Supporting information

Synthesis of fluorescent drug molecules for competitive binding assay based on molecularly imprinted polymers

Experimental details:

Synthesis of compound MNZ-SA:

In a 50 mL flask, metronidazole (1.03 g, 6 mmol) was dissolved in pyridine (4 mL). Then succinic anhydride (SA) (0.90 g, 9 mmol, 1.5 eq) was dissolved in anhydrous pyridine (4 mL) and slowly added dropwise into the above reaction mixture, within 8 minutes under stirring and ice bath condition. After completion of the reaction, the ice bath was removed. Under N_2 atmosphere and avoiding of light, resulting mixture was stirred for 24 h at room temperature. Solvent was removed under reduced pressure. Crude product was dissolved in acetone (25 mL) and then added diethyl ether (50 mL), the mixture was kept overnight at room temperature. Suspension was filtered. The filtrate was treated again by use of the procedure mention above. Filter cake obtained by two filtration steps was combined. The product MNZ-SA was obtained as a white crystal, 1.54 g, yield 95%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 12.22 (s, 1H), 8.04 (s, 1H), 4.57 (t, J = 7.2 Hz, 2H), 4.37 (t, J = 7.2 Hz, 2H), 2.64 (s, 3H), 2.45-2.42 (m, 4H) ppm. IR (KBr): ν_{max} 2534, 1332, 1264 cm^{-1}.

Synthesis of compound AZT-SA:

In a 50 mL flask, AZT (1.07 g, 4 mmol) was dissolved in pyridine (5 mL). Succinic anhydride (SA) (0.60 g, 6 mmol, 1.5 eq) was dissolved in anhydrous pyridine (3 mL) and this mixture was slowly added dropwise into the above reaction, within 5 minutes under stirring and ice bath condition. After completion of the reaction, the ice bath was removed. Under N_2 atmosphere and avoiding of light, resulting mixture was stirred for 24 h at room temperature. Solvent was removed under reduced pressure. Crude product was obtained as yellow powder and was purified by column chromatography over silica gel (30cm×2cm, eluting with V ($CHCl_3$):V (EtOH) = 9:1). AZT-SA product was obtained as a yellow grease 1.35 g, yield: 92%. ^{1}H NMR (400 MHz, $CDCl_3$) δ:12.01(s, 1H), 8.81 (s, 1H), 7.22 (d, J = 1.6 Hz, 1H), 5.78 (d, J = 1.2 Hz, 1H), 4.52 (dd, J = 8.8, 3.2 Hz, 1H), 4.29 (dd, J = 7.6, 4.8 Hz, 1H), 4.21-4.18 (m, 1H), 2.69-2.66 (m, 1H), 2.51-2.48 (m, 1H), 2.01 (s, 3H), 1.48-1.45 (m, 1H) ppm. IR (KBr): ν_{max} 2536, 1707, 1289 cm^{-1}.

Synthesis of compound 3TC-ES:

In a 50 mL flask, lamivudine (3TC) (2.75 g, 12 mmol) was dissolved in anhydrous pyridine (8 mL). Acetic anhydride (1.02 g, 10 mmol) was dissolved in anhydrous pyridine (2 mL) and this mixture was slowly added dropwise into the above reaction, within 3 minutes under stirring and ice bath condition. After completion of the reaction, the ice bath was removed. Under N_2 atmosphere and avoiding of light, resulting mixture was stirred for 12 h at room temperature. Pyridine was removed under reduced pressure, and resulting mixture dried in vacuum. Reactions was monitored by thin-layer chromatography (TLC), result showed that there were three products. Crude product was purified by column chromatography over silica gel (30cm×2cm, eluting with *V* ($CHCl_3$):*V* (EtOH) = 9:1) to collect 3TC-ES, 1.86g, yield 69%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.71 (d, *J* = 7.8 Hz, 1H), 7.28 (d, *J* = 7.8 Hz, 1H), 6.24 (t, *J* = 9.6 Hz, 1H), 5.77 (d, *J* = 11.2 Hz, 1H), 5.36 (dd, *J* = 4.0 Hz, 1H), 4.36-4.35 (m, 2H), 3.40-3.34 (m, 1H), 3.11 (dd, *J* = 1.6 Hz, 1H), 2.06 (s, 3H) ppm. IR (KBr): ν_{max} 3354, 1743, 638 cm^{-1}.

Synthesis of compound 3TC-ES-SA:

In a 50 mL flask, 3TC-ES (0.41g, 1.5 mmoL) was dissolved in anhydrous acetone (15 mL). SA (0.18 g, 1.5 mmol, 1 eq) was dissolved in anhydrous acetone (5 mL) and this mixture was slowly added dropwise into the above reaction, within 5 minutes under stirring and ice bath condition. After completion of the reaction, the ice bath was removed. Under N_2 atmosphere and avoiding of light, resulting mixture was stirred for 24 h at room temperature. During the reaction, white deposit was formed. Anhydrous acetone as solvent, suspension was filtered with vacuum and dried. 3TC-ES-SA product was obtained as white powder, 0.49 g, yield: 86%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.17 (s, 1H), 10.10 (s, 1H), 8.19 (d, *J* = 7.6 Hz, 1H), 7.22 (d, *J* = 7.2 Hz, 1H), 6.24 (t, *J* = 4.0 Hz, 5.2 Hz, 1H), 5.44 (t, *J* = 3.2 Hz, 1H), 4.50-4.40 (m, 2H), 3.61-3.57 (m, 1H), 3.34-3.24 (m, 2H), 2.64 (t, *J* = 8.0, 6.0 Hz, 2H), 2.08 (s, 3H) ppm. IR (KBr): ν_{max} 2657, 1738, 1719 cm^{-1}.

Synthesis of MNZ-F:

In a 50 mL flask, 9-aminoacridine (0.58 g, 3 mmol) was dissolved in anhydrous DMF (9 mL). Dimethylaminopyridine (0.02 g, 0.15 mmol) was added into the mixture. MNZ-SA-HAS was dissolved in anhydrous DMF (9 mL) and slowly added dropwise into the above reaction mixture, within 15 minutes under stirring and ice bath condition. After completion of the reaction, the ice bath was removed. Under N_2 atmosphere and avoiding of light, resulting mixture was stirred for 36 h at room temperature. Under stirring, reaction mixture was poured into the cool distillated water (the flask was kept cold with an ice bath), gradually formed deposit. After 1 h, the suspension was filtrated with vacuum, washed with water and dried at 90°C. Crude product was obtained as yellow powder and was purified by column chromatography over silica gel (30cm×2cm, eluting with *V* ($CHCl_3$):*V* (EtOH) =

40:3). Solvent was removed under reduced pressure, and dried in vacuum. Product was obtained as a yellow powder, 7 mg, yield: 29%. ^{1}H NMR (400 MHz, DMSO-d_6) δ:10.79 (s, 1H), 8.16 (t, J = 8.0 Hz, 8.0 Hz, 4H), 8.04 (s, 1H), 7.89-7.85 (m, 2H), 7.64-7.61 (m, 2H), 4.60 (t, J = 8.0 Hz, 8.0 Hz, 2H), 4.43 (t, J = 8.0 Hz, 4.0 Hz, 2H), 2.94 (t, J = 8.0 Hz, 4.0 Hz, 2H), 2.68 (t, J = 8.0 Hz, 8.0 Hz, 2H), 2.46 (s, 3H) ppm. IR (KBr): ν_{max} 3412, 1740, 1263, 757 cm^{-1}. MS (EMS), *m/z* calcd for $C_{23}H_{21}N_5O_5$, 447.1543, found 448.0 $[M+H]^+$, 301.0 [M–MNZ+Na] $^+$, 277.0 [M–MNZ], 195.0 [Acridine ammonia fragments+2H] $^+$.

Synthesis of AZT-F:

In a 50 mL flask, 9-aminoacridine (0.39 g, 2 mmol) was dissolved in anhydrous DMF (6 mL). Dimethylaminopyridine (0.012 g, 0.10 mmol) was added into the mixture. Previous reaction product was dissolved in anhydrous DMF (6 mL) and slowly added dropwise into the above reaction mixture, within 15 minutes under stirring and ice bath condition. After completion of the reaction, the ice bath was removed. Under N_2 atmosphere and avoiding of light, resulting mixture was stirred for 36 h at room temperature. Under stirring, reaction mixture was poured into the cooled distillation water 60 mL (the flask was kept cold with an ice bath), gradually formed deposit. After 1 h, the suspension was filtrated with vacuum, washed with water and dried at 90°C. Crude product was obtained as yellow powder and was purified by column chromatography over silica gel (30cm×2cm, eluting with V ($CHCl_3$): V (EtOH) = 40:3). Solvent was removed under reduced pressure and dried in vacuum. Product was obtained as a yellow powder, 0.29 g, yield: 27%. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.36 (s, 1H), 10.77 (s, 1H), 8.16 (d, J = 8.0 Hz, 4H), 7.85 (t, J =8.0 Hz, 8.0 Hz, 2H), 7.62 (t, J = 8.0 Hz, 8.0 Hz, 2H), 7.46 (s, 1H), 6.13 (t, J = 8.0 Hz, 4.0 Hz, 1H), 4.34-4.27 (m, 2H), 4.02-3.98 (m, 1H), 3.02 (t, J =8.0 Hz, 8.0 Hz, 2H), 2.83 (dd, J = 8.0 Hz, 4.0 Hz, 2H), 2.44 (dd, J=8.0 Hz, 8.0 Hz, 1H), 2.33 (dd, J=12.0 Hz , 8.0 Hz, 1H), 1.80 (d, J=4.0 Hz, 1H), 1.75 (s, 3H) ppm. IR (KBr): ν_{max} 3245, 1732, 1708, 1272, 751 cm^{-1}. MS (EMS), *m/z* calcd for $C_{27}H_{25}N_7O_6$, 543.1866, found 544.1 $[M+H]^+$, 301.0 [M–AZT+Na]$^+$, 277.0 [M–AZT], 195.0 [Acridine ammonia fragments+2H] $^+$.

Synthesis of 3TC-F:

In a 50 mL flask, 9-aminoacridine (0.19 g, 1 mmol) was dissolved in anhydrous DMF (3 mL). Dimethylaminopyridine (0.01 g, 0.05 mmol) was added into the mixture, and then previous reaction product was slowly added dropwise into the above reaction mixture, within 10 minutes under stirring and ice bath condition. After ice bath was removed. Under N_2 atmosphere and avoiding of light, resulting mixture was stirred for 36 h at room temperature. Under stirring, reaction mixture was poured into the cooled distillation water (50 mL) (the flask was kept cold with an ice bath), gradually formed

deposit. After 1 h, the suspension was filtrated with vacuum, washed with water and dried at 90°C. Crude product was obtained as yellow powder 0.01 g. The filtration was cooled, extracted with chloroform (30×2 mL), and collected them. Chloroform was eliminated under reducing pressure. Crude products were combined 0.10 g. Then crude product was purified by column chromatography over silica gel (30cm×2cm, eluting with *V* ($CHCl_3$):*V* (EtOH) = 40:3) . Solvent was removed under reduced pressure and dried in vacuum. Product was obtained as a yellow powder, 0.29 g, yield: 14%. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.34 (dd, *J* = 8.0 Hz, 8.0 Hz, 2H), 8.13 (d, *J* =8.0 Hz, 1H), 7.82 (s, 1H), 7.74 (dd, *J*=8.0 Hz, 8.0 Hz, 3H), 7.57 (dd, *J*=16.0 Hz, 8.0 Hz, 2H), 7.20 (s, 1H), 6.49 (d, J=4.0 Hz, 1H), 6.32-6.29 (m, 1H), 5.40 (dd, J=8.0 Hz, 4.0 Hz, 1H), 4.63 (dd, J=12.0 Hz, 8.0 Hz, 4.0 Hz, 2H), 3.66 (dt, *J* =8.0 Hz, 8.0 Hz, 8.0 Hz, 1H), 3.33 (dd, J=12.0 Hz, 4.0 Hz, 1H), 3.12 (t, J=8.0 Hz, 4.0 Hz, 2H), 2.32(t, J=8.0 Hz, 4.0 Hz, 2H), 2.15 (s, 3H) ppm. IR (KBr): ν_{max} 3223, 1733, 1694, 1221, 757 cm^{-1}. MS (EMS), *m/z* calcd for $C_{27}H_{25}N_5O_6S$, found 543.2 $[M–CH_3CO+K]^+$, 195.1 [Acridine ammonia fragments+2H]$^+$ and 272 $[M–3–TC–ES+5H]^+$ fragment ion peak.

Fluorescence characteristics:

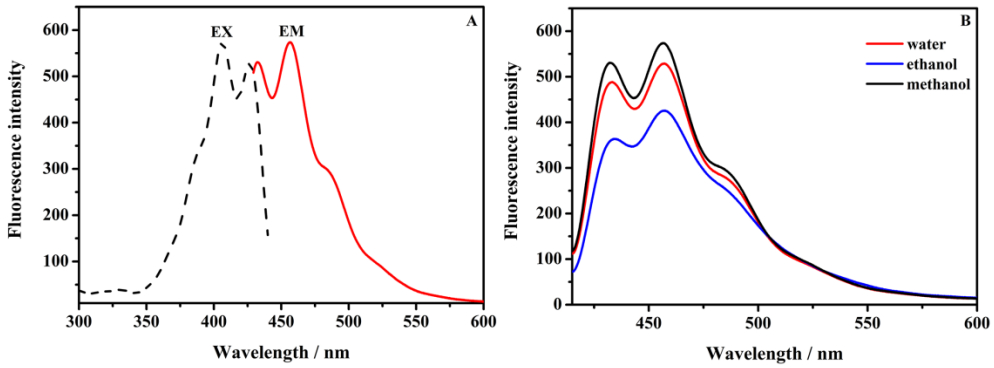

Fig. S1. (A) Fluorescence excitation (EX) and emission (EM) spectra of 9-AA in methanol solution; (B) Fluorescence emission spectra of 9-AA in various solvents. (Condition: λ_{ex}=406 nm, 415-600 nm, sensitivity: 1, slit: 10/10)

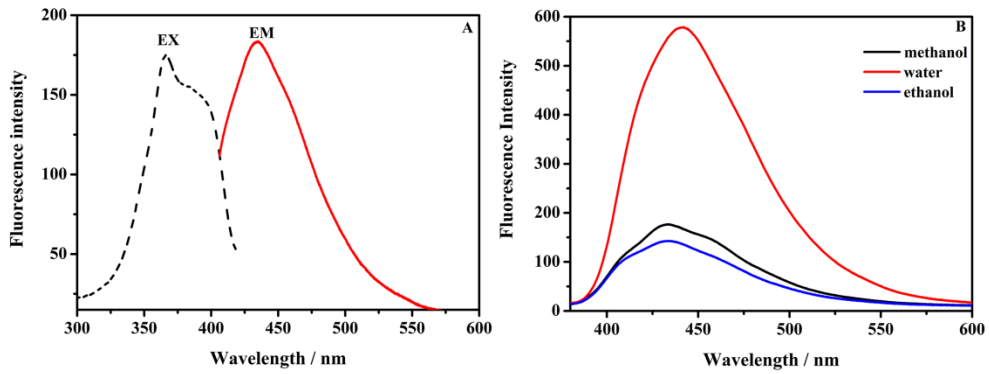

Fig. S2. (A) Fluorescence excitation (EX) and emission (EM) spectra of AZT-F in methanol solution; (B) Fluorescence emission spectra of AZT-F in various solvents. (Condition: λ_{ex}=366 nm,375-600 nm, sensitivity: 2, slit: 10/10)

Synthesis of molecular imprinted polymer:

Molecularly imprinted polymers synthesized according to Ref. [27, 28]. The imprinted polymer of MNZ (AZT) was prepared by dummy-template methods. 0.8 mmol of MNZ-Es (template), 16 mmol of EDMA, 4 mmol of MAA and 40 mg of polymerization initiator (AIBN) were weighed into glass test tubes and dissolved in 5.5 mL chloroform. The solutions were then sonicated for 5 min, purged with nitrogen for 10 min, and placed in a thermostatic oil bath at 50°C for 24 h. The resultant hard bulk polymers was triturated with a mortar and wet screened with methanol to obtain polymer particles in the range of 38 μm to 75 μm. The resulting polymer particles were extracted with methanol: acetic acid (9:1, *V*/*V*) using a Soxhlet extractor until no template was detected. Then, re-wash acetic acid with methanol and the particles were dried in vacuum.

Competitive binding assay of AZT-F:

MIP particles (20 mg) were mixed with 0.05 mmol/L of AZT-F and the AZT-F (final concentration 0-0.25 mmol/L) in the CH_3CN solvent up to a final volume of 4 mL. The mixture was incubated for 12 h at room temperature in polypropylene tubes on a shaking table. After shaking, the solution centrifugated and filtrated. The solution was measured by fluorescence spectrometer. The results are shown in the Fig. S3.

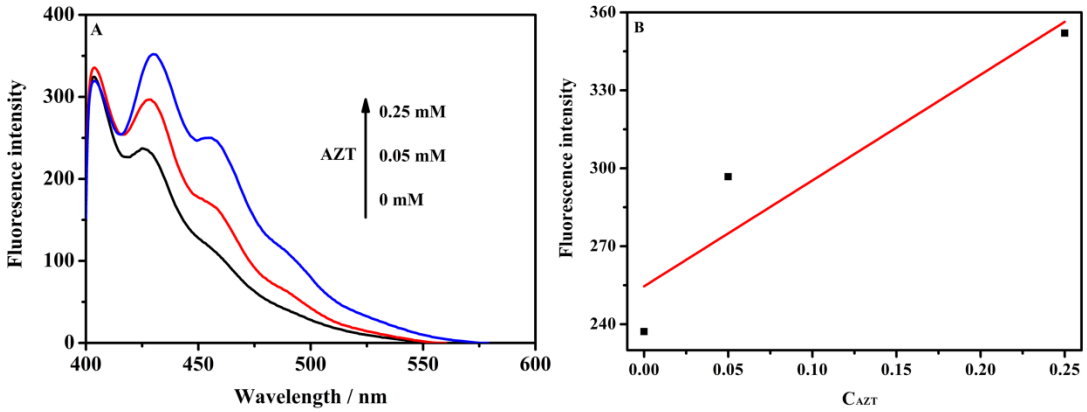

Fig. S3. (A) Fluorescence spectra of competitive binding assay of 0.018 mM MNZ-F in the presence of 0-0.36 mM MNZ in chloroform solution; (B) Relation curve between fluorescence intensity and concentration of AZT, y = 254.603 + 407.208 x, R^2 = 0.7578. (Condition: λ_{ex}=394 nm, 404-600 nm, sensitivity: 1, slit: 20/10)

Measurement of quantum yield

The PL quantum yields of the AZT-F in chloroform (ca. $1 \times 10^{-5} \sim 1 \times 10^{-6}$ M) were measured by comparing to quinine sulfate (ca. $1 \times 10^{-5} \sim 1 \times 10^{-6}$ M) in 0.10 M H_2SO_4 as standard. The quantum efficiencies of AZT-F after refractive index correction can be calculated according to Equation S1[23]:

$$\emptyset_{unk} = \emptyset_{std}\left(\frac{I_{unk}}{I_{std}}\right)\left(\frac{A_{std}}{A_{unk}}\right)\left(\frac{\eta_{unk}}{\eta_{std}}\right)^2 \qquad (S1)$$

where φ_{unk} and φ_{std} are the fluorescent quantum yields, I_{unk} and I_{std} are the integration of the emission intensities, A_{unk} and A_{std} are the absorbances at the excitation wavelength, η_{unk} and η_{std} are the refractive indexes of the corresponding solutions for the samples and the standard. Here we use the refractive indexes of the pure solvents as those of the solutions.

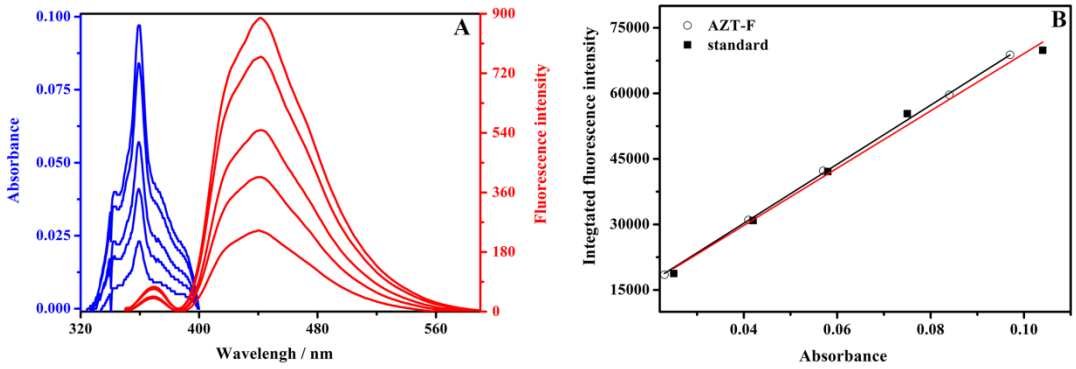

Fig. S4. (A) UV-vis and emission spectra of copolymer AZT-F in water solution with different concentration (B) curve of reference and AZT-F obtained by the integral area and absorbance of fluorescence intensity corresponding to different absorbances.

Characterization of fluorescent labeled products

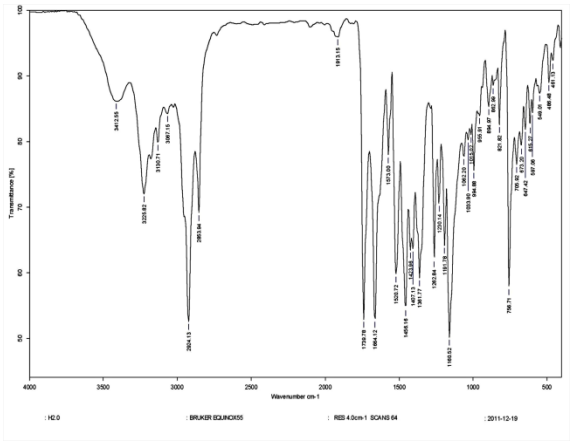

Fig. S5. IR spectrum of MNZ-F.

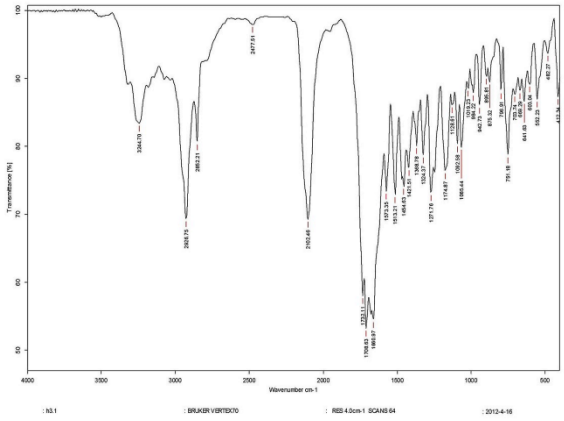

Fig. S6. IR spectrum of AZT-F.

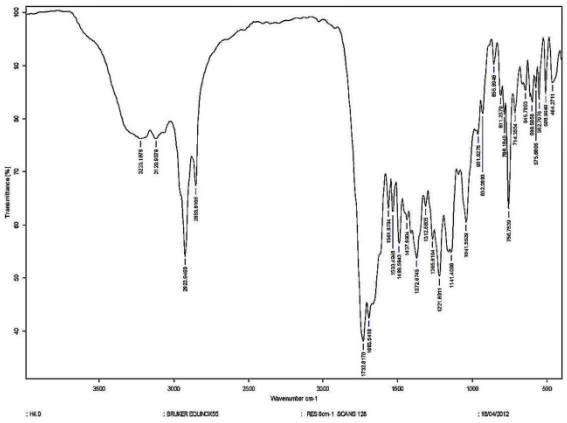

Fig. S7. IR spectrum of 3-TC-F.

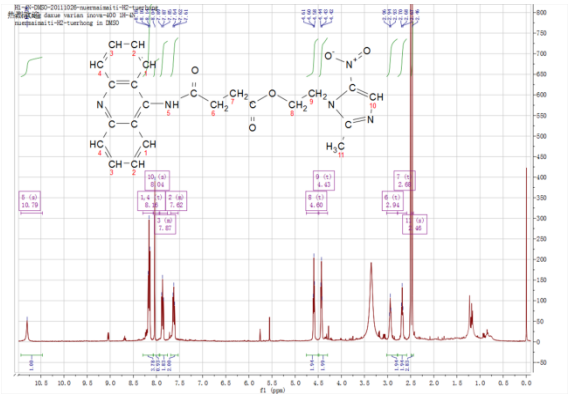

Fig. S8. ^{1}H NMR spectrum of MNZ-F.

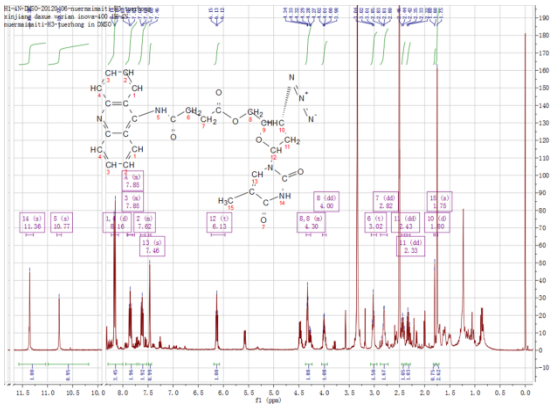

Fig. S9. ^{1}H NMR spectrum of AZT-F.

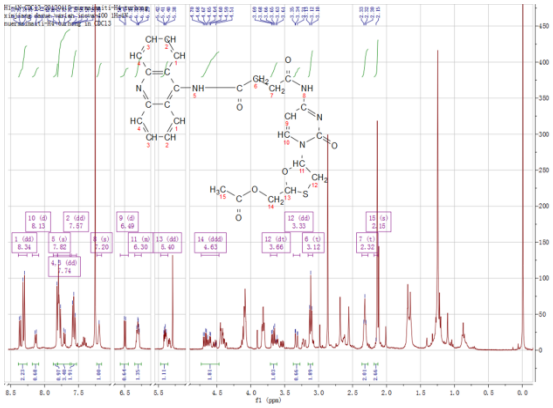

Fig. S10. ^{1}H NMR spectrum of 3TC-F.

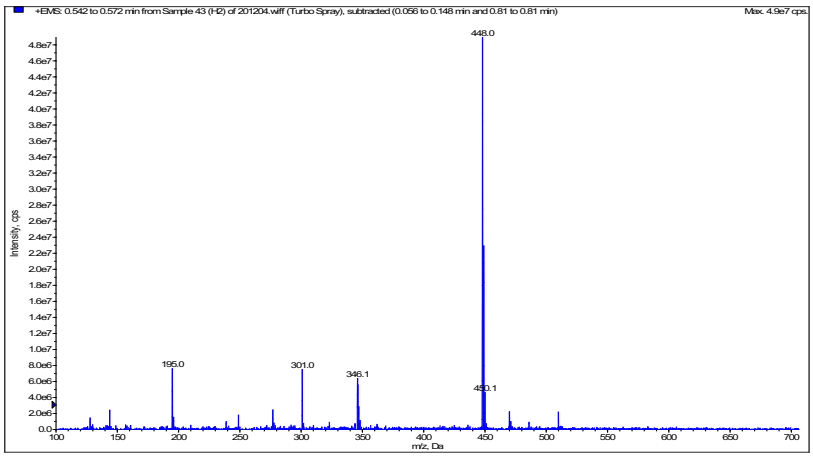

Fig. S11. A mass spectrometry of MNZ-F.

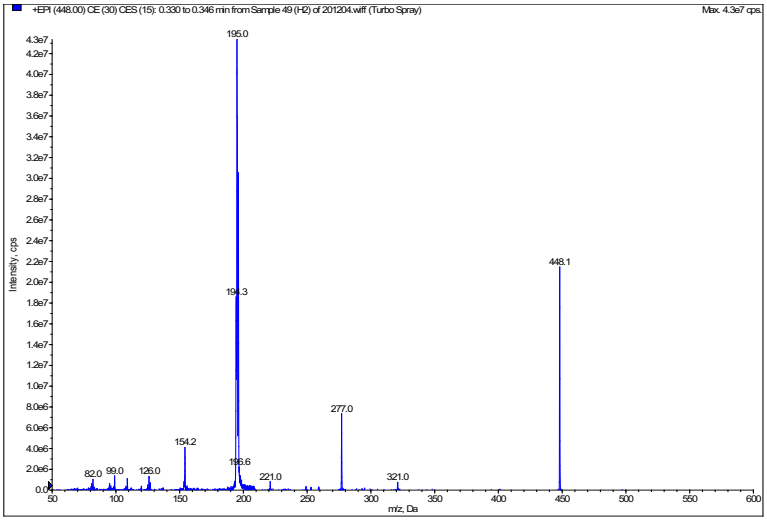

Fig. S12. Two stage mass spectrometry of MNZ-F.

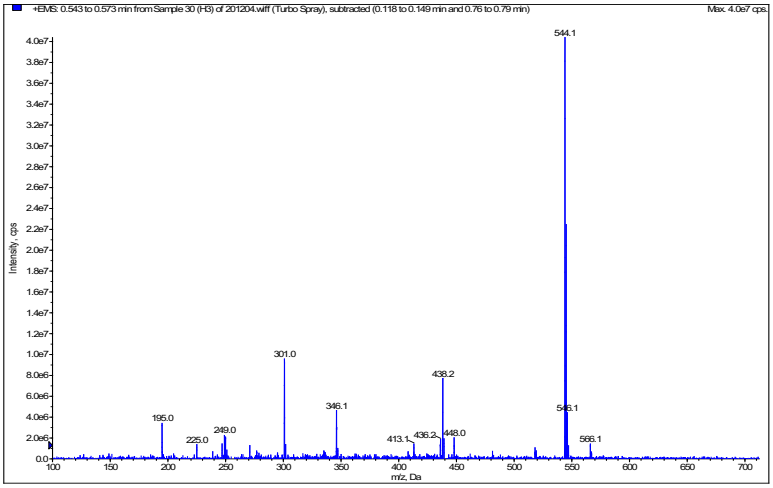

Fig. S13. A mass spectrometry of AZT-F.

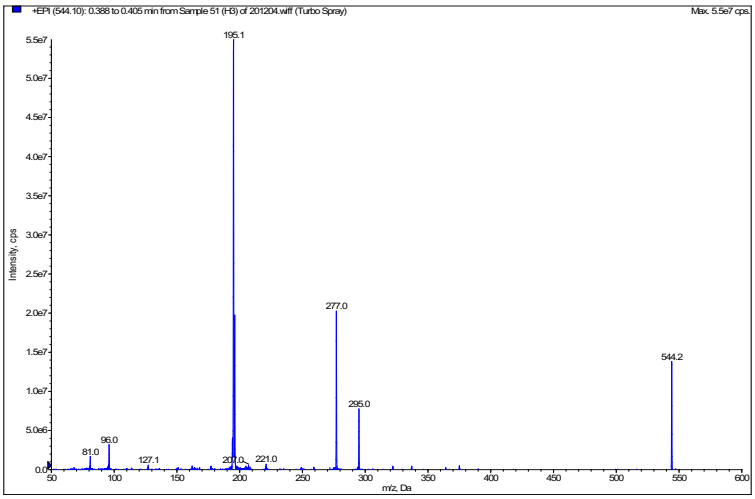

Fig. S14. Two stage mass spectrometry of AZT-F.

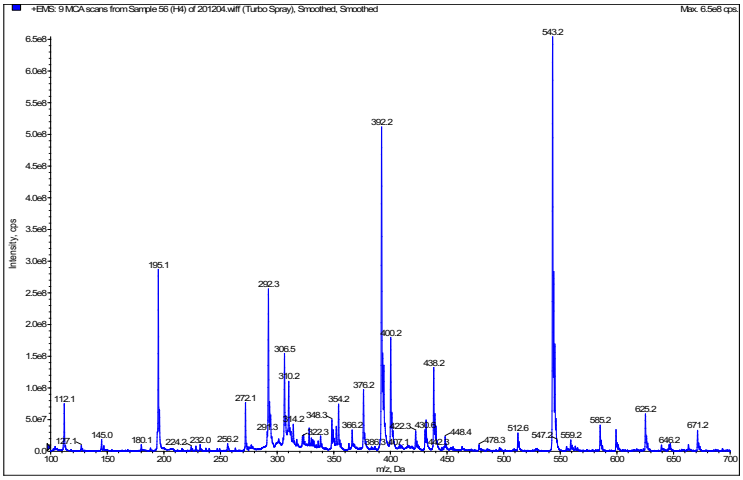

Fig. S15. A mass spectrometry of 3TC-F.

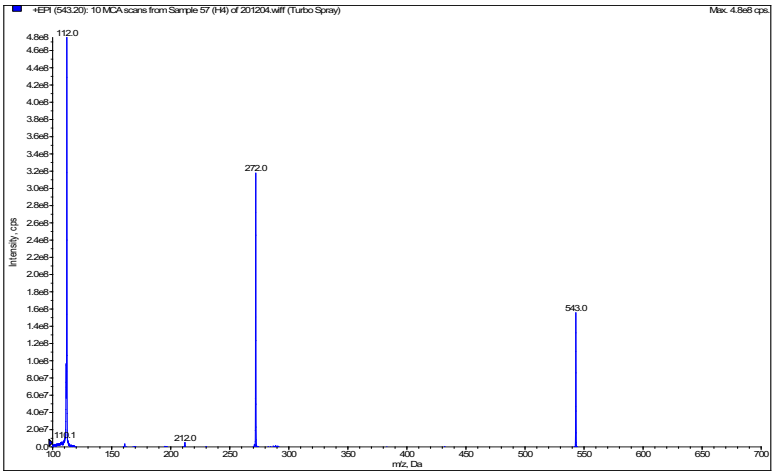

Fig. S16. Two stage mass spectrometry of 3TC-F.