Supplementary Information

Design and synthesis of (aza)indolyl maleimide based covalent inhibitors of glycogen synthase kinase 3β

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1. Synthesis of key intermediates

2-(2-Amino-2-oxoethyl)phenyl *tert*-butylcarbamate (**16c**)

To the mixture of 2-(2-aminophenyl)acetic acid (1.50 g, 9.9 mmol) and NaOH (0.79 g, 19.8 mmol) in the flask containing THF (50 mL) and H_2O (10 mL) was added $(Boc)_2O$ (2.16 g, 9.9 mmol) at room temperature. After 6 h, the reaction mixture was evaporated *in vacuo* and then purified by flash column chromatography to obtain 2-(2-((*tert*-butoxycarbonyl)amino)phenyl)acetic acid as a white solid (2.36 g, yield 95%).

To a solution of 2-(2-((*tert*-butoxycarbonyl)amino)phenyl)acetic acid (1.10 g, 4.4 mmol) in anhydrous DCM (15 mL) and DMF (5 mL) was added triethylamine (2.45 mL, 17.6 mmol), HOBt (0.71 g, 5.28 mmol) and EDCI (1.69 g, 8.8 mmol). The reaction mixture was stirred at ambient temperature for 1 h, then NH_4Cl solid (0.94 g, 17.6 mmol) and NH_3 solution (7 N in methanol, 3.14 mL, 22.0 mmol) were dropwise added. The reaction mixture was stirred for 2 h and then concentrated *in vacuo*, the residue was dissolved in ethyl acetate (100 mL), washed with brine (2 × 30 mL), dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was purified by flash column chromatography (gradient eluent: 1-6% MeOH in DCM), to give compound **16c** as a waxy, off-white solid (0.77 g, yield 70%).^{1}H NMR (400 MHz, Acetone-d_6) δ 9.44 (s, 1H), 7.82 (d, J = 8.0 Hz, 1H), 7.39 (s, 1H), 7.19 (dd, J = 12.2, 4.5 Hz, 2H), 7.00 (td, J = 7.5, 1.2 Hz, 1H), 6.61 (s, 1H), 3.57 (s, 2H), 1.51 (s, 9H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 174.2, 153.2, 138.4, 130.1, 127.2, 126.9, 123.1, 122.3, 78.8, 39.7, 27.6.

Tert-butyl (4-(2-amino-2-oxoethyl) phenyl)carbamate (**16d**)

Compound **16d** was synthesized with a similar procedure that described for **16c** and yielded a waxy, off-white solid (yield 72%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.35 (s, 1H), 7.49 (d, J = 8.5 Hz, 2H), 7.23 (d, J = 8.5 Hz, 2H), 6.71 (s, 1H), 6.17 (s, 1H), 3.43 (s, 2H), 1.48 (s, 9H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 172.3, 152.8, 138.2, 130.2, 129.3, 118.1, 78.9, 41.8, 27.6.

3-(2-Amino-2-oxoethyl)phenyl tert-butylcarbamate (**16e***)*

Compound **16e** was synthesized with a similar procedure that described for **16c** and yielded a waxy, off-white solid (yield 65%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.37 (s, 1H), 7.54 (t, J = 2.0 Hz, 1H), 7.45 – 7.36 (m, 1H), 7.20 (t, J = 7.8 Hz, 1H), 6.96 (ddd, J = 7.6 Hz, 1H), 6.78 (s, 1H), 6.22 (s, 1H), 3.46 (s, 2H), 1.49 (s, 9H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 172.0, 152.8, 139.7, 136.9, 128.4, 123.0, 119.0, 116.4, 78.9, 42.6, 27.6.

3-Bromo-4-(1-methyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione (**23**)

NaH (60% suspension in mineral oil, 0.34 g, 8.4 mmol) was suspended into a solution of 3-bromo-4-(1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione (1.22 g, 4.2 mmol) in anhydrous DMF (10 mL) cooled with an ice bath. After stirring at 0 °C for 1 h, iodomethane(0.39 mL, 6.3 mmol) was dropwise added to the suspended solution. The reaction mixture was stirred until the completion of the reaction. The reaction mixture was dissolved in ethyl acetate (30 mL) and washed with water and brine (2 × 20 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated *in vacuo*. The residue was purified by flash column chromatography (20% ethyl acetate in petroleum ether), to give compound **23** as an orange solid (0.90 g, yield 70%). ^{1}H NMR (400 MHz, DMSO-d_6)) δ 11.35 (s, 1H), 8.06 (s, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.56 (d, J = 8.2 Hz, 1H), 7.29 (ddd, J = 8.2, 6.9, 1.1 Hz, 1H), 7.20 (ddd, J = 8.0, 6.9, 1.0 Hz, 1H), 3.91 (s, 3H). ^{13}C NMR (100 MHz, DMSO-d_6)) δ 170.6, 167.8, 138.0, 137.4, 135.0, 125.3, 122.9, 122.9, 121.1, 114.8, 111.1, 103.1, 33.6.

3-Amino-4-(1-methyl-1*H*-indol-3-yl)-1*H*-pyrrole-2,5-dione (**27**)

To a solution of NH_3 (2.8 mL, 19.7 mmol, 7 N in methanol) was added compound **23** (1.20 g, 3.9 mmol) in a microwave tube. The reaction mixture was microwaved at 117 °C for 2 h. The solvent was evaporated under vacuum, and the residue directly purified by flash column chromatography (20% ethyl acetate in petroleum ether), to give compound **27** as an orange solid (0.80 g, yield 85%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.21 (s, 1H), 7.64 (dd, J = 7.7, 3.6 Hz, 1H), 7.43 (t, J = 3.8 Hz, 2H), 7.14 (t, J = 7.5 Hz, 1H), 7.04 (t, J = 7.5 Hz, 1H), 6.67 (s, 2H), 3.80 (d, J = 4.2 Hz, 3H).^{13}C NMR (100 MHz, DMSO-d_6) δ 173.3, 169.7, 142.9, 136.8, 129.5, 126.5, 121.7, 119.2, 113.6, 110.0, 104.1, 96.3, 32.9.

Methyl 2-(5-fluoro-1-methyl-1*H*-indol-3-yl)-2-oxoacetate (**31a**)

To a solution of 5-fluoroindole (1.00 g, 7.4 mmol) in anhydrous DCM (13 mL) was added Et_2AlCl (11.1 mL, 11.1 mmol, 1 M in hexane) at 0 °C. The mixture was stirred at 0 °C for 30 min. To the mixture was dropwise added a

solution of methyl 2-chloro-2-oxoacetate (1.00 mL, 10.4 mmol) in DCM (13 mL) at 0 °C. The reaction solution was stirred at 0 °C for 3 h. The mixture was quenched and treated with saturated NH_4Cl solution to pH 7.5. After usual workup, the residue product was purified by flash column chromatography (25% ethyl acetate in petroleum ether), to give compound **30a** as a pale-yellow solid (1.15 g, yield 66%).

Compound **31a** was synthesized with a similar procedure that described for **23** and yielded a pale- yellow oil (yield 60%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.43 (s, 1H), 7.95 (dd, J = 9.6, 2.5 Hz, 1H), 7.58 (dd, J = 8.9, 4.3 Hz, 1H), 7.15 (td, J = 9.1, 2.6 Hz, 1H), 4.00 (s, 3H), 3.92 (s, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 177.6, 163.6, 161.1, 158.8, 141.9, 134.2, 127.5, 127.3, 112.1, 112.0, 112.0, 111.9, 111.6, 106.9, 106.7, 104.9, 51.8, 33.3.

Ethyl 2-(6-fluoro-1-methyl-1*H*-indol-3-yl)-2-oxoacetate (**31b**)

Compound **31b** was synthesized with a similar procedure that described for **31a** and yielded a colorless oil (yield 60%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.39 (s, 1H), 8.24 (dd, J = 8.7, 5.5 Hz, 1H), 7.38 (dd, J = 9.6, 2.2 Hz, 1H), 7.14 (ddd, J = 9.7, 8.8, 2.3 Hz, 1H), 4.39 (q, J = 7.1 Hz, 2H), 3.99 (s, 3H), 1.38 (t, J = 7.1 Hz, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 178.3, 163.3, 161.6, 159.3, 141.3, 138.1, 138.0, 123.0, 122.9, 112.1, 111.2, 110.9, 97.5, 97.3, 61.4, 33.1, 13.4.

Methyl 2-(6-methoxy-1-methyl-1*H*-indol-3-yl)-2-oxoacetate (**31c**)

Compound **31c** was synthesized with a similar procedure that described for **31a** and yielded a colorless oil (yield 58%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.22 (s, 1H), 8.15 (dd, J = 8.7, 0.5 Hz, 1H), 7.12 (d, J = 2.2 Hz, 1H), 6.97 (dd, J = 8.7, 2.3 Hz, 1H), 3.95 (s, 3H), 3.90 (s, 3H), 3.89 (s, 3H). ^{13}C NMR (100 MHz, Acetone-d_6) δ177.9, 164.0, 157.8, 139.9, 138.8, 122.4, 120.2, 112.5, 112.2, 94.1, 55.0, 51.7, 33.0.

Methyl 2-(1-isopropyl-1*H*-indol-3-yl)-2-oxoacetate (**31d**)

To a solution of indole (1.00 g, 8.5 mmol) in anhydrous DCM (15 mL) was added Et_2AlCl (12.75 mL, 12.8 mmol, 1 M in hexane) at 0 °C. The mixture was stirred at 0 °C for 30 min. To the mixture was dropwise added a solution of methyl 2-chloro-2-oxoacetate (1.15 mL, 11.9 mmol) in DCM (15 mL) at 0 °C. The reaction solution was stirred at 0 °C for 2 h. The mixture was quenched and treated with saturated NH_4Cl solution to pH 7.5. After usual workup, the residue was purified by flash column chromatography (25% ethyl acetate in petroleum ether), to give compound ethyl 2-(1*H*-indol-3-yl)-2-oxoacetate as a pale-yellow solid (1.22 g, yield 66%).

To a solution of methyl 2-(1*H*-indol-3-yl)-2-oxoacetate (0.80 g, 3.7 mmol) in anhydrous DMF (8 mL) was added Cs_2CO_3 (3.62 g, 11.1 mmol) and 2-iodopropane (0.94 g, 5.55 mmol). The reaction mixture was heated at 55 °C for 6 h, until the completion of the reaction. The reaction mixture was dissolved in ethyl acetate (30 mL), washed with brine (2 × 20 mL), dried over anhydrous Na_2SO_4, filtered and concentrated *in vacuo*. The residue was purified by flash column chromatography (gradient eluent: 25-33% ethyl acetate in petroleum), to give compound **31d** as a colorless oil (0.38 g, yield 40%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.50 (s, 1H), 8.41 – 8.30 (m, 1H), 7.69 – 7.60 (m, 1H), 7.43 – 7.29 (m, 2H), 4.89 (septet, J = 6.7 Hz, 1H), 3.93 (s, 3H), 1.61 (d, J = 6.7 Hz, 6H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 178.1, 164.1, 136.6, 136.1, 126.9, 123.7, 123.1, 122.0, 112.5, 110.9, 51.8, 48.5, 21.6.

Methyl 2-oxo-2-(1-phenethyl-1*H*-indol-3-yl)acetate (**31e**)

Compound **31e** was synthesized with a similar procedure that described for **31d** and yielded a colorless oil (yield 47%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.51 – 8.43 (m, 1H), 8.15 (s, 1H), 7.46 – 7.23 (m, 6H), 7.12 – 7.03 (m, 2H), 4.42 (t, J = 7.3 Hz, 2H), 3.93 (s, 3H), 3.18 (t, J = 7.3 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 176.9, 163.2, 139.5, 137.2, 136.4, 128.8, 128.6, 127.2, 127.1, 124.1, 123.5, 122.9, 112.8, 109.9, 52.6, 49.0, 36.1.

Methyl 2-oxo-2-(1-(pent-4-yn-1-yl)-1*H*-indol-3-yl) acetate (**31f**)

Compound **31f** was synthesized with a similar procedure that described for **31d** and yielded a colorless oil (yield 45%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.46 (s, 1H), 8.38 – 8.29 (m, 1H), 7.65 (dd, J = 6.9, 1.4 Hz, 1H), 7.35 (dqd, J = 14.5, 7.2, 1.3 Hz, 2H), 4.51 (t, J = 7.0 Hz, 2H), 3.92 (s, 3H), 2.51 (t, J = 2.7 Hz, 1H), 2.33 – 2.23 (m, 2H), 2.15 (qd, J = 7.3, 0.7 Hz, 2H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 178.0, 163.9, 139.9, 136.9, 126.8, 123.8, 123.0, 122.0, 112.4, 110.7, 82.5, 70.2, 51.8, 45.6, 28.5, 15.2.

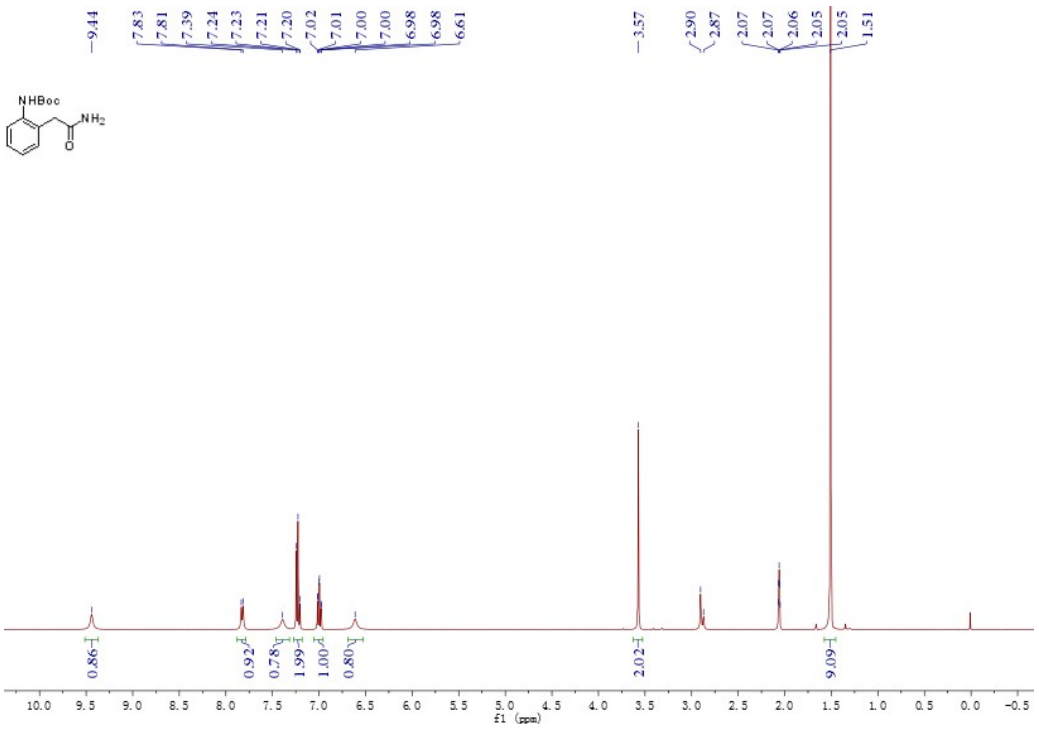
Methyl 2-(1-(3-((tert-butoxycarbonyl) amino) propyl)-1*H*-indol-3-yl)-2-oxoacetate (**31g**)

Compound **31g** was synthesized with a similar procedure that described for **31d** and yielded a colorless oil (yield 46%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.51 (s, 1H), 8.38 – 8.26 (m, 1H), 7.72 – 7.57 (m, 1H), 7.34 (m, 2H), 6.19 (s, 1H), 4.43 (t, J = 6.9 Hz, 2H), 3.92 (s, 3H), 3.19 (q, J = 6.4 Hz, 2H), 2.14 – 2.08 (m, 2H), 1.42 (s, 9H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 178.2, 164.0, 156.0, 140.1, 136.9, 126.8, 123.8, 123.0, 122.0, 112.3, 110.8, 77.8, 51.7, 44.5, 37.5, 30.1, 27.7.

Methyl 2-(1-isopropyl-1*H*-pyrrolo[2,3-b]pyridin-3-yl)-2-oxoacetate (**36**)

Compound **35** was synthesized in a similar manner as compound **23** and yielded an orange solid (yield 88%). To a solution of 1-isopropyl-1*H*-pyrrolo[2,3-b]pyridine (1.36 g, 8.5 mmol) in anhydrous DCM (40 mL) at 0 °C, anhydrous $AlCl_3$ (5.67 g, 42.5 mmol) was added in small partition over 1 h. Methyl 2-chloro-2-oxoacetate (1.18 mL, 12.8 mmol) was dropwise added at ambient temperature. Until acceptable conversion, MeOH was added to quench the reaction, then the mixture poured into saturated $NaHCO_3$ solution (100 mL) and dissolved in ethyl acetate (200 mL). The organic phase was wash with brine (5 × 100 mL), dried over Na_2SO_4 and evaporated under vacuum. Further purification was carried out by flash column chromatography (gradient eluent: 1-10% MeOH in DCM), to give compound **36** as a white solid (1.46 g, yield 70%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.66 (s, 1H), 8.57 (dd, J = 7.9, 1.7 Hz, 1H), 8.44 (dd, J = 4.7, 1.6 Hz, 1H), 7.36 (dd, J = 7.9, 4.7 Hz, 1H), 5.24 (septet, J = 6.8 Hz, 1H), 3.94 (s, 3H), 1.64 (d, J = 6.8 Hz, 6H). ^{13}C NMR (100 MHz, Acetone-d_6) δ 178.0, 163.4, 148.0, 144.7, 136.6, 130.0, 119.2, 119.1, 110.9, 51.9, 47.3, 21.5.

2. ^{1}H-NMR, ^{13}C-NMR spectrums of important intermediates and final compounds



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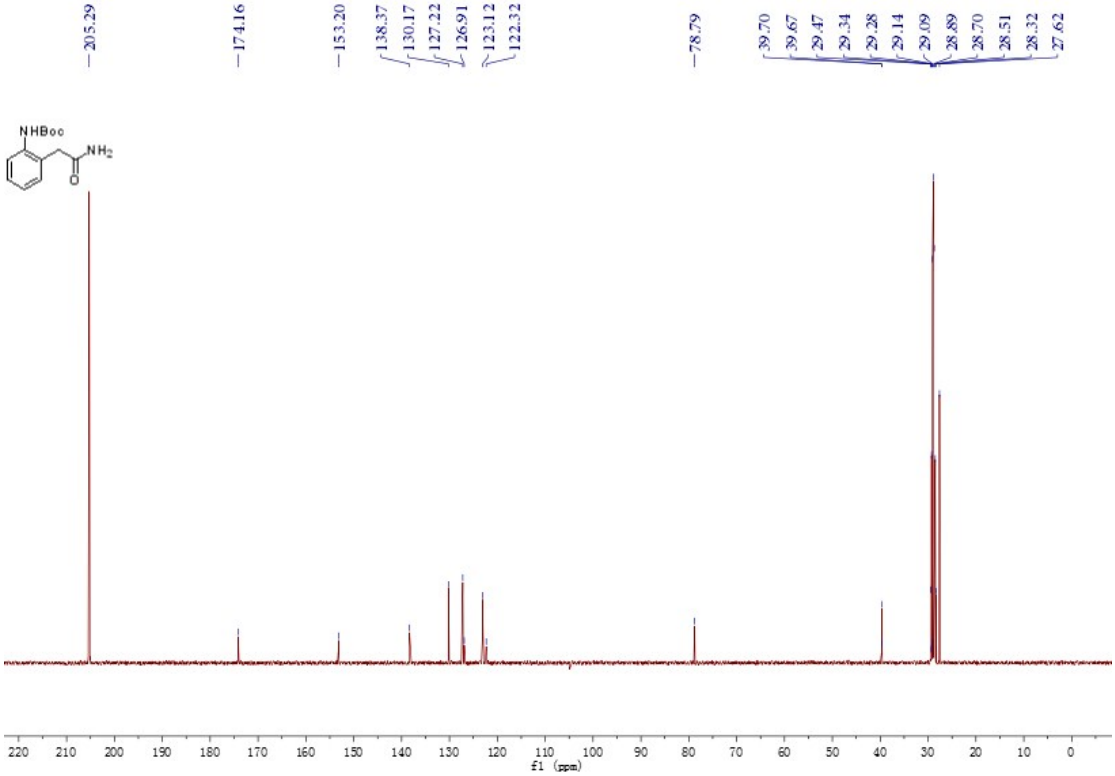

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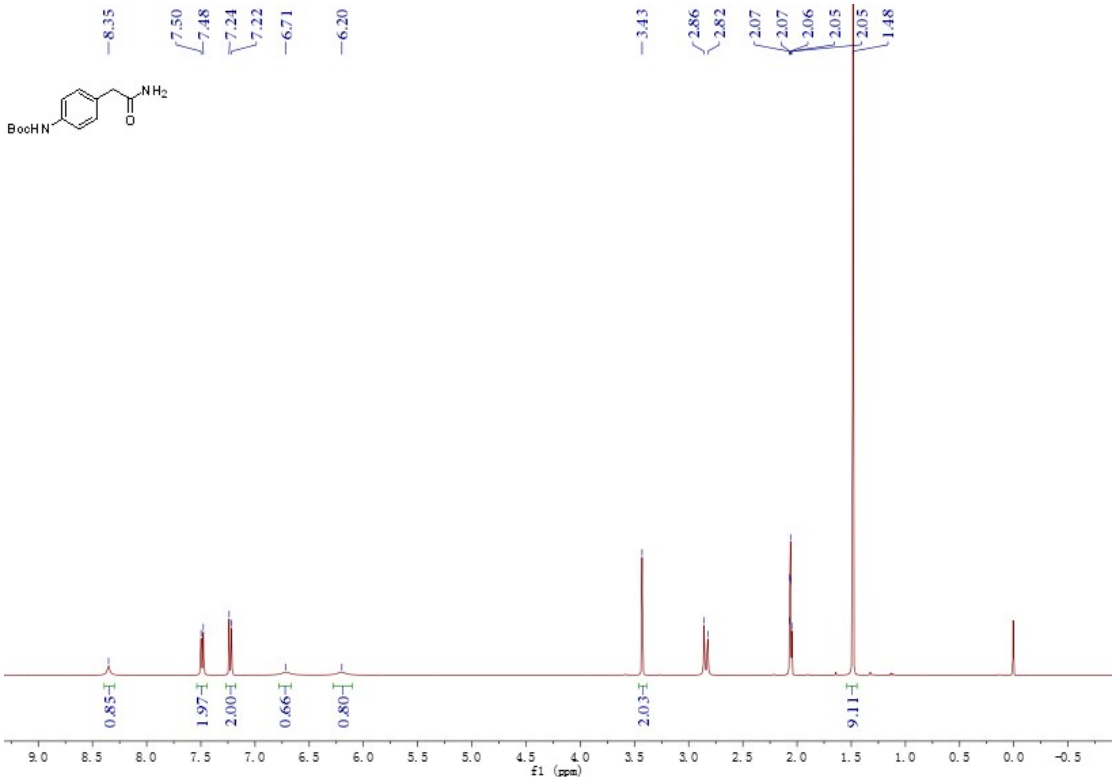

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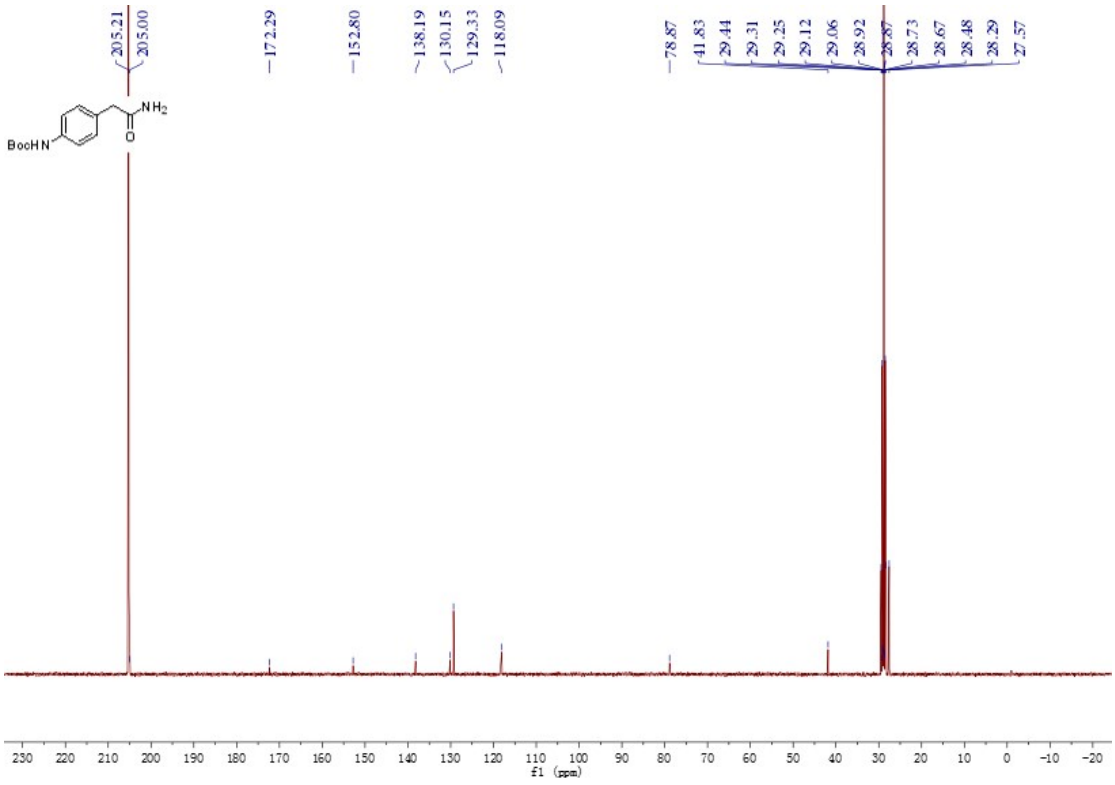

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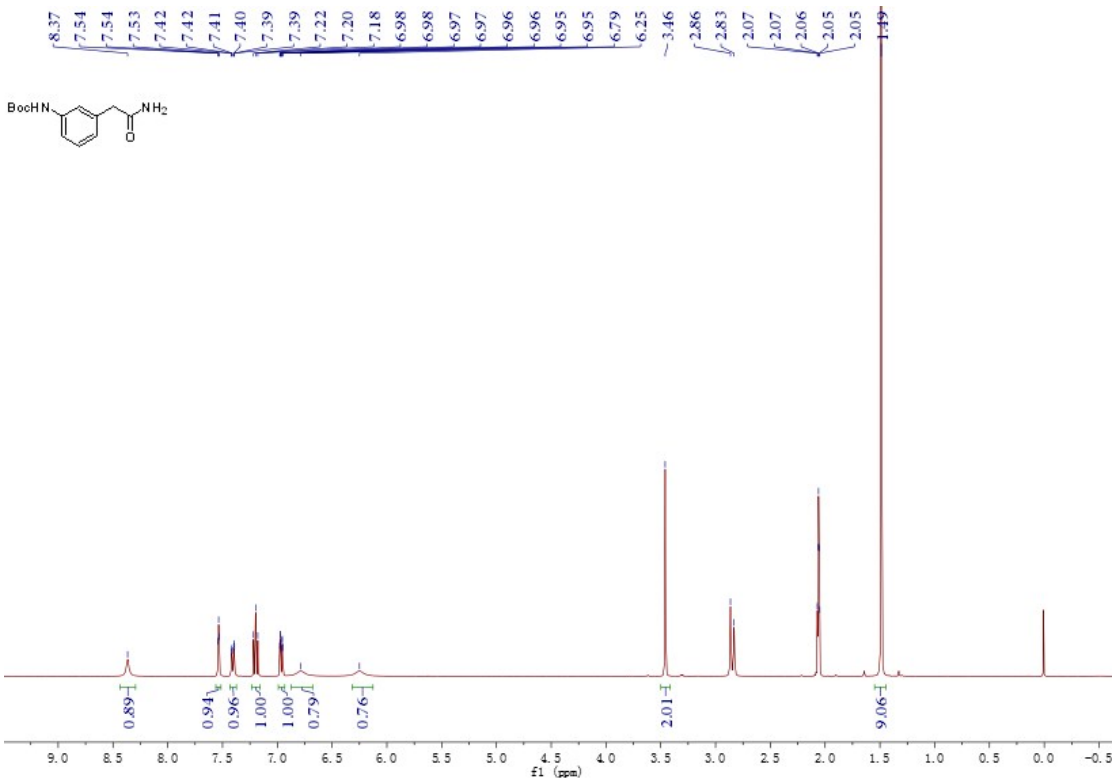

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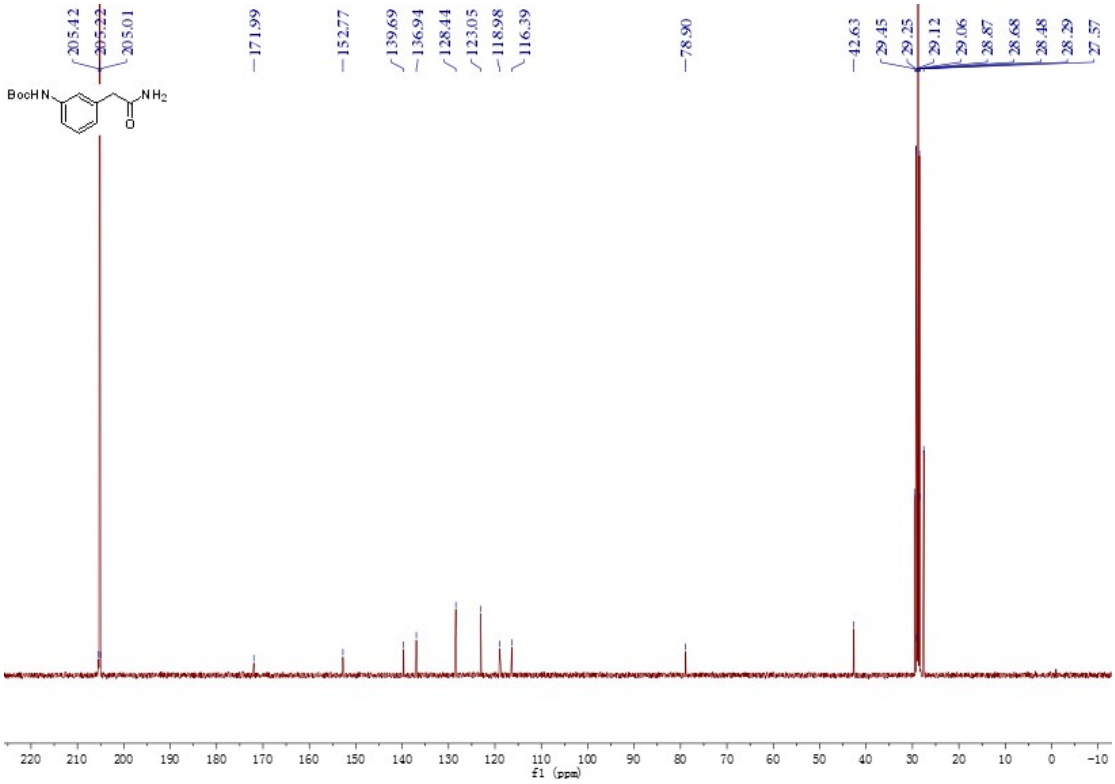

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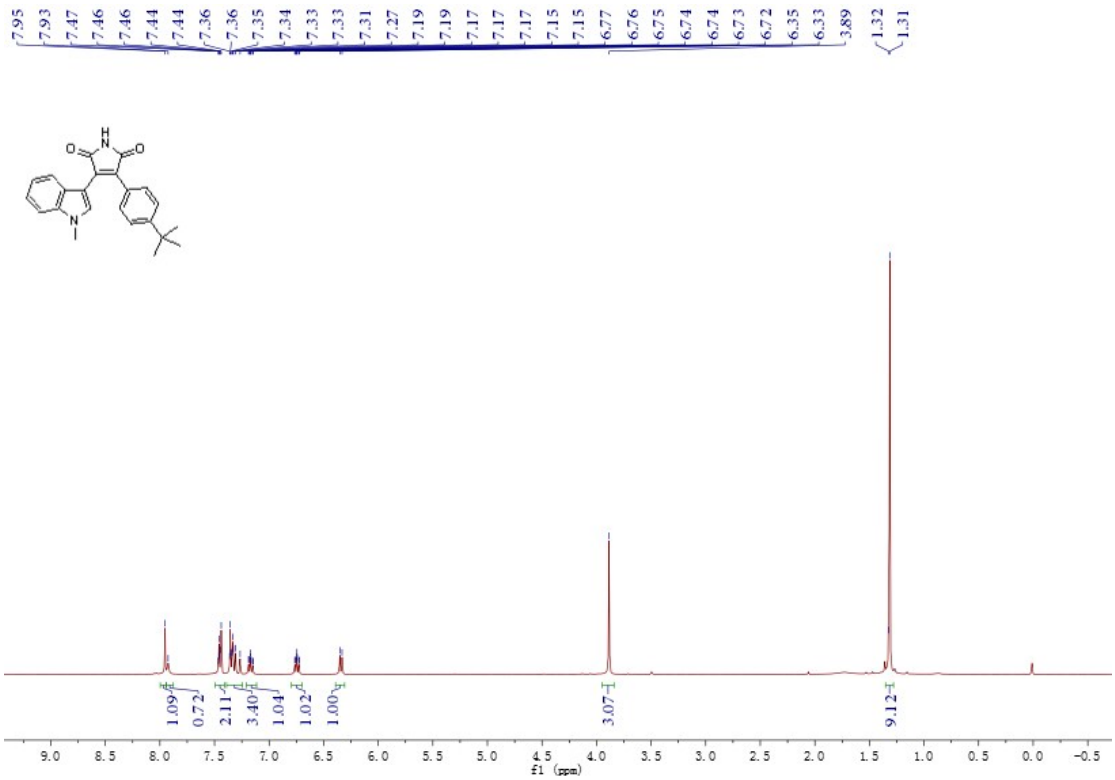

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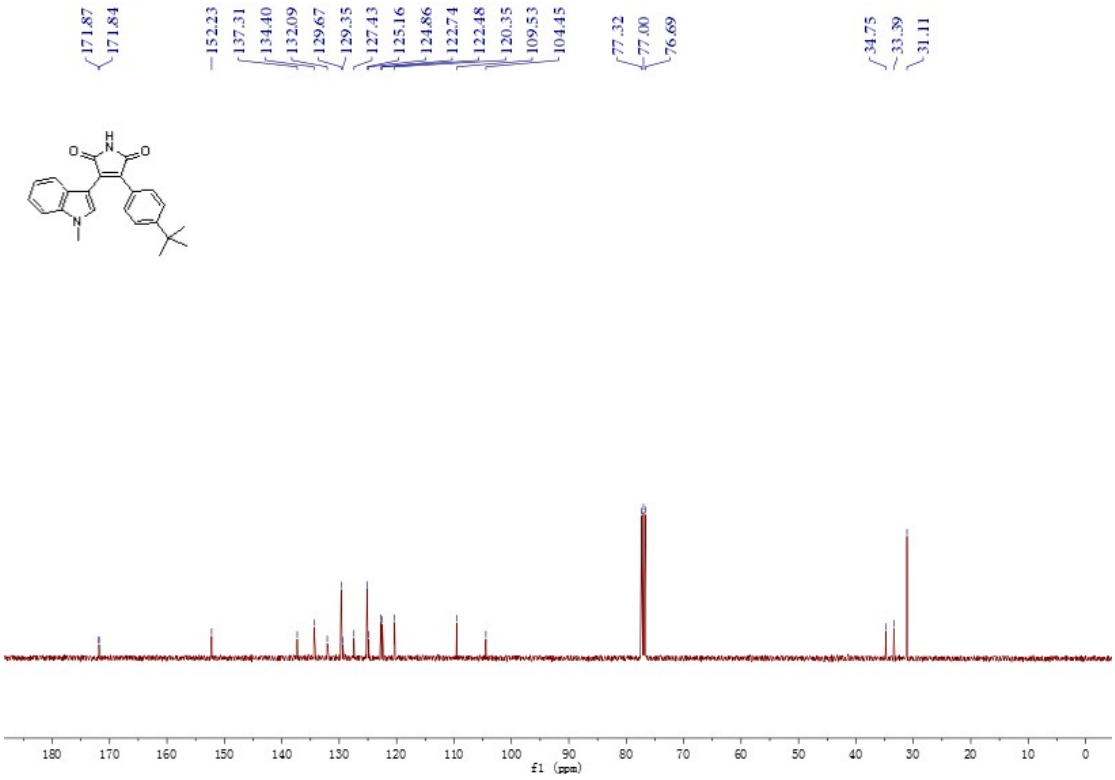

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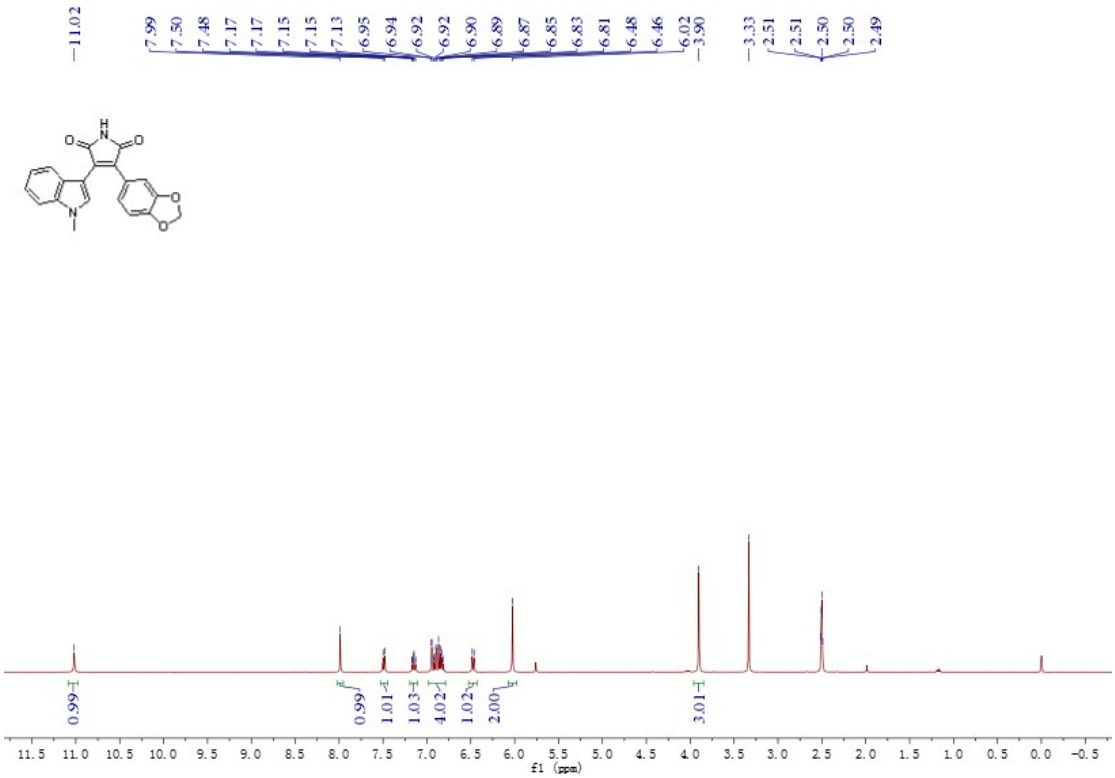

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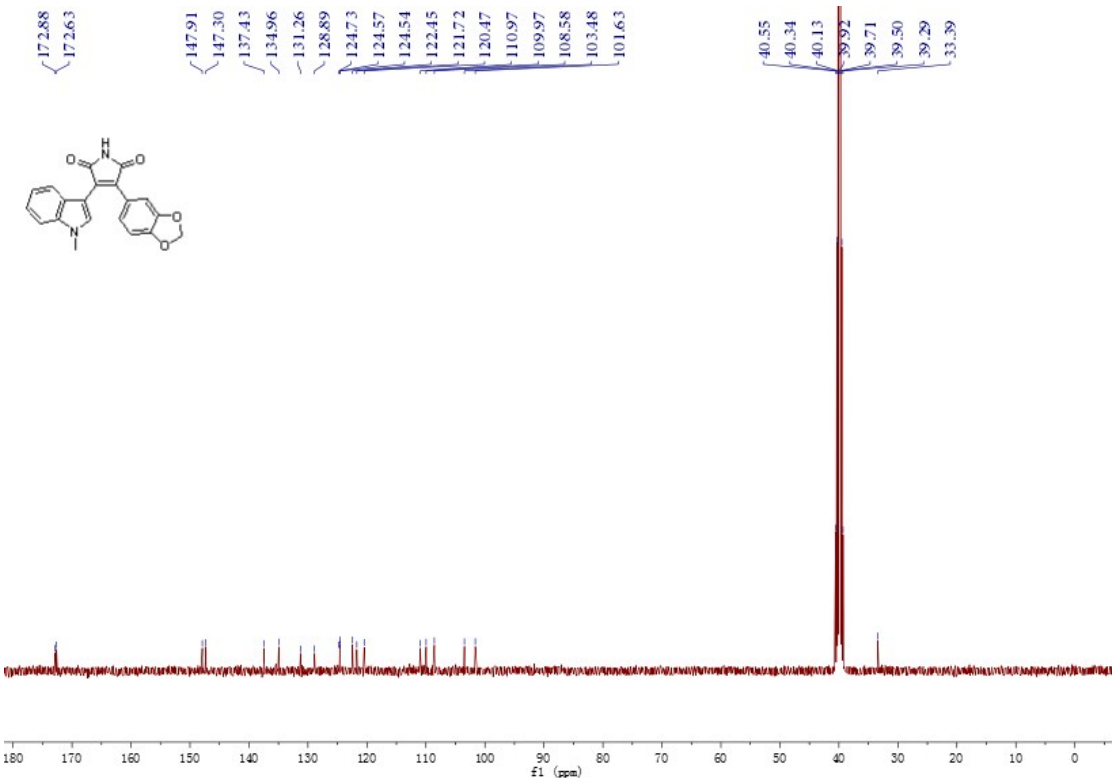

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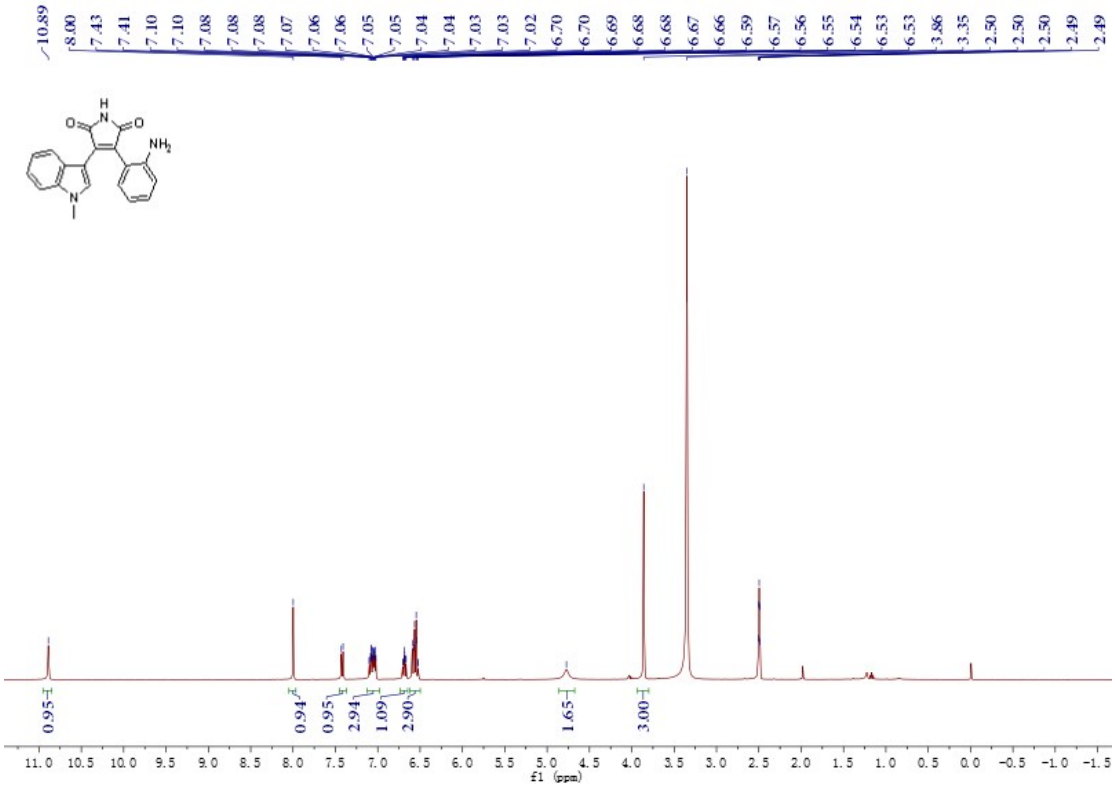

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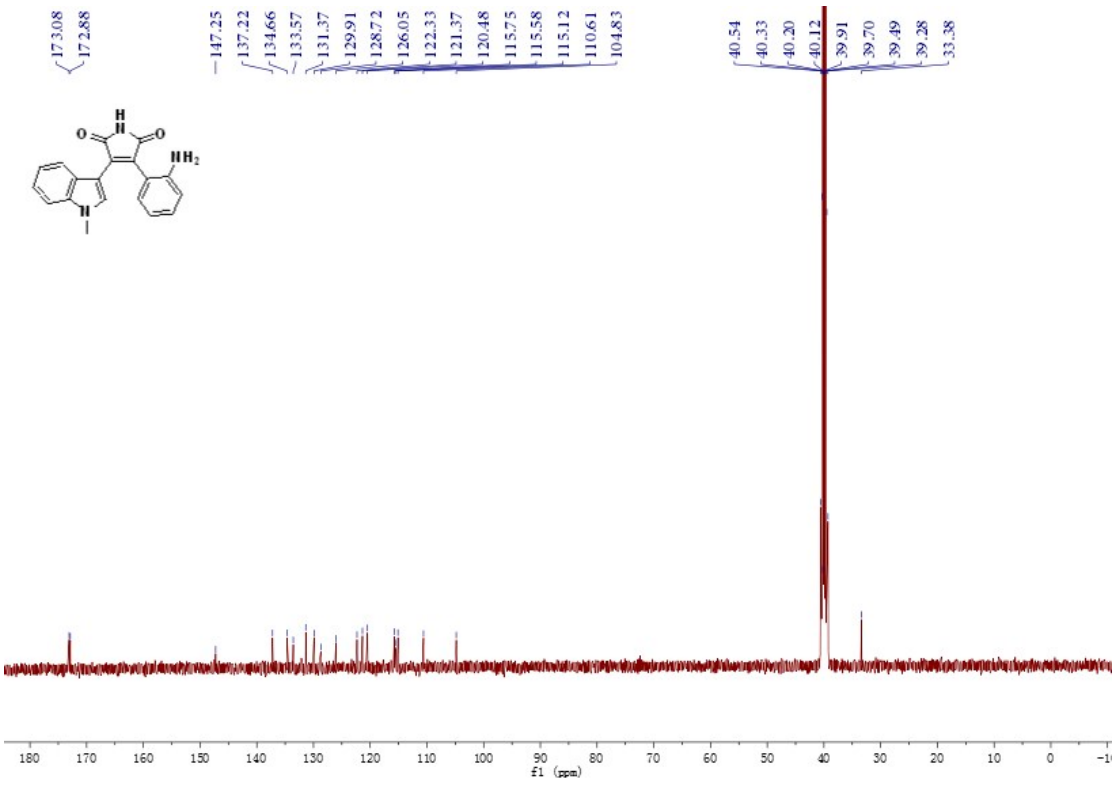

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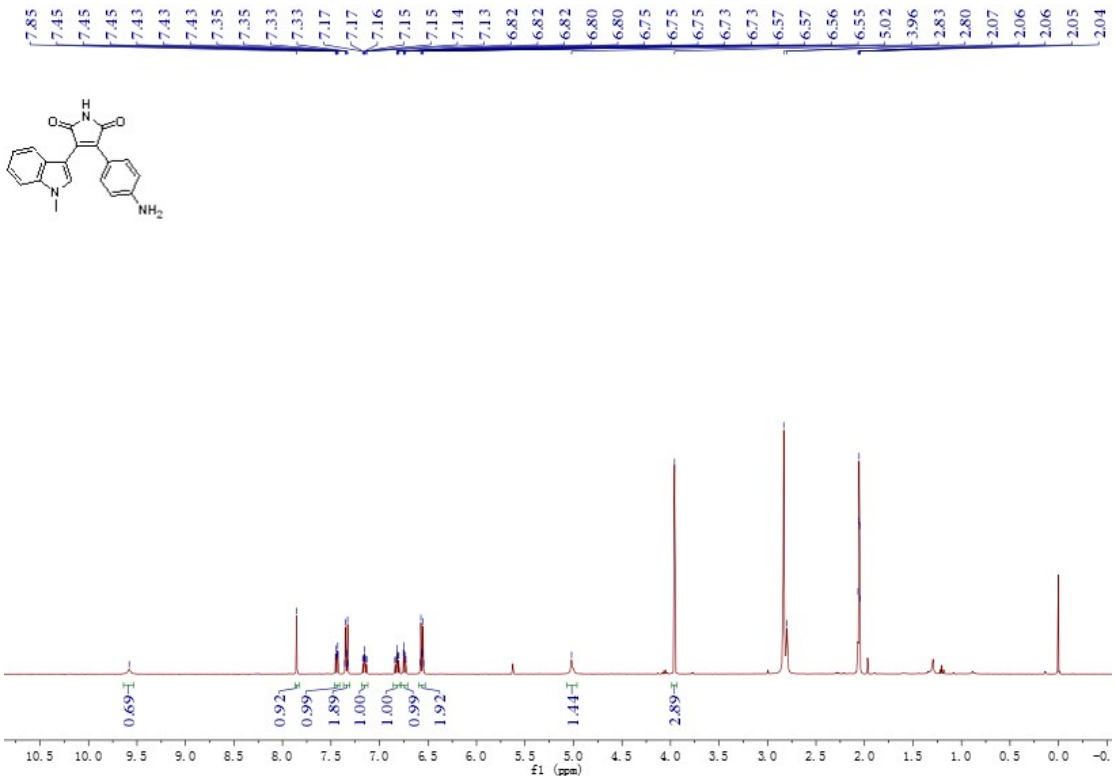

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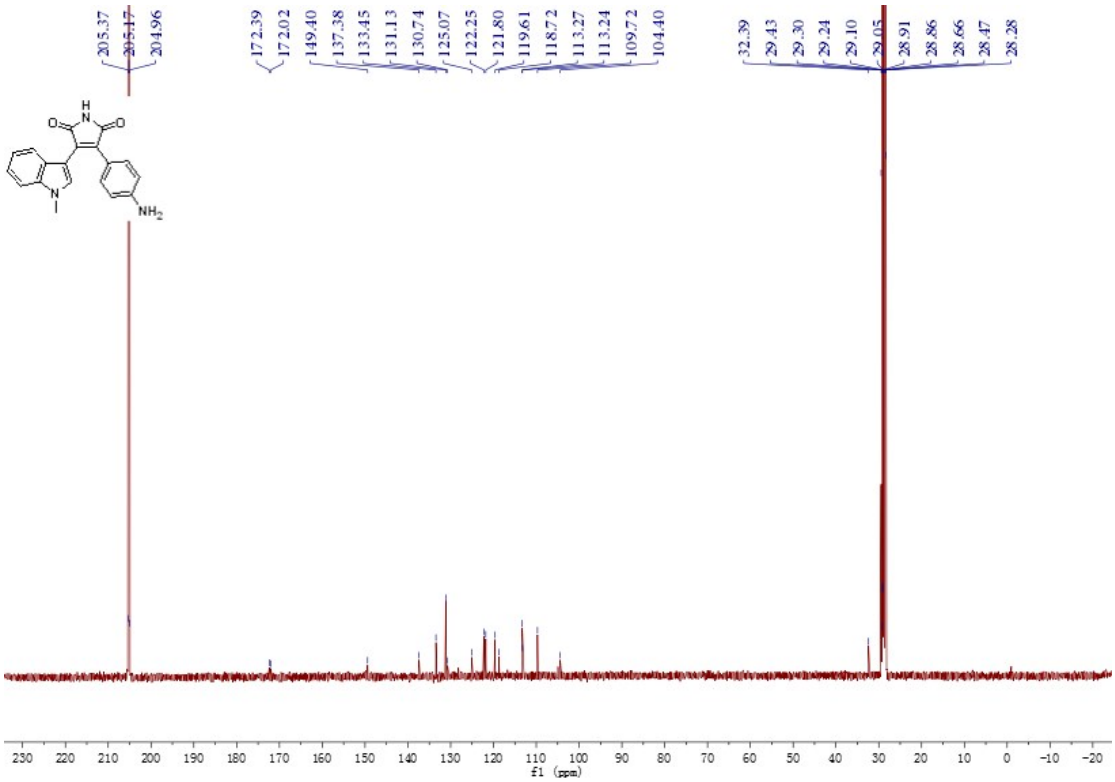

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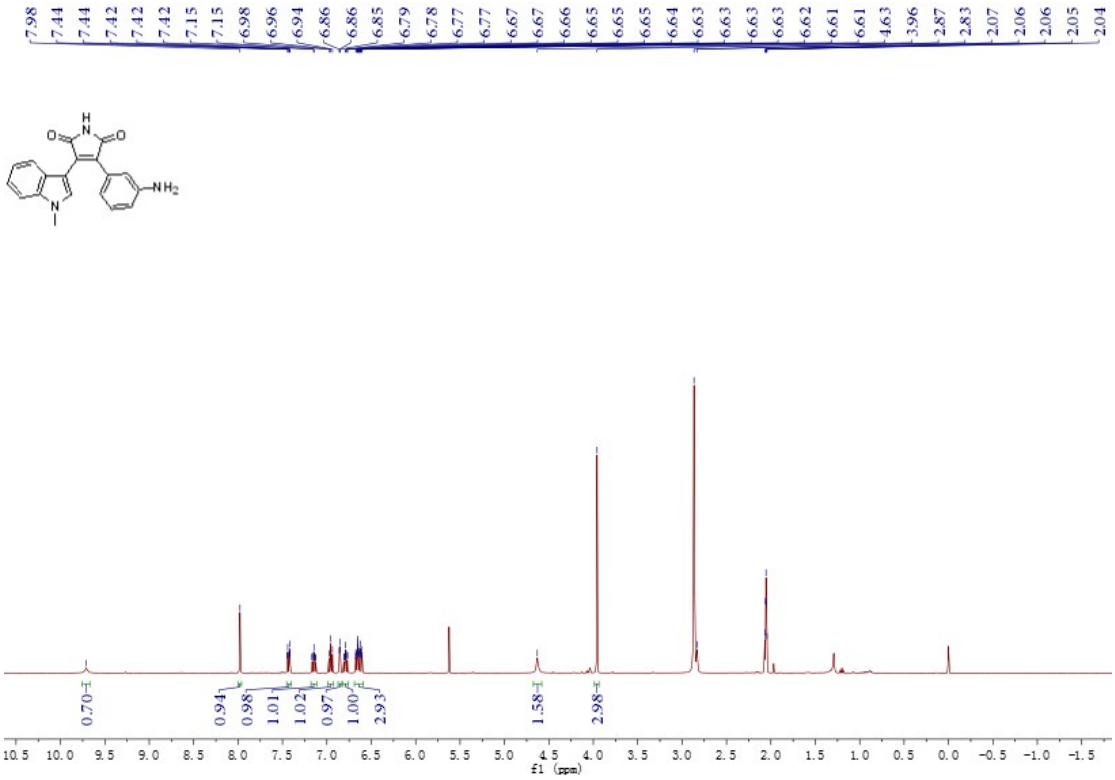

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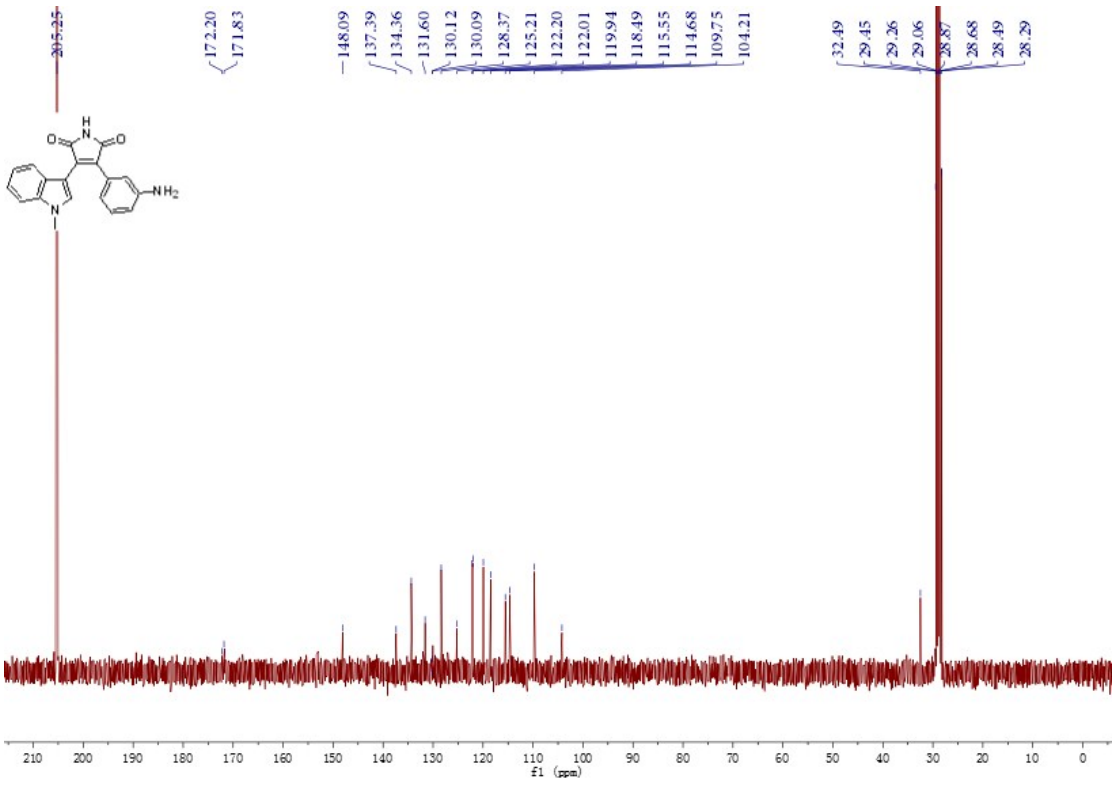

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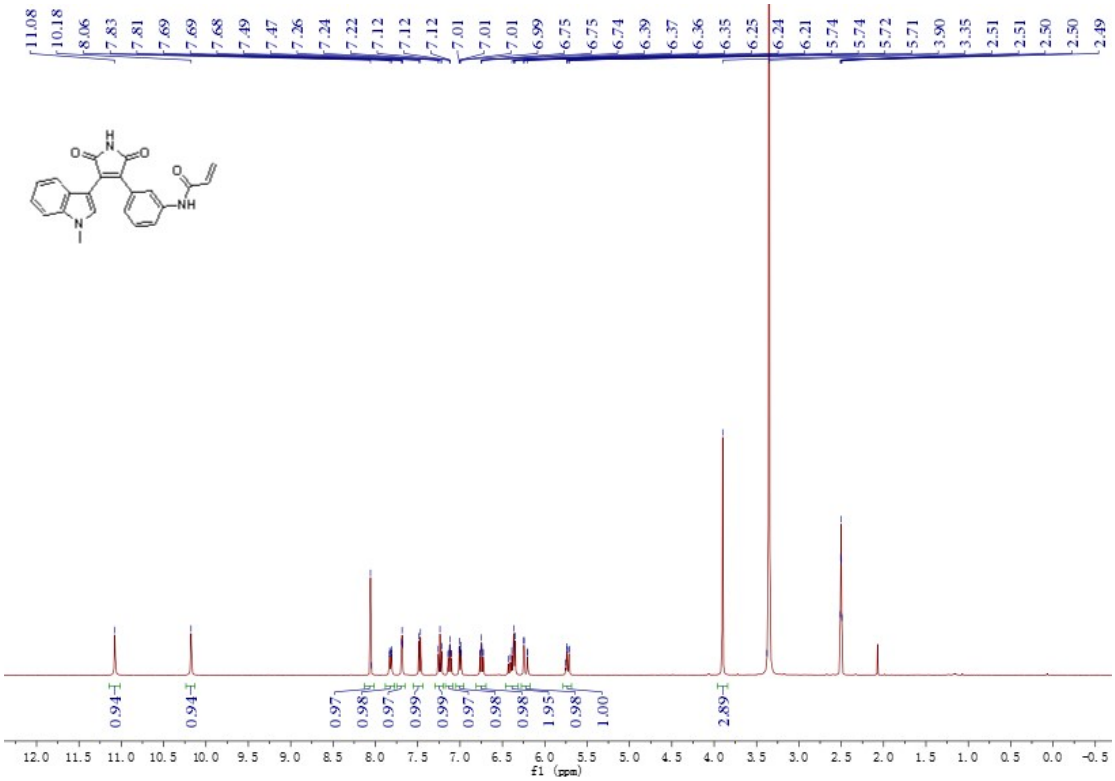

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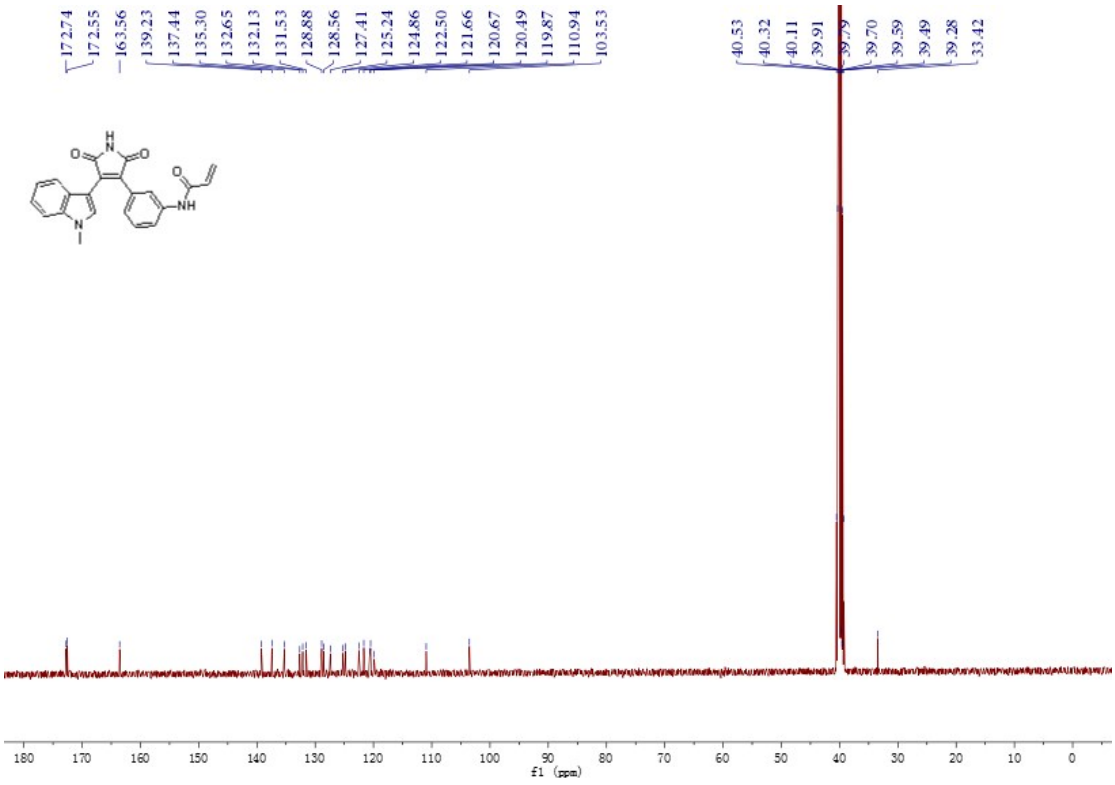

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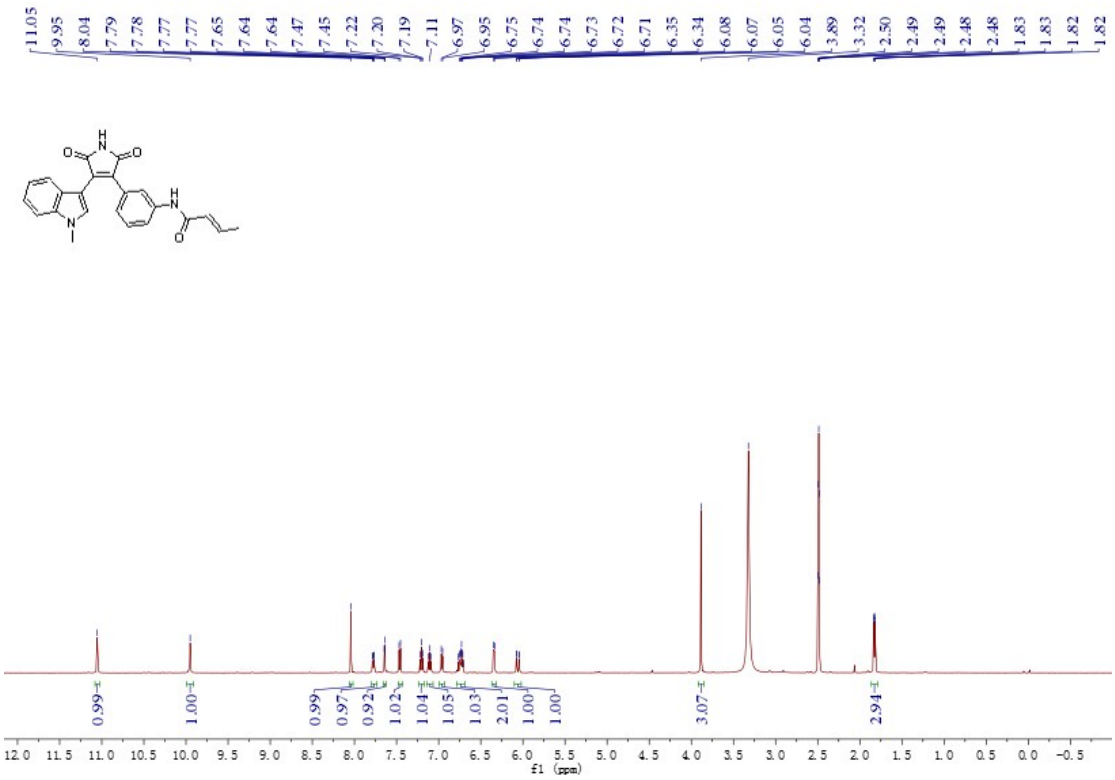

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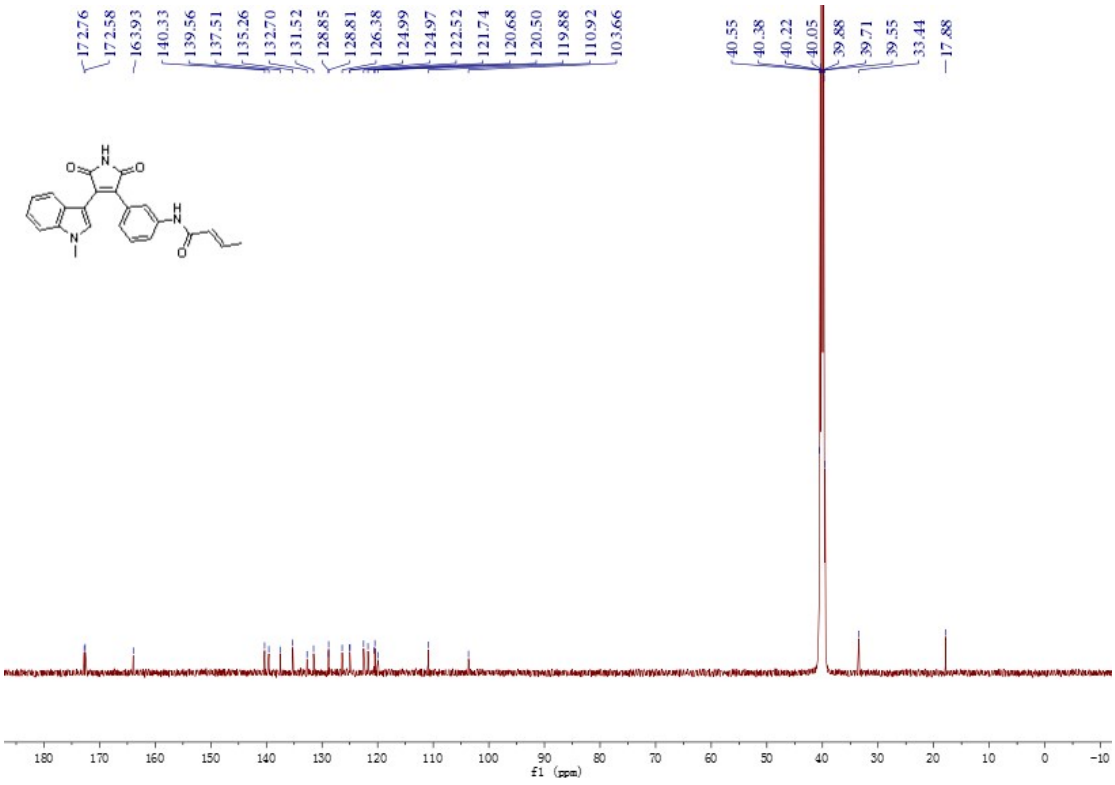

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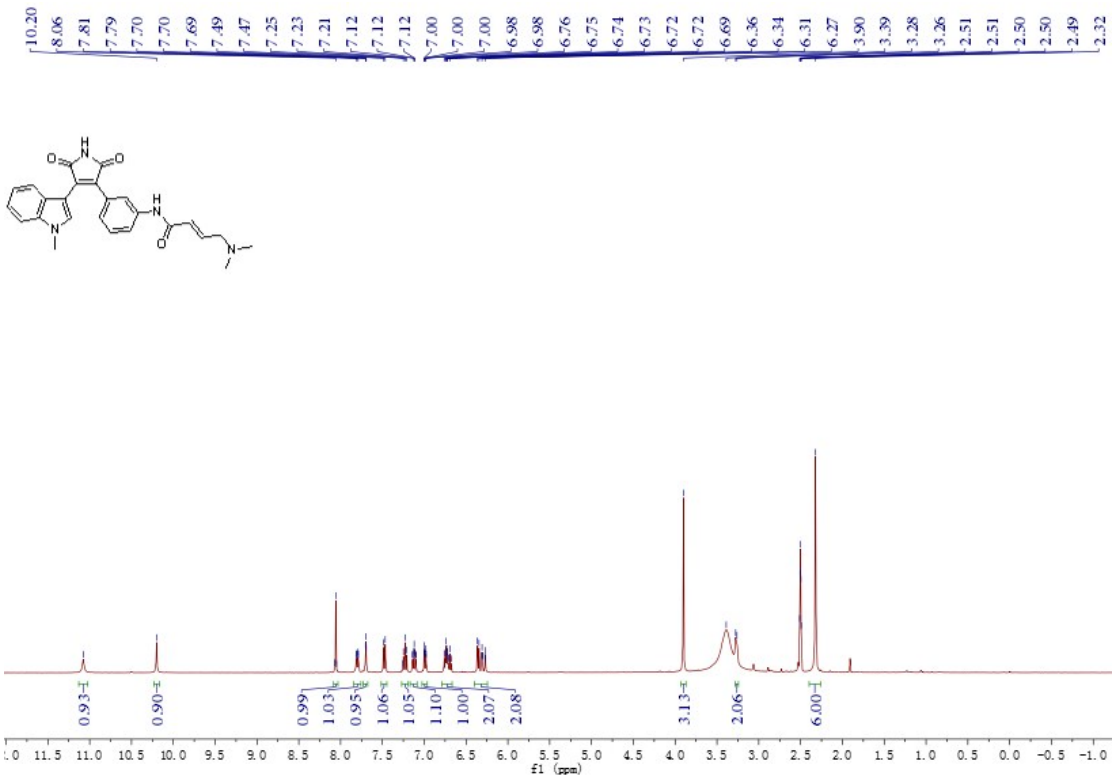

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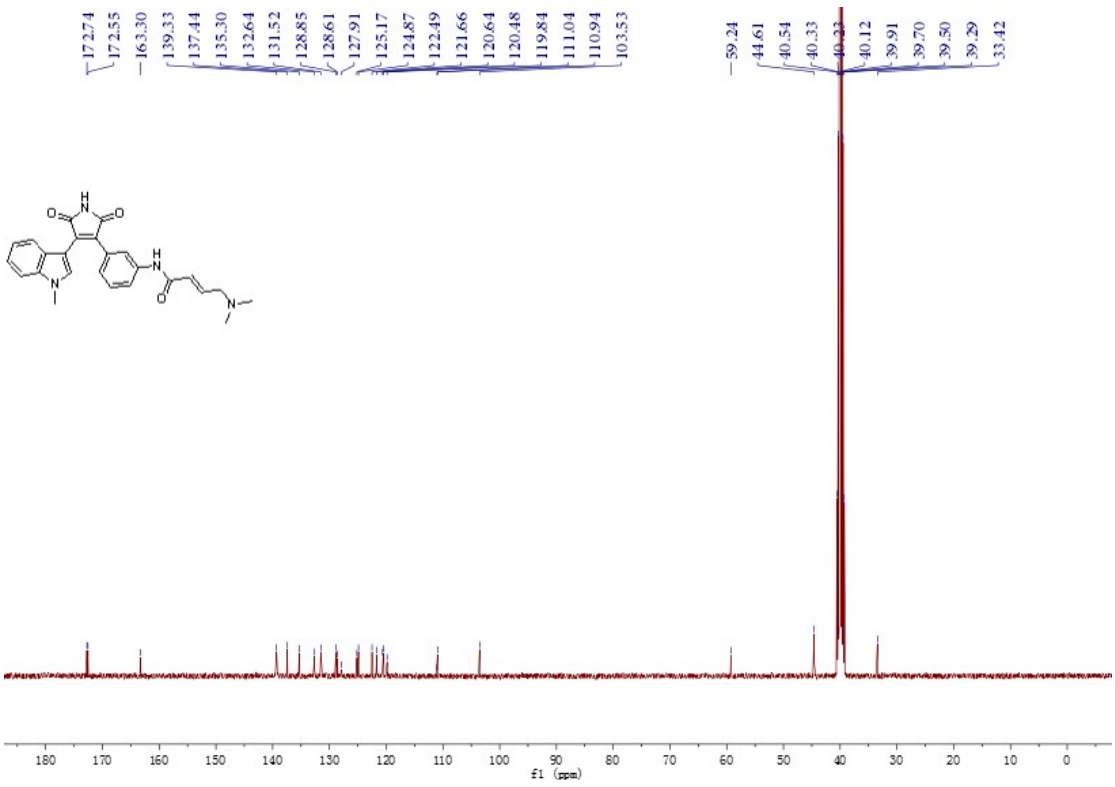
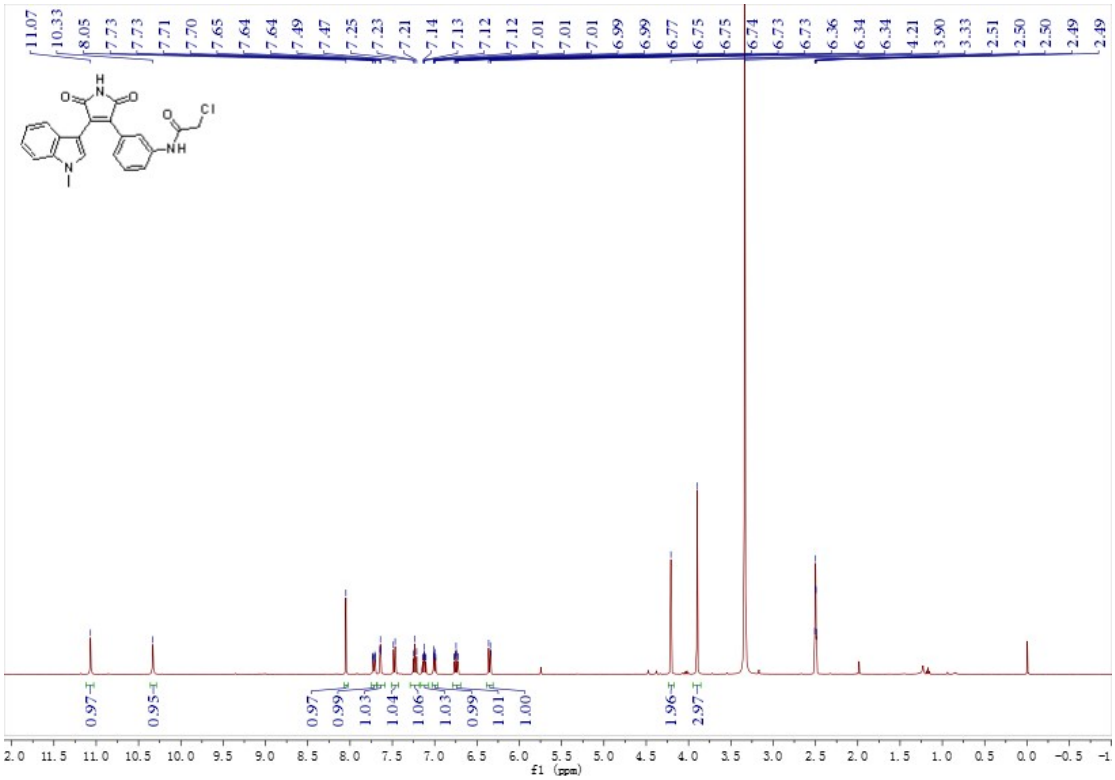
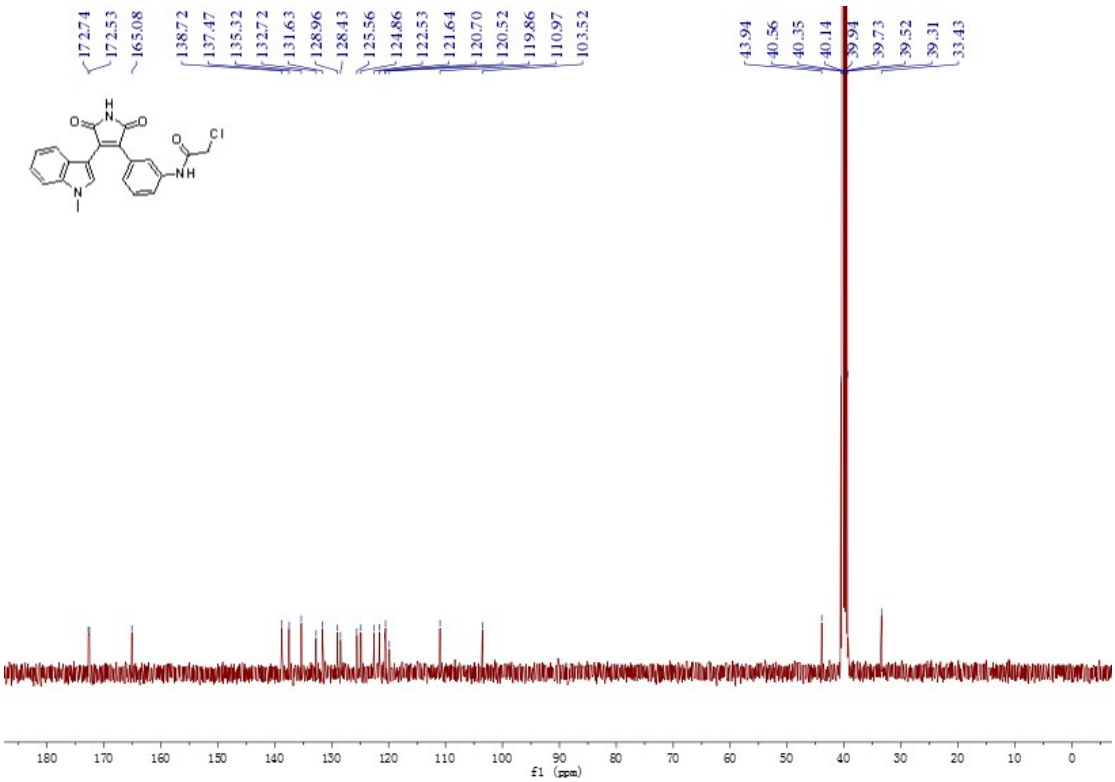


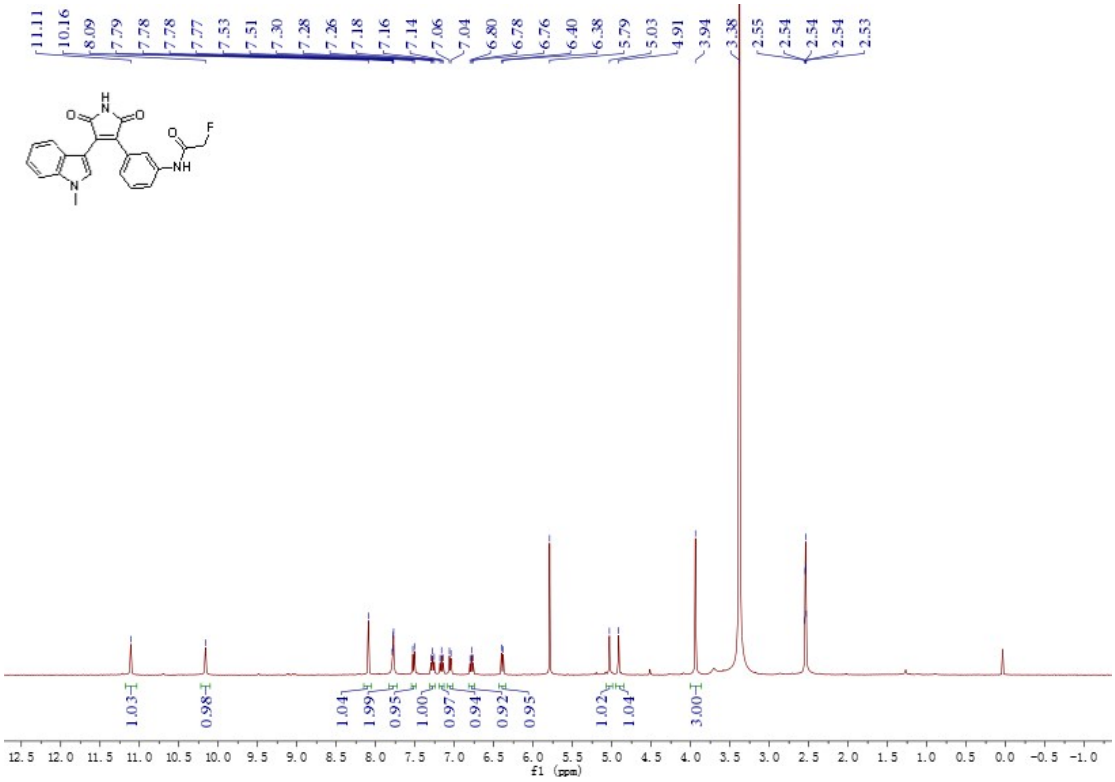
^{13}C-NMR of **20c**



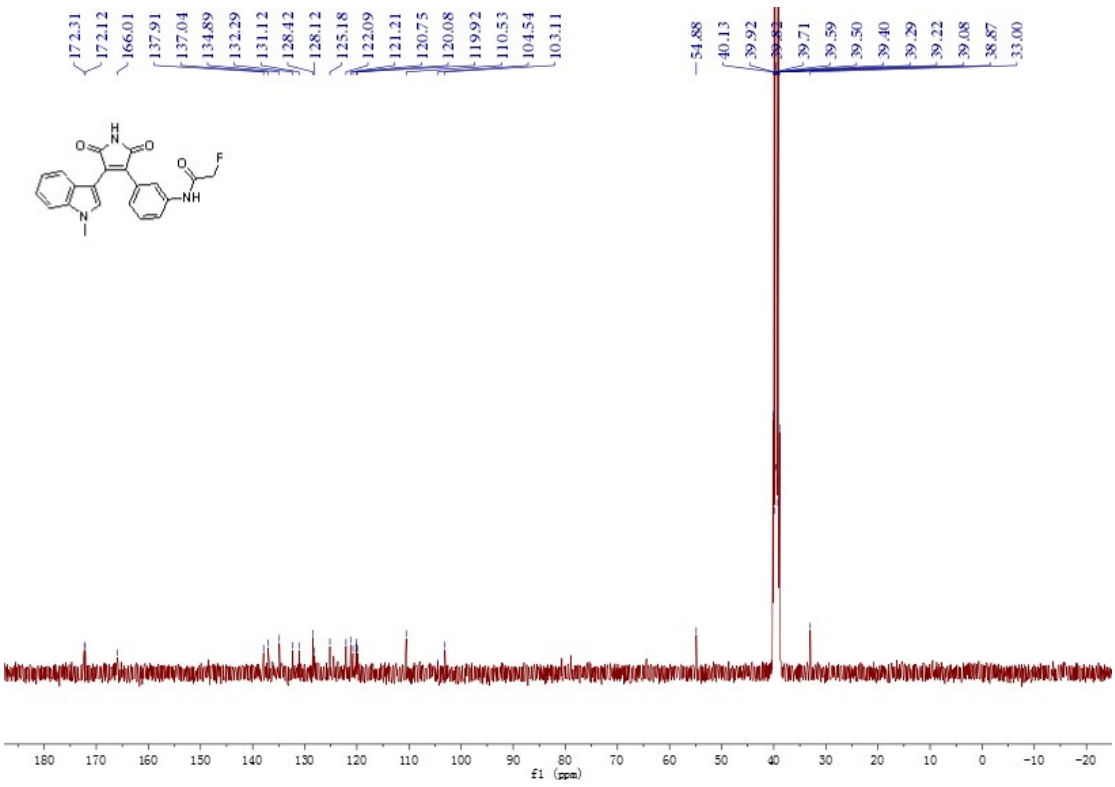
^{1}H-NMR of **20d**



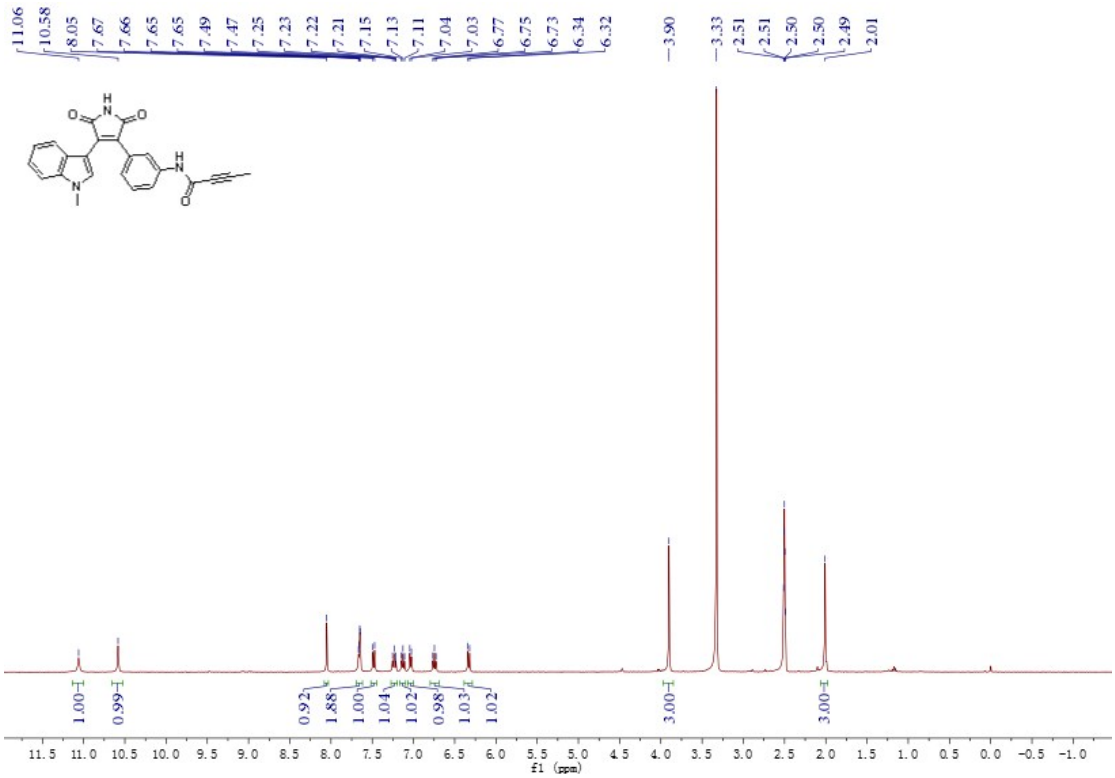
^{13}C-NMR of **20d**



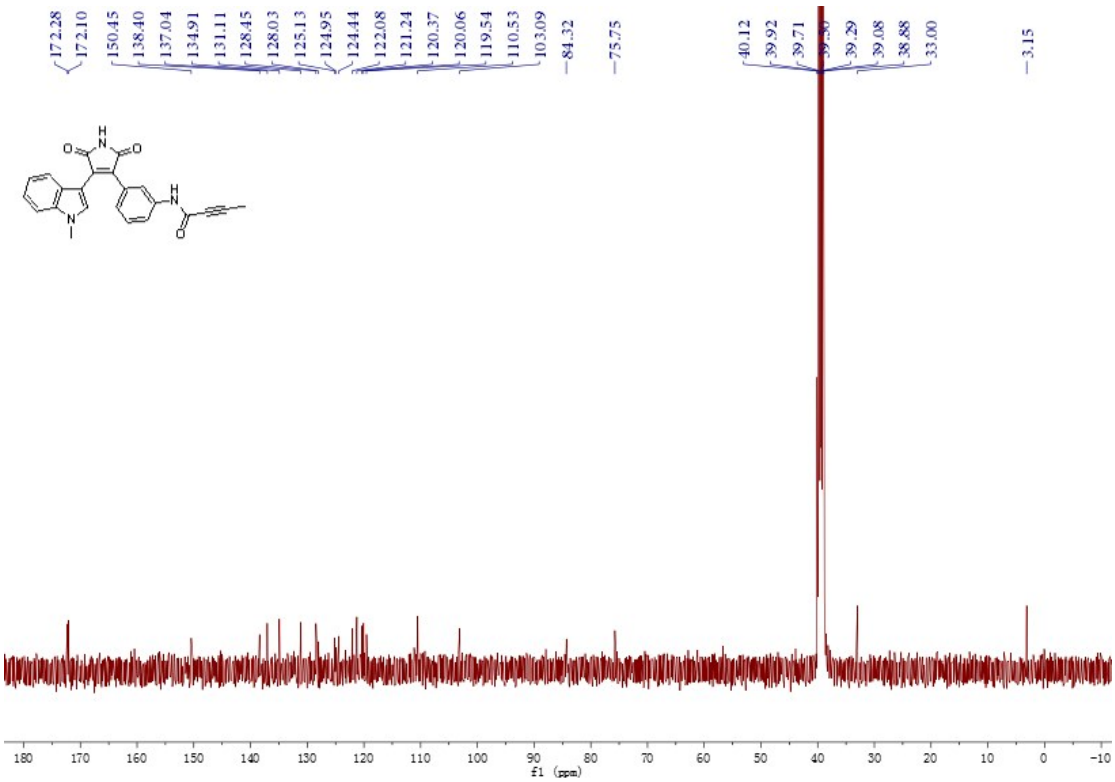
^{1}H-NMR of **20e**



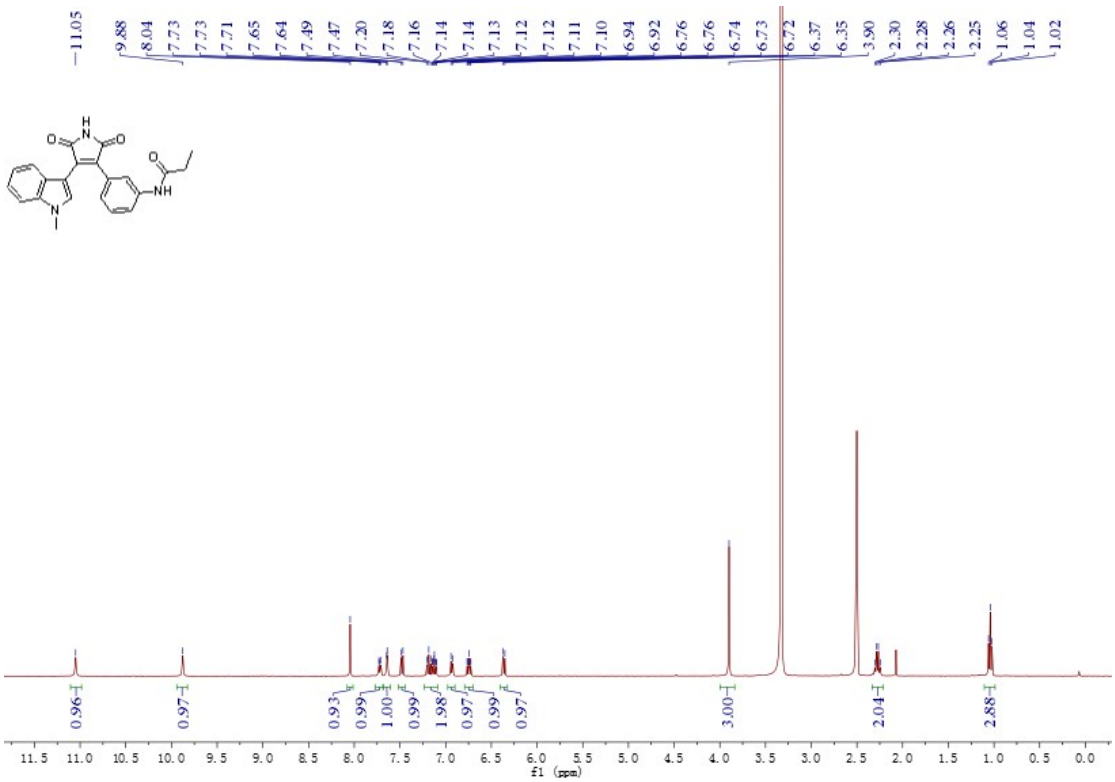
^{13}C-NMR of **20e**



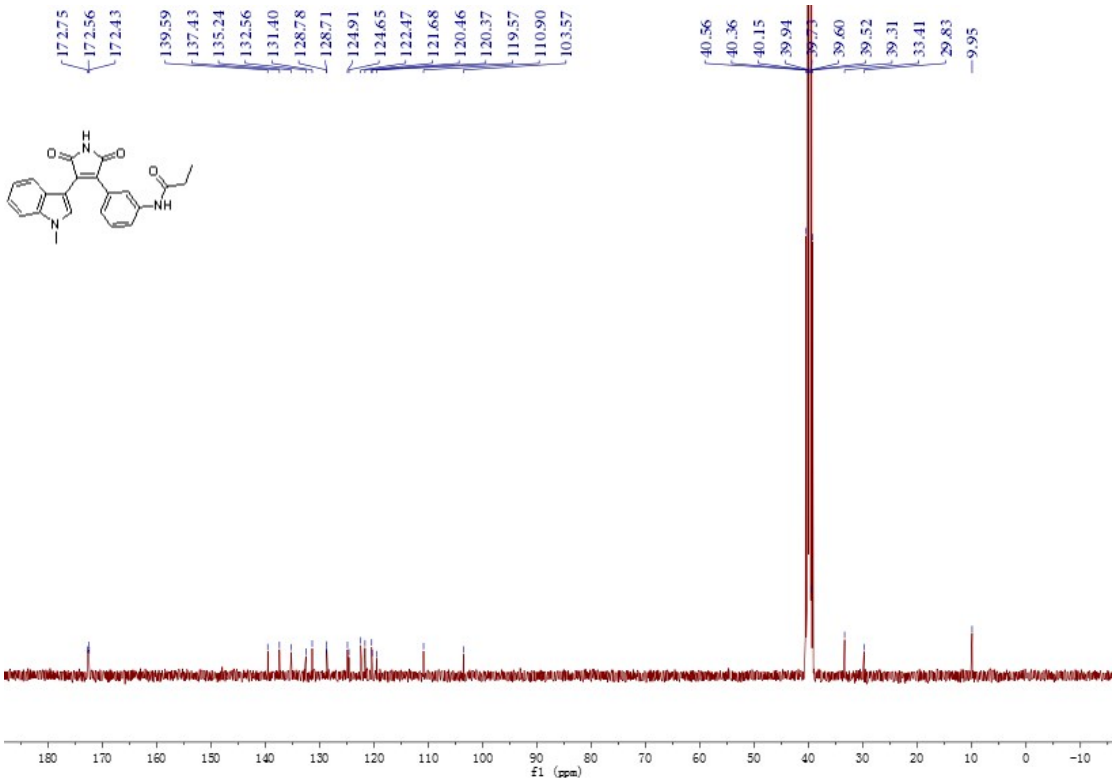
^{1}H-NMR of **20f**



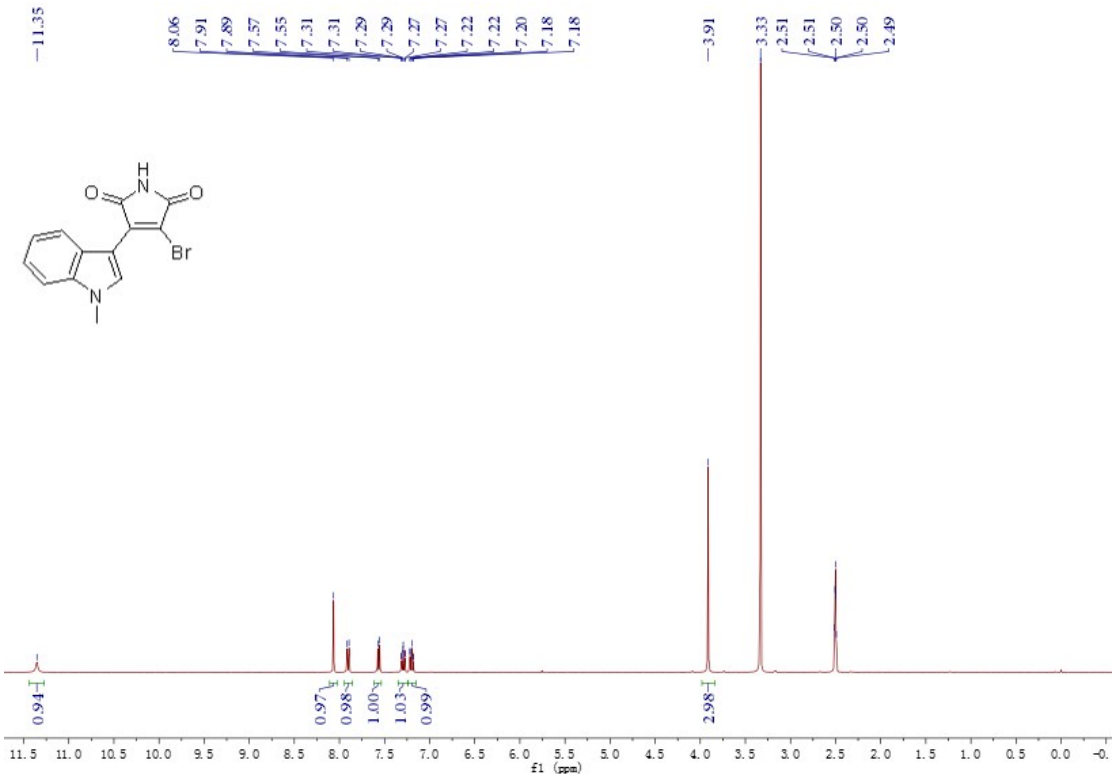
^{13}C-NMR of **20f**



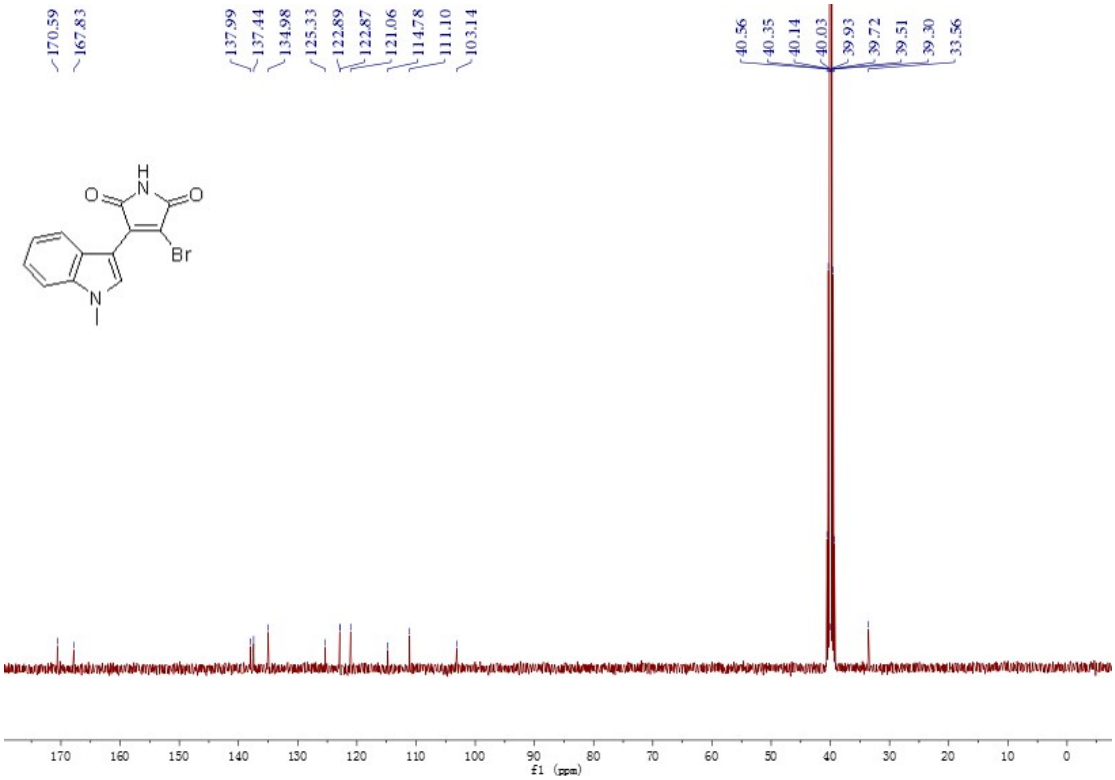
^{1}H-NMR of **20g**



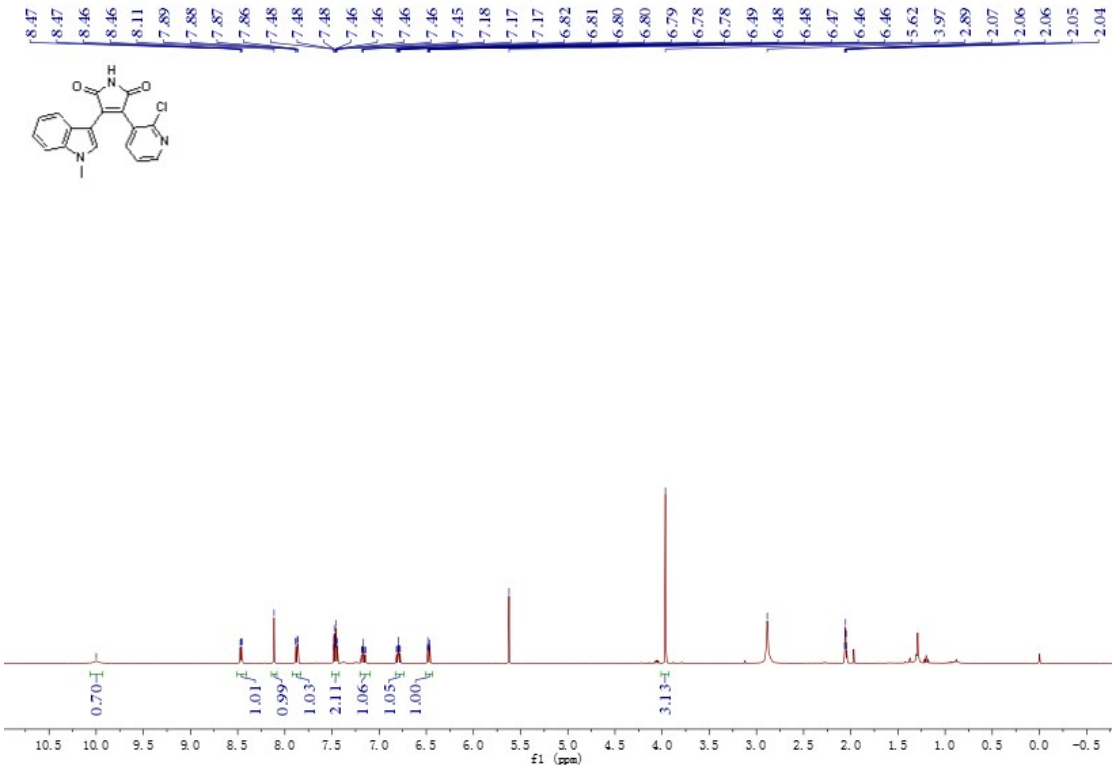
^{13}C-NMR of **20g**



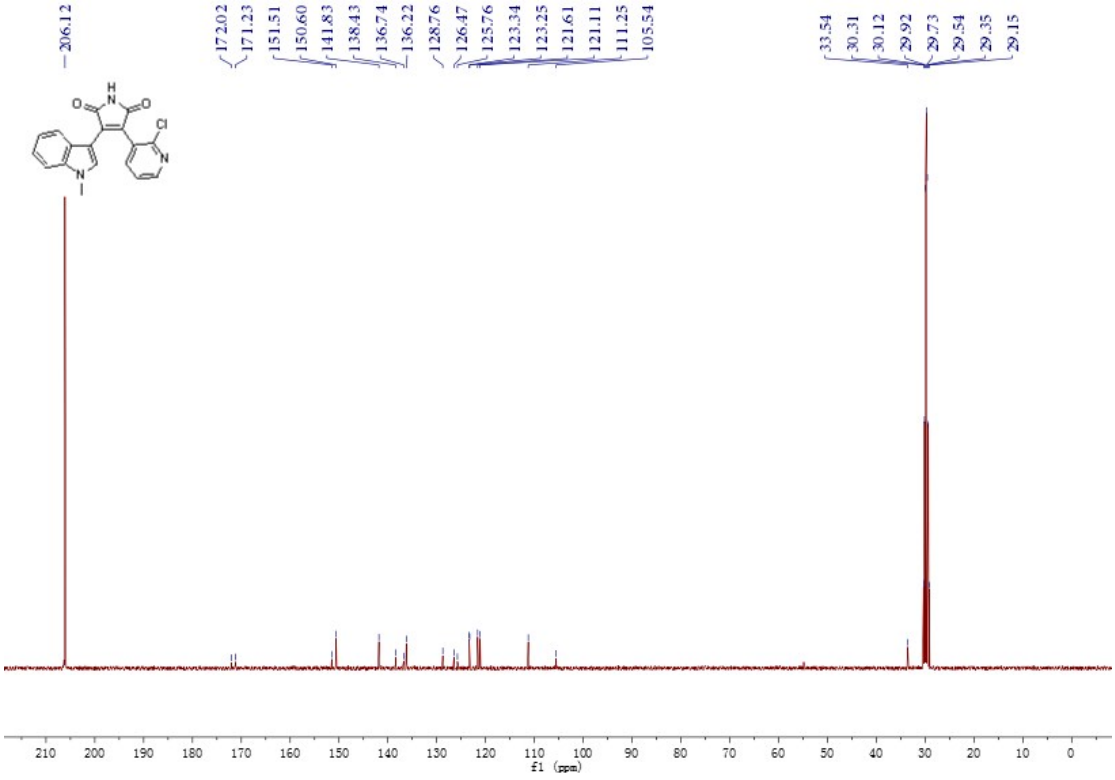
^{1}H-NMR of **23**



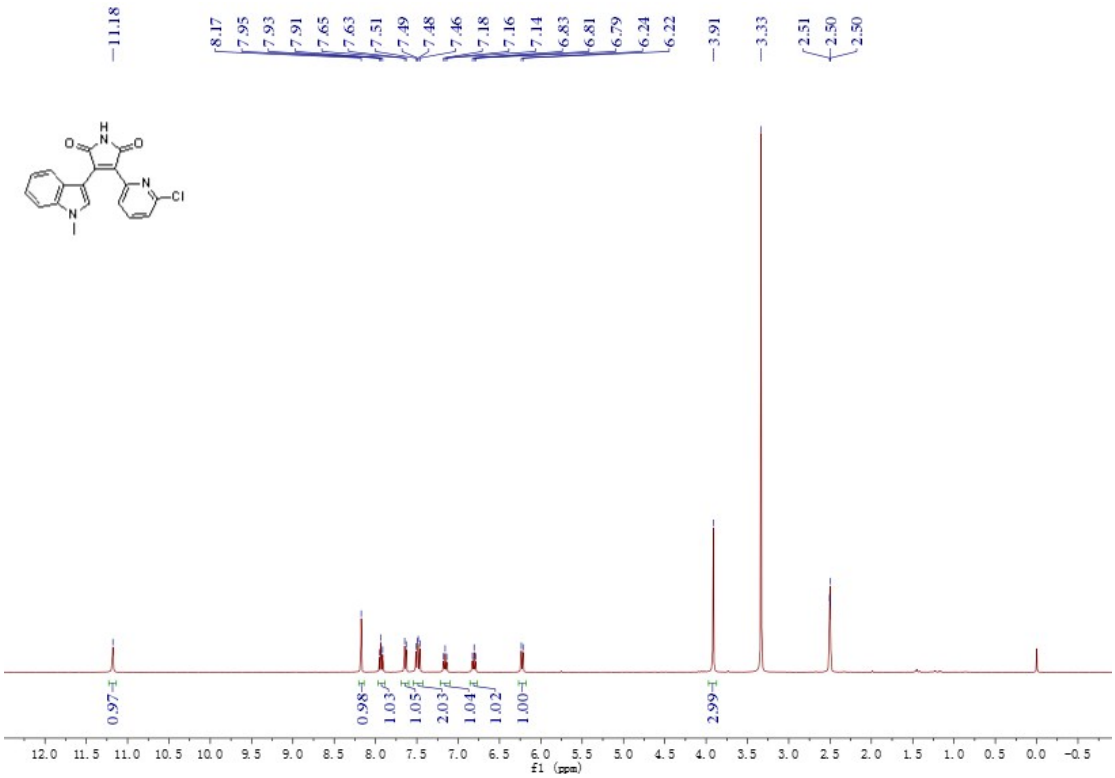
^{13}C-NMR of **23**



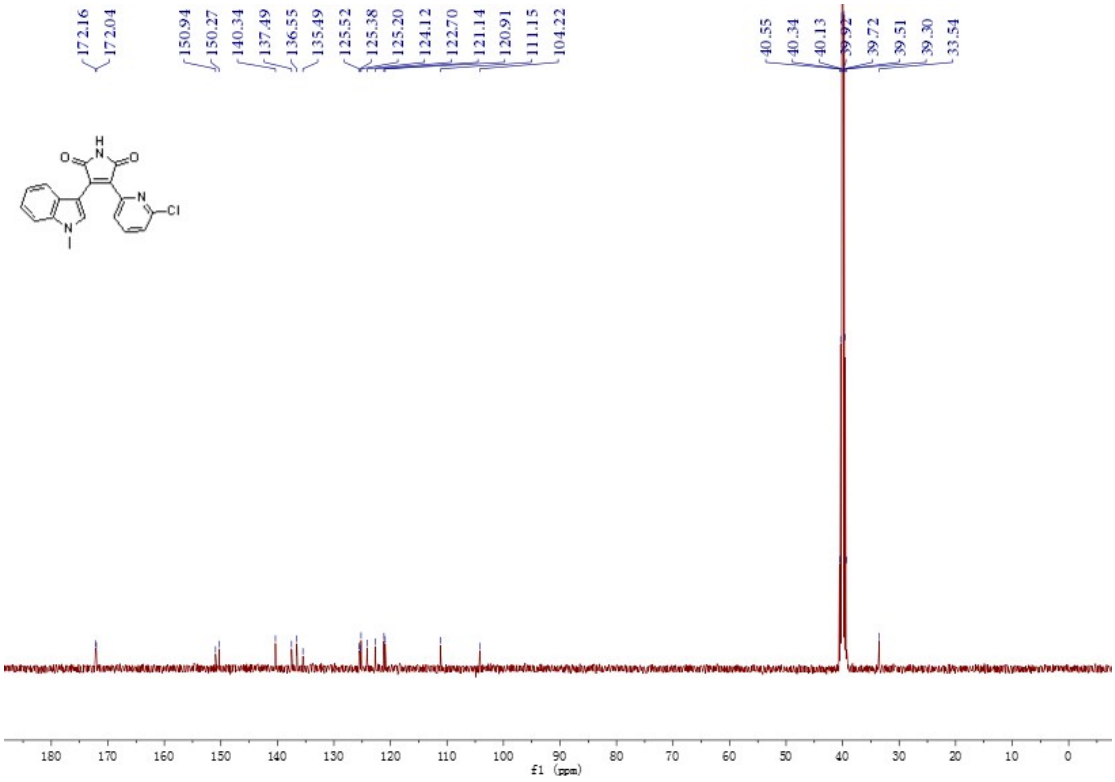
^{1}H-NMR of **24a**



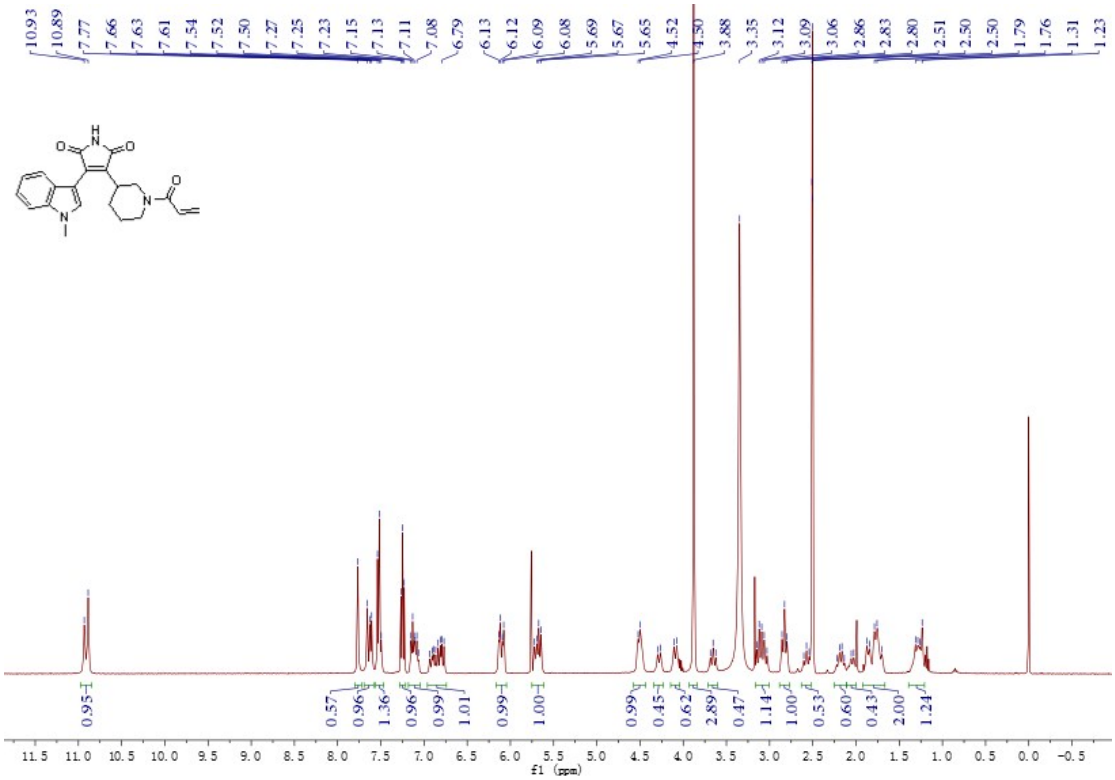
^{13}C-NMR of **24a**



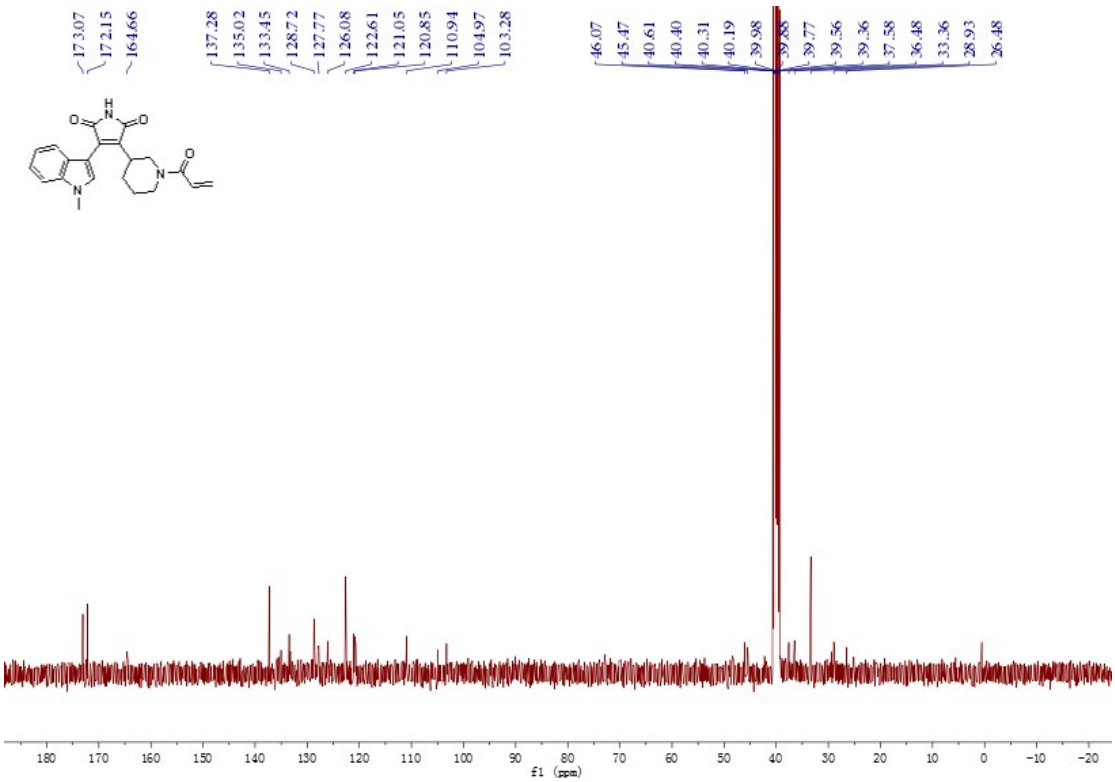
^{1}H-NMR of **24b**



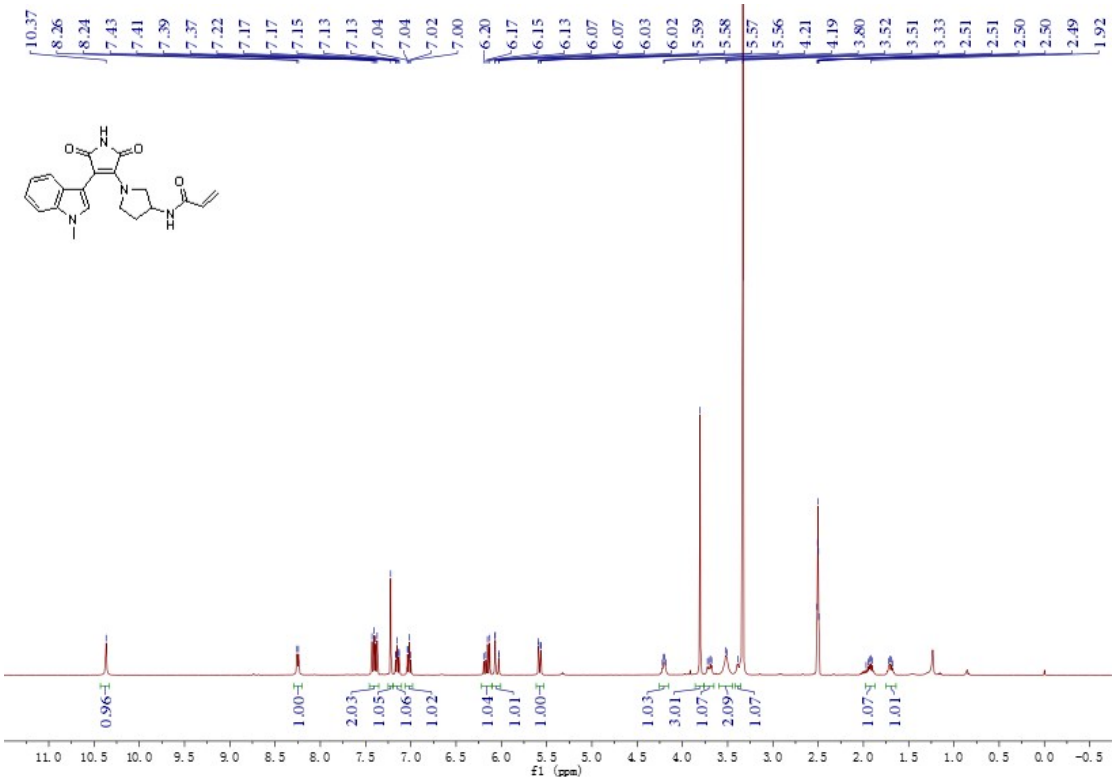
^{13}C-NMR of **24b**



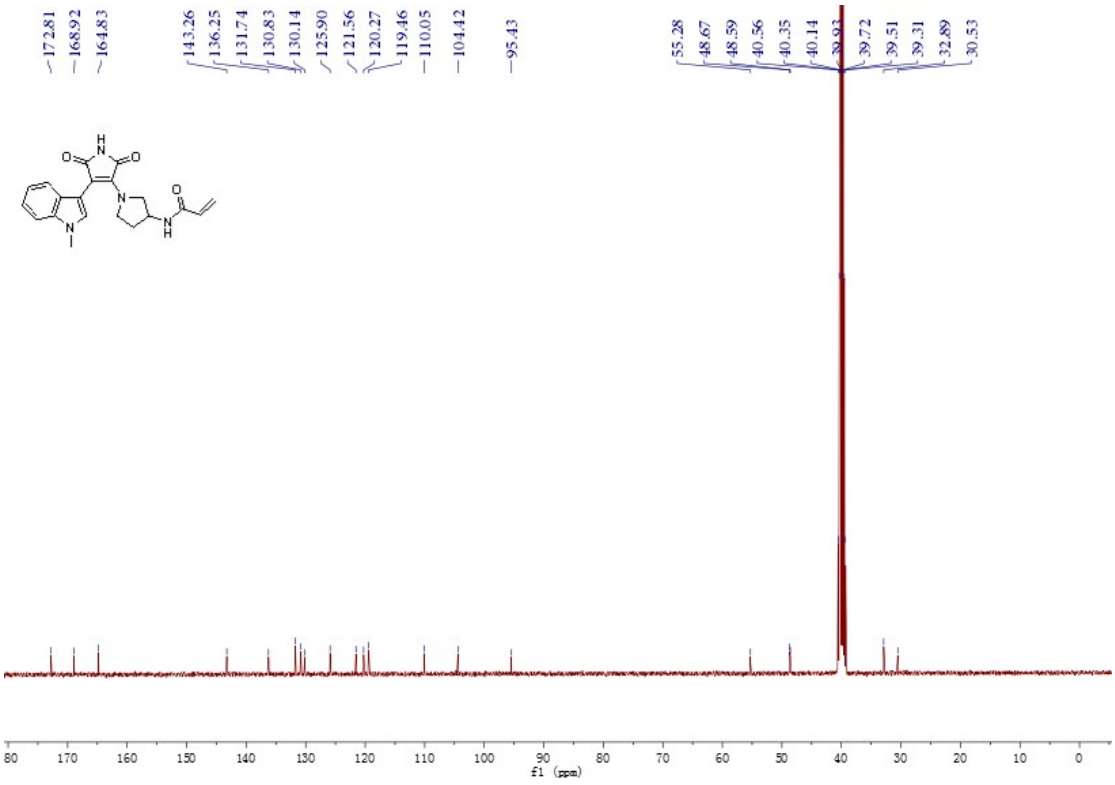
^{1}H-NMR of **26a**



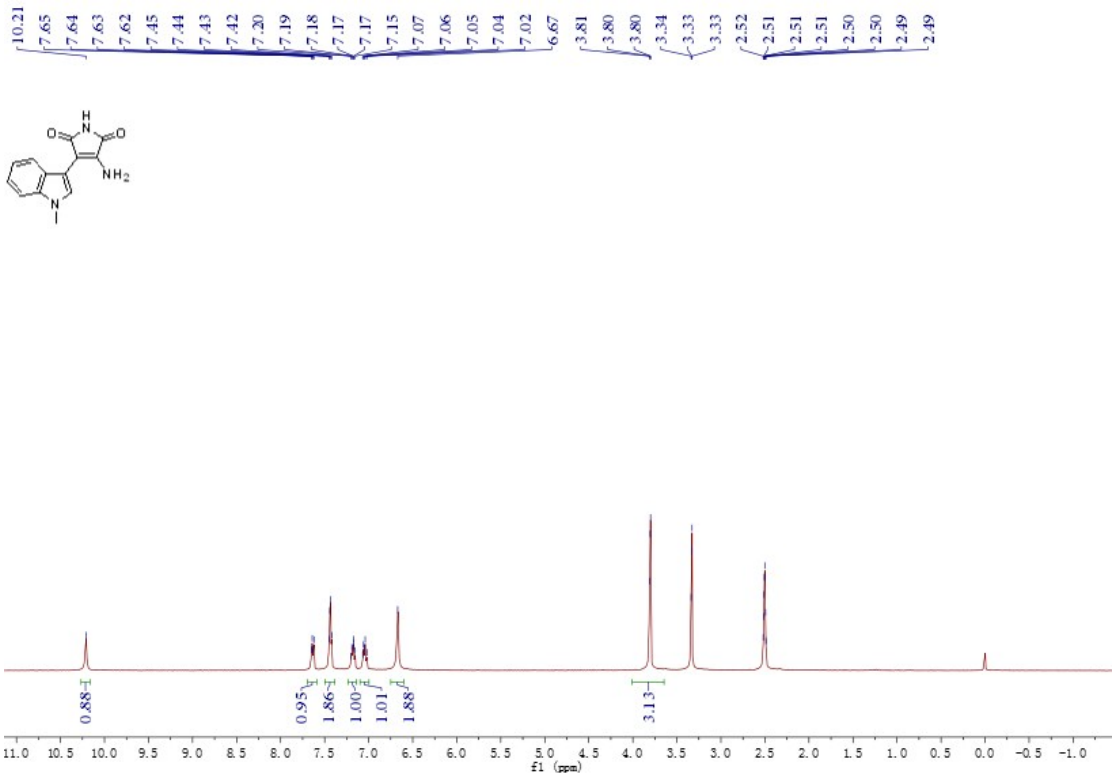
^{13}C-NMR of **26a**



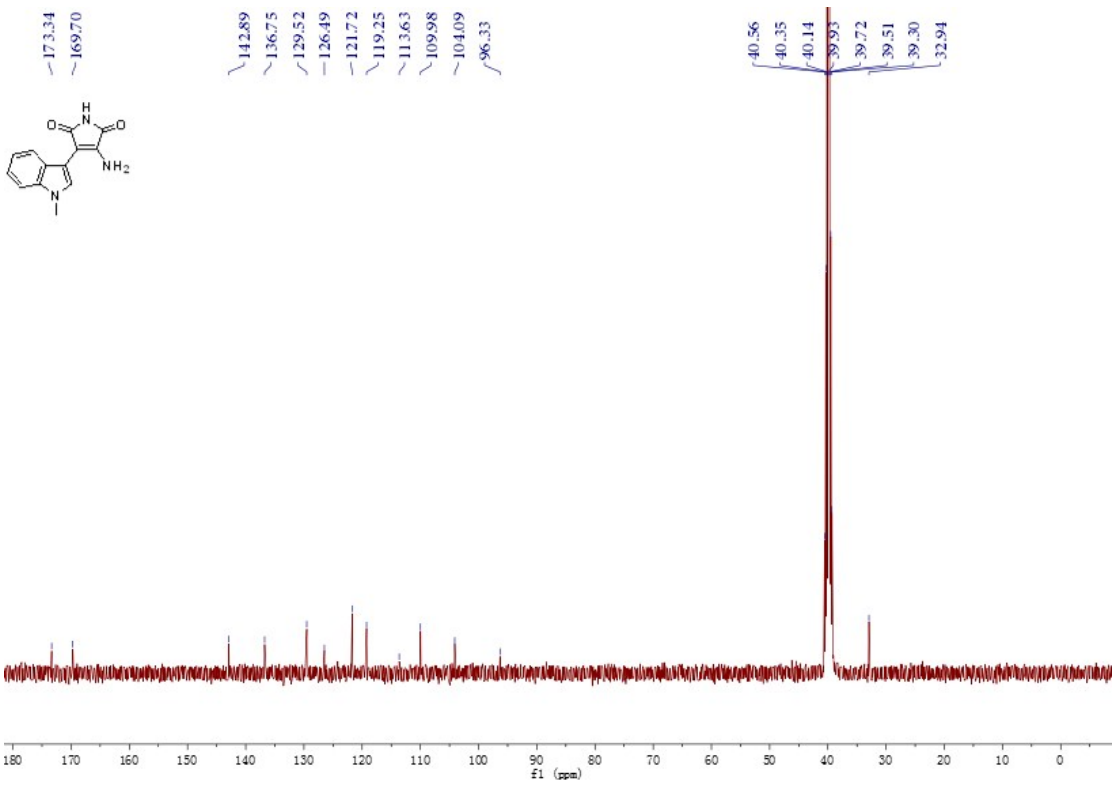
^{1}H-NMR of **26b**



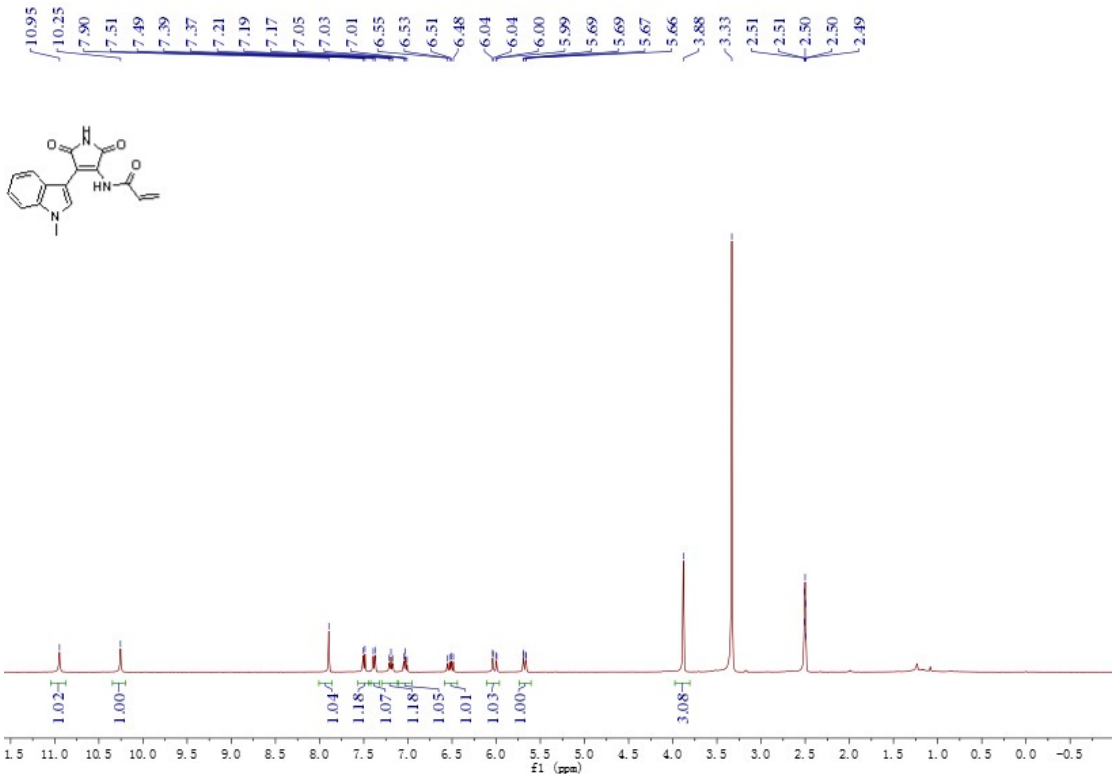
^{13}C-NMR of **26b**



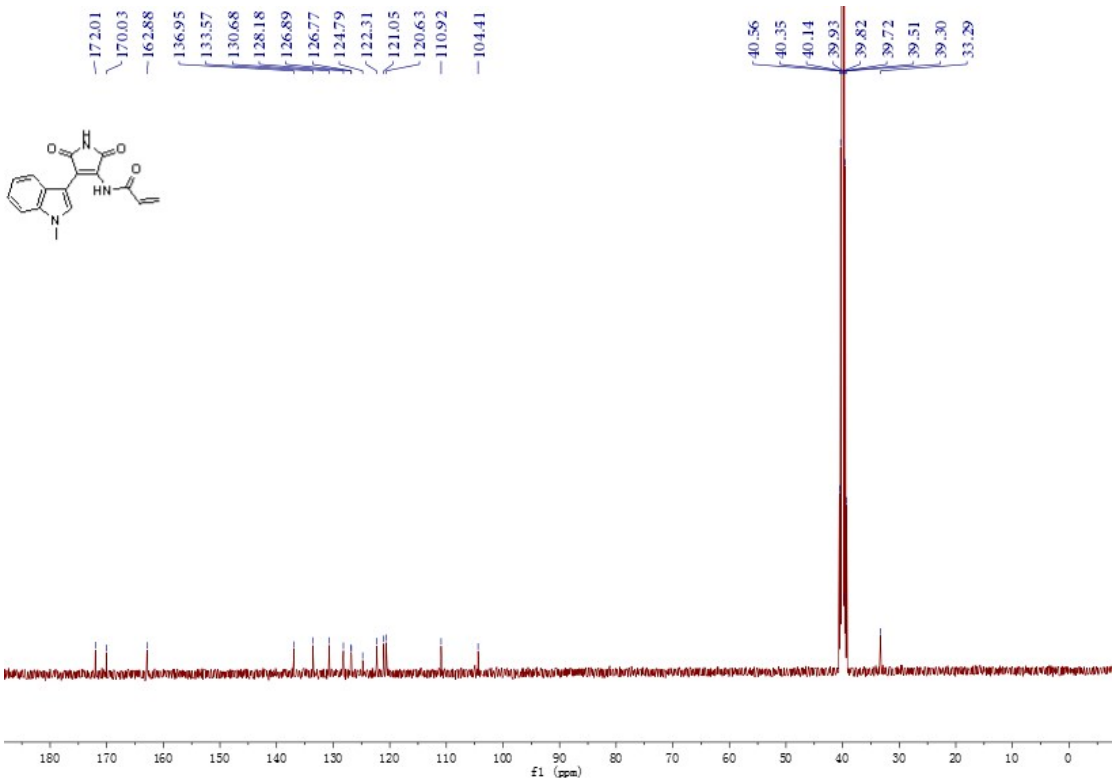
^{1}H-NMR of **27**



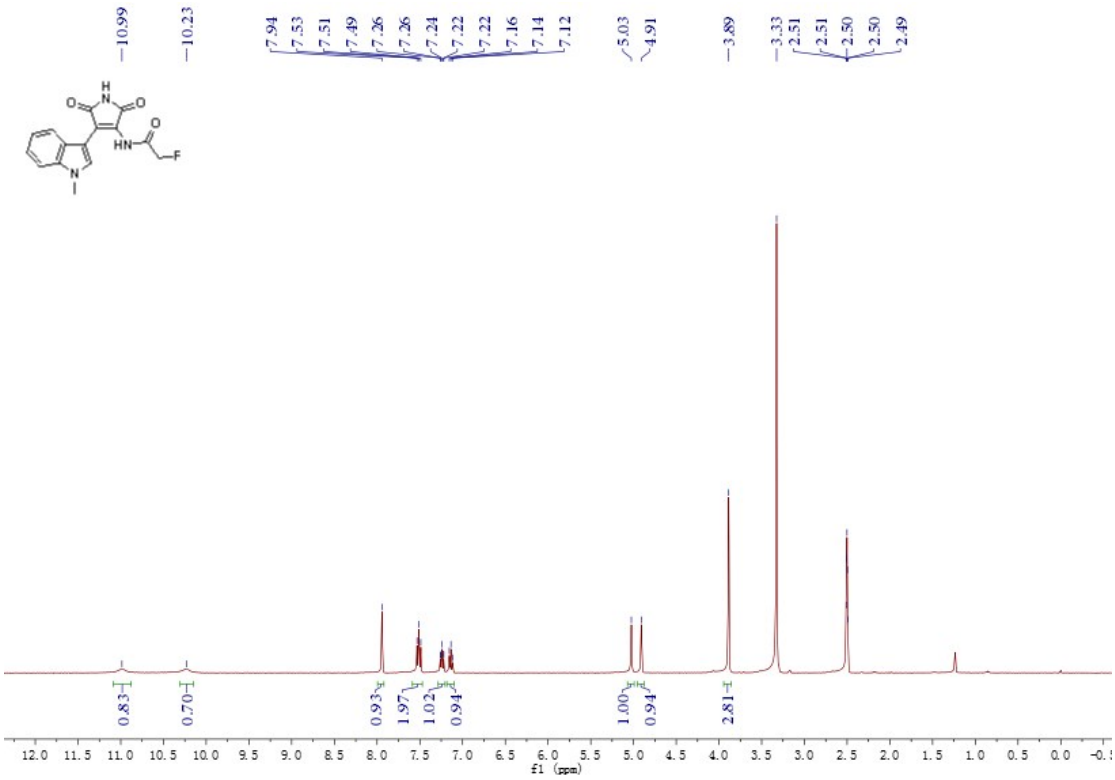
^{13}C-NMR of **27**



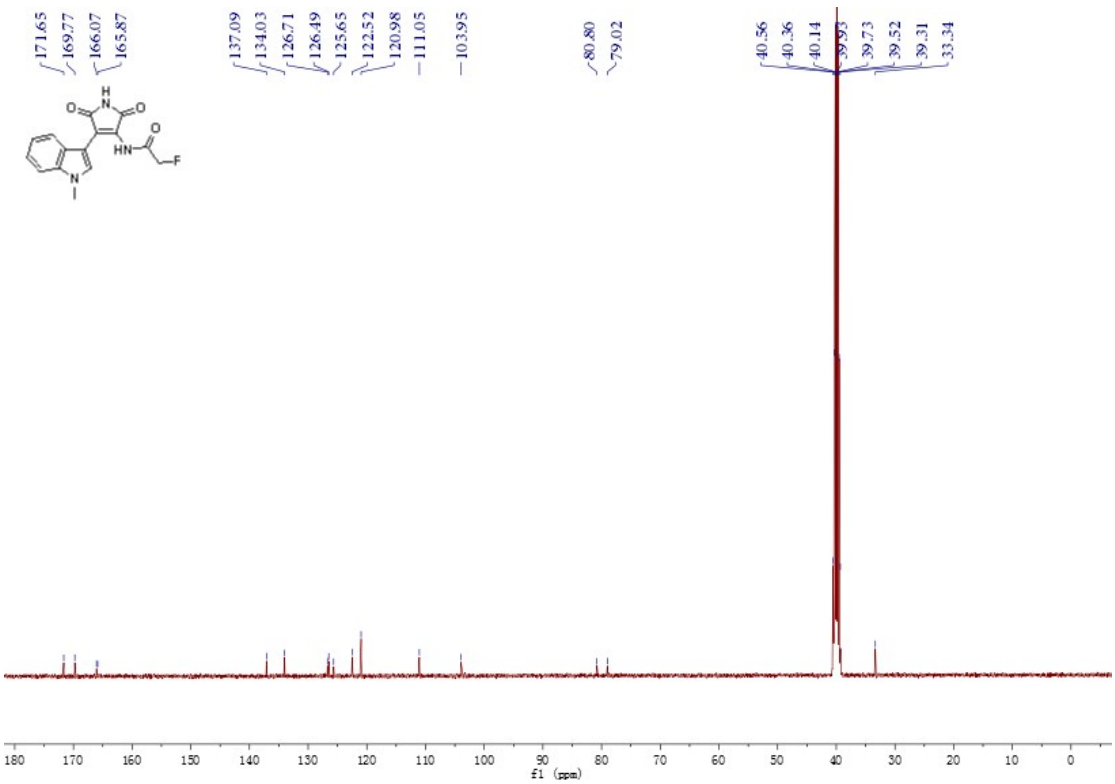
^{1}H-NMR of **28a**



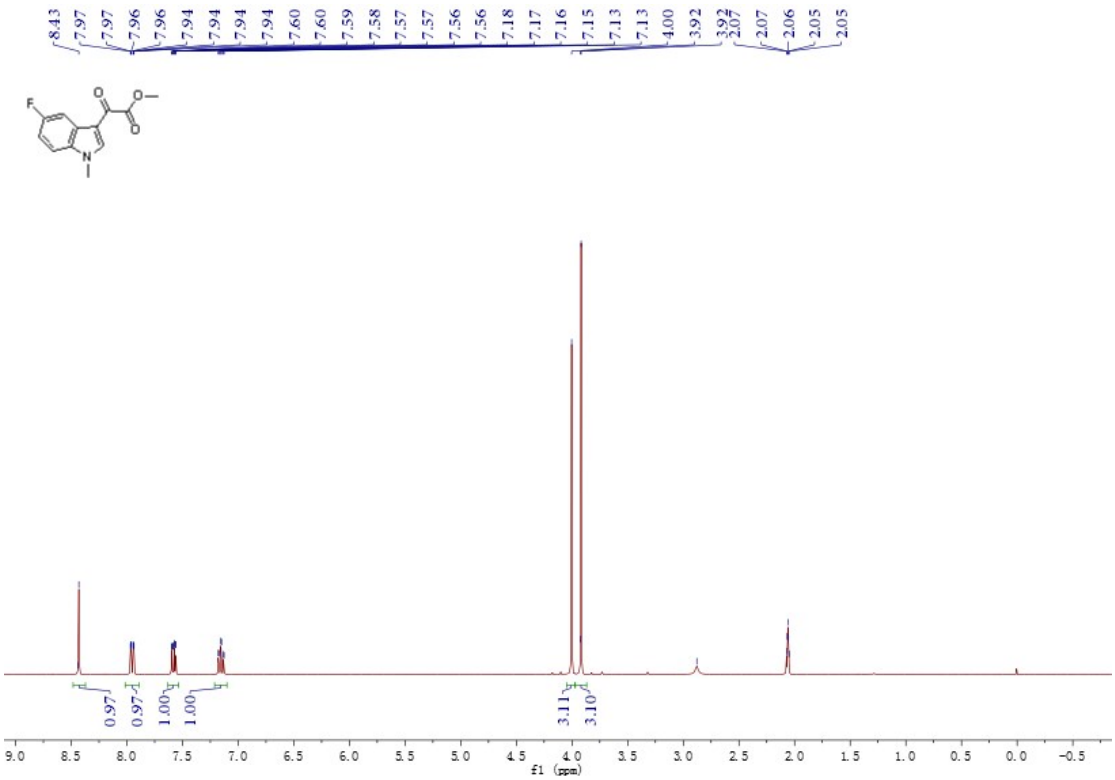
^{13}C-NMR of **28a**



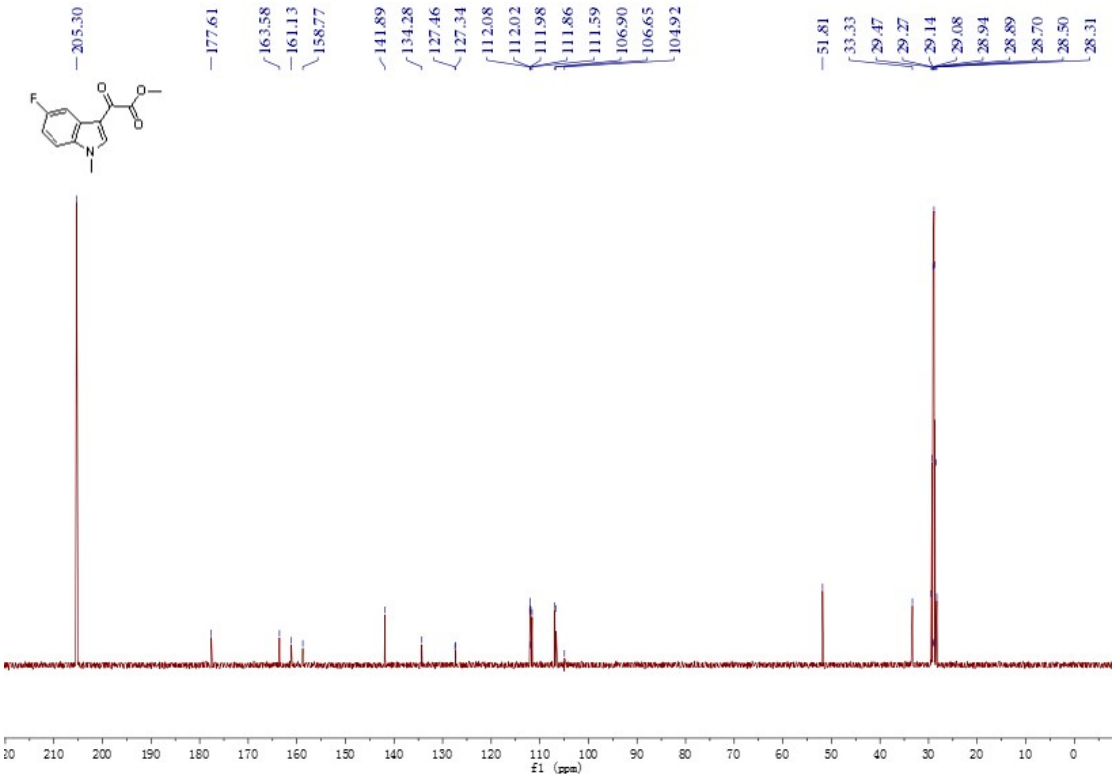
^{1}H-NMR of **28b**



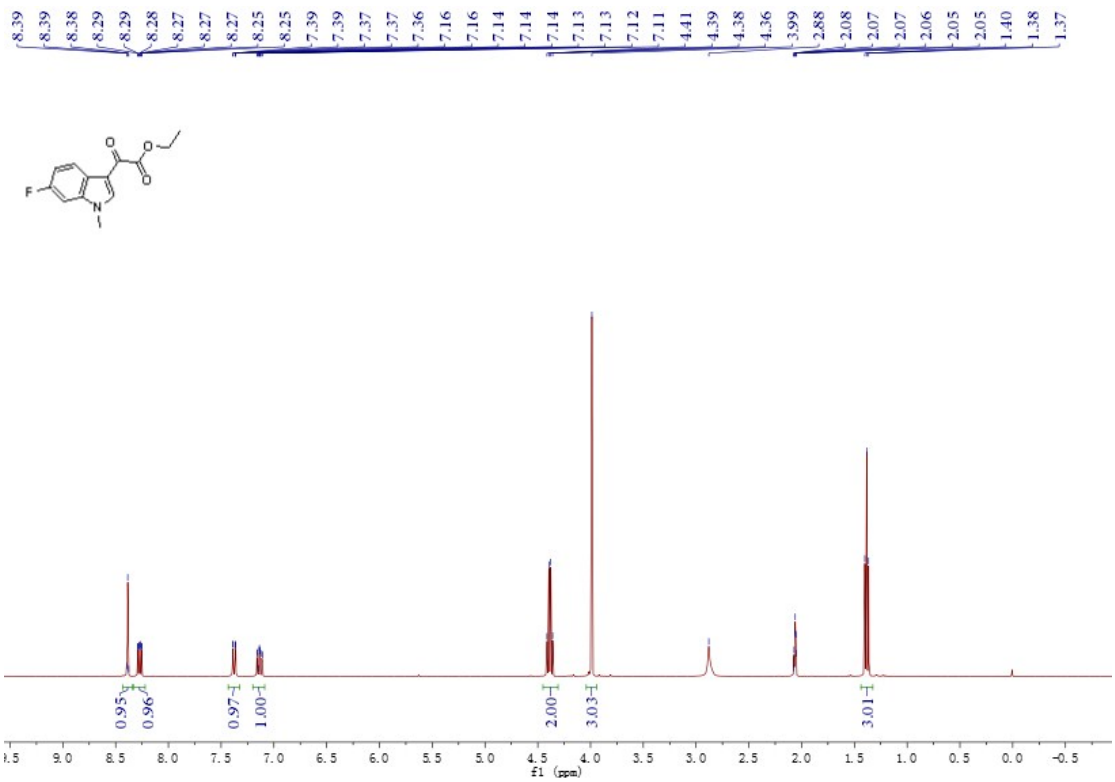
^{13}C-NMR of **28b**



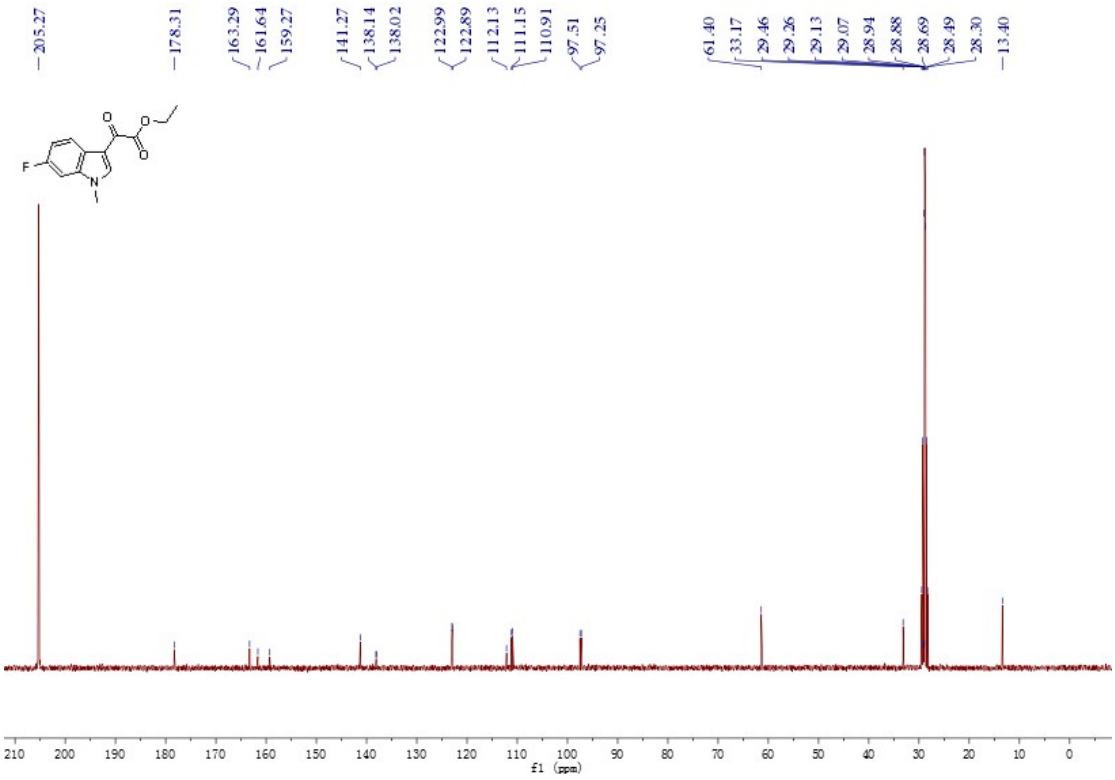
^{1}H-NMR of **31a**



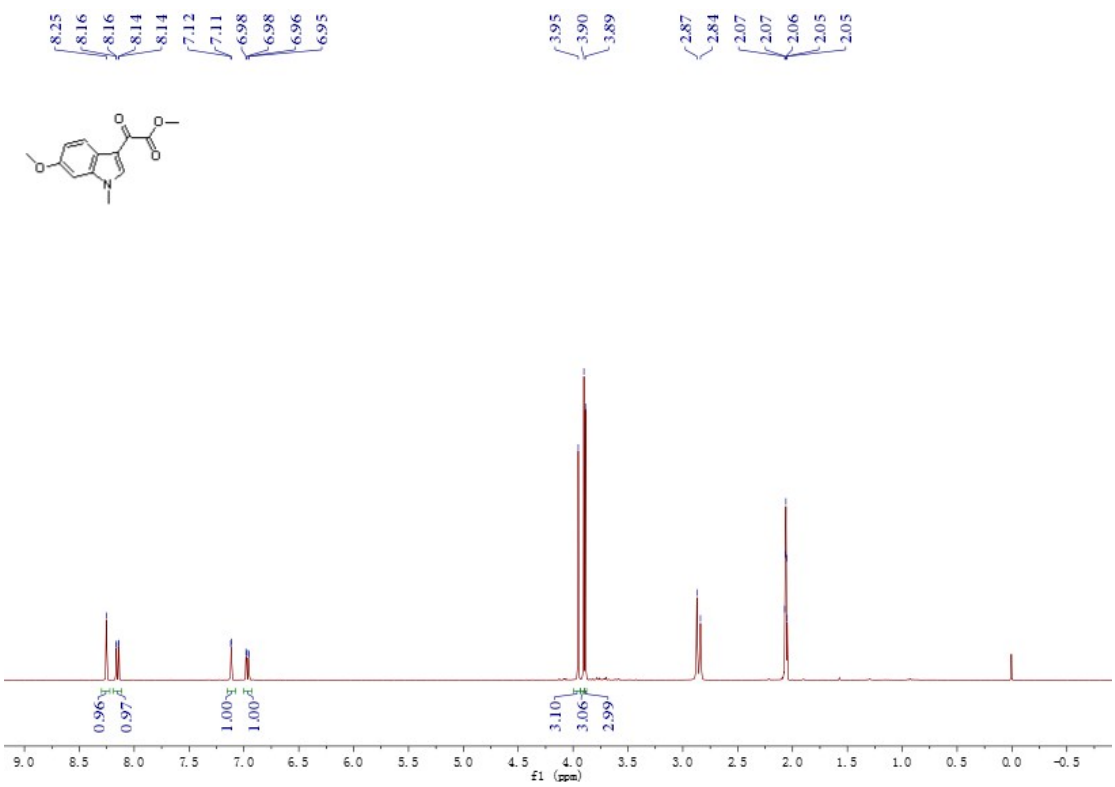
^{13}C-NMR of **31a**



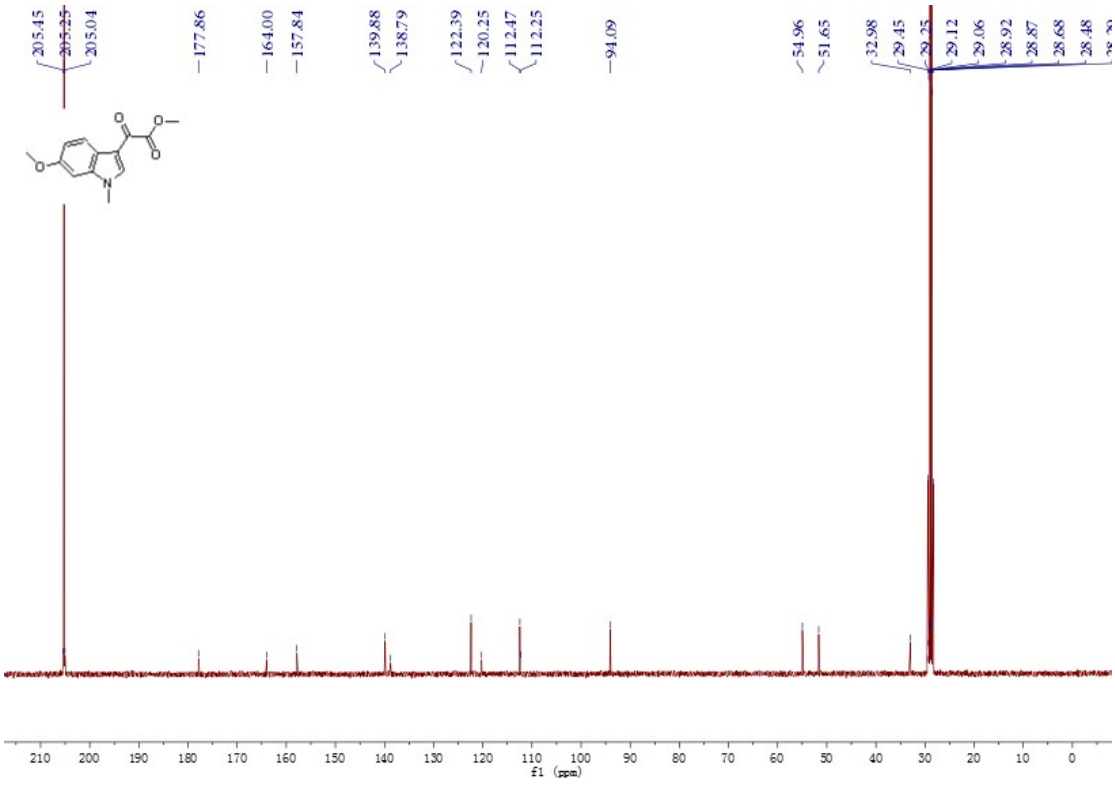
^{1}H-NMR of **31b**



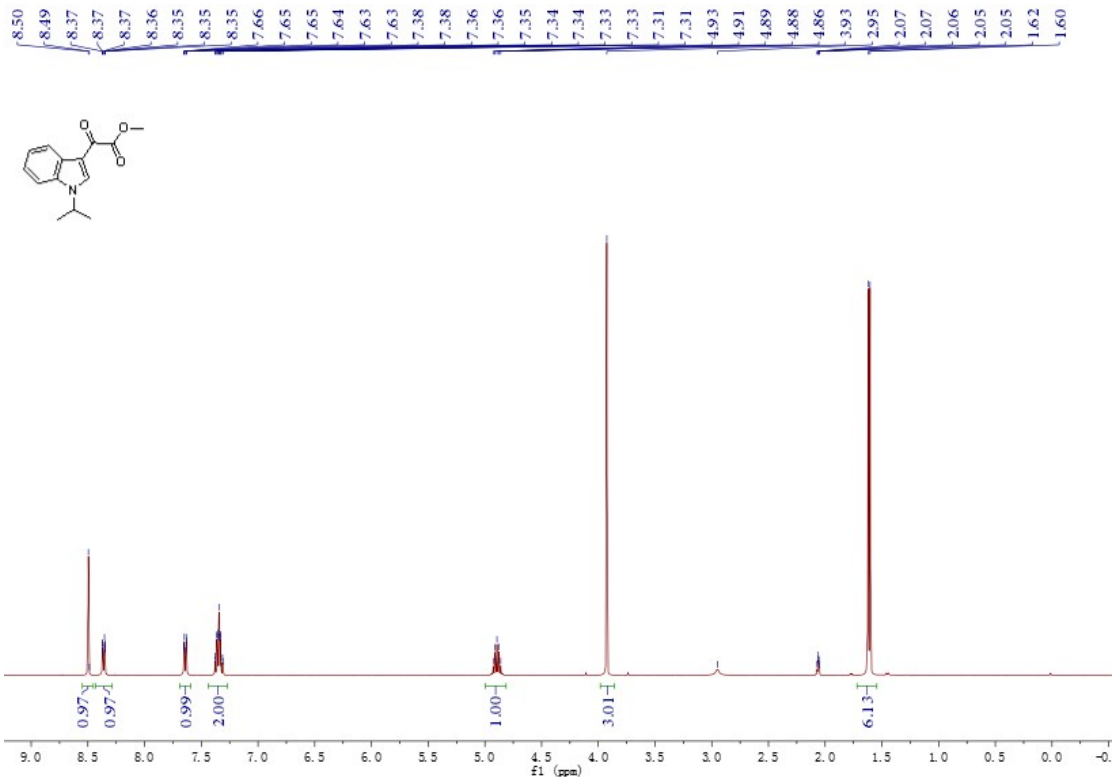
^{13}C-NMR of **31b**



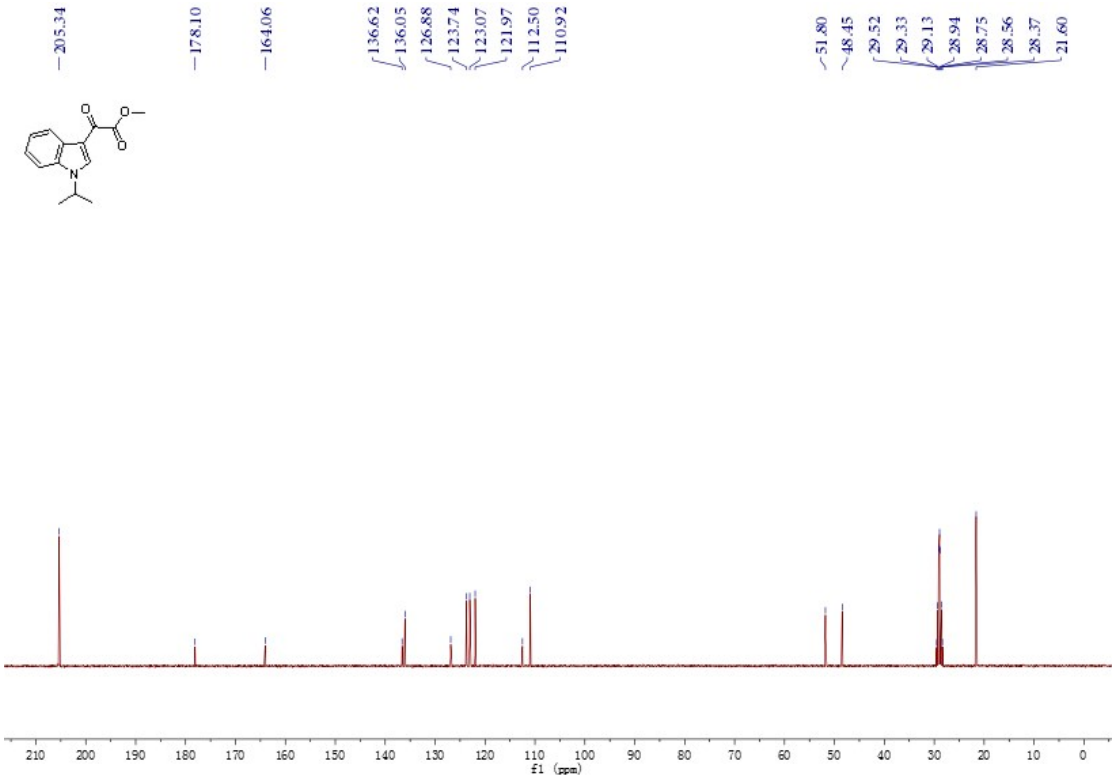
^{1}H-NMR of **31c**



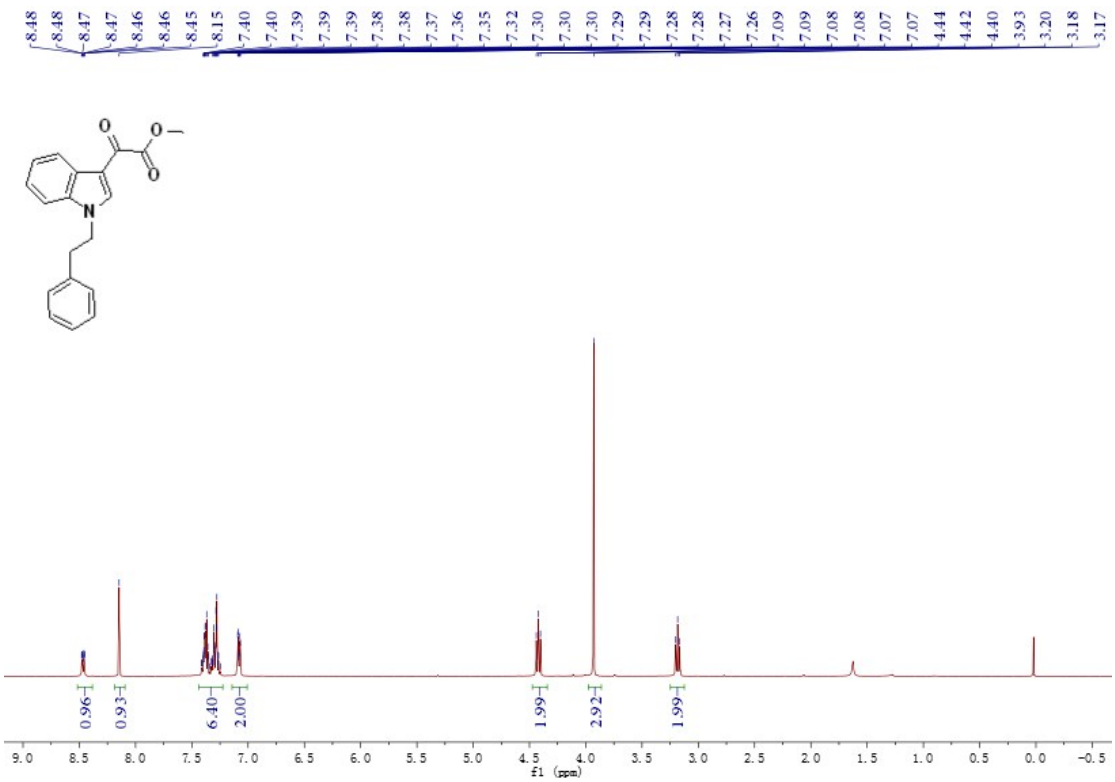
^{13}C-NMR of **31c**



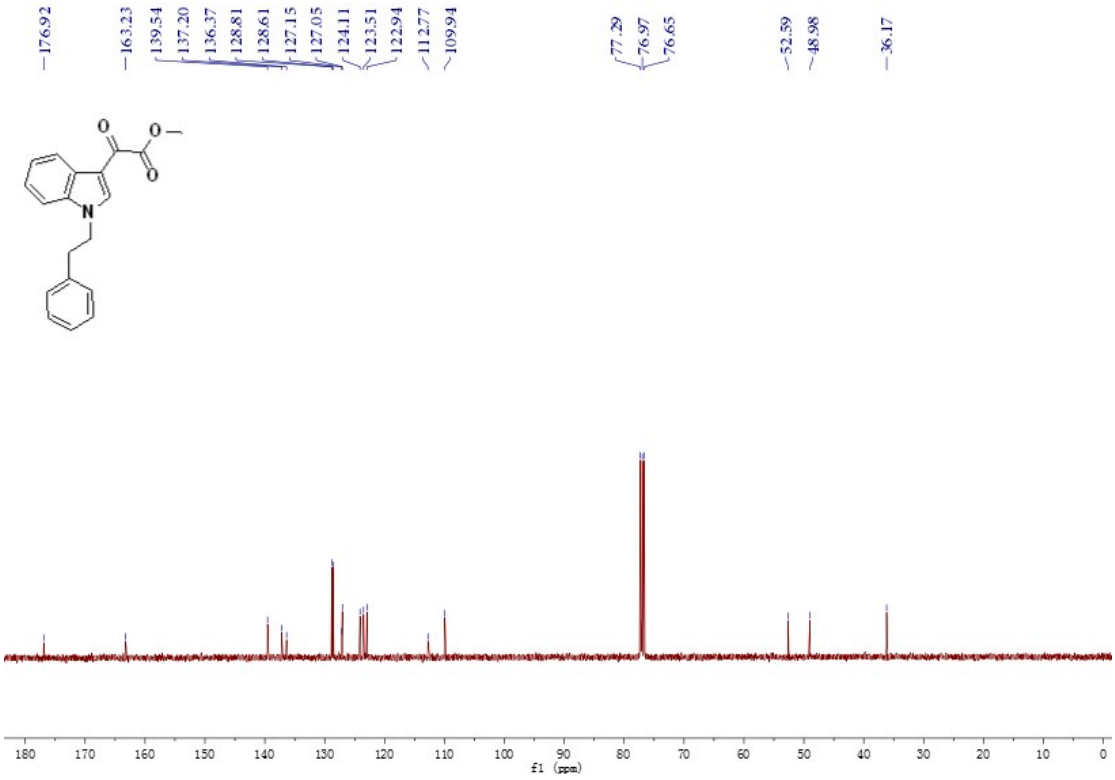
^{1}H-NMR of **31d**



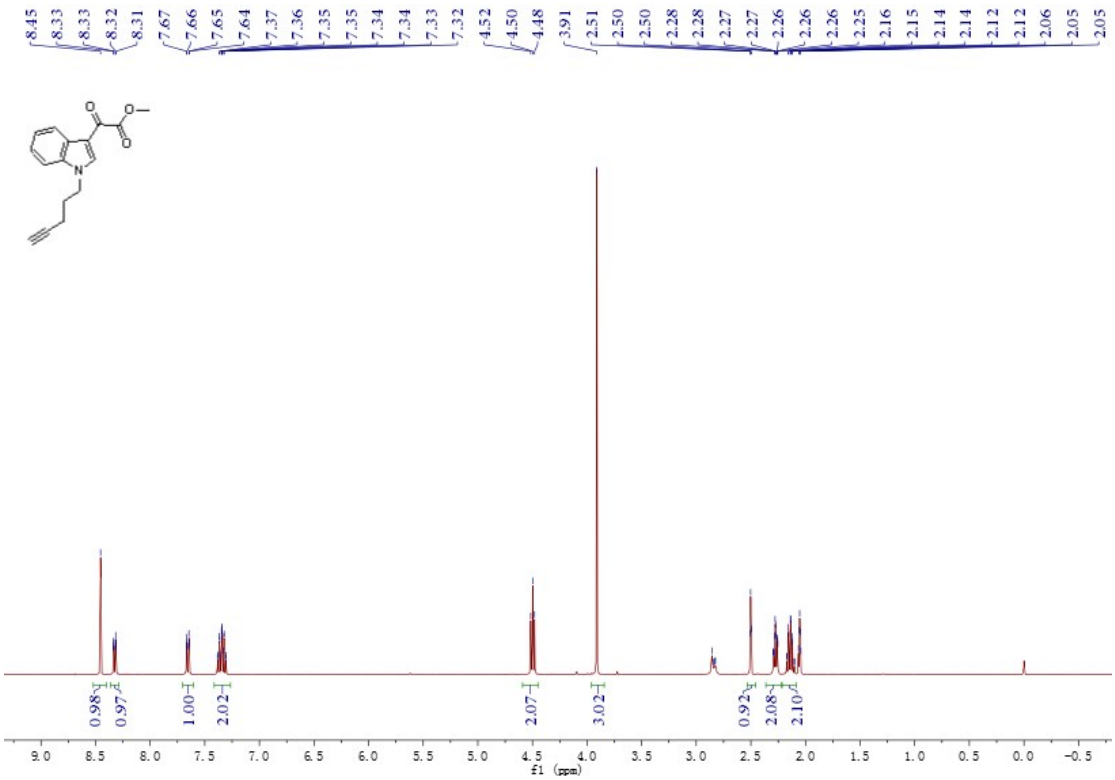
^{13}C-NMR of **31d**



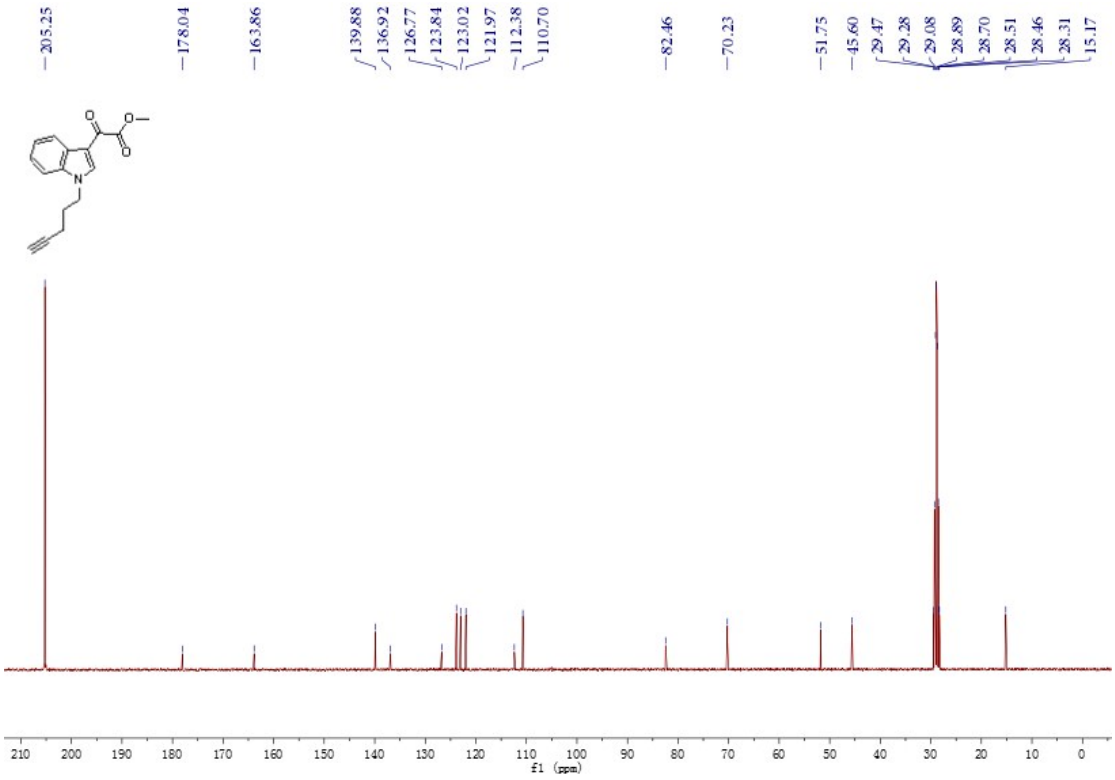
^{1}H-NMR of **31e**



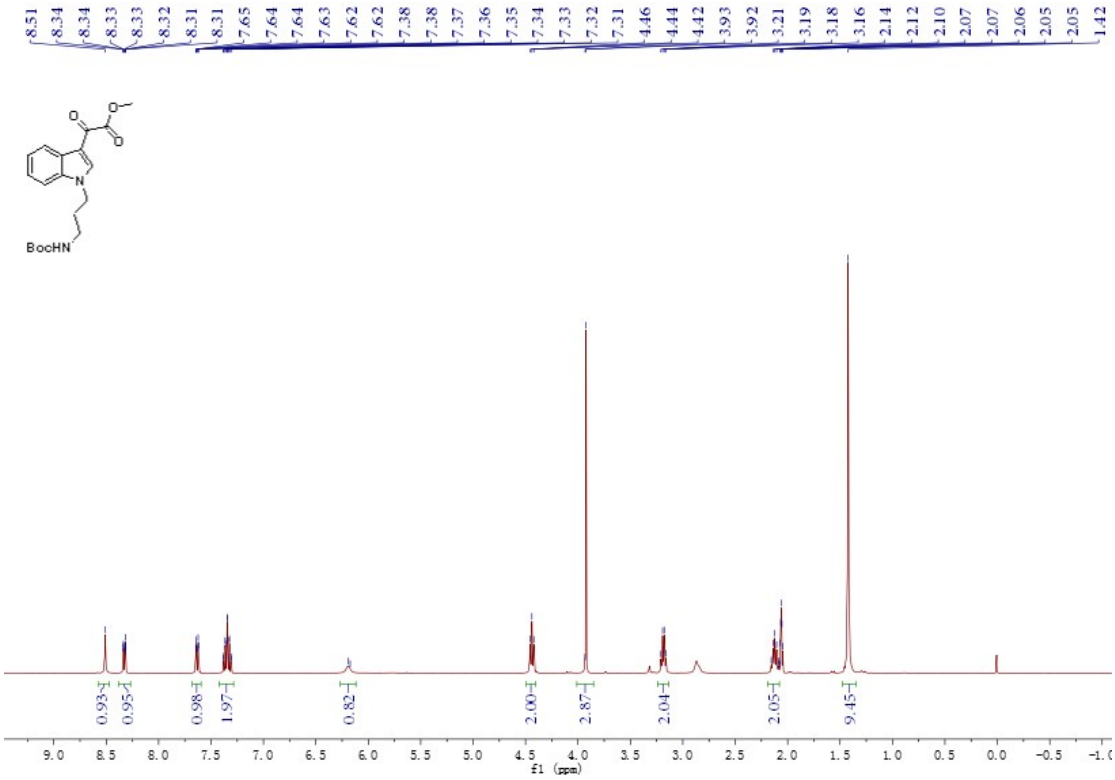
^{13}C-NMR of **31e**



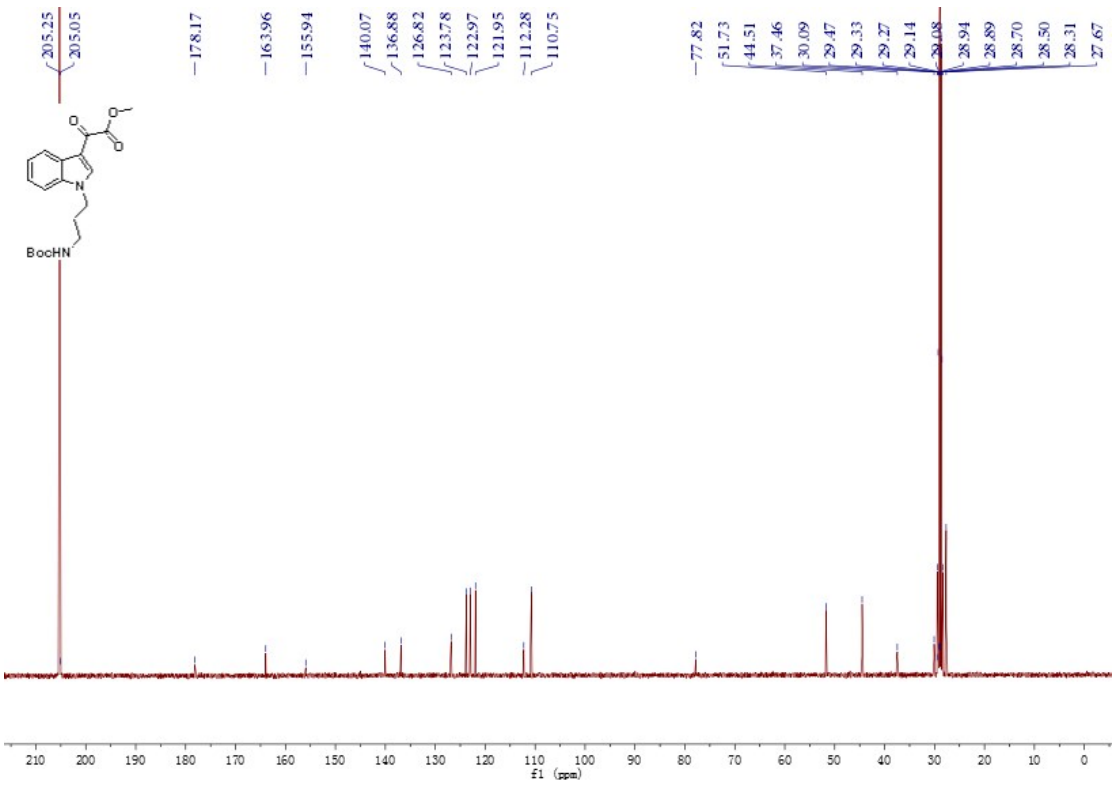
^{1}H-NMR of **31f**



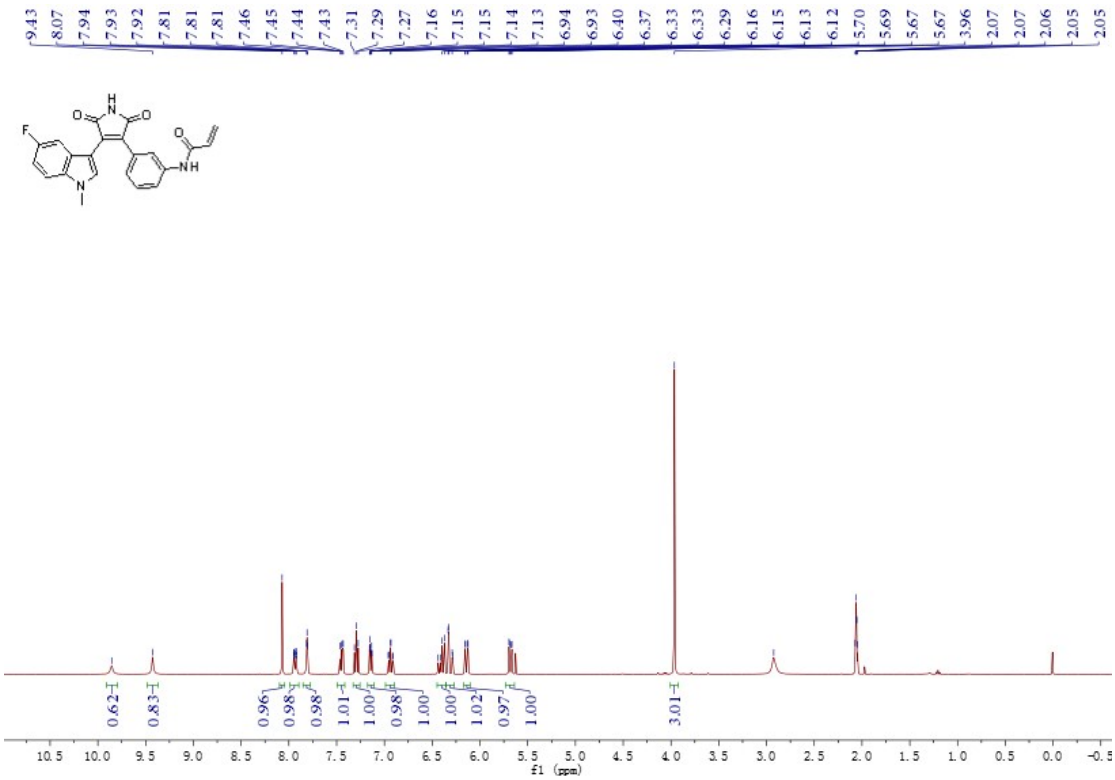
^{13}C-NMR of **31f**



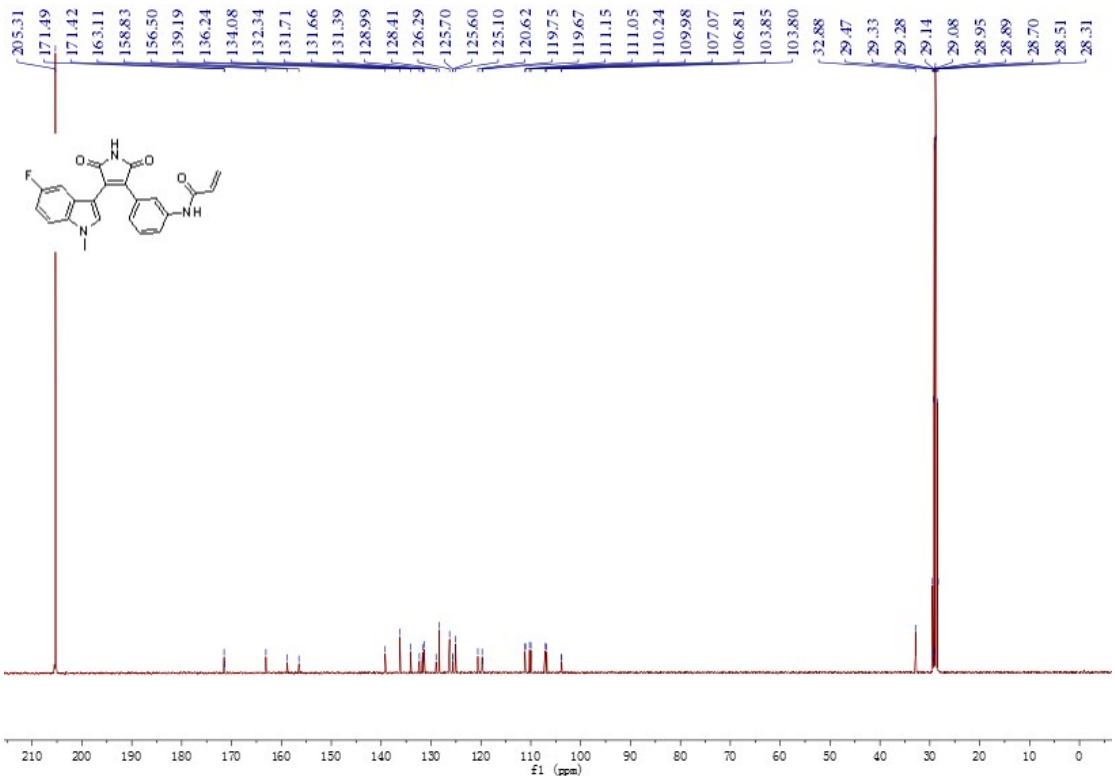
^{1}H-NMR of **31g**



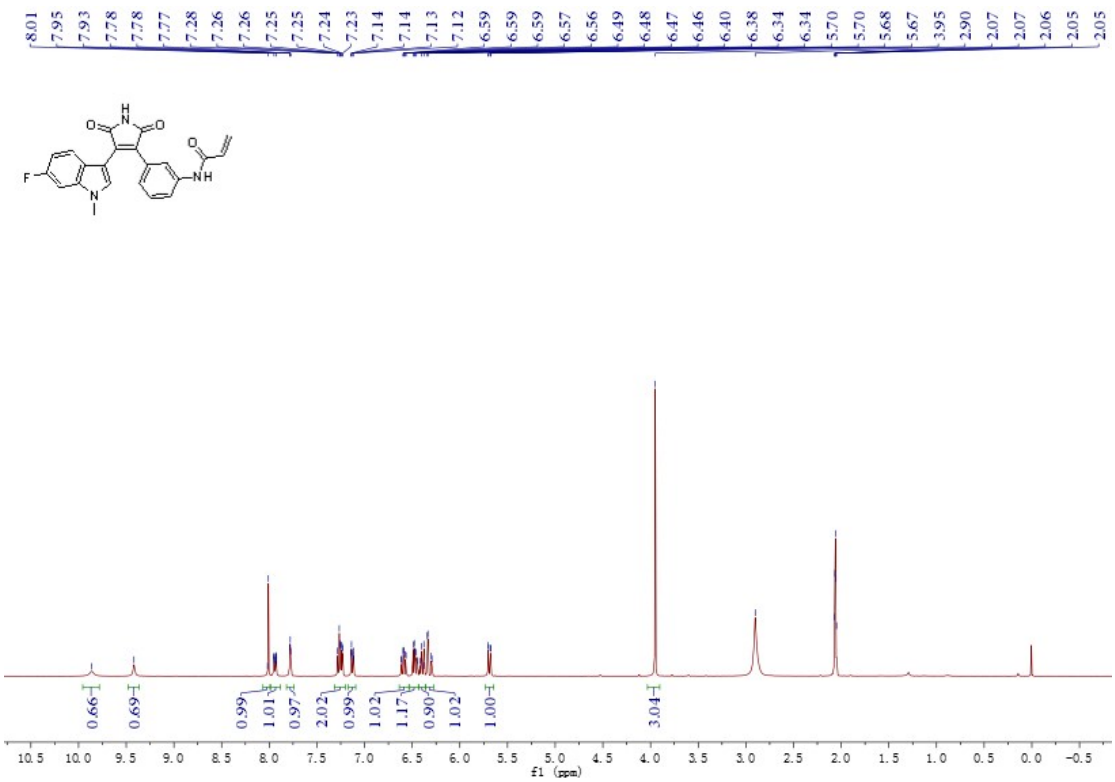
^{13}C-NMR of **31g**



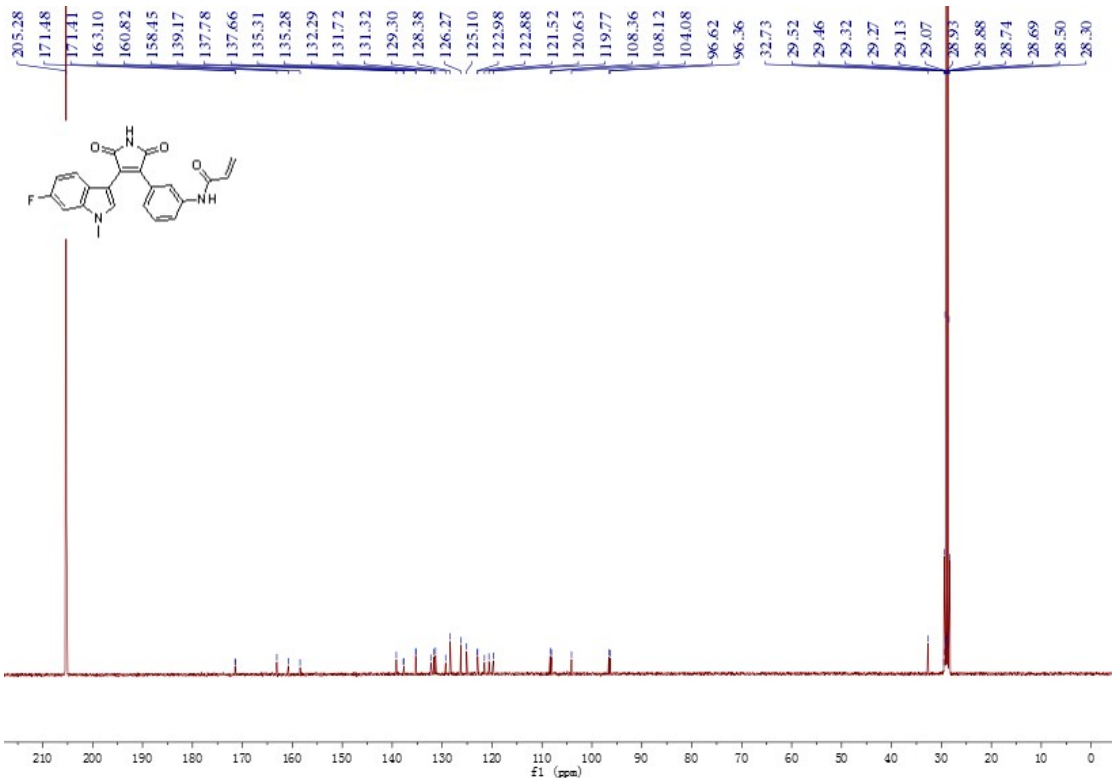
^{1}H-NMR of **33a**



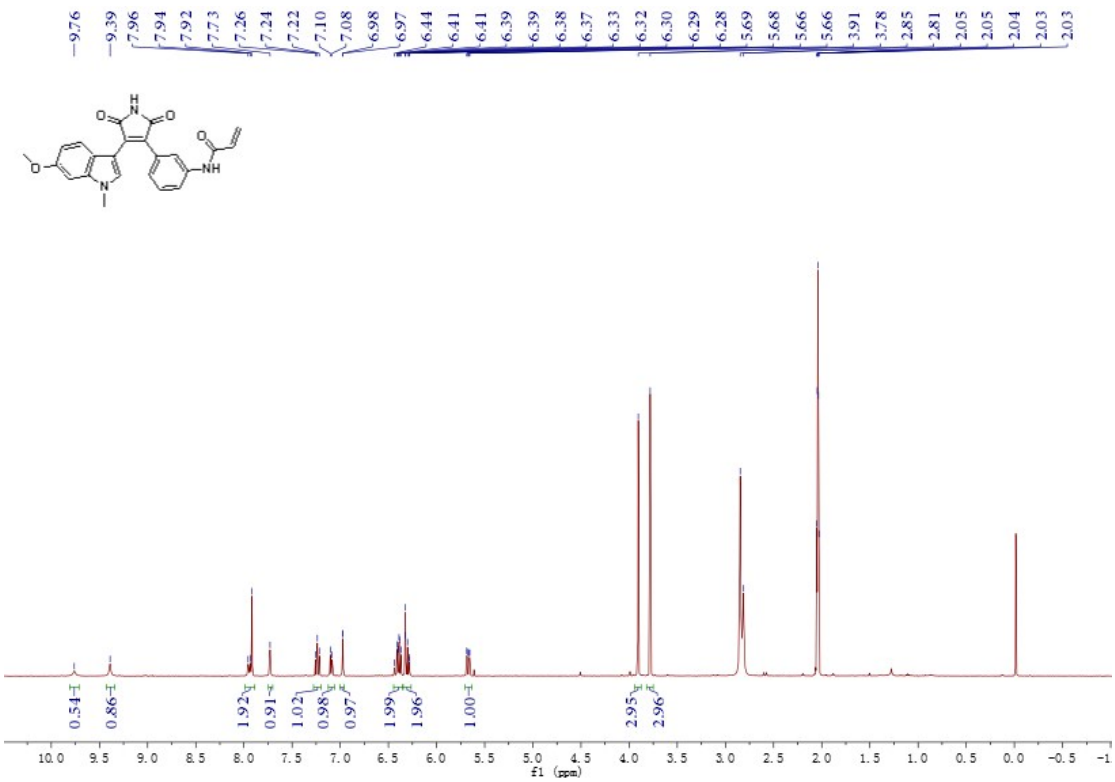
^{13}C-NMR of **33a**



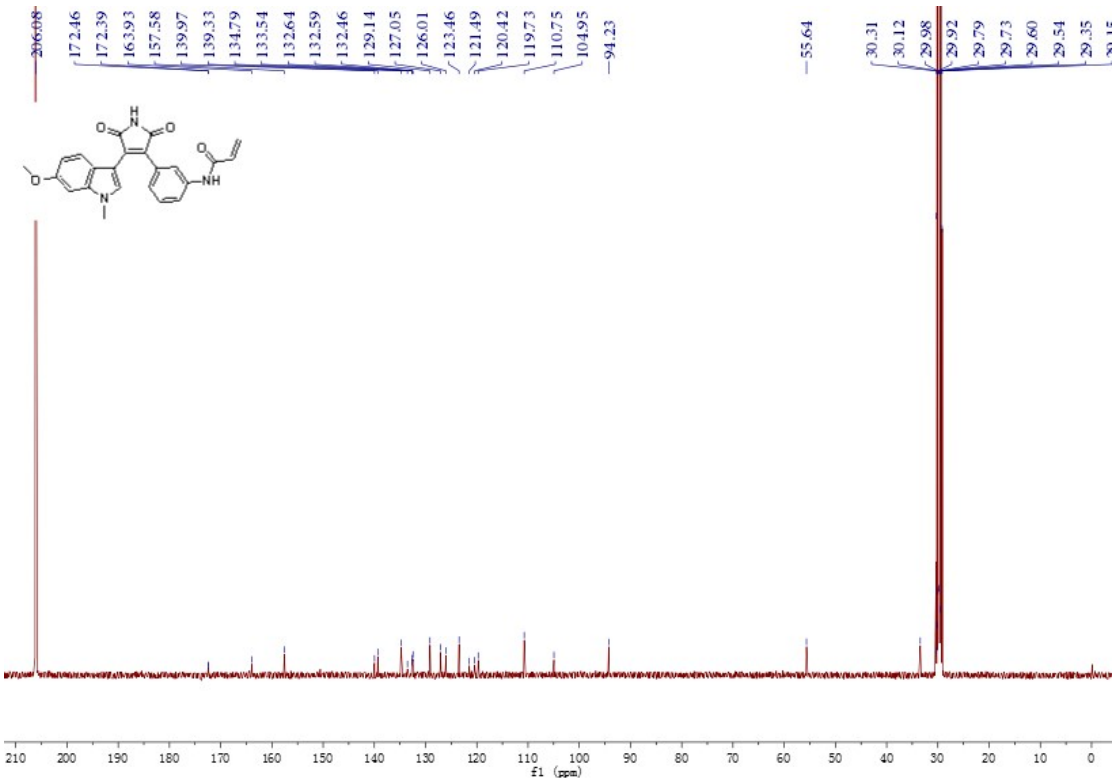
^{1}H-NMR of **33b**



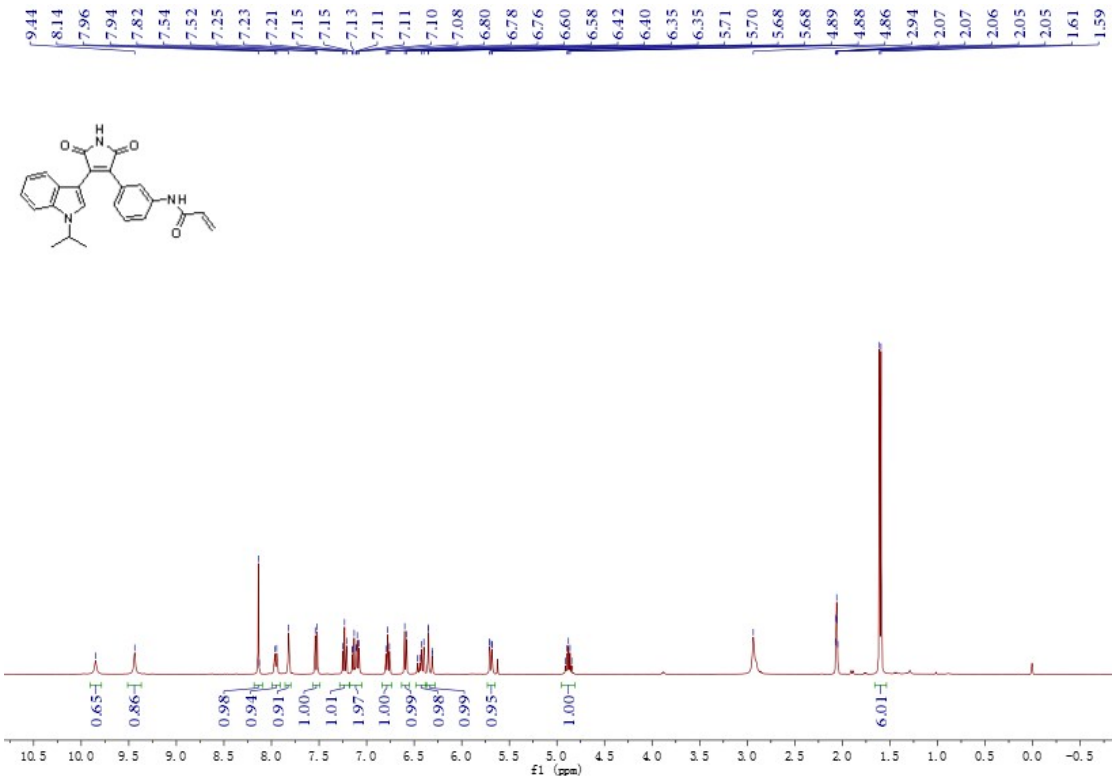
^{13}C-NMR of **33b**



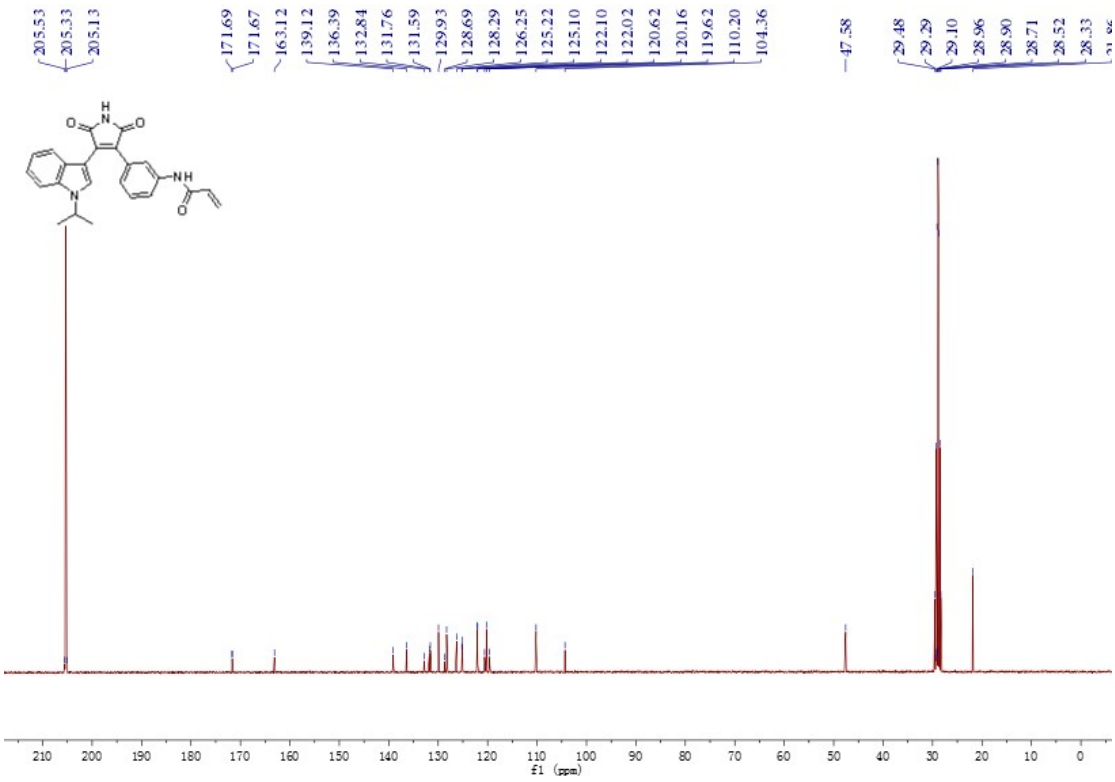
^{1}H-NMR of **33c**



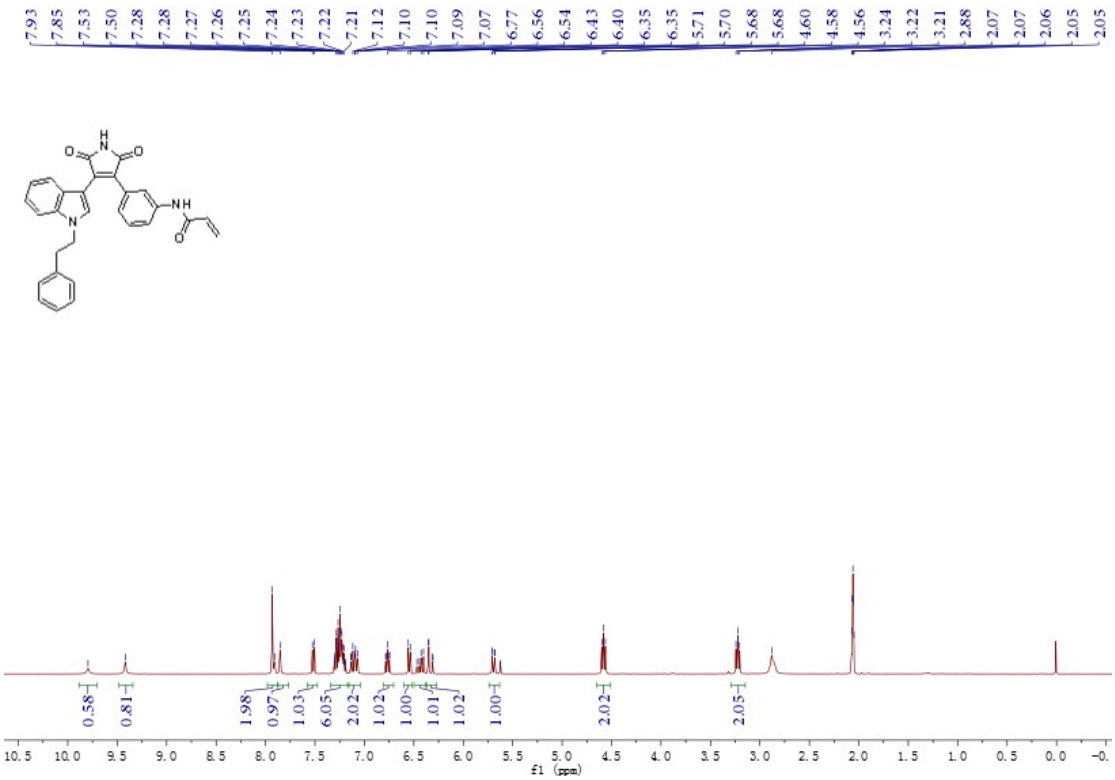
^{13}C-NMR of **33c**



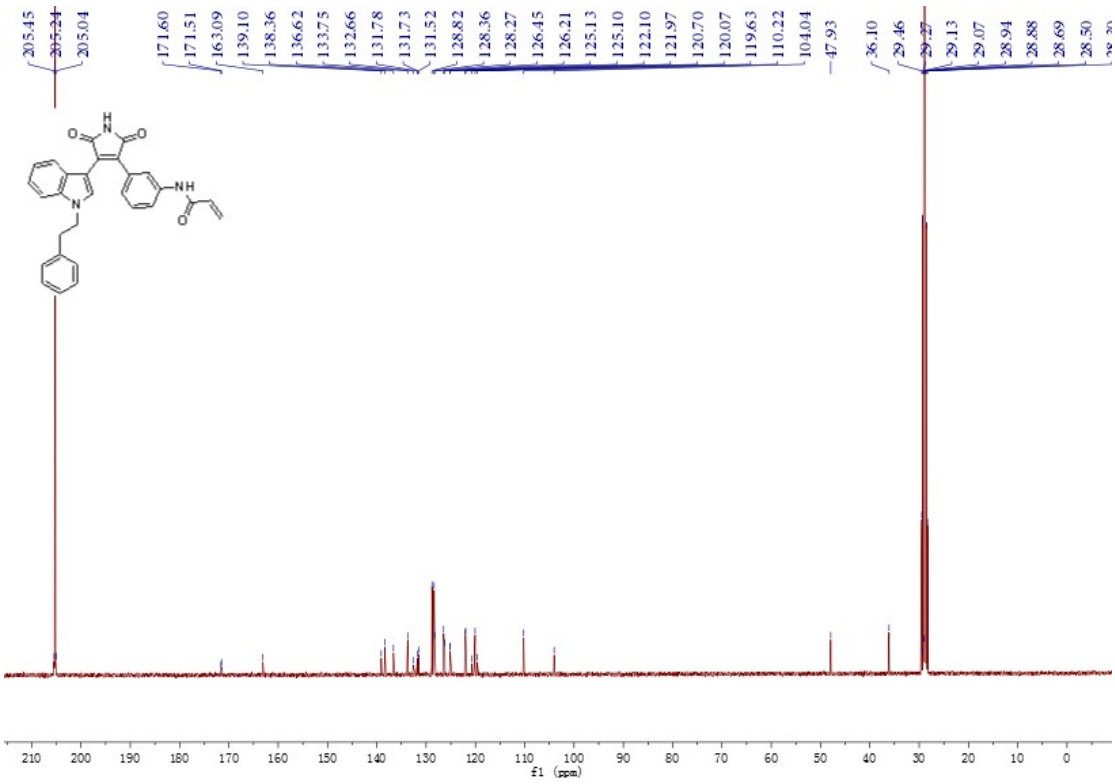
^{1}H-NMR of **33d**



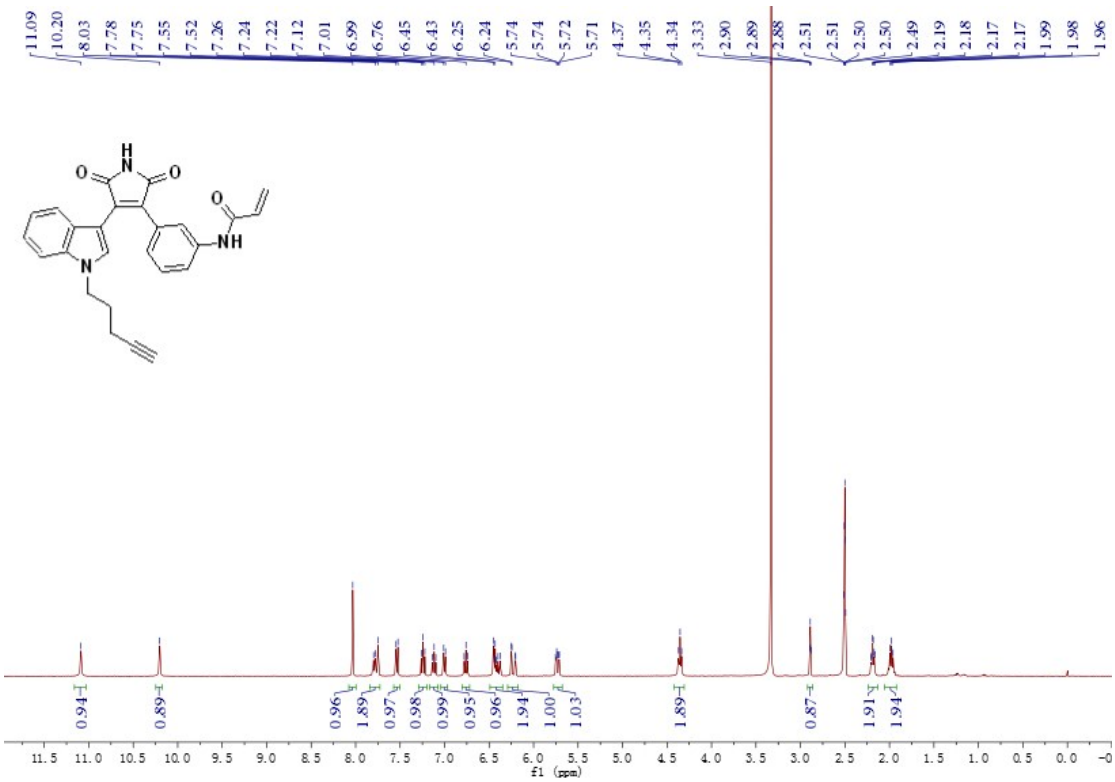
^{13}C-NMR of **33d**



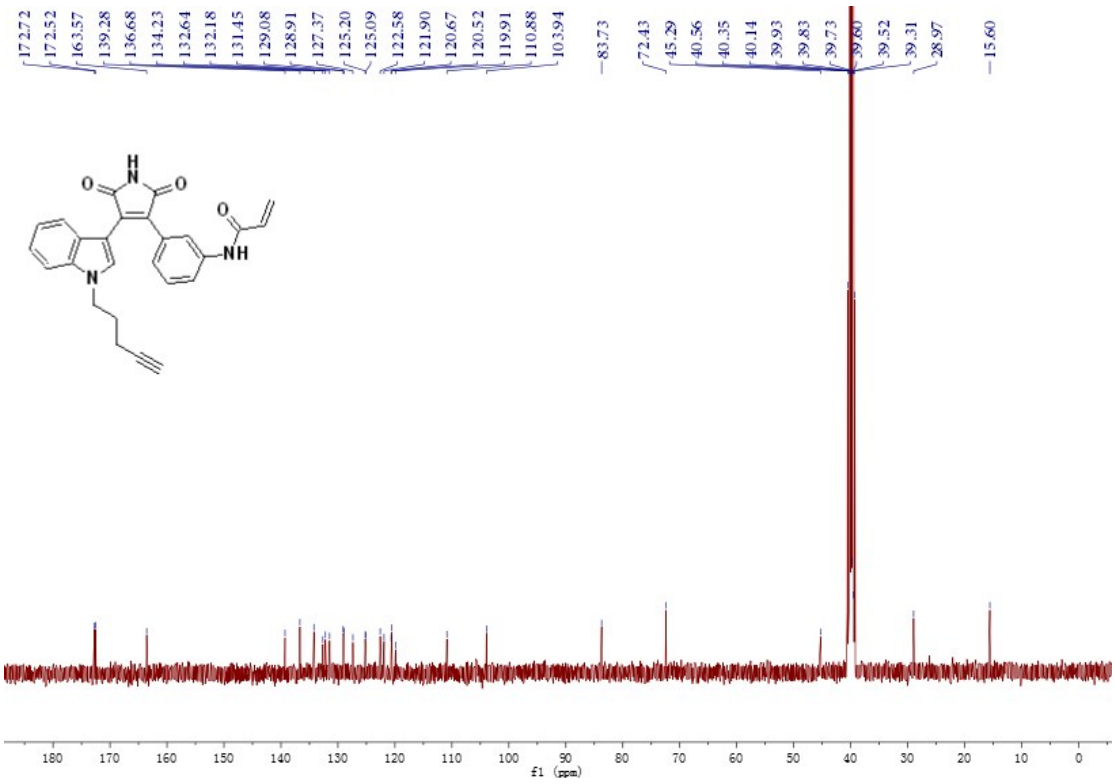
^{1}H-NMR of **33e**



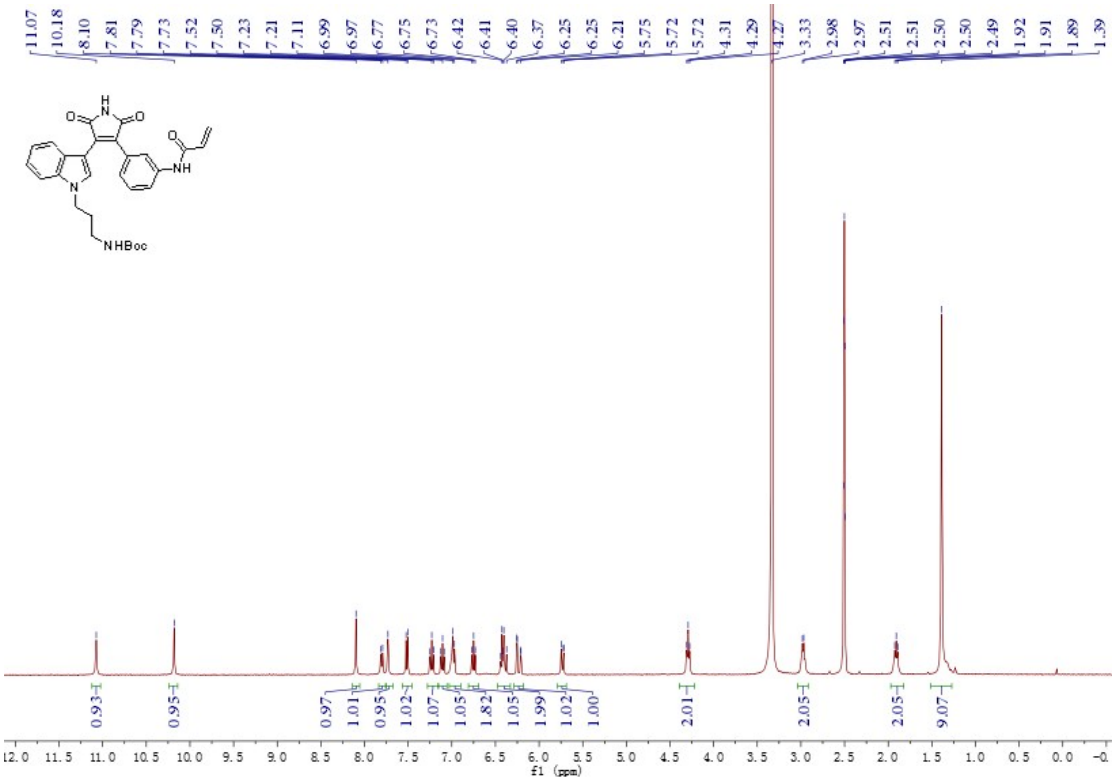
^{13}C-NMR of **33e**



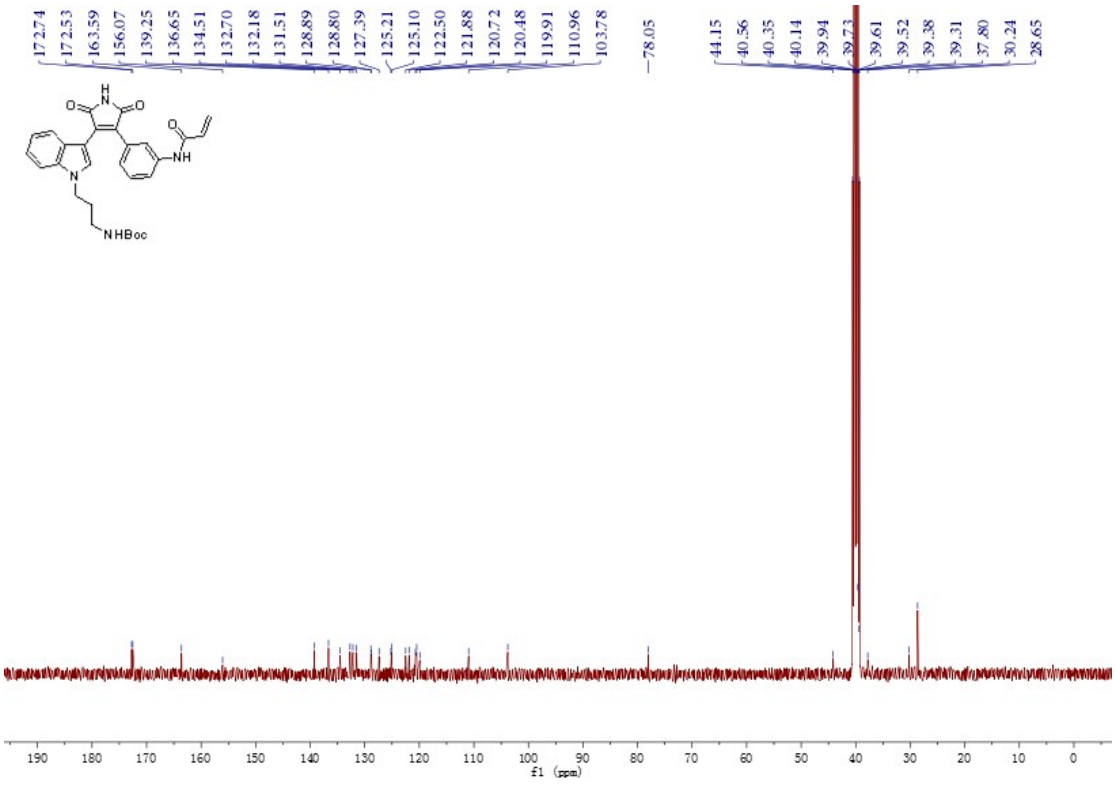
^{1}H-NMR of **33f**



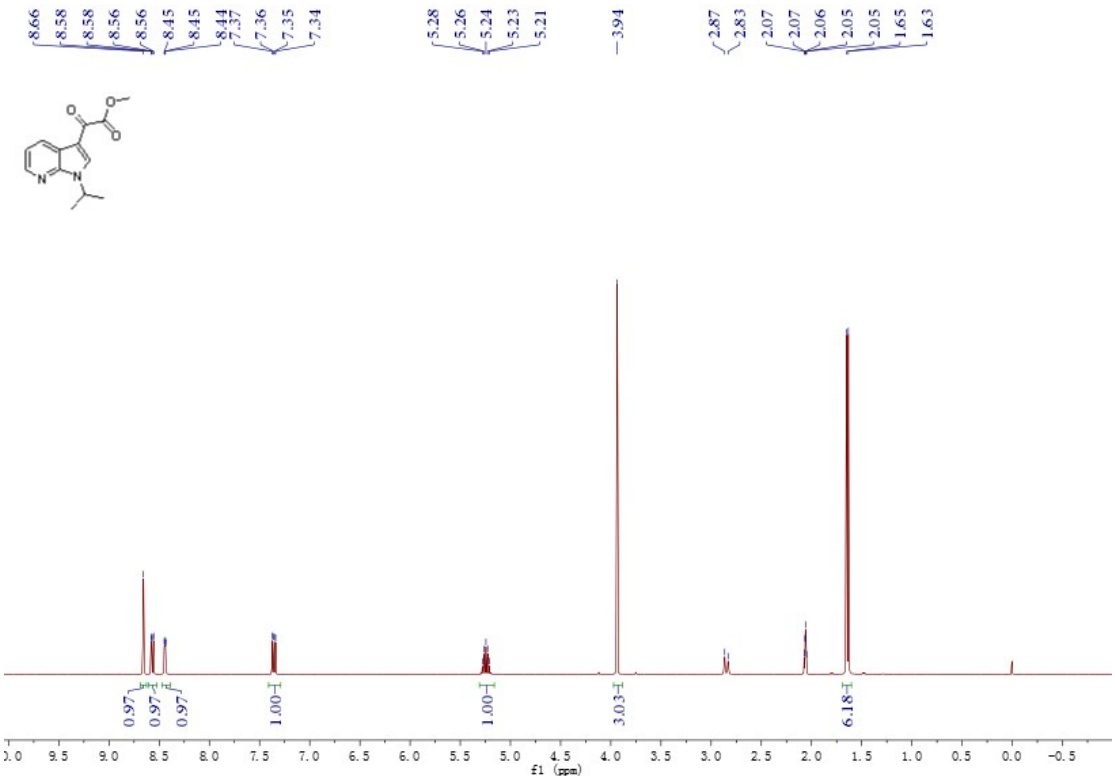
^{13}C-NMR of **33f**



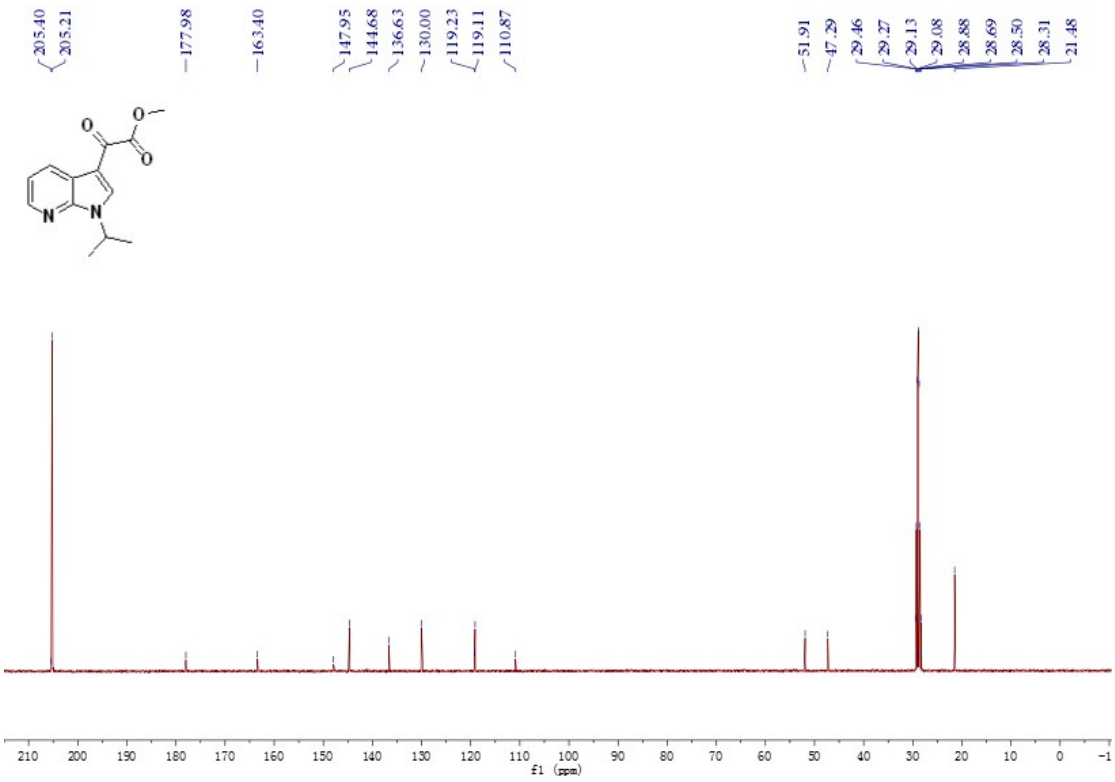
^{1}H-NMR of **33g**



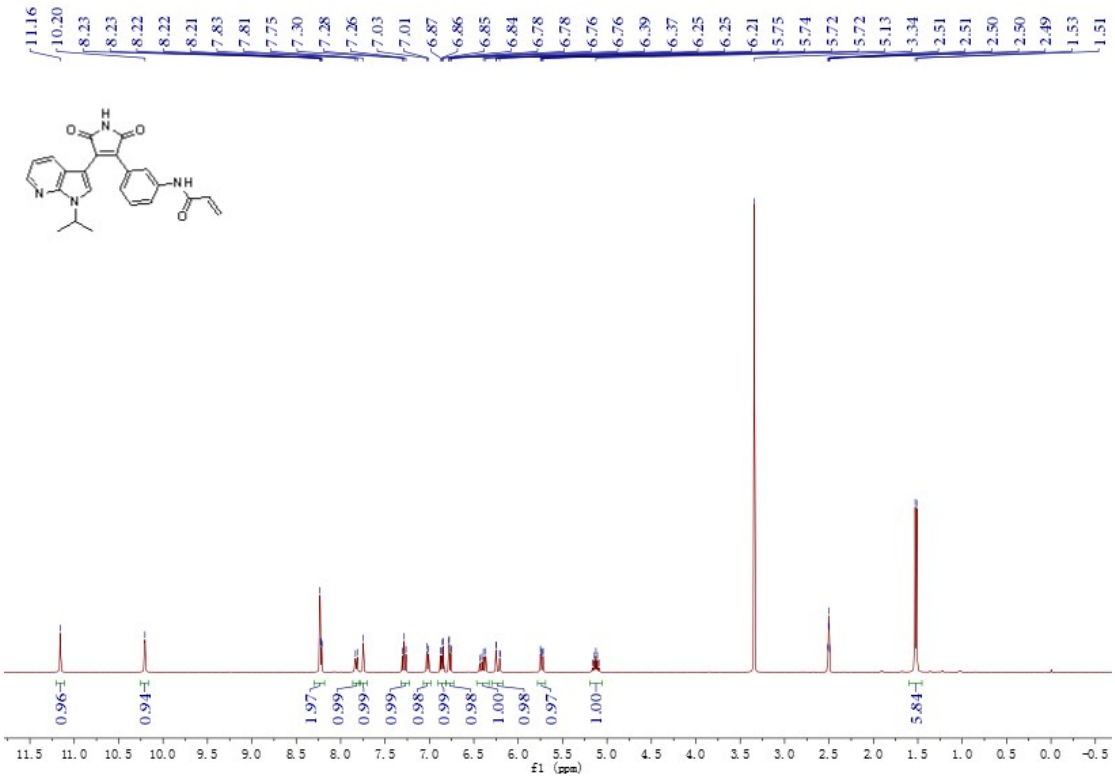
^{13}C-NMR of **33g**



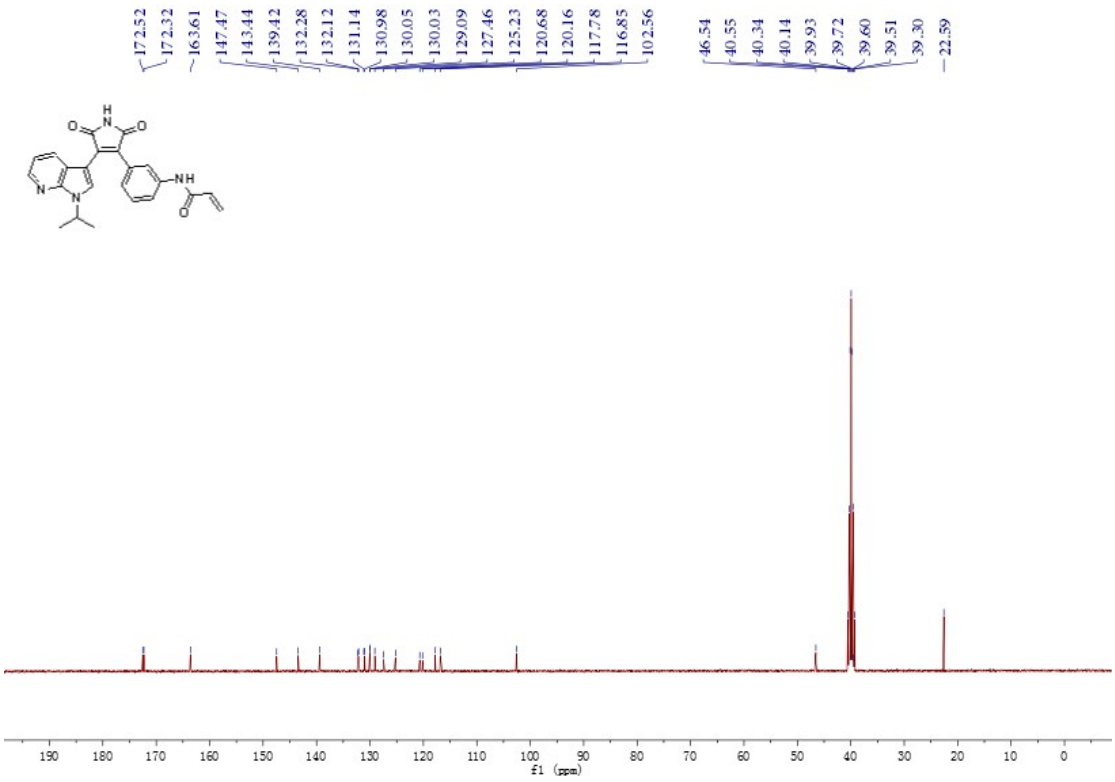
^{1}H-NMR of **36**



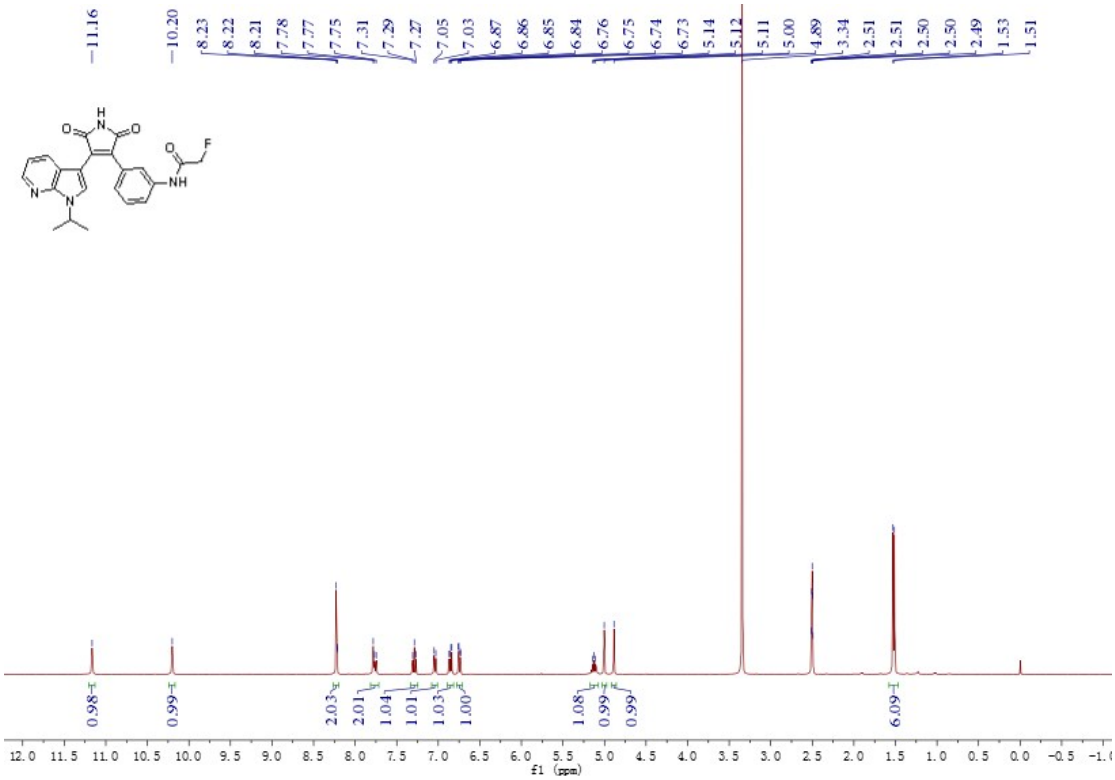
^{13}C-NMR of **36**



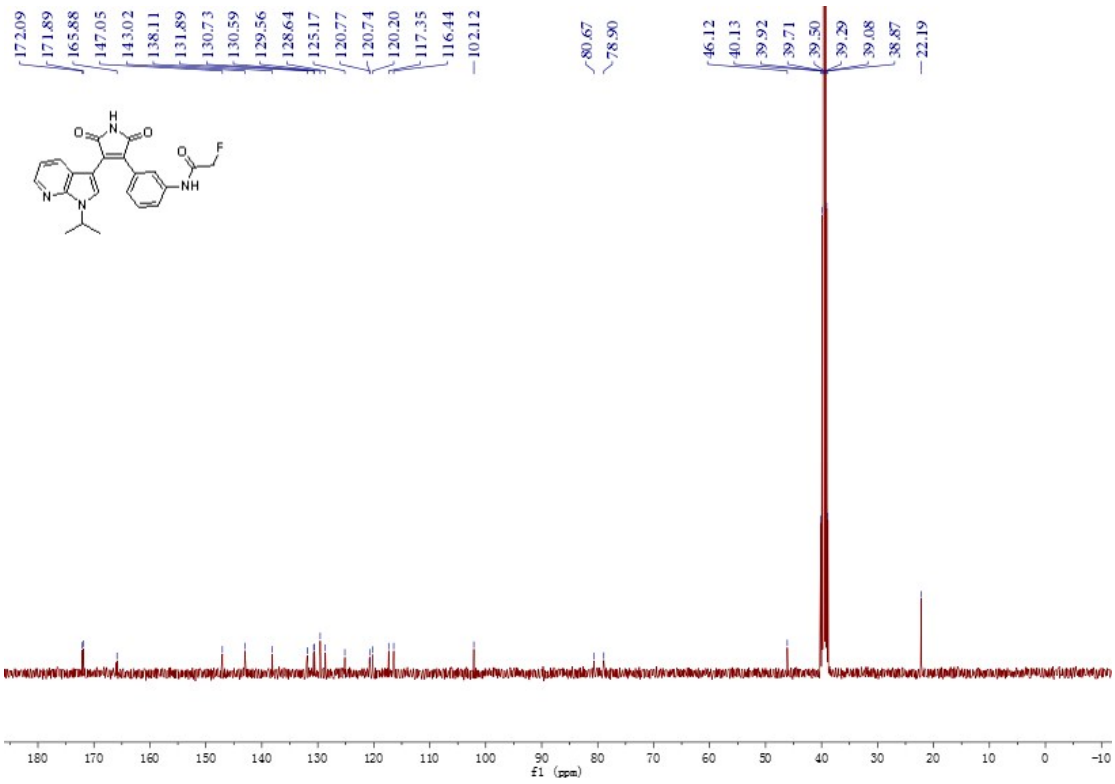
^{1}H-NMR of **38a**



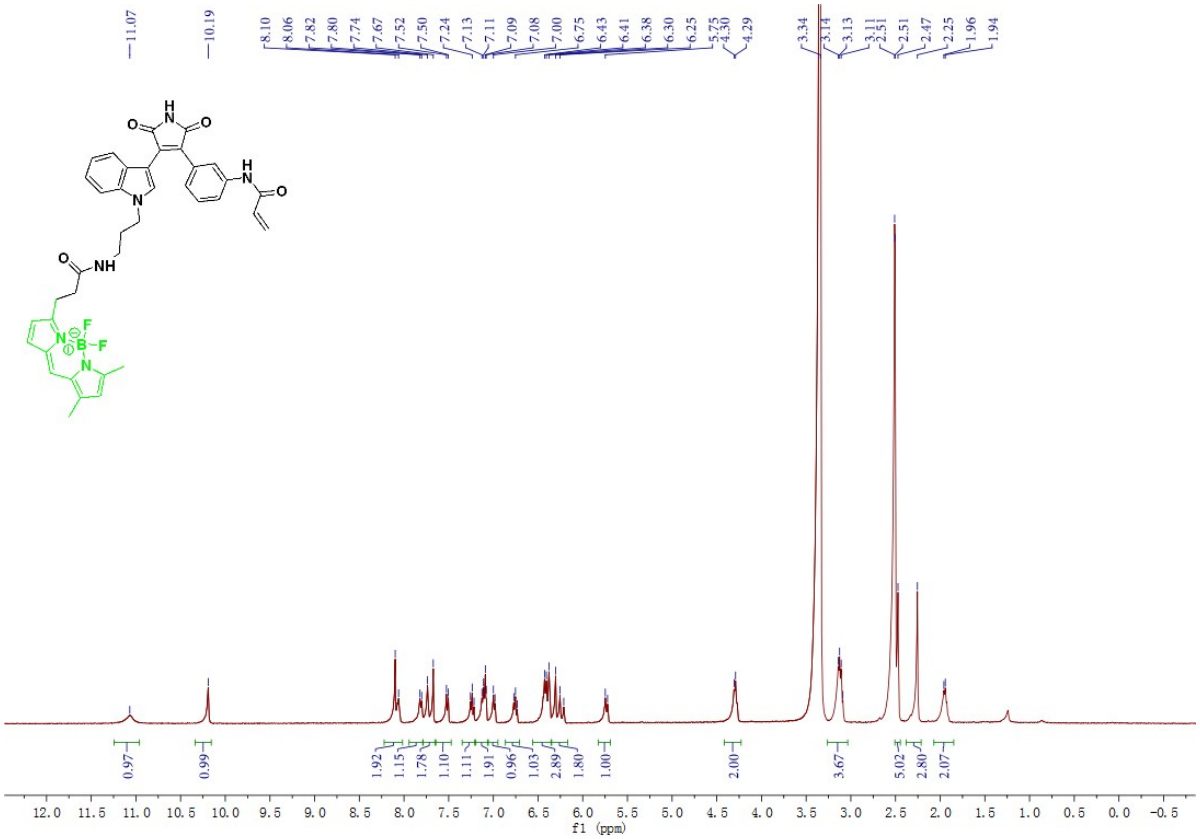
^{13}C-NMR of **38a**



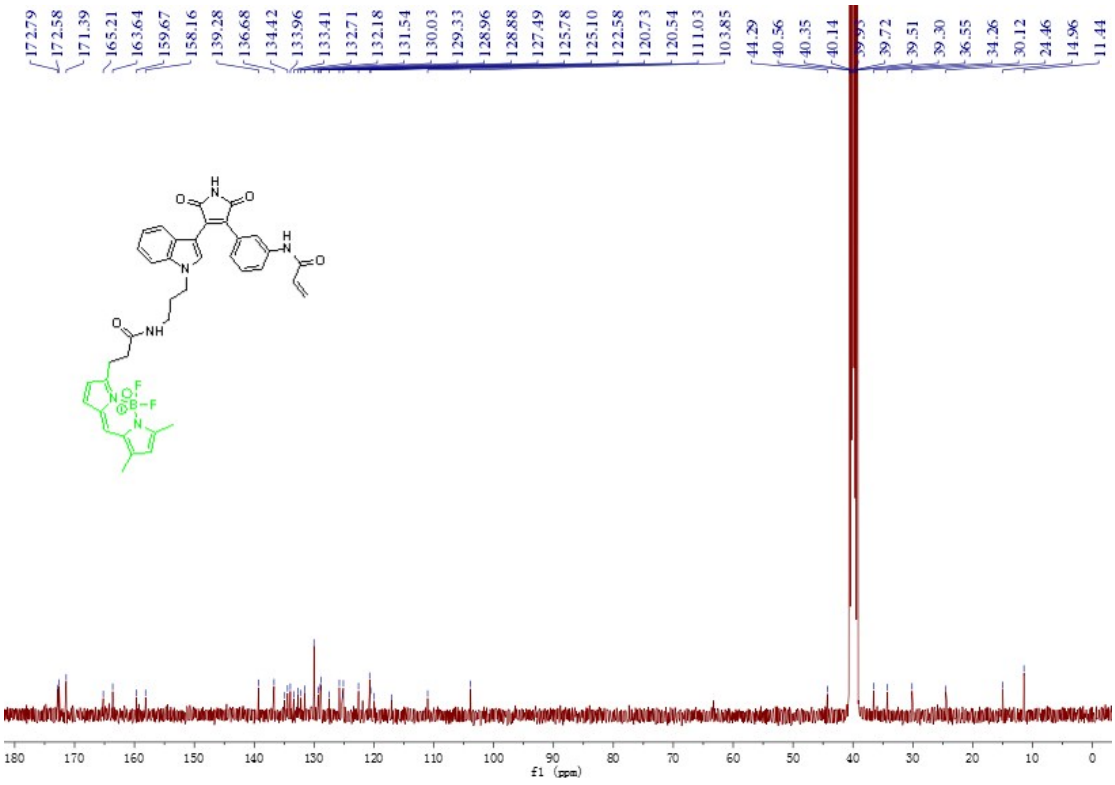
^{1}H-NMR of **38b**



^{13}C-NMR of **38b**



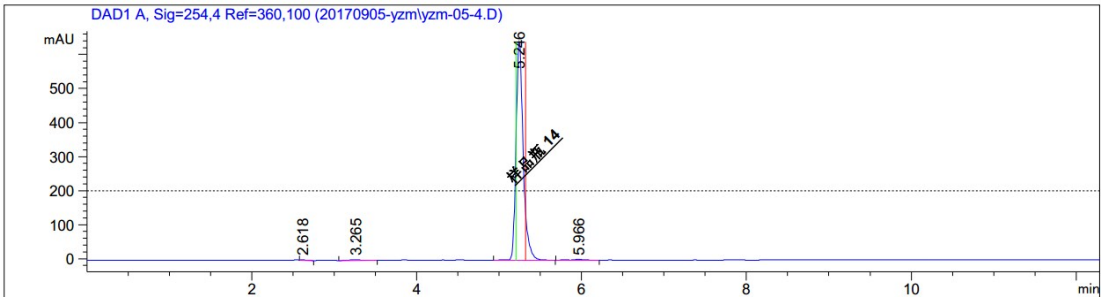
^{1}H-NMR of **probe 39**



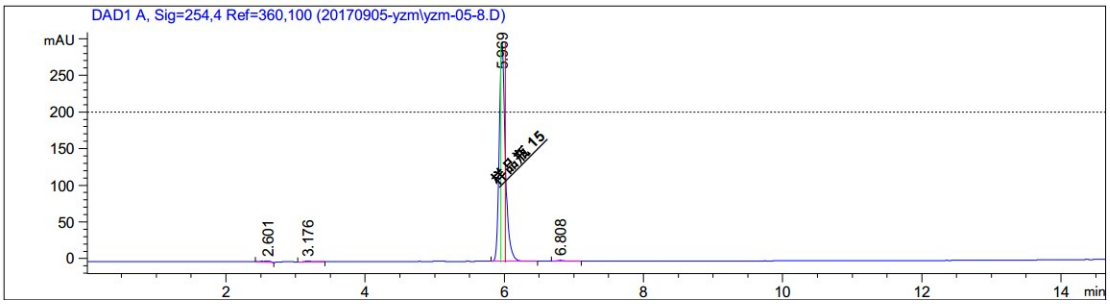
^{13}C-NMR of **probe 39**

3. HPLC analysis chromatograms of final compounds

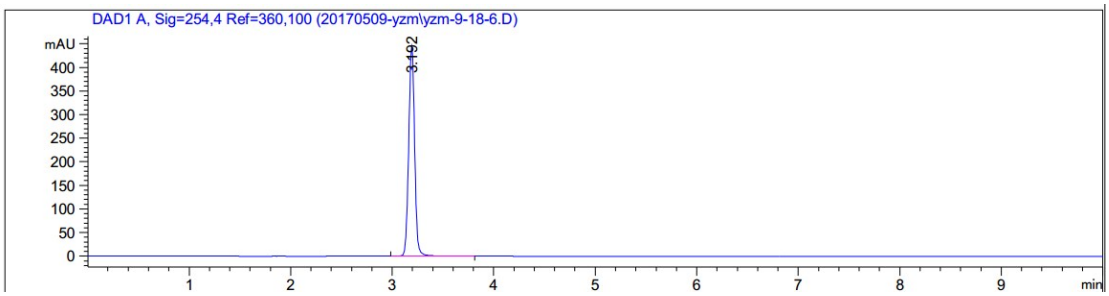
The purities of final compounds were all above 95%.



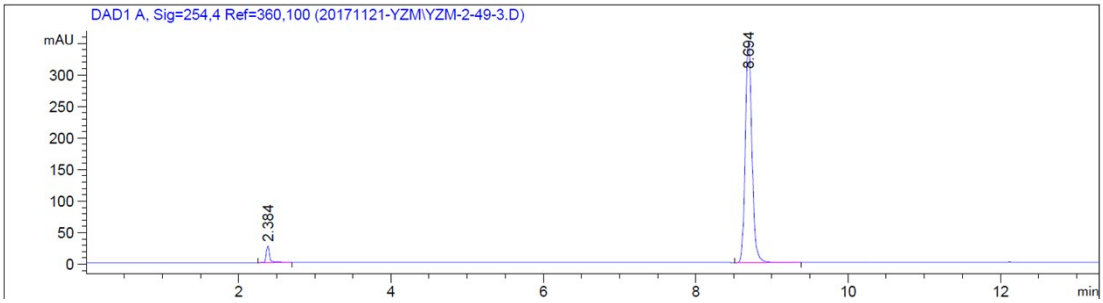
HPLC of **19a** (Retention time: 5.246 min, Purity: 98.80%)



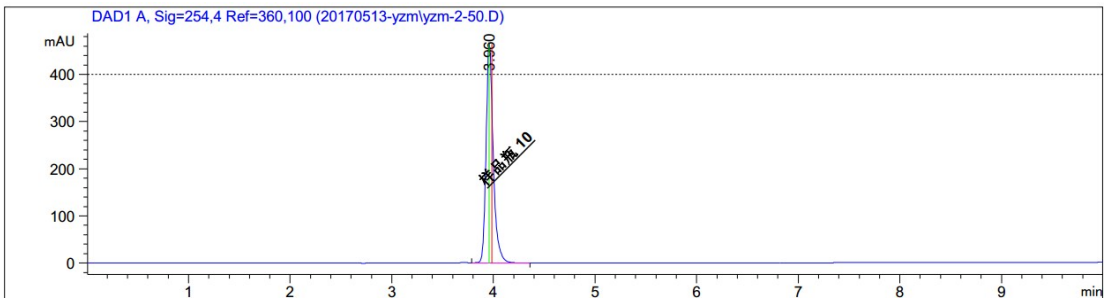
HPLC of **19b**(Retention time: 5.969 min, Purity: 98.39%)



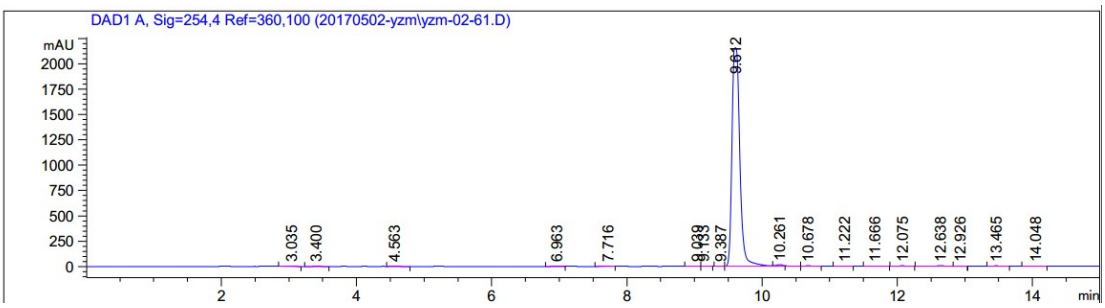
HPLC of **19c** (Retention time: 3.192 min, Purity: 100%)



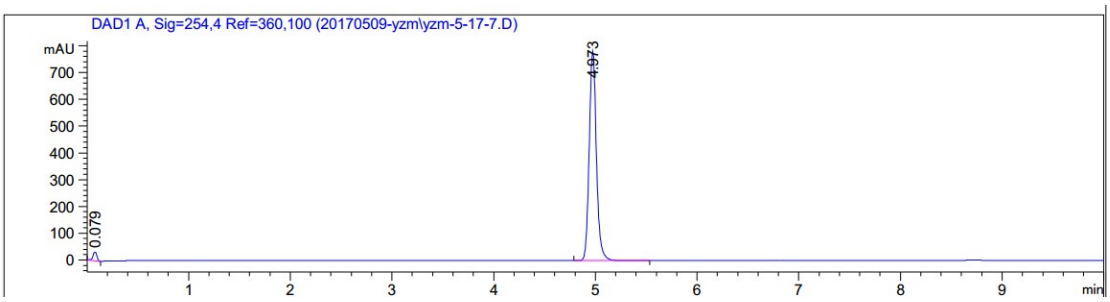
HPLC of **19d** (Retention time: 8.694, Purity: 96.29%)



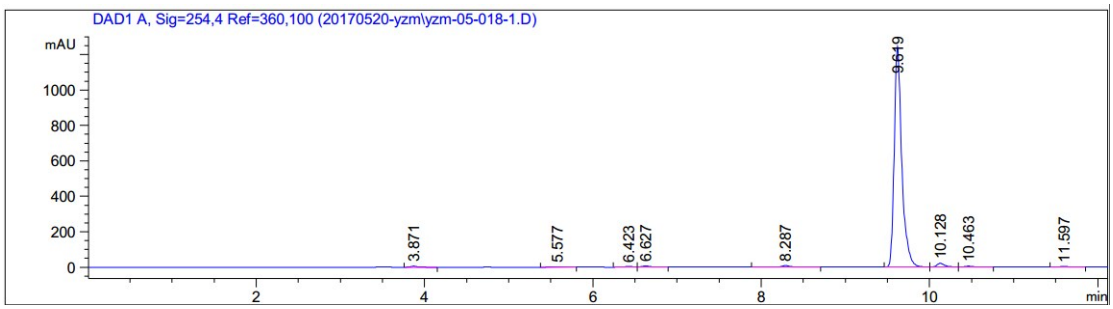
HPLC of **19e** (Retention time: 3.960 min, Purity: 100%)



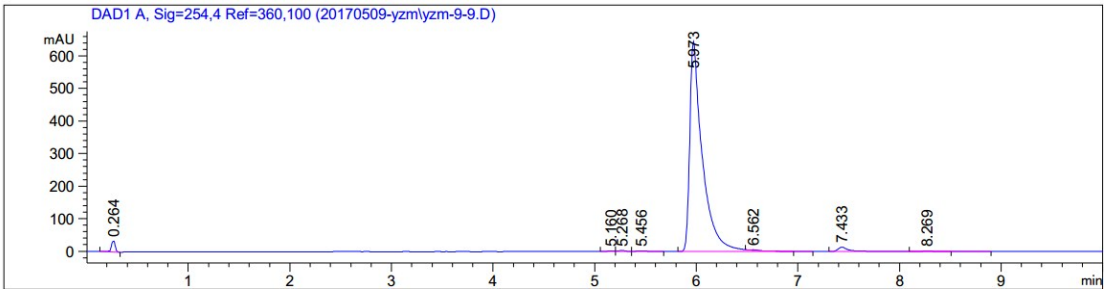
HPLC of **20a** (Retention time: 9.612 min, Purity: 98.11%)



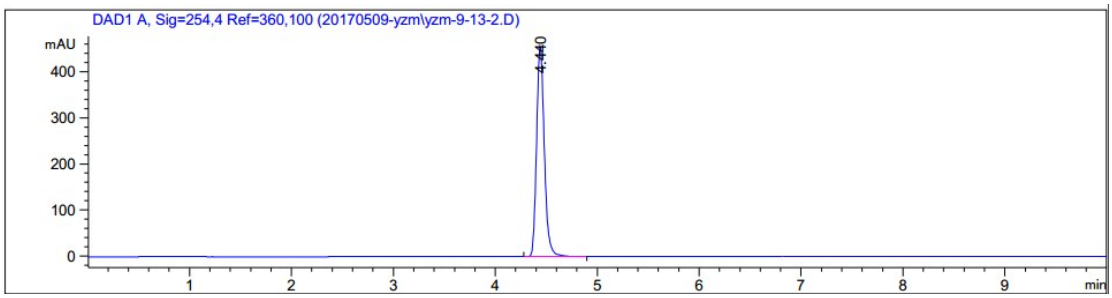
HPLC of **20b** (Retention time: 4.973 min, Purity: 97.88%)



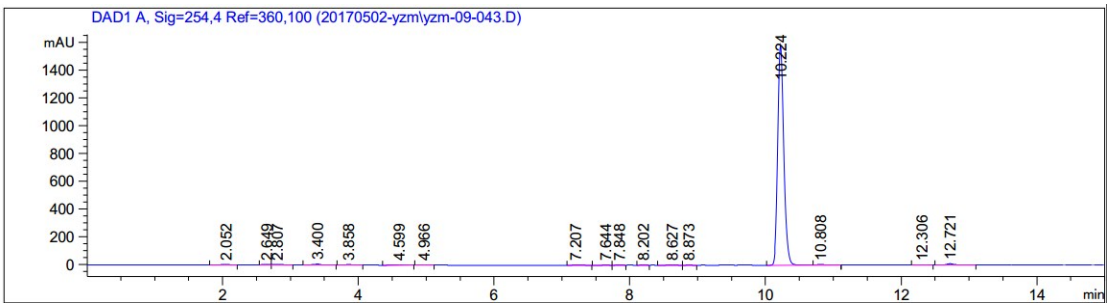
HPLC of **20c** (Retention time: 9.619 min, Purity: 95.61%)



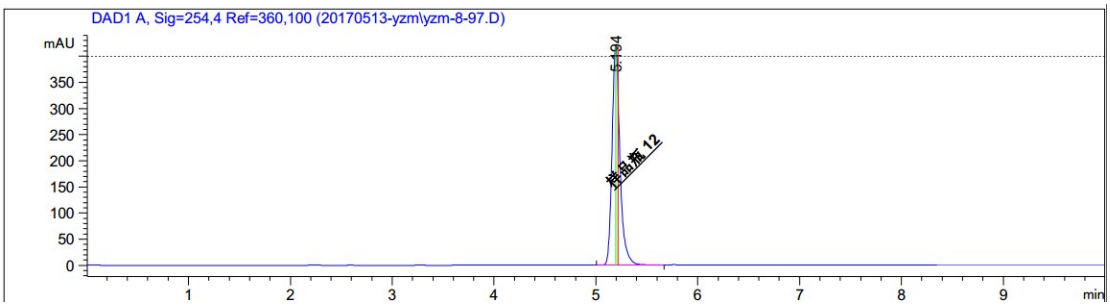
HPLC of **20d** (Retention time: 5.973 min, Purity: 95.62%)



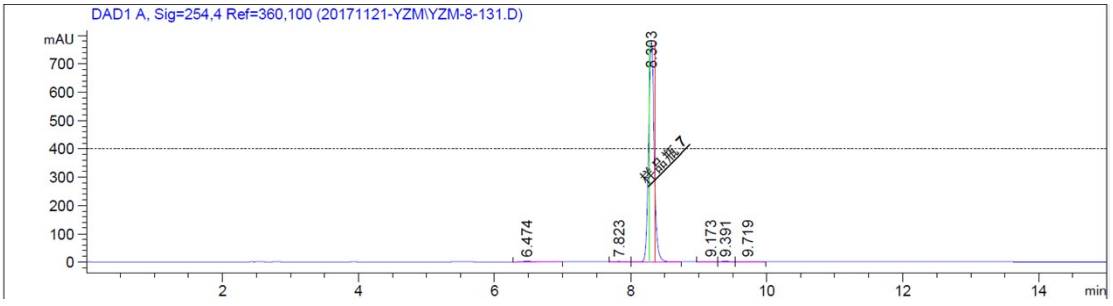
HPLC of **20e** (Retention time: 4.440 min, Purity: 100%)



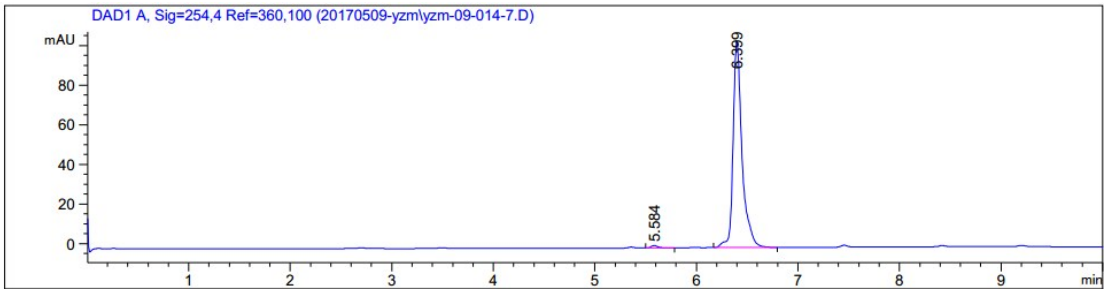
HPLC of **20f** (Retention time: 10.224 min, Purity: 96.55%)



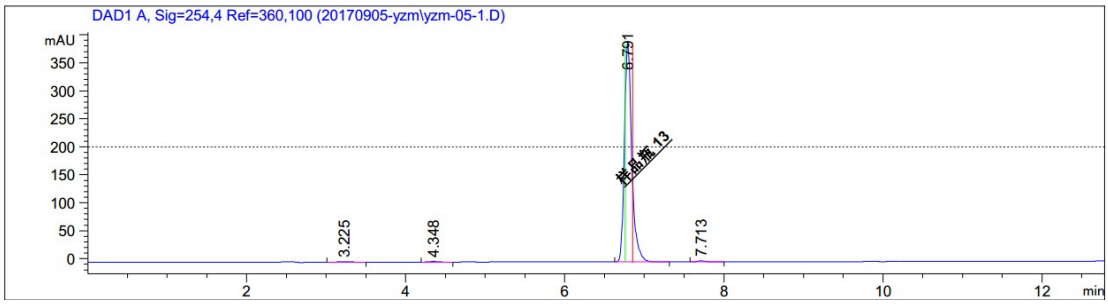
HPLC of **20g** (Retention time: 5.194 min, Purity: 100%)



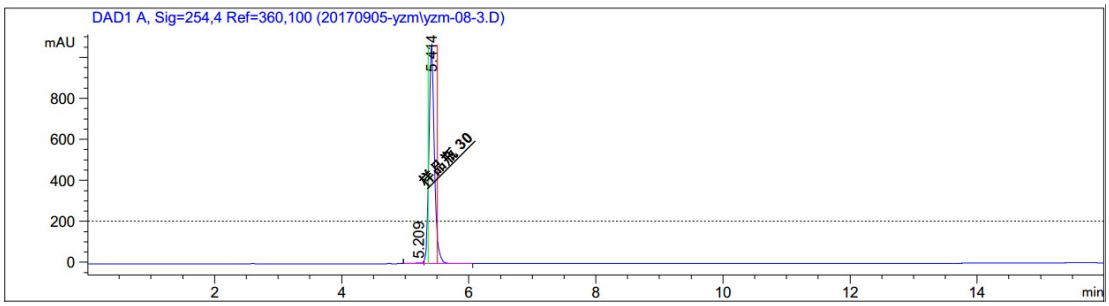
HPLC of **24a** (Retention time: 8.303 min, Purity: 98.47%)



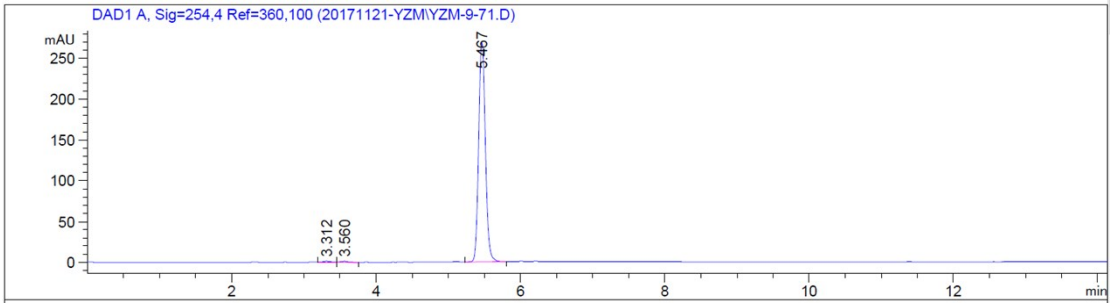
HPLC of **24b** (Retention time: 6.399 min, Purity: 99.04%)



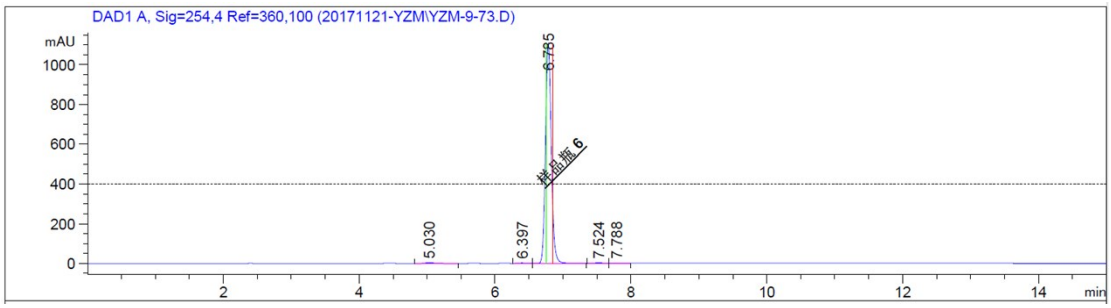
HPLC of **26a** (Retention time: 6.791 min, Purity: 98.75%)



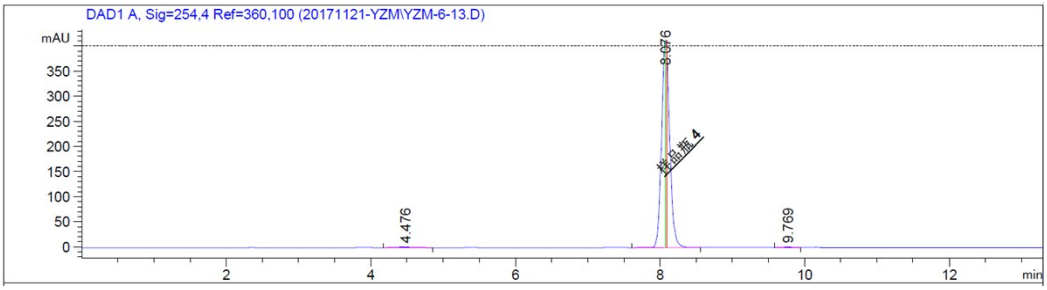
HPLC of **26b** (Retention time: 5.414 min, Purity: 99.62%)



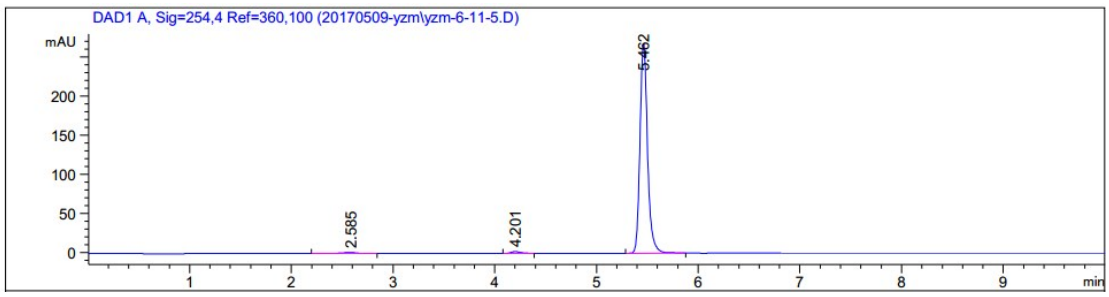
HPLC of **28a** (Retention time: 5.467 min, Purity: 99.09%)



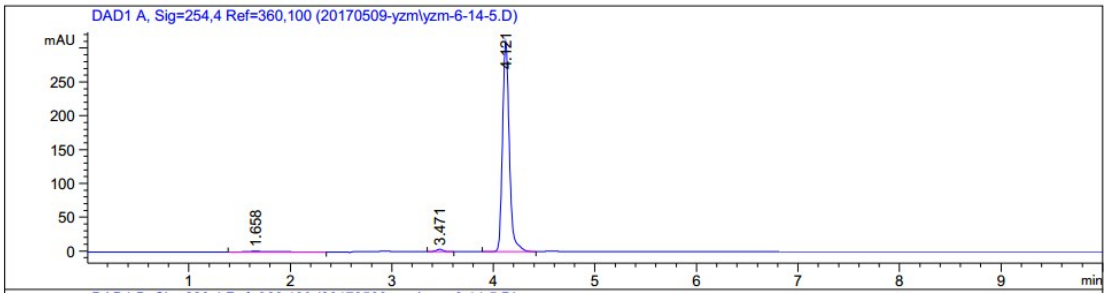
HPLC of **28b** (Retention time: 6.785 min, Purity: 98.57%)



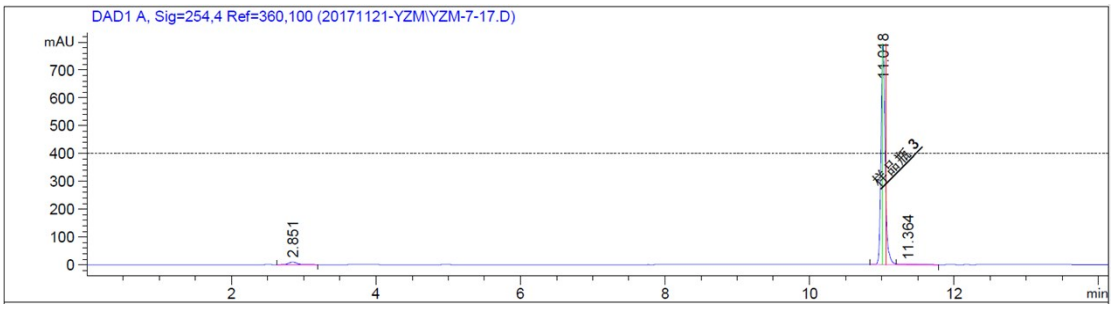
HPLC of **33a** (Retention time: 8.076 min, Purity: 99.06%)



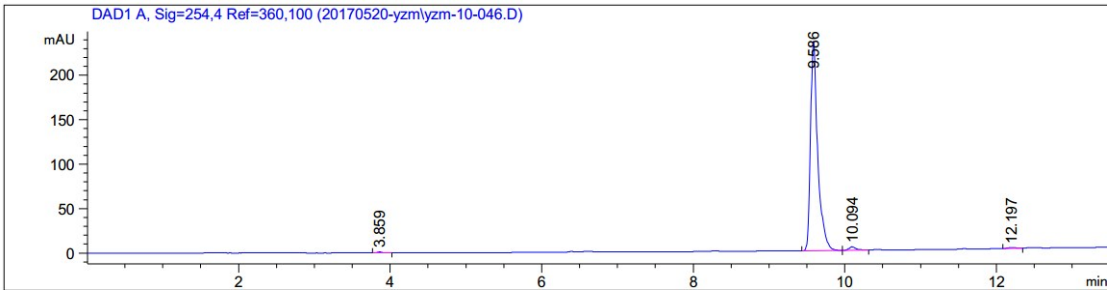
HPLC of **33b** (Retention time: 5.462 min, Purity: 97.91%)



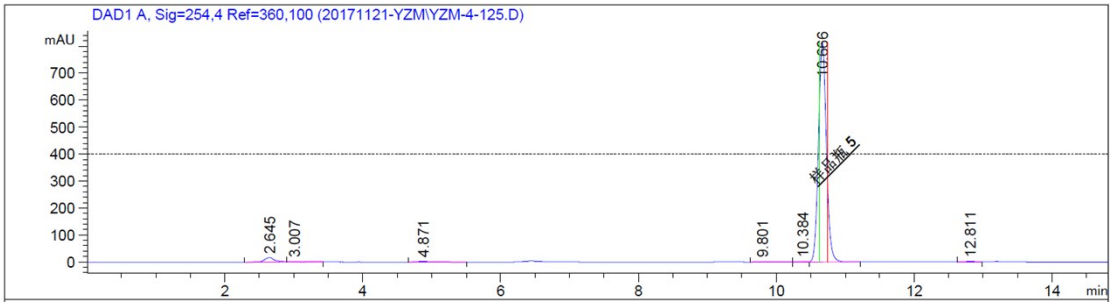
HPLC of **33c** (Retention time: 4.121 min, Purity: 97.54%)



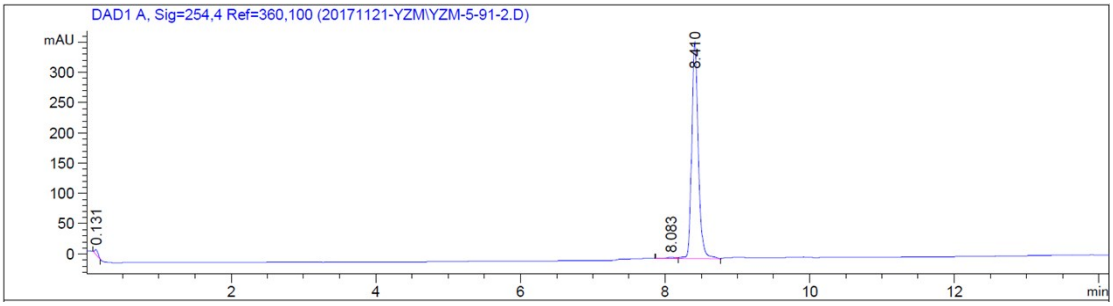
HPLC of **33d** (Retention time: 11.018 min, Purity: 97.23%)



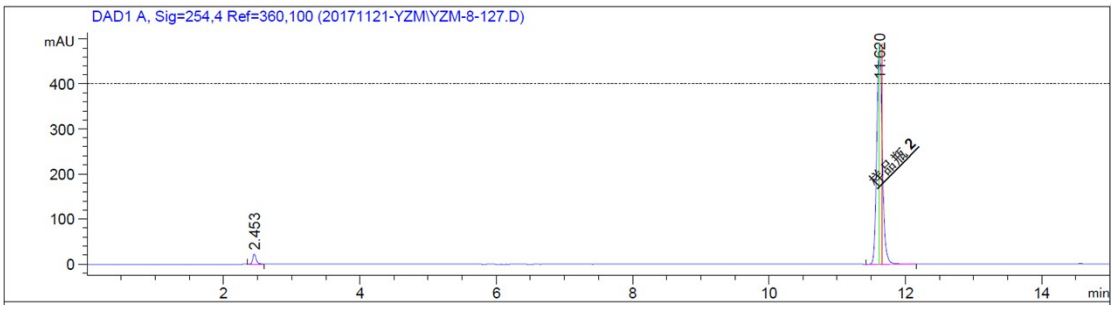
HPLC of **33e** (Retention time: 9.586 min, Purity: 97.67%)



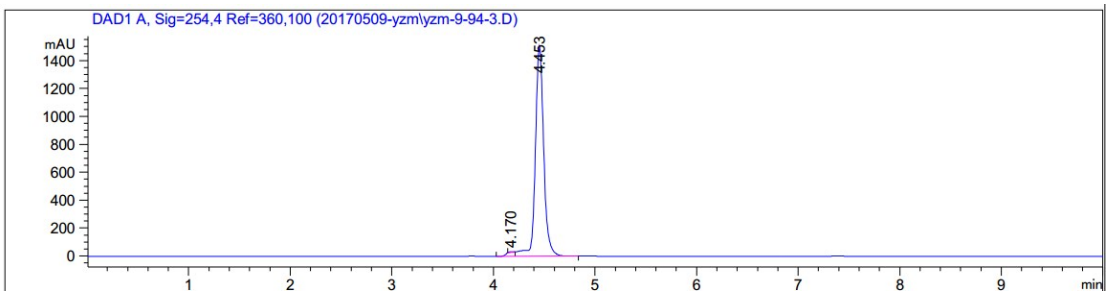
HPLC of **33f** (Retention time: 10.666 min, Purity: 95.96%)



HPLC of **33g** (Retention time: 8.410 min, Purity: 98.42%)



HPLC of **38a** (Retention time: 11.620 min, Purity: 97.09%)

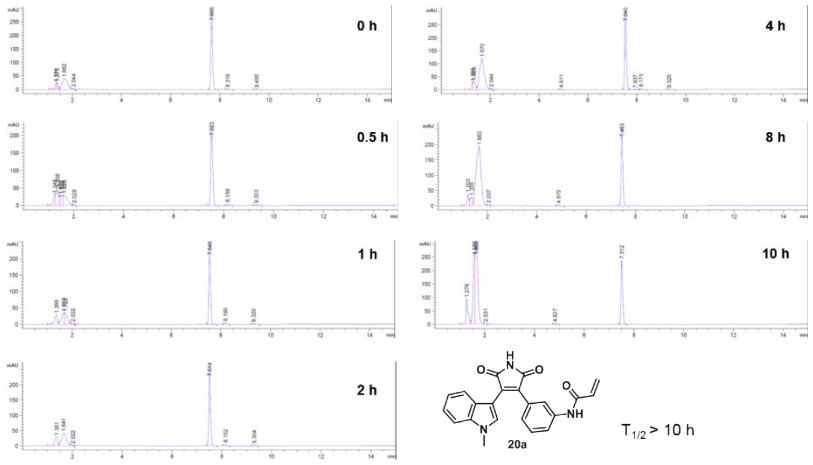


HPLC of **38b** (Retention time: 4.453 min, Purity: 99.91%)

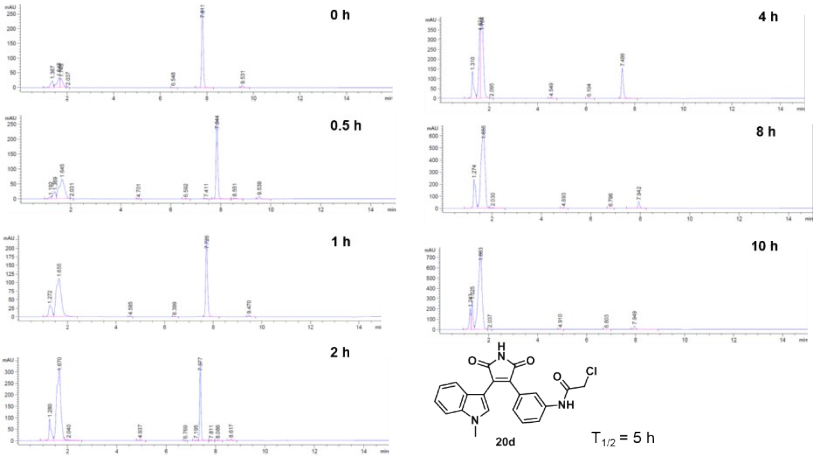
4. Biology

1. Stability of compound 20a, 20d and 38b at 37 °C.

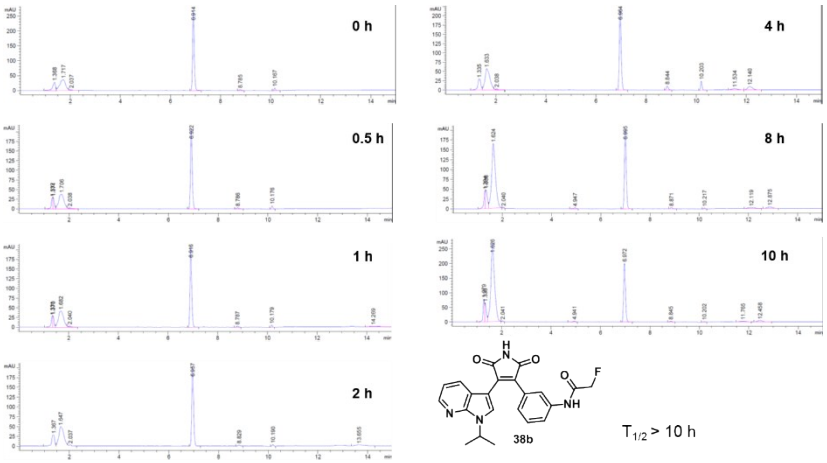
(a)



(b)

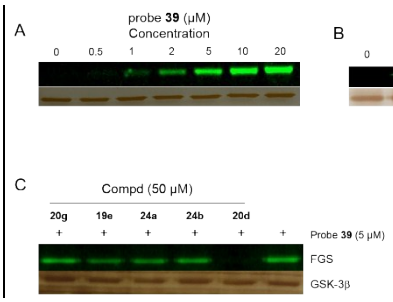


(c)



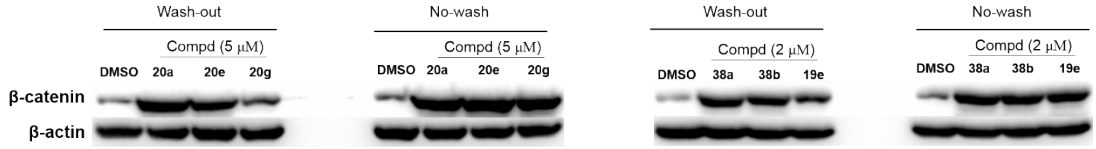
Supplementary Figure S1. Compounds **20a**, **20d** and **38b** (20 μM) were incubated with 20 mM GSH in PBS buffer at pH 7.4 at 37 °C, analysed by HPLC. The compounds with different electrophilic groups showed difference in stability.

2. Competition experiments.



Supplementary Figure S2. (A) Concentration course labelling experiment of recombinant GSK-3β by probe **39** for 2 h. (B) Time course labelling experiment of recombinant GSK-3β by probe **39**. (C) Competition experiments with compound **19e**, **24a**, **24b** and **20d**.

3. Wash-out studies.



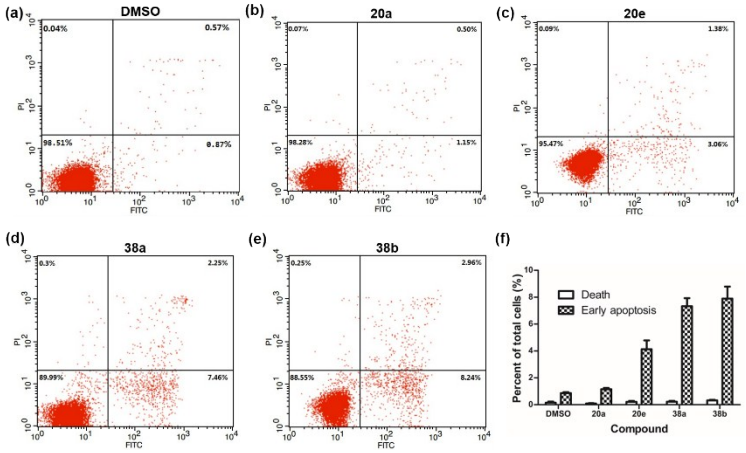
Supplementary Figure S3. The western blotting images of wash-out experiments.

a) 3,000,000 RS4;11 cells were treated with either compounds at 5 μM (or 2 μM) or vehicle control (DMSO) for 2h. Cells were centrifuged and washed twice with fresh culture medium, then re-suspended in 1 mL fresh medium for 6 h.

b) 3,000,000 RS4;11 cells were treated with either compounds at 5 μM (or 2 μM) or vehicle control (DMSO) for 8h.

Then, cells were collected and lysed. Inhibition of substrates phosphorylation were evaluated with wash-out and no-wash cells by western bolt. Western blot analysis was conducted following standard procedure. The blots were quantified by Image-Pro Plus 5.0.

4. Quantitative analysis of apoptotic cells after the treatment of 1 μM compounds for 24 h.

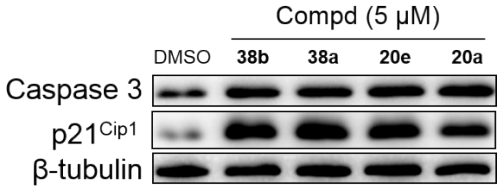


Supplementary Figure S4. Cell apoptosis analysis with Annexin V-FITC and Propidium Iodide staining. (a-e) RS4;11 cells were pre-incubated with different compounds and analyzed with FACS.

(f) The statistical graph of the early apoptosis distribution.

Flow cytometry. The apoptosis assays were performed with TACS Annexin V-FITC kit (Trevigen) and detected by the BD FACS Calibur. More than 1000,000 RS4;11 cells were collected and rinsed once in pre-cold PBS. The cells were stained by Annexin V-FITC and propidium iodide (PI) in 100 μL binding buffer after centrifugation at 1300 g for 5 min. The mixtures were incubated at ambient temperature for 15 min. Then, 400 μL of 1 × binding buffer was added, and the mixture was detected in a polystyrene tube. The experiment was performed triplicate measurements.

5. Western blot analysis of $p21^{Cip1}$ and caspase 3 after the treatment of 5 μM compounds for 24 h.



Supplementary Figure S5. The levels of $p21^{Cip1}$ and caspase 3 in RS4;11 cells were up-regulated upon treatments with GSK-3β inhibitors.

6. Docking study

The docking of synthetic compounds into X-ray crystal structure (PDB code: 1R0E) was carried out as suggested in the MOE manual. The protein-ligand cocrystal structure was loaded into MOE program, and the ligand was modified by the "Builder" to generate compound **20a**, **20e**, **38a** or **38b**. The ATP binding pocket and a modified ligand were first energy-minimized, followed by protein structure preparation including corrections, assignment of ionization states and positioning of hydrogens. Then, docking was performed at the ATP pocket site with the Triangle Matcher and Rigid Receptor methods and ranked with the London dG and GBVI/WSA dG scoring functions. The results were refined by rescoring and the top ranked results were further examined by the combination of scores and visual inspection. The distances between the amino group on the phenyl ring and the thiol group of Cys199 are around 3-5 Å.

7. Permeability Assay in hMDR|-MDCK cells.

After usual operations, the adhered cells were collected, calculated and diluted to 2×10^5 cells/mL. Then the diluted cells were seeded into 24-multiwell Insert Systems with PET (polyethylene terephthalate) membranes (1 micron pore size and 0.3 cm^2 surface area). The cell monolayers are preincubated (at 37 °C, in 5% CO_2 incubator) in transport media, all the apical sides and basolateral sides are preincubated by 0.2 mL and 0.7 mL of the transport media with or without specific P-gp inhibitor cyclosporin A for 40 minutes.

For A to B directional transport, 0.2 mL of donor working solution with test compounds or positive control is added to the A compartment and 0.7 mL of the transport media as receiver working solution then to the B compartment. B to A directional transport was operated with a similar procedure. The cells were incubated (37 °C, in 5% CO_2 incubator) for 90 minutes. 80 μL of samples are taken from both donor and receiver compartments into 96-well assay plates, which pre-added with 160 μL internal standard (IS) solution of acetonitrile each well, and centrifuged (4000× g, 10 min). 80 μL of supernatant are taken into 96-well assay plates, which pre-added with 160 μL ultrapure water and then analyzed by LC-MS/MS. If Papp A→ B > 25× 10^{-6} cm/s, the test compound has high

permeability; 10-25×10^{-6} cm/s means medium permeability and the value below 10× 10^{-6} cm/s means low permeability.

8. Metabolic Stability of compounds in Human Liver Microsomes.

5 µL test compounds stock solutions were diluted with 495 µL (1:1) Methanol/Water (final concentration: 100 µM, 50% MeOH). The solution of diluted compounds (6 µL) was added into 534 µL of liver microsome solution (final concentration: 1.111 µM, 0.555% MeOH), to give working solution. 90 µL liver microsomes solution or 90 µL compound working solution were incubated in a 96-well plate at 37 °C, separately. 10 µL NADPH co-factor solution were added into the plates and incubated at 37 °C. 300 µL cold (4 °C) stop solutions were added at t = 0, 10, 30 and 60 minutes. Samples were centrifuged, and the supernatants were analyzed by LC-MS/MS. First order kinetics was used to calculate $T_{1/2}$ and CL; CL^{liver}= CL× 45mg microsome /g liver × $Liver_{wt}$ ($Liver_{wt}$: 20 g/kg relative liver weight for 70 kg human, 45 g/kg relative liver weight for 0.25 kg rat, 88 g/kg relative liver weight for 0.02 kg mouse, 32 g/kg relative liver weight for 10 kg dog).