SUPPORTING INFORMATION

General information

Ciprofloxacin hydrochloride reference substance (CPLX, 100.0%), danofloxacin mesylate reference substance (DFLX, 99.7%), enrofloxacin reference substance (EFLX, 100.1%), sarafloxacin reference substance (SALX,99.6%), difloxacin hydrochloride reference substance (DIF, 99.9%) were purchased from China Institute of Veterinary Drug Control. 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC.HCl), N,N-dimethylformamide(DMF), N-hydroxysuccinimide (NHS), bovine serum albumin(BSA), ovalbumin(OVA), freund's complete adjuvant(FCA); freund's incomplete adjuvant(FIA) were purchased from sigma-aldrich(St.Louis, MO,USA). Horseradish peroxidase labeled goat anti-mouse IgG is purchased from Santa Cruz Biotechnology Co., Ltd. (Shanghai, china). 3,3',5,5'-Tetramethylbenzidine (TMB) was purchased from the Beijing solarbio science & technology co., ltd. (Beijing, China). 3-Bromopropylamine hydrobromide and glutaraldehyde were purchased from the Shanghai Jingchun Biochemical Technology Co., Ltd. (Shanghai, China). All other chemicals and solvents were of analytical grade or better and were purchased from the National Pharmaceutical Group Chemical Reagent Co., Ltd. (Shanghai, China). Polystyrene microplates were purchased from Corning Inc.(Corning, Michigan, USA).5-week-old SPF female Kunming mice were used in this research [SCXK(jing)2014-0004] (Beijing HFK Bioscience Co. Ltd, China).

Apparatus

The microplate reader was a multiskan MK3 microplate reader (Thermo Fisher Scientific Inc. , USA). UV-visible spectra were obtained by using a U-3900 Ultraviolet-visible spectrophotometer (Hitachi High-Technologies Corporation, Japan). Infrared spectra were acquired with an IR solution spectrometer (Shimadzu Co., Ltd., Kyoto, Japan).An Agilent 1200 series LC/MS System was used(Agilent, Palo Alto, USA). NMR spectra were obtained by using a Bruker AVANCE Ⅲ 500

MHz UltraShield-plusTM digital NMR spectrometer (Bruker Corporation, Switzerland). Reverse osmosis water (RO water) was prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA).

Buffers and solution

0.05 mol/L Carbonate buffer solution (CBS, pH 9.6), 0.01 mol/L Phosphate buffer solution (PBS, pH 7.4), blocking buffer solution (0.2% w/v gelatin in PBS), washing buffer solution (0.05 % v/v Tween 20 in PBS, PBST) , antibody dilution buffer solution (PBS containing 0.1 % w/v gelatin and 0.05 % v/v Tween 20),stopping reagent (2.0 mol/L H_2SO_4).

Synthesis of 3-bromopropylamine derivatives of ciprofloxacin (CPLX-NH_2)

3-Bromopropylamine hydrobromide (218.92 mg) was dissolved in DMF (5.00 mL) to obtain A solution. And ciprofloxacin hydrochloride (385.82 mg) was dissolved in DMF (10.00 mL) to obtain B solution. A solution was initially added dropwise to solution B, and then the mixture was stirred for 3 h at 80 °C. During the reaction, NaOH solution was added dropwise to keep pH = 8. After this reaction, hydrochloric acid solution (1.00 mol/L) was added dropwise to reach pH = 6, and this solution were crystallized, filtrated and dried to obtain crude ciprofloxacin derivative (CPLX-NH_2). The pure ciprofloxacin derivative was obtained by preparing the liquid phase, and sunfire column (3.50 μM, 150.00 mm × 4.60 mm) was taken as the liquid phase. H_2O and acetonitrile was used as the mobile phase at a flow rate of 1.00 mL/min at the column temperature of 40 °C. The content of acetonitrile was changed from 5% to 95% in the first 9 min, and the analyses were performed using 95% acetonitrile for 5 min, and then the acetonitrile was reduced to 5% in 1.0 min. It was identified by infrared spectroscopy (IR), nuclear magnetic resonance (NMR) and electrospray ionization mass spectrometry (ESI-MS).

The analytical results of CPLX-NH_2 by ^{1}H-NMR(500MHz, DMSO-d_6): δ = 15.131 (s, 1H, Ar-COOH), 8.686 (s, 1H, 2-H), 7.976 (d, 1H, J=13, 5-H), 7.628 (d, 1H, *J* =13Hz, 8-H), 3.859 (m, 1H, 11-H), 3.450 (m, 8H, piperazine), 3.240 (m, 4H, 19,

21-H), 2.921 (m, 2H, 20-H), 2.016 (s, 2H, -NH_2), 1.331 (m, 2H, 13-H), 1.200 (m, 2H, 12-H). From the proton signals of the ^{1}H-NMR spectrum it can be inferred that ciprofloxacin successfully conjugated with the 3-propylamino, and the amino was exposed (δ = 2.0 s, 2H).

The analytical results of CPLX-NH_2 by ^{13}C-NMR(125MHz, DMSO-d*6*): δ = 176.33 (C-4), 165.83 (C-14), 153.01 (C-6), 151.83 (C-2), 148.16 (C-7), 143.75 (C-10), 139.01 (C-9), 111.05 (C-5), 106.94 (C-3), 106.78 (C-8), 52.82 (C-16, 17), 50.70 (C-15, 18), 46.53 (C-19), 36.31 (21-C), 35.93 (C-20), 21.72 (C-11), 7.56 (C-12, 13). So from the carbon signals of the ^{13}C-NMR spectrum it can be shown that the carbon number of CPLX-NH_2 was three more than that of ciprofloxacin. Hence, the conjugation between ciprofloxacin and 3-propylamino was successful.

The IR spectra of CPLX-NH_2 : The absorption peak of 3 373 cm^{-1} was the carboxy hydroxy, the absorption peak at 3518 cm^{-1} was the -NH_2, and the absorption peaks at 3012 cm^{-1} and 1628 cm^{-1} were the aromatic nucleus. The absorption peaks at 2711 cm^{-1}, 1 497 cm^{-1} and 805 cm^{-1} were the characteristic absorption of stretching vibration and in-plane rocking vibration of primary amine, and the strong absorption peak at 1709 cm^{-1} represented the characteristic peak of the carbonyl. Therefore, it can be inferred that CPLX and 3-bromo-propylamine conjugated successively.

The full scan results for CPLX-NH_2 by ESI-MS showed that the precursor ion at m/z 389.3 $[M+H]^+$ corresponded to the mass of hapten molecular. The precursor ion at m/z 389.3 $[M+H]^+$ gave product ions at m/z 332.2, m/z 241.0, m/z 213.0, m/z 186.6, m/z 173.1 and other product ions were found and can be reasonably attributable (Fig. S4). Hence, the precursor ion at m/z 389.3 can be attributed to the 3-bromopropylamine derivative of ciprofloxacin.

Synthesis of complete antigens for CPLX-NH_2

Preparation of complete antigen was completed by using glutaraldehyde method. 100.00 mg of CPLX-NH_2 was dissolved in 5.00 mL of HCl solution (0.6 mol/L) and then 30.00 mg of zinc powder was added. The mixture was heated with water bath of

80 °C for 30min to obtain A solution. 100.00 mg of BSA was dissolved in 2.00 mL of 0.02 mol/L PBS solution to obtain B solution. A solution was added dropwise to B solution, and after their intensive mixing 0.10 mL of 25% glutaraldehyde was added dropwise. The mixture was stirred in dark at room temperature for 6 h, and then was filled into a dialysis bag and dialyzed with PBS at 4 °C for 72 h to prepare the immunogen of CPLX-NH_2-BSA, which was repacked and stored at -20 °C.

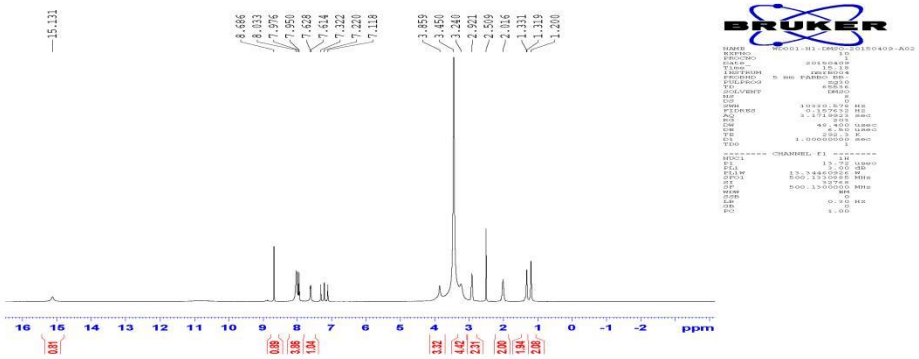
The coating antigen of CPLX-NH_2-OVA conjugate was prepared in a similar method by replacing BSA with OVA.

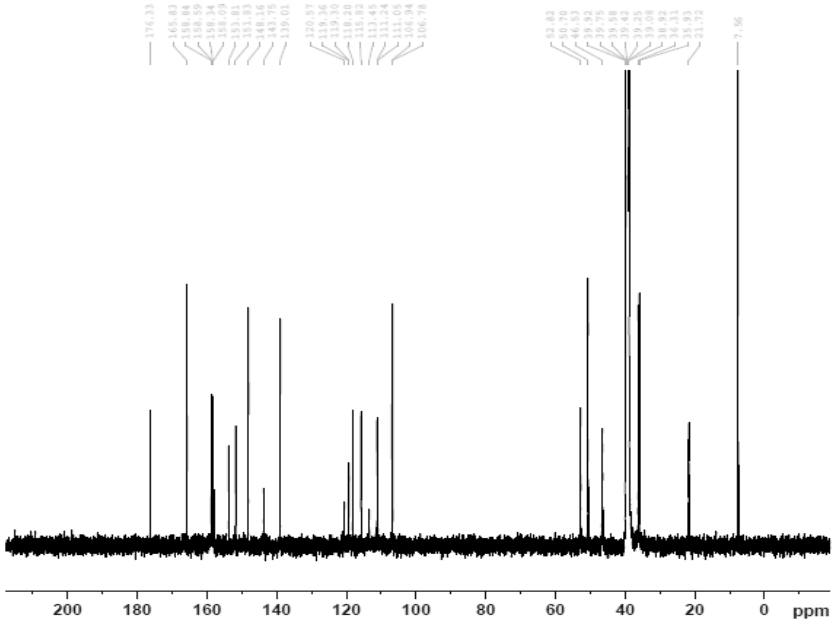
Synthesis of complete antigens for CPLX

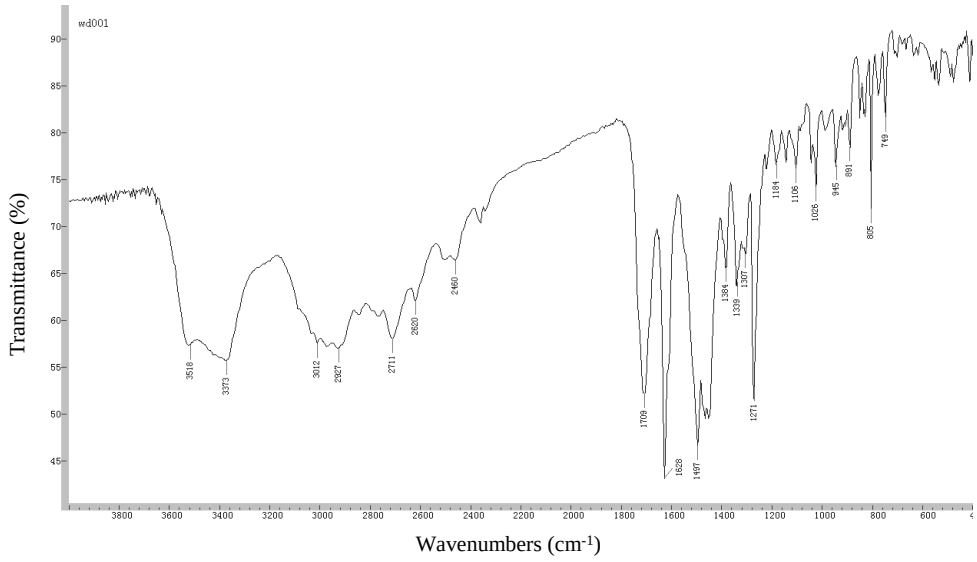
Synthesis of complete antigens were made by N-hydroxyl amber imide possess active ester (NHS) method(Huang, Z. H., 2014) with the following minor modifications. A total of 20.0 mg CPLX, 10.0 mg NHS and 12.5 mg EDC dissolved completely in 1.0 mL DMF in order. The mixture solution was being stirred for 24.0 h at room temperature in dark, and CPLX reaction liquid was got. 50.0 mg BSA dissolved fully in 3 mL PBS (0.01 mol/L, pH 7.4). Then CPLX reaction liquid was added to the BSA solution slowly being stirred, followed by stiring for 3.0 h at room temperature. Finally, the reaction mixture was dialyzed under stirring against PBS for 3 d with PBS solution (changing it three times a day) to remove the unconjugated hapten. The dialysates were centrifuged at 5 000 r/min for 30 min at room temperature, and the supernatant fluid was collected, which was CPLX-BSA immunogen. Then it was repacked and stored at -20℃.

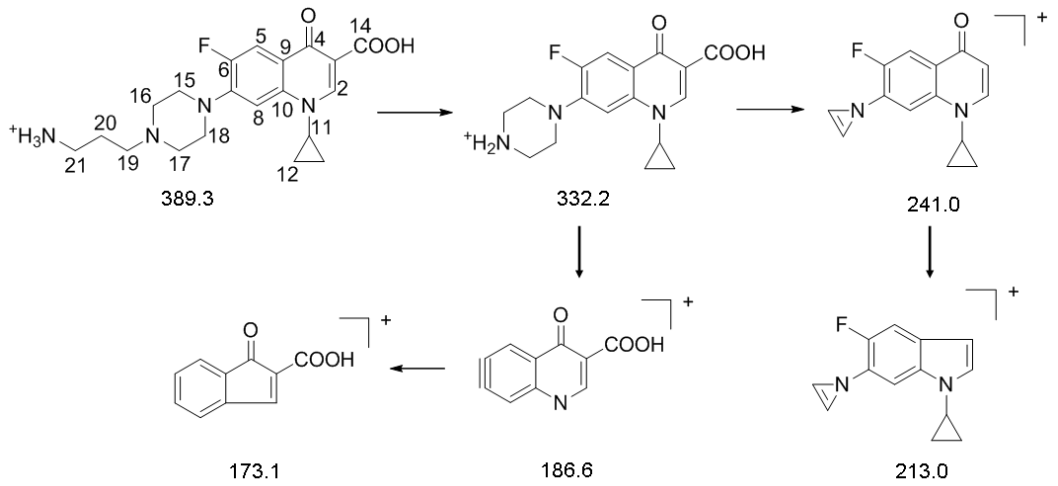
Replace BSA with OVA and do as above, and make a preparation of CPLX-OVA coating antigen.

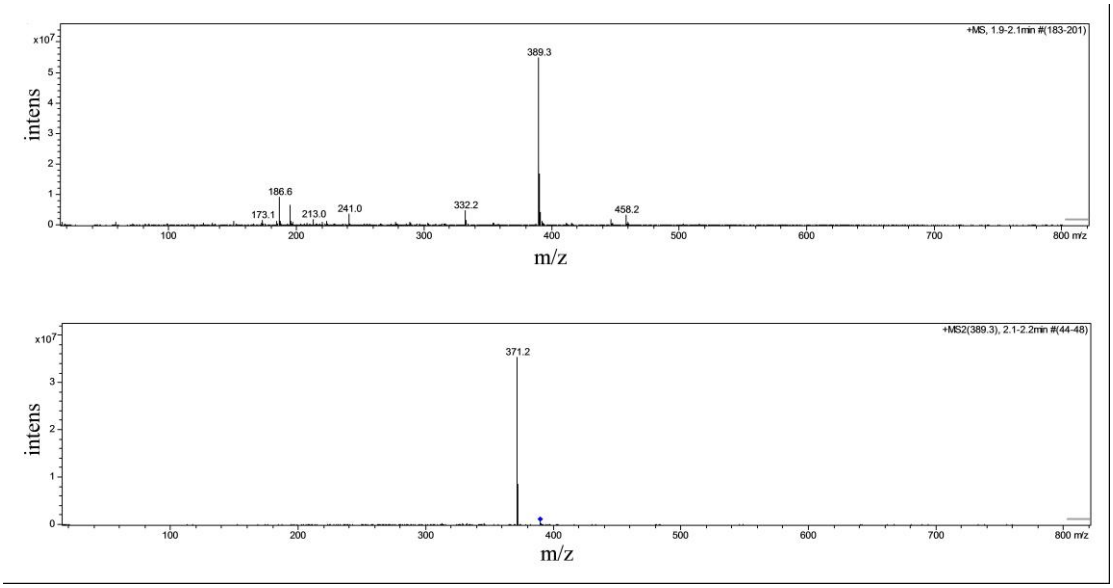
^{1}H NMR, ^{13}C NMR, IR, and ESI-MS of CPLX-NH_2


15.131
8.666
8.033
7.976
7.950
7.628
7.614
7.322
7.220
7.118
3.859
3.456
3.240
2.921
2.509
2.016
1.331
1.319
1.200
BRUKER
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 0 -1 -2 ppm
0.81
0.88
3.86
1.04
3.42
4.42
2.21
2.00
1.94
2.00


176.33
165.83
160.84
158.59
156.34
152.69
152.01
148.16
143.75
139.01
130.57
129.36
129.30
128.80
125.83
123.45
121.24
111.05
106.94
106.78
62.82
60.70
46.53
39.92
39.75
39.58
39.42
39.25
39.08
38.92
14.31
13.93
11.72
7.96
200 180 160 140 120 100 80 60 40 20 0 ppm


wd001
Transmittance (%)
90 85 80 75 70 65 60 55 50 45
3518
3373
3012
2927
2711
2620
2460
1709
1628
1497
1384
1339
1307
1271
1184
1106
1026
945
891
805
749
3800 3600 3400 3200 3000 2800 2600 2400 2200 2000 1800 1600 1400 1200 1000 800 600
Wavenumbers (cm-1)


389.3
332.2
241.0
173.1
186.6
213.0


+MS, 1.9-2.1min #(183-201)
389.3
186.6
173.1
213.0
241.0
332.2
458.2
intens
m/z
+MS2(389.3), 2.1-2.2min #(44-48)
371.2
intens
m/z