UNIVERSITY OF KWAZULU-NATAL

DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF ANTIMICROBIAL AGENTS: BROMOPYRROLE-CINNAMALDEHYDE HYBRIDS

By

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A thesis submitted to the School of Health Science, Discipline of Pharmaceutical science, Department of Pharmaceutical Chemistry, University of KwaZulu-Natal, Westville, for the degree of Master's Degree

This thesis has been prepared according to **Format 4** (Thesis by publications) as outlined in the guidelines of College of Health Sciences, University of KwaZulu-Natal. The chapters consist of an overall of 5 chapters which includes: Thesis overall, introduction; experimental; result and discussion and Conclusion

As the candidate's supervisor, I have approved this thesis for examination/submission.

Supervisor: _____ Signed: _____

ABSTRACT

Antimicrobial resistance threatens the effective prevention and treatment of an increasing range of Infections. The ongoing discovery of newer antimicrobial resistance has been a driving energy in the design, synthesis and development of newer antimicrobial agents. As a contribution to these efforts, we synthesized novel Bromopyrrole-Cinnamaldehyde Hybrids compounds. A series of fifteen Bromopyrrole-Cinnamaldehyde Hybrids molecules were synthesized by molecular hybridization approach. In vitro anti-mycobacterial activity of synthesized compounds was evaluated against Mycobacterium tuberculosis H37Rv strain. Among the series, **4**(**b-e**) exhibited activity (MIC >20 μM; IC_{50} = >20 μM) furthermore the sythesised hybrids displayed promising activity against tested fungal strains, in particular for clinical isolate of C. neoformans with MIC values ranging from 12.5 – 25 μg/mL. All synthesized compounds were confirmed by melting point, FT-IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy. The yield of these compounds obtained ranged from 40% to 80%.

DECLARATION 1: PLAGIARISM

I, **Andile Princess Dlungele** declare that

i. The research reported in this dissertation, except where otherwise indicated, is my original work.

ii. This dissertation has not been submitted for any degree or examination at any other university.

iii. This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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LIST OF ABBREVIATIONS

°C	Degrees Celsius
C NMR	Carbon-13 nuclear magnetic resonance spectroscopy
^{1}H NMR	Proton nuclear magnetic resonance spectroscopy
DCM	Dichloromethane
DMSO	Dimethyl sulfoxide
d	Doublet
EtOAc	Ethyl acetate
EtOH	Ethanol
FT-IR	Fourier transform infrared spectroscopy
Hz	Hertz
m.p.	Melting point
MIC	Minimum inhibitory
min	Minutes
m	Multiplet
RBF	Round bottom flask
S	Singlet
TLC	Thin layer chromatography
t	Triplet
UV-VIS	Ultraviolet-visible spectroscopy

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Thesis overview

CHAPTER 1

1.1. BACKGROUND AND RATIONAL OF THIS STUDY

This work is an effort towards understanding Bromopyrrole-Cinnamaldehyde hybrids in drug discovery and its's antitubercular and antimicrobial activity. TB is a communicable disease caused by the bacillus Mycobacterium tuberculosis. It's known to primarily affect the lungs (pulmonary TB). There are five closely related mycobacteria responsible for tuberculosis: *Mycobacterium. Tuberculosis, Mycobacterium bovis, Mycobacterium africanum, Mycobacterium microt* and *Mycobacterium. Canet. M. tuberculosis*, by far the commonest, is transmitted between humans through the airborne route. [1–5]

A study by Clare V. Smith et al allude to the fact that among the main obstacles to the global control of the disease are emerging multi-drug resistant strains and the recalcitrance of persistent infections to treatment with conventional anti-TB drugs and studies reviewing the recent developments to understanding some of the pathways involved in a persistent infection and pathogenesis of Mycobacterium tuberculosis, reveals the need for development of structure-based drug design as the current TB drug are said to have major adverse reactions that can cause significant morbidity and compromise treatment regimen for TB.[6–9]

Bromopyrroles and cinnamaldehyde respectively are natural occurring products reported as having important synthetic and biological activities. Bromopyrrole derivatives has potent antibacterial and antifungal activity, a study by. Akbar Idhayadhulla et al concludes that synthesized pyrrole compounds screened against *E. coli* and *S. aureus* for antibacterial activity, as well as against *A. Niger* and *C. albicans* for antifungal activity had higher or equal potency to the reference compounds Ciprofloxacin and Clotrimazole. Cinnamaldehyde on the other hand showed significant inhibitory activity against the microbes, using streptomycin as a standard and this study concludes that the synthesized compound shows excellent antibacterial activity especial against Staphylococcus aureus. Activity against only *Escherichia coli* and *Pseudomonas aeraginosa* the synthesized compounds exhibit significant antioxidant activity using ascorbic Acid as a standard.[9–13]

Inspired by the evidence from the literature review above this thesis is directed at investigating the anti-tubercular and antimicrobial activity potential when combining Bromopyrroles and cinnamaldehyde to form novel Bromopyrroles – cinnamaldehyde hybrids.

1.2. AIMS OF THIS STUDY

1.2.1 To synthesize Bromopyrrole-Cinnamaldehyde hybrids

1.2.2 To evaluate the synthesized compounds against anti- microbial and anti-tuberculosis strains

1.3. OBJECTIVES OF THIS STUDY

1.3.1 To synthesize Bromopyrrole-Cinnamaldehyde hybrids by molecular hybridization process

1.3.2 To carry out anti-tubercular testing against *H37Rv*

1.3.3 To carry out antimicrobial testing against a panel of gram-negative, gram-positive bacteria and fungal strains employing a minimum inhibitory concentration (MIC) technique

1.4. OVERVIEW OF THIS THESIS

This has 5 chapters, outlined as follows:

Chapter 1: This chapter addresses the background, aim and objectives, structure of the thesis.

Chapter 2: Provides a historic background of antimicrobials followed by their mechanism of action and resistant, a brief history on tuberculosis and its resistant and the current drug treatment and justification of research

Chapter 3: This chapter provides experimental data, description of different spectroscopic techniques employed to confirm the formation of the desired novel target compounds and their schematic representation. The chapter also displays a concise synthetic strategy and mechanisms of the entire reaction leading to the formation of the novel compounds. Procedures or assay methods employed to evaluate the biological activity have also been explained in this chapter.

Chapter 4: This chapter highlights the results obtained during the course of the research and their discussion. Physio-chemical properties of the synthesized derivatives such as appearance, molecular weight and percentage yield observed have been reported. The chapter also focuses on spectroscopic characterization data to verify the formation of the compounds and lists some examples of spectra obtained with respect to synthesized compounds.

Chapter 5: This chapter focuses on general concluding remarks and future scope of the research work which includes building more library of novel compounds for Bromopyrrole-Cinnamaldehyde hybrids.

Appendix: This section reflects all the NMR and IR spectra obtained for all synthesized derivatives during the course of the research work

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Chapter 2
Introduction

2.1. Review on Antimicrobial Agents:

An antimicrobial is an agent that eradicates or impedes the growth of microbes with minimal or no harm to the host. Antimicrobials are classified based on their activity, effect on microorganisms and mode of action. For instance, agents that act against bacterial are called antibacterial, viruses (antiviral), fungi (antifungal) and protozoa (anti protozoal). [1][2]

Historically, antimicrobials began with the observation made by Pasteur and Koch, who discovered that a particular type of bacteria prevented the growth of the other bacterial. At the time of discovery, they were unable to establish a tangible evidence of the plausible mechanism of action. In the 18th century, a blue-green pigment which was a by-product from culture of Bacillus *pyocyaneus* (*Pseudomonas aeruginosa*) became the first recorded microbial agent to demonstrate antimicrobial activity, but its instability and inherent toxicity in patients resulted in a decline in its usage.[3] Before the 20th century it became evident that the microorganisms were liable for the cause of various microbial infections, consequently this led to an exciting race of discovery of antimicrobial agents. [3] The first chemical compound to have shown proficiency in treatment microbial disease in humans notably syphilis was Salvarsan **(1)** discovered by Paul Ehrlich in 1910. In September 1928, a Scottish scientist called Alexander Fleming discovered that mold (a fungus from the *penenicillium genus*) impeded the growth of *staphylococcus aureus*. The chemical entry was called penicillin **(2)**. It was however only in the 1940's when Florey and Chain made this penicillin available as a therapeutic agent. [4]

In 1935, Domagk's and co-workers developed the first sulfonamide compound as an antimicrobial agent, a synthetic red dye prontosil **(3)** that contains a sulfonamide group. Streptomycin **(4)** was then introduced in 1944; an aminoglycoside antibiotic obtained from the soil bacterium *Streptomyces griseus*, for the treatment of tuberculosis mutant strains of *Mycobacterium tuberculosis*. This was followed by the discovery of Chloramphenicol **(5)** in 1946 from a soil. [5]

Antibiotic Chemical Classification:

Figure 1 Depicts an overview for antibiotic classification, their bactericidal and bacteriostatic action

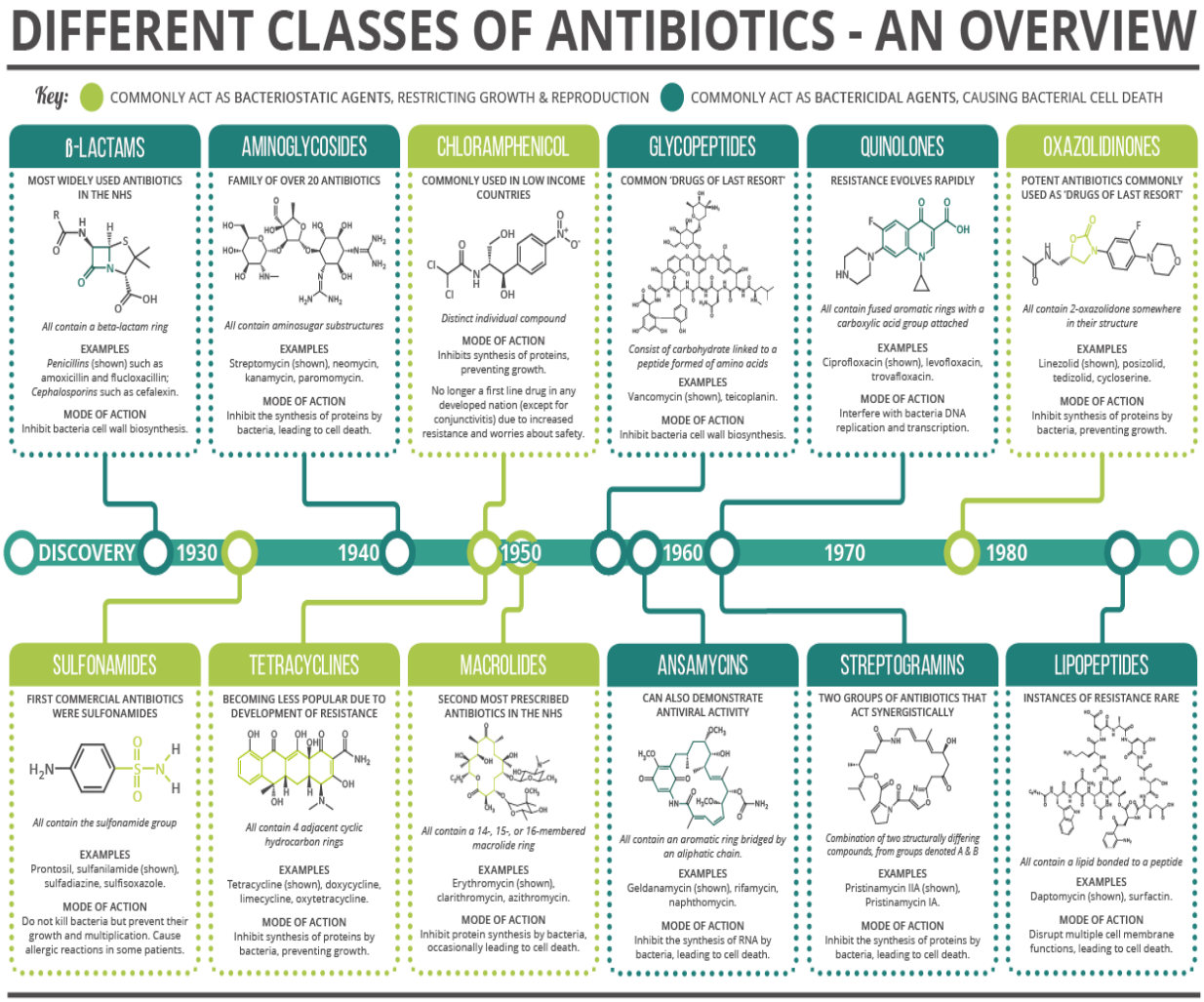


Figure 1: A Brief Overview of Classes of Antibiotics [78]

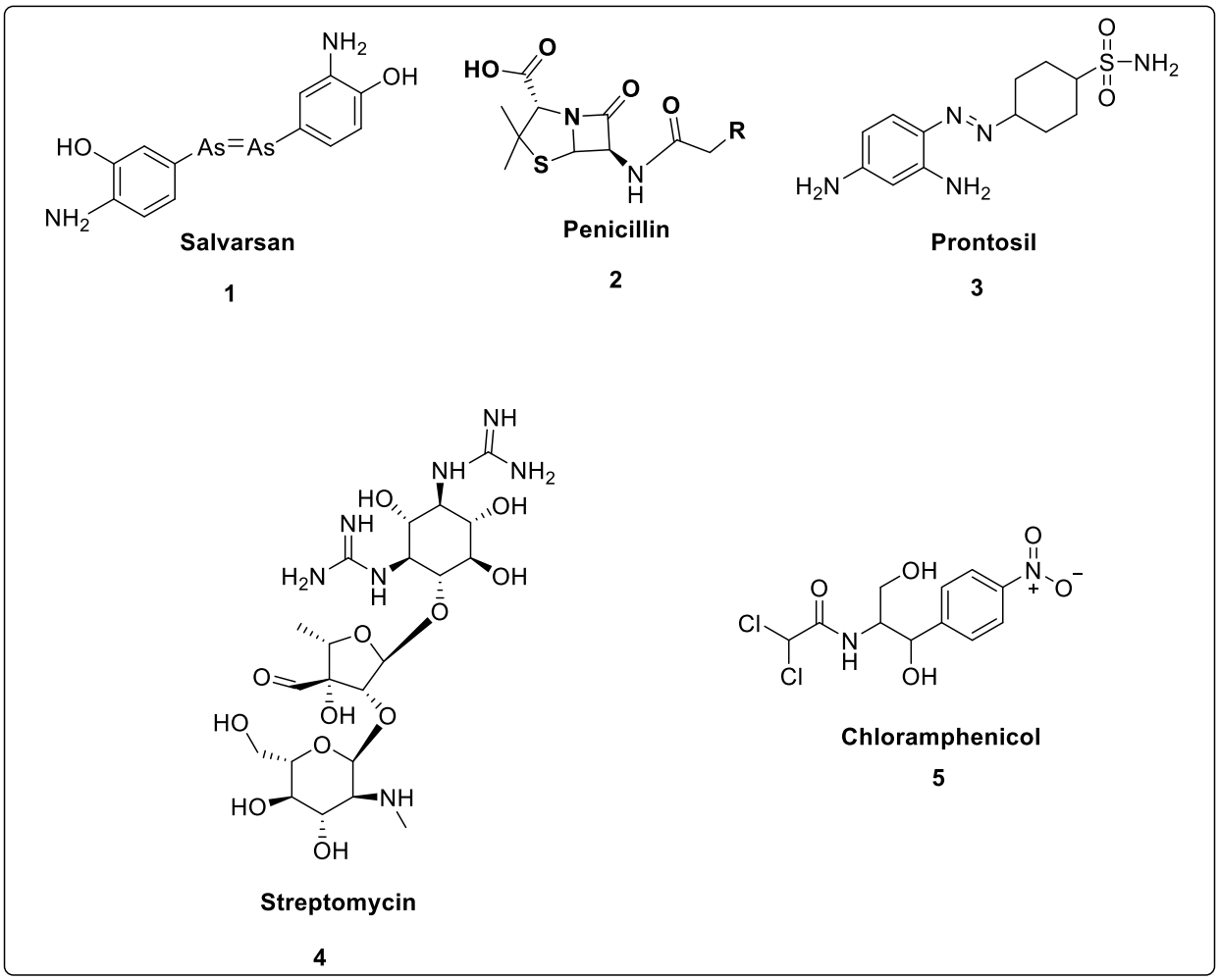


Figure 2: Structures of marketed drugs with antimicrobial activity.

Antimicrobials have since evolved over a period of time and became the key constituents in the treatment of infectious disease caused by micro-organisms thus facilitating a better health for millions around the world. [6][7] Despite the critical role that these agents play in improving the health of patients, Antimicrobial Resistance (AMR) remains a threat in the effective prevention and treatment of the epidemic. The rate of emergence of resistance is higher than that of the antimicrobial agents discovery [7][8]. The threat, therefore poses an urgent call for development of new antimicrobial agents.

2.2. Mechanism of Action

Research states that antimicrobial agents act against microbes by targeting specific receptors, thus having an inhibitory effect on the specific processes that are vital for the microbial advance or division. [9] The goal is to have highly selective toxicity towards these microbes with minimal or no toxicity in humans. The mode by which they execute this action includes: Inhibition of cell wall synthesis, alteration of cell membrane function, inhibition of protein synthesis and that of nucleic acid synthesis. [9] [10]

2.3. Mode of action classification:

2.3.1. Inhibition of cell wall synthesis

The most crucial structure for the existence and endurance of the microbe is the cell wall. It has penicillin-binding proteins (PBPs) a group of enzymes anchored in the cell membrane responsible for the crosslinking of the bacterial cell wall. The group of antibiotics i.e.: penicllins, cephalosporins, carbapenems, monobactams, bacitracin and vancomycin has the beta-lactam ring portion that binds to these different PBPs, preventing them from performing their role in cell wall synthesis thus selectively killing the bacteria. Meanwhile, the binding of glycopeptides to precursors that are responsible for the bacterial cell wall synthesis, leads to the interference with the PBP enzymes, preventing transpeptidases to incorporate the precursors into the growing cell wall, this processes stops the cell wall synthesis and cell death often follows.[3][11]

2.3.2. Alteration of cell membrane function

Cell membranes are important enclosure that separate and control the intra- and extracellular constituents flow. The drugs that inhibits cell membrane function acts by interfering with the functional integrity of the cytoplasmic membrane causing macromolecules and ion escape from the cell which in turn leads to cell death or damage. Both eukaryotic and prokaryotic cells has this enclosure. The class of antibiotics that alter the cell wall membrane can often be poorly selective and often toxic for systemic use, Examples: Amphotericin B and Colistin (polymixin) (**6**) [3].[10][12]

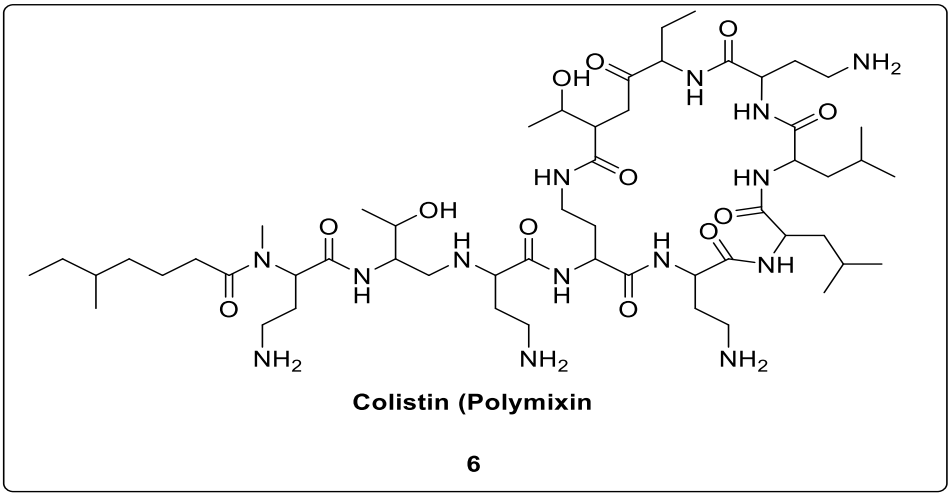


Figure 3: Structures of Colistin (Polymixin) [3]

2.3.3. Inhibition of protein synthesis

The vital process by that the bacterial cells multiply and thus increase their survival is called protein synthesis. Various antibacterial agents inhibits bacterial protein synthesis by binding to either the 30S or 50S subunits of the intracellular ribosomes. Examples of drugs that target the 30's subunit include: aminoglycosides and tetracyclines while the 50's subunit drug targets are macrolides, chloramphenicol (**7**) and Tetracyclines (**8**). This binding subsequently leads to the death of the organism or inhibition of its growth.[3][11][13]

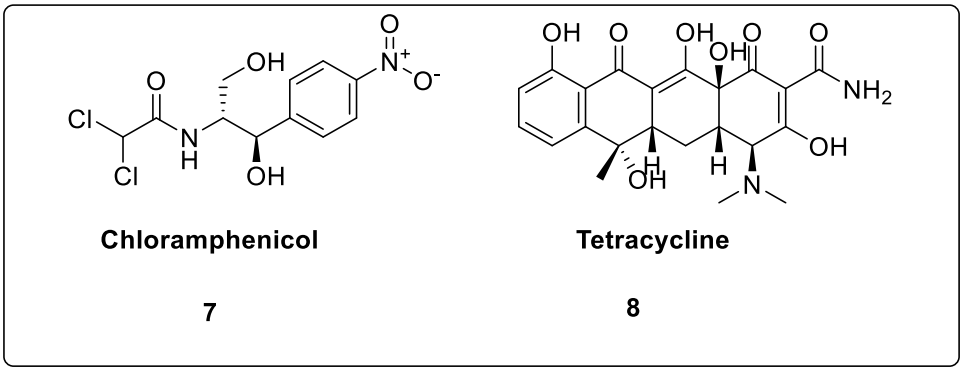


Figure 4: Structures of Chloramphenicol and Tetracycline[3]

2.3.4. Inhibition of nucleic acid synthesis

The mechanism of action includes the inhibition of deoxyribose nucleic acid (DNA) and ribose nucleic acid (RNA) synthesis. These nucleic acids are key in the replication of all living forms, including bacteria. There are antibacterial drugs that work by binding to mechanisms involved in the process of DNA or RNA synthesis[11]. They interfere with folic acid synthesis thus prevention the addition of paraaminobenzoic acid (PABA) into the folic acid molecule through competing for the enzyme dihydropteroate synthetase e.g. Sulfonamides, others interferes with the folic acid pathway by binding to the enzyme dihydrofolate reductase e.g. trimethoprim. Drugs that inhibit the DNA synthesis include fluoroquinolones, ciprofloxacin (**9**), with DNA gyrase being the primary target for fluoroquinolones. Rifampin (**10**) is rifamycin's that blocks bacterial transcription by inhibiting the RNA polymerase, thus preventing bacterial replication. [3][11][14]

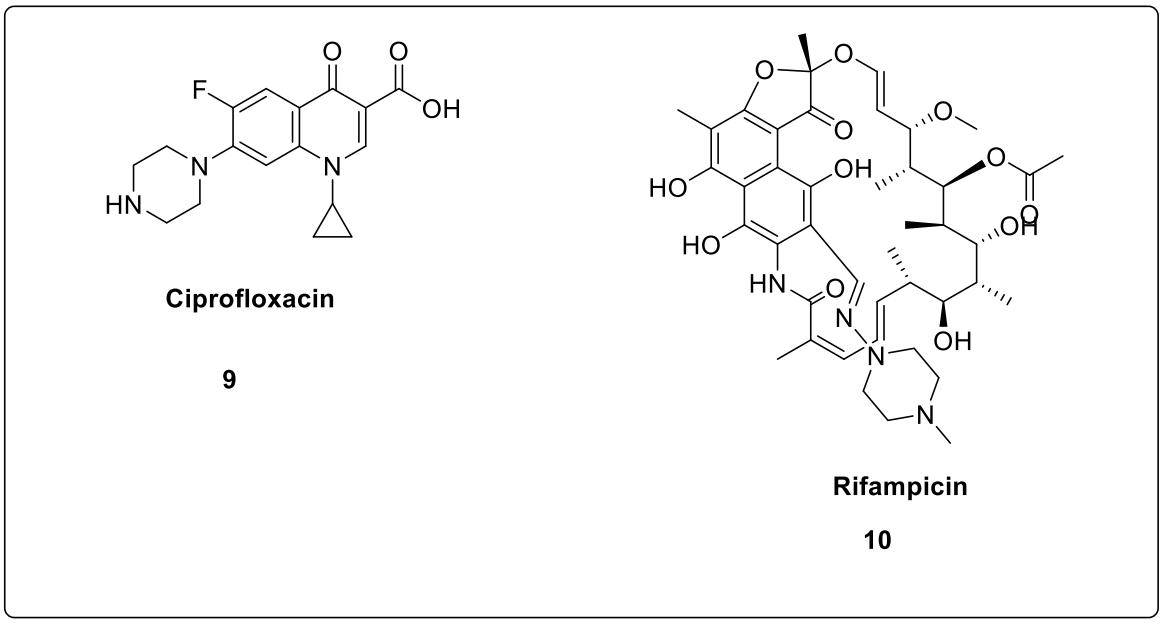


Figure 5: Structures of Ciprofloxacin and Rifampicin

2.4. Antimicrobial resistance (AMR)

The therapeutic agent's curative success is recognized by the potential development of tolerance or resistance to that agent from the time it is first clinically used, and this concept also applies for antibacterial gents. The emergence of resistance in most pathogens has rendered many clinically used antimicrobials ineffective, causing a major global health concern in humans. AMR is defined

as the ability of the pathogen to stay alive in the presence of an antimicrobial agent that would normally inhibit or kill it. In some cases bacterial pathogens progresses into multidrug-resistant (MDR) forms following the multiple antibiotic use, e.g. exposure to first, second and even third line generation of antibiotics.[7][2][15][16]

2.5. Mechanistic basis of antimicrobial resistance

These pathogens may manifest resistance to antimicrobial through three major strategies. To provide a comprehensive classification of the antibiotic resistance mechanisms, we will categorize them according to the biochemical route involved during resistance, as follows: (i) prevention of the compound reaching the antibiotic target (by decreasing penetration or actively extruding the antimicrobial compound), (ii) modifications of the antimicrobial molecule, and (iii) changes to and/or bypassing of target sites. [3][9][11]

2.6. Decreased Antibiotic Penetration and Efflux

2.6.1. Decreased permeability

Decreased permeability due to microbes modifying the cell membrane porin channel frequency, size, and selectivity thus preventing entry of antimicrobials to reaching their intended target sites. Some microbes may acquire gene-encoding enzymes, such as β-lactamases, that destroy the antimicrobial agent before it can have an effect.[17]

2.6.2. Efflux pumps

Efflux pumps can either have the capacity to pump a wide range of unrelated agents or be antimicrobials specific. Active flow out of drugs from the cell is one of the common mechanisms of antimicrobials resistance in bacteria. The process of drug resistance occurs when the percentage of drug flowing out across the membrane exceeds that of drug flowing as bacterial genomes encrypt numerous membrane-bound multidrug out flow systems (**Fig 5**) .[17][18]

2.7. Modifications of the Antibiotic Molecule

This occurs when the bacteria defend themselves by deactivating the active component of the antimicrobial agent. Research confirms that the same tactic has also been observed in *Enterobacteriaceae* against chloramphenicol (acetylation), Gram negative and Gram-positive bacteria against aminoglycosides (phosphorylation, adenylation, and acetylation). [19]

2.8. Target site modification

To circumvent recognition by antimicrobials the bacteria reprogram or mask its critical target sites. This action prevents binding of any active antimicrobial agent to bacteria, this is predominantly noted in *Staphylococci* against methicillin and other beta-lactams (i.e. changes or acquisition of different PBPs that do not sufficiently bind to beta lactams to inhibit cell wall synthesis). It has also been observed in *Enterococci* against vancomycin (i.e. alteration in cell wall precursor components to decrease binding of vancomycin) *Mycobacterium spp.* against streptomycin (i.e. modification of ribosomal proteins or of 16s rRNA). Mutations in RNA polymerase confer resistance to the rifamycins while mutations in DNA gyrase result in resistance to quinolones. Antimicrobial resistance threatens, effective prevention and treatment of increasing range of infection and thus warrants scientific intervention to bring about action plans to curve this threat.[17]. **Figure 6** highlight the therapeutic predominant antimicrobial targets and examples of the drugs for each target.

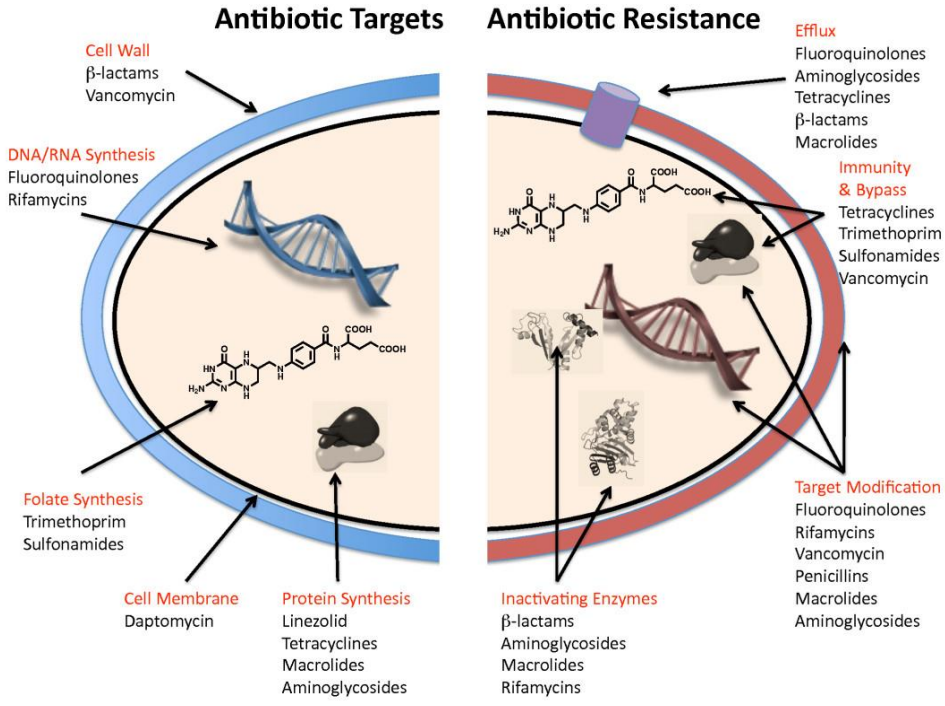


Figure 6: Gerard D Wright. Antibiotic targets and mechanisms of resistance. BMC Biology 2010 8:123 [17]

2.9. Tuberculosis

TB is a communicable disease caused by the bacillus Mycobacterium tuberculosis. It's known to primarily affect the lungs (pulmonary TB), however it has been noted over the years that other site such as (extrapulmonary TB) can be affected too. Researchers still note it to remain a major global health problem, responsible for ill health among millions of people each year and it is ranked as the second leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV).

TB, Mycobacterium tuberculosis, is transmitted by droplets when an infected person coughs or sneezes. The unique clinical characteristics of this dangerous pathogen are a result of its high lipid content. Other M. tuberculosis complex (MTBC) include *M. bovis, M. africanum, M. canetti, and M. microti. M. africanum*, they are however not prevalent in other parts of the world, but they remain a significant cause of tuberculosis in many parts of Africa, especially in Southern Africa. The World Health Organization (WHO) 2014 global tuberculosis report estimates states that in 2013 there were 9.0 million new TB cases and 1.5 million TB deaths (i.e. 1.1 million among HIV-negative people and 0.4 million among HIV-positive people), This indicates an increase from 8·6 million estimated in 2012.

The current standard therapy for drug-sensitive tuberculosis despite being effective, they have longer drug regimen. However, highly effective treatment with shorter regimens are required to reduce the burden of infectious cases. This is even more crucial as the number of new cases of multidrug-resistant (MDR) tuberculosis caused by Mycobacterium tuberculosis resistant strains to at least rifampicin and isoniazid, and the extensively drug-resistant (XDR) tuberculosis to rifampicin, isoniazid, plus any fluoroquinolone and at least one of the three injectable second-line tuberculosis drugs, amikacin, kanamycin and capreomycin) continue to increase.

MDR tuberculosis regimens treatment success rate is still low for both individualized and standard regimens, resulting in high death rates. These bleak treatment outcomes of MDR and XDR tuberculosis highlight the urgent need for the development of new antituberculosis drugs, treatment regimens, and other adjunct treatment approaches to improve treatment outcomes[20][21]

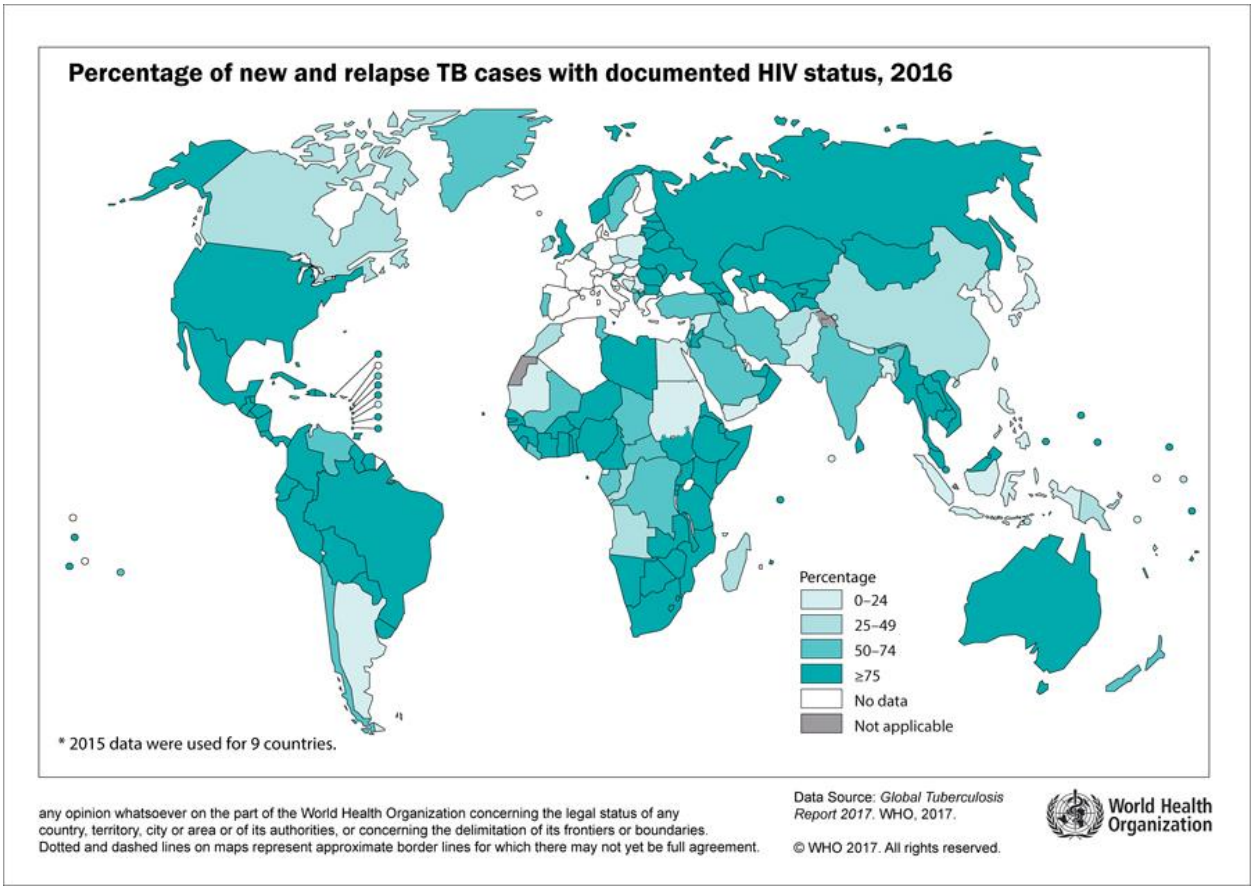


Figure 7: Estimated TB incidence rates in 2016[22]

2.10. Anti- Tuberculosis Drugs

Anti- Tuberculosis Drugs varying properties can be classified as: bactericidal, bacteriostatic (sterilizing) and those with the capability to prevent resistance. Six month is a recommended treatment period, however in some cases such as military meningitis and bone TB treatment may be extended to nine months, however treatment for multi drug resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB), may be as long as twelve months to two years respectively [21][23]

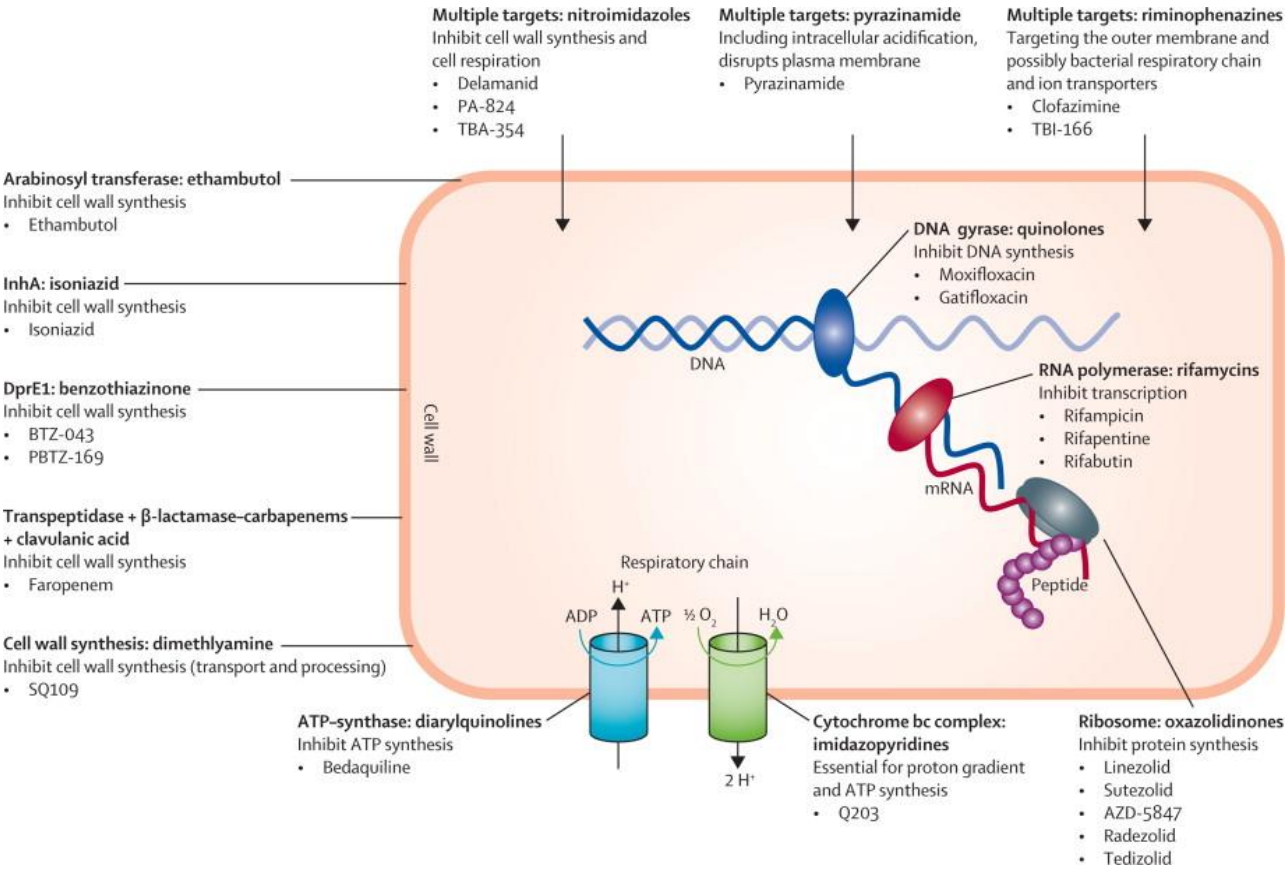


Figure 8: Site of action for TB drugs [24]

2.10.1. First line treatment

The first line TB treatment [**Figure 9**] comprise of **isoniazid (11)** (bactericidal, myolic acid synthesis inhibitor), **rifampicin (12)** (bactericidal, DNA dependent RNA polymerase), **pyrazinamide (13)** (bactericidal, fatty acid synthesis inhibitor), **ethambutol (14)** (bacteriostatic, arabinosyl transferase inhibitor). These are taken with pyridoxine to obviate peripheral neuropathy that may occur as a result of treatment. [21][23][25][26]

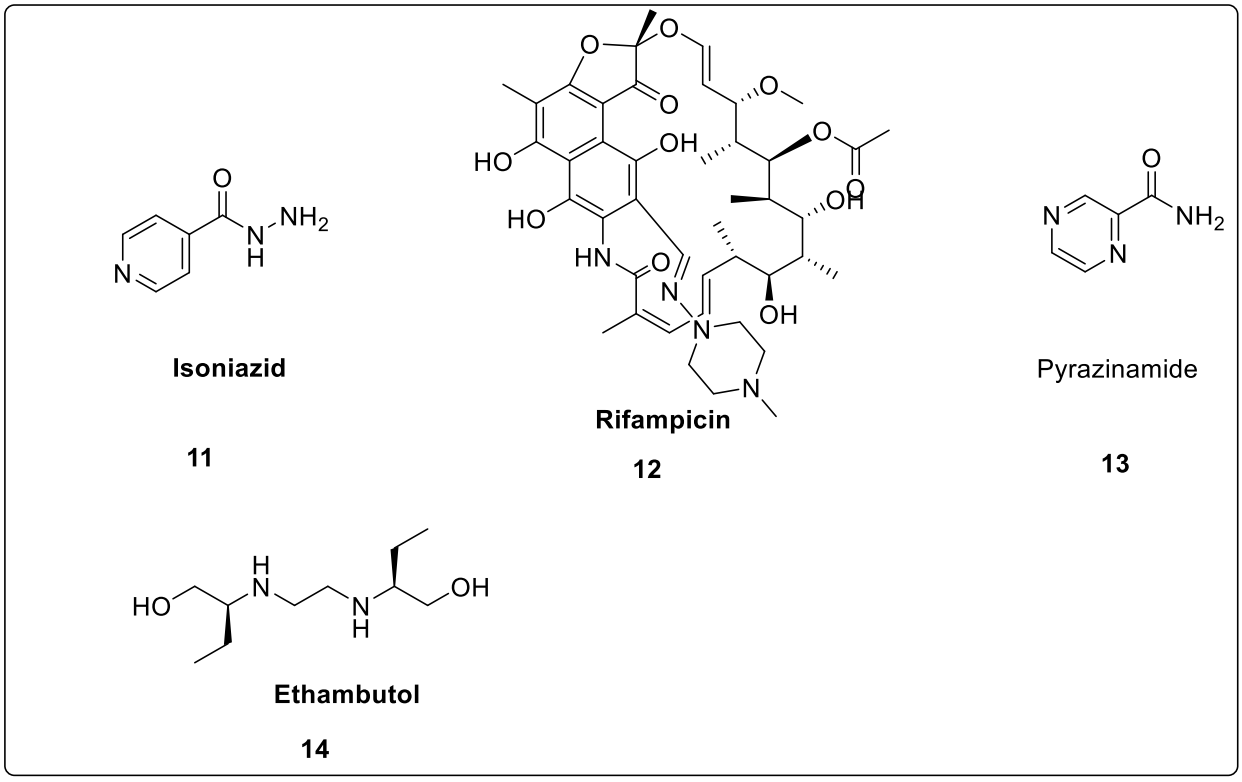


Figure 9: Anti-TB Drugs

2.10.2. Second line treatment

Resistance to either isoniazid or rifampicin may be managed with other first-line drugs, however, resistance to both isoniazid and rifampicin (MDR-TB) demands treatment with second-line drugs. Second line treatment [**Figure 10**] include fluoroquinolones (ciprofloxacin **(15)** and ofloxacin), aminoglycosides (kanamycin **(16)** and amikacin), cyclic peptide antibiotics (capreomycin and viomycin); as well as ethionamide **(17)** and terizidone **(18)**. [21][27][28]

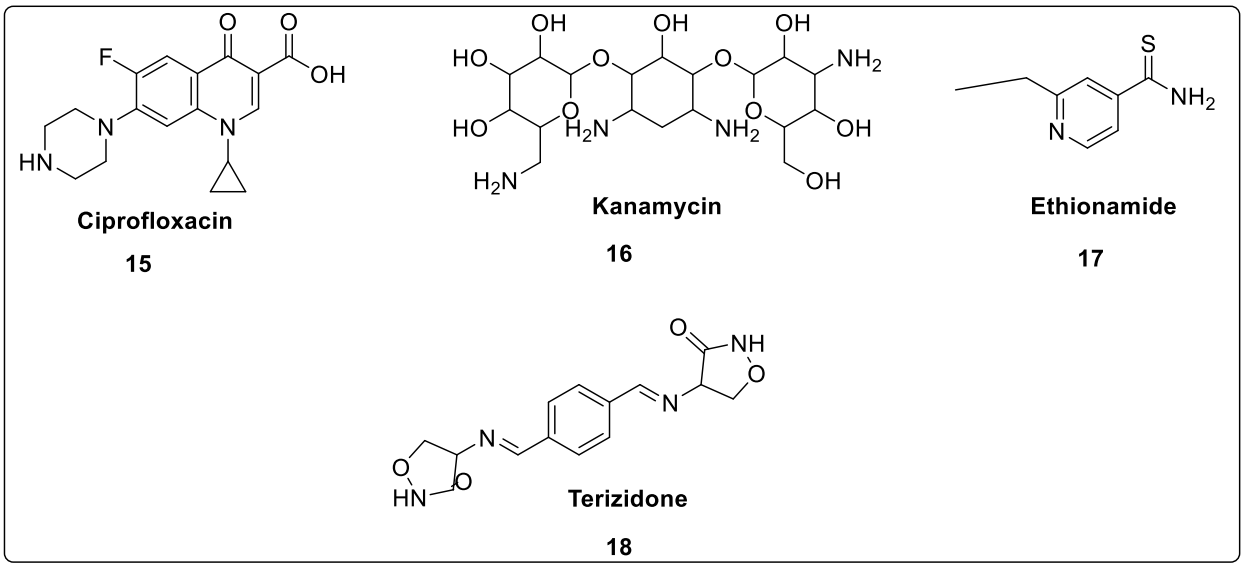


Figure 10: Anti-TB Second line treatment Drugs

2.10.3. Third line treatment

Good management of MDR-TB will prevent extensively drug-resistant TB (XDR-TB), an extremely challenging and require more finance to treat. Based on the previous treatment history and the results of drug susceptibility testing patients with XDR-TB will require an individualized approach to treatment regimes. Treatment [**Figure 11**] amongst others include: linezolid **(19)**, imipenem and cilastatin **(20)** [21][29][30]

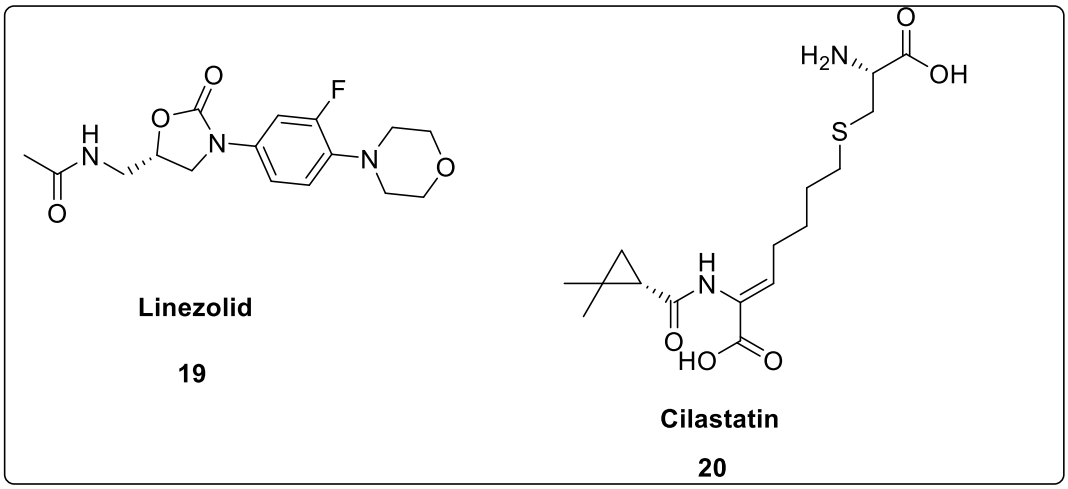


Figure 11: Anti-TB Third line treatment Drugs

2.11. Anti-Tuberculosis Drug: Mechanism of Resistance

The spontaneous mutation in genes that encrypt the enzymes or the drug targets that are involved in drug activation may result in mycobacterium tuberculosis manifesting resistance to anti-tubercular drugs.

Most drug-resistant Mycobacterium tuberculosis are resistant to isoniazid (INH), which is a first-line drug used to treat tuberculosis**.** [31] The M. tuberculosis catalase-peroxidase enzyme KatG activates Isoniazid (a pro-drug)**,** this activation of INH yields oxygen-derived free radicals (i.e. superoxide, hydrogen peroxide, and peroxynitrite) and organic free radicals that hinder the development of mycolic acids of the bacterial cell wall, causing DNA destruction and death of the bacillus. The mutation of KatG is linked to the decreased drug activity thus prevents the pro-drug to convert to it active metabolite leading to INH resistance. The mutation of inhA gene may also lead to INH resistance the, resulting in reduced affinity of the enzyme for NADH without affecting its enoyl reductase activity [32][33]

The mutation in the preserved quinolone determining region of gyrA and gryB , involved in the drug-DNA gyrase interaction are usually associated with *M.tuberculosis* resistant to fluroquinolones. [34]

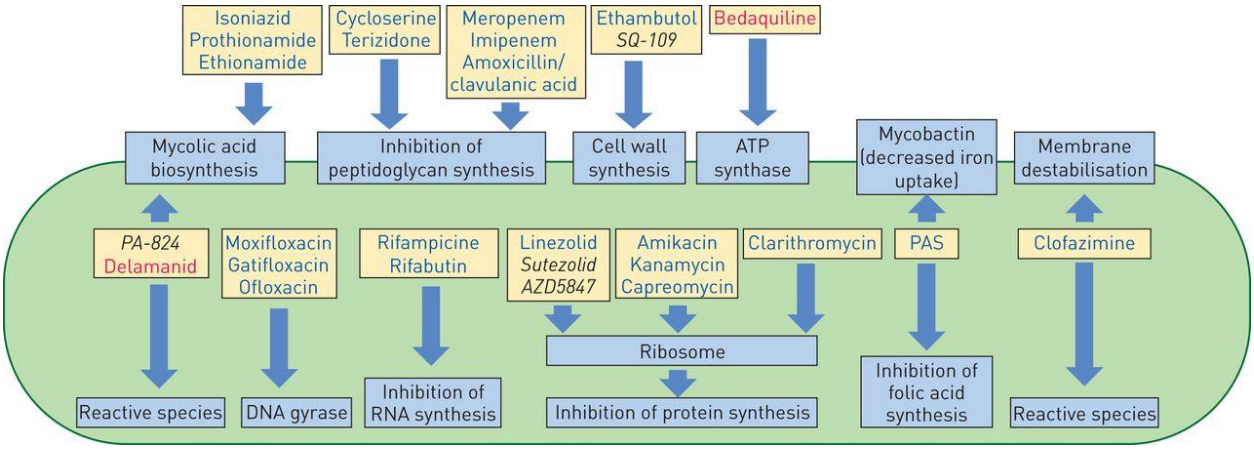


Figure 12: Anti-Tuberculosis Drug: Mechanism of Resistance[35]

2.12. Antimicrobial activity of Bromo-pyrroles and Cinnamaldehyde derivatives

1.12.1. Bromo- Pyrroles

In simple description, a pyrrole is a heterocyclic aromatic five membered compound ring with the chemical formula C_4H_5N. The structure of pyrrole and its substituent positions is shown in **figure 12**

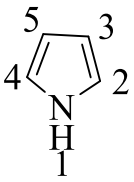


Figure 13: Structure of pyrrole and its possible substituent positions

Pyrroles alkaloids have demonstrated vast significant biological actives such as antimicrobial, antiviral, anti-convulsant, anticancer and anti-inflammatory as shown in **figure 14** [36]. This has qualified a pyrroles to be very importance pharmacophore in medicinal chemistry and organic synthesis. [37]

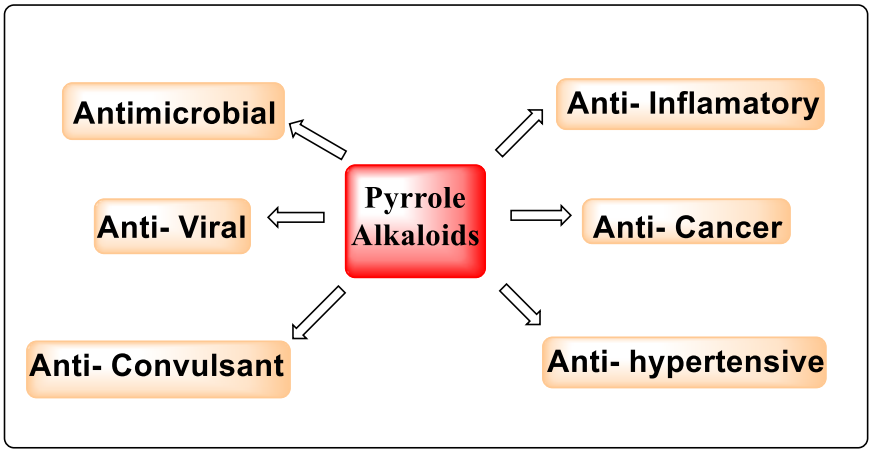


Figure 14: potent biological activity of pyrrole alkaloids [37]

Figure 15 shows commercially available drugs such as Calcimycin, Ageliferin and Atorvastatin that have shown pyrrole as one vital moiety.

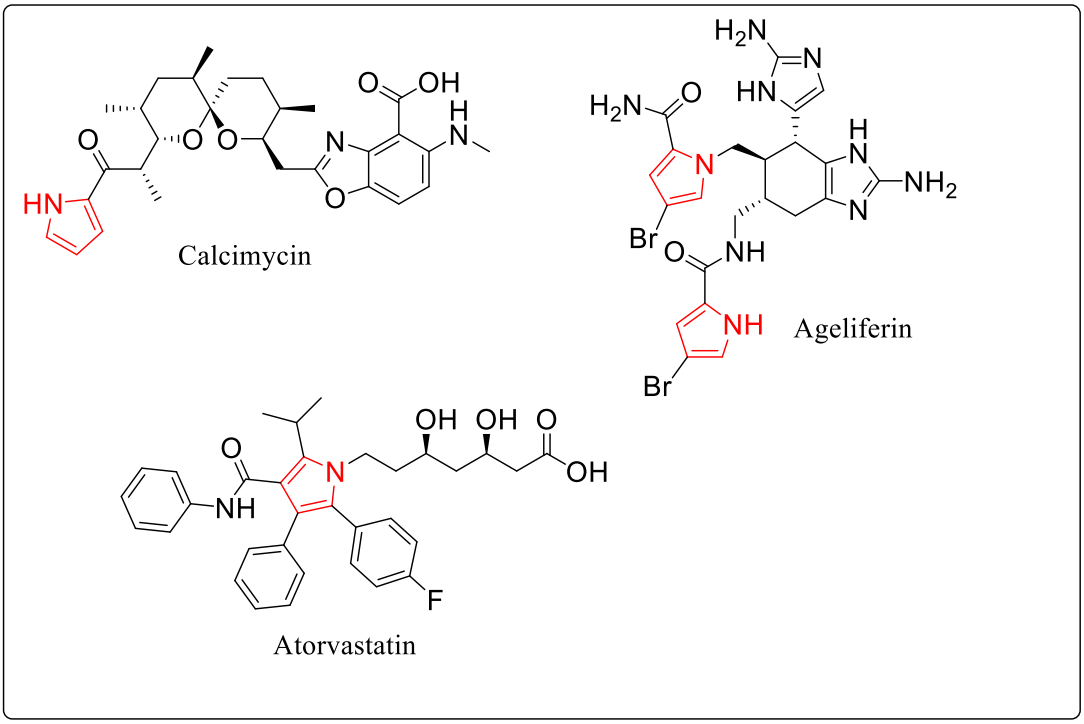


Figure 15: Marketed drugs with pyrrole moiety

Calcimycin possesses antibiotic properties against Gram positive bacteria and fungi. It inhibits mitochondrial ATPase activity and uncouples oxidative phosphorylation. [38] Atorvastatin is used for the treatment of dyslipidemia and prevention of cardiovascular diseases. Ageliferin used antibacterial treatment and induces apoptosis in some cells and prevents it in others.

2.12.2. Bromination of Pyrroles

Bromopyrrole alkaloids are known to be one of the most common and essential metabolites contained in marine sponges. Studies have indicated that, halogenation of pyrrole at position 4 and 5 has significant imparted the biological activity particularly bromination in the synthesis of organic intermediates such as antitumor, antibacterial and antiviral compounds. Bromination has since become a fundamental transformation in pyrrole chemistry and brominated compounds serves as a building blocks which is paramount in organic synthesis.

In a study by (Maria Valeria Raimondi et al) the results of the bromination of 2-(20 hydroxybenzoyl) pyrrole were reported giving a series of derivatives, related to

monodeoxypyoluteorin, whose antimicrobial activity against Staphylococcus aureus increased with the degree of halogenation.

Results from the study by (Rajesh A. Rane et al) further confirms the key role bromination has in antibacterial compounds activity as four of the twenty Hybrids reported in the twenty-hybrid reported in the study exhibited equivalent antibacterial activity (MIC of 1.56g/mL) compared with the standard drug ciprofloxacin against Staphylococcus aureus and Escherichia coli.

The pyrrole moiety containing carboxamide is found to be essential in most cases for antimicrobial activity, recently dihalo-pyrrole carboxamide (Rene A and Telvekar A, 2010) were reported to possess good antibacterial activity and also found to important for the antimicrobial activity on the biofilm, **Figure 15** show other derivative isolated from natural product with their related bio activity where Bromopyrrole was a significant scaffold[39][40][41]

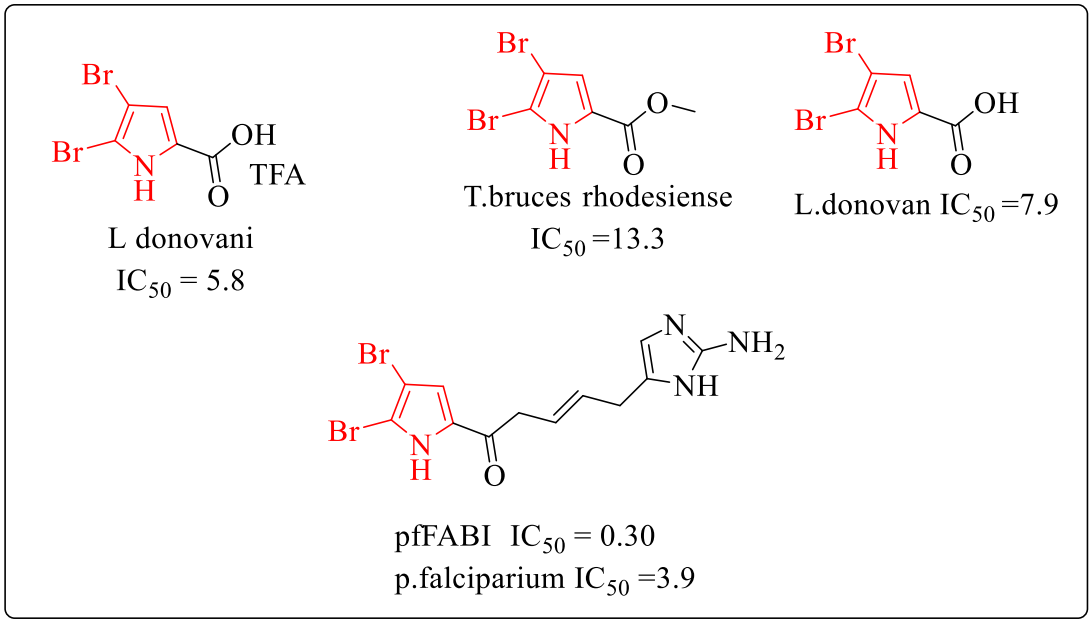


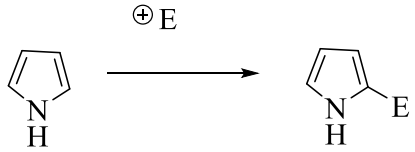
Figure 16: Biologically active Bromo pyrrole natural compounds[42]

Marine sponges are rich sources of natural products and have provided a seemingly infinite supply of bioactive metabolites. Bromo pyrrole alkaloids are a well-known class of sponge metabolites possessing antibacterial, anti-inflammatory, antifungal effect amongst others, with oroidin as their structural prototype. Oroidin analogs emerged as a potent and selective inhibitor of P*f*FabI with IC_{50} values 0.3 g/mL and anti-plasmodial activity, also showed marginal activity of oroidin

towards *M.tuberculosis*. [39][42] Further Balaram S. Takale et al. evaluated Bromo pyrroles molecules for their in vitro antibacterial activities. The compounds exhibited potent activity against both Gram positive and negative pathogens, however the two compounds showed promising activity against both Gram positive Gram negative bacteria with IC_{50} value of 1.56 µM. and 3.12 µM. respectively on comparison with a well know antibiotic Ciprofloxacin with IC_{50} values of 0.05µg/ml to 1.6 µg/ml. [40][43] Subsequently, studies by (Deni- SI Haile and Robert E Johnson 4 1973) pyrroles were sequentially dichloroacetylated or trifluoroacetylated and halogenated. The compounds prepared were assayed for antimicrobial activity and all compounds had in MIC of 15.6 µg/ml or less against Staphylococcus aureus.

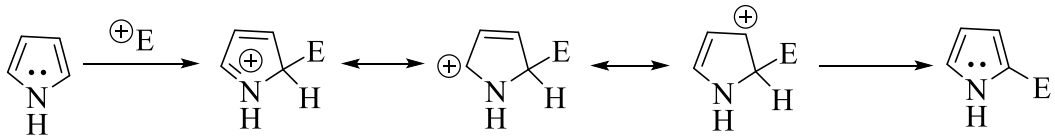
2.12.3. Synthesis of Bromo pyrroles

Bromo pyrrole can easily be synthesized from an electrophilic aromatic substitution EAS reaction. The general reaction [44][45]



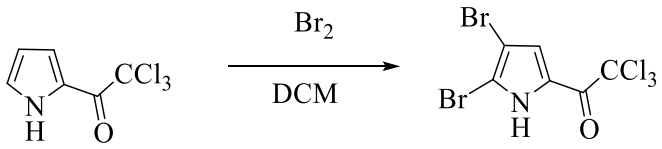
Scheme 1: **EAS for pyrrole**

The proposed general reaction mechanism EAS of pyrrole is shown scheme [44][45]



Scheme 2: proposed reaction mechanism of EAS of pyrrole[44]

The substituent on the pyrrole turns to significantly influence the substitution. An electron withdrawing group as such 1,1,1-trichloroethanone ($COCCl_3$) at position 2 turns to influence electrophilic substitution at position number 4 and 5. For example bromination of 2,2,2-trichloro-1-(1H-pyrrol-2-yl)ethan-1-one by bromine in Dichloromethane [43][46][47]



Scheme 3: Bromination of trichloroacetyl pyrroles[43][46][47]

2.13. Cinnamaldehyde

Natural products still serve as a vital source of therapeutic molecules and one such product is cinnamon. For decades, cinnamon has widely been as spices and treatment of certain infections. It exhibits no side effects at all. The reported medicinal applications for cinnamon include antifungal[48], antibacterial[49], antiviral [50], anti-ulcer [51], anti-oxidant [52] , antidiabetic[53], anti-inflammatory[54] and anticancer properties[55]. Furthermore, it has been used to treat certain cardiovascular [56] and dental infections [57]

Cinnamon is made up 4% essential oils of which 60-75% is cinnamaldehyde.[58] Other vital constituents of cinnamon include trans-cinnamic acid and eugenol.[59][60]

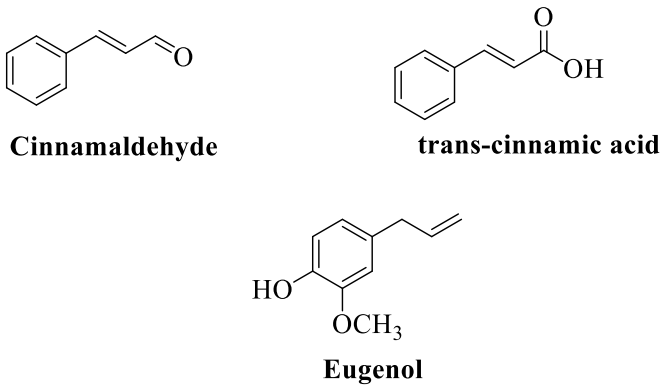


Figure 17: Potent microbial Cinnamaldehyde derivative

Cinnamaldehyde is a principal ingredient which its own has equally demonstrated enormous biological activity such as anti-insecticidal[61] antimicrobial [62][63], antifungal [64], anti-inflammatory [65], anticancer[66], and inhibits tumor growth [67] Other Cinnamaldehyde derivatives that have demonstrated to very important for treating infections include Palpatine, cinromide and Ozagrel.[68]

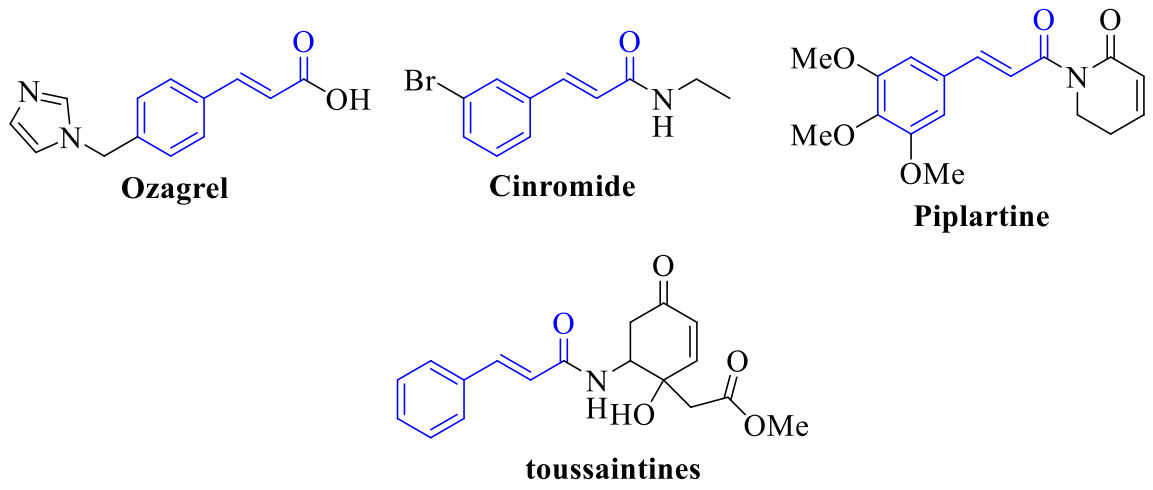
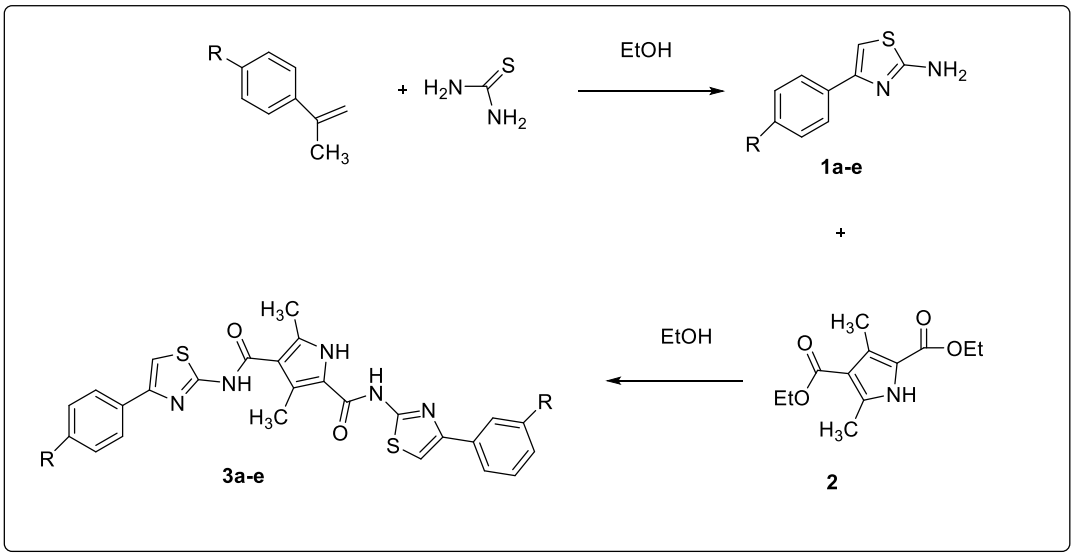


Figure 18: Examples of medically important cinnamaldehyde derivatives [68]

Palpatine an amide alkaloid isolated from seeds of *Piper tuberculatum* [69] demonstrated antibacterial activity against *K. pneumoniae*, *P. aeruginosa* and *S. aureus*, and antifungal activity against *Cladosporium sphaerospermun.* However, for both cases no MIC values were reported. The toussaintines, a cinnamaldehyde derivatives isolated from *Toussaintia orientalis* displayed promising biological activity. Toussaintine **A, B and D** all with a cinnamaldehyde moiety exhibited (**129**) anti E. *coli* at 34 µM, *S. aureus and E. coli* at 67 µM, and *aureus* at 17 µM respectively. In literature there are very few reports pertaining to the antimicrobial activity of natural cinnamic amides.

A new series of pyrrole derivatives with potent antibacterial and antifungal activity were synthesized using standard amination reaction [41]



Scheme 4: Synthetic route of compounds 3a-e

Synthesized compounds were screened against E.coli and S.aureus for antibacterial activity, as well as against A.niger and C.albicans for antifungal activity. Data from the study shows that a 4-hydroxyphenyl ring in this studies most potent compound seems to be responsible for antifungal activity against C.albicans thus the writers concludes that incorporation of a 4-hydroxyphenyl ring as a pharmacophoric feature against C.albicans is a promising prospect.[70]

2.14. Scope of research

In this research work two vital biologically active pharmacophore i.e. bromopyrrole and cinnamaldehyde were coupled to develop a hybrid molecule to improve antimicrobial activity. The two moieties were coupled by a hydrazone linker which its derivatives have equally displayed antibacterial[71], anti-inflammatory, anticancer [72] and antimalaria [73]. For decades, the realms of natural products have served significantly in diverse therapeutic treatments and epic of drug discovery. Our research work centered on this phenomenon not only for their vast bioactive, but for their minimal to non-side effects as well. [43][74]

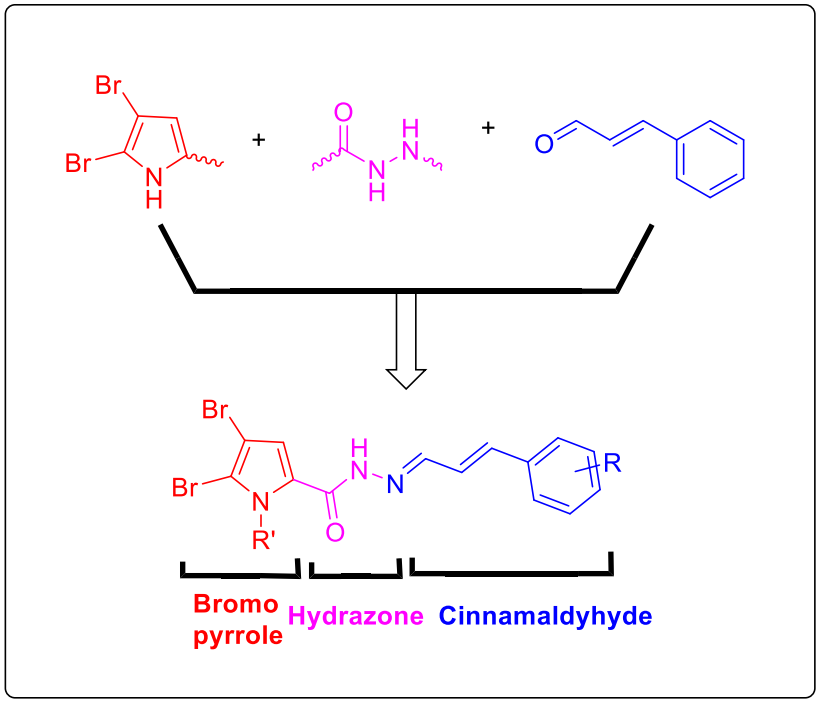


Figure 19 Designing of Bromo pyrrole-cinnamaldehyde hybrid molecule. [75]

Bromopyrroles and Cinnamaldehyde are two naturally occurring product that have displayed profound antimicrobial activity among others that are known for [39][76] Bromopyrroles are secondary metabolites from marine sponges (*genus Angelas sponges*), they are known to have diverse pharmacological activity including antimicrobial. Combining them with cinnamaldehyde, a well-established naturally occurring antimicrobial agent which shows activity against gram positive and gram negative bacteria, *Escherichia coli* being one of the example amongst many.[40][74][7]

The antimicrobial activity displayed by both Bromopyrrole and cinnamaldehyde increased the interest and a need to do molecular hybridization with an intent to produce novel compounds that are potent against microbes. [34][69][77]. In the previous studies the positive mesomeric effect of o-methoxy cinnamaldehyde derivative against antifungal activities increased the activity. In this study a variety of different groups were screened in an effort to increase the hybrid activity [79].

These pyrrole hybrids were synthesized from the condensation reaction of Bromopyrroles and cinnamaldehyde, forming water as the by-product.

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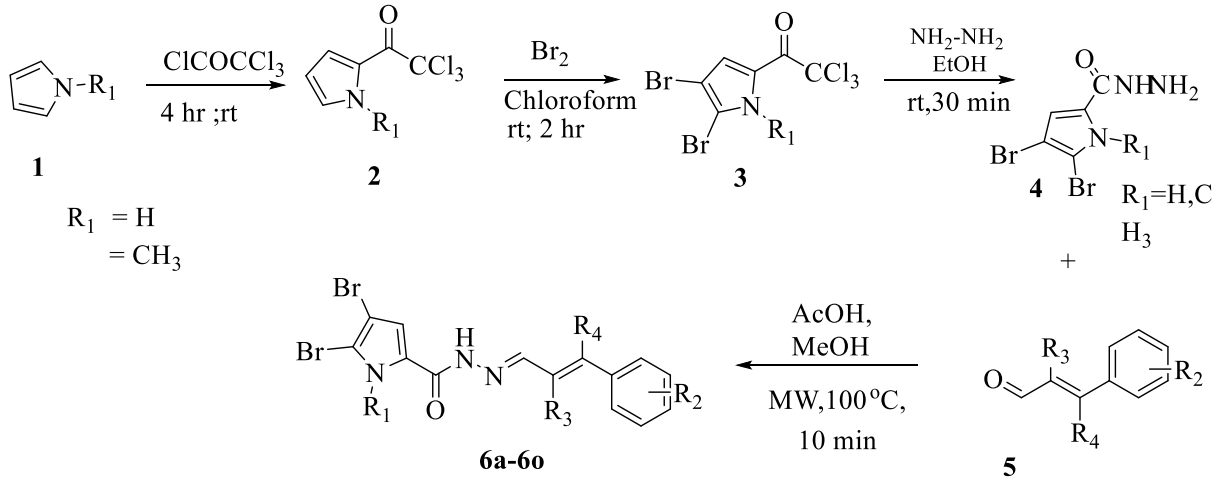
Chapter 3
Experimental

3.1. General Methods

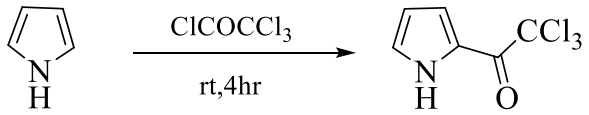
The synthesized compounds were all characterized using the FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopic technique**.** Analytical grade (AR) chemicals and reagents were obtained from Merck and Sigma-Aldrich. The progress of the reactions and the purity of the synthesized compounds were monitored by Thin Layer Chromatography on pre-coated silica gel 60 F254 (mesh) (E. Merck) and spots were visualized under UV light (long and short wavelength). Compounds were purified by recrystallization technique using ethanol. Melting points were determined in open capillaries using (Electro-thermal 9300) digital melting point apparatus. ^{1}H and ^{13}C NMR spectra were recorded on the (Bruker Advance IV) NMR spectrometer at 400 MHz, using DMSO-*d6* as a solvent. Perkin Elmer 100 FT-IR spectrophotometer with universal ATR sampling accessory was used to record all IR spectra[1].



6a: R_1=H;	R_2=4-N(CH_3)$_2$;	R_3=H;	R_4=H
6b: R_1=H;	R_2=H;	R_3=H;	R_4=H
6c: R_1=H;	R_2=H;	R_3=CH_3;	R_4=H
6d: R_1=H;	R=2-OMe;	R_3=H;	R_4=H
6e: R_1=H;	R_2=4-Cl;	R_3=H;	R_4=H
6f: R_1=H;	R_2=2-NO_2;	R_3=H;	R_4=H
6g: R_1=CH_3;	R_2=4-N(CH_3)$_2$;	R_3=H;	R_4=H
6h: R_1=CH_3;	R_2=H;	R_3=H;	R_4=H
6i: R_1=CH_3;	R_2=H;	R_3=CH_3;	R_4=H
6j: R_1=CH_3;	R_2=2-OMe;	R_3=H;	R_4=H
6k: R_1=CH_3;	R_2=4-OMe;	R_3=H;	R_4=H
6l: R_1=CH_3;	R_2=H;	R_3=H;	R_4=H
6m: R_1=CH_3;	R_2=4-Cl;	R_3=H;	R_4=Ph
6n: R_1=CH_3;	R_2=2-NO_2	R_3=H;	R_4=H
6o: R_1=CH_3;	R_2=H;	R_3=CH_3;	R_4=H

Scheme 5: Synthetic route for the novel series of di-bromopyrrole hybrids

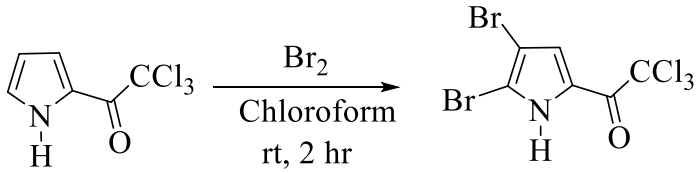
3.1.1. Typical general procedure for the synthesis of **2,2,2-trichloro-1-(1H-pyrrol-2-yl)ethan-1-one (2a)**.



1a **2a**

Trichloroacetyl chloride (13.55g, 74.53 mmol) was dissolving in 50 ml anhydrous diethyl ether. The solution was stirred for 30 minutes at room temperature before dropwise addition of freshly distilled pyrrole (5g, 74.53 mmol). The reaction mixture was further stirred at room temperature for 2 hours. After reaction completion (monitored by the TLC). The reaction was quenched by slowly adding potassium carbonate (5g, 36.18 mmol) in 25 ml water. The compound was collected in an organic layer and dried using magnesium sulfate. The crude compound was dried under rotavap. The crude was purified by washing in Diethyl ether/ Hexane (1:9), filtrating and dried to obtain a pure **2,2,2-trichloro-1-(1H-pyrrol-2-yl) ethan-1-one 2a**. The same procedure was used for methyl pyrrole[2](**2b**).

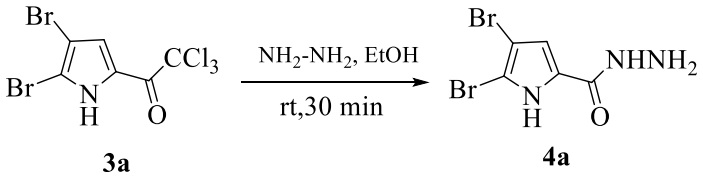
3.1.2. Typical General procedure for synthesis of **2,2,2-trichloro-1-(4,5-dibromo-1H-pyrrol-2-yl)ethan-1-one- Chloroform(3a):**



2a **3b**

0.9 ml of Bromine was added dropwise over period of 1 hour to a solution of 2,2,2-trichloro-1-(1H-pyrrol-2-yl)ethan-1-one (**2a)** (5.5 g, 25.88 mmol) in Chloroform (35ml) at room temperature. The reaction mixture was further stirred at room temperature for 1 hour. A brown effervescence observed. Sodium carbonate was added to the mixture until the efflorescence stopped to release bromine. The reaction completion was monitored by TLC. In a Separating funnel the reaction was partition using 50ml of water, the organic phase was dried over sodium sulfate and the excess solvent was removed using a rotavap. The crude was purified by washing in n-hexane, filtered under vacuum and dried to obtain a pure compound of 2,2,2-trichloro-1-(4,5-dibromo-1H-pyrrol-2-yl)ethan-1-one Chloroform **3a**[3]. The same procedure was used for synthesis of [2](**3b**).

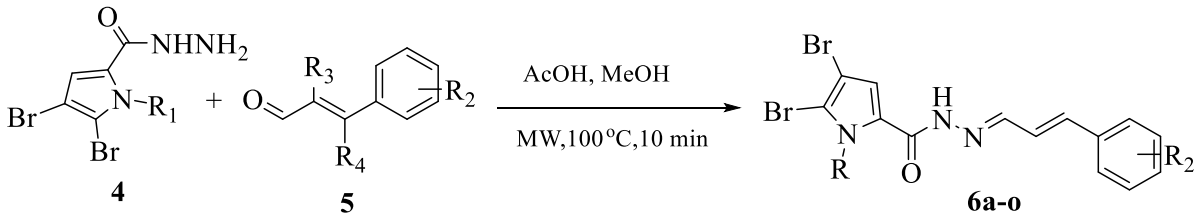
3.1.3. Typical general procedure for synthesis of **4, 5-dibromo-1H-pyrrole-2-carbohydrazide (4a)**



3a **4a**

2,2,2-trichloro-1-(4,5-dibromo-1H-pyrrol-2-yl)ethan-1-one- Chloroform **3** (6 g, 16.21 mmol) was dissolved in of ethanol (30 ml). The solution was stirred at room temperature until homogeneous. 3-4 ml of hydrazide hydrate was then added to the mixture and the reaction mixture was further stirred at room temperature until the precipitate started to appear. The reaction completion was monitored by TLC. The reaction mixture was then poured into ice water, precipitate filtered by vacuum, washed with excess n-hexane and dried to obtain a pure compound of 4,5-dibromo-1H-pyrrole-2-carbohydrazide **4a**. The same procedure was used for synthesis of (**4b**).

3.1.4. Typical procedure for synthesis of **4, 5-dibromo-N'-((1E, 2E)-3-phenylallylidene)-1H-pyrrole-2-carbohydrazide (6a-o)**



4,5-dibromo-1H-pyrrole-2-carbohydrazide **4** (0.20 g, 7.07 mmol), cinnamaldehyde **5** (0.93g, 7.07 mmol), and 3-4 drops of acetic acid in Methanol (4 mL) were charged to microwave for 20 mins at pressure; 150 PSI and power; 100 Watts[4]. The reaction completion was monitored by TLC. The precipitate formed was filtered under vacuum, wash with Diethyl ether/n-hexane (1:9). and dried. Some of the crude compounds were further purified by recrystallization in ethanol. The procedure was used for synthesis of 6(**a-o**)

3.2. The Anti-TB activity

The Anti-TB activity of the synthesized final compounds was carried out on H37Rv strain. This was determined by measuring bacterial growth after 5 days in the presence of test compounds. Compounds were prepared as 10-point two-fold serial dilutions in DMSO and diluted into 7H9-Tw-OADC medium in 96-well plates with a final DMSO concentration of

2%. The highest concentration of compound was 200 µM where compounds were soluble in DMSO at 10 mM. For compounds with limited solubility, the highest concentration was 50X less than the stock concentration e.g. 100 µM for 5 mM DMSO stock, 20 µM for 1 mM DMSO stock. For potent compounds, assays were repeated at lower starting concentrations. Each plate included assay controls for background (medium/DMSO only, no bacterial cells), zero growth (100 µM rifampicin) and maximum growth (DMSO only), as well as a rifampicin dose response curve. Plates were inoculated with *M. tuberculosis* and incubated for 5 days: growth was measured by OD590 and fluorescence (Ex 560/Em 590) using a BioTek™ Synergy 4 plate reader. Growth was calculated separately for OD590 and RFU[5]. The antitubercular assay was carried out in a collaborative effort at NIH, USA.

3.3. Antimicrobial activity

Results obtained from Anti- TB prompted the research to further evaluate the 4, 5-dibromo-N'-((1E, 2E)-3-phenylallylidene)-1H-pyrrole-2-carbohydrazide derivatives for their antimicrobial potential against several other bacterial strains (gram negative, gram positive and fungal strains) by known MIC assay determination method using resazurin dye[6]. All *In vitro* antimicrobial activity was conducted in collaboration with the Department of Microbiology at Inkosi Albert Luthuli Hospital in Durban (RSA).

3.3.1. Microorganisms used

Gram positive microorganism cultures used: *Staphylococcus aureus* (ATCC 25923), *Bacillus Subtilis* (ATCC 6051). Gram negative microorganism cultures used: *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853). Fungal strains used: *Candida albicans* (ATCC 90028), *Cryptococcus neoformans* (ATCC 66031), *Aspergillus Niger* (ATCC 16404). The microorganism culturing and sub culturing was performed a day before the commencement of actual testing.

3.3.2. Preparation of medium

22g of Muller-Hinton Broth containing (Acid Hydrolysate of Casein, Beef Extract and Starch) was dissolved in 1 L of double distilled water (ddH_2O). The pH of this medium was adjusted to 7.4 ± 0.1 and sterilized by autoclave for 15 min at 121°C. The solution was allowed to cool and stored at a temp of 4°C. Sterility check was performed by incubating uninoculated media in an aerobic incubator at 37 °C for 18-24 h. For antifungal activity, RPMI 1640 medium with L-glutamine and 0.165 M MOPS and without sodium bicarbonate (Lonza) was used.

3.3.3. Preparation of test compounds (stock solution and working standard)

A quantity of 4.000 mg of the synthesized final compounds and standard drug (Amoxicillin and /Amphotericin B) were dissolved in 1 ml of DMSO to give stock solution (4000 µg/ml). Further, 100 µl of stock solution was diluted with 900 µl of ddH2O to afford working standard solution at a concentration of 400 µg/ml.

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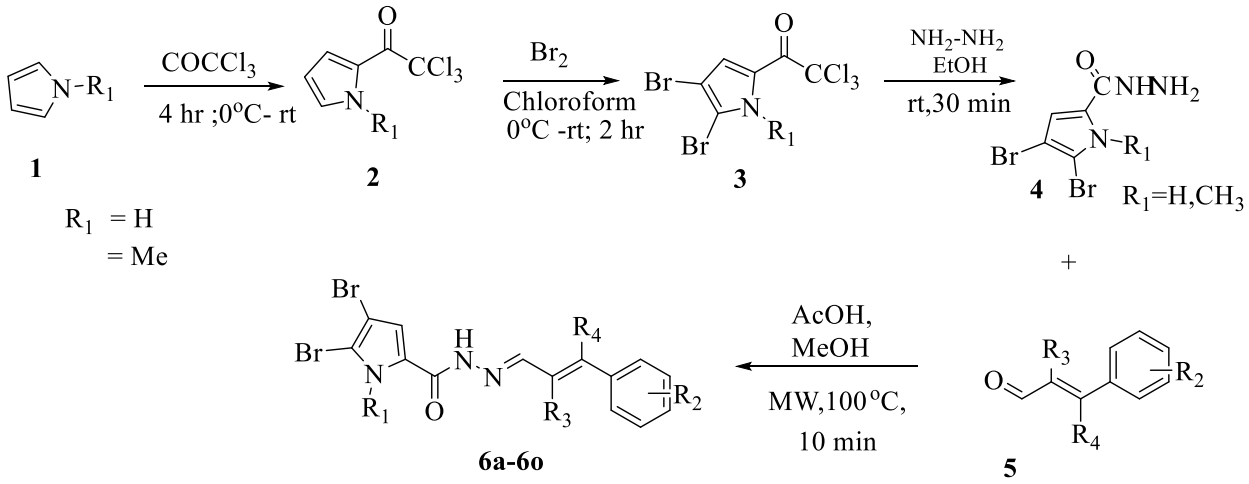
Chapter 4
Results & Discussion

4.1. Chemistry

The research project comprised of 15 novel compounds **(6a-6o)** of di-bromopyrrole hybrids substituted with cinnamaldehyde derivatives **(Scheme 4).** These were synthesized by means of trichloroacetylation of 1H-pyrrole and N-Methyl substituted Pyrrole using equimolar quantity of trichloroacetyl chloride, yielding 2-trichloroacetylpyrrole **2a** & **2b** in excellent yield (89%). Compound **2a and 2b** was then brominated using 2 equivalent of bromine in chloroform for two hours, giving 4, 5-dibromo-2-trichloroacetyl-1H-pyrrole **3a** and **3b** in excellent yield (87%). Bromopyrrole **3a & 3b** was then reacted with excess amount of hydrazine hydrate at room temperature to give its acid hydrazide derivative **4a & 4b** in good yield (80%). The Microwave assisted Schiff base reaction between acid hydrazide **4a & 4b** with cinnamaldehyde derivatives **(5)** proceeds without the requirement of a catalyst, yielding the desired di-bromopyrrole cinnamaldehyde hybrids **6a-o**. All the synthesized compounds were confirmed by the spectral data (IR, ^{1}H NMR, ^{13}C NMR and MS) which was in complete agreement with the proposed structures. $[M]^{+}$ Molecular ion peak confirmed the molecular weight of the parent ions while $[M+2]^{+}$ and $[M+4]^{+}$ peaks confirmed the presence of two bromine atoms in the pyrrole ring with a ratio of 1:1, indicating two bromine isotopes ^{79}Br and ^{81}Br . The ^{1}H NMR spectra of compounds **2-4** showed singlet peak resonating between 12.8–13.0 which corresponds to the N–H of the pyrrole core.



6a: R_1=H; R_2=4-N(CH_3)$_2$; R_3=H; R_4=H
6b: R_1=H; R_2=H; R_3=H; R_4=H
6c: R_1=H; R_2=H; R_3=CH_3; R_4=H
6d: R_1=H; R=2-OMe; R_3=H; R_4=H
6e: R_1=H; R_2=4-Cl; R_3=H; R_4=H
6f: R_1=H; R_2=2-NO_2; R_3=H; R_4=H
6g: R_1=CH_3; R_2=4-N(CH_3)$_2$; R_3=H; R_4=H
6h: R_1=CH_3; R_2=H; R_3=H; R_4=H
6i: R_1=CH_3; R_2=H; R_3=CH_3; R_4=H
6j: R_1=CH_3; R_2=2-OMe; R_3=H; R_4=H
6k: R_1=CH_3; R_2=4-OMe; R_3=H; R_4=H
6l: R_1=CH_3; R_2=H; R_3=H; R_4=H
6m: R_1=CH_3; R_2=4-Cl; R_3=H; R_4=Ph
6n: R_1=CH_3; R_2=2-NO_2 R_3=H; R_4=H
6o: R_1=CH_3; R_2=H; R_3=CH_3; R_4=H

R_2 = 4-N(CH_3)$_2$, 2-OMe,
= 4-Cl, 2-NO_2
R_3 = H, CH_3
R_4 = H, Ph

Scheme 5: Synthetic route for the novel series of di-bromopyrrole hybrids substituted with cinnamaldehyde derivatives

4.1.1. Physicochemical properties

The synthesized compounds appeared as yellowish and brown, colored solids. The percentage (%) yield of the synthesized compounds (6a-o) ranged from 23% - 91 %. The melting points for the synthesized compounds from were found to be between 199 and 228°C **(Table 1).**

Table 1: Physicochemical properties of di-bromopyrrole hybrids substituted with cinnamaldehyde derivatives

Comp.No	R_1(H/Me)	R_2	R_3	R_4	%Yield	M.P.[a](°C)	Appearance	MF	MW
6a	4-$N(CH_3)_2$	R_2=4-$N(CH_3)_2$	R_3=H	R_4=H	90	204-206	Yellow Powder	$C_{16}H_{16}Br_2N_4O$	440.14
6b	-H-	R_2=H	R_3=H	R_4=H	23	217-219	Yellow Powder	$C_{14}H_{11}Br_2N_3O$	397.07
6c	CH_3	R_2=H	R_3= CH3	R_4=H	55	202-204	Yellow Powder	$C_{15}H_{13}Br_2N_3O$	411.1
6d	2-MOe	R_2=2-OMe	R_3=H	R_4=H	63	203-205	Brown Powder	$C_{15}H_{13}Br_2N_3O_2$	427.1
6e	4- Cl	R_2=4-Cl	R_3=H	R_4=H	33	223-225	Yellow Powder	$C_{14}H_{10}Br_2ClN_3O$	431.51
6f	2-NO_2	R_2=2-NO_2	R_3=H	R_4=H	64	206-208	Yellow Powder	$C_{15}H_{12}Br_2N_4O_3$	442.07
6g	4-$N(CH_3)_2$	R_2=4-$N(CH_3)_2$	R_3=H	R_4=H	33	212-214	Yellow Powder	$C_{17}H_{18}Br_2N_4O$	454.17
6h	-H-	R_2=H	R_3=H	R_4=H	46	204-206	Yellow Powder	$C_{15}H_{13}Br_2N_3O$	411.1
6i	CH_3	R_2=H	R_3= CH_3	R_4=H	91	117-199	Yellow Powder	$C_{16}H_{15}Br_2N_3O$	425.12

6j	2-MOe	R_2=2-OMe	R_3=H	R_4=H	66	203-205	Yellow Powder	$C_{16}H_{15}Br_2N_3O_2$	441.12
6k	4-MOe	R_2=4-OMe	R_3=H	R_4=H	80	207-209	Yellow Powder	$C_{16}H_{15}Br_2N_3O_2$	441.12
6l	Ph	R_2=H	R_3=H	R_4=H	70	200-202	Yellow Powder	$C_{21}H_{17}Br_2N_3O$	487.2
6m	4- Cl	R_2=4-Cl	R_3=H	R_4=Ph	70	221-223	Yellow Powder	$C_{15}H_{12}Br_2ClN_3O$	445.54
6n	2-NO_2	R_2=2-NO_2	R_3=H	R_4=H	81	218-221	Yellow Powder	$C_{15}H_{12}Br_2N_4O_3$	456.09
6o	-H-	R_2=H	R_3=CH_3	R_4=H	35	119-201	Yellow Powder	$C_{15}H_{15}Br_2N_3O$	413.11

4.1.2. Spectroscopic Data

Structural interpretation was carried out by means of the following spectroscopic techniques, viz. FT-IR, ^{1}H NMR, ^{13}CNMR and EI-MS) which are depicted in the **Table 2** and the respective spectra for all the compounds were incorporated as appendix.

Table 2 Spectroscopic data of di-bromopyrrole hybrids substituted with cinnamaldehyde derivatives (4a-o)

Compound	IR [ATR, ᵛmax, cm^{-1}]	^{1}H NMR [400 MHz, DMSO-d6, δ, ppm]	^{13}C NMR [101 MHz, DMSOd6, δ, ppm]	EIMS (m/z)
6a	IR γ_{max} (neat): 3227.93, 3160.67, 1603.21, 1559.84, 1359.11, 1315.34, 1226.91, 1055.01, 971.72, 867.44, 803.15, 755.61, 661.55, 596.91, 522.82	^{1}H-NMR (400 MHz, DMSO): δ 12.90 (s, 1H, -NH), 11.28 (s, 1H, -CO-NH-), 8.05 (d, *J*= 8Hz, 1H, -N=CH-C-), 7.43 (m, 2H, Ar-H), 7.07 (m, 1H, Pyrrole 3H), 6.87 (m, 2H, -C=CH-Ar, -C-CH=C-), 6.70 (m, 2H, 2×Ar-H), 2.94 (s, 6H, 2×N-CH_3)	^{13}C NMR (100 MHz DMSO,): δ 150.76, 149.91, 139.75, 139.51, 130.43, 128.46, 128.42, 123.75, 123.70, 122.37, 120.43, 113.32, 112.06, 111.97	437.13 (M^+)
6b	IR γ_{max} (neat): 3127.40, 3026.20, 1623.06, 1539.89, 1477.98, 1347.99, 1246.79, 972.91, 847.47, 800.97, 743.09, 716.39, 677.63, 597.76, 548.32	^{1}H-NMR (400 MHz, DMSO) δ 12.97 (s, 1H, -NH), 11.45 (s, 1H, -CO-NH-), 8.11 (s, 1H, -N=CH-C-), 7.62 (m, 2H, 2×Ar-H), 7.32 (m, 3H, 3×Ar-H), 7.04 (m, 3H, Pyrrole 3H, -C=CH-Ar, -C-CH=C-)	^{13}C NMR (100 MHz, DMSO: δ 155.28, 148.89, 138.84, 135.98, 128.87, 127.12, 125.61, 113.60	394.93 (M^+)
6c	IR γ_{max} (neat): 3143.65, 3027.6, 2952.12, 1625.47, 1541.56, 1479.52, 1414.12, 139.03, 1249.88, 1024.49, 970.35, 847.28,	^{1}H-NMR (400 MHz, DMSO) δ 13.31 (s, 1H, -NH), 11.77 (s, 1H, -CO-NH-), 8.49 (s, 1H, -N=CH-C-), 7.75 (m, 5H, 5×Ar-H), 7.46 (s, 1H, -C-CH=C-)),	^{13}C NMR (100 MHz, DMSO): δ 152.18, 136.74, 136.33, 129.38, 129.29, 128.50, 127.77, 106.86, 98.19	407.93 (M^+)

	747.13, 719.19, 684.98, 603.04	7.21 (s, 1H, Pyrrole 3H), 2.86 (s, 3H, CH_3-C-)		
6d	IR γ_{max} (neat): 3136.16, 3042.82, 2963.80, 2836.50, 1614.36, 1564.57, 1483.17, 1390.37, 1241.96, 1025.43, 977.10, 833.52, 740.39, 600.23	^{1}H-NMR (400 MHz, DMSO) δ 12.95 (s, 1H, -NH), 11.37 (s, 1H, -CO-NH-), 8.07 (d, J= 7.6Hz, 1H, -N=CH-C-), 7.56 (m, 2H, 2×Ar-H), 6.99 (m, 5H, 2×Ar-H, pyrrole 3H, -C=CH-Ar, -C-CH=C-)	^{13}C NMR (100 MHz DMSO,): δ 159.92, 149.32, 138.72, 128.71, 128.67, 123.28, 114.35, 113.48, 106.08, 98.18, 55.28	424.93 (M^+)
6e	IR γ_{max} (neat): 3325.09, 3149.36, 2949.08, 1629.82, 1539.90, 1479.61, 1349.11, 1246.46, 1084.15, 973.11, 852.28, 805.17, 749.70, 699.42	^{1}H-NMR (400 MHz, DMSO): δ 12.98 (s, 1H, -NH), 11.47 (s, 1H, -CO-NH-), 8.10 (s, 1H, -N=CH-C-), 7.65 (m, 2H, 2×Ar-H), 7.43 (m, 2H, 2×Ar-H), 7.06 (m, 3H, Pyrrole 3H, -C=CH-Ar, -C-CH=C-)	^{13}C NMR (100 MHz, DMSO): δ 148.58, 137.30, 134.97, 133.13, 128.83, 128.77, 126.45, 113.61, 106.29, 98.21	427.87 (M^+)
6f	IR γ_{max} (neat): 3639.65, 3440.79, 3296.32, 3150.21, 2956.4, 1642.98, 1572.62, 1544.28, 1515.11, 1347,16, 1255.30, 1081.95, 965.75,	^{1}H-NMR (400 MHz, DMSO): δ 13.00 (s, 1H, -NH), 11.55 (s, 1H, -CO-NH-), 8.14 (s, 1H, -N=CH-C-), 8.05-7.99 (m, 2H, 2×Ar-H), 7.62-7.72 (m, 1H, Ar-H), 7.59-7.55 (m, 1H, Ar-H), 7.14-	^{13}C NMR (100 MHz, DMSO): δ 147.83, 133.86, 133.54, 132.43, 130.46, 130.20, 129.53, 128.30, 128.14, 124.84, 124.58	439.91 (M^+)

	834.01, 737.10, 694.17, 651.97	7.10 (m, 3H, Pyrolle 3H, -C=CH-Ar, -C-CH=C-)		
6g	IR γ_{max} (neat): 3155.57, 2890.00, 2283.50, 1635.88, 1589.80, 1519.11, 1283.03, 1155.46, 1063.00, 942.66, 827.12, 797.21, 707.91, 646.91	^{1}H-NMR (400 MHz, DMSO): δ 11.32 (s, 1H, -CO-NH-), 8.05 (d, *J* = 8Hz, 1H, -N=CH-C-), 7.43 (m, 2H, 2×Ar-H), 7.09 (s, 1H, Pyrrole 3H), 6.86 (d, *J* =16Hz, 1H, -C=CH-Ar), 6.78-6.69 (m, 3H, -C=CH=C-2×Ar-H), 3.88 (s, 3H, -N-CH_3), 2.94 (s, 6H, -N$(CH_3)_2$)	^{13}C NMR (100 MHz, DMSO): δ 155.75, 150.09, 150.15, 139.77, 130.39, 128.41, 126.72, 123.64, 120.39, 144.07, 111.95, 111.5, 97.09, 38.89, 35.49	451.99 (M^+)
6h	IR γ_{max} (neat): 3190.48, 3150.65, 3021.13, 1631.28, 1535.43, 1446.81, 1404.35, 1243.01, 1050.98, 979.94, 885.21, 749.26, 714.63, 685.93	^{1}H-NMR (400 MHz, DMSO): δ 11.44 (s, 1H, -CO-NH-), 8.07 (s, 1H, -N=CH-C-), 7.59-7.57 (m, 2H, 2×Ar-H), 7.36-7.28 (m, 3H, Ar-H), 7.09 (s 1H, Pyrrole 3H), 7.0 (m, 2H, -C=CH-Ar, -C-CH=C-), 3.84 (s, 3H, -N-CH_3)	^{13}C NMR (100 MHz, DMSO): δ 138.88, 135.92, 128.82, 127.09, 126.48, 125.57, 111.90, 97.15, 35.54	407.93 (M^+)
6i	IR γ_{max} (neat): 3382.15, 320.43, 3114.30, 3020.21, 2232.78, 1619.72, 1547.44, 1390.44, 1337.34, 1250.67, 1162.57, 1081.01, 953.44,	^{1}H-NMR (400 MHz, DMSO): δ 11.47 (s, 1H, -CO-NH-), 8.11 (s, 1H, -N=CH-C-), 7.44-7.40 (m, 4H, 4×Ar-H), 7.31 (m, 1H, Ar-H), 7.12 (s, 1H, Pyrrole 3H), 6.83 (s, 1H, -C=CH-Ar),	^{13}C NMR (100 MHz, DMSO): δ 152.52, 136.89, 136.50, 134.58, 129.55, 128.65, 127.92, 115.03, 112.01, 97.34, 35.70, 13.05	422.95 (M^+)

	911.76, 824.09, 778.46, 723.11, 692.93, 616.91	3.89 (s, 3H, -N-CH_3), 2.11 (s, 3H, =CH-CH_3)		
6j	IR γ_{max} (neat): 3206.46, 3034.84, 2955.23, 2830.13, 1621.31, 1540.56, 1483.94, 1451.42, 1411.82, 1243.60, 1036.79, 953.14, 886.40, 860.12, 781.96, 746.96, 653.73	^{1}H-NMR (400 MHz, DMSO): δ 11.44 (s, 1H, -CO-NH-), 8.09 (s, 1H, -N=CH-C-), 7.65-7.63 (m, 1H, Ar-H), 7.34-7.30 (m, 1H, Ar-H), 7.18-7.03 (m, 4H, =CH-Ar , Pyrrole 3H, 2×Ar-H), 6.98 (m, 1H, --C-CH=C-), 3.88-3.86 (s, 6H, -N-CH_3, -OCH_3)	^{13}C NMR (100 MHz, DMSO): δ 156.86, 149.82, 124.20, 120.72, 11.55, 97.16, 55.56, 35.52	438.95 (M^+)
6k	IR γ_{max} (neat): 3748.42, 3207.82, 3032.32, 2967.50, 2835.09, 2337.54, 1696.25, 1649.15, 1572.38, 1534.96, 1504.42, 1452.47, 1354.7, 1248.62, 1168.33, 1110.80, 1019.21, 967.51, 806.08, 717.55, 675.44, 642.13	^{1}H-NMR (400 MHz, DMSO): δ 11.40 (s, 1H, -CO-NH-), 8.08 (s, 1H, -N=CH-C-), 7.57-7.55 (m, 2H, 2×Ar-H), 7.11 (s, 1H, Pyrrole 3H), 6.96-6.85 (m, 4H, 2×Ar-H Pyrrole 3H, -C=CH-Ar, -C-CH=C-), 3.88 (s, 3H,-OCH_3), 3.78 (s, 3H, -N-CH_3)	^{13}C NMR (100 MHz DMSO,): δ 159.88,138.78, 128.64, 123.23, 114.82, 114.30, 111.75, 97.12, 55.23, 35.49	438.95 (M^+)

6l	IR γ_{max} (neat): 3262.96, 3186.64, 3025.37, 1643.24, 1602.75, 1546.45, 1443.67, 1334.48, 1245.26, 1120.15, 1001.90, 958.24, 912.46, 822.98, 771.39, 697.73, 634.80, 566.58	^{1}H-NMR (400 MHz, DMSO): δ 11.40 (s, 1H, -CO-NH-), 7.92 (d, *J* = 8Hz, 1H, -N=CH-C-), 7.52-7.46 (m, 3H, 3×Ar-H), 7.39-7.34 (m, 5H, 5×Ar-H), 7.32-7.30 (m, 2H, 2×Ar-H), 7.04 (s, 1H, Pyrrole 3H), 6.9 (d, *J* = 8Hz, 1H, -HC=C-Ar), 3.87 (s, 3H, -N-CH_3)	^{13}C NMR (100 MHz, DMSO): δ 155.86, 148.0, 147.03, 140.43, 138.09, 129.99, 128.63, 128.59, 128.19, 127.34, 126.3, 124.18, 114.98, 111.9, 97.14, 35.53	483.96 (M^+)
6m	IR γ_{max} (neat): 3149.70, 2955.80, 1643.89, 1523.22, 1485.91, 1422.96, 1295.01, 1210.92, 1086.90, 962.20, 850.44, 765.87, 722.81, 649.03, 604.77	1H-NMR (400 MHz, DMSO): δ 11.50 (s, 1H, -CO-NH-), 8.09 (s, 1H, -N=CH-C-), 7.65-7.63 (m, 2H, 2×Ar-H), 7.44-7.42 (m, 2H, 2×Ar-H), 7.13 (m, 1H, Pyrrole 3H), 7.05-7.03 (m, 2H, -C=CH-Ar, -C-CH=C-), 3.88 (s, 3H, -N-CH_3)	^{13}C NMR (100 MHz, DMSO): δ 148.86, 137.41, 134.92, 133.15, 128.82, 128.77, 126.42, 115.02, 112.00, 97.19, 35.50	441.89 (M^+)
6n	IR γ_{max} (neat): 3156.45, 2956.6, 1645.62, 1615.25, 1511.57, 1417.50, 1346.82, 1250.46, 1147.82, 963.04, 861.07, 829.45, 739.58, 713.80, 612.99, 536.10.	^{1}H-NMR (400 MHz, DMSO): δ 11.58 (s, 1H, -CO-NH-), 8.14 (s, 1H, -N=CH-C-), 8.00-7.98 (m, 2H, 2×Ar-H), 7.75-7.71 (m, 1H, Ar-H), 7.59-7.55 (m, 1H, Ar-H), 7.30-7.28 (d, *J* = 16Hz, 1H, =CH-Ar-H), 7.15-7.07 (m,	^{13}C NMR (100 MHz, DMSO): δ 147.83, 133.57, 132.58, 130.44, 130.17, 129.56, 128.33, 126.30, 124.59, 112.24, 97.26, 30.69	453.92(M^+)

		2H, Pyrrole 3H, -C-CH=C-), 3.89 (s, 3H, -CH_3)		
6o	IR γ_{max} (neat): 3247.70, 3120.64, 3023.67, 2943.86, 1881.45, 1641.72, 1568.80, 1541.57, 1449.51, 1356.89, 1243.74, 155.02, 1041.60, 953.71, 854.42, 817.95, 701.56, 636.10, 608.5, 568.76	^{1}H-NMR (400 MHz, DMSO): δ 11.20 (s, 1H, -CO-NH-), 7.64 (s, 1H, -N=CH-C-), 7.31-7.24 (m, 4H, 4×Ar-H), 7.21-7.19 (m, 1H, Ar-H), 7.02 (m, 1H, Pyrrole 3H), 3.85 (s, 3H, -CH3), 2.81-2.79 (m, 2H, =CH-CH_2-), 2.5 (m, 2H, -CH_2-Ar)	^{13}C NMR (100 MHz, DMSO): δ 155.87, 150.70, 140.88, 128.35, 128.33, 126.40, 125.94, 114.54, 111.43, 96.96, 35.4, 33.64, 31.93	419.95M^+)

4.1.3. Spectral analysis and elucidation

4.1.3.1.Infra-red spectroscopy

Infra-red spectroscopy served as an analytical tool in the identification of different functional groups in all the synthesized novel compounds. The characteristic bands of all compounds were identified to be the amine at N-H stretches which ranged from 31606.07– 3227.93 cm^{-1}, the compounds also reflected a consistent prominent peak around 1600.00 – 1608.00 cm^{-1} which indicated the presence of a carbonyl group (C=O) in all the synthesized compounds.

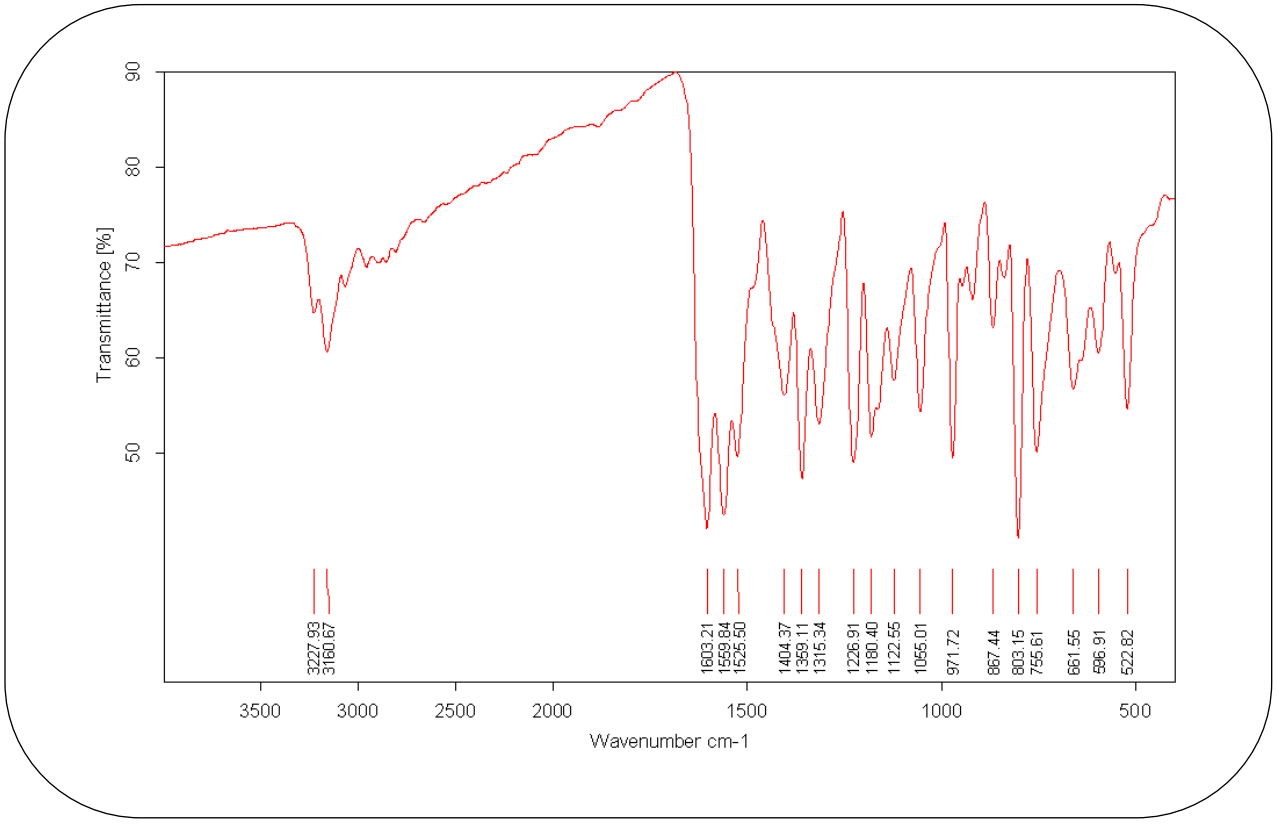


Figure 20: IR spectrum of compound 6a

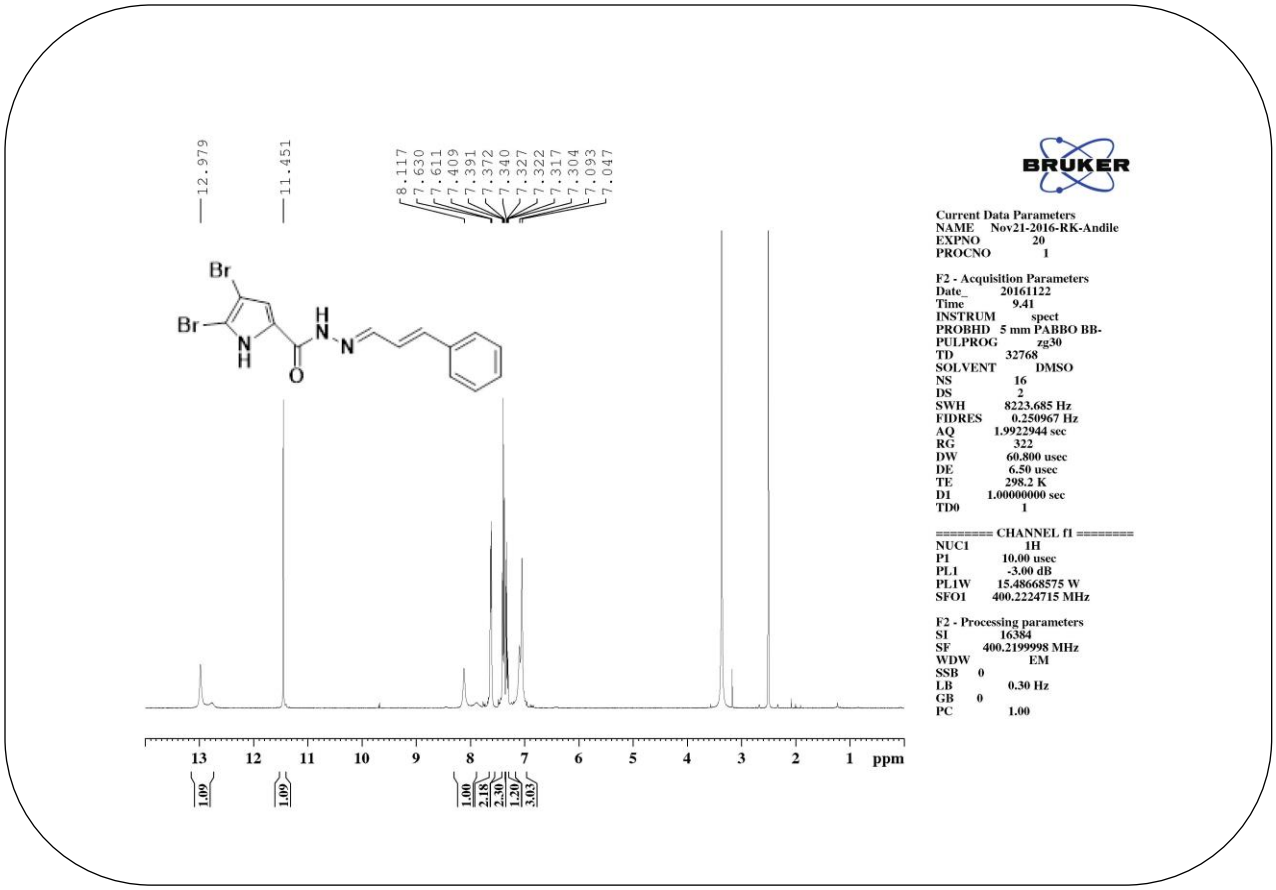


Figure 21: IR spectrum of compound 6b

All synthesized compounds showed resemblance of the characteristic peaks in the IR spectrum which further confirms the presence of the expected functional groups in the newly synthesized compounds

4.1.3.2. ^{1}H NMR Spectroscopy

Proton NMR experiment was conducted to confirm the structure of the newly synthesized compounds. From the ^{1}H NMR spectra of the final compounds (6a-o), the N-Methyl group at 2.95 ppm integrated to 6 protons. There is an inner proton singlet around d 8.1-8.7 in all compounds. The double bond together with the aryl protons from cinnamaldehyde resonate in the range of d 6.7-7.5

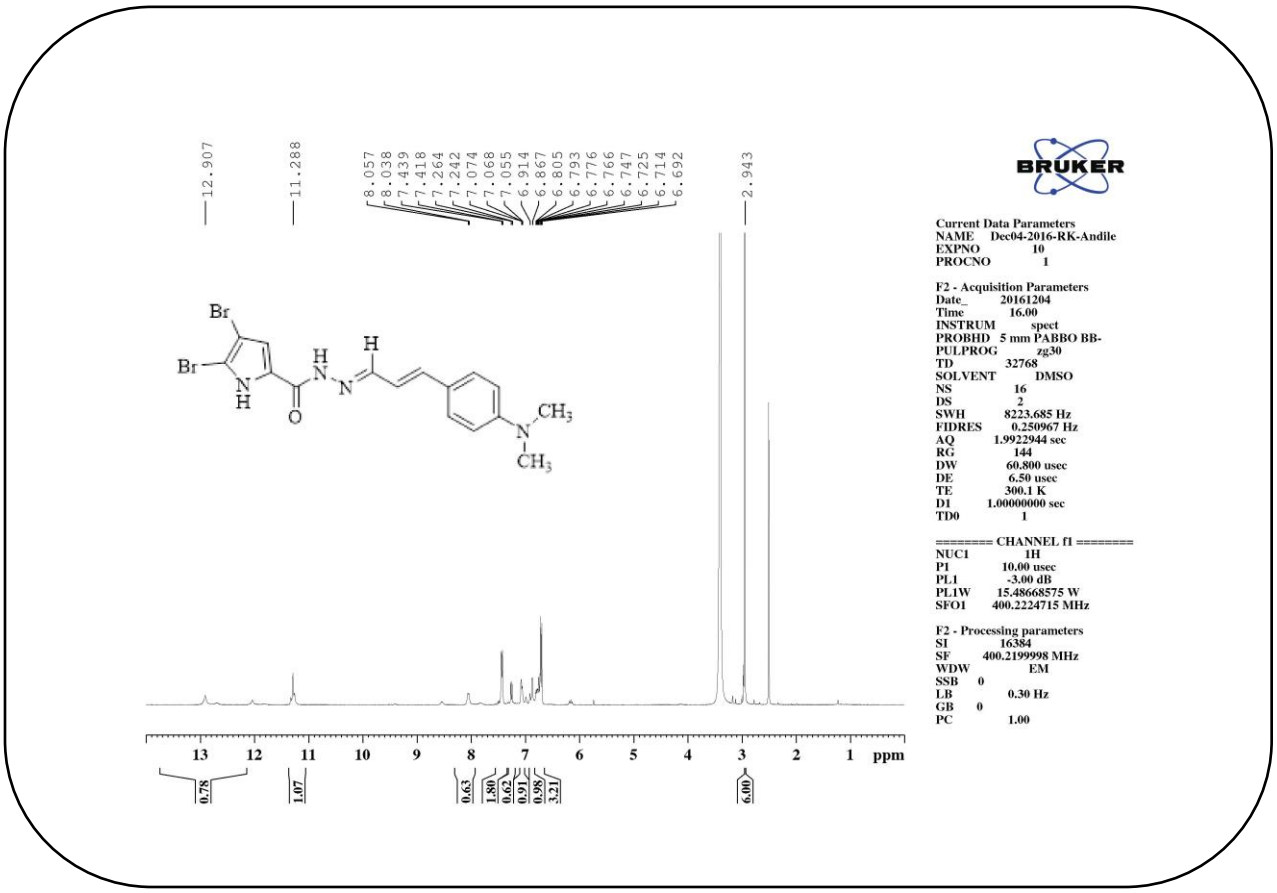


Figure 22: ^{1}H NMR spectrum of compound 6a

4.1.3.3. ^{13}C NMR spectroscopy

^{13}C NMR was also used to evaluate and determine the presence of carbon atoms of the synthesized compounds. Formation of compounds (6a-o) was confirmed by the appearance of N-Methyl groups resonating at 39.8ppm and the amide group at 150.76. The various aromatic carbons resonated between δ 111.97 – 139.75 ppm as shown in the figure 14. All of the synthesized compounds were found to contain the expected number of carbon atoms.

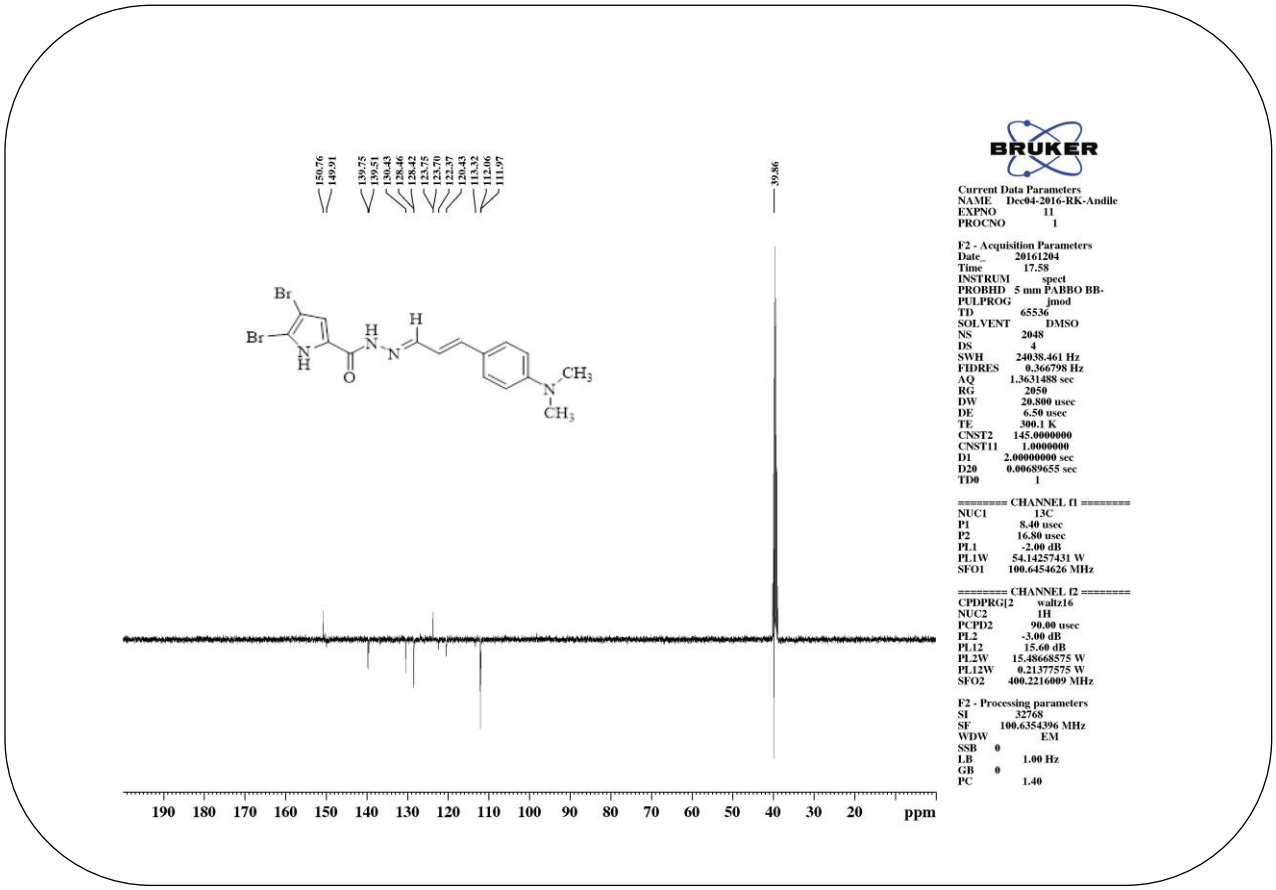


Figure 23: ^{13}C NMR spectrum of compound 6a

4.2. In vitro biological assay

All the synthesized derivatives were evaluated for their antimicrobial potential against several bacterial strains (gram negative and gram positive) by known MIC assay determination method using resazurin dye[1,2] (**Table 3**).

4.2.1. Microorganism used

Gram positive microorganism cultures used: *Staphylococcus aureus* (ATCC 25923), *Bacillus Subtilis* (ATCC 6051). Gram negative microorganism cultures used: *Escherichia coli* (ATCC 35218), *Pseudomonas aeruginosa* (ATCC 27853), Fungal strains used: *Candida albicans* (ATCC90028), *Cryptococcus neoformans* (ATCC 66031), *Aspergillus Niger* (ATCC 16404). The culturing and sub culturing of microorganism were performed a day before the actual testing and

the antimicrobial assay was carried out in a collaborative effort at the Department of Microbiology, Inkosi Albert Luthuli hospital, Durban, South Africa

4.2.2. Discussion of Pharmacological evolution studies:

All the synthesized compounds (6a-6o) were screened for their in vitro antibacterial activity against a series of microorganisms, which comprised of two Gram +ve [*Staphylococcus aureus* ATCC25923, *Bacillus subtilis* ATCC6051] and two Gram –ve [*Escherichia coli* ATCC35218, *Pseudomonas aeruginosa* ATCC27853]. The MIC results were poor as seen from the **table 3** below. Antifungal evaluation was carried out against three fungal strains [*Candida albicans* ATCC90028, *Cryptococcus neoformans* ATCC66031 and *Aspergillus Niger* ATCC16404] and two well characterized clinical isolates strains of *Candida albicans* and *Cryptococcus neoformans*. The MIC results of the synthesized compounds exhibited promising antifungal activity (MICs = 12.56-50 µg/mL), especially against Cryptococcus neoformans as depicted in **table 3** below. The MIC values ranged from 12.5 - >200 µg/mL and all compounds exhibited poor to moderate in-vitro activity against the screened bacterial strains. The reference drug Amoxicillin indicated an MIC of 0.39 µg/mL. However, a systematic analysis of the data also revealed that some of the synthesized compounds exhibited promising antifungal activity (MICs = 12.5-50 µg/mL), especially against *Cryptococcus neoformans*. The reference drug Amphotericin B an MIC of 0.39-1.5µg/mL.

Table 3: Antimicrobial activity data

	ATCC 25923	**ATCC 35218**	**ATCC 27853**	**ATCC 6051**		**ATCC 90028**	**ATCC 66031**	**ATCC 16404**
	Staphylococcus aureus	***Escherichia coli***	***Pseudomonas aeruginosa***	***Bacillus subtilis***		***Candida albicans***	***C. Neoformans***	***Aspergillus Niger***
6a	>200	>200	>200	>200		>200	>200	>200
6b	>200	>200	>200	>200		>200	25	>200
6c	>200	>200	>200	>200		>200	>200	>200
6d	>200	>200	>200	>200		>200	>200	>200
6e	>200	>200	>200	>200		>200	12.5	>200
6f	>200	>200	>200	>200		>200	12.5	>200
6g	>200	>200	>200	>200		>200	25	>200
6h	>200	>200	>200	>200		>200	12.5	>200
6i	>200	>200	>200	>200		>200	>200	>200
6j	>200	>200	>200	>200		>200	25	>200
6k	>200	>200	>200	>200		>200	>200	>200
6l	>200	>200	>200	>200		>200	12.5	>200
6m	>200	>200	>200	>200		>200	>200	>200
6n	>200	>200	>200	>200		>200	>200	>200
6o	>200	>200	>200	>200		50	>200	>200
Amoxicillin	<0.39	<0.39	<0.39	<0.39	***Amphotericin B***	<0.39	1.5	<0.39

The synthesized novel compounds were also evaluated against *M. tuberculosis H37Rv* strain **(Table 4).** The antitubercular activity of these compounds was carried out at National Institute of Allergy and Infectious Diseases (NIAID) screening program, Bethesda, MD, USA. Four of the synthesized compounds **(6b-6e)** displayed moderate activity the rest of the synthesized compound had poor activity against mycobacterial strain.

Table 4: Antitubercular activity data (MIC in µM) of a series of novel derivatives (6a-o)

Compound	***Mycobacterium tuberculosis* (H37Rv)**
6a	>200
6b	>20
6c	>20
6d	>20
6e	>20
6f	>200
6g	>200
6h	>200
6i	>200
6j	>200
6k	>200
6l	>200
6m	>200
6n	>200
6o	>200
Rifampicin	**0.0067**

References:

1. Mann, C.; Markham, J. Journal of Applied Microbiology 1998, 84, 538-544.

2. Palomino, J.-C.; Martin, A.; Camacho, M.; Guerra, H.; Swings, J.; Portaels, F. Antimicrobial agents and chemotherapy 2002, 46, 2720-272

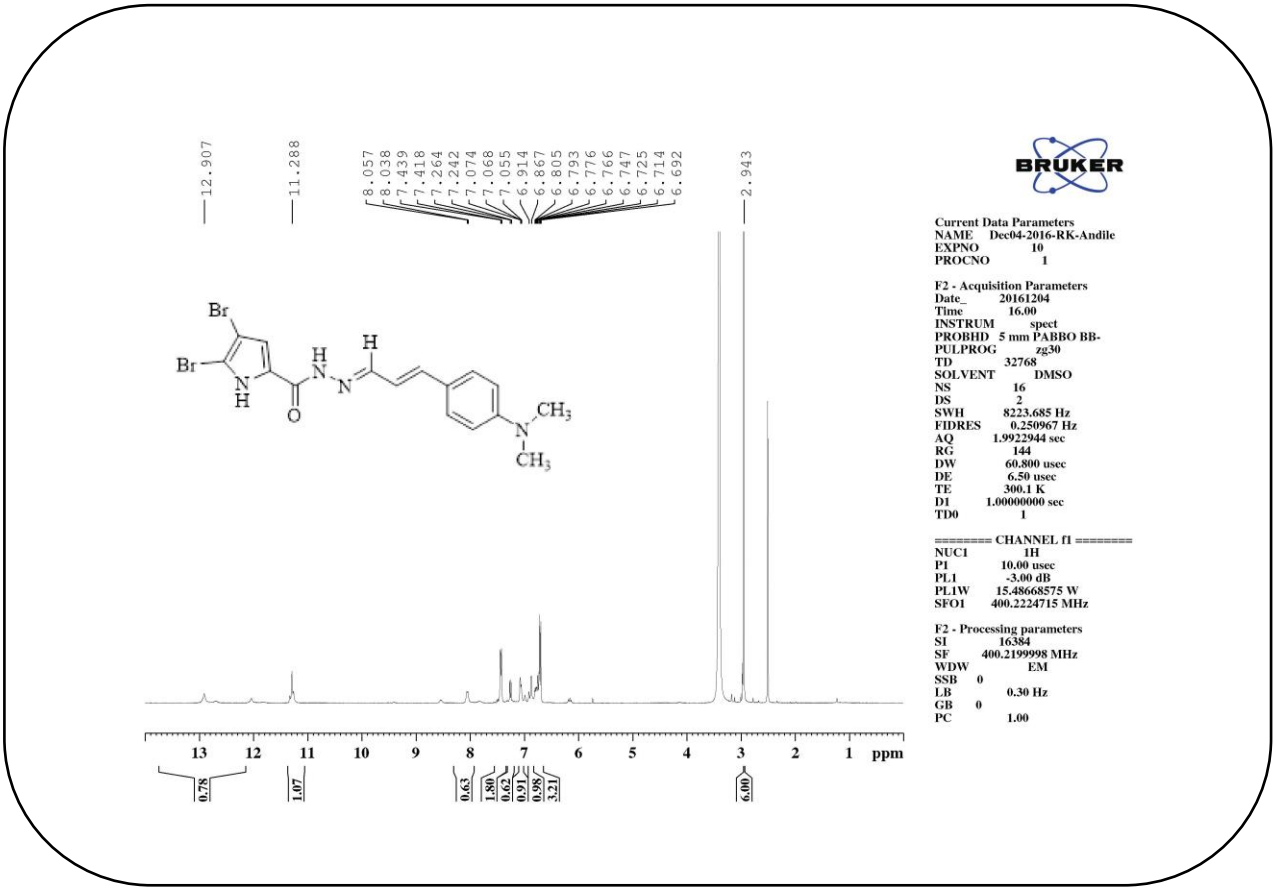
Chapter 5
Conclusion

5.1. Conclusion

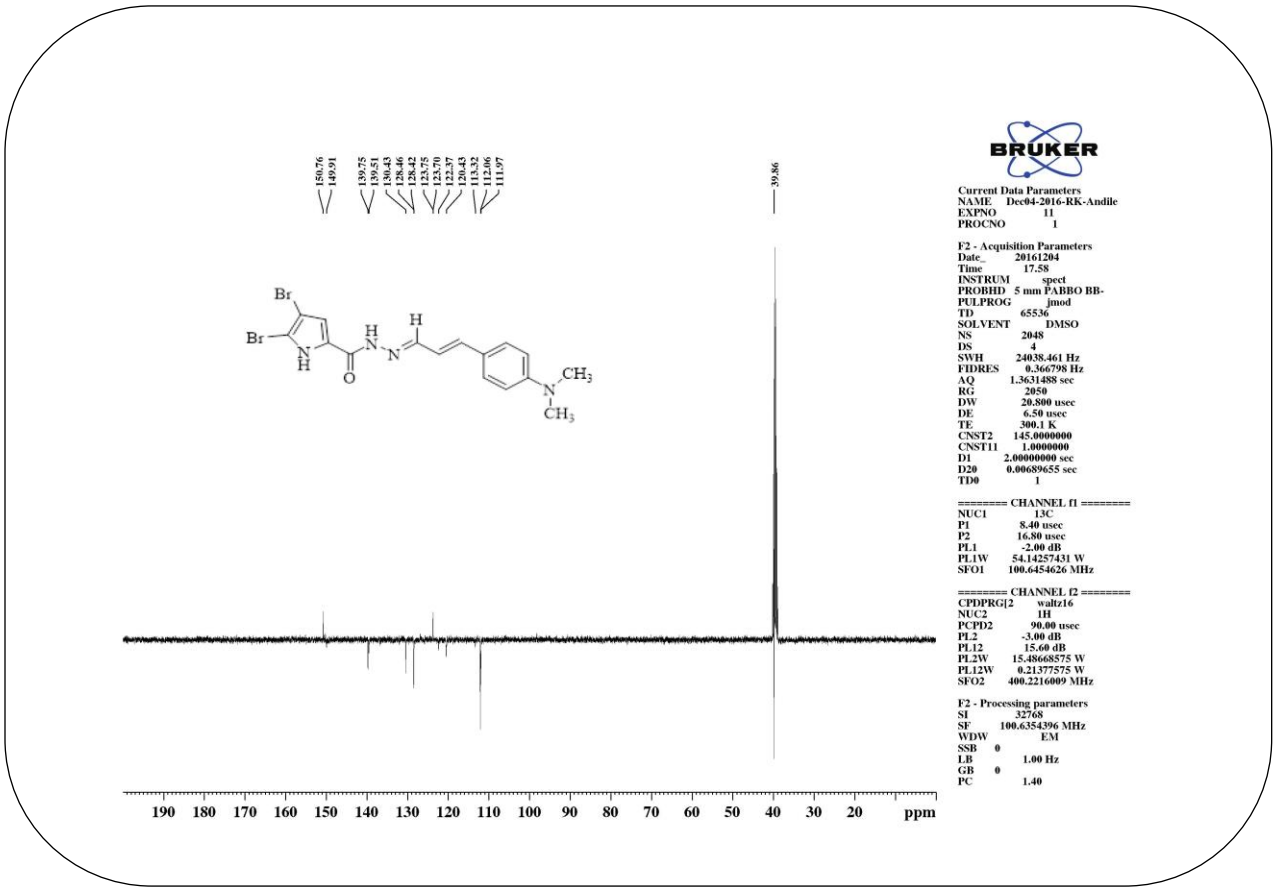
The aim of this study was to investigate the effectiveness of fifteen newly synthesized derivatives of bromopyrroles against antitubercular and antimicrobial potential of these derivatives. To a great extent, this work has accomplished the aims of the study. Results from this work confirmed the following conclusions: These synthesized hybrids displayed promising activity against tested fungal strains, in particular for clinical isolate of *C. neoformans* with MIC values ranging from 12.5 – 25 μg/mL. The sythesised compounds also displayed moderate activity (MIC >20 μM) in four synthesized compounds (4b-e) against *M. tuberculosis H37Rv*.

5.2. Future Studies

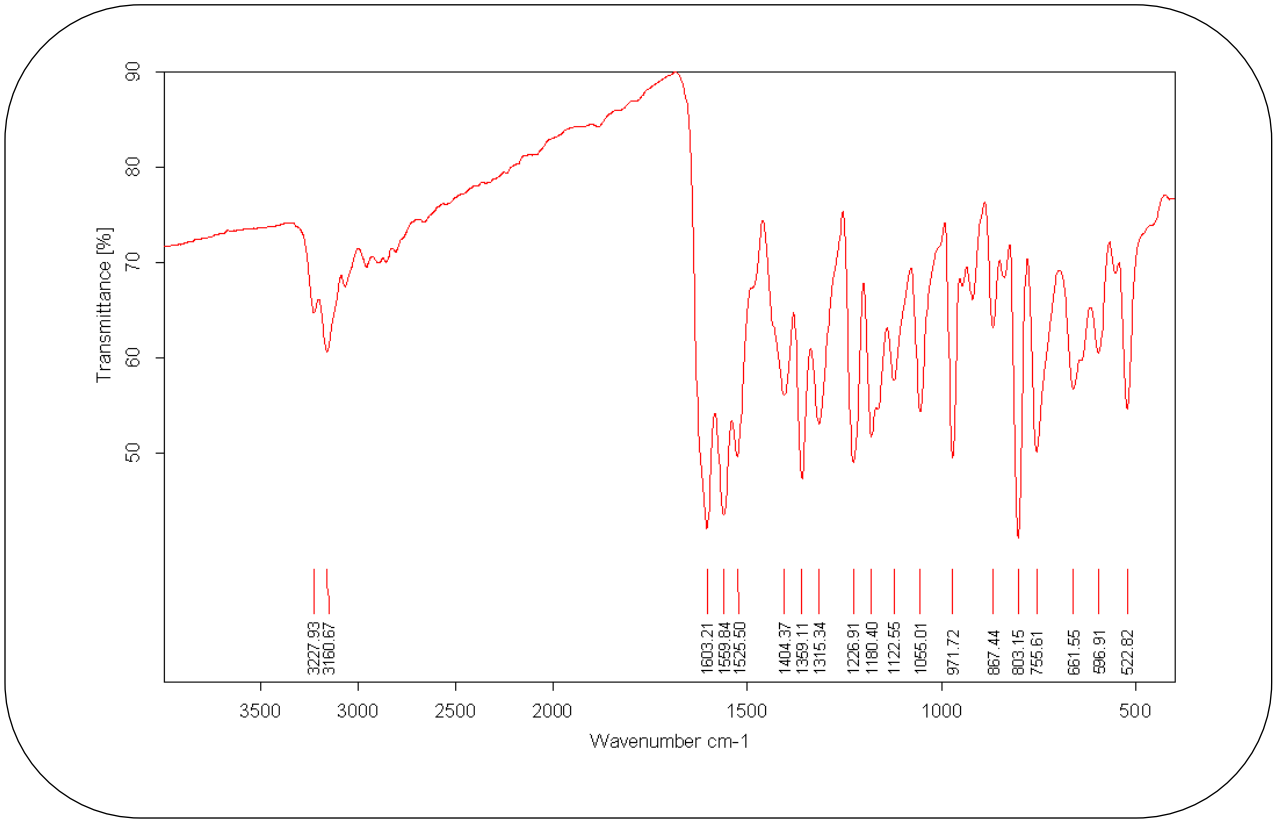
The antifungal activity displayed by compounds **6e-h** and **6l** against *Cryptococcus neoformans* indicates that these substituted hybrids can be further exploited to develop potential antifungal agents. In addition, these active hybrids **6b-6e** also displayed moderate activity (MIC >20 μM) against *M. tuberculosis H37Rv*. The encouraging antifungal and anti-mycobacterial activity of synthesized novel bromopyrrole derivatives indicated the potential for further research into the development antifungal agents against the resistant clinical isolates.



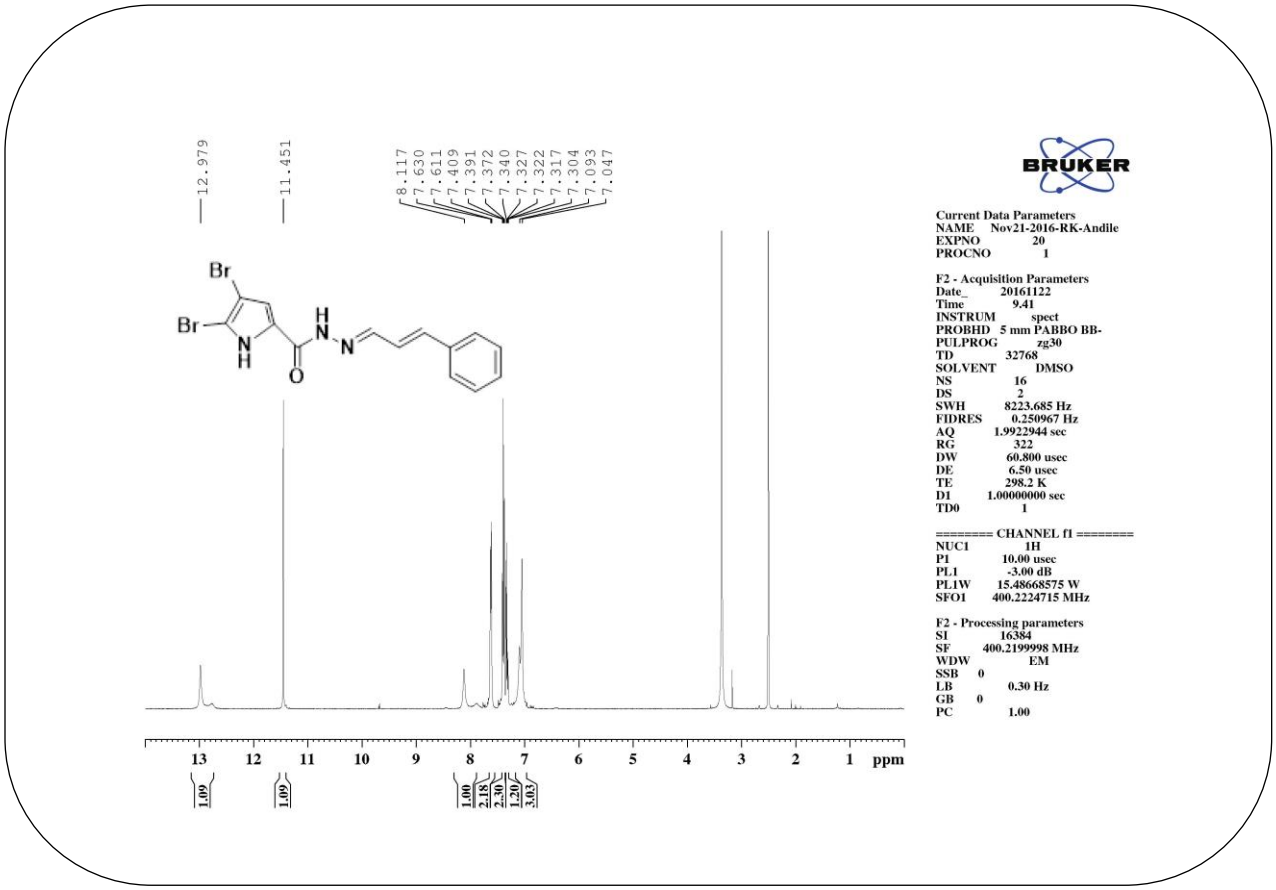
Spectrum 1: 1H NMR spectrum of compound 6a



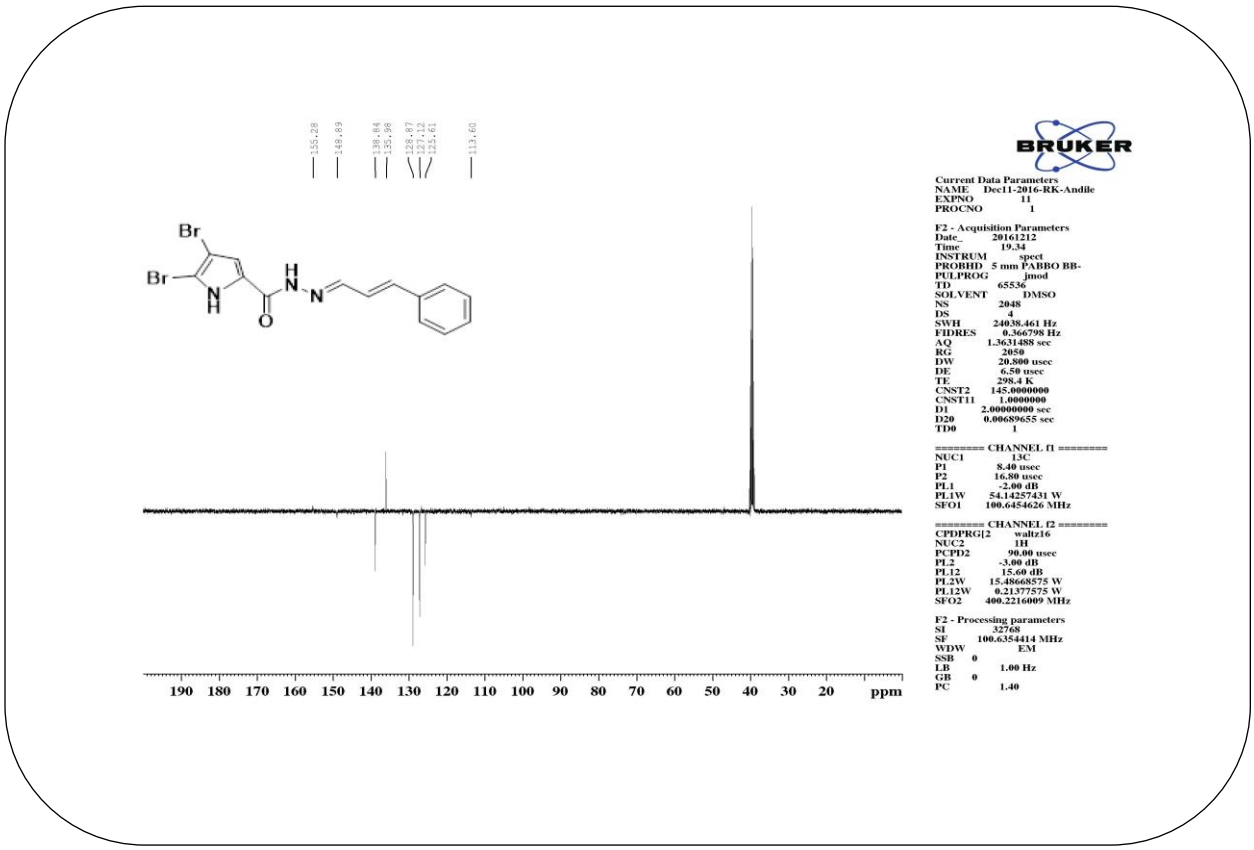
Spectrum 2: ^{13}C NMR spectrum of compound 6a



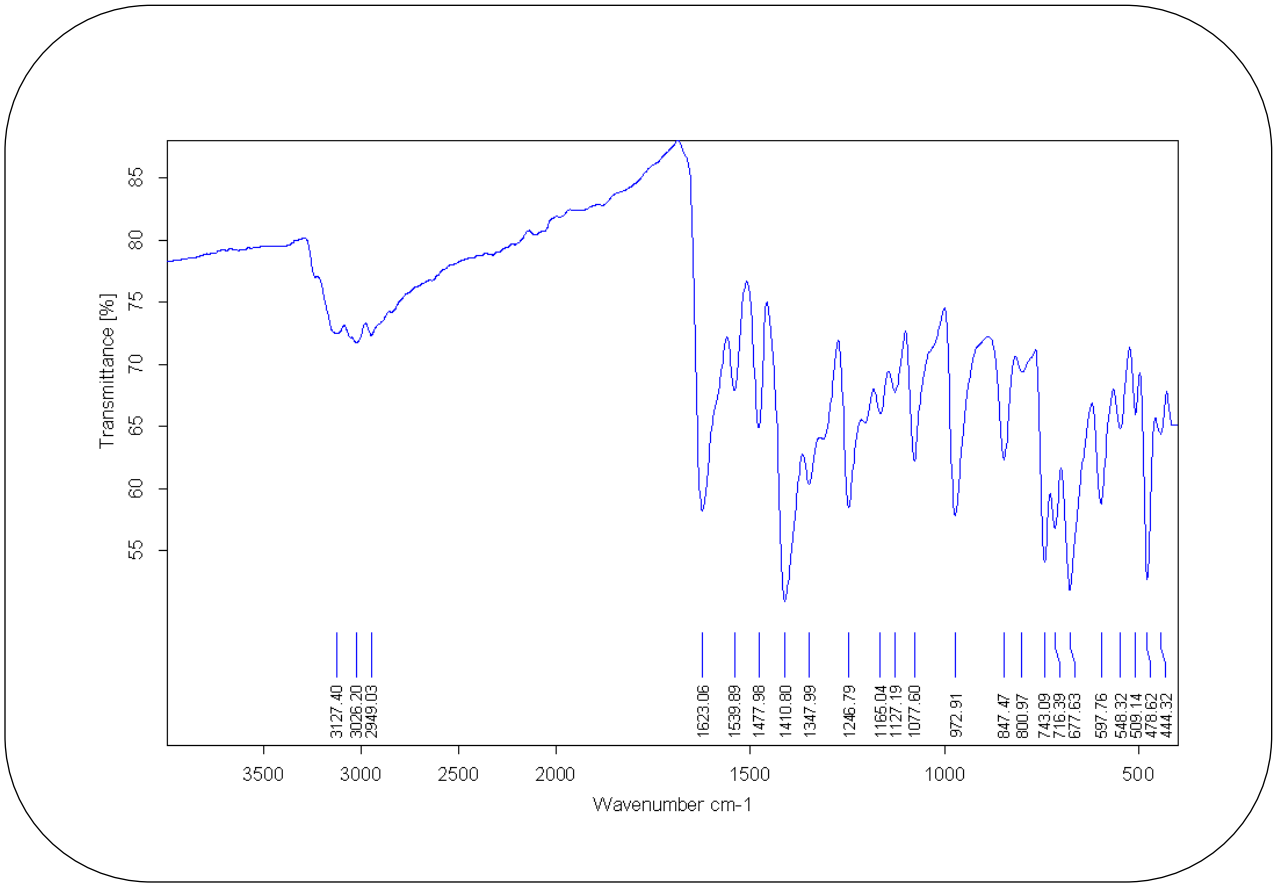
Spectrum 3: IR spectrum of compound 6a



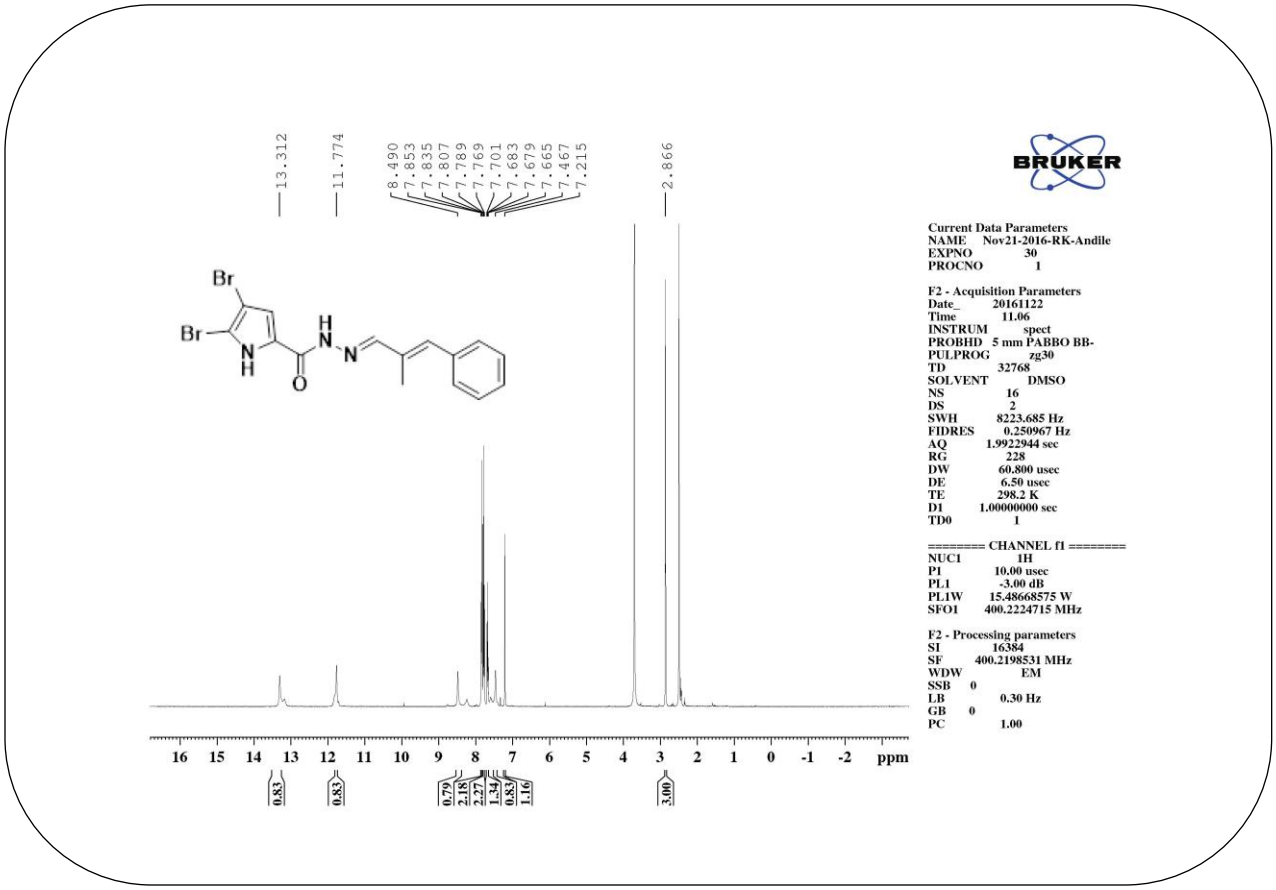
Spectrum 4: 1H NMR spectrum of compound 6b



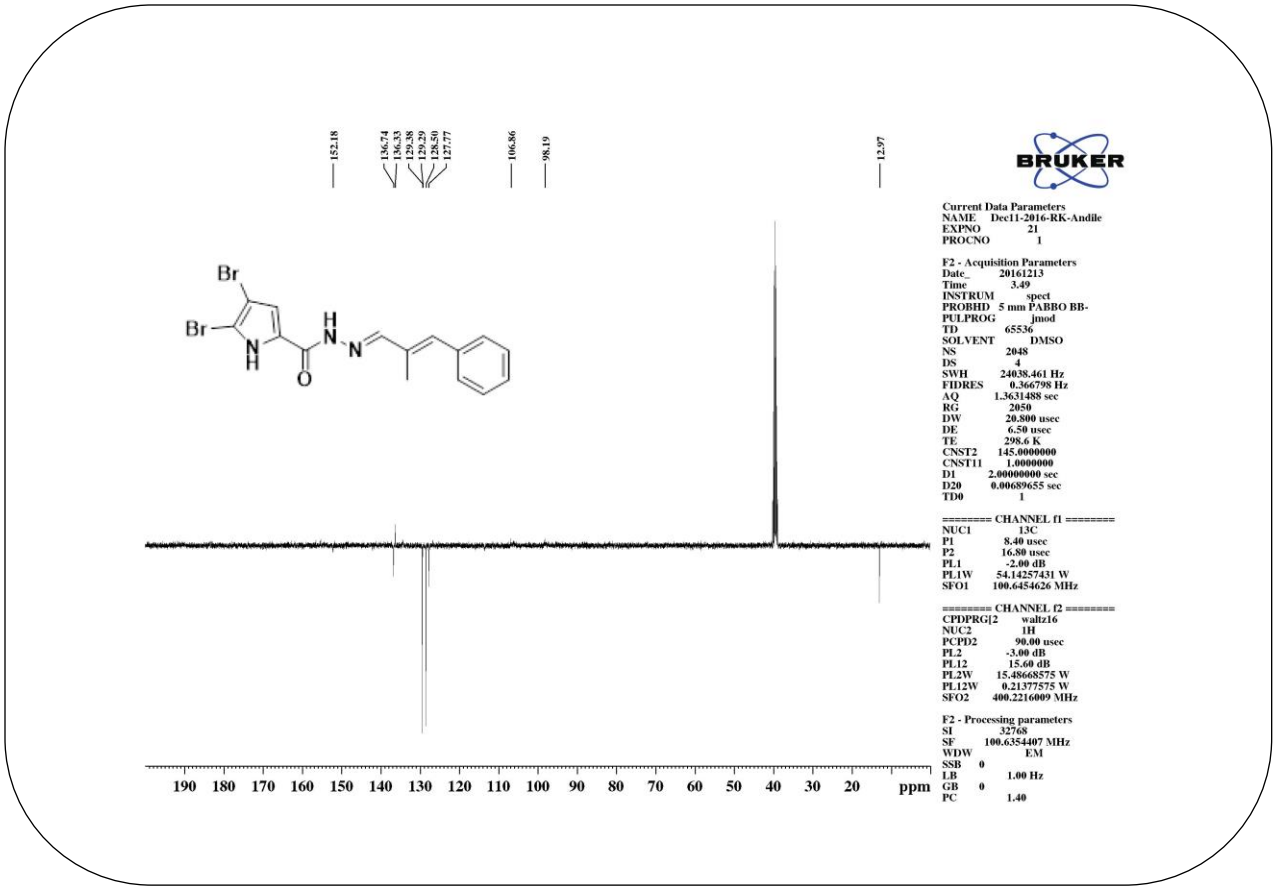
Spectrum 5: 13C NMR spectrum of compound 6b



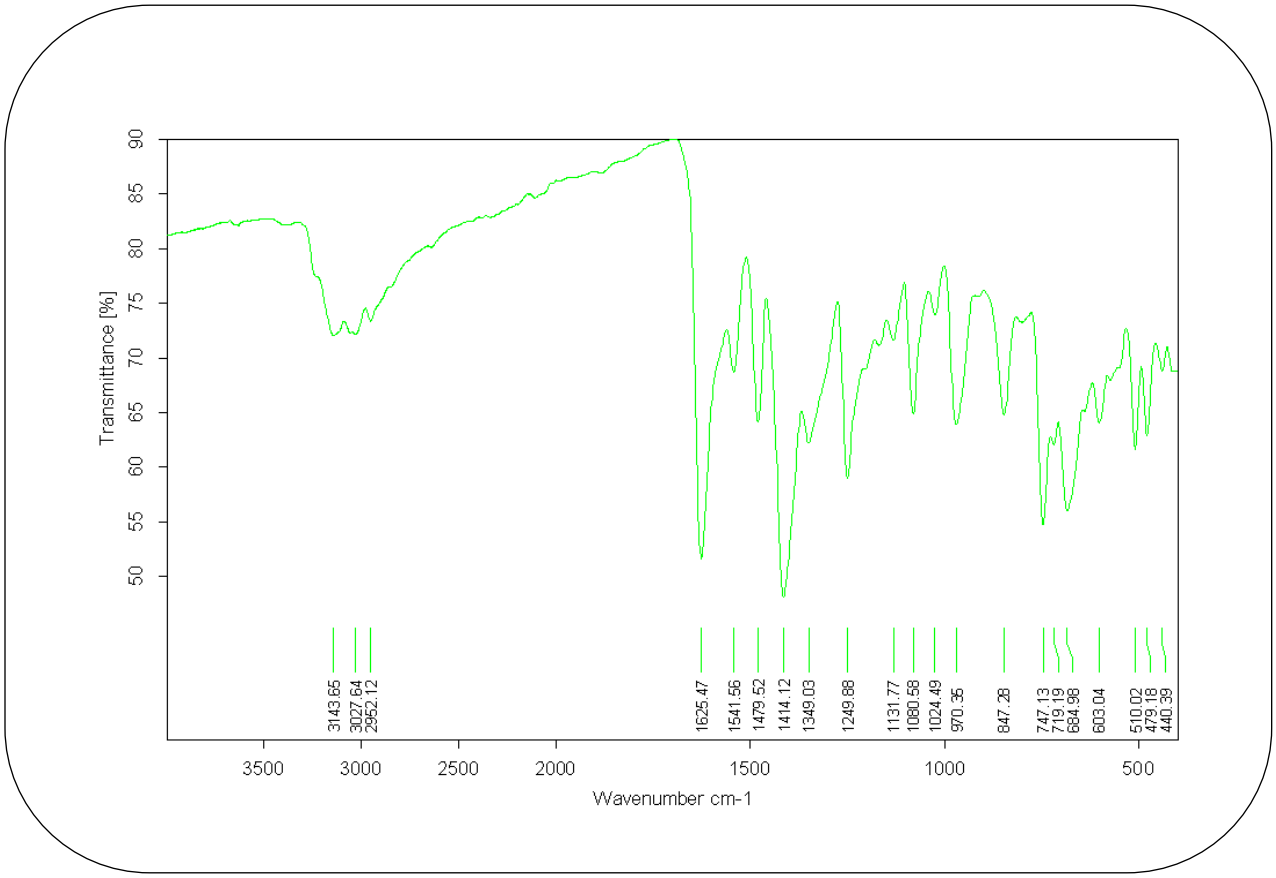
Spectrum 6: IR spectrum of compound 6b



Spectrum 7: 1H NMR spectrum of compound 6c



Spectrum 8: 13C NMR spectrum of compound 6c



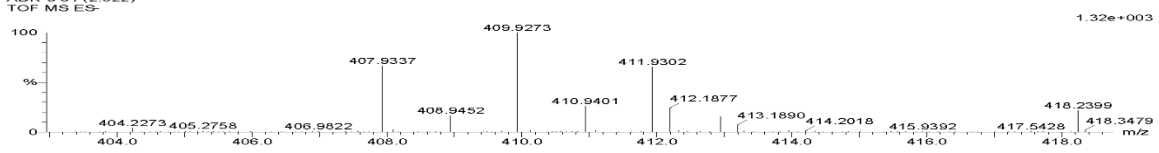
Spectrum 9: IR spectrum of compound 6c

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

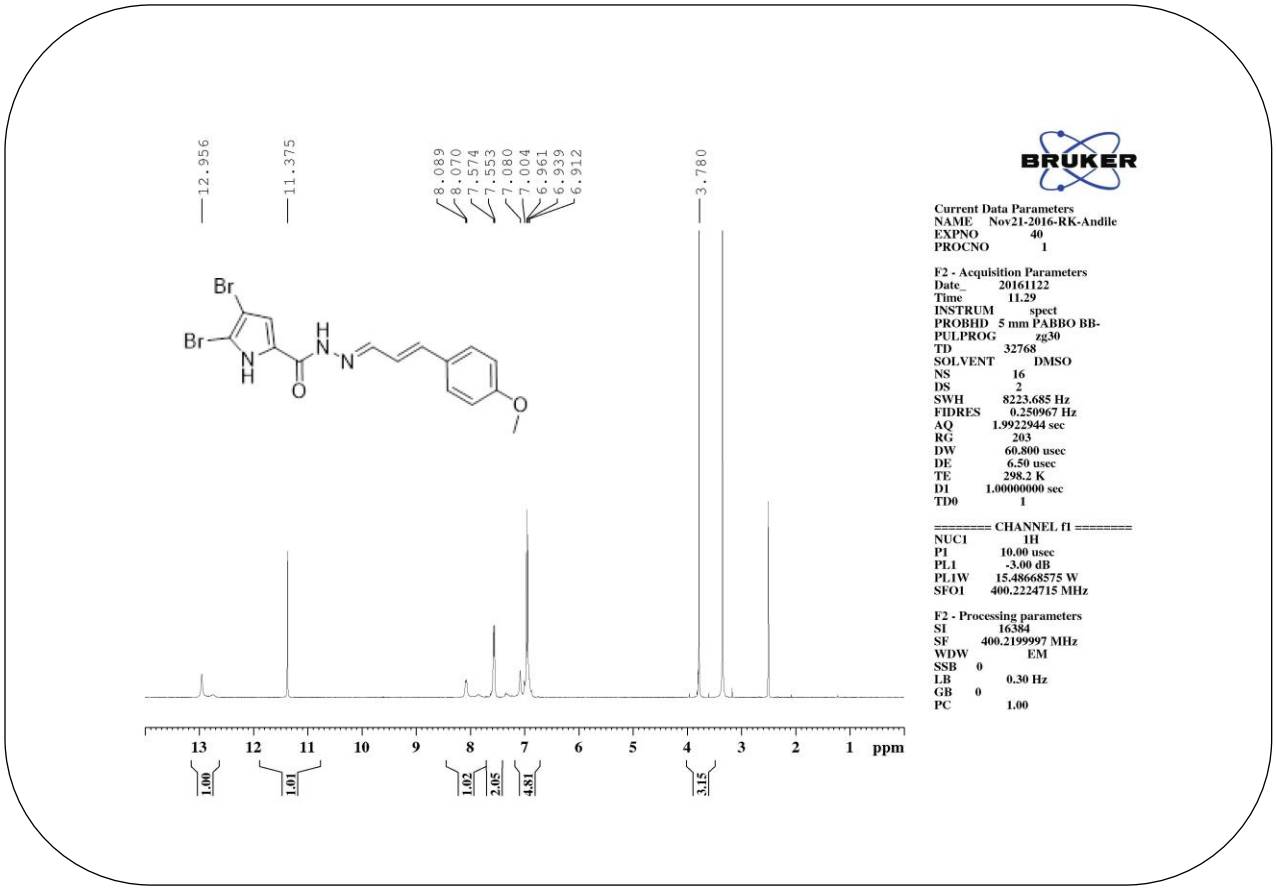
Monoisotopic Mass, Even Electron Ions
150 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 10-15 H: 5-15 N: 0-5 O: 0-5 Br: 0-5

ADR-3 61 (2.022)
TOF MS ES-

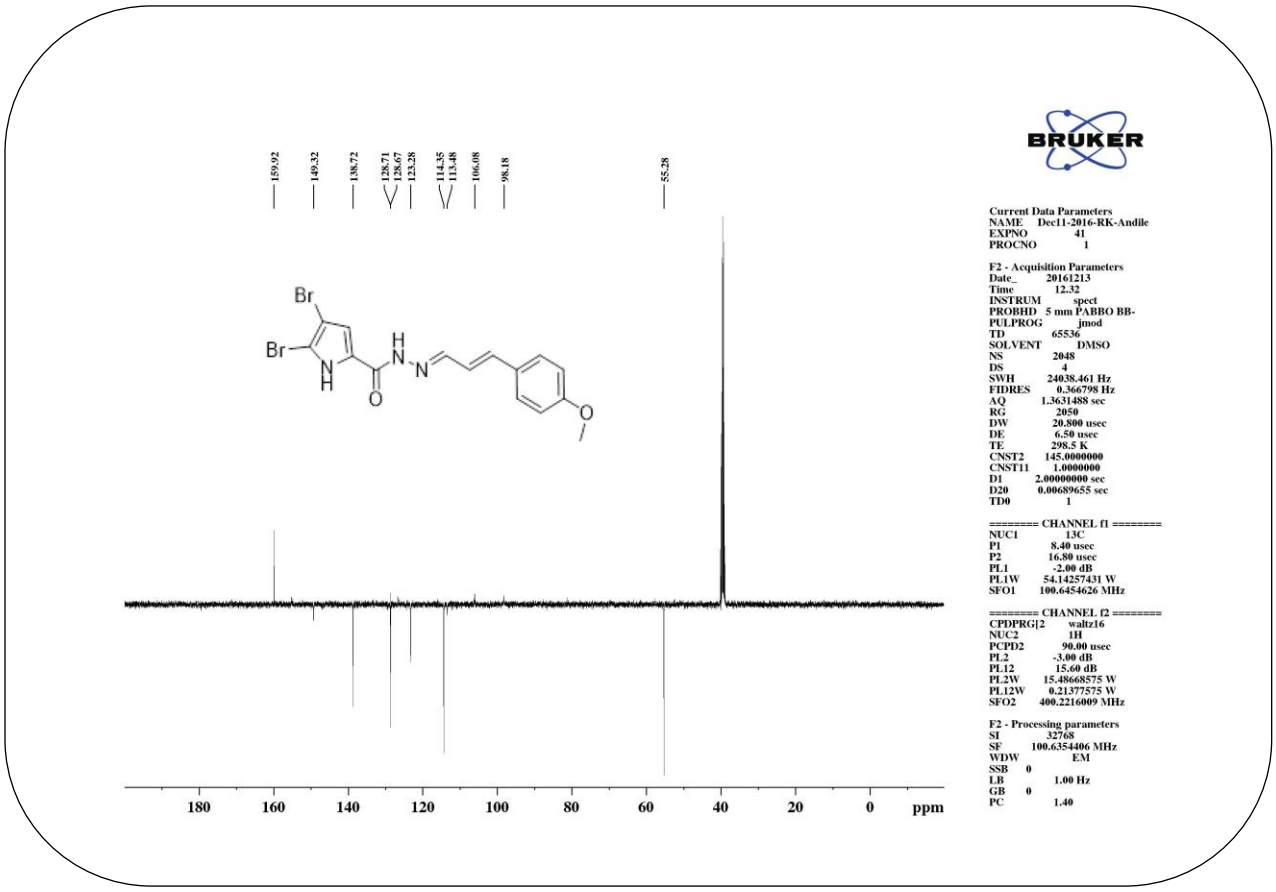


Minimum:			5.0	5.0	-1.5					
Maximum:					100.0					
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula			
407.9337	407.9347	-1.0	-2.5	10.5	64.9	0.0	C15	H12	N3	O Br2

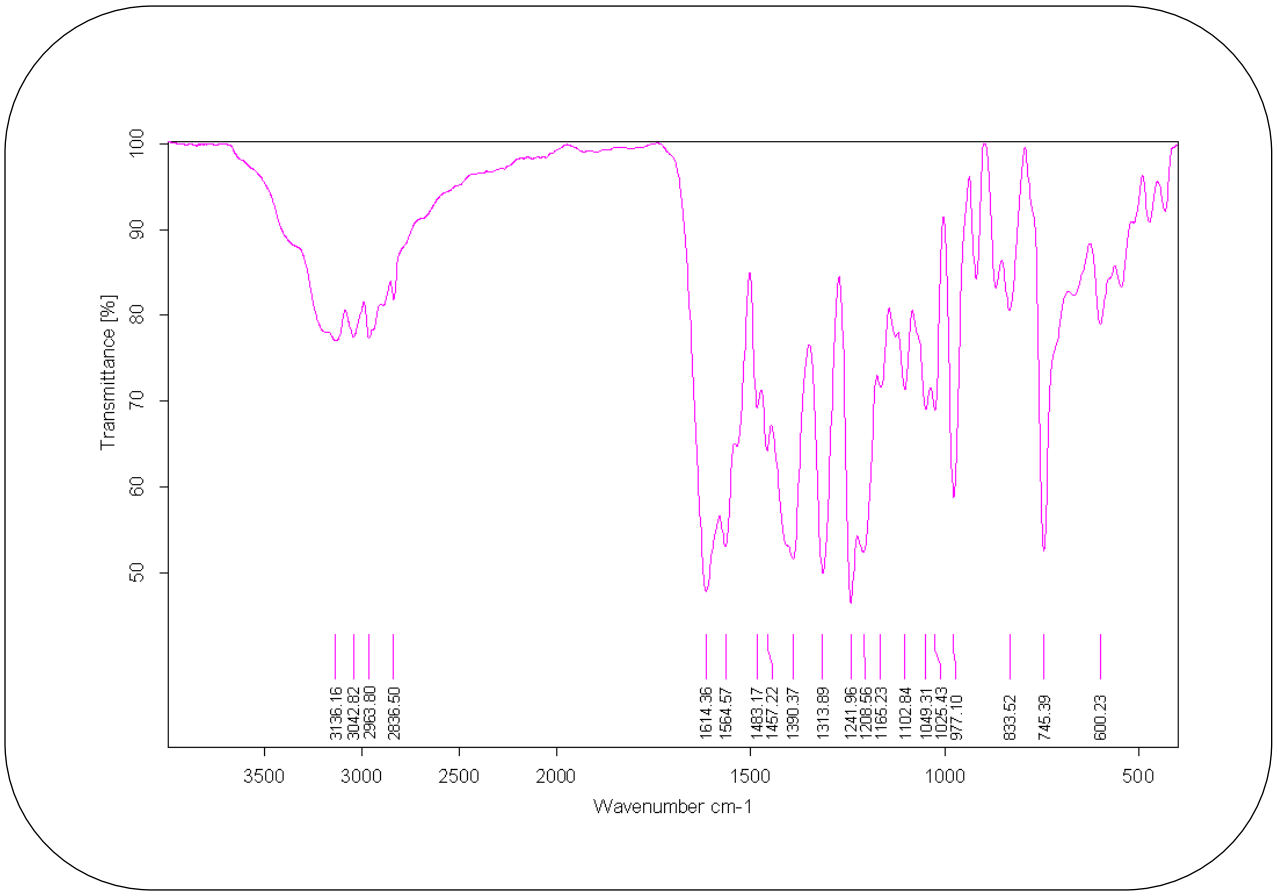
Spectrum 10: Mass spectrum of compound 6c



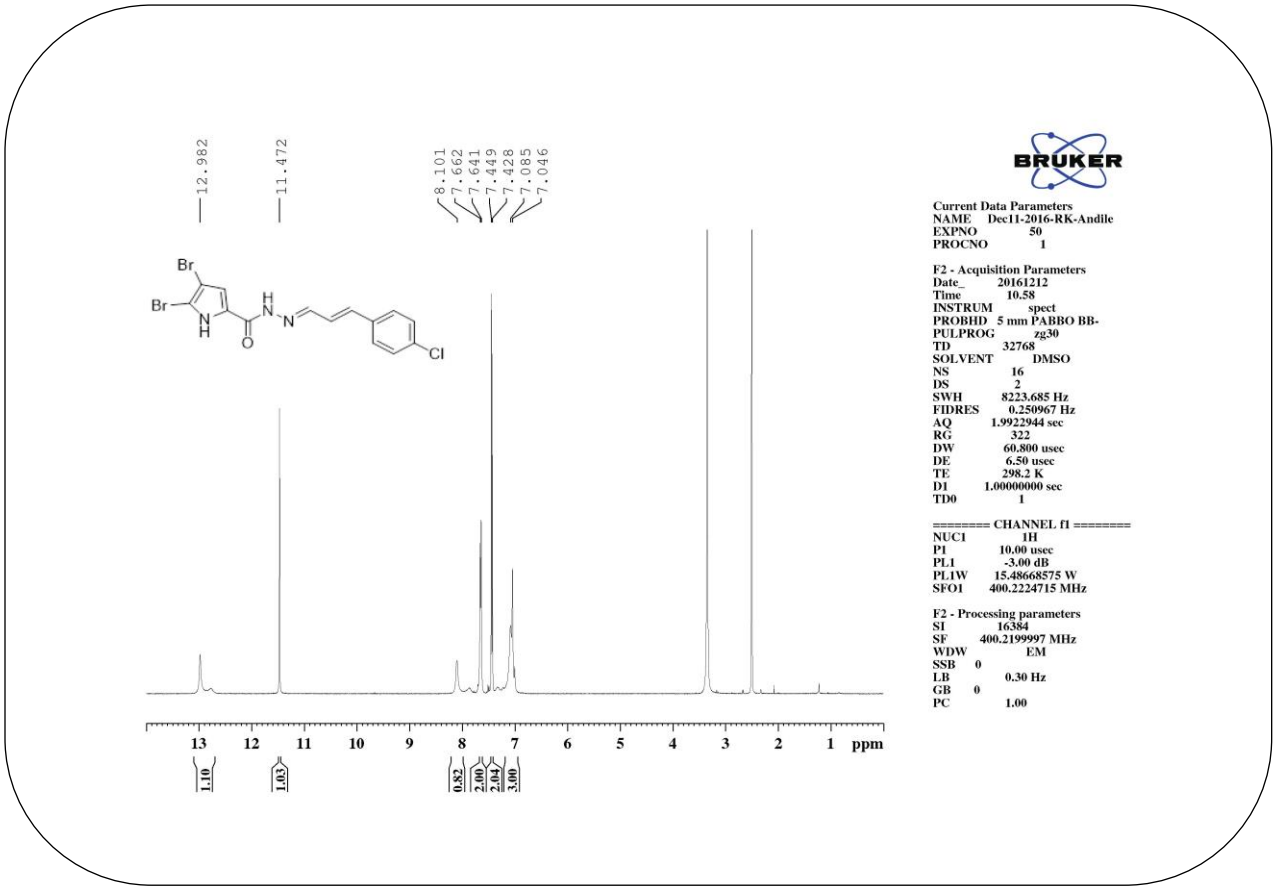
Spectrum 11: 1H NMR spectrum of compound 6d



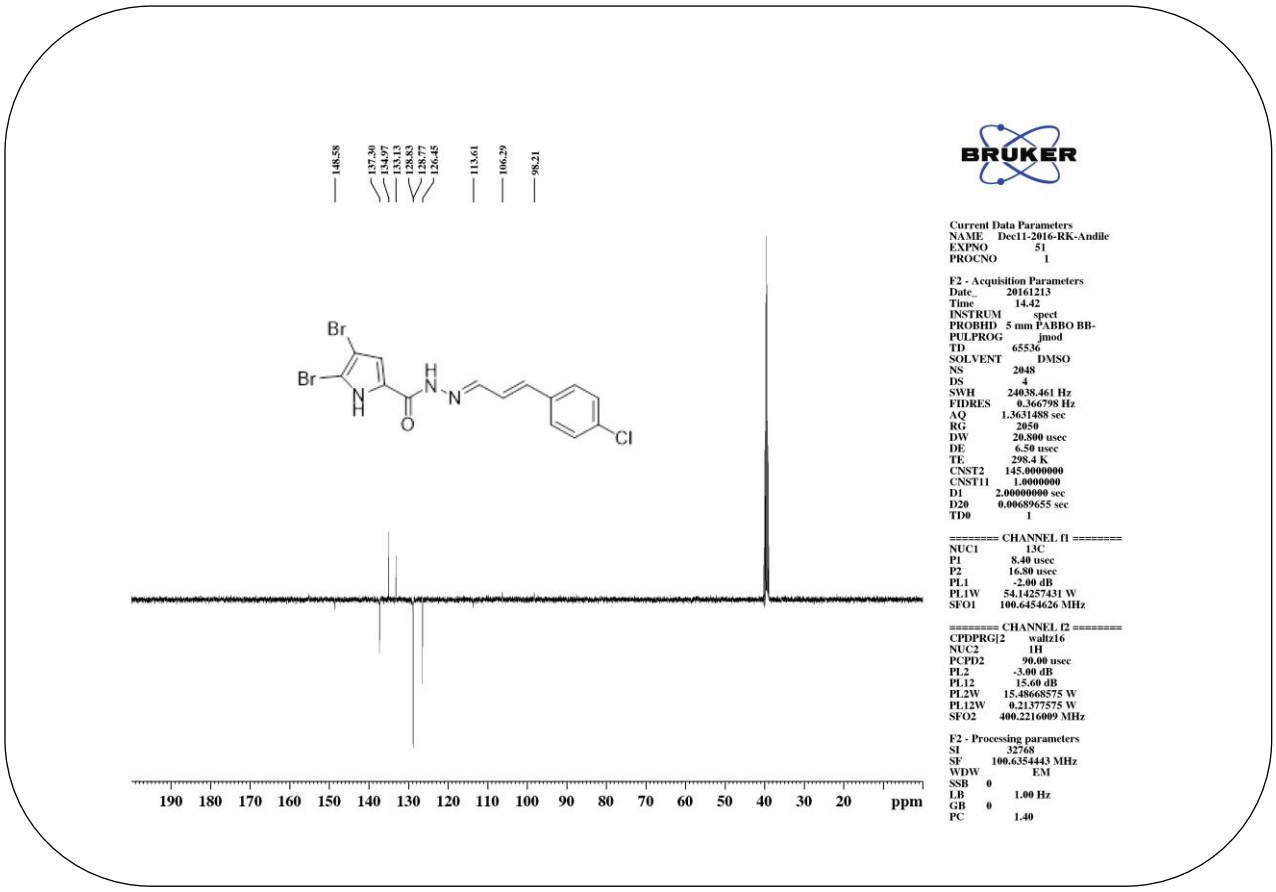
Spectrum 12: 13C NMR spectrum of compound 6d



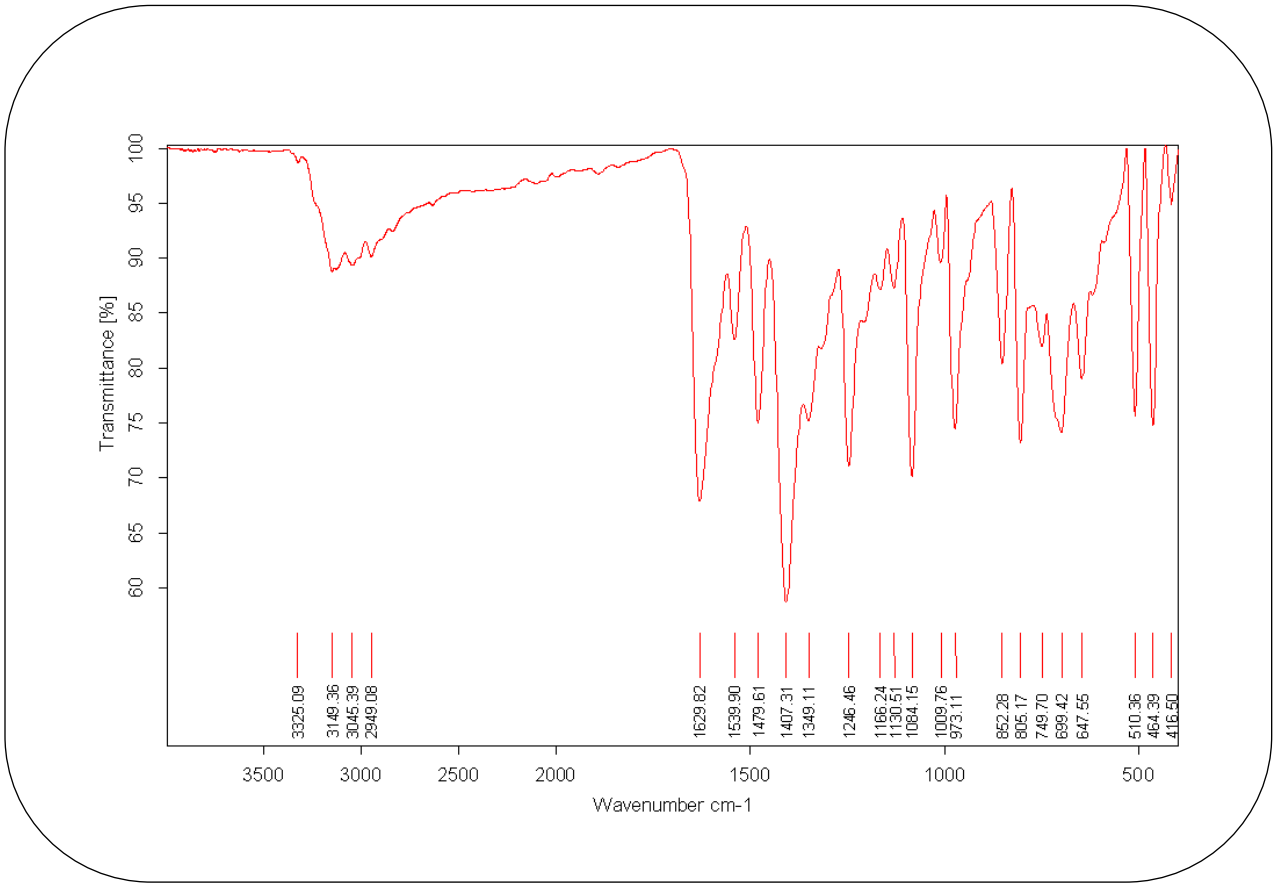
Spectrum 13: IR spectrum of compound 6d



Spectrum 14: 1H NMR spectrum of compound 6e



Spectrum 15: 13C NMR spectrum of compound 6e



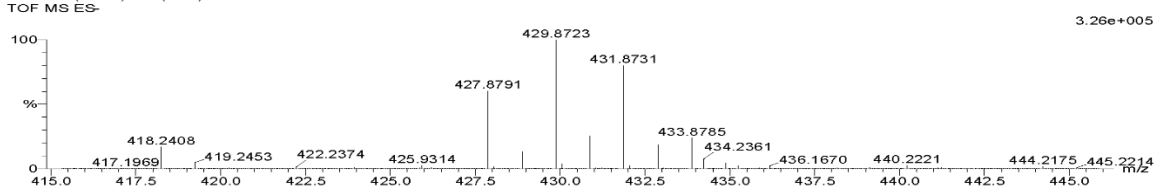
Spectrum 16: IR spectrum of compound 6e

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

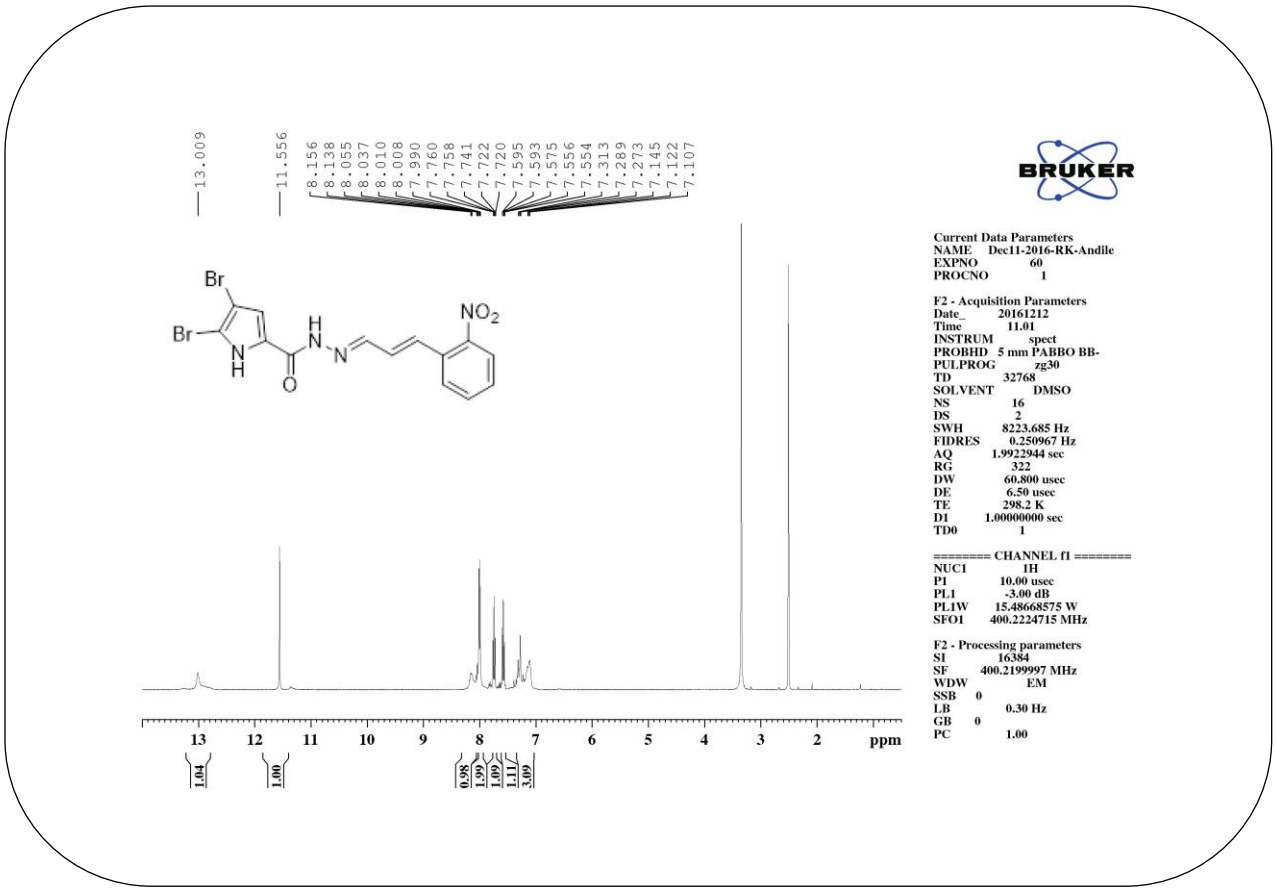
Monoisotopic Mass, Even Electron Ions
186 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 10-15 H: 5-15 N: 0-5 O: 0-5 Cl: 0-1 Br: 0-2

ADR-7 2 (0.034) Cm (1:61)
TOF MS ES-

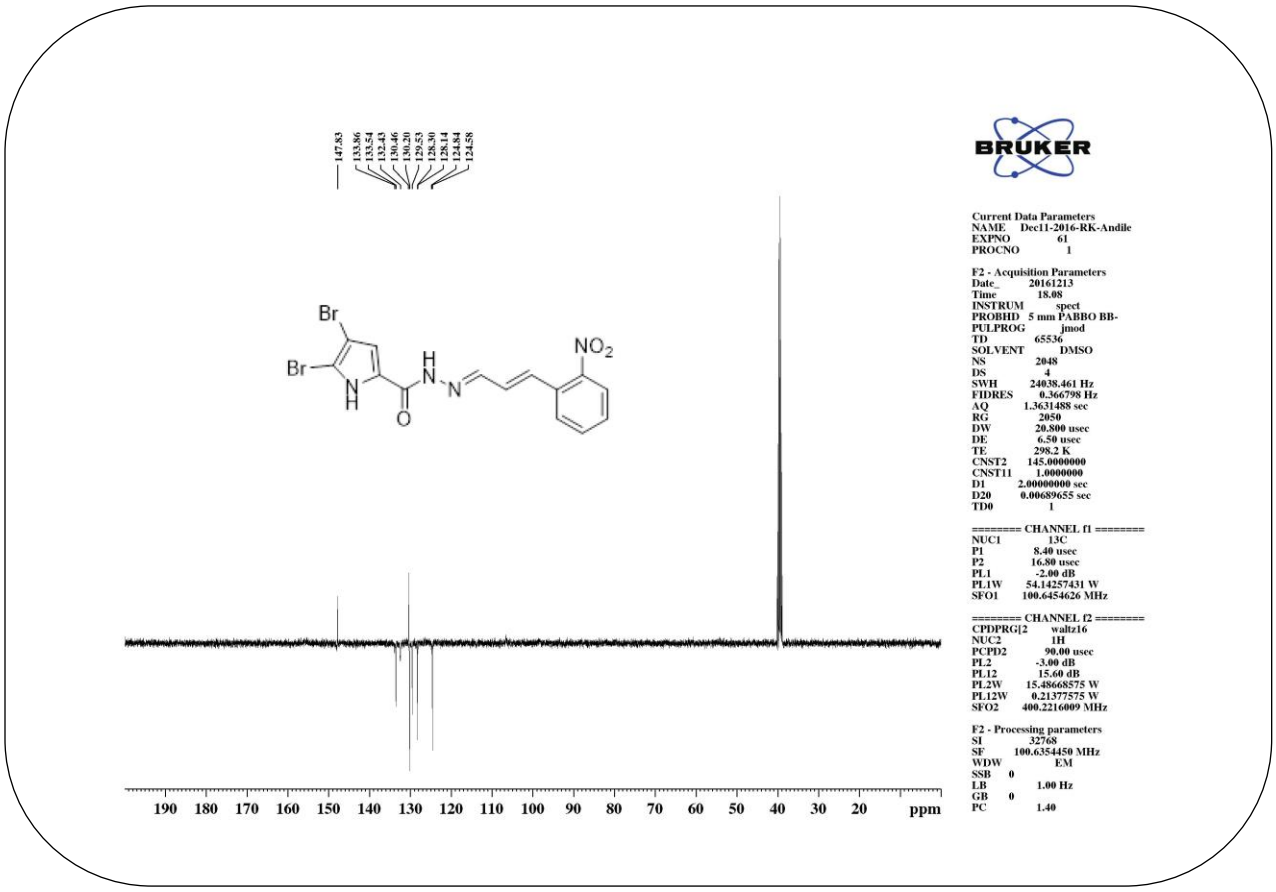


Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
427.8791	427.8801	-1.0	-2.3	10.5	551.3	0.0	C14 H9 N3 O Cl Br2

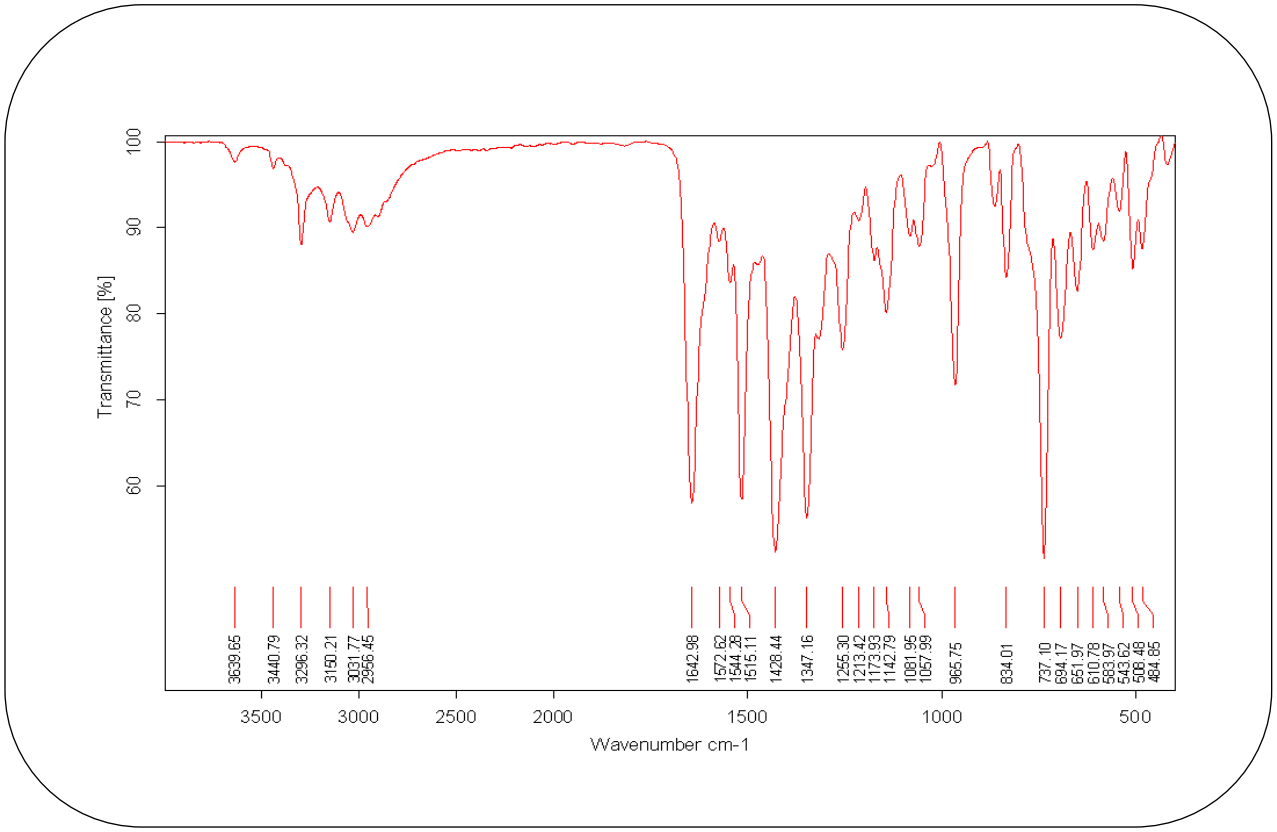
Spectrum 17: Mass spectrum of compound 6e



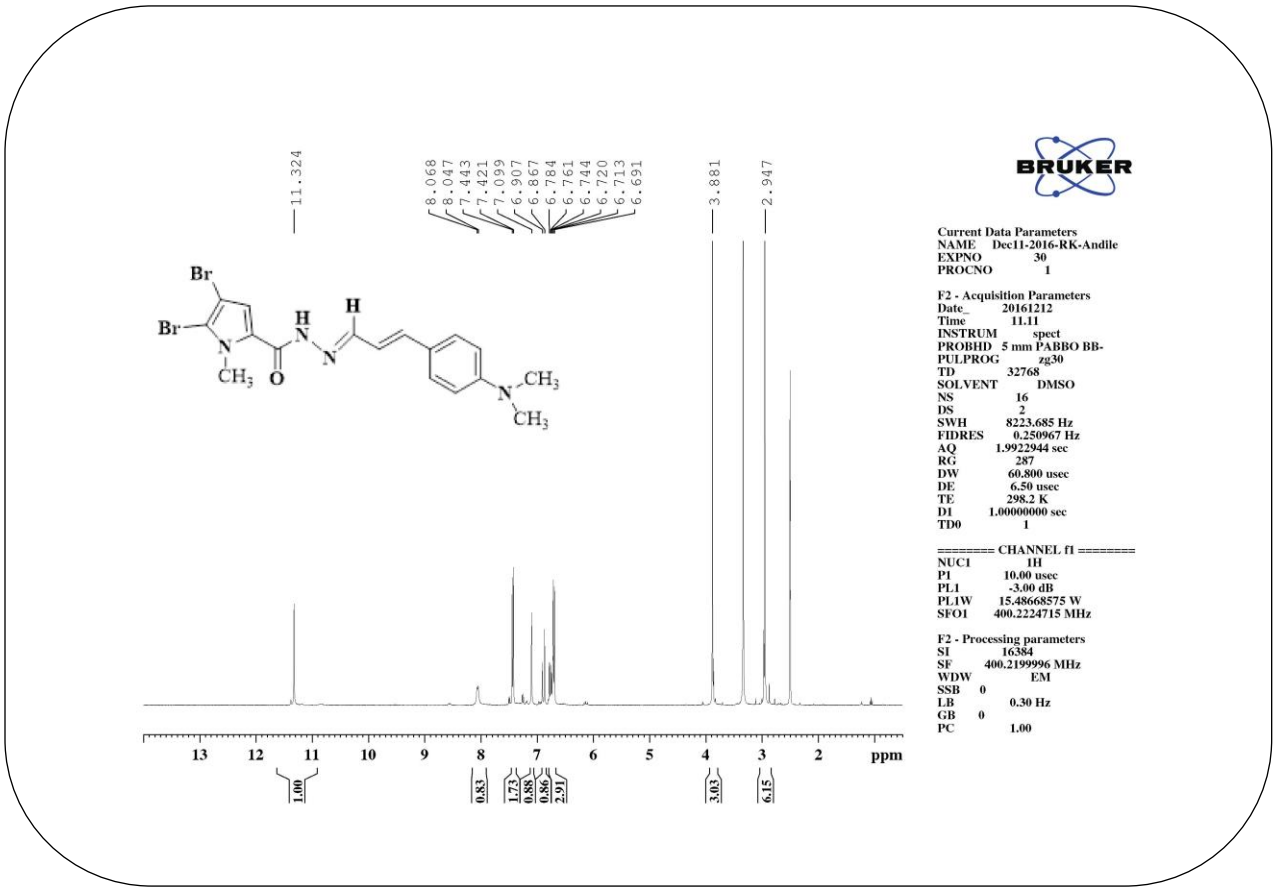
Spectrum 18: 1H NMR spectrum of compound 6f



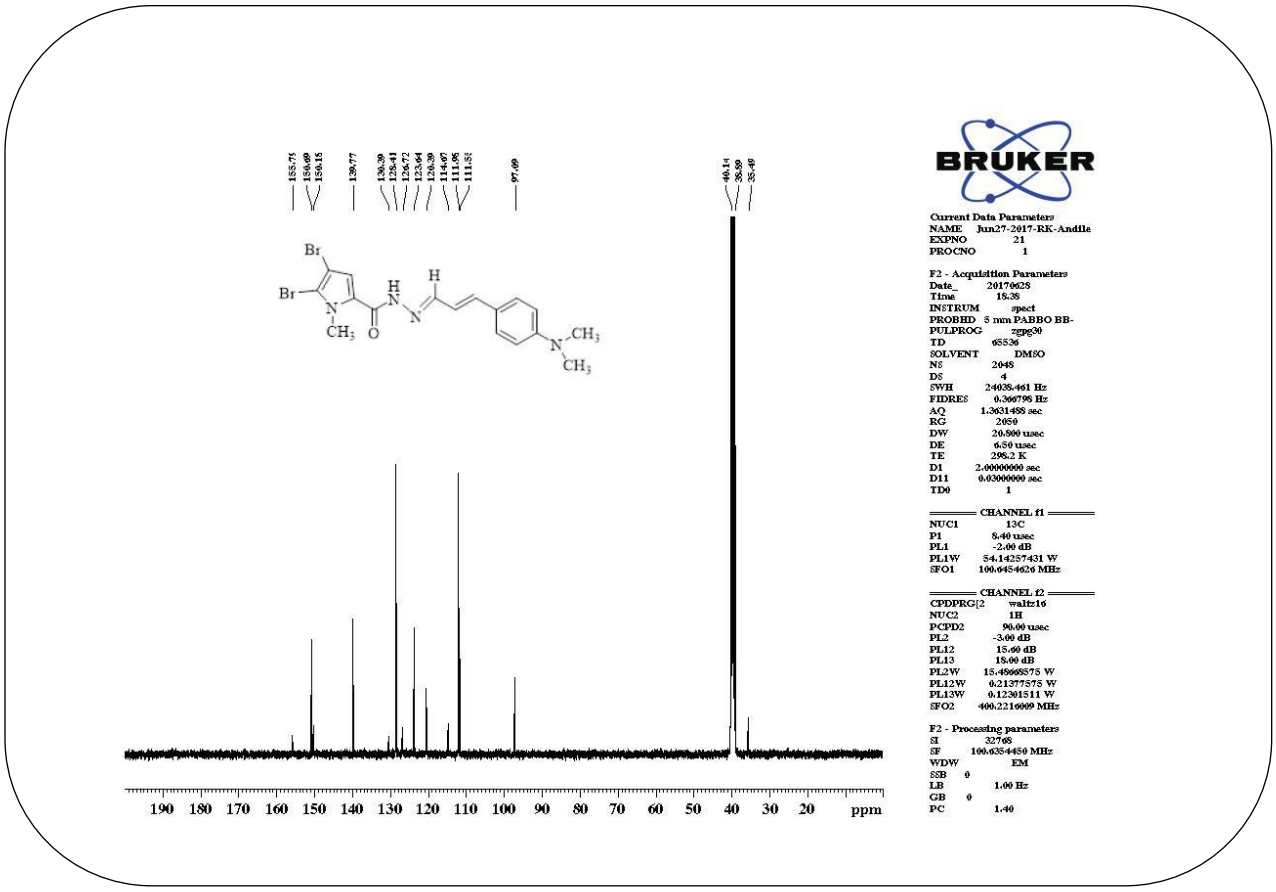
Spectrum 19: 13C NMR spectrum of compound 6f



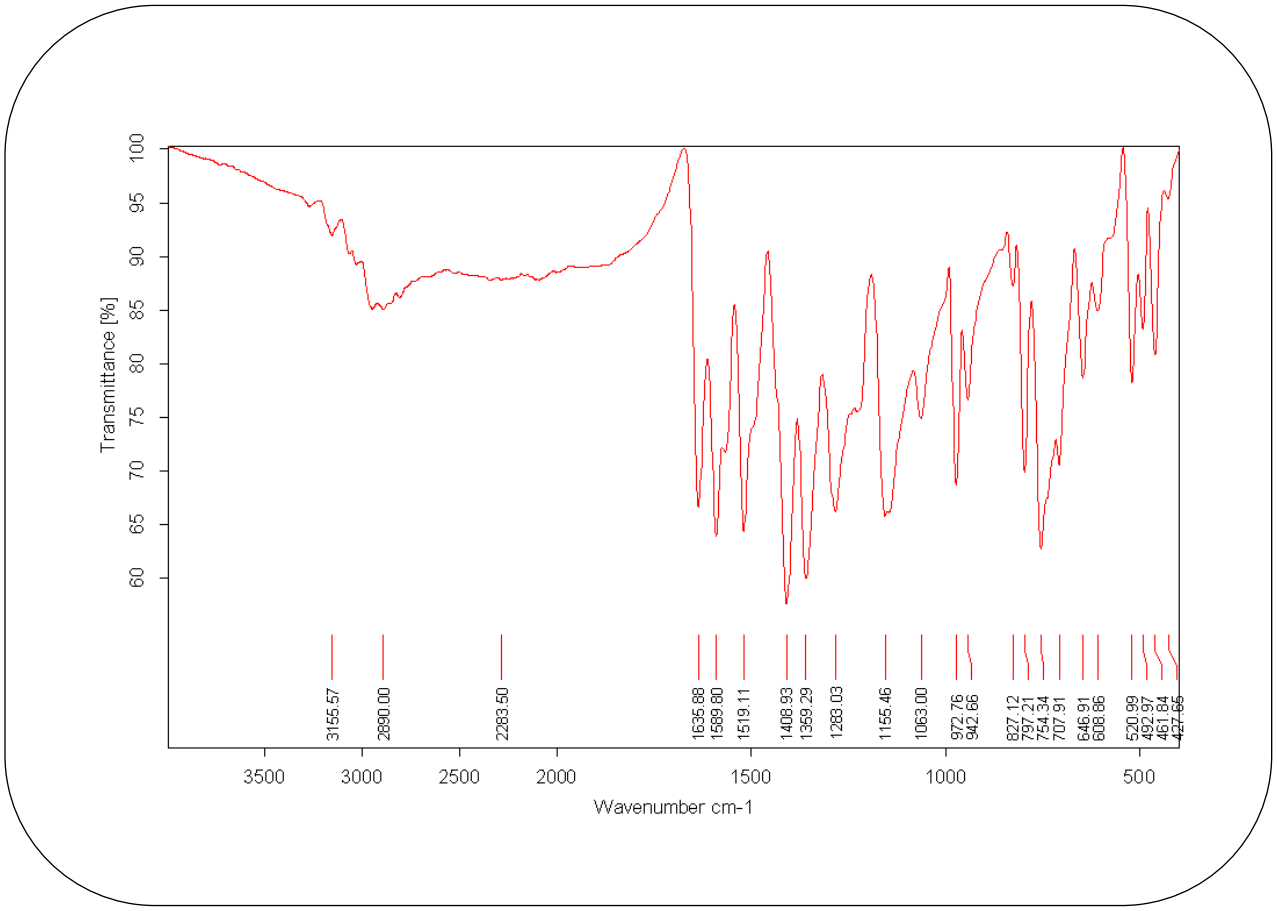
Spectrum 20: IR spectrum of compound 6f



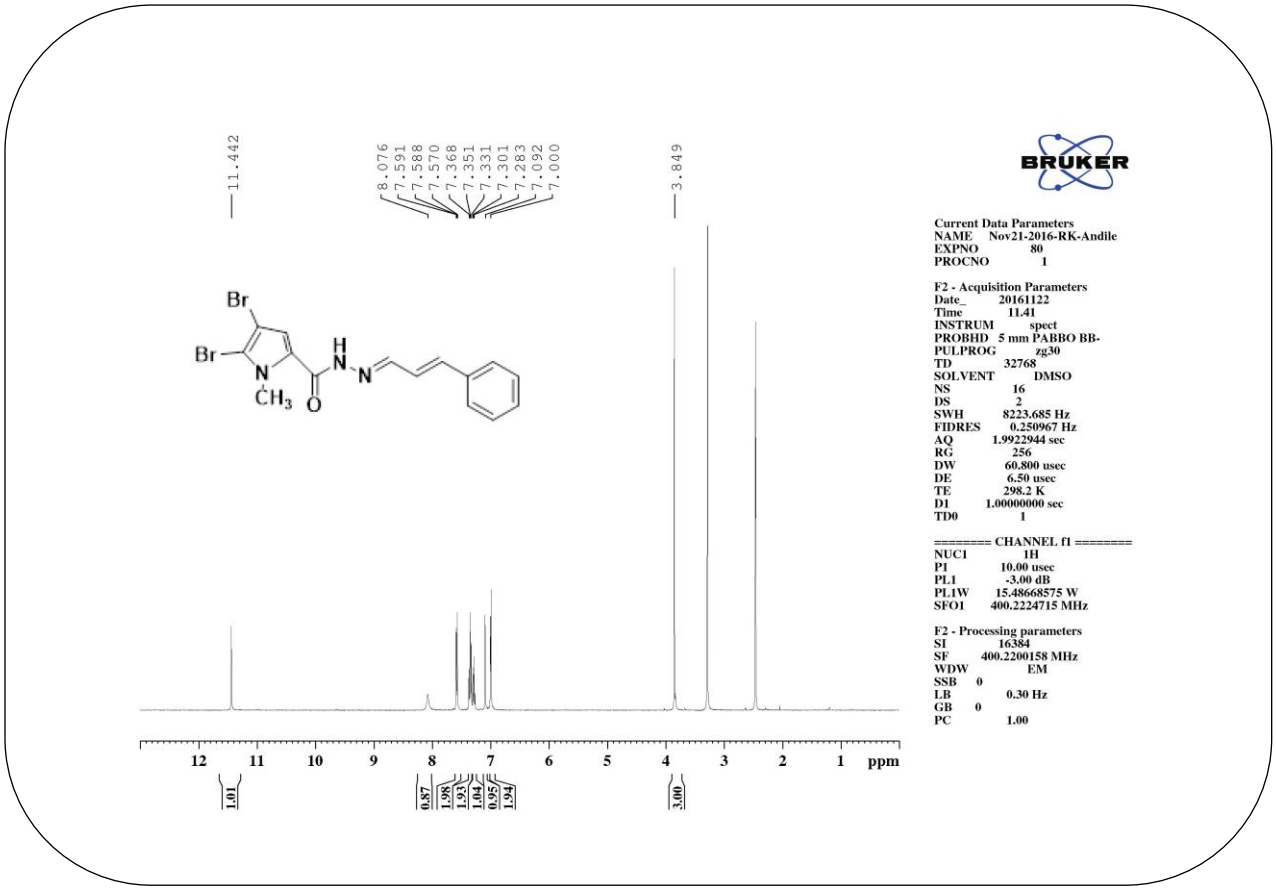
Spectrum 21: 1H NMR spectrum of compound 6g



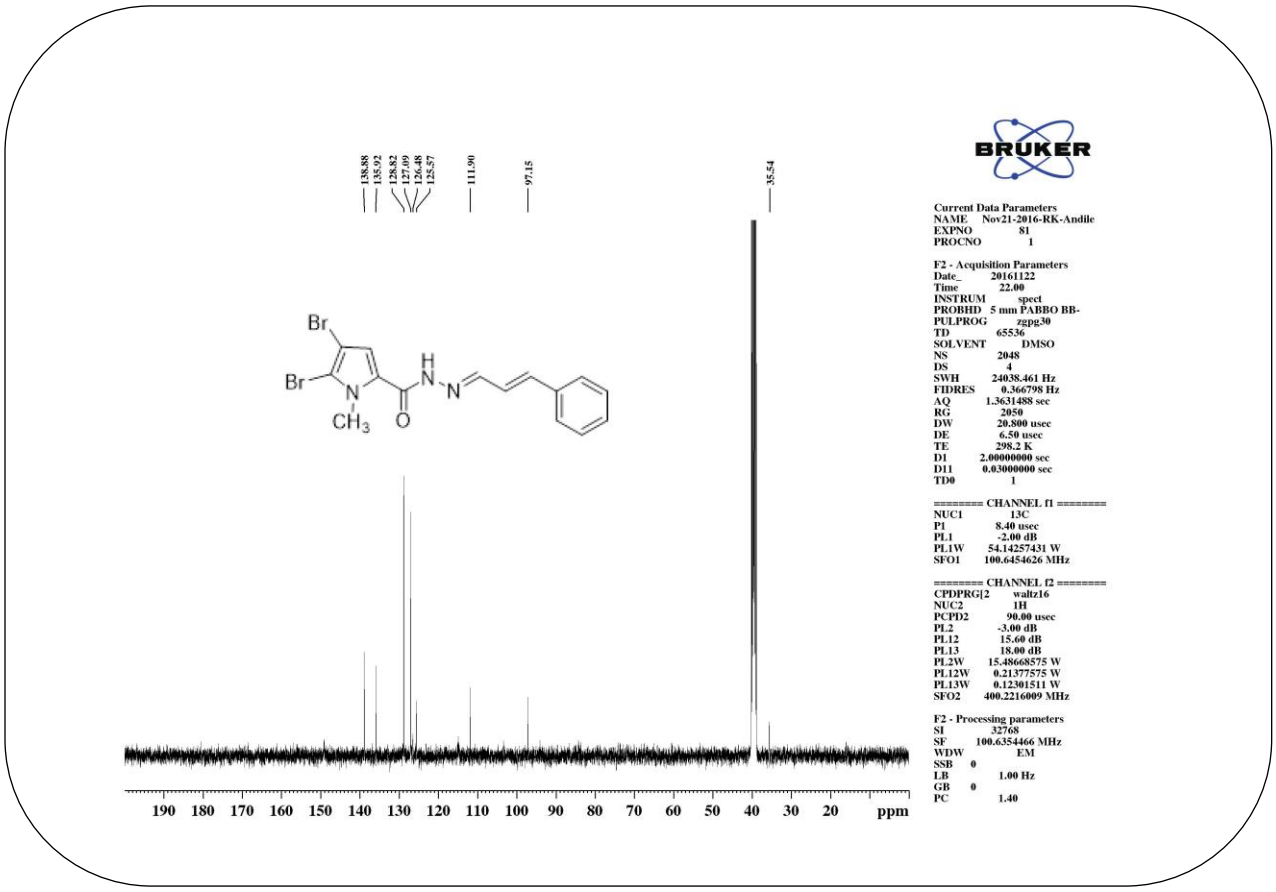
Spectrum 22: 13C NMR spectrum of compound 6g



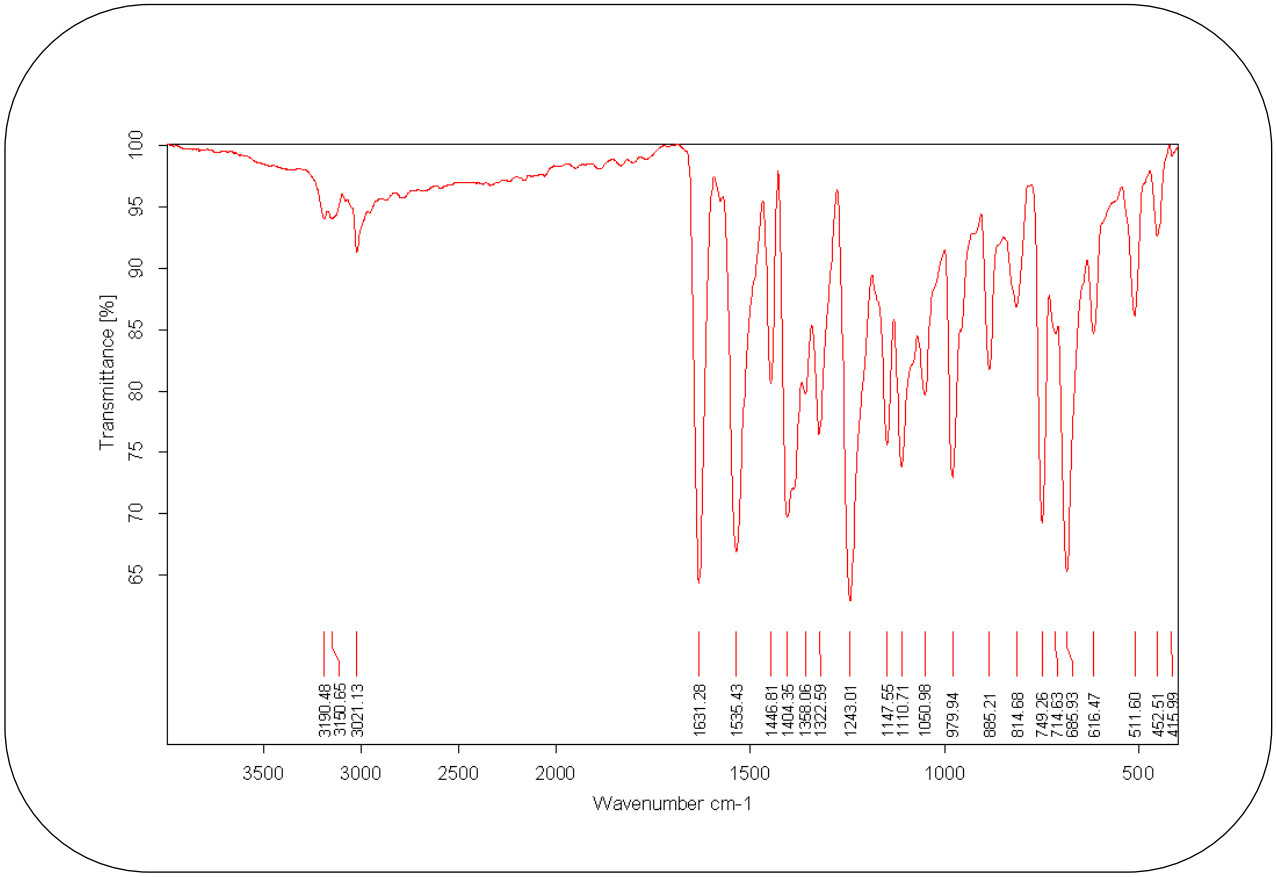
Spectrum 23: IR spectrum of compound 6g



Spectrum 24: 1H NMR spectrum of compound 6h



Spectrum 25: 13C NMR spectrum of compound 6h



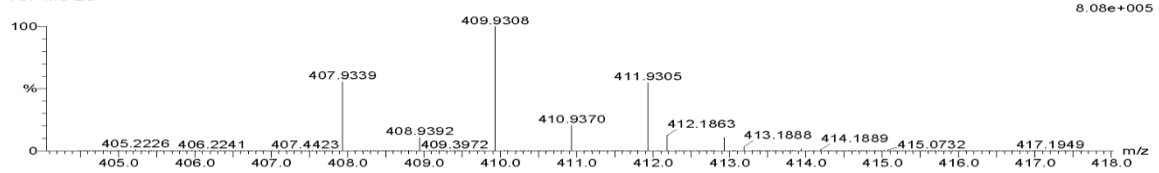
Spectrum 26: IR spectrum of compound 6h

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

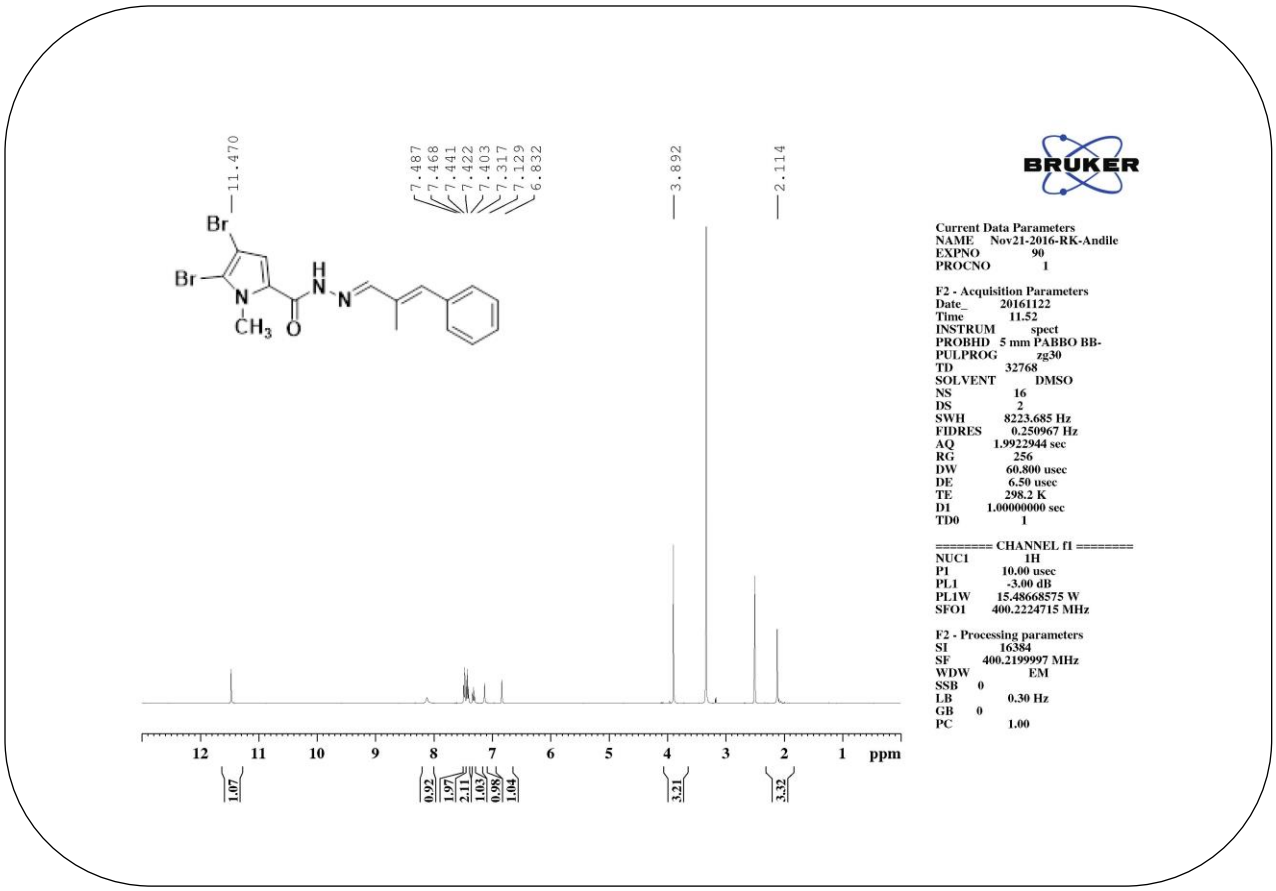
Monoisotopic Mass, Even Electron Ions
70 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-5 Br: 0-2

ADR-12 45 (1.517) Cm (1:60)
TOF MS ES-

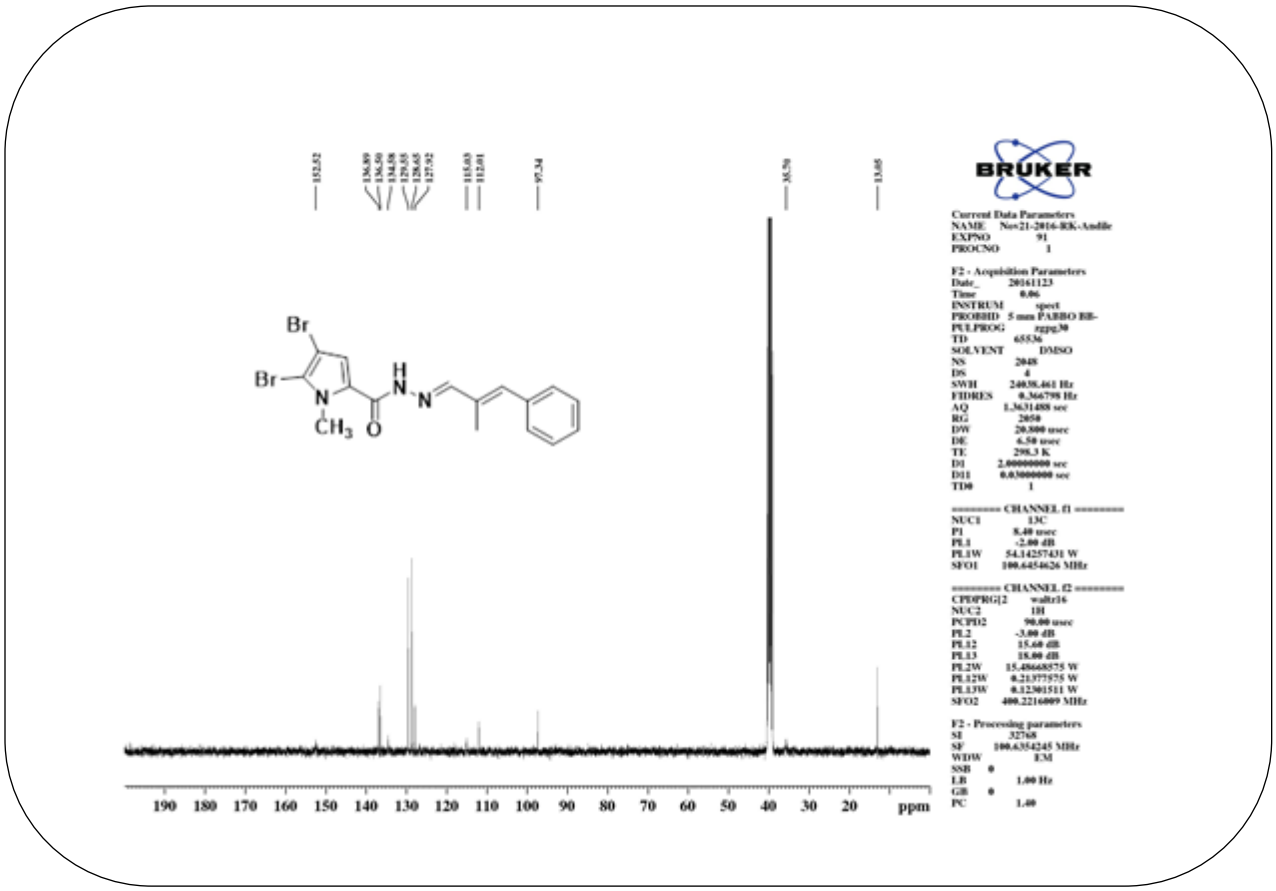


Minimum:			5.0	5.0	-1.5			
Maximum:					100.0			
Mass	Calc. Mass		mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
407.9339	407.9347		-0.8	-2.0	10.5	529.8	0.0	C15 H12 N3 O Br2

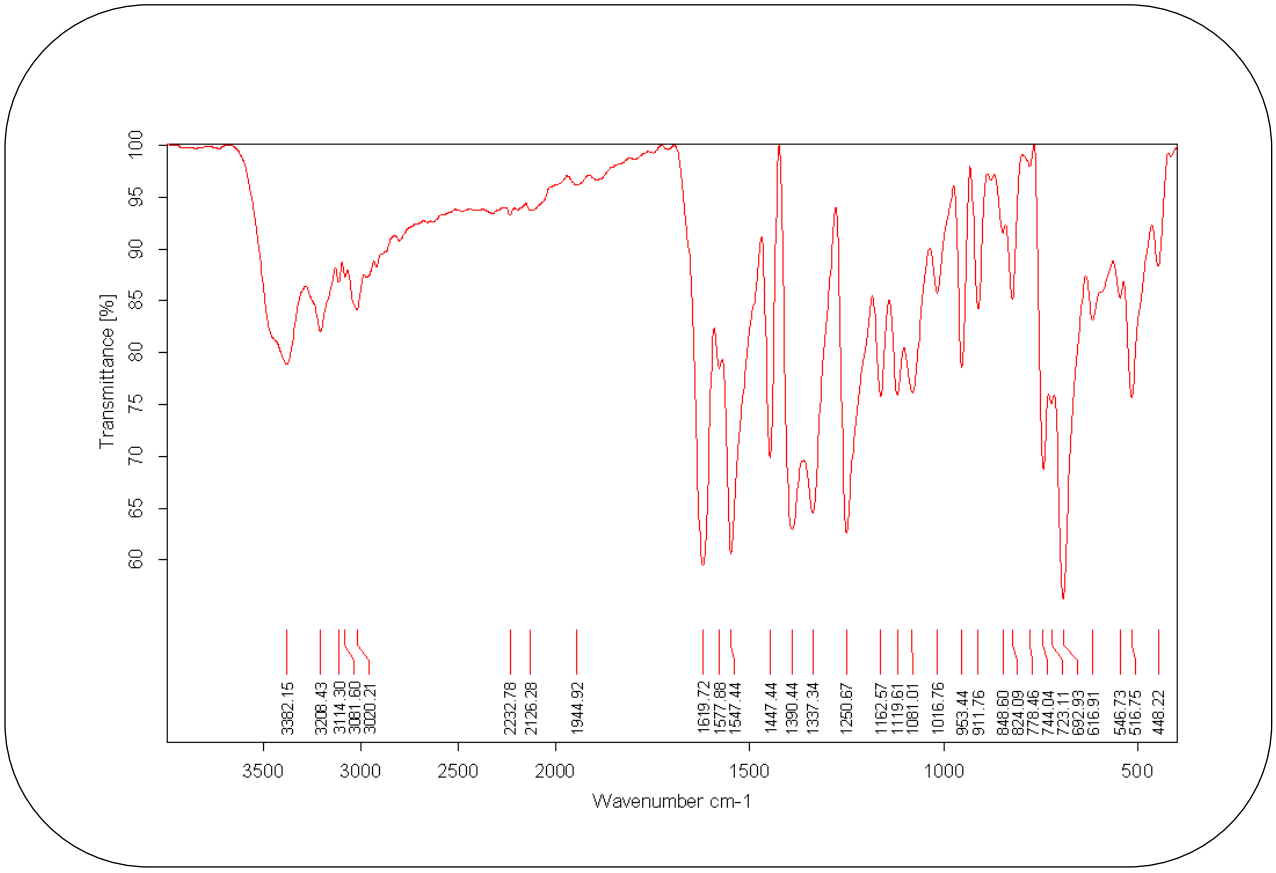
Spectrum 27: Mass spectrum of compound 6h



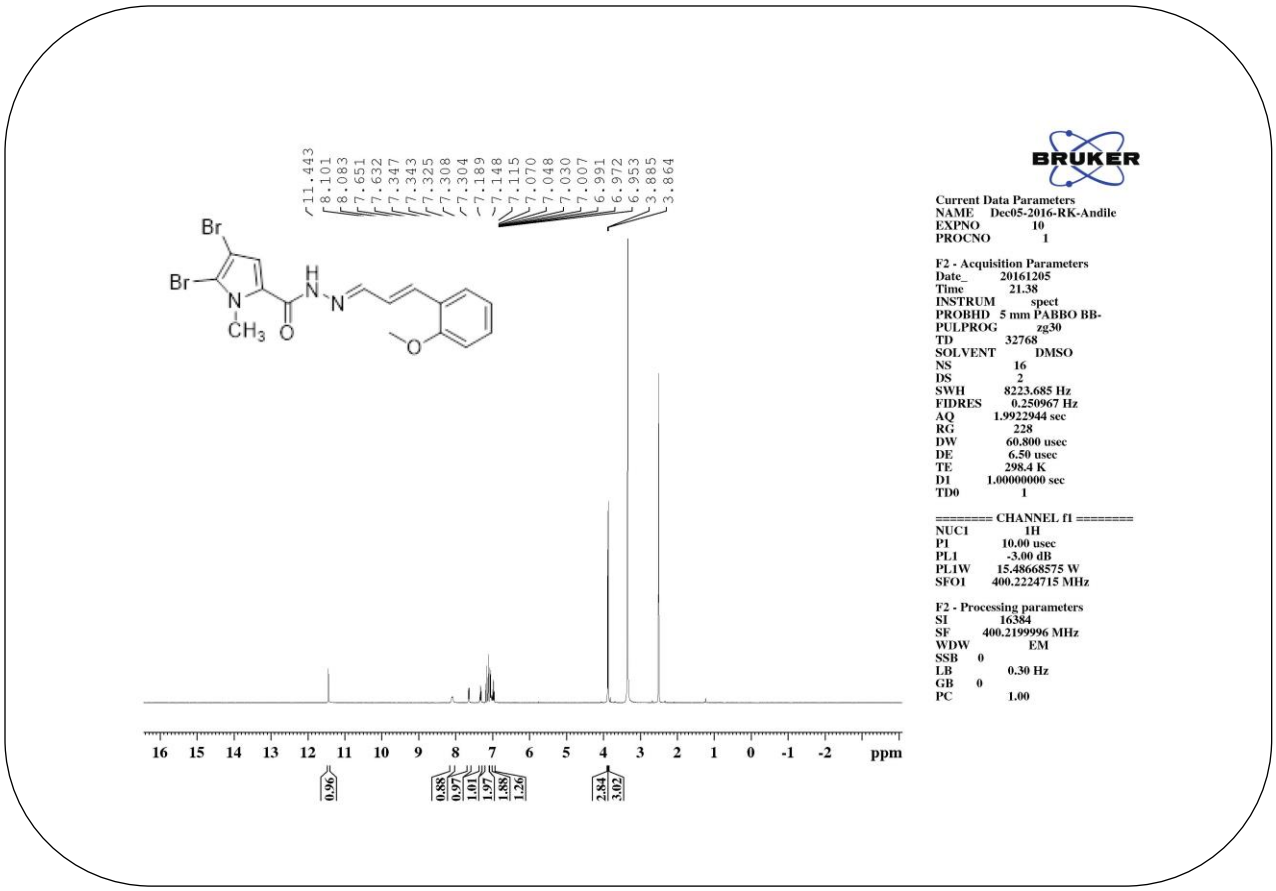
Spectrum 28: 1H NMR spectrum of compound 6i



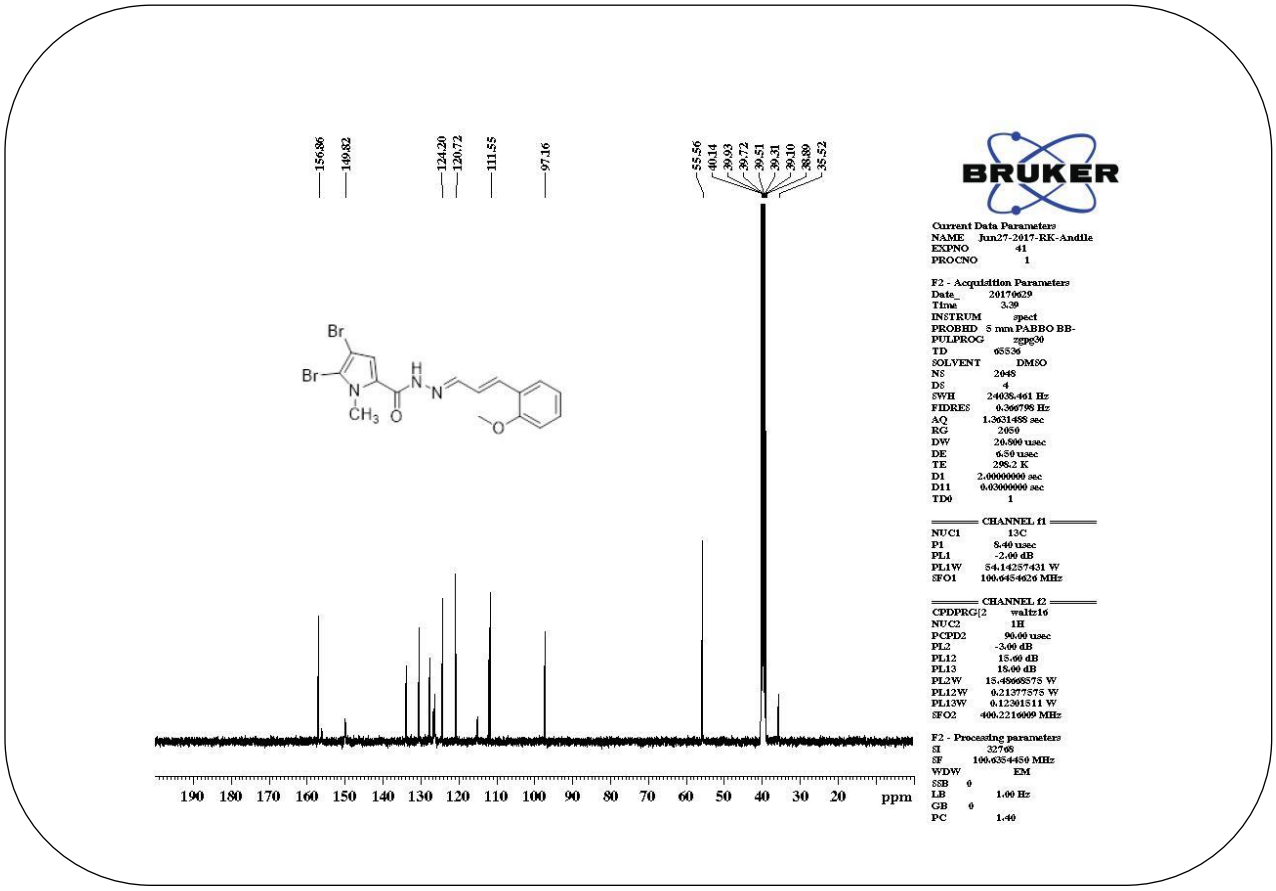
Spectrum 29: 13C NMR spectrum of compound 6i



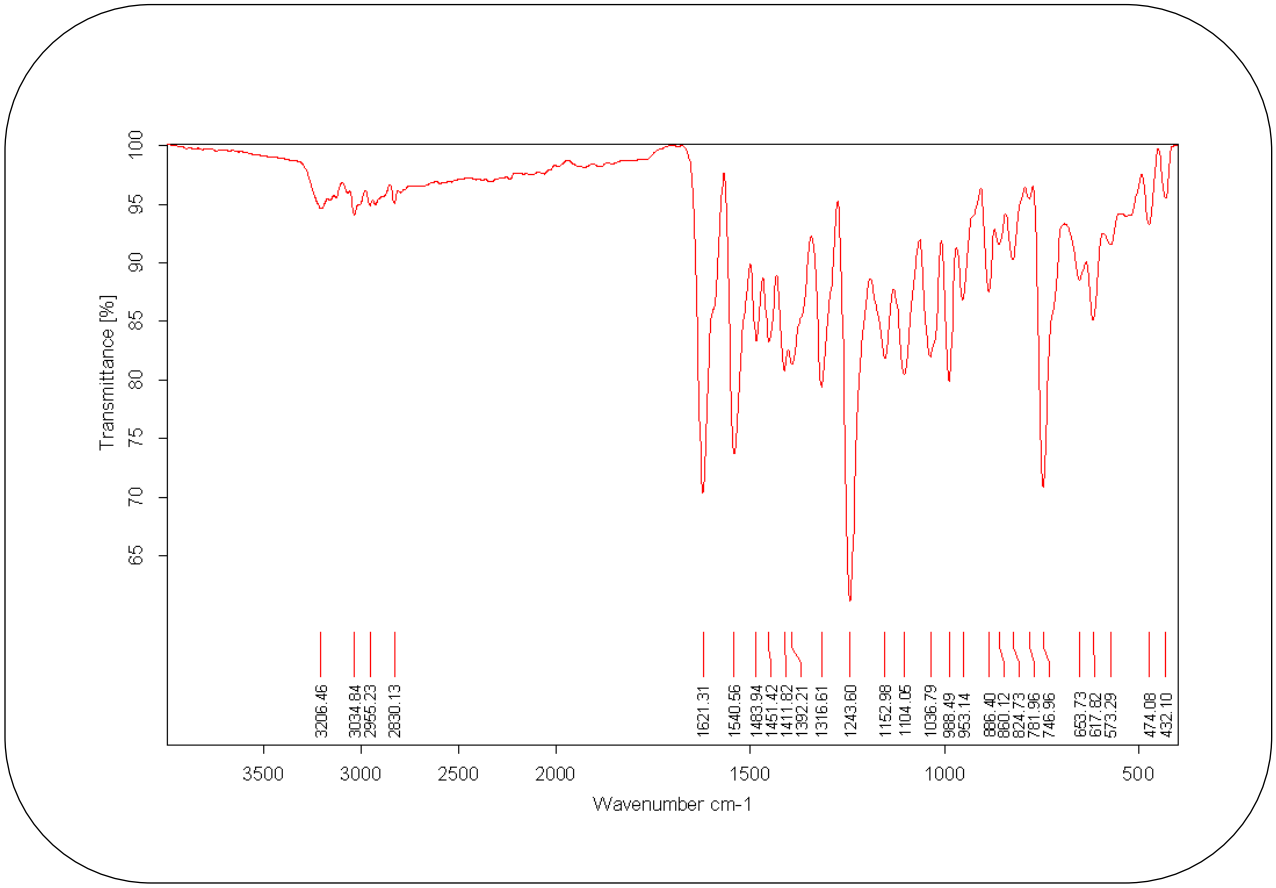
Spectrum 30: IR spectrum of compound 4i



Spectrum 31: 1H NMR spectrum of compound 6j



Spectrum 32: 13C NMR spectrum of compound 6j



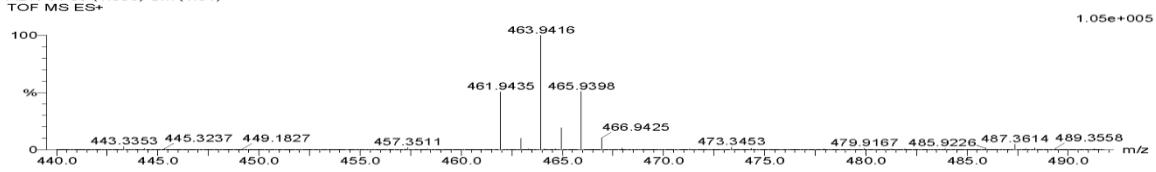
Spectrum 33: IR spectrum of compound 6j

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

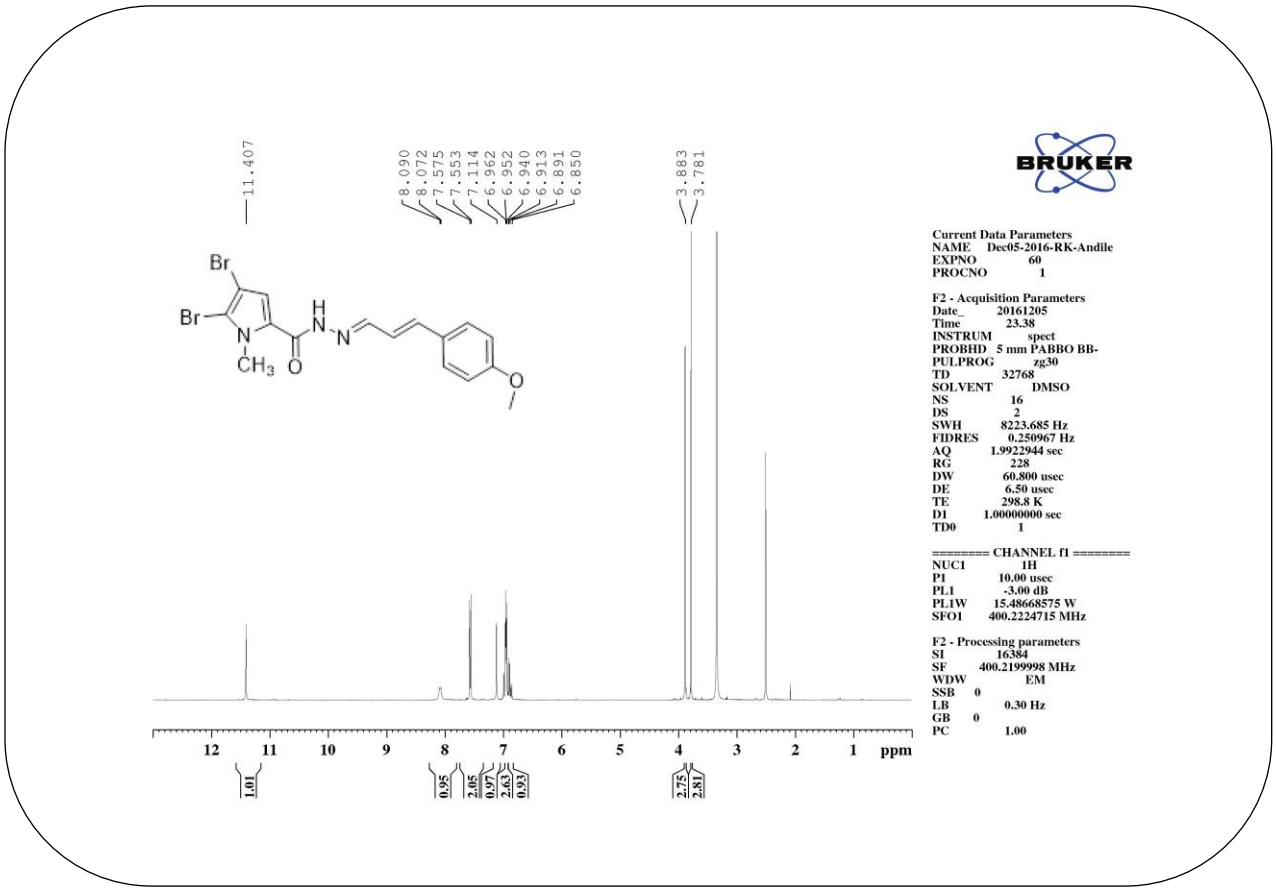
Monoisotopic Mass, Even Electron Ions
73 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-5 Na: 1-1 Br: 0-2

ADR-14 60 (1.990) Cm (1:61)
TOF MS ES+

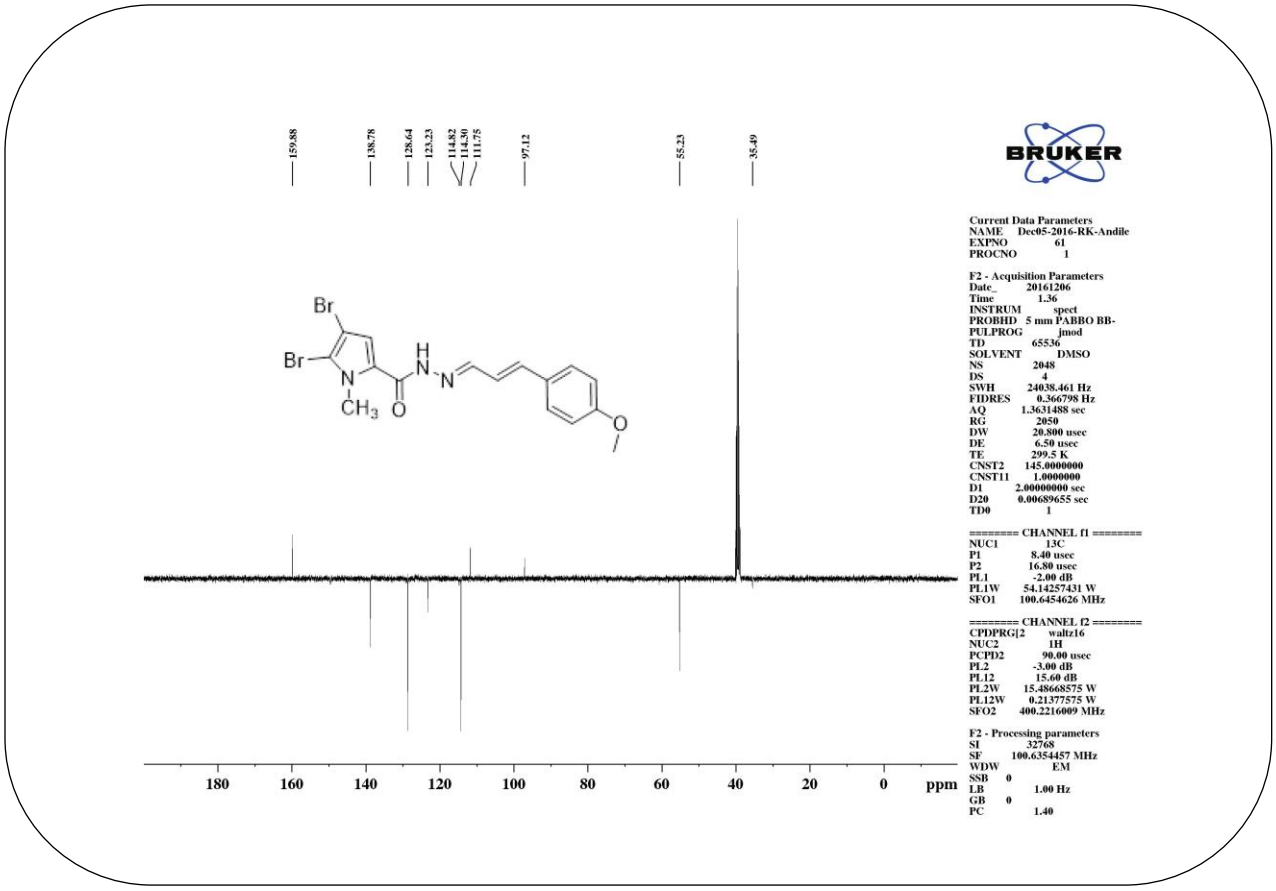


Minimum:		5.0	5.0	-1.5			
Maximum:				100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
461.9435	461.9429	0.6	1.3	9.5	494.4	0.0	C16 H15 N3 O2 Na Br2

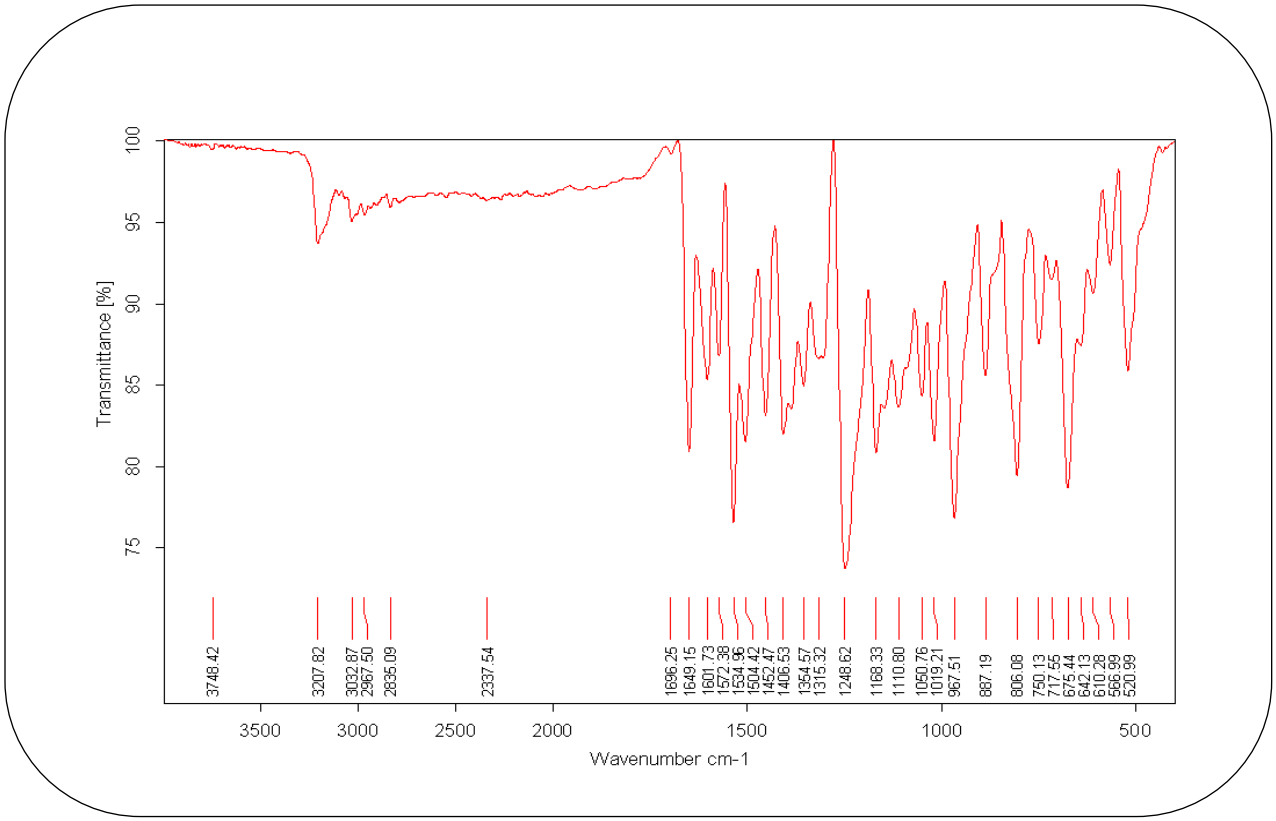
Spectrum 34: Mass spectrum of compound 6j



Spectrum 35: 1H NMR spectrum of compound 6k



Spectrum 36: 13C NMR spectrum of compound 6k



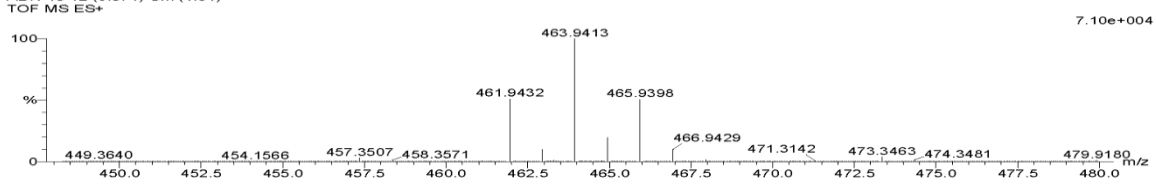
Spectrum 37: IR spectrum of compound 6k

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

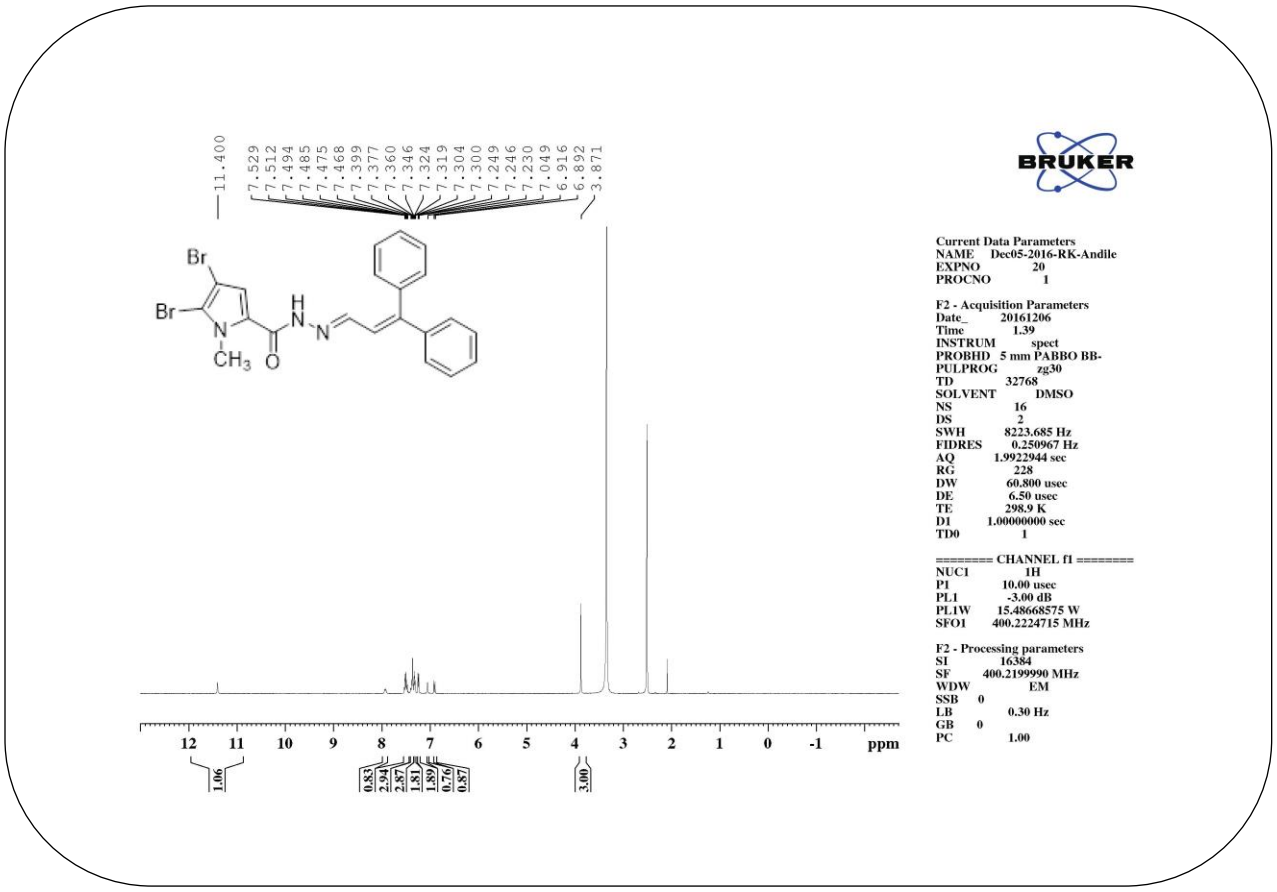
Monoisotopic Mass, Even Electron Ions
73 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-20 H: 10-15 N: 0-5 O: 0-5 Na: 1-1 Br: 0-2

ADR-15 12 (0.371) Cm (1:61)
TOF MS ES+

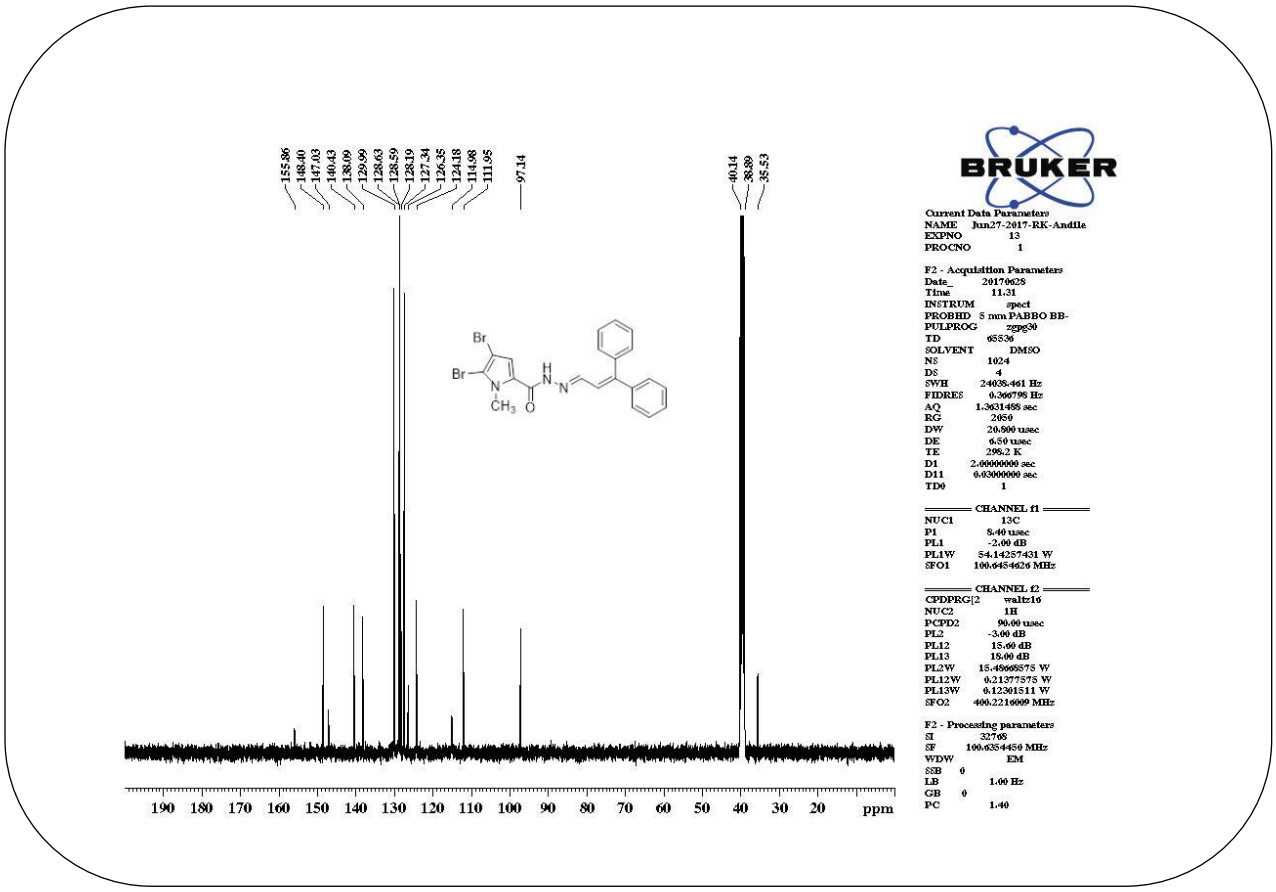


Minimum:		5.0	5.0	-1.5			
Maximum:				100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
461.9432	461.9429	0.3	0.6	9.5	470.4	0.0	C16 H15 N3 O2 Na Br2

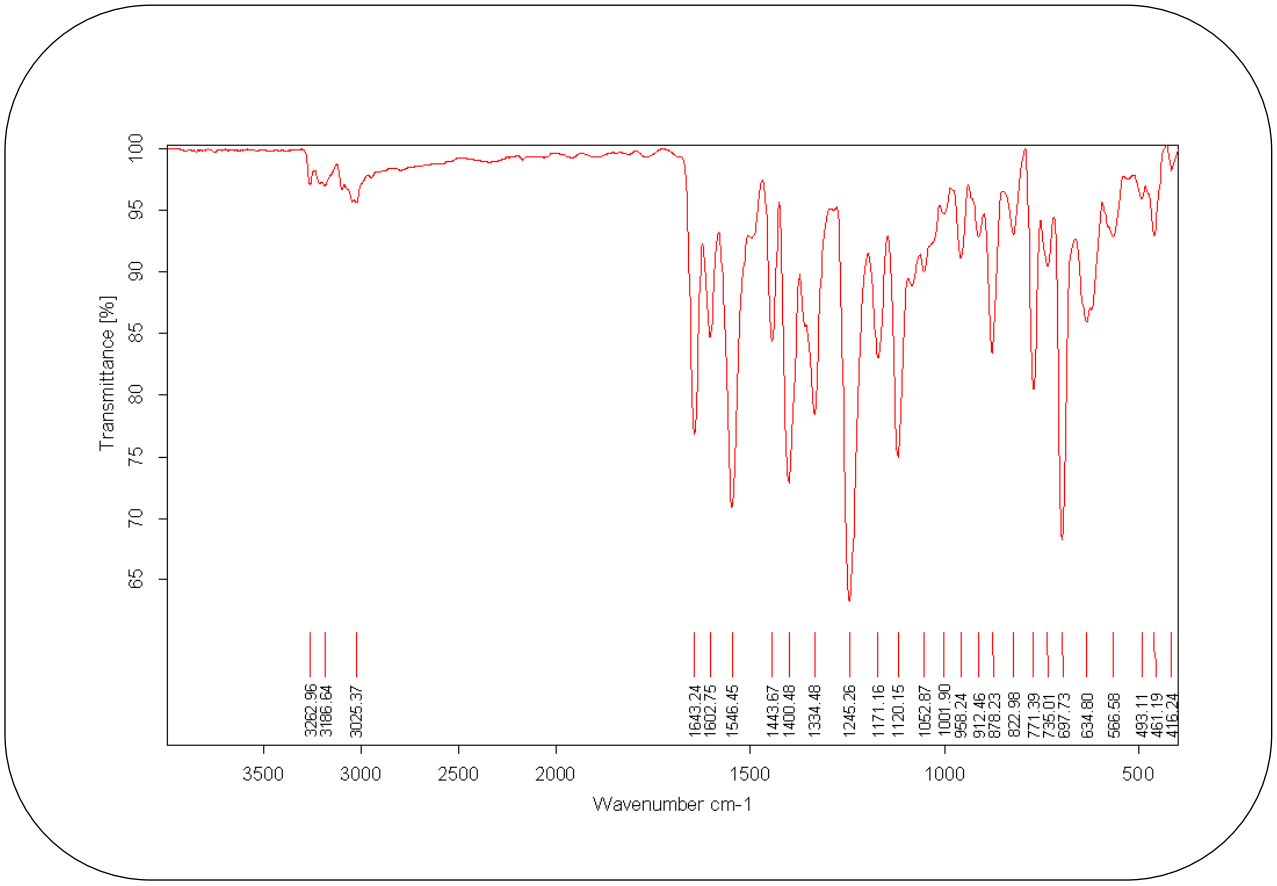
Spectrum 38: Mass spectrum of compound 6k



Spectrum 39: 1H NMR spectrum of compound 6l



Spectrum 40: 13C NMR spectrum of compound 6l



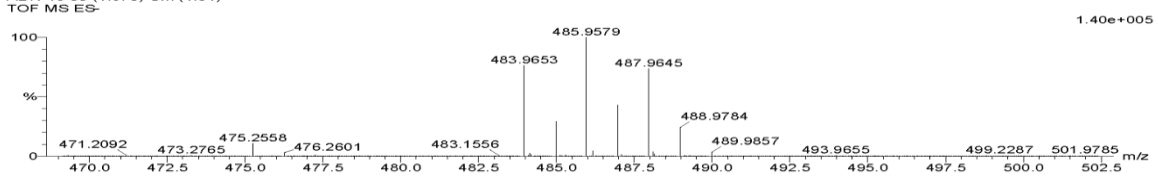
Spectrum 41: IR spectrum of compound 6l

Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

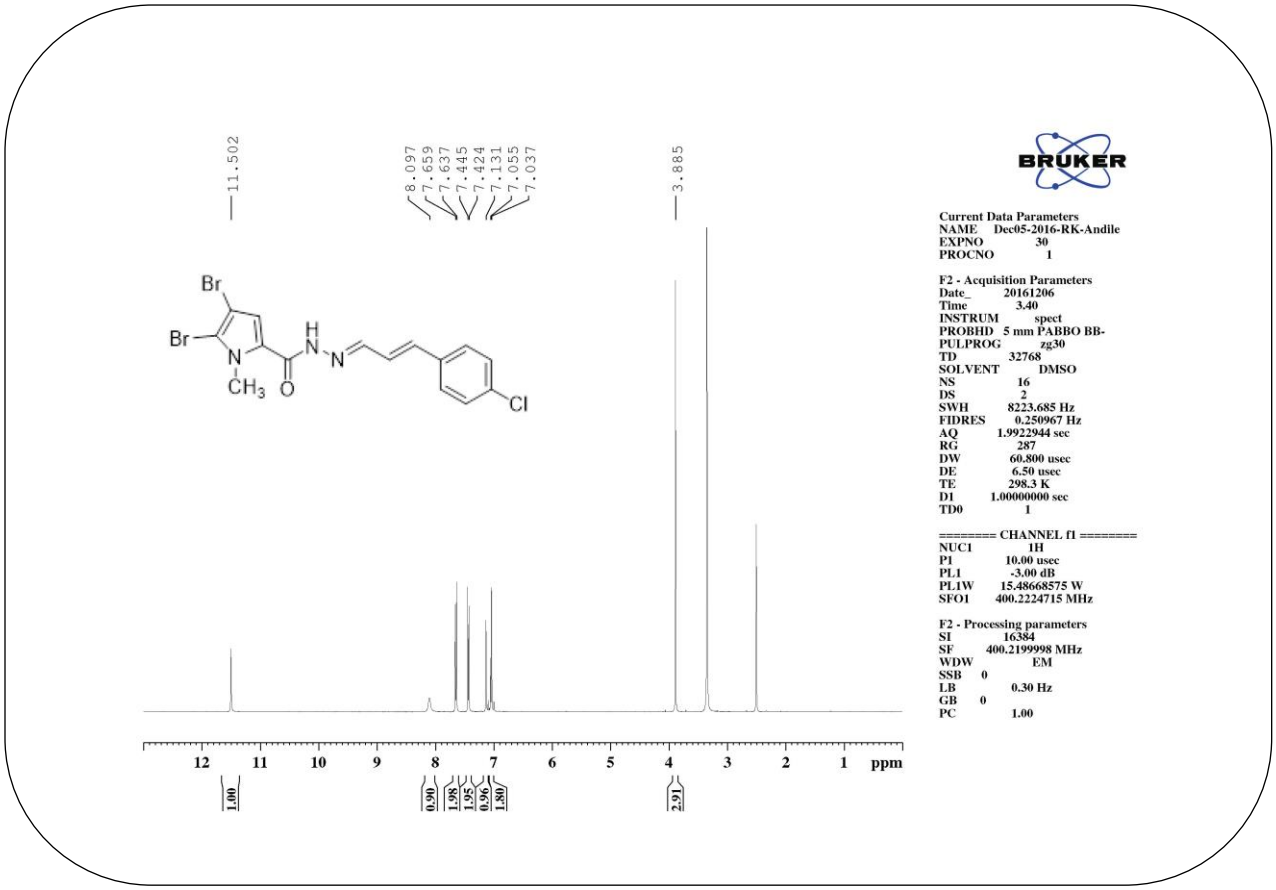
Monoisotopic Mass, Even Electron Ions
63 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 20-25 H: 15-20 N: 0-5 O: 0-5 Br: 0-2

ADR-16 33 (1.079) Cm (1:61)
TOF MS ES-

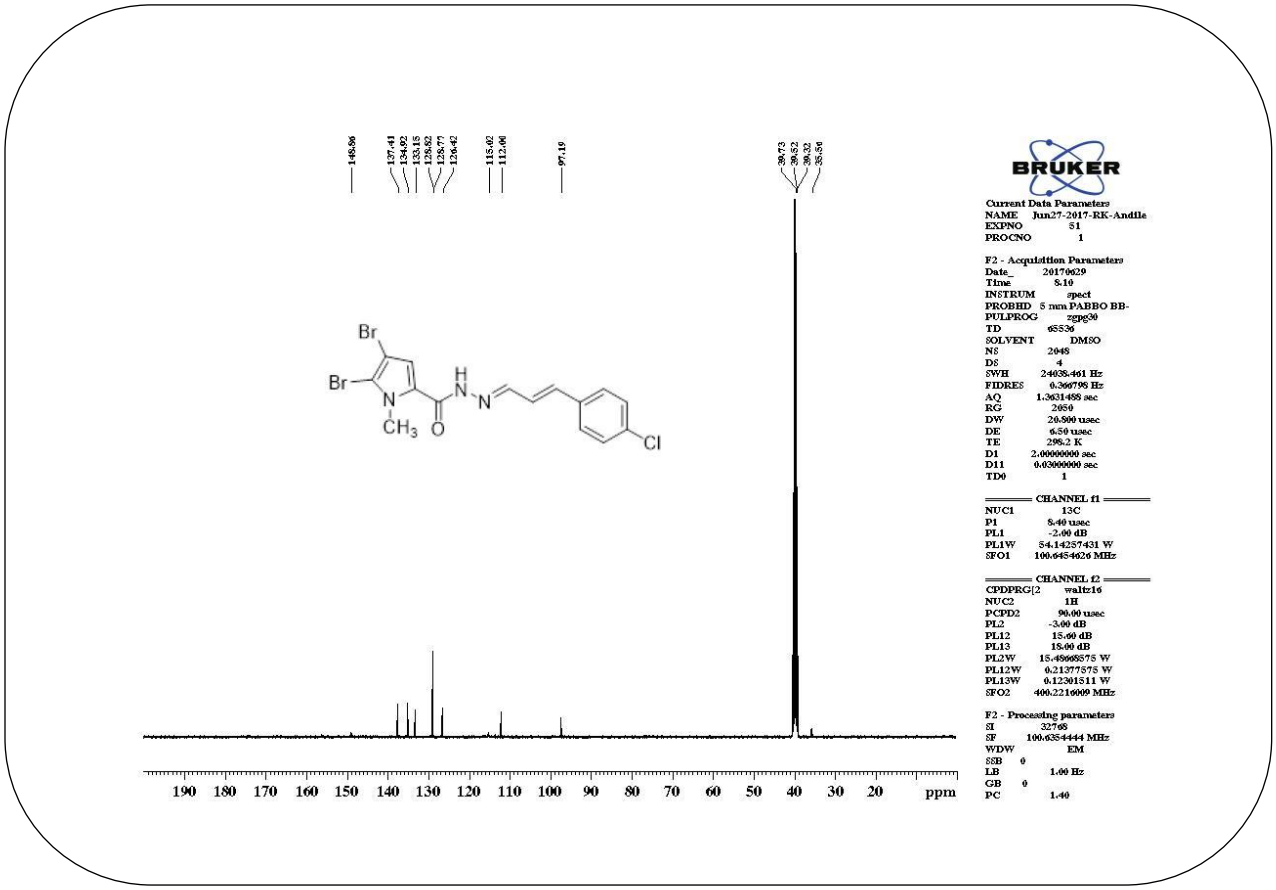


Minimum:				-1.5						
Maximum:		5.0	5.0	100.0						
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula			
483.9653	483.9660	-0.7	-1.4	14.5	466.7	0.0	C21	H16	N3	O Br2

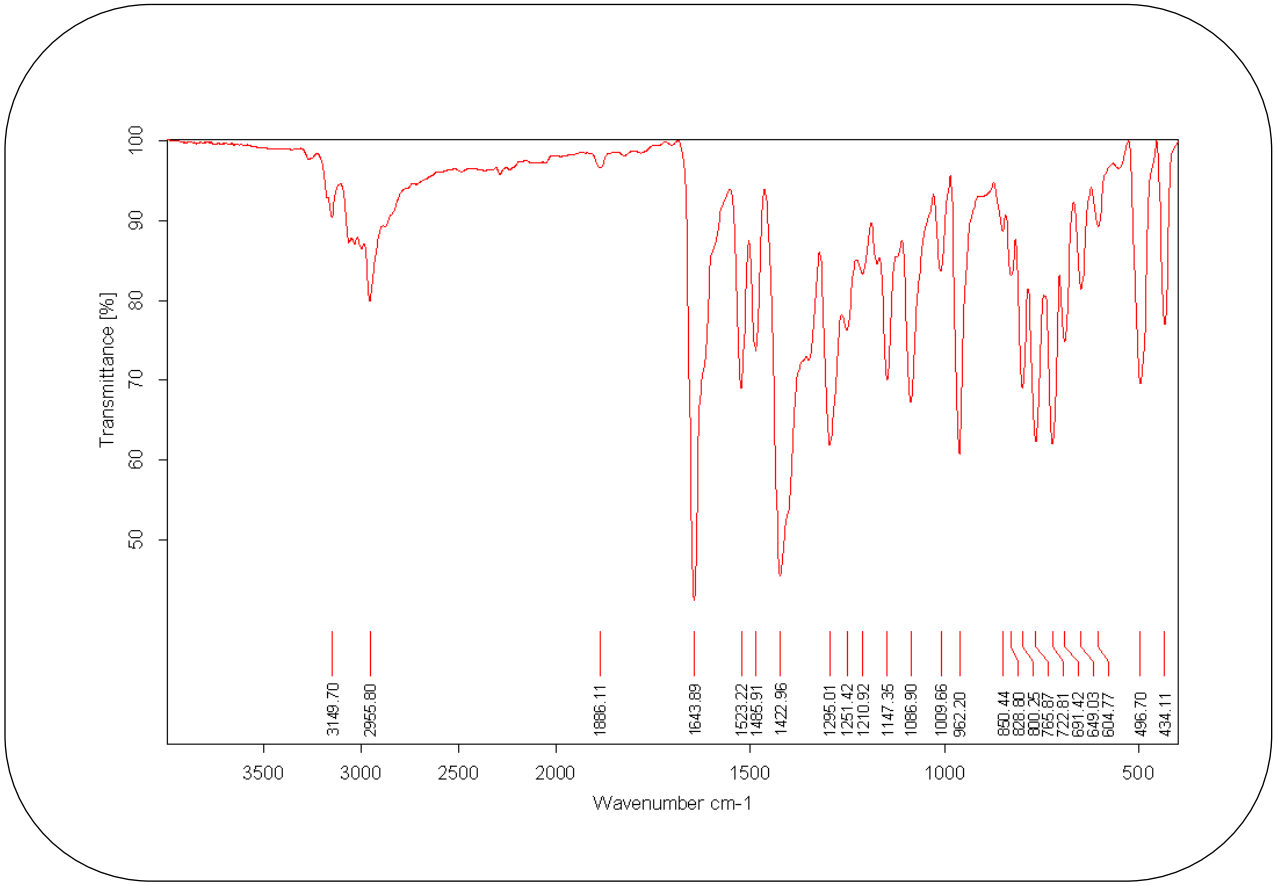
Spectrum 42: Mass spectrum of compound 6l



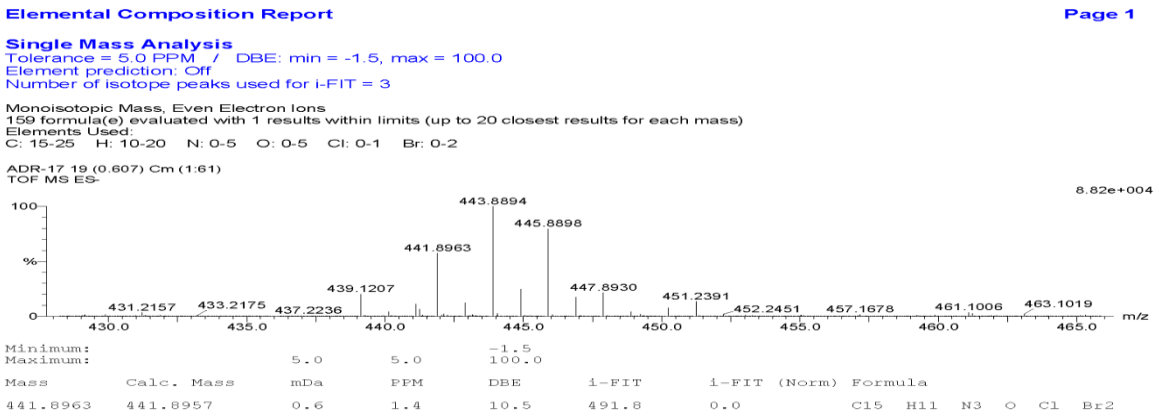
Spectrum 43: 1H NMR spectrum of compound 6m



Spectrum 44: 13C NMR spectrum of compound 6m



Spectrum 45: IR spectrum of compound 6m



Elemental Composition Report Page 1

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

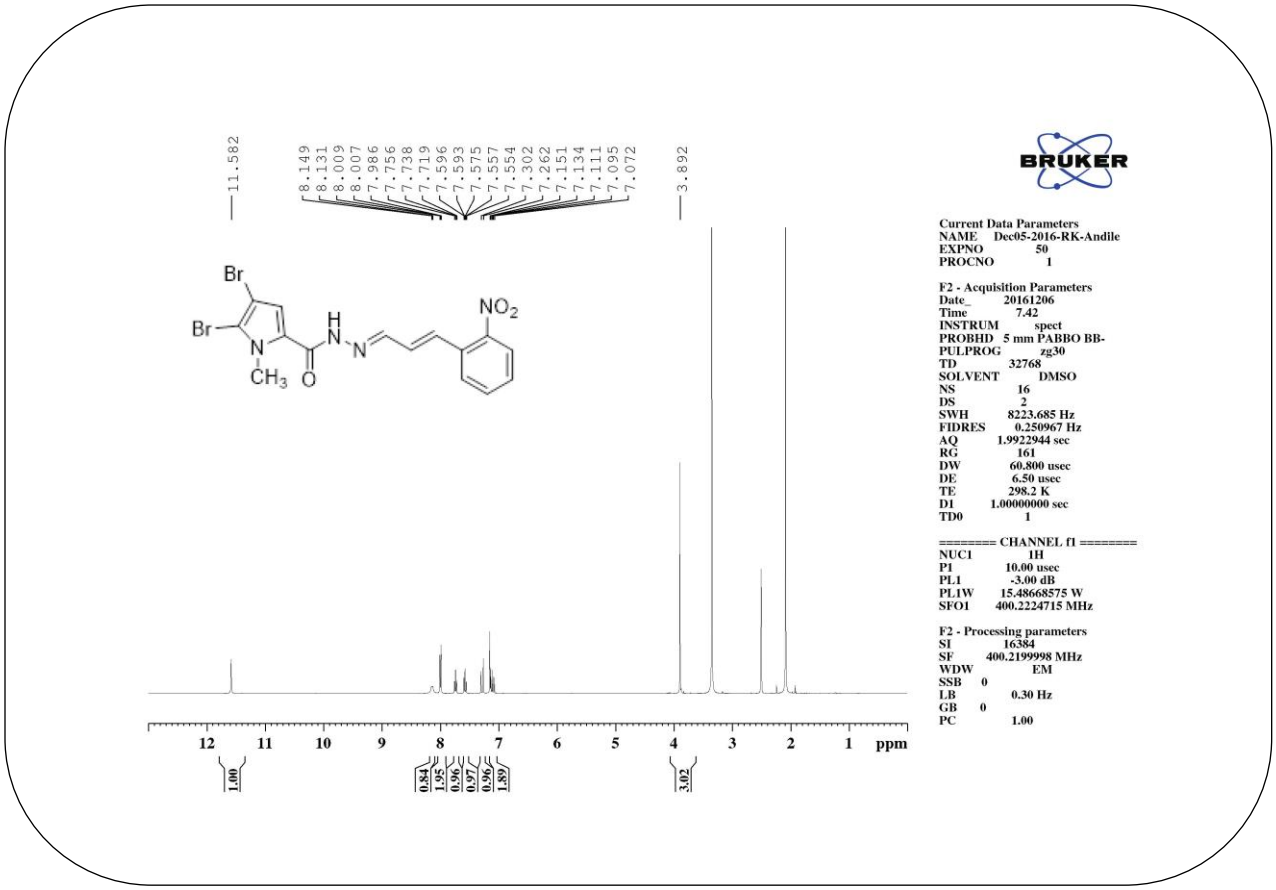
Monoisotopic Mass, Even Electron Ions
159 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-25 H: 10-20 N: 0-5 O: 0-5 Cl: 0-1 Br: 0-2

ADR-17 19 (0.607) Cm (1:61)
TOF MS ES-

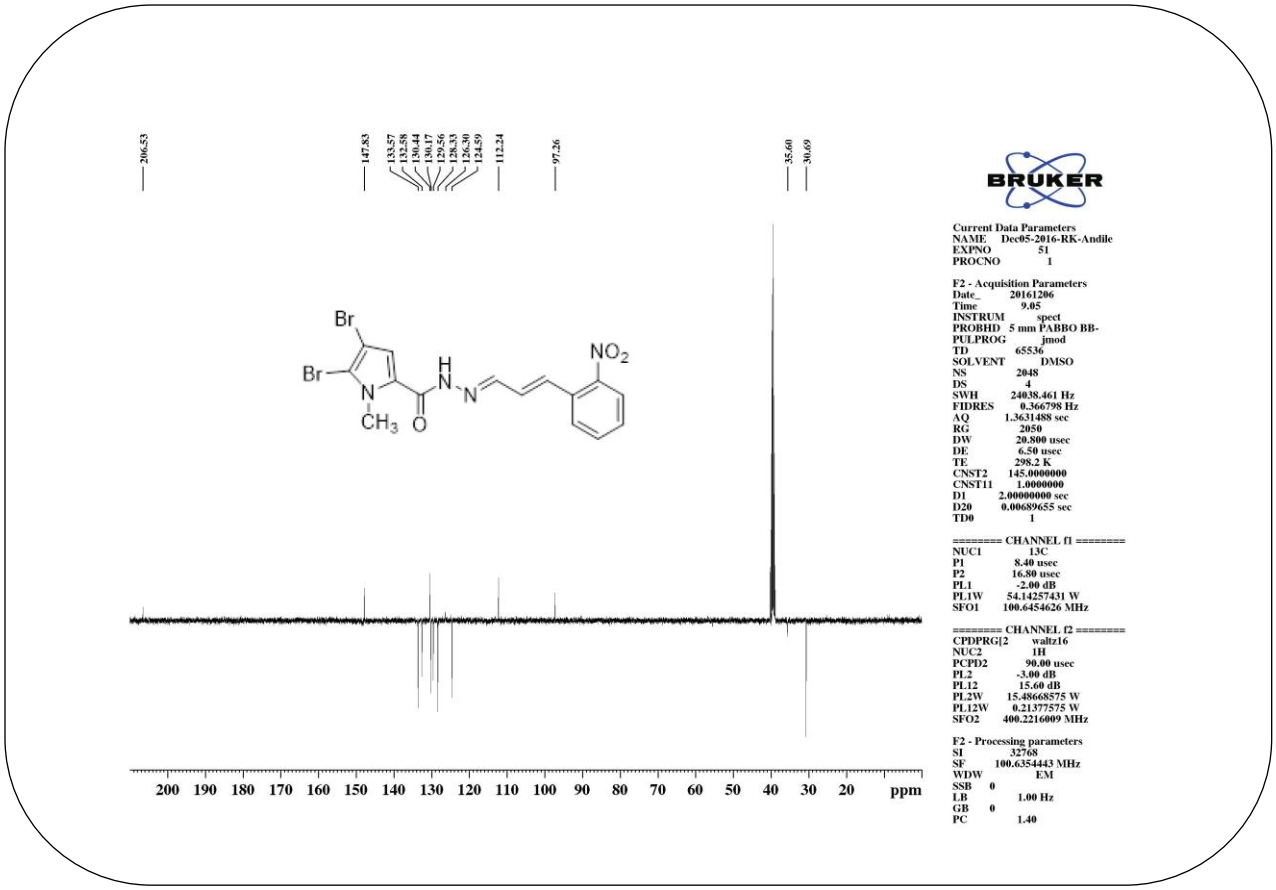
8.82e+004

Minimum:		5.0	5.0	-1.5			
Maximum:				100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
441.8963	441.8957	0.6	1.4	10.5	491.8	0.0	C15 H11 N3 O Cl Br2

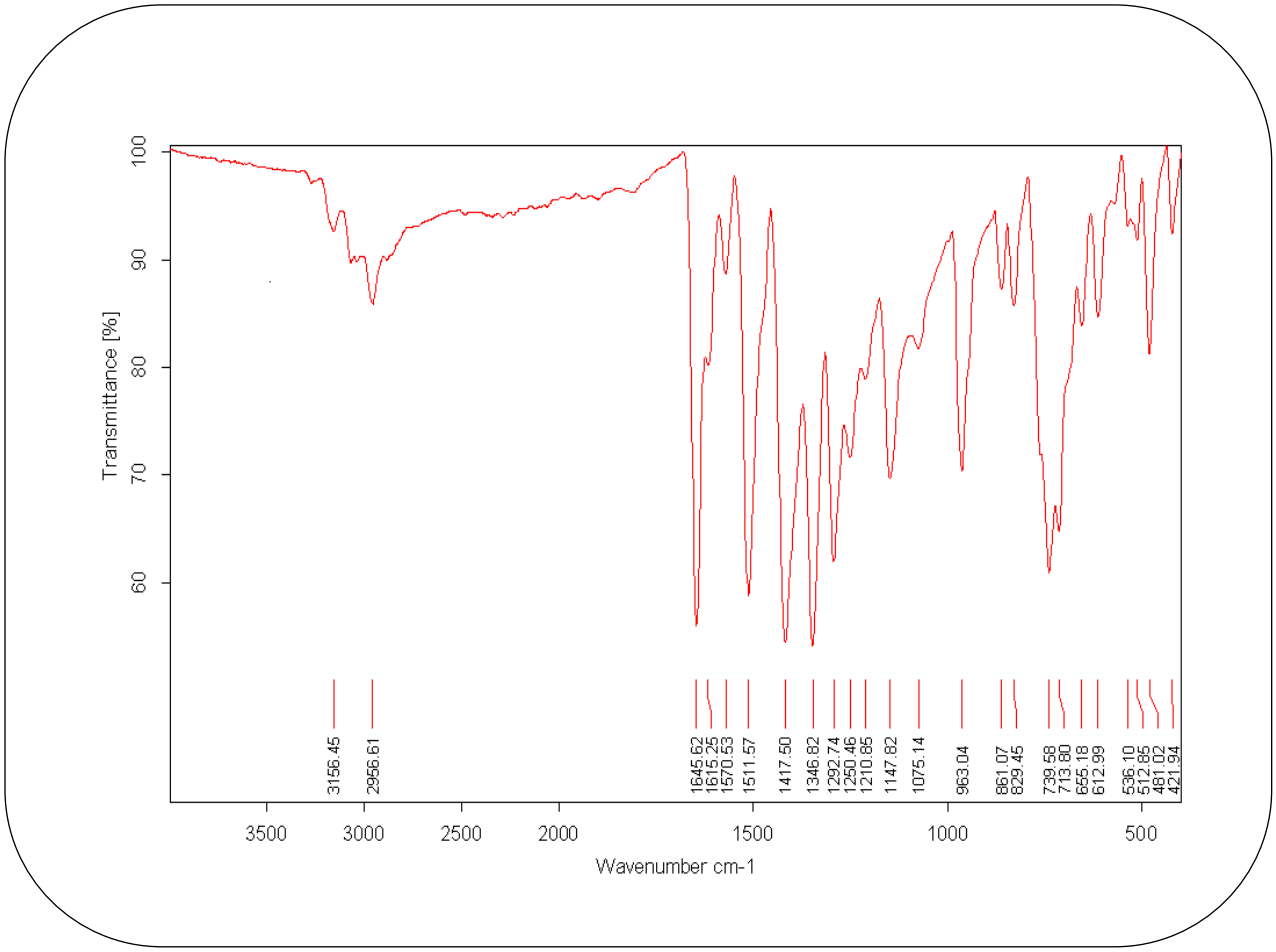
Spectrum 46: Mass spectrum of compound 6m



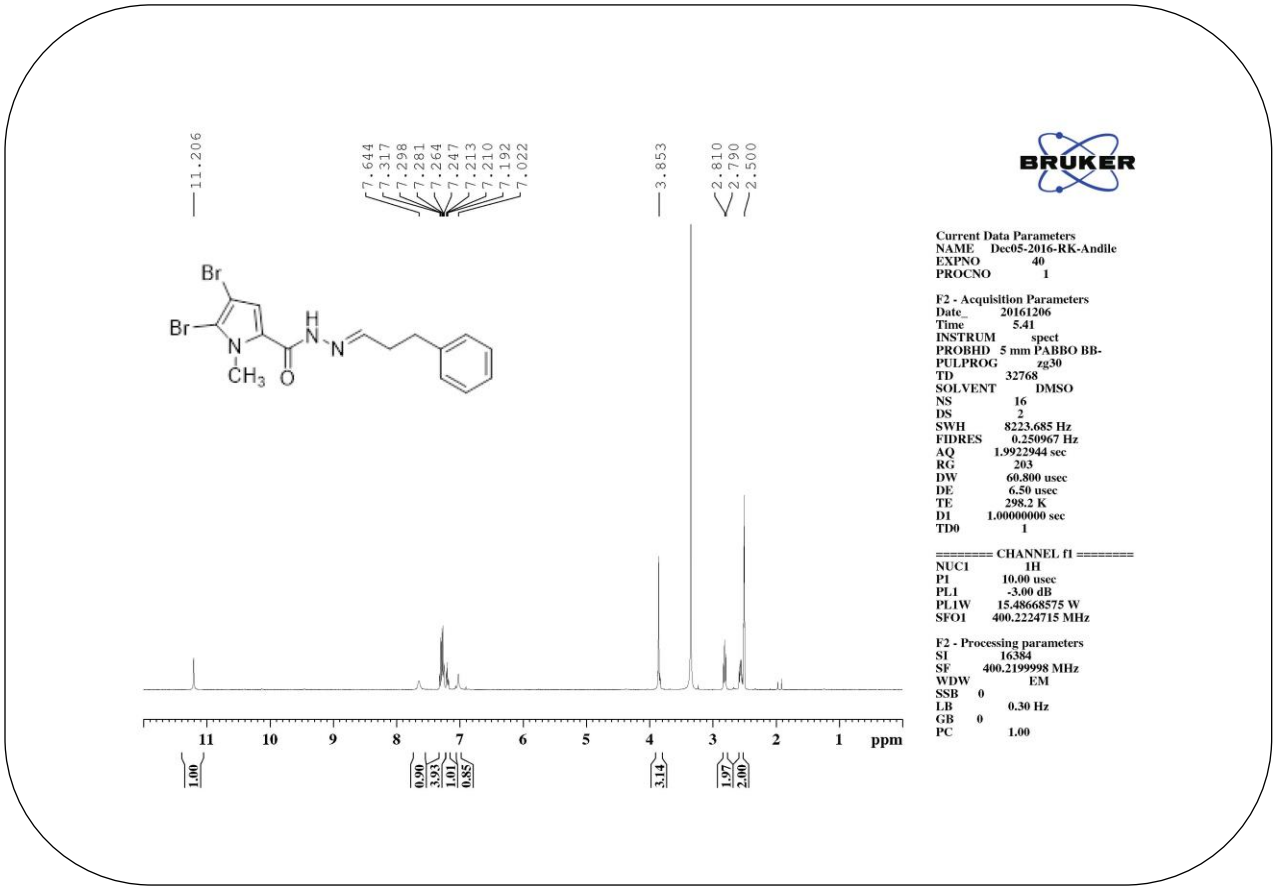
Spectrum 47: 1H NMR spectrum of compound 6n



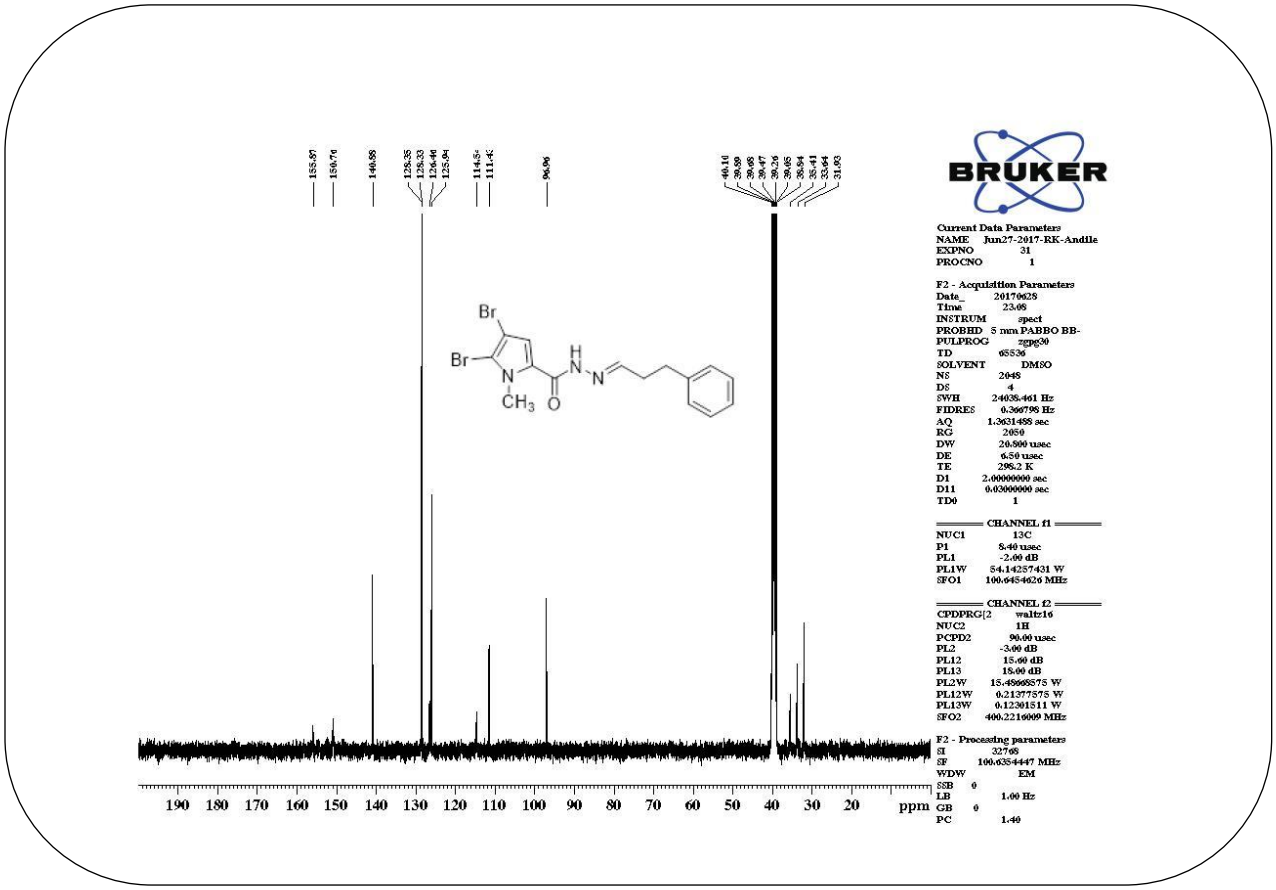
Spectrum 48: 13C NMR spectrum of compound 6n



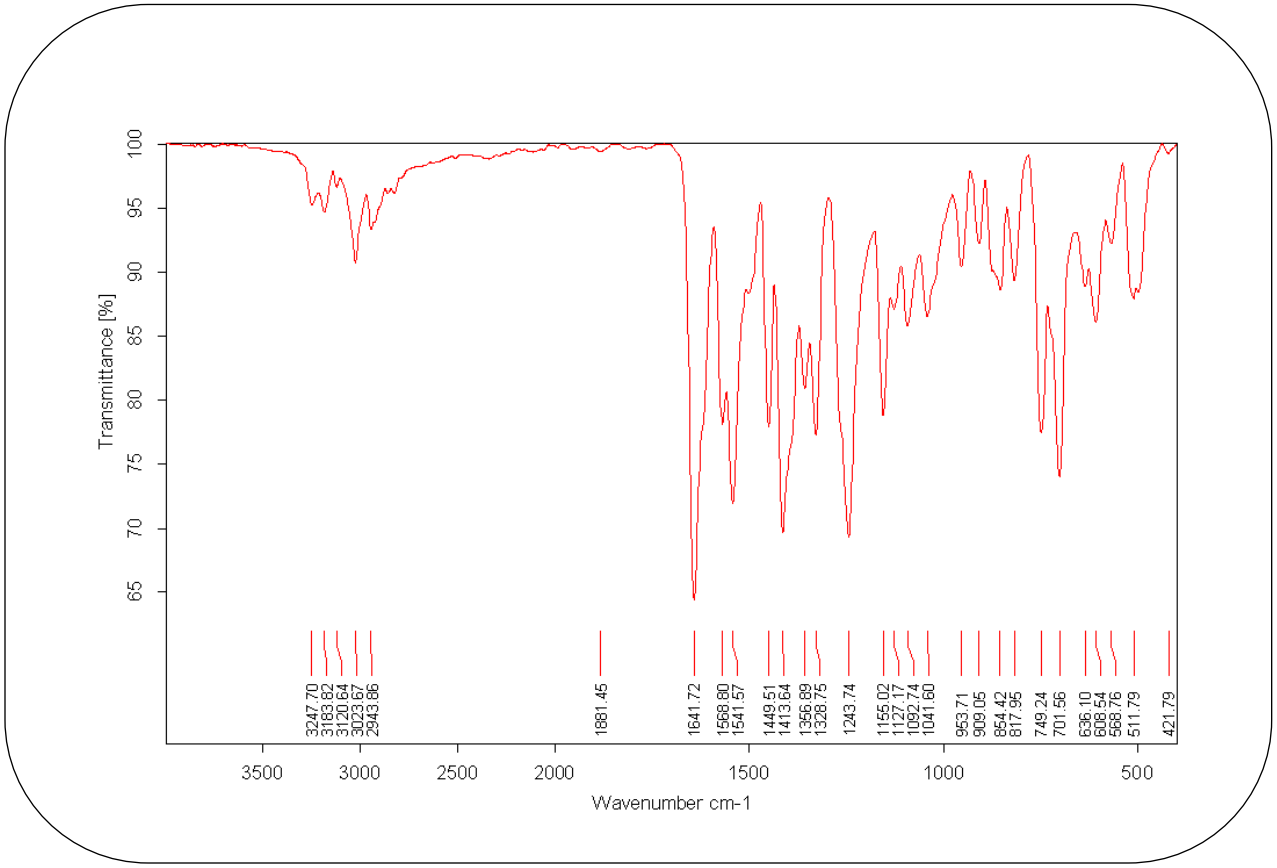
Spectrum 49: IR spectrum of compound 6n



Spectrum 50: 1H NMR spectrum of compound 6o



Spectrum 51: 13C NMR spectrum of compound 6o



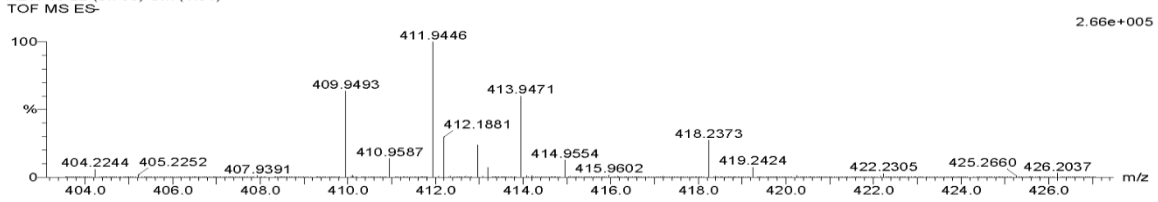
Spectrum 52: IR spectrum of compound 6o

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 100.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
78 formula(e) evaluated with 1 results within limits (up to 20 closest results for each mass)
Elements Used:
C: 15-25 H: 10-20 N: 0-5 O: 0-5 Br: 0-2

ADR-18 22 (0.708) Cm (1:61)
TOF MS ES-



Minimum:				-1.5			
Maximum:		5.0	5.0	100.0			
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	i-FIT (Norm)	Formula
409.9493	409.9504	-1.1	-2.7	9.5	478.9	0.0	C15 H14 N3 O Br2

Spectrum 53: IR spectrum of compound 6o