Supporting Information (1, 2, 3)

Synthesis and biological evaluation of a new class of quinazolinone azoles as potential antimicrobial agents and their interactions with calf thymus DNA and human serum albumin

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Supporting Information 1

1. General methods

Melting points were recorded on X-6 melting point apparatus and uncorrected. TLC analysis was done using pre-coated silica gel plates. FT-IR spectra were carried out on Bruker RFS100/S spectrophotometer (Bio-Rad, Cambridge, MA, USA) using KBr pellets in the 400–4000 cm^{-1} range. NMR spectra were recorded on a Bruker AV 300 or Varian 400 spectrometer using TMS as an internal standard. The chemical shifts were reported in parts per million (ppm), the coupling constants (*J*) were expressed in hertz (Hz) and signals were described as singlet (s), doublet (d), triplet (t), as well as multiplet (m). The mass spectra were recorded on LCMS-2010A and the high-resolution mass spectra (HRMS) were recorded on an IonSpec FT-ICR mass spectrometer with ESI resource.

2. Spectral data of the prepared compounds

*2.1 General procedures for the preparation of intermediates **(1a-b)***

The intermediates **1a-b** were prepared according to the previously reported methods. [1]

*2.2 [3-(2-Bromoethyl)-7-fluoroquinazolin-4(3H)-one] (**2a**)*

To a stirred solution of **1a** (0.20 g, 1.2 mmol) and potassium carbonate (0.28 g, 2.0 mmol) in acetone (3 mL) was added 1,2-dibromoethane (0.38 g, 2.0 mmol). The mixture was stirred at 50 ℃ for 6 h. After the reaction was completed (monitored by TLC, petroleum ether/ethyl acetate, 3/1, V/V), the solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate (20 mL) and extracted with water (3 × 20 mL). After that, the combined organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography eluting with petroleum ether/ethyl acetate (10/1−5/1, V/V) to give the pure compound **2a** (0.26 g) as white solid. Yield: 76.2%; mp: 160−162 °C. IR (KBr, cm^{-1}) ν: 3052 (Ar-H), 2926, 2857 (CH_2), 1659 (C=O), 1608 (C=N), 1480 (aromatic skeleton), 1374, 1232, 1134, 1109, 1039, 873, 814; ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.45 (s, 1H, quinazolinone 2-*H*), 8.24 (dd, 1H, *J* = 8.3, 6.7 Hz, quinazolinone 5-*H*), 7.47 (dd, 2H, *J* = 20.4, 10.2 Hz, quinazolinone 6,8-*H*), 4.39 (t, 2H, *J* = 6.2 Hz, bromoethyl 2-*H*), 3.85 (t, 2H, *J* = 6.2 Hz, bromoethyl 1-*H*) ppm; ESI-MS (m/z): 271 $[M+H]^+$; HRMS (TOF) calcd. for $C_{10}H_8BrFN_2O$ $[M+H]^+$: 270.9804; found: 270.9880.

*2.3 [7-Fluoro-3-(2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethyl)quinazolin-4(3H)-one] (**3a**)*

To a stirred solution of 2-methyl-5-nitro-1*H*-imidazole (0.27 g, 2.1 mmol) and potassium carbonate (0.60 g, 4.4 mmol) in acetonitrile was added **2a** (0.31 g, 1.1 mmol). The mixture was stirred at 50 ℃ for 5 h. After the reaction was completed (monitored by TLC, chloroform/methanol, 30:1, V/V), the solvent was removed under reduced pressure, and the residue was dissolved in chloroform (20 mL) and extracted with water (3 × 20 mL). After that, the combined organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure. The resulting residue was purified by silica gel column chromatography eluting with chloroform/methanol 50/1−80/1, V/V) to give

the pure compound **3a** (0.18 g) as white solid. Yield: 49.8%; mp: 250–251 °C. IR (KBr, cm^{-1}) ν: 3101 (Ar-H), 2965, 2867 (CH_2), 1677 (C=O), 1605 (C=N), 1525 (aromatic skeleton), 1480, 1427, 1359, 1287, 1171, 1108, 964, 868; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.27 (s, 1H, quinazolinone 2-*H*), 8.18 (dd, 1H, *J* = 8.6, 6.4 Hz, quinazolinone 5-*H*), 8.03 (s, 1H, imidazole 4-*H*), 7.45 (dd, 2H, *J* = 17.5, 9.2 Hz, quinazolinone 6,8-*H*), 4.71 (t, 2H, *J* = 5.2 Hz, imidazole-CH_2), 4.41 (t, 2H, *J* = 5.2 Hz, quinazolinone-CH_2), 2.20 (s, 3H, CH_3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ: 167.79, 164.45, 160.16, 151.58, 150.35, 149.44, 133.64, 129.71, 118.61, 116.58, 116.26, 113.02, 112.74, 45.72, 44.86, 13.91 ppm; ESI-MS (m/z): 318 $[M+H]^+$; HRMS (TOF) calcd. for $C_{14}H_{12}FN_5O_3$ $[M+H]^+$: 318.0924; found: 318.0913.

*2.4 [7-Chloro-3-(2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethyl)quinazolin-4(3H)-one] (**3f**)*

Compound **3f** (0.21 g) was obtained as white solid according to general procedure described for **3a** starting from 2-methyl-5-nitro-1*H*-imidazole (0.27 g, 2.1 mmol), potassium carbonate (0.60 g, 4.4 mmol) and **2f** (0.32 g, 1.1 mmol). Yield: 55.4%; mp: 235–237 °C. IR (KBr, cm^{-1}) ν: 3102 (Ar-H), 2915, 2876 (CH_2), 1655 (C=O), 1602 (C=N), 1509 (aromatic frame), 1327, 1241, 1082, 1016, 976, 846; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.27 (s, 1H, quinazolinone 2-*H*), 8.11 (d, 1H, *J* = 8.5 Hz, quinazolinone 5-*H*), 8.02 (s, 1H, imidazole 4-*H*), 7.75 (d, 1H, *J* = 1.5 Hz, quinazolinone 8-*H*), 7.59 (dd, 1H, *J* = 8.5, 1.7 Hz, quinazolinone 6-*H*), 4.71 (t, 2H, *J* = 5.4 Hz, imidazole-CH_2), 4.41 (t, 2H, *J* = 5.4 Hz, quinazolinone-CH_2), 2.21 (s, 3H, CH_3) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ: 160.33, 151.65, 149.75, 149.54, 149.36, 139.77, 133.68, 128.67, 128.15, 126.98, 120.51, 45.87, 44.89, 14.00 ppm; ESI-MS (m/z): 334 $[M+H]^+$; HRMS (TOF) calcd. for $C_{14}H_{12}ClN_5O_3$ $[M+H]^+$: 334.0629; found: 334.0706.

*2.5 [7-Fluoro-3-(2-(4-nitro-1H-imidazol-1-yl)ethyl)quinazolin-4(3H)-one] (**4a**)*

Compound **4a** (0.18 g) was obtained as white solid according to general procedure described for **3a** starting from 4-nitro-1*H*-imidazole (0.14 g, 2.0 mmol), potassium carbonate (0.60 g, 4.4 mmol) and **2a** (0.31 g, 1.1 mmol). Yield: 52.2%; mp: 229–230 °C. IR (KBr, cm^{-1}) ν: 3102 (Ar-H), 2927, 2860 (CH_2), 1662 (C=O), 1609 (C=N), 1508 (aromatic frame), 1318, 1254, 1099, 1021, 961, 853; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.25-8.14 (m, 2H, quinazolinone 2-*H* and quinazolinone 5-*H*), 8.10 (s, 1H, imidazole 2-*H*), 7.90 (s, 1H, imidazole 5-*H*), 7.43 (dd, 2H, *J* = 17.1, 9.2 Hz, quinazolinone 6,8-*H*), 4.76-4.69 (m, 2H, imidazole-CH_2), 4.47-4.39 (m, 2H, quinazolinone-CH_2) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ: 167.67, 164.34, 159.98, 150.34, 149.35, 143.64, 134.13, 129.72, 118.75, 116.28, 115.96, 112.88, 112.59, 46.79, 45.82 ppm; ESI-MS (m/z): 304 $[M+H]^+$; HRMS (TOF) calcd. for $C_{13}H_{10}FN_5O_3$ $[M+H]^+$: 304.0768; found: 304.0843.

*2.6 [7-Chloro-3-(2-(4-nitro-1H-imidazol-1-yl)ethyl)quinazolin-4(3H)-one] (**4f**)*

Compound **4f** (0.21 g) was obtained as white solid according to general procedure described for **3a** starting from 4-nitro-1*H*-imidazole (0.14 g, 2.0 mmol), potassium carbonate (0.60 g, 4.4 mmol) and **2f** (0.32 g, 1.1 mmol). Yield: 58.4%; mp: 210–211 °C. IR (KBr, cm^{-1}) ν: 3105 (Ar-H), 2923, 2862 (CH_2), 1659 (C=O), 1606 (C=N), 1505 (aromatic frame), 1323, 1248, 1089, 1015, 978, 841; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.17 (s, 1H, quinazolinone 2-*H*), 8.09 (s, 2H, quinazolinone 5-*H* and imidazole 2-*H*), 7.90 (s, 1H, imidazole 5-*H*), 7.73 (s, 1H, quinazolinone 8-*H*), 7.58 (d, 1H, *J* = 5.7 Hz, quinazolinone 6-*H*), 4.73 (s, 2H, imidazole-CH_2), 4.43 (s, 2H, quinazolinone-CH_2) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ: 160.17, 149.40, 143.70, 139.56, 139.12, 134.15, 128.71, 127.89, 126.85, 120.64, 118.92, 46.78, 46.03 ppm; ESI-MS (m/z): 320 $[M+H]^+$; HRMS (TOF) calcd. for $C_{13}H_{10}ClN_5O_3$ $[M+H]^+$: 320.0472; found: 320.0547.

*2.7 [3-(2-(1H-Imidazol-1-yl)ethyl)-7-fluoroquinazolin-4(3H)-one] (**5a**)*

Compound **5a** (0.15 g) was obtained as white solid according to general procedure described for **3a** starting from 1*H*-imidazole (0.14 g, 2.0 mmol), potassium carbonate (0.61 g, 4.4 mmol) and **2a** (0.30 g, 1.1 mmol). Yield: 50.8%; mp: 169–170 °C. IR (KBr, cm^{-1}) ν: 3105 (Ar-H), 2926, 2861 (CH_2), 1658 (C=O), 1606 (C=N), 1505 (aromatic frame), 1323, 1249, 1086, 1013, 976, 841; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.23 (dd, 1H, *J* = 8.8, 6.4 Hz, quinazolinone 2-*H*), 7.88 (s, 1H, quinazolinone 5-*H*), 7.55 (s, 1H, imidazole 2-*H*), 7.43 (t, 2H, *J* = 8.2 Hz, quinazolinone 6,8-*H*), 7.17 (s, 1H, imidazole 5-*H*), 6.89 (s, 1H, imidazole 4-*H*), 4.34 (s, 4H, -CH_2CH_2-) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ:

167.68, 164.36, 159.87, 149.08, 137.98, 129.71, 129.16, 119.87, 116.32, 116.01, 112.90, 112.61, 47.25, 44.69 ppm; ESI-MS (m/z): 259 $[M+H]^+$; HRMS (TOF) calcd. for $C_{13}H_{11}FN_4O$ $[M+H]^+$: 259.0917; found: 259.0994.

*2.8 [3-(2-(1H-1,2,4-Triazol-1-yl)ethyl)-7-chloroquinazolin-4(3H)-one] (**5d**)*

Compound **5d** (0.21 g) was obtained as white solid according to general procedure described for **3a** starting from triazole (0.16 g, 2.3 mmol), potassium carbonate (0.61 g, 4.4 mmol) and **2f** (0.32 g, 1.1 mmol). Yield: 67.2%; mp: 176–177 °C. IR (KBr, cm^{-1}) ν: 3131 (Ar-H), 3055, 2957 (CH_2), 1660 (C=O), 1606 (C=N), 1508 (aromatic skeleton), 1468, 1395, 1371, 1319, 1131, 954, 880; ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.48 (s, 1H, quinazolinone 2-*H*), 8.15 (d, 1H, *J* = 8.5 Hz, quinazolinone 5-*H*), 7.96 (s, 1H, triazole 3-*H*), 7.92 (s, 1H, triazole 5-*H*), 7.71 (s, 1H, quinazolinone 8-*H*), 7.59 (d, 1H, *J* = 8.5 Hz, quinazolinone 6-*H*), 4.60 (t, 2H, *J* = 5.6 Hz, triazole-CH_2), 4.39 (t, 2H, *J* = 5.6 Hz, quinazolinone-CH_2) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) δ: 160.11, 152.30, 149.36, 145.22, 139.55, 128.64, 127.87, 126.85, 120.73, 47.21, 46.62 ppm; ESI-MS (m/z): 276 $[M+H]^+$; HRMS (TOF) calcd. for $C_{12}H_{10}ClN_5O$ $[M+H]^+$: 276.0574; found: 276.0649.

2. The NMR and HRMS spectra of some target compounds

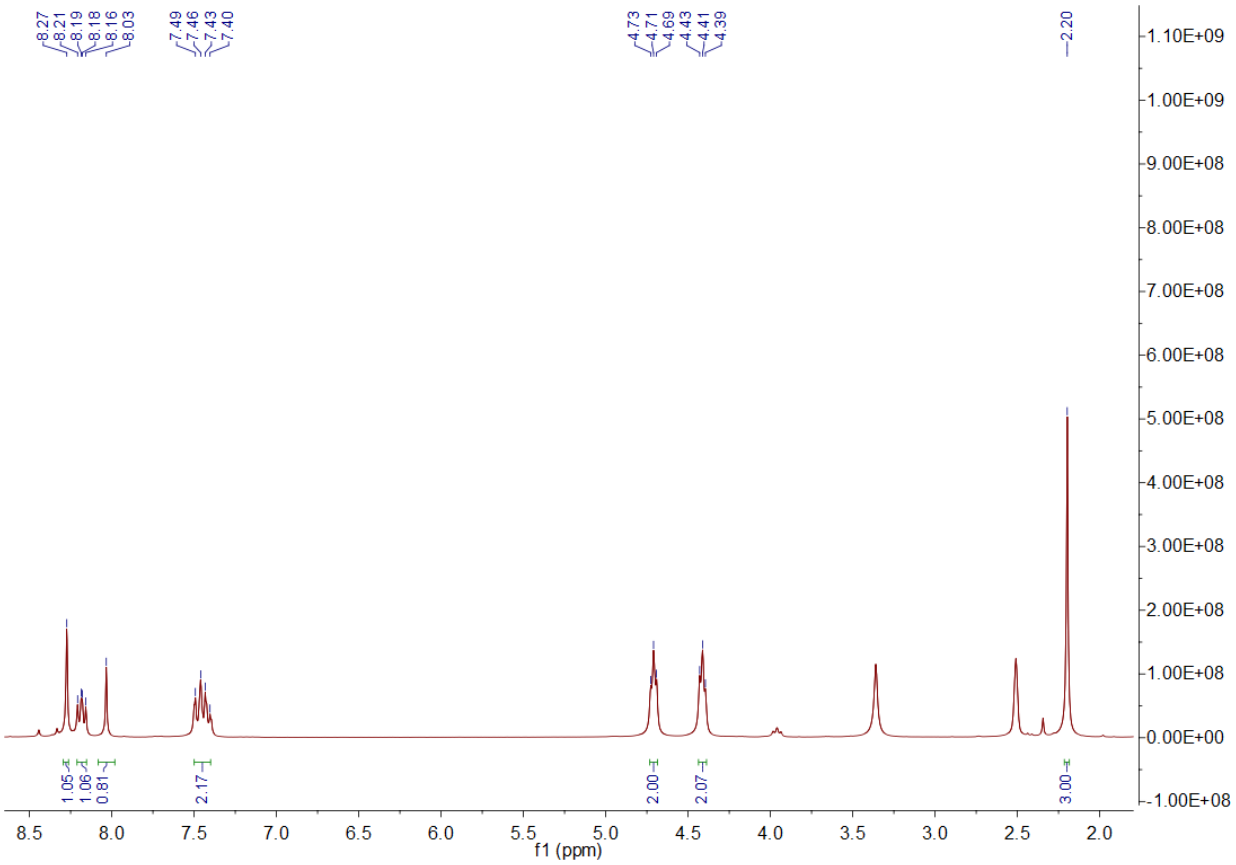

Figure 1 ^{1}H NMR spectrum of compound **3a**

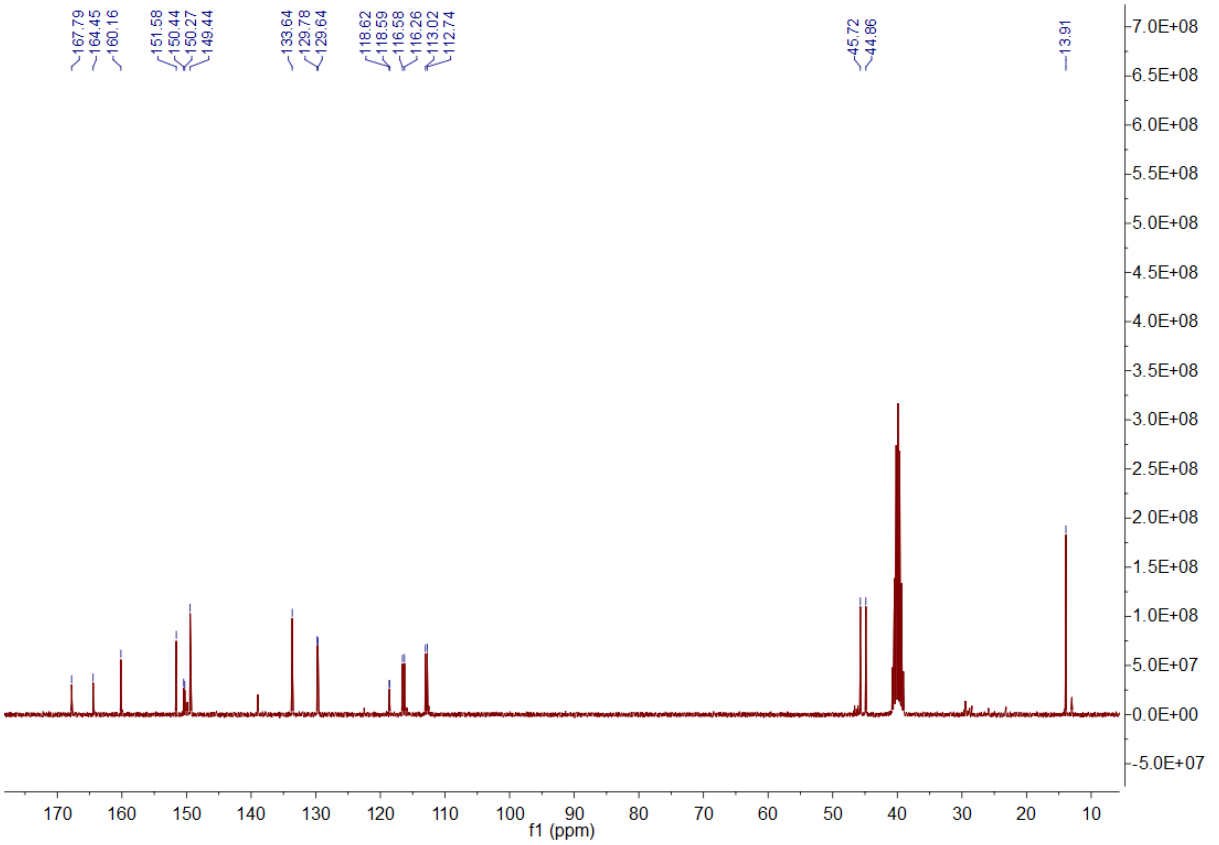

Figure 2 ^{13}C NMR spectrum of compound **3a**

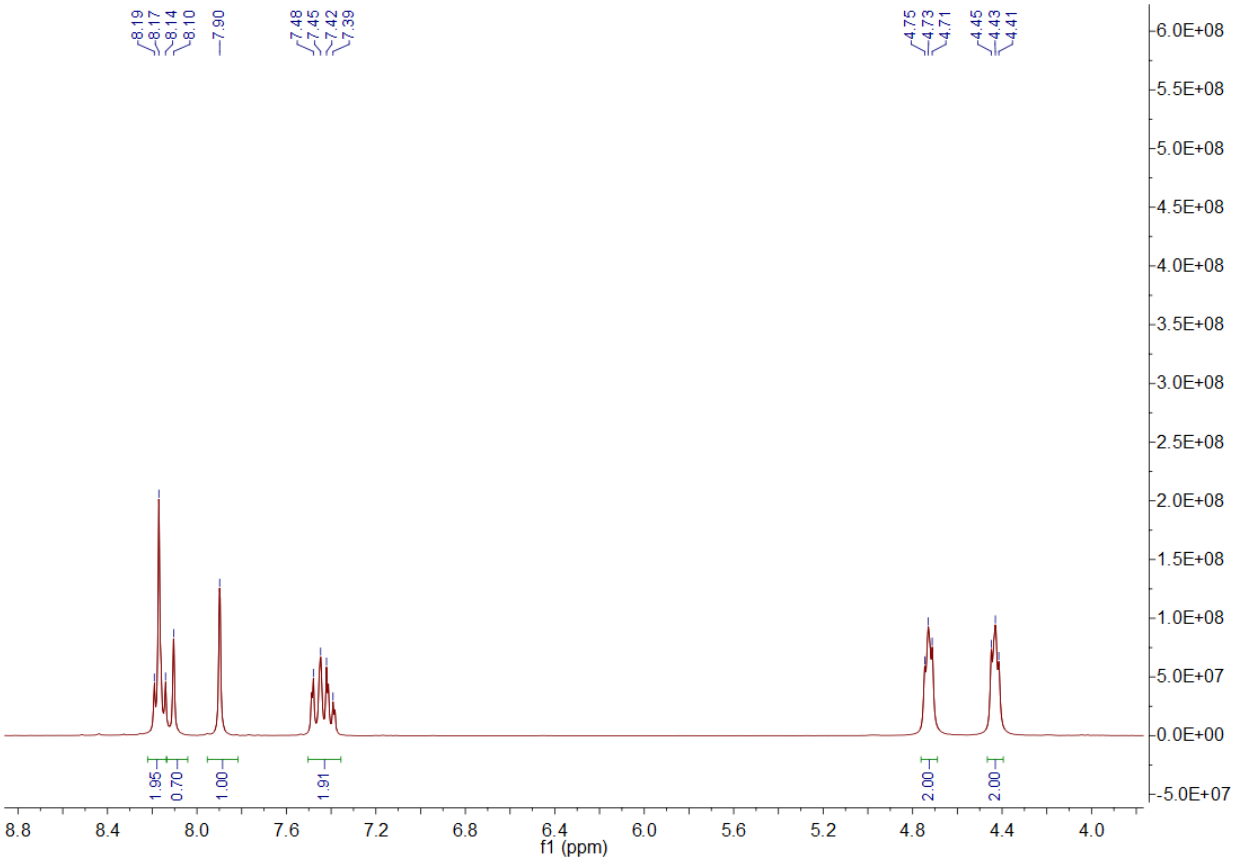

Figure 3 ^{1}H NMR spectrum of compound **4a**

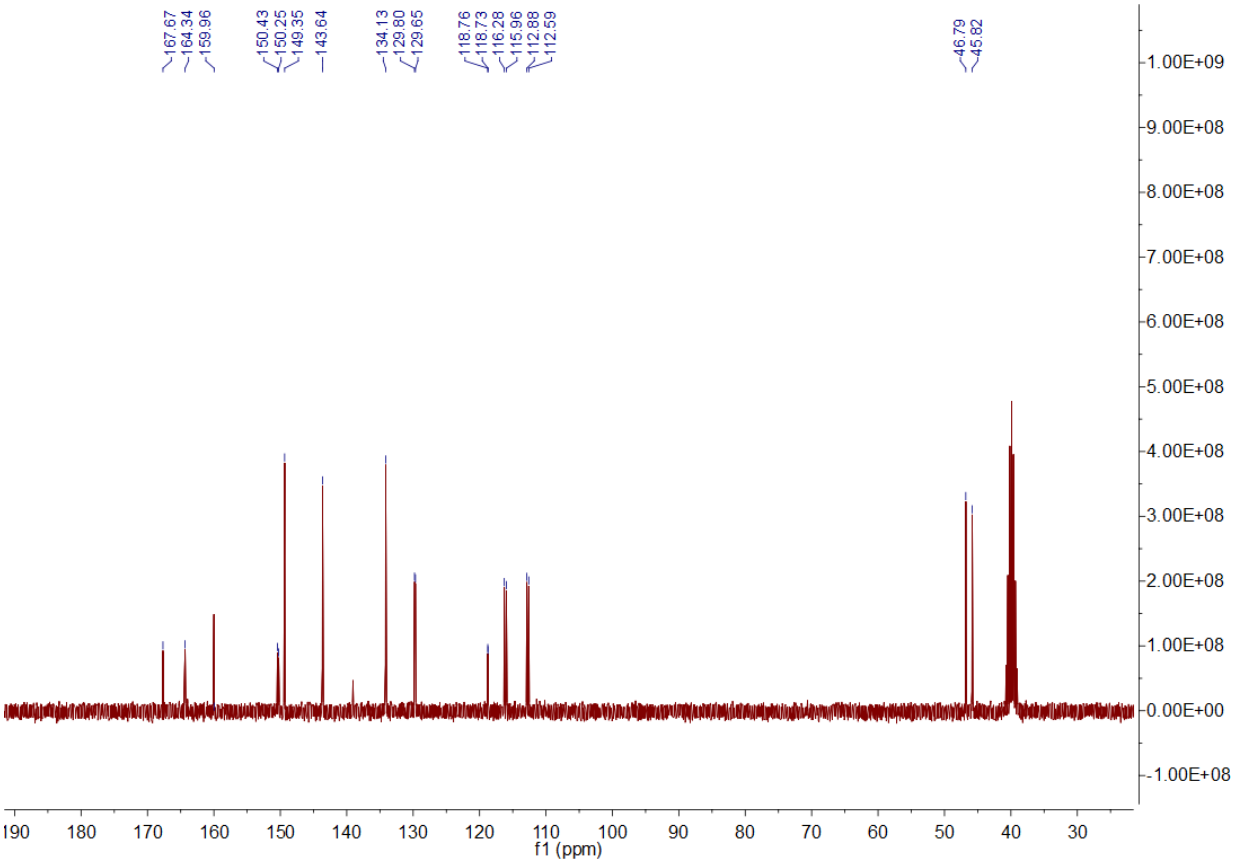

Figure 4 ^{13}C NMR spectrum of compound **4a**

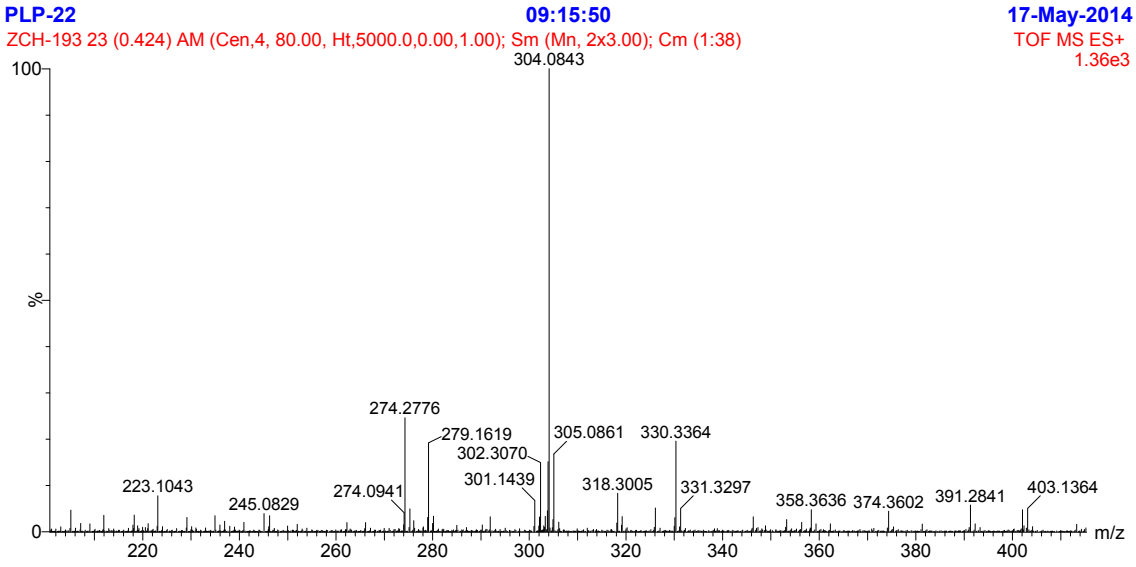

Figure 5 HRMS spectrum of compound **4a**

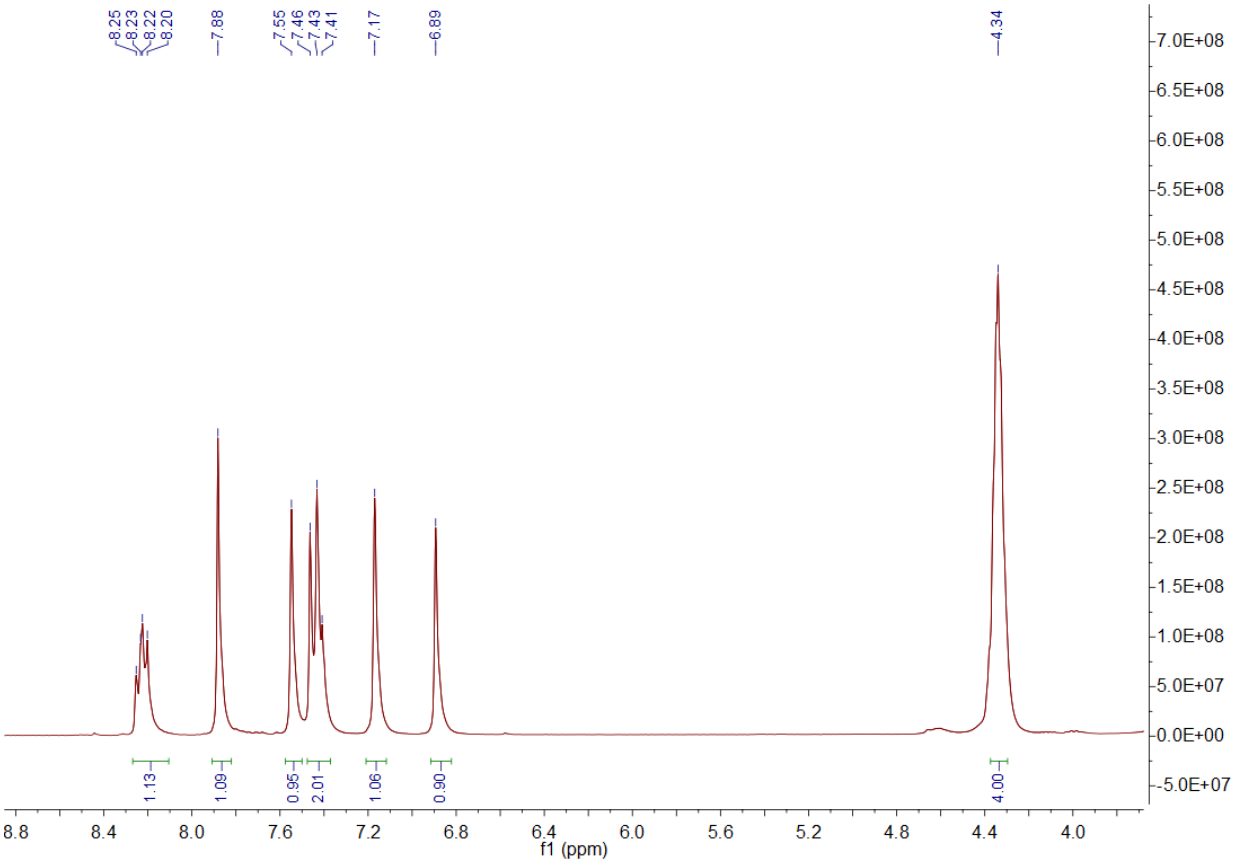

Figure 6 ^{1}H NMR spectrum of compound **5a**

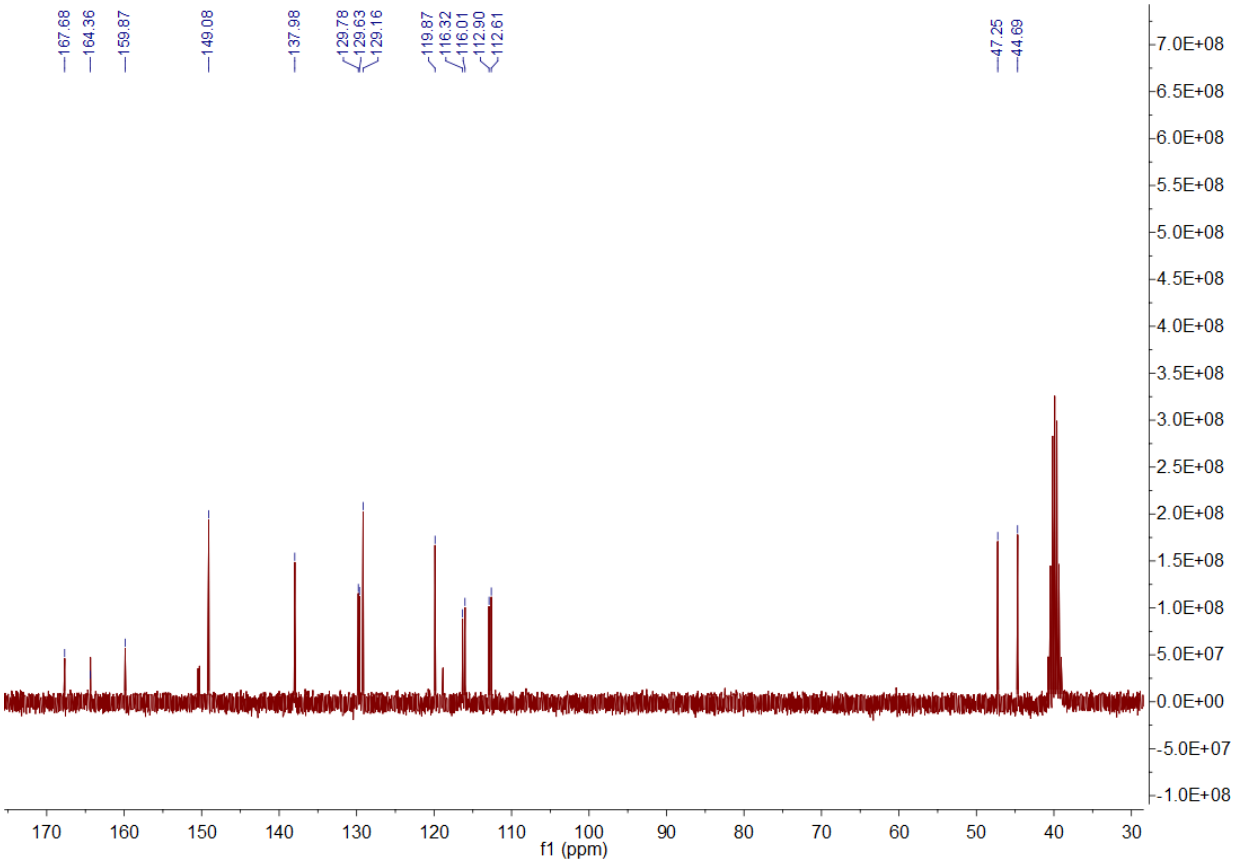

Figure 7 ^{13}C NMR spectrum of compound **5a**

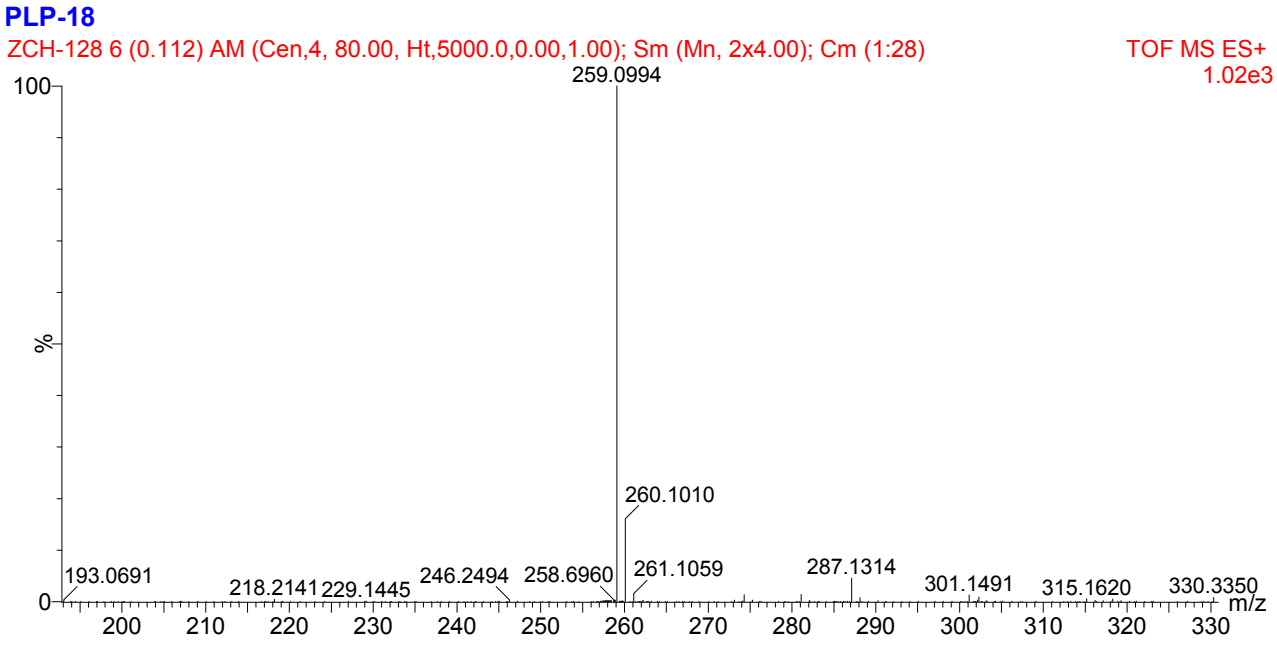

Figure 8 HRMS spectrum of compound **5a**

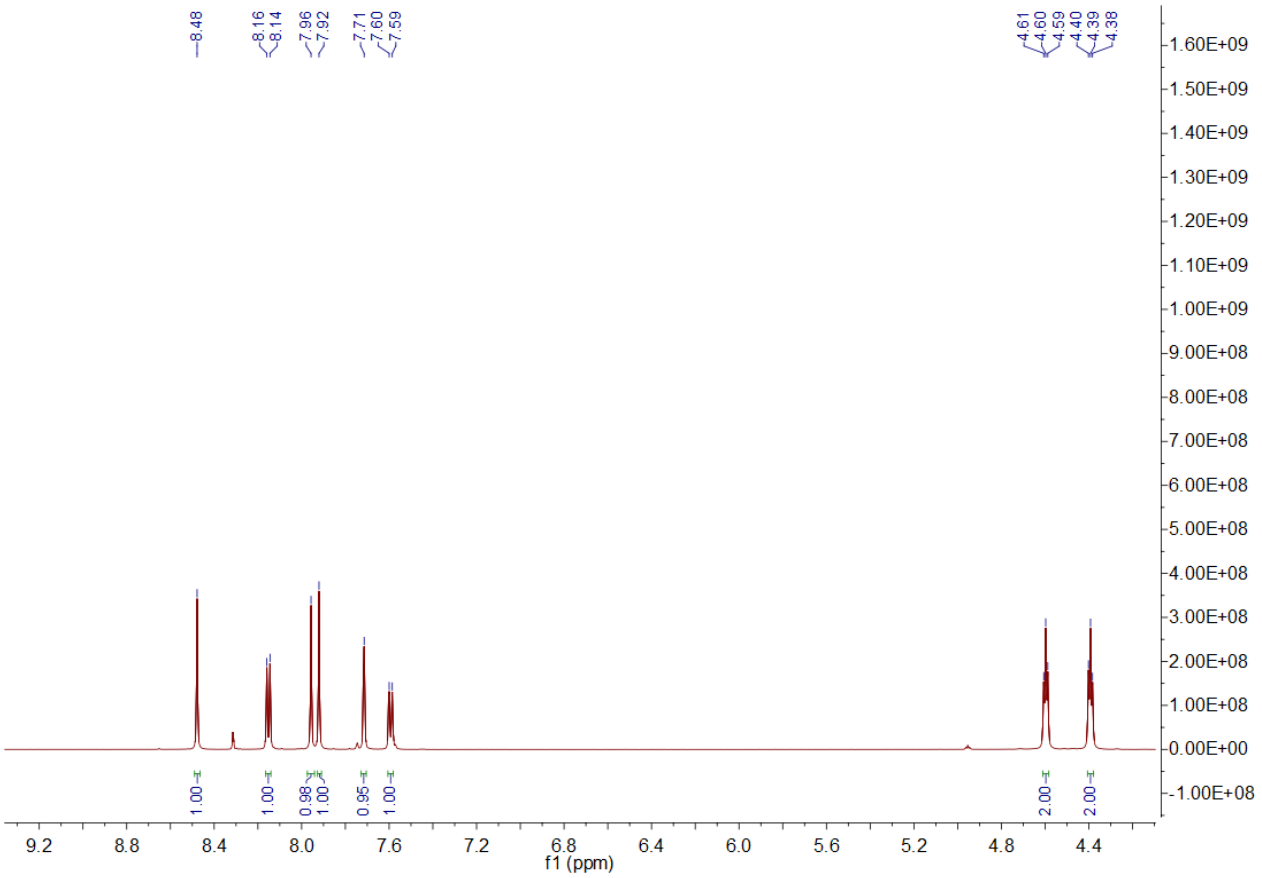

Figure 9 ^{1}H NMR spectrum of compound **5d**

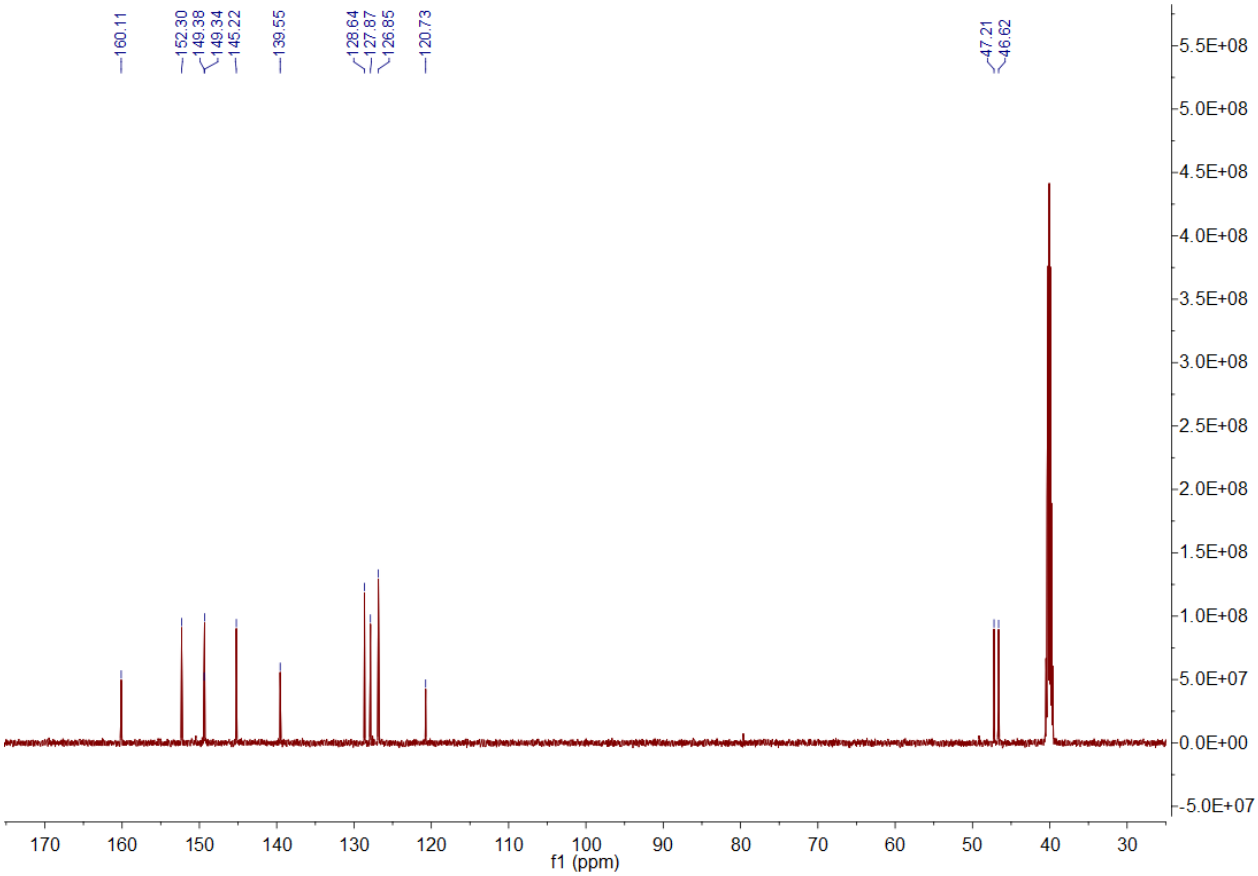

Figure 10 ^{13}C NMR spectrum of compound **5d**

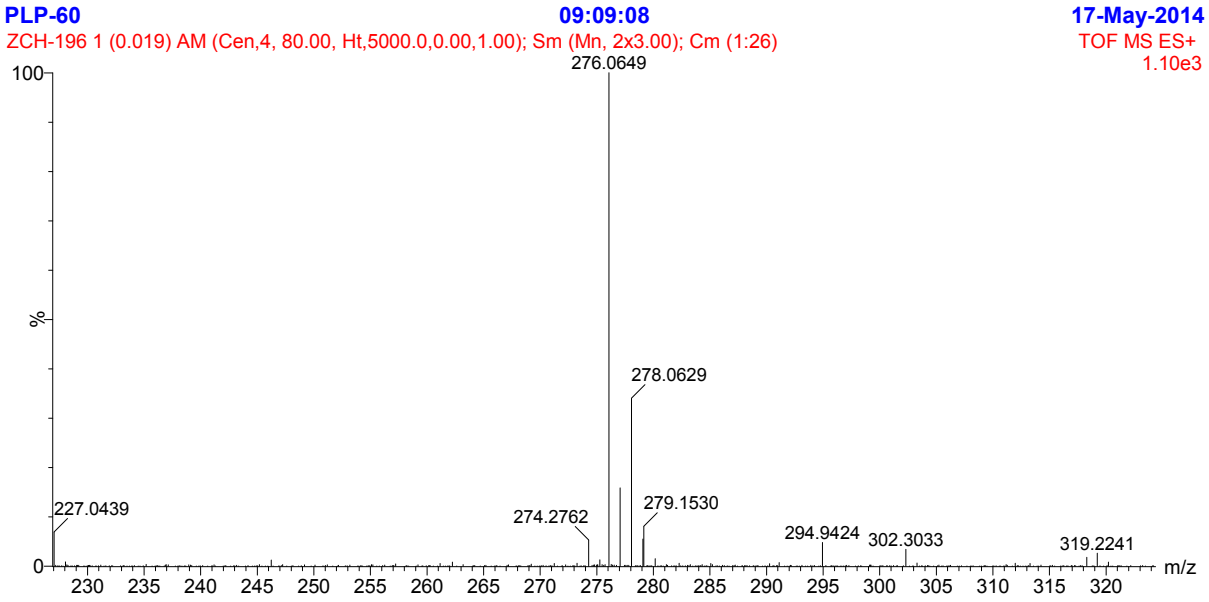

Figure 11 HRMS spectrum of compound **5d**

Supporting Information 2

1. Biological assays procedures

Minimal inhibitory concentration (MIC, μmol/mL) is defined as the lowest concentration of the new compounds that completely inhibited the growth of bacteria, by means of standard two folds serial dilution method in 96-well microtest plates according to the National Committee for Clinical Laboratory Standards (NCCLS). The tested microorganism strains were provided by the School of Pharmaceutical Sciences, Southwest University and the College of Pharmacy, Third Military Medical University. Chloromycin, Norfloxacin and Fluconazole were used as control drugs. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment. All the bacteria and fungi growth was monitored visually and spectrophotometrically. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimal inhibitory concentration (MIC).

1.1 Antibacterial Assays

The prepared compounds **2–5** were evaluated for their antibacterial activities against four Gram-positive bacteria (*Micrococcus luteus* ATCC4698, MRSA, *Staphylococcus aureus* ATCC25923 and *Bacillus subtilis*) and four Gram-negative bacteria (*Pseudomonas aeruginosa*, *Escherichia coli* DH52, *Bacillus proteus* ATCC13315, and *Eberthella typhosa*). The bacterial suspension was adjusted with sterile saline to a concentration of 1×10^5 CFU/mL. The compounds were dissolved in DMSO to prepare the stock solutions. The compounds and reference drugs were prepared in Mueller-Hinton broth (Guangdong huaikai microbial sci.& tech co., Ltd, Guangzhou, Guangdong, China) by twofold serial dilution to obtain the required concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, 1, 0.5 μg/mL. These dilutions were inoculated and incubated at 37 ºC for 24 h. To ensure that the solvent had no effect on bacterial growth, a control test was performed with test medium supplemented with DMSO at the same dilutions as used in the experiment.

1.2 Antifungal Assays

The synthesized compounds were also evaluated for their antifungal activities against five fungi (*Candida utilis*, *Aspergillus flavus*, *beer yeast*, *Candida mycoderma* and *Candida albicans*). A spore suspension in sterile distilled water was prepared from 1-day old culture of the fungi growing on Sabouraud agar (SA) media. The final spore concentration was $1–5\times10^3$ spore/mL. From the stock solutions of the tested compounds and reference antifungal Fluconazole, dilutions in sterile RPMI 1640 medium (Neuronbc Laboraton Technology CO., Ltd, Beijing, China) were made resulting in eleven wanted concentrations (0.5–512 μg/mL) of each tested compounds. These dilutions were inoculated and incubated at 35 ºC for 24 h. The drug's MIC was defined as the first well with an approximate 80% reduction in growth compared to the growth of the drug-free well.

Supporting Information 3

1. Interactions with calf thymus DNA and HSA

1.1 Material

All fluorescence spectra were recorded on F-7000 Spectrofluorimeter (Hitachi, Tokyo, Japan) equipped with 1.0 cm quartz cells, the widths of both the excitation and emission slit were set as 2.5 nm, and the excitation wavelength was 295 nm. UV spectra were recorded at room temperature on a TU-2450 spectrophotometer (Puxi Analytic Instrument Ltd. of Beijing, China) equipped with 1.0 cm quartz cells. Fluorescence spectra were recorded at 298, 304, and 310 K in the range of 300−450 nm. DNA was dissolved in Tris-HCl buffer solution (0.05 M Tris, pH = 7.4). HSA was dissolved in Tris-HCl buffer solution (0.05 M Tris, 0.15 M NaCl, pH = 7.4). Sample masses were weighed on a microbalance with a resolution of 0.1 mg. All other chemicals and solvents were commercially available, and were used without further purification.

Table 2 Stern-Volmer quenching constants for the interactions of compound **3a** with HSA at various temperatures

pH	T (K)	K_{SV} (L/mol)	K_q (L/mol s^{-1})	R[a]	S.D.[b]
7.4	298	8.15×10^4	1.27×10^{13}	0.993	0.077
	303	7.26×10^4	1.13×10^{13}	0.992	0.073
	310	6.25×10^4	9.77×10^{12}	0.996	0.047

R[a] is the correlation coefficient. S.D.[b] is standard deviation

Table 3 Binding constants and sites of **3a**-HSA system at pH = 7.4

T(K)	Modified Stern-Volmer Method			Scatchard Method			
	$10^{-4}K_a$ (L/mol)	R	S.D	$10^{-4}K_b$ (L/mol)	R	S.D	n
298	4.56	0.999	0.046	4.60	0.996	0.008	1.27
303	3.66	0.999	0.028	3.64	0.997	0.005	1.38
310	2.97	0.999	0.042	3.03	0.995	0.006	1.45

Table 4 Thermodynamic parameters of **3a**-HSA system at different temperatures

T (K)	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (J/mol·K)
298		-26.836	
303	-27.351	-26.827	-1.729
310		-26.815	

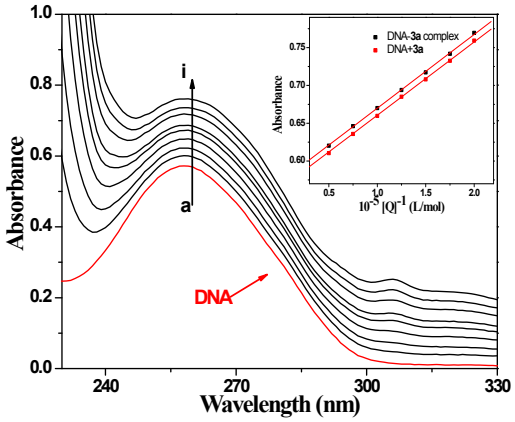

Figure 1 UV absorption spectra of DNA with different concentrations of compound **3a** (pH = 7.4, T = 290 K). Inset: comparison of absorption at 260 nm between the **3a**-DNA complex and the sum values of free DNA and free compound **3a**. *c*(DNA) = 8.62 × 10^{-5} mol/L, and *c*(compound **3a**) = 0–2.0 × 10^{-5} mol/L for curves *a*-*i* respectively at increment 0.25 × 10^{-5}.

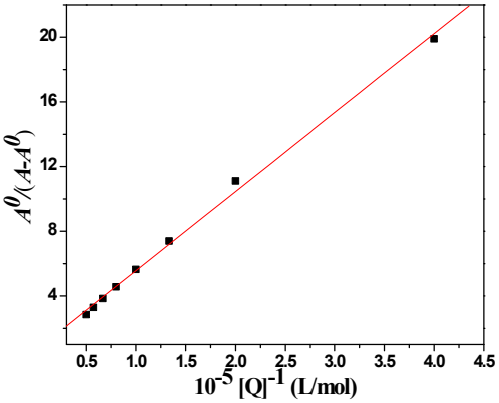

Figure 2 The plot of $A^0/(A-A^0)$ versus 1/[compound **3a**]

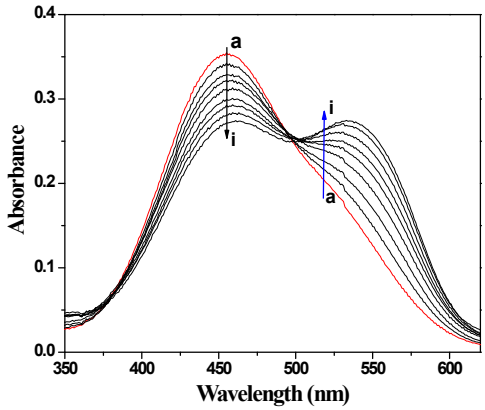

Figure 3 UV absorption spectra of NR in the presence of DNA at pH 7.4 and room temperature. *c*(NR) = 2.0 × 10^{-5} mol/L, and *c*(DNA) = 0–5.25 × 10^{-5} mol/L for curves *a*-*i* respectively at increment 0.656 × 10^{-5}.

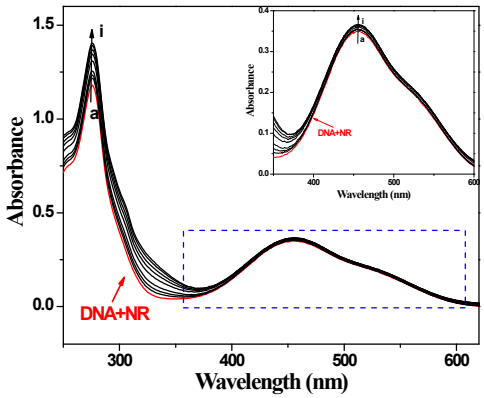

Figure 4 UV Absorption spectra of the competitive reaction between **3a** and neutral red with DNA. *c*(DNA) = 4.31 × 10^{-5} mol/L, *c*(NR) = 2.0 × 10^{-5} mol/L, and *c*(compound **3a**) = 0–2.0 × 10^{-5} mol/L for curves *a*-*i* respectively at increment 0.25 × 10^{-5}. (Inset) Absorption spectra of the system with the increasing concentration of **3a** in the wavelength range of 350–600 nm absorption spectra of competitive reaction between compound **3a** and NR with DNA.

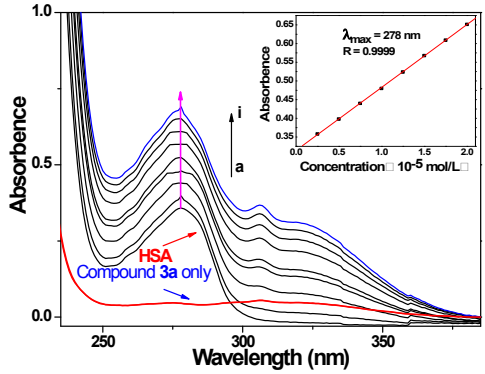

Figure 5 Effect of compound **3a** to HSA UV-vis absorption, *c*(HSA) = 1.0 × 10^{-5} mol/L; *c*(compound **3a**)/(10^{-5} mol/L): 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, (T = 298 K, pH = 7.40). The inset corresponds to the absorbance at 278 nm with different concentrations of compound **3a**.

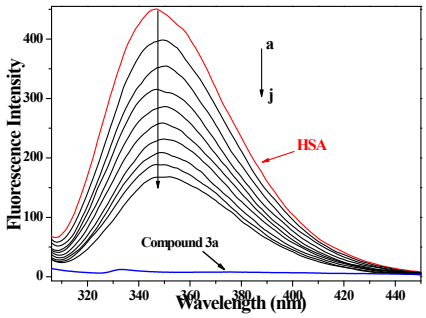

Figure 6 Emission spectra of HSA in the presence of various concentrations of compound **3a**. *c*(HSA) = 1.0 × 10^{-5} mol/L; *c*(compound **3a**)/(10^{-5} mol/L), *a*-*i*: from 0.0 to 2.0 at increments of 0.25; blue line shows the emission spectrum of compound **3a** only; T = 298 K, λ_{ex} = 295 nm.

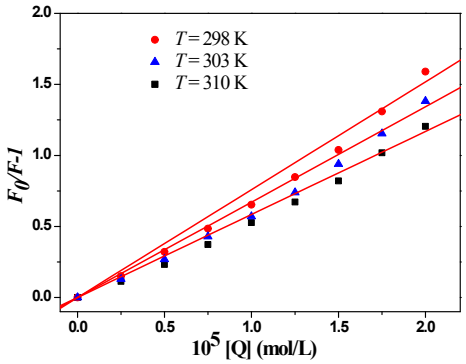

Figure 7 Stern-Volmer plots of **3a**-HSA system at different temperatures

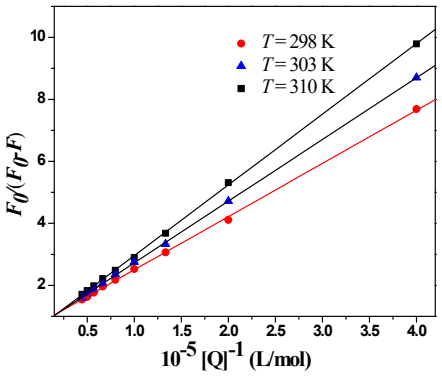

Figure 8 Modified Stern-Volmer plots of **3a**-HSA system at different temperatures

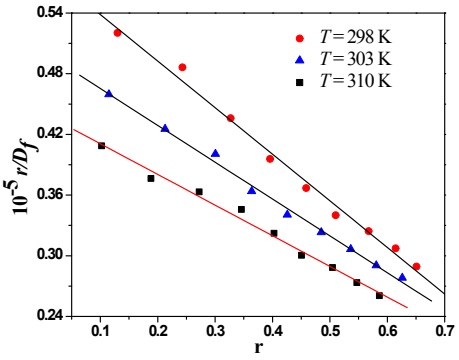

Figure 9 Scatchard plots of **3a**-HSA system at different temperatures

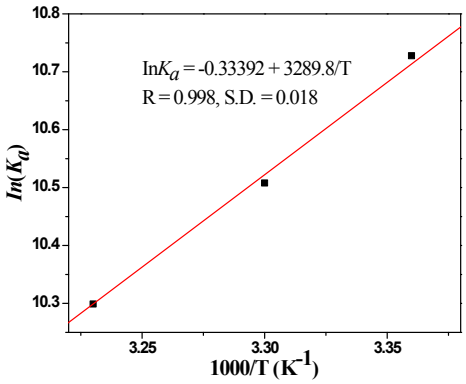

Figure 10 Van't Hoff plots of the **3a**-HSA system

NOTES AND REFERENCES

[1] (a) J. W. Cusic and E. F. Levon, *N*-[2-(nitro-1-imidazolyl)ethyl]naphthal imides, US Pat. 3997572, 1976; (b) K. Lee, J. Kim, K. Jeong, K. W. Lee, Y. Lee, J. Y. Song, M. S. Kim, G. S. Lee and Y. Kim, *Bioorg. Med. Chem.*, 2007, **17**, 3152–3161.