Supplementary Material

Design, Synthesis and Biological Evaluation of Novel 5-Hydroxy-2-methyl-4*H*-pyran-4-one Derivatives as Antiglioma Agent

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Supplementary Material

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1. Experimental Section

1.1. Chemistry

All chemicals were purchased from Alfa Aesar or Sigma Aldrich. The synthetic route to 5-hydroxy-2-methyl-4*H*-pyran-4-one derivatives is shown in **Scheme 1**. All the compounds were fully identified through ^{1}HNMR and ^{13}CNMR spectroscopy, and mass spectrometry (MS) and high resolution mass spectrometry (HRMS) before biological testing. Unless otherwise specified, proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded at 18 ^{o}C in base-filtered $CDCl_3$ with a Varian spectrometer operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Low resolution electrospray ionization (ESI) mass spectra were recorded on a Finnigan LCQ Advantage MAX mass spectrometer fitted with a 4000 Q TRAP or Agilent 6130 Quadruple LC/MS. High resolution mass spectra were obtained on an Agilent 6210 series LC/MSD TOF mass spectrometer. Melting points were determined with a digital melting point apparatus (Briwell/GH60) and are uncorrected.

*1.1.1. General procedure for the reaction of **2a-2h** (procedure A)*

A mixture of allomaltol (5-hydroxy-2-methyl-4H-pyran-4-one, **1**) (7.90 mmol) and an appropriate aldehyde (9.48 mmol) was stirred in the presence of DABCO (9.48 mmol) in dioxane:H_2O (1:1, 20 mL) at room temperature for 12 h. The product was extracted with EtOAc (3 × 20 ml) when the reaction finished. The combined organic layer was dried with anhydrous Na_2SO_4, concentrated *in* vacuo, and purified by column chromatography (MeOH/DCM =0.5:9.5) to afford the white solid.

*1.1.1.1. 3-Hydroxy-2-(hydroxy(phenyl)methyl)-6-methyl-4H-pyran-4-one (**2a**)*

The reaction was performed according to the general procedure A, using **1** (1.00 g, 7.90 mmol) and benzaldehyde (0.97 mL, 9.48 mmol). The crude product was purified by column chromatography (MeOH:DCM=0.5:9.5) to afford the white solid **2a**. (1.56 g, 85%): mp 170.3-171.1 ^{o}C; ^{1}HNMR (300 MHz, DMSO-d_6) δ 2.18 (s, 3H, CH_3), 6.00 (s, 1H, C*H*-OH), 6.20 (s, 1H, C=CH), 7.27-7.43 (m, 5H, Ph-H); ^{13}CNMR (75 MHz, DMSO-d_6) δ 174.28, 164.98, 150.97, 141.81, 141.00, 128.69, 127.81, 126.39, 111.58, 66.26, 19.71; ESI-MS m/z: 233.0 $[M+H]^+$; ESI-HRMS m/z: 233.0804 $[M+H]^+$, calcd for $C_{13}H_{13}O_4$ 233.0808.

1.1.1.2. 2-((4-Fluorophenyl)(hydroxy)methyl)-3-hydroxy-6-methyl-4H-pyran-4-one

(2b)

The reaction was performed according to the general procedure A, using **1** (1.00 g, 7.90 mmol) and *p*-fluorobenzaldehyde (1.00 mL, 9.48 mmol). The crude product was purified by column chromatography (MeOH:DCM=0.5:9.5) to afford the white solid **2b**. (1.79 g, 90%): mp 151.4-152.2 ^{o}C; ^{1}HNMR (300 MHz, DMSO-d_6) δ 2.19 (s, 3H, CH_3), 6.00 (s, 1H, C*H*-OH), 6.20(s, 1H, C=CH), 7.14-7.20 (t, *J* = 9.0 Hz, 2H, Ph-H), 7.41-7.46 (dd, *J* = 8.4 Hz, *J* = 5.4 Hz, 2H, Ph-H); ^{13}CNMR (75 MHz, DMSO-d_6) δ 174.33, 164.98, 150.68, 141.08, 137.96, 128.42, 115.61, 115.33, 111.59, 65.74, 19.69; ESI-MS m/z: 251.0 $[M+H]^+$; ESI-HRMS m/z: 251.0710 $[M+H]^+$, calcd for $C_{13}H_{13}FO_4$ 251.0714.

*1.1.1.3. 2-((4-Chlorophenyl)(hydroxy)methyl)-3-hydroxy-6-methyl-4H-pyran-4-one (**2c**)*

The reaction was performed according to the general procedure A, using **1** (1.00 g, 7.90 mmol) and 4-chlorobenzaldehyde (1.21 g, 9.48 mmol). The crude product was purified by column chromatography (MeOH:DCM=0.5:9.5) to afford the white solid **2c**. (1.85 g, 87%): mp 152.5-153.0 ^{o}C; ^{1}HNMR (300 MHz, DMSO-d_6) δ 2.18 (s, 3H, CH_3), 6.00 (s, 1H, C*H*-OH), 6.20 (s, 1H, C=CH), 7.41 (s, , 4H, Ph-H); ^{13}CNMR (75 MHz, DMSO-d_6) δ 174.34, 165.02, 150.46, 141.22, 140.82, 132.34, 128.69, 128.23, 111.61, 65.74, 19.69; ESI-MS m/z: 267.0 $[M+H]^+$; ESI-HRMS m/z: 267.0418 $[M+H]^+$, calcd for $C_{13}H_{12}ClO_4$ 267.0419.

*1.1.1.4. 3-Hydroxy-2-(hydroxy(4-(trifluoromethyl)phenyl)methyl)-6-methyl-4H-pyran-4-one (**2d**)*

The reaction was performed according to the general procedure A, using **1** (1.00 g, 7.90 mmol) and 4-(trifluoromethyl)benzaldehyde (1.30 ml, 9.48 mmol). The crude product was purified by column chromatography (MeOH:DCM=0.5:9.5) to afford the white solid **2d**. (2.05 g, 89%): mp 179.7-180.6 ^{o}C; ^{1}HNMR (300 MHz, DMSO-d_6) δ 2.17 (s, 3H, CH_3), 6.10 (s, 1H, C*H*-OH), 6.21(s, 1H, C=CH), 7.60-7.63 (d, *J* = 8.4 Hz, 2H, Ph-H), 7.71-7.74 (d, *J* = 8.4 Hz, 2H, Ph-H); ^{13}CNMR (75 MHz, DMSO-d_6) δ 174.30, 165.15, 150.13, 146.46, 141.37, 128.64, 127.11, 126.51, 125.63, 111.66, 65.89, 19.67; ESI-MS m/z: 301.0 $[M+H]^+$; ESI-HRMS m/z: 301.068 $[M+H]^+$, calcd for

$C_{14}H_{12}F_3O_4$ 301.0682.

*1.1.1.5. 2-((4-(Benzyloxy)phenyl)(hydroxy)methyl)-3-hydroxy-6-methyl-4H-pyran-4-one(**2e**)*

The reaction was performed according to the general procedure A, using **1** (1.00 g, 7.90 mmol) and 4-(benzyloxy)benzaldehyde (2.00 g, 9.48 mmol). The crude product was purified by column chromatography (MeOH:DCM=0.5:9.5) to afford the white solid **2e.** (1.92 g, 63%): mp 180.2-180.7 ^oC, ^{1}HNMR (300 MHz, DMSO-d_6) δ 2.20 (s, 3H, CH_3), 5.08(s, 2H, OCH_2-Ph), 6.06 (s, 2H, CH-OH), 6.19 (s, 1H, C=CH), 6.99 (d, J = 8.4 Hz, 2H, Ph-H), 7.31-7.45 (m, 7H, Ph-H); ^{13}CNMR (75 MHz, DMSO-d_6) δ 174.26, 164.91, 158.12, 151.19, 140.77, 137.54, 134.05, 128.87, 128.09, 127.69, 114.97, 111.54, 69.64, 65.95, 19.72; ESI-MS m/z: 339.1 $[M+H]^+$; ESI-HRMS m/z: 339.1226 $[M+H]^+$, calcd for $C_{20}H_{19}O_5$ 339.1227.

*1.1.2. General procedure B for the reaction of **3a-3h***

After dissolving 1.0 equiv of the **2a**-**2h** in dry DCM, 5.0 equiv of triethylsilane was added at 0 ^oC. The homogeneous mixture was treated with 5.0 equiv of trifluoroacetic acid, and stirred at 0 ^oC for 45 min and at room temperature overnight. The reaction mixture were extracted with DCM, and dried over anhydrous $MgSO_4$, provided the crude product which was purified by chromatography[1].

*1.1.2.1. 2-Benzyl-3-hydroxy-6-methyl-4H-pyran-4-one (**3a**)*

According to procedure B, **2a** (1.16 g, 5.00 mmol) was treated with triethylsilane (2.91 g, 25.00 mmol). The crude product was purified by chromatography to provide 1.00 g (92%) of **3a** as white solid: mp 141.6-142.7 ^oC: ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.26 (s, 3H, CH_3), 4.03 (s, 2H, CH_2-Ph), 6.22 (s, 1H, C=CH), 7.26-7.34 (m, 5H, Ph-H); ^{13}CNMR (75 MHz, $CDCl_3$) δ 165.40, 149.42, 136.52, 128.84, 128.68, 126.93, 110.79, 34.40, 20.07; ESI-MS m/z: 217.0 $[M+H]^+$; ESI-HRMS m/z: 217.0857 $[M+H]^+$, calcd for $C_{13}H_{13}O_3$ 217.0859.

*1.1.2.2. 2-(4-Fluorobenzyl)-3-hydroxy-6-methyl-4H-pyran-4-one (**3b**)*

According to procedure B, **2b** (1.25 g, 5.00 mmol) was treated with triethylsilane (2.91 g, 25.00 mmol). The crude product was purified by chromatography to provide 1.10 g (94%) of **3b** as white solid: mp 158.7-159.6 ^oC: ^{1}HNMR (300 MHz, $CDCl_3$) δ

2.26 (s, 3H, CH_3), 4.00 (s, 2H, CH_2-Ph), 6.22 (s, 1H, C=CH), 7.00 (t, *J* = 8.7 Hz, 2H, Ph-H), 7.27 (dd, *J* = 8.7 Hz, *J* = 6.4 Hz, 2H, Ph-H); ^{13}CNMR (75 MHz, $CDCl_3$) δ 165.40, 163.52, 160.27, 149.21, 132.20, 130.28, 115.64, 115.36, 110.80, 33.59, 20.04; ESI-MS m/z: 235.0 $[M+H]^+$; ESI-HRMS m/z: 235.0757 $[M+H]^+$, calcd for $C_{13}H_{12}FO_3$ 235.0765.

*1.1.2.3. 2-(4-Chlorobenzyl)-3-hydroxy-6-methyl-4H-pyran-4-one (**3c**)*

According to procedure B, **2c** (1.33 g, 5.00 mmol) was treated with triethylsilane (2.91 g, 25.00 mmol). The crude product was purified by chromatography to provide 1.24 g (93%) of **3c** as white solid: mp 170.1-171.0 ^oC: ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.25 (s, 3H, CH_3), 3.99 (s, 2H, CH_2-Ph), 6.22 (s, 1H, C=CH), 7.23-7.30 (m, 4H, Ph-H); ^{13}CNMR (75 MHz, $CDCl_3$) δ 165.39, 149.05, 141.55, 135.00, 132.82, 130.20, 128.79, 110.91, 33.74, 20.03; ESI-MS m/z: 251.0 $[M+H]^+$; ESI-HRMS m/z: 251.0460 $[M+H]^+$, calcd for $C_{13}H_{12}ClO_3$ 251.0469.

*1.1.2.4. 3-Hydroxy-6-methyl-2-(4-(trifluoromethyl)benzyl)-4H-pyran-4-one (**3d**)*

According to procedure B, **2d** (1.50 g, 5.00 mmol) was treated with triethylsilane (2.91 g, 25.00 mmol). The crude product was purified by chromatography to provide 1.35 g (90%) of **3d** as white solid: mp 159.8-161.0 ^oC: ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.27 (s, 3H, CH_3), 4.08 (s, 2H, CH_2-Ph), 6.24 (s, 1H, C=CH), 7.43 (d, *J* = 8.1 Hz, 2H, Ph-H), 7.57 (d, *J* = 8.1 Hz, 2H, Ph-H); ^{13}CNMR (75 MHz, $CDCl_3$) δ 165.51, 148.47, 141.73, 140.57, 129.18, 125.64, 122.32, 110.96, 34.18, 20.02; ESI-MS m/z: 285.0 $[M+H]^+$; ESI-HRMS m/z: 285.0731 $[M+H]^+$, calcd for $C_{14}H_{12}F_3O_3$ 285.0733.

*1.1.2.5. 2-(4-(Benzyloxy)benzyl)-3-hydroxy-6-methyl-4H-pyran-4-one (**3e**)*

According to procedure B, **2e** (1.69 g, 5.00 mmol) was treated with triethylsilane (2.91 g, 25.00 mmol). The crude product was purified by chromatography to provide 1.35 g (90%) of **3e** as white solid: mp 188.7-189.7 ^oC: ^{1}HNMR (300 MHz, $CDCl_3$) δ 2.27 (s, 3H, CH_3), 3.96 (s, 2H, CH_2-Ph), 5.07 (s, 2H, OCH_2-Ph), 6.21 (s, 1H, C=CH), 6.95 (d, *J* = 8.4 Hz, 2H, OCH_2Ph-H), 7.26 (t, *J* = 8.4 Hz, 3H, OCH_2Ph-H), 7.34-7.46 (m, 5H, Ph-H); ^{13}CNMR (75 MHz, $CDCl_3$) δ 165.39, 157.83, 149.42, 136.96, 129.87, 128.58, 127.97, 127.45, 115.04, 110.47, 33.59, 20.08; ESI-MS m/z: 323.1 $[M+H]^+$; ESI-HRMS m/z: 323.1276 $[M+H]^+$, calcd for $C_{20}H_{19}O_5$ 323.1278.

*1.1.3. General procedure for the reaction of **4a-4f** (Procedure C)*

Allomaltol (5-hydroxy-2-methyl-4H-pyran-4-one, **1**) (1 eq) and triethylenediamine (DABCO, 1.2 eq) were dissolved in dioxane:H_2O (1:1) and stirred for 15 min. Afterwards, the aldehyde (0.5 eq) was added dropwise to the reaction mixture. The solution was stirred at 50 ^oC for 24 h. The reaction mixture was then cooled to 0 ^oC and the resulting precipitate was collected by filtration. If no precipitation occurred, the reaction mixture was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed twice with saturated $NaHCO_3$ (30 mL) and water (30 mL), dried over Na_2SO_4, filtered and concentrated in vacuo. The crude product was purified by recrystallization.

*1.1.3.1. 6,6'-((4-(Benzyloxy)phenyl)methylene)bis(5-hydroxy-2-methyl-4H-pyran-4-one) (**4a**)*

The reaction was performed according to the general procedure C, using **1** (1.00 g, 7.90 mmol) and 4-(benzyloxy)benzaldehyde (0.84 g, 3.95 mmol).[2] The crude product was recrystallized from isopropanol affording a white powder **4a** (1.53 g, 87%): mp 261.4-262.1 ^oC; ^{1}HNMR (300 MHz, DMSO-d_6) δ 2.22 (s, 6H, CH_3), 5.08 (s, 3H, OCH_2-Ph), 5.96 (s, 1H, CH-Ar), 6.25 (s, 2H, C=CH), , 7.01 (d, J = 9.0 Hz, 2H, Ar-H3'/H5'), 7.22 (d, J = 9.0 Hz, 2H, Ar-H2'/H6'), 7.31-7.45 (m, 5H, Ph-H); ^{13}CNMR (75 MHz, DMSO-d_6) δ 173.95, 165.08, 158.12, 151.20, 147.68, 142.19, 140.77, 137.42, 129.87, 128.91, 128.16, 127.69, 115.46, 114.97, 111.74, 69.69, 19.63; ESI-MS m/z: 447.1 $[M+H]^+$; ESI-HRMS m/z: 447.1438 $[M+H]^+$, calcd for $C_{26}H_{23}O_7$ 447.1438.

*1.1.3.2. 6,6'-((4-((4-Methoxybenzyl)oxy)phenyl)methylene)bis(5-hydroxy-2-methyl-4H-pyran-4-one)(**4b**)*

The reaction was performed according to the general procedure using **1** (1.00 g, 7.90 mmol) and 4-((4-methoxybenzyl)oxy)benzaldehyde (0.96 g, 3.95 mmol) [3]. The crude product was recrystallized from isopropanol affording **4b** as a white powder (1.50 g, 80%): mp 241.6-242.1 ^oC; ^{1}HNMR (300 MHz, DMSO-d_6) δ 2.22 (s, 6H, CH_3), 3.75 (s, 3H, OCH_3), 4.99 (s, 2H, OCH_2-Ph), 5.96 (s, 1H, CH-Ar), 6.25 (s, 2H, CH), 6.93 (d, J = 9.0 Hz, 2H, Ar-H3'/H5'), 6.99 (d, J = 9.0 Hz, 2H, OCH_2Ar-H3'/H5'), 7.20 (d, J = 9.0 Hz, 2H, Ar-H2'/H6'), 7.37 (d, J = 9.0 Hz, 2H, OCH_2Ar-H2'/H6'), 9.22(s, 2H, C=C-

OH); ^{13}CNMR (75 MHz, DMSO-d_6) δ 173.94, 165.07, 159.46, 158.18, 147.69, 142.19, 129.69, 129.83, 129.28, 128.90, 115.47, 114.27, 111.74, 69.45, 55.53, 19.63; ESI-MS m/z:477.1 $[M+H]^+$; ESI-HRMS m/z: 477.1537 $[M+H]^+$, calcd for $C_{27}H_{25}O_8$ 447.1544

*1.1.3.3. 6,6'-((4-((4-Nitrobenzyl)oxy)phenyl)methylene)bis(5-hydroxy-2-methyl-4H-pyran-4-one)(**4c**).*

The reaction was performed according to the general procedure C, using **1** (1.00 g, 7.90 mmol) and 4-((4-nitrobenzyl)oxy)benzaldehyde (1.01 g, 3.95 mmol).[11] The crude product was recrystallized from isopropanol affording **4c** as a white powder (1.45 g, 75%): mp 226.3-226.8 °C; ^{1}HNMR (300 MHz, DMSO-d_6) δ 2.21 (s, 6H, CH_3), 5.27 (s, 2H, OCH_2-Ph), 5.93 (s, 1H, CH-Ar), 6.24 (s, 2H, C=CH), 7.01 (d, J = 9.0 Hz, 2H, Ar-H3'/H5'), 7.24 (d, J = 9.0 Hz, 2H, Ar-H2'/H6'), 7.70(d, J = 9.0 Hz, 2H, OCH_2Ar-H3'/H5'), 8.25 (d, J = 9.0 Hz, 2H, OCH_2Ar-H2'/H6'); ^{13}CNMR (75 MHz, DMSO-d_6) δ 174.27, 164.94, 157.64, 147.77, 147.43, 145.46, 142.60, 129.97, 129.43, 128.68, 124.07, 115.50, 111.76, 68.50, 19.63; ESI-MS m/z: 492.1 $[M+H]^+$; ESI-HRMS m/z: 492.1292 $[M+H]^+$, calcd for $C_{26}H_{22}NO_9$ 492.1289.

*1.1.3.4. 6,6'-((4-Phenethylphenyl)methylene)bis(5-hydroxy-2-methyl-4H-pyran-4-one)(**4d**)*

The reaction was performed according to the general procedure C, using **1** (1.00 g, 7.90 mmol) and 4-phenethylbenzaldehyde (0.83 g, 3.95 mmol). [12] The crude product was recrystallized from isopropanol affording **4d** as a white powder (1.38 g, 79%): mp 202.5-202.9 °C; ^{1}HNMR (300 MHz, DMSO-d_6) δ 2.22 (s, 6H, CH_3), 2.86 (s, 4H, $PhCH_2$-CH_2Ph), 6.00 (s, 1H, CH-Ar), 6.26 (s, 2H, C=CH), 7.01 (d, J = 9.0 Hz, 2H, Ar-H3'/H5'), 7.24 (d, J = 9.0 Hz, 2H, Ar-H2'/H6'), 7.14-7.22 (m, 9H,Ar-H), 9.22 (s, 2H, C=C-OH); ^{13}CNMR (75 MHz, DMSO-d_6) δ 173.91, 165.08, 147.49, 142.35, 141.91, 141.22, 141.22, 134.40, 129.22, 128.78, 128.67, 128.53, 126.29, 111.76, 37.38, 37.63, 19.63; ESI-MS m/z: 445.1 $[M+H]^+$; ESI-HRMS m/z: 445.1646 $[M+H]^+$, calcd for $C_{27}H_{25}O_6$ 445.1646.

1.1.3.5.

*6, 6'-((4-Propoxyphenyl)methylene)bis(5-hydroxy-2-methyl-4H-pyran-4-one)(**4e**)*

The reaction was performed according to the general procedure C, using **1** (1.00

g, 7.90 mmol) and *p*-butoxybenzaldehyde (0.70 mL, 3.95 mmol). The crude product was recrystallized from isopropanol affording **4e** as a white powder (1.40 g, 89%): mp 252.3-252.7 °C; ^{1}HNMR (300 MHz, DMSO-d_6) δ 0.98 (t, 3H, J = 7.5 Hz, CH_2CH_3), 1.65-1.77 (m, 2H, $OCH_2CH_2CH_3$), 2.22 (s, 6H, CH_3), 3.89 (t, 2H, J = 6.6 Hz, OCH_2CH_2), 5.95 (s, 1H, CH-Ar), 6.25 (s, 2H, C=CH), 6.92 (d, J = 8.7 Hz, 2H, Ar-H3'/H5'), 7.20 (d, J = 8.7 Hz, 2H, Ar-H2'/H6'), 9.21 (s, 2H, C=C-OH); ^{13}CNMR (75 MHz, DMSO-d_6) δ 173.93, 165.05, 158.42, 147.71, 142.18, 129.81, 128.64, 115.09, 111.73, 69.35, 22.46, 19.63, 10.86; ESI-MS m/z: 399.1 $[M+H]^+$; ESI-HRMS m/z: 399.1442 $[M+H]^+$, calcd for $C_{22}H_{23}O_7$ 397.1438.

1.1.3.6.

*6,6'-((4-(Allyloxy)phenyl)methylene)bis(5-hydroxy-2-methyl-4H-pyran-4-one)(**4f**)*

The reaction was performed according to the general procedure C, using **1** (1.00 g, 7.90 mmol) and p-(allyloxy)benzaldehyde (0.65 mL, 3.95 mmol). The crude product was recrystallized from isopropanol affording **4f** as a white powder (1.30 g, 84%): mp 232.4-232.8 °C; ^{1}HNMR (300 MHz, DMSO-d_6) δ 2.22 (s, 6H, CH_3), 4.54 (d, 2H, J = 5.1 Hz, $ArOCH_2$-$CH=CH_2$), 5.25 (dd, 1H, J = 9.0 Hz, J = 5.1 Hz, $CH=CH_2$), 5.39 (dd, 1H, J = 9.0 Hz, J = 5.1 Hz, CH=CH*H*), 5.95 (s, 1H, CH-Ar), 5.98-6.09 (m, 1H, $ArOCH_2$-$CH=CH_2$), 6.25 (s, 2H, C=CH), 6.94 (d, J = 9.0 Hz, 2H, Ar-H3'/H5'), 7.22 (d, J = 9.0 Hz, 2H, Ar-H2'/H6'), 9.21 (s, 2H, C=C-OH); ^{13}CNMR (75 MHz, DMSO-d_6) δ 173.93, 165.06, 157.93, 147.67, 142.18, 134.12, 129.82, 128.93, 117.94, 115.34, 111.74, 68.63, 19.63; ESI-MS m/z: 397.1 $[M+H]^+$; ESI-HRMS m/z: 397.1281 $[M+H]^+$, calcd for $C_{22}H_{21}O_7$ 397.1282

1.2. Bioactivity

1.2.1. Antiproliferation assay

First, the cytotoxicity against HT1080 cell line (obtained from ATCC) and U87 cell line (obtained from ATCC) of the synthetic compounds was studied at concentration gradients. The cells were cultured in DMEM medium supplemented with 10% fetal bovine serum and 2 mM L-glutamine and 100 units/mL penicillin/streptomycin in a humidified atmosphere of 5% CO_2 at 37 °C. Cells were

seeded at a density of 5000 cell per well in a 96 well plate for 12 h. Each dose was prepared in DMEM medium by 1000 × dilution of the test compound which was prepared in DMSO solution ensuring that the DMSO concentration is less than 0.1%. Control experiments showed that 0.1% DMSO had no effect on cytotoxicity. Viability was assessed on the basis of cellular conversion of MTT into a formazan product for 4 h. Absorbance was detected by a Gen5 Reader (Bioteck/ Synergy TM HT) at 562 nm. The experiment was performed in 4 replicate wells for each compound or concentration with at least three experimental runs.

1.2.2. Inhibition of D-2HG Production in HT1080 Cells

The *D*-2HG production inhibition assay followed our previous protocol. In brief, 1 × 10^5 HT1080 cells (obtained from ATCC) per well were seeded into wells of a six-well plate and cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% dialyzed fetal bovine serum at 37 ^oC in a 5% CO_2 atmosphere overnight for cell attachment. Cells were treated with an increasing concentration of compounds in 2 mL of culture medium for 48 h. Cells were collected and washed with PBS thrice. After homogenizing the cells, the mixture was centrifuged to remove any precipitate, and the supernatant was subjected to HPLC-MS (ABI/Sciex API 5000) to separate and quantitate the *D*-2HG. HPLC was run using a C_{18} column (COSMOIL) with 0.1% formic acid-water solution and MeOH as an eluent at a flow rate of 0.5 mL/min. Mobile phase buffer A was 0.1% formic acid-water solution. Buffer B was MeOH. HPLC Gradient steps were: 0 min, 10% A, 90% B; 2 min, 50% A, 50% B; 5 min, 50% A, 50% B; 10 min, 95% A, 5% B; 15 min, 95% A, 5% B; 15.1 min, 10% A, 90% B; 25 min, 10% A, 90% B. A single ion monitoring (SIM) for 147 Da was used to detect and quantitate the amount of *D*-2HG (parameters: interface voltage, -4.2 kV; detector voltage, 1.3 kV; nebulizing gas, 1.5 L/min; drying gas, 15 L/min; desolvation line temperature, 250 ^oC; heat block temperature, 200 ^oC; Pirani gauge vacuum, 10^2 Pa; ion gauge vacuum: 5 × 10^{-4} Pa). Authentic *D*-2HG purchased from Sigma-Aldrich was used to validate and calibrate the HPLC-MS assay conditions before measuring the *D*-2HG concentrations secreted by the HT1080 cells untreated or treated with **4a**. The injection volume of control group is 1 μL, while the injection volume of compound **4a**

and **AGI-5198** groups are 5 μL. Quantitative calculations are based on the peak area and the standard curve line (**Fig. S3** in SI).

1.2.3. Measurement of cell migration

A scratch assay was used to assess the difference in migration distance between non-treated and treated cells. Briefly, 2.5×10^5 cells per well were seeded on a 6-well plate. Two linear scratches were made in each well using a 200 μL sterile pipette tip after removal of medium. Wells were washed with PBS to remove any cellular debris created by the scratch. Cells were then treated with a specific concentration of compound **4a** or DMSO in 2 mL media. A microscope system was used to take pictures in four different fields in each sample every 6 h or 12 h. The scratch area were measured using Image J software.

1.2.4. Measurement of colony-forming ability

For colony forming assays, 200 cells per well were seeded on a 6-well plate in 2 mL media and treated with different concentration of DMSO (control), **AGI-5198** (5 μM and 20 μM) compound **4a** (0.5 μM and 1 μM) for 8 days. Then the medium was removed and the cells were washed thrice with pre-cooled PBS. Finally the cells were fixed in 95% pre-cooled MeOH for 15 min with subsequent Giemsa-Wright staining. Colonies were eye-counted at least three times.

1.2.5. Expression/Purification of Homodimeric Mutant IDH1

Mutant human IDH1 genes bearing a His-tag were expressed using *Escherichia coli*. The genes were subcloned into pET28a to produce the expression constructs. The enzyme was expressed in *Rosetta (DE3)* induced by isopropyl-D-thiogalactopyranoside (1 mM). The cleaved proteins were separated through a nickel-NTA column (GE Healthcare) and eluted with a step gradient of imidazole Buffer (50, 100, 250, and 500 mM). The purified protein fractions were filtered and stored in 50 mM Tris,50 mM NaCl, 1 mM DTT, 0.5 mM EDTA, and 10% glycerol, pH 7.5. The purities were verified by SDS-polyacrylamide gel electrophoresis (SDS-PAGE, **Fig. S1**)

1.2.6. Mutant IDH1 inhibitory assay.

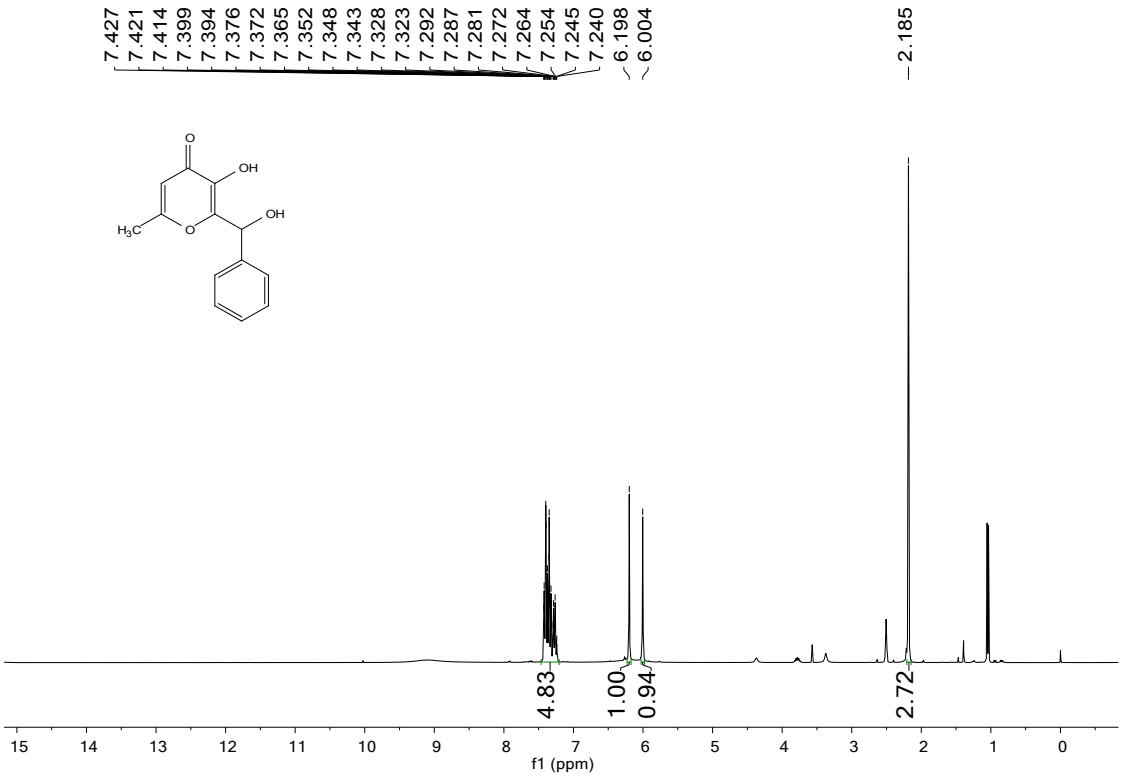
Reaction buffer (50 mM Tris, 200 mM NaCl , 5 mM β-mercaptoethanol, 2 mM

$MgSO_4$, 10% glycerinum), compounds and enzyme were added to a 96 well plate to a total volume of 240 μL for an incubation lasting 30 min at room temperature. Then 96 mM $MgCl_2$, 1 mg/μL NADPH, and 3.51 μg/μL α-KG were subsequently added for another incubation lasting 1 h at 37 ^oC. Finally, absorption at 340 nm was detected using a microplate-reader (Biotek/ Synergy TM HT). Parallel triplicate repetition for each dose was made.

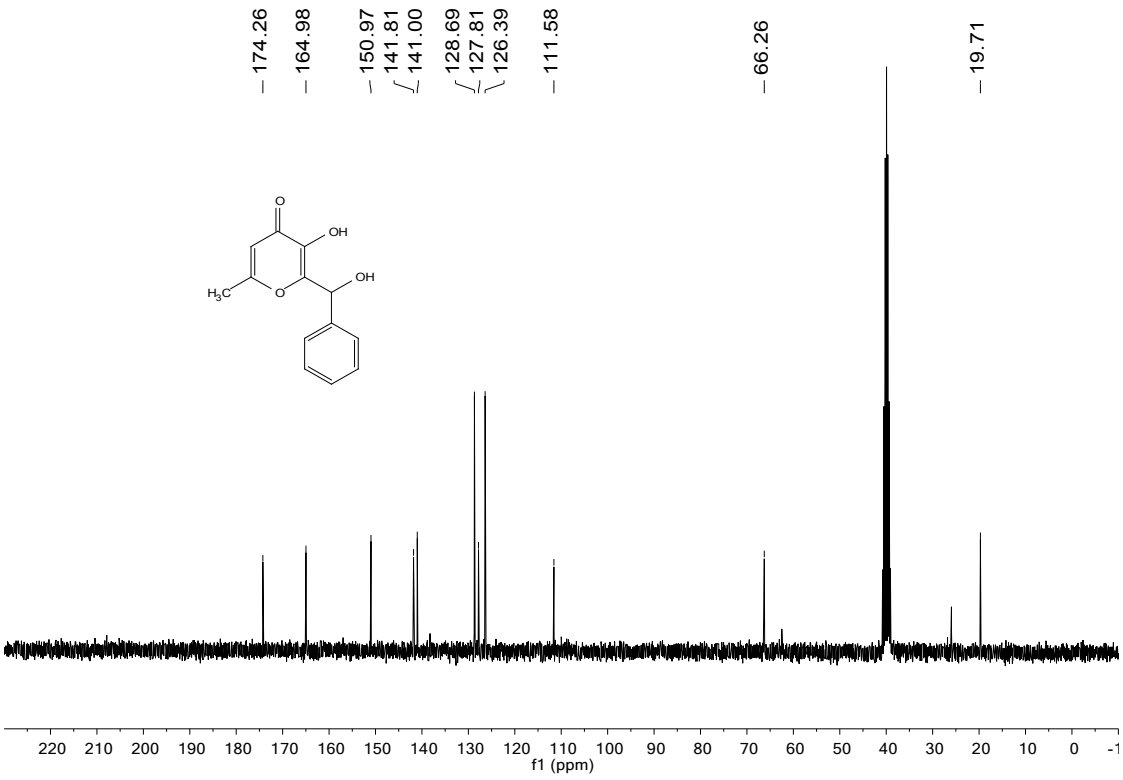
References

1. Brueckner, C.; Holzinger, H.; Reissig, H. U., J. Org. Chem. 1988, **53**, 2450-2456.
2. (a) Kantam, M. L.; Chakravarti, R.; Chintareddy, V. R.; Sreedhar, B.; Bhargava, S., Adv. Synth. Catal., 2008, **350**, 2544-2550; (b) Luo, B.; Wang, J.; Ge, D.; Li, X.; Cao, X.; Pan, Y.; Gu, H., Sci. China Chem., 2014, **57**, 1310-1314.
3. (a) Moreno, L.; Parraga, J.; Galan, A.; Cabedo, N.; Primo, J.; Cortes, D., Bioorg. Med. Chem. 2012, **20**, 6589-6597; (b) Kurosawa, W.; Kan, T.; Fukuyama, T., J. Am. Chem. Soc. 2003, **125**, 8112-8113.

^{1}H NMR spectrum of compound 2a



^{13}C NMR spectrum of compound 2a



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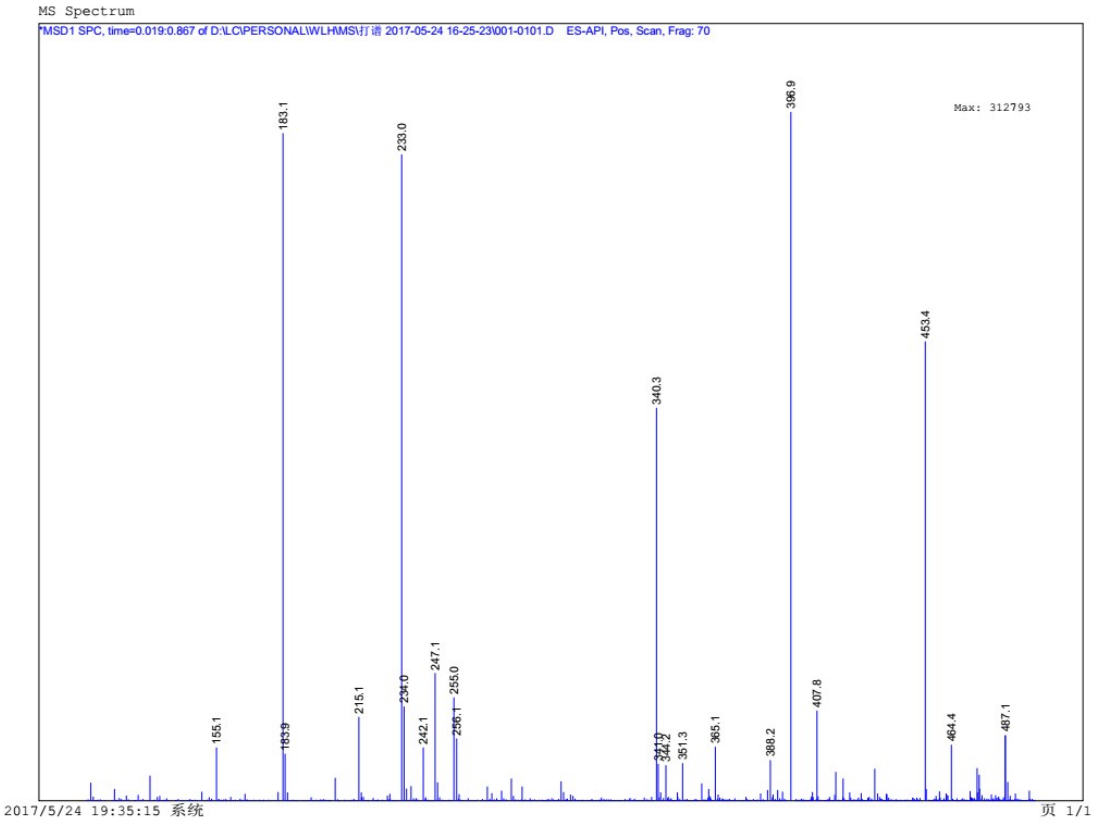

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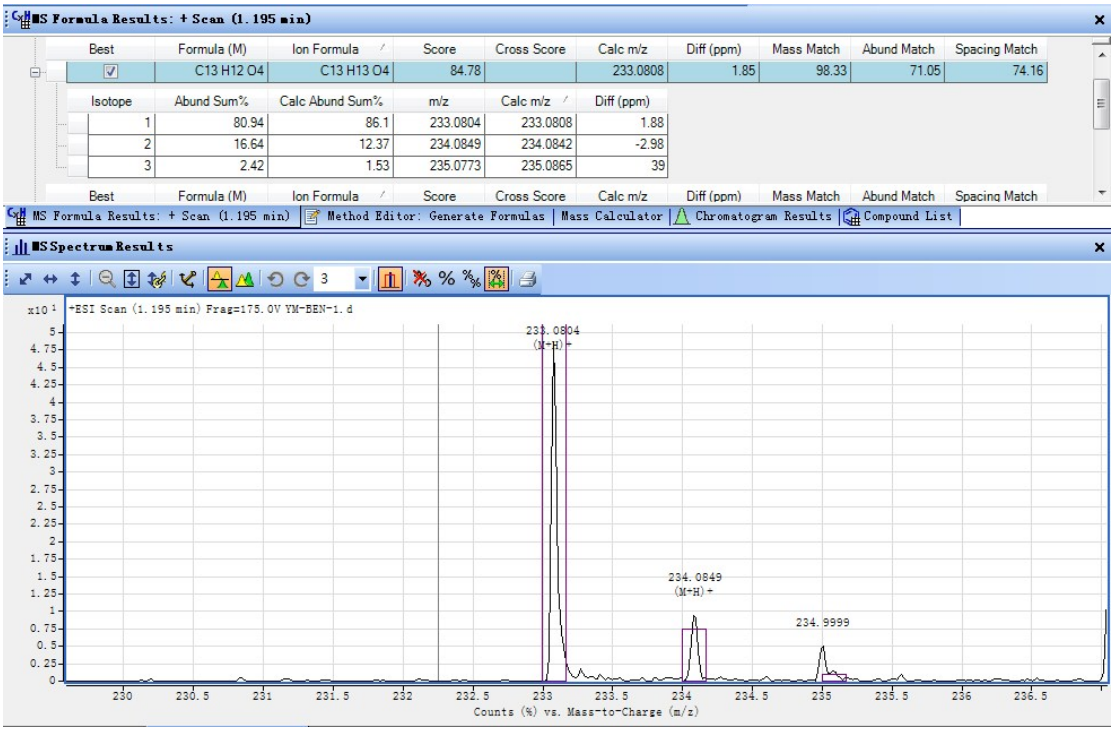

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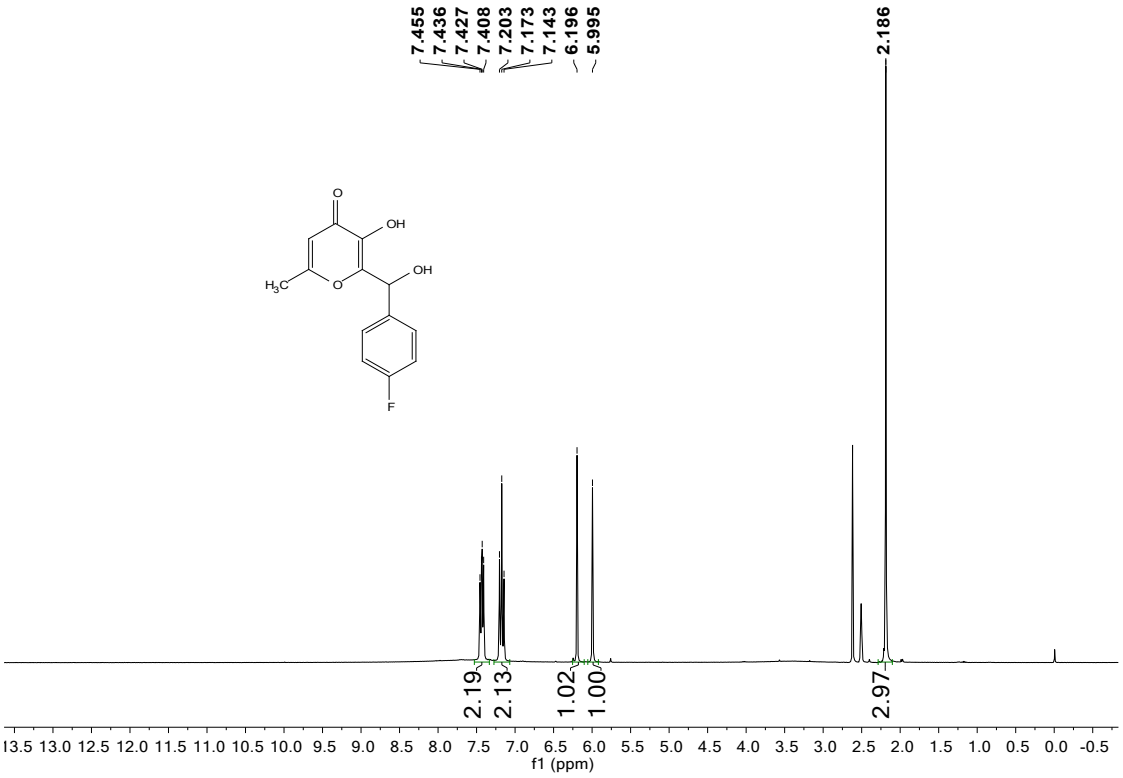

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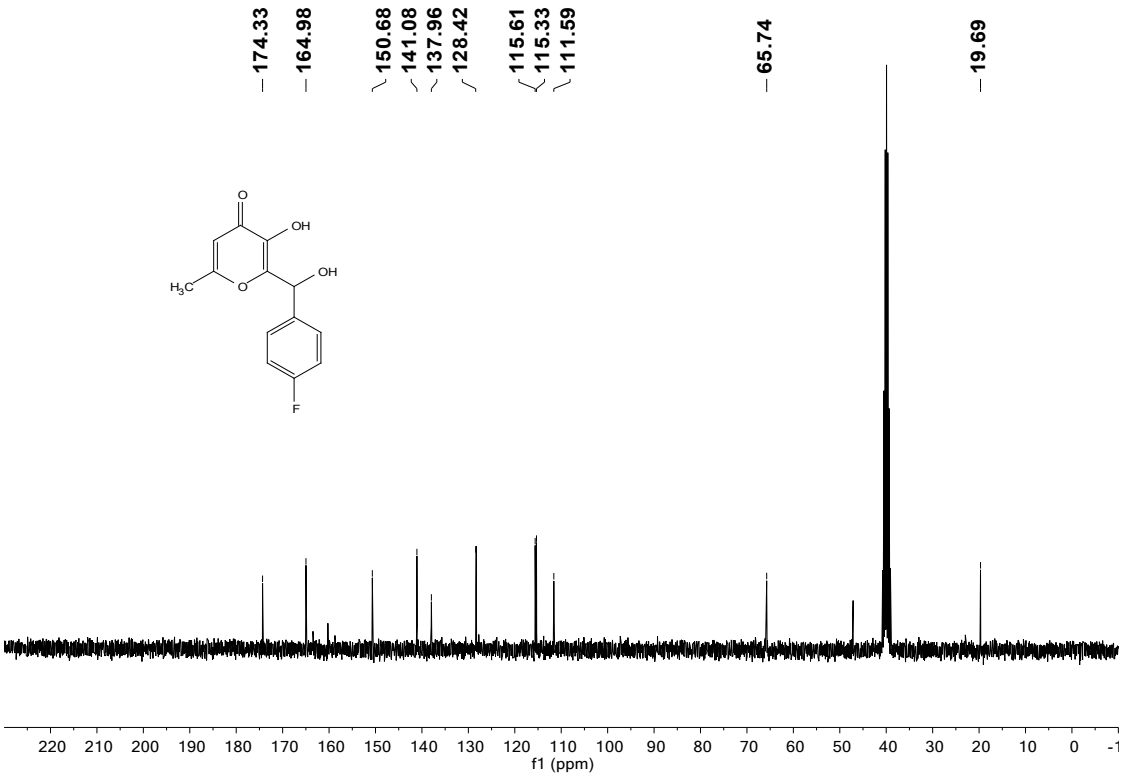

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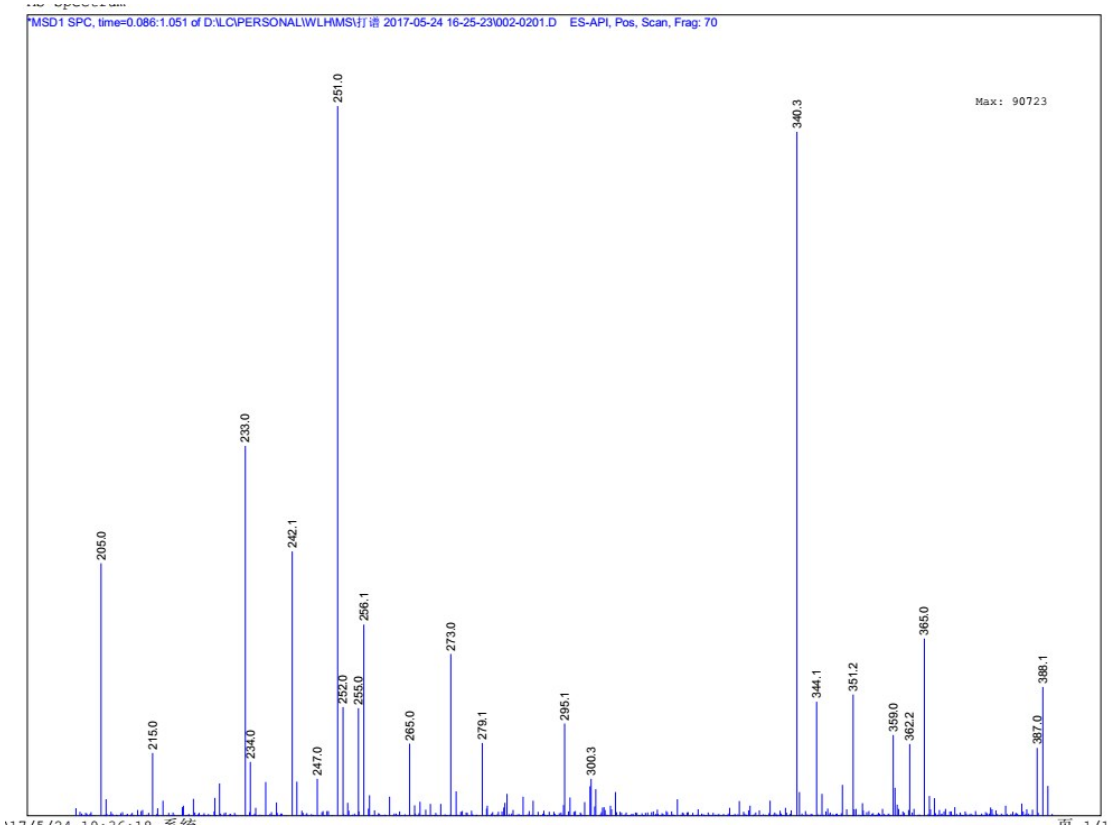

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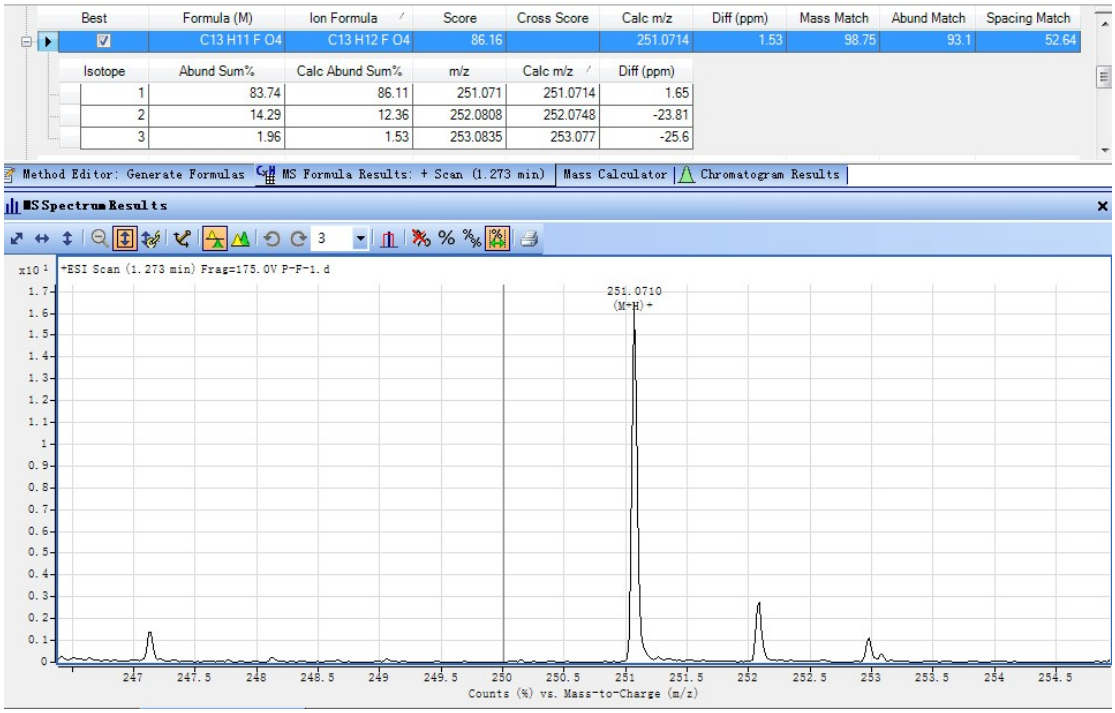

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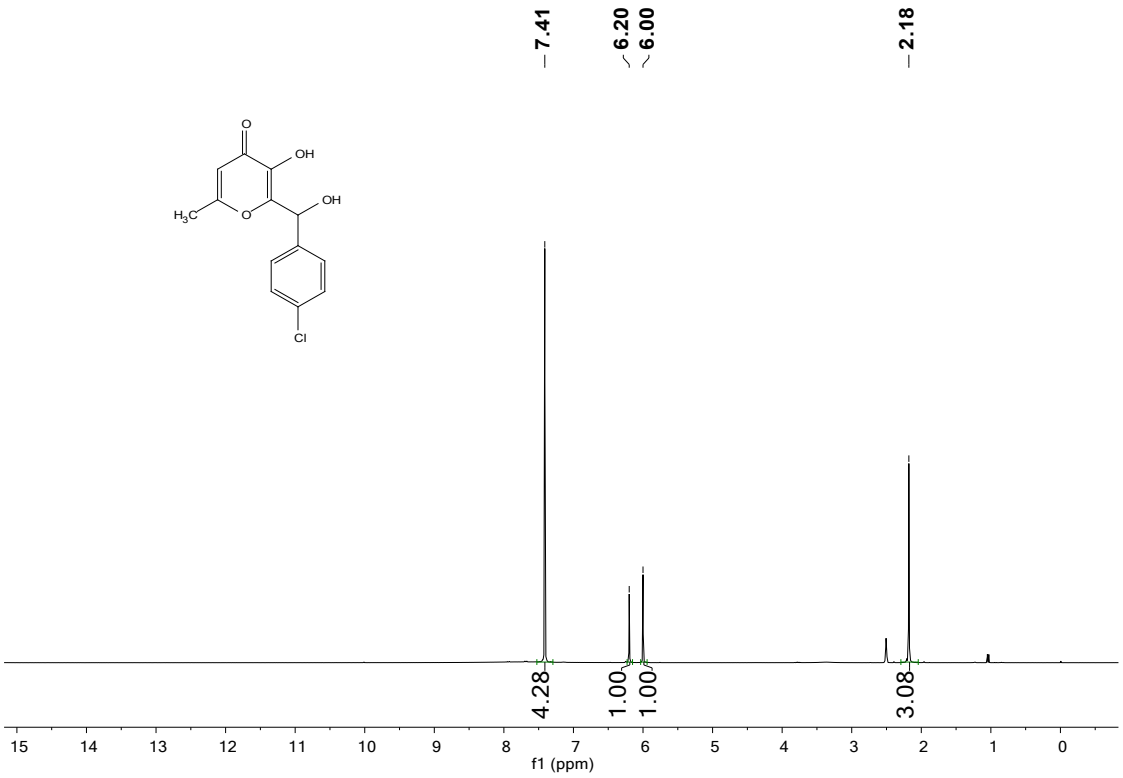
☑	C13 H11 F O4	C13 H12 F O4	86.16		251.0714	1.53	98.75	93.1	52.64

Isotope	Abund Sum%	Calc Abund Sum%	m/z	Calc m/z	Diff (ppm)
1	83.74	86.11	251.071	251.0714	1.65
2	14.29	12.36	252.0808	252.0748	-23.81
3	1.96	1.53	253.0835	253.077	-25.6

^{1}H NMR spectrum of compound 2c



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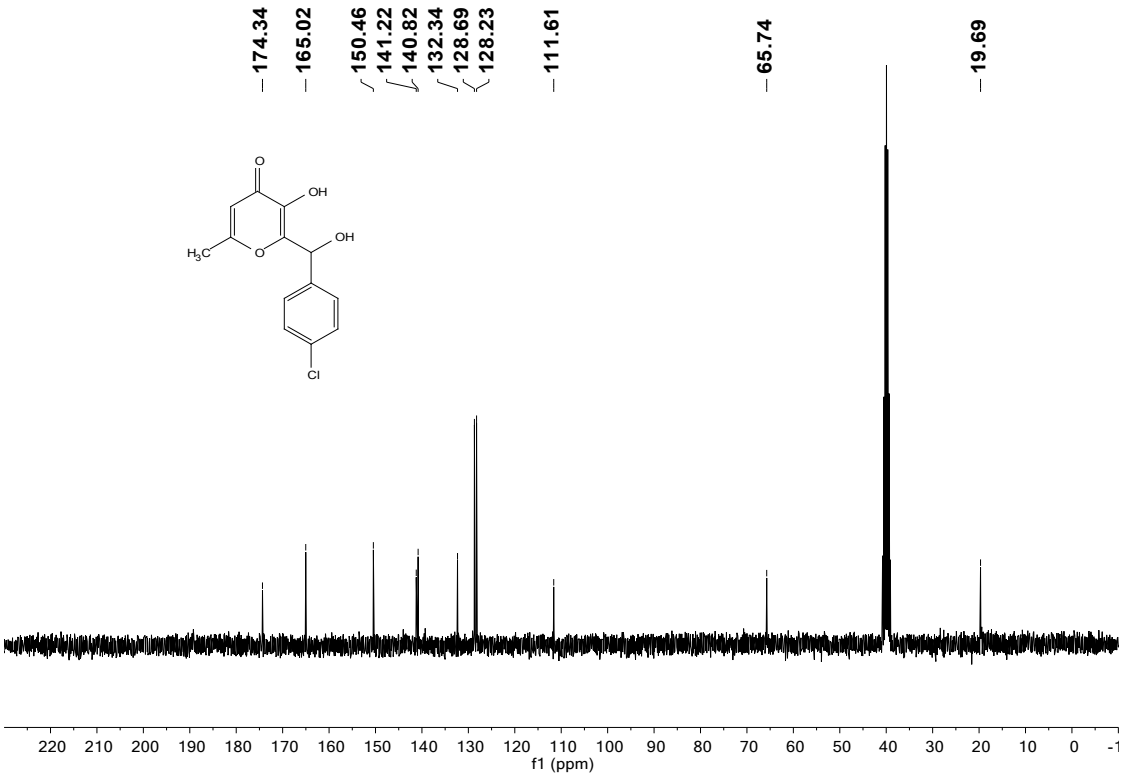

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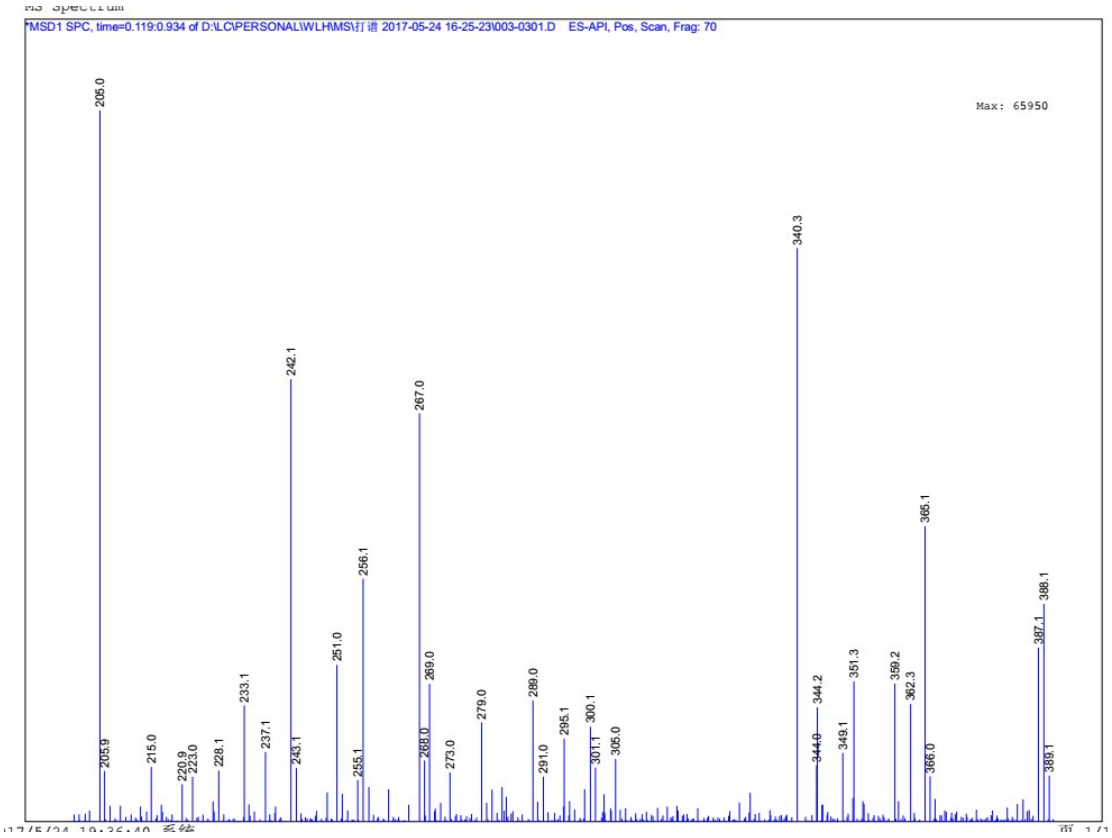

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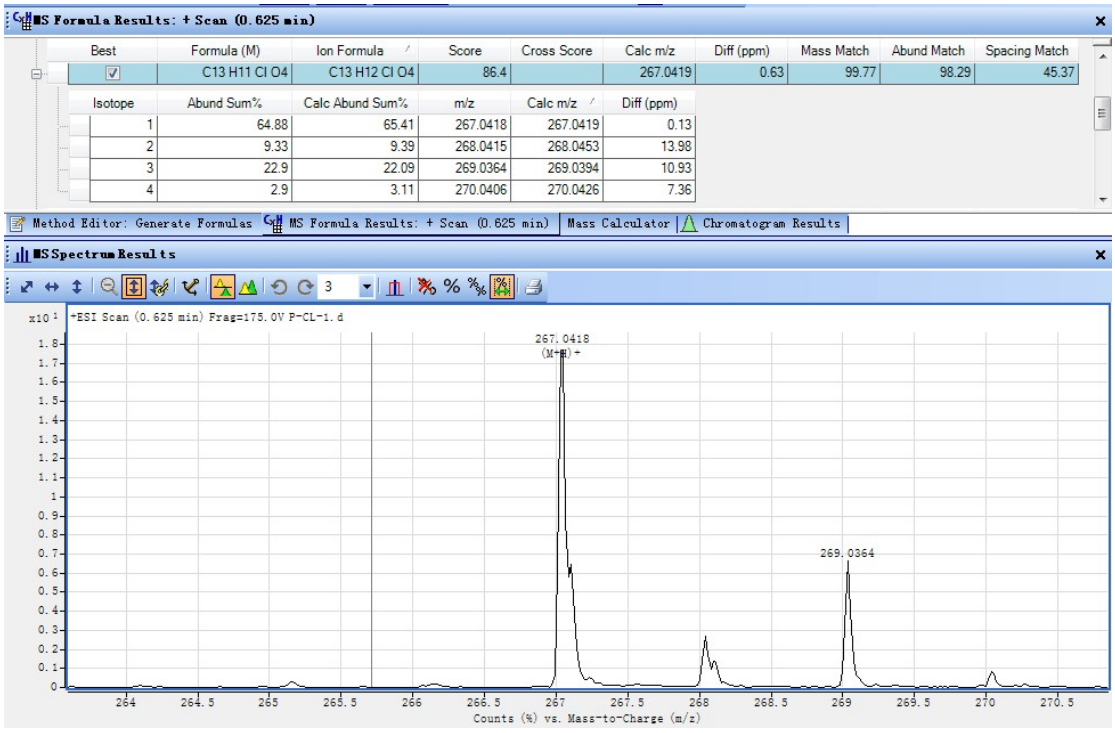

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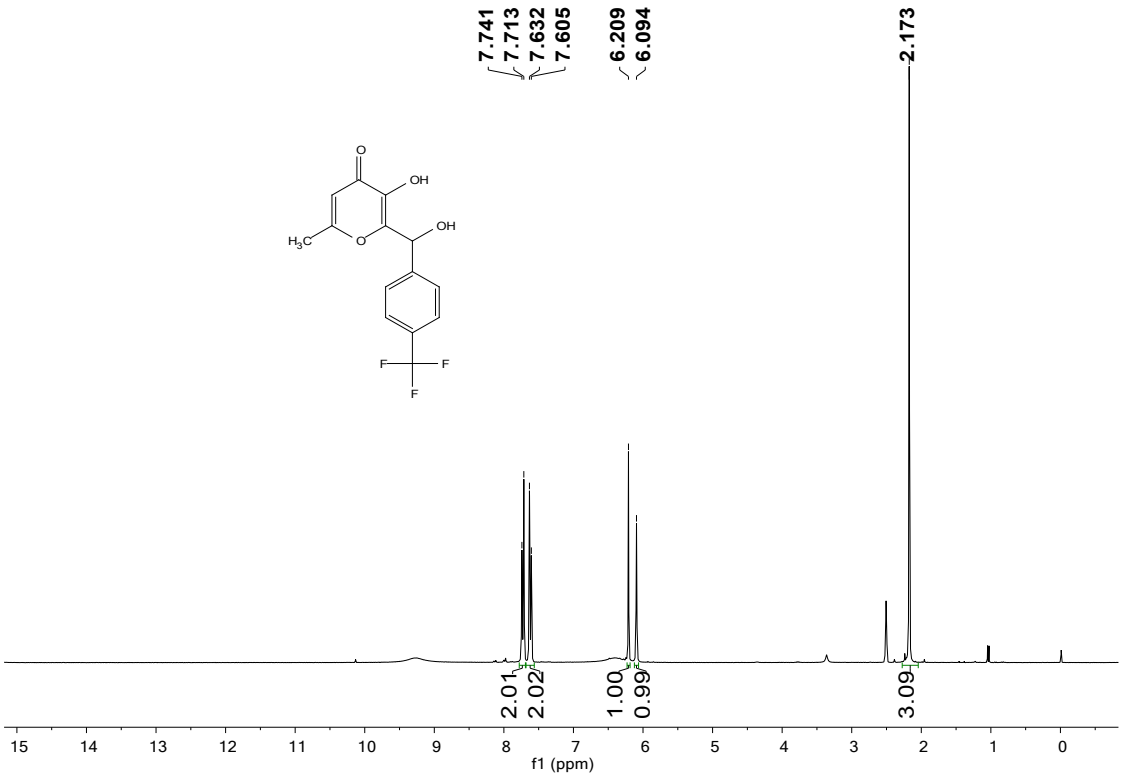

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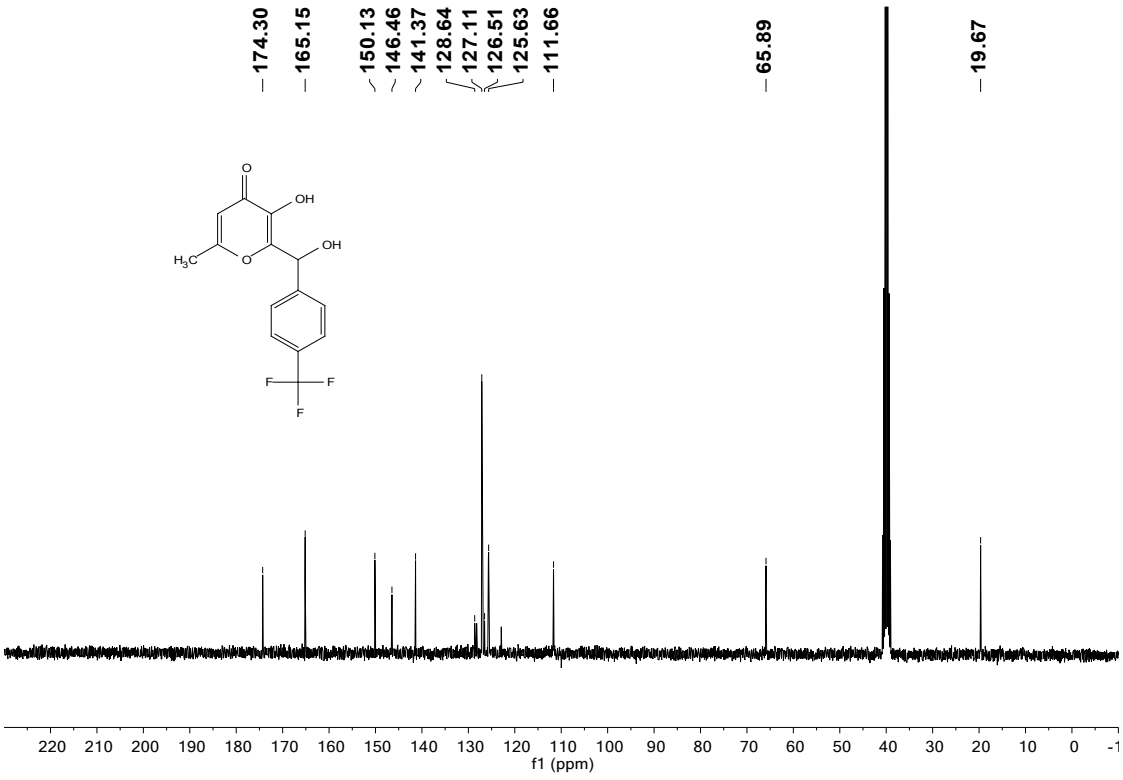

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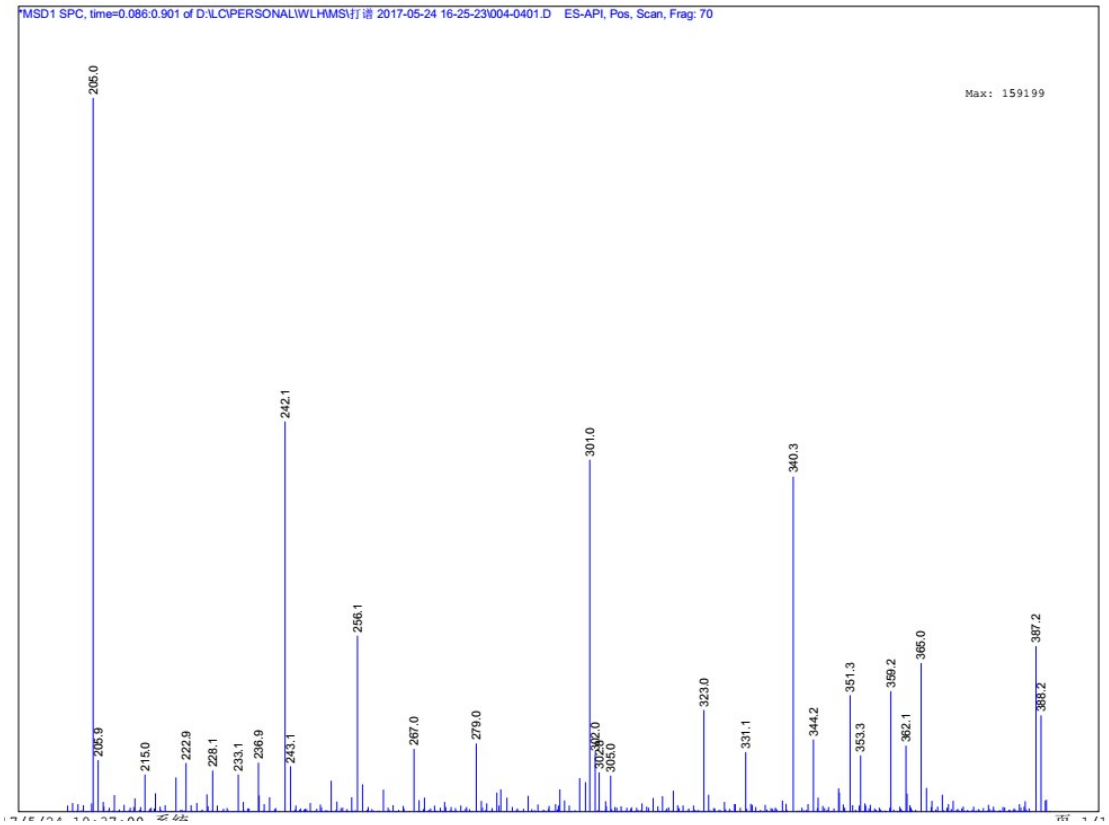

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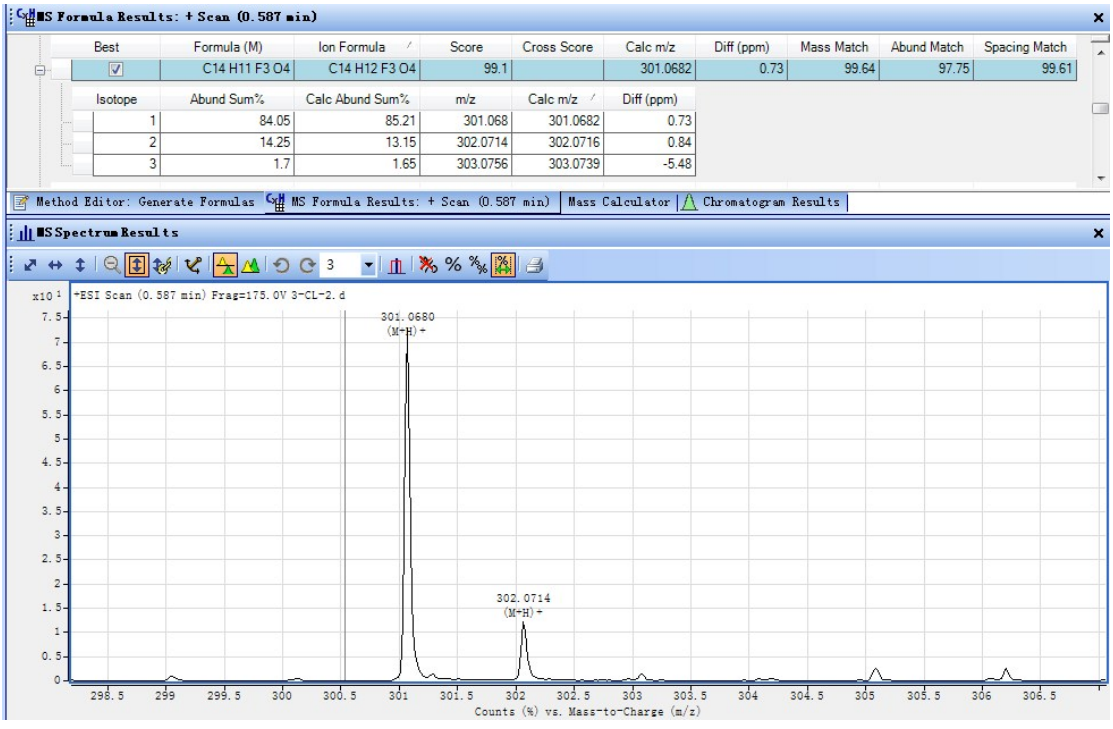

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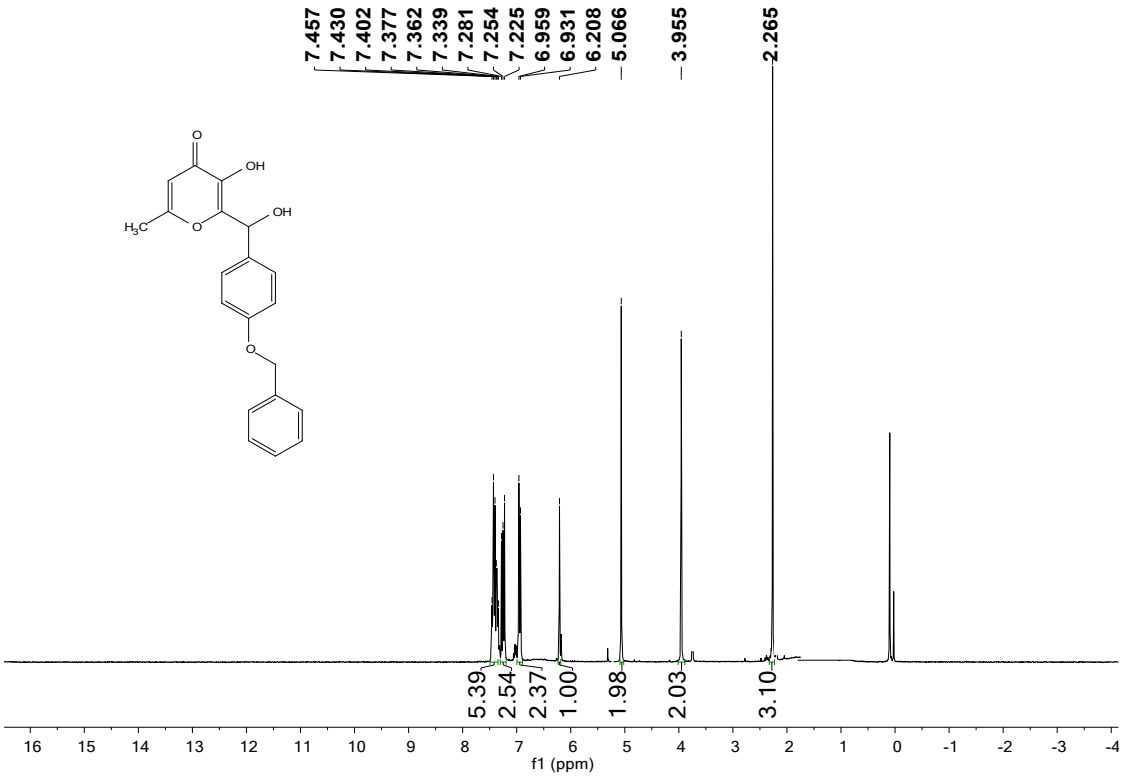

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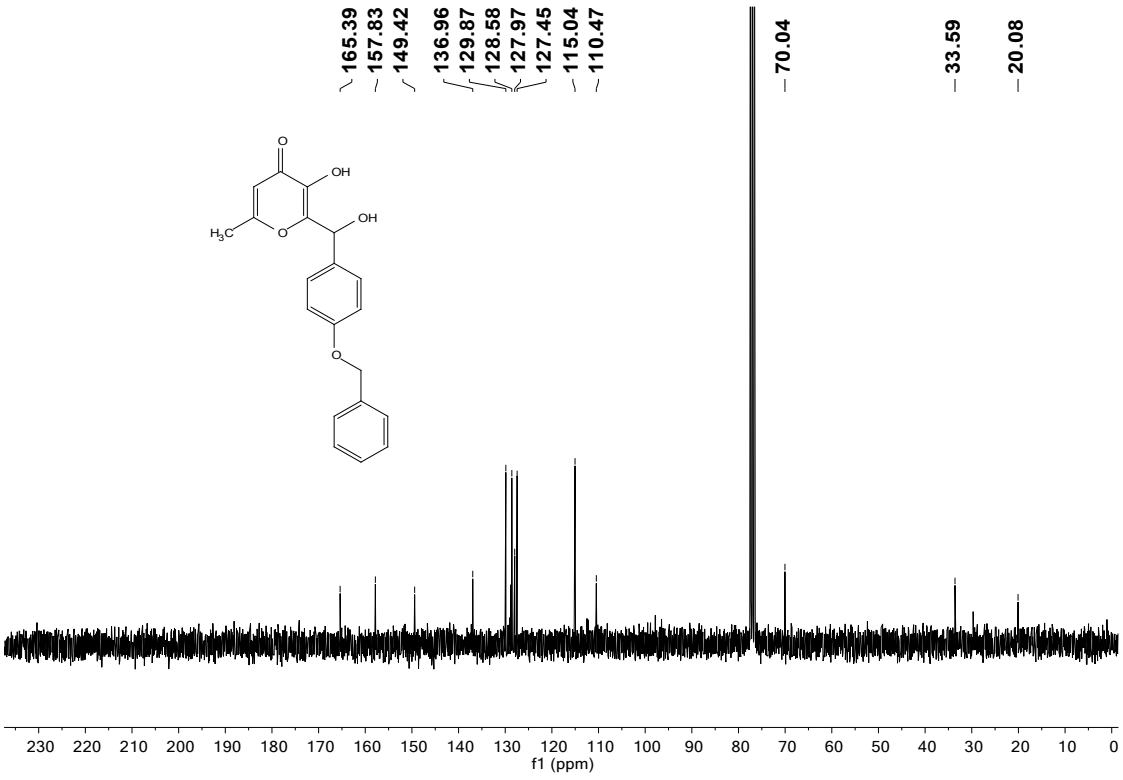

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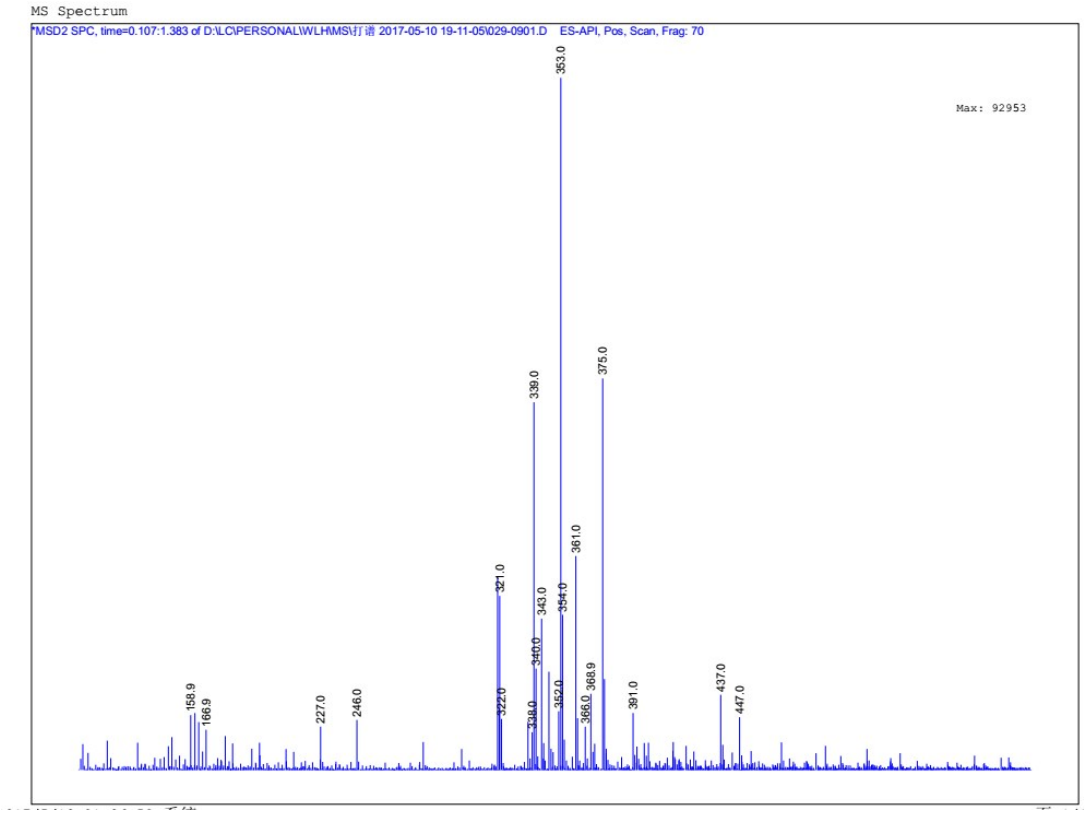

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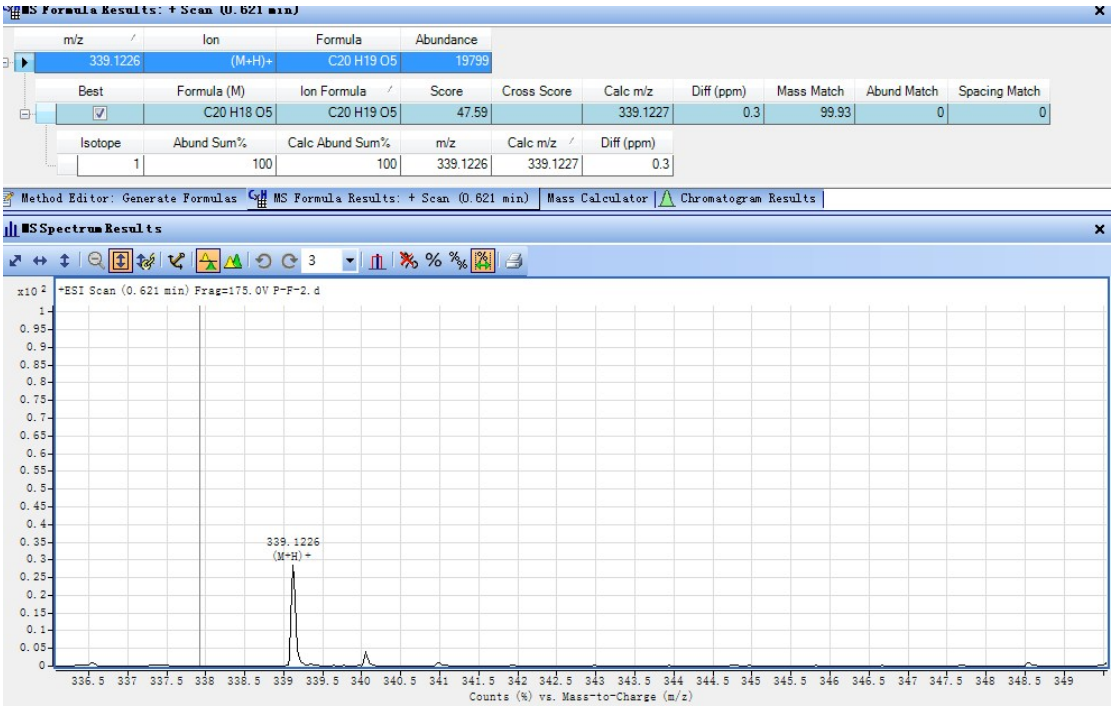

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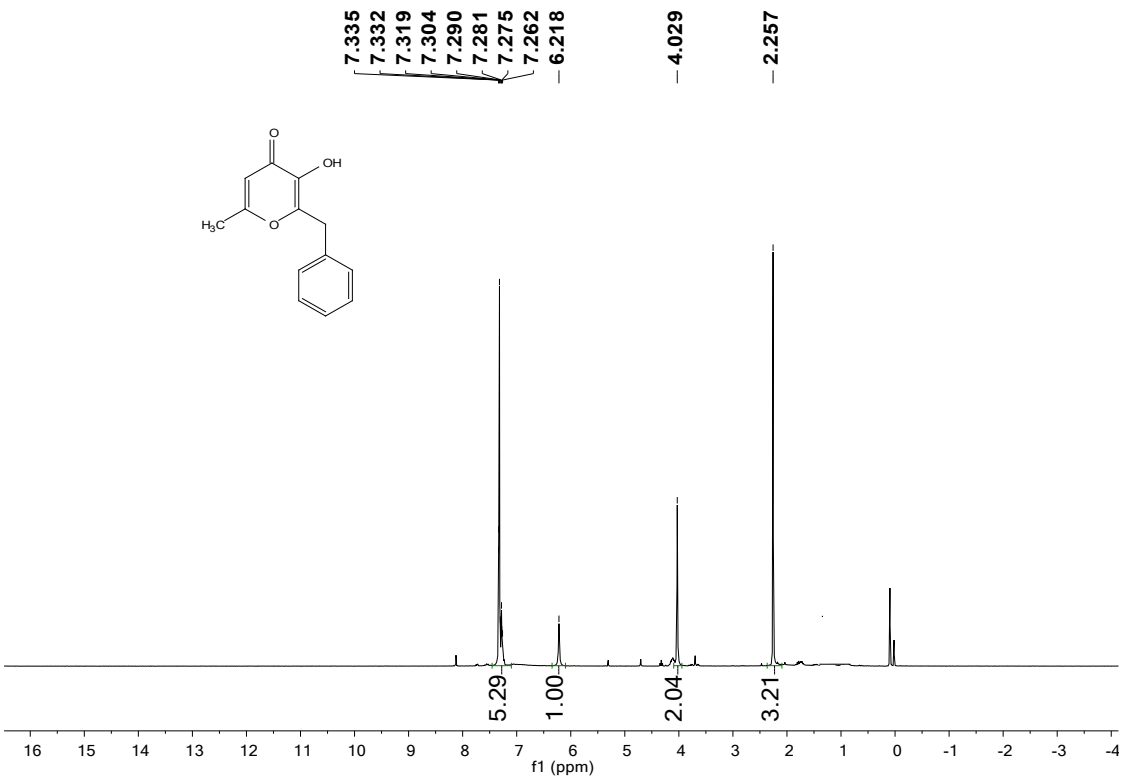

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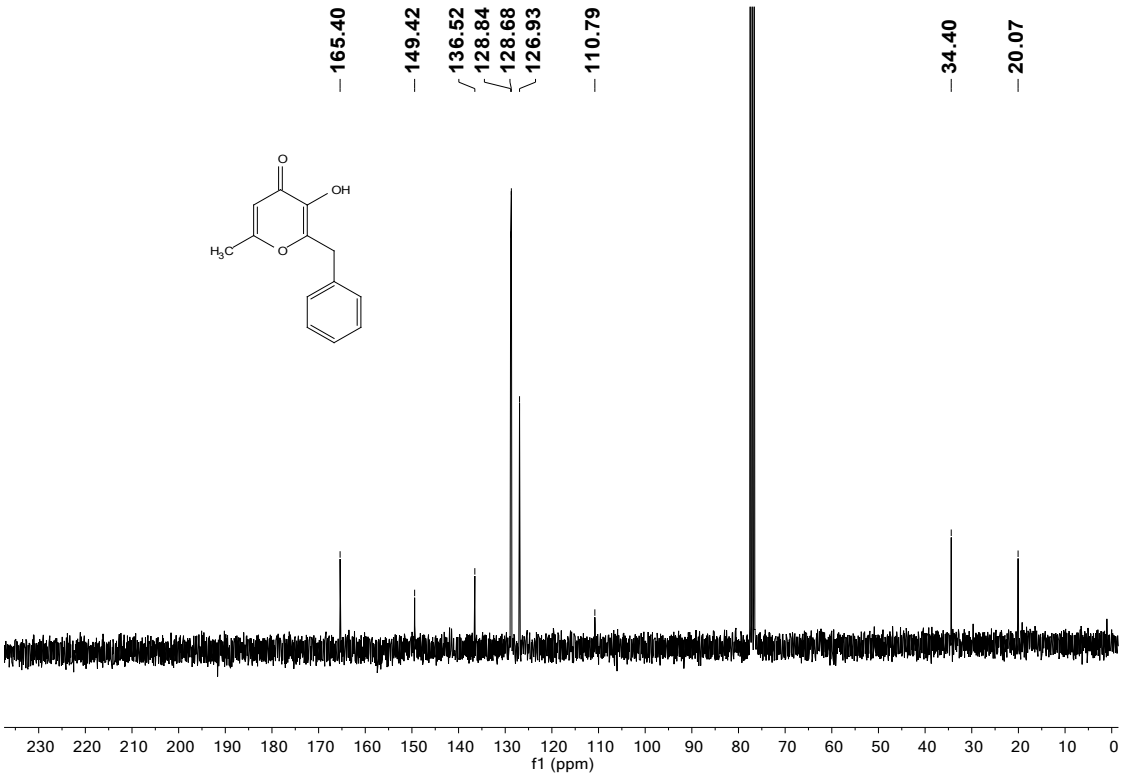

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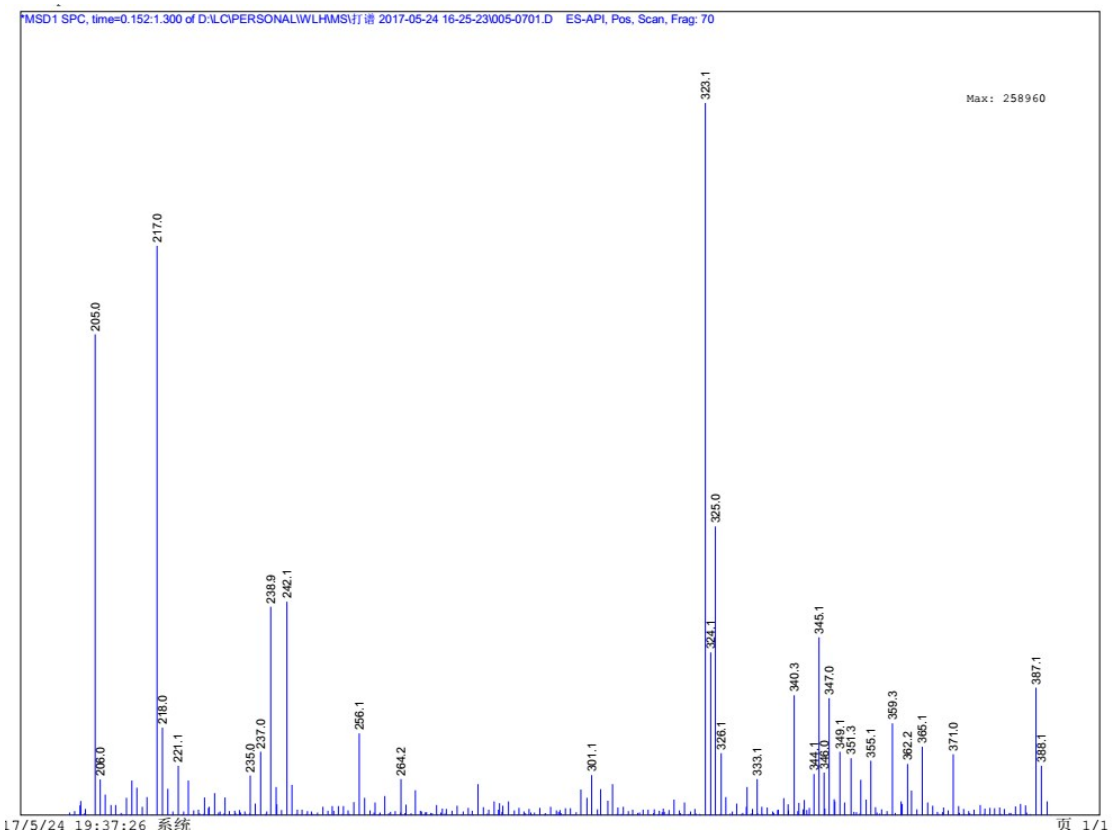

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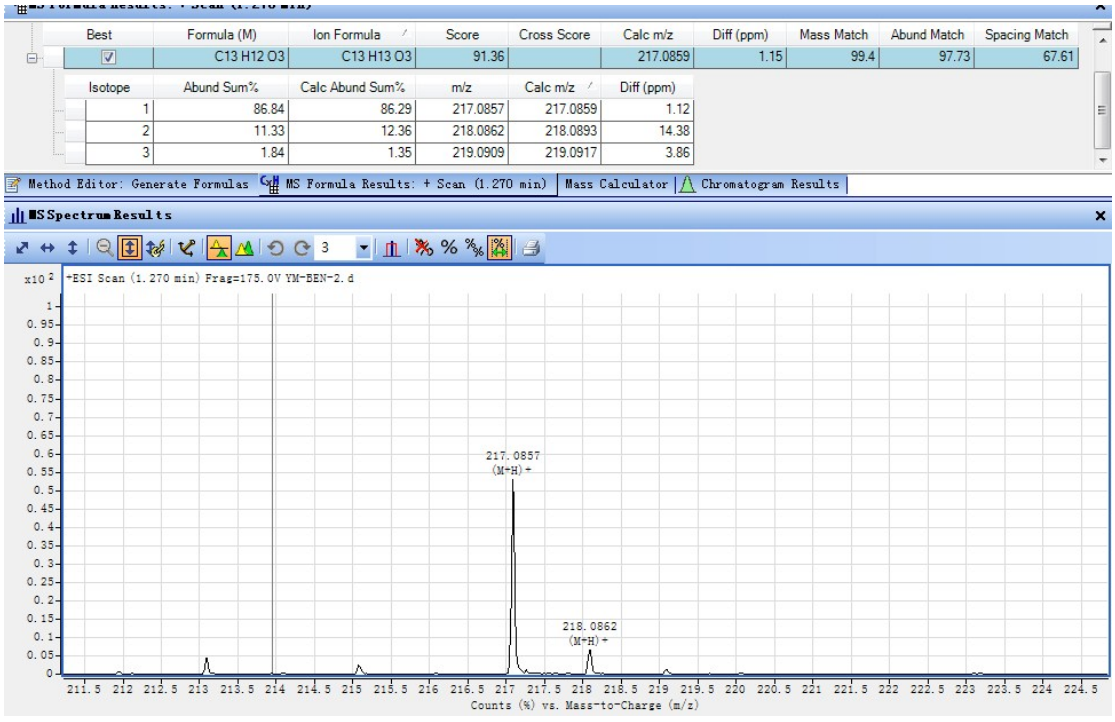

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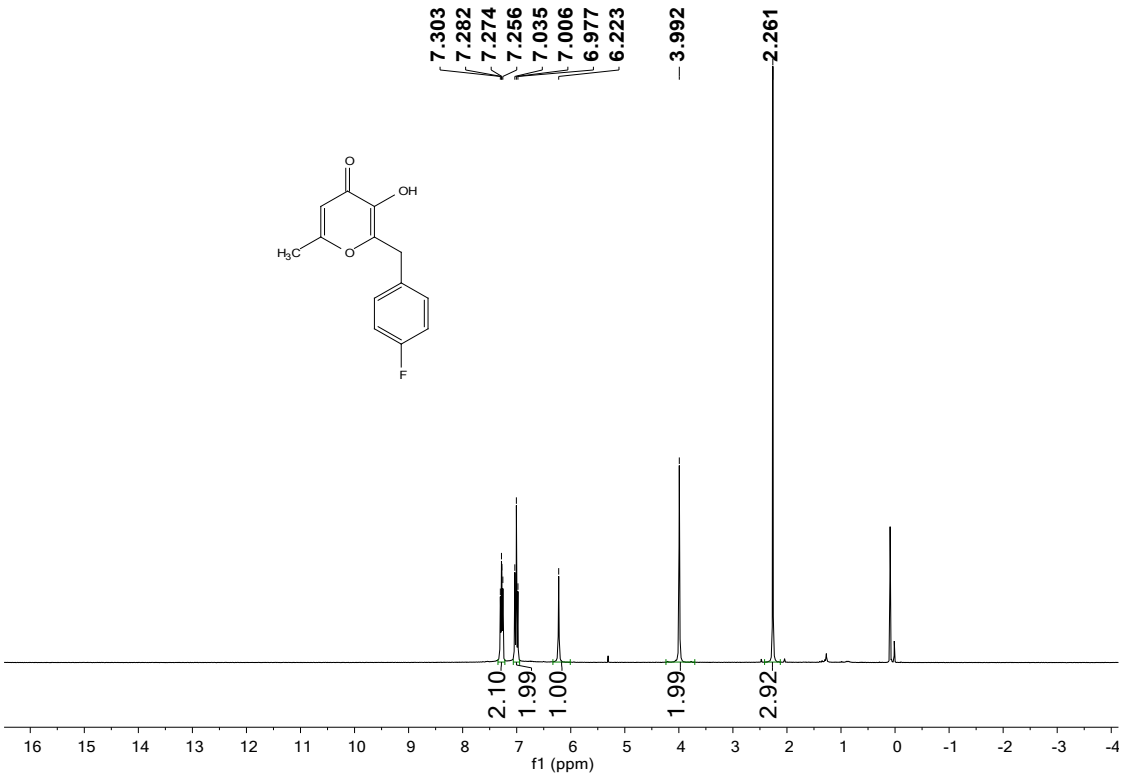

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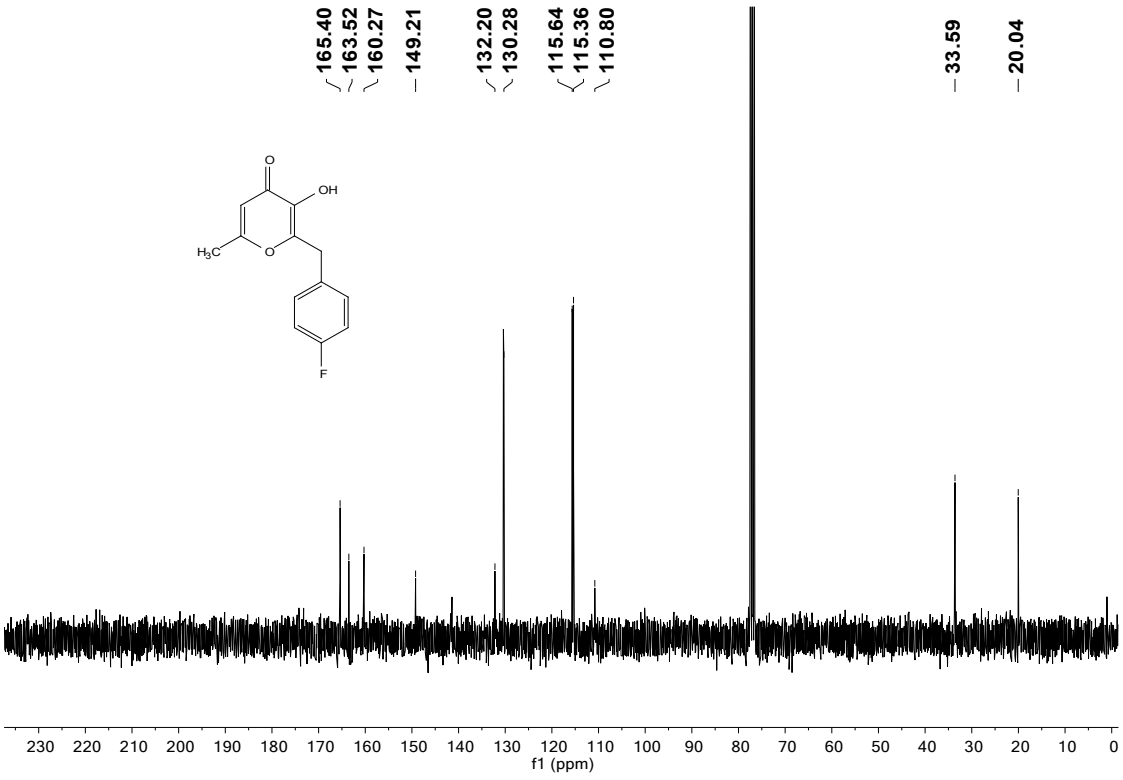

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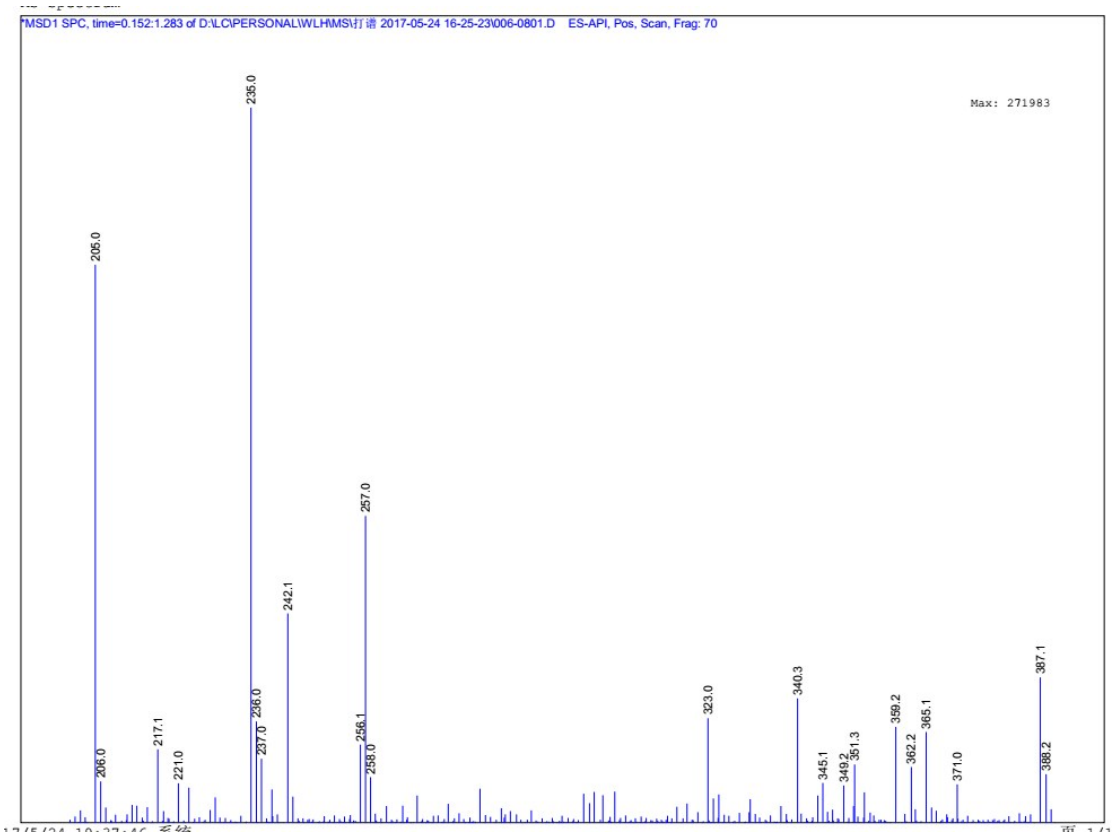

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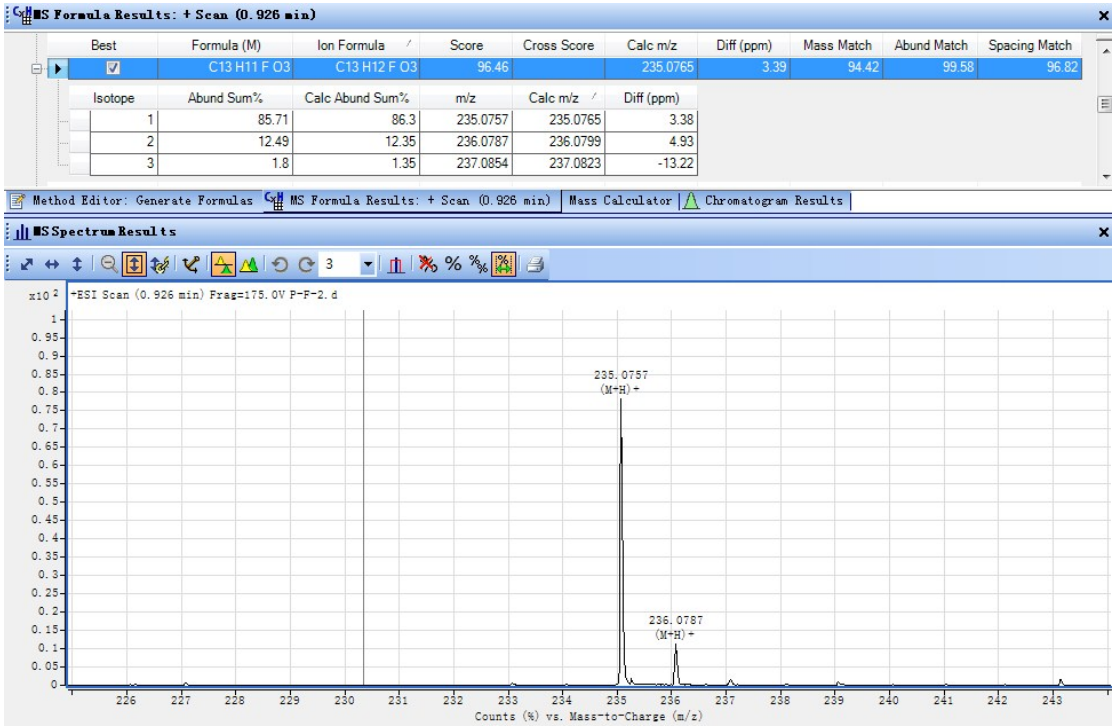

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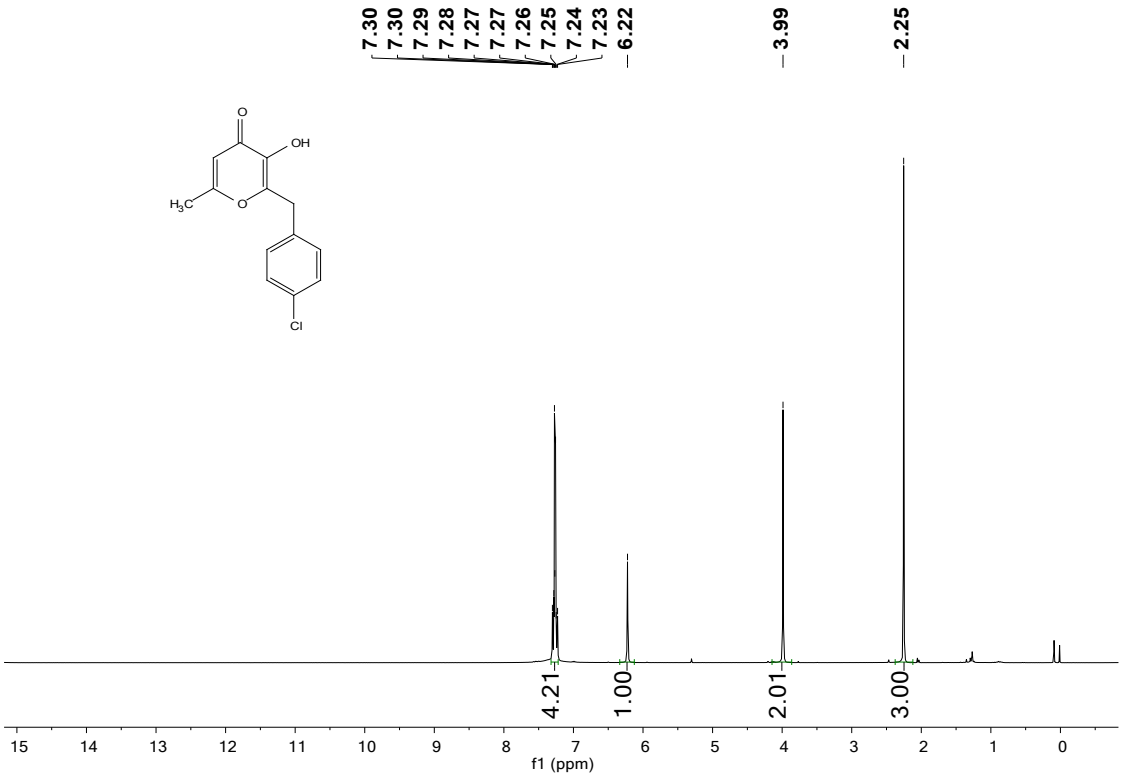

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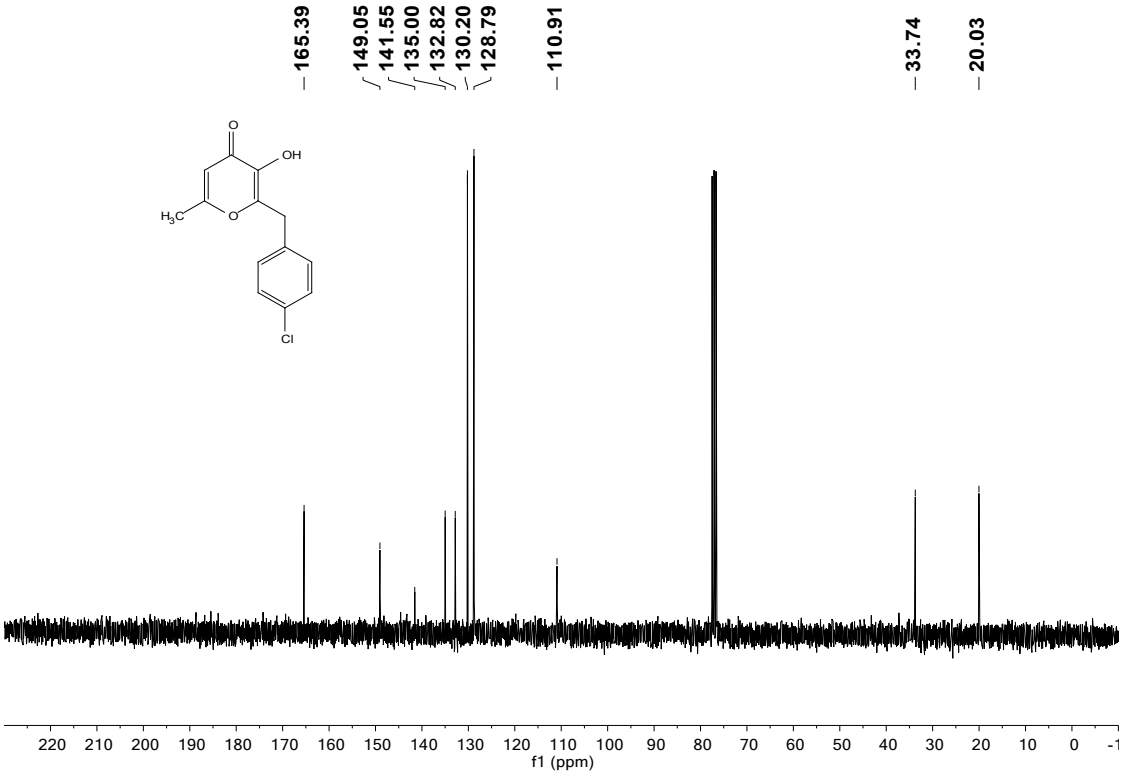

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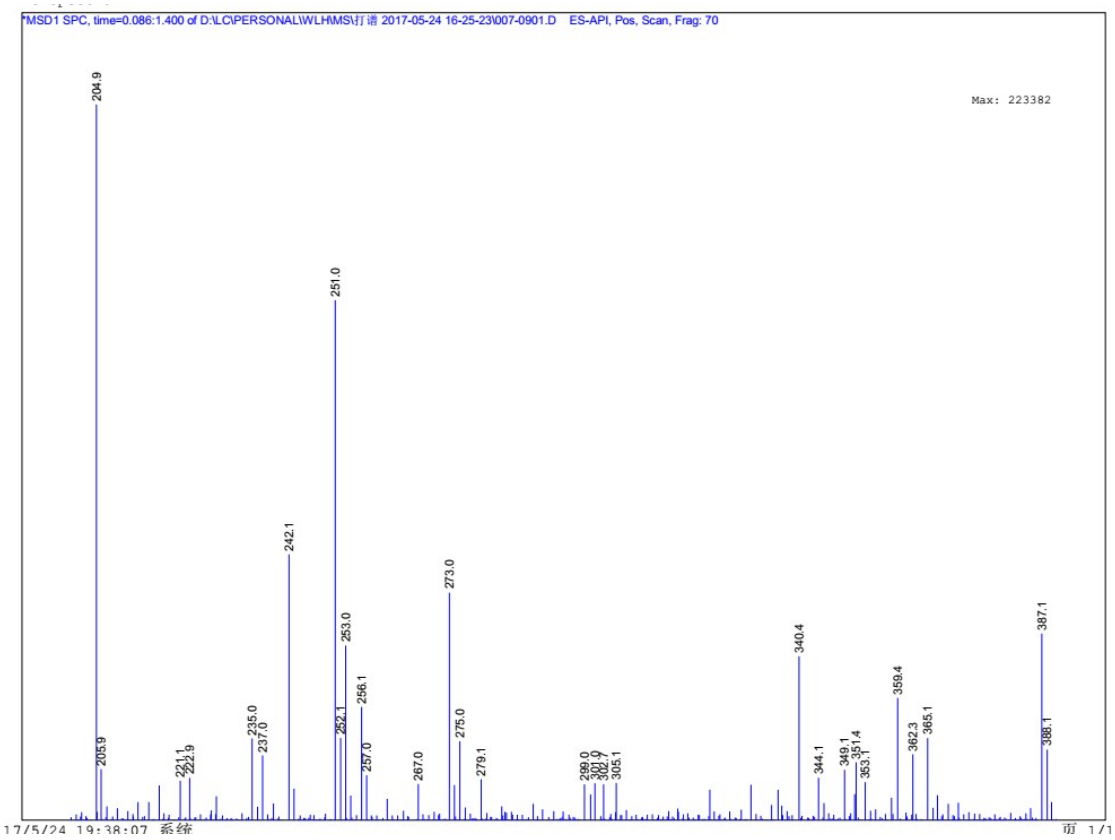

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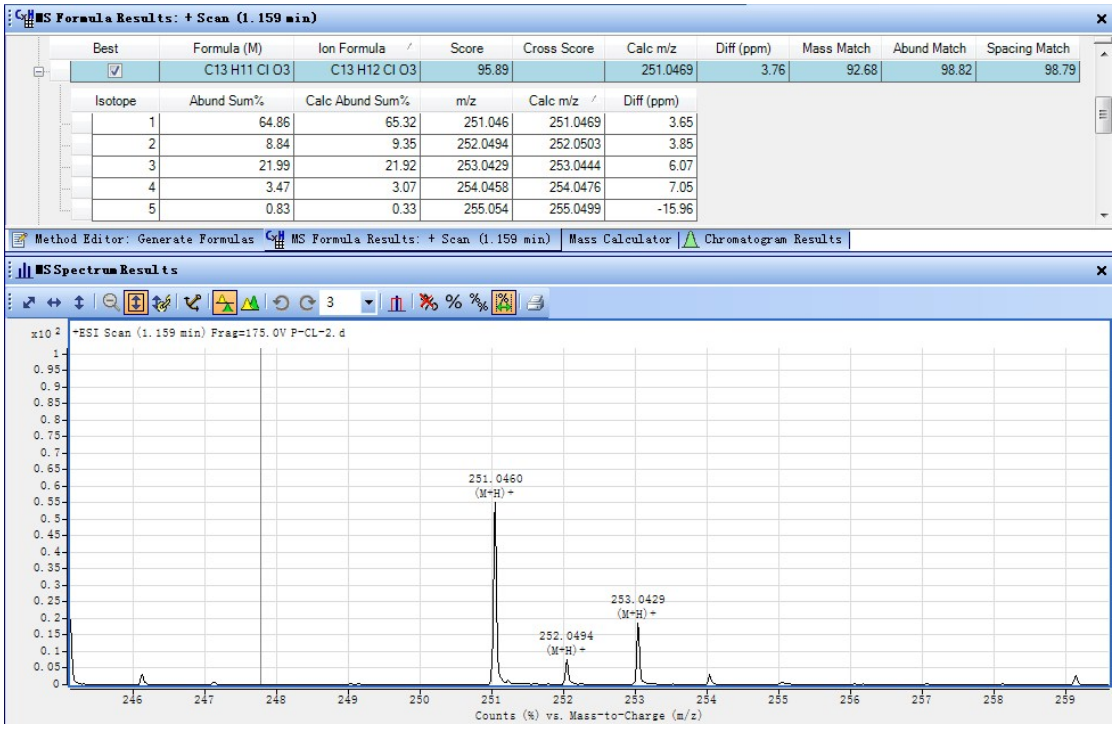

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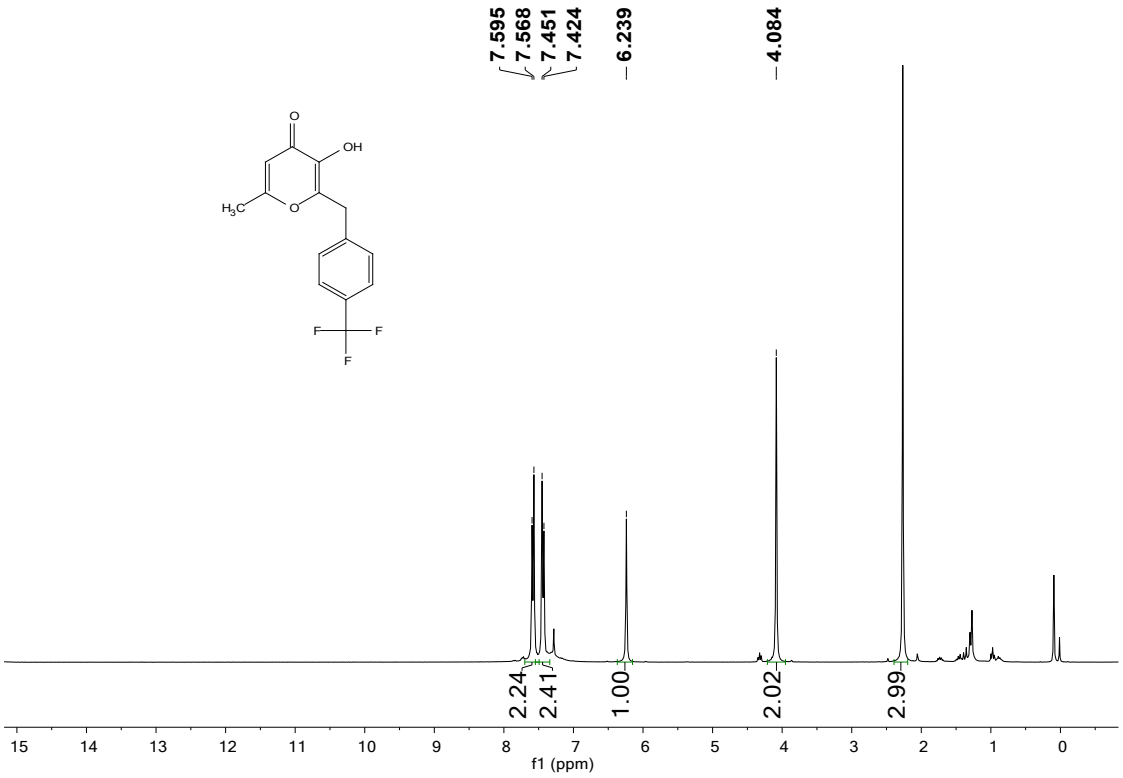

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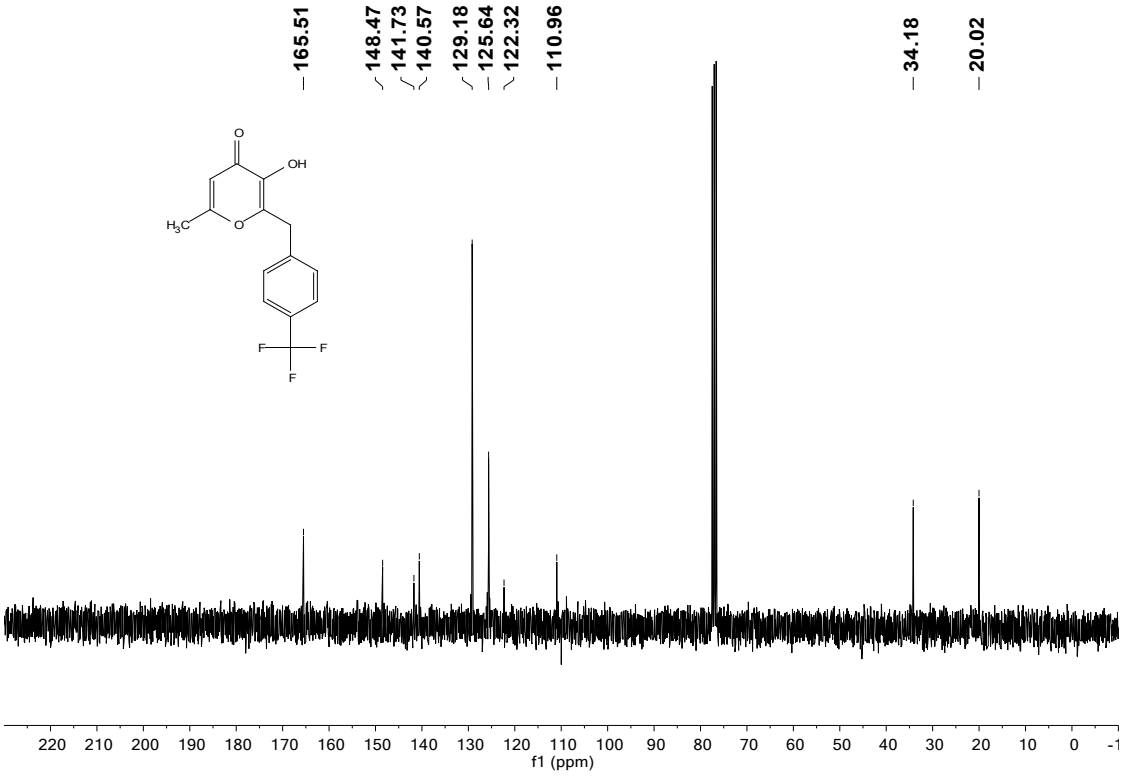

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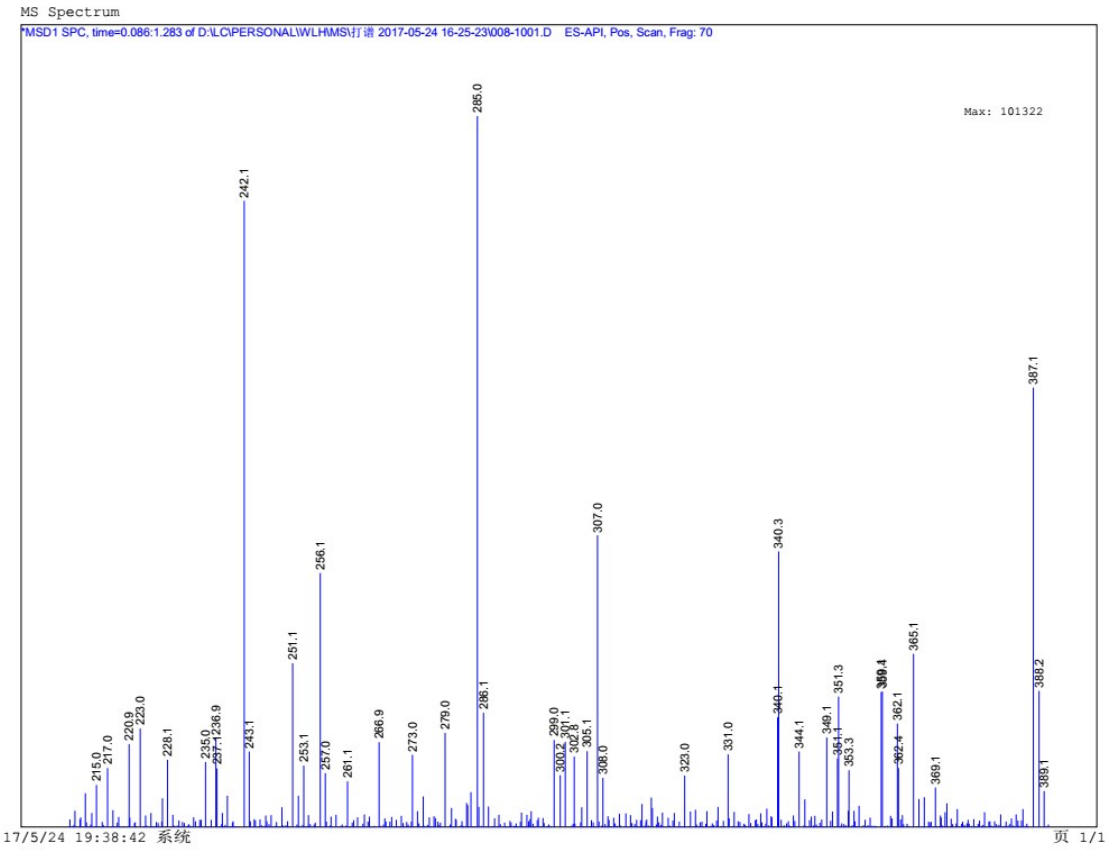

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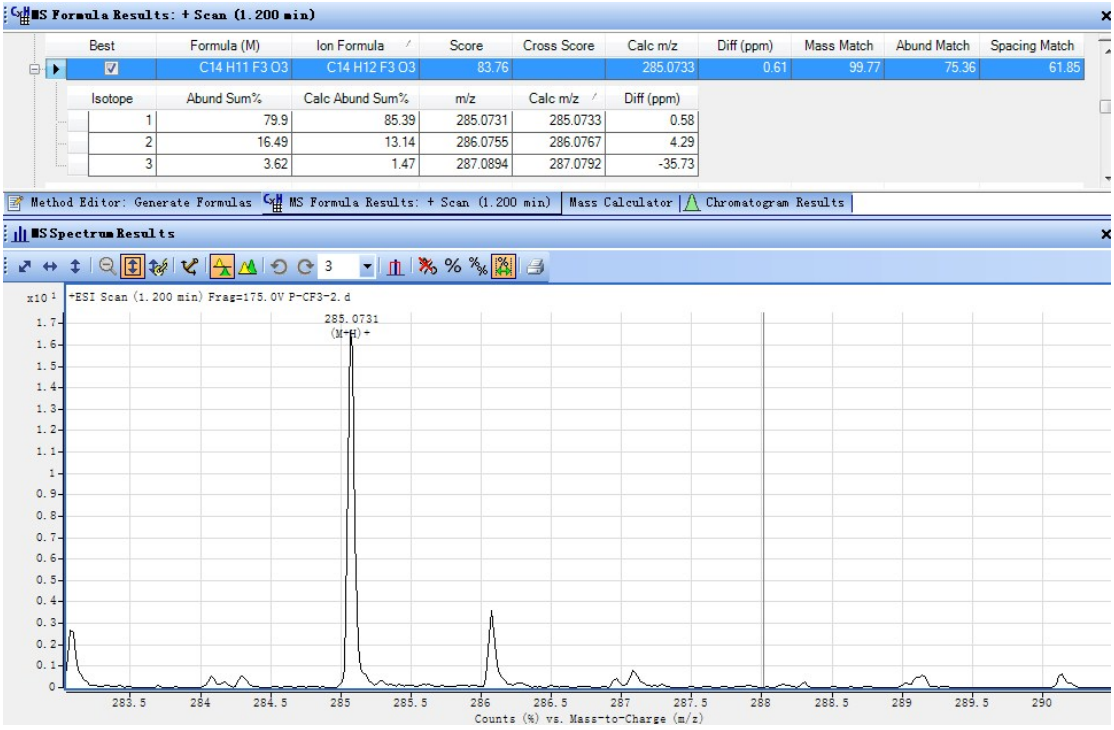

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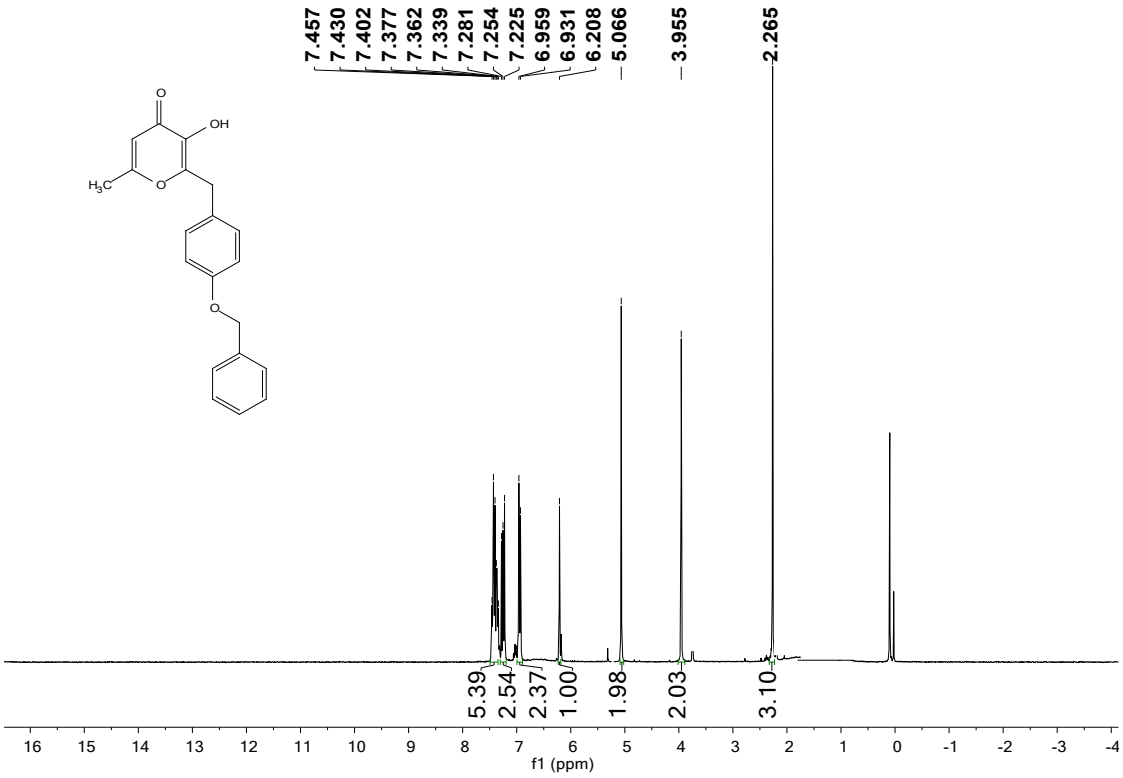

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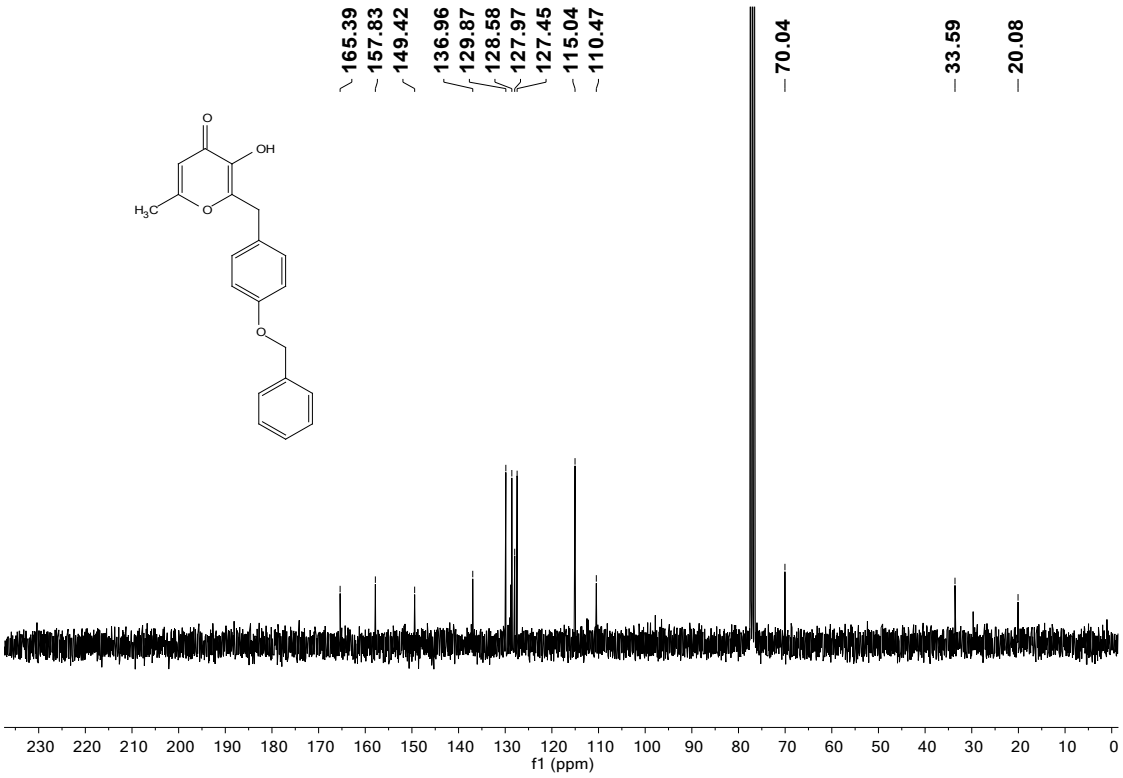

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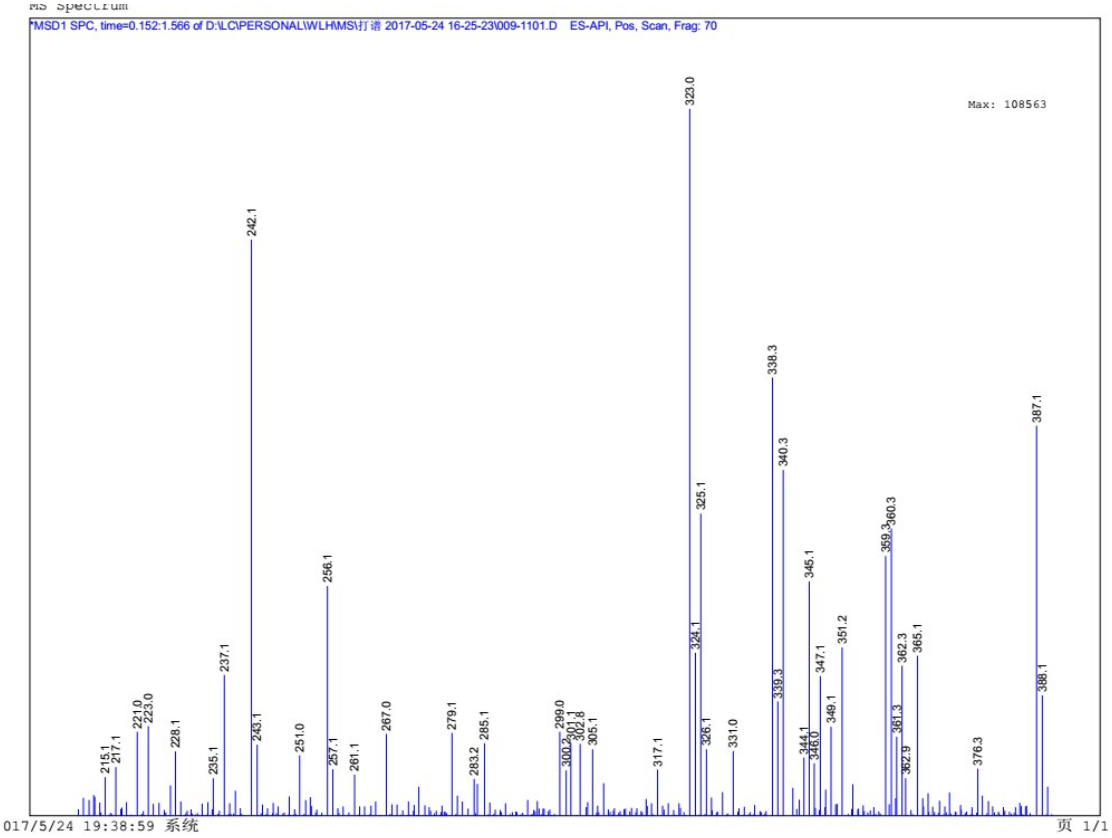

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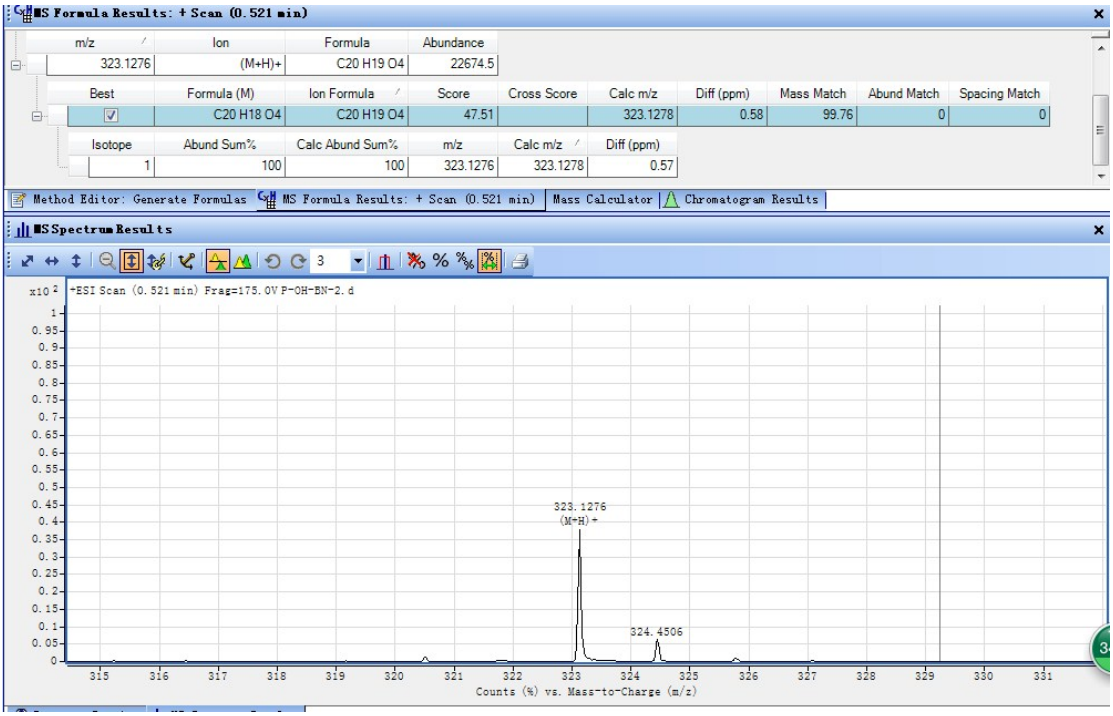

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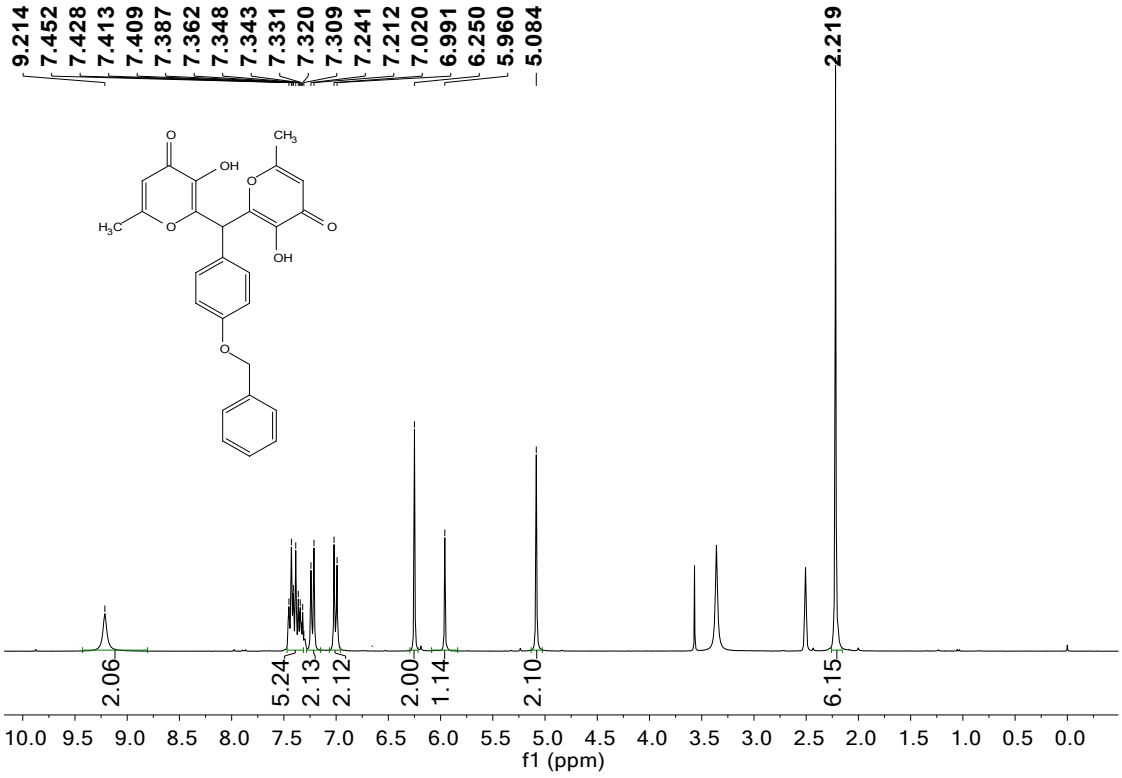

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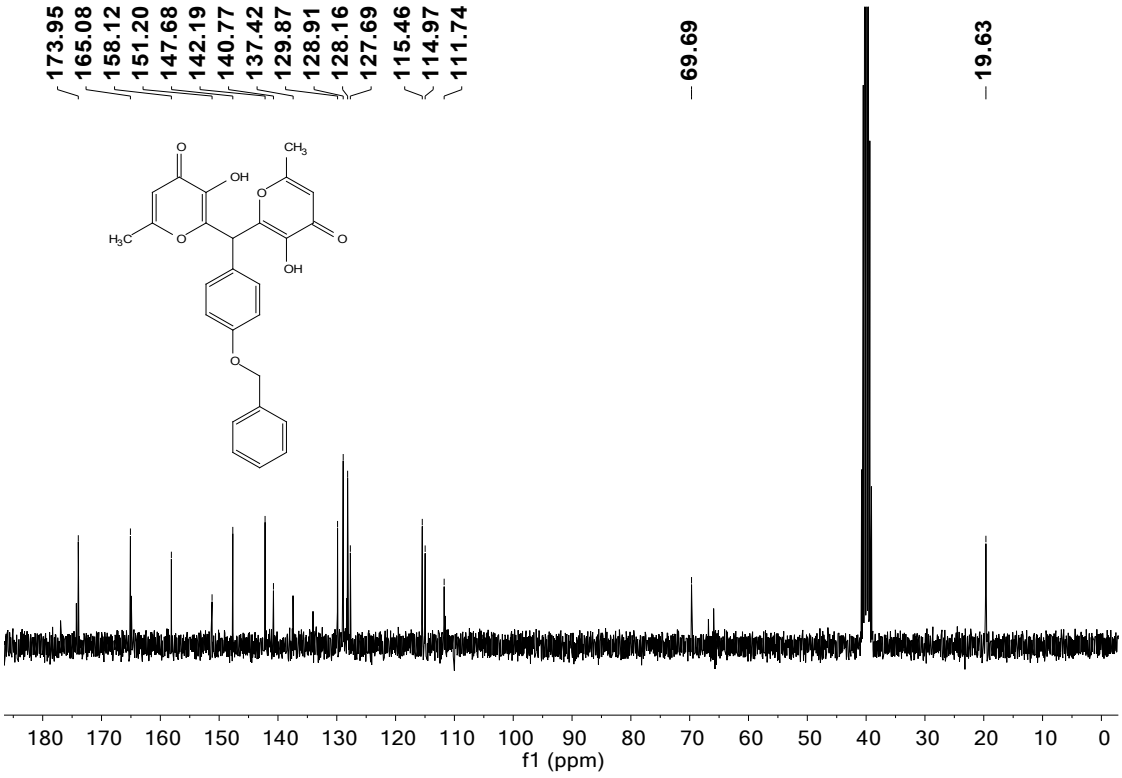

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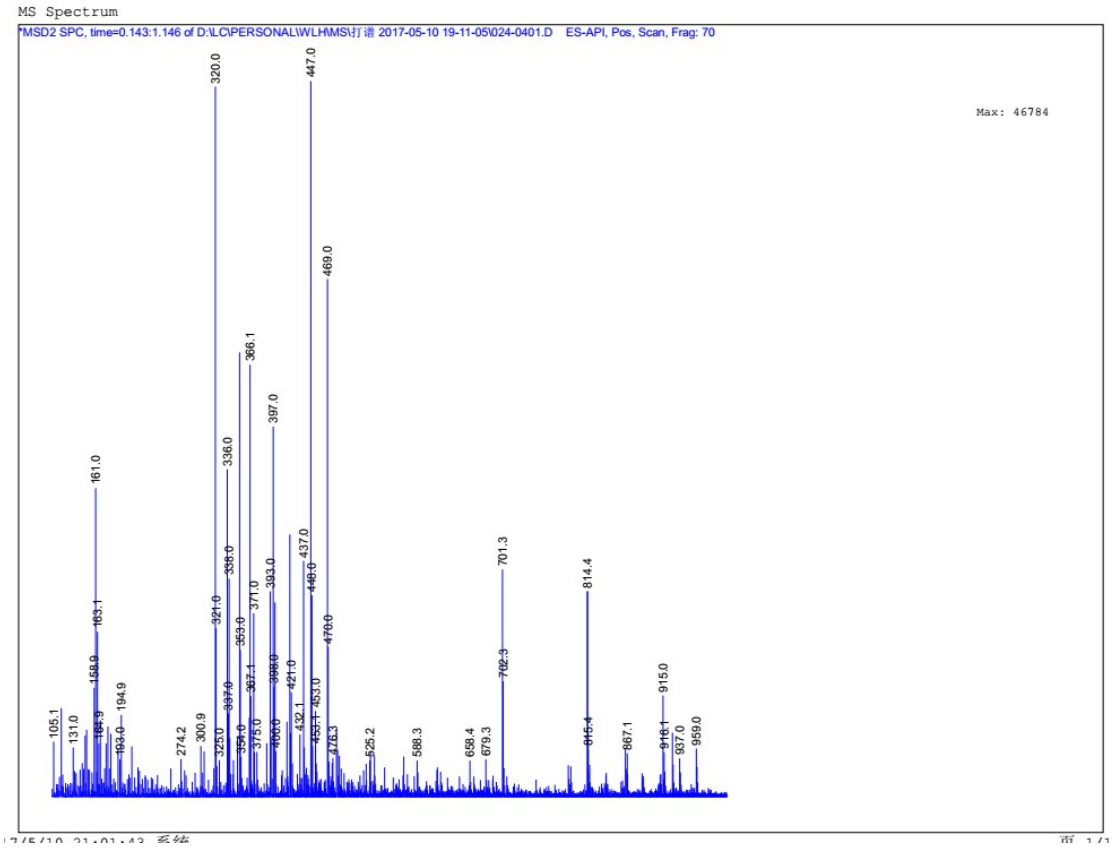

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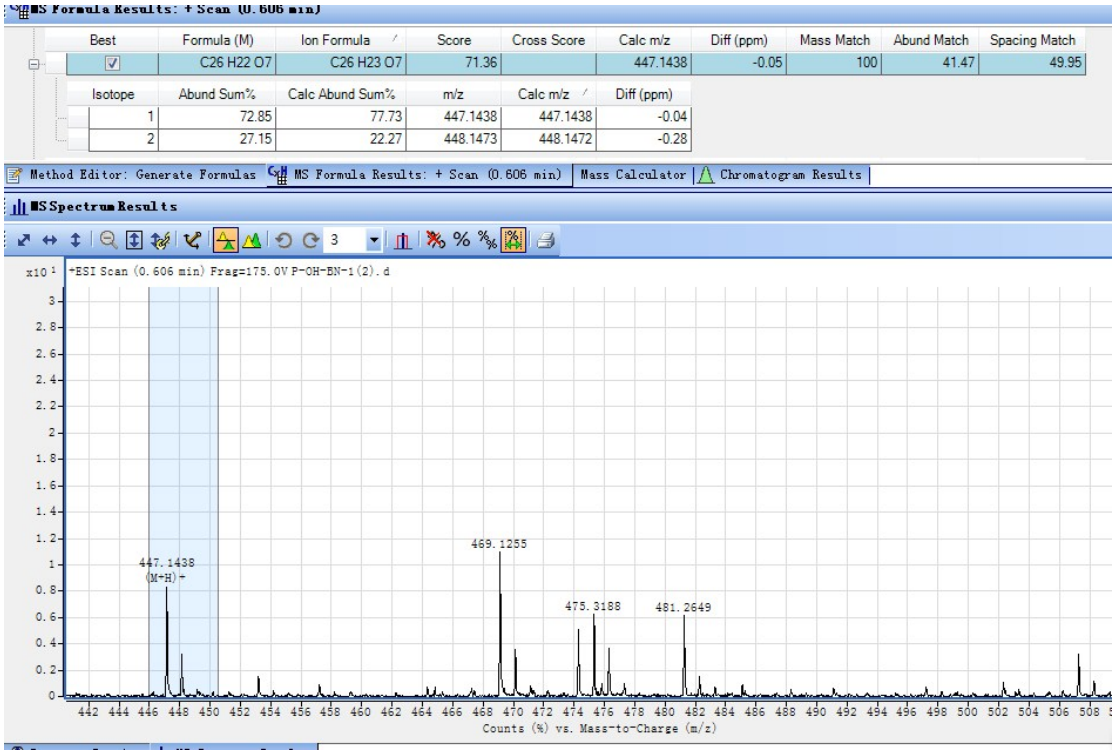

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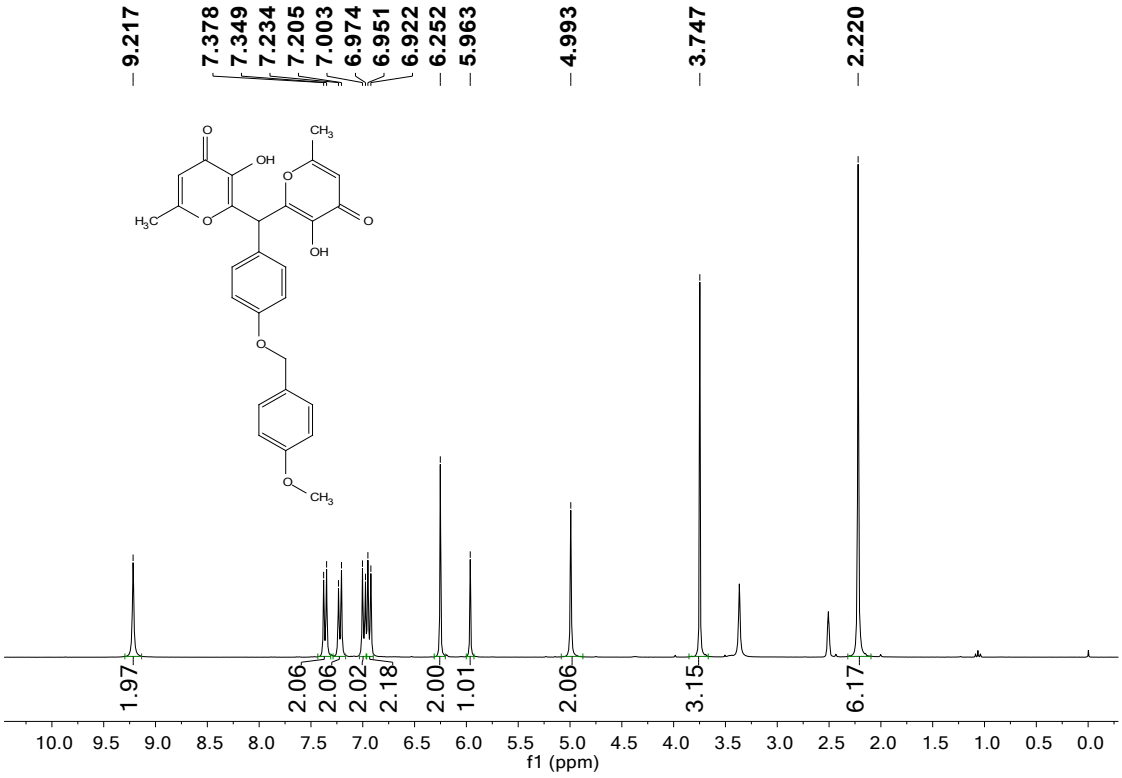

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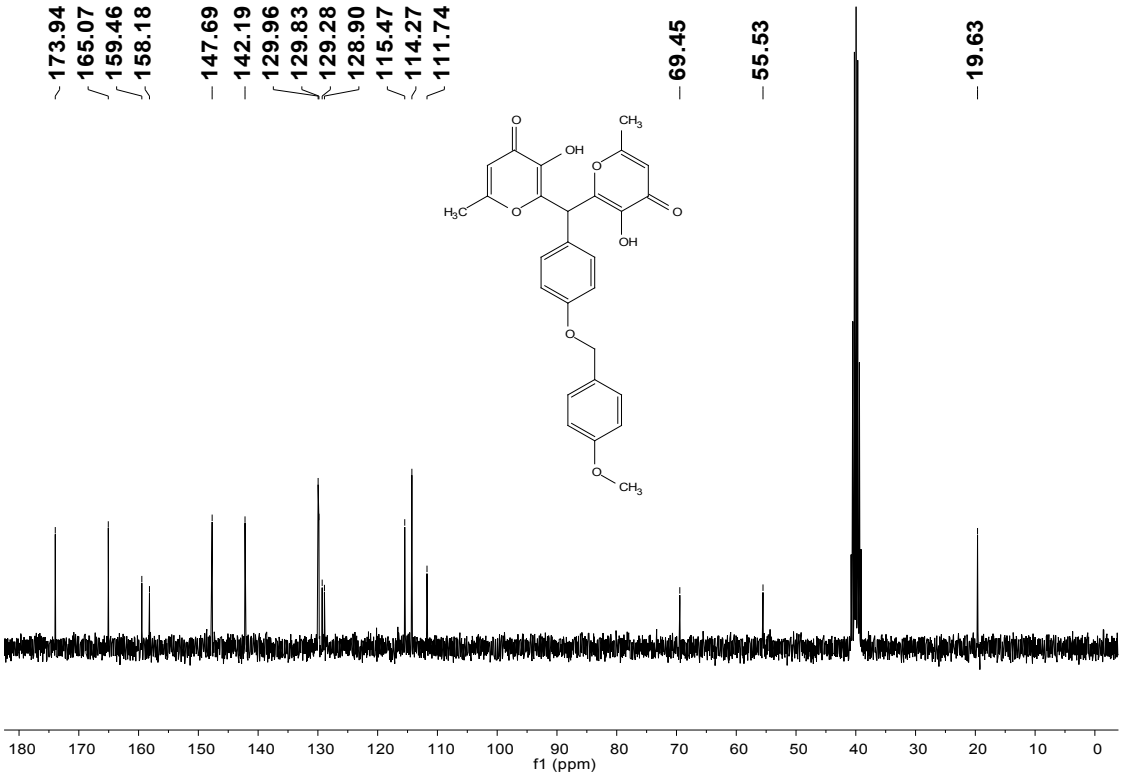
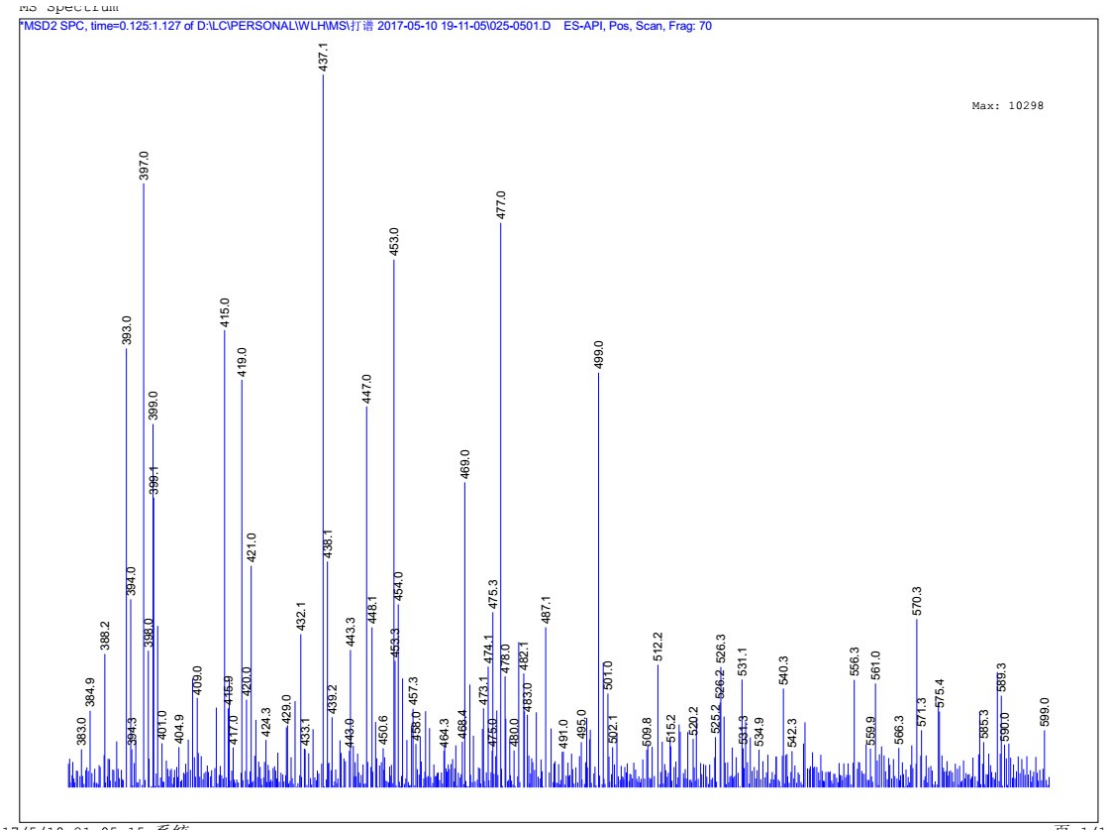
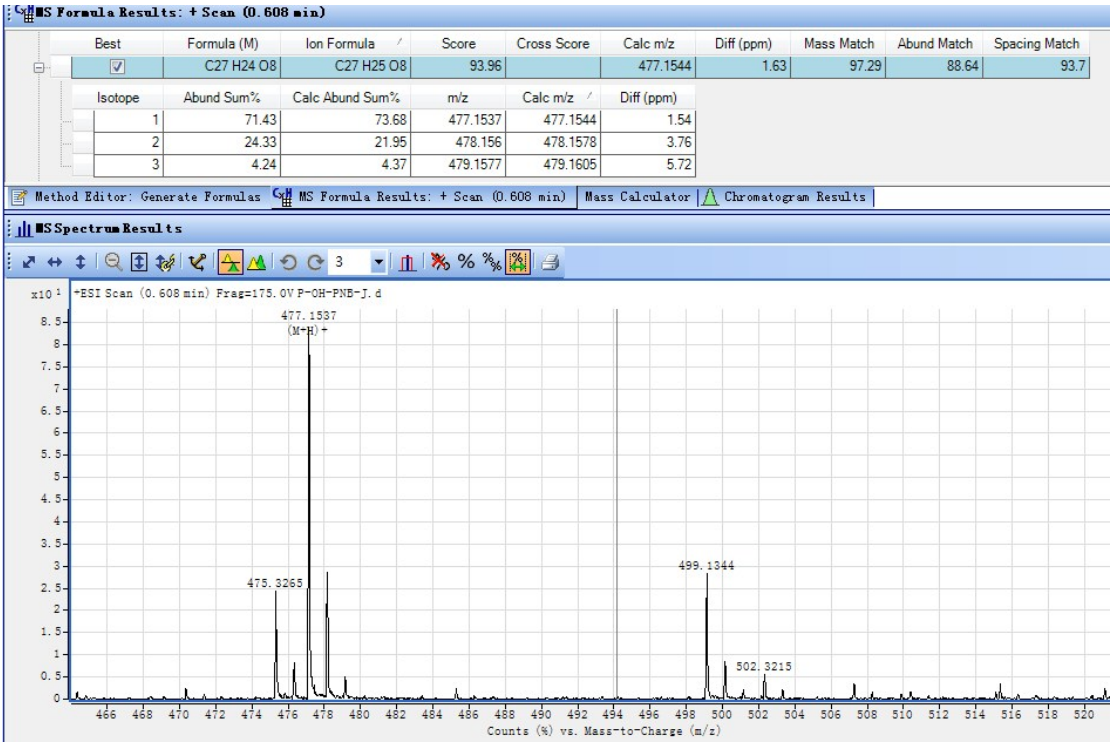


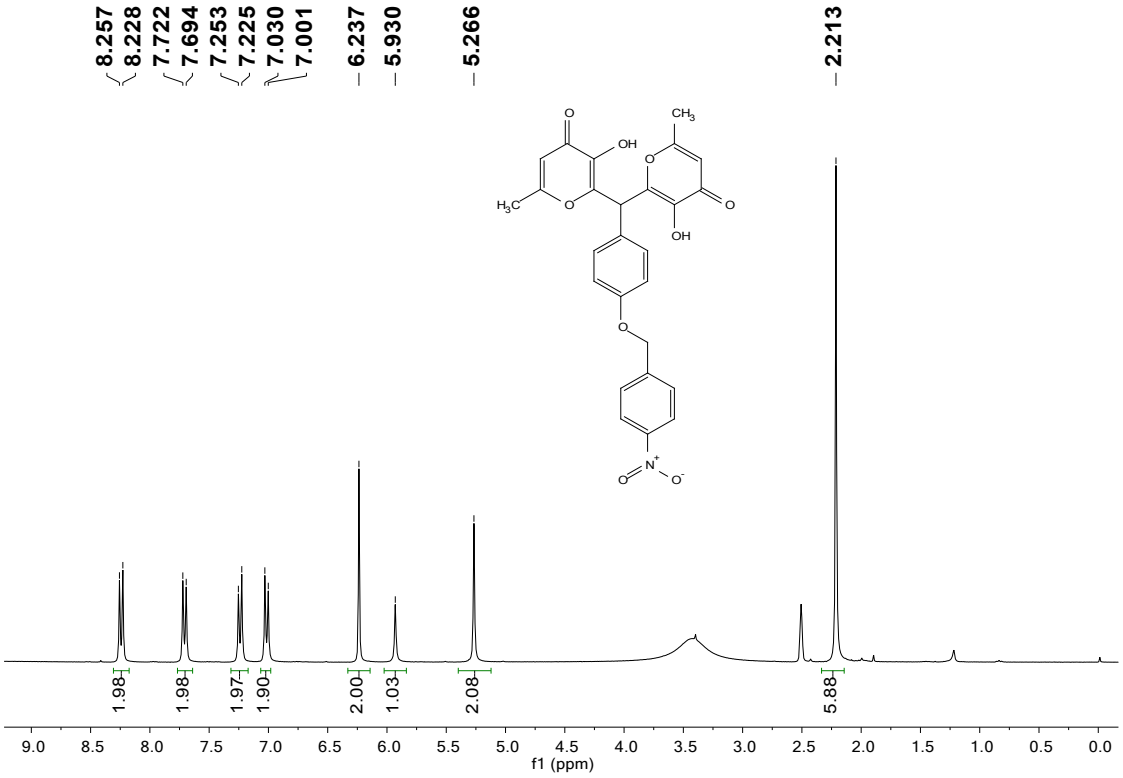
LRMS of compound 2b



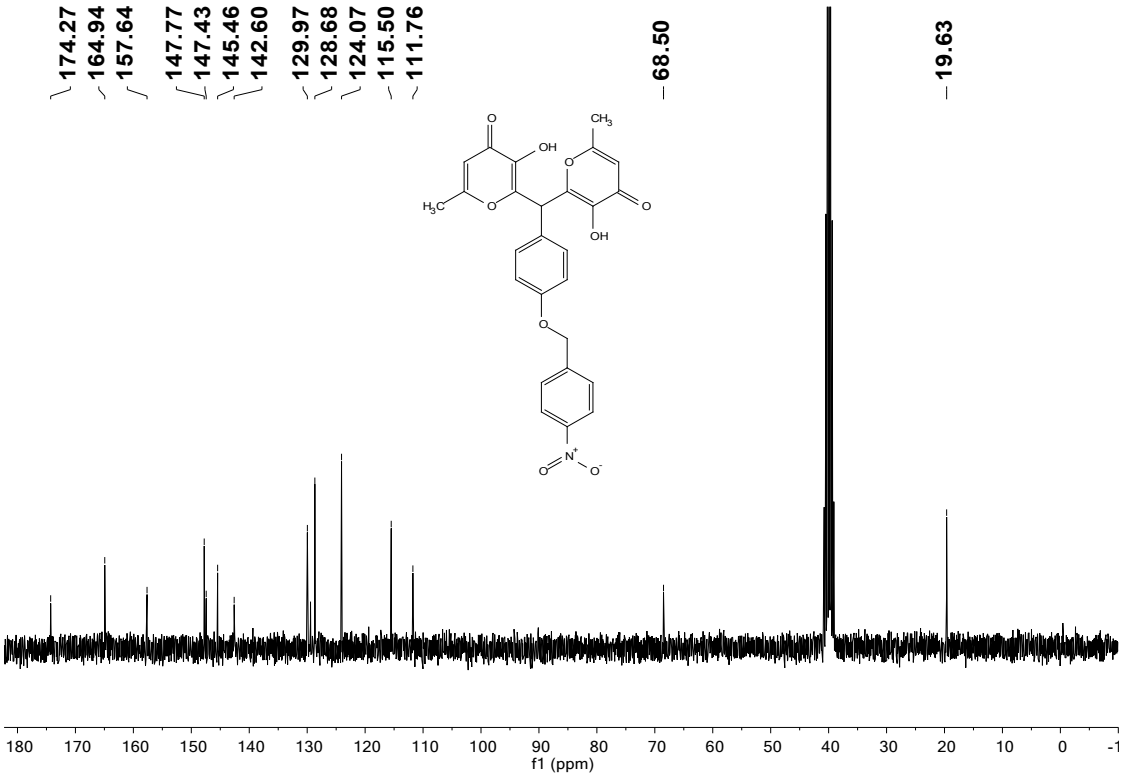
HRMSof compound**2b**



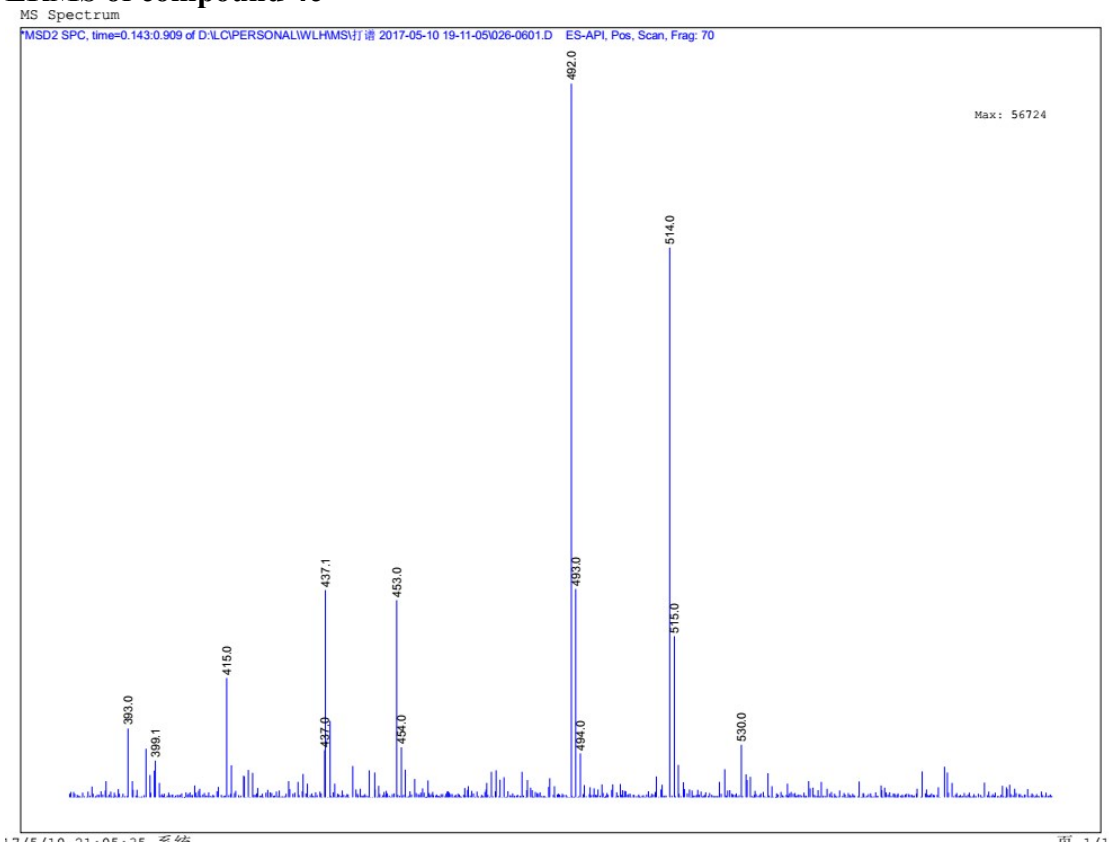
^{1}H NMR spectrum of compound 4c



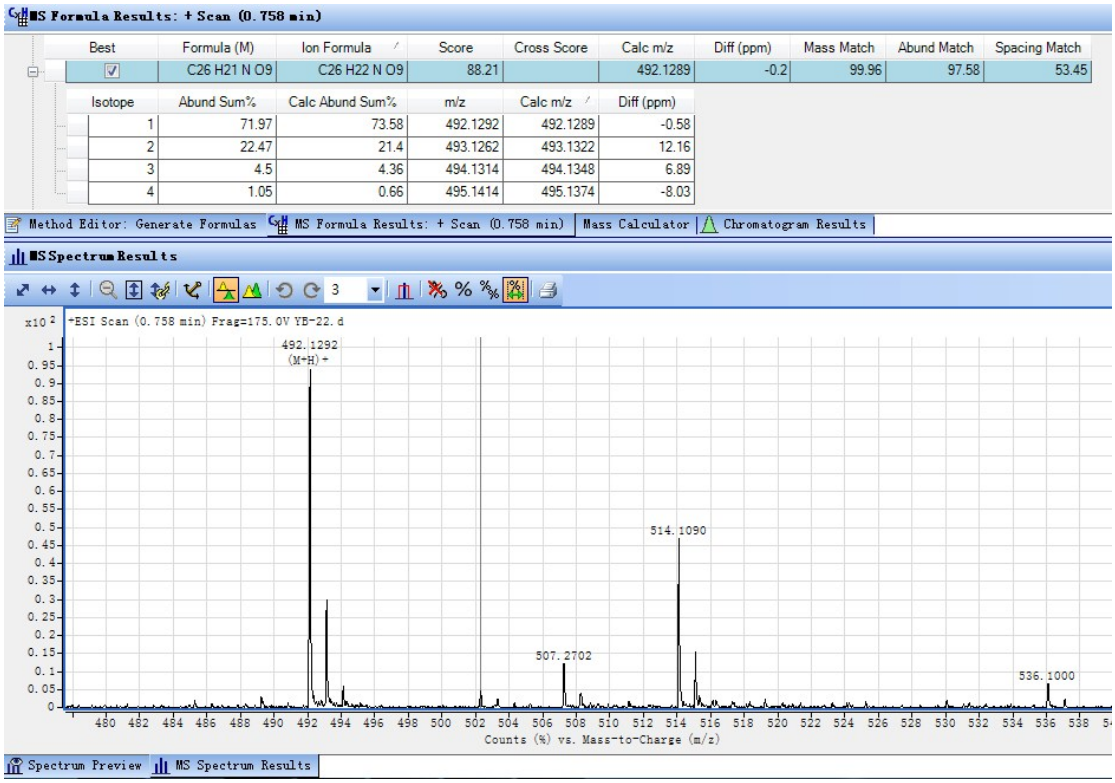
^{13}C NMR spectrum of compound 4c



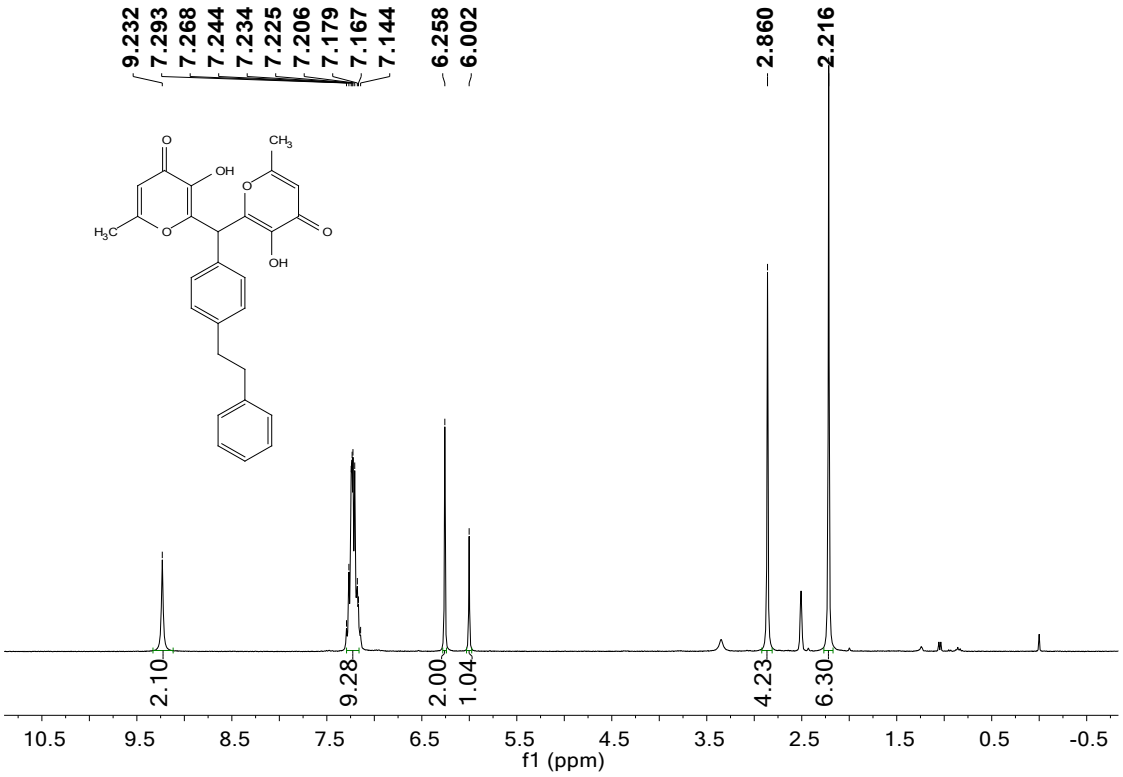
LRMS of compound 4c



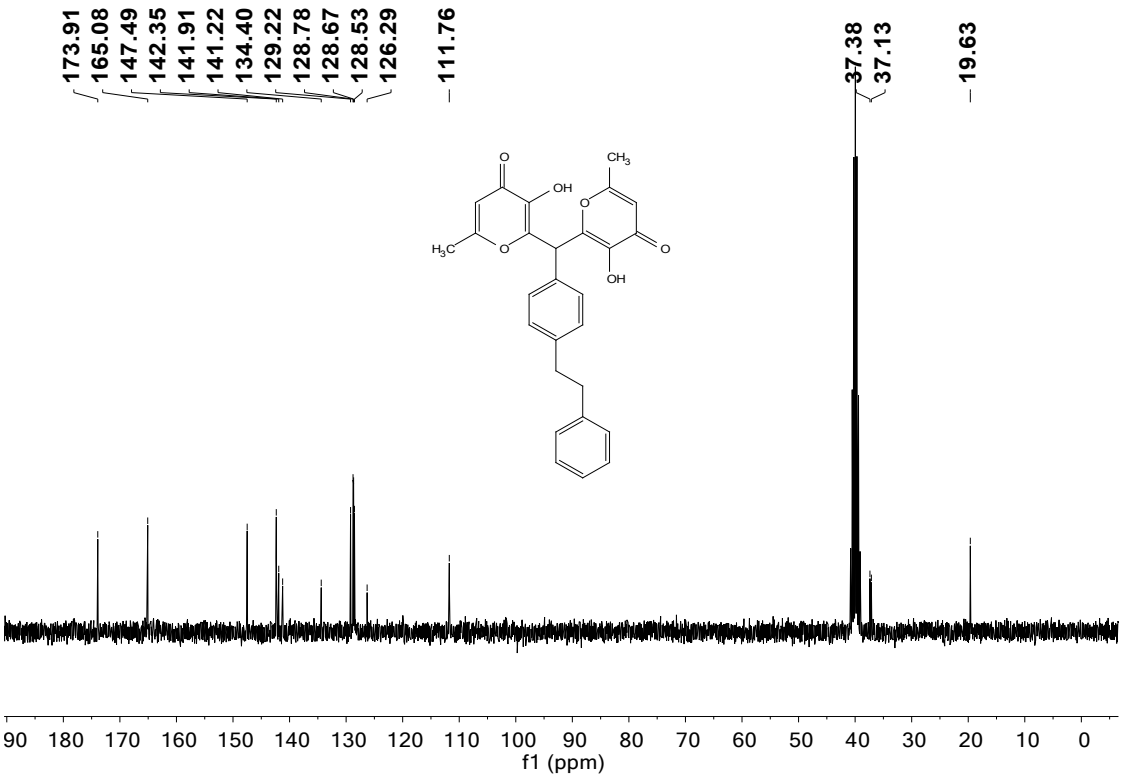
HRMS of compound 4c



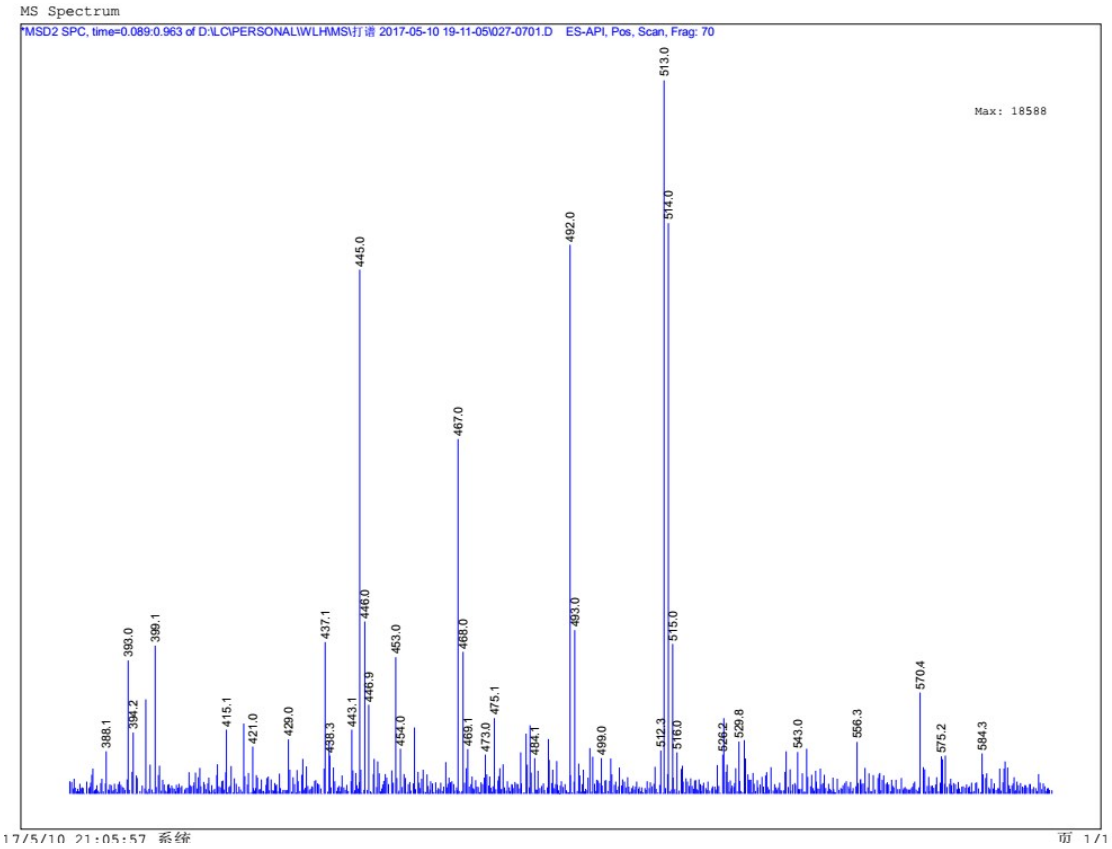
^{1}H NMR spectrum of compound 4d



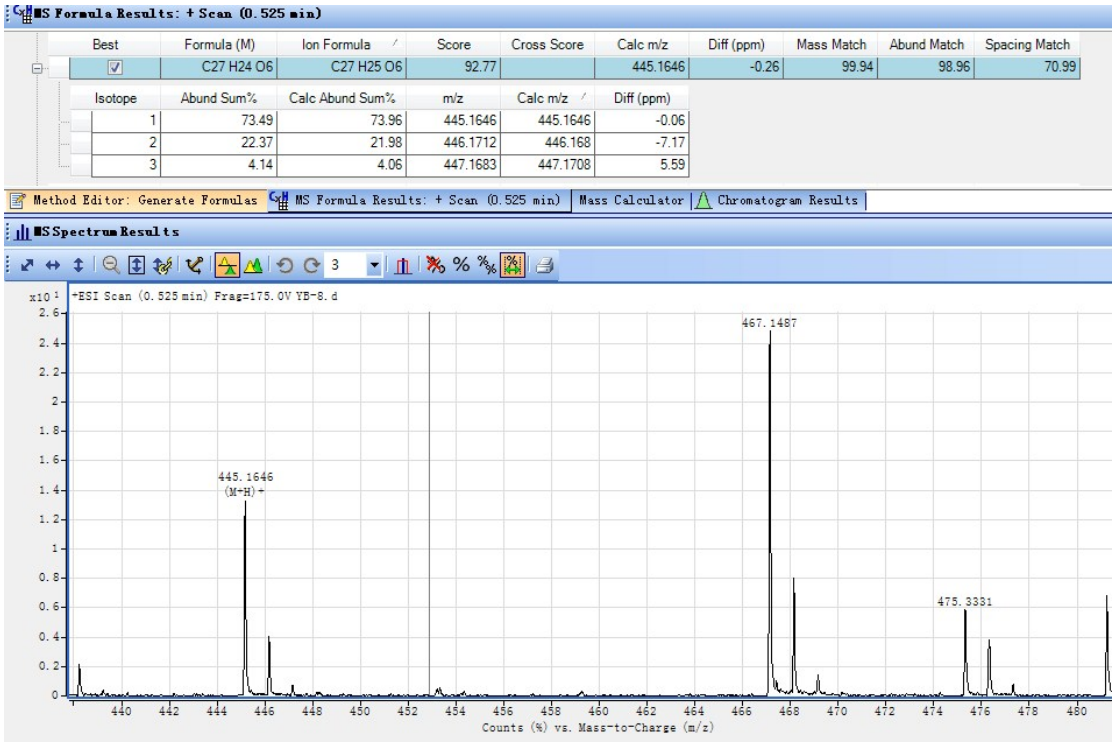
^{13}C NMR spectrum of compound 4d



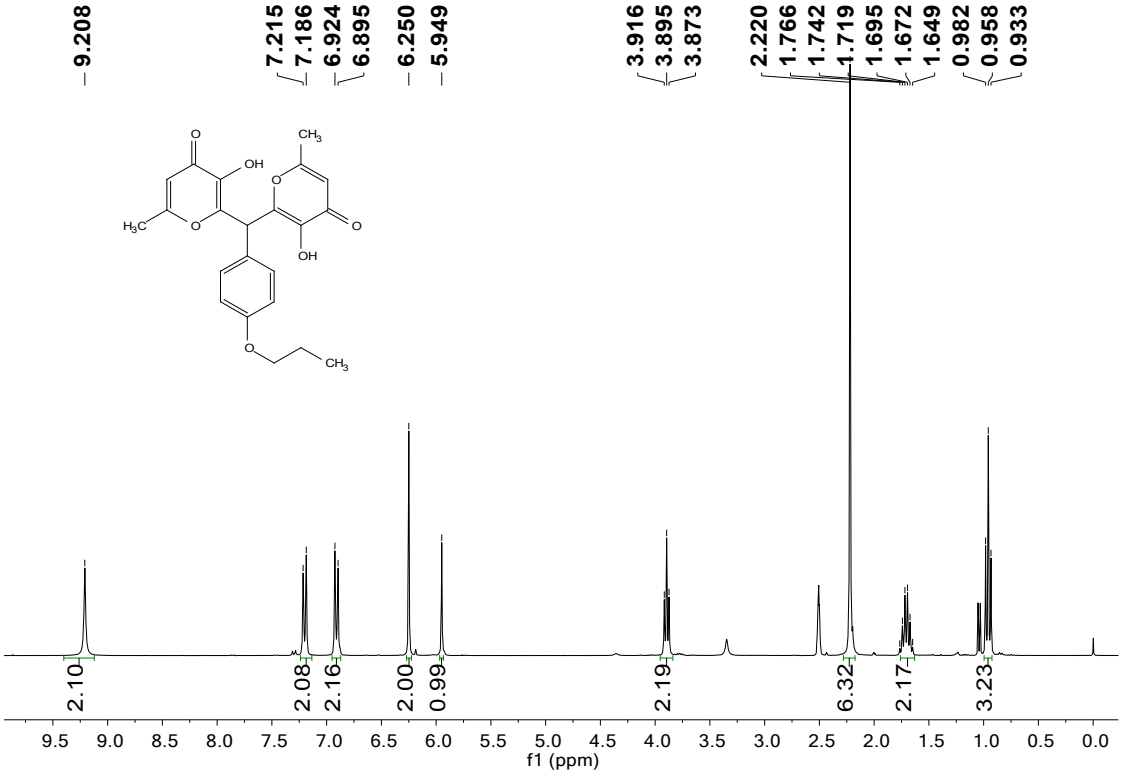
LRMS of compound 4d



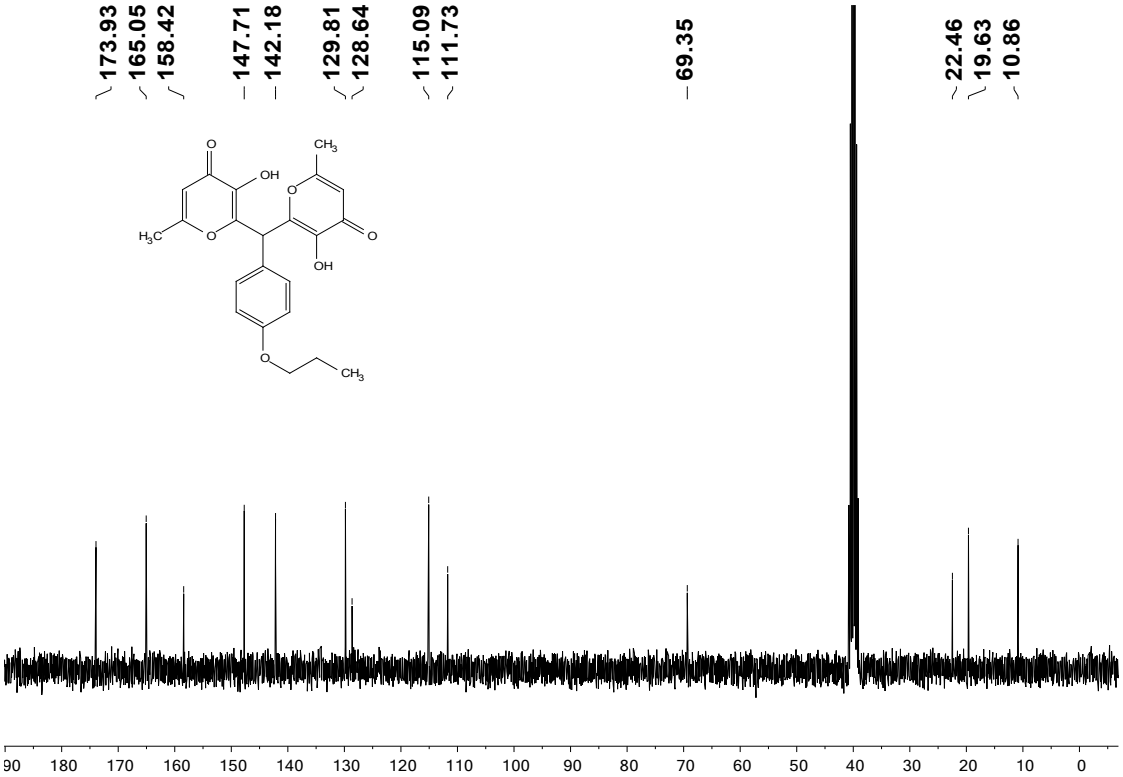
HRMS of compound 4d



^{1}H NMR spectrum of compound 4e



^{13}C NMR spectrum of compound 4e



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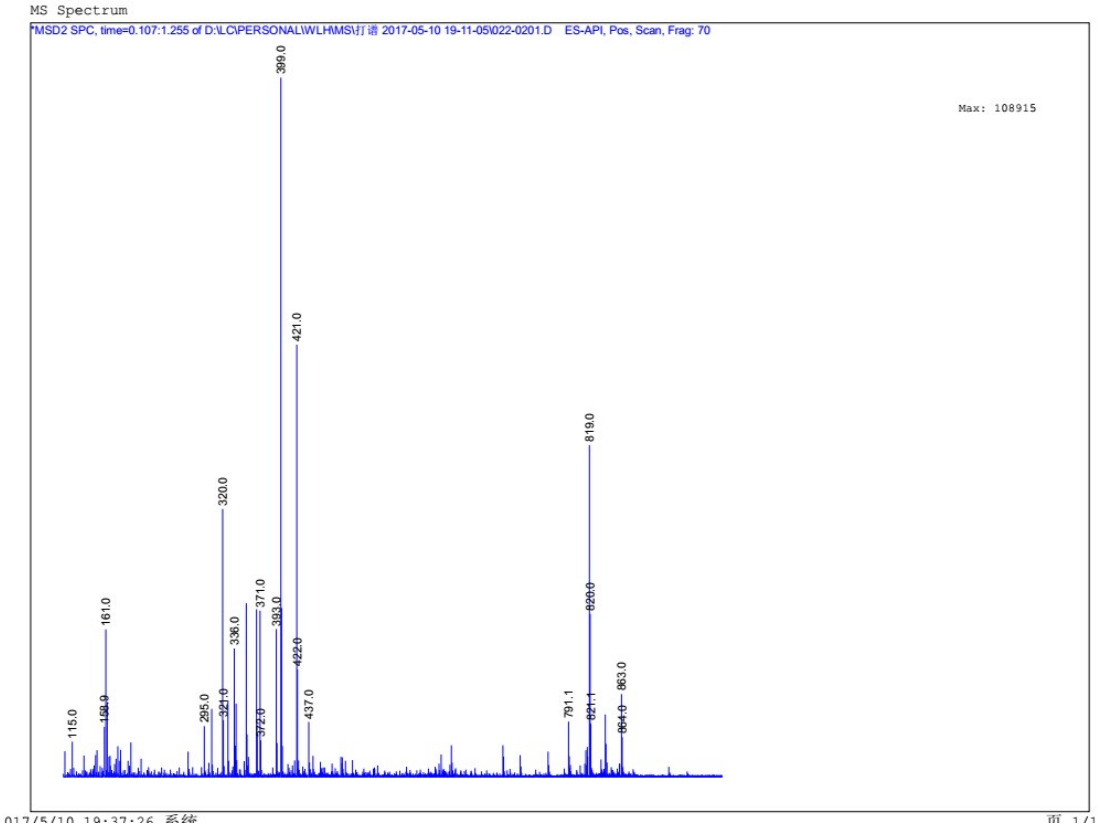

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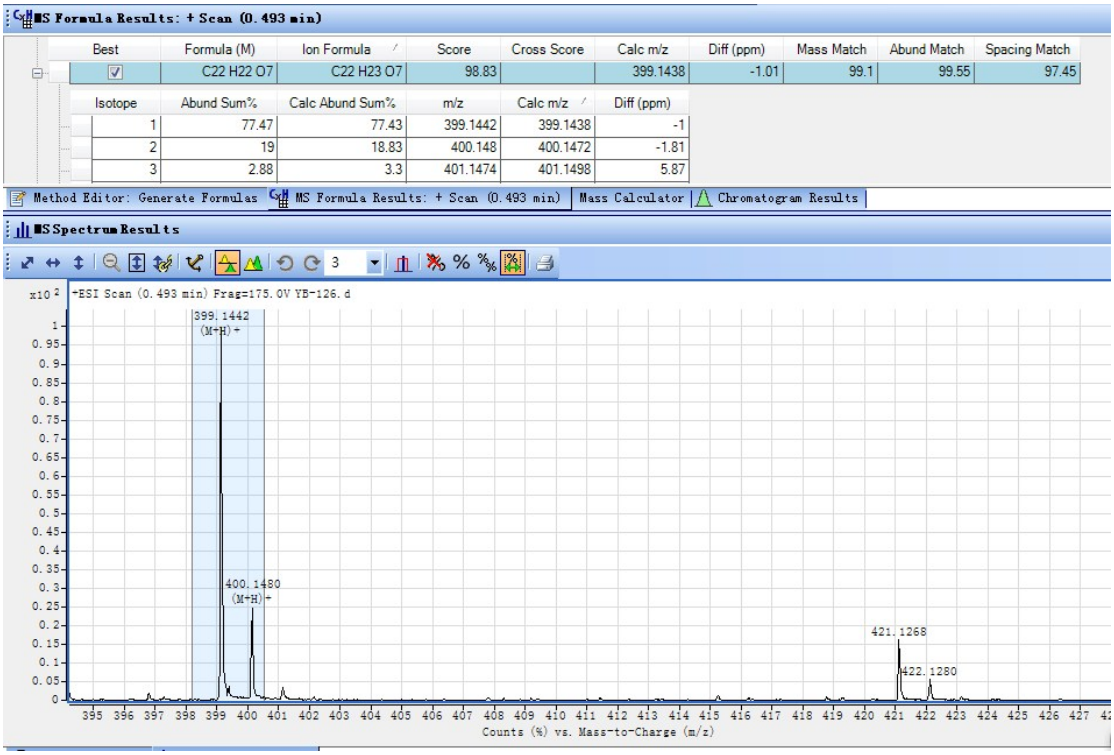
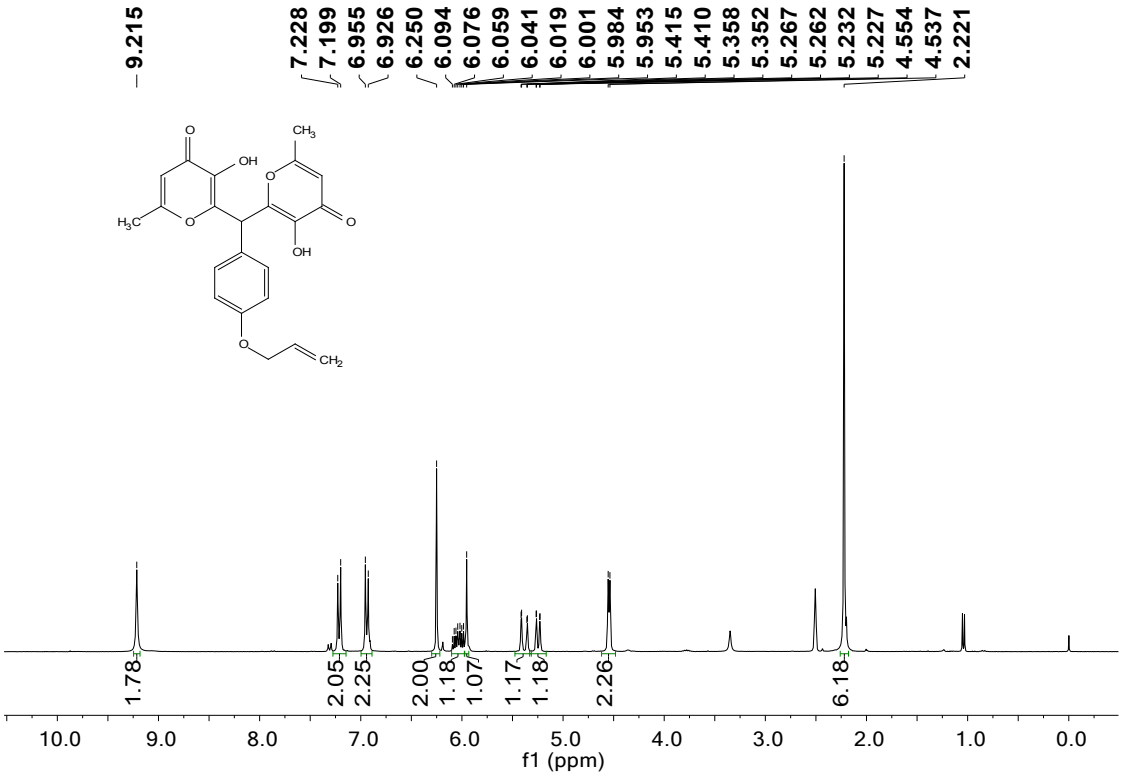
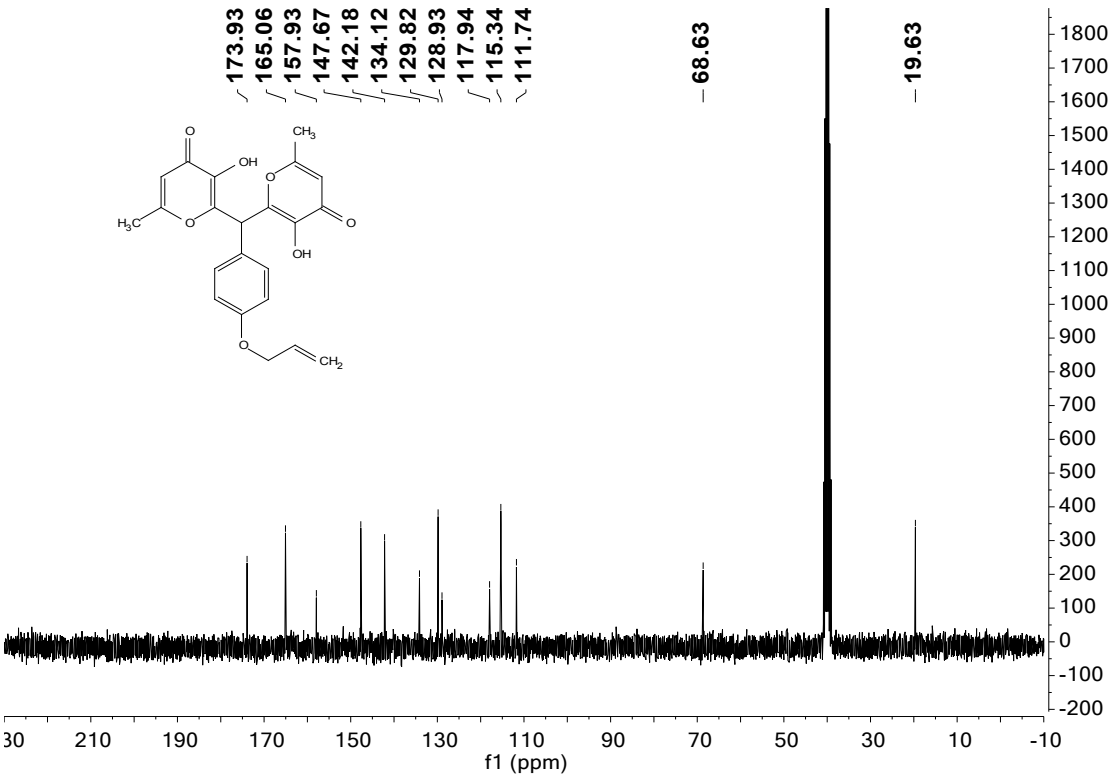


^{1}H NMR spectrum of compound 4f



^{13}C NMR spectrum of compound 4f



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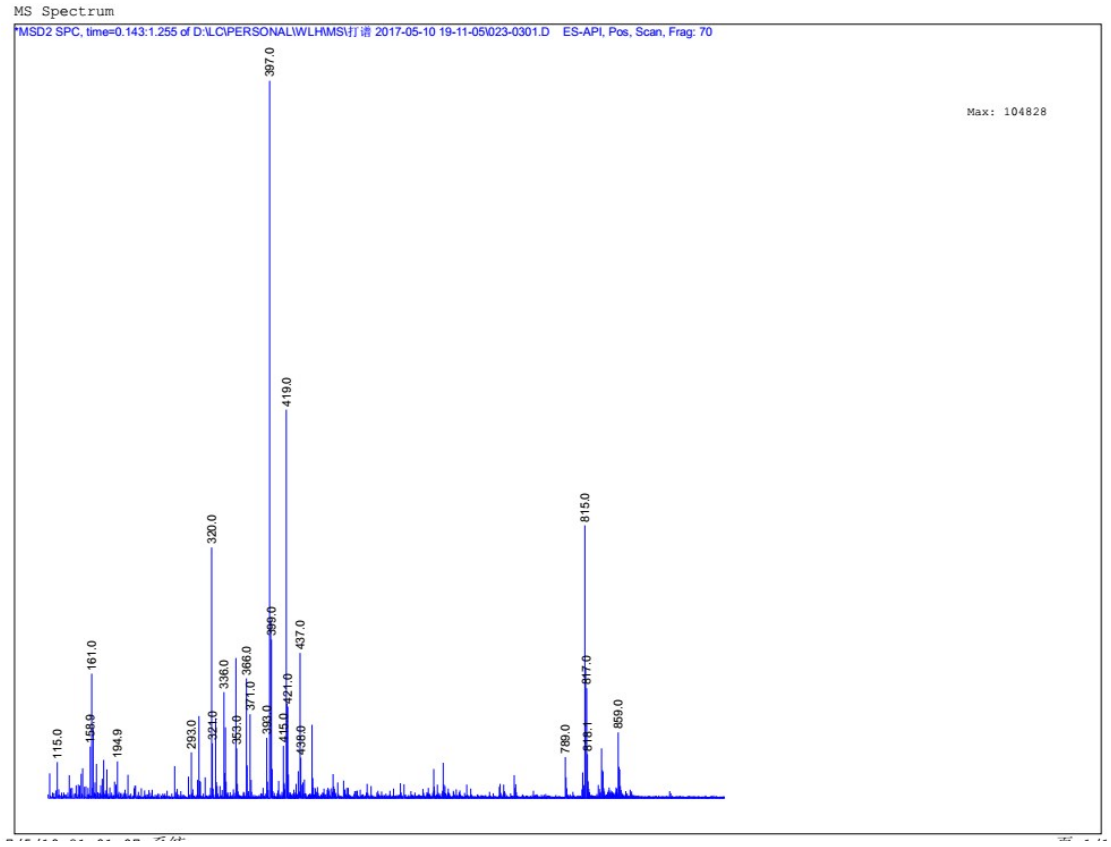
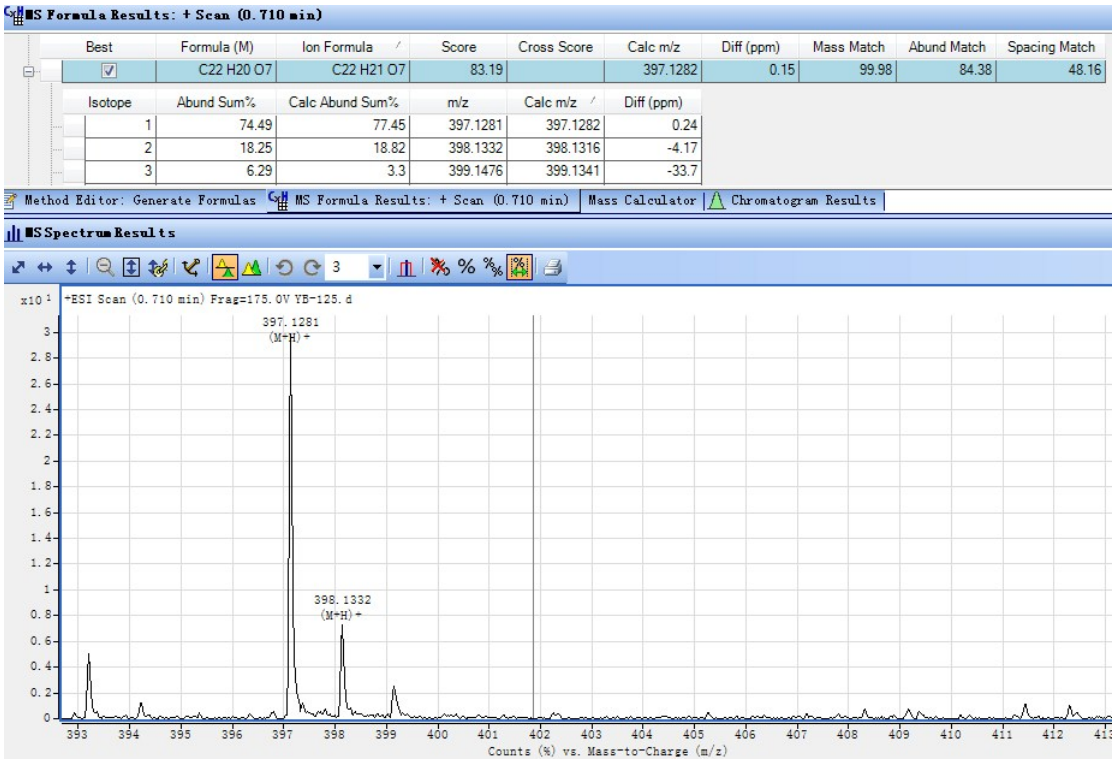


HRMS of compound 4f



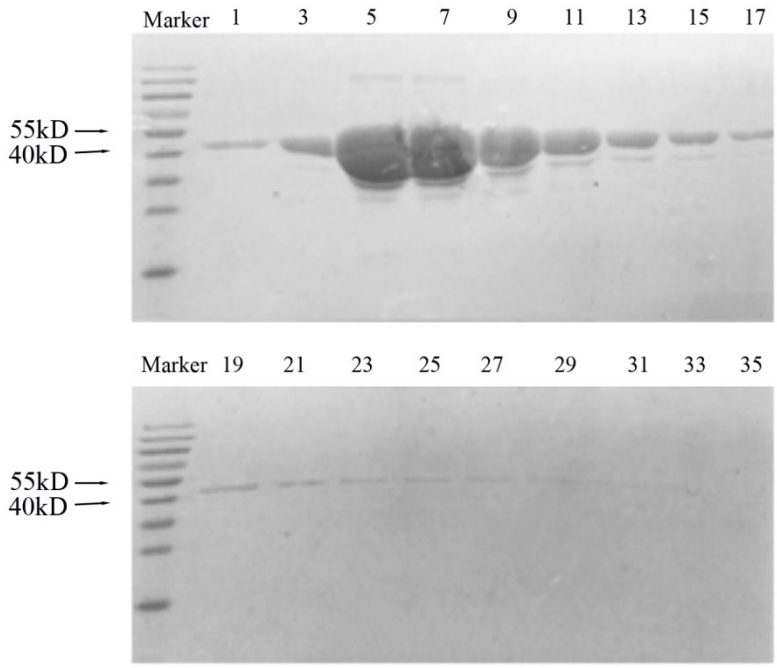


Figure S1. Protein electrophoresis of purified mutant IDH1. 10 μl for each fraction to test by SDS-PAGE gel

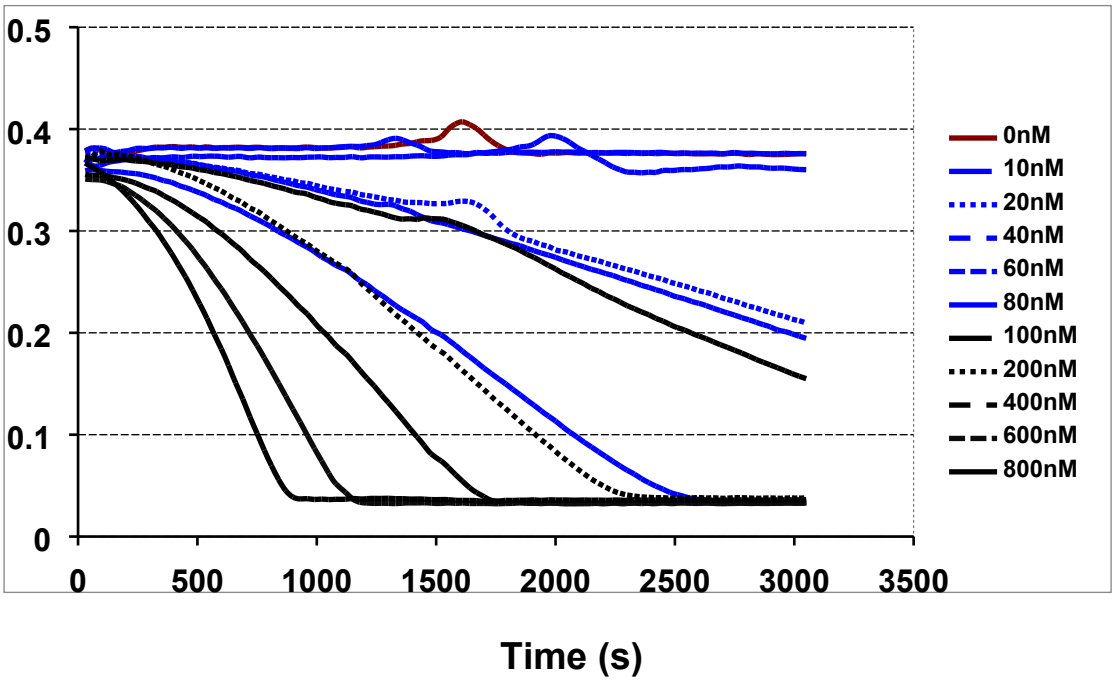


Figure S2. Enzyme activity of purified mutant IDH1 (R132H). Determination of the activity is based on the consumption of NADPH (OD_{340}) in the reaction.

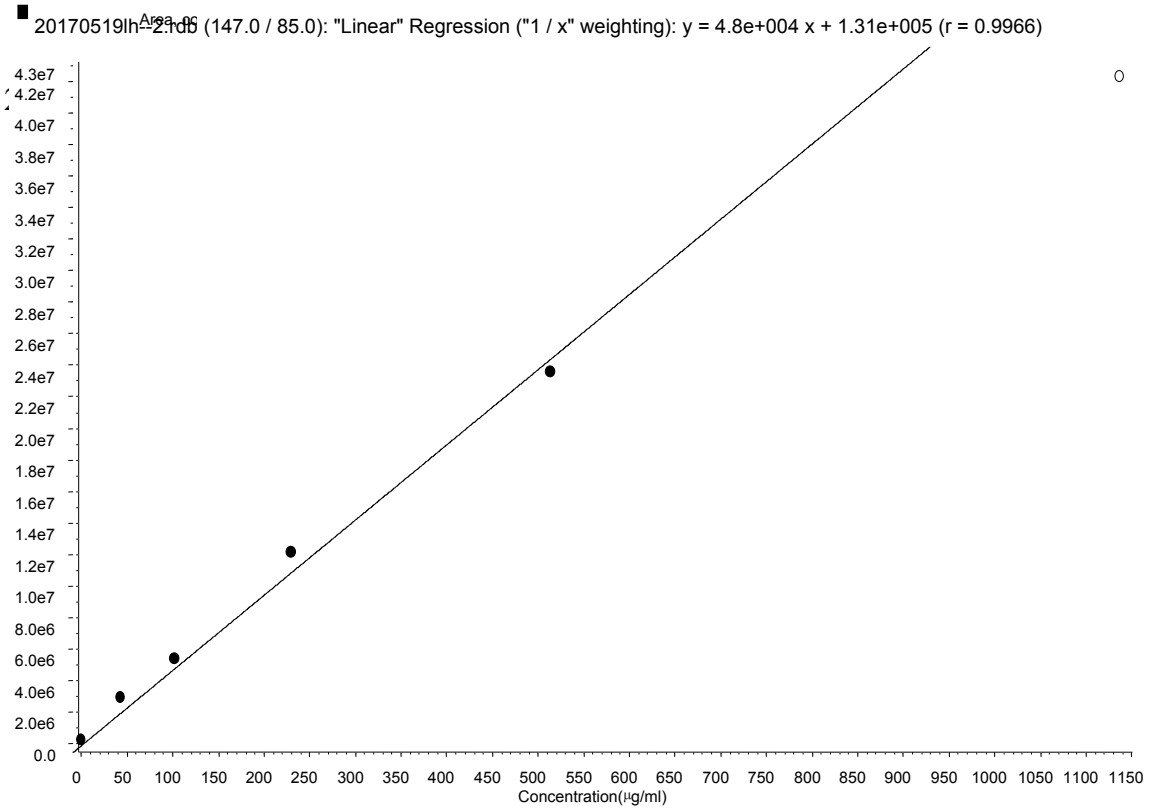


Figure S3. Standard curve line of *D*-2HG. The The concentration of the standard samples are 4.8 μg/ml, 48μg/ml, 105.6μg/ml, 232.3μg/ml, and 511.104μg/ml.