University of Misan

College of Science

Department of Chemistry

Synthesis, Characterization, and Antibacterial Activity Study of Novel Spiro-Cephalosporins

A thesis

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By

Noor H. Zwayyer

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Supervisor

Asst. Prof. Dr. Usama A .Muhsen

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Abbreviations

7-ACA	7-aminocephalosporanic acid
7-ADCA	7-aminodeacetoxycephalosporanic acid
AcOH	Acetic acid
^{t}BuOH	*tert*-butanol
Bn	Benzyl
BSA	Bis-[trimethylsilyl]acetamide
°C	Degrees Celsius
conc.	Concentrated
D-ala-D-ala	D-alanine-D-alanine
DCC	*N,N′*-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DEPT	Distortionless Enhancement by polarization Transfer
DMF	*N,N*-dimethylformamide
DMSO	Dimethyl sulfoxide
E.coli	*Escherichia coli*
EtOAc	Ethyl acetate
Et_3N	Triethylamine
EtOH	Ethanol
FTIR	Fourier Transform Infra-Red spectrophotometer
GCLE	7-phenylacetamide-3-chloromethyl-4-phenylacetamide-*p*- methoxybenzyl ester
g	Gram
h	Hour
Hz	Hertz
H. influenza	*Haemophilus influenza*
HRMS	High-Resolution Mass Spectra
HSQC	Heteronuclear single quantum coherence spectroscopy

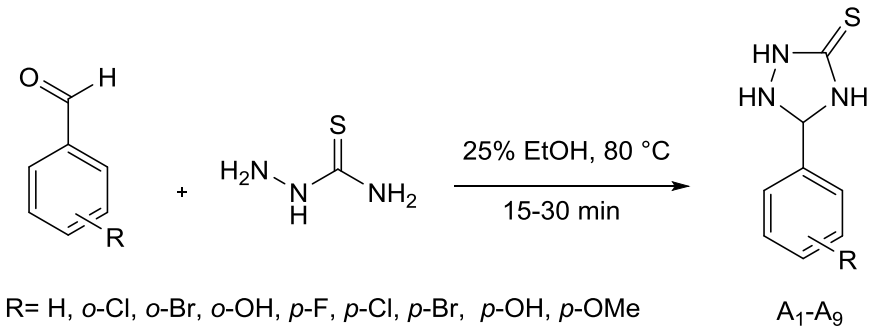
J	Coupling constant
$KOSi(CH_3)_3$	Potassium trimethylsilanolate
KO^tBu	potassium *tert*-butoxide
MBLs	Metallo-β-lactamases
MeCN	Acetonitrile MeOH Methanol
MRSA	Methicillin-resistant *Staphylococcus aureus*
MRSE	Methicillin-resistant *Staphylococcus epidermidis*
$Mn(OAc)_3$	Manganese triacetate
m.p	Melting point min Minute
NMR	Nuclear Magnetic Resonance spectrometer
P. aeruginosa	*Pseudomonas aeruginosa*
PBPs	Penicillin-binding proteins
PDC	Pyridinium dichromate
Ph_3P	Triphenylphosphine
PMB	*p*-methoxybenzyl
PTSA	*p*- Toluenesulfonic acid
^{i}PrONO	Isopropyl nitrite
α- py-CH_2PPh_3Cl	2-((chlorotriphenyl-l5-phosphanyl)methyl)pyridine
S. aureus	*Staphylococcus aureus*
SBLs	Serine β-lactamases
TFA	Trifluoroacetic acid
$Rh_2(OOct)_4$	Rhodium(II) octanote, dimer
TsCl	*p*-toluenesulfonyl chloride
R.B	Round bottom
r.t.	room temperature
μW	Microwave irradiation
δ	Chemical shift

Abstract

The study involves synthesis and characterization of seven novel spiro-cephalosporins starting from commercially available cephalosporin intermediate GCLE. The study can be divided into four main parts:

First part:

This part presents the synthesis of nine triazolidine-3-thione derivatives (**A1- A9**) via an efficient and green synthetic method. Aromatic aldehydes were reacted with thiosemicarbazide in aqueous ethanol under neutral conditions for short time with simple workups and good yields (62-95%). The structures of compounds were verified by FTIR, ^{1}HNMR and ^{13}CNMR.

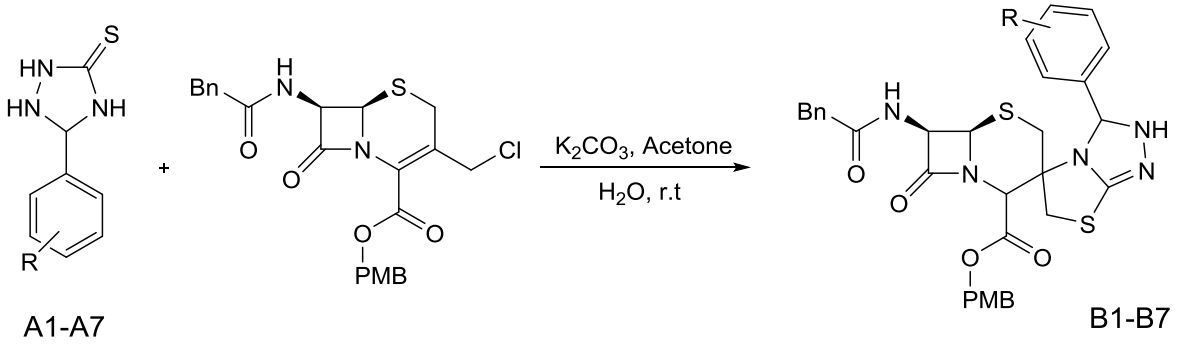


R= H, *o*-Cl, *o*-Br, *o*-OH, *p*-F, *p*-Cl, *p*-Br, *p*-OH, *p*-OMe

A_1-A_9

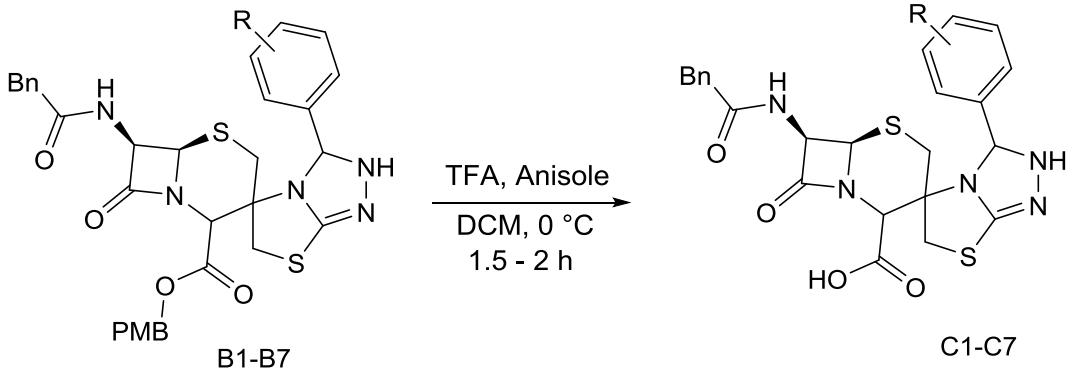
Second part:

This part describes the reaction of 1,2,4-triazolidines-3-thiones (**A1- A7**) with GCLE under basic conditions to furnish protected spiro-cephalosporin products (**B1-B7**) in moderate yields (17.3-32 %). It is suggested that this reaction has been done through a S_N2 type alkylation followed by intramolecular Michael addition to the dihydrothiazine ring. The structures of compounds are verified by FTIR, ^{1}HNMR and ^{13}CNMR.



Third part:

In this part, the *p*-methoxybenzyl (PMB) protecting group of precursors (**B1-B7**) was removed in presence of trifluoroacetic acid (TFA) and anisole to result the target spiro-cephalosporins (**C1-C7**) in good yields (46−80%).
Full spectroscopic characterization details are also included. The structures of compounds were verified by FTIR, ^{1}HNMR, ^{13}CNMR and HRMS.



R= H, *o*-Cl, *o*-Br, *o*-OH, *p*-F, *p*-Cl, *p*-Br

Fourth part:

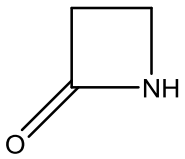
This part summarizes the antibacterial activity of the final spiro-cephalosporins (**C1-C7**). Four organisms, methicillin resistance *S.aureus*, *L.monocytogenes*, *E.coli*, and *Salmonella Newport*, were chosen based on multidrug resistance pathogen, and as representative pathogens of Gram-positive and Gram-negative bacteria, respectively. Compounds (**C1-C7**) were well effective against Gram-positive bacteria especially *S.aureus*; however, they had very little to no effect against Gram-negative bacteria, especially *Salmonella Newport*.

CHAPTER ONE

1. Introduction

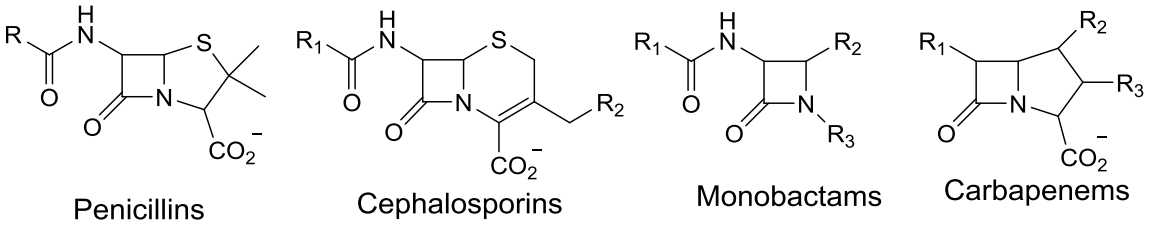
1.1. β-Lactam antibiotics

β-Lactam antibiotics are one of the first antibacterial compounds discovered, and they are also the most benign to humans.[1] They are the most commonly used drugs in clinics around the world due to their high bactericidal activity as a result of cell wall inhibition, low toxicity, and excellent medical efficacy. Since their first use in the 1940s, β-Lactams have been known for their excellent therapeutic efficacy and broad applicability in bacterial infectious diseases. Current β-lactam antibiotics all share the same reactive β-Lactam ring system, which is a highly strained and reactive cyclic amide. (Figure 1-1).[2,3]



(Figure1-1) azetidin-2-one (β-Lactam ring)

The β-lactam ring is found in several antibiotic families, including penicillins, cephalosporins, carbapenems, and monobactams (Figure 1-2).[4]

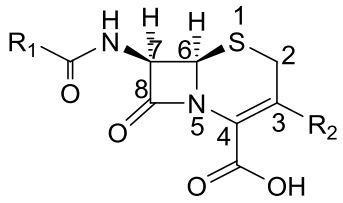


(Figure 1-2) Chemical structures of β-lactam family

These drugs target bacterial cell wall biosynthetic enzymes (so-called penicillin-binding proteins, PBPs),[5] Although the exact mechanism of destruction is still unknown. Unfortunately, resistance to all these agents is common both in Gram-positive and Gram-negative bacteria. Bacterial resistance develops as a result of a drug being inactivated by β-lactamases.[6] The combination of beta-lactamase inhibitors and beta-lactams would be effective on the most resistant organisms. Since the β-lactam ring is so important for these compounds' biological activity, cleavage at any point on this ring results in a complete loss of antibacterial activity.[7,8]

1.2. Cephalosporins

They are one of β-lactam antibiotics that have a broad spectrum of action. They are the second most prescribed class of antibiotics in Europe, as a result of their massive production and consumption. In 1945, the first known member of this class of antibiotics was isolated from the fungus *Cephalosporium acremonium*. Although Guiseppe Brotzu was the first to isolate the drug, Edward Abraham has been credited with patenting it because he was able to obtain the compound.[9,10] Cephalosporins are antibiotics which are used to treat bacterial infections and diseases caused by methicillin-susceptible penicillinase-producing *Streptococci, Staphylococci, Proteus mirabilis*, some *Escherichia coli*, *Haemophilus influenza*, *Klebsiella pneumonia, Enterobacter aerogenes* and some *Neisseria*.[11] Cephalosporins are made up of a fused two-ring system [β-lactam-Δ^3-dihydrothiazine, also known as 7-aminocephalosporanic acid (7-ACA), and their side chains differ at C3 (methylene substituent R2) and C7 (acylamido, R1) (Figure 1-3).[12]



(Figure 1-3) Structure of cephalosporins

The substituents at C3, C4, and C7 are the key factors for their antimicrobial activity. The carboxyl group at C4 must be unsubstituted, while the acylamido side chain at C7 is a key group that mainly determines the hydrophilic/hydrophobic character of these compounds. In addition, the strained β-lactam ring and distortion of planar geometry are considered as essential factors for the high chemical reactivity of this antibiotics.[13]

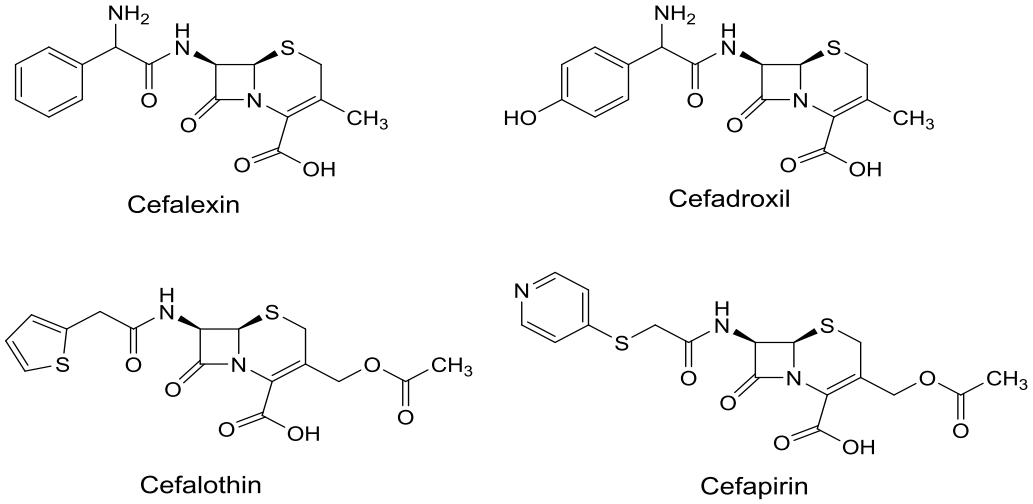
1.2.1. Classification of cephalosporin

Since 1975, various cephalosporins have been introduced, which are described somewhat arbitrarily as generations, according to chronological classification. As a result, rather than providing some benefits at the sacrifice of other or other benefits, each generation that has been presented has added another degree of advantages overall over the generation before it. Although generational differences may seem modest, they are significant.[14] They are divided into five generations, despite of that the fifth generation is still under trial.[15]

1.2.1.1. First generation

Based on whether they are administered orally or intravenously, the first generation of cephalosporin antibiotics can be divided into two classes.

Cefalexin and Cefadroxil are two examples of oral cephalosporins that were first used in medicine in 1967,[16] while cefalothin and cefapirin [17] are the parenteral ones (Figure 1-4). First-generation cephalosporins are more effective against Gram-positive bacteria, including methicillin-susceptible *Staphylococci* and *non-enterococcal Streptococci.* They also have demonstrated strong inhibition activity against bovine *S. aureus species* in vitro.[19] Presence of a leaving group at C3 in the structure of cefalothin and cefapirin renders these antibiotics higher antibacterial potency than cefalexin and cefadroxil.[16]

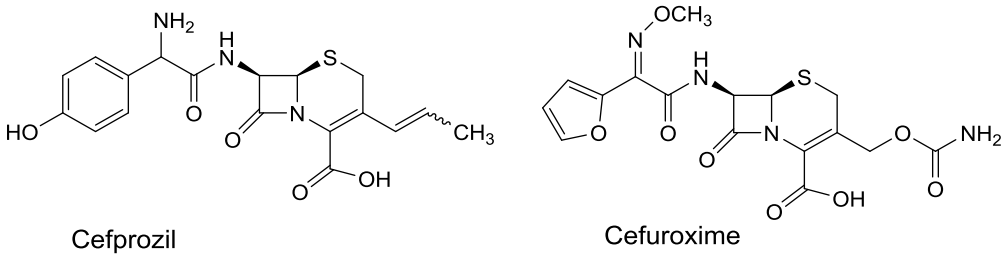


(Figure 1-4) Chemical structure of some 1st generation cephalosporins

1.2.1.2. Second generation

Similarly to the first generation clasification, the second generation cephalosporins are also divided into two classes; oral cephalosporins such as cefprozil, cefaclor and loracarbef,[20] while cefixime, cefuroxime and cefamandole are examples of parenteral ones (Figure 1-5).[21] These medications typically outperform first-generation cephalosporins in their

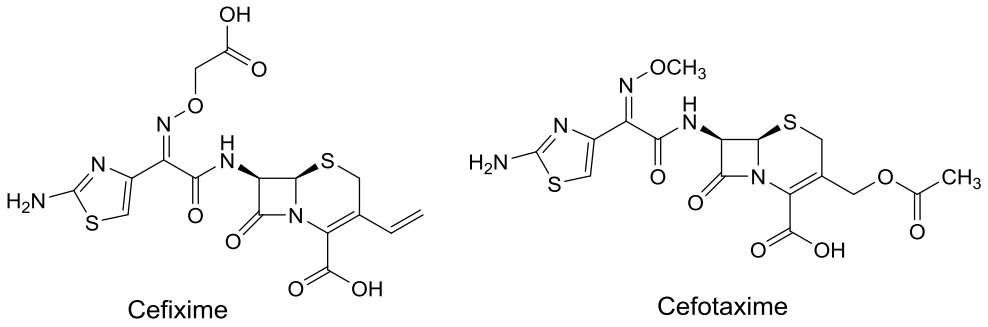
ability to combat gram-negative bacteria.[22] Second-generation cephalosporins are typically more stable towards to the β-lactamases which are produced by many gram-negative bacteria than the earlier medications, making them more potent against several bacteria that are assumed to be resistant toward the older medications. They have greater activity against *H. influenza*, *Moraxella catarralis*, *Neisseria meningitidis* and *Neisseria gonorrhoeae*.[23]



(Figure 1-5) Chemical structure of some 2nd generation cephalosporins

1.2.1.3. Third generation

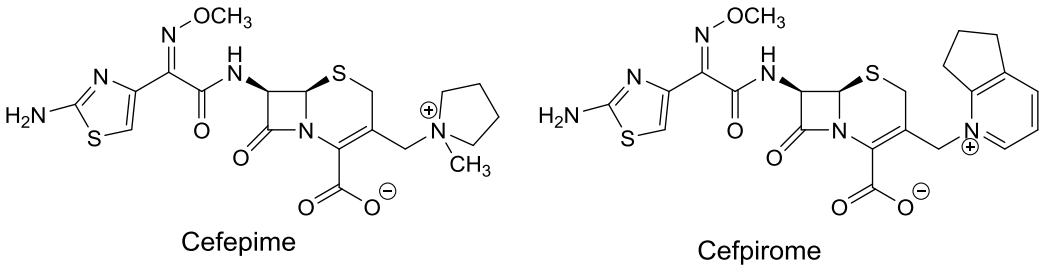
Third-generation cephalosporins offer a wider range of action than earlier generations of cephalosporins, which are less potent against gram-positive bacteria and more active against gram-negative ones like *enterobacteria* and *streptococci*.[24] Cefixime is the only third-generation cephalosporin currently accessible for oral use. It is used to treat urinary tract infections and cervical gonorrhoea as well as bacterial respiratory infections such tonsillitis, pharyngitis, otitis media, sinusitis and acute bronchitis.[25–28] The only difference between cefotaxime, ceftizoxime, and ceftriaxone is the C3-substituent, but they share the same a 7-acyl group with a syn-methoxyimino function that helps maintain affinity for penicillin-binding protein (PBP) (Figure 1-6).[29]



(Figure 1-6) Chemical structure of some 3rd generation cephalosporins

1.2.1.4. Fourth generation

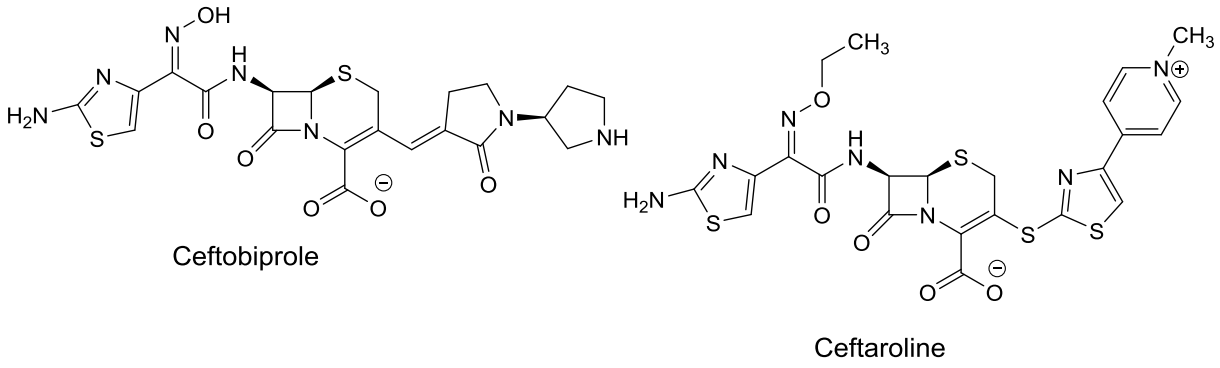
In comparison to third-generation cephalosporins, fourth-generation cephalosporins show stronger antibacterial action against gram-positive pathogens and the same spectrum of activity against gram-negative bacteria. They exhibit good effectiveness against MSSA, *Streptococcus spp.*, *P. aeruginosa*, and enteric gram-negative rods and are less vulnerable to hydrolysis by β-lactamases (Figure 1-7). [30,31] They are structurally related to third-generation cephalosporins. They differ in that they have a quaternary ammonium group at position 3 of the cephem nucleus.[30]



(Figure 1-7) Chemical structure of some 4th generation cephalosporins

1.2.1.5. Fifth generation

The current fifth-generation cephalosporins' preferred medication, ceftaroline, is exceptional in its ability to combat *Staphylococcus aureus's* multidrug resistance. Additionally, it works against *Enterococcus*. Ceftobiprole, is another drug of choice among 5th generation cephalosporins with a very broad-spectrum activity against gram-positive *cocci* including MRSA and methicillin-resistant *Staphylococcus epidermidis* (MRSE), penicillin-resistant *Streptococcus pneumoniae*, and *Enterococcus faecalis* (Figure 1-8).[32–34]

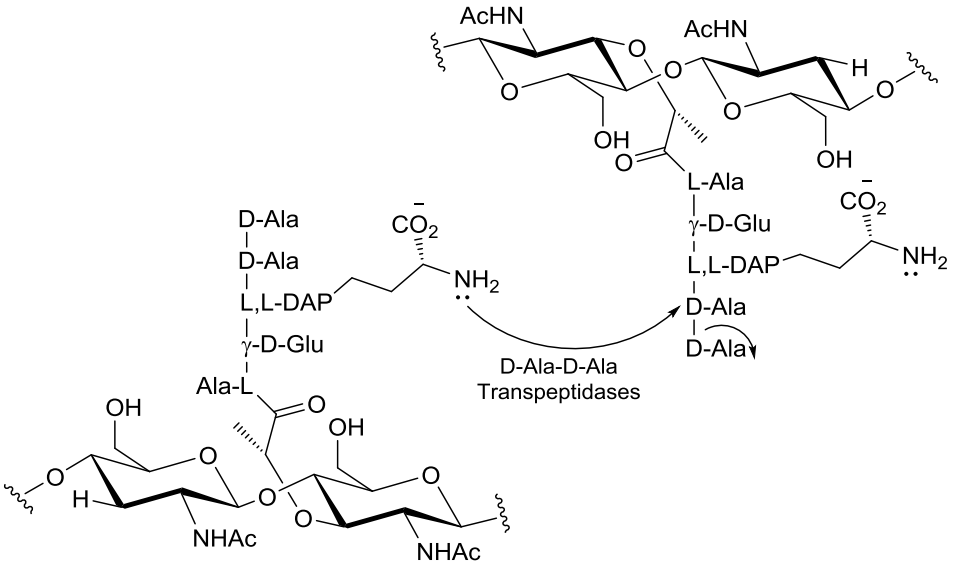


(Figure 1-8) Chemical structure of some 5th generation cephalosporins

1.2.2. Mode of action

The bacterial cell wall consists of cross linked peptidoglycan that keeps the cell rigidity and protects it against harsh environmental conditions. The cross linkages between peptidoglycan chains are D-alainine-D-alanine amide bonds which are made up by transpeptidase enzymes (also known as penicillin binding proteins PBPs) (scheme 1-1).

Because of the structural similarity between β-lactam and D-ala-D-ala, the antibiotic forms a covalent bond with transpeptidase to produce cephalosporin-enzyme complex, which eventually leading to bacterial transpeptidase inhibition. As a result of cell wall synthesis disruption and lacks of its rigidity, the cell contents are released under severe osmotic pressure and eventually burst.[35]

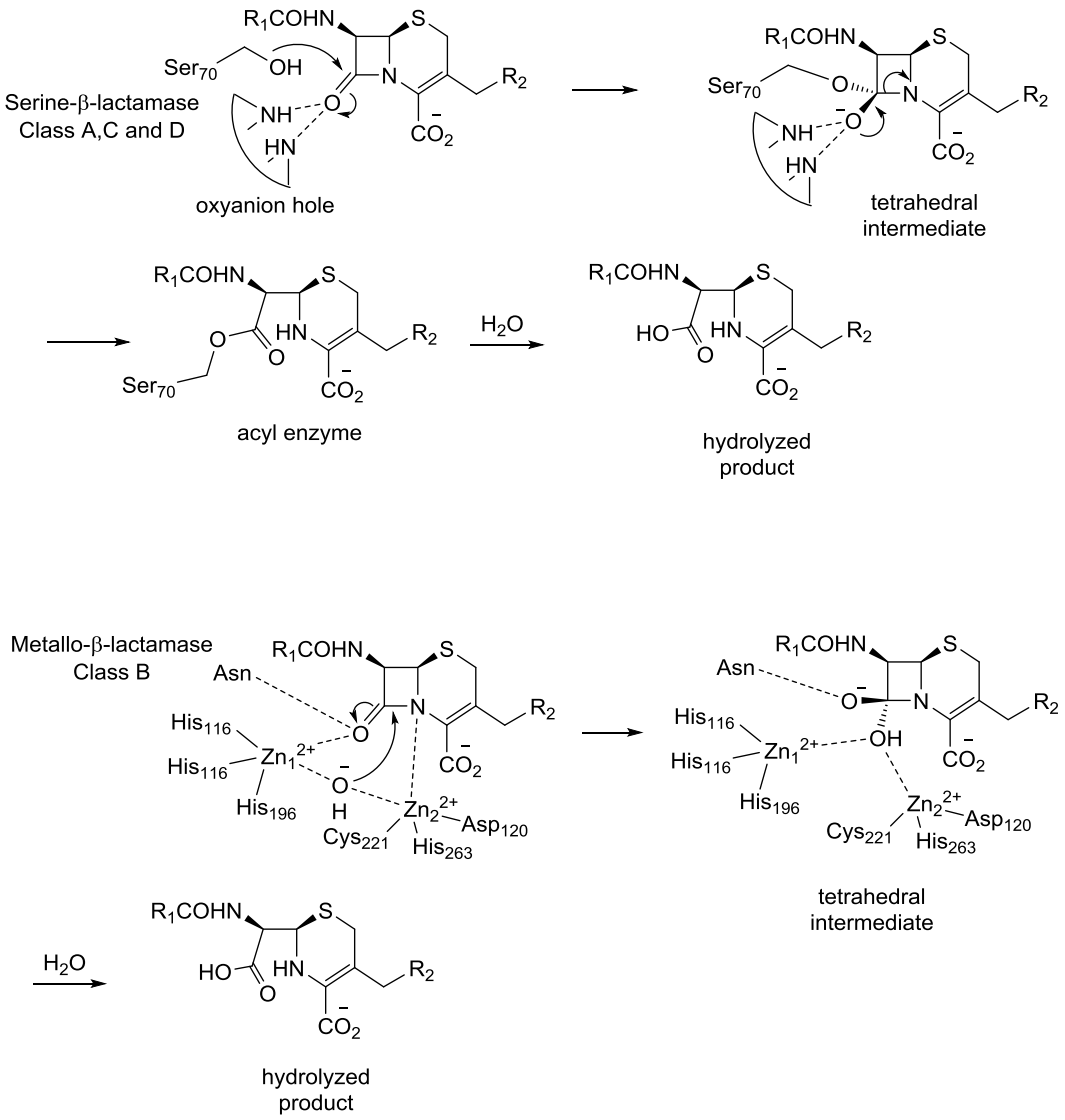


Scheme 1-1: Cross linkage by D-Ala-D-Ala transpeptidases in *E.coli*

1.2.3. Bacterial resistance to cephalosporins

The extensive use of cephalosporins has caused the emergence of resistance.[14] The production of β-lactamases, hydrolytic enzymes that cleavage the amide bond of β-lactam ring, which make the antimicrobial agent ineffective, is a significant mechanism of β-lactam resistance, notably in gram-negative bacteria.[36,37] There are four different classes of β-lactamases: A, B, C, and D. One or two equivalents of Zn^{2+} are needed for each of the class B enzymes known as metallo-β-lactamases (MBLs), and one $Zn^{2+}{}^{-}OH$ is

used for the enzyme's nucleophilic assaults on the β-lactam ring. The class A, C, and D enzymes are serine β-lactamases (SBLs), which cleave the β-lactam ring in cephalosporins by using a serine residue as a nucleophile at the active site. (Scheme 1-2) illustrates the proposed mechanism for the hydrolysis of cephalosporins.[4,7,38–40]

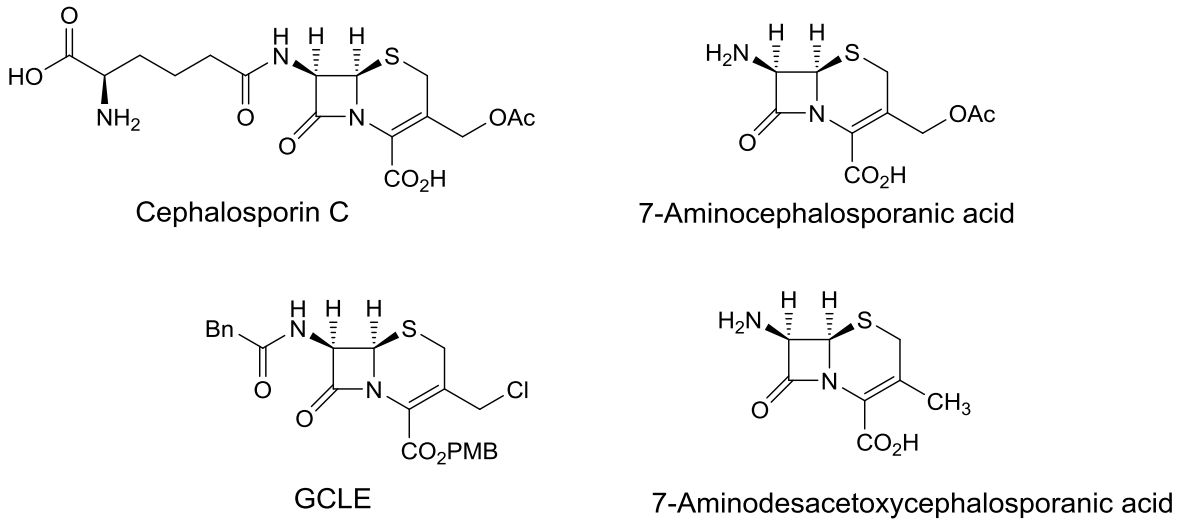


Scheme 1-2: Mechanism of the hydrolysis of cephalosporins

1.2.4. Modification of cephalosporin

The range of action, sensitivity to β-lactamases, protein binding, serum half-life, peak serum concentration, route of elimination, penetration into the central nervous system, and toxicity of cephalosporins are all different. These variations are the result of the cephalosporin molecule being altered in an attempt to stabilize the β-lactam ring.[41–43] Because of beta-lactam antibiotics' ability to fight bacteria depends on the cleavage of their nitrogen-carbonyl bond of β-lactam, so this reaction is considered crucial. In turn, the reactivity of the β-lactam carbonyl is essential for cell wall biosynthesis. The carbonyl must not, however, be too reactive to avoid arriving nucleophiles to hydrolyze it before it can reach the target enzymes. Maximum antibacterial potency can only be attained by achieving a proper balance between the reactivity and stability of the β-lactam ring. Additionally, any method for the complete synthesis or chemical modification of penicillins and cephalosporins must take into consideration the fact that the more reactive β-lactams can be cleaved under very mild circumstances. In general, cephalosporins are comparatively more stable toward β-lactam cleavage than penicillins.[30,44] Cephalosporin C, the prototype cephalosporin was isolated from the fungus *Cephalosporium acremonium* (Figure 9). The structure of cephalosporin C has been modified, leading to variations in the pharmacological, microbiological, and toxicological profiles between the various semi-synthetic cephalosporins. These changes are primarily made by replacing the molecule's two side chains, R1(C-7) and R2 (C-3). Site R1 generally makes a compound more resistant to β-lactamases, which leads to increased activity and a wider antibacterial range. Cefuroxime, cefotaxime, ceftizoxime, ceftriaxone, ceftazidime, and the cephamycins are a few examples of drugs containing R1 replacements. R2 modifications can lengthen the compound's serum half-life. Cefazolin,

Cefamandole, Cefonicid, Cefoperazone, Ceftriaxone, and Ceftazidime all include R2 replacements.[15,45–49] Cephalosporins are synthetically derived from three important intermediates, i.e. 7-aminocephalosporanic acid (7-ACA), 7-aminodeacetoxycephalosporanic acid (7-ADCA), and 7-phenylacetamide-3-chloromethyl-4-phenylacetamide-p-methoxybenzyl ester (GCLE) (Figure 1-9). Many generations of cephalosporins could be produced by altering the side chains of these intermediates at the C3-acetoxy group and/or C7-amino group.[50] Treatment of cephalosporin C with acid produced 7-aminocephalosporonic acid (7-ACA), a crucial precursor for the synthesis of the majority of cephalosporins currently available on the market.[51,52]

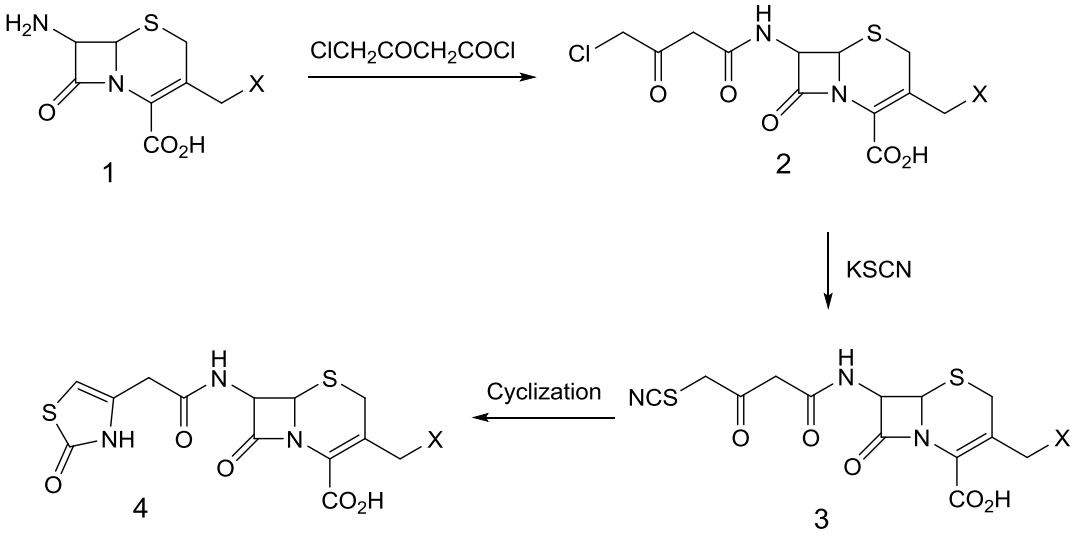


(Figure 1-9) Chemical structure of some cephalosporin's intermediates

1.2.4.1 Modification at position C-7

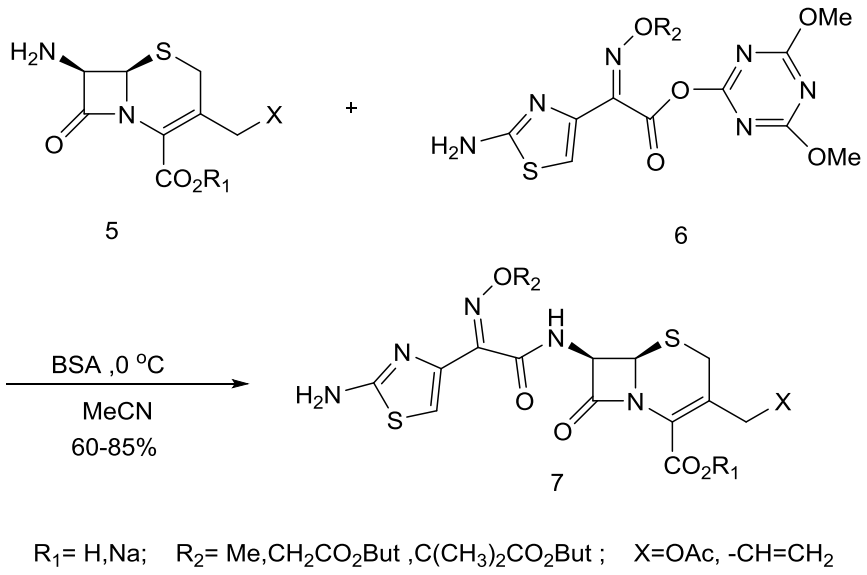
One strategy to develop cephalosporins which are currently in clinical use is by introduction of acyl groups into the amino moiety of 7-aminocephalosporins. These 7-aminoacyl groups together with the

substituents on the methylene in the 3-position impart different antimicrobial spectra, as well as the pharmacokinetic properties of cephalosporins. Acylation of 7-aminocef-3-em-4-carboxylic acids (1) with 4-chloro-3-oxobutanoylchloride gave 7-(4-chloro-3-oxobutyrylamino)-cephalosporins (2), which is then treated with potassium thiocyanate in acetonitrile to generate 7-(3-oxo-4-thiocyanatobutyrylamino)cephalosporins (3). Cyclization of (3) in pH 6.4 phosphate buffer furnished 7-[2-(2-oxo-4-thiazolin-4-yl)acetamido]cephalosporins (4) (Scheme 1-3).[53]



Scheme 1-3: Synthesis of 7-oxothiazolinyl cephalosporins

Another investigation demonstrated that the final 2-aminothiazole-bearing cephalosporins (7) were produced by treating 7-ACA derivatives (5) with active esters (6) after washing the reaction mixture with diluted acid and then sodium bicarbonate solution (Scheme 1-4).[54–56]

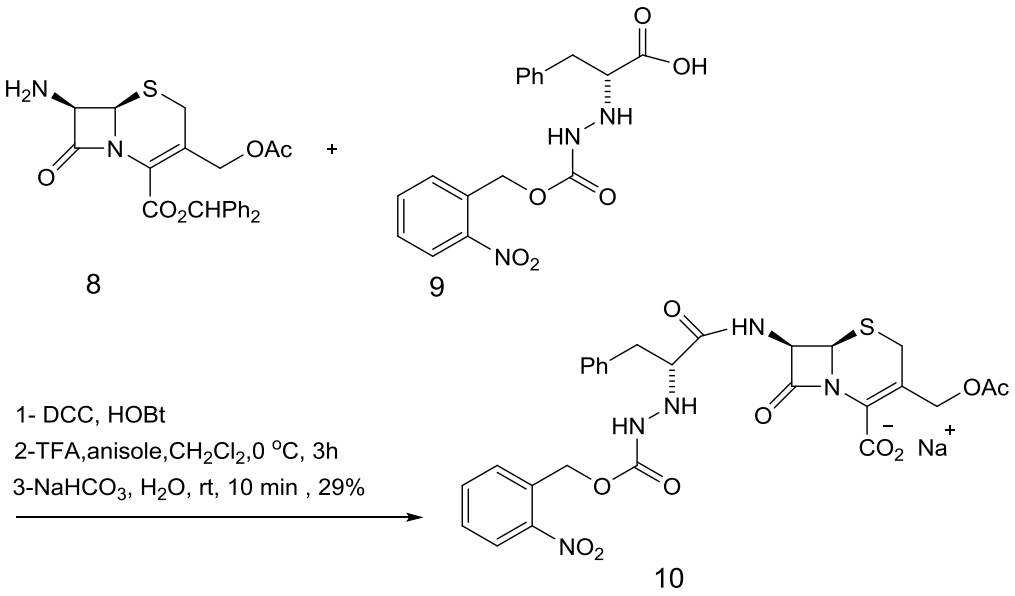


5 6 7

R_1= H,Na; R_2= Me,CH_2CO_2But ,$C(CH_3)_2CO_2But$; X=OAc, -CH=CH_2

Scheme 1-4: Synthesis of 2-aminothiazole cephalosporins

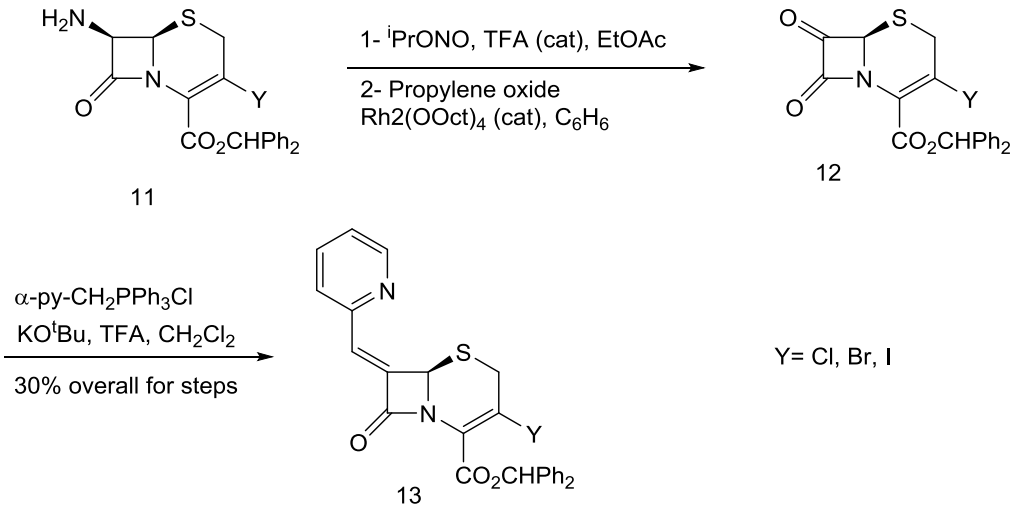
A new class of β-lactam antibiotic, 7-[sodium (R)]-2-(Nb -o-Nitrobenzyloxycarbonyl) hydrazino-3-phenylpropanamido]cephalosporanate (10), was synthesized by coupling 7β-aminocephalosporanic acid diphenylmethyl ester (8) with carboxylic acid (9), followed by removing the diphenylmethyl group from the product with trifluoroacetic acid (TFA). Since this kind of antibiotic can be self-destruct after several hours of exposure to light, there would be no chance for environmental selection of resistance (Scheme 1-5).[57]



8 9 10

Scheme 1-5: Synthesis of phenylpropanamido cephalosporanate

Conversion of the amines (11) to 7-oxocephalosporins (12), then installation of a double bond at the C-7 position through Wittig reaction with pyridine containing phosphonium ylide, produce C-7-alkylidene cephemes (13) (Scheme 1-6).[58–60]



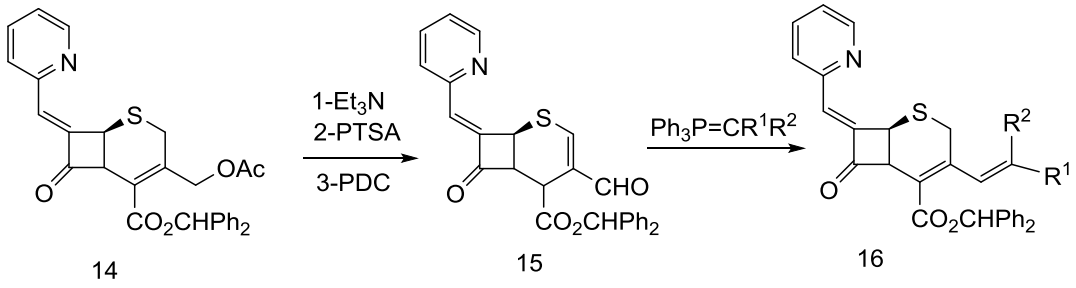
11 12 13

Scheme 1-6: Synthesis of C-7-alkylidene cephemes

1.2.4.2. Modification at position C-3

The main method used to modify the C-3 substituent chemically is to replace the halides of GCLE or the acetoxy group of 7-ACA with nucleophilic heteroatoms.[61]

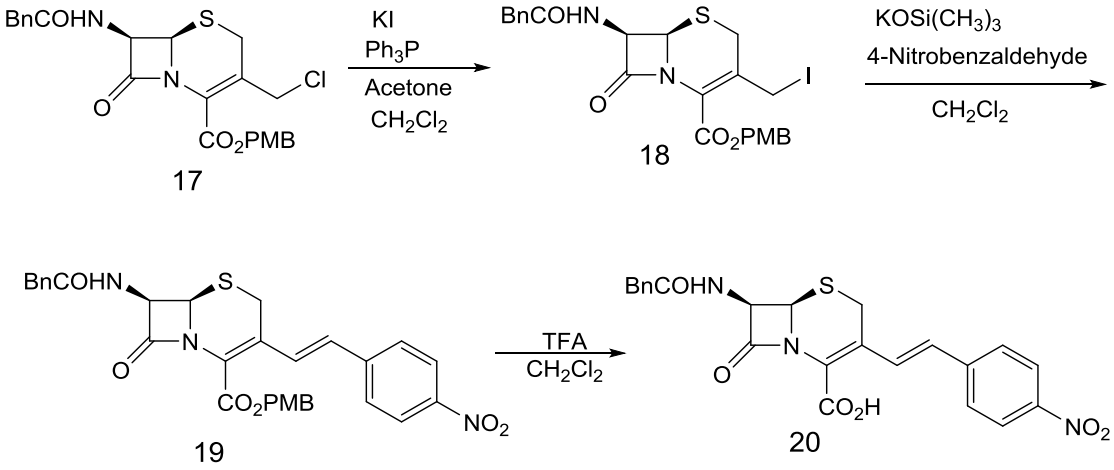
The comparable 3-formyl or 3-carboxy cephalosporin can be produced by oxidation of the alcohols that released during the hydrolysis of 3-acetoxymethyl cephalosporin. The Wittig olefination is one of the several transformations the 3-formyl cephalosporins can go through. For instance, the aldehyde (15) is formed through the hydrolysis of acetate (14), equilibrium of the α-2,3 isomer, and oxidation of the resulting alcohol. The aldehyde (15) then reacts with Wittig reagents to produce alkenes (16) (Scheme 1-7).[62,63]



R^1=CN, CO_2Me, $CONH_2$, $CH=CH_2$; R^2=H, Cl

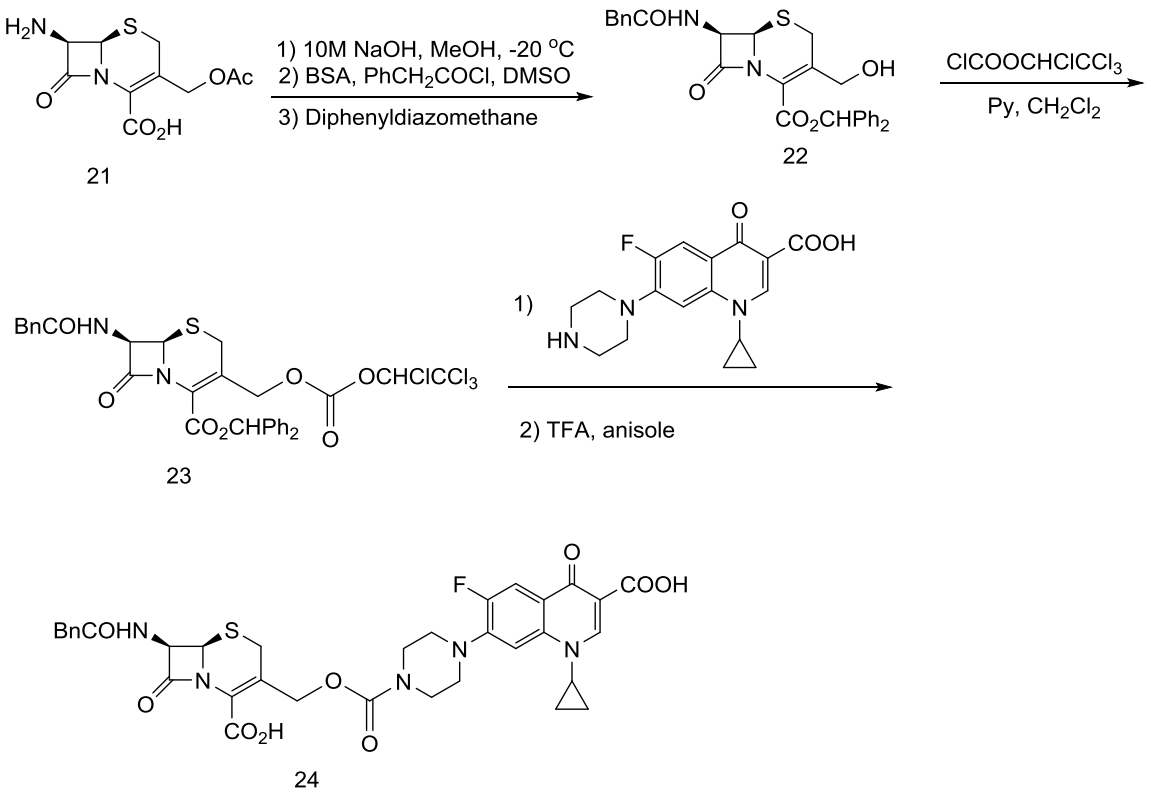
Scheme 1-7: Synthesis of the alkene cephalosporin

Sophia Yu *et al* were able to synthesize chromacef (20) [chromacef a chromogenic cephalosporin derivative has been the predominant assay substrate used for the detection of β-lactamase activity] from the reaction of GCLE (17) with *p*-nitrobenzaldehyde using Wittig reaction (Scheme 1-8).[4]



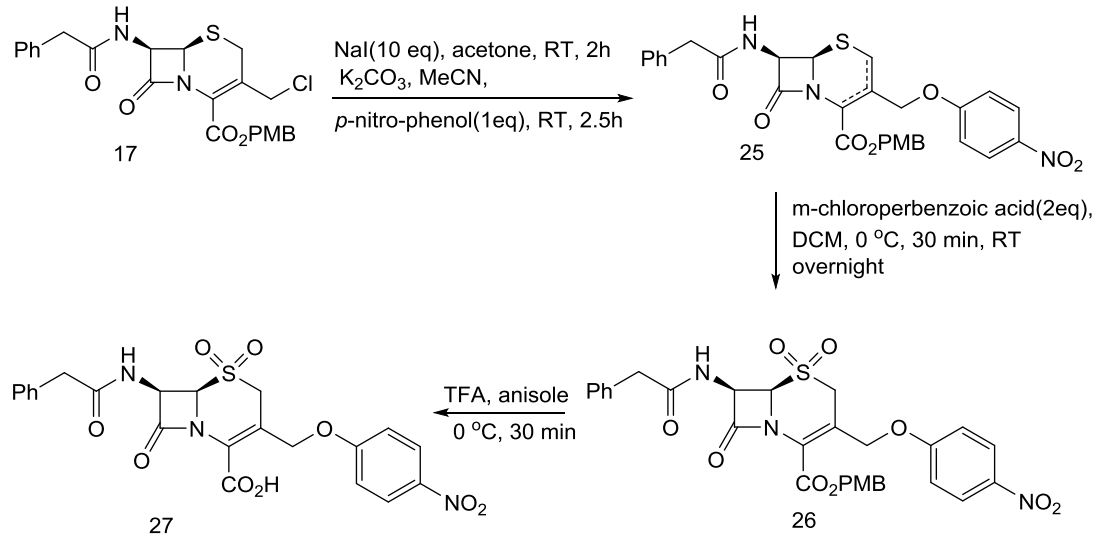
Scheme 1-8: Synthesis of chromacef

It has been found that adding an oxygen or sulfur atom to position C3 improves the antibacterial agent's potency. The synthesis of quinolone cephalosporins (24), which shown a considerable anti-tuberculosis activity, is one example. The first step entailed installing a phenylacetyl group on the C7 position, hydrolyzing the C3 acetyl group, and protecting the C4 carboxylic group. The final product is produced by converting the hydroxyl group to chloroformate at the C3 position, followed by coupling the resultant intermediate (23) with a quinolone derivative and finally deprotection with TFA (scheme 1-9).[64]



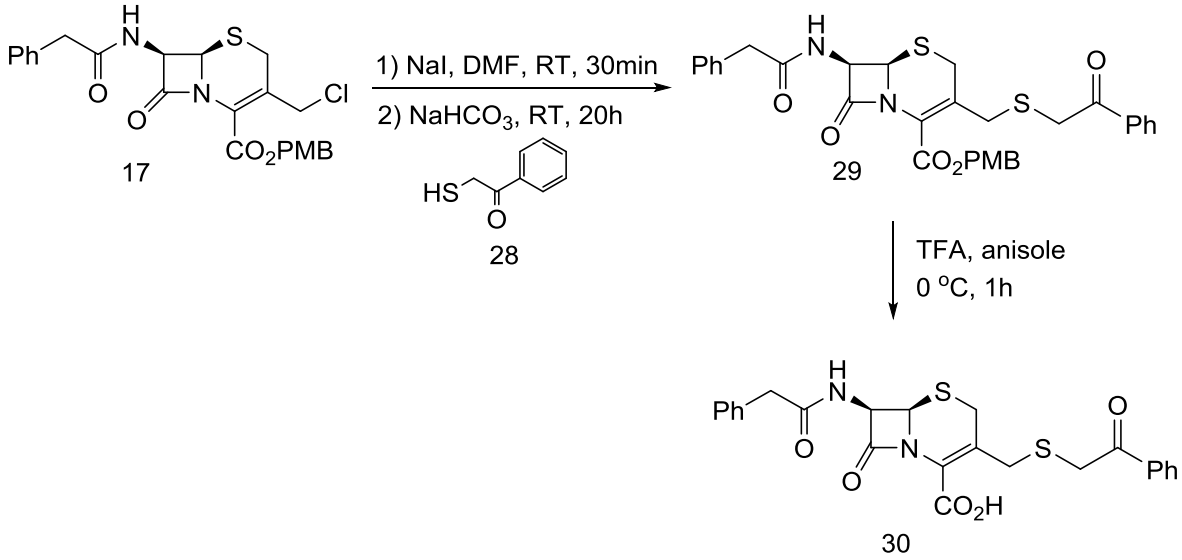
Scheme 1-9: Synthesis of quinolone cephalosporins

Makena *et al* , used a straightforward three-step technique to create chromogenic cephalosporin (27) starting with commercially available (PMB)-protected chlorocephalosporin (GCLE), which was alkylated with *p*-nitrophenol, followed by oxidation the sulfide to sulfone using mCPBA, then deprotection the precursor with TFA (Scheme 1-10).[65]



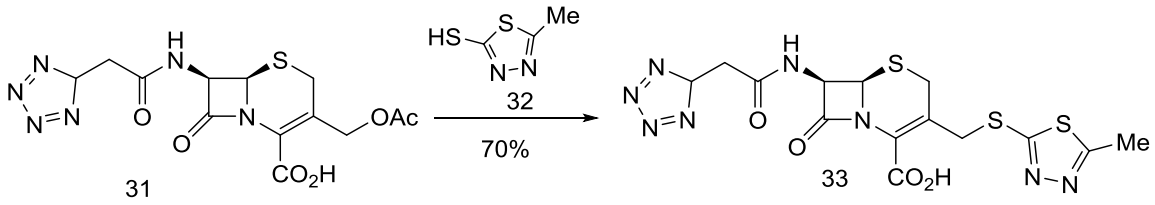
Scheme 1-10: Synthesis of chromogenic cephalosporin

Cephalosporin thiol conjugates were synthesized by thioalkylation of chloromethyl cephalosporin GCLE (17) with mercaptoacetophenone (28) to generate intermediate (29) followed by deprotection with trifluoroacetic acid (TFA) to produce the final compound (30) (Scheme 1-11).[66]



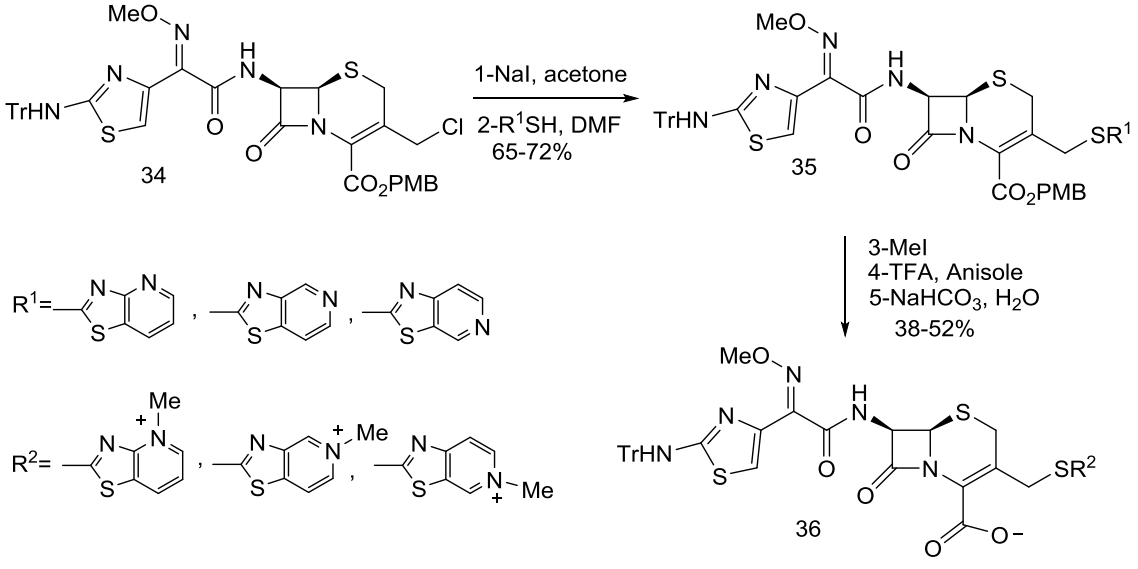
Scheme 1-11: Thioalkylation of cephalosporin

7-[(1H-Tetrazol-1-yl)acetamido]-3-(acetoxymethyl)-β3-cephem-4-carboxylic acid (31) was used as an intermediate for the synthesis of cefazolin (33) by direct displacement of the 3-acetoxy using group with 2-mercapto-5-methyl-1,3,4-thiadiazole(32) (Scheme 1-12).[67]



Scheme 1-12: Synthesis of cefazolin

Compound (34) was converted into sulfides (35) via the corresponding iodide intermediates by substitution with 2-mercaptothiazolo[4,5-c]pyridine, 2-mercaptothiazolo[5,4-c]pyridine, and 2-mercaptothiazolo[4,5-b] pyridine. These sulfides underwent quaternization of the thiazolopyridine group at position 3 by methylation with methyl iodide followed by deprotection with trifluoroacetic acid and anisole to give the final compounds (36) (Scheme 1-13).[68]



Scheme 1-13: Synthesis of cephalosporin thiol conjugates

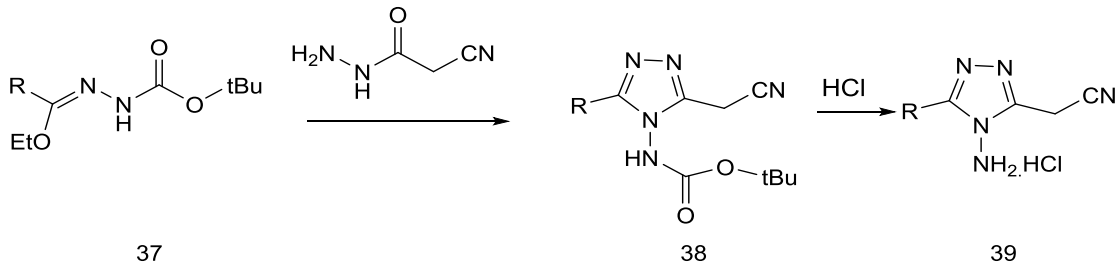
1.3. Triazoles

Triazole is a five-member heterocyclic molecule comprising two carbon atoms and three nitrogen atoms. It has two structural formulae (Figure 1-10).

(Figure 1-10) Structures of triazole

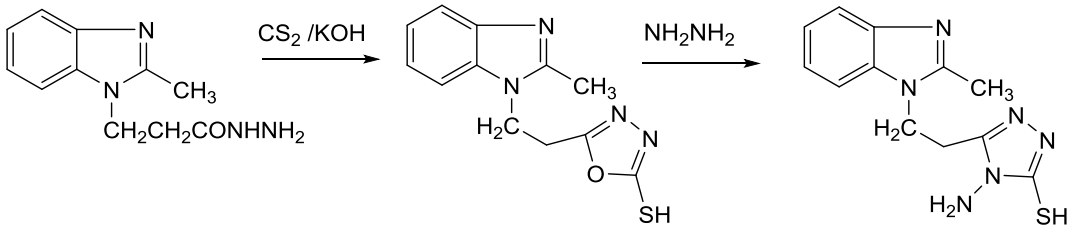
Triazoles, generally, and 1,2,4-triazole derivatives in particular, show a wide spectrum of biological activities among the five-membered heterocyclic compounds, gaining attention for their pesticidal activity, anti-covulsant, antianxiety, anti- cancer, anti-fungal and anti-bacterial properties. Various compounds containing 1,2,4-triazole rings are well known as pharmaceuticals.[69–76]Since first reported triazole synthesis in 1885,[77] many synthetic methods were developed which lead eventually to a vast variety of triazole derivatives.

N-tert-butylcarboxyethoxyhydrazone (37) produced 3-alkyl-4-tert-butoxycarbonylamino-5-cyanomethyl-4*H*-1,2,4-triazole (38) when it reacted with cyanoacetic acid hydrazide. Treatment of compound (38) with HCl, produced the product 3-alkyl-4-amino-5-cyanomethyl-4*H*-1,2,4-triazole hydrochloride (39) in good yield (Scheme 1-14).[78]



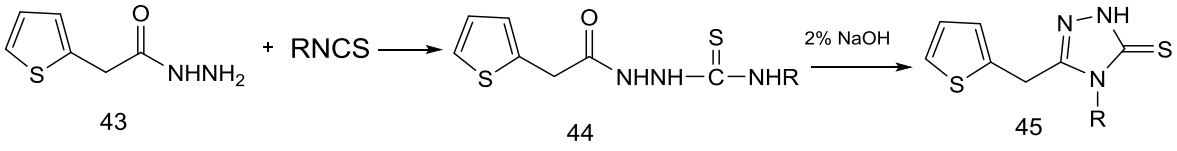
Scheme 1-14: Synthesis of triazole hydrochloride

By using carbon disulfide and potassium hydroxide, 3(2-methyl benzimidazol-1-yl)propanoic acid hydrazide (40) produced 5-[2-(2-methyl benzimidazol-1-yl) ethyl]-1,3,4-Oxadiazole-2(3*H*)-Thione (41). The intermediate (41) yielded the product 5[2-(2-methyl benzimidazole-1-yl) ethyl]-4-amino [1,2,4] triazole-3-thiol (42) upon treatment with hydrazine hydrate in ethanol (Scheme 1-15).[79]



Scheme 1-15: Synthesis of triazole-3-thiol

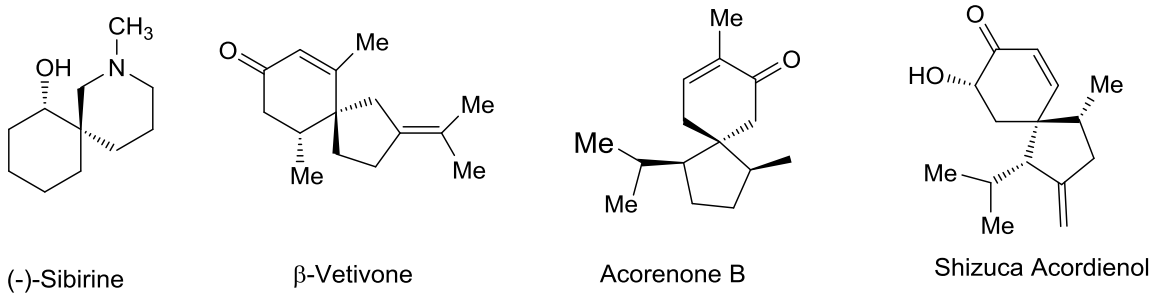
Thiophene-2-acetate (43) and isothiocyanates are mixed to produce new thiosemicarbazide derivatives (44). By using a 2% solution of sodium hydroxide, these compounds produced 4-substituted 3-(thiophene-2-yl-methyl)-1,2,4-triazoline-5-thiones (45) (Scheme 1-16).[80]



Scheme 1-16: Synthesis of 4-substituted triazoline-5-thiones

1.4. Spiro compounds

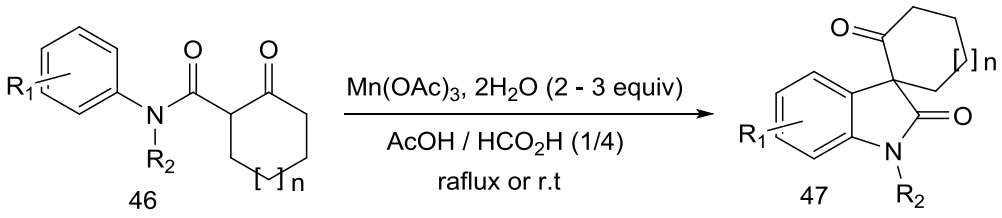
Spiro compounds, also known as spirocycles, are rigid skeletons made up of at least two rings joined together by a single spiro atom. Von Baeyer made the initial discovery of the spirocycle structure in the late 1890s. For chemists, the enantioselective synthesis of spiro compounds still presents significant challenge. Spirocycles are important because of their distinctive structural characteristics as well as the fact that they are found in many natural compounds, including the alkaloid (-) sibirine, Acorenone B, β-Vetivone and Shizuca Acordienol. (Figure 1-11).[81,82]



(Figure 1-11) Some compounds containing a spirocyclic ring

Scientists working in both the medical and synthetic fields are looking for more effective ways to create complex, bioactive compounds. To deal with a range of issues, the drug design process can be approached in a number of different ways. One of them is the addition of new ligands and the conformational limitation caused by the intended molecule's stiffness, which results in a complex and stereo-defined 3D structure. As a result, a wide range of complex and bioactive molecules have been created using spirocyclic chemicals as building blocks. Compared to planar aromatic rings, the installation of a spirocyclic core in the target results in significant molecular diversity and three-dimensionality, which has several benefits.[83–85]

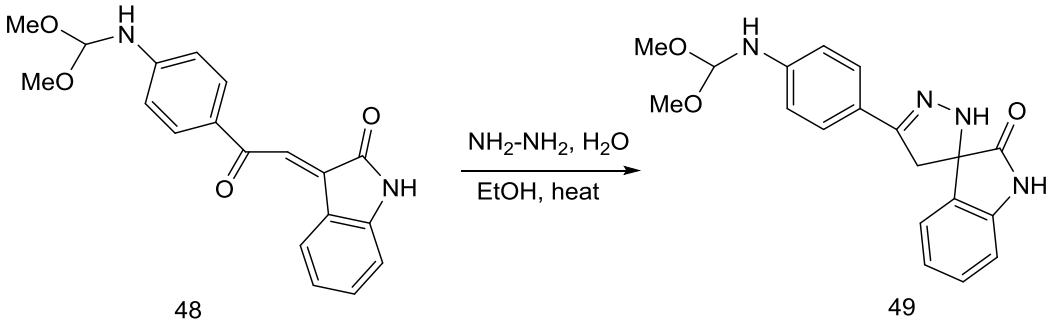
Many research groups have employed $Mn(OAc)_3$ to create some structurally significant spirocyclic molecules. In the synthesis of spiroindolinones (47), Nishino and Katayama described an oxidative cyclization using $Mn(OAc)_3$ of dicarbonyl compounds (46)(Scheme 1-17).[86]



R_1= H, 4_Me, 4_OMe, 4_Cl, 4_F, 3_Me, 2_Me
R_2= Me, Et, n_Pr, i_Pr, n_Bu
n= 0 or 1

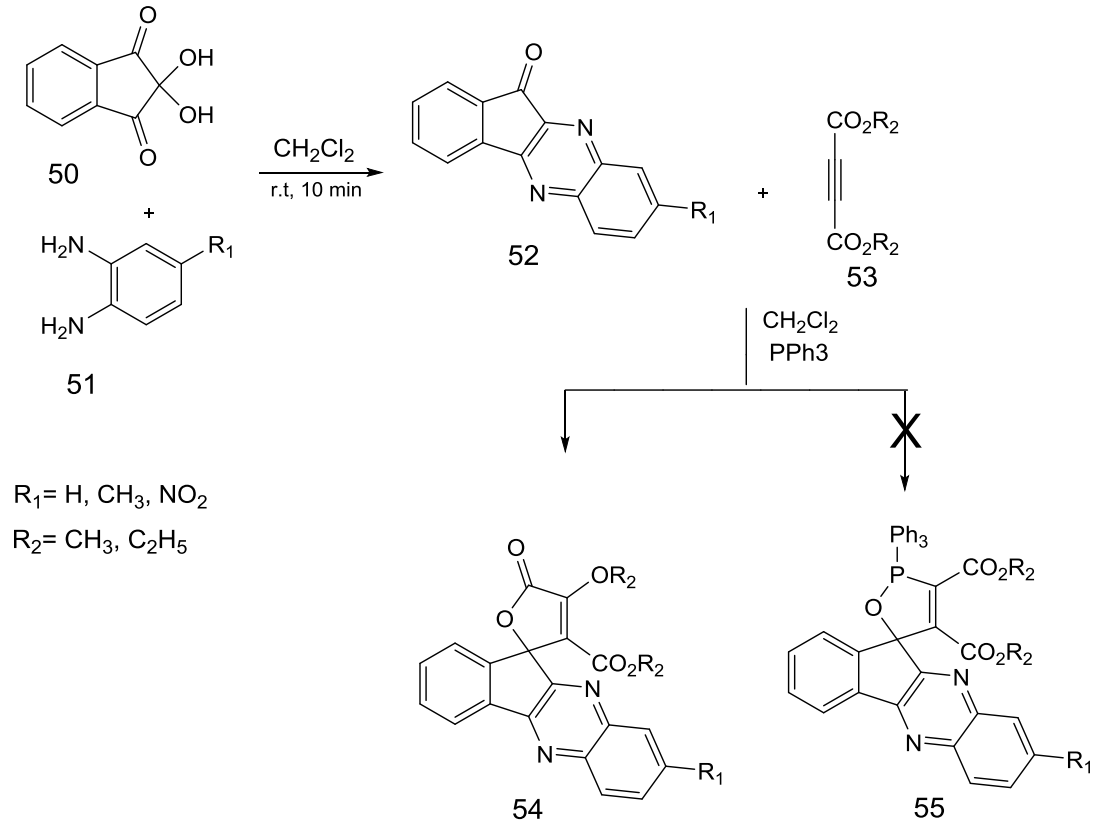
Scheme 1-17: Synthesis of spiroindolinones

Condensation of (E) -3-(2-(4-((dimethoxymethyl)amino)phenyl) -2-oxoethylidene)indolin-2-one (48) and hydrazine hydrate produced 5-(4-methoxycarbonylaminophenyl) -2,4-dihydrospiro[indole-3,3-pyrazol] - 2(1H)-one (49) in excellent yield 93% (Scheme 1-18).[87]



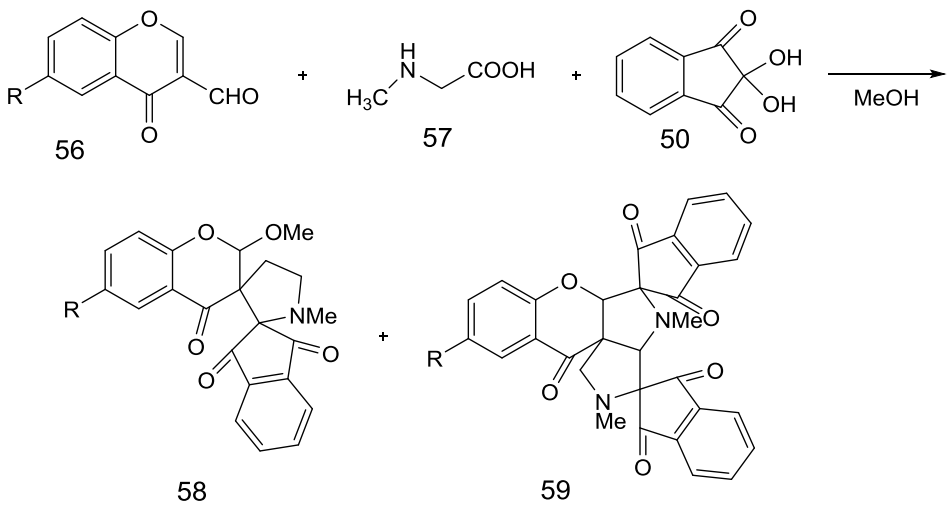
Scheme 1-18: Synthesis of dihydrospiro[indole-3,3-pyrazol]-one

Maghsoodlou *et al*, have studied a domino reaction between ninhydrin (50), benzene-1,2-diammine (51), dialkylethynedicarboxylates (53) and triphenyl phosphine (Ph_3P). The results demonstrated that unexpected product spirofuran-indenoquinoxaline derivatives (54) were successfully formed as opposed to (55) (Scheme 1-19).[88]



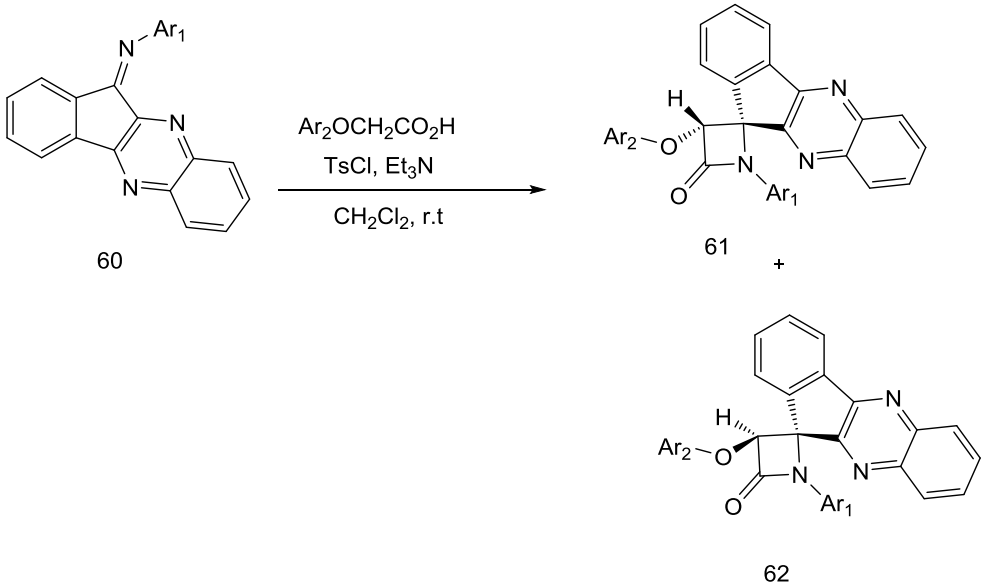
Scheme 1-19: Synthesis of spirofuranindenoquinoxaline derivatives

Chromone 3-carbaldehyde (56), sarcosine (57), and ninhydrine (50) were used in a multicomponent synthesis to create new dispirochromenopyrrolidines (58, 59) (Scheme 1-20).[89]



Scheme 1-20: Synthesis of dispirochromenopyrrolidine

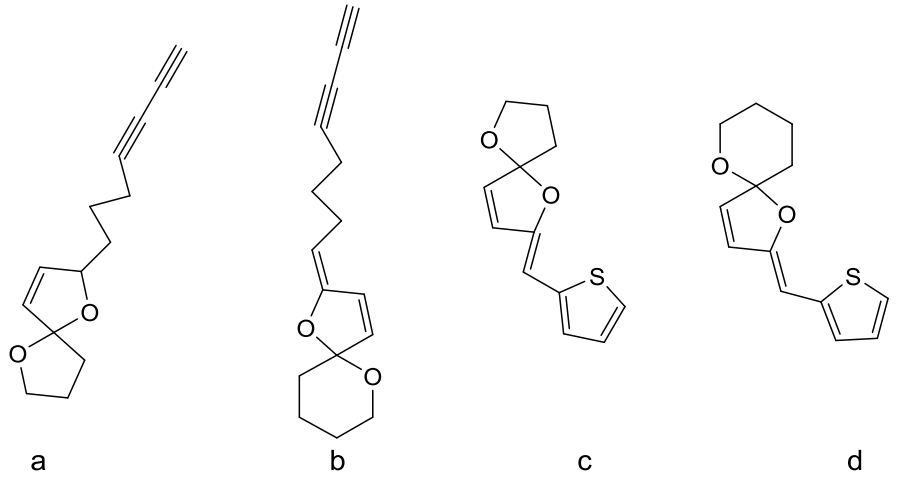
The synthesis of spiro-β-lactam system comprises the [2 + 2] cycloaddition reaction between N-phenyl-11H-indeno[1,2-b]quinoxalin-11-imine derivatives (60) and different phenoxyacetic acid derivatives in the presence of triethylamine, *p*-toluenesulfonyl chloride (TsCl) at room temperature. The two diastereomeric forms (61) and (62) of the resulting spiro-β-lactams were produced in equal amounts (Scheme 1-21).[90]



Scheme 1-21: Synthesis of spiro-β-lactams

1.4.1. Biological importance of spiro compounds

For a very long time, it has been recognised that terpenoids, lactones, or alkaloids found in phytochemicals all contain the spiro functionality. The Columbian poison dart frog, *Dendrobats histrionius*, produces the spirocyclic alkaloid (K)-histrionicotoxin (a), which is a highly effective nicotinic receptor antagonist. Spiroketals are reportedly the building blocks of a variety of biologically interesting naturally occurring compounds, including insect pheromones, antifeedants, and polyether antibiotics. A group of spiroketals (b-d) (Figure 1-12) have been identified from the common vegetable *Chrysanthemum coronanium* in South China. Some of these substances are discovered to exhibit antiphlogistic, spasmolytic, and antifeeding activity against silkworms.[100]



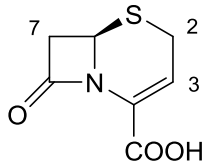
(Figure 1-12) Chemical structure of some spiroketals

Spiro-compounds form a group of generally less investigated compounds. However, recently growing efforts have been made to synthesize and characterize these compounds. Many spirocompounds possess very promising biological activities as anticancer agents[91–94], antibacterial agents[95–97],

anticonvulsant agents, anti-tuberculosis agents, anti-Alzheimer's agents[98–100], pain-relief agents, anti-dermatitis agents, anti-Parkinsonian agents[101–103], antiviral agents[104–107] and β-lactamase inhibitors[108]. In addition to their medical uses, some spiro-compounds have found other uses in the agricultural and industrial fields. For example, they are used as antifungal agents, pesticides, laser dyes and electroluminescent devices. Spiro compounds have also been recently used as antioxidants.[80,109,110]

1.4.2. Spiro-cephalosporins

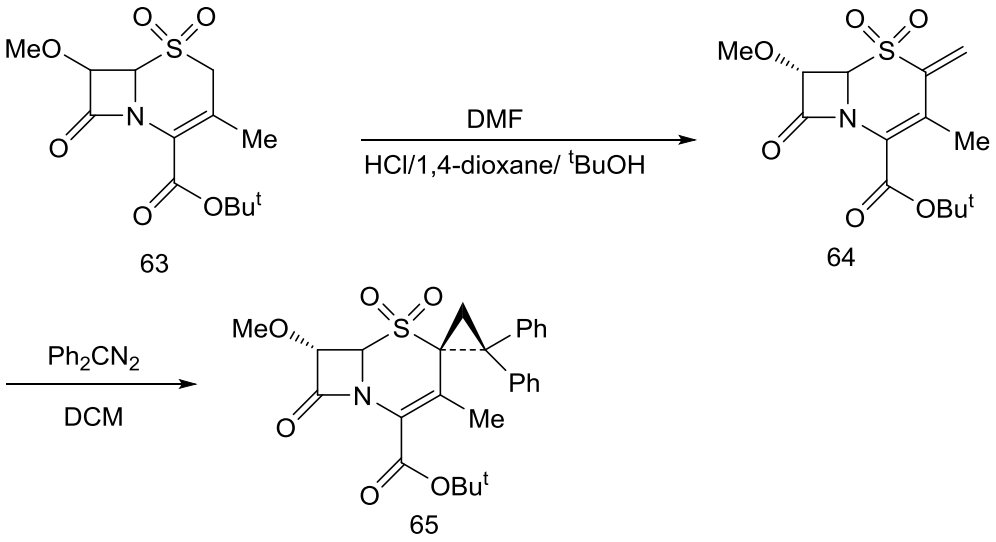
Spirocyclic beta-lactams are one of spirocyclic structures, that have drawn more and more intention since they not only have the desired antibacterial capabilities, but also because they also have other interesting activities, including being enzyme inhibitors and antiviral agents. Multiple reaction sites can be used to modify penicillin- or cephalosporin-based β-lactam antibiotics with spirocyclic moieties without altering their β-lactam core. Spirocycles can be fused directly to the β-lactam ring (site 7) or attached to the dihydrothiazine ring (sites 2 and 3) (Figure 1-13).[111–115]



3-cephem

(Figure 1-13) potential sites of modification in spiro-cephalosporins

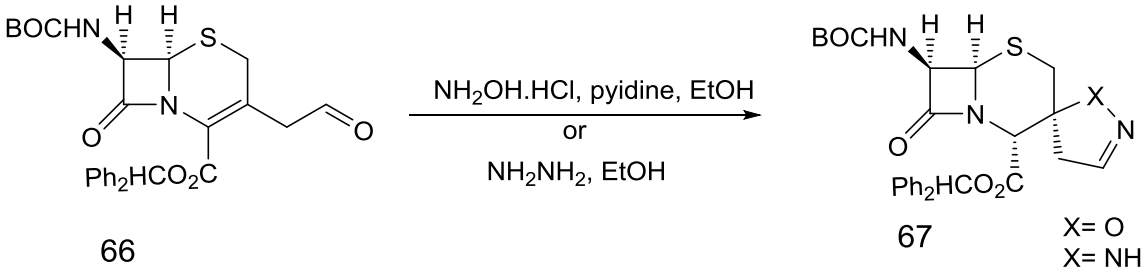
Several different methods to achieve spiro modification at site 7 have been reported, including: (1) 1,3-dipolar cycloaddition approaches; (2) a phosphine-catalyzed [3+2] cycloaddition approach; and (3) a rhodium-catalyzed cyclopropanation approach. However, the methods for 2 and 3-site spiro modifications are quite limited, mostly based on 1,3-dipolar cycloaddition reactions.[2,116–118] One example of modification at site 2 was started by conversion of t-Butyl 7α-methoxy-3-methyl-3-cephem-4-carboxylate 1,1-dioxide (63) to the 2-exomethylene derivative (64) under Mannich conditions. The derivative (64) was then reacted with diphenyldiazomethane to give the product t-butyl 1,1-dioxide-7α-methoxy-2-spiro(2',2'-diphenyl)cyclopropane)-3-methyl-3-cephem-4-carboxylate (65) with a yield of 85% (Scheme 1-22).[114]



Scheme 1-22: Synthesis of spiro-3-cephem

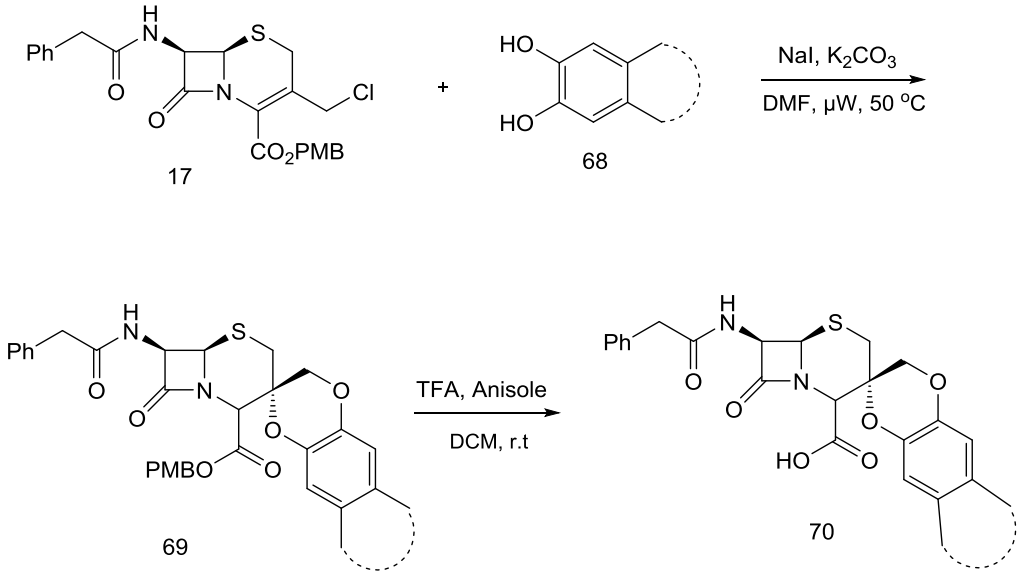
Another example of modification at site 3 was done by Hennequin *et al*., through condensation the aldehyde (66) with hydroxylamine or hydrazine to

obtain the spiro-cephalosporins (67) as single diastereoisomers (Scheme 1-23).[119]



Scheme 1-23: Synthesis of 3-spirocephams

Recently, cephalosporin intermediate GCLE was used to prepare different spiro-cephalosporins (69) through Michael addition reaction with various catechols (68) in the presence of K_2CO_3 under microwave irradiation. The *p*-methoxybenzyl (PMB) protecting group was removed in the presence of trifluoroacetic acid (TFA) and anisole to give functionally active spiro-cephalosporin compounds (70) (Scheme 1-24).[120]



Scheme 1-24: Synthesis of spiro-cephalosporin

1.5. Study aims

The research project aims to:

1- Synthesize of some 5- aryl-1,2,4 triazolidine-3-thiones using green approach and characterization of this compounds using FTIR, ^{1}HNMR and^{13}CNMR spectrometry.

2- Synthesize of novel 1,2,4-triazole bearing spiro-cephalosporins derivatives and characterization of this compounds using FTIR, ^{1}HNMR, ^{13}CNMR and HRMS spectrometry.

3- Evaluation the antibacterial activity of synthesized spiro-cephalosporins against a representative human pathogenic Gram-positive and Gram-negative bacteria.

CHAPTER TWO

2. Experimental

2.1. Chemical Materials

The chemicals used in this study were supplied from the companies showing in Table (2-1).

Table (2-1): The chemicals used in the study

Chemicals	Company
Thiosemicarbazide	Sigma-Aldrich
p-Flourobenzaldehyde	Sigma-Aldrich
p-Chlorobenzaldehyde	TCI
o-Chlorobenzaldehyde	Sigma-Aldrich
Salicylaldehyde	Thomas Baker
p-Hydroxybenzaldehyde	Fluorochem
Benzaldehyde	Loba Chemie
p-Bromobenzaldehyde	Fluorochem
o-Bromobenzaldehyde	Sigma-Aldrich
p-Ansialdehyde	HOPKIN&WILLIAMS
GCLE	Tianfuchem
Potassium carbonate	Thomas Baker
Sodium chloride	Thomas Baker
Anhydrous magnesium sulfate	Scharlau
Silica gel 60-120	Thomas Baker
Absolute ethanol	Scharlau
Acetone	J.T.Baker
Ethyl acetate	SDFCL
Petroleum ether 40-60	SDFCL
Dichloromethane	Merck
Chloroform	Merck
Methanol	Scharlau
Anisole	Himedia
Trifouroacetic acid	Thomas Baker

2.2. Instruments

2.2.1. Melting Point

All melting points are uncorrected and expressed in degree (°C). They were measured at the Department of Chemistry, College of Science, University of Misan, by using Stuart SMP11 melting point apparatus.

2.2.2. Thin Layer Chromatography

TLC was performed using silica gel 60 F^{254} on Merck precoated aluminium sheet (0.2 mm thickness), with visualization by UV light.

2.2.3. Column Chromatography

All synthesized compounds were purified using flash column chromatography on silica gel (60-120 mesh).

2.2.4. Fourier Transform Infrared Spectrophotometer

FTIR spactra of all synthesized compounds were measured as KBr disc for solid samples for the region between (400-4000) cm^{-1} using SHIMADZU IRAffinity-1 (Japan) at BPC-Anlysis Center in Baghdad, Iraq. Only principal absorption bands of interest were reported and expressed in cm^{-1}.

2.2.5. Nuclear Magnetic Resonance Spectrometer

All ^{1}H-NMR, ^{13}C-NMR and HSQC experiments were recorded at the University of Basrah, Iraq using Bruker DRX-400 spectrometer (Germany) and chemical shifts are reported in ppm (δ). DMSO-d_6 was used as a solvent, while TMS was used as an internal standard. The coupling constants (J) are

measured in Hertz and multiplicities are quoted as singlet (s), doublet (d), triplet (t), quartet (q), doublet of doublets (dd), multiplet (m).

2.2.6. Mass Spectrometer

Electrospray ionization (ESI) high-resolution mass spectra (HRMS) were determined using Thermo Scientific Orbitrap Exploris 120 Mass Spectrometer (mass analyzer type: QTOF) at Mass Spectrometry Center operated by the College of Chemistry and Biochemistry, Auburn University, USA.

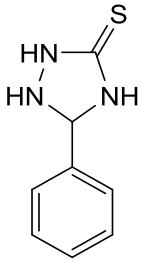
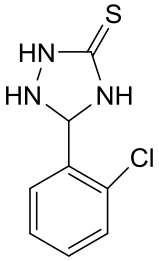
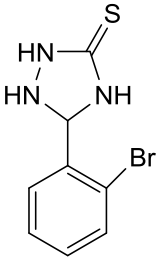
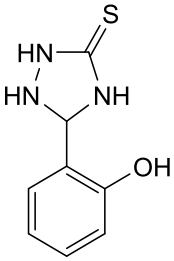
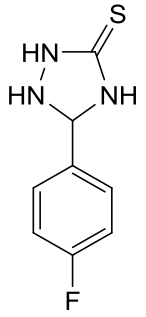
2.3. Synthetic Methods

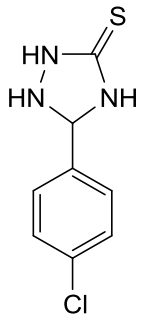
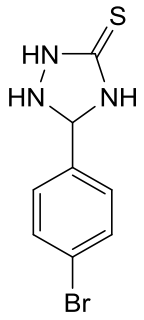
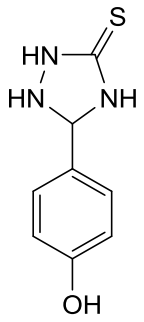
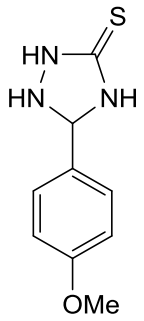
All the experimental reaction was always done in flame and oven-dried glassware under nitrogen atmosphere and at room temperature (r.t), unless otherwise indicated.

2.3.1. Synthesis of 5- aryl-1,2,4 triazolidine-3-thiones (A1-A9)

Thiosemicarbazide (4 mmol) and aromatic aldehyde (4 mmol) were added to RB flask containing 25% ethanol (20 mL). The reaction mixture was stirred at 80 °C for the time indicated in the table (1-2) and monitored by TLC (EtOAc: Petroleum ether; 1:2) until the reaction was completed. After completion of reaction, the mixture was allowed to cool to room temperature. The solids were filtered, and washed with cold ethanol and dried. The crude products were recrystallized from ethanol to give a white/yellow solid 5- aryl-1,2,4 triazolidine-3-thiones in excellent purity (Table 2-2).[121]

Table (2-2): 5- aryl-1,2,4 triazolidine-3-thiones (A1-A9)

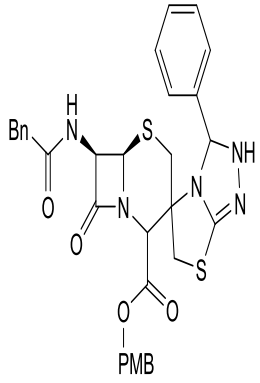
Comp. No.	Structural formula	Molecular formula	Yield (%)	Time (min)	M.P (°C)
A1		$C_8H_9N_3S$	78	30	168
A2		$C_8H_8ClN_3S$	63	15	187-190
A3		$C_8H_8BrN_3S$	95	15	204
A4		$C_8H_9N_3OS$	78	30	211-213
A5		$C_8H_8FN_3S$	65	15	184-186

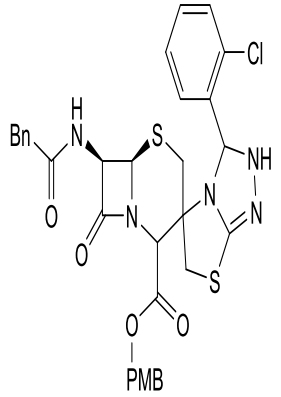
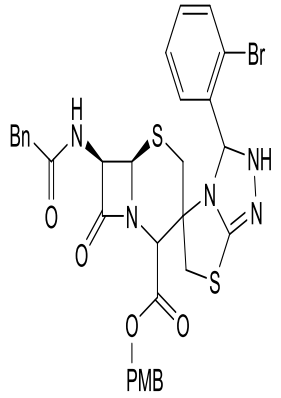
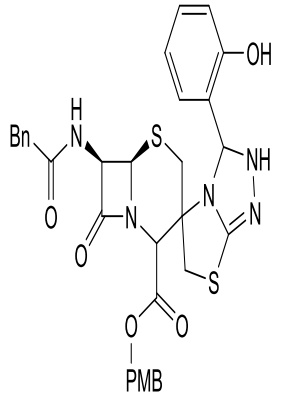
Comp. No.	Structural formula	Molecular formula	Yield (%)	Time (min)	M.P (°C)
A6		$C_8H_8ClN_3S$	74	15	207-209
A7		$C_8H_8BrN_3S$	80	15	196
A8		$C_8H_9N_3OS$	61.8	15	218-220
A9		$C_9H_{11}N_3OS$	66	30	188-190

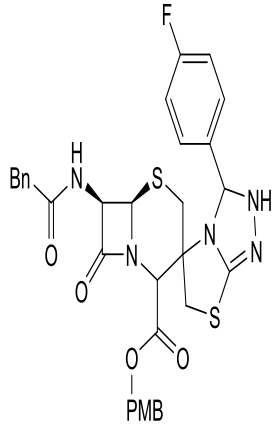
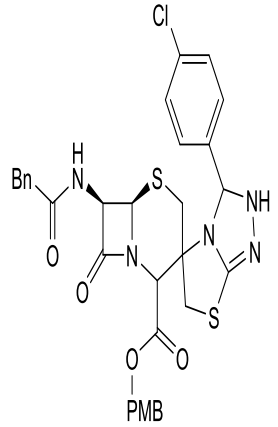
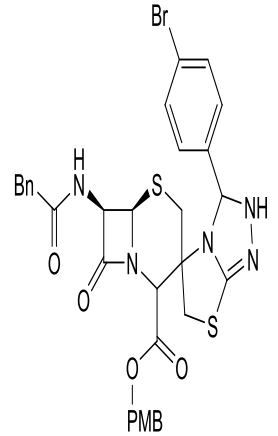
2.3.2. Synthesis of PMB-protected spiro-cephalosporins (B1-B7)

A mixture of GCLE (2.06 mmol), 5- aryl-1,2,4 triazolidine-3-thiones (**A1**-**A7**) (2.36 mmol) and K_2CO_3 (3.9 mmol) in acetone (20 mL) and H_2O (3 mL) were stirred for 16 hours at room temperature. The reaction progress was monitored by TLC (EtOAc: Petroleum ether; 1:2) until the reaction was completed. The reaction mixture was diluted with EtOAc (50 mL), washed with brine (3 × 10 mL), dried over $MgSO_4$, evaporated under reduced pressure by rotary evaporator and the product was purified via silica gel column chromatography using (EtOAc: Petroleum ether; 1:1) as eluent to give the product as a solid in excellent purity (Table 2-3).[122]

Table (2-3): PMB-protected spiro-cephalosporins (B1-B7)

Comp. No.	Structural formula	Molecular formula	M.P (°C)	Yield (%)
B1		$C_{32}H_{31}N_5O_5S_2$	92-93	17.3

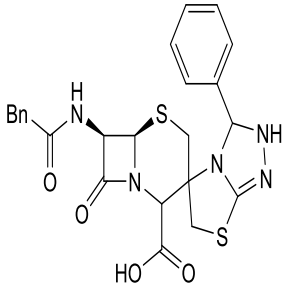
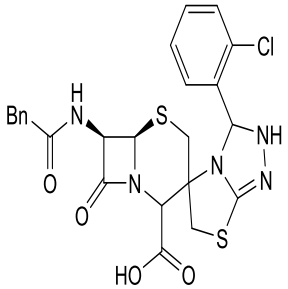
Comp. No.	Structural formula	Molecular formula	M.P (°C)	Yield (%)
B2		$C_{32}H_{30}ClN_5O_5S_2$	98-99	24.2
B3		$C_{32}H_{30}BrN_5O_5S_2$	95-96	20
B4		$C_{32}H_{31}N_5O_6S_2$	117-118	32

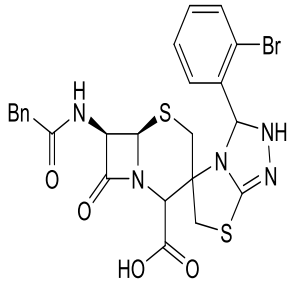
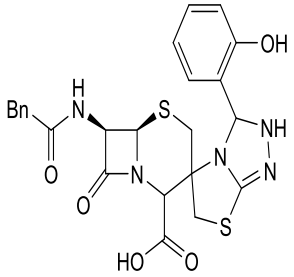
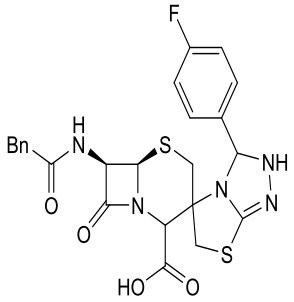
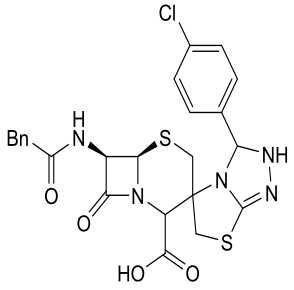
Comp. No.	Structural formula	Molecular formula	M.P (°C)	Yield (%)
B5		$C_{32}H_{30}FN_5O_5S_2$	100-102	24
B6		$C_{32}H_{30}ClN_5O_5S_2$	115-117	20
B7		$C_{32}H_{30}BrN_5O_5S_2$	89-90	23

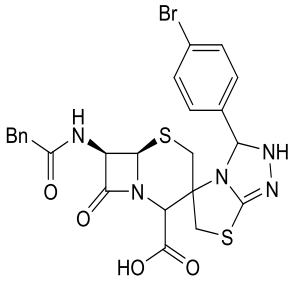
2.3.3. Synthesis of Spiro Cephalosporins (C1-C7)

PMB-protected spiro-cephalosporins (**B1-B7**) (0.28 mmol) were dissolved in CH_2Cl_2 (6 ml) and while stirring in the dark at 0 °C and under a nitrogen atmosphere, TFA (3 ml) and anisole (0.3 ml) were added and the solution was stirred for 1.5-2 hours. The solvents were removed on a rotary evaporator and CH_2Cl_2 (8 ml) was added and removed to co-evaporate the left over TFA. The crude products were purified via silica gel column chromatography (MeOH : CH_2Cl_2, 4:100) to give the products as orange/yellow solid in excellent purity (Table 2-4).[65]

Table (2-4): Synthesized Spiro Cephalosporins (C1-C7)

Comp. No	Structural formula	Molecular formula	M.P (°C)	Yield (%)
C1		$C_{24}H_{23}N_5O_4S_2$	145-147	50
C2		$C_{24}H_{22}ClN_5O_4S_2$	139-140	46

Comp. No	Structural formula	Molecular formula	M.P (°C)	Yield (%)
C3		$C_{24}H_{22}BrN_5O_4S_2$	170	50
C4		$C_{24}H_{23}N_5O_5S_2$	160-162	50
C5		$C_{24}H_{22}FN_5O_4S_2$	138-139	64
C6		$C_{24}H_{22}ClN_5O_4S_2$	150-153	59.3

Comp. No	Structural formula	Molecular formula	M.P (°C)	Yield (%)
C7		$C_{24}H_{22}BrN_5O_4S_2$	168-170	80

2.4. Antibacterial assay

The antibacterial activities of the final spiro-cephalosporins (**C1**-**C7**) were evaluated. Four organisms, methicillin resistance *staphylococcus aureus*, *Listeria monocytogenes, E.coli* O157, and *salmonella Newport*, were chosen based on multidrug resistance pathogen, and as representative pathogens of Gram-positive and Gram-negative bacteria, respectively.

The four bacterial strains were streaked individually onto Tryptic Soy Agar (TSA) plates and incubated at 37 °C to obtain single colonies. About 3 to 5 isolated colonies were transferred from each plate into 15 mL sterile tubes containing 5 mL of Tryptic Soy Broth (TSB) using sterile disposable inoculating loops. Each bacterial culture was vortexed and incubated at 37 °C on a shaker at 350 rpm for 2−4 h. Sterile TSB was used to adjust the turbidity of *Listeria monocytogenes, Salmonella Newport, staphylococcus aureus and E.coli O157* suspensions to obtain approximately equal optical density (OD) to that of 0.5 McFarland Standard (~1.5 x10 ^{8} CFU/ml. The OD of each bacterial suspension was measured at 600 nm (OD600) using an UV−vis spectrophotometer (Nanodrop 2000c, Wilmington, DE, USA). The starting

bacterial concentrations measured at OD600 ranged between 0.098 and 0.112 for all bacterial culture.

2.4.1. Disc Diffusion Method

The disc diffusion method for antimicrobial susceptibility testing was used, as described by Bauer et al. (1966).[123] Using a sterile cotton swab, a bacterium culture (adjusted to the 0.5 McFarland standard) was used to cover Muller Hinton agar plate uniformly. After drying for 15 minutes, the plate was ready for the antibacterial test. 5 mg of synthesized compounds was dissolved in 100 µl DMSO, after that a Whatman paper disc (6 mm) was socked in the solution for one minute to get a final concentration of 750 µg for each disc then all discs were placed on the Mueller Hinton agar surface. Ampicillin (150 µg) and DMSO were served as positive control and negative control, respectively. All the plates were incubated at 37°C for 24 to 48 hours depending on the species of bacteria used in the test. After the incubation period, each plate was analyzed for the inhibition zone. The inhibition zone was measured in millimeters using rulers and recorded. Each test repeated three times to ensure reliability.

CHAPTER THREE

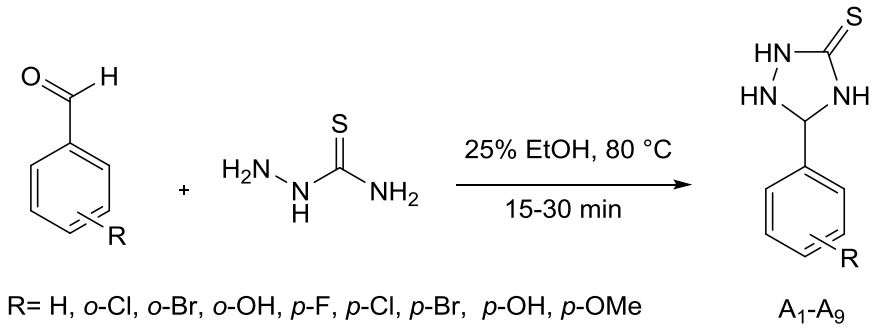
3. Results and Discussion

3.1. 5-aryl-1,2,4-triazolidine-3-thiones (A1-A9)

3.1.1. The synthetic strategy

An efficient and green synthetic method was chosen to prepare the targeted 1,2,4-triazolidine due to its neutral reaction conditions, short reaction time, good product yields and simple works up.[121]

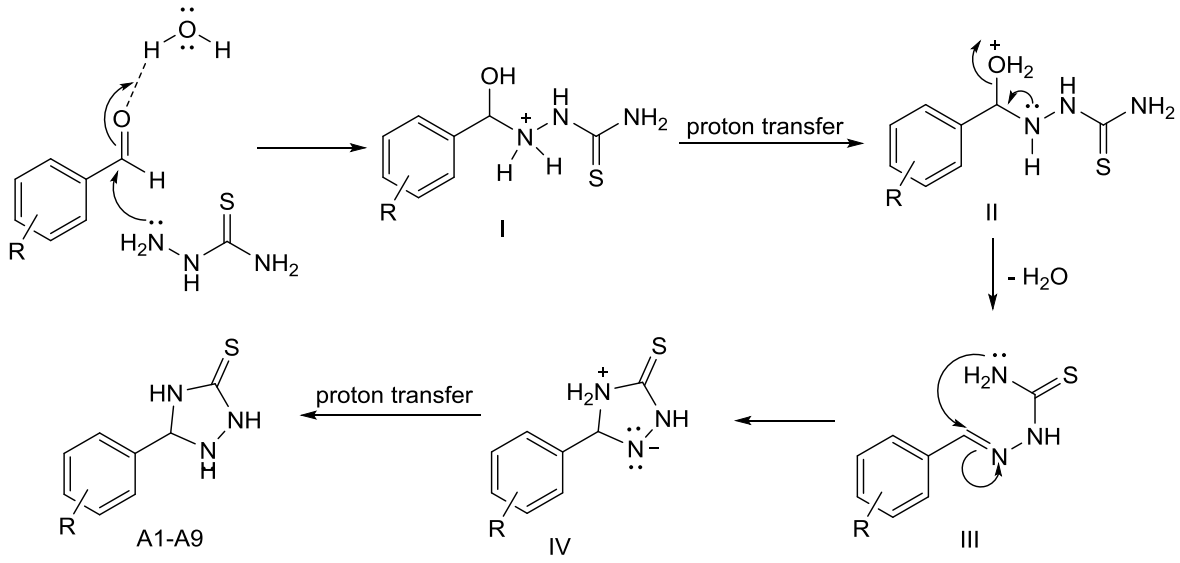
The protocol involves treatment of (4 mmol) thiosemicarbazide with (4 mmol) aromatic aldehydes in a flask containing 25% ethanol (20 mL); the reaction mixture was stirred at 80 °C until the reaction completed (Scheme 3-1).



Scheme 3-1: Synthesis of 5-aryl-1,2,4-triazolidine-3-thiones

3.1.2. The proposed mechanism

The proposed mechanism of the reaction involves activation of aromatic aldehydes through hydrogen bonding between hydrogen of water molecule and carbonyl oxygen atom of aldehydes to increase the electrophilicity of the carbonyl carbon. Then, the thiosemicarbazide added to carbonyl group to form thiosemicarbazone as an intermediate, which undergoes intra-molecular nucleophilic addition by the free -NH_2 to produce 5-aryl-1, 2,4-triazolidine-3-thiones (Scheme 3-2).



Scheme 3-2: mechanism of Synthesis of 5-aryl-1,2,4-triazolidine-3-thiones

3.1.3.Spectroscopic characterization of 1,2,4-triazolidines(A1-A9)

The structure of synthesized compounds were confirmed by spectroscopic techniques such as IR, ^{1}HNMR and ^{13}CNMR, which are in agreement with previous literatures.[124]

IR spectrum of these compounds shows absorption bands in three regions (3360-3443.29), (3232.7-3317.5) and (3197.9-3143.97) cm^{-1} respectively, which belong to three -NH stretching vibration of the heterocyclic ring, while the bands at the region (1600.9-1612.49) cm^{-1} representing the presence of aromatic C=C stretching vibration. The bond (C-N) stretching vibration appears in (1361.74-1381) cm^{-1}, and also a band at (1238-1296.16) cm^{-1} representing the presence of C=S stretching.

^{1}HNMR spectrum of the compounds showed three distinctive singlets at δ (11.33-11.62), (8.13-8.47), and (7.93-8.29) ppm confirming the presence of three -NH protons as well as the singlet at δ (7.91-8.11) ppm highlighting the

presence of benzylic methine proton which is also confirmed by HSQC spectra of **A8** and **A9**. Aromatic proton signals appear in the range (6.81-8.29) ppm.

^{13}CNMR spectra showed the disappearance of carbonyl aldehyde signal, and existence of a peak at δ (177.6-178.3) ppm belong to the presence of thiocarbonyl group.[121,125] The spectrum of **A5** exhibits a distinctive pattern due to strong coupling between carbon and fluorine (spin 1/2), with J_1 coupling constant is 247 Hz and it's getting smaller in longer range coupling.

HSQC spectrum of **A8** and **A9** confirmed the presence of benzylic methine carbon signal at 139.7 and 142.3 ppm, respectively.

5-phenyl-1,2,4-triazolidine-3-thione (**A1**): white crystals, m.p. 168˚C; IR (KBr): 3398 (N-H), 3232 (N-H), 3143 (N-H), 1600 (C=C), 1369 (C-N), 1284 (C=S) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 11.46 (s, 1H, N*H*), , 8.23 (s, 1H, N*H*), 8.05 (s, 1H, C*H*), 8.01 (s, 1H, N*H*), 7.79 (m, 2H, Ar-*H*), 7.39 (m, 3H, Ar-*H*); ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 178.0 (C=S), 142.3 (CH), 134.2 (Ar-C), 129.9 (Ar-CH), 128.7 (Ar-CH×2), 127.3 (Ar-CH×2).

5-(2-chlorophenyl)-1,2,4-triazolidine-3-thione (**A2**): white crystals, mp 187-190 ˚C; IR (KBr): 3414 (N-H), 3248 (N-H), 3151 (N-H), 1608 (C=C), 1377 (C-N), 1292 (C=S) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 11.62 (s, 1H, N*H*), 8.47 (s, 1H, N*H*), 8.29 (dd, 2H, J_1= 7.6 Hz, J_2= 1.8 Hz, Ar-*H*, N*H*), 8.11 (s, 1H, C*H*), 7.46-7.49 (dd, 1H, J_1= 7.6, J_2= 1.0 Hz, Ar-*H*), 7.34-7.42 (m, 2H, Ar-*H*); ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 178.3 (C=S), 138.2 (Ar-C), 133.1 (CH), 131.5 (Ar-C), 131.2 (Ar-CH), 129.8 (Ar-CH), 127.5 (Ar-CH), 127.4 (Ar-CH).

5-(2-bromophenyl)-1,2,4-triazolidine-3-thione (**A3**): white crystals, mp 204 ˚C; IR (KBr): 3414 (N-H), 3244 (N-H), 3155 (N-H), 1608 (C=C), 1373 (C-N), 1292 (C=S) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 11.66 (s, 1H, N*H*), 8.43 (s, 1H, N*H*), 8.32 (s, 1H, N*H*), 8.27 (dd, 1H, J_1=7.8 Hz, J_2=1.8 Hz, Ar-*H*), 8.13 (s, 1H, C*H*), 7.64 (dd, 1H, J_1=8.0 Hz, J_2=0.8 Hz, Ar-*H*), 7.39 (t, 1H, J=7.4 Hz, Ar-*H*), 7.31 (td, 1H, J_1=7.8 Hz, J_2=1.8 Hz, Ar-*H*), ^{13}C-NMR (400 MHz, DMSO-d_6): ppm δ 178.2 (C=S), 140.6 (CH), 132.9 (Ar-C), 131.4 (Ar-CH), 127.83 (Ar-CH), 127.80 (Ar-CH), 123.5 (Ar-C).

5-(2-hydroxyphenyl)-1,2,4-triazolidine-3-thione (**A4**): white crystals, m.p. 111-113 ˚C; IR (KBr): 3441(N-H), 3317 (N-H), 3170 (N-H), 1612 (C=C), 1365 (C-N), 1265 (C=S) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 11.37 (s, 1H, N*H*), 9.88 (s, 1H, O*H*), 8.37 (s, 1H, N*H*), 8.10 (s, 1H, N*H*), 7.91 (d, 2H, J= 6.6 Hz, Ar-*H*, C*H*), 7.21 (m, 1H, Ar-*H*), 6.86 (d, 1H, J= 7.9 Hz, Ar-*H*), 6.81 (t, 1H, J= 7.5 Hz, Ar-*H*); ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 177.7 (C=S), 156.4 (Ar-C), 139.7 (CH), 131.1 (Ar-CH), 126.8 (Ar-CH), 120.4 (Ar-C), 119.3 (Ar-CH), 116.1 (Ar-CH).

5-(4-fluorophenyl)-1,2,4-triazolidine-3-thione (**A5**): white crystals, m.p. 184-186 ˚C; IR (KBr): 3390 (N-H), 3232 (N-H), 3155 (N-H), 1600 (C=C), 1365 (C-N), 1226 (C=S) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 11.43 (s, 1H, N*H*), 8.20 (s, 1H, N*H*), 8.03 (s, 2H, N*H*, C*H*), 7.87 (dd, 2H, J_1=8.7 Hz, J_2=5.7 Hz, Ar-*H*), 7.23 (m, 2H, Ar-*H*); ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 178.0 (C=S), 164.3 and 161.8 (d, J_1= 247 Hz, Ar-C), 141.1 (CH), 130.9 (d, J_4= 2.7 Hz, Ar-C), 129.5 (d, J_3= 8.8 Hz, Ar-CH×2), 115.7 (d, J_2= 22.0 Hz, Ar-CH×2).

5-(4-chlorophenyl)-1,2,4-triazolidine-3-thione (**A6**): white crystals, m.p. 207-209 ˚C; IR (KBr): 3433 (N-H), 3278 (N-H), 3163 (N-H), 1600 (C=C), 1365

(C-N), 1280 (C=S) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 11.50 (s, 1H, N*H*), 8.25 (s, 1H, N*H*), 8.09 (s, 1H, N*H*), 8.02 (s, 1H, C*H*), 7.84 (d, 2H, J=8.4 Hz, Ar-*H*), 7.45 (d, 2H, J=8.4 Hz, Ar-*H*); ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 178.1 (C=S), 140.8 (CH), 134.2 (Ar-C), 133.2 (Ar-C), 129.0 (Ar-CH×2), 128.7 (Ar-CH×2).

5-(4-bromophenyl)-1,2,4-triazolidine-3-thione (**A7**): white crystals, m.p. 196 ˚C; IR (KBr): 3433 (N-H), 3286 (N-H), 3163 (N-H), 1600 (C=C), 1361 (C-N), 1284 (C=S) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 11.50 (s, 1H, N*H*), 8.25 (s, 1H, N*H*), 8.09 (s, 1H, N*H*), 8.0 (s, 1H, C*H*), 7.77 (d, 2H, J=8.6 Hz, Ar-*H*), 7.59 (d, 2H, J=8.6 Hz, Ar-*H*); ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 178.1 (C=S), 140.9 (CH), 133.6 (Ar-C), 131.6 (Ar-CH×2), 129.2 (Ar-CH×2), 123.0 (Ar-C).

5-(4-hydroxyphenyl)-1,2,4-triazolidine-3-thione (**A8**): Dirty white crystals, m.p. 218-220 ˚C; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 11.37 (s, 1H, N*H*), 9.88 (s, 1H, O*H*), 8.37 (s, 1H, N*H*), 8.10 (s, 1H, N*H*), 7.91 (d, 2H, J= 6.6 Hz, Ar-H, C*H*), 7.21 (m, 1H, Ar-*H*), 6.86 (d, 1H, J= 7.9 Hz, Ar-*H*), 6.81 (t, 1H, J= 7.5 Hz, Ar-*H*); ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 177.7 (C=S), 156.4 (Ar-C), 139.7 (CH), 131.1 (Ar-CH), 126.8 (Ar-CH), 120.4 (Ar-C), 119.3 (Ar-CH), 116.1 (Ar-CH).

5-(4-methoxyphenyl)-1,2,4-triazolidine-3-thione (**A9**): white crystals, m.p. 188-190 ˚C; IR (KBr): 3360 (N-H), 3278 (N-H), 3197 (N-H), 1608 (C=C), 1381 (C-N), 1238 (C=S) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 11.33 (s, 1H, N*H*), 8.13 (s, 1H, N*H*), 7.99 (s, 1H, C*H*), 7.93 (s, 1H, N*H*), 7.73 (d, 2H, J=8.7 Hz, Ar-*H*), 6.95 (d, 2H, J=8.7 Hz, Ar-*H*), 3.78 (s, 3H, C*H*$_3$); ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 177.6 (C=S), 160.7 (Ar-C), 142.3 (CH), 129.0 (Ar-CH×2), 126.8 (Ar-C), 114.2 (Ar-CH×2), 55.3 (CH_3).

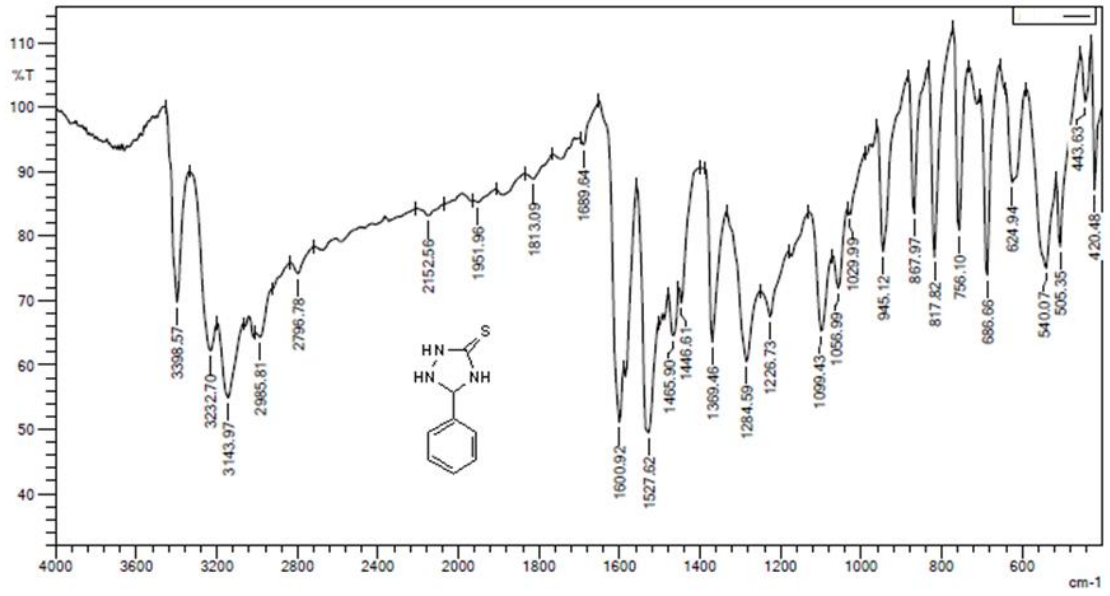


Fig (3-1) IR Spectrum of the compound A1

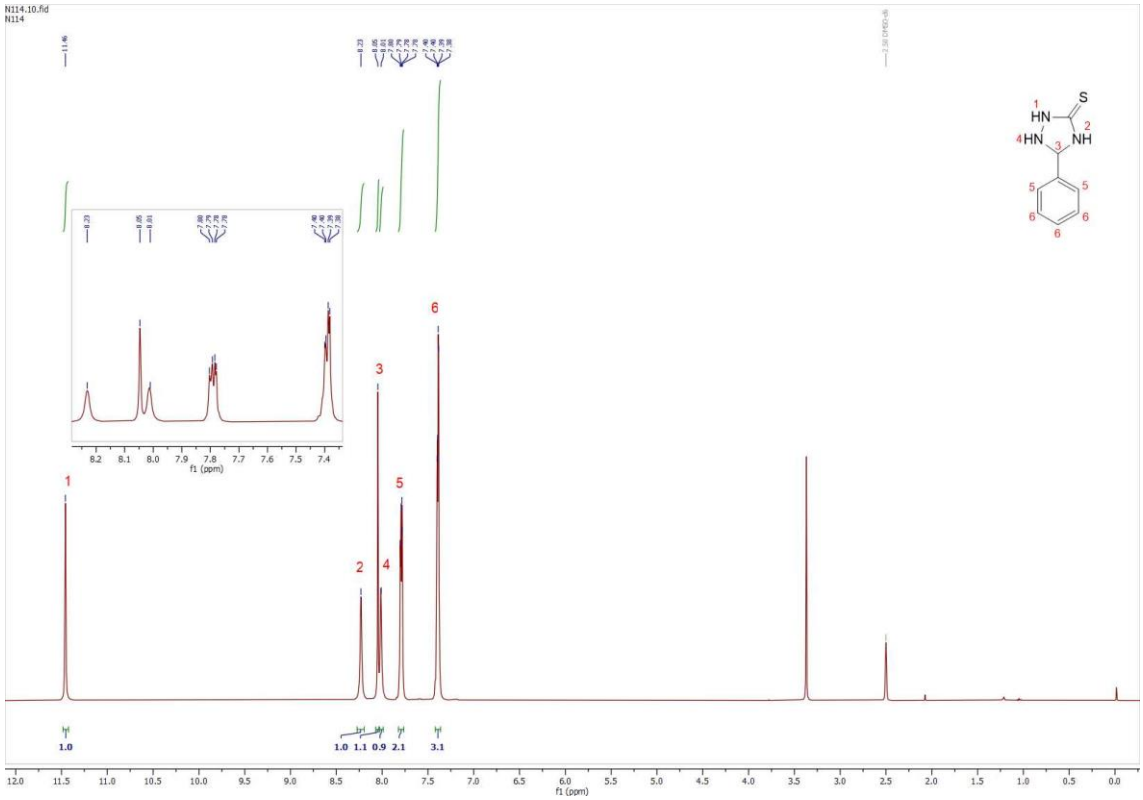


Fig (3-2) ^{1}HNMR Spectrum of the compound A1

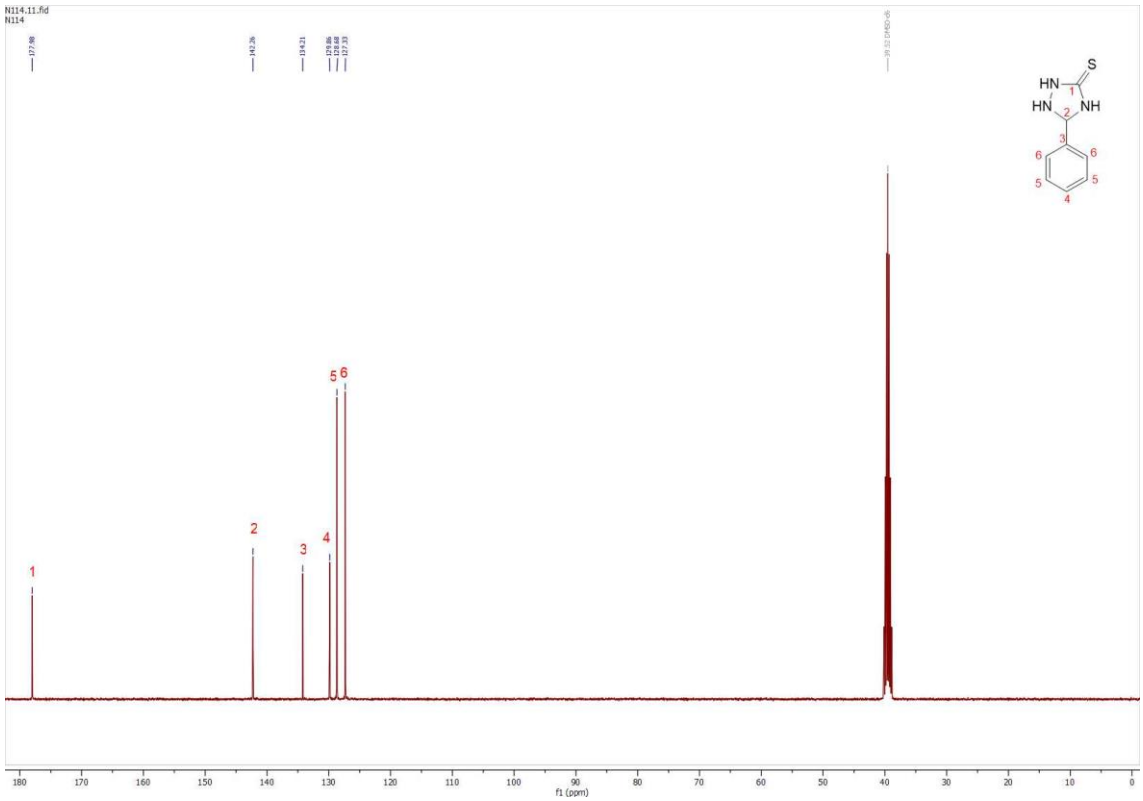


Fig (3-3) ^{13}CNMR Spectrum of the compound A1

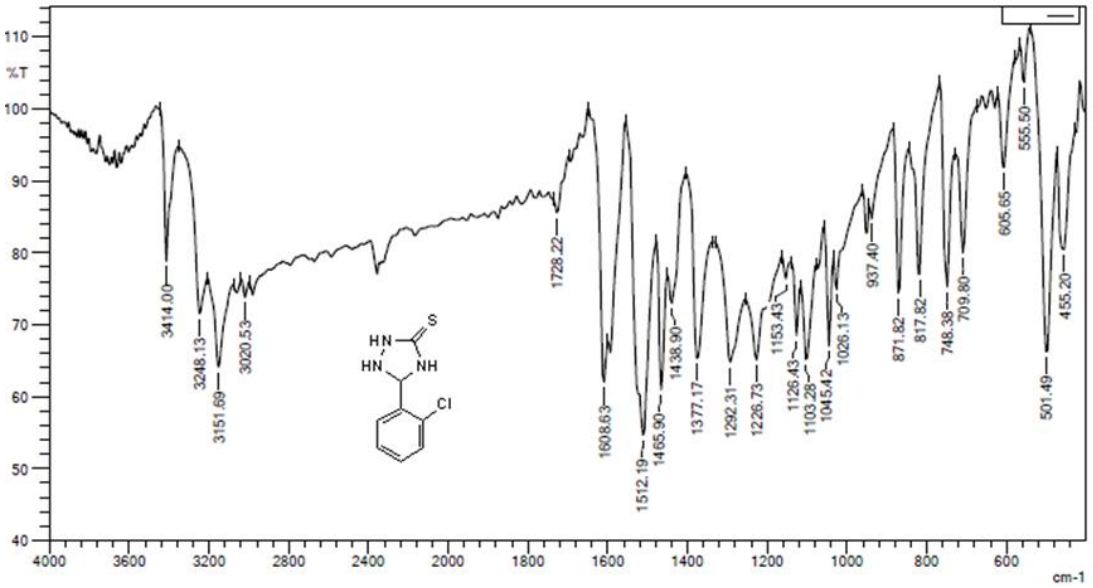


Fig (3-4) IR Spectrum of the compound A2

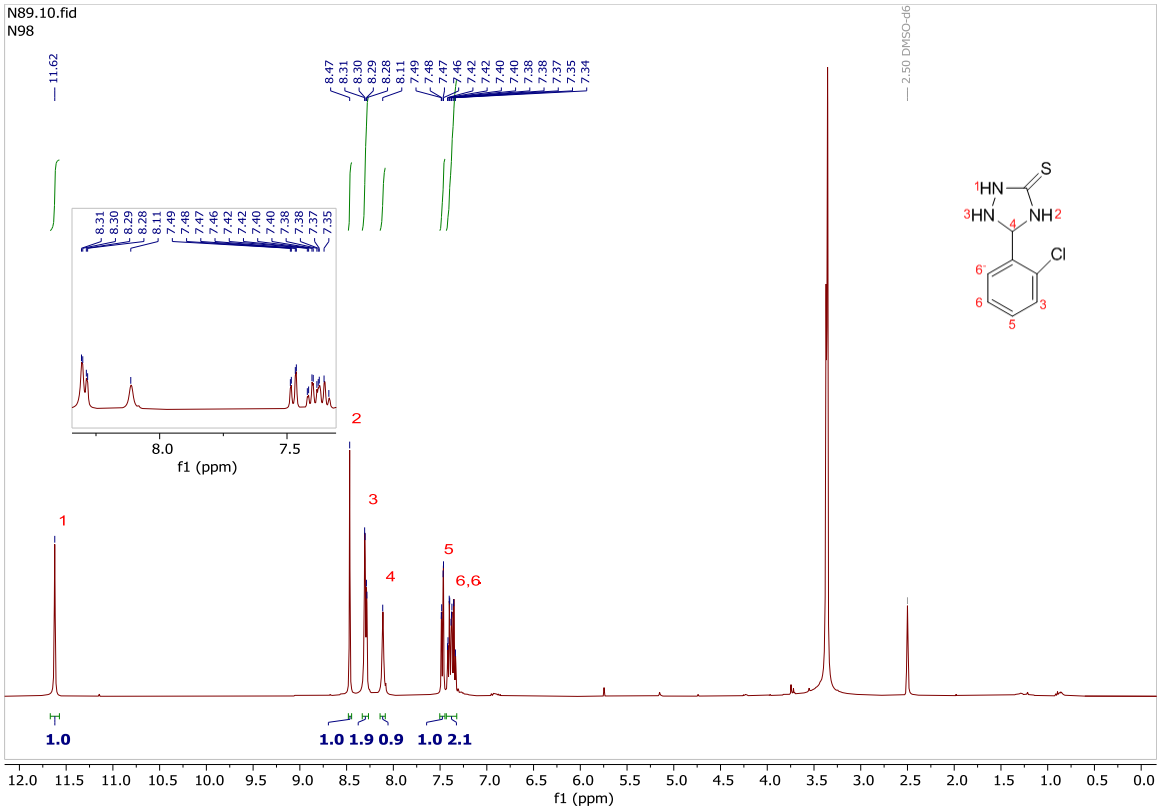


Fig (3-5) ^{1}HNMR Spectrum of the compound A2

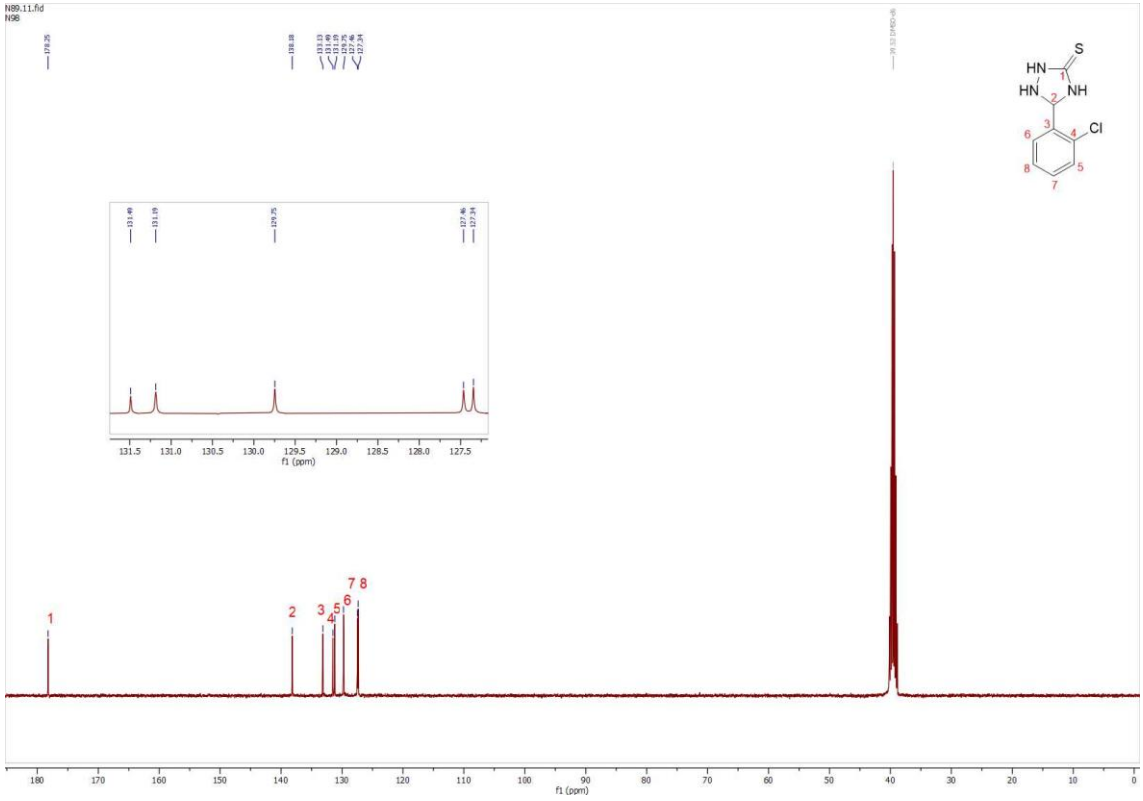


Fig (3-6) ^{13}CNMR Spectrum of the compound A2

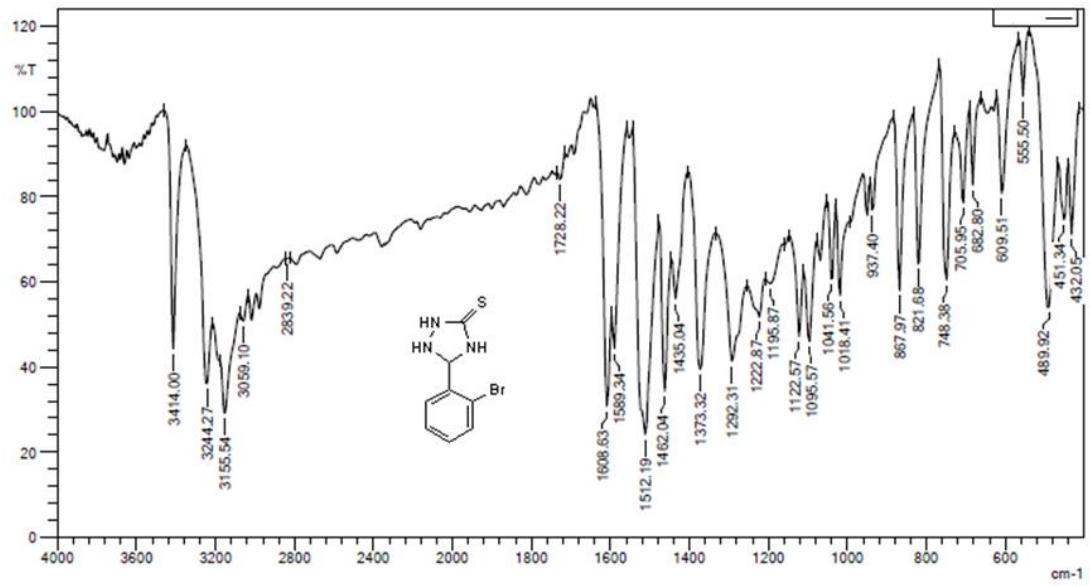


Fig (3-7) IR Spectrum of the compound A3

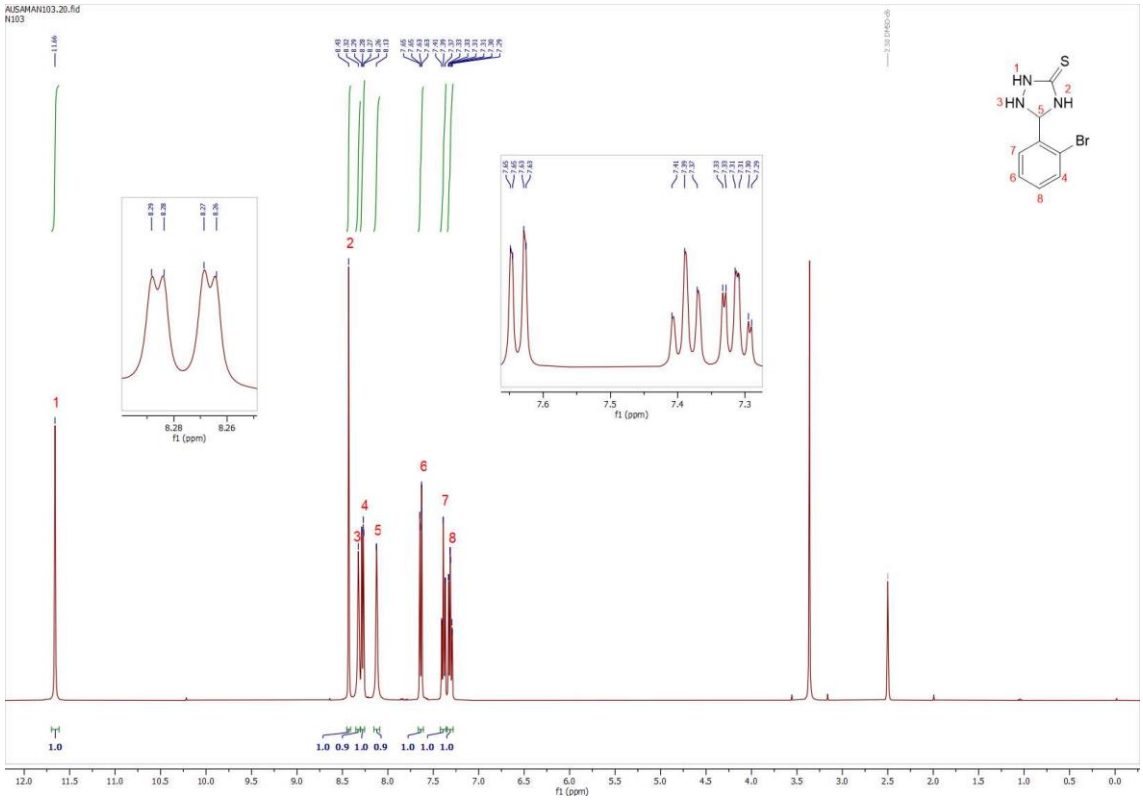


Fig (3-8) ^{1}HNMR Spectrum of the compound A3

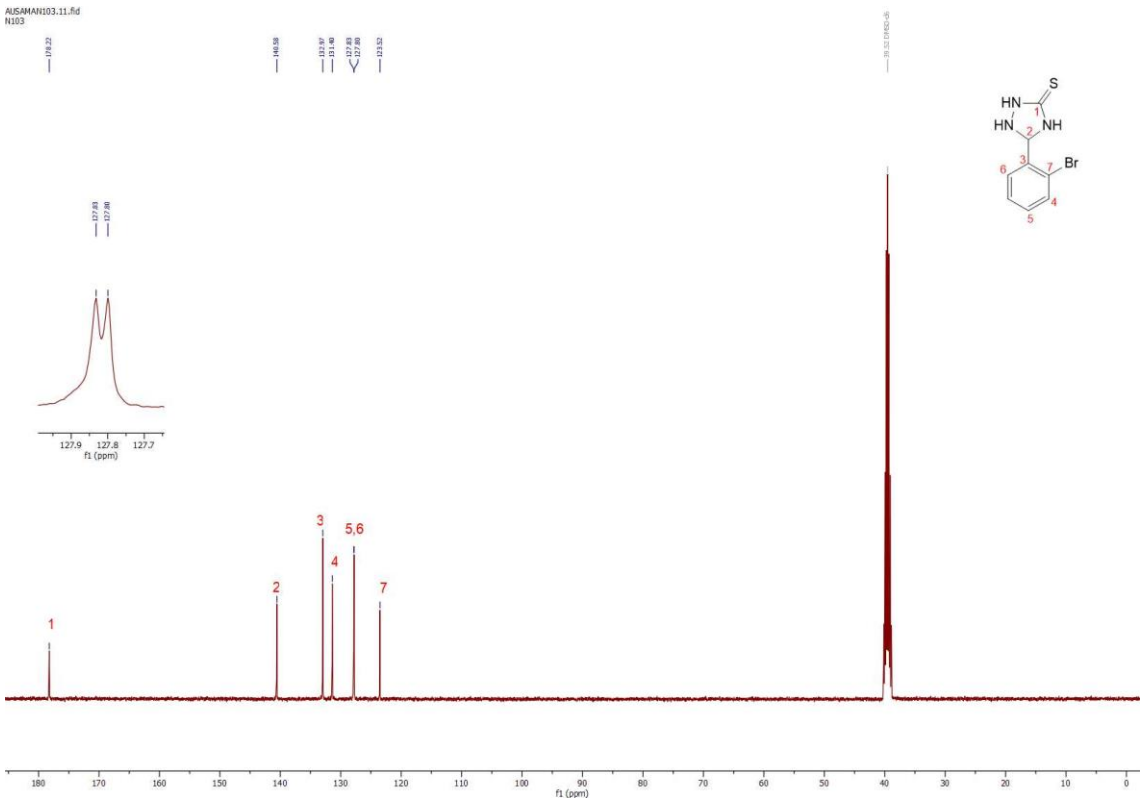


Fig (3-9) ^{13}CNMR Spectrum of the compound A3

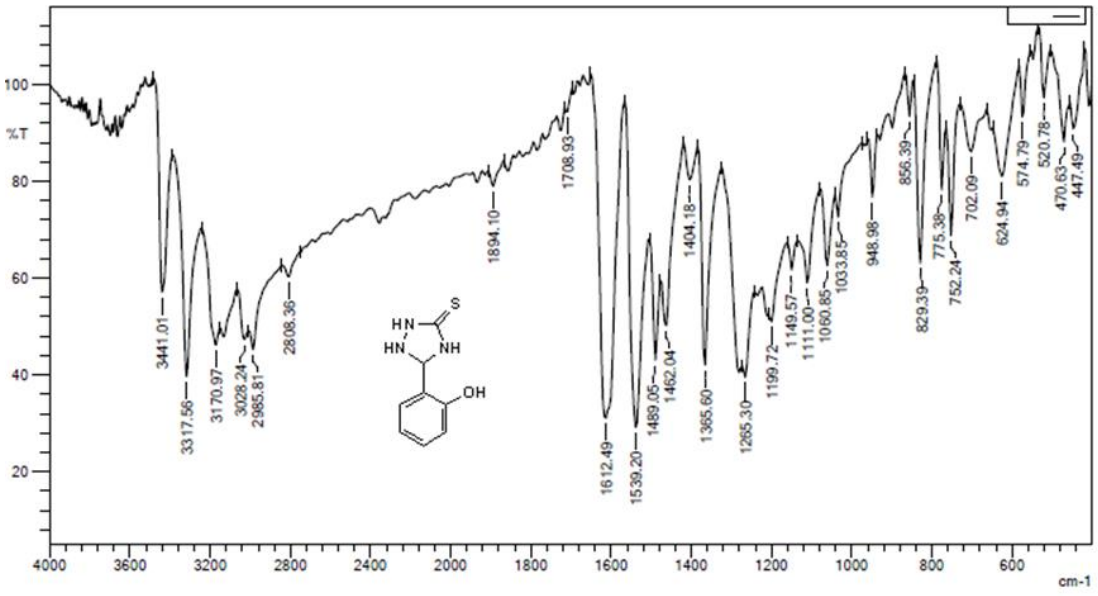


Fig (3-10) IR Spectrum of the compound A4

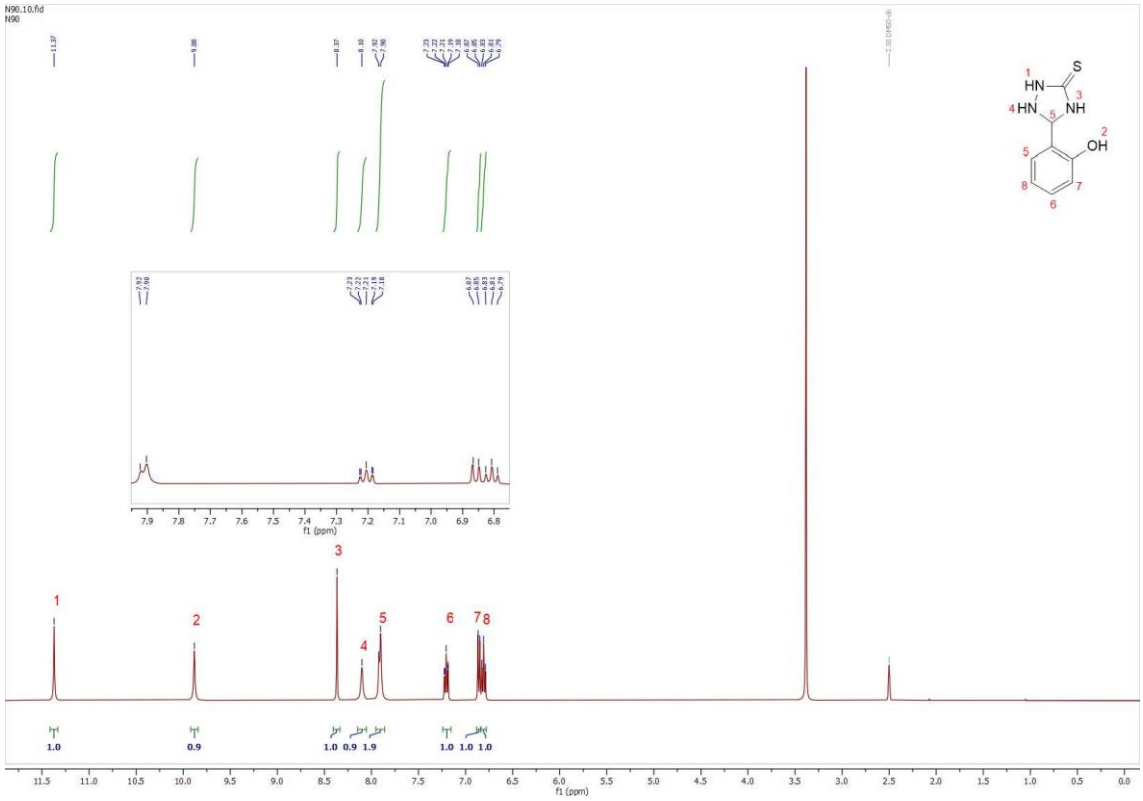


Fig (3-11) ^{1}HNMR Spectrum of the compound A4

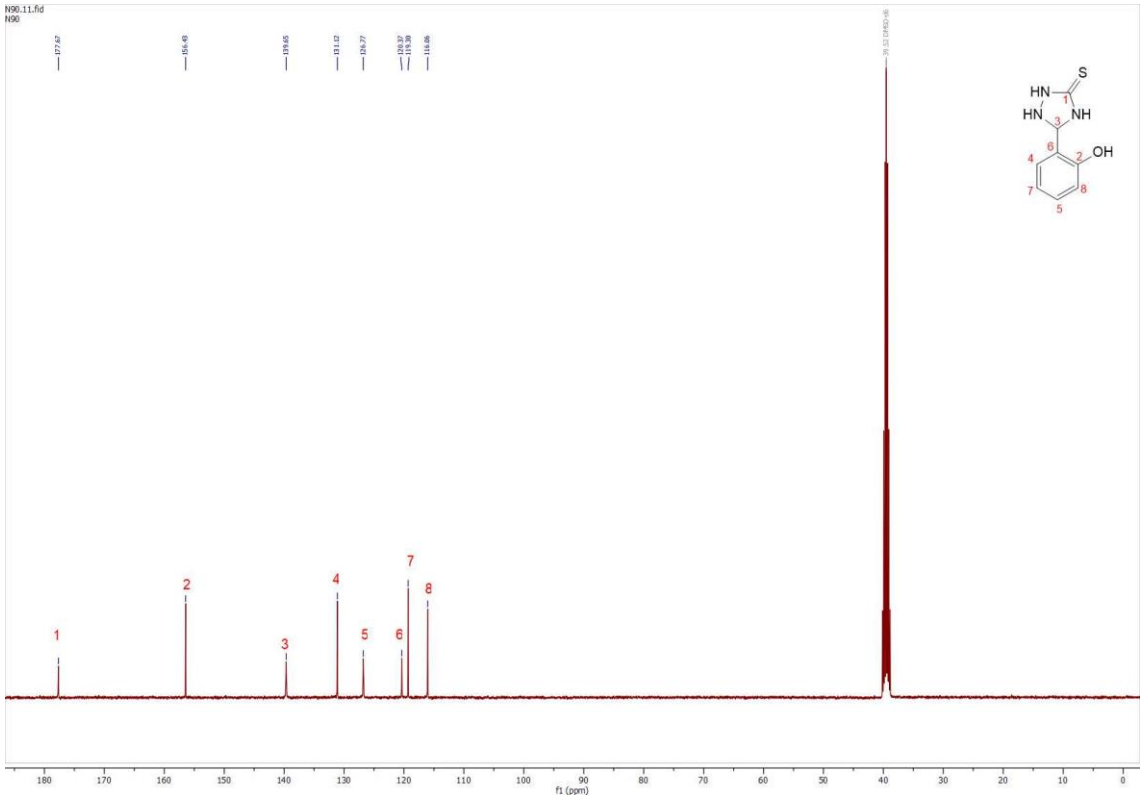


Fig (3-12) ^{13}CNMR Spectrum of the compound A4

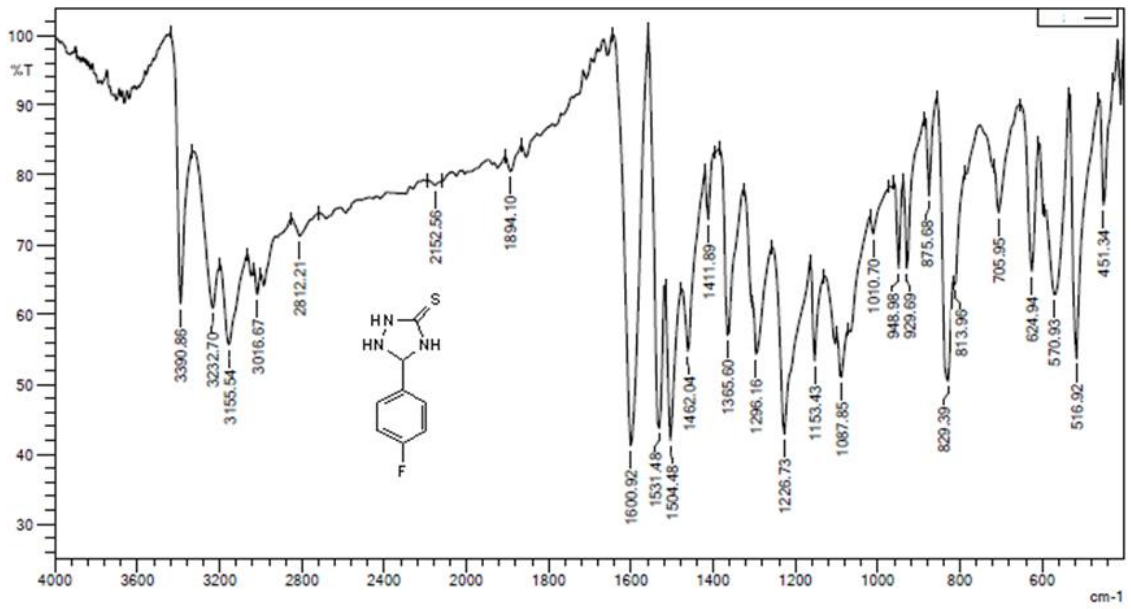


Fig (3-13) IR Spectrum of the compound A5

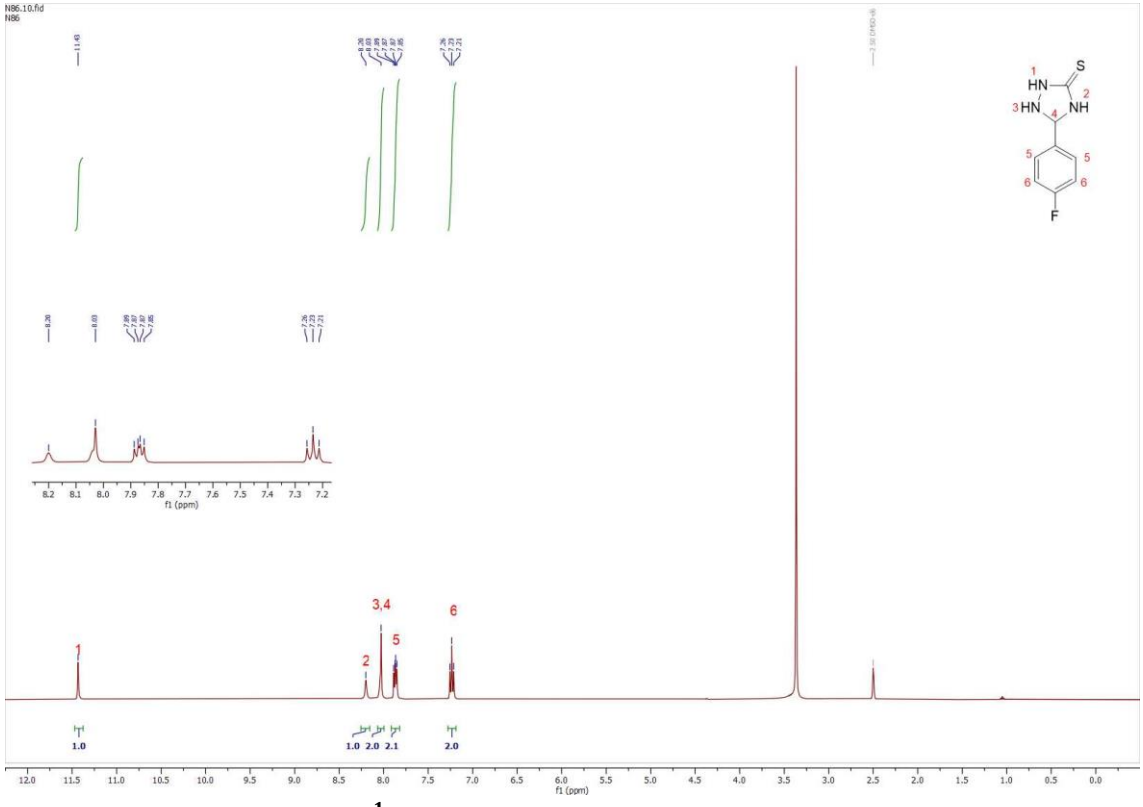


Fig (3-14) ^{1}HNMR Spectrum of the compound A5

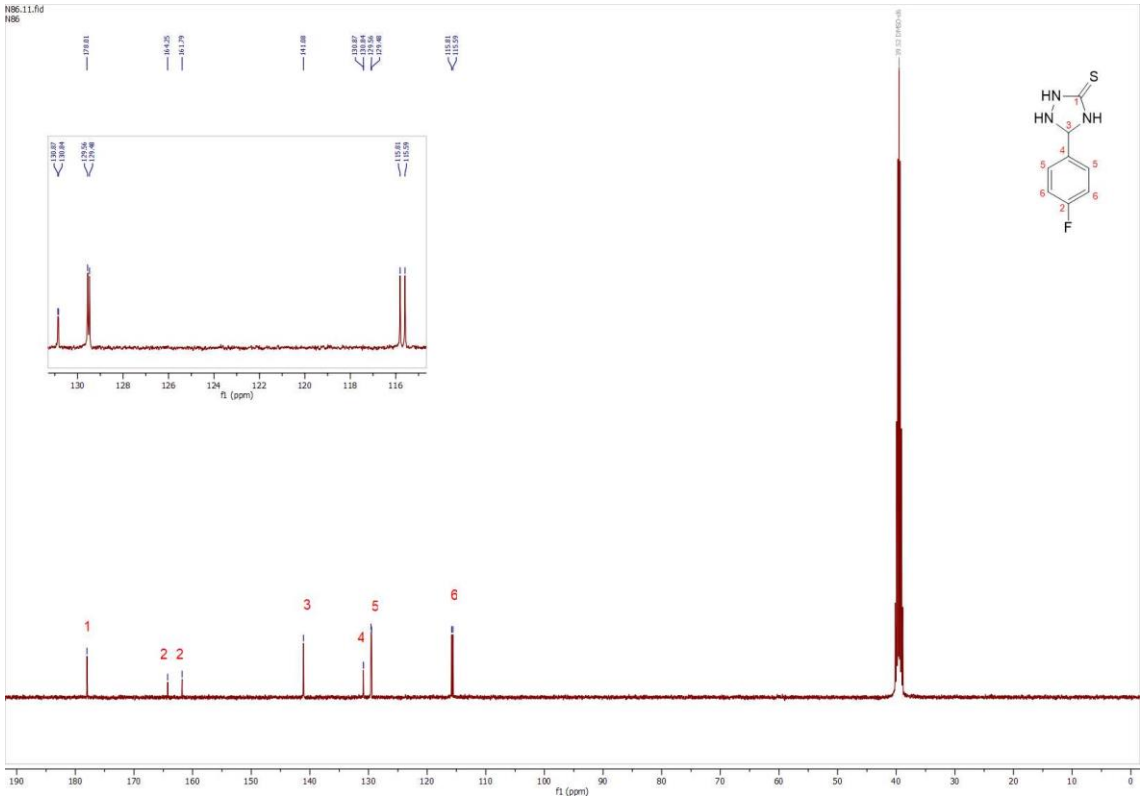


Fig (3-15) ^{13}CNMR Spectrum of the compound A5

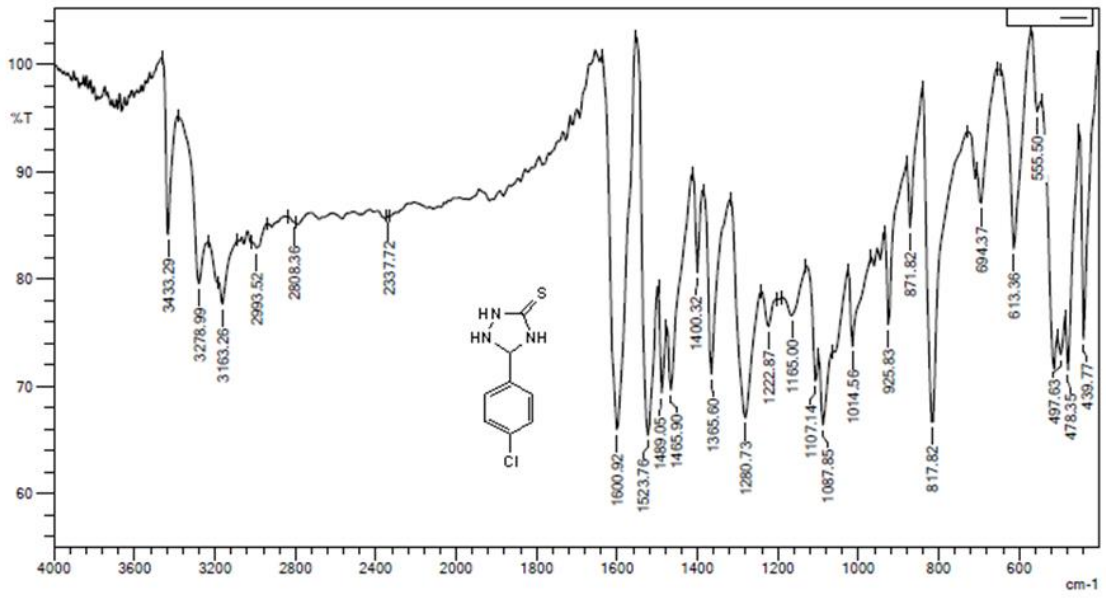


Fig (3-16) IR Spectrum of the compound A6

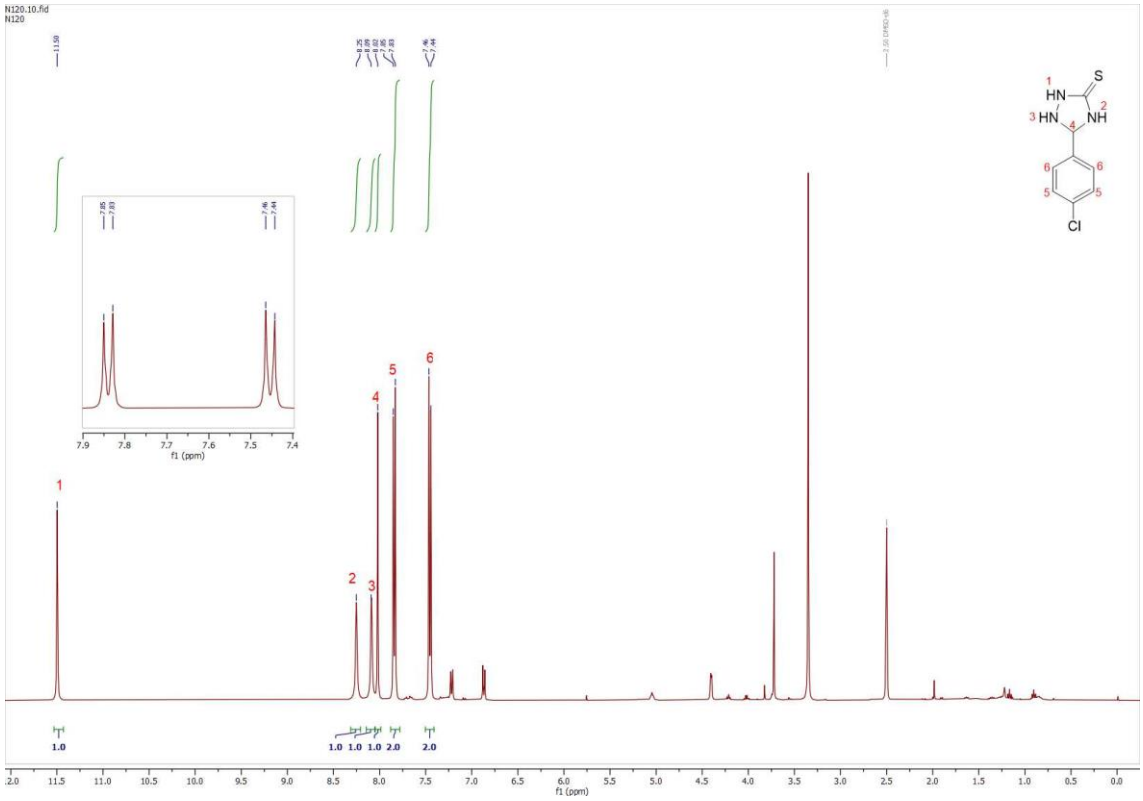


Fig (3-17) ^{1}HNMR Spectrum of the compound A6

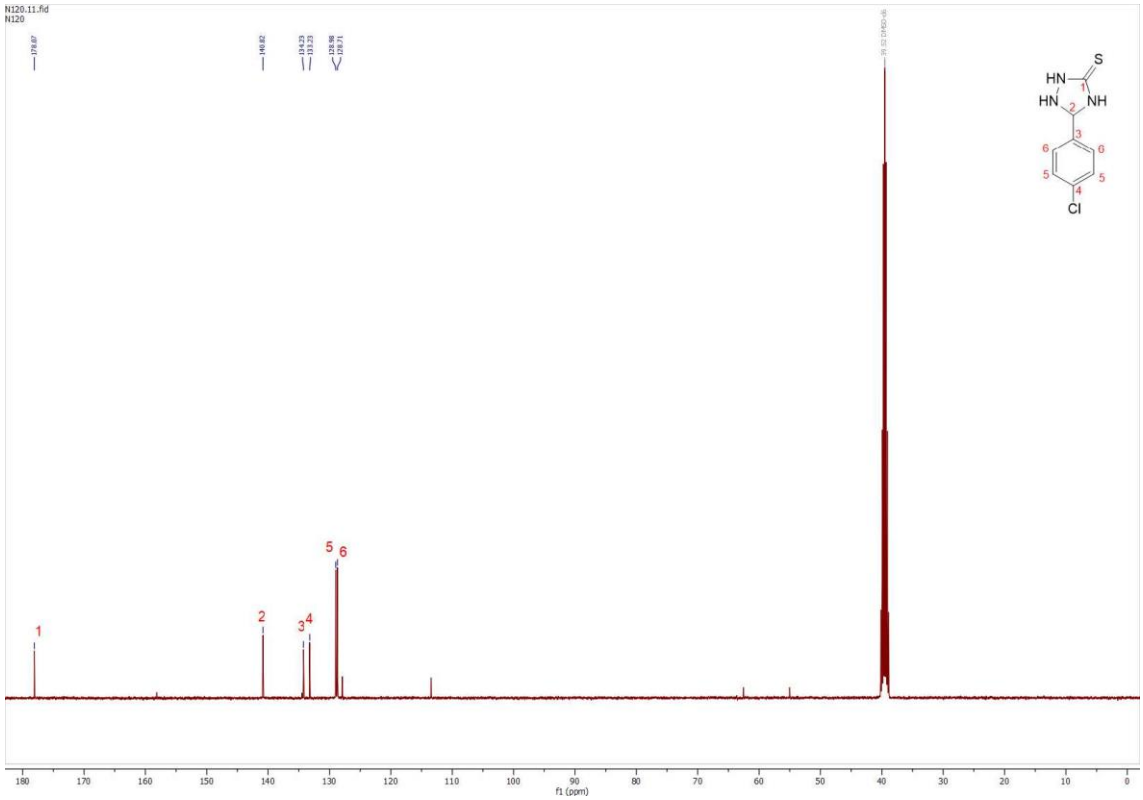


Fig (3-18) ^{13}CNMR Spectrum of the compound A6

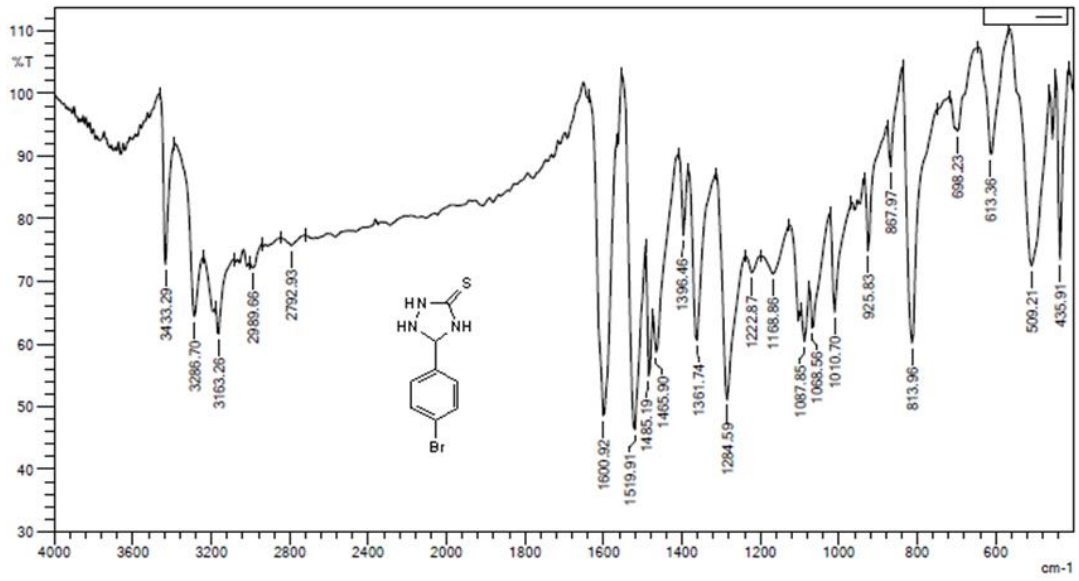


Fig (3-19) IR Spectrum of the compound A7

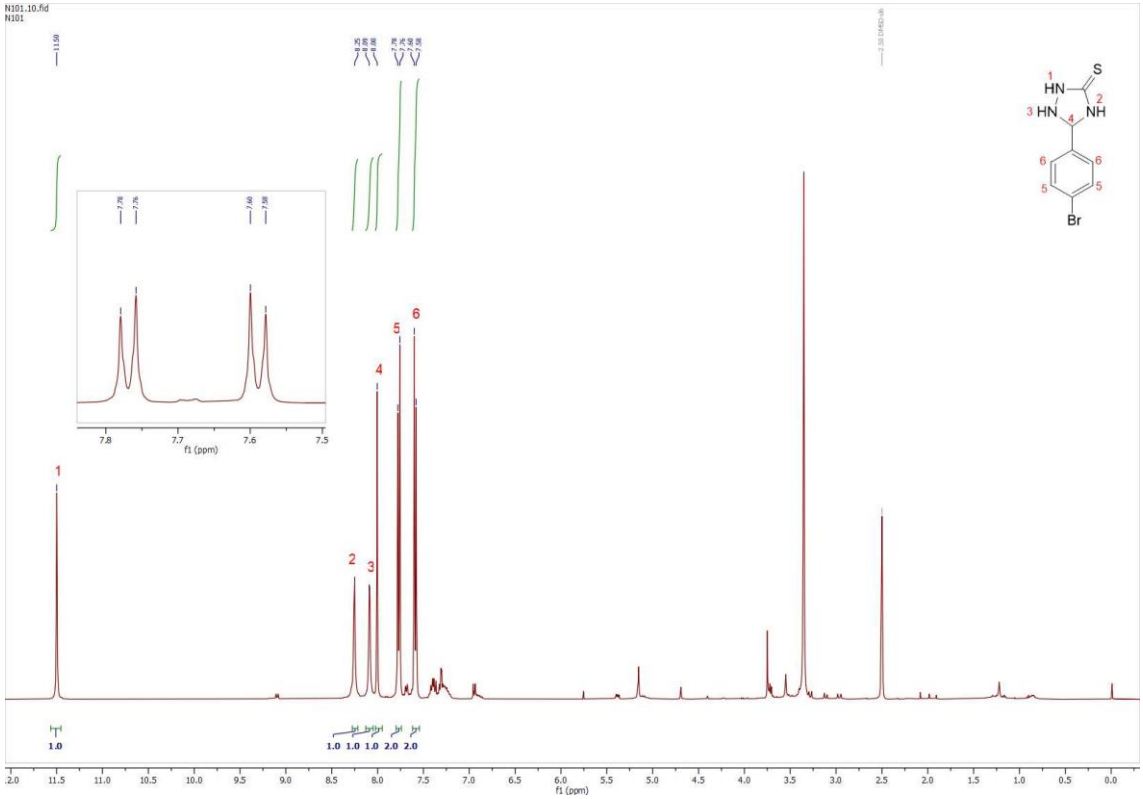


Fig (3-20) ^{1}HNMR Spectrum of the compound A7

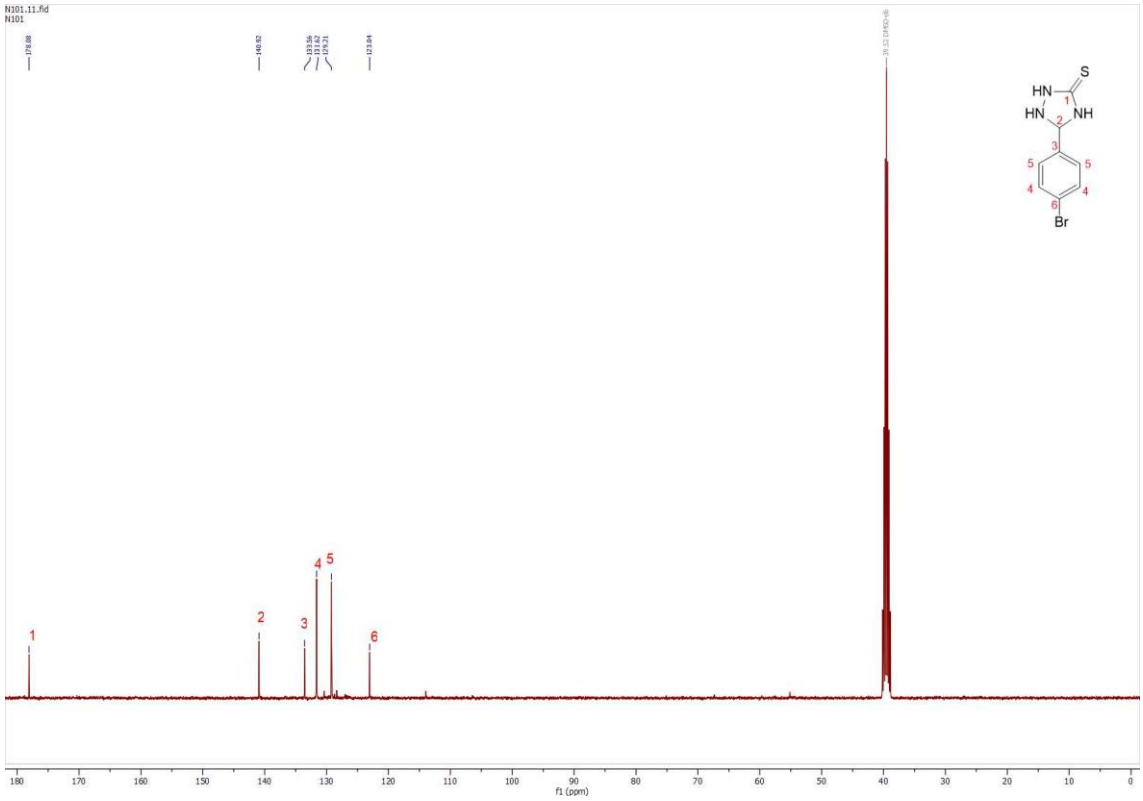


Fig (3-21) ^{13}CNMR Spectrum of the compound A7

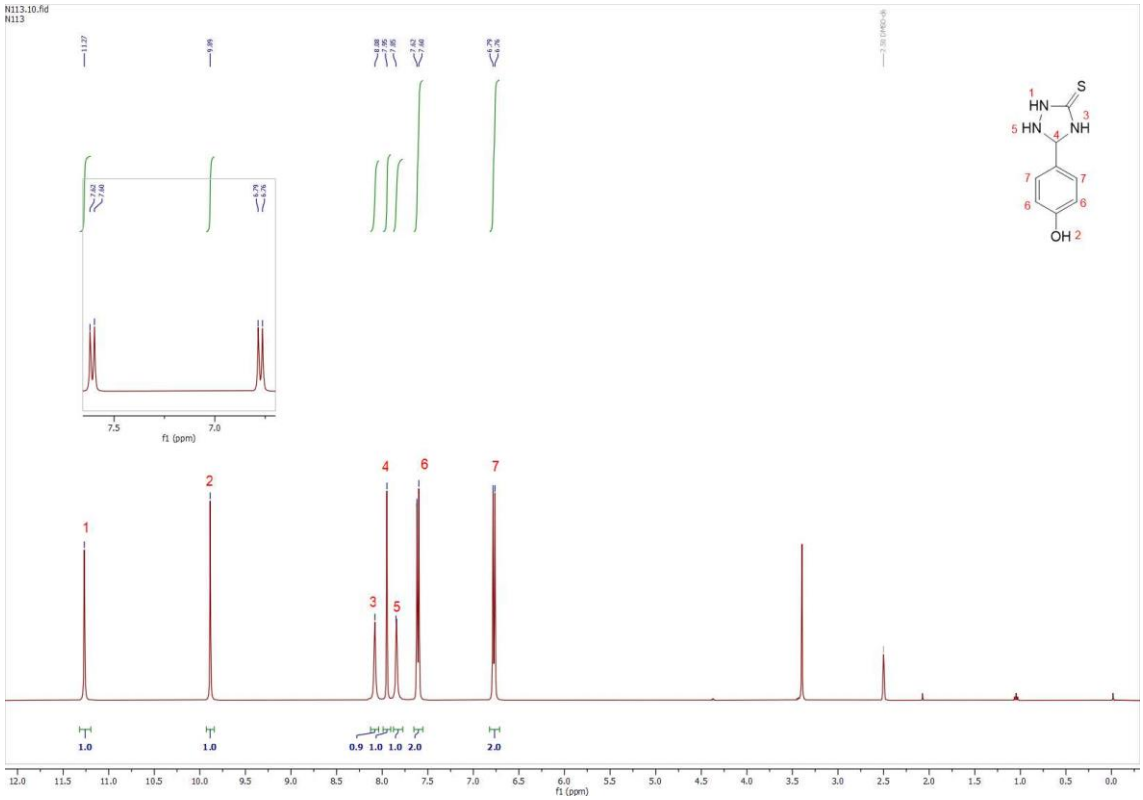


Fig (3-22) ^{1}HNMR Spectrum of the compound A8

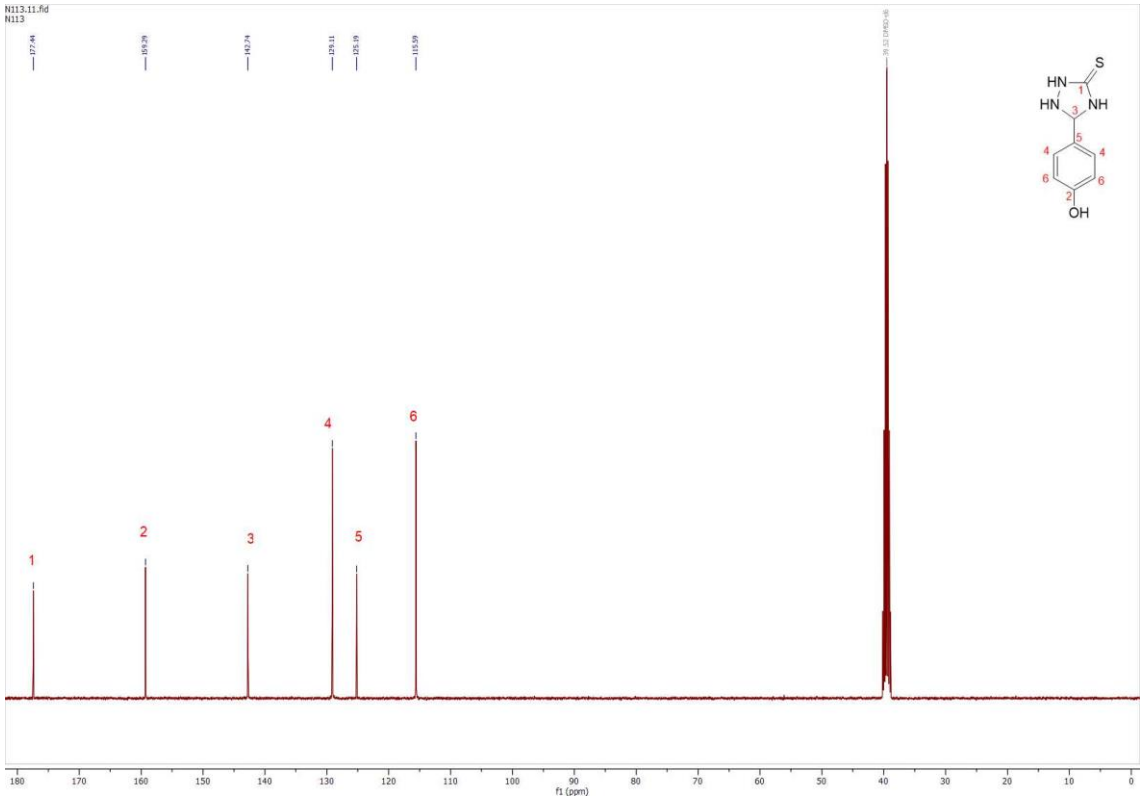


Fig (3-23) ^{13}CNMR Spectrum of the compound A8

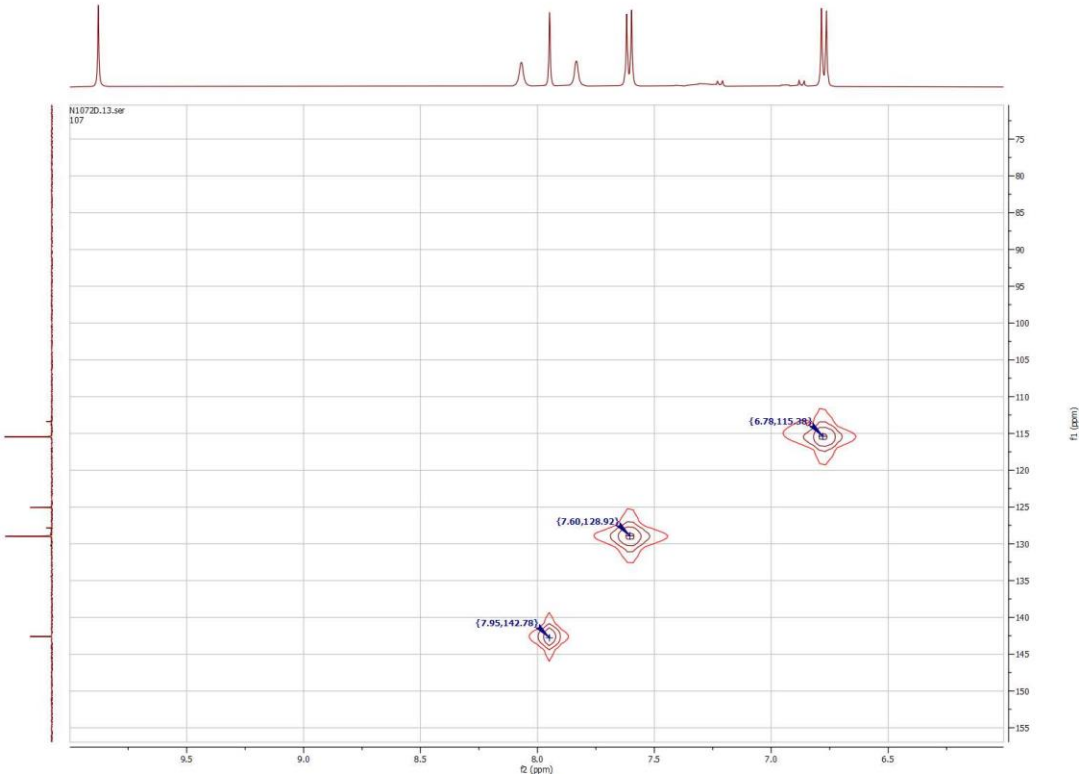


Fig (3-24) HSQC Spectrum of the compound A8

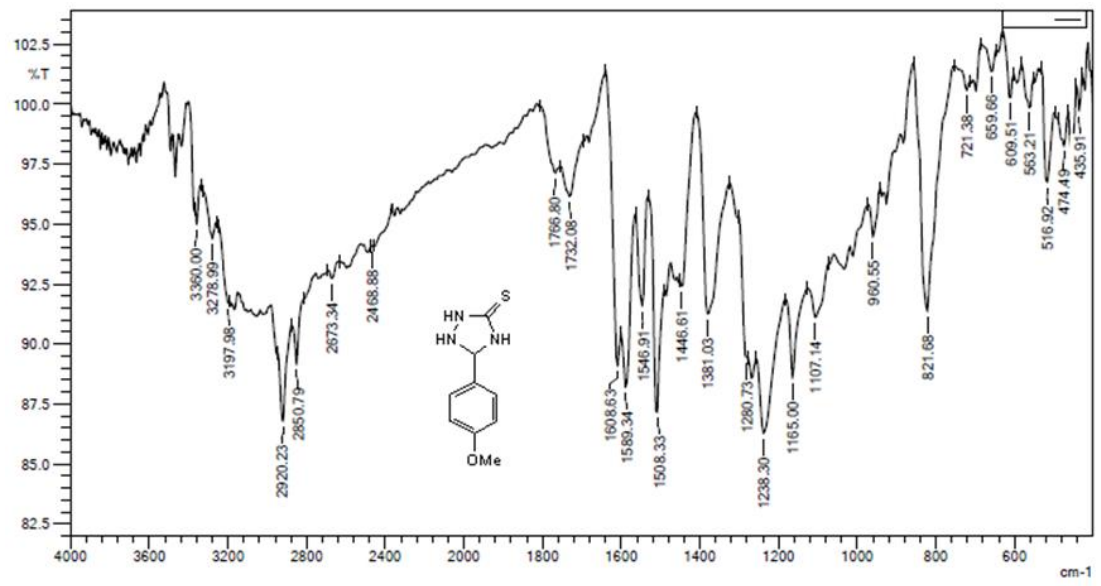


Fig (3-25) IR Spectrum of the compound A9

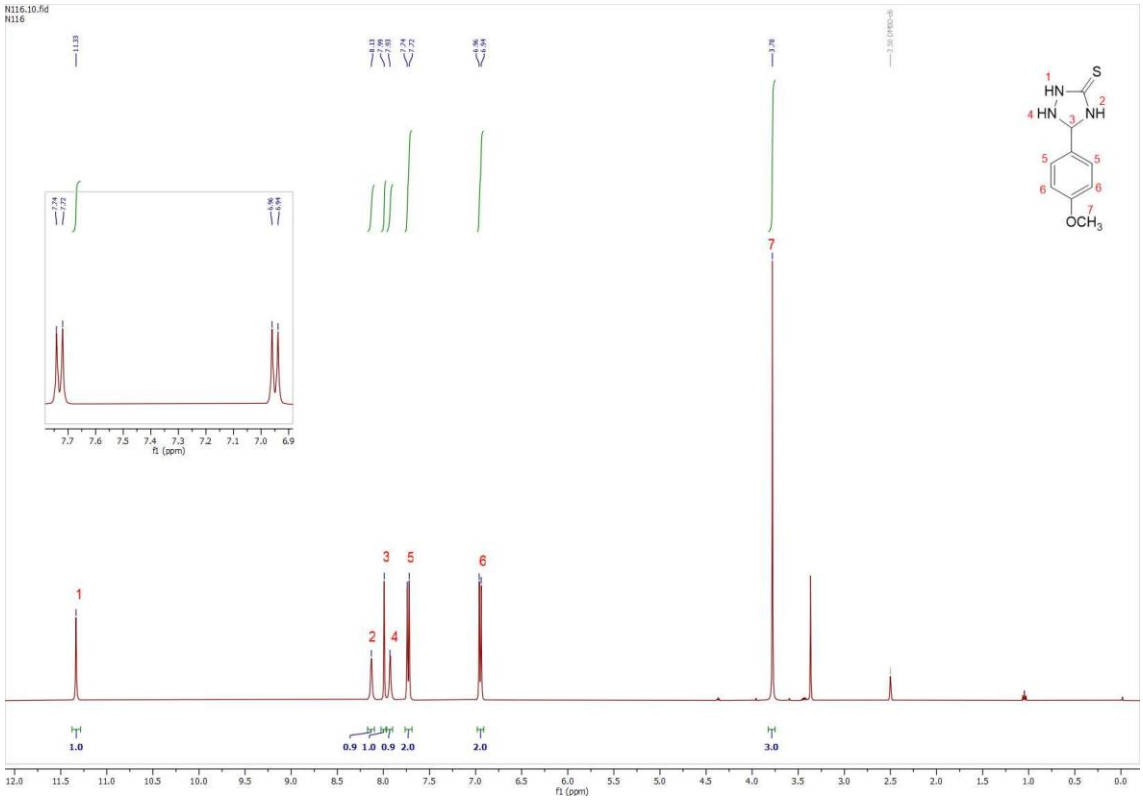


Fig (3-26) ^{1}HNMR Spectrum of the compound A9

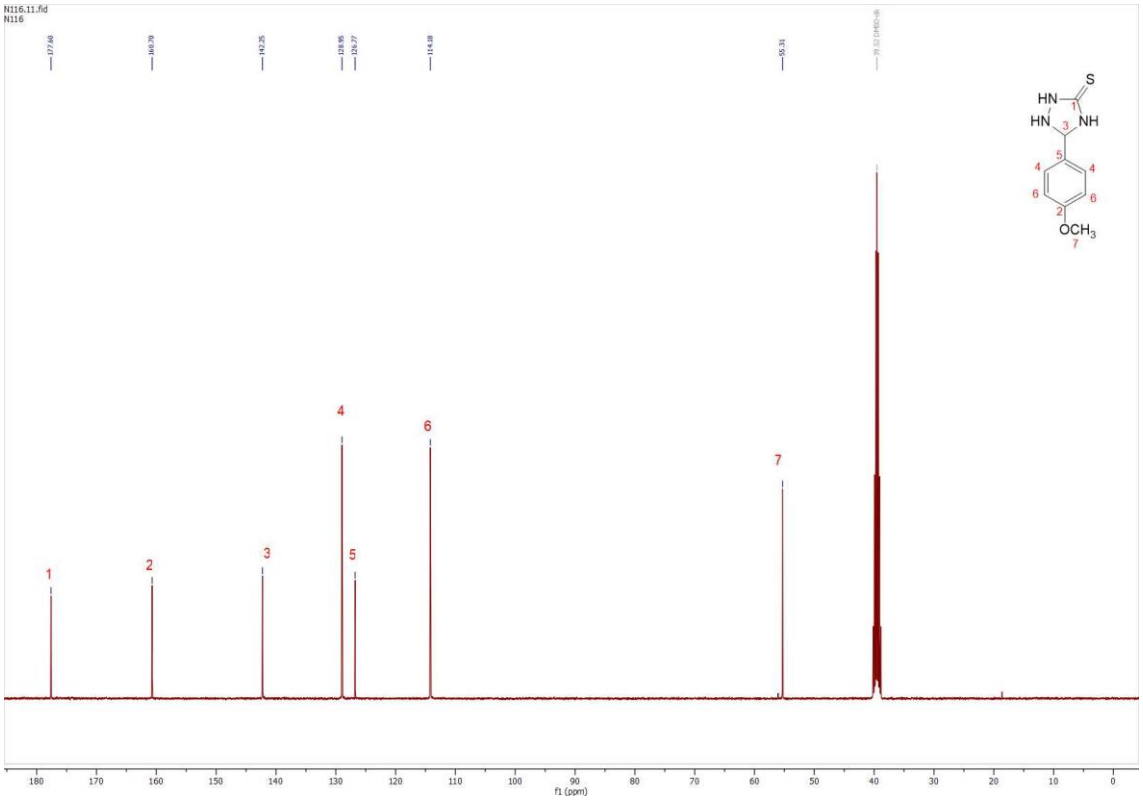


Fig (3-27) ^{13}CNMR Spectrum of the compound A9

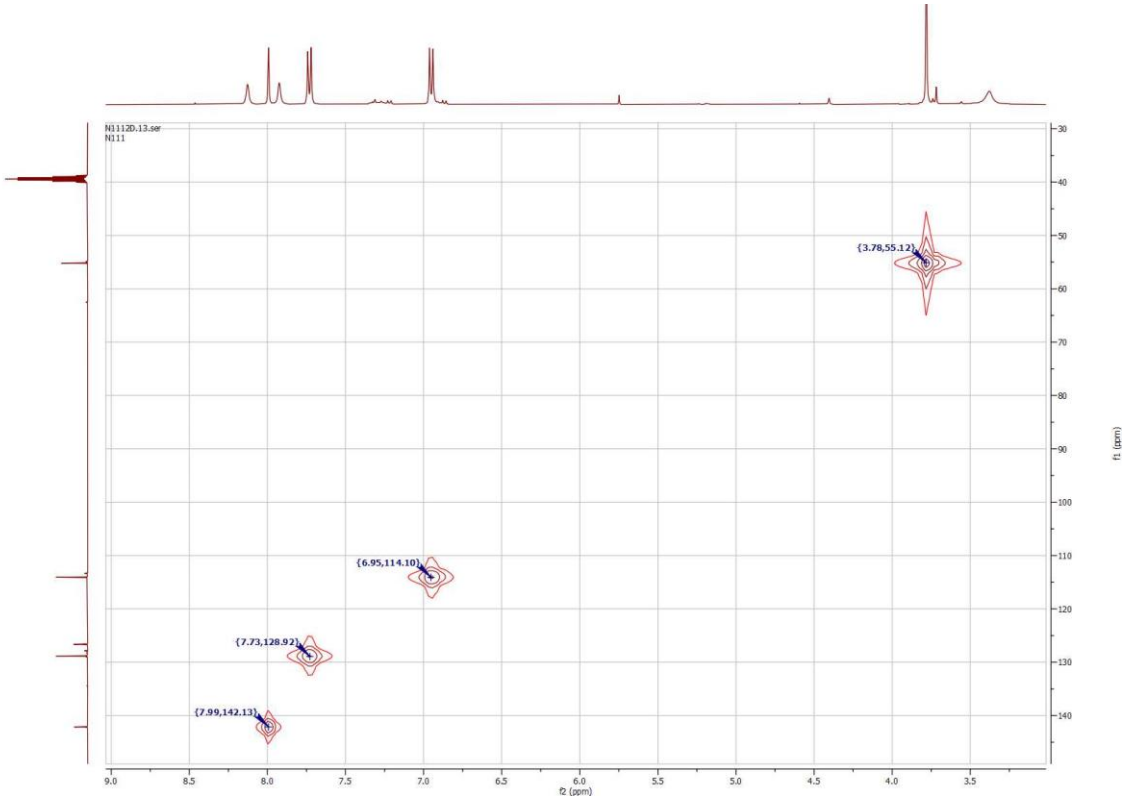


Fig (3-28) HSQC Spectrum of the compound A9

3.2. Protected Spiro-Cephalosporins (B1-B7)

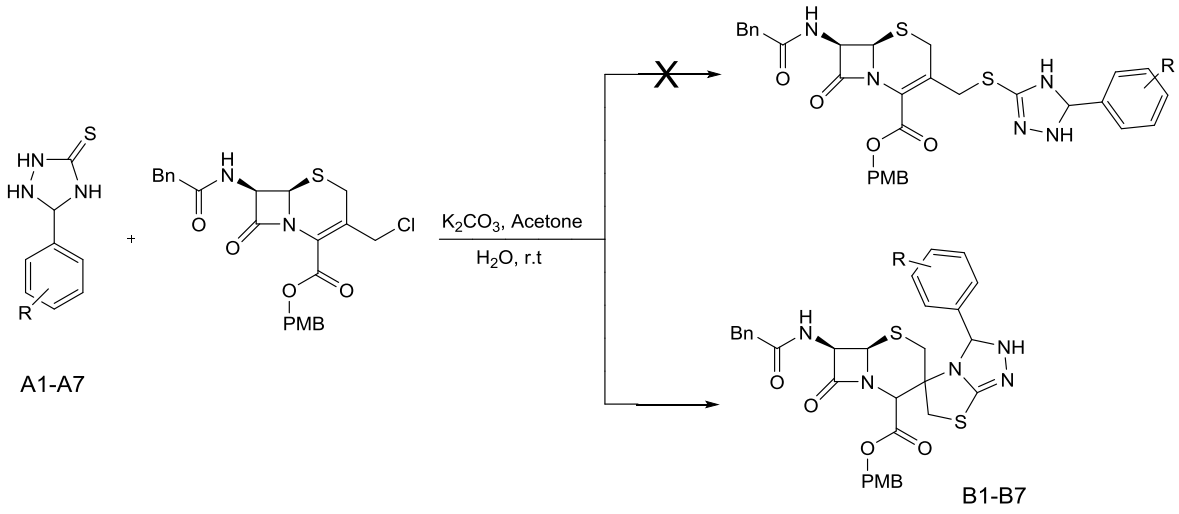
3.2.1. The synthetic strategy

The considerable antibacterial activity of 1,2,4-triazoles have attracted our attention to synthesize novel antibacterial agents by install 5-aryl-1,2,4-triazoles moiety at 3 position of the commercially available cephalosporin intermediate GCLE through S_N2 alkylation under basic conditions (Scheme 3-3).

Surprisingly, none of the expected compounds were formed, instead a novel compounds were isolated which are characterized as spiro-cephalosporins as will see in section 3.2.3.

The products PMB-protected spiro-cephalosporins were isolated from the reaction mixture by column chromatography as a single diastereomer in moderate yields (17.3-32 %).

Unfortunately, no products were obtained when GCLE treated with the triazolidines **A8** and **A9**.



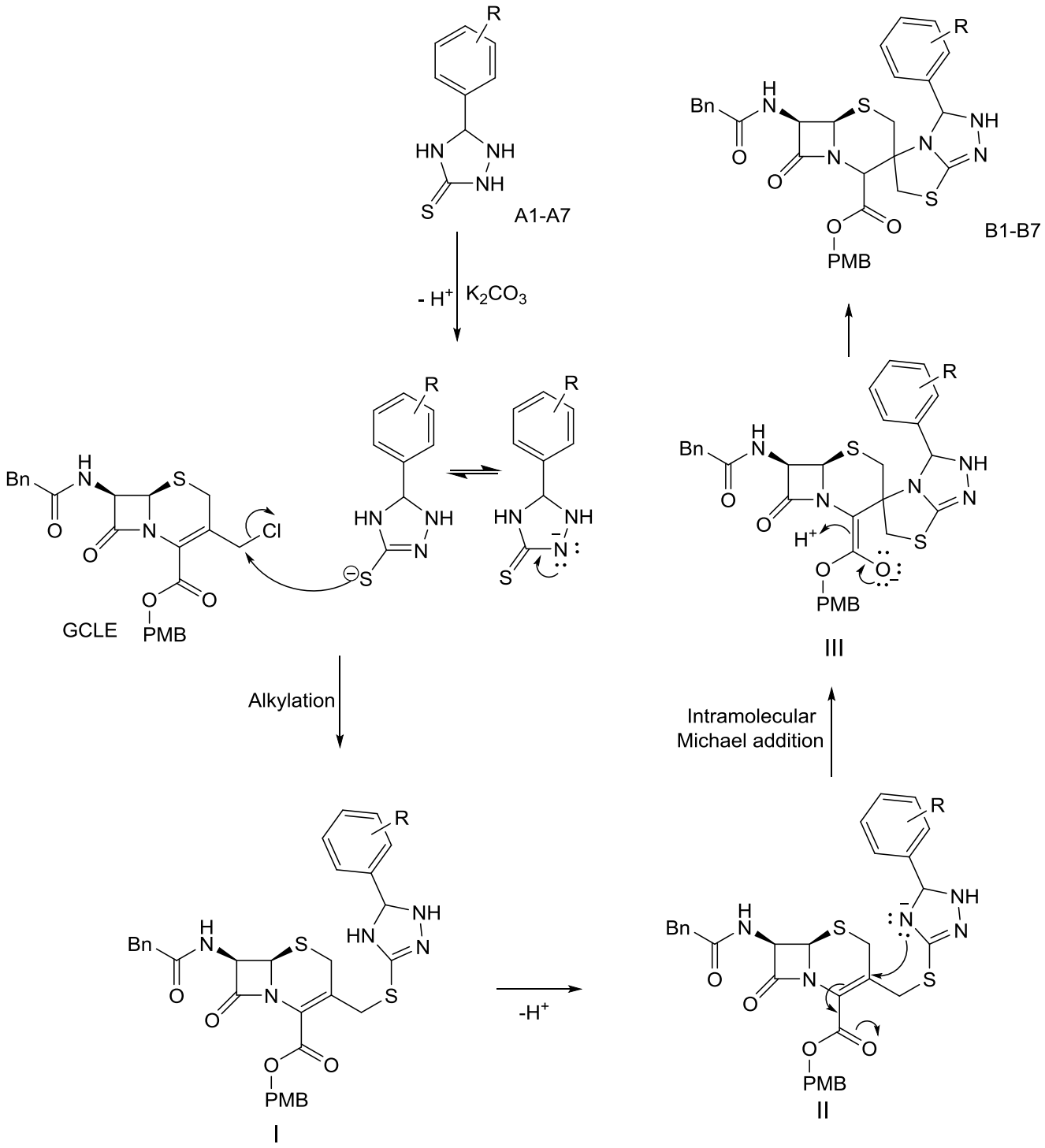
Scheme (3-3) Preparation of Spiro-cephalosporins

Spiro-cephalosporins are considered as attractive targets in drug discovery because of their unique three dimensional structures, which enhance their interaction with proteins. As a result spiro-cephalosporins exhibit desirable activities as antibacterial, antiviral and enzyme inhibitors. To our knowledge there are limited number of spiro-cephalosporins modified at positions C3 which are mostly rely on 1,3-dipolar cycloaddition reaction.[107,120]

Hulme *et. al*., reported recently a method to synthesize spiro-cephalosporins at position 3 through Michael addition reaction by treatment catechols with GCLE in the presence of K_2CO_3 and NaI in DMF.[120]

3.2.2. The proposed mechanism

The suggested mechanism for the formation of protected spiro-cephalosporin is shown in scheme (3-4). Under basic conditions, the more acidic NH group in triazolidine-3-thione is deprotonated to form reactive thiolate, which undergoes S_N2 type alkylation with GCLE to form the intermediate (I). It is expected that the other NH group in intermediate (I) can be also deprotonated and the resulting nitrogen anion attack the double bond at C3 of the dihydrothiazine ring to initiate intramolecular Michael addition reaction which lead ultimately to furnish spiro-cephalosporin products (**B1-B7**).



Scheme (3-4) Proposed mechanism for formation of Spiro-cephalosporins

3.2.3. Characterization of protected Spiro-cephalosporins (B1-B7)

IR spectrum exhibits strong band in the region (3290-3313) cm^{-1} belong to N-H stretching vibration of amide group, while appearance of medium band at (3224-3217) cm^{-1} refers to N-H stretching vibration in heterocyclic ring. In addition, there are three strong bands in the region (1747), (1735) and (1678) cm^{-1}, which indicate the existence of C=O stretching vibration of the β-lactam ring, ester and amide group, respectively. The presence of C=C bond stretching in the aromatic rings is confirmed by the medium band at (1612 - 1620) cm^{-1}.

^{1}HNMR spectra of the compounds shows a doublet at δ (9.03-9.1) ppm confirming the presence of NH proton of amide group, and a singlet at δ (7.25-8.45) ppm refers to NH proton of 1,2,4-triazole ring, while signal of CH proton in triazole ring appears at δ (8.24-8.52) ppm. The characteristic signals of two CH protons in β-lactam ring appear as doublet (overlapped with CH_2 of PMB) and double doublet at 5.16 and 5.38 ppm, respectively. Aromatic protons peaks appear at δ (6.91-7.93) ppm, while methoxy group of PMB protecting group appears as singlet peak at 3.75 ppm. There are two diastereotopic methylene protons in thiazinane ring and thiazolidine ring, each of them produced signals of two doublets (AB system) in the region δ (2.96-3.41) ppm.

One evidence for the occurrence of Michael addition reaction is the appearance of singlet signal at δ (4.69-4.75) ppm, which belong to CH group at position 4 in thiazinane ring.

In ^{13}CNMR spectrum, signals at δ (170.3 -170.6), (169.0-170.4) and (166.4-166.6) ppm denotes the presence of C=O in ester group, β-lactam ring and amide group, respectively. Carbon signal of C=N appears at 164.3 ppm, while signal of CH in triazole ring appears at δ (146.9-153.9) ppm. Aromatic carbon next to methoxy group appears at 159.5 ppm, while the rest of aromatic carbons signals appear at δ (113.9-135.4) ppm.

^{13}CNMR with assistance of DEPT 135 spectra of **B3** and **B6**, have been used to identify the four methylene (CH_2) groups and the methoxy group in the synthesized compounds. β-lactam methine (CH) carbons at positions 6 and 7 appears at 54.8 and 59.7 ppm, respectively, while the signal of methine at position 4 appears at 57.5 ppm.

Assignment of the signal at 59.8 ppm as quaternary carbon (spiro carbon), represent a valid proof to the formation of spiro-cephalosporins. In addition, the disappearance of double bond signals at 125.3 and 125.7 ppm in GCLE ^{13}CNMR spectrum confirm the occurrence of Michael addition reaction.[120]

4-methoxybenzyl 8-oxo-3'-phenyl-7-(2-phenylacetamido)-2',3'-dihydro-6'H-5-thia-1-azaspiro[bicyclo[4.2.0]octane-3,5'-thiazolo[2,3-c][1,2,4]triazole]-2-carboxylate (**B1**): 0.2045 gm (17.3% yield) brown solid; m.p. 92 – 93 °C; IR (KBr) 3305 (N-H), 1747 (C=O), 1735 (C=O), 1678 (C=O), 1616 (C=C) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 9.10 (d, 1H, J=9.2 Hz, N*H*), 8.26 (s, 1H, C*H*), 7.69 (dd, 2H, J_1=7.7 Hz, J_2=1.5 Hz, Ar-*H*), 7.25-7.42 (m, 11H, Ar-*H* and N*H*), 6.95 (d, 2H, J=8.5 Hz, Ar-*H*), 5.39 (dd, 1H, J_1=9.2 Hz, J_2=4.1 Hz, NHC*H*), 5.16 (m, 3H, CH_2 and C*H*), 4.70 (s, 1H, C*H*), 3.75 (s, 3H, CH_3), 3.55 (s, 2H, CH_2), 3.41 (m, 1H, C*Ha*Hb), 3.28 (d, 1H, J=11.8 Hz, C*Hc*Hd), 3.11 (d,

1H, J=11.8 Hz, CHc*Hd*), 2.96 (d, 1H, J=13.5 Hz, CHa*Hb*), ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 170.3 (C=O), 169.1 (C=O), 166.6 (C=O), 164.3 (C=N), 159.5 (Ar-C), 151.4 (CH), 135.4 (Ar-C), 135.0 (Ar-C), 130.4 (Ar-CH ×2), 129.7 (Ar-CH), 129.1 (Ar-CH ×2), 128.7 (Ar-CH ×2), 128.4 (Ar-CH ×2),127.0 (Ar-CH ×2), 126.9 (Ar-C), 126.7 (Ar-CH), 114.0 (Ar-CH ×2), 67.3 (CH_2), 59.8 (C), 59.7 (CH), 57.5 (CH), 55.1 (CH_3), 54.8 (CH), 42.5 (CH_2), 34.5 (CH_2), 31.1 (CH_2).

4-methoxybenzyl 3'-(2-chlorophenyl)-8-oxo-7-(2-phenylacetamido)-2',3'-dihydro-6'H-5-thia-1-azaspiro[bicyclo[4.2.0]octane-3,5'-thiazolo[2,3-c][1,2,4]triazole]-2-carboxylate (**B2**): 0.4551gm (24.2% yield) yellow solid; m.p. 98 – 99 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 9.03 (d, 1H, J=9.1 Hz, N*H*), 8.52 (s, 1H, C*H*), 8.45 (s, 1H, N*H*), 7.93 (m, 1H, Ar-*H*), 7.50 (m, 1H, Ar-*H*), 7.41 (m, 2H, Ar-*H*), 7.38 (d, 2H, J=8.5 Hz, Ar-*H*), 7.25-7.33 (m, 5H, Ar-*H*), 6.95 (d, 2H, J=8.5 Hz, Ar-*H*), 5.38 (dd, 1H, J_1=9.0 Hz, J_2=4.1 Hz, NHC*H*), 5.16 (m, 3H, CH_2 and C*H*), 4.72 (s, 1H, C*H*), 3.75 (s, 3H, CH_3), 3.55 (s, 2H, CH_2), 3.38 (m, 2H, C*Ha*Hb, C*Hc*Hd), 3.15 (d, 1H, J=11.9 Hz, CHc*Hd*), 2.98 (d, 1H, J=13.8 Hz, CHa*Hb*), ^{13}C-NMR (100 MHz, DMSO-d6): ppm δ 170.6 (C=O), 170.4 (C=O), 166.5 (C=O), 164.2 (C=N), 159.4 (Ar-C), 146.9 (CH), 135.4 (Ar-C), 132.8 (Ar-C), 131.9 (Ar-C), 131.1 (Ar-CH), 130.4 (Ar-CH ×2), 129.9 (Ar-CH), 129.1 (Ar-CH ×2), 128.3 (Ar-CH ×2), 127.5 (Ar-CH), 126.98 (Ar-CH), 126.91 (Ar-C), 126.7 (Ar-CH), 113.9 (Ar-CH ×2), 67.3 (CH_2), 59.9 (C), 59.7 (CH), 57.5 (CH), 55.1 (CH_3), 54.7 (CH), 42.4 (CH_2), 34.5 (CH_2), 31.0 (CH_2).

4-methoxybenzyl 3'-(2-bromophenyl)-8-oxo-7-(2-phenylacetamido)-2',3'-dihydro-6'H-5-thia-1-azaspiro[bicyclo[4.2.0]octane-3,5'-thiazolo[2,3-c][1,2,4]triazole]-2-carboxylate (**B3**): 0.4325 gm (20% yield) yellow solid;

m.p. 95 – 96 °C; IR (KBr) 3290 (N-H), 3217 (N-H), 1747 (C=O), 1735 (C=O), 1681 (C=O), 1612 (C=C) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 9.05 (d, 1H, J=9.0 Hz, N*H*), 8.48 (m, 2H, C*H* and N*H*), 7.92 (dd, 1H, J_1=7.9 Hz, J_2=1.4 Hz, Ar-H), 7.67 (m, 1H, Ar-*H*), 7.45 (m, 1H, Ar-*H*), 7.38 (d, 2H, J=8.4 Hz, Ar-*H*), 7.25-7.33 (m, 6H, Ar-*H*), 6.95 (d, 2H, J=8.4 Hz, Ar-*H*), 5.38 (dd, 1H, J_1=9.0 Hz, J_2=4.2 Hz, NHC*H*), 5.16 (m, 3H, CH_2 and C*H*), 4.73 (s, 1H, C*H*), 3.75 (s, 3H, CH_3), 3.56 (s, 2H, CH_2), 3.40 (m, 1H, C*Ha*Hb), 3.32 (m, 1H, C*Hc*Hd), 3.15 (d, 1H, J=11.9 Hz, CHc*Hd*), 2.98 (d, 1H, J=13.9 Hz, CHa*Hb*), ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 170.4 (C=O), 166.5 (C=O), 164.3 (C=N), 159.5 (Ar-C), 149.4 (CH), 135.4 (Ar-C), 133.4 (Ar-C), 133.1 (Ar-CH), 131.4 (Ar-CH), 130.4 (Ar-CH ×2), 129.1 (Ar-CH ×2), 128.4 (Ar-CH ×2),128.0 (Ar-CH), 127.3 (Ar-CH), 126.9 (Ar-C), 126.7 (Ar-CH), 123.3 (Ar-C), 114.0 (Ar-CH ×2), 67.4 (CH_2), 59.8 (C), 59.7 (CH), 57.5 (CH), 55.1 (CH_3), 54.7 (CH), 42.4 (CH_2), 34.6 (CH_2), 31.0 (CH_2).

4-methoxybenzyl 3'-(2-hydroxyphenyl)-8-oxo-7-(2-phenylacetamido)-2',3'-dihydro-6'H-5-thia-1-azaspiro[bicyclo[4.2.0]octane-3,5'-thiazolo[2,3-c][1,2,4]triazole]-2-carboxylate (**B4**): 0.4551 gm (32% yield) yellow solid; m.p. 117 – 118 °C; IR (KBr) 3290 (N-H), 1747 (C=O), 1735 (C=O), 1681 (C=O), 1612 (C=C) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 11.15 (s, 1H, O*H*), 9.05 (d, 1H, J=9.2 Hz, N*H*), 8.46 (s, 1H, C*H*), 8.45 (s, 1H, N*H*), 7.48 (dd, 1H, J_1=7.7 Hz, J_2=1.5 Hz, Ar-*H*), 7.37 (d, 2H, J=8.5 Hz, Ar-*H*), 7.25-7.31 (m, 6H, Ar-*H*), 6.95 (d, 2H, J=8.5 Hz, Ar-*H*), 6.91 (m, 2H, Ar-*H*), 5.38 (dd, 1H, J_1=8.9 Hz, J_2=4.0 Hz, NHC*H*), 5.16 (m, 3H, CH_2 and C*H*), 4.75 (s, 1H, C*H*), 3.75 (s, 3H, CH_3), 3.56 (s, 2H, CH_2), 3.44 (m, 2H, C*Ha*Hb and C*Hc*Hd), 3.24 (d, 1H, J=11.2 Hz, CHc*Hd*), 3.00 (d, 1H, J=13.7 Hz, CHa*Hb*), ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 170.4 (C=O), 168.0 (C=O), 166.4

(C=O), 164.2 (C=N), 159.4 (Ar-C), 157.7 (Ar-C), 153.9 (CH), 135.4 (Ar-C), 131.0 (Ar-CH), 130.5 (Ar-CH), 130.4 (Ar-CH ×2), 129.1 (Ar-CH ×2), 128.4 (Ar-CH ×2), 126.9 (Ar-C), 126.7 (Ar-CH), 119.3 (Ar-CH), 118.8 (Ar-C), 116.1 (Ar-CH), 113.9 (Ar-CH ×2), 67.4 (CH2), 60.1 (C), 59.7 (CH), 57.5 (CH), 55.1 (CH_3), 54.7 (CH), 42.4 (CH_2), 34.9 (CH_2), 31.0 (CH_2).

4-methoxybenzyl 3'-(4-fluorophenyl)-8-oxo-7-(2-phenylacetamido)-2',3'-dihydro-6'H-5-thia-1-azaspiro[bicyclo[4.2.0]octane-3,5'-thiazolo[2,3-c][1,2,4]triazole]-2-carboxylate (**B5**): 0.396 gm (24% yield) orange solid; m.p. 100 – 102 °C; IR (KBr) 3313 (N-H), 3224 (N-H), 1747 (C=O), 1735 (C=O), 1678 (C=O), 1620 (C=C) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 9.08 (d, 1H, J=9.2 Hz, N*H*), 8.26 (m, 2H, C*H* and N*H*), 7.74 (dd, 2H, J_1=8.7 Hz, J_2=5.7 Hz, Ar-*H*), 7.37 (d, 2H, J=8.6 Hz, Ar-*H*), 7.24-7.33 (m, 7H, Ar-*H*), 6.95 (d, 2H, J=8.6 Hz, Ar-*H*), 5.38 (dd, 1H, J_1=9.1 Hz, J_2=4.2 Hz, NHC*H*), 5.17 (m, 3H, CH_2 and C*H*), 4.69 (s, 1H, C*H*), 3.75 (s, 3H, CH_3), 3.55 (s, 2H, CH_2), 3.39 (d, 1H, J=13.9 Hz, C*Ha*Hb), 3.28 (d, 1H, J=11.9 Hz, C*Hc*Hd), 3.11 (d, 1H, J=11.9 Hz, CHc*Hd*), 2.96 (d, 1H, J=13.9 Hz, CHa*Hb*), ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 170.3 (C=O), 169.0 (C=O), 166.5 (C=O), 164.3 (C=N), 161.7 (Ar-C), 159.5 (Ar-C), 150.3 (CH), 135.4 (Ar-C), 131.7 (Ar-C), 130.4 (Ar-CH ×2), 129.1 (Ar-CH ×2), 129.0 (Ar-CH ×2), 128.4 (Ar-CH ×2), 126.9 (Ar-C), 126.7 (Ar-CH), 115.8 (d, J=21.8 Hz, Ar-CH ×2), 113.9 (Ar-CH ×2), 67.3 (CH_2), 59.7 (C), 59.6 (CH), 57.5 (CH), 55.1 (CH_3), 54.7 (CH), 42.4 (CH_2), 34.5 (CH_2), 31.0 (CH_2).

4-methoxybenzyl 3'-(4-chlorophenyl)-8-oxo-7-(2-phenylacetamido)-2',3'-dihydro-6'H-5-thia-1-azaspiro[bicyclo[4.2.0]octane-3,5'-thiazolo[2,3-c][1,2,4]triazole]-2-carboxylate (**B6**): 0.266 gm (20% yield) orange solid; m.p. 115 – 117 °C; IR (KBr) 3309 (N-H), 1747 (C=O), 1735 (C=O), 1678 (C=O),

1616 (C=C) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 9.09 (d, 1H, J=9.3 Hz, N*H*), 8.35 (s, 1H, N*H*), 8.25 (s, 1H, C*H*), 7.70 (d, 2H, J=8.5 Hz, Ar-*H*), 7.49 (d, 2H, J=8.5, Ar-*H*), 7.38 (d, 2H, J=8.5Hz, Ar-*H*), 7.25-7.32 (m, 5H, Ar-*H*), 6.95 (d, 2H, J=8.5 Hz, Ar-*H*), 5.39 (dd, 1H, J_1=9.1 Hz, J_2=4.2 Hz, NHC*H*), 5.15 (m, 3H, CH_2 and C*H*), 4.70 (s, 1H, C*H*), 3.75 (s, 3H, CH_3), 3.55 (s, 2H, CH_2), 3.40 (m, 1H, C*Ha*Hb), 3.30 (d, 1H, J=11.9 Hz, C*Hc*Hd), 3.12 (d, 1H, J=11.9 Hz, CHc*Hd*), 2.97 (d, 1H, J=13.8 Hz, CHa*Hb*), ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 170.3 (C=O), 169.5 (C=O), 166.5 (C=O), 164.3 (C=N), 159.5 (Ar-C), 150.2 (CH), 135.4 (Ar-C), 134.1 (Ar-C), 134.0 (Ar-C), 130.4 (Ar-CH ×2), 129.1 (Ar-CH ×2), 128.8 (Ar-CH ×2), 128.6 (Ar-CH ×2),128.4 (Ar-CH ×2), 126.9 (Ar-C), 126.7 (Ar-CH), 113.9 (Ar-CH ×2), 67.3 (CH_2), 59.8 (C), 59.7 (CH), 57.5 (CH), 55.1 (CH_3), 54.8 (CH), 42.5 (CH_2), 34.5 (CH_2), 31.0 (CH_2).

4-methoxybenzyl 3'-(4-bromophenyl)-8-oxo-7-(2-phenylacetamido)-2',3'-dihydro-6'H-5-thia-1-azaspiro[bicyclo[4.2.0]octane-3,5'-thiazolo[2,3-c][1,2,4]triazole]-2-carboxylate (**B7**): 0.206gm (23% yield) orange solid; m.p. 89 – 90 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 9.09 (d, 1H, J=9.1 Hz, N*H*), 8.24 (s, 1H, C*H*), 7.63 (s, 4H, Ar-*H*), 7.38 (d, 2H, J=8.5 Hz, Ar-*H*), 7.27-7.33 (m, 5H, Ar-*H*), 6.95 (d, 2H, J=8.5 Hz, Ar-*H*), 5.39 (dd, 1H, J_1=9.1 Hz, J_2=4.2 Hz, NHC*H*), 5.15 (m, 3H, CH_2 and C*H*), 4.70 (s, 1H, C*H*), 3.75 (s, 3H, CH_3), 3.55 (s, 2H, CH_2), 3.40 (m, 1H, C*Ha*Hb), 3.30 (d, 1H, J=12.0 Hz, C*Hc*Hd), 3.12 (d, 1H, J=12.0 Hz, CHc*Hd*), 2.96 (d, 1H, J=13.9 Hz, CHa*Hb*), ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 170.3 (C=O), 169.6 (C=O), 166.5 (C=O), 164.3 (C=N), 159.5 (Ar-C), 150.3 (CH), 135.4 (Ar-C), 134.3 (Ar-C), 131.8 (Ar-CH ×2), 130.4 (Ar-CH ×2), 129.1 (Ar-CH ×2), 128.8 (Ar-CH ×2), 128.4 (Ar-CH ×2), 126.9 (Ar-C), 126.7 (Ar-CH), 122.9 (Ar-C), 113.9 (Ar-CH

×2), 67.3 (CH_2), 59.8 (C), 59.7 (CH), 57.5 (CH), 55.1 (CH_3), 54.8 (CH), 42.5 (CH_2), 34.5 (CH_2), 31.0 (CH_2).

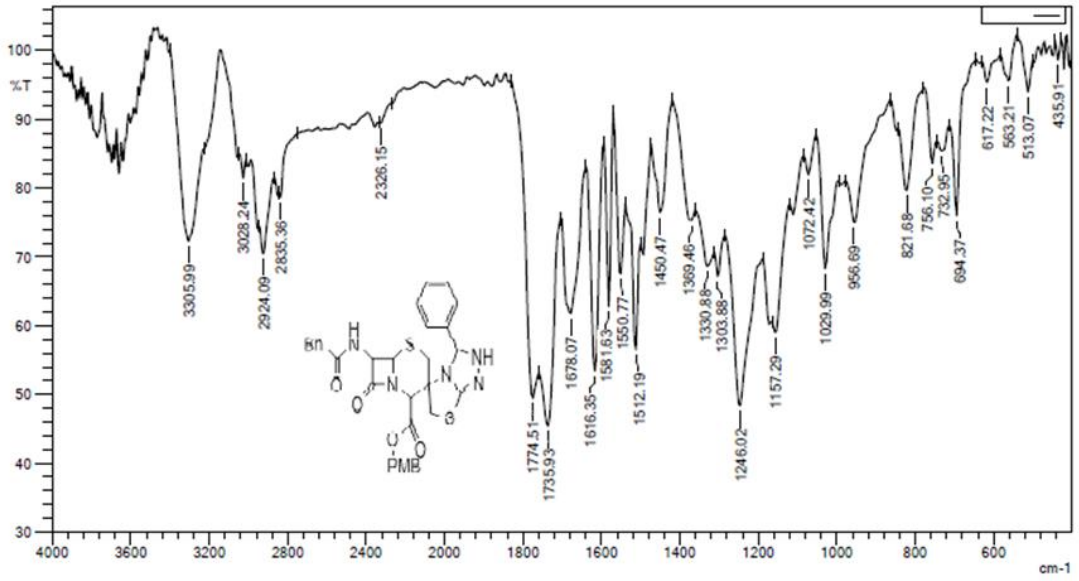


Fig (3-29) IR Spectrum of the compound B1

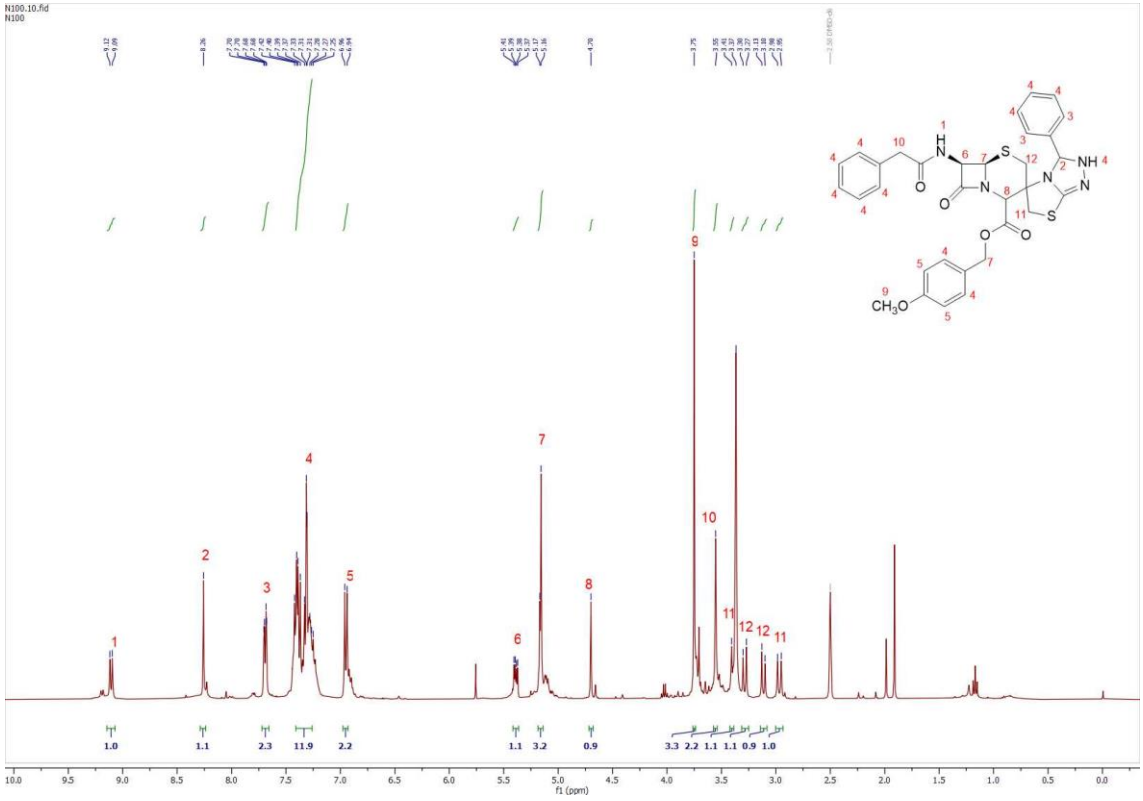


Fig (3-30) ^{1}HNMR Spectrum of the compound B1

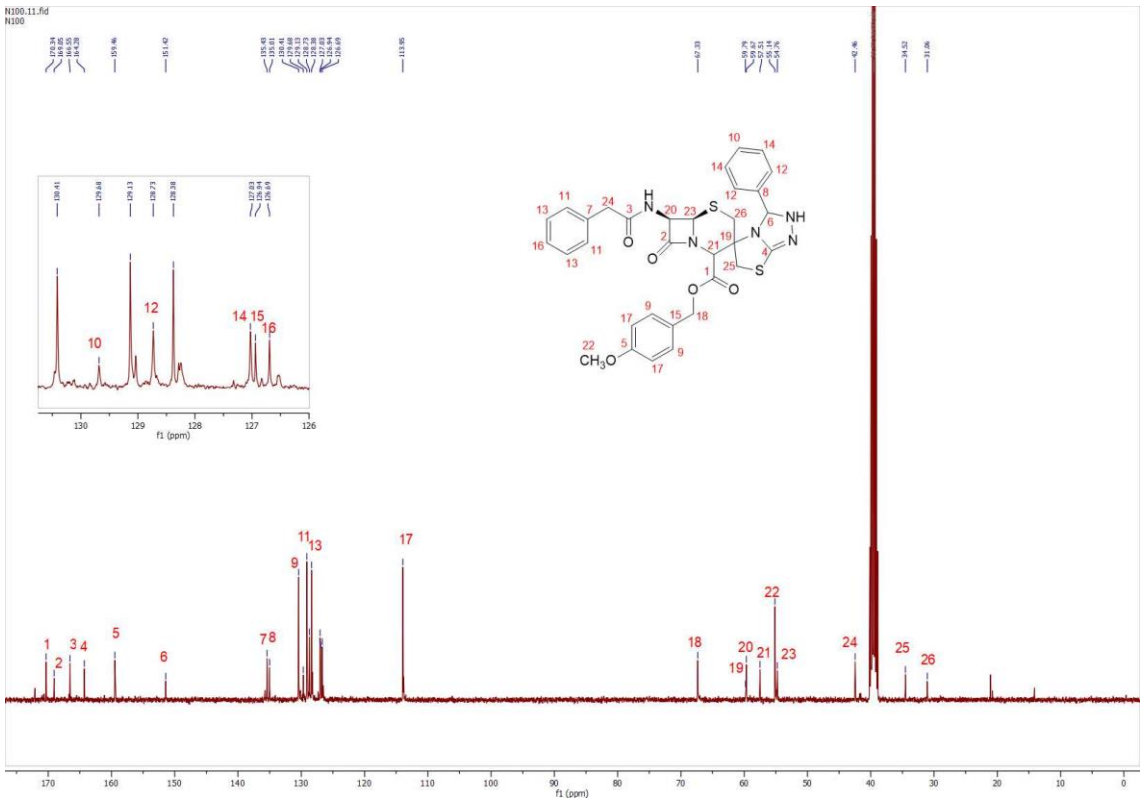


Fig (3-31) ^{13}CNMR Spectrum of the compound B1

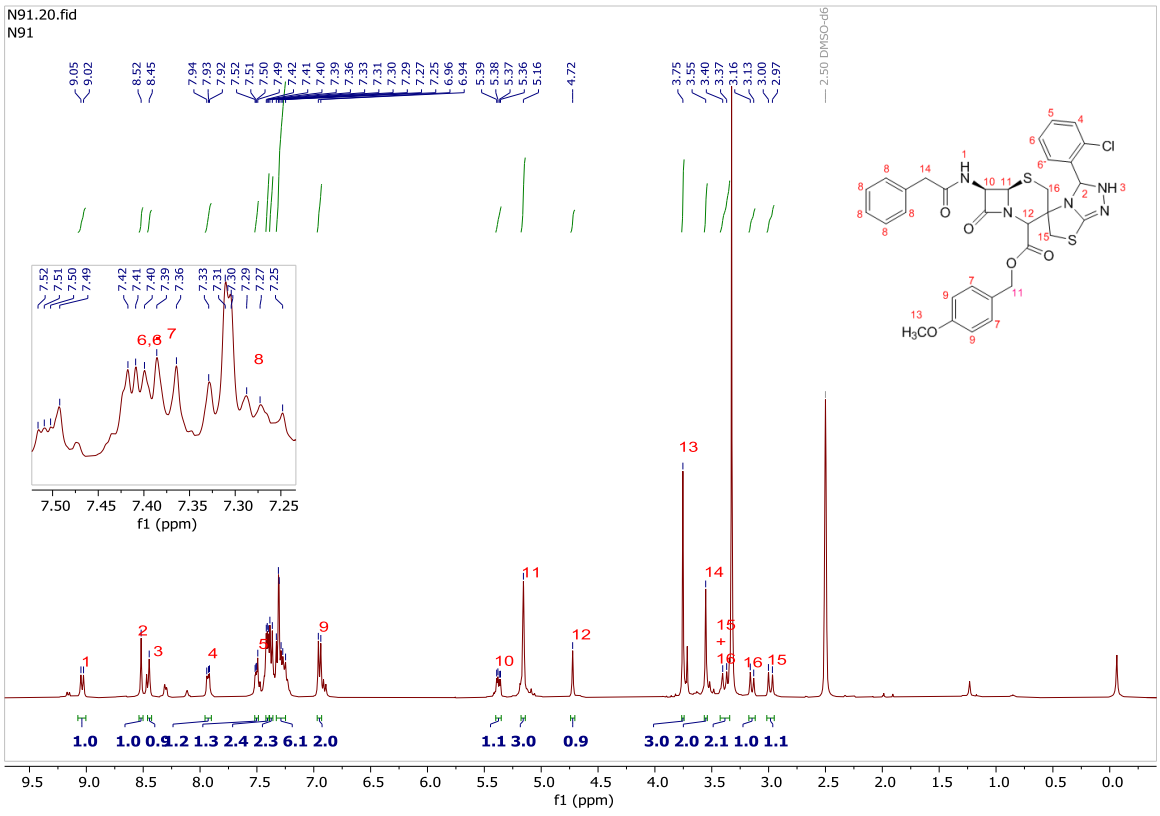


Fig (3-32) ^{1}HNMR Spectrum of the compound B2

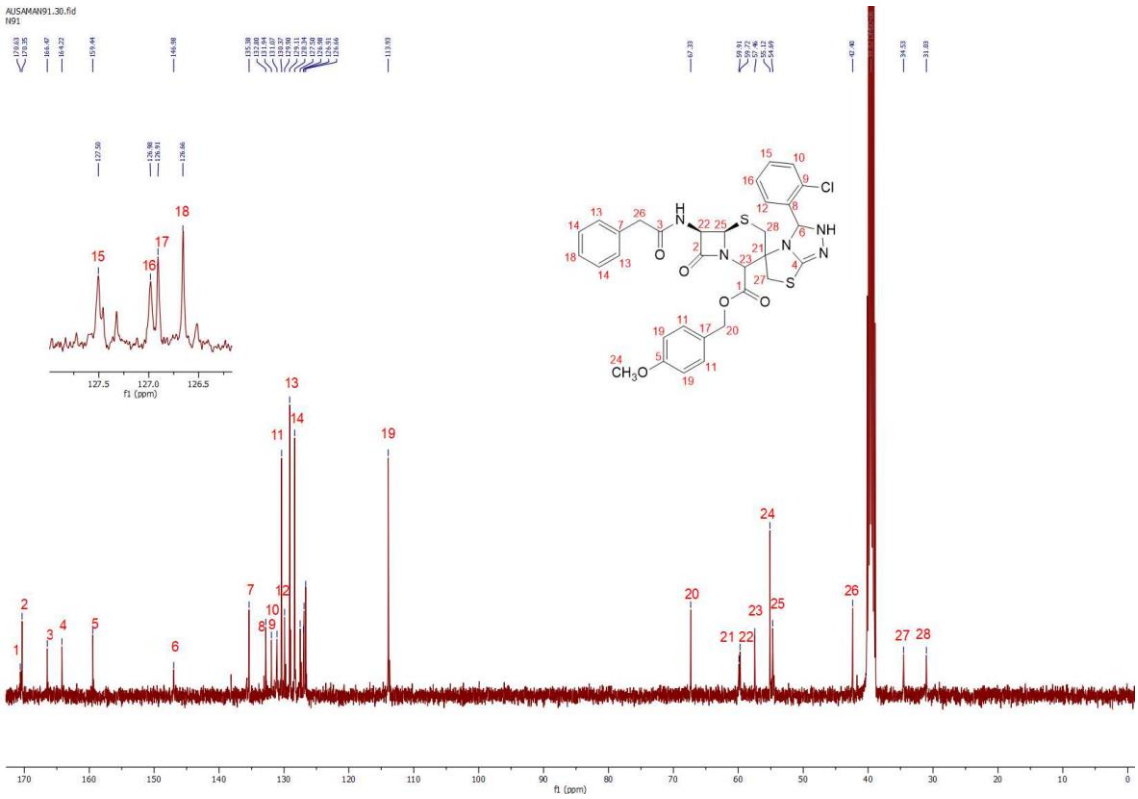


Fig (3-33) ^{13}CNMR Spectrum of the compound B2

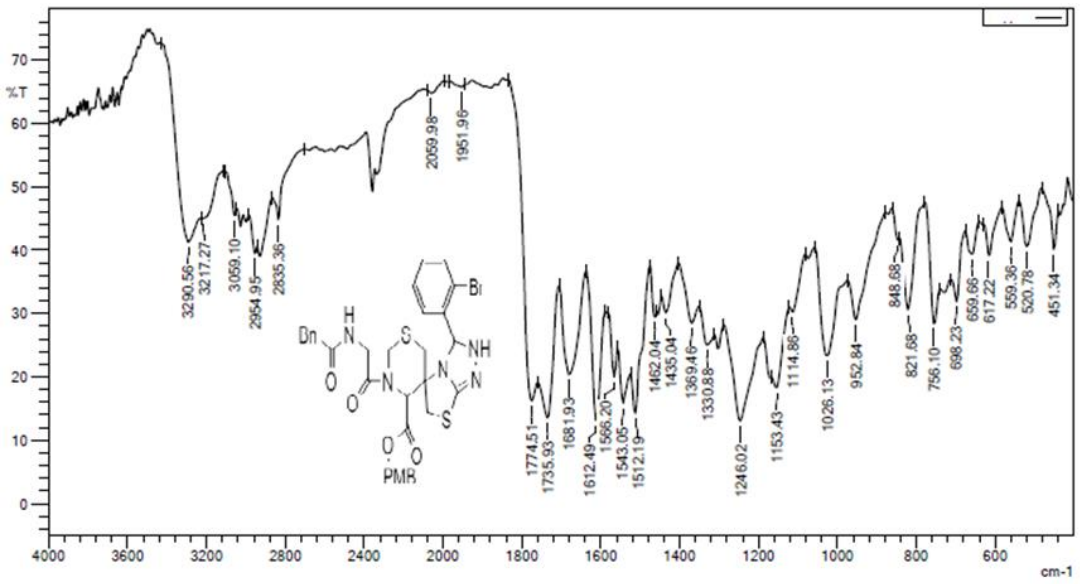


Fig (3-34) IR Spectrum of the compound B3

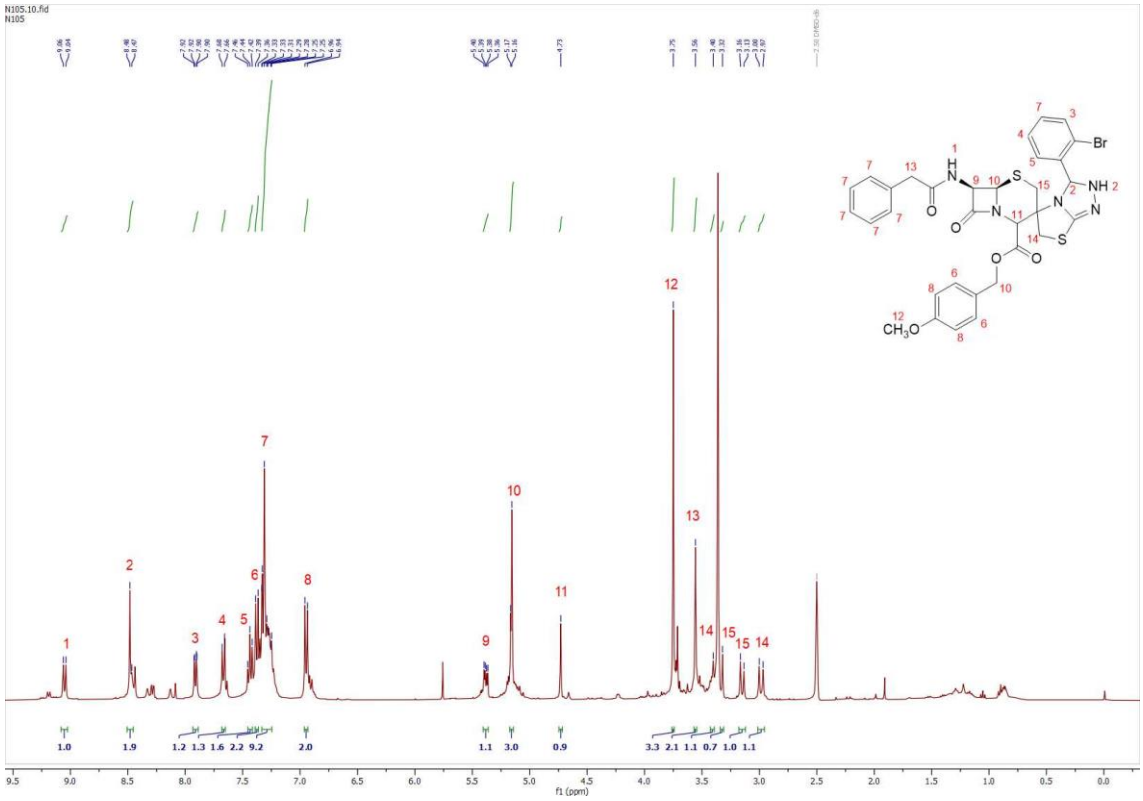


Fig (3-35) ^{1}HNMR Spectrum of the compound B3

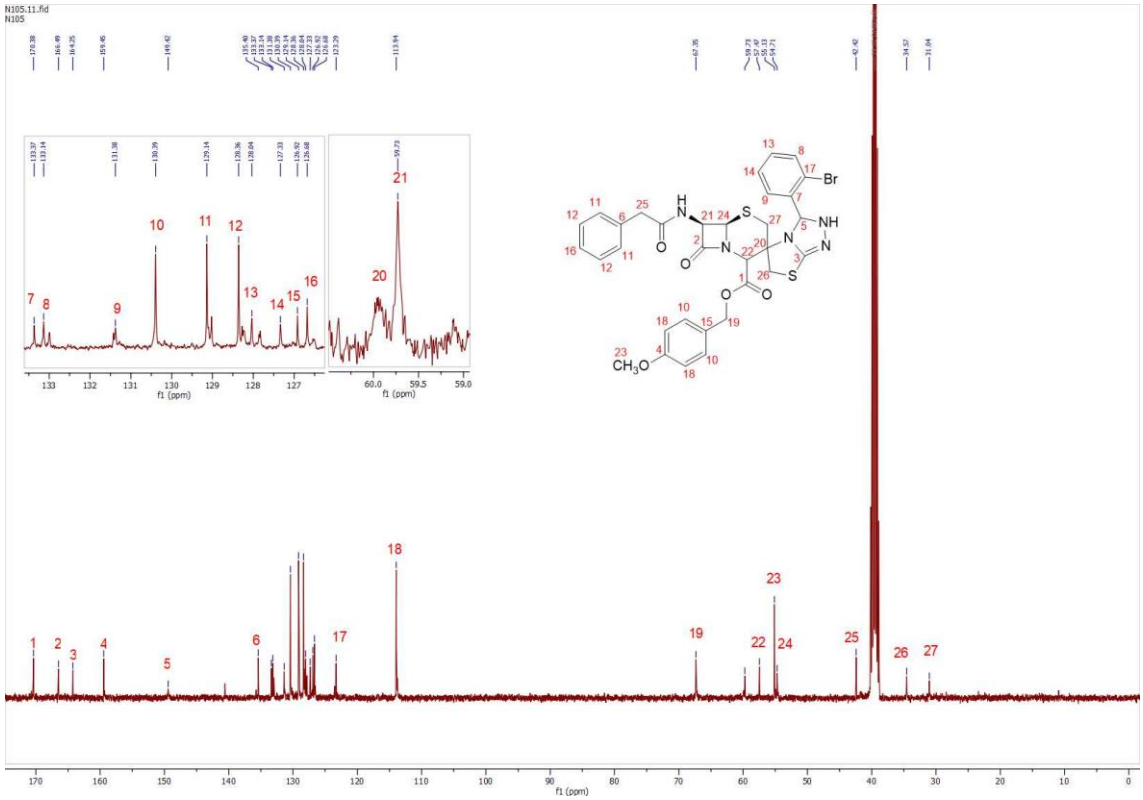


Fig (3-36) ^{13}CNMR Spectrum of the compound B3

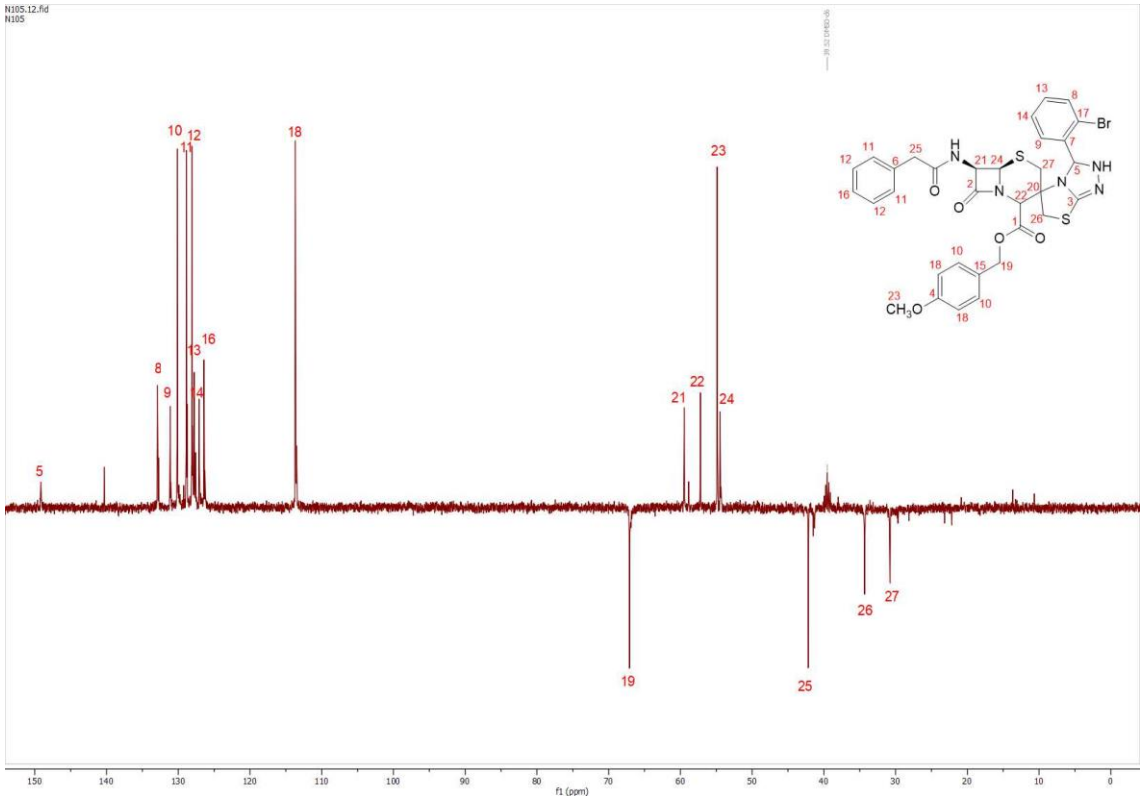


Fig (3-37) DEPT 135 Spectrum of the compound B3

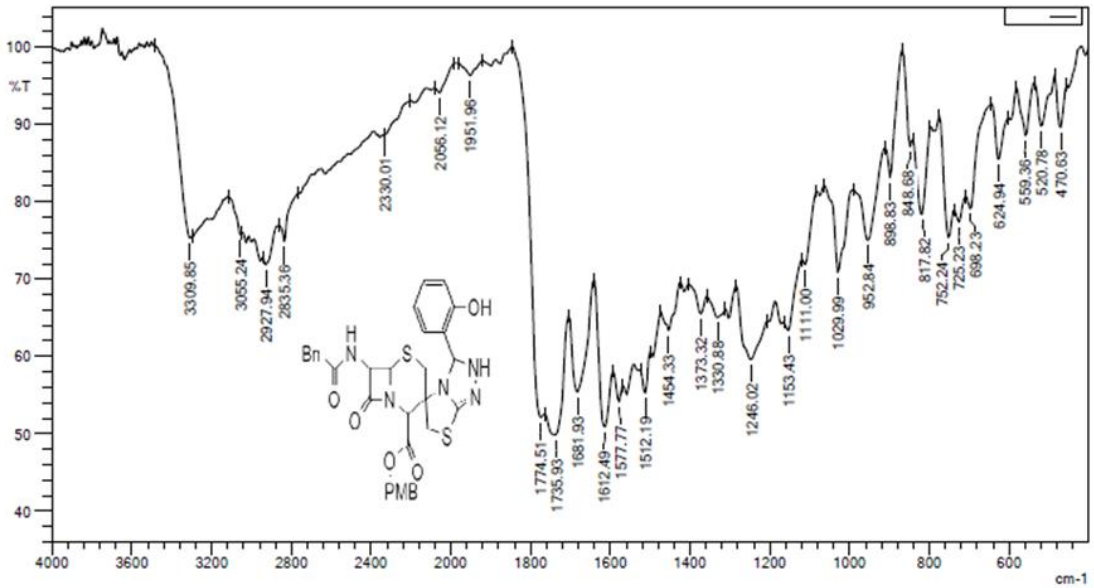


Fig (3-38) IR Spectrum of the compound B4

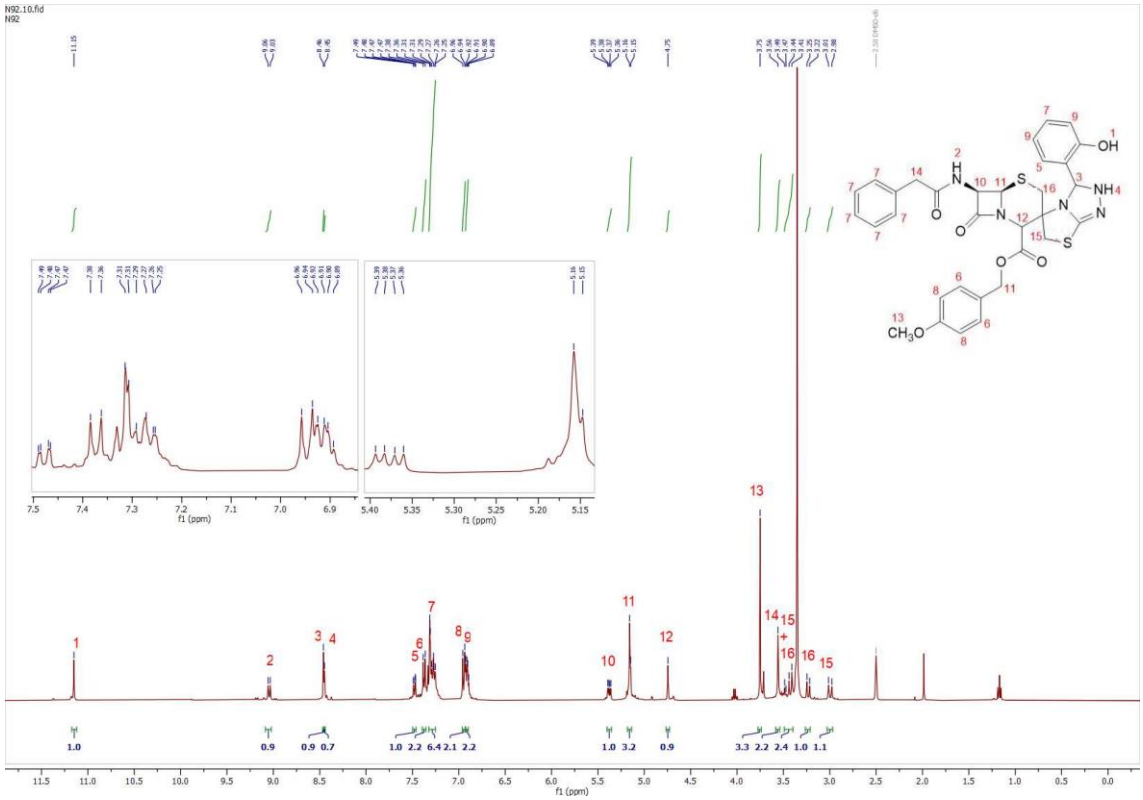


Fig (3-39) ^{1}HNMR Spectrum of the compound B4

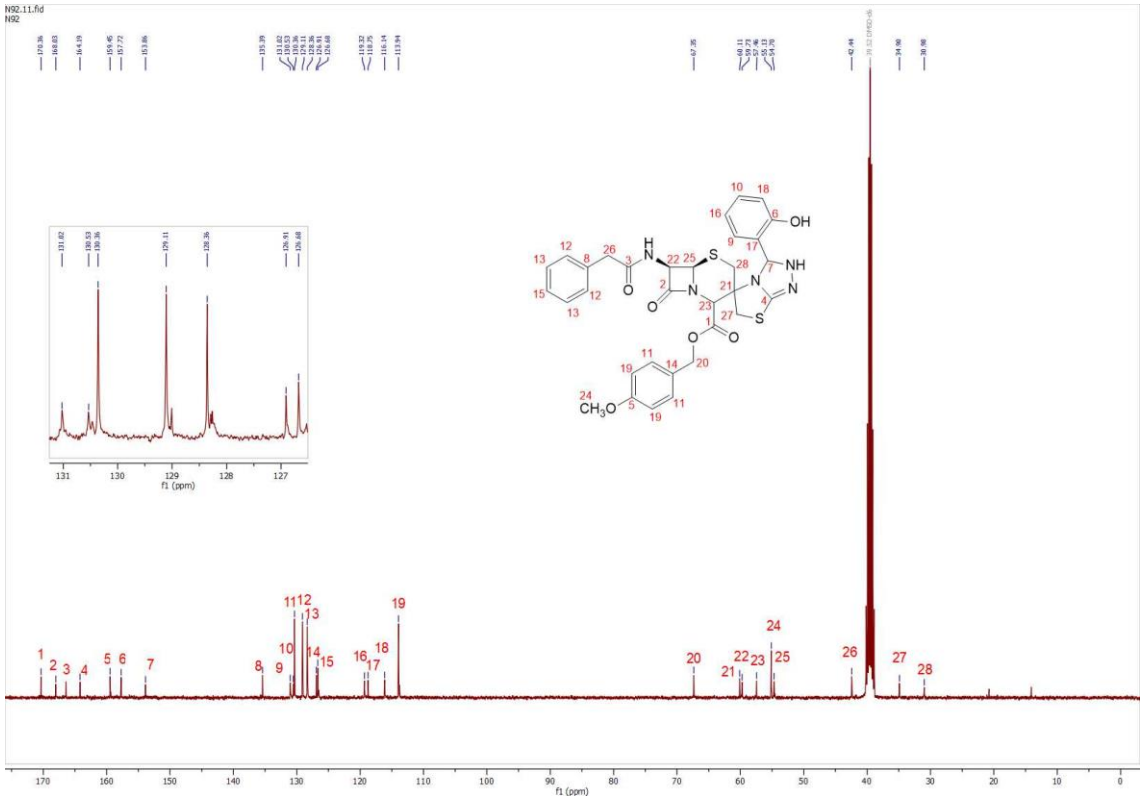


Fig (3-40) ^{13}CNMR Spectrum of the compound B4

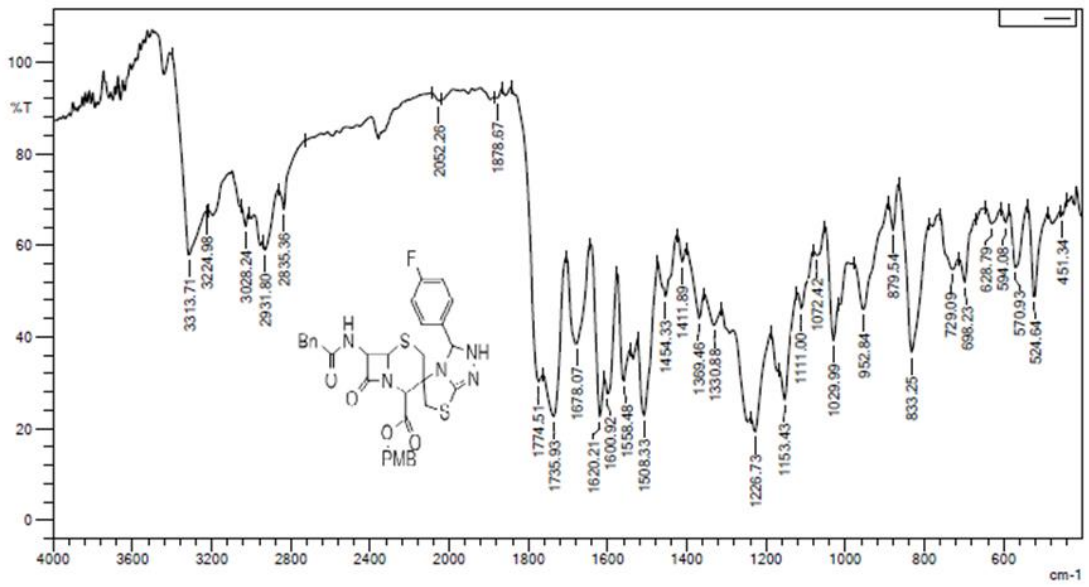


Fig (3-41) IR Spectrum of the compound B5

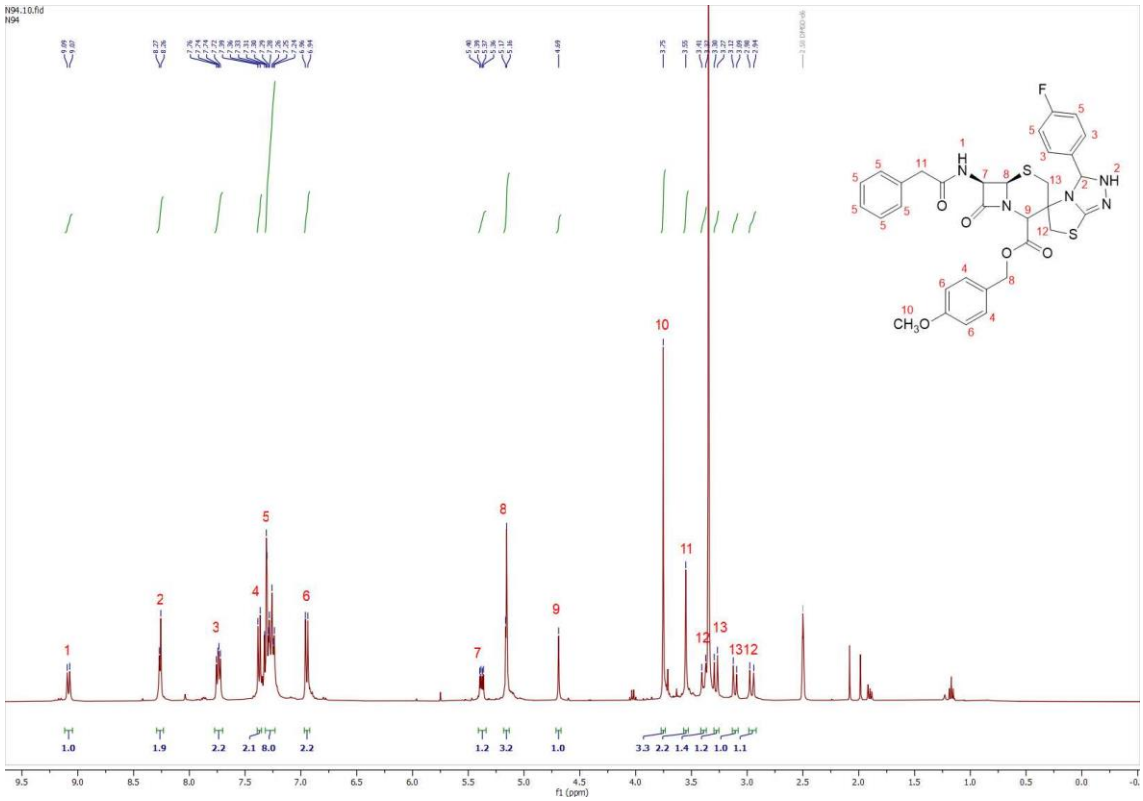


Fig (3-42) ^{1}HNMR Spectrum of the compound B5

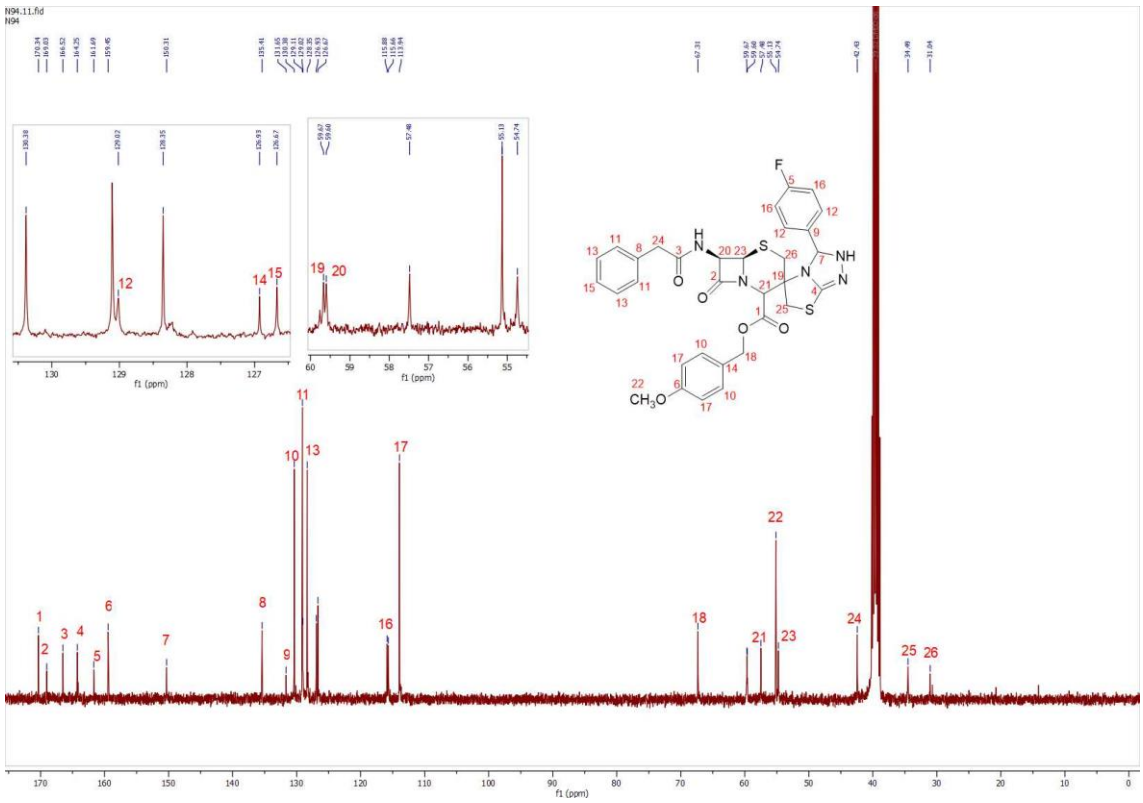


Fig (3-43) ^{13}CNMR Spectrum of the compound B5

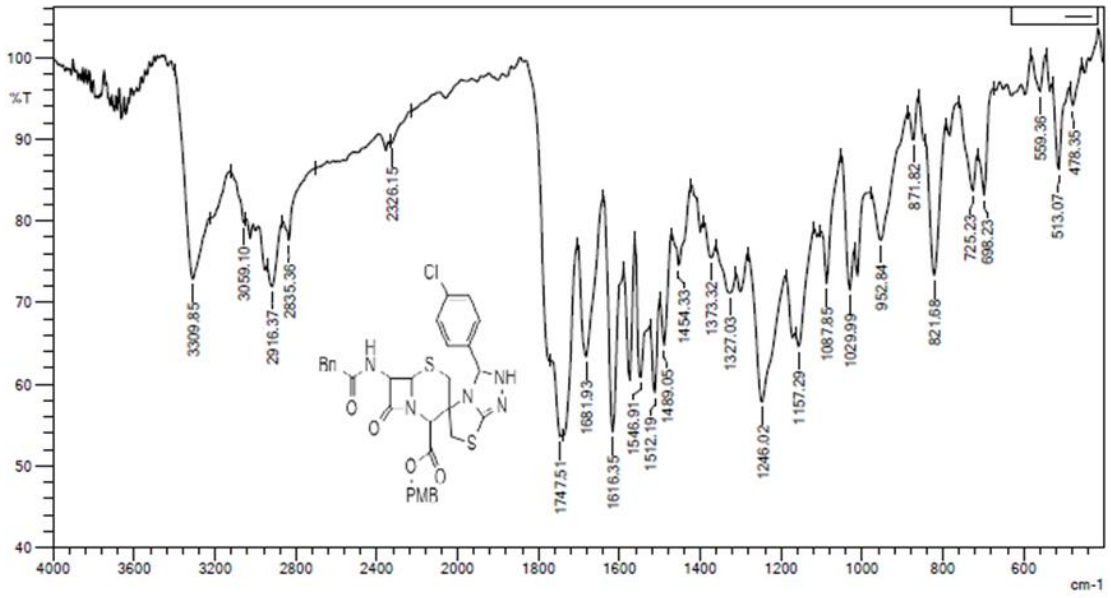


Fig (3-44) IR Spectrum of the compound B6

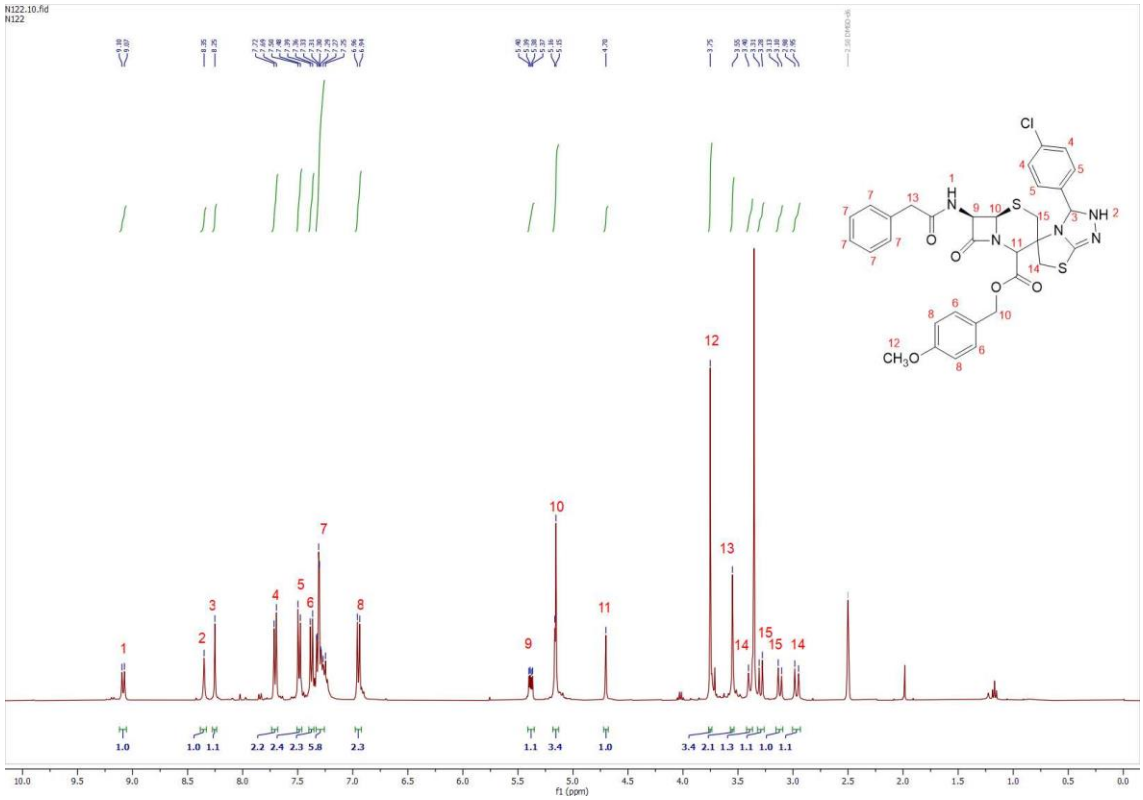


Fig (3-45) ^{1}HNMR Spectrum of the compound B6

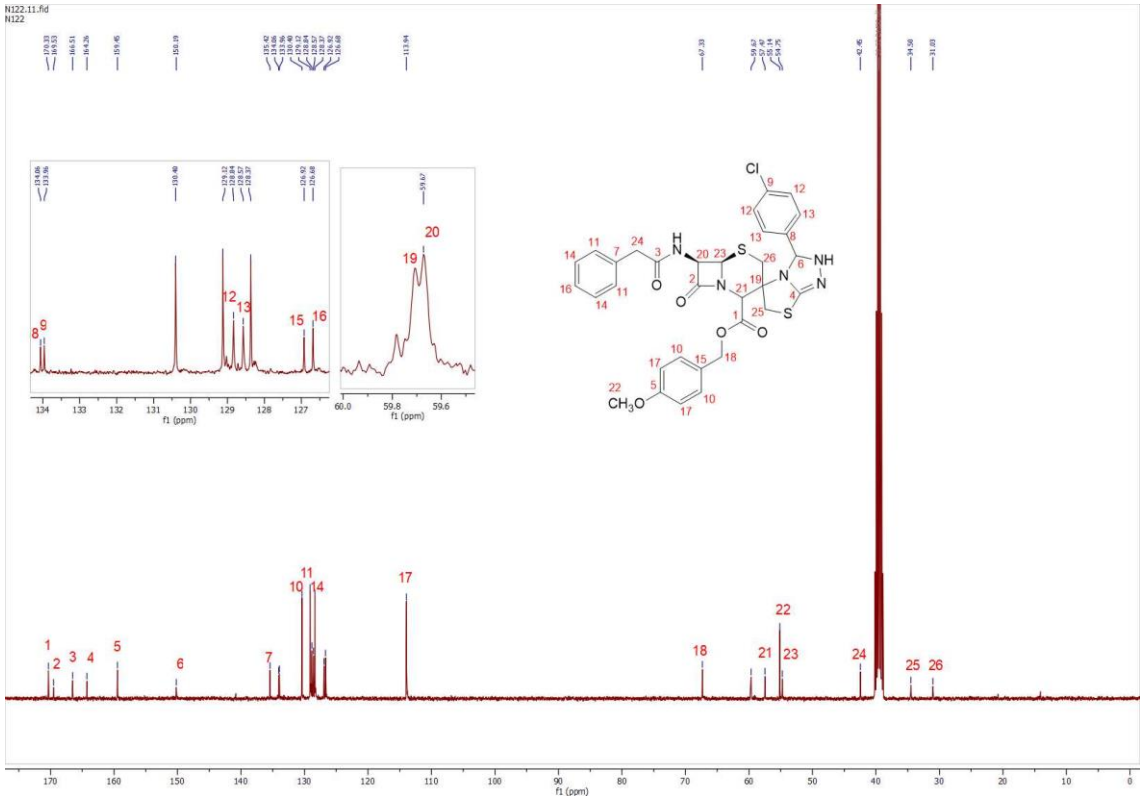


Fig (3-46) ^{13}CNMR Spectrum of the compound B6

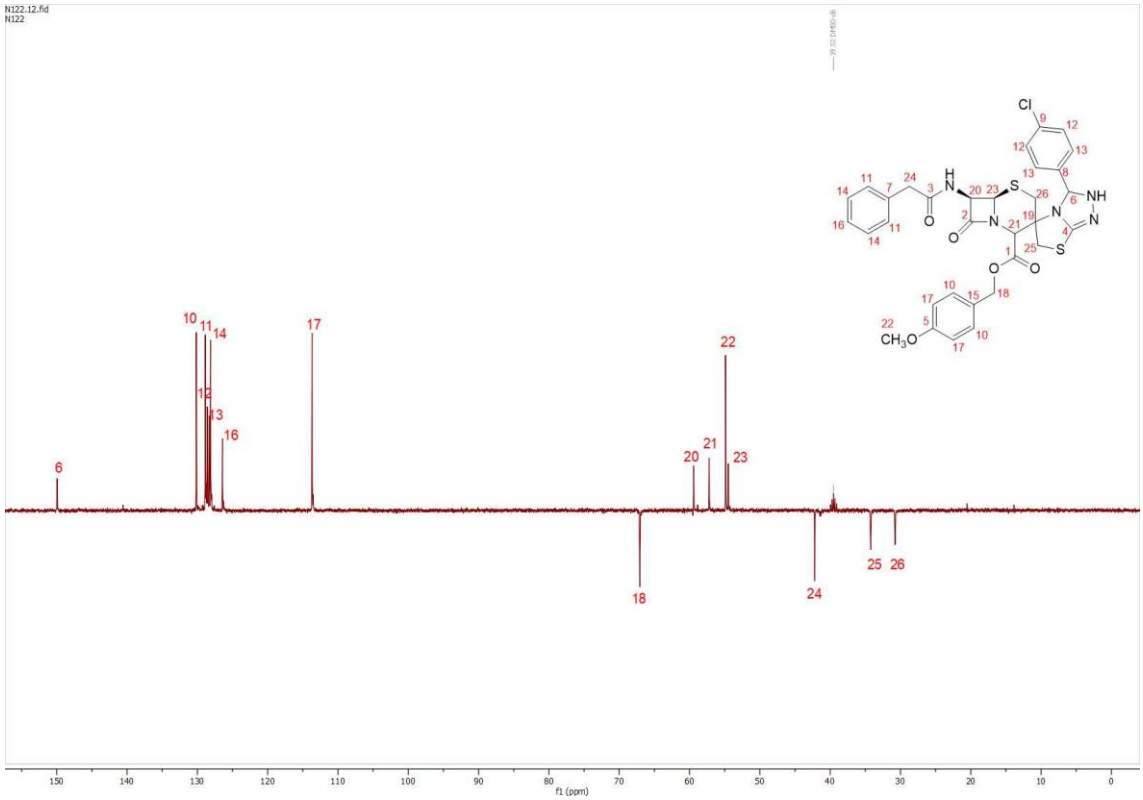


Fig (3-47) DEPT 135 Spectrum of the compound B6

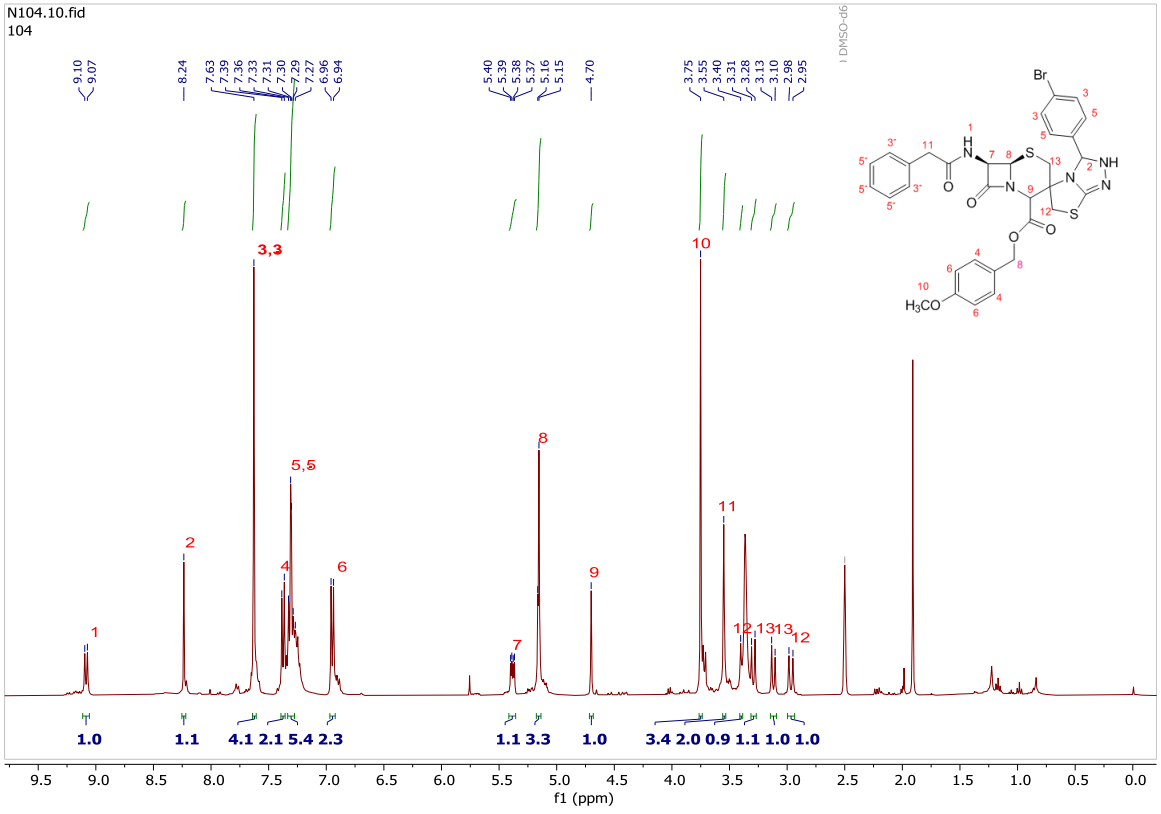


Fig (3-48) ^{1}HNMR Spectrum of the compound B7

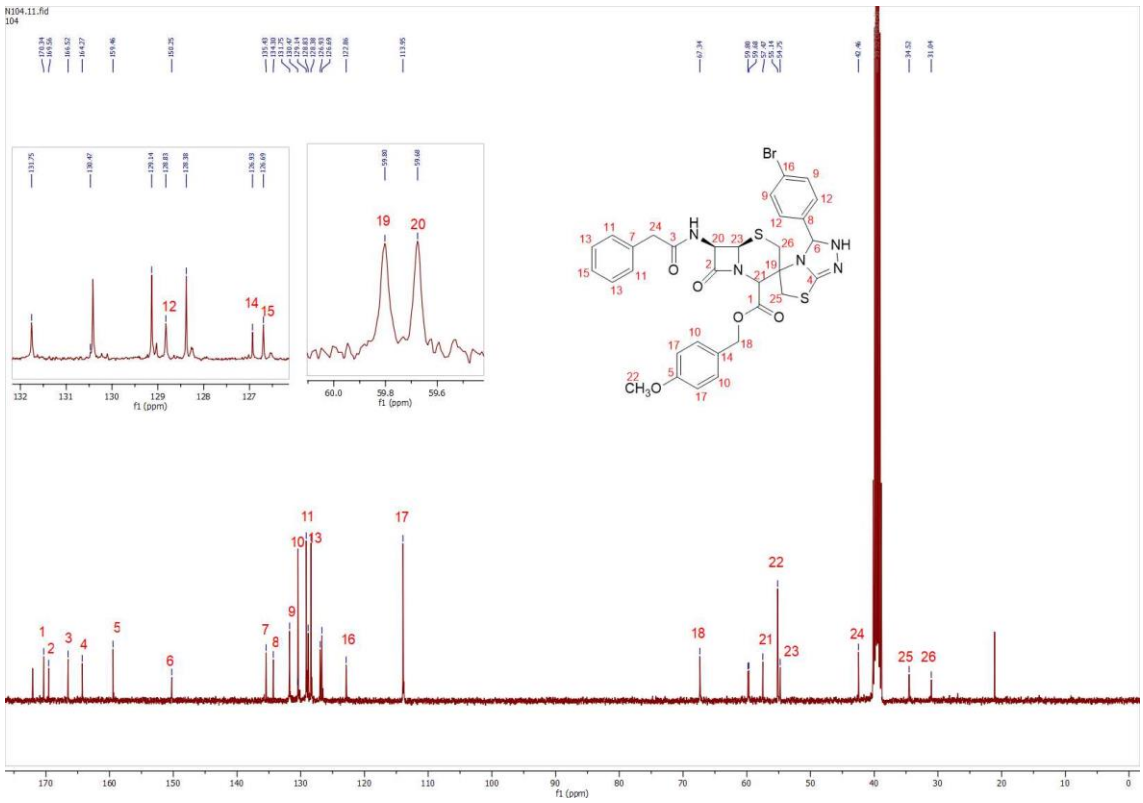
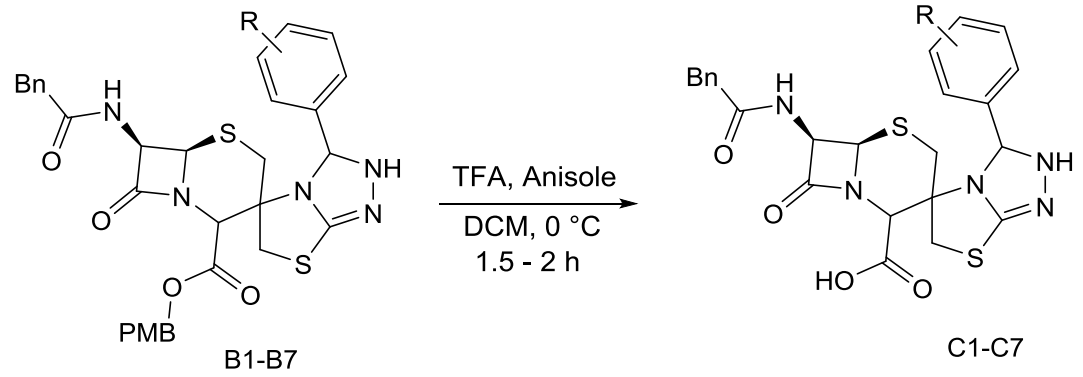


Fig (3-49) ^{13}CNMR Spectrum of the compound B7

3.3. Synthesis of functionally active spiro-cephalosporins (C1-C7)

3.3.1. The synthetic strategy

To provide functionally active spiro-cephalosporin compounds, the *p*-methoxybenzyl (PMB) protecting group was removed in the presence of trifluoroacetic acid (TFA) and anisole. The crude products were purified by column chromatography to give the carboxylic acids as single diastereomers in good yields (46−80%), as shown in Scheme (3-5).



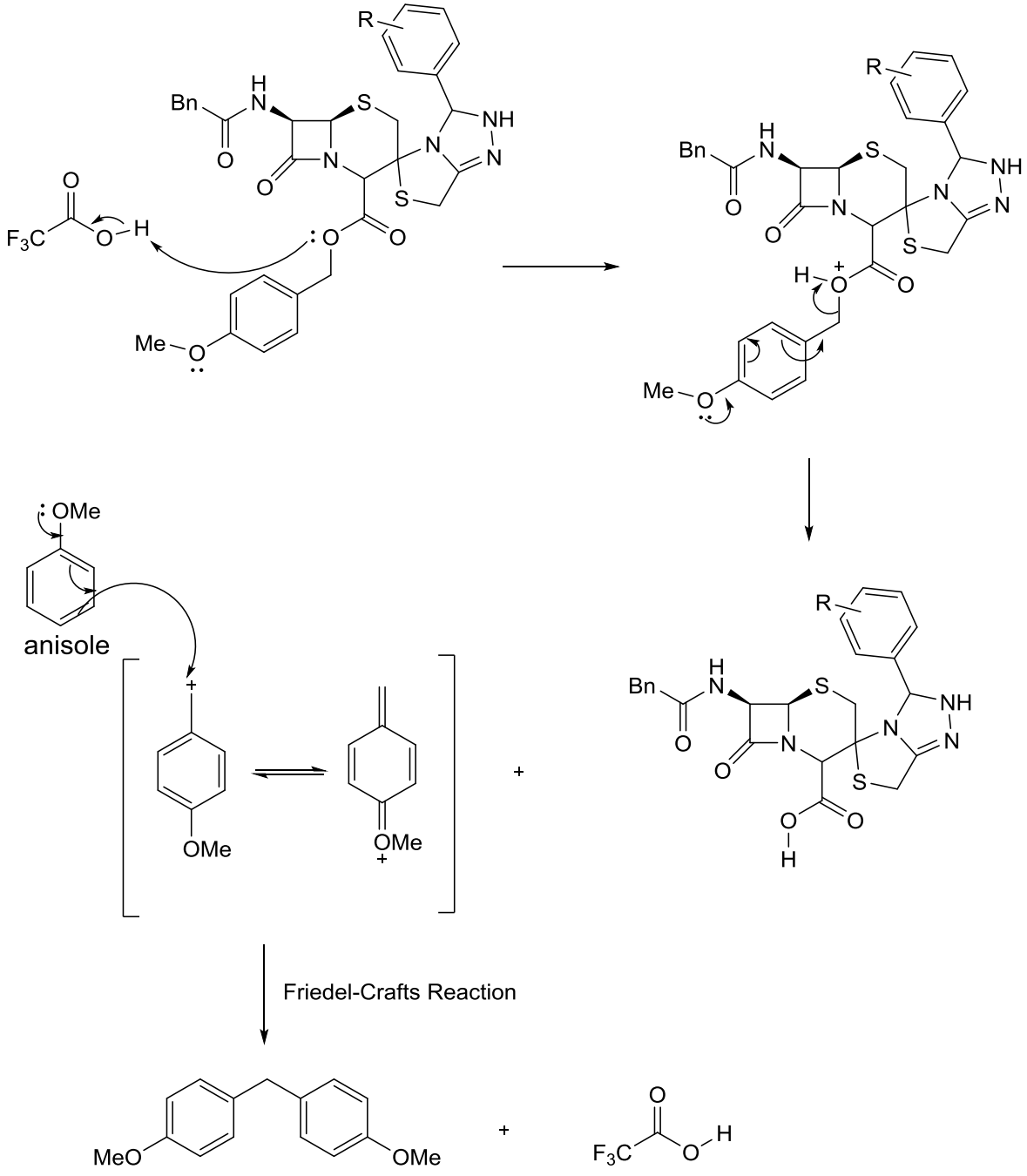
R= H, *o*-Cl, *o*-Br, *o*-OH, *p*-F, *p*-Cl, *p*-Br

Scheme (3-5) PMB deprotection of spiro-cephalosporins

3.3.2. The proposed mechanism

PMB esters were developed explicitly such that the protected carboxylate may be unmasked selectively under acidic conditions. Weygard and Hunger[126,127] were the first to evaluate the stability of the PMB ester in this area and found that PMB esters were readily cleaved from small peptides by exposure to neat trifluoroacetic acid (TFA) at 0 °C. The use of an electron-rich aromatic system as an additive to minimize the formation of side products is common when near stoichiometric amounts of TFA are utilized, with anisole being typically employed for this purpose The nucleophilic additives and/or

solvents are presumed to function as cation scavengers, reacting with the 4-methoxybenzyl cation generated with a Friedel-Craft reaction in the transformation which protects the substrate from side reactions.[122,128]



Scheme (3-6) Mechanism of PMB deprotection of spiro-cephalosporins

3.3.3. Characterization of final spiro-sephalosporins (C1-C7)

The IR spectra of final Spiro-Cephalosporins reveal a strong band at the region (3275-3290) cm^{-1} which is associated with N-H stretching of amide group, while the band at (3143-3186) cm^{-1} belongs to N-H stretching vibration of the triazole ring. Appearance of strong band in the region (1766-1774) and (1662-1666) cm^{-1} indicate the existence of two C=O stretching vibrations. The band at (1577-1616) cm^{-1} representing the presence of C=C stretching, while the band in the region (1527-1535) cm^{-1} belongs to C=N stretching in the heterocyclic ring.

Comparison the ^{1}HNMR spectra of final compounds (**C1**-**C7**) with that of the protected precursors (**B1**-**B7**), reveals the disappearance of 4 signals belonging to the protecting group PMB such as: a singlet at (3.75) ppm of methoxy group, a singlet at (5.16) ppm of CH_2 of benzyl ring, and two signals of CH protons of the benzyl ring in the aromatic region.

Furthermore, appearance of doublet at (9.05-9.10) ppm refers to NH proton of amide group, while appearance of singlet at (8.32-8.52) ppm refers to NH proton of triazole ring.

Methine CH group of triazole ring appears as a singlet at (8.24-8.52) ppm, while aromatic proton signals appear at the region (7.25-7.93) ppm. The characteristic signals of β-lactam ring protons appear as doublet and double doublet at around 5.20 and 5.40 ppm, respectively. By assistance of HSQC spectra, proton of CH group at position 4 appears as singlet at (4.55-4.60) ppm, while the two diastereotopic methylene protons appears as two doublet each at the region (2.95-3.50) ppm.

Similarly, ^{13}CNMR spectra of final spiro-cephalosporins (**C1**-**C7**) exhibit the disappearance of 6 signals comparing with that of precursors (**B1**-**B7**), which include: methoxy group signal at 55.5 ppm, methylene CH_2 signal at 67.6 ppm, three signals in the aromatic region belonging to the benzyl ring, and a signal at 159.5 ppm representing the carbon of the benzyl ring linked methoxy group.[120]

^{13}CNMR spectra show three signals at the region (168.2-170.7) ppm belong to carbon of C=O in carboxylic group, β-lactam and amide group.

In addition, carbon signal of C=N group appears at 164.2 ppm, while the signal of methine group of triazole ring appears at the region (146.8-151.3) ppm. Aromatic signals are found in the region (115.8-135.5) ppm.

Besides, DEPT 135 spectra exhibit three signals in the region (54.7-59.6) ppm which represent the CH groups: two in β-lactam ring and one at position 4, while there are three signals in the region (31.1-42.5) ppm standing for the three CH_2 carbons. Spiro carbon often appears at 59.8 ppm.

Finally, all molecular peaks of intact spiro-cephalosporin molecules (C1-C7) were appeared in high resolution mass spectra, and they are all in agreement with calculated exact mass.

8-oxo-3'-phenyl-7-(2-phenylacetamido)-2',3'-dihydro-6'H-5-thia-1-azaspiro[bicyclo[4.2.0]octane-3,5'-thiazolo[2,3-c][1,2,4]triazole]-2-carboxylic acid (**C1**): 80 mg (50% yield) light brown solid; m.p. 145 – 147 °C; IR (KBr) 3290 (NH), 1774 (C=O), 1666 (C=O), 1577 (C=C), 1531 (C=N) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 9.10 (d, 1H, J=9.0 Hz, N*H*), 8.32 (s, 1H,

N*H*), 8.26 (s, 1H, C*H*), 7.70 (d, 2H, J=6.6 Hz, Ar-*H*), 7.25-7.44 (m, 8H, Ar-*H*), 5.41 (dd, 1H, J_1=9.3 Hz, J_2=4.2 Hz, NHC*H*), 5.20 (d, 1H, J=4.2 Hz, C*H*), 4.56 (s, 1H, C*H*), 3.55 (s, 2H, CH_2), 3.46 (d, 1H, J=13.8 Hz, C*Ha*Hb), 3.39 (d, 1H, J=11.9 Hz, C*Hc*Hd), 3.16 (d, 1H, J=11.9 Hz, CHc*Hd*), 2.96 (d, 1H, J=13.8 Hz, CHa*Hb*). ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 170.3 (C=O), 169.4 (C=O), 168.3 (C=O), 164.2 (C=N), 151.3 (CH), 135.5 (Ar-C), 135.0 (Ar-C), 129.7 (Ar-CH), 129.1 (Ar-CH ×2), 128.7 (Ar-CH×2), 128.4 (Ar-CH ×2),127.0 (Ar-CH ×2), 126.7 (Ar-CH), 59.6 (CH), 57.9 (CH), 54.7 (CH), 42.5 (CH_2), 34.8 (CH_2), 31.1 (CH_2); HRMS (ESI+) *m/z:* (M+H^+) calcd $C_{24}H_{24}N_5O_4S_2^+$ 510.1270 found 510.1261.

3'-(2-chlorophenyl)-8-oxo-7-(2-phenylacetamido)-2',3'-dihydro-6'H-5-thia-1-azaspiro[bicyclo[4.2.0]octane-3,5'-thiazolo[2,3-c][1,2,4]triazole]-2-carboxylic acid (**C2**): 64 mg (46% yield) pale brown solid; m.p. 139-140 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 9.06 (d, 1H, J=9.1 Hz, N*H*), 8.52 (s, 1H, C*H*), 8.45 (s, 1H, N*H*), 7.93 (m, 1H, Ar-*H*), 7.50 (m, 1H, Ar-*H*), 7.41 (m, 2H, Ar-*H*), 7.26-7.33 (m, 5H, Ar-*H*), 5.40 (dd, 1H, J_1=9.2 Hz, J_2=4.1 Hz, NHC*H*), 5.19 (d, 1H, J=4.1 Hz, C*H*), 4.58 (s, 1H, C*H*), 3.56 (s, 2H, CH_2), 3.44 (m, 2H, C*Ha*Hb and C*Hc*Hd), 3.18 (d, 1H, J=11.8 Hz, CHc*Hd*), 2.97 (d, 1H, J=14.0 Hz, CHa*Hb*). ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 170.7 (C=O), 170.3 (C=O), 168.3 (C=O), 164.2 (C=N), 146.8 (CH), 135.4 (Ar-C), 132.8 (Ar-C), 132.0 (Ar-C), 131.1 (Ar-CH), 129.9 (Ar-CH), 129.1 (Ar-CH ×2), 128.4 (Ar-CH ×2), 127.5 (Ar-CH), 127.0 (Ar-CH), 126.7 (Ar-CH), 59.8 (C), 59.6 (CH), 57.8 (CH), 54.7 (CH), 42.5 (CH_2), 34.8 (CH_2), 31.1 (CH_2); HRMS (ESI+) *m/z:* (M+H^+) calcd $C_{24}H_{23}ClN_5O_4S_2^+$ 544.0880, found 544.0871.

2-bromophenyl)-8-oxo-7-(2-phenylacetamido)-2',3'-dihydro-6'H-5-thia-1-azaspiro[bicyclo[4.2.0]octane-3,5'-thiazolo[2,3-c][1,2,4]triazole]-2-carboxylic acid (**C3**): 100 mg (50% yield) dark brown solid; m.p. 170 °C; IR (KBr) 3275 (N-H), 3186 (N-H), 1766 (C=O), 1666 (C=O), 1608 (C=C), 1527 (C=N) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 9.05 (d, 1H, J=9.1 Hz, N*H*), 8.49 (m, 2H, C*H* and N*H*), 7.92 (dd, 1H, J_1=7.7 Hz, J_2=1.5 Hz, Ar-*H*), 7.67 (m, 1H, Ar-*H*), 7.44 (t, 1H, J=7.7 Hz, Ar-*H*), 7.25-7.35 (m, 6H, Ar-*H*), 5.40 (dd, 1H, J_1=9.2 Hz, J_2=4.1 Hz, NHC*H*), 5.20 (d, 1H, J=4.1 Hz, C*H*), 4.58 (s, 1H, C*H*), 3.56 (s, 2H, CH_2), 3.44 (m, 2H, C*Ha*Hb and C*Hc*Hd), 3.20 (d, 1H, J=11.8 Hz, CHc*Hd*), 2.98 (d, 1H, J=13.8 Hz, CHa*Hb*). ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 170.6 (C=O), 170.3 (C=O), 168.3 (C=O), 164.2 (C=N), 149.3 (CH), 135.4 (Ar-C), 133.4 (Ar-C), 133.2 (Ar-CH), 131.4 (Ar-CH), 129.1 (Ar-CH ×2), 128.4 (Ar-CH ×2),128.1 (Ar-CH), 127.3 (Ar-CH), 126.7 (Ar-CH), 123.3 (Ar-C), 59.8 (C), 59.6 (CH), 57.8 (CH), 54.7 (CH), 42.5 (CH_2), 34.8 (CH_2), 31.1 (CH_2); HRMS (ESI+) *m/z:* ($M+H^+$) calcd $C_{24}H_{23}BrN_5O_4S_2^+$ 588.0375, found 588.0364.

3'-(2-hydroxyphenyl)-8-oxo-7-(2-phenylacetamido)-2',3'-dihydro-6'H-5-thia-1-azaspiro[bicyclo[4.2.0]octane-3,5'-thiazolo[2,3-c][1,2,4]triazole]-2-carboxylic acid (**C4**): 70 mg (50% yield) dark brown solid; m.p. 160-162 °C; IR (KBr) 3633 (O-H), 3275 (N-H), 1770 (C=O), 1666 (C=O), 1616.35(C=C), 1527 (C=N) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 11.18 (s, 1H, O*H*), 9.07 (d, 1H, J=9.2 Hz, N*H*), 8.46 (s, 2H, C*H* and N*H*), 7.48 (dd, 1H, J_1=7.8 Hz, J_2=1.4 Hz, Ar-*H*), 7.25-7.35 (m, 6H, Ar-*H*), 6.91 (m, 2H, Ar-*H*), 5.40 (dd, 1H, J_1=9.2 Hz, J_2=4.1 Hz, NHC*H*), 5.18 (d, 1H, J=4.1 Hz, C*H*), 4.60 (s, 1H, C*H*), 3.56 (s, 2H, CH_2), 3.50 (d, 1H, J=11.9 Hz, C*Ha*Hb), 3.45 (d, 1H, J=13.8 Hz, C*Hc*Hd), 3.26 (d, 1H, J=11.9 Hz, CHc*Hd*), 2.98 (d, 1H, J=13.8 Hz,

C*Ha*Hb). ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 170.3 (C=O), 168.4 (C=O), 168.2 (C=O), 164.2 (C=N), 157.8 (Ar-C), 153.7 (CH), 135.5 (Ar-C), 131.1 (Ar-CH), 130.5 (Ar-CH), 129.2 (Ar-CH ×2), 128.4 (Ar-CH ×2), 126.7 (Ar-CH), 119.4 (Ar-CH), 118.8 (Ar-C), 116.2 (Ar-CH), 60.0 (C), 59.6 (CH), 57.8 (CH), 54.7 (CH), 42.5 (CH_2), 35.2 (CH_2), 31.0 (CH_2); HRMS (ESI+) *m/z:* ($M+H^+$) calcd $C_{24}H_{24}N_5O_5S_2^+$ 526.1219, found 526.1212.

3'-(4-fluorophenyl)-8-oxo-7-(2-phenylacetamido)-2',3'-dihydro-6'H-5-thia-1-azaspiro[bicyclo[4.2.0]octane-3,5'-thiazolo[2,3-c][1,2,4]triazole]-2-carboxylic acid (**C5**): 57 mg (64% yield) dark brown solid; m.p. 138-139 °C; IR (KBr) 3402 (O-H), 3290 (N-H), 3143 (N-H), 1770 (C=O), 1604 (C=C), 1535 (C=N) cm^{-1}; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 9.10 (d, 1H, J=9.0 Hz, N*H*), 8.32 (s, 1H, N*H*), 8.26 (s, 1H, C*H*), 7.74 (m, 2H, Ar-*H*), 7.25-7.33 (m, 7H, Ar-*H*), 5.40 (dd, 1H, J_1=9.1 Hz, J_2=4.1 Hz, NHC*H*), 5.20 (d, 1H, J=4.1 Hz, C*H*), 4.56 (s, 1H, C*H*), 3.55 (s, 2H, CH_2), 3.46 (d, 1H, J=13.9 Hz, C*Ha*Hb), 3.39 (d, 1H, J=11.9 Hz, C*Hc*Hd), 3.16 (d, 1H, J=11.9 Hz, CHc*Hd*), 2.95 (d, 1H, J=13.9 Hz, CHa*Hb*). ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 170.3 (C=O), 169.4 (C=O), 168.3 (C=O), 164.2 (C=N), 161.7 (Ar-C), 150.1 (CH), 135.5 (Ar-C), 131.7 (Ar-C), 129.1 (Ar-CH ×2), 129.0 (Ar-CH ×2), 128.4 (Ar-CH ×2), 126.7 (Ar-CH), 115.8 (d, J=21.8 Hz, Ar-CH ×2), 59.8 (C), 59.6 (CH), 57.8 (CH), 54.7 (CH), 42.5 (CH_2), 34.8 (CH_2), 31.1 (CH_2); HRMS (ESI+) *m/z:* ($M+H^+$) calcd $C_{24}H_{23}FN_5O_4S_2^+$ 528.1175, found 528.1167.

3'-(4-chlorophenyl)-8-oxo-7-(2-phenylacetamido)-2',3'-dihydro-6'H-5-thia-1-azaspiro[bicyclo[4.2.0]octane-3,5'-thiazolo[2,3-c][1,2,4]triazole]-2-carboxylic acid (**C6**): 95 mg (59.3% yield) dark brown solid; m.p. 150-153 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 9.10 (d, 1H, J=9.2 Hz, N*H*), 8.36 (s, 1H, N*H*), 8.25 (s, 1H, C*H*), 7.71 (d, 2H, J=8.6 Hz, Ar-*H*), 7.49 (d, 2H, J=8.6, Ar-*H*),

7.25-7.33 (m, 5H, Ar-*H*), 5.40 (dd, 1H, J_1=9.0 Hz, J_2=4.2 Hz, NHC*H*), 5.19 (d, 1H, J=4.2 Hz, C*H*), 4.56 (s, 1H, C*H*), 3.55 (s, 2H, CH_2), 3.46 (d, 1H, J=13.9 Hz, C*Ha*Hb), 3.39 (d, 1H, J=12.0 Hz, C*Hc*Hd), 3.16 (d, 1H, J=12.0 Hz, CHc*Hd*), 2.96 (d, 1H, J=13.9 Hz, CHa*Hb*). ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 170.3 (C=O), 169.8 (C=O), 168.3 (C=O), 164.2 (C=N), 150.0 (CH), 135.5 (Ar-C), 134.0 (Ar-C), 133.9 (Ar-C), 129.1 (Ar-CH ×2), 128.8 (Ar-CH ×2), 128.6 (Ar-CH ×2), 128.4 (Ar-CH ×2), 126.7 (Ar-CH), 59.7 (C), 59.6 (CH), 57.8 (CH), 54.7 (CH), 42.5 (CH_2), 34.8 (CH_2), 31.1 (CH_2); HRMS (ESI+) *m/z:* (M+H^+) calcd $C_{24}H_{23}ClN_5O_4S_2^+$ 544.0880, found 544.0873.

3'-(4-bromophenyl)-8-oxo-7-(2-phenylacetamido)-2',3'-dihydro-6'H-5-thia-1-azaspiro[bicyclo[4.2.0]octane-3,5'-thiazolo[2,3-c][1,2,4]triazole]-2-carboxylic acid (**C7**): 43mg (80% yield) orange solid; m.p. 168-170 °C; ^{1}H-NMR (400 MHz, DMSO-d_6): ppm δ 9.10 (d, 1H, J=9.0 Hz, N*H*), 8.35 (s, 1H, N*H*), 8.24 (s, 1H, C*H*), 7.63 (s, 4H, Ar-*H*), 7.25-7.33 (m, 5H, Ar-*H*), 5.40 (dd, 1H, J_1=9.1 Hz, J_2=4.2 Hz, NHC*H*), 5.19 (d, 1H, J=4.2 Hz, C*H*), 4.55 (s, 1H, C*H*), 3.55 (s, 2H, CH_2), 3.45 (d, J=13.9 Hz, 1H, C*Ha*Hb), 3.39 (d, 1H, J=12.1 Hz, C*Hc*Hd), 3.16 (d, 1H, J=12.1 Hz, CHc*Hd*), 2.95 (d, 1H, J=13.9 Hz, CHa*Hb*). ^{13}C-NMR (100 MHz, DMSO-d_6): ppm δ 170.3 (C=O), 169.8 (C=O), 168.3 (C=O), 164.2 (C=N), 150.1 (CH), 135.5 (Ar-C), 134.3 (Ar-C), 131.7 (Ar-CH ×2), 129.1 (Ar-CH ×2), 128.8 (Ar-CH ×2), 128.4 (Ar-CH ×2), 126.7 (Ar-CH), 122.8 (Ar-C), 59.6 (CH), 57.8 (CH), 54.7 (CH), 42.5 (CH_2), 34.8 (CH_2), 31.1 (CH_2); HRMS (ESI+) *m/z:* (M+H^+) calcd $C_{24}H_{24}BrN_5O_4S_2^+$ 588.0375, found 588.0368.

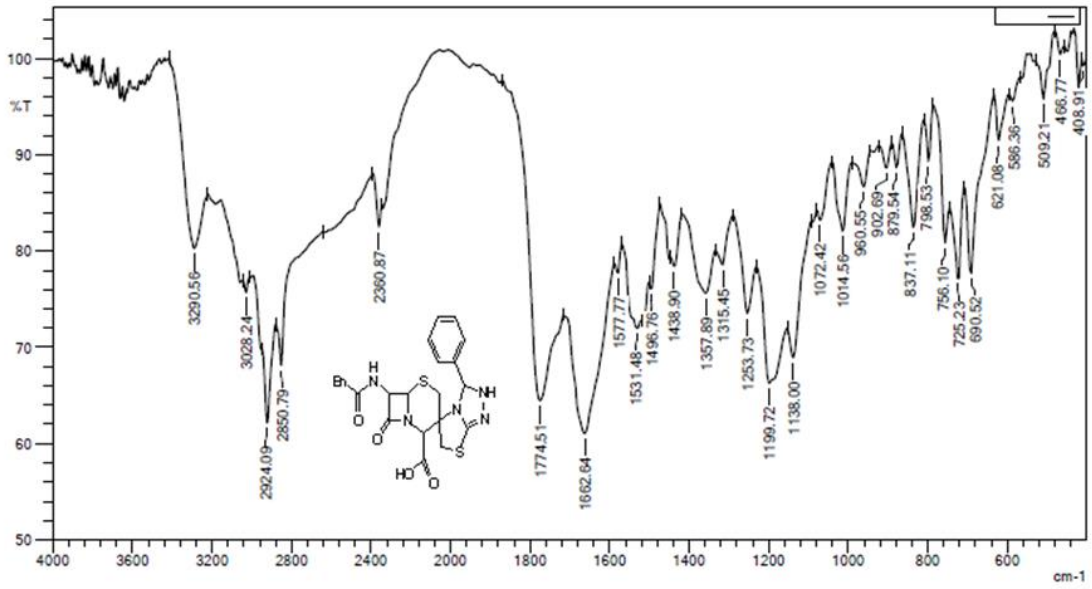


Fig (3-50) IR Spectrum of the compound C1

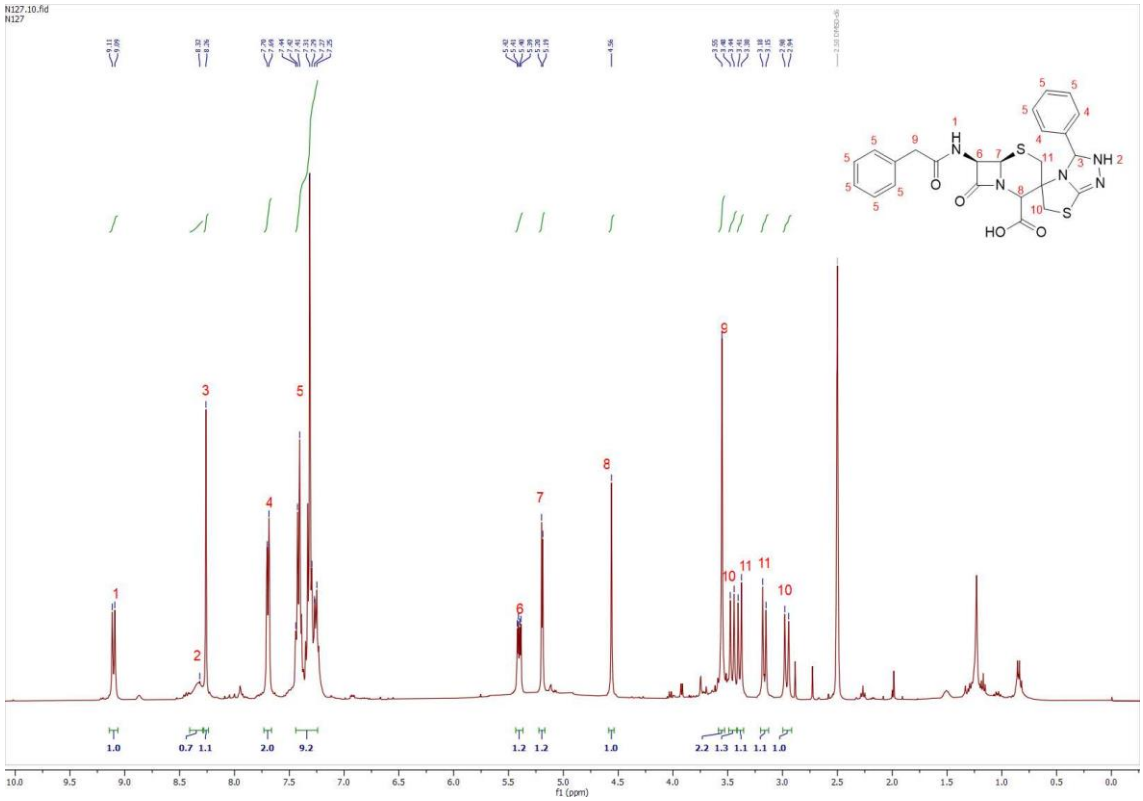


Fig (3-51) ^{1}HNMR Spectrum of the compound C1

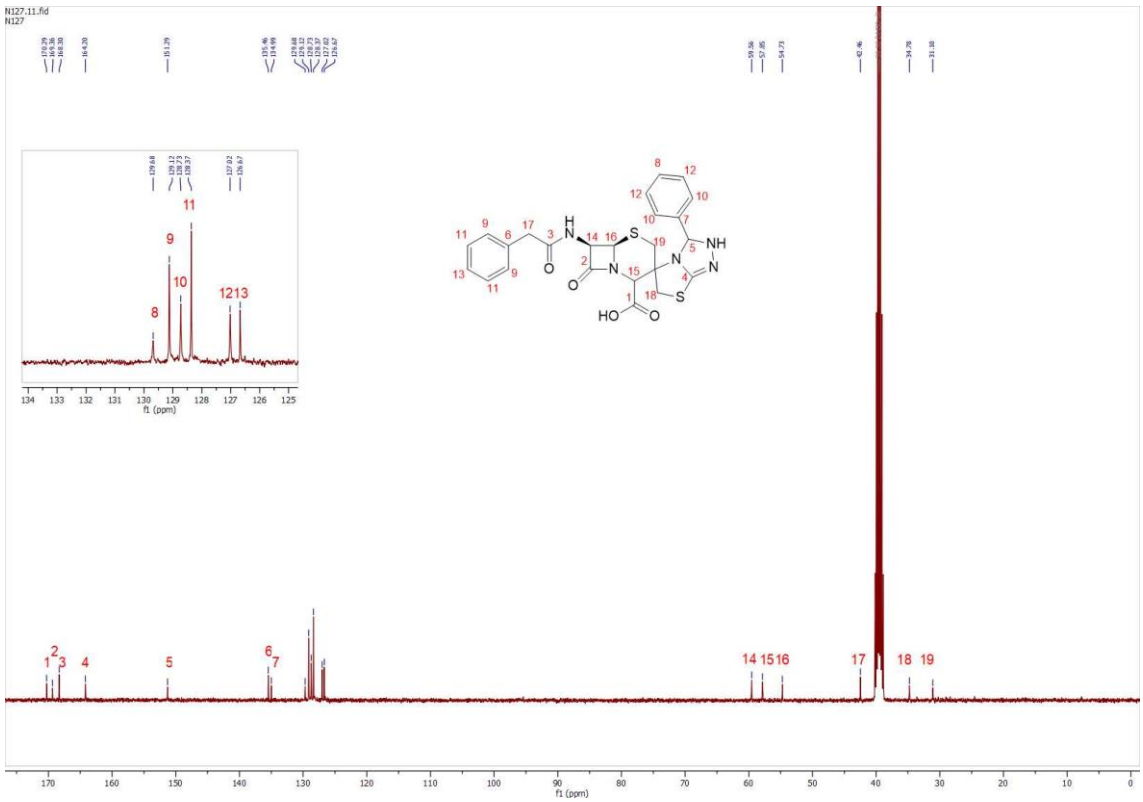


Fig (3-52) ^{13}CNMR Spectrum of the compound C1

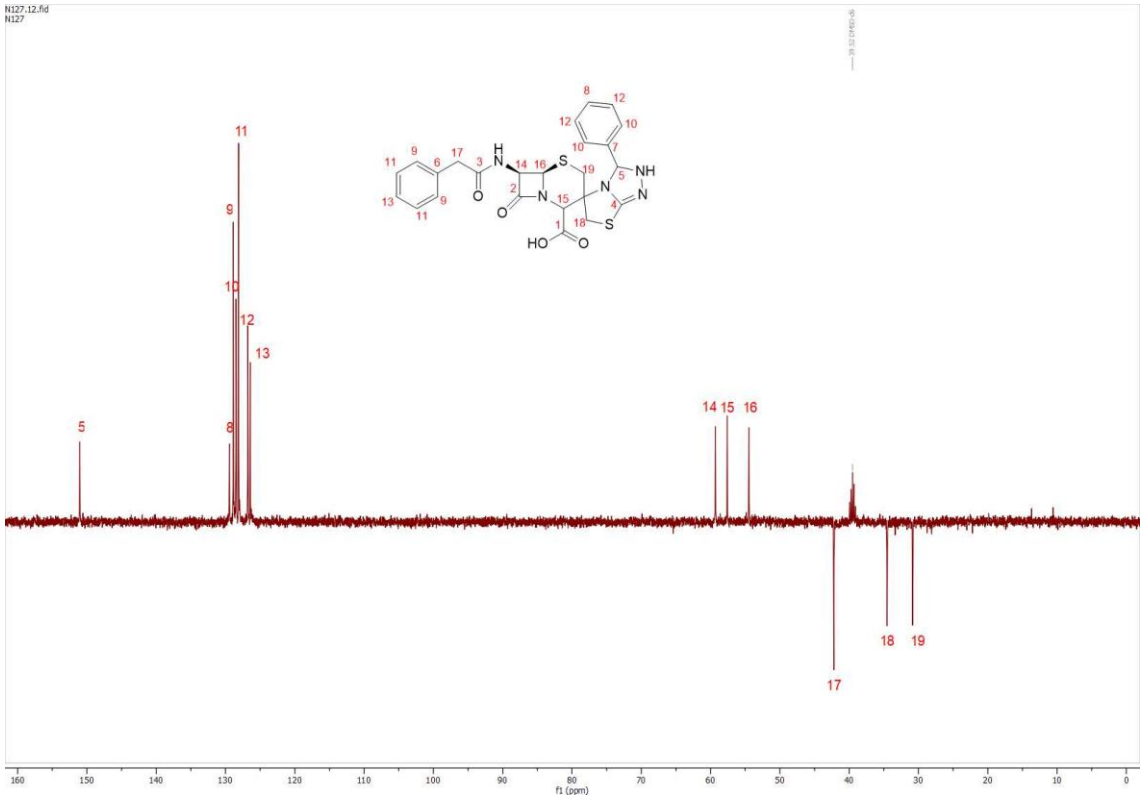


Fig (3-53) DEPT 135 Spectrum of the compound C1

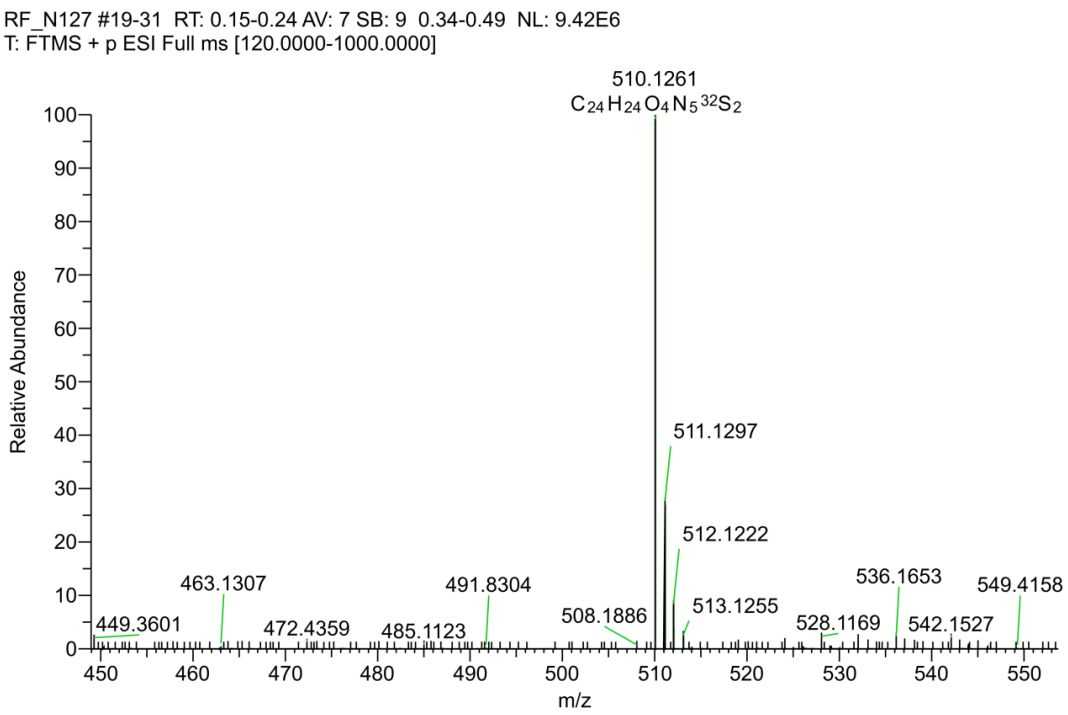


Fig (3-54) Mass Spectrum (ESI) of the compound C1

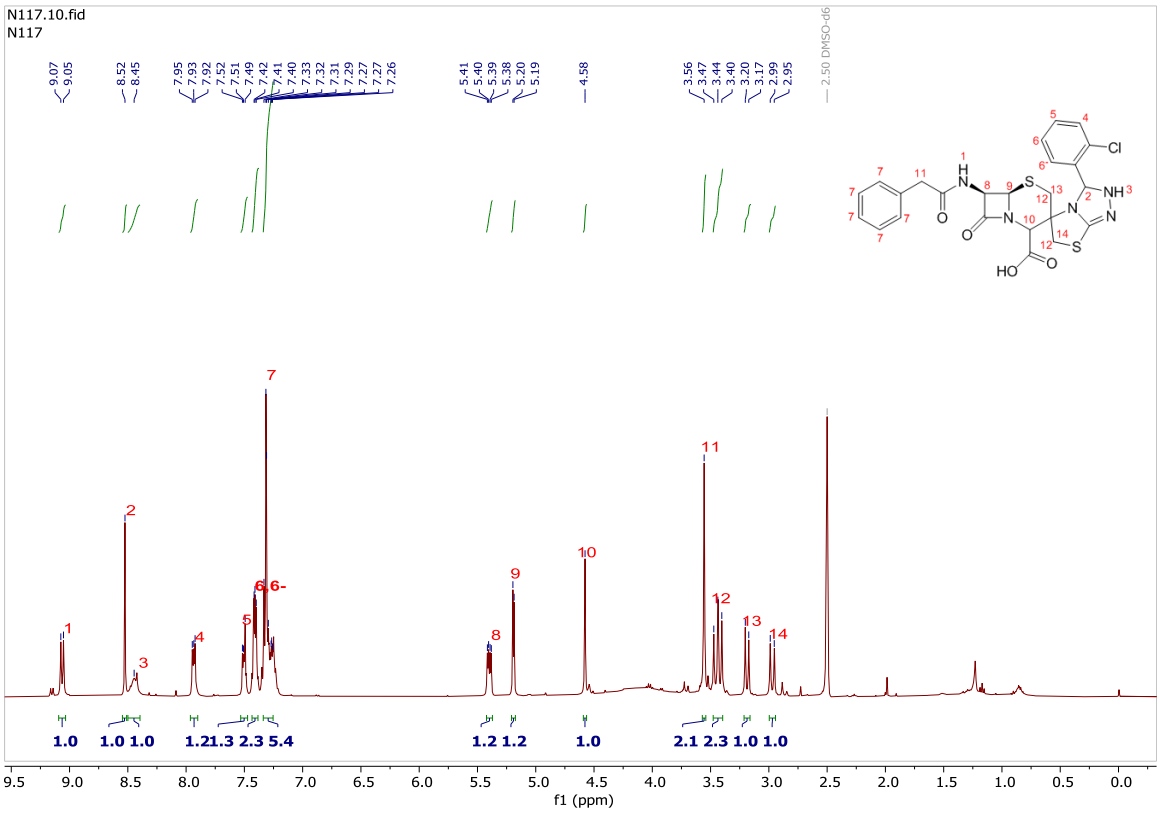


Fig (3-55) ^{1}HNMR Spectrum of the compound C2

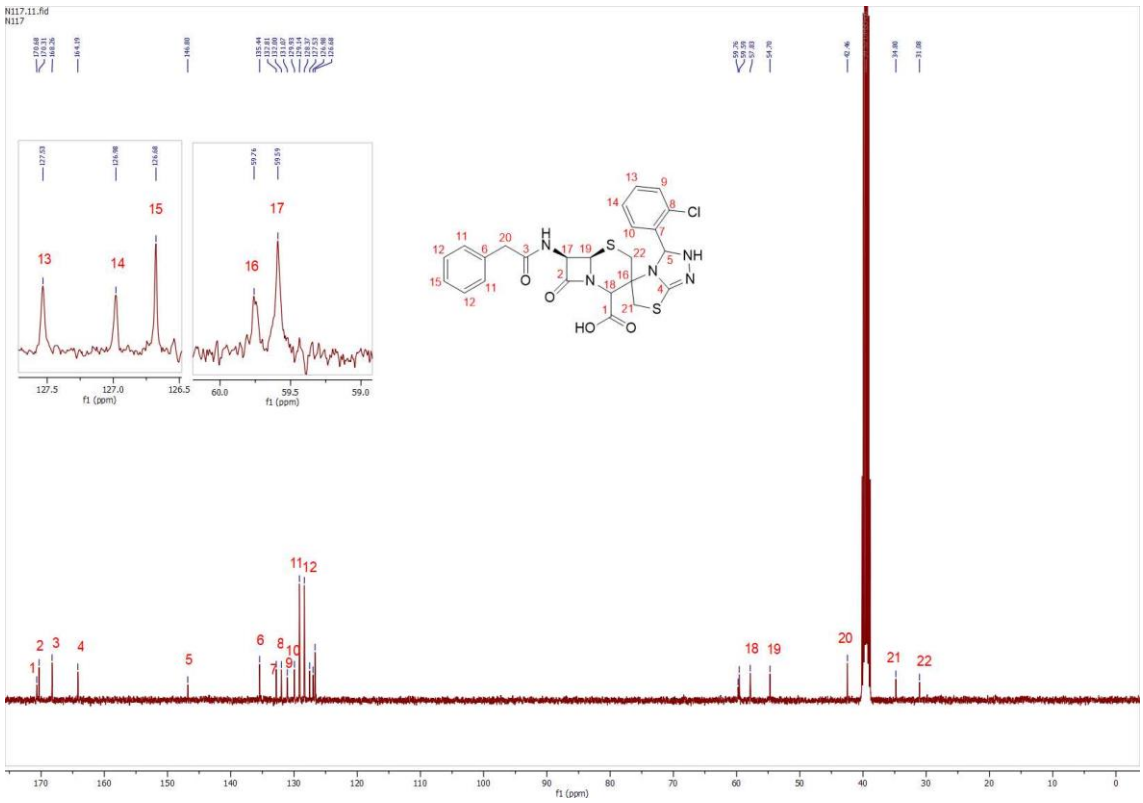


Fig (3-56) ^{13}CNMR Spectrum of the compound C2

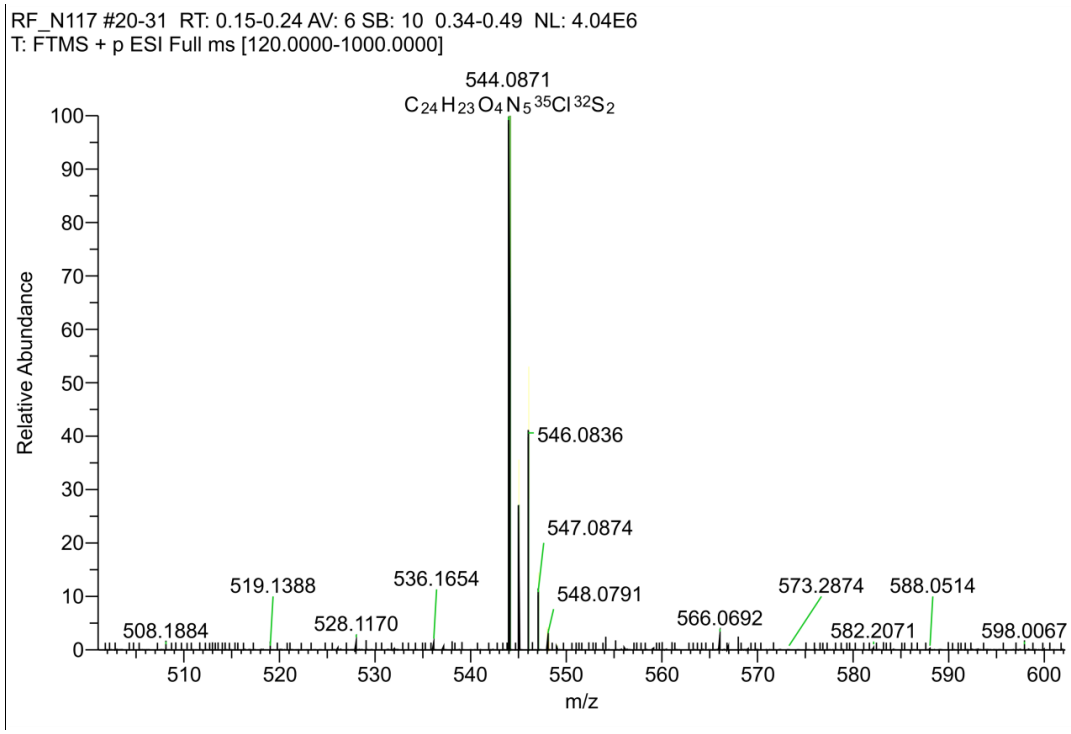


Fig (3-57) Mass Spectrum (ESI) of the compound C2

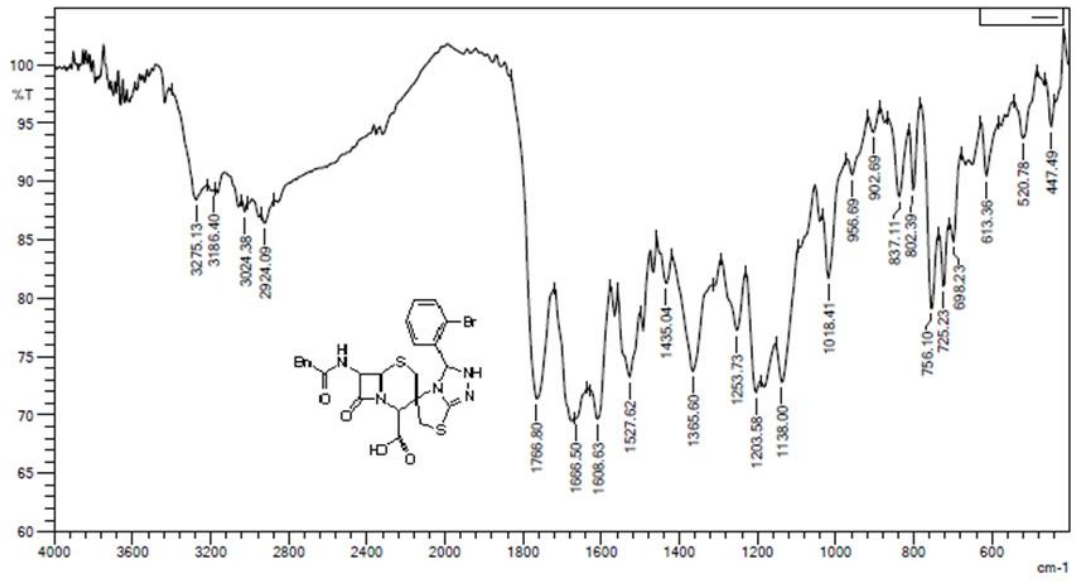


Fig (3-58) IR Spectrum of the compound C3

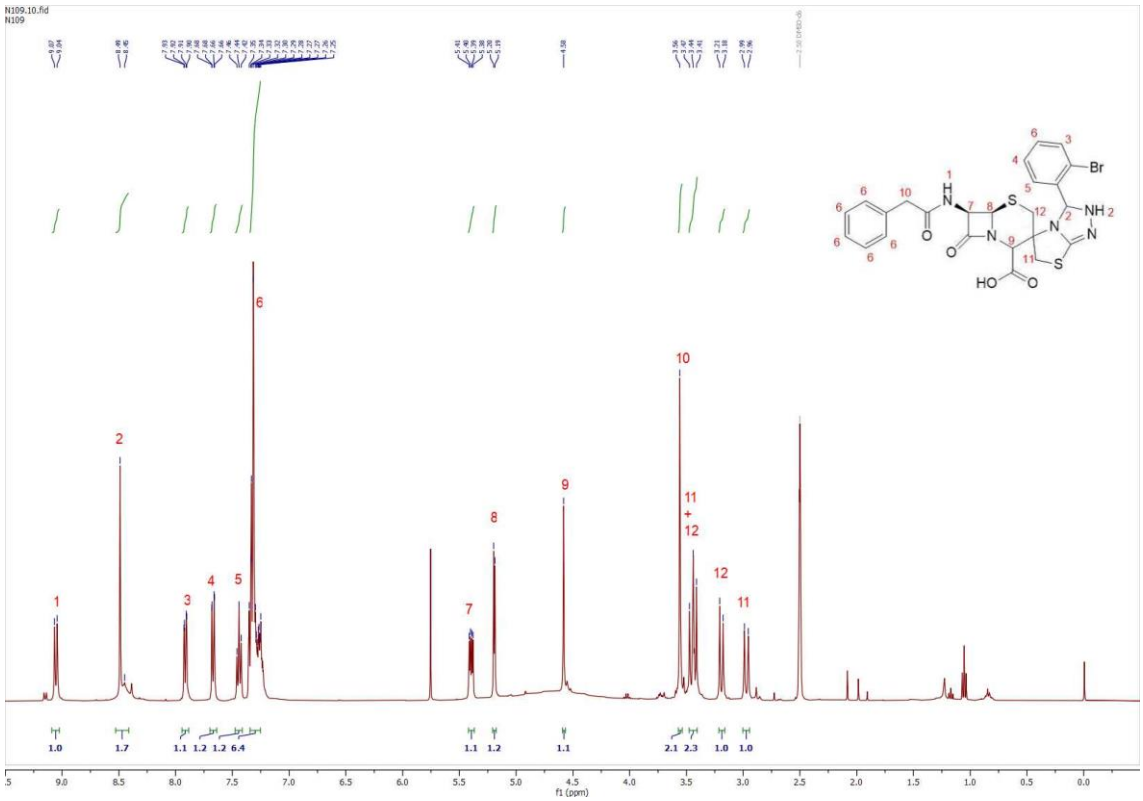


Fig (3-59) ^{1}HNMR Spectrum of the compound C3

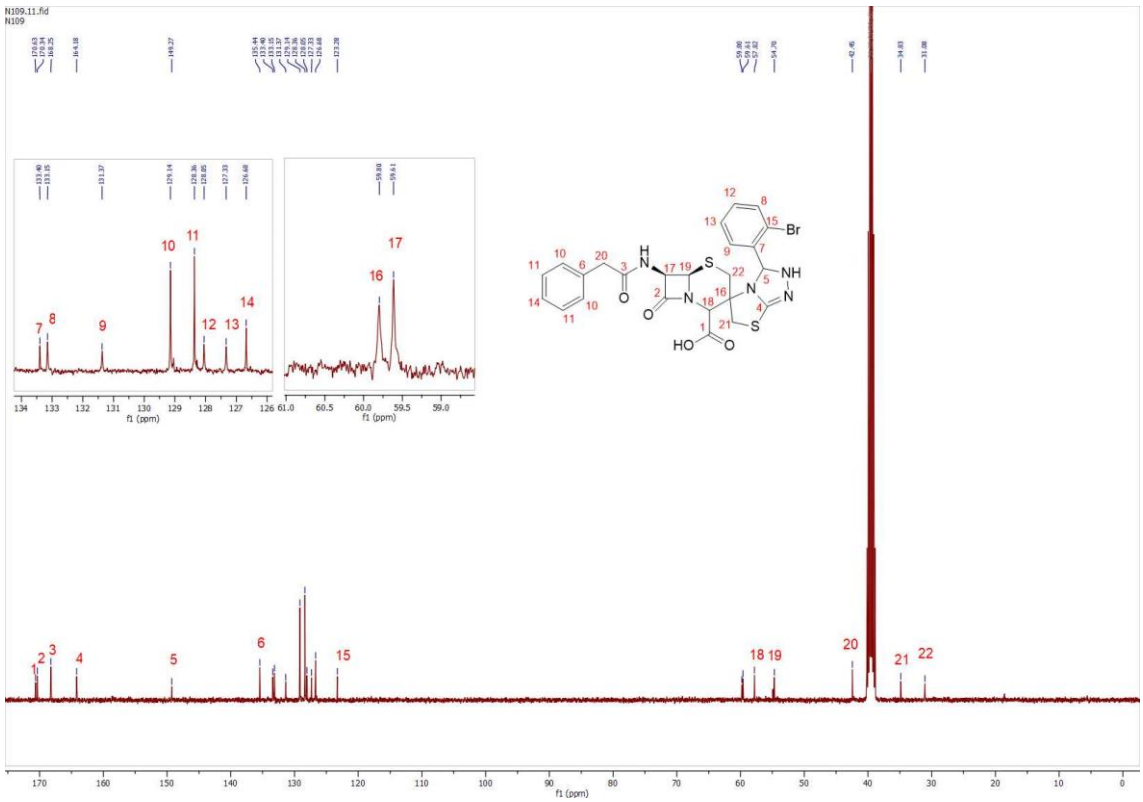


Fig (3-60) ^{13}CNMR Spectrum of the compound C3

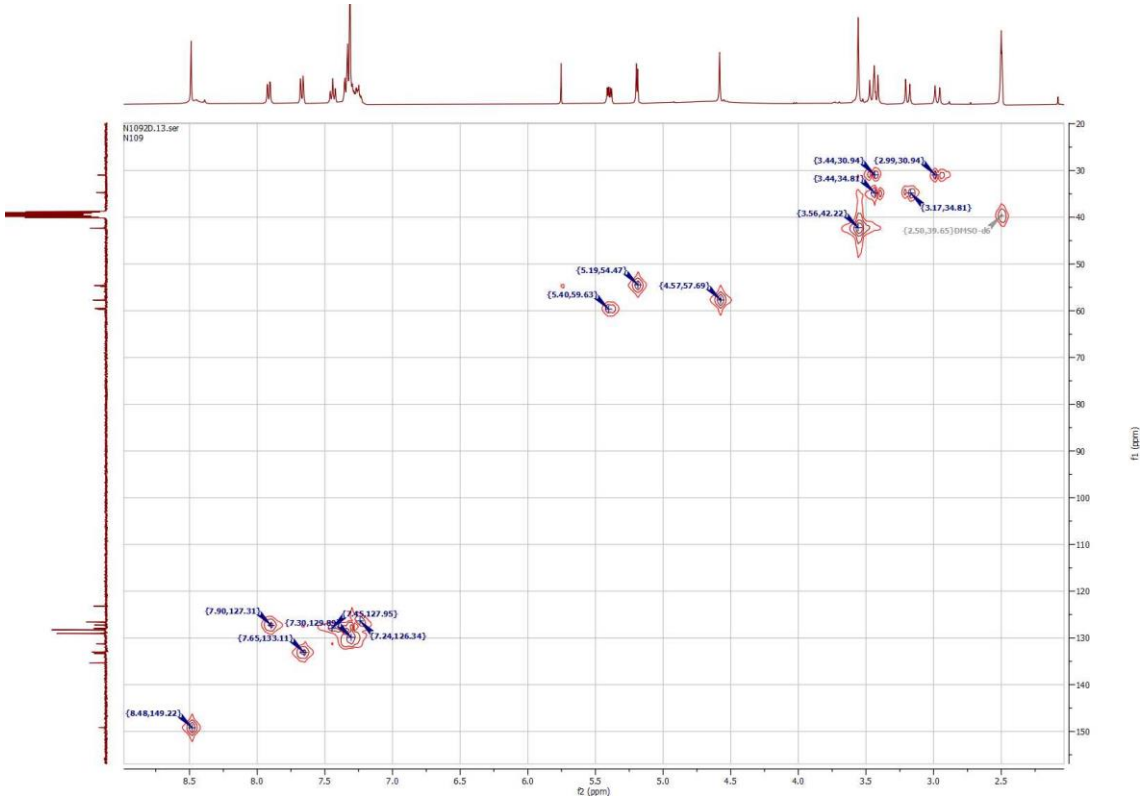


Fig (3-61) HSQC Spectrum of the compound C3

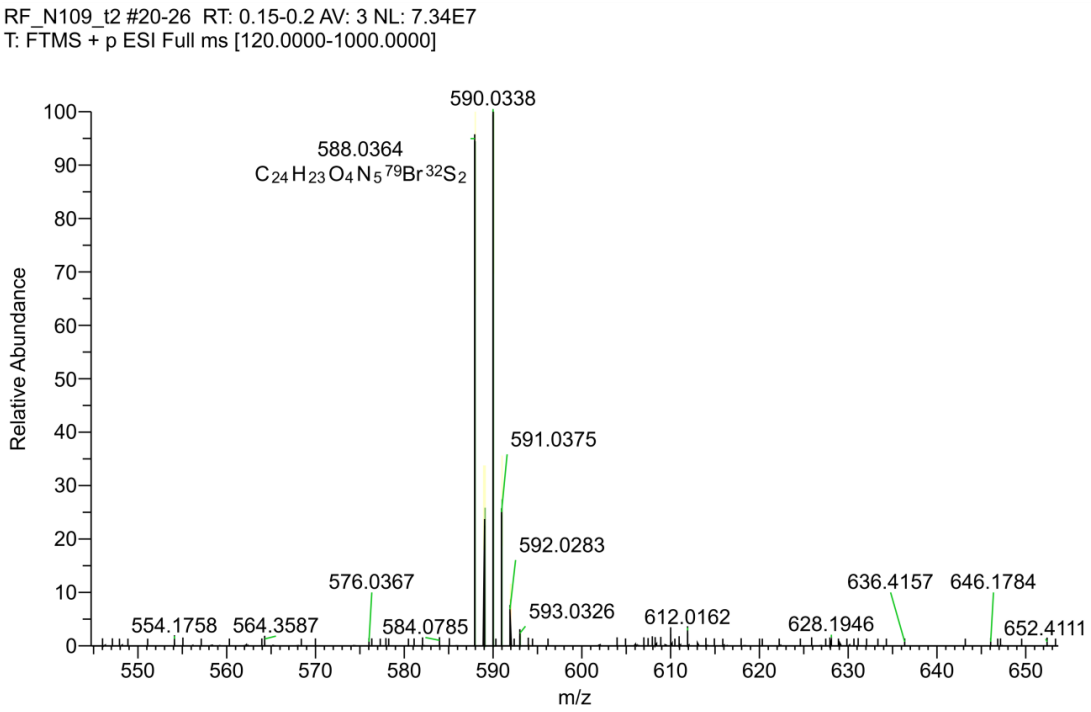


Fig (3-62) Mass Spectrum (ESI) of the compound C3

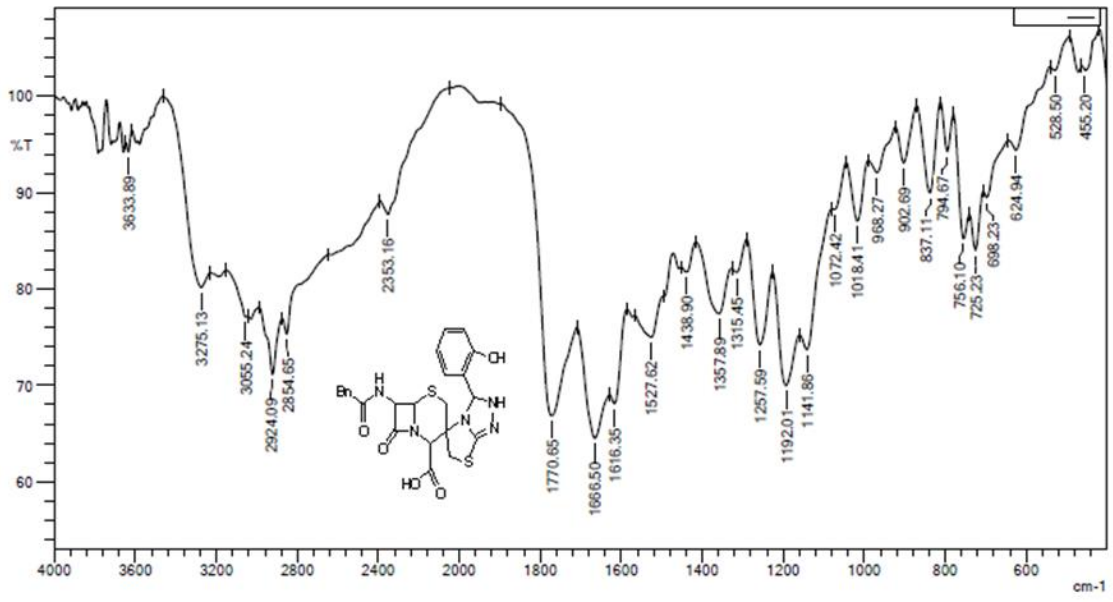


Fig (3-63) IR Spectrum of the compound C4

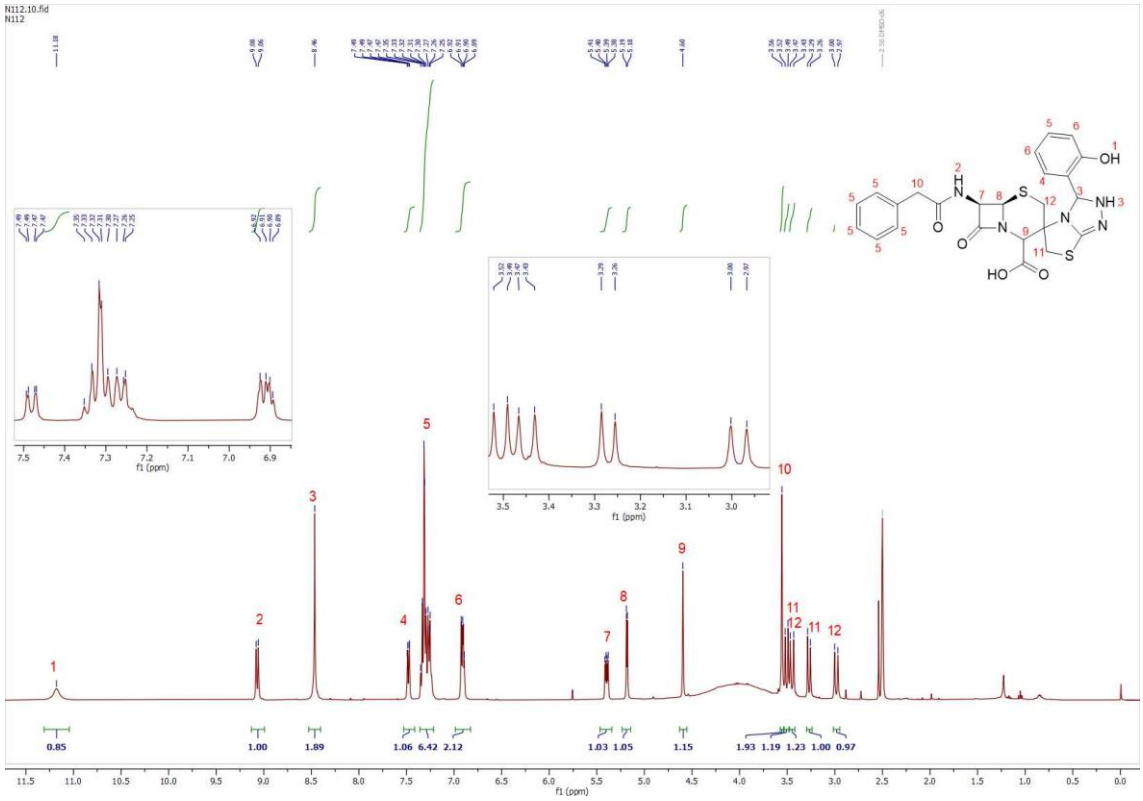


Fig (3-64) ^{1}HNMR Spectrum of the compound C4

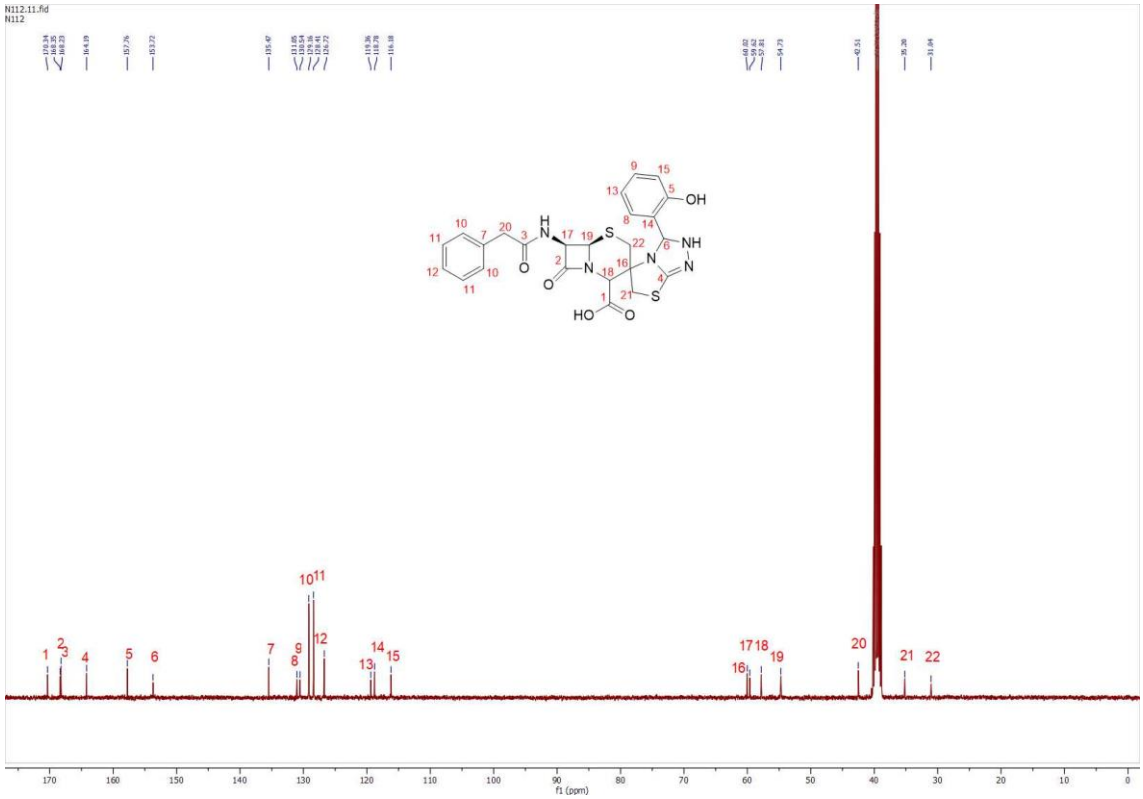


Fig (3-65) ^{13}CNMR Spectrum of the compound C4

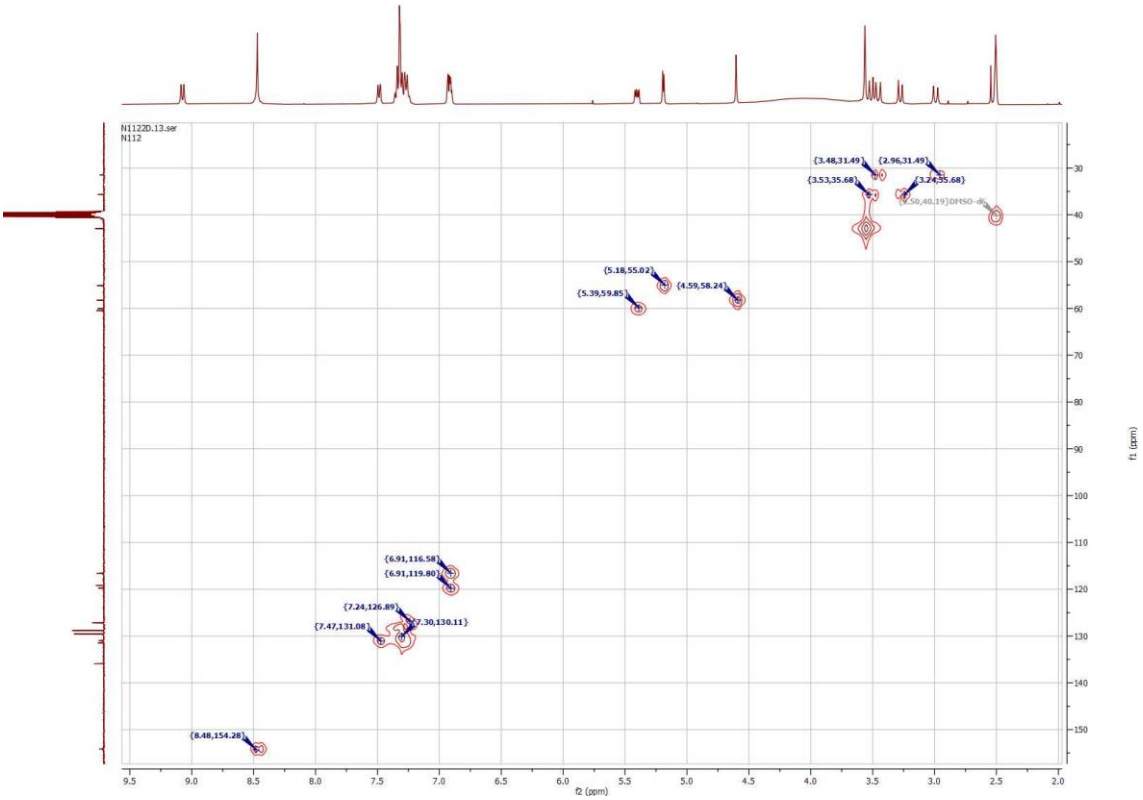


Fig (3-66) HSQC Spectrum of the compound C4

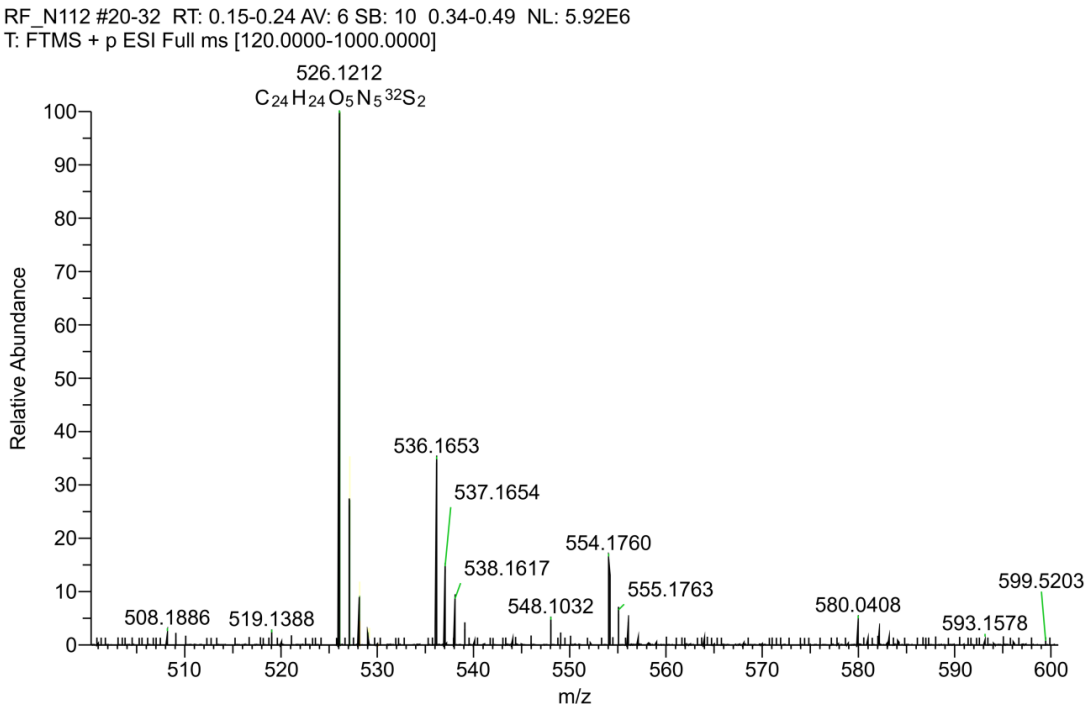


Fig (3-67) Mass Spectrum (ESI) of the compound C4

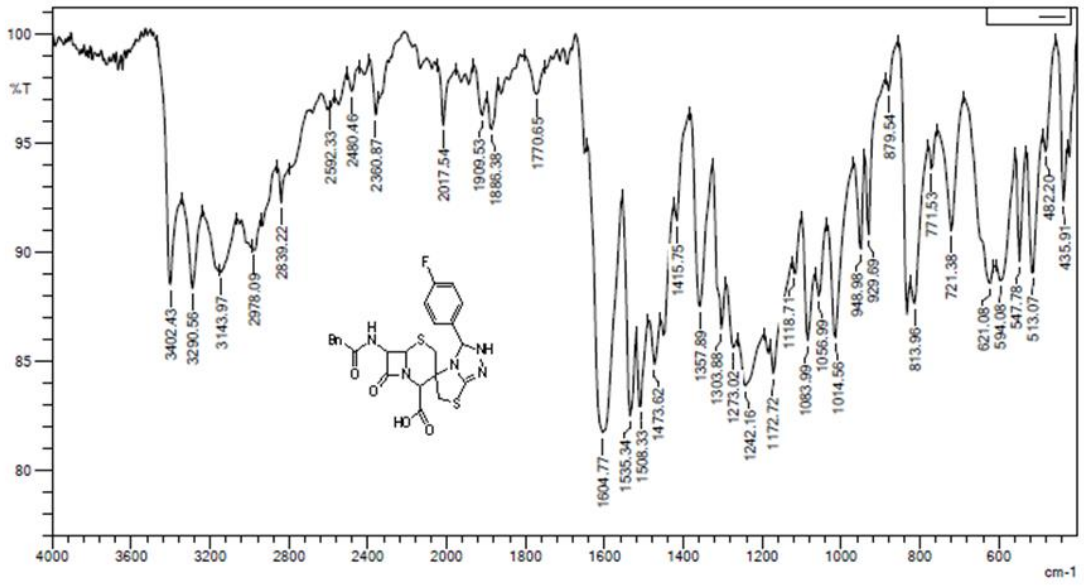


Fig (3-68) IR Spectrum of the compound C5

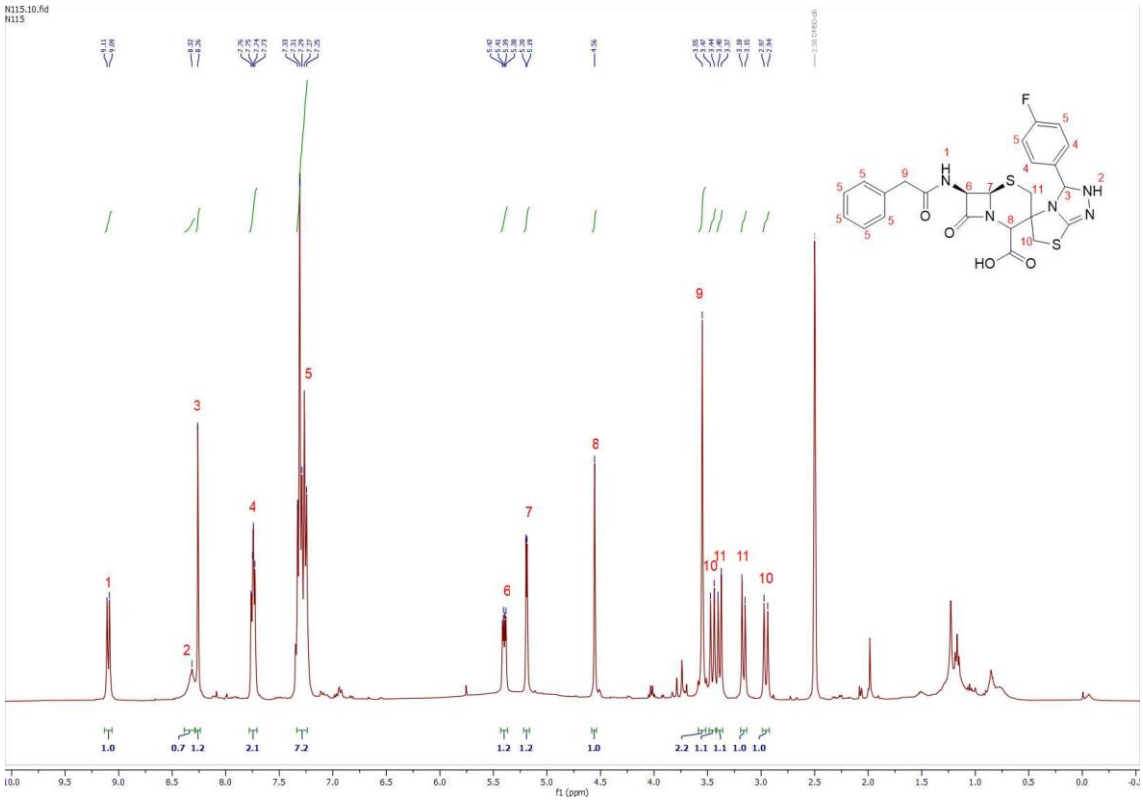


Fig (3-69) ^{1}HNMR Spectrum of the compound C5

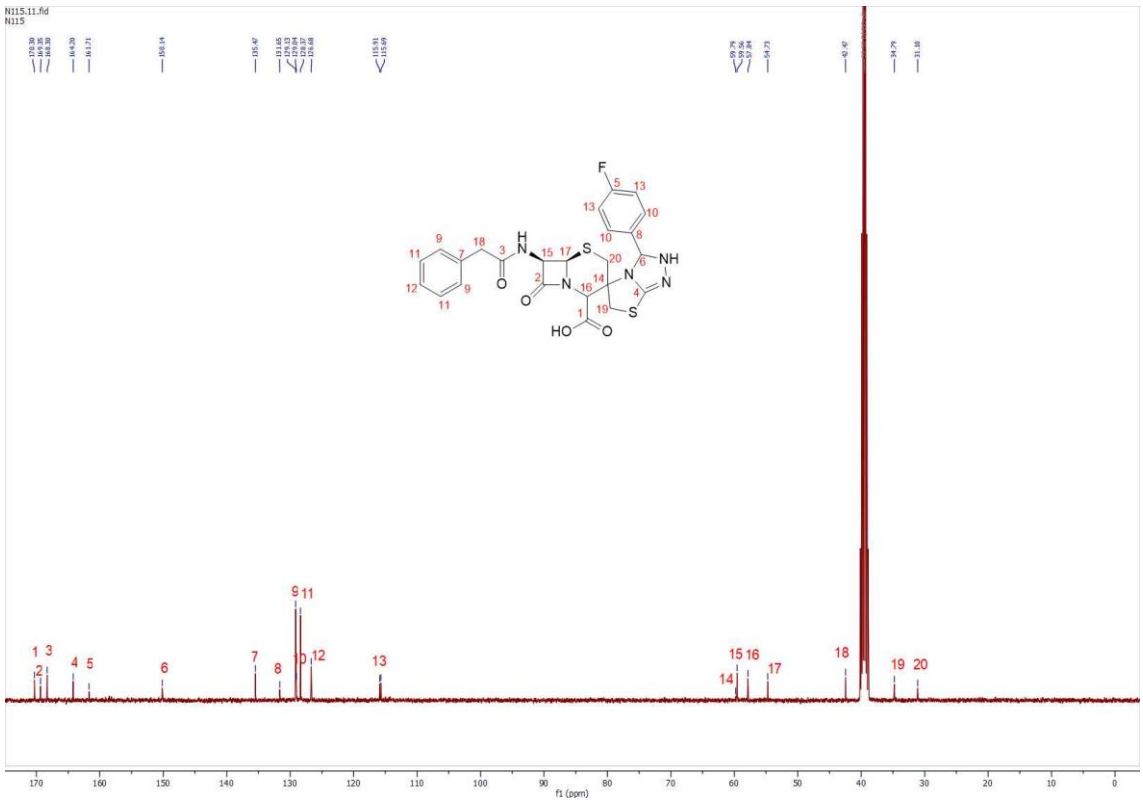


Fig (3-70) ^{13}CNMR Spectrum of the compound C5

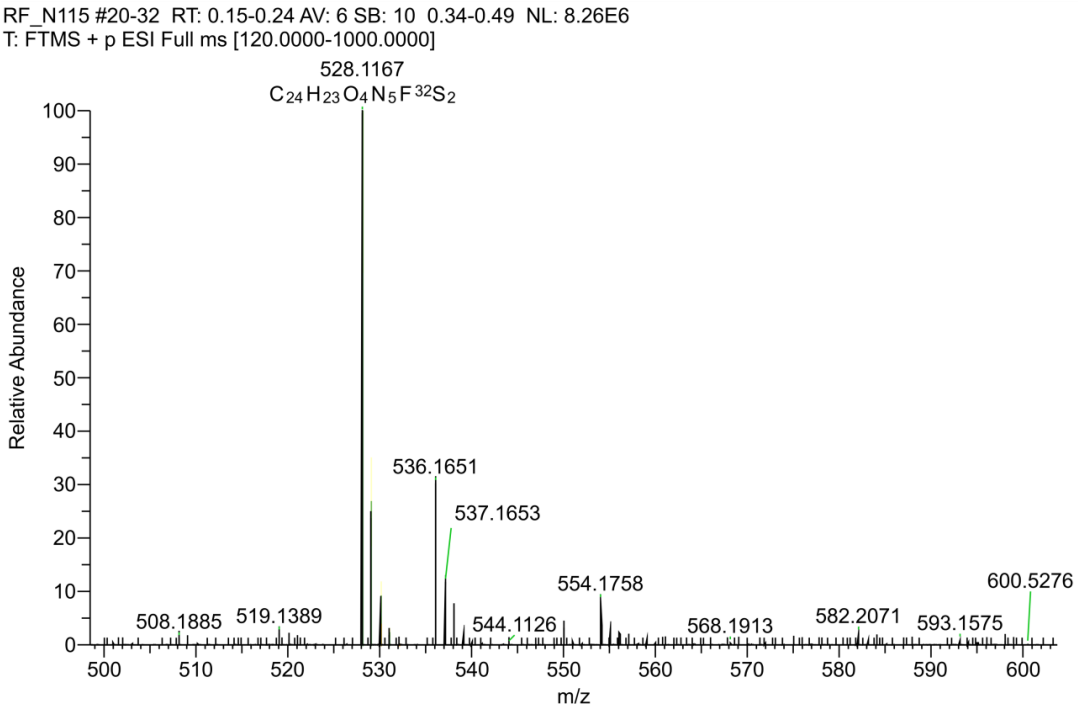


Fig (3-71) Mass Spectrum (ESI) of the compound C5

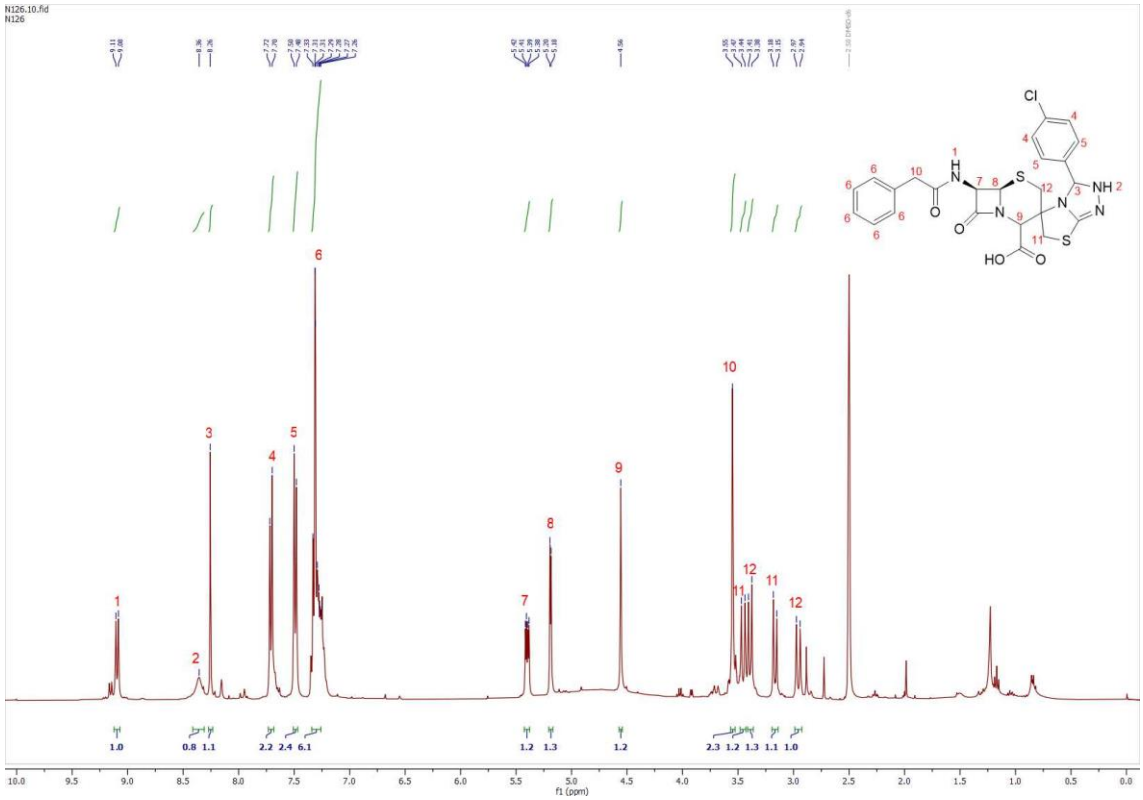


Fig (3-72) ^{1}HNMR Spectrum of the compound C6

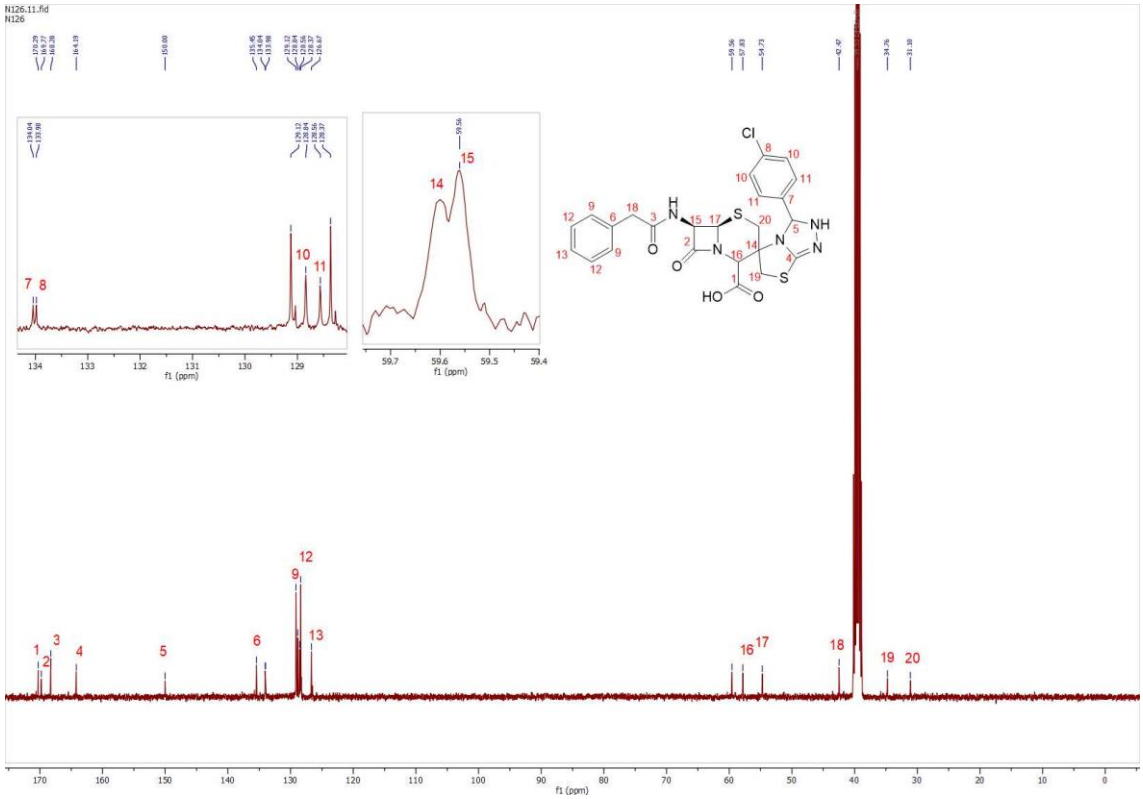


Fig (3-73) ^{13}CNMR Spectrum of the compound C6

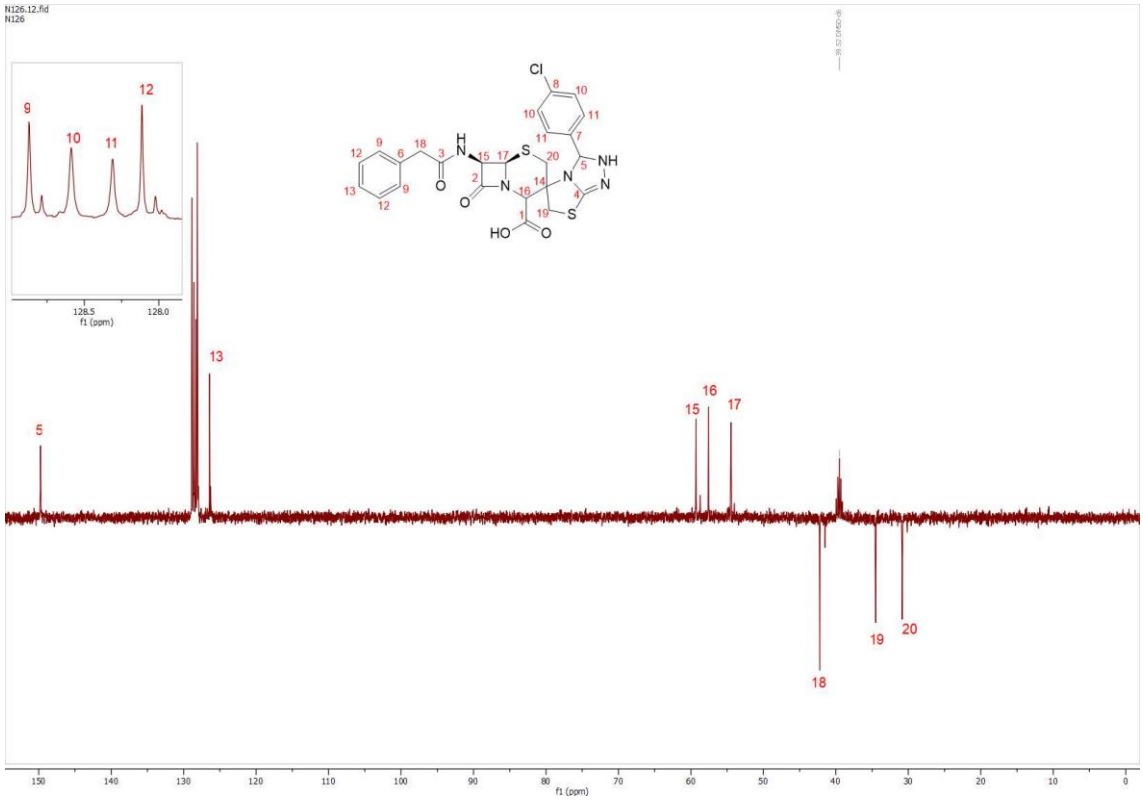


Fig (3-74) DEPT 135 Spectrum of the compound C6

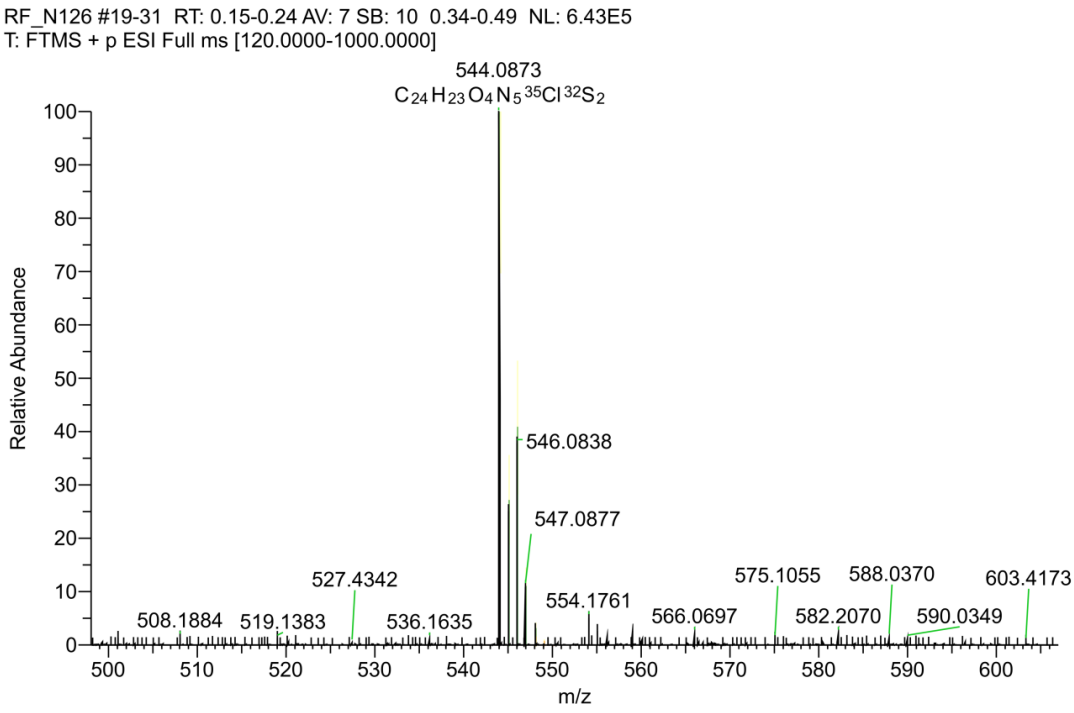


Fig (3-75) Mass Spectrum (ESI) of the compound C6

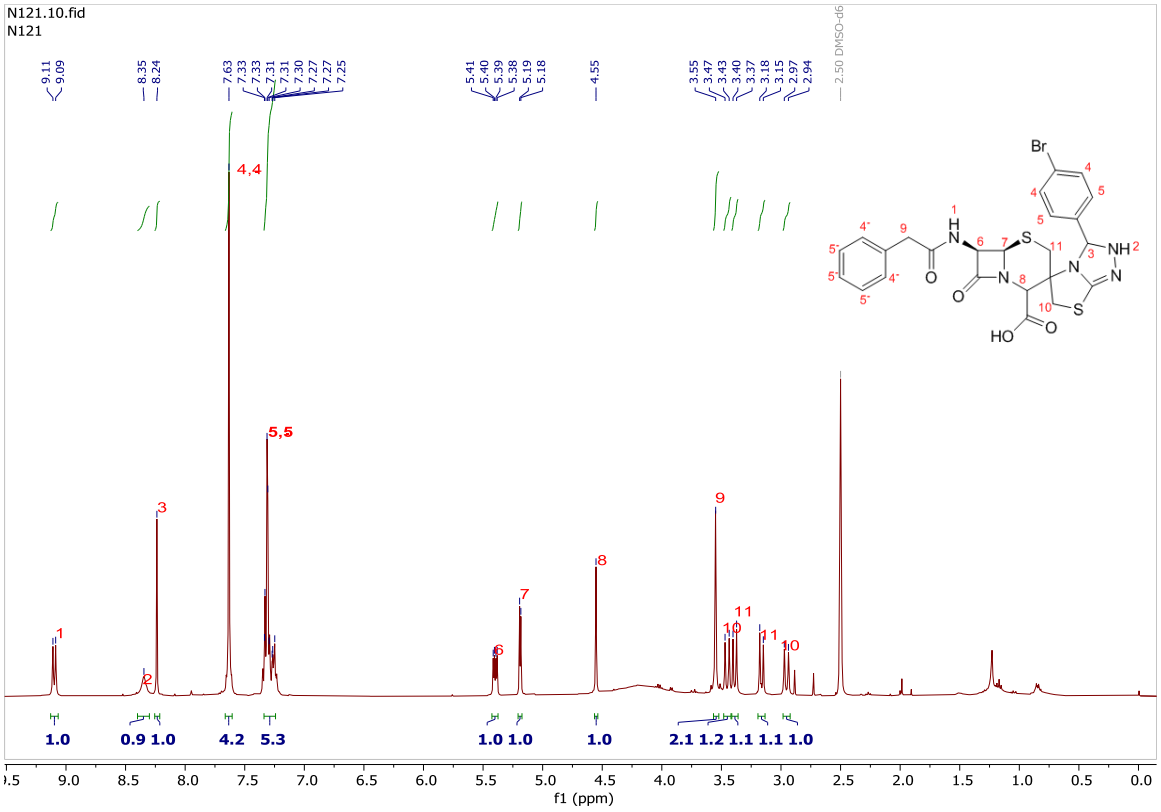


Fig (3-76) ^{1}HNMR Spectrum of the compound C7

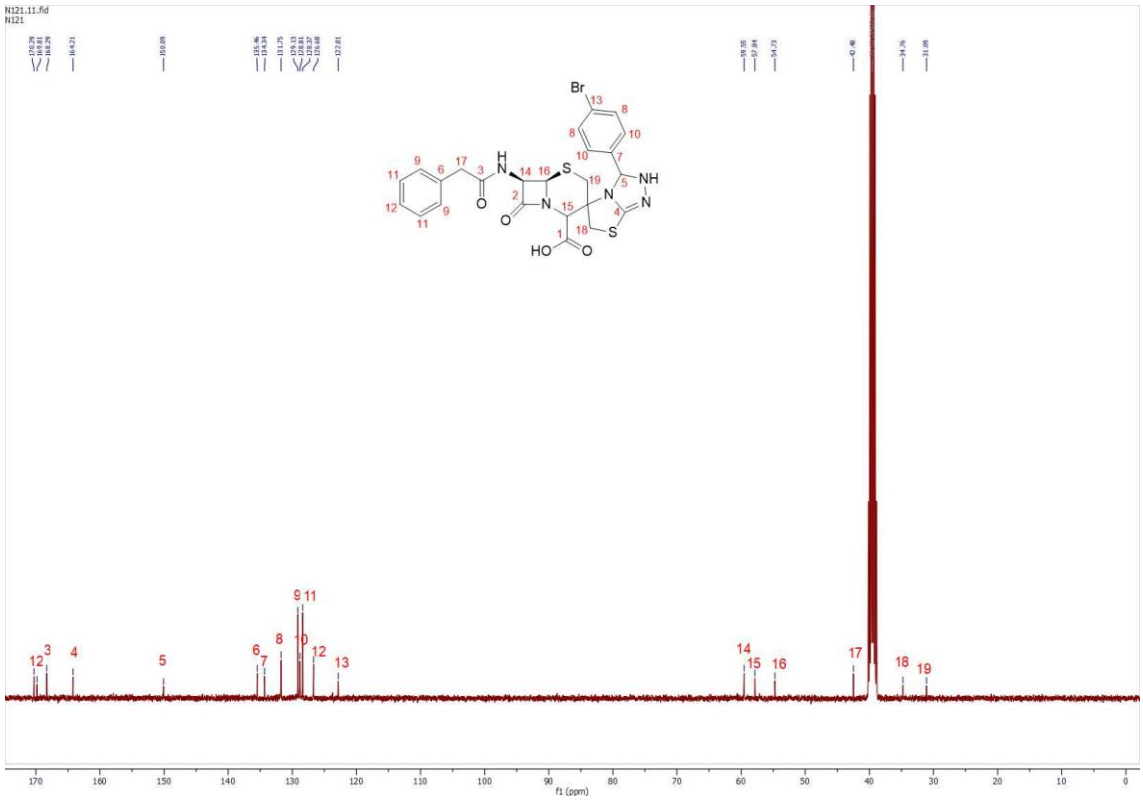


Fig (3-77) ^{13}CNMR Spectrum of the compound C7

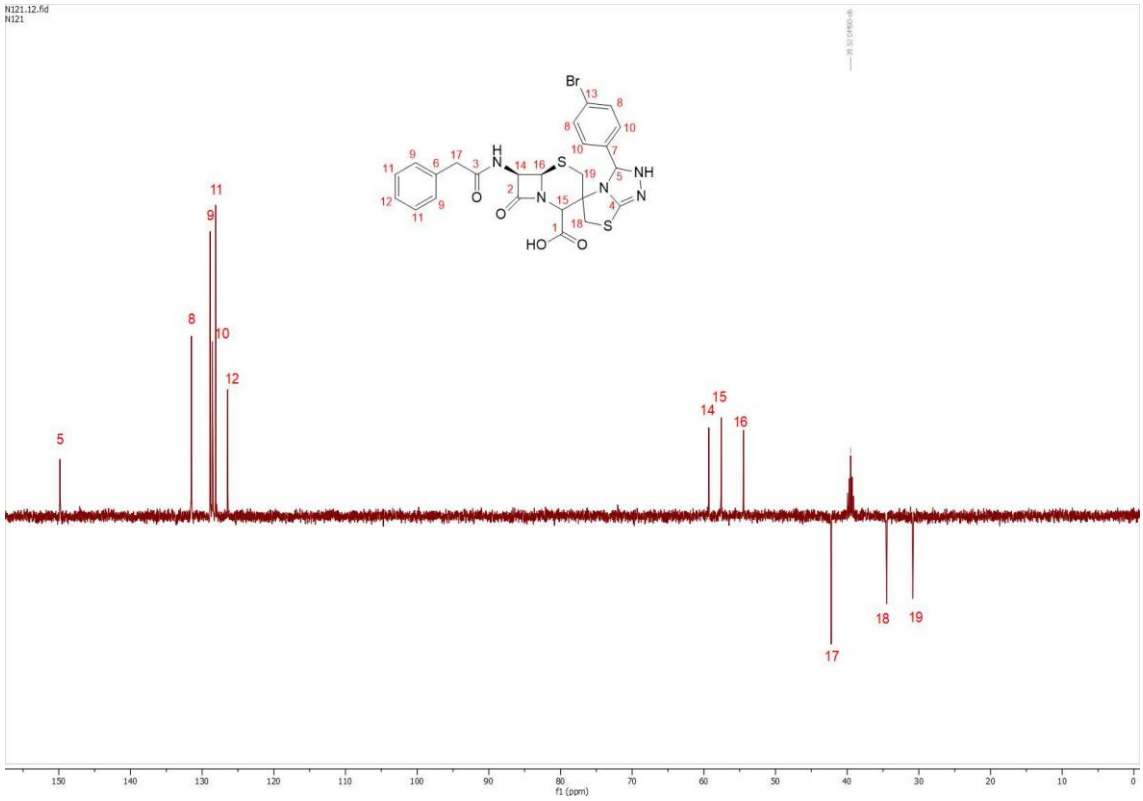


Fig (3-78) DEPT 135 Spectrum of the compound C7

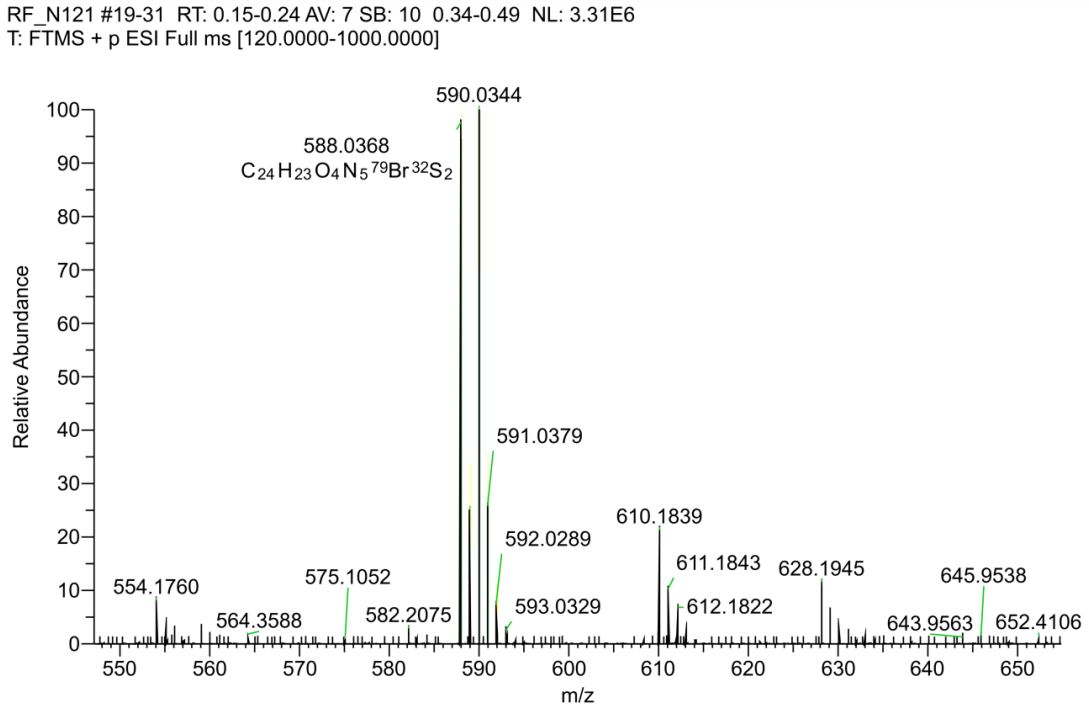


Fig (3-79) Mass Spectrum (ESI) of the compound C7

3.4. Antibacterial activity

Spiro-Cephalosporin compounds were excessive importance in medicinal chemistry, where spirocycles have become an appealing synthetic option in drug development in recent years. Spirocyclic scaffolds are increasingly being used in drug production as core or peripheral structures, and several of them exhibit intriguing biological activity.

Spiro compounds interact with proteins more effectively than their planar (hetero) aromatic analogues because of their intrinsic three-dimensional structure. Spirocyclic scaffolds also feature very stiff structures and a small number of well-defined conformations, which is advantageous for employing in *silico* docking to computationally optimize interactions between medicines and protein targets.

In our study, the antibacterial activities of the final spiro-cephalosporin compounds (C1-C7) were tested against two gram-positive and two gram-negative bacteria, which are methicillin resistance *Staphylococcus aureus*, *Listeria monocytogenes, E.coli* O157, and *Salmonella Newport*, respectively. Anti-bacterial assay was performed using 750 μg concentration for all compounds and 150 μg for ampicillin as positive control; the disc diffusion method for antimicrobial susceptibility testing was used.

All tested compounds showed good inhibition activity against methicillin resistance *Staphylococcus aureus*. Compounds C7, C6 and C2 exhibited the highest antibacterial activity with inhibition zone around 20, 15 and 13 mm, respectively, which are higher than that of ampicillin. All three compounds have phenyl ring substituted with halogen, it seems that presence of halogen atom at para- position is more favorable for antibacterial activity. Compounds

C3 and C5 showed same inhibition zone (10 mm), which is comparable to that of ampicillin, while the compounds C1 and C4 showed the lowest inhibition zone, which is 8 mm. (Figure 3-80) and (Figure 3-81).

For the other Gram-positive bacteria *Listeria monocytogenes*, all compounds were efficient except compound C2, which did not show any efficacy. Compound C5 showed the highest inhibition zone, which is 15 mm, followed by the order of compounds C7, C4, which are 13 mm and 10 mm, respectively, while both compounds C1 and C3 gave the same zone of inhibition, which is 8 mm, and finally, compound C6 gave an inhibition zone of 5 mm. These findings confirm again that presence of halogen atoms at para position of aromatic ring has a significant effect on biological activity (Figure 3-82) and (Figure 3-83).

On the other side, only compounds C1 and C4 showed weak activity against Gram negative bacteria *E. coli* with an inhibition zone of 3 and 4 mm, respectively, while the remaining five compounds did not show any biological activity at all (Figure 3-84) and (Figure 3-85). None of the synthesized spiro-cephalosporins were active against *Salmonella Newport* bacteria (Figure 3-86) and (Figure 3-87).

Although Gram +ve bacteria have a peptidoglycan layer thicker than that of Gram –ve bacteria, but they lack the protective phospholipid outer membrane which is found Gram –ve bacteria.[129] In general, all tested spiro-cephalosporins were more active against Gram +ve bacteria than Gram –ve bacteria. This promising result is in agreement with mode of action of cephalosporins which inhibit transpeptidase enzymes that lead eventually to stop the production of peptidoglycan and kill the bacterial cell.[35] This biological activity assay is just a primarily step, however it reveals that these

synthesized spiro-cephalosporins can be good candidates for development of new class of antibiotics.

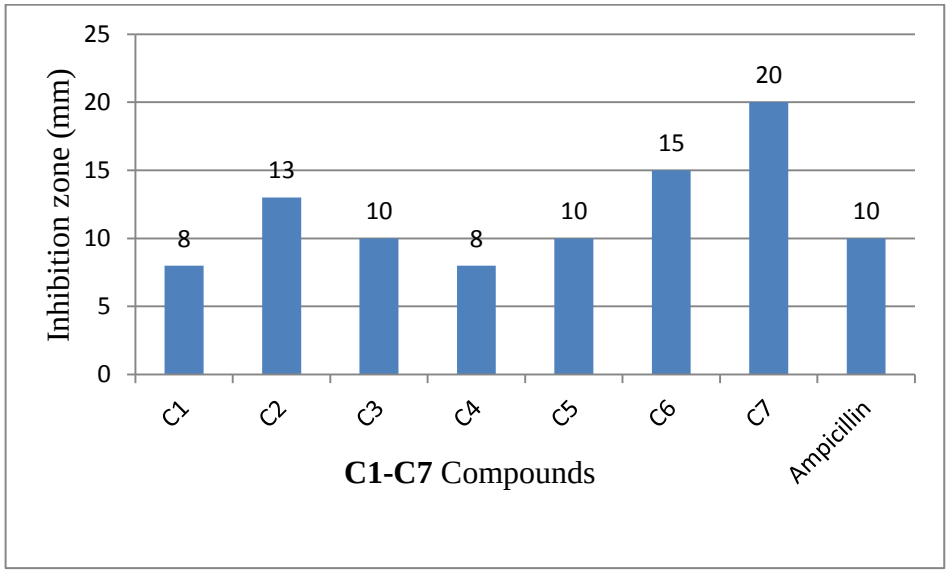


Fig (3-80) Inhibition zone towards *Staphylococcus aureus* bacteria

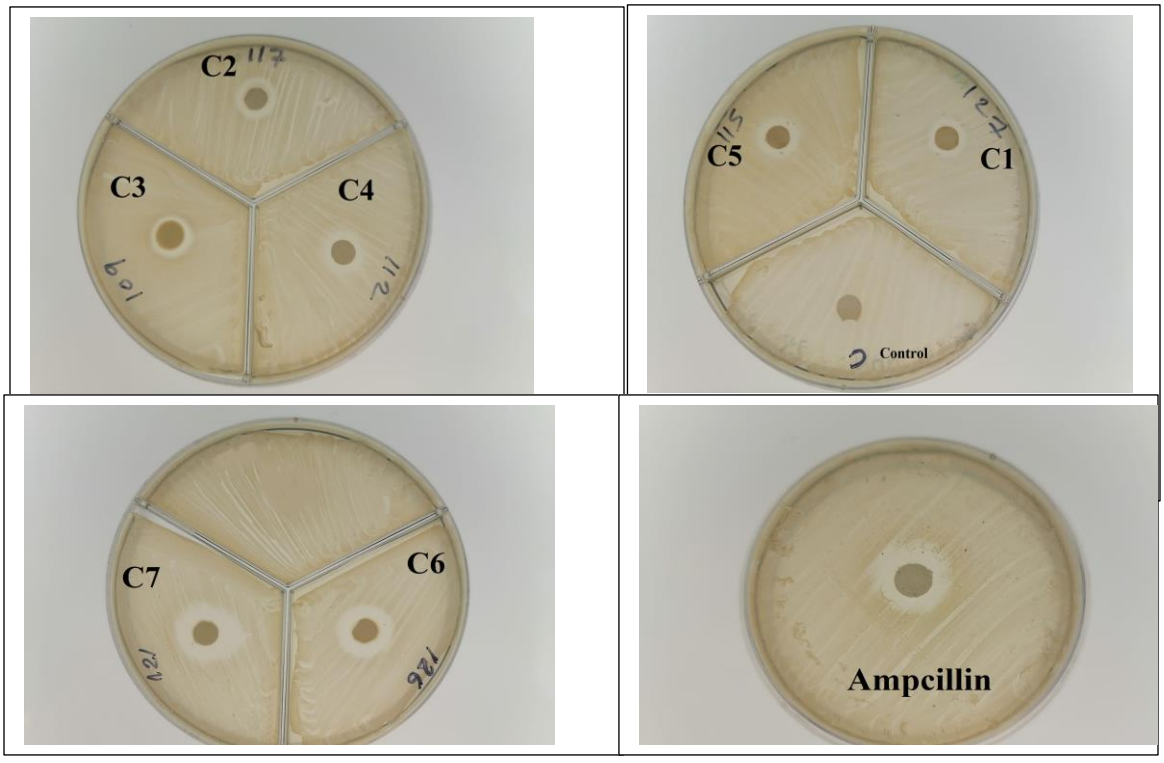


Fig (3-81) Images of inhibition zone produced by C1-C7 against *Staphylococcus aureus*

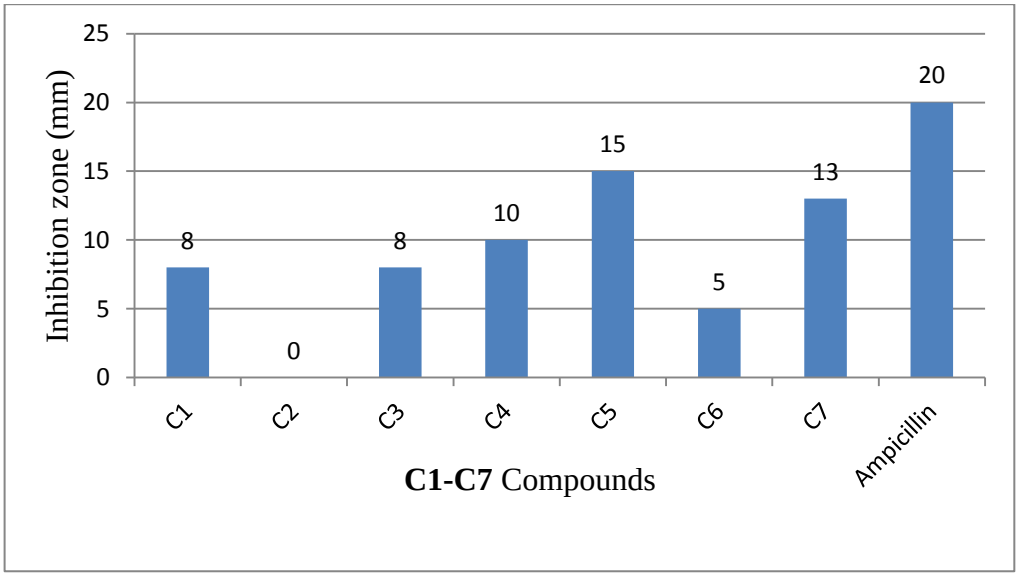


Fig (3-82) Inhibition zone towards *Listeria monocytogenes* bacteria

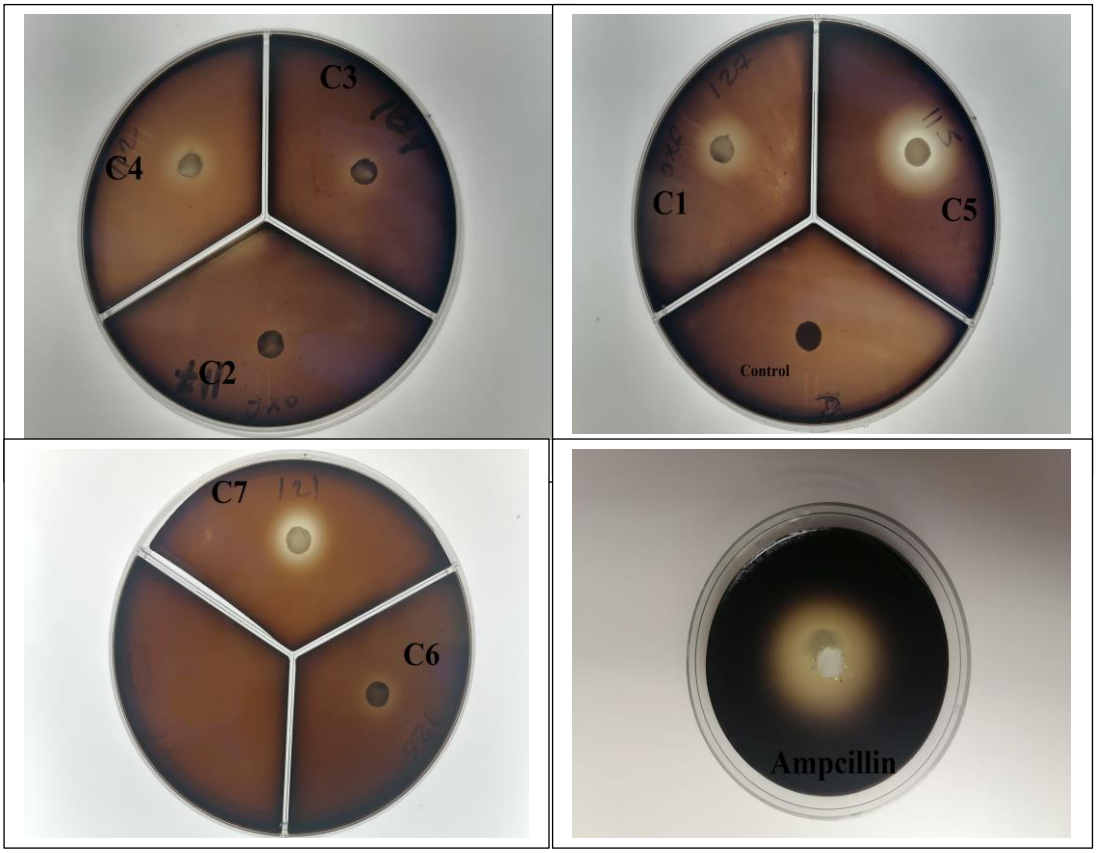


Fig (3-83) Images of inhibition zone produced by C1-C7 against *Listeria monocytogenes*

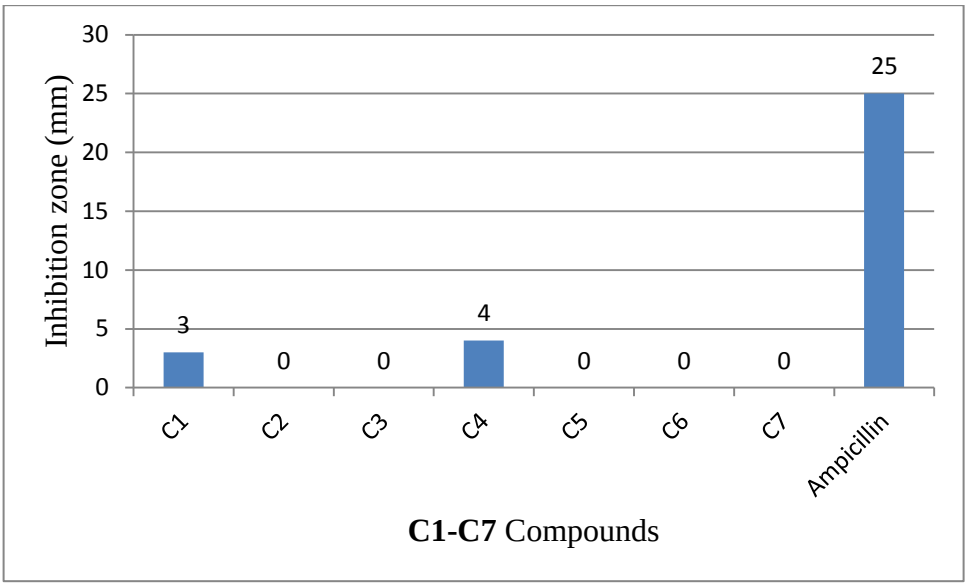


Fig (3-84) Inhibition zone towards *Escherichia coli* bacteria

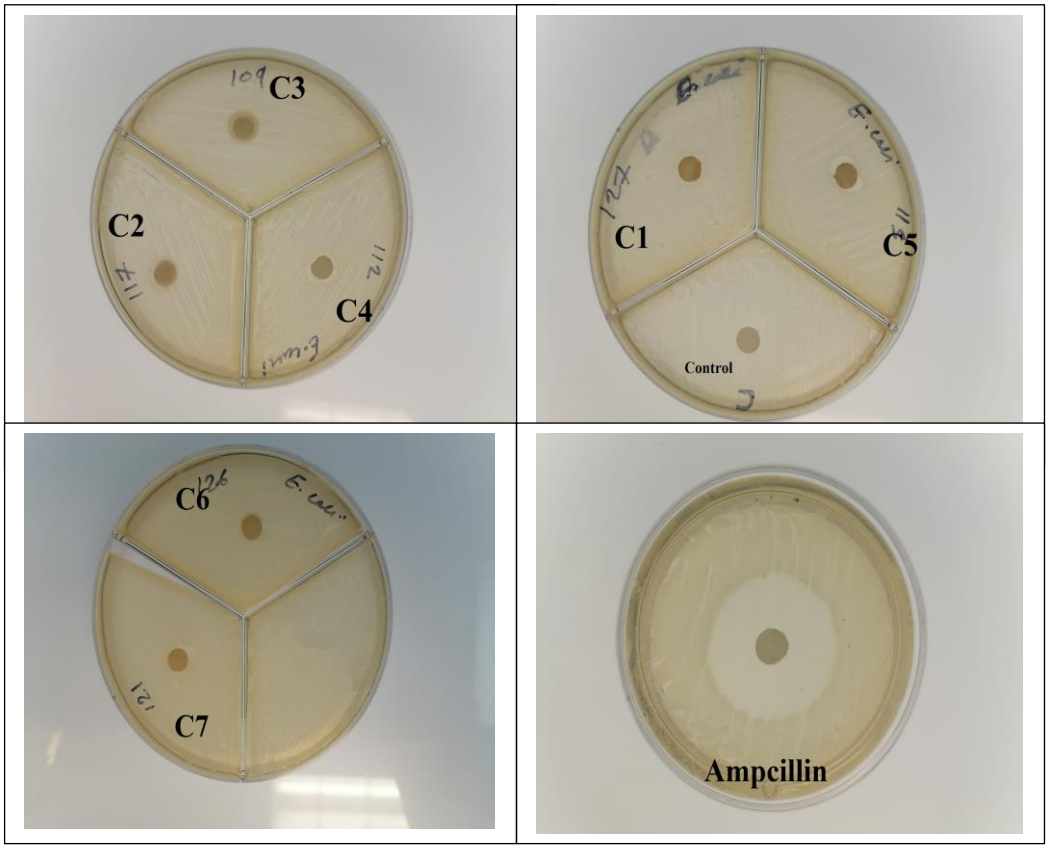


Fig (3-85) Images of inhibition zone produced by C1-C7 against *Escherichia coli*

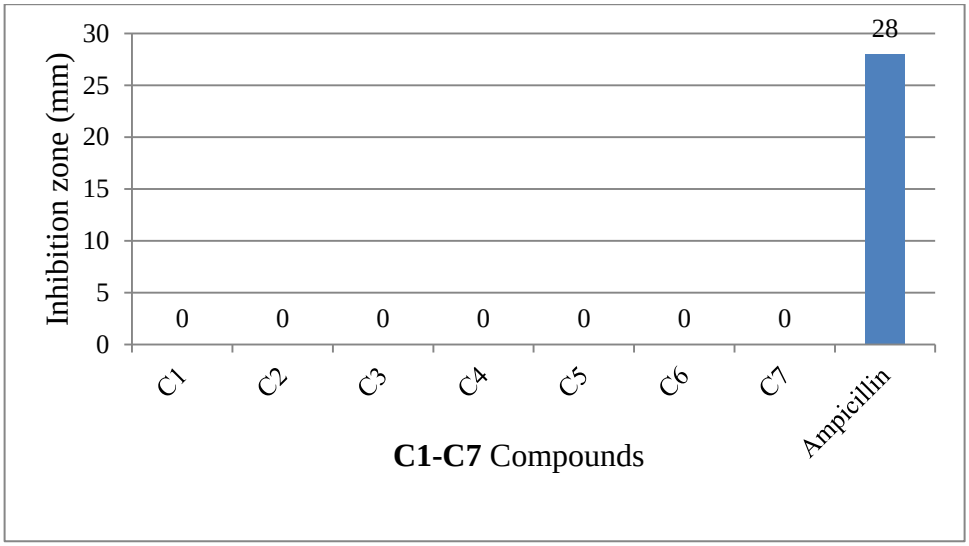


Fig (3-86) Inhibition zone towards *Salmonella Newport* bacteria

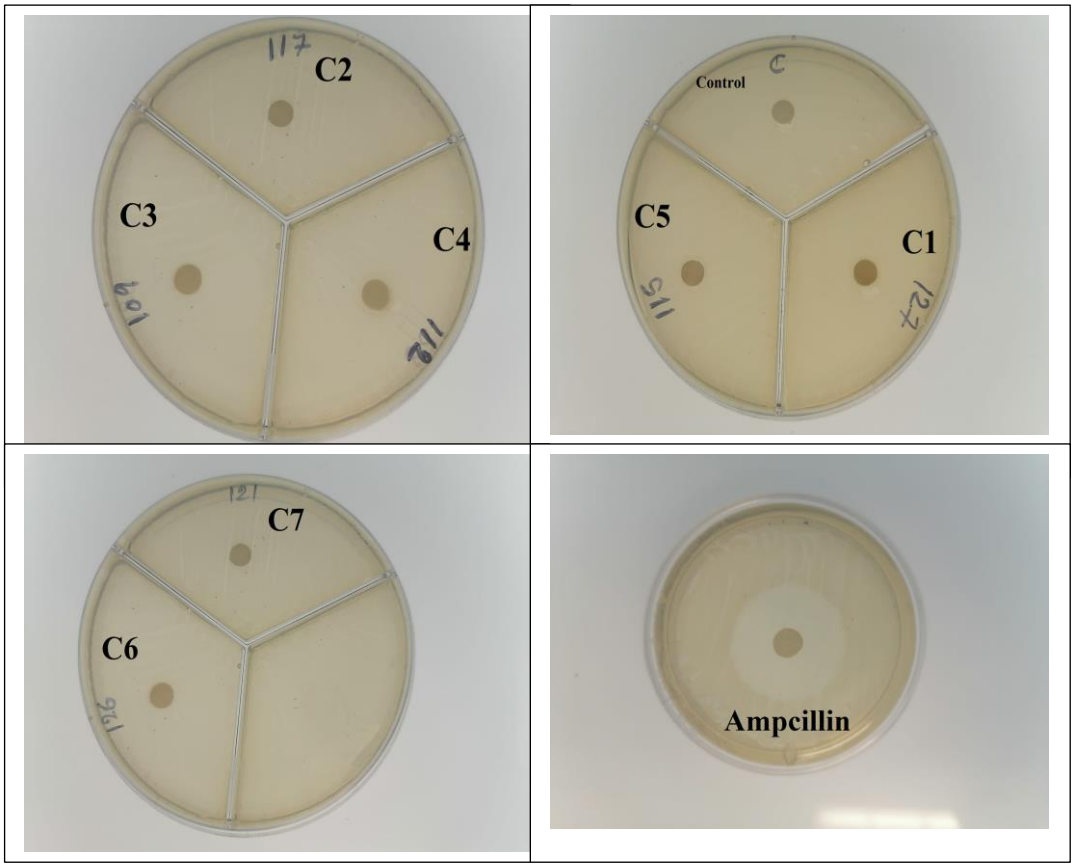


Fig (3-87) Images of inhibition zone produced by C1-C7 against *Salmonella Newport*

3.5. Conclusions

1. Some 5- aryl-1,2,4 triazolidine-3-thiones can be synthesized using green approach.

2. 5- aryl-1,2,4 triazolidine-3-thiones can undergo Michael addition to α,β-unsaturated carbonyl compounds to generate five-membered heterocyclic compounds under basic conditions.

3. No products were obtained when GCLE treated with the triazolidines **A8** and **A9** (which have OH and OCH_3 groups on para- position).

4. The synthesized compounds have significant antibacterial efficacy against Gram-positive bacteria, especially *S.aureus* bacteria.

5. The synthesized compounds did not show any activity against Gram negative bacteria, especially *Salmonella Newport*, except for compounds C1 and C4, which have low efficacy against *Escherichia coli* bacteria.

3.6. Recommendations

1. Synthesis of spiro-cephalosporins containing novel triazole derivatives.

2. Exploiting preparation method of 1,2,4-triazolidine-3-thione to prepare more sophisticated derivatives.

3. Optimization of spirocyclic formation step to increase the yield by varying the reaction conditions.

4. Replacement of 1,2,4-triazolidine-3-thiones with novel heterocycles that act as Michael addition donors in spirocyclic formation reaction.

5. Modification at C7 position of the synthesized spiro-cephalosporins to improve the biological activity.

6. Determination the stereochemistry of spiro carbon.

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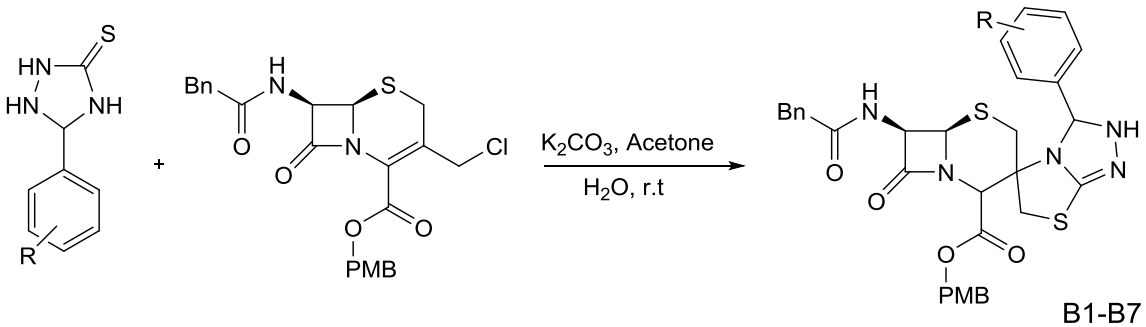
الجزء الرابع:

يلخص هذا الجزء دراسة النشاط المضاد للبكتيريا لمركبات سبايرو- سيفالوسبورين النهائية (C1 - C7). تم اختيار أربع كائنات حية، وهي *S.aureus* methicillin resistance، *L.monocytogenes* ، *E.coli* و*Salmonella Newport* ، كانت المركبات (C1 - C7) فعالة بشكل جيد ضد البكتيريا موجبة الجرام وخاصة *S.aureus*. ومع ذلك ، كان لديهم تأثير ضئيل للغاية أو معدوم ضد البكتيريا سالبة الجرام ، وخاصة بكتيريا *Salmonella Newport*.



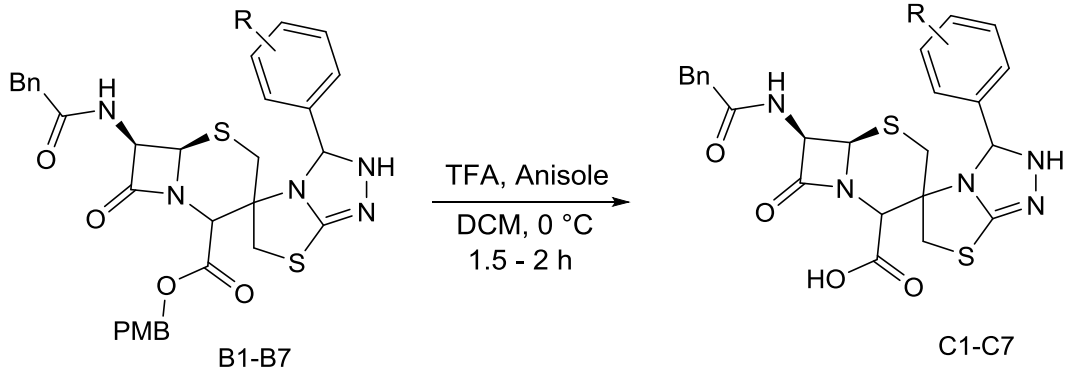
A1-A7

B1-B7

R= H, *o*-Cl, *o*-Br, *o*-OH, *p*-F, *p*-Cl, *p*-Br

الجزء الثالث:

تمت إزالة مجموعة الحماية بارا- ميثوكسي بنزايل (PMB) من السلائف المحمية (B1-B7) بوجود حامض ثلاثي فلورو أسيتيك (TFA) والأنيسول للحصول على مركبات سبايرو - سيفالوسبورين النهائية (C1-C7) بحصيلة جيدة (46-80٪). تم أيضًا تضمين تفاصيل التشخيص الطيفي الكاملة.



B1-B7

C1-C7

R= H, *o*-Cl, *o*-Br, *o*-OH, *p*-F, *p*-Cl, *p*-Br

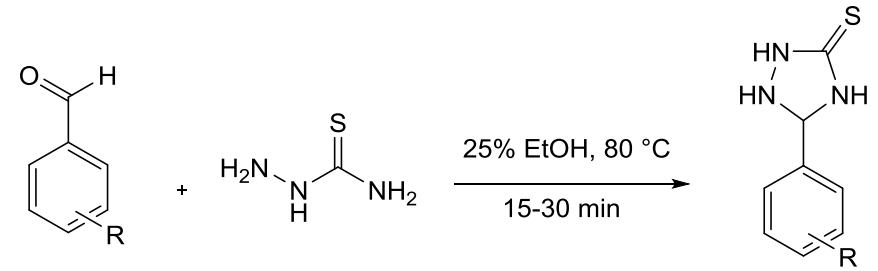
الخلاصة

يتضمن هذا العمل تحضير و تشخيص سبعة انواع جديدة من مركبات سبايرو - سيفالوسبورين بدءاً من مركب السيفالوسبورين الوسطي GCLE المتوفر تجارياً.

يمكن تقسيم هذا العمل إلى أربعة أجزاء رئيسية:

الجزء الأول:

تضمن هذا الجزء تحضير تسعة مشتقات تريازوليدين-3- ثيون (A1- A9) عبر طريقة تركيبية فعالة وصديقة للبيئة. تم تفاعل الألدهيدات الاورماتية مع ثايوسيمكاربازيد في الايثانول المائي في ظروف معتدلة بعمل بسيط وحصيلة جيدة(62-95%). ان تركيب المركبات المحضره تم تشخيصها باستخدام ال FTIR, HNMR , 13CNMR



R= H, *o*-Cl, *o*-Br, *o*-OH, *p*-F, *p*-Cl, *p*-Br, *p*-OH, *p*-OMe

A_1-A_9

الجزء الثاني:

تضمن هذا الجزء تفاعل 4،2،1 - ترايازوليدين-3- ثيون (A1- A9) مع GCLE في ظل ظروف قاعدية لتشكيل مركبات سبايرو - سيفالوسبورين (B1 - B7) بحصيلة معتدلة (17.3 - 32٪). يقترح ان يتم هذا التفاعل من خلال ألكلة من نوع S_N2 متبوعة بتفاعل أضافة ضمنية Michael-type addition إلى حلقة ثنائي هيدروثيازين.

جامعة ميسان

كلية العلوم

قسم الكيمياء

تحضير و تشخيص و دراسة النشاط المضاد للبكتريا لمركبات سبايرو- سيفالوسبورين الجديدة

الرسالة

مقدمة الى كلية العلوم/جامعة ميسان

لأستيفاء متطلبات الحصول على درجة الماجستير في علوم

الكيمياء

من قبل

نور هاشم زوير

بكالوريوس علوم كيمياء/جامعة ميسان(2017)

بأشراف

أ.م. د. أسامة علي محسن

2023