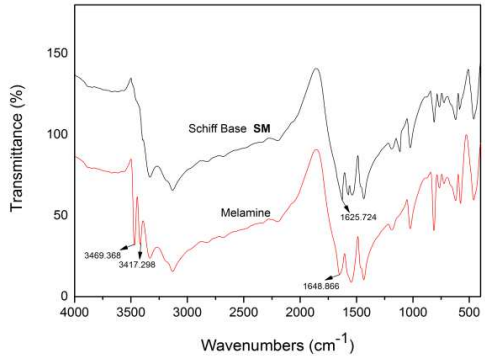

Fig. 4. The IR characterization of SM and Melamine

Figure 3 shows that the peak of 3469.368cm-1, 3417.298cm-1 is the characteristic peak of group-NH2 in the melamine infrared spectrogram.The characteristic peaks of -NH2are not found in the infrared spectrogram of SM, which indicates that -NH2 is involved in the reaction and the characteristic peak disappears. The characteristic peaks appear at 1625.724cm-1 in SM infrared spectrogram, which is the characteristic peak of C=N. The appearance of this characteristic peak indicates that the carbonyl group reacts with the amino group to form the C=N bond. The

disappearance of -NH2 and the generation of C=N indicate the target Schiff Base is generated.

By mass spectrometric analysis, we can find that the molecular mass(438-446)of SM is the same as the theoretical value of the molecular massof the target Schiff Base. The results of elemental analysis(65.78%, 4.15%, 19.57%, 10.60%)arebasically in accordance with the theoretical value (65.75%, 4.14%, 19.17%, 10.94%)of the element analysis of the target product. Itcan be concluded that the molecular formula of SM is C24H18N6O3, which is consistent with the molecular formula (C24H18N6O3) of the target product.NMR analysis shows that there are 8 different carbon environments in the product (167.45ppm, 161.01ppm, 159.74ppm, 134.99ppm, 134.13ppm, 121.00ppm,118.76ppm),which are the same as the 8 carbon environments of the target Schiff base.The characteristic peak of Schiff base C=N appears in the SM infrared spectrum, and there is no characteristic peak of -NH2, which indicates that the condensation reaction between -NH2 and -CHO generates C=N bond.Mass spectrum analysis, elemental analysis, NMR analysis and infrared analysis shows that SM is the target product that melamine salicylaldehyde Schiff base (C24H18N6O3).

4. Study on the bacteriostatic activity of Schiff Base

4.1. Experimental process

The selection of bacteria species:Escherichia coli, Staphylococcus aureus, Bacillus subtilis.

The preparation of SM solution: Dissolving 10mg SM in 10ml DMSO, using DMSO to constant volume, and preparingsolutions that the concentration is 25, 50, 75, 100, 150, 200mg/L.

The culture of bacteriaspecies:Configuringthebrothculture mediumaccording to10.0g peptone,3.0g beef extract powder and 5.0g sodium chloride per liter, and controllingendpoint batch pH=7.2.The prepared culture medium is sterilized by steam sterilizer at 121℃, and then it is reserved.The same amount of broth culture mediumis taken, and inoculating the bacteria on the same broth culture,andrespectively dropping2ml SM solutions with different concentrations, in which the culture solution that no bacteria is used as a blank solution to zero.The blank group 1 is dropped 2ml distilled water, and the blank group 2 is dropped 2mlDMSO. The inoculated bacterial liquid is cultivated 24 hours in the clean worktable at 28℃. The OD600 value is measured every 2 hours, and the OD600 value is recorded. Three experiments are carried outin each group, and the average OD600 value of each group is taken. The experimental results are as follows: 3, 4, 5.

Table 3. Bacteriostatic activity of SM on Escherichia coli

Culture time/h	OD_{600} Average value					
	Blank 1	Blank 2	25	50	100	200
0	0.086±0.003					
2	0.124±0.001	0.111±0.000	0.096±0.001	0.096±0.001	0.092±0.001	0.088±0.001
4	0.361±0.003	0.359±0.001	0.3169±0.002	0.282±0.001	0.222±0.002	0.226±0.001
6	0.456±0.001	0.395±0.003	0.3663±0.001	0.312±0.001	0.302±0.003	0.306±0.002
8	0.567±0.002	0.524±0.002	0.521±0.002	0.411±0.002	0.410±0.001	0.401±0.001
10	0.624±0.001	0.593±0.003	0.586±0.003	0.426±0.003	0.421±0.001	0.462±0.001
12	0.678±0.003	0.612±0.001	0.603±0.001	0.478±0.001	0.462±0.001	0.478±0.001
14	0.712±0.002	0.678±0.000	0.671±0.002	0.488±0.002	0.476±0.002	0.498±0.003
16	0.813±0.002	0.759±0.001	0.749±0.001	0.498±0.001	0.478±0.003	0.521±0.001
18	0.912±0.000	0.883±0.001	0.876±0.002	0.512±0.001	0.488±0.001	0.678±0.002
20	1.025±0.001	0.996±0.003	0.987±0.001	0.522±0.002	0.498±0.003	0.712±0.002
22	1.211±0.002	1.112±0.002	1.107±0.003	0.527±0.002	0.498±0.001	0.722±0.000
24	1.286±0.001	1.118±0.000	1.112±0.001	0.531±0.001	0.498±0.000	0.824±0.001

Table 4. Bacteriostatic activity of SM on Staphylococcus aureus

Culture time/h	OD_{600} Average value					
	Blank 1	Blank 2	25	50	100	200
0	0.078±0.001					
2	0.125±0.001	0.122±0.001	0.101±0.001	0.100±0.001	0.100±0.001	0.095±0.001
4	0.216±0.003	0.210±0.001	0.198±0.003	0.201±0.002	0.221±0.003	0.189±0.003
6	0.312±0.002	0.299±0.001	0.298±0.003	0.297±0.002	0.312±0.003	0.307±0.001
8	0.356±0.001	0.354±0.001	0.346±0.002	0.321±0.001	0.316±0.001	0.310±0.002
10	0.401±0.001	0.399±0.002	0.400±0.001	0.397±0.002	0.353±0.003	0.343±0.003
12	0.411±0.002	0.401±0.002	0.403±0.003	0.401±0.001	0.376±0.001	0.366±0.002
14	0.467±0.001	0.468±0.003	0.459±0.001	0.449±0.001	0.428±0.002	0.417±0.001
16	0.598±0.003	0.588±0.001	0.578±0.001	0.598±0.002	0.567±0.001	0.429±0.001
18	0.612±0.003	0.617±0.002	0.612±0.001	0.622±0.003	0.598±0.001	0.467±0.002
20	0.728±0.001	0.719±0.001	0.712±0.001	0.708±0.001	0.599±0.003	0.512±0.001
22	0.896±0.001	0.901±0.001	0.889±0.001	0.798±0.003	0.599±0.003	0.510±0.003
24	1.023±0.001	1.021±0.003	0.997±0.003	0.971±0.001	0.601±0.002	0.510±0.003

Table 5. Bacteriostatic activity of SM on Bacillus subtilis

Culture time/h	OD_{600} Average value					
	Blank 1	Blank 2	25	50	100	200
0	0.095					
2	0.111±0.001	0.101±0.001	0.101±0.003	0.100±0.001	0.103±0.001	0.095±0.003
4	0.213±0.003	0.201±0.002	0.200±0.001	0.199±0.001	0.201±0.003	0.196±0.001
6	0.312±0.003	0.298±0.001	0.267±0.003	0.266±0.003	0.278±0.003	0.268±0.003
8	0.456±0.003	0.367±0.001	0.365±0.001	0.368±0.003	0.398±0.003	0.333±0.003
10	0.512±0.001	0.467±0.001	0.467±0.003	0.466±0.001	0.467±0.003	0.421±0.001
12	0.555±0.002	0.502±0.001	0.498±0.001	0.503±0.001	0.498±0.001	0.488±0.001
14	0.579±0.001	0.536±0.002	0.535±0.003	0.534±0.001	0.521±0.001	0.496±0.003
16	0.612±0.001	0.563±0.003	0.557±0.002	0.549±0.001	0.579±0.001	0.568±0.003
18	0.712±0.003	0.579±0.001	0.569±0.003	0.571±0.001	0.612±0.001	0.576±0.003
20	0.912±0.003	0.907±0.001	0.889±0.003	0.901±0.001	0.903±0.001	0.901±0.001
22	0.998±0.002	0.908±0.003	0.901±0.002	0.903±0.003	0.903±0.003	0.899±0.002
24	1.103±0.001	0.907±0.003	0.901±0.001	0.902±0.003	0.902±0.001	0.900±0.003

4.2. Experimental results and analysis

As can be seen from Tables 3 and 4, 5, OD600 values of blank group 1 and blank group 2 gradually increase with the culture of the bacterial liquid, and their values are not significantly different. This shows that DMF on E. coli, Staphylococcus aureus, Bacillus subtilis no significant inhibitory effect.

As shown in Table 3, compared with the blank group 2, the OD600 value of the bacterial liquid decrease to a certain extent under the action of different concentrations of SM. When the concentration of SM is 100 mg / L, OD600 value of the broth is 0.498 after 20 hours of culture, and remain stable. Thus, SM has a certain inhibitory effect on Escherichia coli. When the concentration of SM is 100mg / L, the antibacterial effect reaches the best; the OD600 value is 0.620 less than the blank group 2, and the antibacterial effect is 55.46%.

As shown in Table 4, compared with the blank group 2, the OD600 value of the bacterial liquid decrease to a certain extent under the action of different concentrations of SM. When the concentration of SM is 200mg/L, the OD600 value of the bacteria is 0.511 and keep stable after the culture of 22h. It is found that SM had a certain inhibitory effect on Staphylococcus aureus. In the SM concentration of 200mg / L is the best antibacterial effect. The OD600 value is 0.691 smaller than blank group 2, and the antibacterial effect is 67.68%.

As can be seen from Table 5, compared with the blank group 2, under the action of different concentrations of SM, the OD 600 value of the bacterial liquid do not

change much. This shows that the inhibitory effect of SM on Bacillus subtilis is not obvious.

5. Synthetic process of Schiff base

5.1. The effect of solvent on the productivity

Respectively select ethylene glycol, DMF, DMSO melamine dissolved at 150℃; the reaction time is 4h. The synthesis of SM under reflux conditions, calculate the productivity. Each group of experiments is averaged three times. The relationship between solvent and productivity is shown in Table 6:

Table 6. The relationship between the selection of solvent and the productivity

Solvent	Ethanediol	DMF	DMSO
Productivity(%)	10.28%	20.12%	14.23%

According to Table 6, we can see that the productivity of SM has a certain relationship with the solvent of choice. Salicylaldehyde in the reaction process is prone to condensation reaction, and in different solvents has different degrees of condensation. Because salicylaldehyde has different degrees of condensation reaction during the reaction, the condensation of salicylaldehyde reduces the reaction of salicylaldehyde to participate in the Schiff base synthesis, thereby reducing the productivity of Schiff base. The choice of solvent has a great influence on the productivity of SM. With DFM as the solvent, the productivity of SM is the largest, so the suitable solvent is DMF.

5.2. The effect of reaction time on the productivity

The ratio of the mass of melamine and salicylaldehyde is 1: 4; the solvent is DMF; and the reaction temperature is 150℃; the reaction time is changed; the time gradient is 1H, and the experiment is repeated three times in each group. The average productivity is obtained, and the relation between the reaction time and the productivity is shown in Figure 4.

According to figure 5, when the reaction time is 1H and the productivity of SM is 11.03%; the productivity of SM increases with the increase of temperature. When the reaction time is 4h, the maximum productivity is 21.35%. As the reaction time continued to increase, the productivity of SM decrease, so the reaction time of SM is suitable for 4H.

5.3. The effect of reaction temperature on the productivity

The ratio of the mass of melamine to salicylaldehyde is 1:4; the solvent is DMF; the reaction time is 4h; the reaction temperature is changed; the temperature gradient is 5℃. The experiment is repeated three times in each group. The average

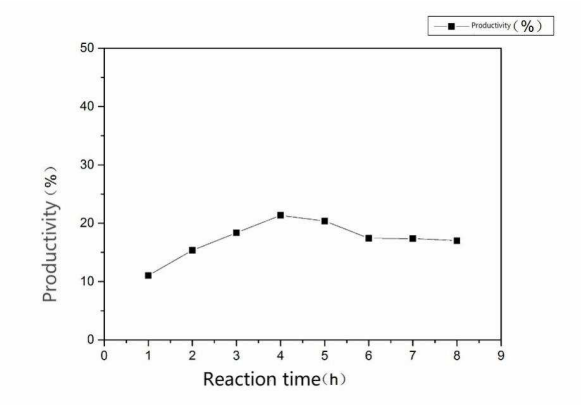

Fig. 5. The effect of reaction time on the productivity

productivity is obtained, and the relation between the productivity and the reaction temperature is shown in Figure 5.

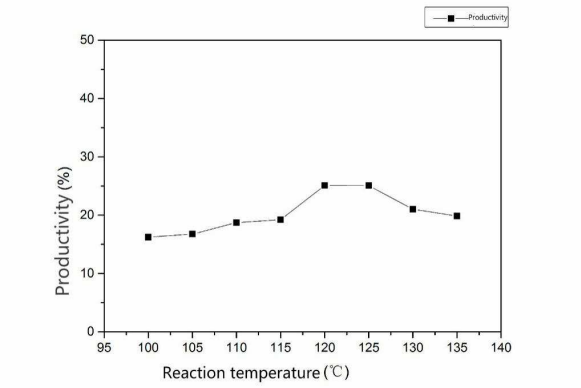

Fig. 6. The effect of reaction temperature on the productivity

The effect of the amount of the reactant substance on the productivity

When the solvent is DMF, the temperature is 120℃ and the reaction time is 4h, the ratio of the amount of salicylaldehyde to melamine is changed (0.5:1; 1.0:1; 1.5:1; 2.0:1; 2.5:1; 3.0??1; 3.5:1; 4.0??1; 4.5:1; 5.0:1); The effect of the amount of the reactants on the productivity of the reactant is investigated. The effect of the amount of the reactant material on the productivity is shown in Figure 7.

As can be seen from FIG. 6, as the ratio of the amount of salicylaldehyde and melamine increase, the SM productivity also increase. When the salicylaldehyde and melamine amount ratio is 4: 1, the productivity of SM reaches the maximum value of 30.12%. When the amount ratio of the material exceeds 4:1, the productivity of the product decreases. Therefore, the most appropriate reaction of the amount of material ratio salicylaldehyde: melamine is equal to 4: 1.

From the above single factor experiments, it can be found that the appropriate

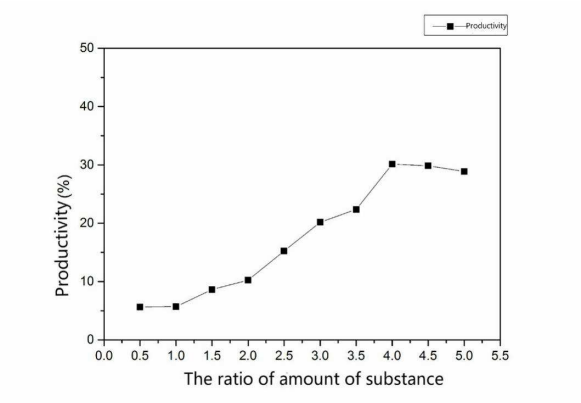

Fig. 7. The effect of the amount of the reactant substance on the productivity

conditions of the synthesis of SM are: The solvent is DMF, the reaction time is 4h, the reaction temperature is 120℃,the ratio of the amount of salicylaldehyde to melamine is 4:1.

6. Conclusion

This paper synthesis melamine salicylaldehyde Schiff base. The Schiff base is characterized by IR, 13CNMR, MS and elemental analysis.By single factor test method, changing the solvent, the reaction time, the reaction temperature, the amount ratio of the reactants, and calculating the SM productivity, it can be seen that the suitable synthesis conditions of SM are: the solvent is DMF; the reaction time is 4h; the reaction temperature is 120℃; and the ratio of the quality of Salicylaldehyde and melamine is 4:1. Under the optimum conditions, the productivity of SM is 30.12%. In this paper, the antibacterial activity of SM was studied to study the inhibitory effect of SM on Escherichia coli, Staphylococcus aureus and Bacillus subtilis. Experiments show that the inhibitory effect of SM on Bacillus subtilis is not obvious; SM on Escherichia coli and Staphylococcus aureus has a good inhibitory effect. The concentrations of SM are 100mg/L and 200mg/L, respectively, which has a better inhibitory effect on Escherichia coli and Staphylococcus aureus, and the inhibitory effects are 55.46% and 67.68% respectively. So, SM is expected to be used for the development of antibacterial drugs.

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