

Supporting Information

Synthesis and fundamental evaluation of radioiodinated rociletinib (CO-1686) as a probe to lung cancer with L858R/T790M mutations of epidermal growth factor receptor (EGFR)

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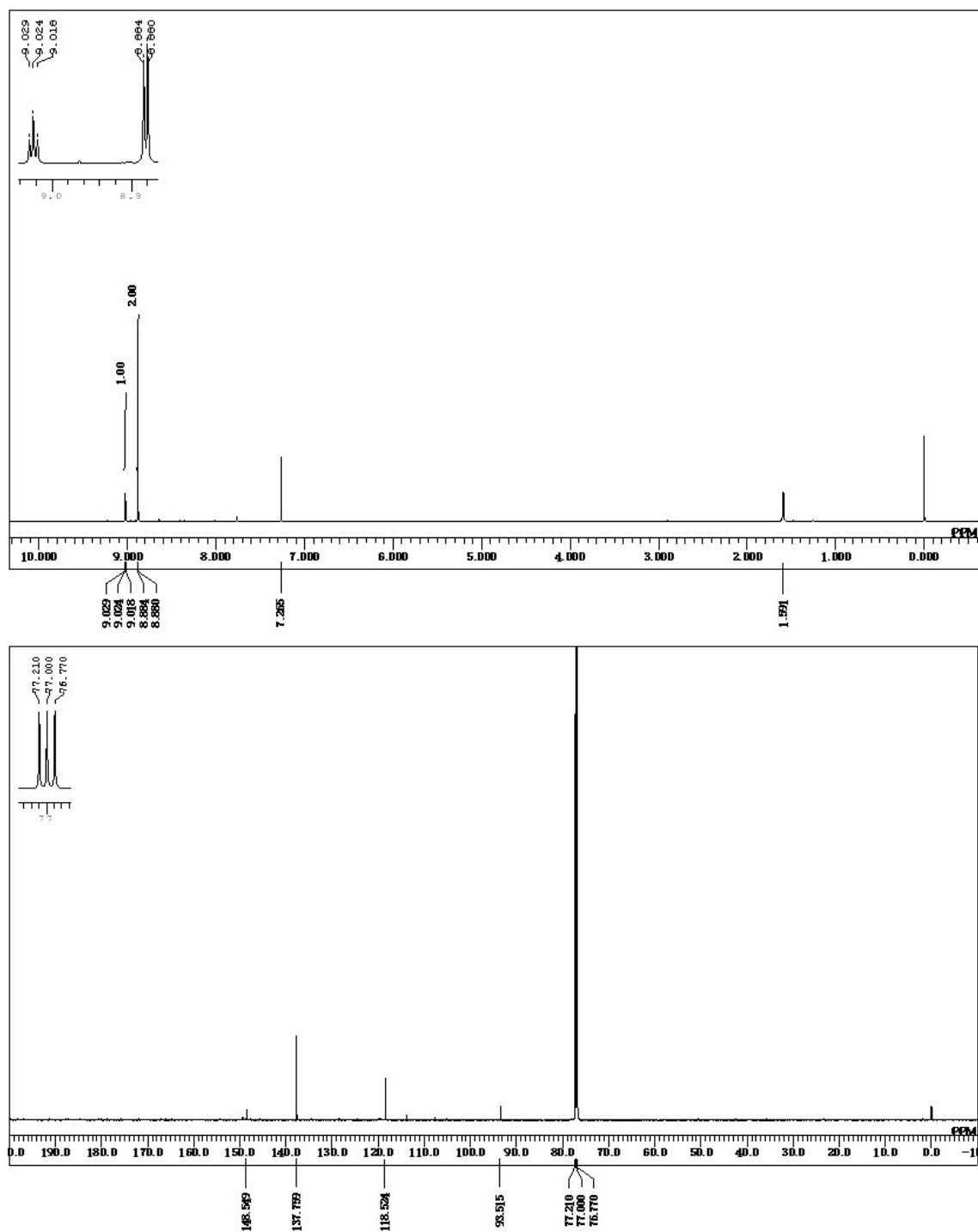


Figure S1. The proton and carbon NMR peak of 1-iodo-3,5-dinitrobenzene (1). ^1H NMR (400 MHz, CDCl_3): δ 8.88 (2H, d, $J = 1.6$ Hz), 9.02 (1H, t, $J = 2.0$ Hz). ^{13}C NMR (100 MHz, CDCl_3): δ 93.5, 118.5, 137.8, 148.5. HRMS (FAB+) calculated for $\text{C}_6\text{H}_3\text{IN}_2\text{O}_4$ $[\text{M} + \text{H}]^+$: $m/z = 294.9216$, found 294.9225.

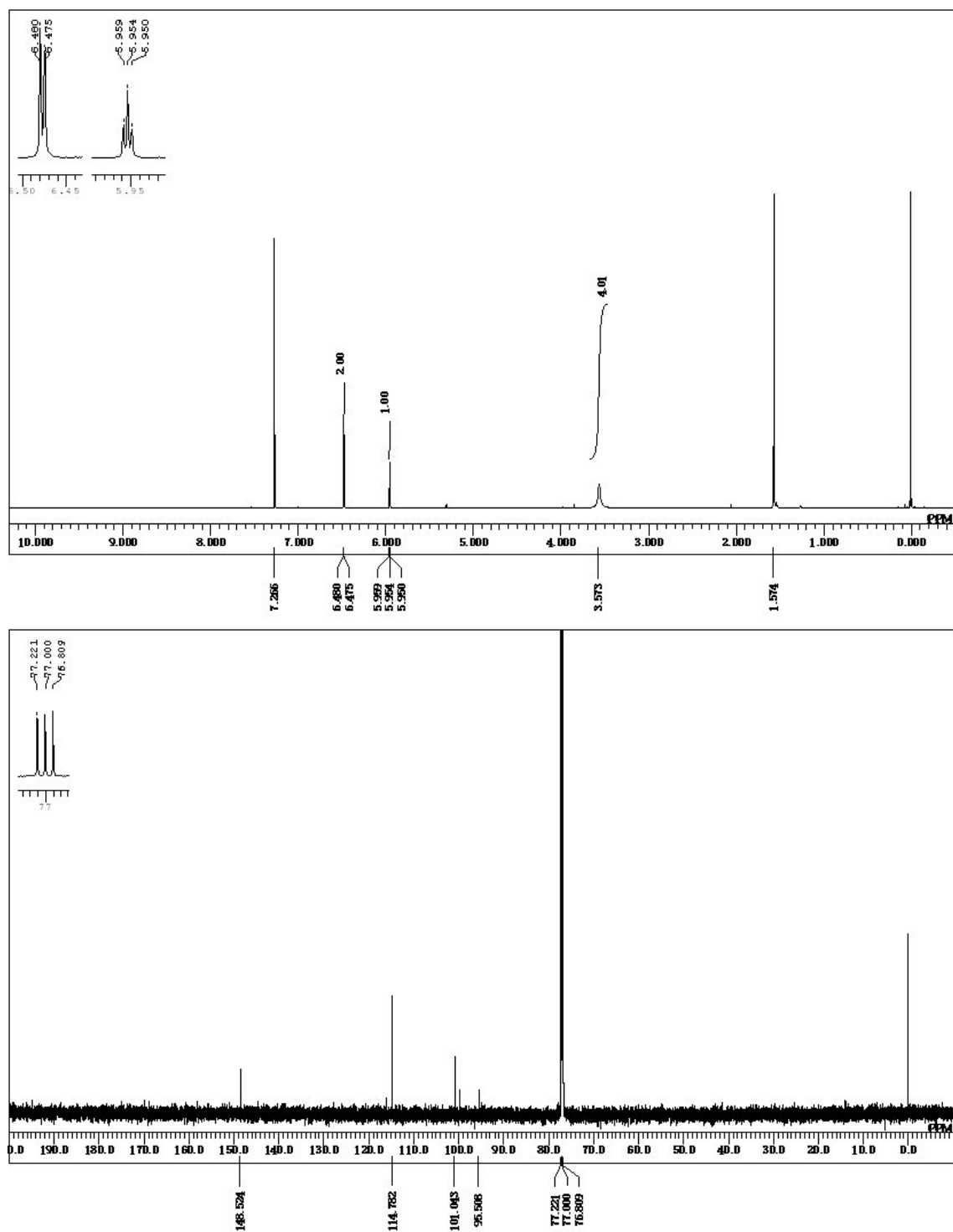


Figure S2. The proton and carbon NMR peak of 5-iodobenzene-1,3-diamine (**2**). ^1H NMR (400 MHz, CDCl_3): δ 3.57 (4H, s), 5.95 (1H, t, $J = 2.0$ Hz), 6.48 (2H, d, $J = 2.0$ Hz). ^{13}C NMR (100 MHz, CDCl_3): δ 95.5, 101.0, 114.8, 148.5. HRMS (FAB+) calculated for $\text{C}_6\text{H}_7\text{IN}_2$ $[\text{M} + \text{H}]^+$: $m/z = 233.9654$, found 233.9645.

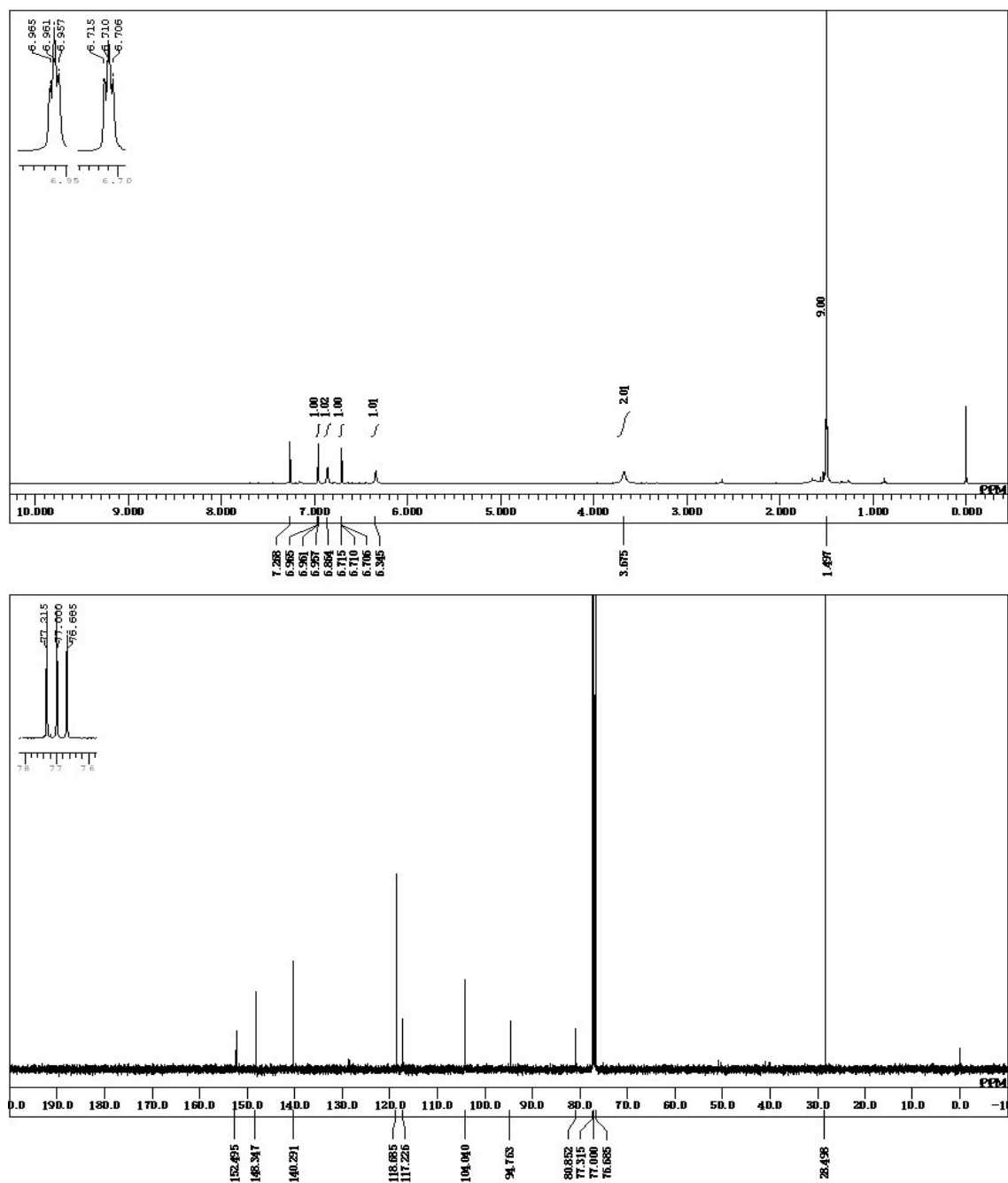


Figure S3. The proton and carbon NMR peak of tert-butyl (3-amino-5-iodophenyl)carbamate (**3**). ¹H NMR (400 MHz, CDCl₃): δ 1.50 (9H, s), 3.68 (2H, s), 6.34 (1H, s), 6.71 (1H, t, *J* = 1.6 Hz), 6.86 (1H, s), 6.96 (1H, t, *J* = 1.6 Hz). ¹³C NMR (100 MHz, CDCl₃): δ 28.5 (3C), 80.9, 94.8, 104.0, 117.2, 118.7, 140.3, 148.3, 152.5. HRMS (FAB+) calculated for C₁₁H₁₅IN₂O₂ [M+ H]⁺: *m/z* = 334.0178, found 334.0168.

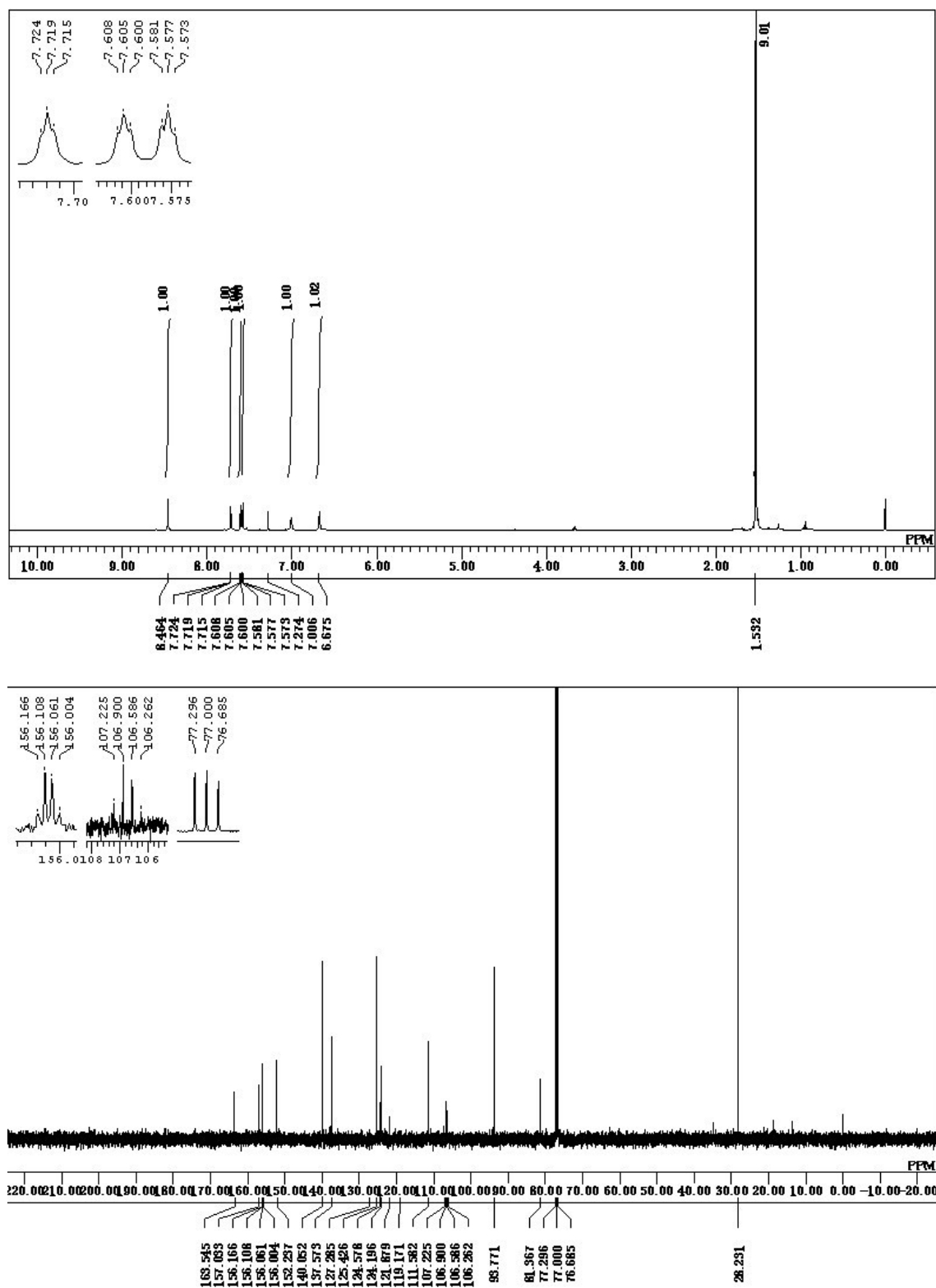


Figure S4. The proton and carbon NMR peak of tert-butyl (3-((2-chloro-5-(trifluoromethyl)pyrimidin-4-yl)amino)-5-iodophenyl)carbamate (**4**). ¹H NMR (400 MHz,

CDCl₃): δ 1.53 (9H, s), 6.68 (1H, s), 7.00 (1H, s), 7.58 (1H, t, *J* = 1.6 Hz), 7.61 (1H, t, *J* = 1.6 Hz), 7.72 (1H, t, *J* = 1.6 Hz), 8.46 (1H, s). ¹³C NMR (100 MHz, CDCl₃): δ 28.2 (3C), 81.4, 93.8, 106.7 (q, *J*_{CF} = 31.4 Hz), 111.6, 123.0 (q, *J*_{CF} = 270 Hz), 124.2, 125.4, 137.6, 140.1, 152.2, 156.1 (q, *J*_{CF} = 4.7 Hz), 157.0, 163.5. HRMS (FAB+) calculated for C₁₆H₁₅ClF₃IN₄O₂ [M+ H]⁺: *m/z* = 513.9880, found 513.9875.

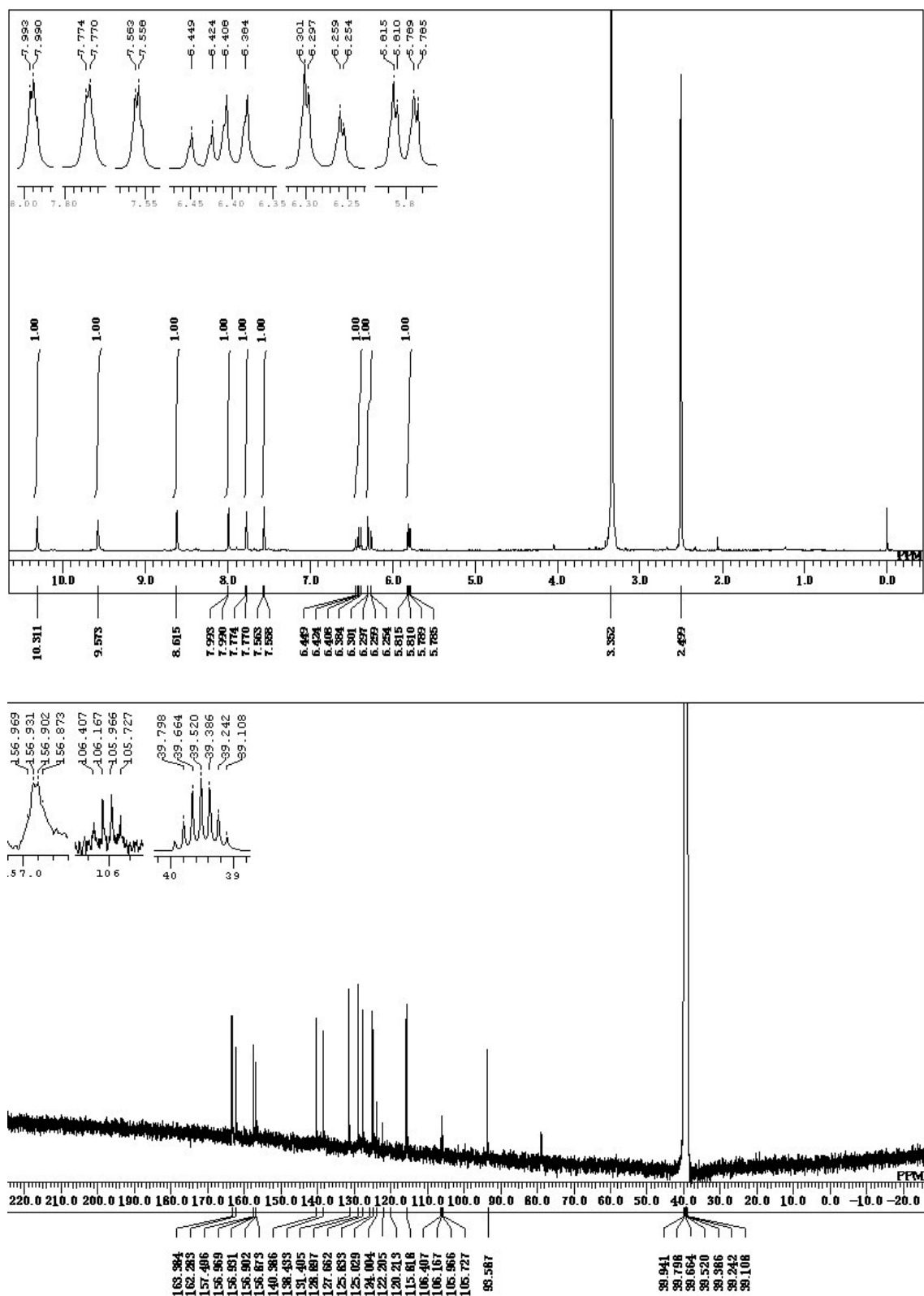


Figure S5. The proton and carbon NMR peak of N-(3-((2-chloro-5-(trifluoromethyl)pyrimidin-4-yl)amino)-5-iodophenyl) acrylamide (**5**). ¹H NMR (400

MHz, (CD₃)₂SO): δ 5.79 (1H, dd, *J* = 10.0, 2.0 Hz), 6.27 (1H, dd, *J* = 16.8, 2.0 Hz), 6.40 (1H, dd, *J* = 16.4, 10.0 Hz), 7.56 (1H, d, *J* = 2.0 Hz), 7.77 (1H, d, *J* = 2.0 Hz), 7.99 (1H, d, *J* = 2.0 Hz), 8.62 (1H, s), 9.57 (1H, s), 10.31 (1H, s). ¹³C NMR (150 MHz, (CD₃)₂SO): δ 93.6, 106.1 (q, *J*_{CF} = 30.2 Hz), 115.8, 123.0 (q, *J*_{CF} = 270 Hz), 125.0, 127.7, 128.9, 131.4, 138.4, 140.4, 156.9 (q, *J*_{CF} = 4.4 Hz), 157.5, 162.3, 163.4. HRMS (FAB+) calculated for C₁₄H₉ClF₃IN₄O [M+H]⁺: *m/z* = 468.9461, found 468.9559.

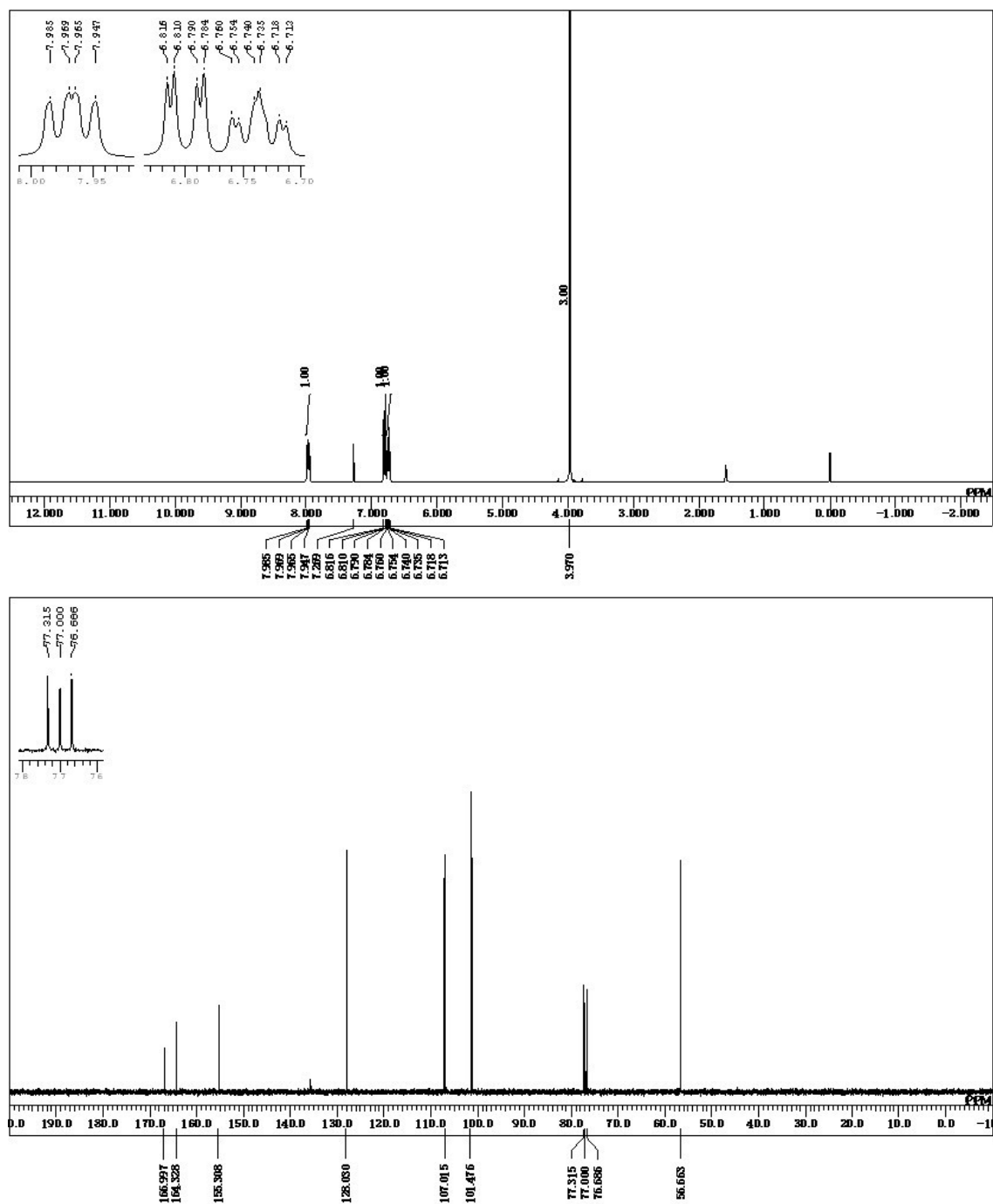


Figure S6. The proton and carbon NMR peak of 4-fluoro-2-methoxy-1-nitrobenzene (**6**). ^1H NMR (400 MHz, CDCl_3): δ 3.97 (3H, s), 6.74 (1H, dt, $J = 8.4, 2.0$ Hz), 6.80 (1H, dd, $J = 10.4, 2.4$ Hz), 7.96 (1H, dd, $J = 8.4, 6.4$ Hz). ^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{SO}$): δ 56.7, 101.5, 107.0, 128.0, 155.3, 164.3, 167.0. HRMS (DART+) calculated for $\text{C}_7\text{H}_6\text{FNO}_3$ $[\text{M} + \text{H}]^+$: $m/z = 172.0331$, found 172.0345.

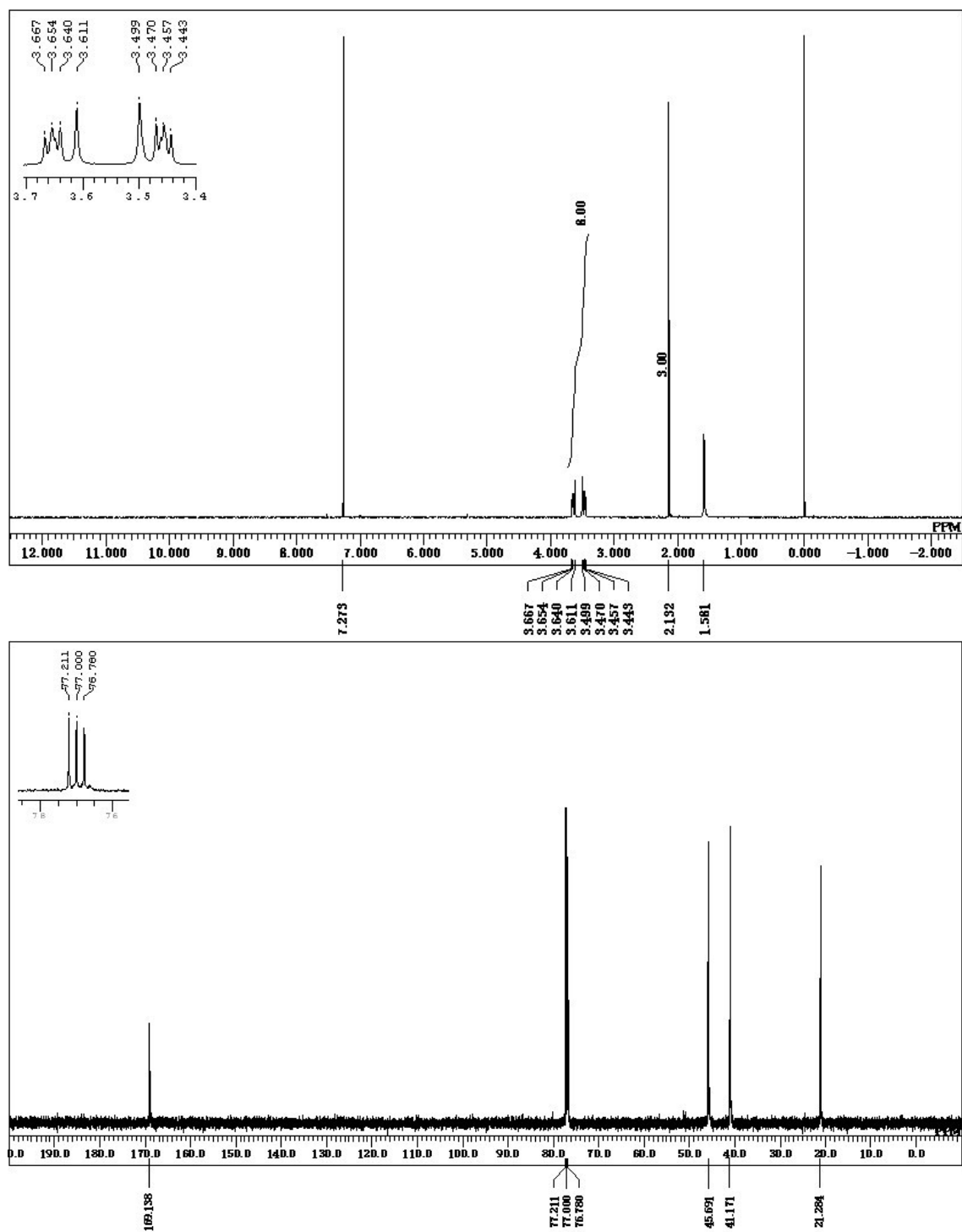


Figure S7. The proton and carbon NMR peak of 1-(piperazine-1-yl)ethan-1-one (**7**). ^1H NMR (400 MHz, CDCl_3): δ 2.13 (3H, s), 3.40-3.70 (8H, m). ^{13}C NMR (100 MHz, CDCl_3): δ 21.3, 41.2 (2C), 45.7 (2C), 169.1. HRMS (FAB+) calculated for $\text{C}_6\text{H}_{12}\text{N}_2\text{O}$ $[\text{M} + \text{H}]^+$: m/z = 129.0949, found 129.1031.

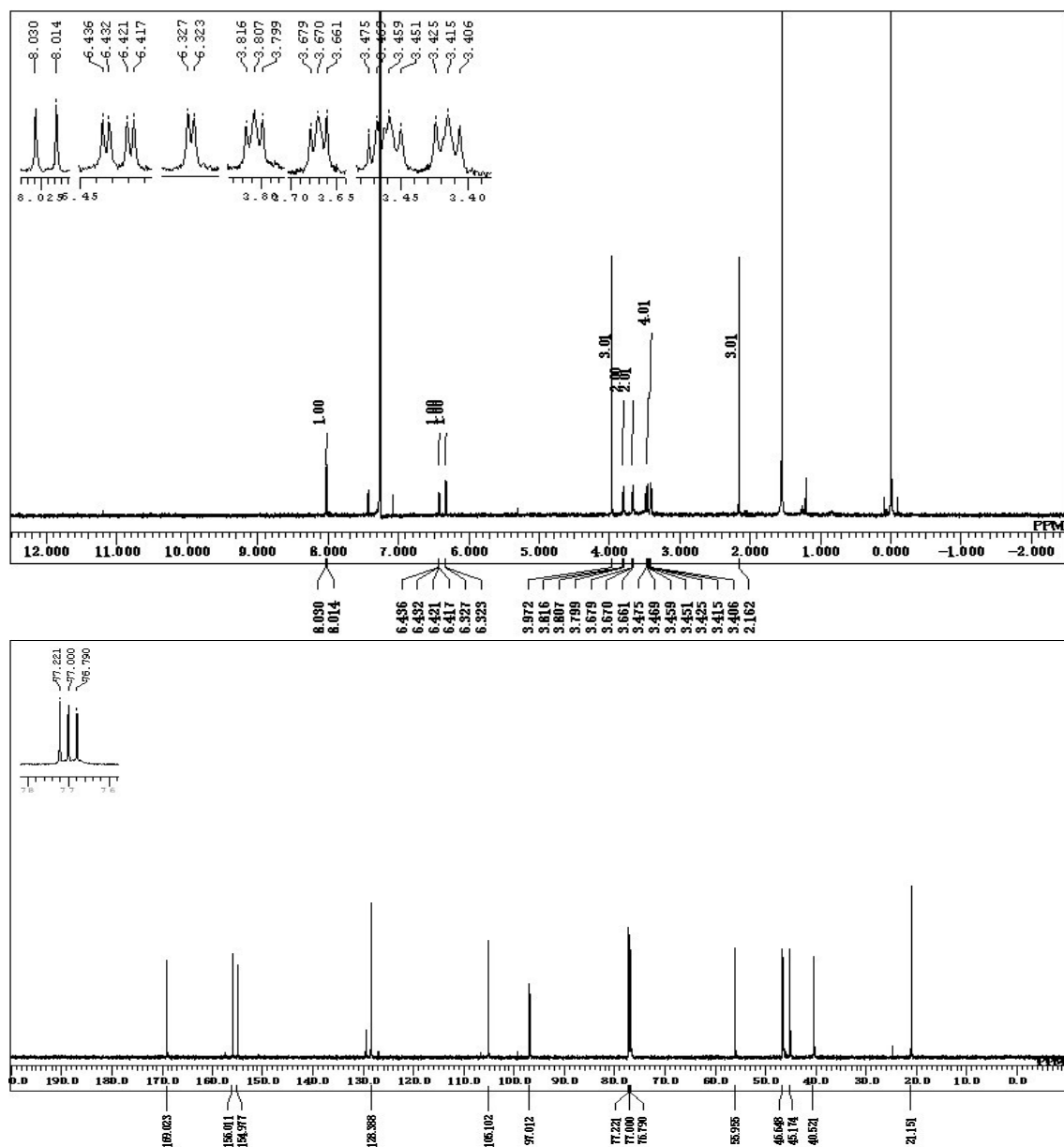


Figure S8. The proton and carbon NMR peak of 1-(4-(3-methoxy-4-nitrophenyl)piperazine-1-yl)ethan-1-one (**8**). ¹H NMR (400 MHz, CDCl₃): δ 2.16 (3H, s), 3.40-3.50 (4H, m), 3.67 (2H, t, *J* = 4.0 Hz), 3.81 (2H, t, *J* = 4.0 Hz), 3.97 (3H, s), 6.33 (1H, d, *J* = 1.6 Hz), 6.42 (1H, dd, *J* = 6.0, 1.6 Hz), 8.02 (1H, d, *J* = 6.4 Hz). ¹³C NMR (100 MHz, CDCl₃): δ, 21.2, 40.5, 45.2, 46.5, 46.6, 56.0, 97.0, 105.1, 128.4, 129.5, 155.0, 156.0, 169.0. HRMS (FAB+) calculated for C₁₃H₁₇N₃O₄ [M+ H]⁺: *m/z* = 280.1219, found 280.1295.

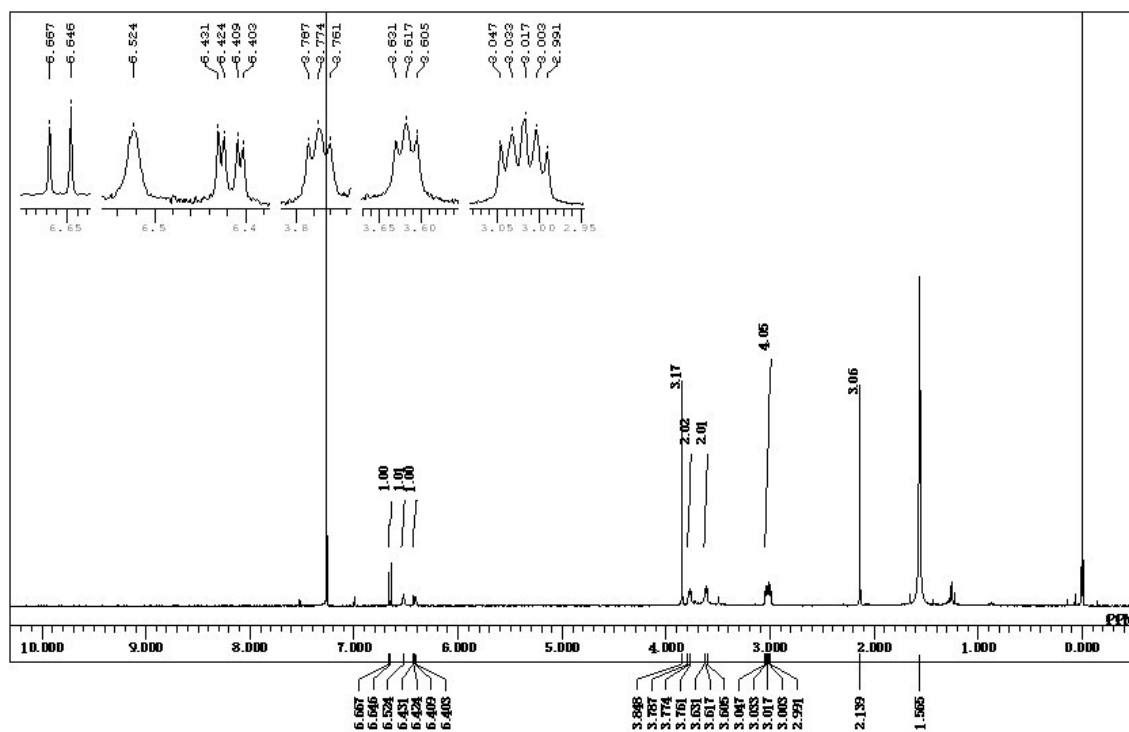


Figure S9. The proton NMR peak of 1-(4-(4-amino-3-methoxyphenyl)piperazine-1-yl)ethan-1-one (**9**). ¹H NMR (400 MHz, CDCl₃): δ 2.13 (3H, s), 2.95-3.08 (4H, m), 3.62 (2H, t, *J* = 5.6 Hz), 3.77 (2H, t, *J* = 5.2 Hz), 3.85 (3H, s), 6.42 (1H, dd, *J* = 8.8, 2.8 Hz), 6.52 (1H, s), 6.66 (1H, d, *J* = 8.4 Hz). HRMS (FAB+) calculated for C₁₃H₁₉N₃O₂ [M+ H]⁺: *m/z* = 249.1477, found 249.1464.

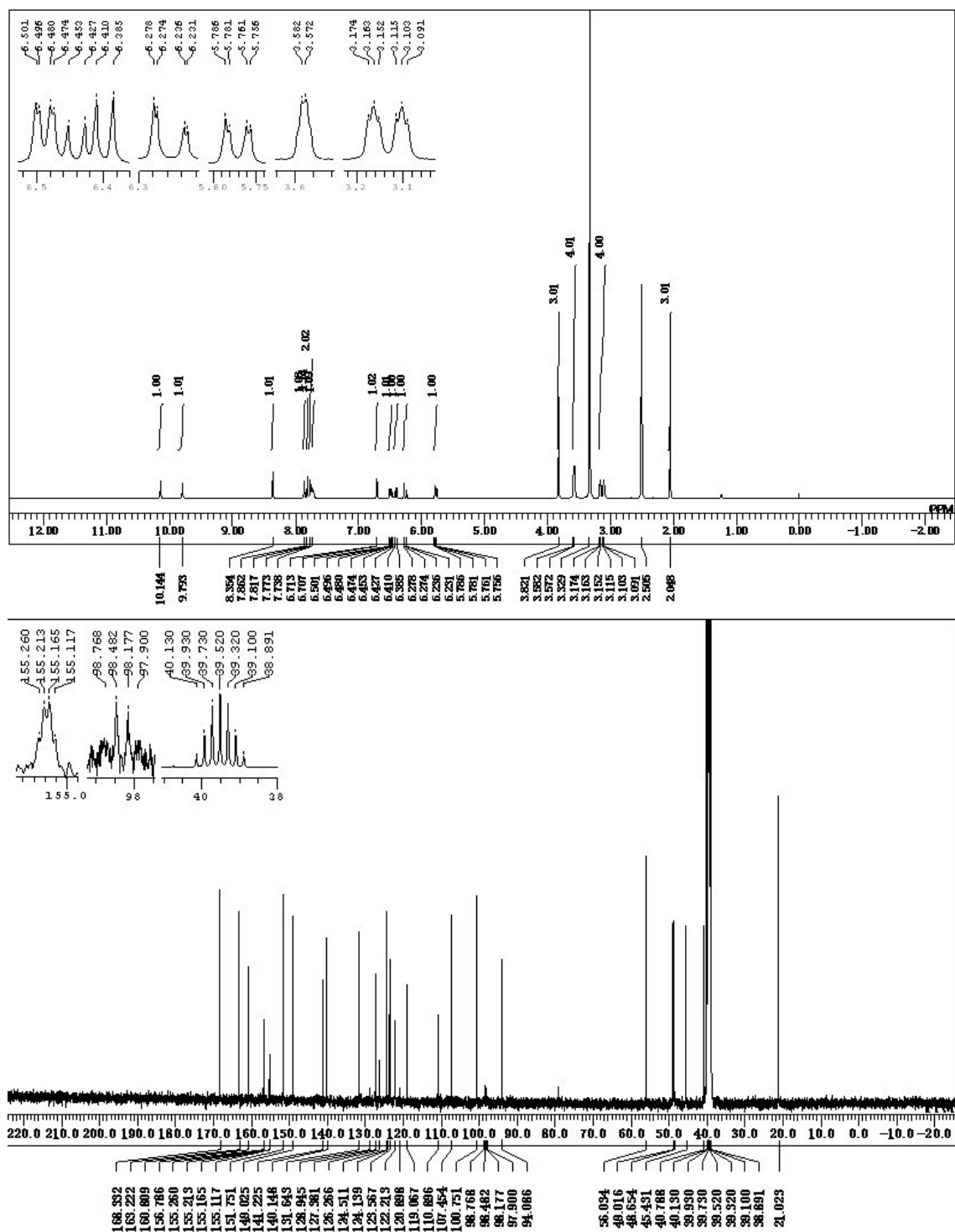


Figure S10. The proton and carbon NMR peak of N-(3-((2-((4-(4-acetylpiperazin-1-yl)-2-methoxyphenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)-5-iodophenyl)acrylamide (**10**). ¹H NMR (400 MHz, (CD₃)₂SO): δ 2.05 (3H, s), 3.10 (2H, t, *J* = 4.8 Hz), 3.16 (2H, t, *J* = 4.4 Hz), 3.53-3.63 (4H, m), 3.82 (3H, s), 5.77 (1H, dd, *J*

= 10.0, 2.0 Hz), 6.25 (1H, dd, $J = 16.8, 2.0$ Hz), 6.41 (1H, dd, $J = 16.8, 10.0$ Hz), 6.49 (1H, dd, $J = 8.8, 2.0$ Hz), 6.71 (1H, s), 7.74 (1H, s), 7.77 (2H, s), 7.82 (1H, s), 7.86 (1H, s), 8.35 (1H, s), 9.79 (1H, s), 10.14 (1H, s). ^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{SO}$): δ 21.0, 40.8, 45.4, 48.7, 49.0, 56.0, 94.1, 98.3 (q, $J_{\text{CF}} = 30.5$ Hz), 100.8, 107.5, 110.9, 119.1, 122.2, 124.1, 124.5, 124.8 (q, $J_{\text{CF}} = 270$ Hz), 127.4, 131.6, 140.1, 141.2, 149.0, 151.8, 155.2 (q, $J_{\text{CF}} = 4.8$ Hz), 156.8, 160.8, 163.2, 168.3. HRMS (FAB+) calculated for $\text{C}_{27}\text{H}_{27}\text{F}_3\text{IN}_7\text{O}_3$ $[\text{M} + \text{H}]^+$: $m/z = 681.1172$, found 681.1186.

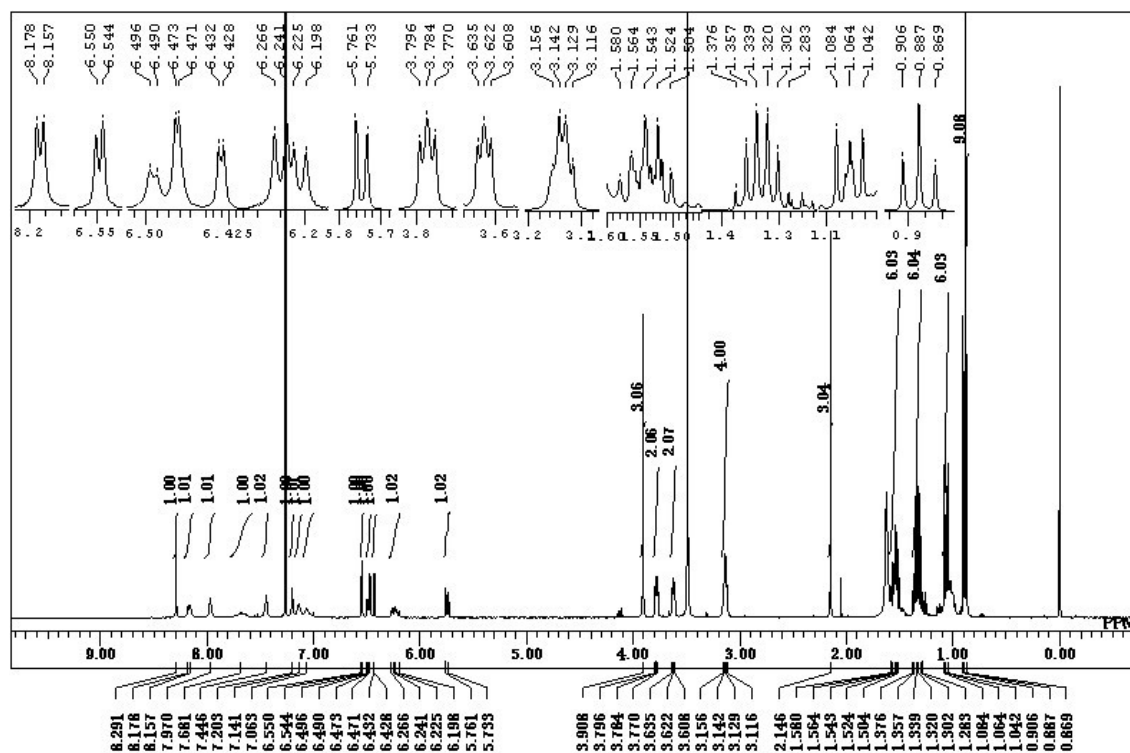


Figure S11. The proton NMR peak of N-(3-((2-((4-(4-acetylpiperazin-1-yl)-2-methoxyphenyl)amino)-5-(trifluoromethyl)pyrimidin-4-yl)amino)-5-(tributylstannyl)phenyl)acrylamide (**11**). ^1H NMR (400 MHz, CDCl_3): δ 0.88 (9H, t, $J = 7.6$ Hz), 1.06 (6H, t, $J = 8.0$ Hz), 1.33 (6H, sex, $J = 7.6$ Hz), 1.54 (6H, quin, $J = 7.6$ Hz), 2.14 (3H, s), 3.10-3.20 (4H, m), 3.62 (2H, t, $J = 5.6$ Hz), 3.78 (2H, t, $J = 5.6$ Hz), 3.91 (3H, s), 5.75 (1H, d, $J = 11.2$ Hz), 6.23 (1H, dd, $J = 17.2, 10.8$ Hz), 6.43 (1H, d, $J = 1.6$ Hz), 6.48 (1H, dd, $J = 7.6, 2.0$ Hz), 6.54 (1H, d, $J = 2.4$ Hz), 7.68 (1H, s), 7.06 (1H, s), 7.14 (1H, s), 7.20 (1H, s), 7.44 (1H, s), 7.97 (1H, s), 8.16 (1H, d, $J = 8.4$ Hz), 8.29 (1H, s). HRMS (FAB+) calculated for $\text{C}_{39}\text{H}_{54}\text{F}_3\text{N}_7\text{O}_3\text{Sn}$ [$\text{M} + \text{H}$] $^+$: $m/z = 845.3267$ found 845.3262.

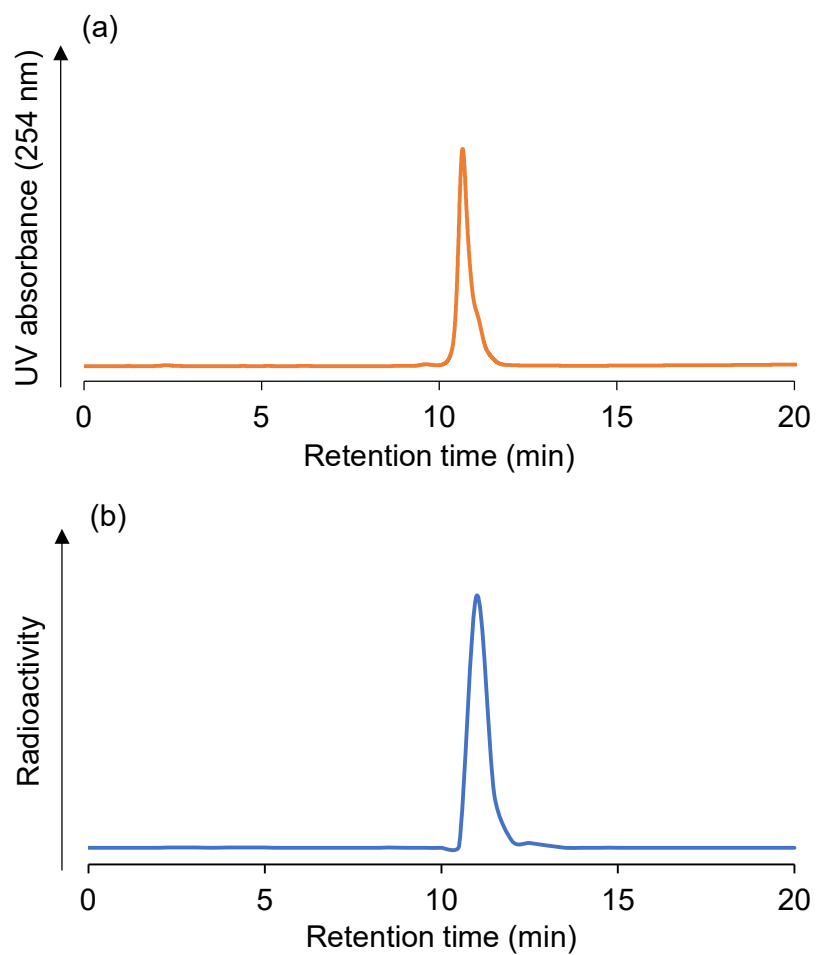


Figure S12. The chromatograms of (a) nonradioactive iodinated compound **10** (ICO1686) and (b) radioactive compound [^{125}I]**10** ([^{125}I]**10**).

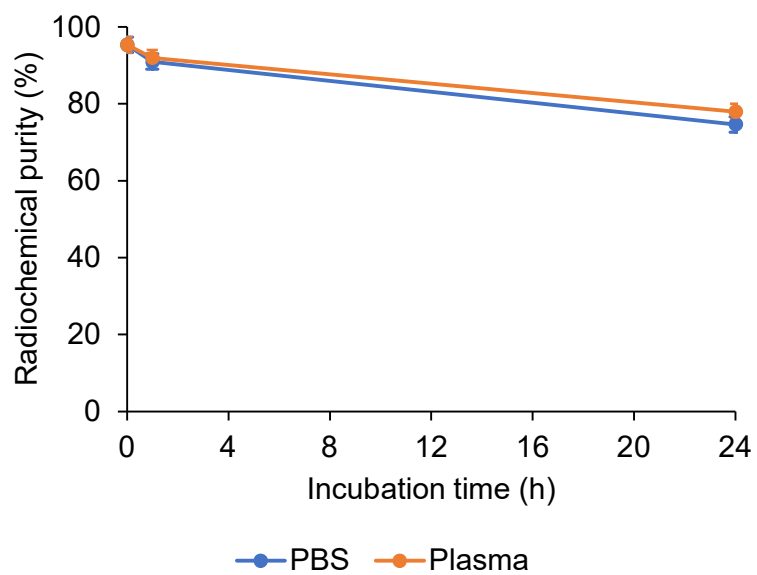


Figure S13. The stability of radiolabeled compound [125I]10 in PBS and plasma.