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Spectroscopic Study on the Interaction between Mobic and Lysozyme

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Abstract— *Under simulated physiological conditions (pH=7.40), the interaction between Mobic and lysozyme was studied by synchronous fluorescence spectroscopy, ultraviolet spectroscopy, circular dichroism spectroscopy and molecular docking simulation technique.The results of spectroscopy showed that the fluorescence of lysozyme was statically quenched by Mobic, the number of binding sites was about 1, and the conformation of lysozyme was changed.The thermodynamic parameters obtained from the van't Hoff equation show that the Gibbs free energy $\Delta G<0$ showed that the reaction between them was spontaneous, and $\Delta H<0$, $\Delta S>0$, indicating that the hydrophobic force plays an important role in the formation of Mobic-lysozyme complex.The results of molecular docking showed that the binding site of Mobic was close to the active site composed of Asp48 and Glu35 residues, indicating that Mobic could change the microenvironment of amino acid residues at the catalytic active center of lysozyme.The results of docking further showed that there was a hydrogen bond between Moby and lysozyme, so the interaction between Mobic and lysozyme was driven by hydrophobic interaction and hydrogen bond.*

Keywords— ***Mobic; lysozyme; spectroscopy; conformational; molecular docking.***

I. INTRODUCTION

Non-steroidal anti-inflammatory drugs can relieve pain and edema to play a role in the treatment of inflammation, however, patients taking non-steroidal anti-inflammatory drugs often cause gastritis, gastric ulcer, kidney and liver damage and other adverse symptoms [1].Mobic is one of the non-steroidal anti-inflammatory drugs [2] and the structural formula is shown in Figure 1. The toxic and side effects of Mobic are much less than those of some other non-steroidal anti-inflammatory drugs (such as dotaline, ibuprofen, etc.). Therefore, the adverse effect of Mobic on gastrointestinal function was much smaller [3].This drug is widely used in daily life and it is a very common drug for the treatment of rheumatoid arthritis. in addition to the treatment of inflammatory diseases, Mobic also has a good effect on relieving physical pain in patients [4].

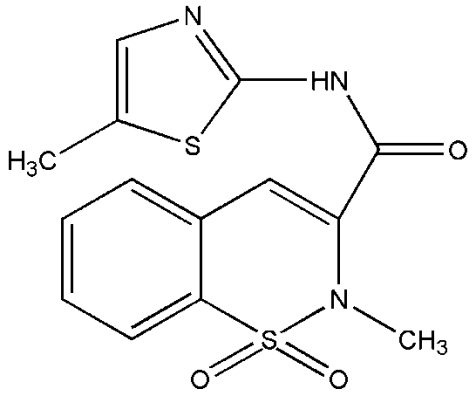

Fig.1: Chemical structure of Mobic.

The relative molecular weight of lysozyme (LYSO) is about 14600 [5]. The main fluorescent groups in LYSO are Trp62 and Trp108 amino acid residues[6].LYSO is widely found in humans and animals. Such as tears, saliva, blood and lymphoid tissue all contain LYSO, which is a very important antibacterial protein in organisms. In addition to antibacterial activity, LYSO also has the ability to treat inflammation, regulate the immune ability of the body, antihistamine and inhibit tumor activity [7]and many other biological functions. Because of its important physiological function, LYSO is often studied as an important protein model.

In recent years, fluorescence spectroscopy has become

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an important means to study the mechanism of ligand-protein system. So far, Sourav Das [8] has explored the relationship between naringin and LYSO by spectroscopy and molecular docking. Yan Juan [9]has studied the mechanism of interaction between vitexin and LYSO by fluorescence spectroscopy. ChenChen[10] has studied the binding of oxaliplatin to LYSO by fluorescence spectroscopy.At present, the methods to explore the mechanism of the reaction between drugs and proteins are mainly to investigate the fluorescence quenching of proteins at the excitation wavelengths of 280nm and 295nm with the change of drug concentration,which is to study the overall interaction between drugs and proteins[11].And synchronous fluorescence has both good selectivity, high sensitivity and it can narrow band and reduce scattering interference[12]. In the study of the reaction mechanism between drug and protein, synchronous fluorescence method is mainly used to investigate the conformational change of protein by the displacement of synchronous fluorescence peak [13].At present, there are few reports on the interaction between drugs and proteins by synchronous fluorescence spectrometry. In this paper, the interaction between Mobic and LYSO was studied by synchronous fluorescence spectroscopy, ultraviolet spectroscopy, circular dichroism spectroscopy and molecular docking simulation. The reaction mechanism between drug molecules and amino acid residues can be revealed at a deeper level, which provides a certain reference for the study of the binding of small molecular drugs and proteins.

II. EXPERIMENTAL

2.1 Apparatus

RF-5301PC fluorometer (Shimadzu, Japan); UV-3600 UV-vis spectrophotometer (Shimadzu, Japan); MOS-450/SFM300 circular dichroism (Bio-Logic, France); SYC-15_B super constant temperature water bath (Nanjing Sanli Electronic equipment Factory); SZ-93 automatic double Pure Water Distiller (Shanghai Yarong biochemical instrument Factory).

2.2 Materials

LYSO (purity grade inferior 99%, Sigma), reserve solution(5.0×10^{-6}mol/L); Mobic (CAS#,71125-28-7), reserve solution(4.0×10^{-4} mol/L).Tris-HCl buffer solutionwas used to keep the pH of the solution at 7.40, and NaCl (0.10 mol/L) solution was used to maintain the ionic strength of the solution.The water used in the experiment was secondary quartz distilled water, and the above storage solution was kept away from light at 277 K.The fluorescence signal measured in the experiment was corrected by the "internal filter effect" Eq. (1)[14]:

$$F_{cor} = F_{obs} \times e^{(A_{ex}+A_{em})/2} \quad (1)$$

Where F_{cor} and F_{obs} are the corrected and observed fluorescence signals, respectively, and A_{ex} and A_{em} are the absorbance values of Mobic-LYSO system at excitation and emission wavelengths, respectively. The fluorescence signal used in this article was corrected.

2.3 Experiment procedure

2.3.1 Synchronous Fluorescence measurements

At 298K, 310K and 318K, 1.0 mL Tris-HCl buffer solution, 1.0 mL LYSO solution and different volume of Mobic solution were added to the 10.0 mL colorimetric tube at constant volume and constant temperature of 30 min. The prepared solution was placed in a 1.0 cm quartz colorimetric dish, and the difference $\Delta\lambda$ between the fixed emission wavelength and the excitation wavelength was set at 5 nm. The synchronous fluorescence spectra of the scanning system were 15 nm and 60 nm, respectively, and the synchronous fluorescence intensity was recorded.

2.3.2 Circular dichroism measurements

At 298K, 1.0 mL Tris-HCl buffer solution, 1.0 mL LYSO solution and different volume of Mobic solution were added to the 10.0 mL colorimetric tube at constant volume and constant temperature of 30 min.Circular dichroism measurements were performed with a 1.0 cm path length quartz cuvette. Each spectrum was recorded at wavelengths between 190 and 300 nm and a scan speed of 1 nm/s.

2.3.3 UV-Vis measurements

At 298 K, 1.0 mL Tris-HCl buffer solution,1.0 mL LYSO solution and different volume Mobic solution was added to the 10.0 mL colorimetric tube at constant volume and constant temperature of 30 min. The absorbance of the system was determined by using the corresponding concentration of Mobic solution as the blank reference, and the UV absorption spectrum of the systemwas drawn.

2.3.4 Molecular docking

The crystal structure (PDB ID: 6LYZ) of LYSO comes

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from the protein database (Protein Data Bank). The ChemDraw Pro 14.0 and ChemBio 3D Ultra 14.0 are used to draw the Mobic structure, and the energy minimization of the three-dimensional structure is carried out. AutoDock 4.2.6 was used to study the molecular docking of Mobic and LYSO, and genetic algorithm was used to calculate the binding conformation of Mobic and LYSO[15].

III. RESULTS AND DISCUSSION

3.1 Synchronous fluorescence studies of Mobic-LYSO system

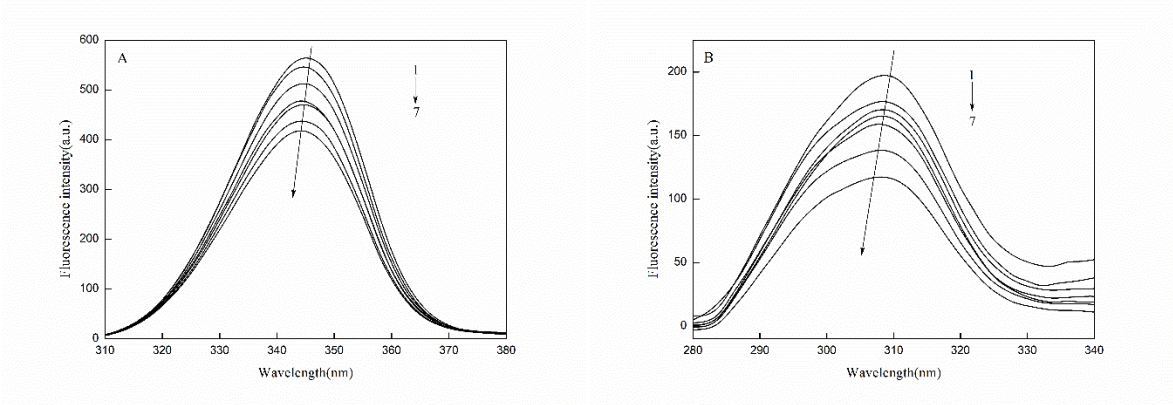

Fig.2: Synchronous fluorescence spectra of Mobic-LYSO system (T=298 K). (A) Δλ=60 nm; (B) Δλ=15nm C_{LYSO}=5.0×10^{-7} mol/L, 1~7 C_{Mobic}=(0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0)×10^{-5}mol/L

Synchronous fluorescence spectra can show the micro environmental information of protein fluorescence groups. When the Δλ value between excitation wavelength and emission wavelength is stable at 15 nm or 60 nm, synchronous fluorescence gives the characteristic information of Tyr residue or Trp residue, respectively [16].As shown in Figure 2, the fluorescence of Tyr residue or Trp residue is quenched by the addition of Mobic, and the quenching degree is further strengthened with the increase of drug concentration, which indicates that Mobic interacts with LYSO to form a stable complex.It can be seen from the synchronous fluorescence spectra that the fluorescence peaks of Trp residues and Tyr residues in LYSO have shifted very weakly. This means that the binding of Mobic to LYSO changes the microenvironment of amino acid residues in small molecular proteins such as LYSO, which reduces its hydrophobicity and increases the extension of peptide chain [17].

Table.1: Quenching reactive parameters of Mobic-LYSO system at different temperatures

Δλ (nm)	*T*/(K)	k_q(L/mol·s)	K_{sv}(L/mol)	r_1	K_a(L/mol)	*n*	r_2
60	298	1.95×10^{12}	1.95×10^4	0.9946	2.13×10^4	1.04	0.9907
	310	1.43×10^{12}	1.43×10^4	0.9980	1.58×10^4	1.05	0.9952
	318	1.23×10^{12}	1.23×10^4	0.9936	1.21×10^4	1.03	0.9955
15	298	1.48×10^{12}	1.48×10^4	0.9939	1.51×10^4	1.06	0.9951
	310	1.16×10^{12}	1.16×10^4	0.9943	1.13×10^4	1.09	0.9956
	318	0.91×10^{12}	0.91×10^4	0.9926	0.84×10^4	1.07	0.9966

r_1 is the linear relative coefficient of F_0/F~[*L*]; r_2 is the linear relative coefficient of lg[(F_0-*F*)/]~lg{[*L*]-*n*[B_t](F_0-*F*)/F_0}

The Stern-Volmer [18] equation was shown below, through which the quenching constant K_{sv} and the quenching

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rate constant k_q can be calculated by using the fluorescence signal data obtained from the experiment:

$$F_0 / F = 1 + k_q \tau_0 [L] = 1 + K_{SV}[L] \quad (2)$$

Where F_0 and F represent the fluorescence signals in the absence and presence of quencher, respectively. τ_0 is the average lifetime of fluorescence without quencher, which is about 10^{-8} s. K_{sv} is the Stern-Volmer quenching constant. k_q is the bimolecular quenching constant, and [L] is the concentration of Mobic.According to the calculation of Eq. (2), the values of K_{sv} and $k_q$were listed in Table 1. The results showed that the k_q values at different temperatures are larger than the maximum diffusion collision quenching constant of 2×10^{10}L/mol·s[19] for biomolecules by various quenching agents. At the same time, it could be seen from the data in Table 1 that with the increase of temperature, k_q of Mobic-LYSO system and K_{sv}'s are decreased. The results showed that the quenching mode of Mobic-LYSO system was static quenching.

For static quenching, the Eq. (3)was generally used to calculate the binding constant K_a and the number of binding sites n:

$$\lg\left(\frac{F_0 - F}{F}\right) = n \lg K_a + n \lg\left\{[L] - n\frac{F_0 - F}{F_0}[B_t]\right\} \quad (3)$$

[B_t] represents the concentration of LYSO, and the results were shown in Table 1.From Table 1, n≈1 at the experimental temperature indicates that there is only one high affinity binding site[20] for Mobic to bind to LYSO, that is, Mobic formed a 1:1 complex with LYSO.The binding constant K_a of Mobic to LYSO decreases with the increased of temperature, which further proved that the fluorescence quenching type of Mobic-LYSO system was static quenching.From the data in the table, it could be further seen that the K_a value at $\Delta\lambda$=15 nm was obviously smaller than that at $\Delta\lambda$=60 nm, which indicated that the reaction between Mobic and Trp residues is stronger.

3.2 Circular dichroism spectra studies of Mobic-LYSO system

As one of the well-known biophysical techniques, circular dichroism spectrum (CD) is usually used to clarify the secondary structure of proteins. In the CD spectrum, the far ultraviolet region (180 nm) mainly shows the α-helix, β-fold and irregular crimping of the protein[21].The peaks at the left and right sides of the 208 nm and 222 nm are characteristic peaks of α-helix. The change of α-helix content can be obtained by using the following equation (4)[22]:

$$K_{\alpha-Helix}(\%) = \frac{-N_{MRE} - 4000}{33000 - 4000} \times 100 \quad (4)$$

The N_{MRE} is the molar ellipticity at 200 nm; the number 4000 is the N_{MRE} value of β-fold and random curl at 200 nm; and the number 33000 is the N_{MRE} value of pure α-helix at 200 nm.

$$N_{MRE} = \frac{I_{CD}}{10 C_p n l} \quad (5)$$

As shown in Eq. (5), I_{CD} is the signal of CD at 208 nm, C_p is the concentration of protein, n is the number of amino acid residues and l is the path length (cm). The circular dichroism spectroscopy of Mobic-LYSO system was shown inFigure 3. When the molar ratio of LYSO to Mobic were 1:0, 1: 5 and 1: 10, the content of α-helix structure of LYSO molecules decreased from 22.76% to 10.79%. The intensity of the negative peaks decreases gradually, without obvious change in position and shape of the peaks. It could be concluded that the interaction between Mobic and LYSO made the α-helix structure of LYSO loose and changed the protein secondary structure, leading to quenching of the LYSO fluorescence. But α-helical structure was still dominant.

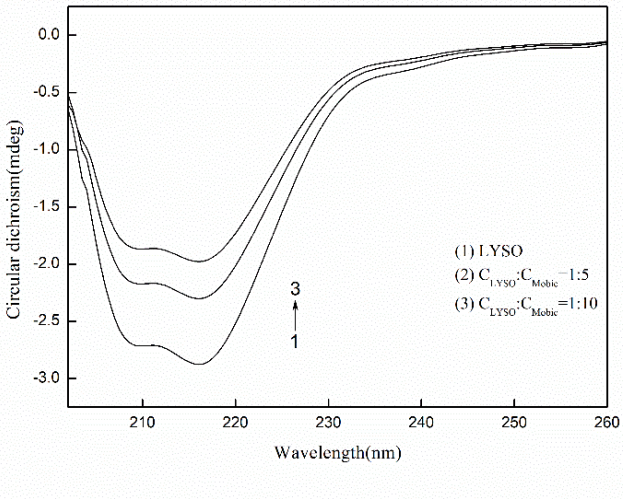

Fig.3: The circular dichroism spectra of Mobic-LYSO system (T=298 K)

C_{LYSO}= 2.0×10^{-6} mol/L;C_{Mobic}= (0, 1.0, 2.0) $\times10^{-5}$ mol/L

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3.3 UV-vis absorption spectra studies of Mobic-LYSO system

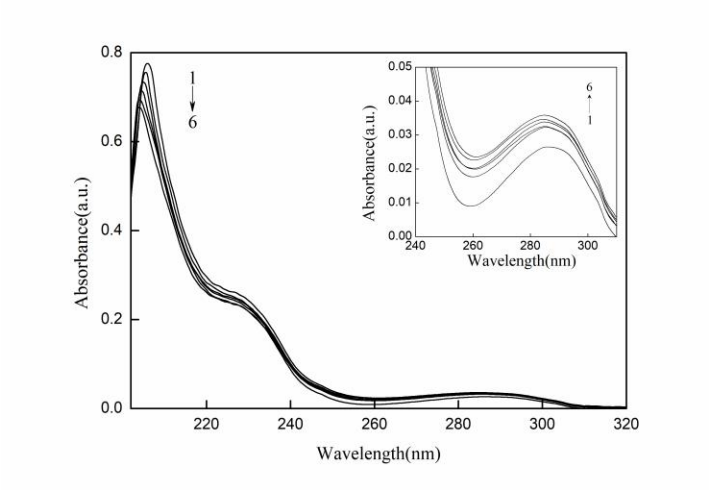

Fig.4: Absorption spectrum of Mobic-LYSO system (T=298 K)

C_{LYSO}=2.0×10^{-6}mol/L, 1~6: C_{Mobic}=(0, 0.2, 0.5, 1.0, 1.5, 2.0)×10^{-5}mol/L

UV-vis absorption spectra can be used to explore the structural changes of proteins and to study the formation of protein-ligand complexes[23]. Figure4was an absorption spectrum of the Mobic-LYSO system. From the figure, it can be seen that LYSO has two absorption peaks, and its strong absorption peak near 208 nm reflects the frame conformation of the protein. The weak absorption peak at about 280 nm is due to aromatic amino acids (Trp,Tyr and Phe)[24]. With the increases of Mobicconcentration, Figure4 showed that the intensity of the absorption peak at 208nm decreased with the blue shift, and the absorption peak at 280nm also decreased slightly. This result indicated that the interaction between Mobic and LYSO led to the formation of new complexes, and the LYSO molecule tended to fold, the hydrophobicity of LYSO microenvironment was enhanced.The UV-Vis absorption spectrum of the system shifted, indicating that the fluorescence quenching of LYSO by Mobic, a new complex formed by the reaction, should belong to the static quenching process [25], which was consistent with the conclusion of synchronous fluorescence experiment.

3.4 Type of interaction force of Mobic-LYSO system

The thermodynamic parameters of Mobic-LYSO systemwere calculated according to van't Hoff equation[26], and the calculated results were shown in Table 2.

$$R\ln K = \Delta S - \Delta H / T \quad (6)$$

$$\Delta G = -RT\ln K = \Delta H - T\Delta S \quad (7)$$

Table 2.The thermodynamic parameters of Mobic-LYSO at different temperatures

System	*T*/(K)	K_a/(L/mol)	Δ*H*/(kJ/mol)	Δ*S*/(J/mol·K)	Δ*G*/(kJ/mol)
Δλ= 60 nm	298	2.13×10^4	-21.98	9.09	-23.69
	310	1.58×10^4		9.47	-24.92
	318	1.21×10^4		9.03	-24.85
Δλ= 15 nm	298	1.51×10^4	-22.68	3.90	-23.84
	310	1.13×10^4		4.43	-24.05
	318	0.84×10^4		3.81	-23.89

Where *R* is a gas constant (Δ*H* and Δ*S* with a value of about 8.314). Mobic and LYSO could be calculated by a linear relationship between the natural logarithm (lnK_a) of the binding constant and the reciprocal (1/T) of the temperature. The results were shown in Table 2.It could be seen from Table 2 that Δ*G*<0 indicated that the binding reaction between Mobic and LYSOwas spontaneous, and Δ*H*<0 indicated that the formation of Mobic-LYSO complex was exothermic.The arrangement of water molecules created a more random configuration around drugs and proteins in a more orderly manner. Therefore, Δ*S*>0 was usually used as evidence of hydrophobic interaction between drug molecules and protein molecules[27]. Based on this, the hydrophobic interaction between Mobic and LYSO could be judged.

Ross and Subramanian [28]believed that when Δ*H*≈0, Δ*S*>0, there was electrostatic attraction between drug molecules and biomolecules, but now some reports thought that when Δ*H*<0, Δ*S*>0 could directly judge that the main type of force between the binding systems was electrostatic

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interaction [29]. In order to further verify whether the main force between Mobic and LYSOwas electrostatic force, the effect of ionic strength on Mobic-LYSO interaction was discussed in this paper.

The concentrations of Mobic and LYSO were fixed, and different concentrations of NaCl were added, and F/F_0 was plotted against C_{NaCl} and the results were shown in Figure5. The experimental results showed that when the concentration of NaCl increases, the ratio of F/F_0 did not change significantly. The results showed that the binding of Mobic to LYSOwas not affected by ionic strength, that was, the electrostatic interaction of Mobic-LYSO system was not obvious. If the electrostatic interaction played a leading role in the binding of protein to ligands, as the increasing of the salt concentration, the interaction intensity between protein and ligand decreases gradually[30]. This also indicated that when $\Delta H<0$, $\Delta S>0$ could not directly judge the main force between Mobic-LYSO system was electrostatic force.

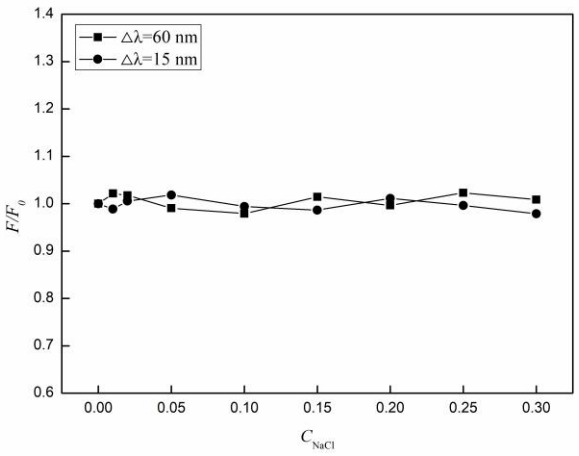

Fig.5: Synchronous fluorescence intensity of Mobic-LYSO system as a function of NaCl concentration (T=298 K)

$C_{LYSO}=5.0\times10^{-7}$ *mol/L*, $C_{Mobic}= 1.0\times10^{-5}$*mol/L*, $C_{Nacl}= (0, 0.1, 0.2, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0) \times10^{-1}$*mol/L*

3.5 Molecular docking

Molecular docking plays an important role in exploring the interaction between ligands and receptors. In order to further determine the binding position of Mobic-LYSO system and the effect of Mobic binding to LYSO on ligands and receptors. In this paper, the binding model of Mobic and LYSOwas established by molecular docking method. By this method, the type of force and the lowest binding energy of Mobic and LYSO binding system could be obtained.Figure6(A) showed the optimal binding position of Mobic to LYSO, where the Trp62 residue and the Trp63 residue formed two hydrogen bonds with Mobic, respectively, with bond lengths of 2.105 Å and 1.809 Å, respectively. This result showed that hydrogen bond played an important role in the binding of Mobic to LYSO. Figure6(B) showed a plurality of hydrophobic amino acid residues such asAla95,Ala107, Val99 and Val109around Mobic, further indicating that there was hydrophobic force in the binding process between Mobic and LYSO.The amino acid residues such as Tyr53, Trp62andTrp63 were relatively close to the binding position of Mobic and LYSO, which led to the binding could effectively quench the endogenous fluorescence of LYSO, which was consistent with the conclusion of fluorescence quenching experiment. Glu and Aspare the key residues of the catalytic active center of LYSO[31]. The results of molecular docking also showed that the binding of Mobic and LYSO could change the microenvironment of the catalytic active center of LYSO.In other words, the binding of the system might affect the catalytic activity of LYSO.

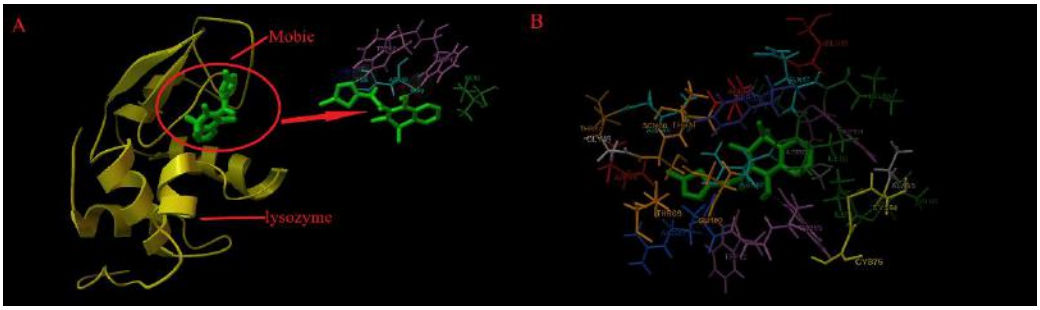

Fig.6: Molecular docking of the Interaction between Mobic and LYSO.
(A) Mobic located within the hydrophobic pocket in LYSO(B) Detailed illustration of the amino acid residues lining the binding site in theMobic and LYSO cavity

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The binding energy obtained from molecular docking for Mobic and LYSO interaction was -26.81kJ/mol. At 310K, it is close to the synchronous fluorescence experiment that ΔG (Trp)=24.92 kJ/mol, ΔG (Tyr) =24.05 kJ/mol.This difference may be due to exclusion of the solvent in docking simulations or rigidity of the receptor other than Trp and Tyr residues[32]. The energy data obtained by docking the molecules were list in Table 3. From Table 3, it could be also seen that the electrostatic energy was very much lower than the sum of van der Waals energy, hydrogen bonding energy and desolvation free energy in the binding process of Mobic with LYSO, indicating that the main interaction mode between Mobic and LYSOwas not electrostatic binding mode.Combined with the data of fluorescence experiments and the results of theoretical modeling, it could be seen that hydrophobic interaction and hydrogen bond were the main forces driving the combination of Mobic molecules with LYSO molecules, which led to the static quenching of LYSO.

Table 3. Docking energy of Mobic-LYSO system (unit: kJ/mol)

Protein PDB ID	ΔG_0	ΔE_1	ΔE_2	ΔE_3
6LYZ	-26.81	-30.53	-30.32	-0.21

ΔG_0 is the binding energy in the binding process.

ΔE_1 denotes intermolecular interaction energy, which is a sum of van der Waals energy, hydrogen bonding energy, desolvation free energy and electrostatic energy.

ΔE_2 is the sum of van der Waals energy, hydrogen bonding energy and desolvation free energy.

ΔE_3 is the electrostatic energy.

IV. CONCLUSION

Under simulated physiological conditions, a variety of spectroscopy and molecular docking techniques were used to explore the interaction between Mobic and LYSO, which is of positive significance for the study of pharmacokinetics, pharmacodynamics and toxicology of Mobic.At present, the use of spectroscopy to study the interaction between proteins and ligands is more and more popular and favored by researchers. Compared with other research methods such as chromatography, this research method has the advantages of low experimental cost and short measurement period. Therefore, this study provides an important reference for the efficient and rapid study of the interaction between proteins and ligands.

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