IJCRT.ORG ISSN : 2320-2882

INTERNATIONAL JOURNAL OF CREATIVE RESEARCH THOUGHTS (IJCRT)

An International Open Access, Peer-reviewed, Refereed Journal

Synthesis And Application Of Hydrazide Derivatives Derived From 2,7 Dichloro Fluorenone

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Abstract: Fluorenyl-based hydrazide derivatives represent a promising class of compounds with potential antimicrobial properties due to their lipophilicity and structural versatility. In this study, a series of Hydrazide — N'-(9H-fluoren-9-ylidene) acetohydrazide, 2-chloro-N'-(9H-fluoren-9-ylidene) acetohydrazide, N'-(9H-fluoren-9-ylidene)-2-methylpropanehydrazide, N'-(9H-fluoren-9-ylidene)-2,2-dimethylpropanehydrazide, N'-(2,7-dichloro-9H-fluoren-9-ylidene) benzohydrazide, and N'-(2,7-dichloro-9H-fluoren-9-ylidene)decanehydrazide were synthesized through condensation of 2,7-dichlorofluorenone hydrazone with various acid chlorides. The compounds were characterized using spectroscopic techniques including IR and NMR. Their antimicrobial activity was assessed against Gram-positive and Gram-negative bacterial strains, as well as *Candida albicans*. Several derived hydrazides exhibited moderate to significant antimicrobial activity, especially against *E. coli* and *Candida albicans*. These results reinforce the potential of fluorenyl-hydrazide as a platform for antimicrobial drug design.

Index Terms **-** Hydrazide derivatives; Fluorenone hydrazone; Acid chlorides; Schiff bases; antimicrobial activity

Introduction

The growing threat of antimicrobial resistance (AMR) poses one of the most pressing challenges to global public health, with drug-resistant infections accounting for an increasing number of deaths and complications each year. The rapid emergence of resistant bacterial and fungal strains has rendered many conventional antibiotics ineffective, leading to treatment failures, prolonged illness, and increased healthcare costs. This alarming trend highlights the urgent need for the discovery and development of novel chemotherapeutic agents with broad-spectrum and targeted antimicrobial activity [1][2]. In recent years, Fluorenone hydrazone have emerged as a class of compounds with considerable pharmacological potential, owing to their structural diversity, ease of synthesis, and broad spectrum of biological activities [3][4]. Among these, hydrazide—formed via the condensation of hydrazone with carbonyl compounds—have been widely studied for their antimicrobial, anti-inflammatory, and anticancer properties [5][6]. These properties are largely attributed to their ability to form stable hydrogen bonds and undergo tautomerism, enhancing their binding to biological targets such as enzymes and receptors [7][8].

Fluorenyl derivatives, particularly those bearing a fluorenone core, offer a valuable framework for drug design due to their rigid, planar tricyclic structure, which enhances molecular stability and facilitates interactions with biological targets [9][10]. When functionalized as hydrazide, these compounds exhibit enhanced lipophilicity and bioavailability, attributes that are favourable for antimicrobial action. Substituent modifications, such as alkyl branching or halogenation (e.g., chlorine atoms), are known to further modulate biological efficacy by influencing electronic properties, solubility, and membrane permeability [11][12]. The synthetic versatility of these hydrazides allows for the creation of a variety of derivatives with potentially enhanced biological activities and specific target interactions. The synthesis of fluorenyl-based hydrazine is typically achieved via condensation reactions between fluorenone or substituted fluorenone derivatives and appropriate hydrazides. In particular, compounds derived from (2,7-dichloro-9H-fluoren-9-ylidene) hydrazine and various acid chlorides yield structurally diverse hydrazide with potential for further chemical modification [13]. These compounds offer a robust platform for further derivatization, including the introduction of alkyl, aryl, or long-chain aliphatic groups that can significantly alter the compound's physicochemical and biological properties. Such structural versatility allows for the rational design of molecules with enhanced selectivity and potency against microbial targets.

In the present study, we report the synthesis and characterization of six novel fluorenyl-hydrazide base derivatives, each featuring distinct side chains—ranging from short aliphatic and branched groups to aromatic and long-chain alkyl moieties. The antimicrobial activity of these compounds was evaluated against a panel of Gram-positive and Gram-negative bacteria, as well as fungal strains, aiming to identify structural features associated with enhanced bioactivity.

I. SYNTHESIS

Experimental Materials

All the chemicals used in this study were of analytical grade and were purchased from Sigma Aldrich made from commercial sources. The organic solvents used in this study were of analytical grade. TLC was performed on Merck grade pre- coated TLC silica gel 60 F254 plates (Merck KGaA, Darmstadt, Germany) & a UV cabinet was used for spot visualization. Fourier Transform Infrared (FT-IR) spectra were scanned using Thermo Fischer Scientific. The 1H NMR spectra of the compounds were recorded on a Bruker instrument (400 MHz for 1 H) in CDCl3 solutions.

General procedure for Synthesis of Hydrazide derivates.

The dichloro fluorenone hydrazone was synthesized (Scheme 1) by condensation reaction using hydrazine with dichloro fluorenone in presence of Acetic acid as catalyst and Methanol as solvent. Initially, 20g. of dichloro fluorenone was taken along with 200 methanol as solvent. Added 7.5 g. of hydrazine hydrate-80%. Added 0.5 g. acetic acid as a catalyst. Above mass was cooked at reflux temp for 6-8 hrs. Further, the completion of the reaction was checked using TLC (Heptane: EA = 90:10). As per TLC requirement, hydrazine hydrate is added if the RM spot is observed. After completion of the reaction (confirmed by TLC), the reaction mass was filtered off & washed with 1 volume of methanol. Wet cake obtained was dried in oven at 80ºC. Final Hydrazone obtained was 18 g.

Further hydrazide derivatives from hydrazone were derived as per reaction Fig-2. 5 g. of Hydrazone was taken along with 50 ml MDC as solvent. Added 10-15 gm of different Acid chloride in presence of 5-10 g of K2CO3 to scavenge generated HCl. Reaction mass was cooked at RT for 2-3 hrs. Further the completion of reaction was checked using TLC (Heptane: EA = 90:10) As per TLC requirement, acid chloride is added if RM spot is observed. After completion of reaction (confirmed by TLC), reaction mass is taken for water wash & 1% soda ash solution. MDC layer taken for evaporation and washed the wet cake with methanol, filtered and dried in oven. Final Dry cake obtained was 3-5 g.

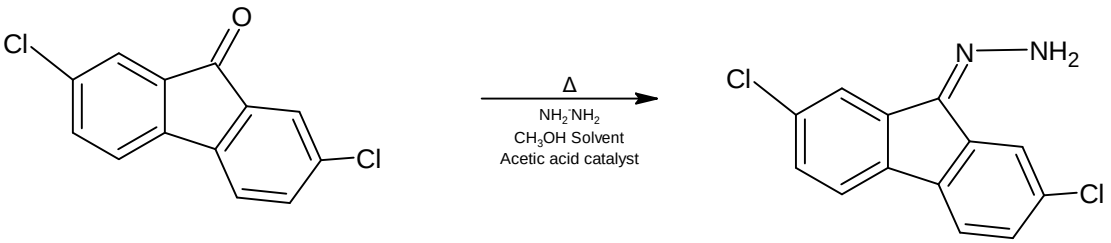

fig-1: synthesis of hydrazone from dichloro fluorenone

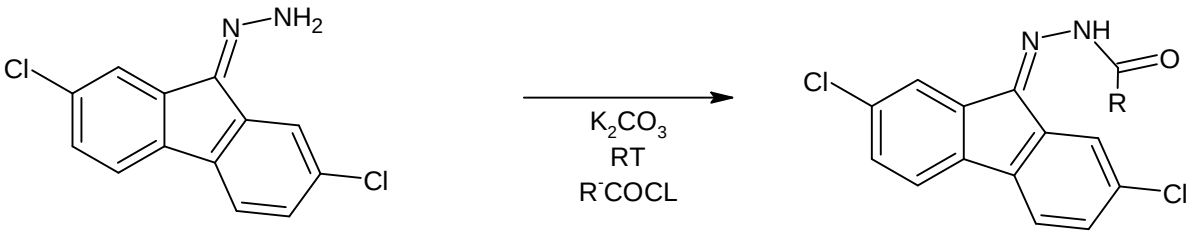

fig-2: synthesis of hydrazide from derived hydrazone

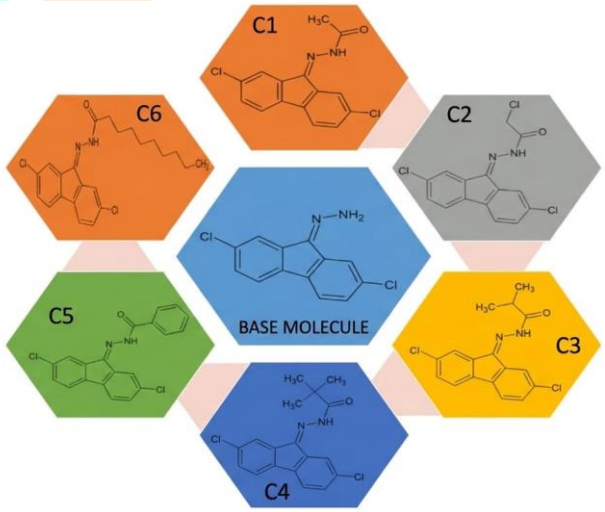

fig-3: novel compound

table-1: novel compounds

Compound	Name
C1	*N*'-(9*H*-fluoren-9-ylidene)acetohydrazide
C2	2-chloro-*N*'-(9*H*-fluoren-9-ylidene)acetohydrazide
C3	*N*'-(9*H*-fluoren-9-ylidene)-2-methylpropanehydrazide
C4	*N*'-(9*H*-fluoren-9-ylidene)-2,2-dimethylpropanehydrazide
C5	*N*'-(2,7-dichloro-9*H*-fluoren-9-ylidene)benzohydrazide
C6	*N*'-(2,7-dichloro-9*H*-fluoren-9-ylidene)undecanehydrazide

(C1) N'-(9H-fluoren-9-ylidene)acetohydrazide

Hydrazone compound (5 gm, 0.02 mol), Soda ash (2.12 gm ,0.02 mol) was mixed at room temperature in 100 ml methylene dichloride as solvent. Acetyl chloride (2.35 gm, 0.03 mol) was added dropwise 10-15 min. Reaction mass was allowed to cooked at room temperature for 4-5 hrs. After completion of reaction, reaction mass was filtered & methylene dichloride was evaporated. Residual Mass is treated with Methanol (20 gm) , filtered and washed with methanol (10 gm). Final Finished product dried in a hot air oven at 120 ^{o}C. Yield:62%

1H NMR δ:2.09 (3H,CH3), 7-9 (6H,Ar-H &1H-NH)
IR [ν, cm-1, KBr]: 1687 cm^{-1}: C=O (amide carbonyl),1593 cm^{-1}: (N–H bending + aromatic C=C) 751 cm^{-1}: C–Cl stretch

(C2) 2-chloro-N'-(9H-fluoren-9-ylidene)acetohydrazide

Hydrazone compound (5 gm, 0.02 mol), Soda ash (2.12 gm ,0.02 mol) was mixed at room temperature in 100 ml methylene dichloride as solvent. Chloro Acetyl chloride (2.39 gm, 0.03 mol) was added dropwise 10-15 min. Reaction mass was allowed to cooked at room temperature for 4-5 hrs. After completion of reaction, reaction mass was filtered & methylene dichloride was evaporated. Residual Mass is treated with Methanol (20 gm) , filtered and washed with methanol (10 gm). Final Finished product dried in a hot air oven at 120 ^{o}C. Yield:65%

1H NMR δ: 4.18 (2H,CH2), 7-9 (6H,Ar-H &1H-NH)
IR [ν, cm-1, KBr]: 1685.75 cm^{-1}: C=O (amide carbonyl),1616 cm^{-1}: (N–H bending + aromatic C=C) 748.43 cm^{-1}: C–Cl stretch

(C3) N'-(9H-fluoren-9-ylidene)-2-methylpropanehydrazide

Hydrazone compound (5 gm, 0.02 mol), Soda ash (2.12 gm ,0.02 mol) was mixed at room temperature in 100 ml methylene dichloride as solvent. Iso Butyryl chloride (3.18gm, 0.03 mol) was added dropwise 10-15 min. Reaction mass was allowed to cooked at room temperature for 4-5 hrs. After completion of reaction, reaction mass was filtered & methylene dichloride was evaporated. Residual Mass is treated with Methanol (20 gm) , filtered and washed with methanol (10 gm). Final Finished product dried in a hot air oven at 120 ^{o}C. Yield:55%

1H NMR : 4.75 (7H,CH3), 7-9 (6H,Ar-H &1H-NH)
IR [ν, cm-1, KBr]: 1683.93 cm^{-1}: C=O (amide carbonyl),1616.64 cm^{-1}: (N–H bending + aromatic C=C)

(C4) N'-(9H-fluoren-9-ylidene)-2,2-dimethylpropanehydrazide

Hydrazone compound (5 gm, 0.02 mol), Soda ash (2.12 gm ,0.02 mol) was mixed at room temperature in 100 ml methylene dichloride as solvent. Pivaloyl chloride (3.6gm, 0.03 mol) was added dropwise 10-15 min. Reaction mass was allowed to cooked at room temperature for 4-5 hrs. After completion of reaction, reaction mass was filtered & methylene dichloride was evaporated. Residual Mass is treated with Methanol (20 gm) , filtered and washed with methanol (10 gm). Final Finished product dried in a hot air oven at 120 ºC. Yield:67%

1H NMR δ: 4.18 (9H,CH3), 7-9 (6H,Ar-H &1H-NH)
IR [ν, cm-1, KBr]: 1683.93 cm^{-1}: C=O (amide carbonyl),1616.64 cm^{-1}: (N–H bending + aromatic C=C)

(C5) N'-(2,7-dichloro-9H-fluoren-9-ylidene)benzohydrazide

Hydrazone compound (5 gm, 0.02 mol), Soda ash (2.12 gm ,0.02 mol) was mixed at room temperature in 100 ml methylene dichloride as solvent. Benzoyl chloride (4.22 gm, 0.03 mol) was added dropwise 10-15 min. Reaction mass was allowed to cooked at room temperature for 4-5 hrs. After completion of reaction, reaction mass was filtered & methylene dichloride was evaporated. Residual Mass is treated with Methanol (20 gm) , filtered and washed with methanol (10 gm). Final Finished product dried in a hot air oven at 120 ºC. Yield:53%

1H NMR δ: 7-9 (11H,Ar-H &1H-NH)
IR [ν, cm-1, KBr]: 1724 cm^{-1}: C=O (amide carbonyl),1626.12cm^{-1}: (N–H bending + aromatic C=C) 761.01 cm^{-1}: C–Cl stretch

(C6) N'-(2,7-dichloro-9H-fluoren-9-ylidene)undecanehydrazide

Hydrazone compound (5 gm, 0.02 mol), Soda ash (2.12 gm ,0.02 mol) was mixed at room temperature in 100 ml methylene dichloride as solvent. Decanoyl chloride (5.72 gm, 0.03 mol) was added dropwise 10-15 min. Reaction mass was allowed to cooked at room temperature for 4-5 hrs. After completion of reaction, reaction mass was filtered & methylene dichloride was evaporated. Residual Mass is treated with Methanol (20 gm) , filtered and washed with methanol (10 gm). Final Finished product dried in a hot air oven at 120 ºC. Yield:53%

1H NMR δ: 0.8-0.9 (3H, CH3),0.9.-1.62(16H,CH2) 7-9 (6H,Ar-H) 9.79(1H-NH)
IR [ν, cm-1, KBr]: 1719 cm^{-1}: C=O (amide carbonyl),1597 cm^{-1}: (N–H bending + aromatic C=C) 757 cm^{-1}: C–Cl stretch

II. INVITRO ANTI-BACTERIAL & ANTI- FUNGAL

The antimicrobial evaluations of the synthesized hydrazide derivatives (C1–C6) were performed at Microcare Laboratory & Tuberculosis Research Centre, Surat, Gujarat, India, using the broth dilution method, a classical, non-automated in vitro bacterial susceptibility assay. This method quantifies the minimum concentration of an antimicrobial agent required to inhibit the visible growth of microbial strains and is commonly conducted using serial dilutions in test tubes.

Procedure

A stock solution of each synthesized compound was prepared at a concentration of 2000 µg/mL. In the primary screening, three concentrations (1000 µg/mL, 500 µg/mL, and 250 µg/mL) were tested against the selected microbial strains. Compounds showing activity at these levels were then selected for secondary screening, in which further serial dilutions were prepared to obtain concentrations of 200, 100, 50, 25, 12.5, and 6.25 µg/mL. Bacterial suspensions were standardized to a 0.5 McFarland standard, corresponding to approximately 1.5×10^8 CFU/mL, and incubated with test compounds under sterile conditions. The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration of the compound that resulted in at least 99% inhibition of bacterial growth, as compared with the control tube. MIC values were determined based on the absence of visible turbidity or microbial growth in the dilution tubes following overnight incubation at 37 °C. Control tubes (without any antibiotic) were sub-cultured on appropriate agar media for all test organisms. Chloramphenicol (6.25–1000 µg/mL) served as the positive control for antibacterial studies, while Griseofulvin (6.25–1000 µg/mL) was used as the positive control for antifungal testing. Sterile double-distilled water served as the negative control in both assays. For antifungal assays, a fungal spore suspension containing 1×10^6 spores/mL was used. The fungal suspensions were treated with each compound across the specified concentration range and incubated for 24 h at 28 ± 2 °C. MIC values were determined in the same manner as the antibacterial evaluation, based on the absence of visible fungal growth when compared to control samples.This method ensures reproducible, quantitative data for evaluating the antimicrobial potential of newly synthesized compounds under standardized in vitro conditions.

III. Result & Conclusion

The formation of hydrazide derivatives was successfully achieved via a condensation reaction between 2,7-dichloro fluorenone hydrazine and various acid chlorides under mild reaction conditions (Fig. 1). The reaction proceeds through a nucleophilic attack by the terminal nitrogen of the hydrazine moiety on the electrophilic carbonyl carbon of the acid chloride, forming a stable C=N–NH–CO– linkage [9,10]. The optimized conditions using acetic acid as a catalyst in methanol provided moderate to excellent yields and clean conversion, with no significant byproduct formation observed [14].

The synthesized hydrazide derivatives (C1–C6) were isolated as yellow to orange solids. All compounds were characterized using FT-IR and ^1H NMR spectroscopy. The spectral data were found to be consistent with the expected structures [15]. FT-IR spectra revealed characteristic amide carbonyl C=O stretching in the range of 1665–1724 cm^{-1}, along with peaks corresponding to N–H bending and aromatic C=C stretching around 1610–1625 cm^{-1}. Additional absorption bands near 750 cm^{-1} confirmed the presence of C–Cl bonds [16].1H NMR spectra (recorded at 600 MHz in $CDCl_3$) showed signals for aromatic protons between δ 7.0–9.0 ppm, while aliphatic protons were observed in the range of δ 0.8–4.0 ppm [17]. Individual compound assignments are as follows

C1: δ 2.1–2.3 ppm (CH_3); IR: 3200–3300 cm^{-1} (N–H), ~1650 cm^{-1} (C=O), ~1590 cm^{-1} (C=N).
C2: δ 4.0–4.5 ppm (CH_2); IR: ~1590 cm^{-1} (C=N), 760–800 cm^{-1} (C–Cl).
C3: Septet at δ 4.5–5.0 ppm; IR: ~1590 cm^{-1} (C=N), ~1650 cm^{-1} (C=O).
C4: δ 1.2–1.4 ppm (CH_3, 9H); IR: ~1590 cm^{-1} (C=N), ~1650 cm^{-1} (C=O).
C5: δ 7.2–8.2 ppm (aromatic); IR: ~1590 cm^{-1} (C=N), ~1650 cm^{-1} (C=O).
C6: δ 1.2–1.6 ppm (CH_2); IR: ~1590 cm^{-1} (C=N), ~1650 cm^{-1} (C=O).

These spectroscopic findings conclusively support the successful formation of the targeted hydrazide derivatives [15–17].

In vitro antimicrobial activities

The in vitro antibacterial activities of the synthesized hydrazide derivatives (C1–C6) were evaluated using the broth dilution method against four bacterial strains: Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus pyogenes. Chloramphenicol and Ciprofloxacin served as standard reference drugs.

The minimum inhibitory concentrations (MICs) are listed in Table 2. Compound C5 demonstrated the highest antibacterial activity, with MICs of 62.5 µg/mL against E. coli and 100 µg/mL against P. aeruginosa and S. aureus, which is comparable to Ciprofloxacin. Compound C6 also showed good activity, particularly against S. pyogenes (MIC = 100 µg/mL). Moderate activity was observed for compounds C1–C4.

These results suggest that structural modifications, particularly the introduction of an additional phenyl ring in the hydrazine moiety (as in C5), may enhance antibacterial activity by facilitating stronger interactions with bacterial enzymes or membrane targets [18]. Further investigation is warranted to elucidate the mechanism of action and to evaluate selectivity, toxicity, and pharmacokinetic profiles for possible therapeutic development.

MINIMAL INHIBITION CONCENTRATION (Micro gm/ml)					
Sr NO	**Code**	**E.COLI**	**P.AERUGINOSA**	**S.AUREUS**	**S.PYOGENUS**
		MTCC 443	**MTCC 1688**	**MTCC 96**	**MT 442**
Std	**Chloramphenicol**	**50**	**50**	**50**	**50**
Std	**Ciprofloxacin**	**25**	**25**	**50**	**50**
1	C1	200	100	200	125
2	C2	125	100	250	200
3	C3	250	200	200	250
4	C4	100	125	250	200
5	C5	62.5	100	100	125
6	C6	125	250	125	100

Table 2: Antibacterial activity of Compounds

In vitro Antifungal activities

The synthesized hydrazide derivatives were also screened for antifungal activity against *Candida albicans* and *Aspergillus niger* using the broth dilution method. Nystatin and Griseofulvin were employed as reference standards.

As shown in Table 2, compounds **C2** and **C3** exhibited the highest antifungal activity against *Candida albicans*, with MIC values of 250 µg/mL—better than the standard drug Griseofulvin (MIC = 500 µg/mL). Compound **C1** displayed moderate antifungal activity comparable to Griseofulvin. All compounds demonstrated relatively poor activity against *Aspergillus niger*, suggesting either intrinsic fungal resistance or insufficient compound efficacy against filamentous species [18].

MINIMAL INHIBITION CONCENTRATION (Micro gm/ml)			
Sr NO	**Code**	**C.ALBICANS**	**A.NIGER**
		MTCC 227	**MTCC 282**
Std	**NYSTANTIN**	**100**	**100**
Std	**GRESEOFULVIN**	**500**	**100**
1	C1	500	500
2	C2	250	1000
3	C3	250	1000
4	C4	1000	500
5	C5	1000	500
6	C6	500	500

Table 3: Antifungal activity of Compounds

The observed antifungal activity, particularly against *Candida albicans*, highlights the influence of hydrazide substitution patterns on bioactivity. Further structure–activity relationship (SAR) studies are needed to optimize antifungal potential.

IV. ACKNOWLEDGMENT

R.A.S. thanks the Chemistry Department of Bhagwan Mahavir University for providing laboratory facilities, resources, and valuable guidance throughout the course of this research. The author also acknowledges the support of faculty members and colleagues whose encouragement and insights contributed to the successful completion of this work.

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V. Supporting Documents

Figure S1: ^{1}H-NMR spectrum of **C1** compound.

Figure S2: FTIR spectrum of **C1** compound.
Figure S3: ^{1}H-NMR spectrum of **C2** compound.
Figure S4: FTIR spectrum of **C2** compound.
Figure S5: ^{1}H-NMR spectrum of **C3** compound.
Figure S6: FTIR spectrum of **C3** compound.
Figure S7: ^{1}H-NMR spectrum of **C4** compound.
Figure S8: FTIR spectrum of **C4** compound.
Figure S9: ^{1}H-NMR spectrum of **C5** compound.
Figure S10: FTIR spectrum of **C5** compound.
Figure S11: ^{1}H-NMR spectrum of **C6** compound.
Figure S12: FTIR spectrum of **C6** compound

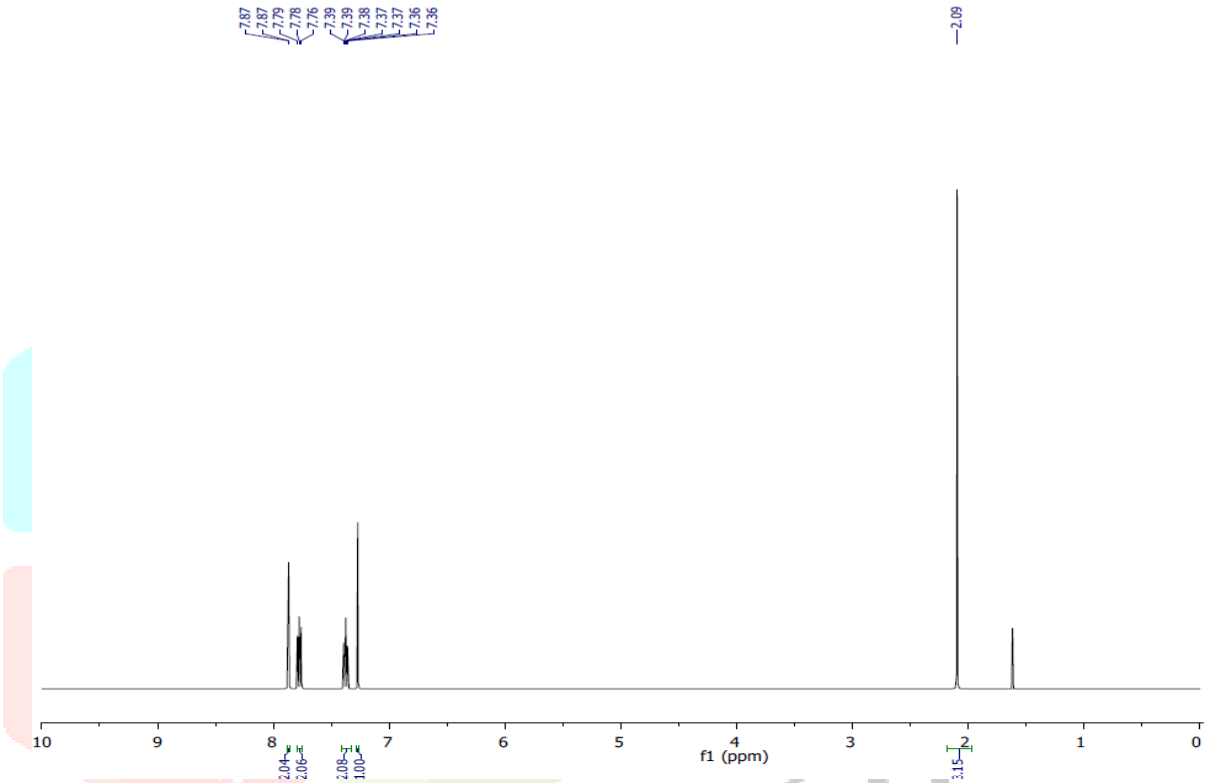

Figure S1 ^{1}H-NMR spectrum of **C1** compound.

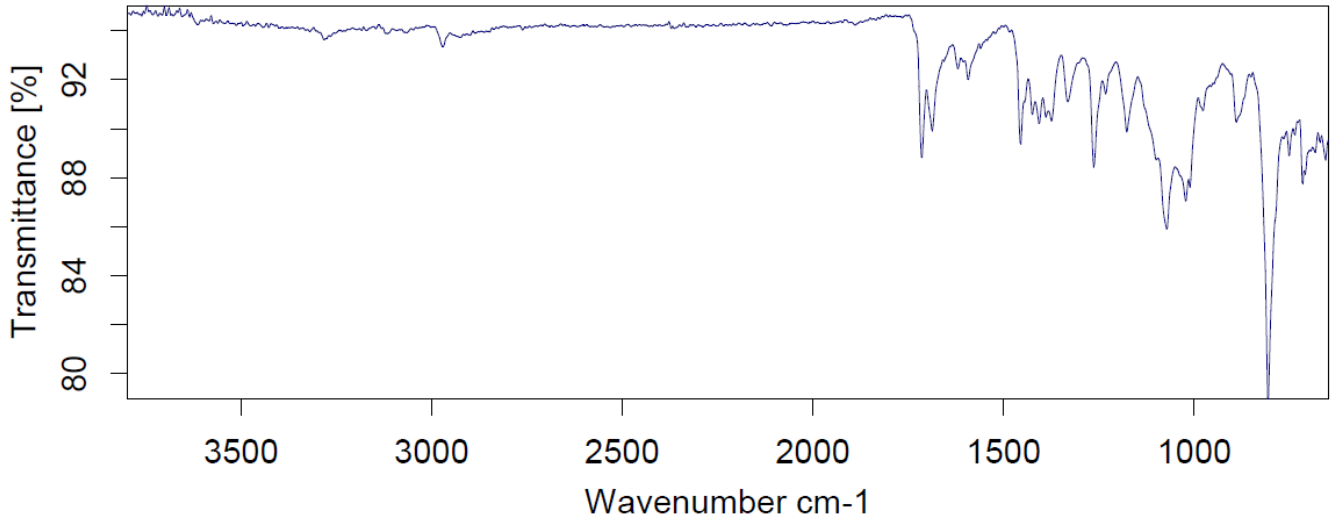

Figure S2: FTIR spectrum of **C1** compound.

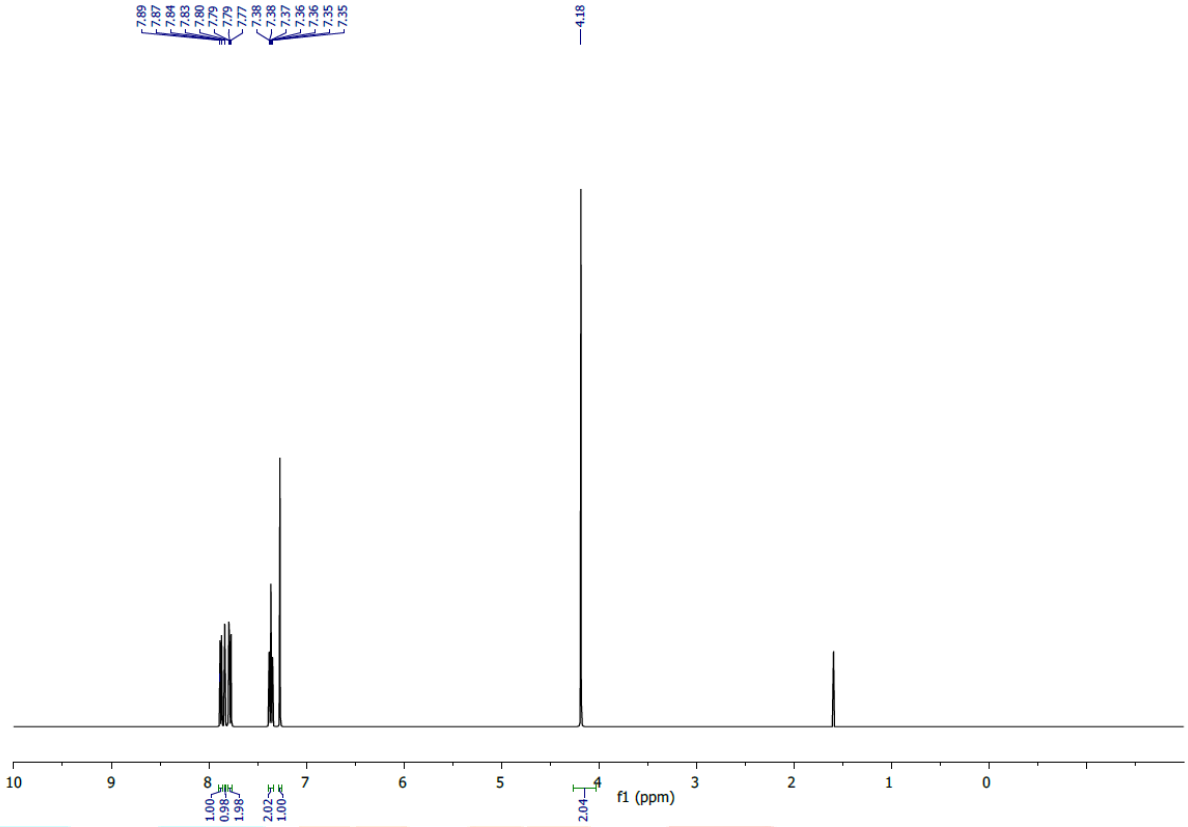

Figure S3: ^{1}H-NMR spectrum of **C2** compound.

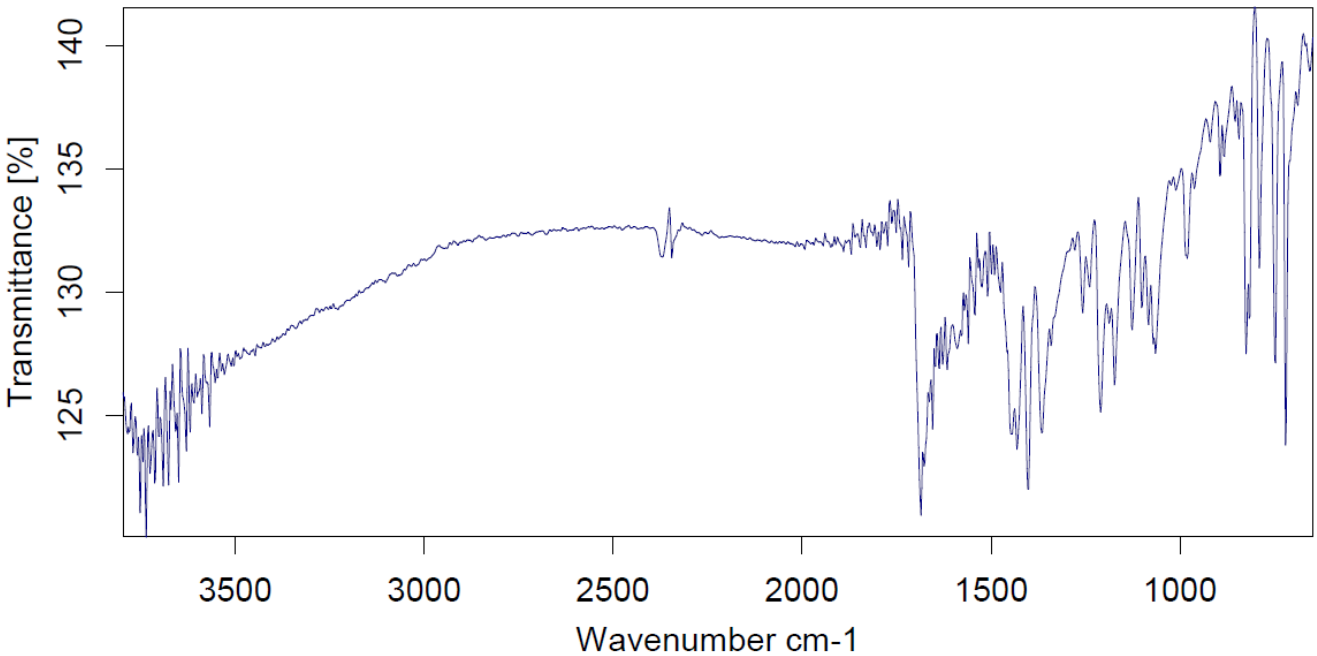

Figure S4: FTIR spectrum of **C2** compound

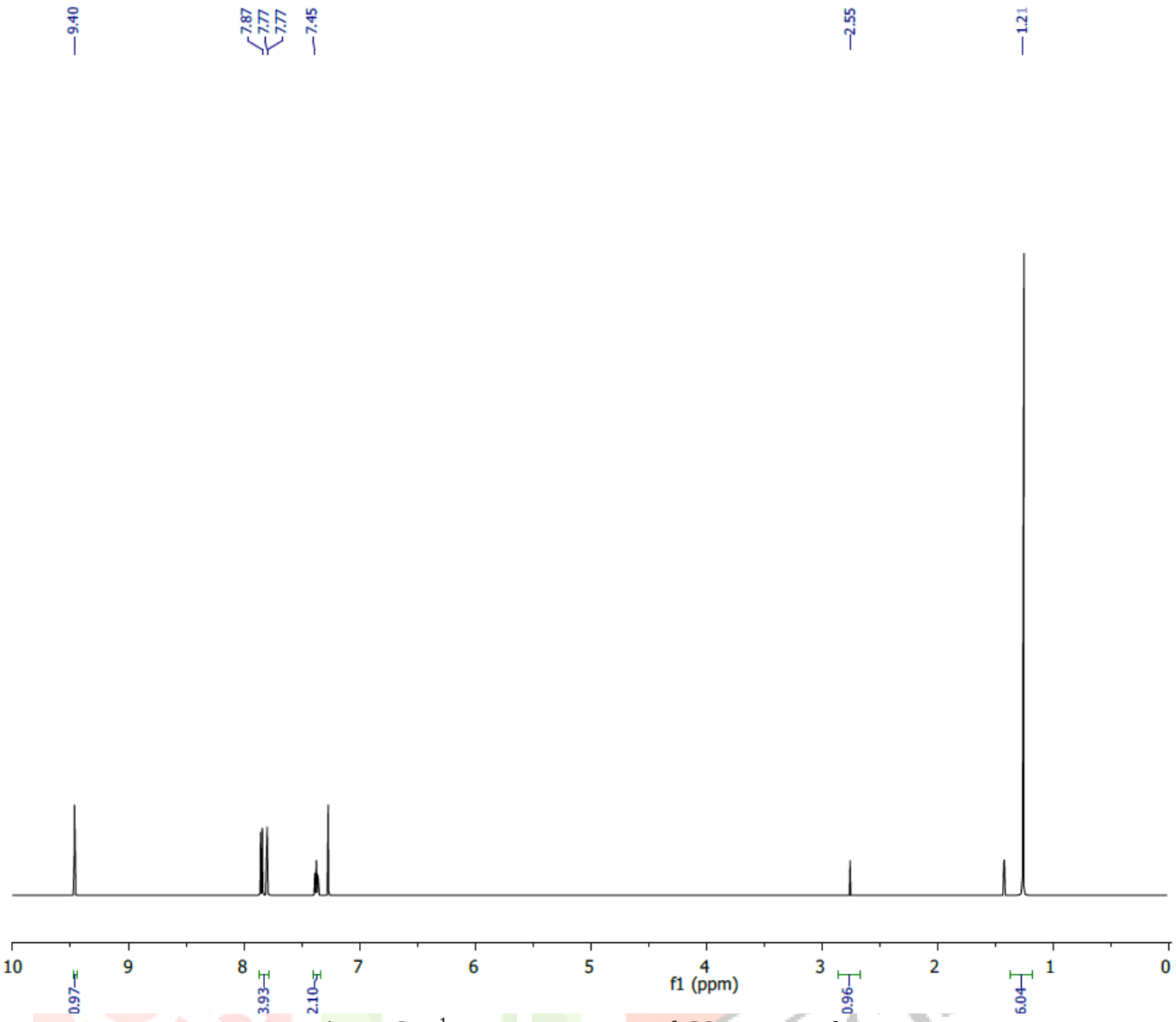

Figure S5: ^{1}H-NMR spectrum of **C3** compound

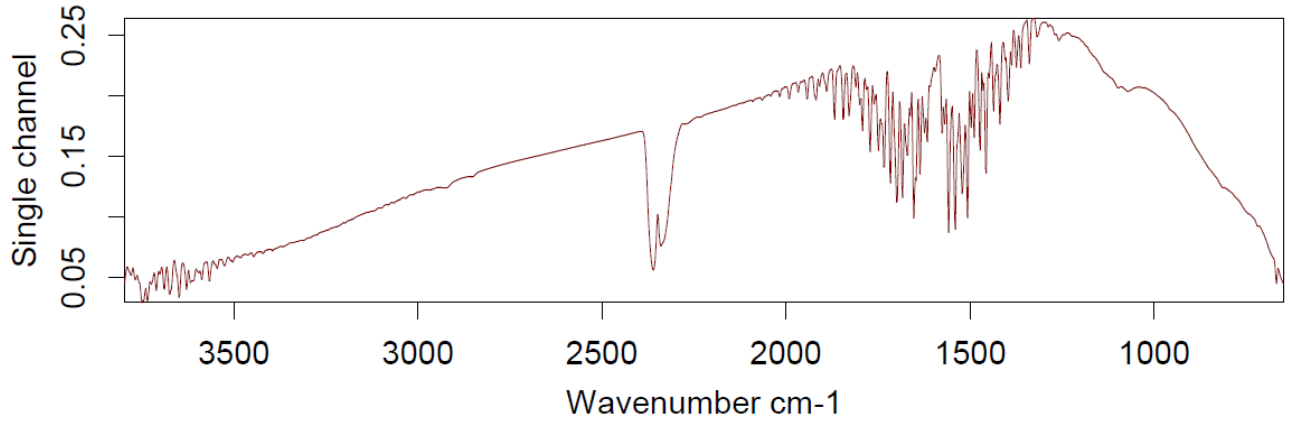

Figure S6: FTIR spectrum of **C3** compound.

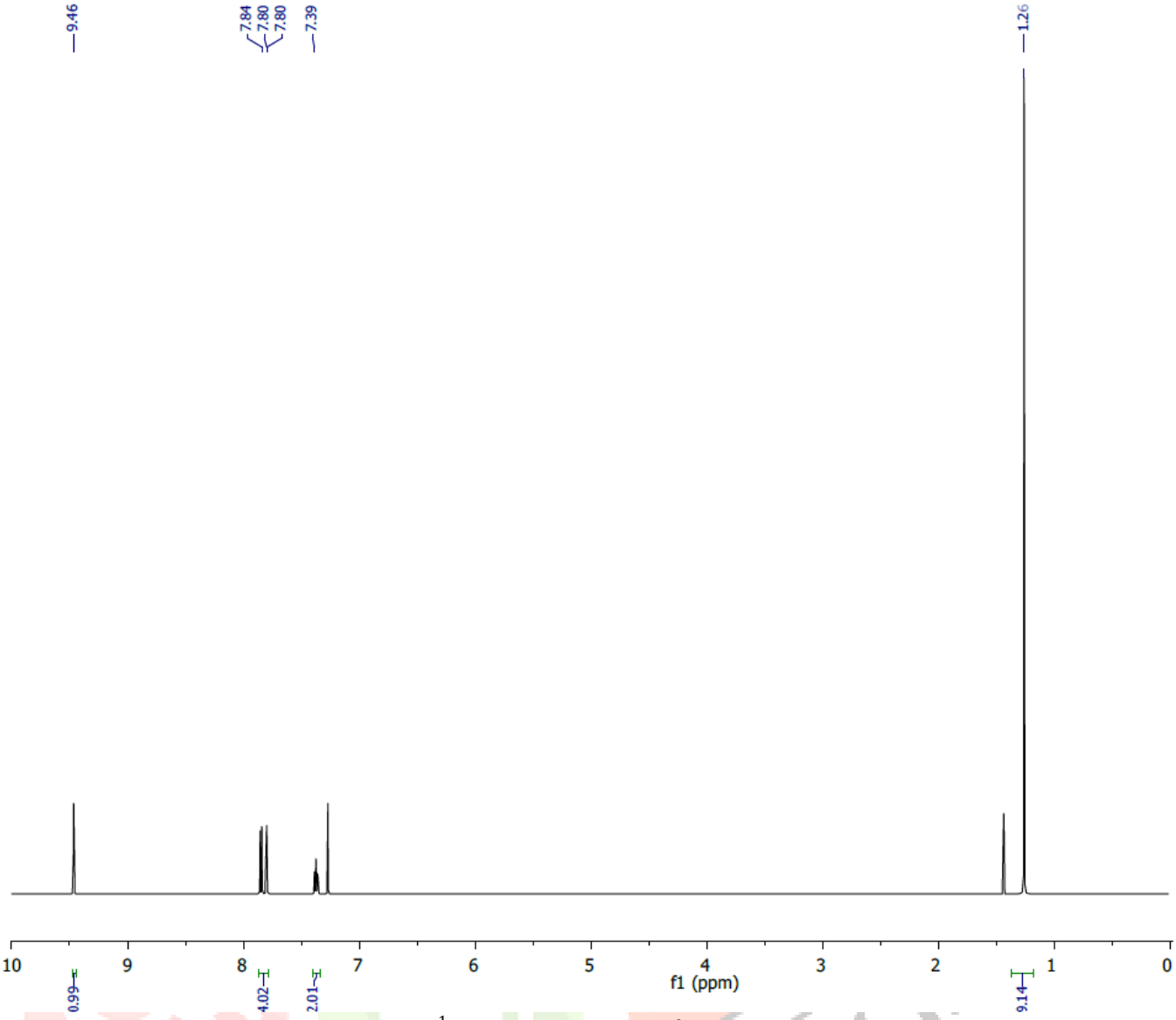

Figure S7: ^{1}H-NMR spectrum of **C4** compound

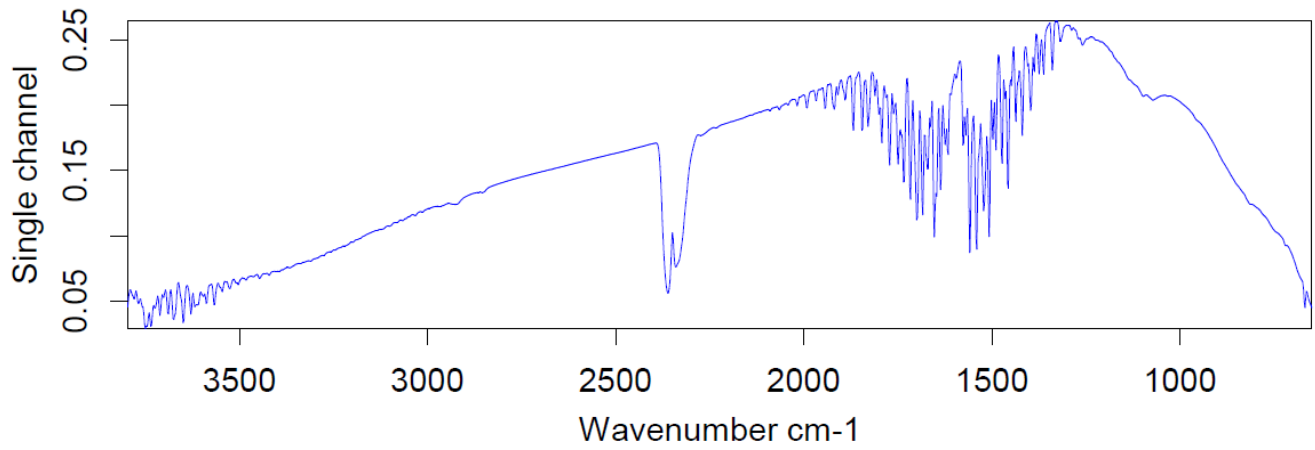

Figure S8: FTIR spectrum of **C4** compound

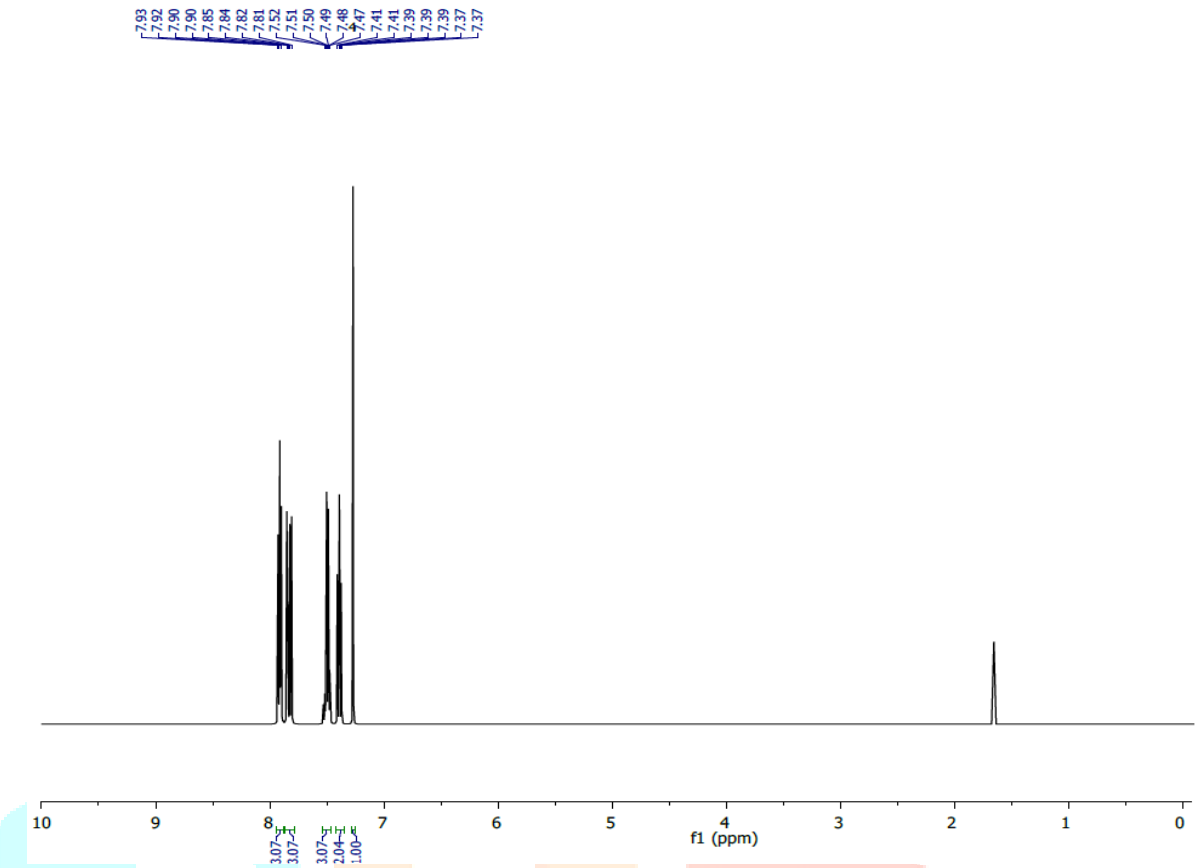

Figure S9: ^{1}H-NMR spectrum of **C5** compound

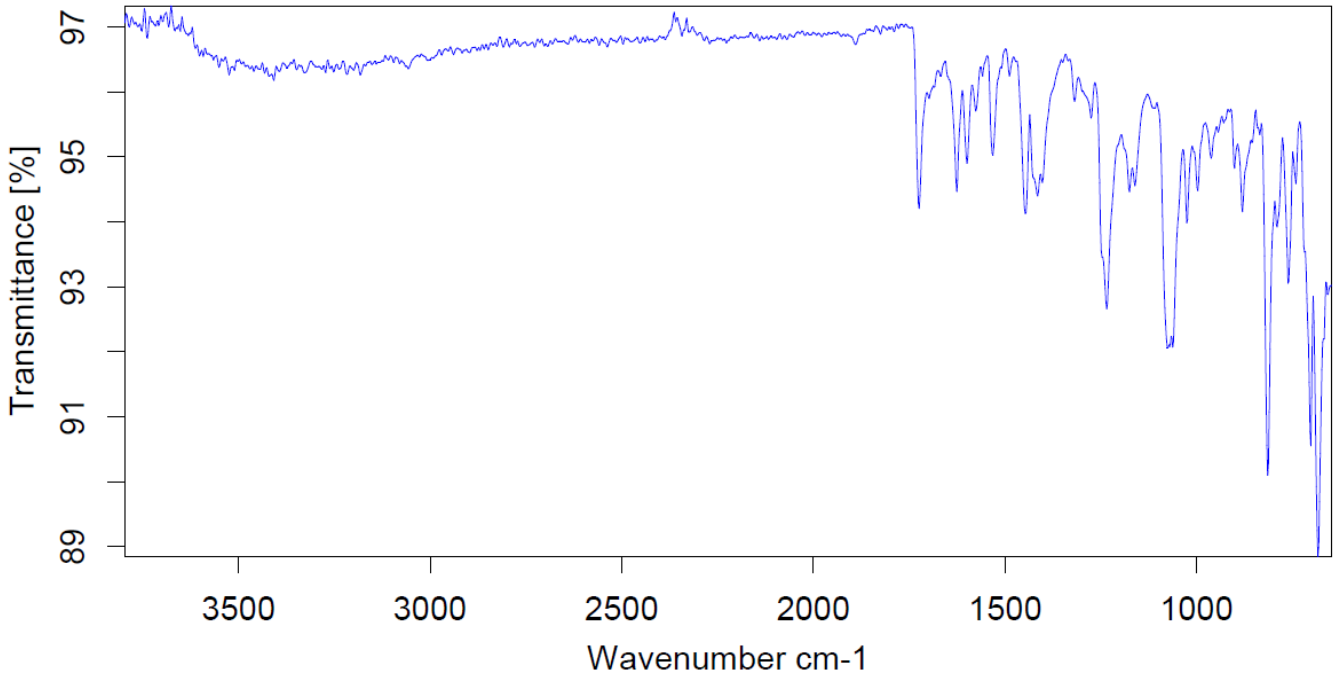

Figure S10: FTIR spectrum of **C5** compound

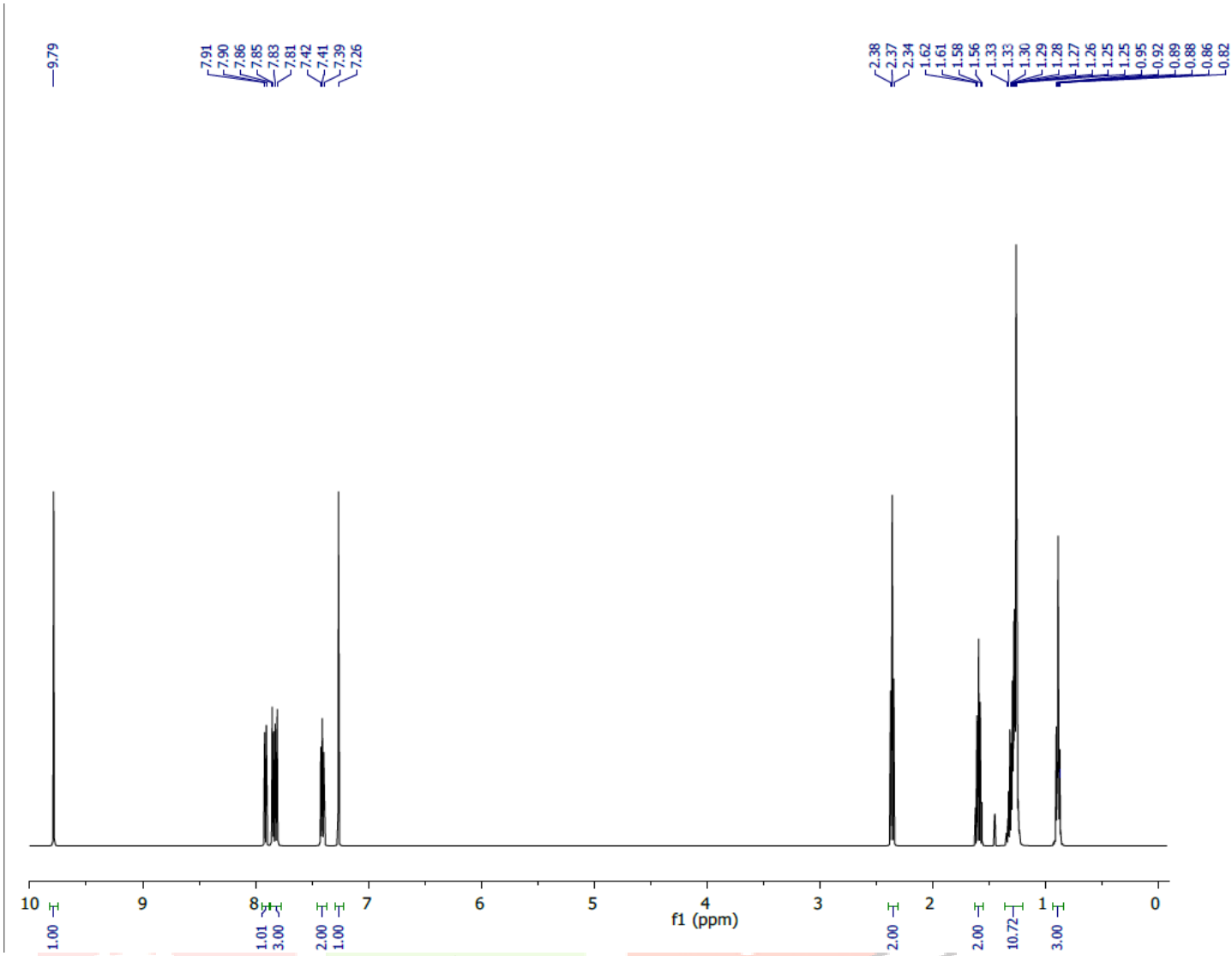

Figure S11: ^{1}H-NMR spectrum of **C6** compound

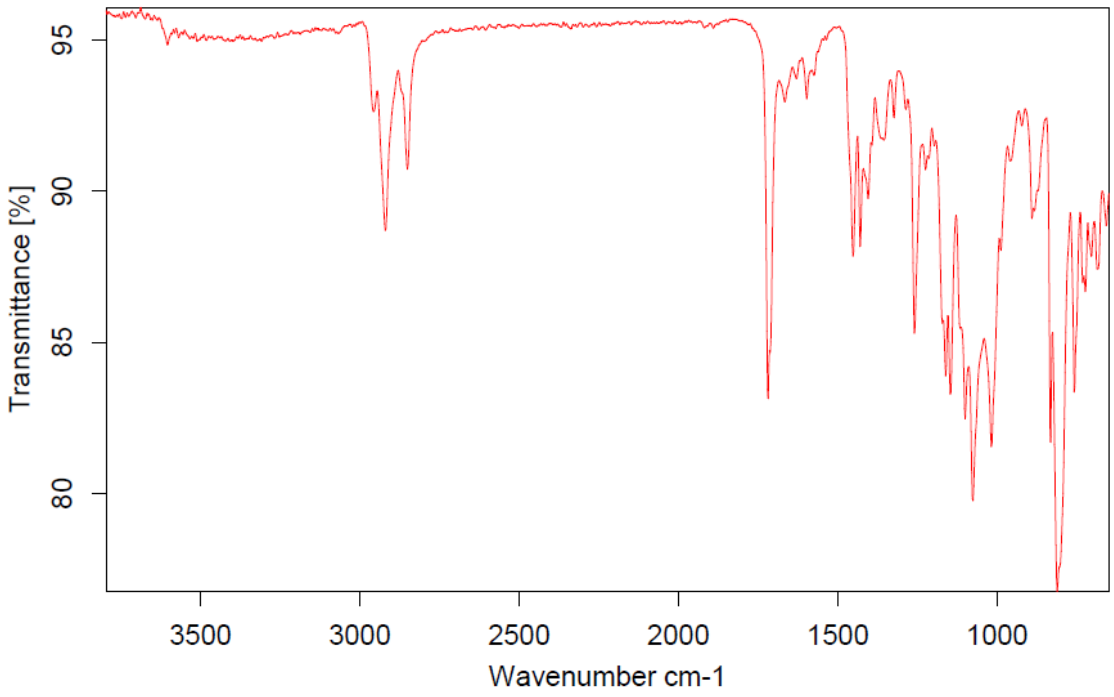

Figure S12: FTIR spectrum of **C6** compound