

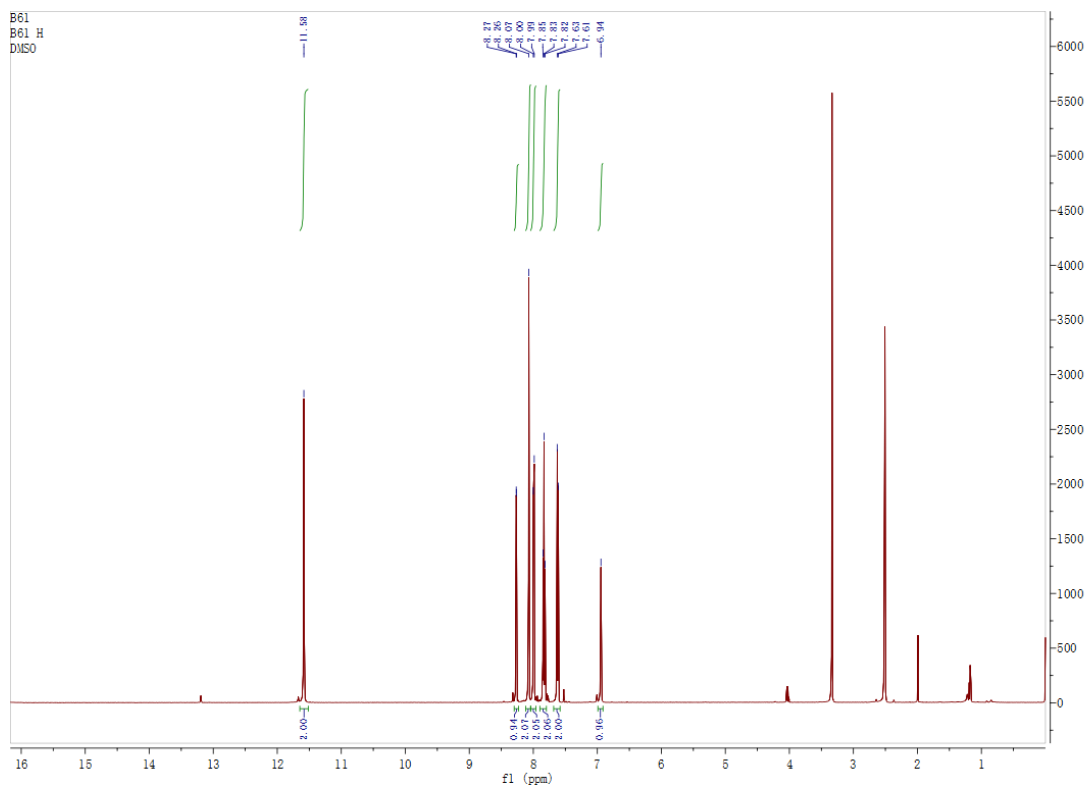
## **Supplementary materials**

# **Synthesis, Biological Evaluation, DNA Binding, and Molecular Docking of Hybrid 4,6-Dihydrazone Pyrimidine Derivatives as Antitumor Agents**

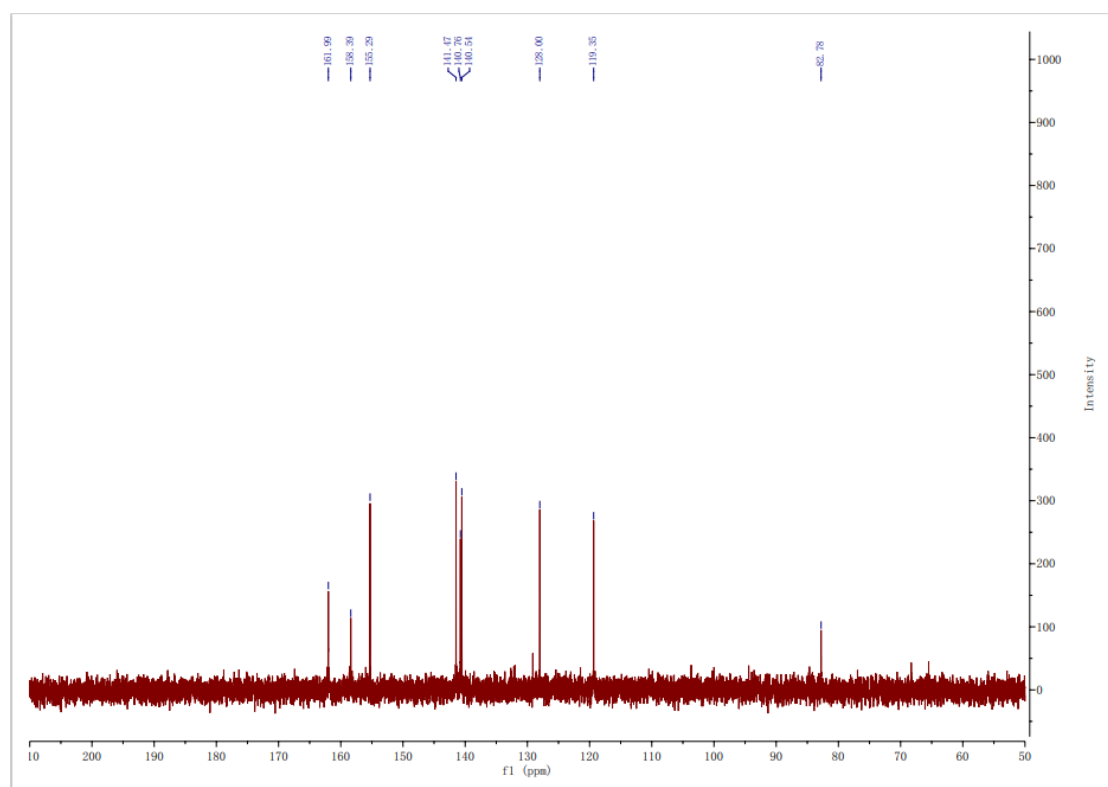
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Zhenqiang Zhang <sup>2,\*</sup> and Shuying Feng <sup>3</sup>

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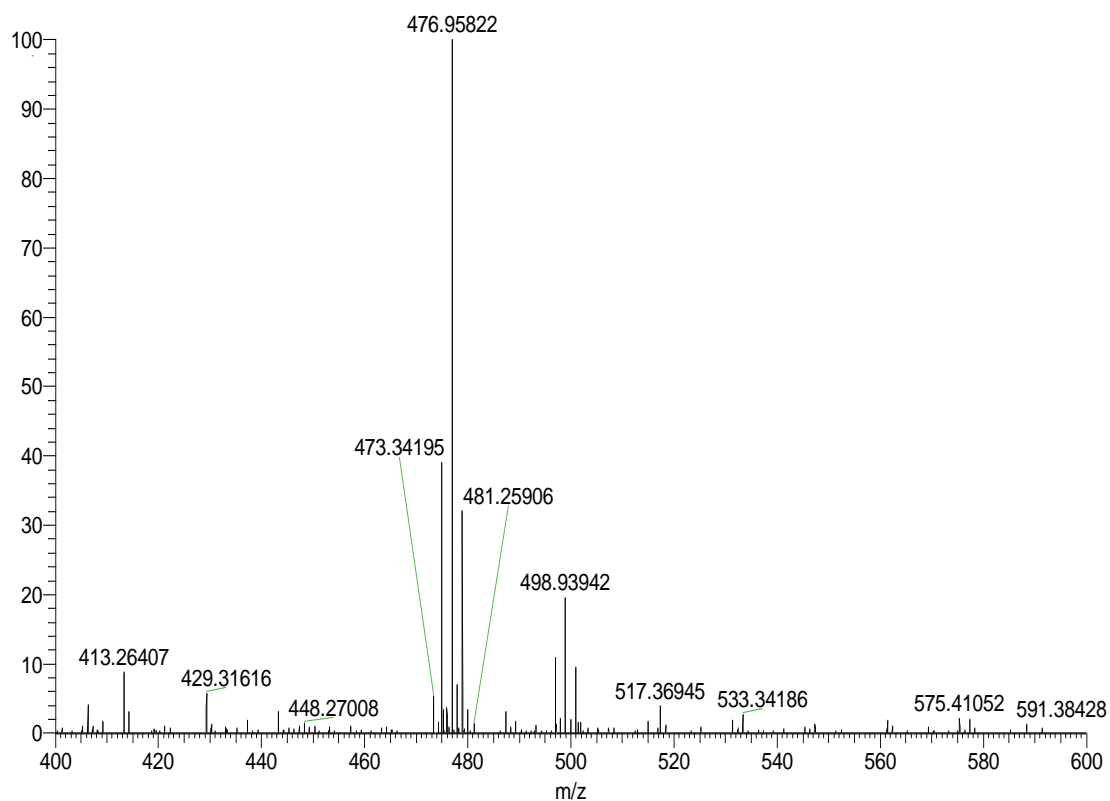
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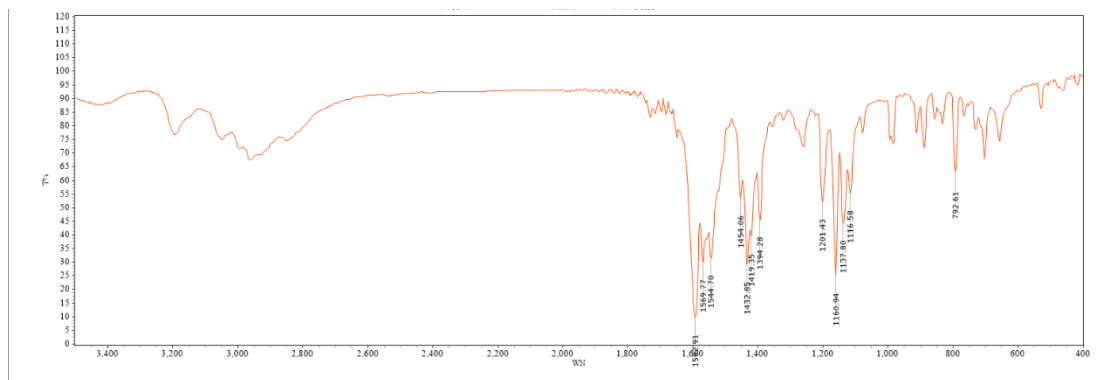
**Figure S1.**  $^1\text{H}$  NMR spectrum of **10a** in DMSO- $d_6$ .



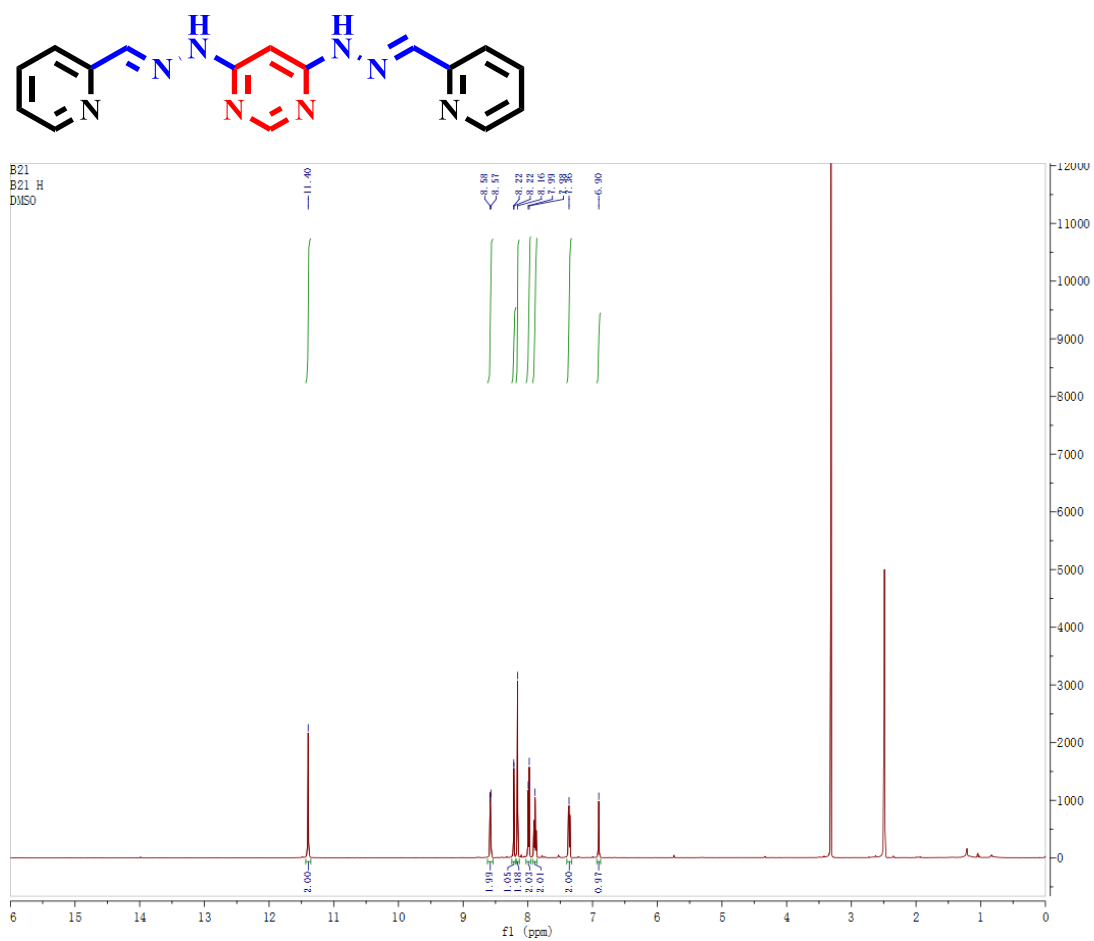
**Figure S2.** <sup>13</sup>C spectrum of **10a** in DMSO-d<sub>6</sub>.



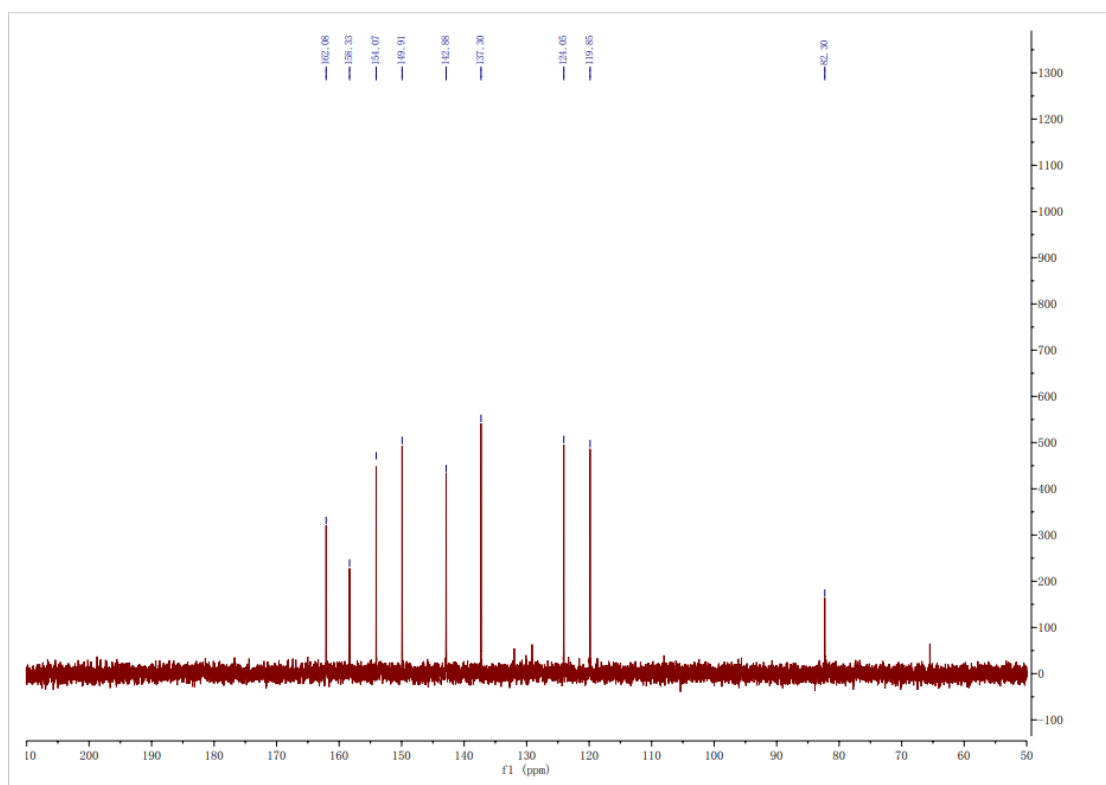
**Figure S3.** ESI-HRMS of **10a** at  $m/z$  476.95822 for  $C_{16}H_{12}Br_2N_8$   $[M+H]^+$ .



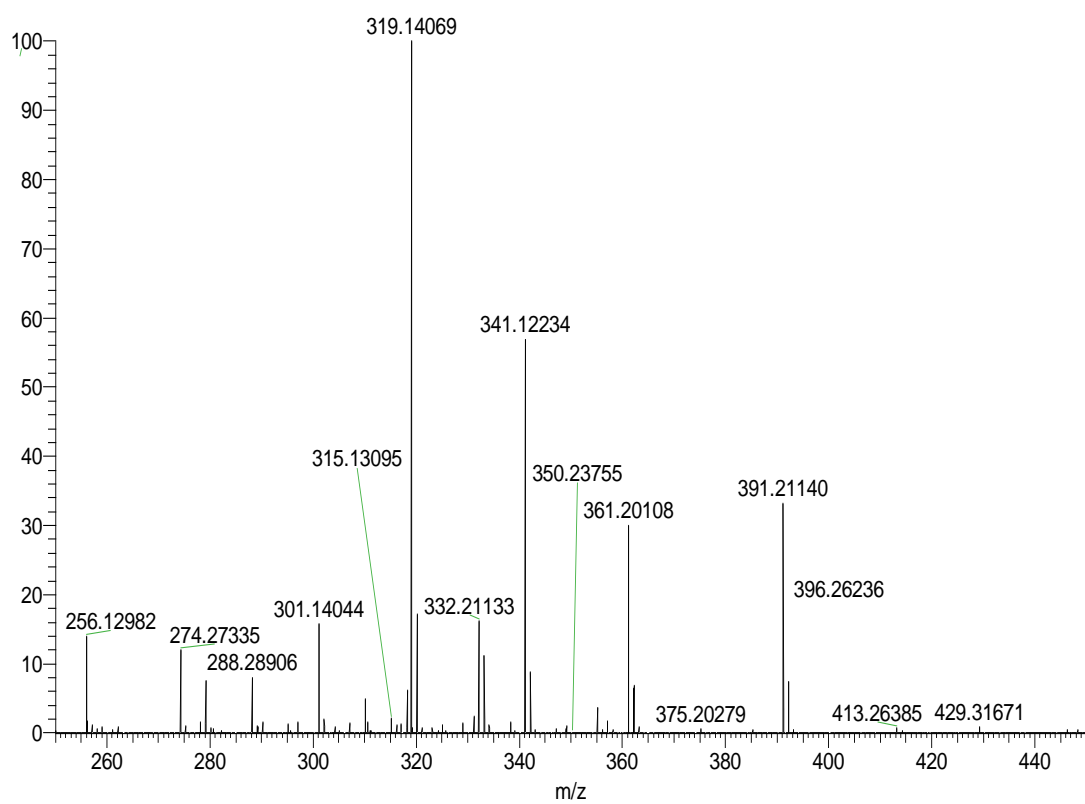
**Figure S4.** IR spectrum of **10a**.



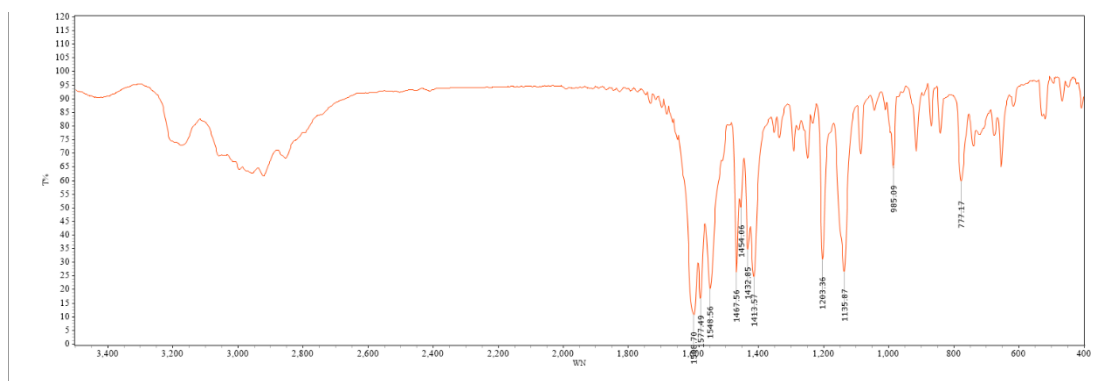
**Figure S5.**  $^1\text{H}$  NMR spectrum of **10b** in DMSO- $d_6$ .



**Figure S6.** <sup>13</sup>C spectrum of **10b** in DMSO-d<sub>6</sub>.

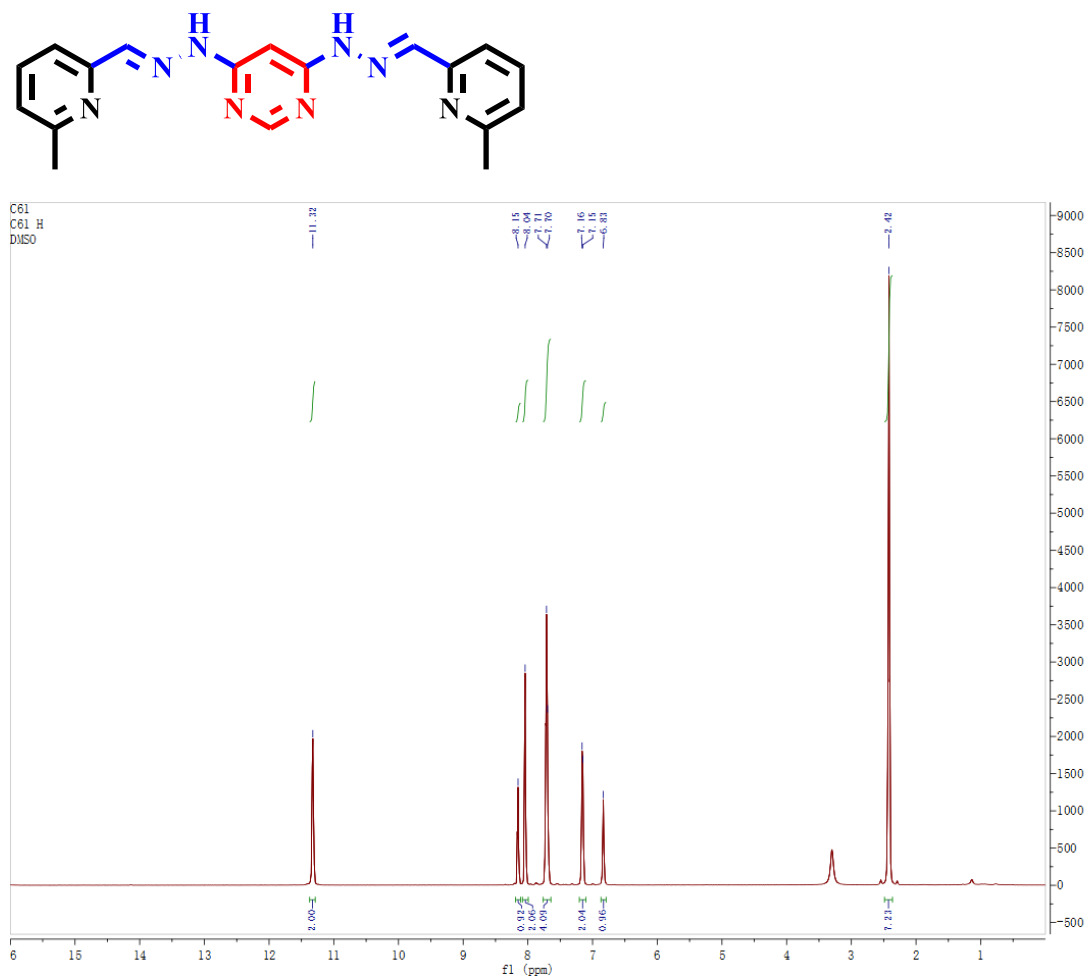


**Figure S7.** ESI-HRMS of **10b** at  $m/z$  319.14069 for C<sub>16</sub>H<sub>14</sub>N<sub>8</sub> [M+H]<sup>+</sup>.

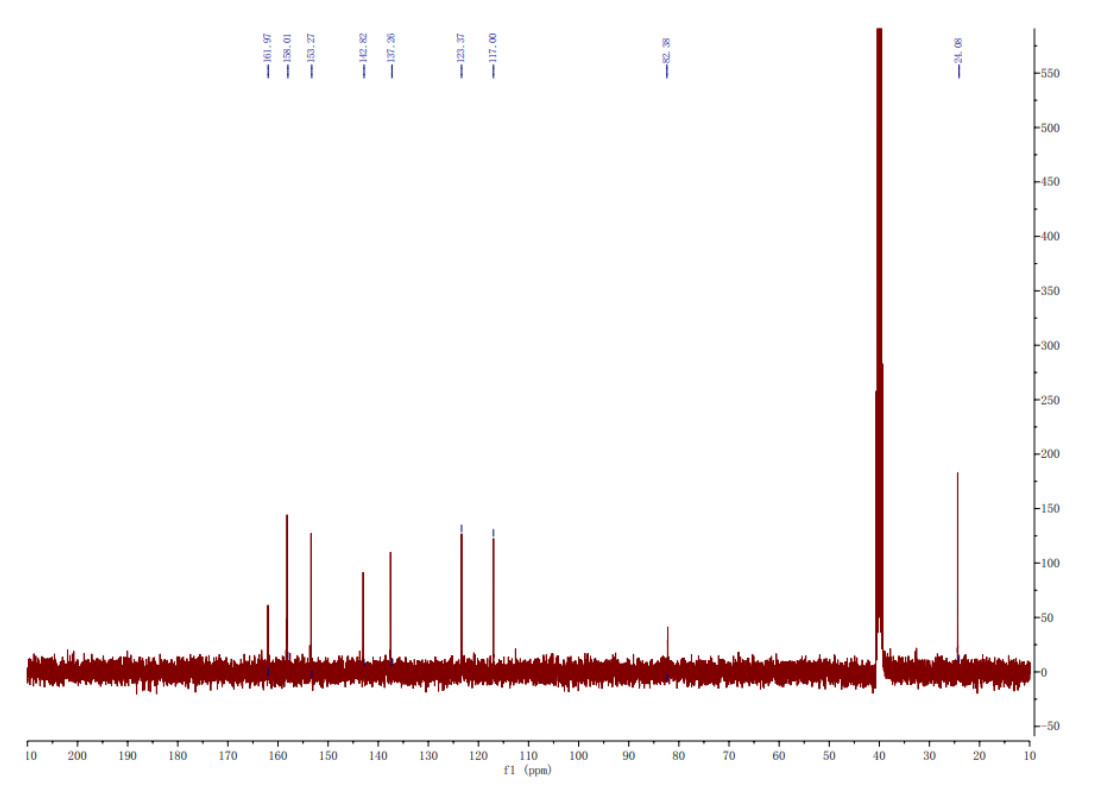


**Figure S8.** IR spectrum of **10b**.

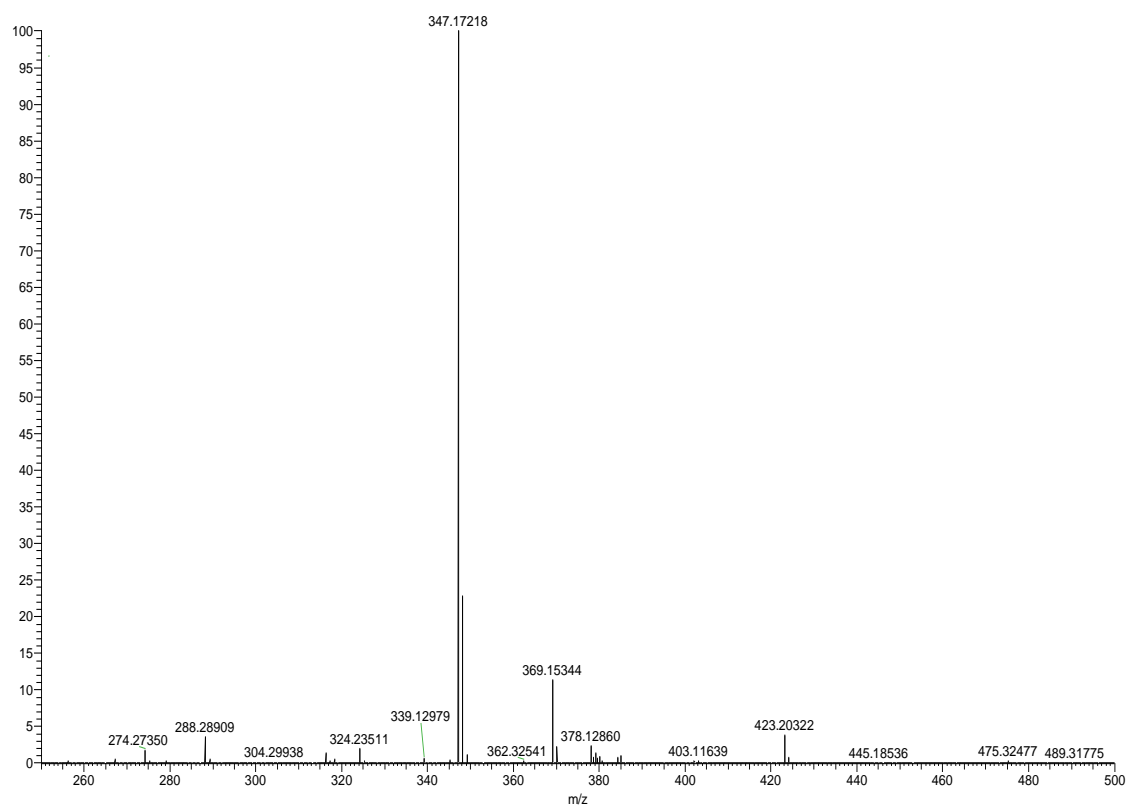




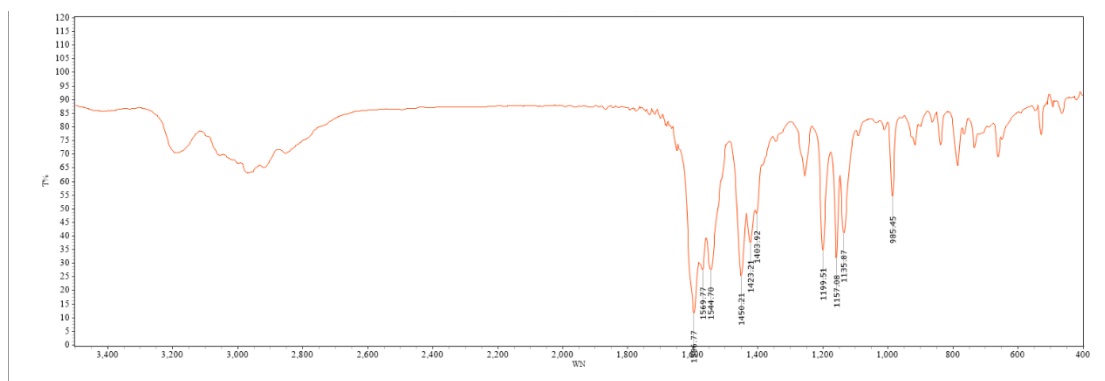
**Figure S9.**  $^1\text{H}$  NMR spectrum of **10c** in DMSO- $d_6$ .



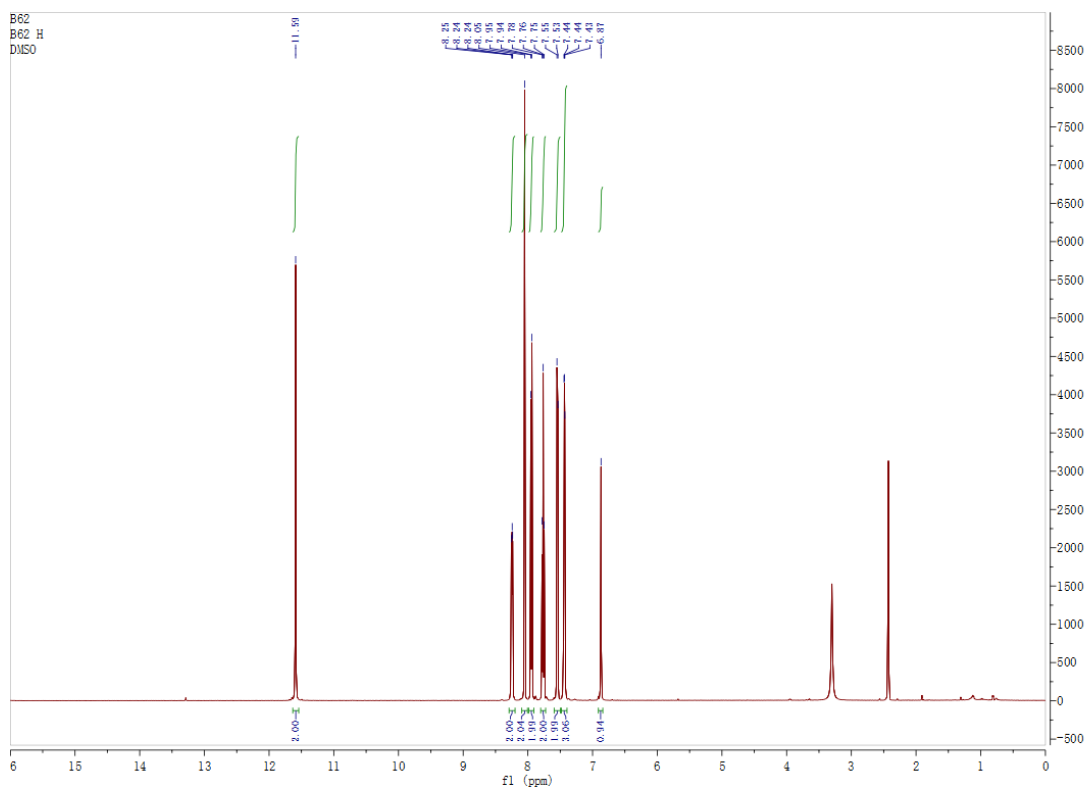
**Figure S10.**  $^{13}\text{C}$  spectrum of **10c** in DMSO- $d_6$ .



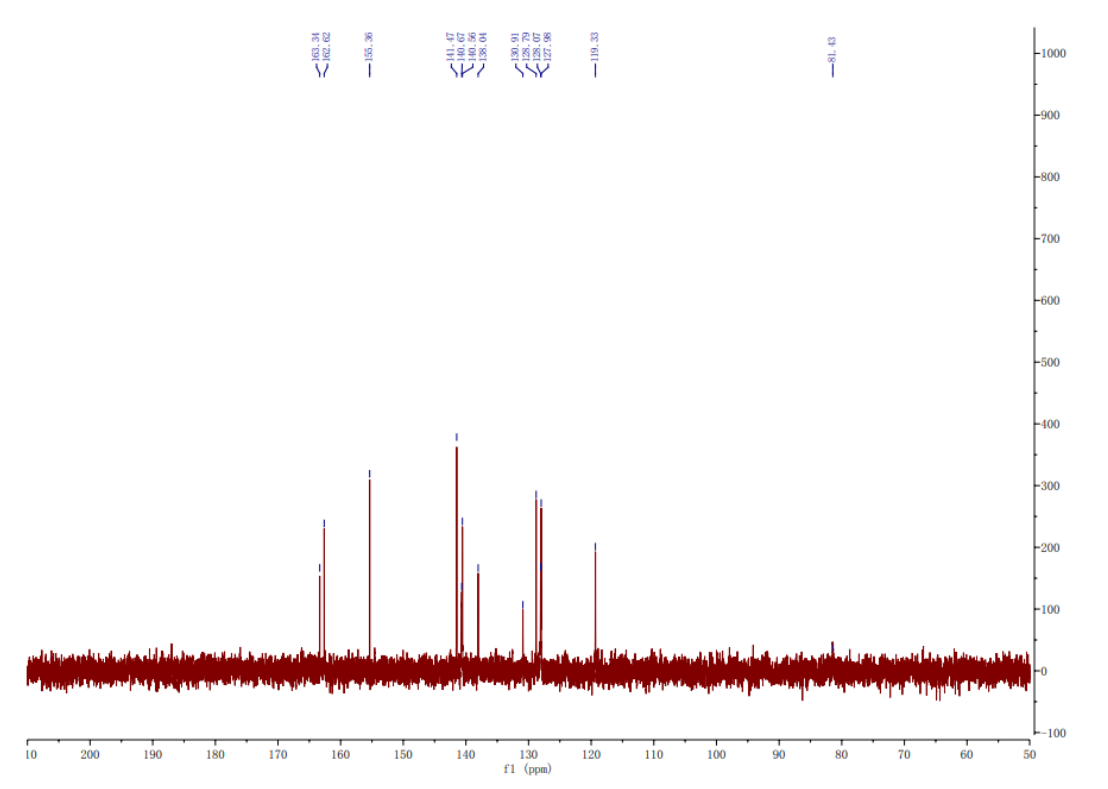
**Figure S11.** ESI-HRMS of **10c** at  $m/z$  347.17218 for  $\text{C}_{18}\text{H}_{18}\text{N}_8$   $[\text{M}+\text{H}]^+$ .



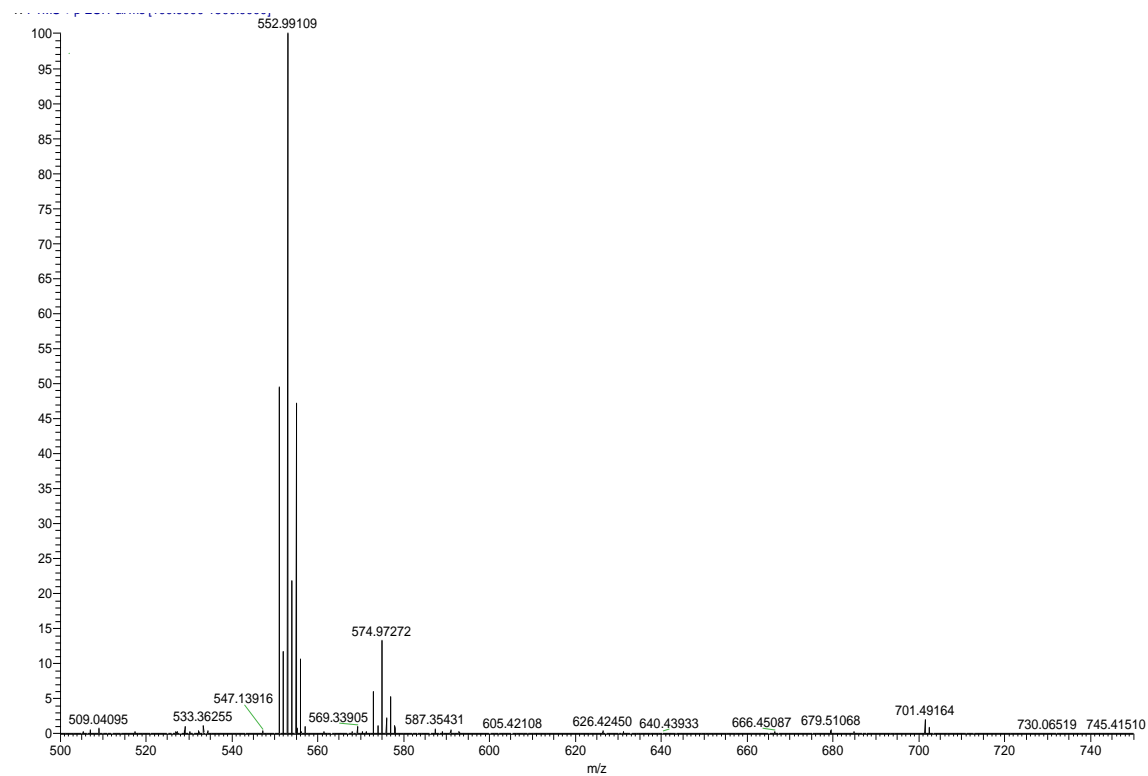
**Figure S12.** IR spectrum of **10c**.



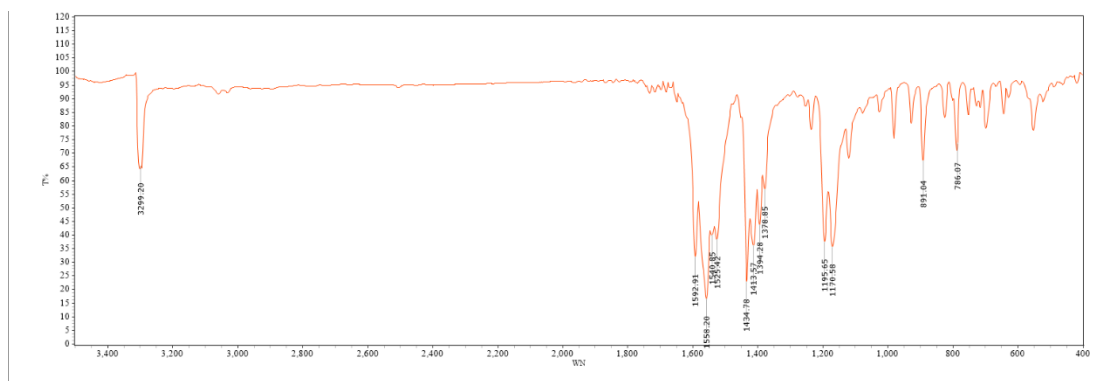
**Figure S13.**  $^1\text{H}$  NMR spectrum of **10d** in DMSO- $d_6$ .



**Figure S14.**  $^{13}\text{C}$  spectrum of **10d** in DMSO- $d_6$ .



**Figure S15.** ESI-HRMS of **10d** at  $m/z$  552.99109 for  $\text{C}_{22}\text{H}_{16}\text{Br}_2\text{N}_8$   $[\text{M}+\text{H}]^+$ .



**Figure S16.** IR spectrum of **10d**.

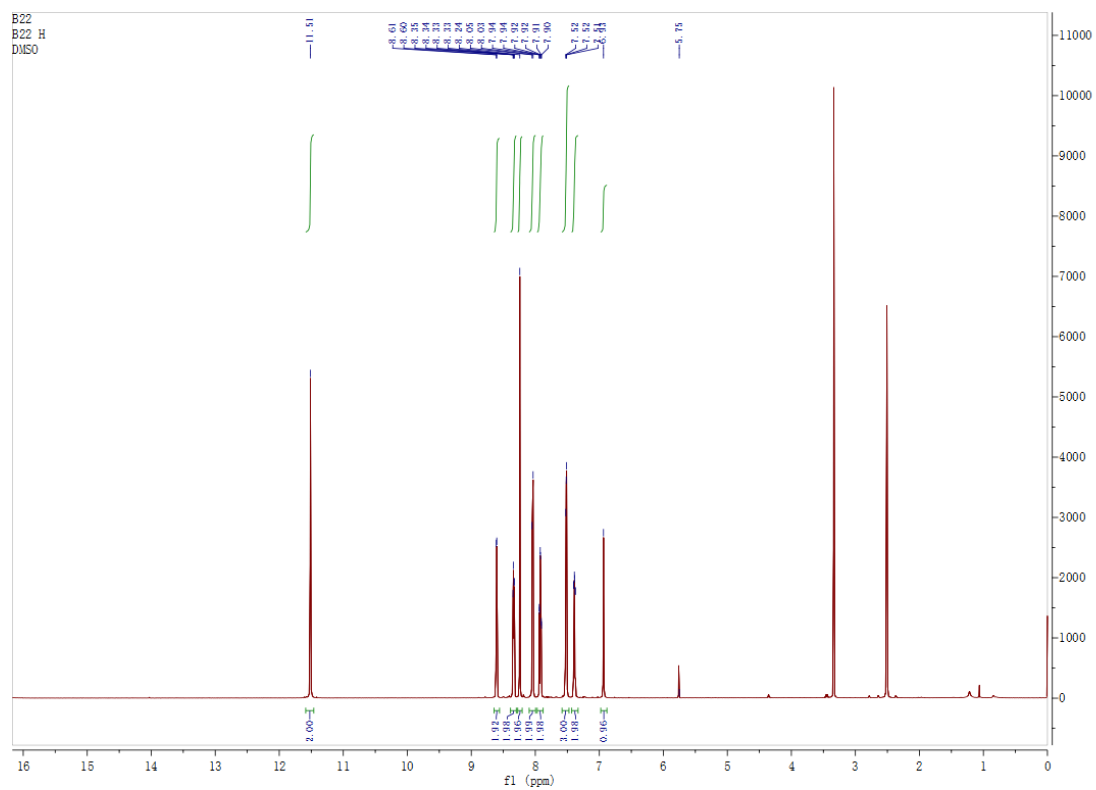
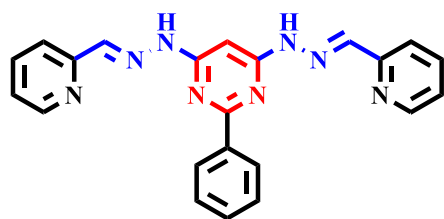
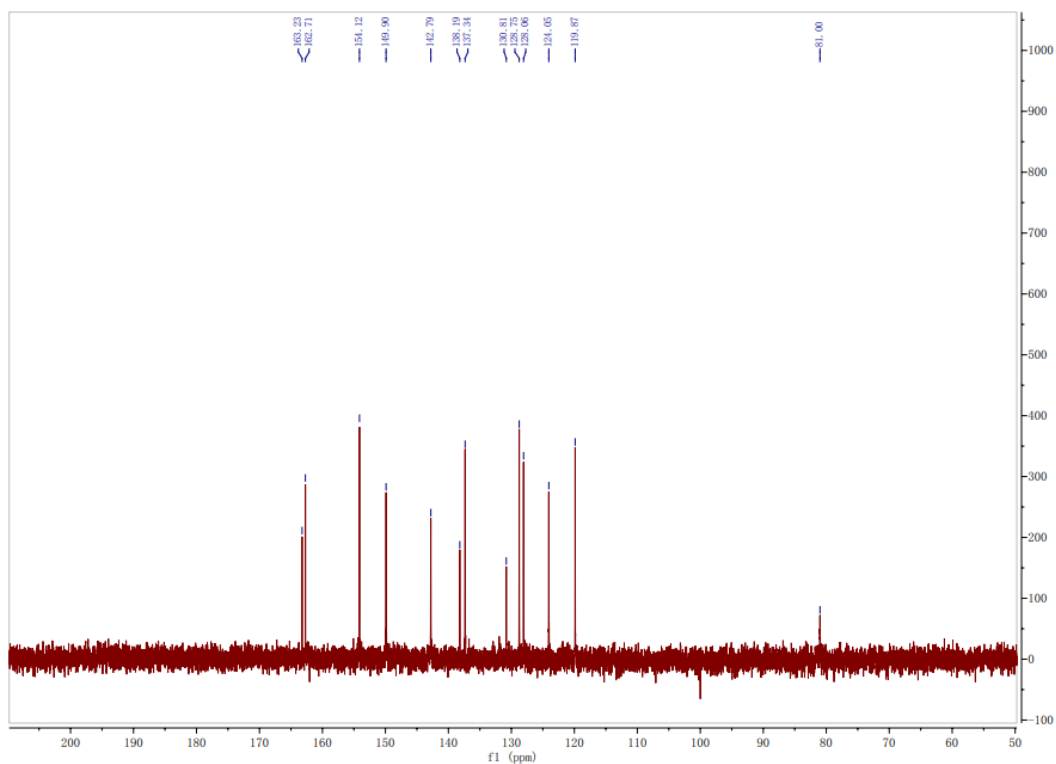
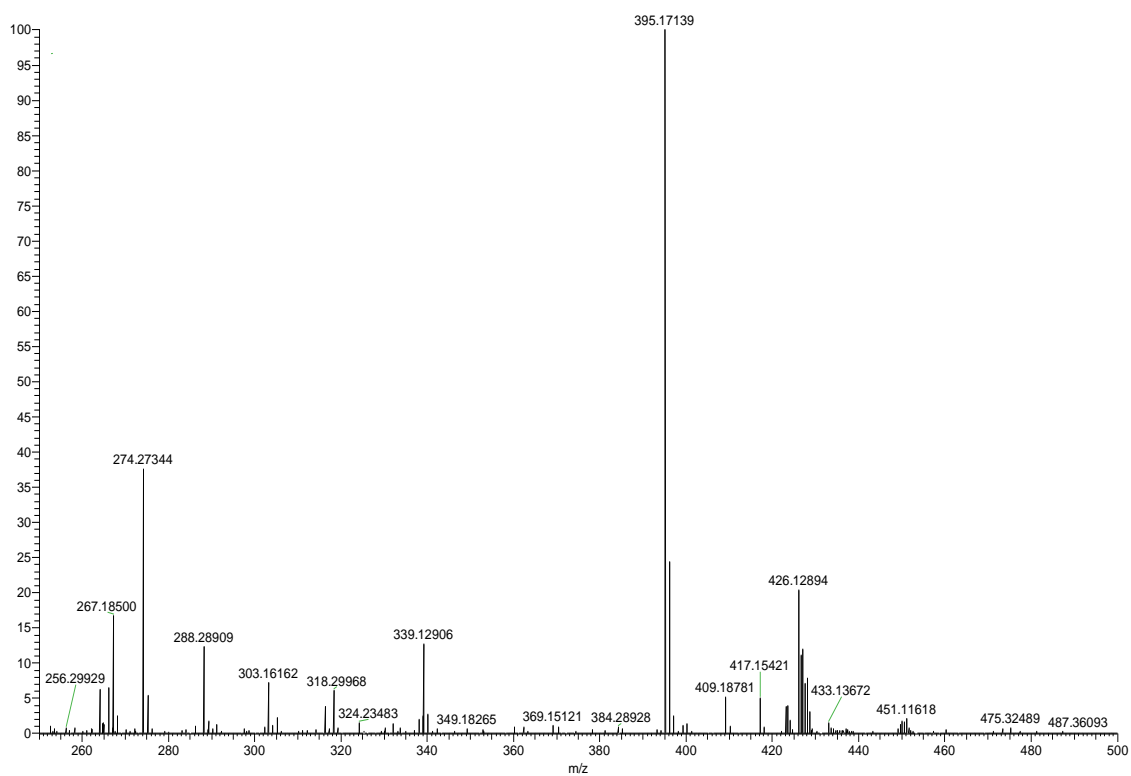


Figure S17.  $^1\text{H}$  NMR spectrum of **10e** in DMSO- $d_6$ .

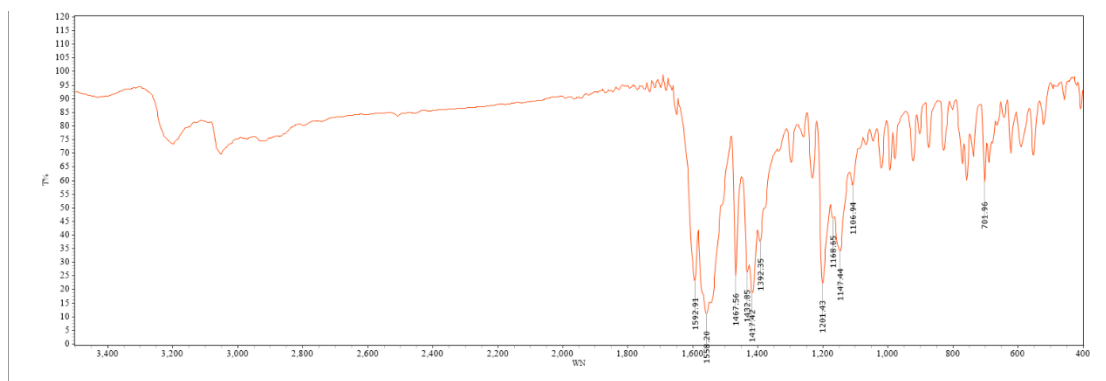


**Figure S18.** <sup>13</sup>C spectrum of **10e** in DMSO-d<sub>6</sub>.

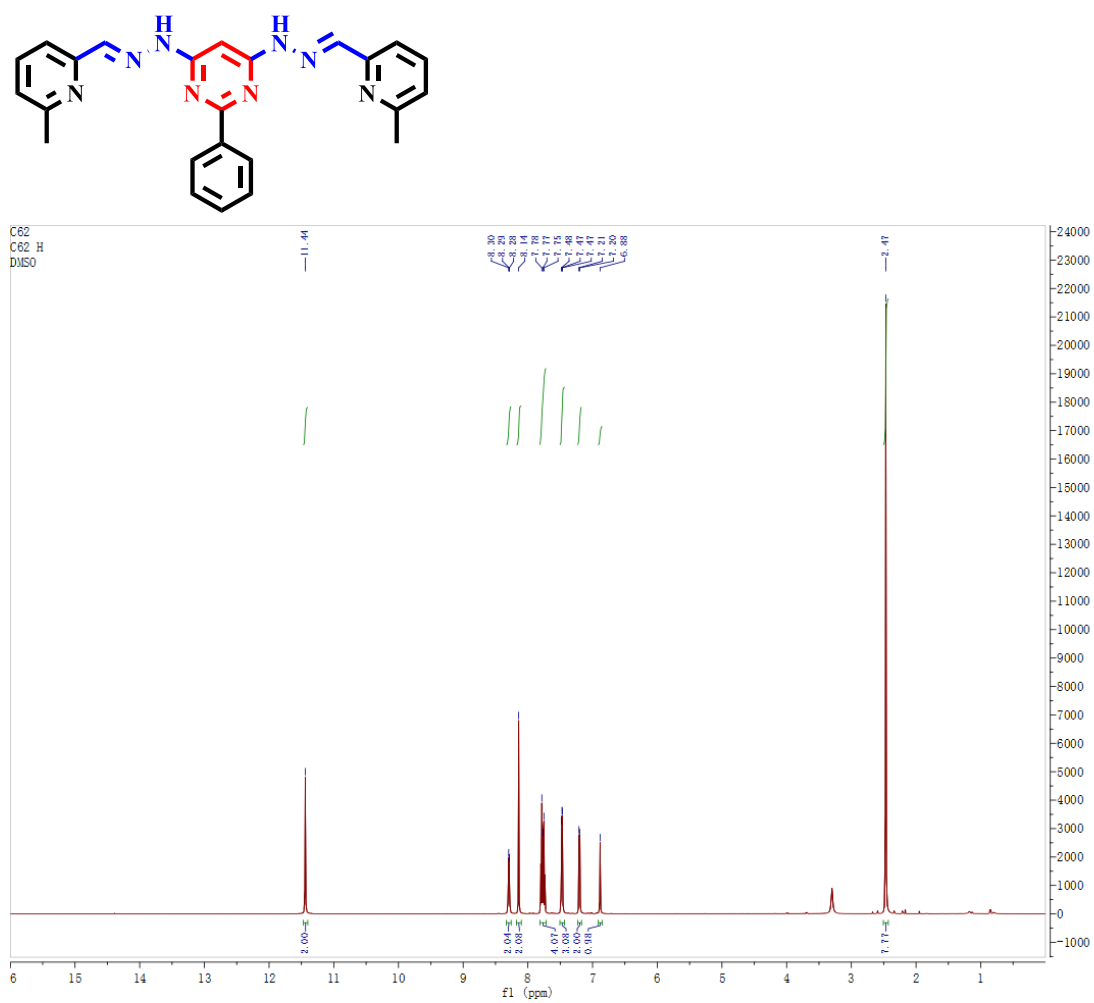


**Figure S19.** ESI-HRMS of **10e** at m/z 395.17139 for C<sub>22</sub>H<sub>18</sub>N<sub>8</sub> [M+H]<sup>+</sup>.

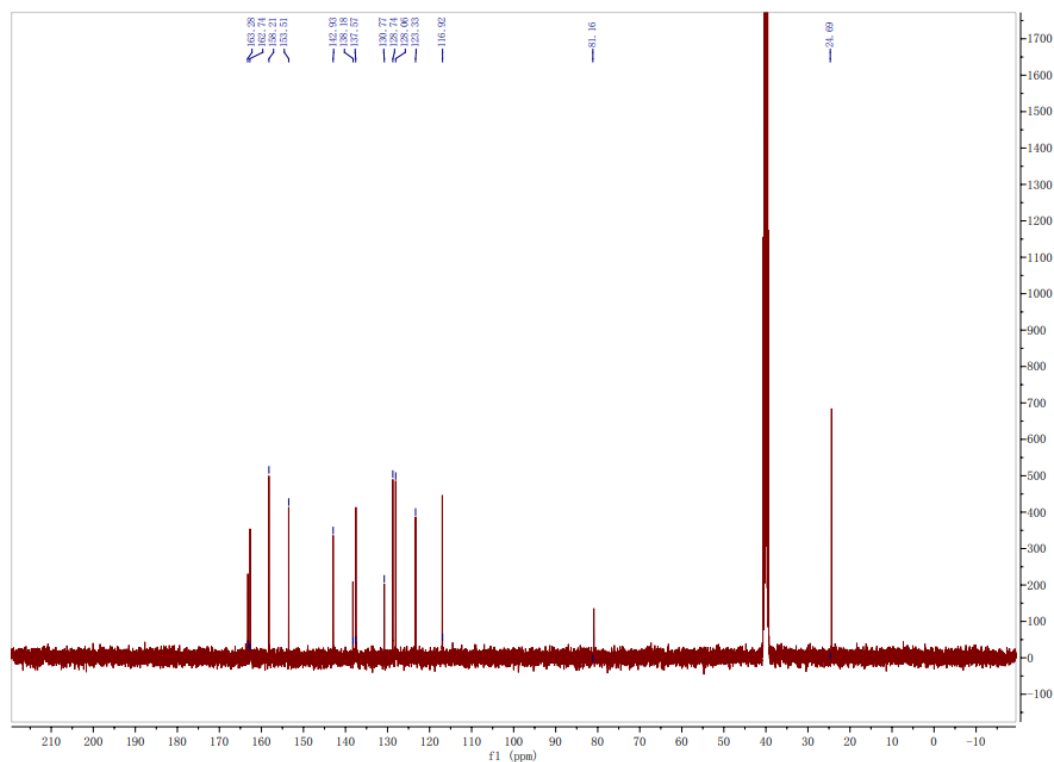




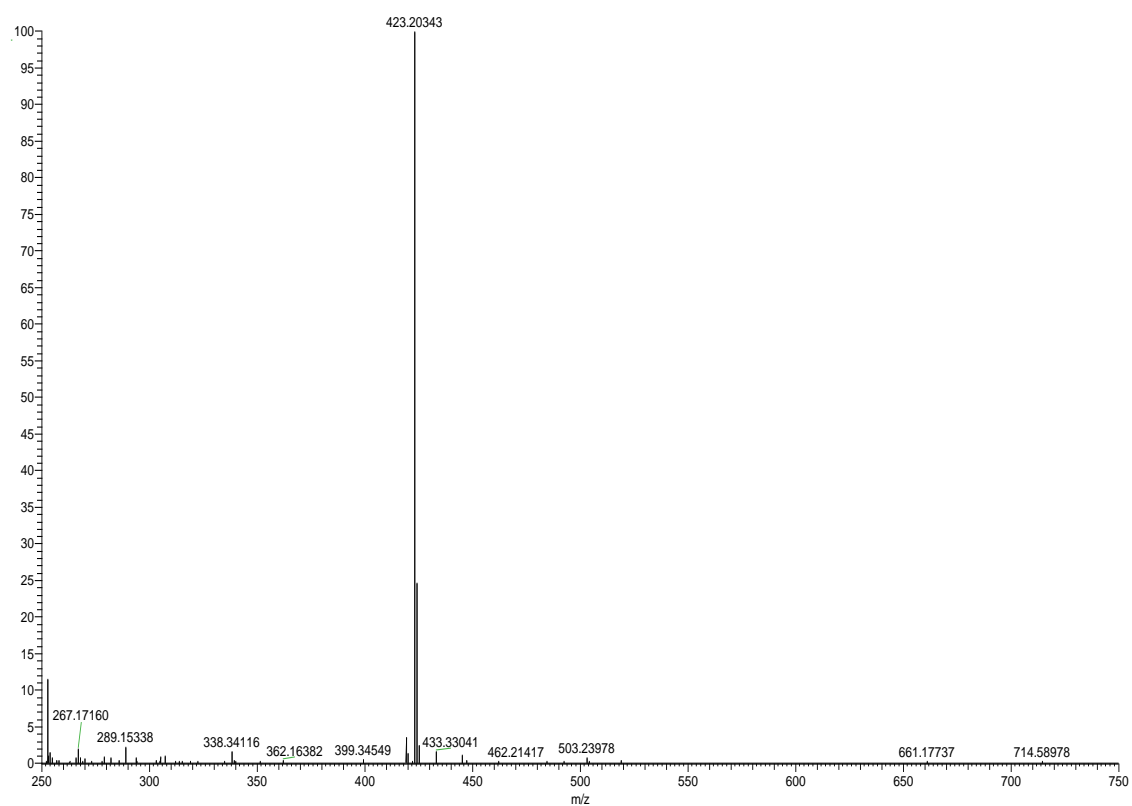
**Figure S20.** IR spectrum of **10e**.



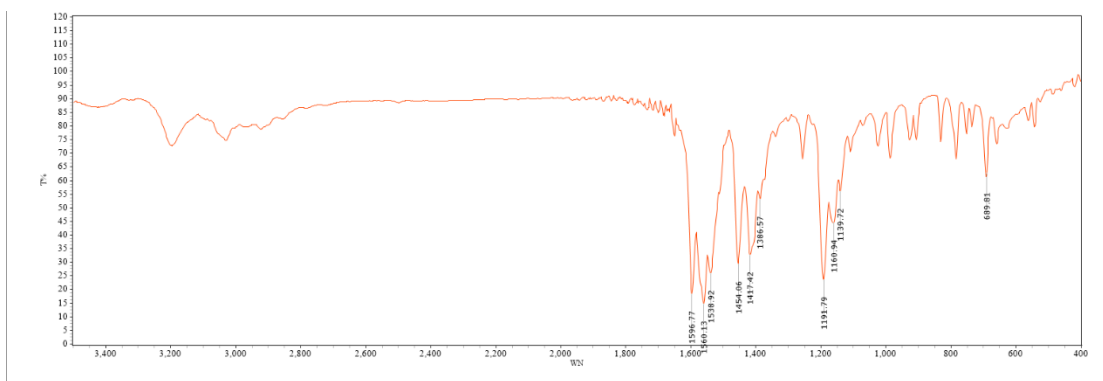
**Figure S21.** <sup>1</sup>H NMR spectrum of **10f** in DMSO-d<sub>6</sub>.



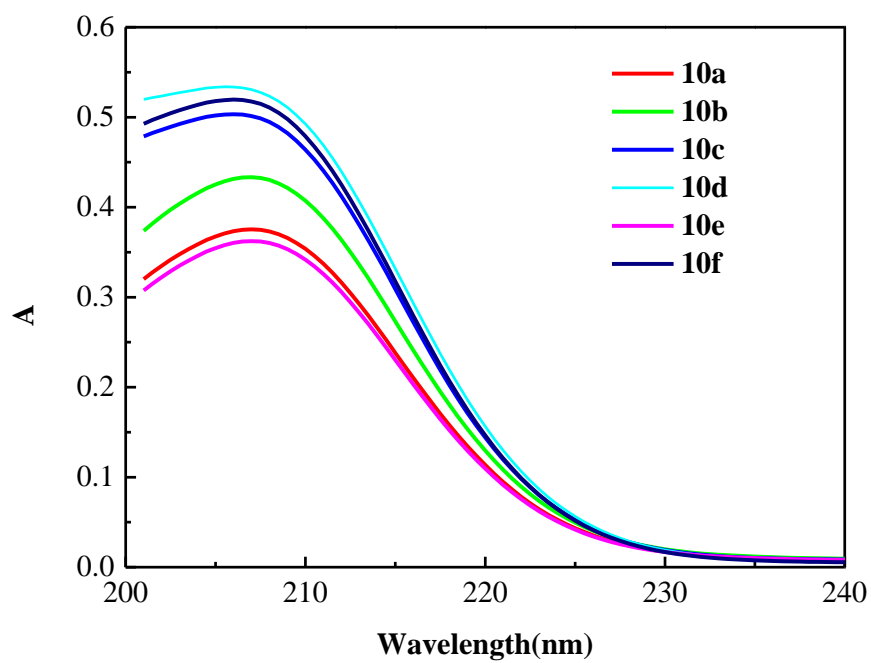
**Figure S22.**  $^{13}\text{C}$  spectrum of **10f** in DMSO- $d_6$ .



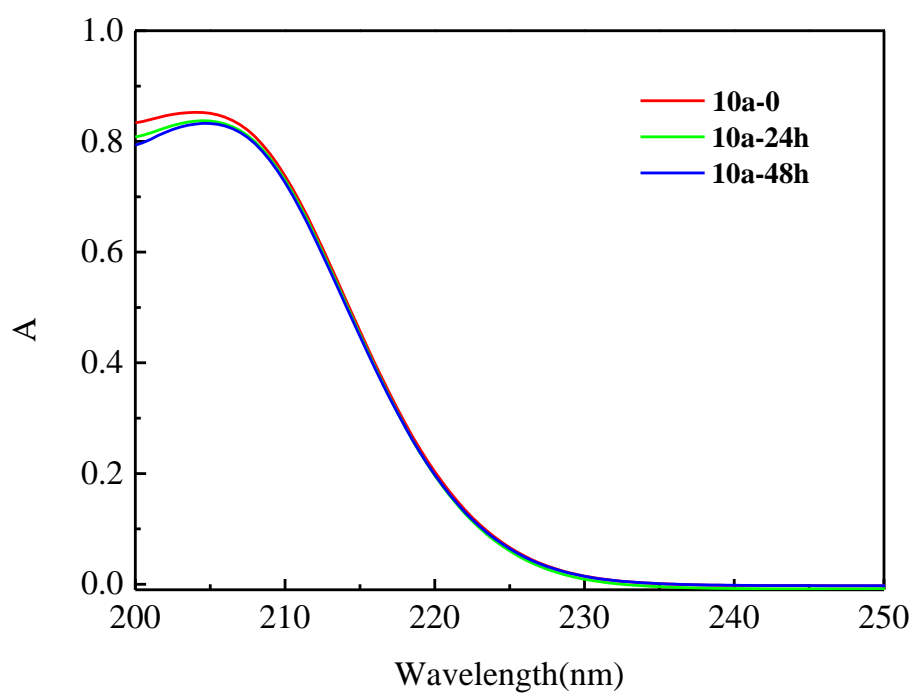
**Figure S23.** ESI-HRMS of **10f** at  $m/z$  423.20343 for  $\text{C}_{24}\text{H}_{22}\text{N}_8$   $[\text{M}+\text{H}]^+$ .



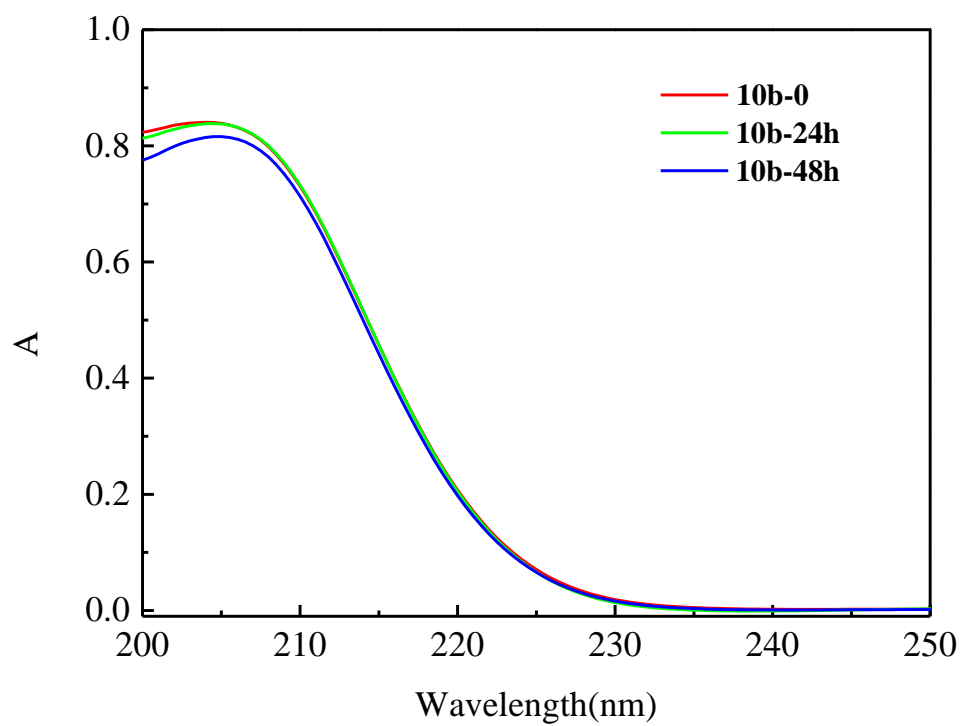
**Figure S24.** IR spectrum of **10f**.



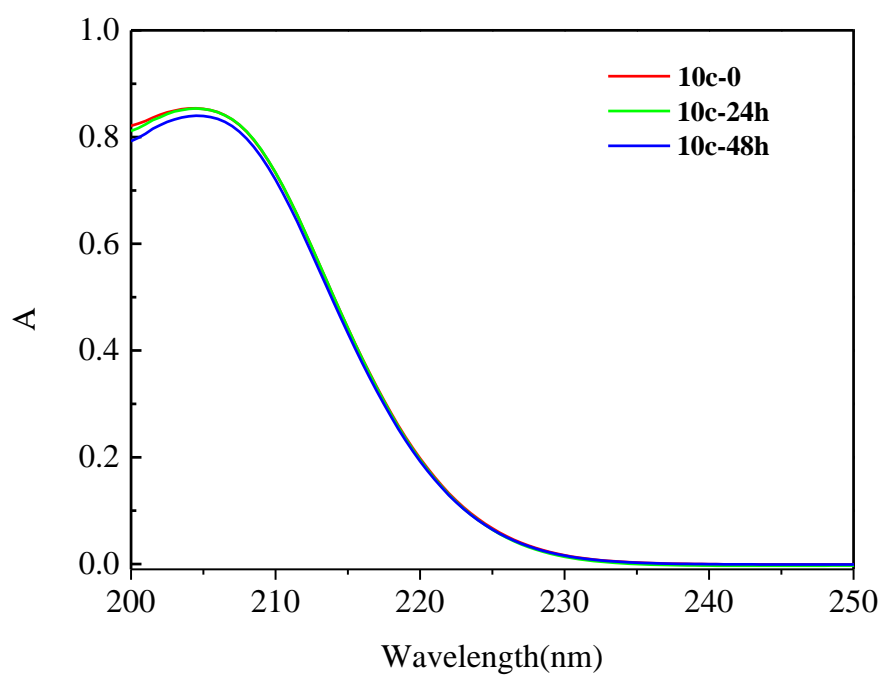
**Figure S25.** UV-vis spectra of **10a~10f** at 0.1  $\mu$ M in Tris-HCl buffer (pH 7.4).



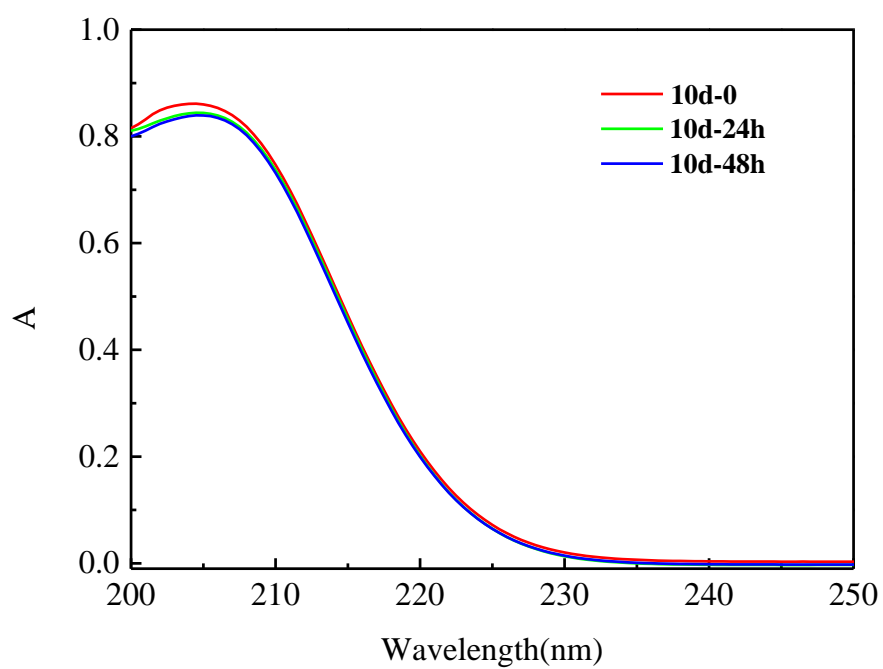
**Figure S26.** The UV-vis spectra of **10a** within 48 h in Tris-HCl buffer (pH 7.4).



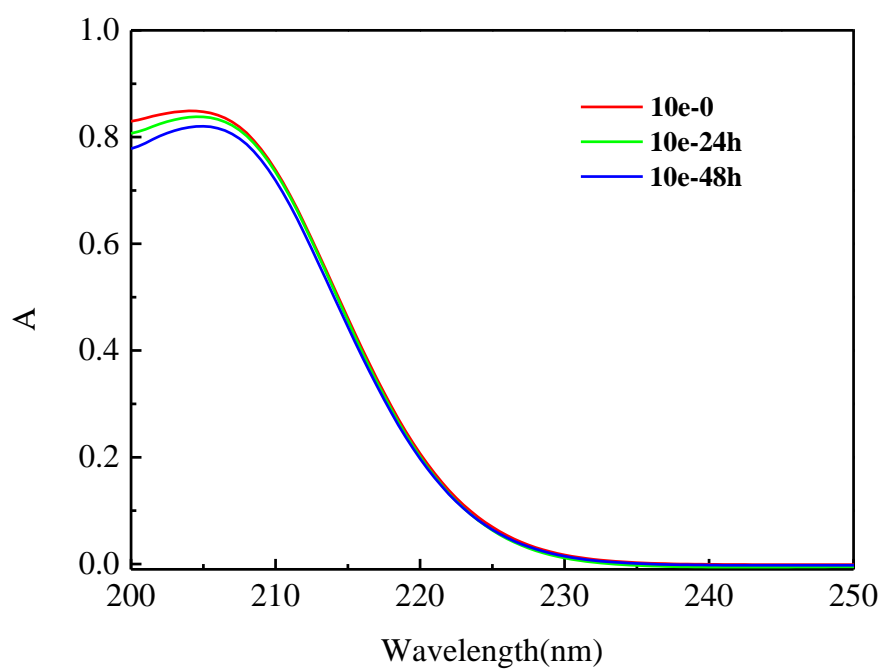
**Figure S27.** The UV-vis spectra of **10b** within 48 h in Tris-HCl buffer (pH 7.4).



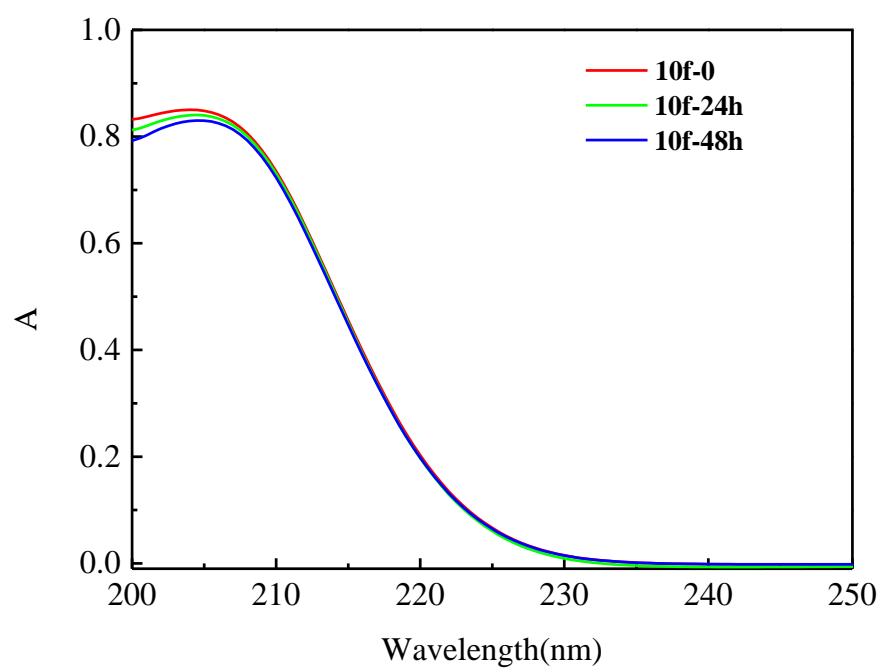
**Figure S28.** The UV-vis spectra of **10c** within 48 h in Tris-HCl buffer (pH 7.4).



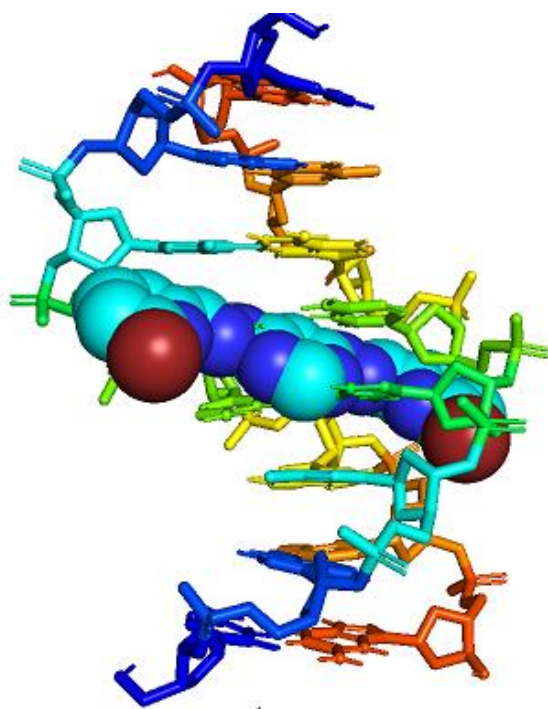
**Figure S29.** The UV-vis spectra of **10d** within 48 h in Tris-HCl buffer (pH 7.4).



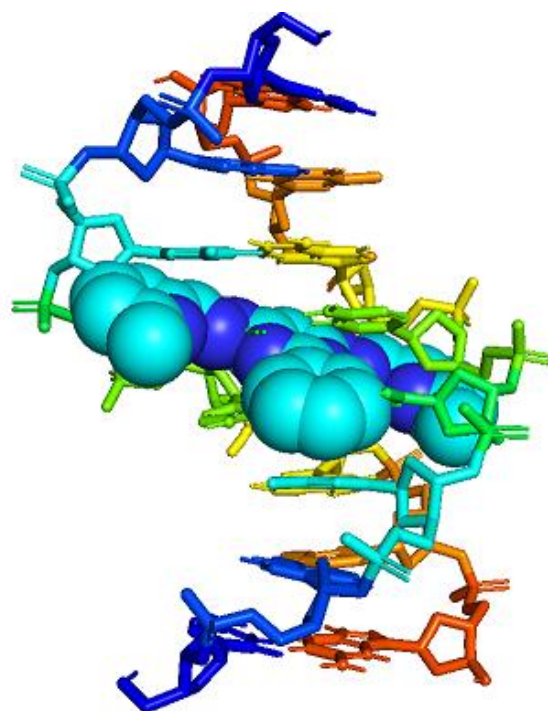
**Figure S30.** The UV-vis spectra of **10e** within 48 h in Tris-HCl buffer (pH 7.4).



**Figure S31.** The UV-vis spectra of **10f** within 48 h in Tris-HCl buffer (pH 7.4).



**Figure S32.** Visualizations of **10a** docking with DNA (PDB ID: 1D18)



**Figure S33.** Visualizations of **10f** docking with DNA (PDB ID: 1D18)



**Table S1.** The  $\Delta G_b^0$  and  $K_b$  of **10a** and **10f** interacting with DNA (PDB ID: 1D18).

Compound	$\Delta G_b^0$ (kcal/ mol)	$K_b$ ( $M^{-1}$ )
10a	−8.0	$7.75 \times 10^5$
10f	−8.1	$9.18 \times 10^5$
5-FU	−5.0	$4.81 \times 10^3$