Apoptosis inducing 1,3,4-oxadiazole conjugates of Capsaicin: Their *in vitro* anti-proliferative and *in silico* studies

Fatima Naaz,[¶] Faiz Ahmad,[†] Bilal Ahmad Lone,[†] Arif Khan,[¶]
Kalicharan Sharma,[#] Intzar Ali,[₮] M. ShaharYar,[#] Yuba Raj Pokharel,[†]* Syed Shafi.[¶]*

[¶]Department of Chemistry, School of Chemical and Life Science, Jamia Hamdard, New Delhi-110062, India;

†Faculty of Life Sciences and Biology, South Asian University, New Delhi-110021;

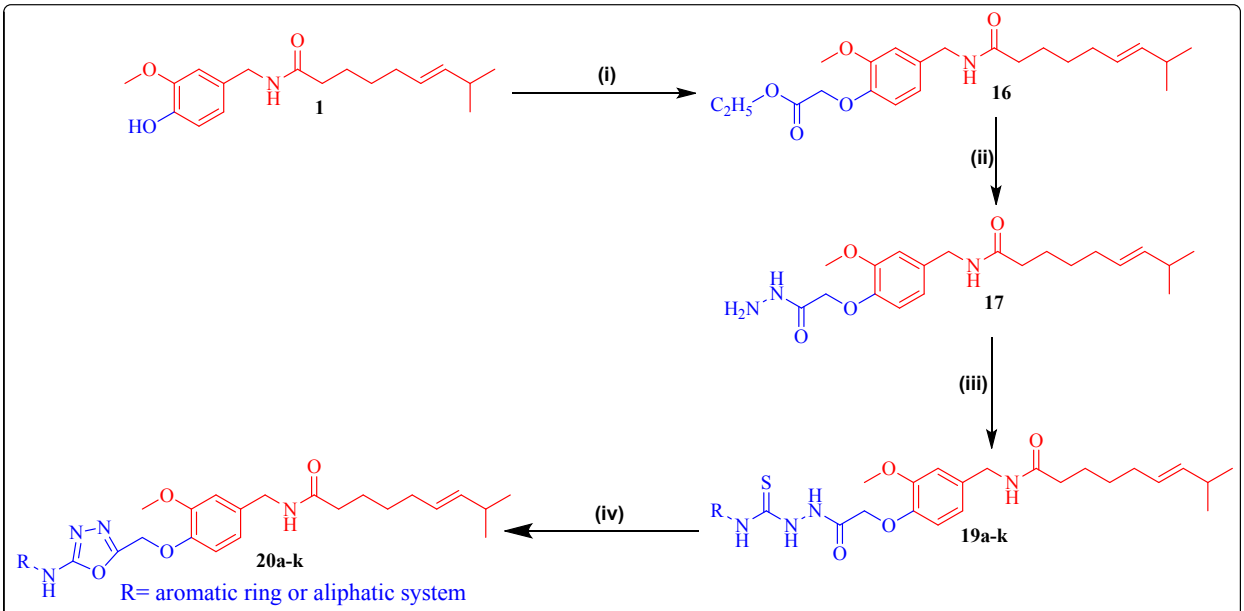
[#]Department of Pharmaceutical Chemistry, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi-110062, India;

[₮]Department of Medical Microbiology Hamdard Institute of Medical Studies and Research, Jamia Hamdard, New Delhi-110062, India.

Content

1. Chemistry

1.1. Scheme S-1. Synthesis of compound 20a-k



Reagents and Condition:

(i) $CH_3COOCH_2CH_2Br$,K_2CO_3, Acetone, reflux at K_2CO_3, Acetone reflux at 60-70 ^oC for 48 h yield: 99%; **(ii)** $NH_2NH_2.H_2O$, RT for 8 h yield: 98%; **(iii)** RNCS (**18a-k**), EtOH, Reflux for 6 h at 60-70 ^oC, yield: 95%; **(iv)** EDC.HCl, Cat.HOBt, Dry DMF, RT for 3-5 h, Yield 78-98%;

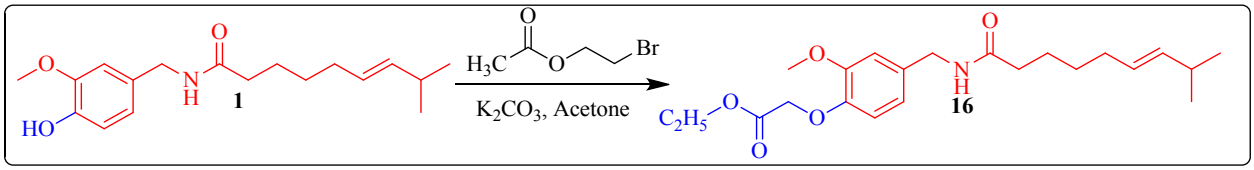
Scheme S-1

1.2. General information

All the chemicals and reagents used in the study were purchased from Merck (India), Spectrochem, and Sigma Aldrich which were of reagent grade. TLC was performed for indication of reaction completion on 0.25 mm silica gel 60-F254 plates. UV light was used to visualize spots. Melting points of all the synthesized compounds were measured using Buchi labortechnik AG 9230 automated melting point apparatus (Switzerland); IR spectra were recorded on Bruker ALPHA FT-IR spectrometer (Germany), ^1H and ^{13}C NMR spectra were determined on a Bruker (300 MHz and 75MHz) spectrometer and chemical shifts were expressed as ppm against TMS as internal reference. Solvent peak of $CDCl_3$ in ^1H NMR was observed at 7.26 ppm and in ^{13}C observed at 77.28-76.77 ppm. A residual DMSO-d_5 ^1H NMR signal is observed at 2.52 ppm. Mass spectra's were recorded on ESI-MS. CHNS elemental analysis of synthesized compounds has been done on Vario EL III Elementar Equipment All compounds prepared in this paper are novel and confirmed with spectral data. All the synthesized compounds were recrystallized in methanol and were purified by column chromatography.

1.3. Synthesis of compound 16-20a-k.

1.3.1. Synthesis of (*E*)-ethyl 2-(2-methoxy-4-((8-methylnon-6-enamido)methyl)phenoxy)acetate (16)

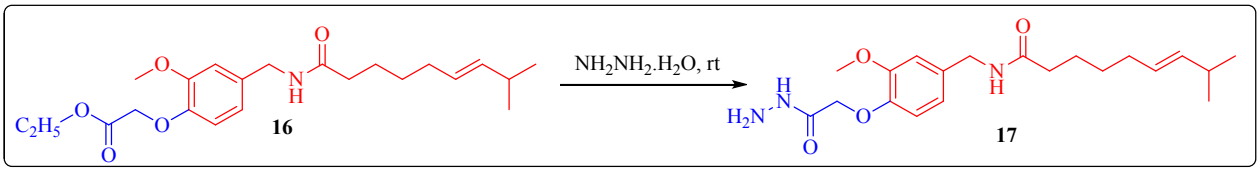


1g of capsaicin **1** was dissolved in 10 mL of dry acetone. Then 0.54 g of activated potassium carbonate was added to the reaction mixture and was allowed to stir for 10 min. after 10 min, 0.5 mL (1.2 equiv.) of bromoethyl acetate was added and refluxed at 60-70 ^{o}C for 48 h. Completion of reaction was monitored by doing TLC (4:6 ratio of ethylacetate:hexane) and anisaldehyde was used as spraying agent for detection of TLC. After completion, reaction mixture was poured into ice and white colour solid was produced which was further filtered and dried under vacuum.

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 8.24 (s, 1H), 6.87 (s, 1H), 6.79 (d, J = 8 Hz, 1H), 6.71 (s, J = 8.4 Hz, 1H), 5.40-5.29 (m, 2H), 4.71 (s, 1H), 4.18-4.10 (m, 4H), 3.75 (s, 3H), 2.11 (t, J = 7.2 Hz, 2H), 1.96-1.91 (m, 2H), 1.55-1.48 (m, 2H), 1.33-1.19 (m, 6H), 0.93 (d, J = 6.8 Hz, 4H), 0.85 (d, J = 6.4 Hz, 2H).

MS (ESI) *m/z*: $[M-1]^{+}$ 390.1.

1.3.2. Synthesis of (*E*)-N-(4-(2-hydrazinyl-2-oxoethoxy)-3-methoxybenzyl)-8-methylnon-6-enamide (17)

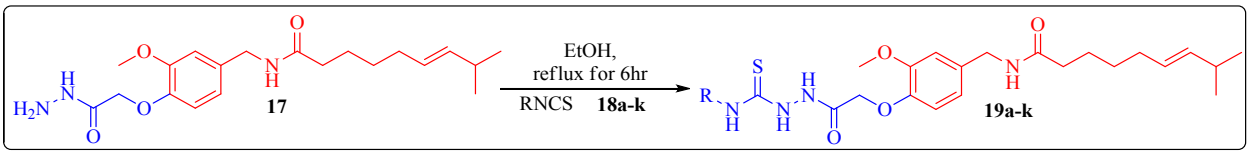


To 2 ml of hydrazine hydrate, 1.4g of compound **16** was added and reaction mixture was stirred for 8 h at room temperature. Completion of reaction was examined by TLC (6:4 ratio of ethylacetate:hexane). A white colour solid was produced within the reaction mixture which was further carried to filter off and dried under high vacuum pump.

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 9.14 (s, 1H), 8.42 (t, J = 5.6 Hz, 1H), 6.86 (d, J = 8.4 Hz, 2H), 6.72 (d, J = 8 Hz, 1H), 4.42 (s, 2H), 4.33 (s,2H), 4.18 (d, J = 6.0 Hz, 2H), 3.75 (s, 3H), 2.11 (t, J = 7.2 Hz, 2H), 1.53-1.48 (m, 3H), 1.23 (s, 6H), 0.84 (d, J = 6.8 Hz, 6H).

MS (ESI) m/z: $[M+3]^+$ 380.4.

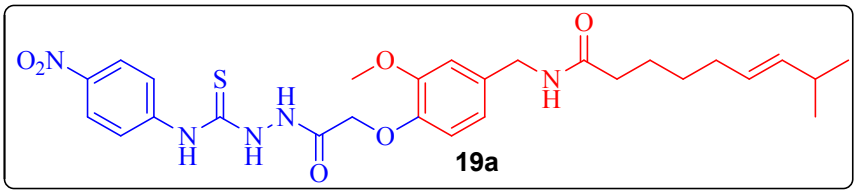
1.3.3. Synthesis of (*E*)-N-(3-methoxy-4-(2-oxo-2-(2-(phenylcarbamothioyl)hydrazinyl)ethoxy)benzyl)-8-methylnon-6-enamide (19a-k)



General procedure A

To ethanolic solution (absolute) of compound **17,** different substituted aryl and alkyl isothiocyanates (**18a-k)** were added to it then the reaction mixture was refluxed for 6 h at 60-70 ^{o}C. Solid precipitate was formed in the reaction mixture was filtered of and washed with cold alcohol to yield compounds (**19a-k**). TLC was run in TEF 4:5:1 which indicate the completion of the reaction.

1.3.3.1. Synthesis of (*E*)-N-(3-methoxy-4-(2-(2-((4-nitrophenyl)carbamothioyl)hydrazinyl)-2-oxoethoxy)benzyl)-8-methylnon-6-enamide (19a)

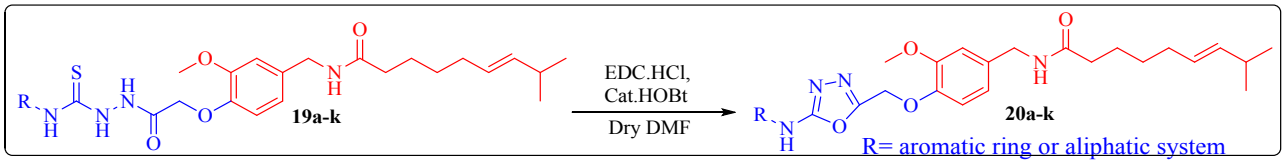


To 10 mL of absolute ethanol, 0.5 g of compound **17** was dissolved, followed by addition of 0.47 g (1.2 equivalents) of 1-isothiocyanato-4-nitrobenzene **18a**. Reaction mixture was refluxed for 6 h at 60-70 ^{o}C and after completion of reaction; solid precipitate was formed in reaction mixture. These Solid precipitate was filtered off and dried to afford compound **19a**.

^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm):11.66 (s, 1H), 10.24 (s, 1H), 10.09 (s , 1H), 9.93 (s, 1H), 8.23 (d, J = 8 Hz, 2H), 7.89 (d, J = 8 Hz, 2H), 6.89 (s, 2H), 6.74 (d, J = 8 Hz, 1H), 4.61 (s, 2H), 4.19 (d, J = 8 Hz, 2H), 3.71 (s, 3H), 2.11 (t, J = 8 Hz, 2H), 1.53-1.48 (m, 3H), 1.39-1.36 (m, 1H), 1.24 (s, 6H), 0.84 (d, J = 8 Hz, 6H).

MS (ESI) m/z: $[M+1]^+$ 558.5.

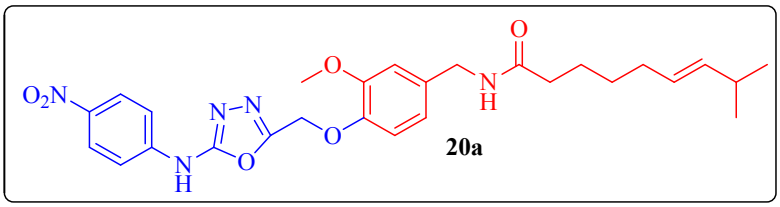
1.3.4. Synthesis of (*E*)-N-(3-methoxy-4-((5-(phenylamino)-1,3,4-oxadiazol-2-yl)methoxy)benzyl)-8-methylnon-6-enamide (20a-k)



General procedure B

To 5 mL of dry dimethylformamide (DMF), 0.5g of compound **19a-k** and 0.52 g (3 equivalents) of *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC.HCl) with catalytic amount of Hydroxybenzotriazole (HOBt) were added to it. Reaction mixture was stirred at room temperature for 3-5 h under inert atmosphere. After the completion of reaction monitored by TLC (TEF, 4:5:1), the reaction mixture was poured onto ice. The precipitate formed was filtered off, washed with excess of water, dried under high vacuum and recrystallized in ethanol to afford the title compound **20a-k** in pure form.

1.3.4.1. Synthesis of (*E*)-N-(3-methoxy-4-((5-((4-nitrophenyl)amino)-1,3,4-oxadiazol-2-yl)methoxy)benzyl)-8-methylnon-6-enamide (20a)



According to general procedure A, To 5 mL of dry dimethylformamide (DMF), 0.5g of compound **19a** and 0.52 g (3 equivalents) of *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC.HCl) with catalytic amount of Hydroxybenzotriazole (HOBt) were added to it. Reaction mixture was stirred at room temperature for 3-5 h under inert atmosphere. After the completion of reaction monitored by TLC (TEF, 4:5:1), the reaction mixture was poured onto ice. The precipitate formed was filtered off, washed with excess of water, dried under high vacuum and recrystallized in ethanol to afford the title compound **20a-k** in pure form.

Creamish yellow; yield: 91%.

Mp: 189.0 °C.

IR cm^{-1}: 3487, 3437, 3384, 3335, 3285, 3231, 3200, 3049, 2990, 2924, 1668, 1639, 1590, 1513, 1459, 1419, 1325, 1266, 1230, 1196, 1142, 1107, 1014, 955, 846, 770, 738, 679, 619, 546.

^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 10.97 (s, 1H), 8.21 (d, J = 8.4 Hz, 2H), 7.76 (d, J = 8.4 Hz, 2H), 7.09 (s, 1H), 6.99 (d, J = 8.1 Hz, 1H), 6.88 (s, 1H), 6.80 (d, J = 8.1 Hz, 1H), 5.22 (s,

2H), 4.34 (d, J = 5.1 Hz, 2H), 3.84 (s, 3H), 2.21 (t, J = 7.5 Hz, 2H), 1.64 (t, J = 6 Hz, 2H), 1.54-1.43 (m, 1H), 1.28 (s, 6H), 0.85 (d, J = 6.3 Hz, 6H).

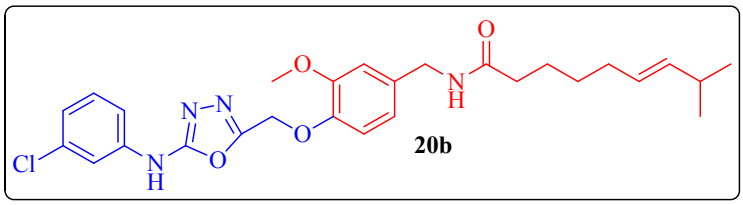
^{13}C NMR (75 MHz, DMSO-d_6) δ: 173.2, 160.2, 156.6, 149.9, 145.6, 144.4, 141.7, 134.4, 125.0, 124.4, 120.6, 119.6, 116.6, 115.7, 111.7, 61.3, 55.6, 42.6, 36.3, 29.4, 29.2, 27.7, 27.0, 25.7, 22.5, 14.0.

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{27}H_{34}N_5O_6$ 524.2509; Found 524.2513.

Anal. Calcd for $C_{27}H_{33}N_5O_6$: C, 61.94; H, 6.35; N, 13.38; O, 18.33%, Found: C, 61.62; H, 6.24; N, 13.28 %.

1.3.4.2. Synthesis of (*E*)-N-(4-((5-((3-chlorophenyl)amino)-1,3,4-oxadiazol-2-yl)methoxy)-3-methoxybenzyl)-8-methylnon-6-enamide (20b)

According to general procedure A, Compound **19b** was cyclized under coupling conditions with EDC.HCl and HOBt to yield desired compound **20b**.



20b

White solid, yield: 89%.

Mp: 158.5 °C.

IR cm^{-1}: 3425, 3387, 3274, 3228, 3120, 3093, 3056, 3006, 2955, 2922, 1649, 1596, 1570, 1544, 1511, 1475, 1420, 1262, 1223, 120, 1082, 1021, 907, 848, 793, 740, 700, 642, 580.

^{1}H NMR (300 MHz,$CDCl_3$) δ (ppm): 7.60-7.57 (m, 1H), 7.43-7.36 (m, 2H), 7.04 (d, J = 7.8 Hz, 1H), 6.96 (d, J = 8.4 Hz, 1H), 6.81 (s, 1H), 6.74 (d, J = 6.3 Hz, 1H), 5.78 (s, 1H), 5.20 (s, 2H), 4.36 (d, J = 5.7 Hz, 2H), 3.83 (s, 3H), 2.21 (t, J = 7.5 Hz, 2H), 1.76 (s, 2H), 1.28-1.22 (m, 7H), 0.85 (d, J = 6.6 Hz, 6H).

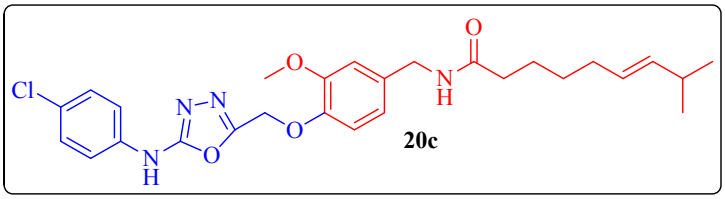
HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{27}H_{34}ClN_4O_4$ 513.2269; Found 513.2272.

Anal. Calcd for $C_{27}H_{33}ClN_4O_4$: C, 63.21; H, 6.48; Cl, 6.91; N, 10.92; O, 12.47%, Found: C, 63.17; H, 6.41; N, 10.83 %.

1.3.4.3. Synthesis of (*E*)-N-(4-((5-((4-chlorophenyl)amino)-1,3,4-oxadiazol-2-yl)methoxy)-3-methoxybenzyl)-8-methylnon-6-enamide (20c)

According to general procedure A, Compound **19c** was cyclized under coupling conditions with EDC.HCl and HOBt to yield desired compound **20c**.

White solid, yield: 98%.



20c

Mp: 175.9 °C.

IR cm^{-1}: 3457, 3419, 3387, 3323, 3223, 3235, 3197, 3120, 3024, 2995, 2926, 1651, 1621, 1572, 1515, 1462, 1416, 1317, 1267, 1224, 1139, 1018, 963, 843, 806, 754, 707, 671, 612.

^{1}H NMR (300 MHz,$CDCl_3$) δ (ppm): 8.31 (d, J = 7.5 Hz, 1H), 7.56 (d, J = 7.5 Hz, 1H), 7.47 (s, 1H), 7.40-7.35 (m, 1H), 7.02-6.94 (m, 1H), 6.86 (s, 1H), 6.79 (d, J = 7.5 Hz, 1H), 5.70 (s, 1H), 5.23 (s, 2H), 4.38 (d, J = 5.1 Hz, 2H), 3.86 (s, 3H), 2.20 (t, J = 6.6 Hz, 2H), 1.65 (s, 2H), 1.53-1.48 (m, 1H), 1.28 (s, 6H), 0.85 (d, J = 6.3 Hz, 6H).

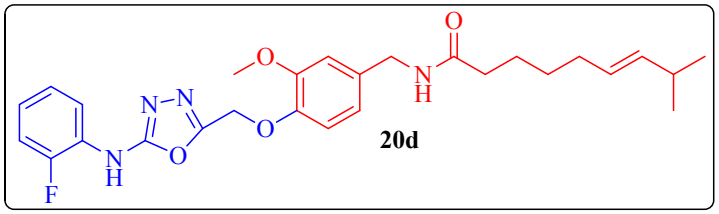
^{13}C NMR (75 MHz, $CDCl_3$) δ: 172.9, 156.8, 146.0, 135.0, 133.8, 132.4, 128.8, 124.1, 120.0, 118.6, 115.7, 111.9, 111.4, 61.3, 55.9, 43.3, 38.9, 36.8, 29.6, 29.3, 27.9, 27.2, 25.7, 22.6.

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{27}H_{34}ClN_4O_4$ 513.2269; Found 513.2270.

Anal. Calcd for $C_{27}H_{33}ClN_4O_4$: C, 63.21; H, 6.48; Cl, 6.91; N, 10.92; O, 12.47%, Found: C, 63.17; H, 6.31; N, 10.83%.

1.3.4.4. Synthesis of (*E*)-N-(4-((5-((2-fluorophenyl)amino)-1,3,4-oxadiazol-2-yl)methoxy)-3-methoxybenzyl)-8-methylnon-6-enamide (20d)

According to general procedure A, Compound **19d** was cyclized under coupling conditions with EDC.HCl and HOBt to yield desired compound **20d**.



20d

White solid, yield: 78%.

Mp: 141.4 °C.

IR cm^{-1}: 3473, 3432, 3375, 3321, 3260, 3201, 3122, 3048, 2922, 1651, 1639, 1581, 1557, 1518, 1460, 1426, 1389, 1271, 1221, 1134, 1029, 851, 814, 730, 690, 645, 618, 560.

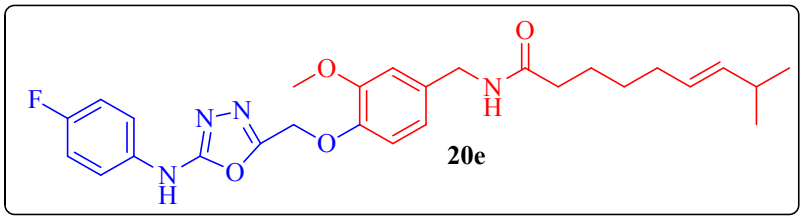
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.13 (t, J = 8.1 Hz, 1H), 7.25 (s, 1H), 7.13-7.05 (m, 2H), 7.02-6.90 (m, 2H), 6.77(s, 1H), 6.70 (d, J = 7.8 Hz, 1H), 5.67 (s, 1H), 5.13 (s, 2H), 4.29 (d, J = 5.7 Hz, 2H), 3.77 (s, 1H), 2.13 (t, J = 7.2 Hz, 2H), 1.91 (q, J = 6.6 Hz, 1H), 1.61-1.56 (m, 4H), 1.04-1.31 (m, 2H), 1.31-1.21 (m, 2H), 0.87 (d, J = 6.6 Hz, 3H), 0.78 (d, J = 6.6 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.3, 160.9, 159.7, 156.3, 150.0, 145.9, 133.7, 119.8, 119.4, 119.3, 116.0, 115.8, 115.6, 111.8, 61.25, 55.8, 43.2, 38.9, 36.8, 29.6, 29.3, 27.9, 27.2, 25.8, 22.6; HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{27}H_{34}FN_4O_4$ 497.2564; Found 497.2565.

Anal. Calcd for $C_{27}H_{33}FN_4O_4$: C, 65.31; H, 6.70; F, 3.83; N, 11.28; O, 12.89%, Found: C, 65.28; H, 6.63; N, 11.19%.

1.3.4.5. Synthesis of (*E*)-N-(4-((5-((4-fluorophenyl)amino)-1,3,4-oxadiazol-2-yl)methoxy)-3-methoxybenzyl)-8-methylnon-6-enamide (20e)

According to general procedure A, Compound **19e** was cyclized under coupling conditions with EDC.HCl and HOBt to yield desired compound **20e**.



20e

White solid; yield: 98%.

Mp: 154.3 °C.

IR cm^{-1}: 3472, 3438, 3385, 3322, 3263, 3205, 3123, 3050, 2928, 1649, 1626, 1579, 1549, 1511, 1455, 1420, 1388, 1266, 1218, 1137, 1020, 843, 808, 722, 683, 634, 610, 552.

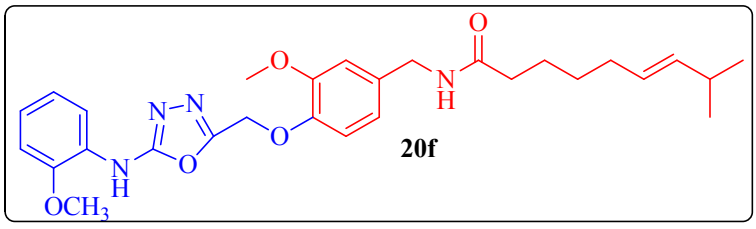
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.26 (s, 1H), 7.46 (q, J = 4.2 Hz, 2H), 7.02 (t, J = 8.4 Hz, 2H), 6.90 (d, J = 8.1 Hz, 1H), 6.78 (s, 1H), 6.70 (d, J = 8.4 Hz, 1H), 5.93 (s, 1H), 5.15 (s, 1H), 4.34 (d, J = 5.4 Hz, 1H), 3.78 (s, 3H), 2.21 (t, J = 7.2 Hz, 2H), 1.65 (t, J = 6.6 Hz, 2H), 1.53-1.41 (m, 1H), 1.26 (s, 1H), 0.84 (d, J = 6.6 Hz, 6H).

^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.3, 160.9, 159.7, 156.3, 150.0, 145.9, 133.7, 119.8, 119.4, 119.3, 116.0, 115.8, 115.6, 111.8, 61.25, 55.8, 43.2, 38.9, 36.8, 29.6, 29.3, 27.9, 27.2, 25.8, 22.6; HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{27}H_{34}FN_4O_4$ 497.2564; Found 497.2568.

Anal. Calcd for $C_{27}H_{33}FN_4O_4$: C, 65.31; H, 6.70; F, 3.83; N, 11.28; O, 12.89%, Found: C, 65.27; H, 6.64; N, 11.21%.

1.3.4.6. Synthesis of (*E*)-N-(3-methoxy-4-((5-((2-methoxyphenyl)amino)-1,3,4-oxadiazol-2-yl)methoxy)benzyl)-8-methylnon-6-enamide (20f)

According to general procedure A, Compound **19f** was cyclized under coupling conditions with EDC.HCl



20f

and HOBt to yield desired compound **20f**.

White solid; Yield 95%.

Mp: 104.7 °C.

IR cm^{-1}: 3491, 3413, 3390, 3353, 3307, 3223, 3194, 3108, 3054, 2998, 2923, 1629, 1577, 1520, 1460, 1421, 1391, 1250, 1217, 1137, 1018, 976, 918, 849, 805, 736, 670, 642, 581.

^1H NMR (300 MHz,$CDCl_3$) δ (ppm): 8.12 (d, J = 3.3 Hz, 1H), 7.55 (s, 1H), 7.01 (d, J = 6.9 Hz, 3H), 6.91-6.88 (m, 1H), 6.84 (s, 1H), 6.78 (d, J =7.8 Hz, 1H), 5.70 (s, 1H), 5.21 (s, 2H), 4.37 (d, J = 5.4 Hz, 2H), 3.87 (d, J = 16.8 Hz, 6H), 2.20 (t, J = 7.8 Hz, 2H), 1.67-1.62 (t, J = 6.3 Hz, 2H), 1.54-1.43 (m, 1H), 1.28 (s, 6H), 0.84 (d, J = 6.6 Hz, 6H).

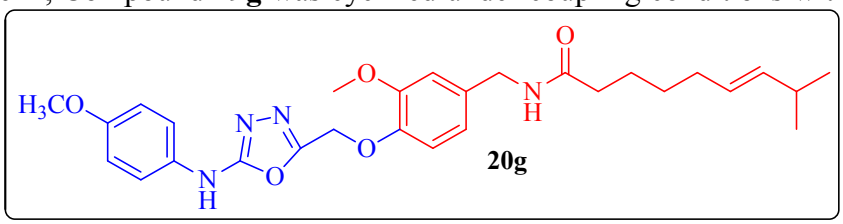
^{13}C NMR (75 MHz, $CDCl_3$) δ: 172.97, 160.5, 156.4, 150.2, 147.0, 146.1, 133.6, 126.9, 122.8, 121.3, 120.1, 117.1, 115.7, 111.9, 110.0, 61.4, 55.9, 55.8, 43.3, 38.9, 36.8, 29.6, 29.3, 27.9, 27.2, 25.8, 22.6.

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{28}H_{37}N_4O_5$ 509.2764; Found 509.2766.

Anal. Calcd for $C_{28}H_{36}N_4O_5$: C, 66.12; H, 7.13; N, 11.02; O, 15.73%, Found: C, 66.06; H, 7.08; N, 10.98%.

1.3.4.7. Synthesis of (*E*)-N-(3-methoxy-4-((5-((4-methoxyphenyl)amino)-1,3,4-oxadiazol-2-yl)methoxy)benzyl)-8-methylnon-6-enamide (20g)

According to general procedure A, Compound **19g** was cyclized under coupling conditions with EDC.HCl and HOBt to yield desired compound **20g**.



20g

White solid, yield: 94%.

Mp: 154.9 °C.

IR cm^{-1}: 3463, 3424, 3380, 3273, 3192, 3138, 3063, 3007, 2924, 1650, 1621, 1576, 1510, 1451, 1420, 1383, 1312, 1239, 1175, 1015, 965, 836, 691, 634, 581, 557, 535.

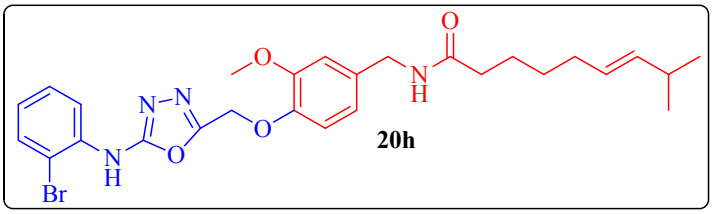
^1H NMR (300 MHz,$CDCl_3$) δ (ppm): 7.37 (d, J = 8.7 Hz, 2H), 7.05-6.98 (m, 2H), 6.88 (d, J = 9.0 Hz, 1H), 6.83 (s, 1H), 6.76 (d, J = 8.4 Hz, 1H), 5.71 (s, 1H), 5.18 (s, 2H), 4.37 (d, J = 5.7 Hz, 2H), 3.82 (d, J = 6 Hz, 6H), 2.20 (t, J = 7.2 Hz, 2H), 1.65-1.60 (m, 2H), 1.52-1.48 (m, 1H), 1.25 (s, 6H), 0.85 (d, J = 6.6 Hz, 6H).

MS (ESI) *m/z*: $[M+1]^+$ 509.

Anal. Calcd for $C_{28}H_{36}N_4O_5$: C, 66.12; H, 7.13; N, 11.02; O, 15.73%, Found: C, 66.07; H, 7.06; N, 10.99%.

1.3.4.8. Synthesis of (*E*)-N-(4-((5-((2-bromophenyl)amino)-1,3,4-oxadiazol-2-yl)methoxy)-3-methoxybenzyl)-8-methylnon-6-enamide (20h)

According to general procedure A, Compound **19h** was cyclized under coupling conditions with EDC.HCl and HOBt to yield desired compound **20h**.



20h

White solid, yield: 83%.

Mp: 145.5 °C.

IR cm^{-1}: 3450, 3410, 3371, 3320, 3239, 3194, 3133, 3089, 3055, 2918, 1628, 1592, 1514, 1451, 1384, 1263, 1222, 1141, 1025, 846, 808, 749, 683, 593, 565.

^{1}H NMR (300 MHz,$CDCl_3$) δ (ppm): 7.71 (d, J = 1.2 Hz, 1H), 7.22-7.19 (m, 3H), 6.94 (d, J = 7.8 Hz, 1H), 6.81 (s, 1H), 6.73 (d, J = 8.1 Hz, 1H), 5.82 (s, 1H), 5.19 (s, 2H), 4.36 (d, J = 5.4 Hz, 2H), 3.82 (s, 3H), 2.20 (t, J = 7.2 Hz, 2H), 1.43 (t, J = 6.9 Hz, 2H), 1.28-1.25 (m, 7H), 0.85 (d, J = 6.3 Hz, 6H).

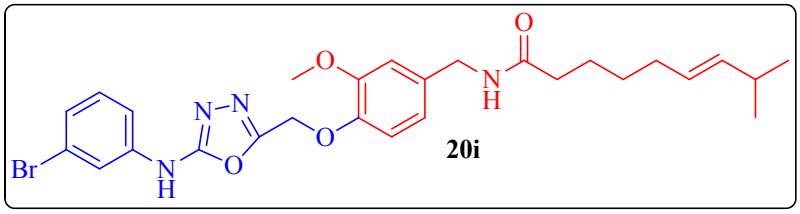
^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.3, 160.3, 156.6, 150.1, 145.9, 138.7, 133.7, 130.6, 128.3, 126.1, 123.0, 122.5, 120.4, 119.8, 116.1, 115.7, 111.8, 61.2, 55.8, 43.2, 38.9, 36.8, 29.6, 27.9, 27.2, 25.8, 22.6, 14.0.

HRMS (ESI) m/z: $[M]^+$ Calcd for $C_{27}H_{33}BrN_4O_4$ 556.1685; Found 556.1688.

Anal. Calcd for $C_{27}H_{33}BrN_4O_4$: C, 58.17; H, 5.97; Br, 14.33, N, 10.05; O, 11.48 %, Found: C, 58.07; H, 5.90; N, 10.01%.

1.3.4.9. Synthesis of (*E*)-N-(4-((5-((3-bromophenyl)amino)-1,3,4-oxadiazol-2-yl)methoxy)-3-methoxybenzyl)-8-methylnon-6-enamide (20i)

According to general procedure A, Compound **19i** was cyclized under coupling conditions with EDC.HCl and HOBt to yield desired compound **20i**.



20i

White solid, Yield 93%.

Mp: 145.7 °C.

IR cm^{-1}: 3482, 3453, 3416, 3379, 3332, 3275, 3275, 3237, 3169, 3072, 3039, 2925, 1644, 1621, 1571, 1543, 1471,1418, 1387, 1333, 1266, 1221, 1137, 1096, 1026, 849, 805, 772, 716, 657, 615, 555.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.71 (s,1H), 7.44-7.42 (m, 1H), 7.20 (d, J = 4.5 Hz, 2H), 6.94 (d, J = 8.1 Hz, 1H), 6.80 (s, 1H), 6.72(d, J = 9.0 Hz, 1H), 5.85 (s, 1H), 5.19 (s, 2H), 4.36 (d, J = 5.1 Hz, 2H), 3.82 (s, 3H), 2.21 (t, J = 7.5 Hz, 2H), 1.67-1.60 (m, 2H), 1.52-1.43 (m, 1H), 1.25 (s, 6H), 0.85 (d, J = 6.3 Hz, 6H).

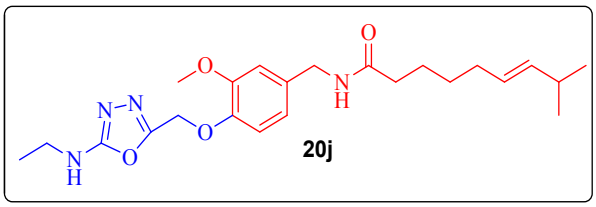
^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.3, 160.3, 156.6, 150.1, 145.9, 138.7, 133.7, 130.6, 128.3, 126.1, 123.0, 122.5, 120.4, 119.8, 116.1, 115.7, 111.8, 61.2, 55.8, 43.2, 38.9, 36.8, 29.6, 27.9, 27.2, 25.8, 22.6, 14.0.

MS (ESI) *m/z*: $[M]^+$ 557.3, $[M+1]^+$ 558, $[M+2]^+$ 559.1.

Anal. Calcd for $C_{27}H_{33}BrN_4O_4$: C, 58.17; H, 5.97; Br, 14.33, N, 10.05; O, 11.48 %, Found: C, 58.11; H, 5.94; N, 9.98%.

1.3.4.10. Synthesis of (*E*)-N-(4-((5-(ethylamino)-1,3,4-oxadiazol-2-yl)methoxy)-3-methoxybenzyl)-8-methylnon-6-enamide (20j)

According to general procedure A, Compound **19j** was cyclized under coupling conditions with EDC.HCl and HOBt to yield desired compound **20j**.



White solid, yield: 87%.

Mp: 106 °C.

IR cm^{-1}: 3483, 3458, 3413, 3379, 3343, 3307, 3259, 3212, 3143, 3107, 2924, 1631, 1557, 1516, 1462, 201, 1260, 1221, 1145, 1090, 1026, 855, 798, 721, 689, 649, 554.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.94 (d, J = 7.8 Hz, 1H), 6.85 (s, 1H), 6.78 (d, J = 7.2 Hz, 1H), 5.80 (s, 1H), 5.33 (s, 1H), 4.38 (d, J = 5.4 Hz, 2H), 4.27-4.20 (m, 2H), 3.83 (s, 3H), 2.20 (t, J = 7.2 Hz, 2H), 1.65 (t, J = 5.4 Hz, 2H), 1.54-1.48 (m, 1H), 1.42 (t, J =7.2 Hz, 3H), 1.27 (s, 6H), 0.85 (d, J = 6.6 Hz, 6H).

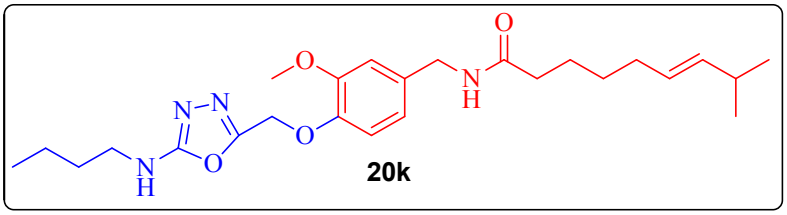
^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.1, 168.2, 150.3, 148.0, 145.6, 134.0, 120.0, 115.9, 111.9, 62.0, 55.7, 43.3, 39.9, 38.9, 36.8, 29.6, 29.3, 27.9, 27.2, 25.8, 22.6, 13.5.

MS (ESI) *m/z*: $[M+1]^+$431.2.

Anal. Calcd for $C_{23}H_{34}N_4O_4$: C, 64.16; H, 7.96; N, 13.01; O, 14.86 %, Found: C, 64.08; H, 7.91, N, 12.99%.

1.3.4.11. Synthesis of (*E*)-N-(4-((5-(butylamino)-1,3,4-oxadiazol-2-yl)methoxy)-3-methoxybenzyl)-8-methylnon-6-enamide (20k)

According to general procedure A, Compound **19k** was cyclized under coupling conditions with EDC.HCl and HOBt to yield desired compound **20k**.



Light grey; solid, yield: 91%.

Mp: 145.6 °C.

IR cm^{-1}: 3449, 3341, 3281, 3161, 3108, 3077, 3003, 2921 2848, 1636, 1558, 1502, 1460, 1355, 1262, 1213, 1147, 1113, 1029, 997, 860, 791, 747, 656, 599, 546.

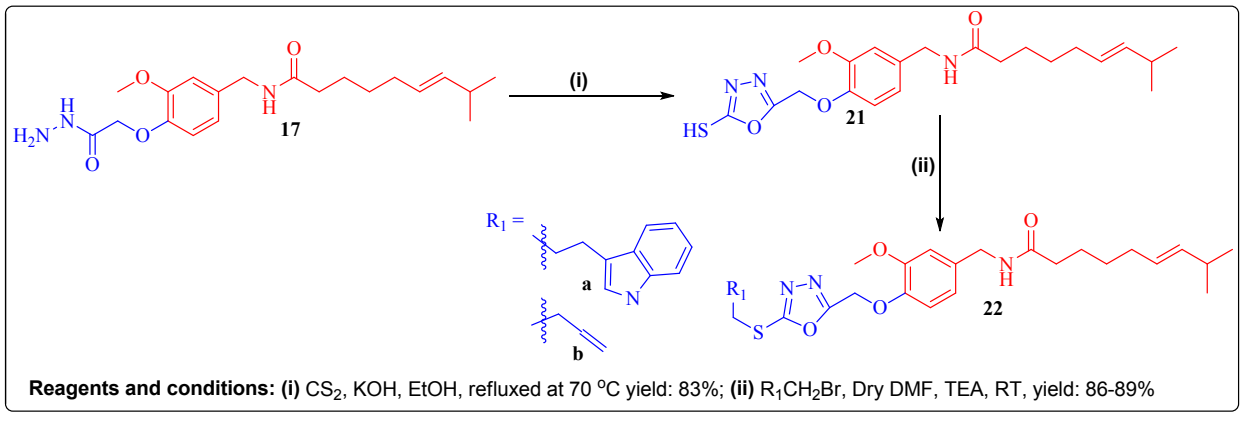
^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.93 (d, J = 8.1 Hz, 1H), 6.85 (s, 1H), 6.77 (d, J = 7.2 Hz, 1H), 5.81 (s, 1H), 5.09 (s, 2H), 4.38 (d, J = 5.1 Hz, 2H), 4.15 (t, J = 7.5 Hz, 2H), 3.83 (s, 3H), 2.22 (t, J = 7.2 Hz, 2H), 1.82 (t, J = 6.3 Hz, 2H), 1.65 (t, J = 5.7 Hz, 2H), 1.54-1.37 (m, 3H), 1.28 (s, 6H), 0.85 (d, J = 6.6 Hz).

^{13}C NMR (75 MHz, $CDCl_3$) δ: 173.1, 168.4, 150.3, 148.1, 145.6, 134.0, 120.0, 116.0, 111.9, 62.0, 55.7, 44.5, 43.3, 39.9, 38.9, 36.8, 30.2, 29.6, 29.3, 27.9, 27.2, 25.8, 22.6, 20.0, 13.6.

MS (ESI) *m/z*: $[M+3]^+$ 461.1.

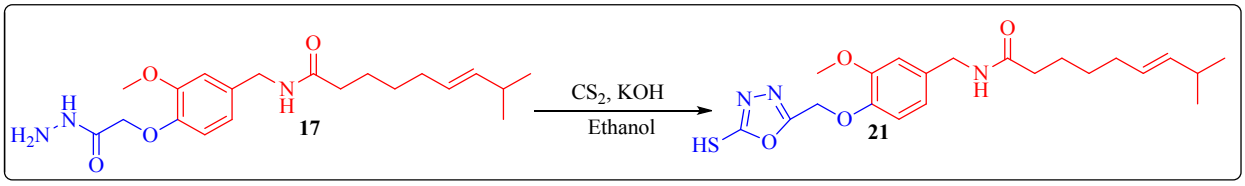
Anal. Calcd for $C_{23}H_{34}N_4O_4$: C, 65.48; H, 8.35; N, 12.22; O, 13.96 %, Found: C, 65.41; H, 8.29; N, 12.18%.

1.4. Scheme S-2. Synthesis of compound 21, 22a-b.



Scheme S-2

1.4.1. Synthesis of (*E*)-N-(4-((5-mercapto-1,3,4-oxadiazol-2-yl)methoxy)-3-methoxybenzyl)-8-methylnon-6-enamide (21)



To ethanolic solution of carbon disulphide (0.21g), 0.16g of potassium hydroxide was added and the reaction mixture was stirred at room temperature for 30 min. After 30 min, 0.5g of capsaicin hydrazide **17** was added to the reaction mixture and was refluxed at 70 ^{o}C. The completion of reaction was monitored by TLC (9:1 ratio of ethyl acetate:methanol). Reaction mixture was concentrated under rota-evaporator and the concentrated reaction mixture was poured into ice. White solid precipitates were formed and filtered under vacuum pump and dried.

White solid, yield: 83%.

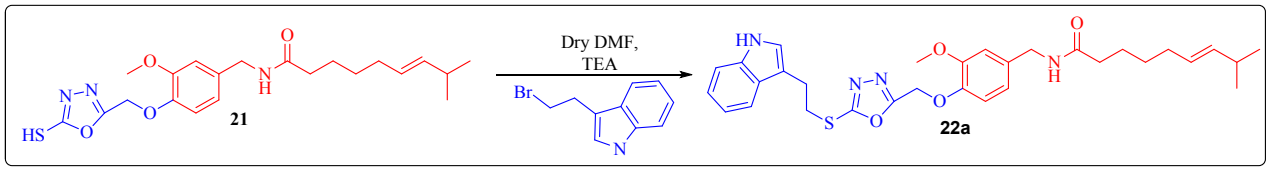
Mp: 160.3 °C.

IR cm^{-1}: 3459, 3395, 3311, 3281, 3224, 3189, 3093, 2955, 2927, 1681, 1638, 1514, 1481, 1377, 1265, 1232, 1143, 1025, 911, 800, 707, 682, 637, 602, 540.

^{1}H NMR (300 MHz,$CDCl_3$) δ (ppm): 9.51 (s, 1H), 6.89-6.76 (m, 3H), 5.60 (s, 1H), 5.33-5.18 (m, 2H), 4.60 (s, 2H), 4.31 (s, 2H), 3.82 (s, 3H), 2.14 (t, J = 6.9 Hz, 2H), 1.93-1.88 (m, 1H), 1.59-1.47 (m, 6H), 0.87 (d, J = 6.3 Hz, 3H), 0.63 (d, J = 6 Hz, 3H).

MS (ESI) *m/z*: [M-1]$^{+}$ 418.4.

1.4.2. Synthesis of (*E*)-N-(4-((5-((2-(1H-indole-3-yl)ethyl)thio)-1,3,4-oxadiazol-2-yl)methoxy)-3-methoxybenzyl)-8-methylnon-6-enamide (22a)



0.2 g of compound **21** was added to dry DMF, 0.12 g of 3-(2-bromoethyl)indole and 9.4 µL of triethylamine were added to the reaction mixture and was stirred at room temperature until the reaction get complete. Completion of reaction was monitored by TLC (7:3 ratio of ethyl

acetate:hexane). Reaction mixture was poured into ice. A white solid precipitates were formed and filtered and dried under vacuum.

White solid, yield: 86%.

Mp: 109.4 °C.

IR cm^{-1}: 3470, 3431, 3385, 3323, 3171, 3122, 3086, 3057, 2932, 1643, 1544, 1513, 1456, 1420, 1318, 1252, 1216, 1143, 1026, 964, 923, 843, 808, 765, 736, 656, 600, 558.

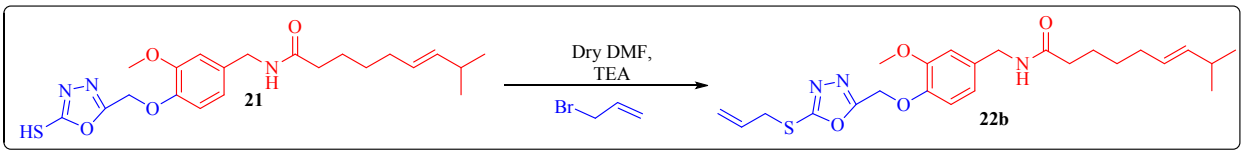
^1H NMR (300 MHz,$CDCl_3$) δ (ppm): 8.27 (s, 1H), 7.63 (d, J = 7.8 Hz, 1H), 7.35 (d, J = 7.8 Hz, 1H), 7.21-7.12 (m, 2H), 7.02 (s, 1H), 6.95 (d, J = 8.1 Hz, 1H), 6.83 (s, 1H), 6.76 (d, J = 8.4 Hz, 1H), 5.72 (s, 1H), 5.41-5.18 (m, 3H), 4.36 (d, J = 5.7 Hz, 2H), 3.82 (s, 3H), 3.54 (t, J = 6.9 Hz, 2H), 3.26 (t, J = 7.2 Hz, 2H), 2.20 (t, J = 7.2 Hz, 2H), 2.01-1.95 (m, 1H), 1.70-1.60 (m, 3H), 1.43-1.25 (m, 4H), 0.94 (d, J = 6.6 Hz, 4H), 0.85 (d, J = 6.6 Hz, 2H).

^{13}C NMR (75 MHz, $CDCl_3$) δ: 172.9, 166.1, 163.3, 150.2, 146.0, 138.1, 136.2, 133.8, 126.9, 126.4, 122.1, 119.9, 119.5, 118.6, 115.9, 113.1, 111.8, 111.3, 61.3, 55.8, 43.2, 38.9, 36.6, 33.2, 32.2, 30.9, 29.6, 29.2, 27.9, 27.2, 25.5, 25.2, 22.6.

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{31}H_{39}N_4O_4S$ 563.2692; Found 563.2692.

Anal. Calcd for $C_{31}H_{38}N_4O_4S$: C, 66.17; H, 6.81; N, 9.96; O, 11.37; S, 5.70 %, Found: C, 66.10; H, 6.76; N, 9.91; S, 5.67%.

1.4.3. Synthesis of (*E*)-N-(4-((5-(allylthio)-1,3,4-oxadiazol-2-yl)methoxy)-3-methoxybenzyl)-8-methylnon-6-enamide (22b)



0.2 g of compound **21** was added to dry DMF, 0.07 g of allylbromide and 9.4 µL of triethylamine was added to the reaction mixture and was stirred at room temperature until the reaction get complete. Completion of reaction was monitored by TLC (7:3 ratio of ethyl acetate:hexane). Reaction mixture was poured into ice. A white solid precipitates were formed and filtered and dried under vacuum.

White solid, yield: 89%.

Mp: 84.7 °C.

IR cm^{-1}: 3478, 3407, 3364, 3292, 3124, 3085, 3005, 2960, 2925, 1633, 1519, 1469, 1419, 1384, 1269, 1221, 1169, 1140, 1026, 979, 918, 849, 796, 730, 700, 667, 632, 585, 532.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 6.97 (d, J = 8.1 Hz, 1H), 6.84 (s, 1H), 6.77 (d, J = 8.1 Hz, 1H), 6.03-5.90 (m, 1H), 5.67 (s, 1H), 5.41-5.31 (m, 2H), 5.24 (s, 2H), 4.37 (d, J = 5.7 Hz, 2H), 3.85 (m, 4H), 2.21 (t, J = 7.5 Hz, 2H), 1.99 (q, J = 7.2 Hz, 1H), 1.71-1.59 (m, 4H), 1.28-1.25 (m, 5H), 0.95 (d, J = 6.9 Hz, 3H), 0.85 (d, J = 6.6 Hz, 3H).

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{24}H_{35}N_3O_4S$ 460.2270; Found 460.2279.

Anal. Calcd for $C_{24}H_{33}N_3O_4S$: C, 62.72; H, 7.24; N, 9.14; O, 13.92; S, 6.98 %, Found: C, 62.69; H, 7.19; N, 9.09; S, 6.94%.

2. Biology

2.1. *In vitro* anti-proliferative assay

2.1.1. *In vitro* anti-proliferative activity at single dose

All the synthesized compounds were screened for their anti-proliferative activity against a panel of 60 cancer cell line at National Cancer Institute, Bethesda, MD, USA as per the standard procedure given at http://www.dtp.nci.nih.gov[1]. RPMI 1640 medium (5% fetal bovine serum and 2 mM L-glutamine) was used to grow the human tumor cell lines. All the tumor cells were incubated into 96-well microtiter plate. Then this plated was placed for incubation at 37 ^oC for 24 hour. After that two plates of each cell line were fixed with TCA *in situ* and optical density was measured at this point which represented the cell population of each cell line at the time of compound addition (ODtzero). On the other hand, all the tested compounds were dissolved in DMSO to yield 400-fold desired final concentration and stored at -80^oC. These frozen compounds were thawed and their aliquot part was diluted to 10^{-4} M concentration with the medium containing 50 µg/mL of gentamicin at the time of compound addition. Control sample was made with DMSO only. 100 µl of the tested compounds from the aliquot parts were added to appropriate 96-well microtiter plate containing 100 µl of medium ensuing in the required final drug concentrations of 10^{-5} M and 0 M (control). After addition of tested compounds, 96-well microtiter plate was incubated for 48 h at 100%, 5% CO_2, 95% air, 100% relative humidity. Cold TCA was used to stop the assay for adherent cells. Further on 50 mL of 50% (w/v) TCA was used to fix the cell and incubated for 1 h at 4 °C. The supernatant was removed, and the 96-well microtiter plates were rinsed five times with water and air dried. 100 mL solution of protein binding dye, Sulforhodamine B (SRB) was made at 0.4% (w/v) in 1% acetic acid and was added to each well of the plates. These Plates were placed at room temperature for incubation for 10

minutes then were washed with 1% acetic acid five times to remove unbound dye. Then the plates were treated with 10 Mm trizma base, so that unbound dye was solubilized with trizma base. The absorbance was measured at a wavelength of 515 nm on an automated plate reader and results for each tested compounds were calculated as the percent of tumor growth of the treated cells in comparison with the untreated control cells. Optical density (OD) was recored for SRB-derived color just before exposing the cells to the test compound (ODtzero) and after 48 h exposure to the test compound (OD test) or the control vehicle (OD ctrl).

Table S1: Growth percentage against NCI panel of 60 human cancer cell lines at 10 μM of all the synthesized compounds

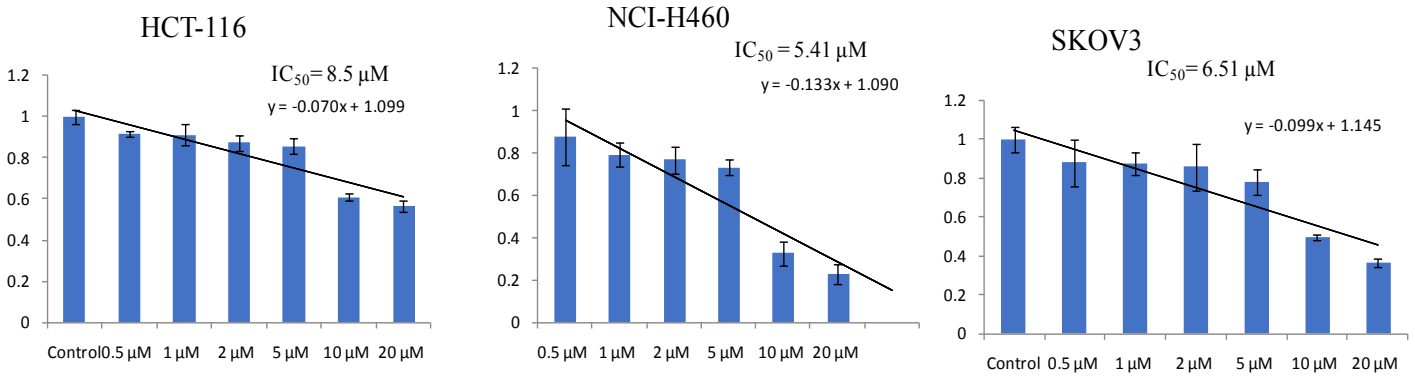
Sub panel cancer cell line	Growth percentage													
	20a	**20b**	**20c**	**20d**	**20e**	**20f**	**20g**	**20h**	**20i**	**20j**	**20k**	**21**	**22a**	**22b**
Leukemia														
CCRF-CEM	78.85	83.38	91.92	82.95	92.76	91.99	nt	97.26	81.76	97.54	91.34	99.62	**34.67**	90.64
HL-60(TB)	101.03	89.41	89.08	89.70	99.69	81.09	98.57	89.55	79.77	98.36	86.64	103.37	**50.23**	98.26
K-562	59.62	70.62	87.28	60.36	70.24	67.95	84.49	81.01	65.02	91.59	85.73	93.12	**40.71**	70.34
MOLT-4	84.22	88.02	89.91	82.05	103.71	76.74	98.19	86.25	83.54	103.67	86.36	Nt	**29.16**	83.83
RPMI-8226	52.34	74.91	84.57	64.91	84.21	70.71	93.01	88.54	70.31	95.52	76.77	99.31	Nt	Nt
SR	64.20	74.53	84.76	67.28	89.46	76.06	95.22	80.43	67.67	89.02	71.54	95.22	**49.30**	80.53
Non-small cell lung cancer														
A549/ATCC	**49.02**	87.33	102.17	105.30	97.53	99.28	99.76	100.52	95.38	97.00	100.32	101.17	79.42	98.75
EKVX	74.70	88.18	101.13	77.14	93.21	85.03	91.18	90.94	78.09	96.74	98.76	96.09	57.36	94.12
HOP-62	**0**	90.21	88.26	56.40	87.34	79.11	106.83	99.73	75.58	98.27	78.66	110.73	92.77	82.37
NCI-H226	62.14	66.28	85.01	64.53	66.49	79.74	85.73	83.50	**50.41**	101.46	84.32	100.61	67.25	91.66
NCI-H23	62.14	77.86	90.57	63.80	82.89	92.25	92.09	87.06	66.01	93.67	93.14	100.87	66.57	96.56
NCI-H322M	54.87	101.74	82.91	86.07	83.98	97.37	85.45	94.14	98.04	107.20	90.56	104.13	88.69	105.55
NCI-H460	**33.48**	97.70	103.15	80.34	97.70	104.67	105.96	93.22	97.55	102.79	102.28	106.50	89.80	105.48
NCI-H522	72.97	73.96	85.69	60.29	78.59	80.95	84.61	76.81	68.60	83.70	83.69	99.00	59.35	89.50
Colon cancer														
COLO 205	70.91	113.34	105.02	104.24	98.70	125.25	123.61	113.16	109.93	110.12	112.79	103.84	71.22	110.75
HCC-2998	83.57	100.28	102.82	90.82	94.58	105.27	103.51	99.78	100.83	102.90	100.87	107.49	97.97	112.22
HCT-116	**24.55**	78.21	95.48	53.45	84.11	86.76	98.15	64.86	67.59	87.16	95.61	102.79	69.99	98.01
HCT-15	81.17	95.64	103.31	90.10	92.38	88.33	94.13	98.87	90.22	96.85	96.64	97.59	68.56	95.32
HT29	71.71	98.66	99.81	89.95	93.76	102.87	109.88	97.63	97.66	104.84	109.45	113.25	69.68	109.65
KM12	71.65	95.09	98.42	78.80	96.75	95.47	92.43	82.88	90.31	98.25	100.69	101.75	73.28	103.88
SW-620	67.32	105.60	103.77	98.94	97.91	105.15	103.10	94.64	95.44	109.92	101.45	100.81	84.70	96.35
CNS cancer														
SF-268	79.65	88.42	98.18	**45.48**	87.38	90.77	89.30	90.35	92.63	97.15	102.42	100.57	78.89	97.08
SF-295	64.90	89.63	98.07	**48.03**	88.34	88.79	89.97	93.76	84.25	97.13	102.61	100.13	68.78	101.54
SF-539	70.24	103.25	94.45	**48.16**	86.63	93.38	98.35	90.97	88.44	103.92	99.53	101.54	80.83	101.59
SNB-19	54.05	99.28	96.48	58.55	86.86	94.57	96.21	90.61	99.55	103.83	94.52	97.90	77.05	95.53
SNB-75	**48.71**	80.51	81.58	57.78	68.43	94.51	80.03	95.25	83.48	86.23	88.42	83.81	83.14	83.94
U251	**48.58**	93.59	103.48	83.96	99.62	101.74	101.89	88.97	101.81	98.29	101.12	102.68	80.52	100.05
Melanoma														
LOX IMVI	55.24	92.37	92.05	83.41	90.96	95.63	92.06	94.00	88.92	93.19	92.01	100.41	55.24	95.91
M14	69.64	98.83	98.52	99.73	90.34	95.41	98.88	101.43	93.30	101.59	94.96	92.91	Nt	Nt
MDA-MB-435	66.39	104.85	103.53	100.01	99.57	103.20	102.02	105.39	101.19	104.46	100.39	100.51	90.22	98.38
SK-MEL-2	71.89	103.66	106.15	111.03	94.60	101.56	104.40	101.46	104.61	103.70	104.03	108.93	86.68	94.98
SK-MEL-28	78.87	98.50	94.43	85.18	90.32	98.06	99.09	99.16	107.28	103.96	97.23	110.77	104.42	106.07
SK-MEL-5	**46.26**	97.12	99.88	86.57	96.15	98.00	101.19	100.83	89.84	102.38	99.66	Nt	105.37	108.83
UACC-257	77.56	98.76	105.06	98.18	99.66	96.70	102.09	106.47	105.03	105.33	99.62	105.61	100.67	103.40
UACC-62	**39.61**	76.93	76.09	65.48	73.92	81.95	78.65	83.60	67.54	94.98	84.54	97.76	67.51	81.27
Ovarian cancer														
IGROV1	53.49	99.02	89.70	58.44	88.25	97.77	86.79	91.27	92.89	102.86	103.25	97.96	73.71	95.03
OVCAR-3	77.31	97.45	103.80	68.29	94.73	89.64	97.64	104.92	87.99	105.82	98.26	110.88	88.32	106.81
OVCAR-4	**11.25**	85.83	96.58	**0.69**	84.23	101.24	95.28	97.29	85.10	103.05	94.12	99.42	71.67	99.46
OVCAR-5	100.97	93.32	94.10	77.67	90.36	89.67	94.23	92.26	91.08	103.14	93.11	105.20	101.99	102.84
OVCAR-8	**24.96**	99.66	100.55	**45.17**	100.44	100.61	97.55	98.66	98.03	105.88	99.16	101.33	71.26	96.48
NCI/ADR-RES	79.62	96.09	100.02	**43.35**	93.66	89.81	95.71	90.56	87.04	102.01	94.80	103.36	56.51	90.53
SK-OV-3	**11.82**	88.19	78.61	**48.61**	85.35	107.43	86.46	97.96	82.58	91.51	94.35	103.46	85.74	107.07
Renal cancer														
786-0	**22.25**	89.17	97.65	**11.98**	85.60	96.22	94.33	95.33	95.60	95.46	103.39	104.31	89.26	98.36
A498	56.81	77.11	114.46	92.53	97.63	102.89	111.00	101.93	77.79	108.94	110.55	105.56	73.28	93.70
ACHN	**41.17**	92.42	91.36	56.05	88.30	95.65	90.34	95.45	85.14	101.38	100.13	100.49	76.55	97.61
CAKI-1	**33.60**	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt	Nt
RXF 393	54.11	109.40	119.47	68.76	105.35	102.60	101.82	96.06	70.61	120.40	117.01	85.08	54.00	Nt
SN 12C	**49.04**	102.91	89.04	70.47	86.50	105.33	97.41	97.94	95.72	104.29	95.08	97.66	83.06	95.06
TK-10	84.10	102.60	146.30	97.67	100.39	103.22	129.89	118.10	104.24	106.57	111.63	106.13	91.69	107.78
UO-31	58.74	77.39	68.08	52.79	68.66	75.24	64.27	74.38	64.01	84.23	79.13	76.93	**45.03**	77.23
Prostate cancer														
PC-3	**37.64**	93.71	93.53	72.50	92.51	84.37	103.14	103.24	87.90	103.73	96.62	98.96	56.85	93.63
DU-145	62.03	101.73	103.12	88.70	98.76	98.97	100.78	101.15	89.79	109.00	103.03	105.86	94.93	103.35
Breast cancer														
MCF7	55.23	93.28	95.59	66.88	90.88	87.12	88.37	82.71	83.70	95.22	90.76	95.31	58.79	98.26
MDA-MB-231/ATCC	67.10	88.44	84.33	63.87	81.41	99.24	81.96	92.54	78.30	103.58	92.62	102.36	70.64	97.59
HS 578T	63.17	92.30	100.61	83.68	91.01	98.03	93.97	96.68	93.67	98.58	95.40	94.96	81.05	89.11
BT-549	108.01	95.78	96.92	76.95	80.99	85.59	88.24	100.93	76.43	102.29	93.67	113.07	75.03	103.85
T-47D	**48.58**	66.28	79.31	65.02	69.56	76.11	92.81	85.60	58.86	81.04	88.82	97.17	**42.98**	93.68
MDA-MB-468	53.75	78.84	105.40	58.92	88.47	99.71	105.45	96.37	48.07	104.24	87.42	111.40	**51.81**	98.31
Mean GP	59.69	91.19	95.99	71.72	89.51	93.21	96.03	93.94	85.48	99.24	95.63	101.05	72.88	96.78

nt = not tested; GP = Growth Percentage

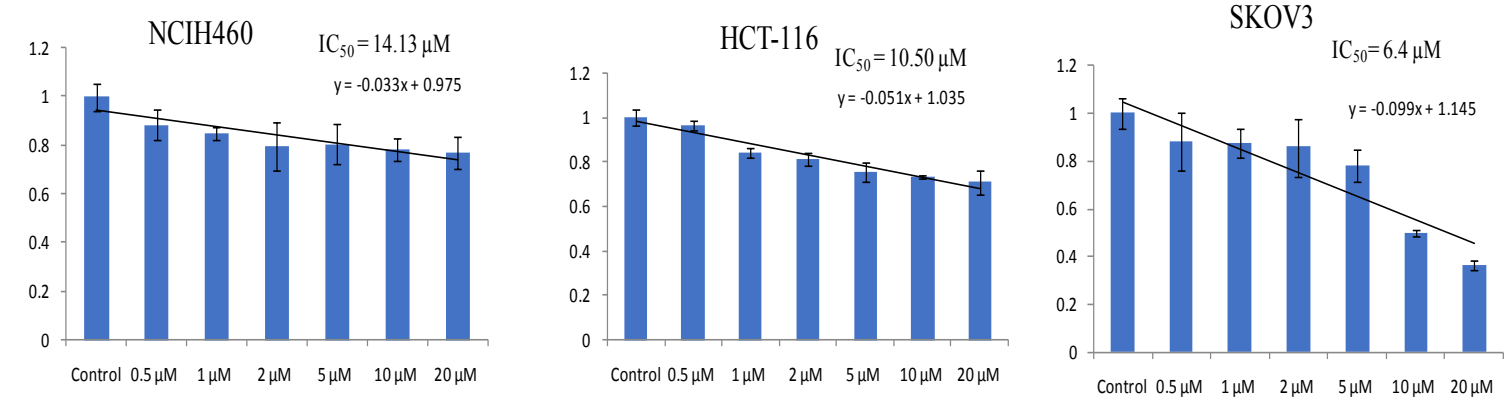
2.1.2. Crystal violet assay

The cell viability of compound **20a** against HCT-116 cancer cell line, NCI-H460 cancer cells, SKOV3 cancer cell line and normal PNT2 cells (normal prostatic epithelial cells) was measured by crystal violet assay. Appox. $4x10^3$ were seeded in each well of 96 well plates. After 24 h, the cells were treated with different concentrations of compound **20a** and further incubated for 72 h. Following this, the media was removed and cells were stained with 0.4% crystal violet solution (prepared in 50% methanol) and the cells were stained for 30 minutes at room temperature. Plates were then washed gently with tape water and air dried at room temperature without lid for 24 h. Next, 100 µl of methanol was added and incubated at room temperature for 15 minutes on bench rocker, followed by measuring the absorbance of dissolved dye at 570 nm. Cell viability was calculated as fold change in absorbance values of compound **20a** treated cells with respect to control[2].

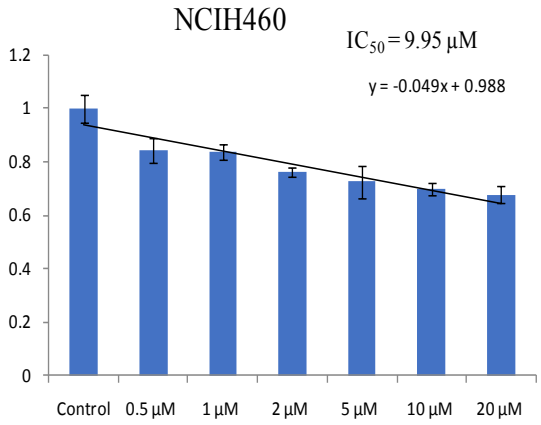
Dose response curves for compound 20a

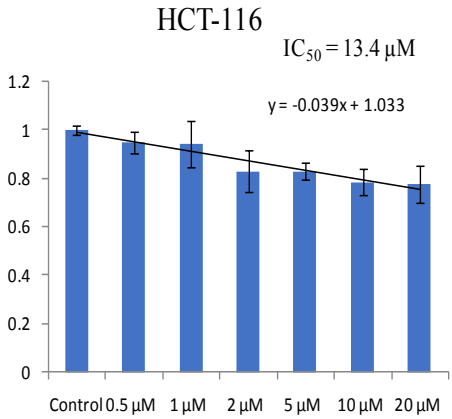


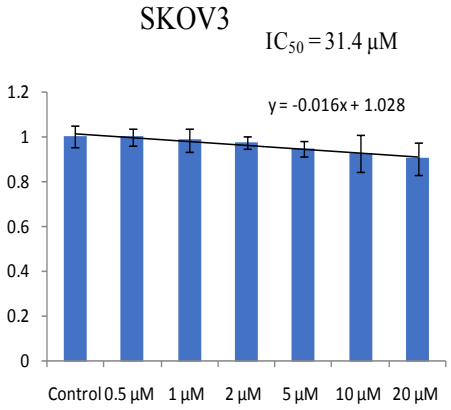
Dose response curves for compound 20d



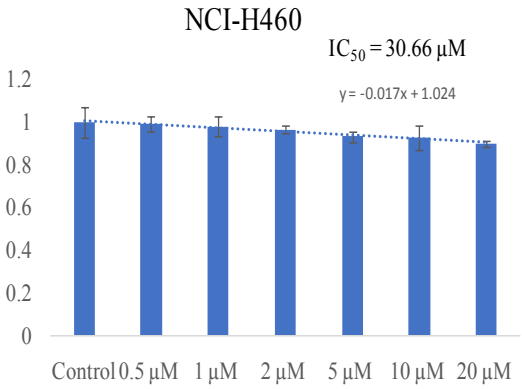
Dose response curves for compound 22a

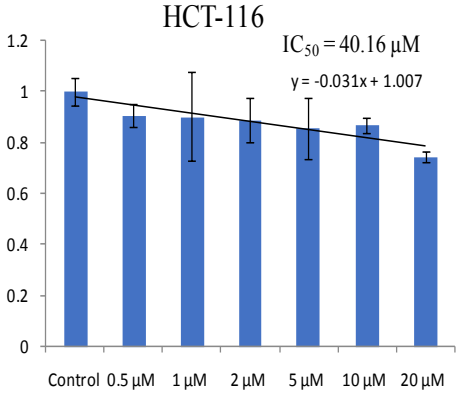


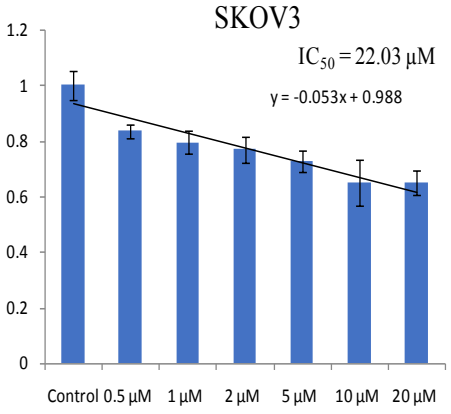




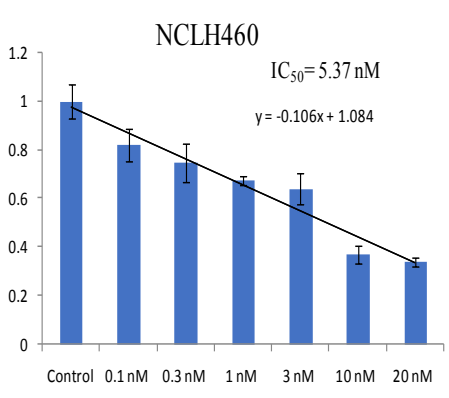
Dose response curve for Capsaicin

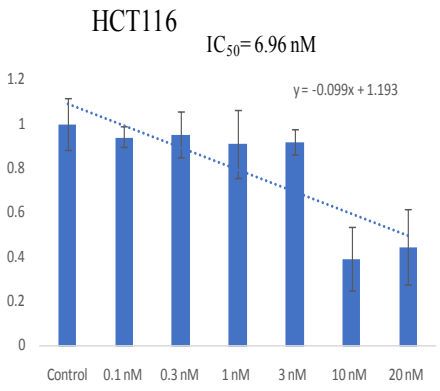


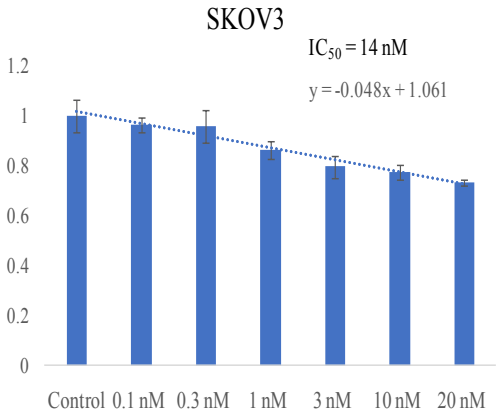




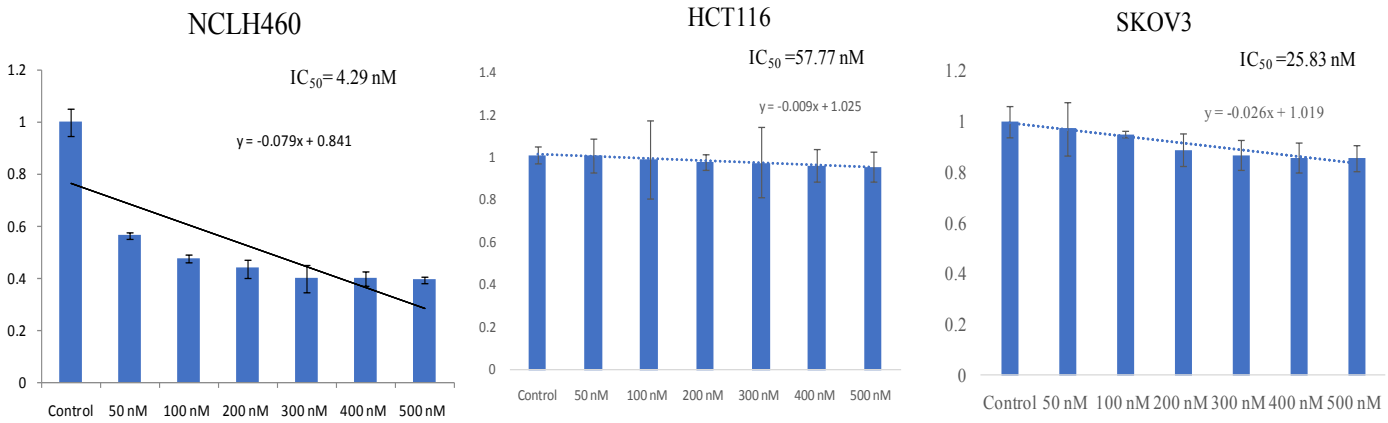
Dose response curve for Combretastatin



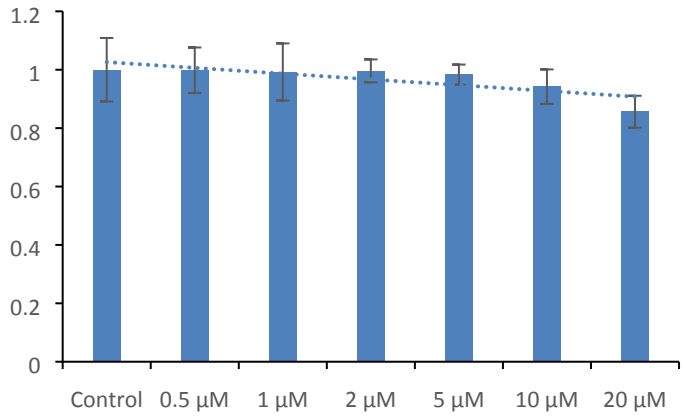




Dose response curve for Doxorubicin



Dose response curve for Compound 20a against normal PNT2 cells (normal prostatic epithelial cells)



2.1.3. Colony formation assay

NCI-H460 cells were seeded in six-well plate at a density of 500 cells per well and caused adherence of the cells (left for overnight), these cells were treated with different concentration of **20a**. After every 72 h, the media was changed and the cells were treated with **20a** and these experiments lasted for 15 days. After the development of colony, the wells were washed with PBS, fixed with 3.7% formaldehyde for 20 min, washed with PBS and then were stained using 0.4% crystal violet followed by rinsing the wells with PBS 4 to 5 times. The colonies were counted by using Image J software [3].

2.2. Evaluation of Mechanistic Studies

2.2.1. Flow cytometry for assessing intracellular ROS

The levels of intracellular reactive oxygen species (ROS) were measured following **20a** treatment. After treatment, the cells were incubated for 24h followed by trypsinization of cells and re-suspension in pre-warmed PBS containing probe CM-H_2DCFDA for 30 minutes in the dark at 37˚C. The fluorescence intensity was measured using flow cytometer [4].

2.2.2. Cell cycle analysis NCI-H460 cells

Effect of **20a** on the cell cycle of cell line was evaluated by using flow cytometry at 5 µM, 10 µM, and 20 µM, concentrations. These cells were seeded into 6 well plates and placed for incubation at 37 °C for 24 h which lead to attachment of cells with 50-60% confluency. Further on it was treated with vehicle control 0.1% DMSO and **20a** (0.5, 1, 2, 5 and 10 µM) and incubated for 72 h. The collected cells were harvested and fixed with 75% ice cold ethanol at 4°C. Excess of ethanol was removed and cells were washed with PBS. Cells were treated and stained with RNase (50 U/mL) and Propidium Iodide (20 µg/mL) staining solution for 20 min at room temperature in dark and was further subjected for flow cytometry (Bectone Dickinson, FACS Verse) [5].

2.2.3. Cell apoptosis assay

To assess the effect of compound **20a** treatment on apoptosis, Annexin V-FITC and Propidium iodide staining was performed and the cells were analyzed using FACS verse (BD). Briefly, NCI-H460 cells were treated with compound **20a** at 5 µM, 10µM and 20µM and the cells were incubated for 72 h at 37˚C and 5% CO_2. Next, the cells were harvested and incubated with 1X binding buffer containing Annexin-V FITC and propidium for 30 minutes. The samples were acquired for cell death analysis using FACS *verse* (BD) [6].

2.2.4. Wound healing assay

NCI-H460 cells (3 x10^5) were seeded and grown to each well of 6 well plates until mono-layer was formed. Then by using 200 μL pipette tip, a scratch was given in confluent mono layer. Cells were washed, to remove non-adherent cell debris. Cells were further treated with 5µM, 10 µM and 20 µM of compound **20a**. Cell migration across the wound area were captured with an inverted microscope and photographed for indicated time (0, and 24 h). The percentage wound healed area was calculated after normalizing the wound area [7].

2.2.5. Western blotting

NCI-H460 Cells treated with compound **20a** were lysed after 72 h of incubation period with 2X SDS lysis buffer containing 0.5 M Tris-HCl, pH 6.8, glycerol, 10 % (w/v) SDS, protease inhibitor cocktail. The lysates were sonicated and centrifuged at 16000 g for 20 min following which supernatant was collected and subjected to protein estimation by Pierce™ BCA assay kit (ThermoFisher). 30-40 µg of proteins were separated on SDS gel and transferred to PVDF membranes. Membranes were exposed to blocking buffer containing 5 % skim milk and probed with specific primary antibodies at 4 ºC overnight. Next, the blots were washed three times for 5 minutes with Tris buffer solution with tween-20 (TBST) and incubated with HRP-conjugated secondary antibody for 1 h. The blots were washed three times with 1x TBST and the bands were detected by ECL Elistar ETA ultra 20 (Cyanagen) [8].

2.26. RNA isolation and quantitative PCR

TRIzol reagent was used to isolate total RNA from the control and drug treated NCI-H460 cells. Following that, cDNA was synthesized from 2g of total RNA using a cDNA synthesis kit (Takara), according to the manufacturer's instructions. **Table S4** lists below the primers used against the genes under investigation.

Table S2: Lists the primers used against the genes under investigation.

GENE	FORWARD PRIMER SEQUENCE	REVERSE PRIMER SEQUENCE
GAPDH	5'-GTCAAGGCTGAGAACGGGAA-3'	5'-AGTGGCTCCATTCACCGC-3'
VEGFR2	5'-GGAACCTCACTATCCGCAGA-3'	5'-CCGCCGTGCCTACTAGAATA-3'

The PCR cycling schedule was 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C, 30 seconds at 60°C, and a melt curve with a single reaction cycle at 95°C for 15 seconds, 60°C for 1 minute, and dissociation at 95°C for 15 seconds. The resulting Ct values were then adjusted to the housekeeping gene actin. The relative expression of genes was determined using the $2^{-\Delta\Delta ct}$ method.

2.27. Molecular docking

To view binding pattern of all the synthesized compounds for their anti-proliferative activity, molecular docking was carried out at the VEGFR2 kinase receptor catalytic ligand binding site (PDBID: 2QU5). Docking simulations of Compounds20a-k, 21, 22a-b were performed using Maestro, version 9.6 implemented from Schrodinger software suite. The ligands were sketched in 3D format using build panel and were prepared for docking using ligprep application. The protein for docking study was obtained from Protein data bank (PDB ID: 2QU5) and prepared by removing solvent, adding hydrogen and further minimization in the presence of bound ligand (276) using protein preparation wizard. Grids for molecular docking were generated with bound co-crystallized ligand. For the validation of docking parameters, the co-crystal ligand (276) was re-docked at the catalytic site of protein and the RMSD between co-crystal and re-docked pose was found to be 0.405 Å. All the synthesized compounds were docked using Glide extra-precision (XP) mode, with up to three poses saved per molecule [9].

3. References

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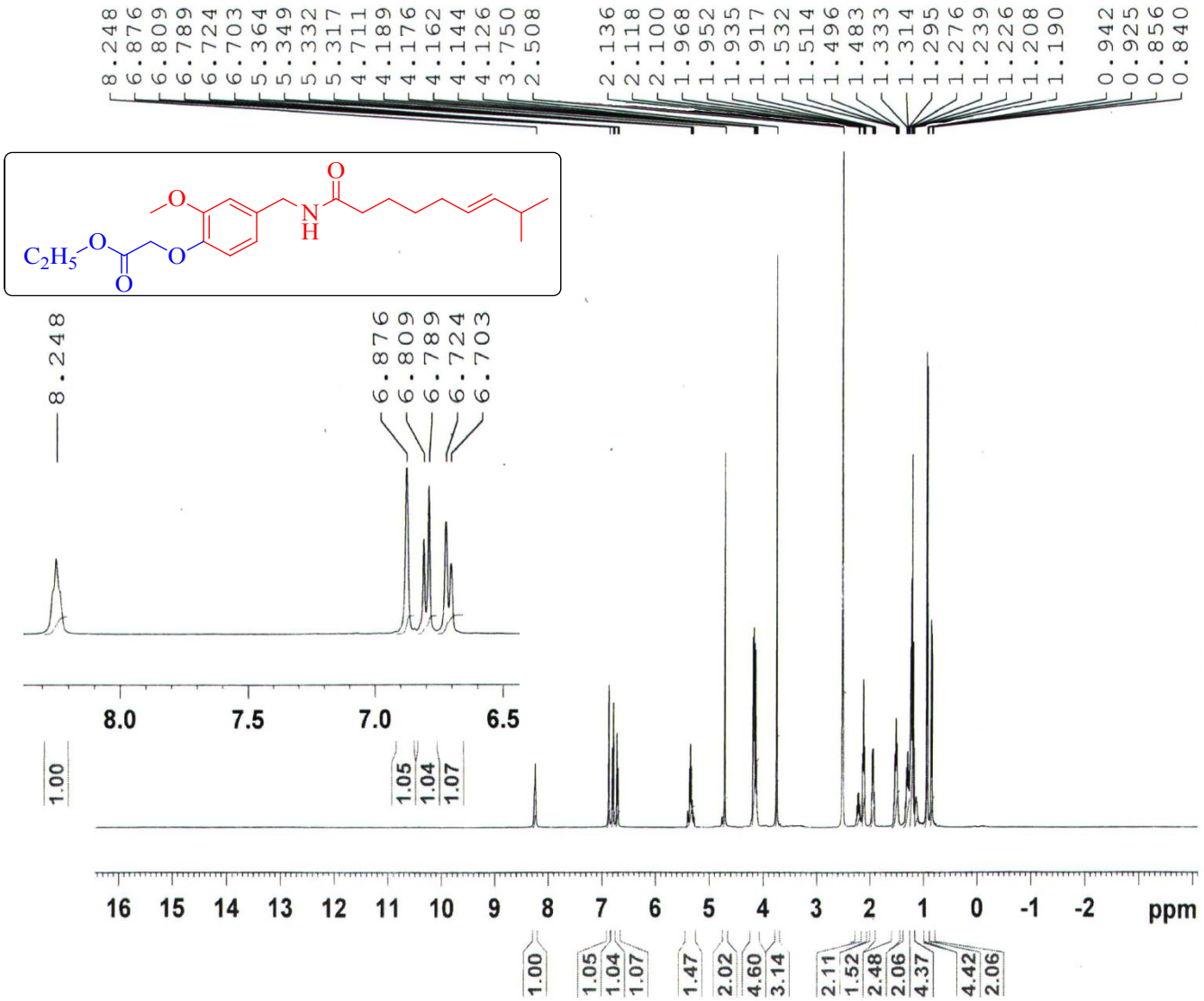
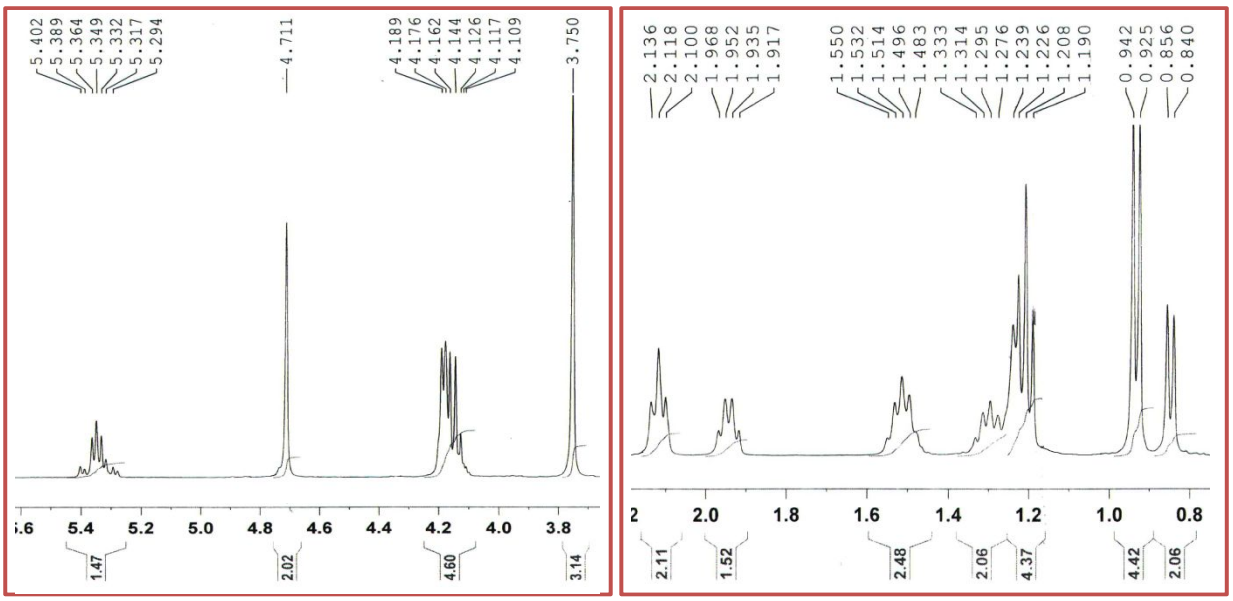
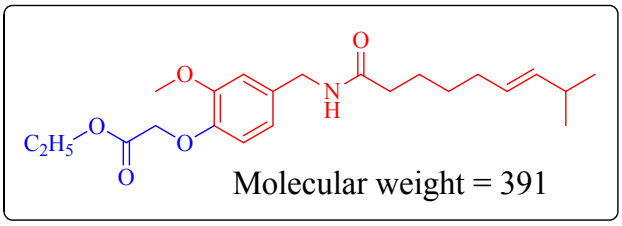
4. Scanned copies of ^{1}H, ^{13}C NMR and Mass spectra's of the representative compounds:

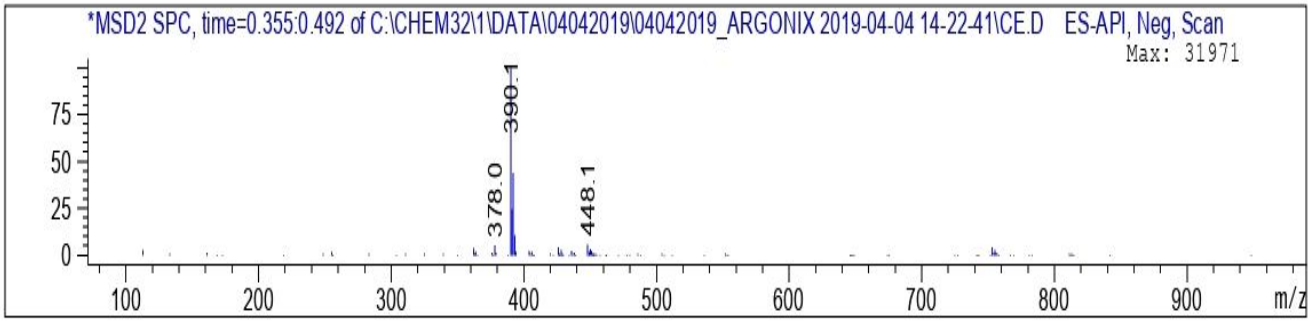


^{1}H NMR of Compound 16

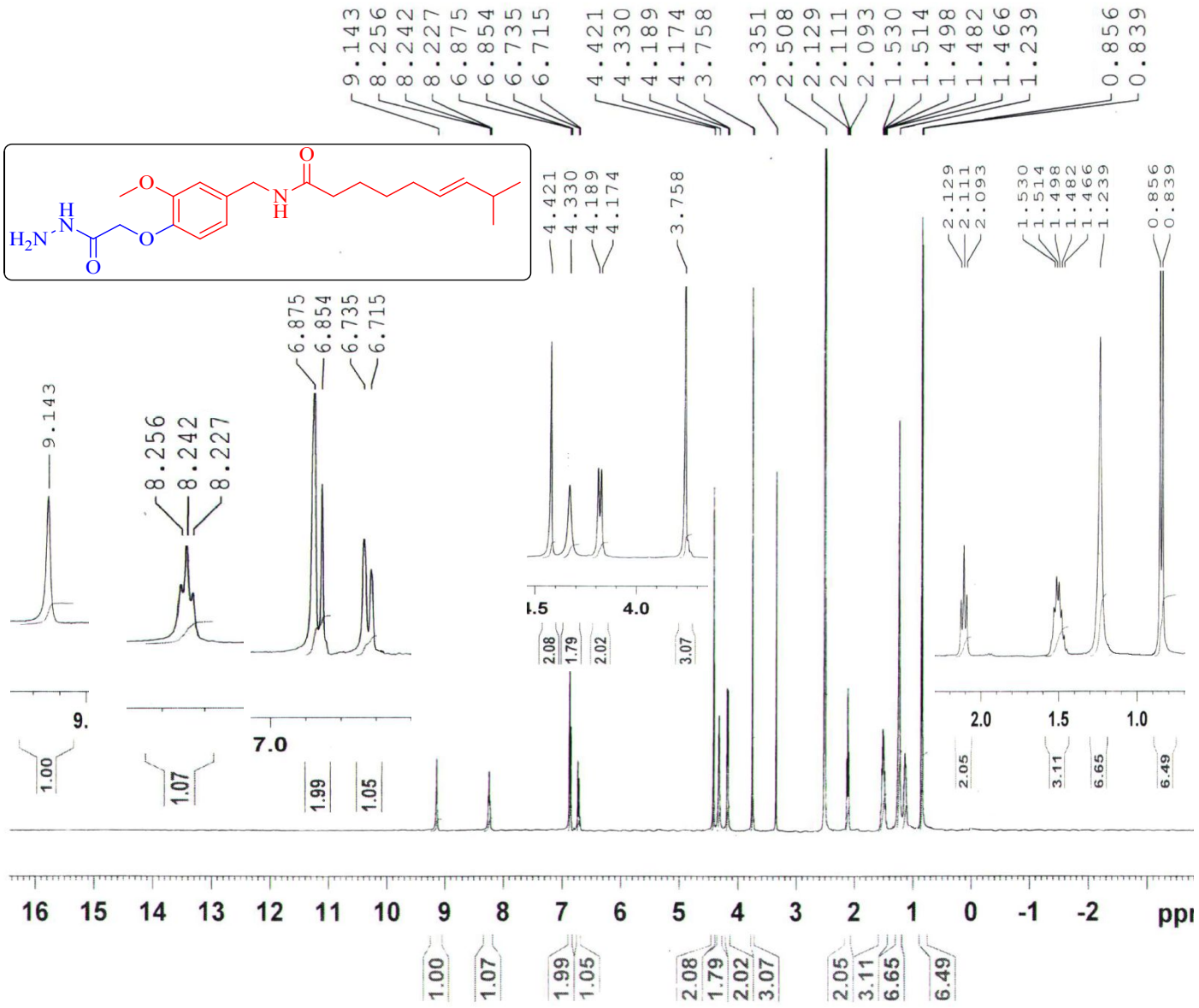


^{1}H NMR of Compound 16

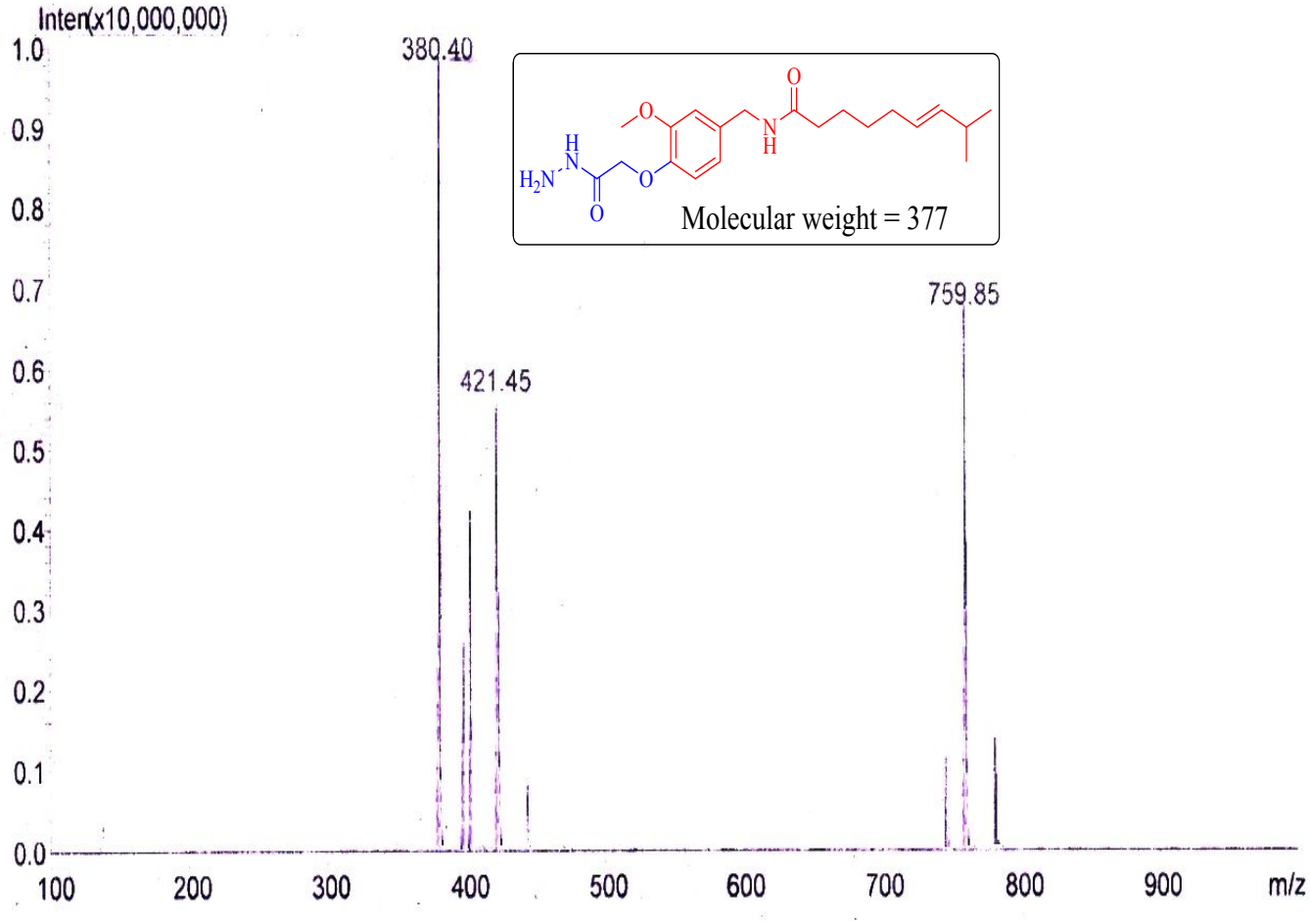




ESI-MS of compound 16



[1]H NMR of Compound 17



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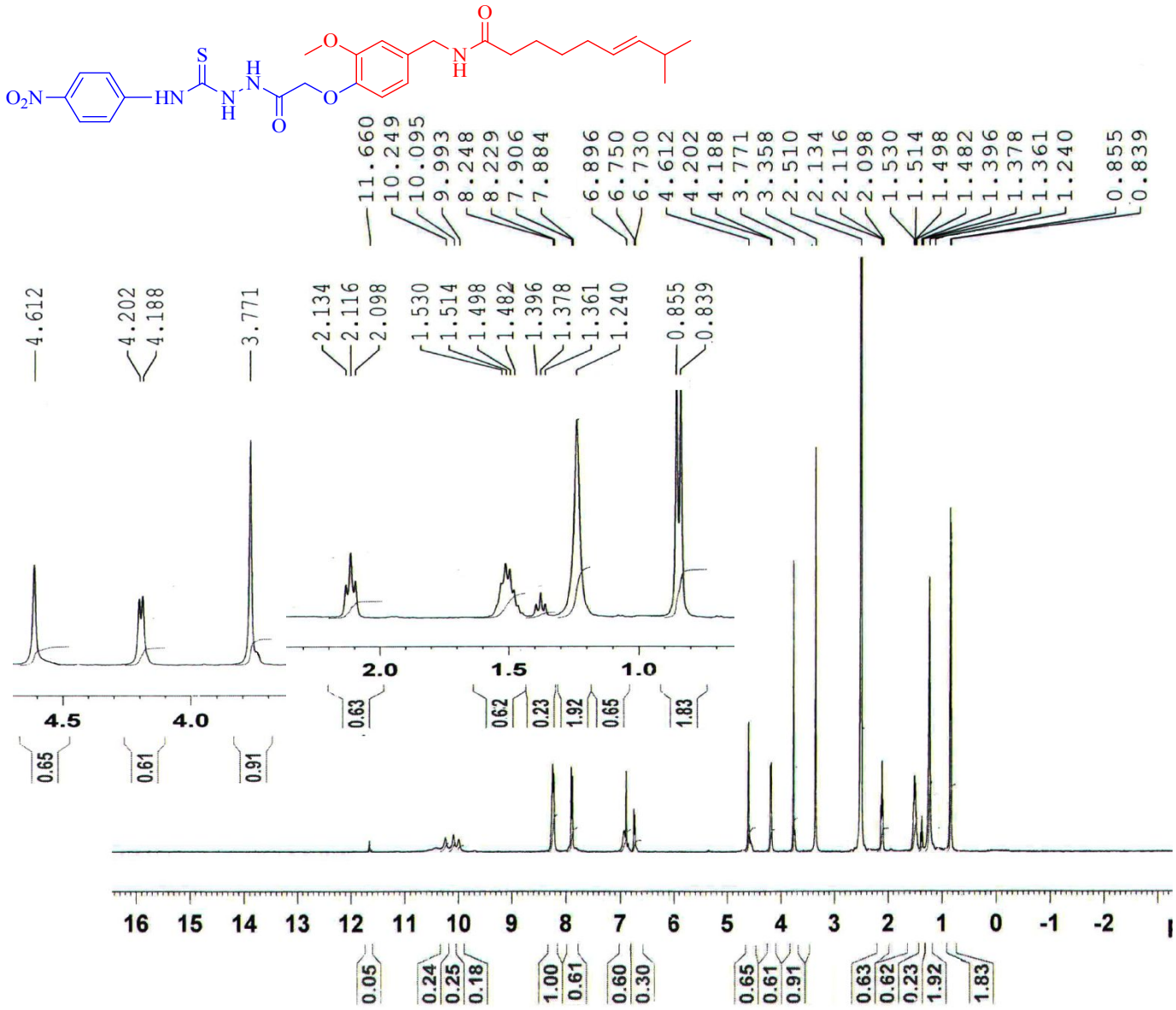
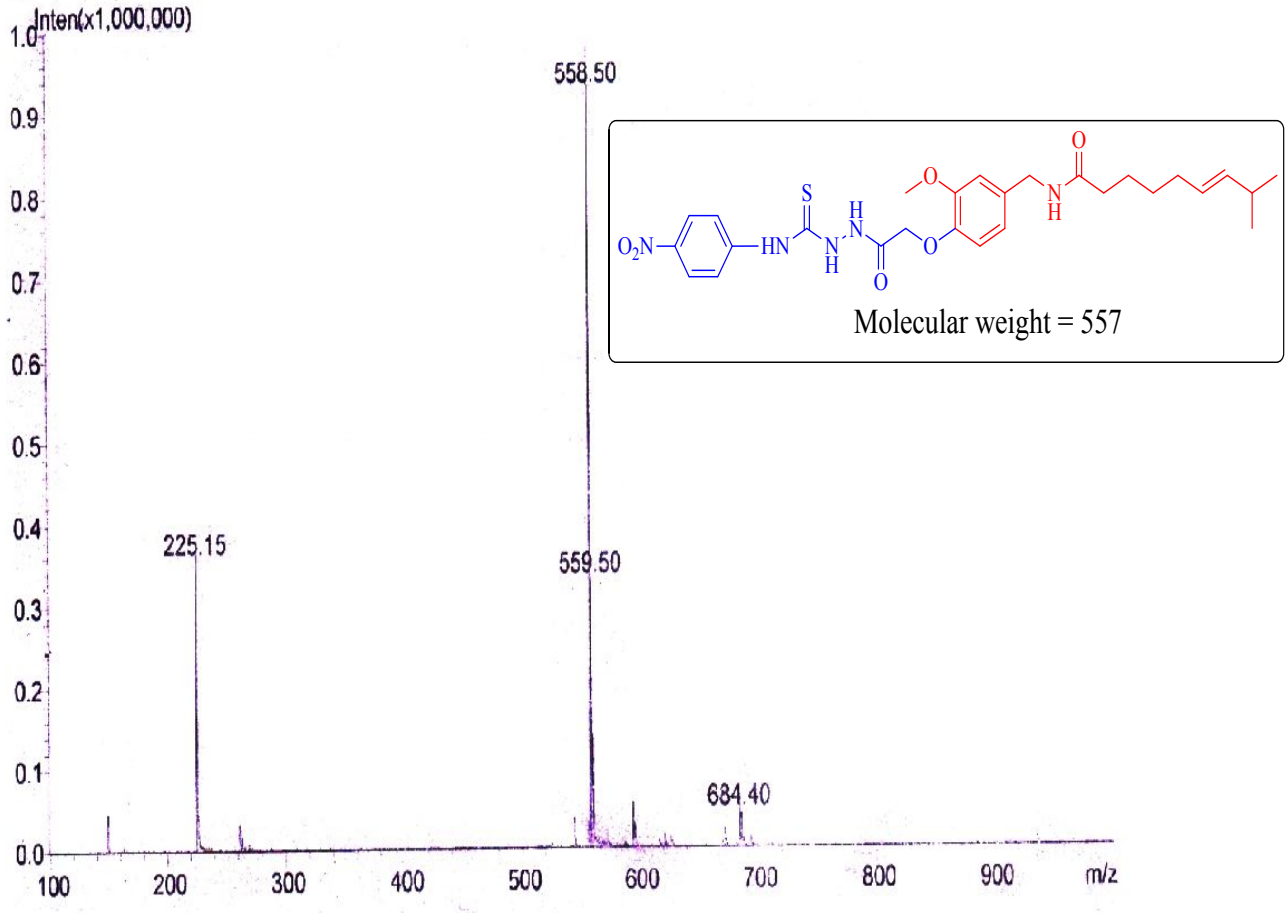


^{1}H NMR of Compound 19a



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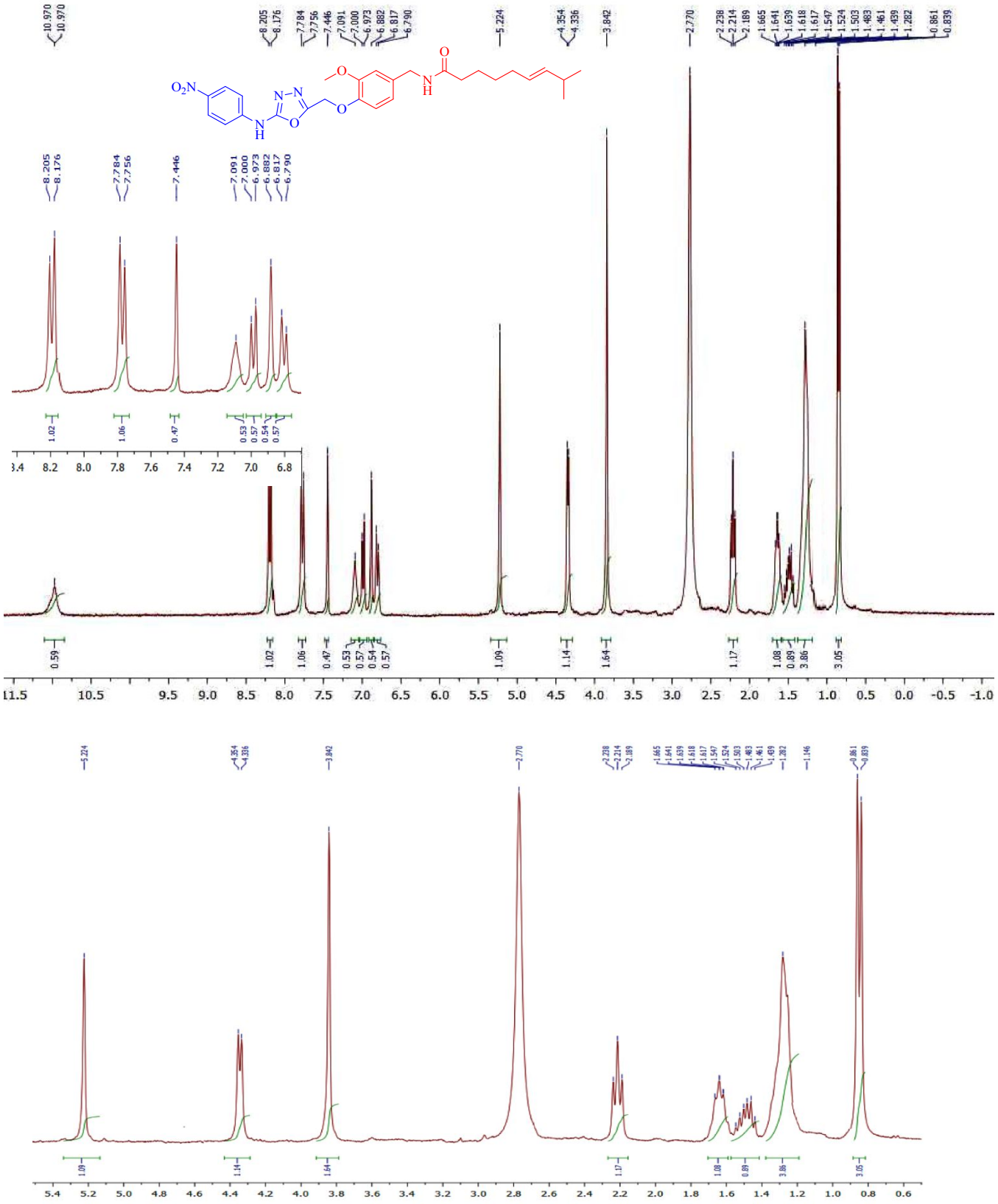
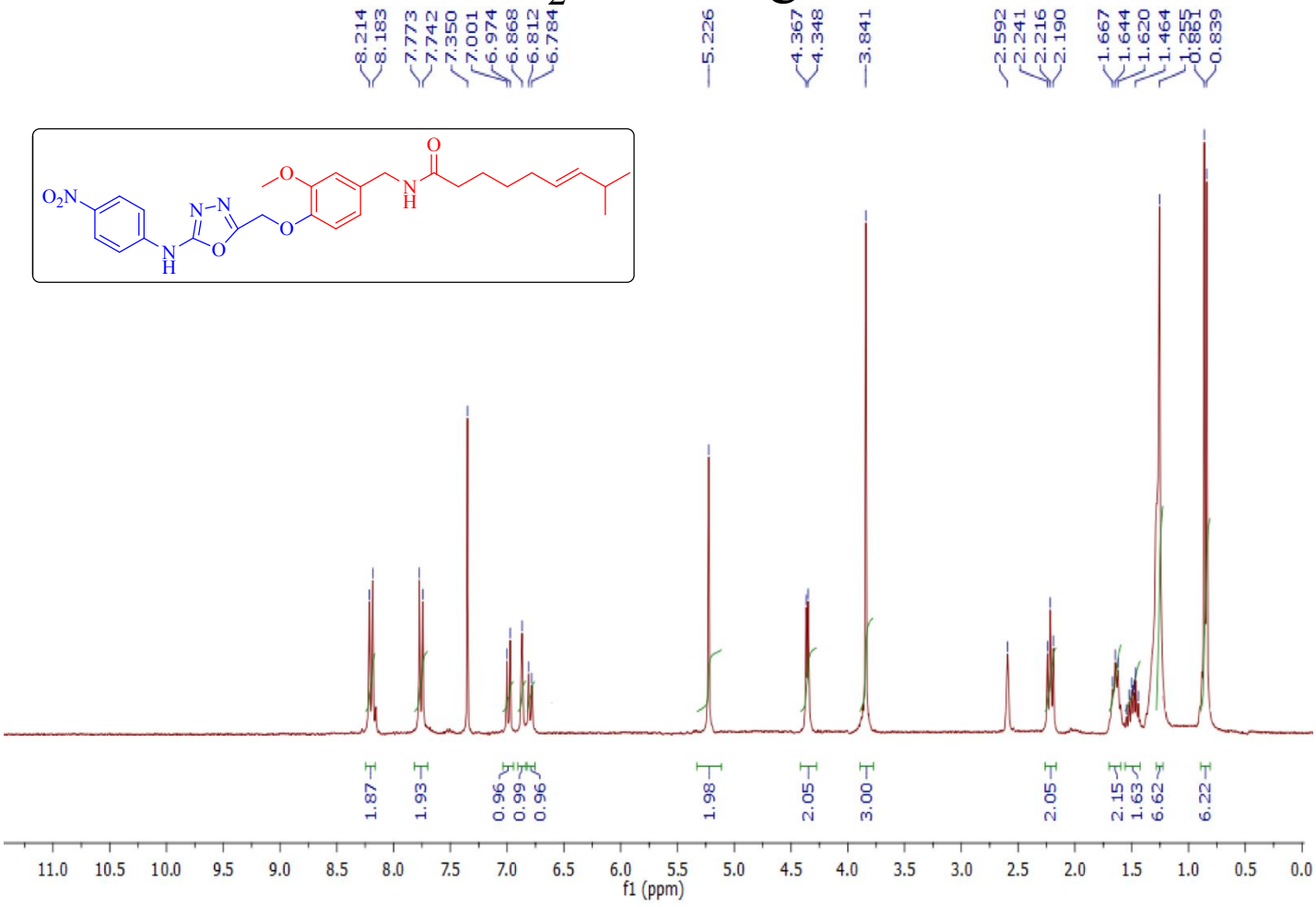
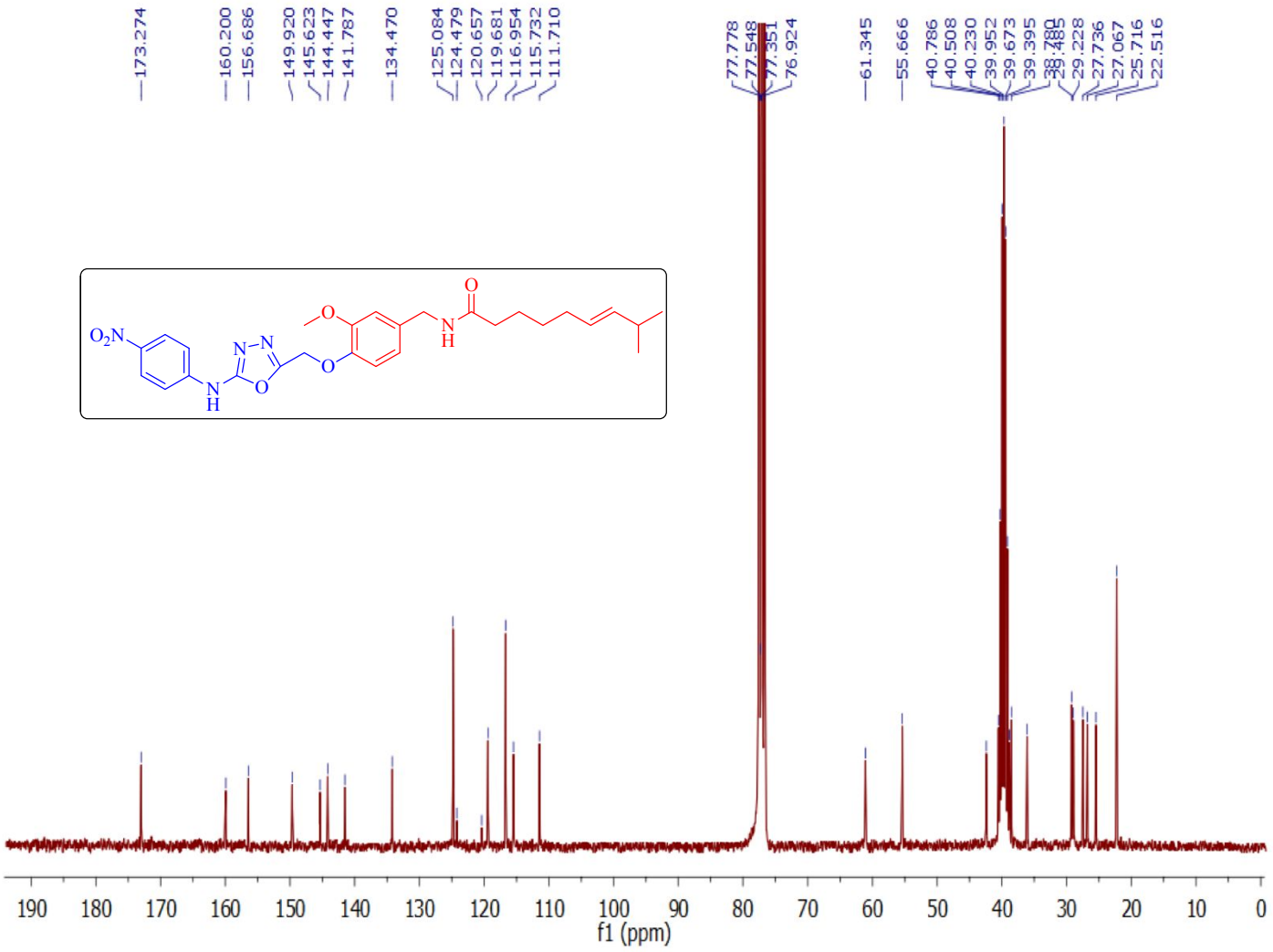


^{1}H NMR of Compound 20a

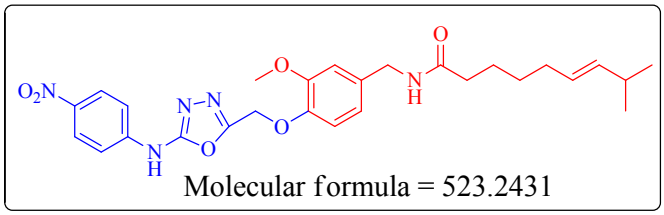
D_2o exchange



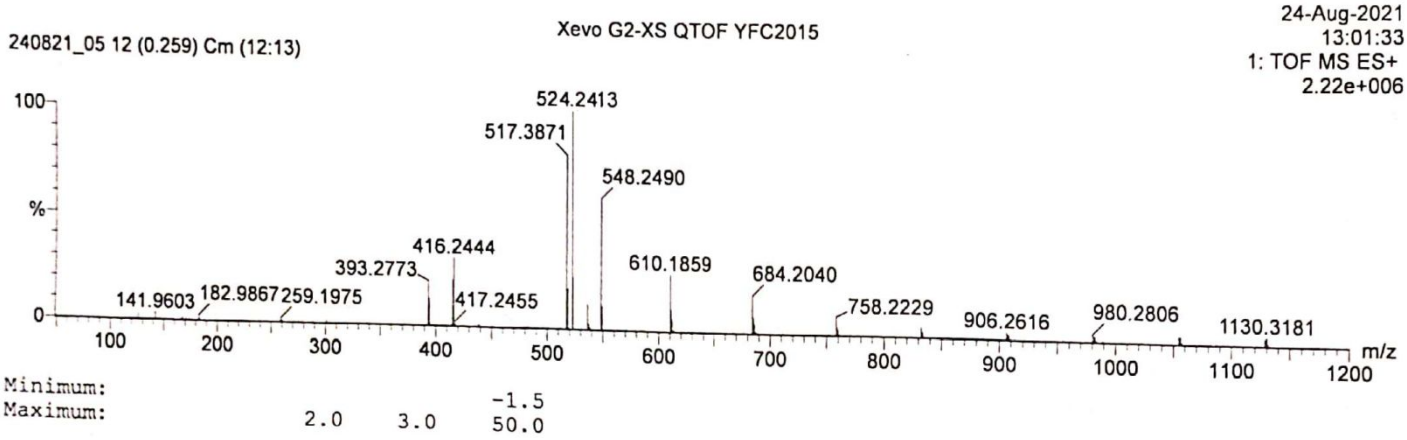
[1]H NMR of Compound 20a



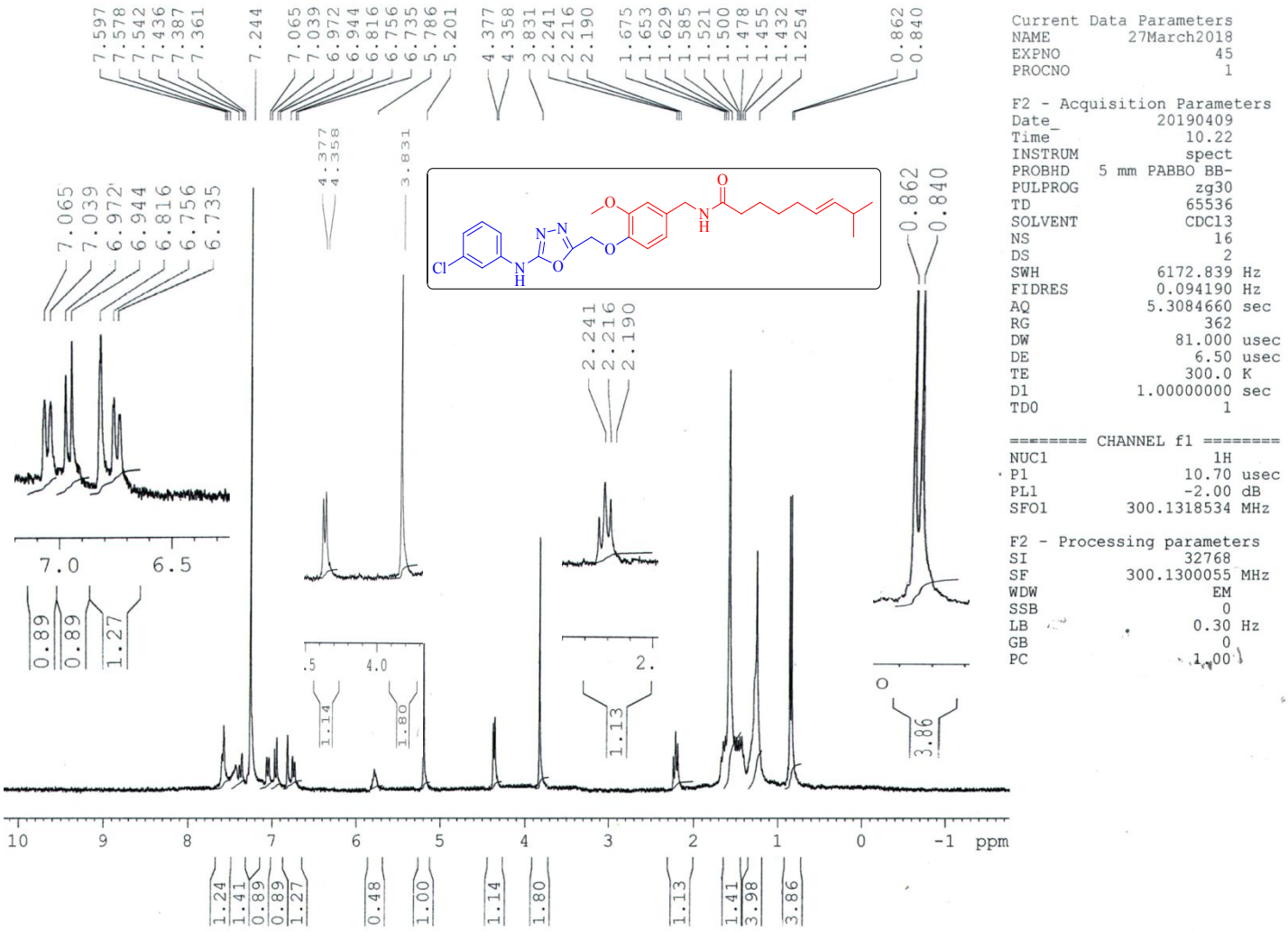
^{13}C NMR of Compound 20a



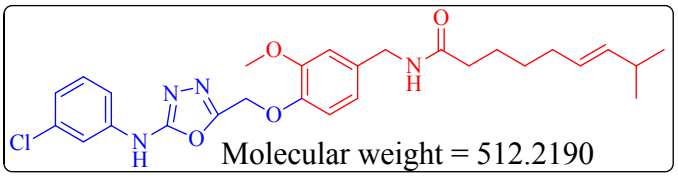
Molecular formula = 523.2431

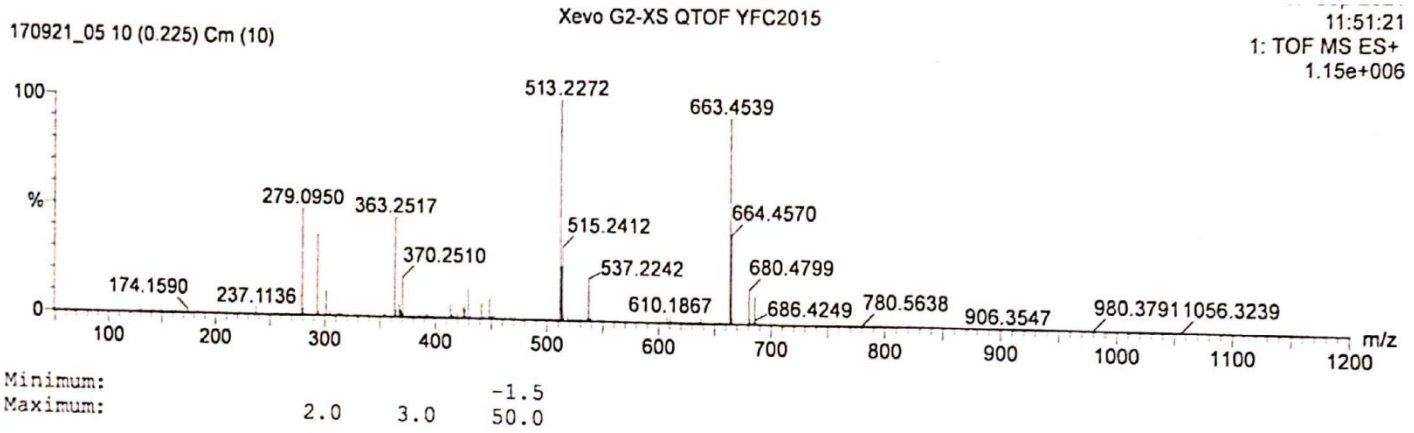


HRMS of compound 20a



^{1}H NMR of Compound 20b





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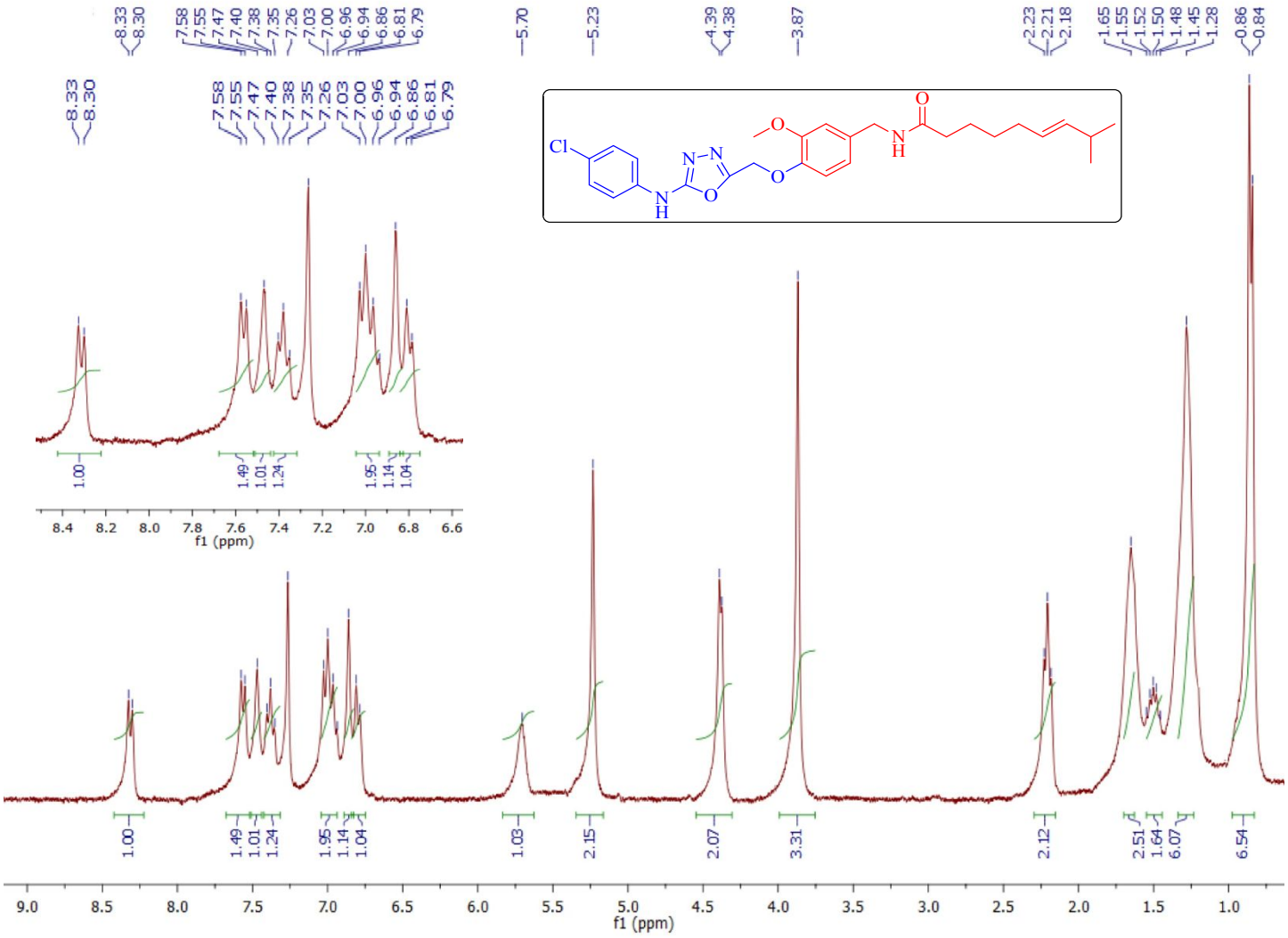
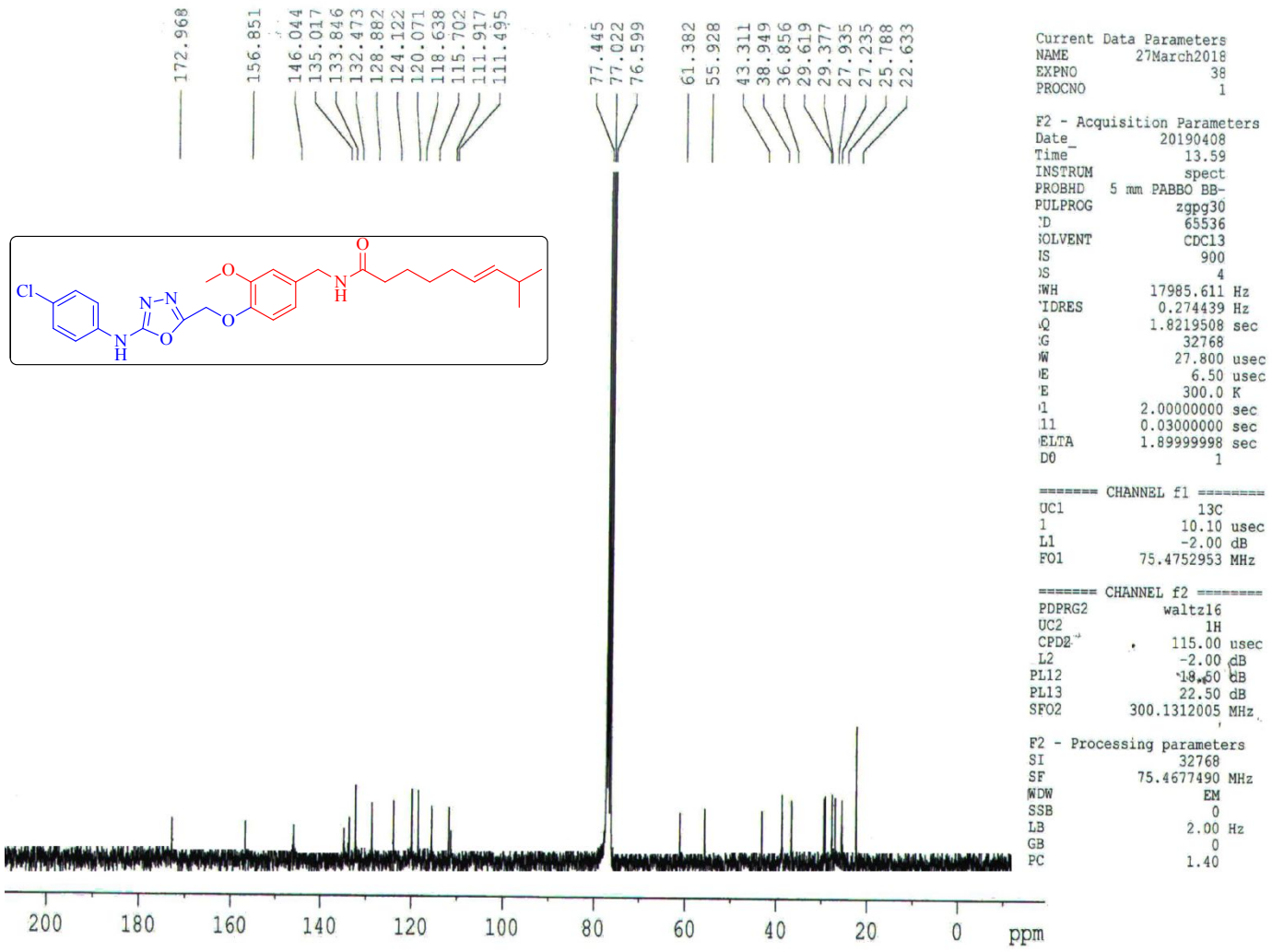


[1]H NMR of Compound 20c



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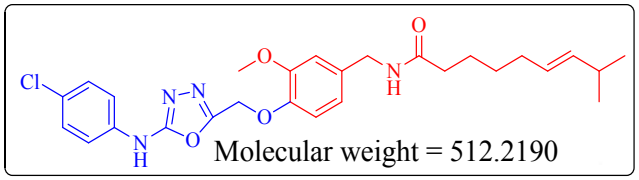
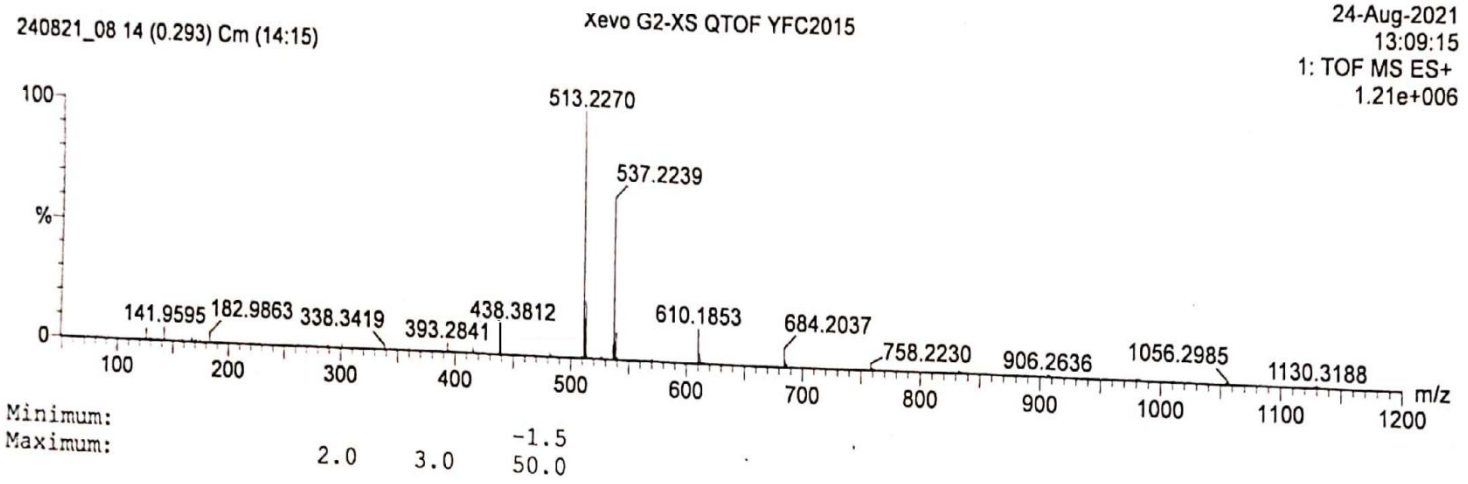
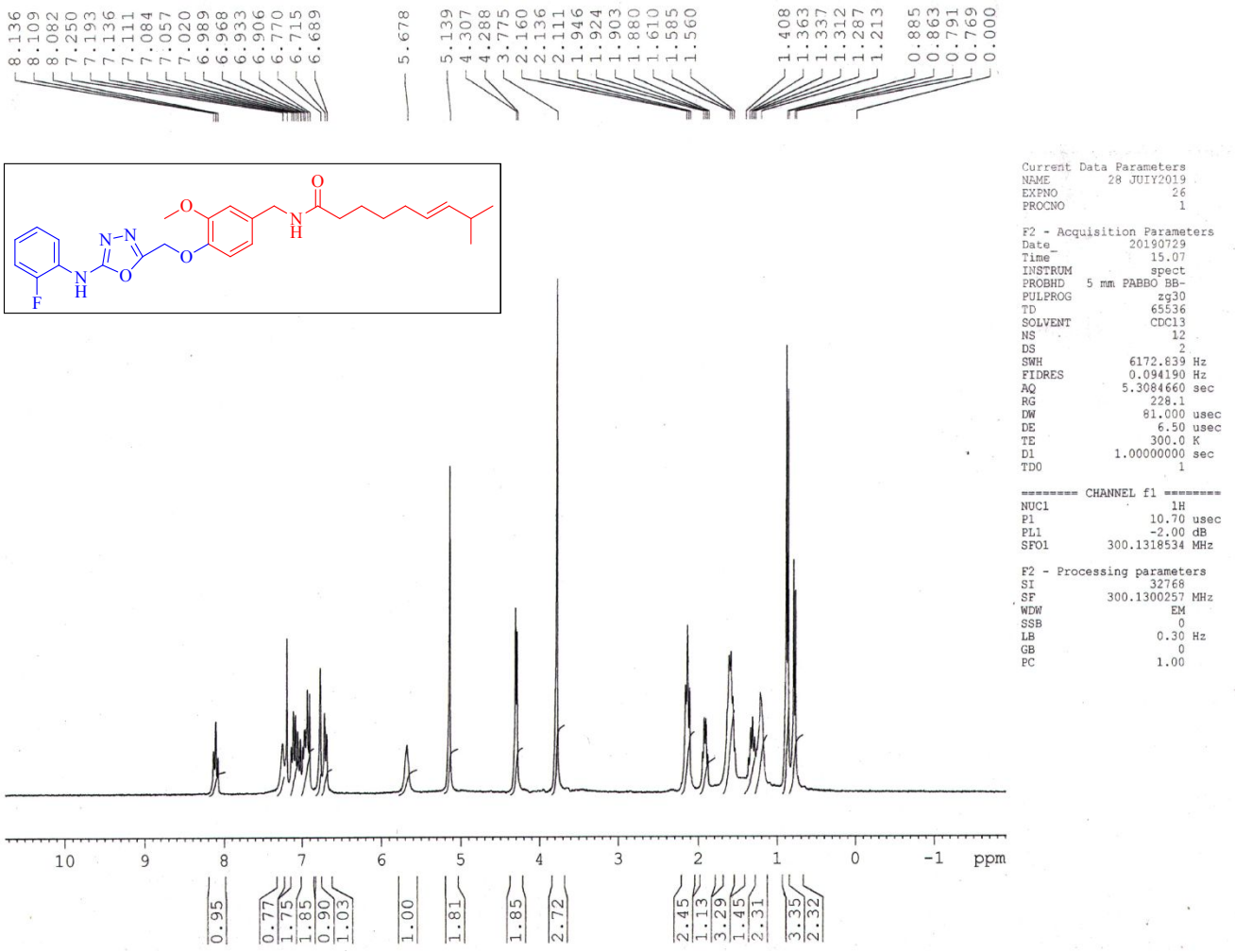
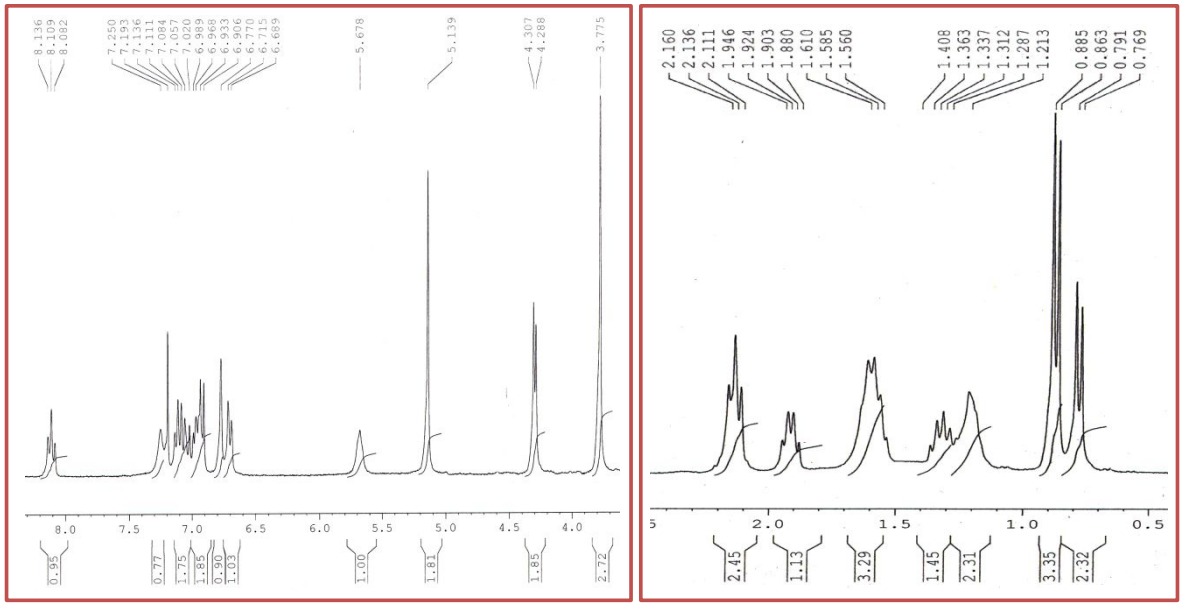


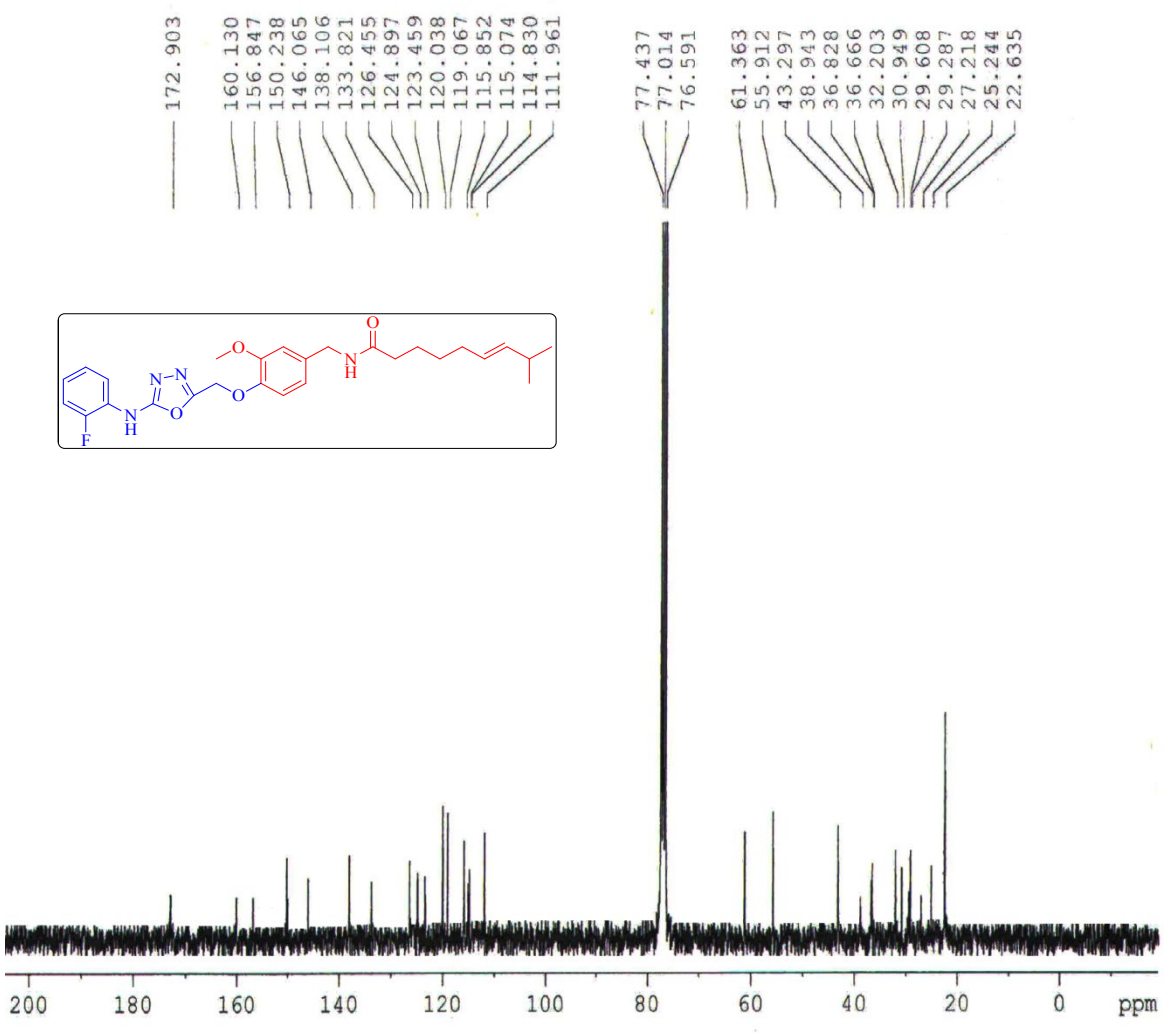


HRMS of Compound 20c





^{1}H NMR of Compound 20d

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EXPNO                          27
PROCNO                          1

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TD                         65536
SOLVENT                    CDCl3
NS                          1123
DS                             4
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FIDRES                0.274439 Hz
AQ                    1.8219508 sec
RG                         32768
DW                        27.800 usec
DE                         6.50 usec
TE                        300.0 K
D1                    2.00000000 sec
d11                   0.03000000 sec
DELTA                 1.89999998 sec
TD0                            1

======== CHANNEL f1 ========
NUC1                         13C
P1                         10.10 usec
PL1                        -2.00 dB
SFO1                75.4752953 MHz

======== CHANNEL f2 ========
CPDPRG2                  waltz16
NUC2                         1H
PCPD2                     115.00 usec
PL2                        -2.00 dB
PL12                       18.50 dB
PL13                       22.50 dB
SFO2                300.1312005 MHz

F2 - Processing parameters
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LB                         2.00 Hz
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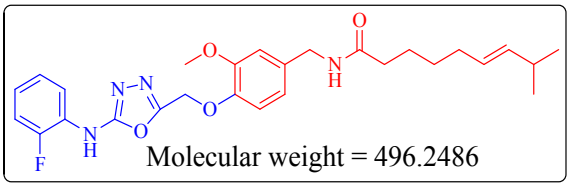
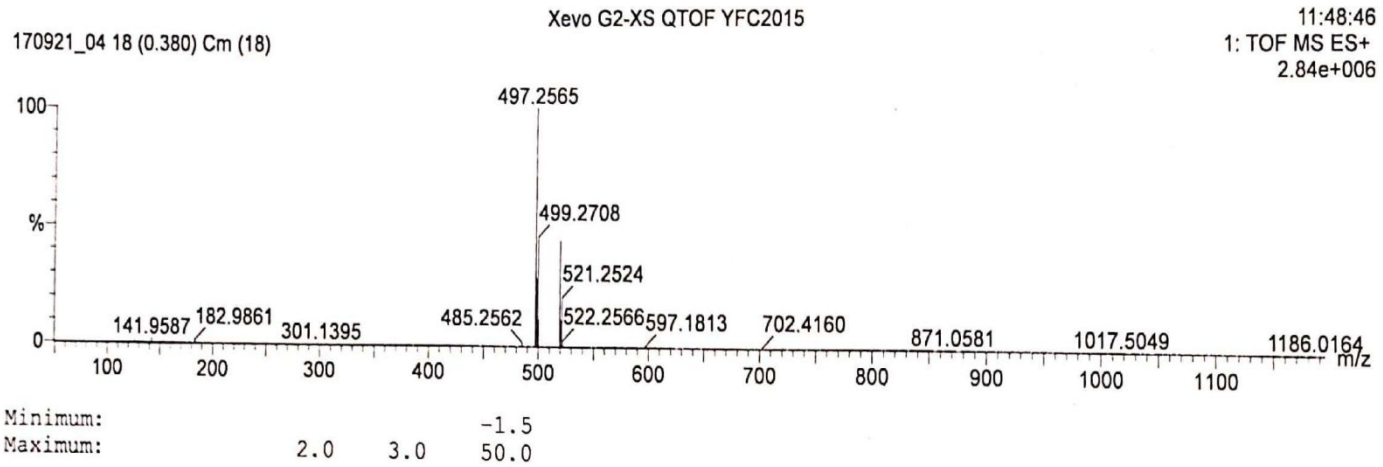
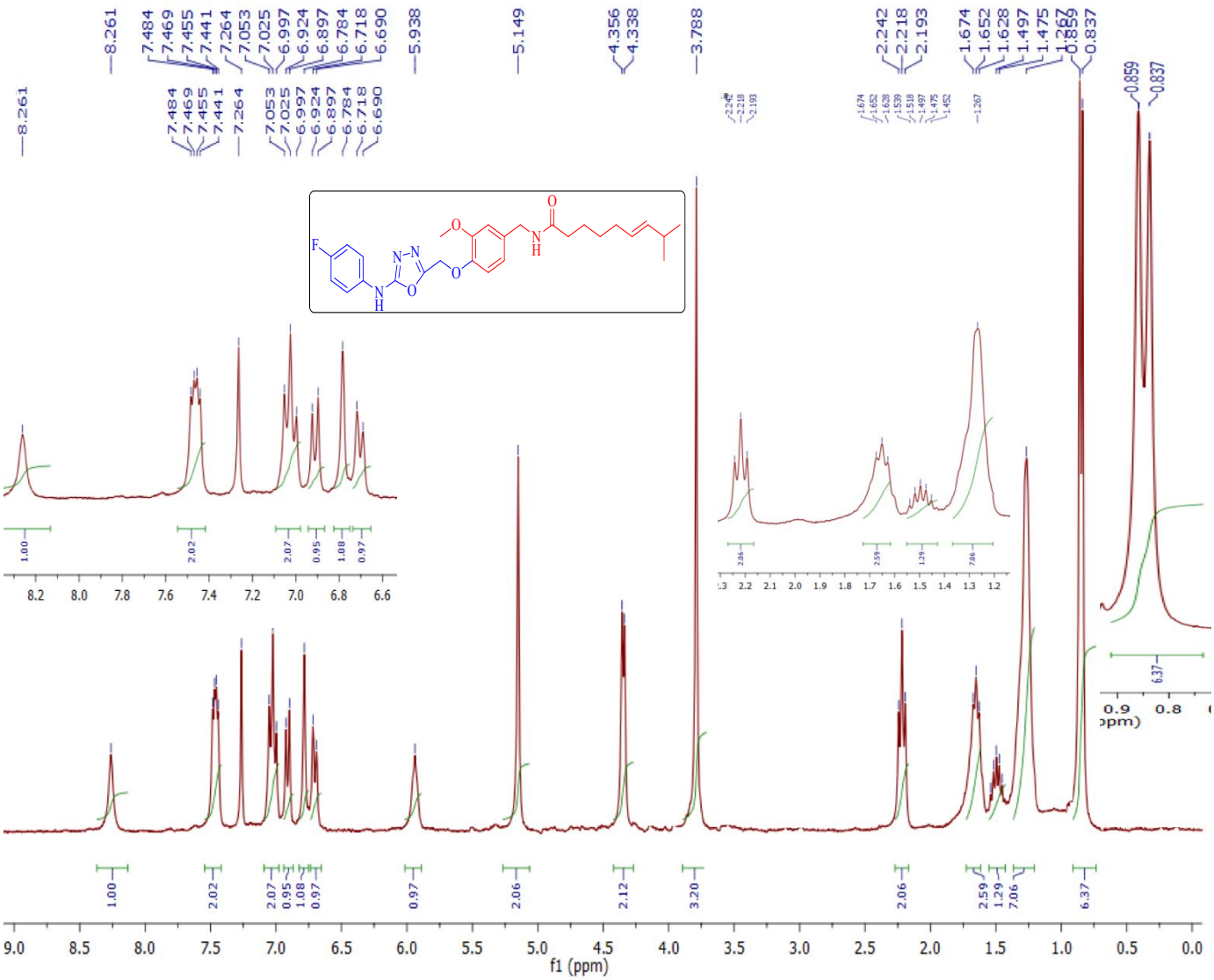
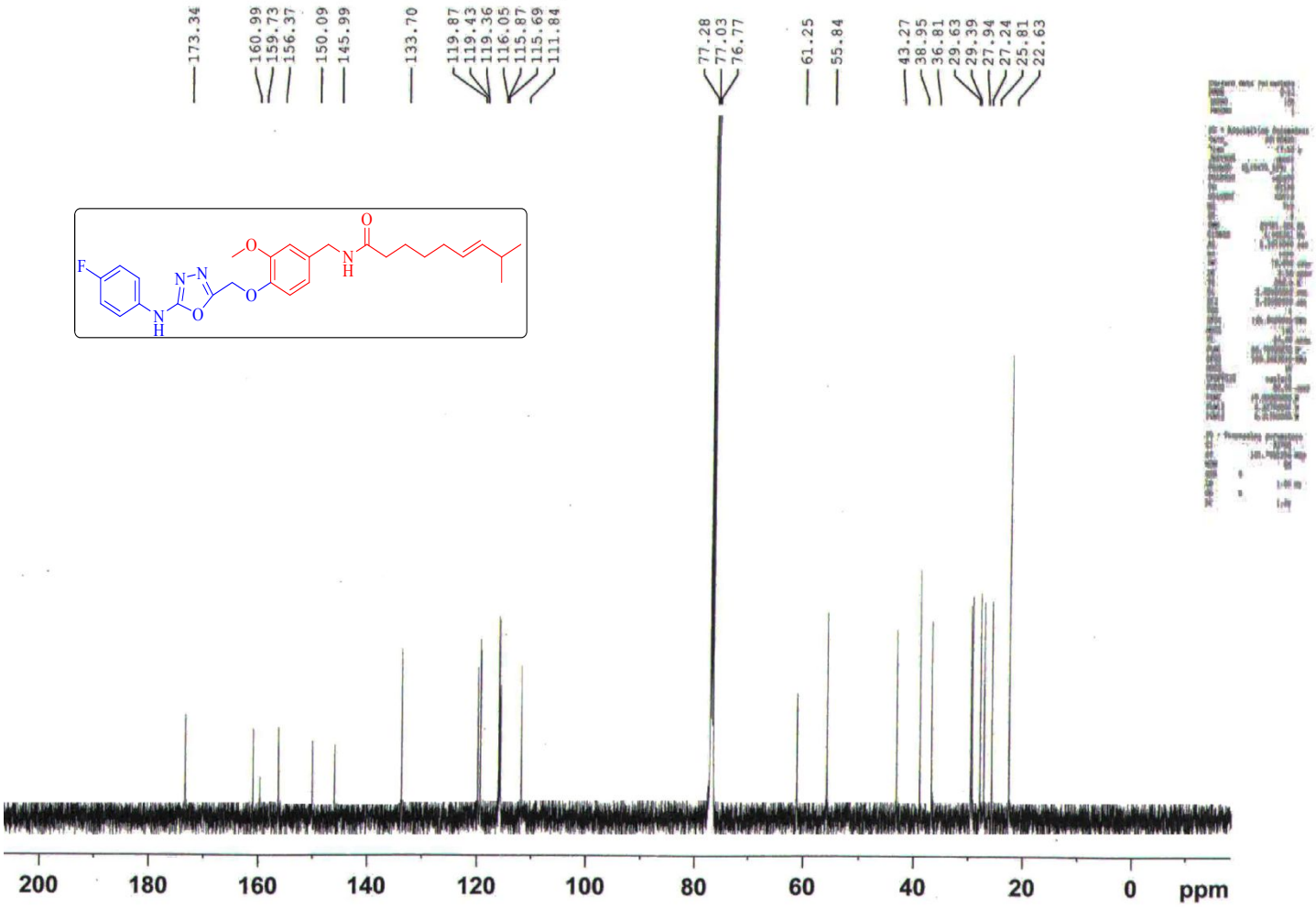




HRMS of Compound 20d



[1]H NMR of Compound 20e



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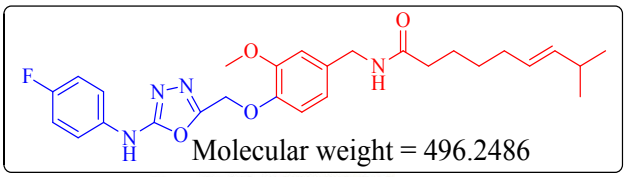
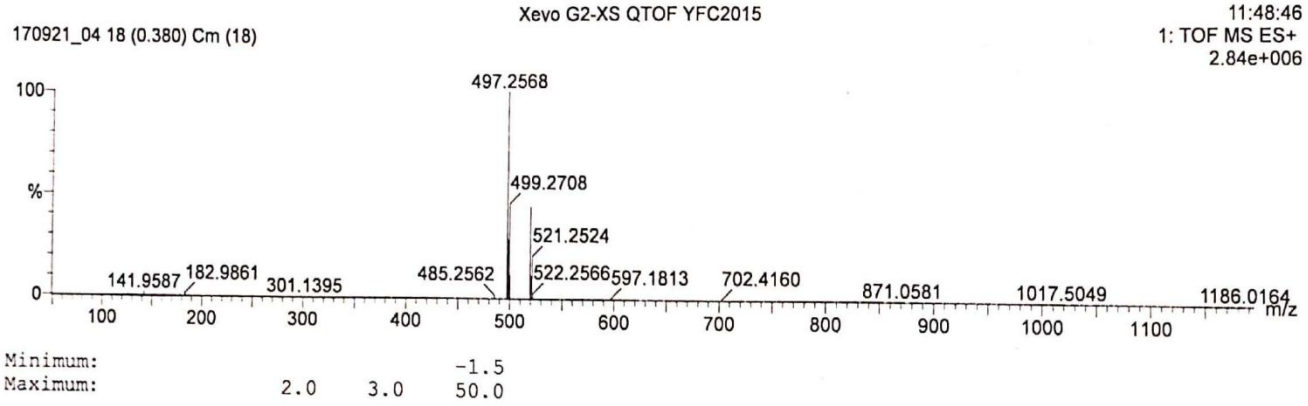
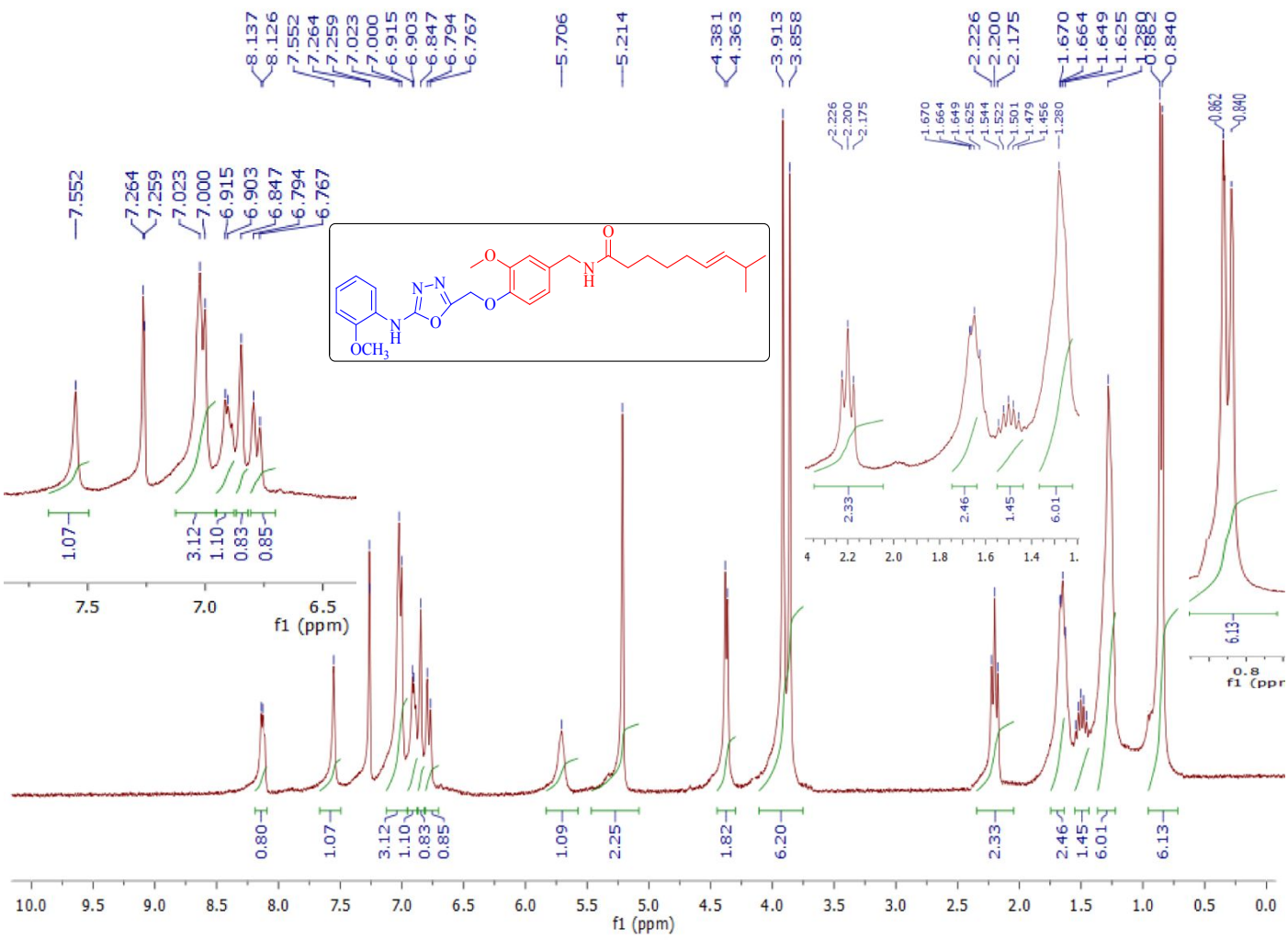




HRMS of Cmpound 20e



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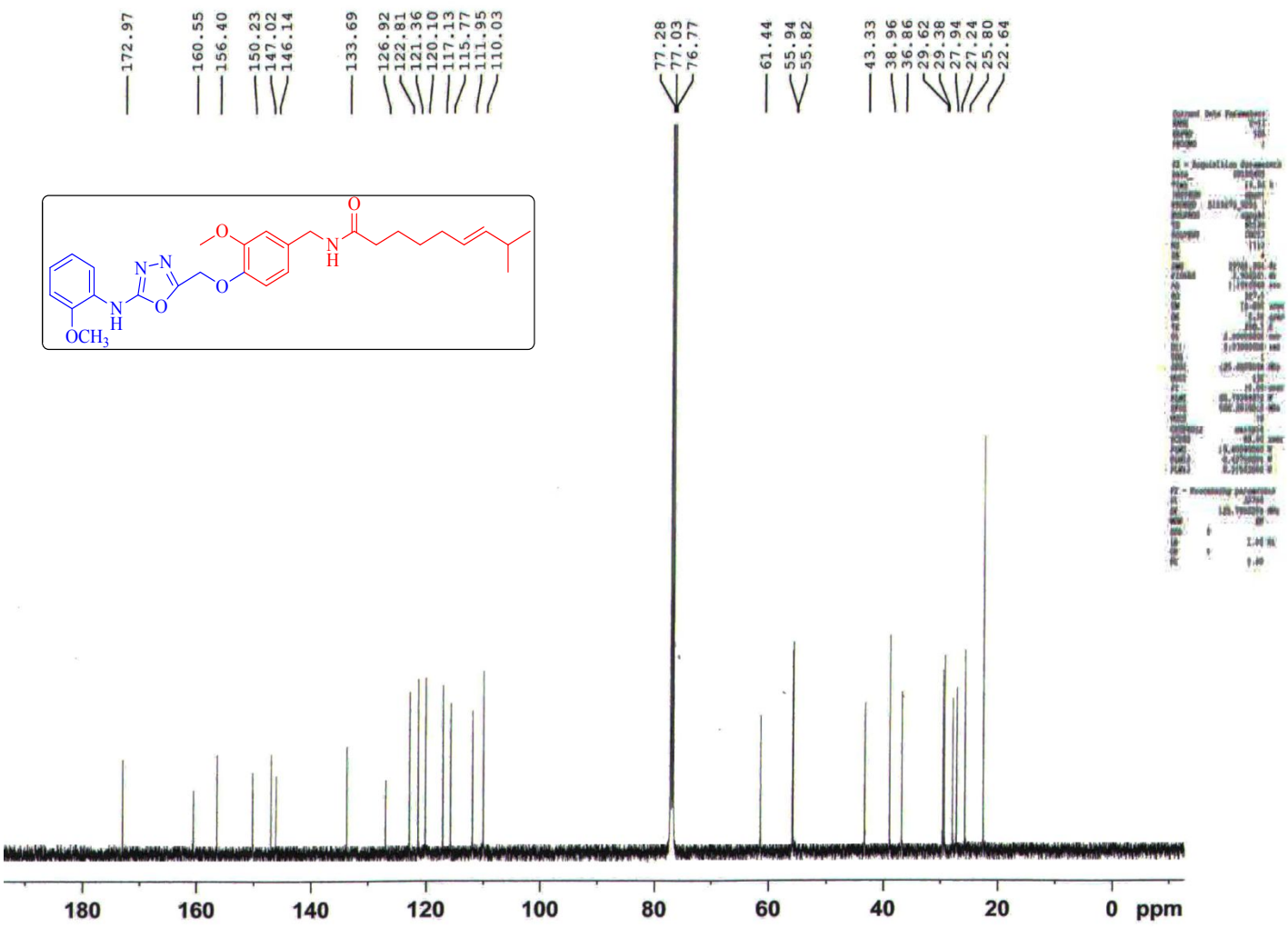

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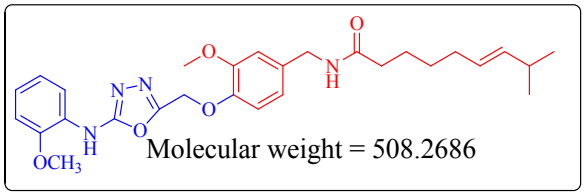
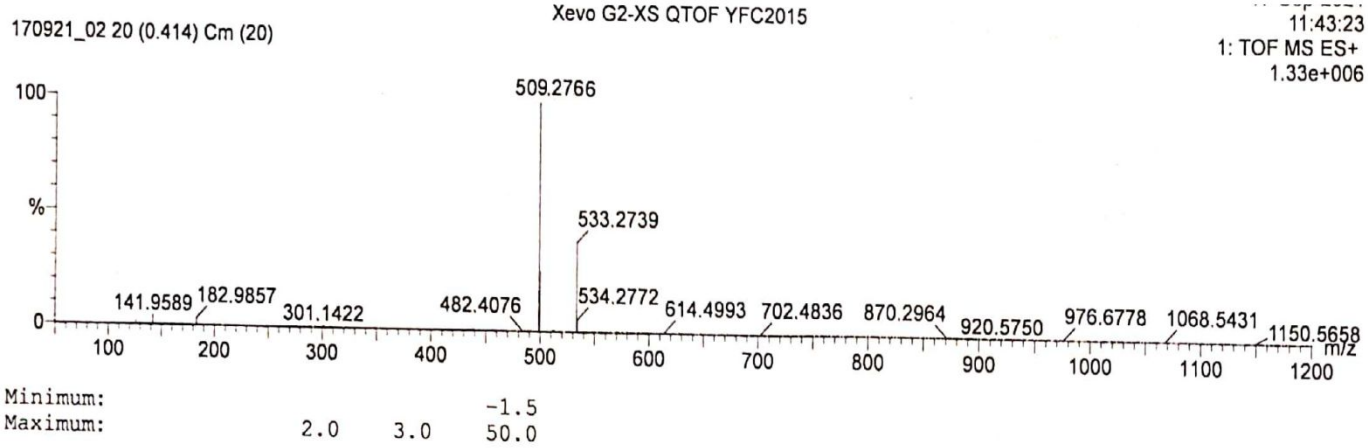


Molecular weight = 508.2686



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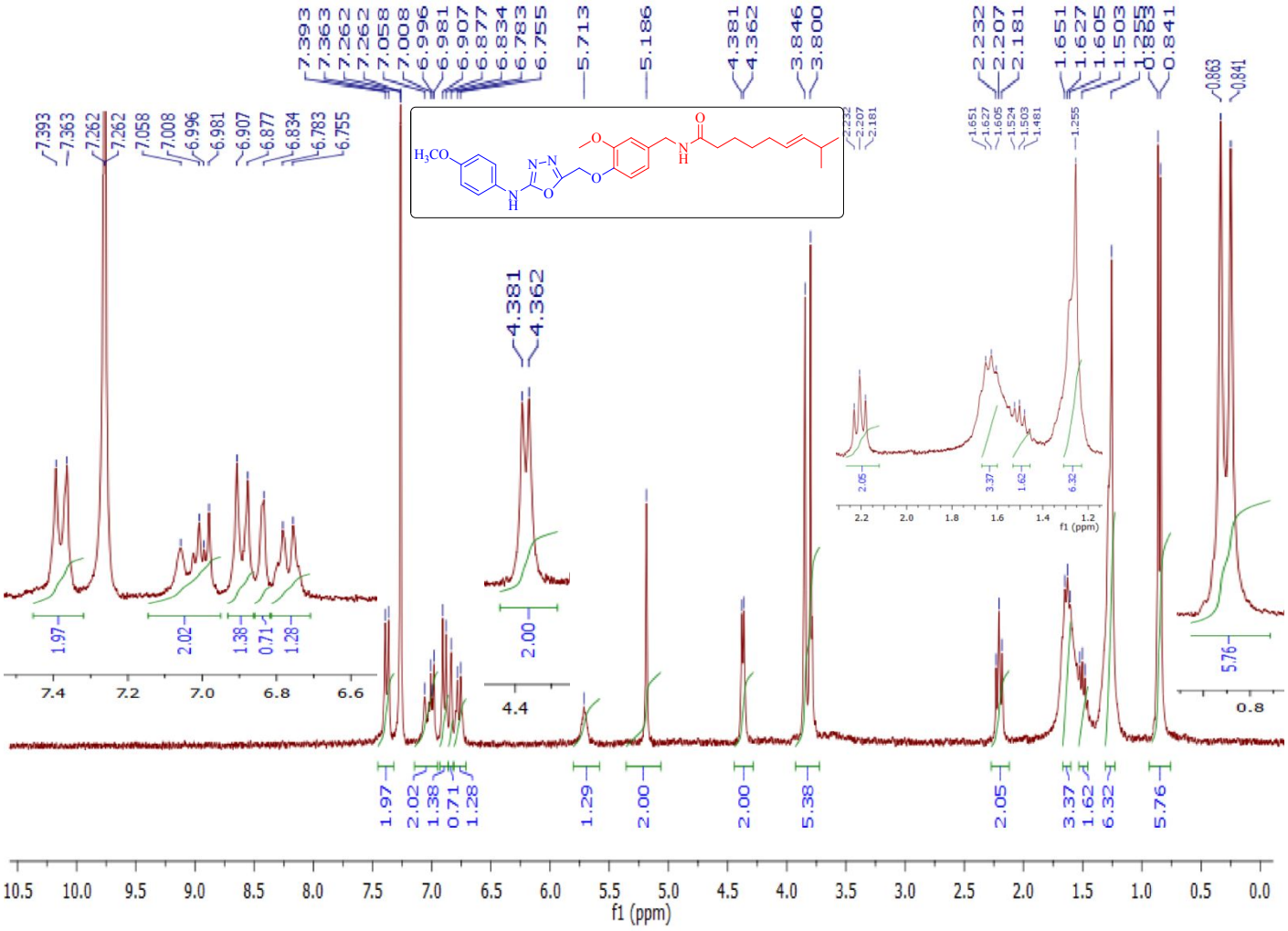
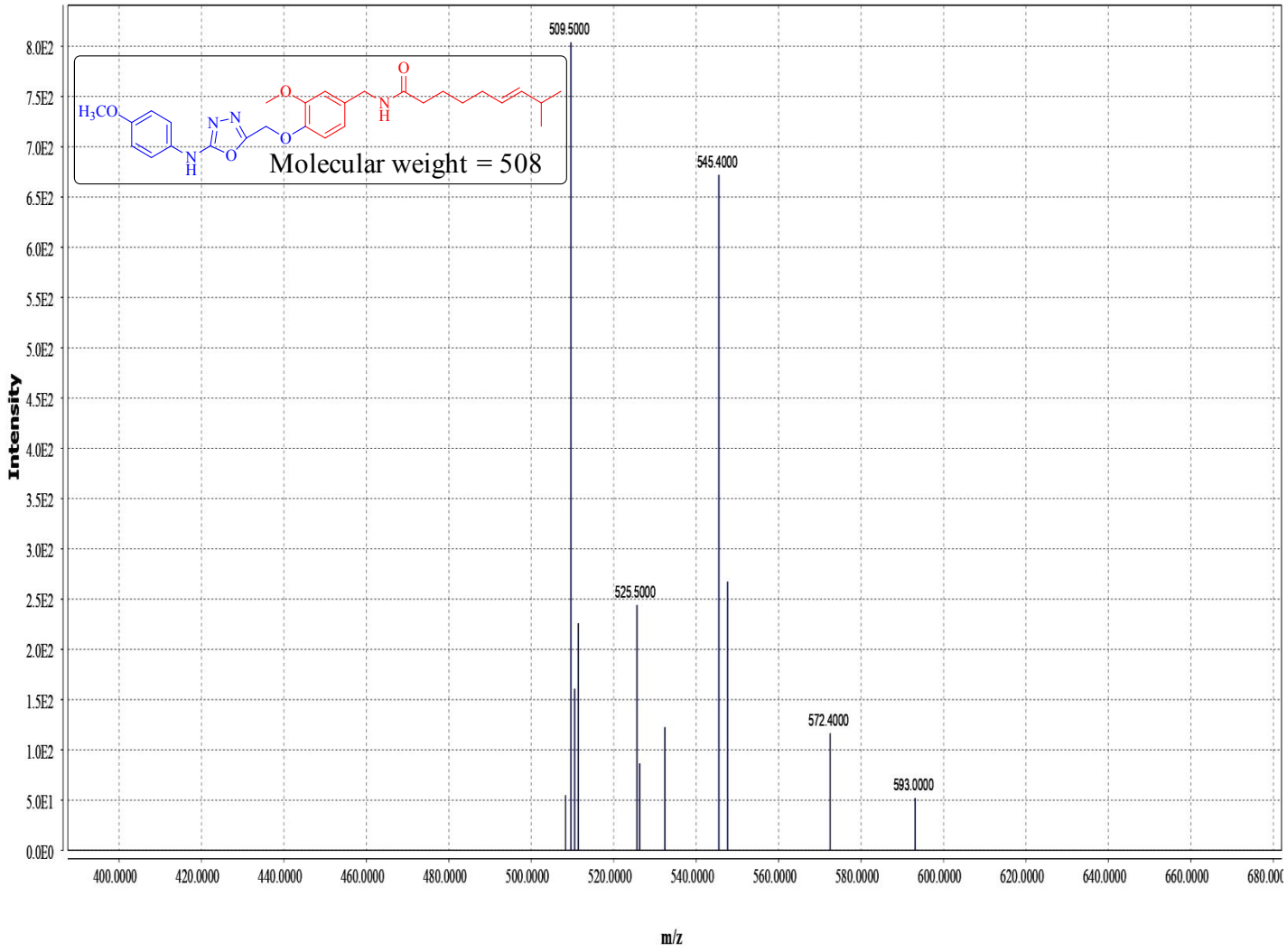


[1]H NMR of Compound 20g



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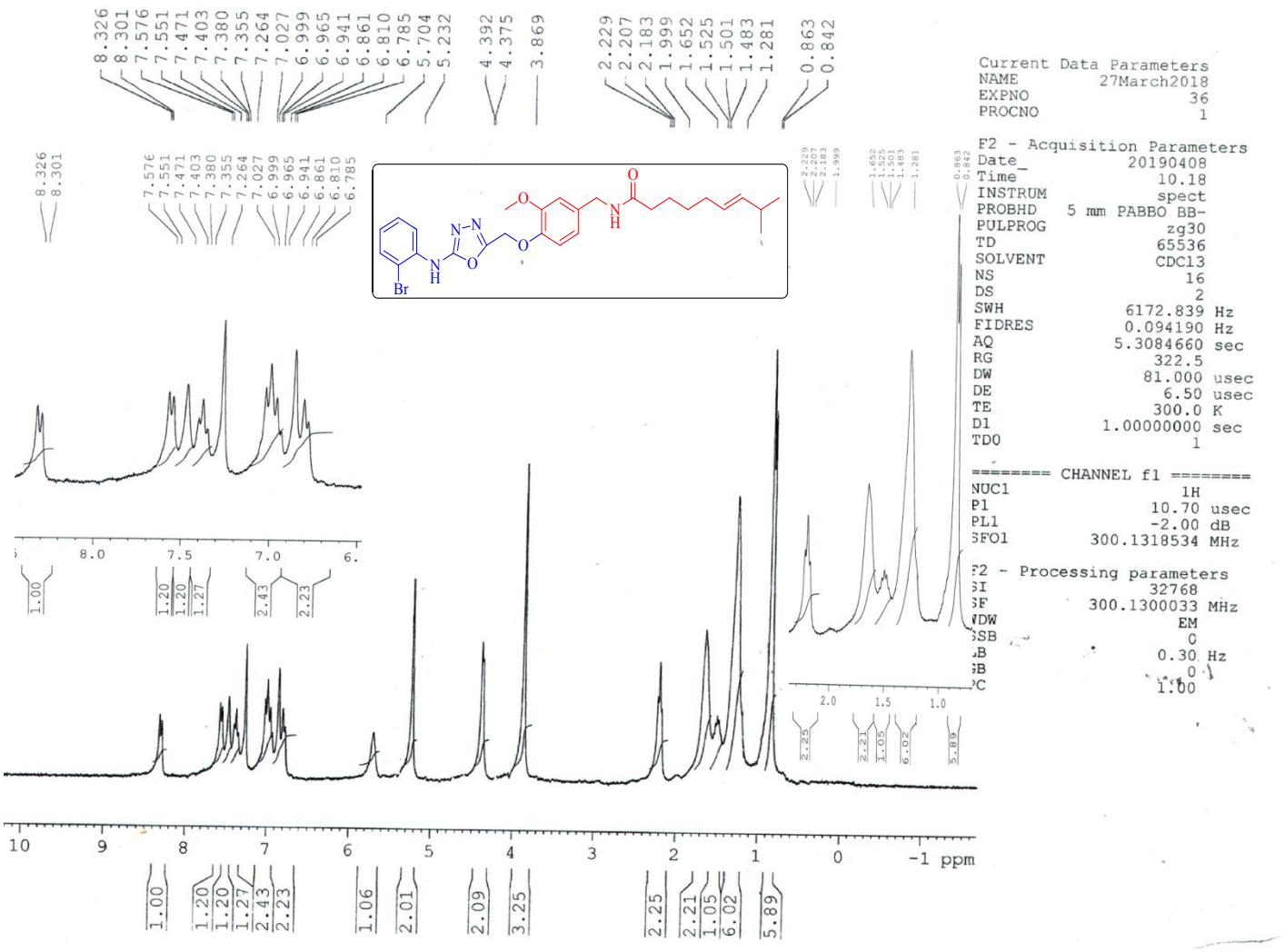
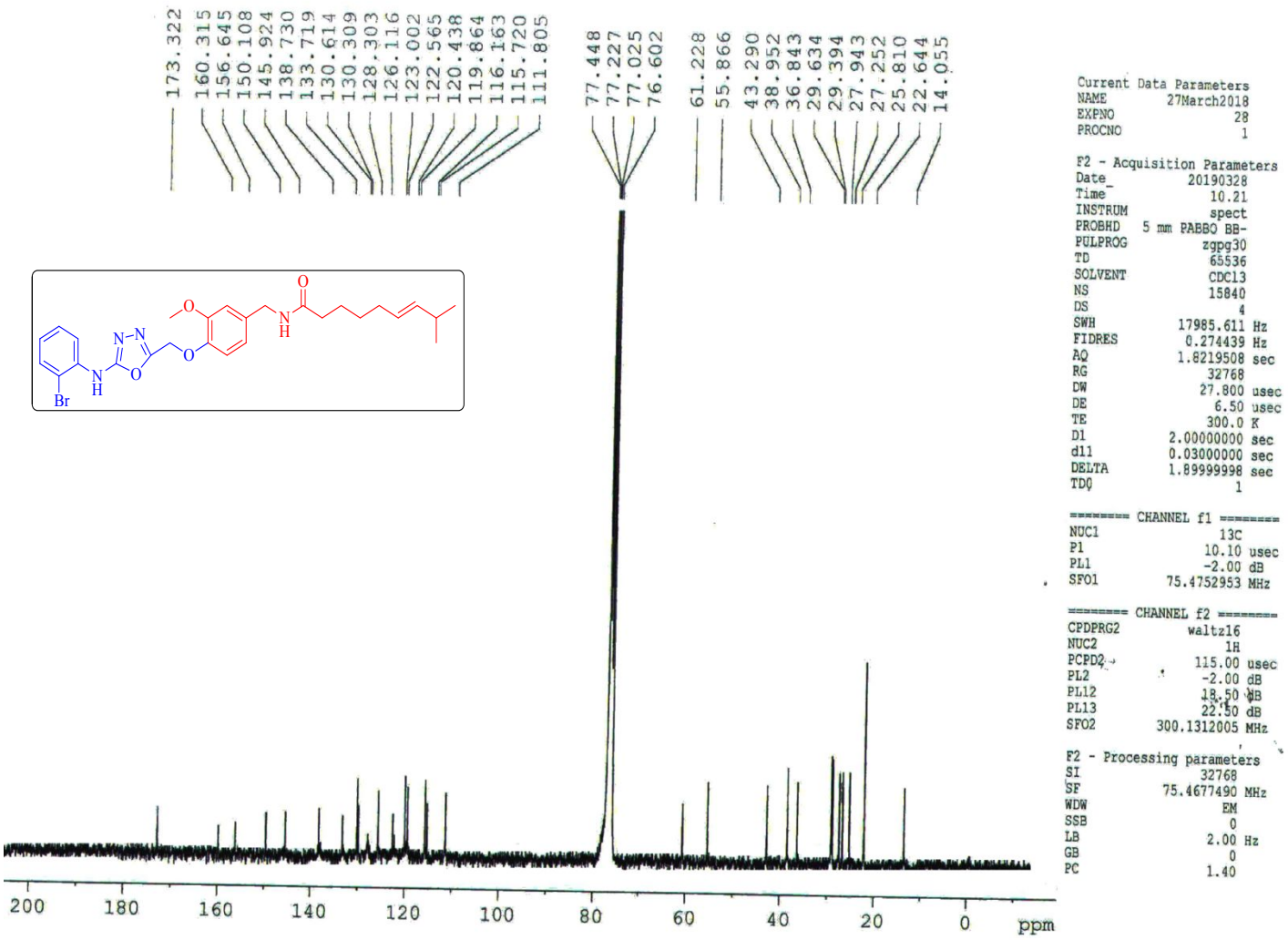


^{1}H NMR of Compound 20h



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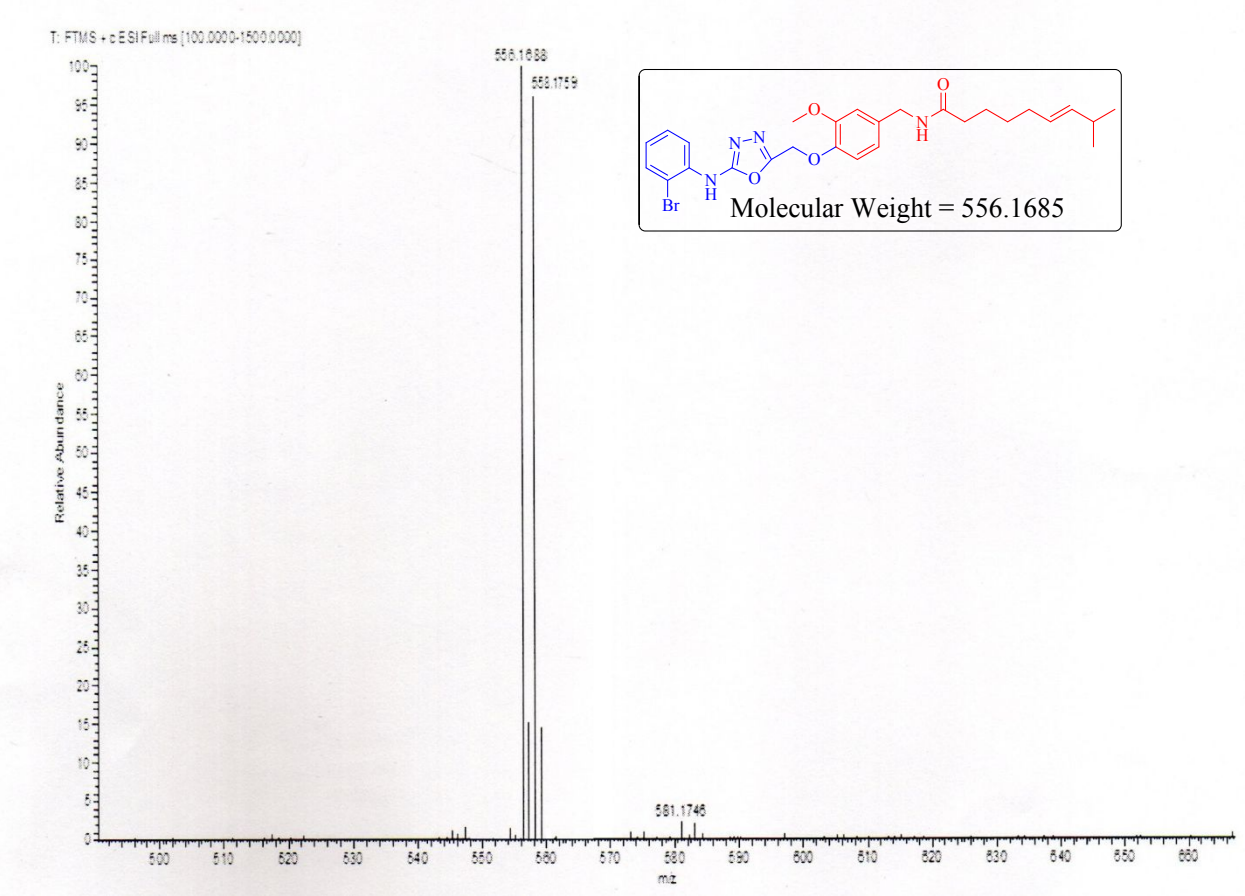

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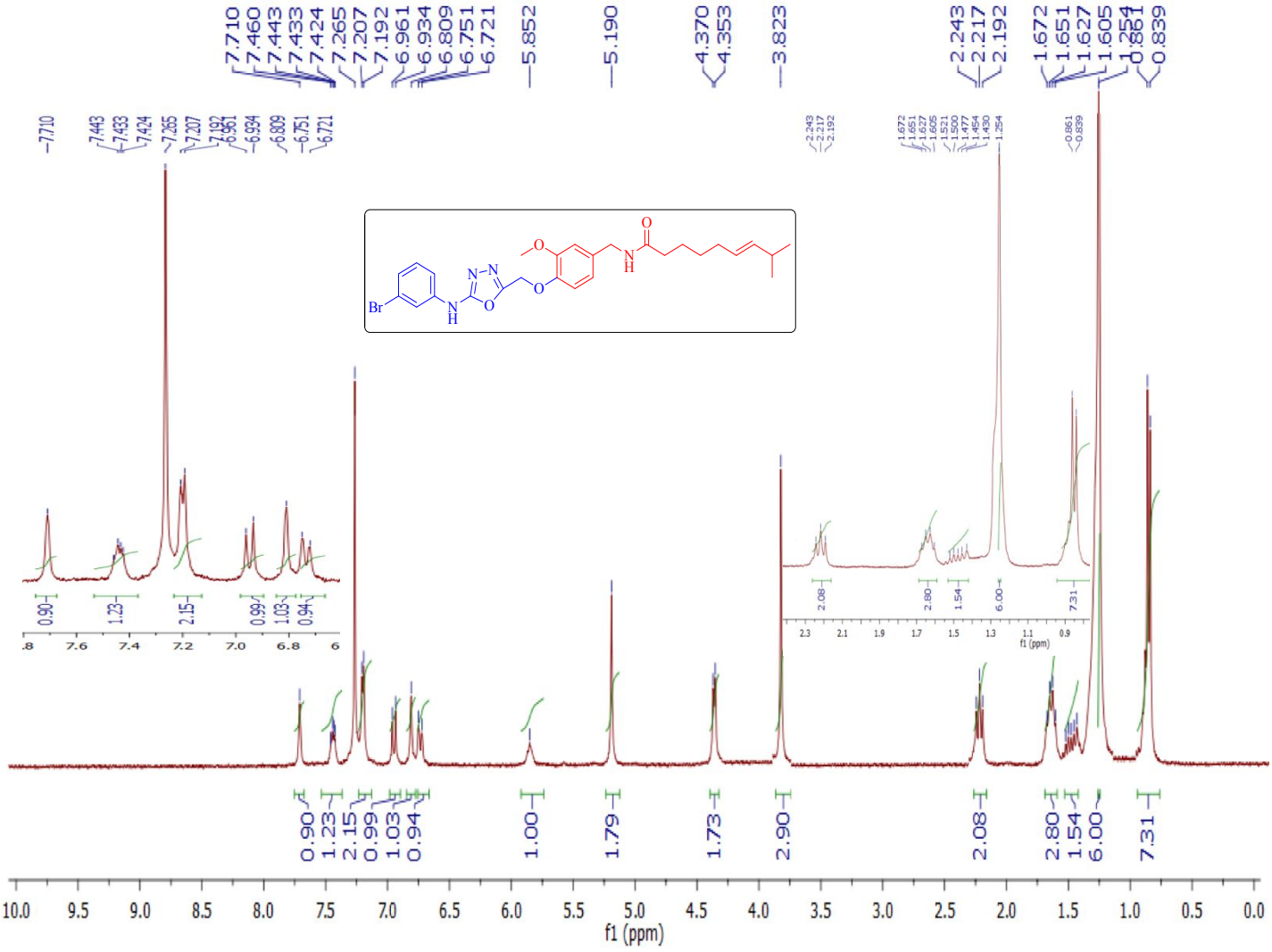

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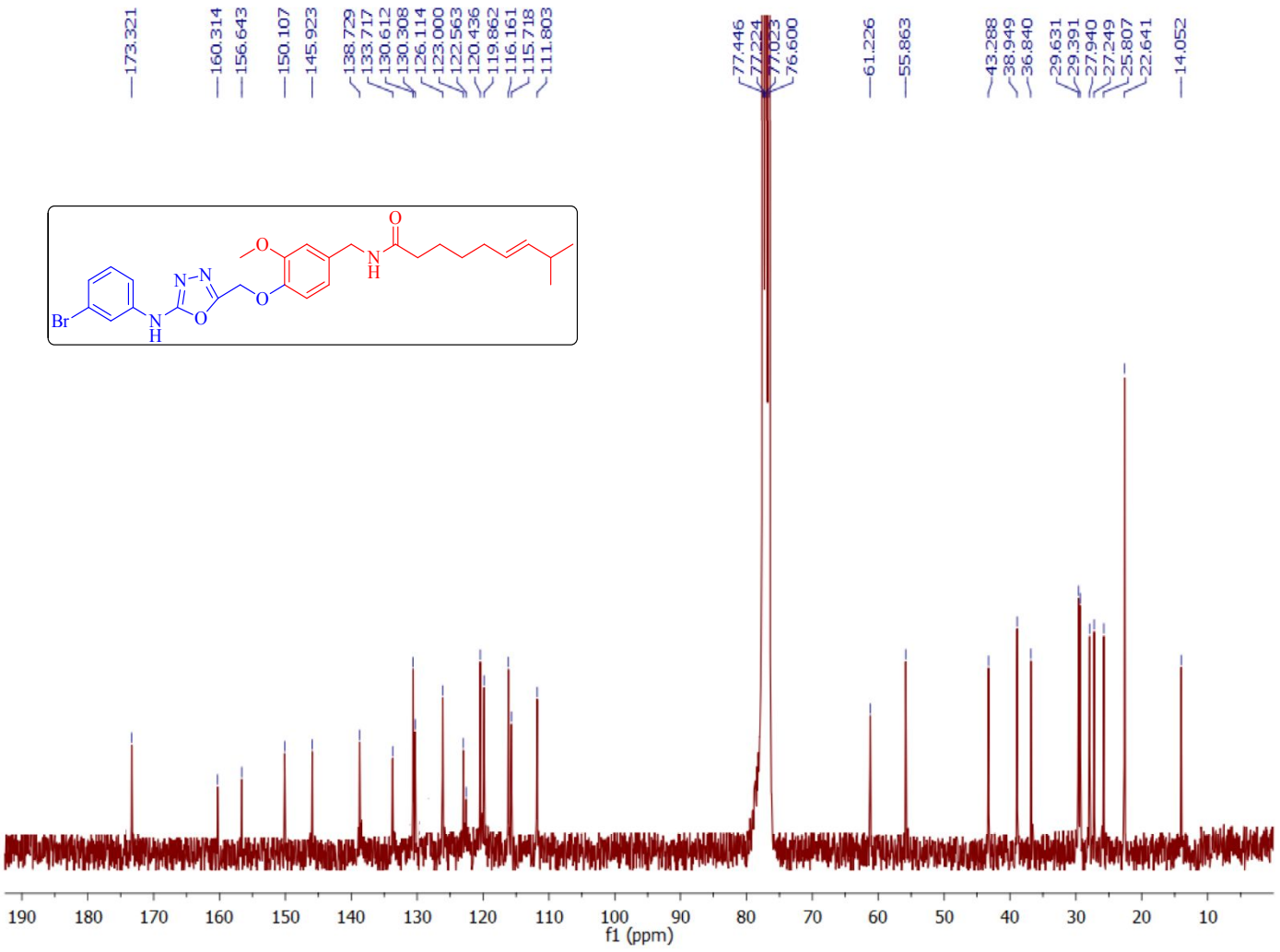

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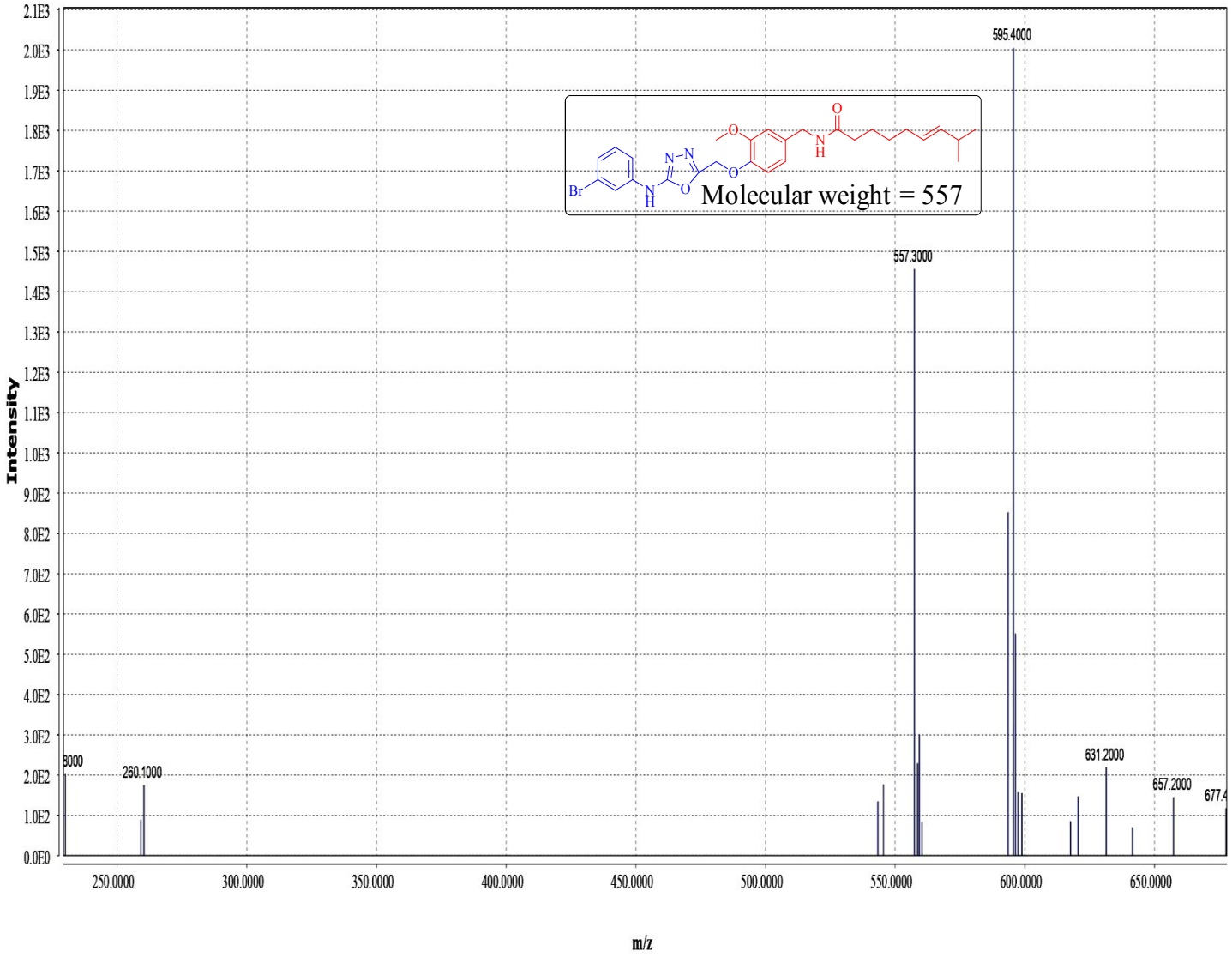

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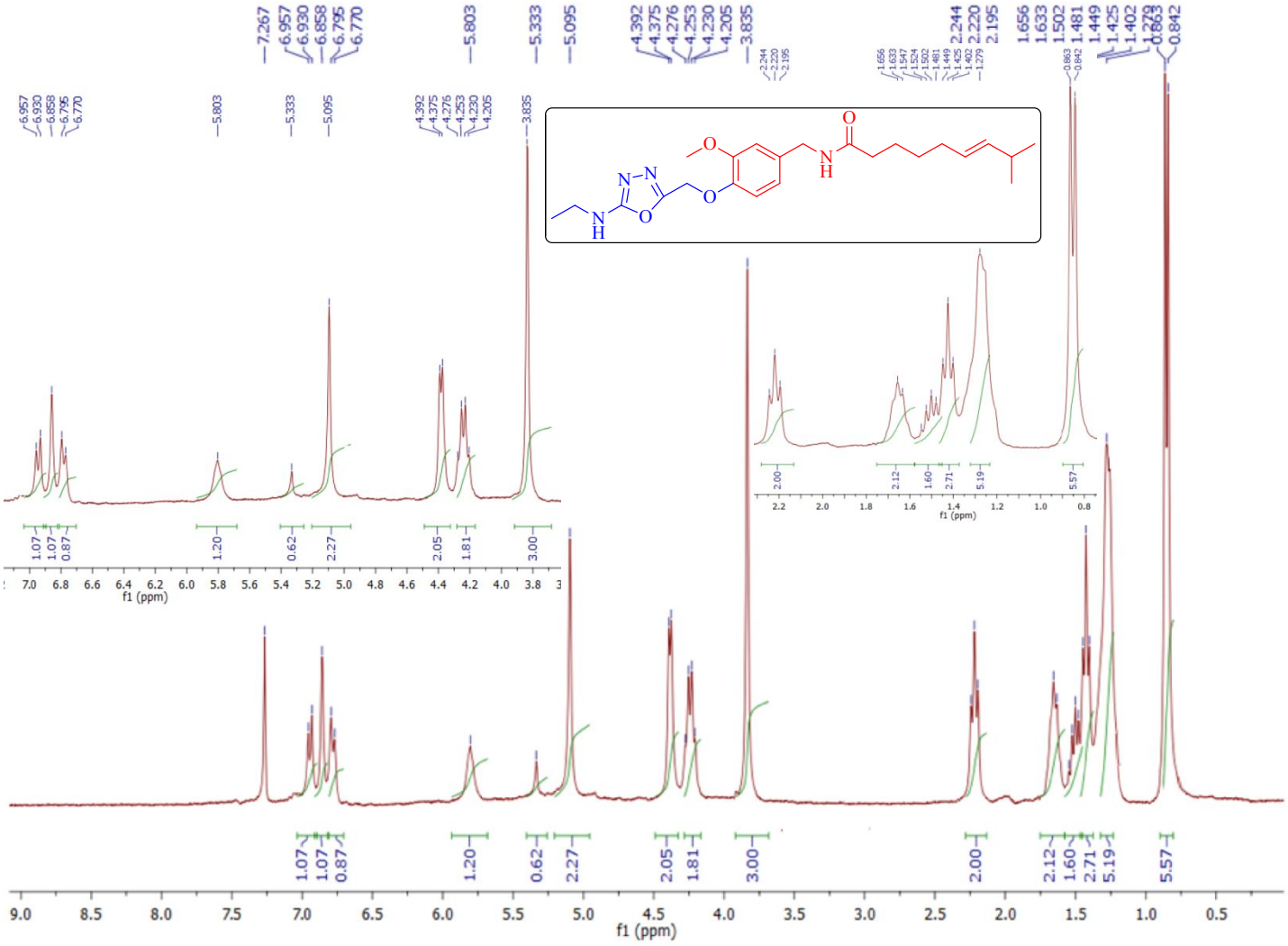

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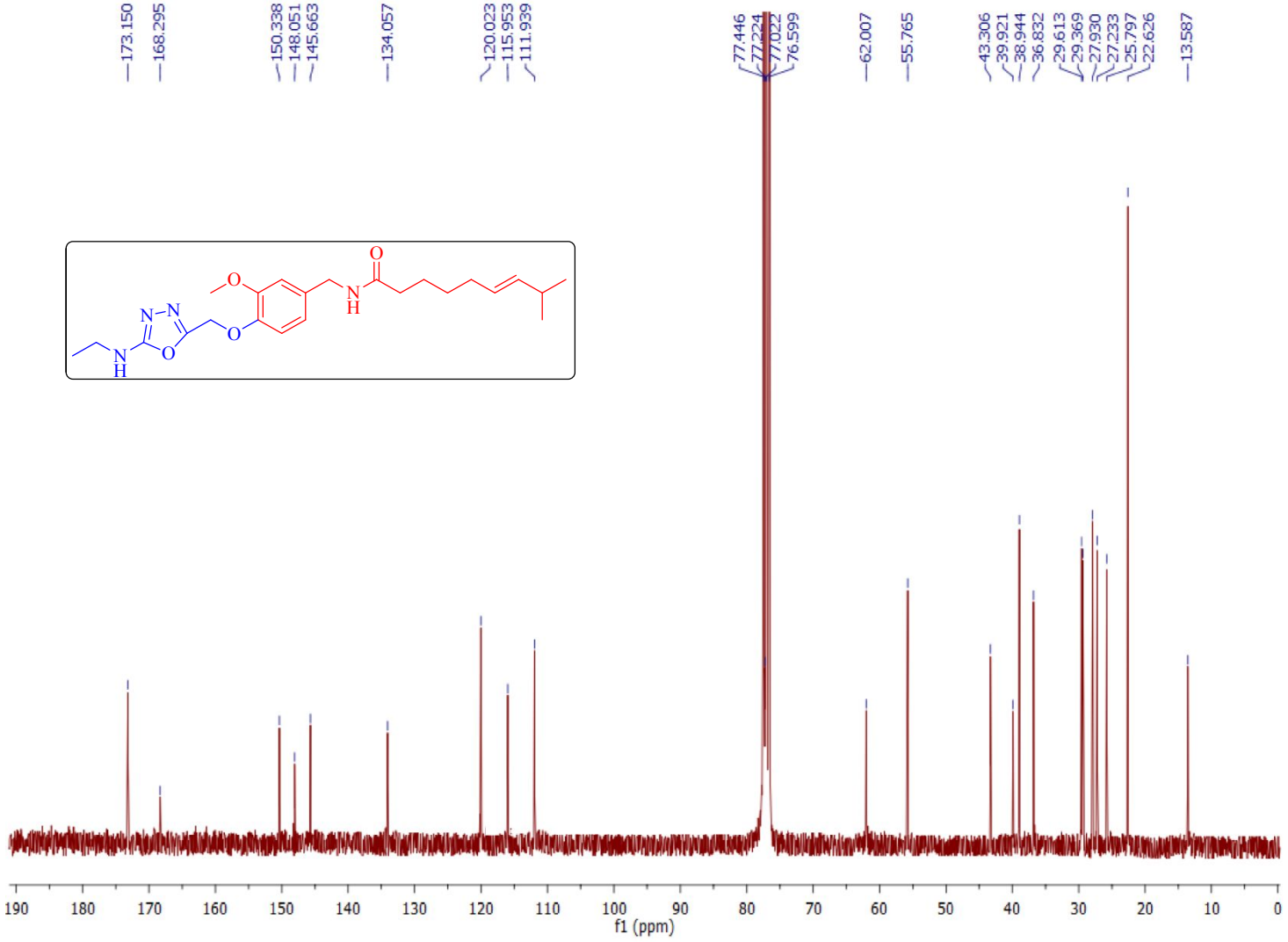

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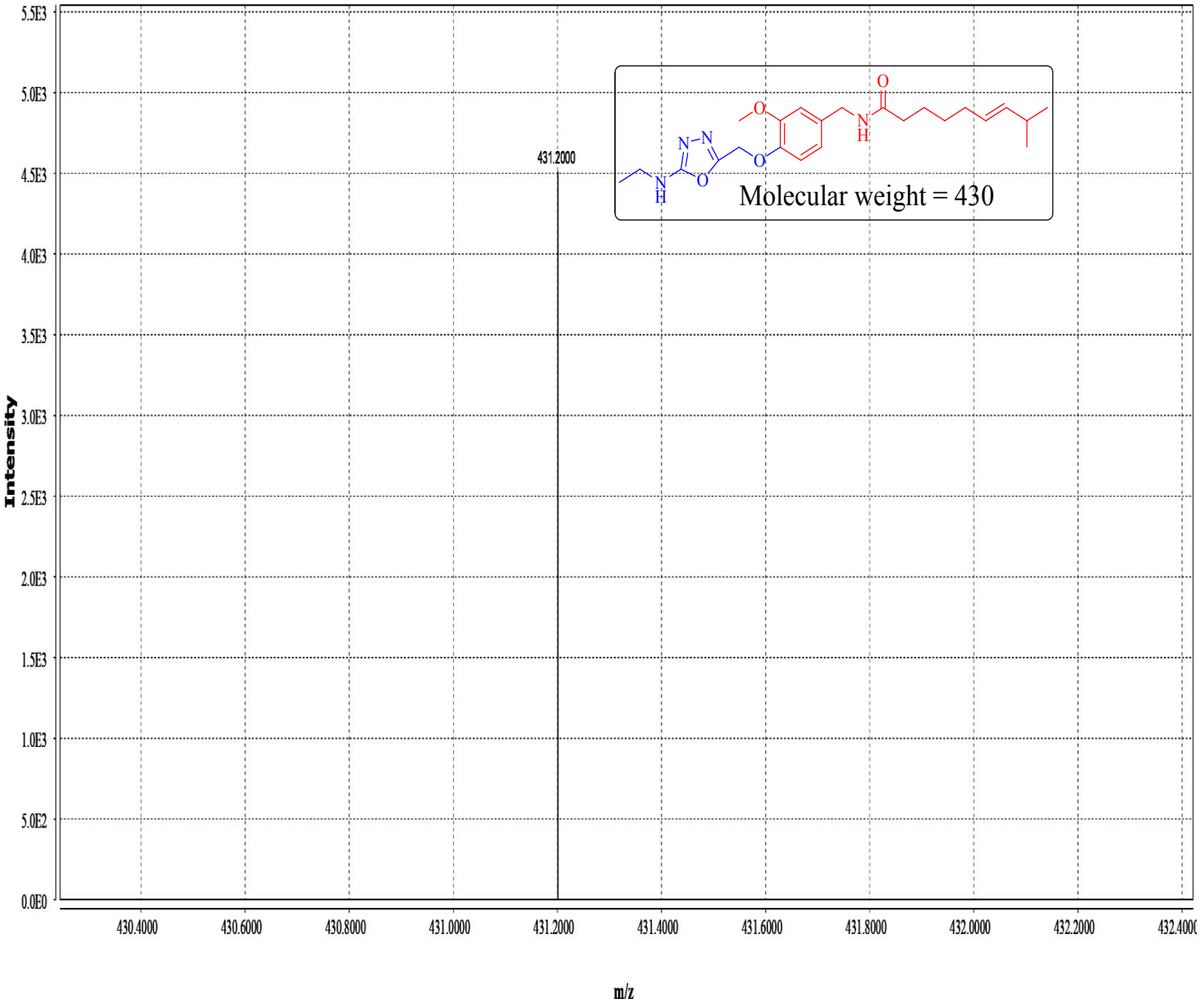

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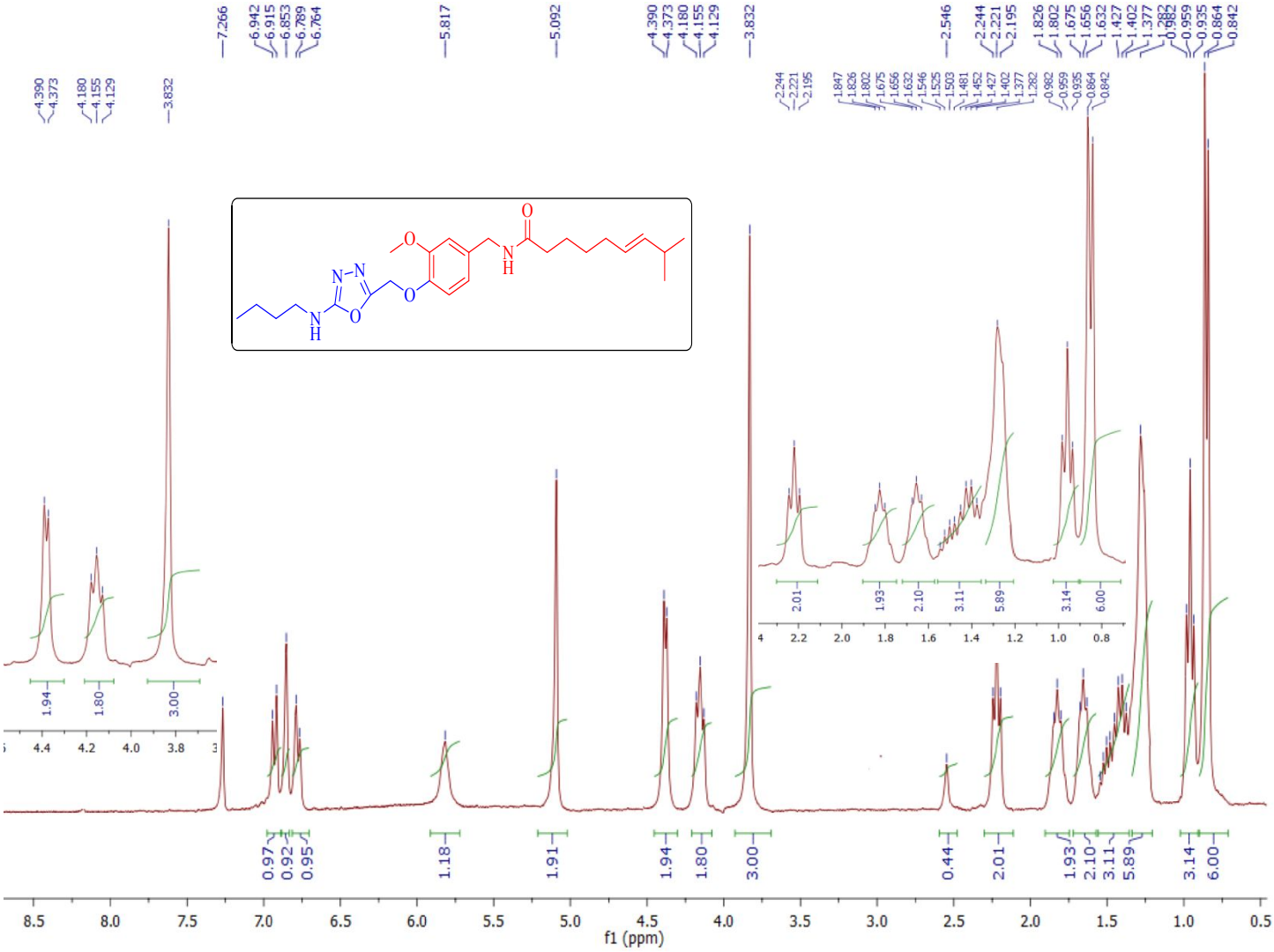

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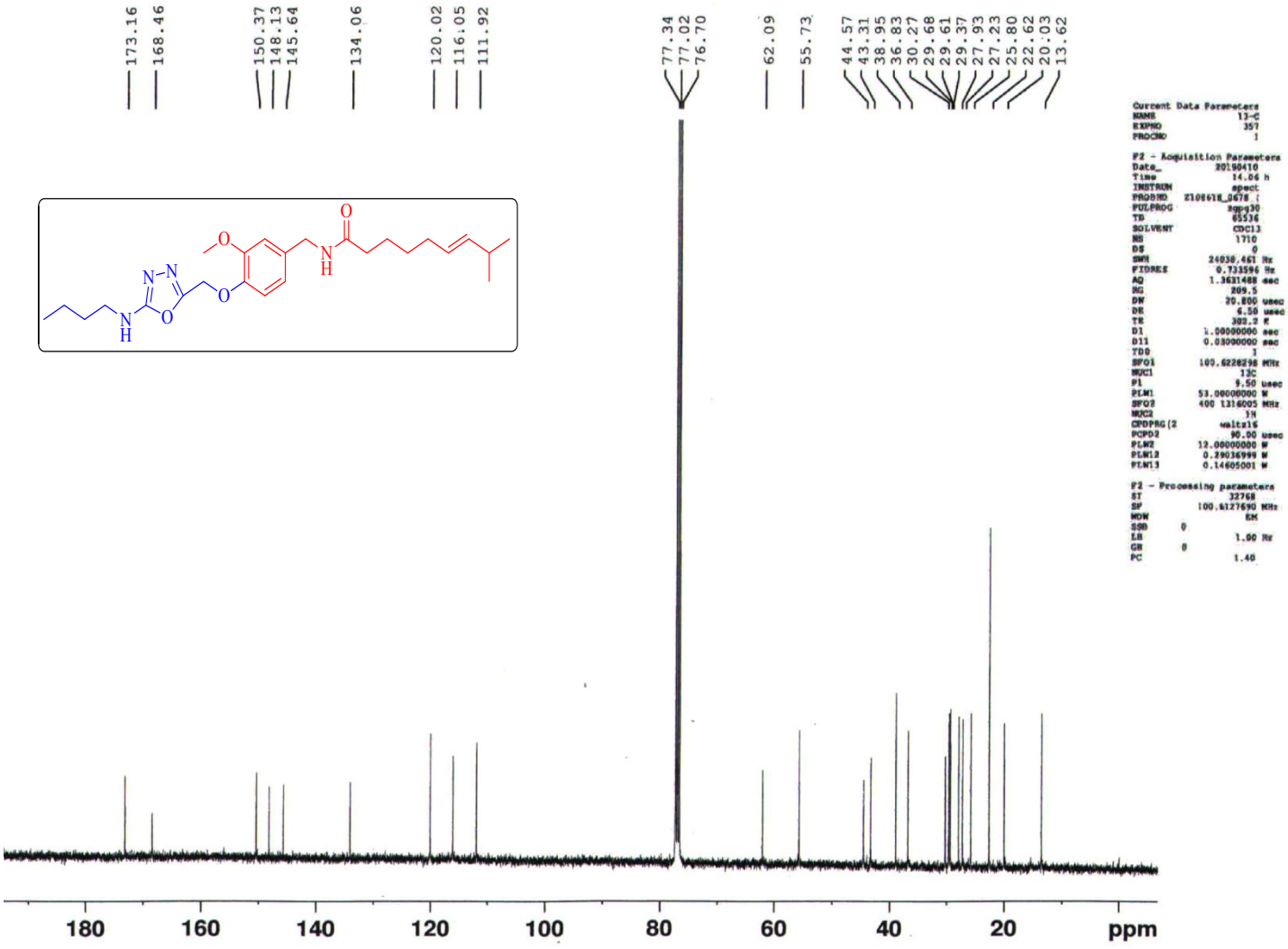

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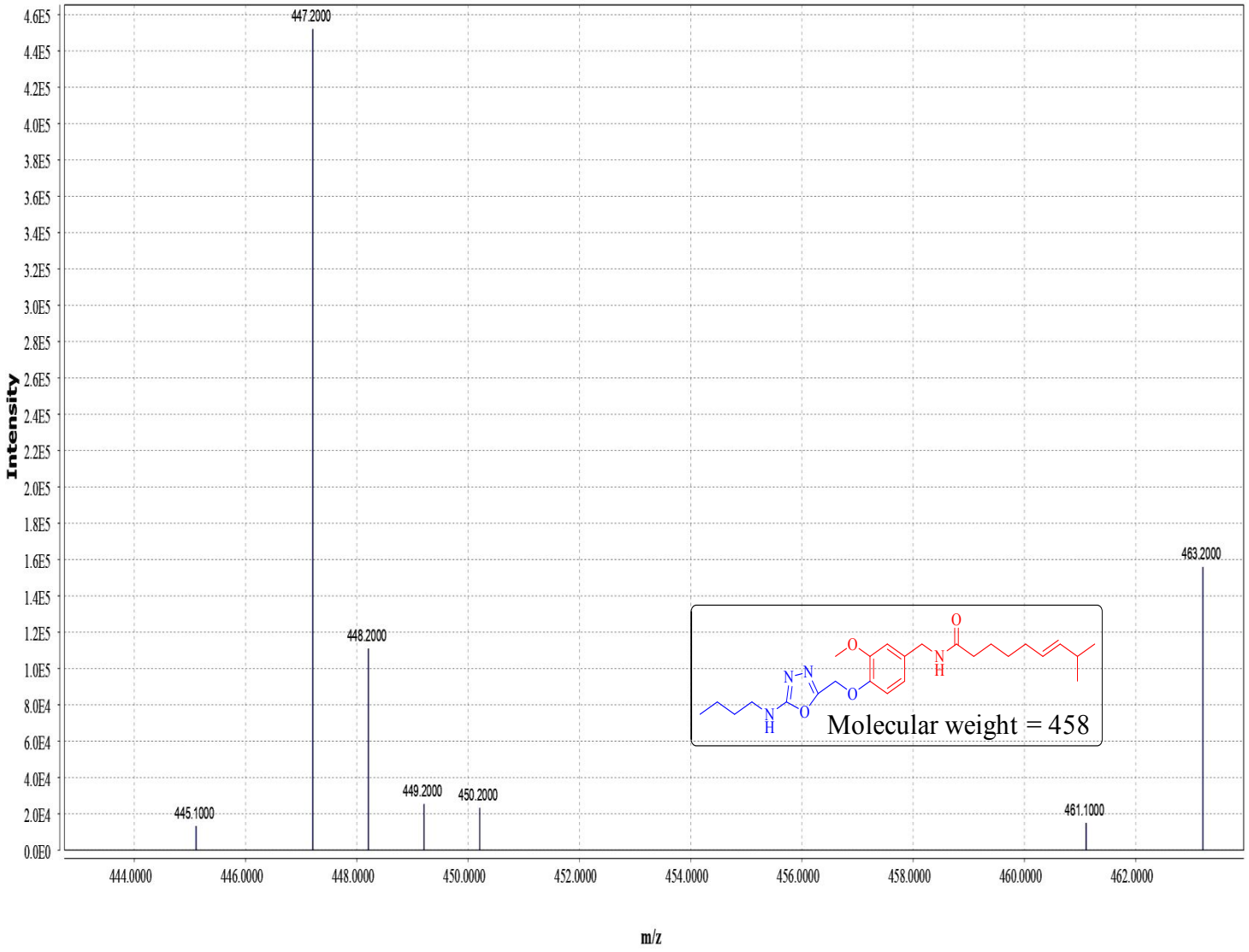

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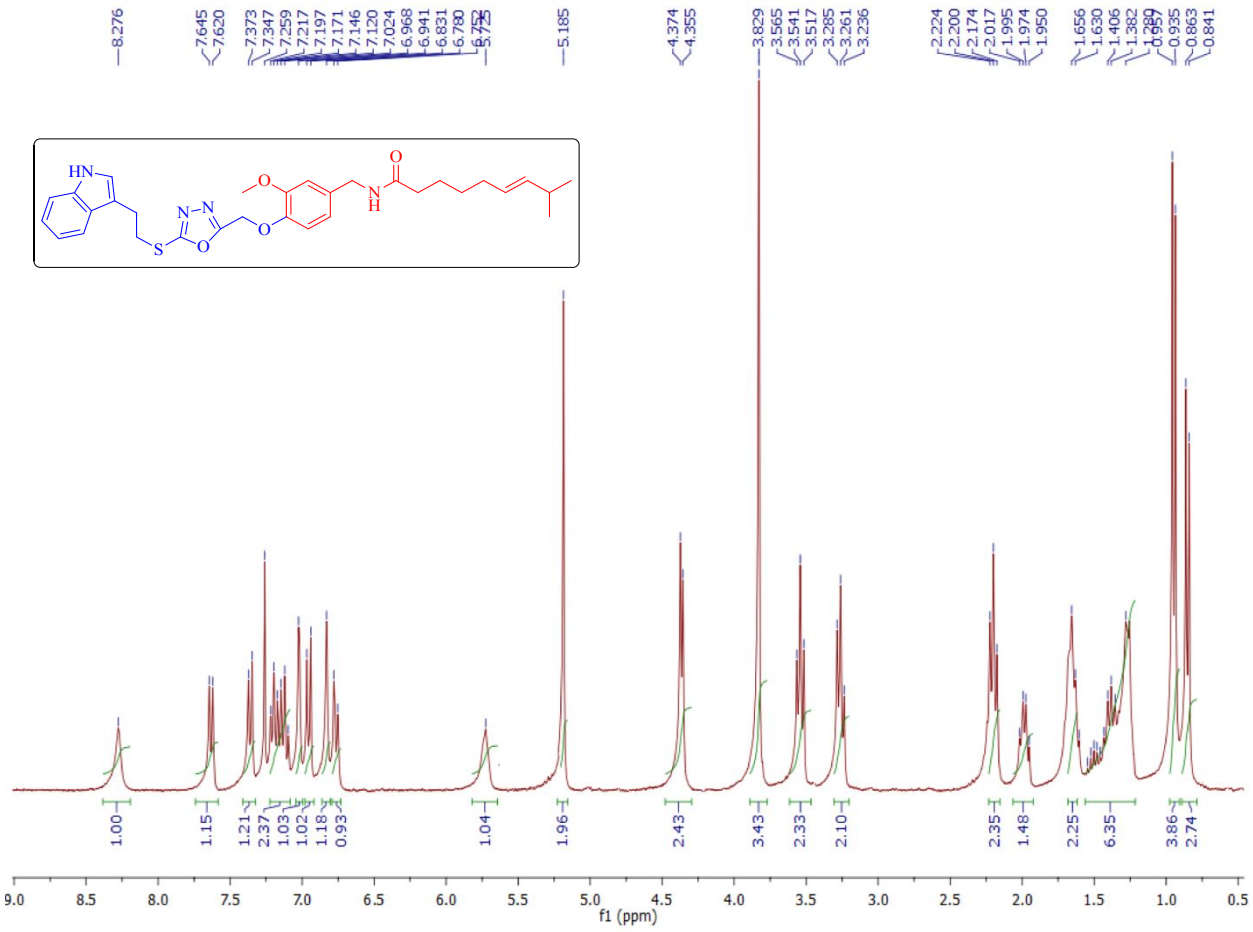

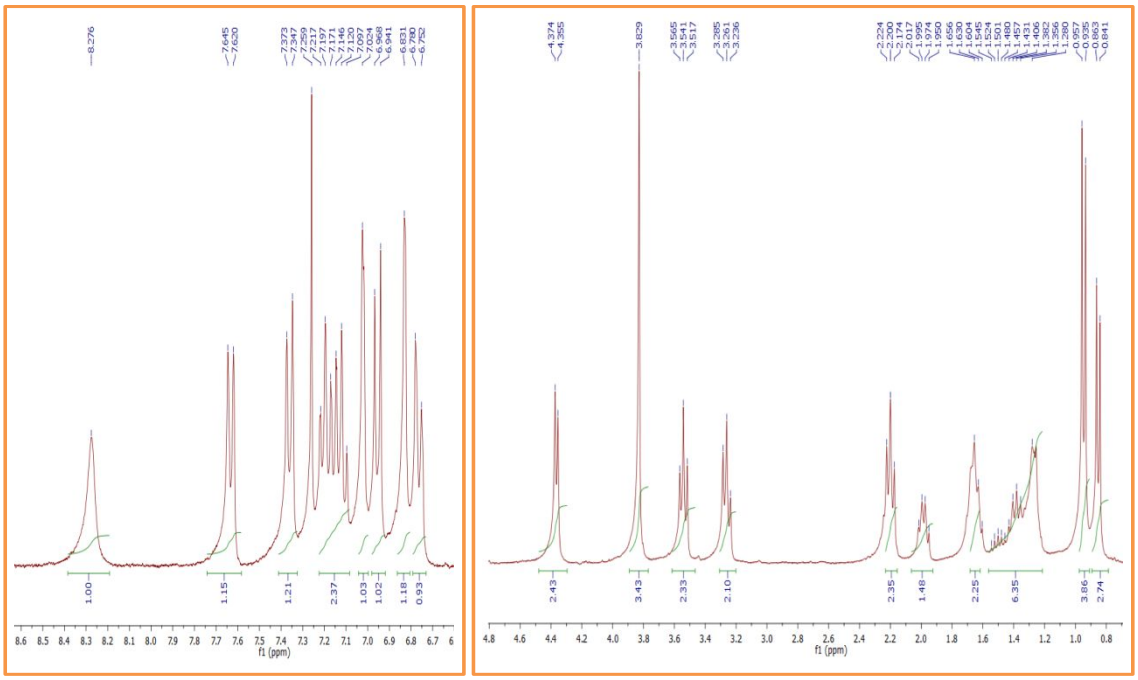

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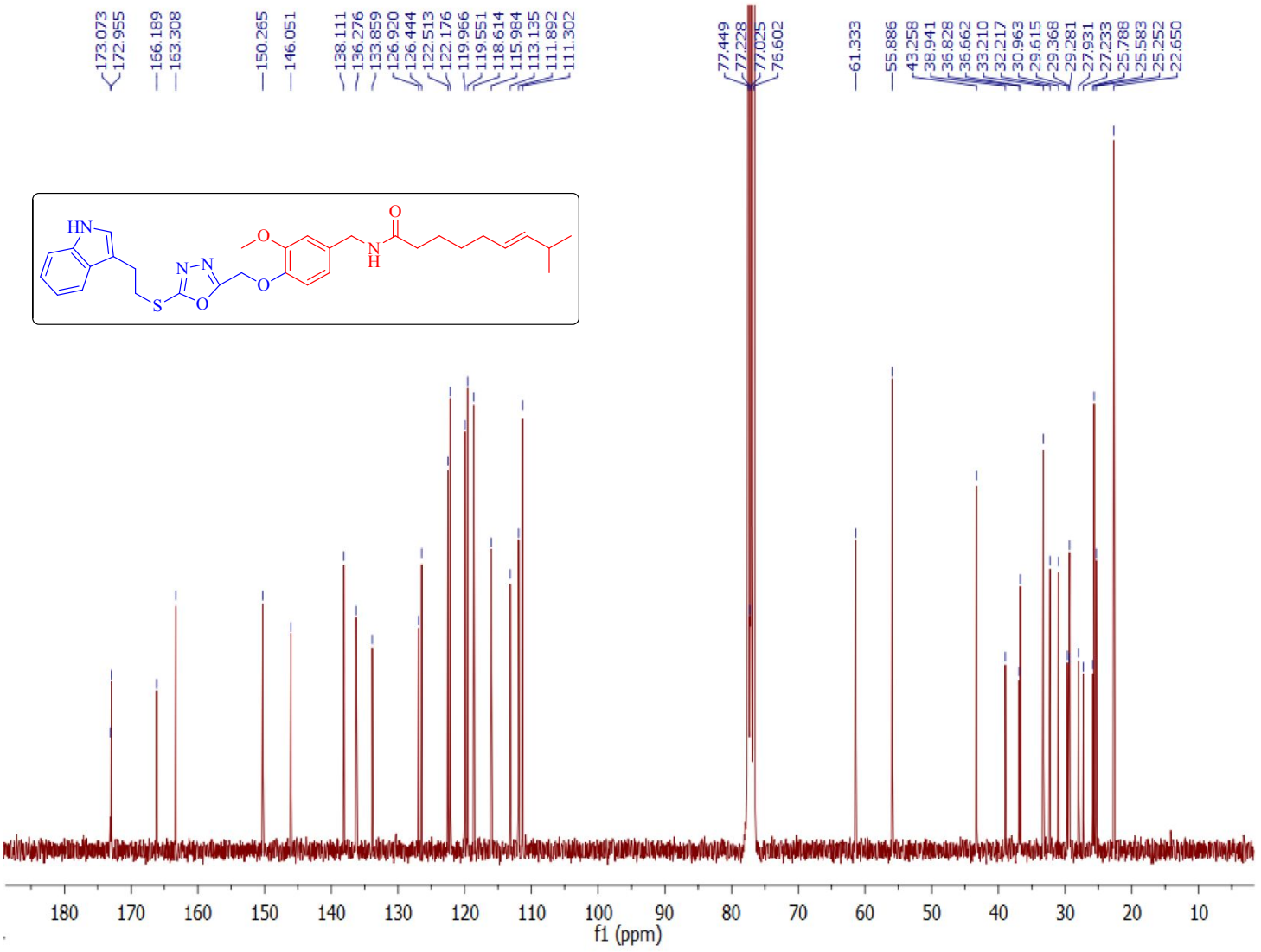

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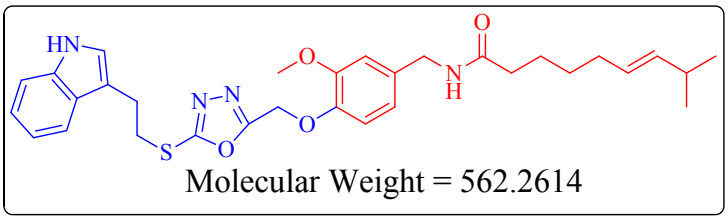
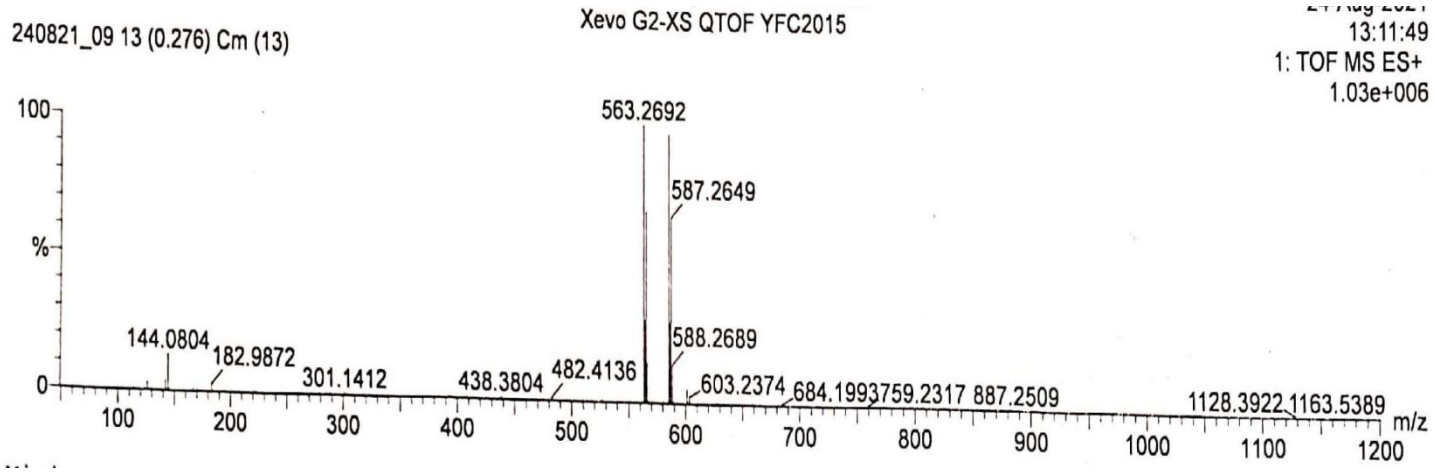


Molecular Weight = 562.2614



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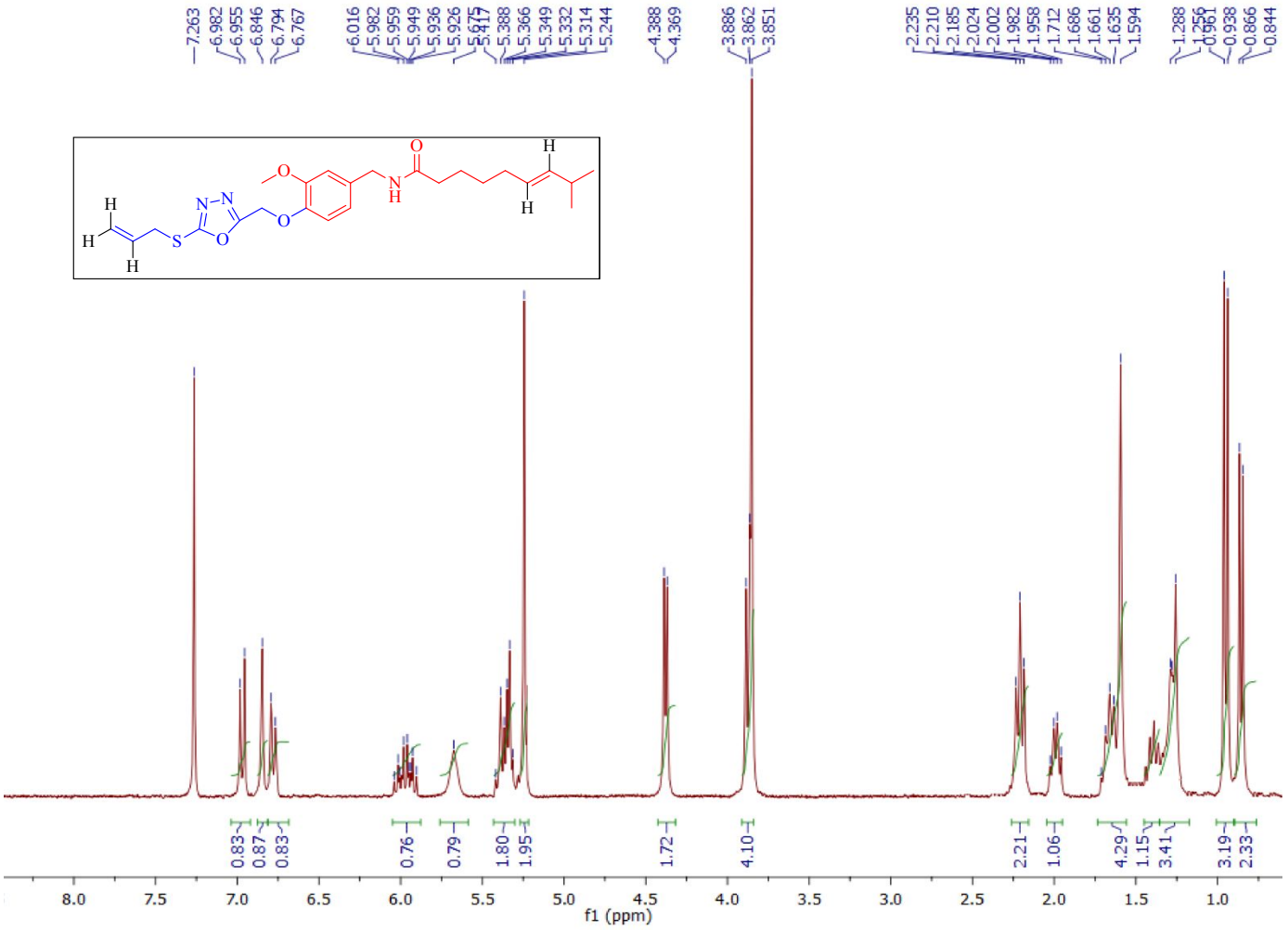

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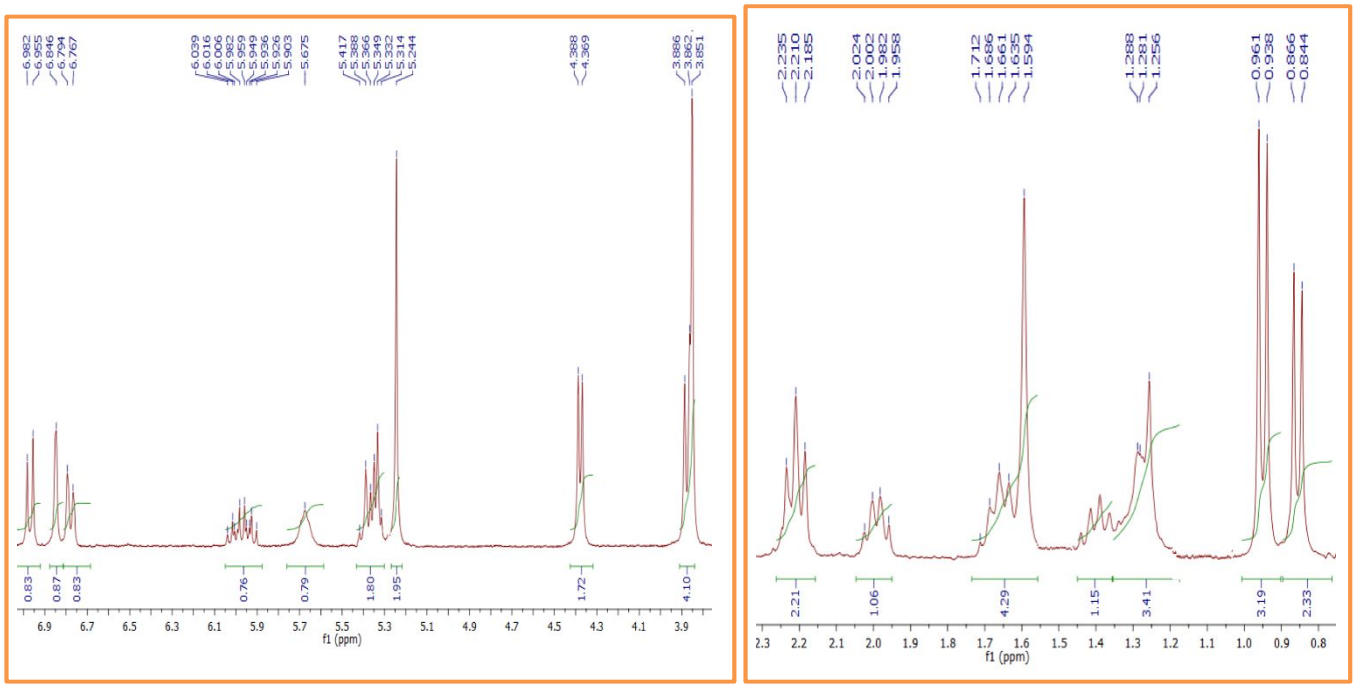

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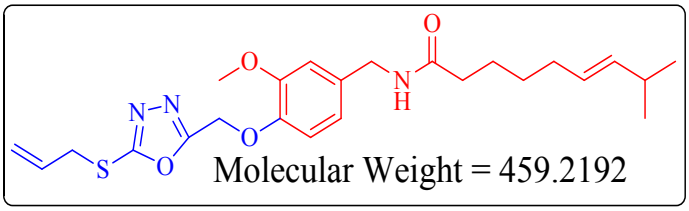

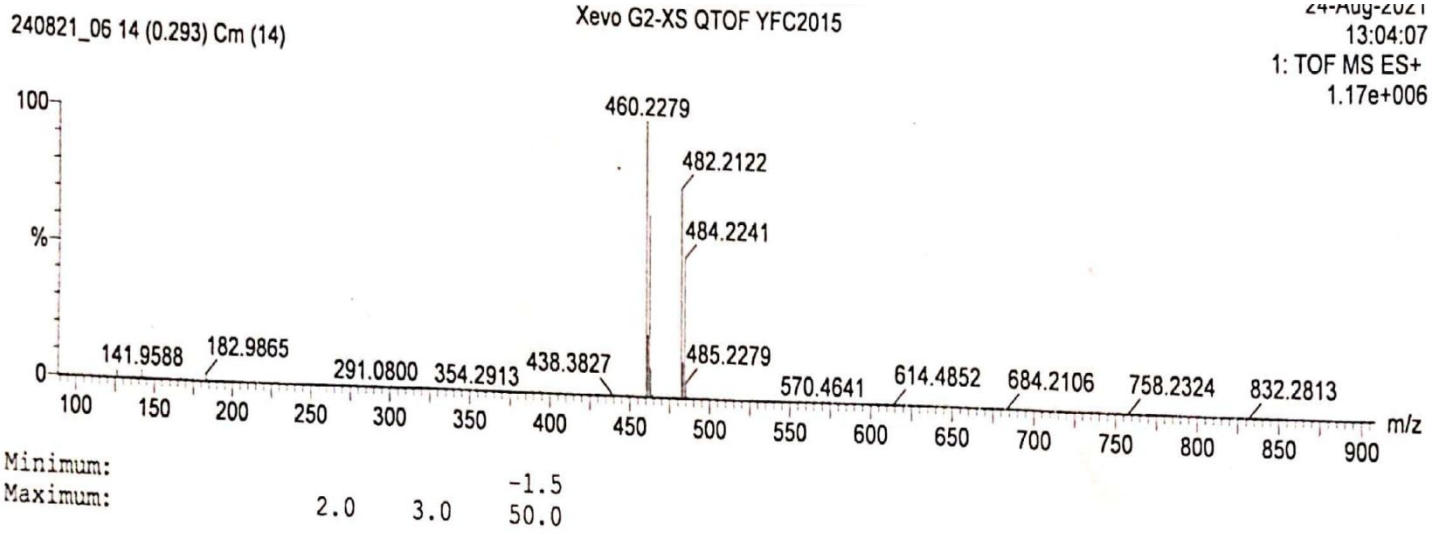

HRMS of compound 22b