Electronic supplementary information

A Step toward Simplified Detection of Serum Albumin on SDS-PAGE Using an Environment-Sensitive Flavone Sensor

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1. Materials and Instruments.

All the chemicals, solvents and reagents were obtained from Fisher Scientific, and used as received without further purification. The bovine serum albumin (BSA), lysozyme (from chicken white), collagen (from bovine Achilles tendon), trypsin (from bovine pancreas), and fibrinogen (from bovine) were purchased from Alfa Aesar. The lipase, gelatin, and human serum albumin (HSA, low heavy metals) were purchased from Ward's Science, AMRESCO, and CalbioChem, respectively. ^{1}H NMR and ^{13}C NMR spectra were obtained by using a Varian Mercury 300 MHz spectrometer. UV−vis spectra were acquired on a Hewlett-Packard 8453 diode-array spectrometer. Fluorescence spectra were measured by using a HORIBA Jobin Yvon NanoLog spectrometer. ESI-MASS spectra were measured by HCTultra ETD II. The fluorescence images of electrophoresis gel were obtained by Canon 60D cameras under hand-hold UV lamp (365 nm).

2. Fluorescence Quantum Yield.

The fluorescence quantum yields were obtained using rhodamine 6G (sigma) as the standard (Φ_{fl} = 0.95, ethanol). The fluorescence quantum yields can be calculated by using the following Equation: $\Phi_s=\Phi_r\times(A_r\times n_s^2\times F_s)/(A_s\times n_r^2\times F_r)$, where the subscripts *s* and *r* refer to the sample and the standard, respectively. Φ is the quantum yield, F is the integrated emission intensity, A is the absorbance, and n is the refractive index.

3. Preparation of Monkey Serum.

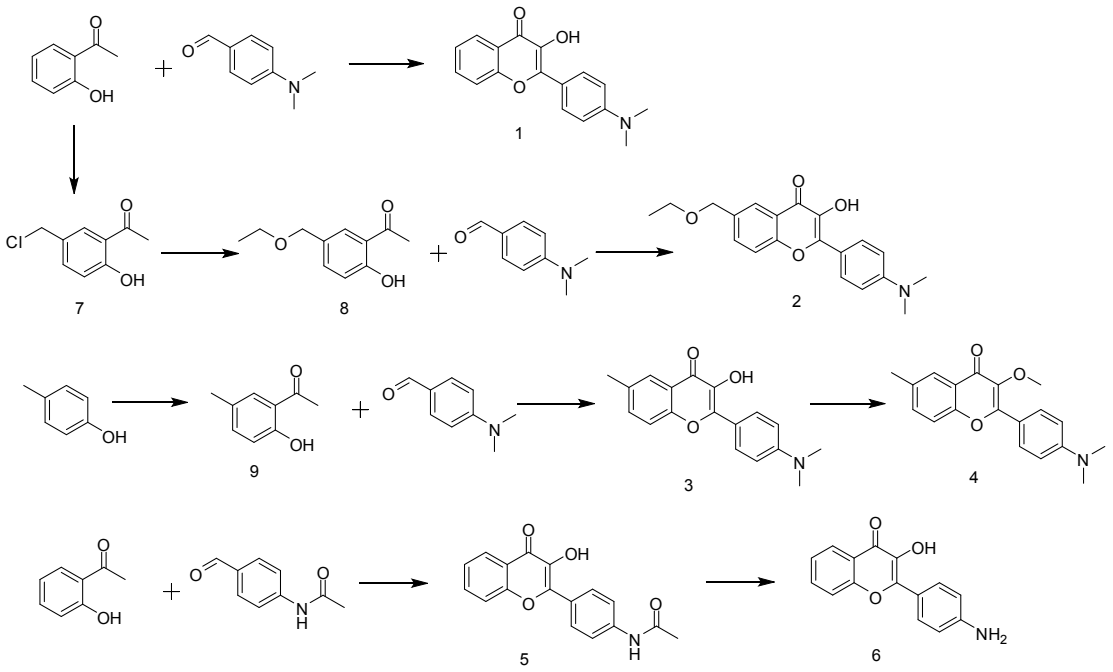
The female Rhesus macaques (Macaca mulatta), aged 2.5–3.5 years and weighing 3.0-4.5 kg, were used in this study.[38] Blood was collected by venipuncture, under general anesthesia. Approximately 5 ml of blood was drawn into collection tubes free of clot activator or other additives. After clotting at room temperature, the blood was centrifuged at 2,500× g for 10 min for serum separation. The collected serum was aliquoted to 1.5 ml tubes and stored at -80 °C until used for protein analysis.

4. Procedure for the Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Gel Image.

Protein samples were diluted 1:1 with loading buffer (Bio-Rad, 65.8 mM Tris HCl pH 6.8, 26.3% glycerol (autoclaved), 2.1% SDS, and 0.01% Bromophenol blue). Then, the samples were loaded into Bio-Rad mini gels (4-15%) and run with running buffer (25 mM Tris, 192 mM glycine, 0.1% SDS pH 8.3), under 100 volts for 1.5 hrs. The staining procedure was shown as follows: Compound 4 was dissolved in AcOH/DMSO/H_2O = 5:10:85 (v/v) at a concentration of 10 mM with 0.3% (wt%) SDS as the dye solution. The protein gel was stained with the dye solution for 2 hour, and imaged by

camera as fluorescent image before washing. Then, the protein gel was washed by MeOH/H_2O solution (v/v = 1/9) for 30 minutes, and imaged by camera as fluorescent image after washing.

5. Synthesis and characterization



Scheme S1. Synthesis routes of flavone dyes **1-6**

2-(4-(dimethylamino)phenyl)-3-hydroxy-4H-chromen-4-one (1):

4-(dimethylamino)benzaldehyde (10 mmol) was added to a solution of the 1-(2-hydroxyphenyl)ethanone (10 mmol) in ethanol (20 mL) and aqueous NaOH (3 g in 10 ml water). The mixture was stirred at 50 °C for 12 h. The reaction mixture was cooled to room temperature, and neutralized with 1M HCl. The solid precipitate was collected by filtration, and then washed with water and a small amount of ethanol. The solid was dissolved in 20 mL ethanol and aqueous NaOH (3 g in 10 ml water). Then reaction mixture was placed in an ice-water bath and 5 mL of 30% H_2O_2 solution was slowly added. The resulting mixture was stirred at room temperature for overnight. Then, the mixture was neutralized with 1M HCl resulting in the gradual formation of precipitation. The crude product was recrystallized from ethanol. Yield= 41%. ^{1}H NMR (d6-DMSO, 300 MHz): δ= 8.13(d, 2H, *J*=9.3), 8.09(d, 1H, *J*=8.4), 7.77(m, 2H), 7.45(m, 1H), 6.85(d, 2H, *J*=9.3), 3.00(s, 6H). ^{13}C NMR (d6-DMSO, 75 MHz): 172.4, 154.7, 151.5, 147.3, 137.7, 133.5, 129.4, 125.1, 124.7, 121.9, 118.6, 118.4, 111.8, 40.1.

1-(5-(chloromethyl)-2-hydroxyphenyl)ethanone (7):

2'-hydroxyacetophenone (100 mmol) was added into 25 ml of concentrated hydrochloric acid containing 150 mmol of paraformaldehyde. The reaction mixture was maintained at room temperature with stirring for 48 h until a precipitate formed. Then the solid product was collected by suction filtration, washed with an aqueous solution of sodium bicarbonate, and then washed with water. Yield= 65%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) = 12.30 (s, 1H, OH), 7.37 (m, 3H), 4.70 (s, 2H), 2.65 (s, 3H).

1-(5-(ethoxymethyl)-2-hydroxyphenyl)ethanone (8)

1-(5-(chloromethyl)-2-hydroxyphenyl)ethanone (10 mmol) was added into 20 ml of ethanol, then 10 ml water solution containing 3 g NaOH was added into the mixture. The mixture was reflux for 2 hours. The product solution was cooled to room temperature and used for next step without further purification.

2-(4-(dimethylamino)phenyl)-6-(ethoxymethyl)-3-hydroxy-4H-chromen-4-one (2)

To the 1-(5-(ethoxymethyl)-2-hydroxyphenyl)ethanone solution, 10 mmol of 4-(dimethylamino)benzaldehyde in 10 ml EtOH was added. The mixture was stirred at 50 °C for 4 h, and then cooled to room temperature. 2 g of NaOH in 5 ml water was added into the mixture. Then reaction mixture was placed in an ice-water bath and 5 mL of 30% H_2O_2 solution was slowly added. The resulting mixture was stirred at room temperature for overnight. Then, the mixture was neutralized with 1M HCl resulting in the gradual formation of precipitation. The crude product was recrystallized twice from ethanol/hexane. Yield= 8%. ^{1}H NMR (d6-DMSO, 300 MHz): δ= 8.13(d, 2H, *J*=9.0), 8.00(s, 1H), 7.68(s, 2H), 6.85(d, 2H, *J*=9.0), 4.57(s, 2H), 3.56 (m, 2H), 3.01(s, 6H), 1.20(t, 3H). ^{13}C NMR (d6-DMSO, 75 MHz): 172.4, 154.0, 151.5, 147.3, 137.7, 135.4, 132.8, 129.4, 121.5, 118.6, 111.8, 111.5, 71.2, 65.6, 40.1, 15.6.

1-(2-hydroxy-5-methylphenyl)ethanone (9)

4-methylphenol (100 mmol) in 20 ml of toluene was heated to melting, and 100 mmol of acetyl chloride was slowly added under vigorously stirring. Then the mixture was cooled to room temperature with an ice-water bath. The anhydrous $AlCl_3$ (200 mmol) was added in three portions. After the addtion of $AlCl_3$, the reaction mixture was heated to 120 °C for 10 hours, and then was hydrolyzed by crushed ice.The oil layer was extracted with CH_2Cl_2 and dried with anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel. Yield= 78%. ^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) = 12.10 (1H, s, OH), 7.52 (1H, d, *J* =1.2), 7.32 (1H, dd, *J* =8.4, 2.1). 6.91 (1H, d, *J* =8.1), 2.63 (3H, s), 2.32 (3H, s).

2-(4-(dimethylamino)phenyl)-3-hydroxy-6-methyl-4H-chromen-4-one (3)

10 mmol of 1-(2-hydroxy-5-methylphenyl)ethanone was added to a solution of the 4-(dimethylamino)benzaldehyde (10 mmol) in ethanol (20 mL) and aqueous NaOH (3 g in 10 ml water). The mixture was stirred at 50 °C for 4 h. The reaction mixture was cooled to room temperature, and neutralized with 1M HCl. The solid precipitate was collected by filtration, washed with water and a small amount of ethanol. The solid was dissolved in 20 mL ethanol and aqueous NaOH (3 g in 10 ml water). Then reaction mixture was placed in an ice-water bath and 5 mL of 30% H_2O_2 solution was slowly added. The resulting mixture was stirred at room temperature for overnight. Then, the mixture was neutralized with 1M HCl resulting in the gradual formation of precipitation. The crude product was recrystallized from ethanol. Yield= 34%. ^{1}H NMR (d6-DMSO, 300 MHz): δ= 8.11(d, 2H, *J*=9.3), 7.86(s, 1H), 7.63(m, 2H), 6.85(d, 2H, *J*=9.3), 3.01(s, 6H), 2.43(s, 3H). ^{13}C NMR (d6-DMSO, 75 MHz): 172.3, 153.1, 151.4, 147.1, 137.6, 134.7, 134.1, 129.3, 124.2, 121.6, 118.5, 111.8, 40.1, 20.9.

2-(4-(dimethylamino)phenyl)-3-methoxy-6-methyl-4H-chromen-4-one (4)

2 mmol of 2-(4-(dimethylamino)phenyl)-3-hydroxy-6-methyl-4H-chromen-4-one was dissolved in 10 ml of acetone, followed by 3 mmol of K_2CO_3. The reaction mixture was placed in the ice-water bath, and then 3 mmol of Me2SO4 was added dropwise. The mixture was stirred for 24 hours until

the mixture color was changed from dark yellow to light yellow. 20 ml of water was added to terminate the reaction. The mixture was extracted with 3 × 20 ml of CH_2Cl_2, dried with Na_2SO_4, and evaporated by reduced pressure. The product was obtained by column chromatography on silica gel(Hexane/CH_2Cl_2=9/1). Yield= 81%. ^{1}H NMR (d6-DMSO, 300 MHz): δ= 8.01(dd, 2H, J_1=9.0, J_2=1.8), 7.83(s, 1H), 7.58(m, 2H), 6.85(dd, 2H, J_1=9.0, J_2=1.8), 3.77(s, 3H) 3.02(s, 6H), 2.42(s, 3H). ^{13}C NMR (d6-DMSO, 75 MHz): 173.5, 153.2, 152.1, 139.6, 135.1, 134.6, 129.9, 124.4, 123.7, 120.8, 118.4, 117.2, 111.9, 59.6, 40.0, 20.9.

N-(4-(3-hydroxy-4-oxo-4H-chromen-2-yl)phenyl)acetamide (5)

10 mmol of N-(4-formylphenyl)acetamide was added to a solution of the 1-(2-hydroxyphenyl)ethanone (10 mmol) in ethanol (20 mL) and aqueous NaOH (3 g in 10 ml water). The mixture was stirred at room temperature for overnight. The reaction mixture was neutralized with 1M HCl, and the solid precipitate was collected by filtration. The solid was dissolved in 20 mL ethanol and aqueous NaOH (3 g in 10 ml water). Then reaction mixture was placed in an ice-water bath and 5 mL of 30% H_2O_2 solution was slowly added. The resulting mixture was stirred at room temperature for overnight. The precipitate was observed and collected by filtration, washed with water. The crude product was recrystallized from ethanol/CH_2Cl_2. Yield= 59%. ^{1}H NMR (d6-DMSO, 300 MHz): δ= 10.2(s, 1H, OH), 8.22(d, 2H, *J*=8.7), 8.10 (d, 1H, *J*=7.8), 7.77(m, 4H), 7.46(m, 1H), 2.08(s, 3H). ^{13}C NMR (d6-DMSO, 75 MHz): 173.2, 169.2, 154.9, 145.7, 141.2, 139.0, 134.0, 128.8, 126.1, 125.2, 124.9, 121.8, 118.9, 118.8, 24.6.

2-(4-aminophenyl)-3-hydroxy-4H-chromen-4-one (6)

5 mmol of N-(4-(3-hydroxy-4-oxo-4H-chromen-2-yl)phenyl)acetamide was dissolved in 5 ml THF, then 25 ml of 37% HCl solution was added into the reaction mixture. The mixture was reflux for 48 hours, and then was neutralized by Na_2CO_3. 3× 20 ml of CH_2Cl_2 was used to extract the mixture, washed with brine and water, then dried under Na_2SO_4. The desired product was purified by column chromatography on silica gel (Hexane/CH_2Cl_2=4/1). Yield= 84%. ^{1}H NMR (d6-DMSO, 300 MHz): δ= 8.12(m, 3H), 7.81 (m, 2H), 7.48(m, 1H), 6.99(d, 2H, *J*=8.4), 5.11(broad peak, hydrogen

bond). ^{13}C NMR (d6-DMSO, 75 MHz):173.1, 154.9, 145.6, 139.8, 138.9, 134.0, 129.5, 126.8, 125.2, 125.0, 121.8, 120.3, 118.7.

6. NMR Spectra

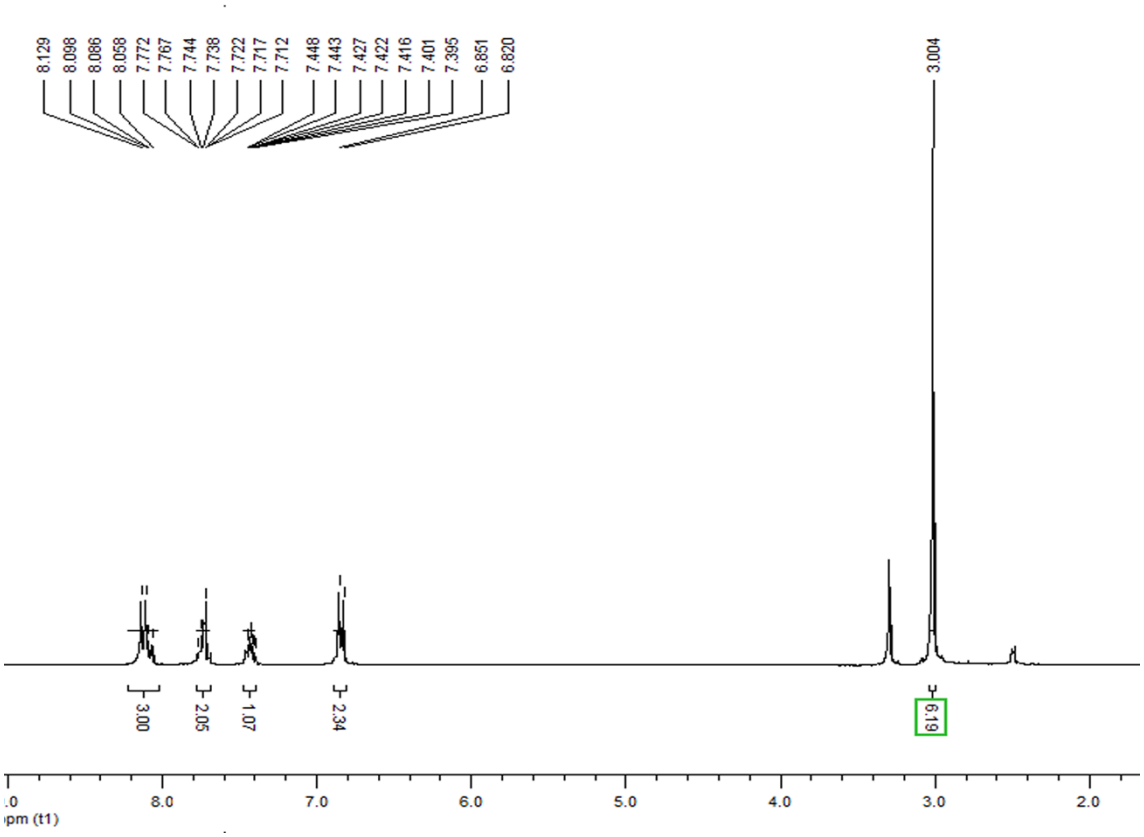


Fig. S1. ^{1}H NMR spectrum of **1**

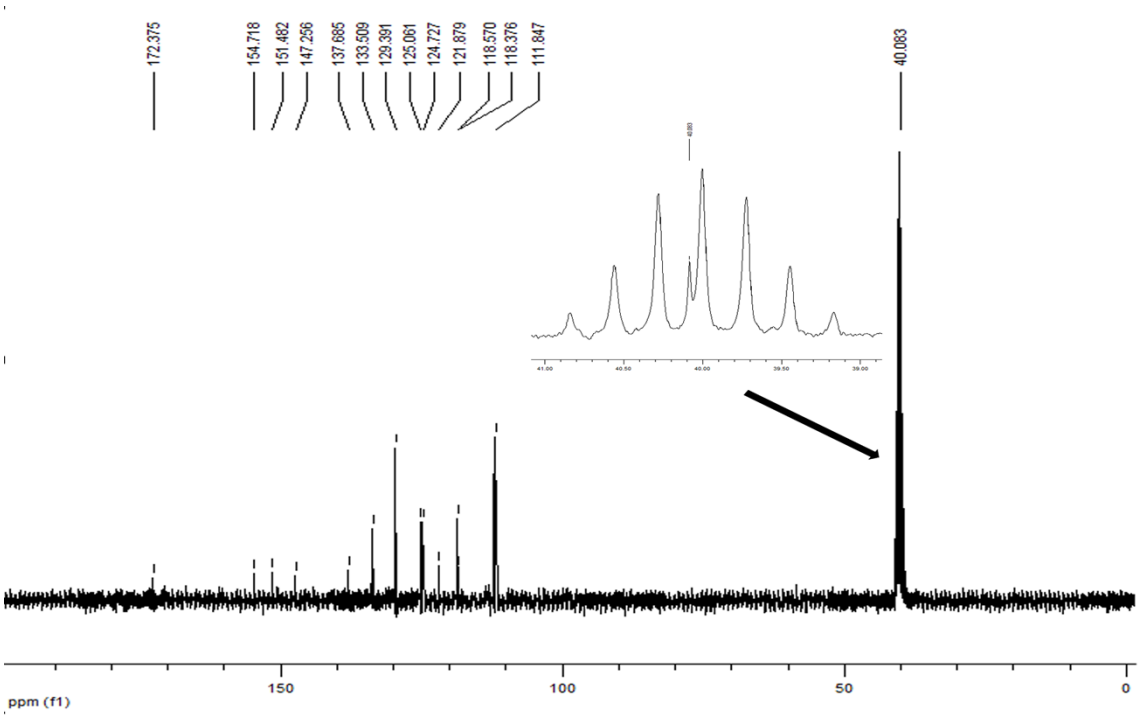


Fig. S2. ^{13}C NMR spectrum of **1**

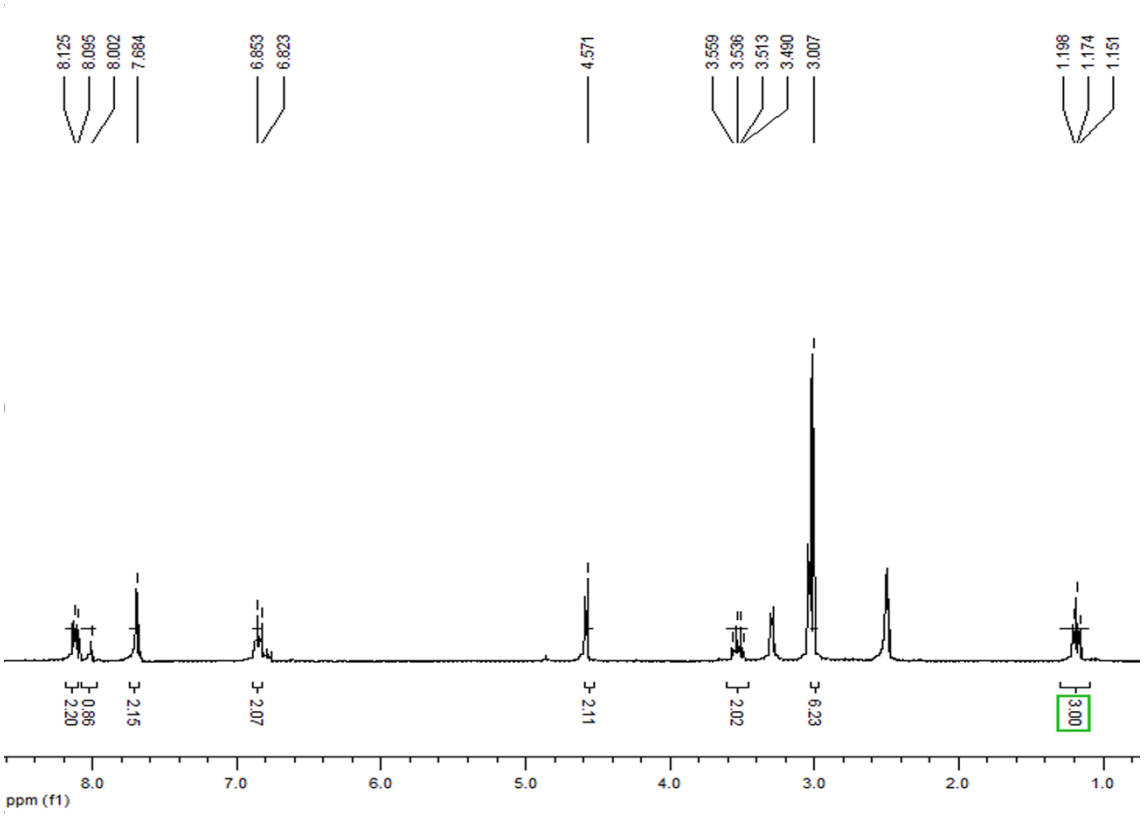


Fig. S3. ^{1}H NMR spectrum of **2**

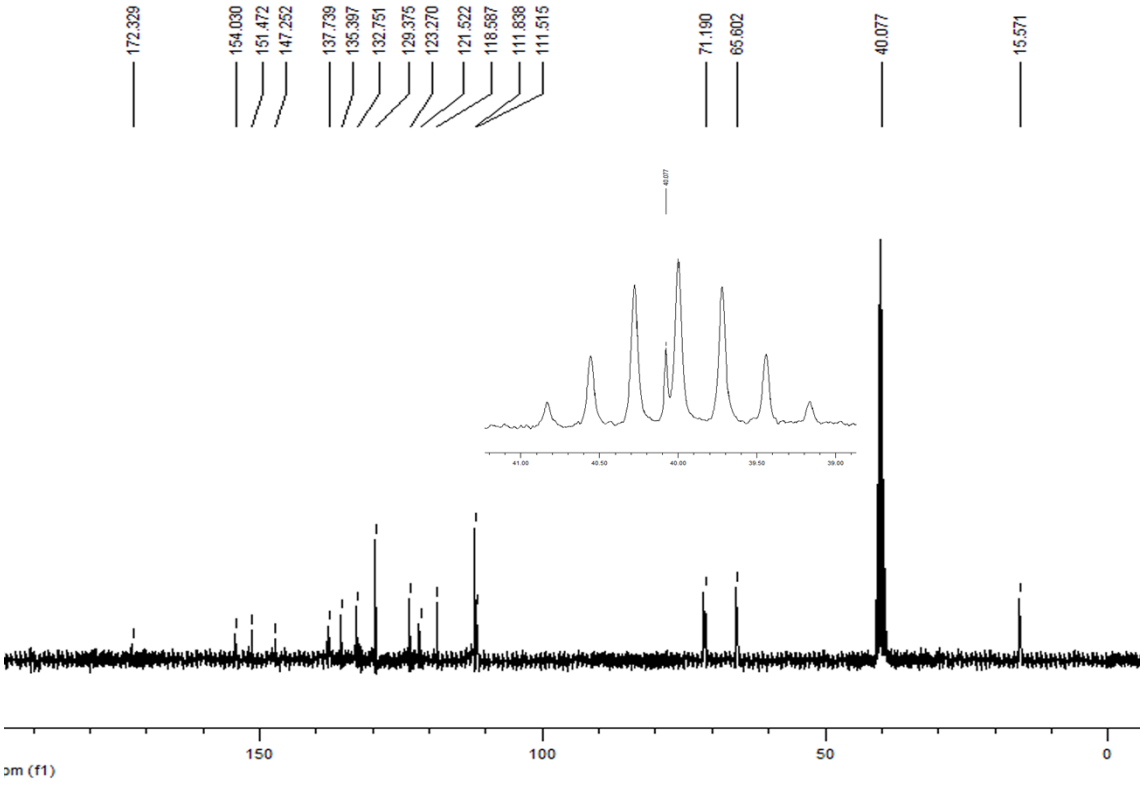


Fig. S4. ^{13}C NMR spectrum of **2**

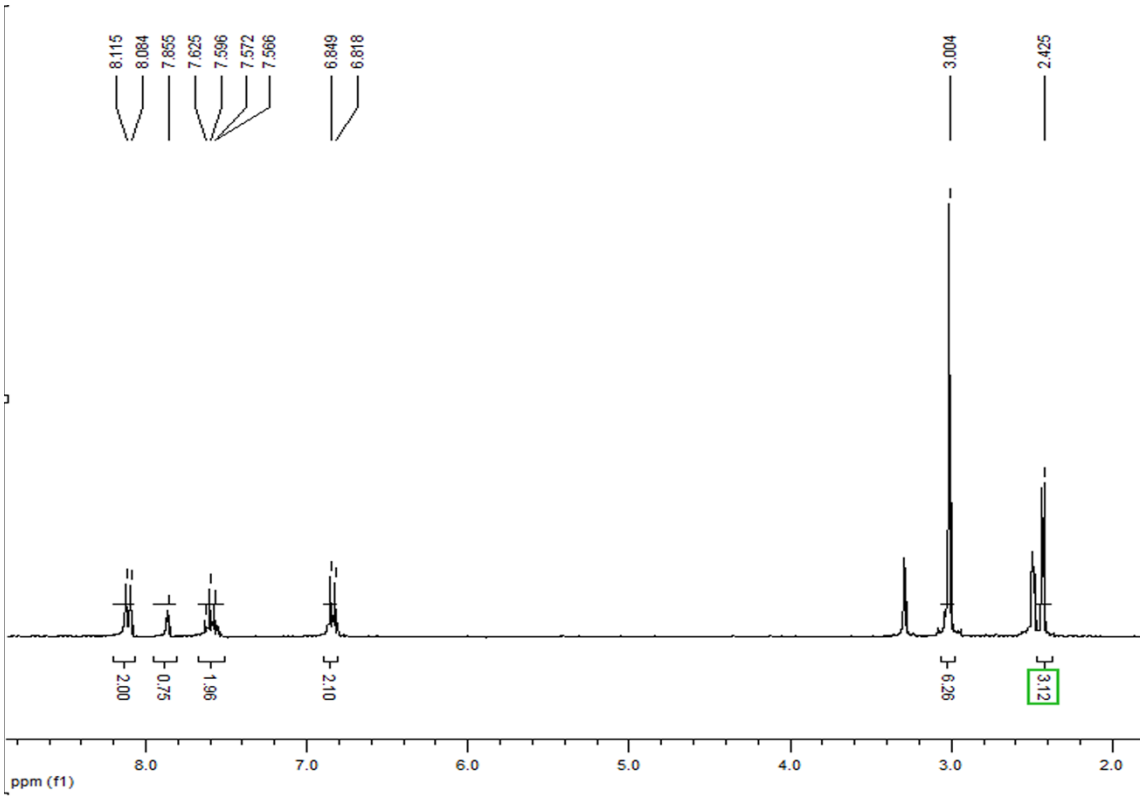


Fig. S5. ^{1}H NMR spectrum of **3**

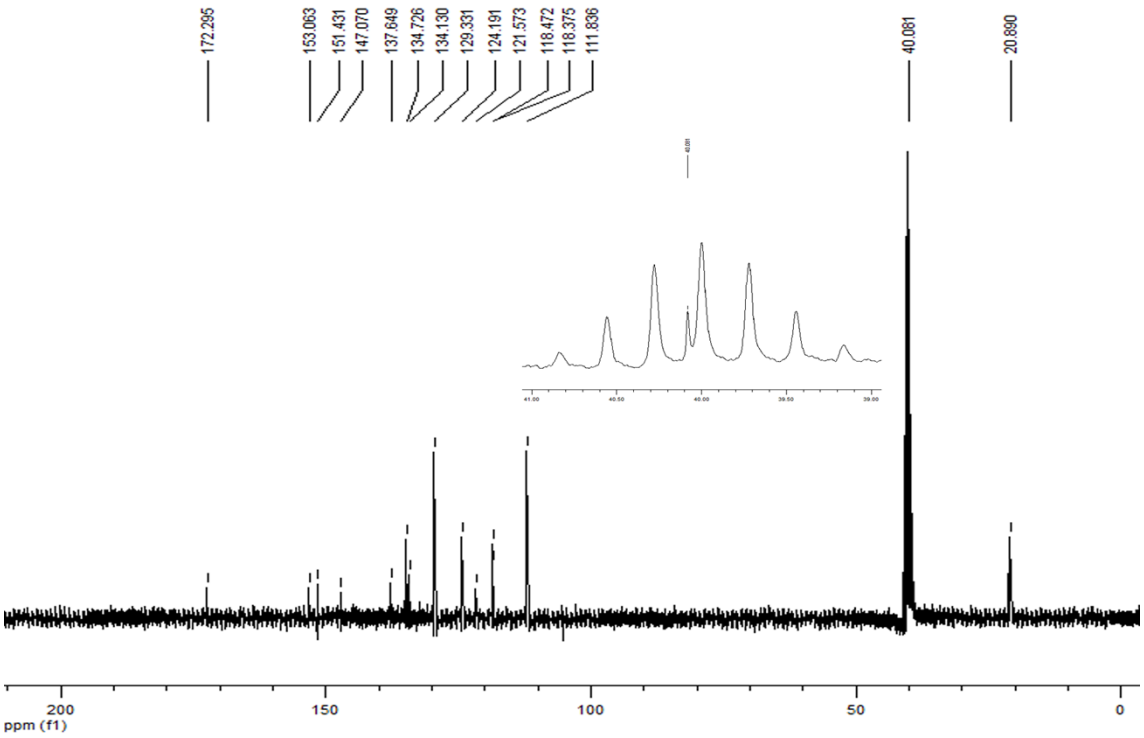


Fig. S6. ^{13}C NMR spectrum of **3**

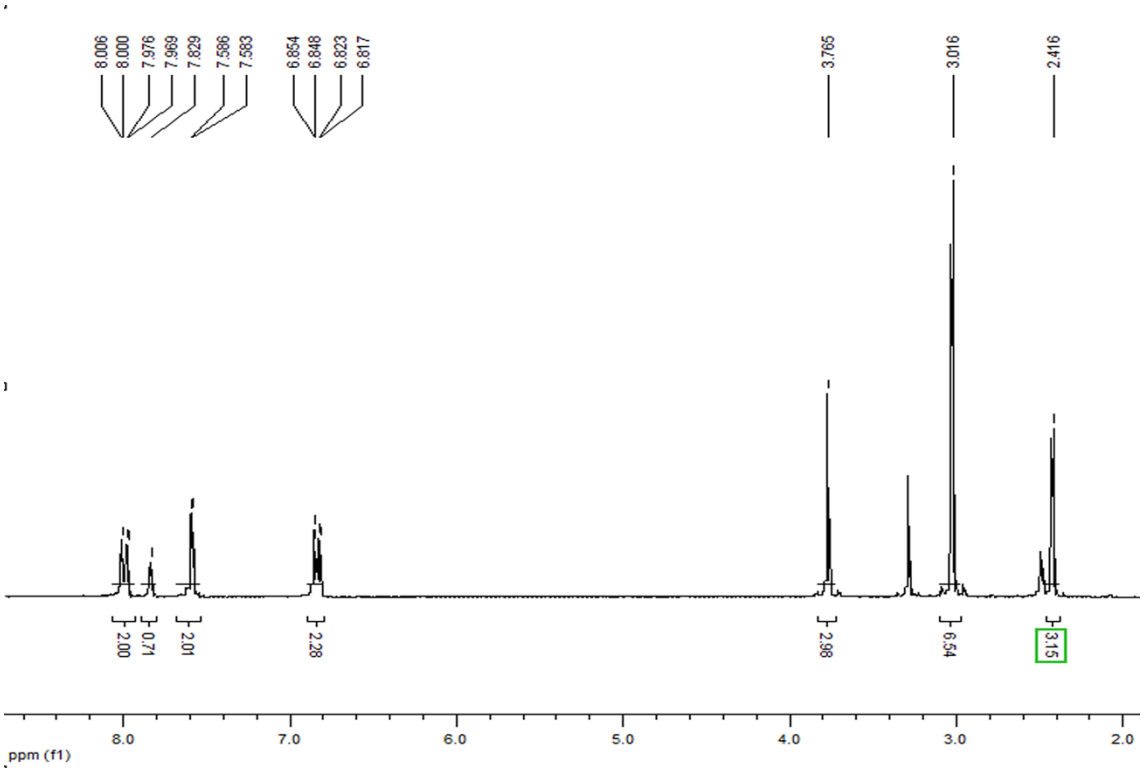


Fig. S7. ^{1}H NMR spectrum of **4**

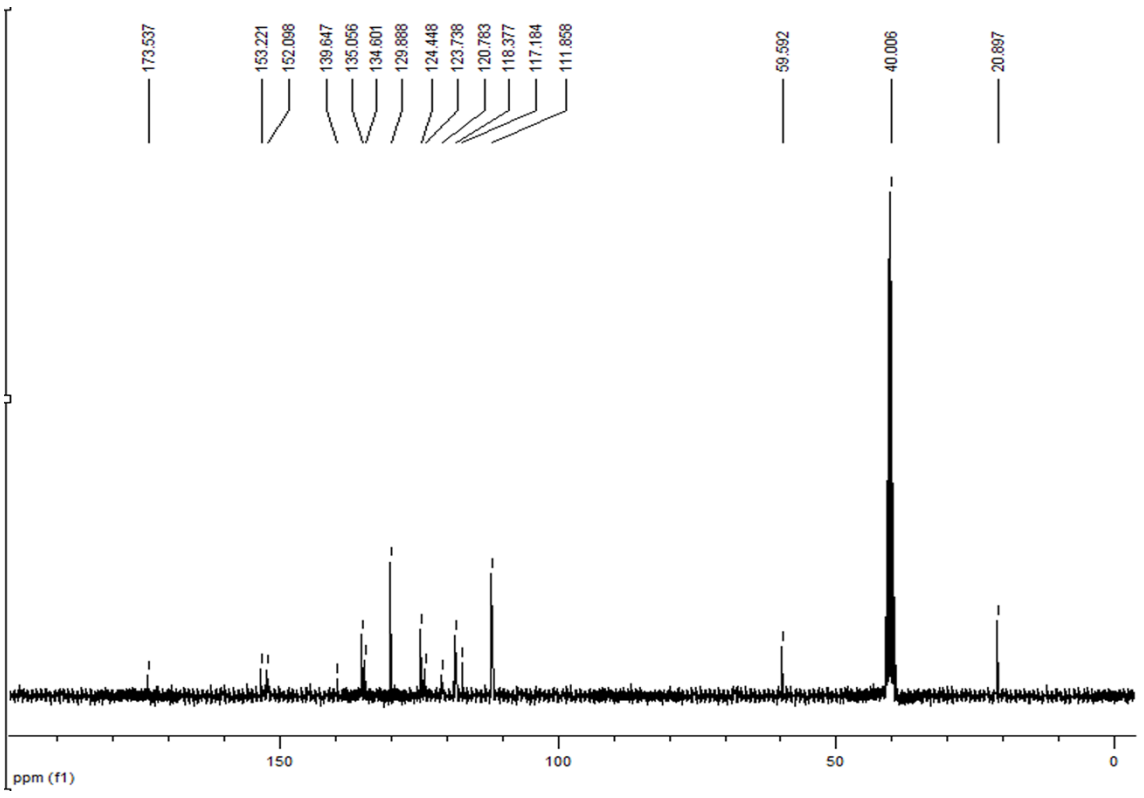


Fig. S8. ^{13}C NMR spectrum of **4**

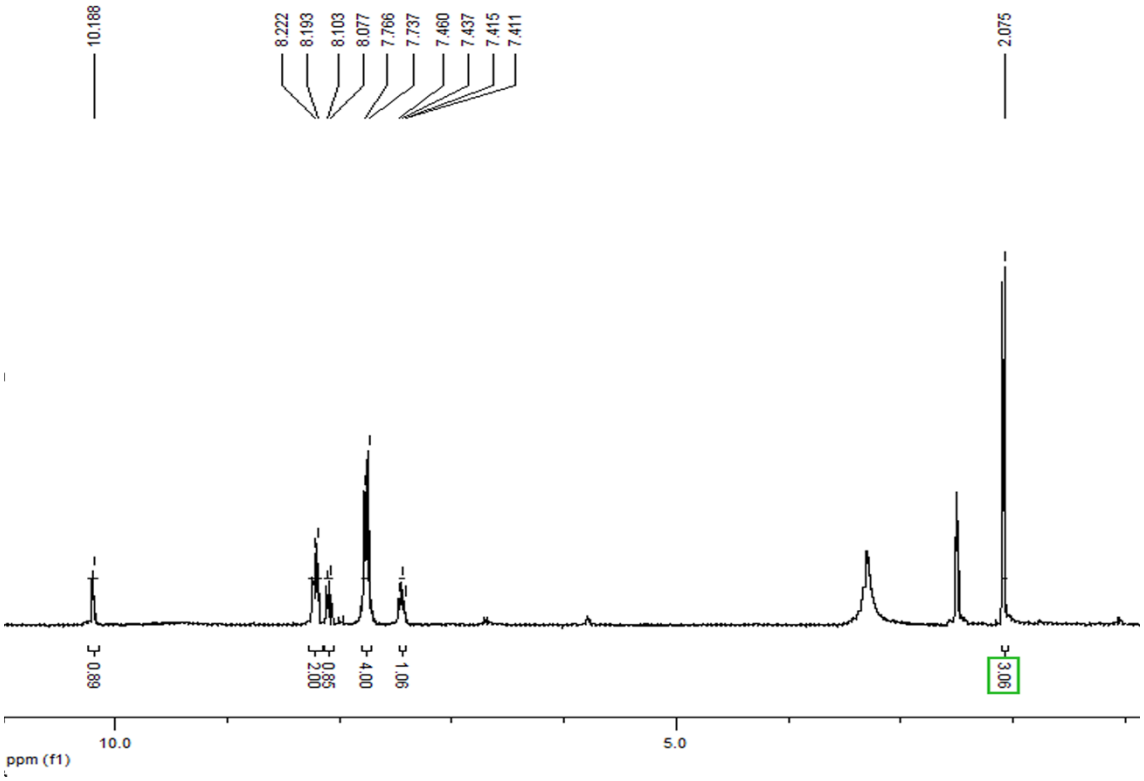


Fig. S9. ^{1}H NMR spectrum of **5**

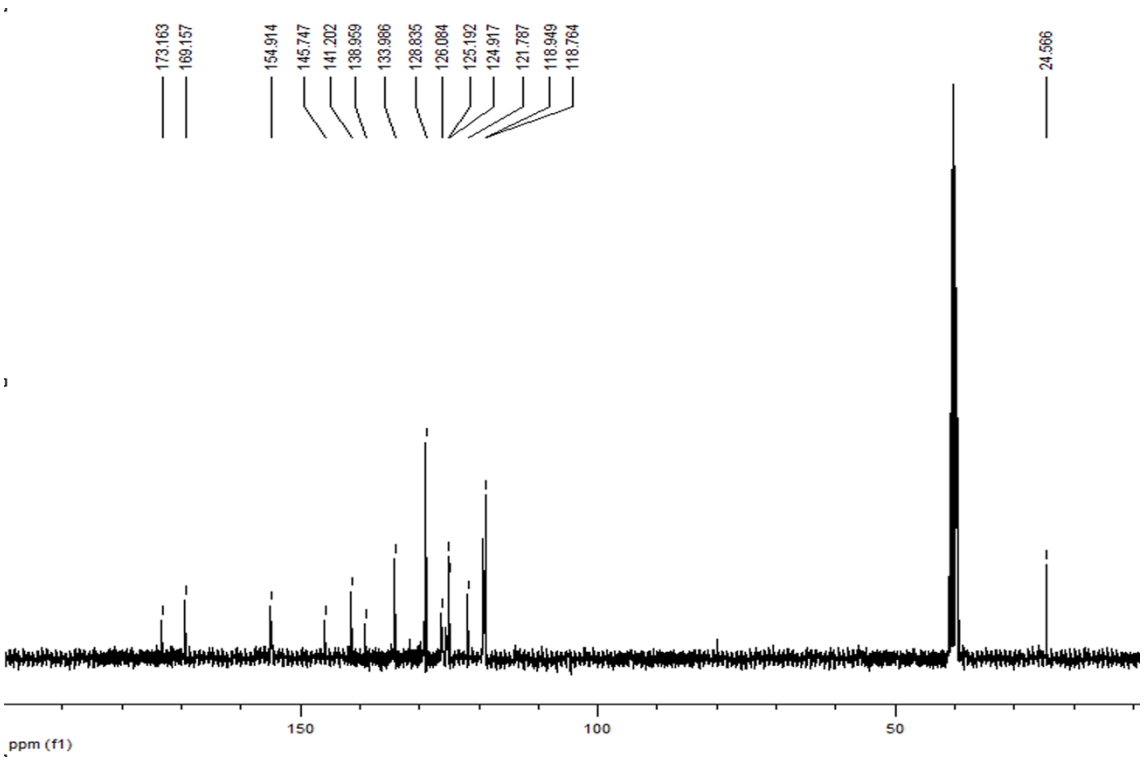


Fig. S10. ^{13}C NMR spectrum of **5**

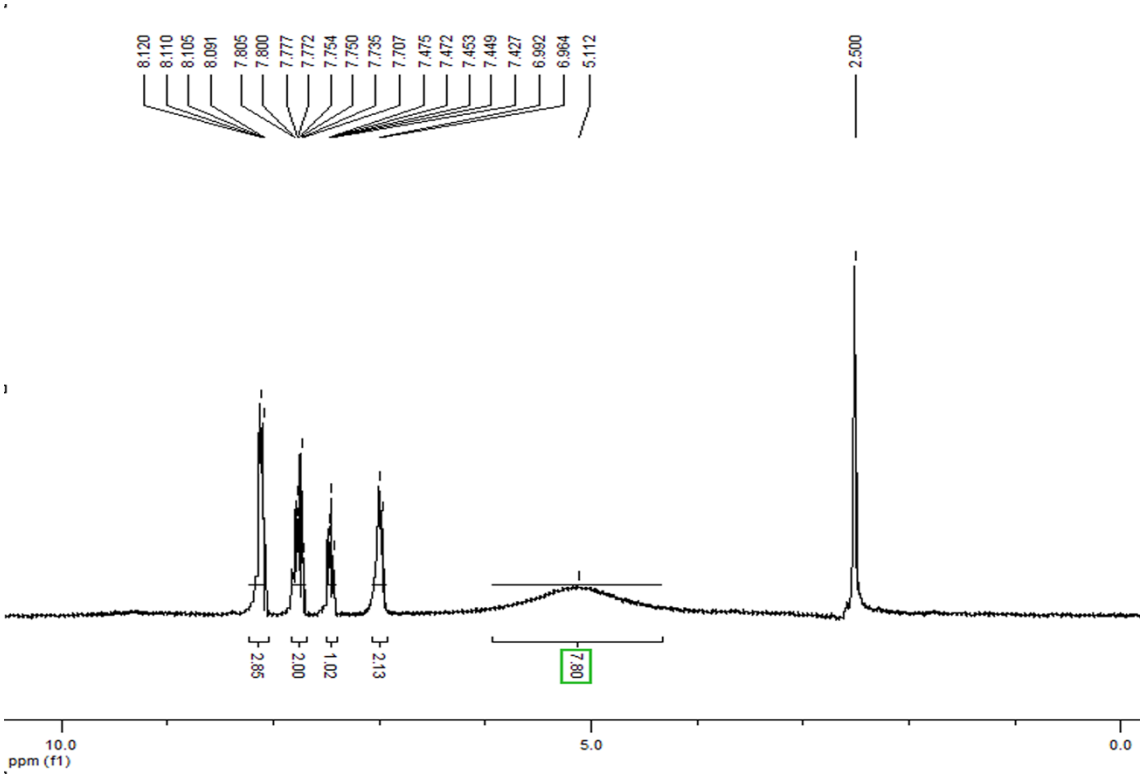


Fig. S11. ^{1}H NMR spectrum of **6**

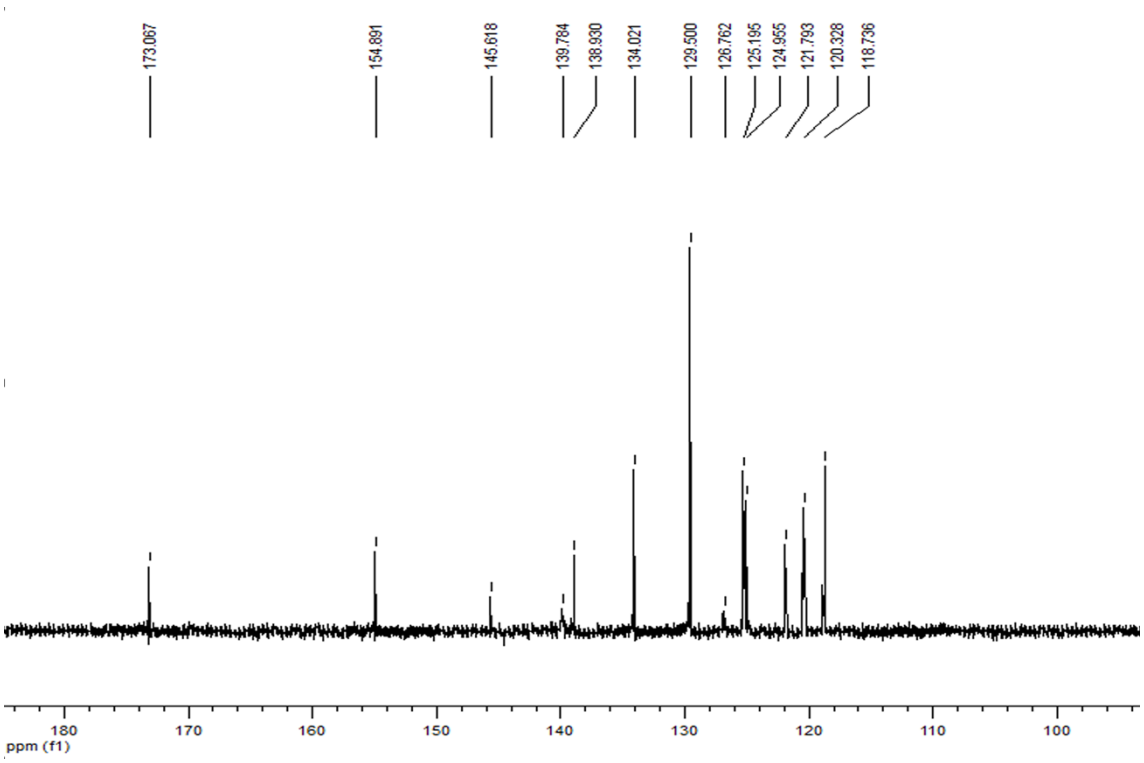


Fig. S12. ^{13}C NMR spectrum of **6**

7. ESI-MASS Spectra

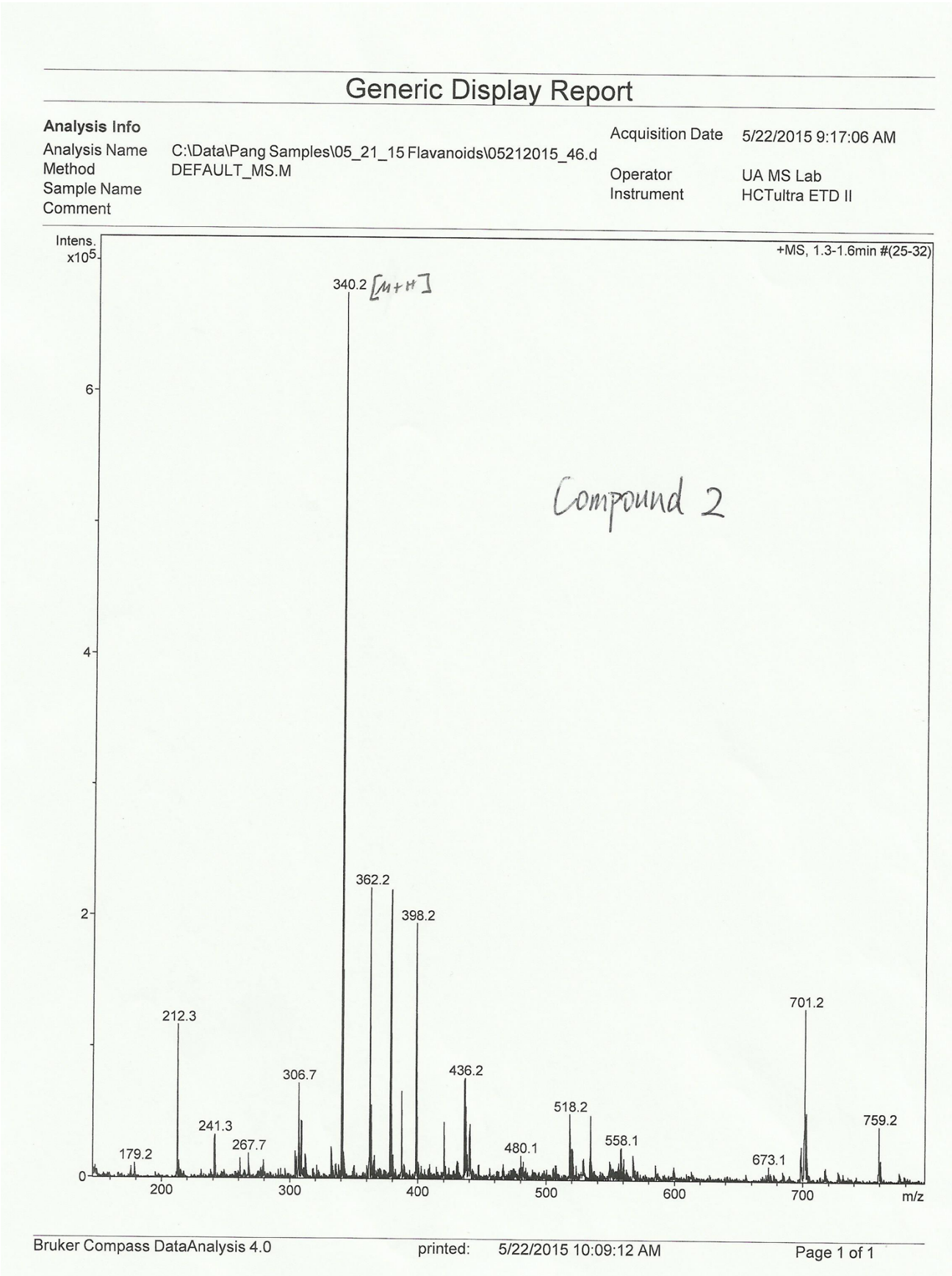


Fig. S13. ESI-MASS spectrum of **2**

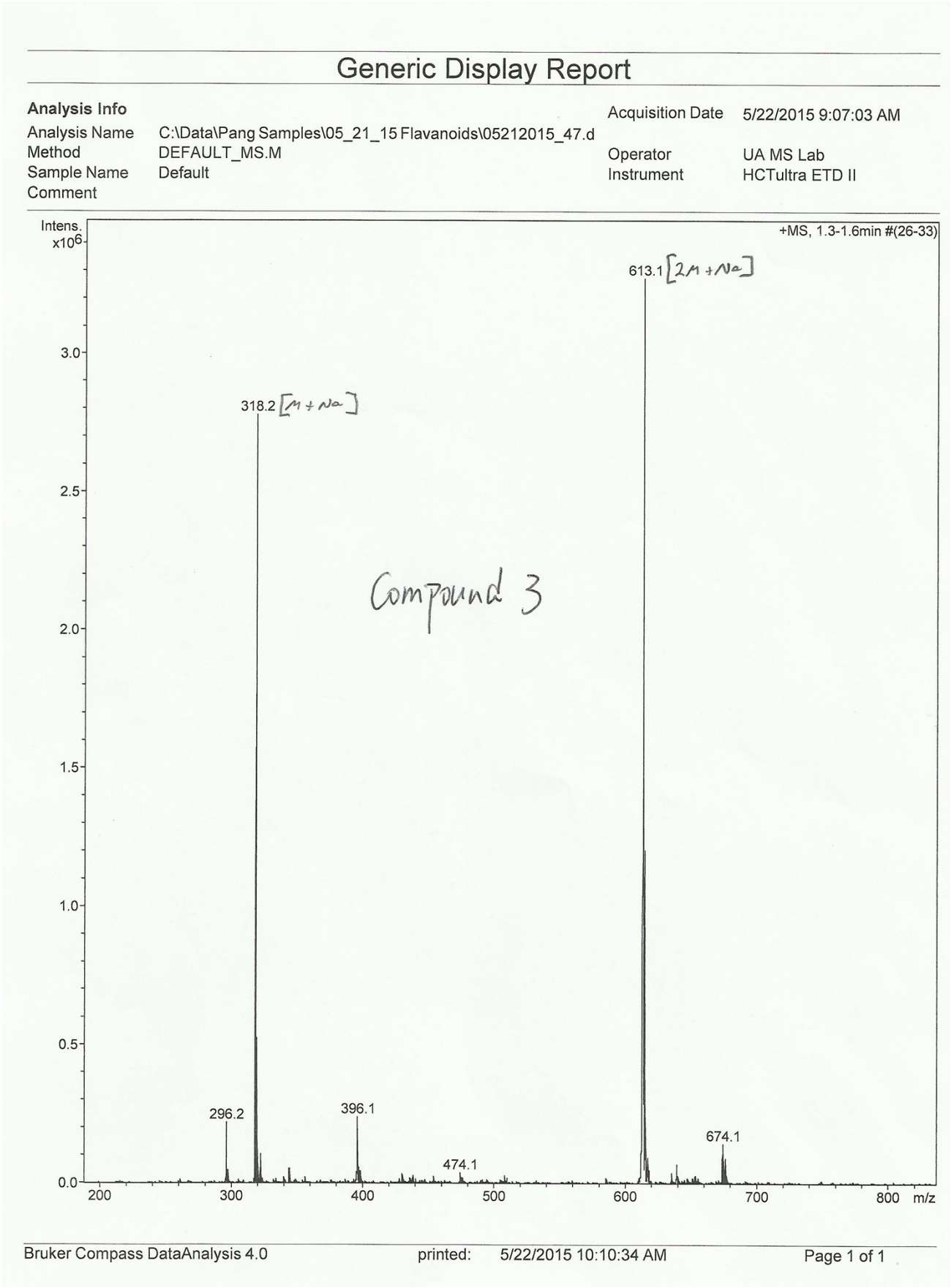


Fig. S14. ESI-MASS spectrum of **3**

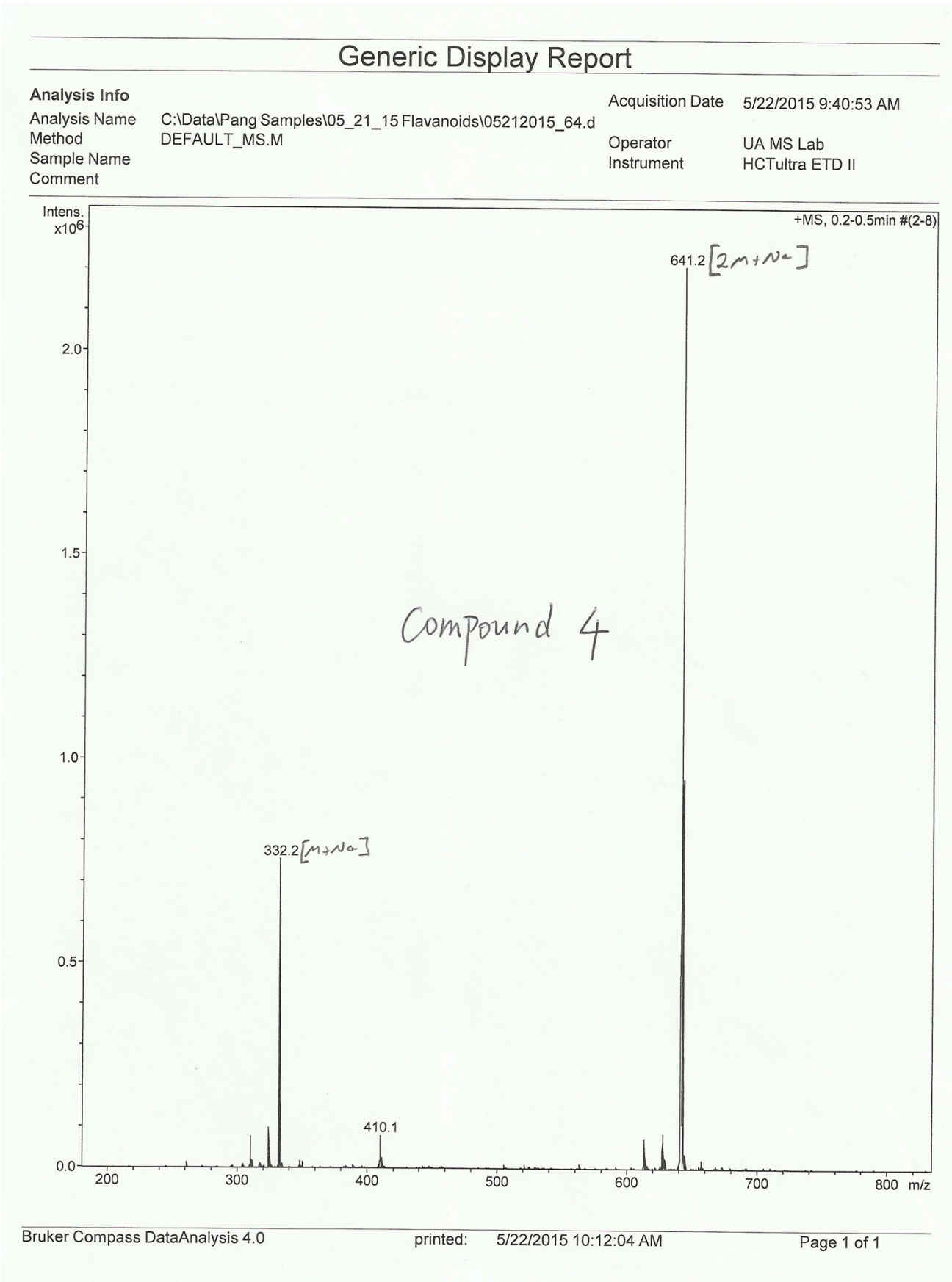


Fig. S15. ESI-MASS spectrum of **4**

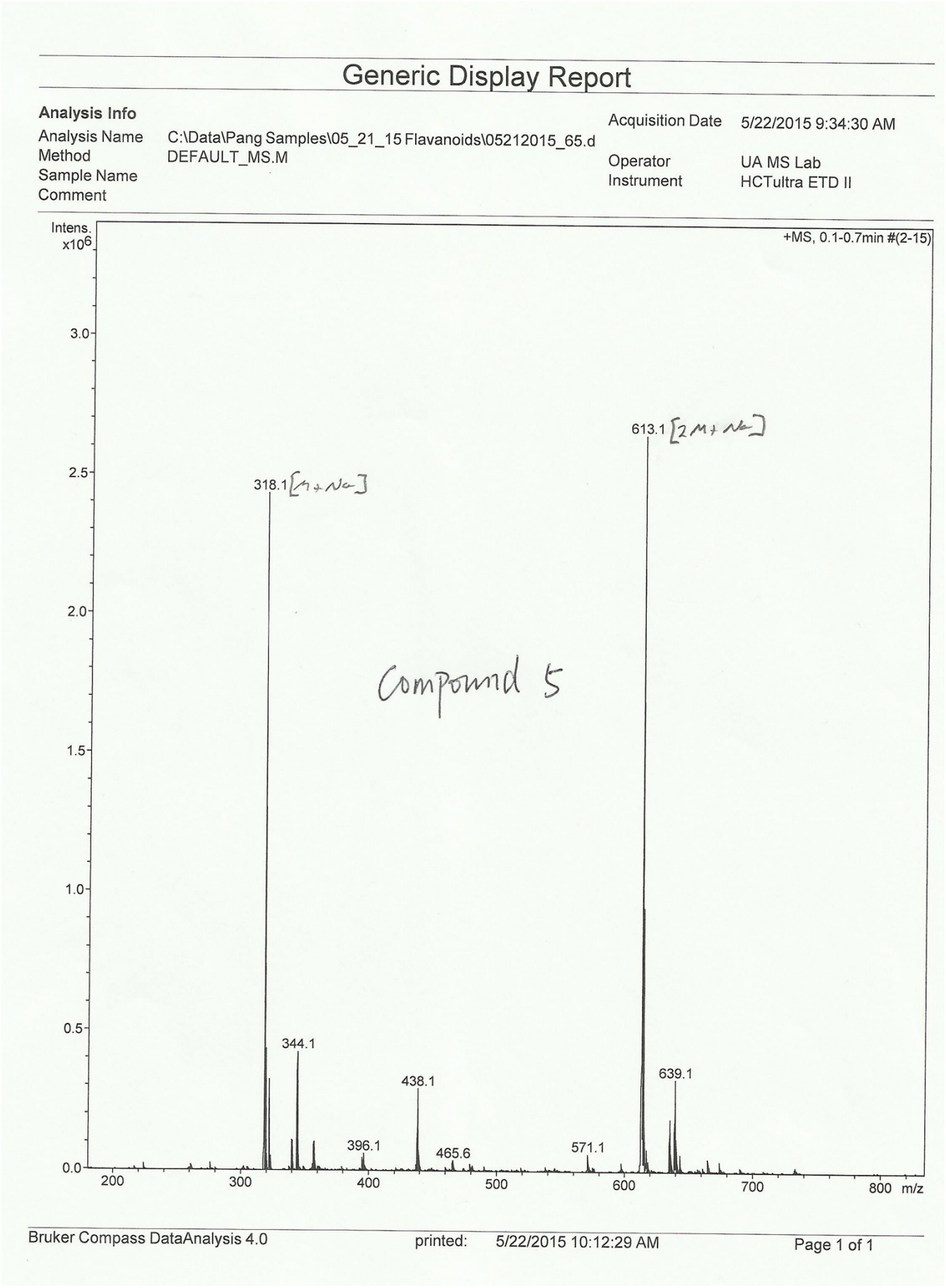


Fig. S16. ESI-MASS spectrum of **5**

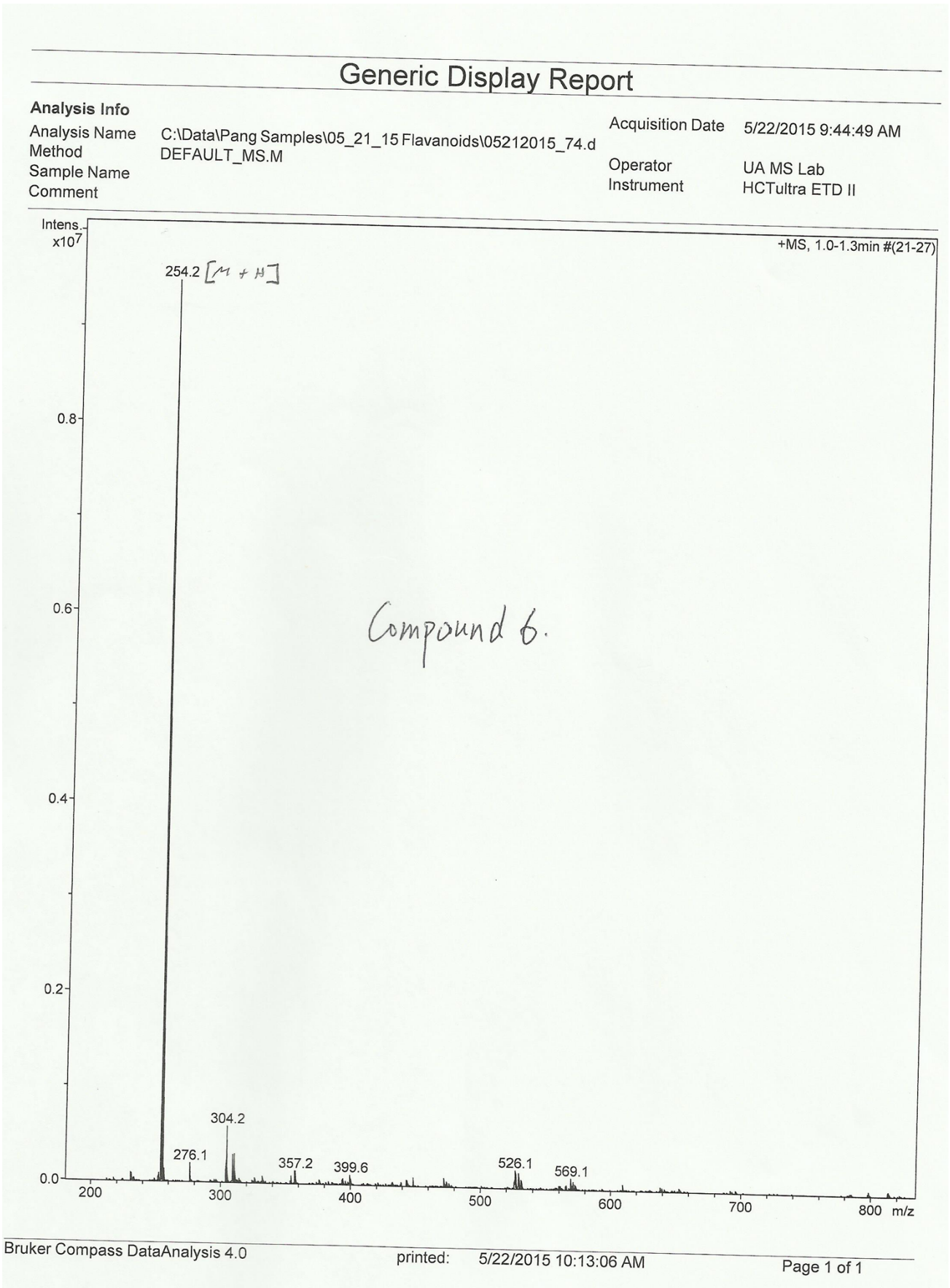


Fig. S17. ESI-MASS spectrum of **6**

8. Additional Data and Spectra

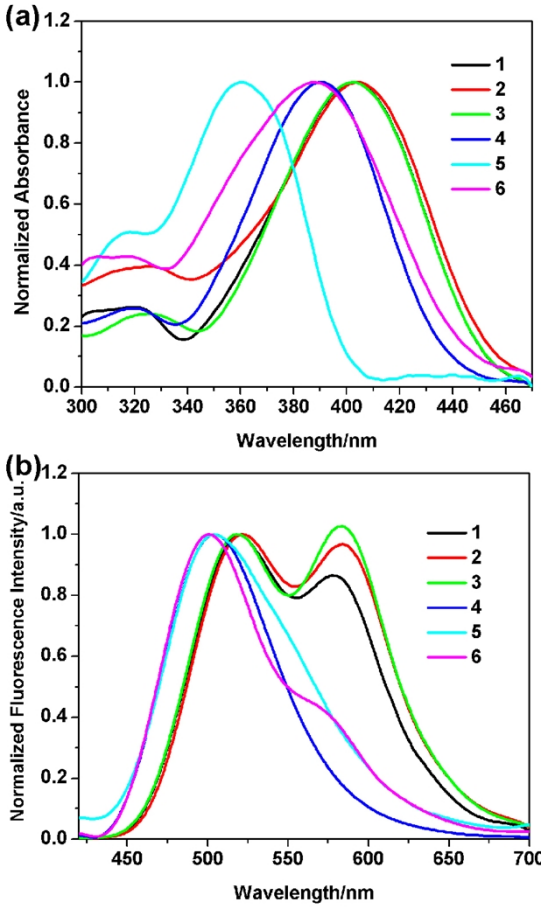


Fig. S18. Normalized absorbance (a) and fluorescence (b) spectra of flavonoid derivates **1-6** in DMSO. Fluorescence was excited at 400 nm.

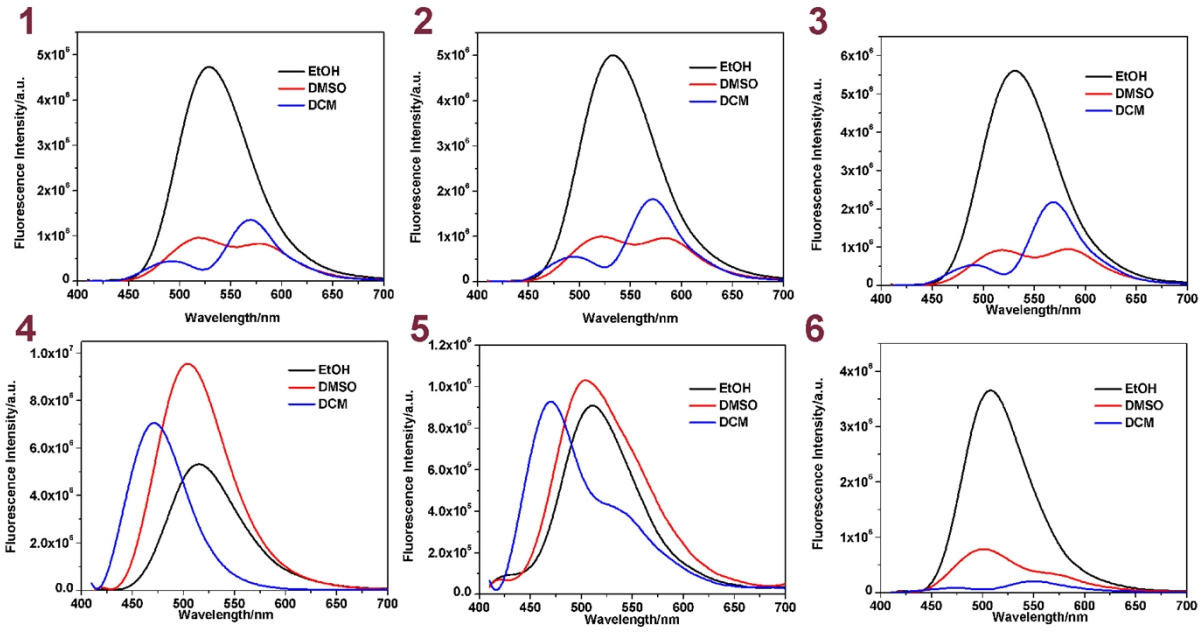


Fig. S19. The solvatochromism of Donor-acceptor (D-A) type flavone dyes **1-6** in EtOH, DMSO, and DCM.

Table S1. Comparison of spectroscopic properties of compounds **1-6** in DMSO. [a] λabs and λem, unit= nm

	λabs[a]	λem[a]	Φ/%
1	403	518, 578	10.0
2	403	521, 584	14.1
3	404	518, 584	11.0
4	390	504	37.6
5	363	505	5.4
6	394	501, 575	7.2

Table S2. Comparison of spectroscopic properties of compounds **1-6** with or without BSA. [**1-6**]=10 μM, [BSA]= 1.0 mg/ml, 10 mM PBS buffer solution containing 0.5% DMSO, pH =7.4. [a] unit= nm, (+)= with BSA, (-)= without BSA; [b] $F_{(+)}$ = fluorescence intensity at $λem_{(+)}$, $F_{(-)}$ = fluorescence intensity at $λem_{(-)}$

	1	**2**	**3**	**4**	**5**	**6**
$λabs_{(-)}$[a]	403	410	400	398	357	373
$λabs_{(+)}$[a]	416	415	409	398	365	386
$λem_{(-)}$[a]	560	555	582	490	452	522
$λem_{(+)}$[a]	556	506	552	484	514	512
$F_{(+)}/F_{(-)}$[b]	38	740	170	1100	7.0	29

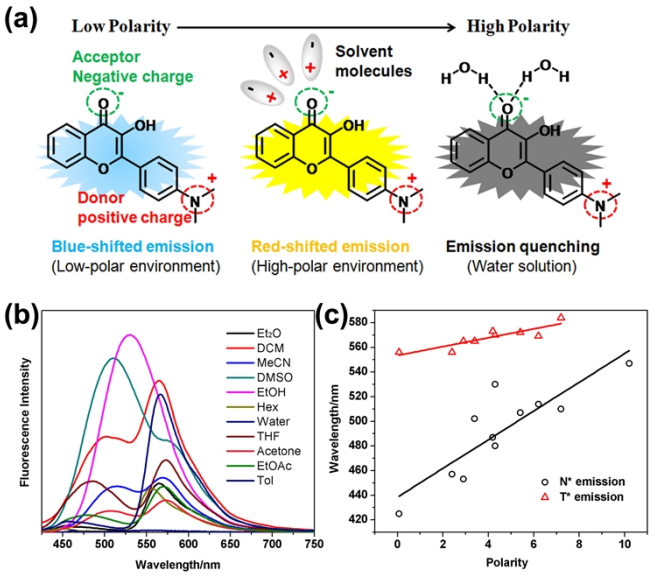


Fig. S20. (a) The solvatochromism of Donor-acceptor (D-A) type flavone dye **1**. (b) Fluorescence spectra and (c) Emission peaks of compound **1** in different solvents.

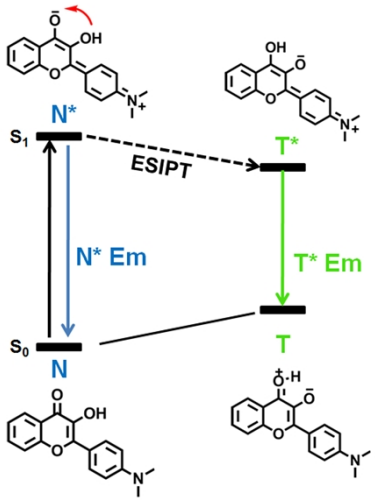


Fig. S21. The ESIPT process of flavone **1**

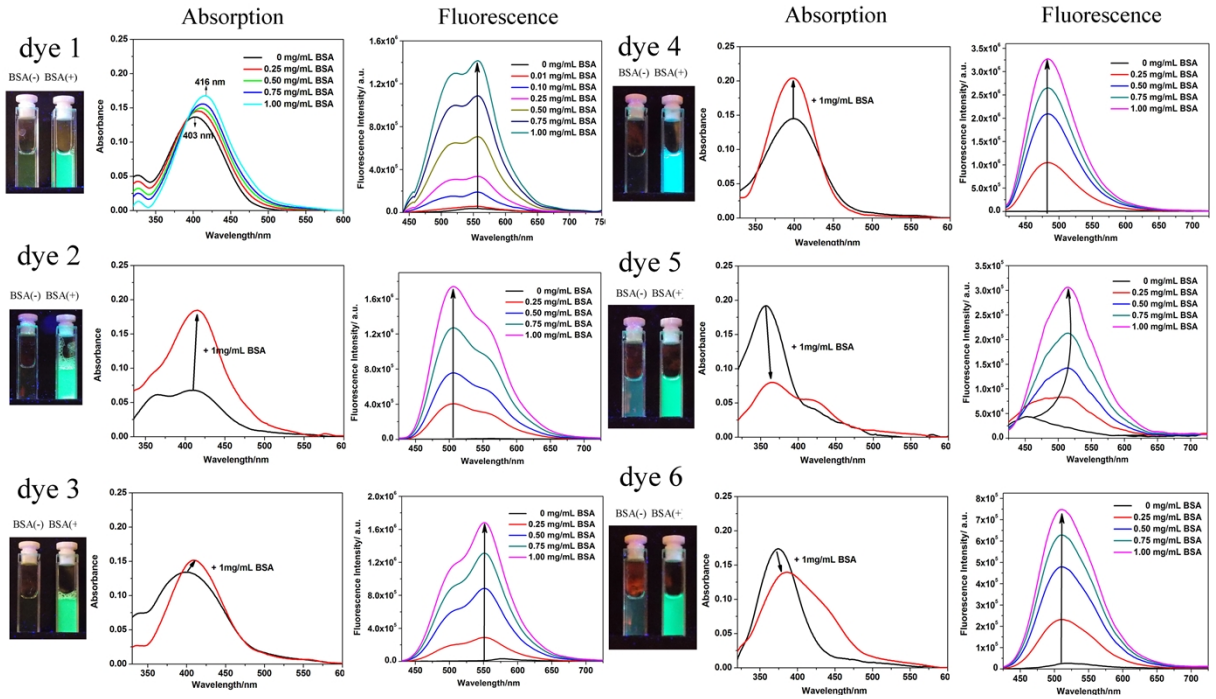


Fig. S22. Absorbance and fluorescence spectra changes of flavone derivatives **1-6** (10 μM, 10 mM of PBS buffered, containing 0.5% DMSO as a cosolvent) upon addition of different concentration of BSA (0~1.0 mg/ml). Fluorescence spectra of flavone derivatives were excited at 400 nm. The photographs of compounds 1-6 solutions in the absence and the presence of BSA were all excited by handheld UV lamp (365 nm).

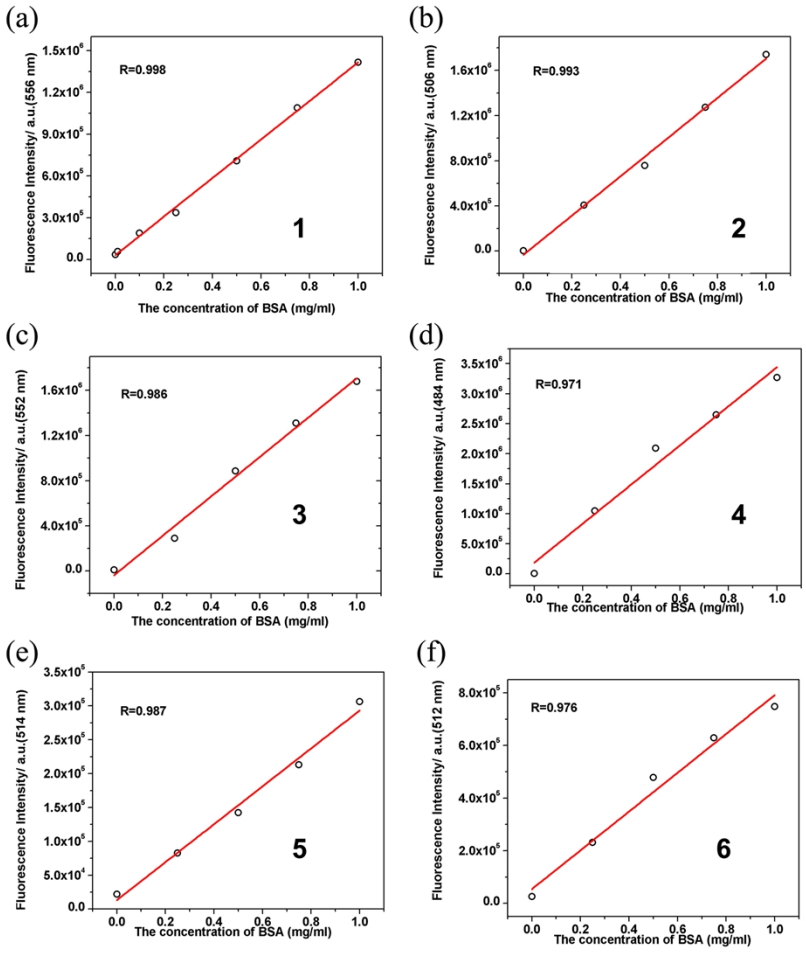


Fig. S23. The fluorescent intensities of compounds **1-6** with different concentration of BSA. [**1-6**]=10 μM, [BSA]= 0~1.0 mg/ml.

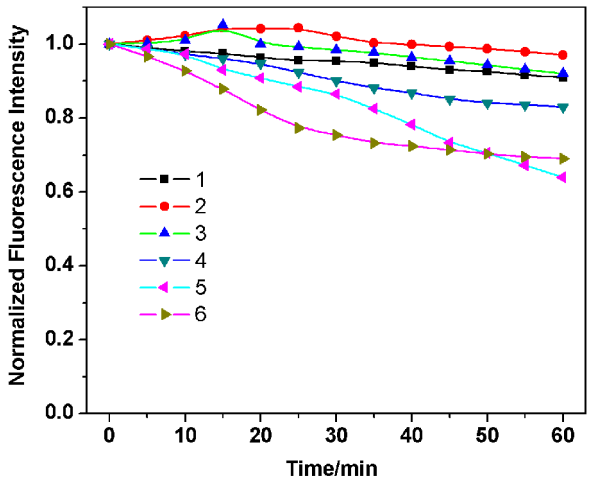


Fig. S24. Photostability of dye-BSA complex (10 μM of compounds 1-6 with 1.0 mg/ml of BSA in 10 mM PBS buffer containing 0.5% DMSO, pH=7.4) under the excitation of UV lamp.

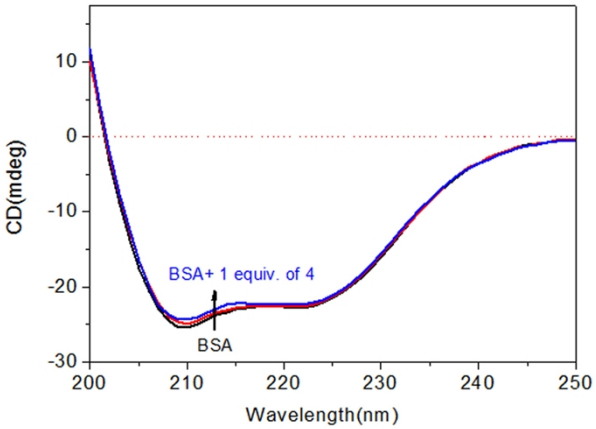


Fig. S25. CD spectra of 1 μM of BSA in PBS buffer (pH=7.4) upon addition of 0, 0.5, and 1 μM of compound 4. Binding of dye with serum albumin is expected to give an induced CD (ICD) signal due to the changes in the local asymmetric environment provided by the amino acid residues at the dye binding sites

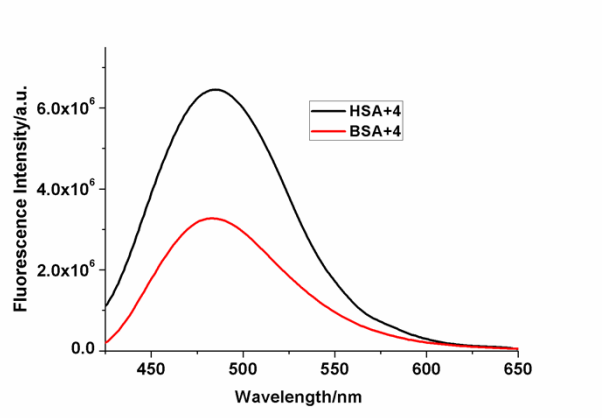


Fig. S26. Fluorescent spectra of HSA-4 complex (1 mg/mL of HSA with addition of 10 μM of **4**) and BSA-4 complex (1 mg/mL of BSA with addition of 10 μM of **4**) in PBS buffer (pH=7.4).