An Efficient Synthesis and Bioactivity Evaluation of Oxazole-Containing Natural Hinduchelin A-D and Their Derivatives

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Supporting Information

Experimental Section

Instrumentation and chemicals. All starting materials and reagents commercially available were used without further purification, unless otherwise specified. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance III 600 MHz FT-NMR spectrometer using $CDCl_3$ or DMSO-d_6 as the solvent and tetramethylsilane (TMS) as the internal standard. Chemical shifts are reported in δ (parts per million) values, and coupling constants ^{n}J are reported in Hz. Mass spectra were performed on a WATERS ACQUITY UPLC® H-CLASS PDA (Waters®) instrument, and Xevo TQD detector was used. Elemental analyses were performed on a Vario EL III elemental analysis instrument. Optical rotations were measured on an Autopol IV, Serial #83376 (Rudolph Research Analytical, Hackettstown, NJ, USA). Analytical thin-layer chromatography was carried out on precoated silica gel plates GF254 (Qindao Haiyang Chemical, China), and spots were visualized with ultraviolet light.

General synthetic procedure for the intermediate 2. To a solution of ethyl acetoacetate **1** (3.25 g, 25 mmol) in acetic acid (15 mL) was added dropwise the solution of sodium nitrite (2.04 g, 30 mmol) in 10 mL of water under ice-bath, which were stirred at 0-5 ^{o}C and detected by thin-layer chromatography.

After the completion of reaction, the mixture was poured into 60 mL of ice water with stirring, and then was extracted with ethyl acetate, and the organic layer was washed with saturated aqueous of $NaHCO_3$ and water, and dried with anhydrous Na_2SO_4. The solvent was removed to give the intermediate **2** as yellowish oil, and which can be directly used for next step reaction without further purification.

General synthetic procedure for the intermediates 5a and 5b. To a solution of intermediate **2** (3.18 g, 20 mmol) and the corresponding aldehyde **3** (22 mmol) in acetic acid (25 mL) was bubbled dry HCl gas under ice-bath, which were stirred at 0-5 °C for 2-3 h. After that, the mixture was diluted with acetone and concentrated, and then the residue was dissolved in acetic acid (20 mL), and zinc (2.34 g, 36 mmol) was added in batches. The mixture was stirred at 45-50 °C for 3-4 h, and then water (60 mL) was added, and which was extracted with ethyl acetate, and the organic layer was washed with H_2O, $NaHCO_3$ aqueous and brine, and dried with anhydrous Na_2SO_4. The solvent was removed to give intermediate **5**. Their basic physico-chemical properties and spectra data are as follows:

*Ethyl 2-(2,3-dimethoxyphenyl)-5-methyloxazole-4-carboxylate **5a***

This compound was obtained following the above method as yellowish oil, yield 88%. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.57 (dd, J = 6 Hz, 1H, Ph-H), 7.13-7.11 (m, 1H, Ph-H), 7.03 (dd, J = 6 Hz, 1H, Ph-H), 4.42 (q, J = 6 Hz, 2H, CH_2), 3.93 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 2.72 (s, 3H, CH_3), 1.41 (t, J = 7.8 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 162.6, 158.3, 156.4, 153.6, 147.9, 128.5, 124.2, 122.0, 121.5, 114.7, 61.4, 60.9, 56.0, 14.4, 12.3; MS (ESI) *m/z* calcd. for $C_{15}H_{18}NO_5^+$ $(M+H)^+$ 292.11, found 292.35.

*Ethyl 2-(2-hydroxy-3-methoxyphenyl)-5-methyloxazole-4-carboxylate **5b***

This compound was obtained following the above method as white floc, yield 82%. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.41 (dd, J = 6 Hz, 1H, Ph-H), 6.98 (dd, J = 6 Hz, 1H, Ph-H), 6.91-6.88 (m, 1H, Ph-H), 4.39 (q, J = 6 Hz, 2H, CH_2), 3.93 (s, 3H, OCH_3), 2.72 (s, 3H, CH_3), 1.40 (t, J = 6 Hz, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 161.7, 159.3, 154.9, 147.6, 127.1, 119.2, 117.5, 114.4, 110.6, 61.0, 56.3, 14.2, 12.0; MS (ESI) *m/z* calcd. for $C_{14}H_{16}NO_5^+$ $(M+H)^+$ 278.10, found 278.40.

General synthetic procedure for the intermediates 6a and 6b. To a solution of intermediate **5** (10 mmol) in methanol (15 mL) was added aqueous (15 mL) of sodium hydroxide (15 mmol), which were stirred at room temperature overnight, and detected by thin-layer chromatography. After the completion

of reaction, the mixture was adjusted to pH 2-3 with dilute hydrochloric acid, and the precipitate was filtered and dried to obtain **6**. Their basic physico-chemical properties and spectra data are as follows:

2-(2,3-Dimethoxyphenyl)-5-methyloxazole-4-carboxylic acid ***6a***

This compound was obtained following the above method as white powder, yield 83%. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.55 (dd, J = 6 Hz, 1H, Ph-H), 7.16-7.13 (m, 1H, Ph-H), 7.05 (dd, J = 6 Hz, 1H, Ph-H), 3.94 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 2.75 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 164.5, 157.9, 157.2, 153.7, 147.9, 127.7, 124.4, 121.6, 120.9, 114.9, 61.3, 56.1, 12.2; MS (ESI) *m/z* calcd. for $C_{13}H_{14}NO_5^+$ $(M+H)^+$ 264.08, found 264.27.

2-(2-Hydroxy-3-methoxyphenyl)-5-methyloxazole-4-carboxylic acid ***6b***

This compound was obtained following the above method as white powder, yield 76%. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 13.29 (br, 1H, COOH), 10.69 (s, 1H, OH), 7.37 (dd, J = 6 Hz, 1H, Ph-H), 7.14 (dd, J = 7.2 Hz, 1H, Ph-H), 6.96-6.94 (m, 1H, Ph-H), 3.83 (s, 3H, OCH_3), 2.66 (s, 3H, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 162.8, 158.9, 155.6, 148.7, 146.9, 127.5, 119.9, 118.1, 115.3, 111.0, 56.0, 12.4; MS (ESI) *m/z* calcd. for $C_{12}H_{12}NO_5^+$ $(M+H)^+$ 250.06, found 250.59.

General synthetic procedure for the conversion of 5a and 7 to Hinduchelin A. To a solution of intermediate **5a** (1 mmol) in EtOH (6 mL) was added compound **7** (0.165 g, 1.2 mmol), and the mixture were heated and stirred at reflux temperature for about 42 h. Then the mixture was concentrated and purified by silica gel column-chromatography (ethyl acetate/petroleum ether) to give pure compound **Hinduchelin A** (18% yield). Its physico-chemical properties and spectra data are consistent with the compound prepared by coupling method as follows.

General synthetic procedure for Hinduchelin A-D. To a solution of intermediate **6a** or **6b** (0.8 mmol) in DMF (8 mL) was added HOBt (0.162 g, 1.2 mmol), EDCI (0.23 g, 1.2 mmol), Et_3N (0.162 g, 1.6 mmol) and appropriate amine (1.2 mmol), which were stirred at room temperature overnight, and detected by thin-layer chromatography. After the completion of reaction, the mixture was extracted with ethyl acetate, and the organic layer was washed with water and brine, and dried with anhydrous Na_2SO_4. The solvent was removed to give crude target compounds. The crude products were purified by silica gel column-chromatography (ethyl acetate/petroleum ether) or recrystallization to give pure compounds. All the compounds were characterized by ESI-MS, ^{1}H NMR and ^{13}C NMR spectroscopic data. Their

physico-chemical properties and spectra data are as follows:

2-(2,3-Dimethoxyphenyl)-N-(2-hydroxy-2-phenylethyl)-5-methyloxazole-4-carboxamide ***Hinduchelin A***

This compound was obtained following the above method as white powder, yield 64%. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.56 (bs, 1H, NH), 7.47-7.43 (m, 3H, Ph-H), 7.36 (t, J = 7.5 Hz, 2H, Ph-H), 7.29 (t, J = 7.2 Hz, 1H, Ph-H), 7.13 (t, J = 7.8 Hz, 1H, Ph-H), 7.03 (d, J = 8.4 Hz, 1H, Ph-H), 4.97 (dd, J = 7.8 Hz, 1H, CH), 3.91 (s, 3H, OCH_3), 3.90 (s, 3H, OCH_3), 3.83-3.79 (m, 1H, CH_2), 3.58-3.53 (m, 1H, CH_2), 2.74 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 163.5, 156.9, 153.8, 153.4, 147.8, 141.9, 129.8, 128.5, 127.8, 125.9, 124.4, 121.3, 121.2, 114.7, 74.2, 61.3, 56.1, 47.5, 11.9; MS (ESI) *m/z* calcd. for $C_{21}H_{23}N_2O_5^+$ $(M+H)^+$ 383.15, found 383.44; Anal. Calcd for $C_{21}H_{22}N_2O_5$: C, 65.96; H, 5.80; N, 7.33; Found: C, 65.84; H, 5.71; N, 7.42; $[\alpha]_D^{25}$ −11.0 (*c* 0.1, MeOH).

2-(2,3-Dimethoxyphenyl)-N-(2-hydroxyphenethyl)-5-methyloxazole-4-carboxamide ***Hinduchelin B***

This compound was obtained following the above method as white powder, yield 65%. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.67 (bs, 1H, NH), 7.41 (d, J = 12 Hz, 1H, Ph-H), 7.09-6.97 (m, 4H, Ph-H), 6.84 (d, J = 6 Hz, 1H, Ph-H), 6.76-6.73 (m, 1H, Ph-H), 3.86 (s, 3H, OCH_3), 3.85 (s, 3H, OCH_3), 3.49 (q, J = 6 Hz, 2H, CH_2), 2.90 (t, J = 6 Hz, 2H, CH_2), 2.68 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 162.4, 155.7, 154.3, 152.7, 152.3, 146.7, 129.3, 128.6, 127.1, 123.6, 123.4, 120.1, 119.0, 115.4, 113.7, 60.2, 55.0, 39.1, 29.9, 10.8; MS (ESI) *m/z* calcd. for $C_{21}H_{23}N_2O_5^+$ $(M+H)^+$ 383.15, found 383.62; Anal. Calcd for $C_{21}H_{22}N_2O_5$: C, 65.96; H, 5.80; N, 7.33; Found: C, 65.81; H, 5.73; N, 7.47.

2-(2-Hydroxy-3-methoxyphenyl)-N-(2-hydroxyphenethyl)-5-methyloxazole-4-carboxamide ***Hinduchelin C***

This compound was obtained following the above method as white powder, yield 74%. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.42 (bs, 1H, NH), 7.37 (d, J = 6 Hz, 1H, Ph-H), 7.08-7.05 (m, 2H, Ph-H), 6.94-6.92 (m, 1H, Ph-H), 6.88-6.85 (m, 2H, Ph-H), 6.81-6.79 (m, 1H, Ph-H), 3.88 (s, 3H, OCH_3), 3.54 (q, J = 6 Hz, 2H, CH_2), 2.92 (t, J = 6 Hz, 2H, CH_2), 2.67 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 160.9, 153.5, 151.4, 147.4, 145.8, 129.7, 127.2, 124.1, 119.6, 118.6, 116.7, 114.9, 113.2, 109.7, 98.9, 55.2, 40.1, 28.6, 10.6; MS (ESI) *m/z* calcd. for $C_{20}H_{21}N_2O_5^+$ $(M+H)^+$ 369.14, found 369.58; Anal. Calcd for $C_{20}H_{20}N_2O_5$: C, 65.21; H, 5.47; N, 7.60; Found: C, 65.34; H, 5.40; N, 7.68.

2-(2-Hydroxy-3-methoxyphenyl)-5-methyl-N-phenethyloxazole-4-carboxamide ***Hinduchelin D***

This compound was obtained following the above method as white powder, yield 82%. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.36 (dd, J = 6 Hz, 1H, Ph-H), 7.28-7.26 (m, 2H, Ph-H), 7.18-7.17 (m, 3H, Ph-H), 6.92 (dd, J = 12 Hz, 1H, Ph-H), 6.86-6.84 (m, 1H, Ph-H), 6.67 (bs, 1H, NH), 3.87 (s, 3H, OCH_3), 3.62 (q, J = 6 Hz, 2H, CH_2), 2.86 (t, J = 8.4 Hz, 2H, Ph-H), 2.68 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 161.0, 158.4, 152.3, 148.5, 146.9, 138.6, 128.8, 128.7, 128.6, 126.7, 119.5, 117.8, 114.4, 110.7, 56.2, 40.4, 35.8, 11.7; MS (ESI) *m/z* calcd. for $C_{20}H_{21}N_2O_4^+$ $(M+H)^+$ 353.14, found 353.56; Anal. Calcd for $C_{20}H_{20}N_2O_4$: C, 68.17; H, 5.72; N, 7.95; Found: C, 68.06; H, 5.63; N, 8.01.

General synthesis of Hinduchelin A-D derivatives 11a-d and 12a-c. The derivatives of natural Hinduchelin A-D have been prepared using a similar method to the aforementioned procedure. All the compounds were characterized by ESI-MS, ^{1}H NMR and ^{13}C NMR spectroscopic data. Their physico-chemical properties and spectra data are as follows:

2-(2,3-Dimethoxyphenyl)-5-methyl-N-phenethyloxazole-4-carboxamide ***11a***

This compound was obtained following the above method as white powder, yield 78%. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.39 (dd, J = 6 Hz, 1H, Ph-H), 7.25 (br, 1H, NH), 7.24-7.22 (m, 1H, Ph-H), 7.18-7.14 (m, 3H, Ph-H), 7.07-7.05 (m, 1H, Ph-H), 6.96 (dd, J = 6 Hz, 1H, Ph-H), 3.83 (s, 3H, OCH_3), 3.80 (s, 3H, OCH_3), 3.61 (q, J = 7.8 Hz, 2H, CH_2), 2.85 (t, J = 7.8 Hz, 2H, CH_2), 2.67 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 161.9, 156.6, 153.8, 152.9, 147.8, 138.9, 129.9, 128.8, 128.6, 126.4, 124.3, 121.3, 121.2, 114.6, 61.2, 56.1, 40.2, 36.0, 11.8; MS (ESI) *m/z* calcd. for $C_{21}H_{23}N_2O_4^+$ $(M+H)^+$ 367.16, found 367.69.

2-(2,3-Dimethoxyphenyl)-N-(2-methoxyphenethyl)-5-methyloxazole-4-carboxamide ***11b***

This compound was obtained following the above method as white powder, yield 73%. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.41 (dd, J = 6 Hz, 1H, Ph-H), 7.34 (br, 1H, NH), 7.14-7.12 (m, 2H, Ph-H), 7.08-7.05 (m, 1H, Ph-H), 6.96 (dd, J = 6 Hz, 1H, Ph-H), 6.83-6.79 (m, 2H, Ph-H), 3.84 (s, 3H, OCH_3), 3.82 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.59 (q, J = 7.8 Hz, 2H, CH_2), 2.88 (t, J = 7.8 Hz, 2H, CH_2), 2.66 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 161.8, 157.6, 156.7, 153.7, 152.9, 147.8, 130.6, 130.0, 127.8, 127.4, 124.3, 121.3, 121.3, 120.5, 114.6, 110.2, 61.2, 56.1, 55.3, 39.2, 30.5, 11.8; MS (ESI) *m/z* calcd. for $C_{22}H_{25}N_2O_5^+$ $(M+H)^+$ 397.17, found 397.84.

2-(2,3-Dimethoxyphenyl)-N-(3-methoxyphenethyl)-5-methyloxazole-4-carboxamide ***11c***

This compound was obtained following the above method as white powder, yield 77%. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.40 (dd, J = 7.8 Hz, 1H, Ph-H), 7.25 (br, 1H, NH), 7.17-7.14 (m, 1H, Ph-H), 7.08-7.05 (m, 1H, Ph-H), 6.96 (dd, J = 7.8 Hz, 1H, Ph-H), 6.79 (d, J = 6 Hz, 1H, Ph-H), 6.73-6.70 (m, 2H, Ph-H), 3.84 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3), 3.61 (q, J = 6 Hz, 2H, CH_2), 2.83 (t, J = 6 Hz, 2H, CH_2), 2.67 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 161.8, 159.8, 156.7, 153.7, 153.0, 147.8, 140.5, 129.8, 129.6, 124.3, 121.2, 121.2, 121.1, 114.7, 114.3, 112.0, 61.2, 56.1, 55.1, 40.1, 36.0, 11.8; MS (ESI) *m/z* calcd. for $C_{22}H_{25}N_2O_5^+$ $(M+H)^+$ 397.17, found 397.57.

2-(2,3-Dimethoxyphenyl)-N-(3-hydroxyphenethyl)-5-methyloxazole-4-carboxamide ***11d***

This compound was obtained following the above method as white powder, yield 58%. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.46 (br, 1H, NH), 7.42 (d, J = 7.8 Hz, 1H, Ph-H), 7.12-7.06 (m, 2H, Ph-H), 6.98 (dd, J = 8.4 Hz, 1H, Ph-H), 6.73-6.64 (m, 3H, Ph-H), 3.84 (s, 3H, OCH_3), 3.83 (s, 3H, OCH_3), 3.60 (q, J = 6 Hz, 2H, CH_2), 2.80 (t, J = 6 Hz, 2H, CH_2), 2.67 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 161.7, 156.6, 156.2, 153.7, 153.1, 147.7, 140.5, 129.8, 129.6, 124.5, 121.1, 120.8, 115.8, 114.9, 113.6, 61.2, 56.1, 40.1, 35.7, 11.8; MS (ESI) *m/z* calcd. for $C_{21}H_{23}N_2O_5^+$ $(M+H)^+$ 383.15, found 383.71.

2-(2-Hydroxy-3-methoxyphenyl)-N-(2-methoxyphenethyl)-5-methyloxazole-4-carboxamide ***12a***

This compound was obtained following the above method as white powder, yield 74%. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.36 (dd, J = 6 Hz, 1H, Ph-H), 7.17-7.14 (m, 1H, Ph-H), 7.11 (dd, J = 6 Hz, 1H, Ph-H), 6.93 (dd, J = 6 Hz, 1H, Ph-H), 6.87-6.82 (m, 4H, Ph-H and OH), 3.87 (s, 3H, OCH_3), 3.81 (s, 3H, OCH_3), 3.60 (q, J = 7.8 Hz, 2H, CH_2), 2.88 (t, J = 7.8 Hz, 2H, CH_2), 2.67 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 161.0, 158.4, 157.5, 152.1, 148.5, 146.9, 130.5, 128.7, 128.0, 127.2, 120.8, 119.5, 117.8, 114.4, 110.8, 110.6, 56.2, 55.6, 39.7, 30.1, 11.7; MS (ESI) *m/z* calcd. for $C_{21}H_{23}N_2O_5^+$ $(M+H)^+$ 383.15, found 383.62.

2-(2-Hydroxy-3-methoxyphenyl)-N-(3-methoxyphenethyl)-5-methyloxazole-4-carboxamide ***12b***

This compound was obtained following the above method as white powder, yield 71%. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.36 (dd, J = 6 Hz, 1H, Ph-H), 7.19 (dd, J = 7.8 Hz, 1H, Ph-H), 6.92 (dd, J = 6 Hz, 1H, Ph-H), 6.86-6.84 (m, 1H, Ph-H), 6.78 (d, J = 6 Hz, 1H, Ph-H), 6.74-6.67 (m, 3H, Ph-H and OH),

3.87 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 3.62 (q, J = 6 Hz, 2H, CH_2), 2.83 (t, J = 6 Hz, 2H, CH_2), 2.68 (s, 3H, CH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ = 161.0, 159.9, 158.4, 152.3, 148.5, 146.9, 140.1, 129.8, 128.5, 121.0, 119.5, 117.8, 114.4, 114.3, 112.1, 110.7, 56.2, 55.1, 40.3, 35.9, 11.7; MS (ESI) *m/z* calcd. for $C_{21}H_{23}N_2O_5^+$ $(M+H)^+$ 383.15, found 383.62.

2-(2-Hydroxy-3-methoxyphenyl)-N-(3-hydroxyphenethyl)-5-methyloxazole-4-carboxamide ***12c***

This compound was obtained following the above method as white powder, yield 62%. ^{1}H NMR (600 MHz, DMSO-d_6): δ = 10.32 (br, 1H, NH), 9.30 (br, 1H, OH), 8.85-8.83 (m, 1H, Ph-H), 7.38 (dd, J = 6 Hz, 1H, Ph-H), 7.14-7.08 (m, 2H, Ph-H), 6.96-6.93 (m, 1H, Ph-H), 6.67-6.60 (m, 3H, Ph-H), 3.84 (s, 3H, OCH_3), 3.44-3.41 (m, 2H, CH_2), 2.77-2.73 (m, 2H, CH_2), 2.67 (s, 3H, CH_3); ^{13}C NMR (150 MHz, DMSO-d_6): δ = 160.8, 157.8, 157.8, 152.1, 148.8, 146.7, 141.2, 129.8, 129.2, 119.9, 119.6, 118.1, 115.9, 115.3, 113.6, 110.9, 56.4, 40.6, 35.8, 11.8; MS (ESI) *m/z* calcd. for $C_{20}H_{21}N_2O_5^+$ $(M+H)^+$ 369.14, found 369.67.

Antibacterial assays. Susceptibility tests were conducted by the standard broth dilution method in accordance with the National Committee for Clinical Laboratory Standards (2008) in Mueller–Hinton broth (MHB, Oxoid Ltd., Basingstoke, UK) with an inoculum of approximately 10^5 colony-forming units (CFU)/Ml. Standard test strains included: *Staphylococcus aureus subsp. aureus Rosenbach* ATCC25923, *Erysipelothrix rhusiopathiae* ATCC 19414, *Escherichia coli* ATCC25922, and *Pasteurella multocida subsp. multocida* ATCC 43137. The MHB was supplemented with the compounds (testing samples or positive-controls), concentrations ranging from 30 to 60 μg/mL. Data were reported as MIC values, the lowest concentration of the compound inhibiting visible growth after 24 h of incubation at 37 °C.

In vitro **cytotoxicity assays.** The *in vitro* cytotoxicity of the synthesized compounds against different cell lines (SGC-7901, A875, HepG2, and MARC) were measured with the MTT assay. All data were analyzed with SPSS software, and the 50% inhibitory concentrations (IC_{50}) of each compound for the different cell lines were determined. A control was run for each test, and all assays were performed in triplicate on three independent experiments, and measurement data were expressed as the mean ± S.D.

In vitro **fungicidal assays.** All natural Hinduchelin A-D and their derivatives were also screened for their potential antifungal activities against seven plant fungal pathogens including *Ralstonia solanacearum*, *Botrytis cinereal*, *Septoria nodorum*, *Alternaria Solani*, *Fusarium culmorum*,

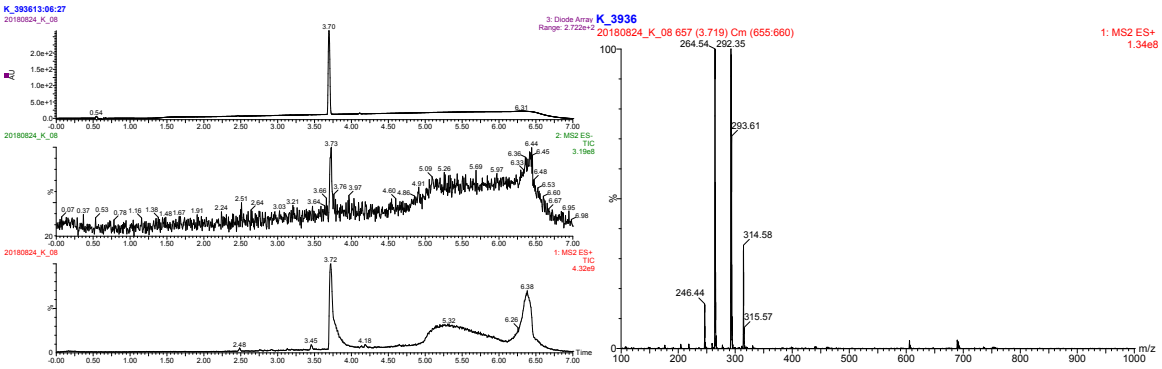
Rhizoctonia solani, and *Uromyces fabae* which was evaluated according to the mycelium growth rate method at the concentration of 50 μg/mL.

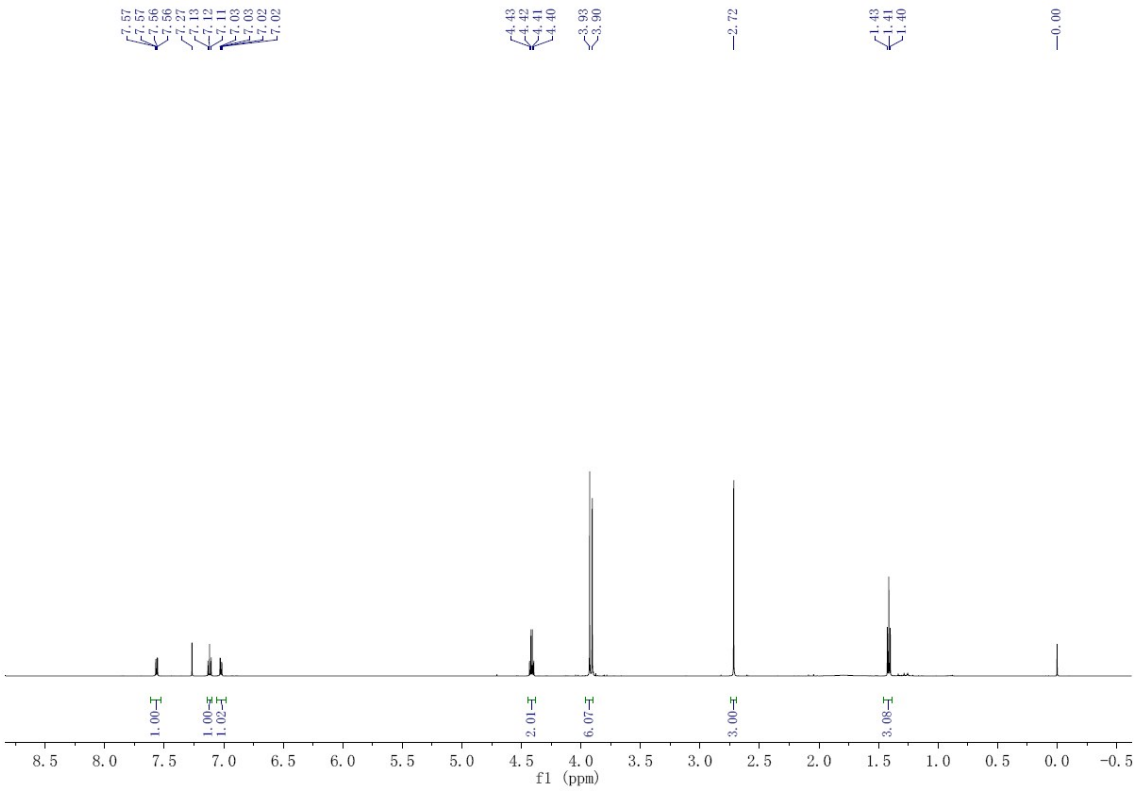
Insecticidal activity screening. *Helicoverpa armigera* and *Aphis craccivora Koch* from our laboratory cultivate were used as general tested species. For *H. armigera*, the assay was performed by placing artificial diets (300 μL) in a 24-well microtiter plates, which were covered with the solution of different tested concentration compounds in ethanol (20 μL). When the ethanol was completely evaporated leaving the effective compounds as a coat on the surface of the artificial diets, the tested species were introduced and the culture plates was closed with a plastic cover. Ethanol alone was used as a control. For *Aphis craccivora Koch*, the standard FAO dipping method was adopted. Mortality in these assays was recorded at 72 h, and each treatment was replicated 3 times.

Spectroscopy for intermediates

The structures of obtained intermediates **5a-b**, and **6a-b** were confirmed by LC-MS, ^{1}H NMR, and ^{13}C NMR spectra analyses, and the typical spectra are described as follows.

Compd. 5a




7.57
7.57
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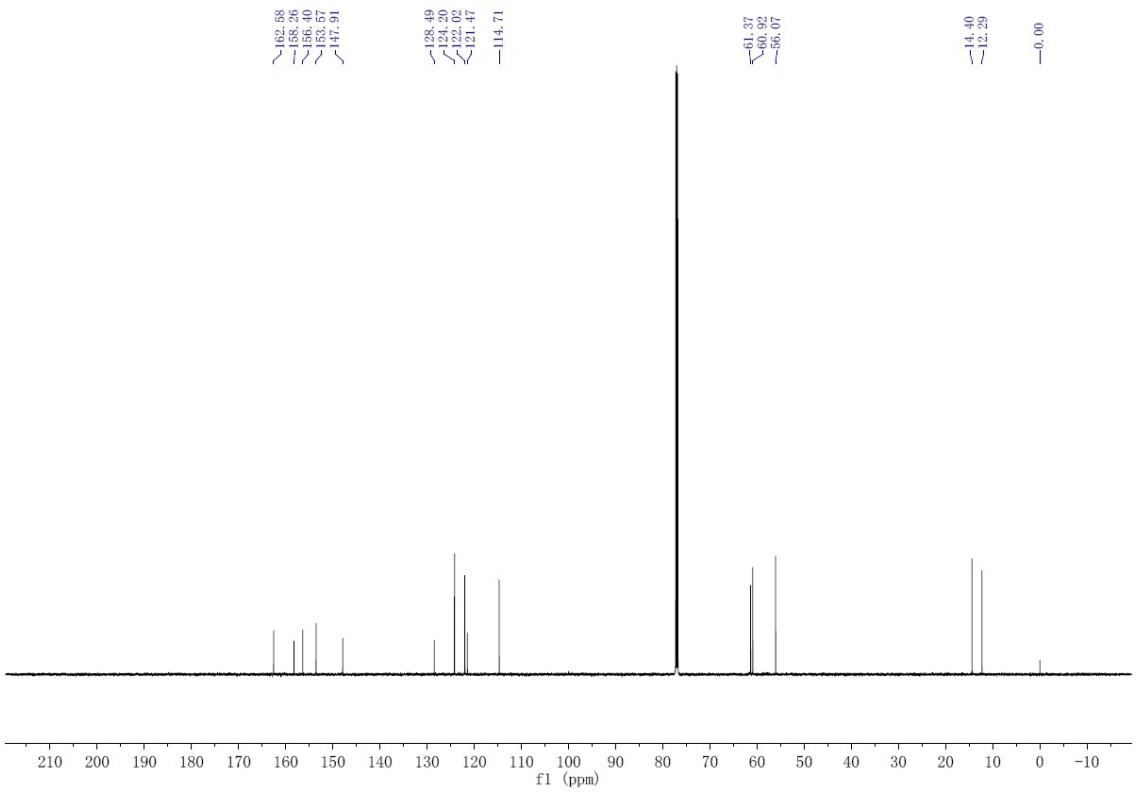
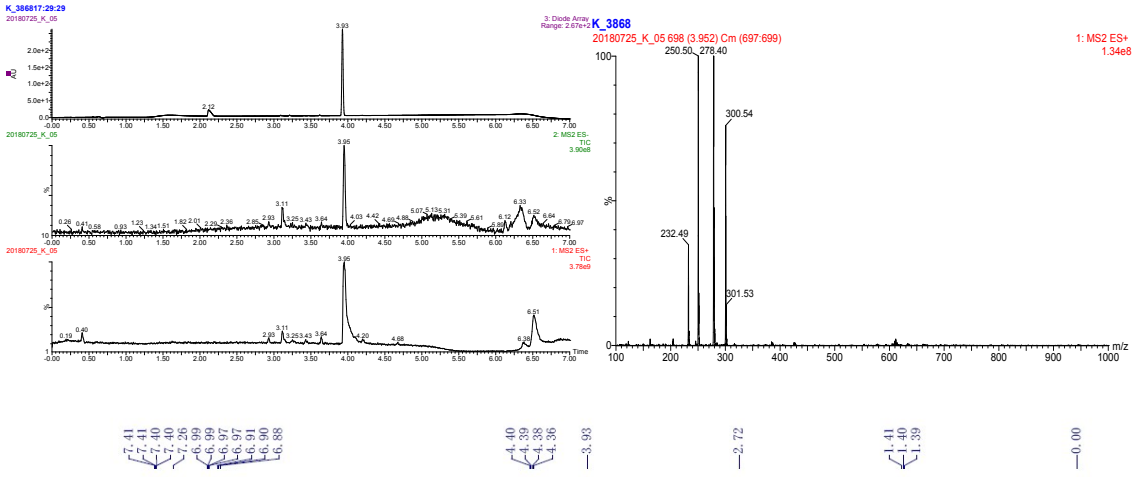
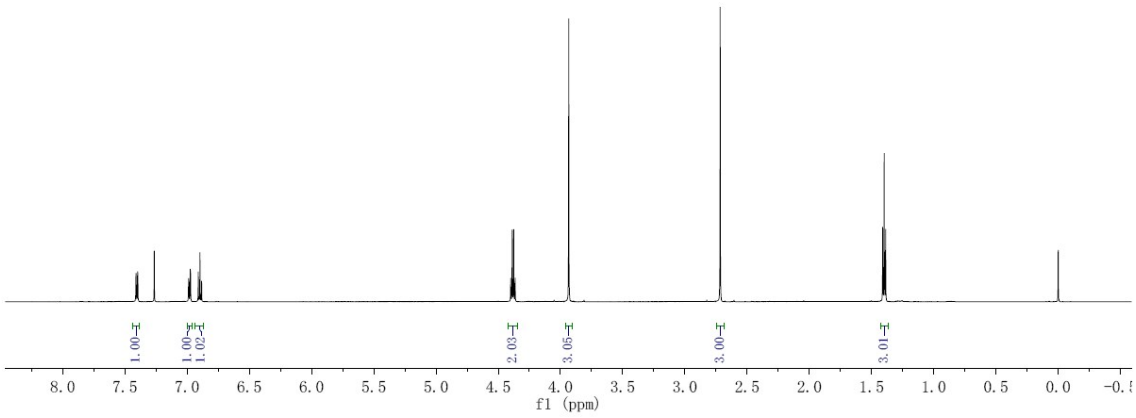
7.02
4.43
4.42
4.41
4.40
3.93
3.90
2.72
1.43
1.41
1.40
0.00
1.00
1.00
1.02
2.01
6.07
3.00
3.08
f1 (ppm)

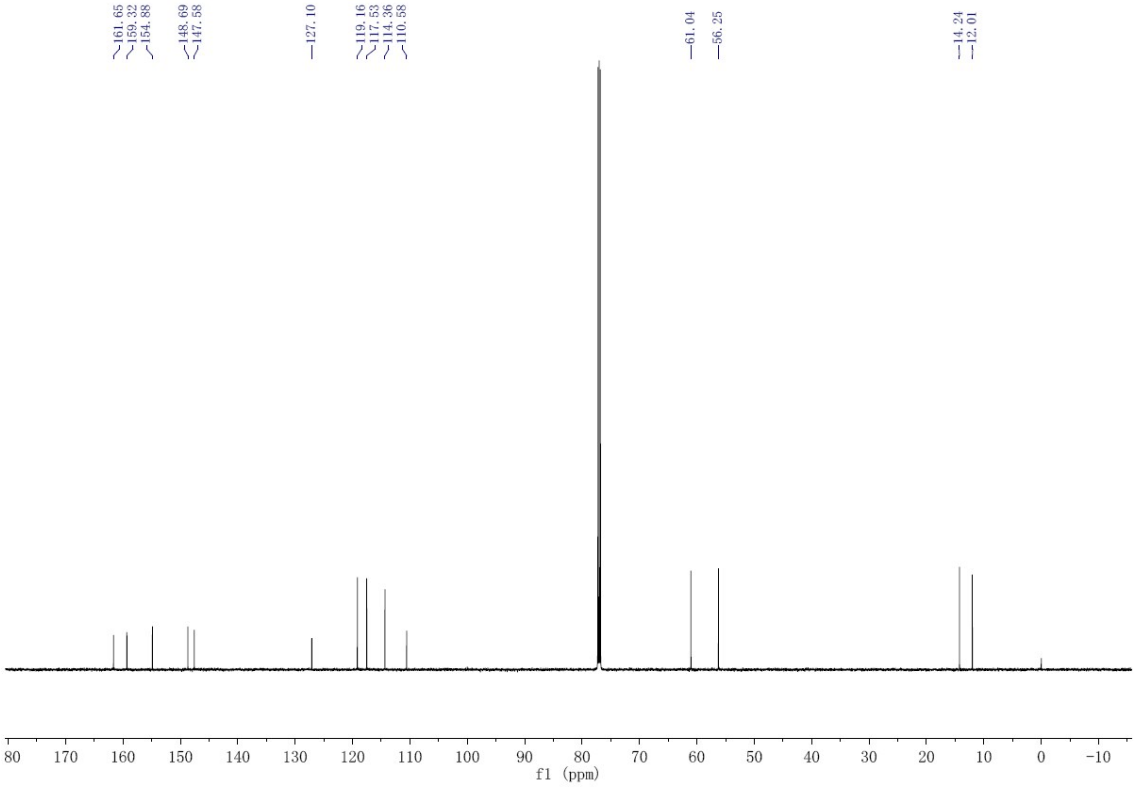

162.58
158.26
156.40
153.57
147.91
128.49
124.20
122.02
121.47
114.71
61.37
60.92
56.07
14.40
12.29
0.00
f1 (ppm)

Compd. 5b

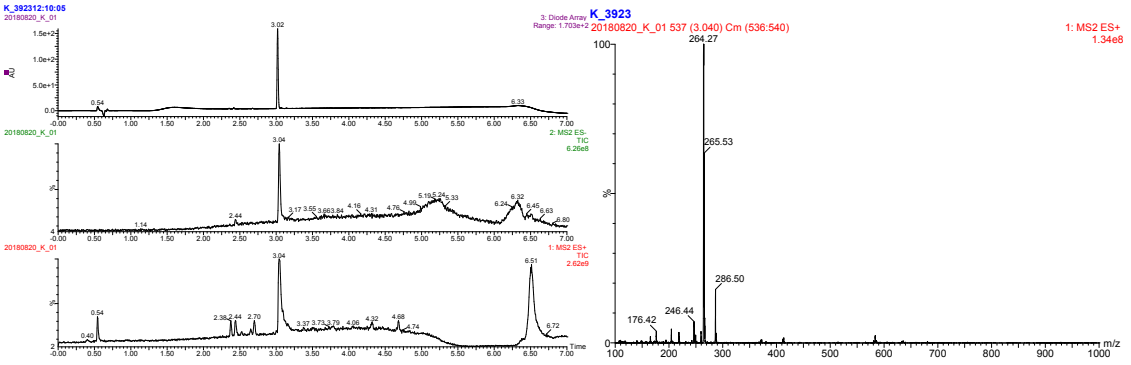

K_386817:29:29
20180725_K_05
3: Diode Array
Range: 2.67e+2
3.93
2.22
20180725_K_05
2: MS2 ES-
TIC
3.90e8
3.95
3.11
6.33
20180725_K_05
1: MS2 ES+
TIC
3.78e9
3.95
6.51
0.19
0.40
3.11
4.20
4.88
6.80
Time
K_3868
20180725_K_05 698 (3.952) Cm (697:699)
1: MS2 ES+
1.34e8
100
%
250.50
278.40
300.54
232.49
301.53
0
100
200
300
400
500
600
700
800
900
1000
m/z

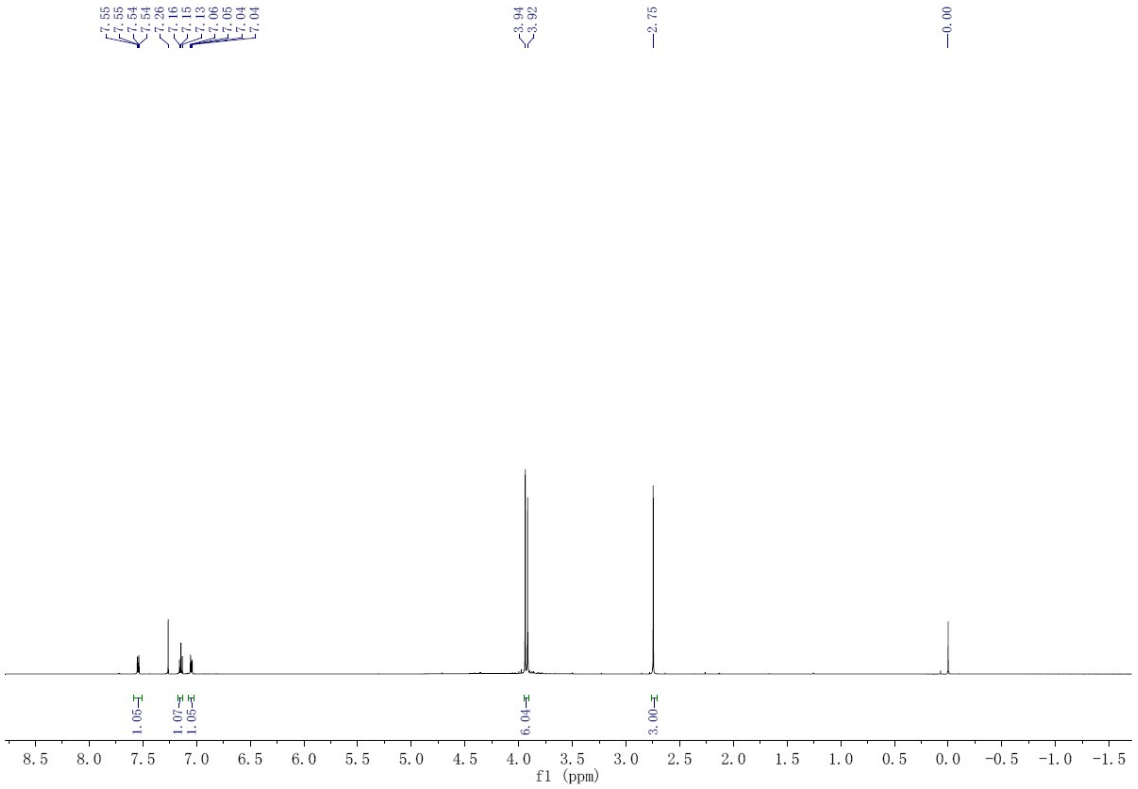
7.41
7.41
7.40
7.40
7.26
6.99
6.99
6.97
6.97
6.91
6.90
6.88
4.40
4.39
4.38
4.36
3.93
2.72
1.41
1.40
1.39
0.00

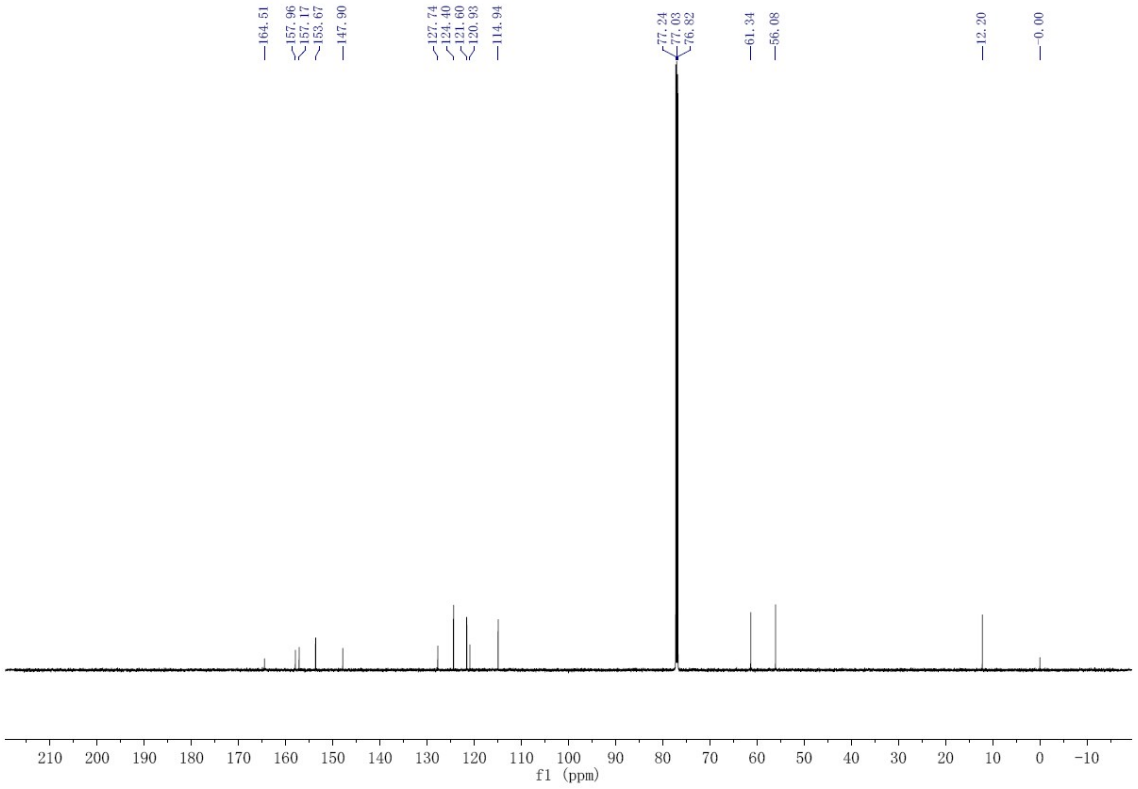

1.00
1.00
1.02
2.03
3.05
3.00
3.01
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


161.65
159.32
154.88
148.69
147.58
127.10
119.16
117.53
114.36
110.58
61.04
56.25
14.24
12.01
f1 (ppm)

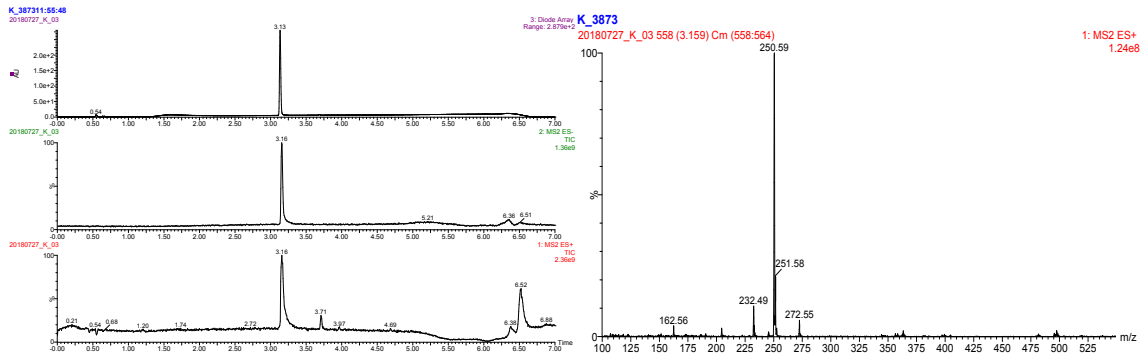
Compd. 6a


K_392312:10:05
20180820_K_01
3: Diode Array
Range: 1.703e+2
K_3923
20180820_K_01 537 (3.040) Cm (536:540)
1: MS2 ES+
1.34e8
2: MS2 ES-
TIC
6.26e8
1: MS2 ES+
TIC
2.62e9
264.27
265.53
286.50
246.44
176.42
m/z
Time

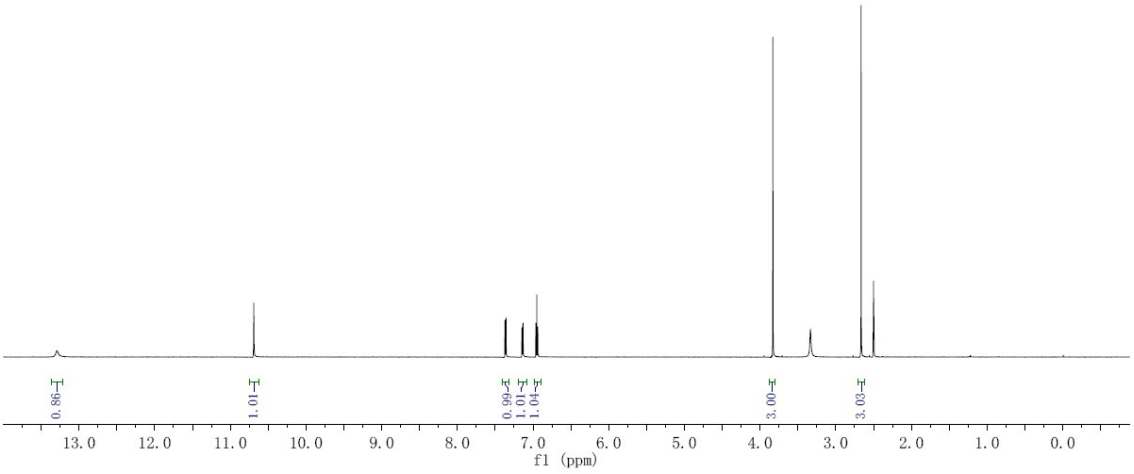

7.55
7.55
7.54
7.54
7.26
7.16
7.15
7.13
7.06
7.05
7.04
7.04
3.94
3.92
2.75
0.00
1.05
1.07
1.05
6.04
3.00
f1 (ppm)

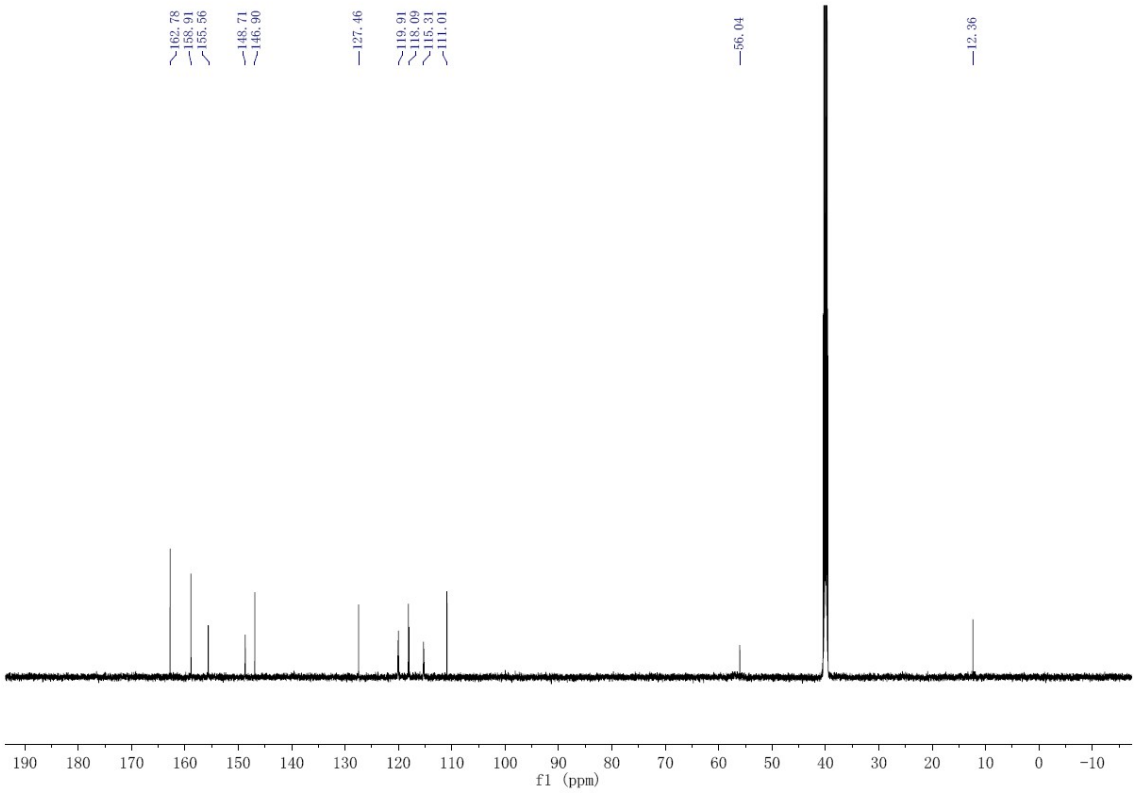

164.51
157.96
157.17
153.67
147.90
127.74
124.40
121.60
120.93
114.94
77.24
77.03
76.82
61.34
56.08
12.20
0.00
f1 (ppm)

Compd. 6b


K_387311:55:48
20180727_K_03
3.13
0.84
20180727_K_03
3.16
5.21
6.36
6.51
20180727_K_03
3.16
6.52
3.71
3.97
4.49
0.21
0.54
0.68
1.20
1.74
2.72
6.38
6.88
Time
K_3873
20180727_K_03 558 (3.159) Cm (558:564)
1: MS2 ES+
1.24e8
250.59
251.58
232.49
272.55
162.56
%
m/z


13.29
10.69
7.37
7.37
7.36
7.36
7.15
7.15
7.13
6.96
6.95
6.94
3.83
3.33
2.66
2.50

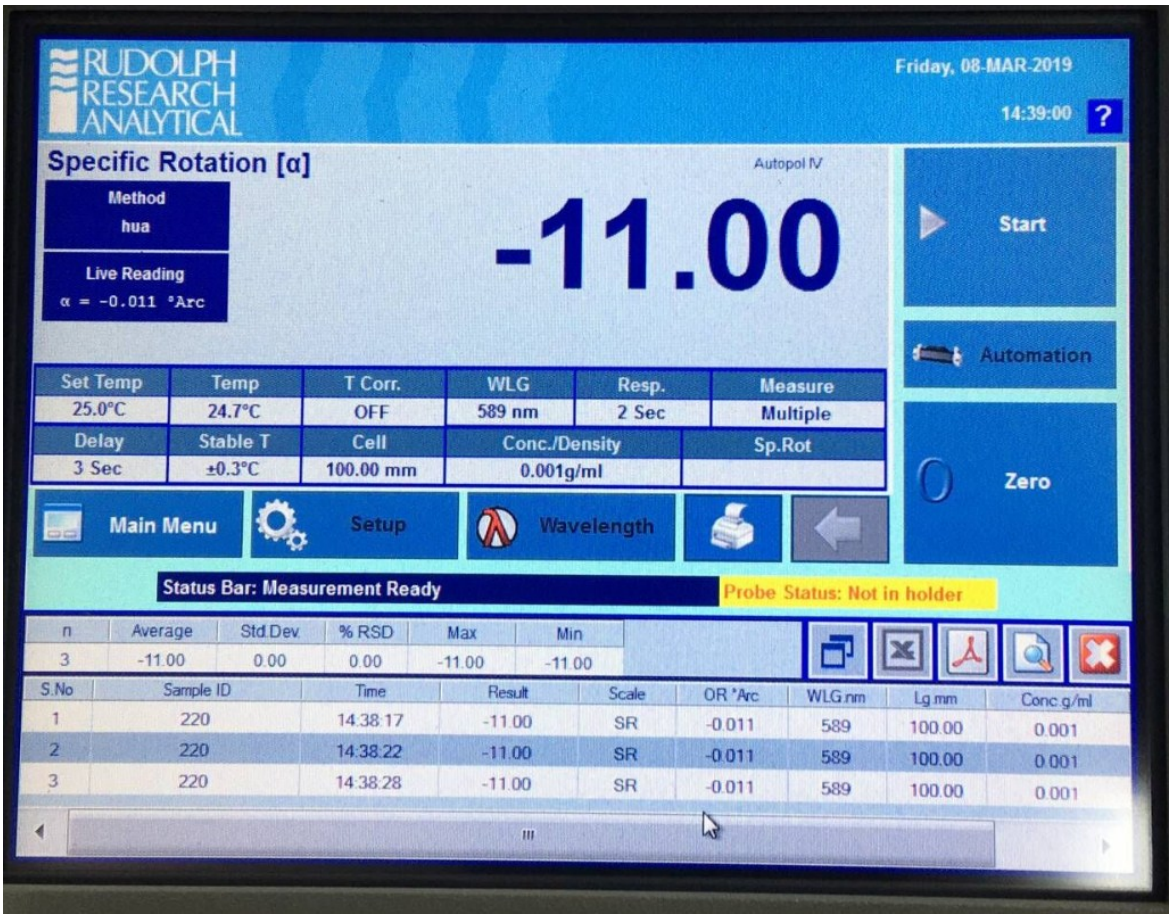

0.86
1.01
0.99
1.01
1.04
3.00
3.03
13.0
12.0
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

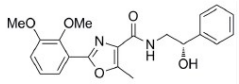


Spectroscopy for target compounds

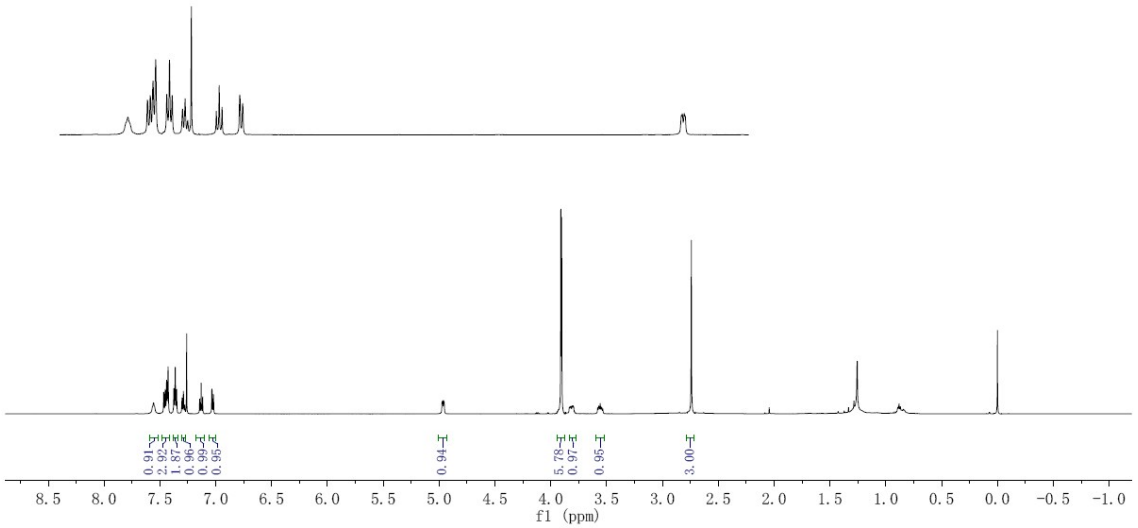
The structures of target compounds were confirmed by their ^{1}H NMR, ^{13}C NMR and ESI-MS spectra analyses, and all the ^{1}H NMR, ^{13}C NMR and ESI-MS spectra analyses were in good agreement with the proposed structures. The typical ^{1}H NMR, ^{13}C NMR and ESI-MS spectra for all target compounds have been presented in the following, which can further confirm the result.

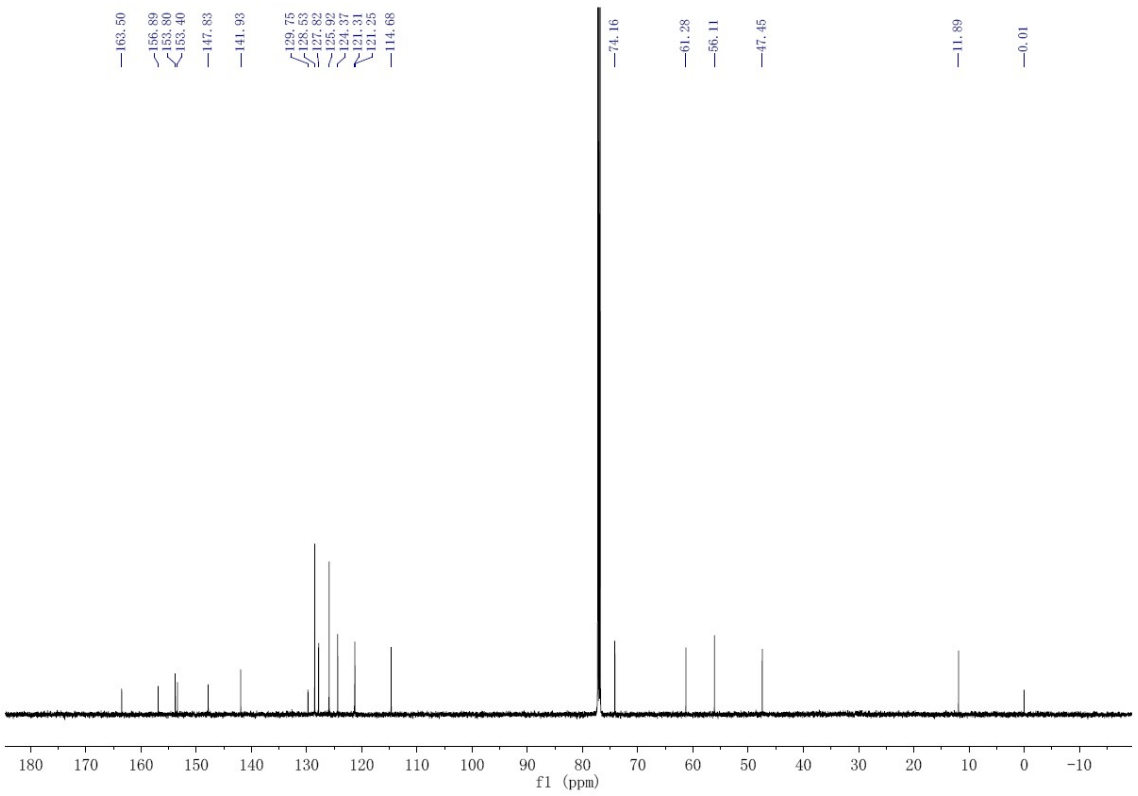
Compd. Hinduchelin A

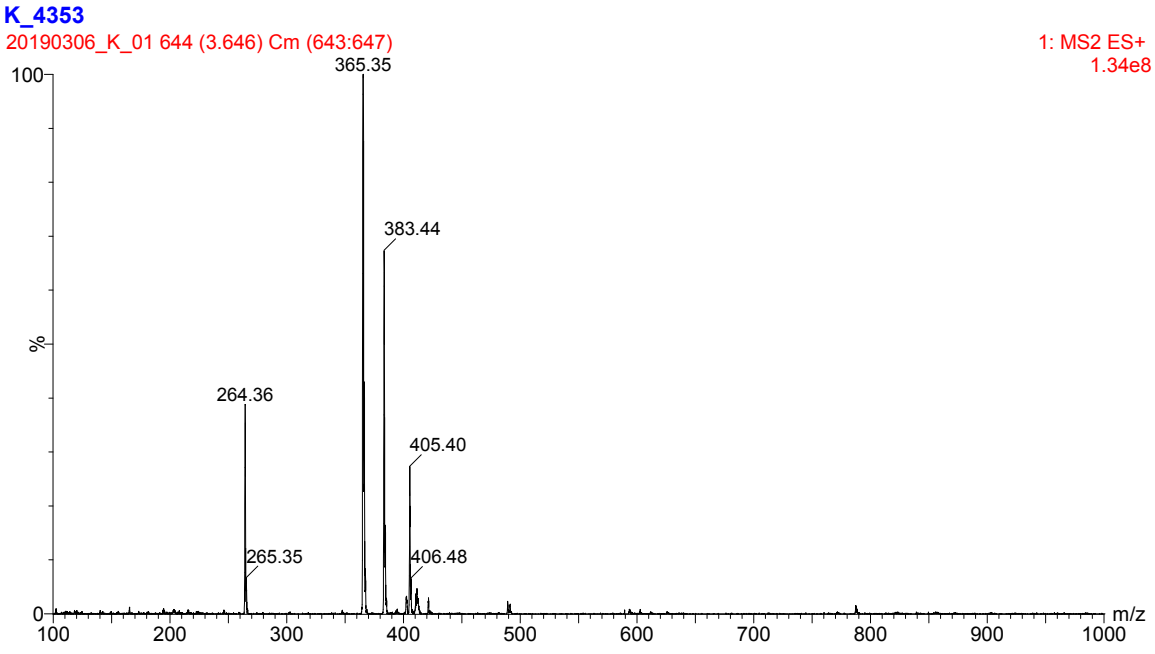




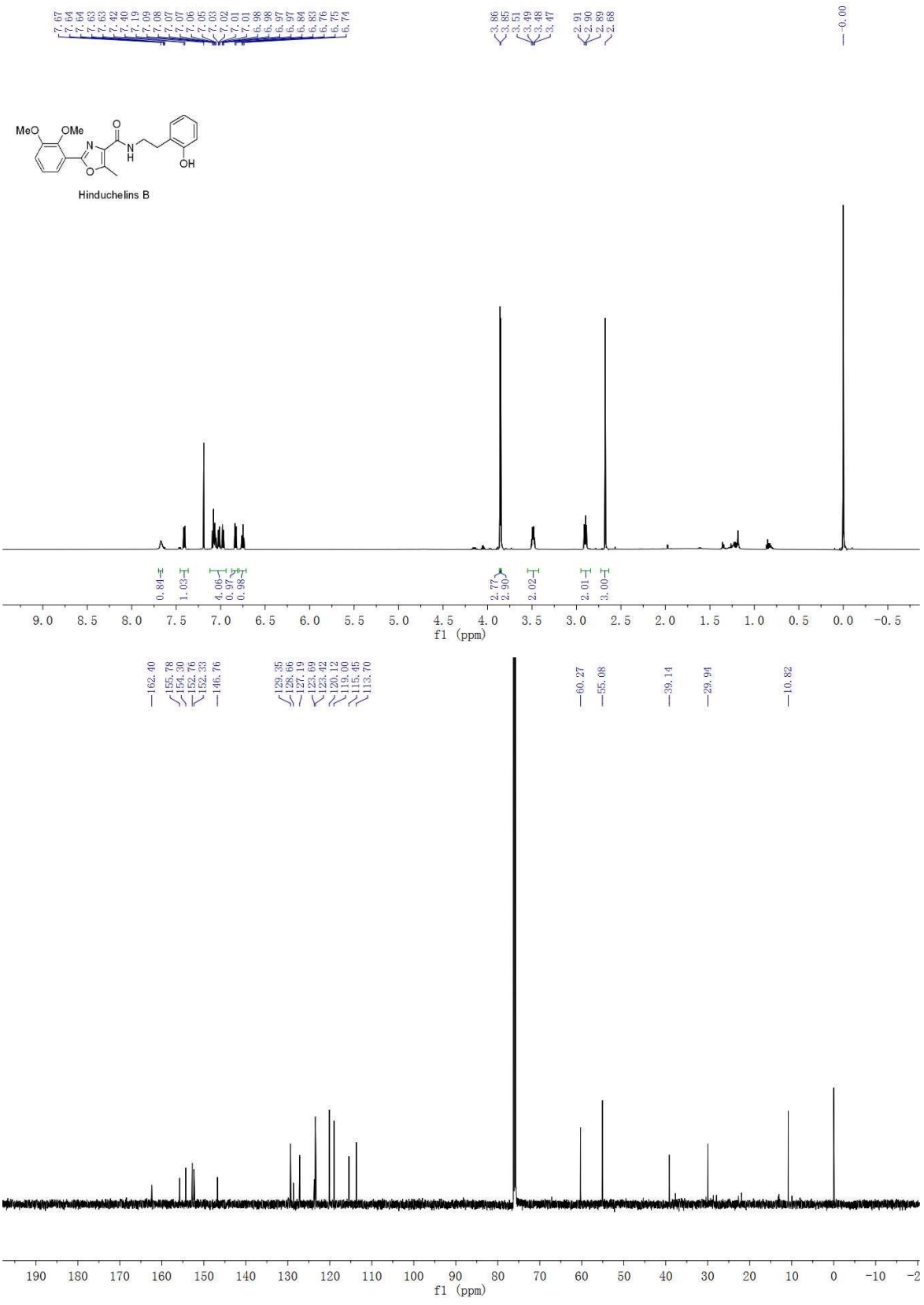
Hinduchelins A

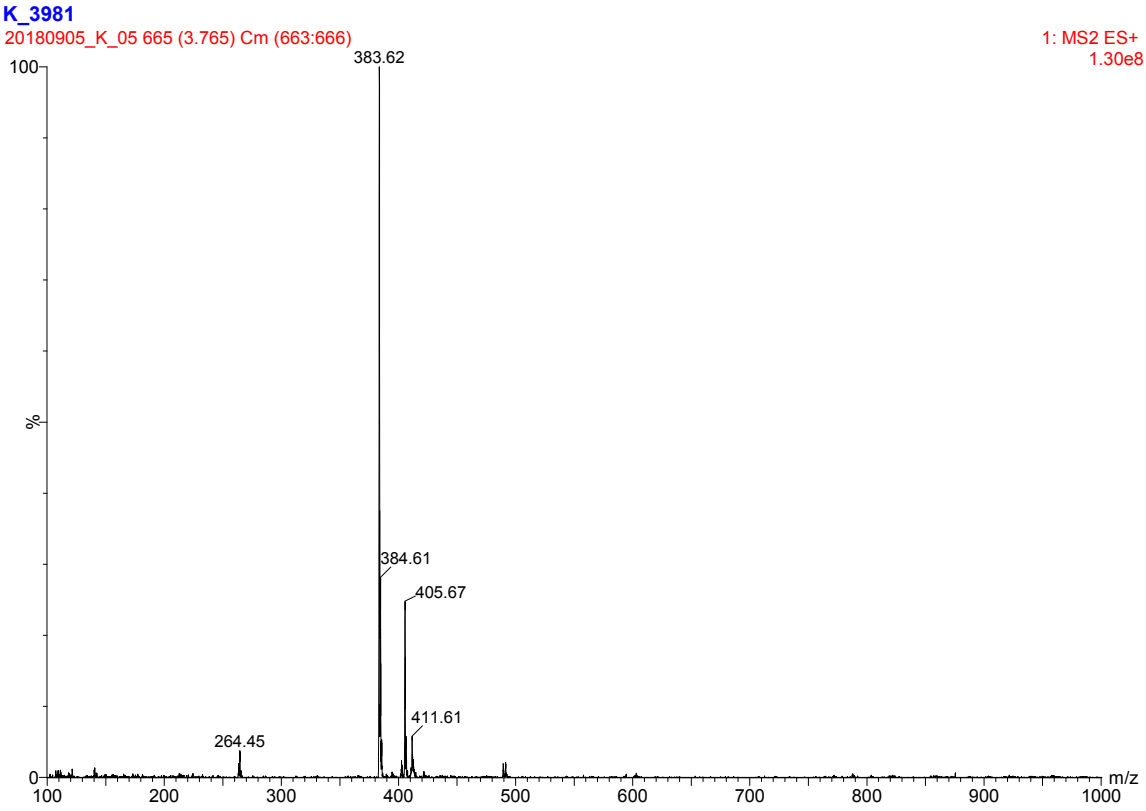




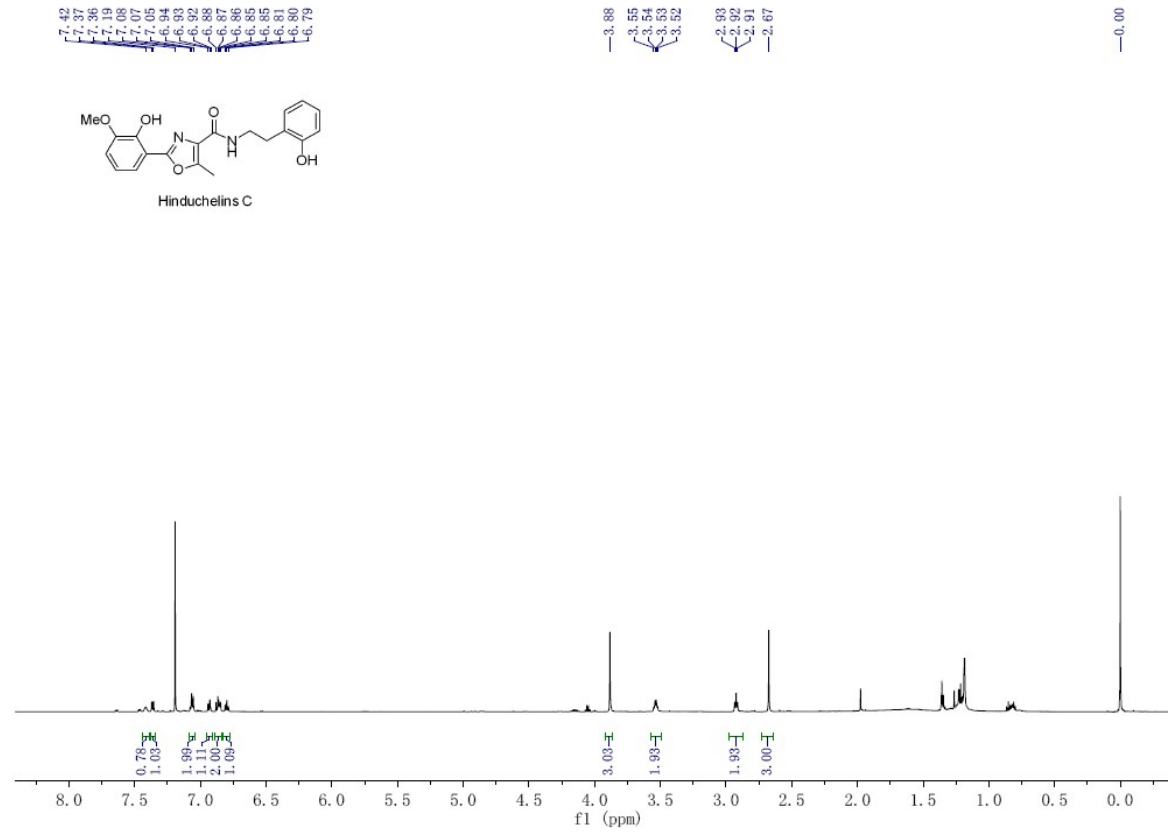


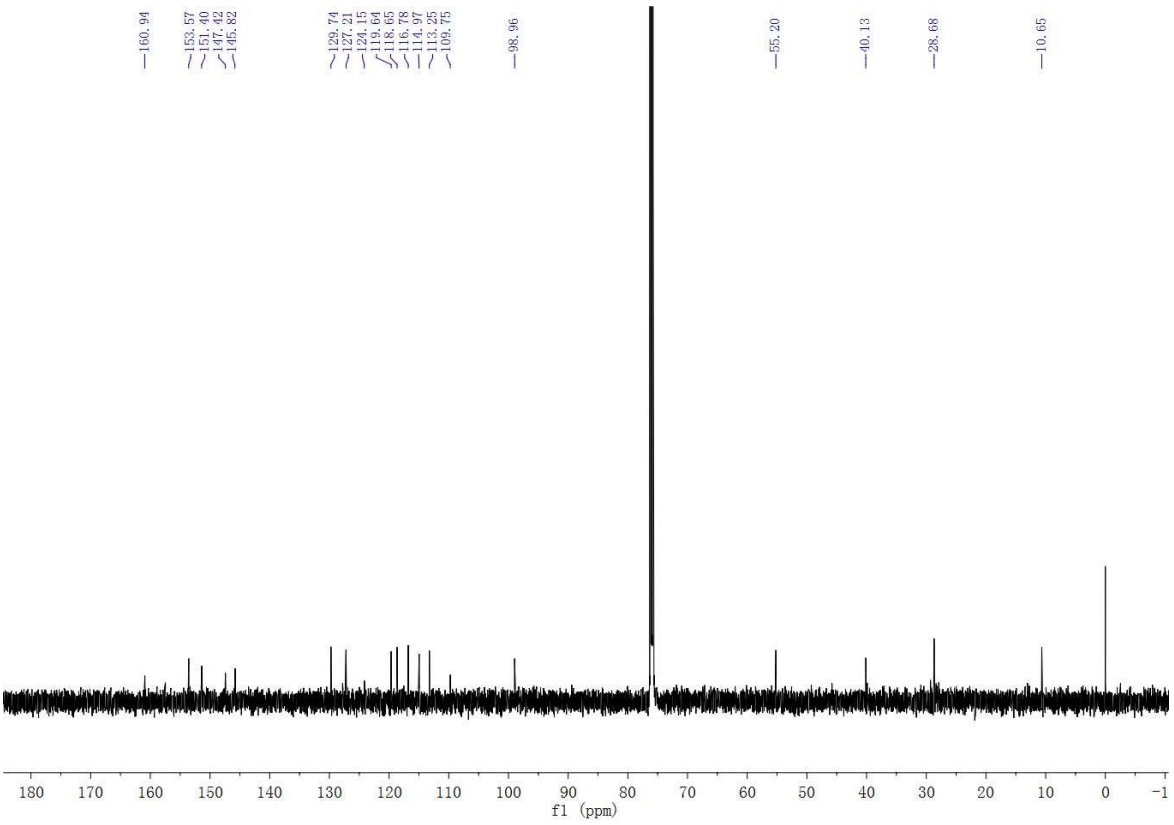
Compd. Hinduchelin B


7.67
7.64
7.64
7.63
7.63
7.42
7.40
7.19
7.09
7.08
7.07
7.07
7.06
7.05
7.03
7.02
7.01
7.01
6.98
6.98
6.97
6.97
6.84
6.83
6.76
6.75
6.74
3.86
3.85
3.51
3.49
3.48
3.47
2.91
2.90
2.89
2.68
0.00
MeO
OMe
N
O
N
H
O
OH
Hinduchelins B
0.84
1.03
4.06
0.97
0.98
2.77
2.90
2.02
2.01
3.00
f1 (ppm)
162.40
155.78
154.30
152.76
152.33
146.76
129.35
128.66
127.19
123.69
123.42
120.12
119.00
115.45
113.70
60.27
55.08
39.14
29.94
10.82
f1 (ppm)



Compd. Hinduchelin C

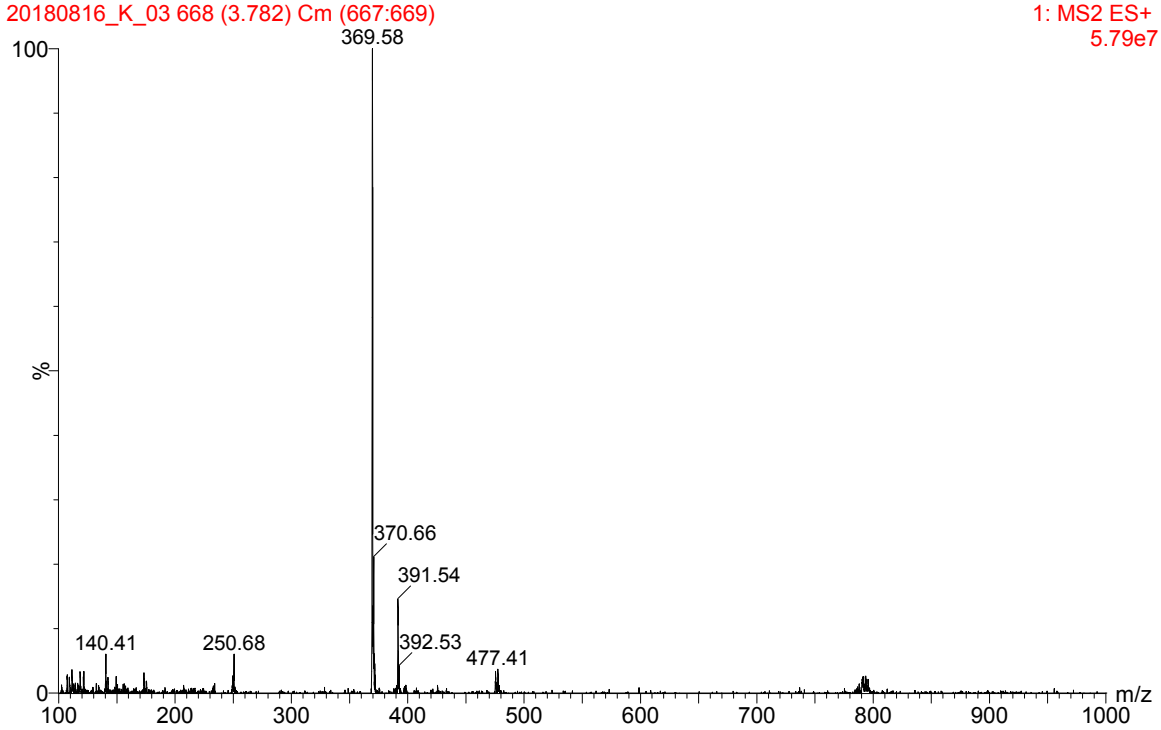


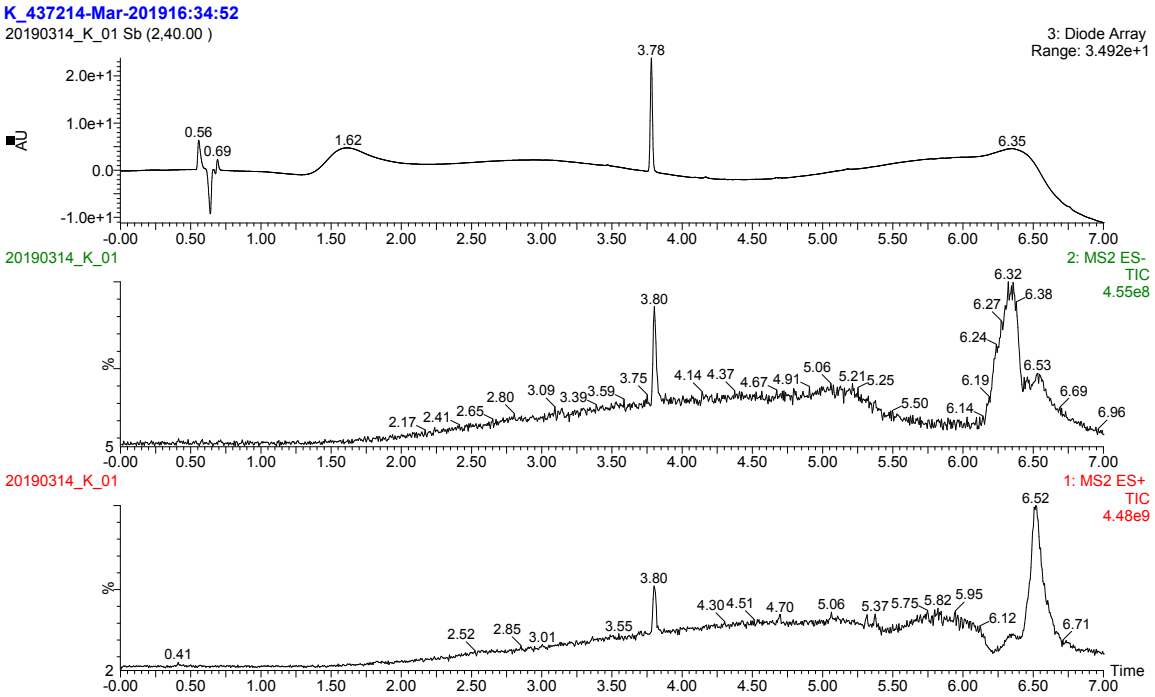


K_3921

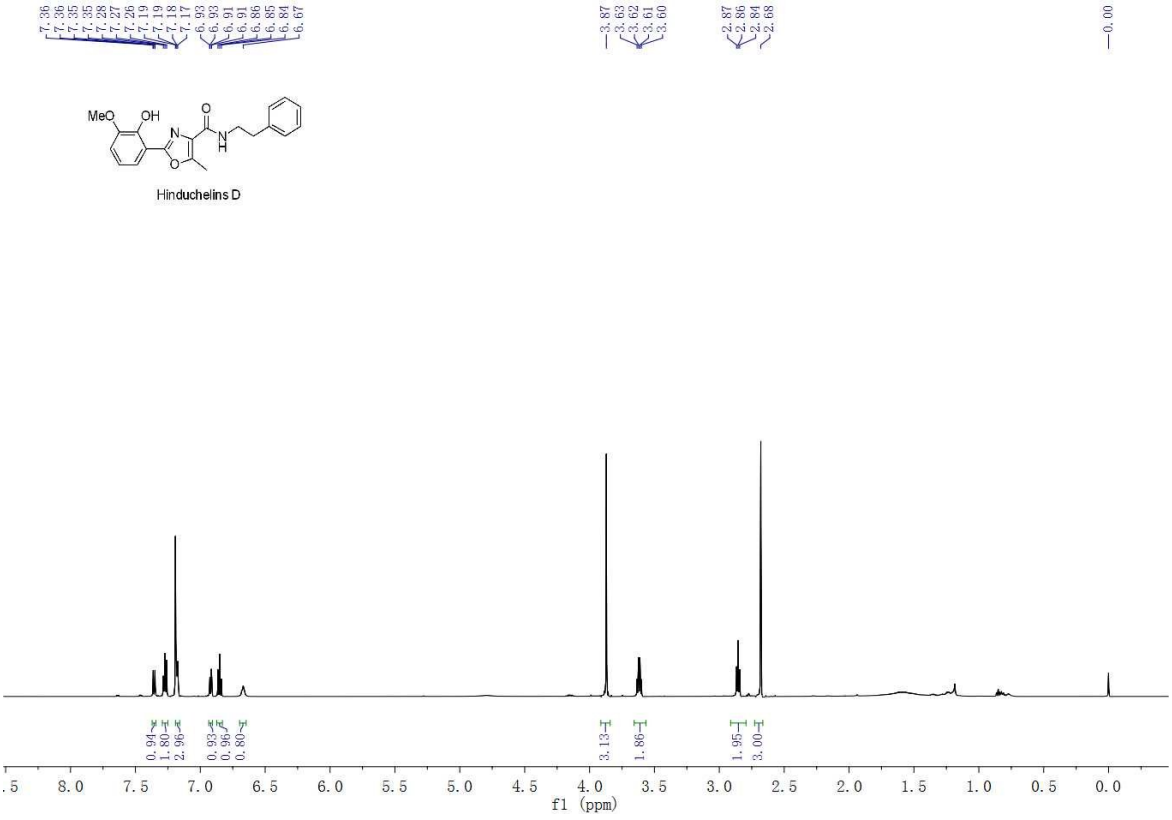
20180816_K_03 668 (3.782) Cm (667:669)

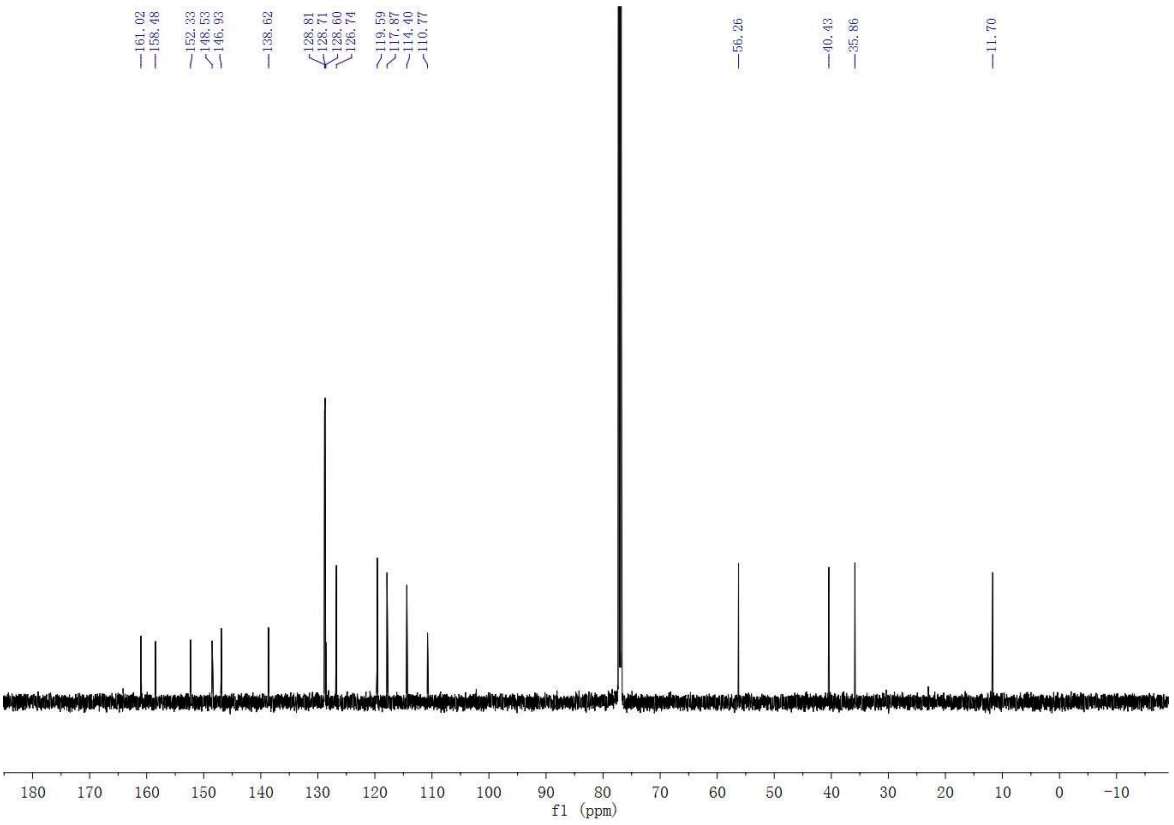
1: MS2 ES+
5.79e7

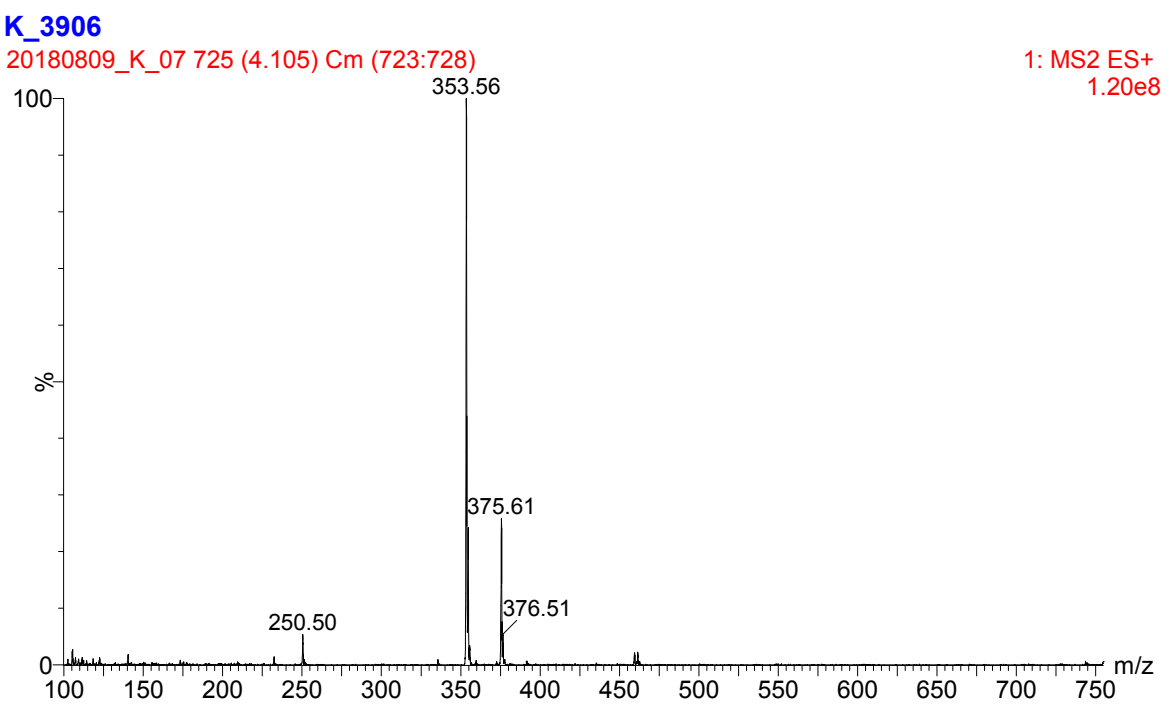




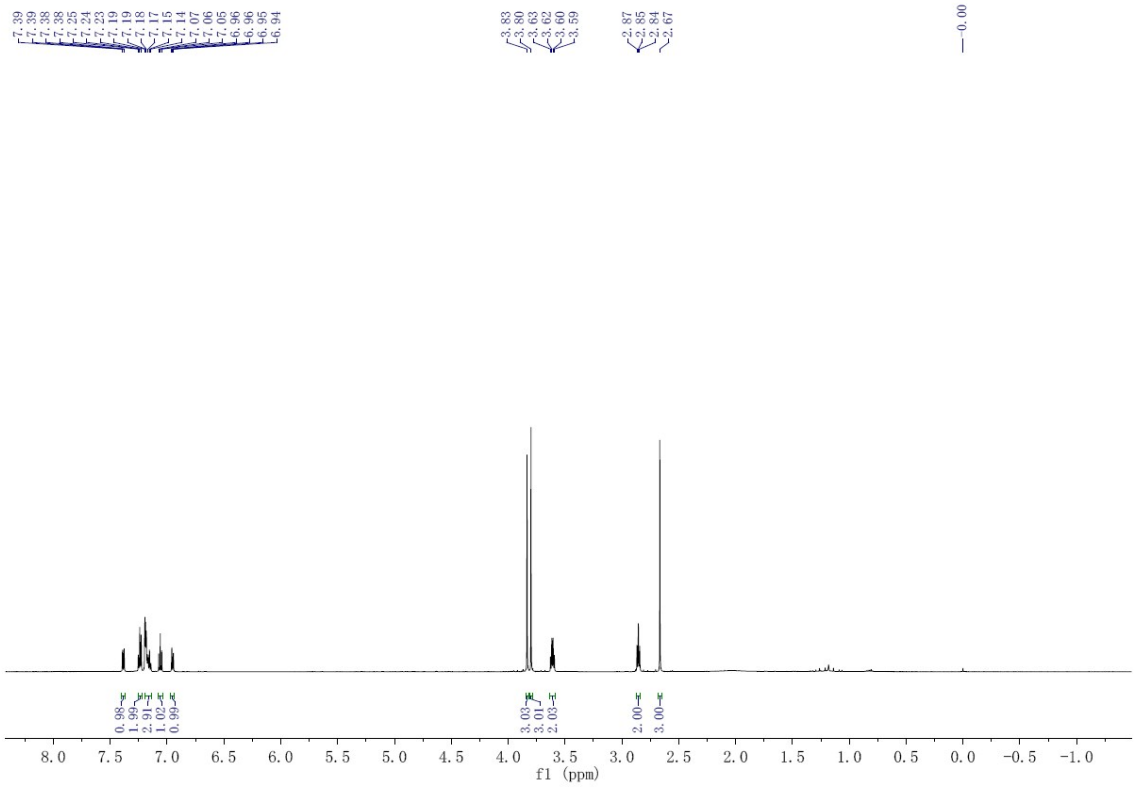
Compd. Hinduchelin D

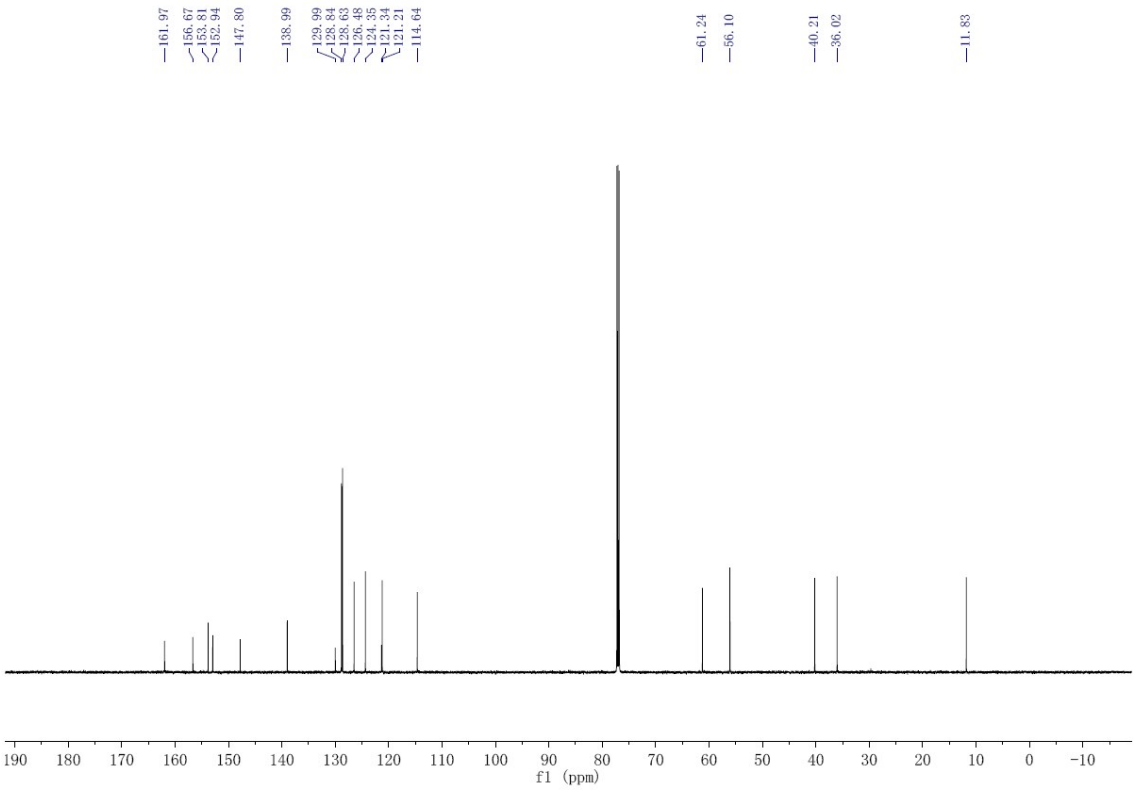



161.02
158.48
152.33
148.53
146.93
138.62
128.81
128.71
128.60
126.74
119.59
117.87
114.40
110.77
56.26
40.43
35.86
11.70
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)


K_3906
20180809_K_07 725 (4.105) Cm (723:728)
1: MS2 ES+
1.20e8
100
%
0
353.56
375.61
376.51
250.50
100 150 200 250 300 350 400 450 500 550 600 650 700 750
m/z

Compd. 11a

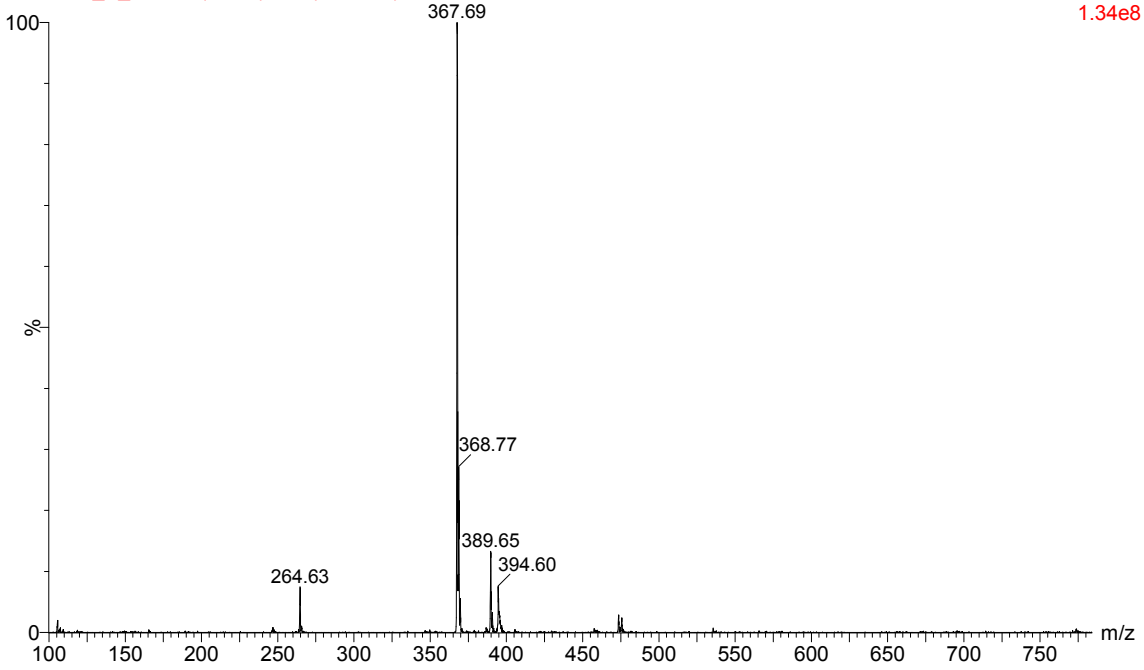

7.39
7.39
7.38
7.38
7.25
7.24
7.23
7.19
7.19
7.18
7.17
7.15
7.14
7.07
7.06
7.05
6.96
6.96
6.95
6.94
3.83
3.80
3.63
3.62
3.60
3.59
2.87
2.85
2.84
2.67
0.00
0.98
1.99
2.91
1.02
0.99
3.03
3.01
2.03
2.00
3.00
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)


161.97
156.67
153.81
152.94
147.80
138.99
129.99
128.84
128.63
126.48
124.35
121.34
121.21
114.64
61.24
56.10
40.21
36.02
11.83
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

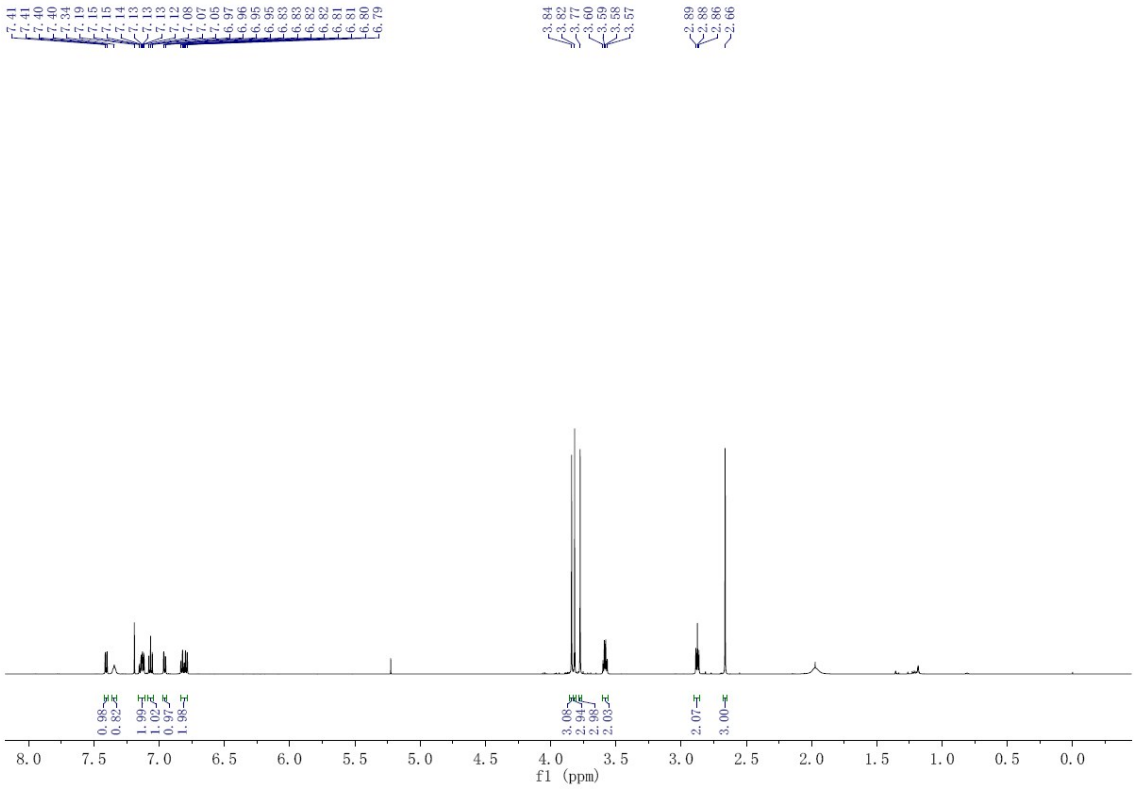
K_3949

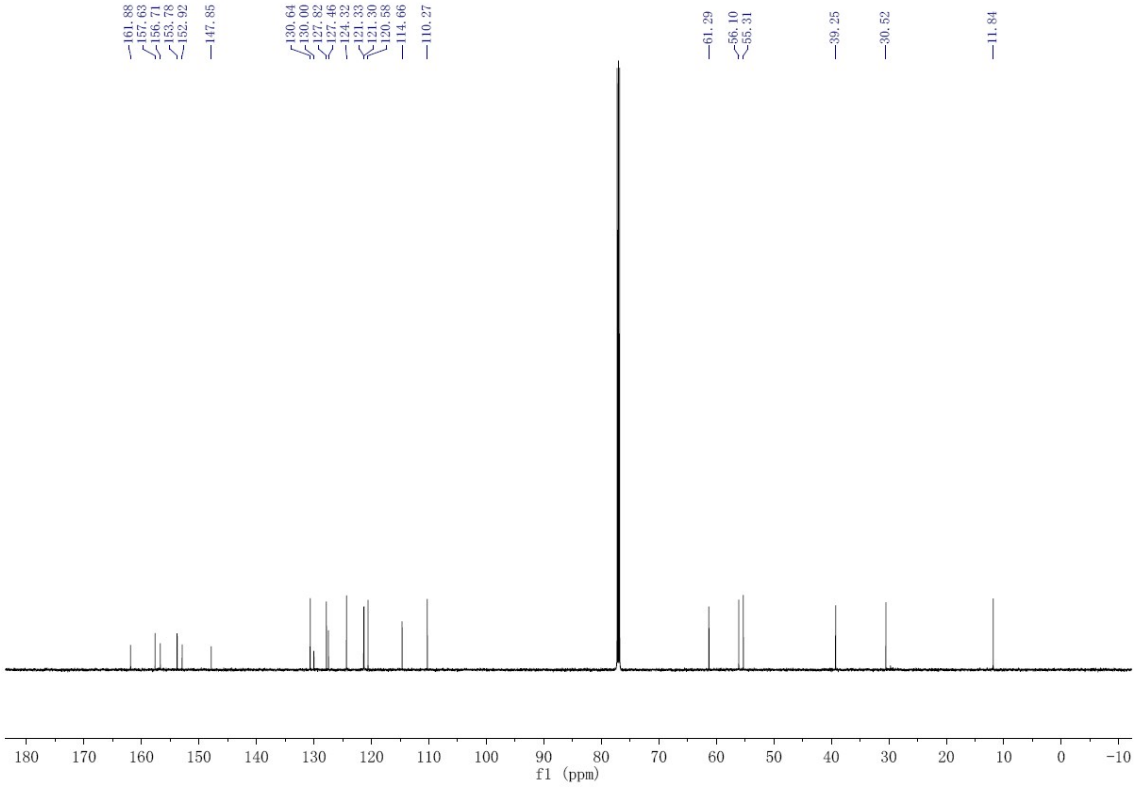
20180830_K_01 730 (4.133) Cm (728:732)

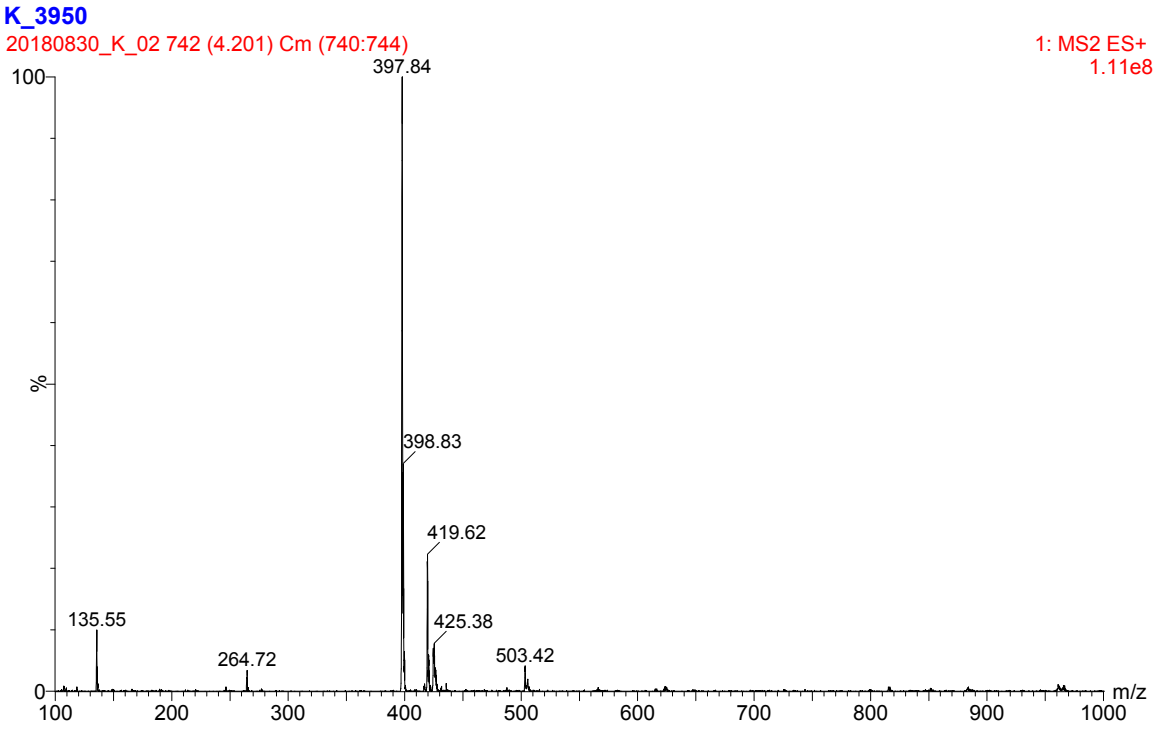
1: MS2 ES+
1.34e8



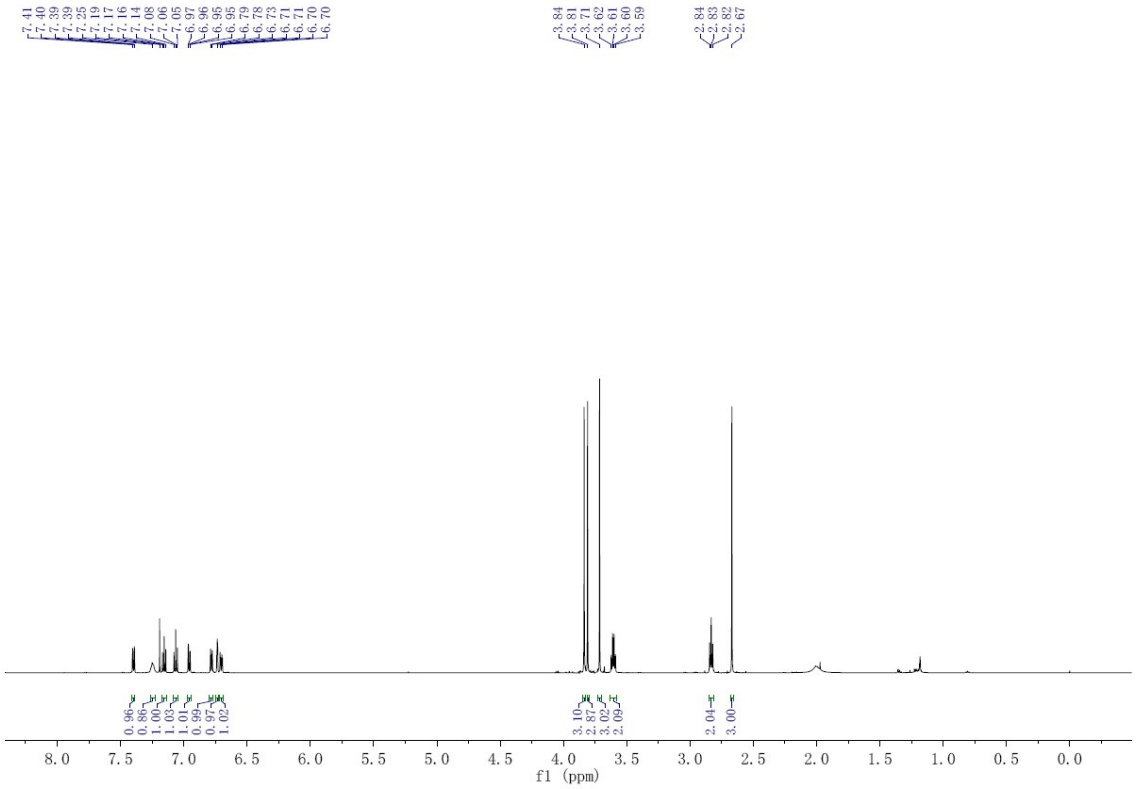
Compd. 11b

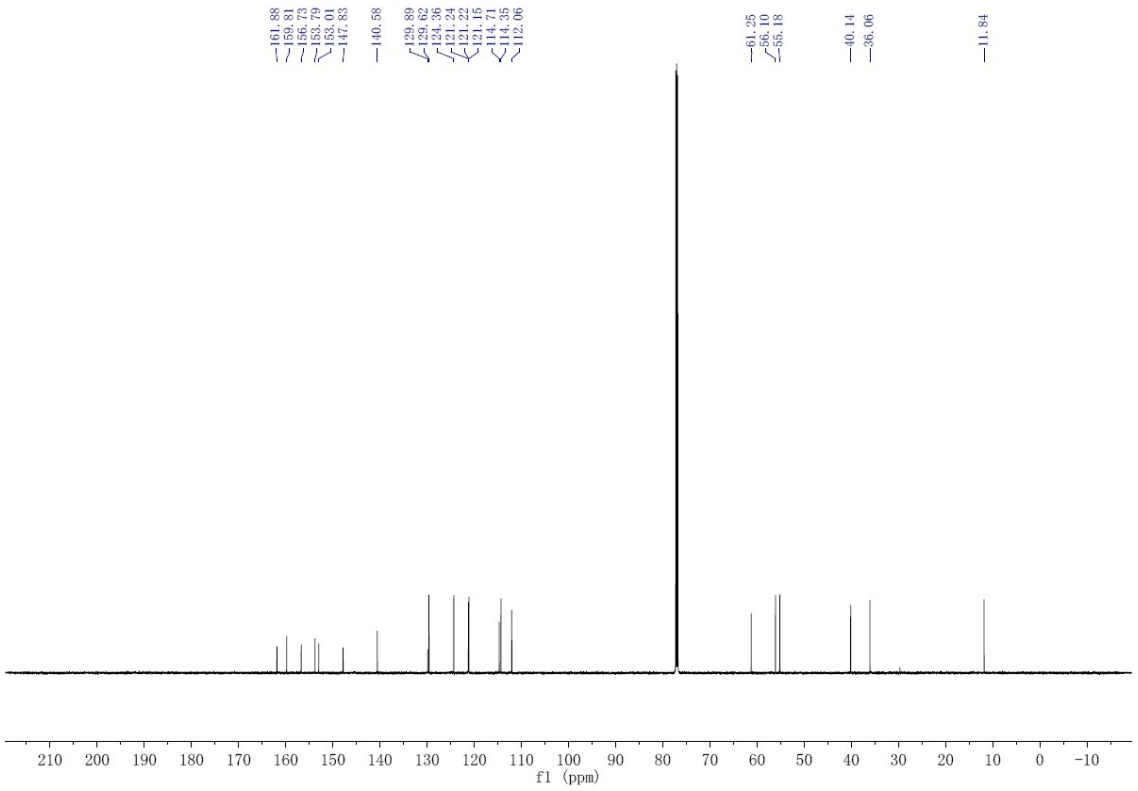


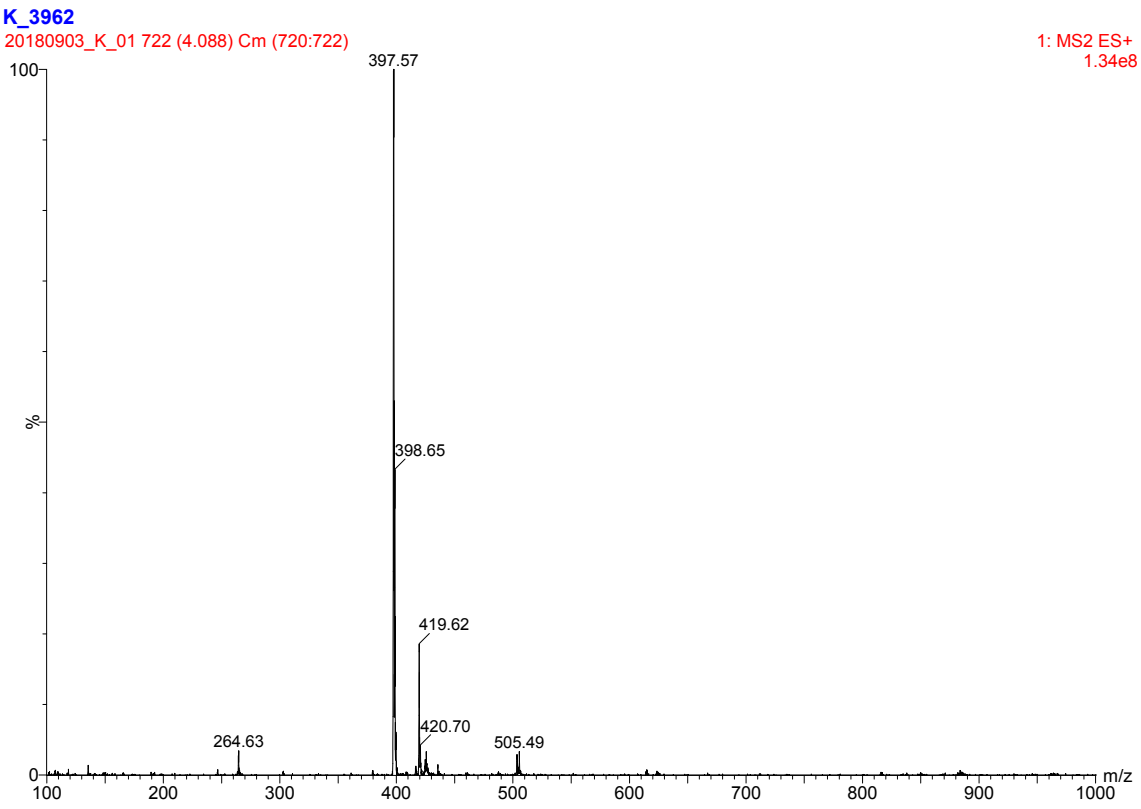

161.88
157.63
156.71
153.78
152.92
147.85
130.64
130.00
127.82
127.46
124.32
121.33
121.30
120.58
114.66
110.27
61.29
56.10
55.31
39.25
30.52
11.84
180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 −10
f1 (ppm)


K_3950
20180830_K_02 742 (4.201) Cm (740:744)
1: MS2 ES+
1.11e8
100
%
0
397.84
398.83
419.62
425.38
135.55
264.72
503.42
100 200 300 400 500 600 700 800 900 1000
m/z

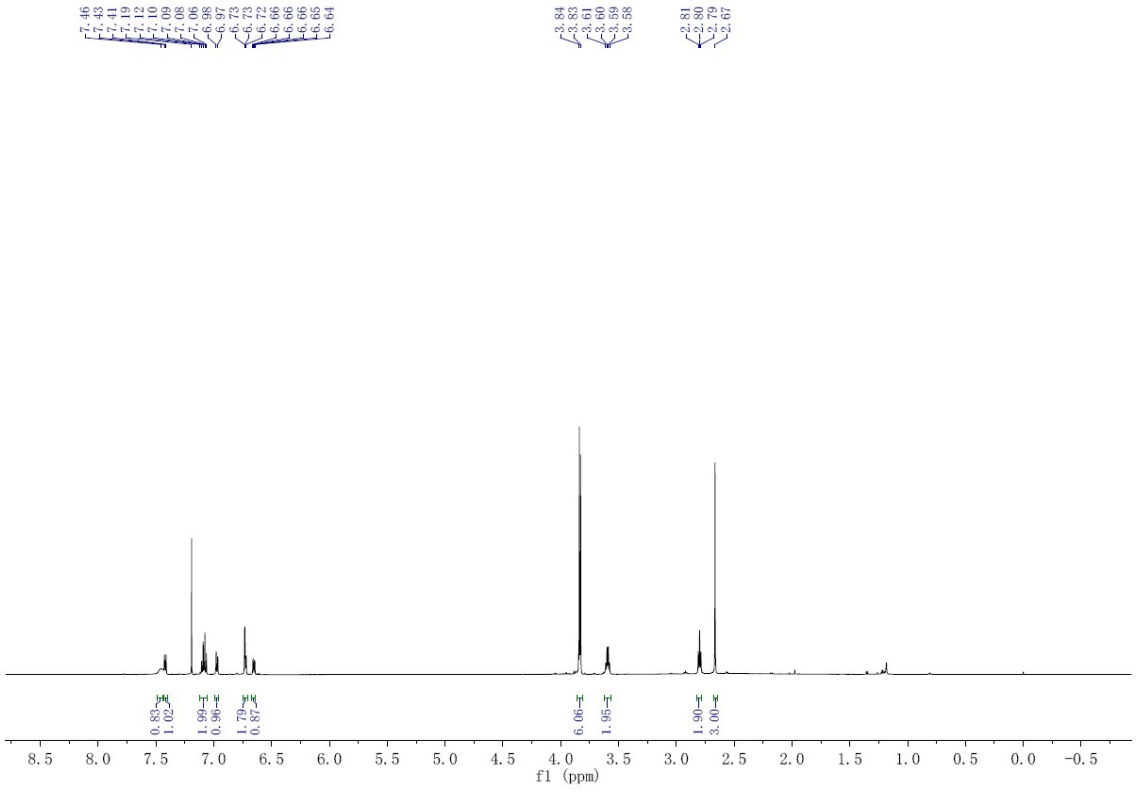
Compd. 11c

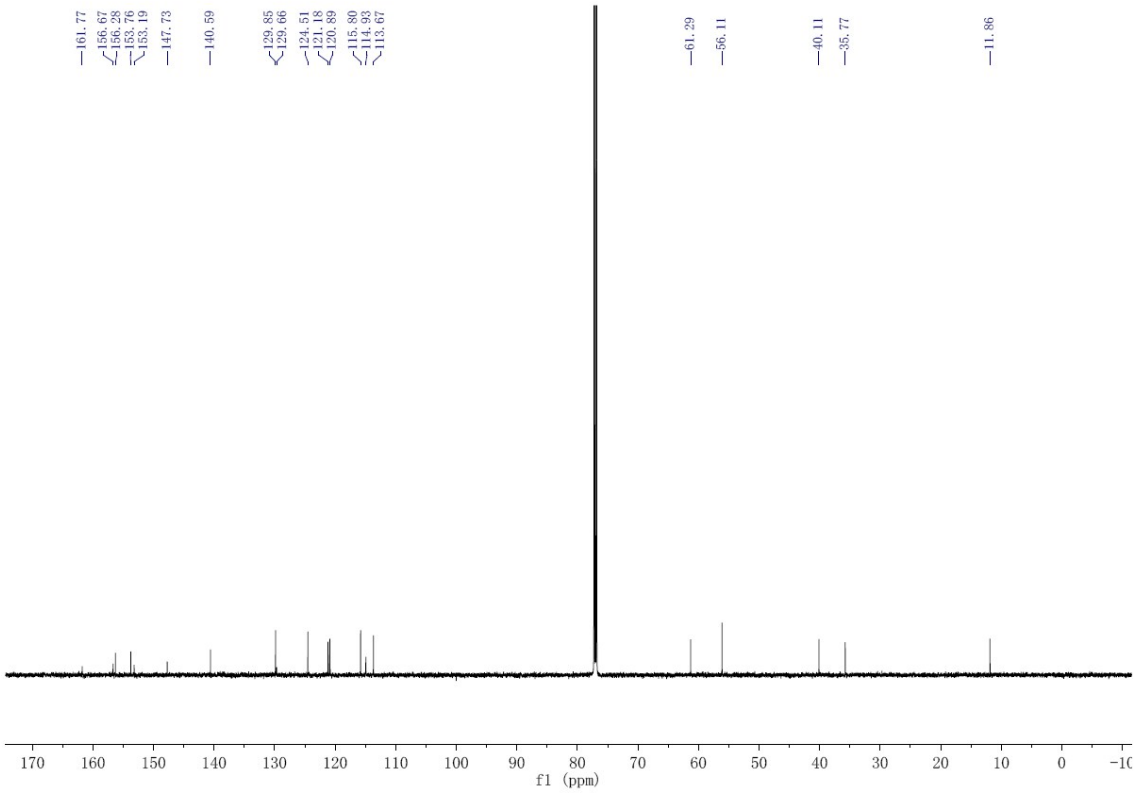

7.41
7.40
7.39
7.39
7.25
7.19
7.17
7.16
7.14
7.08
7.06
7.05
6.97
6.96
6.95
6.95
6.79
6.78
6.73
6.71
6.71
6.70
6.70
3.84
3.81
3.71
3.62
3.61
3.60
3.59
2.84
2.83
2.82
2.67
0.96
0.86
1.00
1.03
1.01
0.99
0.97
1.02
3.10
2.87
3.02
2.09
2.04
3.00
f1 (ppm)

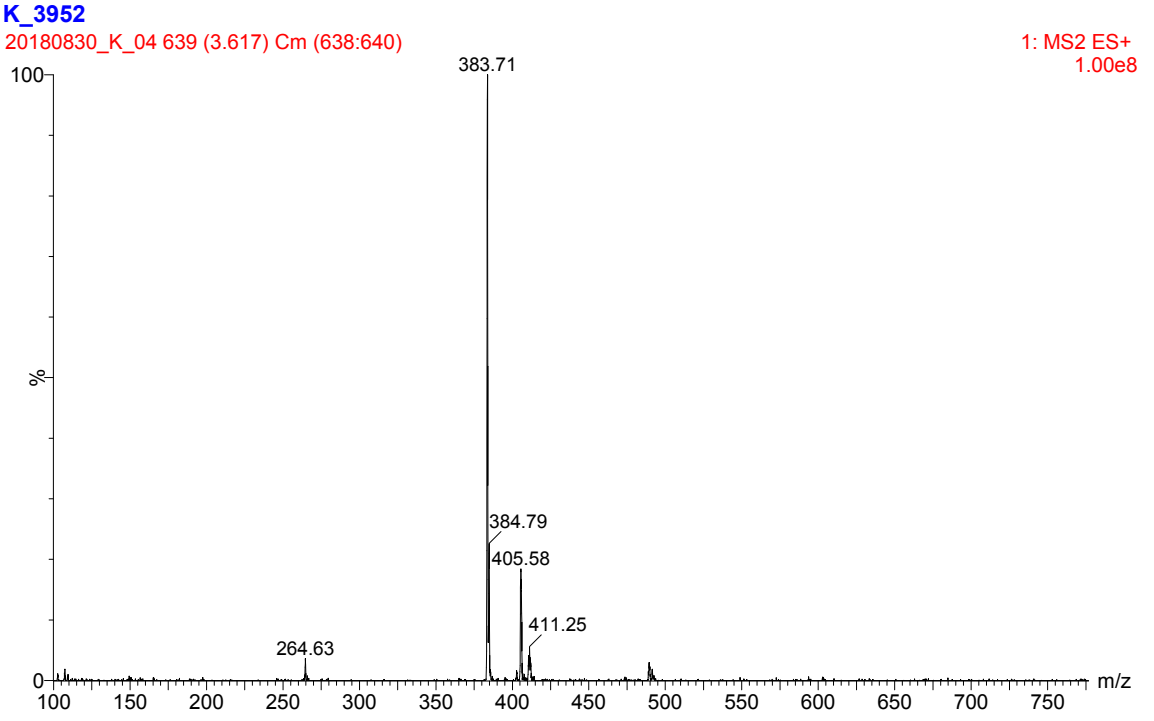

161.88
159.81
156.73
153.79
153.01
147.83
140.58
129.89
129.62
124.36
121.24
121.22
121.15
114.71
114.35
112.06
61.25
56.10
55.18
40.14
36.06
11.84
f1 (ppm)


K_3962
20180903_K_01 722 (4.088) Cm (720:722)
1: MS2 ES+
1.34e8
100
%
0
397.57
398.65
419.62
420.70
264.63
505.49
100
200
300
400
500
600
700
800
900
1000
m/z

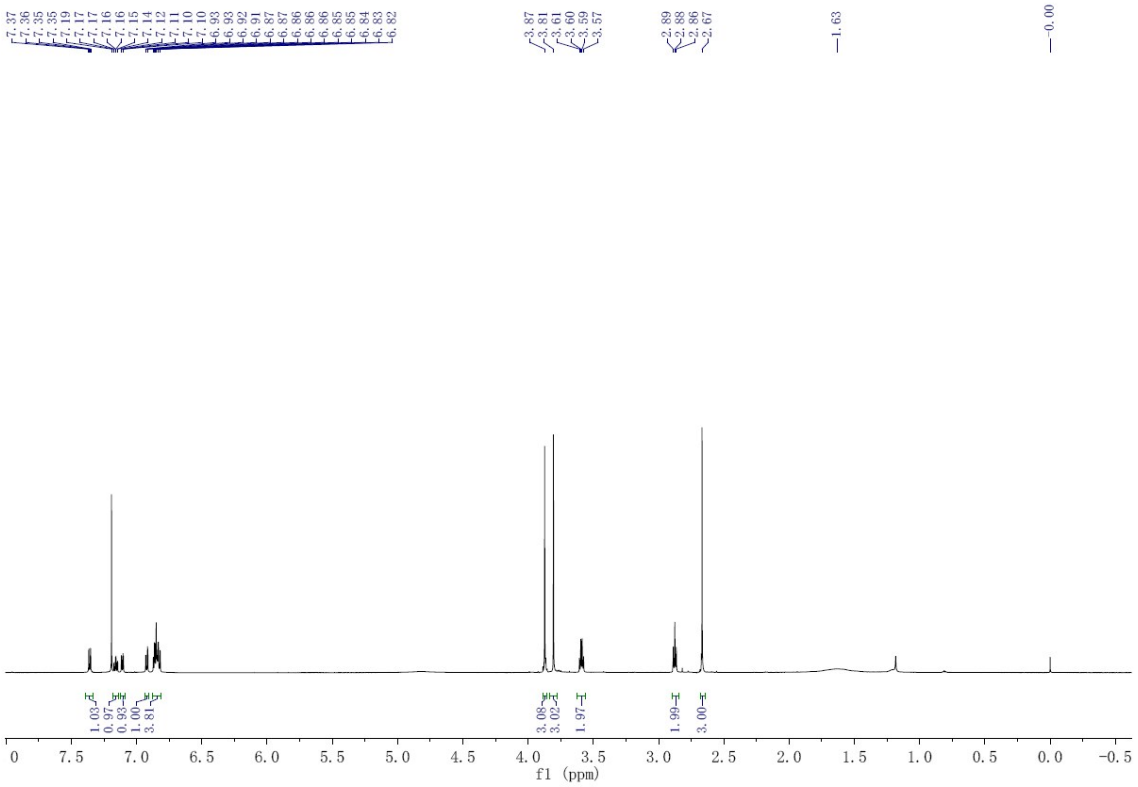
Compd. 11d

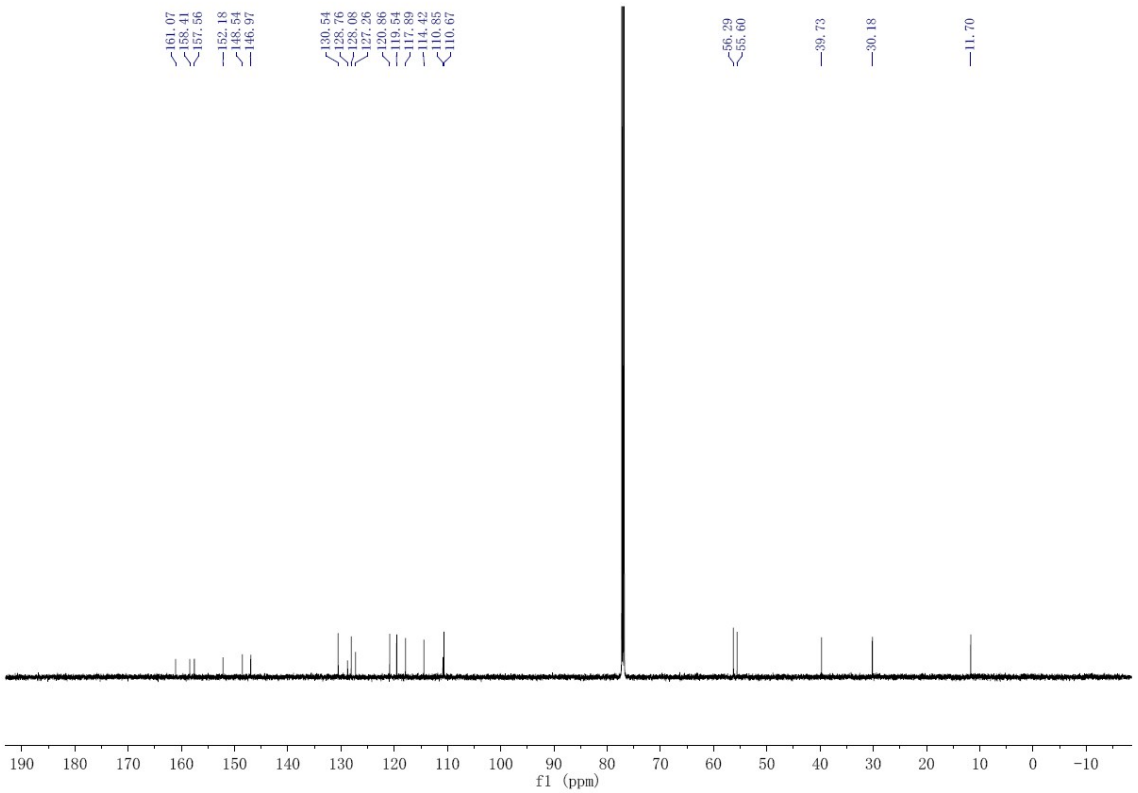

7.46
7.43
7.41
7.19
7.12
7.10
7.09
7.08
7.06
6.98
6.97
6.73
6.73
6.72
6.66
6.66
6.66
6.65
6.64
3.84
3.83
3.61
3.60
3.59
3.58
2.81
2.80
2.79
2.67
0.83
1.02
1.99
0.96
1.79
0.87
6.06
1.95
1.90
3.00
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)


161.77
156.67
156.28
153.76
153.19
147.73
140.59
129.85
129.66
124.51
121.18
120.89
115.80
114.93
113.67
61.29
56.11
40.11
35.77
11.86
170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)

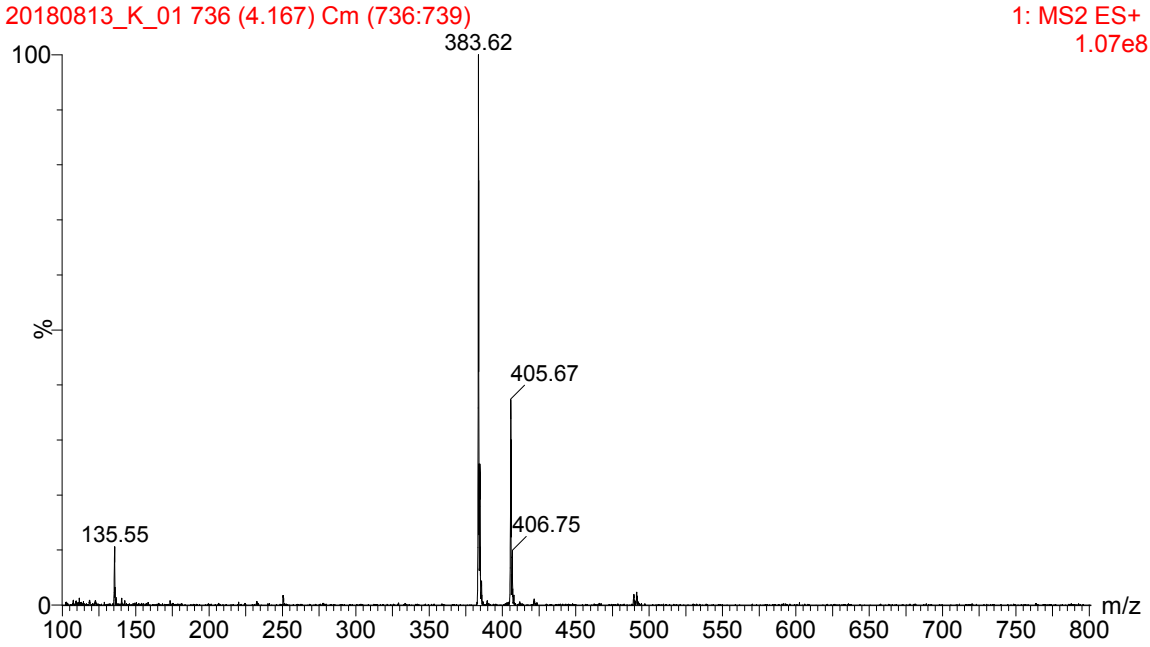

K_3952
20180830_K_04 639 (3.617) Cm (638:640)
1: MS2 ES+
1.00e8
100
%
0
383.71
384.79
405.58
411.25
264.63
100 150 200 250 300 350 400 450 500 550 600 650 700 750
m/z

Compd. 12a

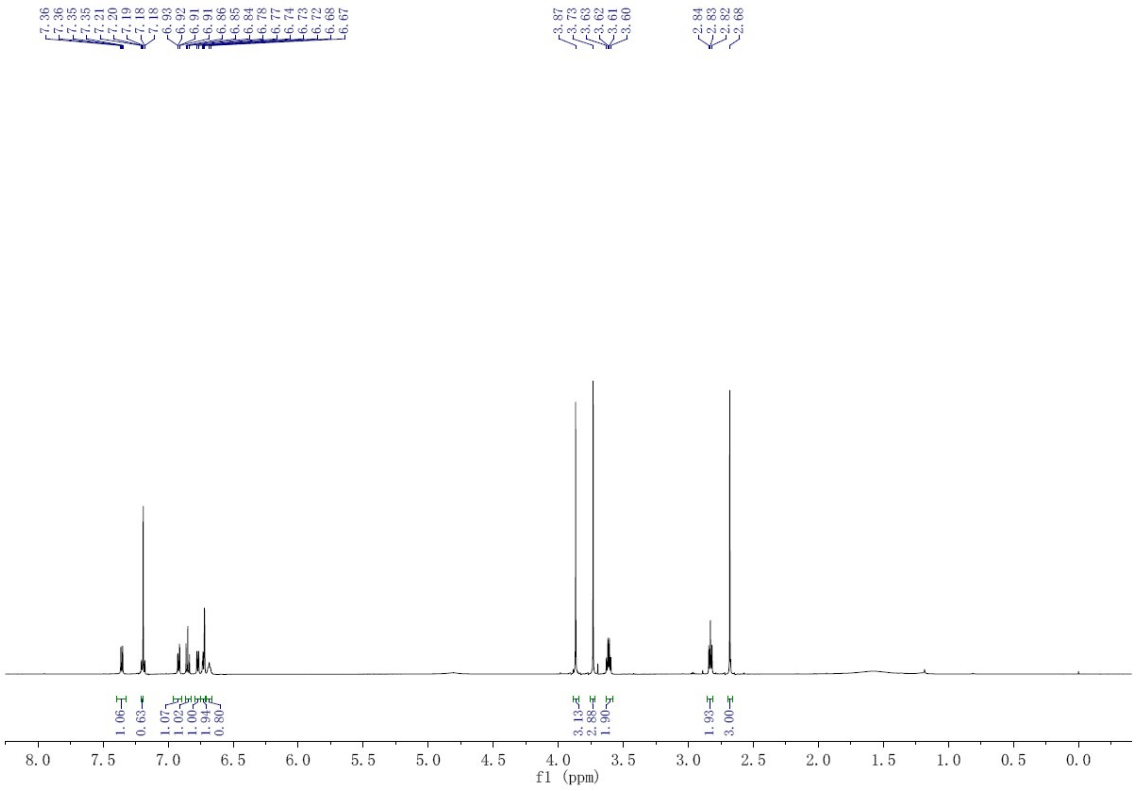

7.37
7.36
7.35
7.35
7.19
7.17
7.17
7.16
7.16
7.15
7.14
7.12
7.11
7.10
7.10
6.93
6.93
6.92
6.91
6.87
6.87
6.86
6.86
6.86
6.85
6.85
6.84
6.83
6.82
3.87
3.81
3.61
3.60
3.59
3.57
2.89
2.88
2.86
2.67
1.63
0.00
1.03
0.97
0.93
1.00
3.81
3.08
3.02
1.97
1.99
3.00
f1 (ppm)

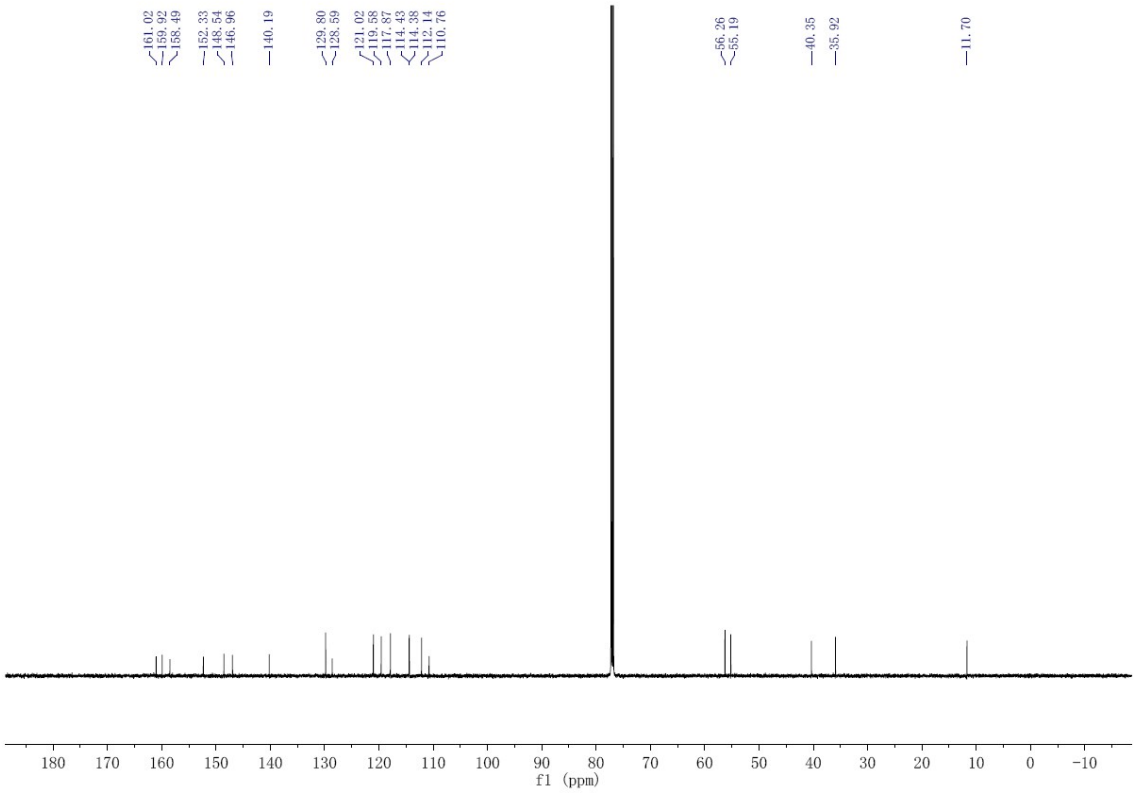

161.07
158.41
157.56
152.18
148.54
146.97
130.54
128.76
128.08
127.26
120.86
119.54
117.89
114.42
110.85
110.67
56.29
55.60
39.73
30.18
11.70
f1 (ppm)

K_3911



Compd. 12b

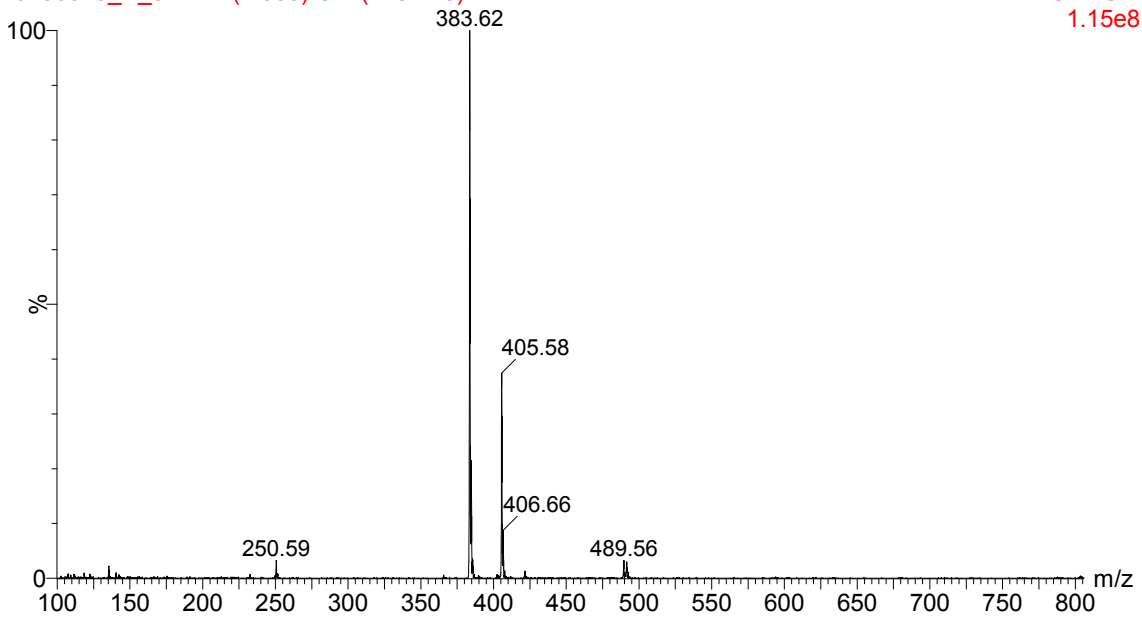




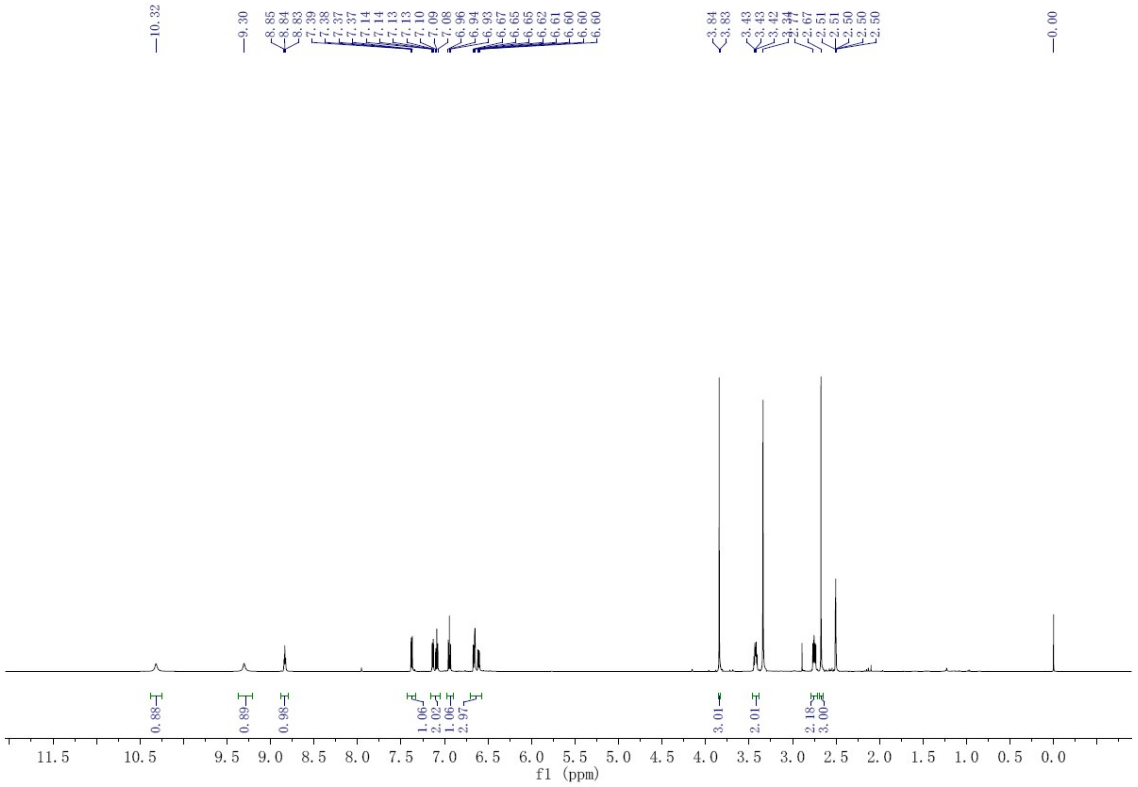
K_3912

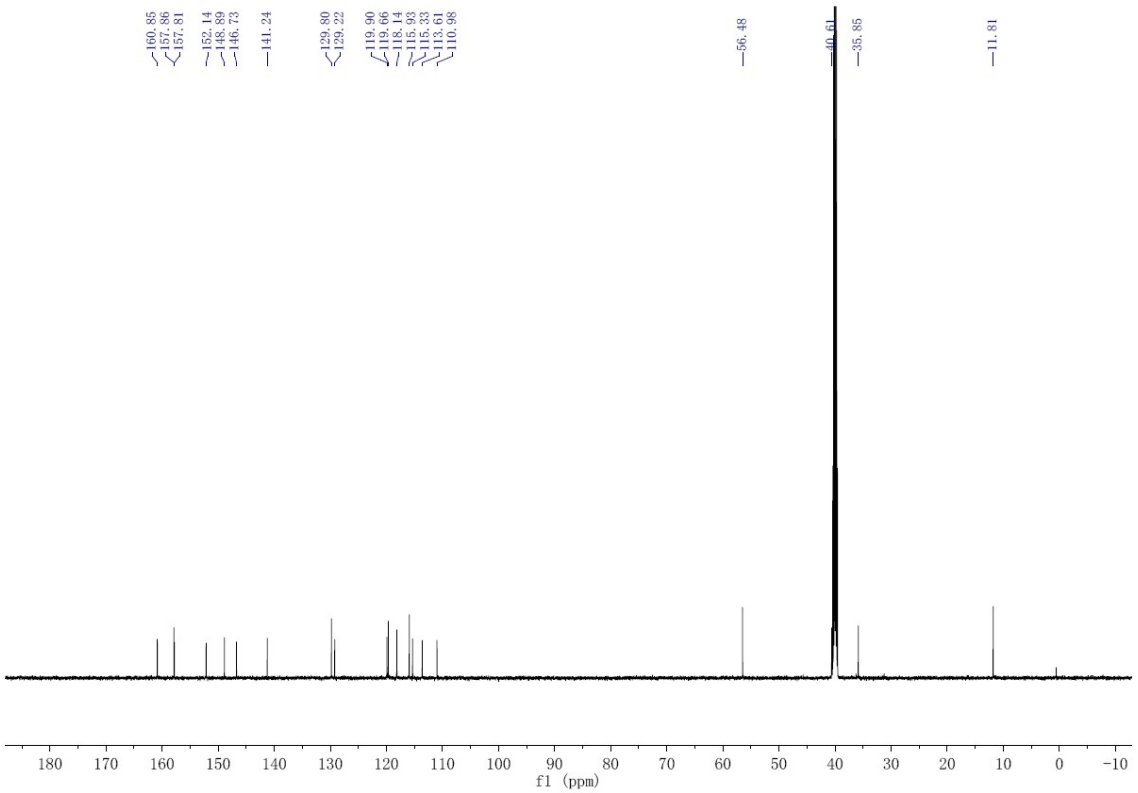
20180813_K_02 722 (4.088) Cm (719:723)

1: MS2 ES+
1.15e8



Compd. 12c


10.32
9.30
8.85
8.84
8.83
7.39
7.38
7.37
7.37
7.14
7.14
7.13
7.13
7.10
7.09
7.08
6.96
6.94
6.93
6.67
6.65
6.62
6.61
6.60
6.60
6.60
3.84
3.83
3.43
3.43
3.42
2.67
2.51
2.51
2.50
2.50
2.50
0.00
0.88
0.89
0.98
1.06
2.02
1.06
2.97
3.01
2.01
2.18
3.00
f1 (ppm)


160.85
157.86
157.81
152.14
148.89
146.73
141.24
129.80
129.22
119.90
119.66
118.14
115.93
115.33
113.61
110.98
56.48
40.61
35.85
11.81
f1 (ppm)

K_3913

20180813_K_03 642 (3.634) Cm (641:643)

1: MS2 ES+
1.15e8