International Journal Multidisciplinary
Homepage : https://journal.antispublisher.com/index.php/IJMI
Email : admin@antispublisher.com

e-ISSN : 3031-9870
IJMI, Vol. 3, No. 2, May 2026
Page 363-375

Microwave-Assisted Synthesis and Biological Evaluation of Novel Pyrazole–Thiosemicarbazone Schiff Bases as Potential Anticancer Agents

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DOI : https://doi.org/10.61796/ijmi.v3i2.478

Sections Info

Article history:
Submitted: March 15, 2026
Final Revised: April 05, 2026
Accepted: April 20, 2026
Published: May 02, 2026

Keywords:
Lung cancer
Microwave assisted
Pyrazole–thiosemicarbazone
Schiff bases

ABSTRACT

Objective: *Primary processes were involved in the synthesis and identification of Schiff bases derivatives obtained from thiosemicarbazide.* ***Method:*** *Three compounds were synthesized using a microwave assisted method, ((E)-2-((3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)hydrazine-1 carbothioamide (BT); (E)-2-((1-(4-chlorophenyl)-3-(m-tolyl)-1H-pyrazol-4-yl)methylene)hydrazine-1-carbothioamide (CMT); and (E)-2-((1-(4-chlorophenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl)methylene)hydrazine-1-carbothioamide (CNT). FT-IR, ^{1}H-NMR, ^{13}C-NMR, mass spectrometry, recrystallization of the generated compounds, and TLC monitoring of the chemical process were used to identify the chemical structure of the compounds. Anticancer activity using human lung cancer cells A549 through MTT assay to estimate IC50 value for each compound.* ***Results:*** *The results showed that compound BT (1) had a lower IC50 value 53.03 µM.* ***Novelty:*** *Perspective research required to develop compound BT with a mechanistic investigation.*

INTRODUCTION

Lung cancer is one of the most common and deadly malignancies worldwide, posing a significant health burden due to its high incidence and mortality rates [1]. This disease is of significant epidemiological importance, ranking first in terms of incidence and mortality, with a growing trend of its occurrence at younger ages, exacerbating the social and economic burdens [2]. Treatment strategies for lung cancer have witnessed remarkable advancements, now including surgical interventions and radiotherapy [3, 4]. Lung cancer arises from the uncontrolled growth of abnormal cells within lung tissue and is a leading cause of cancer-related deaths globally, with approximately 1.8 million deaths recorded in 2020, representing 18% of all cancer deaths [5]. Recent studies have established smoking as the leading cause of lung cancer, alongside other risk factors including secondhand smoke exposure, chronic lung diseases, air pollution, poor diet, excessive radiation exposure, and genetic predispositions to cancer [6, 7] Lung cancer prevention strategies encompass two main levels: primary and secondary prevention. Primary prevention aims to reduce incidence rates by minimizing risk factors and promoting healthy lifestyles through smoking cessation, smoke-free environments, strict tobacco control policies, and environmental pollution reduction [8]. Secondary prevention focuses on early detection before symptoms appear, significantly improving treatment and survival rates. [9] Regarding treatment, surgery is the preferred option in the early stages of the disease, provided the tumor has not spread to other organs.

However, many patients may not be candidates for surgery due to their poor overall health. Therefore, there is a need to develop safer and more effective alternative treatment strategies. Chemotherapy is used to shrink or eliminate tumors, but it can negatively affect healthy cells and cause significant side effects [10]. In contrast, targeted therapies have emerged as a promising option, as they selectively target cancer cells, reducing damage to healthy tissues and limiting disease spread [11]. Heterocyclic compounds are fundamental to organic chemistry, representing a large proportion of known molecular structures [12]. They are characterized by a remarkable diversity in their physical, chemical, and biological properties [13, 14], reflected in their wide range of reactions and structural stability [15]. Structurally, they are important as efficient reaction mediators, protective groups, and chiral catalysts, as well as for their role as organic catalysts and metal-metal bonds in asymmetric catalytic systems, particularly in pharmaceutical applications. [16, 17] Among these compounds, five-membered nitrogen-containing rings are of particular interest due to their structural diversity and ability to exhibit a wide range of biological activities [18]. This group includes several important rings such as pyrazole, imidazole, oxazole, triazole, tetrazole, oxadiazole, thiazole, and isoxazole, which have demonstrated notable activity as antibacterial and antifungal agents. The pyrazole ring is defined as a five-membered heterocyclic ring system containing two adjacent nitrogen atoms and is a common structural unit in many molecules of applied importance. Natural pyrazole-containing compounds, along with their synthetic derivatives, have demonstrated a wide range of biological activities, enhancing their importance in the field of pharmaceutical chemistry. [17-19] In recent years, pyrazole derivatives have attracted increasing attention in drug development, such as Fezolamine, Celecoxib [20], and Sulfaphenazole [21]. The Vilsmeier-Hack reaction is one of the most common and efficient methods for preparing 4-formylpyrazole derivatives [22]. Microwaved Schiff bases derived from aldehydes or ketones, when treated with a mixture of dimethylformamide and phosphorus chloride oxide, undergo cyclic reactions followed by formalinization of the pyrazole ring. Both aliphatic and aromatic methyl ketone hydrazones can also be converted to pyrazole-4-carboxyaldehydes upon reaction with the Vilsmeier reagent, further demonstrating the efficiency of this synthetic pathway. These bases are then reacted with thiosemicarbazides via microwave to obtain new Schiff bases [23]. Therefore, the aim of this study was to synthesize new heterocyclic compounds of Schiff bases from the Vilsmeier aldehyde reaction with thiosemicarbazide using microwave assisted instrument.

RESEARCH METHOD

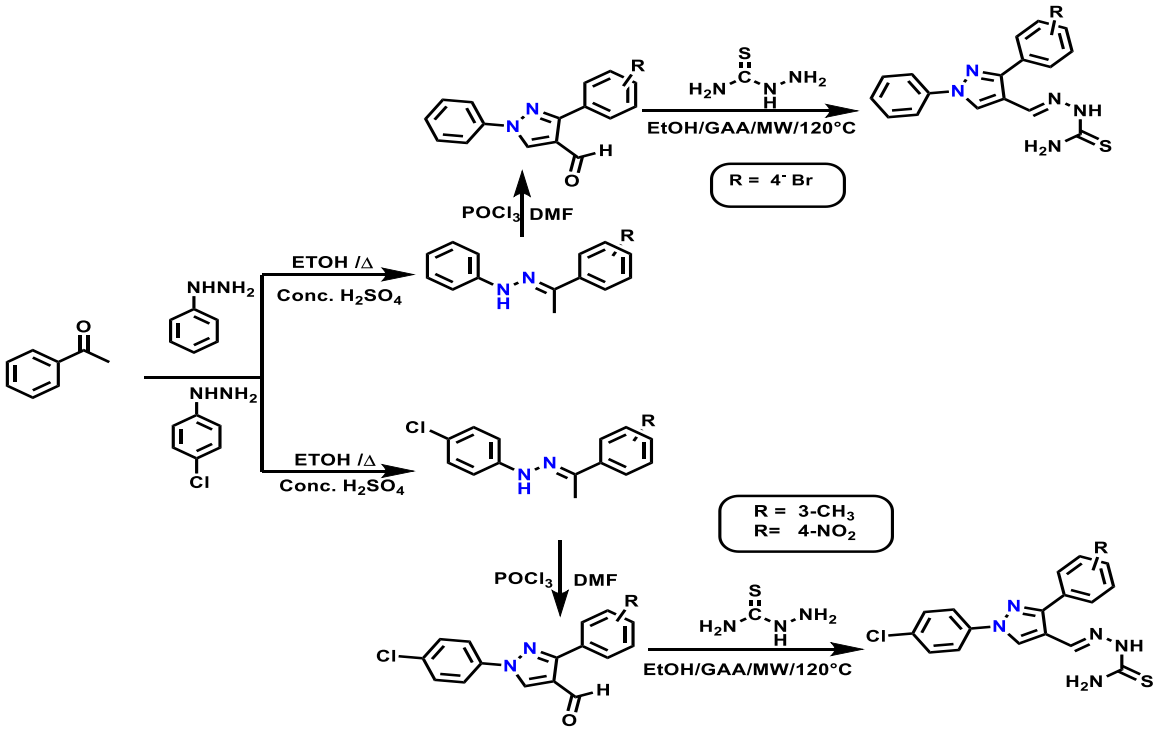
The method involved the synthesis of pyrazole–thiosemicarbazone Schiff base derivatives using a microwave-assisted approach. Initially, substituted acetophenones were reacted with phenylhydrazine or 4-chlorophenylhydrazine in ethanol with a few drops of concentrated sulfuric acid under microwave irradiation to form intermediate compounds, followed by Vilsmeier aldehyde formation. These intermediates were then condensed with thiosemicarbazide in ethanol containing glacial acetic acid under

microwave conditions to yield three target compounds (BT, CMT, and CNT). The products were purified by filtration and recrystallization, and the reactions were monitored using thin-layer chromatography (TLC). Structural characterization was performed using FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry. The anticancer activity was evaluated in vitro against A549 human lung cancer cells cultured in RPMI-1640 medium using the MTT assay, and IC50 values were determined based on absorbance measurements at 570 nm.

RESULTS AND DISCUSSION

Chemistry

A new Schiff bases derived from thiosemicarbazide were synthesized in a microwave to obtain the final three Schiff bases derivatives: ((E)-2-((3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)hydrazine-1- carbothioamide (BT); (E)-2-((1-(4-chlorophenyl)-3-(m-tolyl)-1H-pyrazol-4-yl)methy -lene)hydrazine-1-carbothioamide (CMT); and (E)-2-((1-(4-chlorophenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl) methylene)hydrazine-1-carbothioamide (CNT), as shown in Scheme 1. The compounds were characterized using FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrum, as shown in Supplementary Information. BT compound has a bromine functional group, CMT compound has a chloro and methyl functional groups, and CNT has a chloro and nitroso functional groups in various positions. These various functional groups paved to examine their anticancer activity using A549 lung cancer cells, and MTT assay to estimate IC_{50} values for each compound.



Scheme 1. The steps of synthesis Schiff bases compounds.

Cytotoxicity of Schiff bases compounds

The IC_{50} values were estimated via MTT assay for compounds BT, CMT, and CNT, 53.03, 97.00, and 945.36, respectively, Table 1. Compound BT was found to have the lower IC_{50} value. Bromine group may play a role in increasing the anticancer activity through increased the binding affinity, affect the reactivity of compound toward nucleophilic or electrophilic sites in the cells. Hence, the bromine group is large and hydrophobic, therefore, adding it within the structure will increase the lipophilicity of compound, and increase the metabolic stability [24].

Table 1. IC_{50} values of compounds against lung cancer cells A549.

Symbol	IC_{50} (μM)
BT	53.03
CMT	97.00
CNT	945.36

Experemental Section

Chemistry

Melting points are measured and uncorrected. The IR spectrum is recorded using FT-IR Shimadzu (affinity-1) in the range 4000–400 cm^{-1}. 1H and 13C NMR spectra are also recorded on a Brucker (400 MHz) spectrometer. Chemical shifts are reported by reference to the solvent DMSO-d_6 or decomposing peaks ($^{\delta}H$ 2.5 and 3.3 and $^{\delta}C$ 49 ppm); coupling constants were reported in Hertz. The abbreviations for a sign you are using are as follows: s: single, d: doublet, t: triple, m: multiple. Mass spectra are recorded on an Agilent mass spectrometer 5975 quadrupole analyzer with electronic impact (EI) at energy (70 eV)

Synthesis of Schiff's bases derived from acetophenone substitutes

The solution was prepared by dissolving 0.01 mol of acetophenone substitutes with 0.01 mol of phenylhydrazine or 4-chlorophenylhydrazine in 10 mL of ethanol. Two drops of concentrated sulfuric acid were then added, and the mixture was microwaved for 2 to 8 minutes at 60% strength. The solution was then cooled to room temperature and allowed to precipitate. The resulting solution was filtered and dried, and its melting point was measured. The reaction was monitored by thin-layer chromatography (TLC) using a mixture of hexane: ethanol: ethyl acetate [25].

Synthesis of Schiff bases derived from thiosemicarbazide and Vilsmeier aldehydes

0.001 mol of thiosemicarbazide is placed with 0.001 mol of Velsmeier aldehydes prepared in the previous step in a round-bottomed flask. 15 ml of ethanol and 2-3 drops of glacial acetic acid are added. Microwave the mixture for 12-24 minutes at 432-864 kW. The solution is then removed and allowed to cool to room temperature until it precipitates. The resulting solution is filtered and purified with appropriate solvents. It is then dried and its melting point measured. The reaction is monitored using thin-layer chromatography (TLC) using a mixture of hexane, ethanol, and ethyl acetate (1:1:4) [26].

(E)-2-((3-(4-bromophenyl)-1-phenyl-1H-pyrazol-4-yl)methylene)hydrazine-1-carbothioamide (BT)

White Crystals, yield: 47% , m.p 230-232 C . IR (KBr) cm^{-1}, v:3449,3337 (NH_2 Asym , sym) , 3189 (N=C-H $_{shiff\ base}$), 3013 (N-C-H py), 1597 (C=N $_{shiff\ base}$), 1543 (C=N py), 1501 (C=C Ar), 1277 (C-S). ^{1}HNMR (300 MHz, DMSO-d6) , 11.34(s , 1H ,H-N-C=S) , 9.19(s, 1H , H-C=C) , 8.29(s, 1H , N-Ha) , 8.19 (s, 1H ,H-C=N) , 7.8(s, 1H , N-Hb) , 7.93-7.35 (m , 9H , Ar-H) . ^{13}CNMR spectrum (300 MHz, DMSO-d6) , C=S = 178.01 , C=N shiff base= 151.61 .C=N py= 139.42 , N-C=C py = 135.09 , C-Ar = 132.22-119.02 .C-Br = 123.66 . C=C py = 117.83 . MS (ESI) m/z= 400.30$[M]^+$.

(E)-2-((1-(4-chlorophenyl)-3-(m-tolyl)-1H-pyrazol-4-yl)methylene)hydrazine-1-carbothioamide (**CMT**)

White Crystals, yield: 40% , m.p 226-227 C . . IR (KBr) cm^{-1}, v:3366,3261 (NH_2 Asym , sym) , 3172 (N=C-H $_{shiff\ base}$), 2973 (N-C-H py), 1642 (C=N $_{shiff\ base}$), 1615 (C=N py), 1496 (C=C Ar), 1283 (C-S).^{1}H NMR (300 MHz, DMSO) , 11.37(s , 1H ,H-N-C=S) , 9.20(s, 1H , H-C=C) , 8.29(s, 1H , N-Ha) , 8.21 (s, 1H ,H-C=N) , 7.77(s, 1H , N-Hb) , 7.94-7.27 (m , 8H , Ar-H) , 2.41(s , 1H , CH_3) . ^{13}CNMR spectrum (300 MHz, DMSO-d6) . C=S = 178.05 , C=N py = 151.13 , C=N shiff base = 138.56 , C-Cl= 129.18 , N-C=C py = 128.30 , C-Ar = 135.31-118.13 . C=C py = 118.07 , C-CH_3 = 21.52 . MS (ESI) m/z= 369.87 $[M]^+$.

(E)-2-((1-(4-chlorophenyl)-3-(4-nitrophenyl)-1H-pyrazol-4-yl)methylene)hydrazine-1-carbothioamide (**CNT**)

Yellowish powder, yield: 47% , . IR (KBr) cm^{-1}, v:3423,3251 (NH_2 Asym , sym) , 3140 (N=C-H $_{shiff\ base}$), 3131 (N-C-H py), 1661 (C=N $_{shiff\ base}$), 1598 (C=N py), 1547 (C=C Ar), 1290 (C-S).^{1}H NMR (300 MHz, DMSO) , 11.39(s , 1H ,H-N-C=S) , 9.26(s, 1H , H-C=C) , 8.34(s, 1H , N-Ha) , 8.25 (s, 1H ,H-C=N) , 7.76(s, 1H , N-Hb) , 8.0-7.65 (m , 8H , Ar-H) . ^{13}CNMR spectrum (300 MHz, DMSO-d6) . C=S = 178.14 , C=N shiff base= 149.67 , C-NO_2 = 147.75 , C=N py= 138.12 , C-Cl= 129.64 , N-C=C py = 129.08 , C-Ar = 134.53-120.80 , C=C py = 118.80 . MS (ESI) m/z= 400.05 $[M]^+$.

Cell lines and culture

A549 (human lung cancer cell line) was purchased from the National Cell Bank of Iran (Pasteur Institute, Iran). Cells were grown in RPMI-1640 medium (Gibco) with 10% FBS (Gibco) supplemented with antibiotics (100 U/ml penicillin and 100 µg/ml streptomycin), respectively. Cells were maintained at 37°C under humidified air containing 5% CO_2 and were passaged using trypsin/EDTA (Gibco) and phosphate-buffered saline (PBS) solution.

MTT cell viability assay

The cytotoxicity of compounds was determined by the MTT test, in which cells were grown in 96-well plates (5×10^{-3} cells/well) and allowed to adhere overnight at standard culture conditions (37 °C, 5% CO_2). The next day, the test chemicals were subjected to the cells in varying amounts for 24 hours. Post-incubation, 20 µL of MTT solution (5 mg/mL in PBS) was incorporated into each well. Subsequently, the plates were incubated at 37 °C for 4 hours so that the mitochondrial dehydrogenases had sufficient time to develop MTT into soluble purple Formosan crystals with sufficient time. then carefully remove

the medium and dissolve the Formosan in 150 µL of DMSO. Absorbance at 570 nm was measured using an ELISA reader (Model wave xs2, BioTek, USA). Cell viability was calculated as a percentage with respect to untreated control cells. IC_{50} values were calculated [27-29].

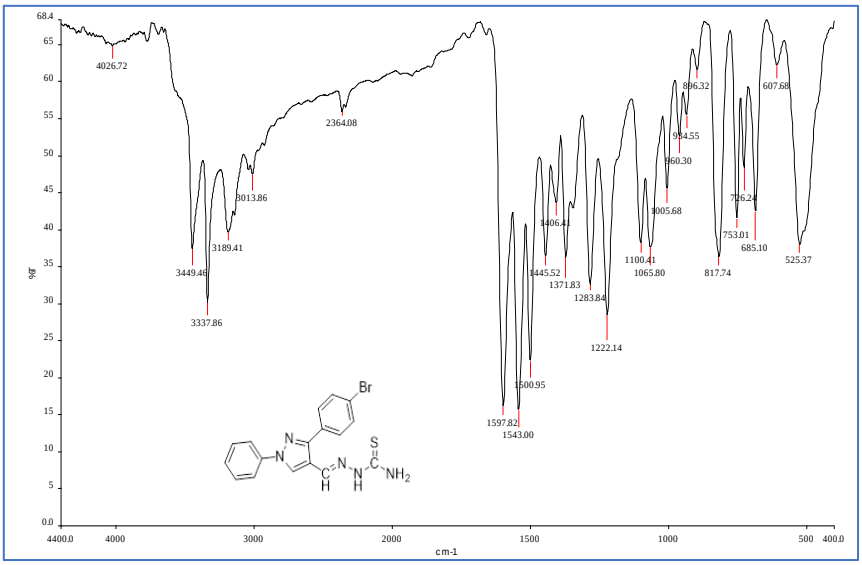


Figure 1. FT-IR Spectra for compound BT (1)

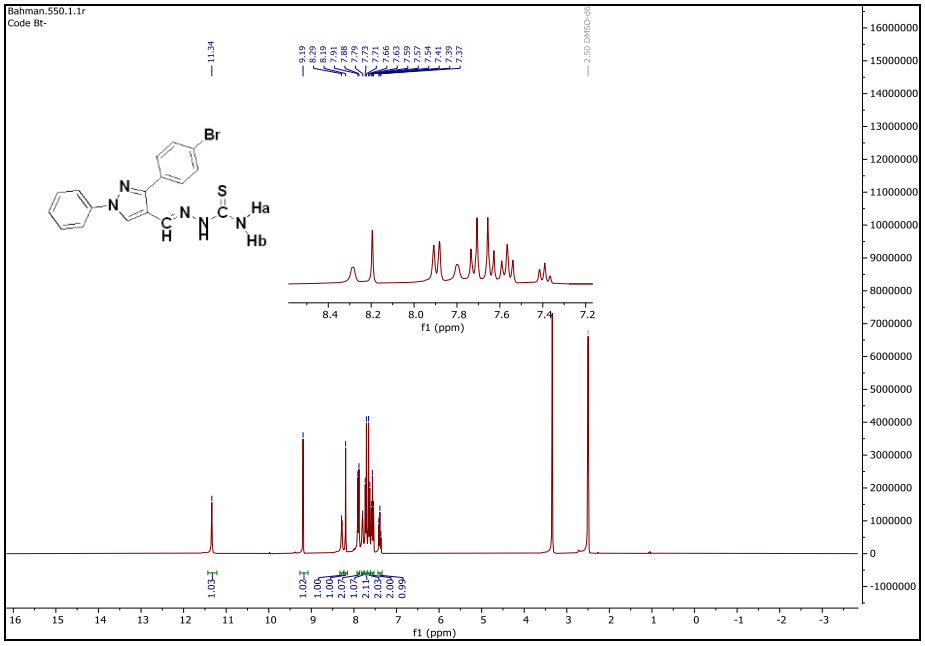


Figure 2. ^{1}H-NMR Spectrum for compound BT (1)

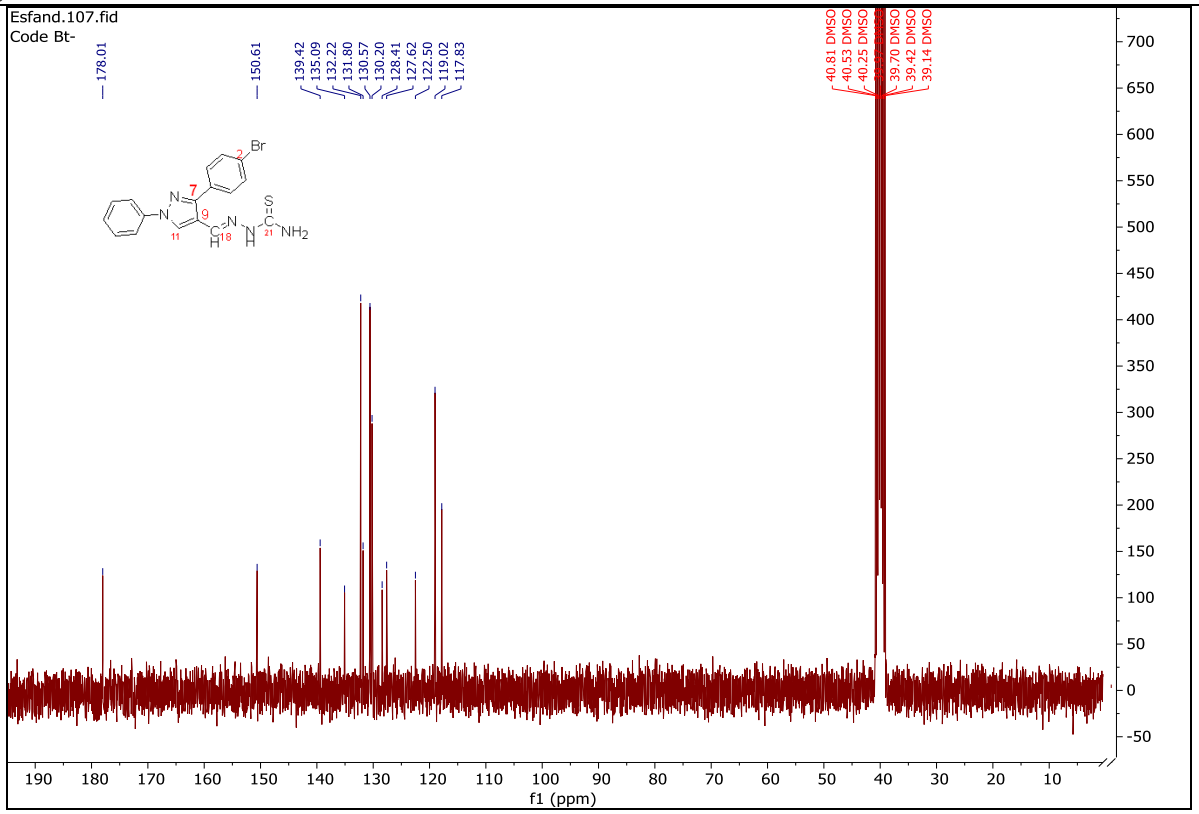


Figure 3. ^{13}C-NMR Spectrum for compound BT (1)

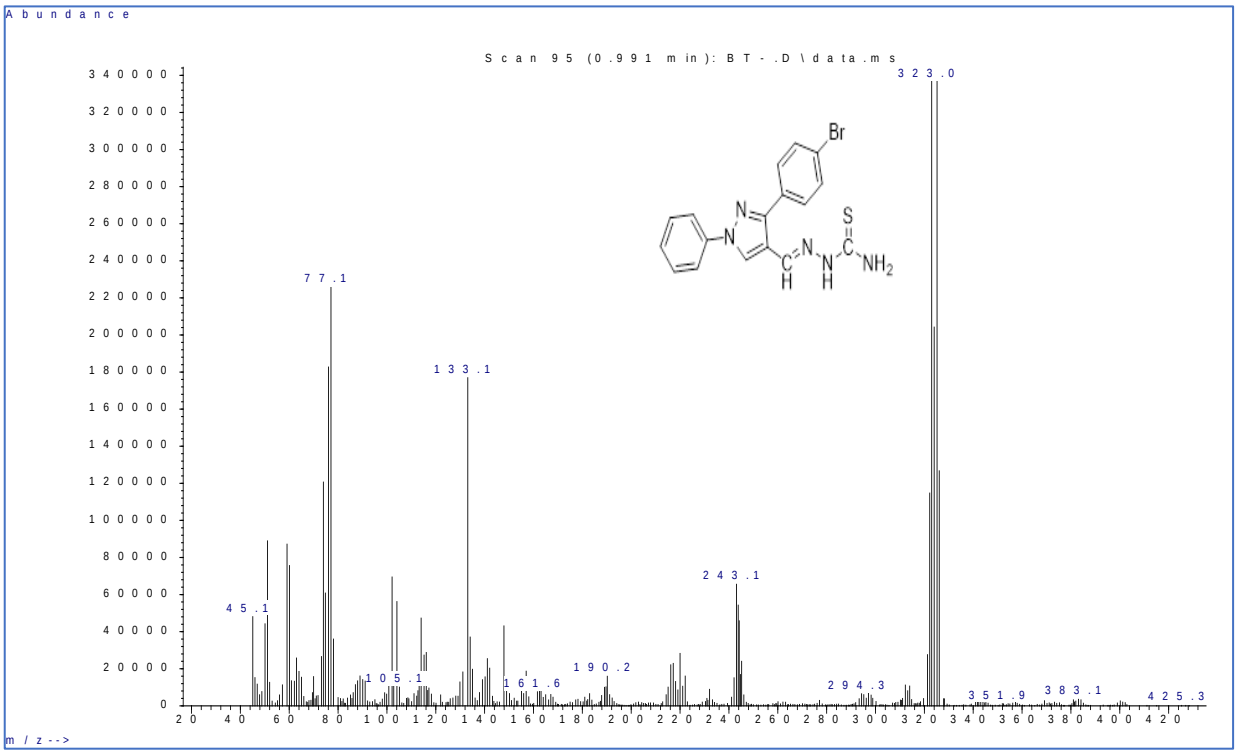


Figure 4. Mass Spectra for compound BT (1)

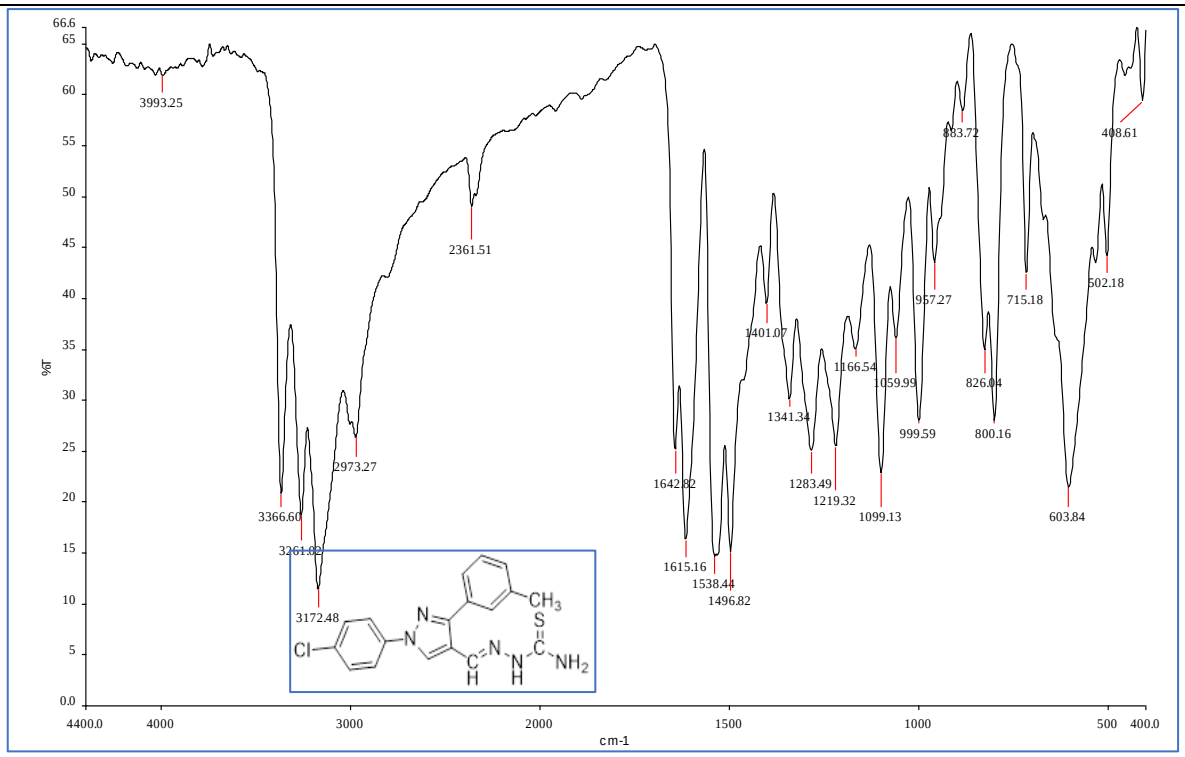


Figure 5. FT-IR Spectra for compound CMT (2)

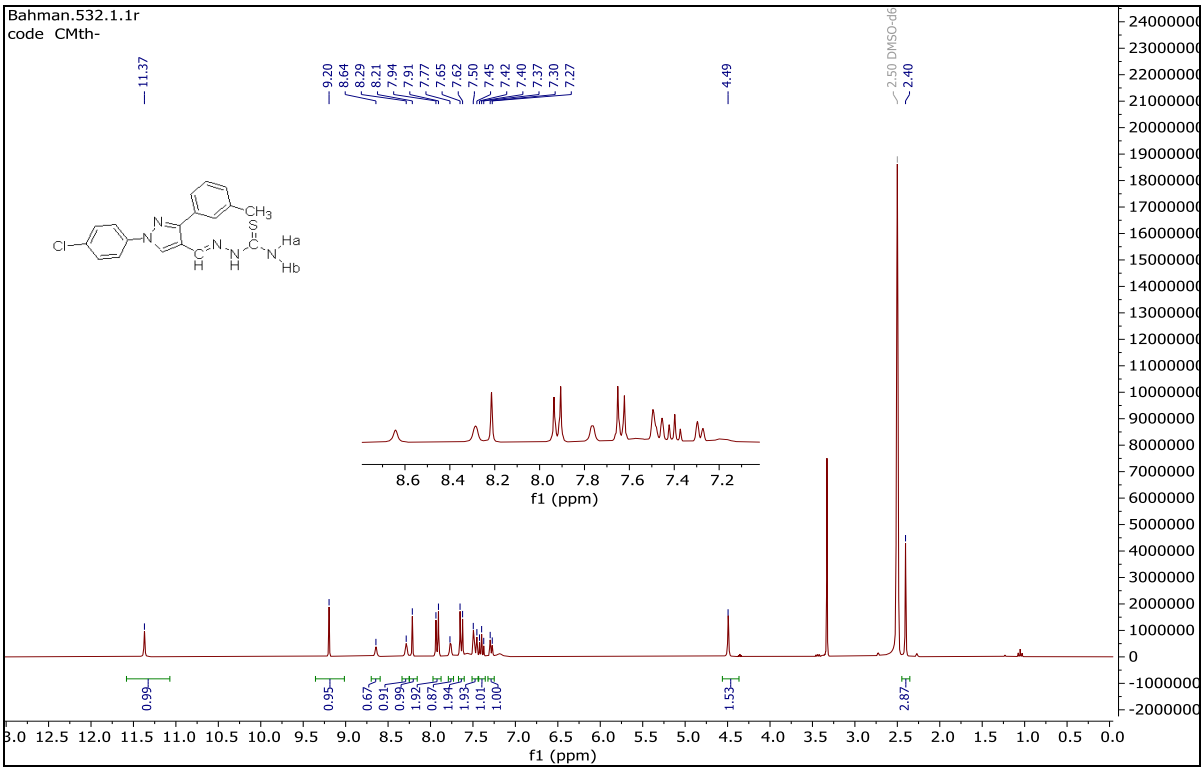


Figure 6. ^{1}H-NMR Spectrum for compound CMT (2)

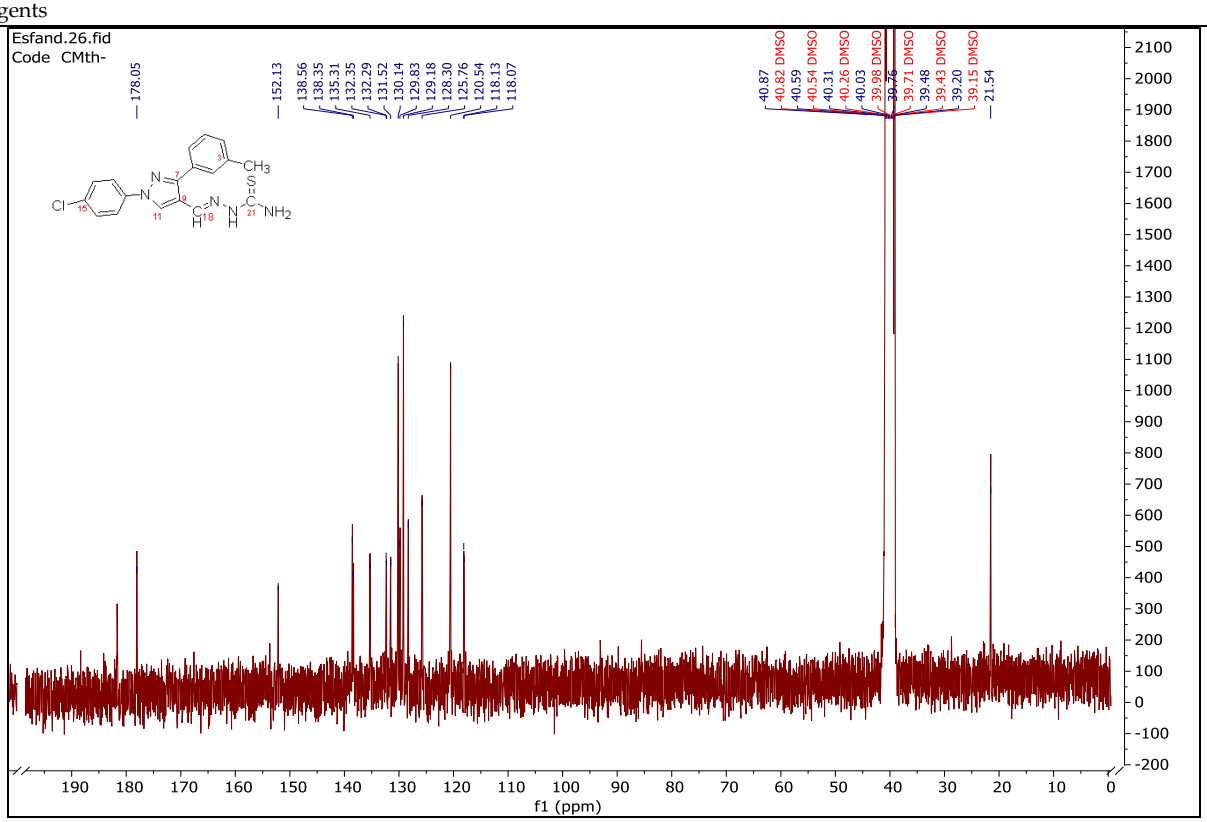


Figure 7. ^{13}C-NMR Spectrum for compound CMT (2)

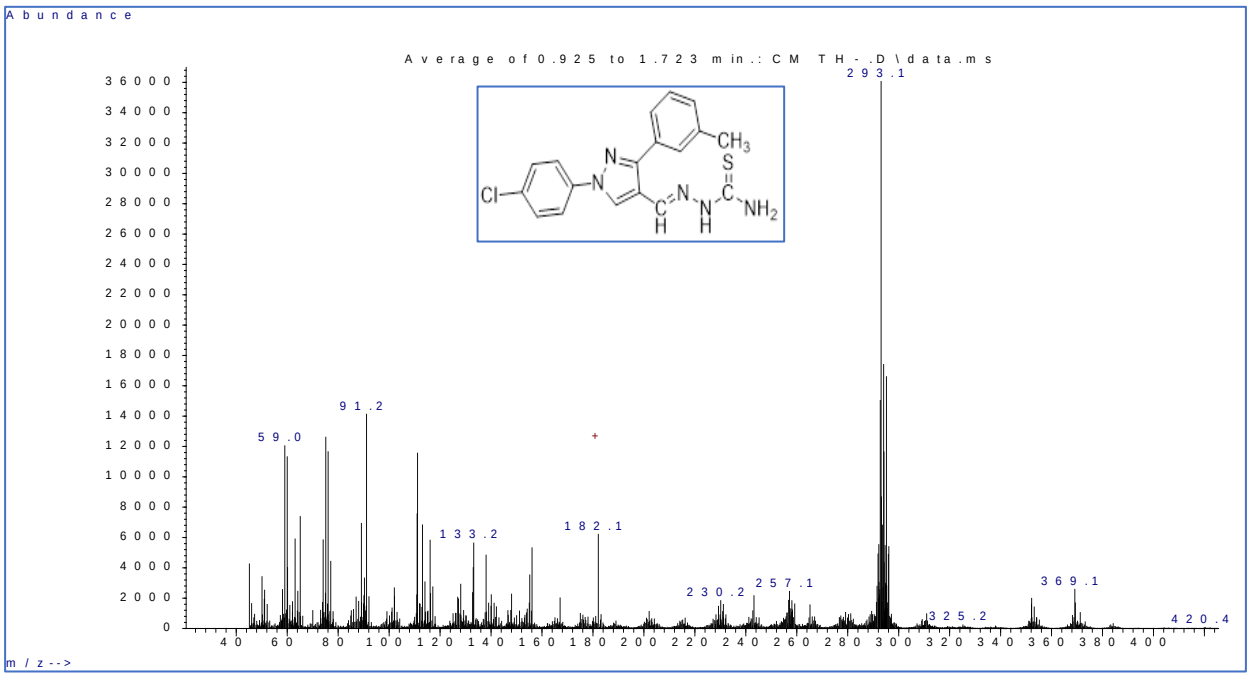


Figure 8. Mass Spectra for compound CMT (2).

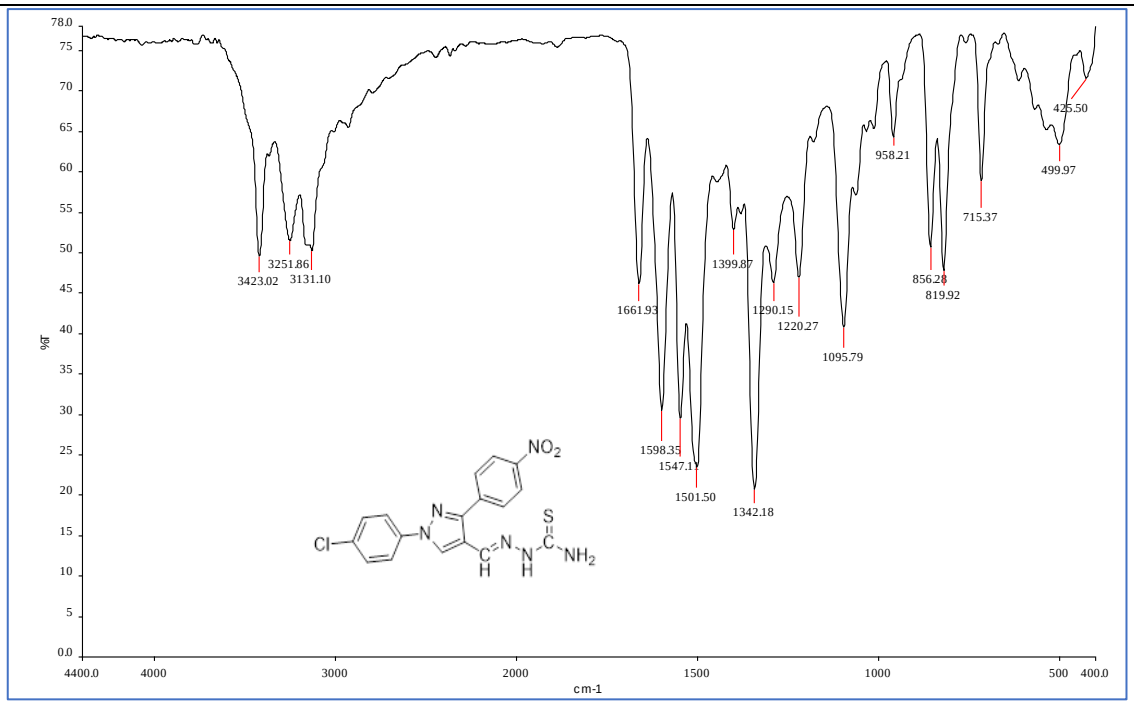


Figure 9. FT-IR Spectra for compound CNT (3).

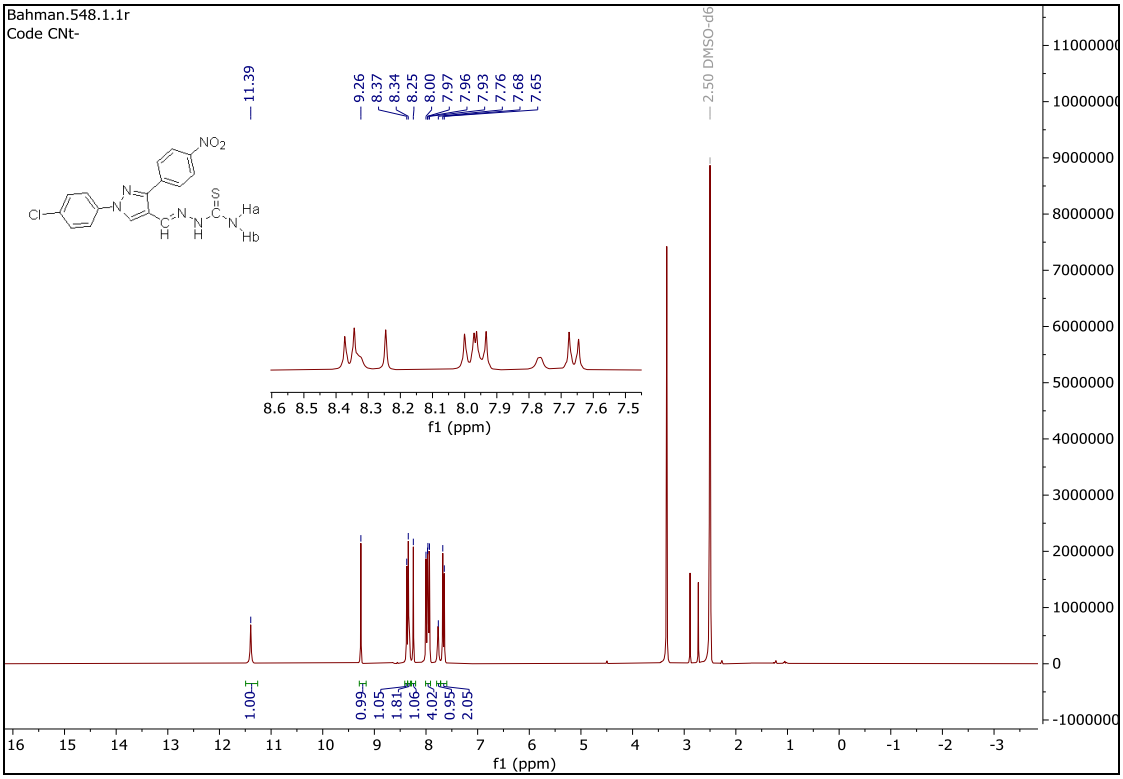


Figure 10. ^{1}H-NMR Spectrum for compound CNT (3)

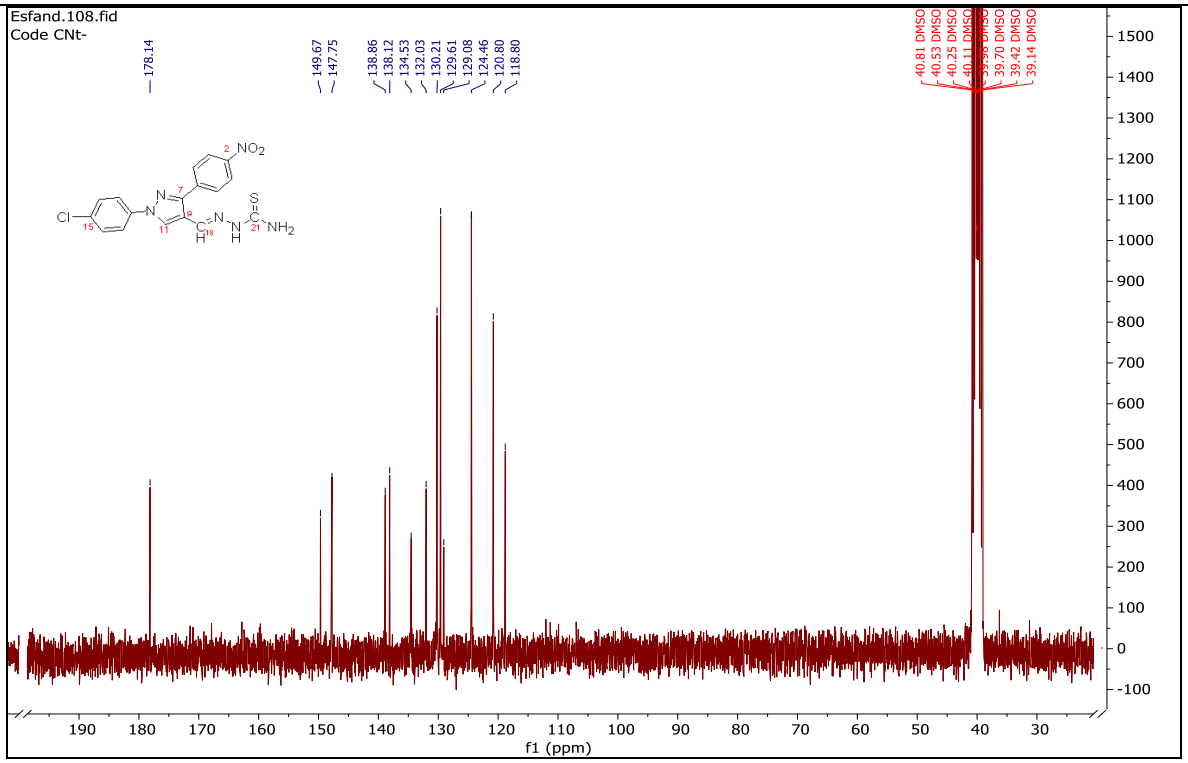


Figure 11. ^{13}C-NMR Spectrum for compound CNT (3)

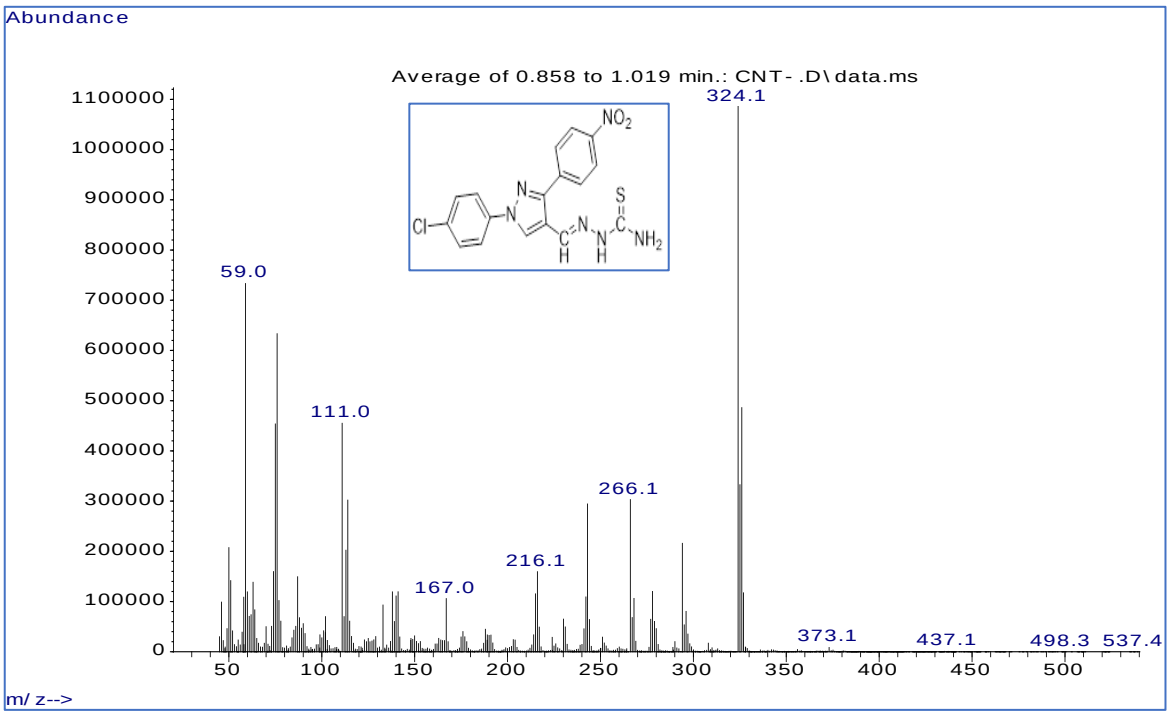


Figure 12. Mass Spectra for compound CNT (3)

CONCLUSION

Fundamental Finding : Three Schiff base derivatives (BT, CMT, and CNT) were successfully synthesized via microwave-assisted condensation and structurally confirmed using FT-IR, ^{1}H-NMR, ^{13}C-NMR, and mass spectrometry; among them, BT exhibited the lowest IC_{50} value against lung cancer cells. **Implication :** The superior cytotoxic activity of compound BT highlights its potential as a promising lead candidate

for anticancer drug development, particularly targeting lung cancer. **Limitation :** The study is limited to preliminary cytotoxic evaluation, without detailed mechanistic investigations or comprehensive biological pathway analysis. **Future Research :** Further studies should explore the anticancer mechanism of BT through advanced assays, including apoptosis analysis, cell cycle evaluation, Western blotting, and molecular docking studies.

ACKNOWLEDGEMENTS

The authors express their profound appreciation to the University of Basrah for offering an exemplary work environment and cutting-edge facilities that were crucial for the practical components of this research. This research is part of the Master graduation requirements.

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