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Original Article

Design and Synthesis of Novel Series of Thiophene-2, 5-dicarbohydrazide Derivatives as Potential Anticancer Agents

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ABSTRACT

Background: The biological activity of thiophene and carbohydrazide derivatives is well known; some of them are included in various anticancer drugs. **Objectives:** The main goal of this work was to develop effective, effective and non-toxic compounds with anti-cancer properties. To achieve this goal, we synthesized a new series of N2, N5-bis(1E)-ethylidene thiophene-2,5-dicarbohydrazide derivatives. **Materials and Methods:** Thiophene-2,5-dicarbohydrazide reacts with aromatic aldehydes and ethanol to form a new series of derivatives. This work examined the compounds obtained for them *in vitro* cytotoxic activity against the breast cancer lines MCF-7. **Results:** The results of antiproliferative cell inhibition studies showed that some compounds showed high activities compared to standard imatinib drugs. **Conclusion:** The most promising compounds D1-D10 were selected to study their inhibitory effects on the MCF-7 cell line, where compound D5 showed the highest activity. All synthesized compounds have no adverse effect on normal cell lines.

Keywords: Thiophene-2,5-dicarbohydrazide, Anticancer, MCF-7 cell lines, Derivatives.

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INTRODUCTION

According to the World Health Organization, cancer is the second biggest cause of mortality worldwide. Despite being the most frequent disease in women, research on breast cancer has had a favorable effect on public health. Surgical removal of cancerous tissue, radiation therapy, and chemotherapy are now the gold standards, with targeted therapies and immunotherapies increasingly gaining ground. Thus, modern cancer treatment focuses on chemotherapeutic agents, biologics, and immune-mediated treatments. Therefore, it is crucial to discover and synthesize more potent active substances with fewer adverse effects.[1] In both their natural and synthetic forms, thiophenes are sulfur-containing heterocycles. The pharmacological effects they have vary widely. These days, scientists are trying to find heterocycles containing Schiff bases to boost activity and effectiveness. Similar to aldehydes and ketones, but with an imine or azomethine group instead of a carbonyl, these compounds are called Schiff bases. They're employed in many different industries because of their diverse biological activities. New drug discovery relies heavily on azomethine proton molecules (-NHN=CH-). Hydrazone is formed when an aldehyde or ketone reacts with hydrazine or hydrazide. The C=N bond in the hydrazone and the lone pair of electrons on the terminal nitrogen atom are responsible for the chemical and physical properties.[2] Crystalline solids, common Schiff bases may be insoluble salts when combined with strong acids. The synthesis of amino acids requires the use of Schiff bases as intermediates and a wide variety of metal complexes may be produced using ligands derived from Schiff bases.[3] The C=N-imine bond offers great binding potential with different nucleophiles and electrophiles, which may block certain illnesses, enzymes, or DNA replication. C=N hydrazones are useful in drug design as ligands for metal complexes, in organo-catalysis, and the synthesis of organic molecules.[4] Thiophene and its substituted derivatives, such as hydrazine, are an important family of heterocyclic molecules with exciting applications in medicinal chemistry.[5,6] Anti-inflammatory, anti-psychotic, anti-arrhythmic, anti-anxiety, anti-fungal, antioxidant, estrogen receptor regulating, anti-mitotic, anti-microbial, kinase inhibitory, and anti-cancer are only a few of their pharmacological and physiological qualities.[7-9] These compounds are quite potent. Many people with cancer may not respond well to standard chemotherapy because resistance has developed in their bodies. There is an obvious and pressing need for new, effective, and Nontoxic (NT) medications to treat cancer since many existing chemotherapeutic treatments are ineffective and have major side effects.[10] These researches have shown the feasibility of

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synthesizing thiophene-2,5-dicarbohydrazide derivatives.[11-13] To this end, we tested ten different thiophene-2,5-dicarbohydrazide derivatives (D1-D10) in the MCF-7 cell line for their cytotoxic and inhibitory activities. In this investigation, ten different thiophene-2,5-dicarbohydrazide derivatives (D1-D10) were tested in the MCF-7 cell line for their cytotoxic and inhibitory activities. In the following article, we describe how to synthesize a bunch of different series of thiophene-2,5- dicarbohyrazide, then they were chemically characterized by utilizing Nuclear Magnetic Resonance (NMR), and high-resolution mass spectroscopy, Infrared spectroscopy (IR) and determining their anti-proliferative property and *in vitro* cytotoxic activity against on MCF-7 cancer cell lines by using MTT assay.

MATERIALS AND METHODS

Synthesis of the compounds

The synthesis pathway is shown in Scheme 1.

General Procedure for the Synthesis

The thiophene-2,5-dicarboxylic acid substrate was dissolved in methanol (15 mL), and 0.1 M H_2SO_4 was added dropwise while the mixture was continuously shaken during a typical experimental technique. When finished, the mixture was refluxed in an oil bath at a temperature of less than 80ºC. The substance was cooled to room temperature after the reaction was complete. After several rinses with water and a final rinse with a 10% $NaHCO_3$ solution, the product was dried over anhydrous Na_2SO_4 (1.38 g, yield: 88 percent, powder substance). Overnight, at a temperature just below 80°C, a combination of thiophene-2,5-dicrbohydrazide and hydrazine hydrate (85%, 15 mL) in methanol (20 mL) was refluxed. After 12 hr, the solution had become a light-yellow color. During this time, a colorless product called thiophene-2,5-dicarbonate crystallized out of the reaction mixture and was then employed in the following process after being dried and recrystallized in ethanol. After steeping aromatic aldehydes and thiophene-2,5-dicrbohydrazide in 50 mL of 100% ethanol for 6-12 hr, the mixture was allowed to reflux. The compounds were obtained by filtering off the solid precipitate after cooling, washing it with ethanol, drying it, and then crystallizing it from the ethanol (Figure 1). 10 Derivatives prepared by synthetic route (Table 1).

RESULTS

Characterization of the synthesized compounds

(10E,12E)-N5'-((3-methyl-1H-pyrrol-2-yl)methylene)-N2'-((4-methyl-1H-pyrrol-2-yl) methylene)thiophene-2,5-dicarbohydrazide(1): "Yellow powder, IR (KBr,cm^{-1}):(3464) C=N-H, ((2201)-C=C,(698)C-S,(1570)C=N,1659(C=O) (Figure 2); MS: *m/z* 383.12;^{1}H NMR (500 MHz, DMSO): δ 2.16-2.28 (6H, 2.21 (s), 2.23 (s)), 6.00 (1H, d,*J* = 1.2 Hz), 6.14 (1H, d,*J*=2.7 Hz), 6.97 (1H, d,*J*=1.2 Hz), 7.40 (1H, d,*J*=2.7 Hz), 7.80-7.94 (4H, 7.86 (d,*J*=8.0 Hz), 7.86 (d,*J*=8.0 Hz), 7.87 (s), 7.89 (s)) (Figure 3). Exact mass calcd. For $C_{18}H_{18}N_6O_2S$: 382; found: 382.0945 (Figure 4).

(10E,12E)-N2', N5'-dibenzylidenethiophene-2,5-dicarbohydrazide(2): Yellow powder, IR(KBr,cm^{-1}):(3190) C=N-H,(2404)-C=C,(698)C-S,(1636)C=N, 1922 (C=O) (Figure 5); MS: *m/z* 377.89;^{1}H NMR(500MHz, DMSO): δ 7.22-7.48 (10H, 7.28 (tt,*J*=7.4, 1.3 Hz), 7.37 (D,*J*=7.9, 7.4, 1.9, 0.4 Hz), 7.42 (DTD,*J*=7.9, 1.3, 0.4 Hz)), 7.87 (2H, d,*J*=8.0 Hz), 8.11 (2H, s) (Figure 6). Exact mass calcd. For $C_{20}H_{16}N_4O_2S$: 376.13; found: 376.13 (Figure 7).

(10E,12E)-N2',N5'-bis(2-fluorobenzylidene)thiophene-2,5-dicarbohydrazide(3): Yellow powder, IR(KBr,cm^{-1}): (3360) C=N-H,(2851)-C=C,(846)C-S,1688(C=N), 1688 (C=O) (Figure 8); MS: *m/z* 413.89;^{1}H NMR(500MHz,DMSO): δ 7.00 (2H, ddd,*J*=8.3, 1.2, 0.5 Hz), 7.22 (2H, ddd,*J*=7.9, 7.4, 1.2 Hz), 7.49 (2H, ddd,*J*=8.3, 7.4, 1.4 Hz), 7.72 (2H, ddd,*J*=7.9, 1.4, 0.5 Hz), 7.87 (2H, d,*J*=8.0 Hz), 8.14 (2H,s) (Figure 9). Exact mass calcd. For $C_{20}H_{14}F_2N_4O_2S$: 412.37; found: 412.37 (Figure 10).

(10E,12E)-N2',N5'-bis((5-methylfuran-2-yl)methylene) thiophene-2,5-dicarbohydrazide (4): Yellow powder IR (KBr,cm^{-1}): (3255)C=N-H,(904)-C-O-,(2851)-C=C,(846) C-S,1620(C=N),1688.37 (C=O);MS (Figure 11): *m/z* 385.95; ^{1}H NMR(500MHz,DMSO): δ 2.30 (6H, s), 6.13 (2H, d,*J*=3.5 Hz), 6.78 (2H, d,*J*=3.5 Hz), 7.78 (2H, s), 7.94 (2H, d,*J*=8.0 Hz) (Figure 12). Exact mass calcd. For $C_{18}H_{16}N_4O_4S$: 384.93; found: 384.93 (Figure 13).

(10E,12E)-N2'-((oxazol-2-yl)methylene)-N5'-((oxazol-5-yl) methylene)thiophene-2,5-dicarbohydrazide (5); Yellow powder, IR (KBr,cm^{-1}):(3269.72)C=N-H,(963.2)-C-O-,(2487.72)-C=C,(676.892)C-S,(1632)C=N,1659 (C=O) (Figure 14).; MS:

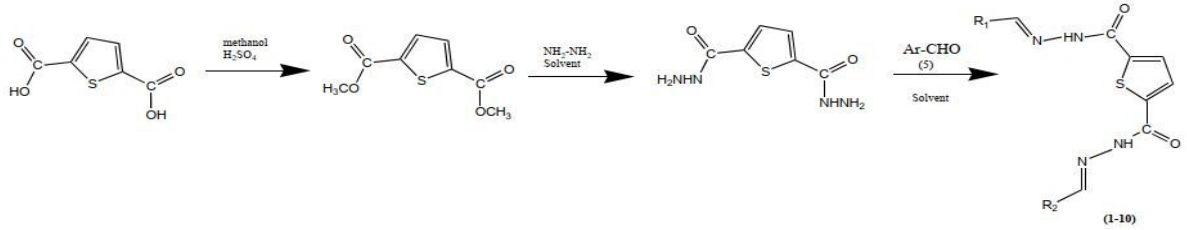
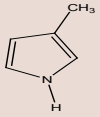
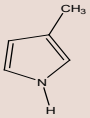
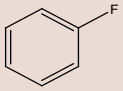
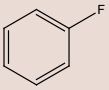
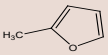
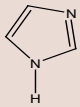
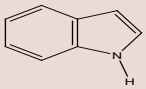
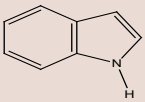
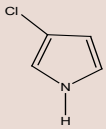
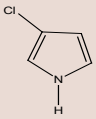


Figure 1: The synthesis route of the compounds.

Table 1: Derivatives Prepared by synthetic route.

Sl. No.	Derivatives	R1	R2
1	D1		
2	D2		
3	D3		
4	D4		
5	D5		
6	D6		
7	D7		
8	D8		
9	D9		
10	D10		

m/z 359.39;[1]H NMR(500MHz, DMSO) δ 6.89 (1H, d,*J*=2.6 Hz), 7.03 (1H, d,*J*=0.9 Hz), 7.14 (1H, d,*J*=2.6 Hz), 7.67-7.93 (4H, 7.71 (s), 7.81 (s), 7.87 (d,*J*=8.0 Hz), 7.87 (d,*J*=8.0 Hz)), 8.05 (1H, d,*J*= 0.9 Hz) (Figure 15). Exact mass calcd. For $C_{14}H_{10}N_6O_4S$: 358.93; found: 358.93 (Figure 16).

(10E,12E)-N2'-((thiazol-2-yl)methylene)-N5'-((thiazol-5-yl) methylene)thiophene-2,5-dicarbohydrazide (6); Yellow powder, IR (KBr,cm^{-1}): (3323)C=N-H,(2755)-C=C,(715)C-S, 1655(C=N), 1731 (C=O) (Figure 17).; MS: *m/z* 391.49; [1]H NMR(500MHz, DMSO) δ 7.13 (1H, d,*J*=6.6 Hz), 7.47 (1H, d,*J*=6.6 Hz), 7.80-7.93 (3H, 7.86 (d,*J*=7.8 Hz), 7.87 (d,*J*=7.8 Hz), 7.87 (s)), 8.00 (1H, s), 8.32-8.52 (2H, 8.38 (d,*J*=1.9 Hz), 8.47 (d,*J*=1.9 Hz)) (Figure 18). Exact mass calcd. For $C_{14}H_{10}N_6O_2S_3$: 390.15; found: 390.15 (Figure 19).

(10E,12E)-N2'-((1H-imidazol-2-yl)methylene)-N5'-((1H-imidazol-5-yl)methylene)thiophene-2,5-dicarbohydrazide (7); Yellow powder, IR (KBr,cm^{-1}):(3308)C=N-H,(1094)-C-O-,(2364)-C=C,(725)C-S, 1698 (C=N),1626 (C=O) 1698.02 (C=O) (Figure 20).; MS:*m/z*357.93; [1]H NMR (500MHz, DMSO) δ 7.02 (1H, d,*J*=4.0 Hz), 7.37-7.50 (2H, 7.42 (d,*J*=1.3 Hz), 7.44 (d,*J*=4.0 Hz)), 7.65 (1H, s), 7.81-8.00 (3H, 7.87 (d,*J*=8.0 Hz), 7.94 (d,*J*=8.0 Hz), 7.93 (s)), 8.11 (1H, d,*J*=1.3 Hz) (Figure 21): Exact mass calcd. For $C_{14}H_{12}N_8O_2S$: 356.39; found: 356.39 (Figure 22).

(10E,12E)-N2', N5'-bis((indolin-2-yl)methylene) thiophene-2,5-dicarbohydrazide **(8)**; Yellow powder, IR (KBr,cm^{-1}): (3343-C=N-H), (2223)-C=C,(1011)C-S,(1551)C=N, 1638 (C=O) (Figure 23); MS: *m/z* 457; [1]H NMR(500MHz, DMSO) δ 3.03-3.19 (4H, 3.11 (dd,*J*=15.8, 6.1 Hz), 3.11 (dd,*J*=15.8, 6.1 Hz)), 4.17 (2H, td,*J*=6.1, 3.8 Hz), 6.71-7.01 (8H, 6.78 (ddd,*J*=7.9, 7.6, 1.2 Hz), 6.84 (ddd,*J*=8.0, 1.2, 0.5 Hz), 6.86 (ddd,*J*=7.9, 1.3, 0.5

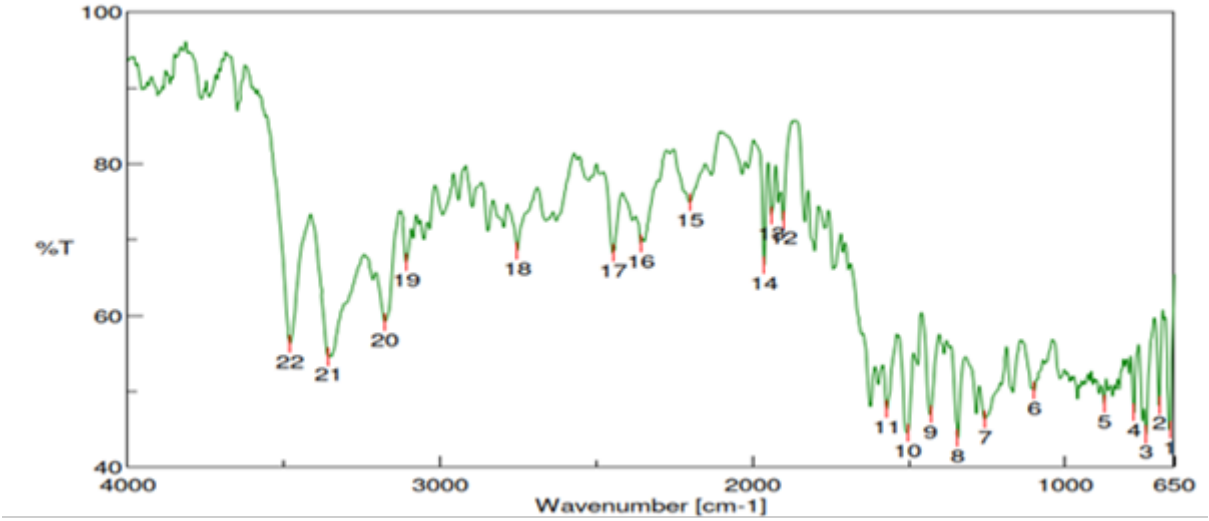


Figure 2: IR of D1 Compound.

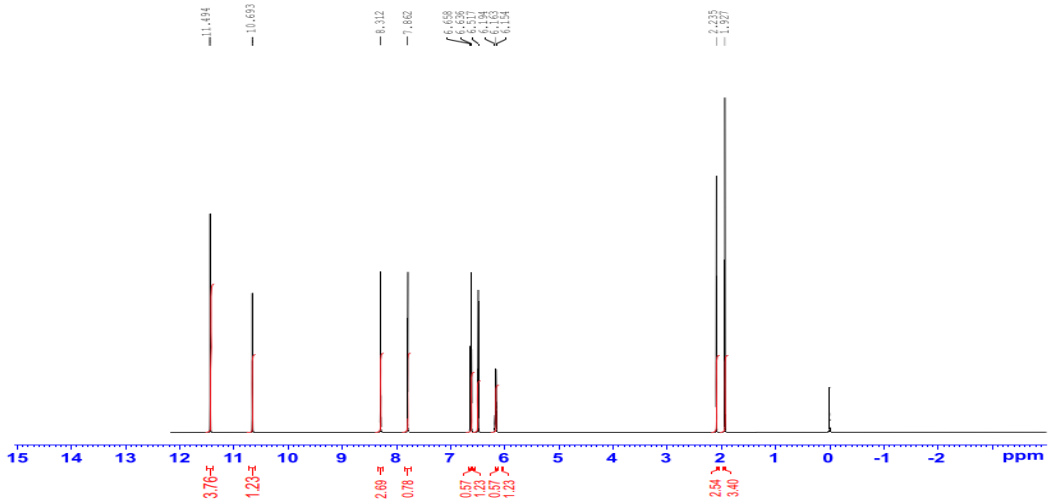


Figure 3: ^{1}H NMR of D1 Compound.

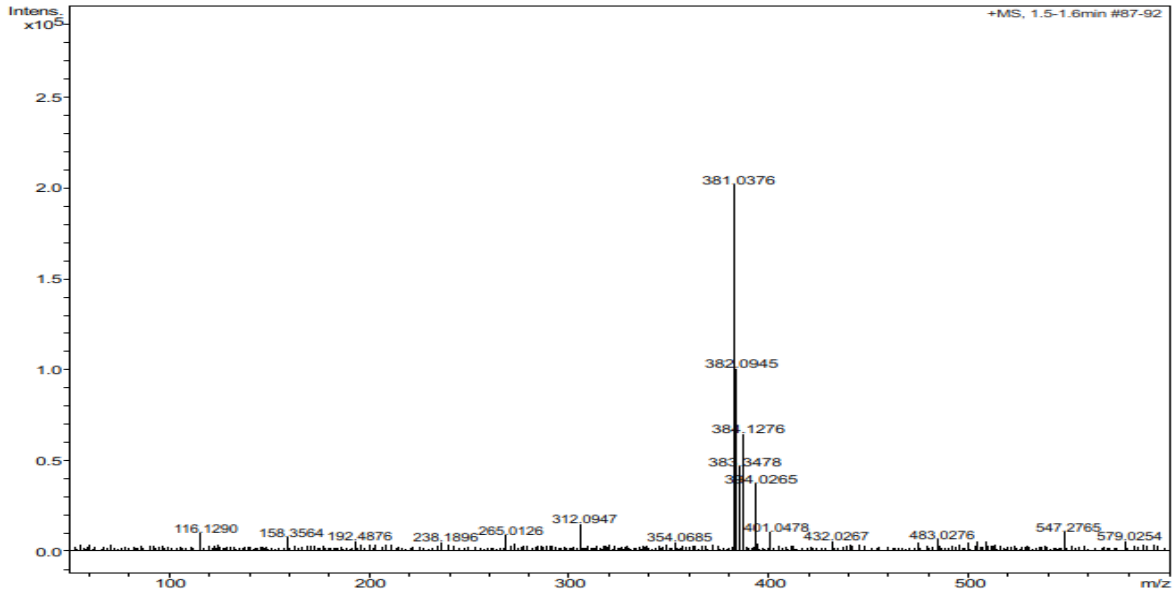


Figure 4: MASS of D1 Compound.

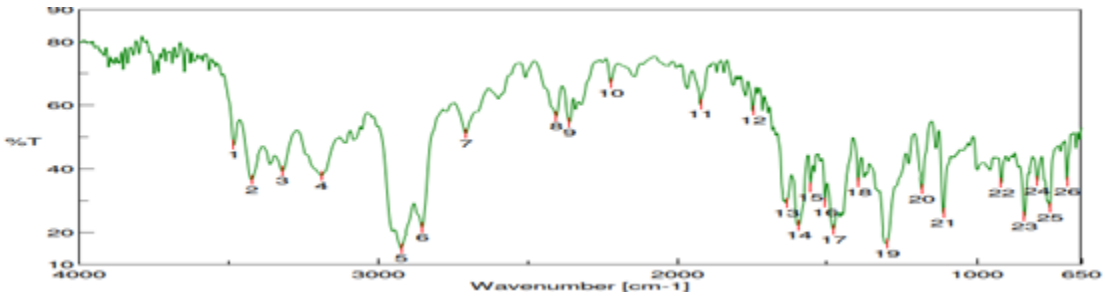


Figure 5: IR D2 Compound.

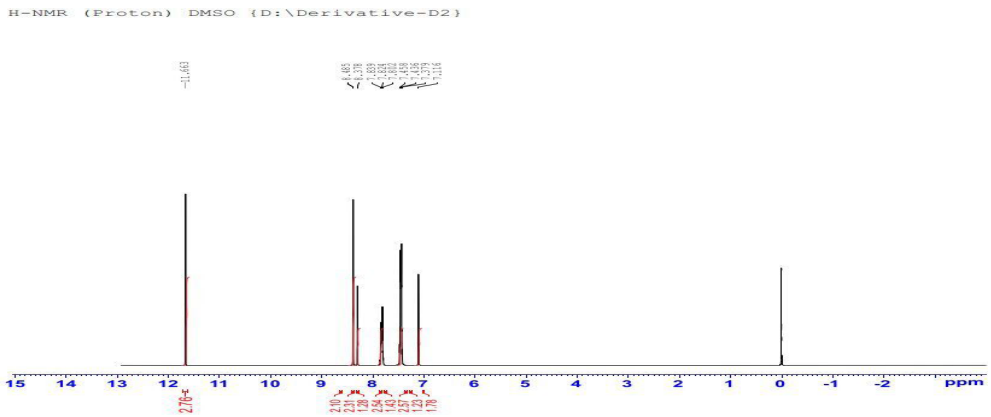


Figure 6: ^{1}H NMR D2 Compound.

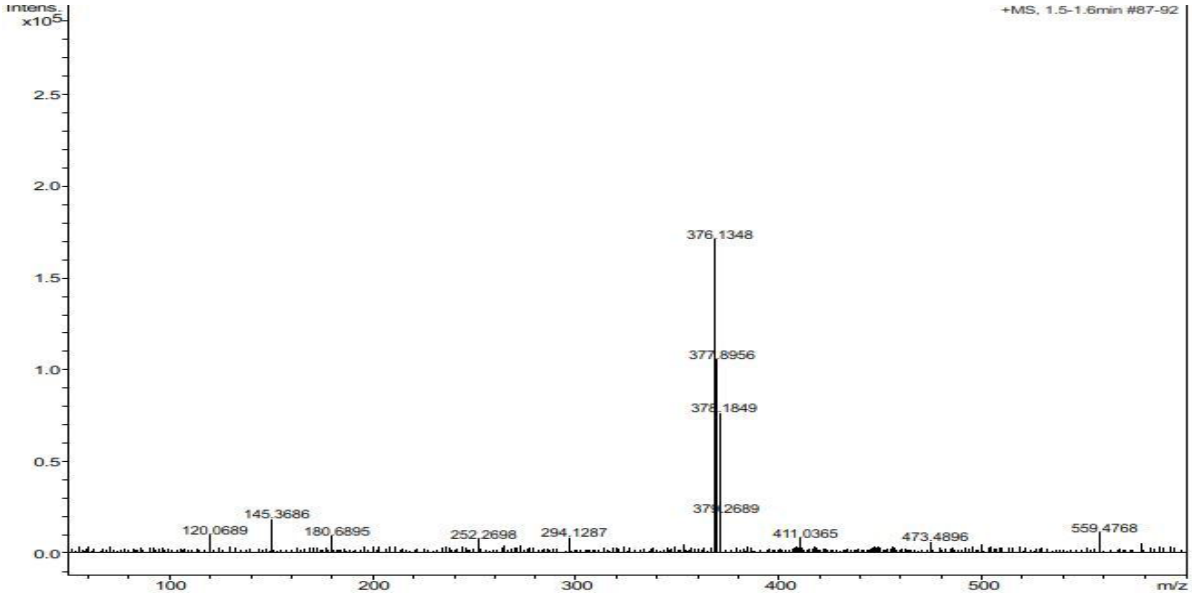


Figure 7: MASS of D2 Compound.

Hz), 6.94 (ddd,*J* = 8.0, 7.6, 1.3 Hz)), 7.19 (2H, d,*J*=3.8 Hz), 7.93 (2H, d,*J*=8.0 Hz) (Figure 24): Exact mass calcd. For $C_{24}H_{22}N_6O_2S$: 456.39; found: 456.39 (Figure 25).

(10E,12E)-N2', N5'-bis((4-chloro-1H-pyrrol-2-yl)methylene) thiophene-2,5-dicarbohydrazide (9); Yellow powder, IR (KBr,cm^{-1}):(3296-C=N-H), (2223)-C=C,(1011)C-S,(1551) C=N,1658.48 (C=O) (Figure 26); MS: *m/z* 423.4; ^{1}H NMR(500MHz, DMSO) δ 6.43 (2H, d,*J*=1.2 Hz), 7.03 (2H, d,*J*=1.2 Hz), 7.80-7.99 (4H, 7.86 (d,*J*=8.0 Hz), 7.94 (s)) (Figure 27): Exact mass calcd. For $C_{24}H_{22}N_6O_2S$: 422.4; found: 422.4 (Figure 28).

(10E,12E)-N2', N5'-bis((4H-1,2,4-triazol-3-yl)methylene) thiophene-2,5-dicarbohydrazide (10); Yellow powder, IR (KBr,cm^{-1}):(3370)-C=N-H, (2338)-C=C,(689)C-S,(1808)C=N, 2250 (C=O) (Figure 29); MS: *m/z* 423.4; ^{1}H NMR(500 MHz, DMSO) δ 6.43 (2H, d,*J*=1.2 Hz), 7.03 (2H, d,*J*=1.2 Hz), 7.80-7.99 (4H, 7.86 (d,*J*=8.0 Hz), 7.80-7.99 (4H, 7.86 (d,*J*=8.0 Hz), 7.94 (s))

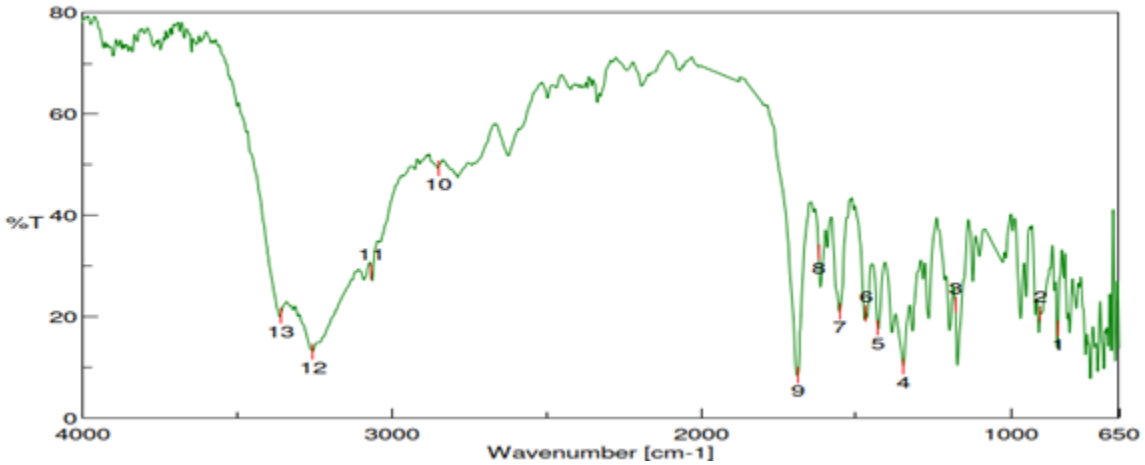


Figure 8: IR of D3 Compound.

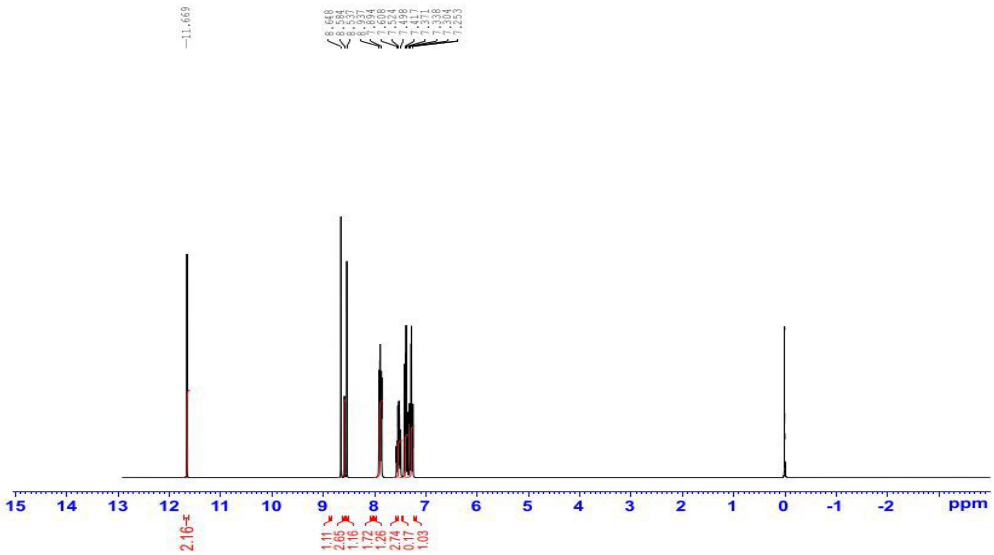


Figure 9: ^{1}H NMR of D3 Compound.

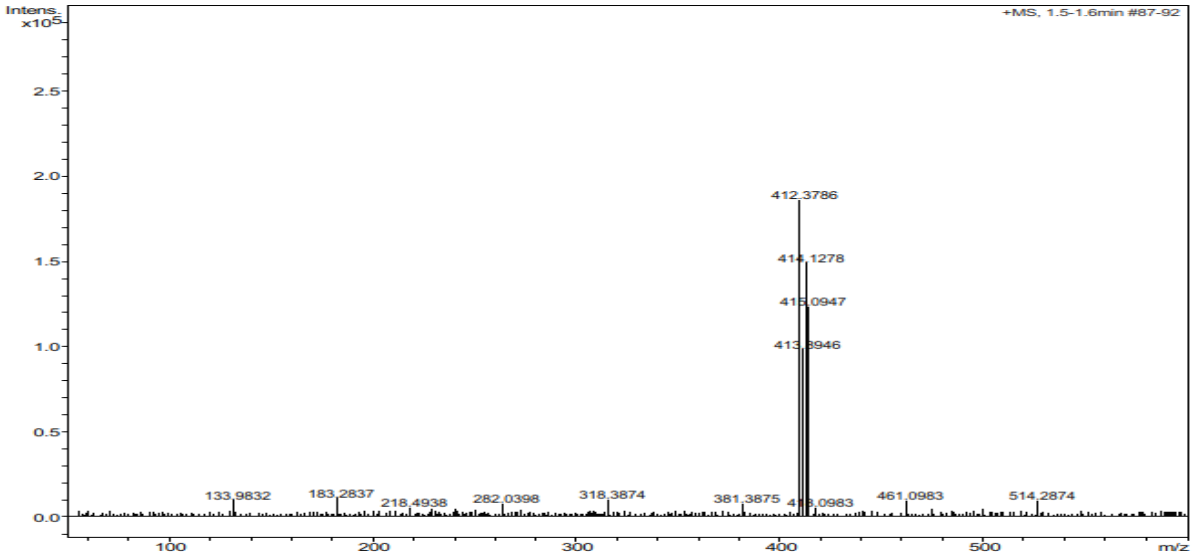


Figure 10: MASS of D3 Compound.

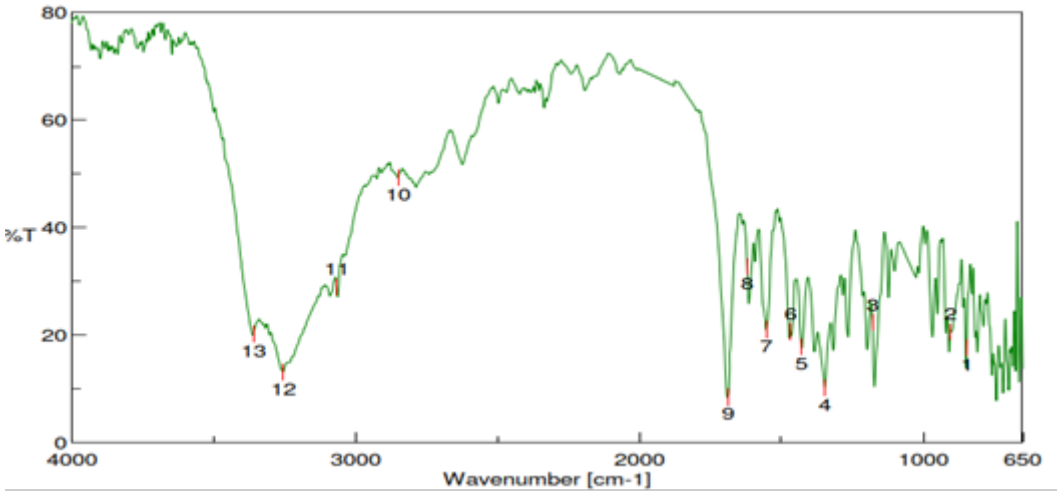


Figure 11: IR of D4 Compound.

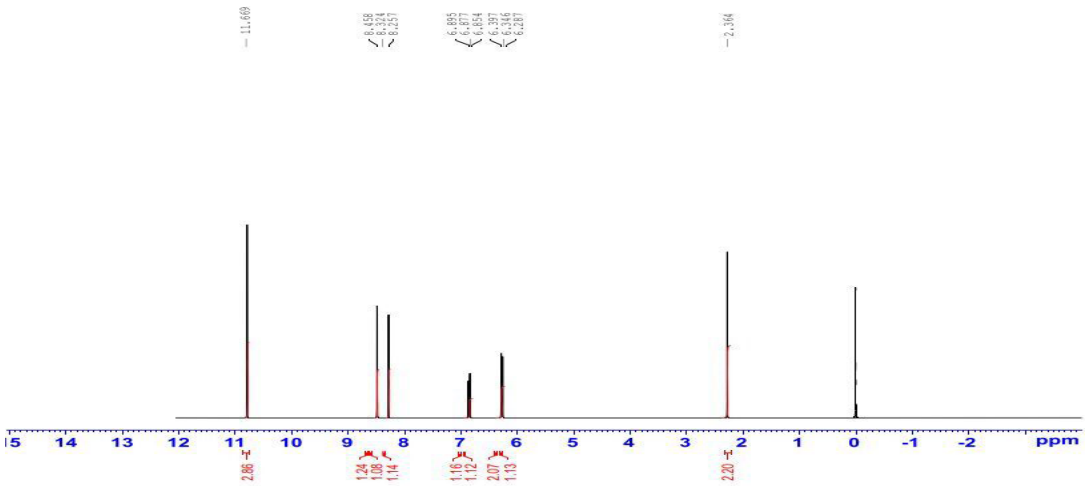


Figure 12: ^{1}H NMR of D4 Compound.

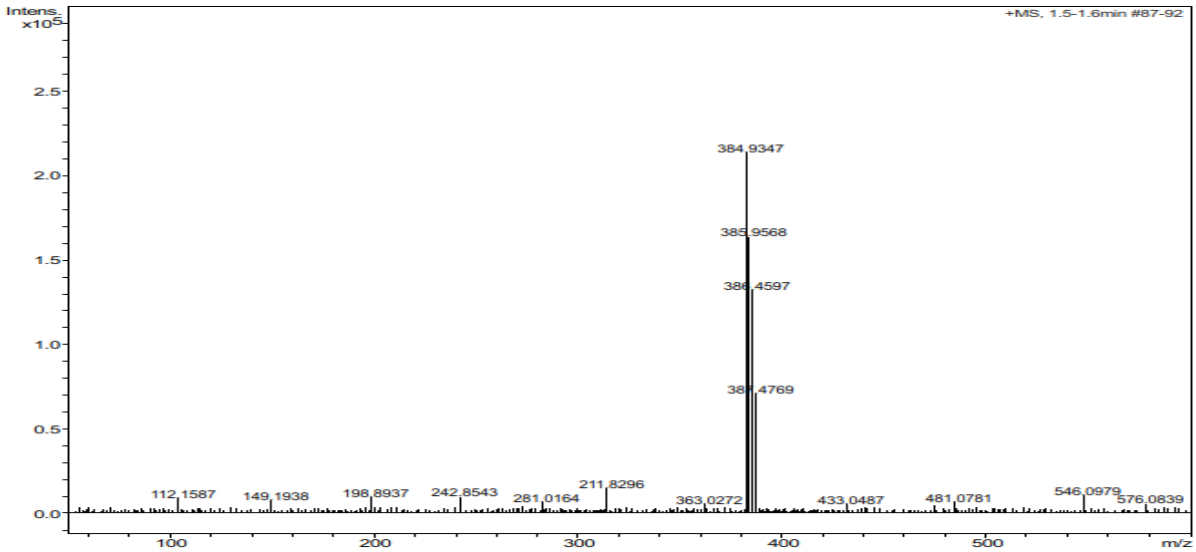


Figure 13: MASS of D4 Compound.

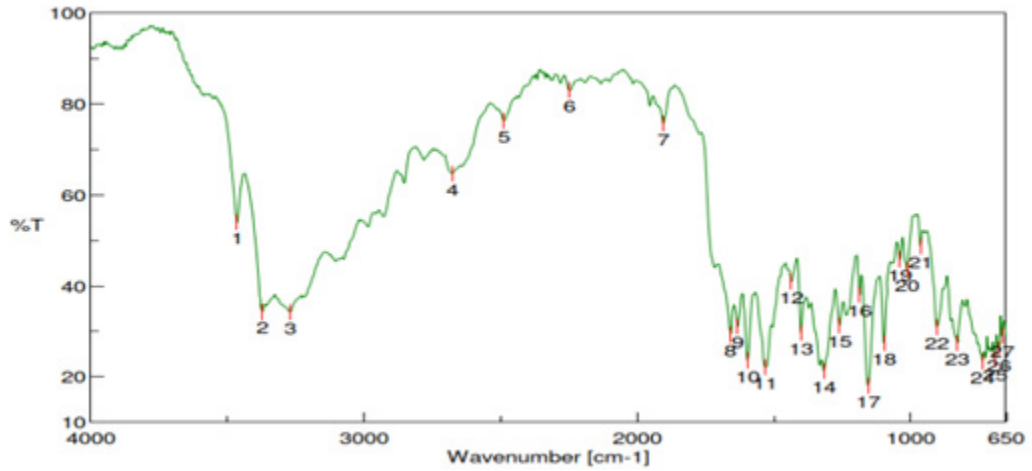


Figure 14: IR of D5 Compound.

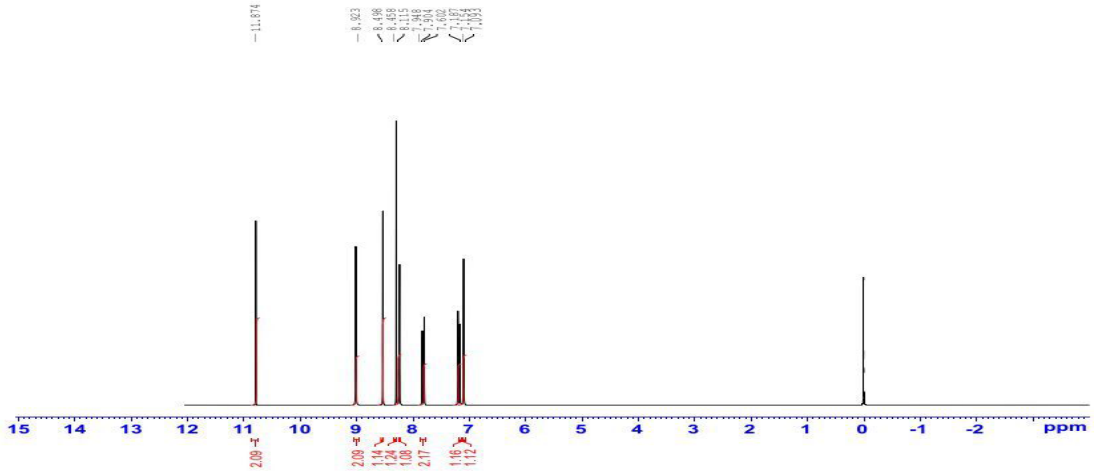


Figure 15: ^{1}H NMR of D5 Compound.

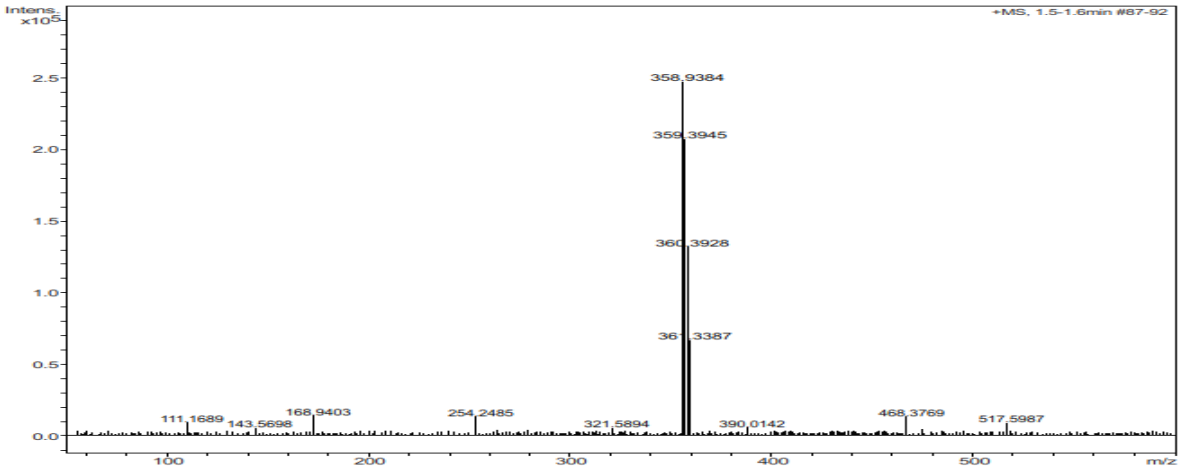


Figure 16: MASS of D5 Compound.

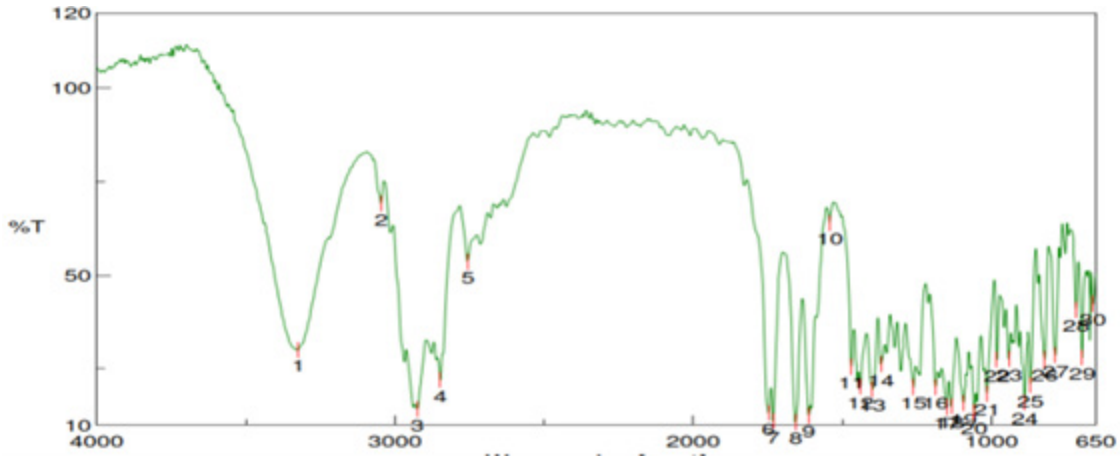


Figure 17: IR of D6 Compound.

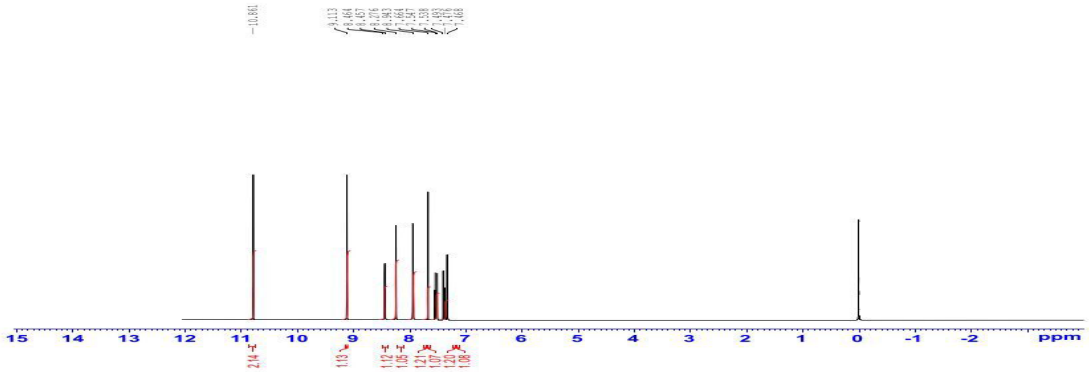


Figure 18: ^{1}H NMR of D6 Compound.

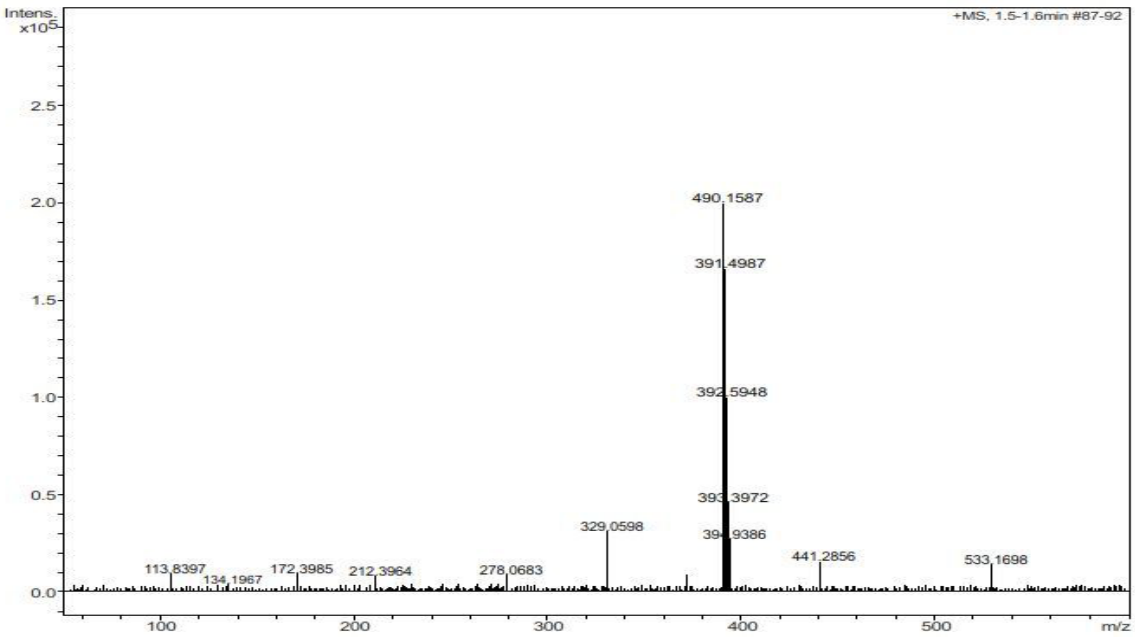


Figure 19: MASS of D6 Compound.

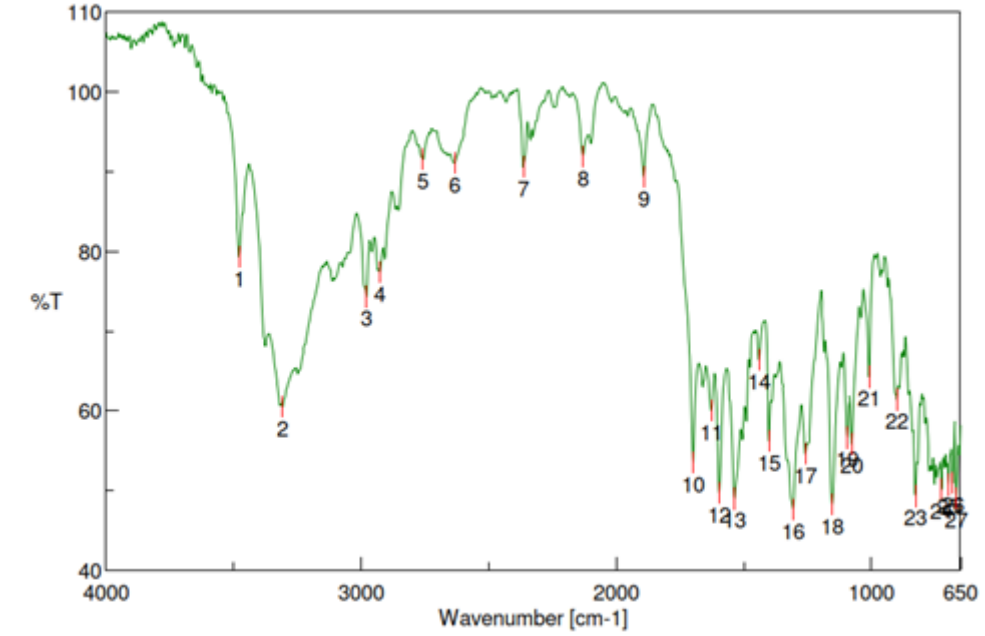


Figure 20: IR of D7 Compound.

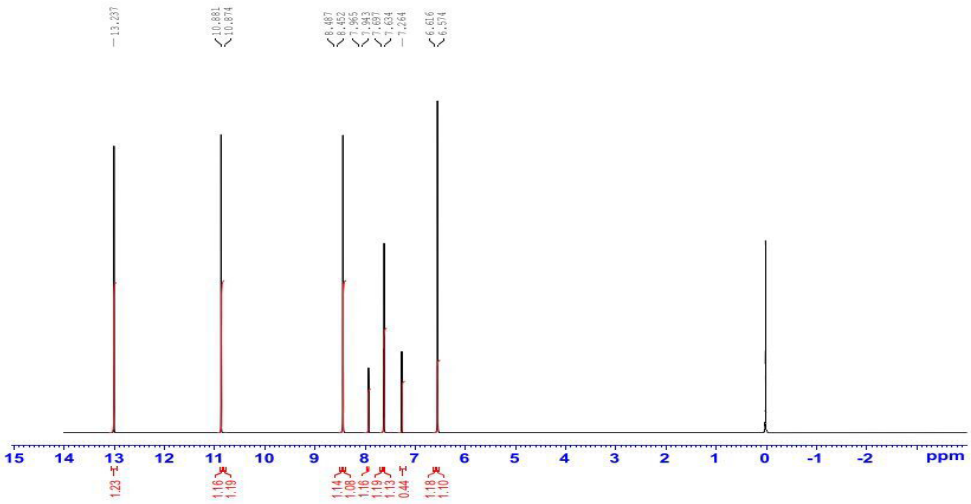


Figure 21: ^{1}H NMR of D7 Compound.

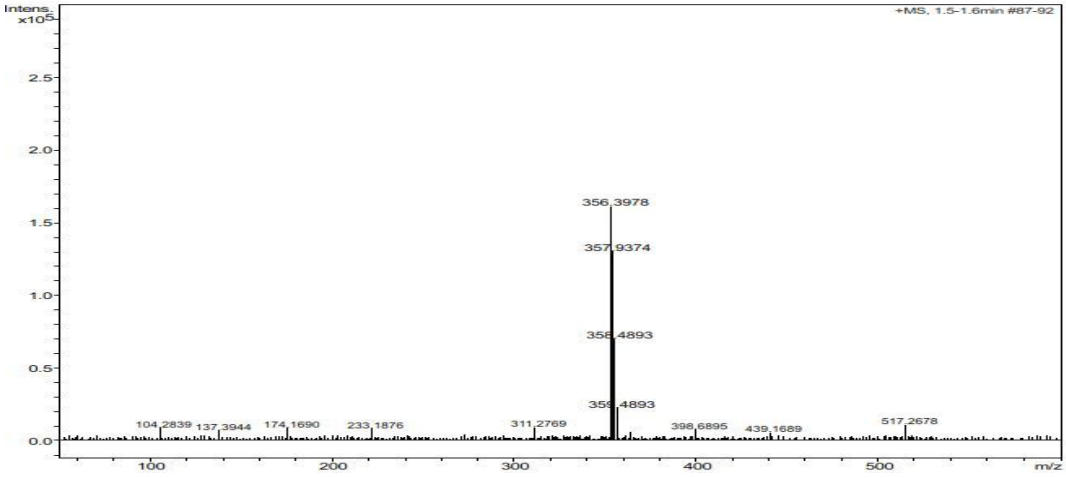


Figure 22: MASS of D7 Compound.

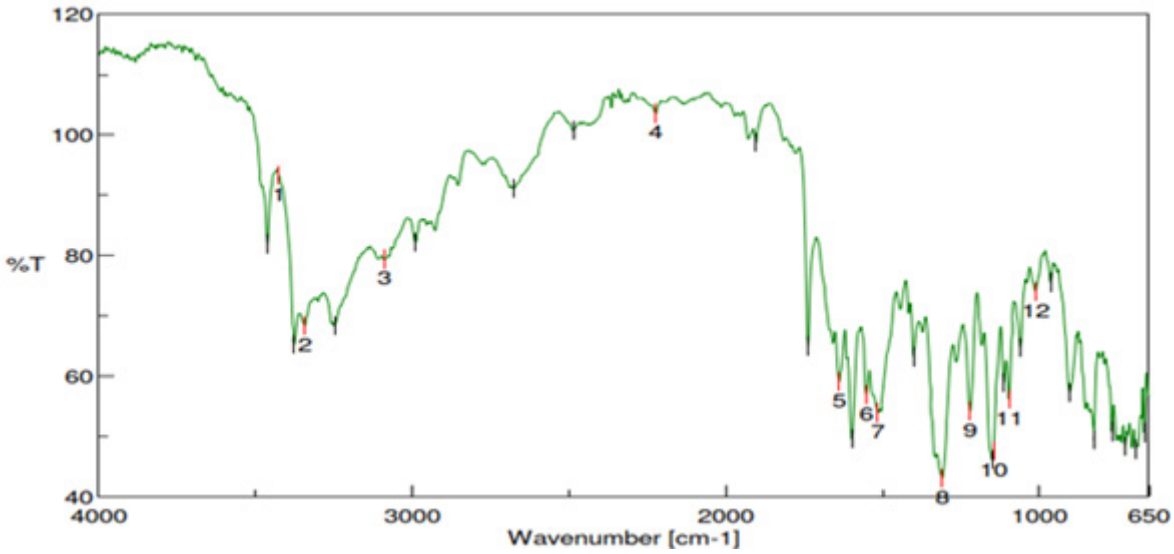


Figure 23: IR of D8 Compound.

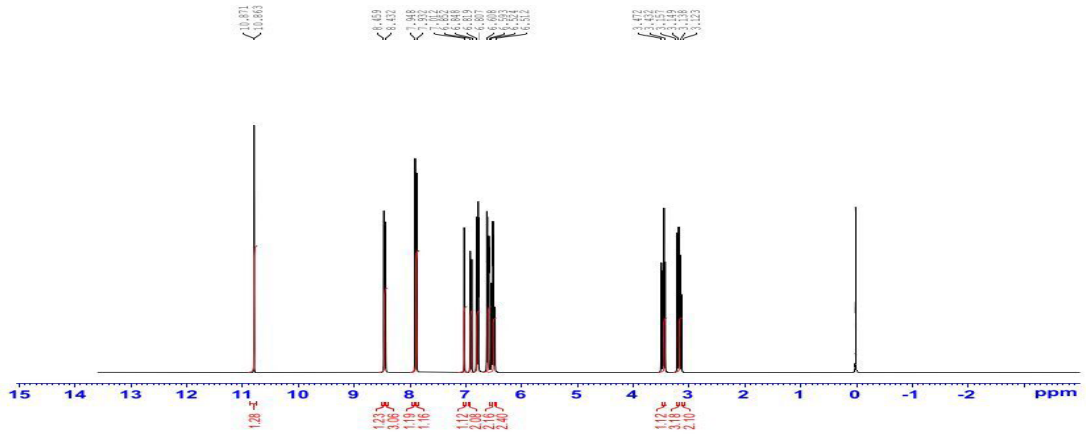


Figure 24: ^{1}H NMR of D8 Compound.

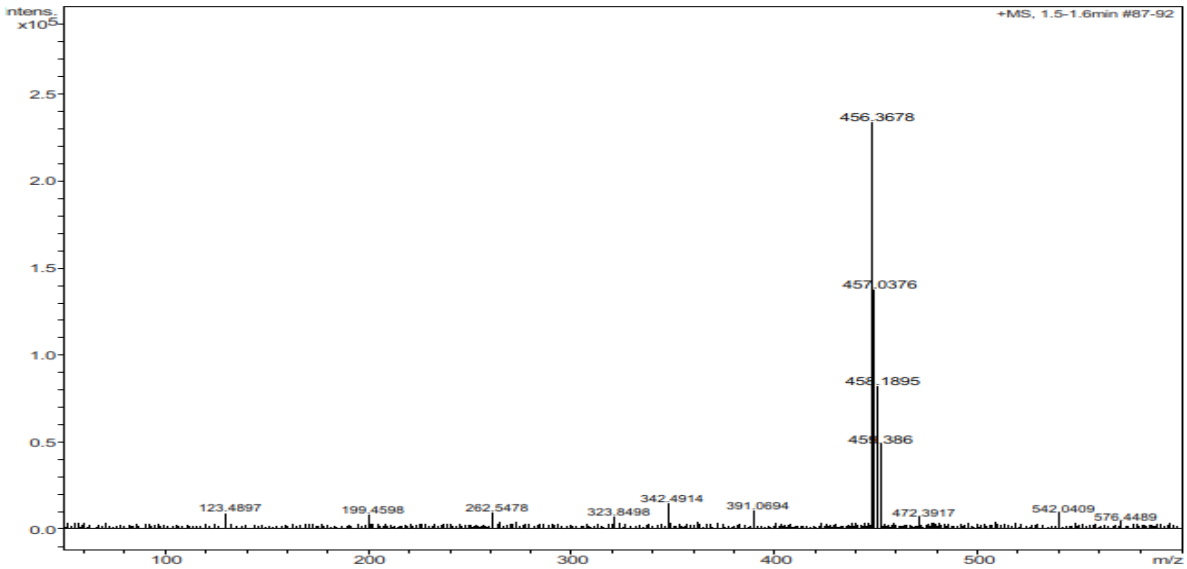


Figure 25: MASS of D8 Compound.

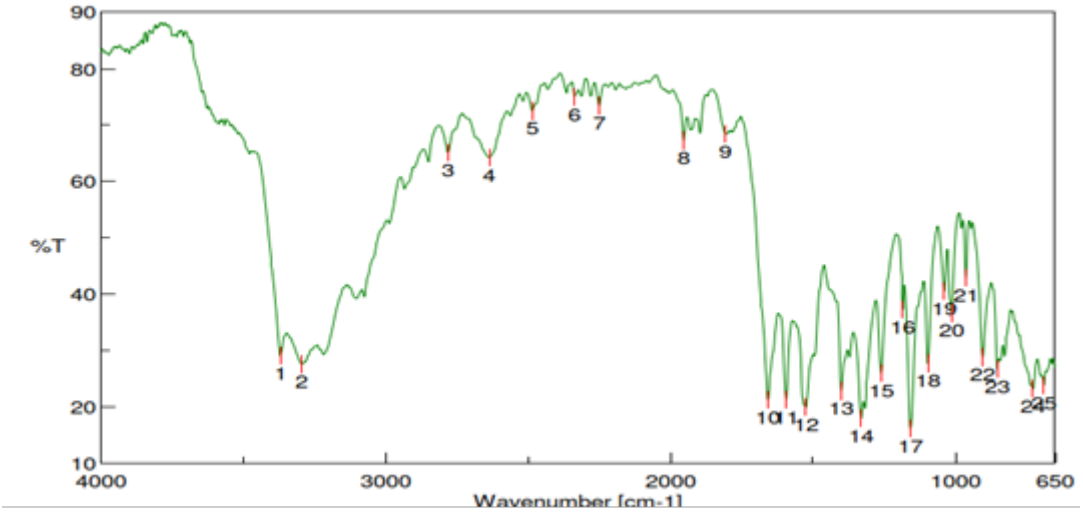


Figure 26: IR of D9 Compound.

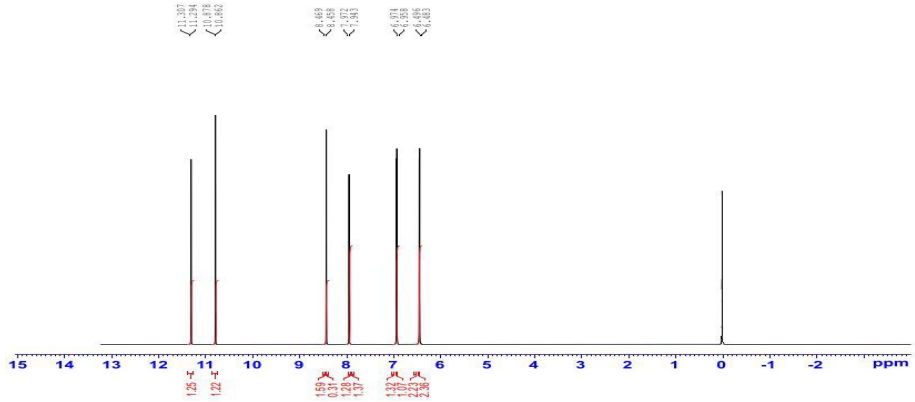


Figure 27: ^{1}H NMR of D9 Compound.

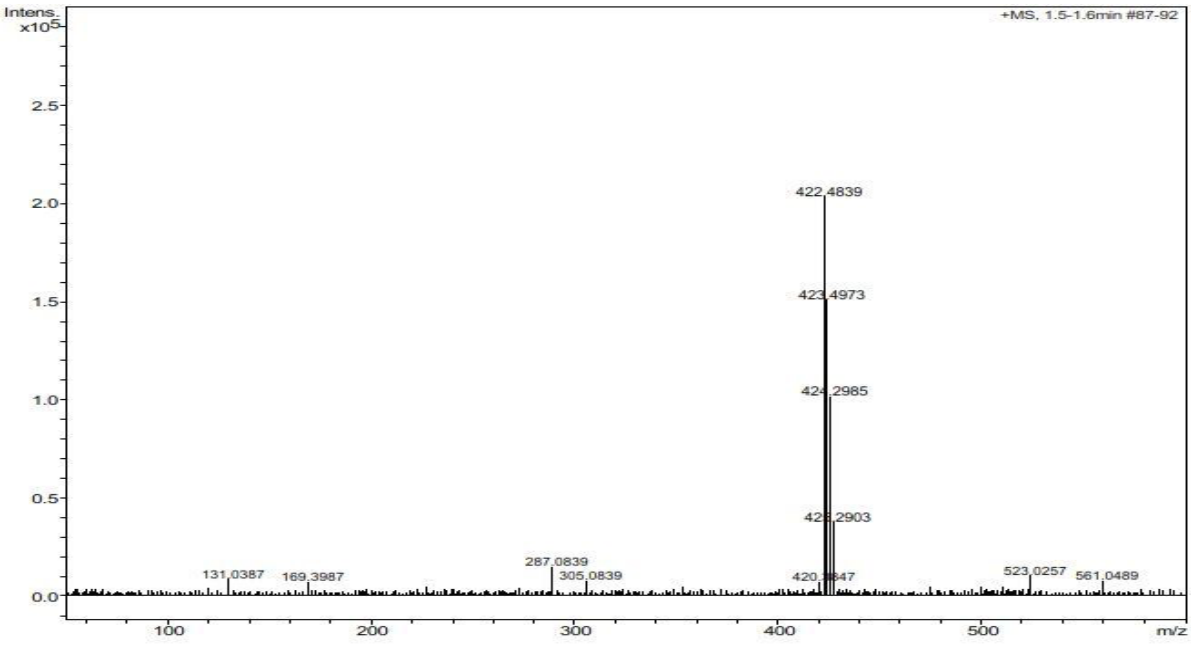


Figure 28: MASS of D9 Compound.

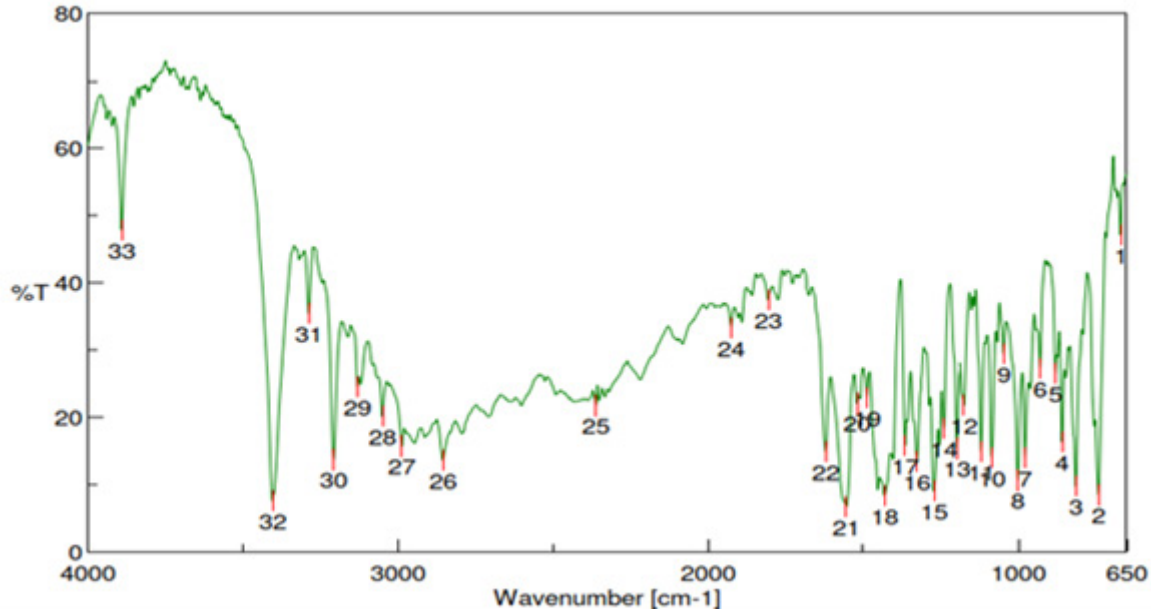


Figure 29: IR of D10 Compound.

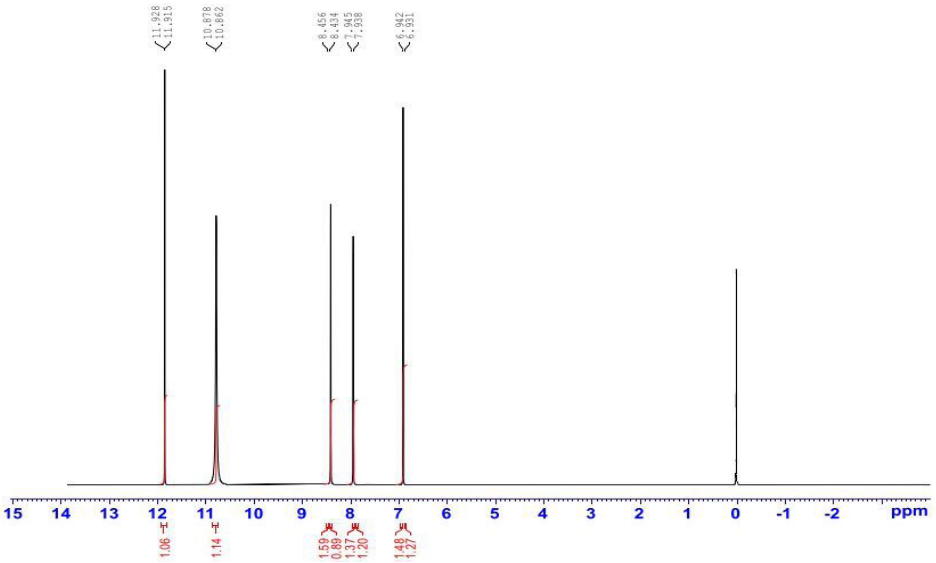


Figure 30: ^{1}H NMR of D10 Compound.

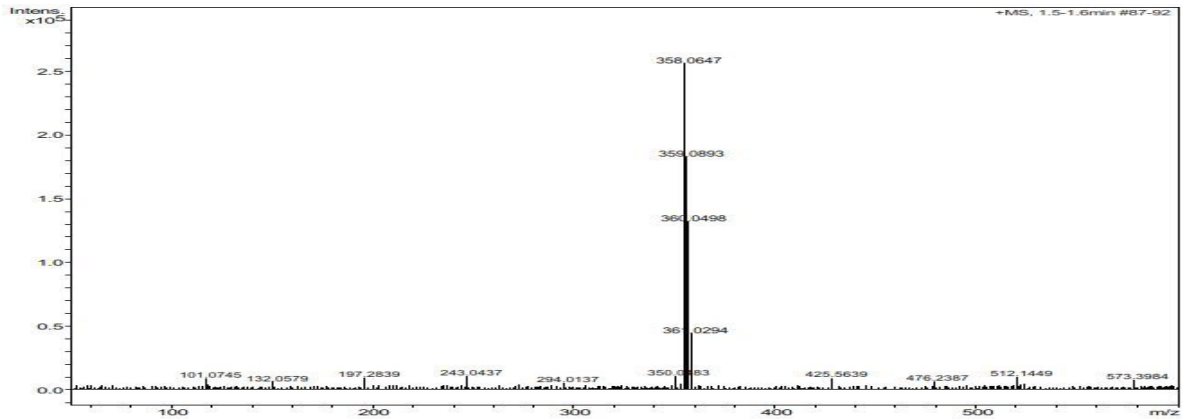


Figure 31: MASS of D10 Compound.

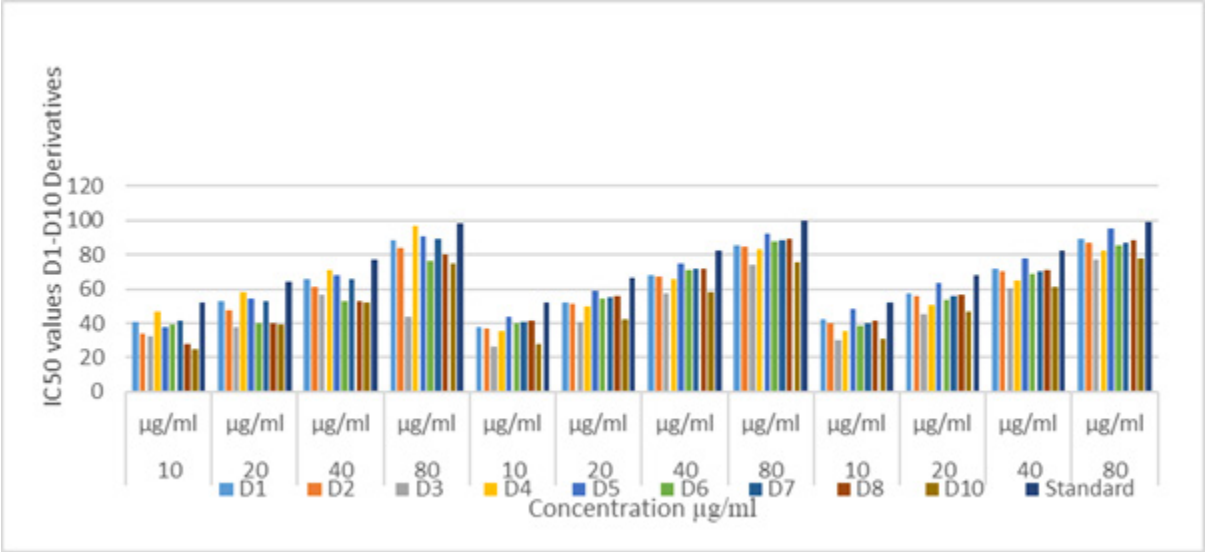


Figure 32: Graph of Conc Vs IC_{50}.

Table 2: IC_{50} Values of D1-D10 on Human Breast Cancer Cell Line (MCF-7).

Human Breast Cancer Cell Line (MCF-7)												
Percentage of inhibitions on MCF-7ata concentration of µg/mL (IC_{50})												
Sample Concentrations(µg/mL)												
Derivatives	Experiment1				Experiment2				Experiment3			
	10 µg/mL	20 µg/mL	40 µg/mL	80 µg/mL	10 µg/mL	20 µg/mL	40 µg/mL	80 µg/mL	10 µg/mL	20 µg/mL	40 µg/mL	80 µg/mL
D1	40.45	52.63	65.63	88.73	37.64	52.04	68.24	85.44	42.03	57.43	72.03	89.03
D2	39.66	42.63	58.63	78.73	27.64	32.04	78.24	95.44	32.03	67.43	82.03	79.03
D3	33.75	47.92	60.92	84.02	36.62	51.02	67.22	84.42	40.25	55.65	70.25	87.25
D4	46.88	58.09	71.09	97.19	35.48	49.88	66.08	83.28	35.27	50.67	65.27	82.27
D5	37.95	54.78	67.78	90.88	44.17	58.57	74.77	91.97	48.23	63.63	78.23	95.23
D6	39.28	40.06	53.06	76.16	40.17	54.57	70.77	87.97	38.56	53.96	68.56	85.56
D7	41.19	53.07	66.07	89.17	40.95	55.35	71.55	88.75	40.27	55.67	70.27	87.27
D8	28.15	40.15	53.15	80.25	41.15	55.55	71.75	88.95	41.36	56.76	71.36	88.36
D9	31.45	43.63	56.63	79.73	24.64	39.04	55.24	72.44	29.03	44.43	59.03	76.03
D10	24.75	38.92	51.92	75.02	27.62	42.02	58.22	75.42	31.25	46.65	61.25	78.25
Control	41.15	53.15	66.15	93.25	26.48	40.88	57.08	74.28	26.27	41.67	56.27	73.27
Standard (Imatinib)	52.19	64.07	77.07	98.54	52.15	66.55	82.75	99.95	52.36	67.76	82.36	99.36

(Figure 30): Exact mass calcd. For $C_{24}H_{22}N_6O_2S$: 358; found: 358

(Figure 31).

Anticancer Activity

Biological method

Cytotoxicity test by MTT Assay

Materials

Cell line-MCF-7 Culture media- MEM medium with Antibiotics and 10% FBS 96 well Tissue culture plate, Neubauer's chamber, MTT Reagent (sigma), Phosphate buffer saline, Acidic isopropanol, 96 well Plate reader".

Procedure

The first day involves seeding 96-well plates with 11-05 cells/mL (the cell count was determined in the Neubauer chamber). After that, the plate goes into a CO_2 incubator for 24 hr at 370°C. A 24

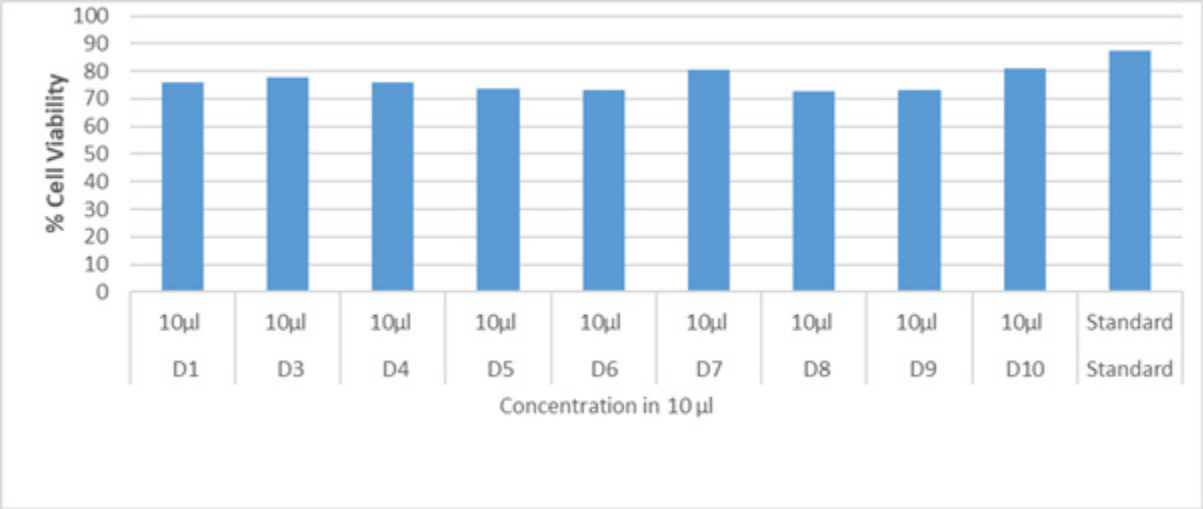


Figure 33: Graph of % Cell viability compound D1-D10.

hr incubation period is followed by a microscopic inspection of the plate with an inverted microscope on Day 2. A clean, triplicate scaffold test specimen is included. The scaffold sample extract medium is added in triplicate, totaling 100 L. After that, you put the plate in a 370°C CO_2 incubator for 24 hr. Three days later, after an incubation period of 24 hr, the plate is inspected using an inverted microscope. Scaffold samples are collected for testing, and 100 L of new DMEM media is added to the mix. Each well is then supplied with 5 mD/mL of MTT reagent. A CO_2 incubator is used for 4 hr while the plate is covered in aluminum foil. After 4 hr, the plate is taken out of the incubator, put on an inverted microscope, and photographed. Swirling the plate thoroughly eliminates any remaining medium, and 200 L of acid isopropanol is poured into each well. Absorbance is measured at 492 nm on a 96-well plate reader after 1 hour. Histopathological tests with regular imatinib and compounds D1, D2, D3, D4, D5, D6, D7, D8, and D10 were conducted at a concentration of 80 D/mL. 10 Formula for IC 50: 10 µg /mL, 20 µg /mL, 40 µg /mL, and 80 µg/mL at the aforementioned concentration (Figure 32), it was determined that all samples were non-toxic (Figure 33). Results are shown in Table 2.

CONCLUSION

The creation of new chemical compounds has significant relevance in the quest for more efficient and less cytotoxic anticancer treatments. In the present investigation, we undertook the synthesis of derivatives of Thiophene-2,5-dicarbohydrazide by the incorporation of diverse aromatic rings, both through substitution and without substitution, to augment their efficacy and mitigate their inherent toxicity.

Compounds D1, D2, D3, D4, D5, D6, D7, D8, D9, and D10 exhibited significant anticancer activity, as shown by their respective IC_{50} values of 54.03, 49.48, 51.53, 52.88, 58.99, 54.69, 50.82, 42.36, and 42.53 µg/mL. The IC_{50} values observed in this study closely approximate the average IC_{50} value often associated with widely used anticancer medications. These findings suggest that compounds D1-D10 have significant potential as efficacious therapeutics against cancer cells. When cell viability optical density ranges from 70-90% the test sample is considered to be non-toxic (Figure 33). All of the compounds are non-toxic and have a range of 70-90%; the D5 compound has a range of 73.57%, while some other compounds like D10, D7 have a range of cell viability optical density that is greater than D5 compound however, D5 compound is consider to be more active due to its higher IC_{50} value than those of D7 and D10 compounds. One significant discovery made in this study is that the D1-D10 class of chemicals demonstrated noteworthy anticancer efficacy while not causing apoptosis in normal cells. The observed selective cytotoxicity towards cancer cells has significant value, as it implies that these chemicals may exhibit the desired attributes of being efficacious against cancer while minimizing harm to healthy cells. In summary, the process of synthesizing derivatives of Thiophene-2,5-dicarbohydrazide combined with different aromatic rings has resulted in the production of a range of compounds, namely D1-D10. These compounds have shown significant effectiveness in inhibiting cancer growth while exhibiting no harmful effects on healthy cells. The aforementioned results highlight the possibility of these compounds as viable candidates for future examination and advancement as anticancer medicines, exhibiting improved effectiveness and diminished adverse effects. The exploration of these innovative chemical compounds represents a crucial advancement in enhancing the overall efficacy and safety of anticancer pharmaceuticals.

ACKNOWLEDGEMENT

I wish to express my sincere gratitude to my co-authors for accepting me as the main author and for their guidance and encouragement.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

IR: Infrared Radiation, **NMR:** Nuclear Magnetic Resonance, **IC_{50}:** Half-Maximal Inhibitory Concentration, **µg/mL:** Micro-gram per milliliter.

SUMMARY

Cancer poses a significant global health burden, occupying the second position among the primary causes of mortality on a global scale. Breast cancer, which is prevalent among women, has seen notable progress in its treatment, including traditional approaches such as surgical intervention, radiation therapy, and chemotherapy. In contemporary cancer therapy, targeted treatments and immunotherapies are key components. In light of these advancements, there is an increasing need for more efficacious and less harmful anti-neoplastic agents. The present research emphasizes the imperative nature of identifying novel and effective anti-cancer medications, while concurrently mitigating the occurrence of detrimental side effects. The background portion of this study emphasizes the recognized biological activity of thiophene and carbohydrazide derivatives, some of which have previously been used in several anti-cancer therapeutics. The aforementioned context establishes the foundation for the principal aim of the research. The primary objective of this study is to synthesize molecules that exhibit robust anti-cancer effects while maintaining a non-toxic profile. To accomplish this objective, a new series of derivatives of N2, N5-bis[(1E)-ethylidene] thiophene-2,5-dicarbohydrazide was produced. The primary goal is evident: to identify and amalgamate molecules that possess the ability to successfully treat cancer while minimizing any potential negative consequences. The materials and methods part of the paper provides a detailed description of the approach used to synthesize the newly developed chemicals. The synthesis of a novel series of derivatives is achieved by subjecting thiophene-2,5-dicarbohydrazide to a reaction with aromatic aldehydes and ethanol. Subsequently, these compounds underwent *in vitro* testing to evaluate their cytotoxic efficacy against the MCF-7 breast cancer cell line. The findings of this study indicate that some newly created compounds exhibited significant anti-proliferative activity in comparison to the established anti-cancer medication imatinib. This finding underscores the potential efficacy of these chemicals in combating breast cancer. In summary, this study showcases a significant accomplishment in the synthesis of a range of derivatives of Thiophene-2,5-dicarbohydrazide, which exhibit substantial effectiveness in impeding the proliferation of cancer cells. The chemicals D1, D2, D3, D4, D5, D6, D7, D8, and D10 exhibited notable IC50 values, suggesting their potential effectiveness in suppressing the development of cancer cells. These compounds demonstrated a selective cytotoxicity against cancer cells while showing little influence on normal cells. This suggests that these compounds have the potential to be effective anti-cancer drugs with minimal side effects. The research is characterized by the presence of relevant terms, such as Thiophene-2,5-dicarbohydrazide, anticancer properties, MCF-7 cell lines, and derivatives, which effectively capture the focus of the investigation. The study's context underscores the global significance of cancer in public health and the need for the development of anti-cancer therapeutics that exhibit enhanced efficacy and reduced toxicity. The primary aim of this research is to conduct chemical synthesis with a specific emphasis on breast cancer, to address this substantial health concern. The present research aims to examine the pharmacological effects of thiophene derivatives, which have been well acknowledged for their diverse array of activities. The primary focus is on the investigation of the prospective applications of Schiff bases formed from thiophene within the realm of pharmaceutical research since they have shown encouraging outcomes. The significance of this finding lies in the possibility of these compounds providing a targeted and improved approach to cancer therapy. This issue has special significance in light of the detrimental side effects and diminishing efficacy of conventional chemotherapy, which may be ascribed to the development of drug resistance. The materials and methods section is a detailed description of the synthesis procedure used for the production of these compounds. The synthesis route involves a series of chemical reactions and subsequent purification steps, eventually leading to the generation of these novel compounds. The methodology ensures the integrity and consistency of the substances for further analysis. The results provide empirical support for the effectiveness of this research. The tested compounds exhibited significant anti-proliferative activity, surpassing the effectiveness of the well-established anti-cancer drug imatinib. This finding implies that certain chemical compounds have promise as therapeutic agents for the treatment of cancer. The conclusion has great relevance in our pursuit of knowledge. The Thiophene-2,5-dicarbohydrazide derivatives listed above have considerable potential as effective pharmacological agents in the treatment of cancer. Remarkably, these chemical compounds demonstrated selective cytotoxicity against cancerous cells while maintaining the viability of normal cells, a crucial attribute for minimizing detrimental consequences. The selective targeting of these compounds differentiates them as viable candidates for further investigation and progress in the field of anticancer treatments. The results of this research provide a positive outlook for the development of more efficient and secure treatment strategies for cancer in the next years. In conclusion, this work represents a significant progression in the ongoing endeavor to develop enhanced and less toxic anti-cancer drugs. The synthesis of derivatives of Thiophene-2,5-dicarbohydrazide, accompanied by extensive experimentation, has led to the identification of several compounds that have substantial promise in the realm

of anti-cancer treatments. The shown selectivity towards cancer cells underscores the potential of these chemical compounds to provide heightened effectiveness and increased safety within the field of cancer treatment. The findings of this study provide a foundation for prospective investigations and advancements, perhaps culminating in the production of anti-cancer medications that exhibit enhanced efficacy and reduced bad effects. This research signifies a significant progression in enhancing the overall efficacy and safety of anticancer drugs, ultimately offering benefits to those grappling with this debilitating disease.

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