Supporting Information

Synthesis and Evaluation of Quinazolone Derivatives as a New Class of *c-KIT* G-Quadruplex Binding Ligands

Xiaoxiao Wang,[‡] Chen-Xi Zhou,[‡] Jin-Wu Yan, Jin-Qiang Hou, Shuo-Bin Chen, Tian-Miao Ou, Lian-Quan Gu, Zhi-Shu Huang, and Jia-Heng Tan*

School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, China

[‡]These authors contributed equally.

*Corresponding authors: Jia-Heng Tan
Tel: 8620-39943053. E-mail: tanjiah@mail.sysu.edu.cn

EXPERIMENTAL SECTION

1. Chemistry

All chemicals are commercially available without further purification unless otherwise specified. ^{1}H NMR and ^{13}C NMR spectra were recorded using TMS as the internal standard in MeOD or DMSO-*d6* or $CDCl_3$ on a Bruker BioSpin GmbH spectrometer at 400.132 and 100.614MHz, respectively. Mass spectra (MS) were recorded on a Shimadzu LCMS-2010A instrument with an ESI or ACPI mass selective detector, and high resolution mass spectra (HRMS) on Shimadzu LCMS-IT-TOF. Melting points (m.p.) were determined using a SRS-OptiMelt automated melting point instrument without correction. Flash column chromatography was performed with silica gel (200-300 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd. The purities of synthesized compounds were confirmed to be higher than 95% by using analytical HPLC with a dual pump Shimadzu LC-20AB system equipped with a Ultimate XB-C18 column (4.6×250 mm, 5 m) and eluted with methanol/water (35:65 to 45:55) containing 0.1% TFA at a flow rate of 0.5 mL/min.

6, 7-difluoro-2-methyl-4H-benzo[d][1,3]oxazin-4-one (2). A solution of 2-amino-4, 5-difluorobezoic acid (17.3 g, 0.1 mol) in acetic anhydride (50 ml) was made to reflux at 130 ℃ for 2 h. The mixture was then cooled to room temperature as a large amount of white precipitate appeared. The precipitate was separated from solvent by filtration and rinsed with diethyl ether to afford a white solid. The solid was recrystallized from ethyl acetate to give compound **2** in the form of long, white, dense needles in 61% yield. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.89 (dd, J = 9.2, 8.3 Hz, 1H), 7.28 (dd, J = 10.3, 6.9 Hz, 1H), 2.40 (s, 3H).

6, 7-difluoro-2-methyl-3-phenylquinazolin-4(3H)-one (3). Compound **2** (6 g, 30 mmol) dissolved at room temperature in aniline (19.5 g) to give a clear solution, which soon became turbid as a white precipitate began to form throughout the solution. Precipitation appeared to be complete within 4 h. The mixture was separated by filtration and rinsed with diethyl ether to yield 5.4 g of intermediate as a white powder. The intermediate was dissolved at room temperature in a minimum amount of 2% aqueous NaOH and then diluted twofold. The clear solution became cloudy within 15 min and precipitation appeared to be complete within 8 h. The mixture was filtered and rinsed with water to afford a white solid (5.7 g, 62%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.05-7.98 (m, 1H), 7.77 (dd, J = 11.4, 7.3 Hz, 1H), 7.55 (m, 3H), 7.46 (d, J = 7.1 Hz, 2H), 2.12 (s, 3H).

6-fluoro-2-methyl-7-(4-methylpiperazin-1-yl)-3-phenylquinazolin-4(3H)-one (4). Compound **3** (5.7 g, 21 mmol) and N-methylpiperazine (10.5 g, 0.105 mol) were stirred in DMF (10 ml) with a minimum amount of sodium carbonate. The mixture was refluxed at 140 ℃ for 2 h and then put into 20 ml water as a white precipitate appeared. The mixture was separated by filtration and rinsed with water to give a white solid (6.9 g, 94%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 12.9 Hz, 1H), 7.54

(dt, *J* = 14.0, 7.0 Hz, 3H), 7.25 (d, *J* = 7.0 Hz, 2H), 7.11 (d, *J* = 7.8 Hz, 1H), 3.37-3.28 (m, 4H), 2.71-2.60 (m, 4H), 2.39 (s, 3H), 2.21 (s, 3H).

(*E*)-2-(4-aminostyryl)-6-fluoro-7-(4-methylpiperazin-1-yl)-3-phenylquinazolin-4(3H)-one (5). Compound **4** (6.9 g, 19.6 mmol) and 4-nitrobenzaldehyde (8.9 g, 58.8 mmol) were dissolved in glacial acetic acid (20 mL) with a minimum amount of NaOAc. The mixture was made to reflux at 130 ℃ for 8h and then cooled to room temperature. The precipitate was separated from solvent by filtration and rinsed with diethyl ether to afford a pale-yellow solid with a yield of 97%. The product (3 g, 6.6 mmol) was dissolved in ethanol (20 mL), while $Na_2S·9H_2O$ (4.8 g, 20 mmol) and NaOH (4 g, 0.1 mol) in water (30 mL). The mixture was made to reflux at 90 ℃ for 6 h. The solvent was removed under vacuum, and the residue was cooled to room temperature. The precipitate was separated by filtration and washed with water to afford compound **5** as an orange-red solid (1.8 g, 69%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.63 (s, 1H), 7.60-7.43 (m, 5H), 7.40-7.28 (m, 2H), 7.00 (s, 1H), 6.71 (s, 1H), 6.36-6.22 (m, 2H), 4.28 (s, 2H), 3.75-3.71 (m, 2H), 3.52-3.48 (m, 2H), 2.64-2.60 (m, 2H), 2.48-2.44 (m, 2H), 2.29 (s, 3H).

(*E*)-4-(2-(6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)vinyl)benzoic acid (6). A mixture of compound 5 (1.2 g, 3.4 mmol), 4-carboxylbenzaldehyde (1.5 g, 10 mmol) and a minimum amount of NaOAc were dissolved in glacial acetic acid (8 ml) and made to reflux at 115 ℃ for 10 h. The mixture was then cooled to room temperature and the pH value was adjusted to 8.0 with ammonia water. The precipitate was separated by filtration and washed with water to give a pale-yellow solid (1.4 g, 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.12 (s, 1H), 7.90-7.76 (m, 2H), 7.60 (m, 2H), 7.54-7.49 (m, 4H), 7.40-7.30 (m, 2H), 6.98 (m, 2H), 3.75-3.71 (m, 2H), 3.52-3.48 (m, 2H), 2.64-2.60 (m, 2H), 2.48-2.44 (m, 2H), 2.29 (s, 3H).

(*E*)-N-(4-(2-(6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)vinyl)phenyl)-2-(pyrrolidin-1-yl)acetamide (7a). A suspension of compound **5** (1 g, 2.2 mmol) in 2-chloracetyl chloride (10 mL) was made to reflux at 105 ℃ for 4 h until TLC indicated completion of reaction. After cooled to 0-5 ℃, the mixture was filtered and washed with diethyl ether to give an orange intermediate. Tetrahydropyrrole (0.4 mL, 5 mmol) in EtOH (2 ml) was dropped into a stirred suspension of the intermediate (0.25 g, 0.47 mmol) and a minimum amount of NaI in EtOH (3 mL). The mixture was made to reflux at 90 ℃ for 3 h and then cooled to room temperature. The precipitate was filtered, and washed with diethyl ether. The crude product was further purified by column chromatography to give compound **7a** as a pale-yellow solid (0.07 g, 27%). Mp: 212-215 ℃. ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 9.19 (s, 1H), 7.90-7.79 (m, 2H), 7.58-7.52 (m, 5H), 7.38-7.24 (m, 5H), 6.27 (d, *J* = 15.4 Hz, 1H), 3.35-3.27 (m, 6H), 2.67 (s, 8H), 2.39 (s, 3H), 1.85 (s, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.30, 161.34, 155.46, 151.77, 149.96, 146.66, 145.83, 138.94, 136.98, 131.08, 129.87, 129.32, 128.73, 128.62, 119.45, 118.79, 115.29, 112.87, 112.62, 59.72, 54.87, 54.59, 49.94,

49.89, 46.07, 24.06. Purity: 97% by HPLC. HRMS(ESI) m/z calcd $C_{33}H_{35}FN_6O_2$ $[M + H]^+$ 567.2884, found 567.2878.

(*E*)-2-(diethylamino)-N-(4-(2-(6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)vinyl)phenyl)acetamide (7b). The method for the preparation of compound **7a** was used by replacing tetrahydropyrrole with diethylamine. Compound **7b** was synthesized as a pale-yellow solid (0.14 g, 52%). Mp: 182-185 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.49 (s, 1H), 7.84 (t, J = 14.0 Hz, 2H), 7.59-7.50 (m, 5H), 7.35-7.25 (m, 4H), 7.20 (d, J = 8.0 Hz, 1H), 6.26 (d, J = 15.5 Hz, 1H), 3.37-3.30 (m, 4H), 3.13 (s, 2H), 2.66-2.60 (m, 8H), 2.39 (s, 3H), 1.07 (t, J = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.31, 161.31, 155.41, 152.93, 151.76, 146.66, 145.82, 138.91, 136.98, 131.04, 129.85, 129.30, 128.74, 128.64, 119.28, 118.76, 115.26, 114.35, 112.71, 58.09, 54.86, 49.88, 48.87, 46.07, 12.43. Purity: 100% by HPLC. HRMS(ESI) m/z calcd $C_{33}H_{37}FN_6O_2$ $[M + H]^+$ 569.3040, found 569.3035.

(*E*)-N-(4-(2-(6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)vinyl)phenyl)-2-(piperidin-1-yl)acetamide (7c). The method for the preparation of compound **7a** was used by replacing tetrahydropyrrole with piperidine. Compound **7c** was synthesized as a pale-yellow solid (0.24 g, 85%). Mp: 205-208 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.36 (s, 1H), 7.84 (dd, J = 14.1, 11.5 Hz, 2H), 7.60-7.51 (m, 5H), 7.32-7.25 (m, 4H), 7.21 (d, J = 7.8 Hz, 1H), 6.27 (d, J = 15.5 Hz, 1H), 3.42-3.28 (m, 4H), 3.06 (s, 2H), 2.66 (t, J = 4.0 Hz, 4H), 2.52 (s, 4H), 2.39 (s, 3H), 1.66-1.60 (m, 4H), 1.51-1.46 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 169.12, 161.32, 155.43, 152.95, 151.76, 146.72, 145.83, 138.92, 136.99, 131.06, 129.86, 129.31, 128.74, 128.64, 119.35, 118.79, 115.28, 114.38, 112.73, 62.72, 54.89, 54.87, 49.95, 49.90, 46.07, 26.29, 23.55. Purity: 99% by HPLC. HRMS(ESI) m/z calcd $C_{34}H_{37}FN_6O_2$ $[M + H]^+$ 581.3040, found 581.3037.

(*E*)-N-(4-(2-(6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)vinyl)phenyl)-3-(pyrrolidin-1-yl)propanamide (7d). The method for the preparation of compound **7a** was used by replacing 2-chloracetyl chloride with 3-chloropropanoyl chloride. Compound **7d** was synthesized as a pale-yellow solid (0.2 g, 77%). Mp: 256-257 °C. ^{1}H NMR (400 MHz, MeOD) δ 7.81 (d, J = 15.6 Hz, 1H), 7.72 (d, J = 12.7 Hz, 1H), 7.59-7.46 (m, 5H), 7.33-7.30 (m, 3H), 7.19 (d, J = 8.6 Hz, 2H), 6.21 (d, J = 15.6 Hz, 1H), 3.84 (d, J = 12.7 Hz, 2H), 3.59 (d, J = 11.1 Hz, 4H), 3.45 (t, J = 6.6 Hz, 2H), 3.36-3.27 (m, 4H), 3.09-3.02 (m, 2H), 2.93 (s, 3H), 2.83 (t, J = 6.6 Hz, 2H), 2.11-2.04 (m, 2H), 1.95 (dd, J = 12.9, 7.4 Hz, 2H). ^{13}C NMR (101 MHz, MeOD) δ 169.96, 162.29, 154.78, 148.62, 145.40, 142.12, 141.65, 139.51, 137.87, 132.07, 131.13, 130.93, 129.93, 129.76, 123.83, 121.05, 118.53, 115.66, 113.99, 55.53, 54.54, 52.17, 43.69,32.95, 24.02. Purity: 98% by HPLC. HRMS(ESI) m/z calcd $C_{34}H_{37}FN_6O_2$ $[M + H]^+$ 581.3040, found 581.3033.

(*E*)-3-(diethylamino)-N-(4-(2-(6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxo-3-phenyl-3,4-dihyd

roquinazolin-2-yl)vinyl)phenyl)propanamide (7e). The method for the preparation of compound **7a** was used by replacing 2-chloracetyl chloride with 3-chloropropanoyl chloride and tetrahydropyrrole with diethylamine. Compound **7e** was synthesized as a pale-yellow solid (0.17 g, 63%). Mp: 181-182 °C. ^{1}H NMR (400 MHz, MeOD) δ 7.82 (d, *J* = 15.6 Hz, 1H), 7.72-7.60 (m, 4H), 7.54 (d, *J* = 8.6 Hz, 2H), 7.39 (dd, *J* = 8.0, 1.5 Hz, 2H), 7.24 (dd, *J* = 8.1, 6.0 Hz, 3H), 6.26 (d, *J* = 15.5 Hz, 1H), 3.37 (t, *J* = 6.0 Hz, 4H), 3.31-3.27 (m, 2H), 3.08 (q, *J* = 7.3 Hz, 4H), 2.79 (t, *J* = 6.8 Hz, 2H), 2.74 (t, *J* = 4.0 Hz, 4H), 2.43 (s, 3H), 1.28 (t, *J* = 7.3 Hz, 6H). ^{13}C NMR (101 MHz, MeOD) δ 164.39, 161.38, 155.11, 152.66, 151.80, 146.24, 145.66, 138.69, 136.71, 130.62, 129.30, 128.35, 127.65, 126.43, 119.56, 119.30, 118.05, 114.55, 111.51, 54.09, 48.90, 44.36, 41.82, 9.86, 8.88. Purity: 98% by HPLC. HRMS(ESI) m/z calcd $C_{34}H_{39}FN_6O_2$ $[M + H]^+$ 583.3197, found 583.3190.

(*E*)-N-(4-(2-(6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)vinyl)phenyl)-3-(piperidin-1-yl)propanamide (7f). The method for the preparation of compound **7a** was used by replacing 2-chloracetyl chloride with 3-chloropropanoyl chloride and tetrahydropyrrole with piperidine. Compound **7f** was synthesized as a pale-yellow solid (0.21 g, 78%). Mp: 229-231 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (dd, *J* = 14.2, 11.4 Hz, 2H), 7.60-7.48 (m, 5H), 7.37-7.15 (m, 5H), 6.26 (d, *J* = 15.5 Hz, 1H), 3.34 (t, *J* = 4.0 Hz, 4H), 2.71-2.62 (m, 6H), 2.58-2.45 (m, 6H), 2.39 (s, 3H), 1.70-1.64 (m, 4H), 1.59-1.52 (m, 2H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 170.92, 161.32, 155.03, 154.66, 151.88, 146.69, 145.86, 140.33, 139.12, 137.03, 130.46, 129.86, 129.28, 128.71, 119.42, 118.35, 115.26, 114.37, 112.73, 54.91, 54.20, 53.56, 49.97, 46.13, 32.50, 26.24, 24.15. Purity: 99% by HPLC. HRMS(ESI) m/z calcd $C_{35}H_{39}FN_6O_2$ $[M + H]^+$ 595.3197, found 595.3195.

(*E*)-N-(2-(dimethylamino)ethyl)-4-(2-(6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxo-3-phenyl-3, 4-dihydroquinazolin-2-yl)vinyl)benzamide (7g). N,N-Dimethylethane-1,2-diamine (0.12 ml, 0.9 mmol) in DMF (2 ml) was dropped into a stirred suspension of compound **6** (0.2 g, 0.4 mmol) and BOP (0.3 g, 0.6 mmol) in DMF (6 ml). The mixture was made to reflux at 65 °C overnight. The mixture was then put into water as a yellow precipitate formed throughout the solvent. The precipitate was filtered, and washed with water. The crude product was further purified by column chromatography to give compound **7g** as a pale-yellow solid (0.09 g, 41%). Mp: 217-219 °C. ^{1}H NMR (400 MHz, $CDCl_3$) *δ* 7.90 (d, *J* = 15.5 Hz, 1H), 7.83 (d, *J* = 12.9 Hz, 1H), 7.74 (d, *J* = 8.2 Hz, 2H), 7.62-7.54 (m, 3H), 7.32-7.28 (m, 4H), 7.22 (d, *J* = 7.8 Hz, 1H), 7.04 (s, 1H), 6.40 (d, *J* = 15.5 Hz, 1H), 3.52 (dd, *J* = 10.9, 5.3 Hz, 2H), 3.37-3.32 (m, 4H), 2.67-2.63 (m, 4H), 2.54 (t, *J* = 5.7 Hz, 2H), 2.39 (s, 3H), 2.28 (s, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.65, 161.17, 155.60, 153.12, 151.17, 146.78, 145.64, 138.22, 136.80, 135.08, 129.93, 129.46, 128.71, 127.56, 121.60, 115.39, 114.53, 112.89, 112.65, 57.80, 54.86, 49.90, 46.09, 45.05, 37.06. Purity: 100% by HPLC. HRMS(ESI) m/z calcd $C_{32}H_{35}FN_6O_2$ $[M + H]^+$ 555.2884, found 555.2877.

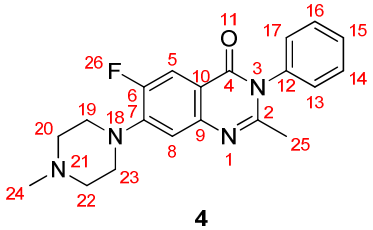
(*E*)-N-(2-(diethylamino)ethyl)-4-(2-(6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxo-3-phenyl-3,4-

dihydroquinazolin-2-yl)vinyl)benzamide (7h). The method for the preparation of compound **7g** was used by replacing N,N-dimethylethane-1,2-diamine with N,N-diethylethane-1,2-diamine. Compound **7h** was synthesized as a pale-yellow solid (0.07 g, 30%). Mp: 152-156 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.90 (d, *J* = 15.5 Hz, 1H), 7.84 (d, *J* = 12.9 Hz, 1H), 7.72 (d, *J* = 8.3 Hz, 2H), 7.59 (dt, *J* = 14.7, 5.1 Hz, 3H), 7.33 (dd, *J* = 8.7, 5.1 Hz, 4H), 7.22 (d, *J* = 7.8 Hz, 1H), 7.05 (s, 1H), 6.41 (d, *J* = 15.5 Hz, 1H), 3.48 (dd, *J* = 11.2, 5.3 Hz, 2H), 3.40 – 3.31 (m, 4H), 2.70 – 2.51 (m, 10H), 2.39 (s, 3H), 1.03 (t, *J* = 7.1 Hz, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.45, 161.18, 151.18, 146.80, 146.70, 145.66, 138.25, 138.01, 136.82, 135.30, 129.94, 129.44, 128.71, 127.73, 127.42, 121.58, 115.40, 112.91, 112.67, 54.88, 51.32, 49.97, 46.75, 46.10, 37.23, 11.81. Purity: 95% by HPLC. HRMS(ESI) m/z calcd $C_{34}H_{39}FN_6O_2$ $[M + H]^+$ 583.3197, found 583.3188.

(*E*)-4-(2-(6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)vinyl)-N-(2-(pyrrolidin-1-yl)ethyl)benzamide (7i). The method for the preparation of compound **7g** was used by replacing N,N-dimethylethane-1,2-diamine with 2-(pyrrolidin-1-yl)ethanamine. Compound **7i** was synthesized as a pale-yellow solid (0.11 g, 48%). Mp: 169-173 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.83 (d, *J* = 15.5 Hz, 1H), 7.77 (d, *J* = 12.9 Hz, 1H), 7.66 (d, *J* = 8.3 Hz, 2H), 7.54-7.46 (m, 3H), 7.29-7.21 (m, 4H), 7.15 (d, *J* = 7.8 Hz, 1H), 6.94 (s, 1H), 6.34 (d, *J* = 15.5 Hz, 1H), 3.48 (dd, *J* = 11.1, 5.4 Hz, 2H), 3.27 (t, *J* = 6.0 Hz, 4H), 2.66 (t, *J* = 5.8 Hz, 2H), 2.61-2.48 (m, 8H), 2.32 (s, 3H), 1.79-1.68 (m, 4H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.66, 160.15, 152.13, 150.19, 145.70, 144.65, 137.21, 137.05, 135.82, 134.21, 128.93, 128.44, 127.71, 126.68, 126.55, 120.63, 114.38, 113.52, 111.79, 53.89, 53.60, 52.92, 48.95, 45.12, 37.34, 22.49. Purity: 95% by HPLC. HRMS(ESI) m/z calcd $C_{34}H_{37}FN_6O_2$ $[M + H]^+$ 581.3040, found 581.3032.

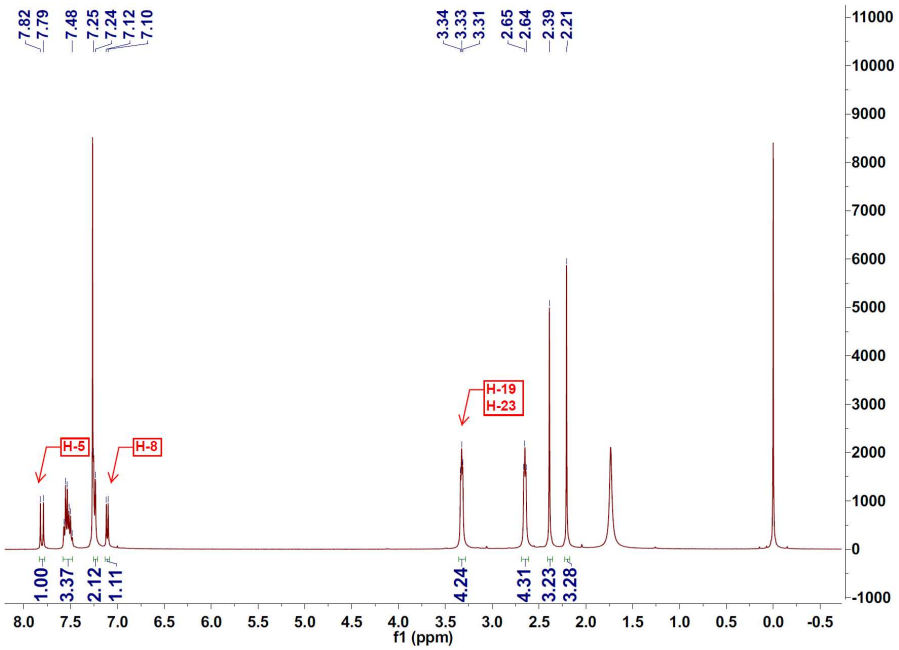
(*E*)-4-(2-(6-fluoro-7-(4-methylpiperazin-1-yl)-4-oxo-3-phenyl-3,4-dihydroquinazolin-2-yl)vinyl)-N-(3-(pyrrolidin-1-yl)propyl)benzamide (7j). The method for the preparation of compound **7g** was used by replacing N,N-dimethylethane-1,2-diamine with 3-(pyrrolidin-1-yl)propan-1-amine. Compound **7j** was synthesized as a pale-yellow solid (0.12 g, 50%). Mp: 131-135 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.76 (s, 1H), 7.89 (d, *J* = 15.5 Hz, 1H), 7.83 (d, *J* = 12.9 Hz, 1H), 7.69 (d, *J* = 8.2 Hz, 2H), 7.62-7.53 (m, 3H), 7.35-7.28 (m, 4H), 7.21 (d, *J* = 7.8 Hz, 1H), 6.39 (d, *J* = 15.5 Hz, 1H), 3.55 (dd, *J* = 10.8, 5.4 Hz, 2H), 3.40-3.30 (m, 4H), 2.71 (t, *J* = 6.0 Hz, 2H), 2.68-2.56 (m, 8H), 2.39 (s, 3H), 1.86-1.74 (m, 6H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.35, 161.21, 155.61, 151.23, 146.73, 145.67, 138.29, 137.86, 136.80, 135.50, 130.86, 129.92, 128.71, 127.56, 127.34, 121.50, 115.38, 114.48, 112.76, 55.71, 54.86, 54.06, 49.92, 46.09, 40.68, 26.07, 23.44. Purity: 99% by HPLC. HRMS(ESI) m/z calcd $C_{35}H_{39}FN_6O_2$ $[M + H]^+$ 595.3197, found 595.3192.

NMR spectra of compound 4

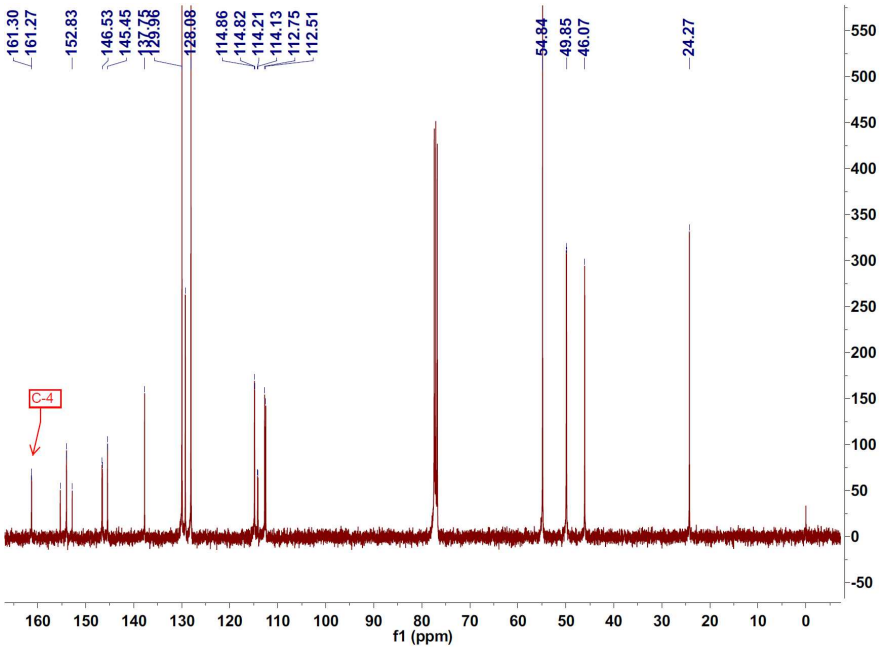


4

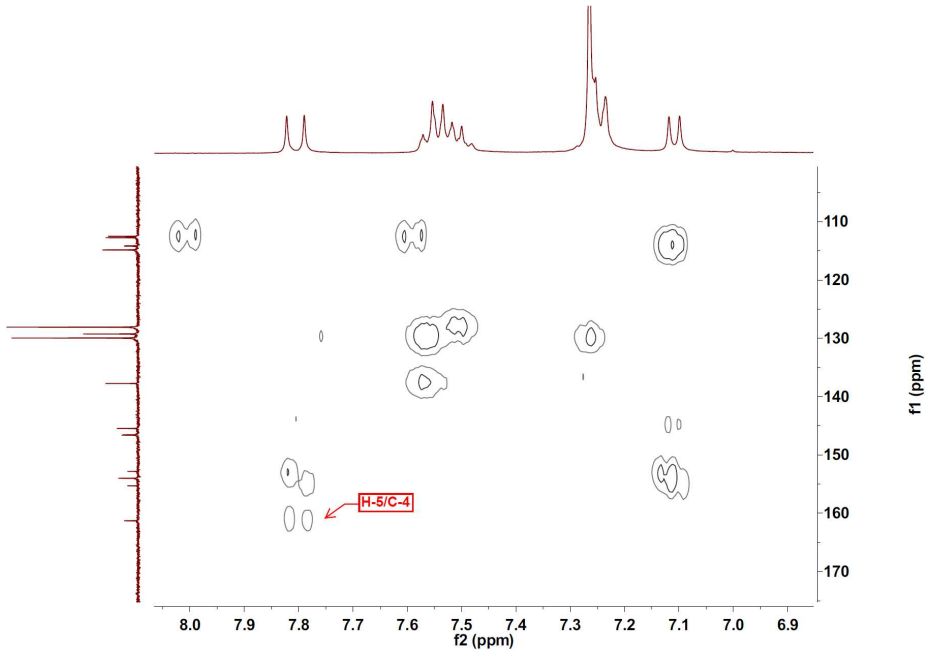
^{1}H NMR spectrum of **4**



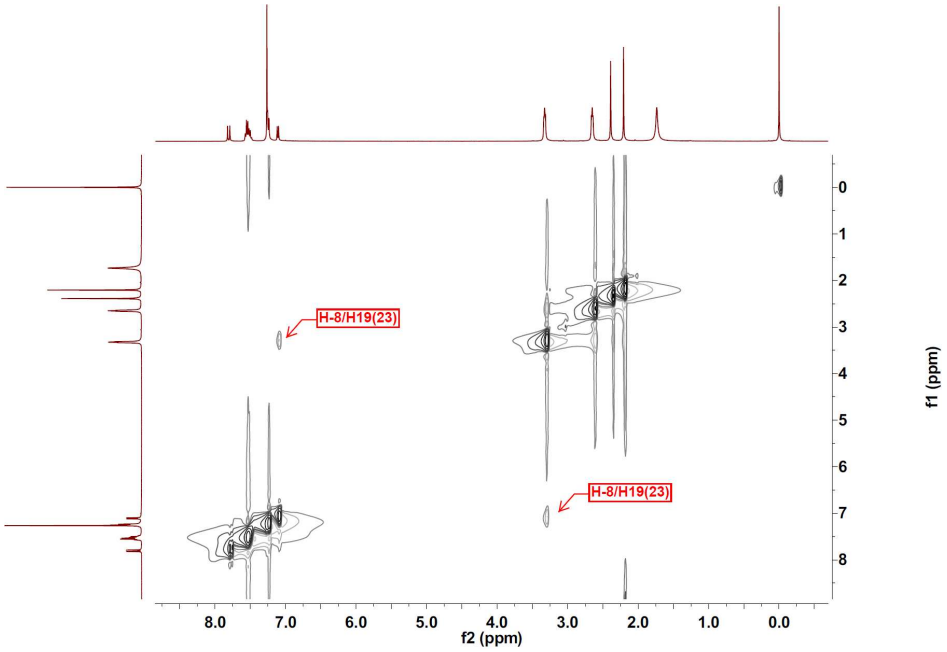
^{13}C NMR spectrum of **4**



HMBC spectrum of **4**



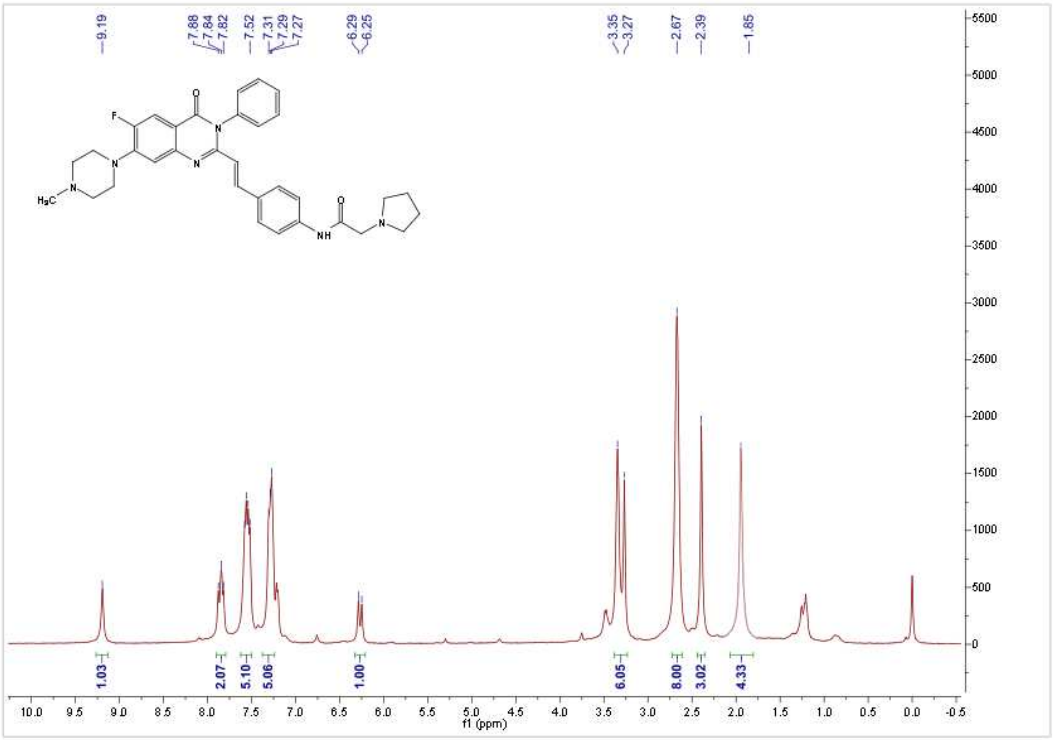
NOESY spectrum of **4**



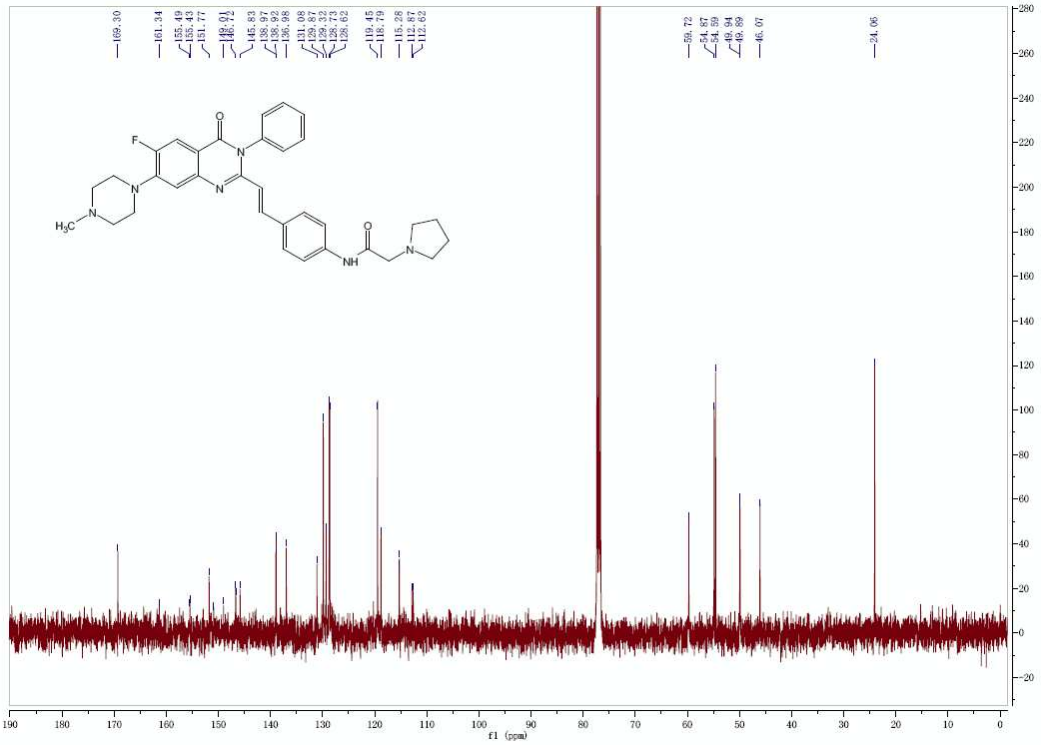
Structural identification of **4**: The structure of compound **4** was determined by HMBC and NOESY in two-dimensional NMR. In the HMBC spectrum, a correlation was observed between H-5 and C-4, suggesting that H-5 was proximal to the carbonyl group. Furthermore, in the NOESY spectrum, NOE correlation was observed between H-8 and H-19(23), suggesting that H-8 was proximal to the N-methylpiperazine group.

NMR spectra of compounds (7a-7j)

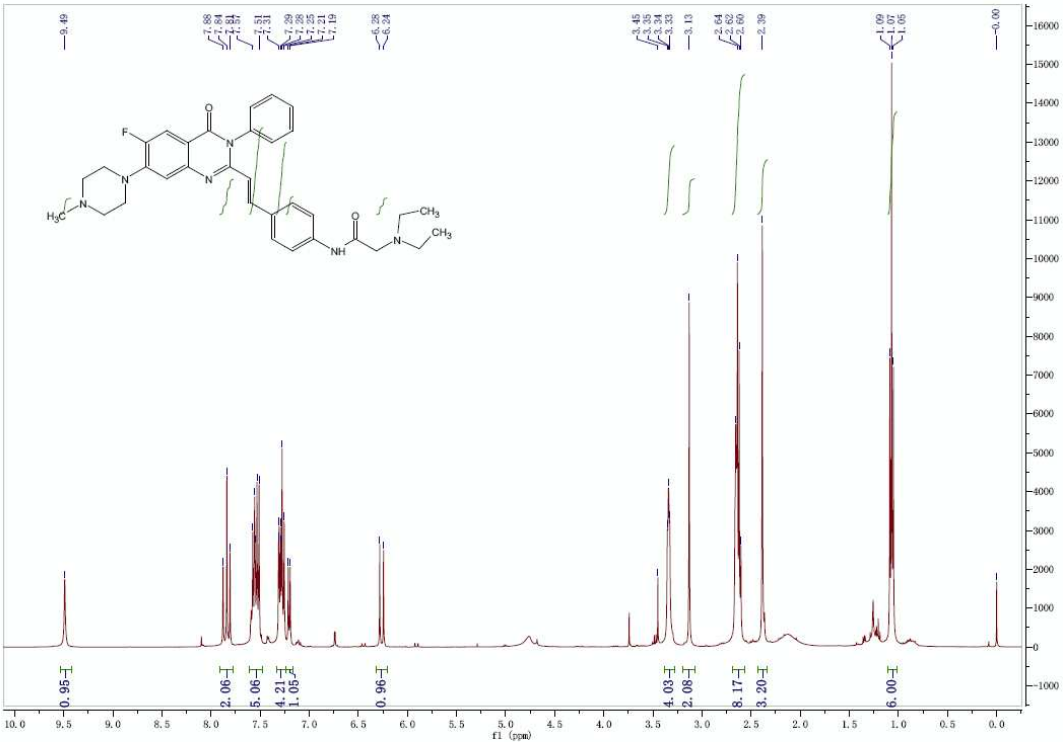
^{1}H NMR spectrum of **7a**



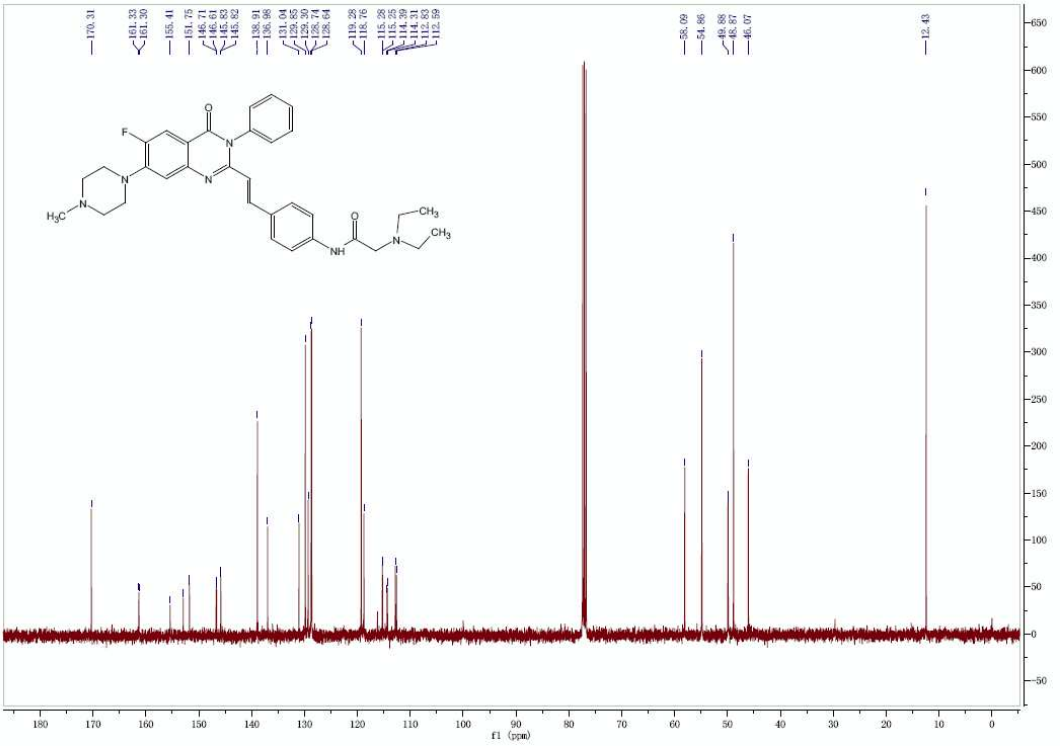
^{13}C NMR spectrum of **7a**



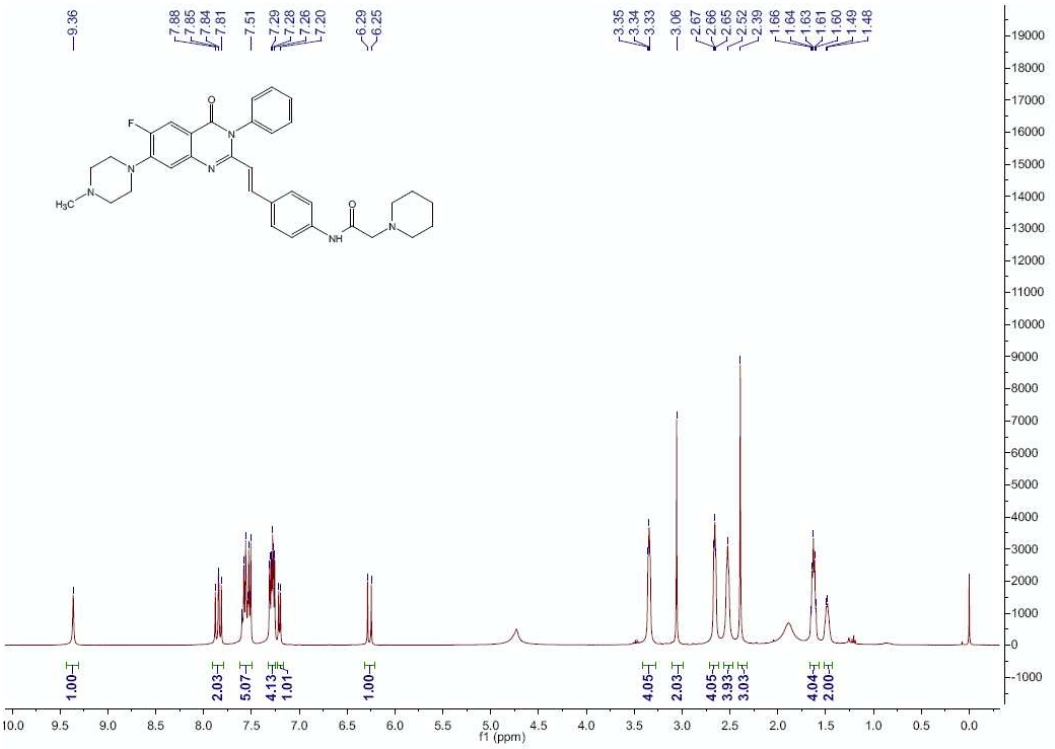
^{1}H NMR spectrum of **7b**



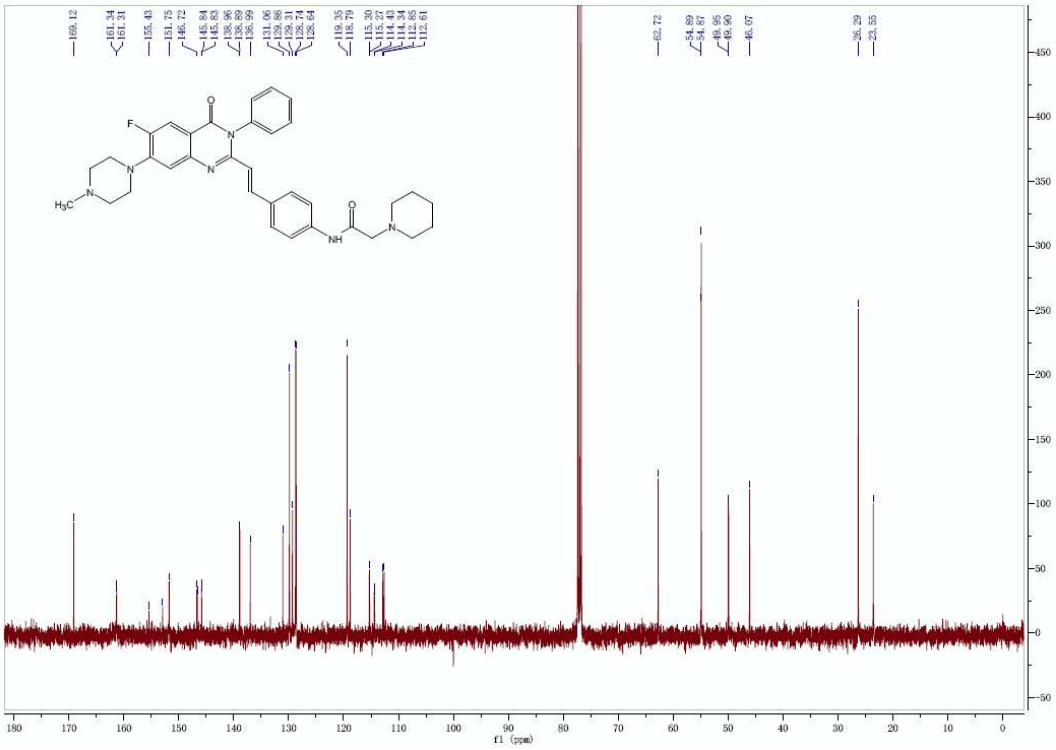
^{13}C NMR spectrum of **7b**



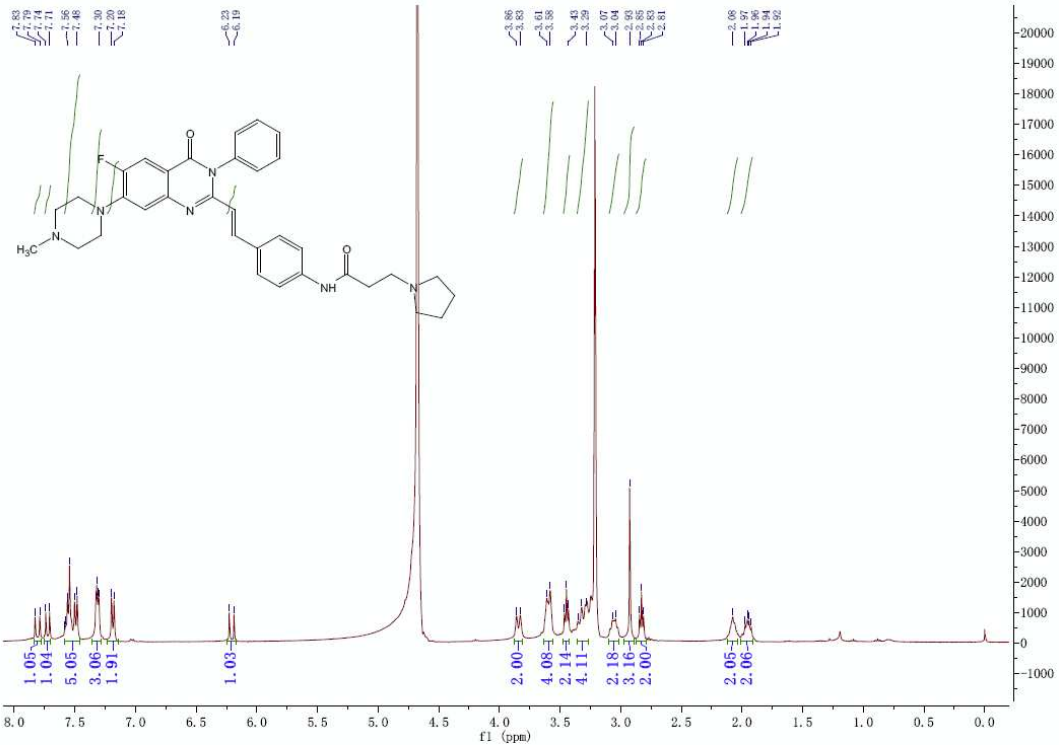
^{1}H NMR spectrum of **7c**



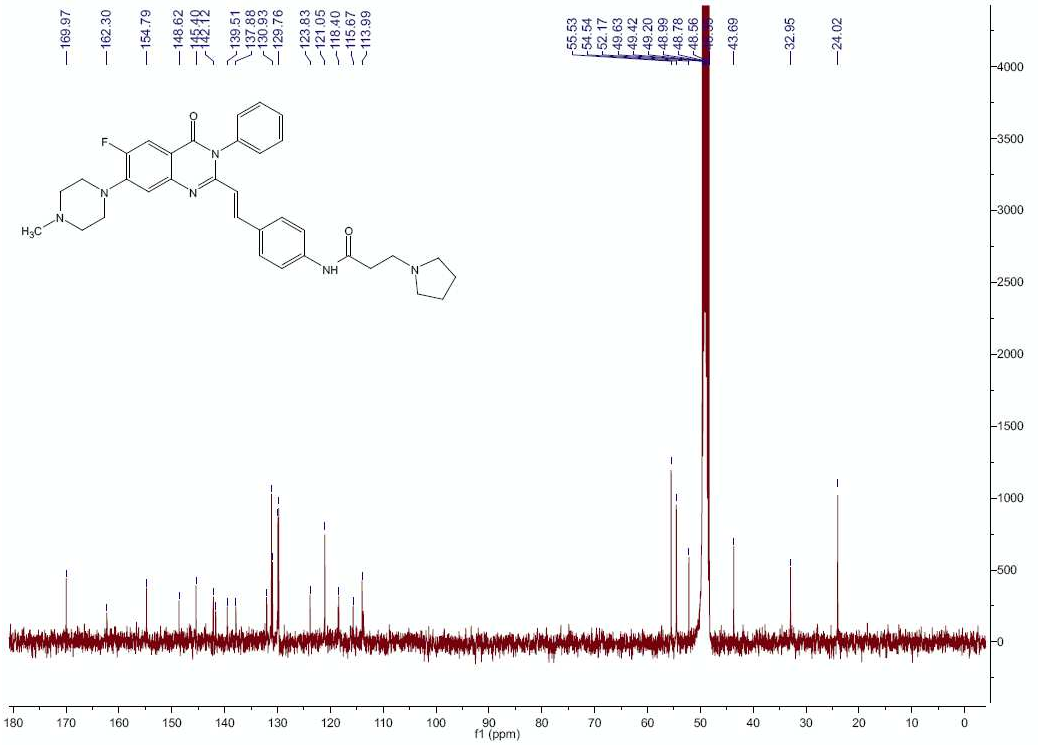
^{13}C NMR spectrum of **7c**



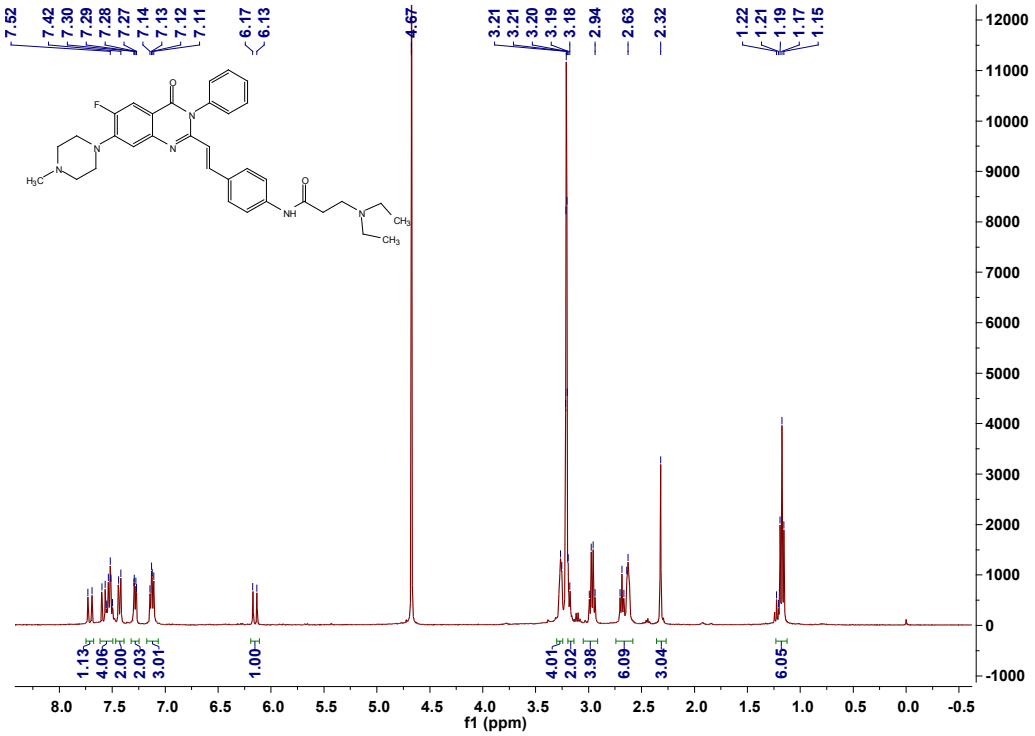
^{1}H NMR spectrum of **7d**



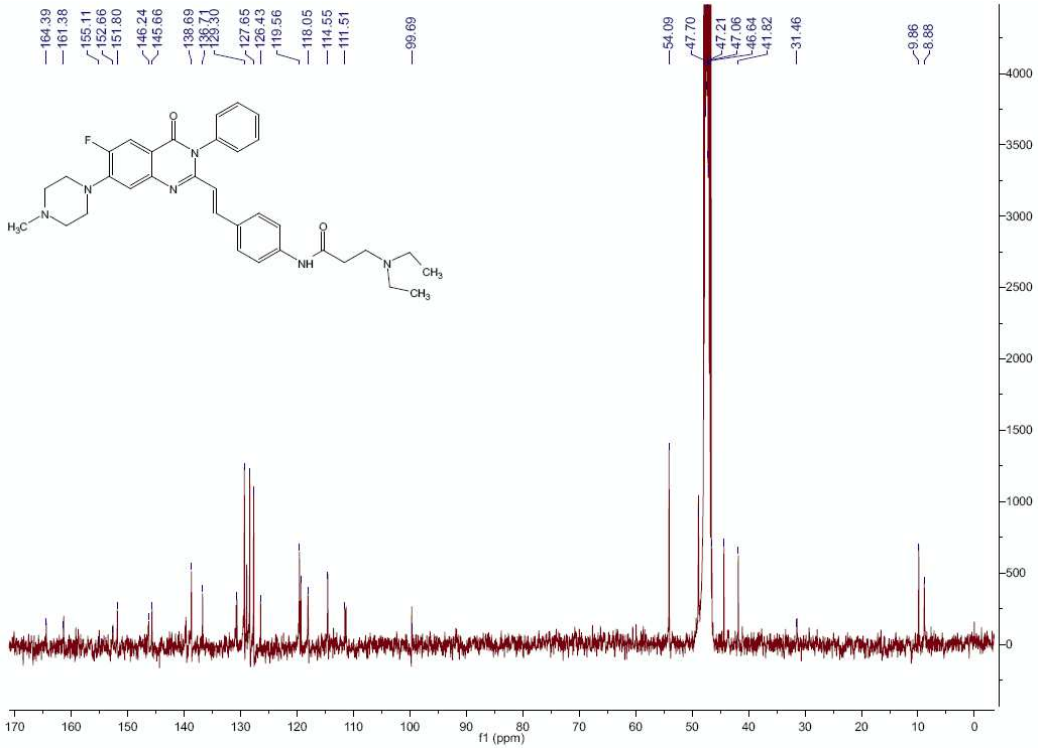
^{13}C NMR spectrum of **7d**



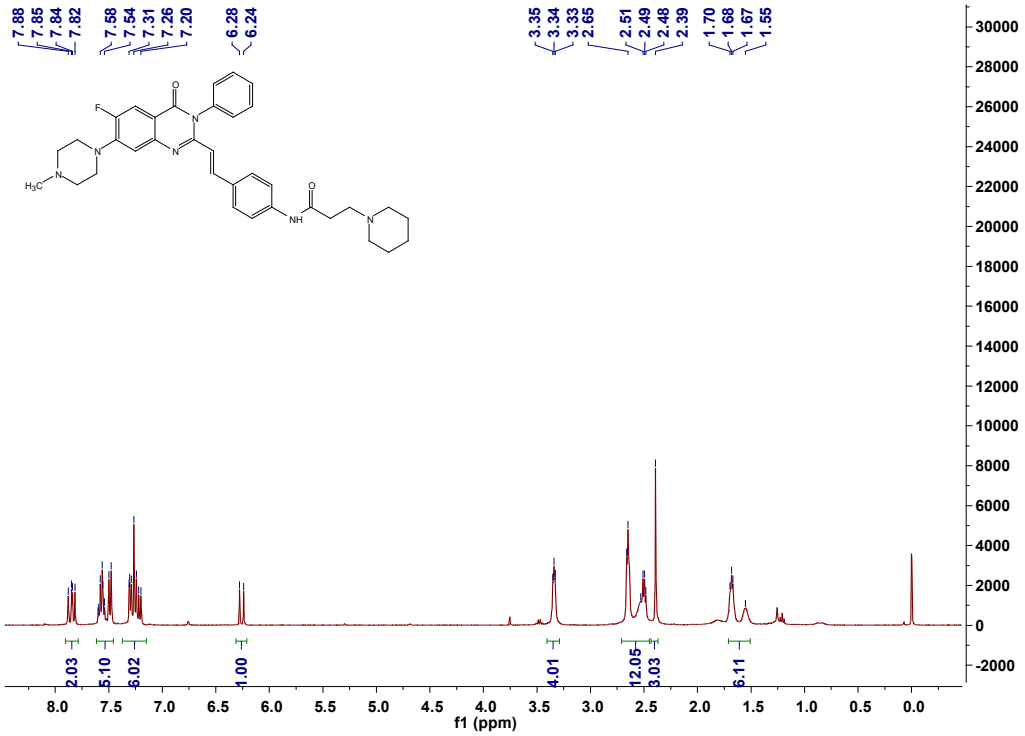
^{1}H NMR spectrum of **7e**



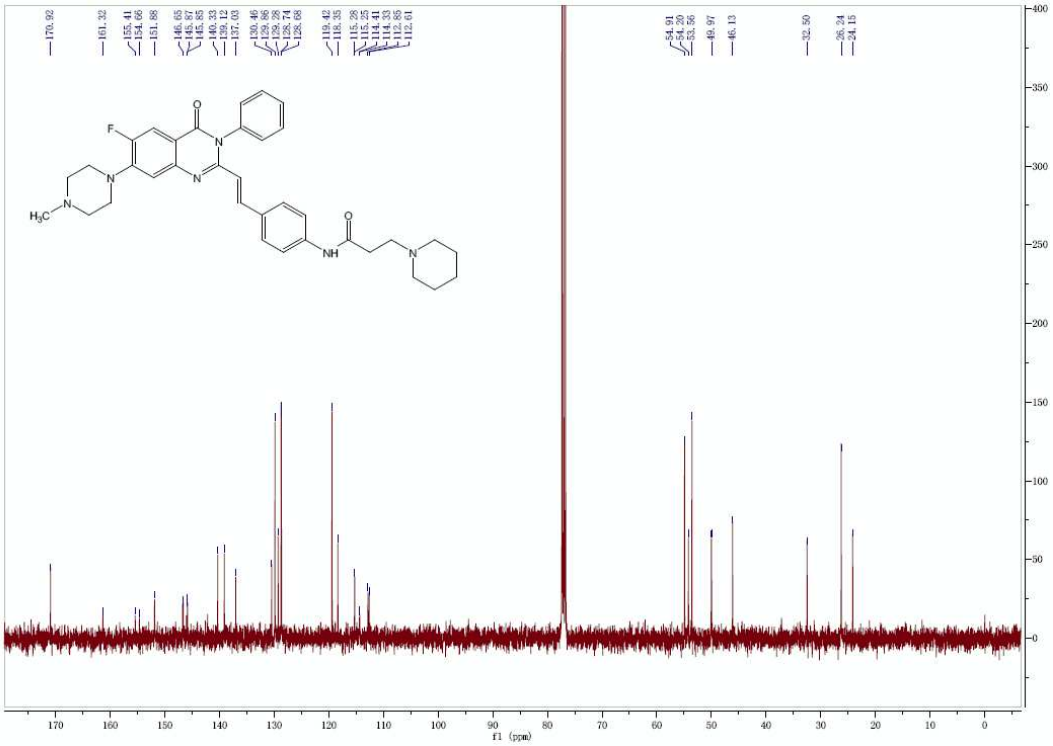
^{13}C NMR spectrum of **7e**



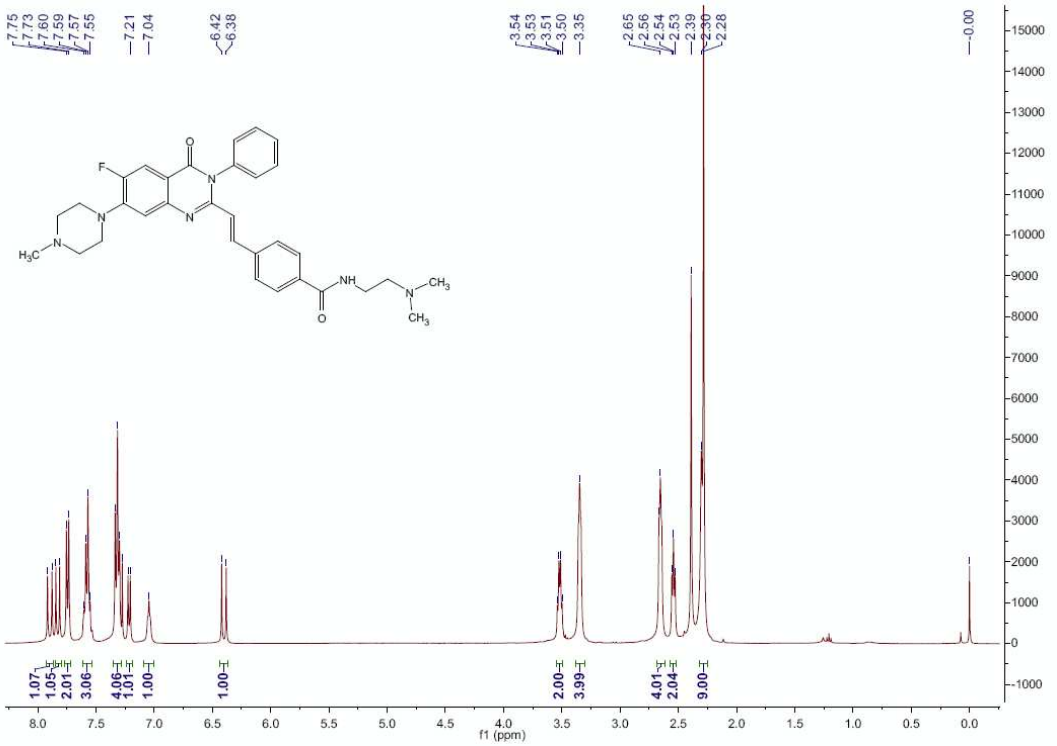
^{1}H NMR spectrum of **7f**



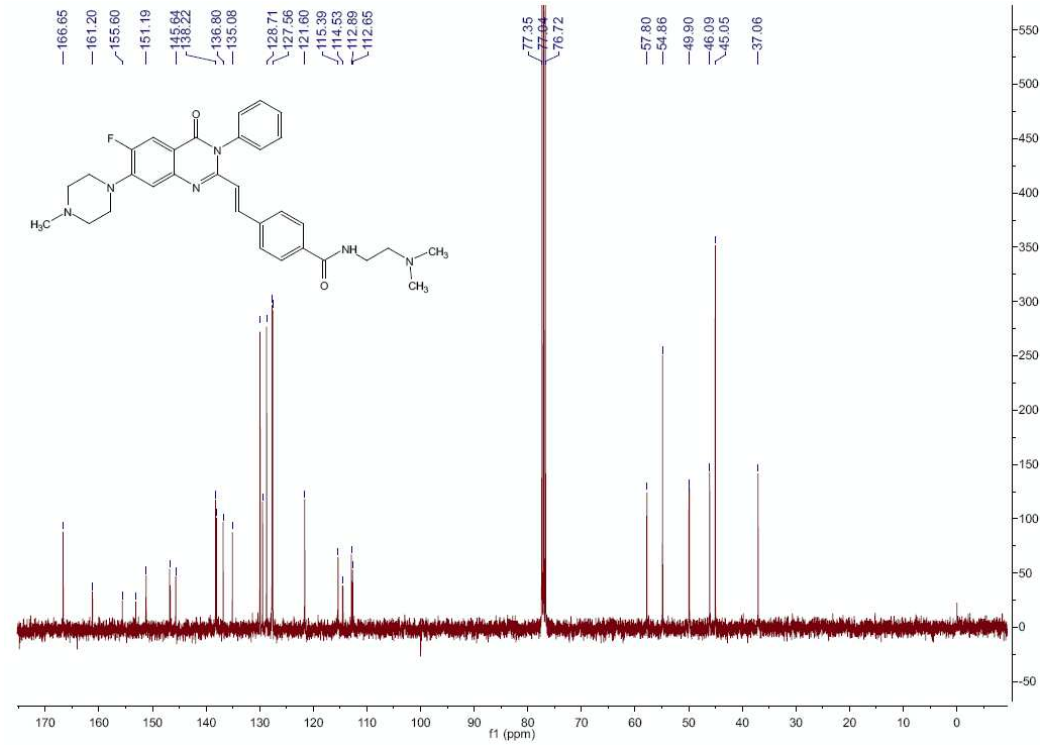
^{13}C NMR spectrum of **7f**



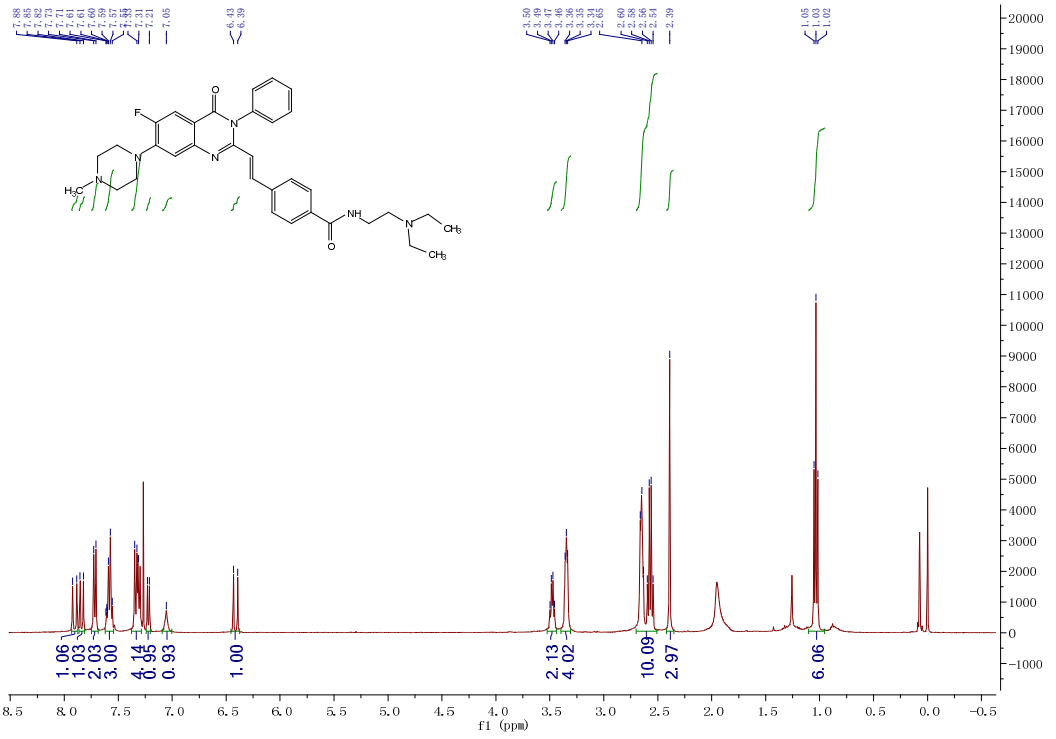
^{1}H NMR spectrum of **7g**



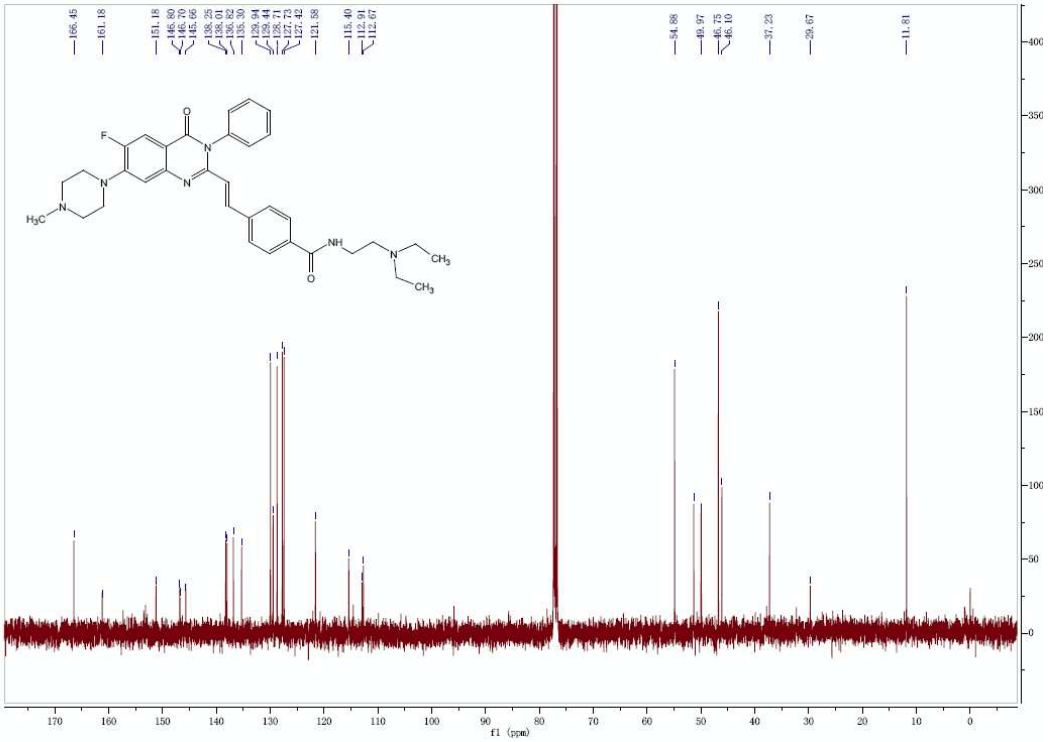
^{13}C NMR spectrum of **7g**



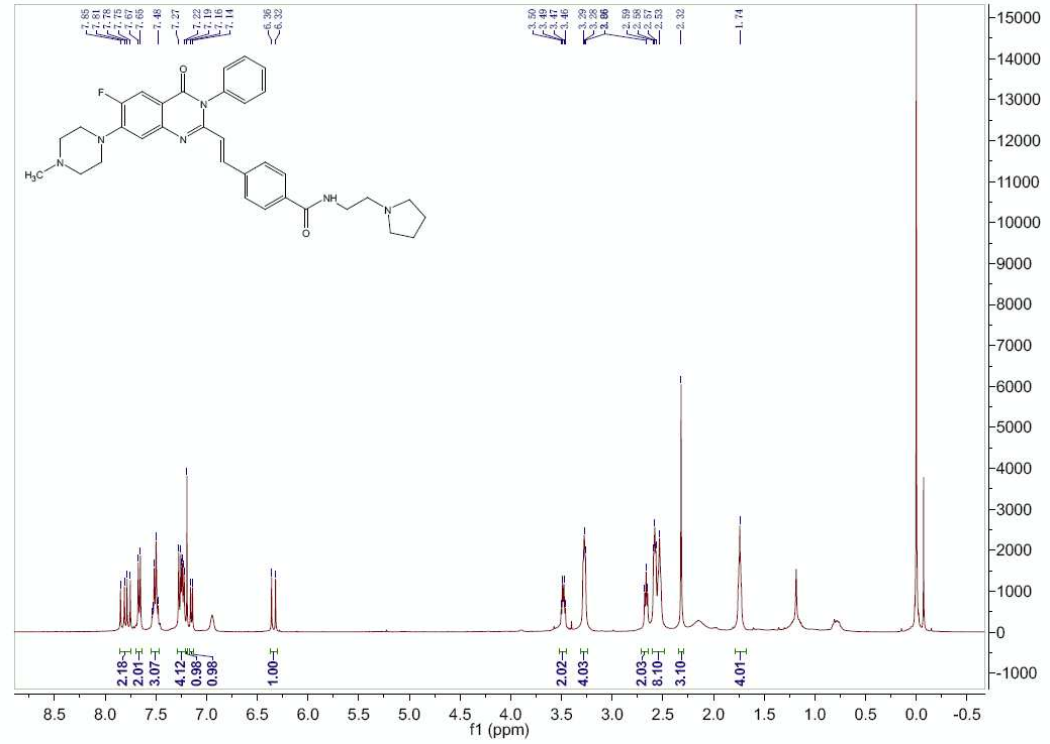
^{1}H NMR spectrum of **7h**



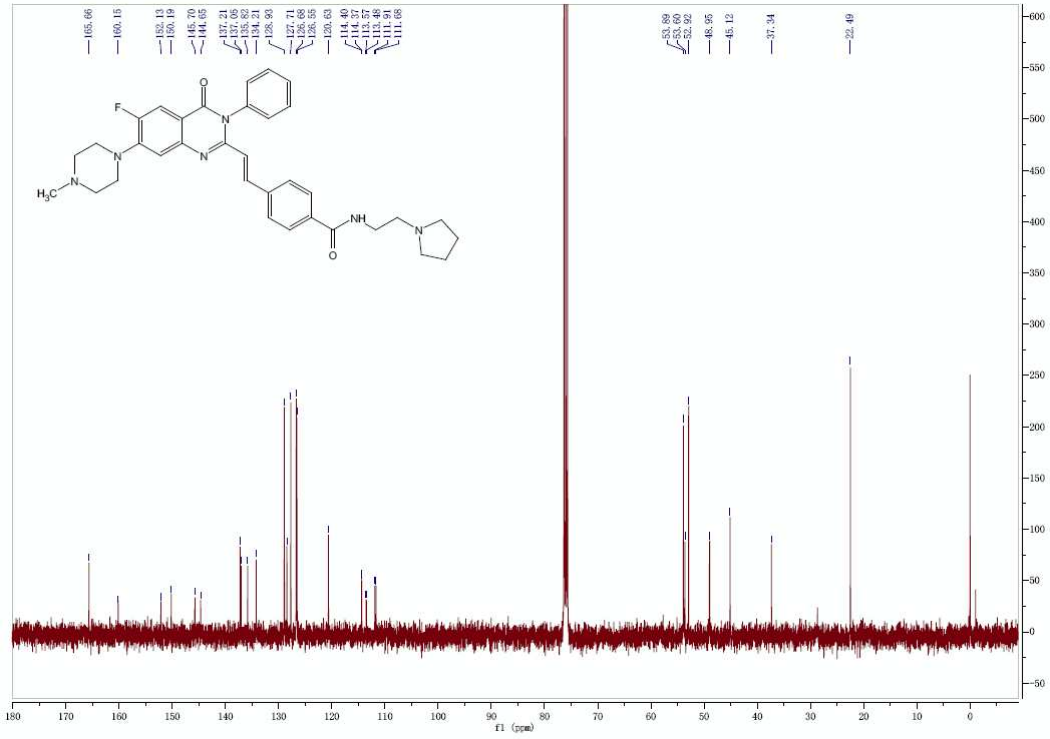
^{13}C NMR spectrum of **7h**



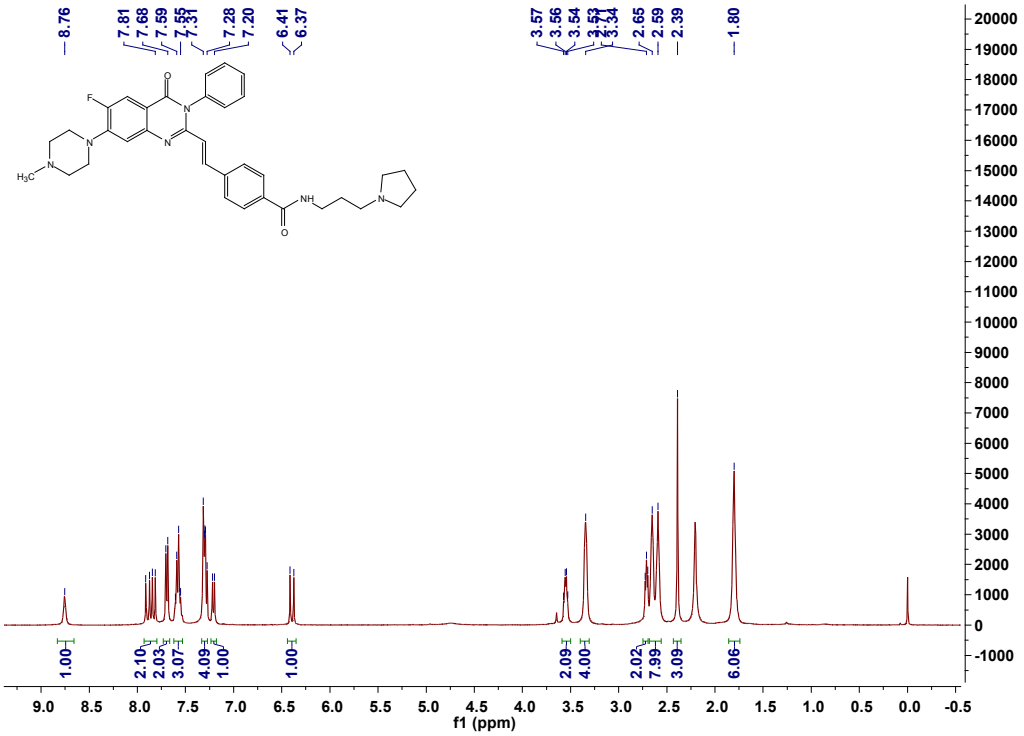
^{1}H NMR spectrum of **7i**



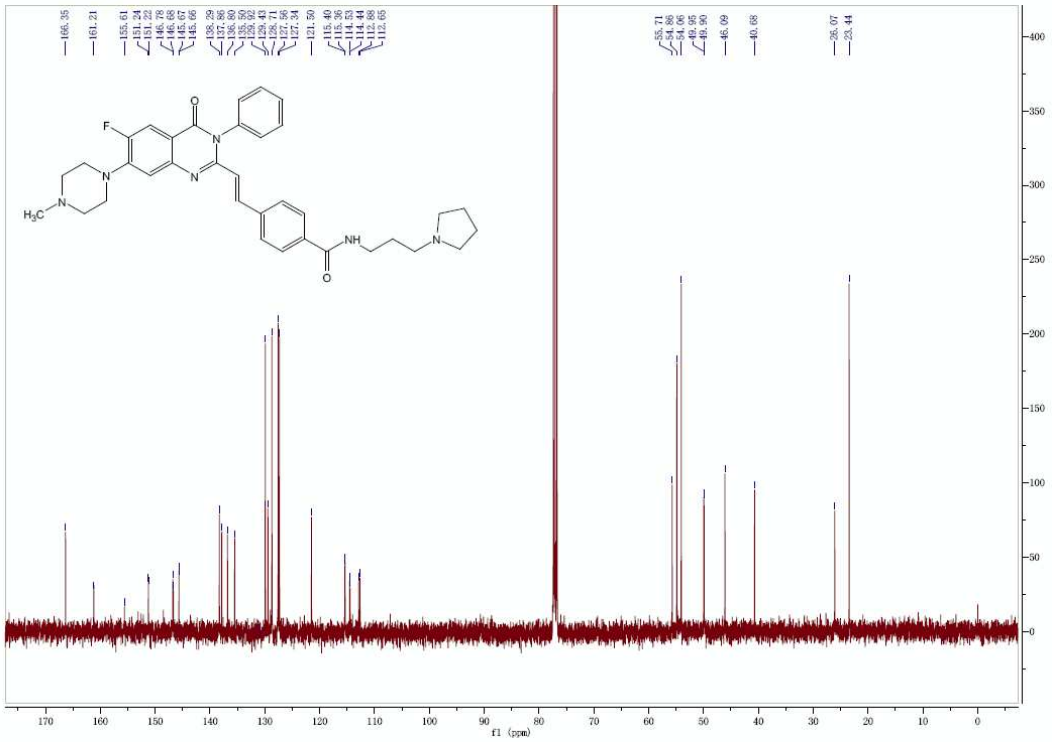
^{13}C NMR spectrum of **7i**



^{1}H NMR spectrum of **7j**



^{13}C NMR spectrum of **7j**



HPLC Purity Analysis of Compounds 7a-7j [a]

Compound	Purity(%)	Methanol/Water	Retention time (min)
7a	97	55/45	8.09
7b	100	55/45	8.48
7c	99	55/45	9.04
7d	98	55/45	8.1
7e	98	55/45	8.56
7f	99	55/45	8.87
7g	100	55/45	7.37
7h	95	55/45	8.15
7i	95	55/45	7.85
7j	99	55/45	8.02

[a] 0.1% TFA in MeOH and 0.1% aqueous TFA over 30 min (1 ml/min); Purity at 254 nm.

2. Biophysical Assay

FRET-melting assay. The oligonucleotide labeled with *FAM* and *TAMRA* (with *FAM*: 6-carboxyfluorescein and *TAMRA*: 6-carboxytetramehtylrhodamine) was purchased from Invitrogen (China). Fluorescence melting curves were determined by a Roche LightCycler 2 real-time PCR machine, using a total reaction volume of 20 μL, with 1 μM of labeled oligonucleotide in 10 mM Tris-HCl buffer, pH 7.2, 60 mM KCl. Fluorescence readings with excitation at 470 nm and detection at 530 nm were taken at intervals of 1 ºC over the range 37 - 99 °C, with a constant temperature being maintained for 30 s prior to each reading to ensure a stable value. The melting of the G-quadruplex was monitored alone or in the presence of various concentrations of compounds. Final analysis of the data was carried out using Origin 8 (OriginLab Corp.).

Surface plasmon resonance. SPR measurements were performed on a ProteOn XPR36 Protein Interaction Array system (BioRad Laboratories, Hercules, CA) using a Neutravidincoated GLH sensor chip. In a typical experiment, biotinylated *c-KIT*1 (5'-biotin-$AG_3AG_3CGCTG_3AGGAG_3$-3') and duplex DNA (5'-biotin-T_9CGAATTCGT_5CGAATTCG-3') were folded in filtered and degassed running buffer (50 mM TrisHCl, pH 7.2, 100 mM KCl). The DNA samples were then captured (~1000 RU) in flow cell 1, leaving the fourth flow cell as a blank. Ligand solutions (at 10, 5, 2.5, 1.25, 0.625, 0.3125μM) were prepared with running buffer by serial dilutions from stock solution. Six concentrations were injected simultaneously at a flow rate of 100 μL/min for 150 s of association phase, followed with 300 s of dissociation phase at 25 ºC. The GLH sensor chip was regenerated with short injection of 50 mM NaOH between consecutive measurements. The final graphs were obtained by subtracting blank sensorgrams from quadruplex sensorgrams. Data are analyzed with ProteOn

manager software, using the Equilibrium method for fitting kinetic data.

CD measurements. The oligomers *c-KIT*1 (5'-d[$AG_3AG_3CGCTG_3AGGAG_3$]-3') and *c-KIT*2 (5'-d[$G_3CG_3CGCGAG_3AG_4$]-3') were diluted from stock to the required concentration (5 μM) in Tris-HCl buffer (10 mM, pH 7.2) with or without 150 mM KCl, and then annealed by heating to 90 °C for 5 min, gradually cooled to room temperature, and incubated at 4 °C overnight. CD experiments were performed on a Chirascan circular dichroism spectrophotometer (Applied Photophysics). A quartz cuvette with 4 mm path length was used for the spectra recorded over a wavelength range of 230-450 nm at 1 nm bandwidth, 1 nm step size, and 0.5 s per point. A buffer baseline was collected in the same cuvette, and subtracted from the sample spectra. Final analysis of the data was carried out using Origin 8 (OriginLab Corp.).

3. Cellular Assays

Cell culture. The human gastric carcinoma cell line HGC-27 was purchased from the Center of Experiment Animal of Sun Yat-sun University. Imatinib was purchased from Energy Chemical Company. Cells are cultured in DMEM medium and supplemented with 10% fetal bovine serum. Cells are passaged at 70-80% confluence at 37 °C in a 5% CO_2 humidified atmosphere.

Short-term cell viability. Short-term growth inhibition was measured using MTT assay. HGC-27 cells were seeded into 96-well plates at 5,000 cells/well and cultured overnight. Afterward, the compounds were added in a solution of fresh media at concentrations ranging from 1 μM to 100 μM in triplicate and cells were incubated at 37 °C for 48 h. Subsequently, cells were stained with MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) for 4 h and then dissolved in 100 μL of DMSO. The optical density (OD) was measured at 570 nm using Bio-Tek PowerWave XS. The IC_{50} values were determined with the mean OD of the tests versus drug concentration curves.

Plasmid Construction. A DNA fragment of the human *c-KIT* gene promoter region, containing wild-type *c-KIT*1 and *c-KIT*2 sequence, was extracted from MCF-7 cell total DNA by PCR. This fragment was then inserted into pMetLuc-Reporter Vector (Clontech), named *c-KIT* native construct. Then the *c-KIT* native construct was site-mutagenesis as *c-KIT* mutated construct (see below).

***c-KIT* native construct**

——— CCGGGCGGGCGCGAGGGAGGGGAGGCGAGGAGGGGCGTGGCCGGCGCGCAGAGGGAGGGCGCTGGGAGGAGGGCT ———

wild-type *c-KIT*2 wild-type *c-KIT*1

***c-KIT* mutated construct**

——— CCGGGCGGGCGCGAGGGAGTTTAGGCGAGGAGGGGCGTGGCCGGCGCGCAGAGGGAGGGCGCTGGGCGGCGGGCT ———

mutated *c-KIT*2 mutated *c-KIT*1

Transfection and Luciferase Activity Assay. *c-KIT* native construct or mutated construct and pRL-TK were cotransfected into MCF-7 cell with Lipofectamine 2000 reagent (Invitrogen) as

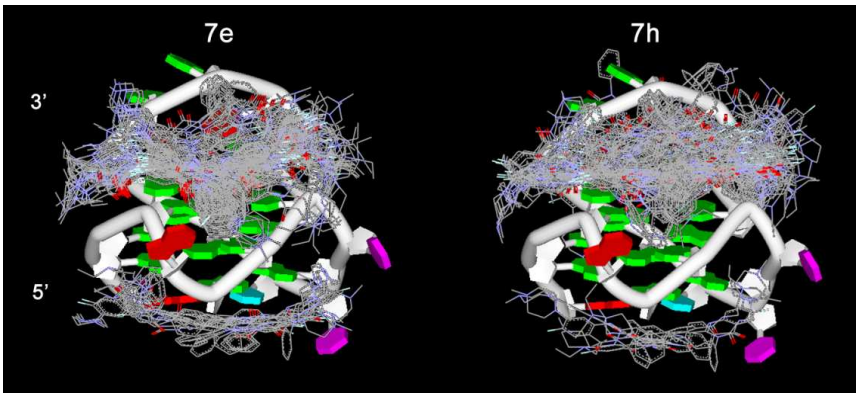
manufacturer's instructions. Then after 4 h of transfection, different concentrations of the compounds were added into medium. Cells were incubated for another 48 h at 37 ℃ in 5% CO_2, then the luciferase activity was evaluated by Ready-To-Glow Secreted Luciferase Reporter System (Clontech) and Renilla Luciferase Assay System (Promega).

RNA extraction. For RNA extraction, about 1×10^5 HGC-cells were seeded in 6-well plates with appropriate concentrations of compounds and incubated for 24 h. Cell pellets were harvested and lysed in TRIpure solution. Total RNA was extracted from the pellets according to manufacture's protocol and eluted in distilled, deionized water with 0.1% diethyl pyrocarbonate (DEPC) to a final volume of 10-50 μL. RNA was stored at -80 ℃.

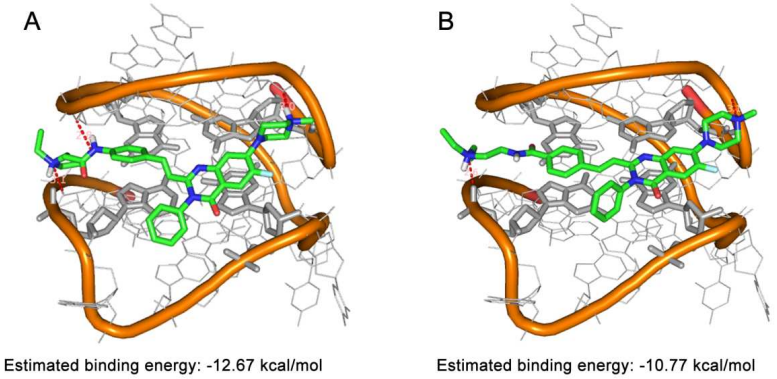
RT-PCR. The quantity of RNA was measured by using UV spectrometry. The cDNA was synthesized with 1 μg of total RNA. RNA was used as a template for reverse transcription according to the manufacture's protocol. For each cDNA sample, a 20 μL reaction, containing 1 × PCR buffer, 500 μM dNTPs, 0.15 μM *β-actin* primers, 0.15 μM *c-kit* primers, 1 U of Taq polymerase, and 2 μL of the cDNA template, was performed. Both *β-actin* and *c-KIT* were amplified by using PCR. The PCR products were resolved in 1.5% agarose gel, and photographs were taken on an AlphaImager.

4. Docking Studies

The previous built *c-KIT*1 G-quadruplex structure (PDB ID: 2O3M) was used as the initial model to study the interaction between compounds and human *c-KIT* G-quadruplex DNA.[1] Ligands were constructed and minimized in SYBYL 7.3.5 (Tripos Inc., St. Louis, MO, USA). Docking studies were carried out using the AUTODOCK 4.0 program.[2] The G-quadruplex structure was used as an input for the AUTOGRID program. The grid box was placed at the center of the G-quadruplex. The dimensions of the active site box were set at 70×70×70 Å. Docking calculations were carried out using the Lamarckian genetic algorithm (LGA). Other parameters were used as default. One hundred of independent docking runs were carried out and the conformers of ligand-quadruplex complexes are shown below.



The 100 docking conformers of the complexes from docking studies. Ligands predominantly stacked on the 3' end of the *c-KIT* G-quadruplex DNA. Therefore, the most probable conformation of each ligand on 3' end was chosen and shown below, considering more negative estimated binding free energy as well as a good stacking arrangement with the G-tetrads.



Top view of compound (A) **7e** and (B) **7h** making π–π stacking interactions with the 3' G-quartet. Hydrogen bonding is shown in red dotted line. Pictures are generated with PyMOL. (DeLano, W. L.)

REFERENCES

(1) Phan, A. T.; Kuryavyi, V.; Burge, S.; Neidle, S.; Patel, D. J. Structure of an unprecedented G-quadruplex scaffold in the human c-kit promoter. *J. Am. Chem. Soc.* **2007**, *129*, 4386-4392.

(2) Haider, S.; Neidle, S. Molecular modeling and simulation of G-quadruplexes and quadruplex-ligand complexes. *Methods Mol. Biol.* **2010**, *608*, 17-37.