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Substituted Camphor-Hydrazide Schiff Bases: Synthesis, Characterization, and Anticancer Activity

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ABSTRACT

The present study covers synthesis of 2-phenylacetohydrazide linked camphor Schiff base. Very few studies have been conducted on synthesis of camphor derivatives, highlighting the significance of my research in this area. For this research, 2-phenylacetic acid converted into their ester form, ethyl 2-phenylacetate using ethanol. Further, reaction with hydrazine hydrate forms the 2-phenylacetohydrazide. At final step, reaction with camphor yields (*E*)-2-phenyl-*N*'-(1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)acetohydrazide. Structure identification is verified by IR spectroscopy, mass spectroscopy, elemental analysis, ^{1}H and ^{13}C NMR. All the compounds were evaluated for anticancer activity against the MCF-7 breast cancer cell line, showing cytotoxicity in the range of 10 mg/ml to 40 mg/ml and MBL-104 exhibited the highest cytotoxic potency. These results open the door for further detailed applications in medicinal chemistry.

***Keywords*:** Anticancer activity, camphor, MCF-7 cell line, 1,7,7-trimethylbicyclo[2.2.1]heptan-2-one.

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1. INTRODUCTION

Plants have been utilize for medical applications for centuries. They have been traded commercially and evaluated scientifically. Camphor (1,7,7-trimethylbicyclo[2.2.1]heptan-2-one) is one of the first plant metabolites isolated in the chemically pure state [1]. Camphor is a waxy, colorless solid with a strong aroma [2]. It is classified as a terpenoid (monoterpenoid) and a cyclic ketone. Camphor possesses a range of useful application such as Anti-inflammatory [3], Insecticidal [4, 5], Allelopathic [5], antibacterial activity [6], as antiviral agent [7] as antimicrobial [8], Acaricidal [9], Antioxidative, [10]. Algicidal [11], insects repellent [12], mosquito repellent [13]. For this research 2-phenylacetic acid converted into their respective ester form, ethyl phenylacetate using ethanol in the presence of conc. H_2SO_4 [14, 15]. Further, reaction with hydrazine hydrate forms the 2-phenylacetohydrazide at 0°C in ethanol [16]. Final product, (*E*)-2-phenyl-*N*'-(1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)acetohydrazide derivatives was afford by the reaction with camphor with catalytic amount of glacial acetic acid in hot ethanol [17]. The synthesis scheme is illustrated in **Scheme 1** and physiochemical data in **Figure 1**. Structure identification is fulfilled by several analytical evolution, like Mass Spectroscopy, Elemental Analysis, ^{1}H and ^{13}C NMR, and IR Spectroscopy.

2. EXPERIMENTAL

2.1. General Materials and Methods

All chemical reactions were carried out by Sigma–Aldrich and Loba Chemie chemicals. The progress of reactions was monitored by thin-layer chromatography (TLC) on pre-coated silica gel GF254 plates from E-Merck Co., and compound filmed by exposure to Ultra-Violet light. Melting point of compound was found in open capillary. The IR spectra of compound was captured on Shimadzu FT-IR 8400 spectrophotometer using the KBr pellet technique and Tetramethylsilane (TMS) used as an internal standard in NMR spectra of compound were captured on Jeol JNM- ECZ 400S 400 MHz FT-NMR Spectrometer in $CDCL_3$-d solvent. Mass spectra were recorded on Shimadzu LC-MS.

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 [18]

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 Substituted Ethyl 2-phenylacetate (M-101 to M-106)

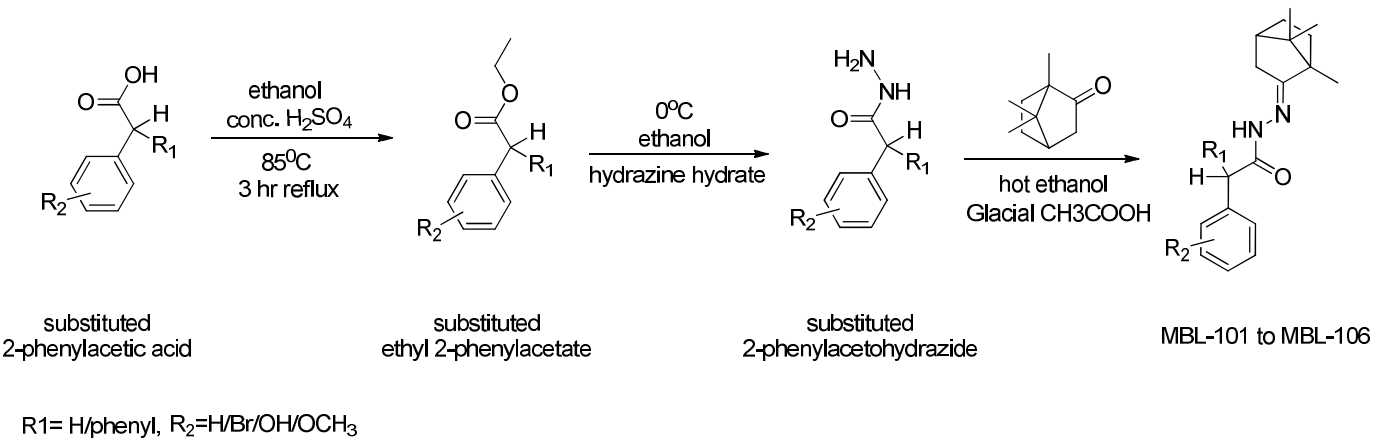
Various substituted 2-phenylacetic acid (1 mole) treated with 50 ml ethanol in the presence of conc. H_2SO_4 reflux for 180 minutes at 85°C on a magnetic stirrer results in substituted ethyl 2-phenylacetate derivatives (M-101 to M-106) conformed by TLC plate Ethyl acetate and Hexane (6:4) ratio in the UV chamber. The crude product was poured on ice and a dilute solution of Sodium bicarbonate ($NaHCO_3$) to remove unreacted substituted 2-phenylacetic acid. The resultant solid is filtered and allowed to dry in a desiccator.

2.4.2. General Synthesis of Substituted 2-phenylacetohydrazide (MB-101 to MB-106)

Substituted ethyl 2-phenylacetate (M-101 to M-106) is kept in ice-cold (0°C) ethanol solution for 30 minutes with vigorous stirring. Treating the solution with hydrazine hydrate 80% resulted in substituted 2-phenylacetohydrizide derivatives (MB-101 to MB-106).

2.4.3. General Synthesis of Various Substituted (*E*)-2-phenyl-*N*'-(1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)acetohydrazide (MBL-101 to MBL-106)

Substituted ethyl 2-phenylacetate (MB-101 to MB-106) (0.005 mole) is in 50 ml flask dissolved in 25 ml hot ethanol with a catalytic amount of Glacial CH_3COOH after dissolution, add 1,7,7-trimethylbicyclo[2.2.1]heptan-2-one (Camphor) (0.006 mole). Reflux the solution mixture for 120 minutes at 70-80°C temperature. Monitor reaction with TLC (Ethyl acetate: Hexane) 5:5. After completion of reaction pour into cold water. Excessive Camphor is dissolved in dilute CH_3COOH resulting in removal during filtration. For further purification wash with cold methanol.



Scheme 1. Synthesis route for titled derivatives MBL-101 to MBL-106.

2.4.3.1. Spectral Data of (MBL-101)

(*E*)-2-phenyl-*N*'-(1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)acetohydrazide:
White, yield (87%), m.p. 60-62°C. IR spectrum, ν, cm^{-1}: 3190, 1651 (NH, C=O, secondary amide), 1555 (C=N), 1485, 1446 (C=C). ^{1}H NMR (400 MHz, $CHCl_3$-d) ppm: 8.21-8.04 (s, 1H), 7.44-7.19 (m, 5H), 4.10-3.87 (d, 2H), 2.36-2.22 (d, 1H) 2.05-1.96 (d, 1H), 1.95-1.65 (m, 3H), 1.49-1.32 (m, 1H), 1.28-1.13 (m, 1H), 1.11-1.03 (s, 3H), 0.99-0.90 (s, 3H), 0.81-0.62 (s, 3H); ^{13}C NMR (101 MHz, $CHCl_3$-d) δ = 173.270, 165.327, 135.490, 129.741, 129.636, 129.300, 128.428, 126.723, 52.706, 47.982, 44.092, 39.627, 33.255, 33.006, 32.603, 32.326, 27.334, 27.161, 19.544, 18.710, 11.332, 11.150; Mass: Obs. (m/z) 285.0, calcd. (m/z) 284.4; Elemental Analysis (%): ($C_{18}H_{24}N_2O$); C, 76.02; H, 8.51; N, 9.85; O, 5.63; Found: C, 76.03; H, 8.50; N, 9.87; O, 5.61.

2.4.3.2. Spectral Data of (MBL-102)

(*E*)-2-(4-bromophenyl)-*N*'-(1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)acetohydrazide: White, yield (86%), m.p. 64-66°C. IR spectrum, ν, cm^{-1}: 3190, 1651 (NH, C=O, secondary amide), 1555 (C=N), 1481, 1420 (C=C). ^{1}H NMR (400 MHz, $CHCl_3$-d) ppm: 8.20-8.14 (s, 1H), 7.30-7.27 (s, 1H), 7.14-7.04 (m, 3H), 3.81-3.77 (s, 2H), 2.19-2.07 (d, 1H), 1.88-1.81 (t, 1H), 1.79-1.69 (m, 1H), 1.66-1.57 (ds, 2H), 1.27-1.18 (m, 1H), 1.09-0.99 (m, 1H), 0.91-0.84 (s, 3H), 0.82-0.78 (s, 3H), 0.62-0.53 (s, 3H); ^{13}C NMR (101 MHz, $CHCl_3$-d) δ = 172.762, 165.844, 134.465, 132.290, 131.504, 131.466, 120.715, 52.744, 48.011, 44.073, 39.081, 32.380, 32.603, 31.243, 27.314, 26.759, 19.544, 18.710, 16.775, 11.352; Mass: Obs. (m/z) 363.0, calcd. (m/z) 363.3; ($C_{18}H_{23}BrN_2O$).

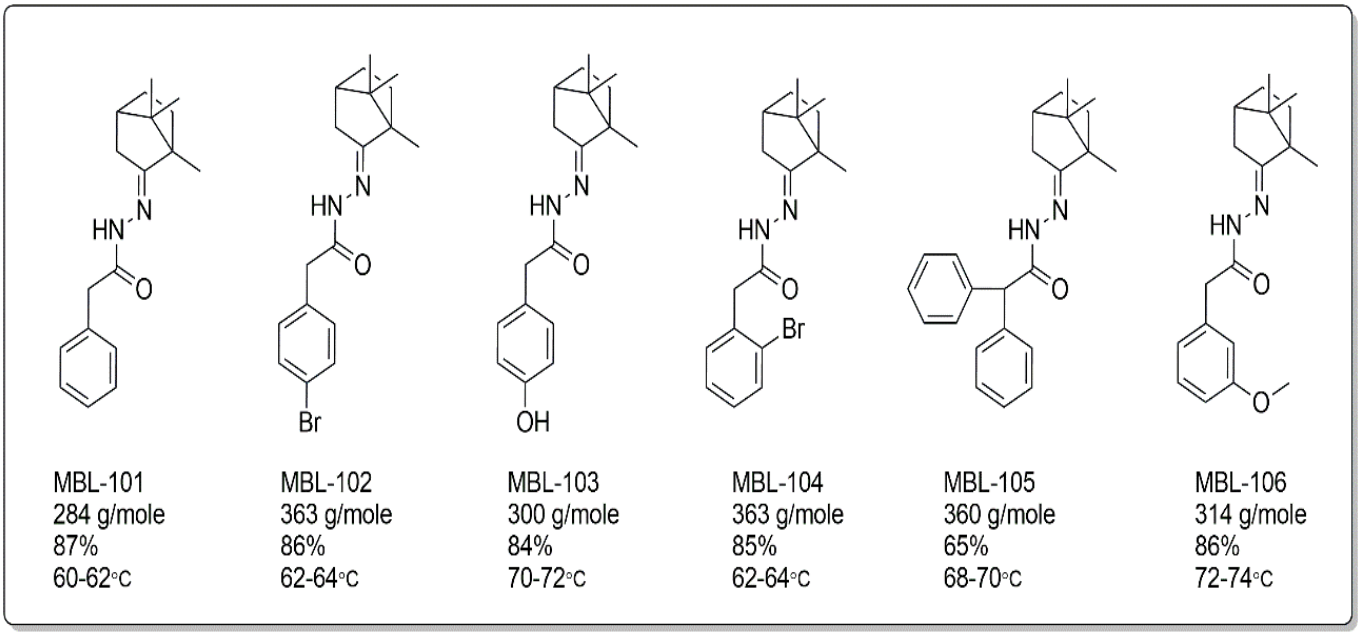


Figure 1. Physiochemical data of MBL Synthesis library

3. RESULTS AND DISCUSSION

The title derivatives of (*E*)-2-phenyl-*N*'-(1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylidene)acetohydrazide, was synthesized through a three-step reaction sequence starting from 2-phenylacetic acid. Esterification with ethanol yielded ethyl 2-phenylacetate, followed by hydrazinolysis to obtain 2-phenylacetohydrazide. The final condensation with camphor under reflux conditions in the presence of glacial acetic acid afforded the desired camphor-derived Schiff bases with 65-87% yield and 60–74°C melting point range. Structural verification was achieved through a combination of spectroscopic and analytical techniques. FT-IR spectrum exhibited characteristic peaks at 3190 cm^{-1} (NH), 1651 cm^{-1} (C=O), and 1555 cm^{-1} (C=N), confirming the presence of hydrazide and imine functionalities. ^{1}H NMR and ^{13}C NMR spectra ($CDCl_3$-d, 400 MHz) were consistent with the proposed structure, displaying signals corresponding to aromatic protons, aliphatic protons of the camphor moiety, and hydrazone linkage. Mass spectrometry showed a molecular ion peak at m/z = 285.0 (calcd. 284.4), confirming the molecular weight of MBL-101. The anticancer activities of a series of MBL derivatives (MBL-101 to MBL-106) were evaluated against the MCF-7 breast cancer cell line and illustrated in **Figure 2**.

The IC_{50} values (± standard deviation) obtained from the cytotoxicity assay were as follows: MBL-101: 27.44 ± 2.52 mg, MBL-102: 17.68 ± 1.94 mg, MBL-103: 13.94 ± 1.12 mg, MBL-104: 10.24 ± 1.13 mg, MBL-105: 16.03 ± 3.57 mg, and MBL-106: 40.05 ± 5.02 mg. Among the tested compounds, MBL-104 exhibited the highest cytotoxic potency, with the lowest IC_{50} value of 10.24 mg. This was followed by MBL-103 and MBL-105, with IC_{50} values of 13.94 mg and 16.03 mg, respectively. MBL-106 showed the weakest activity (IC_{50} = 40.05 mg), indicating significantly lower potency compared to the other derivatives. The standard deviations were generally low, suggesting good reproducibility of the assay results. These findings indicate that structural modifications among the MBL derivatives influence anticancer activity, warranting further investigation of MBL-104 and related analogs as potential therapeutic candidates.

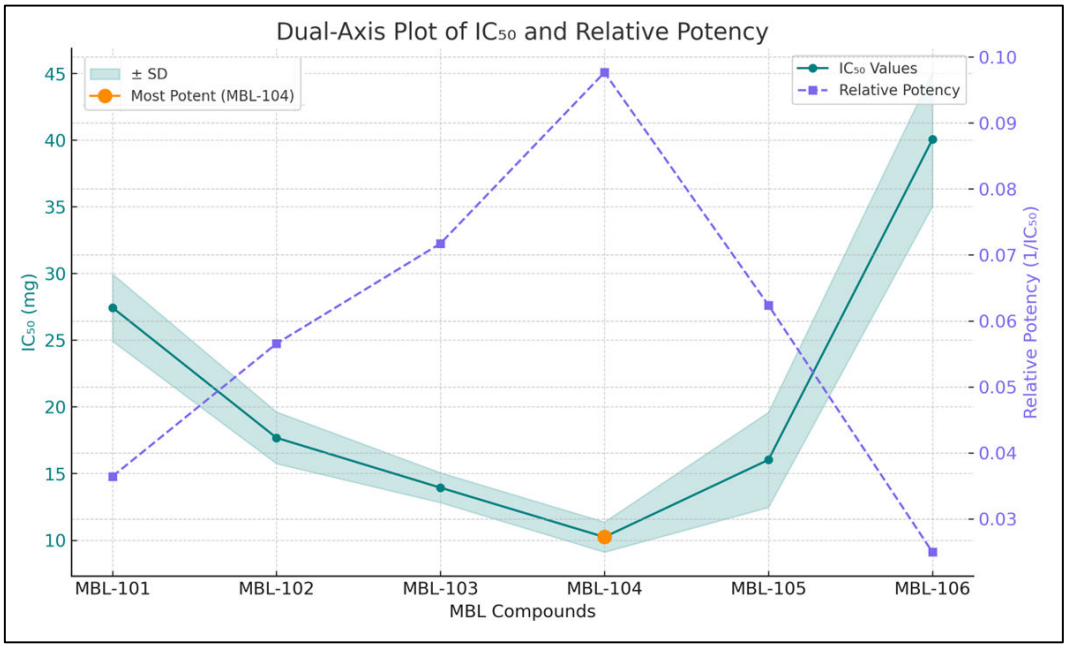


Figure 2. The line and relative potency plot of IC_{50} values for MBL compounds against MCF-7 human breast cells line.

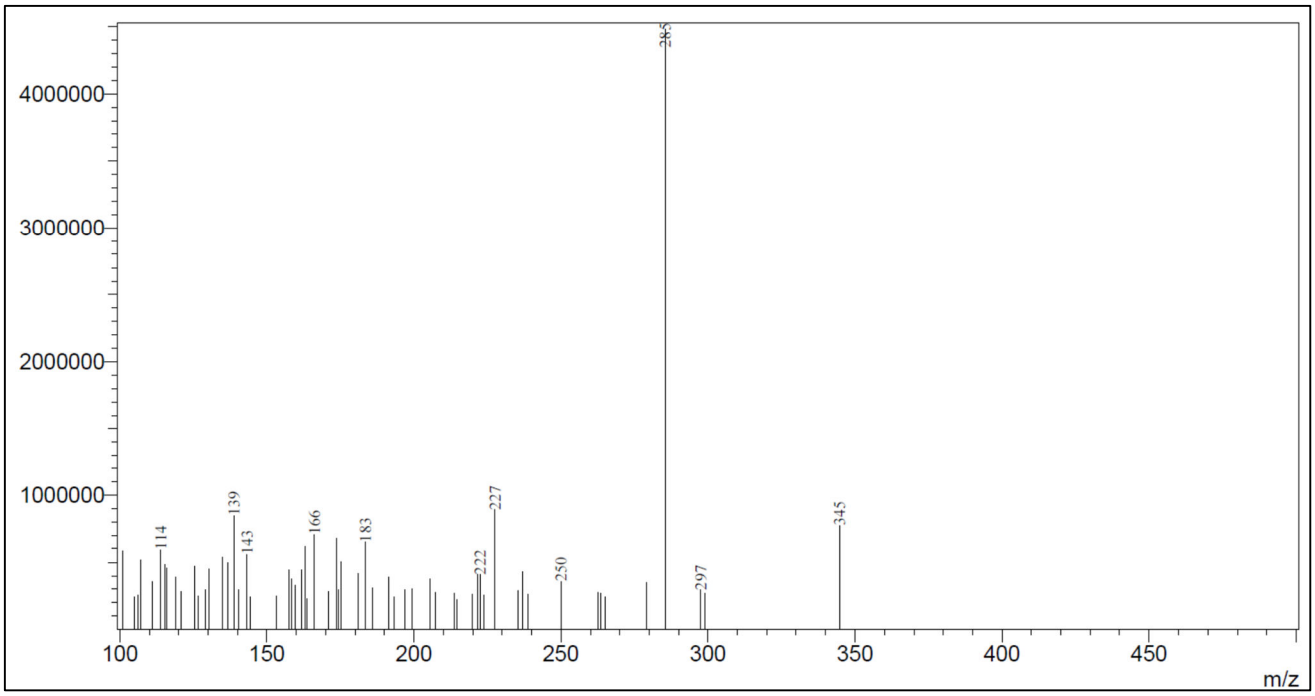


Figure 3. Mass spectra of MBL-101.

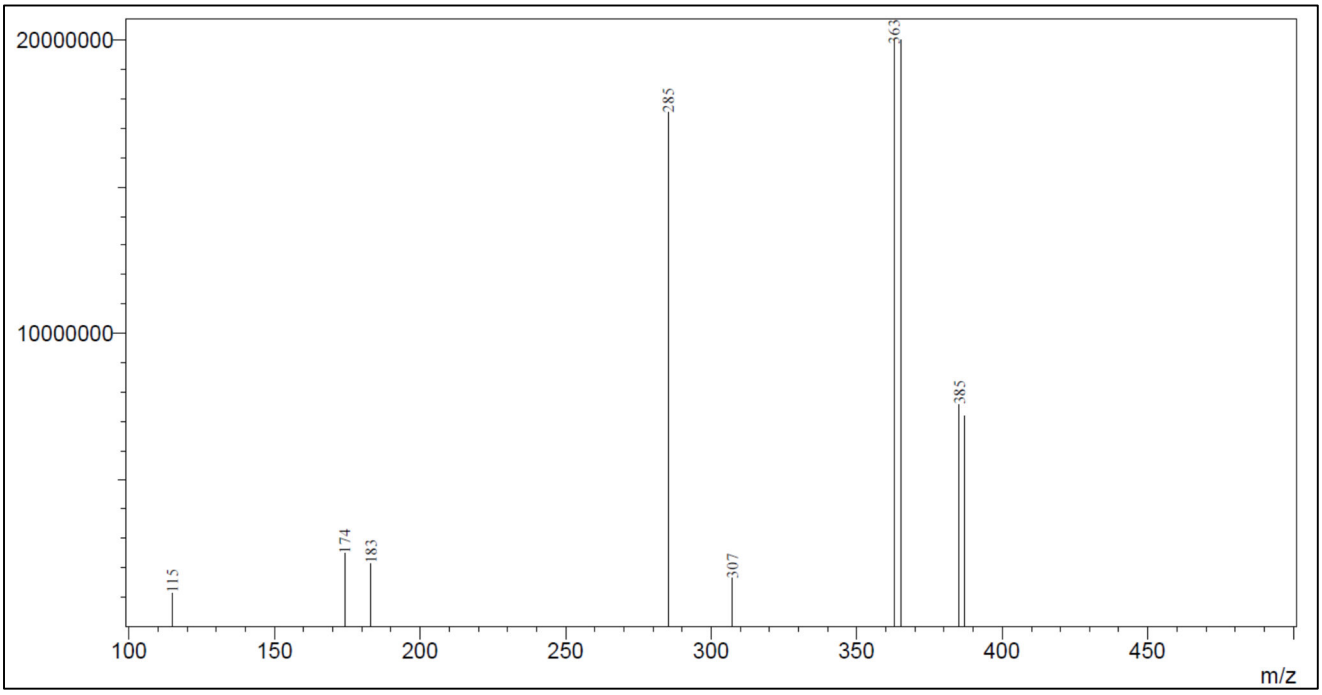


Figure 4. Mass spectra of MBL-102.

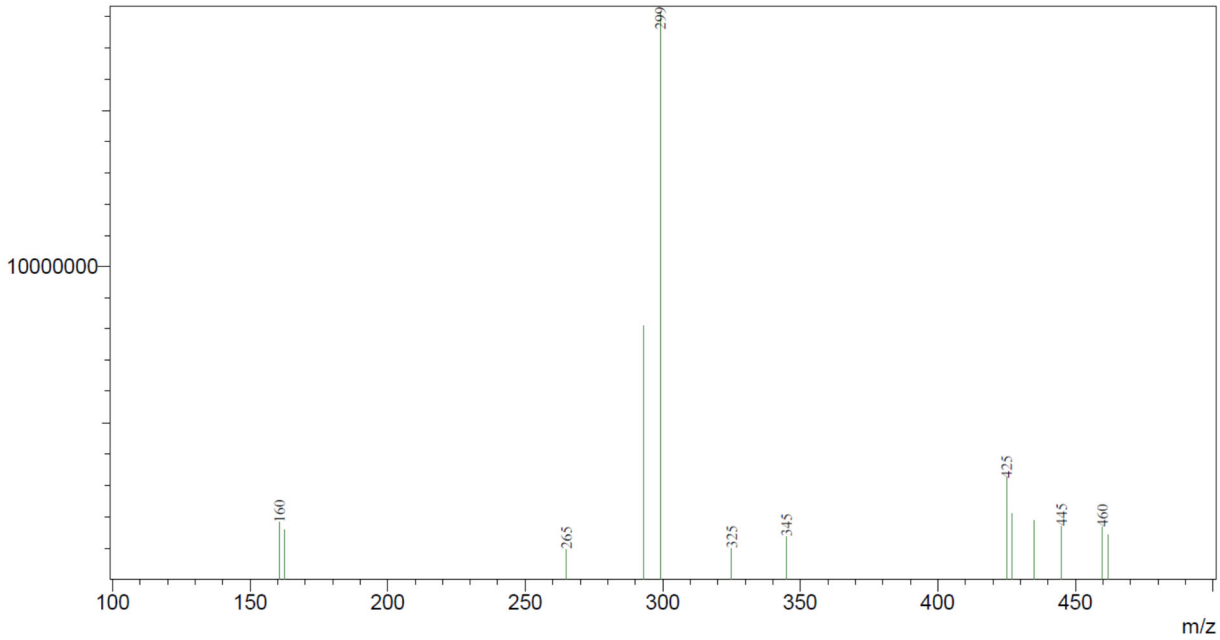


Figure 5. Mass spectra of MBL-103.

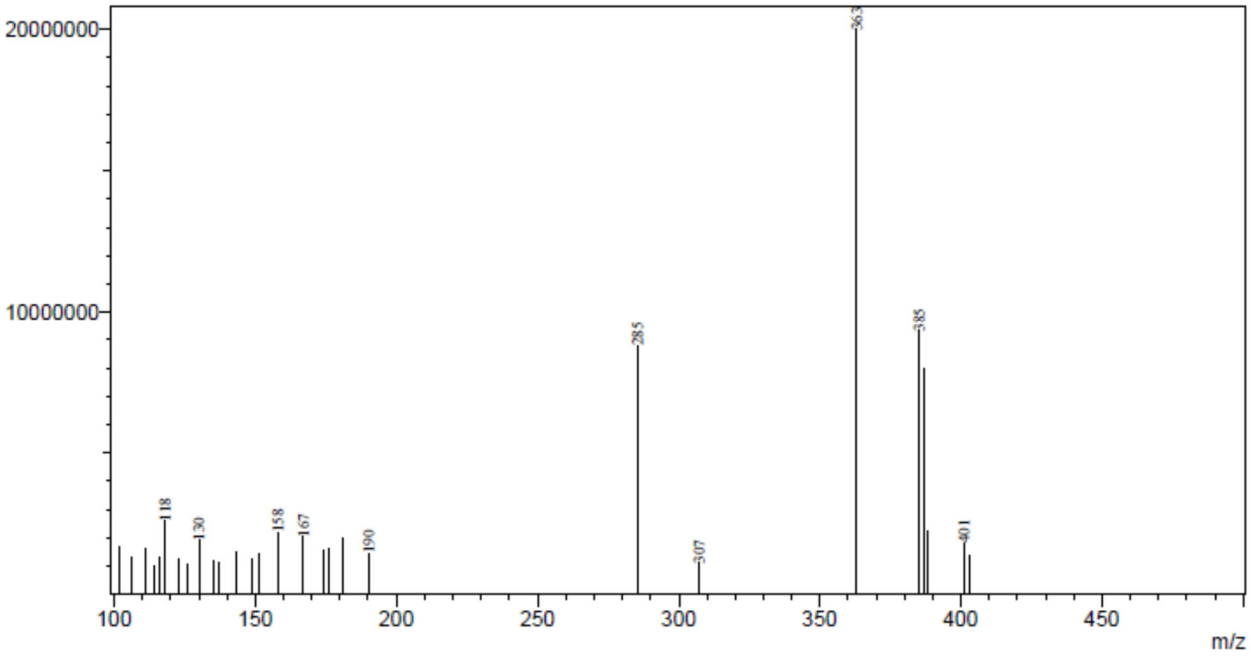


Figure 6. Mass spectra of MBL-104.

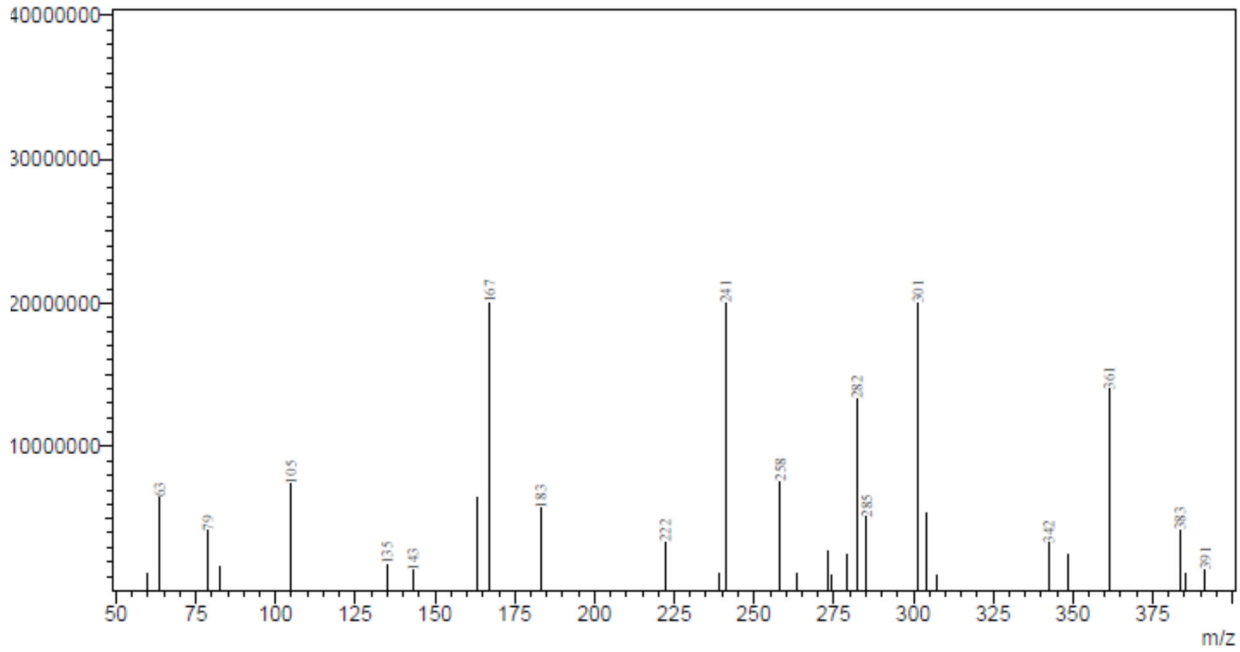


Figure 7. Mass spectra of MBL-105.

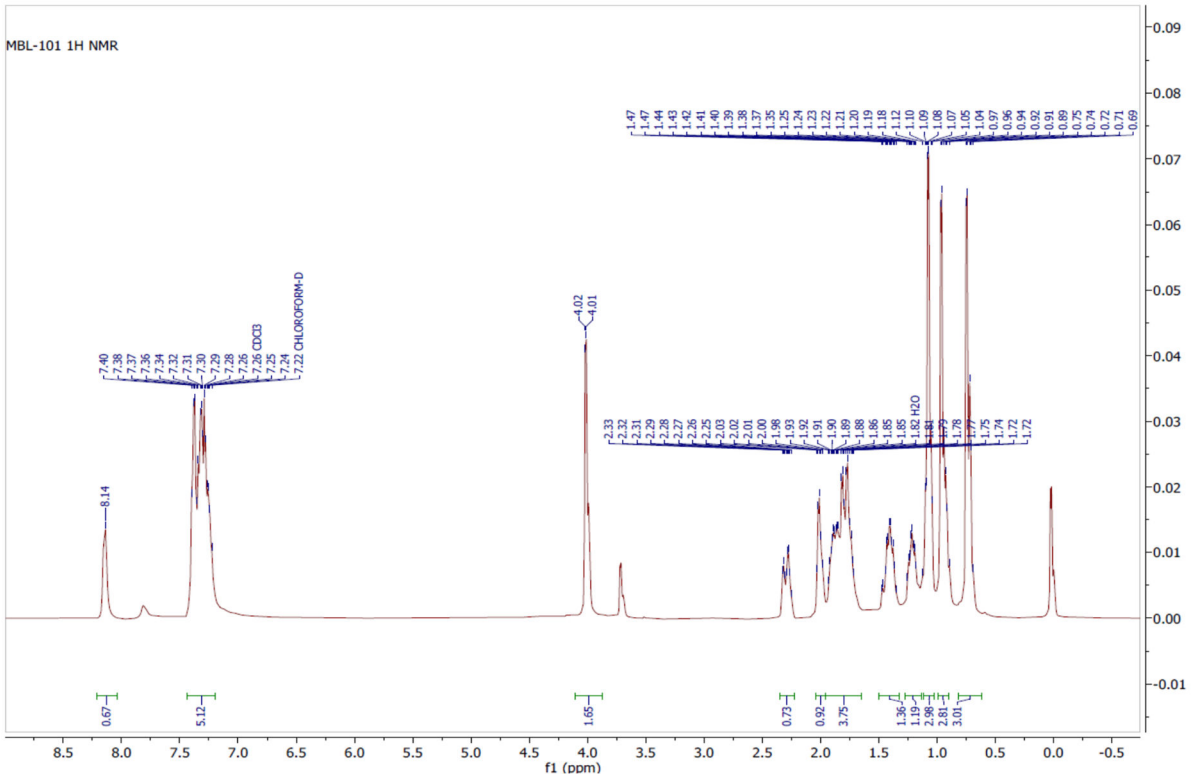


Figure 8. ^{1}H NMR spectra of MBL-101.

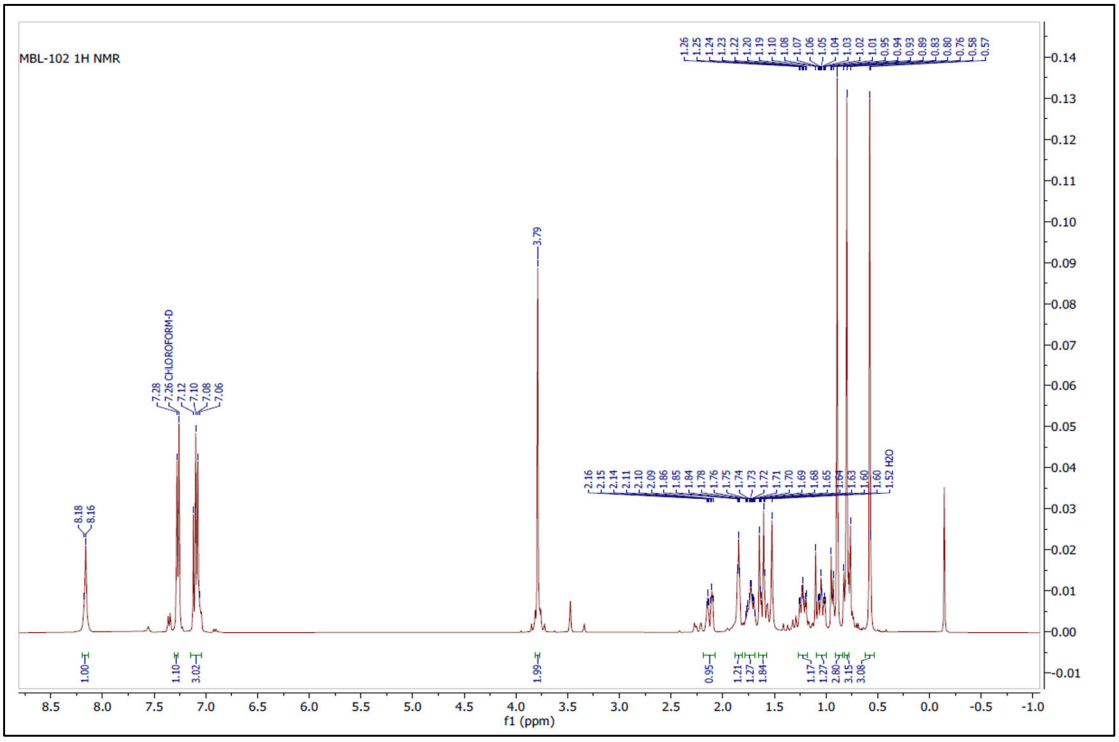


Figure 9. ^{1}H NMR spectra of MBL-102.

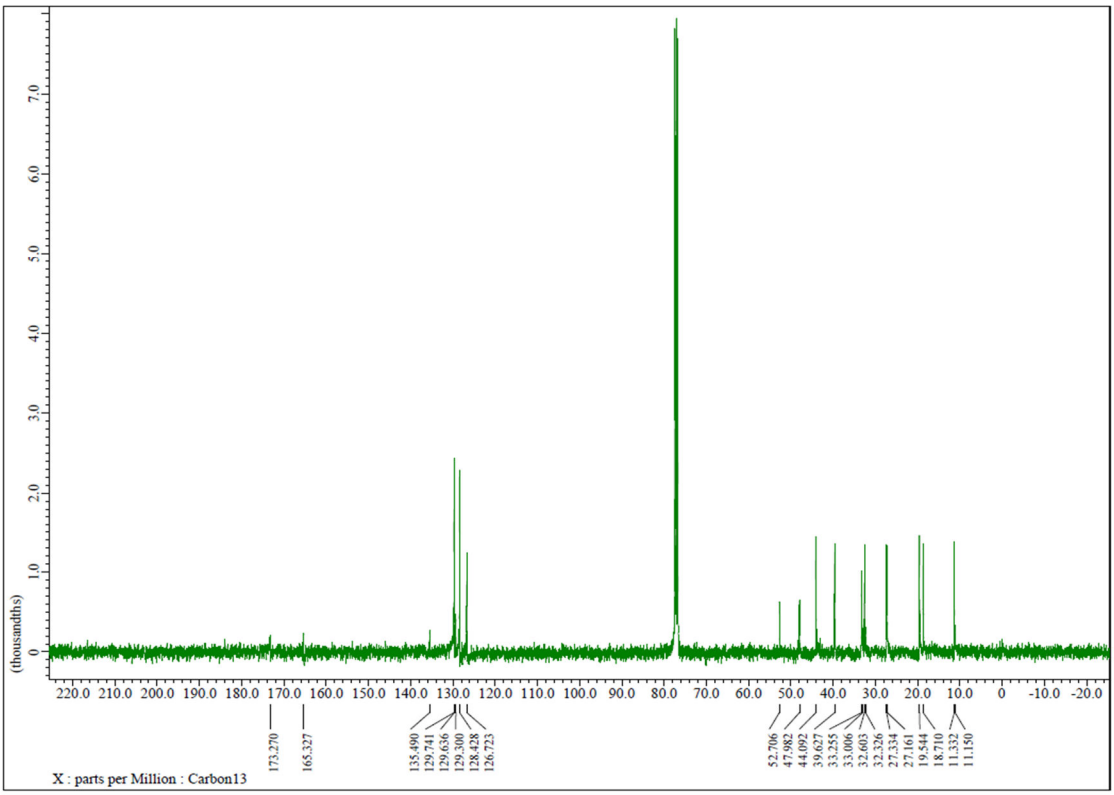


Figure 10. ^{13}C NMR spectra of MBL-101

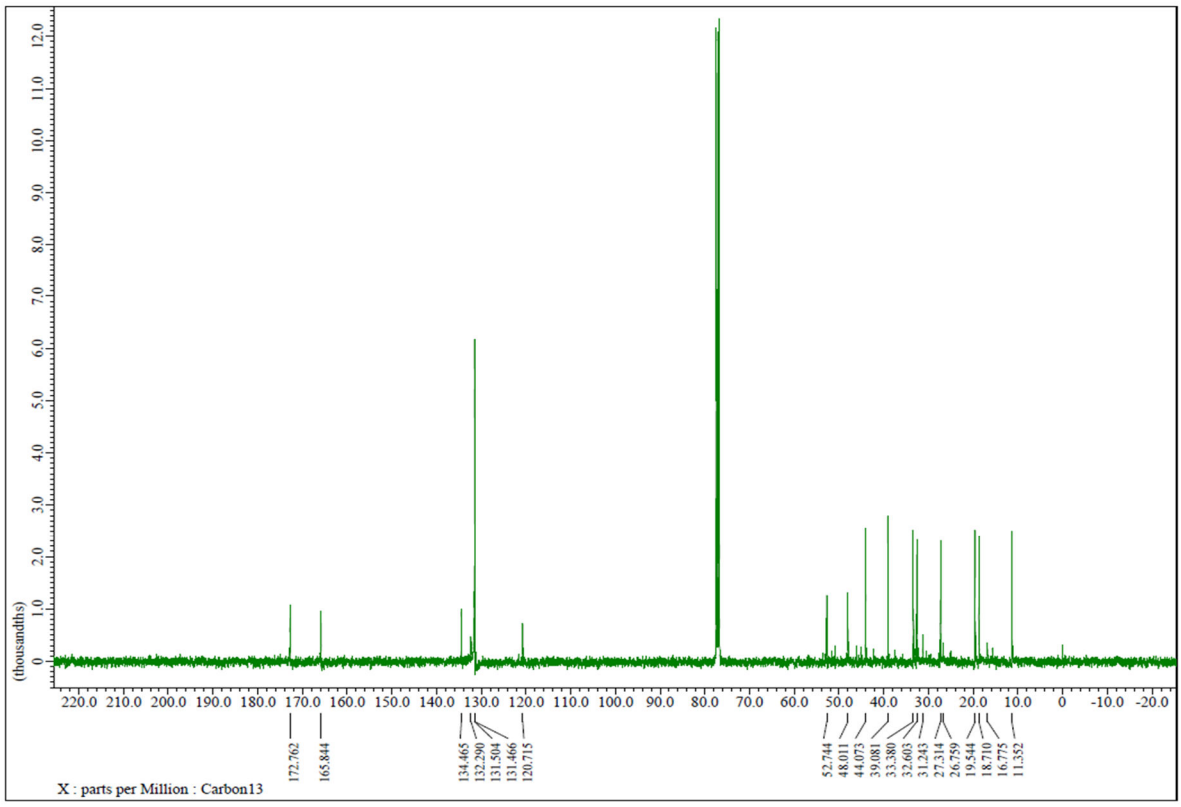


Figure 11. ^{13}C NMR Spectra of MBL-102.

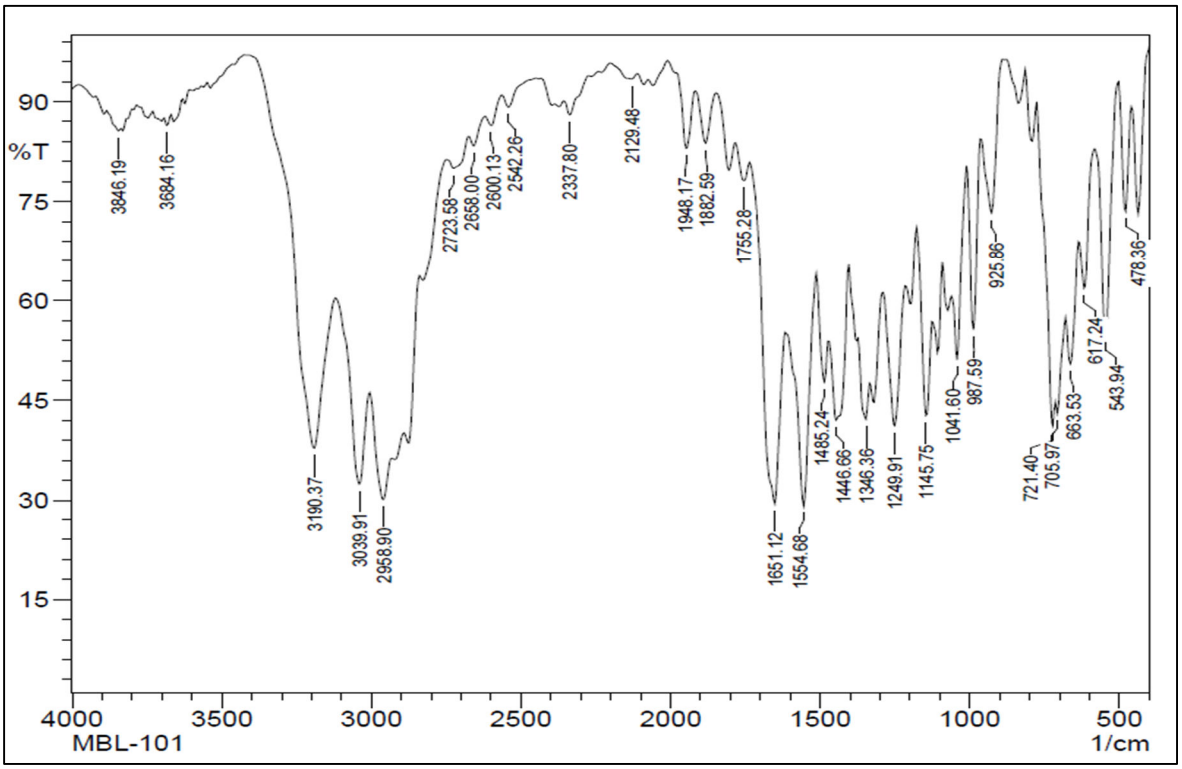


Figure 12. IR spectrum of MBL-101.

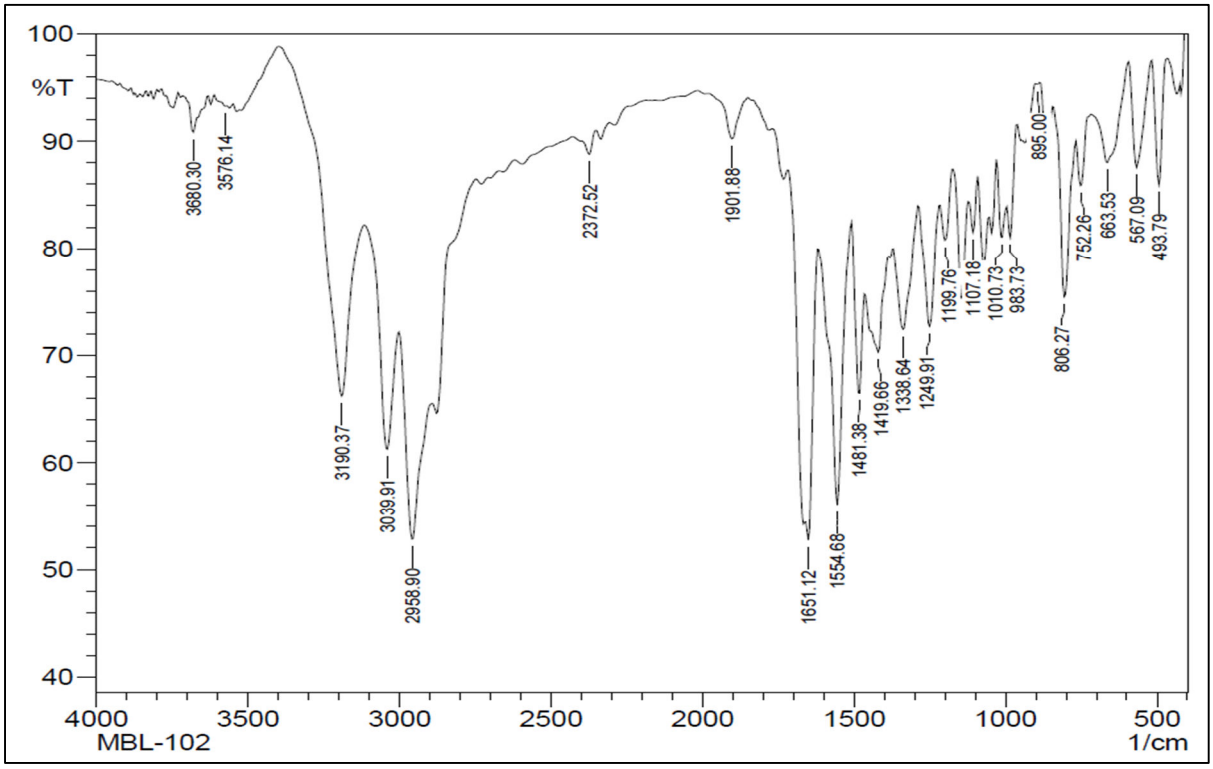


Figure 13. IR spectrum of MBL-102.

4. CONCLUSIONS

This synthetic route demonstrates a straightforward and efficient methodology for generating camphor-based Schiff bases, which are well-known for their wide range of biological properties. The anticancer activity results against MCF-7 breast cancel cell line shows the potentials for further investigation. The structurally characterized product provides a platform for further derivatization and bioactivity screening, potentially extending into antimicrobial, anti-inflammatory, or insecticidal applications, as suggested by prior literature on camphor derivatives.

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