Supplementary Material (ESI) for Medicinal Chemistry Communications

< Supporting Information >

Discovery of a Novel Benzopyranyl Compound as a Potent *In Vitro* and *In Vivo* Osteogenic Agent

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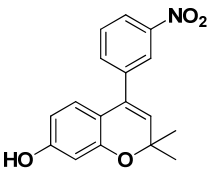
I. General Information about Synthesis

^{1}H and ^{13}C NMR spectra were recorded on a Varian Inova-500 [Varian Assoc., Palo Alto, USA], and chemical shifts were measured in ppm relative to internal tetramethylsilane (TMS) standard or specific solvent signal. Multiplicity was indicated as follows: s (singlet); d (doublet); t (triplet); q (quartet); m (multiplet); dd (doublet of doublet); bs (broad singlet), etc. Coupling constants were reported in Hz. Routine mass analyses were performed on LC/MS system equipped with a reverse phase column (C-18, 50 × 2.1 mm, 5 μm) and photodiode array detector using electron spray ionization (ESI). The HRMS analyses were conducted at the Mass Spectrometry Laboratory of Seoul National University by direct injection on JEOL JMS AX505WA spectrometer using electron impact (EI) method. The purity was determined used by Shimadzu SCL-10AVP HPLC [Japan] instrument with Shimadzu Shim pack VP-ODS (C18, 150 × 4.6 mm) column.

All reagents in this synthetic procedure were purchase from Sigma-Aldrich [MO, USA] and TCI [Japan]. The progress of reaction was monitored using thin-layer chromatography (TLC) (silica gel 60 F_{254} 0.25 mm), and components were visualized by observation under UV light (254 and 365 nm) or by treating the TLC plates with anisaldehyde staining solution followed by heating. Silica gel 60 (0.040–0.063 mm) used in flash column chromatography was purchased from Merck [Germany]. All reactions were conducted in oven-dried glassware under dry argon atmosphere, unless otherwise specified. CH_2Cl_2 was distilled from CaH_2 immediately prior to use. Other solvents and organic reagents were purchased from commercial venders and used without further purification unless otherwise mentioned.

II. Full Characterization of 6b as a Novel Osteogenic Agent

*2,2-Dimethyl-4-(3-nitrophenyl)-2H-chromen-7-ol (***Compound 6b***)*

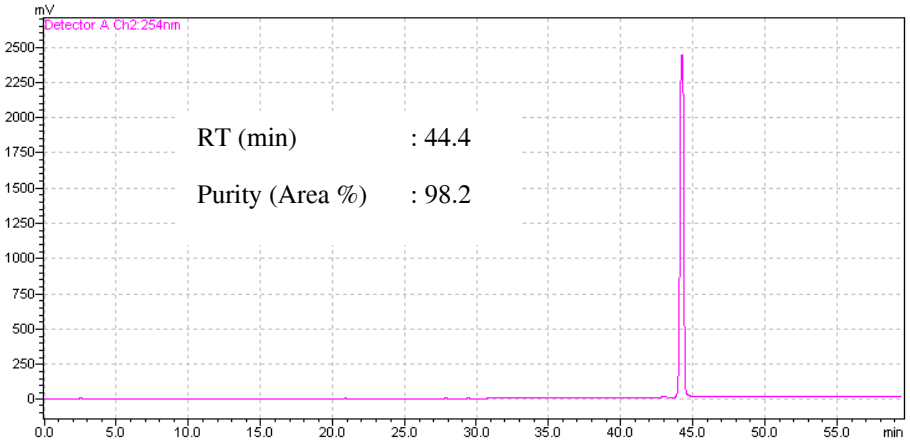


Yellow solid (83% from compound **4a**); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.22–8.20 (m, 2H), 7.67 (d, *J* = 8.0 Hz, 1H), 7.56 (t, *J* = 8.0 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 6.45 (d, *J* = 2.5 Hz, 1H), 6.35 (dd, *J* = 8.5 and 2.5 Hz, 1H), 5.56 (s, 1H), 5.42 (bs, 1H), 1.50 (s, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 157.3, 155.0, 148.6, 140.6, 135.0, 133.2, 129.5, 128.1, 126.3, 123.8, 122.8, 115.1, 108.2, 104.7, 76.5, 27.6; HRMS (EI+) *m/z* calculated for $C_{17}H_{15}NO_4$ $[M]^+$: 297.1001; Found: 297.1003.

HPLC Spectrum

[Condition]

	Time		
	0~10 min (Isocratic condition)	10~50 min (Gradient condition)	50~60 min (Isocratic condition)
Mobile phase A (H_2O / 0.1% TFA)	90%	90 → 0%	0%
Mobile phase B (MeOH / 0.1% TFA)	10%	10 → 100%	100%



MASS Data

LRMS

File: 5773
Sample: 6b

Date Run: 03-15-2010

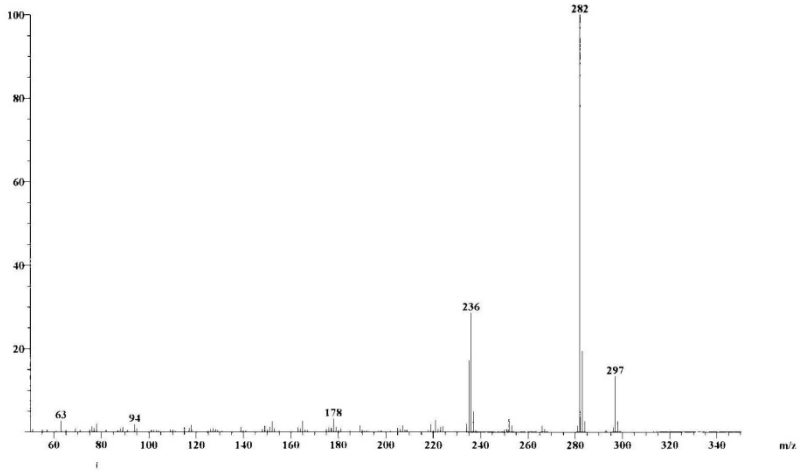
Time Run: 17:51:47

Instrument: JEOL JMS600
Inlet: Direct Probe

Ionization mode: EI+

Scan: 20-24
Base: m/z 282; 79.9%FS TIC: 2323019

R.T.: 1:04.26



HRMS

File: 5795
Sample: 6b

Date Run: 03-18-2010

Time Run: 16:38:43

Instrument: JEOL JMS600
Inlet: Direct Probe

Ionization mode: EI+

Scan: 78-101
Base: m/z 282; 16.9%FS TIC: 1699276

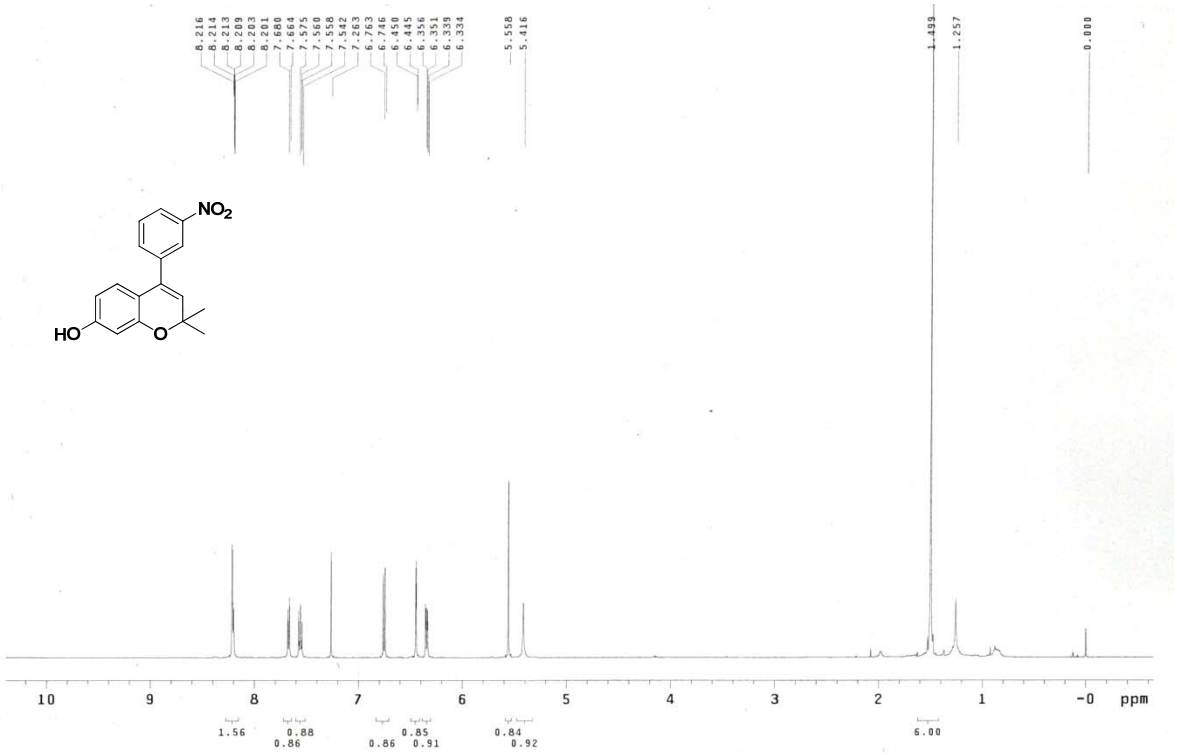
R.T.: 1:02.45

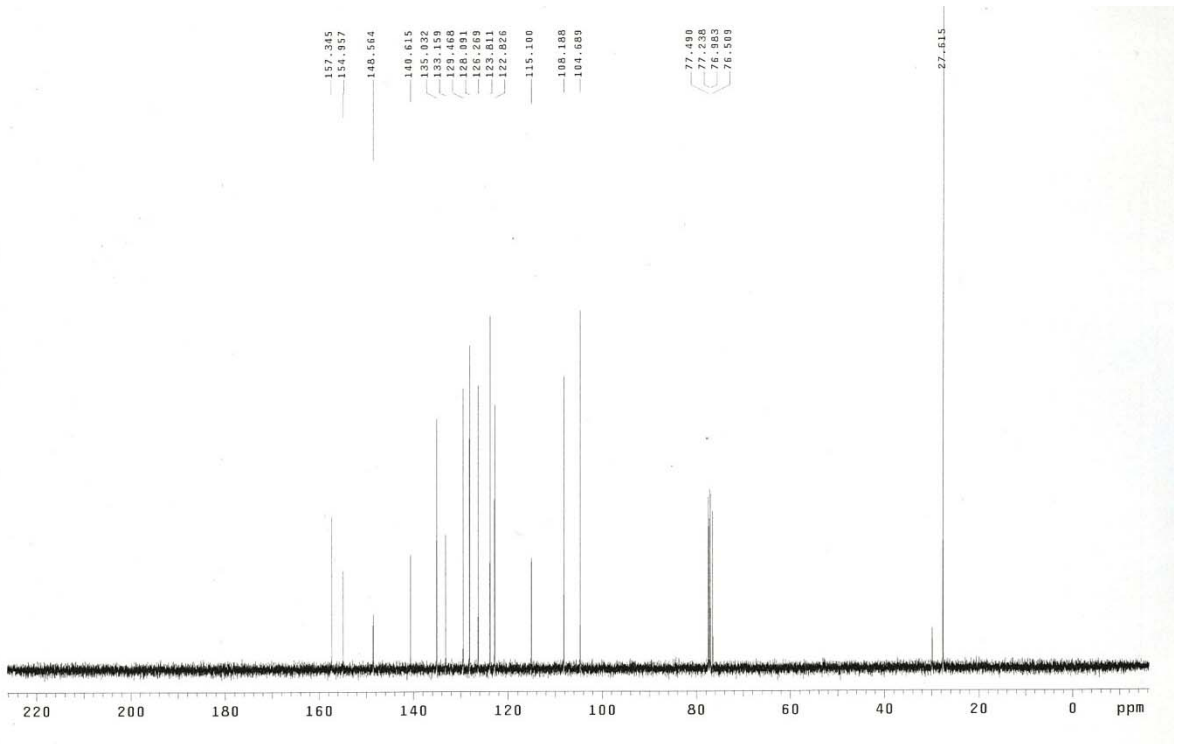
Selected Isotopes : $C_{0-50}H_{0-100}O_{0-10}N_{0-5}$

Error Limit : 20 ppm

Measured Mass	*% Base*	*Formula*	*Calculated Mass*	*Error*
297.1003	17.5%	$C_{14}H_{17}O_7$	297.0974	-9.7
		$C_{17}H_{15}O_4N$	297.1001	-0.7
		$C_{10}H_{19}O_9N$	297.1059	19.0
		$C_{20}H_{13}O\,N_2$	297.1028	8.3
		$C_{12}H_{15}O_6N_3$	297.0960	-14.0
		$C_{15}H_{13}O_3N_4$	297.0987	-5.3
		$C_8H_{17}O_8N_4$	297.1046	14.0
		$C_{18}H_{11}N_5$	297.1014	3.8

^{1}H and ^{13}C NMR Data





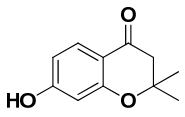
III. Synthetic Procedure[1] and Compound Characterizations

General procedure for the cyclization of hydroxyacetophenone

A solution of hydroxyacetophenone (1.0 equiv.) in EtOH was treated with pyrrolidine (3.0 equiv.) and then ketone (cyclopentanone 3.0 equiv., acetone 10.0 equiv., or ethyl levulinate 3.0 equiv.) and then heated at reflux for about 48 h. After the completion of reaction monitored by TLC, the reaction mixture was concentrated *in vacuo*. Evaporated residue was redissolved in ethyl acetate and washed several times with 1N HCl solution. After washing with brine, the combined organic layer was dried over anhydrous $MgSO_4$, filtrated, and evaporated *in vacuo*. Purification by silica-gel flash column chromatography gave the desired compound **2a~2d**.

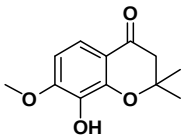
To a solution of purified compound **2** (1.0 equiv.) and imidazole (1.5 equiv.) dissolved in CH_2Cl_2, *tert*-butyldimethylsilyl chloride (TBDMSCl, 1.2 equiv.) was added and then stirred for about 3 h at room temperature. After the reaction completion monitored by TLC, the reaction mixture was diluted with ethylacetate and then washed with brine. The separated organic layer was dried over anhydrous $MgSO_4$, filtrated and evaporated *in vacuo*. The resulting mixture was purified with silica-gel flash column chromatography to provide desired product **3a~3d**.

Compound 2a



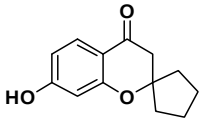
Colorless crystal (69% from 2',4'-dihydroxyacetophenone); this compound was previously reported.[1]

Compound 2b



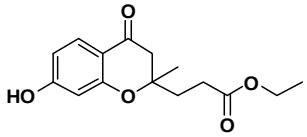
Colorless crystal (64% from 2',3'-dihydroxy-4'-methoxyacetophenone); this compound was previously reported.[1]

Compound 2c



Colorless crystal (58% from 2',4'-dihydroxyacetophenone); this compound was previously reported.[1]

Compound 2d



Colorless crystal (68% from 2',4'-dihydroxyacetophenone); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.74 (bs, 1H), 7.77 (d, *J* = 8.5 Hz, 1H), 6.55 (dd, *J* = 8.5 and 2.0 Hz, 1H), 6.39 (d, *J* = 2.0 Hz, 1H), 4.15 (q, *J* = 7.0 Hz, 2H), 2.79–2.60 (AB q, J_{AB}= 16.5 Hz, 2H), 2.53–2.49 (m, 2H), 2.21–2.15 (m, 1H), 2.05–1.99 (m, 1H), 1.40 (s, 3H), 1.26 (t, *J* = 7.0 Hz, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 192.2, 173.9, 165.0, 162.2, 129.1, 113.8, 110.7, 103.8, 80.5, 61.2, 47.0, 34.6, 29.1, 23.8, 14.4.

General procedure for the formation of vinyl triflate

A solution of compound **3** (1.0 equiv.) and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP, 1.3 equiv.) in anhydrous CH_2Cl_2 under N_2 atmosphere at 0 °C was treated with triflic anhydride

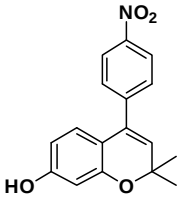
(Tf_2O, 1.2 equiv.). The reaction was stirred for 10 min at the same temperature. The reaction mixture was filtered to remove any solid and the filtrate was concentrated *in vacuo*. The residue was redissolved in ethyl acetate and washed with sat. $NaHCO_3$ solution and brine. The combined organic layer was dried over anhydrous $MgSO_4$, filtrated, and evaporated *in vacuo*. Purification by silica-gel flash column chromatography gave the desired compound **4a~4d**.

General procedure of Suzuki coupling for compound 6

Compound **4** (1.0 equiv.), arylboronic acid derivative (1.1 equiv.), $Pd(PPh_3)_4$ (5 mol%) and Na_2CO_3 (3.0 equiv.) were suspended in solvent mixture of EtOH:toluene:H_2O (1:1:0.5) and the reaction mixture was stirred at 70 °C for about 3 h. After reaction completion monitored by TLC, the resulting mixture was diluted with ethyl acetate and then washed with brine. The organic layer was dried over anhydrous $MgSO_4$, filtrated, and evaporated *in vacuo*. Purification by silica-gel flash column chromatography gave the desired compound **5a~5m**.

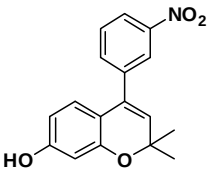
To a solution of purified compound **5** (1.0 equiv.) dissolved in tetrahydrofuran (THF), tetrabutylammonium fluoride (1M solution in THF, 1.5 equiv.) was added at 0 °C and then stirred for about 30 min at the same temperature. After the reaction completion monitored by TLC, the reaction mixture was diluted with ethylacetate and then washed with brine. The separated organic layer was dried over anhydrous $MgSO_4$, filtrated and evaporated *in vacuo*. Purification by silica-gel flash column chromatography gave the desired compound **6a~6m**.

Compound 6a



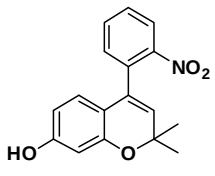
Yellow solid (87% from compound **4a**); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.25 (dd, *J* = 7.0 and 2.0 Hz, 2H), 7.51 (dd, *J* = 7.0 and 2.0 Hz, 2H), 6.77 (d, *J* = 8.5 Hz, 1H), 6.44 (d, *J* = 2.5 Hz, 1H), 6.34 (dd, *J* = 8.5 and 2.5 Hz, 1H), 5.56 (s, 1H), 5.16 (s, 1H), 1.50 (s, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 157.3, 155.0, 147.5, 145.8, 133.5, 129.7, 128.4, 126.4, 123.9, 115.1, 108.1, 104.7, 76.5, 27.6; LRMS (ESI) *m/z* calculated for $C_{17}H_{16}NO_4$ $[M+H]^+$: 298.11; Found: 298.08.

Compound 6b



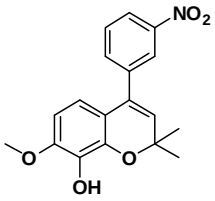
Yellow solid (83% from compound **4a**); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.22–8.20 (m, 2H), 7.67 (d, *J* = 8.0 Hz, 1H), 7.56 (t, *J* = 8.0 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 6.45 (d, *J* = 2.5 Hz, 1H), 6.35 (dd, *J* = 8.5 and 2.5 Hz, 1H), 5.56 (s, 1H), 5.42 (bs, 1H), 1.50 (s, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 157.3, 155.0, 148.6, 140.6, 135.0, 133.2, 129.5, 128.1, 126.3, 123.8, 122.8, 115.1, 108.2, 104.7, 76.5, 27.6; HRMS (EI+) *m/z* calculated for $C_{17}H_{15}NO_4$ $[M]^+$: 297.1001; Found: 297.1003.

Compound 6c



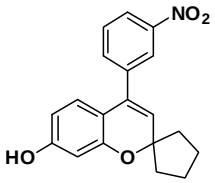
Yellow solid (77% from compound **4a**); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.95 (d, *J* = 7.5 Hz, 1H), 7.60 (t, *J* = 7.5 Hz, 1H), 7.49 (t, *J* = 7.5 Hz, 1H), 7.35 (d, *J* = 7.5 Hz, 1H), 6.44 (d, *J* = 8.5 Hz, 1H), 6.40 (d, *J* = 2.0 Hz, 1H), 6.25 (dd, *J* = 8.5 and 2.0 Hz, 1H), 5.35 (s, 1H), 1.48 (s, 3H), 1.45 (s, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 157.6, 154.3, 149.7, 133.5, 133.0, 132.2, 131.5, 128.9, 126.1, 125.8, 124.3, 115.1, 108.3, 104.4, 76.5, 28.1, 26.8; LRMS (ESI) *m/z* calculated for $C_{17}H_{16}NO_4$ $[M+H]^+$: 298.11; Found: 298.19.

Compound 6d



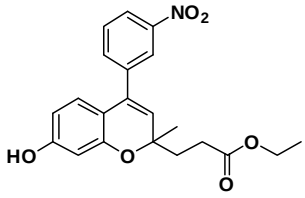
Yellow solid (81% from compound **4b**); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.23–8.20 (m, 2H), 7.69 (d, *J* = 7.5 Hz, 1H), 7.57 (t, *J* = 7.5 Hz, 1H), 6.45–6.40 (m, 2H), 5.62 (s, 1H), 5.58 (bs, 1H), 3.90 (s, 3H), 1.55 (s, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 148.5, 148.3, 140.7, 140.4, 135.0, 134.7, 133.4, 129.4, 128.8, 123.8, 122.8, 115.9, 103.8, 77.0, 56.3, 27.6; LRMS (ESI) *m/z* calculated for $C_{18}H_{18}NO_5$ $[M+H]^+$: 328.12; Found: 327.99.

Compound 6e



Yellow solid (79% from compound **4c**); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.22–8.19 (m, 2H), 7.68 (d, *J* = 8.0 Hz, 1H), 7.55 (t, *J* = 8.0 Hz, 1H), 6.75 (d, *J* = 8.5 Hz, 1H), 6.42 (d, *J* = 2.5 Hz, 1H), 6.33 (dd, *J* = 8.5 and 2.5 Hz, 1H), 5.62 (s, 1H), 5.31 (bs, 1H), 2.23–2.19 (m, 2H), 1.96–1.90 (m, 2H), 1.77–1.64 (m, 4H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 157.2, 155.0, 148.6, 140.6, 135.0, 133.9, 129.5, 127.3, 126.1, 123.8, 122.8, 115.9, 108.1, 104.8, 87.4, 39.0, 23.7; LRMS (ESI) *m/z* calculated for $C_{19}H_{18}NO_4$ $[M+H]^+$: 324.12; Found: 324.14.

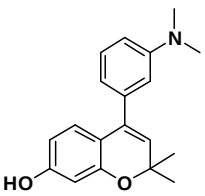
Compound 6f



Yellow solid (83% from compound **4d**); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 8.22–8.20 (m, 2H), 7.67 (d, *J* = 7.5 Hz, 1H), 7.56 (t, *J* = 7.5 Hz, 1H), 6.72 (d, *J* = 8.5 Hz, 1H), 6.44 (d, *J* = 2.5 Hz, 1H), 6.36 (dd, *J* = 8.5 and 2.5 Hz, 1H), 5.45 (s, 1H), 4.12 (q, *J* = 7.0 Hz, 2H), 2.60–2.47 (m, 2H), 2.16–2.08 (m, 2H), 1.46 (s, 3H), 1.22 (t, *J* = 7.0 Hz, 3H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 174.2,

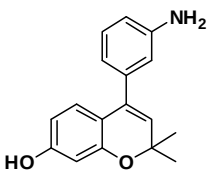
158.5, 154.7, 148.6, 148.5, 140.4, 135.0, 134.4, 129.5, 126.2, 125.7, 123.7, 122.8, 113.9, 108.4, 104.5, 78.0, 60.9, 35.9, 29.6, 26.0, 14.3; LRMS (ESI) *m/z* calculated for $C_{21}H_{22}NO_6$ $[M+H]^+$: 384.14; Found: 384.22.

Compound 6g



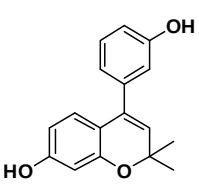
White solid (81% from compound **4a**); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.25 (t, *J* = 8.5 Hz, 1H), 6.91(d, *J* = 8.5 Hz, 1H), 6.76–6.74 (m, 1H), 6.71–6.70 (m, 2H), 6.38 (d, *J* = 2.5 Hz, 1H), 6.23 (dd, *J* = 8.0 and 2.5 Hz, 1H), 5.47 (s, 1H), 2.95 (s, 6H), 1.47 (s, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 156.8, 155.0, 150.8, 139.7, 135.4, 129.2, 127.1, 126.1, 117.8, 116.3, 113.4, 112.5, 107.7, 104.2, 76.5, 41.1, 27.9; LRMS (ESI) *m/z* calculated for $C_{19}H_{22}NO_2$ $[M+H]^+$: 296.17; Found: 296.34.

Compound 6h



White solid (64% from compound **4a**); ^{1}H-NMR (500 MHz, MeOD): δ 7.11 (t, *J* = 7.5 Hz, 1H), 6.83 (d, *J* = 8.5 Hz, 1H), 6.71 (d, *J* = 7.5 Hz, 1H), 6.69 (s, 1H), 6.63 (d, *J* = 7.5 Hz, 1H), 6.29 (d, *J* = 2.5 Hz, 1H), 6.26 (dd, *J* = 8.5 and 2.5 Hz, 1H), 5.41 (s, 1H), 1.41 (s, 6H); ^{13}C-NMR (125 MHz, MeOD): δ 159.8, 156.1, 148.8, 141.2, 136.7, 130.1, 127.8, 126.3, 119.9, 117.0, 116.2, 116.0, 108.7, 104.9, 77.1, 28.0; LRMS (ESI) *m/z* calculated for $C_{17}H_{18}NO_2$ $[M+H]^+$: 268.13; Found: 267.98.

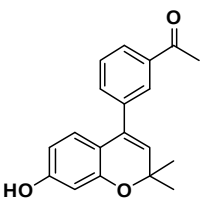
Compound 6i



White solid (83% from compound **4a**); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.24 (t, *J* = 8.0 Hz, 1H), 6.89 (d, *J* = 7.5 Hz, 1H), 6.86 (d, *J* = 8.0 Hz, 1H), 6.83 (dd, *J* = 8.5 and 2.5 Hz, 1H), 6.77–6.76 (m, 1H), 6.40 (d, *J* = 2.5 Hz, 1H),

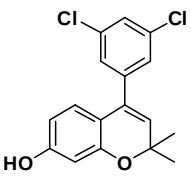
6.29 (dd, *J* = 8.5 and 2.5 Hz, 1H), 5.49 (bs, 2H), 5.47 (s, 1H), 1.46 (s, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 156.8, 155.6, 154.8, 140.5, 134.4, 129.8, 126.9, 126.5, 121.4, 116.0, 115.8, 114.9, 107.9, 104.4, 76.6, 27.7; LRMS (ESI) *m/z* calculated for $C_{17}H_{17}O_3$ $[M+H]^+$: 269.12; Found: 269.13.

Compound 6j



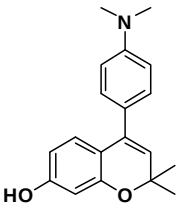
White solid (77% from compound **4a**); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.95–7.93 (m, 2H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.48 (t, *J* = 7.5 Hz, 1H), 6.77 (d, *J* = 8.0 Hz, 1H), 6.45 (d, *J* = 2.5 Hz, 1H), 6.33 (dd, *J* = 8.0 and 2.5 Hz, 1H), 5.81 (bs, 1H), 5.51 (s, 1H), 2.64 (s, 3H), 1.49 (s, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 199.0, 157.3, 155.0, 139.5, 137.4, 134.1, 133.8, 128.8, 127.9, 127.2, 126.5, 115.6, 108.0, 104.5, 76.5, 27.7, 27.0; LRMS (ESI) *m/z* calculated for $C_{19}H_{19}O_3$ $[M+H]^+$: 295.13; Found: 295.11.

Compound 6k



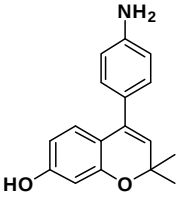
White solid (85% from compound **4a**); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.34 (t, *J* = 1.5 Hz, 1H), 7.22 (d, *J* = 1.5 Hz, 2H), 6.80 (d, *J* = 8.5 Hz, 1H), 6.41 (d, *J* = 2.5 Hz, 1H), 6.34 (dd, *J* = 8.5 and 2.5 Hz, 1H), 5.49 (s, 1H), 5.17 (bs, 1H), 1.47 (s, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 157.2, 154.9, 141.9, 135.1, 132.9, 127.9, 127.8, 127.4, 126.4, 115.1, 108.1, 104.6, 76.4, 27.6; LRMS (ESI) *m/z* calculated for $C_{17}H_{15}ClO_2$ $[M+H]^+$: 321.04; Found: 321.10.

Compound 6l



White solid (82% from compound **4a**); ^{1}H-NMR (500 MHz, $CDCl_3$): δ 7.22 (d, *J* = 8.5 Hz, 2H), 6.91 (d, *J* = 8.5 Hz, 1H), 6.77 (d, *J* = 8.5 Hz, 2H), 6.39 (d, *J* = 2.5 Hz, 1H), 6.22 (dd, *J* = 8.5 and 2.5 Hz, 1H), 5.41 (s, 1H), 2.98 (s, 6H), 1.45 (s, 6H); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 156.7, 155.1, 150.3, 134.4, 129.7, 126.9, 125.2, 116.5, 112.8, 107.6, 104.3, 76.5, 41.0, 27.9; LRMS (ESI) *m/z* calculated for $C_{19}H_{22}NO_2$ $[M+H]^+$: 296.17; Found: 296.07.

Compound 6m



White solid (70% from compound **4a**); ^{1}H-NMR (500 MHz, MeOD): δ 7.05 (d, *J* = 8.5 Hz, 2H), 6.83 (d, *J* = 8.5 Hz, 1H), 6.73 (d, *J* = 8.5 Hz, 2H), 6.28–6.25 (m, 2H), 5.36 (s, 1H), 1.40 (s, 6H); ^{13}C-NMR (125 MHz, MeOD): δ 159.7, 156.3, 148.5, 136.3, 130.6, 129.9, 127.8, 125.4, 116.6, 116.4, 108.7, 104.9, 77.1, 28.0; LRMS (ESI) *m/z* calculated for $C_{17}H_{18}NO_2$ $[M+H]^+$: 268.13; Found: 268.27.

IV. General Information about Biological Procedure

ALP assay and staining

To assess ALP activities, C3H10T1/2 cells were seeded into 24-well plate at 3×10^4 cells/well. After 24 hrs, each small molecule (0.25, 0.5, 1, 2 and 4 μM) or vehicle was treated and Wnt-3a (100 ng/ml) was used as a control. After incubation for 72 hrs, cell lysates were prepared and ALP activities were measured via the previously described manners. All data were expressed as means ± SDs of triplicate wells. ALP staining was carried out using an alkaline phosphatase kit according to the manufacturer's instructions (Promega, Southampton, U.K.). Briefly, after 5 min fixation with 10% formalin, the osteogenic culture was incubated in a mixture of nitroblue tetrazolium chloride and 5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt within 1 h. The resulting blue, insoluble, and granular dye deposit indicated sites of alkaline phosphatase activity.

Establishment of TOPflash transfected C3H10T1/2 cells

To generate the high-throughput screening system, 3×10^5 C3H10T1/2 cells were plated onto a 60-mm culture dish. After 24 h incubation at 37°C, TOPflash plasmid was transfected by using Lipofectamine Plus system (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's instructions. To set up for the stable cell line selection, standard media was replaced by media containing 1,000 μg/ml G418 after 48 h. Stably transfected TOPflash-C3H10T1/2 cells were established 3 weeks later, based upon this selection process. 1×10^4 TOPflash-C3H10T1/2 cells were plated and incubated for 24 h followed by treatment of small molecules (2 μM) or Wnt-3a (100 ng/mL) for 48 h before cell harvest. The dual luciferase assay was performed using the Promega Luciferase assay system and activity was

measured using a luminometer (Lumat LB 9507, Berthold, Germany). All data were expressed as means ± SDs of triplicate wells.

Animal experiments

Female C57BL/6 10-week old mice were purchased from Japan SLC Lab (Hamamatsu, Japan). All animal experiments were performed under approval from the Institutional Animal Care and Use Committee of Seoul National University and complied with the National Research Council's "Guidelines for the Care and Use of Laboratory Animals" (revised 1996). 12-Week-old female C57BL/6 mice were divided into five groups, as described above. On study day 0, bilateral OVX or Sham-op was performed by the standard method for "OVX" or "Sham-op" groups, respectively. OVX mice were permitted to lose bone mass for 4 weeks, in order to establish osteopenia, before the initiation of treatments. Compound **6b**, at a dose of 5 or 10 mg/kg/d, was administered orally, using an oral zonde needle, from study day 28 to day 56 (for 4 weeks, 5 days per week). The compound was prepared in a 40% PEG400 / 5% DMSO vehicle (Sigma-Aldrich, MO). For OVX + estradiol group, 60 mg/kg of 17β-estradiol was weekly injected intramuscularly. BMD (g/cm^2) and body composition was measured, using a Lunar PIXImus densitometer (software version 2.0; GE Lunar, Madison, WI), on weeks 0, 4, and 8, for the remaining mice. For three-dimensional measurement with Micro-CT, femurs were obtained 4 weeks after treatment, fixed overnight in 70% ethanol and analyzed using a Micro-CT scanner and associated analysis software (model 1076, Skyscan, Antwerp, Belgium) at 9-μm voxel size.

V. Supporting Figure

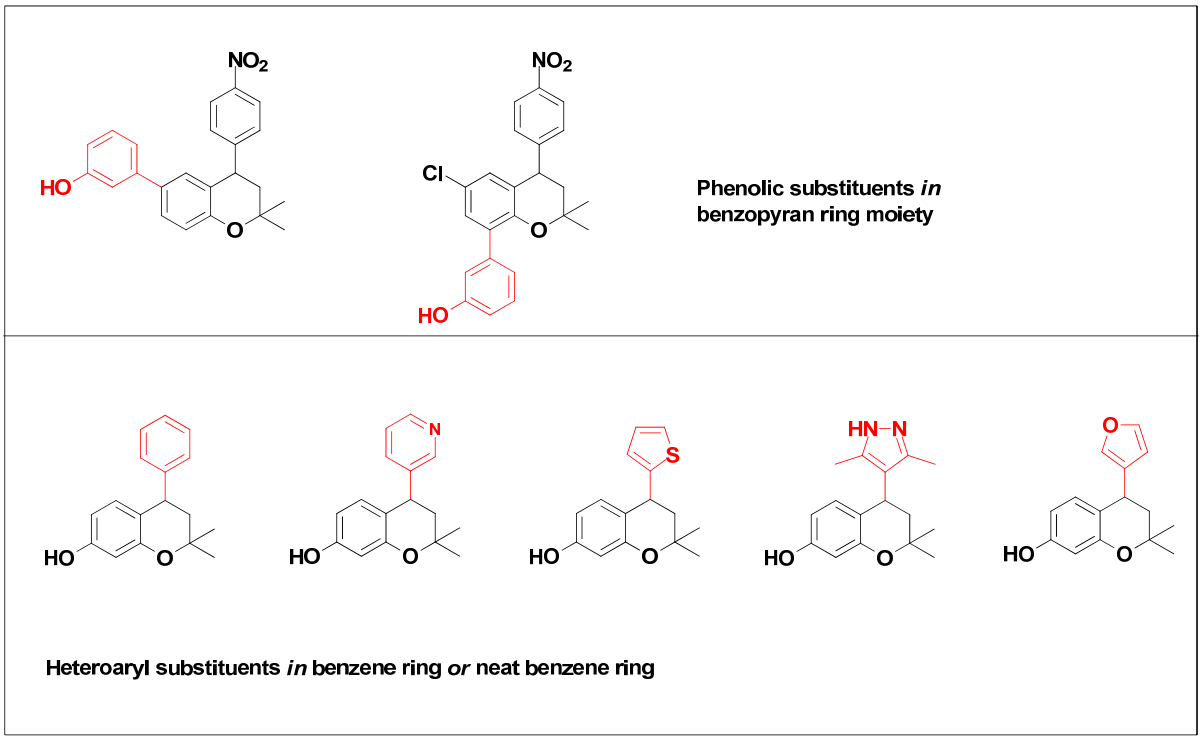


Fig. S1 Representative compounds without osteogenic activity based on our initial library screening.

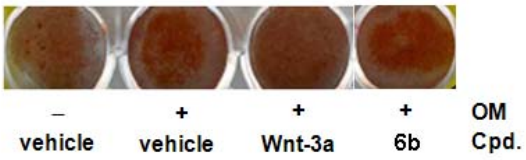


Fig. S2 Alizarin red staining of cell calcification treated with **6b** (4 μM) in osteogenic medium (OM) containing L-ascorbic acid and β-glycerophosphate.

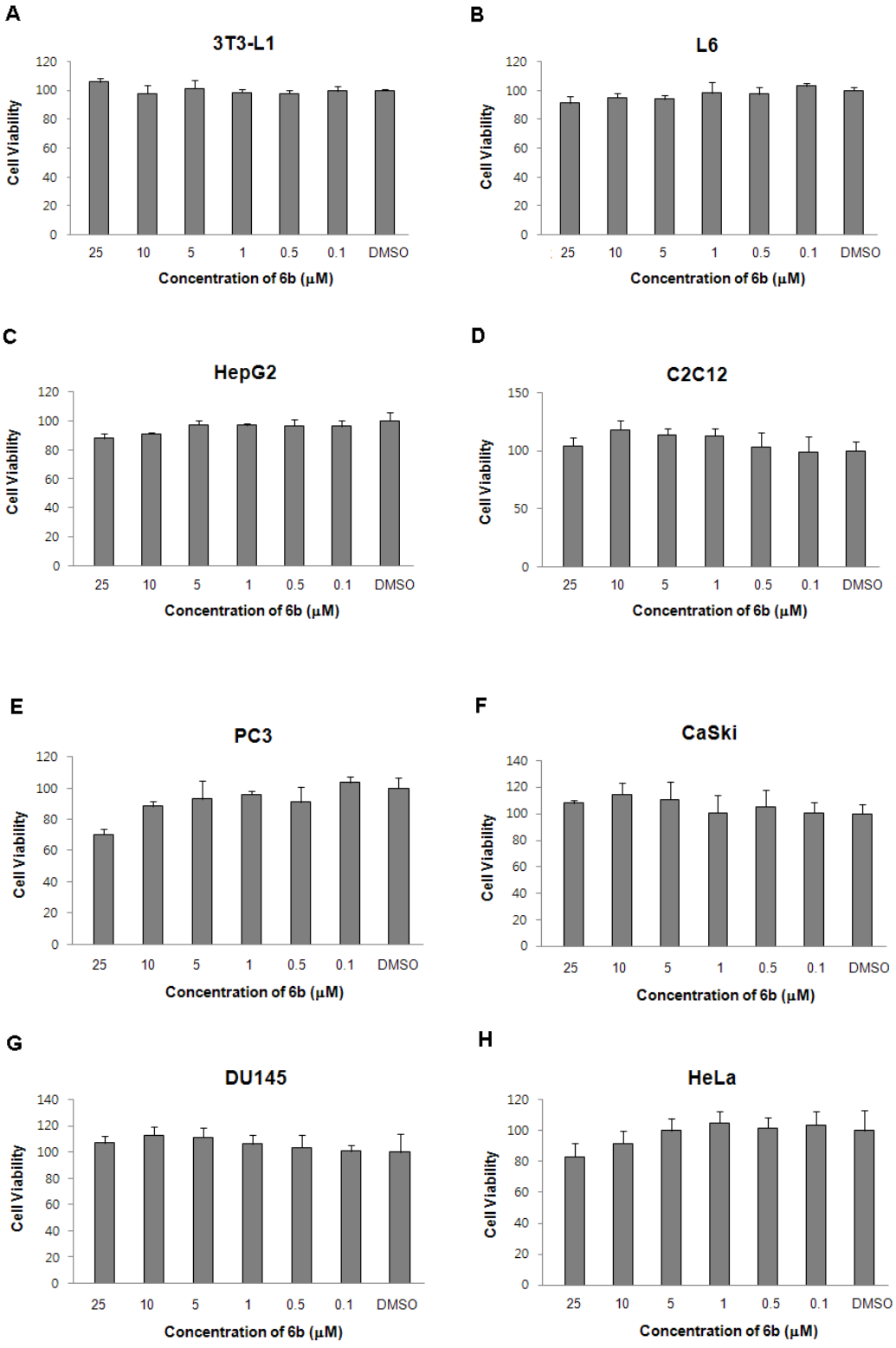
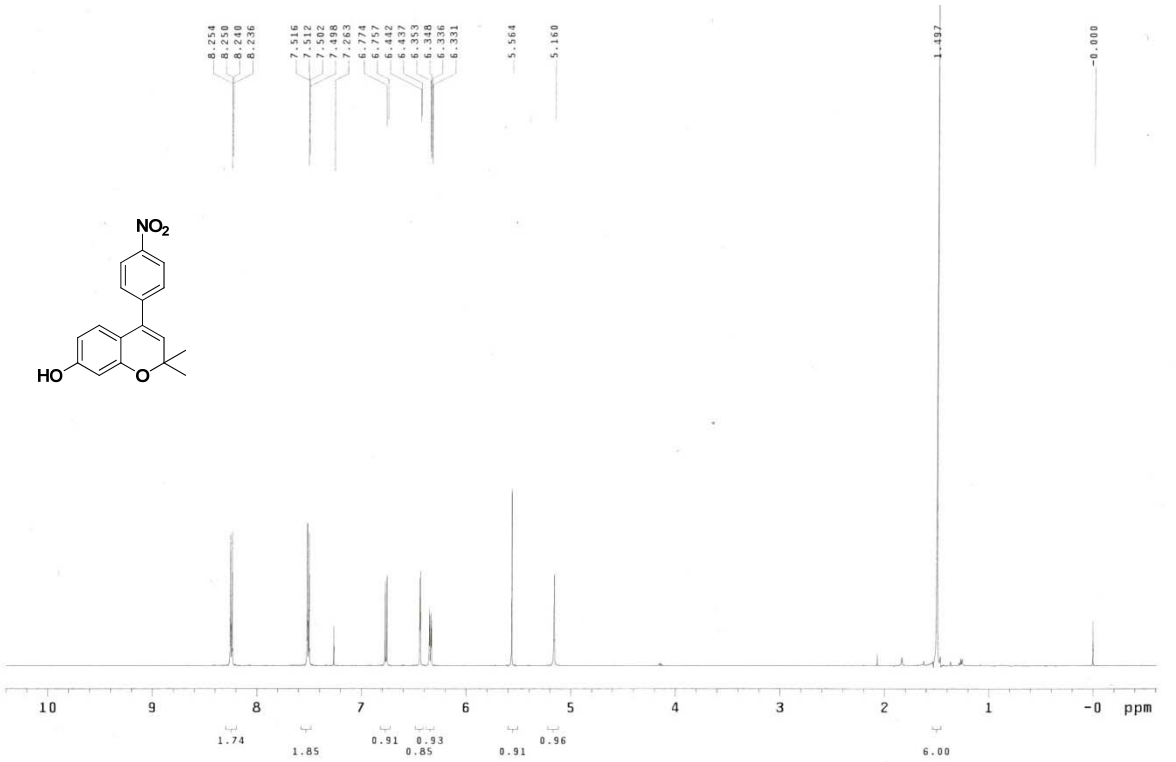
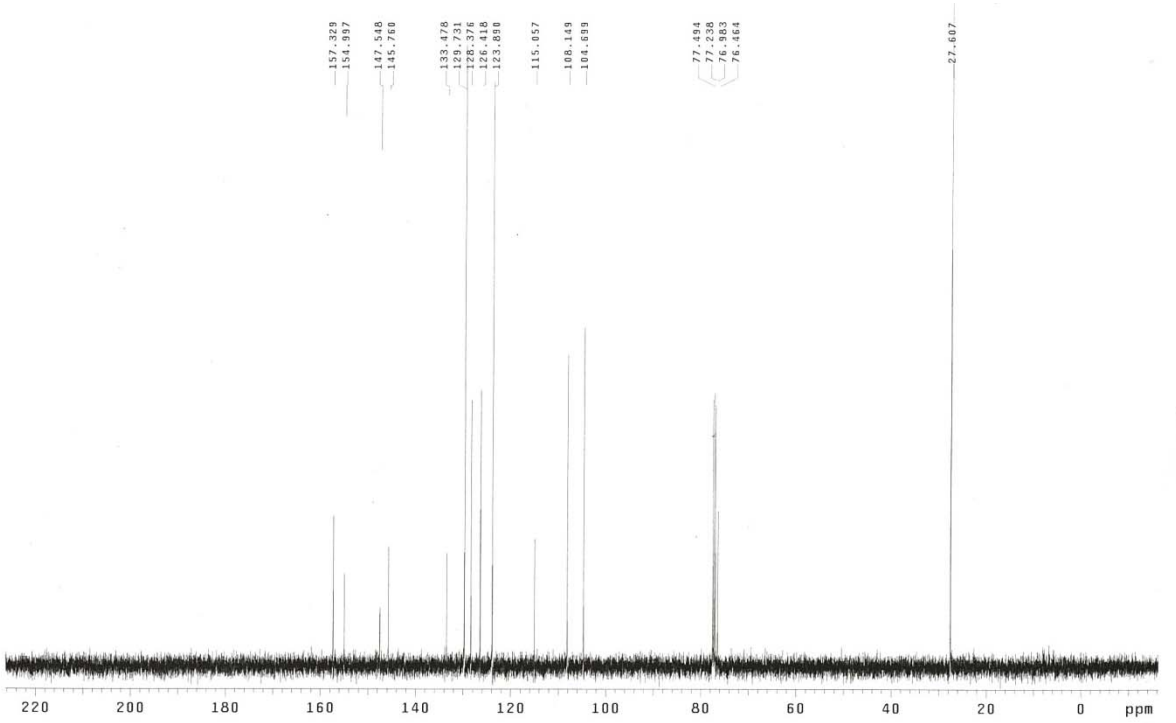


Fig. S3 **6b** does not affect the cell viability in various cell lines monitored by mitochondrial activity using WST-1 assay at the varied concentration for 24 h incubation.

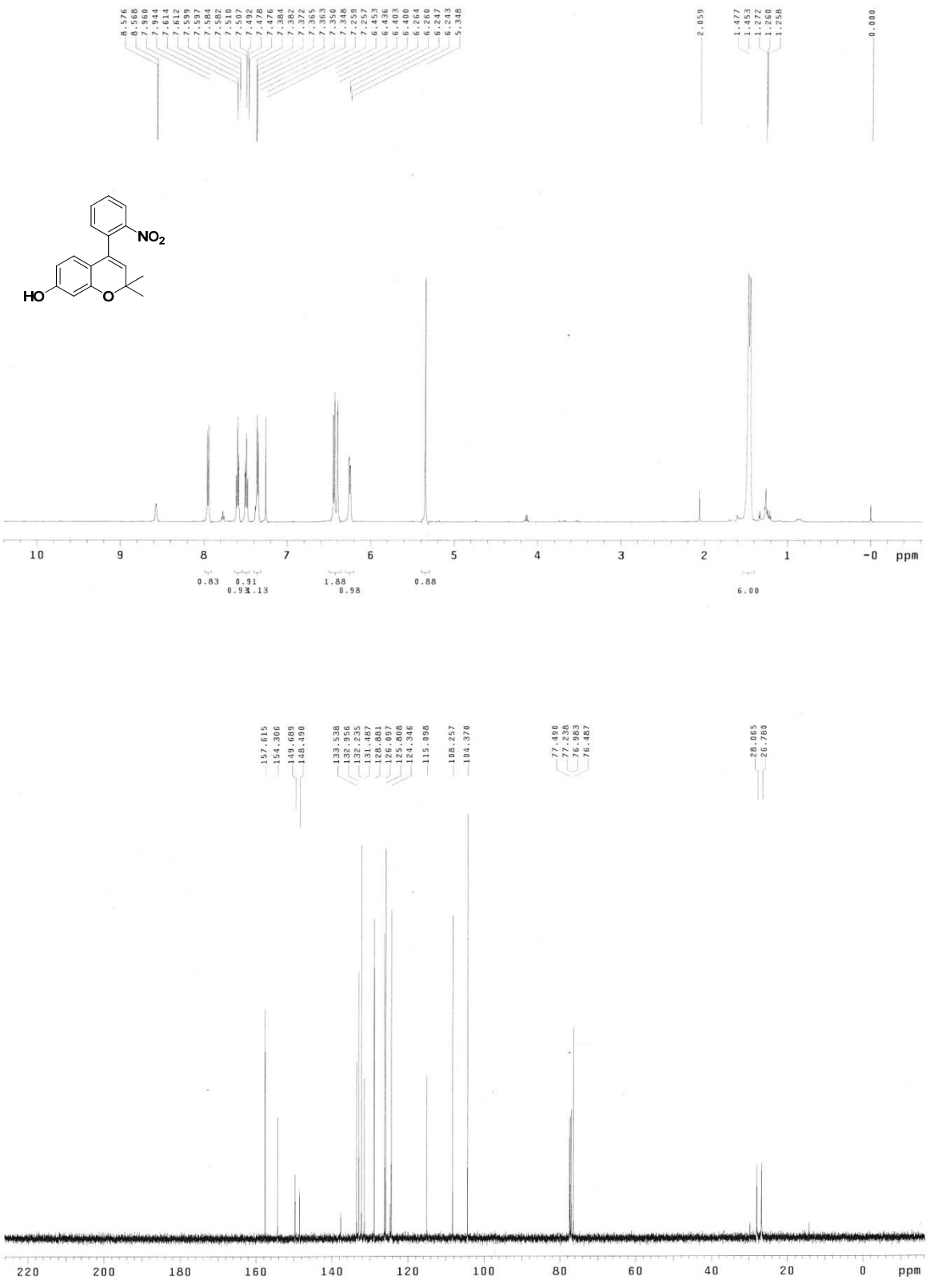
VI. NMR Data

6a





6c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7.612
7.599
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7.582
7.510
7.507
7.492
7.478
7.476
7.384
7.382
7.372
7.365
7.363
7.350
7.348
7.259
7.257
6.453
6.436
6.403
6.400
6.264
6.260
6.247
6.243
5.348
2.059
1.477
1.453
1.272
1.260
1.258
0.000
NO2
HO
O
10
9
8
7
6
5
4
3
2
1
-0
ppm
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0.91
0.93
1.13
1.88
0.98
0.88
6.00
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154.306
149.689
148.490
133.538
132.956
132.235
131.487
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126.697
126.008
124.346
115.098
108.257
104.370
77.490
77.238
76.983
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26.780
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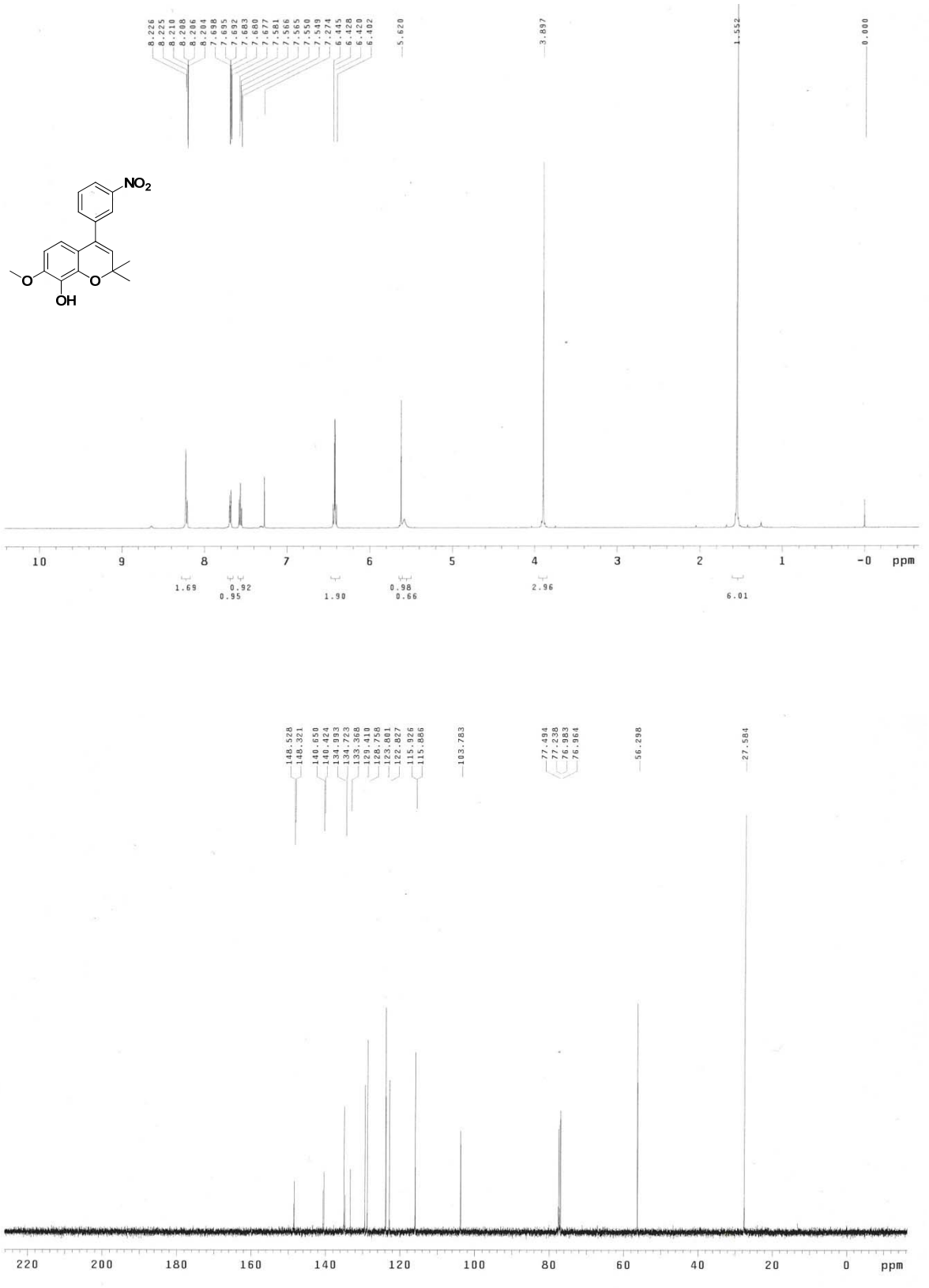
100
80
60
40
20
0
ppm

6d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7.680
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7.581
7.566
7.565
7.550
7.549
7.274
6.445
6.428
6.420
6.402
5.620
3.897
1.552
0.000
NO2
O
O
OH
10
9
8
7
6
5
4
3
2
1
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ppm
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0.92
0.95
1.90
0.98
0.66
2.96
6.01
148.528
148.321
140.650
140.424
134.993
134.723
133.368
129.410
128.758
123.601
122.827
115.926
115.886
103.783
77.494
77.238
76.983
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140
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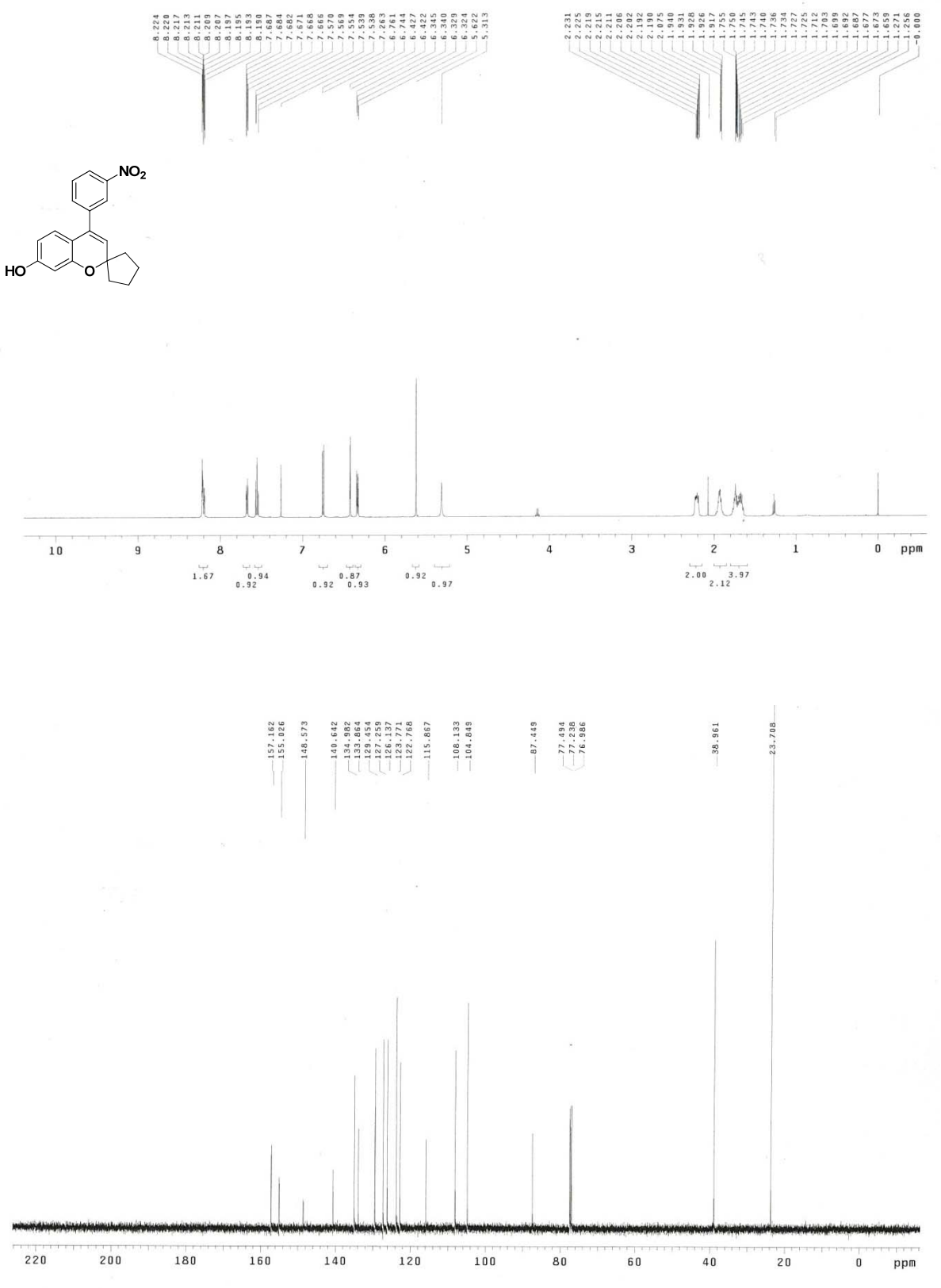
100
80
60
40
20
0
ppm

6e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8.197
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8.190
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7.684
7.682
7.671
7.668
7.666
7.570
7.569
7.554
7.539
7.538
6.763
6.761
6.744
6.427
6.422
6.345
6.340
6.329
6.324
5.622
5.313
2.231
2.225
2.219
2.215
2.211
2.206
2.202
2.192
2.190
2.075
2.040
1.931
1.928
1.926
1.917
1.755
1.750
1.745
1.743
1.740
1.736
1.734
1.727
1.725
1.712
1.703
1.699
1.692
1.687
1.677
1.673
1.659
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1.256
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HO
O
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9
8
7
6
5
4
3
2
1
0
ppm
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0.92
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0.92
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2.12
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129.454
127.259
126.137
123.771
122.768
115.867
108.133
104.849
87.449
77.494
77.238
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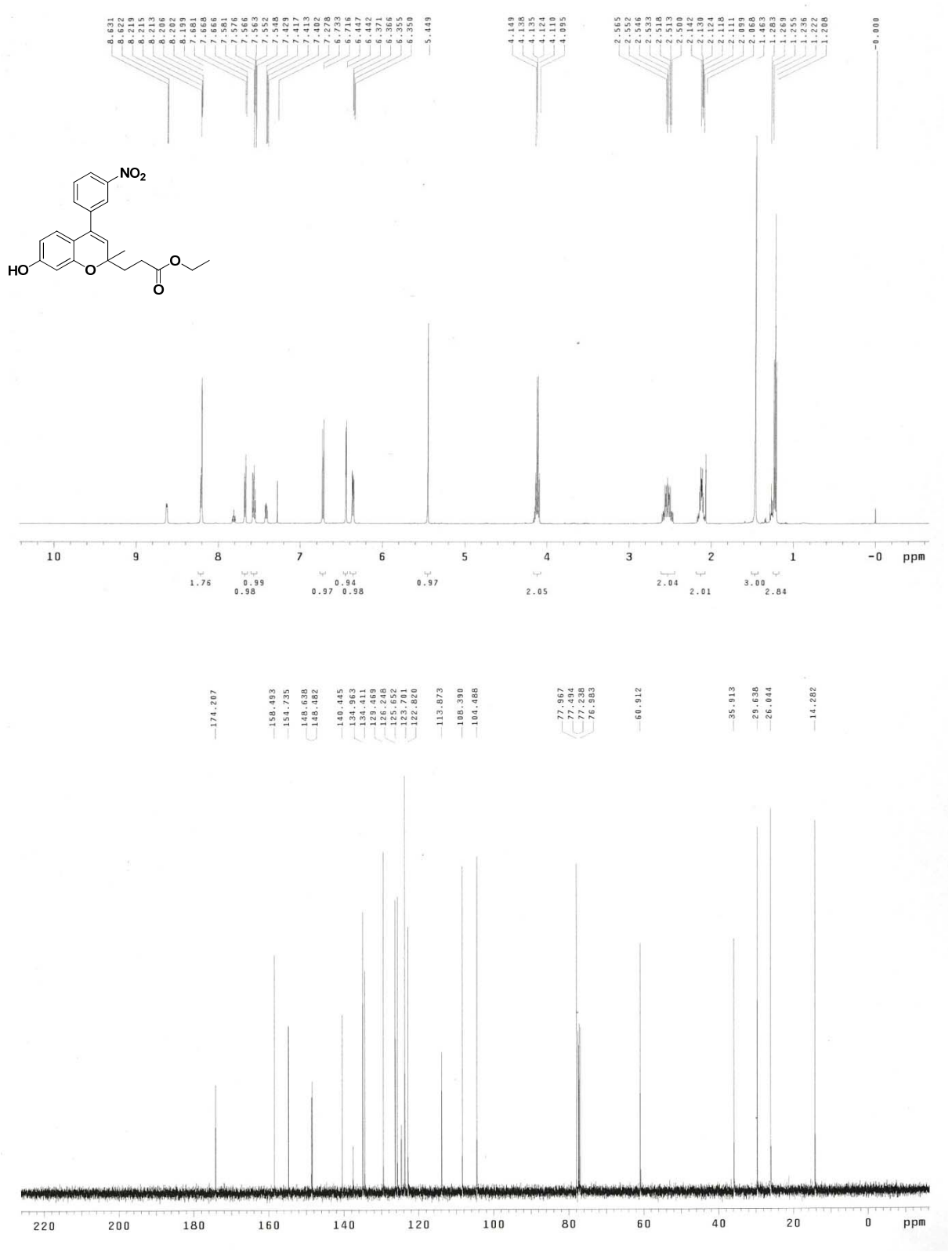
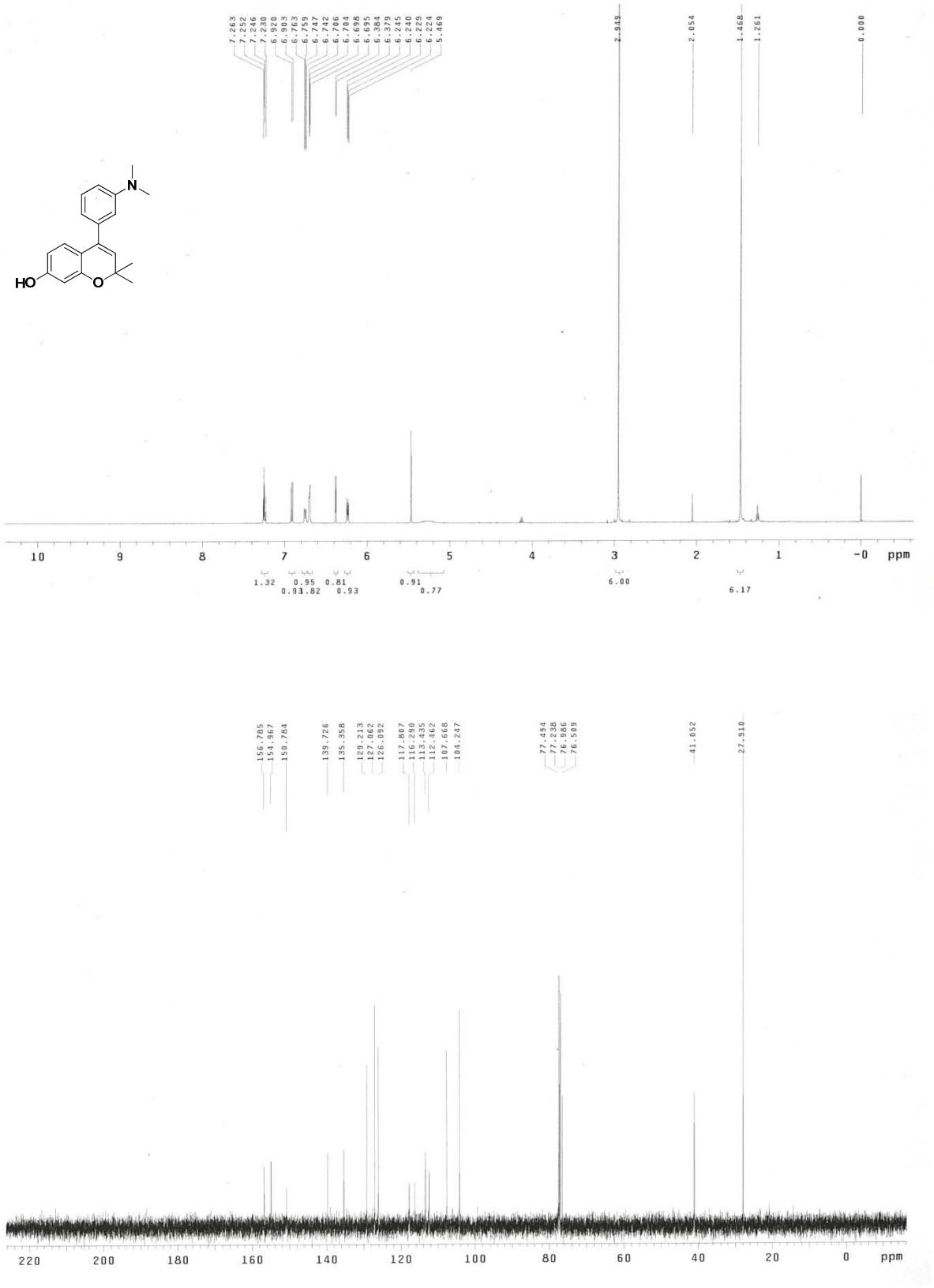
40
20
0
ppm

6f



6g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6.245
6.240
6.229
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HO
O
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9
8
7
6
5
4
3
2
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ppm
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0.91
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77.238
76.986
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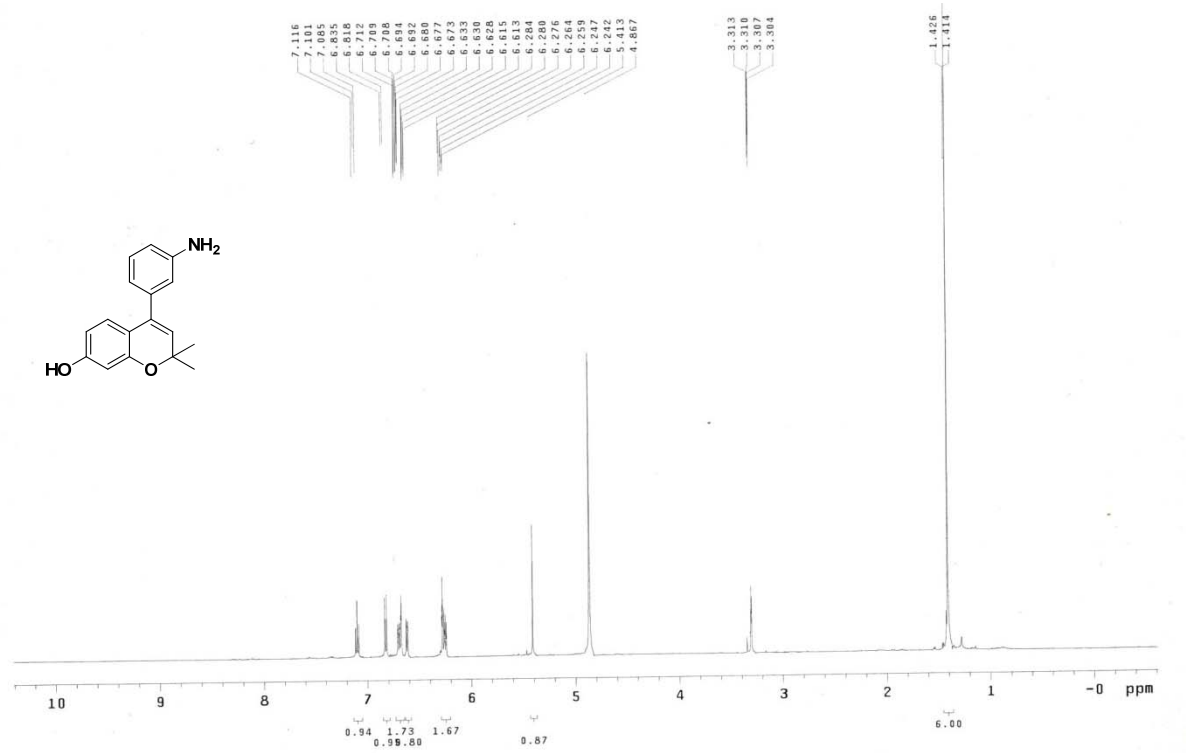
ppm

6h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6.708
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6.630
6.628
6.615
6.613
6.284
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6.275
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6.242
5.413
4.867
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3.307
3.304
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HO
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8
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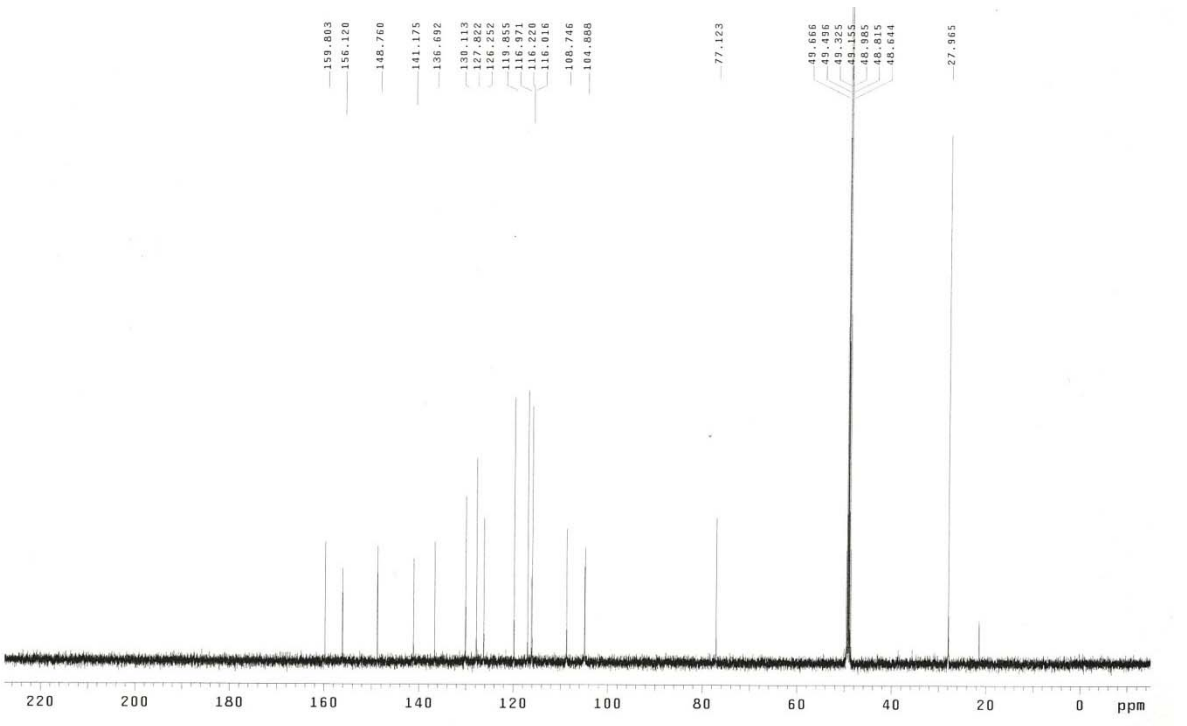
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77.123
49.666
49.496
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49.155
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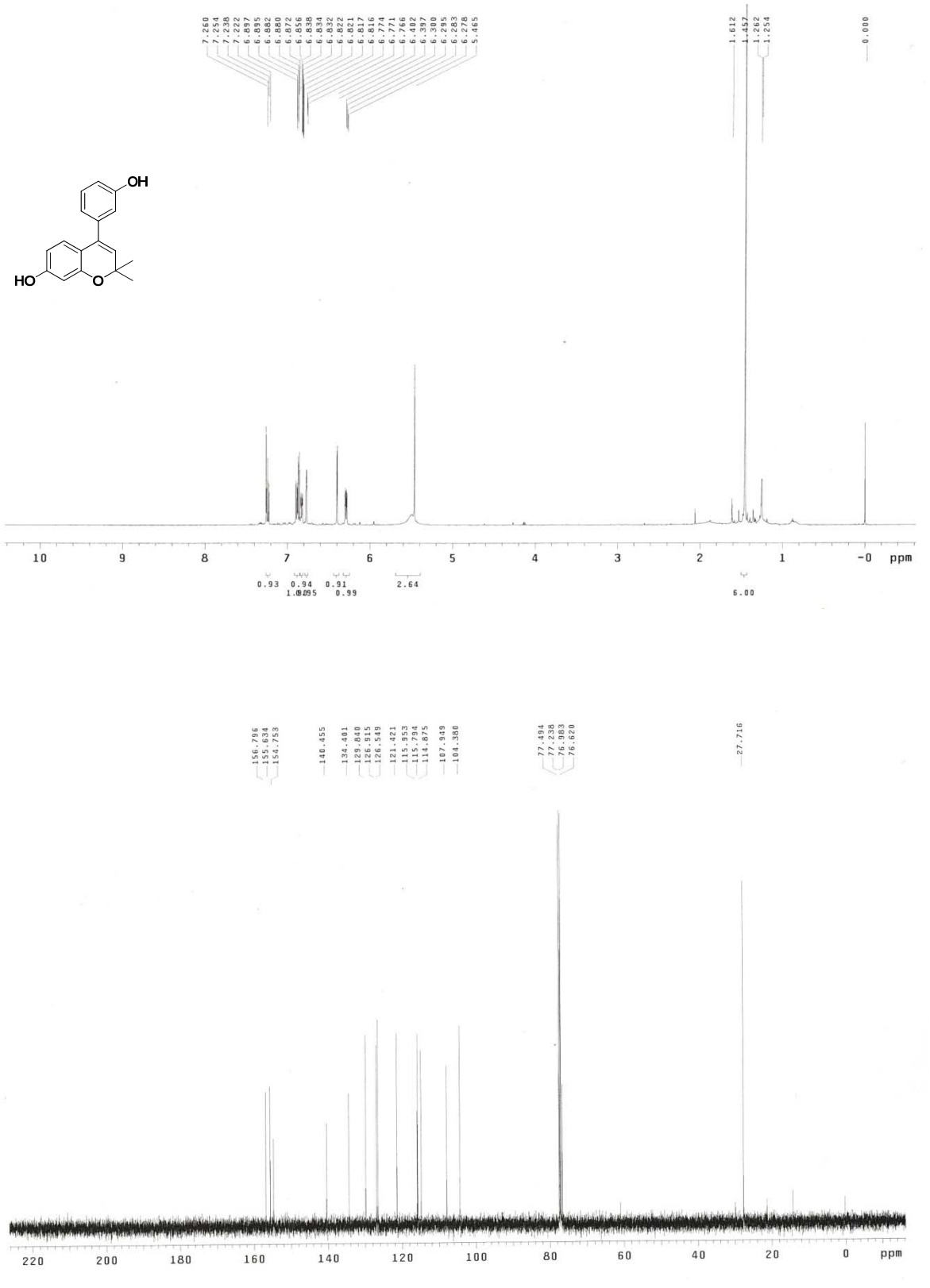
6i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6.834
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6.822
6.821
6.817
6.816
6.774
6.771
6.766
6.402
6.397
6.300
6.295
6.283
6.278
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1.262
1.254
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10
9
8
7
6
5
4
3
2
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0.94
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0.99
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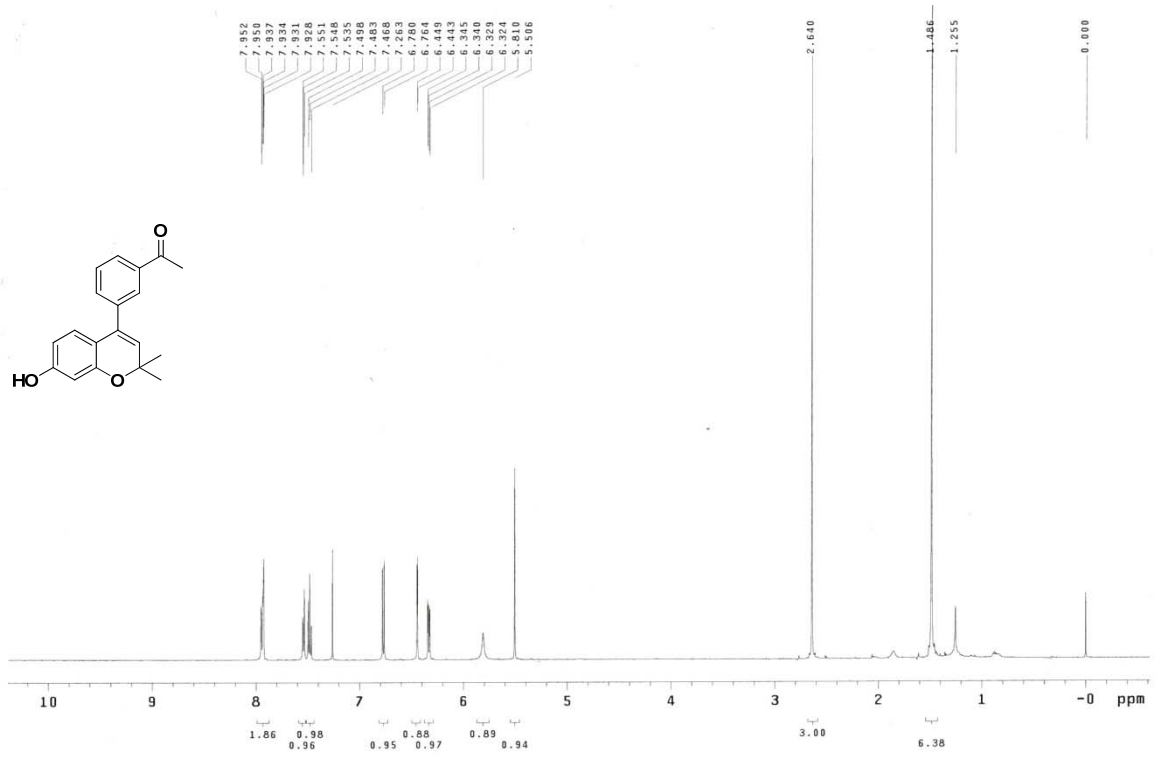
ppm

6j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6.443
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6.340
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O
HO
O
10
9
8
7
6
5
4
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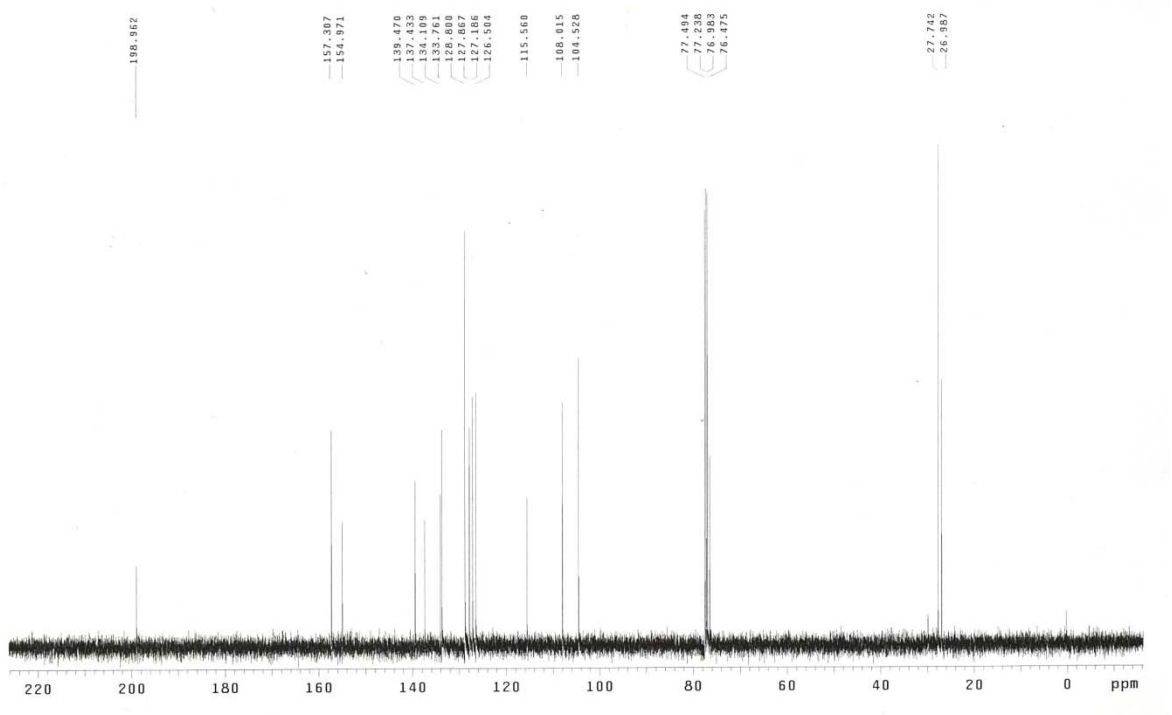
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104.528
77.494
77.238
76.983
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180
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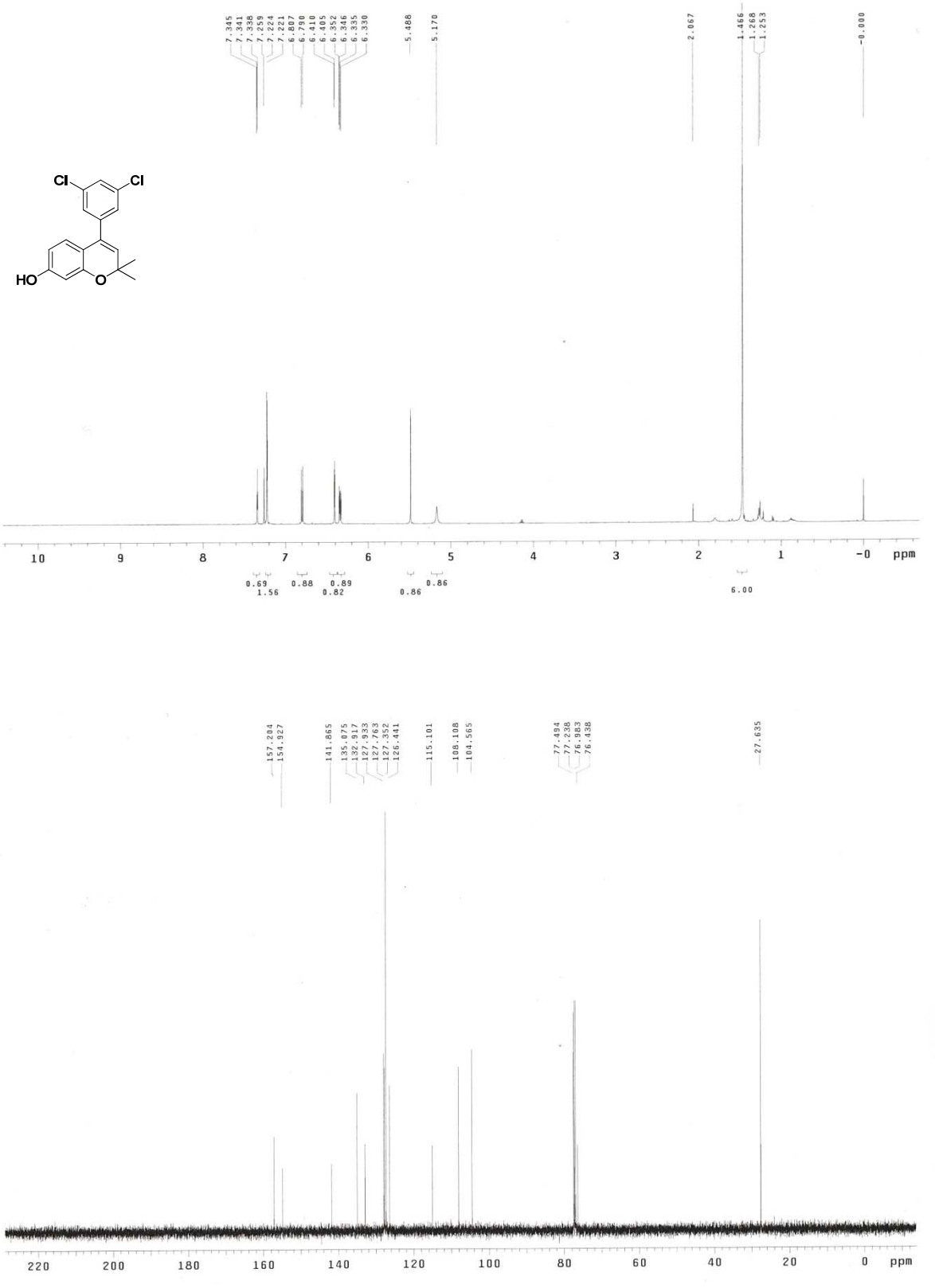
140
120
100
80
60
40
20
0
ppm

6k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5.170
2.067
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Cl
HO
O
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9
8
7
6
5
4
3
2
1
-0
ppm
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0.88
0.89
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0.86
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120
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80
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40
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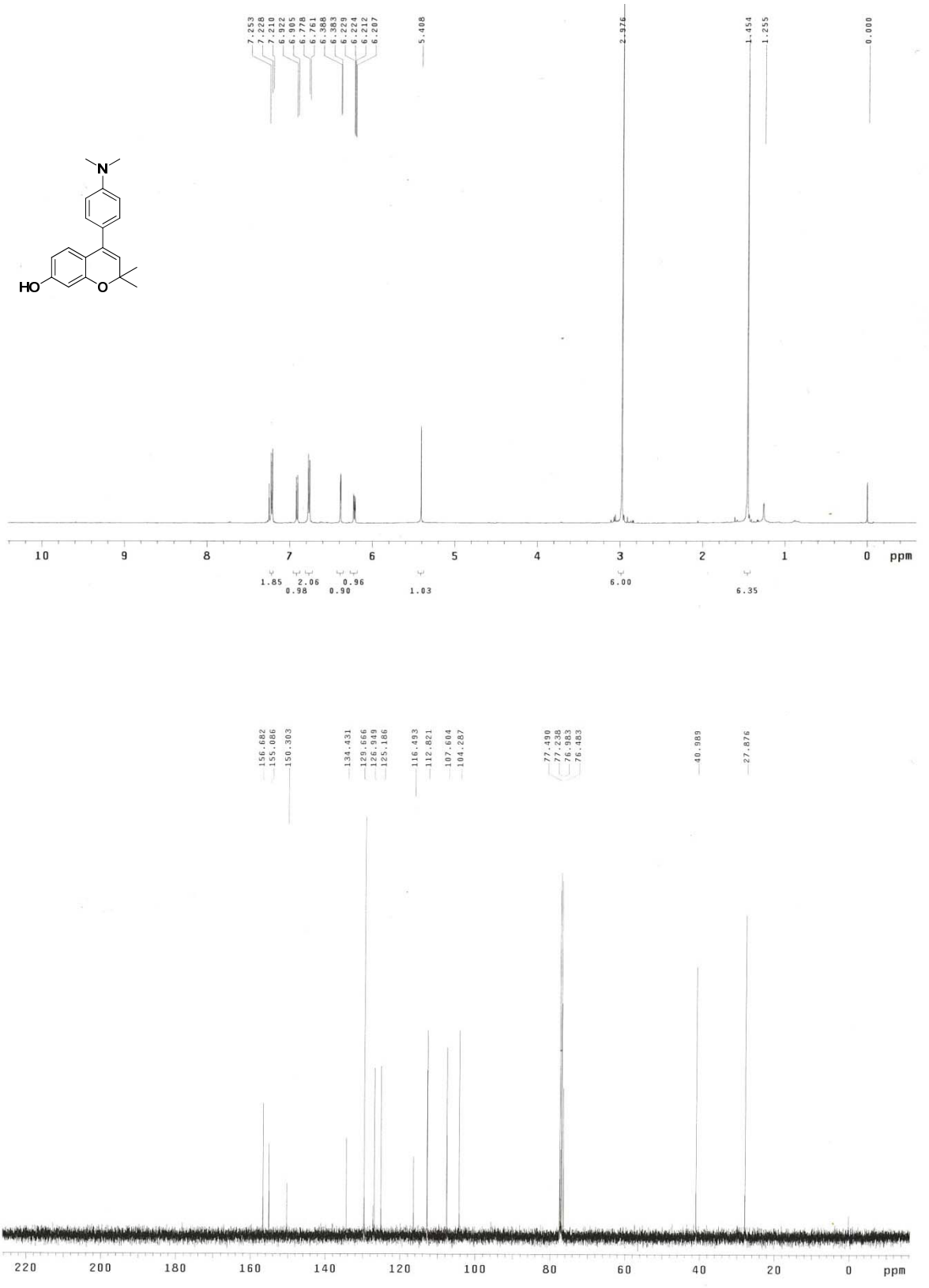
0
ppm

6l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5.408
2.976
1.454
1.255
0.000
HO
O
N
10
9
8
7
6
5
4
3
2
1
0
ppm
1.85
2.06
0.96
0.98
0.90
1.03
6.00
6.35
156.682
155.066
150.303
134.431
129.666
126.949
125.186
116.493
112.821
107.604
104.287
77.490
77.238
76.983
76.483
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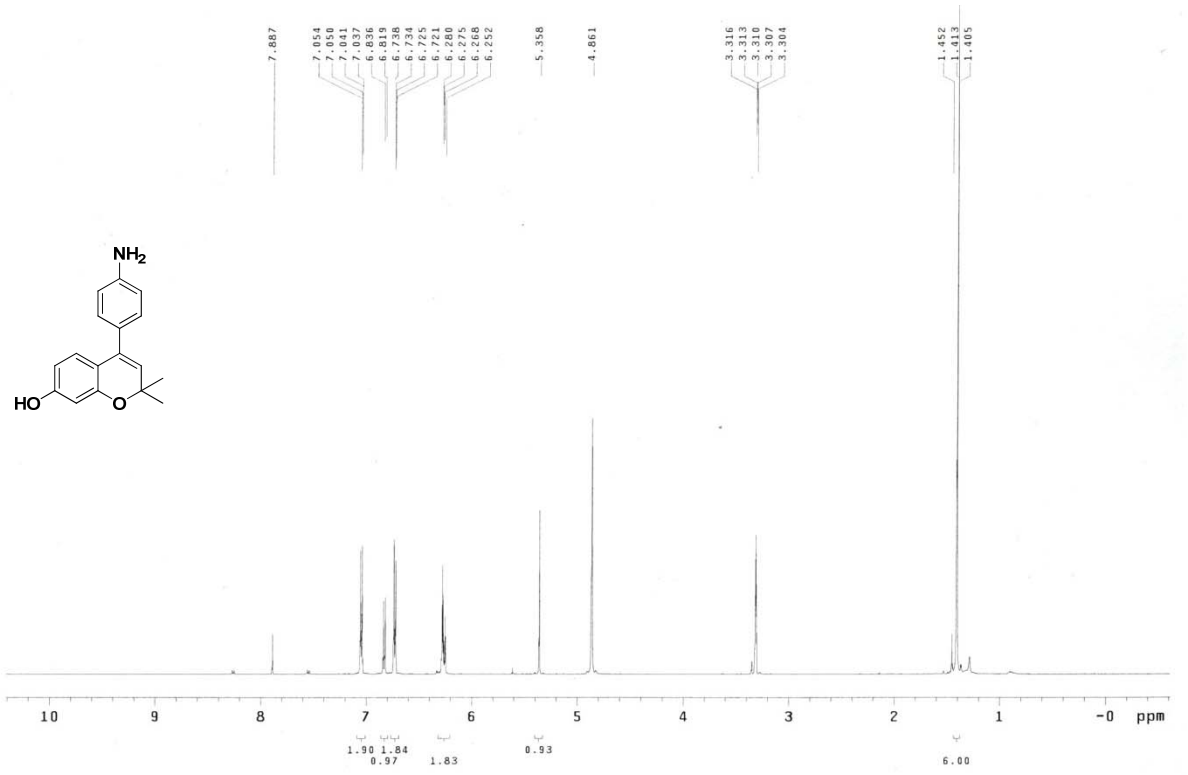
27.876
220
200
180
160
140
120
100
80
60
40
20
0
ppm

6m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7.041
7.037
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6.819
6.738
6.734
6.725
6.721
6.280
6.275
6.268
6.252
5.358
4.861
3.316
3.313
3.310
3.307
3.304
1.452
1.413
1.405
NH2
HO
O
10
9
8
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6
5
4
3
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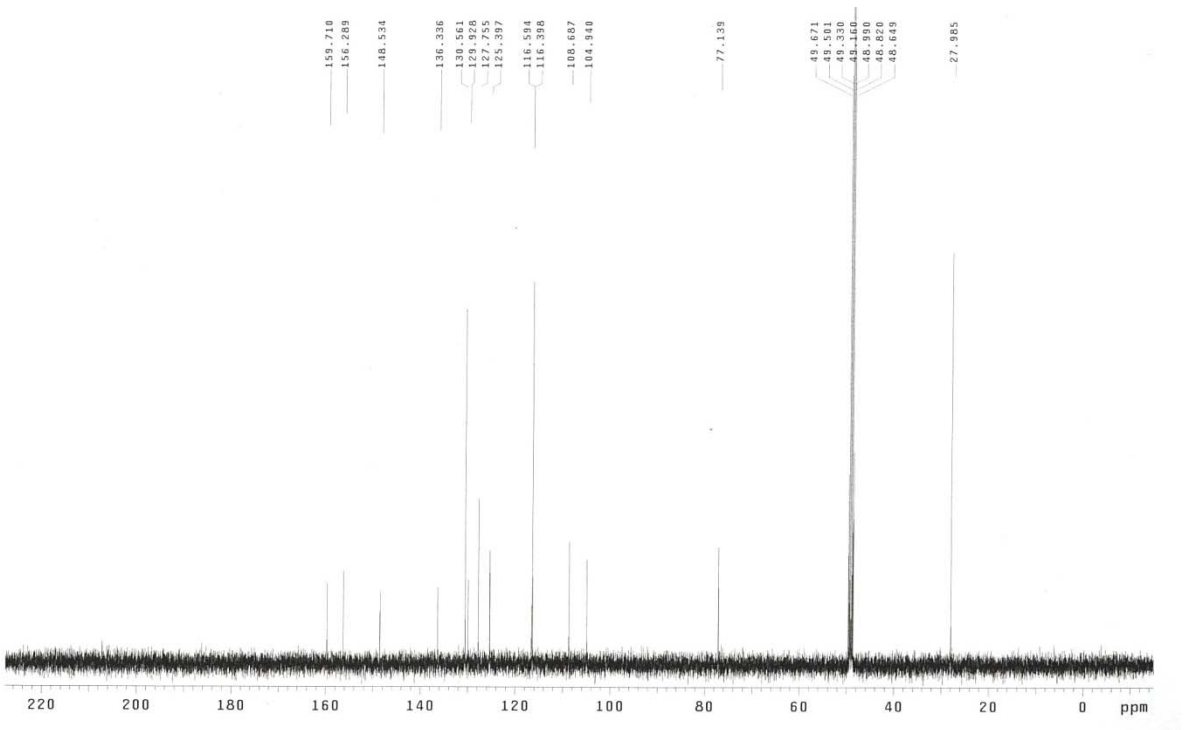
6.00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77.139
49.671
49.501
49.330
49.160
48.990
48.820
48.649
27.985
220
200
180
160
140
120
100
80
60
40
20
0
ppm

VII. Reference

1. Ko, S. K.; Jang, H. J.; Kim, E.; Park, S. B. *Chem. Commun*. **2006**, 2962–2964.