

Supporting Information

Design, synthesis, and biological evaluation of novel pyrimidin-2-amine derivatives as potent PLK4 inhibitors

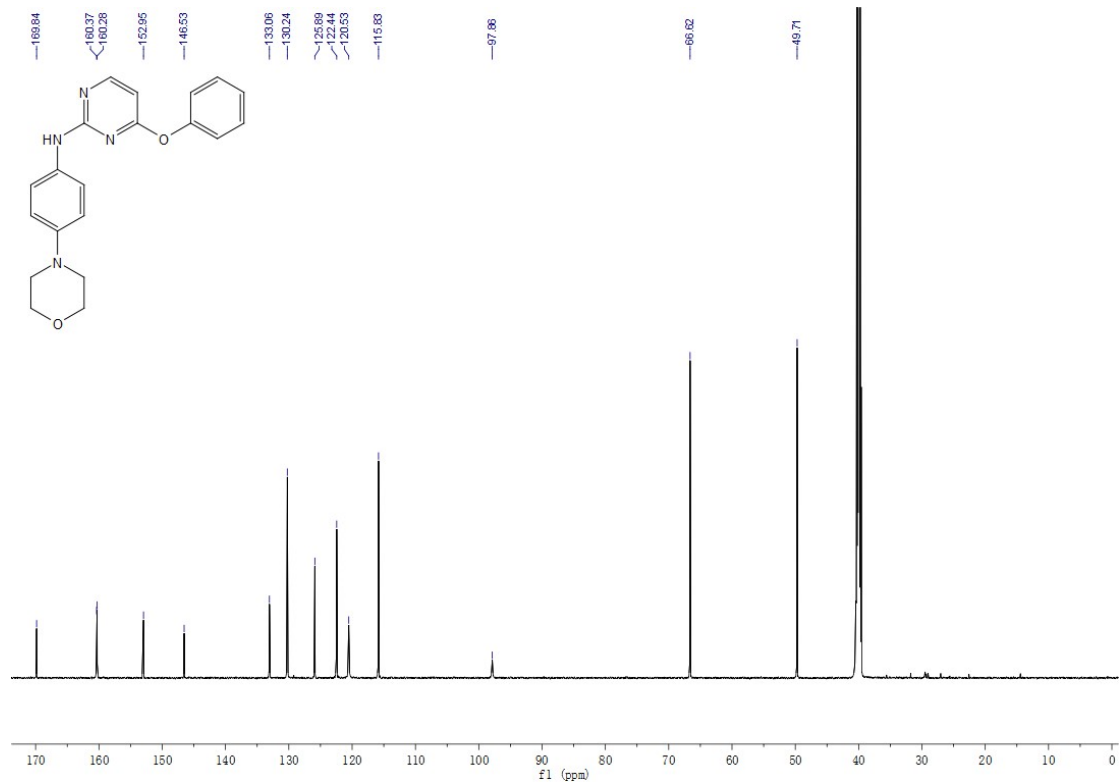
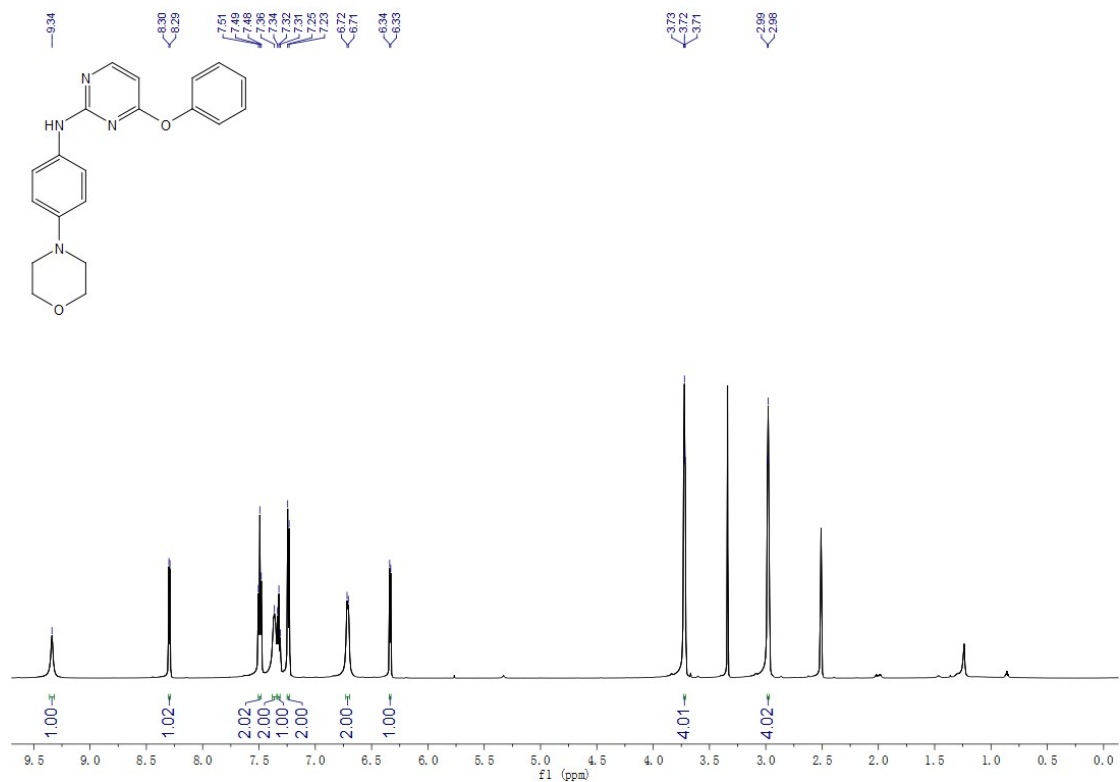
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Maosheng Cheng

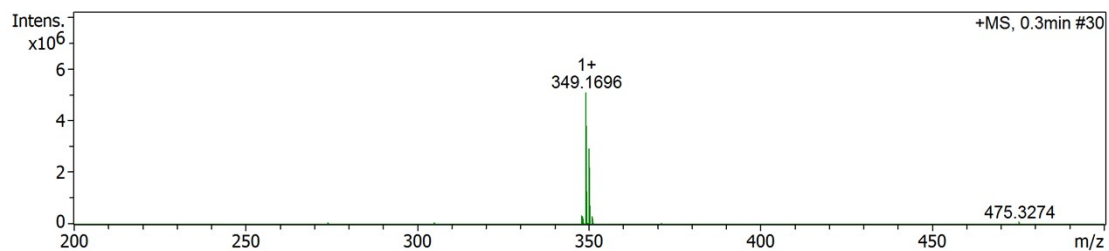
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Pharmaceutical Engineering, Shenyang Pharmaceutical University, 103 Wenhua Road, Shenhe
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Yanli Xue, Shuyi Mu, Pengkun Sun and Yin Sun contributed equally to this study.

3a



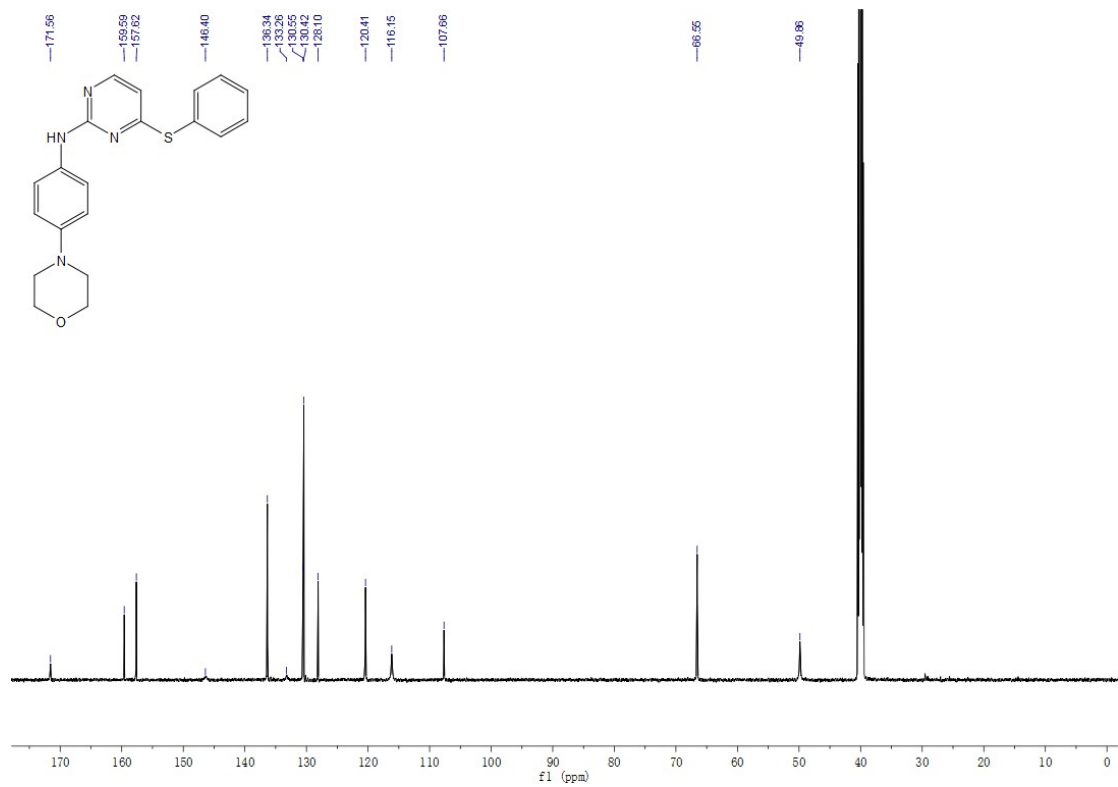
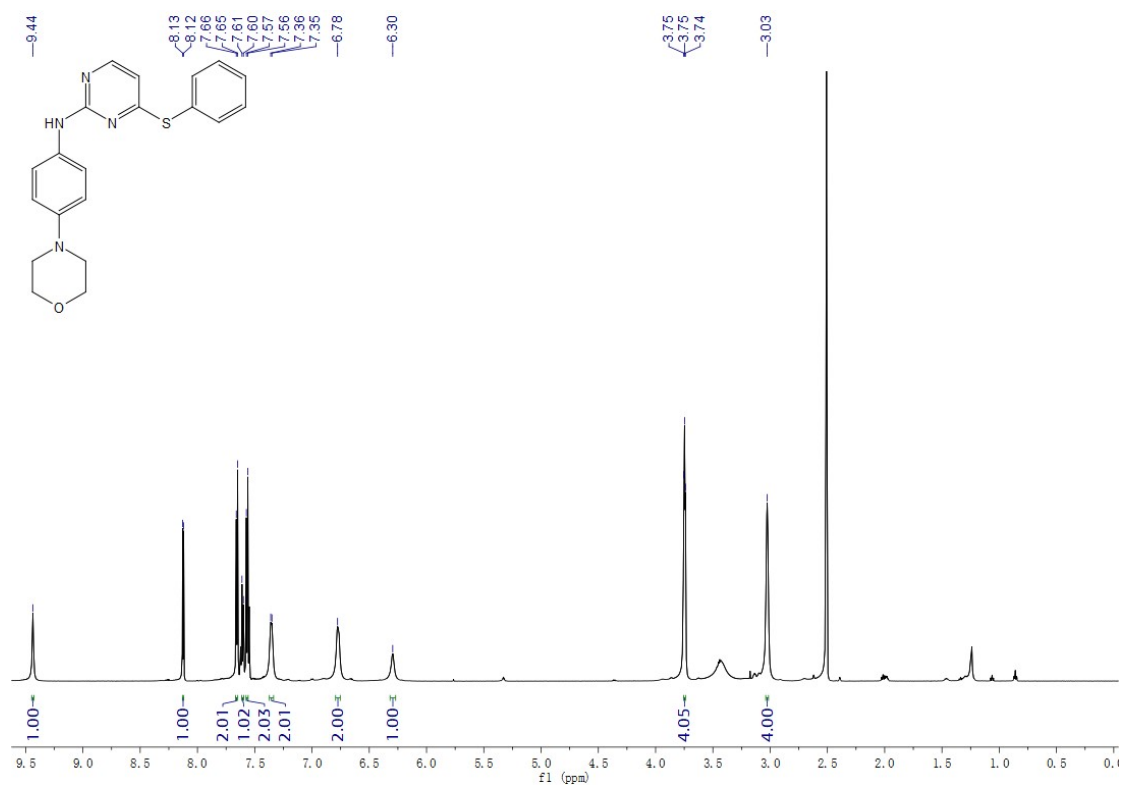


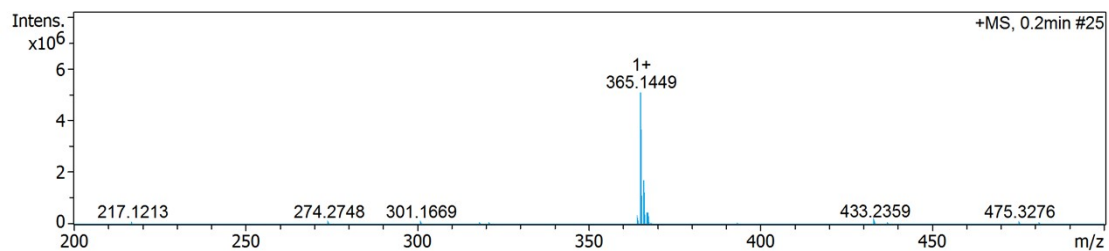
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.34 (s, 1H), 8.30 (d, *J* = 5.5 Hz, 1H), 7.50 (t, *J* = 7.7 Hz, 2H), 7.36 (s, 2H), 7.33 (t, *J* = 12 Hz, 1H), 7.24 (d, *J* = 6 Hz, 2H), 6.71 (d, *J* = 7.1 Hz, 2H), 6.33 (d, *J* = 5.5 Hz, 1H), 3.73 – 3.71 (m, 4H), 2.99 – 2.97 (m, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 169.8, 160.4, 160.3, 152.9, 146.5, 133.1, 130.2, 125.9, 122.4, 120.5, 115.8, 97.9, 66.6, 49.7.

HRMS (ESI, *m/z*) calcd for C₂₀H₂₀N₄O₂, [M+H]⁺: 349.1659; found: 349.1696.

3b



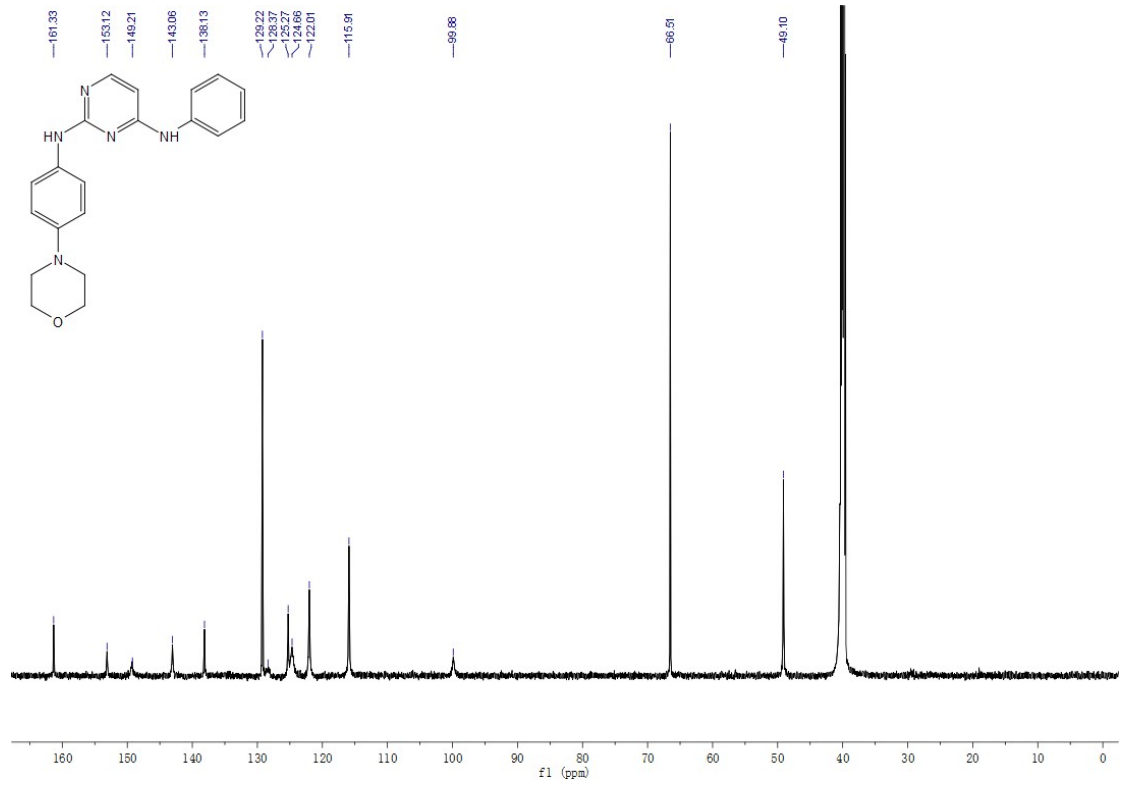
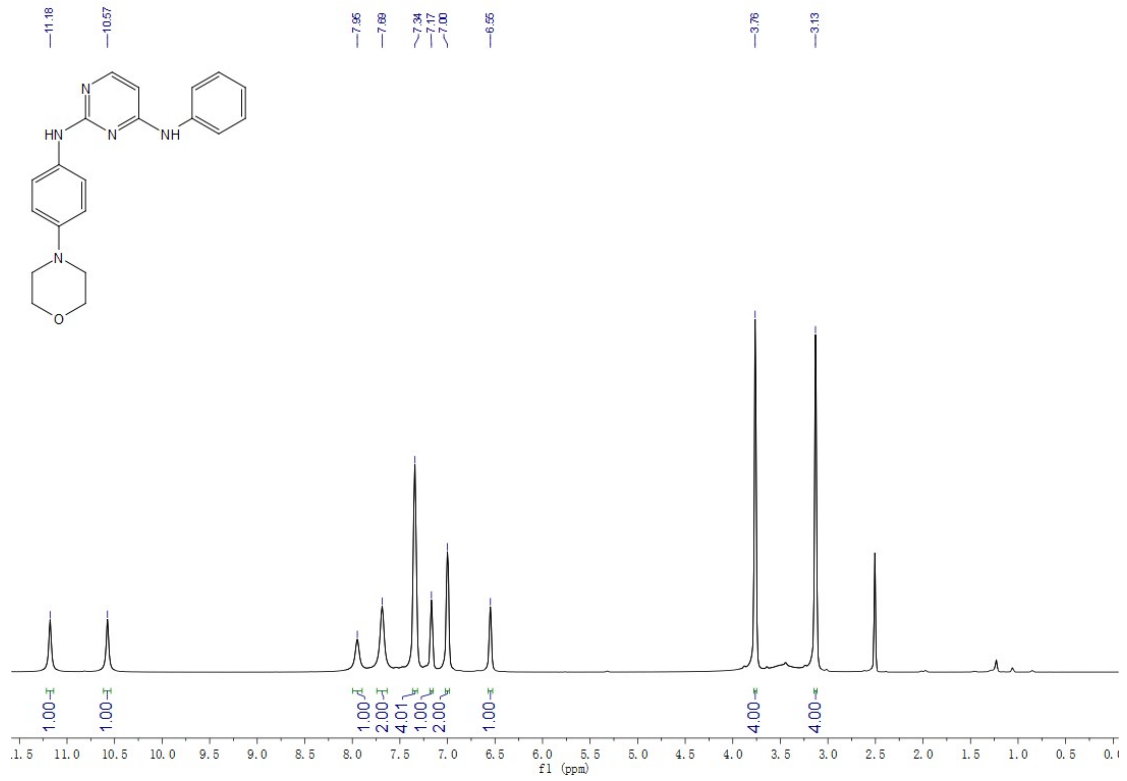


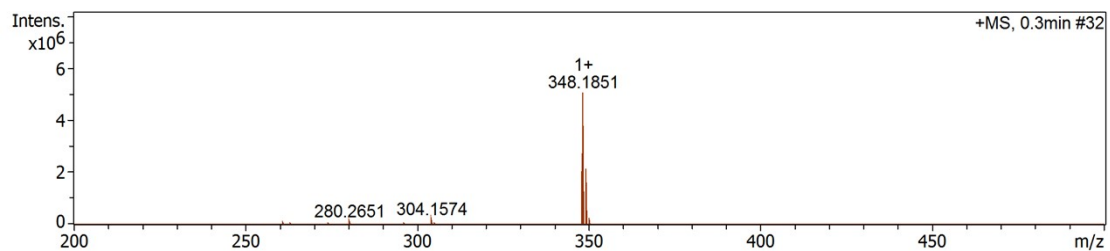
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.44 (s, 1H), 8.12 (d, *J* = 5.3 Hz, 1H), 7.66 (t, *J* = 7.0 Hz, 2H), 7.61 (t, *J* = 7.4 Hz, 1H), 7.57 (t, *J* = 7.6 Hz, 2H), 7.35 (d, *J* = 6.1 Hz, 2H), 6.78 (s, 2H), 6.30 (s, 1H), 3.76 – 3.74 (m, 4H), 3.03 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 171.6, 159.6, 157.6, 146.4, 136.3, 133.3, 130.6, 130.4, 128.1, 120.4, 116.2, 107.7, 66.6, 49.9.

HRMS (ESI, *m/z*) calcd for C₂₀H₂₀N₄OS, [M+H]⁺: 365.1431; found: 365.1449.

3c



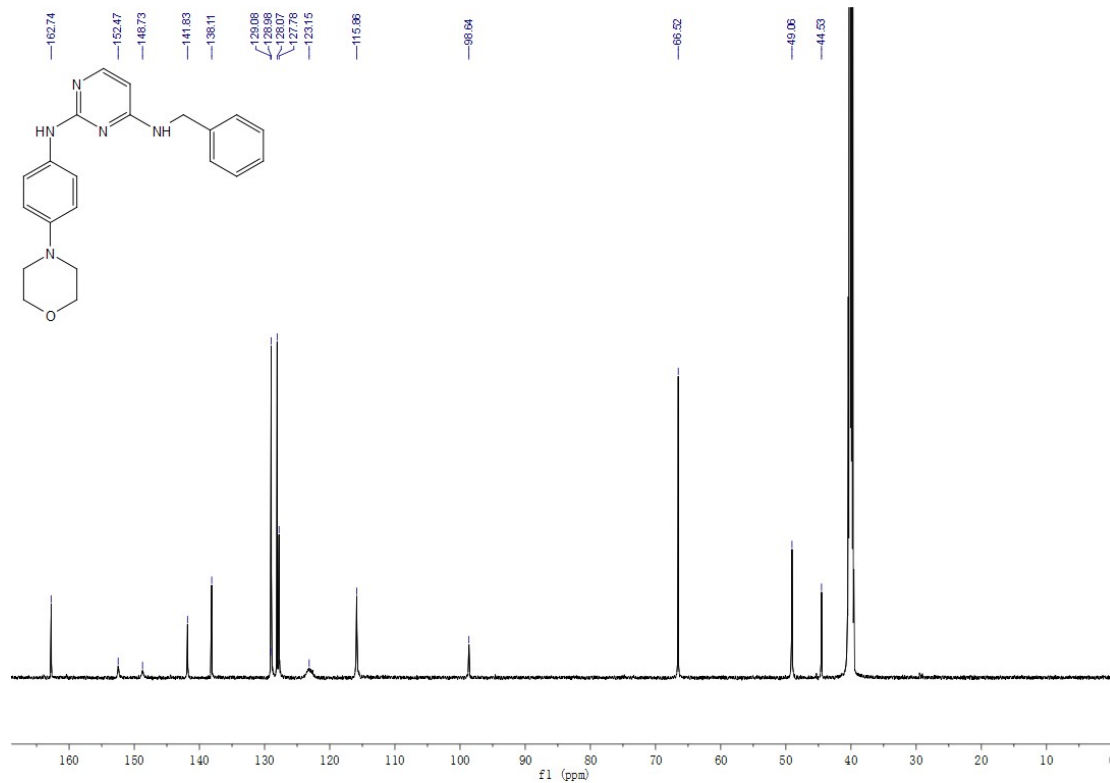
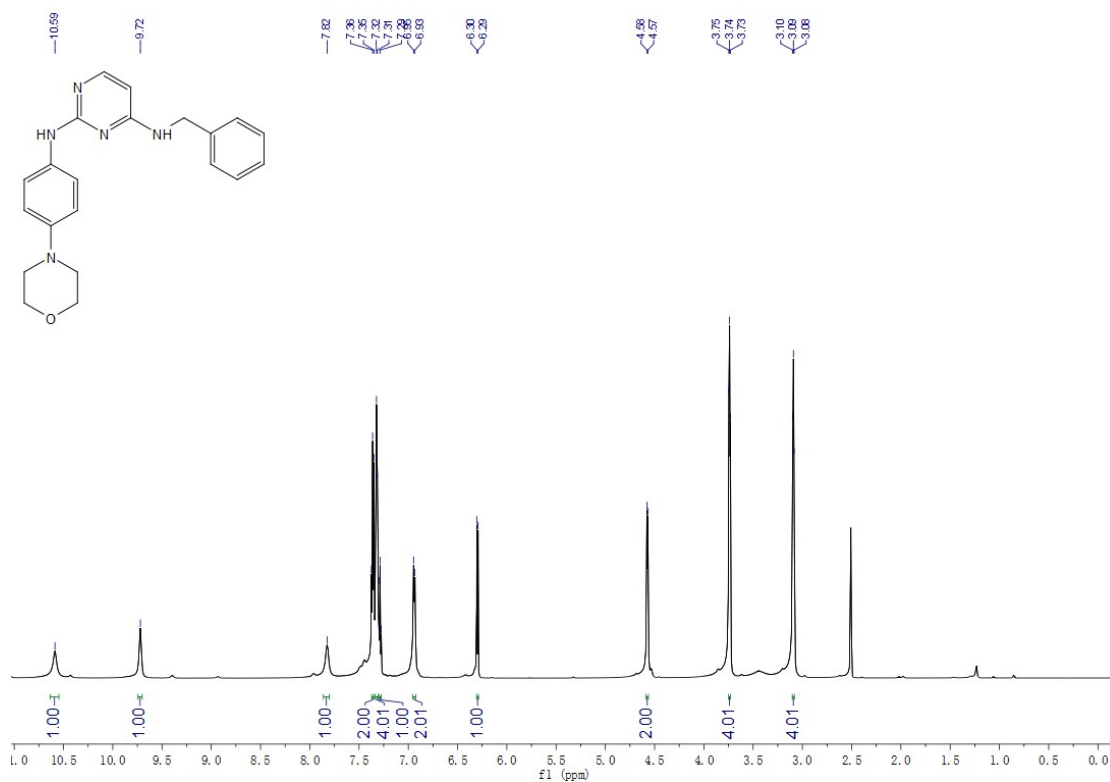


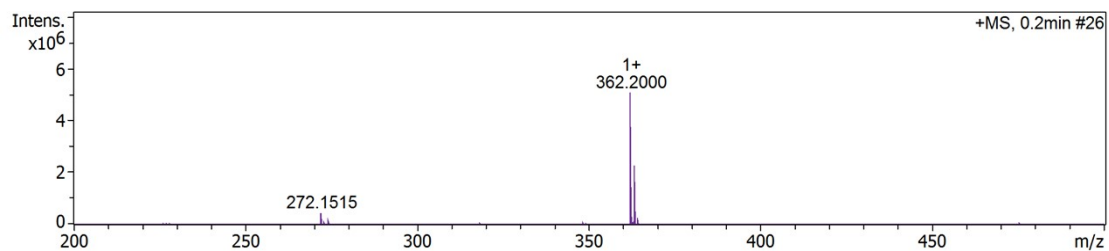
¹H NMR (600 MHz, DMSO-*d*₆) δ 11.18 (s, 1H), 10.57 (s, 1H), 7.95 (s, 1H), 7.69 (s, 2H), 7.34 (s, 4H), 7.17 (s, 1H), 7.00 (s, 2H), 6.55 (s, 1H), 3.76 (s, 4H), 3.13 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 161.3, 153.1, 149.2, 143.1, 138.1, 129.2, 128.4, 125.3, 124.7, 122.0, 115.9, 99.9, 66.5, 49.1.

HRMS (ESI, *m/z*) calcd for C₂₀H₂₁N₅O, [M+H]⁺: 348.1819; found: 348.1851.

3d



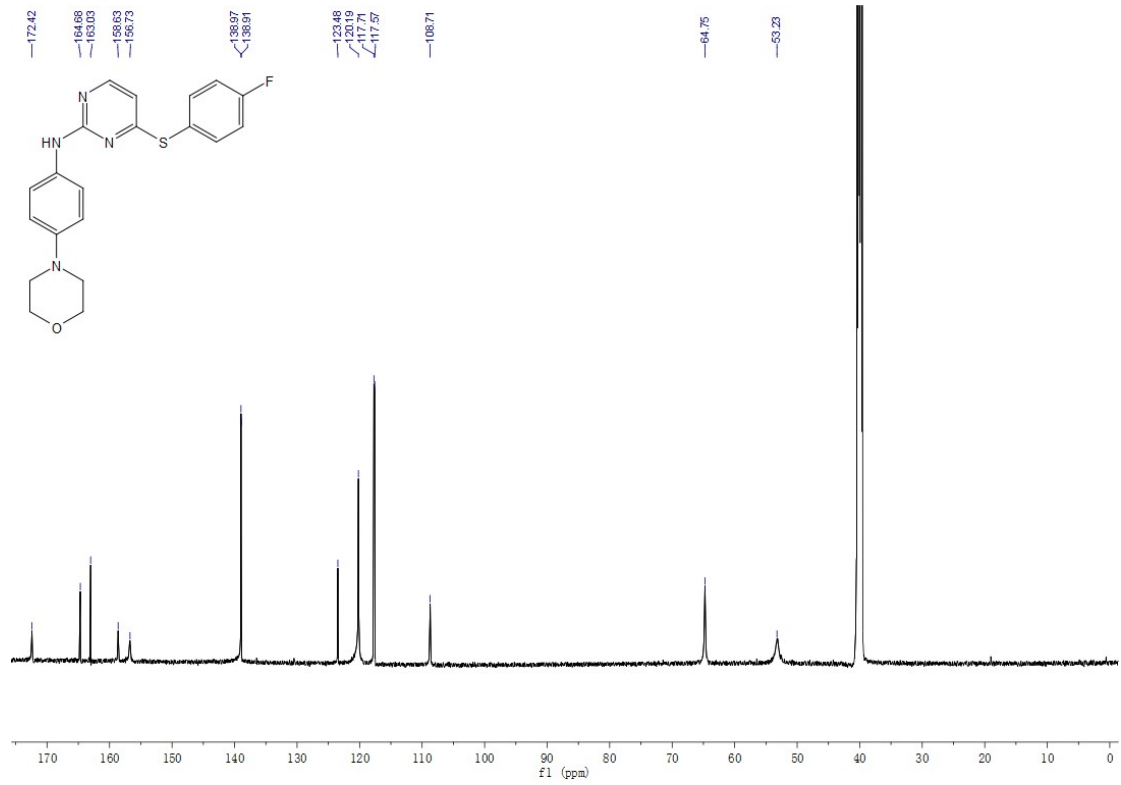
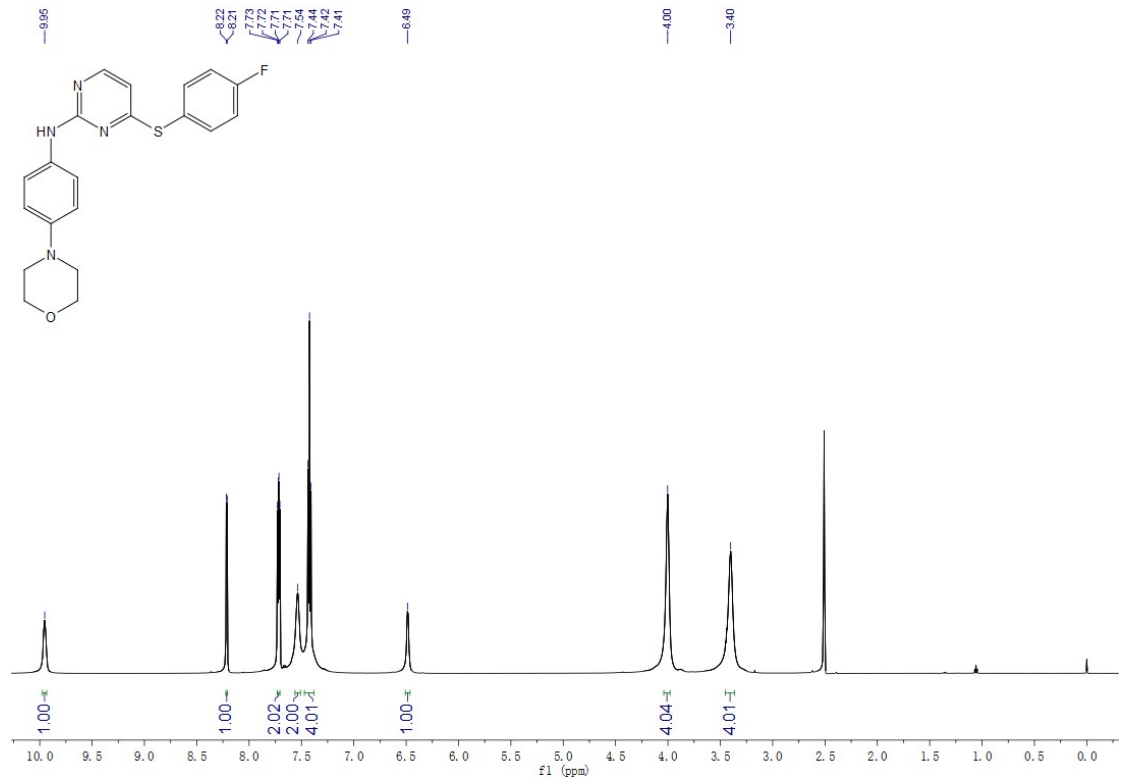


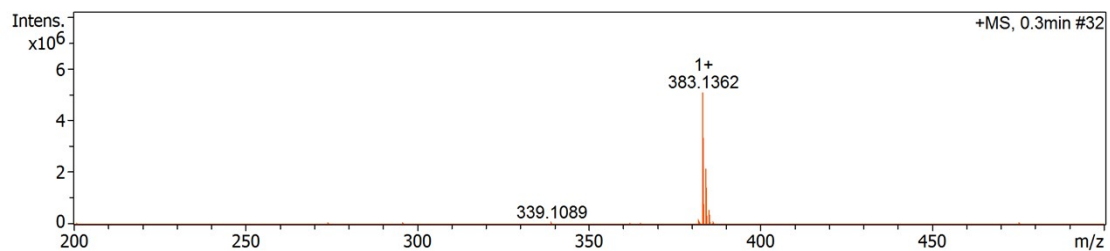
¹H NMR (600 MHz, DMSO-*d*₆) δ 10.59 (s, 1H), 9.72 (s, 1H), 7.82 (s, 1H), 7.36 (s, 2H), 7.32 (d, *J* = 5.4 Hz, 4H), 7.29 (t, *J* = 7.2 Hz, 1H), 6.94 (d, *J* = 8.3 Hz, 2H), 6.30 (d, *J* = 7.2 Hz, 1H), 4.57 (d, *J* = 5.6 Hz, 2H), 3.75 – 3.73 (m, 4H), 3.10 – 3.08 (m, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 162.7, 152.5, 148.7, 141.8, 138.1, 129.1, 129.0, 128.1, 127.8, 123.2, 115.9, 98.6, 66.5, 49.1, 44.5.

HRMS (ESI, *m/z*) calcd for C₂₁H₂₃N₅O, [M+H]⁺: 362.1975; found: 362.2000.

3e



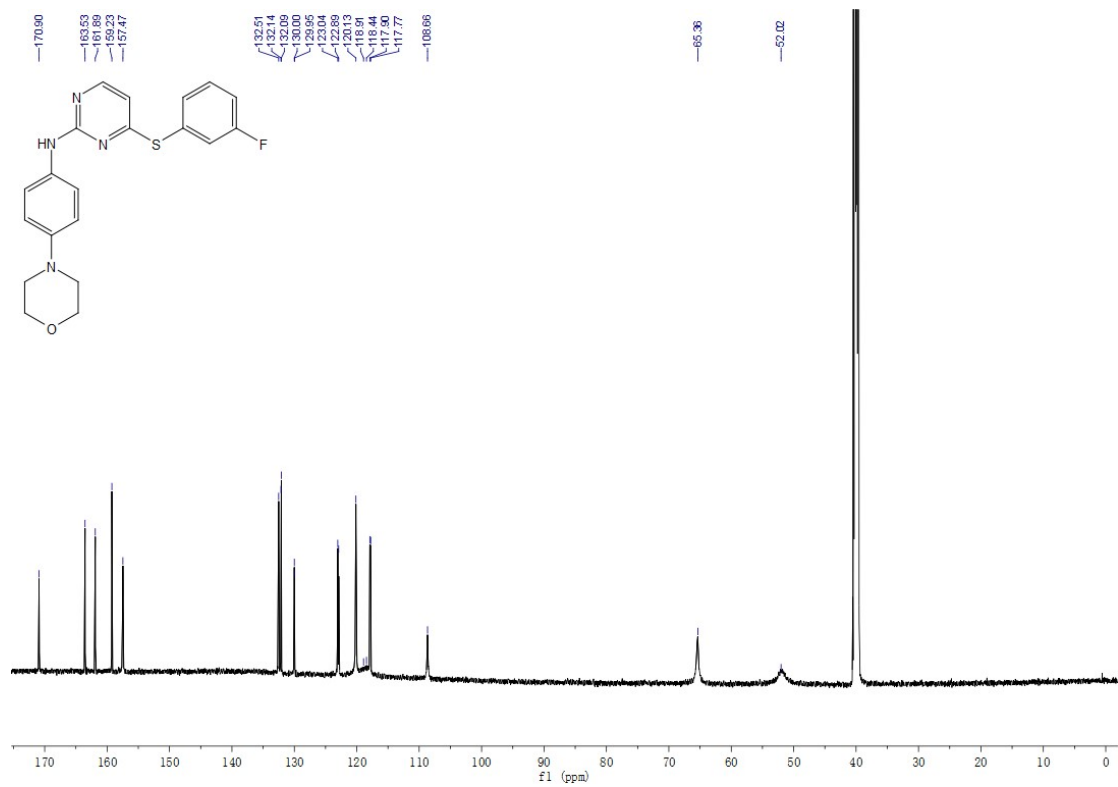
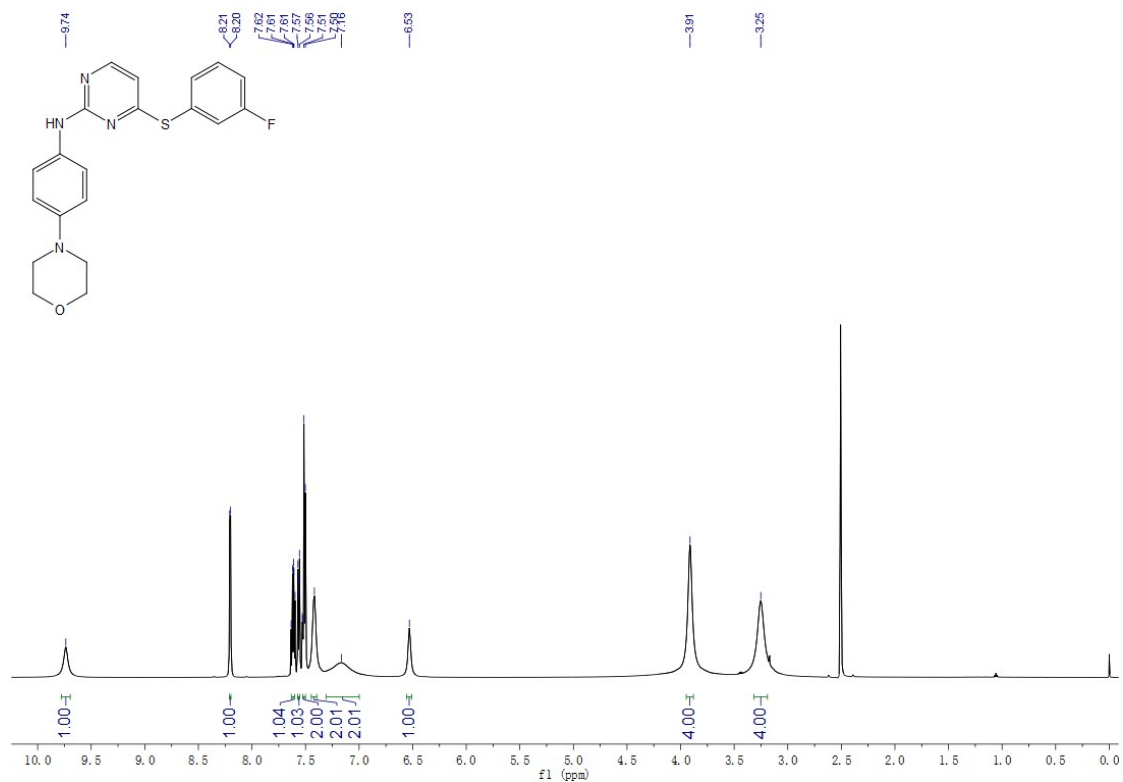


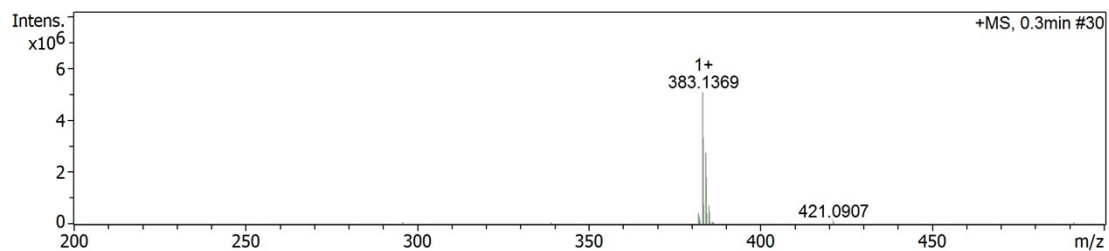
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.95 (s, 1H), 8.21 (d, *J* = 5.4 Hz, 1H), 7.72 (dd, *J* = 8.6, 5.4 Hz, 2H), 7.54 (s, 2H), 7.42 (t, *J* = 8.8 Hz, 4H), 6.49 (s, 1H), 4.00 (s, 4H), 3.40 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 172.4, 164.7, 163.0, 158.6, 156.7, 138.94 (d, *J* = 8.8 Hz), 130.5, 123.5, 120.2, 117.7, 117.6, 108.7, 64.8, 53.2.

HRMS (ESI, *m/z*) calcd for C₂₀H₁₉FN₄OS, [M+H]⁺: 383.1336; found: 383.1362.

3f



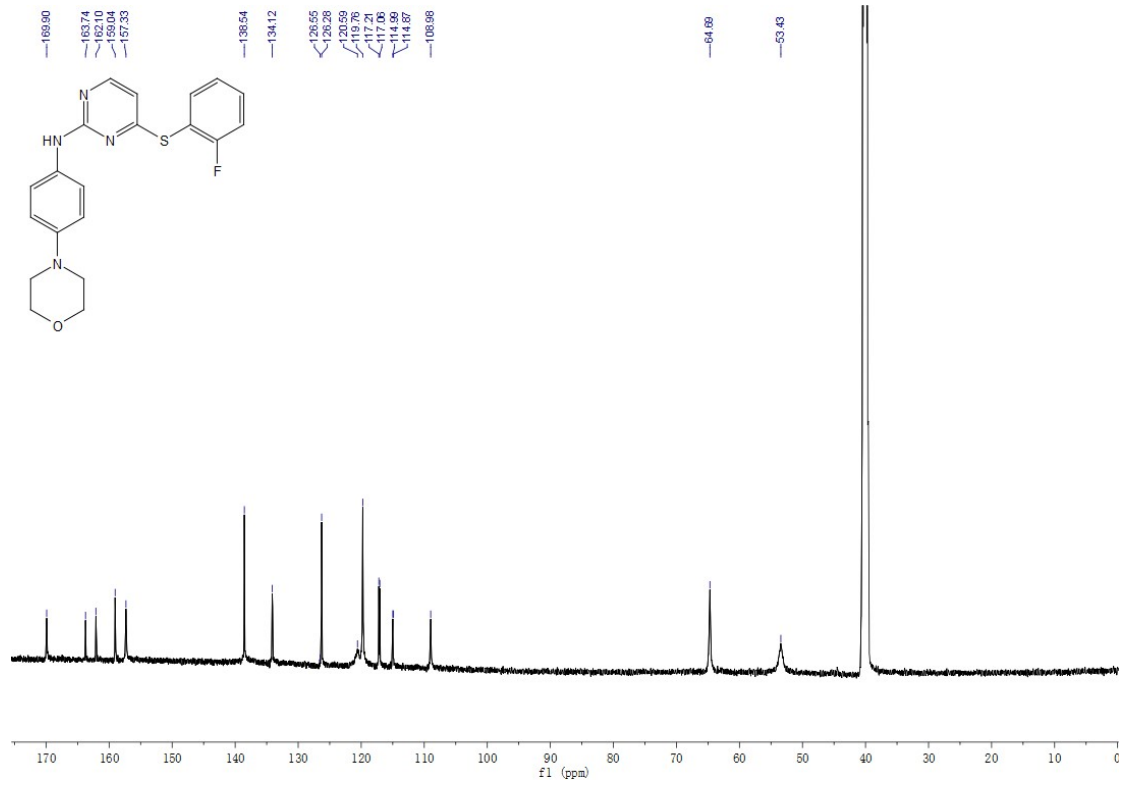
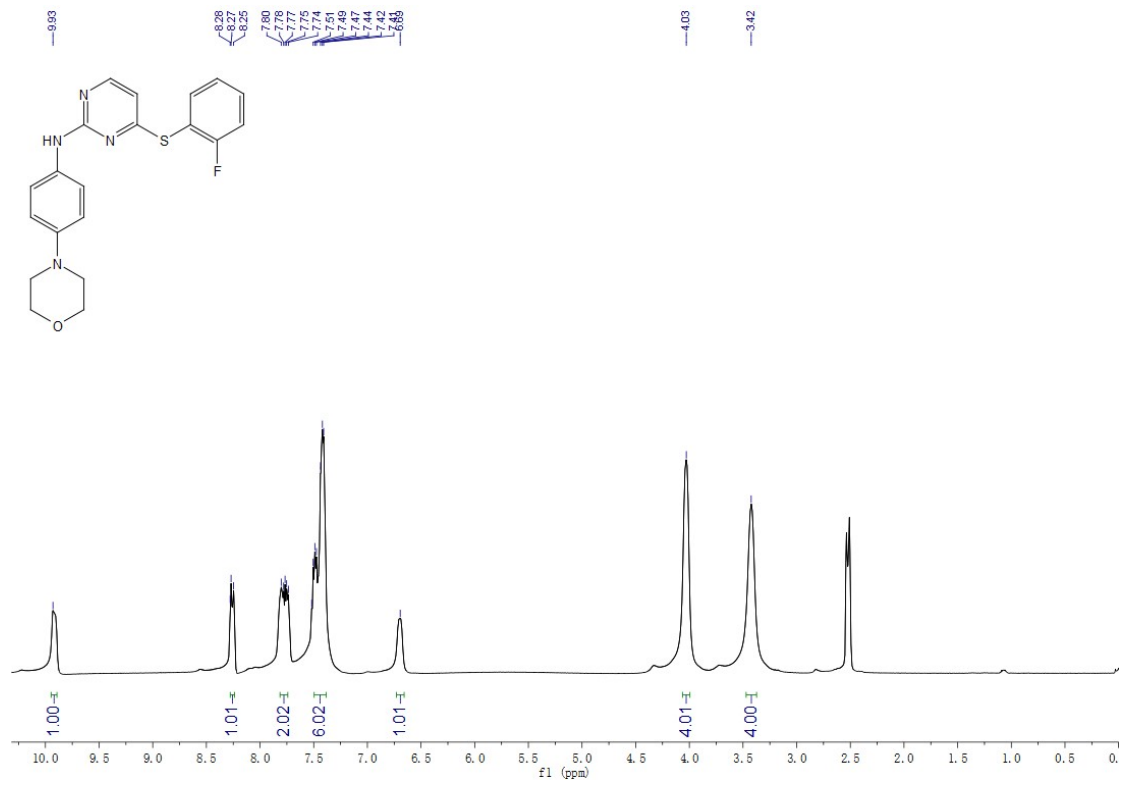


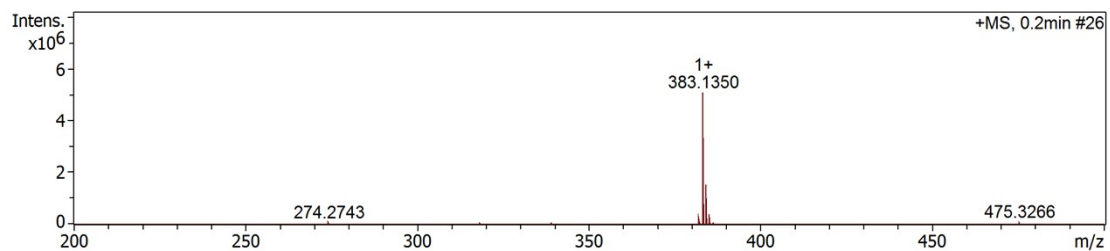
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.74 (s, 1H), 8.20 (d, *J* = 5.3 Hz, 1H), 7.63 – 7.60 (m, 1H), 7.57-7.55 (m, 1H), 7.53-7.50 (m, 2H), 7.42 (s, 2H), 7.16 (s, 2H), 6.53 (s, 1H), 3.91 (s, 4H), 3.25 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 170.9, 163.5, 161.9, 159.2, 157.5, 132.5, 132.1 (d, *J* = 8.2 Hz), 130.0 (d, *J* = 8.2 Hz), 123.0 (d, *J* = 10.1 Hz), 120.1, 118.9, 118.4, 117.8 (d, *J* = 20.9 Hz), 108.7, 65.4, 52.0.

HRMS (ESI, *m/z*) calcd for C₂₀H₁₉FN₄OS, [M+H]⁺: 383.1336; found: 383.1369.

3g



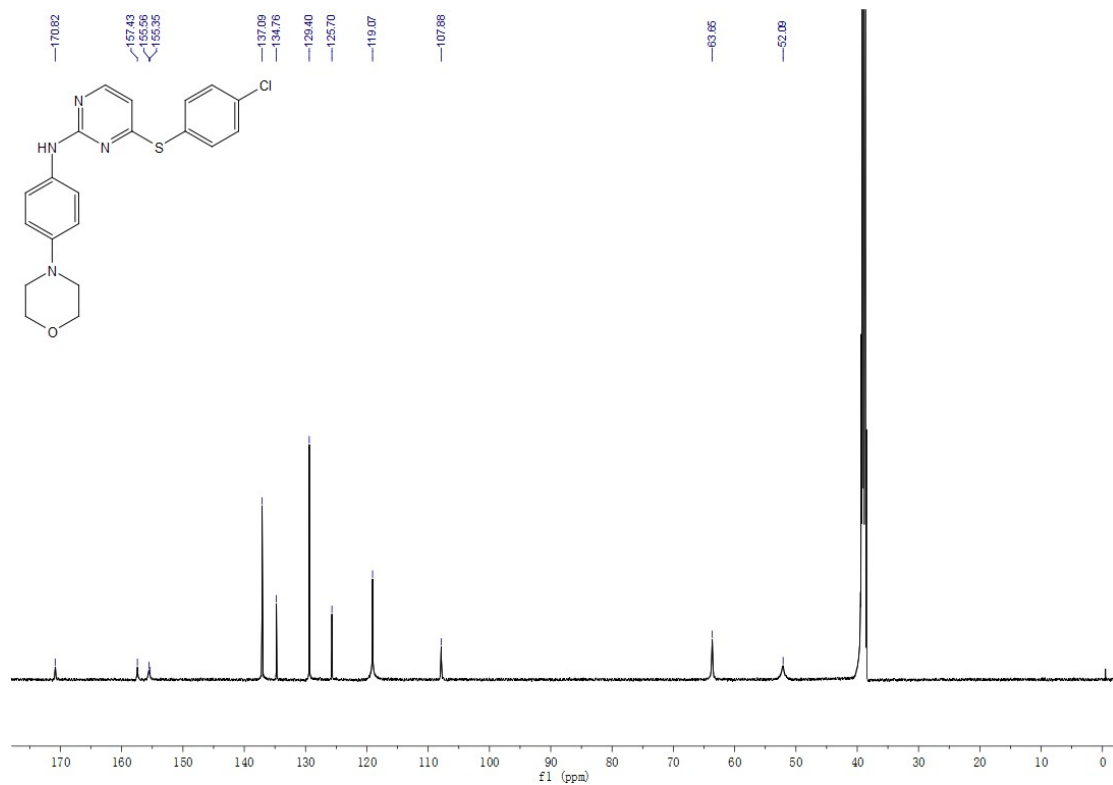
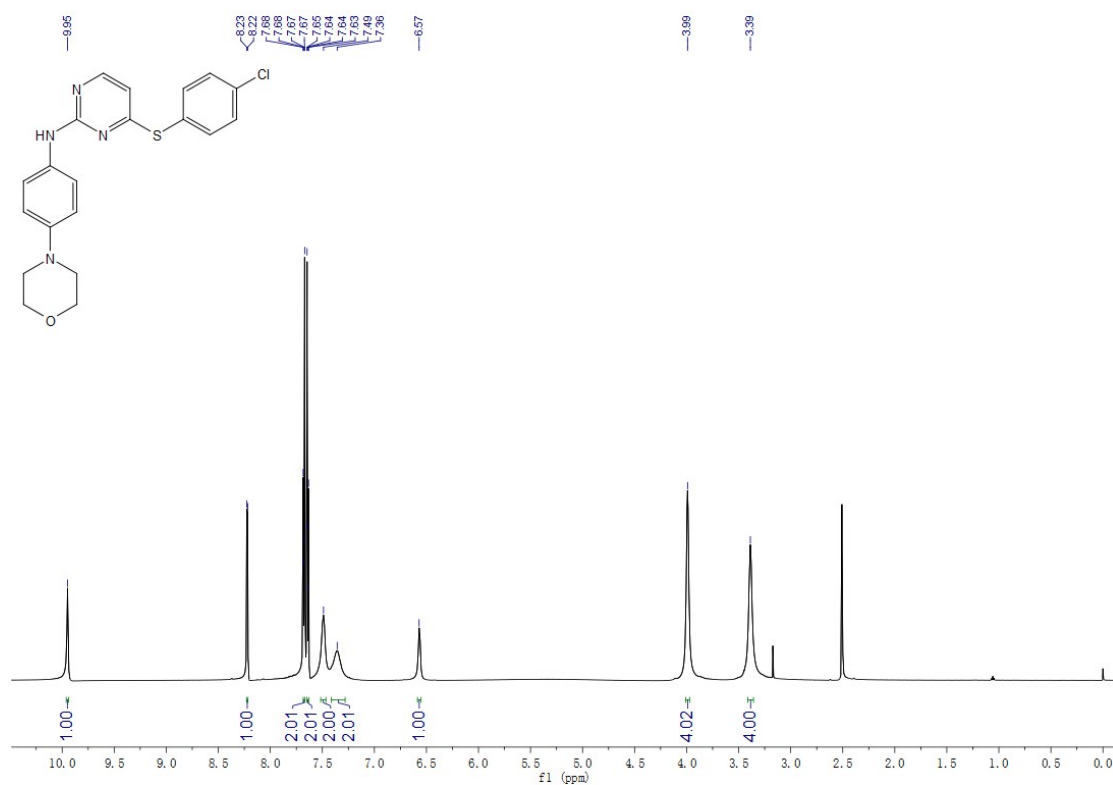


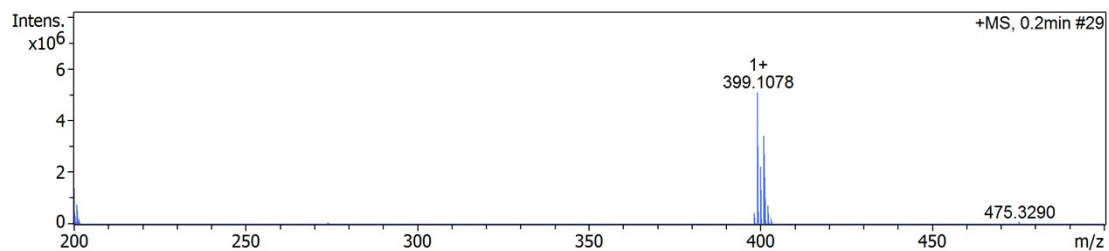
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.93 (s, 1H), 8.26 (d, *J* = 14.1 Hz, 1H), 7.80-7.74 (m 2H), 7.52 – 7.41 (m, 6H), 6.69 (s, 1H), 4.03 (s, 4H), 3.42 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 169.9, 163.7, 162.1, 159.0, 157.3, 138.5, 134.1, 126.3, 120.6, 119.8, 117.2(d, *J* = 22.6 Hz), 114.9 (d, *J* = 18.2 Hz), 108.9, 64.7, 53.4.

HRMS (ESI, *m/z*) calcd for C₂₀H₁₉FN₄OS, [M+H]⁺: 383.1336; found: 383.1350.

3h



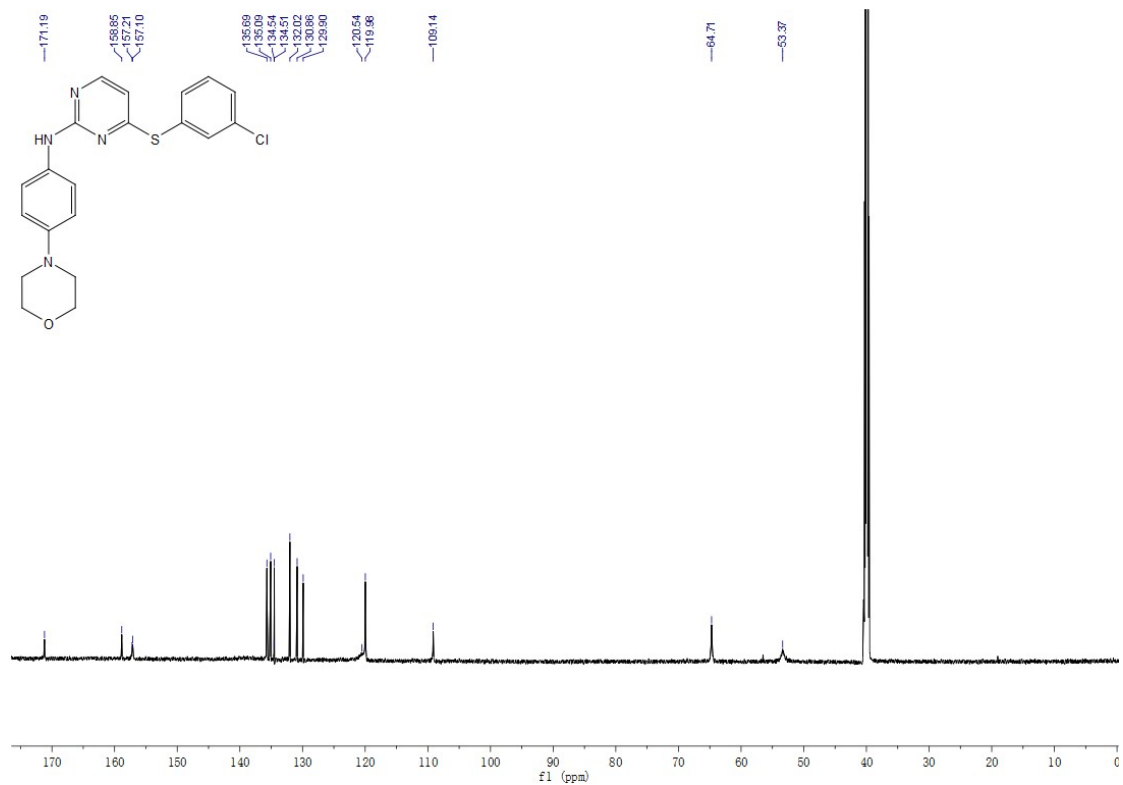
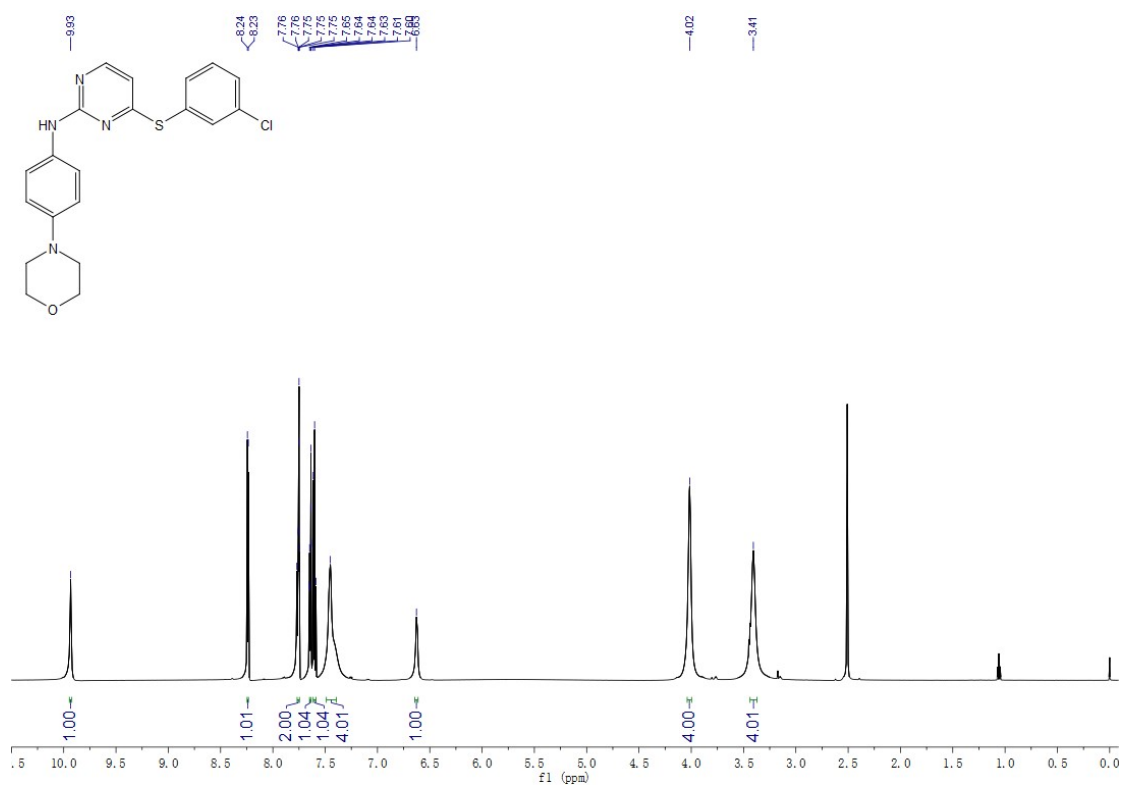


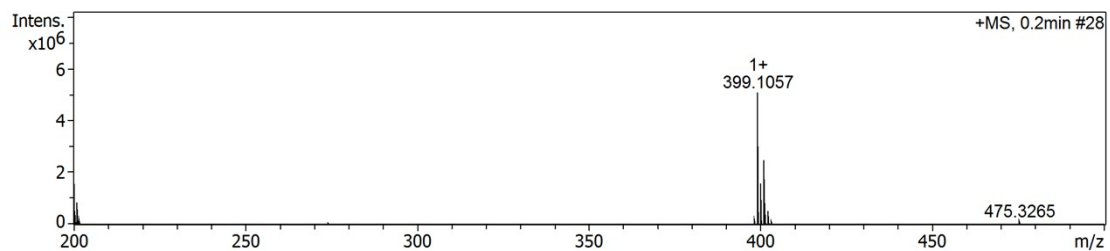
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.95 (s, 1H), 8.22 (d, *J* = 5.5 Hz, 1H), 7.68 – 7.67 (m, 2H), 7.65 – 7.63 (m, 2H), 7.49 (s, 2H), 7.36 (s, 2H), 6.57 (s, 1H), 3.99 (s, 4H), 3.39 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 170.8, 157.4, 155.6, 155.4, 137.1, 134.8, 129.4, 125.7, 119.1, 107.9, 63.7, 52.1.

HRMS (ESI, *m/z*) calcd for C₂₀H₁₉ClN₄OS, [M+H]⁺: 399.1041; found: 399.1078.

3i



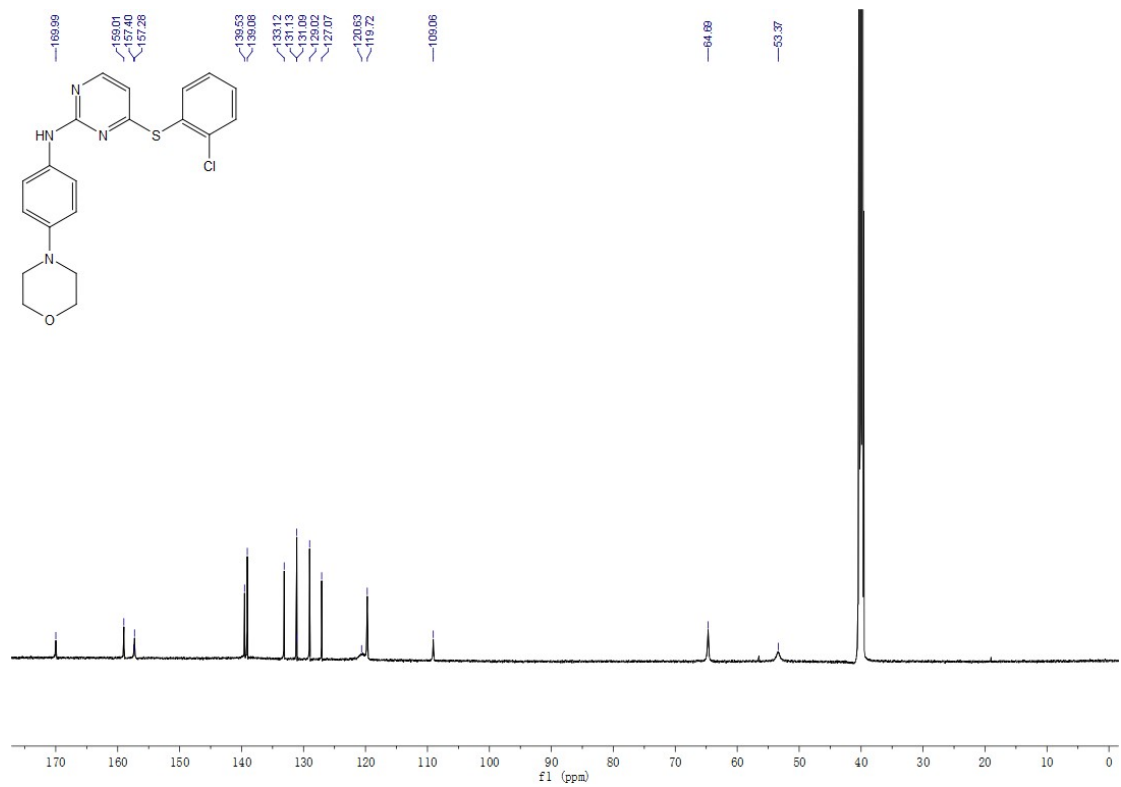
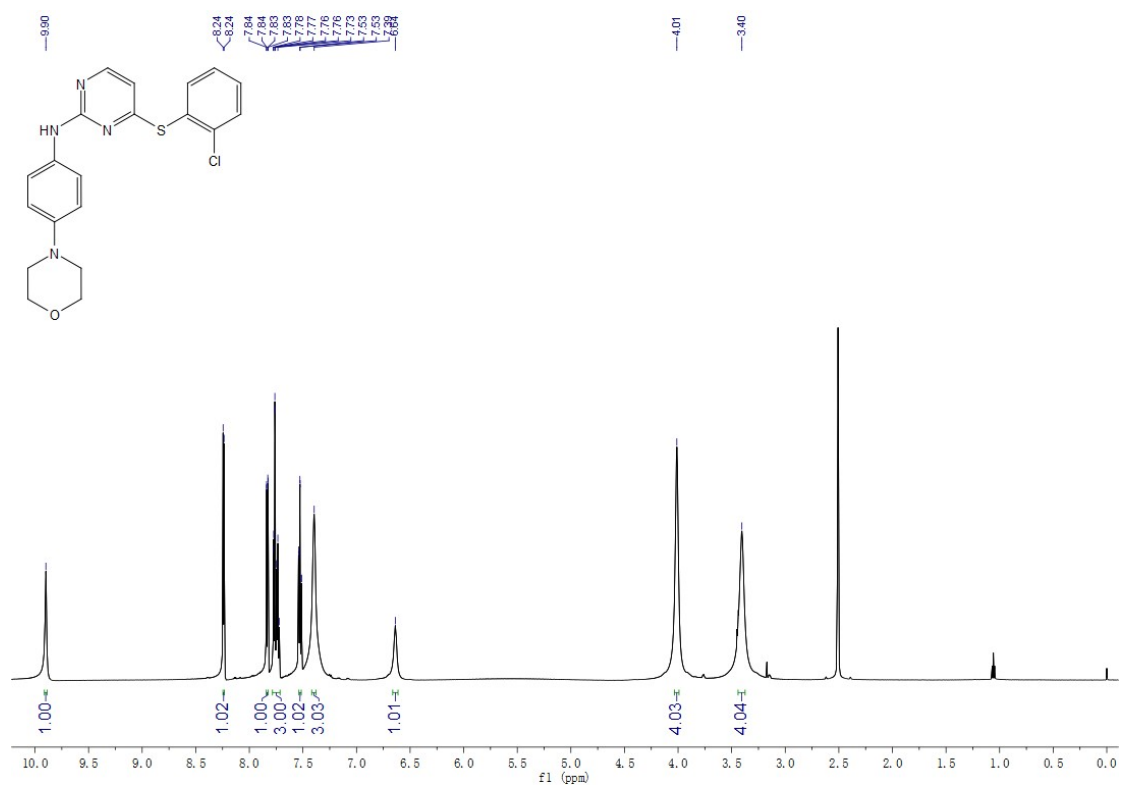


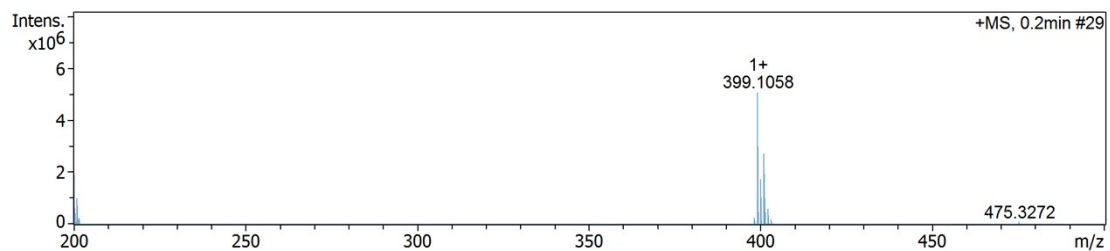
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.93 (s, 1H), 8.24 (d, *J* = 5.4 Hz, 1H), 7.77 – 7.75 (m, 2H), 7.64 (dd, *J* = 6.4, 1.4 Hz, 1H), 7.61 (d, *J* = 7.8 Hz, 1H), 7.45 (s, 4H), 6.63 (s, 1H), 4.02 (s, 4H), 3.41 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 171.2, 158.9, 157.2, 157.1, 135.7, 135.1, 134.6, 134.5, 132.0, 130.9, 129.9, 120.5, 119.9, 109.1, 64.7, 53.4.

HRMS (ESI, *m/z*) calcd for C₂₀H₁₉ClN₄OS, [M+H]⁺: 399.1041; found: 399.1057.

3j



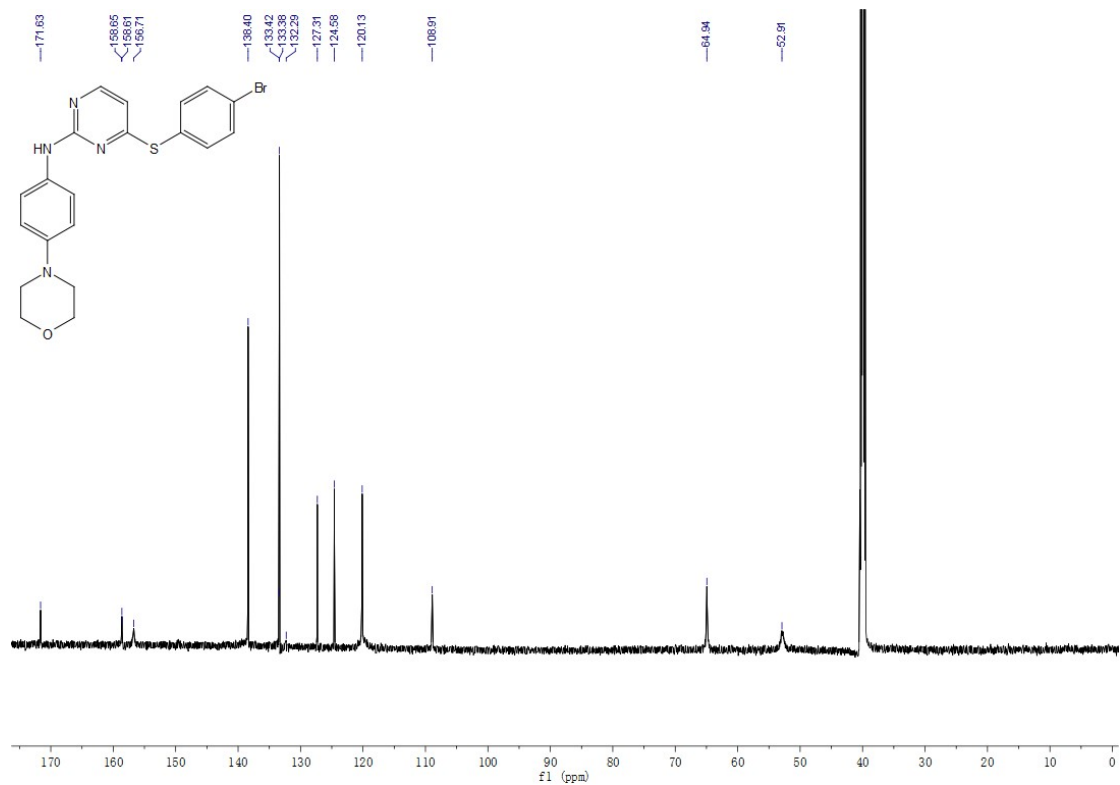
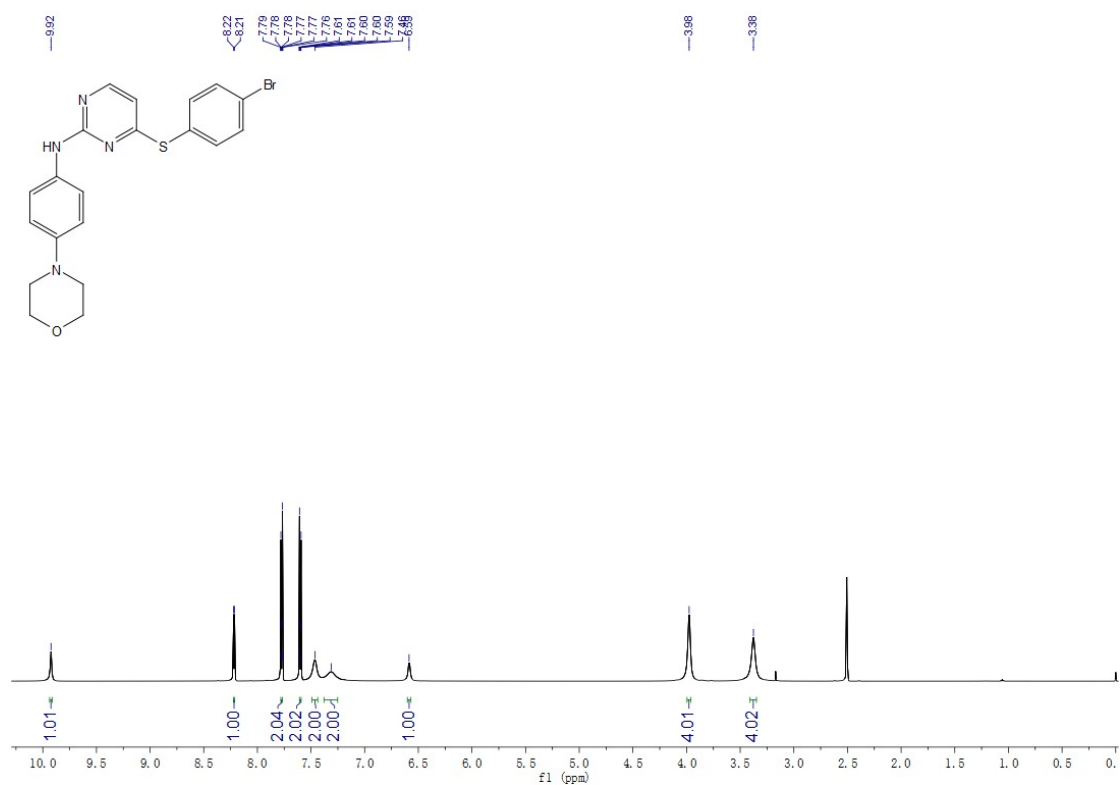


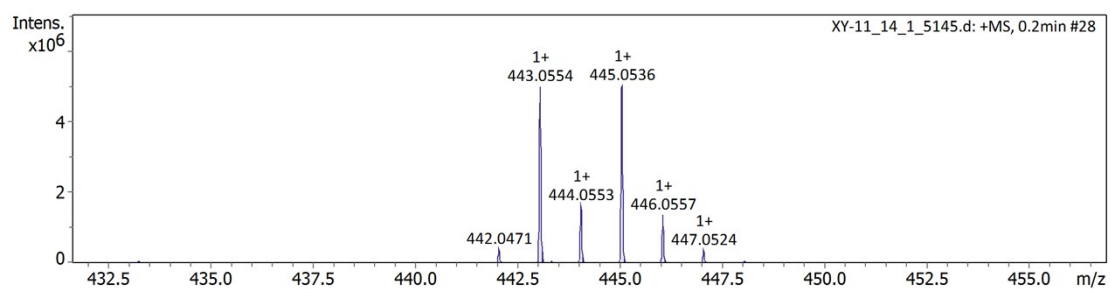
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.90 (s, 1H), 8.24 (d, *J* = 5.4 Hz, 1H), 7.84 – 7.83 (m, 1H), 7.78 – 7.71 (m, 3H), 7.54 – 7.52 (m, 1H), 7.39 (s, 3H), 6.64 (s, 1H), 4.01 (s, 4H), 3.40 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 167.0, 159.0, 157.4, 157.3, 139.5, 139.1, 133.1, 131.1, 131.0, 129.0, 127.1, 120.6, 119.7, 109.1, 64.7, 53.4.

HRMS (ESI, *m/z*) calcd for C₂₀H₁₉ClN₄OS, [M+H]⁺: 399.1041; found: 399.1058.

3k

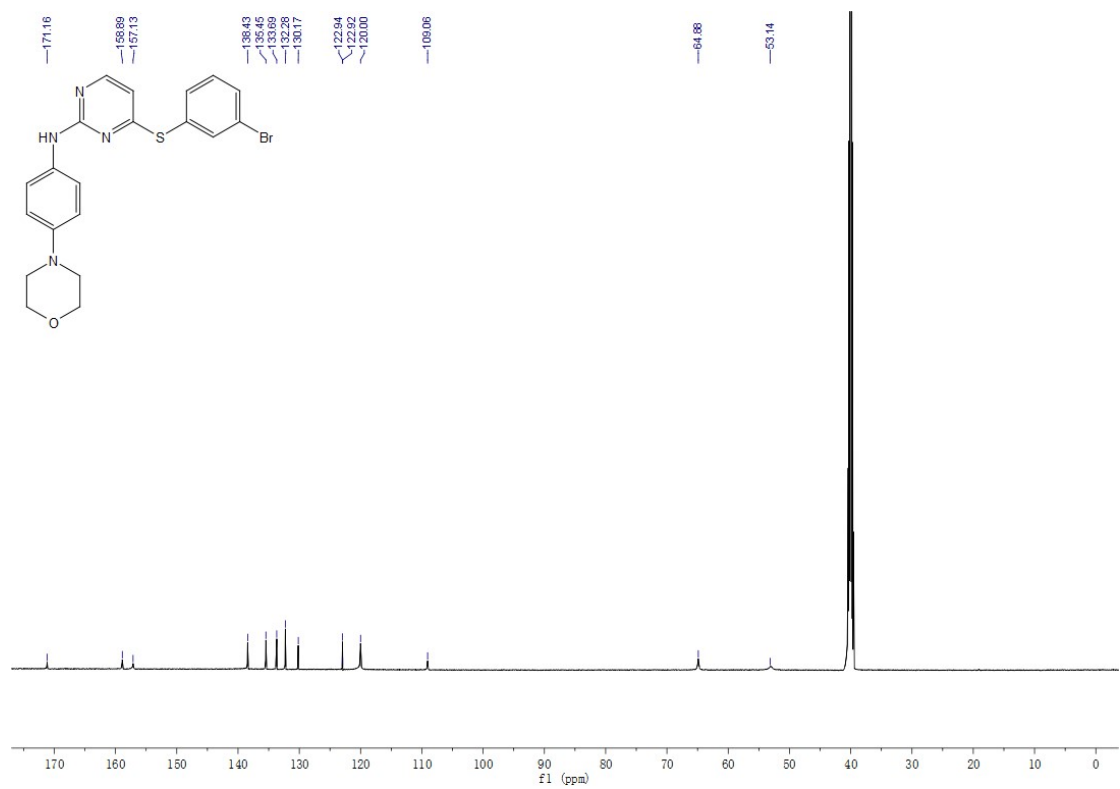
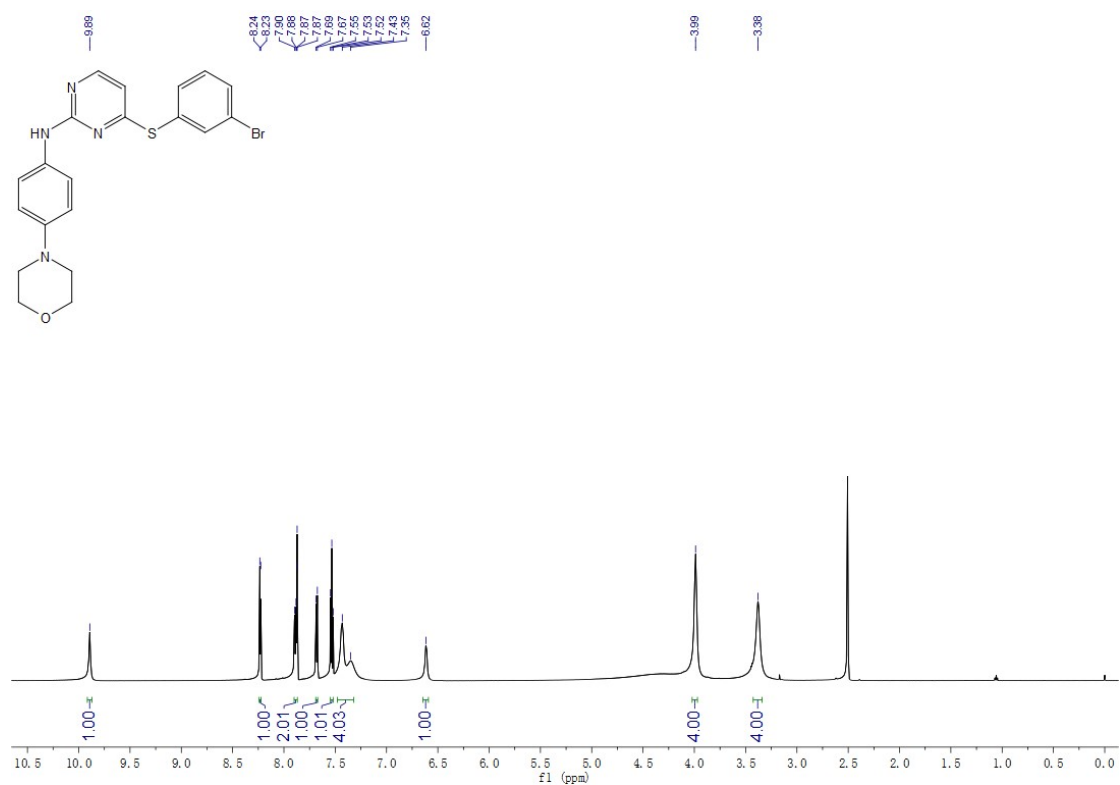


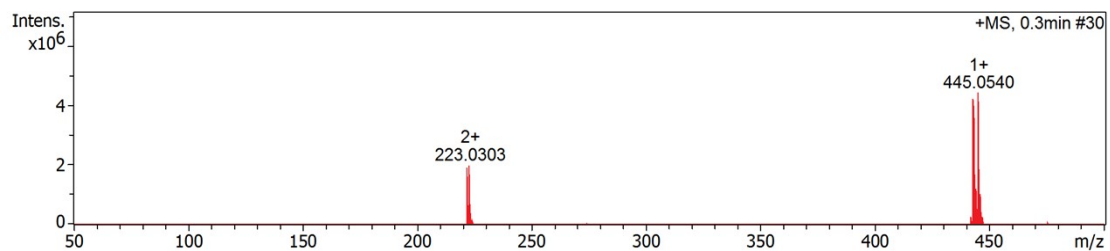


^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 9.92 (s, 1H), 8.22 (d, $J = 5.4$ Hz, 1H), 7.78 – 7.77 (m, 2H), 7.61 – 7.59 (m, 2H), 7.46 (s, 2H), 7.31 (s, 2H), 6.59 (s, 1H), 3.98 (s, 4H), 3.38 (s, 4H).

^{13}C NMR (151 MHz, $\text{DMSO-}d_6$) δ 171.6, 158.7, 158.6, 156.7, 138.4, 133.4, 133.3, 132.3, 127.3, 124.6, 120.1, 108.9, 64.9, 52.9.

HRMS (ESI, m/z) calcd for $\text{C}_{20}\text{H}_{19}\text{BrN}_4\text{OS}$, $[\text{M}+\text{H}]^+$: 443.0536, 445.0517; found: 443.0554, 445.0536.



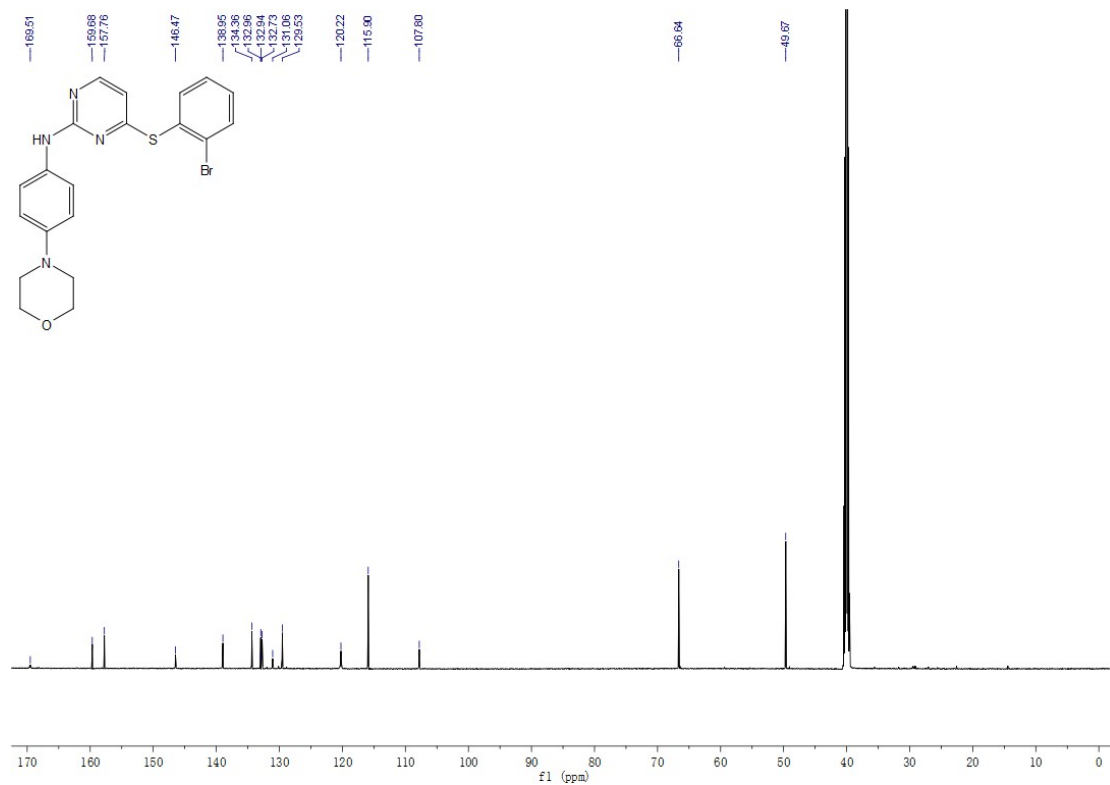
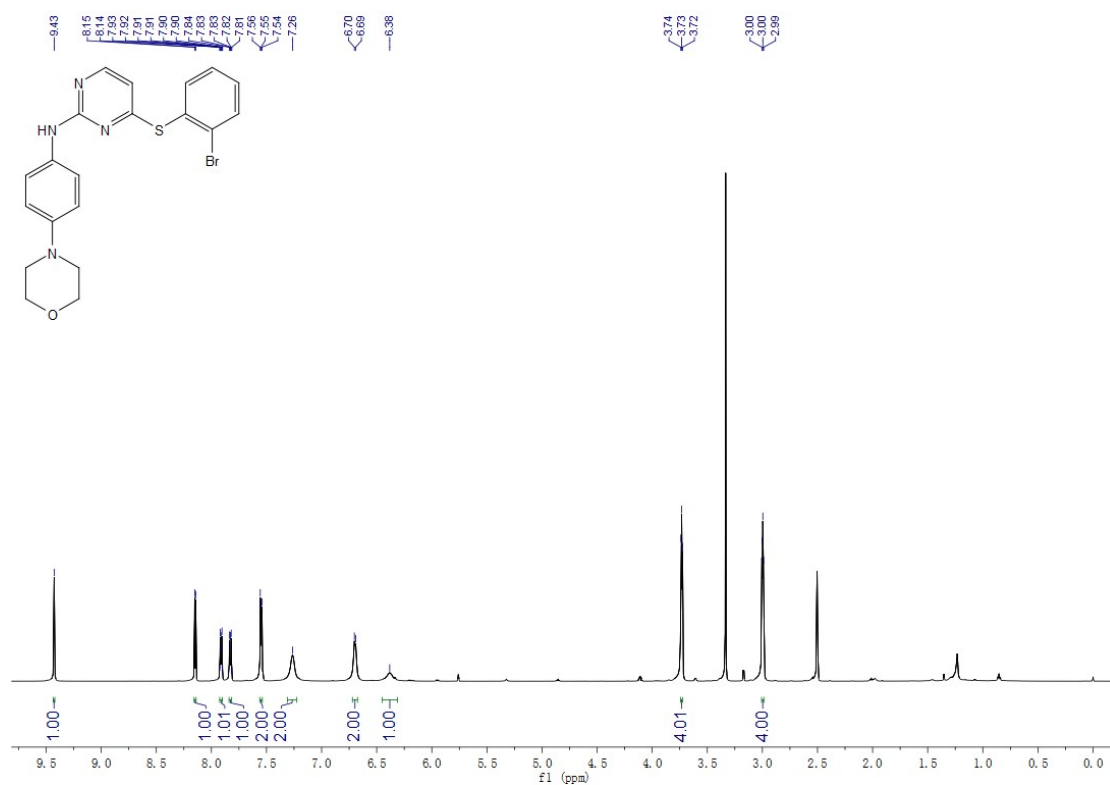


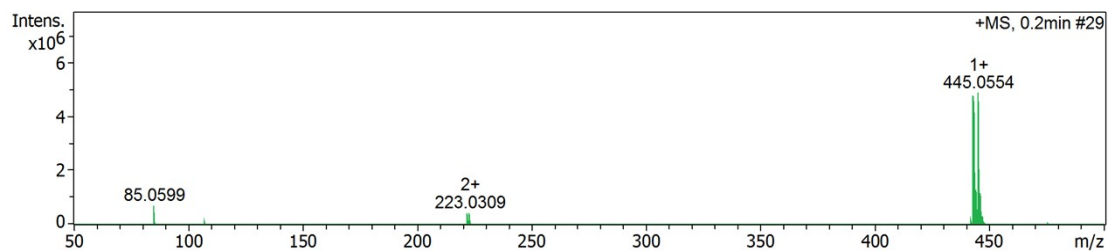
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.89 (s, 1H), 8.23 (d, *J* = 5.4 Hz, 1H), 7.88 (dd, *J* = 9.7, 4.9 Hz, 2H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.54 (d, *J* = 7.9 Hz, 1H), 7.39 (d, *J* = 49.3 Hz, 4H), 6.62 (s, 1H), 3.99 (s, 4H), 3.38 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 171.2, 158.9, 157.1, 138.4, 135.5, 133.7, 132.3, 130.2, 122.9, 122.9, 120.0, 109.1, 64.9, 53.14.

HRMS (ESI, *m/z*) calcd for C₂₀H₁₉BrN₄OS, [M+H]⁺: 445.0517; found: 445.0540.

3m



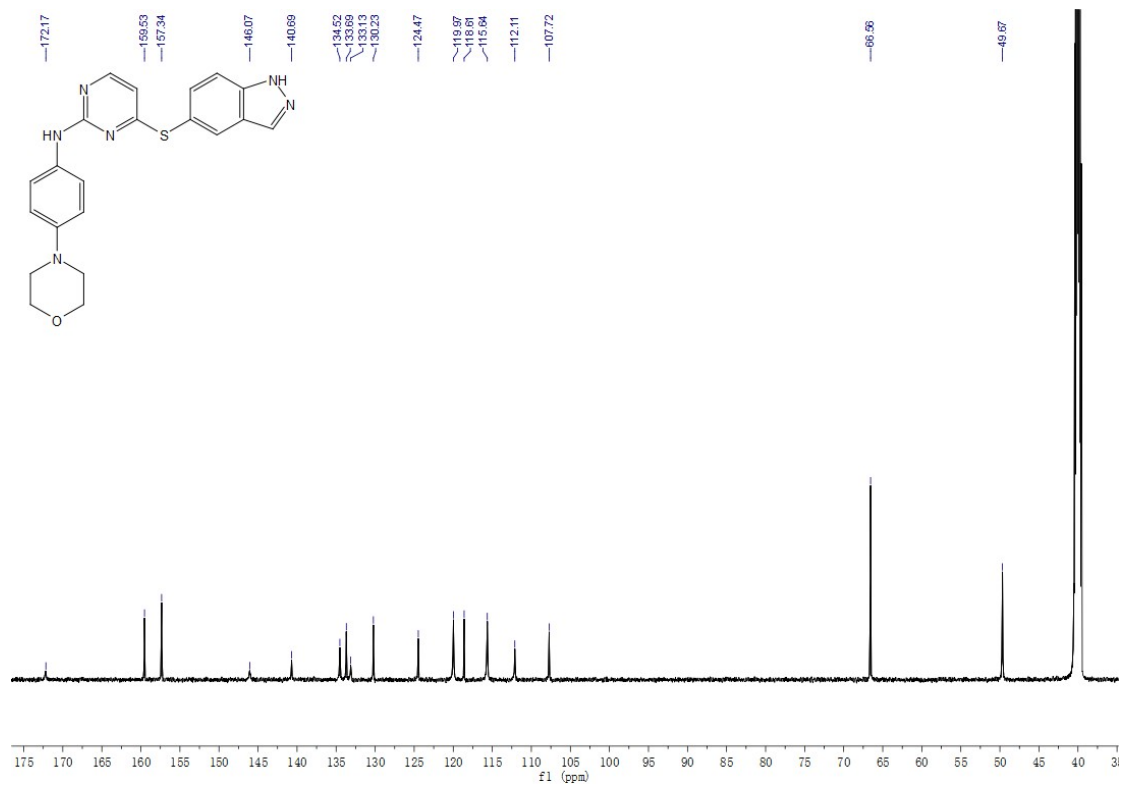
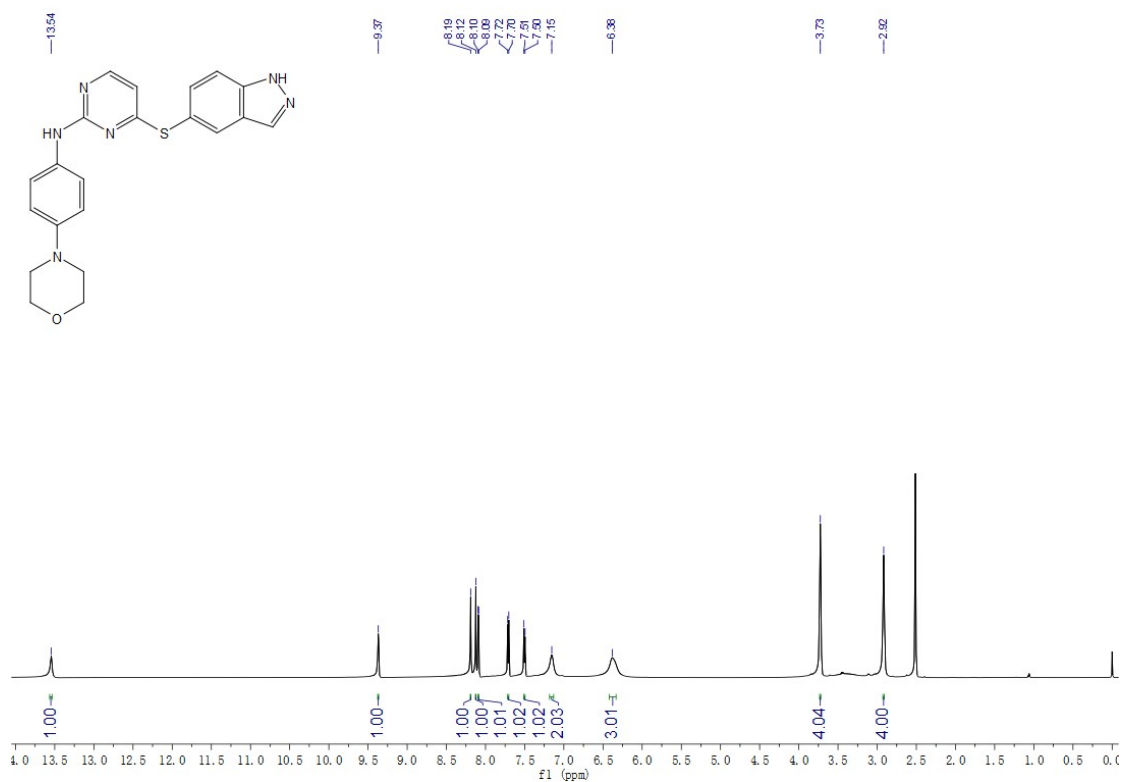


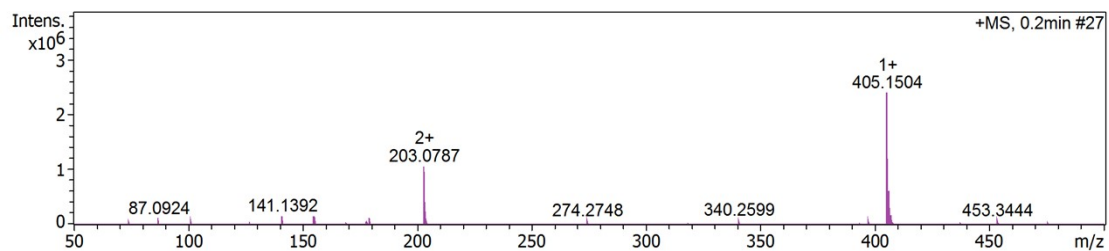
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.43 (s, 1H), 8.15 (d, *J* = 5.3 Hz, 1H), 7.91 (dd, *J* = 5.8, 3.5 Hz, 1H), 7.83 (dd, *J* = 5.8, 3.5 Hz, 1H), 7.56 – 7.54 (m, 2H), 7.26 (s, 2H), 6.70 (d, *J* = 7.3 Hz, 2H), 6.38 (s, 1H), 3.74 – 3.72 (m, 4H), 3.01 – 2.99 (m, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 169.5, 159.7, 157.8, 146.5, 138.9, 134.4, 133.0, 132.9, 132.8, 131.1, 129.5, 120.2, 115.9, 107.8, 66.6, 49.7.

HRMS (ESI, *m/z*) calcd for C₂₀H₁₉BrN₄OS, [M+H]⁺: 445.0517; found: 445.0554.

3n



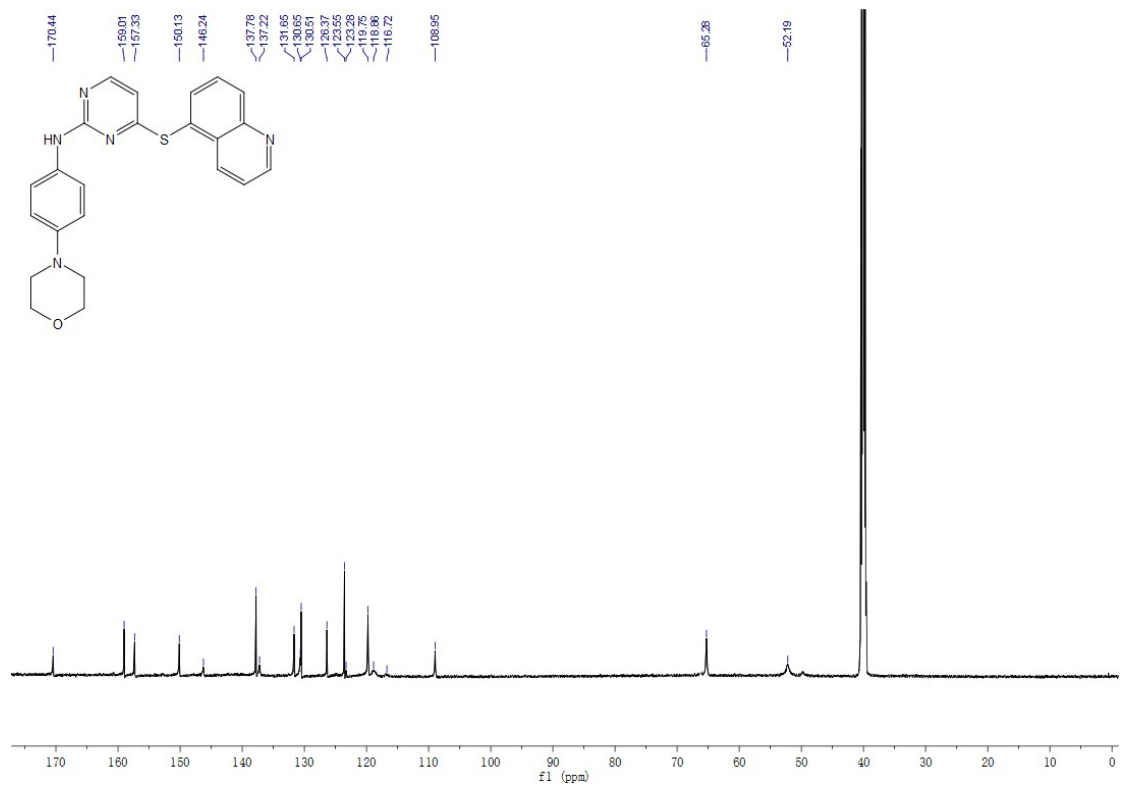
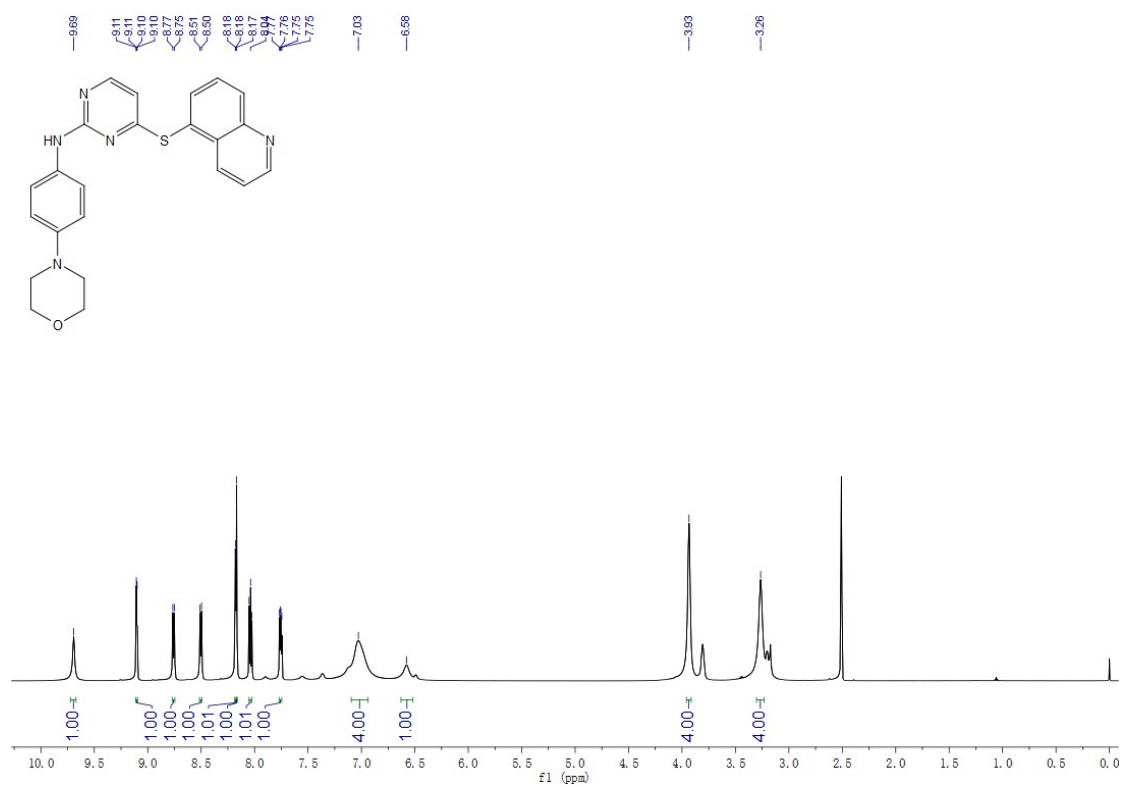


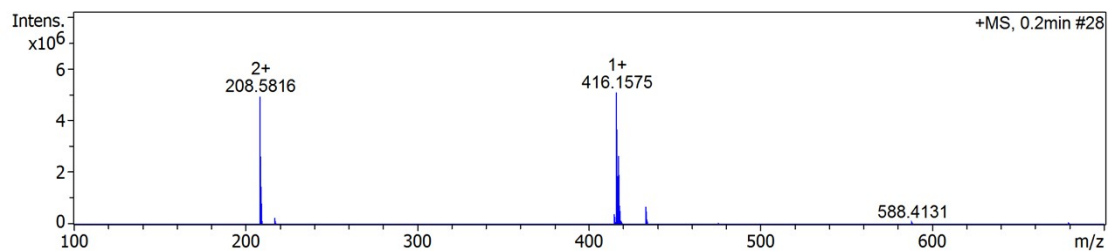
¹H NMR (600 MHz, DMSO-*d*₆) δ 13.54 (s, 1H), 9.37 (s, 1H), 8.19 (s, 1H), 8.12 (s, 1H), 8.09 (d, *J* = 5.3 Hz, 1H), 7.71 (d, *J* = 8.6 Hz, 1H), 7.50 (d, *J* = 8.6 Hz, 1H), 7.15 (s, 2H), 6.38 (s, 3H), 3.73 (s, 4H), 2.92 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 172.3, 159.5, 157.3, 146.1, 140.7, 134.5, 133.7, 133.1, 130.2, 124.5, 120.0, 118.6, 115.6, 112.1, 107.7, 66.6, 49.7.

HRMS (ESI, *m/z*) calcd for C₂₁H₂₀N₆OS, [M+H]⁺: 405.1492; found: 405.1504.

3o



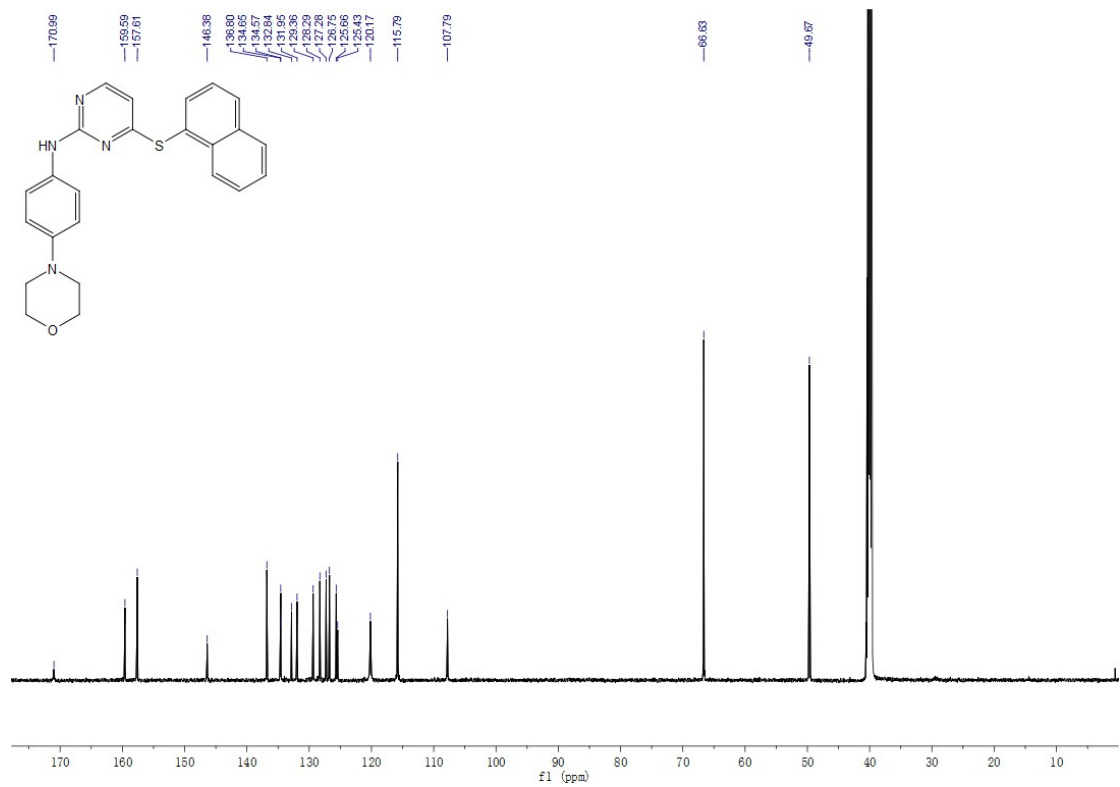
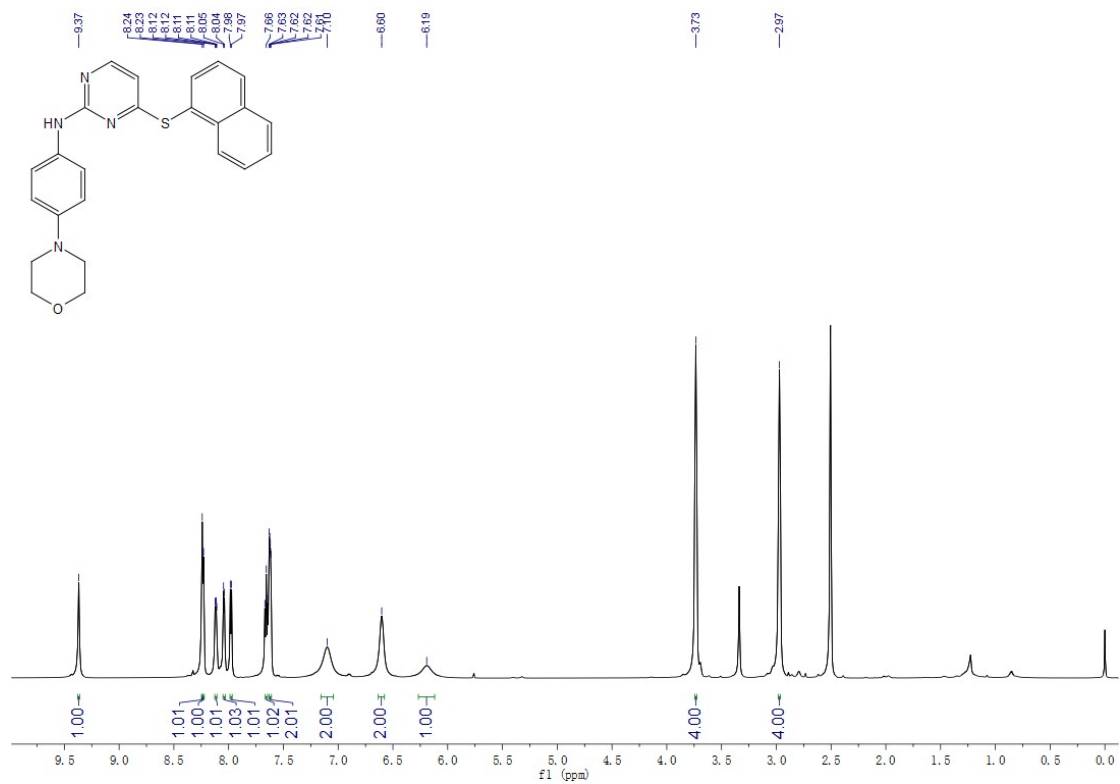


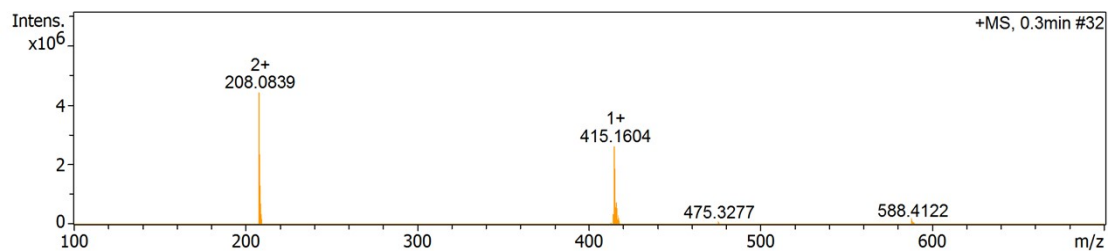
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.69 (s, 1H), 9.10 (dd, *J* = 4.3, 1.2 Hz, 1H), 8.76 (d, *J* = 8.5 Hz, 1H), 8.50 (d, *J* = 8.5 Hz, 1H), 8.18 (d, *J* = 1.7 Hz, 1H), 8.17 (s, 1H), 8.05 – 8.02 (m, 1H), 7.76 (dd, *J* = 8.6, 4.4 Hz, 1H), 7.03 (s, 4H), 6.58 (s, 1H), 3.93 (s, 4H), 3.26 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 170.4, 159.0, 157.3, 150.1, 146.2, 137.8, 137.2, 131.6, 130.6, 130.5, 126.4, 123.6, 123.3, 119.8, 118.9, 116.7, 108.9, 65.3, 52.2.

HRMS (ESI, *m/z*) calcd for C₂₃H₂₁N₃OS, [M+H]⁺: 416.1540; found: 416.1575.

3p



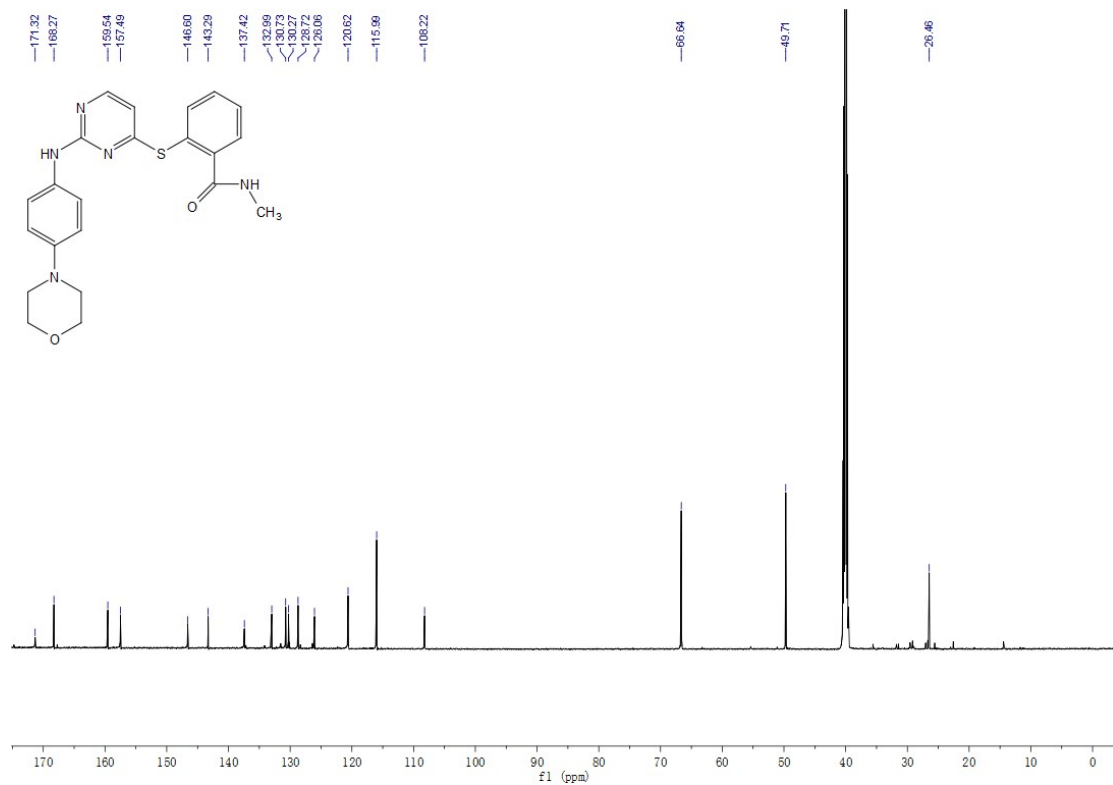
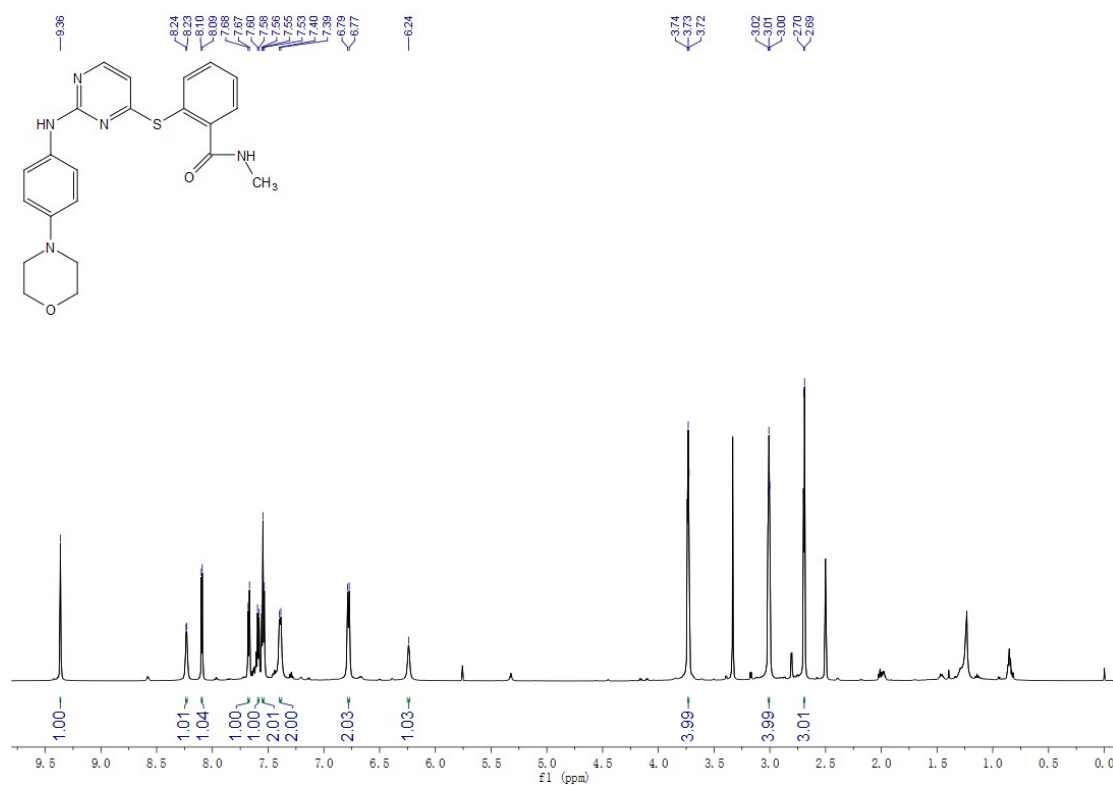


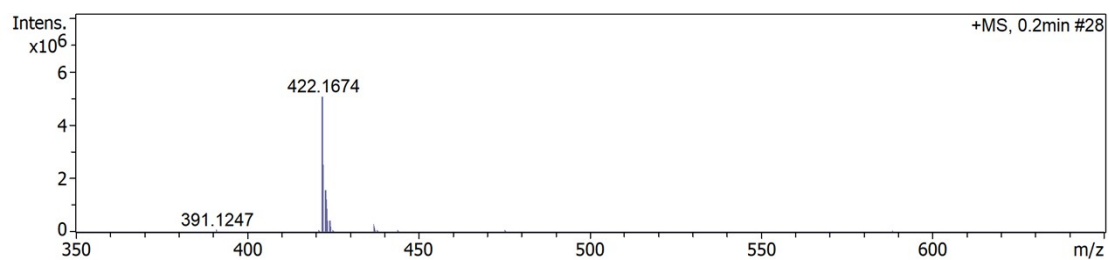
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.37 (s, 1H), 8.24 (s, 1H), 8.23 (s, 1H), 8.12-8.12 (m, 1H), 8.04 (d, *J* = 4.9 Hz, 1H), 7.98 (d, *J* = 6.9 Hz, 1H), 7.66 (t, *J* = 9 Hz, 1H), 7.63-7.61 (m, 2H), 7.10 (s, 2H), 6.60 (s, 2H), 6.19 (s, 1H), 3.73 (s, 4H), 2.97 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 171.0, 159.6, 157.6, 146.4, 136.8, 134.7, 136.6, 132.8, 132.0, 129.4, 128.3, 127.3, 126.8, 125.7, 125.4, 120.2, 115.8, 107.8, 66.6, 49.8.

HRMS (ESI, *m/z*) calcd for C₂₄H₂₂N₄OS, [M+H]⁺: 415.1587; found: 415.1604.

3q



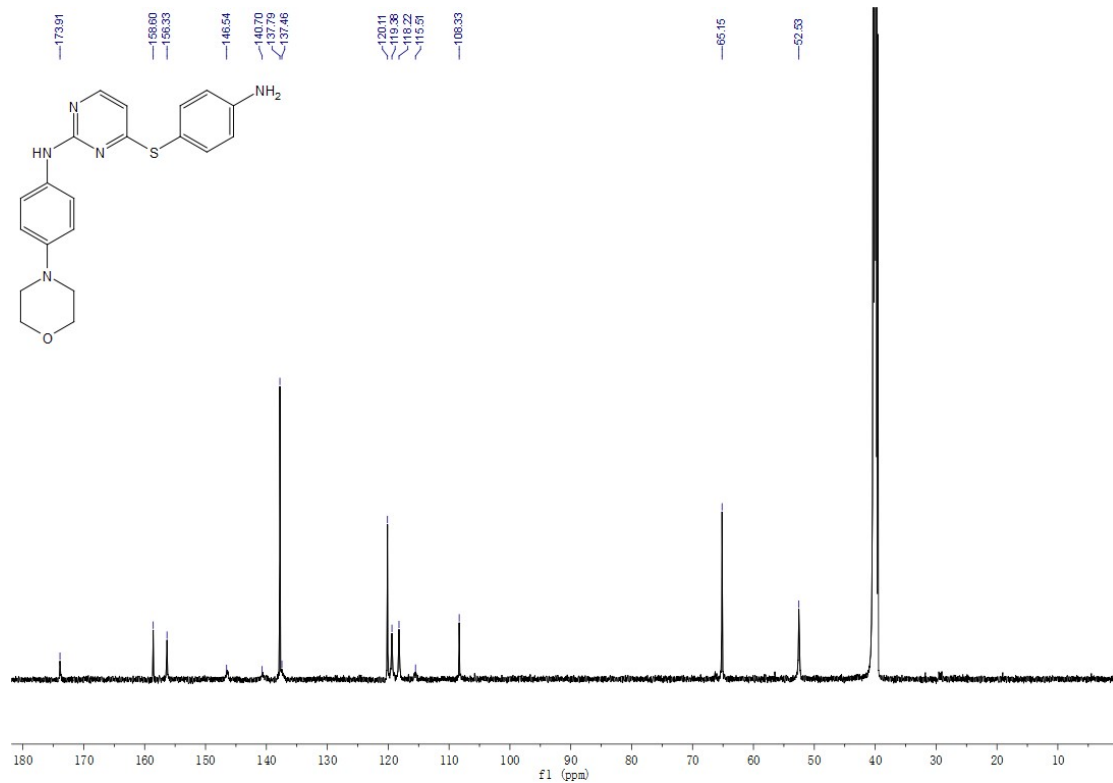
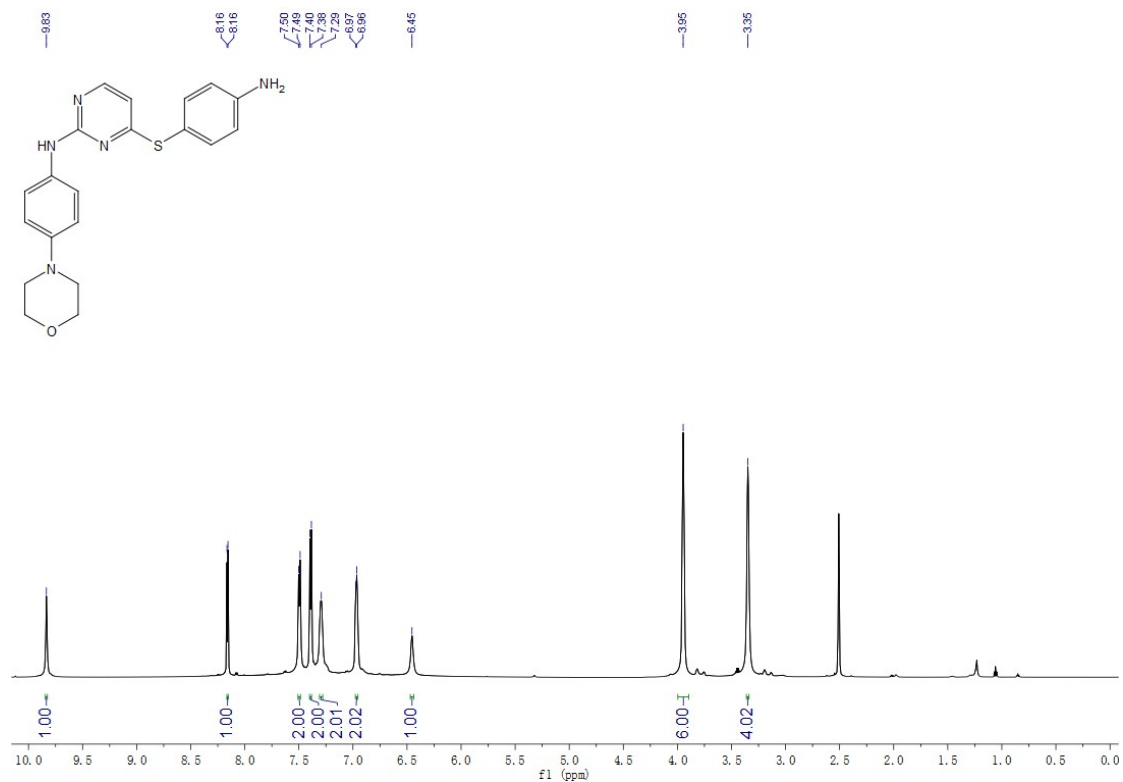


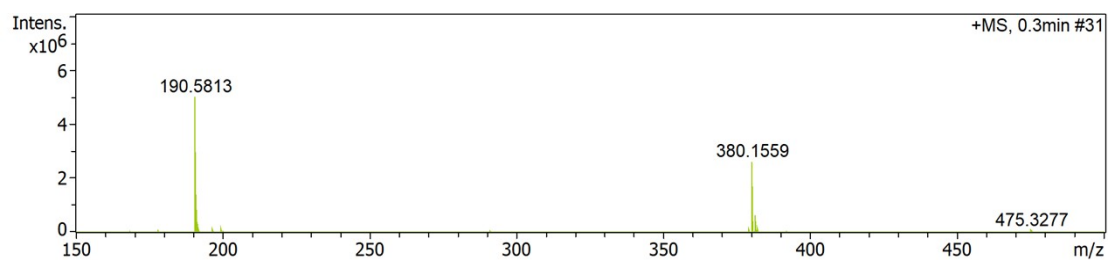
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.36 (s, 1H), 8.23 (d, *J* = 4.4 Hz, 1H), 8.10 (d, *J* = 5.3 Hz, 1H), 7.68 (s, 1H), 7.58 (s, 1H), 7.55 (s, 2H), 7.39 (d, *J* = 7.4 Hz, 2H), 6.77 (s, 2H), 6.24 (s, 1H), 3.73 (s, 4H), 3.01 (s, 4H), 2.69 (s, 3H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 171.3, 168.3, 159.5, 157.5, 146.6, 143.3, 137.4, 133.0, 130.7, 130.3, 128.7, 126.1, 120.6, 116.0, 108.2, 66.6, 49.7, 26.5.

HRMS (ESI, *m/z*) calcd for C₂₂H₂₃N₅O₂S, [M+H]⁺: 422.1645; found: 422.1674.

3r



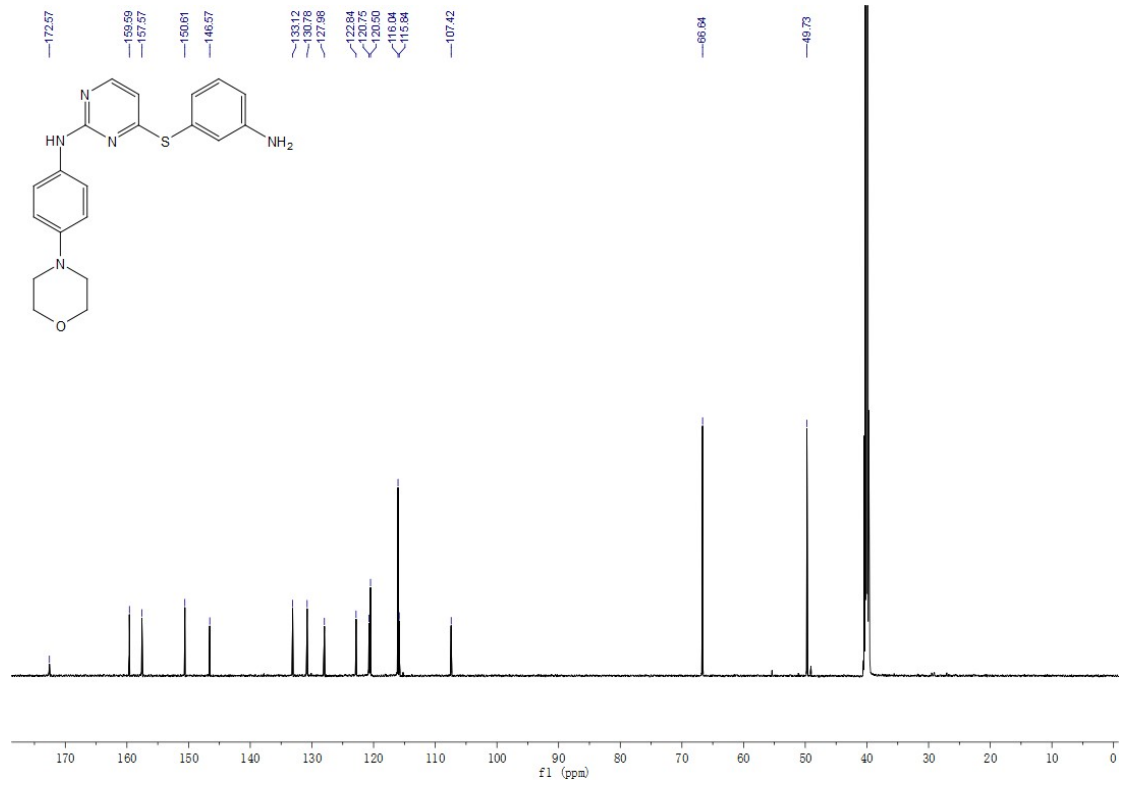
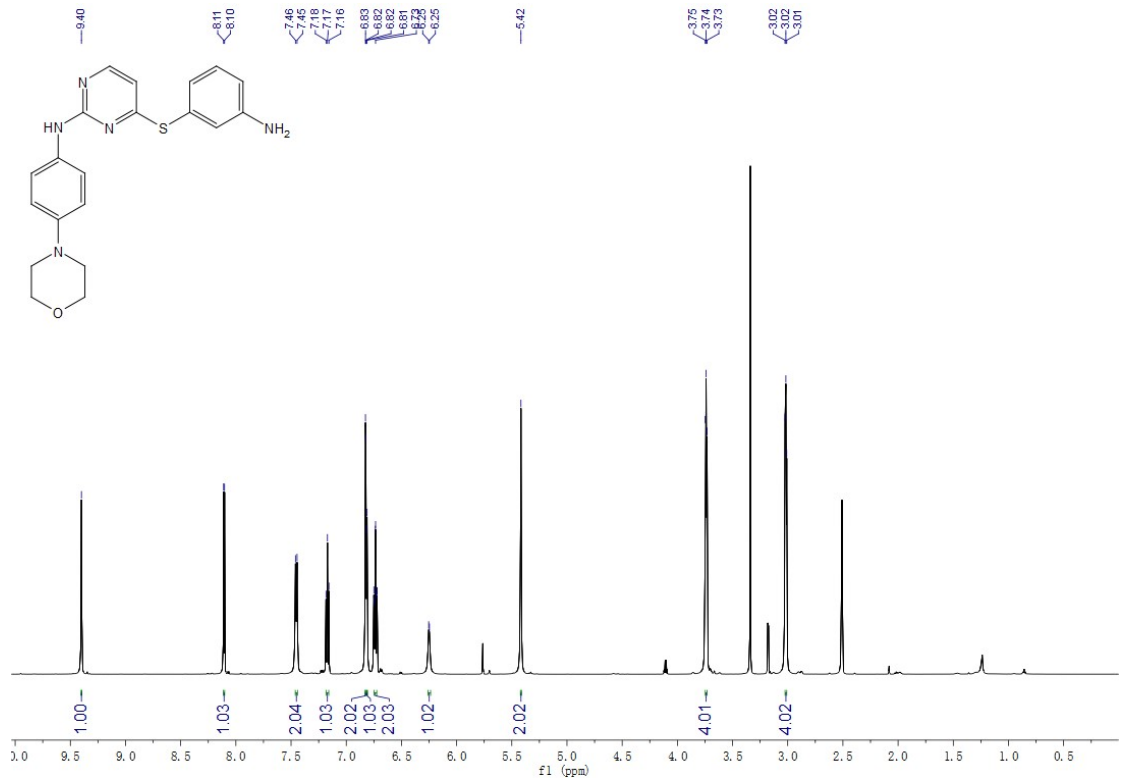


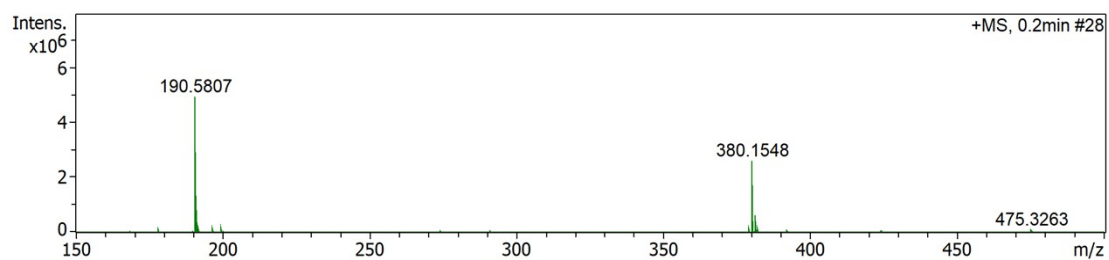
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.83 (s, 1H), 8.16 (d, *J* = 5.4 Hz, 1H), 7.50 (d, *J* = 8.5 Hz, 2H), 7.39 (d, *J* = 8.2 Hz, 2H), 7.29 (s, 2H), 6.97 (d, *J* = 4.6 Hz, 2H), 6.45 (s, 1H), 3.95 (s, 6H), 3.35 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 173.9, 158.6, 156.3, 146.5, 140.7, 137.8, 137.5, 120.1, 119.4, 118.2, 115.5, 108.3, 65.2, 52.5.

HRMS (ESI, *m/z*) calcd for C₂₀H₂₁N₅OS, [M+H]⁺: 380.1540; found: 380.1559.

3s



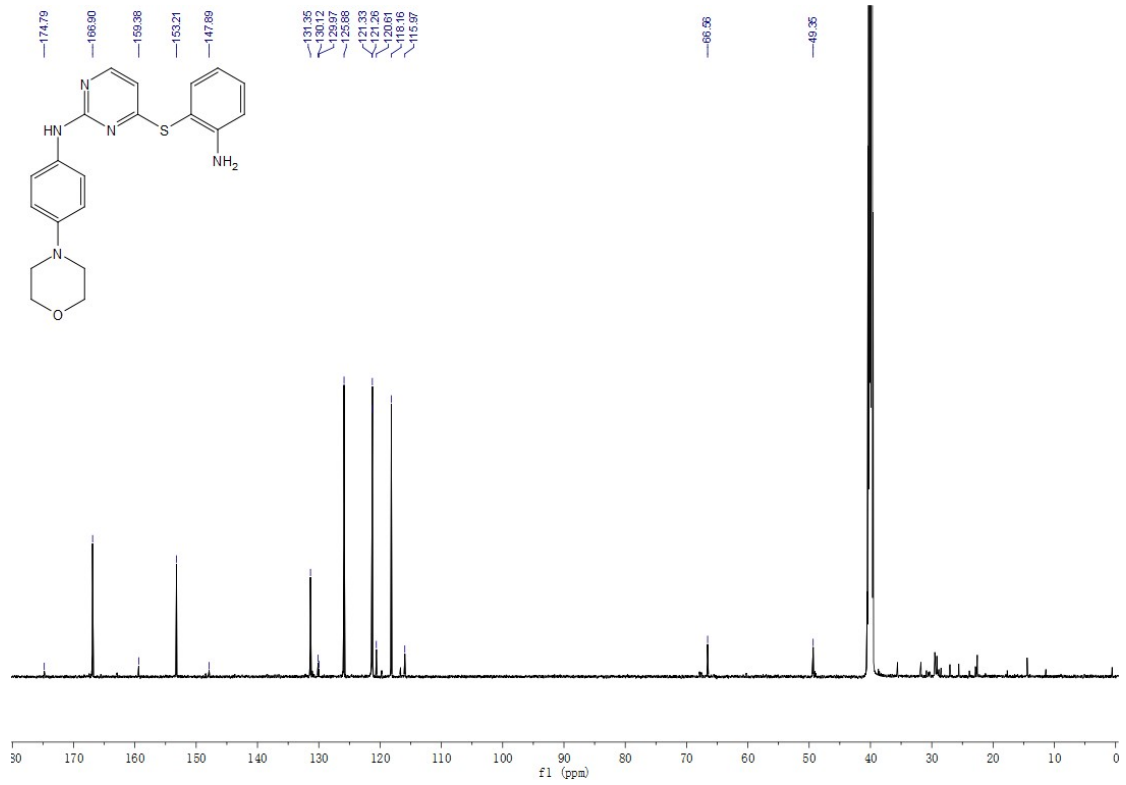
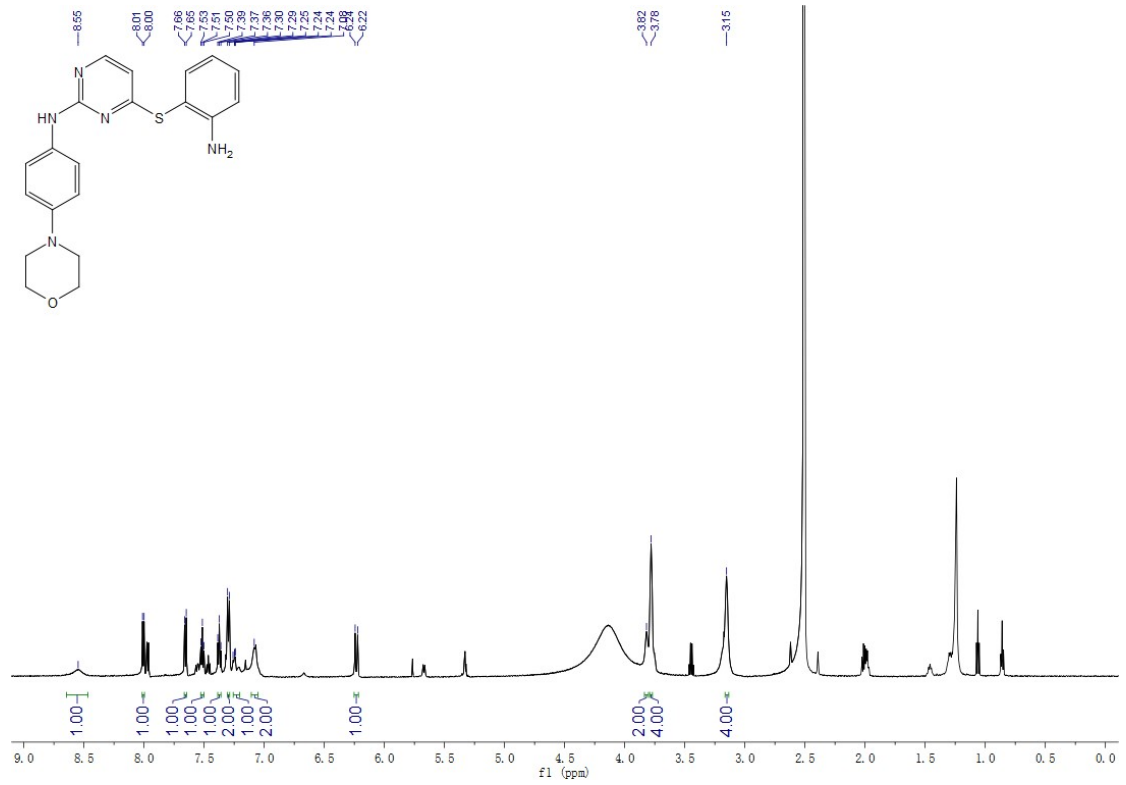


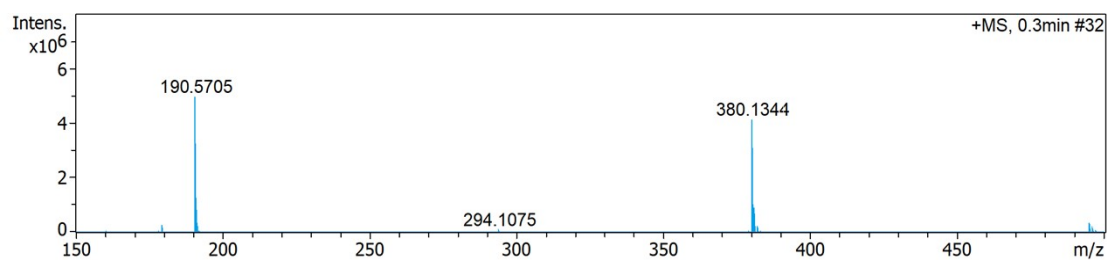
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.40 (s, 1H), 8.11 (d, *J* = 5.3 Hz, 1H), 7.45 (d, *J* = 8.7 Hz, 2H), 7.18 (d, *J* = 7.8 Hz, 1H), 6.82 (d, *J* = 2.0 Hz, 2H), 6.81 (s, 1H), 6.74 (dd, *J* = 7.2, 0.9 Hz, 2H), 6.25 (d, *J* = 3.9 Hz, 1H), 5.42 (s, 2H), 3.74 (s, 4H), 3.01 (d, *J* = 4.8 Hz, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 172.6, 159.6, 157.6, 150.6, 146.6, 133.1, 130.8, 128.0, 122.8, 120.8, 120.5, 116.1, 115.9, 107.4, 66.6, 49.7.

HRMS (ESI, *m/z*) calcd for C₂₀H₂₁N₃OS, [M+H]⁺: 380.1540; found: 380.1548.

3t



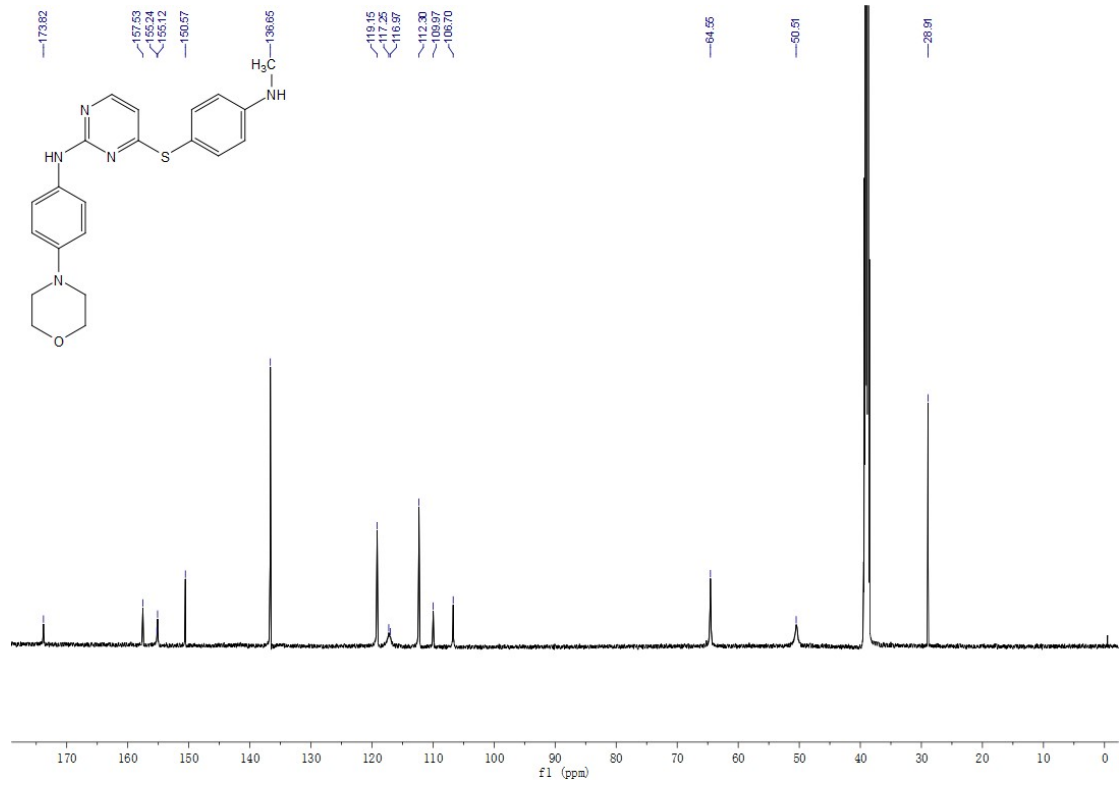
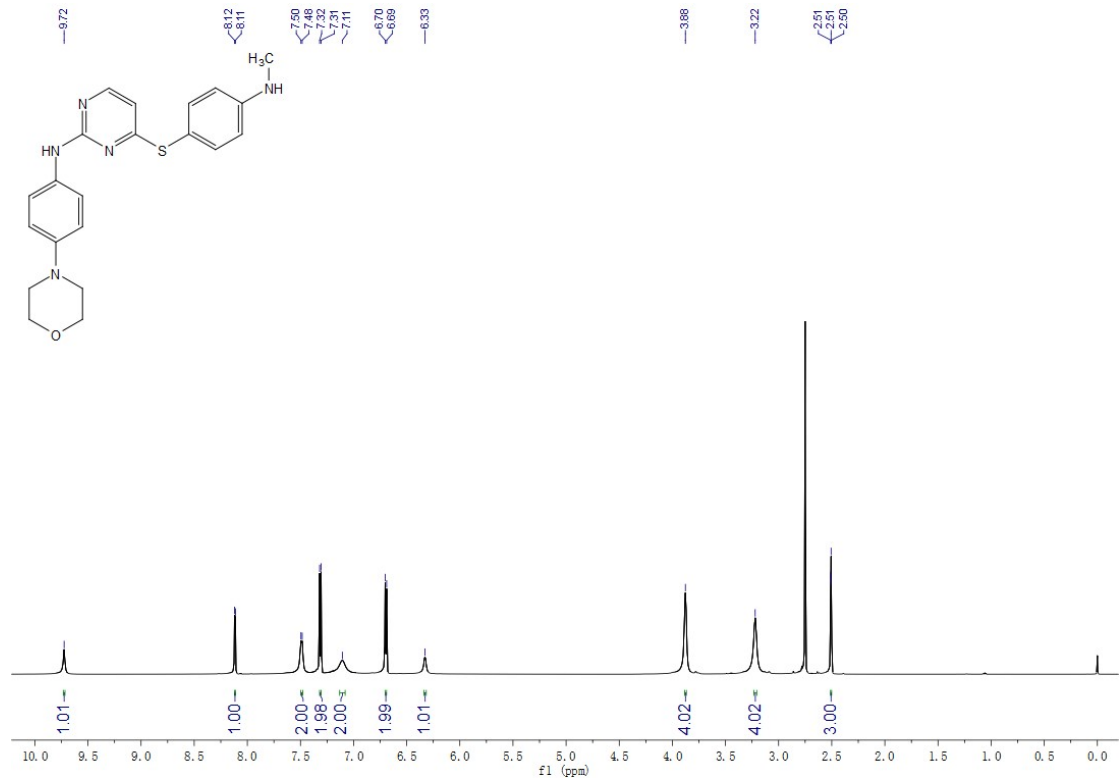


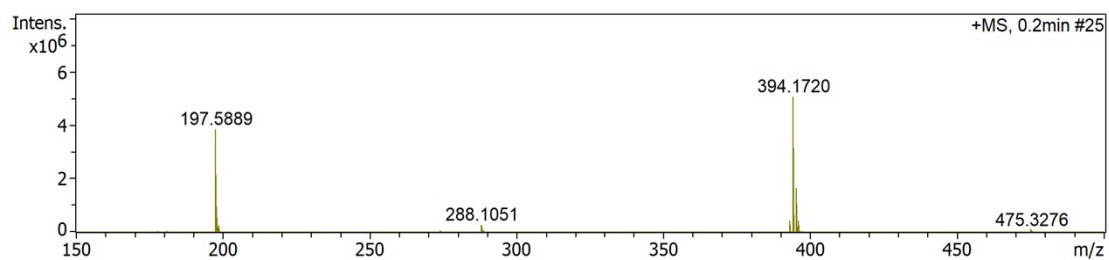
^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 8.55 (s, 1H), 8.01 (d, $J = 7.9$ Hz, 1H), 7.66 (d, $J = 8.1$ Hz, 1H), 7.51 (t, $J = 7.8$ Hz, 1H), 7.37 (t, $J = 7.8$ Hz, 1H), 7.30 (d, $J = 9.2$ Hz, 2H), 7.26 – 7.20 (m, 1H), 7.08 (s, 2H), 6.23 (d, $J = 12.6$ Hz, 1H), 3.82 (s, 2H), 3.78 (s, 4H), 3.15 (s, 4H).

^{13}C NMR (151 MHz, $\text{DMSO-}d_6$) δ 174.8, 166.9, 159.4, 153.2, 147.9, 131.4, 130.1, 130.0, 125.9, 121.3, 121.2, 120.6, 118.2, 116.0, 66.6, 49.4.

HRMS (ESI, m/z) calcd for $\text{C}_{20}\text{H}_{21}\text{N}_5\text{OS}$, $[\text{M}+\text{H}]^+$: 380.1540; found: 380.1344.

3u



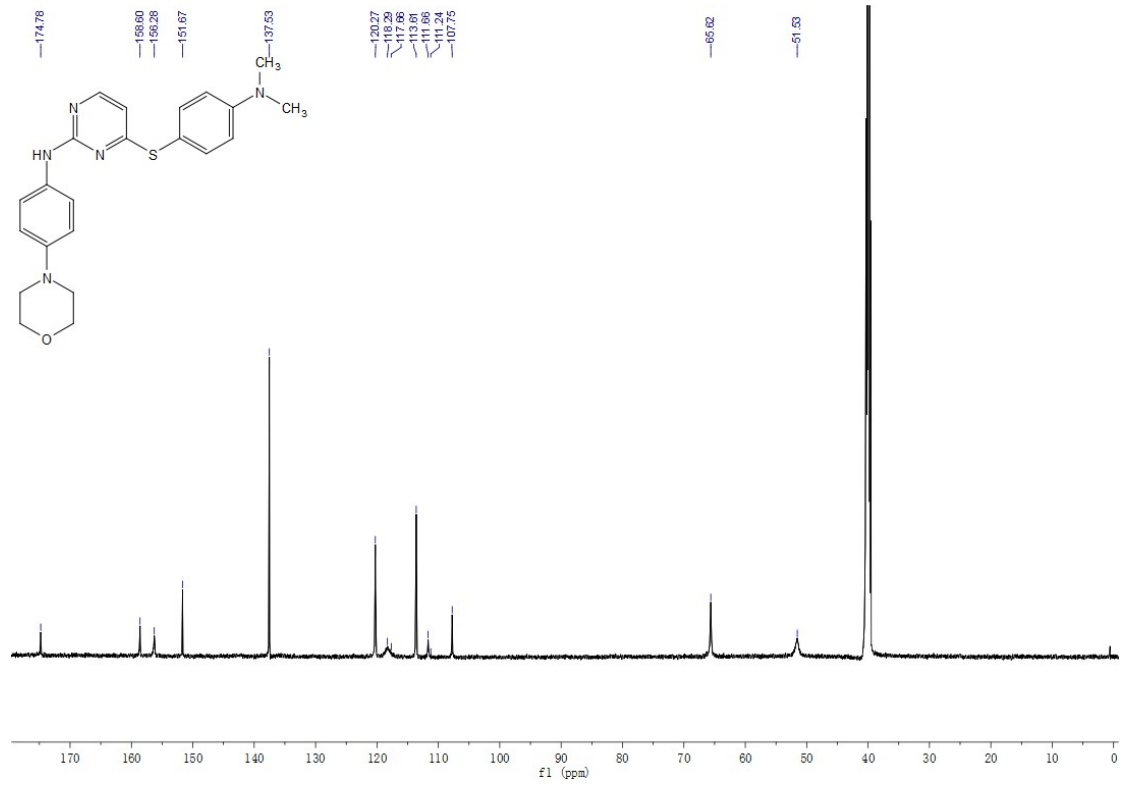
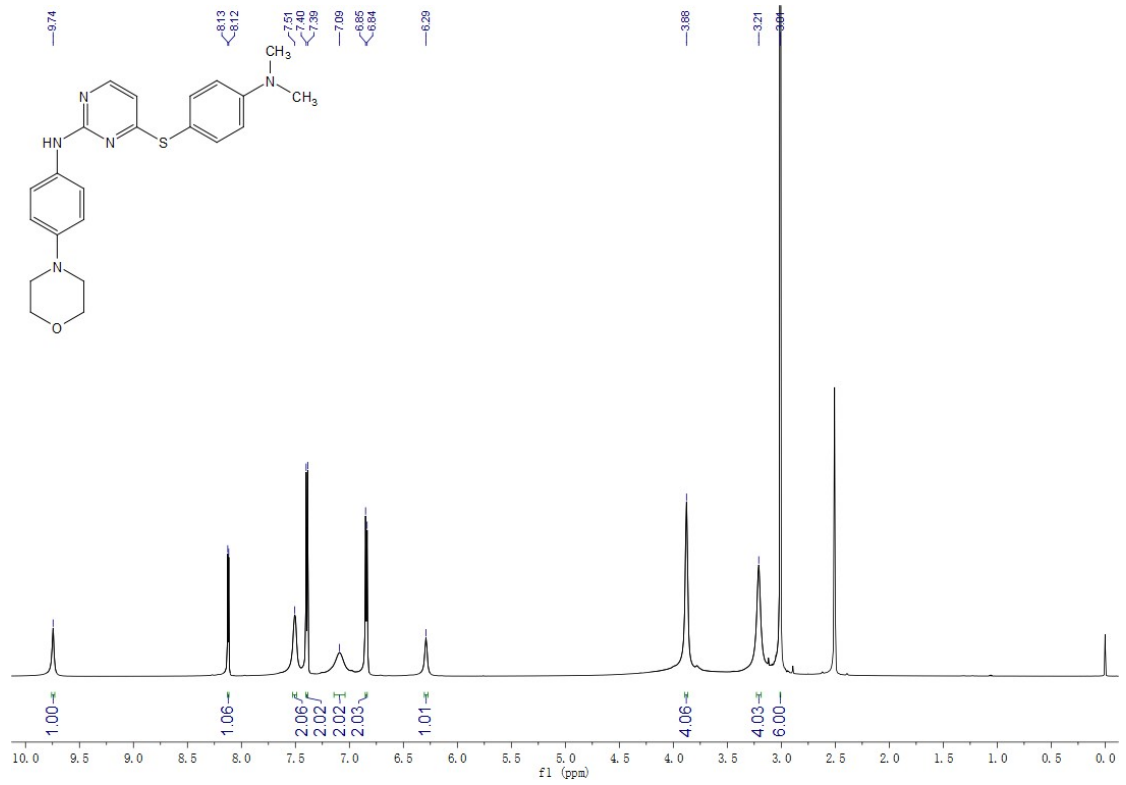


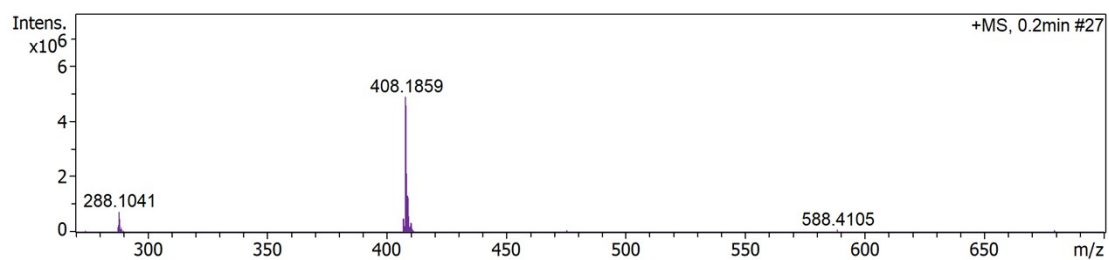
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.72 (s, 1H), 8.12 (d, *J* = 5.5 Hz, 1H), 7.49 (d, *J* = 7.0 Hz, 2H), 7.32 (s, 2H), 7.11 (s, 2H), 6.70 (s, 2H), 6.33 (s, 1H), 3.88 (s, 4H), 3.22 (s, 4H), 2.51 – 2.50 (m, 3H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 173.8, 157.5, 155.1, 155.1, 150.6, 136.7, 119.2, 117.23, 117.0, 112.3, 110.0, 106.7, 64.6, 50.5, 28.9.

HRMS (ESI, *m/z*) calcd for C₂₁H₂₃N₅OS, [M+H]⁺: 394.1696; found: 394.1720.

3v



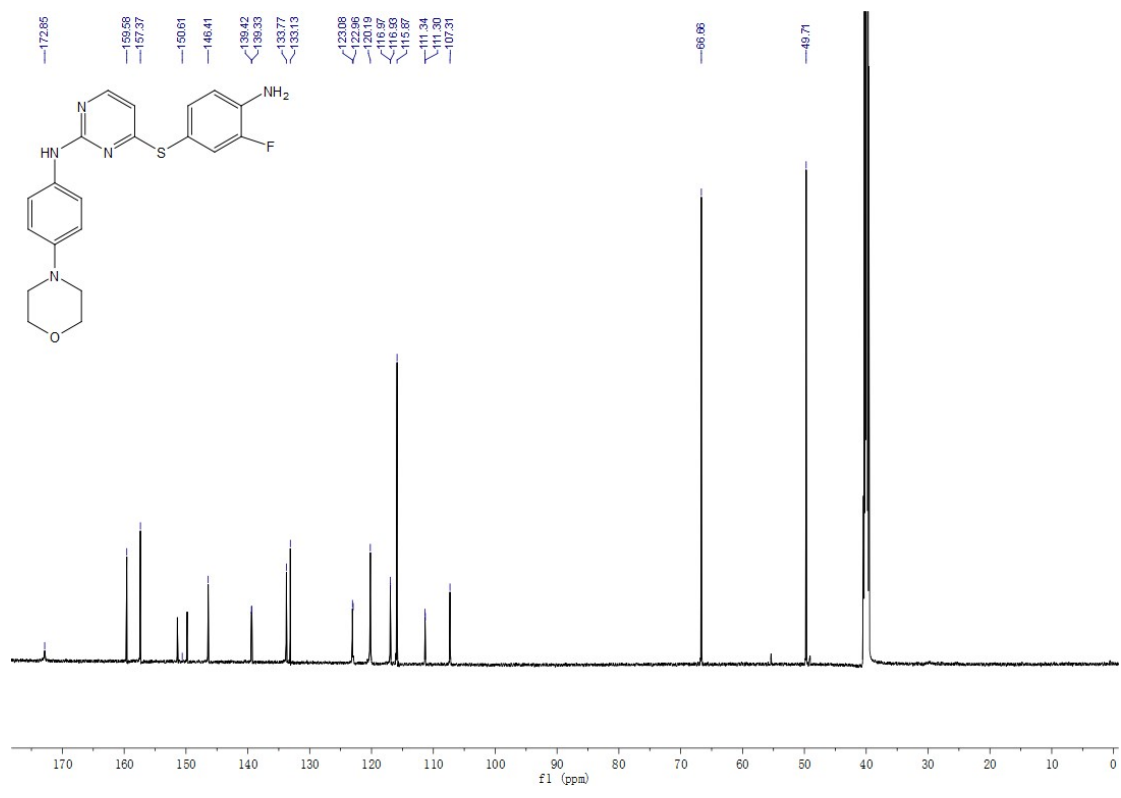
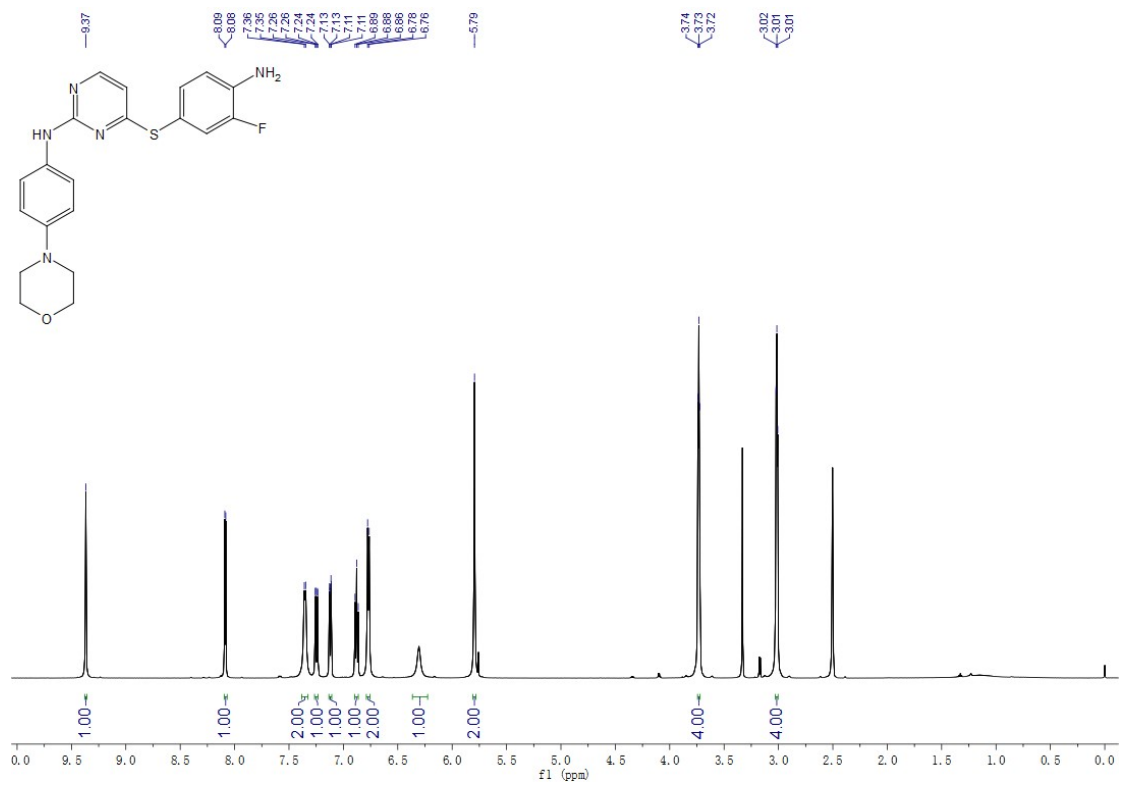


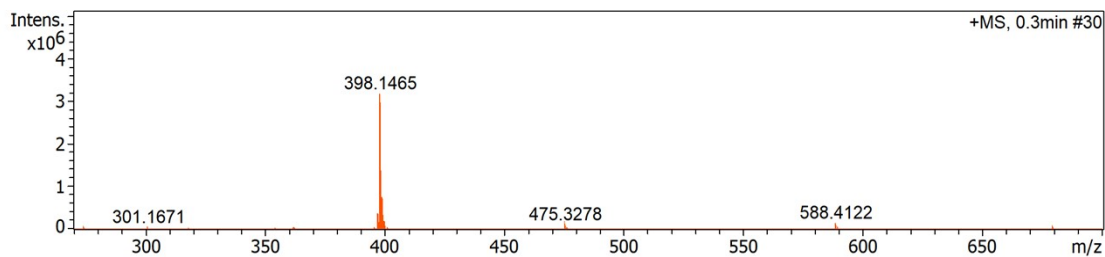
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.74 (s, 1H), 8.12 (d, *J* = 5.5 Hz, 1H), 7.51 (s, 2H), 7.39 (d, *J* = 8.7 Hz, 2H), 7.09 (s, 2H), 6.84 (d, *J* = 8.7 Hz, 2H), 6.29 (s, 1H), 3.88 (s, 4H), 3.21 (s, 4H), 3.01 (s, 6H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 173.8, 157.5, 155.2, 155.1, 150.6, 136.7, 119.2, 117.3, 117.0, 112.3, 111.2, 106.7, 64.6, 50.5, 28.9.

HRMS (ESI, *m/z*) calcd for C₂₂H₂₅N₅OS, [M+H]⁺: 408.1853; found: 408.1859.

3w



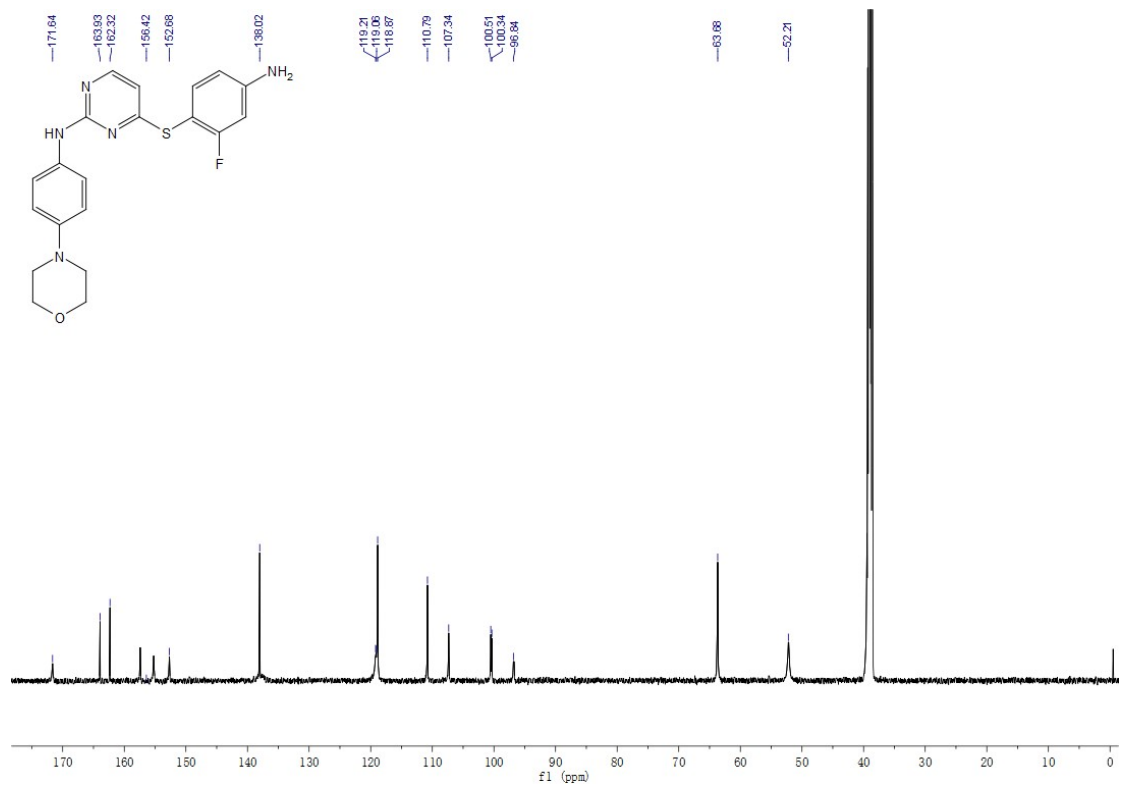
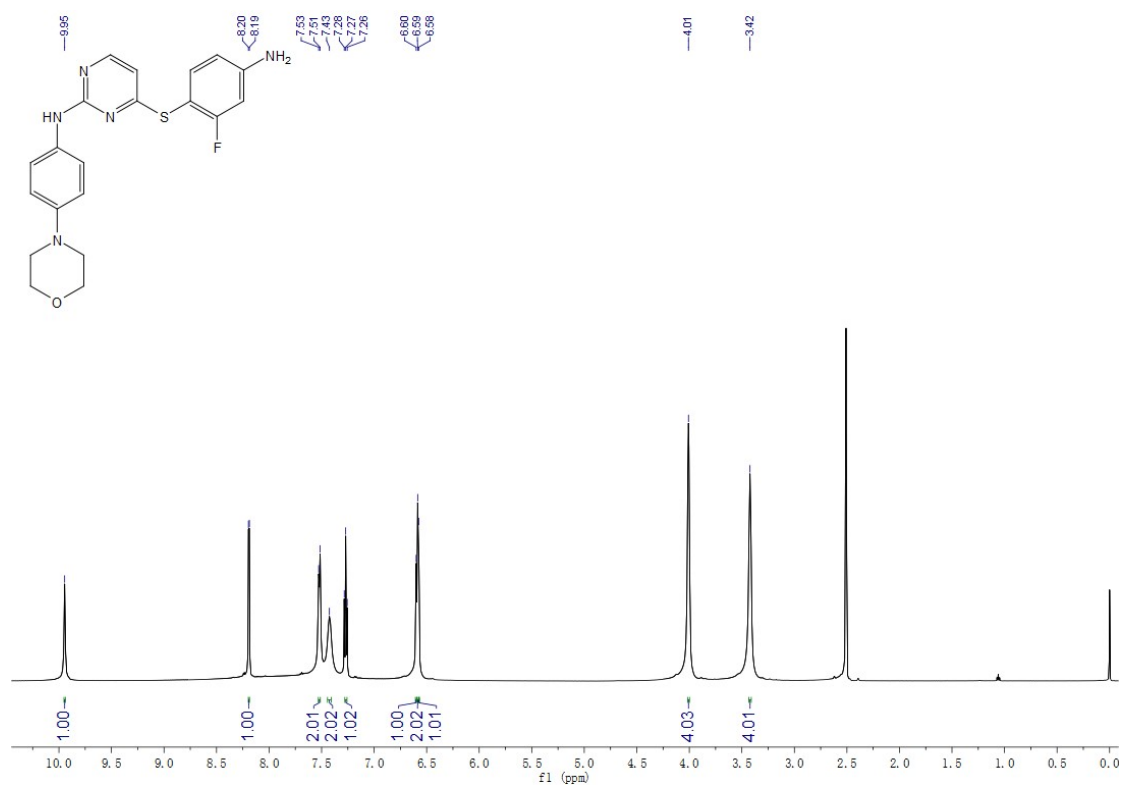


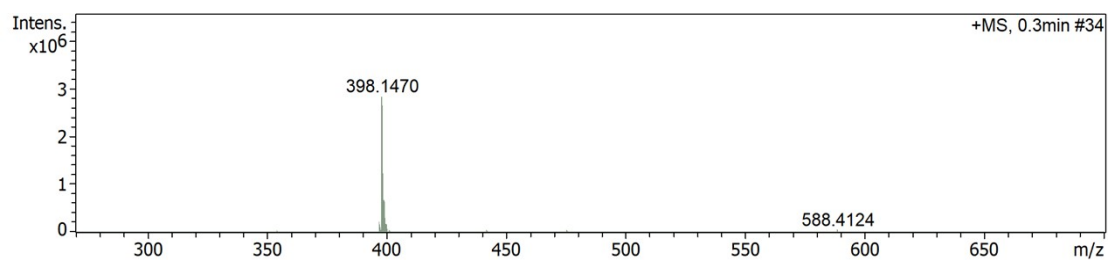
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.37 (s, 1H), 8.09 (d, *J* = 5.3 Hz, 1H), 7.35 (d, *J* = 7.4 Hz, 2H), 7.25 (dd, *J* = 11.2, 1.5 Hz, 1H), 7.12 (dd, *J* = 8.2, 1.6 Hz, 1H), 6.88 (t, *J* = 8.8 Hz, 1H), 6.77 (d, *J* = 8.7 Hz, 2H), 6.31 (s, 1H), 5.79 (s, 2H), 3.74 – 3.72 (m, 4H), 3.03 – 3.00 (m, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 172.9, 159.6, 157.4, 150.6, 146.4, 139.4 (d, *J* = 12.7 Hz), 133.8, 133.1, 123.0 (d, *J* = 18.3 Hz), 120.2, 116.9, 115.9, 111.32 (d, *J* = 6.7 Hz), 107.3, 66.7, 49.7.

HRMS (ESI, *m/z*) calcd for C₂₀H₂₀FN₅OS, [M+H]⁺: 398.1445; found: 398.1465.

3x



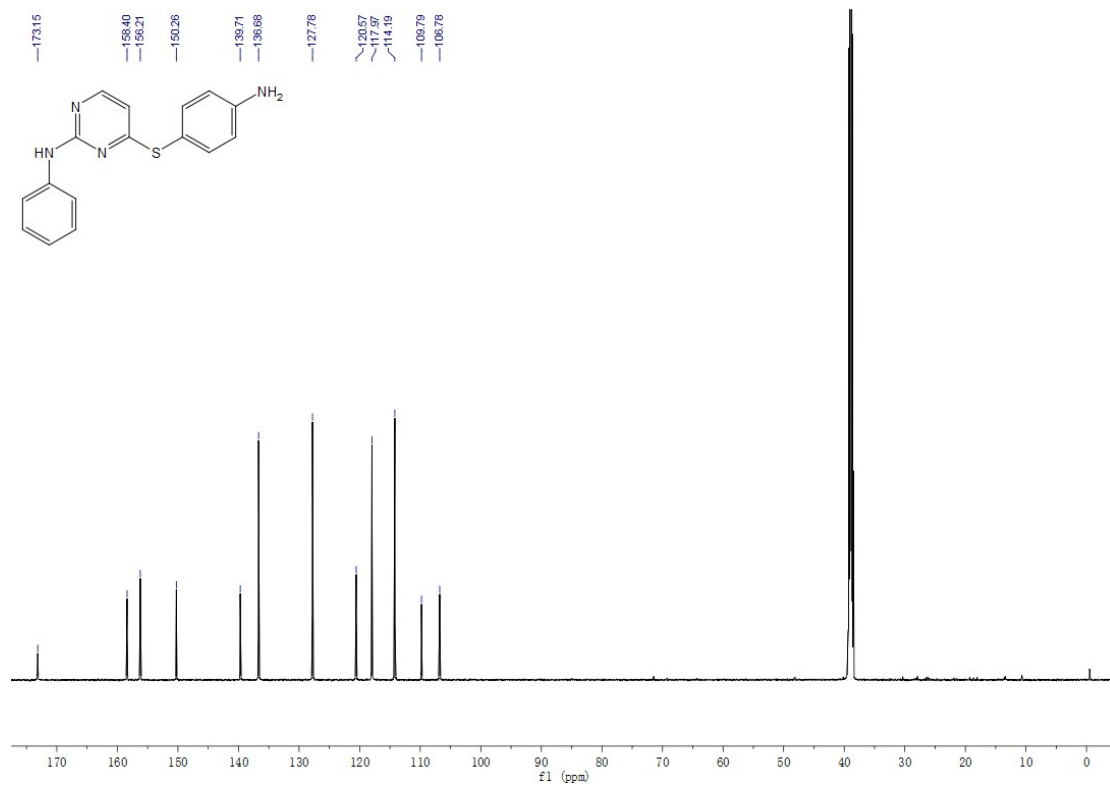
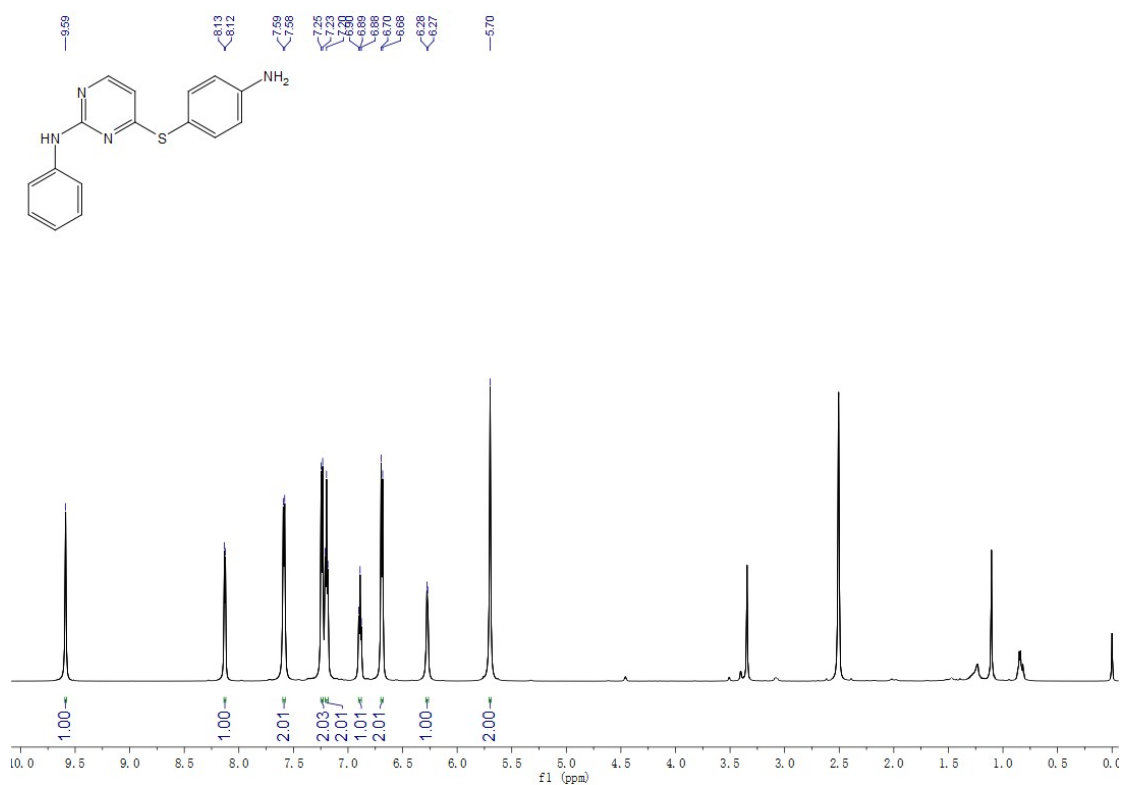


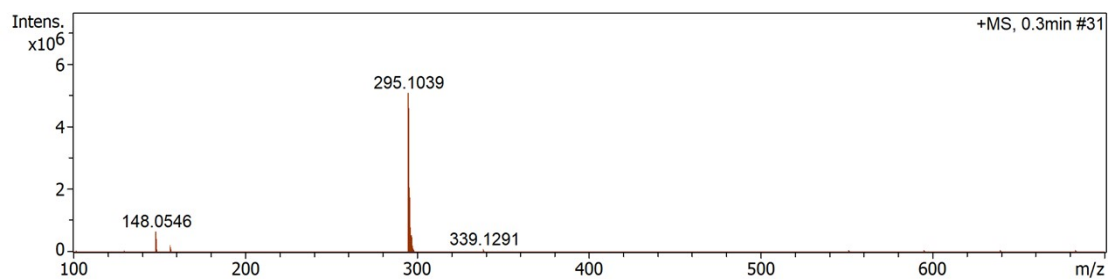
^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 9.95 (s, 1H), 8.19 (d, $J = 5.4$ Hz, 1H), 7.52 (d, $J = 8.0$ Hz, 2H), 7.43 (s, 2H), 7.27 (s, 1H), 6.60 (s, 1H), 6.59 (s, 2H), 6.58 (s, 1H), 4.01 (s, 4H), 3.42 (s, 4H).

^{13}C NMR (151 MHz, $\text{DMSO-}d_6$) δ 171.6, 163.9, 162.3, 156.4, 152.7, 138.0, 119.7, 119.0, 118.4, 110.8, 107.3, 100.4 (d, $J = 25.3$ Hz), 96.8, 63.7, 52.2.

HRMS (ESI, m/z) calcd for $\text{C}_{20}\text{H}_{20}\text{FN}_5\text{OS}$, $[\text{M}+\text{H}]^+$: 398.1445; found: 398.1470.

8a



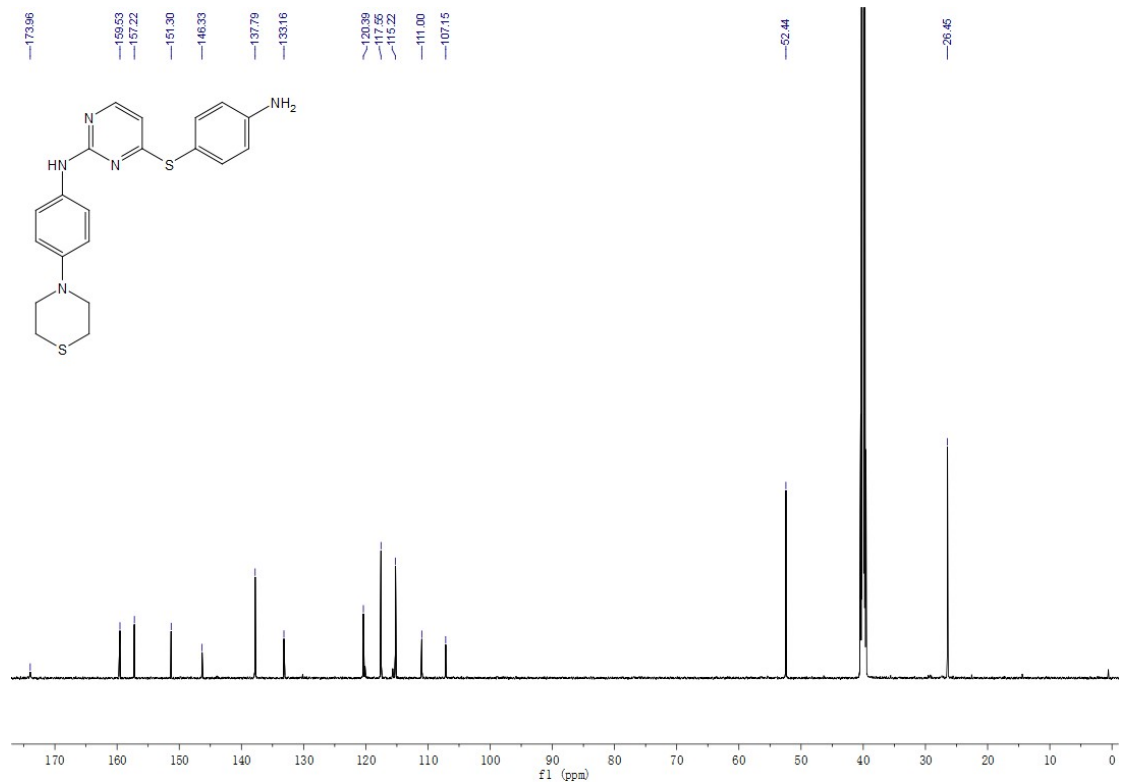
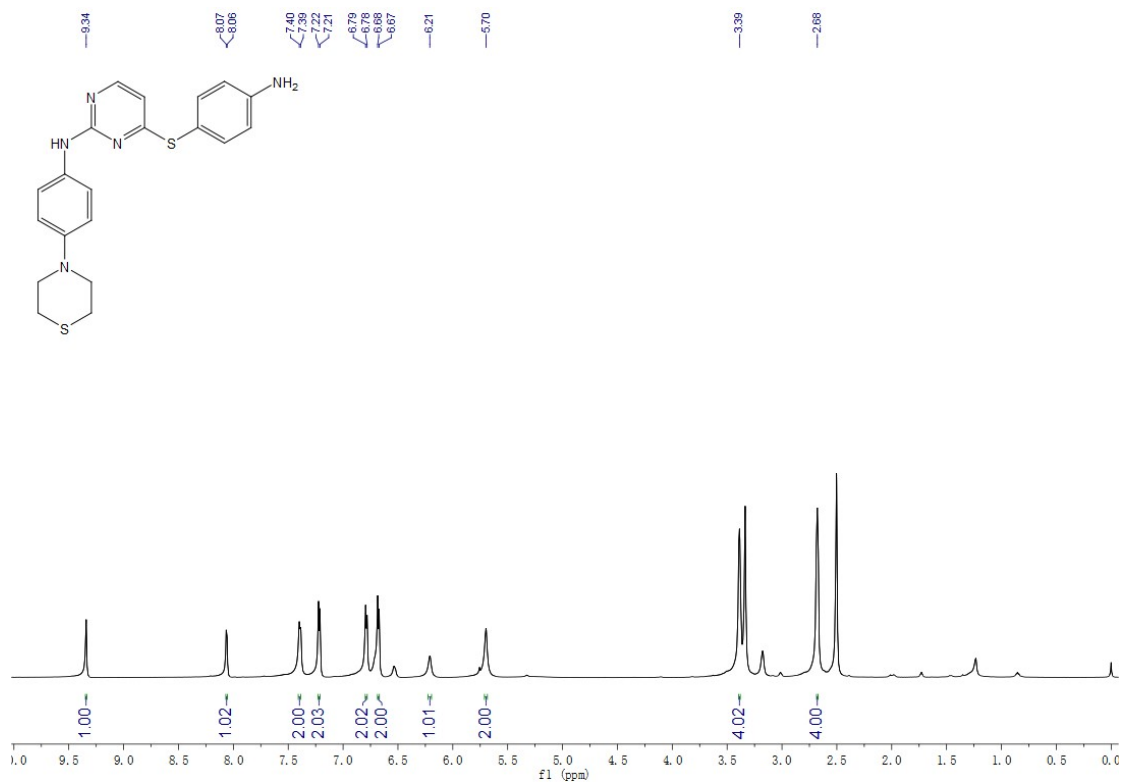


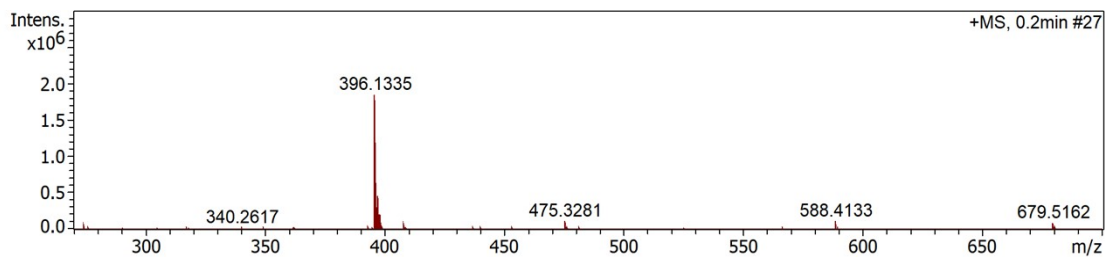
^1H NMR (600 MHz, $\text{DMSO-}d_6$) δ 9.59 (s, 1H), 8.13 (d, $J = 5.2$ Hz, 1H), 7.59 (d, $J = 7.8$ Hz, 2H), 7.24 (d, $J = 8.1$ Hz, 2H), 7.19 (d, $J = 7.7$ Hz, 2H), 6.90 (d, $J = 7.1$ Hz, 1H), 6.69 (d, $J = 8.2$ Hz, 2H), 6.27 (d, $J = 4.5$ Hz, 1H), 5.70 (s, 2H).

^{13}C NMR (151 MHz, $\text{DMSO-}d_6$) δ 173.2, 158.4, 156.2, 150.3, 139.7, 136.7, 127.8, 120.6, 118.0, 114.2, 109.8, 106.8.

HRMS (ESI, m/z) calcd for $\text{C}_{16}\text{H}_{14}\text{N}_4\text{S}$, $[\text{M}+\text{H}]^+$: 295.1012; found: 295.1039.

8b



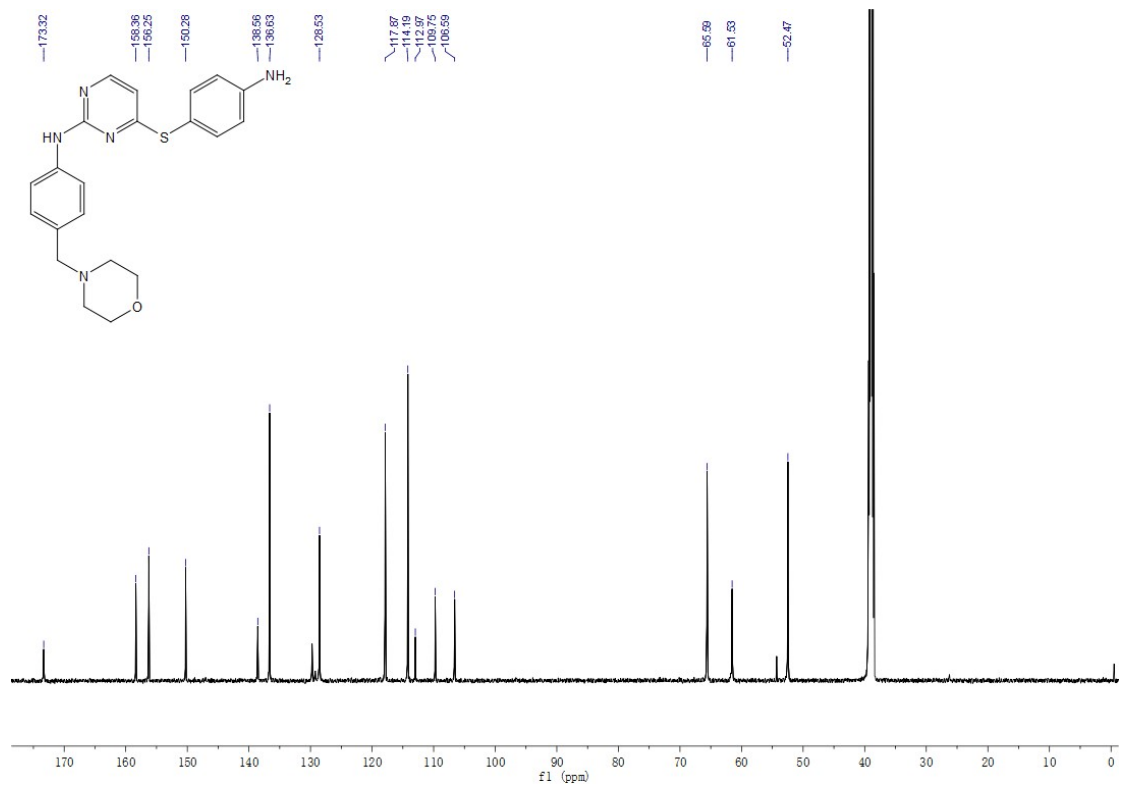
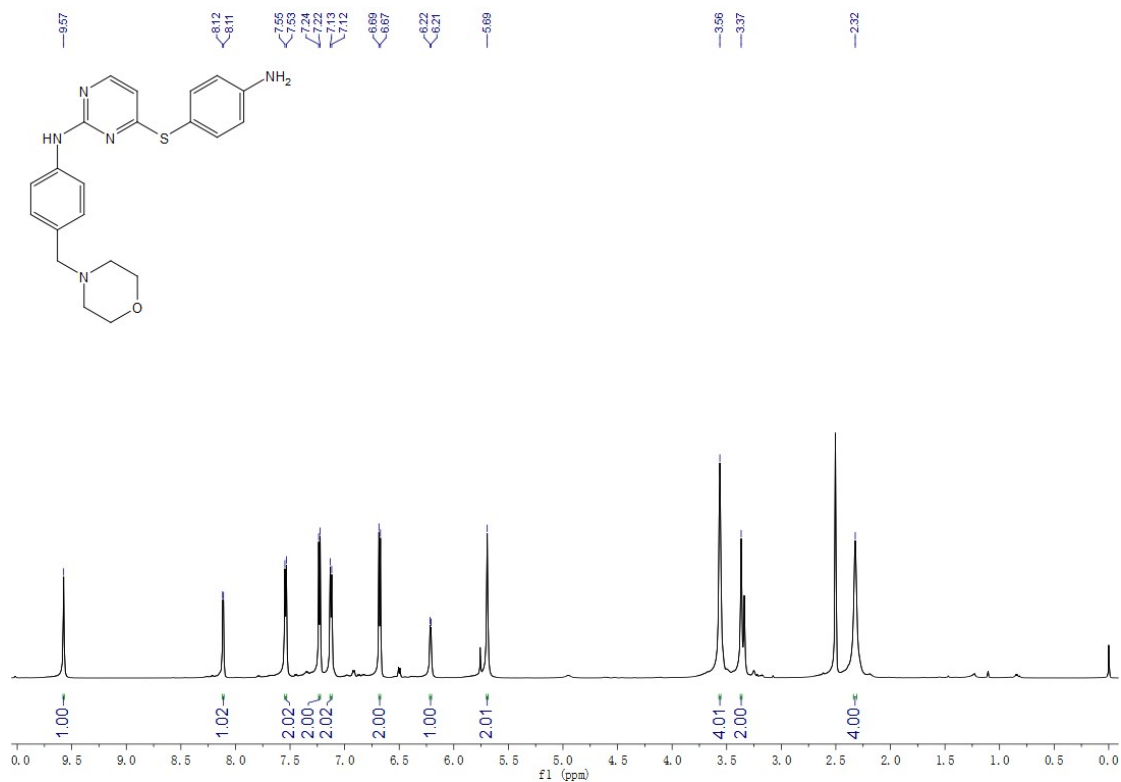


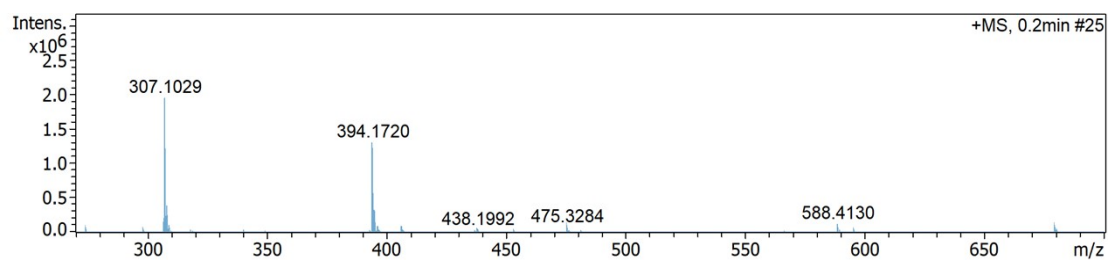
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.34 (s, 1H), 8.06 (d, *J* = 5.1 Hz, 1H), 7.39 (d, *J* = 7.4 Hz, 2H), 7.22 (s, 2H), 6.79 (d, *J* = 8.4 Hz, 2H), 6.68 (d, *J* = 8.1 Hz, 2H), 6.21 (s, 1H), 5.70 (s, 2H), 3.39 (s, 4H), 2.68 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 174.0, 159.5, 157.2, 151.3, 146.3, 137.8, 133.2, 120.4, 117.6, 115.2, 111.0, 107.2, 52.4, 26.5.

HRMS (ESI, *m/z*) calcd for C₂₀H₂₁N₅S₂, [M+H]⁺: 396.1335; found: 396.1335.

8c



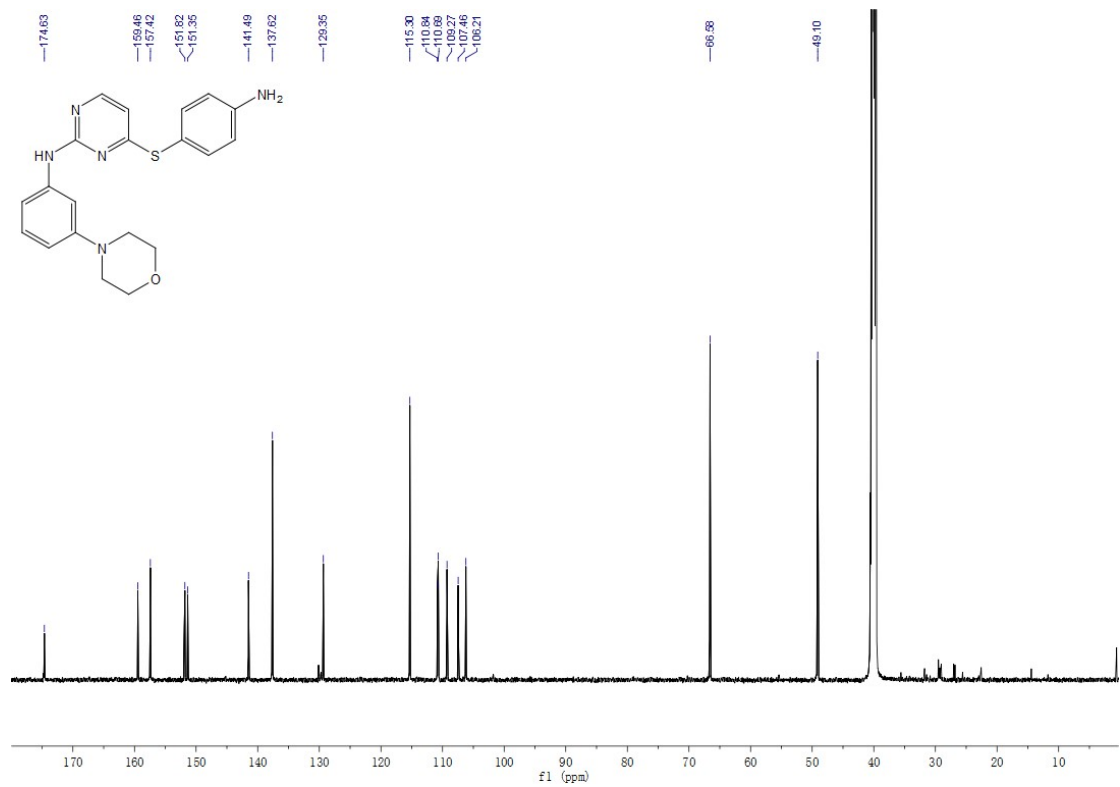
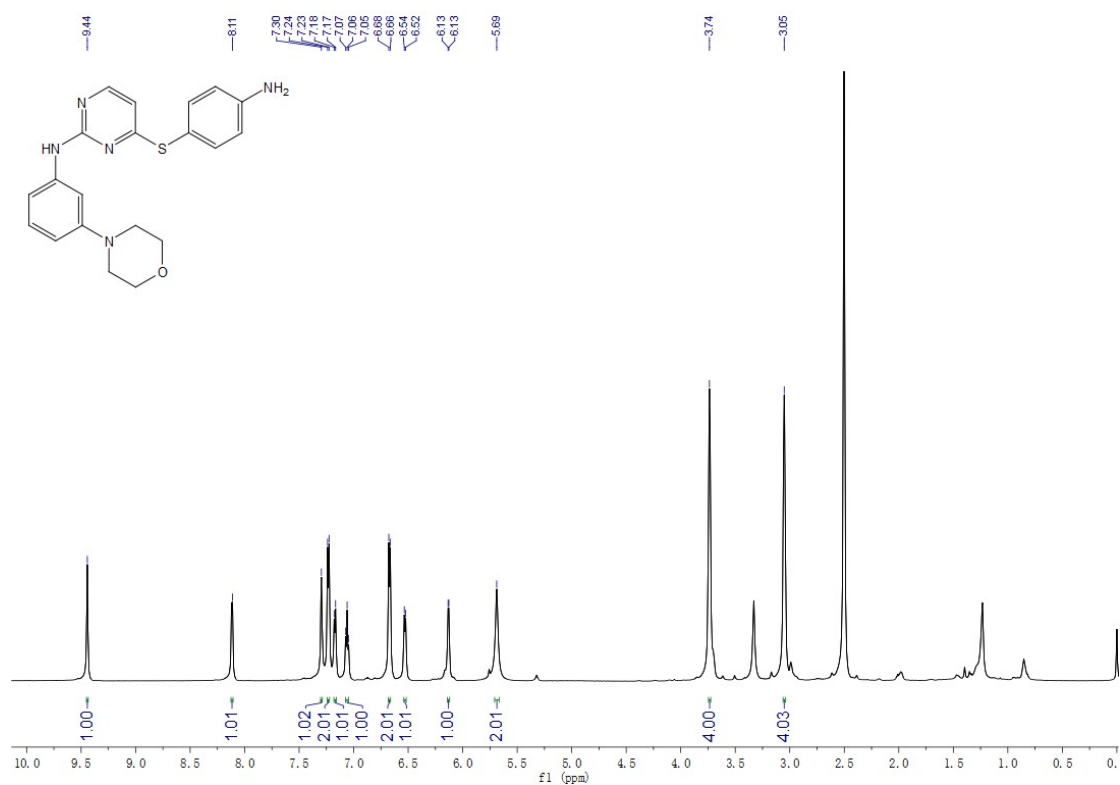


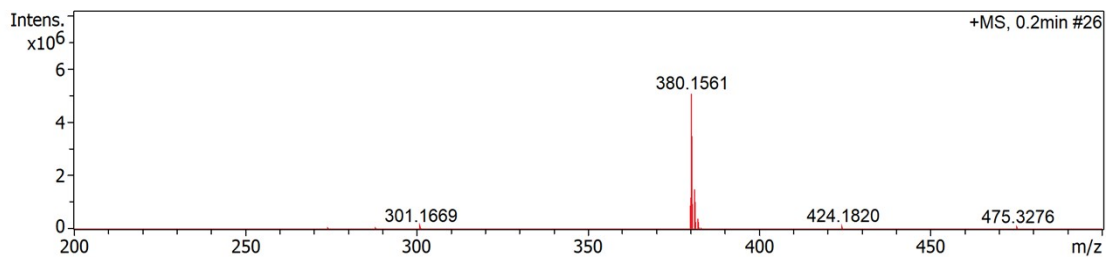
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.57 (s, 1H), 8.11 (d, *J* = 5.2 Hz, 1H), 7.54 (d, *J* = 8.1 Hz, 2H), 7.23 (d, *J* = 8.2 Hz, 2H), 7.12 (d, *J* = 8.0 Hz, 2H), 6.68 (d, *J* = 8.3 Hz, 2H), 6.21 (d, *J* = 4.7 Hz, 1H), 5.69 (s, 2H), 3.56 (s, 4H), 3.37 (s, 2H), 2.32 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 173.3, 158.4, 156.3, 150.3, 138.6, 136.6, 128.5, 117.9, 114.2, 113.0, 109.8, 106.6, 65.6, 61.5, 52.5.

HRMS (ESI, *m/z*) calcd for C₂₁H₂₃N₃OS, [M+H]⁺: 394.1696; found: 394.1720.

8d



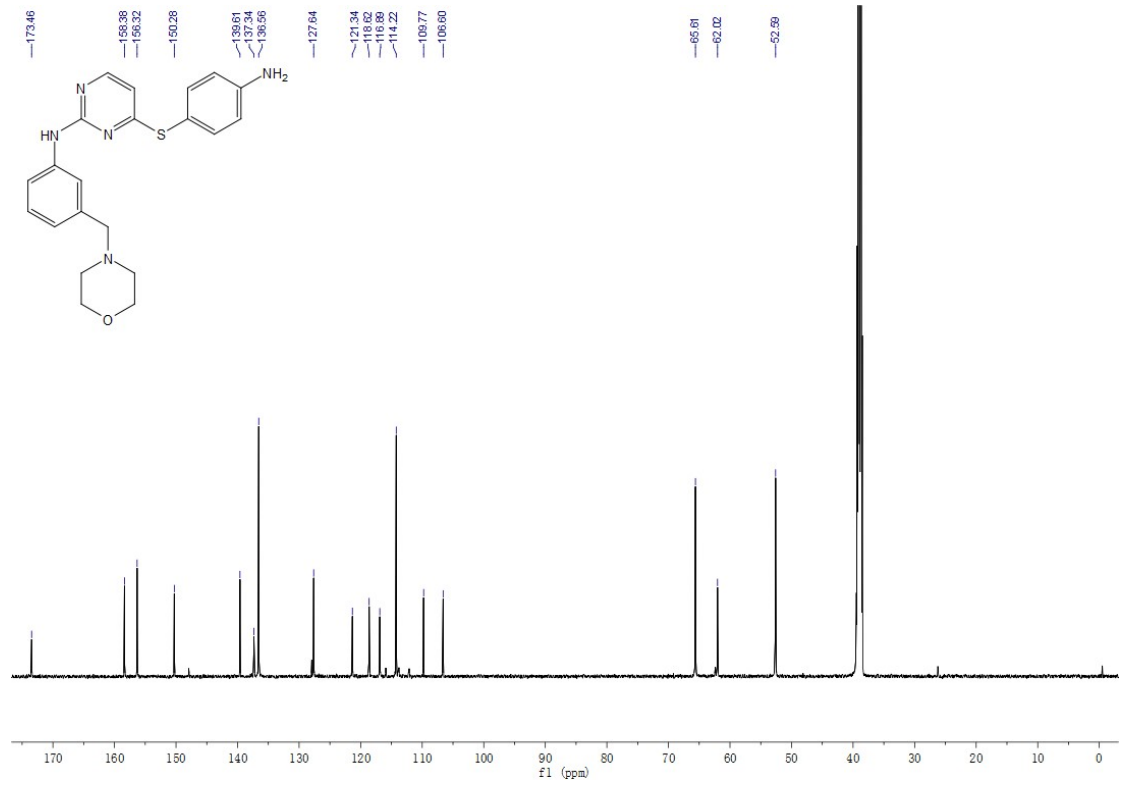
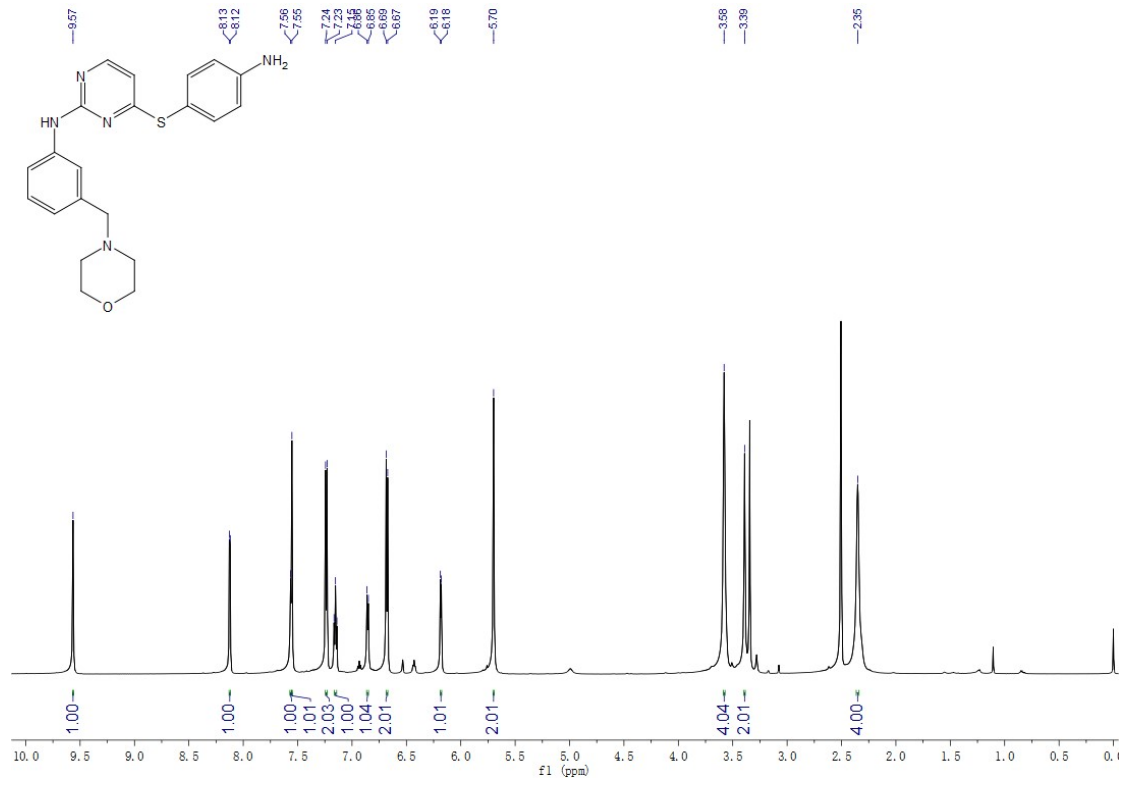


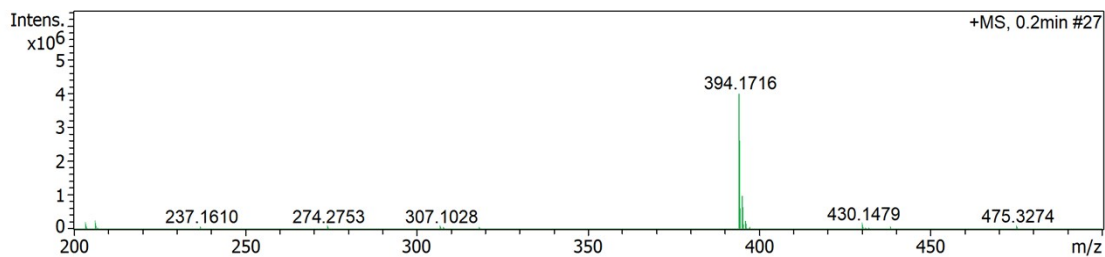
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.44 (s, 1H), 8.11 (s, 1H), 7.30 (s, 1H), 7.23 (d, *J* = 7.7 Hz, 2H), 7.17 (d, *J* = 7.7 Hz, 1H), 7.06 (t, *J* = 7.8 Hz, 1H), 6.67 (d, *J* = 7.7 Hz, 2H), 6.53 (d, *J* = 7.6 Hz, 1H), 6.13 (d, *J* = 4.3 Hz, 1H), 5.69 (s, 2H), 3.74 (s, 4H), 3.05 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 174.6, 159.5, 157.4, 151.8, 151.4, 141.5, 137.6, 129.4, 115.3, 110.8, 110.7, 109.3, 107.5, 106.2, 66.6, 49.1.

HRMS (ESI, *m/z*) calcd for C₂₀H₂₁N₃OS, [M+H]⁺: 380.1540; found: 380.1561.

8e



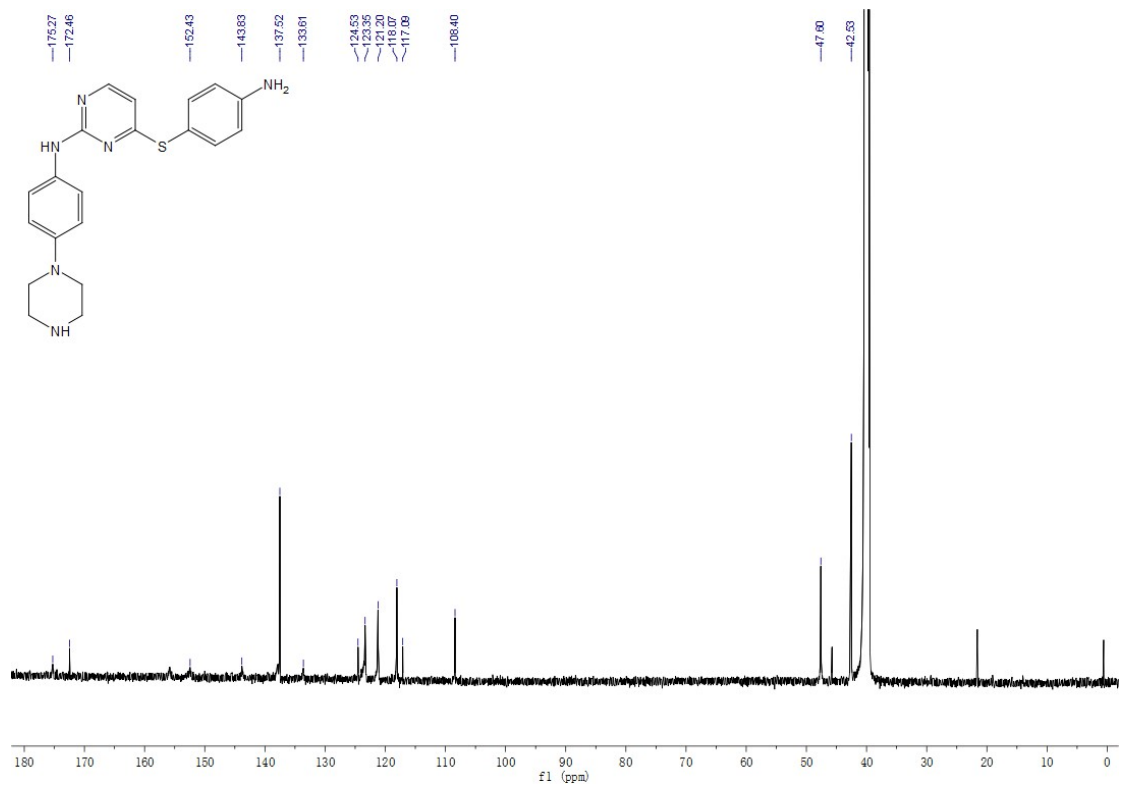
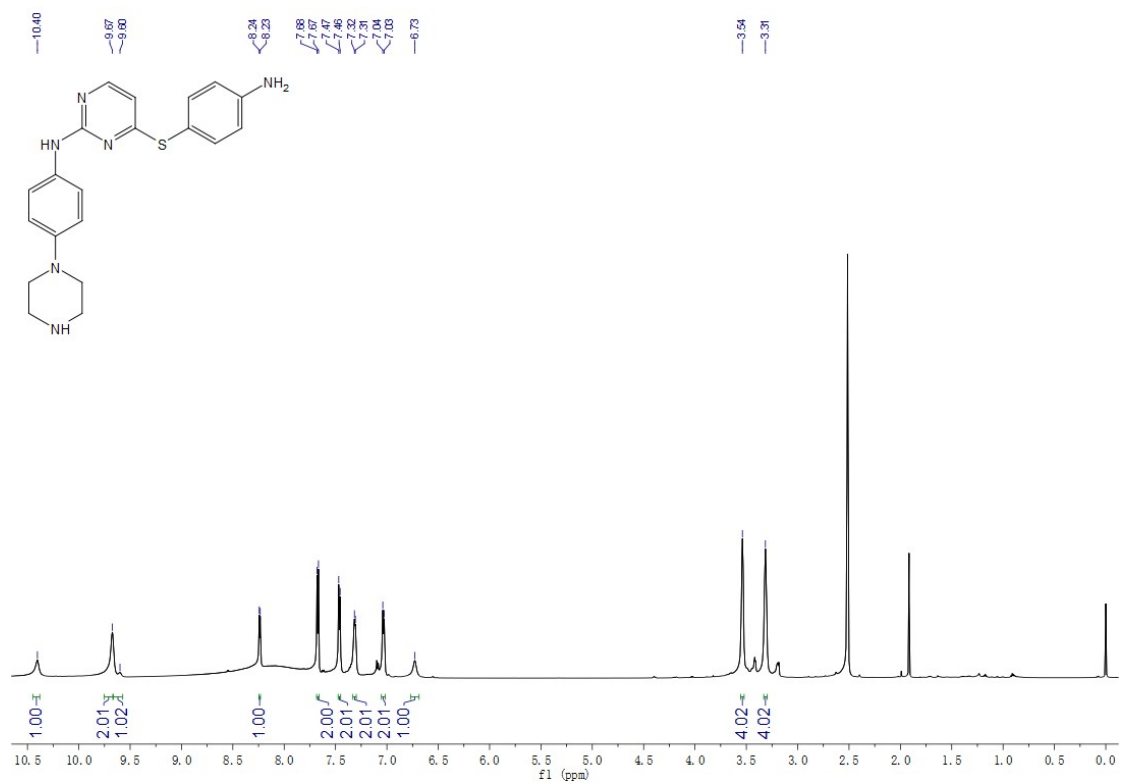


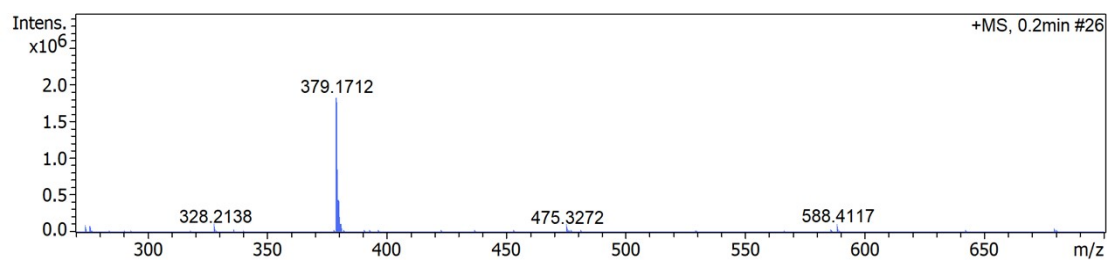
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.57 (s, 1H), 8.12 (d, *J* = 5.3 Hz, 1H), 7.56 (s, 1H), 7.55 (s, 1H), 7.24 (d, *J* = 8.3 Hz, 2H), 7.15 (s, 1H), 6.86 (d, *J* = 7.3 Hz, 1H), 6.68 (d, *J* = 8.4 Hz, 2H), 6.18 (d, *J* = 5.2 Hz, 1H), 5.70 (s, 2H), 3.58 (s, 4H), 3.39 (s, 2H), 2.35 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 173.5, 158.4, 156.3, 150.3, 139.6, 137.3, 136.6, 127.6, 121.3, 118.6, 116.9, 114.2, 109.8, 106.6, 65.6, 62.0, 52.6.

HRMS (ESI, *m/z*) calcd for C₂₁H₂₃N₃OS, [M+H]⁺: 394.1696; found: 394.1716.

8f



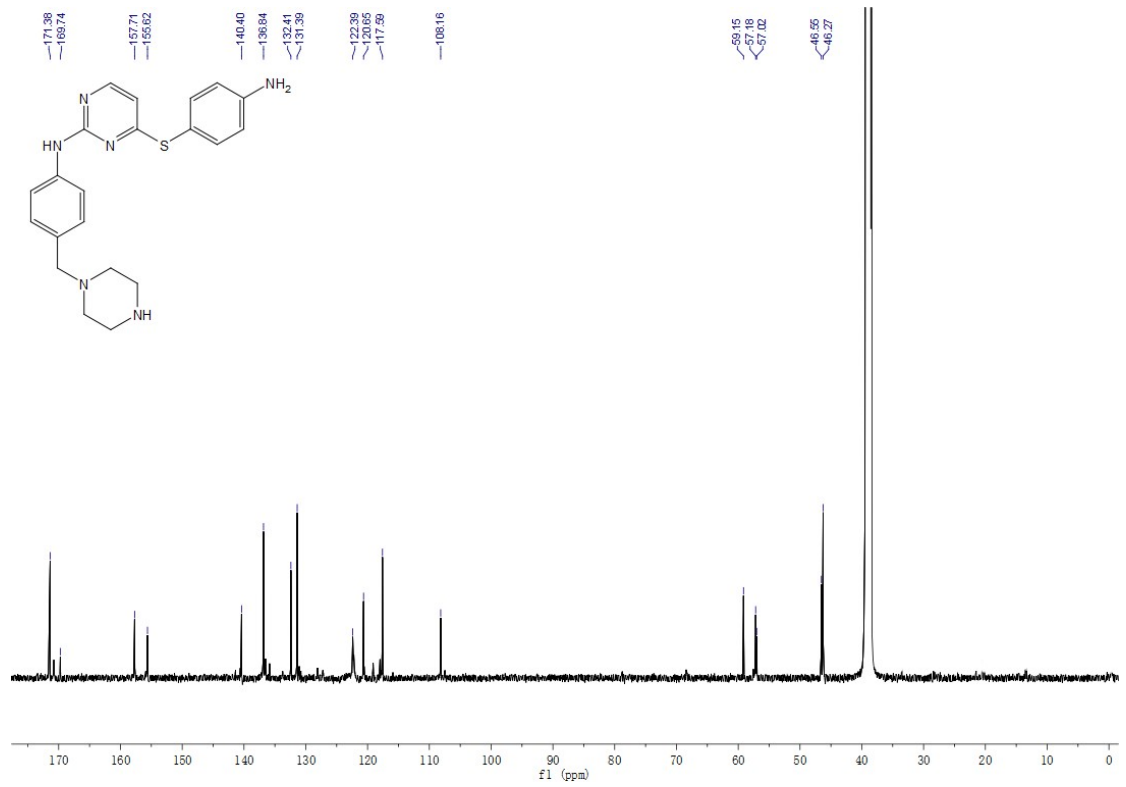
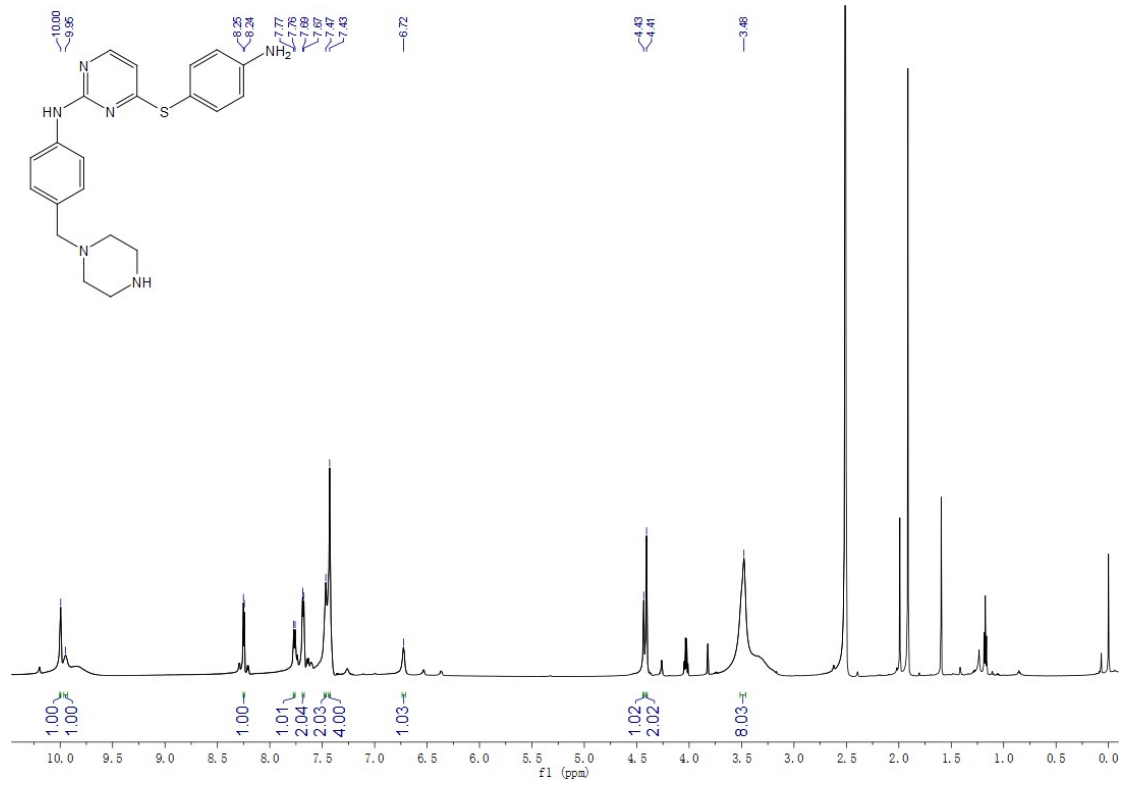


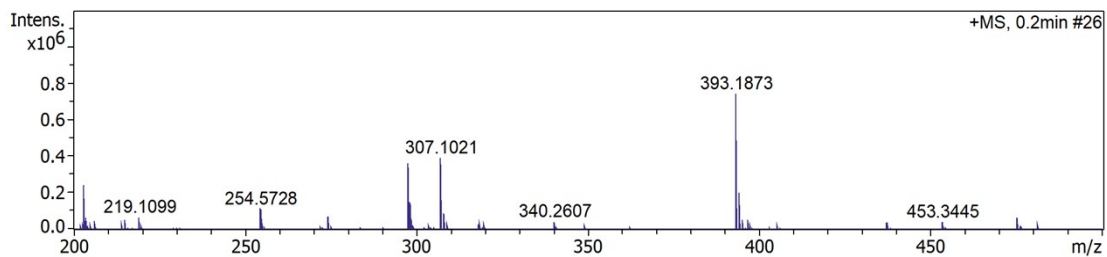
¹H NMR (600 MHz, DMSO-*d*₆) δ 10.40 (s, 1H), 9.67 (s, 2H), 9.60 (s, 1H), 8.24 (d, *J* = 5.8 Hz, 1H), 7.67 (d, *J* = 8.3 Hz, 2H), 7.46 (d, *J* = 8.1 Hz, 2H), 7.31 (d, *J* = 5.5 Hz, 2H), 7.03 (d, *J* = 8.4 Hz, 2H), 6.73 (s, 1H), 3.54 (s, 4H), 3.31 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 175.3, 172.5, 152.4, 143.8, 137.5, 133.6, 124.5, 123.4, 121.2, 118.1, 117.1, 108.4, 47.6, 42.5.

HRMS (ESI, *m/z*) calcd for C₂₀H₂₂N₆S, [M+H]⁺: 379.1699; found: 379.1712.

8g



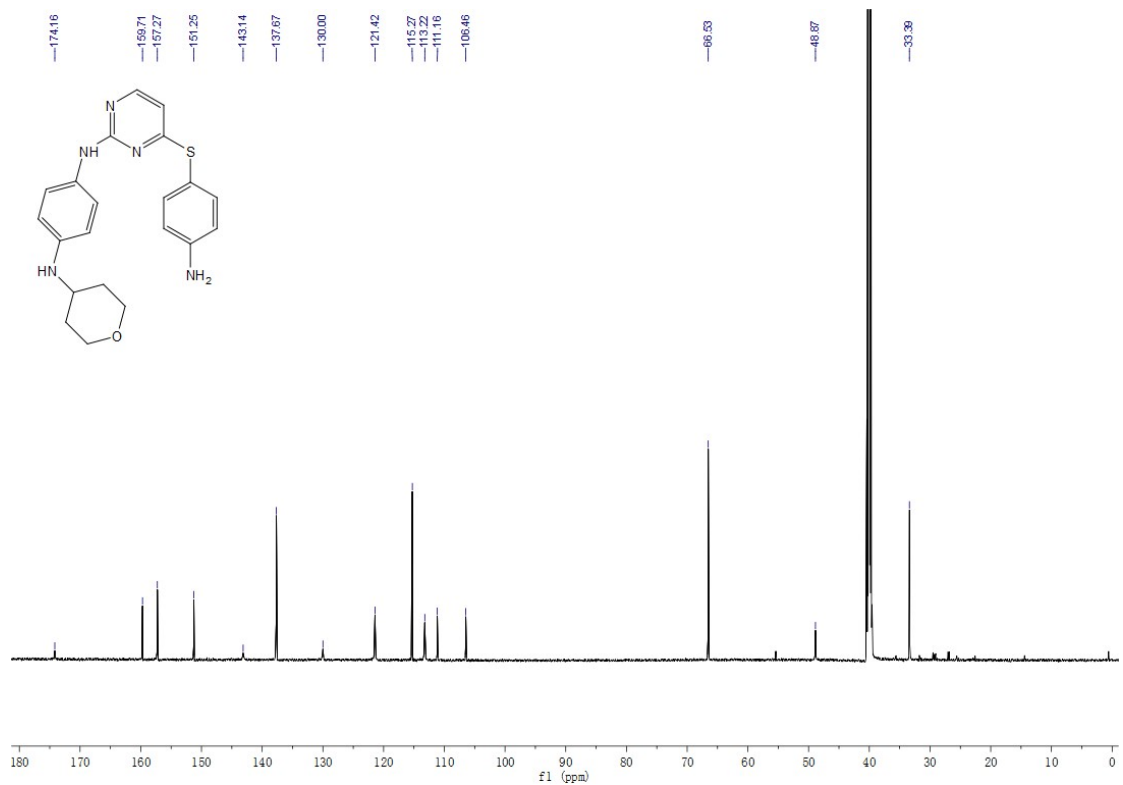
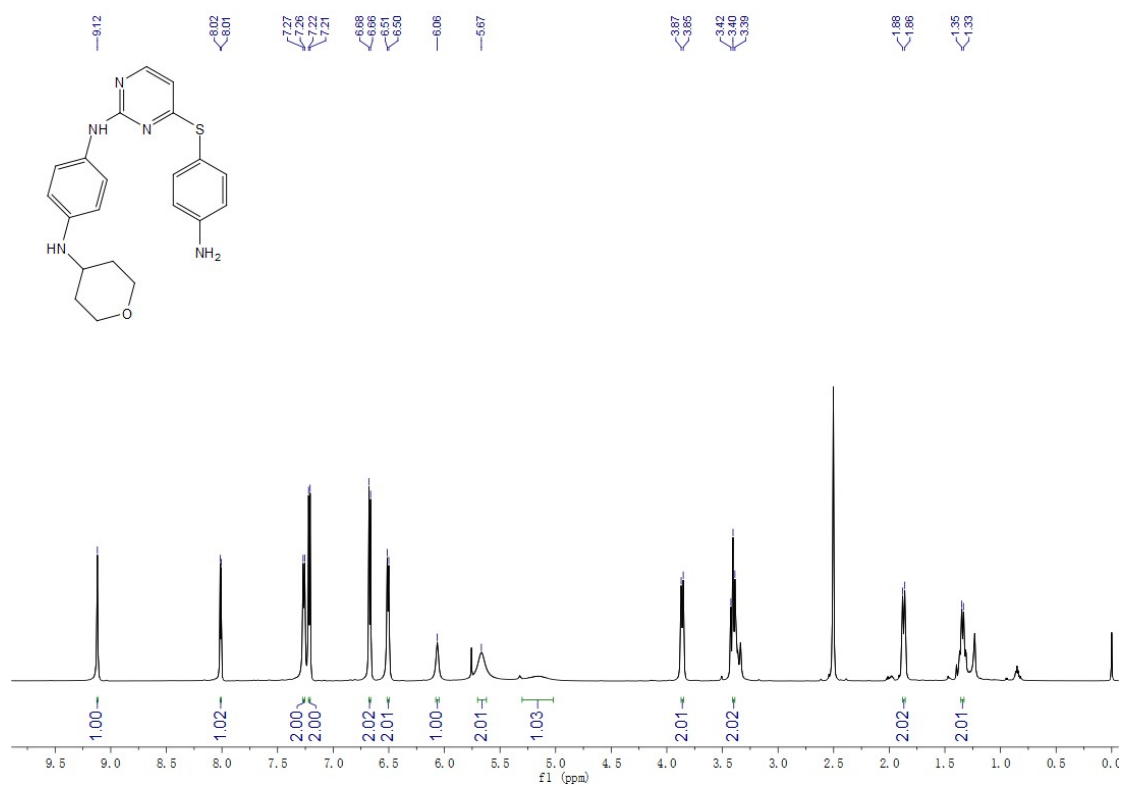


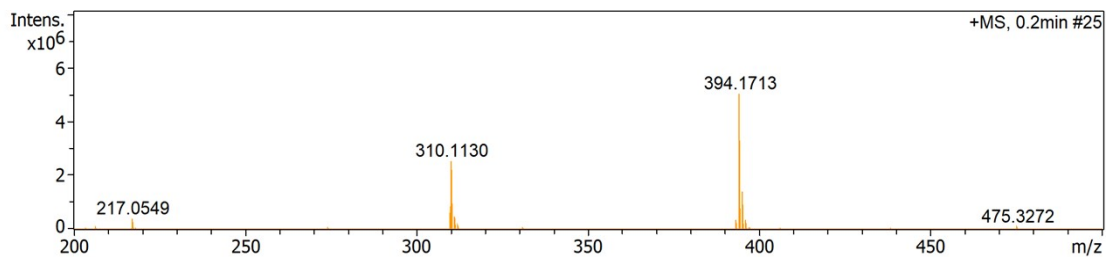
¹H NMR (600 MHz, DMSO-*d*₆) δ 10.00 (s, 1H), 9.95 (1H), 8.25 (d, *J* = 5.4 Hz, 1H), 7.77 (d, *J* = 7.9 Hz, 1H), 7.68 (d, *J* = 7.1 Hz, 2H), 7.47 (s, 2H), 7.43 (s, 4H), 6.72 (s, 1H), 4.43 (s, 1H), 4.41 (s, 2H), 3.48 (s, 8H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 171.4, 169.7, 157.7, 155.6, 140.4, 136.8, 132.4, 131.4, 122.4, 120.7, 117.6, 108.2, 59.2, 57.2, 46.4.

HRMS (ESI, *m/z*) calcd for C₂₁H₂₄N₆S, [M+H]⁺: 393.1856; found: 393.1873.

8h



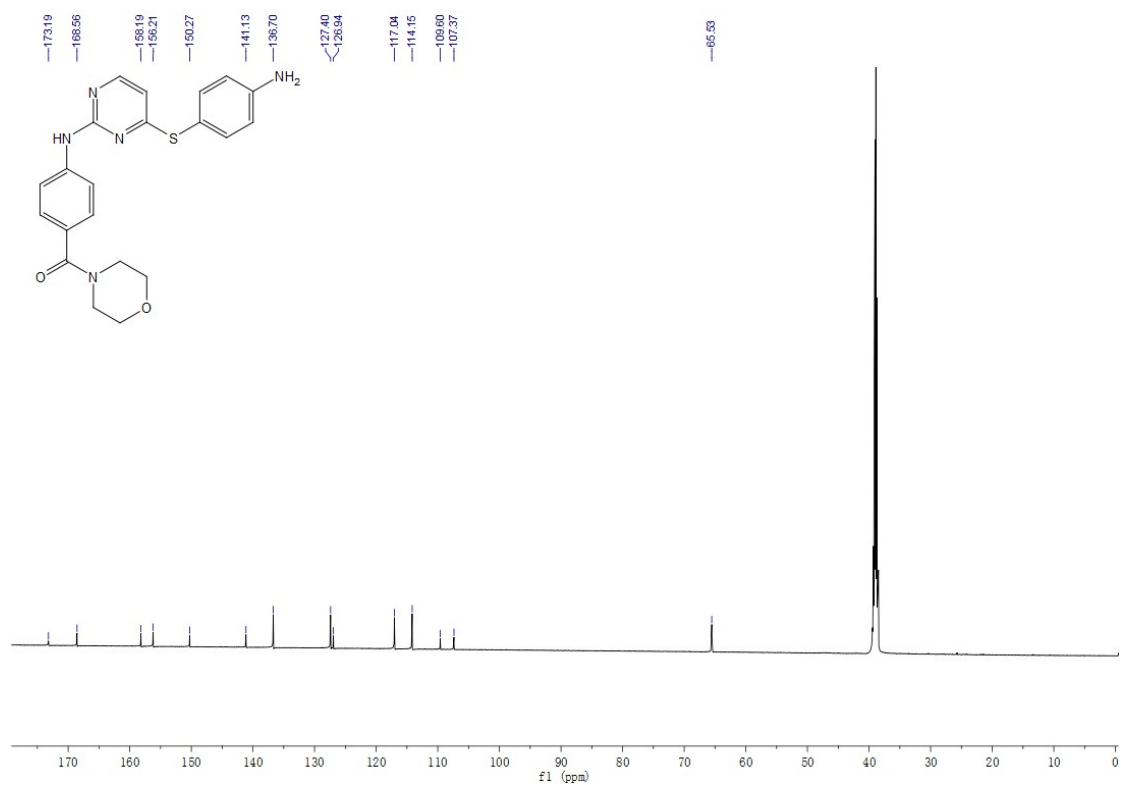
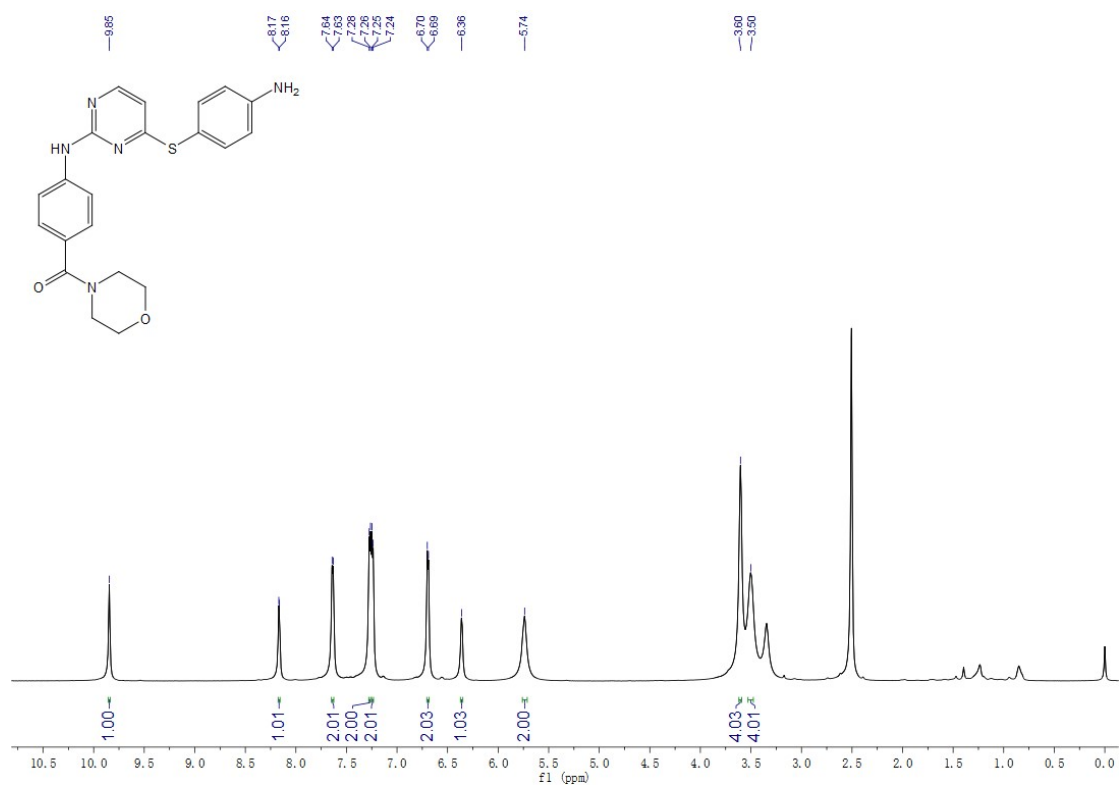


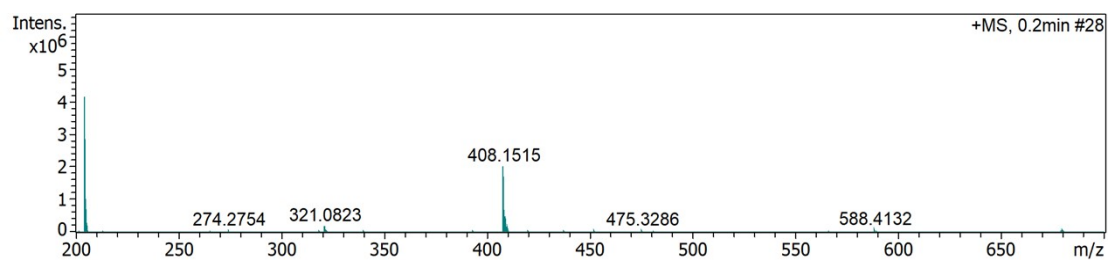
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.12 (s, 1H), 8.01 (d, *J* = 5.3 Hz, 1H), 7.26 (d, *J* = 8.3 Hz, 2H), 7.21 (d, *J* = 8.4 Hz, 2H), 6.67 (d, *J* = 8.4 Hz, 2H), 6.51 (d, *J* = 8.4 Hz, 2H), 6.06 (s, 1H), 5.67 (s, 2H), 5.15 (s, 1H), 3.86 (d, *J* = 11.4 Hz, 2H), 3.40 (s, 2H), 1.87 (d, *J* = 12.0 Hz, 2H), 1.34 (d, *J* = 9.2 Hz, 2H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 174.2, 159.7, 157.3, 151.3, 143.1, 137.7, 130.0, 121.4, 115.3, 113.2, 111.2, 106.5, 66.5, 48.9, 33.4.

HRMS (ESI, *m/z*) calcd for C₂₁H₂₃N₃OS, [M+H]⁺: 394.1696; found: 394.1713.

8i



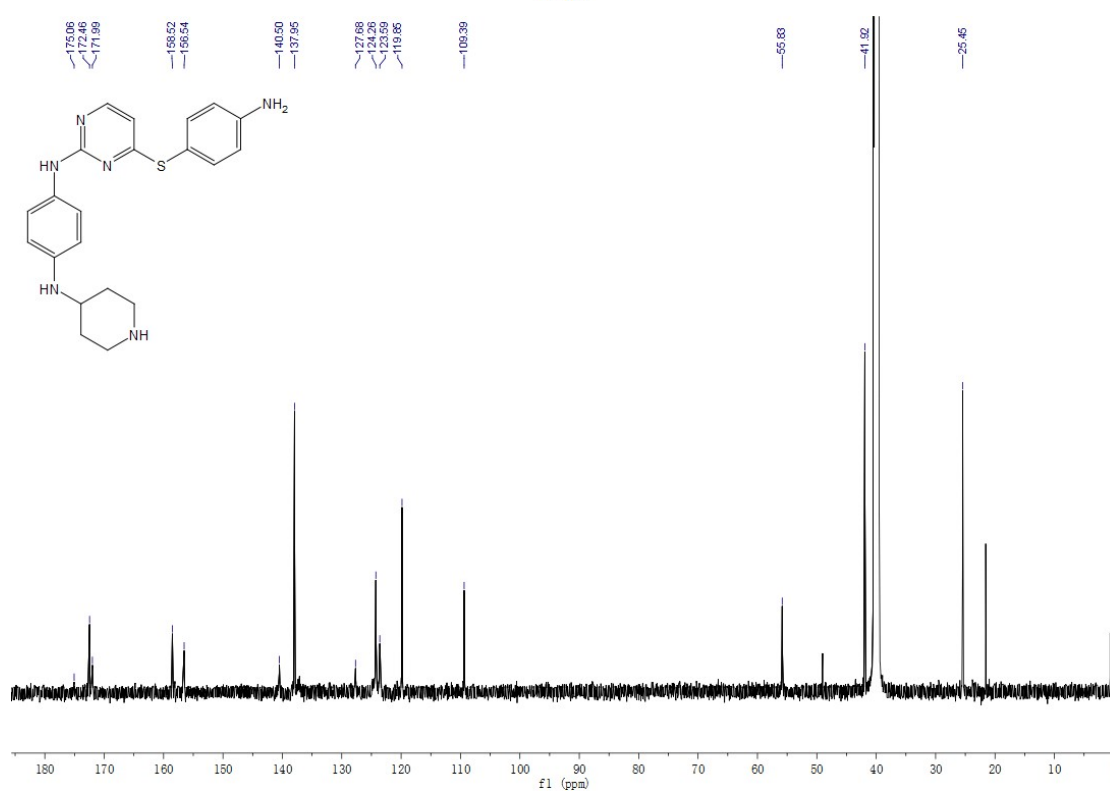
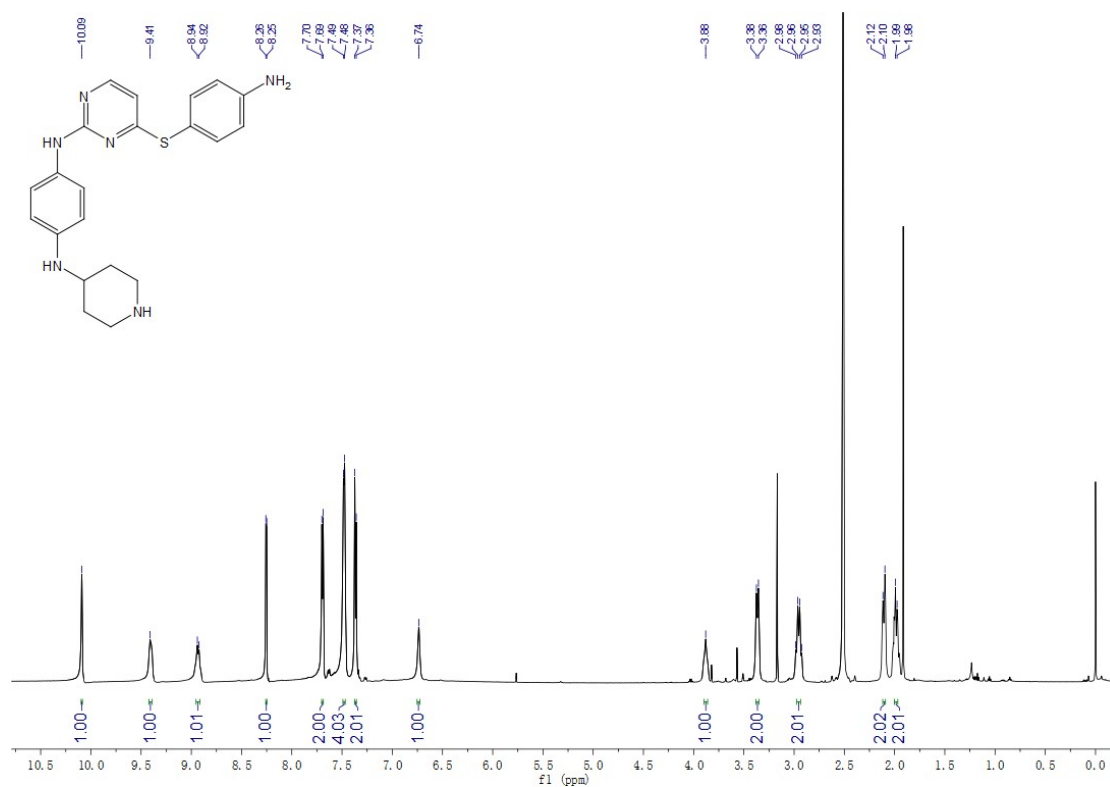


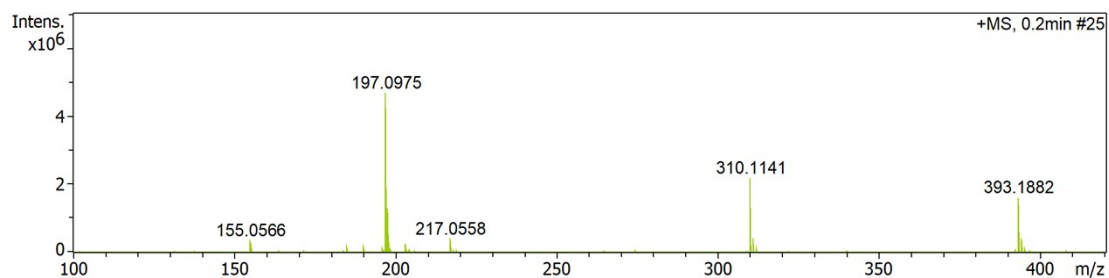
¹H NMR (600 MHz, DMSO-*d*₆) δ 9.85 (s, 1H), 8.17 (d, *J* = 3.9 Hz, 1H), 7.64 (d, *J* = 6.7 Hz, 2H), 7.27 (d, *J* = 7.7 Hz, 2H), 7.24 (d, *J* = 7.4 Hz, 2H), 6.69 (d, *J* = 7.1 Hz, 2H), 6.36 (s, 1H), 5.74 (s, 2H), 3.60 (s, 4H), 3.50 (s, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 170.9, 168.6, 158.2, 156.2, 150.3, 141.1, 136.7, 127.4, 126.9, 117.0, 114.2, 109.6, 107.4, 65.5.

HRMS (ESI, *m/z*) calcd for C₂₁H₂₁N₅O₂S, [M+H]⁺: 408.1489; found: 408.1515.

8j



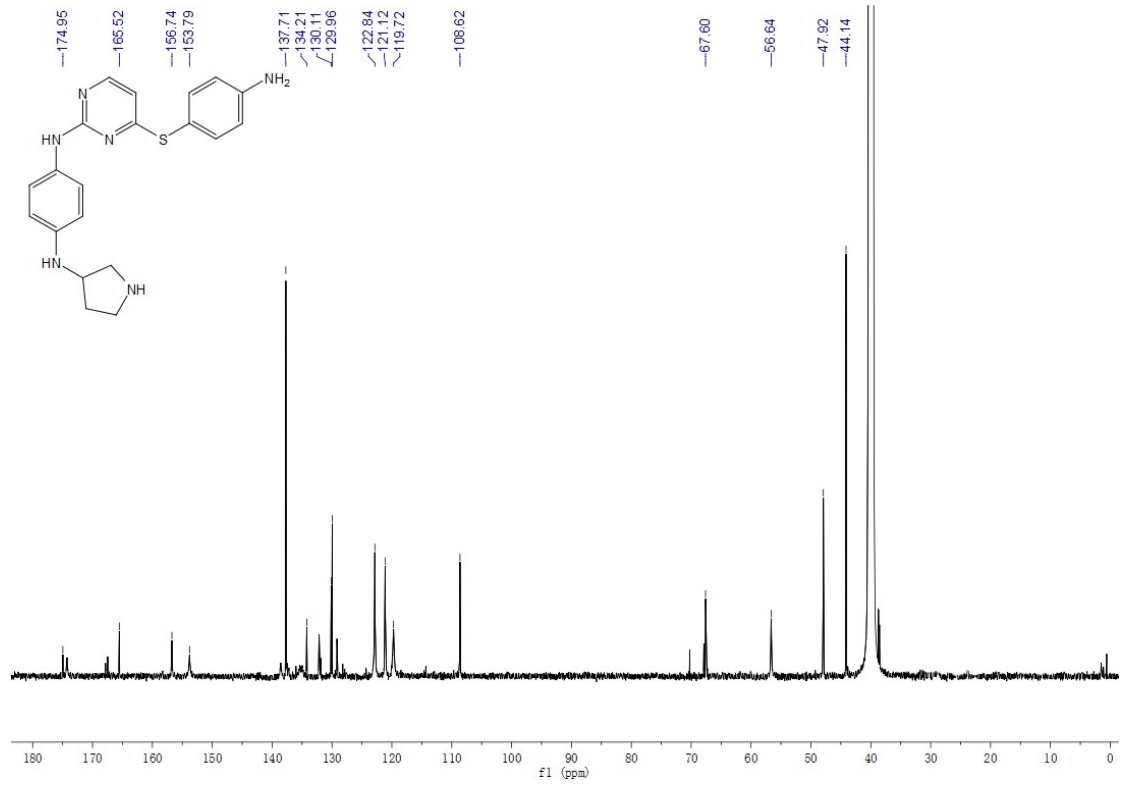
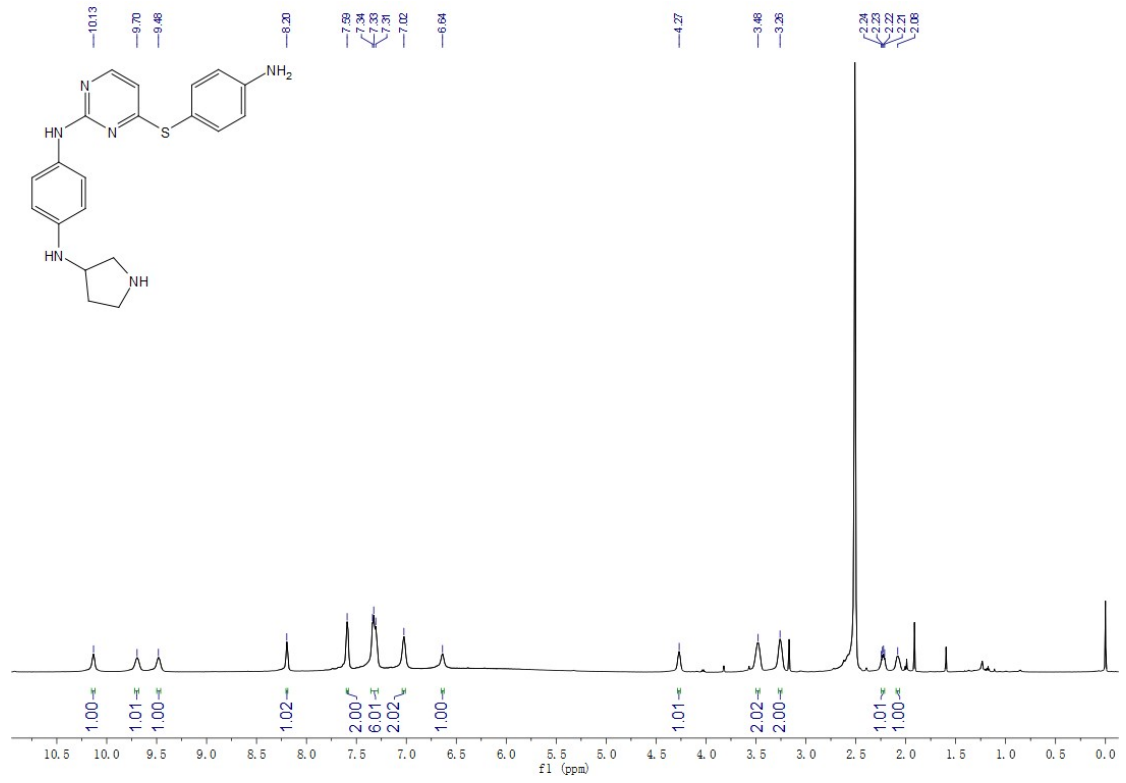


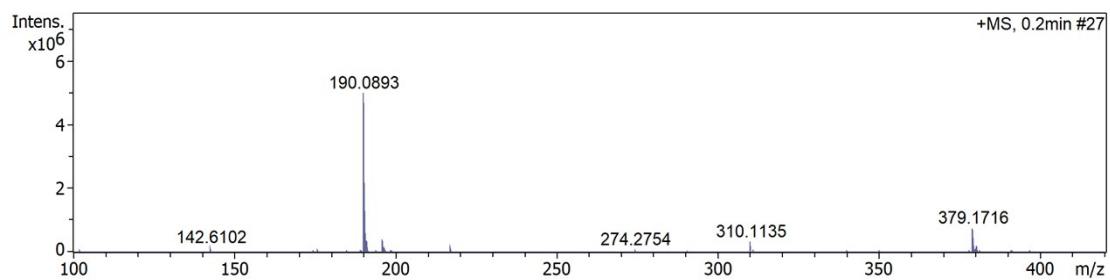
¹H NMR (600 MHz, DMSO-*d*₆) δ 10.09 (s, 1H), 9.41 (s, 1H), 8.93 (d, *J* = 9.8 Hz, 1H), 8.25 (d, *J* = 5.4 Hz, 1H), 7.69 (d, *J* = 7.4 Hz, 2H), 7.48 (d, *J* = 6.7 Hz, 4H), 7.37 (d, *J* = 8.8 Hz, 2H), 6.74 (s, 1H), 3.88 (s, 1H), 3.37 (d, *J* = 12.0 Hz, 2H), 2.95 (m, 2H), 2.11 (d, *J* = 11.7 Hz, 2H), 1.98 (d, *J* = 9.5 Hz, 2H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 172.5, 172.5, 172.0, 158.5, 156.5, 140.5, 137.9, 127.9, 124.3, 123.6, 119.9, 109.4, 55.8, 41.9, 25.5.

HRMS (ESI, *m/z*) calcd for C₂₁H₂₄N₆S, [M+H]⁺: 393.1856; found: 393.1882.

8k

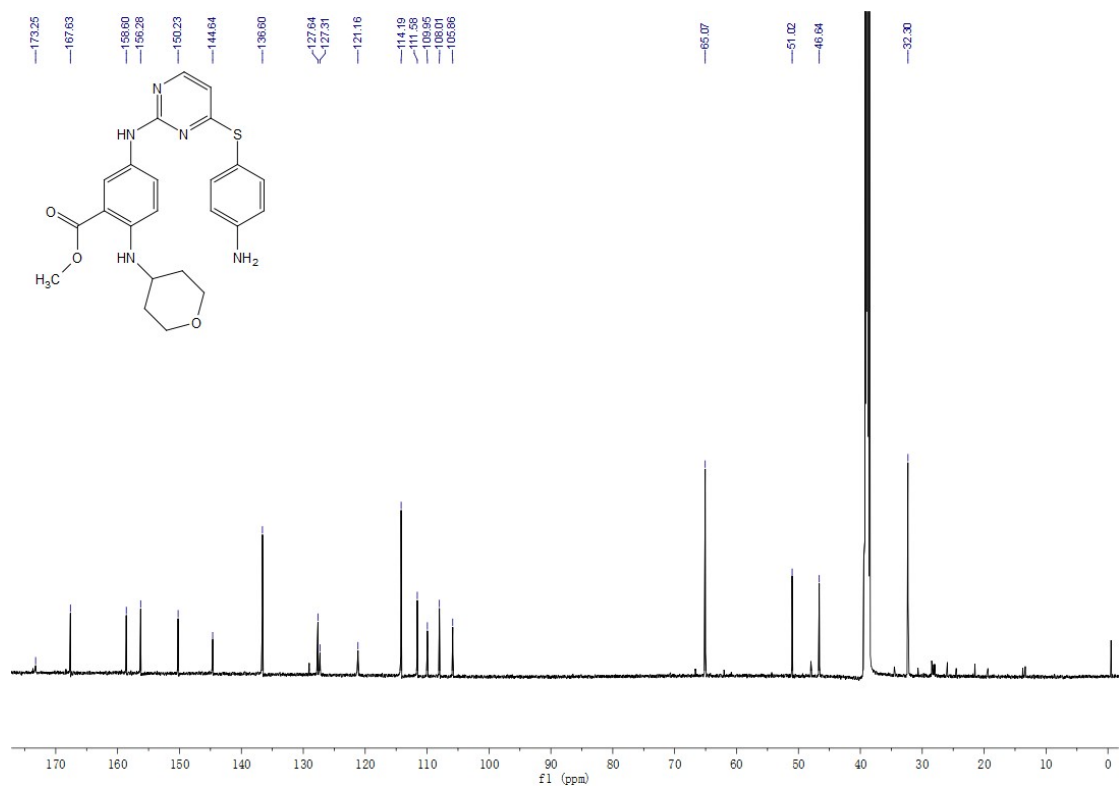
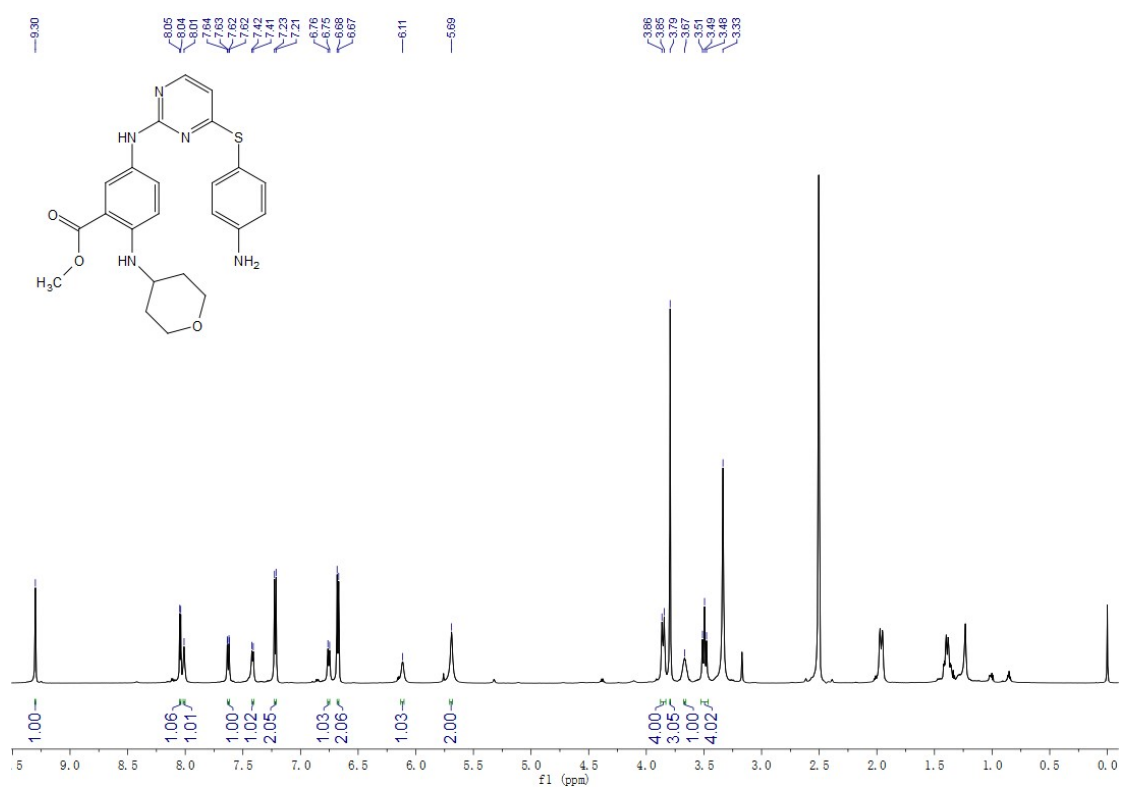


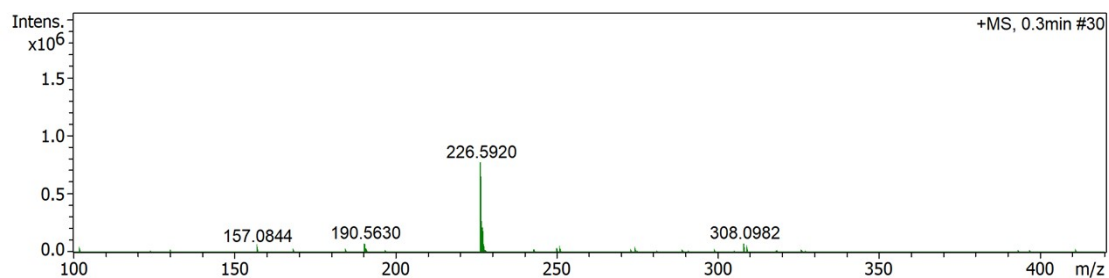


¹H NMR (600 MHz, DMSO-*d*₆) δ 10.13 (s, 1H), 9.70 (s, 1H), 9.48 (s, 1H), 8.20 (s, 1H), 7.59 (s, 2H), 7.36 – 7.28 (m, 6H), 7.02 (s, 2H), 6.64 (s, 1H), 4.27 (s, 1H), 3.48 (s, 2H), 3.26 (s, 2H), 2.25 – 2.21 (m, 1H), 2.08 (s, 1H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 174.9, 165.5, 156.7, 153.8, 137.7, 134.2, 130.1, 123.0, 122.8, 121.1, 119.7, 108.6, 67.6, 56.6, 47.9, 44.1.

HRMS (ESI, *m/z*) calcd for C₂₀H₂₂N₆S, [M+H]⁺: 379.1699; found: 379.1716.



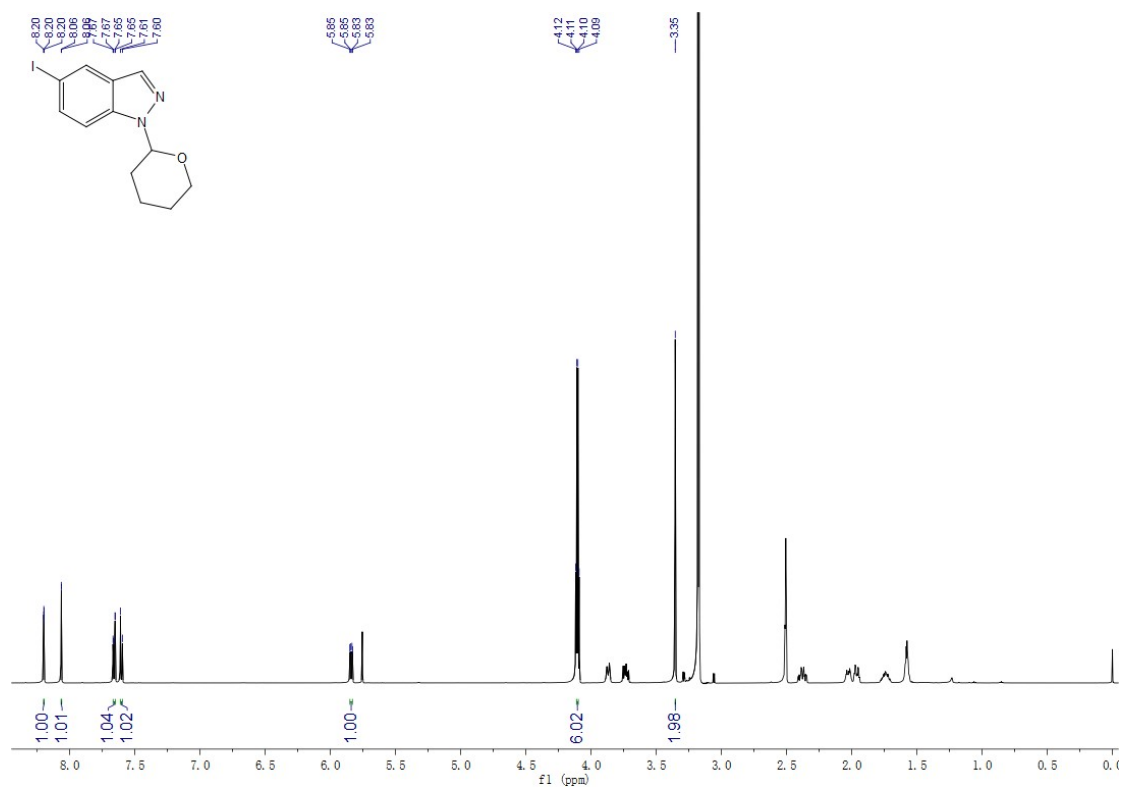


¹H NMR (600 MHz, DMSO-*d*₆) δ 9.30 (s, 1H), 8.04 (d, *J* = 5.3 Hz, 1H), 8.01 (s, 1H), 7.63 (d, *J* = 6.8 Hz, 1H), 7.42 (d, *J* = 7.5 Hz, 1H), 7.22 (d, *J* = 8.2 Hz, 2H), 6.76 (d, *J* = 9.1 Hz, 1H), 6.68 (d, *J* = 8.2 Hz, 2H), 6.11 (s, 1H), 5.69 (s, 2H), 3.85 (d, *J* = 11.5 Hz, 4H), 3.79 (s, 3H), 3.67 (s, 1H), 3.49 (t, *J* = 10.5 Hz, 4H).

¹³C NMR (151 MHz, DMSO-*d*₆) δ 173.3, 167.6, 158.6, 156.3, 150.2, 144.6, 136.6, 127.6, 127.3, 121.2, 114.2, 111.6, 110.0, 108.0, 105.9, 65.1, 51.0, 46.6, 32.3.

HRMS (ESI, *m/z*) calcd for C₂₃H₂₅N₅O₃S, [M/2+H]⁺: 226.5839; found: 226.5920.

5



¹H NMR (600 MHz, DMSO-*d*₆) δ 8.20 (dd, *J* = 1.5, 0.6 Hz, 1H), 8.07 – 8.06 (m, 1H), 7.66 (d, *J* = 7.2 Hz, 1H), 7.60 (d, *J* = 8.8 Hz, 1H), 5.84 (d, *J* = 7.1 Hz, 1H), 4.11 (m, 6H), 3.35 (s, 2H).

Table S1 In vitro CYPs inhibition of compound **centrinone**^a.

Isozyme	CYP1A2	CYP2C9	CYP2C19	CYP2D6	CYP3A4
Inhibition at 10 μM (%)	44.7	91.1	78.7	27.0	62.9

^a α -Naphthoflavone (CYP1A2, 83.5% inhibition), sulfaphenazole (CYP2C9, 85.1% inhibition), (+)-N-3-benzylrivanol (CYP2C19, 82.6% inhibition), quinidine (CYP2D6, 94.8% inhibition), and ketoconazole (CYP3A4, 98.8% inhibition) were used as the positive controls.

Table S2 Liver microsomal stability of compound **centrinone**^a.

Compound	R ²	$t_{1/2}$	CL _{int(mic)} (μ L/min/mg)	CL _{int(liver)} (μ L/min/mg)	Remaining (%) (T = 60min)	Remaining (%) (NCF ^b , T = 60min)
centrinone	0.94	5.8	240.1	216.1	1.7	98.6

^a Testosterone ($t_{1/2}$ = 11.4 min), diclofenac ($t_{1/2}$ = 3.2 min), and propafenone ($t_{1/2}$ = 4.8 min) were used as the positive controls. ^b NCF: abbreviation of no cofactor. No NADPH was added to NCF samples (replaced by buffer) during the 60 min incubation.

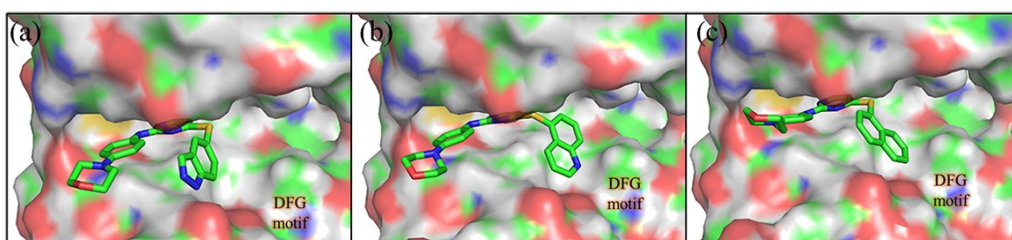


Fig. S1 (a) Binding mode of **3n** in the ATP-binding site of PLK4 (PDB code: 4YUR, surface mode). (e) Binding mode of **3o** in the ATP-binding site of PLK4 (PDB code: 4YUR, surface mode). (f) Binding mode of **3p** in the ATP-binding site of PLK4 (PDB code: 4YUR, surface mode).

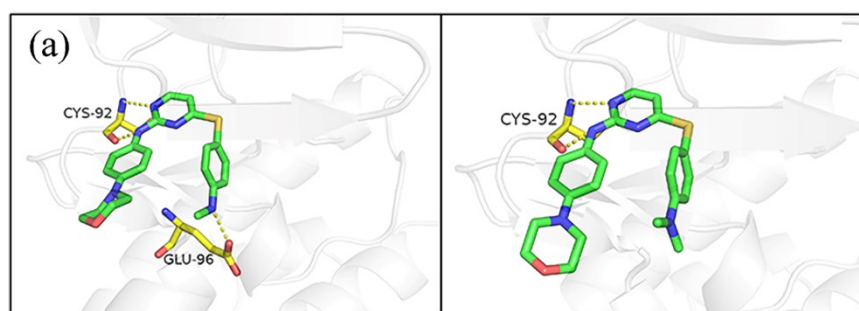


Fig. S2 (a) Binding mode of **3u** in the ATP-binding site of PLK4 (PDB code: 4YUR, colored grey). (b) Binding mode of **3v** in the ATP-binding site of PLK4 (PDB code: 4YUR, colored grey). Interactions are illustrated with yellow dashed lines. ligands are depicted by the element with carbons in green.

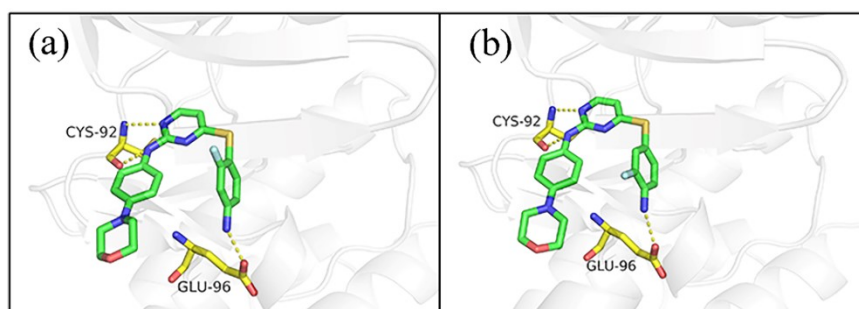


Fig. S3 (a) Binding mode of **3w** in the ATP-binding site of PLK4 (PDB code: 4YUR, colored grey). (b) Binding mode of **3x** in the ATP-binding site of PLK4 (PDB code: 4YUR, colored grey). Interactions are illustrated with yellow dashed lines. ligands are depicted by the element with carbons in green.