Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Development of a highly sensitive fluorescent light-up probe for G-quadruplexes

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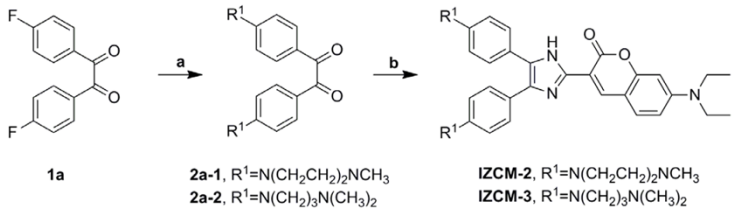
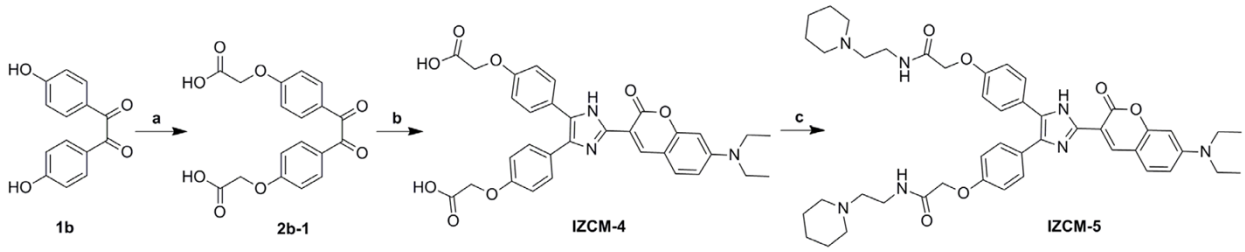
Experimental procedures for the synthesis of triarylimidazole fluorescent probes

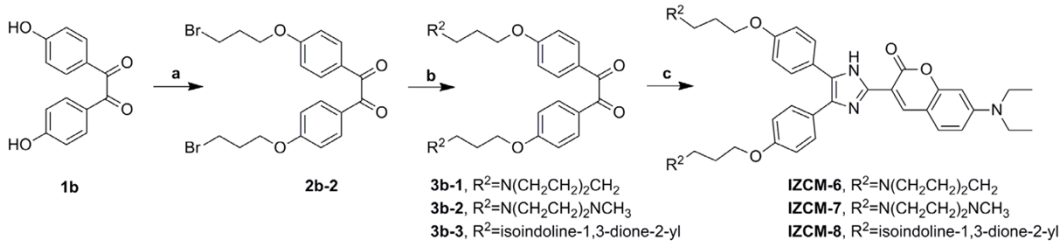
Scheme S1. The synthesis of triarylimidazole fluorescent probes



Reagents and conditions: (a) *N*-methylpiperazine or *N,N,N′*-trimethyl-1,3-propanediamine, K_2CO_3, DMSO, 90 °C, 20 hours; (b) 7-diethylaminocoumarin-3-aldehyde, NH_4OAc, AcOH, reflux, 2 hours.



Reagents and conditions: (a) bromoacetic acid, K_2CO_3, acetonitrile, reflux, 8 hours; (b) 7-diethylaminocoumarin-3-aldehyde, NH_4OAc, AcOH, reflux, 2 hours. (c) NHS, DIC, chloroform, rt, 24 hours; then, Et_3N, *N*-(2-aminoethyl)piperidine, rt, 24 hours.



Reagents and conditions: (a) 1,3-dibromopropane, K_2CO_3, acetonitrile, reflux, 8 hours; (b) piperidine or *N*-methylpiperazine or phthalimide, K_2CO_3, acetonitrile, reflux, 6 hours. (c) 7-diethylaminocoumarin-3-aldehyde, NH_4OAc, AcOH, reflux, 2 hours.

General remarks: ^1H and ^{13}C NMR spectra were recorded by using TMS as the internal standard in methanol-d_4 or $CDCl_3$ at 400 MHz and 100 MHz, respectively, with a Bruker BioSpin GmbH spectrometer. 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) were recorded on a Shimadzu LCMS-IT-TOF. Melting points (m.p.) were determined by using an 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 purity of the synthesized compound was confirmed to be higher than 95% by using analytical HPLC performed with a dual pump Shimadzu LC-20 AB system equipped with a Ultimate XB-C18 column (4.6 × 250 mm, 5 μm) and eluted with methanol-water (80: 20) containing 0.1% TFA at a flow rate of 1.0 mL/min. All

chemicals were purchased from commercial sources unless otherwise specified. All the solvents were of analytical reagent grade and were used without further purification.

1,2-bis(4-(4-methylpiperazin-1-yl)phenyl)ethane-1,2-dione (2a-1): To the solution of 4,4'-difluorobenzil (2.0 mmol, 0.49 g) in DMSO (10 mL) was added *N*-methylpiperazine (10.0 mmol, 1.11 mL) and K_2CO_3 (6.0 mmol, 0.82 g). The reaction mixture was stirred at 90 °C for 20 hours, and was then extracted with EtOAc and water. The organic layer was dried over Na_2SO_4 and concentrated to give the compound **2a-1** (0.65 g, yield 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (d, J = 8.9 Hz, 4H), 6.77 (d, J = 9.0 Hz, 4H), 3.34 (m, 8H), 2.40 – 2.52 (m, 8H), 2.30 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.59, 154.84, 132.14, 123.32, 113.21, 54.58, 46.84, 46.05. ESI-MS m/z: 407.2 $[M+H]^+$.

1,2-bis(4-((3-(dimethylamino)propyl)(methyl)amino)phenyl)ethane-1,2-dione (2a-2): To the solution of 4,4'-difluorobenzil (2.0 mmol, 0.49 g) in DMSO (10 mL) was added *N,N,N'*-trimethyl-1,3-propanediamine (10.0 mmol, 1.45 mL) and K_2CO_3 (6.0 mmol, 0.82 g). The reaction mixture was stirred at 90 °C for 20 hours, and was then extracted with EtOAc and water. The organic layer was dried over Na_2SO_4 and concentrated to give the compound **2a-2** (0.70 g, yield 80%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84 (d, J = 9.0 Hz, 4H), 6.66 (d, J = 9.0 Hz, 4H), 3.47 (t, J = 7.2 Hz, 4H), 3.04 (s, 6H), 2.29 (t, J = 6.9 Hz, 4H), 2.23 (s, 12H), 1.66 – 1.80 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.81, 153.42, 132.36, 121.44, 110.74, 56.67, 50.11, 45.43, 38.38, 25.10. ESI-MS m/z: 439.3 $[M+H]^+$.

2,2'-((oxalylbis(4,1-phenylene))bis(oxy))diacetic acid (2b-1): To a solution of 4,4'-dihydroxybenzil (8.0 mmol, 1.94 g) and anhydrous K_2CO_3 (20.0 mmol, 2.76 g) in 20 mL dry acetonitrile was added bromoacetic acid (40.0 mmol, 5.56 g). The resulting mixture was heated under reflux for 8 hours, and then the remaining solution was filtered. After that, the solvent was removed under reduced pressure and then the mixture was treated with 3.0 mol/L HCl (aq) to reach the pH of 4, and the mixture was filtered to get a pale yellow solid (2.63 g, yield 92%). ^{1}H NMR (400 MHz, DMSO) δ 13.26 (s, 2H), 7.86 (d, J = 8.3 Hz, 4H), 7.12 (d, J = 8.3 Hz, 4H), 4.85 (s, 4H). ^{13}C NMR (100 MHz, DMSO) δ 193.96, 169.95, 163.73, 132.44, 126.22, 115.79, 65.12. ESI-MS m/z: 357.1 $[M-H]^-$.

1,2-bis(4-(3-bromopropoxy)phenyl)ethane-1,2-dione (2b-2): To a solution of 4,4'-dihydroxybenzil (8.0 mmol, 1.94 g) and anhydrous K_2CO_3 (20.0 mmol, 2.76 g) in 20 mL dry acetonitrile was added 1,3-dibromopropane (40.0 mmol, 4.08 mL). The resulting mixture was heated under reflux for 8 hours, and then the remaining solution was filtered. After concentration, the crude product was purified by flash gel chromatography with petroleum ether/EtOAc (2:1) to give a white solid (3.29 g, yield 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, J = 8.5 Hz, 4H), 6.98 (d, J = 8.5 Hz, 4H), 4.20 (t, J = 5.7 Hz, 4H), 3.60 (t, J = 6.3 Hz, 4H), 2.30 – 2.40 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.43, 163.96, 132.43, 126.44, 114.75, 65.67, 31.98, 29.60. ESI-MS m/z: 484.0 $[M+H]^+$.

1,2-bis(4-(3-(piperidin-1-yl)propoxy)phenyl)ethane-1,2-dione (3b-1): To a stirred suspension of **2b-2** (4.0 mmol, 1.94 g) and anhydrous K_2CO_3 (10.0 mmol, 1.38 g) in dry acetonitrile (20 mL) was added excess piperidine (20.0 mmol, 1.98 mL), and the resulting mixture was heated under reflux for 6 h until the starting material disappeared. The K_2CO_3 was removed through filtration, and the remaining solution was concentrated under reduced pressure. The crude product was purified by flash gel chromatography with CH_2Cl_2/MeOH (30:1) as elution solvents to give the desired product **3b-1** (1.38 g, yield 70%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J* = 8.9 Hz, 4H), 6.96 (d, *J* = 8.9 Hz, 4H), 4.09 (t, *J* = 6.4 Hz, 4H), 2.38 – 2.49 (m, 12H), 1.95 – 2.05 (m, 4H), 1.59 (dt, *J* = 11.0, 5.6 Hz, 8H), 1.53 – 1.34 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.55, 164.41, 132.32, 126.13, 114.74, 66.93, 55.66, 54.64, 26.58, 25.94, 24.39. ESI-MS m/z: 493.3 $[M+H]^+$.

1,2-bis(4-(3-(4-methylpiperazin-1-yl)propoxy)phenyl)ethane-1,2-dione (3b-2): To a stirred suspension of **2b-2** (4.0 mmol, 1.94 g) and anhydrous K_2CO_3 (10.0 mmol, 1.38 g) in dry acetonitrile (20 mL) was added excess *N*-methylpiperazine (20.0 mmol, 2.22 mL), and the resulting mixture was heated under reflux for 6 h until the starting material disappeared. The K_2CO_3 was removed through filtration, and the remaining solution was concentrated under reduced pressure. The crude product was purified by using gel chromatography with CH_2Cl_2/MeOH (30:1) as elution solvents to give the desired product **3b-2** (1.25 g, yield 60%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J* = 8.7 Hz, 4H), 6.96 (d, *J* = 8.7 Hz, 4H), 4.10 (t, *J* = 6.3 Hz, 4H), 2.72 – 2.32 (m, 20H), 2.32 (s, 6H), 2.07 – 1.92 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.55, 164.35, 132.35, 126.11, 114.73, 66.66, 55.08, 54.82, 53.16, 46.02, 26.50. ESI-MS m/z: 523.3 $[M+H]^+$.

1,2-bis(4-(3-(isoindoline-1,3-dione-2-yl)propoxy)phenyl)ethane-1,2-dione (3b-3): To a solution of **2b-2** (8.0 mmol, 1.94 g) and anhydrous K_2CO_3 (20.0 mmol, 2.76 g) in 20 mL dry acetonitrile was added phthalimide (20.0 mmol, 2.94 g). The resulting mixture was heated under reflux for 6 hours, and then the remaining solution was filtered. After concentration, the crude product was purified by using flash gel chromatography with CH_2Cl_2 to give the desired product **3b-3** (4.19 g, yield 85%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.87 (d, *J* = 8.9 Hz, 4H), 7.84 (dd, *J* = 5.5, 3.0 Hz, 4H), 7.72 (dd, *J* = 5.5, 3.0 Hz, 4H), 6.84 (d, *J* = 9.0 Hz, 4H), 4.11 (t, *J* = 6.0 Hz, 4H), 3.92 (t, *J* = 6.7 Hz, 4H), 2.18 – 2.25 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 193.45, 168.34, 163.94, 134.04, 132.32, 132.10, 126.36, 123.30, 114.62, 66.12, 35.30, 28.10. ESI-MS m/z: 617.2 $[M+H]^+$.

3-(4,5-bis(4-(4-methylpiperazin-1-yl)phenyl)-1*H*-imidazol-2-yl)-7-(diethylamino)-2*H*-chromen-2-one (IZCM-2): A mixture of 1,2-bis(4-(4-methylpiperazin-1-yl)phenyl)ethane-1,2-dione (**2a-1**, 1.0 mmol, 0.40 g), 7-diethylaminocoumarin-3-aldehyde (1.5 mmol, 0.36 g), NH_4OAc (20.0 mmol, 1.54 g) and AcOH (8 mL) was stirred at reflux temperature for 2 hours. After cooling, the mixture was treated with 3.0 mol/L NaOH (aq) to reach the pH

of 8, and the product was exacted by CH_2Cl_2 (20 mL×5). The combined organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by using flash column chromatography with CH_2Cl_2/MeOH (30:1) elution to afford a dark red solid **IZCM-2** (0.41 g, yield 65%). m.p. 184-185 °C. ^{1}H NMR (400 MHz, CD_3OD) δ 8.21 (s, 1H), 7.24 (d, J = 8.9 Hz, 1H), 7.13 (d, J = 8.6 Hz, 4H), 6.70 (d, J = 8.7 Hz, 4H), 6.53 (d, J = 9.0 Hz, 1H), 6.31 (s, 1H), 3.26 (q, J = 7.0 Hz, 4H), 3.04 (t, J = 4.2 Hz, 8H), 2.46 (t, J = 4.5 Hz, 8H), 2.22 (s, 6H), 1.04 (t, J = 7.0 Hz, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 162.97, 157.29, 152.73, 151.46, 142.41, 140.39, 131.20, 129.80, 116.81, 111.25, 109.99, 109.22, 97.66, 55.94, 49.41, 46.10, 45.84, 12.95. Purity: 98% by HPLC. HRMS (ESI) m/z: calcd for $C_{38}H_{45}N_7O_2$: 632.3713 $[M+H]^+$. Found 632.3720 $[M+H]^+$.

3-(4,5-bis(4-((3-(dimethylamino)propyl)(methyl)amino)phenyl)-1*H*-imidazol-2-yl)-7-(diethylamino)-2*H*-chromen-2-one (IZCM-3): A mixture of 1,2-bis(4-((3-(dimethylamino)propyl)(methyl)amino)phenyl)ethane- 1,2-dione (**2a-2**, 1.0 mmol, 0.44 g), 7-diethylaminocoumarin-3-aldehyde (1.5 mmol, 0.36 g), NH_4OAc (20.0 mmol, 1.54 g) and AcOH (8 mL) was stirred at reflux temperature for 2 hours. After cooling, the mixture was treated with 3.0 mol/L NaOH (aq) to reach the pH of 8, and the product was exacted by CH_2Cl_2 (20 mL×5). The combined organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude product was purified by using flash column chromatography with CH_2Cl_2/MeOH (20:1) elution to afford a dark red solid **IZCM-3** (0.40 g, yield 60%). m.p. 128-130 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.71 (s, 1H), 7.70 – 7.31 (m, 5H), 6.66 – 6.75 (m, 4H), 6.67 (d, *J* = 8.9 Hz, 1H), 6.57 (s, 1H), 3.46 (q, *J* = 7.2 Hz, 4H), 3.40 – 3.42 (m, 4H), 2.97 (s, 6H), 2.33 (t, *J* = 7.2 Hz, 4H), 2.26 (s, 12H), 1.78 (dt, *J* = 14.1, 7.1 Hz, 4H), 1.25 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.10, 155.82, 150.72, 148.32, 140.62, 138.17, 129.59, 128.59, 112.07, 109.73, 109.24, 109.05, 96.97, 57.14, 50.58, 45.44, 44.91, 38.23, 24.92, 12.49. Purity: 98% by HPLC. HRMS (ESI) m/z: calcd 332.7203 for $C_{40}H_{53}N_7O_2$: $[M+2H]^{2+}$, found 332.7200 $[M+2H]^{2+}$.

2,2'-(((2-(7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)-1*H*-imidazole-4,5-diyl)bis(4,1-phenylene))bis(oxy))diacetic acid (IZCM-4): A mixture of 2,2'-((oxalylbis(4,1-phenylene))bis(oxy))diacetic acid (**2b-1**, 1.0 mmol, 0.36 g), 7-diethylaminocoumarin-3-aldehyde (1.5 mmol, 0.36 g), NH_4OAc (20.0 mmol, 1.54 g) and AcOH (8 mL) was stirred at reflux temperature for 2 hours. After cooling, the mixture was treated with 3.0 mol/L NaOH (aq) to reach the pH of 6, and the product was exacted by CH_2Cl_2 (20 mL×5). The combined organic phase was dried over Na_2SO_4 and solvent was removed by rotary evaporation. The crude product was purified by using flash column chromatography with CH_2Cl_2/MeOH/HCOOH (100:5:1) elution to afford an orange solid **IZCM-4** (0.23 g, yield 40%). m.p. 257-258 °C. ^{1}H NMR (400 MHz, DMSO) δ 12.96 (s, 1H), 11.73 (s, 1H), 8.55 (s, 1H), 8.14 (s, 1H), 7.66 (d, *J* = 9.0 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 4H), 6.91 (d, *J* = 7.6 Hz, 4H), 6.78 (d, *J* = 9.0 Hz,

1H), 6.63 (s, 1H), 4.69 (s, 4H), 3.47 (t, *J* = 6.4 Hz, 4H), 1.15 (t, *J* = 6.9 Hz, 6H). ^{13}C NMR (100 MHz, DMSO) δ 170.11, 160.17, 156.90, 155.77, 150.87, 140.80, 139.60, 130.11, 128.83, 114.38, 109.71, 108.77, 108.06, 96.27, 64.52, 44.15, 12.29. Purity: 97% by HPLC. HRMS (ESI) m/z: calcd 582.1882 for $C_{32}H_{29}N_3O_8$: $[M-H]^-$, found 582.1861 $[M-H]^-$.

2,2'-(((2-(7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)-1*H*-imidazole-4,5-diyl)bis(4,1-phenylene))bis(oxy))bis(*N*-(2-(piperidin-1-yl)ethyl)acetamide) (IZCM-5): A mixture of **IZCM-4** (0.5 mmol, 0.29 g), NHS (0.6 mmol, 0.07 g), DIC (0.6 mmol, 0.09 mL) and chloroform (5 mL) was stirred at room temperature for 24 hours. After that, Et_3N (0.8 mmol, 0.11 mL) and *N*-(2-aminoethyl)piperidine (0.8 mmol, 0.10 g) were added into the reaction mixture and then stirred at room temperature for another 24 hours. The solvent was removed by rotary evaporation and the crude product was purified by using flash column chromatography with CH_2Cl_2/MeOH (20:1) elution to afford an orange solid (0.20 g, yield 50%). m.p. 158-160 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.07 (s, 1H), 8.70 (s, 1H), 7.60 (d, *J* = 7.7 Hz, 2H), 7.48 – 7.38 (m, 3H), 7.29 (s, 2H), 6.88 – 6.96 (m, 4H), 6.67 (d, *J* = 7.6 Hz, 1H), 6.56 (s, 1H), 4.52 (s, 4H), 3.40 – 3.48 (m, 8H), 2.47 (t, *J* = 5.9 Hz, 4H), 2.35 – 2.42 (m, 8H), 1.48 – 1.52 (m, 8H), 1.44 – 1.48 (m, 4H), 1.24 (t, *J* = 6.7 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 167.85, 162.13, 156.45, 156.10, 151.12, 141.74, 139.11, 129.81, 129.22, 114.62, 109.90, 108.91, 108.70, 97.03, 67.41, 56.88, 54.23, 44.99, 35.60, 26.02, 24.29, 12.48. Purity: 98% by HPLC. HRMS (ESI) m/z: calcd 402.7258 for $C_{46}H_{57}N_7O_6$: $[M+2H]^{2+}$, found 402.7248 $[M+2H]^{2+}$.

3-(4,5-bis(4-(3-(piperidin-1-yl)propoxy)phenyl)-1*H*-imidazol-2-yl)-7-(diethylamino)-2*H*-chromen-2-one (IZCM-6): A mixture of 1,2-bis(4-(3-(piperidin-1-yl)propoxy)phenyl)ethane-1,2-dione (**3b-1**, 1.0 mmol, 0.49 g), 7-diethylaminocoumarin-3-aldehyde (1.5 mmol, 0.36 g), NH_4OAc (20.0 mmol, 1.54 g) and AcOH (8 mL) was stirred at reflux temperature for 2 hours. After cooling, the mixture was treated with 3.0 mol/L NaOH (aq) to reach the pH of 8, and the product was exacted by CH_2Cl_2 (20 mL×5). The combined organic phase was dried over Na_2SO_4 and solvent was removed by rotary evaporation. The crude product was purified by using flash column chromatography with CH_2Cl_2/MeOH (30:1) elution to afford an orange solid **IZCM-6** (0.33 g, yield 46%). m.p. 145-147 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.03 (s, 1H), 8.69 (s, 1H), 7.71 – 7.28 (m, 5H), 7.04 – 6.75 (m, 4H), 6.64 (d, *J* = 7.9 Hz, 1H), 6.54 (s, 1H), 4.02 (s, 4H), 3.45 (q, *J* = 6.7 Hz, 4H), 2.61 – 2.47 (m, 4H), 2.46 – 2.22 (m, 8H), 2.11 – 1.91 (m, 4H), 1.81 – 1.49 (m, 8H), 1.57 – 1.32 (m, 4H), 1.23 (t, *J* = 6.7 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 162.07, 158.54, 155.99, 150.95, 141.25, 138.73, 129.68, 128.93, 114.80, 109.80, 109.04, 109.00, 97.05, 66.52, 56.00, 54.62, 44.92, 26.81, 25.92, 24.40, 12.47. Purity: 99% by HPLC. HRMS (ESI) m/z: calcd 359.7200 for $C_{44}H_{55}N_5O_4$: $[M+2H]^{2+}$, found 359.7193 $[M+2H]^{2+}$.

3-(4,5-bis(4-(3-(4-methylpiperazin-1-yl)propoxy)phenyl)-1*H*-imidazol-2-yl)-7-(diethylamino)-2*H*-chromen-2-one (IZCM-7): A mixture of 1,2-bis(4-(3-(4-methylpiperazin-1-yl)propoxy)phenyl)ethane-1,2-dione (**3b-2**, 1.0 mmol, 0.52 g), 7-diethylaminocoumarin-3-aldehyde (1.5 mmol, 0.36 g), NH_4OAc (20.0 mmol, 1.54 g) and AcOH (8 mL) was stirred at reflux temperature for 2 hours. After cooling, the mixture was treated with 3.0 mol/L NaOH (aq) to reach the pH of 8, and the product was exacted by CH_2Cl_2 (20 mL×5). The combine organic phase was dried over Na_2SO_4 and solvent was removed by rotary evaporation. The crude product was purified by using flash column chromatography with CH_2Cl_2/MeOH (30:1) elution to afford an orange solid **IZCM-7** (0.35 g, yield 47%). m.p. 146-149 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.72 (s, 1H), 7.40 – 7.48 (m, 5H), 6.86 (d, *J* = 8.7 Hz, 4H), 6.66 (d, *J* = 8.9 Hz, 1H), 6.55 (s, 1H), 4.04 (t, *J* = 6.0 Hz, 4H), 3.45 (q, *J* = 6.9 Hz, 4H), 2.85 – 2.92 (m, 16H), 2.68 – 2.75 (m, 4H), 2.58 (s, 6H), 2.05 (dt, *J* = 16.9, 8.3 Hz, 4H), 1.24 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (100 MHz, DMSO) δ 160.26, 157.62, 155.73, 150.82, 140.77, 139.44, 130.10, 128.85, 114.32, 109.72, 108.87, 108.07, 96.25, 65.52, 53.66, 52.80, 50.38, 44.16, 43.40, 25.68, 12.31. Purity: 98% by HPLC. HRMS (ESI) m/z: calcd for $C_{44}H_{57}N_7O_4$: 374.7309 $[M+2H]^{2+}$, found 374.7298 $[M+2H]^{2+}$.

2,2'-((((2-(7-(diethylamino)-2-oxo-2*H*-chromen-3-yl)-1*H*-imidazole-4,5-diyl)bis(4,1-phenylene))bis(oxy))bis(propane-3,1-diyl))bis(isoindoline-1,3-dione) (IZCM-8): A mixture of 1,2-bis(4-(3-(isoindoline-1,3-dione-2- yl)propoxy)phenyl)ethane-1,2-dione (**3b-3**, 1.0 mmol, 0.62 g), 7-diethylaminocoumarin-3-aldehyde (1.5 mmol, 0.36 g), NH_4OAc (20.0 mmol, 1.54 g) and AcOH (8 mL) was stirred at reflux temperature for 2 hours. After cooling, the mixture was treated with 3.0 mol/L NaOH (aq) to reach the pH of 8, and the product was exacted by CH_2Cl_2 (20 mL×5). The combine organic phase was dried over Na_2SO_4 and solvent was removed by rotary evaporation. The crude product was purified by using flash column chromatography with petroleum ether/EtOAc (2:1) elution to afford a yellow solid **IZCM-8** (0.50 g, yield 60%). m.p. 160-162 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (s, 1H), 7.85 – 7.69 (m, 4H), 7.70 – 7.51 (m, 4H), 7.46 – 7.17 (m, 5H), 6.70 (d, *J* = 8.1 Hz, 4H), 6.57 (d, *J* = 8.6 Hz, 1H), 6.46 (s, 1H), 3.98 (t, *J* = 5.1 Hz, 4H), 3.84 (t, *J* = 6.4 Hz, 4H), 3.36 (q, *J* = 7.0 Hz, 4H), 2.21 – 2.02 (m, 4H), 1.15 (t, *J* = 7.0 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 168.35, 162.03, 158.08, 156.04, 151.09, 141.22, 139.18, 133.92, 132.17, 129.86, 128.94, 123.23, 114.56, 109.85, 108.98, 108.53, 97.02, 65.73, 44.95, 35.53, 28.31, 12.47. Purity: 97% by HPLC. HRMS (ESI) m/z: calcd 842.3184 for $C_{50}H_{43}N_5O_8$: $[M+H]^+$, found 842.3168 $[M+H]^+$.

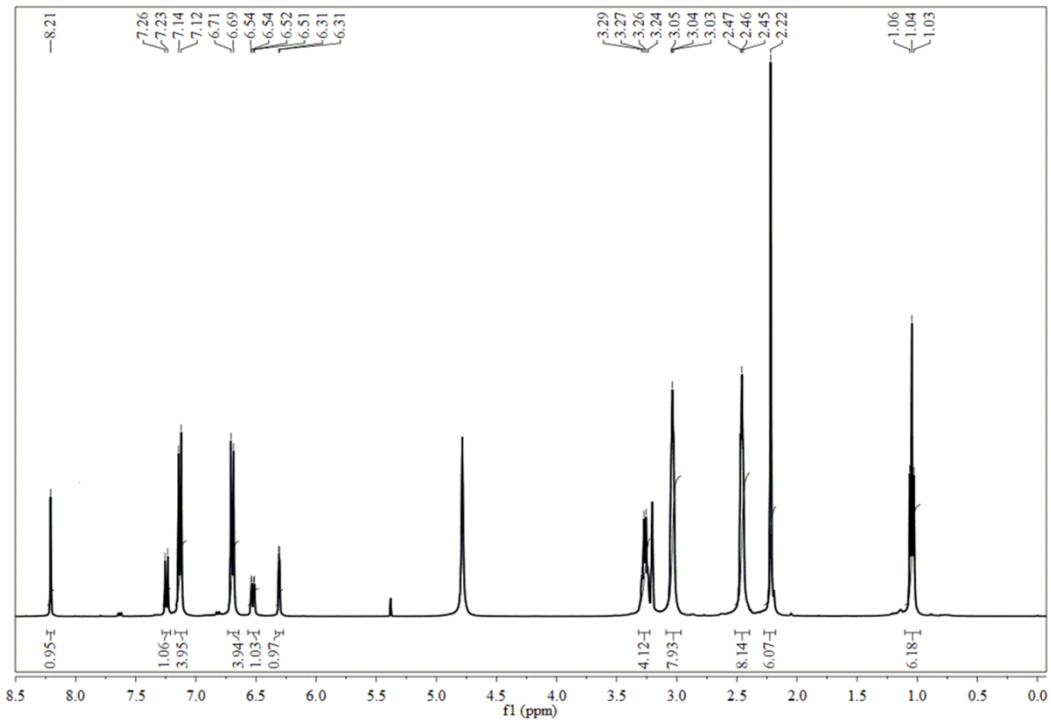


Figure S1. ^{1}H NMR spectrum of **IZCM-2**

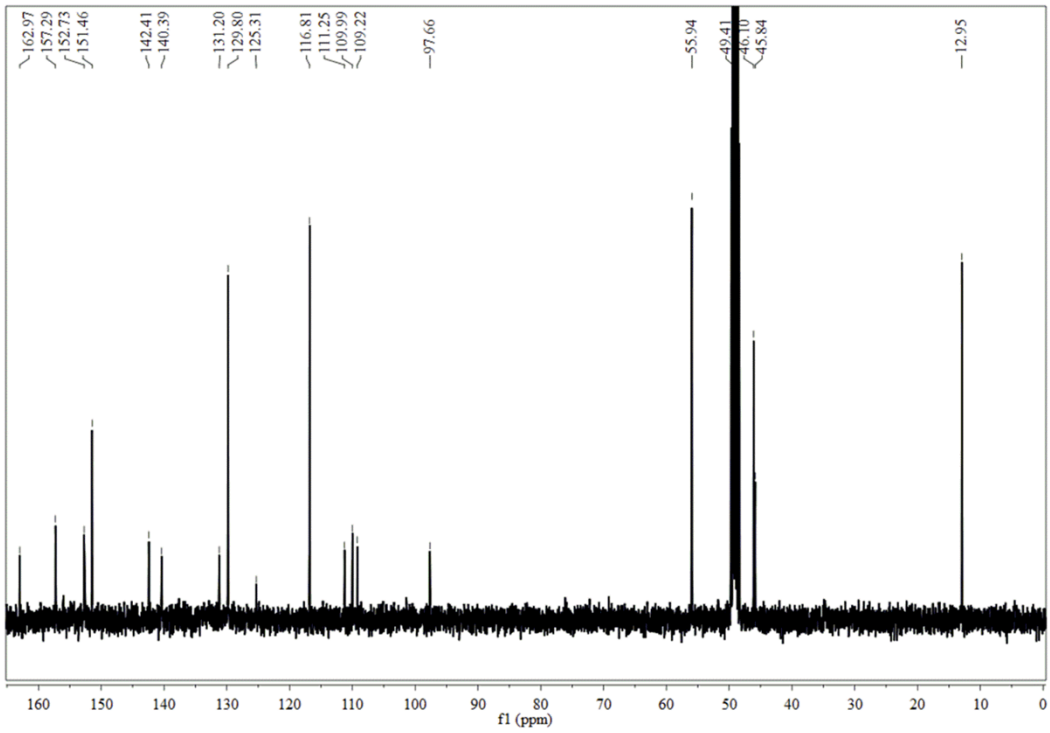
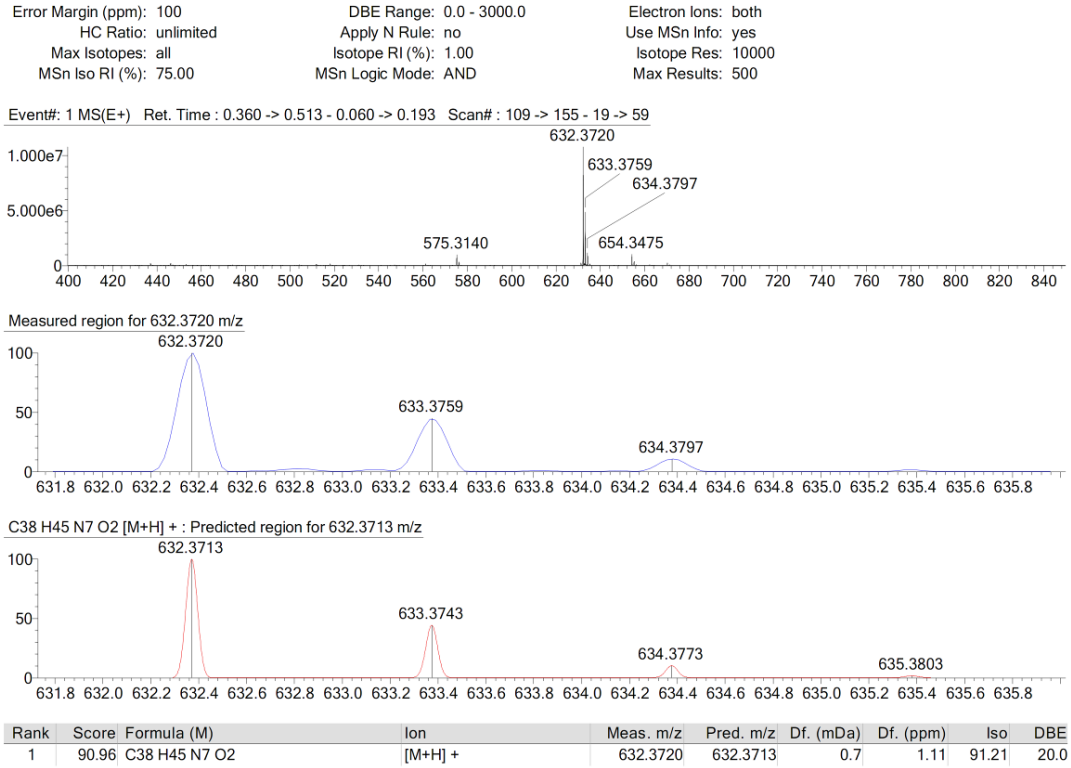


Figure S2. ^{13}C NMR spectrum of **IZCM-2**



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	90.96	C38 H45 N7 O2	[M+H] +	632.3720	632.3713	0.7	1.11	91.21	20.0

Figure S3. HRMS spectrum of **IZCM-2**

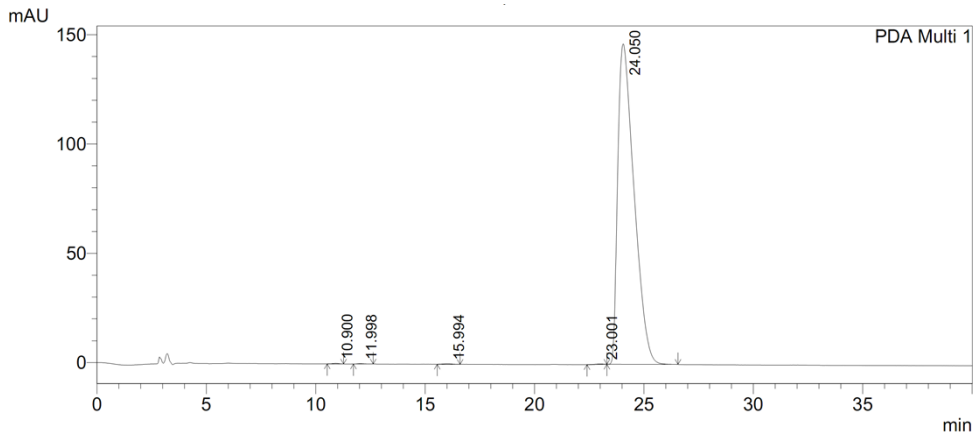


Figure S4. HPLC analysis of **IZCM-2**

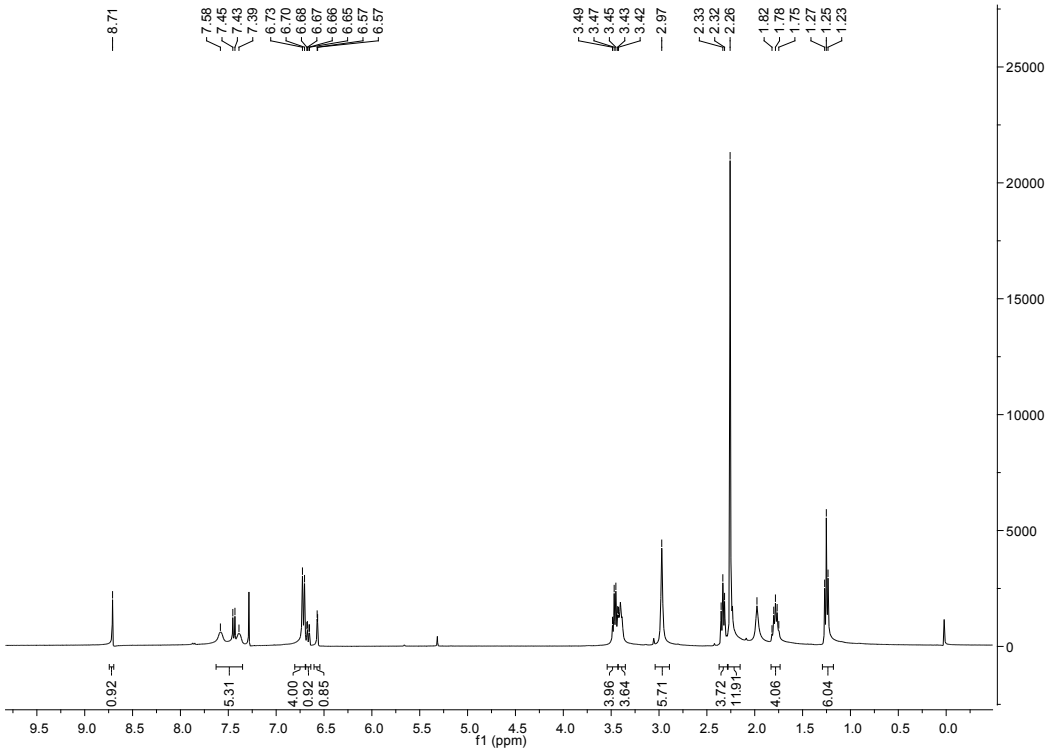


Figure S5. ^{1}H NMR spectrum of **IZCM-3**

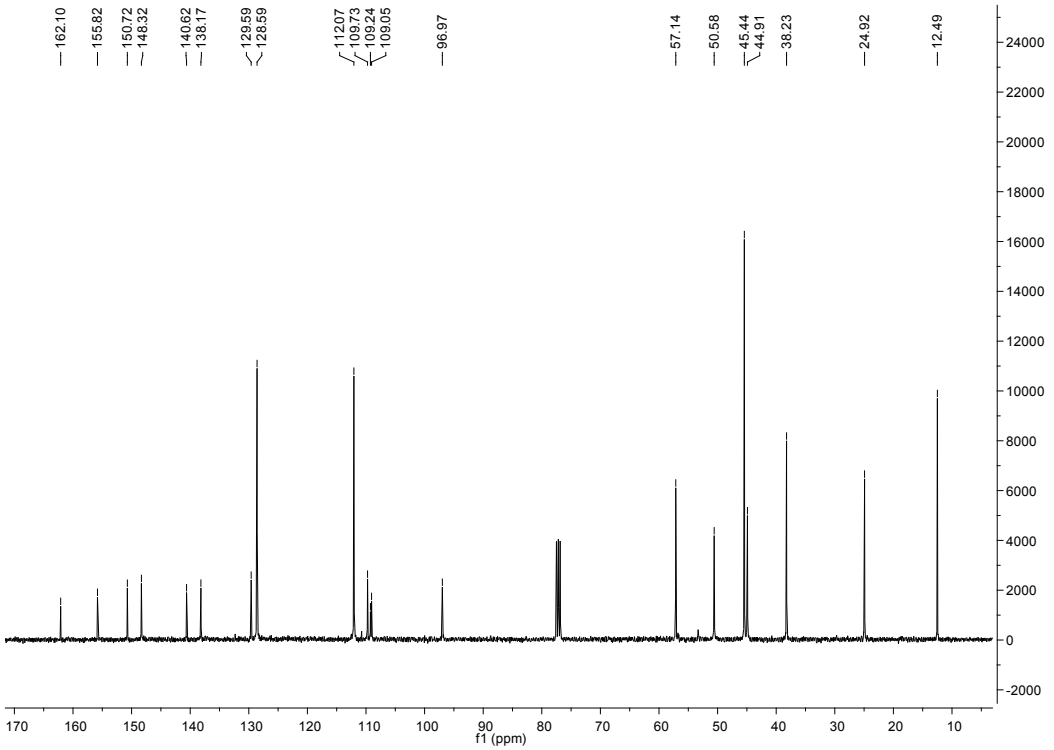


Figure S6. ^{13}C NMR spectrum of **IZCM-3**

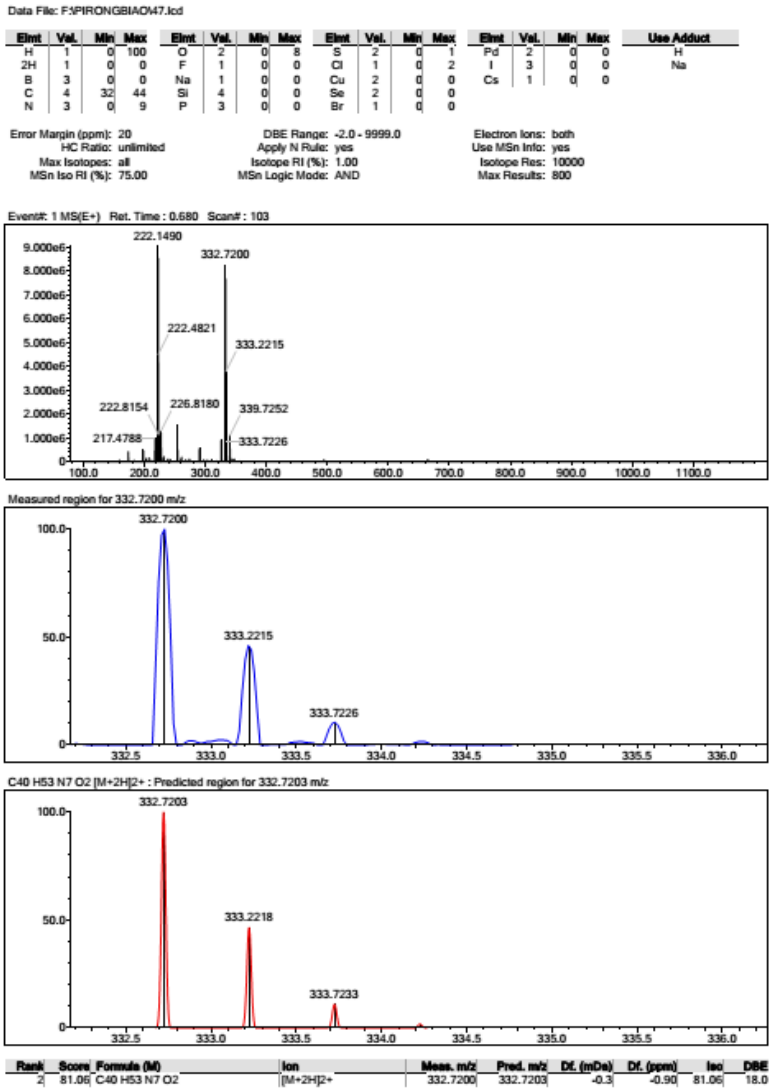


Figure S7. HRMS spectrum of **IZCM-3**

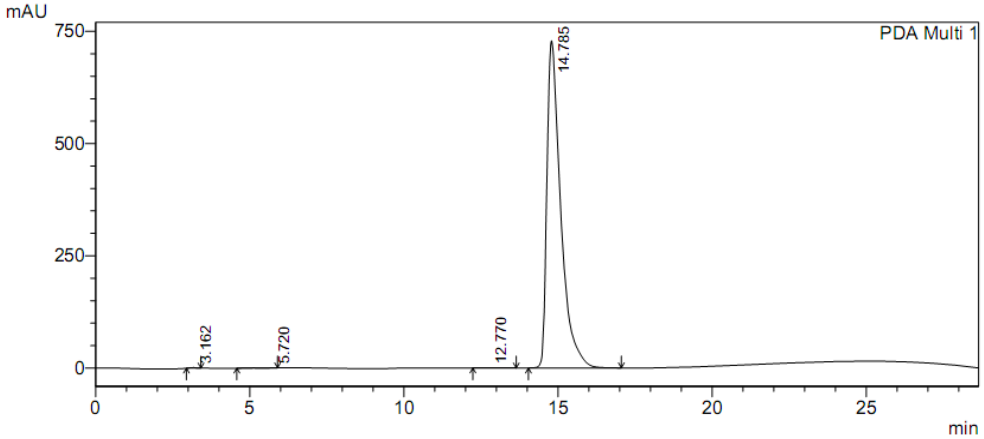


Figure S8. HPLC analysis of **IZCM-3**

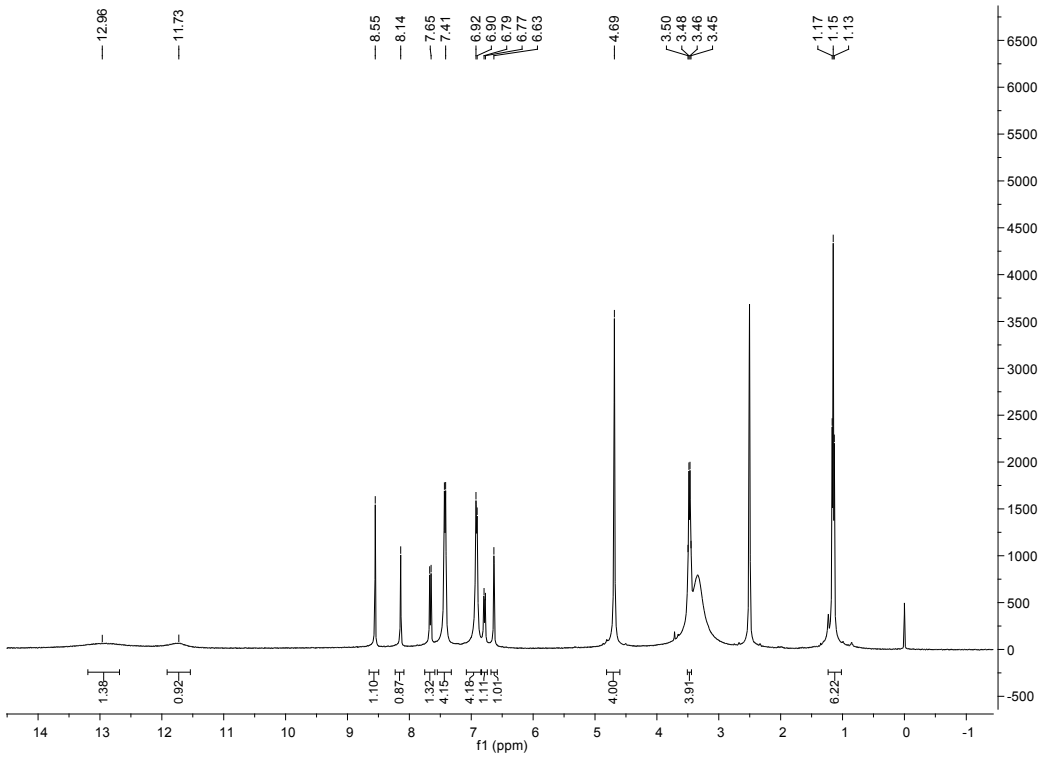


Figure S9. ^{1}H NMR spectrum of **IZCM-4**

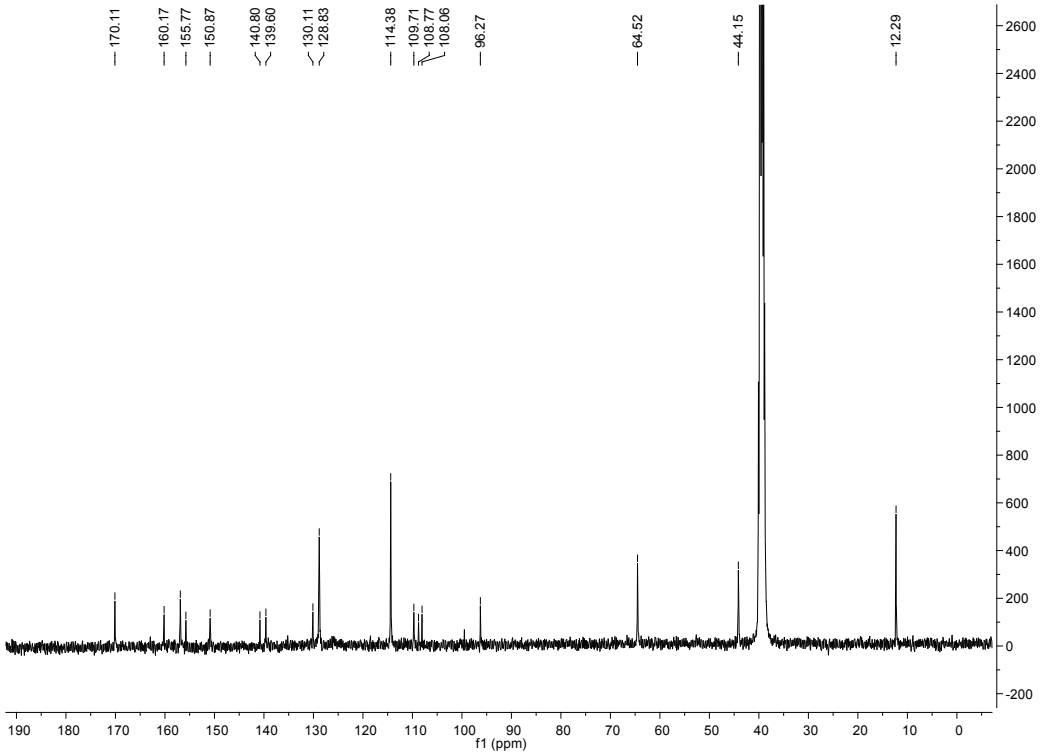


Figure S10. ^{13}C NMR spectrum of **IZCM-4**

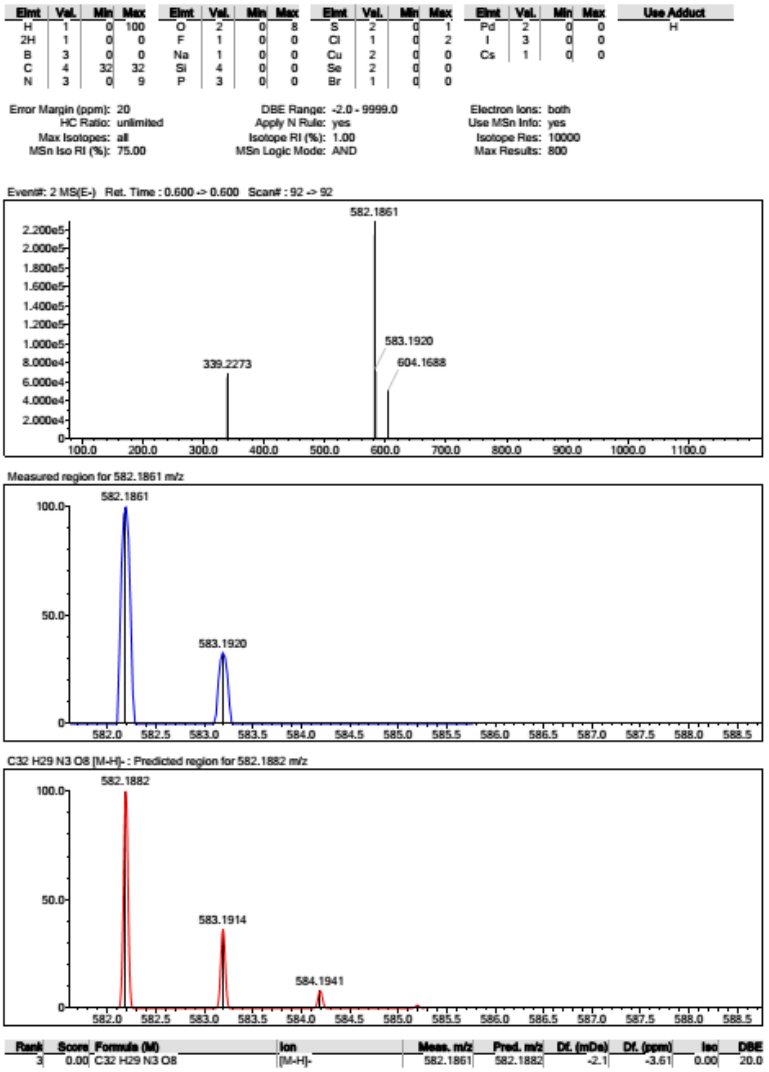


Figure S11. HRMS spectrum of **IZCM-4**

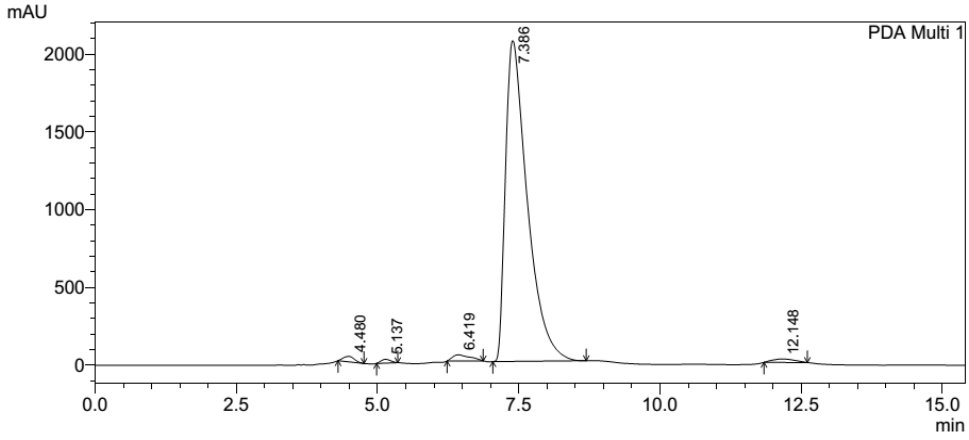


Figure S12. HPLC analysis of **IZCM-4**

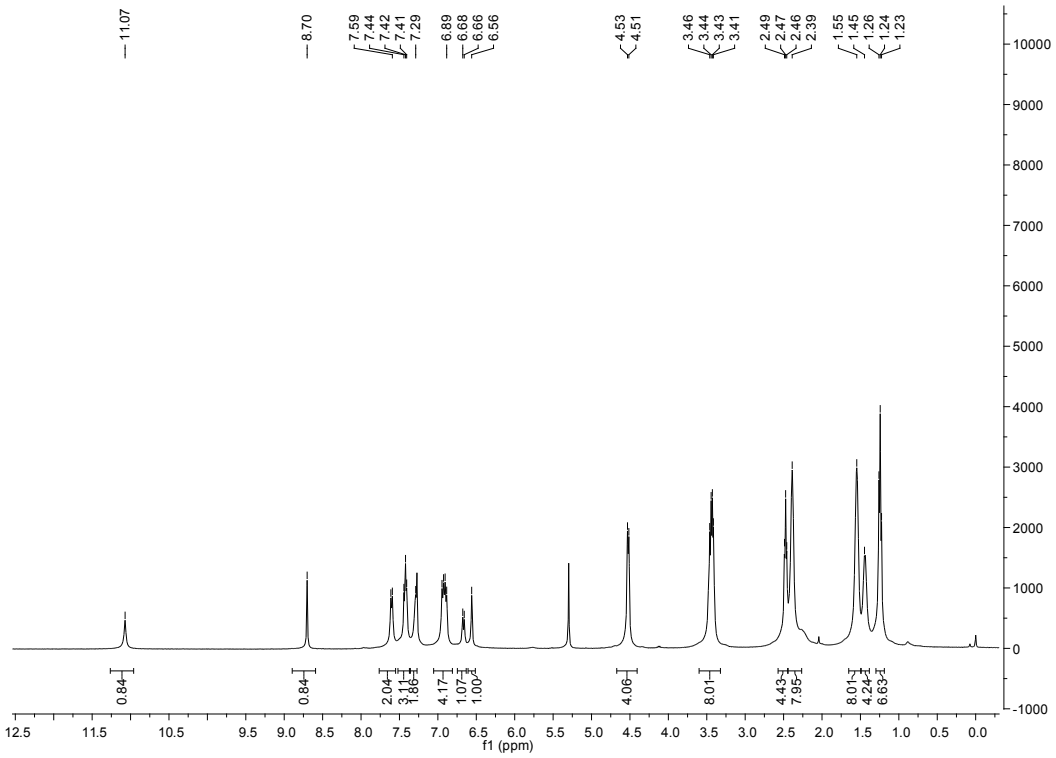


Figure S13. ^{1}H NMR spectrum of **IZCM-5**

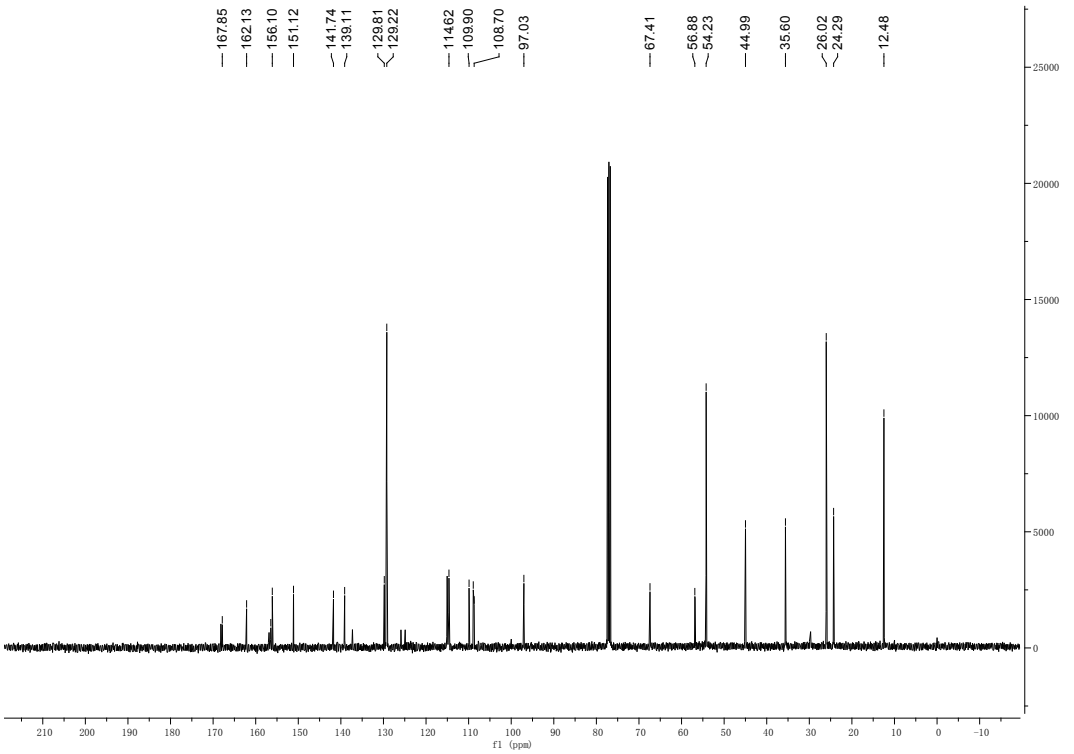


Figure S14. ^{13}C NMR spectrum of **IZCM-5**

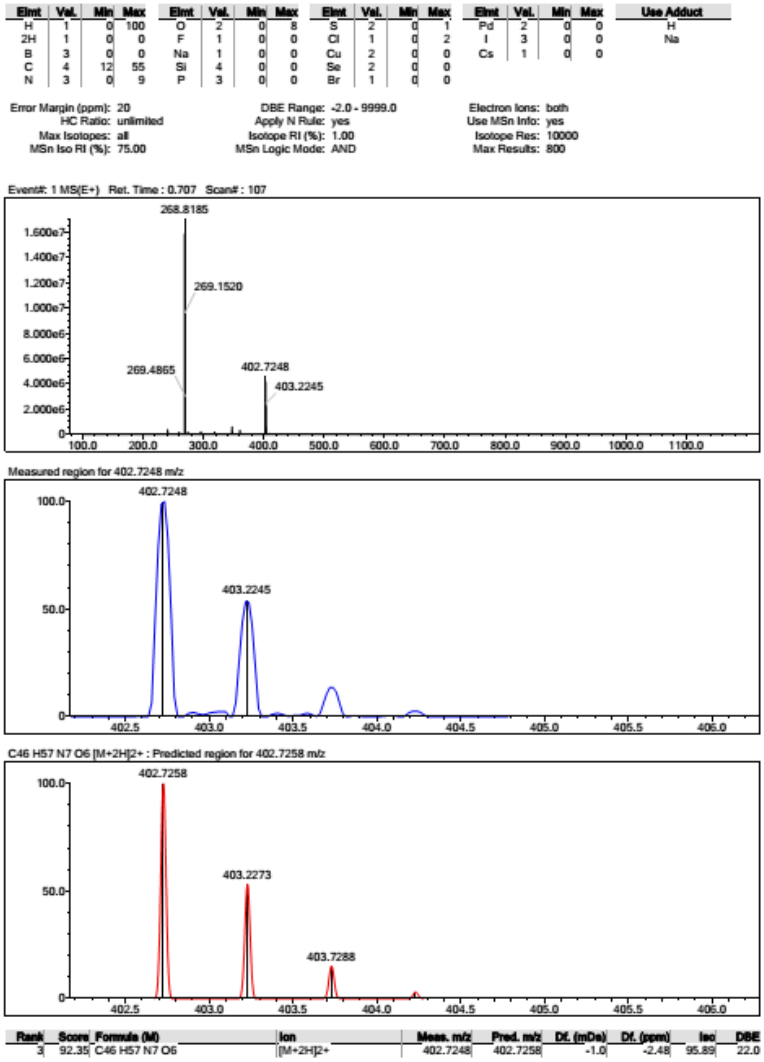


Figure S15. HRMS spectrum of **IZCM-5**

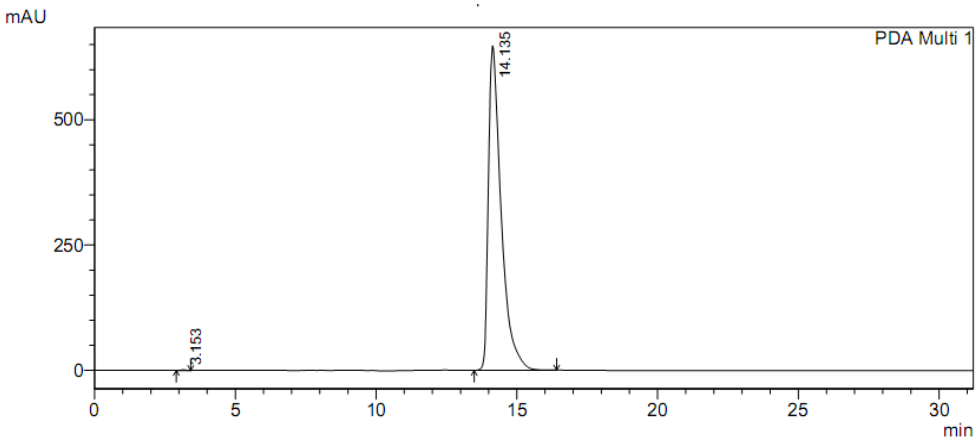


Figure S16. HPLC analysis of **IZCM-5**

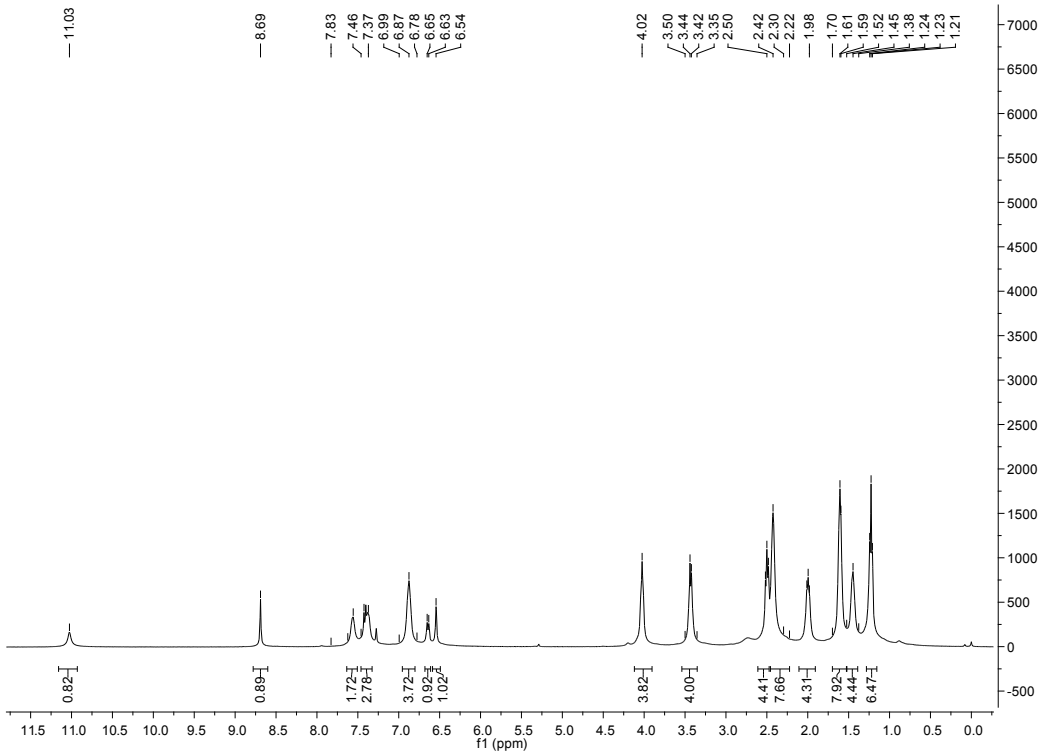


Figure S17. ^{1}H NMR spectrum of **IZCM-6**

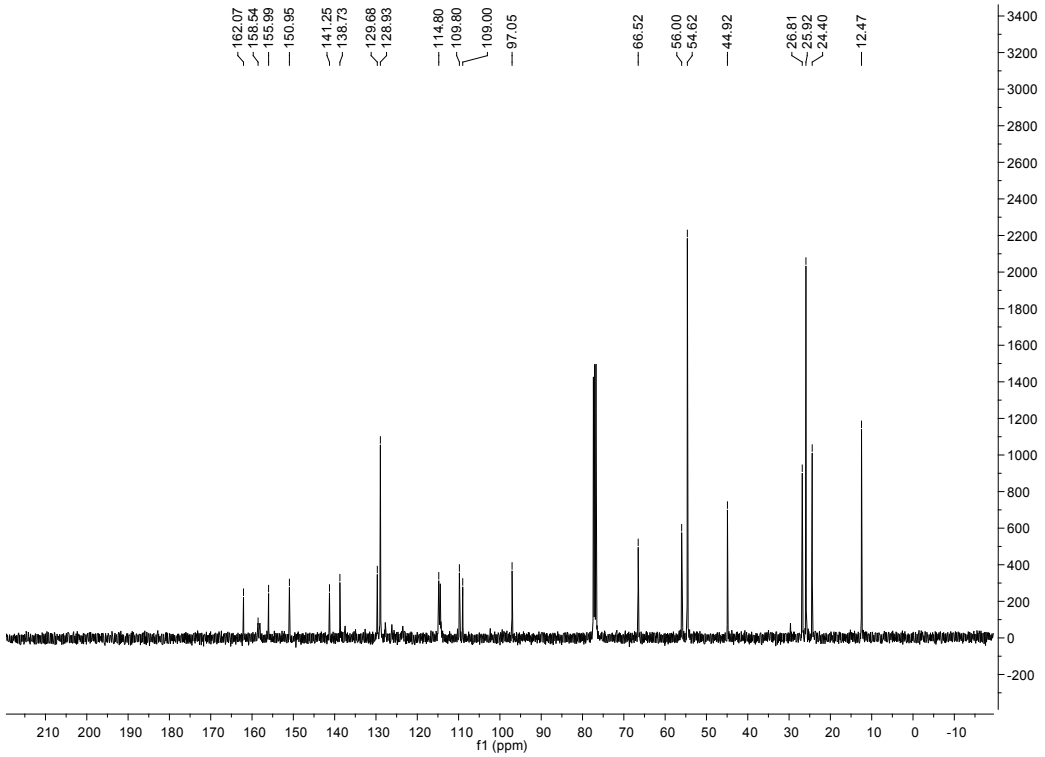


Figure S18. ^{13}C NMR spectrum of **IZCM-6**

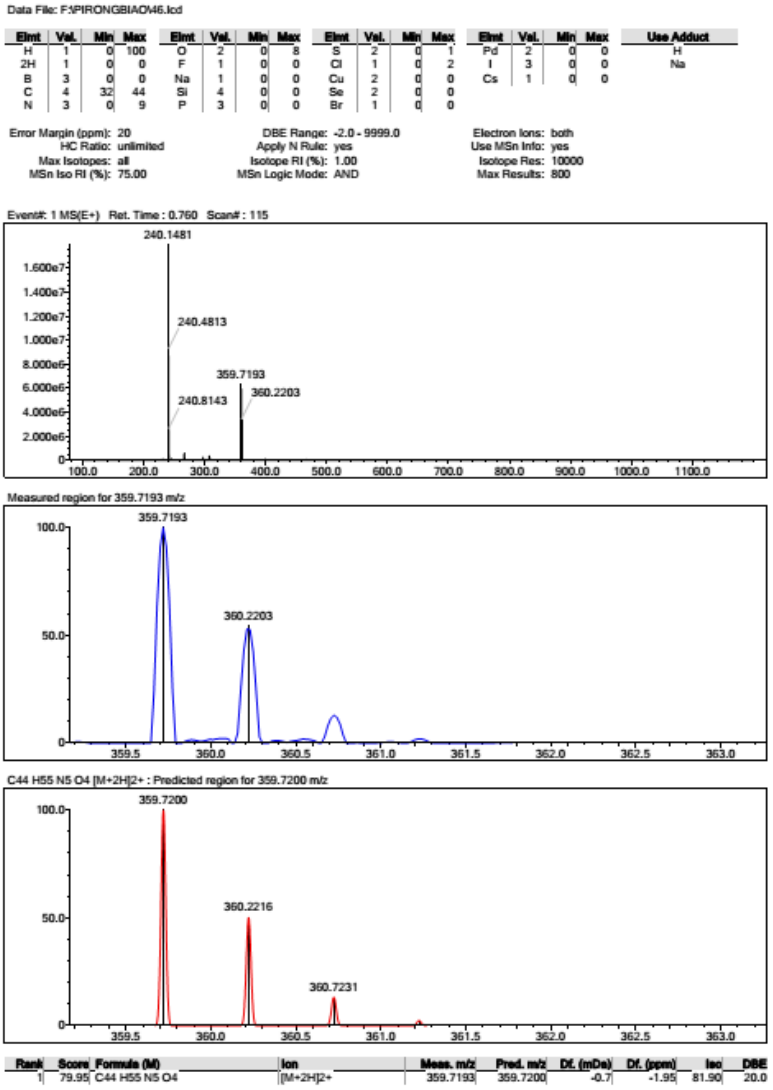


Figure S19. HRMS spectrum of **IZCM-6**

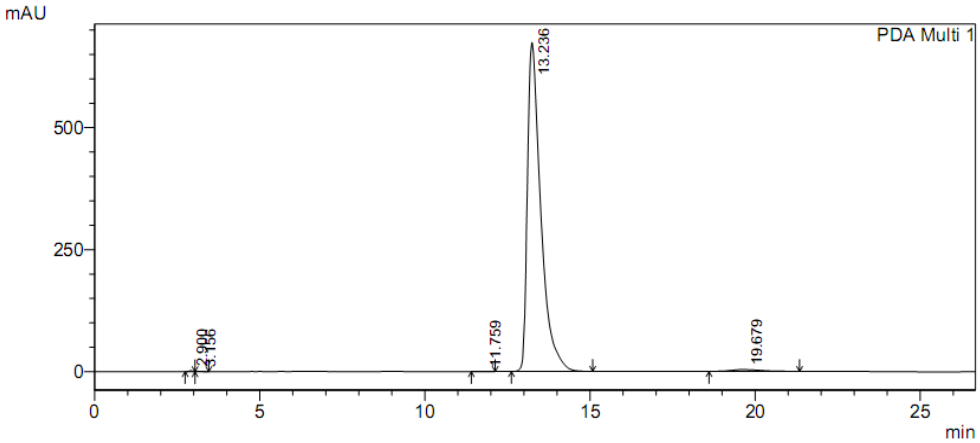


Figure S20. HPLC analysis of **IZCM-6**

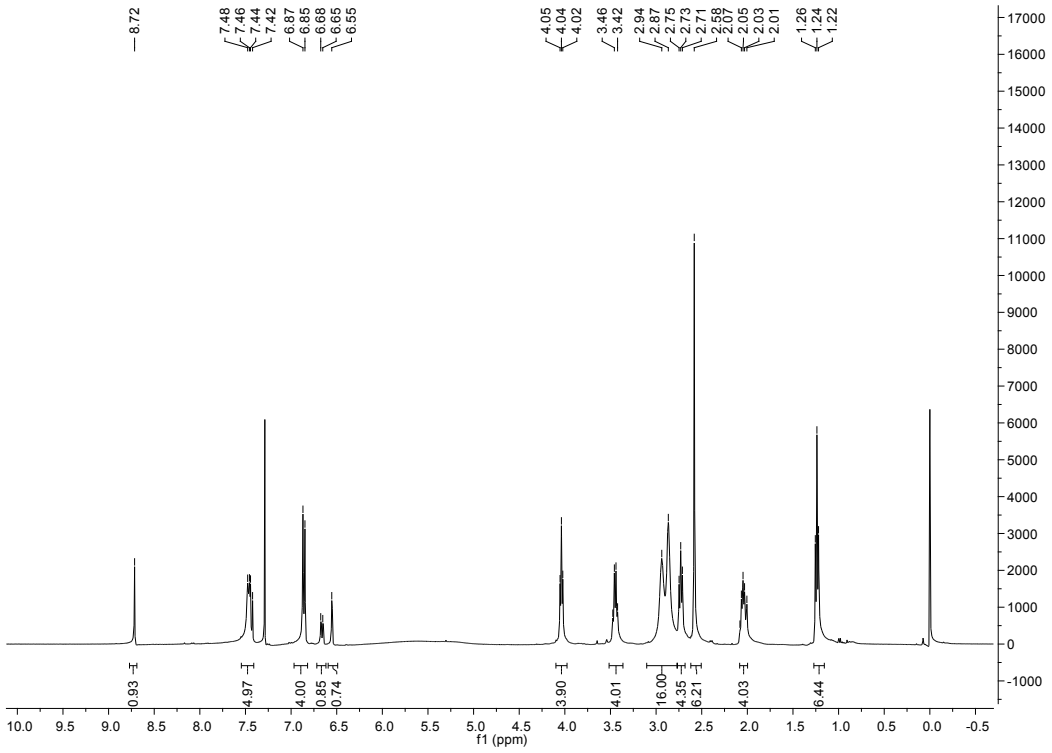


Figure S21. ^{1}H NMR spectrum of **IZCM-7**

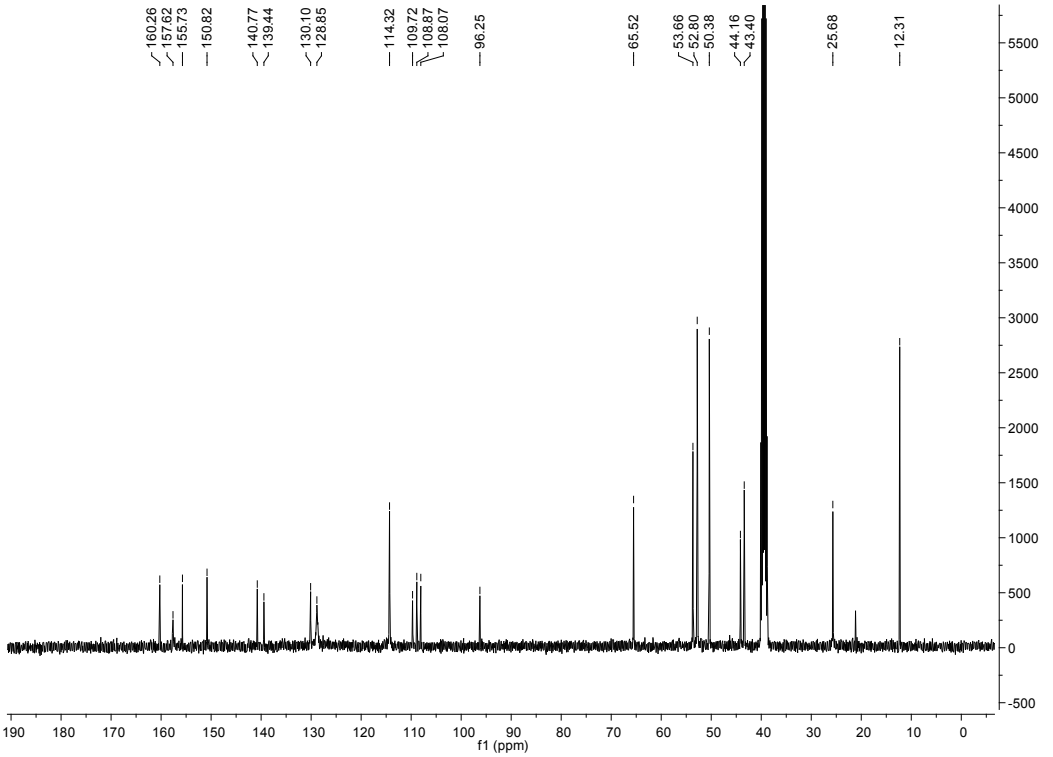


Figure S22. ^{13}C NMR spectrum of **IZCM-7**

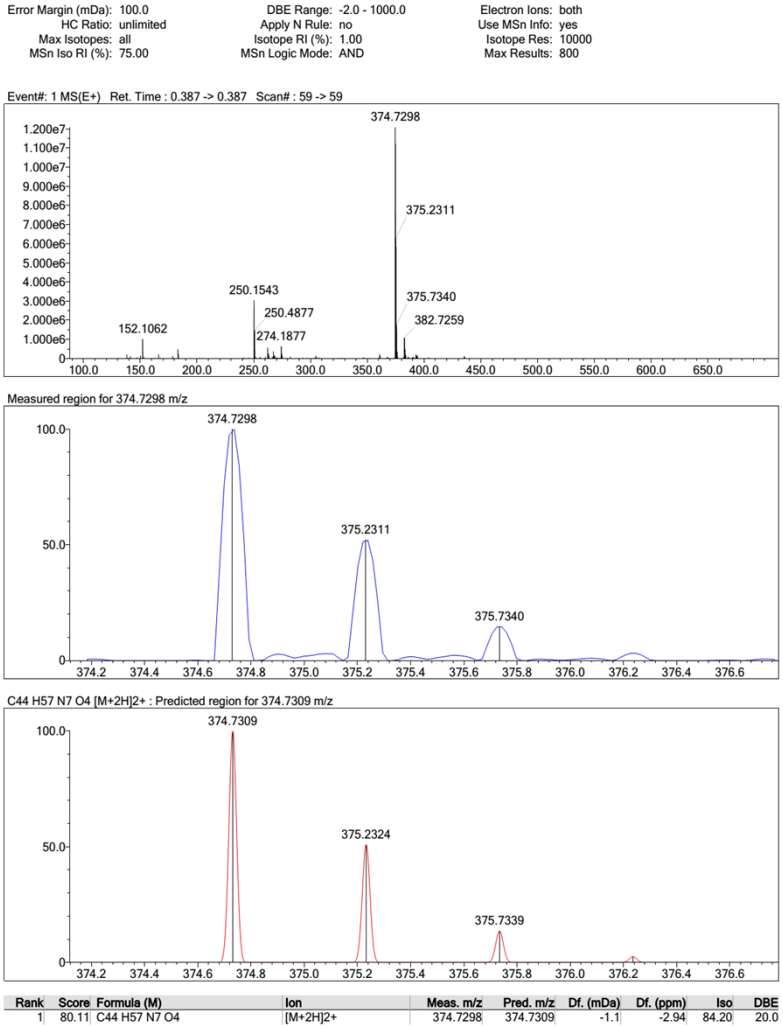


Figure S23. HRMS spectrum of **IZCM-7**

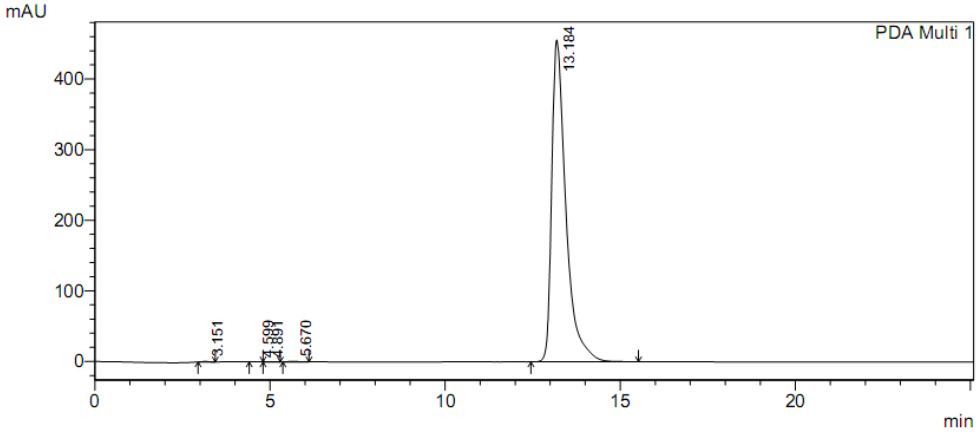


Figure S24. HPLC analysis of **IZCM-7**

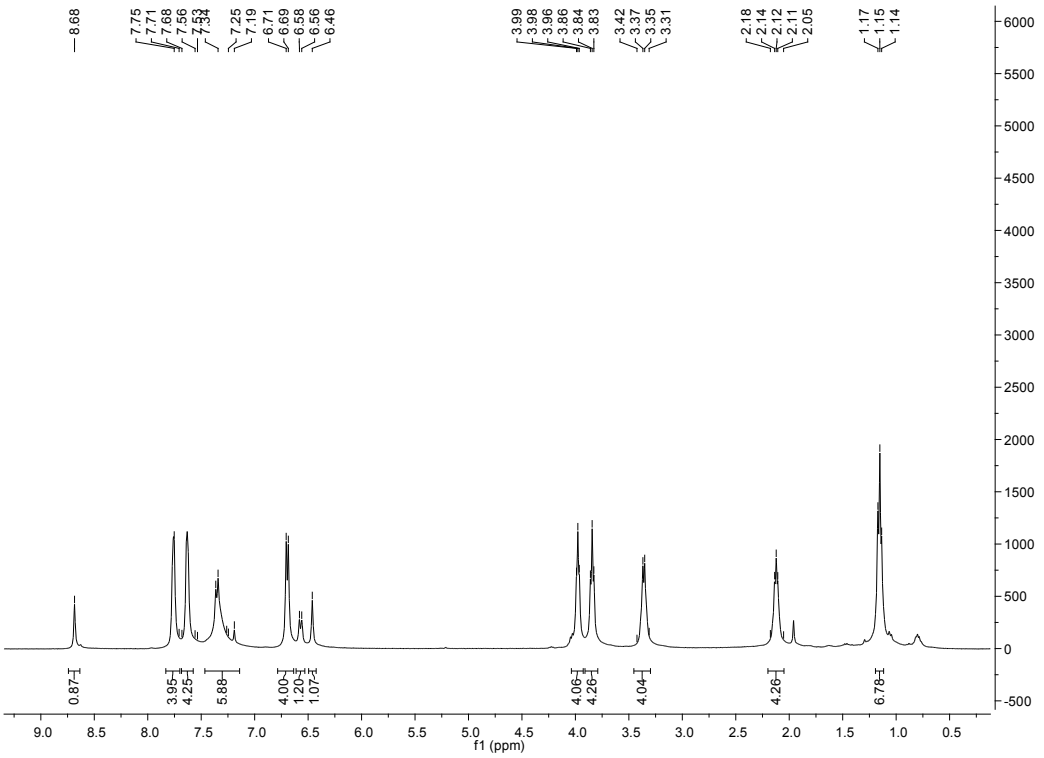


Figure S25. ^{1}H NMR spectrum of **IZCM-8**

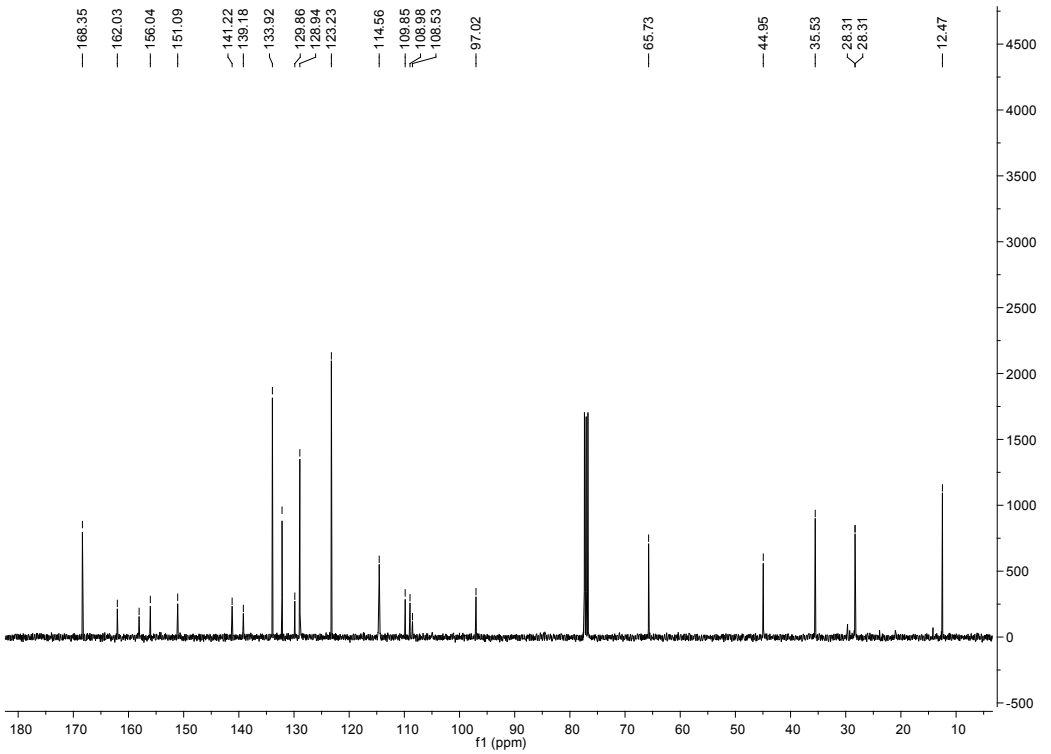


Figure S26. ^{13}C NMR spectrum of **IZCM-8**

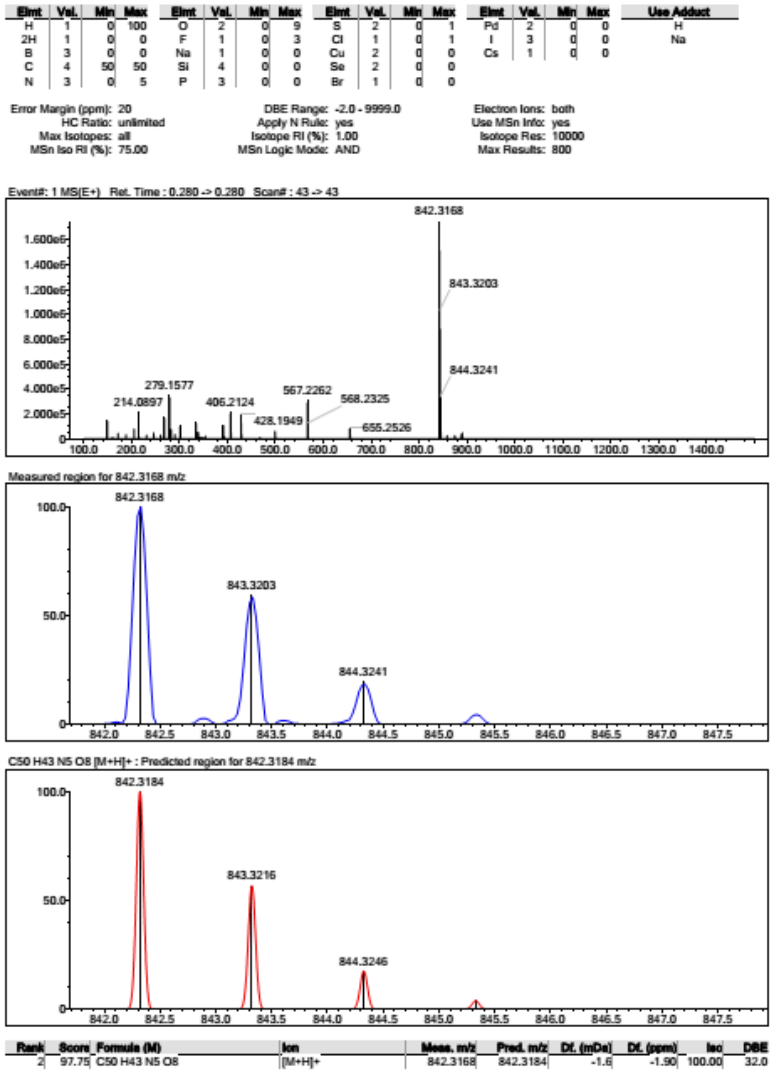


Figure S27. HRMS spectrum of **IZCM-8**

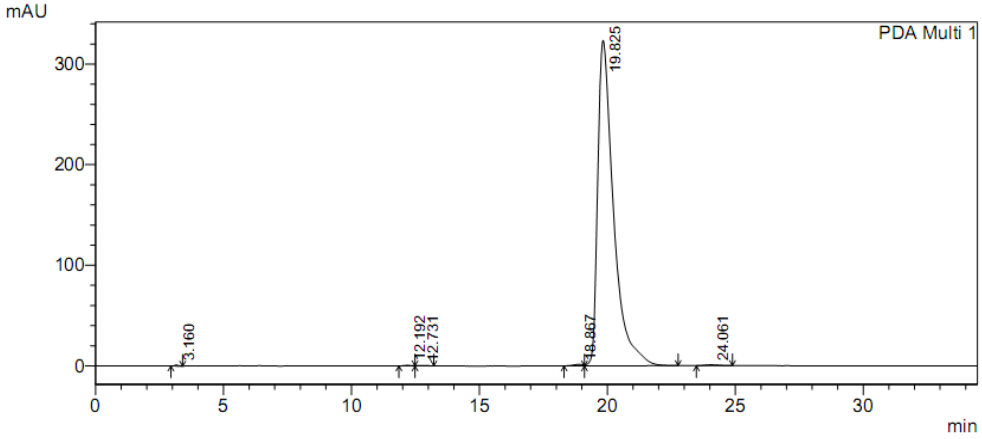


Figure S28. HPLC analysis of **IZCM-8**

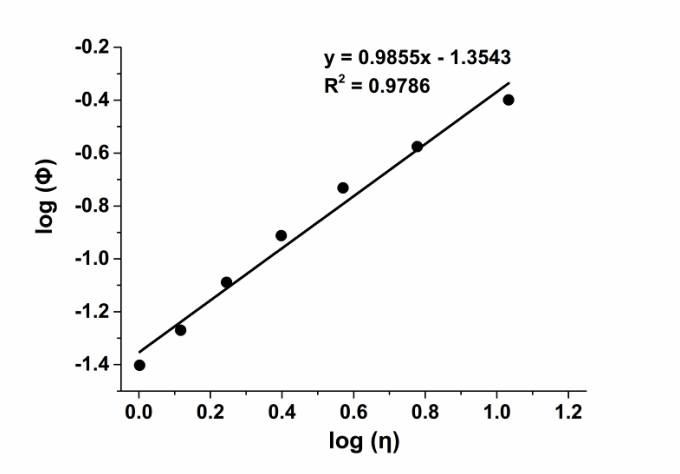


Figure S29. Viscosity dependence of the fluorescence quantum yield for **IZCM-7** in glycerol-water mixed solution.

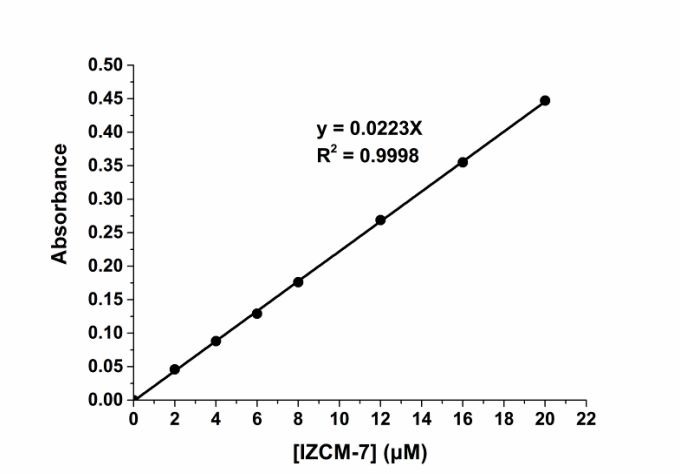


Figure S30. Concentration-dependent UV-Vis absorbance of **IZCM-7**. All of the spectra were collected in 10 mM Tris-HCl buffer, 100 mM KCl, pH 7.2. Concentration dependent UV-Vis absorbance spectra of **IZCM-7** showed that the variations follow the Beer-Lambert law.

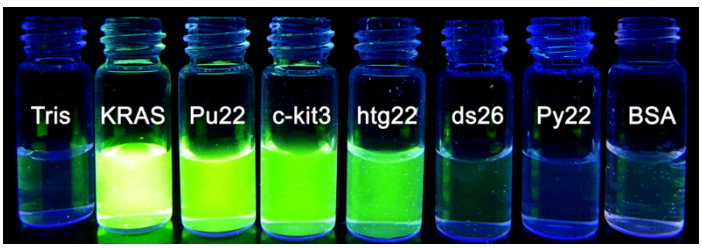


Figure S31. Photograph of 1 μM **IZCM-7** with and without 5 μM DNA or BSA samples in 10 mM Tris-HCl buffer, 100 mM KCl, pH 7.2, taken under UV light.

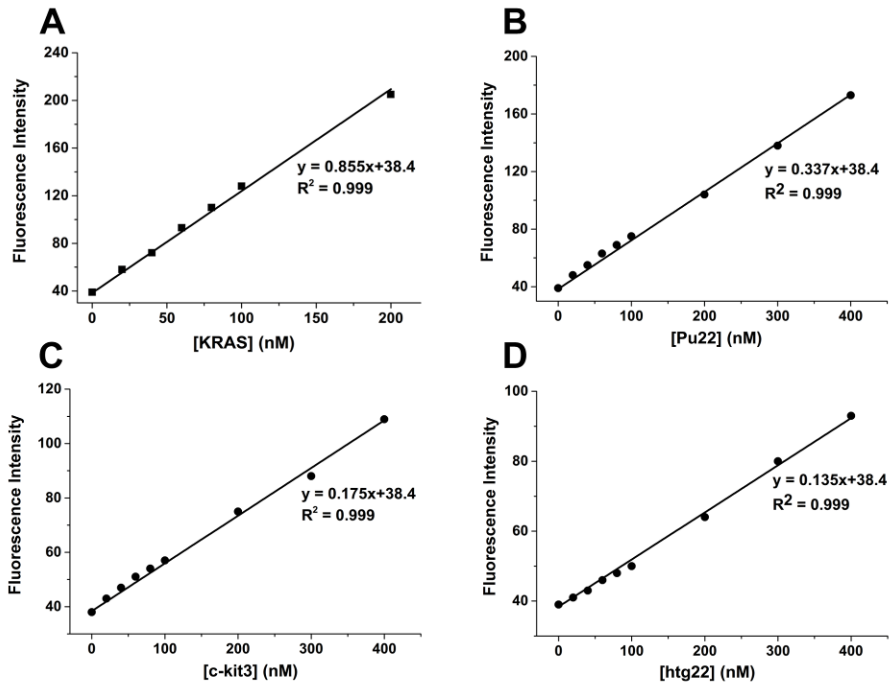


Figure S32. Linear fit equations for calculating LOD values of **IZCM-7** for (A) KRAS, (B) Pu22, (C) c-kit3 and (D) htg22 in solution. The concentration of **IZCM-7** was fixed at 1.0 μM in 10 mM Tris-HCl buffer, 100 mM KCl, and pH 7.2. Fluorescence intensities at 525 nm were collected.

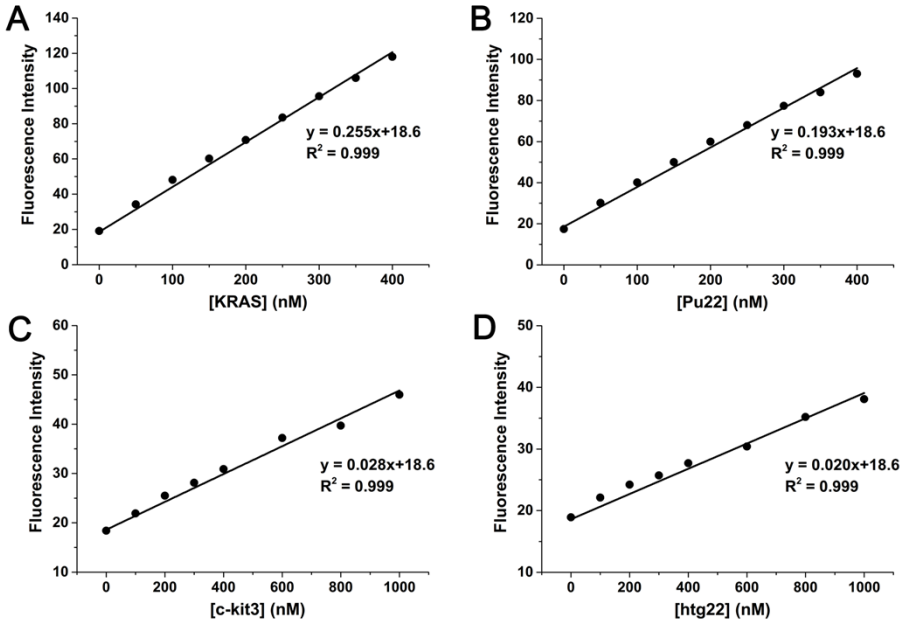


Figure S33. Linear fit equations for calculating LOD values of **IZCM-1** for (A) KRAS, (B) Pu22, (C) c-kit3 and (D) htg22 in solution. The concentration of **IZCM-1** was fixed at 1.0 μM in 10 mM Tris-HCl buffer, 100 mM KCl, and pH 7.2. Fluorescence intensities at 525 nm were collected.

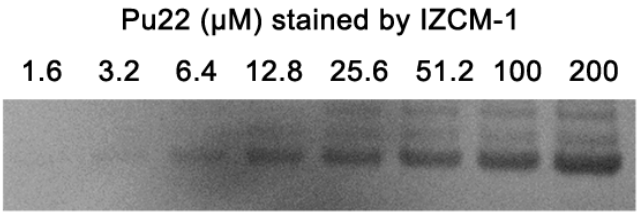


Figure S34. Dose-response staining of G-quadruplex Pu22 by **IZCM-1.** The detection limit of **IZCM-1** for Pu22 was 224 ng.

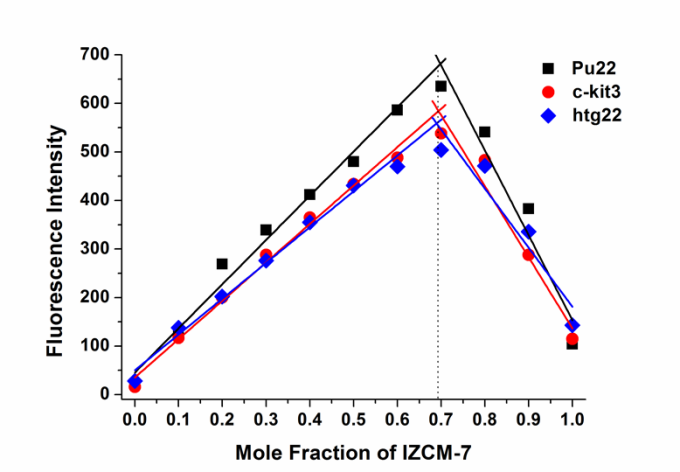


Figure S35. Job plot analysis for the binding stoichiometry of **IZCM-7** with G-quadruplexes. The points of intersection of the best fit lines for the Job plots are near 0.67, showing **IZCM-7** exhibited a 2:1 stoichiometry binding to the G-quadruplex. The fluorescence intensity was plotted as a function of the molar ratio of [**IZCM-7**] / ([**IZCM-7**] + [DNA]). Excitation was set at 450 nm and emission was measured at 525 nm.

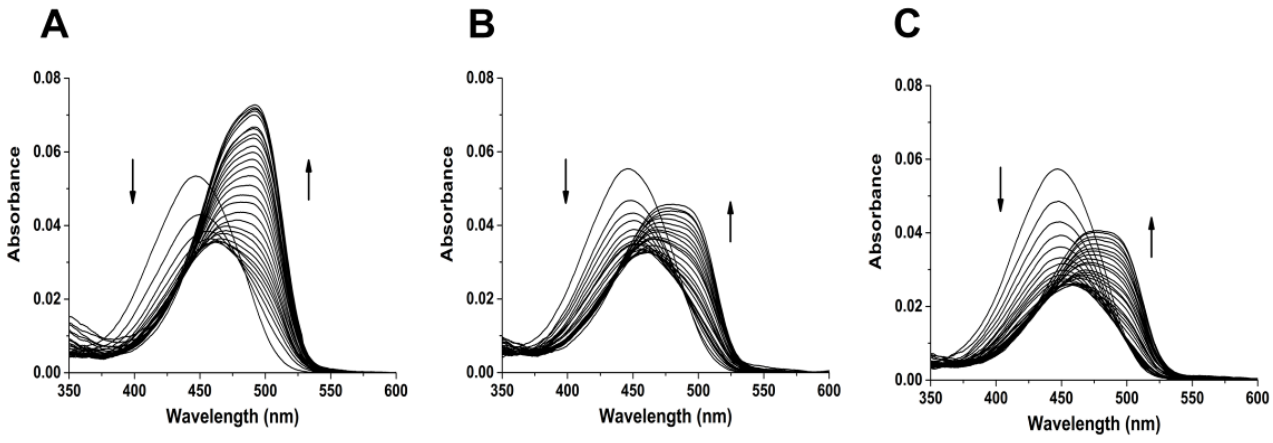


Figure S36. UV-Vis absorption titrations of 5 μM **IZCM-7** with stepwise addition of (A) KRAS, (B) c-kit3 and (C) htg22 in 10 mM Tris-HCl buffer, 100 mM KCl, pH 7.2, arrows: 0 – 10 μM.

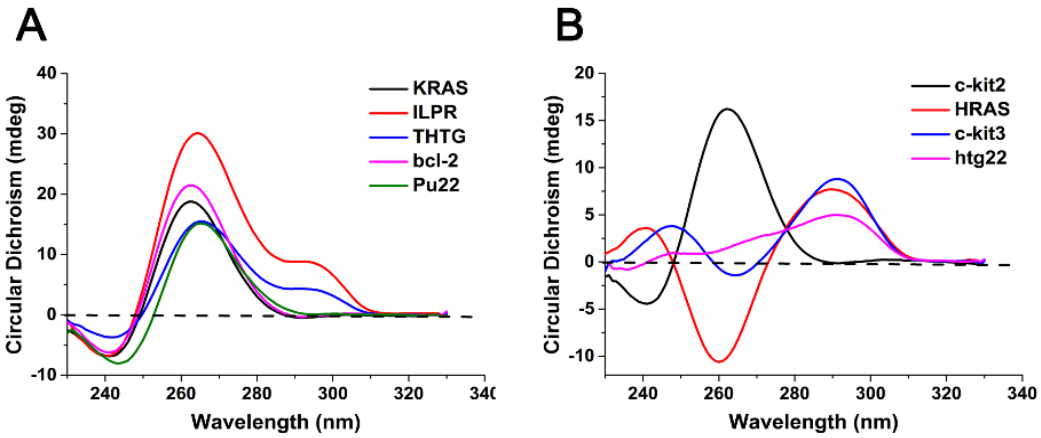


Figure S37. CD spectra of 5 μM G-quadruplex samples in 10 mM Tris-HCl buffer, 100 mM KCl, pH 7.2. CD studies were performed on a Chirascan circular dichroism spectrophotometer (Applied Photophysics, UK). A quartz cuvette with a 4 mm path length was used for the recording of spectra over a wavelength range of 230–340 nm with a 1 nm bandwidth, 1 nm step size and time of 0.5 s per point.