Available online at www.worldscientificnews.com

World Scientific News

An International Scientific Journal

WSN 202 (2025) 34-64 EISSN 2392-2192

Synthesis and evaluation of novel derivatives with anticancer activities: benzoxazole-linked 1,3,5-triazine thioethers, Isatin-based compounds

Mukesh B. Parmar[1,2], Jignesh H. Pandya[1]*, Manisha K. Vara[2], Manisha Modak[3], Ghanshyam L. Jadav[1], Krishna K. Joshi[4]

[1]Department of Chemistry, D. K. V. Arts & Science College, Jamnagar, Gujarat-361008, India

[2]Envitro Laboratories Private Limited, Envitro Group of Companies, Rajkot, Gujarat-360004, India

[3]Department of Zoology, Sir Parashurambhau College, Pune, Maharashtra-411030, India

[4]Department of Microbiology, Atmiya University, Rajkot, Gujarat – 360005, India

*Corresponding E-mail address: jhpandya@gmail.com (J. H. Pandya)

ABSTRACT

This study integrates the synthesis, characterization, and biological evaluation of two classes of compounds: Isatin derivatives, and benzoxazole-linked 1,3,5-triazine thioethers. For synthesis of substituted 4,6-bis(benzo[*d*]oxazol-2-ylthio)-*N*-phenyl-1,3,5-triazine-2-amine derivatives, condensation of 2-aminophenol using carbon disulfide in the presence of potassium hydroxide gave the benzo[*d*]oxazole-2-thiol. On the other side, 2,4,6-trichloro-1,3,5-triazine and various substituted aniline were put into the reaction, yielding various substituted 4,6-dichloro-N-phenyl-1,3,5-triazine-2-amine derivatives (P-01 to P-12). Finally, both intermediates react with each other and forms final triazine derivatives (JHP-01 to JHP-12). For the synthesis of Isatin derivatives, Isatin and 2-chloro-*N*-(4-(3-oxomorpholino)phenyl)acetamide was used to yield 2-(2,3-dioxoindolin-1-yl)-*N*-(4-(3-oxomorpholino) phenyl)acetamide. Further reaction with various substituted anilines to form substituted (*E*)-2-(2-oxo-3-(phenylimino)indolin-1-yl)-*N*-(4-(3-oxomorpholino)phenyl)acetamide (MMP-01 to MMP-12). A detailed comparison of their anticancer activities against the MCF-7 human breast cancer cell line highlights their therapeutic potential. Isatin and triazine derivatives demonstrated IC50 values as low as ~1.7 mg/mL. This comprehensive study underscores the structural features critical for anticancer efficacy and provides a framework for future medicinal chemistry research. Synthesis is confirmed using mass spectra, IR spectra, ^{1}H NMR and ^{13}C NMR.

***Keywords*:** Anticancer activity, benzo[*d*]oxazole-2-thiol, *s*-Triazine, Isatin, MCF-7 cell line.

(Received 10 January 2025; Accepted 15 March 2025; Date of Publication 9 April 2025)

1. INTRODUCTION

Cancer continues to pose a significant global health challenge, with breast cancer being the most prevalent among women. Chemotherapeutic agents, while effective, often come with severe side effects, driving the need for novel therapeutic compounds. It's interesting to note that *s*-Triazine-based compounds have emerged as a crucial element of medical and pharmaceutical chemistry. Clinically successful triazine and isatin-based drugs are shown in **Figure 1**, such as enasidenib, simazine, atrazine, cyanazine, propazine, altretamine, tretamine, sunitinib, nintedanib, orantinib, semaxanib, metisazone, and toceranib.

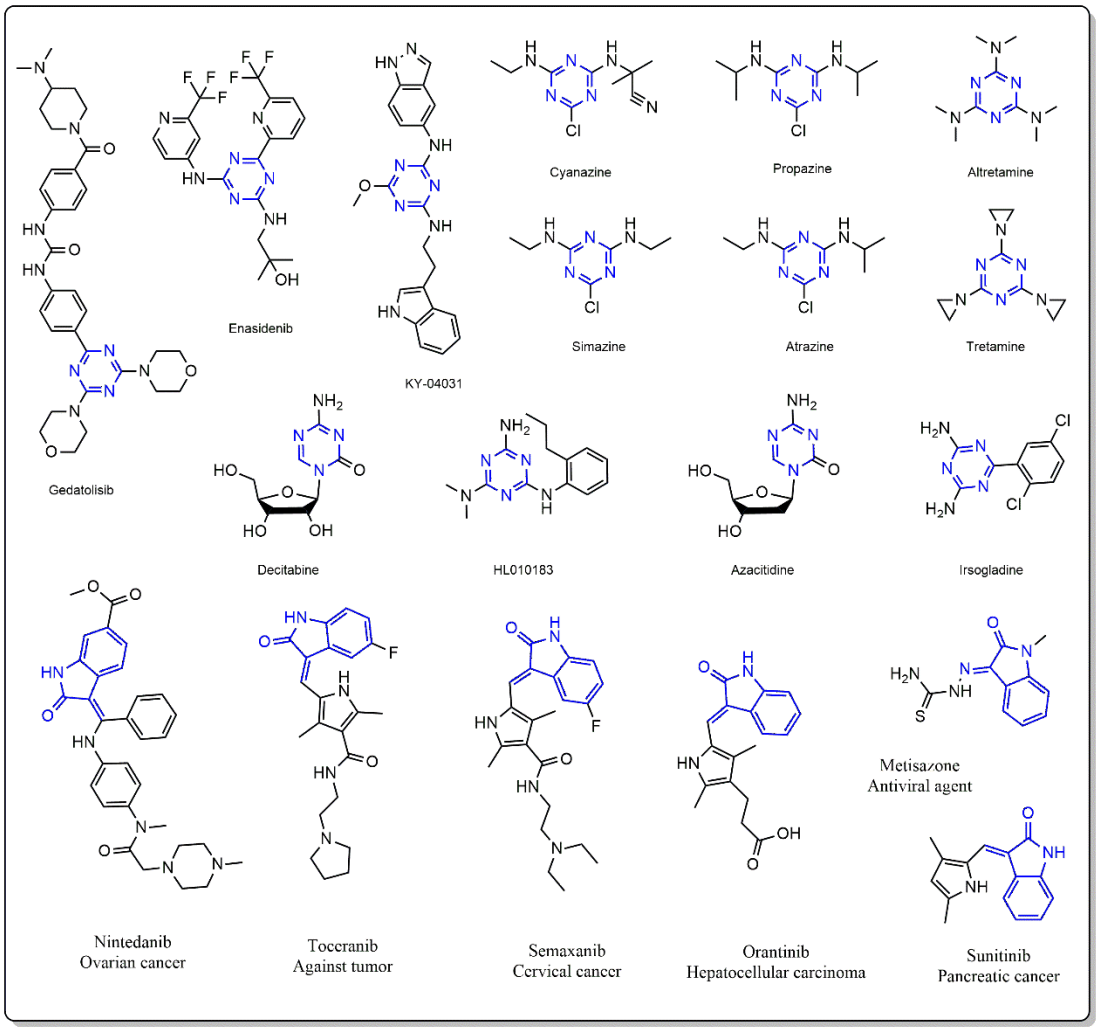


Figure 1. Commercially available drugs containing *s*-Triazine and Isatin scaffold.

Owing to *s*-Triazinessymmetrical scaffold, has attracted a lot of research for the design of new compounds as it facilitates the synthesis of diverse types of analogs which has an active biological history [1]. Similarly, Isatin derivatives have demonstrated a broad spectrum of pharmacological activities, including anticancer properties [1].

Contributing to the synthesis library of *s*-Triazines, we develop a convenient synthesis route for substituted 4,6-bis(benzo[*d*]oxazol-2-ylthio)-*N*-phenyl-1,3,5-triazine-2-amine derivatives (JHP-01 to JHP-12) as shown in **scheme-1**. Compound benzo[*d*]oxazole-2-thiol also known as 2-mercaptobenzoxazole was synthesized using 2-aminophenol and carbon disulphide [2, 3]. 2,4,6-trichloro-1,3,5-triazine and various substituted aniline were put into the reaction and yielded various substituted 4,6-dichloro-*N*-phenyl-1,3,5-triazine-2-amine derivatives (P-01 to P-12) [4]. Further, both intermediate react and form final products (JHP-01 to JHP-12) [4, 5].

In our **scheme-2**, we developed an efficient methodology for the synthesis of Isatin related derivatives. We use 4-(4-aminophenyl)morpholin-3-one and chloroacetyl chloride to yield 2-chloro-*N*-(4-(3-oxomorpholino)phenyl)acetamide (INT-01) [6]. Further reaction with Isatin yields 2-(2,3-dioxoindolin-1-yl)-*N*-(4-(3-oxomorpholino)phenyl)acetamide (MM) [6]. Further reaction with various substituted anilines forms final compounds (MMP-01 to MMP-12) [7-10]. Physiochemical data, cytotoxicity result of synthesized compounds are given in **Table 1**.

2. EXPERIMENTAL

2.1. General Materials and Methods

Chemicals were procured from Sigma-Aldrich and Loba Chemie. Reactions were monitored using thin-layer chromatography (TLC) with E-Merck silica gel GF254 plates. In open capillaries, the melting points of synthesized substances were checked. The IR spectra of compounds were captured on an FTIR spectrophotometer using the KBr pellet technique. Tetramethylsilane (TMS) was used as an internal standard in ^{1}H NMR & ^{13}C NMR spectra of synthesized compounds captured on Bruker 400-MHz NMR spectrometer in DMSO-d6 solvent. Mass spectra were recorded on GC-MS and Shimadzu LC-MS/MS. Elemental data was recorded by Vario microcube elemental analyzer.

2.2. Cell Culture Procedure

MCF-7 cells (Breast cancer cell line) were procured from National Centre for Cell Sciences (NCCS), Pune. The cells were maintained at 37°C under 5% CO_2 in complete DMEM medium.

2.3. Cytotoxicity by MTT Assay [11]

Cytotoxicity of the nanoparticles was checked by MTT assay against MCF-7 cell line. Briefly, 1 x 10^4 cells were plated per well with 0.2ml medium in 96 well culture plates and were incubated at 37 °C under 5% CO_2 conditions for 24 hrs. Five different concentrations of compounds all derivatives (1, 10, 100, 1000, 10000 µg/ml) were added to the well. DMSO was kept as negative control and cells were incubated for 24 hrs. After 24 hrs, the media was carefully removed, and 200ul MTT reagent (5mg/10ml PBS) was added. The plates were incubated at 37°C under 5% CO_2 conditions for 4 hrs. MTT reagent was discarded, and 150ul of DMSO was added to solubilise purple formazan crystals. Absorbance of the colour was then measured at 570 nm using a microplate reader (Thermo Scientific). Control untreated were considered as 100 % survival, and accordingly, the % survival for rest was calculated. Standard graph was plotted by taking concentration of the nanoparticles on X axis and percent cell survival on Y axis and IC50 values calculated.

2.4. Synthesis Procedure

2.4.1. General synthesis of benzo[*d*]oxazole-2-thiol

To a solution of 2-aminophenol (100 mmol) in ethanol (150 ml) was added aqueous sodium hydroxide (130 mmol) in water (30 ml). Followed by the addition of carbon disulfide (150 mmol). The resulting mixture was refluxed at 65°C for 5 hr. After the completion of the reaction, the reaction mixture was poured into the crushed ice which was neutralized with conc. HCl and mixture were filtered and washed with hexane to afford benzo[*d*]oxazole-2-thiol.

2.4.2. General synthesis of substituted 4,6-dichloro-*N*-phenyl-1,3,5-triazin-2-amine derivatives (P-01 to P-12)

Into a stirred solution of 2,4,6-trichloro-1,3,5-triazine (10 g, 0.054 mol) in anhydrous Acetone (150 mL) substituted anilines (10.09 g, 0.054 mol) was added portion wise at 0°C. The resulting reaction mixture was stirred at this temperature for 2 hr. then aqueous Solution of $NaHCO_3$ (5.48 g, 0.054 mol) was added and stirring was continued for another 5 hr. The reaction mixture was then poured into crushed ice, followed by neutralization with dilute HCl, and filtered, dried, and recrystallized from acetone to afford (P-01 to P-12).

2.4.2.1. Spectral data of (P-03)

4,6-dichloro-*N*-(4-methoxyphenyl)-1,3,5-triazin-2-amine: Off white, ^{1}H NMR (400 MHz, DMSO-d6) ppm: 11.29-11.15 (s, 1H), 7.60-7.49 (dd, 2H), 7.10-6.85 (dd, 2H), 3.82-3.72 (s, 3H); ^{13}C NMR (101 MHz, DMSO) δ = 149.88, 129.55, 123.65, 123.36, 114.04, 55.26; Mass: Obs. (m/z) 269.0, calcd. (m/z) 270.01.

2.4.3. General synthesis of substituted 4,6-bis(benzo[*d*]oxazol-2-ylthio)-*N*-phenyl-1,3,5-triazin-2-amine derivatives (JHP-01 to JHP-12)

To a solution of various P-01 to P-12 (1 equivalent) in 1,4-dioxane (30 mL), benzo[*d*]oxazole-2-thiol was added (2 equivalent) and the reaction mixture was stirred at room temperature for 24 hrs. Potassium carbonate (2 equivalents) was used for the neutralization of the reaction mixture. Progress of the reaction was monitored by TLC using toluene:acetone (8:2) as a solvent system. The mixture was then treated with crushed ice and neutralized by dilute HCl. The precipitate thus obtained was filtered off, dried, and recrystallized from THF to afford the desired compounds (JHP-01 to JHP-12).

2.4.3.1. Spectral data of (JHP-01)

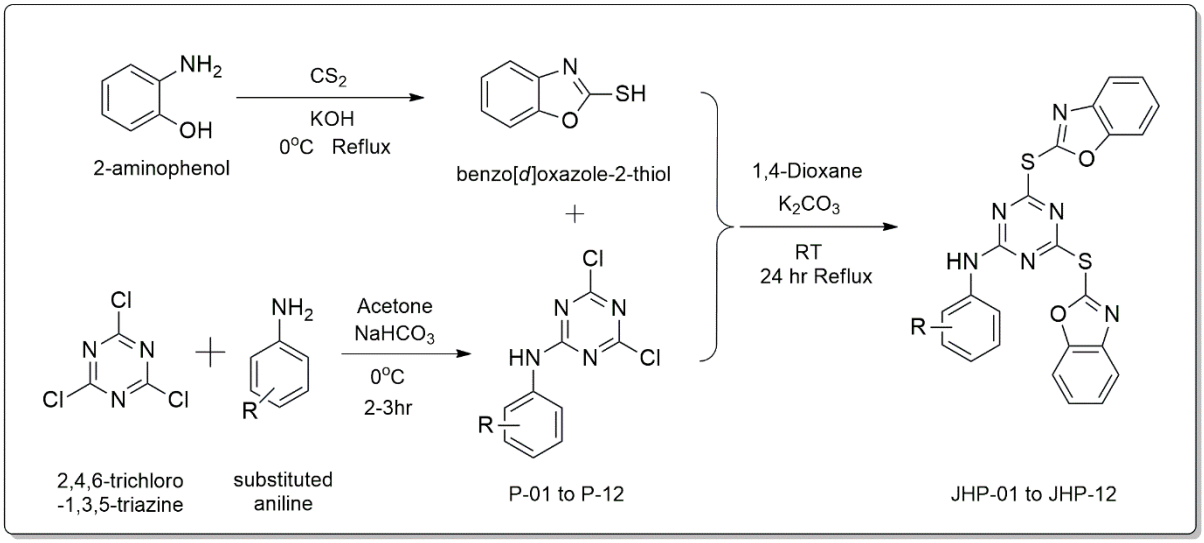
4,6-bis(benzo[*d*]oxazol-2-ylthio)-*N*-(4-chlorophenyl)-1,3,5-triazin-2-amine: Off white, yield (79%), m.p. 192-194°C. IR spectrum, ν, cm^{-1}: 3368, 3283 (NH, Secondary), 1599, 1520 (C=N), 1456, 1408 (C=C). ^{1}H NMR (400 MHz, DMSO-d6) ppm: 10.77-10.51 (s, 1H), 7.94-7.73 (s, 4H), 7.62-7.42 (m, 4H), 7.13-6.97 (d, 2H), 6.68-6.43 (d, 2H). ^{13}C NMR (101 MHz, DMSO) δ = 178.04, 176.86, 160.94, 154.08, 152.46, 141.39, 136.15, 127.92, 127.29, 125.44, 122.32, 120.81, 111.52. Mass: Obs. (m/z) 505.0, calcd. (m/z) 504.02. Elemental Analysis (%): ($C_{23}H_{13}ClN_6O_2S_2$): C, 54.71; H, 2.59; Cl, 7.02; N, 16.64; O, 6.34; S, 12.70. Found; C, 54.69; H, 2.58; Cl, 7.03; N, 16.66; O, 6.31; S, 12.73.

2.4.3.2. Spectral data of (JHP-02)

4,6-bis(benzo[*d*]oxazol-2-ylthio)-*N*-(2-chlorophenyl)-1,3,5-triazin-2-amine: white, yield (70%), m.p. 208-210°C. IR spectrum, ν, cm^{-1}: 3352, 3202 (NH, Secondary), 1519 (C=N), 1607, 1406 (C=C). ^{1}H NMR (400 MHz, DMSO-d6) ppm: 10.93-10.03 (s, 1H), 8.54-6.35 (m, 12H). ^{13}C NMR (101 MHz, DMSO) δ = (180-175), (165-110). Mass: Obs. (m/z) 505.0, calcd. (m/z) 504.02. Elemental Analysis (%): ($C_{23}H_{13}ClN_6O_2S_2$): C, 54.71; H, 2.59; Cl, 7.02; N, 16.64; O, 6.34; S, 12.70. Found; C, 54.70; H, 2.59; Cl, 7.05; N, 16.65; O, 6.32; S, 12.69.

2.4.3.3. Spectral data of (JHP-03)

4,6-bis(benzo[*d*]oxazol-2-ylthio)-*N*-(4-methoxyphenyl)-1,3,5-triazin-2-amine: Off white, yield (79%), m.p. 188-190°C. IR spectrum, ν, cm^{-1}: 3119 (NH, Secondary), 1549, 1512 (C=N), 1597, 1480, 1449 (C=C). ^{1}H NMR (400 MHz, DMSO-d6) ppm: 10.83-10.14 (s, 1H), 8.12-7.72 (m, 3H), 7.70-7.31 (m, 4H), 7.07-6.77 (m, 3H), 6.22-5.91 (d, 2H), 3.57-3.37 (s, 3H). ^{13}C NMR (101 MHz, DMSO) δ = 179.37, 177.82, 167.71, 161.70, 160.50, 155.77, 154.25, 152.55, 141.48, 130.12, 127.20, 125.45, 121.93, 120.75, 114.04, 113.24, 111.41, 55.345. Mass: Obs. (m/z) 501.0, calcd. (m/z) 500.07. Elemental Analysis (%): ($C_{24}H_{16}N_6O_3S_2$): C, 57.59; H, 3.22; N, 16.79; O, 9.59; S, 12.81. Found; 57.55; H, 3.21; N, 16.81; O, 9.60; S, 12.83.



Scheme 1. Synthesis route for triazine derivatives (JHP-01 to JHP-12).

2.4.4. General synthesis of 2-chloro-*N*-(4-(3-oxomorpholino)phenyl)acetamide (INT-1)

To a solution of 4-(4-aminophenyl)morpholin-3-one (1 equivalent) in acetone, chloroacetyl chloride (1 equivalent) was added dropwise at 0-5°C and the resulting mixture was stirred at the same temperature for a further 2-3 hrs. The reaction mixture was then shifted to crushed ice, and the solid intermediate product was separated, which was filtered and washed with water. Dry it and use it in the next step without further purification.

2.4.5. General synthesis of 2-(2,3-dioxoindolin-1-yl)-*N*-(4-(3-oxomorpholino)phenyl) acetamide (MM)

A flask equipped with a magnetic stirring bar was charged with potassium carbonate (1.8 g, 13 mmol) and 100 mL of DMF. At room temperature, the compound mixture was stirred for 5 minutes. Afterward, the appropriate isatin (10 mmol) was added. Stirring was continued for 45 minutes, and then 2-chloro-*N*-(4-(3-oxomorpholino)phenyl)acetamide (1.39 g, 11 mmol) was added. Stirring was continued at 80°C for 12 hrs and the mixture was diluted with 200 mL of water. The mixture was extracted with diethyl ether in 100 mL three times. The combined organic layers were washed with water (3 x 50 mL), dried over calcium chloride, and the solvent was removed at slightly reduced pressure to yield 2-(2,3-dioxoindolin-1-yl)-*N*-(4-(3-oxomorpholino)phenyl)acetamide.

2.4.6. General synthesis of Isatin derivatives (MMP-01 to MMP-12)

Conventional method: A mixture of appropriate Aniline (1 mmol) and appropriate isatin intermediate MM (1 mmol) in ethanol (25 mL) was refluxed in the presence of glacial acetic acid (1 mL) for an appropriate time (4-6 hours for complete reactions). The reaction mixture was cooled, and the precipitated solid was filtered, purified, and crystallized in the appropriate solvent (ethanol or ethanol/DMF mixtures).

2.4.6.1. Spectral data of (MMP-01)

(*E*)-2-(3-((4-methoxyphenyl)imino)-2-oxoindolin-1-yl)-*N*-(4-(3-oxomorpholino)phenyl)acetamide: Yellow-orange, yield (79%), m.p. 144-146°C. IR spectrum, ν, cm^{-1}: 3306, 1732 (NH, C=O, 2° amide), 1693 (C=O, 3°amide five member ring), 1643 (C=O, 3° amide six member ring), 1543 (C=N), 1474 (C=C). ^{1}H NMR (400 MHz, DMSO-d6) ppm: 10.59-10.20 (s, 1H), 7.75-7.52 (m, 4H), 7.47-7.27 (m, 3H), 7.26-6.99 (m, 4H), 6.98-6.69 (m, 1H) 4.71-4.56 (s, 2H), 4.32-4.06 (s, 2H), 3.99-3.91 (m, 2H), 3.88-3.79 (s, 3H), 3.74-3.64 (m, 2H). ^{13}C NMR (101 MHz, DMSO) δ = 183.36, 166.32, 165.49, 165.31, 163.13, 158.85, 157.69, 153.86, 151.20, 147.71, 143.26, 138.75, 137.64, 137.51, 137.08, 136.87, 134.55, 126.31, 126.27, 125.06, 124.91, 123.92, 122.81, 120.22, 119.98, 119.92, 117.93, 115.72, 115.23, 113.91, 111.40, 110.87, 68.13, 63.89, 55.74, 49.42, 43.57, 43.47. Mass: Obs. (m/z) 485.30, calcd. (m/z) 484.17. Elemental Analysis (%): $C_{27}H_{24}N_4O_5$; C, 66.93; H, 4.99; N, 11.56; O, 16.51; Found; C, 66.90; H, 4.99; N, 11.58; O, 16.52.

2.4.6.2. Spectral data of (MMP-02)

(*E*)-2-(3-((4-chlorophenyl)imino)-2-oxoindolin-1-yl)-*N*-(4-(3-oxomorpholino)phenyl)acetamide: Yellow-orange, yield (80%), m.p. 134-136°C. 3298, 1736 (NH, C=O, 2° amide), 1690 (C=O, 3° amide five member ring), 1651 (C=O, 3° amide six member ring), 1547 (C=N), 1474 (C=C). ^{1}H NMR (400 MHz, DMSO-d6) ppm: 10.35-10.25 (s, 1H), 7.72-7.64 (t, 2H), 7.63-7.52 (m, 4H), 7.40-7.29 (d, 4H), 7.23-7.10 (m, 2H), 4.64-4.54 (s, 2H), 4.22-4.15 (s, 2H), 4.00-3.91 (t, 2H), 3.75-3.64 (t, 2H) . ^{13}C NMR (101 MHz, DMSO) δ = 183.16, 166.13, 165.11, 158.65, 151.00, 138.55, 137.44, 136.67, 126.14, 126.07, 124.71, 123.72, 120.02, 117.73, 111.19, 67.92, 63.68, 49.21, 43.37. Mass: Obs. (m/z) 489.20, calcd. (m/z) 488.13. Elemental Analysis (%):$C_{26}H_{21}ClN_4O_4$; C, 63.87; H, 4.33; Cl, 7.25; N, 11.46; O, 13.09; Found; C, 63.88; H, 4.34; Cl, 7.27; N, 11.44; O, 13.07.

2.4.6.3. Spectral data of (MMP-03)

(*E*)-2-(3-((2-nitrophenyl)imino)-2-oxoindolin-1-yl)-*N*-(4-(3-oxomorpholino)phenyl)acetamide: Yellow-orange, yield (74%), m.p. 128-130°C. 3302, 1732 (NH, C=O, 2° amide), 1693 (C=O, 3° amide five member ring), 1608 (C=O, 3° amide six member ring), 1543 (C=N), 1473(C=C). ^{1}H NMR (400 MHz, DMSO-d6) ppm: 10.38-10.34 (s, 1H), 7.89-7.49 (m, 6H), 7.44-7.28 (m, 3H), 7.26-7.03 (m, 3H), 4.61-4.56 (s, 2H), 4.20-4.15 (s, 2H), 3.97-3.92 (s, 2H), 3.71-3.66 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ = 182.76, 165.73, 164.70, 158.23, 150.59, 138.16, 137.02, 136.28, 125.66, 124.30, 123.31, 119.61, 117.31, 110.78, 67.51, 63.27, 48.81, 42.95. Mass: Obs. (m/z) 500.10, calcd. (m/z) 499.15. Elemental Analysis (%): $C_{26}H_{21}N_5O_6$; C, 62.52; H, 4.24; N, 14.02; O, 19.22; Found; C, 62.53; H, 4.25; N, 14.02; O, 19.20.

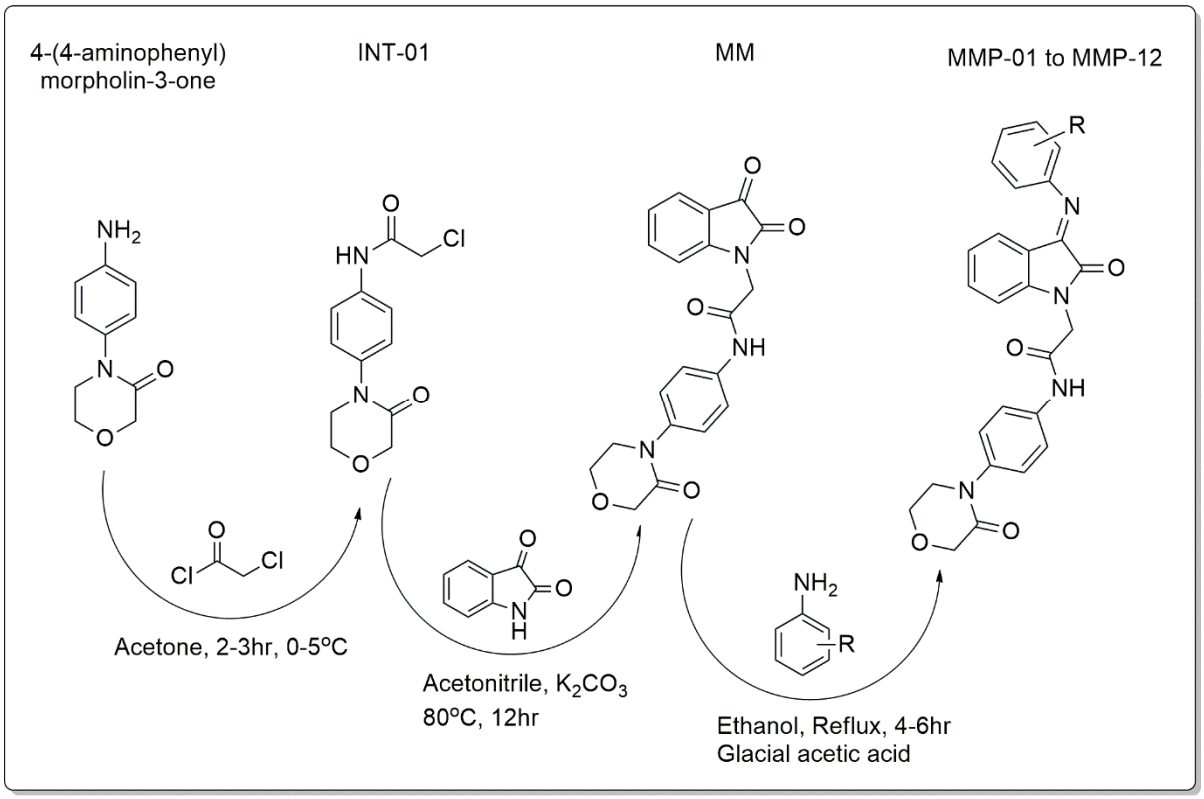
2.4.6.4. Spectral data of (MMP-04)

(*E*)-2-(3-((4-nitrophenyl)imino)-2-oxoindolin-1-yl)-*N*-(4-(3-oxomorpholino)phenyl)acetamide: Yellow-orange, yield (77%), m.p. 122-124°C. ^{1}H NMR (400 MHz, DMSO-d6) ppm: 10.45-10.39 (s, 1H), 8.01-7.47 (m, 6H), 7.46-6.87 (m, 6H), 4.64-4.57 (s, 2H), 4.22-4.15 (s, 2H), 3.97-3.93 (s, 2H), 3.72-3.67 (s, 2H). ^{13}C NMR (101 MHz, DMSO) δ = 182.96, 165.91, 164.90, 158.43, 150.80, 138.35, 137.21, 136.50, 125.85, 124.49, 123.50, 119.80, 117.50, 110.99, 67.72, 63.47, 49.00, 43.16. Mass: Obs. (m/z) 498.10, calcd. (m/z) 499.15. Elemental Analysis (%): $C_{26}H_{21}N_5O_6$; C, 62.52; H, 4.24; N, 14.02; O, 19.22; Found; C, 62.52; H, 4.26; N, 14.01; O, 19.21.

Table 1. Physiochemical data of Synthesis library, triazine and Isatin derivatives.

Compound Code	Mol. Wt. (g/mole)	Substitution					Yield (%)	Melting Point (°C)	Cytotoxicity	
		R1	R2	R3	R4	R5			IC50 in mg	STDEV
MMP-01	484	H	H	OCH_3	H	H	79	144-146	1.66	0.44
MMP-02	488	H	H	CL	H	H	80	134-136	29.37	5.20
MMP-03	499	NO_2	H	H	H	H	74	128-130	8.22	0.34
MMP-04	499	H	H	NO_2	H	H	77	122-124	16.31	1.44
MMP-05	502	CH_3	CL	H	H	H	74	158-160	-	-
MMP-06	482	H	CH_3	CH_3	H	H	79	134-136	8.88	0.56
MMP-07	499	H	NO_2	H	H	H	78	136-138	1.69	0.10
MMP-08	522	H	CL	CL	H	H	76	130-132	10.21	0.66
MMP-09	533	H	NO_2	CL	H	H	72	122-124	4.71	0.09
MMP-10	490	F	H	F	H	H	71	138-140	-	-
MMP-11	488	CL	H	H	H	H	73	132-134	11.22	2.96

MMP-12	488	H	CL	H	H	H	71	136-138	6.95	2.63
JHP-01	504	H	H	Cl	H	H	79	192-194	10.94	1.75
JHP-02	504	Cl	H	H	H	H	70	208-210	1.71	0.20
JHP-03	500	H	H	OCH_3	H	H	79	188-190	5.00	0.66
JHP-04	470	H	H	H	H	H	76	206-208	-	-
JHP-05	538	H	Cl	Cl	H	H	72	190-192	-	-
JHP-06	516	H	H	NO_2	H	H	71	188-190	-	-
JHP-07	516	H	NO_2	H	H	H	73	188-190	-	-
JHP-08	516	NO_2	H	H	H	H	75	192-194	-	-
JHP-09	550	H	NO_2	Cl	H	H	76	208-210	-	-
JHP-10	506	F	H	F	H	H	75	200-202	-	-
JHP-11	530	H	CH_3	CH_3	H	H	76	196-198	-	-
JHP-12	504	H	Cl	H	H	H	75	204-206	-	-



Scheme 2. Synthesis route for Isatin derivatives (MMP-01 to MMP-12).

3. RESULTS AND DISCUSSION

All final compounds are stable at room temperature, yield in the range of 70-80% and physiochemical data are given in **Table 1**. Various spectroscopic study has been recorded for the structural identification of the synthesized compounds. Mass spectra of intermediate and final compounds favour the synthesis. Further ^{1}H NMR & ^{13}C NMR spectra have been recorded for some of the derivatives. Compound JHP-01 shows good ^{1}H NMR spectra, with all protons attached to benzene showing peaks in the range of 6 - 8 ppm. A sharp singlet of (NH) was observed at 10.6 ppm. ^{13}C NMR also supports the structural requirement for JHP-01. A peak of 1,4-dioxane was also observed in the NMR spectra. Some of the compounds are partially soluble in the DMSO solvent, which leads to poor resolution and S/N ratio, with bad line shape and baseline. JHP-02 shows the same issue, but all the peak ranges are similar to the first derivative JHP-01 which is also identical in structure.

A peak of (NH) was observed at 10.93 - 10.03 ppm, and remaining aromatic protons present between 8.54 - 6.35 ppm clubbed together. Similarly, JHP-02 ^{13}C NMR (NH) peak was observed at 180 – 175 ppm, and the remaining phenyl carbon ranges from 165 - 110 ppm clubbed together. Also, JHP-03 has high noise peaks and in ^{1}H NMR spectra broad suppressed singlet of (NH) has been observed at 10.5 ppm. A peak of methoxy proton is observed at 3.5 ppm. Residual peaks of water and 1,4-Dioxane were observed at 3.3 ppm and 3.7 ppm respectively. The remaining phenyl proton peaks are ranges from 6 – 8 ppm which support the synthesis. Overall, all the spectral data lead to the successful synthesis of *s*-Triazine derivatives.

The ^{1}H NMR spectra of MMP-01 show a (NH) proton peak between 10-11 ppm. All aromatic protons have a peak between 6-8 ppm, which is the aromatic protons range. All the remaining protons, like methoxy proton and (CH2) are present between 3-5 ppm. Similarly, MMP-02 and MMP-03 show peaks at the same ppm, which supports the synthesis of targeted compounds. It was observed that JHP series was showing cytotoxicity even at low concentration. JHP-02 was showing lowest IC50 i.e. 1.7 mg/ml. MMP series compounds were also showing good cytotoxicity activity, with MMP-01 as lowest 1.65 mg/ml.

4. CONCLUSIONS

In conclusion, we use privileged scaffolds like *s*-Triazine, Isatin, and camphor, and developed an efficient methodology for the synthesis of a series of respective derivatives. The pros of this presently developed method are synthesis complete in simple conditions, higher yields, and low costs. The suitable synthesis reaction condition of targeted compounds was studied. Compounds are well characterized and supported by various analytical techniques like Mass, IR, ^{1}H NMR, and ^{13}C NMR. Cytotoxicity study was evaluated against MCF-7 breast cancer cell line, and showing cytotoxicity even at low concentration. Hence, the realization of this research has indeed led to a better understanding of the structural requirements for a synthesis of *s*-Triazine and Isatin related compounds.

Acknowledgement

The authors are very much thankful to the Department of Chemistry of D. K. V. Arts and Science College, Jamnagar for providing laboratory facilities. Also thankful to Envitro Laboratories Private Limited and CoE NFDD – Saurashtra University for providing an instrumental analysis facility. Cytotoxicity was evaluated at Shri Parashurambhau College, Pune. SXRD facility is provided by STIC-Cochin Sponsored Jointly by KSCSTE and CUSAT. NMR facility is provided by SAIF-KUD, funded by DST. Cytotoxicity study was evaluated at Shri Parashurambhau College, Pune.

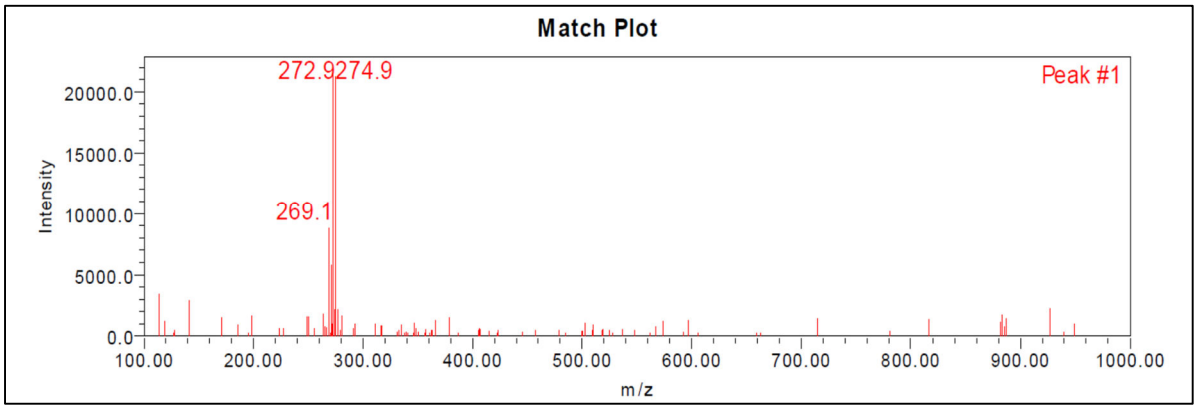


Figure 2. Mass spectra of P-01.

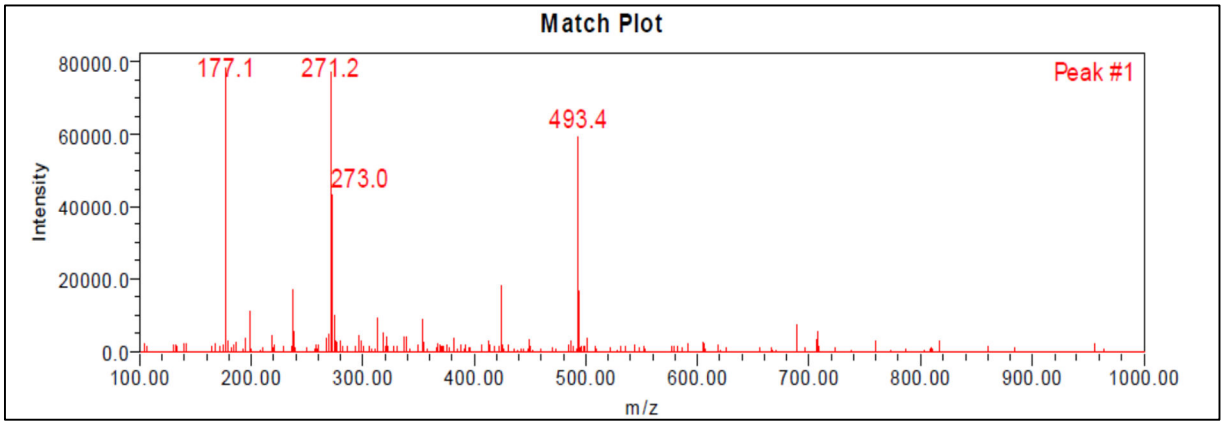


Figure 3. Mass (+) spectra of P-02.

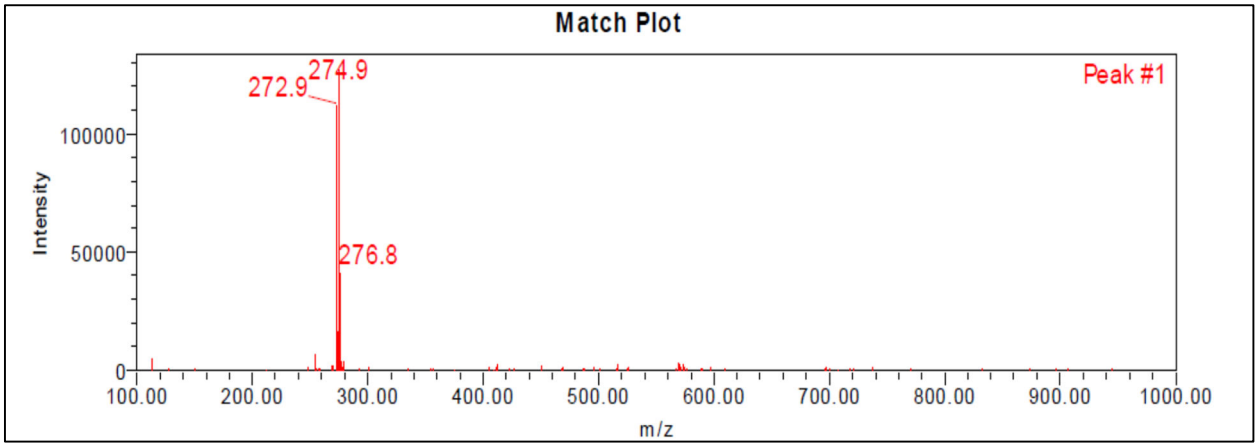


Figure 4. Mass (-) spectra of P-02.

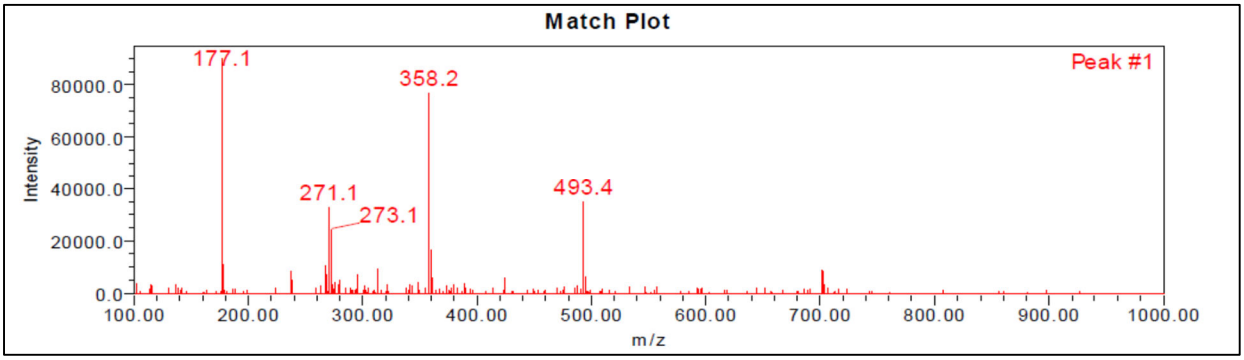


Figure 5. Mass (+) spectra of P-03.

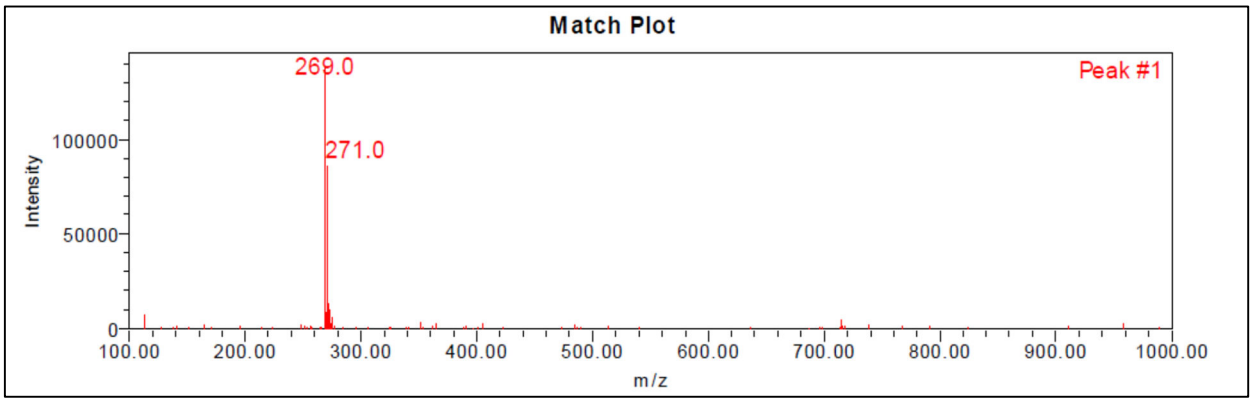


Figure 6. Mass (-) spectra of P-03.

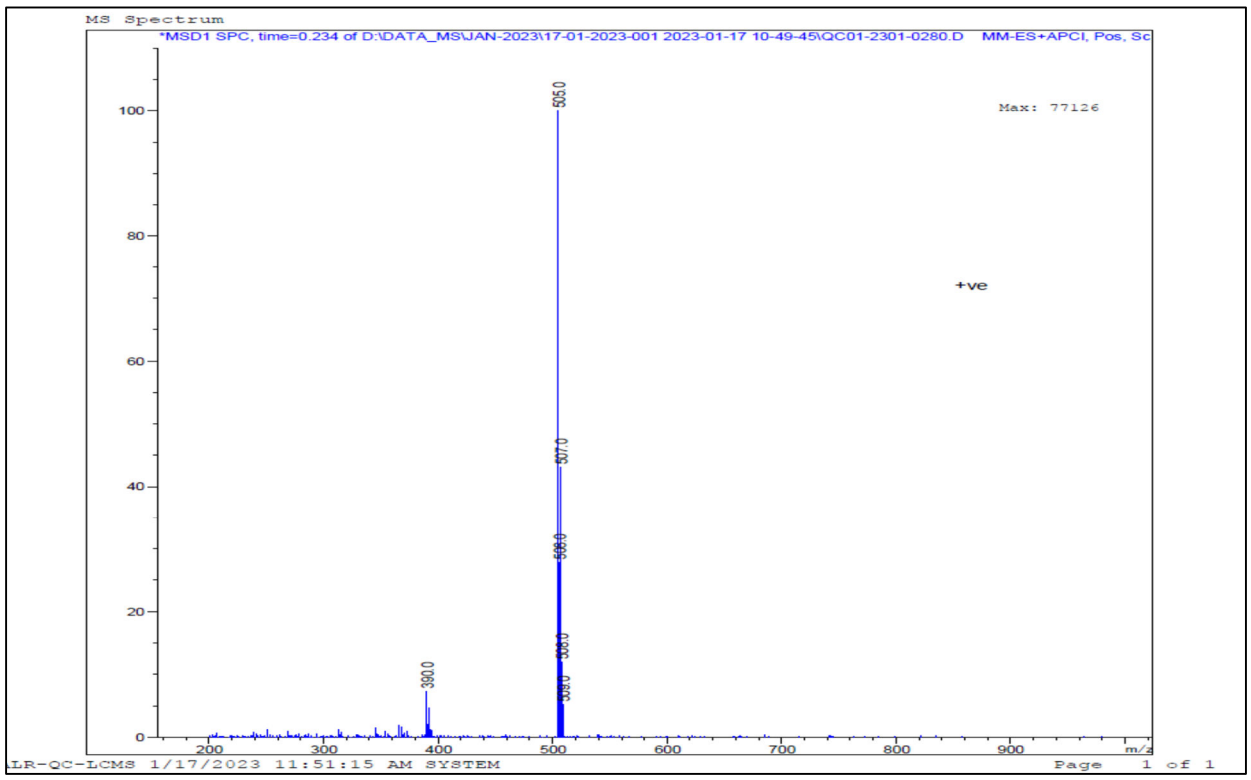


Figure 7. Mass (+) spectra of JHP-01.

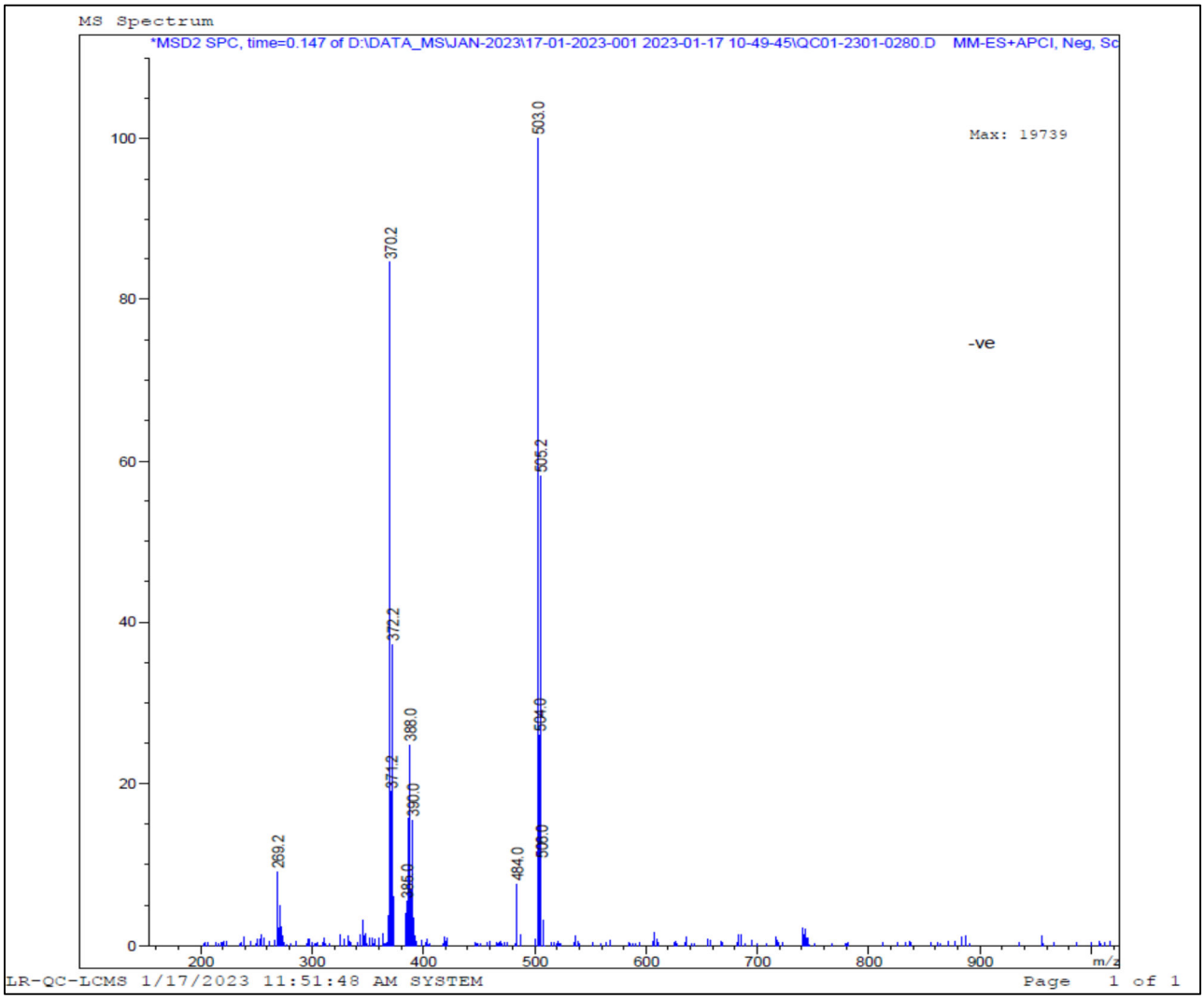


Figure 8. Mass (-) spectra of JHP-01.

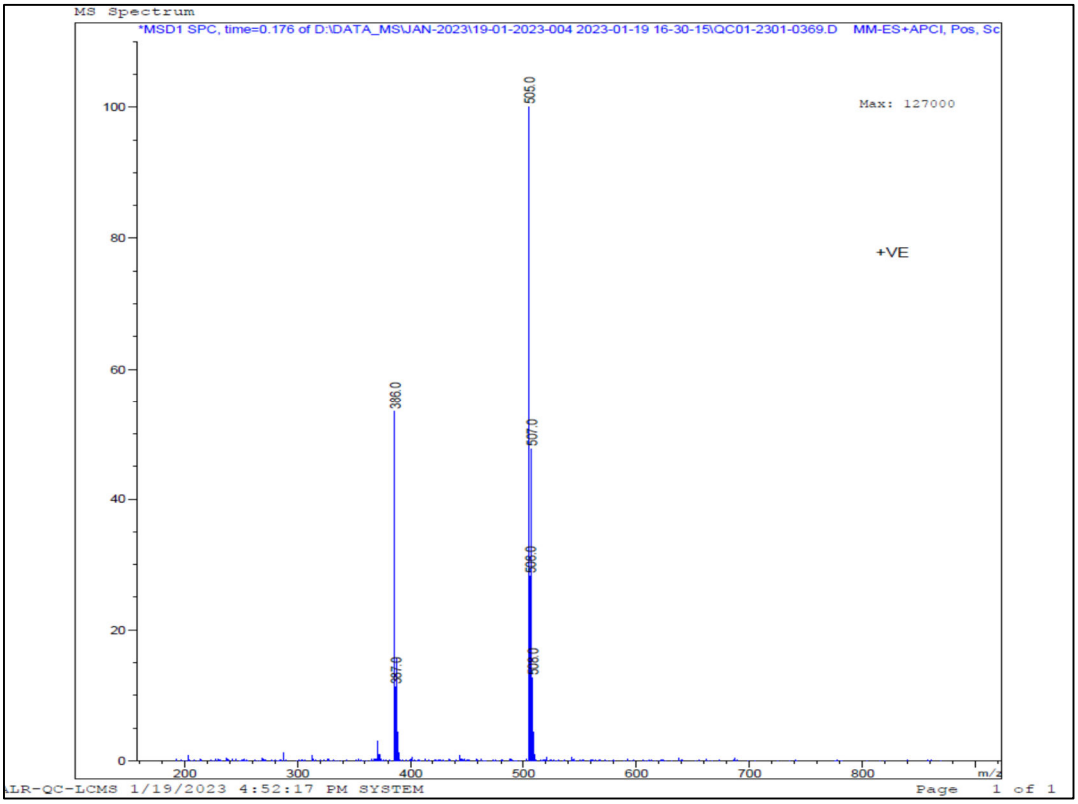


Figure 9. Mass (+) spectra of JHP-02.

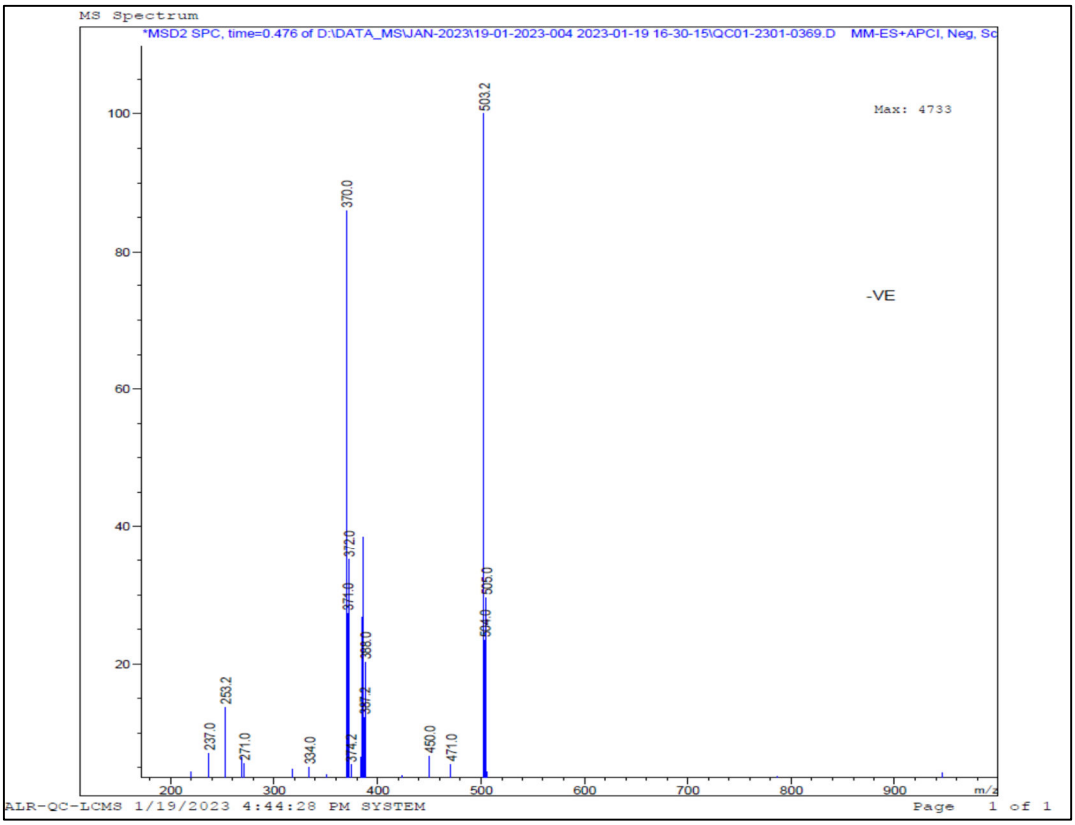


Figure 10. Mass (-) spectra of JHP-02.

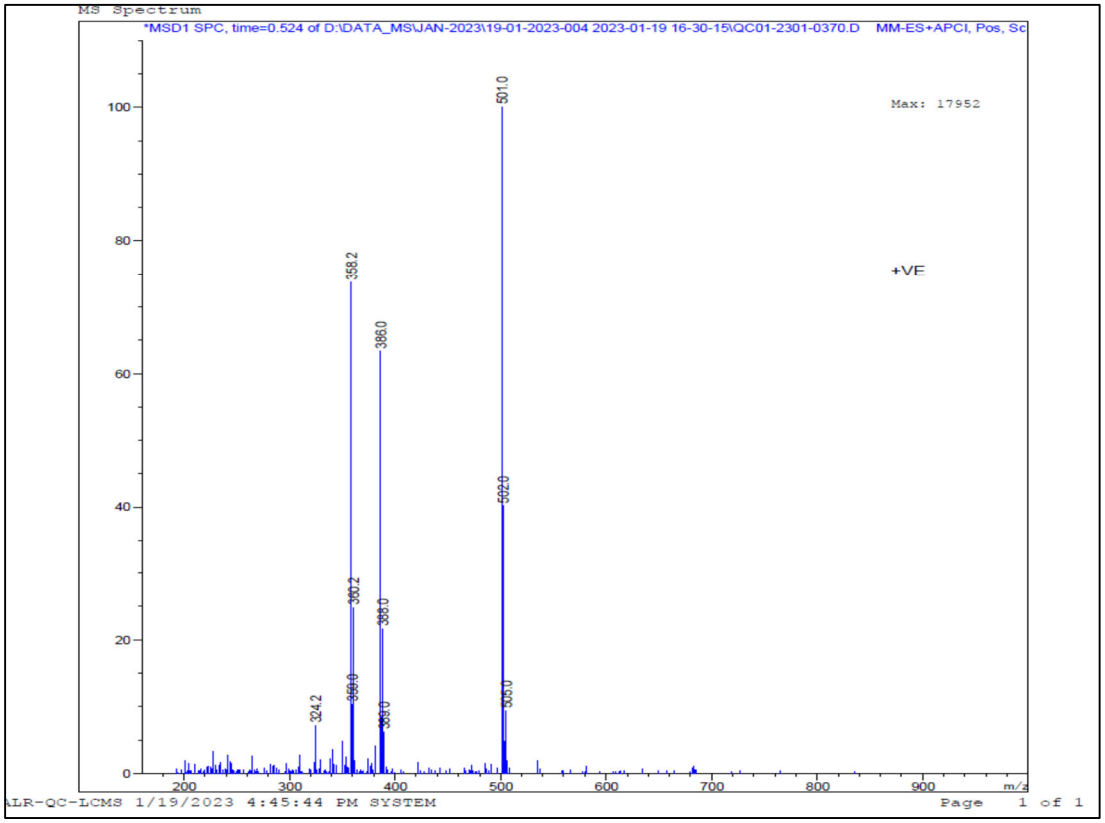


Figure 11. Mass (+) spectra of JHP-03.

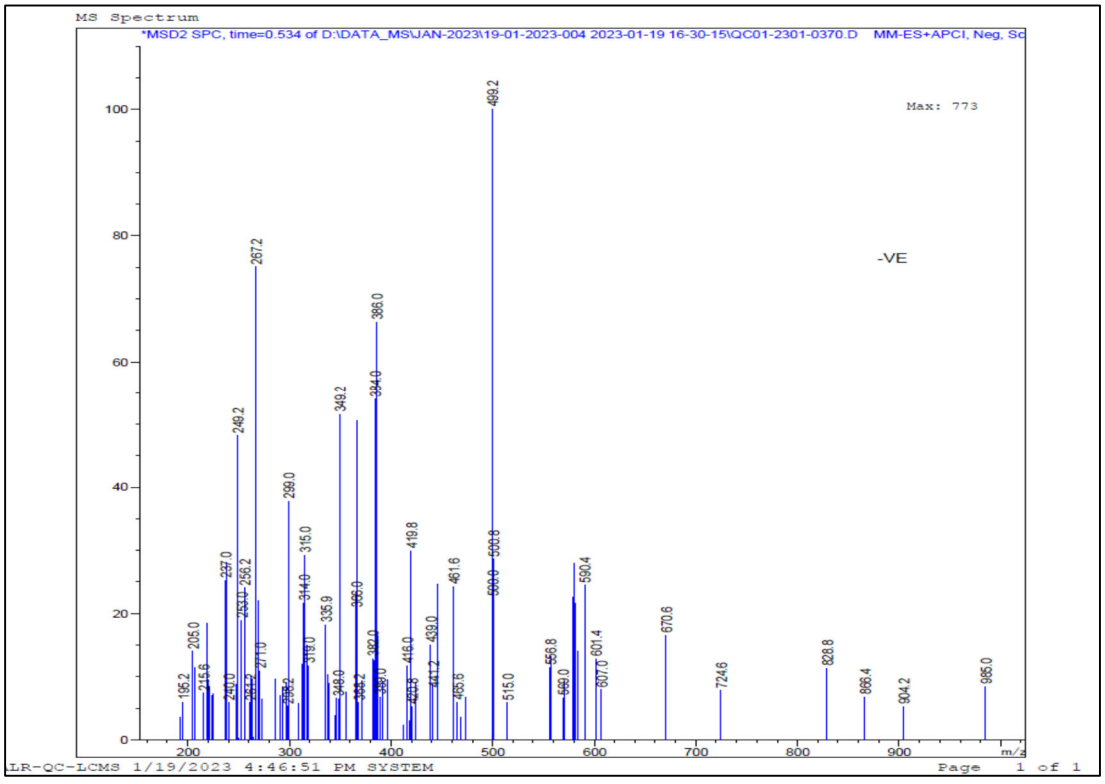


Figure 12. Mass (-) spectra of JHP-03.

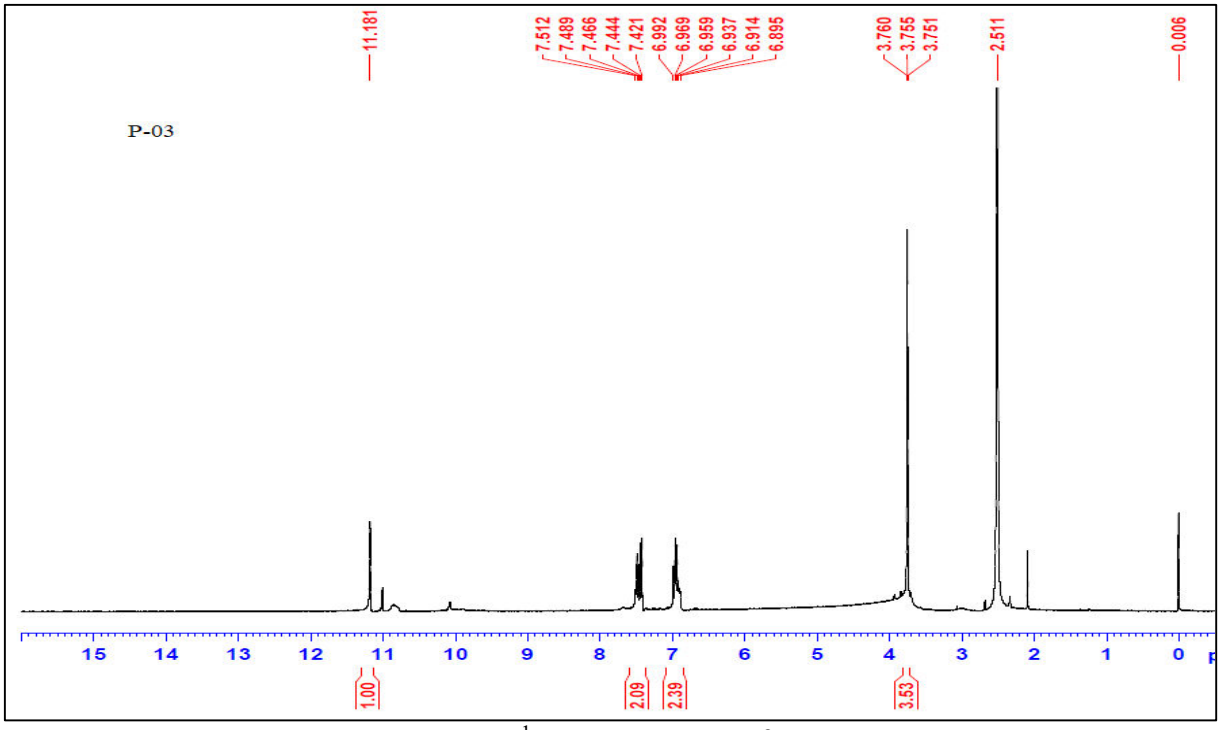


Figure 13. ^{1}H NMR spectra of P-03.

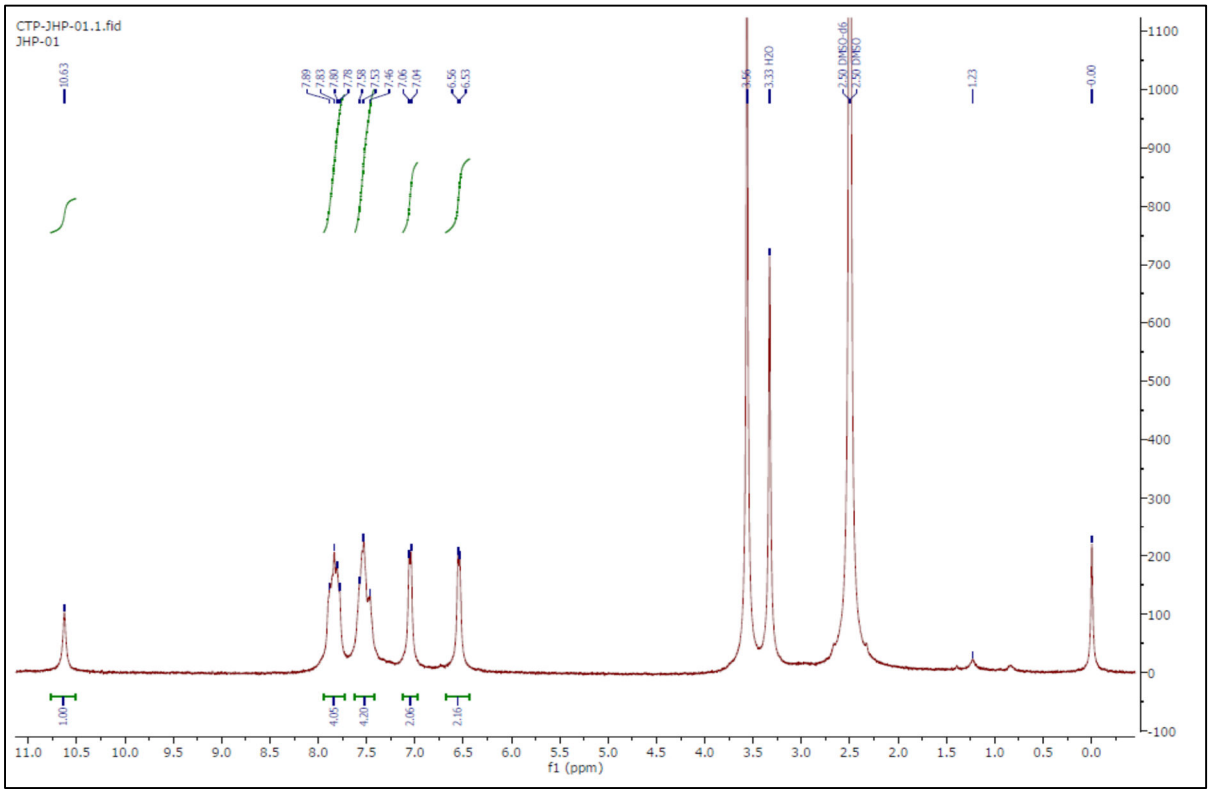


Figure 14. ^{1}H NMR spectra of JHP-01.

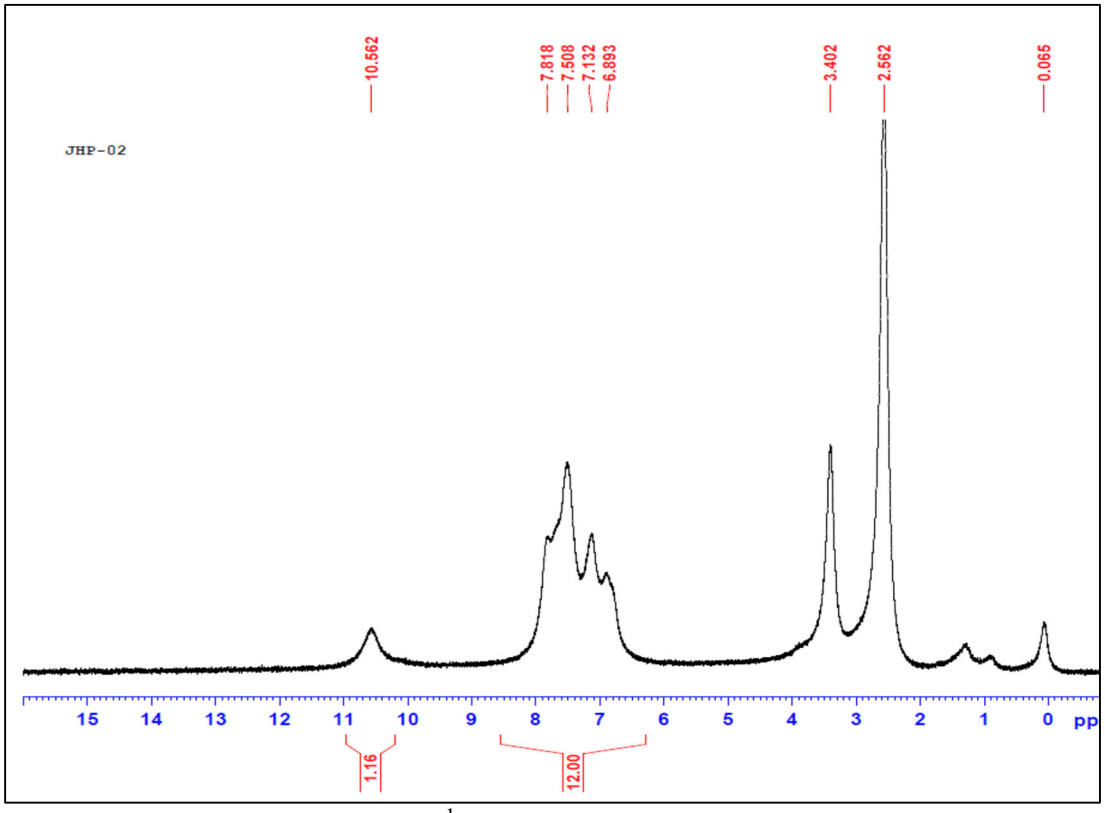


Figure 15. [1]H NMR spectra of JHP-02.

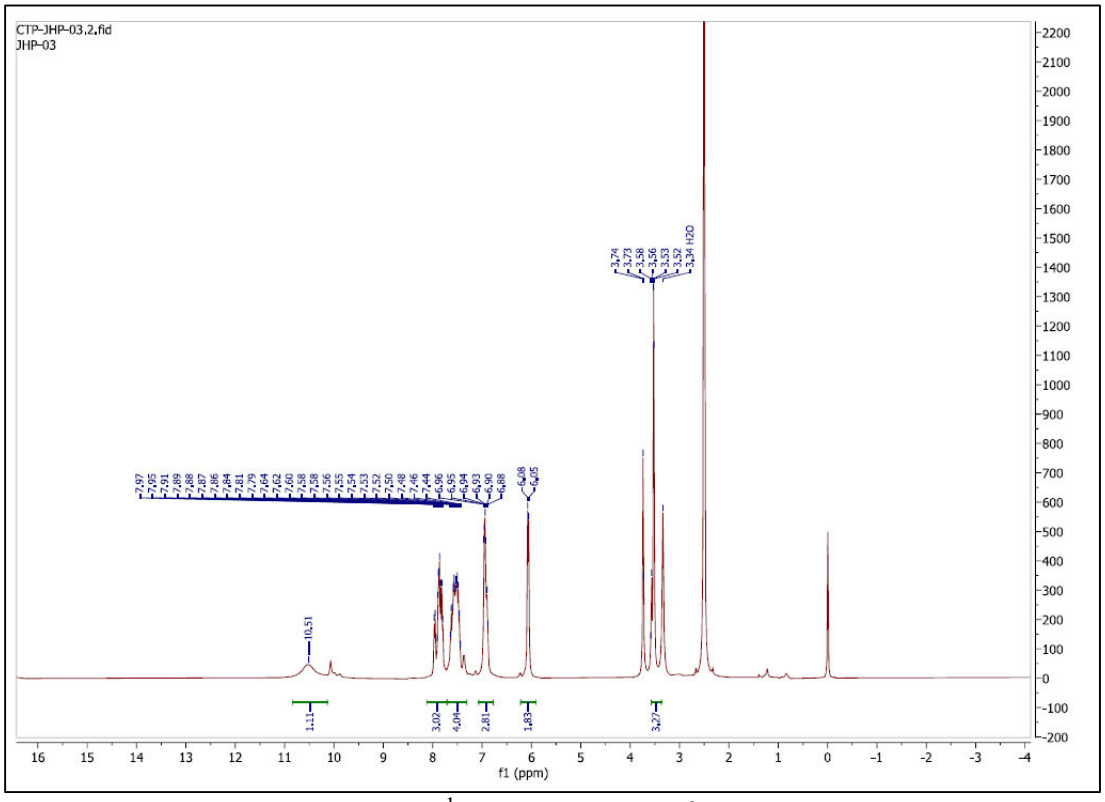


Figure 16. [1]H NMR spectra of JHP-03.

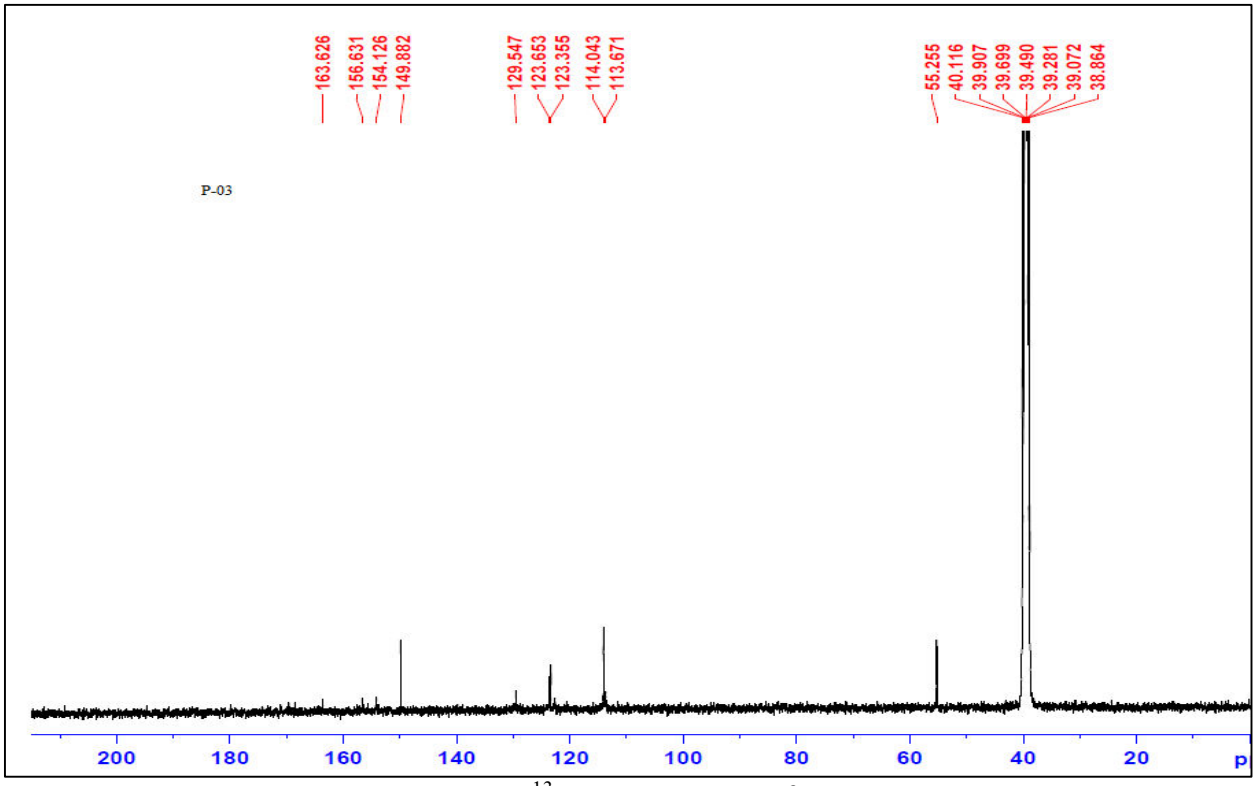


Figure 17. ^{13}C NMR Spectra of P-03.

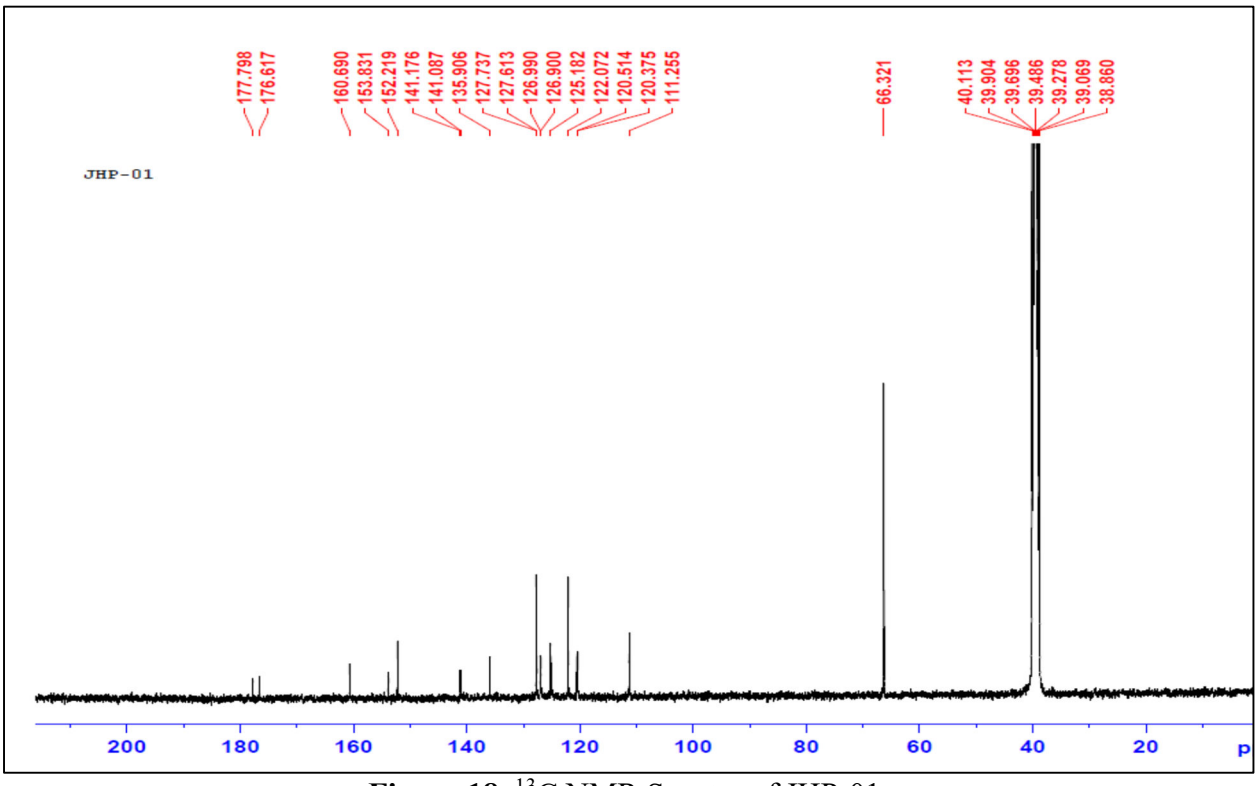


Figure 18. ^{13}C NMR Spectra of JHP-01.

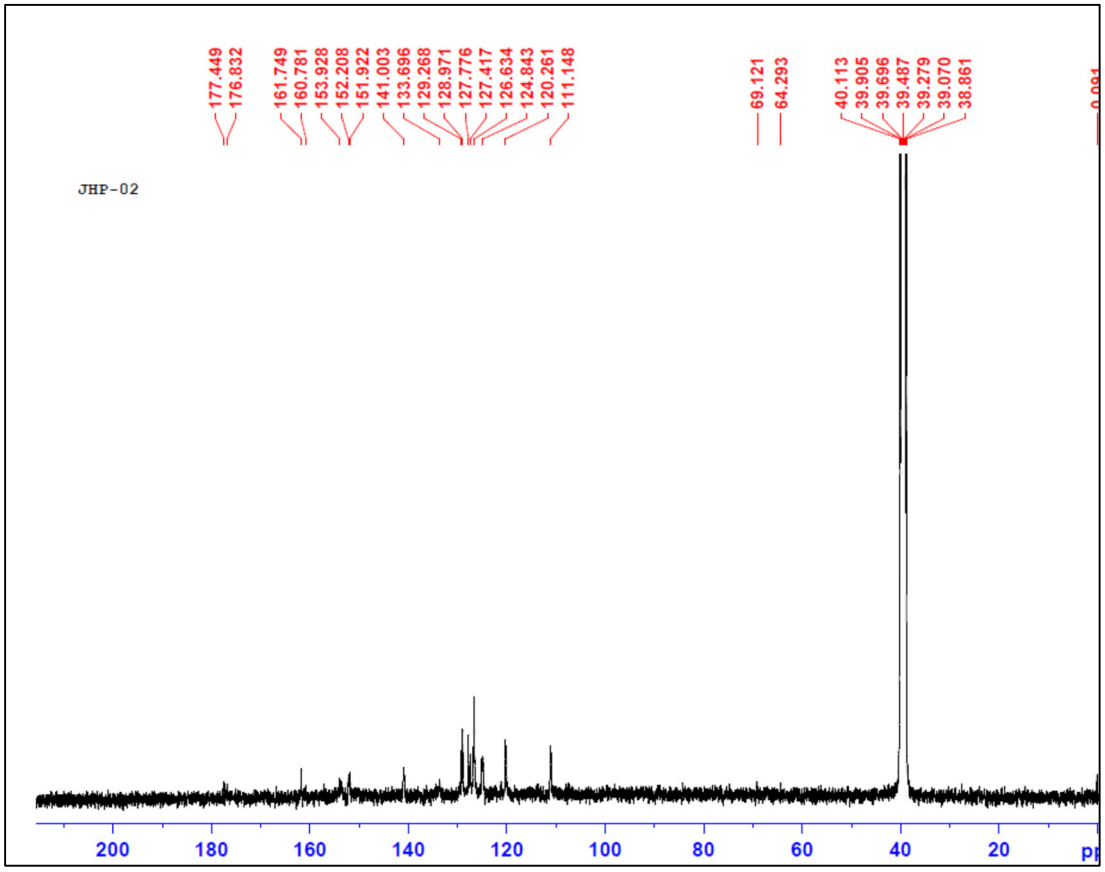


Figure 19. ^{13}C NMR Spectra of JHP-02.

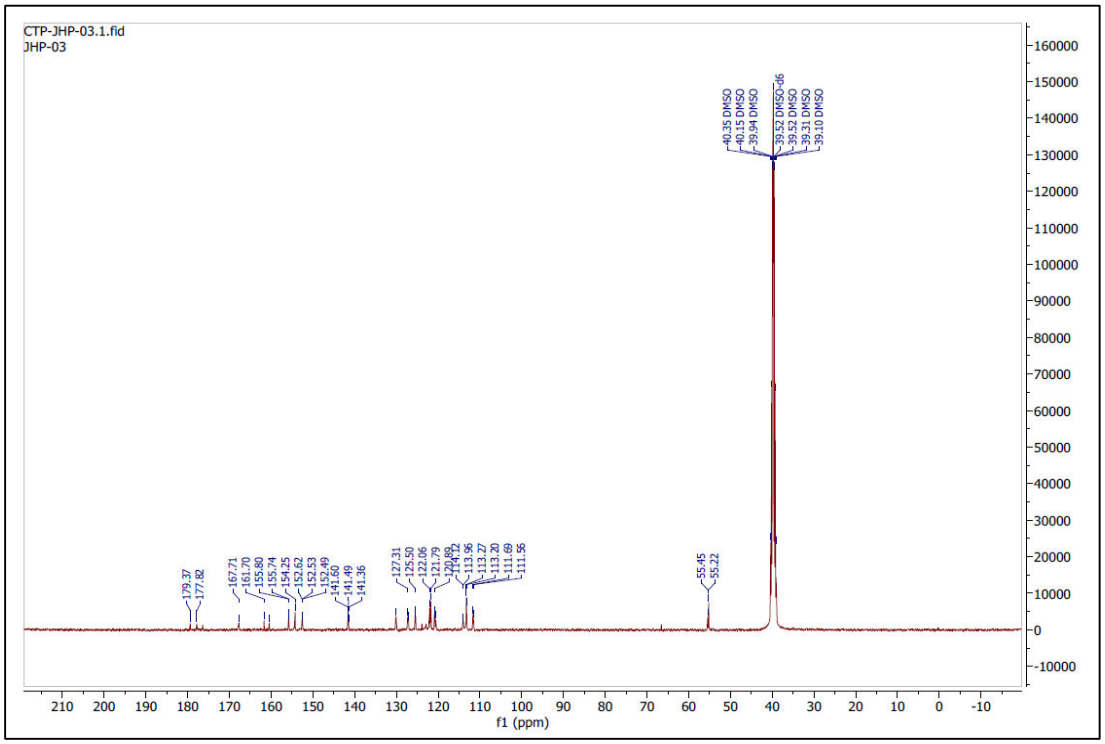


Figure 20. ^{13}C NMR Spectra of JHP-03.

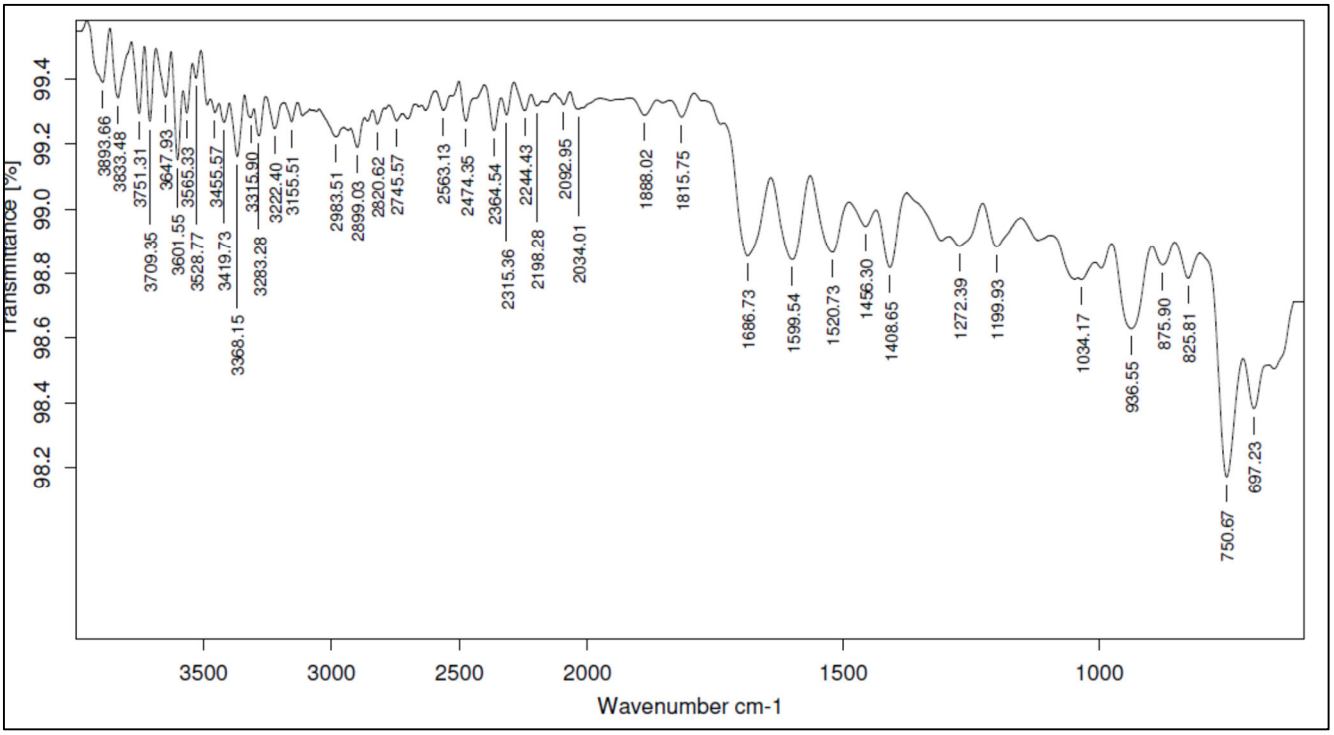


Figure 21. IR spectrum of JHP-01.

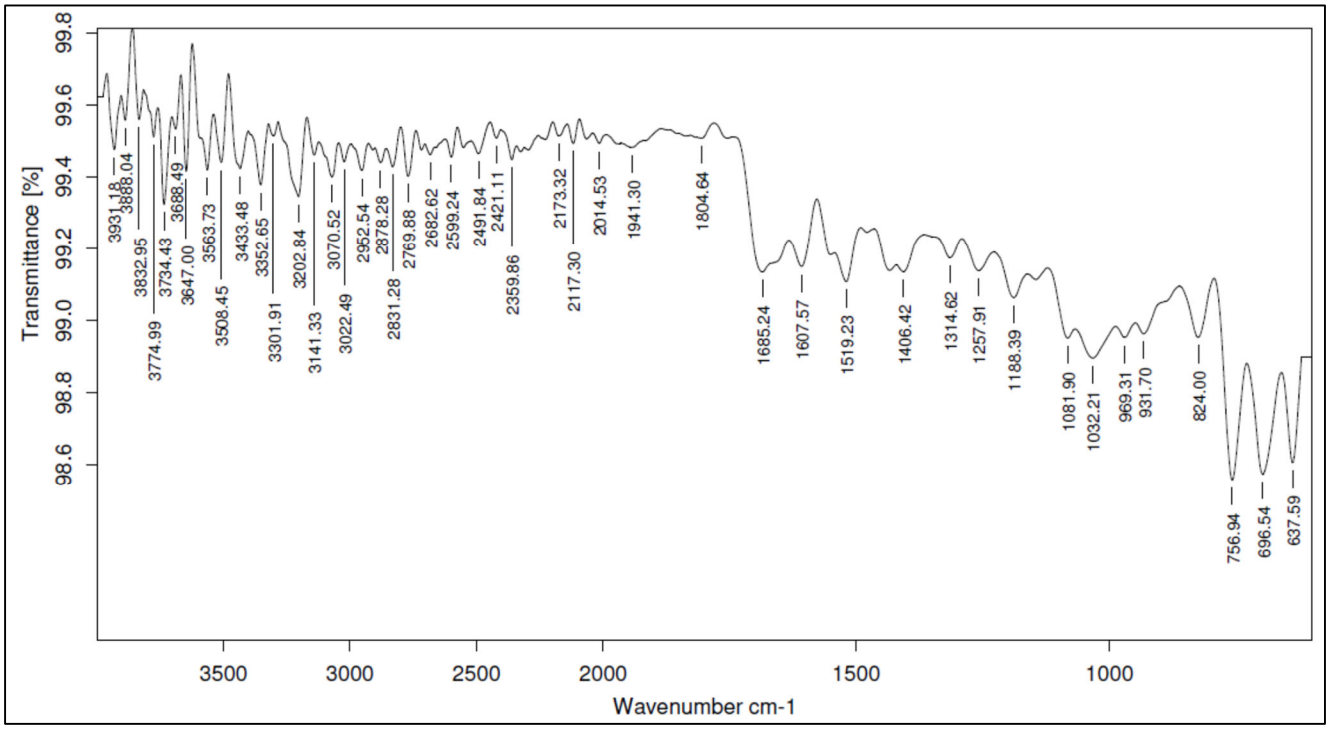


Figure 22. IR spectrum of JHP-02.

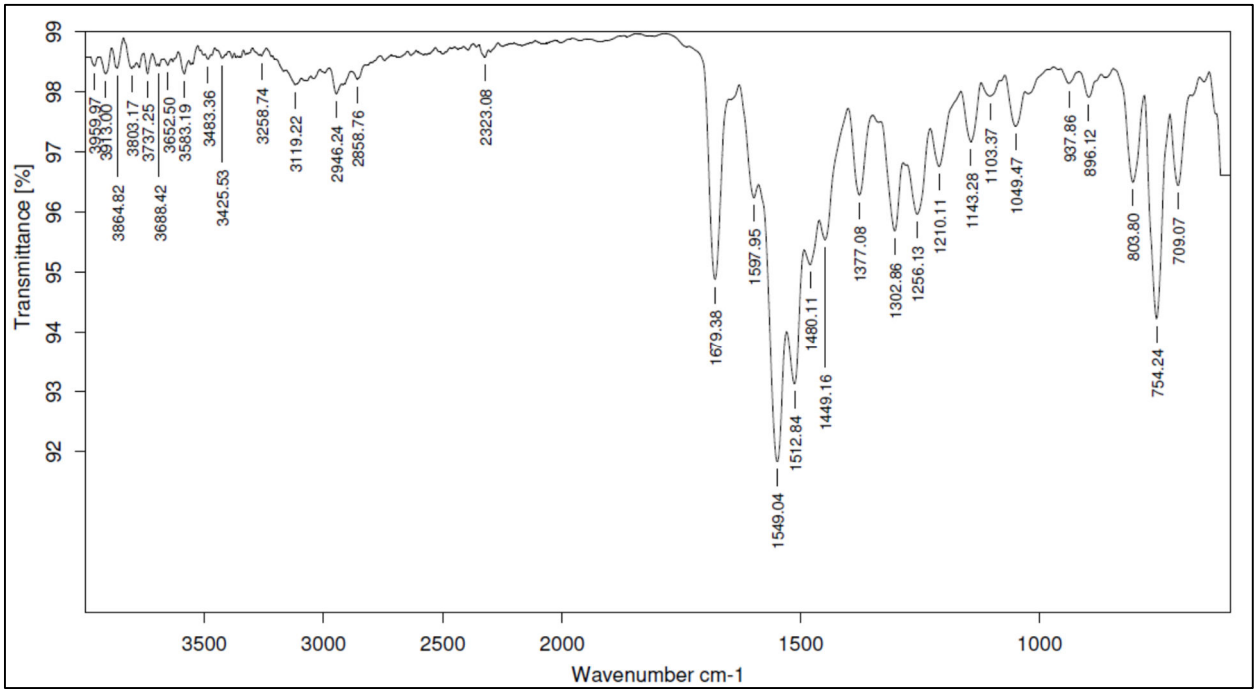


Figure 23. IR spectrum of JHP-03.

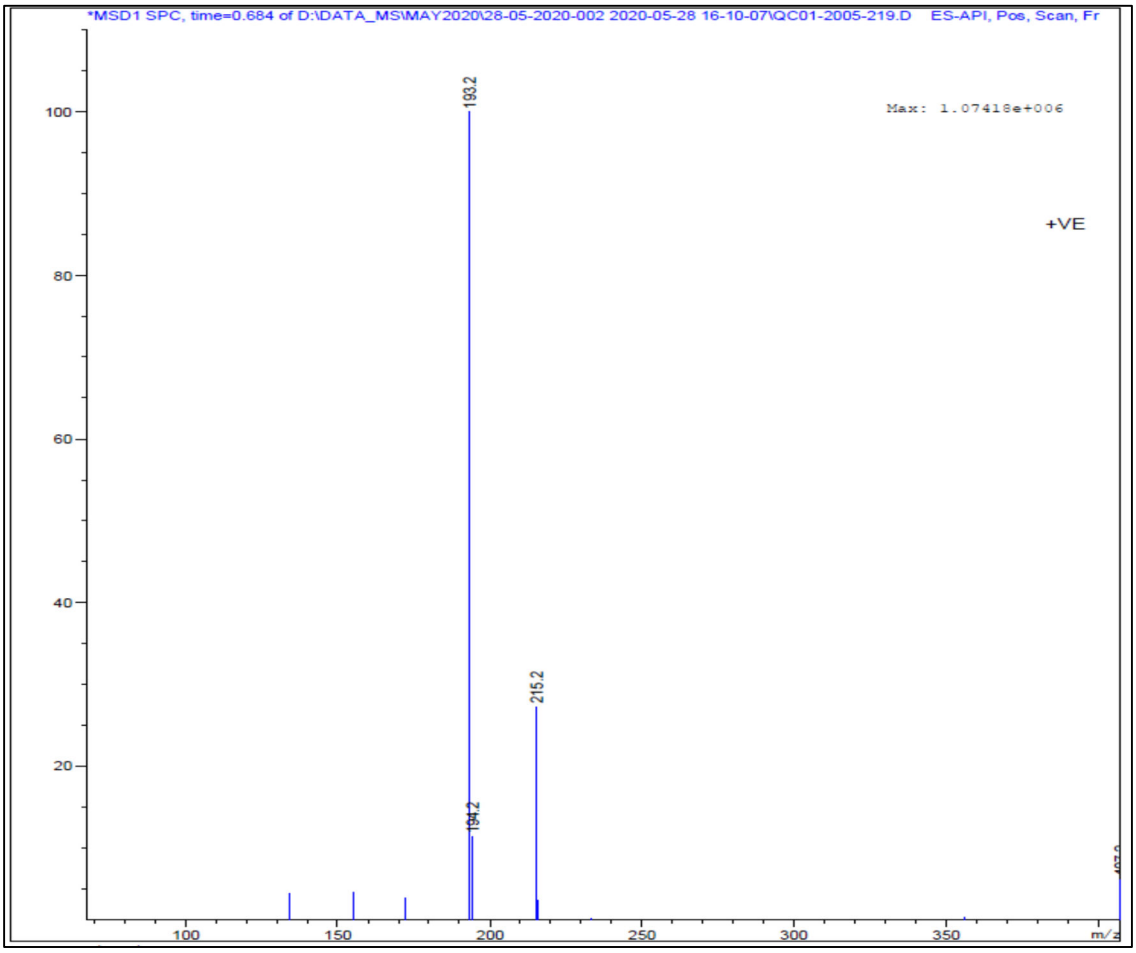


Figure 24. Mass Spectra of 4-(4-aminophenyl)morpholin-3-one.

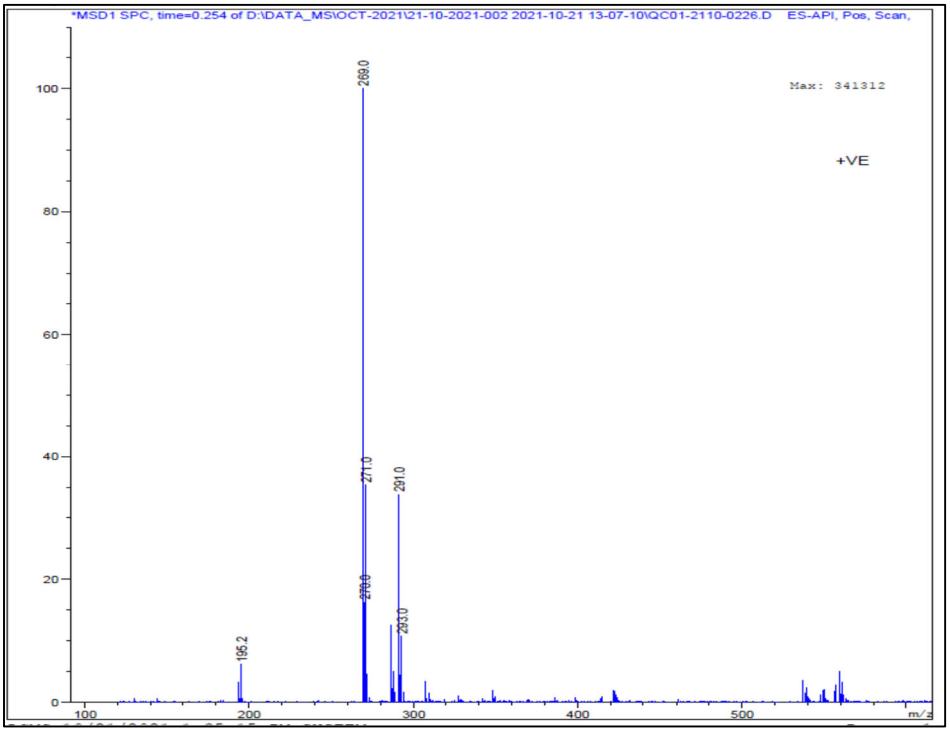


Figure 25. Mass (+) Spectra of INT-01.

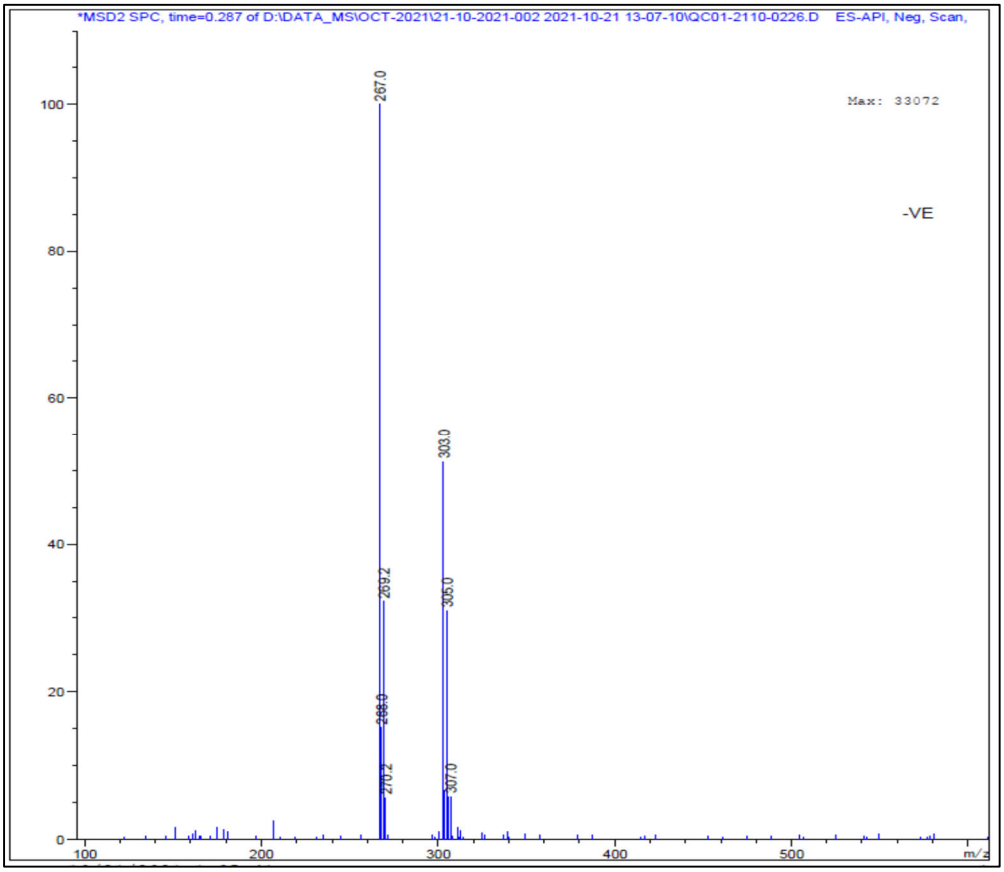


Figure 26. Mass (-) Spectra of INT-01.

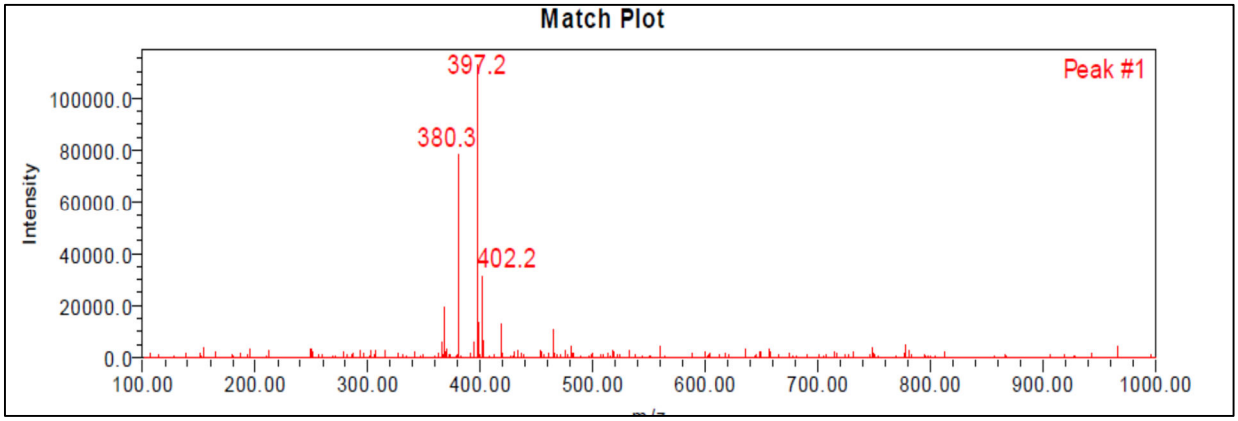


Figure 27. Mass (+) spectrum of MM.

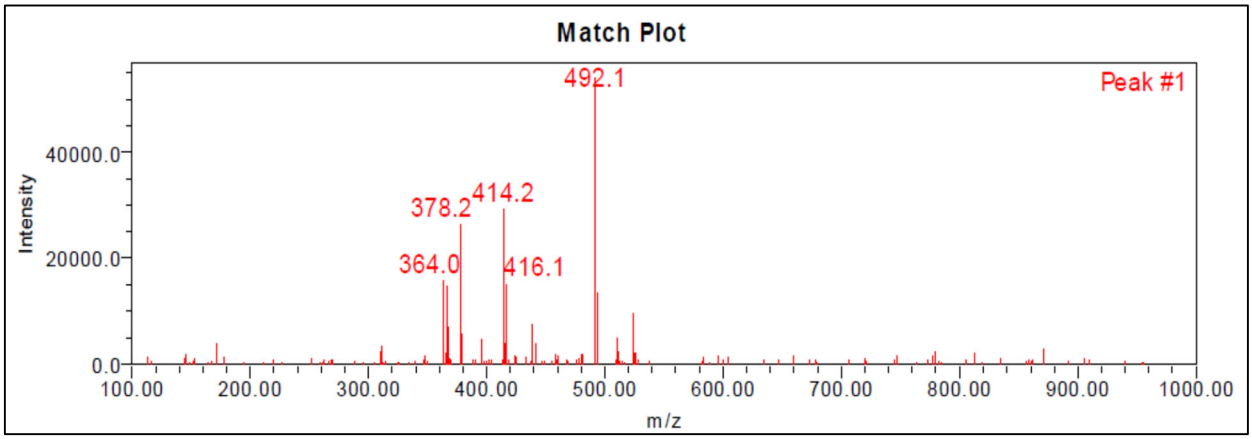


Figure 28. Mass (-) spectrum of MM.

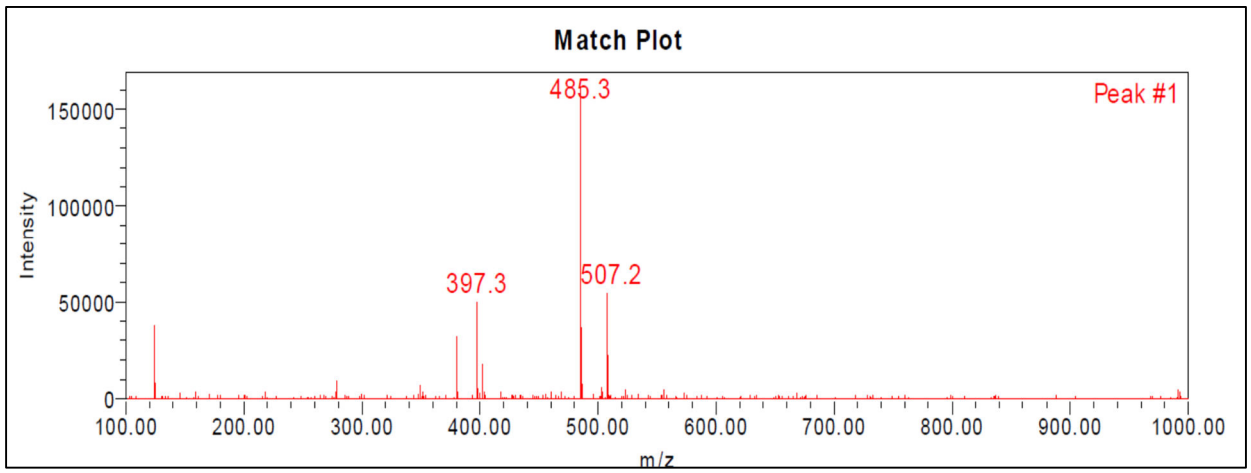


Figure 29. Mass (+) spectrum of MMP-01.

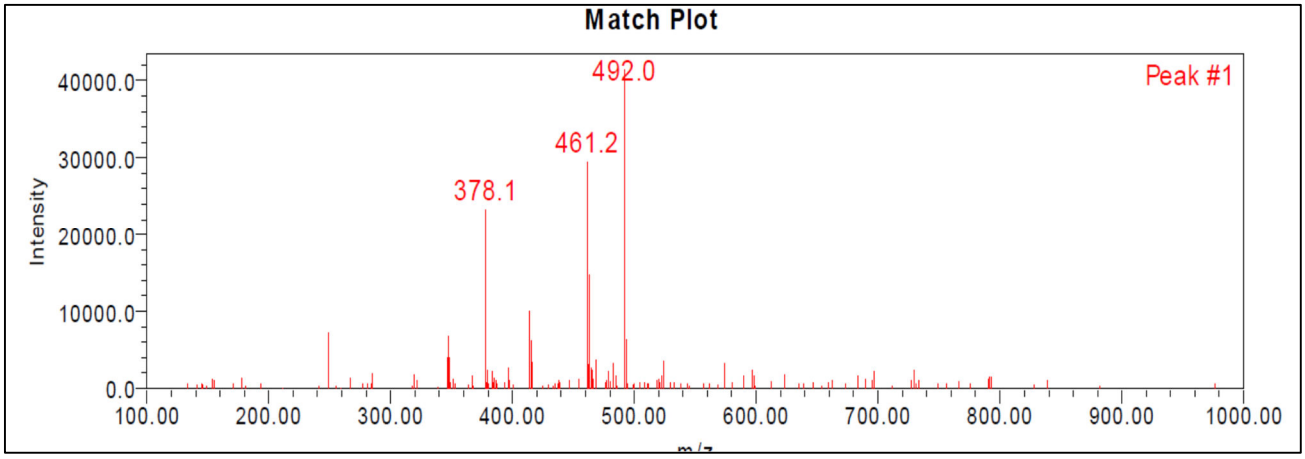


Figure 30. Mass (-) spectrum of MMP-01.

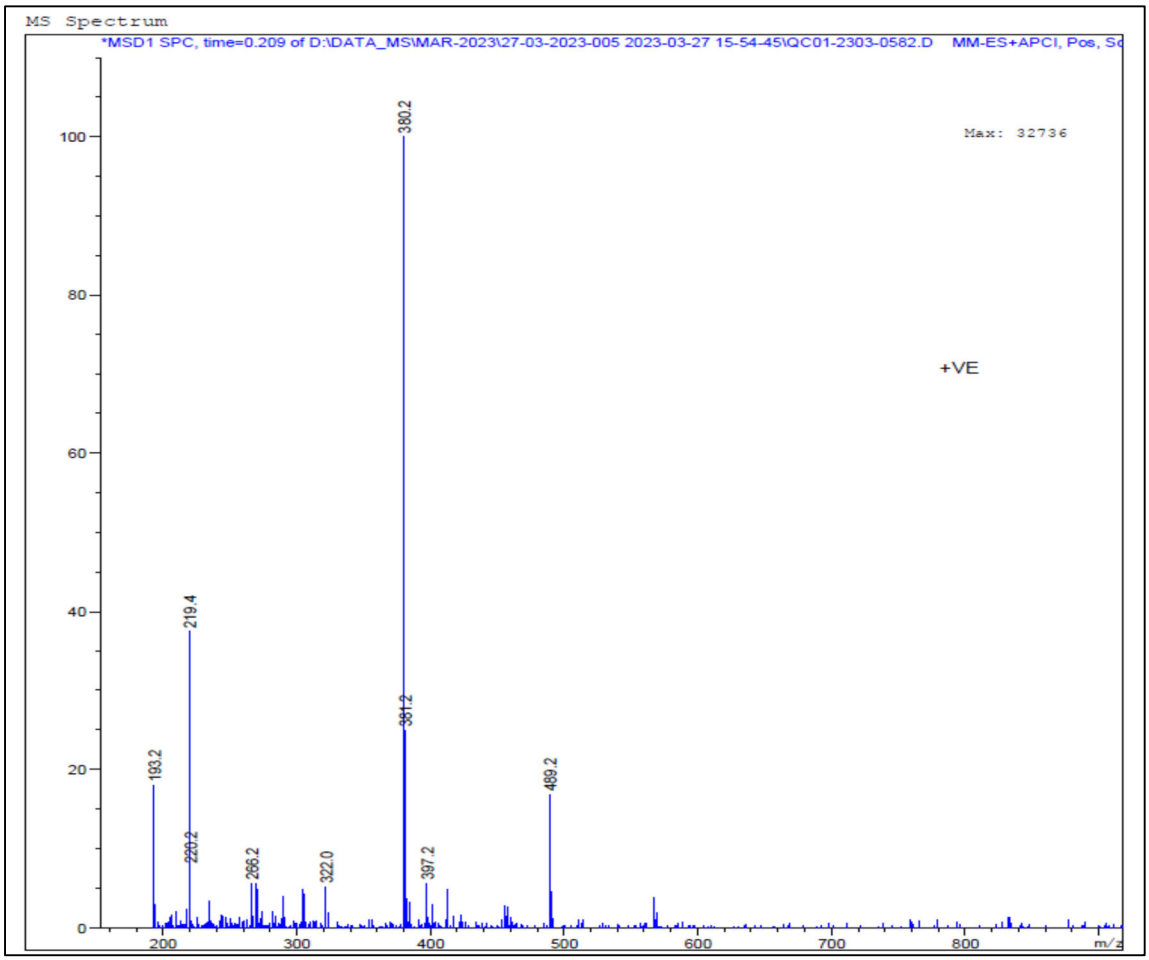


Figure 31. Mass (+) spectrum of MMP-02.

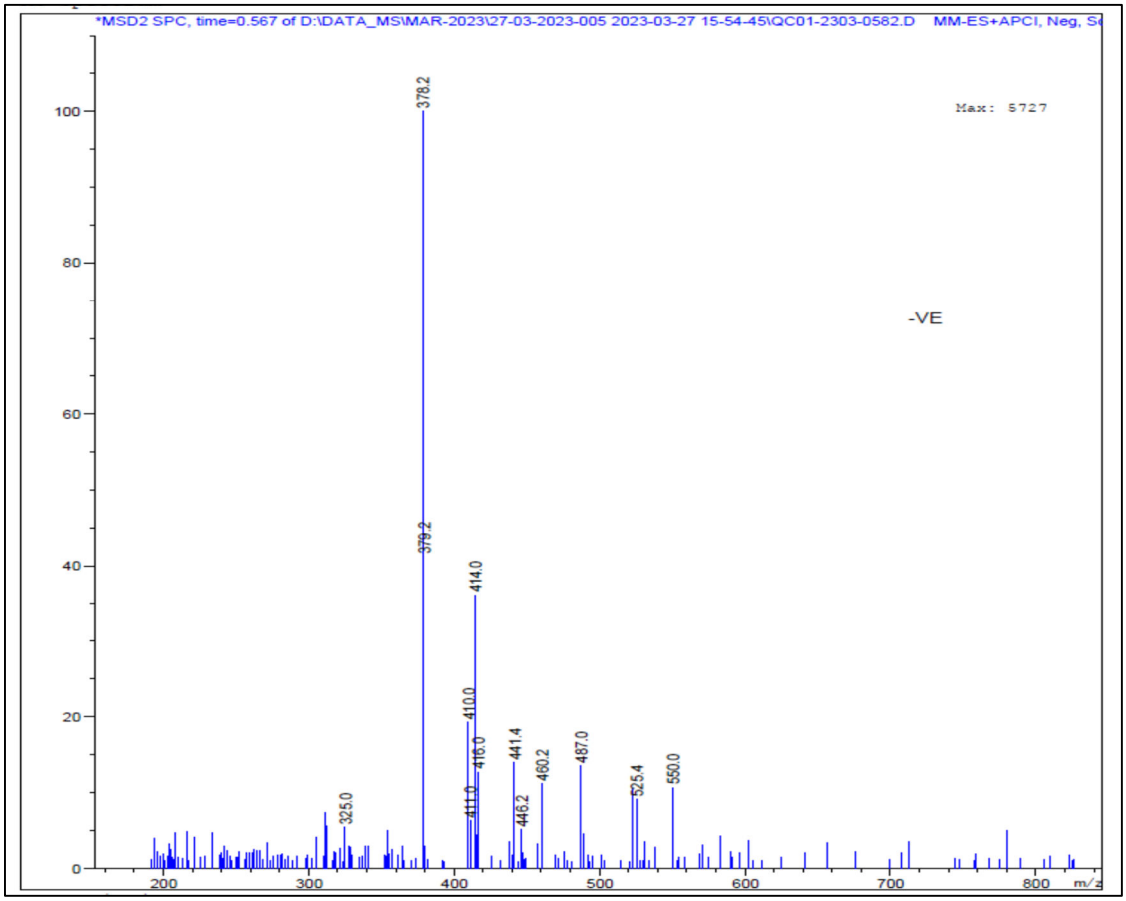


Figure 32. Mass (-) spectrum of MMP-02.

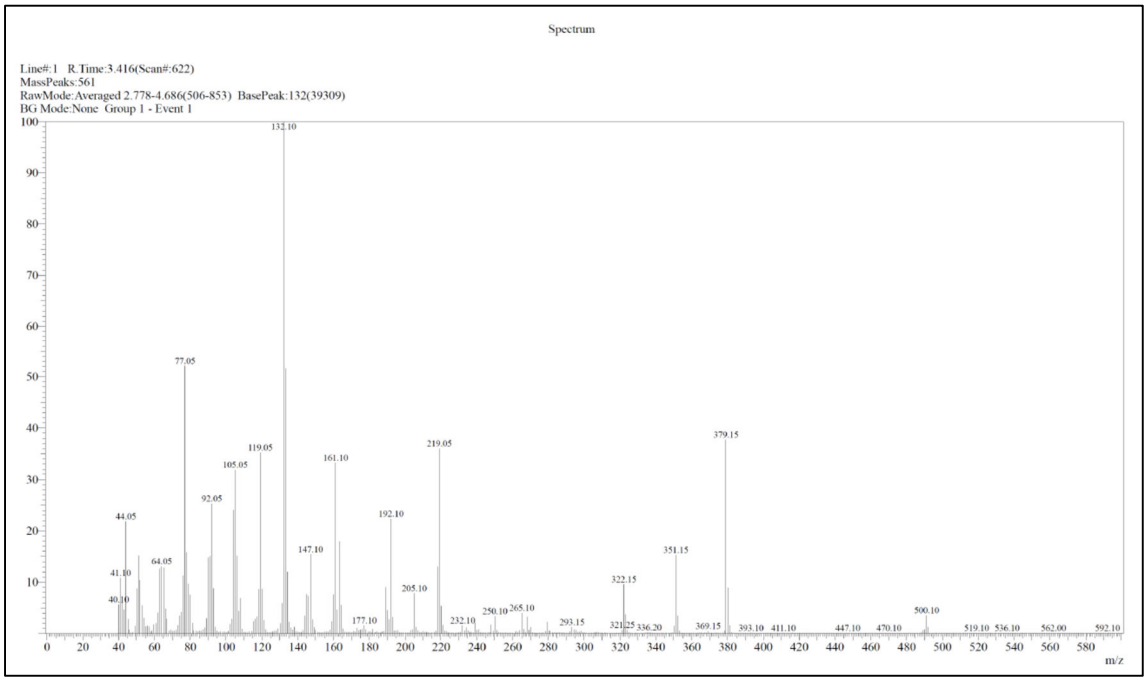


Figure 33. Mass spectrum of MMP-03.

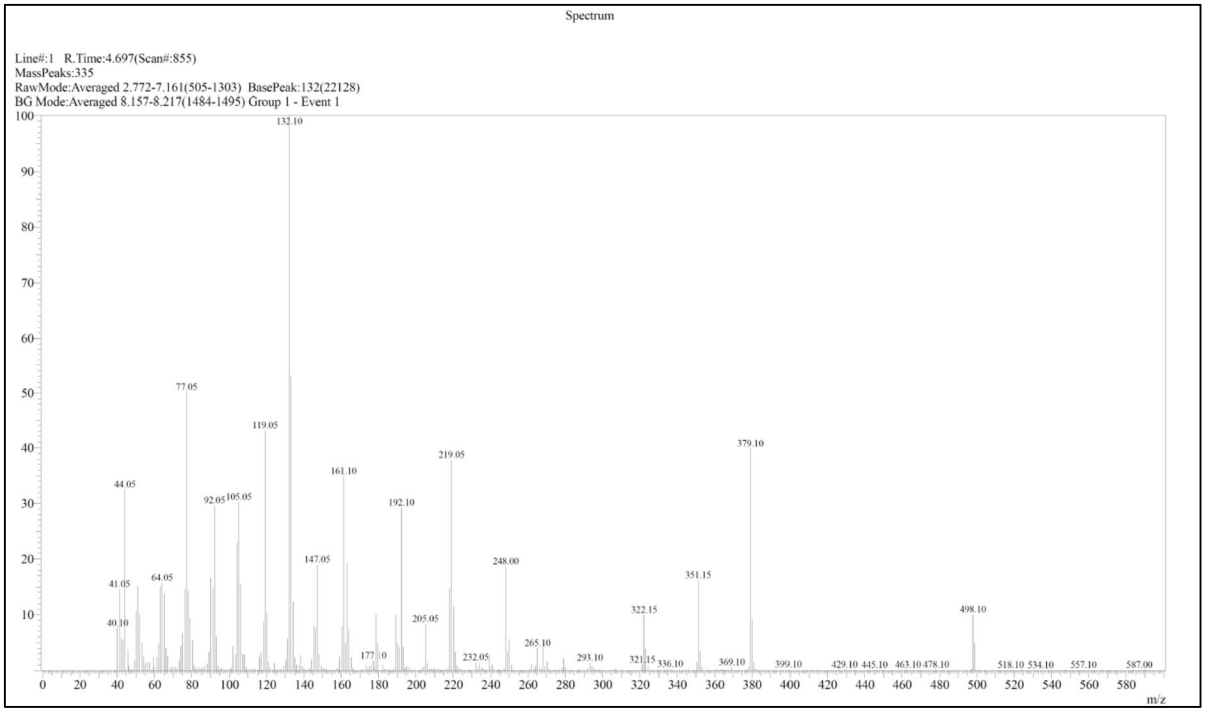


Figure 34. Mass spectrum of MMP-04.

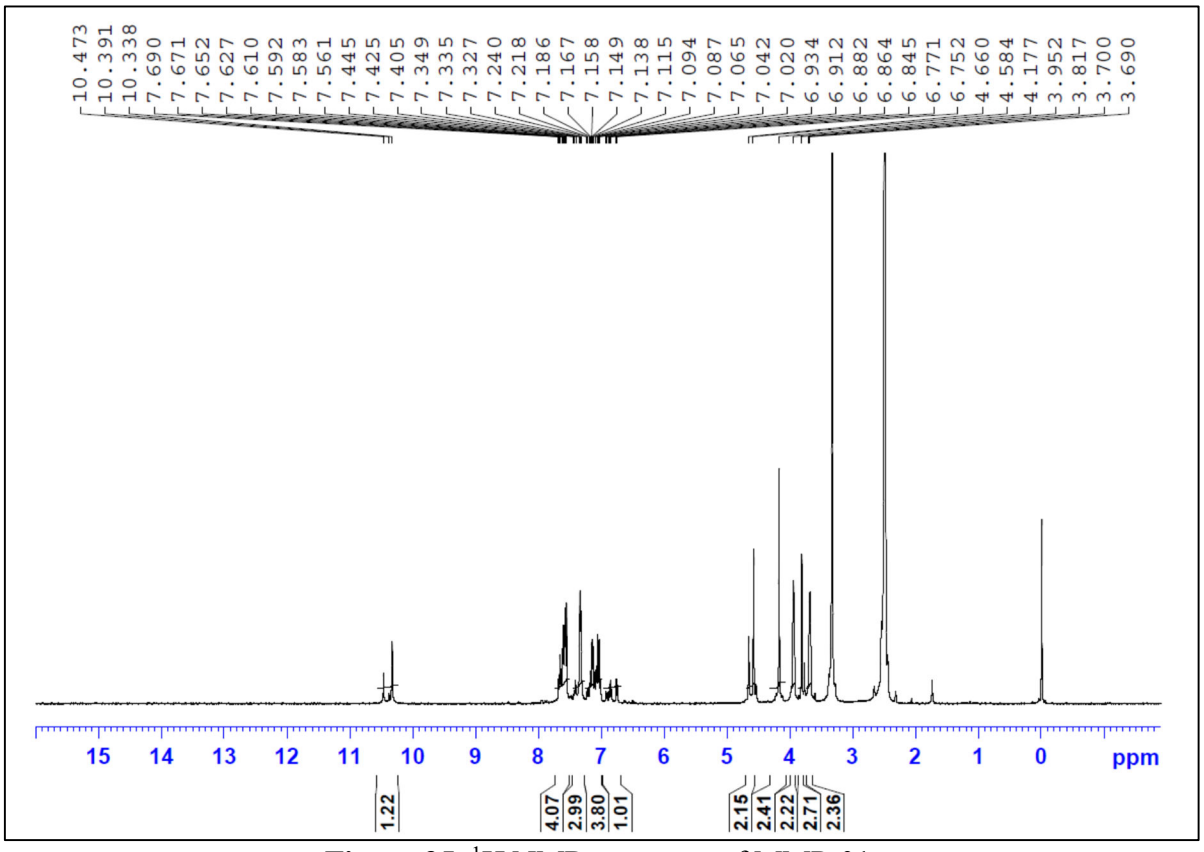


Figure 35. ^{1}H NMR spectrum of MMP-01.

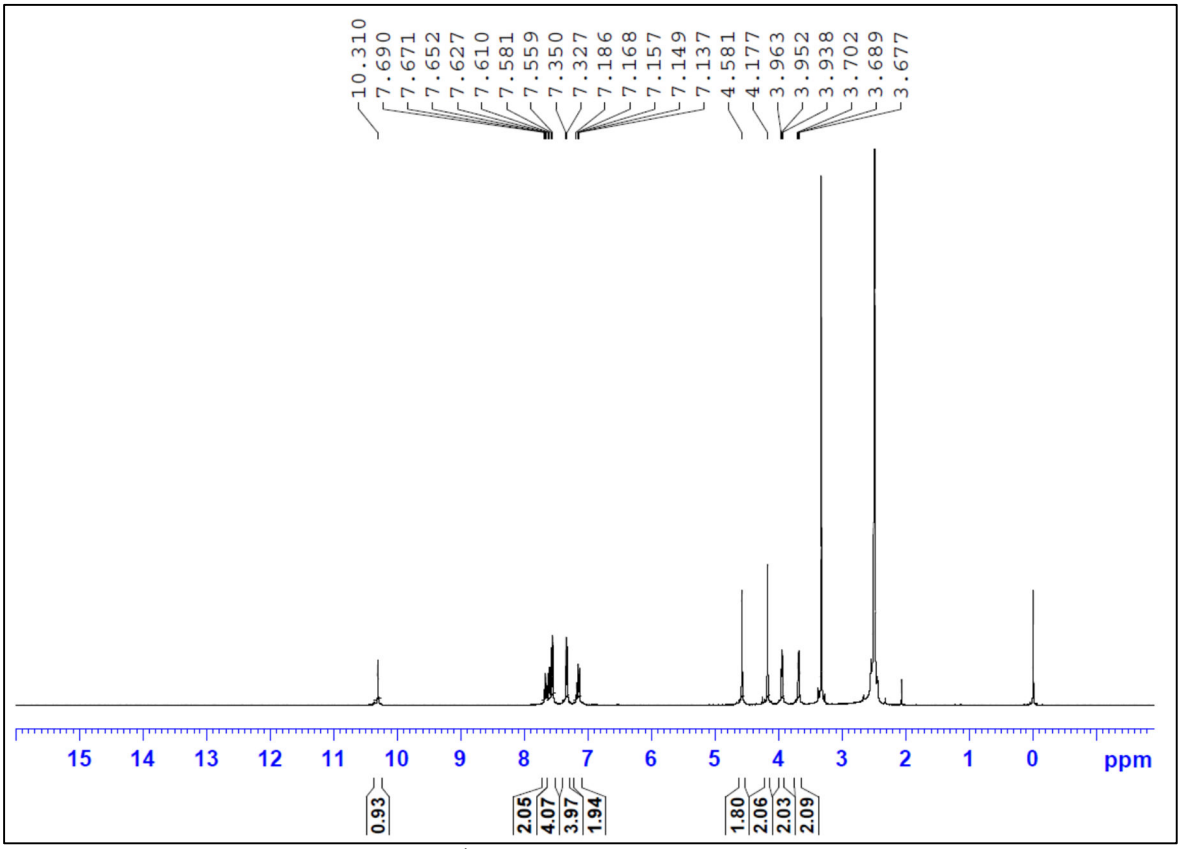


Figure 36. ^{1}H NMR spectrum of MMP-02.

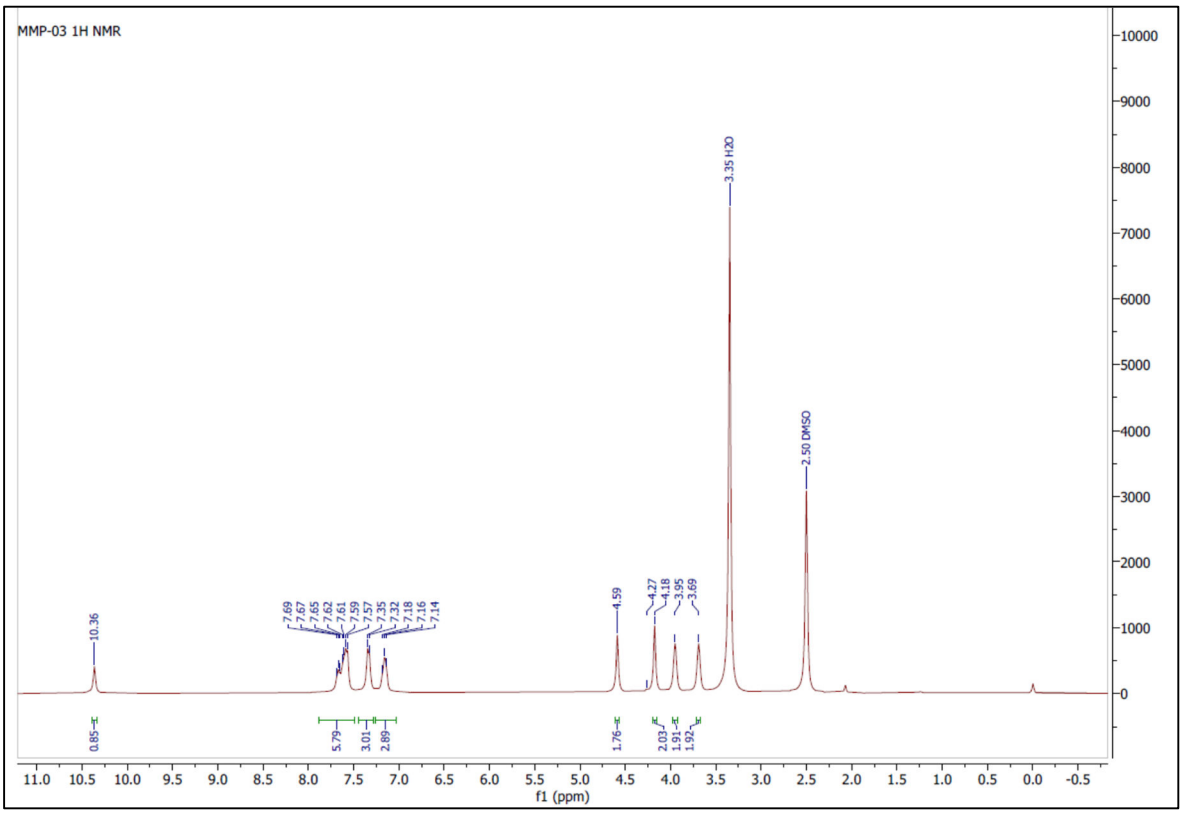


Figure 37. ^{1}H NMR spectrum of MMP-03.

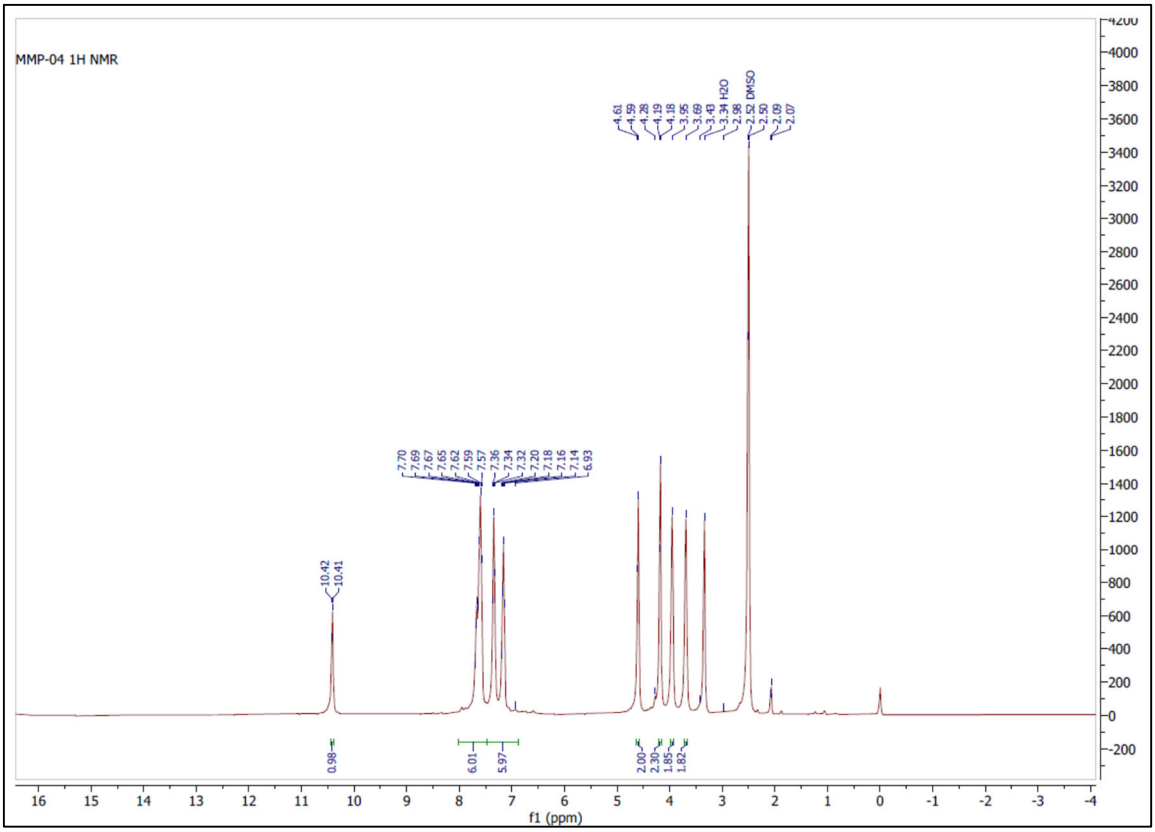


Figure 38. ^{1}H NMR spectrum of MMP-04.

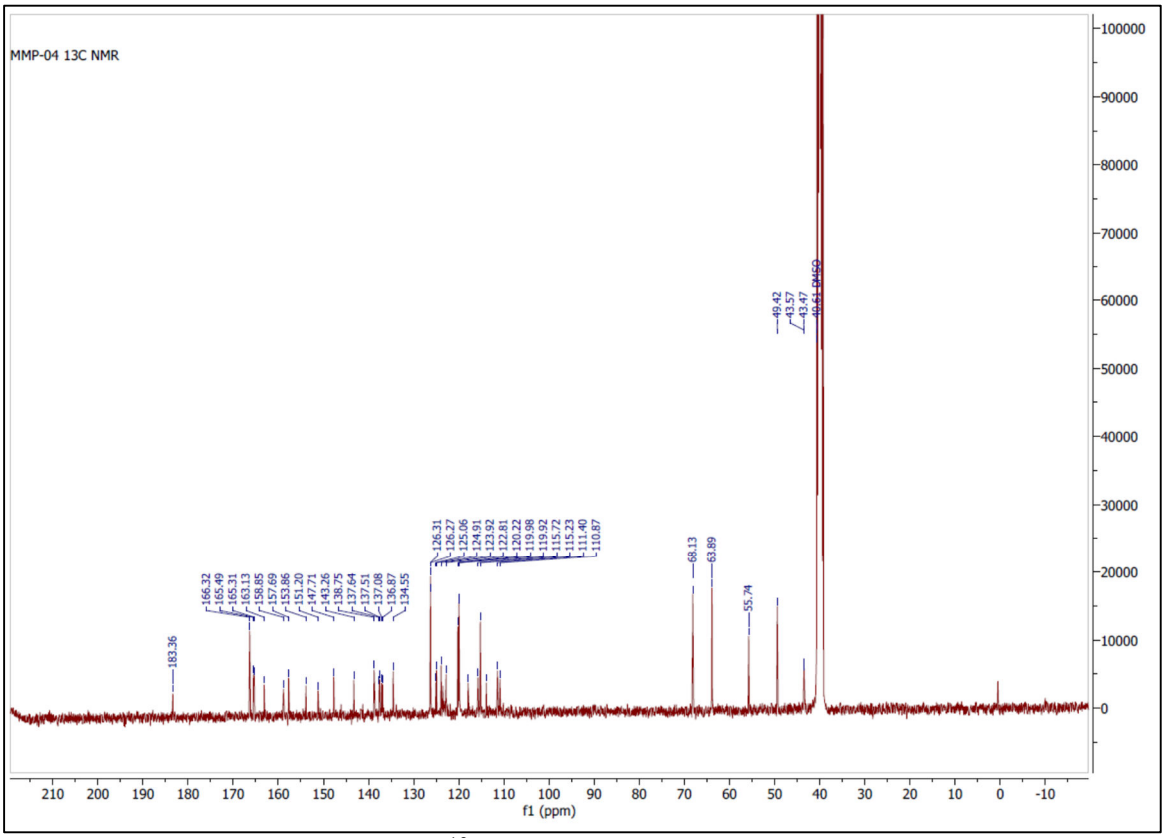


Figure 39. ^{13}C NMR spectrum of MMP-01.

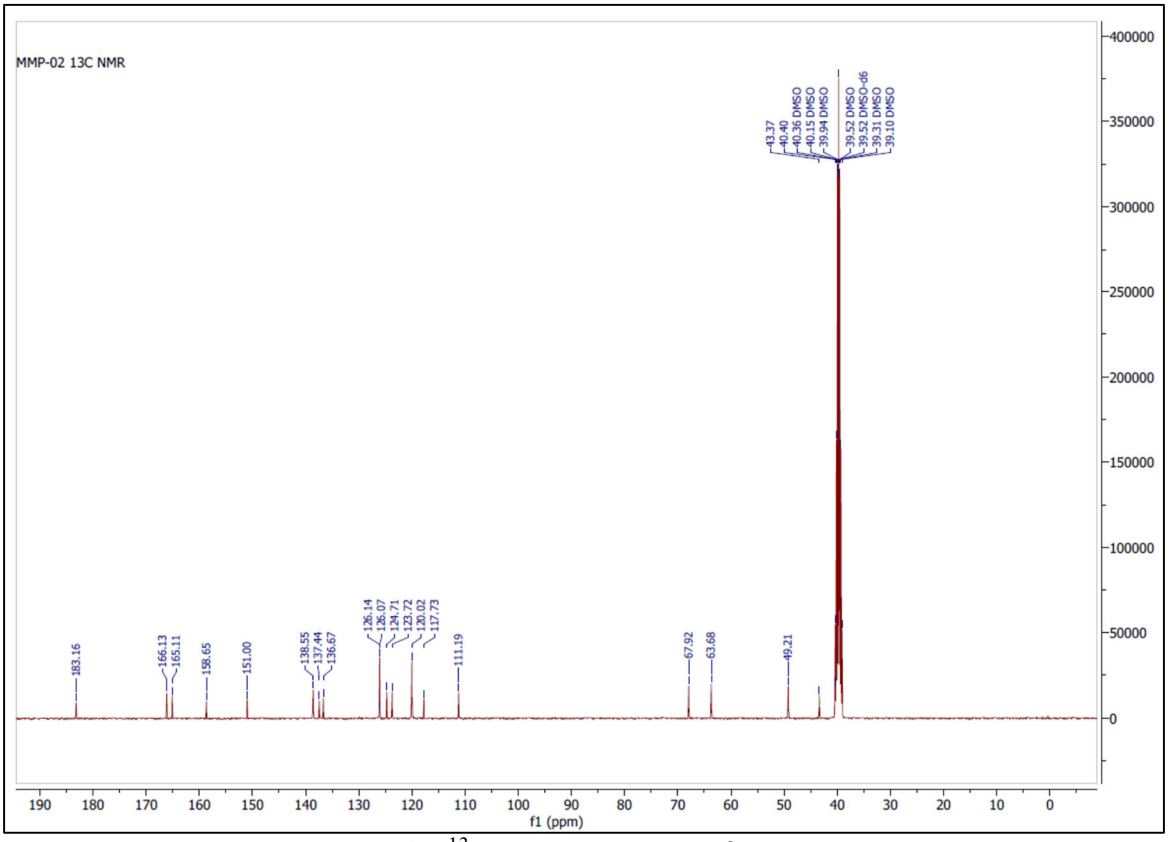


Figure 40. ^{13}C NMR spectrum of MMP-02.

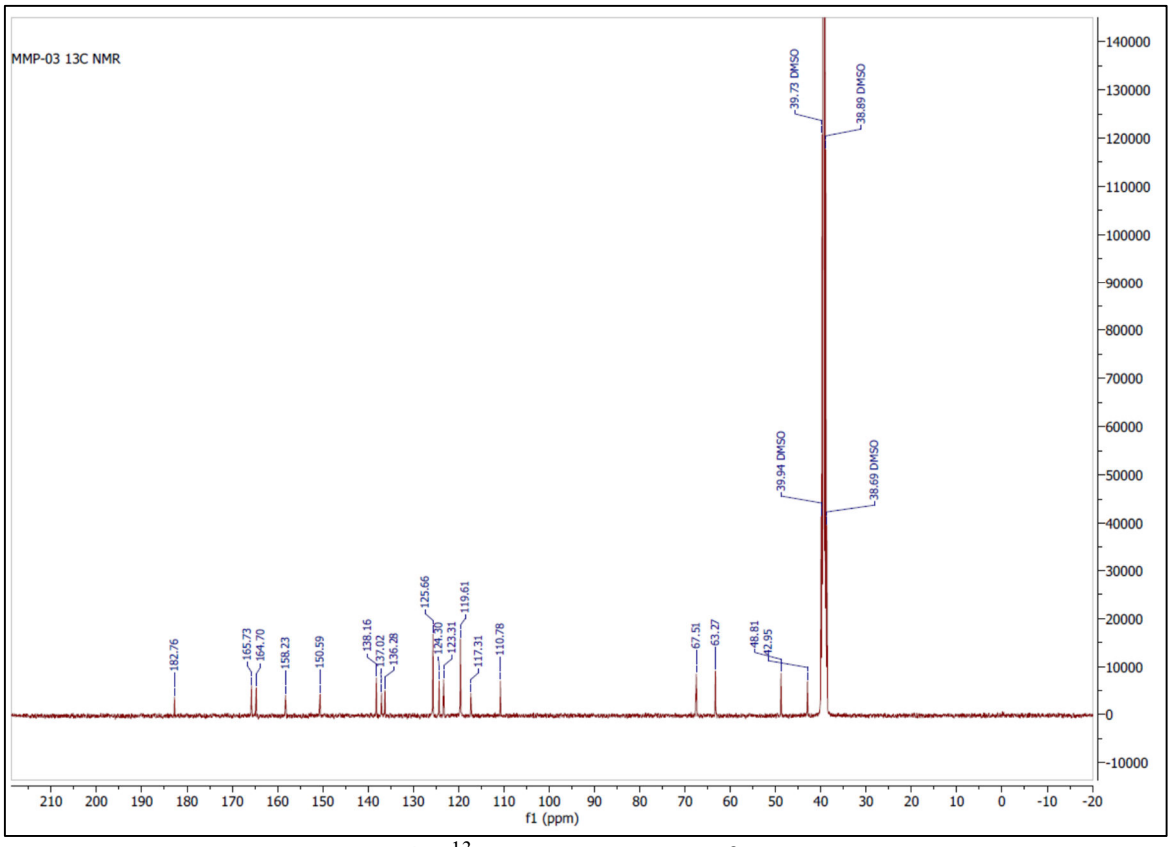


Figure 41. ^{13}C NMR spectrum of MMP-03.

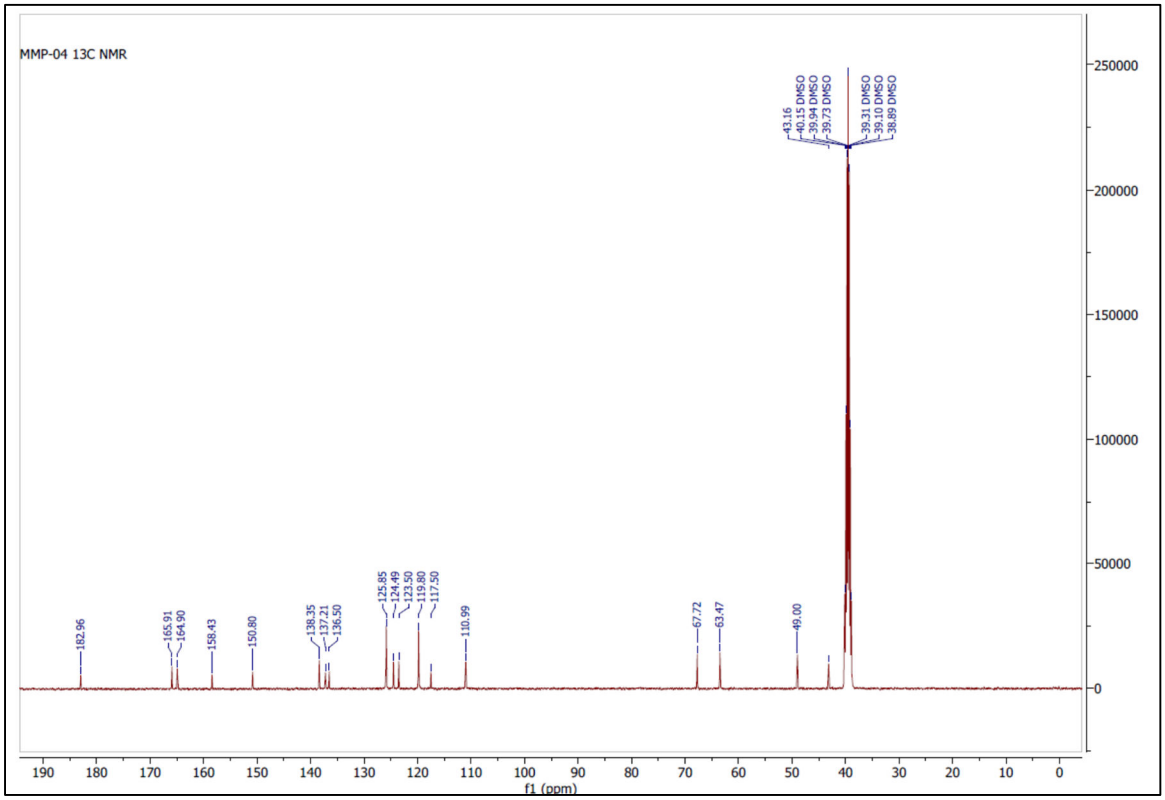


Figure 42. ^{13}C NMR spectrum of MMP-04.

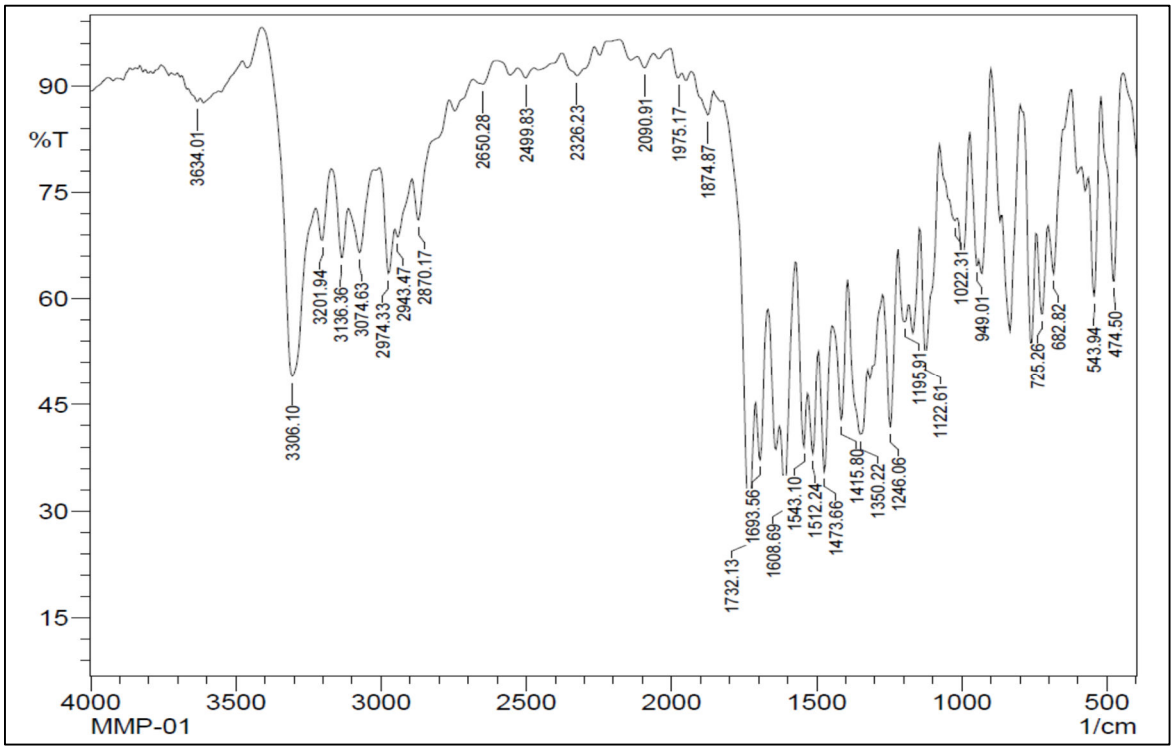


Figure 43. IR spectral analysis of MMP-01.

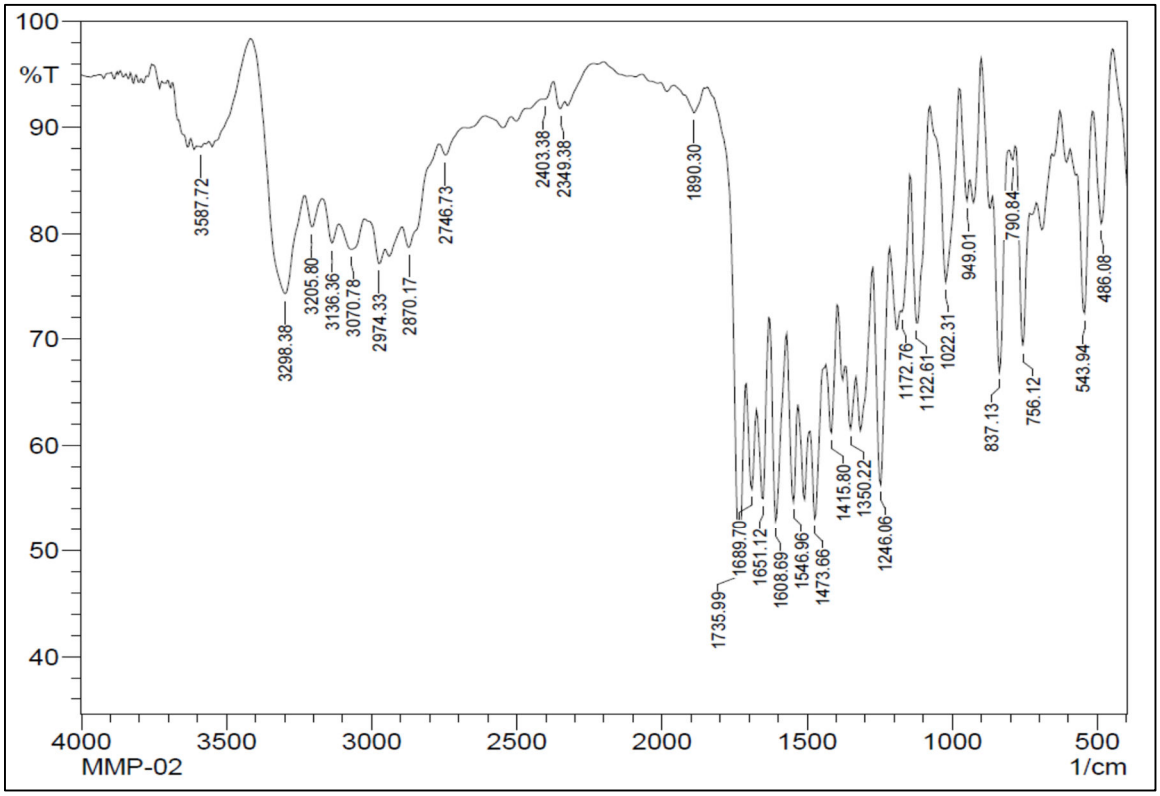


Figure 44. IR spectral analysis of MMP-02.

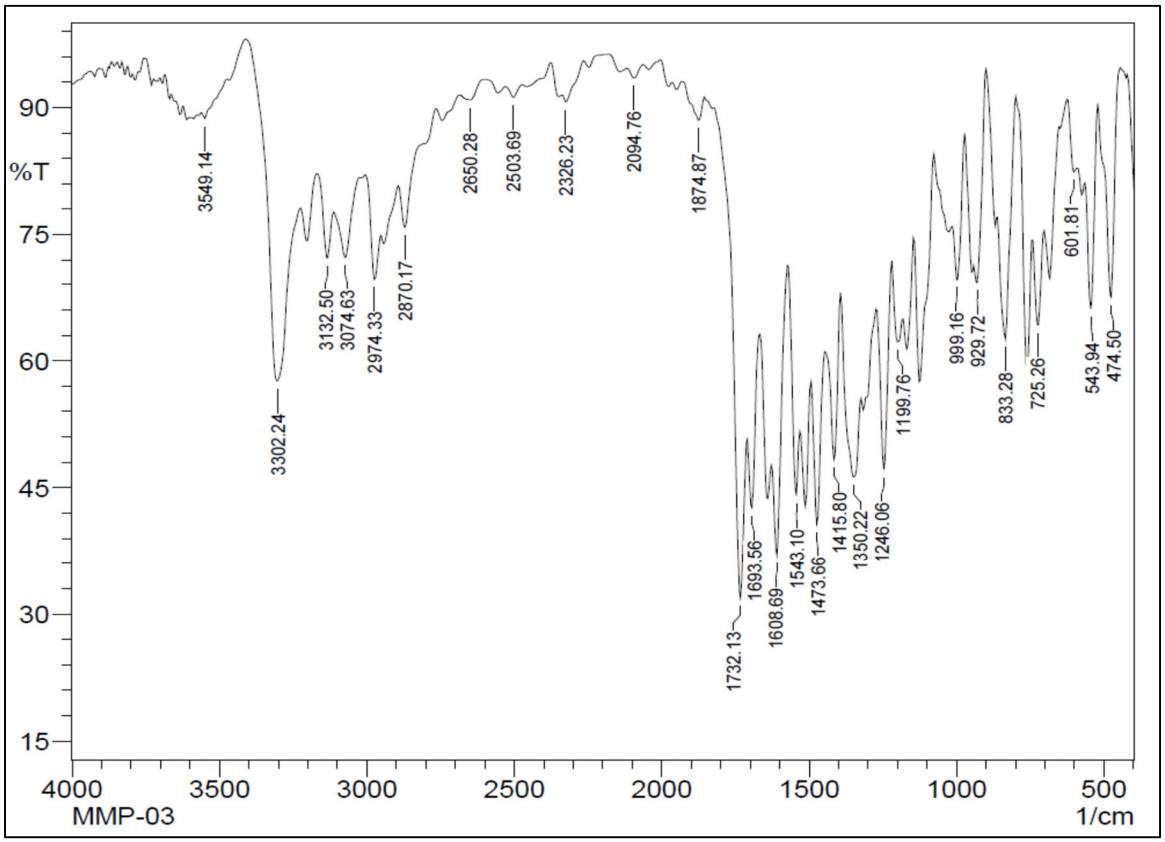


Figure 45. IR spectral analysis of MMP-03.

References

[1] M. B. Parmar, M. K. Vara and J. H. Pandya, A brief review on imidazole, triazine, and isatin derivatives: synthesis approaches and their applications. *Discover Chemistry* 1(56) (2024). https://doi.org/10.1007/s44371-024-00057-z

[2] S. H. Shreedhara, H. M. Vagdevi, N. D. Jayanna, R. Ramappa, K. Pamidimukkala, D. Prabhu and S. Mohammed, *Journal of Chemical and Pharmaceutical Research.* 9(5) (2017) 108-119.

[3] S. Kakkar, S. Tahlan, S. M. Lim, K. Ramasamy, V. Mani, S. A. A. Shah and B. Narasimhan, Benzoxazole derivatives: design, synthesis and biological evaluation. *Chemistry Central Journal* 12(92) (2018). https://doi.org/10.1186/s13065-018-0459-5

[4] J. Hu, Y. Zhang, N. Tang, Y. Lu, P. Guo and Z. Huang, Discovery of novel 1,3,5-triazine derivatives as potent inhibitor of cervical cancer via dual inhibition of PI3K/mTOR. *Bioorganic & Medicinal Chemistry* 32 (2021). https://doi.org/10.1016/j.bmc.2021.115997

[5] M. A. Motaleb, I. T. Ibrahim, M. O. Sarhan and W. A. Zaghary, Radioiodination and biological distribution of a new s-triazine derivative for tumor uptake evaluation. *Journal of Labelled Compounds and Radiopharmaceutical* 61(14) (2018) 1058-1068. https://doi.org/10.1002/jlcr.3682

[6] M. B. Parmar, J. H. Pandya and M. K. Vara. Synthesis, Characterization of Various Substituted (E)-2-(2-butyl-5-chloro-4-((phenylimino)methyl)-1H-imidazol-1-yl)-N-(4-(3-oxomorpholino)phenyl)acetamide. *Journal of Scientific Research* 15(2) (2023) 509-518. https://doi.org/10.3329/jsr.v15i2.61736

[7] A. A. Kulkarni, S. B. Wankhede, N. D. Dhawale, P. B. Yadav, V. V. Deore and I. D. Gonjari, Synthesis, characterization and biological behavior of some Schiff's and Mannich base derivatives of Lamotrigine. *Arabian Journal of Chemistry* 10(1) (2017) S184-S189. https://doi.org/10.1016/j.arabjc.2012.07.020

[8] P. V. Gandhi, S. R. Burande, M. S. Charde and R. D. Chakole, A review on different approaches to isatin synthesis. *International journal of creative research thoughts* 9(10) (2021) d348-d356.

[9] R. K. Thakur, P. Joshi, P. Baranwal, G. Sharma, S. K. Shukla, R. Tripathi and R. P. Tripathi, Synthesis and antiplasmodial activity of glyco-conjugate hybrids of phenylhydrazono-indolinones and glycosylated 1,2,3- triazolyl-methyl-indoline-2,3-diones. *European Journal of Medicinal Chemistry* 155 (2018) 764–771. https://doi.org/10.1016/j.ejmech.2018.06.042

[10] H. M. Osman, T. Elsaman, B. A. Yousef, E. Elhadi, A. A. E. Ahmed, E. M. Eltayib, M. S. Mohamed and M. A. Mohamed, Schiff bases of isatin and adamantane-1-carbohydrazide: synthesis, characterization and anticonvulsant activity. *Journal of Chemistry* 2021 (2021) 1-11. https://doi.org/10.1155/2021/6659156

[11] Tim Mosmann, Rapid colorimetric assay for cellular growth and survival: Application to proliferation and cytotoxicity assays. *Journal of Immunological Methods* 65(1–2) (1983) 55-63. https://doi.org/10.1016/0022-1759(83)90303-4