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Synthesis and Antibacterial Activity of New Indole-Based Hydrazide Hydrazone Derivatives

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P A P E R I N F O

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A b s t r a c t

In this research, six new hydrazone derivatives (ET1-ET6) were synthesized, focusing on two indole-2-carboxylic acid derivatives (5-bromoindole-2-carbhydrazide and indole-2-carbhydrazide) with three different aromatic heterocyclic aldehydes (2-pyrrolecarbaldehyde, 2-imidazole carbaldehyde, and 3-indole carbaldehyde). The new derivatives were synthesized by the reaction of hydrazide with aldehyde in ethanol with reflux to produce the target compounds in good yield and high purity. The structure of the prepared compound was elucidated using FT-IR, H-NMR, and C-NMR, confirming the synthesis of the hydrazones.

The study of anti-bacterial activity of the hydrazone compounds was examined against two pathogenic bacterial strain (S. aureus, and E. coli) using the agar diffusing method. The outcomes of antibacterial activity showed that ET3 demonstrate highest growth inhibition against S. aureus (MIC = 0.348), while ET6 derivative showed the most activity toward E. coli (MIC = 0.595 mM). The differences in activity noted between Gram-positive and Gram-negative bacteria may be due to the variations in cell wall structure. Overall, the incorporation of indole and heterocyclic rings into the hydrazone structure yields promising compounds for further investigation into their antibacterial properties.

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1. INTRODUCTION

Hydrazone derivatives constitute an important and well-studied family of organic compounds assigned to their structural diversity, simple synthesis, and high biological activity (Pai & Bhat, 2026), (Rollas & Küçükgüzel, 2007). The unique azomethine functional group (-C=N-), generated during condensation reactions between hydrazides and carbonyl compounds, is a key element in govern the chemical and biological properties of these molecules (Raczuk et al., 2022) (Popiołek, 2021).Hydrazones are renowned for their diverse pharmacological activity, such as antibacterial, antifungal, antiviral, anti-inflammatory, and anticancer properties (Popiołek, 2017). Furthermore, their ability to act as both hydrogen bond donors and acceptors, along with their structural flexibility, makes them desirable compounds in medicinal chemistry and the synthesis of heterocyclic compounds (Ibraheem et al., 2024) (Sable et al., 2025). The indole nucleus is the most important heterocyclic system in chemical and medicinal chemistry. Indole derivatives are widespread in natural products, alkaloids, and medicinal compounds, where they play a significant role in biological activity (Drăgoi et al., 2026) (Kumar & Ritika, 2020). The indole ring promotes molecule planarity, π-π stacking interactions, and hydrogen bonding with biological targets (Drăgoi et

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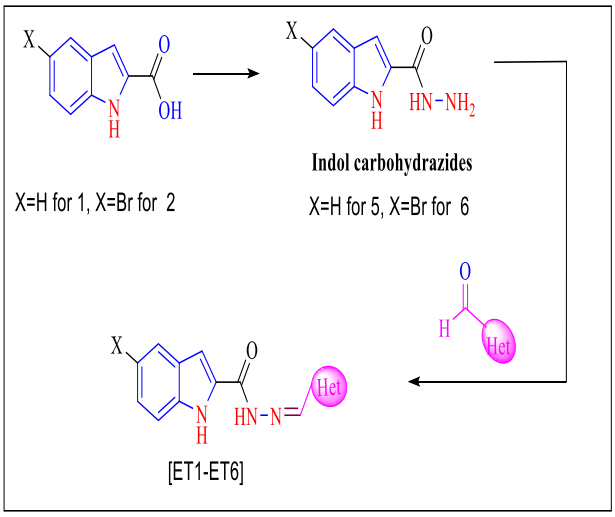
al., 2026) (Yakkala et al., 2025). Indole-carbohydrazide derivative, in particular, is a key synthetic step in the formation of hydrazone derivatives via Schiff base reactions with aldehydes (Mirfazli et al., 2014 (Bhagwat et al., 2025) .Structural changes in this compound, such as the substitution of bromine at the 5-position to form 5-bromoindole -2-carbhydrazide, can modify the molecule's electrical configuration and lipid solubility, potentially affecting its reactivity and biological properties. The potential biological significance of these molecules may be enhanced by combining pyrrole, imidazole, and indole units into a single chemical structure. Accordingly, this study focuses on the synthesis of six novel hydrazone derivatives by condensing two important hydrazide precursors, 5-bromoindole -2-carbhydrazide, and indole-2-carbhydrazide, with three different heterocyclic aldehydes: pyrrole-2-carbaldehyde, imidazole-2-carbaldehyde, and indole-3-carbaldehyde. This synthetic approach yields structurally diverse compounds by combining several nitrogen-containing heterocyclic systems into a single hydrazone structure. This initiative aims to expand the range of indole-based hydrazone derivatives and lay the foundation for future chemical and biological studies.



Scheme 1. Preparation of ET1-ET6 derivatives

2. EXPERIMENTS

2.1. Material methods

The chemicals used were analytical grade and supplied by commercially accessible companies. Imidazole-2-carbaldehyde and pyrrole-2-carbaldehyde were acquired from Leyan, China, whereas 5-bromoindole-2-carboxylic acid and 3-indole carbaldehyde were obtained from Macklin, China. Melting points were calculated using a Gallenkamp MFB-600 Stuart instrument, and FTIR spectra were acquired with a Shimadzu IRAffinity-1S. 1HNMR was performed with a Bruker AC 400 NMR spectrometer tuned to 400 MHz.

2.2. Synthesis of Hydrazones [ET1-ET6]

2.2.1. Synthesis of Ethyl 1H-indole-2-carboxylates (3 and 4) (Boraei et al., 2016)

A solution of 2 mmol of 1H-indole-2-carboxylic acids In1 and In2 in 25 mL ethanol was mixed with 1ml of H2SO4 and agitated at 80 °C for two hours. After TLC monitoring, the reaction was stopped with a saturated $NaHCO_3$ aqueous solution and extracted three times with EtOAc (3 × 30 mL). The mixed organic phase was dried over anhydrous MgSO4 before vacuum suction filtering. The crude product was then chromatographically separated on silica gel (1:5 v/v ethyl acetate/petroleum ether), yielding a white solid for 3 In 95% yield, m.p.= (121-123) ºC, and a light brown for 4 and 98% yield, m.p.= (165-167) ºC.

2.2.2. Synthesis of Indole Carbohydrazides (5, and 6) (Boraei et al., 2016)

In two distinct reactions, Hydrazine hydrate (2.5 mmol) was added dropwise to a solution of indole esters (3, 4), (2 mmol) in 20 mL of EtOH and refluxed for 8 hours. Following this time span, the mixture was allowed to cool to room temperature. The resulting solid product underwent recrystallization in ethanol, yielding pure white crystals of (5) in 96% yeild, m.p = (243-245) ºC. and light brown crystals of (6) in 92% yield, m.p = (222-225) ºC.

2.2.3. General procedure for the Synthesis of Indole-linked Hydrazide-hydrazone (ET1-ET6) (Demir-Yazıcı et al., 2019)

To a solution of indole carbohydrazide (5, and 6), (1 mmol) and heterocyclic aromatic aldehydes in EtOH (10 mL), three drops of AcOH were added and stirred under refluxed conditions for five hours. After completion of the reaction, as confirmed by TLC analysis, the formed precipitate was filtered and washed several times with hot ethanol. The residue obtained was gathered and subsequently refined via recrystallization in ethanol, resulting pure products of hydrazones, (ET1-ET6) which were then characterized using FT-IR, ^{1}H, and ^{13}C NMR spectroscopy.

2.2.3.1. Spectral Data of Indole-linked Hydrazide-hydrazone (ET1-ET6)

N'-((1H-pyrrol-2-yl)methylene)-5-bromo-1H-indole-2-carbohydrazide (ET1)

Off weight, yield = 77 %, m.p. = 248-250 °C., Rf = 0.57 (ethyl acetate, 100%), IR (υ/cm^{-1}): 3382, 3339,

and 3224 (N-H), 3056 (Csp2-H), 1643 (C=O), 1606 (C=N), 1543 (C=C).

^{1}H NMR (300 MHz, DMSO) δ 12.93 (s, 1H), 12.15 (s, 1H), 12.03 (s, 1H), 8.41 (s, 1H), 7.95 (s, 1H), 7.48 – 7.29 (m, 4H), 7.21 (s, 1H). ^{13}C NMR (75 MHz, DMSO) δ 157.27, 141.09, 135.76, 132.26, 129.31, 127.44, 126.64, 124.30, 123.15, 114.81, 114.03, 112.84, 109.84, 102.91, 40.76, 40.48, 40.20, 39.93, 39.65, 39.37, 39.09.

N'-((1H-imidazol-2-yl)methylene)-5-bromo-1H-indole-2-carbohydrazide [ET2]

Yellow, yield = 82 %, m.p. = 315-317 °C., Rf = 0.53 (ethyl acetate, 100%), IR (υ/cm^{-1}): 3301, and 3205 (N-H), 1632 (C=O), 1570 (C=N), 1525 (C=C). ^{1}H NMR (300 MHz, DMSO) δ 12.93 (s, 1H), 12.15 (s, 1H), 12.03 (s, 1H), 8.41 (s, 1H), 7.95 (s, 1H), 7.48 – 7.29 (m, 4H), 7.21 (s, 1H).

^{13}C NMR (75 MHz, DMSO) δ 157.71, 142.86, 139.83, 135.94, 131.73, 130.05, 129.21, 126.99, 124.46, 119.40, 114.89, 112.96, 103.61, 40.75, 40.47, 40.20, 39.92, 39.64, 39.36, 39.08.

N'-((1H-indol-2-yl)methylene)-5-bromo-1H-indole-2-carbohydrazide [ET3]

yellow, yield = 82 %, m.p. = 270-272 °C., Rf = 0.56 (ethyl acetate, 100%), IR (υ/cm^{-1}): 3365, and 3224 (N-H), 1592 (C=O), 1561 (C=N) and (C=C). ^{1}H NMR (300 MHz, DMSO) δ 12.01 (s, 1H), 11.70 (d, J = 13.3 Hz, 2H), 8.68 (s, 1H), 8.34 (d, J = 7.1 Hz, 1H), 7.93 (d, J = 13.1 Hz, 2H), 7.48 (d, J = 9.4 Hz, 2H), 7.42 – 7.30 (m, 2H), 7.29 – 7.13 (m, 2H). ^{13}C NMR (75 MHz, DMSO) δ 157.19, 145.33, 137.54, 135.75, 132.56, 131.03, 129.39, 126.55, 124.80, 124.25, 123.16, 122.47, 120.94, 114.85, 112.82, 112.36, 112.13, 102.78, 56.54, 40.76, 40.48, 40.20, 39.93, 39.65, 39.37, 39.09, 19.05.

N'-((1H-pyrrol-2-yl)methylene)-1H-indole-2-carbohydrazide [ET4]

Off-white, yield = 80 %, m.p. = 230-231°C., Rf = 0.62 (ethyl acetate, 100%), IR (υ/cm^{-1}): 3419, 3335 and 3253 (N-H), 1631 (C=O), 1600 (C=N), 1546 (C=C). ^{1}H NMR (300 MHz, DMSO) δ 11.84 (s, 1H), 11.64 (s, 1H), 11.60 (s, 1H), 8.32 (s, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.47 (d, J = 8.2 Hz, 1H), 7.30 (s, 1H), 7.22 (d, J = 15.1 Hz, 1H), 7.08 (t, J = 7.5 Hz, 1H), 6.95 (s, 1H), 6.54 (s, 1H), 6.18 (s, 1H). ^{13}C NMR (75 MHz, DMSO) δ 157.70, 140.78, 137.18, 130.91, 127.52, 124.09, 123.04, 122.13, 120.39, 113.87, 112.83, 109.81, 103.50, 40.76, 40.48, 40.20, 39.93, 39.65, 39.37, 39.09.

N'-((1H-imidazol-2-yl)methylene)-1H-indole-2-carbohydrazide [ET5]

Off-white, yield = 80 %, m.p. = 298-300 °C., Rf = 0.42 (ethyl acetate, 100%), IR (υ/cm^{-1}): 3321 (N-H), 1634 (C=O), 1573 (C=N), 1526 (C=C). ^{1}H NMR (300 MHz, DMSO) δ 12.96 (s, 1H), 11.96 (s, 1H), 11.94 (s, 1H), 8.43 (s, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.48 (d, J = 8.3 Hz, 1H), 7.34 (s, 1H), 7.31 – 7.12 (m, 3H), 7.07 (t, J = 7.5 Hz, 1H). ^{13}C NMR (75 MHz, DMSO) δ 158.14, 142.96, 139.49, 137.38, 130.43, 127.45, 124.44, 122.30, 120.52, 119.99, 112.91, 104.25, 40.72, 40.44, 40.17, 39.89, 39.61, 39.33, 39.05.

N'-((1H-indol-2-yl)methylene)-1H-indole-2-carbohydrazide [ET6]

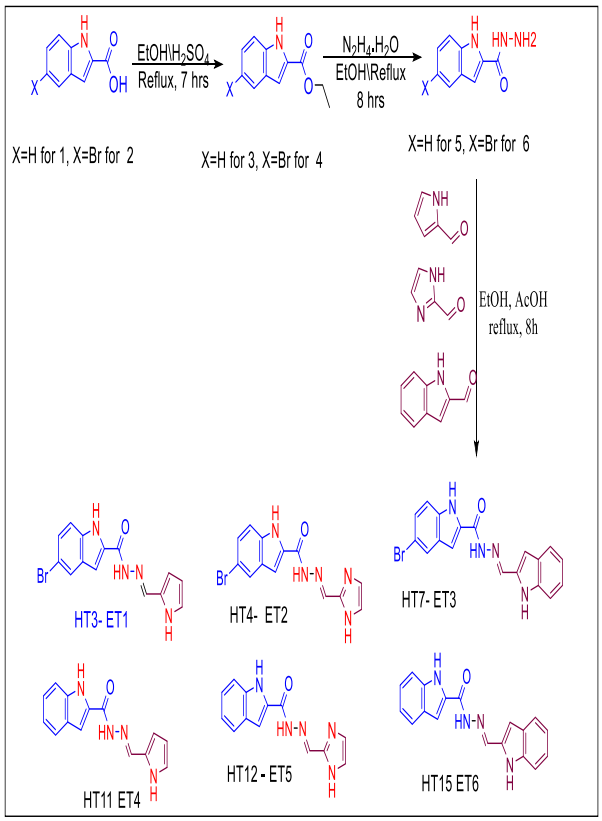
Off-white, yield = 80 %, m.p. = 260 °C. dec., Rf = 0.48 (ethyl acetate, 100%), IR (υ/cm^{-1}): 3384, 3253 (N-H), 1636 (C=O), 1609 (C=N), 1536 (C=C). ^{1}H NMR (300 MHz, DMSO) δ 11.81 (s, 1H), 11.67 (s, 2H), 8.70 (s, 1H), 8.37 (d, J = 7.6 Hz, 1H), 7.90 (d, J = 2.7 Hz, 1H), 7.70 (d, J = 8.0 Hz, 1H), 7.52 (dd, J = 13.2, 7.9 Hz, 2H), 7.36 (d, J = 2.1 Hz, 1H), 7.23 (q, J = 7.9 Hz, 3H), 7.10 (t, J = 7.5 Hz, 1H). ^{13}C NMR (75 MHz, DMSO) δ 157.66, 145.04, 137.56, 137.18, 131.22, 130.89, 127.60, 124.83, 124.02, 123.17, 122.50, 122.11, 120.93, 120.39, 112.88, 112.37, 112.20, 103.40, 40.77, 40.49, 40.21, 39.93, 39.65, 39.38, 39.10.

3. RESULTS AND DISCUSSION

3.1. Chemistry

3.1.1. Preparation of ET1-ET6 derivatives

Scheme 2 depicts the synthesis process for ET1-ET6 derivatives. The products ethyl 1H-indole-2-carboxylates (3,4) were produced by reacting 1H-indole-2-carboxylic acids (1,2) with sulphuric acid in EtOH under reflux conditions. The treatment of ester derivatives (3,4) with hydrazine results in the production of indole carbohydrazides (5,6), which when combined with heterocyclic aromatic aldehydes yields indole-based hydrazide-hydrazones ET1-ET6 derivatives.



Scheme 2. Synthetic pathway of ET1-ET6 derivatives

3.2. Antibacterial study

The MIC technique was used to assess ET1-ET6 antibacterial activities against S. aureus, a Gram-positive bacterium, and E. coli, a Gram-negative bacterium, with Cefixim acting as a reference antibiotic for comparison in DMSO as negative control which shows no inhibtion of bacterial growth. As shown in Table 2, the produced compounds ET1-ET2 were more effective against all tested bacteria. Furthermore, all tested compounds demonstrated stronger antibacterial activity against Gram-positive bacteria than Gram-negative bacteria. ET3 had the best activity against S. aureus (MIC = 0.348 mM), while ET6 had the greatest activity against E. coli (MIC = 0.595 mM).

TABLE 1. Antibacterial assay of the ET1-ET6

Entry	**Sample**	***S. aureus***	***E. coli***
1	**ET1**	1.15	2.43
2	**ET2**	0.715	2.33
3	**ET3**	0.348	1.19
4	**ET4**	0.598	1.31
5	**ET5**	1.315	2.47
6	**ET6**	0.588	0.595
7	Cefixim	0.01	0.02

[a]Values as mM.

4. CONCLUSION

A novel series of ET1-ET6 was created, which was derivatives by preparing indole-containing hydrazines. Then, they reacted with heterocyclic aromatic aldehydes to produce the matching indole-hydrazide-hydrazones. The FTIR, 1H, and 13C NMR spectroscopy were used to confirm the structure of the produced ET1-ET6 derivatives. The antibacterial properties of the studied ET1-ET6 were tested against S. aureus and E. coli using the MIC technique. Compound ET3 had the highest activity against S. aureus (MIC = 0.348 mM), whereas ET6 was most effective against E. coli (MIC = 0.595 mM). In the ongoing heterocyclic chemistry study, attention is payed on novel ways for synthesizing increasingly complex compounds with biological functions.

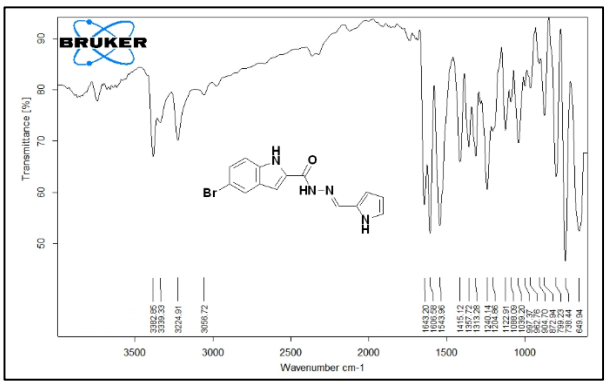


Fig.1: FT-IR spectrum Of ET1

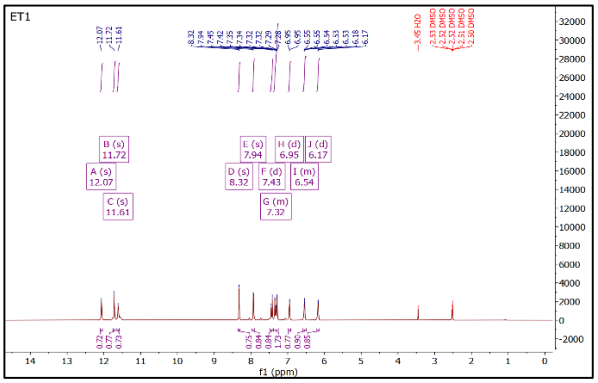


Figure 2: ^{1}H-NMR spectrum Of ET1

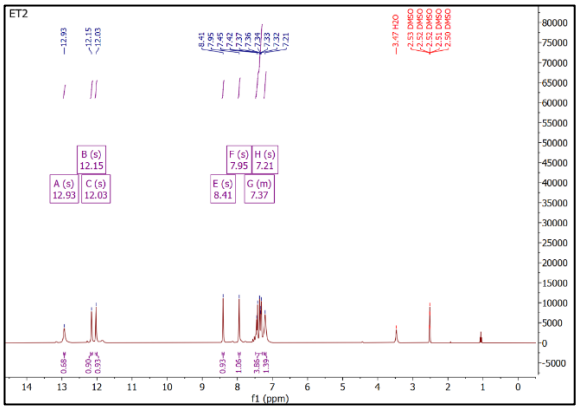


Figure 5: ^{1}H-NMR spectrum Of ET2

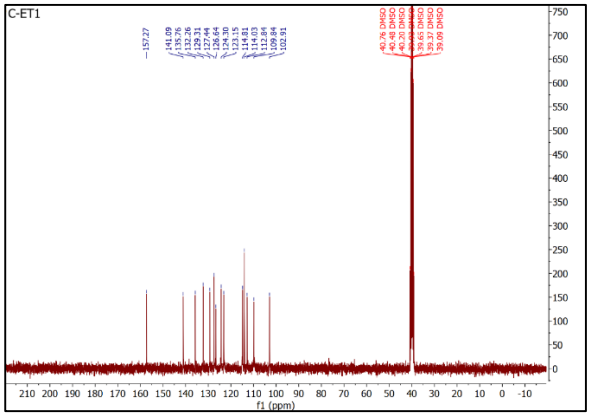


Figure 3: ^{13}C-NMR spectrum Of ET1

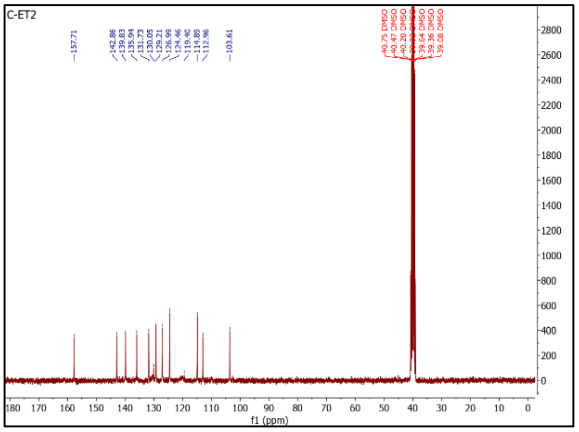


Figure 6: ^{13}C-NMR spectrum Of ET2

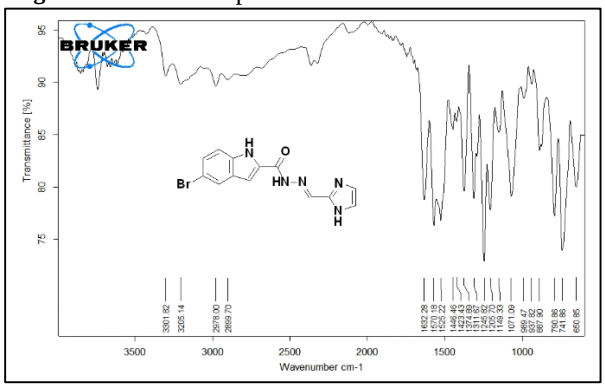


Figure 4: FT-IR spectrum Of ET2

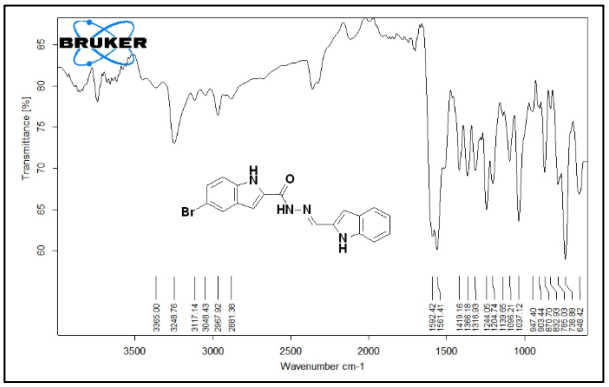


Fig.7: FT-IR spectrum Of ET3

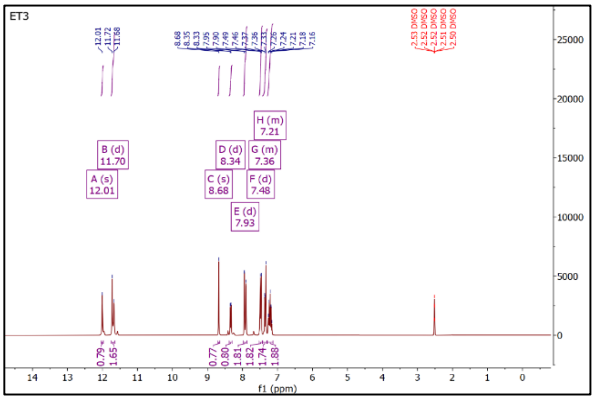


Figure 8: ^{1}H-NMR spectrum Of ET3

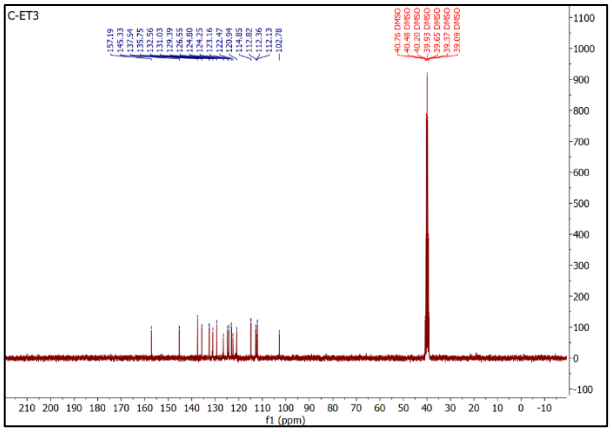


Figure 9: ^{13}C-NMR spectrum Of ET3

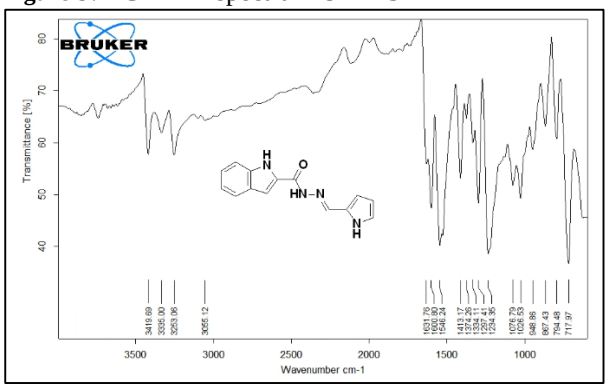


Figure 10: FT-IR spectrum Of ET4

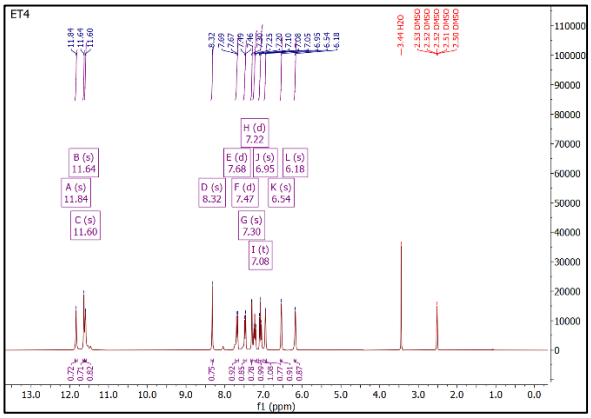


Figure 11: ^{1}H-NMR spectrum Of ET4

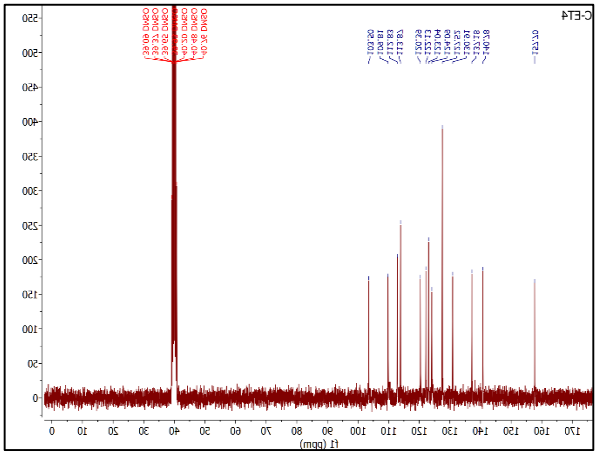


Figure 12: ^{13}C-NMR spectrum Of ET4

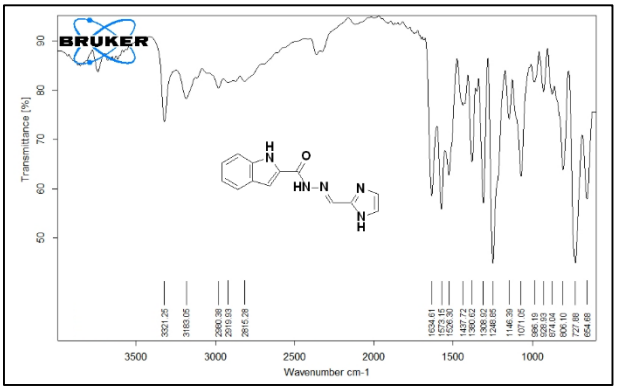


Figure 13: FT-IR spectrum Of ET5

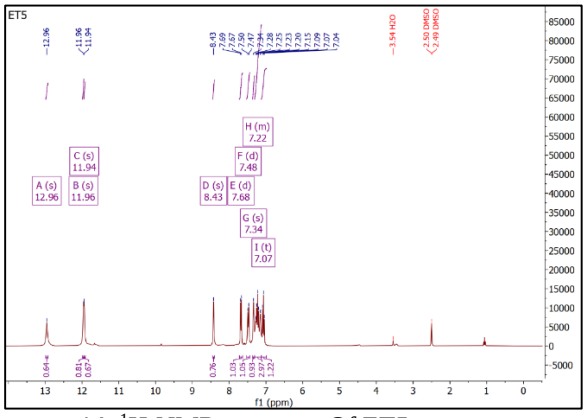


Figure 14: ^{1}H-NMR spectrum Of ET5

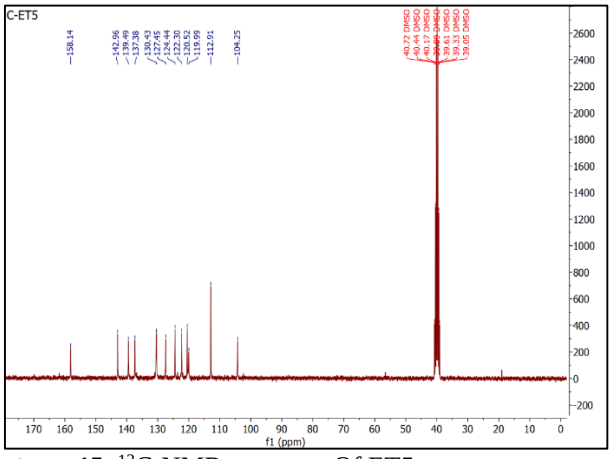


Figure 15: ^{13}C-NMR spectrum Of ET5

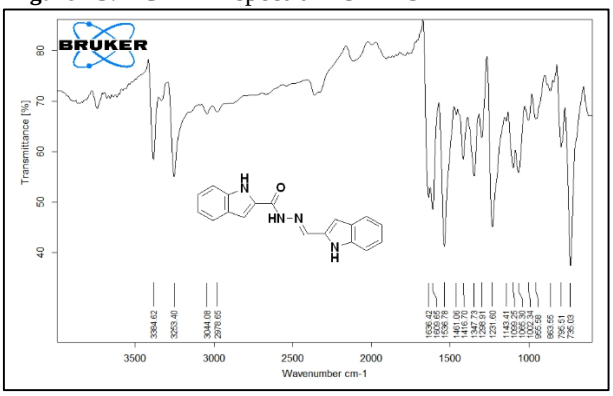


Figure 16: FT-IR spectrum Of ET6

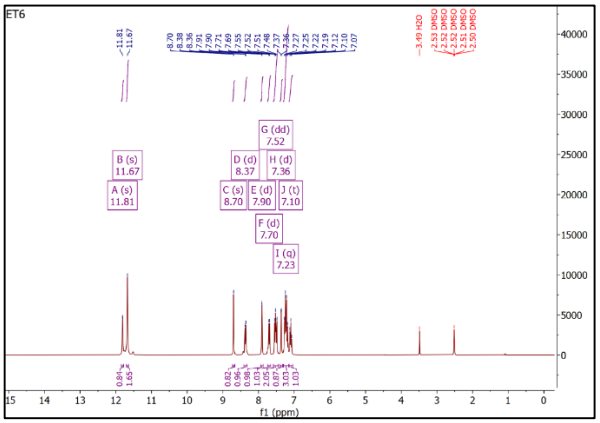


Figure 17: ^{1}H-NMR spectrum Of ET6

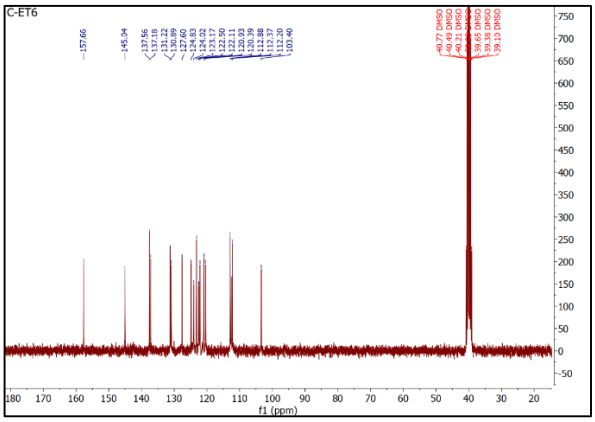


Figure 18: ^{13}C-NMR spectrum Of ET6

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